**A Drosophila model to study retinitis pigmentosa pathology associated with mutations in the core splicing factor Prp8**

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**Fig. S1. Expression patterns of Gal4 drivers used in the study.**

(A) *phm-Gal4* driver is active in the *Drosophila* prothoracic gland as depicted by GFP expressed from the UAS-based transgene. (B) *ey-Gal4* is active in the eye primordium of the larval EAD in cells both anterior and posterior to the morphogenetic furrow (arrowhead) as depicted by RFP expression. (C) The late acting *GMR-Gal4* driver expresses in cells posterior to the morphogenetic furrow (arrowhead) of larval EAD as indicated by the RFP signal. (A-C) Micrographs show projections of multiple confocal sections of respective tissues dissected from third instar larvae 7 days AEL. Scale bars: 50 µm (A) and 100 µm (B, C).
Fig. S2. Prp8 antibody detects endogenous as well as transgenic *Drosophila* Prp8 proteins. (A-B) *eyFLP*-mediated mitotic recombination was used to generate clones (GFP) in EADs of the indicated genotypes. Immunostaining of EADs with the self-made antibody directed against the CTD of the *Drosophila* Prp8 protein revealed reduction of Prp8 levels in clones where *prp8* transcript was knocked down by RNAi relative to non-clonal tissue (A’). In contrast, Prp8 levels were increased in cells overexpressing Prp8\(^{wt}\) (B’). Note the cytoplasmic localization of the endogenous and transgenic Prp8 proteins (B’). Discs were counterstained with DAPI. Micrographs are single confocal slices of EADs 7 days after egg laying. Scale bars: 10 \(\mu\)m.
**Figure S3**

| GMR-Gal4 |  |
|----------|---|
| **A** | **B** | **C** | **D** | **E** | **F** |
| control | Prp8<sup>WT</sup> | Prp8<sup>S</sup>-<sup>E</sup> | Prp8<sup>R</sup>-<sup>G</sup> | Prp8<sup>R</sup>-<sup>K</sup> | Prp8<sup>F</sup>-<sup>L</sup> |
| **G** | **H** | **I** | **J** | **K** | **L** |
| Prp8<sup>V</sup>-<sup>N</sup> | Prp8<sup>R</sup>-<sup>P</sup> | Prp8<sup>H</sup>-<sup>R</sup> | Prp8<sup>R</sup>-<sup>S</sup> | Prp8<sup>R</sup>-<sup>T</sup> | prp8<sup>RNAi</sup> |

**Fig. S3.** Expression of RP-Prp8 variants under the **GMR-Gal4** driver is asymptomatic.

(A-L) Overexpression of wild-type Prp8 (B) or any of the nine different RP-Prp8 mutant variants (C-K) or RNAi-mediated knockdown (**prp8**<sup>RNAi</sup>) (L) using the late-acting **GMR-Gal4** driver did not have any detrimental effect on the development of the adult eye and were comparable to control (A).
Fig. S4. Differential impact of early induction of RP-Prp8 mutations on the adult eye development.

(A-K) Expression of Prp8<sup>S>F</sup> (C) and Prp8<sup>H>R</sup> (I) in the EADs using the early acting ey-Gal4 driver resulted in rough and irregularly shaped adult eyes compared to control (A) and those expressing Prp8<sup>wt</sup> (B) or other RP-Prp8 variants (D-H, J, K).
Fig. S5. Prp8 loss is incompatible with cell survival and halving the prp8 gene dose enhances RP-Prp8-induced phenotypes.

(A-D) eyFLP-mediated mitotic recombination was used to generate homozygous clones (GFP) in EADs of the indicated genotypes. In contrast to control (A), cells homozygous for the prp8\textsuperscript{del14} deletion allele (B) or the prp8\textsuperscript{KG03188} insertion allele (C) do not survive and do not contribute to the total volume of the EAD tissue (D). (E-L) RP-Prp8 mutant variants were expressed in prp8\textsuperscript{KG03188/+} heterozygous
background using the ey-Gal4 driver. In contrast to controls (w^{1118}+/+, E; prp8\textsuperscript{KG03188}+/+, F) and asymptomatic overexpression of Prp8\textsubscript{wt} (G) and Prp8\textsubscript{F>L} (H), Prp8\textsubscript{S>F} disrupts morphology and reduces size of the adult eyes (I, L) mimicking phenotypes observed when overexpressed in prp8\textsubscript{del14}+/+ heterozygotes (J, L). (D, L) Data represent means ± s.d., n=6-9 (D), n=22-59 (L). Statistical significance was determined using ordinary one-way ANOVA with Tukey’s multiple comparisons test; **P<0.01, ****P<0.0001, n.s. = non-significant. The exact number of biological replicates per genotype (n) and P-values are specified in Supplementary Dataset 2.
Fig. S6. Blocking apoptosis does not suppress Prp8<sup>S>F</sup>-mediated GstD1 induction.

