Supporting Information

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SI Materials and Methods

Antibodies, Immunohistochemistry, and X-Gal Staining. For immunohistochemistry, guts were dissected in PBS, fixed with 4% paraformaldehyde and 0.1% glutaraldehyde in PBS, and washed in PBS. The guts were treated in an ascending series of ethanol concentrations for dehydration and embedded in LRWhite (London Resin). The guts were cut at 500 nm with an ultramicrotome (Leica) and attached to slide glasses. The samples were then stained with anti-Dcy serum as described by Micheva et al. (1). A secondary staining was performed with Alexa 594 coupled with an anti-rat antibody (Invitrogen) and mounted with DABCO (Invitrogen). Samples were scanned with an LSM 700 confocal laser microscope (Zeiss). β-Galactosidase was visualized by X-gal staining, as previously described (2), then mounted in a 50:50 mix of ethanol and glycerol. Images were captured with a Leica DFC300FX camera and the Leica Application Suite.

Electron Microscopy. *Drosophila* adults were dissected into PBS, and the guts were immediately fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in PBS for 4 h at 4 °C. The samples were rinsed three times in 0.1 M cacodylate buffer, then postfixed with 1% osmium tetroxide and 1.5% potassium ferrocyanide solution in 0.1 M cacodylate buffer for 40 min at room temperature, followed by 1% osmium tetroxide solution in 0.1 M cacodylate buffer for 40 min at room temperature. The samples were then treated with 1% uranyl acetate in water for 40 min at room temperature. Dehydration of the guts was performed in an ascending series of ethanol concentrations, and then the samples were embedded in Durcupan (Fluka). Guts were cut at 50 nm for transmission electron microscopy with a Leica ultramicrotome. Ultrathin sections were contrasted with lead citrate and observed with a transmission electron microscope.

Bacterial Persistence. Bacterial persistence was measured in wild-type and dcy^1 mutant flies by plating appropriate dilutions of homogenates of five surface-sterilized adults on LB-agar plates.

Fat and Glycogen Assays. For fat assay, 10 males were homogenized in 0.2 mL PBS 0.1% Tween-20. After heat-inactivation for 10 min at 70 °C, the lysate was centrifuged for 5 min at 15,700 × *g*. The supernatant (0.02 mL) was subjected to fat quantification using a Free Glycerol Determination Kit (Sigma). Fat amount was determined by adding the quantity of glycerol to the amount of triglycerides. For glycogen assay, 10 male flies were homogenized in 1.0 mL of cold lysis buffer [0.01 M KH_2PO_4 and 1 mM EDTA (pH 7.4)], and the lysate was centrifuged for 2 min at 400 × *g*. The supernatant (0.025 mL) was used to measure glycogen using a Glucose (HK) Assay Kit (Sigma), in which 1 U/mL of amyloglucosidase was supplemented. This reaction was blanked against a reaction in which amyloglucosidase had not been added. All reactions were carried out in 96-well plates with 0.2 mL reagent per well.

Phototaxis. A tube apparatus was used for testing flies' vision. A fly tube coated with black tape was joined to an empty fly tube. Forty female flies were placed in the dark tube, and a source light was applied at the distal end of the empty tube for 5 min. At the end of the test, the distribution of flies in each tube was quantified.

1. Micheva KD, Smith SJ (2007) Array tomography: A new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron* 55:25–36.

2. Romeo Y, Lemaitre B (2008) *Drosophila* immunity: Methods for monitoring the activity of toll and imd signaling pathways. *Methods Mol Biol* 415:379–394.

