Supporting Information

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SI Text

Materials and Methods

Immunohistochemistry and Western Blots. All fluorescent images were obtained with a Zeiss LSM510 confocal microscope, using ×20 or ×40 objective lenses. The following antibodies were used: monoclonal anti-rhodopsin1 (1:50 for immunohistochemistry; 1:200 for Western blot; Developmental Studies Hybridoma Bank, University of Iowa), mouse anti-profilin (1;50 for Western blot; Developmental Studies Hybridoma Bank, University of Iowa), monoclonal 9E10 (1:500 for immunohistochemistry; Roche) and rabbit anti-Myc (1:500 for tissue-labeling, Santa Cruz) antibodies for Myc-tag detection, rabbit anti-cleaved caspase-3 antibody (1:50; Cell Signaling Technologies), guinea-pig anti-Hsc3 (1:50 for immunohistochemistry;) and rabbit anti-GFP (1:2000 for tissue-labeling, Molecular Probes) antibodies. Rhodamine phalloidin (Molecular Probes) was used to detect rhabdomeres. Immunoprecipitation was performed with anti–myc (Roche), anti-HA antibody (Roche), and protein G–coupled Sepharose beads (Roche).

Analysis of Retinal Degeneration. Flies with the relevant genotypes were crossed into *cn, br* -/- background to eliminate eye pigments, which may otherwise affect the course of retinal degeneration. These flies were selected and reared in vials (30–50 flies in each vial), in permanent light. The vials were changed frequently to avoid mixing the flies with eventual progeny. The quantification of pseudopupils was performed on a pad under blue fluorescent light after anesthetizing the flies with CO_2. Semithin plastic sections were performed as described previously and toluidine blue was used as a dye to increase the contrast.

Statistics. Statistical significance was applied through unpaired Student's *t*-test analyses.

1. Ryoo HD, Domingos PM, Kang MJ, Steller H (2007) Unfolded protein response in a *Drosophila* model for retinal degeneration. *EMBO J* 26:242–252.

2. Tomlinson A, Ready DF (1987) Cell fate in the *Drosophila* ommatidium. *Dev Biol* 123:264–275.

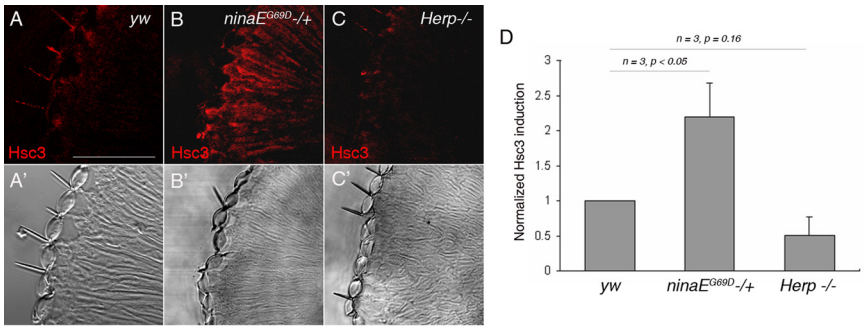

Fig. S1. Loss of *Herp* does not induce *hsc3* expression. (*A*–*C*) Horizontal sections of 1-day-old adult eyes labeled with anti-Hsc3 antibody (red). (*A*′-*C*′) Bright field image of the samples shown in *A*–*C*. Compared to the basal level of Hsc3 detected in a control fly (*A*), *ninaE*^{G69D} -/+ retina have significantly higher levels of Hsc3 (*B*), indicative of UPR activation. Hsc3 levels remain at a basal level in *Herp* -/- retina (*C*). (*D*) Comparison of the anti-Hsc3 labeling intensity in these retina (average of $n = 3$), with the value from the control fly (*y,w*) retina set at 1. Scale bar represents 50 μM (*A*). Genotypes: *y,w* (*A*, *A*′), *w; ninaE*^{G69D}-/+ (*B*, *B*′), *w; Herp*^{G13463}/*Herp*^{G13463} (*C*, *C*′).