(A-D) Eye-specific expression of Prp8<sup>S>F</sup> in the <i>prp8<sup>del14</sup></i>/+ heterozygous background using the ey-Gal4 driver induces apoptosis (A') and activity of the GstD1-GFP reporter (A''). While co-expression of p35 effectively suppresses Prp8<sup>S>F</sup>-induced apoptosis (B') it does not block upregulation of the GstD1-GFP reporter (B''). Expression of the mock Flag tripeptide alone does not induce cell death or the GstD1-GFP reporter (C). Flag co-expression does not remedy the phenotypic consequences of Prp8<sup>S>F</sup> (D).
Table S1. List of *Drosophila* lines.

| Name                              | Genotype                                           | Source                                              | Identifier               |
|-----------------------------------|----------------------------------------------------|-----------------------------------------------------|--------------------------|
| ey>                               | *w*; *P*[*w*^m*=GAL4-ey.H]*3-8                   | Bloomington Drosophila Stock Center                 | RRID:BDSC_5534           |
| UAS-mCD8.ChRFP                    | *w*; *P*[*w*^m*=UAS-mCD8.ChRFP]*2               | Bloomington Drosophila Stock Center                 | RRID:BDSC_27391          |
| ey>mCD8.ChRFP                     | *w*; *P*[*w*^m*=GAL4-ey.H]*3-8, UAS-mCD8.ChRFP/CyO | this study                                          | N/A                      |
| GstD1-GFP, ey>mCD8.ChRFP          | *w*; *P*[*w*^m*=GAL4-ey.H]*3-8, UAS-mCD8.ChRFP, GstD1-GFP/CyO | this study                                          | N/A                      |
| GstD1-GFP                         | *w*; GstD1-GFP/CyO                                 | Sykiotis and Bohmann (2008)                         | N/A                      |
| GstD1-GFP, ey>mCD8.ChRFP, p35     | *w*; *P*[*w*^m*=GAL4-ey.H]*3-8, UAS-mCD8.ChRFP, pTFW, GstD1-GFP/CyO | this study                                          | N/A                      |
| GstD1-GFP, ey>mCD8.ChRFP, Flag    | *w*; *P*[*w*^m*=GAL4-ey.H]*3-8, UAS-mCD8.ChRFP, pTFW, GstD1-GFP/CyO | this study                                          | N/A                      |
| dpp>                             | *y^7*; *w*^67c23, *P*[*w*^mc*=dpp-GAL4.PS]*6A/TM3, Ser^1 | Bloomington Drosophila Stock Center                 | RRID:BDSC_7007           |
| dpp>mCD8.ChRFP                    | *w*; ey-Gal4, UAS-mCD8.ChRFP/CyO                  | this study                                          | N/A                      |
| phm>                             | *y^1*; *w*[^*]; *P*[*w*^mc*=phm-GAL4.O]*22       | Ono et al. (2006)                                  | RRID:BDSC_80577          |
| phm>mCD8::GFP                     | *w*; phm-GAL4, UAS-mCD8::GFP.L/TM6B               | Ono et al. (2006)                                  | N/A                      |
| GMR>                             | *w*; *P*[*w*^mc*=GAL4-ninaE.GMR]*12              | Bloomington Drosophila Stock Center                 | RRID:BDSC_1104           |
| UAS-yr-mRFP                      | *w*[^118*, *P*[*w*^mc*=UAS-yr-mRFP]*1           | Bloomington Drosophila Stock Center                 | RRID:BDSC_7118           |
| GMR>yr-mRFP                      | *w*; GMR-Gal4, UAS-yr-mRFP/CyO                    | this study                                          | N/A                      |
| ey MARCM>>FRT82B Green           | eyFLP; act> ey>*Gal4, UAS-GFP; *P*[*ry*^17.2*=neoFRT]82B tub-Gal80 | Pagliarini and Xu (2003)                            | N/A                      |
| ey MARCM>>FRT42D Green           | eyFLP; *P*[*ry*^17.2*=neoFRT]42D tub-Gal80/T(2;3)B3, CyO:TM6 Tb^1/act> ey>*Gal4, UAS-GFP | this study                                          | N/A                      |
| FRT42D                           | *P*[*ry*^17.2*=neoFRT]42D; *ry*[^205*]             | Bloomington Drosophila Stock Center                 | RRID:BDSC_1802           |
| Strain | Description | Source | RRID |
|--------|-------------|--------|------|
| **FRT82B** | $P(r^{y^{17.2}=neoFRT})82B\ ry^{60S}$ | Bloomington Drosophila Stock Center | RRID:BDSC_2035 |
| **Act5C-cas9, Lig4[169]** | $y^{1}, M(Act5C-Cas9)ZH-2A, w^{119}, Lig4^{169}$ | Zhang et al. (2014) | RRID:BDSC_54590 |
| **sgRNA-Prp8** | $v^{1};; pCFD4-U6:1-U6:3\ sgRNA-Prp8/\ TM6b$ | this study | N/A |