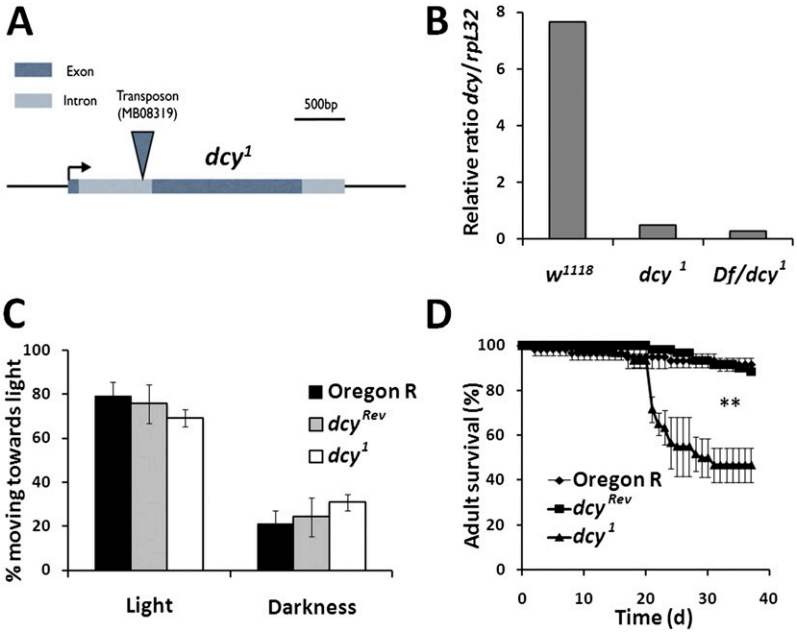

Fig. S1. *Dcy*^{1} is a strong loss-of-function mutant affecting the *drosocrystallin* gene. (*A*) Schematic representation of the *dcy* locus with the position of the *MB08319 Minos* transposon. (*B*) Real-time quantitative PCR analysis of *dcy* mRNA in *white*^{1118}, *dcy*^{1} homozygous, and *Df(dcy)/dcy*^{1}hemizygous flies. (*C*) Phototaxis of the *dcy*^{1} flies. (*D*) Lifespan analysis of unchallenged flies reveals an increase in mortality rate of *dcy*^{1} flies ($P < 0.0001$). Each survival curve corresponds to at least three independent experiments of three tubes of 20 flies each. *P* values were calculated using a log–rank test.

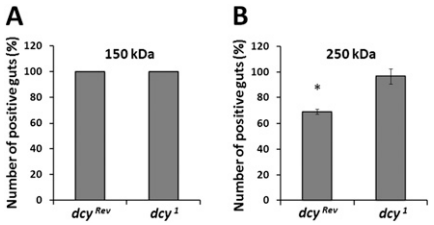

Fig. S2. Dextran-feeding assay of wild-type or *dcy*^{1} flies. Adult flies were fed with (*A*) 150-kDa or (*B*) 250-kDa FITC-labeled dextran beads. Guts were dissected and examined under a fluorescence microscope. The FITC signal is retained in the lumen if the dextran beads cannot pass through PM. The FITC signal is observed in contact with epithelial cells (indicated as positive) if beads can cross the PM. Bar graph shows the number of "positive" guts for each genotype when dextran molecules were fed. Means and SEs from three independent experiments are shown. *$P < 0.05$.

