

# A new *Drosophila melanogaster* research resource: CRISPR-induced mutations for clonal analysis of fourth chromosome genes

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As part of an ongoing effort to generate comprehensive resources for the experimental analysis of fourth chromosome genes in *Drosophila melanogaster*, the Fourth Chromosome Resource Project has used CRISPR mutagenesis with single guide RNAs to isolate mutations in 62 of the 80 fourth chromosome, protein-coding genes. These mutations were induced on a fourth chromosome bearing a basal FRT insertion to facilitate experimental approaches involving FLP recombinase-induced mitotic recombination. To permit straightforward comparisons among mutant stocks, most of the mutations were generated on isogenic fourth chromosomes, which were then crossed into a common genetic background. Of the 119 mutations, 84 are frameshift mutations likely to be null alleles, 29 are small, in-frame deletions, and 6 have yet to be characterized molecularly. The mutations were tested for recessive lethal, female-sterile, and visible phenotypes. Stable stocks for most of the mutations have been submitted to repositories in the United States and Japan for public distribution.

**Keywords:** *Drosophila melanogaster*; CRISPR; fourth chromosome; mutation; Fourth Chromosome Resource Project

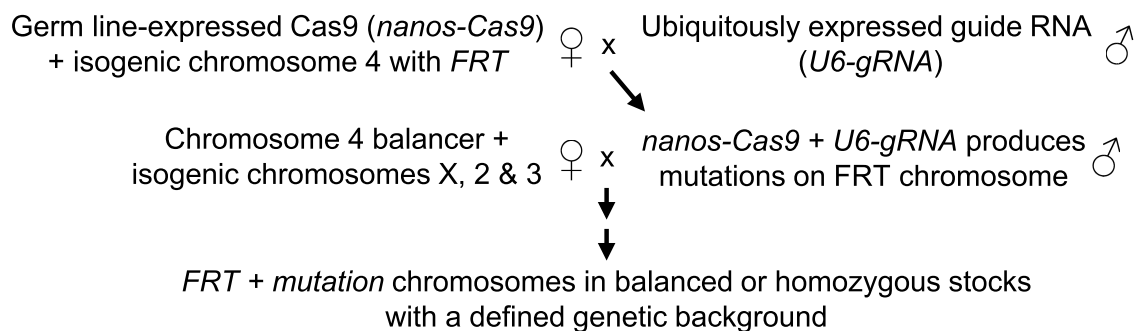
## Introduction

The specific roles a gene plays in the normal functioning of a cell are often inferred from the phenotypes seen when its activity is reduced or eliminated, and loss-of-function mutations are important for such experimental approaches. While the functional significance of a gene can sometimes be determined from phenotypes seen in individuals composed entirely of homozygous mutant cells, it is often more informative to examine the phenotypes of homozygous mutant cells embedded in an otherwise heterozygous individual. Indeed, experimental approaches involving mitotic recombination to generate small clones of mutant cells are particularly important when homozygosity results in zygotic lethality.

Most current clonal analysis experiments in *Drosophila* involve the use of the GAL4–UAS–GAL80 cell-specific expression system to control FLP recombinase-induced mitotic recombination between FRT sequences positioned at the bases of chromosome arms. These methods permit clone formation to be targeted spatially and temporally, and they allow investigators to determine the contributions of individual genes to the development, homeostasis, and adaptation of specific tissues (Weasner et al. 2017). These methods have become especially important in exploring

cell-to-cell communication. For example, they are used extensively in studying competition among cells (Kiparaki and Baker 2023).

Here, we describe the efforts of the Fourth Chromosome Resource Project to develop *Drosophila* stocks for the clonal analysis of loss-of-function phenotypes for the majority of fourth chromosome, protein-coding genes. This work was prompted by the paucity of research resources related to the fourth chromosome and by 2 experimental developments. First, Goldsmith et al. (2022) used CRISPR site-specific genome-editing technology to insert an FRT sequence at the base of the long arm of the fourth chromosome (the short arm carries no genes), which they then used to generate mitotic clones. Second, a set of stocks bearing guide RNA-expressing transgenes for most protein-coding genes on the fourth chromosome was generated at the National Institute of Genetics in Japan. We recognized that the easiest way to place mutations distal to this basal FRT was to use these guide RNAs to induce mutations directly on the FRT-bearing chromosome. To add experimental value to the stock collection, we have taken care to generate the mutations on fourth chromosomes derived from a single progenitor chromosome and to place the mutagenized chromosomes into a common genetic background. The new mutation-bearing stocks are now being distributed by stock centers in both Japan and the United States.



**Fig. 1.** Conceptual outline of the CRISPR screens producing new fourth chromosome mutations. In males, a transgene expressing Cas9 in the germ line (*nanos-Cas9*) was combined with a transgene ubiquitously expressing a guide RNA targeting a specific fourth chromosome gene (*U6-gRNA*) and a transgene carrying an FRT sequence inserted near the base of an isogenized fourth chromosome. Many of the gametes produced by these males carried mutations in the targeted gene caused by Cas9-induced double-stranded DNA breaks. A series of crosses involving stocks with balancer chromosomes and isogenic X, second, and third chromosomes produced balanced or homozygous stocks with a defined genetic background. [Supplementary File 1](#) provides a detailed description of the screens and the development of the stocks used.

## Materials and methods

An extensive description of our experimental methods is provided in [Supplementary File 1](#). It provides details of the screens isolating CRISPR-induced mutations on a single-origin fourth chromosome carrying the FRT transgene *Tl{Ti}FRT101F* (Goldsmith et al. 2022); the crosses placing the mutagenized chromosomes into a common, isogenic background; the construction of stocks necessary for these crosses; the molecular characterization of the mutations; and the preliminary characterization of associated phenotypes. Mutations in 4 genes were generated by alternative crosses, which are also outlined. [Supplementary Table 1](#) gives a list of fourth chromosome genes. [Supplementary Table 2](#) provides details of the guide RNA transgenes used in the screens. [Supplementary Table 3](#) gives primers utilized to identify and characterize new mutations. [Supplementary Table 4](#) is the Reagent Table listing the progenitor stocks used to develop the strains employed in the screens, the stocks used to characterize mutant phenotypes, and the sources of biological and chemical reagents.

All fly cultures were reared under standard conditions. All genomic coordinates cited in this report refer to *D. melanogaster* genome release 6. Gene models, annotations, and nomenclature reflect FlyBase release 2024\_4. Information about the new mutations in publicly distributed stocks has been archived in FlyBase (Öztürk-Çolak et al. 2024). Information about the stocks may be obtained via the websites of the Bloomington Drosophila Stock Center at Indiana University (<https://bdsc.indiana.edu/>) and the Department of Drosophila Genomics and Genetic Resources at the Kyoto Institute of Technology (<https://www.dgcr.kit.ac.jp/>). A preliminary report of this work was presented by Stinchfield et al. (2024).

## Results and discussion

As mentioned, our effort to generate CRISPR-induced mutations in fourth chromosome genes on an FRT-bearing chromosome depended on the availability of a set of guide RNA-expressing stocks from the Japan National Institute of Genetics. These stocks were generated as part of a large-scale project to construct guide RNA-expressing stocks for all *D. melanogaster* protein-coding genes, which will be described elsewhere. The transgenes in these stocks were constructed by the methods of Kondo and Ueda (2013), and they ubiquitously express guide RNAs with 20-base targeting sequences under the control of *snRNA:U6:96Ab* regulatory

sequences. Stocks targeting 77 of the 80 protein-coding genes on the fourth chromosome were available for our efforts (those we used are listed in [Supplementary Table 2](#)). In addition, we used 1 guide RNA stock originating in the work of Zirin et al. (2020).

As outlined in [Fig. 1](#) and shown in detail in [Supplementary File 1](#), the guide RNA-expressing transgenes were combined in males with a transgene expressing the Cas9 nuclease in the germ line and a fourth chromosome carrying the FRT transgene *Tl{Ti}FRT101F*. All FRT-bearing chromosomes used in screens were derived from a single chromosome to minimize sequence variation across all screens. Putative mutation-bearing fourth chromosomes were isolated in balanced or homozygous stocks and tested for alterations at the guide RNA target sites by PCR amplification followed by Sanger sequencing. We generally found it necessary to sequence fewer than 10 mutagenized chromosomes per gene to identify 1 or more independent mutations. [Table 1](#) summarizes the results of the 69 screens undertaken using this method and [Supplementary Table 5](#) describes the mutations chosen for phenotypic characterization. In several instances, we recovered independent mutations with identical molecular lesions—reflecting the predictability of CRISPR mutagenesis and DNA repair ([Table 1](#)).

The crosses to establish new mutations in balanced or homozygous stocks simultaneously placed the mutagenized fourth chromosomes into a defined genetic background so that, to the extent practical, the mutation-bearing stocks would differ at the sequence level only by the induced mutations. As shown in [Supplementary File 1](#), the isogenic background consists of second and third chromosomes derived from the genetic background used for the DrosDel deletions (Ryder et al. 2007), the *Dp(1;Y)BSC* chromosomes (Cook et al. 2010) and their progenitor insertions (Ryder et al. 2004), and an X chromosome marked with *y*<sup>1</sup> and *w*<sup>1118</sup> derived from a heavily used Bloomington stock. The Y chromosomes in the final stocks are derived from the guide RNA stocks and cannot be assumed to be uniform. Fourth chromosomes bearing dominant mutations with visible phenotypes (e.g. *ci*<sup>D</sup> or *Gat*<sup>ey</sup>) or transgenes expressing fluorescent reporter proteins (e.g. *P[ActGFP]*unc-13*<sup>CJ</sup>*) were used to balance the new, fourth chromosome mutations. [Supplementary Table 6](#) lists the stocks that have been placed into public distribution to date at the Drosophila stock centers in Bloomington and Kyoto.

We encountered idiosyncratic lethality and sterility in the early crosses of 3 screens. While these problems were unrelated to the mutageneses per se and are likely attributable to cryptic mutations

**Table 1.** Recovery of fourth chromosome mutations in screens.

| Gene                                              | Mutagenized chromosomes recovered <sup>a</sup> | Frameshift mutations | In-frame deletions | No mutation |
|---------------------------------------------------|------------------------------------------------|----------------------|--------------------|-------------|
| <b>Primary screening approach<sup>b,c</sup></b>   |                                                |                      |                    |             |
| 4E-T                                              | 3                                              | 2                    | 0                  | 0           |
| Abcd1                                             | 7                                              | 3                    | 1                  | 0           |
| Actbeta                                           | 2                                              | 1                    | 1                  | 0           |
| Ank                                               | 5                                              | 1                    | 0                  | 0           |
| anne                                              | 3                                              | 3                    | 0                  | 0           |
| apolpp                                            | 4                                              | 3 Identical          | 1                  | 0           |
| Arf4                                              | 4                                              | 1                    | 0                  | 0           |
| Arl4                                              | 2                                              | 0                    | 1                  | 0           |
| ATPsynbeta                                        | 5                                              | 1                    | 0                  | 1           |
| Cadps                                             | 8 <sup>d</sup>                                 | 1                    | 0                  | 3           |
| Cals                                              | 4                                              | 0                    | 1                  | 2           |
| CaMKI                                             | 8                                              | 2                    | 0                  | 0           |
| CG11360                                           | 5                                              | 1                    | 0                  | 0           |
| CG1674                                            | 4                                              | 2                    | 1                  | 0           |
| CG1909                                            | 2                                              | 1                    | 1                  | 0           |
| CG31998                                           | 3                                              | 2                    | 0                  | 0           |
| CG31999                                           | 4                                              | 3 (2 identical)      | 1                  | 0           |
| CG32017                                           | 8                                              | 2                    | 1                  | 4           |
| CG33521                                           | 3                                              | 3 Identical          | 0                  | 0           |
| CG33978                                           | 1                                              | 1                    | 0                  | 0           |
| ci                                                | 4                                              | 1                    | 2                  | 0           |
| Crk                                               | 11                                             | 4 Identical          | 1                  | 6           |
| dpr7                                              | 3                                              | 0                    | 2 Identical        | 0           |
| Dyrk3                                             | 3 <sup>d</sup>                                 | 0                    | 0                  | 0           |
| elF4G1                                            | 6                                              | 2                    | 1                  | 3           |
| Ekar                                              | 2                                              | 0                    | 2                  | 0           |
| Eph                                               | 5                                              | 4 (2 identical)      | 0                  | 0           |
| Ephrin                                            | 1                                              | 1                    | 0                  | 0           |
| ey                                                | 5                                              | 5 (3 identical)      | 0                  | 0           |
| fd102C                                            | 3                                              | 1                    | 0                  | 0           |
| Gyf                                               | 7                                              | 2                    | 0                  | 2           |
| Hcf                                               | 5                                              | 3 Identical          | 2 Identical        | 0           |
| Kif3C                                             | 2                                              | 0                    | 1                  | 1           |
| lgs                                               | 3                                              | 1                    | 1                  | 1           |
| mav                                               | 4                                              | 2                    | 0                  | 2           |
| MED26                                             | 3                                              | 0                    | 2 Identical        | 1           |
| mGluR                                             | 4                                              | 4                    | 0                  | 0           |
| Mitf                                              | 3 <sup>d</sup>                                 | 0                    | 0                  | 0           |
| myo                                               | 3                                              | 1                    | 2 Identical        | 0           |
| ND-49                                             | 3                                              | 0                    | 3 Identical        | 0           |
| Nfl                                               | 5                                              | 5 (2 identical)      | 0                  | 0           |
| oncut                                             | 4                                              | 3                    | 0                  | 1           |
| pan                                               | 2                                              | 2                    | 0                  | 0           |
| pho                                               | 5                                              | 1                    | 0                  | 2           |
| PMCA                                              | 4                                              | 1                    | 0                  | 1           |
| Pur-alpha                                         | 5                                              | 1                    | 0                  | 4           |
| Rad23                                             | 9                                              | 3                    | 2 Identical        | 1           |
| RhoGAP102A                                        | 5                                              | 3 (2 identical)      | 0                  | 2           |
| Rnf11                                             | 6                                              | 6 (4 identical)      | 0                  | 0           |
| Slip1                                             | 2                                              | 0                    | 1                  | 1           |
| Sox102F                                           | 5                                              | 2 Identical          | 1                  | 2           |
| sv                                                | 4                                              | 0                    | 2                  | 2           |
| Syt7                                              | 3                                              | 1                    | 2                  | 0           |
| Taf3                                              | 3                                              | 2 Identical          | 1                  | 0           |
| Tdg                                               | 8                                              | 4 (2 identical)      | 4 (3 identical)    | 0           |
| toy                                               | 5                                              | 4 (3 identical)      | 1                  | 0           |
| ukar                                              | 1                                              | 0                    | 1                  | 0           |
| yellow-h                                          | 3                                              | 2                    | 0                  | 0           |
| zfh2                                              | 5                                              | 5 (4 identical)      | 0                  | 0           |
| Zyx                                               | 3                                              | 3 Identical          | 0                  | 0           |
| <b>Alternative screening approach<sup>c</sup></b> |                                                |                      |                    |             |
| bt                                                | 8                                              | 6 Identical          | 2 Identical        | 0           |
| JYalpha                                           | 9                                              | 2                    | 2                  | 4           |
| unc-13                                            | 4                                              | 0                    | 1                  | 0           |
| zfh2                                              | 2                                              | 1                    | 1                  | 0           |

<sup>a</sup> In general, we sequenced target sites on mutagenized chromosomes from a screen only until we identified desirable mutations; consequently, some mutagenized chromosomes were never analyzed.

<sup>b</sup> Screens for 9 genes (*Asator*, *CamKII*, *CG32006*, *dati*, *fuss*, *Gat*, *gw*, *Mpv17*, and *PIP4 K*) using the primary screening approach produced either no mutations or no viable stocks.

<sup>c</sup> Screens for *zfh2* mutations were undertaken using both screening approaches.

<sup>d</sup> The molecular lesions associated with *Cadps*<sup>G</sup>, *Dyrk3*<sup>A</sup>, *Dyrk3*<sup>B</sup>, *Dyrk3*<sup>C</sup>, *Mitf*<sup>A</sup>, and *Mitf*<sup>C</sup> have not yet been identified.

**Table 2.** Loss-of-function phenotypes of the fourth chromosome, protein-coding genes.<sup>a</sup>**Lethality**

*Actbeta*, *anne*, *apolpp*, *Arf4*, *Asator*, *ATPSynbeta*, *bt*, *Cadps*, *CamKII*, CG31998, CG32006, CG33978, *ci*, *Crk*, *dati*, *Dyrk3*, *eIF4G1*, *ey*, *Gat*, *gw*, *Hcf*, *Kif3C*, *lgs*, *MED26*, *Mitf*, *myo*, *oncut*, *pan*, *pho*, *PIP4 K*, *PlexA*, *PlexB*, *PMCA*, *RpS3A*, *sv*, *Taf3*, *toy*, *unc-13*, *zfh2*

**Male sterility**

*JYalpha*

**Viability**

4E-T, *Abcd1*, *Ank*, *CamKI*, CG1674, CG1909, CG11360, CG32017, CG33521, CG33941, *dpr7*, *Ekar*, *Eph*, *Ephrin*, *fd102C*, *fuss*, *Gyf*, *mav*, *mGluR*, *Mpv17*, *Nfl*, *Polr1G*, *Pur-alpha*, *Rad23*, *RhoGAP102A*, *Rnf11*, *Sox102F*, *Syt7*, *Tdg*, *ukar*, *yellow-h*, *Zip102B*, *Zyx*

**Unknown**

*Ar14*, *Cals*, CG31997, CG31999, CG46466<sup>b</sup>, ND-49, *Slip1*

<sup>a</sup> [Supplementary Table 11](#) summarizes the evidence for categorizing each gene. We did not attempt to quantify intermediate levels of mortality. With the exception of *ey* mutations, which have well-characterized partial lethality, we categorized “lethality” as the presence of few if any viable escapers.

<sup>b</sup> New gene annotations by FlyBase scientists and collaborators added CG46466 in 2023, changing the number of protein-coding genes from 79 to 80.

in the guide RNA stocks, [Table 1](#) shows that we were able to recover mutations using the same guide RNA stocks in alternative crosses (described in [Supplementary File 1](#)). [Supplementary Table 5](#) describes the mutations and [Supplementary Table 6](#) lists the stocks. Note that these screens and subsequent stock constructions did not rigorously control the genetic backgrounds and that future screens by the Fourth Chromosome Resource Project will use variations of this approach and will, likewise, not control for genetic background in a disciplined way.

The predominant outcome of the mutageneses was the induction of small deletions ([Supplementary Table 5](#)). In any mutagenesis, deletions that result in frameshifted translation lead to the production of truncated proteins, while in-frame deletions lead to the loss of a small number of protein residues. Frameshifted alleles that eliminate substantial portions of proteins are likely to be amorphic, while in-frame deletion alleles may or may not affect protein function depending on the consequences of removing particular amino acids. Our goal was to recover at least 1 truncation mutation for each gene screened. We were largely successful as we recovered truncation mutations in 52 of the 72 genes for which we undertook screens ([Table 1](#)). Since the median position of the truncations was 14% of normal protein length ([Supplementary Table 5](#)), the mutations likely shorten proteins enough to result in null phenotypes. For 51 of the 52 genes with truncation mutations, all predicted protein isoforms are affected (2 of the 7 predicted *Crk* isoforms are unaffected by *Crk<sup>E</sup>* even though, as described in the following, we showed it is a loss-of-function allele). The Fourth Chromosome Resource Project will revisit the genes for which we recovered no truncation alleles. We identified mutations in 3 genes from their lethality in complementation tests even though we have yet to identify the molecular lesions ([Table 1](#)).

While loss-of-function phenotypes have been characterized for many fourth chromosome genes, they are unknown for others. To evaluate, at least superficially, the effects of disrupting fourth chromosome genes, we assessed the viability, female fertility, and gross morphology of flies in which the new alleles were homozygous and in flies in which the new alleles were combined with preexisting mutations or chromosomal deficiencies that delete the targeted genes (the deficiencies are described in [Supplementary Table 7](#), control crosses validating the deficiency and preexisting mutation stocks are shown in [Supplementary Table 8](#), and the crosses evaluating the new mutations are detailed in [Supplementary Table 9](#)).

Our results are summarized in [Supplementary Table 10](#), which shows that the phenotypes of mutation homozygotes and hemizygotes are consistent. Homozygotes occasionally have more severe phenotypes than hemizygotes as one might expect if linked, deleterious mutations—preexisting or generated in the course of the crosses—were homozygosed along with the targeted mutations. Our results are presented in the context of previous phenotypic studies in [Supplementary Table 11](#). This analysis allowed us to define the loss-of-function phenotypes for 35 genes that had never been characterized: 12 are necessary for viability while 23 have no overt loss-of-function phenotypes. It also allowed us to confirm and extend the previous characterizations of 29 genes and provide evidence that 3 in-frame deletions from our screens are loss-of-function alleles.

Several decades ago, Benjamin Hochman and colleagues attempted to determine the number of fourth chromosome loci needed for viability or female fertility via mutagenesis and complementation analyses [reviewed in Hochman (1976)]. They identified 37 loci, but they did not know how close they were to isolating mutations in all vital loci. They were aware that factors such as complex intragenic complementation patterns and the low mutability of some genes make it difficult to arrive at a definitive count of genes using their approach. Our analysis of protein-coding genes ([Table 2](#)) shows 39 genes needed for viability, 1 gene needed strictly for male fertility, 33 genes with no overt phenotypes, and 7 genes for which there is no information. We have seen no evidence that any fourth chromosome genes have phenotypes affecting only external morphology or female fertility. (The absence of genes devoted exclusively to female fertility is not surprising: Perrimon et al. (1986) estimated that the X chromosome, which has more than 20 times as many genes as the fourth chromosome, carries only 15 genes needed solely for female reproduction.) There is a growing consensus that noncoding RNA genes usually have subtle loss-of-function phenotypes (Liu et al. 2017; Schor et al. 2018; Camilleri-Robles et al. 2024). The lack of obvious phenotypes associated with deletion of the antisense RNA gene *asRNA:CR44031* ([Supplementary Tables 8 and 11](#)) and increased male-to-male courtship observed as the only apparent effect associated with deletion of the long noncoding RNA gene *lncRNA:sphinx* (Dai et al. 2008) are consistent with this view. If we assume that the remaining 25 noncoding RNA genes are unlikely to have lethal or sterile phenotypes, our relatively complete inventory agrees well with the count of Hochman and colleagues, suggesting that their studies were closer to identifying mutations in all vital loci than Hochman concluded in his most comprehensive review (Hochman 1976). Completion of the Fourth Chromosome Resource Project will facilitate a comprehensive assessment of loss-of-function phenotypes (including phenotypes more subtle than we assessed in our tests), and it will then be possible to determine how the Hochman vital complementation groups, most of which are still represented in extant stocks, correspond to molecularly defined genes.

We have confirmed the presence of the basal FRT-bearing transgene *TI(TI)FRT101F* in all the final stocks by PCR amplification. Although there is no reason to suspect the mutagenesis procedure methodically corrupted the FRT-bearing transgene, we have not systematically verified that the mutation-bearing chromosomes are capable of participating in FLP-mediated mitotic recombination. Nonetheless, Stinchfield et al. (2024) successfully recovered mitotic clones with fourth chromosomes from 2 stocks (*Pur-alpha<sup>B</sup>* and *zfh2<sup>51A</sup>*).

To assess whether the mutagenesis procedure produced off-target mutations on the fourth chromosome, we sampled stocks for the presence of unexpected mutations producing lethal,

female-sterile, or visible phenotypes (Supplementary Table 12). We tested twelve chromosomes with targeted mutations that were lethal (*Actbeta<sup>B</sup>*, *apolpp<sup>B</sup>*, *Arf4<sup>A</sup>*, *ATPsynbeta<sup>C</sup>*, *bt<sup>A</sup>*, *CG31998<sup>C</sup>*, *CG31998<sup>D</sup>*, *CG33978<sup>B</sup>*, *ci<sup>C</sup>*, *ey<sup>B</sup>*, *pho<sup>F</sup>*, *sv<sup>F</sup>*, *toy<sup>C</sup>*) for the presence of additional deleterious mutations by combining these chromosomes via crosses with a panel of deficiencies and mutations that together delete or disrupt all fourth chromosome genes. None of these chromosomes showed evidence of additional mutations. Similarly, we tested 5 chromosomes with targeted mutations that produced no overt phenotypes in combination with deficiencies or preexisting mutations yet showed appreciable recessive lethality or sterility in stocks (*CG32017<sup>K</sup>*, *Gyf<sup>F</sup>*, *Hcf<sup>A</sup>*, *Pur-alpha<sup>B</sup>*, *Slip1<sup>A</sup>*). None of these chromosomes had additional mutations. The poor viability in these and similar stocks may come from sublethal effects of the targeted mutations combined with sublethal effects of other mutations that were induced during the mutagenesis or that arose spontaneously in subsequent culture. The conclusions we can draw from all these crosses are limited: we did not test all mutated chromosomes, and it was impossible to test for off-target mutations in every fourth chromosome gene because some genes are deleted only by the same deficiencies as targeted genes. Nevertheless, these results indicate that off-target mutations were not common.

In summary, the work described here has provided a new resource for examining the cellular functions of most protein-coding genes on the fourth chromosome. It complements the other resources being generated by the Fourth Chromosome Resource Project (Stinchfield et al. 2024) including UAS constructs for fourth chromosome gene misexpression and phenotypic rescue, GAL4 knock-ins for expressing UAS constructs in the normal patterns of fourth chromosome genes, UAS constructs for the human homologs of *Drosophila* fourth chromosome genes for creating “humanized” flies with human gene expression substituted for fly gene expression, GFP knock-ins into fourth chromosome genes for expressing fluor-tagged proteins in native gene patterns, and UAS-RNAi transgenes for GAL4-directed, tissue-specific knockdown of fourth chromosome genes. Our hope is that these resources will alleviate many of the difficulties that have historically hampered investigations of fourth chromosome gene functions.

## Data availability

The accompanying tables contain complete mutation-characterization data. Stocks for mutations chosen for public distribution may be obtained from the Bloomington *Drosophila* Stock Center or the Department of *Drosophila* Genomics and Genetics Resources of the Kyoto Institute of Technology as indicated in Supplementary Table 6.

Supplemental material available at G3 online.

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## Conflicts of interest

The author(s) declare no conflict of interest.

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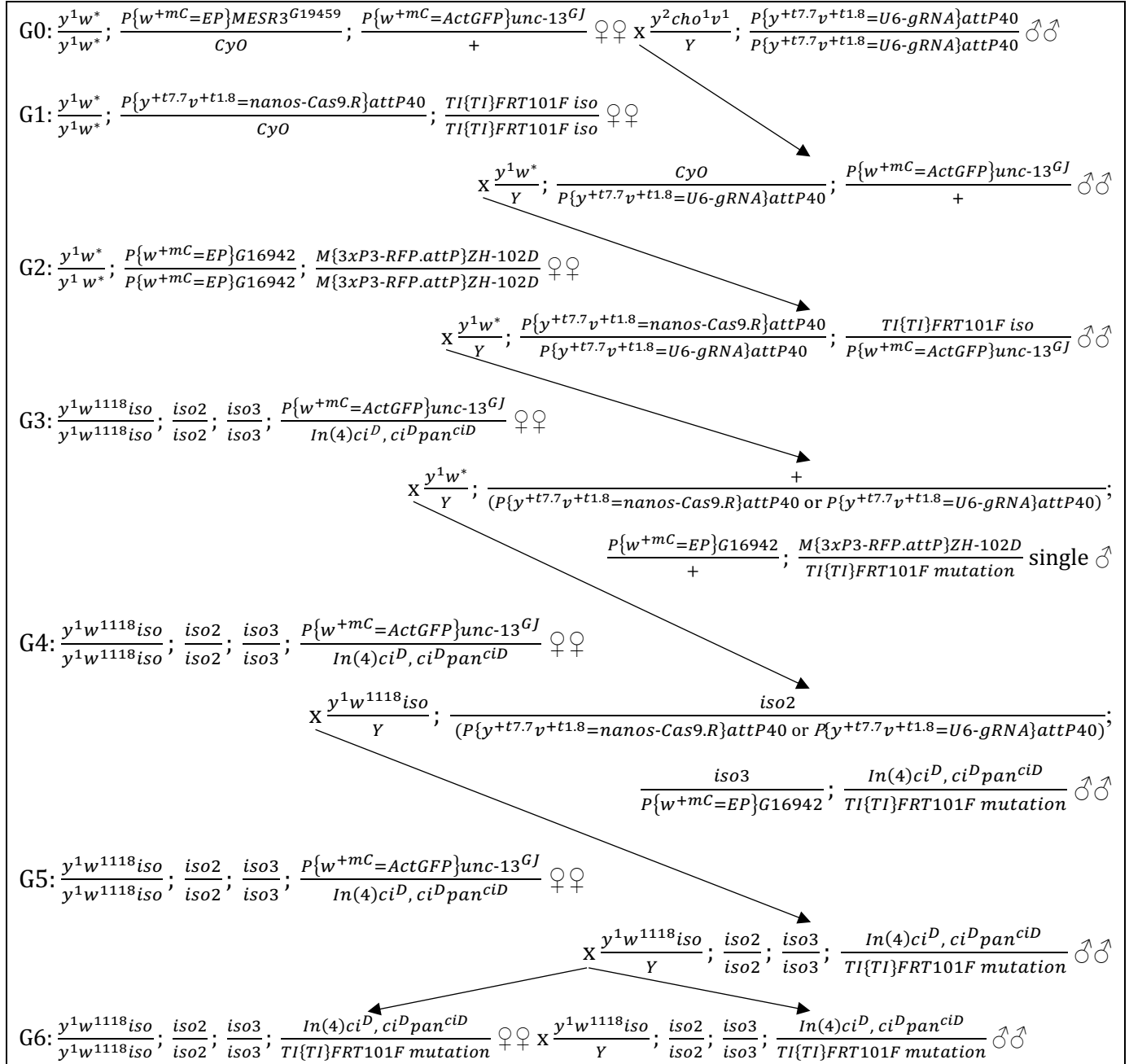
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## Supplementary File 1. Mutagenesis and mutation characterization

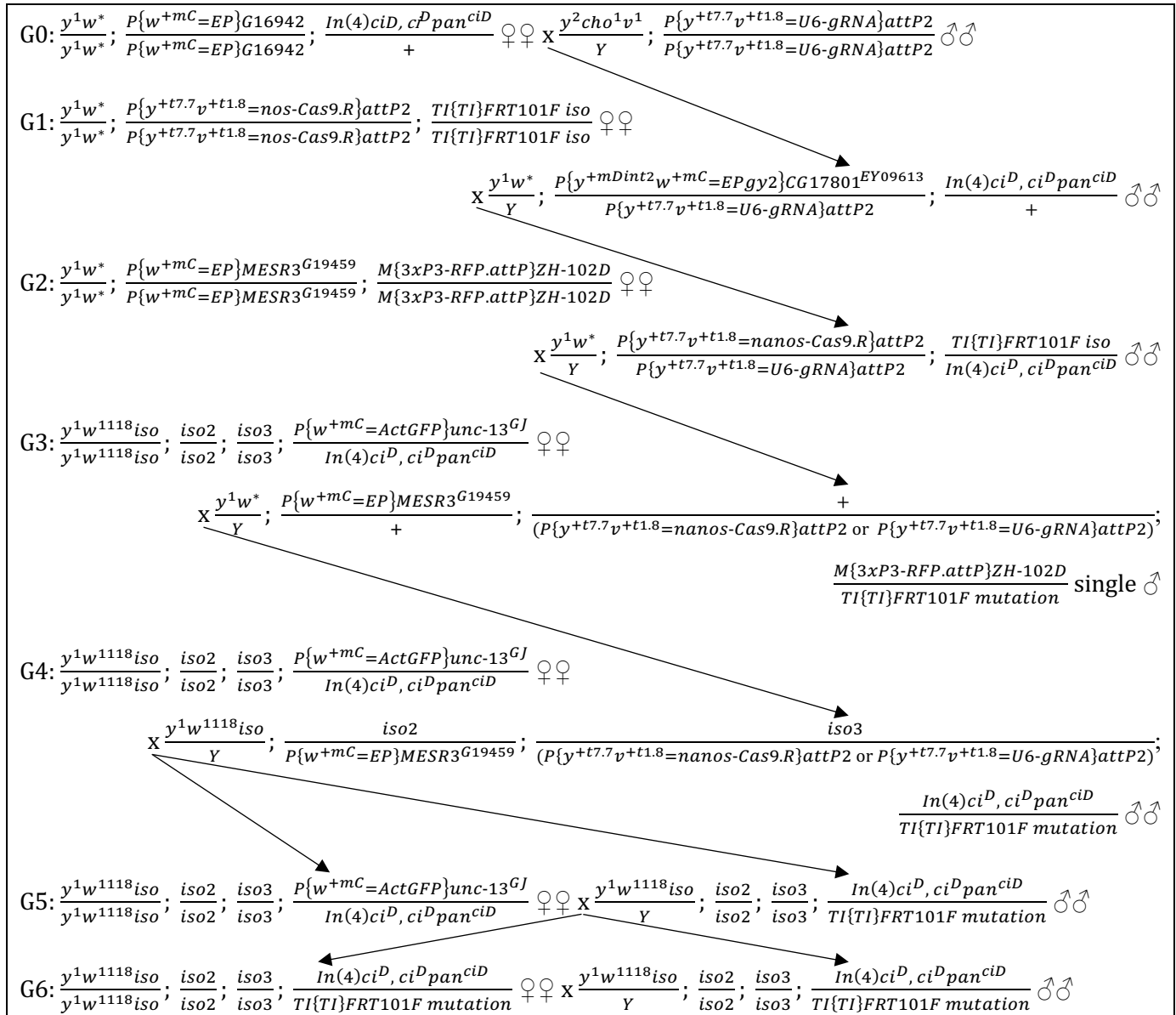
### Primary approach for CRISPR mutagenesis and the construction of isogenic stocks

The following genetic scheme shows how we generated mutations on an isogenic, FRT-bearing fourth chromosome in the male germ line using guide RNA constructs located on the second chromosome, and how we established the mutations in stocks sharing a defined genetic background. The guide RNA constructs (Supplementary Table 1) are shown generically as  $P\{U6-gRNA\}$  to indicate that the guide RNA was under the control of *snRNA:U6:96Ab* regulatory sequences.

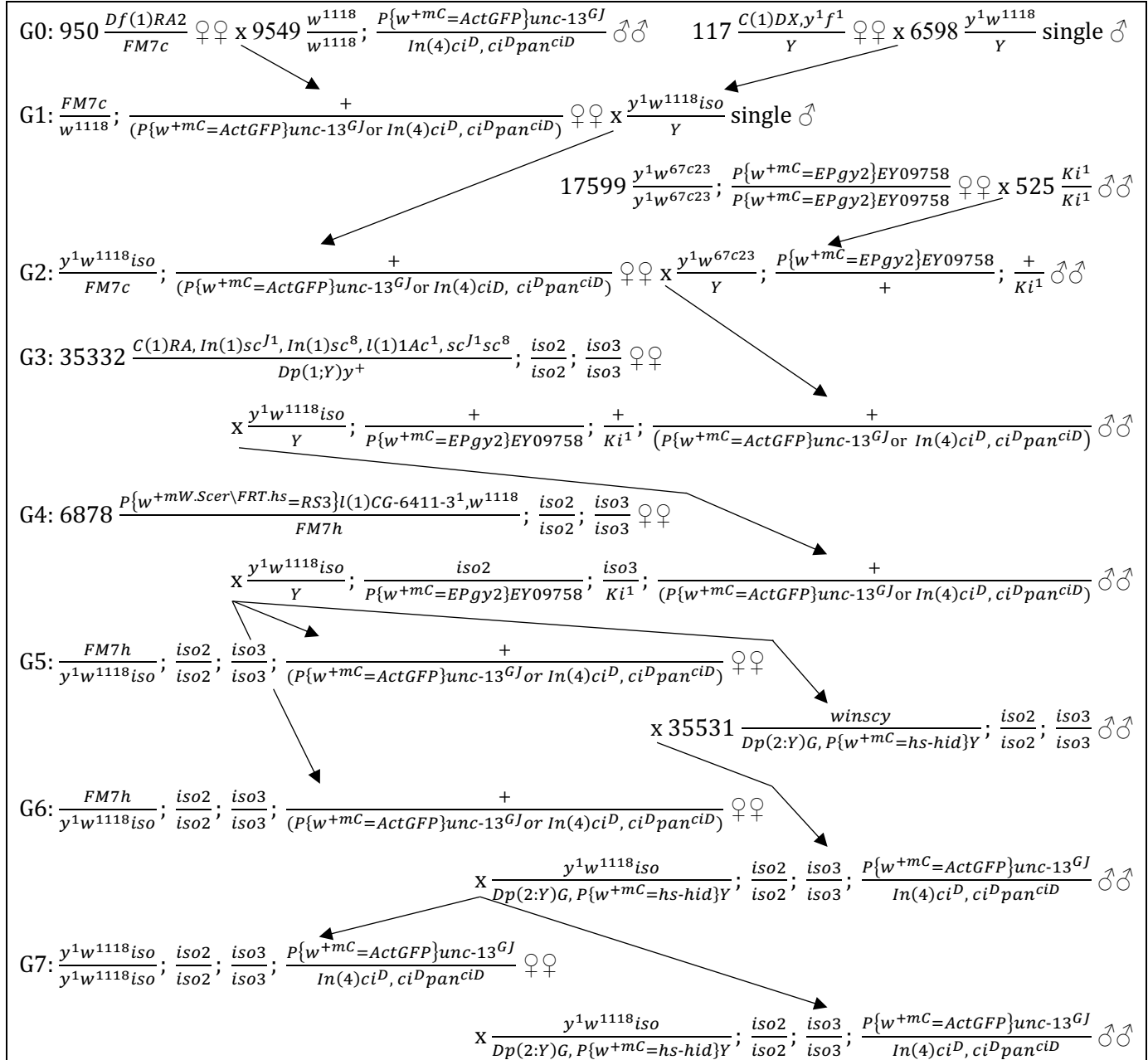


Note that in G1 we introduced a target FRT-bearing fourth chromosome that had been isogenized in crosses we will show below. This assured, as much as possible, that all mutation-bearing chromosomes have the same sequence other than the mutation at the CRISPR target site. After isolation of the mutation-bearing fourth chromosomes in G3, they were crossed into a genetic background with defined X, second and third chromosomes (indicated as “iso” in the crosses). In G3, multiple single-male crosses established independent lines, which were carried through the remaining crosses. The CRISPR target sites were sequenced for each line as described below. Typically, few than 10 lines were established for each set of crosses involving a particular guide RNA construct. If the progeny of a G6 cross were viable and fertile, we established a homozygous stock.

Similarly, the following crosses show how we screened for mutations using guide RNA constructs on the third chromosome.

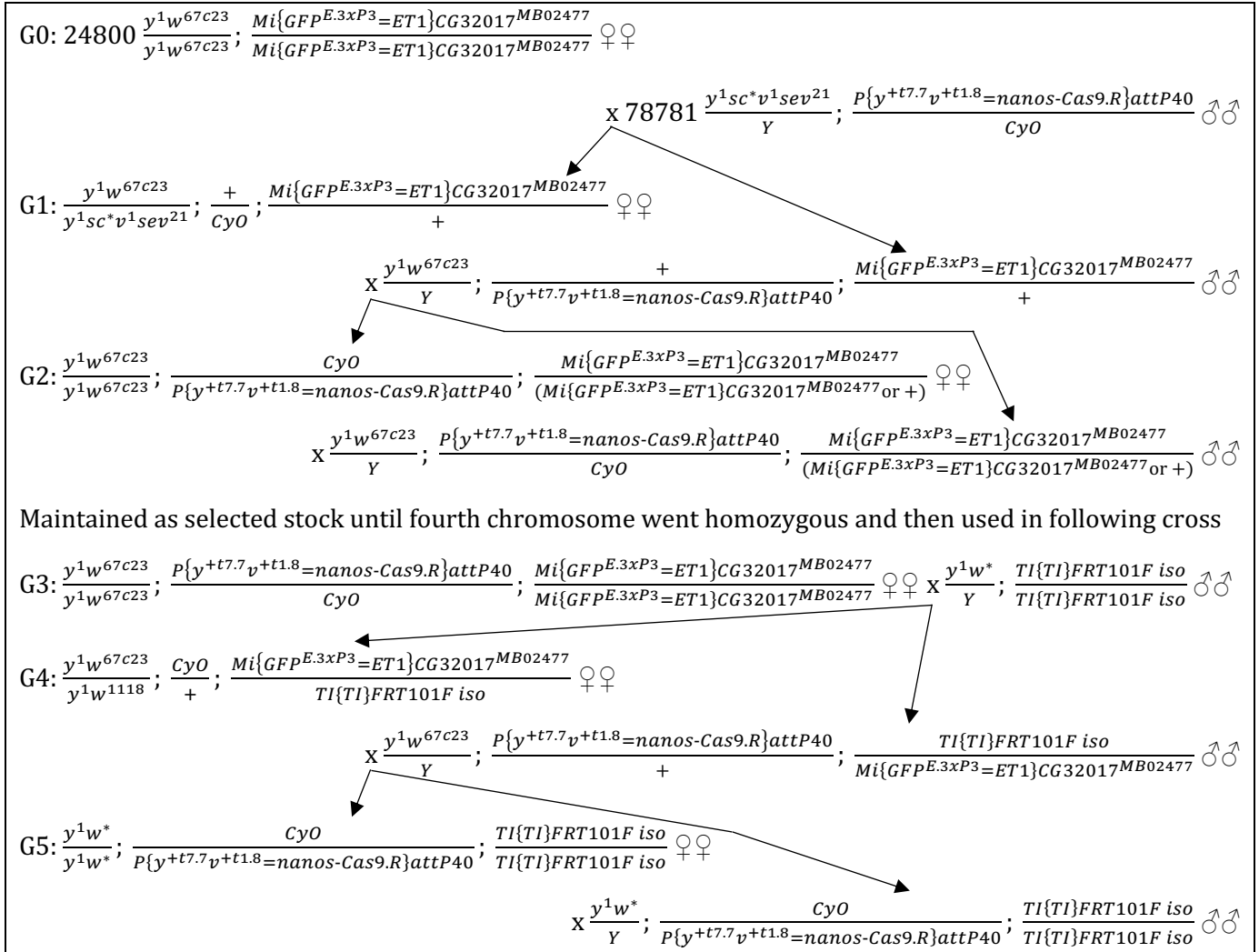


Each of these modes of screening for fourth chromosome mutations required us to generate stocks beforehand. The following genetic scheme shows how we generated stocks for use in G3, G4 and G5 of both of the screening schemes above with defined X, second and third chromosomes and fourth chromosomes carrying dominant markers. The X chromosome was derived from a stock with a commonly used  $y^1 w^{1118}$  chromosome. The second and third chromosomes are derived from stocks that were constructed by Ryder *et al.* (RYDER *et al.* 2004) and Cook *et al.* (COOK *et al.* 2010) to have various chromosomes introgressed into a common isogenic background. This assures that the second and third chromosomes in the final fourth chromosome mutation stocks have minimal genetic variability, containing only those polymorphisms that may have arisen since the progenitor stocks (6878, 35531 and 35332) were established. The numbers here and below refer to Bloomington Stock Center stock numbers.