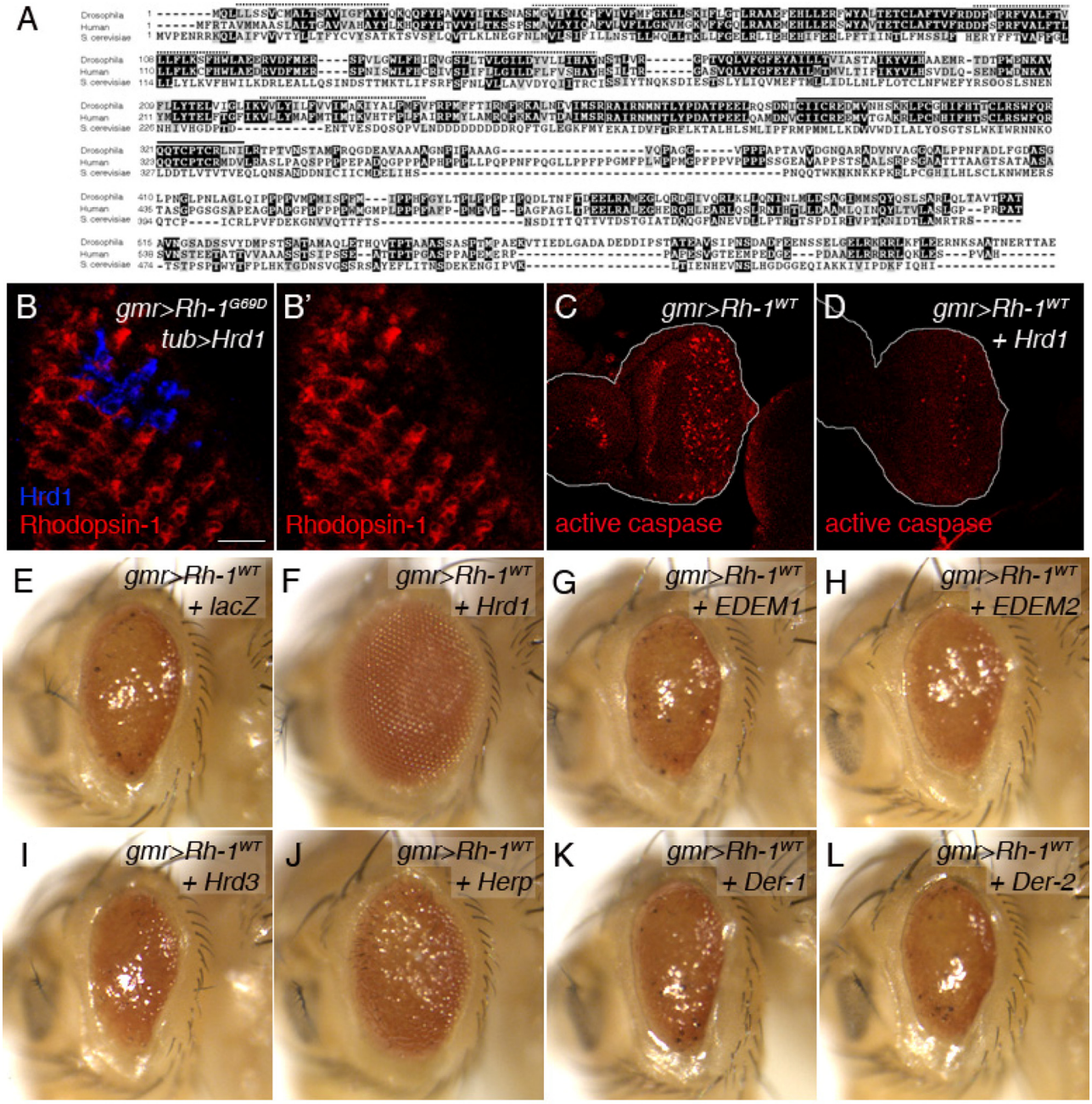

Fig. S2. Overexpression of putative ERAD components in *Drosophila*. (*A*) ClustalW amino acid alignment between *Drosophila* hrd1 and its human homolog. Dark shading indicates identity, whereas light shading indicates similarity. The numbers refer to amino acids. The solid line indicates the RING-finger domain (amino acid numbers 289–331). The regions with the predicted transmembrane domains are marked by dotted lines. (*B*, *B'*) The effect of Hrd1 on misexpressed Rh-1^{G69D}, as determined through Hrd1 expressing mosaic clones. Here, Rh-1^{G69D} (red) was expressed through the *gmr* promoter while Hrd1 (blue) was expressed in mosaic clones through the *tubulin* promoter. (*C*, *D*) The misexpression of Rh-1^{WT} led to massive apoptosis as detected by anti-cleaved caspase labeling (*C*, red), which was almost completely