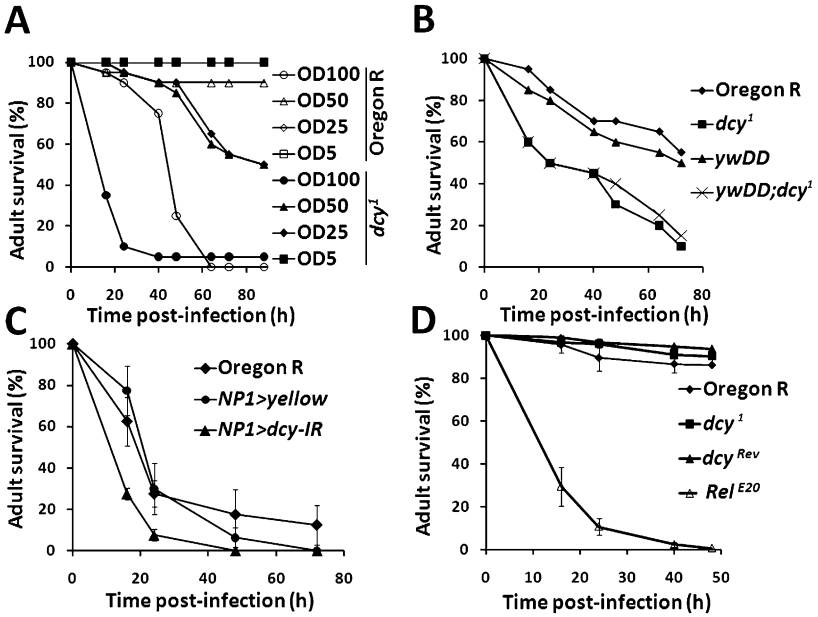

Fig. S3. Survival assays of the *dcy*^{1} mutant upon various challenges. (*A*) Survival analysis of wild-type and homozygous *dcy*^{1} flies upon oral infection with *P. entomophila* at different concentrations: OD of the *P. entomophila* feeding solution was 100, 50, 25, or 5. This experiment was repeated three times and yielded similar results ($P < 0.0003$, log–rank test). (*B*) Survival analysis of wild-type, *dcy*^{1}, *y,w, Dpt-LacZ, Drs-GFP* (referred to as *ywDD*), and *ywDD*;*dcy*^{1} flies upon oral infection with *P. entomophila* ($OD_{600} = 200$). This experiment was repeated three times and yielded similar results ($P = 0.0207$, log–rank test). (*C*) Survival analysis of wild-type, *NP1-Gal4 UAS-yellow* (*NP1>yellow*), and *NP1-Gal4 UAS-dcy-IR* (*NP1>dcy-IR*) flies upon oral infection with *P. entomophila* ($OD_{600} = 200$). Means and SEs of four independent experiments are shown ($P < 0.0003$, log–rank test). (*D*) Survival analysis of Oregon R, Canton S, *w*^{1118}, *Rel*^{E20},*dcy*^{1}, and *dcy*^{Rev} flies upon septic injury with *Ecc15*. This experiment was repeated three times and yielded similar results (not significant).

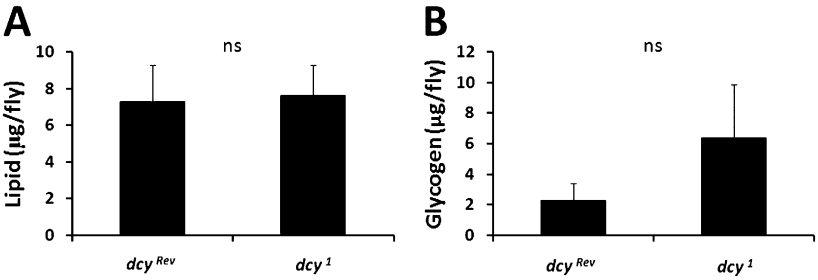

Fig. S4. *Dcy*^{1} mutation did not affect the triglyceride and glycogen stores. Quantification of fat (*A*) and glycogen (*B*) amount in *dcy*^{1} and *dcy*^{Rev} flies. Amount of fat and glycogen are in micrograms per fly. This experiment was repeated three times and yielded similar results.

