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

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ABSTRACT The CORL family of CNS-specific proteins share a Smad-binding region with mammalian SsnN 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 dCORL1 and dCORL2 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.

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 (Takesu 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.

KEYWORDS ataxia, climbing, courtship, Fussel/SKOR, phototaxis

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Our hypothesis was that the association of Fusell15 and Fusell18 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 dCORL21B and dCORL23C two CRISPR generated mutations. Overall, the finding that mutant adult 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 sphinx20Rw is as described (Dai et al., 2008). The Pbac[Rb]e02096 insertion disrupts all predicted transcripts of the elf4E-Transporter (CG32016), a gene whose primary expression is in oogenesis (Flybase; Thurmond et al. 2019). The P excision alleles Glu-RA112B (a large deletion; noted here as Glu-RA-null) and Glu-RA28 (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 shown in Figure S1. Canton-S, yw and w118 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 defects were phenocopied by dCORL21B and dCORL23C two CRISPR generated mutations. Overall, the finding that mutant adult defects due to loss of dCORL are subject to age-dependent plasticity suggests new hypotheses for CORL functions in flies and mammals.

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°C 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°C and...
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 \( \sim 10 \, \text{M} \Omega \). 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.
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 dCORL show age-dependent plasticity
In two previous studies we employed six control strains to determine if a phenotype observed for Df(4)dCORL was attributable to the loss of dCORL 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)dCORL and four lines carry mutations in the other three genes. Tested alleles for two of the genes were previously shown to be null: Glu-RA-null is a deletion resulting from an imprecise excision and spx20GRW is an engineered mutation disabling splicing. For the third gene, Pbac{RB}e02096 is predicted to disrupt all transcripts of the elf4E-Transporter and should also be a null (Fig. S1). We showed that Df(4)dCORL 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)dCORL adult behavior (8 lines total).

The project began with a climbing assay in 5 day old adults of both sexes separately for Df(4)dCORL 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)dCORL females require significantly longer time to complete the task than all other lines in both measures. Df(4)dCORL males require more time than all strains but are only significantly slower than 4 of them. We observed that Df(4)dCORL flies exhibit longer climbing times because they stop frequently and wander horizontally rather than climbing vertically. In Figure 1C we show that Df(4)dCORL 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)dCORL flies is attributable to loss of dCORL. Numerical data are shown in Table S1.

To ascertain if there was any age-dependent plasticity in the Df(4)dCORL 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)dCORL 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)dCORL now match all controls in both sexes. Age-dependent plasticity is also seen for the climbing failure rate of Df(4)dCORL 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)dCORL did not catch up as they did in climbing time. The results demonstrate that the dCORL mediated climbing defects in speed and completion of Df(4)dCORL 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)dCORL flies of both sexes were significantly impaired in their light response. The average number of photopositive Df(4)dCORL flies was roughly 10% the number in all other lines. As shown in Figure 2B, for six of the fifteen trials no Df(4)dCORL flies moved toward the light. This was
observed in the climbing and phototaxis assays that Df(4)dCORL
fls demonstrate that the light response defect of Df(4)dCORL
flies is attributable to loss of dCORL. The climbing and phototaxis data for Df(4)dCORL
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)dCORL
flies was due to a vision defect, rather than a motor defect, we compared
electroretinograms of Df(4)dCORL 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 poten-
tial (as measured in millivolts) in Df(4)dCORL flies is significantly
more robust than in the Glu-RA-control (Figure 2C). Contrast between
Df(4)dCORL and Glu-RA-control performance in the phototaxis assay
(Figure 2A with Df(4)dCORL significantly worse than Glu-RA-control)
and the retinogram assay (Figure 2C with Df(4)dCORL significantly
better than Glu-RA-control) indicates that the phototaxis defect in
Df(4)dCORL flies is not due to a vision deficit. Numerical data in
Table S3. The data support our initial hypothesis that dCORL adult
mutants will display movement-based defects.