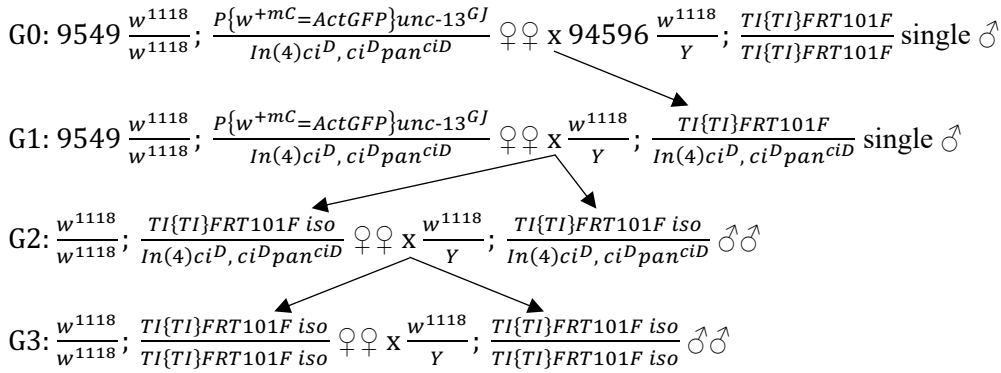


Here we will detail the stock constructions needed for screens involving second chromosome insertions of guide RNA constructs.

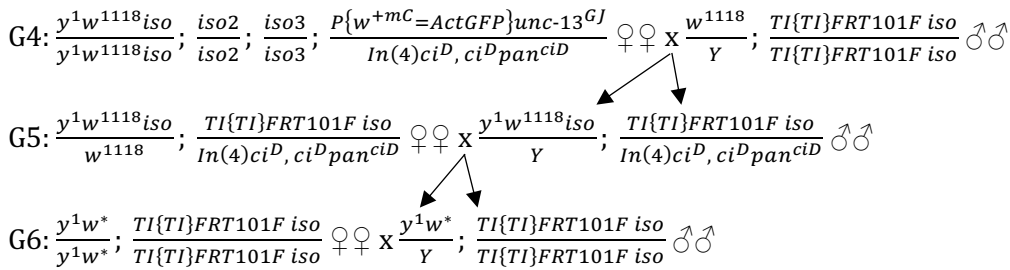
The following genetic scheme shows how we generated the stock used in G1 with an isogenic *FRT*-bearing fourth chromosome and a second chromosome *nanos-Cas9* insertion.



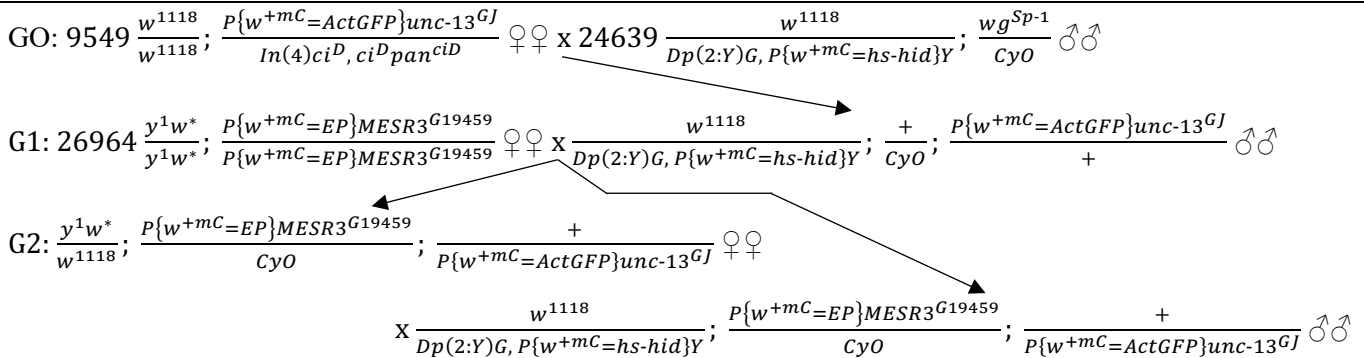
The following crosses show how we generated the stock used in G3 with an isogenic, FRT-bearing fourth chromosome.



Maintained as a stock and used in following cross

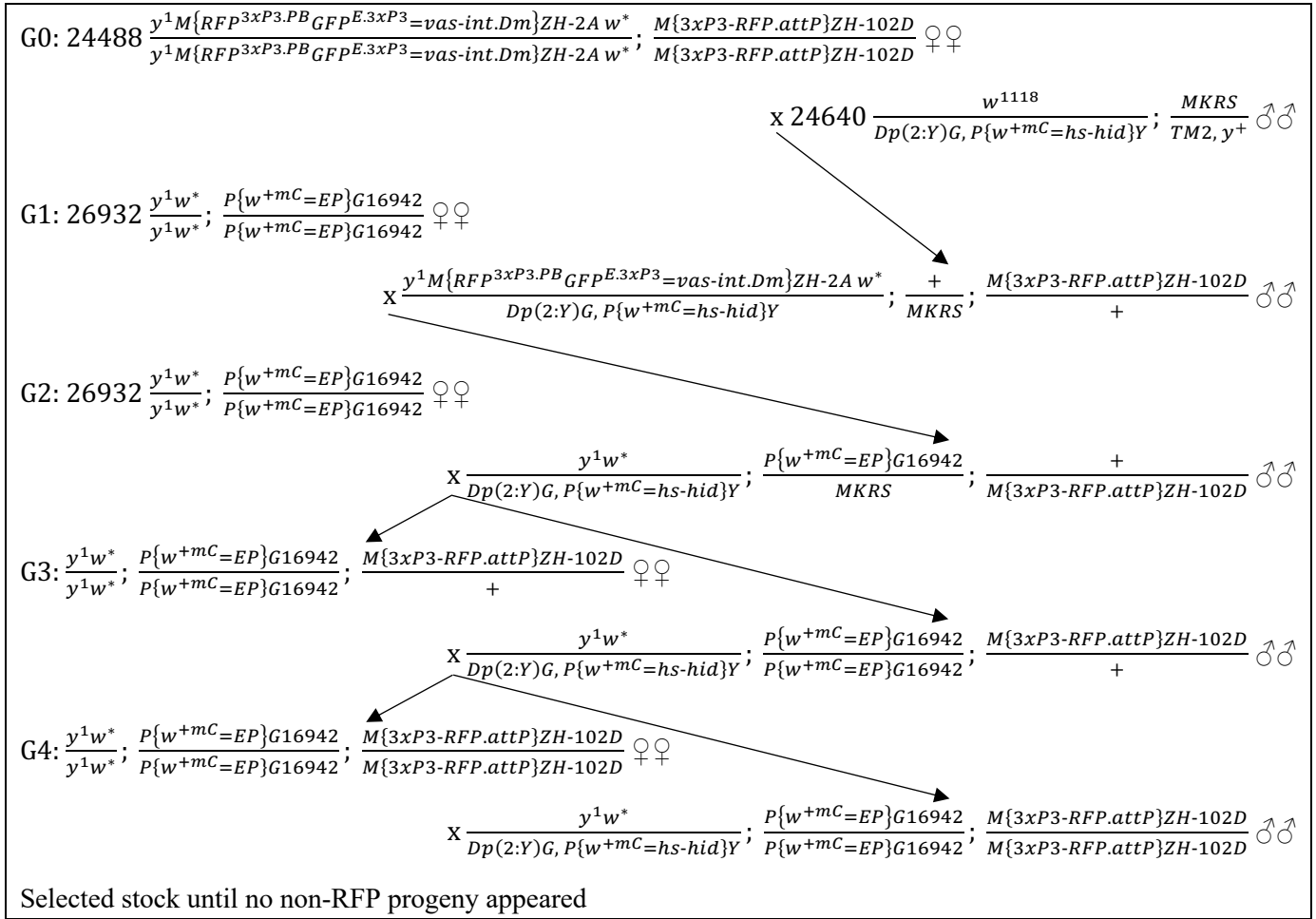


The following genetic scheme shows how we generated the stock used in G0 of the screen with a miniwhite-marked transgene insertion on the second chromosome (chosen simply for the visible marker and the vigor of the stock) and a dominant fourth chromosome marker on the fourth chromosome.



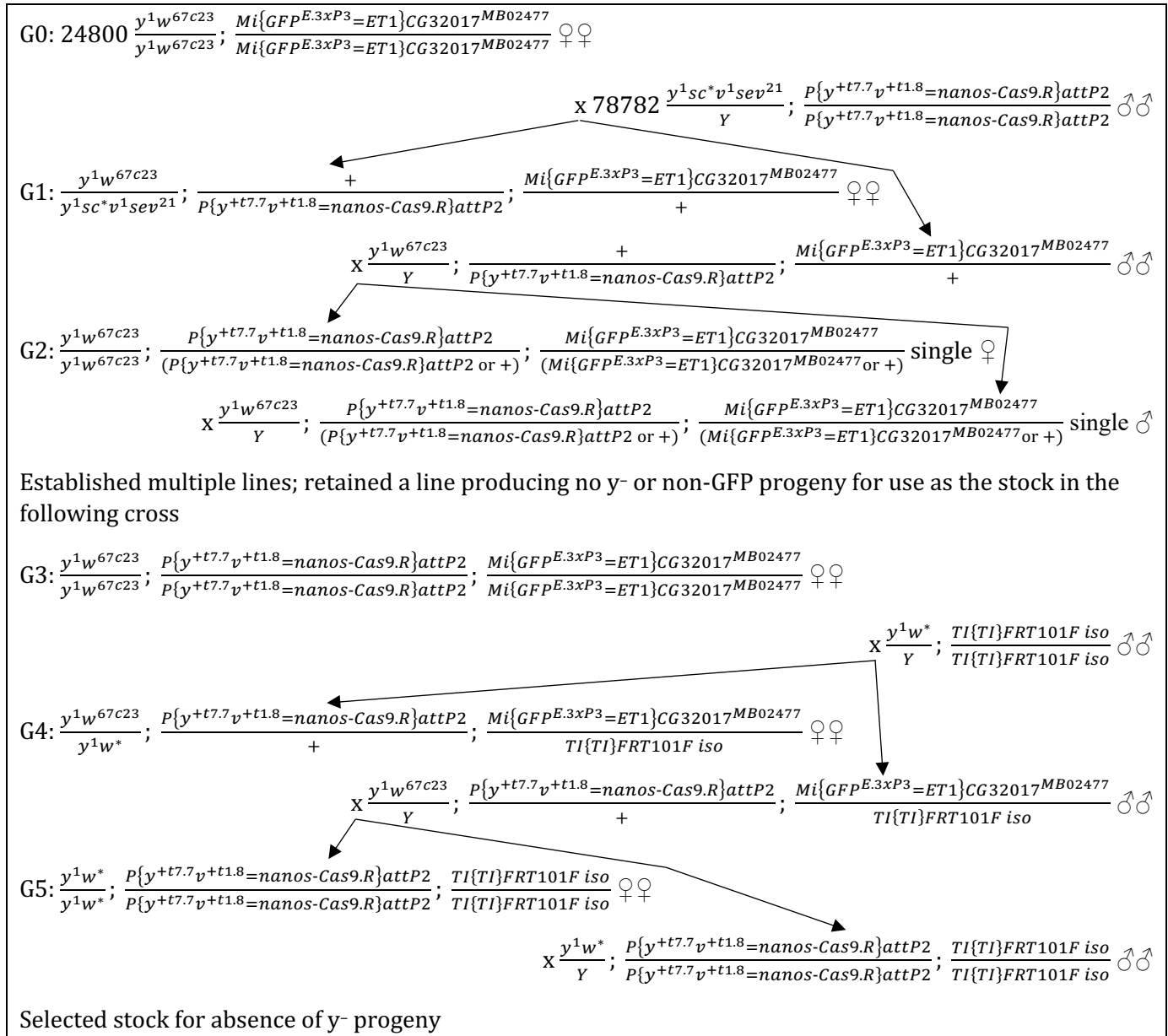
Selected stock for y- and GFP

The following crosses generated the stock used in G2 of the screen with a homozygous viable and fertile, miniwhite-marked, third chromosome insertion (chosen for the visible marker and the vigor of the stock) and a fourth chromosome insertion expressing RFP.

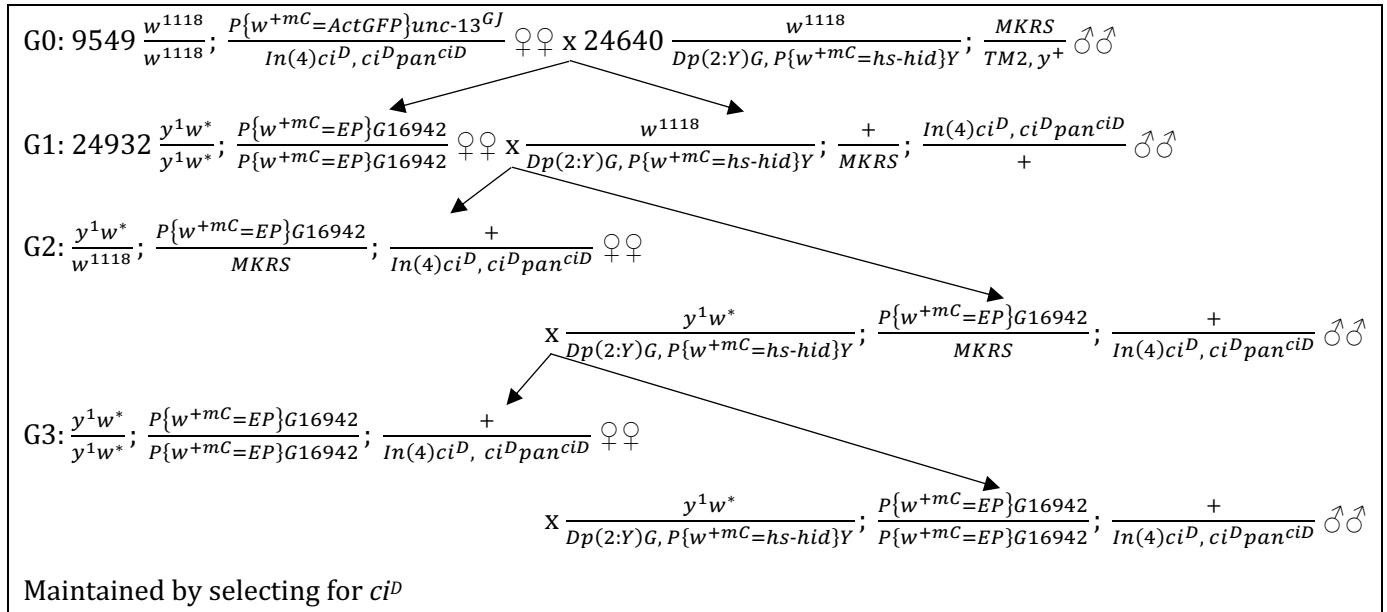


Here we will detail the stock constructions needed for screens involving third chromosome insertions of guide RNA constructs. The stock generated in the genetic scheme shown immediately above was also used in these screens.

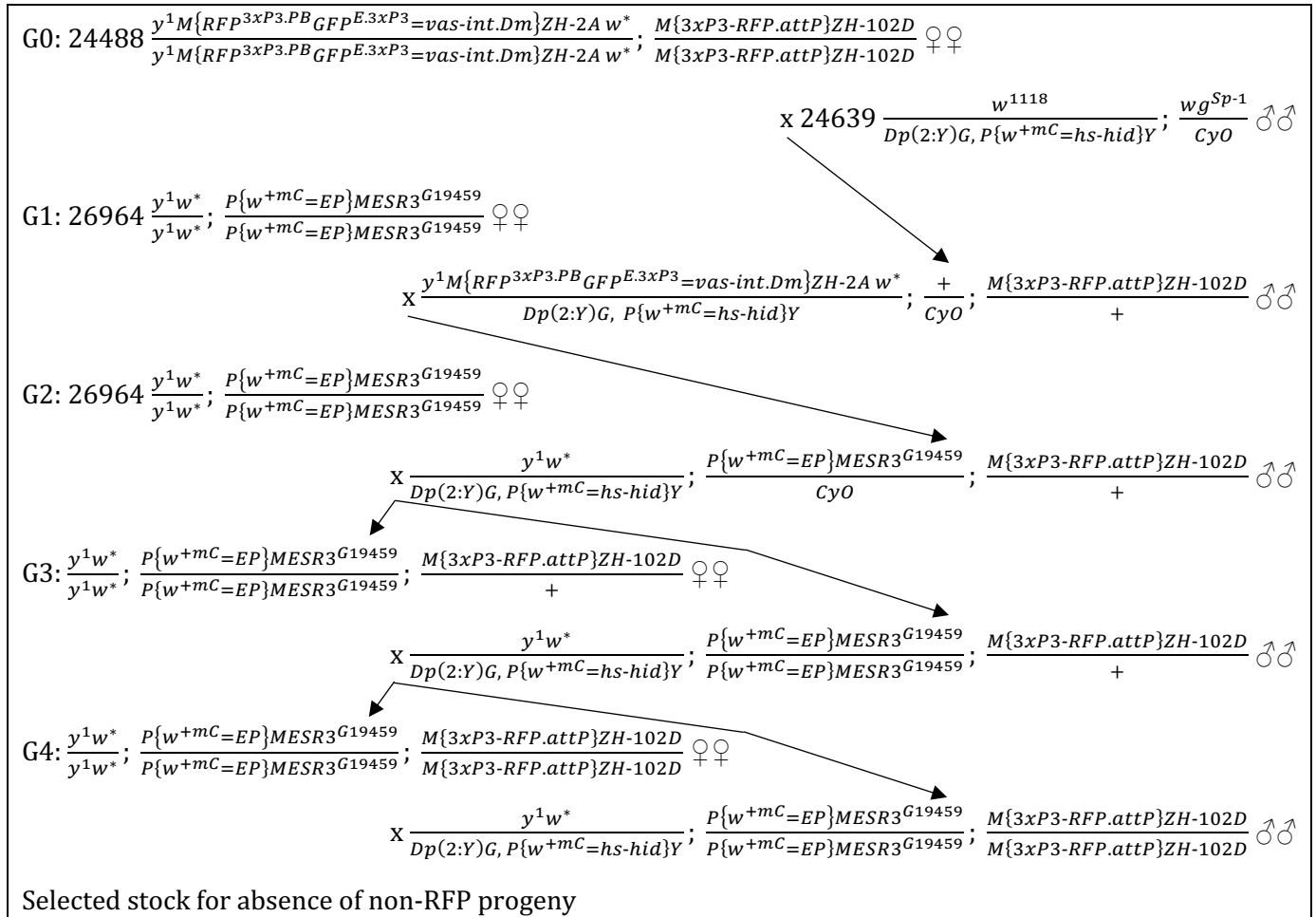
The following genetic scheme shows how we generated the stock used in G1 of the screen with an isogenic *FRT*-bearing fourth chromosome and a third chromosome *nanos-Cas9* insertion.



The following genetic scheme shows how we generated the stock used in G0 of the screen with a miniwhite-marked transgene insertion on the third chromosome (chosen for the visible marker and the vigor of the stock) and a dominant fourth chromosome marker on the fourth chromosome

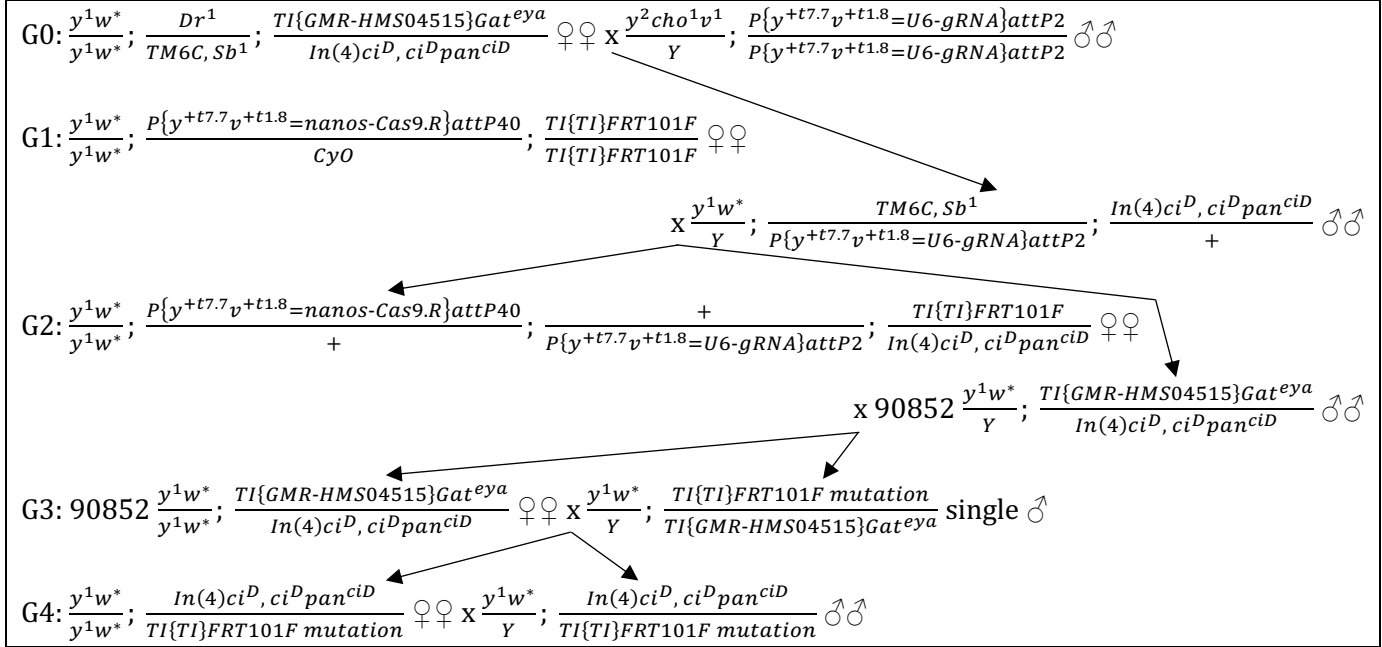


The following genetic scheme shows how we generated the stock used in G2 with a homozygous viable and fertile, miniwhite-marked transgene insertion on the second chromosome (chosen for the visible marker and the vigor of the stock) and a dominant fourth chromosome marker on the fourth chromosome.



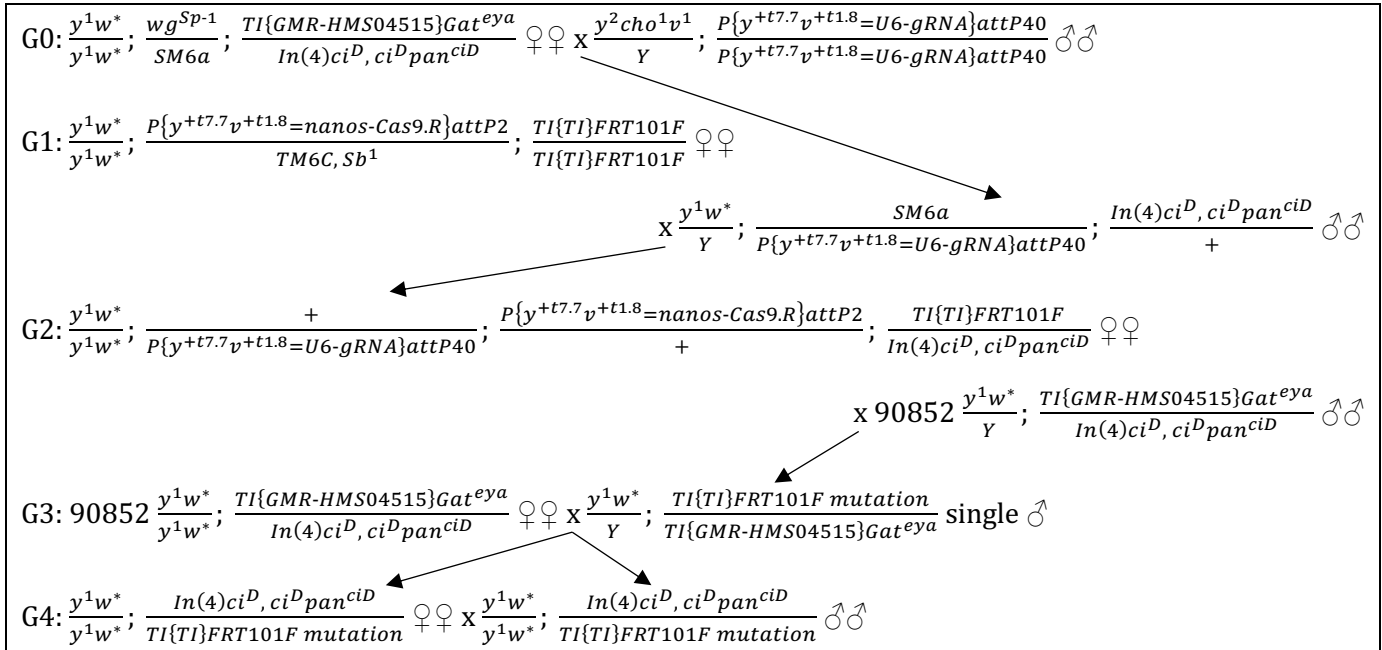
## Alternative approach for CRISPR mutagenesis

The following genetic scheme shows how we generated mutations on an FRT-bearing fourth chromosome in the female germ line using guide RNA constructs located on the third chromosome and established the new mutations in stable stocks. Again, the guide RNA constructs (Supplementary Table 1) are shown generically as  $P\{U6-gRNA\}$ .



Mutation-bearing fourth chromosomes were established in homozygous stocks when possible.

The screen involving a guide RNA construct on the second chromosome was similar.



Again, mutation-bearing fourth chromosomes were established in homozygous stocks when possible.

In all these screens, the FRT-bearing fourth chromosome was derived from a stock related to the stock used in the crosses in the first section, but no effort was made to isolate a single fourth chromosome from the progenitor stocks for these crosses. Likewise, the X, Y, second and third chromosomes in the final stocks have mixed origins. The stable stocks providing females for the G0 and G1 crosses were generated ahead of time by standard crosses with readily available stocks and will not be detailed here.

### **Molecular characterization of mutations**

We extracted genomic DNA from five flies from each stock potentially carrying a new mutation by crushing the flies in 75 µl of a buffered solution containing Proteinase K (10 mM Tris-Cl pH 8.0, 25 mM NaCl, 1 mM EDTA, 0.2 µg/µl Proteinase K), incubating the mixture at 37° for 30 minutes and then heat-killing the enzyme by incubating the mixture for 5 minutes at 95°. The supernatant containing genomic DNA was used for all subsequent steps. DNA concentrations were determined using a Thermo Scientific NanoDrop One spectrophotometer. For PCR amplification, the genomic preps were diluted in 10 mM Tris-Cl pH 8.0 to a DNA concentration of 150 ng/µl. We used LongAmp Taq 2X Master Mix from New England Biolabs (NEB) and reaction conditions recommended by the NEB Tm Calculator tool (<https://tmcaculator.neb.com/>). We confirmed the presence of the FRT-bearing transgene on the fourth chromosome by PCR amplification of the DNA preparation using a genomic primer (TTCGGTAAGGGGCGATACATATTT) and a primer within the FRT sequence (GGACAACACGGAAGTTCCTATTCTC) followed by electrophoresis in a 1% agarose gel. To determine if a mutation had been induced by CRISPR mutagenesis, we used primers flanking the target sites to produce ~600 base-pair products. PCR products were cleaned up using the Thermo Scientific GeneJET PCR Purification Kit (Product #K0701) and quantified using the NanoDrop One spectrophotometer. The purified PCR products were diluted to 30 ng/µl in 10 mM Tris-Cl pH8.0 in preparation for Sanger sequencing by Eurofins Genomics using the SimpleSeq Premixed Kit. We typically used the same primers for sequencing that we used to amplify the PCR products, but Supplementary Table 2 shows instances in which we used alternative primers for this step. Sequence from the mutated fourth chromosomes was aligned to the reference genomic sequence surrounding the gRNA target site using SnapGene software (Dotmatics). We could determine the sequences of mutations in DNA samples from homozygotes with no further steps. In DNA samples from heterozygotes, the presence of a mutation was detected by the divergence of sequence traces from the wild type and mutation-bearing chromosomes near the site of the mutation, leading to double-peak calls. The sequences of the normal and mutation-bearing chromosomes could often be extracted with the assistance of Poly Peak Parser (HILL *et al.* 2014), software that computationally separates mixed sequence traces in relation to a reference sequence. We retained stocks with mutations for further analysis.

### **Characterization of lethal, female sterile and morphological phenotypes**

To provide a preliminary phenotypic characterization of the new fourth chromosome mutations, we assessed the viability of homozygous adults—generally with any survival judged as “viable”. In most cases, we did not attempt to measure relative viability. Viable adults were examined for grossly abnormal morphological defects (rough eyes, disturbed bristles or veins, abnormal coloration, etc.) and females were tested for fertility with the production of any progeny judged as “fertile”, i.e. we did not measure relative fecundity. We assessed male fertility for only a few mutations.

We also assessed viability, morphological phenotypes and female fertility of adults with the new fourth chromosome mutations combined with preexisting mutations or chromosomal deletions for the regions of the targeted genes using the same broad criteria (summarized in Supplementary Table 9 with cross details provided in Supplementary Table 8). Again, we assessed male fertility for only a few genotypes.

For several genes, the phenotypes associated with preexisting mutations had never been described in publications. When a phenotypic analysis of such mutations was judged informative, we assessed viability, morphological phenotypes and female fertility of adults with these mutations combined with chromosomal deletions for the

regions of the mutations. These results are reported in Supplementary Table 7 and mentioned, when relevant, in Supplementary Table 10.

### **References cited in this supplementary file**

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**Supplementary Table 1. Fourth chromosome genes<sup>a</sup>**

| Gene                  | Annotation symbol | FlyBase ID  | Genomic location and orientation | Cytological order <sup>b</sup> |
|-----------------------|-------------------|-------------|----------------------------------|--------------------------------|
| <i>JYalpha</i>        | CG45760           | FBgn0267363 | 4:879..5039 [+]                  | 1                              |
| <i>PlexB</i>          | CG17245           | FBgn0025740 | 4:32478..43778 [-]               | 2                              |
| <i>asRNA:CR45124</i>  | CR45124           | FBgn0266617 | 4:42774..43374 [+]               | 3                              |
| <i>IncRNA:CR44440</i> | CR44440           | FBgn0265633 | 4:44774..46074 [+]               | 4                              |
| <i>ci</i>             | CG2125            | FBgn0004859 | 4:47710..57041 [-]               | 5                              |
| <i>asRNA:CR43957</i>  | CR43957           | FBgn0264617 | 4:56497..63124 [+]               | 6                              |
| <i>RpS3A</i>          | CG2168            | FBgn0017545 | 4:65225..67207 [-]               | 7                              |
| <i>snoRNA:CD68</i>    | CR43712           | FBgn0263851 | 4:65587..65647 [-]               | 8                              |
| <i>pan</i>            | CG34403           | FBgn0085432 | 4:69326..114270 [+]              | 9                              |
| <i>Ank</i>            | CG1651            | FBgn0011747 | 4:115074..129849 [-]             | 10                             |
| <i>anne</i>           | CG32000           | FBgn0052000 | 4:132220..143123 [+]             | 11                             |
| <i>IncRNA:CR43956</i> | CR43956           | FBgn0264616 | 4:132674..132874 [-]             | 12                             |
| <i>CG32006</i>        | CG32006           | FBgn0052006 | 4:150764..153538 [+]             | 13                             |
| <i>CG31997</i>        | CG31997           | FBgn0051997 | 4:154654..156060 [-]             | 14                             |
| <i>CG33978</i>        | CG33978           | FBgn0053978 | 4:157575..175639 [-]             | 15                             |
| <i>asRNA:CR45198</i>  | CR45198           | FBgn0266725 | 4:175455..175874 [+]             | 16                             |
| <i>Arl4</i>           | CG2219            | FBgn0039889 | 4:177348..179389 [-]             | 17                             |
| <i>IncRNA:CR46488</i> | CR46488           | FBgn0287619 | 4:180529..182098 [-]             | 18                             |
| <i>Abcd1</i>          | CG2316            | FBgn0039890 | 4:184076..193829 [-]             | 19                             |
| <i>IncRNA:CR44441</i> | CR44441           | FBgn0265634 | 4:194732..195900 [+]             | 20                             |
| <i>CG31998</i>        | CG31998           | FBgn0051998 | 4:196675..205945 [-]             | 21                             |
| <i>Crk</i>            | CG1587            | FBgn0024811 | 4:210224..213165 [+]             | 22                             |
| <i>CG31999</i>        | CG31999           | FBgn0051999 | 4:215009..227748 [-]             | 23                             |
| <i>yellow-h</i>       | CG1629            | FBgn0039896 | 4:227922..230428 [+]             | 24                             |
| <i>asRNA:CR46065</i>  | CR46065           | FBgn0267734 | 4:228576..229112 [-]             | 25                             |
| <i>CG1674</i>         | CG1674            | FBgn0039897 | 4:230730..245903 [+]             | 26                             |
| <i>dpr7</i>           | CG33481           | FBgn0053481 | 4:247124..253329 [-]             | 27                             |
| <i>RhoGAP102A</i>     | CG42316           | FBgn0259216 | 4:254997..265574 [+]             | 28                             |
| <i>Nfl</i>            | CG2380            | FBgn0042696 | 4:268175..276369 [-]             | 29                             |
| <i>IncRNA:CR44023</i> | CR44023           | FBgn0264793 | 4:276523..278174 [+]             | 30                             |
| <i>Syt7</i>           | CG2381            | FBgn0039900 | 4:278685..306481 [-]             | 31                             |
| <i>Rad23</i>          | CG1836            | FBgn0026777 | 4:308093..310822 [+]             | 32                             |
| <i>Zip102B</i>        | CG2177            | FBgn0039902 | 4:310930..314087 [-]             | 33                             |
| <i>Rnf11</i>          | CG32850           | FBgn0052850 | 4:314923..331387 [+]             | 34                             |
| <i>PMCA</i>           | CG42314           | FBgn0259214 | 4:328193..358917 [-]             | 35                             |
| <i>Hcf</i>            | CG1710            | FBgn0039904 | 4:359938..375554 [+]             | 36                             |
| <i>dati</i>           | CG2052            | FBgn0262636 | 4:375640..395605 [-]             | 37                             |
| <i>IncRNA:CR45125</i> | CR45125           | FBgn0266618 | 4:380724..381275 [+]             | 38                             |
| <i>asRNA:CR44027</i>  | CR44027           | FBgn0264819 | 4:395396..395954 [+]             | 39                             |
| <i>mir-9385</i>       | CR46291           | FBgn0283557 | 4:400703..400765 [-]             | 40                             |
| <i>IncRNA:CR43437</i> | CR43437           | FBgn0263385 | 4:426067..426601 [+]             | 41                             |
| <i>IncRNA:CR44024</i> | CR44024           | FBgn0264794 | 4:427331..431769 [+]             | 42                             |
| <i>lgs</i>            | CG2041            | FBgn0039907 | 4:436957..443911 [-]             | 43                             |
| <i>CaMKI</i>          | CG1495            | FBgn0016126 | 4:445504..453947 [+]             | 44                             |
| <i>Taf3</i>           | CG2009            | FBgn0026262 | 4:459887..466828 [-]             | 45                             |
| <i>asRNA:CR45126</i>  | CR45126           | FBgn0266619 | 4:459768..461413 [+]             | 46                             |
| <i>CG33941</i>        | CG33941           | FBgn0262731 | 4:467497..471476 [+]             | 47                             |

|                       |         |             |                        |    |
|-----------------------|---------|-------------|------------------------|----|
| <i>Asator</i>         | CG11533 | FBgn0039908 | 4:469409..488994 [-]   | 48 |
| <i>zfh2</i>           | CG1449  | FBgn0004607 | 4:501810..540874 [+]   | 49 |
| <i>Tdg</i>            | CG1981  | FBgn0026869 | 4:538332..557854 [-]   | 50 |
| <i>lncRNA:CR43958</i> | CR43958 | FBgn0264618 | 4:557025..557854 [-]   | 51 |
| <i>Pur-alpha</i>      | CG1507  | FBgn0022361 | 4:558016..567257 [+]   | 52 |
| <i>ND-49</i>          | CG1970  | FBgn0039909 | 4:568296..571554 [-]   | 53 |
| <i>Ephrin</i>         | CG1862  | FBgn0040324 | 4:571755..580122 [+]   | 54 |
| <i>CG1909</i>         | CG1909  | FBgn0039911 | 4:583108..589348 [+]   | 55 |
| <i>lncRNA:CR45199</i> | CR45199 | FBgn0266726 | 4:597624..598274 [-]   | 56 |
| <i>onecut</i>         | CG1922  | FBgn0028996 | 4:598555..607649 [-]   | 57 |
| <i>Eph</i>            | CG1511  | FBgn0025936 | 4:610684..622470 [+]   | 58 |
| <i>mav</i>            | CG1901  | FBgn0039914 | 4:623219..626031 [-]   | 59 |
| <i>mir-954</i>        | CR43009 | FBgn0262302 | 4:626126..626212 [+]   | 60 |
| <i>Gat</i>            | CG1732  | FBgn0039915 | 4:629691..635350 [+]   | 61 |
| <i>Ekar</i>           | CG9935  | FBgn0039916 | 4:636131..646556 [-]   | 62 |
| <i>gw</i>             | CG31992 | FBgn0051992 | 4:649041..660608 [-]   | 63 |
| <i>CG46466</i>        | CG46466 | FBgn0287597 | 4:661009..663124 [-]   | 64 |
| <i>Slip1</i>          | CG1783  | FBgn0024728 | 4:663621..669884 [+]   | 65 |
| <i>CG11360</i>        | CG11360 | FBgn0039920 | 4:671142..690650 [+]   | 66 |
| <i>myo</i>            | CG1838  | FBgn0026199 | 4:690686..696055 [-]   | 67 |
| <i>ey</i>             | CG1464  | FBgn0005558 | 4:697689..721173 [+]   | 68 |
| <i>bt</i>             | CG32019 | FBgn0005666 | 4:724400..776474 [+]   | 69 |
| <i>lncRNA:CR45200</i> | CR45200 | FBgn0266727 | 4:776475..777146 [-]   | 70 |
| <i>MED26</i>          | CG1793  | FBgn0039923 | 4:778516..788978 [-]   | 71 |
| <i>Sox102F</i>        | CG11153 | FBgn0039938 | 4:800436..834393 [-]   | 72 |
| <i>fd102C</i>         | CG11152 | FBgn0039937 | 4:836986..844529 [-]   | 73 |
| <i>lncRNA:CR45123</i> | CR45123 | FBgn0266616 | 4:851174..851624 [+]   | 74 |
| <i>Gyf</i>            | CG11148 | FBgn0039936 | 4:853557..867025 [-]   | 75 |
| <i>unc-13</i>         | CG2999  | FBgn0025726 | 4:867824..912331 [-]   | 76 |
| <i>asRNA:CR43425</i>  | CR43425 | FBgn0263344 | 4:867824..871426 [+]   | 77 |
| <i>asRNA:CR44029</i>  | CR44029 | FBgn0264821 | 4:879374..884752 [+]   | 78 |
| <i>elF4G1</i>         | CG10811 | FBgn0023213 | 4:915297..930715 [-]   | 79 |
| <i>mGluR</i>          | CG11144 | FBgn0019985 | 4:936819..949441 [+]   | 80 |
| <i>4E-T</i>           | CG32016 | FBgn0052016 | 4:951542..958392 [-]   | 81 |
| <i>lncRNA:CR45201</i> | CR45201 | FBgn0266728 | 4:959224..959434 [-]   | 82 |
| <i>asRNA:CR44030</i>  | CR44030 | FBgn0264822 | 4:959675..962692 [+]   | 83 |
| <i>fuss</i>           | CG11093 | FBgn0039932 | 4:962341..978489 [-]   | 84 |
| <i>lncRNA:sphinx</i>  | CR34154 | FBgn0083990 | 4:975168..977261 [+]   | 85 |
| <i>toy</i>            | CG11186 | FBgn0019650 | 4:989725..1007922 [+]  | 86 |
| <i>PlexA</i>          | CG11081 | FBgn0025741 | 4:1009895..1027101 [-] | 87 |
| <i>asRNA:CR44031</i>  | CR44031 | FBgn0264823 | 4:1027863..1029359 [+] | 88 |
| <i>Mpv17</i>          | CG11077 | FBgn0039930 | 4:1028675..1029361 [-] | 89 |
| <i>Polr1G</i>         | CG11076 | FBgn0039929 | 4:1029711..1031757 [-] | 90 |
| <i>ATPsynbeta</i>     | CG11154 | FBgn0010217 | 4:1031484..1034917 [+] | 91 |
| <i>CaMKII</i>         | CG18069 | FBgn0264607 | 4:1035288..1053703 [-] | 92 |
| <i>Zyx</i>            | CG32018 | FBgn0011642 | 4:1057365..1065001 [-] | 93 |
| <i>apolpp</i>         | CG11064 | FBgn0087002 | 4:1060969..1077273 [+] | 94 |
| <i>Actbeta</i>        | CG11062 | FBgn0024913 | 4:1077331..1084796 [-] | 95 |
| <i>sv</i>             | CG11049 | FBgn0005561 | 4:1088798..1113317 [+] | 96 |

|                |                |             |                        |     |
|----------------|----------------|-------------|------------------------|-----|
| <i>Cals</i>    | <i>CG11059</i> | FBgn0039928 | 4:1112874..1123357 [-] | 97  |
| <i>Arf4</i>    | <i>CG11027</i> | FBgn0013749 | 4:1124167..1125325 [+] | 98  |
| <i>ukar</i>    | <i>CG11155</i> | FBgn0039927 | 4:1126602..1137919 [+] | 99  |
| <i>CG32017</i> | <i>CG32017</i> | FBgn0052017 | 4:1137830..1162158 [-] | 100 |
| <i>Kif3C</i>   | <i>CG17461</i> | FBgn0039925 | 4:1147466..1169239 [+] | 101 |
| <i>pho</i>     | <i>CG17743</i> | FBgn0002521 | 4:1172469..1181628 [-] | 102 |
| <i>CG33521</i> | <i>CG33521</i> | FBgn0250819 | 4:1185768..1192985 [+] | 103 |
| <i>PIP4K</i>   | <i>CG17471</i> | FBgn0039924 | 4:1192419..1196848 [-] | 104 |
| <i>Mitf</i>    | <i>CG43369</i> | FBgn0263112 | 4:1198852..1224467 [+] | 105 |
| <i>Dyrk3</i>   | <i>CG40478</i> | FBgn0027101 | 4:1225089..1230713 [-] | 106 |
| <i>Cadps</i>   | <i>CG33653</i> | FBgn0053653 | 4:1230837..1271759 [+] | 107 |

<sup>a</sup> Pseudogenes not listed. Annotation symbols, genomic coordinates and gene orientations from FlyBase.

