

Adult Movement Defects Associated with a CORL Mutation in *Drosophila* Display Behavioral Plasticity

Agapi Dimitriadou,* Nasia Chatzianastasi,* Panagiota I. Zacharaki,* MaryJane O'Connor,†

Samuel L. Goldsmith,‡ Michael B. O'Connor,† Christos Consoulas,* and Stuart J. Newfeld*,¹

*Medical School, National and Kapodistrian University of Athens, 11527 Greece, †Dept. Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN 55455, and ‡School of Life Sciences, Arizona State University, Tempe AZ 85287

ORCID IDs: 0000-0002-3067-5506 (M.B.O.); 0000-0003-1400-7978 (S.J.N.)

ABSTRACT The CORL family of CNS-specific proteins share a Smad-binding region with mammalian SnoN and c-Ski protooncogenes. In this family *Drosophila* CORL has two mouse and two human relatives. Roles for the mouse and human CORL proteins are largely unknown. Based on genome-wide association studies linking the human CORL proteins *Fussel15* and *Fussel18* with ataxia, we tested the hypothesis that *dCORL* mutations will cause adult movement disorders. For our initial tests, we conducted side by side studies of adults with the small deletion *Df(4)dCORL* and eight control strains. We found that deletion mutants exhibit three types of behavioral plasticity. First, significant climbing defects attributable to loss of *dCORL* are eliminated by age. Second, significant phototaxis defects due to loss of *dCORL* are partially ameliorated by age and are not due to faulty photoreceptors. Third, *Df(4)dCORL* males raised in groups have a lower courtship index than males raised as singles though this defect is not due to loss of *dCORL*. Subsequent tests showed that the climbing and phototaxis defects were phenocopied by *dCORL*^{21B} and *dCORL*^{23C} two CRISPR generated mutations. Overall, the finding that adult movement defects due to loss of *dCORL* are subject to age-dependent plasticity suggests new hypotheses for CORL functions in flies and mammals.

KEYWORDS

ataxia
climbing
courtship
Fussel/SKOR
phototaxis

Studies of a *dCORL* deletion (*fussel* in Flybase) revealed roles in TGF- β /Activin signaling during larval mushroom body development and in insulin expressing neurons in adults (Takaesu *et al.* 2012, Tran *et al.* 2018a,b). *dCORL* has two mouse relatives *mCORL1* (*mSKOR1*) and *mCORL2* (*mSKOR2*). *mCORL1* transcription in embryos is found only in dorsal interneurons of the cerebellum (Mizuhara *et al.* 2005), while *mCORL2* is expressed only in Purkinje neurons of the cerebellum (Minaki *et al.* 2008). *mCORL2* knockout studies revealed a requirement for Purkinje cell differentiation via the inhibition of interneuron fate (Miyata *et al.* 2010; Wang *et al.* 2011; Nakatani *et al.* 2014). No *mCORL1* knockout has been reported. In adults *mCORL1* is primarily, though not exclusively, expressed in the

cerebellum while *mCORL2* is restricted to the cerebellum (Yue *et al.* 2014). In adults, the specific neurons expressing the *mCORL* genes are unknown. A recent study of *mCORL* transgenes in *Drosophila* demonstrated that *mCORL2* and *dCORL* have a common function with *mCORL1* fulfilling a distinct function (Stinchfield *et al.* 2019).

dCORL has two human relatives *Fussel15* (*hSKOR1*) and *Fussel18* (*hSKOR2*). RT-PCR studies report that the *Fussel15* expression pattern is the same as its homolog *mCORL1*, primarily though not exclusively in the adult cerebellum. The *Fussel18* expression pattern is the same as its homolog *mCORL2*, restricted to the adult cerebellum (Fagerberg *et al.* 2014). Genome-wide association studies have linked single nucleotide polymorphisms in *Fussel15* to three ataxias - Essential Tremor, Periodic Leg Movement and Restless Leg (Kemlink *et al.* 2009; Moore *et al.* 2014; Li *et al.* 2017; Chen *et al.* 2018). An association of *Fussel18* with ataxia has also been reported (Valence *et al.* 2019). Ataxias are movement disorders arising from dysfunction in the cerebellum, where *Fussel15* and *Fussel18* are expressed.

Taking cues from: 1) the study of the *mCORL2* knockout in Purkinje neurons of the cerebellum, 2) *Fussel15* and *Fussel18* associations with ataxia, 3) the demonstration that *dCORL* functions in adult brains and 4) the similarity of transgenic *mCORL2* and *dCORL* functions, we examined *dCORL* mutant adults for movement defects.

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¹Corresponding author: E-mail: newfeld@asu.edu

Our hypothesis was that the association of *Fussell15* and *Fussell18* with ataxia will be conserved and visible in *dCORL* adult mutants as defects in one or more movement-based behaviors.

Here we report adult behavioral studies of the small deletion *Df(4)dCORL* and eight control strains. We found that *Df(4)dCORL* flies exhibit three types of behavioral plasticity. First, significant climbing defects attributable to loss of *dCORL* are eliminated by age. Second, significant phototaxis defects due to loss of *dCORL* are partially ameliorated by age and are not due to faulty photoreceptors. Third, *Df(4)dCORL* males raised in groups have a lower courtship index than males raised as singles though this defect is not due to loss of *dCORL*. Subsequent tests showed that the climbing and phototaxis defects were phenocopied by *dCORL*^{21B} and *dCORL*^{23C} two CRISPR generated mutations. Overall, the finding that adult movement defects due to loss of *dCORL* are subject to age-dependent plasticity suggests new hypotheses for CORL functions in flies and mammals.

MATERIALS AND METHODS

Fly stocks

The *Df(4)dCORL* parental insertions *Pbac{WH}f07015* and *Pbac{WH}f06253* are as described (Takaesu *et al.* 2012). The null allele *sphinx*^{720RW} is as described (Dai *et al.*, 2008). The *Pbac{RB}e02096* insertion disrupts all predicted transcripts of the eIF4E-Transporter (CG32016), a gene whose primary expression is in oogenesis (Flybase; Thurmond *et al.* 2019). The P excision alleles *Glu-RA*^{112B} (a large deletion; noted here as *Glu-RA-null*) and *Glu-RA*^{2B} (a precise excision reverting to wild type; noted here as *Glu-RA-control*) are as described (Bogdanik *et al.* 2004). The genomic positions of these lines in relation to the only functional *dCORL* start site (Tran *et al.* 2018a) is in Figure S1. Canton-S, *yw* and *w*¹¹¹⁸ are common stocks in fly labs. Two *dCORL* CRISPR mutant lines and a line that went through CRISPR mutagenesis but has a wild type *dCORL* sequence are described below.

dCORL CRISPR mutagenesis

Guide RNAs for *dCORL* were designed using the CRISPR Optimal Target Finder (<http://targetfinder.flycrispr.neuro.brown.edu/>) using the following parameters: guide length 20 base pairs, 5'GG only. Oligos to targets 1 and 4 were cloned into pU6 BbsI chi RNA resulting in two gRNAs that were co-injected by BestGene Inc. (Chino Hills, CA) into embryos carrying maternally expressed Cas9 (*PBac{y[+mDint2]=vas-Cas9}VK00027*; BDSC #51324). Thirty-nine G0 flies were mated to flies from a transheterozygous 4th chromosome stock (*P{w[+mC]=ActGFP}unc-13[GJ]/In(4)ci[D]*; BDSC #9549). After successful mating, G0 flies were screened for deletions by PCR analysis using the target 4 forward and target 1 reverse oligos that flank both targeted sites. Five G0 lines yielded additional fragments, suggestive of a deletion. From these lines, 8 single males carrying a putative *dCORL* deletion over *ci*^D were crossed to parental females and rescreened by PCR for the presence of a deletion. Offspring from two of the five G0 founders yielded independent deletions in the Sno homology domain of exon 3 (*dCORL*^{21B} and *dCORL*^{23C}). These were confirmed by sequencing homozygotes. A line that went through the same process but showed no errors in *dCORL* was kept as a control for off-target phenotypes (*dCORL*⁴).

gRNA oligos:

dCORL target 1 for 5'-P-CTTCGGTGTTCCTTTAACTTACA-3'
dCORL target 1 rev 5'-P-AAACTGTAAGTTAAAGGGAACACC-3'
dCORL target 4 for 5'-P-CTTCGCGTAATCATGCCACATCGT-3'
dCORL target 4 rev 5'-P-AAACACGATGTGGCATGATTACCC-3'

PCR and sequencing oligos

dCORL target 1 rev 5'-GTTTGAGTCTGGTTTAAACCCAG-3'

dCORL target 4 for 5'-GGAGTGCAGATTGTATCCCTG-3'

Behavior assays

Adult climbing: 20 males or 20 females at 5 or 10 days old were examined individually in 14 ml tubes with an interior coating of propylene that facilitates climbing (Falcon). Every fly was tested six times in two rounds of three trials at a temperature of 22° and humidity greater than 60%. The Falcon tubes were marked at 6cm from the bottom and the test was to estimate the climbing time for the 6cm distance. If a fly failed to complete the test within ten seconds, then that was considered a failure. From this experiment three parameters were estimated. 1) The average climbing time (average score) per genotype was estimated by finding the average time of each fly (average of six trials) and then calculating the overall average scores of all flies. 2) The average best score was calculated by averaging the best score (the shortest climbing time from six trials/fly) of all flies per genotype. 3) The average failure rate was calculated by the same methods as average time across all individuals of a given genotype.

Adult phototaxis: 20-30 males or females at 5 or 10 days old were transferred into 25ml sterilized plastic pipettes with both sides blocked by cotton. The pipette was then placed vertically on the surface of the bench top causing the flies to be concentrated at the lower end. Then the pipette was placed horizontally and the end opposite to the flies was illuminated. After 30 sec the other end of the pipette was illuminated. This procedure was repeated fifteen times. The number of flies crossing the midline of the pipette within fifteen seconds by moving either toward or away from the light source was counted.

Adult male courtship: Five day old males were tested. In the "singles" assays, males were collected as late pupae and isolated in vials (one fly per vial) until the age of 5 days. Each individual was examined for courtship with a virgin female of same age and genotype (between 10 and 25 males were examined for each genotype). In the "group" assays, 10 to 25 males were collected as late pupae and placed together in the same vial. When the group reached 5 days old, each individual was examined for courtship with a virgin female of same age and genotype. All virgin females were stored in groups before testing (20 females per vial). The assay was conducted with one male and one virgin female in a courtship chamber (1.2cm diameter × 0.8cm high), which contained a layer of yeast media. Courtship index is the percentage of time that the male spent successfully courting the female during a 10 min period (e.g., orientation, vibrating wings and attempting to copulate).

Larval crawling: Five groups of ten early third instar larvae were collected, washed in dH₂O and placed at the center of an agar-coated 10cm petri dish. One minute videos of spontaneous crawling activity were recorded and the average and best scores estimated from bouts of continuous crawling. There were a total of 50 videos for each genotype. A template at the bottom of the dish (2mm of increasing diameter for seven concentric circles) was used to facilitate measurements of the distance traveled. At the end of a minute a snapshot was taken and the distribution of the larvae quantitated via the concentric circles.