Df(4)dCORL defects in male courtship are not attributable to loss of dCORL
We next turned to courtship assays to determine if Df(4)dCORL
flies displayed a phenotype attributable to loss of dCORL 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)dCORL was not different than Canton-S. There was a
significant reduction in courtship in 2 strains compared to Df(4)dCORL
(Figure 4A top). For males raised and mated in groups, Df(4)dCORL
was not different than Canton-S. There was a significant reduction in
courtship index for 5 strains compared to Df(4)dCORL (Figure 4A
middle). When looking within genotypes at the courtship of singles
vs. group raised males, all strains including Df(4)dCORL 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)dCORL
males raised in groups was the highest of all strains in this test except
for Canton-S. Males from the parental line Pbac{WH}f06253 and
from Pbac{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)dCORL and Canton-S, 2) strength
between group raised male courtship for Df(4)dCORL 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 Pbac{WH}f06253 and
Pbac{RB}e02096 suggest that the group raised male courtship defect
in Df(4)dCORL was not due to loss of dCORL.

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)dCORL was not different than Canton-S. There was a
significant difference of single and group raised male courtship for Df(4)dCORL and Canton-S, 2) strength
between group raised male courtship for Df(4)dCORL 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 Pbac{WH}f06253 and
Pbac{RB}e02096 suggest that the group raised male courtship defect
in Df(4)dCORL was not due to loss of dCORL.

not seen in any other line in any trial. We observed that Df(4)dCORL
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)dCORL flies is attributable to loss of
dCORL. Numerical data are shown in Table S3.

Examining 10 day old Df(4)dCORL flies for phototaxis defects
again revealed age-dependent plasticity. Df(4)dCORL 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)dCORL did not catch up. This line still
contained significantly fewer photopositive flies. The results dem-
strate that the dCORL mediated defects in light response of
Df(4)dCORL flies are subject to age-dependent plasticity. Overall we
observed in the climbing and phototaxis assays that Df(4)dCORL flies
initiate normally but are unable to sustain either behavior due to the
loss of dCORL. The climbing and phototaxis data for Df(4)dCORL
flies 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.
all strains. Df(4)dCORL was not different than Canton-S. There was a significant increase in time to orientation for 3 strains compared to Df(4)dCORL (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)dCORL) 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)dCORL time to orientation or courtship index are not attributable to loss of dCORL. 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)dCORL flies are dCORL related. **Df(4)dCORL 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)dCORL 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 Phac(RB)e02096 and Glu-RA-null. Df(4)dCORL 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)dCORL 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)dCORL adults are not developmental but are instead due to errors in adult physiology.