<sup>b</sup> These numbers are used in other supplementary tables to allow rows to be sorted by cytological order

Supplementary Table 2. Guide RNA transgenes

| Gene                                                  | FlyBase gene ID | Construct                         | Guide RNA sequence    | Stock genotype <sup>a</sup>                                                               | Cytological order <sup>b</sup> |
|-------------------------------------------------------|-----------------|-----------------------------------|-----------------------|-------------------------------------------------------------------------------------------|--------------------------------|
| <b>Stocks from the National Institute of Genetics</b> |                 |                                   |                       |                                                                                           |                                |
| <i>4E-T</i>                                           | FBgn0052016     | <i>P{U6-4E-T.NIG.sgRNA}</i>       | GTACGACAATCGTCGTGCA   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-4E-T.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$       | 81                             |
| <i>Abcd1</i>                                          | FBgn0039890     | <i>P{U6-Abcd1.NIG.sgRNA}</i>      | GAGAGGATTTCAAGCTGCGAG | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Abcd1.NIG.sgRNA\}attP40/CyO$                 | 19                             |
| <i>Actbeta</i>                                        | FBgn0024913     | <i>P{U6-Actbeta.NIG.sgRNA}</i>    | GCACTGTCCTACTGCTGGCT  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Actbeta.NIG.sgRNA\}attP40$                   | 95                             |
| <i>Ank</i>                                            | FBgn0011747     | <i>P{U6-Ank.NIG.sgRNA}</i>        | GCTGACATCGTTTCCAAATC  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Ank.NIG.sgRNA\}attP40/CyO$                   | 10                             |
| <i>anne</i>                                           | FBgn0052000     | <i>P{U6-anne.NIG.sgRNA}</i>       | GACGCCACCAATGTAACACG  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-anne.NIG.sgRNA\}attP40/CyO$                  | 11                             |
| <i>apolpp</i>                                         | FBgn0087002     | <i>P{U6-apolpp.NIG.sgRNA}</i>     | GATAAAGTCTCACTATCAGAT | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-apolpp.NIG.sgRNA\}attP40/CyO$                | 94                             |
| <i>Arf4</i>                                           | FBgn0013749     | <i>P{U6-Arf4.NIG.sgRNA}</i>       | GTGGAAATAGTGCGCCACAA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Arf4.NIG.sgRNA\}attP40$                      | 98                             |
| <i>Arl4</i>                                           | FBgn0039889     | <i>P{U6-Arl4.NIG.sgRNA}</i>       | GGATTCTCGGGAAAAACAA   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Arl4.NIG.sgRNA\}attP2$                       | 17                             |
| <i>Asator</i>                                         | FBgn0039908     | <i>P{U6-Asator.NIG.sgRNA}</i>     | GCACTGAATGGATGGATTCAA | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Asator.NIG.sgRNA\}attP2$                     | 48                             |
| <i>ATPsynbeta</i>                                     | FBgn0010217     | <i>P{U6-ATPsynbeta.NIG.sgRNA}</i> | GGAAAGATTGTGGCCGTAAT  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-ATPsynbeta.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$ | 91                             |
| <i>bt</i>                                             | FBgn0005666     | <i>P{U6-bt.NIG.sgRNA}</i>         | GATGAAGGAGACGGTCGAGA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-bt.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$         | 69                             |
| <i>Cadps</i>                                          | FBgn0053653     | <i>P{U6-Cadps.NIG.sgRNA}</i>      | GAGCCGACAGATAGCTAAG   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Cadps.NIG.sgRNA\}attP2$                      | 107                            |
| <i>Cals</i>                                           | FBgn0039928     | <i>P{U6-Cals.NIG.sgRNA}</i>       | GATCTGTGCAATCTATTGTGA | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Cals.NIG.sgRNA\}attP2$                       | 97                             |
| <i>CaMKI</i>                                          | FBgn0016126     | <i>P{U6-CaMKI.NIG.sgRNA}</i>      | GTTGATCGCATTGTCGAAAAG | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CaMKI.NIG.sgRNA\}attP2$                      | 44                             |
| <i>CG11360</i>                                        | FBgn0039920     | <i>P{U6-CG11360.NIG.sgRNA}</i>    | GGCATTGGTAGCGTTGAGC   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG11360.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$    | 66                             |
| <i>CG1674</i>                                         | FBgn0039897     | <i>P{U6-CG1674.NIG.sgRNA}</i>     | GGCCGATAAATCAGATGCAA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG1674.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$     | 26                             |
| <i>CG1909</i>                                         | FBgn0039911     | <i>P{U6-CG1909.NIG.sgRNA}</i>     | GAAAGGGACGTGCCAAAGAG  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG1909.NIG.sgRNA\}attP2$                     | 55                             |
| <i>CG31998</i>                                        | FBgn0051998     | <i>P{U6-CG31998.NIG.sgRNA}</i>    | GCGCAGATGTGGGCAATGCA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG31998.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$    | 21                             |
| <i>CG31999</i>                                        | FBgn0051999     | <i>P{U6-CG31999.NIG.sgRNA}</i>    | GGTACTCGCTGTGACGGAGA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG31999.NIG.sgRNA\}attP2$                    | 23                             |
| <i>CG32006</i>                                        | FBgn0052006     | <i>P{U6-CG32006.NIG.sgRNA}</i>    | GTTCCGTTGGGTTGTAATCAA | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG32006.NIG.sgRNA\}attP2$                    | 13                             |
| <i>CG32017</i>                                        | FBgn0052017     | <i>P{U6-CG32017.NIG.sgRNA}</i>    | GCGCGGAGCTGTCGCATGT   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG32017.NIG.sgRNA\}attP40/CyO$               | 100                            |
| <i>CG33521</i>                                        | FBgn0250819     | <i>P{U6-CG33521.NIG.sgRNA}</i>    | GAAACCCGTGTACAAATGG   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG33521.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$    | 103                            |
| <i>CG33978</i>                                        | FBgn0053978     | <i>P{U6-CG33978.NIG.sgRNA}</i>    | GGAGTGTGGTAAACAGAAC   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-CG33978.NIG.sgRNA\}attP40$                   | 15                             |
| <i>ci</i>                                             | FBgn0004859     | <i>P{U6-ci.NIG.sgRNA}</i>         | GCATTCATCTTGCCGACT    | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-ci.NIG.sgRNA\}attP40/CyO$                    | 5                              |
| <i>Crk</i>                                            | FBgn0024811     | <i>P{U6-Crk.NIG.sgRNA}</i>        | GAAAAGACGAGGATCAATGG  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Crk.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$        | 22                             |
| <i>dati</i>                                           | FBgn0262636     | <i>P{U6-dati.NIG.sgRNA}</i>       | GCGCCGCTGCGCTGCTCGAAC | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-dati.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$       | 37                             |
| <i>dpr7</i>                                           | FBgn0053481     | <i>P{U6-dpr7.NIG.sgRNA}</i>       | GGTATCATGGATGCGTAAGC  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-dpr7.NIG.sgRNA\}attP2$                       | 27                             |
| <i>Dyrk3</i>                                          | FBgn0027101     | <i>P{U6-Dyrk3.NIG.sgRNA}</i>      | GTTCGTGAAATAAAAGGTG   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Dyrk3.NIG.sgRNA\}attP40$                     | 106                            |
| <i>elF4G1</i>                                         | FBgn0023213     | <i>P{U6-elF4G1.NIG.sgRNA}</i>     | GCCAGGAGCCAGCACTGTAA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-elF4G1.NIG.sgRNA\}attP2$                     | 79                             |
| <i>Ekar</i>                                           | FBgn0039916     | <i>P{U6-Ekar.NIG.sgRNA}</i>       | GCACTGGACATCCCAACAT   | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Ekar.NIG.sgRNA\}attP2$                       | 62                             |
| <i>Eph</i>                                            | FBgn0025936     | <i>P{U6-Eph.NIG.sgRNA}</i>        | GAGGAAACGCCTTGTCATGCA | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Eph.NIG.sgRNA\}attP2$                        | 58                             |
| <i>Ephrin</i>                                         | FBgn0040324     | <i>P{U6-Ephrin.NIG.sgRNA}</i>     | GGAAGCAGGGATAGAATCAA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Ephrin.NIG.sgRNA\}attP40/CyO$                | 54                             |
| <i>ey</i>                                             | FBgn0005558     | <i>P{U6-ey.NIG.sgRNA}</i>         | GGCGCAAGCAACTCCGGGGA  | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-ey.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$         | 68                             |
| <i>fd102C</i>                                         | FBgn0039937     | <i>P{U6-fd102C.NIG.sgRNA}</i>     | GCGGCCATTTCGATTAAGTGA | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-fd102C.NIG.sgRNA\}attP2$                     | 73                             |
| <i>fuss</i>                                           | FBgn0039932     | <i>P{U6-fuss.NIG.sgRNA}</i>       | GTTGTAAACGCGAGGCTAGAA | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-fuss.NIG.sgRNA\}attP2$                       | 84                             |
| <i>Gat</i>                                            | FBgn0039915     | <i>P{U6-Gat.NIG.sgRNA}</i>        | GAGTGGGCCTAGCTATAGGTT | $y^2 cho^1 v^1$ ; $P\{y^{+7.7} v^{+1.8} = U6-Gat.NIG.sgRNA\}attP2$                        | 61                             |

|                                                            |             |                                   |                       |                                                                                     |     |
|------------------------------------------------------------|-------------|-----------------------------------|-----------------------|-------------------------------------------------------------------------------------|-----|
| <i>gw</i>                                                  | FBgn0051992 | <i>P{U6-gw.NIG.sgRNA}</i>         | GAGCGGAACTCCAGCAACGC  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-gw.NIG.sgRNA\}attP2$                      | 63  |
| <i>Gyf</i>                                                 | FBgn0039936 | <i>P{U6-Gyf.NIG.sgRNA}</i>        | GTGGCTCTGGTCTCTAGAT   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Gyf.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$     | 75  |
| <i>Hcf</i>                                                 | FBgn0039904 | <i>P{U6-Hcf.NIG.sgRNA}</i>        | GGTCACAGCTTTACAATGGT  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Hcf.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$     | 36  |
| <i>JYalpha</i>                                             | FBgn0267363 | <i>P{U6-JYalpha.NIG.sgRNA}</i>    | GCAATTCTATGCATCGATAT  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-JYalpha.NIG.sgRNA\}attP40/CyO$            | 1   |
| <i>Kif3C</i>                                               | FBgn0039925 | <i>P{U6-Kif3C.NIG.sgRNA}</i>      | GCTTACGGTCAAAACAGTTG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Kif3C.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$   | 101 |
| <i>lgs</i>                                                 | FBgn0039907 | <i>P{U6-lgs.NIG.sgRNA}</i>        | GCAAAAGGTCAAGCAGCTGG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-lgs.NIG.sgRNA\}attP2$                     | 43  |
| <i>mav</i>                                                 | FBgn0039914 | <i>P{U6-mav.NIG.sgRNA}</i>        | GCCTATTACTGATAATGCTG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-mav.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$     | 59  |
| <i>MED26</i>                                               | FBgn0039923 | <i>P{U6-MED26.NIG.sgRNA}</i>      | GTCATCGGTCAAGTTAATCA  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = MED26.NIG.sgRNA\}attP40$                     | 71  |
| <i>Mitf</i>                                                | FBgn0263112 | <i>P{U6-Mitf.NIG.sgRNA}</i>       | GACAGACATTAGACTGCTA   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Mitf.NIG.sgRNA\}attP2$                    | 105 |
| <i>mGluR</i>                                               | FBgn0019985 | <i>P{U6-mGluR.NIG.sgRNA}</i>      | GGCATATGTGTCTCTGAAC   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-mGluR.NIG.sgRNA\}attP2$                   | 80  |
| <i>Mpv17</i>                                               | FBgn0039930 | <i>P{U6-Mpv17.NIG.sgRNA}</i>      | GCCAAAACCTACTCGCCAATG | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Mpv17.NIG.sgRNA\}attP2$                   | 89  |
| <i>myo</i>                                                 | FBgn0026199 | <i>P{U6-myo.NIG.sgRNA}</i>        | GCCGTGACATATCGAGTTACA | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-myo.NIG.sgRNA\}attP2$                     | 67  |
| <i>ND-49</i>                                               | FBgn0039909 | <i>P{U6-ND-49.NIG.sgRNA}</i>      | GCATGAAGCAGTTAGCGGCC  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-ND-49.NIG.sgRNA\}attP40$                  | 53  |
| <i>Nfl</i>                                                 | FBgn0042696 | <i>P{U6-Nfl.NIG.sgRNA}</i>        | GACTGAGGTGAAGCAAAAGT  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Nfl.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$     | 29  |
| <i>oncut</i>                                               | FBgn0028996 | <i>P{U6-oncut.NIG.sgRNA}</i>      | GCATATGCAGCATGGCGGTG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-oncut.NIG.sgRNA\}attP2$                   | 57  |
| <i>pan</i>                                                 | FBgn0085432 | <i>P{U6-pan.NIG.sgRNA}</i>        | GGCGTTTCATGCTCTATATGA | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-pan.NIG.sgRNA\}attP2$                     | 9   |
| <i>pho</i>                                                 | FBgn0002521 | <i>P{U6-pho.NIG.sgRNA}</i>        | GATATTAATACCGAAGAGTC  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-pho.NIG.sgRNA\}attP2$                     | 102 |
| <i>PIP4K</i>                                               | FBgn0039924 | <i>P{U6-PIP4K.NIG.sgRNA}</i>      | GCCGTCACATTCAAAGTCA   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-PIP4K.NIG.sgRNA\}attP2$                   | 104 |
| <i>PMCA</i>                                                | FBgn0259214 | <i>P{U6-PMCA.NIG.sgRNA}</i>       | GAGGTGTGCCAATATCAGT   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-PMCA.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$    | 35  |
| <i>Pur-alpha</i>                                           | FBgn0022361 | <i>P{U6-Pur-alpha.NIG.sgRNA}</i>  | GGATAATGGTCACGAAATT   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Pur-alpha.NIG.sgRNA\}attP40/CyO$          | 52  |
| <i>Rad23</i>                                               | FBgn0026777 | <i>P{U6-Rad23.NIG.sgRNA}</i>      | GTGACGAATTGATTGGCGAGT | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Rad23.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$   | 32  |
| <i>RhoGAP102A</i>                                          | FBgn0259216 | <i>P{U6-RhoGAP102A.NIG.sgRNA}</i> | GAGCATTCAGCTGTCTAAG   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-RhoGAP102A.NIG.sgRNA\}attP2$              | 28  |
| <i>Rnf11</i>                                               | FBgn0052850 | <i>P{U6-Rnf11.NIG.sgRNA}</i>      | GTTGTGCAAGACGACGTGGA  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Rnf11.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$   | 34  |
| <i>Slip1</i>                                               | FBgn0024728 | <i>P{U6-Slip1.NIG.sgRNA}</i>      | GGTTTGAGTTTCTTCGATG   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Slip1.NIG.sgRNA\}attP40/CyO$              | 65  |
| <i>Sox102F</i>                                             | FBgn0039938 | <i>P{U6-Sox102F.NIG.sgRNA}</i>    | GCGGCTGTAGGAAGGCCGGA  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Sox102F.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$ | 72  |
| <i>sv</i>                                                  | FBgn0005561 | <i>P{U6-sv.NIG.sgRNA}</i>         | GGAGGCGTTAATCAACTTGG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-sv.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$      | 96  |
| <i>Syt7</i>                                                | FBgn0039900 | <i>P{U6-Syt7.NIG.sgRNA}</i>       | GACCGATCCAAGTGAAATGT  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Syt7.NIG.sgRNA\}attP40/CyO$               | 31  |
| <i>Taf3</i>                                                | FBgn0026262 | <i>P{U6-Taf3.NIG.sgRNA}</i>       | GCTCCCAACGTCCAATTCAA  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Taf3.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$    | 45  |
| <i>Tdg</i>                                                 | FBgn0026869 | <i>P{U6-Tdg.NIG.sgRNA}</i>        | GTGACCAGTGCCTGCTCTGG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Tdg.NIG.sgRNA\}attP2$                     | 50  |
| <i>toy</i>                                                 | FBgn0019650 | <i>P{U6-toy.NIG.sgRNA}</i>        | GAGAATACTACAAGTGCCAA  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-toy.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$     | 86  |
| <i>ukar</i>                                                | FBgn0039927 | <i>P{U6-ukar.NIG.sgRNA}</i>       | GGTATATTTGAACTGGACGA  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-ukar.NIG.sgRNA\}attP2/TM6C, Sb^1 Tb^1$    | 99  |
| <i>unc-13</i>                                              | FBgn0025726 | <i>P{U6-unc-13.NIG.sgRNA}</i>     | GTTGACAGTATGCCGGACATA | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-unc-13.NIG.sgRNA\}attP2$                  | 76  |
| <i>yellow-h</i>                                            | FBgn0039896 | <i>P{U6-yellow-h.NIG.sgRNA}</i>   | GTTGCACAAAAGTTGAGTAT  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-yellow-h.NIG.sgRNA\}attP40/CyO$           | 24  |
| <i>zfh2</i>                                                | FBgn0004607 | <i>P{U6-zfh2.NIG.sgRNA}</i>       | GGGACATGGGCTTGAAATA   | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-zfh2.NIG.sgRNA\}attP40$                   | 49  |
| <i>Zyx</i>                                                 | FBgn0011642 | <i>P{U6-Zyx.NIG.sgRNA}</i>        | GGCTTTGTAAAGCTGTTGAG  | $y^2 cho^1 v^1; P\{y^{+7.7} v^{+1.8} = U6-Zyx.NIG.sgRNA\}attP2$                     | 93  |
| <b>Stocks from the Bloomington Drosophila Stock Center</b> |             |                                   |                       |                                                                                     |     |
| <i>CaMKII</i>                                              | FBgn0264607 | <i>P{TKO.GS03310}</i>             | GCGGCTACATGACAGTATAC  | $y^1 v^1; P\{y^{+7.7} v^{+1.8} = TKO.GS03310\}attP40$                               | 92  |

<sup>a</sup> Balancers are noted in genotypes when they were present in stocks, even if insertion homozygotes were also present. Since these stocks are not in public distribution, no RRIDs are available.

<sup>b</sup> This column may be used to sort row/genes by cytological order (see Supplementary Table 1)

Supplementary Table 3. PCR primers

| Gene              | FlyBase ID  | Forward primer                   | Reverse primer                   | Forward sequencing primer <sup>a</sup> | Reverse sequencing primer <sup>a</sup> | Cytological order <sup>b</sup> |
|-------------------|-------------|----------------------------------|----------------------------------|----------------------------------------|----------------------------------------|--------------------------------|
| <i>4E-T</i>       | FBgn0052016 | TCCAAGTGTTAAGGATCTGGAAGCTCAAATG  | CAGCGCAGGGTATAAAAGCATTAATAAAG    |                                        |                                        | 81                             |
| <i>Abcd1</i>      | FBgn0039890 | TGTTGACCGGATTGCAGAAAAGTGTGAAC    | TGTTATTTACCGAAACGCCAGAGACAG      |                                        |                                        | 19                             |
| <i>Actbeta</i>    | FBgn0024913 | CCGGAAGTCATTTCACAACCCGCTGC       | GACACCAGTAGGCTTCTCTGACTGAC       |                                        |                                        | 95                             |
| <i>Ank</i>        | FBgn0011747 | CTTGCGGTAGCAATGCAGCAGGGACAC      | TGTTGTCCAACAATAATGAGCTGCTTC      |                                        |                                        | 10                             |
| <i>anne</i>       | FBgn0052000 | CCATGTATTGGACTATTAATCCAGATCAGG   | GGCACGTACTTTTGAATTTGGCCGAGG      |                                        |                                        | 11                             |
| <i>apolpp</i>     | FBgn0087002 | CCAAGCGGTACCAAGGCAAACCTACCAG     | CACCTAGTGCAGCTACCGTATTGCCAC      |                                        |                                        | 94                             |
| <i>Arf4</i>       | FBgn0013749 | GGGACTAACAATATCTAGTCTTTTGACACG   | GGTTAAGGCGCAACTTGTCCGTAAGCTC     |                                        |                                        | 98                             |
| <i>Arl4</i>       | FBgn0039889 | TCGACAATATTTTCATTGAAGATGGGTGCTAC | CCTGGTTATCAGGACATTTAGCGGTACG     |                                        |                                        | 17                             |
| <i>Asator</i>     | FBgn0039908 | ATTCATCGGATGTGGGCGGAATGATCG      | TGCTTTAATTTTCGCCATGGCAGTTG       |                                        |                                        | 48                             |
| <i>ATPsynbeta</i> | FBgn0010217 | TGCTGCGGTGGCCTTCGACAAGTCCTG      | CGAATTCAGGAGCCTCAGCATGAATGG      |                                        |                                        | 91                             |
| <i>bt</i>         | FBgn0005666 | GAACCTGCGGTTCTTGACAGAAAAATGG     | AGTACAGTTGGTTGTGCGGCGTGCTATG     |                                        |                                        | 69                             |
| <i>Cadps</i>      | FBgn0053653 | TCTCTTTCCCGAAAACAACCCCT          | CCAGTGCGGGACTATTACGG             | TCTCTTTCCCGAAAACAACCCCT                | CCATCAAAGTTCATTGCACGACAACTGG           | 107                            |
| <i>Cals</i>       | FBgn0039928 | CCGTATCACGAAATTCATTAAAGGTAAG     | ACGCACCGTGTAGTCAATGCGTTCTGG      |                                        |                                        | 97                             |
| <i>CaMKI</i>      | FBgn0016126 | TTACGGTTAACTCATCCAAATATAGTGCAAC  | GAACCTGTACGCTCAACCCCAACCTTC      |                                        |                                        | 44                             |
| <i>CaMKII</i>     | FBgn0264607 | GTAGATATATCAATCCGCATCTGCAAGC     | CGAACGGAGGGAAAAGGATGAAGTATG      |                                        |                                        | 92                             |
| <i>CG11360</i>    | FBgn0039920 | CATTACCATGGCGGCTCAGGTCACACC      | AGACGGACTCGGCCAGATCGACTACGG      |                                        |                                        | 66                             |
| <i>CG1674</i>     | FBgn0039897 | TTTGAAACATTTCATTACGTGCACCTTC     | TCAGATTTTCATTGACAGACGACGACGAG    |                                        |                                        | 26                             |
| <i>CG1909</i>     | FBgn0039911 | GTCCTGCCAGCAAAGCCTAAGACAGTAC     | GTCCACTTCTGCATTTAGTGCCGCACTC     |                                        |                                        | 55                             |
| <i>CG31998</i>    | FBgn0051998 | GATTCAACAGACAGAAGGGAGTGTGTG      | CCATCGTACTTACTCGATGGTATTGGC      |                                        |                                        | 21                             |
| <i>CG31999</i>    | FBgn0051999 | TTCGACATGCTCGTACGCCGCTCTT        | CCCGGGGTGGCACTTACATTGGTATGC      |                                        |                                        | 23                             |
| <i>CG32006</i>    | FBgn0052006 | TGTCATACCACCTTACTTCTTCGGCAAAACAG | TCGCCCATTTTGTAAACATAGCCATTCC     |                                        |                                        | 13                             |
| <i>CG32017</i>    | FBgn0052017 | TGGCAACTTAAAGGCTTGGAATGCAC       | CGAAATTACTCACTTCTACATGACAATC     |                                        |                                        | 100                            |
| <i>CG33521</i>    | FBgn0250819 | ACGTAATGTTATTCATGTACGTTTCACGAG   | GTGCATAGAGCAATATAGACTTCCATCATG   |                                        |                                        | 103                            |
| <i>CG33978</i>    | FBgn0053978 | TTCAGATAGACGTGCGGTGTTTCTCACAC    | TTGCAGGCTGAAGCCGGCTGTTTCTATG     |                                        |                                        | 15                             |
| <i>ci</i>         | FBgn0004859 | CCCGGCCTATAGAATCCCGGATACATGG     | GCAGACGGGTTGAATGAACATGAGACATGG   |                                        |                                        | 5                              |
| <i>Crk</i>        | FBgn0024811 | CGGCTGCGGTGTAAGGGTGGAAAAAG       | CCCGCTAGTCATAAAATAGGCGTGGGGAAAAG |                                        |                                        | 22                             |
| <i>dati</i>       | FBgn0262636 | GCAAGGGAAACAGGTATCATCTTTCGACG    | CGTAATAGATTCAAGAATGGGCTATAATTCTG |                                        |                                        | 37                             |
| <i>dpr7</i>       | FBgn0053481 | ACGTGGGAACTTGCCCTCCATTTCATC      | TGCAITTCAGCTATTTATTGCCCAACTC     |                                        |                                        | 27                             |
| <i>elF4G1</i>     | FBgn0023213 | ACTCACAAACCCCTTACCAG             | TCTCGTAACACCTTAAACGAAACG         | CATGCCATACTACCACTACCAATATGTC           | GATTCTGATCCTGTGATGATGTACTGG            | 79                             |
| <i>Ekar</i>       | FBgn0039916 | GACAAAAGCCTTTTGCTGAGACTACCG      | CGTTGCTTACGTTACTACTATGACTGACG    |                                        |                                        | 62                             |
| <i>Eph</i>        | FBgn0025936 | CGATCGTGGATCAGCAAACCGACTTTACATTG | TGGCGTCTCATACGGTTCTGCGTTATC      |                                        |                                        | 58                             |
| <i>Ephrin</i>     | FBgn0040324 | ACGTGCCAGGAAGTATTGAGAGTATCC      | GCCACGCGTGTATACGCGACATTAGGG      |                                        |                                        | 54                             |
| <i>ey</i>         | FBgn0005558 | GGCTGCGCAAAAGGAGCAGCAAAGCAC      | TTGCAACGGGGCAGTTTCTCTGGTGAC      |                                        |                                        | 68                             |
| <i>fd102C</i>     | FBgn0039937 | CTACCCACTTGGAACGACAAGAACTTCAC    | CGGACCTTACGCTGCGCCTTGCAGCGTC     |                                        |                                        | 73                             |
| <i>fuss</i>       | FBgn0039932 | CAGTTACAACGAGATACACAACAGGAGAG    | CGCAGTGGATTGTGTTGGTGAGCCAGG      |                                        |                                        | 84                             |
| <i>Gat</i>        | FBgn0039915 | GCGGCTTGTTGGGTATTAGACTGGACATGG   | GTAATAATACCGCCACGCAAGAATGAC      |                                        |                                        | 61                             |
| <i>gw</i>         | FBgn0051992 | AGCTTTTCCCTCAAATGTGACTAACAATAC   | CCACTGTGTTGGGATATTGCTGATACTG     |                                        |                                        | 63                             |
| <i>Gyf</i>        | FBgn0039936 | ACCAACGCCCAAGCGGTCTATATGATG      | ACAACAGATTGTAGCATGAATGGTATCG     |                                        |                                        | 75                             |
| <i>Hcf</i>        | FBgn0039904 | TGTTTTTGGGGGAATGATCGAATACGG      | GCCTCCCTGAAGTTTTCGGCTTCGAC       |                                        |                                        | 36                             |
| <i>JYalpha</i>    | FBgn0267363 | GCAGGCGGCAGATATGATTCTATTGGATG    | CGCGGGTCTAATGTCAAACCGCCATTTC     |                                        |                                        | 1                              |
| <i>Kif3C</i>      | FBgn0039925 | TCCGTGACTAATCCATCAGCTCGCATATC    | TCATGCGGTGGACATAGCGACGGTAAC      |                                        |                                        | 101                            |
| <i>lgs</i>        | FBgn0039907 | GCCGTGCTACGCAGATGCTACTAGACGC     | CGTAGAACAAATGTAGTCCGCTTGAAGGG    |                                        |                                        | 43                             |
| <i>mav</i>        | FBgn0039914 | AGGAAACAGCTGTGTGGTGCGACTCT       | TGGTACGGGTGTTATCCACTGAACTGAC     |                                        |                                        | 59                             |
| <i>MED26</i>      | FBgn0039923 | CGGGTGAACATACCTTACTTCATCCGAAC    | AAAACTAATTGGTGGGCACACGATTCC      |                                        |                                        | 71                             |
| <i>mGluR</i>      | FBgn0019985 | ATTTCCGGTCCATGAAAAAGGTGAAGG      | CTGCTTTCAGAACATTGATTAAGACTCACC   |                                        |                                        | 80                             |
| <i>Mpv17</i>      | FBgn0039930 | CCAATCACTTTGCGATTATTAGGCAACC     | GGCGATGAAGTGGGCGTAGAGCACCTG      |                                        |                                        | 89                             |
| <i>myo</i>        | FBgn0026199 | TGCTGCGGTTGGAGAAAATATAAAACC      | CCACTTTGGCTTTCTACGCAACTGACG      |                                        |                                        | 67                             |

|            |             |                                  |                                  |                      |                             |    |
|------------|-------------|----------------------------------|----------------------------------|----------------------|-----------------------------|----|
| ND-49      | FBgn0039909 | CGTTGATTCTCTGGACTTTCACACTTGAGG   | TACGGCAATGCCTGGGTGTATGTCCTTG     |                      | 53                          |    |
| Nfi        | FBgn0042696 | AACATACCTCACATATCTTTGGGATTGTC    | TAACCATGACCAAATCAAGCCGCCATAC     |                      | 29                          |    |
| onecut     | FBgn0028996 | GTGAAACACCGGACCTGCTCTGCATCTG     | GATCTCTGGTGCAAGCCATGGATGATCTG    |                      | 57                          |    |
| pan        | FBgn0085432 | CGCATTGTTTAGTTTTATAACACAGGGAAG   | TTATCTCGTGCCTCCACCCCGGATAC       |                      | 9                           |    |
| pho        | FBgn0002521 | CATGAAAACGACAAAATAAAGCGGCTGAC    | TGAAGAATCCGGAAGCAAGATTGGCTCTTC   |                      | 102                         |    |
| PIP4K      | FBgn0039924 | GTAATGCTTTTGCCGGACGATTTTCGAGC    | CTCATGACAACGAAGTAATATTGCACGC     |                      | 104                         |    |
| PMCA       | FBgn0259214 | GCTGTGTTGCAGGAGGAGGAAGAACACC     | GTTCTGCAACTGCCGACCAAGGAGCG       |                      | 35                          |    |
| Pur-alpha  | FBgn0022361 | GCGTCGAACAGGAATTGGCTACGAAAATG    | GGGCAAACGATTTACTCTCTGATTAGACC    |                      | 52                          |    |
| Rad23      | FBgn0026777 | CGCGATTCTGTCTAGTTCAAATCGTAATCAAC | TGAAGTGTGCAAATTTAGTACTTCTGGGTAG  |                      | 32                          |    |
| RhoGAP102A | FBgn0259216 | CCTGGAGATATATGACAGATGCAGGCCG     | CTTCGAGAATGCTTAGTGGAGCATCGCC     |                      | 28                          |    |
| Rnf11      | FBgn0052850 | CAATGTGGCATCTAGCCCGTCTCAACG      | GGATTTCAAATTTCTCAGCTCCTTAGTATTGC |                      | 34                          |    |
| Slip1      | FBgn0024728 | GCCAGTGCCTCTCAACAAATAAAATTGC     | CCGTCCGACCGCTCGATCATTCAAAGTC     |                      | 65                          |    |
| Sox102F    | FBgn0039938 | GCATGACCGACATTGATGCGAGTGCTC      | GGATTCTCGTACGTTGGGGACTCTGGC      |                      | 72                          |    |
| sv         | FBgn0005561 | TTCCACTAGTTGATGATCAGGGAACTCG     | TGAGAGCACTAATTGTTACCGCAGCTGTCC   |                      | 96                          |    |
| Syt7       | FBgn0039900 | GAATAAAAGGCGACGCCCTCGCAGCAG      | TTCGTCAGTCCATGCTTAAATCACTC       |                      | 31                          |    |
| Taf3       | FBgn0026262 | GCCACCAATGCAGGACCCCGAACTTCG      | CAGTTAGCATCGTATGTTGTTAGACGC      |                      | 45                          |    |
| Tdg        | FBgn0026869 | TGGATCCGAAATCAAAATGTCAGACACC     | ACTCGATGCTGTTCTGTGCGTTGCTG       |                      | 50                          |    |
| toy        | FBgn0019650 | GCATCCCCACTCGTCAGTCGGGCAGAG      | GGTCAACAACTTGCCTACGTCCATATC      |                      | 86                          |    |
| ukar       | FBgn0039927 | TGGCCCAACAGATGCTCTTTAGCTAGTC     | CGGACAAACGGACATGGCTAGATCGAC      |                      | 99                          |    |
| unc-13     | FBgn0025726 | GATTTATTCACTGGCAAGTAACGCCTTTTCG  | CAGTCTATACCTTGAGAACCTGCCGTCC     |                      | 76                          |    |
| yellow-h   | FBgn0039896 | CGTTGGTAGATCTGGTCGATTGATTCC      | AAAGATATGGATGAGACGCACTGAATG      |                      | 24                          |    |
| zfh2       | FBgn0004607 | CGGATGTTCCATGCTCAGGGTTTTTGC      | TGCCCATCCATAAAAACCTATCCATCC      |                      | 49                          |    |
| Zyx        | FBgn0011642 | CCGGCAATTTTGGAACAGCA             | ATCAATCGACCATGCAAAAATTACA        | CCGGCAATTTTGGAACAGCA | TCAATTGGTTTGTGAATTCCAAAGTGG | 93 |

<sup>a</sup> For most genes, we used the same primers to amplify the genomic region surrounding each mutation and sequence the PCR product. In these columns, we note genes for which we used a different primer pair for sequencing.