suppressed by Hrd1 co-expression (*D*, red). (*E–L*) External adult eyes misexpressing Rh-1^{WT} together with the indicated *Drosophila* ERAD factors or control genes. Shown are representative images of flies co-expressing lacZ (*E*), Hrd1 (*F*), EDEM1 (*G*), EDEM2 (*H*), Hrd3 (*I*), Herp (*J*), Derlin-1 (*K*), Derlin-2 (*L*). Only Hrd1 was sufficient to suppress the eye ablation phenotype (*F*). Scale bar represents 20 μM (*B*). Genotypes: *hs-flp;UAS-Hrd1/+;tub>GFP>Gal4/gmr-Rh-1^{G69D}* (*B*, *B'*), *gmr-Gal4/+;UAS-Rh-1^{WT}/+* (*C*), *gmr-Gal4/UAS-Hrd1;UAS-Rh-1^{WT}/+* (D, F), *gmr-Gal4/UAS-lacZ;UAS-Rh-1^{WT}/+* (E), *gmr-Gal4/+;UAS-Rh-1^{WT}/UAS-EDEM1* (G), *gmr-Gal4/UAS-EDEM2;UAS-Rh-1^{WT}/+* (H), *gmr-Gal4/UAS-Hrd3;UAS-Rh-1^{WT}/+* (I), *gmr-Gal4/+;UAS-Rh-1^{WT}/UAS-Herp* (J), *gmr-Gal4/+;UAS-Rh-1^{WT}/UAS-Der-1* (*K*), *gmr-Gal4/+;UAS-Rh-1^{WT}/UAS-Der-2* (*L*).