Electroretinogram recordings

Recordings were collected from 10 day old male and female *Df(4)dCORL* (n = 12) and *Glu-RA-control* (n = 8) flies at 22° and

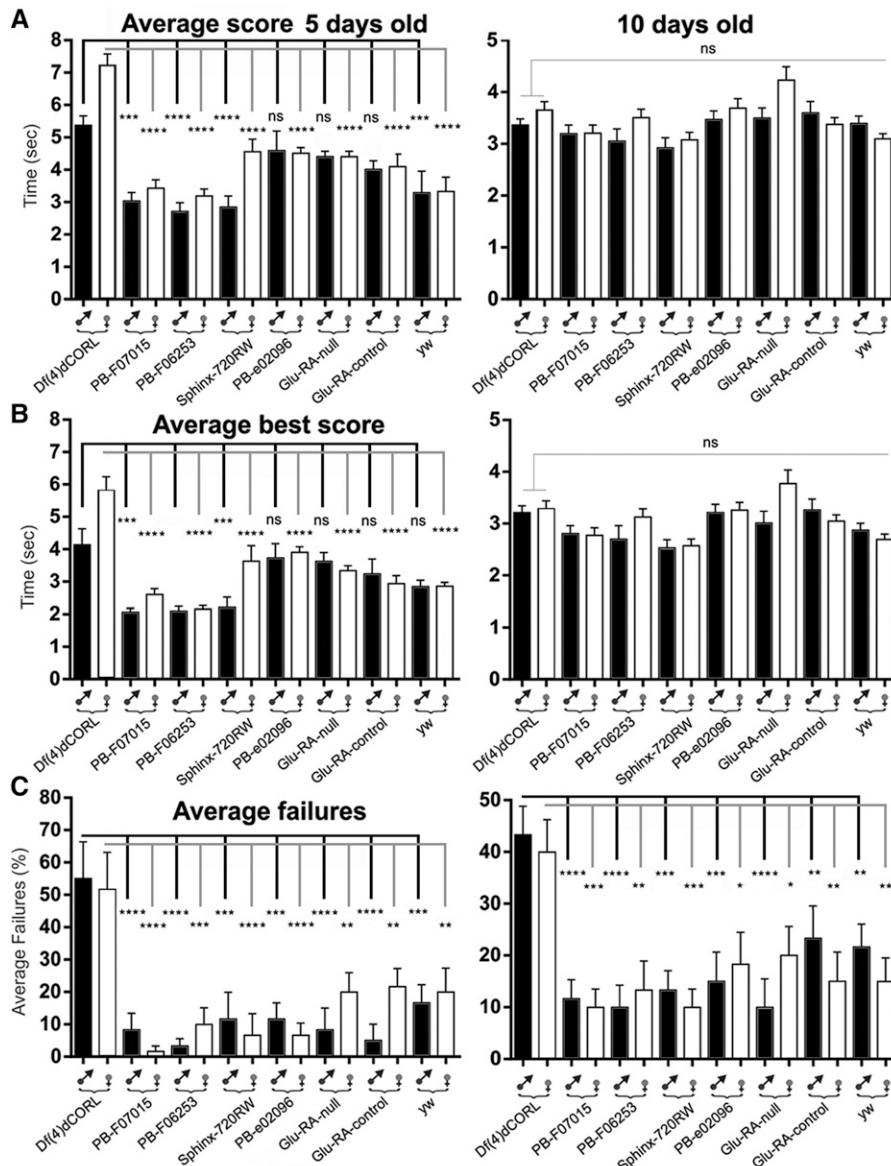


Figure 1 Climbing defects in *Df(4)dCORN* adults display age-dependent plasticity attributable to *dCORN*. A-C) Climbing assays for 5 day (left side) and 10 day old males and females analyzed separately from eight strains: from left *Df(4)dCORN*, its parental lines *Pbac{WH}f07015* and *Pbac{WH}f06253*, four lines associated with lesions in the other three genes in the deletion and *yw* as a control for the genetic background of *Df(4)dCORN*. A genomic map identifying the location of the relevant mutation in each strain (except *yw*) is in Fig. S1. Bar graphs in the top two rows display the mean of elapsed time (extending bars report standard error), while the bottom row shows the mean number of incomplete trials for each genotype. Numerical data for 5 day olds in Table S1 and 10 day olds in Table S2. A) Average score (average time to climb a 6cm vertical distance) for 5 day olds. *Df(4)dCORN* females have a significantly slower average speed than all others, while males are significantly slower than 4 lines and slower but not significantly than 3 lines. Average score for 10 day olds. *Df(4)dCORN* males and females have increased their average speed and are not distinguishable from all others, while all others have maintained the same average speed. B) Average best score (the minimum time to climb a 6cm vertical distance) for 5 and 10 day olds show exactly the same pattern. *Df(4)dCORN* females have a significantly slower maximum speed than all others while males are significantly slower than 4 lines and slower but not significantly than 3 lines. Average best score for 10 day olds. *Df(4)dCORN* males and females have increased their top speed and are not distinguishable from all others, while all others have maintained the same top speed. C) Average failure rate (percentage of trials an individual fails to cover the 6cm distance within ten seconds) for 5 day olds. *Df(4)dCORN* males and females have a significantly higher failure rate than all

the others. Average failure rate for 10 day olds. All flies reduced their failure rate by roughly the same amount leaving *Df(4)dCORN* males and females with a significantly higher failure rate. Significant results are noted as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ with nonsignificant (ns) $P > 0.05$.

40–70% relative humidity as described (Wu and Wong 1977). Electrodes were created from filamented glass micropipettes (1.2 mm OD, 0.8 mm ID, WPI, Sarasota, FL) with an electrode-puller (P97 Flaming/Brown Micropipette Puller, Shutter Inst.). Electrodes were filled with a saline solution (0.7% w/v NaCl) and inserted into an electrode holder containing a chloridized silver wire. The electrode resistance was ~ 10 M Ω . The recording electrode was inserted into the cornea, while the ground electrode, filled with 3M NaCl, was inserted into the proboscis. Signals were recorded with a DC amplifier (Axoclamp-2B, Molecular Devices). The signals were sampled at 10 kHz by a data acquisition device (Digidata 1200, Molecular Devices) and without filtering were analyzed and displayed with Clampex v8.1 software (Molecular Devices). A generic cool-white LED driven at a constant voltage delivered light flashes. The LED was positioned toward the eye, approximately 1 cm away, and every fly received three light flashes of 500 ms separated by 60 sec of dark

adaptation. Means for each genotype were compared with an unpaired, two-tailed Student's T-test.

Longevity assays

Measurement of the lifespan of males and females, as virgins and after mating, was conducted as described (Tran *et al.* 2018b).

Statistical analysis

In Figures 1,2,6,7 data are presented in bar graphs representing the mean with extending bars indicating the standard error of the mean. In Figure 3, 4 data are presented as box blots representing the median, 25th and 75th percentiles with extending bars indicating the 10th and 90th percentiles. Initial results were evaluated via Graphpad6 software with D'Agostino-Pearson omnibus normality tests. Results passing the normality test were evaluated by non-parametric Kruskal-Wallis one-way ANOVA followed by Dunn's multiple comparison tests.

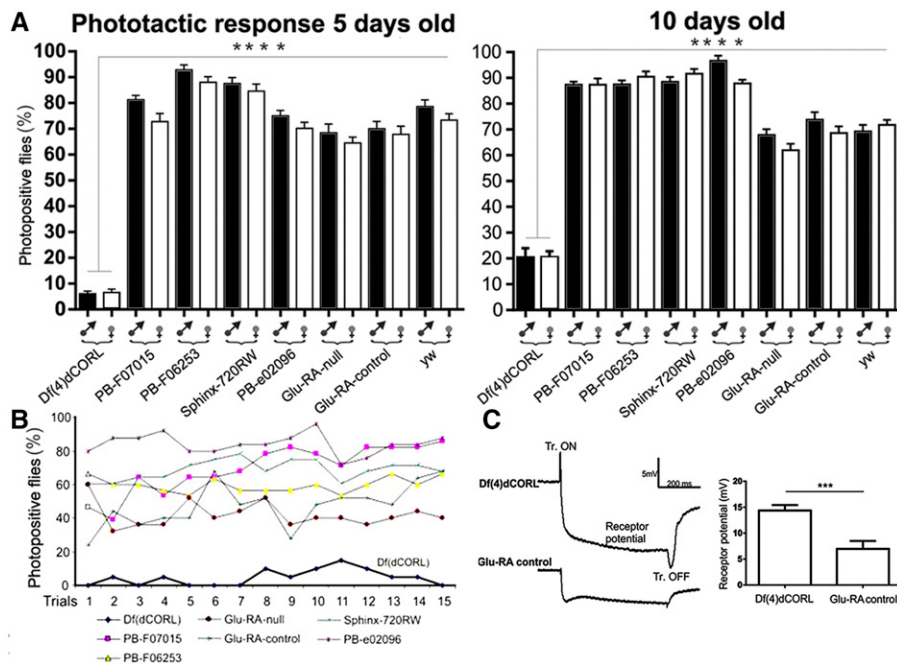


Figure 2 Phototaxis defects in *Df(4)dCORN* adults display age-dependent plasticity attributable to dCORN but are not due to faulty photoreceptors. A) Phototaxis assay of 5 day and 10 day old males and females from 8 strains. Graphs report the percent of flies of each genotype that move toward light when given a binary choice. Numerical data in Table S3. Phototaxis assays reveal an effect of age on performance similar to that seen with climbing. 10 day old *Df(4)dCORN* flies are significantly improved compared to 5 day olds. For the other strains no obvious pattern is visible with some strains improved with age while others decreased. The distinction between *Df(4)dCORN* and all other strains at both ages was highly significant. B) Detailed results from the assay of 5 day old males. Of fifteen trials with *Df(4)dCORN*, six had no *Df(4)dCORN* flies move toward the light. In contrast males of the same age in all other lines show strongly phototactic responses in all trials. C) Electrophysiological traces from *Df(4)dCORN* and *Glu-RA-control* males. Receptor potential (measured in millivolts) in *Df(4)dCORN* flies is significantly more robust than in the *Glu-RA-control*.

The contrast between *Df(4)dCORN* and *Glu-RA-control* in the phototaxis (*Df(4)dCORN* worse) and retinogram assays (*Df(4)dCORN* better) indicates that the phototaxis defect in *Df(4)dCORN* is not due to a photoreceptor deficit.

When the data did not pass the normality test then they were evaluated by an ordinary one-way ANOVA followed by Tukey's multiple comparison tests. Statistical significance in graphs and tables is noted as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ and not significant (ns) $P > 0.05$.

Data availability

Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the text, figures and supplemental materials. Supplemental material available at figshare: <https://doi.org/10.25387/g3.11968362>.

RESULTS

Adult movement defects attributable to loss of dCORN show age-dependent plasticity

In two previous studies we employed six control strains to determine if a phenotype observed for *Df(4)dCORN* was attributable to the loss of dCORN and not one of the other three genes in the deletion (Takaesu *et al.* 2012, Tran *et al.* 2018b). Two of the six are the parental PiggyBac insertions employed to generate *Df(4)dCORN* and four lines carry mutations in the other three genes. Tested alleles for two of the genes were previously shown to be nulls: *Glu-RA-null* is a deletion resulting from an imprecise excision and *spx^{720RW}* is an engineered mutation disabling splicing. For the third gene, *Pb{RB}e02096* is predicted to disrupt all transcripts of the eIF4E-Transporter and should also be a null (Fig. S1). We showed that *Df(4)dCORN* does not affect the adjacent *twin of eyeless* (Takaesu *et al.* 2012, Tran *et al.* 2018b). We employed these six strains, plus yw as a control for genetic background, in studies of *Df(4)dCORN* adult behavior (8 lines total).

The project began with a climbing assay in 5 day old adults of both sexes separately for *Df(4)dCORN* and the seven control lines. In

Figure 1A we show the average score (climbing time) and in Figure 1B the average best score (fastest climbing time). *Df(4)dCORN* females require significantly longer time to complete the task than all other lines in both measures. *Df(4)dCORN* males require more time than all strains but are only significantly slower than 4 of them. We observed that *Df(4)dCORN* flies exhibit longer climbing times because they stop frequently and wander horizontally rather than climbing vertically. In Figure 1C we show that *Df(4)dCORN* flies fail to complete the climbing test significantly more often than all other flies of both sexes. The results of these assays suggest that the climbing defect of *Df(4)dCORN* flies is attributable to loss of dCORN. Numerical data are shown in Table S1.

To ascertain if there was any age-dependent plasticity in the *Df(4)dCORN* climbing phenotype we examined 10 day old males and females separately. It is easily visible in Figure 1A,B that age significantly improved both climbing average score and average best score of *Df(4)dCORN* flies from those of 5 day old mutants. As none of the control strains showed any difference with age, both scores for 10 day old *Df(4)dCORN* now match all controls in both sexes. Age-dependent plasticity is also seen for the climbing failure rate of *Df(4)dCORN* flies shown in Figure 1C. There is improvement of roughly 10% in the failure rate strictly due to age. However, this remained significantly higher than in all the other lines, which did not show improvement. As a result, *Df(4)dCORN* did not catch up as they did in climbing time. The results demonstrate that the dCORN mediated climbing defects in speed and completion of *Df(4)dCORN* flies are subject to age-dependent plasticity. Numerical data are shown in Table S2.