| **nos-phiC31int;;attP2** | $y^{1}\ sc^{1}\ v^{1}\ P(y^{17.7}=nos-\ phiC31int.NLS)X;\ P(y^{17.7}=CaryP)\ attP2$ | Bloomington Drosophila Stock Center | RRID:BDSC_25710 |
| **Prp8wt** | $w;; pUAST-attB-Prp8^{wt}\ attP-9A/\ TM6B$ | this study | N/A |
| **Prp8H>R** | $w;; pUAST-attB-prp8^{H2369R}\ attP-9A/\ TM6B$ | this study | N/A |
| **Prp8H>P** | $w;; pUAST-attB-prp8^{H2369P}\ attP-9A/\ TM6B$ | this study | N/A |
| **Prp8Y>N** | $w;; pUAST-attB-prp8^{Y2395N}\ attP-9A/\ TM6B$ | this study | N/A |
| **Prp8F>L** | $w;; pUAST-attB-prp8^{F2374L}\ attP-9A/\ TM6B$ | this study | N/A |
| **Prp8R>G** | $w;; pUAST-attB-prp8^{H2370G}\ attP-9A/\ TM6B$ | this study | N/A |
| **Flag::Prp8wt** | $w;; pUAST-attB-Flag::Prp8^{wt}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8H>R** | $w;; pUAST-attB-Flag::Prp8^{H2369R}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8H>P** | $w;; pUAST-attB-Flag::Prp8^{H2369P}\ attP2/\ TM3$ | this study | N/A |
| **Flag::Prp8F>L** | $w;; pUAST-attB-Flag::Prp8^{F2374L}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8R>G** | $w;; pUAST-attB-Flag::Prp8^{H2370G}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8Y>N** | $w;; pUAST-attB-Flag::Prp8^{Y2395N}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8F>L** | $w;; pUAST-attB-Flag::Prp8^{F2374L}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8R>G** | $w;; pUAST-attB-Flag::Prp8^{H2370G}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8Y>N** | $w;; pUAST-attB-Flag::Prp8^{Y2395N}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8F>L** | $w;; pUAST-attB-Flag::Prp8^{F2374L}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8R>G** | $w;; pUAST-attB-Flag::Prp8^{H2370G}\ attP2/\ TM6B$ | this study | N/A |
| **Flag::Prp8Y>N** | $w;; pUAST-attB-Flag::Prp8^{Y2395N}\ attP2/\ TM6B$ | this study | N/A |
| Genotype | Stock Description | Notes |
|----------|-------------------|-------|
| FRT42D, prp8<sup>del14</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup>, prp8<sup>del14</sup>/CyO, P[ActGFP]<sup>JMR1</sup> | this study, N/A |
| FRT42D, prp8<sup>del14</sup>, Prp8<sup>S>F</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup>, prp8<sup>del14</sup>/<i>T</i>(2;3)B3, CyO:TM6<sup>Tb</sup>/<i>pUAST-attB-Prp8</i><sup>S2178F</sup>attP-9A | this study, N/A |
| FRT42D, prp8<sup>del14</sup>, Prp8<sup>wt</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup>, prp8<sup>del14</sup>/<i>T</i>(2;3)B3, CyO:TM6<sup>Tb</sup>/<i>pUAST-attB-Prp8</i><sup>del14</sup>attP-9A | this study, N/A |
| FRT42D, prp8<sup>del14</sup>, Prp8<sup>F>L</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup>, prp8<sup>del14</sup>/<i>T</i>(2;3)B3, CyO:TM6<sup>Tb</sup>/<i>pUAST-attB-Prp8</i><sup>F2374L</sup>attP-9A | this study, N/A |
| prp8<sup>RNAi</sup> | w; UAS-prp8<sup>RNAi</sup>[GD18555] | Vienna Drosophila Resource Center, N/A |
| prp8<sup>RNAi</sup>, FRT82B | w; UAS-prp8<sup>RNAi</sup>[GD18555];<i>P</i>[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>82B</sup> | this study, N/A |
| FRT42D;Prp8<sup>wt</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup>;<i>pUAST-attB-Prp8</i><sup>del14</sup>attP-9A/ TM6B | this study, N/A |
| p35 | w; UAS-p35 | Bloomington Drosophila Stock Center, RRID: BDSC_5072 |
| FRT42D prp8<sup>KG03188</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup> P[y<sup>mDint2</sup>BR.E.BR=SUPor-P]<i>Prp8</i><sup>KG03188</sup>/CyO, y<sup>+</sup> | Kyoto stock Center 111506 |
| prp8<sup>KG03188</sup> | P[SUPor-P]<i>Prp8</i><sup>Prp8</sup><sup>KG03188</sup>/CyO, P[ActGFP]<sup>JMR1</sup> | Bloomington Drosophila Stock Center, RRID:BDSC_13006 |
| FRT42D prp8<sup>KG03188</sup>, Prp8<sup>F>L</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup> P[y<sup>mDint2</sup>BR.E.BR=SUPor-P]<i>Prp8</i><sup>KG03188</sup>/<i>T</i>(2;3)B3, CyO:TM6<sup>Tb</sup>/<i>pUAST-attB-Prp8</i><sup>F2374L</sup>attP-9A | Kyoto stock Center 111506 |
| FRT42D prp8<sup>KG03188</sup>, Prp8<sup>wt</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup> P[y<sup>mDint2</sup>BR.E.BR=SUPor-P]<i>Prp8</i><sup>KG03188</sup>/<i>T</i>(2;3)B3, CyO:TM6<sup>Tb</sup>/<i>pUAST-attB-Prp8</i><sup>wt</sup>attP-9A | Bloomington Drosophila Stock Center, RRID:BDSC_13006 |
| FRT42D, prp8<sup>KG03188</sup>, Prp8<sup>S>F</sup> | w; P[<i>ry</i><sup>17.2</sup>=neoFRT]<sup>42D</sup>, prp8<sup>del14</sup>/<i>T</i>(2;3)B3, CyO:TM6<sup>Tb</sup>/<i>pUAST-attB-Prp8</i><sup>S2178F</sup>attP-9A | this study, N/A |
Table S2. List of vectors and plasmids.