<sup>b</sup> This column may be used to sort rows/genes by cytological order (see Supplementary Table 1)

Supplementary Table 4. Stocks and reagents used in this study

A. *Drosophila melanogaster* stocks obtained from the Bloomington Drosophila Stock Center (BDSC)

| Stock number                                   | Stock genotype                                                                                                                                                                                                                                          | Research Resource Identifier (RRID) |
|------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| <i>Stocks used in screens</i>                  |                                                                                                                                                                                                                                                         |                                     |
| 117                                            | <i>sn</i> <sup>360</sup> / <i>C(1)DX</i> , <i>y</i> <sup>1</sup> <i>f</i> <sup>1</sup>                                                                                                                                                                  | RRID:BDSC_117                       |
| 525                                            | <i>Kl</i> <sup>1</sup>                                                                                                                                                                                                                                  | RRID:BDSC_525                       |
| 950                                            | <i>Df(1)RA2/FM7c</i>                                                                                                                                                                                                                                    | RRID:BDSC_950                       |
| 5906                                           | <i>w</i> <sup>1118</sup> / <i>Dp(1;Y)y</i> <sup>+</sup> ; <i>TM2/TM6C</i> , <i>Sb</i> <sup>1</sup>                                                                                                                                                      | RRID:BDSC_5906                      |
| 6598                                           | <i>y</i> <sup>1</sup> <i>w</i> <sup>1118</sup>                                                                                                                                                                                                          | RRID:BDSC_6598                      |
| 6878                                           | <i>P{w<sup>+</sup>mmW.Scer<sup>+</sup>FRT.hs=RS3}l(1)CB-6411-3<sup>1</sup>, <i>w</i><sup>1118</sup> /<i>FM7h</i></i>                                                                                                                                    | RRID:BDSC_6878                      |
| 9549                                           | <i>w</i> <sup>1118</sup> ; <i>P{w<sup>+</sup>mmC=ActGFP}unc-13<sup>GJ</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                             | RRID:BDSC_9549                      |
| 17599                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>67c23</sup> ; <i>P{y<sup>+</sup>mmDint2 <i>w<sup>+</sup>mmC=EPgy2}EY09758</i></i>                                                                                                                                   | RRID:BDSC_17599                     |
| 24488                                          | <i>y</i> <sup>1</sup> <i>M{RFP<sup>3xP3.PB</sup> GFP<sup>E.3xP3</sup>=vas-int.Dm}ZH-2A w<sup>+</sup>; M{3xP3-RFP.attP}ZH-102D</i>                                                                                                                       | RRID:BDSC_24488                     |
| 24639                                          | <i>w</i> <sup>1118</sup> / <i>Dp(2;Y)G</i> , <i>P{w<sup>+</sup>mmC=hs-hid}y; <i>wg</i><sup>Sp-1</sup> /<i>CyO</i></i>                                                                                                                                   | RRID:BDSC_24639                     |
| 24640                                          | <i>w</i> <sup>1118</sup> / <i>Dp(2;Y)G</i> , <i>P{w<sup>+</sup>mmC=hs-hid}y; <i>MKRS/TM2</i>, <i>y</i><sup>+</sup></i>                                                                                                                                  | RRID:BDSC_24640                     |
| 24800                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>67c23</sup> ; <i>Mi{GFP<sup>E.3xP3</sup>=ET1}CG32017</i> <sup>MB02477</sup>                                                                                                                                         | RRID:BDSC_24800                     |
| 26932                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>P{w<sup>+</sup>mmC=EP}G16942</i>                                                                                                                                                                       | RRID:BDSC_26932                     |
| 26964                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>P{w<sup>+</sup>mmC=EP}MESR3</i> <sup>G19459</sup>                                                                                                                                                      | RRID:BDSC_26964                     |
| 35531                                          | <i>winscy/Dp(2;Y)G</i> , <i>P{w<sup>+</sup>mmC=hs-hid}Y</i>                                                                                                                                                                                             | RRID:BDSC_35531                     |
| 35532                                          | <i>C(1)RA</i> , <i>ln(1)sc</i> <sup>II</sup> , <i>ln(1)sc</i> <sup>8</sup> , <i>l(1)1Ac</i> <sup>1</sup> , <i>sc</i> <sup>II</sup> <i>sc</i> <sup>8</sup> / <i>winscy/Dp(1;Y)y</i> <sup>+</sup>                                                         | RRID:BDSC_35532                     |
| 78781                                          | <i>y</i> <sup>1</sup> <i>sc</i> <sup>+</sup> <i>v</i> <sup>1</sup> <i>sev</i> <sup>21</sup> ; <i>P{y<sup>+</sup>tt7.7 v<sup>+</sup>tt1.8=nanos-Cas9.R}attP40</i>                                                                                        | RRID:BDSC_78781                     |
| 78782                                          | <i>y</i> <sup>1</sup> <i>sc</i> <sup>+</sup> <i>v</i> <sup>1</sup> <i>sev</i> <sup>21</sup> ; <i>P{y<sup>+</sup>tt7.7 v<sup>+</sup>tt1.8=nanos-Cas9.R}attP2</i>                                                                                         | RRID:BDSC_78782                     |
| 81692                                          | <i>y</i> <sup>1</sup> <i>v</i> <sup>1</sup> ; <i>P{y<sup>+</sup>tt7.7 v<sup>+</sup>tt1.8=TKO.GS03310}attP40</i>                                                                                                                                         | RRID:BDSC_81692                     |
| 94596                                          | <i>w</i> <sup>1118</sup> ; <i>Tl(Tl)FRT101F</i>                                                                                                                                                                                                         | RRID:BDSC_94596                     |
| <i>Stocks used in characterizing mutations</i> |                                                                                                                                                                                                                                                         |                                     |
| 638                                            | <i>bt</i> <sup>D</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                                                                                                 | RRID:BDSC_638                       |
| 642                                            | <i>ci</i> <sup>1</sup> <i>sv</i> <sup>n</sup>                                                                                                                                                                                                           | RRID:BDSC_642                       |
| 648                                            | <i>ey</i> <sup>2</sup>                                                                                                                                                                                                                                  | RRID:BDSC_648                       |
| 658                                            | <i>sv</i> <sup>spa-1</sup>                                                                                                                                                                                                                              | RRID:BDSC_658                       |
| 1082                                           | <i>Df(4)M101-63a/ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                                                                                                            | RRID:BDSC_1082                      |
| 1197                                           | <i>Df(4)38/ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup> <i>sv</i> <sup>spa-pol</sup>                                                                                                                                     | RRID:BDSC_1197                      |
| 2515                                           | <i>st</i> <sup>1</sup> <i>ry</i> <sup>2</sup> ; <i>sv</i> <sup>spa-pol</sup>                                                                                                                                                                            | RRID:BDSC_2515                      |
| 4055                                           | <i>C(1)DX</i> , <i>y</i> <sup>1</sup> <i>f</i> <sup>1</sup> ; <i>T(1;4)sc</i> <sup>H</sup> , <i>sc</i> <sup>H</sup>                                                                                                                                     | RRID:BDSC_4055                      |
| 4231                                           | <i>w</i> <sup>1118</sup> ; <i>bt</i> <sup>1-b</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                                                                    | RRID:BDSC_4231                      |
| 4759                                           | <i>w</i> <sup>1118</sup> ; <i>pan</i> <sup>2</sup> / <i>P{w<sup>+</sup>mmC=ActGFP}unc-13<sup>GJ</sup></i>                                                                                                                                               | RRID:BDSC_4759                      |
| 4760                                           | <i>pan</i> <sup>3</sup> / <i>Dp(2;4)ey</i> <sup>D</sup> , <i>Ablp</i> <sup>eyD</sup> : <i>ey</i> <sup>D</sup>                                                                                                                                           | RRID:BDSC_4760                      |
| 6212                                           | <i>pho</i> <sup>1</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                                                                                                | RRID:BDSC_6212                      |
| 7082                                           | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Df(4)J2/ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup> <i>sv</i> <sup>spa-pol</sup>                                                                                       | RRID:BDSC_7082                      |
| 7083                                           | <i>w</i> <sup>+</sup> ; <i>Df(4)C3/ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup> <i>sv</i> <sup>spa-pol</sup>                                                                                                             | RRID:BDSC_7083                      |
| 7084                                           | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Df(4)O2/ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup> <i>sv</i> <sup>spa-pol</sup>                                                                                       | RRID:BDSC_7084                      |
| 8067                                           | <i>w</i> <sup>1118</sup> ; <i>Df(4)ED6366</i> , <i>P{w<sup>+</sup>mmW.Scer<sup>+</sup>FRT.hs3=3'.RSS+3.3'}<i>Abcd1</i><sup>ED6366</sup> <i>pan</i><sup>ED6366</sup> /<i>Dp(2;4)ey</i><sup>D</sup>, <i>Ablp</i><sup>eyD</sup>: <i>ey</i><sup>D</sup></i> | RRID:BDSC_8067                      |
| 9422                                           | <i>w</i> <sup>1118</sup> ; <i>Df(4)ED6369</i> , <i>P{w<sup>+</sup>mmW.Scer<sup>+</sup>FRT.hs3=3'.RSS+3.3'}<i>ED6369/l(4)102Etf</i><sup>1</sup></i>                                                                                                      | RRID:BDSC_9422                      |
| 9433                                           | <i>w</i> <sup>+</sup> ; <i>Df(4)M101-62f/Dp(2;4)ey</i> <sup>D</sup> , <i>Ablp</i> <sup>eyD</sup> : <i>ey</i> <sup>D</sup>                                                                                                                               | RRID:BDSC_9433                      |
| 9549                                           | <i>w</i> <sup>1118</sup> ; <i>P{w<sup>+</sup>mmC=ActGFP}unc-13<sup>GJ</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                             | RRID:BDSC_9549                      |
| 14379                                          | <i>w</i> <sup>1118</sup> ; <i>P{y<sup>+</sup>mmDint2 <i>w<sup>+</sup>BR.E.BR=SUPor-P}dat<sup>KG01667</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i></i>                                                          | RRID:BDSC_14379                     |
| 14579                                          | <i>y</i> <sup>1</sup> ; <i>ry</i> <sup>506</sup> ; <i>P{y<sup>+</sup>mmDint2 <i>w<sup>+</sup>BR.E.BR=SUPor-P}PlexB<sup>KG00878</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i></i>                                | RRID:BDSC_14579                     |
| 22661                                          | <i>y</i> <sup>1</sup> ; <i>Mi{GFP<sup>E.3xP3</sup>=ET1}Ekar</i> <sup>MB00001</sup>                                                                                                                                                                      | RRID:BDSC_22661                     |
| 23097                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>67c23</sup> ; <i>P{y<sup>+</sup>mmDint2 <i>w<sup>+</sup>mmC=EPgy2}PlexA<sup>EY16548</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i></i>                                       | RRID:BDSC_23097                     |
| 23727                                          | <i>y</i> <sup>1</sup> ; <i>Mi{GFP<sup>E.3xP3</sup>=ET1}MED26</i> <sup>MB00704</sup>                                                                                                                                                                     | RRID:BDSC_23727                     |
| 31010                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}Gyf<sup>MI00455</sup></i>                                                                                                                                                 | RRID:BDSC_31010                     |
| 42700                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>1118</sup> ; <i>lgs</i> <sup>17E</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                                             | RRID:BDSC_42700                     |
| 60760                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}CG31998<sup>MI02727</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                       | RRID:BDSC_60760                     |
| 60790                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}CG31998<sup>MI06163</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                       | RRID:BDSC_60790                     |
| 60804                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}dpr7<sup>MI07186</sup></i>                                                                                                                                                                      | RRID:BDSC_60804                     |
| 60814                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}CG33978<sup>MI08362</sup></i>                                                                                                                                                                   | RRID:BDSC_60814                     |
| 60815                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}CG33978<sup>MI08413</sup></i>                                                                                                                                                                   | RRID:BDSC_60815                     |
| 60816                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}Cadps<sup>MI08448</sup> /<i>Dp(2;4)ey</i><sup>D</sup>, <i>Ablp</i><sup>eyD</sup>: <i>ey</i><sup>D</sup></i>                                                                                     | RRID:BDSC_60816                     |
| 60832                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}CG1674<sup>MI10152</sup> /<i>Dp(2;4)ey</i><sup>D</sup>, <i>Ablp</i><sup>eyD</sup>: <i>ey</i><sup>D</sup></i>                                                                                    | RRID:BDSC_60832                     |
| 60856                                          | <i>y</i> [1]; <i>Mi{y<sup>+</sup>mmDint2 =MIC}PMCA<sup>MI12515</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                                    | RRID:BDSC_60856                     |
| 61706                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}CG33978<sup>MI03508</sup></i>                                                                                                                                                                   | RRID:BDSC_61706                     |
| 61711                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}anne<sup>MI04450</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                          | RRID:BDSC_61711                     |
| 61741                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Mi{GFP<sup>E.3xP3</sup>=ET1}PlexA</i> <sup>MB09499</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                              | RRID:BDSC_61741                     |
| 62013                                          | <i>y</i> <sup>1</sup> ; <i>Mi{y<sup>+</sup>mmDint2 =MIC}Hcf<sup>MI03718</sup> /<i>ln(4)ci</i><sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                           | RRID:BDSC_62013                     |
| 62020                                          | <i>w</i> <sup>1118</sup> ; <i>Mi{GFP<sup>E.3xP3</sup>=ET1}Ank</i> <sup>MB09683</sup>                                                                                                                                                                    | RRID:BDSC_62020                     |
| 76037                                          | <i>w</i> <sup>+</sup> ; <i>lgs</i> <sup>2-8</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                                                                      | RRID:BDSC_76037                     |
| 76667                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Mi{Trojan-GAL4.0}eIF4G1</i> <sup>MI05019-TG4.0</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                  | RRID:BDSC_76667                     |
| 76741                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Mi{Trojan-GAL4.2}PMCA</i> <sup>MI12515-TG4.2</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                    | RRID:BDSC_76741                     |
| 78385                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Mi{Trojan-GAL4.1}dpr7</i> <sup>MI05719-TG4.1</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                                                    | RRID:BDSC_78385                     |
| 79256                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>+</sup> ; <i>Tl{GFP<sup>3xP3.cla</sup>=CRIMIC.TG4.1}Gyf</i> <sup>CR00412-TG4.1</sup> / <i>ln(4)ci</i> <sup>D</sup> , <i>ci</i> <sup>D</sup> <i>pan</i> <sup>ciD</sup>                                               | RRID:BDSC_79256                     |
| 84132                                          | <i>y</i> <sup>1</sup> <i>w</i> <sup>67c23</sup> ; <i>Df(4)8-M318</i> , <i>P{w<sup>+</sup>mmW.hs=hsp26-pt-Tj8-M318/ln(4)ci<sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                               | RRID:BDSC_84132                     |
| 84133                                          | <i>w</i> <sup>+</sup> ; <i>Df(4)5-M244</i> , <i>P{w<sup>+</sup>mmW.hs=hsp26-pt-Tj5-M244/ln(4)ci<sup>D</sup>, <i>ci</i><sup>D</sup> <i>pan</i><sup>ciD</sup></i>                                                                                         | RRID:BDSC_84133                     |

|        |                                                                                                                          |                  |
|--------|--------------------------------------------------------------------------------------------------------------------------|------------------|
| 84134  | $y^1 w^{67c23}; Df(4)8-M175, P\{w^{mW.hs} = hsp26-pt-Tj8-M175/ln(4)ci^D, ci^D pan^{ciD}$                                 | RRID:BDSC_84134  |
| 84135  | $w^{1118}; Df(4)8-M91, P\{w^{mW.hs} = hsp26-pt-Tj8-M91/ln(4)ci^D, ci^D pan^{ciD}$                                        | RRID:BDSC_84135  |
| 84136  | $w^*; Df(4)5-M263, P\{w^{mW.hs} = hsp26-pt-Tj5-M263/ln(4)ci^D, ci^D pan^{ciD}$                                           | RRID:BDSC_84136  |
| 85105  | $w^{1118}; P\{w^{mC} = XP\}CG33941^{d09357}$                                                                             | RRID:BDSC_85105  |
| 86431  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.0\}Zyx^{CR01526-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                              | RRID:BDSC_86431  |
| 86471  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.2\}Ephrin^{CR01621-TG4.2}/ln(4)ci^D, ci^D pan^{ciD}$                           | RRID:BDSC_86471  |
| 90850  | $y^1 w^*; Tl\{RFP^{DsRed.3xP3.cla} = Tl\}Crk^{dsRed}/Tl\{GMR-HMS04515\}Gat^{eya}$                                        | RRID:BDSC_90850  |
| 90852  | $y^1 w^*; Tl\{GMR-HMS04515\}Gat^{eya}/ln(4)ci^D, ci^D pan^{ciD}$                                                         | RRID:BDSC_90852  |
| 92207  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.1\}myo^{CR02262-TG4.1}$                                                        | RRID:BDSC_92207  |
| 93687  | $y^1 w^{1118}; Mi\{DH.1\}Cadps^{M105877-DH.GT-TG4.1}/ln(4)ci^D, ci^D pan^{ciD}$                                          | RRID:BDSC_93687  |
| 93690  | $y^1 w^{1118}; Mi\{DH.0\}Hcf^{M111162-DH.GT-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                                            | RRID:BDSC_93690  |
| 93692  | $y^1 w^{1118}; Mi\{DH.0\}Rnf11^{M14327-DH.GT-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                                           | RRID:BDSC_93692  |
| 93693  | $y^1 w^{1118}; Mi\{DH.1\}Actbeta^{M14795-DH.GT-TG4.1}/ln(4)ci^D, ci^D pan^{ciD}$                                         | RRID:BDSC_93693  |
| 93694  | $y^1 w^{1118}; Mi\{DH.1\}PlexB^{M15559-DH.GT-TG4.1}/ln(4)ci^D, ci^D pan^{ciD}$                                           | RRID:BDSC_93694  |
| 94483  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.0\}Slip1^{CR02779-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                            | RRID:BDSC_94483  |
| 94599  | $y^1 w^{67c23}; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.1\}anne^{CR70036-TG4.1}/ln(4)ci^D, ci^D pan^{ciD}$                       | RRID:BDSC_94599  |
| 94691  | $y^1 w^{67c23}; Df(4)8-M167, P\{w^{mW.hs} = hsp26-pt-Tj8-M167/ln(4)ci^D, ci^D pan^{ciD}$                                 | RRID:BDSC_94691  |
| 94692  | $y^1 w^{67c23}; Df(4)4-M529, P\{w^{mW.hs} = hsp26-pt-Tj4-M529/ln(4)ci^D, ci^D pan^{ciD}$                                 | RRID:BDSC_94692  |
| 95461  | $w^{1118}; PBac\{w^{mC} = RB\}CG33941^{e02647}$                                                                          | RRID:BDSC_95461  |
| 97180  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.1\}ukar^{CR70473-TG4.1}/ln(4)ci^D, ci^D pan^{ciD}$                             | RRID:BDSC_97180  |
| 97184  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.0\}gw^{CR70482-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                               | RRID:BDSC_97184  |
| 97185  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.0\}Yalpha^{CR70483-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                           | RRID:BDSC_97185  |
| 97186  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.0\}PIP4K^{CR70484-TG4.0}/ln(4)ci^D, ci^D pan^{ciD}$                            | RRID:BDSC_97186  |
| 97187  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.1\}PlexA^{CR70485-TG4.1}/ln(4)ci^D, ci^D pan^{ciD}$                            | RRID:BDSC_97187  |
| 97216  | $w^*; zfh2^{w^{1118}}/ln(4)ci^D, ci^D pan^{ciD}$                                                                         | RRID:BDSC_97216  |
| 97333  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = SA-KozakGAL4\}CG32006^{CR70476-KG4}/ln(4)ci^D, ci^D pan^{ciD}$                            | RRID:BDSC_97333  |
| 97334  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.0\}Zip102B^{CR70486-KO-TG4.0}$                                                 | RRID:BDSC_97334  |
| 98482  | $y^1 w^*; Tl\{GFP^{3xP3.cla} = CRIMIC.TG4.1\}CG33978^{CR70478-TG4.1}/Tl\{GMR-HMS04515\}Gat^{eya}$                        | RRID:BDSC_98482  |
| 99055  | $y^1 w^{67c23}; Dr^1/TM3, Sb^1 Ser^1; P\{y^{mDln2} w^{BR.EBR} = SUPor-P\}ATPsynbeta^{KG07618}/ln(4)ci^D, ci^D pan^{ciD}$ | RRID:BDSC_99055  |
| 600120 | $y^1 w^*; Tl\{GFP^{3xP3.cla} = KozakGAL4\}Polr1G^{CR70943-KO-KG4}/ln(4)ci^D, ci^D pan^{ciD}$                             | RRID:BDSC_600120 |
| 600121 | $y^1 w^*; Df(4)CRIMIC-CR70944 Tl\{GFP^{3xP3.cla} = KozakGAL4\}Mpv17^{CR70944-KO-KG4}$                                    | RRID:BDSC_600121 |
| 600122 | $y^1 w^*; Df(4)CRIMIC-CR70944 Tl\{GFP^{3xP3.cla} = KozakGAL4\}fd102C^{CR70946-KO-KG4}$                                   | RRID:BDSC_600122 |
| 600127 | $y^1 w^*; Tl\{GFP^{3xP3.cla} = KozakGAL4\}Arf4^{CR71015-KO-KG4}/ln(4)ci^D, ci^D pan^{ciD}$                               | RRID:BDSC_600127 |
| 603165 | $y^1 w^*; Tl\{GFP^{3xP3.cla} = KozakGAL4\}Pur-alpha^{CR71382-KO-KG4}$                                                    | RRID:BDSC_603165 |

#### B. *Drosophila melanogaster* stocks obtained from the Kyoto Stock Center

| Stock number | Genotype                                                                                                        | Research Resource Identifier (RRID) |
|--------------|-----------------------------------------------------------------------------------------------------------------|-------------------------------------|
| 150531       | $w^{1118}; Df(4)ED6382, P\{w^{mW.Scer\backslash FRT.hs3} = 3'.RS5+3.3'\}ED6382/P\{w^{mC} = ActGFP\}unc-13^{G1}$ | RRID:DGGR_150531                    |
| 150529       | $w^{1118}; Df(4)ED6380, P\{w^{mW.Scer\backslash FRT.hs3} = 3'.RS5+3.3'\}ED6380/l(4)102Eff^1$                    | RRID:DGGR_150529                    |

#### C. Biological and chemical reagents

| Source              | Item                         | Product number |
|---------------------|------------------------------|----------------|
| New England Biolabs | LongAmp Taq 2X Master Mix    | M0287L         |
| Eurofins            | SimpleSeq Premixed Kit       |                |
| Thermo Scientific   | GeneJET PCR Purification Kit | K0701          |

#### D. Software

| Source                          | Item             |
|---------------------------------|------------------|
| Dotmatics                       | SnapGene         |
| Joseph Yost, University of Utah | Poly Peak Parser |



|                            |            |                                                                                                                                                                                                                                                                                                         |                                                                                         |                                                                      |                                                  |                                              |                                    |     |
|----------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------|----------------------------------------------|------------------------------------|-----|
| CG31998 <sup>D</sup>       | F8a0405958 | Frameshift deletion of bases 4:201579 resulting in the addition of CPHLRPTQIF after alanine 245 of the CG31998 PA isoform (which corresponds to A149 of PB) and premature truncation after these novel amino acids                                                                                      | 4:201599<br>ACTGTGAGAGGACCATTGCTCCCA<br>TCTGCCGCCAAC 4:201559                           | 4:201599 ACTGTGAGAGGACCATTGCA<br>TGCCCACTCTGCCGCCAAC 4:201559        | AA236 YELDLERDHALPTSAPNADIL<br>AA256             | AA236 YELDLERDHALPTSAPNADIL<br>AA256         | PA 17%; PB 11%                     | 21  |
| CG31999 <sup>C</sup>       | F8a0406192 | In-frame deletion of bases 4:224219 through 4:224224 resulting in the loss of aspartic acid 97 through glycine 98 from all CG31999 isoforms                                                                                                                                                             | 4:224244<br>CTTTAGACGGTACTCGTGTGACGAGAA<br>GGAATGTAACTATCATCTAG 4:224199                | 4:224244 CTTTAGACGTACTCGTGTG—<br>—GGAAGAAATGTAACTATCATCTAG 4:224199  | AA87 RLALDGTGRCDEGNVTSSSYA<br>AA108              | AA87 RLALDGTGRC—EGNVTSSSYA AA108             |                                    | 23  |
| CG32017 <sup>B</sup>       | F8a0406212 | In-frame deletion of bases 4:1143661 through 4:1143661 resulting in the loss of glutamic acid 77 through cysteine 80 from all CG32017 isoforms                                                                                                                                                          | 4:1143681<br>CCTTTTCACTTAACACAGGAACCGACAT<br>GCGAACAGCTCCGCGCATGTG                      | 4:1143681 CCTTTTCACTTAACACAG—<br>—GGAACAGCTCCGCGCATGTG               | AA67 VPKTFSLSNQPTCEQLRAMWIFS<br>AA90             | AA67 VPKTFSLSNQ—EQLRAMWIFS<br>AA90           |                                    | 100 |
| CG32017 <sup>E</sup>       | F8a0405959 | Frameshift deletion of bases 4:1143652 resulting in the addition of ANSSAPCGFFGRNLELQK after threonine 79 of all CG32017 isoforms and premature truncation after these novel amino acids                                                                                                                | 4:1143672<br>TTAACTACAGGAACGACATGCGAACAG<br>CTCCGCCGCATG 4:1143632                      | 4:1143672 TTAACTACAGGAACGACAC<br>GCGAACAGCTCCGCCGCATG 4:1143632      | TFSLNSQPTCEQLRAMWIFSQRGRABE<br>AA99              | TFSLNSQPTCEQLRAMWIFSQRGRABE<br>AA99          | PB, PC, PD 16%                     | 100 |
| CG32017 <sup>K</sup>       | F8a0405960 | Frameshift deletion of bases 4:1187789 and 4:1187790 resulting in the addition of NGGSHS after tyrosine 88 of the CG3221 PA and PG isoforms (which corresponds to Y74 of PC and PD) and premature truncation after these novel amino acids                                                              | 4:1187769<br>AATGCAAGAACCCGTGTGCAAAATGGAG<br>GAATGATCTCTTA 4:1187810                    | 4:1187769 AATGCAAGAACCCGTGTAC—<br>AATGGAGGAAGTCTCTTA 4:1187810       | AA67 VPKTFSLSNQPTCEQLRAMWIFSQRABE<br>AE AA98     | AA67 VPKTFSLSNQPTCEQLRAMWIFSQRABE<br>AE AA98 | PB, PC, PD 16%                     | 100 |
| CG33521 <sup>C</sup>       | F8a0404798 | Frameshift deletion of bases 4:174838 through 4:174844 resulting in the addition of LPHVSYSQ after arginine 39 of all CG33978 isoforms and premature truncation after these novel amino acids                                                                                                           | 4:174864<br>TTACAATTTCACTTGACCGTGTCTCTTT<br>ACCACACTCTTATTA 4:174818                    | 4:174864 TTACAATTTCACTTGACCG—<br>—GTTTACACACTCTTATTA 4:174818        | AA30 STYNNFNLRLVFTLLF AA47<br>AA140              | AA30 STYNNFNLRLPHVSYSQ* AA47<br>AA140        | PA 13%; PC, PD 11%; PG 15%         | 103 |
| CG33978 <sup>B</sup>       | F8a0404782 | Insertion of one base (G) between bases 4:51916 and 4:51917 and deletion of bases 4:51912 through 4:51913 resulting in the addition of MVWDQLFWAAEV after glycine 149 of the <i>ci</i> PA and PC isoforms (which corresponds to G31 of PB) and premature truncation after these novel amino acids       | 4:51936<br>GATGATCTTCCATCTTGCCG<br>GGACTGTGGTGGATCAGCTGATT<br>4:51892                   | 4:51936 CATGACATCTTCTTGCCGGA—<br>TGGTGTGGGATCAGCTGATT 4:51892        | AA140 NCASAFHLAGLGLGSADFLGSRGL<br>AA163          | AA140 NCASAFHLAGMVDQLFWAAEV*<br>AA163        | PC, PE 2%; PD 2%; PF 2%; PG 2%     | 15  |
| <i>ci</i> <sup>C</sup>     | F8a0404782 |                                                                                                                                                                                                                                                                                                         | 4:51943<br>AATTGTGATCAGCATCTTCTTGCCGGA<br>CTTGGTTGGGATCAGCTGATTTT<br>4:51889            | 4:51943 AATTGTGATCAGCATCTTCA—<br>—TTTGGGATCAGCTGATTTT 4:51889        | AA137 PYNVCASAFHLAGLGLGSADFLGSR<br>AA161         | AA137 PYNVCASAF—LGSADFLGSR<br>AA161          | PA 11%; PB 2%; PC 26%              | 5   |
| <i>ci</i> <sup>F</sup>     | F8a0406189 | In-frame deletion of bases 4:51909 through 4:51923 resulting in the loss of leucine 147 through glycine 151 from the <i>ci</i> RA and RC isoforms (which corresponds to L29 through G33 of PB)                                                                                                          | 4:51936<br>AGTTCGAAAGACGAGATCAATGGTGGG<br>CTGCGGTGAATC 4:211939                         | 4:51936 AGTTCGAAAGACGAGGATC—<br>TGTGGTGAATCAGCTGATC 4:211939         | AA132 VLTIVRKDEDQWWTAR AA147<br>AA132            | AA132 VLTIVRKDEDQWWTAR AA147<br>AA132        | PA, PC 52%; PB 49%; PD 52%; PE 51% | 22  |
| <i>Crk</i> <sup>E</sup>    | F8a0405961 | Frameshift deletion of bases 4:211918 and 4:211919 resulting in the addition of LVDCAs after aspartic acid 141 of the <i>Crk</i> PA, PC and PD isoforms (which corresponds to D123 of PB and D133 of PE) and premature truncation after these novel amino acids. The PG and PH isoforms are unaffected. | 4:211895<br>AATGATCTGAAAAGACGAGGATCAATGGT<br>GGACTGCGGTAAC 4:211937                     | 4:211895 AATGATCTGAAAAGACGAGG—<br>AATGGTGAATCAGCTGTAAC 4:211937      | AA131 EVLTVRKDEDQWWTARNSSGK<br>AA152             | AA131 EVLTVRKDE—EWWWTARNSSGK<br>AA152        |                                    | 22  |
| <i>Crk</i> <sup>G</sup>    | F8a0406191 | In-frame deletion of bases 4:211915 through 4:211917 resulting in the loss of aspartic acid 141 and a Q142E amino acid change in the <i>Crk</i> PA, PC and PD isoforms (which correspond to D123 and Q124 in PB and D133 and Q134 in PE). The PG and PH isoforms are unaffected.                        | 4:251534<br>TTATTCAGGATCATGATGCTGAAGCGG<br>GATCTTCACATCTTA 4:251489                     | 4:251534 TTATTCAGGATCATGATGAT—<br>GCGGGATCTTCACATCTTA 4:251489       | AA77 NKGNRVTSWMRRKDLHILTTNI<br>AA98              | AA77 NKGNRVTSWM—RDLHILTTNI AA98              |                                    | 27  |
| <i>dpr</i> <sup>A</sup>    | F8a0406194 | In-frame deletion of bases 4:251509 through 4:251514 resulting in the loss of arginine 87 and lysine 88 from the <i>dpr</i> <sup>7</sup> PF isoform (which correspond to R61 and K62 of PG)                                                                                                             | 4:926225<br>TGACGCCAGGACGACATGTAATGGG<br>CTGTGCGGCTCT 4:926185                          | 4:926225 TGACGCCAGGACGACGACTC—<br>TAATGGGCGTGTGGCGTCT 4:926185       | AA422 TSTVQPGASTV AA432<br>AA422                 | AA422 TSTVQPGASTV AA432<br>AA422             | PA, PC 11%; PB 23%                 | 79  |
| <i>ef4G1</i> <sup>A</sup>  |            | Frameshift deletion of base 4:926205 resulting at premature truncation at amino acid position 179 of the <i>ef4G1</i> PA and PC isoforms (which corresponds to 432 of PB)                                                                                                                               | 4:926224<br>GCAGCCAGGACGACGAGCTG—<br>—TAATGGGCTGTGGCGTCT 4:926185                       | 4:926224 GCAGCCAGGACGACGAGCTGCTTAACT<br>GGGCTGTGGCGTCT 4:926185      | AA422 TSTVQPGASTV AA432<br>AA422                 | AA422 TSTVQPGASTV AA432<br>AA422             | PA, PC 11%; PB 22%                 | 79  |
| <i>ef4G1</i> <sup>E</sup>  |            | Insertion of bases GCTCC between bases 4:926204 and 4:926205 resulting in the addition of GS after threonine 178 of the <i>ef4G1</i> PA and PC isoforms (which corresponds to T431 of PB) and premature truncation following these novel amino acids                                                    | 4:646110<br>ATTCAATTTGCGACGACTGGACATCCCAA<br>ACATAGGCGCGTCTG 4:646065                   | 4:646110 ATTCAATTTGCGACGACGACTG—<br>—CAACAATAGGCGCGTCTG 4:646065     | AA101 THINISCDALDIPNIGRASHDF<br>AA122            | AA101 THINISCDAL—DIPNIGRASHDF<br>AA122       |                                    | 62  |
| <i>Ekar</i> <sup>C</sup>   | F8a0406203 | In-frame deletion of bases 4:646085 through 4:646090 resulting in the loss of aspartic acid 111 and isoleucine 112 from all <i>Ekar</i> isoforms                                                                                                                                                        | 4:613915<br>AAAATGTGTTCAATTTTTAGGAACGCCT<br>TGTCATGCAAGGAACATTTAGCTTACT<br>4:613974     | 4:613915 AAAATGTGTTCAATTTTTT—<br>—AGGAACATTTAGCTTACT<br>4:613974     | AA164 IRDCLSPFNLGAL AA175<br>AA164               | AA164 IRDCLSPFNGNI* AA175<br>AA164           | PA, PC, PD, PE 16%; PB 13%; PF 14% | 58  |
| <i>Eph</i> <sup>A</sup>    | F8a0405962 | Frameshift deletion of bases 4:613935 through 4:613954 resulting in the addition of an isoleucine after asparagine 173 of the <i>Eph</i> PA, PC, PD and PE isoforms (which corresponds to N140 of PB and PF) and premature truncation after this novel amino acid                                       | 4:613926<br>AATTTTTAGGAACGCTGTGTCGAA<br>GGAAATCAATTTAGCT 4:613970                       | 4:613926 AATTTTTAGGAACGCTGT—<br>TGCAAGGAACATTTAGCT 4:613970          | AA166 DCSLFPNGALSKET AA180<br>AA167              | AA166 DCSLFPNGALQSGNI* AA180<br>AA167        | PA, PC, PD, PE 16%; PB 13%; PF 14% | 58  |
| <i>Eph</i> <sup>G</sup>    | F8a0404793 | Frameshift deletion of bases 4:613949 through 4:613952 resulting in the addition of RKLHAYFTSLMRLLNLHHGKQIATG after serine 176 of the <i>Eph</i> PA, PC, PD and PE isoforms (which corresponds to S143 of PB and PF) and premature truncation after these novel amino acids                             | 4:613929<br>TTTTTAGGAACGCTGTGTCAGCAAGGA<br>AACATTTAGCTTAC 4:613972                      | 4:613929 TTTTTAGGAACGCTGTG—<br>CAAGGAACATTTAGCTTAC 4:613972          | CSLFPNGALSKETFTSLLYFEFDAATREPP<br>WQTDYSYR AA204 | CSLFPNGALSKHAYFTSLMRLLNLHH<br>GKQIATG* AA204 | PA, PC, PD, PE 16%; PB 13%; PF 14% | 58  |
| <i>Ephrin</i> <sup>A</sup> | F8a0392555 | Frameshift deletion of bases 4:574194 through 4:574201 resulting in the addition of GITAFWC after isoleucine 148 of all <i>Ephrin</i> protein isoforms and premature protein truncation after these novel amino acids                                                                                   | 4:574174<br>ACTGGTGGGACGAGGATGAATCAAG<br>GACATCGGCAATTTGTGG 4:574221                    | 4:574174 ACTGGTGGGACGAGGATG—<br>—GACATCGGCAATTTGTGG 4:574221         | AA139 HSSVLVEAGIESKASRHV AA156<br>AA139          | AA139 HSSVLVEAGIGITAFWC* AA156<br>AA139      | PA, PB, PC 23%                     | 54  |
| <i>ey</i> <sup>A</sup>     | F8a0404795 | Frameshift deletion of base 4:714098 resulting in the addition of RVNRRRFRSFGC after glycine 293 of the <i>ey</i> PD isoform (which corresponds to G233 of PA, G19 of PB and 252 of PC) and premature truncation after these novel amino acids                                                          | 4:714078<br>GCGGTGGCGCAAGCACTCGGGGAGG<br>GTAGTGAAAGAGGAG 4:714118                       | 4:714078 GCGGTGGCGCAAGCAACTCC—<br>GGAGGGTAGTGAAAGAGGAG 4:714118      | AA284 SESGASNSGEGSEQEIYELRL<br>AA308             | AA284 SESGASNSGSRVNRFRFRSFGC*<br>AA308       | PA 28%; PB 3%; PC 29%; PD 33%      | 68  |
| <i>ey</i> <sup>B</sup>     | F8a0397354 | Frameshift deletion of bases 4:714100 and 4:714101 resulting in an E to G residue change at position 294 of the PD isoform (which corresponds to E234 of PA, E20 of PB and E253 of PC) followed by a premature truncation in the <i>ey</i> RD isoform                                                   | 4:714078<br>GCGGTGGCGCAAGCACTCGGGGAGG<br>GTAGTGAAAGAGGAG 4:714119                       | 4:714078 GCGGTGGCGCAAGCAACTCC—<br>GGAGGGTAGTGAAAGAGGAG 4:714119      | AA284 SESGASNSGEG AA295<br>AA94                  | AA284 SESGASNSGEG* AA295<br>AA94             | PA 28%; PB 3%; PC 30%; PD 33%      | 68  |
| <i>fd102C</i> <sup>D</sup> | F8a0405963 | Frameshift deletion of bases 4:838696 through 4:838703 resulting in the addition of NGRSFNNPLTNATHST after serine 103 of the only <i>fd102C</i> isoform (PA) and premature truncation after these novel amino acids                                                                                     | 4:838723<br>CATGGCTCAGGACCTTCTCATCTTAAT<br>CGAATGGCGCTCTTCA 4:838676                    | 4:838723 CATGGCTCAGGACGCTCTCC—<br>—ATCGAATGGCGCTCTTCA 4:838676       | GHGSGSLPSNRSFNNPLTNATHST*<br>AA123               | GHGSGSLPSNRSFNNPLTNATHST*<br>AA123           | PA 17%                             | 73  |
| <i>Gyf</i> <sup>A</sup>    | F8a0404796 | Frameshift deletion of bases 4:865561 and 4:865562 resulting in the addition of LEWNSKLKSKRLF after leucine 196 of the <i>Gyf</i> PG and PH isoforms (which corresponds to L189 of PD and PF) and premature truncation after these novel amino acids                                                    | 4:865582<br>ACTAGTGCTGTGCTCTAGATTGGAAT<br>GGATCTCAAGC 4:865541                          | 4:865582 ACTAGTGCTGTGCTGCTCT—<br>ATTGGAATGGAATCTCAAGC 4:865541       | AA187 TTSISGSGALDWNTPSSSPKDY<br>AA211            | AA187 TTSISGSGALDWNTPSSSPKDY<br>AA211        | PD, PF 12%; PG, PH 12%             | 75  |
| <i>Gyf</i> <sup>F</sup>    | F8a0405964 | Frameshift deletion of bases 4:865559 through 4:865563 resulting in the addition of EWNSKLKSKRLF after leucine 196 of the <i>Gyf</i> PG and PH isoforms (which corresponds to L189 of PD and PF) and premature truncation after these novel amino acids                                                 | 4:865583<br>CAGTAGTGCTGTGCTCTAGATTGGA<br>ATGGAATCTCAAGCTC 4:865539                      | 4:865583 CAGTAGTGCTGTGCTGCTCT—<br>TGGAATGGAATCTCAAGCTC 4:865539      | AA187 TTSISGSGALDWNTPSSSPKDY<br>AA210            | AA187 TTSISGSGALEWNSKLKSKRLF*<br>AA210       | PD, PF 12%; PG, PH 12%             | 75  |
| <i>Hcf</i> <sup>A</sup>    | F8a0406198 | In-frame deletion of bases 4:362535 through 4:362543 resulting in the loss of threonine 184 through valine 186 from all <i>Hcf</i> isoforms                                                                                                                                                             | 4:362522<br>TCTCGCTGTGCTACAGCTTACAAATGGT<br>TGGTAAAAAATATTTCT 4:362563                  | 4:362522 TCTCGCTGTGCTACAGCT—<br>—TGGTAAAAAATATTTCT 4:362563          | AA174 SPCPLGHSFTMGKIFLFGGL<br>AA196              | AA174 SPCPLGHSFTMGKIFLFGGL<br>AA196          |                                    | 36  |
| <i>Hcf</i> <sup>C</sup>    | F8a0397363 | A single base insertion between 4:362541 and 4:362542 (R6) resulting in addition of IGW after threonine 184 of all <i>Hcf</i> protein isoforms and premature protein truncation after these novel amino acids                                                                                           | 4:362522<br>TCTCGCTGTGCTACAGCTTACAAATGGT<br>TGGTGTGAAAAAATATTTCT 4:362561               | 4:362522 TCTCGCTGTGCTACAGCT—<br>—TGGTGTGAAAAAATATTTCT 4:362561       | AA174 SPCPLGHSFTMGVGE AA188<br>AA102             | AA174 SPCPLGHSFTMGW* AA188<br>AA102          | PA, PB, PG 12%; PE 12%; PF 13%     | 36  |
| <i>KjβC</i> <sup>A</sup>   | F8a0404800 | In-frame deletion of bases 4:1149173 through 4:1149193 resulting in the loss of AVGTGCG (residues 86 through 92) in the only <i>KjβC</i> isoform (PA)                                                                                                                                                   | 4:1149153<br>ATAATGGAACCAATTTTGTCTACGGTCA<br>AACAGGTGTGGGAAACACACAATGCG<br>AG 4:1149213 | 4:1149153 ATAAATGGAACCAATTTTGT—<br>—GGAACACACACAATGCGAG<br>4:1149213 | AA76 TIEGNYGTIF—GKTHTMQGD<br>AA102               | AA76 TIEGNYGTIF—GKTHTMQGD<br>AA102           |                                    | 101 |