Next we deployed phototaxis assays examining the sexes separately from the same eight strains. Figure 2A shows that *Df(4)dCORN* flies of both sexes were significantly impaired in their light response. The average number of photopositive *Df(4)dCORN* flies was roughly 10% the number in all other lines. As shown in Figure 2B, for six of the fifteen trials no *Df(4)dCORN* flies moved toward the light. This was

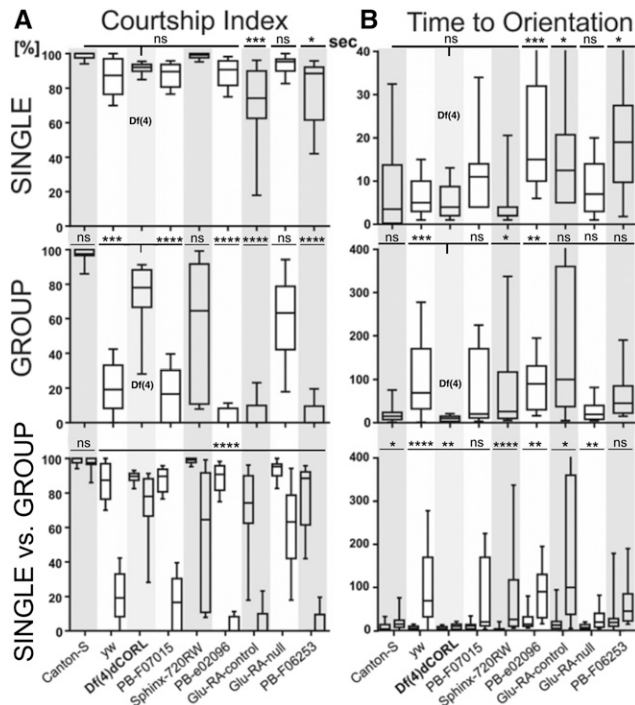


Figure 3 *Df(4)dCORN* defects in male courtship are not attributable to loss of *dCORN*. Column A) Courtship index is the percent of time that a 5 day old male spends successfully courting during a 10 min interval. Top row Single. Males from 8 strains that were raised in isolation from the pupal stage. Males from 6 of the strains including *Df(4)dCORN* courted 5 day old congenic virgin females at wild type levels as represented by Canton-S. Two strains spent less time courting. Numerical data in Table S4. Middle row Group. Males were raised in groups of 10-25 from eclosion. Males from 3 of the strains including *Df(4)dCORN*, courted 5 day old congenic virgin females at wild type levels. Four strains spent less time courting. Bottom row Single vs. Group. Males from all 8 strains, including *Df(4)dCORN* showed significant reductions in courtship index when raised in groups vs. when raised as singles, but not Canton-S. Column B) Orientation is the time it takes for a 5 day old male to orient its body toward a 5 day old congenic virgin female (representing courtship initiation). Top row Single. Males from five strains including *Df(4)dCORN* oriented toward 5 day old congenic virgin females at wild type levels. Three strains took more time to orient themselves. Middle row Group. Males from five strains including *Df(4)dCORN* oriented toward 5 day old congenic virgin females at wild type levels. Bottom row Single vs. Group—Males of all strains including *Df(4)dCORN* took longer to orient themselves in groups than when raised as singles, including Canton-S.

not seen in any other line in any trial. We observed that *Df(4)dCORN* flies initially react positively but then wander randomly reminiscent of their climbing phenotype. The results of these assays suggest that the light response defect of *Df(4)dCORN* flies is attributable to loss of *dCORN*. Numerical data are shown in Table S3.

Examining 10 day old *Df(4)dCORN* flies for phototaxis defects again revealed age-dependent plasticity. *Df(4)dCORN* males and females consistently performed better than 5 day olds (Figure 2A). As with climbing failures, even though the results for the other lines did not change *Df(4)dCORN* did not catch up. This line still contained significantly fewer photopositive flies. The results demonstrate that the *dCORN* mediated defects in light response of *Df(4)dCORN* flies are subject to age-dependent plasticity. Overall we observed in the climbing and phototaxis assays that *Df(4)dCORN* flies

initiate normally but are unable to sustain either behavior due to the loss of *dCORN*. The climbing and phototaxis data for *Df(4)dCORN* displayed age-dependent plasticity with improvement in all 4 tests. Improvement was sufficient to erase the climbing speed defects but not the completion or phototaxis defects.

To address the hypothesis that the phototaxis defect in *Df(4)dCORN* flies was due to a vision defect, rather than a motor defect, we compared electrophoretograms of *Df(4)dCORN* 10 day old males with the same age males from one of the control lines. We chose the *Glu-RA-control* strain that contains a precise excision reverting to wild type at the sequence and phenotype levels. We found that photoreceptor potential (as measured in millivolts) in *Df(4)dCORN* flies is significantly more robust than in the *Glu-RA-control* (Figure 2C). Contrast between *Df(4)dCORN* and *Glu-RA-control* performance in the phototaxis assay (Figure 2A with *Df(4)dCORN* significantly worse than *Glu-RA-control*) and the retinogram assay (Figure 2C with *Df(4)dCORN* significantly better than *Glu-RA-control*) indicates that the phototaxis defect in *Df(4)dCORN* flies is not due to a vision deficit. Numerical data in Table S3. The data support our initial hypothesis that *dCORN* adult mutants will display movement-based defects.

Df(4)dCORN* defects in male courtship are not attributable to loss of *dCORN

We next turned to courtship assays to determine if *Df(4)dCORN* males displayed a phenotype attributable to loss of *dCORN* and if so, whether there was any behavioral plasticity. In this case we examined social context dependent plasticity in the courtship index (the percent of time spent in courtship leading to mating success within a ten minute period) for 5 day old virgin males with a virgin female of the same age and genotype. We added an additional control strain, the true wild type Canton-S. For males raised singly from eclosion to 5 days old, *Df(4)dCORN* was not different than Canton-S. There was a significant reduction in courtship in 2 strains compared to *Df(4)dCORN* (Figure 4A top). For males raised and mated in groups, *Df(4)dCORN* was not different than Canton-S. There was a significant reduction in courtship index for 5 strains compared to *Df(4)dCORN* (Figure 4A middle). When looking within genotypes at the courtship of singles vs. group raised males, all strains including *Df(4)dCORN* showed significant reductions in group raised males but not Canton-S. Numerical data in Table S4.

Interestingly, the median for the courtship index of *Df(4)dCORN* males raised in groups was the highest of all strains in this test except for Canton-S. Males from the parental line *Pba{WH}f06253* and from *Pba{RB}e02096* were the worst at group courtship completion. Curiously *Glu-RA-null* males performed better than *Glu-RA-control* males. Taken together the: 1) insignificant difference of single and group raised male courtship for *Df(4)dCORN* and Canton-S, 2) strength between group raised male courtship for *Df(4)dCORN* vs. other mutant strains, 3) unexpectedly strong performance of group raised male courtship for *Glu-RA-null* vs. *Glu-RA-control* and 4) the poor performance of group raised male courtship for *Pba{WH}f06253* and *Pba{RB}e02096* suggest that the group raised male courtship defect in *Df(4)dCORN* was not due to loss of *dCORN*.

To determine if the reduction in courtship index for group raised males was due to failure to initiate or failure to complete courtship, the initial step of courtship (time to orientation) was evaluated for social context dependent plasticity. For males raised singly *Df(4)dCORN* was not different than Canton-S. There was a significant increase in time to orientation for 3 strains compared to *Df(4)dCORN* (Figure 4B top). Males raised and mated in groups on average took an order of magnitude longer to orient than their single counterparts in

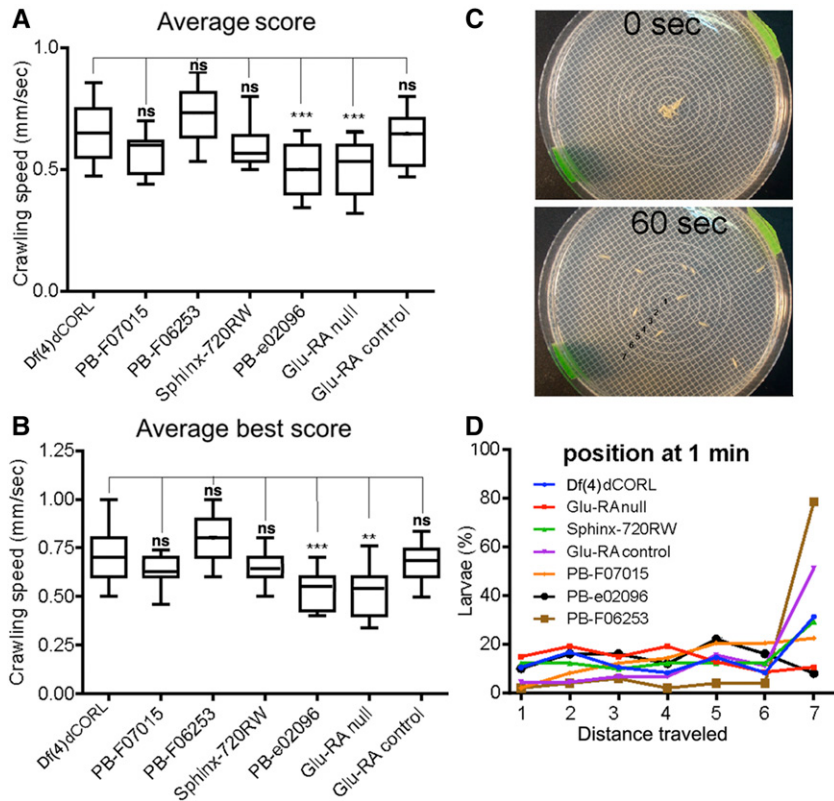


Figure 4 *Df(4)dCORN* larvae do not display defects in crawling speed or crawling distance. A,B) Average score and average best score for crawling speed of *Df(4)dCORN* larvae and six control strains. *Df(4)dCORN* is not significantly different from four strains but *Pbac{RB}e02096* and *Glu-RA-null* larvae are significantly slower than *Df(4)dCORN*. Numerical data in Table S6. C) Representative snapshot of the larval distance traveled assay at time 0 and 60 sec. D) Frequency distributions of distance traveled after 60 sec of crawling for all strains. No significant differences in distance traveled were detected. Numerical data in Table S7.

all strains. *Df(4)dCORN* was not different than Canton-S. There was a significant increase in time to orientation for 3 strains compared to *Df(4)dCORN* (Figure 4B middle). When looking within genotypes at the time to orientation of singles vs. group raised males, all strains (including Canton-S and *Df(4)dCORN*) showed significant increases with 2 exceptions. Six strains showed increases in time to orientation and reductions in courtship index when comparing males raised as singles to those raised in groups. We conclude that males when raised in groups fail to begin courtship normally resulting in a reduced courtship index. Numerical data in Table S5.

Overall, the effects of social context on *Df(4)dCORN* time to orientation or courtship index are not attributable to loss of *dCORN*. As courtship behavior is an innate motor behavior (like climbing and phototaxis), the data on courtship reveal that not all innate behavioral phenotypes of *Df(4)dCORN* flies are *dCORN* related

***Df(4)dCORN* larvae do not display defects in crawling speed or distance traveled**

To ascertain whether the defects in adult climbing were due to defects in development (manifest in larvae and persist in adults) or physiology (only manifest in adults), we examined larval crawling average speed, maximum speed and distance traveled. We tested *Df(4)dCORN* and the six original control lines. In Figure 4A we show the average score (average crawling speed) and in Figure 4B the average best score (maximum crawling speed). We found that two lines were slower than all other strains in average score and average best score. These were *Pbac{RB}e02096* and *Glu-RA-null*. *Df(4)dCORN* was not significantly different than the other four lines. Numerical data in Table S6. We measured crawling ability as distance traveled in 60 sec (example in Figure 4C). In this assay *Df(4)dCORN* larvae were not significantly different from any of the other strains (Figure 4D). Numerical data in Table S7. Overall, larval crawling data shows that

the climbing defects of *Df(4)dCORN* adults are not developmental but are instead due to errors in adult physiology.

***dCORN*^{21B} and *dCORN*^{23C} phenocopy *Df(4)dCORN* adult climbing and phototaxis defects**

To be sure that the climbing and phototaxis defect seen in *Df(4)dCORN* adults were due to loss of *dCORN* and not another gene in the deletion or a synergistic effect of the loss of multiple genes we generated mutations in *dCORN* by CRISPR. Two independent mutations were identified. A non-mutant line that went through the CRISPR process (*dCORN*⁴) was kept to serve as a control for off target effects. *dCORN*^{21B} and *dCORN*^{23C} are large in-frame deletions of 218 or 217 amino acids respectively starting in the highly conserved Sno homology domain (mutant amino acid sequences Figure 5 and mutant nucleotide sequences Fig. S2). Specifically, *dCORN*^{21B} removes amino acids between Arg¹⁰⁴ and His³²², while *dCORN*^{23C} removes amino acids between Arg¹⁰⁵ and His³²² (numbering from GenBank JX126878.1). In both the Zinc finger domain is lost while the APC recognition site that allows a protein to be marked by ubiquitylation for degradation is intact. The deletions remove 62% of the Sno homology domain and likely create null alleles.