**dCORL<sup>21B</sup> and dCORL<sup>23C</sup> phenocopy Df(4)dCORL adult climbing and phototaxis defects**

To be sure that the climbing and phototaxis defect seen in Df(4)dCORL adults were due to loss of dCORL and not another gene in the deletion or a synergistic effect of the loss of multiple genes we generated mutations in dCORL by CRISPR. Two independent mutations were identified. A non-mutant line that went through the CRISPR process (dCORL<sup>4</sup>) was kept to serve as a control for off target effects. dCORL<sup>21B</sup> and dCORL<sup>23C</sup> 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, dCORL<sup>21B</sup> removes amino acids between Arg<sup>104</sup> and His<sup>322</sup>, while dCORL<sup>23C</sup> removes amino acids between Arg<sup>105</sup> and His<sup>322</sup> (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 dCORL<sup>21B</sup>, dCORL<sup>23C</sup> and dCORL<sup>4</sup> for climbing and phototaxis defects. We employed the w<sup>1118</sup> parental line as a further control. In the first climbing assay, average score, we found that dCORL<sup>23C</sup> and dCORL<sup>21B</sup> males and females are significantly slower than w<sup>1118</sup> and dCORL<sup>4</sup> 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)dCORL males and females as shown in Figure 1A. The second climbing assay, average best score, shows the
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)dCORL. The analysis of average score and average best score showed that dCORL21B and Df(4)dCORL males and females have a significantly different response to light than w1118 and dCORL4 at 5 days and this defect persists at 10 days (Figure 6D, Table S12). These results are the same as seen for Df(4)dCORL (Figure 2A). Overall in 7 of 8 behavioral studies (males and females in four assays) dCORL23C and dCORL21B match the results for Df(4)dCORL, 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)dCORL. The analysis of average score and average best score showed that dCORL23C and Df(4)dCORL males and females are significantly faster at 10 than 5 days, just like Df(4)dCORL (Figure 7A,B, numerical data in Table S12). The third climbing assay, average failure rate, shows the same results as both speed assays for dCORL23C and dCORL21B females at 5 and 10 days. The mutant females caught up to the controls. Alternatively, mutant males catch up to dCORL4 but not quite to w1118 (Figure 6C, Table S10). Reduction in failures for both sexes was also seen for Df(4)dCORL (Figure 1C). However, mutant males’ inability to catch up to the others is not. In the phototaxis assay dCORL23C and Df(4)dCORL males and females have a significantly weaker response to light than w1118 and dCORL4 at 5 days and this defect persists at 10 days (Figure 6D, Table S11). These results are the same as seen for Df(4)dCORL (Figure 1C). How do the data impact our hypothesis that the association of Fussel15 and Fussel18 with ataxia will be conserved and visible in...
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 dCORL23C and dCORL21B 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, are there implications for Fussel15 and Fussel18 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 Fussel18/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 Fussel18/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 6** dCORL21B and dCORL23C 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: w1118 parental, dCORL4 CRISPR control and two CRISPR deletion mutants dCORL23C and dCORL21B. Numerical data in Tables S8-S10. A) Average score shows that dCORL23C and dCORL21B males and females are significantly slower than w1118 and dCORL4 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 dCORL23C and dCORL21B males and females are significantly slower than w1118 and dCORL4 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 dCORL23C and dCORL21B males and females do not complete the climb significantly more often than w1118 and dCORL4 at 5 days old. At 10 days there is no difference between the strains for females as seen for Df(4)dCORL. dCORL23C and dCORL21B males improve but do not quite match the w1118 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 dCORL23C and dCORL21B males and females have a significantly weaker response to light than w1118 and dCORL4 at 5 days old and this defect persists at 10 days old. This is the same result as Df(4)dCORL in Fig. 2A.
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 Fussen18/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 un gainly 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 dCORL21B and dCORL23C 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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Figure 7 Quantification of age-dependent plasticity for climbing and longevity defects in dCORL23C and dCORL21B. 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 dCORL23C and dCORL21B 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. dCORL23C and dCORL21B 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 dCORL21B females improve but not significantly. D) Longevity assays of virgin and mated males and females from Df(4)dCORL, dCORL4, dCORL23C and dCORL21B. 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 dCORL23C mated males. dCORL23C and dCORL21B shortened lifespans do not show reductions of the magnitude of Df(4)dCORL.
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**Supplemental data - two figures and thirteen tables**

**Fig. S1.** dCORM genomic region displaying the *Df(4)dCORM* deletion and associated mutations. The *dCORM* cDNA (GenBank JX126878) is shown with exons shown as boxes (white - untranslated; red - protein coding). This cDNA is also known as isoformB (Genbank ACZ950983). *dCORM*, Glu-RA and *CG2316* transcript directions are shown by a poly-A tail. *sphinx* transcript direction and toy transcript direction (opposite from *dCORM*) are indicated by arrowheads. The only functional *dCORM* 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)dCORM* is shown in red. The locations of the two FRT-bearing Piggy-bac insertions employed to generate *Df(4)dCORM*, 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).

**Fig. S2.** cDNA junction sequences from two CRISPR mutated dCORM alleles. Nucleotide numbers match GenBank. A) *dCORM*²¹⁺ is missing 654bp encoding an in frame loss of 218aa. B) *dCORM*²³⁺ 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      | 0.8146  |
| *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 73.38 | 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).

| Test           | Mean Diff.       | 95% CI of Diff. | Significant? | Summary | P Value |
|----------------|------------------|-----------------|--------------|---------|---------|
| **Average score Males** |                  |                 |              |         |         |
| Df(4)dCORL vs. PB-107015 | 0.1670          | -0.5346 to 0.8686 | No           | ns      | 0.9848  |
| Df(4)dCORL vs. PB-106253 | 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-RAcontrol | -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-107015 | 0.4460          | -0.1873 to 1.079 | No           | ns      | 0.2825  |
| Df(4)dCORL vs. PB-106253 | 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-RAcontrol | -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-107015 | 0.4111          | -0.2925 to 1.115 | No           | ns      | 0.4758  |
| Df(4)dCORL vs. PB-106253 | 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-RAcontrol | 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-107015 | 0.5094          | -0.09815 to 1.117 | No           | ns      | 0.1387  |
| Df(4)dCORL vs. PB-106253 | 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-RAcontrol | -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-107015 | 31.67           | 13.08 to 50.26  | Yes          | ****    | < 0.0001 |
| Df(4)dCORL vs. PB-106253 | 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-RAcontrol | 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-107015 | 30.00           | 10.55 to 49.45  | Yes          | ***     | 0.0005  |
| Df(4)dCORL vs. PB-106253 | 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-RAcontrol | 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).