[illegible]

|                               |            |                                                                                                                                                                                                                                                                                              |                                                                                                                   |                                                                                  |                                                                                                            |                                                                                                         |                                                       |    |
|-------------------------------|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-------------------------------------------------------|----|
| <i>Rad23<sup>δ</sup></i>      | FBa0397360 | Insertion of three bases (ATA) between bases 4:308779 and 4:308780 resulting in premature protein truncation at amino acid position 140 of the <i>Rad23</i> PA and PC protein isoforms and amino acid position 69 of PB protein isoform                                                      | 4:308760 TAGTGCAGAAATTGATTGGCG—<br>AGTTGGCCAGGGCTTCCTTA 4:308799                                                  | 4:308760 TAGTGCAGAAATTGATTGGCGAATAGTTGG<br>CCAGGCTTCCTTA 4:308799                | AA130 PLSSDELIGEL AA140                                                                                    | AA130 PLSSDELIGE* AA140                                                                                 | PA, PC 34%; PB 20%                                    | 32 |
| <i>Rad23<sup>δ</sup></i>      | FBa0406197 | In-frame deletion of bases 4:308772 through 4:308780 resulting in the loss of isoleucine 137 through glutamic acid 139 from the <i>Rad23</i> PA and PC isoforms (which correspond to 166 through E68 of PB)                                                                                  | 4:308752 CCTTATCTAGTAGCAGAAATTGATGGCGAG<br>TTGGCCAGGCTTCCTTAC 4:308800                                            | 4:308752 CCTTATCTAGTAGCAGAAATT—<br>—TTGGCCAGGCTTCCTTAC 4:308800                  | AA127 ETRPLSSDELIGELAQSLSRA<br>AA149                                                                       | AA127 ETRPLSSDEL—LAQASLSRA<br>AA149                                                                     |                                                       | 32 |
| <i>RhoGAP102A<sup>Δ</sup></i> | FBa0397359 | Frameshift deletion of bases 4:255962 through 4:255963 resulting in premature protein truncation at amino acid position 149 of all <i>RhoGAP102A</i> protein isoforms                                                                                                                        | 4:255941 ACCCAGGAGCATTACAGCTGTTCTAAGCGG<br>AGATTAACCAT 4:255982                                                   | 4:255941 ACCCAGGAGCATTACAGCTGT—<br>TAAGCGGAGATTAACCAT 4:255982                   | AA139 RTSYPGAFSCS AA149                                                                                    | AA139 RTSYPGAFSC* AA149                                                                                 | PC 14%; PD 12%; PE 13%; PF 12%                        | 28 |
| <i>RhoGAP102A<sup>Δ</sup></i> | FBa0406195 | In-frame deletion of bases 4:255955 through 4:256002 resulting in the loss of serine 147 through alanine 162 from all <i>RhoGAP102A</i> isoforms                                                                                                                                             | 4:255935 CATCATACCCAGGAGCATTGCTGTTCTTA<br>AGCGGAGATTAAACCATTTGTGAGGCAT<br>GCAAGTGTAGCCGAGAAAACAGACAGT<br>4:256022 | 4:255935 CATCATACCCAGGAGCATT—<br>SRRTSYPGAFSCSKRRLLPLRHASARRKQ<br>TVDAT AA172    | AA137<br>SRRTSYPGAFSCSKRRLLPLRHASARRKQ<br>SRRKQTVDAT AA172                                                 |                                                                                                         | PC 14%; PD 12%; PE 13%; PF 12%                        | 28 |
| <i>RhoGAP102A<sup>Δ</sup></i> | FBa0405970 | Frameshift deletion of base 4:255963 resulting in the addition of SGD after serine 149 of all <i>RhoGAP102A</i> isoforms and premature truncation after these novel amino acids                                                                                                              | 4:255943 CCAGGAGCATTACGCTGTTCTAAGCGGAG<br>ATTAAACCAT 4:255983                                                     | 4:255943 CCAGGAGCATTACGCTGTT—<br>AAGCGGAGATTAAACCAT 4:255983                     | AA140 TSYPGAFSCSKRR AA153                                                                                  | AA140 TSYPGAFSCSSGD* AA153                                                                              | PC 14%; PD 12%; PE 13%; PF 12%                        | 28 |
| <i>Rnf11<sup>A</sup></i>      | FBa0404789 | Frameshift deletion of bases 4:319485 and 4:319486 resulting in the addition of VVFDNANTIF after serine 43 of the <i>Rnf11</i> PA isoform [542 of the PB isoform] and premature truncation after these novel amino acids                                                                     | 4:319465 AAGCAGAATTGTACCTTCACGTCTGCTCT<br>CGACAACGCTAA 4:319506                                                   | 4:319465 AAGCAGAATTGTACCTTCCTCC—<br>GTGCTGTTCCGACAACGCTAA 4:319506               | AA34 EHYQRELYPSTSSSTLTPSS AA54                                                                             | AA34 EHYQRELYPSVVDNANTIF* AA54                                                                          | PA 29%; PB 29%                                        | 34 |
| <i>Rnf11<sup>Δ</sup></i>      |            | Frameshift insertion of an A between bases 4:319485 and 4:319486 resulting in the addition of NVVFDNANTIF after serine 43 of the <i>Rnf11</i> PA isoform (which corresponds to S42 of PB) and premature truncation after these novel amino acids                                             | 4:319465 AAGCAGAATTGTACCTTCCTCC—<br>ACGTCGCTCTCGACAACGT 4:319504                                                  | 4:319465 AAGCAGAATTGTACCTTCACGTCTCT<br>TCGACAACGCT 4:319504                      | AA34 EHYQRELYPSTSSSTLTPSS AA55                                                                             | AA34 EHYQRELYPSVVDNANTIF* AA55                                                                          | PA 29%; PB 29%                                        | 34 |
| <i>Rnf11<sup>Δ</sup></i>      | FBa0404790 | Frameshift deletion of bases 4:319480 through 4:319502 resulting in the addition of NTIF after proline 42 of the <i>Rnf11</i> PA isoform (P41 of the PB isoform) and premature truncation after these novel amino acids                                                                      | 4:319460 TTATCAACGAGAATTGTACCTTCACGTC<br>GTCTTCGACAACGTAAACATCATCTTAA<br>CAA 4:319522                             | 4:319460 TTATCAACGAGAATTGTACCTTCACGTC—<br>—CTAACCATCTCTTAACAA                    | AA33 GEHYQRELYPSTSS AA47                                                                                   | AA33 GEHYQRELYPNTIF* AA47                                                                               | PA 29%; PB 28%                                        | 34 |
| <i>Slp1<sup>A</sup></i>       | FBa0392557 | In-frame deletion of bases 4:664200 through 4:664202 resulting in the loss of serine 130 from all <i>Slp1</i> isoforms                                                                                                                                                                       | 4:664180 TGGGAAGTCTACAAGCCGATCGAAGAA<br>ACTCAAAACCAATC 4:664222                                                   | 4:664180 TGGGAAGTCTACAAGCCGCA—<br>AAGGAAACTCAAAACCAATC 4:664222                  | AA120 EDRLGSLQAAKETQTQSVVG<br>AA140                                                                        | AA120 EDRLGSLQAA-KETQTQSVVG<br>AA140                                                                    |                                                       | 65 |
| <i>Sox102F<sup>Δ</sup></i>    | FBa0406207 | In-frame deletion of bases 4:803101 through 4:803124 resulting in the loss of proline 64 through glutamine 71 from the <i>Sox102F</i> PA, PC and PD isoforms (which correspond to P102 through Q109 of PB)                                                                                   | 4:803144 GTATCTTCGGAAACTGATGCGGTCCTCT<br>CGGGCTCTCTACAGCGGCCAACCTCCAA<br>TGCTC 4:803081                           | 4:803144 GTATCTTCGGAAACTGAT—<br>—GCCGCAATGCCCAATGCTC                             | AA54<br>AVSVSSGNLMPFLAPLQPPMPNAQQL<br>AA81                                                                 | AA54 AVSVSSGNLM—PPMPNAQQL<br>AA81                                                                       |                                                       | 72 |
| <i>Sox102F<sup>Δ</sup></i>    | FBa0400123 | Frameshift deletion of bases 4:803109 through 4:803121 resulting in the addition of SYSRQCPMLNSSCSFPMRMPNLSQLITTVTRKVKHSAHTRKH after proline 64 of the <i>Sox102F</i> PA, PC and PD isoforms (which corresponds to P102 of PB) and premature truncation after these novel amino acids        | 4:803144 GTATCTTCGGAAACTGATGCGGTCCTCT<br>CGGGCTCTCTACAGCGGCCAAT<br>4:803092                                       | 4:803144 GTATCTTCGGAAACTGAT—<br>—GCCCTCTACAGCGCCAAT                              | AA55<br>VSVSSGNLMPFLAPLQPPMPNAQQL<br>PGHENACBVPVTHHSHSPQSEAFSTH<br>AA109                                   | AA55<br>VSVSSGNLMPYSYRQCPMLNSSCSFPMV<br>RMPNLSQLITTVTRKVKHSAHTRKH*<br>AA109                             | PA, PC, PD 11%; PB 17%                                | 72 |
| <i>sv<sup>Δ</sup></i>         |            | Frameshift deletion of base 4:1101955 resulting in the addition of VAYSLMVAHFMPSYVSLWSWPMGSAHYTHYNYLVTVCLKYFQDMLRLVLKLVLAGPNLKWPLRL after leucine 182 of the sv PA, PC, PD, PF, PG and PH isoforms (which corresponds to L26 of PI) and premature truncation after these novel amino acids   | 4:1101935 TCATGGAGGCGTTAATCAACTGTGTGGCG<br>TATTCGTTAATC 4:1101975                                                 | 4:1101935 TCATGGAGGCGTTAATCAAC—<br>TGTGTGGCGTATTCGTTAATC 4:1101975               | AA173<br>GDGHGGVNLQGLGVFVNGRPLPDVVRQRI<br>VELAHNGVVRPCDISRQLRVSHGCVSKLSRY<br>YETGSKFAGVIGGSKPKVATPPV AA255 | AA173<br>GDGHGGVNLQGLVAYSLMVAHFMPSYVSL<br>WSWPMGSAHYTHYNYLVTVCLKYFQ<br>DIMRLVLKLVLAGPNLKWPLRL*<br>AA255 | PA 22%; PC 28%; PD 27%; PF 23%; PG 29%; PH 22%; PI 5% | 96 |
| <i>sv<sup>F</sup></i>         | FBa0405971 | In-frame deletion of bases 4:1101938 through 4:1101955 resulting in the loss of amino acids 177 through 182 (GGVNLQ) of sv isoforms PA, PC, PD, PF, PG and PH (which correspond to amino acids 21 through 26 of PI)                                                                          | 4:1101918 GATTTCCTCTAATTAGTCTAGCGAGGCGTT<br>AATCAACTTGTGTGGCGTATTCGTTAATG<br>4:1101975                            | 4:1101918 GATTTCCTCTAATTAGTGTC—<br>—TGTGTGGCGTATTCGTTAATG                        | AA167<br>SQGISGSGHGGVNLQGLGVFVNGRPL<br>AA192                                                               | AA167 SQGISGSGDGH—GGVFNVRPL<br>AA192                                                                    |                                                       | 96 |
| <i>Syt7<sup>Δ</sup></i>       | FBa0400122 | Frameshift deletion of bases 4:282681 through 4:282708 resulting in the addition of GKYSIPWSMISKTH after lysine 139 of the Syt7 PA, PB, PI and PM isoforms (which corresponds to K6 of PF) and premature truncation after these novel amino acids                                            | 4:282728 TCTCAATGACAGACATGATTTAGTGAAGCA<br>CGGATCCCAAGTAAAGTTGGGCCAAATC<br>CATTCTTC 4:282661                      | 4:282728 TCTCAATGACAGACATGTAT—<br>—                                              | AA130<br>KISIMTDMYLDSTDPSENVGQIHSF<br>AA155                                                                | AA130<br>KISIMTDMYLGKYSIPWSMISKTH*<br>AA155                                                             | PA 33%; PB 43%; PF 2%; PI 40%; PM 33%                 | 31 |
| <i>Taf3<sup>Δ</sup></i>       | FBa0400120 | Frameshift deletion of bases 4:464911 through 4:464914 resulting in addition of WTLGAQSAK after phenylalanine 190 of the only <i>Taf3</i> isoform (PA) and premature truncation after these novel amino acids                                                                                | 4:464935 AATTTCCGGTCAAGTCTTGAATGTGAC<br>GTTGGGACTGAGT 4:464892                                                    | 4:464935 AATTTCCGGTCAAGTGCCTT—<br>GTTGGGACTGAGGAGCTAGT 4:464892                  | AA149<br>AA181 TVINFRSNAFELDVGSVRE AA200                                                                   | AA181 TVINFRSNAFWTLGAQSAK*<br>AA200                                                                     | PA 14%                                                | 45 |
| <i>Taf3<sup>F</sup></i>       | FBa0406200 | In-frame deletion of bases 4:464909 through 4:464920 resulting in the loss of alanine 189 through leucine 192 from the only <i>Taf3</i> isoform (PA)                                                                                                                                         | 4:464940 TGATAAATTTCCGCTGAATGCTTGAAT<br>TGGACGTTGGGAGCTAGTCCG<br>4:464889                                         | 4:464940 TGATAAATTTCCGCTGCAAT—<br>—GACGTTGGGAGCTAGTCCG                           | AA179 PNTVINFRSNAFELDVGSVREMS<br>AA202                                                                     | AA179 PNTVINFRSN—DVGSVREMS<br>AA202                                                                     |                                                       | 45 |
| <i>Tdg<sup>Δ</sup></i>        | FBa0404801 | Frameshift deletion of bases 4:552973 and 4:552974 resulting in the addition of AGTGH after proline 201 of all <i>Tdg</i> isoforms and premature truncation after these novel amino acids                                                                                                    | 4:552994 AAGGATGCTATATGCCCAAGCAGCAGCG<br>ACTGGTCACTAGC 4:552953                                                   | 4:552994 AAGGATGCTATATGCCCCCC—<br>AGCAGGCACTGCTGCTAGC 4:552953                   | AA192 PYDNDCYMPPEQALVT AA207                                                                               | AA192 PYDNDCYMPAGTGH* AA207                                                                             | PA, PB, PC 12%                                        | 50 |
| <i>Tdg<sup>Δ</sup></i>        |            | Frameshift deletion of base 4:552974, base 4:552971 changed from G to C, base 4:552972 changed from A to C, and base 4:552973 changed from G to C resulting in the addition of PRHWSLAWS after proline 201 of all <i>Tdg</i> isoforms and premature truncation after these novel amino acids | 4:552994 AAGGATGCTATATGCCCAAGCAGCAGCG<br>ACTGGTCACTAGC 4:552951                                                   | 4:552994 AAGGATGCTATATGCCCCCC—<br>CCCCAGGCACTGCTGCTAGC 4:552951                  | AA192 PYDNDCYMPPEQALVTSVEL<br>AA211                                                                        | AA192 PYDNDCYMPPRHWSLAWS*<br>AA211                                                                      | PA, PB, PC 12%                                        | 50 |
| <i>Tdg<sup>F</sup></i>        | FBa0405972 | Frameshift deletion of bases 4:552972 through 4:552987 resulting in the addition of RHWSLAWS after cysteine 197 of all <i>Tdg</i> isoforms and premature truncation after these novel amino acids                                                                                            | 4:553007 AGTGCCATATGATAACGATTGCTATATGCC<br>CCAGAGCAGGCATGCTGCTACTAGCG<br>4:552952                                 | 4:553007 AGTGCCATATGATAACGATT—<br>—GCAGGCACTGCTGCTAGCG                           | AA188 EKLVPYDNDCYMPPEQALV AA206                                                                            | AA188 EKLVPYDNDCHWSLAWS*<br>AA206                                                                       | PA, PB, PC 11%                                        | 50 |
| <i>Tdg<sup>I</sup></i>        | FBa0406201 | In-frame deletion of bases 4:552968 through 4:552973 resulting in the loss of glutamic acid 202 and glutamine 203 from all <i>Tdg</i> isoforms                                                                                                                                               | 4:552993 ACGATTGCTATATGCCCAAGCAGCAGCA<br>CTGTGCTAGCTAGCTGGA 4:552948                                              | 4:552993 ACGATTGCTATATGCCCCCA—<br>—GCACTGCTAGCTAGCTGGA 4:552948                  | AA192 PYDNDCYMPPEQALVTSVELV<br>AA213                                                                       | AA192 PYDNDCYMP—ALVTSVELV<br>AA213                                                                      |                                                       | 50 |
| <i>toy<sup>A</sup></i>        | FBa0404797 | Frameshift deletion of bases 4:990465 through 4:990472 resulting in the addition of RLKQNFQQL after glutamine 72 of all <i>toy</i> isoforms and premature truncation after these novel amino acids                                                                                           | 4:990445 GTGATATTCAAGAATACTACAAGTGTCCA<br>ACGGTTGCTGAAGCAAAA 4:990492                                             | 4:990445 GTGATATTCAAGAATACTA—<br>—CAACGGTTGCTGAAGCAAAA 4:990492                  | AA63 RPDISRILQVSNCGVSKILGR AA84                                                                            | AA63 RPDISRILQRLKQNFQQL* AA84                                                                           | PA 13%; PC 14%; PD 11%                                | 86 |
| <i>toy<sup>B</sup></i>        | FBa0406209 | In-frame deletion of bases 4:990468 through 4:990473 and base 4:990474 changed from A to T resulting in the change of valine 73 to tyrosine and loss of serine 74 and asparagine 75 from all <i>toy</i> isoforms                                                                             | 4:990448 ATATTTCGAAGAATACTACAGTGTCCAAG<br>TGTGCTGAAGCAAAAT 4:990493                                               | 4:990448 ATATTTCGAAGAATACTACA—<br>TACGTTGCTGCTGAAGCAAAAT 4:990493                | AA63 RPDISRILQVSNCGVSKILGR<br>AA85                                                                         | AA63 RPDISRILQY—GCVSKILGR AA85                                                                          |                                                       | 86 |
| <i>toy<sup>C</sup></i>        | FBa0400121 | Insertion of eight bases (AGTGACT) between bases 4:990471 and 4:990472 and base 4:990472 changed from C to A resulting in premature truncation at amino acid position 74 of all <i>toy</i> isoforms                                                                                          | 4:990451 TTTCAGAATACTACAAGTGT—<br>—CCAACGGTTGCTGAAGCAA 4:990490                                                   | 4:990451 TTTCAGAATACTACAAGTGTAGTGACTA<br>CAACGGTTGCTGAAGCAA 4:990490             | AA64 PCDISRILQVS AA74                                                                                      | AA64 PCDISRILQV* AA74                                                                                   | PA 14%; PC 14%; PD 11%                                | 86 |
| <i>ukar<sup>Δ</sup></i>       | FBa0406211 | In-frame deletion of bases 4:1127381 through 4:1127383 resulting in the loss of threonine 173 from all <i>ukar</i> isoforms                                                                                                                                                                  | 4:1127361 ATATAGTGATATTGAATGCTGACGAAGGT<br>GGCAATAATTATG 4:1127403                                                | 4:1127361 ATATAGTGATATTGAATG—<br>GAAGGTGCAATAATTATG 4:1127403                    | AA163 YRDIMVYLNWTKVAIYEEDY<br>AA183                                                                        | AA163 YRDIMVYLNW—KVAIYEEDY<br>AA183                                                                     |                                                       | 99 |
| <i>yellow-h<sup>A</sup></i>   | FBa0397357 | A 19 base insertion between bases 4:228201 and 4:228202 and base pair change C228202T resulting in addition of IIFHIYFNFCATPTIYFKWRLCTK after tyrosine 66 of the only <i>yellow-h</i> protein isoform (PA) and premature truncation after these novel amino acids                            | 4:228182 AAATATTGGATTCTCTATA—<br>—CTCAACTATTGTGCAACGCCA<br>4:228222                                               | 4:228182 AAATATTGGATTCTCTATATTAATTCTA<br>TATATTCTCAACTATTGTGCAACGCCA<br>4:228222 | AA57<br>YEWKYLDFLYFVQRQSLINGDFVFNKNN<br>LPLGID AA92                                                        | AA57<br>YEWKYLDFLYFIHFIYFNFCATPTIYFKWRLC<br>TKK* AA92                                                   | PA 14%                                                | 24 |

|                              |             |                                                                                                                                                                                                                                                                                                                |                                                                                                                   |                                                                           |                                                                                                                                             |                                                                                                                                          |                                        |    |
|------------------------------|-------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|----|
| <i>yellow-h</i> <sup>D</sup> | FBal0404787 | Multiple sequence changes between 4:228201 and 4:228209 result in premature truncation at residue 66 of the only <i>yellow-h</i> isoform (PA).                                                                                                                                                                 | 4:228181<br>GAAATATTGGATTCTCTACTCAACTTT<br>GTGCACGCGCAACAATCT 4:228229                                            | 4:228181<br>GAAATATTGGATTCTCTAGAGTATG--<br>TTGTGCAACGCCCAACAATCT 4:228229 | AA57 YEWKYLDLY AA66                                                                                                                         | AA57 YEWKYLDL* AA66                                                                                                                      | PA 14%                                 | 24 |
| <i>zfh2</i> <sup>A</sup>     | FBal0405973 | Frameshift deletion of bases 4:522783 through 4:522789 resulting in the addition of SHVPITPLMVMWTAKPANSLTSSSGQSRRLPHTSFLNLPSCKFSRSRKPPIAHLAATVLPCTYPHPAPPRWPALMRRPP HPALQSVVPSTSMVVPKE after leucine 426 of all <i>zfh2</i> isoforms and premature truncation after these novel amino acids                    | 4:522763<br>GCAACATCAGAGTTCCTTATTCCAGCCC<br>ATGTCCTCGATCACCCCG 4:522809                                           | 4:522763 GCAACATCAGAGTTCCTTAT--<br>-CCCATGTCCCGATCACCCCG 4:522809         | AA417<br>QQQLQQHQSLLFPSPCPDHPDLNGVDCKTC<br>ELLDIQQRSKSPSSSHHQFQSLPQLQIQSQ<br>PQQTPIHRSPSCSNVALPVSPASSVASVG<br>ASTATSSFTIGACSEHINGRPQ AA528  | AA417<br>QQQLQQHQSLLSHVPITPLMVMWTAKPANS<br>LTSSSGQSRRLPHTSFLNLPSCKFSRSRKP<br>PIAHLAATVLPCTYPHPAPPRWPALMRR<br>PPHPALQSVVPSTSMVVPKE* AA528 | PA 14%; PB 14%; PC 15%                 | 49 |
| <i>zfh2</i> <sup>D</sup>     | FBal0404792 | Frameshift deletion of bases 4:522777 through 4:52279 resulting in the addition of HVPITPLMVMWTAKPANSLTSSSGQSRRLPHTSFLNLPSCKFSRSRKPPIAHLAATVLPCTYPHPAPPRWPALMRRPP HPALQSVVPSTSMVVPKE after serine 425 of all <i>zfh2</i> isoforms and premature truncation after these novel amino acids                       | 4:522757<br>GCTGCAGCAACATCAGAGTTCCTTATTC<br>AAGCCCATGTCCCGATCACCCCG<br>4:522809                                   | 4:522757 GCTGCAGCAACATCAGAGTT--<br>-CCCATGTCCCGATCACCCCG<br>4:522809      | AA416<br>QQQLQQHQSLLFPSPCPDHPDLNGVDCKTC<br>CELLDIQQRSKSPSSSHHQFQSLPQLQIQSQ<br>QPQQTPIHRSPSCSNVALPVSPASSVASVG<br>NASTATSSFTIGACSEHINGR AA526 | AA416<br>QQQLQQHQSLLSHVPITPLMVMWTAKPANS<br>LTSSSGQSRRLPHTSFLNLPSCKFSRSRKP<br>PIAHLAATVLPCTYPHPAPPRWPALMRR<br>PPHPALQSVVPSTSMVVPKE* AA526 | PA 14%; PB 14%; PC 15%                 | 49 |
| <i>Zyx</i> <sup>D</sup>      |             | Frameshift deletion of bases 4:1059571 through 4:1059583 resulting in the addition of ALQSPFORL after threonine 150 of the <i>Zyx</i> PA, PB and PL isoforms (which corresponds to T88 of PG and T61 of PH) and premature truncation following these novel amino acids                                         | 4:1059603<br>TTTGGGAGTACAAGCTAAGCCACACAACT<br>CTCTCAACAGCTTTACAAAGCCCC<br>4:1059551                               | 4:1059603 TTTGGGAGTACAAGCTAAGC--<br>-CAACAGCTTTACAAAGCCCC<br>4:1059551    | AA141 GLLGVQAKPTQPLNFTKPL<br>AA160                                                                                                          | AA141 GLLGVQAKPTALQSPFORL*<br>AA160                                                                                                      | PA 27%; PB 14%; PG 17%; PH 12%         | 93 |
| <i>bt</i> <sup>A</sup>       | FBal0400124 | Base change T735988A and frameshift deletion of bases 4:735989 resulting in the addition of EMALCSNQSSLNPRRKYWISEIKIISTPTNQLY after glycine 887 of the <i>bt</i> P1 isoform (which corresponds to G1003 of PC, G1218 of PF, T857 of PH and T1221 of PI) and premature truncation after these novel amino acids | 4:735968<br>CTTCTCGTGAAGGAGAGCGTCGAGATGG<br>CGCTGTGCTCAAA 4:736009                                                | 4:735968 CTCTCGTGAAGGAGAGCGGA-<br>GAGATGGCGTGTGCTCAAA 4:736009            | AA878<br>KGKLPDEGDGRDGAVALKPVIEPEKEILDG<br>NKKNNQHADKPTVL AA922                                                                             | AA878<br>KGKLPDEGDGEMALCSNQSSLNPRRKYWI<br>SEIKIISTPTNQLY* AA922                                                                          | PC 12%; PF 14%; PH 10%; PI 14%; PJ 10% | 69 |
| <i>Jyalpha</i> <sup>I</sup>  | FBal0404783 | Frameshift deletion of bases 4:3732 through 4:3750 resulting in the addition of ICFRQYR after leucine 794 of the only <i>Jyalpha</i> isoform (PB) and premature truncation after these novel amino acids                                                                                                       | 4:3712<br>CTGGGCACATAGCAATTCTATGCAATCATC<br>4:3770                                                                | 4:3712 CTGGGCACATAGCAATTCT--<br>-GATAGTCTTCCGGCAATATC<br>4:3770           | AA785 PLALGTIALCIDIGTDM AA802                                                                                                               | AA785 PLALGTIALICFRQYR* AA802                                                                                                            | PB 79%                                 | 1  |
| <i>Jyalpha</i> <sup>I</sup>  | FBal0406188 | In-frame deletion of bases 4:3730 through 4:3735 resulting in the loss of leucine 794 and cysteine795 from the only <i>Jyalpha</i> isoform (PB)                                                                                                                                                                | 4:3710<br>CTCTGGGCACATAGCAATTCTATGCAATCG<br>ATATCGGCACATGATAT 4:3755                                              | 4:3710 CTCTGGGCACATAGCAATT--<br>ATCGATATCGGCACATGATAT 4:3755              | AA784 IPLALGTIALCIDIGTDMPLA<br>AA805                                                                                                        | AA784 IPLALGTIAL-IDIGTDMPLA AA805                                                                                                        |                                        | 1  |
| <i>Jyalpha</i> <sup>O</sup>  | FBal0404784 | Frameshift deletion of bases 4:3739 and 4:3740 resulting in the addition of YRH after isoleucine 796 of the only <i>Jyalpha</i> isoform (PB) and premature truncation after these novel amino acids                                                                                                            | 4:3719<br>CTATAGCAATTCTATGCAATGATATCGGCA<br>CTGATATGCTTC 4:3760                                                   | 4:3719 CTATAGCAATTCTATGCAATC-<br>TATCGGCACATGATATGCTTC 4:3760             | AA787 ALGTIALICIDIGT AA800                                                                                                                  | AA787 ALGTIALICIRH* AA800                                                                                                                | PB 79%                                 | 1  |
| <i>unc-13</i> <sup>C</sup>   | FBal0406208 | In-frame deletion of bases 4:885781 through 4:885816 resulting in the loss of aspartic acid 1658 through serine 1669 from the <i>unc-13</i> PA, PC, PD and PG isoforms (which correspond to D1970 through S1981 of PB and PE and D1553 through S1564 of PF)                                                    | 4:885836<br>ATCCATTCTATAGCAATATGACAGTATGC<br>CGGACATAAGGCTCGAAGAAATCCATT<br>CTCTTGTGCTGAGCT 4:885761              | 4:885836 ATCCATTCTATAGCAATATT--<br>-----<br>ATTCCTCTGCTGCTGAGCT 4:885761  | AA1648<br>PGDNPFYSNIDSMPIRPRKSIPLVSELTMA<br>AA1679                                                                                          | AA1648 PGDNPFYSNI-----<br>IPLVSELTMA AA1679                                                                                              |                                        | 76 |
| <i>zfh2</i> <sup>AA</sup>    |             | Frameshift deletion of bases 4:524875 through 4:524920 resulting in the addition of AGTGGILLRL after alanine 594 of all <i>zfh2</i> isoforms and premature truncation after these novel amino acids                                                                                                            | 4:524855<br>GAGTTTAAATCAGTACCAATTATTTAAAC<br>CCTTTTCTTATGCGACAGCAACAGCATC<br>CTCGGCTGGCACGGGGGAATCCTA<br>4:524940 | 4:524855 GAGTTTAAATCAGTACCAA--<br>-----<br>CTGGCACGGGGGAATCCTA 4:524940   | AA585<br>GESACGYCLAGQQHPRLARGES<br>AA606                                                                                                    | AA585 GESACGYCLAAGTGGILLRL*<br>AA606                                                                                                     | PA 20%; PB 20%; PC 21%                 | 49 |
| <i>zfh2</i> <sup>2A</sup>    | FBal0392552 | In-frame deletion of bases 4:524907 through 4:524933 resulting in loss of amino acids 597 through 605 (QHPRLARGE) of all <i>zfh2</i> protein isoforms                                                                                                                                                          | 4:524901<br>TGGTATTGCTTGACGAGCAACAGCATCC<br>TCGGCTGGCACGGGGGAATCCTACTCTT<br>GCGGTTAT 4:524953                     | 4:524901 TGGTATTGCTTGCGAGGAC--<br>-----<br>AATCCTACTCTTGCGGTTAT 4:524953  | AA586<br>ESACGYCLAGQQHPRLARGESYCGYKPY<br>AA614                                                                                              | AA586 ESACGYCLAGQ-----SYSCGYKPY<br>AA614                                                                                                 |                                        | 49 |

<sup>D</sup> FlyBase IDs have not yet been assigned for all alleles

<sup>A</sup> This column shows the percentage of the wild type protein left after truncation. Identical polypeptides are grouped together.

<sup>C</sup> This column may be used to sort alleles/rows by the cytological order of the genes mutated (see Supplementary Table 1)

Supplementary Table 6. Stocks with new fourth chromosome mutations

| Allele                  | FlyBase ID  | Stock Center | Stock number      | Genotype                                                              | Stock RRID        | Cytological order <sup>a</sup> |
|-------------------------|-------------|--------------|-------------------|-----------------------------------------------------------------------|-------------------|--------------------------------|
| 4E-T <sup>A</sup>       | FBal0397362 | Bloomington  | 602203            | $y^1 w^{1118}; Ti(Ti)FRT101F 4E-T^A$                                  | RRID:BDSC_602203  | 81                             |
| 4E-T <sup>A</sup>       | FBal0397362 | Kyoto        | 119169            | $y^1 w^{1118}; Ti(Ti)FRT101F 4E-T^A$                                  | RRID:DGGR_119169  | 81                             |
| 4E-T <sup>D</sup>       | FBal0405951 | Bloomington  | 605780            | $y^1 w^{1118}; Ti(Ti)FRT101F 4E-T^D$                                  | RRID:BDSC_605780  | 81                             |
| 4E-T <sup>D</sup>       | FBal0405951 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F 4E-T^D$                                  | Not yet available | 81                             |
| Abcd1 <sup>A</sup>      | FBal0397358 | Bloomington  | 602199            | $y^1 w^{1118}; Ti(Ti)FRT101F Abcd1^A$                                 | RRID:BDSC_602199  | 19                             |
| Abcd1 <sup>A</sup>      | FBal0397358 | Kyoto        | 119165            | $y^1 w^{1118}; Ti(Ti)FRT101F Abcd1^A$                                 | RRID:DGGR_119165  | 19                             |
| Actbeta <sup>A</sup>    | FBal0406210 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F Actbeta^A$                               | Not yet available | 95                             |
| Actbeta <sup>B</sup>    | FBal0405952 | Bloomington  | 605781            | $y^1 w^{1118}; Ti(Ti)FRT101F Actbeta^B /ln(4)ci^D, ci^D pan^{ciD}$    | RRID:BDSC_605781  | 95                             |
| Actbeta <sup>B</sup>    | FBal0405952 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F Actbeta^B /ln(4)ci^D, ci^D pan^{ciD}$    | Not yet available | 95                             |
| Ank <sup>A</sup>        | FBal0397355 | Bloomington  | 602194            | $y^1 w^{1118}; Ti(Ti)FRT101F Ank^A$                                   | RRID:BDSC_602194  | 10                             |
| Ank <sup>A</sup>        | FBal0397355 | Kyoto        | 119160            | $y^1 w^{1118}; Ti(Ti)FRT101F Ank^A$                                   | RRID:DGGR_119160  | 10                             |
| anne <sup>B</sup>       | FBal0397356 | Bloomington  | 602197            | $y^1 w^{1118}; Ti(Ti)FRT101F anne^B /ln(4)ci^D, ci^D pan^{ciD}$       | RRID:BDSC_602197  | 11                             |
| anne <sup>B</sup>       | FBal0397356 | Kyoto        | 119163            | $y^1 w^{1118}; Ti(Ti)FRT101F anne^B /ln(4)ci^D, ci^D pan^{ciD}$       | RRID:DGGR_119163  | 11                             |
| anne <sup>D</sup>       | FBal0404786 | Bloomington  | 605312            | $y^1 w^{1118}; Ti(Ti)FRT101F anne^D /ln(4)ci^D, ci^D pan^{ciD}$       | RRID:BDSC_605312  | 11                             |
| anne <sup>D</sup>       | FBal0404786 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F anne^D /ln(4)ci^D, ci^D pan^{ciD}$       | Not yet available | 11                             |
| apolpp <sup>B</sup>     | FBal0392553 | Bloomington  | 600262            | $y^1 w^{1118}; Ti(Ti)FRT101F apolpp^B /ln(4)ci^D, ci^D pan^{ciD}$     | RRID:BDSC_600262  | 94                             |
| apolpp <sup>B</sup>     | FBal0392553 | Kyoto        | 119073            | $y^1 w^{1118}; Ti(Ti)FRT101F apolpp^B /ln(4)ci^D, ci^D pan^{ciD}$     | RRID:DGGR_119073  | 94                             |
| apolpp <sup>J</sup>     | FBal0404799 | Bloomington  | 605313            | $y^1 w^{1118}; Ti(Ti)FRT101F apolpp^J /ln(4)ci^D, ci^D pan^{ciD}$     | RRID:BDSC_605313  | 94                             |
| apolpp <sup>J</sup>     | FBal0404799 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F apolpp^J /ln(4)ci^D, ci^D pan^{ciD}$     | Not yet available | 94                             |
| Arf4 <sup>A</sup>       | FBal0405953 | Bloomington  | 605782            | $y^1 w^{1118}; Ti(Ti)FRT101F Arf4^A /ln(4)ci^D, ci^D pan^{ciD}$       | RRID:BDSC_605782  | 98                             |
| Arf4 <sup>A</sup>       | FBal0405953 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F Arf4^A /ln(4)ci^D, ci^D pan^{ciD}$       | Not yet available | 98                             |
| ATPsynbeta <sup>C</sup> | FBal0404781 | Bloomington  | 605314            | $y^1 w^{1118}; Ti(Ti)FRT101F ATPsynbeta^C /ln(4)ci^D, ci^D pan^{ciD}$ | RRID:BDSC_605314  | 91                             |
| ATPsynbeta <sup>C</sup> | FBal0404781 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F ATPsynbeta^C /ln(4)ci^D, ci^D pan^{ciD}$ | Not yet available | 91                             |
| bt <sup>A</sup>         | FBal0400124 | Bloomington  | 603547            | $y^1 w^*; Ti(Ti)FRT101F bt^A /Ti(GMR-HMS04515)Gat^{eye}$              | RRID:BDSC_603547  | 69                             |
| bt <sup>A</sup>         | FBal0400124 | Kyoto        | 119193            | $y^1 w^*; Ti(Ti)FRT101F bt^A /Ti(GMR-HMS04515)Gat^{eye}$              | RRID:DGGR_119193  | 69                             |
| bt <sup>C</sup>         | FBal0406205 | Kyoto        | Not yet available | $y^1 w^*; Ti(Ti)FRT101F bt^C$                                         | Not yet available | 69                             |
| CaMKI <sup>F</sup>      | FBal0404780 | Bloomington  | 605315            | $y^1 w^{1118}; Ti(Ti)FRT101F CaMKI^F$                                 | RRID:BDSC_605315  | 44                             |
| CaMKI <sup>F</sup>      | FBal0404780 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CaMKI^F$                                 | Not yet available | 44                             |
| CaMKI <sup>I</sup>      | FBal0405954 | Bloomington  | 605783            | $y^1 w^{1118}; Ti(Ti)FRT101F CaMKI^I$                                 | RRID:BDSC_605783  | 44                             |
| CaMKI <sup>I</sup>      | FBal0405954 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CaMKI^I$                                 | Not yet available | 44                             |
| CG1674 <sup>E</sup>     | FBal0405955 | Bloomington  | 605784            | $y^1 w^{1118}; Ti(Ti)FRT101F CG1674^E$                                | RRID:BDSC_605784  | 26                             |
| CG1674 <sup>E</sup>     | FBal0405955 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG1674^E$                                | Not yet available | 26                             |
| CG1909 <sup>A</sup>     | FBal0406202 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG1909^A$                                | Not yet available | 55                             |
| CG1909 <sup>B</sup>     | FBal0405956 | Bloomington  | 605785            | $y^1 w^{1118}; Ti(Ti)FRT101F CG1909^B$                                | RRID:BDSC_605785  | 55                             |
| CG1909 <sup>B</sup>     | FBal0405956 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG1909^B$                                | Not yet available | 55                             |
| CG11360 <sup>D</sup>    | FBal0404794 | Bloomington  | 605316            | $y^1 w^{1118}; Ti(Ti)FRT101F CG11360^D$                               | RRID:BDSC_605316  | 66                             |
| CG11360 <sup>D</sup>    | FBal0404794 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG11360^D$                               | Not yet available | 66                             |
| CG31998 <sup>C</sup>    | FBal0405957 | Bloomington  | 605786            | $y^1 w^{1118}; Ti(Ti)FRT101F CG31998^C /ln(4)ci^D, ci^D pan^{ciD}$    | RRID:BDSC_605786  | 21                             |
| CG31998 <sup>C</sup>    | FBal0405957 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG31998^C /ln(4)ci^D, ci^D pan^{ciD}$    | Not yet available | 21                             |
| CG31998 <sup>D</sup>    | FBal0405958 | Bloomington  | 605787            | $y^1 w^{1118}; Ti(Ti)FRT101F CG31998^D /ln(4)ci^D, ci^D pan^{ciD}$    | RRID:BDSC_605787  | 21                             |
| CG31998 <sup>D</sup>    | FBal0405958 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG31998^D /ln(4)ci^D, ci^D pan^{ciD}$    | Not yet available | 21                             |
| CG31999 <sup>C</sup>    | FBal0406192 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG31999^C$                               | Not yet available | 23                             |
| CG32017 <sup>B</sup>    | FBal0406212 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG32017^B$                               | Not yet available | 100                            |
| CG32017 <sup>E</sup>    | FBal0405959 | Bloomington  | 605788            | $y^1 w^{1118}; Ti(Ti)FRT101F CG32017^E /ln(4)ci^D, ci^D pan^{ciD}$    | RRID:BDSC_605788  | 100                            |
| CG32017 <sup>E</sup>    | FBal0405959 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG32017^E /ln(4)ci^D, ci^D pan^{ciD}$    | Not yet available | 100                            |
| CG32017 <sup>K</sup>    | FBal0405960 | Bloomington  | 605789            | $y^1 w^{1118}; Ti(Ti)FRT101F CG32017^K /ln(4)ci^D, ci^D pan^{ciD}$    | RRID:BDSC_605789  | 100                            |
| CG32017 <sup>K</sup>    | FBal0405960 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG32017^K /ln(4)ci^D, ci^D pan^{ciD}$    | Not yet available | 100                            |
| CG33521 <sup>C</sup>    | FBal0404798 | Bloomington  | 605317            | $y^1 w^{1118}; Ti(Ti)FRT101F CG33521^C$                               | RRID:BDSC_605317  | 103                            |
| CG33521 <sup>C</sup>    | FBal0404798 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F CG33521^C$                               | Not yet available | 103                            |
| ci <sup>C</sup>         | FBal0404782 | Bloomington  | 605318            | $y^1 w^{1118}; Ti(Ti)FRT101F ci^C /P(w^{+mc}=ActGFP)unc-13^{ci}$      | RRID:BDSC_605318  | 5                              |
| ci <sup>C</sup>         | FBal0404782 | Kyoto        | Not yet available | $y^1 w^{1118}; Ti(Ti)FRT101F ci^C /P(w^{+mc}=ActGFP)unc-13^{ci}$      | Not yet available | 5                              |