We tested homozygous escaper males and females at 5 and 10 days old from *dCORN*^{21B}, *dCORN*^{23C} and *dCORN*⁴ for climbing and phototaxis defects. We employed the *w*¹¹¹⁸ parental line as a further control. In the first climbing assay, average score, we found that *dCORN*^{23C} and *dCORN*^{21B} males and females are significantly slower than *w*¹¹¹⁸ and *dCORN*⁴ at 5 days. The two control lines were not different from each other nor were the two mutant lines. At 10 days there is no difference between any of the strains for either sex (Figure 6A, numerical data Table S8). The age-dependent plasticity for climbing average speed is the same result seen for *Df(4)dCORN* males and females as shown in Figure 1A. The second climbing assay, average best score, shows the

mCORN1	1	MALLCGLQVTLRLVPLFPQSENRIQFLAAGAFRLRSGGMEALTTQLGPGREGSSPNSK
dCORN1	1	-----MDLNENFKMASISVA
mCORN1	61	QELQPYSGSSALKPNQVGETSLYGVIVSLVIDGQRLCLAQISNTLLKNYSYNEIHNR
dCORN1	16	HFRDMENCQTSQSNHVSLLYGVIVSLVIDGQRLCLAQISNTLLKNYSYNEIHNR
		↑ exon3
mCORN1	121	VALGITCVQCTPVQLEILRRAGAMPSSRRCGMITKREAERLCKSFLEHKKPKLPENFA
dCORN1	76	VALGITCVQCTPVQLEILRRAGAMPSSRRCGMITKREAERLCKSFLEHKKPKLPENFA
		AA21B/23C deletion begin -
mCORN1	181	FDVVHECAWGSRGSTIPARYNSSRAKCIKGYCSMFFSPNKFIFSHRTP-DAKYIQPDA
dCORN1	136	FNVOHFCWAGCRGSFIPARYNSSRAKCIKSYCSMFFSPNKFIFSHRITNDYVQPDA
mCORN1	240	ANFNWRRHKLSDKSATDELSHAWEDVKAMFNGGTRKRTFSLQGGGGGANGSGGGAGK
dCORN1	196	ANFNWRRHMSLSDGNDYDEKTHAWEDVKAMFNGGTRKR-----
mCORN1	300	GGAGGGGGPGCGSEMAPGPPPHKSLRCGEDEAGPPGPPPHQALGLAAASCPAGPG
dCORN1	235	-----LVSSINPRSEGSPTQSTAVSSVCGSS--
mCORN1	360	GPGGSAGVRSYPVIVPVSXGFLLOKLPPLFPHPYCFPTAFGLCPKKDPPVLVAGEPKG
dCORN1	263	-----CGSPTSEFRTDNEVDGKSGSSLPQR
mCORN1	420	GPGTGSSGG-AGTAACAGCPGAGHLP-PACGPPCGGTFMWHQPSGAAKDAAVAAAAA
dCORN1	288	-----SQIDSQYNYCTVAAAAGVGVTAAGATVGVFNLHGSTVLSPLHSLRNERDLS
		A21B/23C resume
mCORN1	478	AA--IVYPTFMFWPAAGSLPVPYPAAQSQKAVAAAAAAGGGPESD
dCORN1	342	IMPIGRNFVVDYMWQKQDQHYQQPYARKIPSCDSDSSDFDSWVKPDSNNPIPNANSVR
mCORN1	535	GAPPAKEGSLGTEERCPSAISLGPLDEDGADEALPPSLGPLPPPPPPARKSSYVSAFRP
dCORN1	402	TSDNCSFNKINSHSSSIYSHIKNHKLSGVPNSGLNLSIDYNPISILN-----CSAFKP
mCORN1	595	VVKDAESIAYKLSAREAYGSGPARGPVPGTGTGGGYVSPDFLSEGSSTVHSSSPVDTA
dCORN1	454	VVASTAIVS-----TSLYTRSNESRTDCCNNP
mCORN1	655	DEPEVD--VENRFPDEEGA-QDDTEPRAPSTGGGPDGQAPGPPSVTSSGADGPTDSAD
dCORN1	481	AQSTRTTTVLHNSISASSHQIVGHKTFSEILCKIHSNPEKNRIVSVFASSTGNADND
mCORN1	712	GDSRPRRRLGPPPAIRSAFGLVADDVRRTERSPSPSGGYELREPCGLGGPGAQVYA
dCORN1	541	DDDEVVDIETTE-----DECKFTVQHADSPKTYDAISGSDSPSRSP-----
mCORN1	772	PERDEHVKSTAAALGPAASYICTPETHEPKEDNHSITADDLETKRSESDORS-VSQP
dCORN1	583	-----STSTNIDVEQDVVDIITDITDQLLSVGRYSTENTSHLSLFFQAEKIQISDL
mCORN1	831	SPANTDRGEDGLTLDVTGTOLWEKDLENLAREELOKLLLEOMELRKKLEEFQSLKDNFQ
dCORN1	637	SPNTKTSSGNETNTEKDAKGNNGNLOILARRKRNKLQKDRCLSKRTVSKCVSDKS-LIK
mCORN1	891	DQMKRELAYREEMVQOLQIVRDTLCNELDQERKARYAIQOKLK--EAHDALHHSCKML
dCORN1	696	GLAANELSSSLQIQHVAFFVF-LKSQHLRLPQQAQQVVSITNSVAESNKWSYPVGNL
mCORN1	948	TERHCTGNCSEKPPPLP 964
dCORN1	755	EPLCQYQTSNGKNSLV 770

Figure 5 Loss of the majority of the Sno homology domain in two dCORN CRISPR mutants. An alignment between mCORN1 and dCORN is shown. A black arrow indicates the splice acceptor site for dCORN exon3. The highly conserved Sno homology domain begins six amino acids downstream. The starting points for two CRISPR induced deletions in adjacent Arginine residues within the Sno homology domain are shown with red arrowheads. In both mutations the Zinc finger domain (green) is lost while the APC recognition site that allows a protein to be marked by ubiquitylation for degradation is intact. dCORN^{21B} and dCORN^{23C} are deletions of 218 or 217 amino acids respectively beginning at Arg¹⁰⁴ or Arg¹⁰⁵ and ending at His³²². The deletions remove 62% of the Sno homology domain. DNA sequence data for these mutations are shown in Fig. S2.

same pattern as average score (Fig. 6B, Table S9). The CRISPR mutants match the results for *Df(4)dCORN* (Figure 1B).

The third climbing assay, average failure rate, shows the same results as both speed assays for dCORN^{23C} and dCORN^{21B} females at 5 and 10 days. The mutant females caught up to the controls. Alternatively, mutant males catch up to dCORN⁴ but not quite to *w*¹¹¹⁸ (Figure 6C, Table S10). Reduction in failures for both sexes was also seen for *Df(4)dCORN* (Figure 1C). However, mutant males' inability to catch up is similar to *Df(4)dCORN* but females' ability to catch up to the others is not. In the phototaxis assay dCORN^{23C} and dCORN^{21B} males and females have a significantly weaker response to light than *w*¹¹¹⁸ and dCORN⁴ at 5 days and this defect persists at 10 days (Figure 6D, Table S11). These results are the same as seen for *Df(4)dCORN* (Figure 2A). Overall in 7 of 8 behavioral studies (males and females in four assays) dCORN^{23C} and dCORN^{21B} match the results for *Df(4)dCORN*, including age-dependent plasticity.

We examined the effect of age within each genotype for these four strains, rather than between genotypes at the same age, with a goal of quantifying the age-dependent plasticity of each behavior for comparison to *Df(4)dCORN*. The analysis of average score and average best score showed that dCORN^{23C} and dCORN^{21B} males and females are significantly faster at 10 than 5 days, just like *Df(4)dCORN* (Figure 7A,B, numerical data in Table S12). The average failure rate also shows the

same pattern with one difference, dCORN^{21B} females improve but not significantly (Figure 7C). Alternatively, *Df(4)dCORN* females and males did not improve significantly, leading only to a match with dCORN^{21B} females but not dCORN^{23C} females or either set of males. Quantification shows that the distinction in the failure assay between *Df(4)dCORN* and the CRISPR mutants is one of degree, as all genotypes improved with age just not to exactly the same extent. The discrepancy does not negate the fact that the overall finding is similarity between dCORN^{23C} and dCORN^{21B} and *Df(4)dCORN*.

Lastly, we checked the longevity of virgin and mated males and females from dCORN^{23C} and dCORN^{21B} and *Df(4)dCORN* against the CRISPR control (Figure 7D). While the lifespans of all tested strains are significantly shorter than dCORN⁴, except dCORN^{21C} mated males, neither of the dCORN CRISPR mutations showed reductions of the magnitude of *Df(4)dCORN*. Numerical data in Table S13. Quantification shows that the distinction in lifespan between *Df(4)dCORN* and the CRISPR mutants is one of degree. Thus, in virtually all assays the dCORN^{23C} and dCORN^{21B} phenocopied *Df(4)dCORN* adding confidence to the association of dCORN loss with movement disorders displaying age-dependent plasticity.

DISCUSSION

How do the data impact our hypothesis that the association of *Fussell15* and *Fussell18* with ataxia will be conserved and visible in

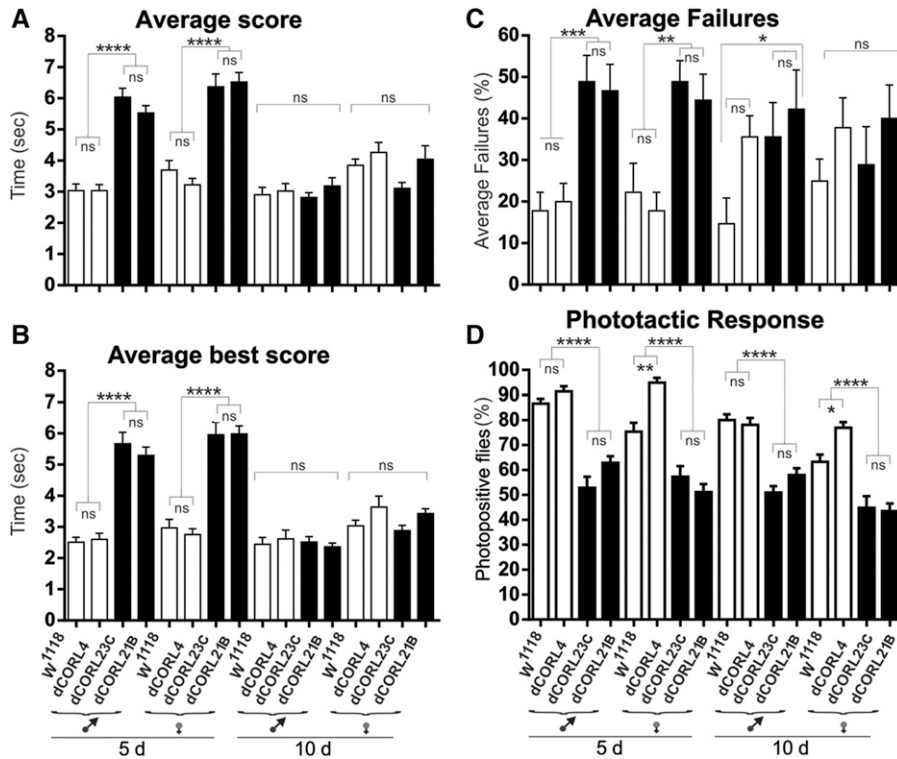


Figure 6 *dCORL*^{21B} and *dCORL*^{23C} phenocopy *Df(4)dCORL* adult climbing and phototaxis defects. A-C) Climbing assays for adults at ages 5 and 10 days old, separated by sex from four strains: *w*¹¹¹⁸ parental, *dCORL*⁴ CRISPR control and two CRISPR deletion mutants *dCORL*^{23C} and *dCORL*^{21B}. Numerical data in Tables S8-S10. A) Average score shows that *dCORL*^{23C} and *dCORL*^{21B} males and females are significantly slower than *w*¹¹¹⁸ and *dCORL*⁴ at 5 days old. At 10 days old there is no difference between the strains for either sex. This is the same result as *Df(4)dCORL* in Fig. 1A. B) Average best score shows that *dCORL*^{23C} and *dCORL*^{21B} males and females are significantly slower than *w*¹¹¹⁸ and *dCORL*⁴ at 5 days old. At 10 days old there is no difference between the strains for either sex. This is the same result as *Df(4)dCORL* in Fig. 1B. C) Average failure rate shows that *dCORL*^{23C} and *dCORL*^{21B} males and females do not complete the climb significantly more often than *w*¹¹¹⁸ and *dCORL*⁴ at 5 days old. At 10 days there is no difference between the strains for females as seen for *Df(4)dCORL*. *dCORL*^{23C} and *dCORL*^{21B} males improve but do not quite match the *w*¹¹¹⁸ completion rate at the lowest level of significance. This is the same result as *Df(4)dCORL* males in Fig. 1C. D) Phototaxis assays show that *dCORL*^{23C} and *dCORL*^{21B} males and females have a significantly weaker response to light than *w*¹¹¹⁸ and *dCORL*⁴ at 5 days old and this defect persists at 10 days old. This is the same result as *Df(4)dCORL* in Fig. 2A.

dCORL adult mutants as defects in movement-based behaviors? First, we are encouraged by the absence of a larval crawling phenotype for *Df(4)dCORL*. Most ataxias are not pediatric (Orr 2010) and thus the absence of a developmental component to the *Df(4)dCORL* adult climbing defect is consistent with the hypothesis. Second, the absence of any courtship defect attributable to *dCORL* in *Df(4)dCORL* adult males is not inconsistent with the hypothesis. The complex nature of courtship that includes modalities that are not movement-based such as olfaction and singing provide other potential causes for this defect. In addition, *sphinx* and *Glu-RA* that lie within *Df(4)dCORL* have already been implicated in courtship (Dai *et al.* 2008, Chen *et al.* 2011; Schoenfeld *et al.* 2013). Third, the phototaxis defect attributable to *dCORL* is not caused by a photoreceptor defect and phototaxis is regulated by the same neurotransmitters that regulate movement - dopamine and octopamine (Gorostiza *et al.* 2016). This result is also consistent with our hypothesis.