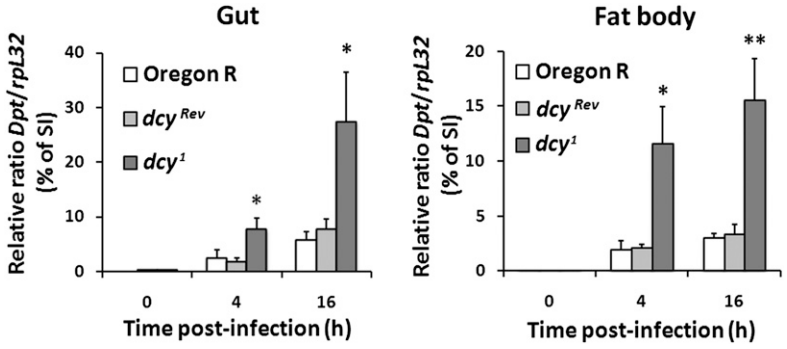

Fig. S5. *Dpt* expression upon oral *Ecc15* infection is higher in *dcy*^{1} flies compared with wild-type flies. *Dpt* expression in the midgut (*Left*) or the fat body (*Right*) of wild-type and *dcy*^{1} flies upon oral infection with *Ecc15* was measured by real-time quantitative PCR at the indicated time points. Data are the mean of four to seven independent experiments, and error bars show the SE. *$P < 0.05$, **$P < 0.01$ vs. Oregon R.

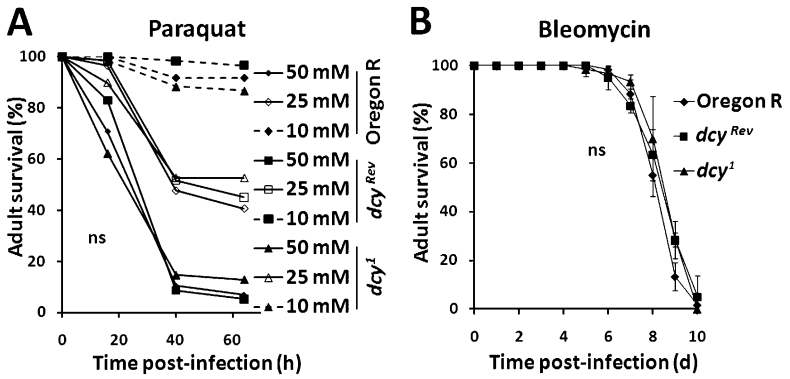

Fig. S6. *Dcy*^{1} mutation does not affect resistance to damaging agents. (*A*) Survival analysis of wild-type, *dcy*^{Rev}, and *dcy*^{1} flies upon feeding of paraquat as the indicated concentration. Data show means and SEs from three cohorts and are representative of two independent experiments. (*B*) Survival analysis of wild-type, *dcy*^{Rev}, and *dcy*^{1} flies upon feeding of 250 μg/mL of bleomycin. Data are representative of two independent experiments. ns, not significant.

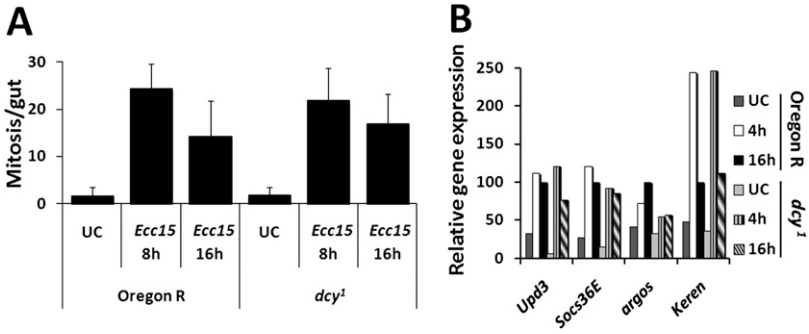

Fig. S7. *Dcy*^{1} mutation does not affect gut repair in response to *Ecc15*. (*A*) Quantification of PH3-positive cells per midgut was monitored in wild-type and *dcy* mutant flies upon *Ecc15* oral infection. Mean values from three experiments (n = 15–20 guts each) ± SE are shown. (*B*) Real-time quantitative PCR analysis of gut extracts shows that genes regulated by the JAK/STAT (*upd3*, *Socs36E*) and EGFR (*argos*, *Keren*) pathways are induced at the same levels in wild-type and *dcy*^{1} flies upon oral ingestion with *Ecc15*. Values are ratio over *RpL32* that were normalized to their maximum expression levels.