| Name                        | Source                      | Catalog Number | Identifier |
|-----------------------------|-----------------------------|----------------|------------|
| pDest17                     | Thermo Scientific           |                | N/A        |
| pDest17 Prp8-CTD            | this study                  |                | N/A        |
| pCFD4-U6:1_U6:3tandemgRNAs  | Port et al. (2014), Addgene |                | N/A        |
| CFD4-U6:1-U6:3 Prp8 sgRNA   | this study                  |                | N/A        |
| pAW-GAL4                    | Y. Hiromi                   |                | N/A        |
| pIE-EGFP                    |                             |                | N/A        |
| pUAST-attB                  | Drosophila Genomics Resource Center |        | N/A        |
| pTFW                        | Drosophila Genomics Resource Center |        | N/A        |
| pUAST-attB-Prp8[H2369R]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[H2369P]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[Y2395N]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[F2374L]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[H2370G]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[wt]         | this study                  |                | N/A        |
| pUAST-attB-Prp8[S2178F]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[R2370K]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[R2370S]     | this study                  |                | N/A        |
| pUAST-attB-Prp8[P2361T]     | this study                  |                | N/A        |
| pUAST-attB-Flag::Prp8[H2369R] | this study              |                | N/A        |
| pUAST-attB-Flag::Prp8[H2369P] | this study              |                | N/A        |
| pUAST-attB Flag::Prp8[wt]   | this study                  |                | N/A        |
| pUAST-attB-Flag::Prp8[R2370K] | this study              |                | N/A        |
| pUAST-attB-Flag::Prp8[P2361T] | this study              |                | N/A        |
| pUAST-attB-Flag::Prp8[S2178F] | this study              |                | N/A        |
| pUAST-attB-Flag::Prp8[R2370S] | this study              |                | N/A        |
| pENTR4-Prp8[H2369R]         | this study                  |                | N/A        |
| pENTR4-Prp8[R2370K]         | this study                  |                | N/A        |
| pENTR4-Prp8[R2370S]         | this study                  |                | N/A        |
| pENTR4-Prp8[F2374L]         | this study                  |                | N/A        |