Which brings us to the direct evidence in support of the hypothesis, our identification of adult climbing defects in two independently generated *dCORL* mutants and their age-dependent plasticity. The defects in climbing are attributable to loss of *dCORL* due to assays with *dCORL*^{23C} and *dCORL*^{21B} and supported by the absence of climbing defects in six control strains with mutations in the region. We conclude that the hypothesis is true, *dCORL* mutations cause deficits in adult movement-based behaviors.

Given this, there are implications for *Fussell15* and *Fussell18* in our data? First to clarify, data from transgene assays in flies showed that *mCORL2/SKOR2* and *dCORL* are both capable of fully rescuing an

endogenous function in *Df(4)dCORL* larvae while *mCORL1/SKOR1* cannot (Stinchfield *et al.* 2019). This fly transgene data indicates that movement defects in *dCORL* mutants are most relevant to *Fussell18/SKOR2* associated ataxias. Second, it is important to consider our observations of “attention-deficit” as the cause of the climbing and phototaxis defects. We noted that initiation of both behaviors appeared normal but then *Df(4)dCORL* flies would wander randomly. Third, the age-dependent increases in climbing speed that erase the initial deficits in average score and average best score in males and females of all *dCORL* mutant genotypes could reflect “hyperactivation” of the locomotor circuitry underlying walking. The last piece to consider is that the inbred spontaneously hypertensive rat is the most widely studied rodent model of Attention Deficit Hyperactivity Disorder (ADHD) and that these rats display both motor impairments and loss of Purkinje cells (Bruchhage *et al.* 2018). In the absence of a genetic model for ADHD, we propose that the *mCORL2/SKOR2* knockout mouse is a candidate. Further, the data suggest that polymorphisms in *Fussell18/SKOR2* be examined for association with ADHD in humans.

In an initial attempt to identify the mechanism behind the movement defects in *dCORL* mutants we pre-fed flies with yohimbine, an antagonist of tyramine receptors. In previous reports, yohimbine feeding rescued adult climbing and flight maintenance defects in a mutant characterized by low octopamine and high tyramine levels (Saraswati *et al.* 2004; Schützler *et al.* 2019). Tyramine is a metabolic precursor and functional antagonist of octopamine, the fly counterpart to norepinephrine in mammals. In each of the three climbing

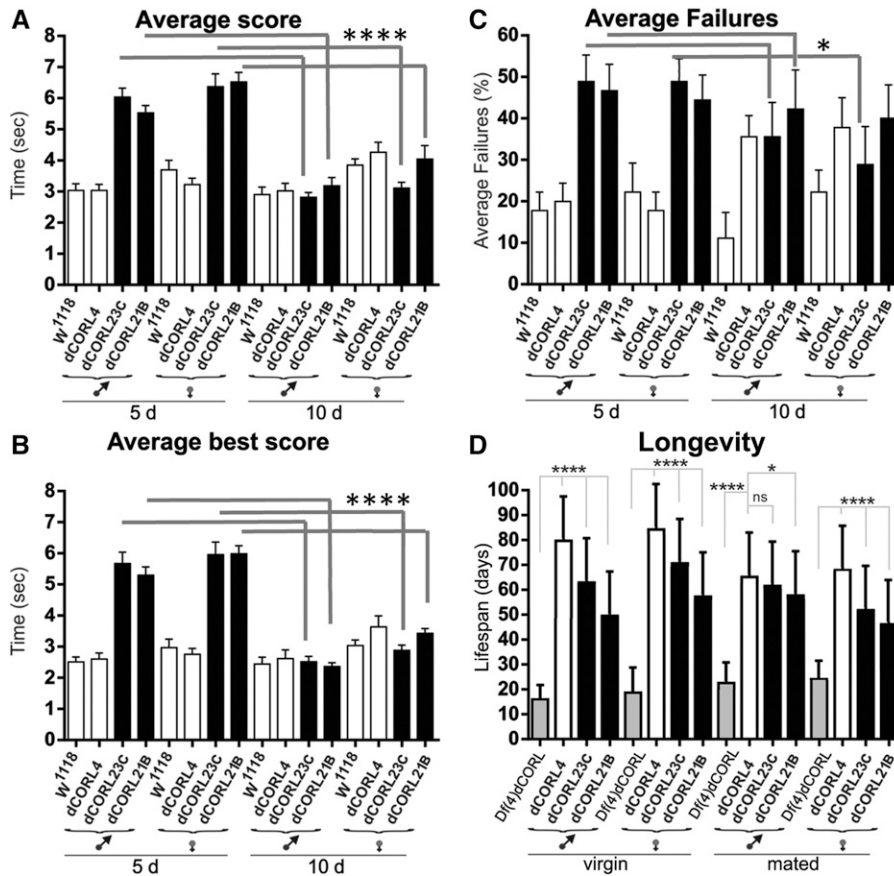


Figure 7 Quantification of age-dependent plasticity for climbing and longevity defects in *dCORL^{23C}* and *dCORL^{21B}*. A-C) Graphs from Fig. 6 reporting the analysis of age effects within each genotype, rather than comparisons between genotypes at the same age as before. Numerical data in Table S12. A) Average score shows that *dCORL^{23C}* and *dCORL^{21B}* males and females are significantly faster at 10 days than 5 days. *w¹¹¹⁸* and *dCORL⁴* are unaffected by age or show declines in performance. B) Average best score shows the same results. *dCORL^{23C}* and *dCORL^{21B}* males and females are significantly faster at 10 days than 5 days while *w¹¹¹⁸* and *dCORL⁴* are unaffected by age or show declines in performance. C) Average failure rate shows the same pattern with two differences. The significance of the improvement is not as robust as for climbing speed and *dCORL^{21B}* females improve but not significantly. D) Longevity assays of virgin and mated males and females from *Df(4)dCORL*, *dCORL⁴*, *dCORL^{23C}* and *dCORL^{21B}*. Numerical data in Table S13. Lifespans of virgin and mated males and females from the three *dCORL* mutant strains are significantly shorter than *dCORL⁴* except *dCORL^{21C}* mated males. *dCORL^{23C}* and *dCORL^{21B}* shortened lifespans do not show reductions of the magnitude of *Df(4)dCORL*.

assays, the combination of age and yohimbine improved the scores of *Df(4)dCORL* flies over age alone. How *dCORL* might interact with a tyramine/octopamine locomotion circuit awaits further experimentation

Thinking broadly, a new hypothesis derived from the preliminary yohimbine data are that treatment of *Fussell18/SKOR2* associated ataxia with a norepinephrine antagonist would be therapeutic. A first step would be to test this hypothesis in *mCORL2/SKOR2* knockout mice with an ungainly gait from birth to adulthood (Wang *et al.* 2011). Rescue of the gait defect, analogous to the rescue of the climbing defects in *dCORL* mutants by age and yohimbine, is predicted if the hypothesis is true.

Overall, our data shows that *Df(4)dCORL* mutants exhibit three types of behavioral plasticity. First, significant climbing defects attributable to loss of *dCORL* are eliminated by age. Second, significant phototaxis defects due to loss of *dCORL* are partially ameliorated by age and are not due to faulty photoreceptors. Third, *Df(4)dCORL* males raised in groups have a lower courtship index than males raised as singles though this defect is not due to loss of *dCORL*. Subsequent tests showed that the climbing and phototaxis defects were phenocopied by *dCORL^{21B}* and *dCORL^{23C}* two CRISPR generated mutations. Overall, the finding that adult movement defects due to loss of *dCORL* are subject to age-dependent plasticity suggests new hypotheses for CORL functions in flies and mammals.

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LITERATURE CITED

- Bogdanik, L., R. Mohrmann, A. Ramaekers, J. Bockaert, Y. Grau *et al.*, 2004 The drosophila metabotropic glutamate receptor DmGluRA regulates activity-dependent synaptic facilitation and fine synaptic morphology. *J. Neurosci.* 24: 9105–9116. <https://doi.org/10.1523/JNEUROSCI.2724-04.2004>
- Bruchhage, M., M. Bucci, and E. Becker, 2018 Cerebellar involvement in autism and ADHD. *Handb. Clin. Neurol.* 155: 61–72. <https://doi.org/10.1016/B978-0-444-64189-2.00004-4>
- Chen, J., P. Huang, Y. He, J. Shen, J. Du *et al.*, 2018 MAP2K5/SKOR1 was associated with essential tremor in a Chinese population. *Parkinsonism Relat. Disord.* 53: 118–119. <https://doi.org/10.1016/j.parkreldis.2018.05.017>
- Chen, Y., H. Dai, S. Chen, L. Zhang, and M. Long, 2011 Highly tissue specific expression of Sphinx supports its male courtship related role in *Drosophila*. *PLoS One* 6: e18853. <https://doi.org/10.1371/journal.pone.0018853>
- Dai, H., Y. Chen, S. Chen, Q. Mao, D. Kennedy *et al.*, 2008 The Evolution of courtship behaviors through the origination of a new gene in *Drosophila*. *Proc. Natl. Acad. Sci. USA* 105: 7478–7483. <https://doi.org/10.1073/pnas.0800693105>
- Fagerberg, L., B. Hallström, P. Oksvold, C. Kampf, and D. Djureinovic, 2014 Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol. Cell. Proteomics* 13: 397–406. <https://doi.org/10.1074/mcp.M113.035600>
- Gorostiza, E., J. Colomb and B. Brembs, 2016 A decision underlies phototaxis in an insect. *Open Biology* 6: rsob160229. <https://doi.org/10.1098/rsob.160229>

- Kemlink, D., O. Polo, B. Frauscher, V. Gschliesser, B. Högl *et al.*, 2009 Replication of restless legs syndrome loci in three European populations. *J. Med. Genet.* 46: 315–318. <https://doi.org/10.1136/jmg.2008.062992>
- Li, G., H. Tang, C. Wang, X. Qi, J. Chen *et al.*, 2017 Association of BTBD9 and MAP2K5/SKOR1 With Restless Legs Syndrome in Chinese Population. *Sleep (Basel)* 40: zsx028. <https://doi.org/10.1093/sleep/zsx028>
- Minaki, Y., T. Nakatani, E. Mizuhara, T. Inoue, and Y. Ono, 2008 Identification of a novel transcriptional corepressor, CORL2, as a cerebellar Purkinje cell-selective marker. *Gene Expr. Patterns* 8: 418–423. <https://doi.org/10.1016/j.gep.2008.04.004>
- Miyata, T., Y. Ono, M. Okamoto, M. Masaoka, A. Sakakibara *et al.*, 2010 Migration, early axonogenesis, and Reelin-dependent layer-forming behavior of early/posterior-born Purkinje cells in the developing mouse lateral cerebellum. *Neural Dev.* 5: 23. <https://doi.org/10.1186/1749-8104-5-23>
- Mizuhara, E., T. Nakatani, Y. Minaki, Y. Sakamoto, and Y. Ono, 2005 CORL1 a novel neuronal lineage-specific transcriptional corepressor for the homeodomain transcription factor Lbx1. *J. Biol. Chem.* 280: 3645–3655. <https://doi.org/10.1074/jbc.M411652200>
- Moore, H., J. Winkelmann, L. Lin, L. Finn, R. Peppard *et al.*, 2014 Periodic leg movements during sleep are associated with polymorphisms in BTBD9, TOX3/BC034767, MEIS1, MAP2K5/SKOR1 and PTPRD. *Sleep (Basel)* 37: 1535–1542. <https://doi.org/10.5665/sleep.4006>
- Nakatani, T., Y. Minaki, M. Kumai, C. Nitta, and Y. Ono, 2014 The c-Ski family member and transcriptional regulator CORL2/SKOR2 promotes early differentiation of cerebellar Purkinje cells. *Dev. Biol.* 388: 68–80. <https://doi.org/10.1016/j.ydbio.2014.01.016>
- Orr, H. T., 2010 Nuclear ataxias. *Cold Spring Harb. Perspect. Biol.* 2: a000786. <https://doi.org/10.1101/cshperspect.a000786>
- Saraswati, S., L. Fox, D. Soll, and C. Wu, 2004 Tyramine and octopamine have opposite effects on the locomotion of *Drosophila* larvae. *J. Neurobiol.* 58: 425–441. <https://doi.org/10.1002/neu.10298>
- Schoenfeld, B., R. Choi, C. Choi, A. Terlizzi, P. Hinchey *et al.*, 2013 The *Drosophila* DmGluRA is required for social interaction and memory. *Front. Pharmacol.* 4: 64. <https://doi.org/10.3389/fphar.2013.00064>
- Schützler, N., C. Girwert, I. Hügli, G. Mohana, J. Roignant *et al.*, 2019 Tyramine action on motoneuron excitability and adaptable tyramine/octopamine ratios adjust *Drosophila* locomotion to nutritional state. *Proc. Natl. Acad. Sci. USA* 116: 3805–3810. <https://doi.org/10.1073/pnas.1813554116>
- Stinchfield, M., K. Miyazawa, and S. J. Newfeld, 2019 Transgenic analyses in *Drosophila* reveal that mCORL1 is functionally distinct from mCORL2 and dCORL. *G3 (Bethesda)* 9: 3781–3789. <https://doi.org/10.1534/g3.119.400647>
- Takaesu, N., M. Stinchfield, K. Shimizu, M. Arase, J. Quijano *et al.*, 2012 *Drosophila* CORL is required for Smad2-mediated activation of Ecdysone Receptor expression in the mushroom body. *Development* 139: 3392–3401. <https://doi.org/10.1242/dev.079442>
- Thurmond, J., J. Goodman, V. Strelets, H. Attrill, L. S. Gramates *et al.*, 2019 FlyBase 2.0: the next generation. *Nucleic Acids Res.* 47: D759–D765. <https://doi.org/10.1093/nar/gky1003>
- Tran, N., S. Goldsmith, A. Dimitriadou, N. Takaesu, C. Consoulas *et al.*, 2018a CORL expression and function in insulin producing neurons reversibly influences adult longevity in *Drosophila*. *G3 (Bethesda)* 8: 2979–2990. <https://doi.org/10.1534/g3.118.200572>
- Tran, N., N. Takaesu, E. Cornell, and S. J. Newfeld, 2018b CORL expression in the *Drosophila* central nervous system is regulated by stage specific interactions of intertwined activators and repressors. *G3 (Bethesda)* 8: 2527–2536. <https://doi.org/10.1534/g3.118.200282>
- Valence, S., E. Cochet, R. Rougeot, C. Garel, S. Chantot-Bastaraud *et al.*, 2019 Exome sequencing in congenital ataxia identifies two new candidate genes and highlights a pathophysiological link between some congenital ataxias and early infantile epileptic encephalopathies. *Genet. Med.* 21: 553–563. <https://doi.org/10.1038/s41436-018-0089-2>
- Wang, B., W. Harrison, P. Overbeek, and H. Zheng, 2011 Transposon mutagenesis with coat color genotyping identifies an essential role for Skor2 in sonic hedgehog signaling and cerebellum development. *Development* 138: 4487–4497. <https://doi.org/10.1242/dev.067264>
- Wu, C. F., and F. Wong, 1977 Frequency characteristics in the visual system of *Drosophila*: Genetic dissection of electroretinogram components. *J. Gen. Physiol.* 69: 705–724. <https://doi.org/10.1085/jgp.69.6.705>
- Yue, F., Y. Cheng, A. Breschi, J. Vierstra, W. Wu *et al.*, 2014 A comparative encyclopedia of DNA elements in the mouse genome. *Nature* 515: 355–364. <https://doi.org/10.1038/nature13992>