| Phototactic response Males 5 days old | Mean Diff. | 95% CI of Diff. | Significant? | Summary | P Value |
|--------------------------------------|------------|----------------|--------------|---------|---------|
| 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 | Mean Diff. | 95% CI of Diff. | Significant? | Summary | P Value |
|-----------------------------------------|------------|----------------|--------------|---------|---------|
| 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                      | -72.56     | -81.43 to -63.68 | Yes         | ****    | < 0.0001|

| Phototactic response Males 10 days old | Mean Diff. | 95% CI of Diff. | Significant? | Summary | P Value |
|----------------------------------------|------------|----------------|--------------|---------|---------|
| 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 | Mean Diff. | 95% CI of Diff. | Significant? | Summary | P Value |
|-----------------------------------------|------------|----------------|--------------|---------|---------|
| 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 | 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).

| Courtship Index (5 day old males as singles prior to courtship) | Mean Diff. | Significant? | Summary | P Value |
|---------------------------------------------------------------|------------|--------------|---------|---------|
| *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-RAnull*                              | 69.82      | Yes          | ****    | < 0.0001|
| *Df*(4)*dCORL* vs. *PB*-f06253                                | 15.56      | No           | ns      | > 0.9999|
| *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) | U value   | Significant? | Summary | P Value |
|----------------------------------------------------------------|-----------|--------------|---------|---------|
| *Df*(4)*dCORL* vs. Canton-S                                  | 120.5     | 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                                | 103.10    | Yes          | ****    | < 0.0001|
| *Df*(4)*dCORL* vs. *PB*-f06253                                | 103.10    | Yes          | ****    | < 0.0001|

| Courtship Index (5 day old males single vs. group) | 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).

| Orientation (5 day old males as singles prior to courtship) | Mean Diff. | Significant? | Summary | P Value |
|------------------------------------------------------------|------------|---------------|---------|---------|
| 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-I07015                                   | -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-I07015                                   | -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                                   |
| Canton S                                                    | 110.0      | Yes *         |         | 0.0220  |
| yw                                                          | 62.0       | Yes ****      |         | < 0.0001|
| Df(4)dCORL                                                  | 224.5      | Yes **        |         | 0.0088  |
| PB-I07015                                                   | 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 0.221 | 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 \textit{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 19.42 | 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      | -7.929 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).

| Average score Males (improvement of 10 day olds vs 5 day olds) | t value, dg fr* | Significant? | Summary | P Value |
|---------------------------------------------------------------|-----------------|---------------|---------|---------|
| 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) | t value, dg fr* | Significant? | Summary | P Value |
|----------------------------------------------------------------|-----------------|---------------|---------|---------|
| 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) | t value, dg fr* | Significant? | Summary | P Value |
|-------------------------------------------------------------------|-----------------|---------------|---------|---------|
| 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) | t value, dg fr* | Significant? | Summary | P Value |
|---------------------------------------------------------------------|-----------------|---------------|---------|---------|
| 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)  | t value, dg fr* | Significant? | Summary | P Value |
|------------------------------------------------------------------|-----------------|---------------|---------|---------|
| 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 Failures Females (improvement of 10 day olds vs 5 day olds) | t value, dg fr* | Significant? | Summary | P Value |
|--------------------------------------------------------------------|-----------------|---------------|---------|---------|
| 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 \textit{Df(4)dCORL} (data for Fig. 7D).

| Genotype     | Virgin Males | P-value vs. dCORL-4 | Genotype     | Mated Males | P-value vs. dCORL-4 |
|--------------|--------------|---------------------|--------------|-------------|---------------------|
| Virgin Males |              |                     | Mated Males  |             |                     |
| 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              |
| Mated Males  |              |                     | Mated Males  |             |                     |
| 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            |