|                            |             |             |                   |                                                                         |                   |     |
|----------------------------|-------------|-------------|-------------------|-------------------------------------------------------------------------|-------------------|-----|
| <i>ci<sup>F</sup></i>      | FBal0406189 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F ci^F$                                    | Not yet available | 5   |
| <i>Crk<sup>E</sup></i>     | FBal0405961 | Bloomington | 605790            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Crk^E / \ln(4) ci^D, ci^D pan^{cD}$      | RRID:BDSC_605790  | 22  |
| <i>Crk<sup>E</sup></i>     | FBal0405961 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Crk^E / \ln(4) ci^D, ci^D pan^{cD}$      | Not yet available | 22  |
| <i>Crk<sup>G</sup></i>     | FBal0406191 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Crk^G$                                   | Not yet available | 22  |
| <i>dpr7<sup>A</sup></i>    | FBal0406194 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F dpr7^A$                                  | Not yet available | 27  |
| <i>Ekar<sup>C</sup></i>    | FBal0406203 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Ekar^C$                                  | Not yet available | 62  |
| <i>Eph<sup>A</sup></i>     | FBal0405962 | Bloomington | 605791            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Eph^A$                                   | RRID:BDSC_605791  | 58  |
| <i>Eph<sup>A</sup></i>     | FBal0405962 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Eph^A$                                   | Not yet available | 58  |
| <i>Eph<sup>G</sup></i>     | FBal0404793 | Bloomington | 605319            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Eph^G$                                   | RRID:BDSC_605319  | 58  |
| <i>Eph<sup>G</sup></i>     | FBal0404793 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Eph^G$                                   | Not yet available | 58  |
| <i>Ephrin<sup>A</sup></i>  | FBal0392555 | Bloomington | 602196            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Ephrin^A$                                | RRID:BDSC_602196  | 54  |
| <i>Ephrin<sup>A</sup></i>  | FBal0392555 | Kyoto       | 119162            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Ephrin^A$                                | RRID:DGGR_119162  | 54  |
| <i>ey<sup>A</sup></i>      | FBal0404795 | Bloomington | 605320            | $y^1 w^{1118}; Tl\{Ti\}FRT101F ey^A / \ln(4) ci^D, ci^D pan^{cD}$       | RRID:BDSC_605320  | 68  |
| <i>ey<sup>A</sup></i>      | FBal0404795 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F ey^A / \ln(4) ci^D, ci^D pan^{cD}$       | Not yet available | 68  |
| <i>ey<sup>B</sup></i>      | FBal0397354 | Bloomington | 602195            | $y^1 w^{1118}; Tl\{Ti\}FRT101F ey^B / \ln(4) ci^D, ci^D pan^{cD}$       | RRID:BDSC_602195  | 68  |
| <i>ey<sup>B</sup></i>      | FBal0397354 | Kyoto       | 119161            | $y^1 w^{1118}; Tl\{Ti\}FRT101F ey^B / \ln(4) ci^D, ci^D pan^{cD}$       | RRID:DGGR_119161  | 68  |
| <i>fd102C<sup>D</sup></i>  | FBal0405963 | Bloomington | 605792            | $y^1 w^{1118}; Tl\{Ti\}FRT101F fd102C^D$                                | RRID:BDSC_605792  | 73  |
| <i>fd102C<sup>D</sup></i>  | FBal0405963 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F fd102C^D$                                | Not yet available | 73  |
| <i>Gyjf<sup>B</sup></i>    | FBal0404796 | Bloomington | 605321            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Gyjf^B / \ln(4) ci^D, ci^D pan^{cD}$     | RRID:BDSC_605321  | 75  |
| <i>Gyjf<sup>B</sup></i>    | FBal0404796 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Gyjf^B / \ln(4) ci^D, ci^D pan^{cD}$     | Not yet available | 75  |
| <i>Gyjf<sup>F</sup></i>    | FBal0405964 | Bloomington | 605793            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Gyjf^F / \ln(4) ci^D, ci^D pan^{cD}$     | RRID:BDSC_605793  | 75  |
| <i>Gyjf<sup>F</sup></i>    | FBal0405964 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Gyjf^F / \ln(4) ci^D, ci^D pan^{cD}$     | Not yet available | 75  |
| <i>Hcf<sup>A</sup></i>     | FBal0406198 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Hcf^A / \ln(4) ci^D, ci^D pan^{cD}$      | Not yet available | 36  |
| <i>Hcf<sup>C</sup></i>     | FBal0397363 | Bloomington | 602204            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Hcf^C / \ln(4) ci^D, ci^D pan^{cD}$      | RRID:BDSC_602204  | 36  |
| <i>Hcf<sup>C</sup></i>     | FBal0397363 | Kyoto       | 119170            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Hcf^C / \ln(4) ci^D, ci^D pan^{cD}$      | RRID:DGGR_119170  | 36  |
| <i>Jyalpha<sup>I</sup></i> | FBal0404783 | Bloomington | 605322            | $y^1 w^*; Tl\{Ti\}FRT101F Jyalpha^I / \ln(4) ci^D, ci^D pan^{cD}$       | RRID:BDSC_605322  | 1   |
| <i>Jyalpha<sup>I</sup></i> | FBal0404783 | Kyoto       | Not yet available | $y^1 w^*; Tl\{Ti\}FRT101F Jyalpha^I / \ln(4) ci^D, ci^D pan^{cD}$       | Not yet available | 1   |
| <i>Jyalpha<sup>L</sup></i> | FBal0406188 | Kyoto       | Not yet available | $y^1 w^*; Tl\{Ti\}FRT101F Jyalpha^L$                                    | Not yet available | 1   |
| <i>Jyalpha<sup>P</sup></i> | FBal0404784 | Bloomington | 605323            | $y^1 w^*; Tl\{Ti\}FRT101F Jyalpha^P / Tl\{GMR-HMS04515\}Gat^{cya}$      | RRID:BDSC_605323  | 1   |
| <i>Jyalpha<sup>P</sup></i> | FBal0404784 | Kyoto       | Not yet available | $y^1 w^*; Tl\{Ti\}FRT101F Jyalpha^P / Tl\{GMR-HMS04515\}Gat^{cya}$      | Not yet available | 1   |
| <i>Kif3C<sup>A</sup></i>   | FBal0404800 | Bloomington | 605324            | $y^1 w^{1118}; Tl\{Ti\}FRT101F Kif3C^A / \ln(4) ci^D, ci^D pan^{cD}$    | RRID:BDSC_605324  | 101 |
| <i>Kif3C<sup>A</sup></i>   | FBal0404800 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F Kif3C^A / \ln(4) ci^D, ci^D pan^{cD}$    | Not yet available | 101 |
| <i>lgs<sup>C</sup></i>     | FBal0406199 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F lgs^C$                                   | Not yet available | 43  |
| <i>mav<sup>B</sup></i>     | FBal0397361 | Bloomington | 602202            | $y^1 w^{1118}; Tl\{Ti\}FRT101F mav^B$                                   | RRID:BDSC_602202  | 59  |
| <i>mav<sup>B</sup></i>     | FBal0397361 | Kyoto       | 119168            | $y^1 w^{1118}; Tl\{Ti\}FRT101F mav^B$                                   | RRID:DGGR_119168  | 59  |
| <i>mav<sup>D</sup></i>     | FBal0405965 | Bloomington | 605794            | $y^1 w^{1118}; Tl\{Ti\}FRT101F mav^D$                                   | RRID:BDSC_605794  | 59  |
| <i>mav<sup>D</sup></i>     | FBal0405965 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F mav^D$                                   | Not yet available | 59  |
| <i>MED26<sup>A</sup></i>   | FBal0406206 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F MED26^A$                                 | Not yet available | 71  |
| <i>mGluR<sup>B</sup></i>   | FBal0405966 | Bloomington | 605795            | $y^1 w^{1118}; Tl\{Ti\}FRT101F mGluR^B$                                 | RRID:BDSC_605795  | 80  |
| <i>mGluR<sup>B</sup></i>   | FBal0405966 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F mGluR^B$                                 | Not yet available | 80  |
| <i>mGluR<sup>E</sup></i>   | FBal0405967 | Bloomington | 605796            | $y^1 w^{1118}; Tl\{Ti\}FRT101F mGluR^E$                                 | RRID:BDSC_605796  | 80  |
| <i>mGluR<sup>E</sup></i>   | FBal0405967 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F mGluR^E$                                 | Not yet available | 80  |
| <i>myo<sup>A</sup></i>     | FBal0406204 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F myo^A$                                   | Not yet available | 67  |
| <i>ND-49<sup>A</sup></i>   | FBal0392556 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F ND-49^A$                                 | Not yet available | 53  |
| <i>onecut<sup>G</sup></i>  | FBal0404779 | Bloomington | 605325            | $y^1 w^{1118}; Tl\{Ti\}FRT101F onecut^G$                                | RRID:BDSC_605325  | 57  |
| <i>onecut<sup>G</sup></i>  | FBal0404779 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F onecut^G$                                | Not yet available | 57  |
| <i>onecut<sup>H</sup></i>  | FBal0405968 | Bloomington | 605797            | $y^1 w^{1118}; Tl\{Ti\}FRT101F onecut^H$                                | RRID:BDSC_605797  | 57  |
| <i>onecut<sup>H</sup></i>  | FBal0405968 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F onecut^H$                                | Not yet available | 57  |
| <i>pan<sup>F</sup></i>     | FBal0397364 | Bloomington | 660205            | $y^1 w^{1118}; Tl\{Ti\}FRT101F pan^F / P\{w^{mC} = ActGFP\}unc-13^{G1}$ | RRID:BDSC_660205  | 9   |
| <i>pan<sup>F</sup></i>     | FBal0397364 | Kyoto       | 119171            | $y^1 w^{1118}; Tl\{Ti\}FRT101F pan^F / P\{w^{mC} = ActGFP\}unc-13^{G1}$ | RRID:DGGR_119171  | 9   |
| <i>pan<sup>I</sup></i>     | FBal0404785 | Bloomington | 605326            | $y^1 w^{1118}; Tl\{Ti\}FRT101F pan^I / P\{w^{mC} = ActGFP\}unc-13^{G1}$ | RRID:BDSC_605326  | 9   |
| <i>pan<sup>I</sup></i>     | FBal0404785 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl\{Ti\}FRT101F pan^I / P\{w^{mC} = ActGFP\}unc-13^{G1}$ | Not yet available | 9   |
| <i>pho<sup>F</sup></i>     | FBal0405969 | Bloomington | 605798            | $y^1 w^{1118}; Tl\{Ti\}FRT101F pho^F / \ln(4) ci^D, ci^D pan^{cD}$      | RRID:BDSC_605798  | 102 |

|                               |             |             |                   |                                                                                                                                 |                   |     |
|-------------------------------|-------------|-------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------|-------------------|-----|
| <i>pho<sup>F</sup></i>        | FBal0405969 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F pho^F /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | Not yet available | 102 |
| <i>PMCA<sup>D</sup></i>       | FBal0404791 | Bloomington | 605327            | $y^1 w^{1118}; Tl(Tl)FRT101F PMCA^D /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:BDSC_605327  | 35  |
| <i>PMCA<sup>D</sup></i>       | FBal0404791 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F PMCA^D /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | Not yet available | 35  |
| <i>Pur-a<sup>B</sup></i>      | FBal0392554 | Bloomington | 600263            | $y^1 w^{1118}; Tl(Tl)FRT101F Pur-alpha^B /ln(4)ci^D, ci^D pan^{ciD}$                                                            | RRID:BDSC_600263  | 52  |
| <i>Pur-a<sup>B</sup></i>      | FBal0392554 | Kyoto       | 119074            | $y^1 w^{1118}; Tl(Tl)FRT101F Pur-alpha^B /ln(4)ci^D, ci^D pan^{ciD}$                                                            | Not yet available | 52  |
| <i>Rad23<sup>B</sup></i>      | FBal0406197 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Rad23^B$                                                                                           | Not yet available | 32  |
| <i>Rad23<sup>3</sup></i>      | FBal0404788 | Bloomington | 605328            | $y^1 w^{1118}; Tl(Tl)FRT101F Rad23^3$                                                                                           | RRID:BDSC_605328  | 32  |
| <i>Rad23<sup>3</sup></i>      | FBal0404788 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Rad23^3$                                                                                           | Not yet available | 32  |
| <i>Rad23<sup>B</sup></i>      | FBal0397360 | Bloomington | 602201            | $y^1 w^{1118}; Tl(Tl)FRT101F Rad23^B$                                                                                           | RRID:BDSC_602201  | 32  |
| <i>Rad23<sup>B</sup></i>      | FBal0397360 | Kyoto       | 119167            | $y^1 w^{1118}; Tl(Tl)FRT101F Rad23^B$                                                                                           | RRID:DGGR_119167  | 32  |
| <i>RhoGAP102A<sup>A</sup></i> | FBal0397359 | Bloomington | 602200            | $y^1 w^{1118}; Tl(Tl)FRT101F RhoGAP102^A$                                                                                       | RRID:BDSC_602200  | 28  |
| <i>RhoGAP102A<sup>A</sup></i> | FBal0397359 | Kyoto       | 119166            | $y^1 w^{1118}; Tl(Tl)FRT101F RhoGAP102^A$                                                                                       | RRID:DGGR_119166  | 28  |
| <i>RhoGAP102A<sup>B</sup></i> | FBal0406195 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F RhoGAP102^B$                                                                                       | Not yet available | 28  |
| <i>RhoGAP102A<sup>E</sup></i> | FBal0405970 | Bloomington | 605799            | $y^1 w^{1118}; Tl(Tl)FRT101F RhoGAP102^E$                                                                                       | RRID:BDSC_605799  | 28  |
| <i>RhoGAP102A<sup>E</sup></i> | FBal0405970 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F RhoGAP102^E$                                                                                       | Not yet available | 28  |
| <i>Rnf11<sup>A</sup></i>      | FBal0404789 | Bloomington | 605329            | $y^1 w^{1118}; Tl(Tl)FRT101F Rnf11^A$                                                                                           | RRID:BDSC_605329  | 34  |
| <i>Rnf11<sup>A</sup></i>      | FBal0404789 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Rnf11^A$                                                                                           | Not yet available | 34  |
| <i>Rnf11<sup>F</sup></i>      | FBal0404790 | Bloomington | 605330            | $y^1 w^{1118}; Tl(Tl)FRT101F Rnf11^F$                                                                                           | RRID:BDSC_605330  | 34  |
| <i>Rnf11<sup>F</sup></i>      | FBal0404790 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Rnf11^F$                                                                                           | Not yet available | 34  |
| <i>Slip1<sup>A</sup></i>      | FBal0392557 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Slip1^A$                                                                                           | Not yet available | 65  |
| <i>Sox102F<sup>B</sup></i>    | FBal0406207 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Sox102F^B$                                                                                         | Not yet available | 72  |
| <i>Sox102F<sup>C</sup></i>    | FBal0400123 | Bloomington | 603546            | $y^1 w^{1118}; Tl(Tl)FRT101F Sox102F^C$                                                                                         | RRID:BDSC_603546  | 72  |
| <i>Sox102F<sup>C</sup></i>    | FBal0400123 | Kyoto       | 119192            | $y^1 w^{1118}; Tl(Tl)FRT101F Sox102F^C$                                                                                         | RRID:DGGR_119192  | 72  |
| <i>sv<sup>F</sup></i>         | FBal0405971 | Bloomington | 605800            | $y^1 w^{1118}; Tl(Tl)FRT101F sv^F$                                                                                              | RRID:BDSC_605800  | 96  |
| <i>sv<sup>F</sup></i>         | FBal0405971 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F sv^F$                                                                                              | Not yet available | 96  |
| <i>Syt7<sup>B</sup></i>       | FBal0400122 | Bloomington | 603545            | $y^1 w^{1118}; Tl(Tl)FRT101F Syt7^B /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:BDSC_603545  | 31  |
| <i>Syt7<sup>B</sup></i>       | FBal0400122 | Kyoto       | 119191            | $y^1 w^{1118}; Tl(Tl)FRT101F Syt7^B /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:DGGR_119191  | 31  |
| <i>Taf3<sup>A</sup></i>       | FBal0400120 | Bloomington | 603543            | $y^1 w^{1118}; Tl(Tl)FRT101F Taf3^A /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:BDSC_603543  | 45  |
| <i>Taf3<sup>A</sup></i>       | FBal0400120 | Kyoto       | 119189            | $y^1 w^{1118}; Tl(Tl)FRT101F Taf3^A /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:DGGR_119189  | 45  |
| <i>Taf3<sup>F</sup></i>       | FBal0406200 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Taf3^F$                                                                                            | Not yet available | 45  |
| <i>Tdg<sup>C</sup></i>        | FBal0404801 | Bloomington | 605331            | $y^1 w^{1118}; Tl(Tl)FRT101F Tdg^C /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | RRID:BDSC_605331  | 50  |
| <i>Tdg<sup>C</sup></i>        | FBal0404801 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Tdg^C /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | Not yet available | 50  |
| <i>Tdg<sup>F</sup></i>        | FBal0405972 | Bloomington | 605801            | $y^1 w^{1118}; Tl(Tl)FRT101F Tdg^F$                                                                                             | RRID:BDSC_605801  | 50  |
| <i>Tdg<sup>F</sup></i>        | FBal0405972 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Tdg^F$                                                                                             | Not yet available | 50  |
| <i>Tdg<sup>I</sup></i>        | FBal0406201 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F Tdg^I$                                                                                             | Not yet available | 50  |
| <i>toy<sup>A</sup></i>        | FBal0404797 | Bloomington | 605332            | $y^1 w^{1118}; Tl(Tl)FRT101F toy^A /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | RRID:BDSC_605332  | 86  |
| <i>toy<sup>A</sup></i>        | FBal0404797 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F toy^A /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | Not yet available | 86  |
| <i>toy<sup>B</sup></i>        | FBal0406209 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F toy^B /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | Not yet available | 86  |
| <i>toy<sup>C</sup></i>        | FBal0400121 | Bloomington | 603544            | $y^1 w^{1118}; Tl(Tl)FRT101F toy^C /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | RRID:BDSC_603544  | 86  |
| <i>toy<sup>C</sup></i>        | FBal0400121 | Kyoto       | 119190            | $y^1 w^{1118}; Tl(Tl)FRT101F toy^C /ln(4)ci^D, ci^D pan^{ciD}$                                                                  | RRID:DGGR_119190  | 86  |
| <i>ukar<sup>D</sup></i>       | FBal0406211 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F ukar^D$                                                                                            | Not yet available | 99  |
| <i>unc-13<sup>C</sup></i>     | FBal0406208 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F unc-13^C$                                                                                          | Not yet available | 76  |
| <i>yellow-h<sup>A</sup></i>   | FBal0397357 | Bloomington | 602198            | $y^1 w^{1118}; Tl(Tl)FRT101F yellow-h^A$                                                                                        | RRID:BDSC_602198  | 24  |
| <i>yellow-h<sup>A</sup></i>   | FBal0397357 | Kyoto       | 119164            | $y^1 w^{1118}; Tl(Tl)FRT101F yellow-h^A$                                                                                        | RRID:DGGR_119164  | 24  |
| <i>yellow-h<sup>D</sup></i>   | FBal0404787 | Bloomington | 605333            | $y^1 w^{1118}; Tl(Tl)FRT101F yellow-h^D$                                                                                        | RRID:BDSC_605333  | 24  |
| <i>yellow-h<sup>D</sup></i>   | FBal0404787 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F yellow-h^D$                                                                                        | Not yet available | 24  |
| <i>zfh2<sup>51A</sup></i>     | FBal0392552 | Bloomington | 600261            | $y^1 w^{1118}; P\{w^{mc}=Act5C-GAL4\}25FO1, P\{w^{mc}=UAS-GFP.U\}2/SM6a; Tl(Tl)FRT101F zfh2^{51A} /Tl\{GMR-HMS04515\}Gat^{ova}$ | RRID:BDSC_600261  | 49  |
| <i>zfh2<sup>51A</sup></i>     | FBal0392552 | Kyoto       | 119072            | $y^1 w^{1118}; P\{w^{mc}=Act5C-GAL4\}25FO1, P\{w^{mc}=UAS-GFP.U\}2/SM6a; Tl(Tl)FRT101F zfh2^{51A} /Tl\{GMR-HMS04515\}Gat^{ova}$ | RRID:DGGR_119072  | 49  |
| <i>zfh2<sup>A</sup></i>       | FBal0405973 | Bloomington | 605802            | $y^1 w^{1118}; Tl(Tl)FRT101F zfh2^A /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:BDSC_605802  | 49  |
| <i>zfh2<sup>A</sup></i>       | FBal0405973 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F zfh2^A /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | Not yet available | 49  |
| <i>zfh2<sup>D</sup></i>       | FBal0404792 | Bloomington | 605334            | $y^1 w^{1118}; Tl(Tl)FRT101F zfh2^D /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | RRID:BDSC_605334  | 49  |
| <i>zfh2<sup>D</sup></i>       | FBal0404792 | Kyoto       | Not yet available | $y^1 w^{1118}; Tl(Tl)FRT101F zfh2^D /ln(4)ci^D, ci^D pan^{ciD}$                                                                 | Not yet available | 49  |

<sup>a</sup> This column may be used to sort alleles/rows by the cytological order of the genes mutated (see Supplementary Table 1)

Supplementary Table 7. Chromosomal deletions used in complementation tests

| Deletion                                 | FlyBase ID  | Proximal breakpoint                                                                                    | References                                                                      | Position                                                                                                                                                        | Distal breakpoint                                                                              | References                                                                      | Stock RRID <sup>a</sup> |
|------------------------------------------|-------------|--------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------|
| <i>Df(4)M101-62f</i>                     | FBab0002960 | Proximal to <i>JYalpha</i>                                                                             | Podemski <i>et al.</i> (2004); Masly <i>et al.</i> (2006)                       | 4:236230–236585                                                                                                                                                 |                                                                                                | Podemski <i>et al.</i> (2004)                                                   | RRID:BDSC_9433          |
| <i>Df(4)M101-63a</i>                     | FBab0002961 | Distal to <i>ci</i> but disrupting <i>ci</i> expression via position effects; proximal to <i>RpS3A</i> | Locke <i>et al.</i> (1994)                                                      | Within or distal to <i>RpS3A</i>                                                                                                                                |                                                                                                | Hochman (1971); Dooijes <i>et al.</i> (1998); Kronhamn <i>et al.</i> (1999)     | RRID:BDSC_1082          |
| <i>Df(4)ED6366</i>                       | FBab0037590 | 4:69710                                                                                                | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | 4:193485                                                                                                                                                        |                                                                                                | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | RRID:BDSC_8067          |
| <i>Df(4)ED6369</i>                       | FBab0037593 | 4:69710                                                                                                | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | 4:454141                                                                                                                                                        |                                                                                                | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | RRID:BDSC_9422          |
| <i>Df(4)S-M244</i>                       | FBab0046981 | 4:315921                                                                                               | Sun <i>et al.</i> (2004)                                                        | 4:385441                                                                                                                                                        |                                                                                                | Sun <i>et al.</i> (2004)                                                        | RRID:BDSC_84133         |
| <i>Df(4)S-M263</i>                       | FBab0046980 | 4:359573                                                                                               | Sun <i>et al.</i> (2004)                                                        | 4:466776                                                                                                                                                        |                                                                                                | Sun <i>et al.</i> (2004)                                                        | RRID:BDSC_84136         |
| <i>Df(4)M-M529</i>                       | FBab0046970 | 4:359573                                                                                               | Sun <i>et al.</i> (2004)                                                        | 4:393798                                                                                                                                                        |                                                                                                | Sun <i>et al.</i> (2004)                                                        | RRID:BDSC_94692         |
| <i>Df(4)ED6382</i>                       | FBab0037606 | 4:411150                                                                                               | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | 4:867028                                                                                                                                                        |                                                                                                | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | RRID:DGGR_150531        |
| <i>Df(4)ED6380</i>                       | FBab0037604 | 4:411150                                                                                               | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | 4:714403                                                                                                                                                        |                                                                                                | Ryder <i>et al.</i> (2004; 2007) and associated DrosDel Project data in FlyBase | RRID:DGGR_150529        |
| <i>Df(4)O2</i>                           | FBab0029601 | 4:732402–777385                                                                                        | Podemski <i>et al.</i> (2004)                                                   | 4:976768–990535                                                                                                                                                 |                                                                                                | Podemski <i>et al.</i> (2004)                                                   | RRID:BDSC_7084          |
| <i>Df(4)8-M318</i>                       | FBab0047548 | 4:912919                                                                                               | Riddle <i>et al.</i> (2008)                                                     | 4:1098782                                                                                                                                                       |                                                                                                | Riddle <i>et al.</i> (2008)                                                     | RRID:BDSC_84132         |
| <i>Df(4)8-M91</i>                        | FBab0047542 | 4:910421                                                                                               | Riddle <i>et al.</i> (2008)                                                     | 4:1098782                                                                                                                                                       |                                                                                                | Riddle <i>et al.</i> (2008)                                                     | RRID:BDSC_84135         |
| <i>Df(4)8-M167</i>                       | FBab0047543 | 4:1031865                                                                                              | Riddle <i>et al.</i> (2008)                                                     | 4:1098782                                                                                                                                                       |                                                                                                | Riddle <i>et al.</i> (2008)                                                     | RRID:BDSC_94691         |
| <i>Df(4)8-M175</i>                       | FBab0047544 | 4:1062401                                                                                              | Riddle <i>et al.</i> (2008)                                                     | 4:1098782                                                                                                                                                       |                                                                                                | Riddle <i>et al.</i> (2008)                                                     | RRID:BDSC_84134         |
| <i>Df(4)C3</i>                           | FBab0028454 | 4:732424–777385                                                                                        | Podemski <i>et al.</i> (2004)                                                   |                                                                                                                                                                 | <i>Df(4)C3</i> is a terminal deletion disrupting <i>bt</i> and deleting all genes distal to it | Podemski <i>et al.</i> (2004)                                                   | RRID:BDSC_7083          |
| <sup>a</sup> <i>Df(4)jsc<sup>h</sup></i> | FBab0023008 | Within <i>PlexA</i>                                                                                    | This study                                                                      | All genes distal to the <i>T(1;4)jsc<sup>h</sup></i> breakpoint are absent when <i>Df(4)jsc<sup>h</sup></i> is separated from <i>Ts(1Rt;4Rt)jsc<sup>h</sup></i> |                                                                                                |                                                                                 | RRID:BDSC_4055          |

<sup>a</sup> *Df(4)jsc<sup>h</sup>* is our nickname for the *Ts(1Lt;4Lt)jsc<sup>h</sup>* segregant from *T(1;4)jsc<sup>h</sup>* that bears the fourth chromosome centromere and the left end of the X chromosome. We isolated *Df(4)jsc<sup>h</sup>* in a balanced stock in the absence of the *Ts(1Rt;4Rt)jsc<sup>h</sup>* segregant, which carries the right end of the 4th chromosome and the X chromosome centromere.

<sup>b</sup> As explained in footnote a of Supplementary Table 8, we built new *Df(4)ED6380* and *Df(4)jsc<sup>h</sup>* stocks from Kyoto and Bloomington stocks before using the deletions in complementation crosses

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Supplementary Table 8. Control crosses

| Mutations or deletions crossed <sup>a</sup> |             |                  |                               |             |                  | Phenotype |                |                                   | Cytological order <sup>b</sup> |
|---------------------------------------------|-------------|------------------|-------------------------------|-------------|------------------|-----------|----------------|-----------------------------------|--------------------------------|
| Mutation or deletion                        | FlyBase ID  | Stock RRID       | Mutation or deletion          | FlyBase ID  | Stock RRID       | Viability | Fertility      | Adult morphology                  |                                |
| Controls for chromosomal deletions          |             |                  |                               |             |                  |           |                |                                   |                                |
| Df(4)4-M529                                 | FBab0046970 | RRID:BDSC_94692  | Df(4)5-M263                   | FBab0046980 | RRID:BDSC_84136  | Lethal    |                |                                   | G                              |
| Df(4)5-M244                                 | FBab0046981 | RRID:BDSC_84133  | Df(4)4-M529                   | FBab0046970 | RRID:BDSC_94692  | Lethal    |                |                                   | E                              |
| Df(4)5-M244                                 | FBab0046981 | RRID:BDSC_84133  | Df(4)ED6382                   | FBab0037606 | RRID:DGGR_150531 | Lethal    |                |                                   | E                              |
| Df(4)5-M263                                 | FBab0046980 | RRID:BDSC_84136  | Df(4)ED6380                   | FBab0037604 | RRID:DGGR_150529 | Lethal    |                |                                   | F                              |
| Df(4)5-M263                                 | FBab0046980 | RRID:BDSC_84136  | Df(4)ED6382                   | FBab0037606 | RRID:DGGR_150531 | Lethal    |                |                                   | F                              |
| Df(4)5-M263                                 | FBab0046980 | RRID:BDSC_84136  | lgs <sup>17E</sup>            | FBal0135672 | RRID:BDSC_42700  | Lethal    |                |                                   | F                              |
| Df(4)5-M263                                 | FBab0046980 | RRID:BDSC_84136  | lgs <sup>2-8</sup>            | FBal0325838 | RRID:BDSC_76037  | Lethal    |                |                                   | F                              |
| Df(4)8-M167                                 | FBab0047543 | RRID:BDSC_94691  | ATPsynbeta <sup>K007618</sup> | FBal0148507 | RRID:BDSC_99055  | Lethal    |                |                                   | M                              |
| Df(4)8-M167                                 | FBab0047543 | RRID:BDSC_94691  | Df(4)8-M175                   | FBab0047544 | RRID:BDSC_84134  | Lethal    |                |                                   | M                              |
| Df(4)8-M167                                 | FBab0047543 | RRID:BDSC_94691  | Df(4)C3                       | FBab0028454 | RRID:BDSC_7083   | Lethal    |                |                                   | M                              |
| Df(4)8-M167                                 | FBab0047543 | RRID:BDSC_94691  | sv <sup>spo-1</sup>           | FBal0015985 | RRID:BDSC_658    | Viable    | Female fertile | Rough eye (disarranged ommatidia) | M                              |
| Df(4)8-M167                                 | FBab0047543 | RRID:BDSC_94691  | sv <sup>spo-pol</sup>         | FBal0015991 | RRID:BDSC_2515   | Viable    | Female fertile | Glossy, misshapened eye           | M                              |
| Df(4)8-M167                                 | FBab0047543 | RRID:BDSC_94691  | Zyx <sup>CRO1526-TG4.0</sup>  | FBal0356730 | RRID:BDSC_86431  | Lethal    |                |                                   | M                              |
| Df(4)8-M175                                 | FBab0047544 | RRID:BDSC_84134  | Df(4)C3                       | FBab0028454 | RRID:BDSC_7083   | Lethal    |                |                                   | N                              |
| Df(4)8-M175                                 | FBab0047544 | RRID:BDSC_84134  | sv <sup>spo-1</sup>           | FBal0015985 | RRID:BDSC_658    | Viable    | Female fertile | Rough eye (disarranged ommatidia) | N                              |
| Df(4)8-M175                                 | FBab0047544 | RRID:BDSC_84134  | sv <sup>spo-pol</sup>         | FBal0015991 | RRID:BDSC_2515   | Viable    | Female fertile | Glossy, misshapened eye           | N                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | ATPsynbeta <sup>K007618</sup> | FBal0148507 | RRID:BDSC_99055  | Lethal    |                |                                   | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | Df(4)8-M167                   | FBab0047543 | RRID:BDSC_94691  | Lethal    |                |                                   | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | Df(4)8-M175                   | FBab0047544 | RRID:BDSC_84134  | Lethal    |                |                                   | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | Df(4)8-M91                    | FBab0047542 | RRID:BDSC_84135  | Lethal    |                |                                   | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | Df(4)C3                       | FBab0028454 | RRID:BDSC_7083   | Lethal    |                |                                   | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | PlexA <sup>MB09499</sup>      | FBal0280754 | RRID:BDSC_61741  | Lethal    |                |                                   | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | sv <sup>spo-1</sup>           | FBal0015985 | RRID:BDSC_658    | Viable    | Female fertile | Rough eye (disarranged ommatidia) | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | sv <sup>spo-pol</sup>         | FBal0015991 | RRID:BDSC_2515   | Viable    | Female fertile | Glossy, misshapened eye           | K                              |
| Df(4)8-M318                                 | FBab0047548 | RRID:BDSC_84132  | Zyx <sup>CRO1526-TG4.0</sup>  | FBal0356730 | RRID:BDSC_86431  | Lethal    |                |                                   | K                              |
| Df(4)8-M91                                  | FBab0047542 | RRID:BDSC_84135  | ATPsynbeta <sup>K007618</sup> | FBal0148507 | RRID:BDSC_99055  | Lethal    |                |                                   | L                              |
| Df(4)8-M91                                  | FBab0047542 | RRID:BDSC_84135  | Df(4)8-M167                   | FBab0047543 | RRID:BDSC_94691  | Lethal    |                |                                   | L                              |
| Df(4)8-M91                                  | FBab0047542 | RRID:BDSC_84135  | Df(4)8-M175                   | FBab0047544 | RRID:BDSC_84134  | Lethal    |                |                                   | L                              |
| Df(4)8-M91                                  | FBab0047542 | RRID:BDSC_84135  | Df(4)C3                       | FBab0028454 | RRID:BDSC_7083   | Lethal    |                |                                   | L                              |
| Df(4)8-M91                                  | FBab0047542 | RRID:BDSC_84135  | sv <sup>spo-1</sup>           | FBal0015985 | RRID:BDSC_658    | Viable    | Female fertile | Rough eye (disarranged ommatidia) | L                              |
| Df(4)8-M91                                  | FBab0047542 | RRID:BDSC_84135  | Zyx <sup>CRO1526-TG4.0</sup>  | FBal0356730 | RRID:BDSC_86431  | Lethal    |                |                                   | L                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | ATPsynbeta <sup>K007618</sup> | FBal0148507 | RRID:BDSC_99055  | Lethal    |                |                                   | O                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | bt <sup>1-b</sup>             | FBal0027984 | RRID:BDSC_4231   | Lethal    |                |                                   | O                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | Cadps <sup>M108448</sup>      | FBal0303070 | RRID:BDSC_60816  | Lethal    |                |                                   | O                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | sv <sup>spo-1</sup>           | FBal0015985 | RRID:BDSC_658    | Viable    | Female fertile | Rough eye (disarranged ommatidia) | O                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | sv <sup>spo-pol</sup>         | FBal0015991 | RRID:BDSC_2515   | Viable    | Female fertile | Glossy, misshapened eye           | O                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | unc-13 <sup>G1</sup>          | FBal0198125 | RRID:BDSC_9549   | Lethal    |                |                                   | O                              |
| Df(4)C3                                     | FBab0028454 | RRID:BDSC_7083   | Zyx <sup>CRO1526-TG4.0</sup>  | FBal0356730 | RRID:BDSC_86431  | Lethal    |                |                                   | O                              |
| Df(4)ED6366                                 | FBab0037590 | RRID:BDSC_8067   | pan <sup>2</sup>              | FBal0059982 | RRID:BDSC_4759   | Lethal    |                |                                   | C                              |
| Df(4)ED6366                                 | FBab0037590 | RRID:BDSC_8067   | pan <sup>3</sup>              | FBal0059981 | RRID:BDSC_4760   | Lethal    |                |                                   | C                              |
| Df(4)ED6369                                 | FBab0037593 | RRID:BDSC_9422   | lgs <sup>2-8</sup>            | FBal0325838 | RRID:BDSC_76037  | Lethal    |                |                                   | D                              |
| Df(4)ED6369                                 | FBab0037593 | RRID:BDSC_9422   | pan <sup>3</sup>              | FBal0059981 | RRID:BDSC_4760   | Lethal    |                |                                   | D                              |
| Df(4)ED6380                                 | FBab0037604 | RRID:DGGR_150529 | Df(4)ED6382                   | FBab0037606 | RRID:DGGR_150531 | Lethal    |                |                                   | I                              |
| Df(4)ED6380                                 | FBab0037604 | RRID:DGGR_150529 | ey <sup>2</sup>               | FBal0003921 | RRID:BDSC_648    | Viable    | Female fertile | Small eye                         | I                              |
| Df(4)ED6380                                 | FBab0037604 | RRID:DGGR_150529 | Gat <sup>eyo</sup>            | FBal0361674 | RRID:BDSC_90852  | Lethal    |                |                                   | I                              |
| Df(4)ED6380                                 | FBab0037604 | RRID:DGGR_150529 | lgs <sup>17E</sup>            | FBal0135672 | RRID:BDSC_42700  | Lethal    |                |                                   | I                              |
| Df(4)ED6380                                 | FBab0037604 | RRID:DGGR_150529 | lgs <sup>2-8</sup>            | FBal0325838 | RRID:BDSC_76037  | Lethal    |                |                                   | I                              |
| Df(4)ED6380                                 | FBab0037604 | RRID:DGGR_150529 | zfh2 <sup>vii-2</sup>         | FBal0367988 | RRID:BDSC_97216  | Lethal    |                |                                   | I                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | bt <sup>D</sup>               | FBal0001328 | RRID:BDSC_638    | Lethal    |                |                                   | H                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | bt <sup>1-b</sup>             | FBal0027984 | RRID:BDSC_4231   | Lethal    |                |                                   | H                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | Df(4)C3                       | FBab0028454 | RRID:BDSC_7083   | Lethal    |                |                                   | H                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | Df(4)O2                       | FBab0029601 | RRID:BDSC_7084   | Lethal    |                |                                   | H                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | ey <sup>2</sup>               | FBal0003921 | RRID:BDSC_648    | Viable    | Female fertile | Small eye                         | H                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | lgs <sup>17E</sup>            | FBal0135672 | RRID:BDSC_42700  | Lethal    |                |                                   | H                              |
| Df(4)ED6382                                 | FBab0037606 | RRID:DGGR_150531 | lgs <sup>2-8</sup>            | FBal0325838 | RRID:BDSC_76037  | Lethal    |                |                                   | H                              |

|                             |             |                |                                            |             |                 |        |                               |                                   |   |
|-----------------------------|-------------|----------------|--------------------------------------------|-------------|-----------------|--------|-------------------------------|-----------------------------------|---|
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>ci</i> <sup>1</sup>                     | FBal0001644 | RRID:BDSC_642   | Viable | Female fertile                | Discontinuous wing veins          | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>Df(4)ED6366</i>                         | FBab0037590 | RRID:BDSC_8067  | Lethal |                               |                                   | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>Df(4)M101-63a</i>                       | FBab0002961 | RRID:BDSC_1082  | Lethal |                               |                                   | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>    | FBal0386736 | RRID:BDSC_97185 | Viable | Female fertile. Male sterile. | No overt visible phenotypes       | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>pan</i> <sup>2</sup>                    | FBal0059982 | RRID:BDSC_4759  | Lethal |                               |                                   | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>pan</i> <sup>3</sup>                    | FBal0059981 | RRID:BDSC_4760  | Lethal |                               |                                   | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>PlexB</i> <sup>K000878</sup>            | FBal0216740 | RRID:BDSC_14579 | Lethal |                               |                                   | A |
| <i>Df(4)M101-62f</i>        | FBab0002960 | RRID:BDSC_9433 | <i>PlexB</i> <sup>M15559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694 | Lethal |                               |                                   | A |
| <i>Df(4)M101-63a</i>        | FBab0002961 | RRID:BDSC_1082 | <i>ci</i> <sup>1</sup>                     | FBal0001644 | RRID:BDSC_642   | Viable | Female fertile                | Discontinuous wing veins          | B |
| <i>Df(4)O2</i>              | FBab0029601 | RRID:BDSC_7084 | <i>bt</i> <sup>D</sup>                     | FBal0001328 | RRID:BDSC_638   | Lethal |                               |                                   | J |
| <i>Df(4)O2</i>              | FBab0029601 | RRID:BDSC_7084 | <i>Df(4)8-M318</i>                         | FBab0047548 | RRID:BDSC_84132 | Lethal |                               |                                   | J |
| <i>Df(4)O2</i>              | FBab0029601 | RRID:BDSC_7084 | <i>Df(4)8-M91</i>                          | FBab0047542 | RRID:BDSC_84135 | Lethal |                               |                                   | J |
| <i>Df(4)O2</i>              | FBab0029601 | RRID:BDSC_7084 | <i>Df(4)C3</i>                             | FBab0028454 | RRID:BDSC_7083  | Lethal |                               |                                   | J |
| <i>Df(4)O2</i>              | FBab0029601 | RRID:BDSC_7084 | <i>unc-13</i> <sup>GI</sup>                | FBal0198125 | RRID:BDSC_9549  | Lethal |                               |                                   | J |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4055 | <i>ATPsynbeta</i> <sup>K007618</sup>       | FBal0148507 | RRID:BDSC_99055 | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4056 | <i>bt</i> <sup>I-b</sup>                   | FBal0027984 | RRID:BDSC_4231  | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4057 | <i>Cadps</i> <sup>M108448</sup>            | FBal0303070 | RRID:BDSC_60816 | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4058 | <i>Df(4)8-M167</i>                         | FBab0047543 | RRID:BDSC_94691 | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4059 | <i>Df(4)8-M318</i>                         | FBab0047548 | RRID:BDSC_84132 | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4060 | <i>Df(4)O2</i>                             | FBab0029601 | RRID:BDSC_7084  | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4061 | <i>PlexA</i> <sup>EY16548</sup>            | FBal0192220 | RRID:BDSC_23097 | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4062 | <i>PlexA</i> <sup>MB09499</sup>            | FBal0280754 | RRID:BDSC_61741 | Lethal |                               |                                   | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4063 | <i>sv</i> <sup>spa-1</sup>                 | FBal0015985 | RRID:BDSC_658   | Viable | Female fertile                | Rough eye (disarranged ommatidia) | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4064 | <i>sv</i> <sup>spa-pol</sup>               | FBal0015991 | RRID:BDSC_2515  | Viable | Female fertile                | Glossy, misshapened eye           | P |
| <i>Df(4)sc</i> <sup>H</sup> | FBab0023008 | RRID:BDSC_4065 | <i>Zyx</i> <sup>CRO1526-TG4.0</sup>        | FBal0356730 | RRID:BDSC_86431 | Lethal |                               |                                   | P |