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Supplemental data - two figures and thirteen tables

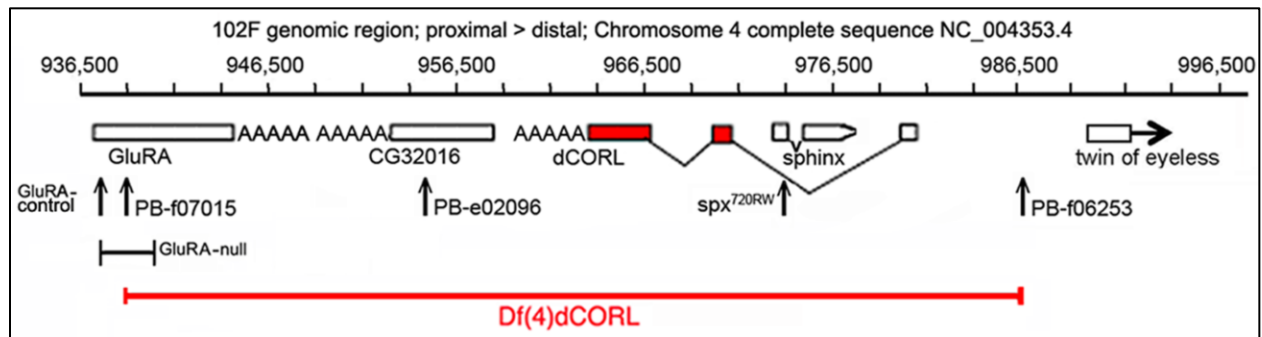


Fig. S1. *dCORL* genomic region displaying the *Df(4)dCORL* deletion and associated mutations. The *dCORL* cDNA (GenBank JX126878) is shown with exons shown as boxes (white - untranslated; red - protein coding). This cDNA is also known as isoformB (Genbank ACZ950983). *dCORL*, *Glu-RA* and *CG2316* transcript directions are shown by a poly-A tail. *sphinx* transcript direction and toy transcript direction (opposite from *dCORL*) are indicated by arrowheads. The only functional *dCORL* start site (as shown in reporter studies; Tran et al. 2018a) is located distal to *sphinx* at 986,265bp. The region precisely deleted via FLP-FRT mediated intrachromosomal recombination resulting in *Df(4)dCORL* is shown in red. The locations of the two FRT-bearing Piggy-bac insertions employed to generate *Df(4)dCORL*, a Piggy-bac insertion in *CG32016*, a splicing mutation in *sphinx* and two mutations impacting *Glu-RA* are also shown (*Glu-RA-control* is a precise excision and *Glu-RA-null* is a large deletion).

A. <i>dCORL</i>^{21B}			
572	597	1251 in frame	654bp deleted
I	I	I	
GGCGCCATGCCAGTGAGTTCCCGAC.....ATGGCTCAACCGTCTTA			
B. <i>dCORL</i>^{23C}			
572	599	1250 in frame	651bp deleted
I	I	I	
GGCGCCATGCCAGTGAGTTCCCGACGA.....CATGGCTCAACCGTCTTA			

Fig. S2. cDNA junction sequences from two CRISPR mutated *dCORL* alleles. Nucleotide numbers match GenBank. A) *dCORL*^{21B} is missing 654bp encoding an in frame loss of 218aa. B) *dCORL*^{23C} is missing 651bp encoding an in frame loss of 217aa.

Table S1. *Df(4)dCORL* 5 day old adults display defects in climbing speed and completion attributable to *dCORL* (data for Fig. 1 left column).

Dunnett's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Average score Males					
Df(4)dCORL vs. PB-f07015	2.337	0.941 to 3.733	Yes	***	0.0003
Df(4)dCORL vs. PB-f06253	2.665	1.269 to 4.060	Yes	****	< 0.0001
Df(4)dCORL vs. Spx-720RW	2.525	1.130 to 3.921	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	0.779	-0.617 to 2.174	No	ns	0.4902
Df(4)dCORL vs. Glu-RANull	0.971	-0.425 to 2.366	No	ns	0.2752
Df(4)dCORL vs. Glu-RAcontrol	1.364	-0.101 to 2.830	No	ns	0.0772
Df(4)dCORL vs. yw	2.081	0.7251 to 3.437	Yes	***	0.0007
Average score Females					
Df(4)dCORL vs. PB-f07015	3.791	2.655 to 4.927	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	4.035	2.899 to 5.171	Yes	****	< 0.0001
Df(4)dCORL vs. Spx-720RW	2.665	1.529 to 3.801	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	2.712	1.576 to 3.848	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RANull	2.822	1.686 to 3.957	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	3.127	1.965 to 4.288	Yes	****	< 0.0001
Df(4)dCORL vs. yw	3.892	2.797 to 4.986	Yes	****	< 0.0001
Average Best score Males					
Df(4)dCORL vs. PB-f07015	2.075	0.738 to 3.411	Yes	***	0.0007
Df(4)dCORL vs. PB-f06253	2.039	0.702 to 3.375	Yes	***	0.0009
Df(4)dCORL vs. Spx-720RW	1.926	0.589 to 3.262	Yes	**	0.0019
Df(4)dCORL vs. PB-e02096	0.407	-0.929 to 1.743	No	ns	0.9209
Df(4)dCORL vs. Glu-RANull	0.511	-0.826 to 1.847	No	ns	0.8145
Df(4)dCORL vs. Glu-RAcontrol	0.902	-0.464 to 2.269	No	ns	0.3235
Df(4)dCORL vs. yw	1.295	-0.005051 to	No	ns	0.0513
Average Best score Females					
Df(4)dCORL vs. PB-f07015	3.203	2.111 to 4.296	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	3.651	2.559 to 4.744	Yes	****	< 0.0001
Df(4)dCORL vs. Spx-720RW	2.176	1.084 to 3.269	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	1.906	0.814 to 2.999	Yes	***	0.0001
Df(4)dCORL vs. Glu-RANull	2.471	1.379 to 3.564	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	2.868	1.751 to 3.985	Yes	****	< 0.0001
Df(4)dCORL vs. yw	2.949	1.901 to 3.997	Yes	****	< 0.0001
Average failure rate Males					
Df(4)dCORL vs. PB-f07015	46.68	21.30 to 72.06	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	51.68	26.30 to 77.06	Yes	****	< 0.0001
Df(4)dCORL vs. Spx-720RW	43.35	17.97 to 68.73	Yes	***	0.0002
Df(4)dCORL vs. PB-e02096	43.34	17.96 to 68.72	Yes	***	0.0002
Df(4)dCORL vs. Glu-RANull	46.67	21.30 to 72.05	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	50.00	24.62 to 75.38	Yes	****	< 0.0001
Df(4)dCORL vs. yw	38.33	13.12 to 63.55	Yes	***	0.0008
Average failure rate Females					
Df(4)dCORL vs. PB-f07015	50.01	26.42 to 73.60	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	41.67	18.08 to 65.26	Yes	***	0.0001
Df(4)dCORL vs. Spx-720RW	45.00	21.41 to 68.59	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	45.01	21.42 to 68.61	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RANull	31.69	8.102 to 55.29	Yes	**	0.0041
Df(4)dCORL vs. Glu-RAcontrol	30.01	6.423 to 53.61	Yes	**	0.0071
Df(4)dCORL vs. yw	31.67	7.191 to 56.14	Yes	**	0.0056

Table S2. *Df(4)dCORL* 10 day old adults display defects only in climbing completion attributable to *dCORL* (data for Fig. 1 right column).

Dunnett's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Average score Males					
Df(4)dCORL vs. PB-f07015	0.1670	-0.5346 to 0.8686	No	ns	0.9848
Df(4)dCORL vs. PB-f06253	0.3103	-0.3913 to 1.012	No	ns	0.7492
Df(4)dCORL vs. Spx-720RW	0.4416	-0.2600 to 1.143	No	ns	0.3963
Df(4)dCORL vs. PB-e02096	-0.1078	-0.8094 to 0.5938	No	ns	0.9978
Df(4)dCORL vs. Glu-RAnull	-0.1339	-0.8442 to 0.5764	No	ns	0.9949
Df(4)dCORL vs. Glu-RAcontrol	-0.2363	-0.9466 to 0.4740	No	ns	0.9167
Df(4)dCORL vs. yw	-0.02953	-0.7311 to 0.6721	No	ns	0.9999
Average score Females					
Df(4)dCORL vs. PB-f07015	0.4460	-0.1873 to 1.079	No	ns	0.2825
Df(4)dCORL vs. PB-f06253	0.1447	-0.4965 to 0.7858	No	ns	0.9890
Df(4)dCORL vs. Spx-720RW	0.5768	-0.05649 to 1.210	No	ns	0.0898
Df(4)dCORL vs. PB-e02096	-0.03673	-0.6779 to 0.6044	No	ns	0.9998
Df(4)dCORL vs. Glu-RAnull	-0.5774	-1.219 to 0.06370	No	ns	0.0957
Df(4)dCORL vs. Glu-RAcontrol	0.2750	-0.3582 to 0.9083	No	ns	0.7637
Df(4)dCORL vs. yw	0.5530	-0.08033 to 1.186	No	ns	0.1131
Average Best score Males					
Df(4)dCORL vs. PB-f07015	0.4111	-0.2925 to 1.115	No	ns	0.4758
Df(4)dCORL vs. PB-f06253	0.5166	-0.1870 to 1.220	No	ns	0.2437
Df(4)dCORL vs. Spx-720RW	0.6861	-0.01751 to 1.390	No	ns	0.0592
Df(4)dCORL vs. PB-e02096	0.0006110	-0.7030 to 0.7042	No	ns	> 0.9999
Df(4)dCORL vs. Glu-RAnull	0.2061	-0.4975 to 0.9097	No	ns	0.9542
Df(4)dCORL vs. Glu-RAcontrol	-0.05020	-0.7625 to 0.6621	No	ns	0.9997
Df(4)dCORL vs. yw	0.3421	-0.3615 to 1.046	No	ns	0.6653
Average Best score Females					
Df(4)dCORL vs. PB-f07015	0.5094	-0.09815 to 1.117	No	ns	0.1387
Df(4)dCORL vs. PB-f06253	0.1581	-0.4570 to 0.7732	No	ns	0.9765
Df(4)dCORL vs. Spx-720RW	0.7114	0.1038 to 1.319	Yes	*	0.0141
Df(4)dCORL vs. PB-e02096	0.02284	-0.5922 to 0.6379	No	ns	0.9999
Df(4)dCORL vs. Glu-RAnull	-0.4871	-1.095 to 0.1204	No	ns	0.1707
Df(4)dCORL vs. Glu-RAcontrol	0.2369	-0.3707 to 0.8444	No	ns	0.8395
Df(4)dCORL vs. yw	0.5899	-0.01765 to 1.197	No	ns	0.0609
Average failure rate Males					
Df(4)dCORL vs. PB-f07015	31.67	13.08 to 50.26	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	33.33	14.74 to 51.92	Yes	****	< 0.0001
Df(4)dCORL vs. Spx-720RW	30.00	11.41 to 48.59	Yes	***	0.0002
Df(4)dCORL vs. PB-e02096	28.33	9.745 to 46.92	Yes	***	0.0006
Df(4)dCORL vs. Glu-RAnull	33.33	14.74 to 51.92	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	20.00	1.412 to 38.59	Yes	*	0.0291
Df(4)dCORL vs. yw	21.67	3.078 to 40.26	Yes	*	0.0147
Average failure rate Females					
Df(4)dCORL vs. PB-f07015	30.00	10.55 to 49.45	Yes	***	0.0005
Df(4)dCORL vs. PB-f06253	26.67	7.219 to 46.11	Yes	**	0.0025
Df(4)dCORL vs. Spx-720RW	30.00	10.55 to 49.45	Yes	***	0.0005
Df(4)dCORL vs. PB-e02096	21.67	2.219 to 41.11	Yes	*	0.0219
Df(4)dCORL vs. Glu-RAnull	20.00	0.5522 to 39.45	Yes	*	0.0410
Df(4)dCORL vs. Glu-RAcontrol	25.00	5.552 to 44.45	Yes	**	0.0054
Df(4)dCORL vs. yw	25.00	5.552 to 44.45	Yes	**	0.0054