Disease Models & Mechanisms: doi:10.1242/dmm.043174: Supplementary information
| Construct               | Source                | Reference   |
|------------------------|-----------------------|-------------|
| pENTR4-Prp8[S2178F]    | this study            | N/A         |
| pENTR4-Prp8[F2374L]    | this study            | N/A         |
| pENTR4-Prp8[Y2395N]    | this study            | N/A         |
| pENTR4-Prp8[H2370G]    | this study            | N/A         |
| pENTR-Prp8[H2369P]     | this study            | N/A         |
| pENTR4-Prp8[P2361T]    | this study            | N/A         |
| pENTR4-Prp8[wt]        | this study            | N/A         |
| PTFW-Prp8[wt]          | Claudius et al. (2014)| N/A         |
Table S3. List of oligonucleotides.

| Name | Sequence | Purpose |
|------|----------|---------|
| sgRNA_pCFD4_Prp 8_Intron_12_For | TATATAGAAAGATATCCGGTGAACTGTACTAGTACATATGCTAAGTGTTTTAGAGCTAGAAATAGCAAG | cloning |
| sgRNA_pCFD4_Prp 8_3'UTR_1_Rev | ATTTTAACTTGCTATTTCTAGCTCTAAAAACTAAGACTCCATA | cloning |
| Spok iQ For | GCTCTTTTGCGGTGATCGAAACAA | qPCR |
| Spok iQ Rev | CGCCGAGCTAAATTTCTCCGCTTT | qPCR |
| Spok iQ For intron | GCCATCCTCTTTAAGGAGTGTGGTCAT | qPCR |
| Spok iQ Rev intron | TGCGCACCGACGTTAAATTGAAAATAGGTC | qPCR |
| Rpl49 For | TCCTACCAGCTTCAAGTGAC | qPCR |
| Rpl49 Rev | CACGTTGTCGCCAGGACT | qPCR |
| Prp8_H2369R_For | ATTCACGAGTTGCGTCACCGGCGAAGCCCA | mutagenesis |
| Prp8_H2369R_Rev | TGGCAGGTCGACCGCAACTCGTGAT | mutagenesis |
| Prp8_H2309P_For | ATTCACGAGTTGCGTCACCGGCGAAGCCCA | mutagenesis |
| Prp8_H2309P_Rev | GAGGTGCGAGGCAACTCGTGAT | mutagenesis |
| Prp8_Y2334N_for | CGGGAGGATGTGAACGCGTAAGCGC | mutagenesis |
| Prp8_Y2334N_rev | GCGCTTACGCGTTCACATCCTCCC | mutagenesis |
| Prp8_F2374L_For | ACCTCGCATTTTACTGCTCTTCTCG | mutagenesis |
| Prp8_F2374L_Rev | AATGCGAGGTCTTATGCAACTCGT | mutagenesis |
| Prp8_R2310G_For | ACGATGTCATCTTCTTCGGCG | mutagenesis |
| Prp8_R2310G_Rev | AATGCGAGGTCTTATGCAACTCGT | mutagenesis |
| PRP8 cDNA Bcl1 Forward | AAATGATCACGATGTCCATTCCGCCGTACATG | cloning |
| PRP8 cDNA Not1 Rev | AAAGCGGGCGGCGCTTACGCGTACACATCCTCCC | cloning |
| PRP8 CTD EcoRI For | AAGAATTTTCATGCGACACAGGGAAGGCAACAA | cloning |
| ets21c iQ For | ATTAATGCGCATCGACAGGTGATGGCC | qPCR |
| ets21c iQ Rev | GGGAGGCGGTACGTCTCCTCCC | qPCR |
| GstE6 iQ For | CCAAGGACGCGATACGAGCCCA | qPCR |
| GstE6 iQ Rev | CCACGAAAGGCGTCAAGGGAG | qPCR |
Supplementary Dataset 1

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