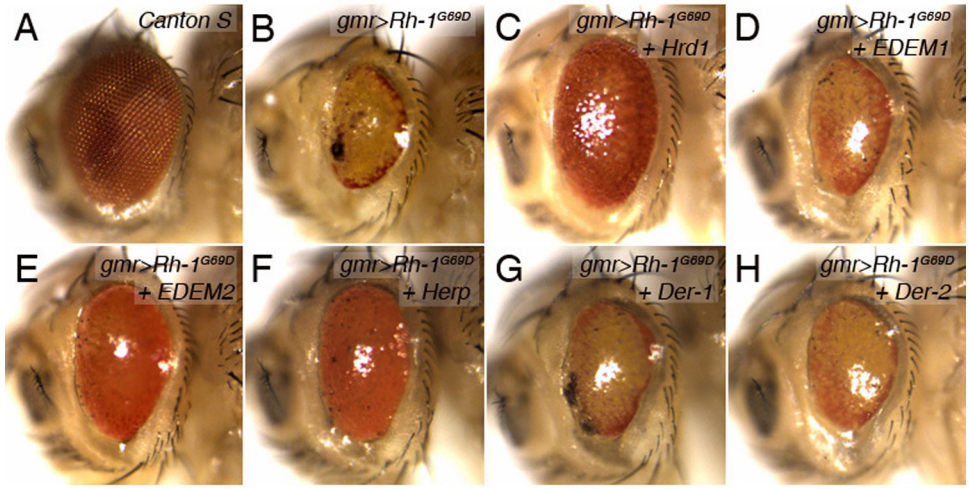

Fig. S3. The eye phenotype caused by misexpression of Rh-1^{G69D} was partially rescued by Hrd1, EDEM2, and Herp. Stress levels are reflected by the degree of eye size reduction and loss of pigmentation due to pigment cell death. (*A*) A control wild type fly. (*B*) A fly expressing Rh-1^{G69D} alone. (*C–H*) Representative images of fly eyes misexpressing Rh-1^{G69D} together with the indicated *Drosophila* ERAD factors, Hrd1 (*C*), EDEM1 (*D*), EDEM2 (*E*), Herp (*F*), Derlin-1 (*G*), Derlin-2 (*H*). Hrd1, EDEM2, and Herp partially suppressed the eye phenotype caused by Rh-1^{G69D}. Genotypes: *Canton S* (*A*), *gmr-Gal4, UAS-Rh-1^{G69D}/+* (*B*), *gmr-Gal4, UAS-Rh-1^{G69D}/UAS-Hrd1* (*C*), *gmr-Gal4,UAS-Rh-1^{G69D}/+;UAS-EDEM1/+* (*D*), *gmr-Gal4, UAS-Rh-1^{G69D}/UAS-EDEM2* (*E*), *gmr-Gal4, UAS-Rh-1^{G69D}/+;UAS-Herp/+* (*F*), *gmr-Gal4, UAS-Rh-1^{G69D}/UAS-Der-1* (*G*),*gmr-Gal4, UAS-Rh-1^{G69D}/+;UAS-Der-2/+* (*H*).

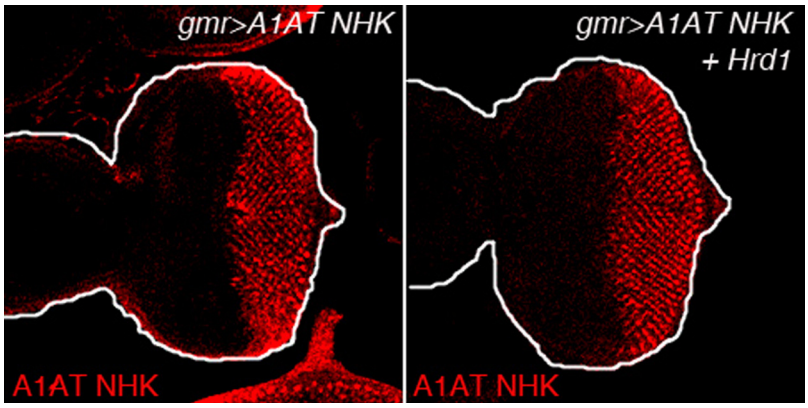

Fig. S4. *Drosophila* Hrd1 does not affect NHK stability. The level of NHK was detected by anti-A1AT labeling (red). Genotypes: *gmr-Gal4/UAS-NHK* (*Left*), *gmr-Gal4/UAS-NHK;UAS-Hrd1/+* (*Right*).