Table S3. 5 and 10 day old *Df(4)dCORL* adults display persistent defects in phototaxis attributable to *dCORL* that are not due to a vision defect (data for Fig. 2).

Dunnett's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Phototactic response Males 5 days old					
Df(4)dCORL vs. PB-f07015	-75.14	-84.01 to -66.27	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	-86.75	-95.62 to -77.87	Yes	****	< 0.0001
Df(4)dCORL vs. SpX-720RW	-81.44	-90.32 to -72.57	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	-68.89	-77.76 to -60.02	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAnull	-62.33	-71.21 to -53.46	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	-63.89	-72.76 to -55.02	Yes	****	< 0.0001
Df(4)dCORL vs. yw	-72.56	-81.43 to -63.68	Yes	****	< 0.0001
Phototactic response Females 10 days old					
Df(4)dCORL vs. PB-f07015	-66.11	-75.60 to -56.62	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	-81.33	-90.83 to -71.84	Yes	****	< 0.0001
Df(4)dCORL vs. SpX-720RW	-77.92	-87.41 to -68.42	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	-63.53	-73.02 to -54.04	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAnull	-57.89	-67.39 to -48.40	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	-61.18	-70.67 to -51.68	Yes	****	< 0.0001
Df(4)dCORL vs. yw	-66.67	-76.16 to -57.17	Yes	****	< 0.0001
Phototactic response Males 10 days old					
Df(4)dCORL vs. PB-f07015	-66.96	-76.93 to -56.99	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	-67.08	-77.05 to -57.11	Yes	****	< 0.0001
Df(4)dCORL vs. SpX-720RW	-68.07	-78.04 to -58.10	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	-76.30	-86.27 to -66.33	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAnull	-47.55	-57.52 to -37.58	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	-53.41	-63.38 to -43.44	Yes	****	< 0.0001
Df(4)dCORL vs. yw	-48.90	-57.53 to -40.26	Yes	****	< 0.0001
Phototactic response Females 10 days old					
Df(4)dCORL vs. PB-f07015	-66.96	-75.59 to -58.33	Yes	****	< 0.0001
Df(4)dCORL vs. PB-f06253	-70.03	-78.66 to -61.40	Yes	****	< 0.0001
Df(4)dCORL vs. SpX-720RW	-71.22	-79.85 to -62.59	Yes	****	< 0.0001
Df(4)dCORL vs. PB-e02096	-67.47	-76.10 to -58.84	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAnull	-41.52	-50.15 to -32.89	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	-48.18	-56.81 to -39.55	Yes	****	< 0.0001
Df(4)dCORL vs. yw	-51.36	-58.84 to -43.89	Yes	****	< 0.0001
Electroretinogram					
Unpaired 2-tailed Student's t test	t value	Degree Freedom	Significant?	Summary	P Value
Df(4)dCORL vs. Glu-RAcontrol	4.119	18	Yes	***	0.0006

Table S4. *Df(4)dCORL* males display social-context dependent plasticity in courtship index not attributable to *dCORL* (data for Fig. 3A).

Dunn's multiple comparisons test	Mean Diff.	Significant?	Summary	P Value
Courtship Index (5 day old males as singles prior to courtship)				
Df(4)dCORL vs. Canton-S	-33.08	No	ns	0.1544
Df(4)dCORL vs. yw	37.05	No	ns	0.0703
Df(4)dCORL vs. PB-f07015	46.65	No	ns	0.1763
Df(4)dCORL vs. Spx-720RW	-29.66	No	ns	0.3240
Df(4)dCORL vs. PB-e02096	35.23	No	ns	0.1574
Df(4)dCORL vs. Glu-RAcontrol	69.82	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAnull	15.56	No	ns	> 0.9999
Df(4)dCORL vs. PB-f06253	59.24	Yes	*	0.0125
Courtship Index (5 day old males in groups prior to courtship)				
Df(4)dCORL vs. Canton-S	-34.69	No	ns	0.3899
Df(4)dCORL vs. yw	61.04	Yes	***	0.0008
Df(4)dCORL vs. PB-f07015	68.37	Yes	****	< 0.0001
Df(4)dCORL vs. Spx-720RW	19.15	No	ns	> 0.9999
Df(4)dCORL vs. PB-e02096	104.40	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAcontrol	103.00	Yes	****	< 0.0001
Df(4)dCORL vs. Glu-RAnull	12.27	No	ns	> 0.9999
Df(4)dCORL vs. PB-f06253	103.10	Yes	****	< 0.0001
Courtship Index (5 day old males single vs. group)				
2-tailed Mann-Whitney test	U value	Significant?	Summary	P Value
Canton S	120.5	No	ns	0.0730
yw	0.0	Yes	****	< 0.0001
Df(4)dCORL	37.5	Yes	****	< 0.0001
PB-f07015	0.0	Yes	****	< 0.0001
Spx-720RW	48.5	Yes	****	< 0.0001
PB-e02096	0.0	Yes	****	< 0.0001
Glu-RAcontrol	6.0	Yes	****	< 0.0001
Glu-RAnull	21.0	Yes	****	< 0.0001
PB-f06253	0.0	Yes	****	< 0.0001

Table S5. *Df(4)dCORL* males display social-context dependent plasticity in time to orientation during courtship not attributable to *dCORL* (data for Fig. 3B).

Dunn's multiple comparisons test	Mean Diff.	Significant?	Summary	P Value
Orientation (5 day old males as singles prior to courtship)				
Df(4)dCORL vs. Canton-S	-8.247	No	ns	> 0.9999
Df(4)dCORL vs. yw	-13.81	No	ns	> 0.9999
Df(4)dCORL vs. PB-f07015	-50.89	No	ns	> 0.9999
Df(4)dCORL vs. Spx-720RW	13.04	No	ns	> 0.9999
Df(4)dCORL vs. PB-e02096	-86.58	Yes	***	0.0004
Df(4)dCORL vs. Glu-Racontrol	-65.10	Yes	*	0.0254
Df(4)dCORL vs. Glu-RAnull	-25.48	No	ns	> 0.9999
Df(4)dCORL vs. PB-f06253	-81.64	Yes	*	0.0376
Orientation (5 day old males in groups prior to courtship)				
Df(4)dCORL vs. Canton-S	-19.14	No	ns	> 0.9999
Df(4)dCORL vs. yw	-68.56	Yes	***	0.0009
Df(4)dCORL vs. PB-f07015	-51.01	No	ns	0.0716
Df(4)dCORL vs. Spx-720RW	-52.78	Yes	*	0.0211
Df(4)dCORL vs. PB-e02096	-78.52	Yes	**	0.0019
Df(4)dCORL vs. Glu-RAcontrol	-80.70	No	ns	0.0964
Df(4)dCORL vs. Glu-RAnull	-29.02	No	ns	> 0.9999
Df(4)dCORL vs. PB-f06253	-65.21	No	ns	0.3503
Orientation (5 day old males single vs. group)				
2-tailed Mann-Whitney test	U value	Significant?	Summary	P Value
Canton S	110.0	Yes	*	0.0220
yw	62.0	Yes	****	< 0.0001
Df(4)dCORL	224.5	Yes	**	0.0088
PB-f07015	34.0	No	ns	0.612
Spx-720RW	49.5	Yes	****	< 0.0001
PB-e02096	34.5	Yes	**	0.0017
Glu-RAcontrol	23.5	Yes	*	0.0459
Glu-RAnull	80.0	Yes	**	0.0027
PB-f06253	13.00	No	ns	0.0682

Table S6. *Df(4)dCORL* larvae do not display defects in crawling speed (data for Fig. 4A,B).

Dunn's multiple comparisons test	Mean Diff.	Significant	Summary	P Value
Average score				
Df(4)dCORL vs. PB-f07015	36.06	No	ns	0.1874
Df(4)dCORL vs. PB-f06253	-35.12	No	ns	0.1725
Df(4)dCORL vs. SpX-720RW	22.17	No	ns	> 0.9999
Df(4)dCORL vs. PB-e02096	63.44	Yes	***	0.0003
Df(4)dCORL vs. Glu-RAnull	60.13	Yes	***	0.0002
Df(4)dCORL vs. Glu-RAcontrol	00.20	No	ns	> 0.9999
Average Best score				
Df(4)dCORL vs. PB-F07015	35.31	No	ns	0.1890
Df(4)dCORL vs. PB-F06253	-38.53	No	ns	0.0868
Df(4)dCORL vs. SpX-720RW	23.28	No	ns	0.8359
Df(4)dCORL vs. PB-e02096	57.70	Yes	**	0.0010
Df(4)dCORL vs. Glu-RAnull	58.85	Yes	***	0.0002
Df(4)dCORL vs. Glu-RAcontrol	02.75	No	ns	> 0.9999

Table S7. *Df(4)dCORL* larvae do not display defects in crawling distance (data for Fig. 4D).

Spearman Rank-Order Correlation	Spearman R	P value	Summary	Number of pairs
Df(4)dCORL vs. PB-f07015	0.174	0.700	ns	7
Df(4)dCORL vs. PB-f06253	0.590	0.195	ns	7
Df(4)dCORL vs. SpX-720RW	0.476	0.381	ns	7
Df(4)dCORL vs. PB-e02096	-0.132	0.676	ns	7
Df(4)dCORL vs. Glu-RAnull	-0.046	0.898	ns	7
Df(4)dCORL vs. Glu-RAcontrol	0.231	0.622	ns	7

Table S8. Two dCORL CRISPR mutations phenocopy *Df(4)dCORL* climbing defects for Average score (data for Fig. 6A).

Tukey's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Average score Males 5 days old					
w-1118 vs. dCORL-4 control	0.005222	-1.294 to 1.305	No	ns	> 0.9999
w-1118 vs. dCORL-21B	-2.999	-4.298 to -1.699	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	-2.493	-3.793 to -1.193	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	0.5054	-0.7942 to 1.805	No	ns	0.9935
dCORL-21B vs. dCORL-4 control	3.004	1.704 to 4.303	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	2.498	1.199 to 3.798	Yes	****	< 0.0001
Average score Females 5 days old					
w-1118 vs. dCORL-4 control	0.4774	-0.8452 to 1.800	No	ns	0.9970
w-1118 vs. dCORL-21B	-2.672	-3.995 to -1.349	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	-2.826	-4.149 to -1.504	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	-0.1541	-1.454 to 1.146	No	ns	> 0.9999
dCORL-21B vs. dCORL-4 control	3.150	1.850 to 4.449	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	3.304	2.004 to 4.603	Yes	****	< 0.0001
Average score Males 10 days old					
w-1118 vs. dCORL-4 control	-0.1160	-1.416 to 1.184	No	ns	> 0.9999
w-1118 vs. dCORL-21B	0.08871	-1.234 to 1.411	No	ns	> 0.9999
w-1118 vs. dCORL-23C	-0.2744	-1.653 to 1.104	No	ns	> 0.9999
dCORL-21B vs. dCORL-23C	-0.3631	-1.763 to 1.037	No	ns	> 0.9999
dCORL-21B vs. dCORL-4 control	-0.2047	-1.527 to 1.118	No	ns	> 0.9999
dCORL-23C vs. dCORL-4 control	0.1584	-1.220 to 1.537	No	ns	> 0.9999
Average score Females 10 days old					
w-1118 vs. dCORL-4 control	-0.4090	-1.732 to 0.9136	No	ns	0.9995
w-1118 vs. dCORL-21B	0.7398	-0.6089 to 2.089	No	ns	0.8723
w-1118 vs. dCORL-23C	-0.1917	-1.540 to 1.157	No	ns	> 0.9999
dCORL-21B vs. dCORL-23C	-0.9315	-2.328 to 0.4645	No	ns	0.6140
dCORL-21B vs. dCORL-4 control	-1.149	-2.520 to 0.2221	No	ns	0.2211
dCORL-23C vs. dCORL-4 control	-0.2173	-1.588 to 1.154	No	ns	> 0.9999

Table S9. Two dCORL CRISPR mutations phenocopy *Df(4)dCORL* climbing defects for Average Best score (data for Fig. 6B).