#### Controls for preexisting transgene insertions

|                                               |             |                  |                                  |             |                  |        |                               |                             |     |
|-----------------------------------------------|-------------|------------------|----------------------------------|-------------|------------------|--------|-------------------------------|-----------------------------|-----|
| <i>Actbeta</i> <sup>M114795-DH.GT-TG4.1</sup> | FBal0371013 | RRID:BDSC_93693  | <i>Df(4)8-M167</i>               | FBab0047543 | RRID:BDSC_94691  | Viable | Female fertile                | No overt visible phenotypes | 95  |
| <i>Actbeta</i> <sup>M114795-DH.GT-TG4.1</sup> | FBal0371013 | RRID:BDSC_93693  | <i>Df(4)8-M175</i>               | FBab0047544 | RRID:BDSC_84134  | Viable | Female fertile                | No overt visible phenotypes | 95  |
| <i>Actbeta</i> <sup>M114795-DH.GT-TG4.1</sup> | FBal0371013 | RRID:BDSC_93693  | <i>Df(4)8-M318</i>               | FBab0047548 | RRID:BDSC_84132  | Viable | Female fertile                | No overt visible phenotypes | 95  |
| <i>Actbeta</i> <sup>M114795-DH.GT-TG4.1</sup> | FBal0371013 | RRID:BDSC_93693  | <i>Df(4)8-M91</i>                | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile                | No overt visible phenotypes | 95  |
| <i>Actbeta</i> <sup>M114795-DH.GT-TG4.1</sup> | FBal0371013 | RRID:BDSC_93693  | <i>Df(4)sc</i> <sup>H</sup>      | FBab0023008 | RRID:BDSC_4059   | Viable | Female fertile                | No overt visible phenotypes | 95  |
| <i>Ank</i> <sup>MB09683</sup>                 | FBal0304618 | RRID:BDSC_62020  | <i>Ank</i> <sup>MB09683</sup>    | FBal0304618 | RRID:BDSC_62020  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 10  |
| <i>anne</i> <sup>CR70036-TG4.1</sup>          | FBal0368514 | RRID:BDSC_94599  | <i>Df(4)ED6366</i>               | FBab0037590 | RRID:BDSC_8067   | Lethal |                               |                             | 11  |
| <i>anne</i> <sup>M104450</sup>                | FBal0304701 | RRID:BDSC_61711  | <i>Df(4)ED6366</i>               | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 11  |
| <i>Arf4</i> <sup>CR71015-KO-4G4</sup>         | FBal0392678 | RRID:BDSC_600127 | <i>Df(4)C3</i>                   | FBab0028454 | RRID:BDSC_7083   | Lethal |                               |                             | 98  |
| <i>Arf4</i> <sup>CR71015-KO-4G4</sup>         | FBal0392678 | RRID:BDSC_600127 | <i>Df(4)sc</i> <sup>H</sup>      | FBab0023008 | RRID:BDSC_4059   | Lethal |                               |                             | 98  |
| <i>Cadps</i> <sup>M105877-DH.GT-TG4.1</sup>   | FBal0371025 | RRID:BDSC_93687  | <i>Df(4)C3</i>                   | FBab0028454 | RRID:BDSC_7083   | Lethal |                               |                             | 107 |
| <i>Cadps</i> <sup>M105877-DH.GT-TG4.1</sup>   | FBal0371025 | RRID:BDSC_93687  | <i>Df(4)sc</i> <sup>H</sup>      | FBab0023008 | RRID:BDSC_4059   | Lethal |                               |                             | 107 |
| <i>CG1674</i> <sup>M110152</sup>              | FBal0303083 | RRID:BDSC_60832  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 26  |
| <i>CG31998</i> <sup>M106163</sup>             | FBal0303039 | RRID:BDSC_60790  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 21  |
| <i>CG32006</i> <sup>CR70476-4G4</sup>         | FBal0386648 | RRID:BDSC_97333  | <i>Df(4)ED6366</i>               | FBab0037590 | RRID:BDSC_8067   | Lethal |                               |                             | 13  |
| <i>CG32006</i> <sup>CR70476-4G4</sup>         | FBal0386648 | RRID:BDSC_97333  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 13  |
| <i>CG33941</i> <sup>d09357</sup>              | FBal0228530 | RRID:BDSC_85105  | <i>CG33941</i> <sup>d09357</sup> | FBal0228530 | RRID:BDSC_85105  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 47  |
| <i>CG33941</i> <sup>d09357</sup>              | FBal0228530 | RRID:BDSC_85105  | <i>Df(4)ED6380</i>               | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 47  |
| <i>CG33941</i> <sup>d09357</sup>              | FBal0228530 | RRID:BDSC_85105  | <i>Df(4)ED6382</i>               | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 47  |
| <i>CG33941</i> <sup>e02647</sup>              | FBal0228526 | RRID:BDSC_95461  | <i>CG33941</i> <sup>e02647</sup> | FBal0228526 | RRID:BDSC_95461  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 47  |
| <i>CG33978</i> <sup>CR70478-TG4.1</sup>       | FBal0390518 | RRID:BDSC_98482  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 15  |
| <i>CG33978</i> <sup>M103508</sup>             | FBal0304696 | RRID:BDSC_61706  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 15  |
| <i>CG33978</i> <sup>M108362</sup>             | FBal0303068 | RRID:BDSC_60814  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 15  |
| <i>Crk</i> <sup>d5Red</sup>                   | FBal0361675 | RRID:BDSC_90850  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 22  |
| <i>dati</i> <sup>K001667</sup>                | FBal0221341 | RRID:BDSC_14379  | <i>Df(4)4-M529</i>               | FBab0046970 | RRID:BDSC_94692  | Viable | Female fertile                | No overt visible phenotypes | 37  |
| <i>dati</i> <sup>K001667</sup>                | FBal0221341 | RRID:BDSC_14379  | <i>Df(4)5-M263</i>               | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile                | No overt visible phenotypes | 37  |
| <i>dati</i> <sup>K001667</sup>                | FBal0221341 | RRID:BDSC_14379  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 37  |
| <i>dati</i> <sup>K001667</sup>                | FBal0221341 | RRID:BDSC_14379  | <i>Df(4)5-M244</i>               | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 37  |
| <i>dpr7</i> <sup>M105719-TG4.1</sup>          | FBal0337327 | RRID:BDSC_78385  | <i>Df(4)5-M244</i>               | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 27  |
| <i>dpr7</i> <sup>M105719-TG4.1</sup>          | FBal0337327 | RRID:BDSC_78385  | <i>Df(4)ED6369</i>               | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 27  |
| <i>dpr7</i> <sup>M107186</sup>                | FBal0303055 | RRID:BDSC_60804  | <i>dpr7</i> <sup>M107186</sup>   | FBal0303055 | RRID:BDSC_60804  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 27  |

|                                                             |             |                  |                                                             |             |                  |        |                               |                             |     |
|-------------------------------------------------------------|-------------|------------------|-------------------------------------------------------------|-------------|------------------|--------|-------------------------------|-----------------------------|-----|
| <i>dpr7</i> <sup>MI07186</sup>                              | FBal0303055 | RRID:BDSC_60804  | <i>Df(4)ED6369</i>                                          | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 27  |
| <i>elF4G1</i> <sup>MI05019-TG4.0</sup>                      | FBal0336085 | RRID:BDSC_76667  | <i>Df(4)8-M318</i>                                          | FBab0047548 | RRID:BDSC_84132  | Lethal |                               |                             | 79  |
| <i>elF4G1</i> <sup>MI05019-TG4.0</sup>                      | FBal0336085 | RRID:BDSC_76667  | <i>Df(4)8-M91</i>                                           | FBab0047542 | RRID:BDSC_84135  | Lethal |                               |                             | 79  |
| <i>elF4G1</i> <sup>MI05019-TG4.0</sup>                      | FBal0336085 | RRID:BDSC_76667  | <i>Df(4)O2</i>                                              | FBab0029601 | RRID:BDSC_7084   | Lethal |                               |                             | 79  |
| <i>Ephrin</i> <sup>CR01621-TG4.2</sup>                      | FBal0356673 | RRID:BDSC_86471  | <i>Df(4)ED6380</i>                                          | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 54  |
| <i>fd102C</i> <sup>CR70946-KO-4G4</sup>                     | FBal0392714 | RRID:BDSC_600122 | <i>Df(4)ED6380</i>                                          | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 73  |
| <i>fd102C</i> <sup>CR70946-KO-4G4</sup>                     | FBal0392714 | RRID:BDSC_600122 | <i>Df(4)ED6382</i>                                          | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 73  |
| <i>gw</i> <sup>CR70482-TG4.0</sup>                          | FBal0386753 | RRID:BDSC_97184  | <i>Df(4)ED6380</i>                                          | FBab0037604 | RRID:DGGR_150529 | Lethal |                               |                             | 63  |
| <i>Gyf</i> <sup>CR00412-TG4.1</sup>                         | FBal0340959 | RRID:BDSC_79256  | <i>Df(4)ED6382</i>                                          | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 75  |
| <i>Gyf</i> <sup>CR00412-TG4.1</sup>                         | FBal0340959 | RRID:BDSC_79256  | <i>Df(4)O2</i>                                              | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 75  |
| <i>Gyf</i> <sup>CR70565-TG4.1</sup>                         | FBal0390539 | RRID:BDSC_79256  | <i>Df(4)O2</i>                                              | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 75  |
| <i>Gyf</i> <sup>MI00455</sup>                               | FBal0243105 | RRID:BDSC_31010  | <i>Df(4)ED6382</i>                                          | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 75  |
| <i>Gyf</i> <sup>MI00455</sup>                               | FBal0243105 | RRID:BDSC_31010  | <i>Df(4)O2</i>                                              | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 75  |
| <i>Hcf</i> <sup>MI03718</sup>                               | FBal0304698 | RRID:BDSC_62013  | <i>Df(4)4-M529</i>                                          | FBab0046970 | RRID:BDSC_94692  | Viable | Female fertile                | No overt visible phenotypes | 36  |
| <i>Hcf</i> <sup>MI03718</sup>                               | FBal0304698 | RRID:BDSC_62013  | <i>Df(4)5-M244</i>                                          | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 36  |
| <i>Hcf</i> <sup>MI03718</sup>                               | FBal0304698 | RRID:BDSC_62013  | <i>Df(4)5-M263</i>                                          | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile                | No overt visible phenotypes | 36  |
| <i>Hcf</i> <sup>MI11162-DH-GT-TG4.0</sup>                   | FBal0371029 | RRID:BDSC_93690  | <i>Df(4)4-M529</i>                                          | FBab0046970 | RRID:BDSC_94692  | Viable | Female fertile                | No overt visible phenotypes | 36  |
| <i>Hcf</i> <sup>MI11162-DH-GT-TG4.0</sup>                   | FBal0371029 | RRID:BDSC_93690  | <i>Df(4)5-M244</i>                                          | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 36  |
| <i>Hcf</i> <sup>MI11162-DH-GT-TG4.0</sup>                   | FBal0371029 | RRID:BDSC_93690  | <i>Df(4)5-M263</i>                                          | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile                | No overt visible phenotypes | 36  |
| <i>MED26</i> <sup>MB00704</sup>                             | FBal0212488 | RRID:BDSC_23727  | <i>Df(4)ED6382</i>                                          | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 71  |
| <i>MED26</i> <sup>MB00704</sup>                             | FBal0212488 | RRID:BDSC_23727  | <i>Df(4)O2</i>                                              | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 71  |
| <i>Df(4)CRIMIC-CR70944, Mpv17</i> <sup>CR70944-KO-4G4</sup> | FBab0049402 | RRID:BDSC_600121 | <i>Df(4)CRIMIC-CR70944, Mpv17</i> <sup>CR70944-KO-4G4</sup> | FBab0049402 | RRID:BDSC_600121 | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 89  |
| <i>Df(4)CRIMIC-CR70944, Mpv17</i> <sup>CR70944-KO-4G4</sup> | FBab0049402 | RRID:BDSC_600121 | <i>Df(4)8-M318</i>                                          | FBab0047548 | RRID:BDSC_84132  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 89  |
| <i>Df(4)CRIMIC-CR70944, Mpv17</i> <sup>CR70944-KO-4G4</sup> | FBab0049402 | RRID:BDSC_600121 | <i>Df(4)8-M91</i>                                           | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 89  |
| <i>Df(4)CRIMIC-CR70944, Mpv17</i> <sup>CR70944-KO-4G4</sup> | FBab0049402 | RRID:BDSC_600121 | <i>Df(4)C3</i>                                              | FBab0028454 | RRID:BDSC_7083   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 89  |
| <i>Df(4)CRIMIC-CR70944, Mpv17</i> <sup>CR70944-KO-4G4</sup> | FBab0049402 | RRID:BDSC_600121 | <i>Df(4)sc<sup>H</sup></i>                                  | FBab0023008 | RRID:BDSC_4065   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 89  |
| <i>myo</i> <sup>CR02262-TG4.1</sup>                         | FBal0366707 | RRID:BDSC_92207  | <i>Df(4)ED6380</i>                                          | FBab0037604 | RRID:DGGR_150529 | Lethal |                               |                             | 67  |
| <i>myo</i> <sup>CR02262-TG4.1</sup>                         | FBal0366707 | RRID:BDSC_92207  | <i>Df(4)ED6382</i>                                          | FBab0037606 | RRID:DGGR_150531 | Lethal |                               |                             | 67  |
| <i>PIP4K</i> <sup>CR70484-TG4.0</sup>                       | FBal0386738 | RRID:BDSC_97186  | <i>Df(4)C3</i>                                              | FBab0028454 | RRID:BDSC_7083   | Lethal |                               |                             | 104 |
| <i>PIP4K</i> <sup>CR70484-TG4.0</sup>                       | FBal0386738 | RRID:BDSC_97186  | <i>Df(4)sc<sup>H</sup></i>                                  | FBab0023008 | RRID:BDSC_4055   | Lethal |                               |                             | 104 |
| <i>PIP4K</i> <sup>CR70484-TG4.0</sup>                       | FBal0386738 | RRID:BDSC_97186  | <i>Df(4)8-M318</i>                                          | FBab0047548 | RRID:BDSC_84132  | Lethal |                               |                             | 104 |
| <i>PlexA</i> <sup>MB09499</sup>                             | FBal0280754 | RRID:BDSC_61741  | <i>Df(4)8-M318</i>                                          | FBab0047548 | RRID:BDSC_84132  | Lethal |                               |                             | 87  |
| <i>PlexA</i> <sup>MB09499</sup>                             | FBal0280754 | RRID:BDSC_61741  | <i>Df(4)8-M91</i>                                           | FBab0047542 | RRID:BDSC_84135  | Lethal |                               |                             | 87  |
| <i>PMCA</i> <sup>MI12515</sup>                              | FBal0303100 | RRID:BDSC_60856  | <i>Df(4)5-M244</i>                                          | FBab0046981 | RRID:BDSC_84133  | Lethal |                               |                             | 35  |
| <i>PMCA</i> <sup>MI12515</sup>                              | FBal0303100 | RRID:BDSC_60856  | <i>Df(4)ED6369</i>                                          | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 35  |
| <i>PMCA</i> <sup>MI12515-TG4.2</sup>                        | FBal0336050 | RRID:BDSC_76741  | <i>Df(4)5-M244</i>                                          | FBab0046981 | RRID:BDSC_84133  | Lethal |                               |                             | 35  |
| <i>PMCA</i> <sup>MI12515-TG4.2</sup>                        | FBal0336050 | RRID:BDSC_76741  | <i>Df(4)ED6369</i>                                          | FBab0037593 | RRID:BDSC_9422   | Lethal |                               |                             | 35  |
| <i>Poir1G</i> <sup>CR70943-KO-4G4</sup>                     | FBal0392681 | RRID:BDSC_600120 | <i>Df(4)8-M318</i>                                          | FBab0047548 | RRID:BDSC_84132  | Viable | Female fertile                | No overt visible phenotypes | 90  |
| <i>Rnf11</i> <sup>MI14327-DH-GT-TG4.0</sup>                 | FBal0371041 | RRID:BDSC_93692  | <i>Df(4)5-M244</i>                                          | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 34  |
| <i>Rnf11</i> <sup>MI14327-DH-GT-TG4.0</sup>                 | FBal0371041 | RRID:BDSC_93692  | <i>Df(4)ED6369</i>                                          | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 34  |
| <i>Slip1</i> <sup>CR02779-TG4.0</sup>                       | FBal0375346 | RRID:BDSC_94483  | <i>Df(4)ED6380</i>                                          | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 65  |
| <i>ukar</i> <sup>CR70473-TG4.1</sup>                        | FBal0386720 | RRID:BDSC_97180  | <i>Df(4)C3</i>                                              | FBab0028454 | RRID:BDSC_7083   | Viable | Female fertile                | No overt visible phenotypes | 99  |
| <i>uka</i> <sup>CR70473-TG4.1</sup>                         | FBal0386720 | RRID:BDSC_97180  | <i>Df(4)sc<sup>H</sup></i>                                  | FBab0023008 | RRID:BDSC_4055   | Viable | Female fertile                | No overt visible phenotypes | 99  |
| <i>Zip102B</i> <sup>CR70486-KO-TG4.0</sup>                  | FBal0386713 | RRID:BDSC_97334  | <i>Df(4)ED6369</i>                                          | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 33  |
| <i>Zip102B</i> <sup>CR70486-KO-TG4.0</sup>                  | FBal0386713 | RRID:BDSC_97334  | <i>Zip102B</i> <sup>CR70486-KO-TG4.0</sup>                  | FBal0386713 | RRID:BDSC_97334  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 33  |

<sup>a</sup> The *Df(4)ED6380* and *Df(4)ED6369* stocks obtained from the Kyoto and Bloomington stock centers were trisomic for chromosome 4. The deletions were isolated in disomic stocks prior to their use in complementation tests. The *Df(4)ED6382* stock obtained from the Kyoto Stock Center was trisomic for chromosome 4, but could be used in complementation tests without converting it to a disomic stock. The *Mpv17*<sup>CR70944-KO-4G4</sup> mutation was caused by the breakpoints of the two-gene deletion *Df(4)CRIMIC-CR70944*, which also deletes most of the large exon of the noncoding RNA gene *asRNA:CR44031*.

<sup>b</sup> This column may be used to sort the deletions in column A by the cytological order shown in Supplementary Table 6 and/or to sort the alleles in column A by the cytological order of the genes mutated (see Supplementary Table 1)







Supplementary Table 10. Homozygous and hemizygous mutant phenotypes

| Allele                         | FlyBase ID <sup>a</sup> | Allele type                 | Homozygous phenotype <sup>b</sup> |                |                             | Hemizygous phenotype <sup>b</sup> |                |                             | Cytological order <sup>c</sup> |
|--------------------------------|-------------------------|-----------------------------|-----------------------------------|----------------|-----------------------------|-----------------------------------|----------------|-----------------------------|--------------------------------|
|                                |                         |                             | Viability                         | Fertility      | Adult morphology            | Viability                         | Fertility      | Adult morphology            |                                |
| <i>4E-T</i> <sup>A</sup>       | FBal0397362             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 81                             |
| <i>4E-T</i> <sup>D</sup>       | FBal0405951             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 81                             |
| <i>Abcd1</i> <sup>A</sup>      | FBal0397358             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 19                             |
| <i>Actbeta</i> <sup>A</sup>    | FBal0406210             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBal0405952             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 95                             |
| <i>Ank</i> <sup>A</sup>        | FBal0397355             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 10                             |
| <i>anne</i> <sup>B</sup>       | FBal0397356             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 11                             |
| <i>anne</i> <sup>D</sup>       | FBal0404786             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 11                             |
| <i>anne</i> <sup>E</sup>       |                         | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 11                             |
| <i>apolpp</i> <sup>B</sup>     | FBal0392553             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 94                             |
| <i>apolpp</i> <sup>J</sup>     | FBal0404799             | In-frame deletion           | Lethal                            |                |                             | Lethal                            |                |                             | 94                             |
| <i>Arf4</i> <sup>A</sup>       | FBal0405953             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 98                             |
| <i>Arl4</i> <sup>A</sup>       | FBal0406190             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 17                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBal0404781             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 91                             |
| <i>bt</i> <sup>A</sup>         | FBal0400124             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 69                             |
| <i>bt</i> <sup>C</sup>         | FBal0406205             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 69                             |
| <i>Cadps</i> <sup>B</sup>      |                         | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 107                            |
| <i>Cadps</i> <sup>G</sup>      |                         | Molecularly uncharacterized | Lethal                            |                |                             | Lethal                            |                |                             | 107                            |
| <i>CAMK1</i> <sup>F</sup>      | FBal0404780             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 44                             |
| <i>CAMK1</i> <sup>I</sup>      | FBal0405954             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 44                             |
| <i>CG11360</i> <sup>D</sup>    | FBal0404794             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 66                             |
| <i>CG1674</i> <sup>E</sup>     | FBal0405955             | Truncation                  | Lethal                            |                |                             | Viable                            | Female fertile | No overt visible phenotypes | 26                             |
| <i>CG1909</i> <sup>A</sup>     | FBal0406202             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 55                             |
| <i>CG1909</i> <sup>B</sup>     | FBal0405956             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 55                             |
| <i>CG31998</i> <sup>A</sup>    |                         | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 21                             |
| <i>CG31998</i> <sup>C</sup>    | FBal0405957             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 21                             |
| <i>CG31998</i> <sup>D</sup>    | FBal0405958             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 21                             |
| <i>CG31999</i> <sup>C</sup>    | FBal0406192             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 23                             |
| <i>CG32017</i> <sup>B</sup>    | FBal0406212             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 100                            |
| <i>CG32017</i> <sup>E</sup>    | FBal0405959             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 100                            |
| <i>CG32017</i> <sup>K</sup>    | FBal0405960             | Truncation                  | Partial lethality                 | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 100                            |
| <i>CG33521</i> <sup>C</sup>    | FBal0404798             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 103                            |
| <i>CG33978</i> <sup>B</sup>    |                         | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 15                             |
| <i>ci</i> <sup>C</sup>         | FBal0404782             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 5                              |
| <i>ci</i> <sup>F</sup>         | FBal0406189             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 5                              |
| <i>Crk</i> <sup>E</sup>        | FBal0405961             | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 22                             |
| <i>Crk</i> <sup>G</sup>        | FBal0406191             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 22                             |
| <i>dpr7</i> <sup>A</sup>       | FBal0406194             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 27                             |
| <i>Dyrk3</i> <sup>A</sup>      |                         | Molecularly uncharacterized | Lethal                            |                |                             | Lethal                            |                |                             | 106                            |
| <i>Dyrk3</i> <sup>B</sup>      |                         | Molecularly uncharacterized | Lethal                            |                |                             | Lethal                            |                |                             | 106                            |
| <i>Dyrk3</i> <sup>C</sup>      |                         | Molecularly uncharacterized | Lethal                            |                |                             | Lethal                            |                |                             | 106                            |
| <i>elF4G1</i> <sup>B</sup>     |                         | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 79                             |
| <i>elF4G1</i> <sup>E</sup>     |                         | Truncation                  | Lethal                            |                |                             | Lethal                            |                |                             | 79                             |
| <i>Ekar</i> <sup>C</sup>       | FBal0406203             | In-frame deletion           | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 62                             |
| <i>Eph</i> <sup>A</sup>        | FBal0405962             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 58                             |
| <i>Eph</i> <sup>G</sup>        | FBal0404793             | Truncation                  | Viable                            | Female fertile | No overt visible phenotypes | Viable                            | Female fertile | No overt visible phenotypes | 58                             |

|                               |             |                             |        |                               |                             |               |                               |                             |     |
|-------------------------------|-------------|-----------------------------|--------|-------------------------------|-----------------------------|---------------|-------------------------------|-----------------------------|-----|
| <i>Eph<sup>I</sup></i>        |             | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 58  |
| <i>Ephrin<sup>A</sup></i>     | FBal0392555 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 54  |
| <i>ey<sup>A</sup></i>         | FBal0404795 | Truncation                  | Lethal |                               |                             | Mostly lethal |                               | Small or absent eye         | 68  |
| <i>ey<sup>B</sup></i>         | FBal0397354 | Truncation                  | Lethal |                               |                             | Mostly lethal |                               | Small or absent eye         | 68  |
| <i>fd102C<sup>D</sup></i>     | FBal0405963 | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 73  |
| <i>Gyf<sup>B</sup></i>        | FBal0404796 | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 75  |
| <i>Gyf<sup>F</sup></i>        | FBal0405964 | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 75  |
| <i>Hcf<sup>A</sup></i>        | FBal0406198 | In-frame deletion           | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 36  |
| <i>Hcf<sup>C</sup></i>        | FBal0397363 | Truncation                  | Lethal |                               |                             | Lethal        |                               |                             | 36  |
| <i>JYalpha<sup>I</sup></i>    | FBal0404783 | Truncation                  | Viable | Female fertile. Male sterile. | No overt visible phenotypes | Viable        | Female fertile. Male sterile. | No overt visible phenotypes | 1   |
| <i>JYalpha<sup>L</sup></i>    | FBal0406188 | In-frame deletion           | Viable | Female fertile. Male Fertile. | No overt visible phenotypes | Viable        | Female fertile. Male fertile. | No overt visible phenotypes | 1   |
| <i>JYalpha<sup>P</sup></i>    | FBal0404784 | Truncation                  | Viable | Female fertile. Male sterile. | No overt visible phenotypes | Viable        | Female fertile. Male sterile. | No overt visible phenotypes | 1   |
| <i>Kif3C<sup>A</sup></i>      | FBal0404800 | In-frame deletion           | Lethal |                               |                             | Lethal        |                               |                             | 101 |
| <i>lgs<sup>C</sup></i>        | FBal0406199 | In-frame deletion           | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 43  |
| <i>mav<sup>B</sup></i>        | FBal0397361 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 59  |
| <i>mav<sup>D</sup></i>        | FBal0405965 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 59  |
| <i>MED26<sup>A</sup></i>      | FBal0406206 | In-frame deletion           | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 71  |
| <i>mGluR<sup>A</sup></i>      |             | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 80  |
| <i>mGluR<sup>B</sup></i>      | FBal0405966 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 80  |
| <i>mGluR<sup>C</sup></i>      |             | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 80  |
| <i>mGluR<sup>E</sup></i>      | FBal0405967 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 80  |
| <i>Mitf<sup>A</sup></i>       |             | Molecularly uncharacterized | Lethal |                               |                             | Lethal        |                               |                             | 105 |
| <i>Mitf<sup>C</sup></i>       |             | Molecularly uncharacterized | Lethal |                               |                             | Lethal        |                               |                             | 105 |
| <i>myo<sup>A</sup></i>        | FBal0406204 | In-frame deletion           | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 67  |
| <i>myo<sup>C</sup></i>        |             | Truncation                  | Lethal |                               |                             | Lethal        |                               |                             | 67  |
| <i>ND-49<sup>A</sup></i>      | FBal0392556 | In-frame deletion           | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 53  |
| <i>Nfj<sup>A</sup></i>        |             | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 29  |
| <i>Nfj<sup>B</sup></i>        |             | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 29  |
| <i>Nfj<sup>C</sup></i>        |             | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 29  |
| <i>Nfj<sup>D</sup></i>        |             | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 29  |
| <i>Nfj<sup>E</sup></i>        |             | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 29  |
| <i>oncut<sup>G</sup></i>      | FBal0404779 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 57  |
| <i>oncut<sup>H</sup></i>      | FBal0405968 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 57  |
| <i>oncut<sup>I</sup></i>      |             | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 57  |
| <i>pan<sup>F</sup></i>        | FBal0397364 | Truncation                  | Lethal |                               |                             | Lethal        |                               |                             | 9   |
| <i>pan<sup>I</sup></i>        | FBal0404785 | Truncation                  | Lethal |                               |                             | Lethal        |                               |                             | 9   |
| <i>pho<sup>F</sup></i>        | FBal0405969 | Truncation                  | Lethal |                               |                             | Lethal        |                               |                             | 102 |
| <i>PMCA<sup>D</sup></i>       | FBal0404791 | Truncation                  | Lethal |                               |                             | Lethal        |                               |                             | 35  |
| <i>Pur-a<sup>B</sup></i>      | FBal0392554 | Truncation                  | Lethal |                               |                             | Viable        | Female fertile                | No overt visible phenotypes | 52  |
| <i>Rad23<sup>3</sup></i>      | FBal0404788 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 32  |
| <i>Rad23<sup>6</sup></i>      |             | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 32  |
| <i>Rad23<sup>8</sup></i>      | FBal0397360 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 32  |
| <i>Rad23<sup>8</sup></i>      | FBal0406197 | In-frame deletion           | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 32  |
| <i>RhoGAP102A<sup>A</sup></i> | FBal0397359 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 28  |
| <i>RhoGAP102A<sup>B</sup></i> | FBal0406195 | In-frame deletion           | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 28  |
| <i>RhoGAP102A<sup>E</sup></i> | FBal0405970 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 28  |
| <i>Rnf11<sup>A</sup></i>      | FBal0404789 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 34  |
| <i>Rnf11<sup>E</sup></i>      |             | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 34  |
| <i>Rnf11<sup>F</sup></i>      | FBal0404790 | Truncation                  | Viable | Female fertile                | No overt visible phenotypes | Viable        | Female fertile                | No overt visible phenotypes | 34  |

|                              |             |                   |        |                               |                             |        |                               |                             |    |
|------------------------------|-------------|-------------------|--------|-------------------------------|-----------------------------|--------|-------------------------------|-----------------------------|----|
| <i>Slip1</i> <sup>A</sup>    | FBal0392557 | In-frame deletion | Viable | Female fertile. Male sterile. | No overt visible phenotypes | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65 |
| <i>Sox102F</i> <sup>B</sup>  | FBal0406207 | In-frame deletion | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 72 |
| <i>Sox102F</i> <sup>C</sup>  | FBal0400123 | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 72 |
| <i>sv</i> <sup>B</sup>       |             | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 96 |
| <i>sv</i> <sup>F</sup>       | FBal0405971 | In-frame deletion | Lethal |                               |                             | Lethal |                               |                             | 96 |
| <i>Syt7</i> <sup>B</sup>     | FBal0400122 | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 31 |
| <i>Taf3</i> <sup>A</sup>     | FBal0400120 | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 45 |
| <i>Taf3</i> <sup>F</sup>     | FBal0406200 | In-frame deletion | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 45 |
| <i>Tdg</i> <sup>C</sup>      | FBal0404801 | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 50 |
| <i>Tdg</i> <sup>D</sup>      |             | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 50 |
| <i>Tdg</i> <sup>F</sup>      | FBal0405972 | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 50 |
| <i>Tdg</i> <sup>I</sup>      | FBal0406201 | In-frame deletion | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 50 |
| <i>toy</i> <sup>A</sup>      | FBal0404797 | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 86 |
| <i>toy</i> <sup>B</sup>      | FBal0406209 | In-frame deletion | Lethal |                               |                             | Lethal |                               |                             | 86 |
| <i>toy</i> <sup>C</sup>      | FBal0400121 | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 86 |
| <i>ukar</i> <sup>D</sup>     | FBal0406211 | In-frame deletion | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 99 |
| <i>unc-13</i> <sup>C</sup>   | FBal0406208 | In-frame deletion | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 76 |
| <i>yellow-h</i> <sup>A</sup> | FBal0397357 | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 24 |
| <i>yellow-h</i> <sup>D</sup> | FBal0404787 | Truncation        | Viable | Female fertile                | No overt visible phenotypes | Viable | Female fertile                | No overt visible phenotypes | 24 |
| <i>zfh2</i> <sup>3A</sup>    |             | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 49 |
| <i>zfh2</i> <sup>51A</sup>   | FBal0392552 | In-frame deletion | Lethal |                               |                             | Lethal |                               |                             | 49 |
| <i>zfh2</i> <sup>A</sup>     | FBal0405973 | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 49 |
| <i>zfh2</i> <sup>D</sup>     | FBal0404792 | Truncation        | Lethal |                               |                             | Lethal |                               |                             | 49 |
| <i>Zyx</i> <sup>D</sup>      |             | Truncation        | Lethal |                               |                             | Viable | Female fertile                | No overt visible phenotypes | 93 |

<sup>a</sup> FlyBase IDs have not yet been assigned to some alleles.

<sup>b</sup> Some escapers may be present for mutations with lethal phenotypes

<sup>c</sup> This column may be used to sort alleles/rows by the cytological order of the genes mutated (see Supplementary Table 1)

Supplementary Table 11. Evidence for loss-of-function phenotypes of fourth chromosome genes

| Gene                 | FlyBase ID  | Type           | Phenotypes                                          | Evidence for phenotypes from previous publications <sup>a</sup>                                                                                                                 | Evidence for phenotypes from this study                                                                                                                                                                                                                                                                                                                         | Cytological order <sup>b</sup> |
|----------------------|-------------|----------------|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Protein-coding genes |             |                |                                                     |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                 |                                |
| <i>4E-T</i>          | FBgn0052016 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation alleles <i>4E-T</i> <sup>A</sup> or <i>4E-T</i> <sup>B</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                       | 81                             |
| <i>Abcd1</i>         | FBgn0039890 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation allele <i>Abcd1</i> <sup>A</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                   | 19                             |
| <i>Actbeta</i>       | FBgn0024913 | Protein coding | Lethality                                           | Zhu <i>et al.</i> (2008) showed that an <i>Actbeta</i> truncation allele was homozygous lethal.                                                                                 | Hemizygotes for the truncation allele <i>Actbeta</i> <sup>B</sup> are lethal.                                                                                                                                                                                                                                                                                   | 95                             |
| <i>Ank</i>           | FBgn0011747 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Homozygotes for the coding-exon insertion <i>Mi[ET1]Ank</i> <sup>M009683</sup> (Bellen <i>et al.</i> , 2011) show no obvious phenotypes. Hemizygotes for the truncation allele <i>Ank</i> <sup>A</sup> showed no obvious phenotypes.                                                                                                                            | 10                             |
| <i>anne</i>          | FBgn0052000 | Protein coding | Lethality                                           |                                                                                                                                                                                 | Hemizygotes for the <i>Ti[CRIMIC.TG4.1]anne</i> <sup>CR70036-TG4.1</sup> insertion (Kanca <i>et al.</i> , 2022) or the truncation alleles <i>anne</i> <sup>B</sup> , <i>anne</i> <sup>C</sup> and <i>anne</i> <sup>E</sup> are lethal.                                                                                                                          | 11                             |
| <i>apolpp</i>        | FBgn0087002 | Protein coding | Lethality                                           | <i>apolpp</i> null alleles are recessive lethal (Tanaka <i>et al.</i> , 2021).                                                                                                  | Hemizygotes for the truncation allele <i>apolpp</i> <sup>B</sup> or the in-frame deletion allele <i>apolpp</i> <sup>I</sup> are homozygous lethal.                                                                                                                                                                                                              | 94                             |
| <i>Arf4</i>          | FBgn0013749 | Protein coding | Lethality                                           |                                                                                                                                                                                 | Hemizygotes for the <i>Ti[KozakGAL4]Arf4</i> <sup>CR71035-KO-4G4</sup> (Kanca <i>et al.</i> , 2022) insertion or the truncation allele <i>Arf4</i> <sup>A</sup> are lethal.                                                                                                                                                                                     | 98                             |
| <i>Arl4</i>          | FBgn0039889 | Protein coding | Unknown                                             |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                 | 17                             |
| <i>Asator</i>        | FBgn0039908 | Protein coding | Lethality                                           | Qi <i>et al.</i> (2009) showed that an intragenic deletion allele of <i>Asator</i> was recessive lethal.                                                                        |                                                                                                                                                                                                                                                                                                                                                                 | 48                             |
| <i>ATPSynbeta</i>    | FBgn0010217 | Protein coding | Lethality                                           |                                                                                                                                                                                 | Hemizygotes for the <i>P[Supor-P]ATPSynbeta</i> <sup>K007618</sup> insertion (Bellen <i>et al.</i> , 2004) or the truncation allele <i>ATPSynbeta</i> <sup>C</sup> are lethal.                                                                                                                                                                                  | 91                             |
| <i>bt</i>            | FBgn0005666 | Protein coding | Lethality                                           | Ayme-Southgate <i>et al.</i> (1995) identified lethal <i>bt</i> mutations molecularly.                                                                                          | Hemizygotes for the truncation allele <i>bt</i> <sup>A</sup> are lethal.                                                                                                                                                                                                                                                                                        | 69                             |
| <i>Cadps</i>         | FBgn0053653 | Protein coding | Lethality                                           | Renden <i>et al.</i> (2001) showed a null <i>Cadps</i> allele was embryonic lethal.                                                                                             | Hemizygotes for the <i>Mi[DH.1]Cadps</i> <sup>M005877-DH.GT-TG4.1</sup> insertion (Stinchfield <i>et al.</i> , 2024), the coding-exon <i>Mi[MIC]Cadps</i> <sup>M08448</sup> insertion (Nagarkar-Jaiswal <i>et al.</i> , 2015), the truncation allele <i>Cadps</i> <sup>B</sup> and the molecularly uncharacterized allele <i>Cadps</i> <sup>D</sup> are lethal. | 107<br>97                      |
| <i>Cals</i>          | FBgn0039928 | Protein coding | Unknown                                             |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                 |                                |
| <i>CaMKI</i>         | FBgn0016126 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation alleles <i>CaMKI</i> <sup>F</sup> or <i>CaMKI</i> <sup>I</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                     | 44                             |
| <i>CaMKII</i>        | FBgn0264607 | Protein coding | Lethality                                           | Johnson <i>et al.</i> (2002) documented that a <i>CaMKII</i> mutation was recessive lethal.                                                                                     |                                                                                                                                                                                                                                                                                                                                                                 | 92                             |
| <i>CG1674</i>        | FBgn0039897 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation allele <i>CG1674</i> <sup>E</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                  | 26                             |
| <i>CG1909</i>        | FBgn0039911 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation allele <i>CG1909</i> <sup>B</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                  | 55                             |
| <i>CG11360</i>       | FBgn0039920 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation allele <i>CG11360</i> <sup>D</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                 | 66                             |
| <i>CG31997</i>       | FBgn0051997 | Protein coding | Unknown                                             |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                 | 14                             |
| <i>CG31998</i>       | FBgn0051998 | Protein coding | Lethality                                           |                                                                                                                                                                                 | Hemizygotes for the <i>Mi[MIC]CG31998</i> <sup>M02727</sup> or <i>Mi[MIC]CG31998</i> <sup>M06163</sup> insertions (Nagarkar-Jaiswal <i>et al.</i> , 2015) or the truncation alleles <i>CG31998</i> <sup>A</sup> , <i>CG31998</i> <sup>C</sup> or <i>CG31998</i> <sup>B</sup> are lethal.                                                                        | 21<br>23                       |
| <i>CG31999</i>       | FBgn0051999 | Protein coding | Unknown                                             |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                 |                                |
| <i>CG32006</i>       | FBgn0052006 | Protein coding | Lethality                                           |                                                                                                                                                                                 | Hemizygotes for the <i>Ti[SA-KozakGAL4]CG32006</i> <sup>CR70476-4G4</sup> insertion (Kanca <i>et al.</i> , 2022) are lethal.                                                                                                                                                                                                                                    | 13                             |
| <i>CG32017</i>       | FBgn0052017 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation alleles <i>CG32017</i> <sup>E</sup> or <i>CG32017</i> <sup>K</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                 | 100                            |
| <i>CG33521</i>       | FBgn0250819 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Hemizygotes for the truncation allele <i>CG33521</i> <sup>C</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                 | 103                            |
| <i>CG33941</i>       | FBgn0262731 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Homozygotes for the coding-exon insertions <i>PBac[RB]CG33941</i> <sup>402647</sup> or <i>P[XP]CG33941</i> <sup>409357</sup> (Thibault <i>et al.</i> , 2004) show no obvious phenotypes.                                                                                                                                                                        | 47                             |
| <i>CG33978</i>       | FBgn0053978 | Protein coding | Lethality                                           |                                                                                                                                                                                 | Homozygotes and hemizygotes for the coding-exon insertions <i>Mi[MIC]CG33978</i> <sup>M08362</sup> and <i>Mi[MIC]CG33978</i> <sup>M03508</sup> (Nagarkar-Jaiswal <i>et al.</i> , 2015) are lethal. Hemizygotes for the insertion <i>Ti[CRIMIC.TG4.1]CG33978</i> <sup>CR70478-TG4.1</sup> and the truncation mutation <i>CG33978</i> <sup>B</sup> are lethal.    | 15<br>64                       |
| <i>CG46466</i>       | FBgn0287597 | Protein coding | Unknown                                             |                                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                                 |                                |
| <i>ci</i>            | FBgn0004859 | Protein coding | Lethality                                           | Locke <i>et al.</i> (1994) mapped recessive lethal <i>ci</i> mutations map to the <i>ci</i> gene.                                                                               | Hemizygotes for the truncation allele <i>ci</i> <sup>C</sup> are lethal.                                                                                                                                                                                                                                                                                        | 5                              |
| <i>Crk</i>           | FBgn0024811 | Protein coding | Lethality                                           | Ishimaru <i>et al.</i> (2004) and Spracklen <i>et al.</i> (2019) showed that loss-of-function <i>Crk</i> alleles are recessive lethal.                                          | Hemizygotes for the truncation allele <i>Crk</i> <sup>E</sup> are lethal.                                                                                                                                                                                                                                                                                       | 22                             |
| <i>dati</i>          | FBgn0262636 | Protein coding | Lethality with uncoordinated escapers               | Schinaman <i>et al.</i> (2014) showed that <i>dati</i> <sup>K002689</sup> and <i>dati</i> <sup>K001667</sup> are recessive lethal; escapers are uncoordinated & female sterile. |                                                                                                                                                                                                                                                                                                                                                                 | 37                             |
| <i>dpr7</i>          | FBgn0053481 | Protein coding | Viable, female fertile, no overt visible phenotypes |                                                                                                                                                                                 | Homozygotes and hemizygotes for the coding-exon insertion <i>Mi[MIC]dpr7</i> <sup>M07186</sup> (Nagarkar-Jaiswal <i>et al.</i> , 2015) show no obvious phenotypes.                                                                                                                                                                                              | 27                             |