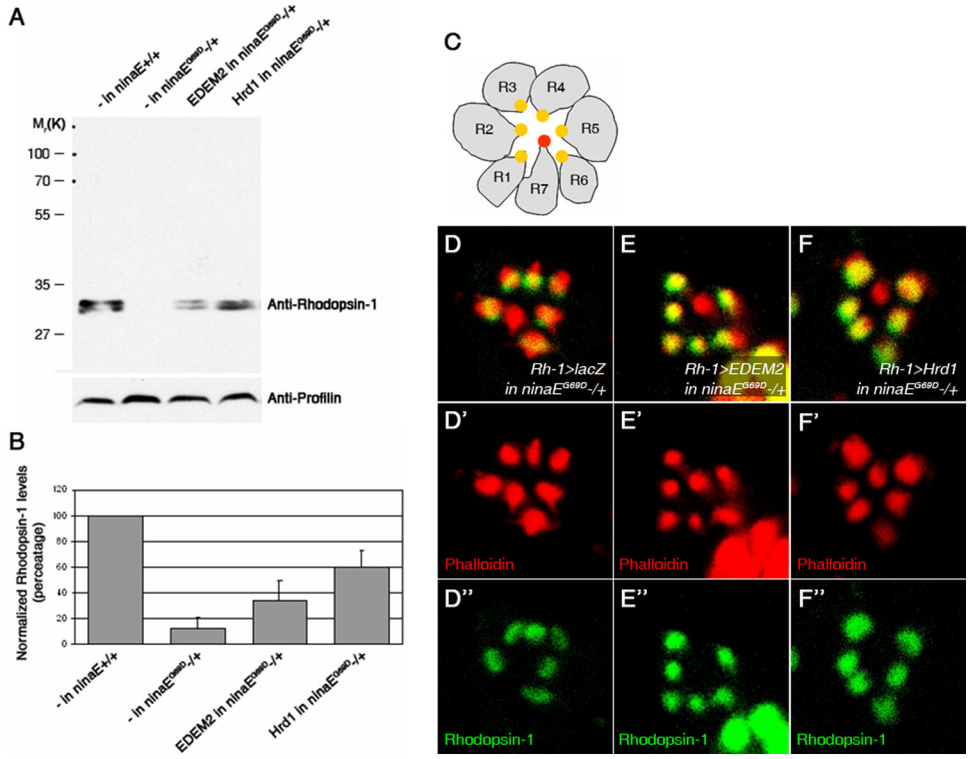

Fig. S5. Overexpression of ERAD regulators partially restores the level of Rh-1 in the retina of *ninaE*^{G69D}-/+ flies. (*A*) The protein level of Rh-1 was probed with anti-Rh-1(4C5) antibody (upper gel). Anti-profilin blots were used as loading controls (lower gel). (*B*) Quantification of the average normalized Rh-1 band intensity (average of $n = 4$), with the value from wild type of Rh-1+/+ heads extracts set at 100%. Overexpression of EDEM2 ($P = 0.017$) and Hrd1 ($P < 0.001$) in *ninaE*^{G69D} -/+ flies makes a statistically meaningful difference from those without gene overexpression. (*C*) A schematic representation of R1–7 photoreceptor cells and their rhabdomeres in adult retina. While the rhabdomeres of the R1–6 photoreceptors are shown in yellow, the rhabdomere of the R7 photoreceptor is shown in red. Rh-1 protein is only expressed in the R1–6 photoreceptors. (*D–F*) Overexpression of EDEM2 or Hrd1 enhances the localization of Rh-1 to rhabdomeres in *ninaE*^{G69D} -/+ retina. Phalloidin, which labels rhabdomeric F-Actin, was labeled in red. Rh-1 was shown in green. Many rhabdomeres of *ninaE*^{G69D} -/+ retina expressing a control lacZ gene showed limited anti-Rh-1 labeling within the phalloidin labeling region, as evident by largely red rhabdomeres (*D*, *D''*; $n = 18$, only 44.4% of the examined rhabdomeres show full loverlap between red and green channels). Rh-1 targeting to the rhabdomeres was significantly improved when EDEM2 (*E*, $n = 18$, 100% of rhabdomeres with full overlap between the rhabdomere marker and Rh-1) or Hrd1 (F, $n = 25$, 96% of rhabdomeres with full overlap) was overexpressed in this background, as evident by the yellow color of rhabdomeres. Genotypes: (*A*) (lane 1) *Rh1-Gal4;; ninaE*+/+, (lane 2) *Rh1-Gal4;;ninaE*^{G69D}/+, (lane 3) *Rh1-Gal4;UAS-EDEM2/+;ninaE*^{G69D}/+, (lane 4) *Rh1-Gal4;UAS-Hrd1/+;ninaE*^{G69D}/+. (*D-D''*) *Rh1-Gal4;cn.br, uas-lacZ/cn.br;ninaE*^{G69D}/+, (*E-E''*) *Rh1-Gal4;cn.br, uas-EDEM2/cnbr;ninaE*^{G69D}/+,(*F-F''*) *Rh1-Gal4;cn.br, uas-Hrd1/cn.br;ninaE*^{G69D}/+.