Tukey's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Average Best score Males 5 days old					
w-1118 vs. dCORL-4 control	-0.09533	-1.317 to 1.126	No	ns	> 0.9999
w-1118 vs. dCORL-21B	-3.161	-4.383 to -1.940	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	-2.785	-4.007 to -1.564	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	0.3760	-0.8453 to 1.597	No	ns	0.9995
dCORL-21B vs. dCORL-4 control	3.066	1.845 to 4.287	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	2.690	1.469 to 3.911	Yes	****	< 0.0001
Average Best score Females 5 days old					
w-1118 vs. dCORL-4 control	0.2107	-1.032 to 1.454	No	ns	> 0.9999
w-1118 vs. dCORL-21B	-2.987	-4.230 to -1.744	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	-3.009	-4.252 to -1.766	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	-0.0220	-1.243 to 1.199	No	ns	> 0.9999
dCORL-21B vs. dCORL-4 control	3.198	1.977 to 4.419	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	3.220	1.999 to 4.441	Yes	****	< 0.0001
Average Best score Males 10 days old					
w-1118 vs. dCORL-4 control	-0.1760	-1.419 to 1.067	No	ns	> 0.9999
w-1118 vs. dCORL-21B	-0.07446	-1.342 to 1.193	No	ns	> 0.9999
w-1118 vs. dCORL-23C	0.07817	-1.217 to 1.374	No	ns	> 0.9999
dCORL-21B vs. dCORL-23C	0.1526	-1.186 to 1.492	No	ns	> 0.9999
dCORL-21B vs. dCORL-4 control	-0.1015	-1.390 to 1.187	No	ns	> 0.9999
dCORL-23C vs. dCORL-4 control	-0.2542	-1.570 to 1.062	No	ns	> 0.9999
Average Best score Females 10 days old					
w-1118 vs. dCORL-4 control	-0.5973	-1.819 to 0.6240	No	ns	0.9458
w-1118 vs. dCORL-21B	0.1544	-1.113 to 1.422	No	ns	> 0.9999
w-1118 vs. dCORL-23C	-0.3926	-1.660 to 0.8748	No	ns	0.9995
dCORL-21B vs. dCORL-23C	-0.5469	-1.859 to 0.7649	No	ns	0.9870
dCORL-21B vs. dCORL-4 control	-0.7517	-2.019 to 0.5157	No	ns	0.7899
dCORL-23C vs. dCORL-4 control	-0.2048	-1.472 to 1.063	No	ns	> 0.9999

Table S10. Two dCORL CRISPR mutations phenocopy *Df(4)dCORL* climbing defects for Average Failures score (data for Fig. 6C).

Tukey's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Average Failures score Males 5 days old					
w-1118 vs. dCORL-4 control	-2.222	-22.73 to 18.28	No	ns	0.9917
w-1118 vs. dCORL-21B	-31.11	-51.62 to -10.61	Yes	***	0.0010
w-1118 vs. dCORL-23C	-28.89	-49.39 to -8.384	Yes	**	0.0025
dCORL-21B vs. dCORL-23C	2.222	-18.28 to 22.73	No	ns	0.9917
dCORL-21B vs. dCORL-4 control	28.89	8.384 to 49.39	Yes	**	0.0025
dCORL-23C vs. dCORL-4 control	26.67	6.162 to 47.17	Yes	**	0.0059
Average Failures score Females 5 days old					
w-1118 vs. dCORL-4 control	4.444	-17.57 to 26.46	No	ns	0.9503
w-1118 vs. dCORL-21B	-26.67	-48.68 to -4.650	Yes	*	0.0116
w-1118 vs. dCORL-23C	-22.22	-44.24 to -0.2056	Yes	*	0.0471
dCORL-21B vs. dCORL-23C	4.444	-17.57 to 26.46	No	ns	0.9503
dCORL-21B vs. dCORL-4 control	31.11	9.094 to 53.13	Yes	**	0.0024
dCORL-23C vs. dCORL-4 control	26.67	4.650 to 48.68	Yes	*	0.0116
Average Failures score Males 10 days old					
w-1118 vs. dCORL-4 control	-24.44	-52.40 to 3.511	No	ns	0.1067
w-1118 vs. dCORL-21B	-24.44	-52.40 to 3.511	No	ns	0.1067
w-1118 vs. dCORL-23C	-31.11	-59.07 to -3.156	Yes	*	0.0235
dCORL-21B vs. dCORL-23C	-6.667	-34.62 to 21.29	No	ns	0.9215
dCORL-21B vs. dCORL-4 control	2.035e-006	-27.96 to 27.96	No	ns	> 0.9999
dCORL-23C vs. dCORL-4 control	6.667	-21.29 to 34.62	No	ns	0.9215
Average Failures score Females 10 days old					
w-1118 vs. dCORL-4 control	-15.56	-43.86 to 12.75	No	ns	0.4711
w-1118 vs. dCORL-21B	-6.667	-34.97 to 21.64	No	ns	0.9241
w-1118 vs. dCORL-23C	-17.78	-46.08 to 10.53	No	ns	0.3527
dCORL-21B vs. dCORL-23C	-11.11	-39.42 to 17.20	No	ns	0.7272
dCORL-21B vs. dCORL-4 control	-8.889	-37.20 to 19.42	No	ns	0.8393
dCORL-23C vs. dCORL-4 control	2.222	-26.08 to 30.53	No	ns	0.9968

Table S11. Two dCORL CRISPR mutations phenocopy *Df(4)dCORL* phototaxis defects (data for Fig. 6D).

Tukey's multiple comparisons test	Mean Diff.	95% CI of Diff.	Significant?	Summary	P Value
Phototactic response Males 5 days old					
w-1118 vs. dCORL-4 control	-4.667	-15.70 to 6.370	No	ns	0.6791
w-1118 vs. dCORL-21B	23.81	12.77 to 34.85	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	33.81	22.77 to 44.85	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	-10.00	-21.04 to 1.037	No	ns	0.0889
dCORL-21B vs. dCORL-4 control	28.48	17.44 to 39.51	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	38.48	27.44 to 49.51	Yes	****	< 0.0001
Phototactic response Females 5 days old					
w-1118 vs. dCORL-4 control	-19.54	-32.13 to -6.950	Yes	***	0.0007
w-1118 vs. dCORL-21B	24.22	11.63 to 36.81	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	18.00	5.412 to 30.59	Yes	**	0.0021
dCORL-21B vs. dCORL-23C	6.222	-6.366 to 18.81	No	ns	0.5613
dCORL-21B vs. dCORL-4 control	43.76	31.17 to 56.35	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	37.54	24.95 to 50.13	Yes	****	< 0.0001
Phototactic response Males 10 days old					
w-1118 vs. dCORL-4 control	1.852	-7.792 to 11.50	No	ns	0.9576
w-1118 vs. dCORL-21B	22.03	12.39 to 31.67	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	29.05	19.40 to 38.69	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	-7.019	-18.15 to 4.117	No	ns	0.3533
dCORL-21B vs. dCORL-4 control	20.18	9.041 to 31.31	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	27.20	16.06 to 38.33	Yes	****	< 0.0001
Phototactic response Females 10 days old					
w-1118 vs. dCORL-4 control	-13.64	-25.69 to -1.598	Yes	*	0.0201
w-1118 vs. dCORL-21B	25.05	13.00 to 37.10	Yes	****	< 0.0001
w-1118 vs. dCORL-23C	23.68	11.63 to 35.73	Yes	****	< 0.0001
dCORL-21B vs. dCORL-23C	1.373	-12.54 to 15.28	No	ns	0.9938
dCORL-21B vs. dCORL-4 control	38.70	24.79 to 52.61	Yes	****	< 0.0001
dCORL-23C vs. dCORL-4 control	37.32	23.41 to 51.23	Yes	****	< 0.0001

Table S12. Quantification of age-dependent plasticity for climbing defects in two dCORL CRISPR mutations (data for Fig. 7. A,B,C).

2-tailed unpaired Student's t test	t value, dg fr*	Significant?	Summary	P Value
Average score Males (improvement of 10 day olds vs 5 day olds)				
w-1118	t=0.418 df=28	No	ns	0.6792
dCORL-4 control	t=0.037 df=28	No	ns	0.9706
dCORL-21B	t=9.846 df=27	Yes	****	< 0.0001
dCORL-23C	t=6.639 df=25	Yes	****	< 0.0001
Df(4)dCORL	t=7.693 df=23	Yes	****	< 0.0001
Average score Females (improvement of 10 day olds vs 5 day olds)				
w-1118	t=0.433 df=27	No	ns	0.0668
dCORL-4 control (got worse)	t=2.768 df=27	No	ns	0.1010
dCORL-21B	t=6.819 df=26	Yes	****	< 0.0001
dCORL-23C	t=6.639 df=25	Yes	****	< 0.0001
Df(4)dCORL	t=10.47 df=23	Yes	****	< 0.0001
Average Best score Males (improvement of 10 day olds vs 5 day olds)				
w-1118	t=0.240 df=28	No	ns	0.8121
dCORL-4 control	t=0.044 df=27	No	ns	0.9652
dCORL-21B	t=7.432 df=26	Yes	****	< 0.0001
dCORL-23C	t=6.608 df=26	Yes	****	< 0.0001
Df(4)dCORL	t=2.530 df=23	Yes	*	0.0187
Average Best score Females (improvement of 10 day olds vs 5 day olds)				
w-1118	t=0.206 df=27	No	ns	0.8384
dCORL-4 control (got worse)	t=2.198 df=28	Yes	*	0.0364
dCORL-21B	t=6.608 df=26	Yes	****	< 0.0001
dCORL-23C	t=7.974 df=26	Yes	****	< 0.0001
Df(4)dCORL	t=6.928 df=23	Yes	****	< 0.0001
Average Failures Males (improvement of 10 day olds vs 5 day olds)				
w-1118	t=0.390 df=28	No	ns	0.6994
dCORL-4 control (got worse)	t=1.275 df=28	No	ns	0.2128
dCORL-21B	t=2.198 df=28	Yes	*	0.0364
dCORL-23C	t=2.315 df=28	Yes	*	0.0282
Df(4)dCORL	t=1.050 df=28	No	ns	0.3025
Average Falures Females (improvement of 10 day olds vs 5 day olds)				
w-1118	t=2.3e-7 df=28	No	ns	>0.9999
dCORL-4 control (got worse)	t=1.877 df=28	No	ns	0.0710
dCORL-21B	t=0.4350 df=28	No	ns	0.6120
dCORL-23C	t=2.369 df=28	Yes	*	0.0249
Df(4)dCORL	t=0.9880 df=28	No	ns	0.3316

* degrees of freedom

Table S13. Two dCORL CRISPR mutations display longevity defects that are not as severe as *Df(4)dCORL* (data for Fig. 7D).

Virgin Males			Mated Males		
Genotype	Mean Lifespan	P-value vs. dCORL-4	Genotype	Mean Lifespan	P-value vs. dCORL-4
Df(4)dCORL	15.33+/-07.91	9.53E-46	Df(4)dCORL	21.17+/-10.64	1.92E-36
dCORL-4	79.92+/-26.39	n/a	dCORL-4	65.28+/-21.51	n/a
dCORL-21B	49.38+/-20.40	1.30E-16	dCORL-21B	57.52+/-24.01	0.021
dCORL-23C	64.61+/-20.83	1.27E-05	dCORL-23C	63.02+/-29.55	0.557
Virgin Females			Mated Females		
Df(4)dCORL	16.70+/-09.05	1.45E-51	Df(4)dCORL	23.76+/-13.00	4.06E-37
dCORL-4	85.65+/-25.23	n/a	dCORL-4	67.28+/-21.82	n/a
dCORL-21B	57.32+/-15.50	2.19E-17	dCORL-21B	47.37+/-21.78	1.18E-9
dCORL-23C	71.78+/-18.05	1.87E-5	dCORL-23C	53.65+/-26.32	1.70E-4