|                |                            |                                                      |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |     |
|----------------|----------------------------|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <i>Dyrk3</i>   | FBgn0027101 Protein coding | Lethality                                            |                                                                                                                                                                                                                                                    | Hemizygotes for the molecularly uncharacterized alleles <i>Dyrk3</i> <sup>A</sup> , <i>Dyrk3</i> <sup>B</sup> or <i>Dyrk3</i> <sup>C</sup> are recessive lethal.                                                                                                                                                                                                                                                                                                                                    | 106 |
| <i>elF4G1</i>  | FBgn0023213 Protein coding | Lethality                                            |                                                                                                                                                                                                                                                    | Hemizygotes for the <i>Mi</i> [ <i>Trojan-GAL4.0</i> ]elF4G1 <sup>M105019-TG4.0</sup> insertion (Lee <i>et al.</i> , 2018) or the truncation alleles elF4G1 <sup>B</sup> or elF4G1 <sup>C</sup> are lethal.                                                                                                                                                                                                                                                                                         | 79  |
| <i>Ekar</i>    | FBgn0039916 Protein coding | Viable, female fertile, no overt visible phenotypes  | Hu <i>et al.</i> (2015) showed that homozygotes of the null <i>Ekar</i> <sup>M800001</sup> allele are viable, but have neurophysiological defects.                                                                                                 | Homozygotes and hemizygotes for <i>Ekar</i> <sup>M800001</sup> (Bellen <i>et al.</i> , 2011) are viable and fertile and show no obvious visible phenotypes.                                                                                                                                                                                                                                                                                                                                         | 62  |
| <i>Eph</i>     | FBgn0025936 Protein coding | Viable, female fertile, no overt visible phenotypes  | Boyle <i>et al.</i> (2006) showed that a null <i>Eph</i> allele was homozygous viable and fertile.                                                                                                                                                 | Hemizygotes for the truncation alleles <i>Eph</i> <sup>A</sup> , <i>Eph</i> <sup>B</sup> or <i>Eph</i> <sup>C</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                                                                                                   | 58  |
| <i>Ephrin</i>  | FBgn0040324 Protein coding | Viable, female fertile, no overt visible phenotypes  |                                                                                                                                                                                                                                                    | Hemizygotes for the truncation allele <i>Ephrin</i> <sup>A</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                                                                                                                                                      | 54  |
| <i>ey</i>      | FBgn0005558 Protein coding | Reduced or absent eyes, reduced viability            | Quiring <i>et al.</i> (1994) showed that homozygotes for loss-of-function <i>ey</i> mutations have small eyes. Callaerts <i>et al.</i> (2001) showed homozygous and hemizygous null <i>ey</i> alleles lead to reduced, but not complete lethality. | Hemizygotes for the truncation alleles <i>ey</i> <sup>A</sup> and <i>ey</i> <sup>B</sup> are mostly lethal. Escapers have reduced eyes.                                                                                                                                                                                                                                                                                                                                                             | 68  |
| <i>fd102C</i>  | FBgn0039937 Protein coding | Viable, female fertile, no overt visible phenotypes  |                                                                                                                                                                                                                                                    | Homozygotes and hemizygotes for the intragenic deletion <i>Ti</i> [ <i>KozakGAL4</i> ]fd102C <sup>CR70484-KO-ΔG4</sup> (Kanca <i>et al.</i> , 2022) and hemizygotes for the truncation allele fd102C <sup>D</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                     | 73  |
| <i>fuss</i>    | FBgn0039932 Protein coding | Viable, female fertile, no overt visible phenotypes  | Takaesu <i>et al.</i> (2012) showed that a multigene deletion encompassing <i>fuss</i> is homozygous viable. Rass <i>et al.</i> (2019) showed that the intragenic deletion <i>fuss</i> <sup>6625</sup> is homozygous viable.                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 84  |
| <i>Gat</i>     | FBgn0039915 Protein coding | Lethality                                            | Stork <i>et al.</i> (2014) showed that the intragenic deletion <i>Gat</i> <sup>22-1</sup> is embryonic lethal.                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 61  |
| <i>gw</i>      | FBgn0051992 Protein coding | Lethality                                            | Schneider <i>et al.</i> (2006) showed that the truncation allele <i>gw</i> <sup>1</sup> is zygotic lethal.                                                                                                                                         | Hemizygotes for the insertion <i>Ti</i> [ <i>CRIMIC.TG4.0</i> ]gw <sup>CR70482-TG4.0</sup> (Kanca <i>et al.</i> , 2022) are lethal.                                                                                                                                                                                                                                                                                                                                                                 | 63  |
| <i>Gyf</i>     | FBgn0039936 Protein coding | Viable, female fertile, no overt visible phenotypes  | Kim <i>et al.</i> (2015) showed adults homozygotes for the null, coding-exon insertion <i>Mi</i> ( <i>MC</i> ) <i>Gyf</i> <sup>M100455</sup> are viable, but short-lived.                                                                          | Hemizygotes for <i>Gyf</i> <sup>M100455</sup> (Nagarkar-Jaiswal <i>et al.</i> , 2015) and the truncation alleles <i>Gyf</i> <sup>B</sup> and <i>Gyf</i> <sup>C</sup> are viable and female fertile and show no obvious visible phenotypes.                                                                                                                                                                                                                                                          | 75  |
| <i>Hcf</i>     | FBgn0039904 Protein coding | Reduced viability, female sterility                  | Rodríguez-Jato <i>et al.</i> (2011) showed that homozygotes for a <i>Hcf</i> intragenic deletion are female sterile and have reduced viability.                                                                                                    | Hemizygotes for the truncation allele <i>Hcf</i> <sup>C</sup> are lethal.                                                                                                                                                                                                                                                                                                                                                                                                                           | 36  |
| <i>JYalpha</i> | FBgn0267363 Protein coding | Male sterility                                       | Masly <i>et al.</i> (2006) showed that disruption of <i>JYalpha</i> results in male sterility.                                                                                                                                                     | Hemizygotes for the truncation alleles <i>JYalpha</i> <sup>1</sup> or <i>JYalpha</i> <sup>B</sup> are recessive male sterile.                                                                                                                                                                                                                                                                                                                                                                       | 1   |
| <i>Kif3C</i>   | FBgn0039925 Protein coding | Lethality                                            |                                                                                                                                                                                                                                                    | Hemizygotes for the internal deletion allele <i>Kif3C</i> <sup>A</sup> are lethal.                                                                                                                                                                                                                                                                                                                                                                                                                  | 101 |
| <i>lgs</i>     | FBgn0039907 Protein coding | Lethality                                            | Kramps <i>et al.</i> (2002) showed that <i>lgs</i> point mutations are recessive lethal.                                                                                                                                                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 43  |
| <i>mav</i>     | FBgn0039914 Protein coding | Viable, female fertile, no overt visible phenotypes  | Hoyer <i>et al.</i> (2018) showed the intragenic deletion <i>mav</i> <sup>ko</sup> is homozygous viable and fertile.                                                                                                                               | Hemizygotes for the truncation alleles <i>mav</i> <sup>A</sup> or <i>mav</i> <sup>D</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                                                                                                                             | 59  |
| <i>MED26</i>   | FBgn0039923 Protein coding | Lethality                                            | Marr <i>et al.</i> (2014) showed that the coding-exon insertion <i>Mi</i> ( <i>ET1</i> ) <i>MED26</i> <sup>M800704</sup> and imprecise-excision derivatives are recessive lethal.                                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 71  |
| <i>mGluR</i>   | FBgn0019985 Protein coding | Viable, female fertile, no overt visible phenotypes  | Bogdanik <i>et al.</i> (2004) showed the intragenic deletion <i>mGluR</i> <sup>112b</sup> is homozygous viable and fertile.                                                                                                                        | Hemizygotes for the truncation alleles <i>mGluR</i> <sup>A</sup> , <i>mGluR</i> <sup>B</sup> , <i>mGluR</i> <sup>C</sup> or <i>mGluR</i> <sup>S</sup> showed no obvious phenotypes.                                                                                                                                                                                                                                                                                                                 | 80  |
| <i>Mitf</i>    | FBgn0263112 Protein coding | Lethality                                            | Zhang <i>et al.</i> (2015) shows that <i>Mitf</i> loss-of-function mutations are recessive lethal.                                                                                                                                                 | Hemizygotes for the molecularly uncharacterized alleles <i>Mitf</i> <sup>A</sup> and <i>Mitf</i> <sup>C</sup> alleles are lethal.                                                                                                                                                                                                                                                                                                                                                                   | 105 |
| <i>Mpv17</i>   | FBgn0039930 Protein coding | Viable, female fertile, no overt visible phenotypes  | Corrà <i>et al.</i> (2023) showed that homozygotes for a null <i>Mpv17</i> allele are viable, but have altered energy metabolism.                                                                                                                  | Individuals carrying both <i>Df</i> (4) <i>CRIMIC-CR70944</i> (Kanca <i>et al.</i> , 2022), which deletes both <i>Mpv17</i> and <i>asRNA:CR44031</i> , and other deletions for the region are viable, female fertile and show no obvious phenotypes.                                                                                                                                                                                                                                                | 89  |
| <i>myo</i>     | FBgn0026199 Protein coding | Lethality                                            |                                                                                                                                                                                                                                                    | Hemizygotes for the <i>Ti</i> ( <i>CRIMIC.TG4.1</i> )myo <sup>CR02262-TG4.1</sup> insertion (Kanca <i>et al.</i> , 2022) or the truncation allele myo <sup>C</sup> are lethal.                                                                                                                                                                                                                                                                                                                      | 67  |
| <i>ND-49</i>   | FBgn0039909 Protein coding | Unknown                                              |                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 53  |
| <i>Nfl</i>     | FBgn0042696 Protein coding | Viable, female fertile, no overt visible phenotypes  |                                                                                                                                                                                                                                                    | Hemizygotes for the truncation alleles <i>Nfl</i> <sup>A</sup> , <i>Nfl</i> <sup>B</sup> , <i>Nfl</i> <sup>C</sup> , <i>Nfl</i> <sup>D</sup> and <i>Nfl</i> <sup>E</sup> show no obvious phenotypes.                                                                                                                                                                                                                                                                                                | 29  |
| <i>onecut</i>  | FBgn0028996 Protein coding | Lethality                                            | Boyle <i>et al.</i> (2006) showed that loss-of-function <i>onecut</i> alleles removing upstream regulatory sequences and the transcription start site are recessive lethal.                                                                        | Homozygotes and hemizygotes for <i>onecut</i> <sup>E</sup> , <i>onecut</i> <sup>H</sup> and <i>onecut</i> <sup>I</sup> show no obvious phenotypes even though the mutations should result in <i>onecut</i> proteins being truncated to 11% of their normal length. We do not understand the disagreement between our results and those of Boyle <i>et al.</i> (2006). Perhaps differences in genetic background determine the viability versus lethality of <i>onecut</i> loss-of-function alleles. | 57  |
| <i>pan</i>     | FBgn0085432 Protein coding | Lethality                                            | van de Wetering <i>et al.</i> (1997), Brunner <i>et al.</i> (1997) and Kronhamn & Rasmuson-Lestander (1999) showed that loss-of-function <i>pan</i> alleles are recessive lethal.                                                                  | Hemizygotes for the truncation alleles <i>pan</i> <sup>A</sup> or <i>pan</i> <sup>I</sup> are lethal.                                                                                                                                                                                                                                                                                                                                                                                               | 9   |
| <i>pho</i>     | FBgn0002521 Protein coding | Lethality                                            | Brown <i>et al.</i> (1998) showed that loss-of-function <i>pho</i> alleles are recessive lethal.                                                                                                                                                   | Hemizygotes for the truncation allele <i>pho</i> <sup>F</sup> are lethal.                                                                                                                                                                                                                                                                                                                                                                                                                           | 102 |
| <i>PIP4K</i>   | FBgn0039924 Protein coding | Viable, but low body weight and delayed development. | Gupta <i>et al.</i> (2013) showed that homozygotes for a null <i>PIP4K</i> allele have low body weight and delayed development (relative viability was not stated, but is presumably reduced).                                                     | Hemizygotes for the <i>Ti</i> ( <i>CRIMIC.TG4.0</i> )PIP4K <sup>CR70484-TG4.0</sup> insertion (Kanca <i>et al.</i> , 2022) are lethal.                                                                                                                                                                                                                                                                                                                                                              | 104 |
| <i>PlexA</i>   | FBgn0025741 Protein coding | Lethality                                            |                                                                                                                                                                                                                                                    | Hemizygotes for the <i>Mi</i> ( <i>ET1</i> ) <i>PlexA</i> <sup>M800499</sup> (Bellen <i>et al.</i> , 2011) insertion are lethal.                                                                                                                                                                                                                                                                                                                                                                    | 87  |
| <i>PlexB</i>   | FBgn0025740 Protein coding | Lethality                                            | Ayoob <i>et al.</i> (2006) showed that the <i>P</i> [ <i>SUPor-P</i> ]PlexB <sup>K600878</sup> insertion is homozygous semilethal and hemizygous lethal.                                                                                           | Hemizygotes for the <i>Mi</i> ( <i>DH.1</i> )PlexB <sup>M115559-DH.PF-GPSTE.1</sup> insertion (Stinchfield <i>et al.</i> , 2024) are lethal.                                                                                                                                                                                                                                                                                                                                                        | 2   |

|                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                         |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                |                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| <i>PMCA</i>                                                                                                                                                                                                                                                                                                                                                                                                                       | FBgn0259214 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Lethality                                                                                                                                                                               |                                                                                                                                                                                                                                                                   | Hemizygotes for the <i>Mi(MIC)PMCA</i> <sup>MI12515</sup> (Nagarkar-Jaiswal <i>et al.</i> , 2015) or <i>Mi(Trajan-GAL4.2)PMCA</i> <sup>MI12515-TG4.2</sup> (Lee <i>et al.</i> , 2018) insertions or the truncation allele <i>PMCA</i> <sup>D</sup> are lethal. | 35                                                                                               |
| <i>Polr1G</i>                                                                                                                                                                                                                                                                                                                                                                                                                     | FBgn0039929 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the intragenic deletion <i>Ti(KozakGAL4)Polr1G</i> <sup>CR70943-KO-NG4</sup> (Kanca <i>et al.</i> , 2022) showed no obvious phenotypes.                                                                                                        | 90                                                                                               |
| <i>Pur-alpha</i>                                                                                                                                                                                                                                                                                                                                                                                                                  | FBgn0022361 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Homozygotes and hemizygotes for the intragenic deletion <i>Pur-alpha</i> <sup>CR71382-KO-NG4</sup> (Kanca <i>et al.</i> , 2022) and hemizygotes for the truncation allele <i>Pur-alpha</i> <sup>B</sup> showed no obvious phenotypes.                          | 52                                                                                               |
| <i>Rad23</i>                                                                                                                                                                                                                                                                                                                                                                                                                      | FBgn0026777 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the truncation alleles <i>Rad23</i> <sup>3</sup> , <i>Rad23</i> <sup>6</sup> or <i>Rad23</i> <sup>8</sup> showed no obvious phenotypes.                                                                                                        | 32                                                                                               |
| <i>RhoGAP102A</i>                                                                                                                                                                                                                                                                                                                                                                                                                 | FBgn0259216 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the truncation alleles <i>RhoGap102A</i> <sup>A</sup> or <i>RhoGap102A</i> <sup>F</sup> showed no obvious phenotypes.                                                                                                                          | 28                                                                                               |
| <i>Rnf11</i>                                                                                                                                                                                                                                                                                                                                                                                                                      | FBgn0052850 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the truncation alleles <i>Rnf11</i> <sup>A</sup> , <i>Rnf11</i> <sup>E</sup> or <i>Rnf11</i> <sup>F</sup> showed no obvious phenotypes.                                                                                                        | 34                                                                                               |
| <i>RpS3A</i>                                                                                                                                                                                                                                                                                                                                                                                                                      | FBgn0017545 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Haploinsufficient Minute, recessive lethality                                                                                                                                           | Hochman <i>et al.</i> (1964) showed that the Minute mutation <i>RpS3A</i> <sup>179</sup> is homozygous and hemizygous lethal. Kronhamn and Rasmuson-Lestander (1999) showed that <i>RpS3A</i> <sup>179</sup> is a transposon insertion upstream of <i>RpS3A</i> . |                                                                                                                                                                                                                                                                | 7<br>65                                                                                          |
| <i>Slip1</i>                                                                                                                                                                                                                                                                                                                                                                                                                      | FBgn0024728 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Unknown                                                                                                                                                                                 |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                |                                                                                                  |
| <i>Sox102F</i>                                                                                                                                                                                                                                                                                                                                                                                                                    | FBgn0039938 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     | Li <i>et al.</i> (2017) showed that RNAi-mediated silencing of <i>Sox102F</i> leads to a variety of morphological, physiological and behavioral defects as well as neurodegeneration, but not full lethality or female sterility.                                 | Hemizygotes for the truncation allele <i>Sox102F</i> <sup>C</sup> were homozygous viable and female fertile and showed no obvious phenotypes.                                                                                                                  | 72                                                                                               |
| <i>sv</i>                                                                                                                                                                                                                                                                                                                                                                                                                         | FBgn0005561 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Lethality                                                                                                                                                                               | Kavaler <i>et al.</i> (1999) showed that strong loss-of-function <i>sv</i> alleles are recessive lethal.                                                                                                                                                          | Hemizygotes for the truncation allele <i>sv</i> <sup>B</sup> or the internal deletion allele <i>sv</i> <sup>F</sup> are lethal.                                                                                                                                | 96                                                                                               |
| <i>Syt7</i>                                                                                                                                                                                                                                                                                                                                                                                                                       | FBgn0039900 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     | Guan <i>et al.</i> (2020) saw no obvious phenotypes in <i>Syt7</i> null mutants.                                                                                                                                                                                  | Hemizygotes for the truncation allele <i>Syt7</i> <sup>B</sup> showed no obvious phenotypes                                                                                                                                                                    | 31                                                                                               |
| <i>Taf3</i>                                                                                                                                                                                                                                                                                                                                                                                                                       | FBgn0026262 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Lethality                                                                                                                                                                               | Prince <i>et al.</i> (2008) showed that the intragenic deletions <i>Taf3</i> <sup>3</sup> and <i>Taf3</i> <sup>4</sup> are late embryonic lethals.                                                                                                                | Hemizygotes for the truncation allele <i>Taf3</i> <sup>A</sup> are lethal.                                                                                                                                                                                     | 45                                                                                               |
| <i>Tdg</i>                                                                                                                                                                                                                                                                                                                                                                                                                        | FBgn0026869 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the truncation alleles <i>Tdg</i> <sup>C</sup> , <i>Tdg</i> <sup>D</sup> or <i>Tdg</i> <sup>F</sup> showed no obvious phenotypes.                                                                                                              | 50                                                                                               |
| <i>toy</i>                                                                                                                                                                                                                                                                                                                                                                                                                        | FBgn0019650 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Lethality                                                                                                                                                                               | Kronhamn <i>et al.</i> (2002) showed that deletion of <i>toy</i> coding sequences results in lethality.                                                                                                                                                           | Hemizygotes for the truncation alleles <i>toy</i> <sup>A</sup> or <i>toy</i> <sup>C</sup> are lethal.                                                                                                                                                          | 86                                                                                               |
| <i>ukar</i>                                                                                                                                                                                                                                                                                                                                                                                                                       | FBgn0039927 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     | Nguyen <i>et al.</i> (2024) showed that homozygotes for a null <i>ukar</i> allele are viable and fertile, but have mild locomotory defects.                                                                                                                       |                                                                                                                                                                                                                                                                | 99                                                                                               |
| <i>unc-13</i>                                                                                                                                                                                                                                                                                                                                                                                                                     | FBgn0025726 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Lethality                                                                                                                                                                               | Aravamudan <i>et al.</i> (1999) showed that <i>unc-13</i> <sup>P84200</sup> is a null, embryonic-lethal mutation.                                                                                                                                                 |                                                                                                                                                                                                                                                                | 76                                                                                               |
| <i>yellow-h</i>                                                                                                                                                                                                                                                                                                                                                                                                                   | FBgn0039896 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the truncation alleles <i>yellow-h</i> <sup>A</sup> or <i>yellow-h</i> <sup>D</sup> show no obvious phenotypes.                                                                                                                                | 24                                                                                               |
| <i>zfh2</i>                                                                                                                                                                                                                                                                                                                                                                                                                       | FBgn0004607 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Lethality                                                                                                                                                                               | Guntur <i>et al.</i> (2021) showed that <i>zfh2</i> <sup>MI12</sup> , which deletes a <i>zfh2</i> upstream regulatory region, is embryonic lethal.                                                                                                                | Hemizygotes for the truncation alleles <i>zfh2</i> <sup>A</sup> , <i>zfh2</i> <sup>D</sup> and <i>zfh2</i> <sup>3A</sup> and the in-frame deletion allele <i>zfh2</i> <sup>31A</sup> are lethal.                                                               | 49                                                                                               |
| <i>Zip102B</i>                                                                                                                                                                                                                                                                                                                                                                                                                    | FBgn0039902 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Hemizygotes for the intragenic deletion <i>Zip102B</i> <sup>CR70486-KO-TG4.0</sup> (Kanca <i>et al.</i> , 2022) show no obvious phenotypes.                                                                                                                    | 33                                                                                               |
| <i>Zyx</i>                                                                                                                                                                                                                                                                                                                                                                                                                        | FBgn0011642 Protein coding                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Viable, female fertile, no overt visible phenotypes                                                                                                                                     | Gaspar <i>et al.</i> (2015) showed that the truncation allele <i>Zyx</i> <sup>CR1041</sup> resulted in no antibody-detectable protein and small, fertile adults.                                                                                                  | Hemizygotes for the truncation allele <i>Zyx</i> <sup>D</sup> showed no obvious phenotypes.                                                                                                                                                                    | 93<br>0                                                                                          |
| <i>asRNA:CR43425</i><br><i>asRNA:CR43957</i><br><i>asRNA:CR44027</i><br><i>asRNA:CR44029</i><br><i>asRNA:CR44030</i>                                                                                                                                                                                                                                                                                                              | FBgn0263344 Noncoding RNA<br>FBgn0264617 Noncoding RNA<br>FBgn0264819 Noncoding RNA<br>FBgn0264821 Noncoding RNA<br>FBgn0264822 Noncoding RNA                                                                                                                                                                                                                                                                                                                                                             | Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown                                                                                                                                     | <b>Noncoding RNA genes</b>                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                | 77<br>6<br>39<br>78<br>83                                                                        |
| <i>asRNA:CR44031</i>                                                                                                                                                                                                                                                                                                                                                                                                              | FBgn0264823 Noncoding RNA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Viable, female fertile, no overt visible phenotypes                                                                                                                                     |                                                                                                                                                                                                                                                                   | Individuals carrying both <i>Df(4)CRIMIC-CR70944</i> (Kanca <i>et al.</i> , 2022), which deletes both <i>Mpv17</i> and <i>asRNA:CR44031</i> , and other deletions for the region are viable, female fertile and show no obvious phenotypes.                    | 88                                                                                               |
| <i>asRNA:CR45124</i><br><i>asRNA:CR45126</i><br><i>asRNA:CR45198</i><br><i>asRNA:CR46065</i><br><i>lncRNA:CR43437</i><br><i>lncRNA:CR43956</i><br><i>lncRNA:CR43958</i><br><i>lncRNA:CR44023</i><br><i>lncRNA:CR44024</i><br><i>lncRNA:CR44440</i><br><i>lncRNA:CR44441</i><br><i>lncRNA:CR45123</i><br><i>lncRNA:CR45125</i><br><i>lncRNA:CR45199</i><br><i>lncRNA:CR45200</i><br><i>lncRNA:CR45201</i><br><i>lncRNA:CR46488</i> | FBgn0266617 Noncoding RNA<br>FBgn0266619 Noncoding RNA<br>FBgn0266725 Noncoding RNA<br>FBgn0267734 Noncoding RNA<br>FBgn0263385 Noncoding RNA<br>FBgn0264616 Noncoding RNA<br>FBgn0264618 Noncoding RNA<br>FBgn0264793 Noncoding RNA<br>FBgn0264794 Noncoding RNA<br>FBgn0265633 Noncoding RNA<br>FBgn0265634 Noncoding RNA<br>FBgn0266616 Noncoding RNA<br>FBgn0266618 Noncoding RNA<br>FBgn0266726 Noncoding RNA<br>FBgn0266727 Noncoding RNA<br>FBgn0266728 Noncoding RNA<br>FBgn0287619 Noncoding RNA | Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown<br>Unknown |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                | 3<br>46<br>16<br>25<br>41<br>12<br>51<br>30<br>42<br>4<br>20<br>74<br>38<br>56<br>70<br>82<br>18 |

|                     |                           |                         |                                                                                                                                                                                                                                  |    |
|---------------------|---------------------------|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <i>lncRNA:spinx</i> | FBgn0083990 Noncoding RNA | Male behavioral defects | Dai <i>et al.</i> (2008) showed that homozygotes for the null allele <i>lncRNA:spinx</i> <sup>720RW</sup> are viable and fertile and have no obvious morphological defects, but homozygous males have abnormal mating behaviors. | 85 |
| <i>mir-954</i>      | FBgn0262302 Noncoding RNA | Unknown                 |                                                                                                                                                                                                                                  | 60 |
| <i>mir-9385</i>     | FBgn0283557 Noncoding RNA | Unknown                 |                                                                                                                                                                                                                                  | 40 |
| <i>snoRNA:CD68</i>  | FBgn0263851 Noncoding RNA | Unknown                 |                                                                                                                                                                                                                                  | 8  |

<sup>a</sup> We have attempted to identify the first publications associating loss-of-function mutations with molecularly defined genes.

<sup>b</sup> This column may be used to sort row/genes by cytological order (see Supplementary Table 1)

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Supplementary Table 12. Crosses to look for off-target mutations on mutagenized fourth chromosomes

|                                | Allele on mutagenized chromosome <sup>a</sup> |                  |                   |            | Deletions and mutations testing as many nontarget genes as possible <sup>b</sup> |             |                  |                     | Phenotype                     |                             | Cytological order <sup>c</sup> |
|--------------------------------|-----------------------------------------------|------------------|-------------------|------------|----------------------------------------------------------------------------------|-------------|------------------|---------------------|-------------------------------|-----------------------------|--------------------------------|
|                                | Allele                                        | FlyBase ID       | Bloomington RRID  | Kyoto RRID | Deletion or mutation                                                             | FlyBase ID  | Stock RRID       | Viability           | Fertility                     | Adult morphology            |                                |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>                                          | FBaI0386736 | RRID:BDSC_97185  | Viable              | Female fertile. Male fertile. | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>PlexB</i> <sup>KGO0878</sup>                                                  | FBaI0216740 | RRID:BDSC_14579  | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>PlexB</i> <sup>M15559-OH.GT-TG4.1</sup>                                       | FBaI0371037 | RRID:BDSC_93694  | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)M101-63a</i>                                                             | FBaB0002961 | RRID:BDSC_1082   | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)ED6366</i>                                                               | FBaB0037590 | RRID:BDSC_8067   | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)ED6369</i>                                                               | FBaB0037593 | RRID:BDSC_9422   | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)5-M244</i>                                                               | FBaB0046981 | RRID:BDSC_84133  | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)5-M263</i>                                                               | FBaB0046980 | RRID:BDSC_84136  | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)ED6380</i>                                                               | FBaB0037604 | RRID:DGGR_150529 | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)ED6382</i>                                                               | FBaB0037606 | RRID:DGGR_150531 | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>Actbeta</i> <sup>B</sup>    | FBaI0405952                                   | RRID:BDSC_605781 | Not yet available |            | <i>Df(4)O2</i>                                                                   | FBaB0029601 | RRID:BDSC_7084   | Viable              | Female fertile                | No overt visible phenotypes | 95                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>                                          | FBaI0386736 | RRID:BDSC_97185  | Viable              | Female fertile. Male fertile. | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>PlexB</i> <sup>KGO0878</sup>                                                  | FBaI0216740 | RRID:BDSC_14579  | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>PlexB</i> <sup>M15559-OH.GT-TG4.1</sup>                                       | FBaI0371037 | RRID:BDSC_93694  | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)M101-63a</i>                                                             | FBaB0002961 | RRID:BDSC_1082   | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)ED6366</i>                                                               | FBaB0037590 | RRID:BDSC_8067   | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)ED6369</i>                                                               | FBaB0037593 | RRID:BDSC_9422   | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)5-M244</i>                                                               | FBaB0046981 | RRID:BDSC_84133  | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)5-M263</i>                                                               | FBaB0046980 | RRID:BDSC_84136  | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)ED6380</i>                                                               | FBaB0037604 | RRID:DGGR_150529 | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)ED6382</i>                                                               | FBaB0037606 | RRID:DGGR_150531 | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>apolpp</i> <sup>B</sup>     | FBaI0392553                                   | RRID:BDSC_600262 | RRID:DGGR_119073  |            | <i>Df(4)O2</i>                                                                   | FBaB0029601 | RRID:BDSC_7084   | Viable              | Female fertile                | No overt visible phenotypes | 94                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>                                          | FBaI0386736 | RRID:BDSC_97185  | Viable              | Female fertile. Male fertile. | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>PlexB</i> <sup>KGO0878</sup>                                                  | FBaI0216740 | RRID:BDSC_14579  | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>PlexB</i> <sup>M15559-OH.GT-TG4.1</sup>                                       | FBaI0371037 | RRID:BDSC_93694  | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)M101-63a</i>                                                             | FBaB0002961 | RRID:BDSC_1082   | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)ED6366</i>                                                               | FBaB0037590 | RRID:BDSC_8067   | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)ED6369</i>                                                               | FBaB0037593 | RRID:BDSC_9422   | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)5-M244</i>                                                               | FBaB0046981 | RRID:BDSC_84133  | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)5-M263</i>                                                               | FBaB0046980 | RRID:BDSC_84136  | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)ED6380</i>                                                               | FBaB0037604 | RRID:DGGR_150529 | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)ED6382</i>                                                               | FBaB0037606 | RRID:DGGR_150531 | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)O2</i>                                                                   | FBaB0029601 | RRID:BDSC_7084   | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>Arf4</i> <sup>A</sup>       | FBaI0405953                                   | RRID:BDSC_605782 | Not yet available |            | <i>Df(4)8-M91</i>                                                                | FBaB0047542 | RRID:BDSC_84135  | Viable              | Female fertile                | No overt visible phenotypes | 98                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>                                          | FBaI0386736 | RRID:BDSC_97185  | Viable              | Female fertile. Male fertile. | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>PlexB</i> <sup>KGO0878</sup>                                                  | FBaI0216740 | RRID:BDSC_14579  | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>PlexB</i> <sup>M15559-OH.GT-TG4.1</sup>                                       | FBaI0371037 | RRID:BDSC_93694  | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)M101-63a</i>                                                             | FBaB0002961 | RRID:BDSC_1082   | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)ED6366</i>                                                               | FBaB0037590 | RRID:BDSC_8067   | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)ED6369</i>                                                               | FBaB0037593 | RRID:BDSC_9422   | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)5-M244</i>                                                               | FBaB0046981 | RRID:BDSC_84133  | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)5-M263</i>                                                               | FBaB0046980 | RRID:BDSC_84136  | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)ED6380</i>                                                               | FBaB0037604 | RRID:DGGR_150529 | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)ED6382</i>                                                               | FBaB0037606 | RRID:DGGR_150531 | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>ATPsynbeta</i> <sup>C</sup> | FBaI0404781                                   | RRID:BDSC_605314 | Not yet available |            | <i>Df(4)O2</i>                                                                   | FBaB0029601 | RRID:BDSC_7084   | Viable              | Female fertile                | No overt visible phenotypes | 91                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>                                          | FBaI0386736 | RRID:BDSC_97185  | Viable              | Female fertile. Male fertile. | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>PlexB</i> <sup>KGO0878</sup>                                                  | FBaI0216740 | RRID:BDSC_14579  | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>PlexB</i> <sup>M15559-OH.GT-TG4.1</sup>                                       | FBaI0371037 | RRID:BDSC_93694  | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)M101-63a</i>                                                             | FBaB0002961 | RRID:BDSC_1082   | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)ED6366</i>                                                               | FBaB0037590 | RRID:BDSC_8067   | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)ED6369</i>                                                               | FBaB0037593 | RRID:BDSC_9422   | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)5-M244</i>                                                               | FBaB0046981 | RRID:BDSC_84133  | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)5-M263</i>                                                               | FBaB0046980 | RRID:BDSC_84136  | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)ED6380</i>                                                               | FBaB0037604 | RRID:DGGR_150529 | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)ED6382</i>                                                               | FBaB0037606 | RRID:DGGR_150531 | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)8-M318</i>                                                               | FBaB0047548 | RRID:BDSC_84132  | Viable              | Female fertile                | No overt visible phenotypes | 69                             |
| <i>bt</i> <sup>A</sup>         | FBaI0400124                                   | RRID:BDSC_603547 | RRID:DGGR_119193  |            | <i>Df(4)jsc<sup>H</sup></i>                                                      | FBaB0023008 | RRID:BDSC_4055   | Lethal <sup>d</sup> |                               |                             | 69                             |

|                      |             |                                    |                                             |             |                  |        |                               |                             |     |
|----------------------|-------------|------------------------------------|---------------------------------------------|-------------|------------------|--------|-------------------------------|-----------------------------|-----|
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>     | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>PlexB</i> <sup>KG00878</sup>             | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>PlexB</i> <sup>M115559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)JM101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)ED6366</i>                          | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)J5-M244</i>                         | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)ED6380</i>                          | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)ED6382</i>                          | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)J8-M318</i>                         | FBab0047548 | RRID:BDSC_84132  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)J8-M91</i>                          | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)C3</i>                              | FBab0028454 | RRID:BDSC_7083   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>C</sup> | FBal0405957 | RRID:BDSC_60578f Not yet available | <i>Df(4)Jsc<sup>H</sup></i>                 | FBab0023008 | RRID:BDSC_4055   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>     | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>PlexB</i> <sup>KG00878</sup>             | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>PlexB</i> <sup>M115559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)JM101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)ED6366</i>                          | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)J5-M244</i>                         | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)ED6380</i>                          | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)ED6382</i>                          | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)J8-M318</i>                         | FBab0047548 | RRID:BDSC_84132  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)J8-M91</i>                          | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)C3</i>                              | FBab0028454 | RRID:BDSC_7083   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG31998 <sup>D</sup> | FBal0405958 | RRID:BDSC_605787 Not yet available | <i>Df(4)Jsc<sup>H</sup></i>                 | FBab0023008 | RRID:BDSC_4055   | Viable | Female fertile                | No overt visible phenotypes | 21  |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>     | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 100 |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>PlexB</i> <sup>KG00878</sup>             | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile                | No overt visible phenotypes | 100 |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>PlexB</i> <sup>M115559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile                | No overt visible phenotypes | 100 |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>Df(4)JM101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile                | No overt visible phenotypes | 100 |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>Df(4)ED6366</i>                          | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 100 |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>Df(4)ED6369</i>                          | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 100 |
| CG32017 <sup>K</sup> | FBal0405960 | RRID:BDSC_60578f Not yet available | <i>Df(4)J5-M244&lt;/</i>                    |             |                  |        |                               |                             |     |

|                  |            |                                      |                           |              |                   |        |                 |                             |    |
|------------------|------------|--------------------------------------|---------------------------|--------------|-------------------|--------|-----------------|-----------------------------|----|
|                  | FBa0404782 | RRIID:BDSC_605531£ Not yet available | Df(4)C3                   | FBAab0028454 | RRIID:BDSC_7083   | Viable | Female fertile  | No overt visible phenotypes | 5  |
| cI <sup>C</sup>  | FBa0404782 | RRIID:BDSC_60531£ Not yet available  | Df(4)Jsc <sup>H</sup>     | FBAab0023008 | RRIID:BDSC_4055   | Viable | Female fertile  | No overt visible phenotypes | 5  |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | JYalrho CR70483-TG4.0     | FBA0386736   | RRIID:BDSC_97185  | Viable | Female fertile. | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | PlexB KG00878             | FBA0216740   | RRIID:BDSC_14579  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | PlexB MI15559-DH.GT.TG4.1 | FBA0371037   | RRIID:BDSC_93694  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)JM101-63a            | FBAab0002961 | RRIID:BDSC_1082   | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)ED6366               | FBAab0037590 | RRIID:BDSC_8067   | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)ED6369               | FBAab0037593 | RRIID:BDSC_9422   | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)J5-M244              | FBAab0046981 | RRIID:BDSC_84133  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)J5-M263              | FBAab0046980 | RRIID:BDSC_84136  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)J4-M529              | FBAab0046970 | RRIID:BDSC_94692  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)JO2                  | FBAab0029601 | RRIID:BDSC_7084   | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)J8-M318              | FBAab0047548 | RRIID:BDSC_84132  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)J8-M167              | FBAab0047543 | RRIID:BDSC_94691  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)J8-M175              | FBAab0047544 | RRIID:BDSC_84134  | Viable | Female fertile  | No overt visible phenotypes | 68 |
| eY <sup>B</sup>  | FBA0397354 | RRIID:BDSC_60219£ RRIID:DGGK_119161  | Df(4)Jsc <sup>H</sup>     | FBAab0023008 | RRIID:BDSC_4055   | Viable | Female fertile  | No overt visible phenotypes | 68 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | JYalrho CR70483-TG4.0     | FBA0386736   | RRIID:BDSC_97185  | Viable | Female fertile. | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | PlexB KG00878             | FBA0216740   | RRIID:BDSC_14579  | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | PlexB MI15559-DH.GT.TG4.1 | FBA0371037   | RRIID:BDSC_93694  | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)JM101-63a            | FBAab0002961 | RRIID:BDSC_1082   | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)ED6366               | FBAab0037590 | RRIID:BDSC_8067   | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)ED6369               | FBAab0037593 | RRIID:BDSC_9422   | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)J5-M244              | FBAab0046981 | RRIID:BDSC_84133  | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)J5-M263              | FBAab0046980 | RRIID:BDSC_84136  | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)ED6380               | FBAab0037604 | RRIID:DGGK_150529 | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)J8-M318              | FBAab0047548 | RRIID:BDSC_84132  | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)J8-M91               | FBAab0047542 | RRIID:BDSC_84135  | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)JC3                  | FBAab0028454 | RRIID:BDSC_7083   | Viable | Female fertile  | No overt visible phenotypes | 75 |
| GyF <sup>F</sup> | FBA0405964 | RRIID:BDSC_60579£ Not yet available  | Df(4)Jsc <sup>H</sup>     | FBAab0023008 | RRIID:BDSC_4055   | Viable | Female fertile  | No overt visible phenotypes | 75 |
| Hcf <sup>A</sup> | FBA0406198 | Not yet available                    | JYalrho CR70483-TG4.0     | FBA0386736   | RRIID:BDSC_97185  | Viable | Female fertile. | No overt visible phenotypes | 36 |
| Hcf <sup>A</sup> | FBA0406198 | Not yet available                    | PlexB KG00878             | FBA0216740   | RRIID:BDSC_14579  | Viable | Female fertile  | No overt visible phenotypes | 36 |
| Hcf <sup>A</sup> | FBA0406198 | Not yet available                    | PlexB MI15559-DH.GT.TG4.1 | FBA0371037   | RRIID:BDSC_93694  | Viable | Female fertile  | No overt visible phenotypes | 36 |
| Hcf <sup>A</sup> | FBA0406198 | Not yet available                    | Df(4)JM101-63a            | FBAab0002961 | RRIID:BDSC_1082   | Viable |                 |                             |    |

|                           |             |                                    |                                            |             |                  |        |                               |                             |     |
|---------------------------|-------------|------------------------------------|--------------------------------------------|-------------|------------------|--------|-------------------------------|-----------------------------|-----|
| <i>pha</i> <sup>F</sup>   | FBal0405969 | RRID:BDSC_60579E Not yet available | <i>Df(4)ED6382</i>                         | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 102 |
| <i>pha</i> <sup>F</sup>   | FBal0405969 | RRID:BDSC_60579E Not yet available | <i>Df(4)O2</i>                             | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 102 |
| <i>pha</i> <sup>F</sup>   | FBal0405969 | RRID:BDSC_60579E Not yet available | <i>Df(4)8-M91</i>                          | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile                | No overt visible phenotypes | 102 |
| <i>pha</i> <sup>F</sup>   | FBal0405969 | RRID:BDSC_60579E Not yet available | <i>Df(4)8-M175</i>                         | FBab0047544 | RRID:BDSC_84134  | Viable | Female fertile                | No overt visible phenotypes | 102 |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>    | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>PlexB</i> <sup>KG00878</sup>            | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>PlexB</i> <sup>M15559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)M101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)ED6366</i>                         | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)ED6369</i>                         | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)5-M244</i>                         | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)4-M529</i>                         | FBab0046970 | RRID:BDSC_94692  | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)5-M263</i>                         | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)O2</i>                             | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)8-M91</i>                          | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Pur-a</i> <sup>B</sup> | FBal0392554 | RRID:BDSC_600263 Not yet available | <i>Df(4)Jsc<sup>H</sup></i>                | FBab0023008 | RRID:BDSC_4055   | Viable | Female fertile                | No overt visible phenotypes | 52  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>    | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>PlexB</i> <sup>KG00878</sup>            | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>PlexB</i> <sup>M15559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)M101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)ED6366</i>                         | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)ED6369</i>                         | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)5-M244</i>                         | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)5-M263</i>                         | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)O2</i>                             | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)8-M318</i>                         | FBab0047548 | RRID:BDSC_84132  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)8-M91</i>                          | FBab0047542 | RRID:BDSC_84135  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>Slip1</i> <sup>A</sup> | FBal0392557 | Not yet available                  | <i>Df(4)Jsc<sup>H</sup></i>                | FBab0023008 | RRID:BDSC_4055   | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 65  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>    | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>PlexB</i> <sup>KG00878</sup>            | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>PlexB</i> <sup>M15559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)M101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)ED6366</i>                         | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)ED6369</i>                         | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)5-M244</i>                         | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)5-M263</i>                         | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)ED6380</i>                         | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)ED6382</i>                         | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>sv</i> <sup>F</sup>    | FBal0405971 | RRID:BDSC_60580C Not yet available | <i>Df(4)O2</i>                             | FBab0029601 | RRID:BDSC_7084   | Viable | Female fertile                | No overt visible phenotypes | 96  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>JYalpha</i> <sup>CR70483-TG4.0</sup>    | FBal0386736 | RRID:BDSC_97185  | Viable | Female fertile. Male fertile. | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>PlexB</i> <sup>KG00878</sup>            | FBal0216740 | RRID:BDSC_14579  | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>PlexB</i> <sup>M15559-DH.GT-TG4.1</sup> | FBal0371037 | RRID:BDSC_93694  | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)M101-63a</i>                       | FBab0002961 | RRID:BDSC_1082   | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)ED6366</i>                         | FBab0037590 | RRID:BDSC_8067   | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)ED6369</i>                         | FBab0037593 | RRID:BDSC_9422   | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)5-M244</i>                         | FBab0046981 | RRID:BDSC_84133  | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)5-M263</i>                         | FBab0046980 | RRID:BDSC_84136  | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)ED6380</i>                         | FBab0037604 | RRID:DGGR_150529 | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)ED6382</i>                         | FBab0037606 | RRID:DGGR_150531 | Viable | Female fertile                | No overt visible phenotypes | 86  |
| <i>toy</i> <sup>C</sup>   | FBal0400121 | RRID:BDSC_603544 RRID:DGGR_119190  | <i>Df(4)Jsc<sup>H</sup></i>                | FBab0023008 | RRID:BDSC_4055   | Viable | Female fertile                | No overt visible phenotypes | 86  |

<sup>a</sup> FlyBase IDs have not yet been assigned to some alleles. In general, new mutations were complementation tested before stock samples were sent to the Kyoto or Bloomington stock centers for distribution; nevertheless, we have shown the Kyoto and Bloomington RRIDs for convenience. Alleles with RRIDs listed as "Not yet available" have stocks being prepared for distribution at the corresponding stock center. Those alleles without RRIDs will either not be distributed by the corresponding stock center or the stocks have yet to be processed into distribution.

<sup>b</sup> Table S9 shows control crosses demonstrating the integrity of tester stocks. The footnotes to Table S9 provide relevant information about the *Df(4)ED6369*, *Df(4)ED6380*, *Df(4)ED6382* and *Df(4)Jsc<sup>H</sup>* stocks.

<sup>c</sup> This column may be used to sort alleles/rows by the cytological order of the genes mutated (see Supplementary Table 1)

<sup>d</sup> The noncomplementation of *bt*<sup>A</sup> and *Df(4)Jsc<sup>H</sup>* reflects the presence of a *bt* mutation on the *Df(1)Jsc<sup>H</sup>* chromosome, not noncomplementation of an off-target mutation on the *bt*<sup>A</sup> chromosome within the region deleted by *Df(1)Jsc<sup>H</sup>* (compare Tables S9 and S11).