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

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Figure S1

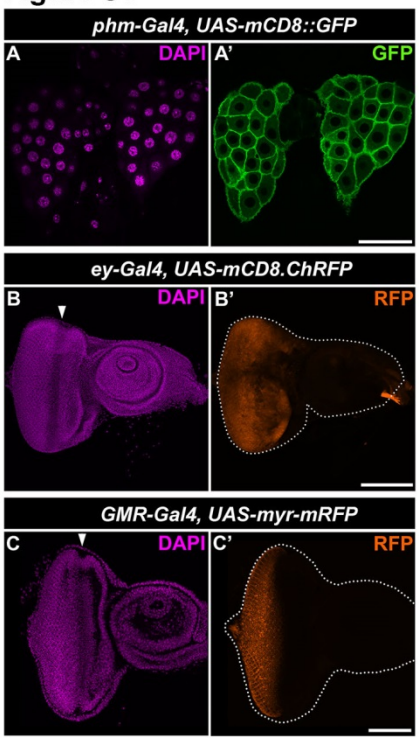

Fig. S1. Expression patterns of Gal4 drivers used in the study.

(**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).

Figure S2

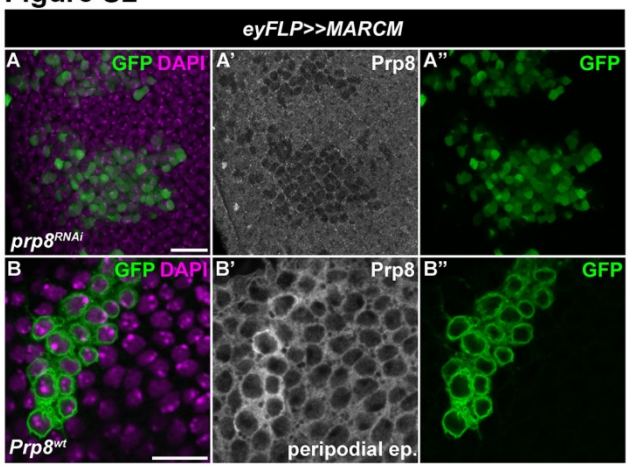

Fig. S2. Prp8 antibody detects endogenous as well as transgenic *Drosophila* Prp8 proteins. (**A-B**) *eyFLP*-mediated mitotic recombination was used to generate clones (GFP) in EADs of the indicated genotypes. Immunostaining of EADs with the self-made antibody directed against the CTD of the *Drosophila* 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 μm.

Figure S3

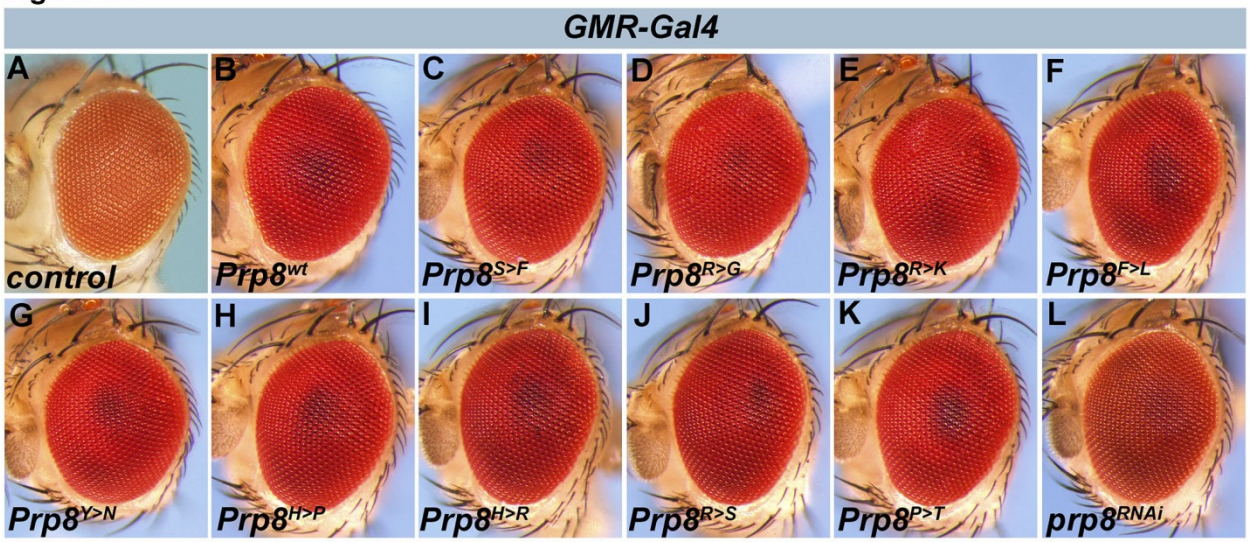

Fig. S3. Expression of RP-Prp8 variants under the *GMR-Gal4* driver is asymptomatic.

(**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*^{RNAi}) (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).

Figure S4

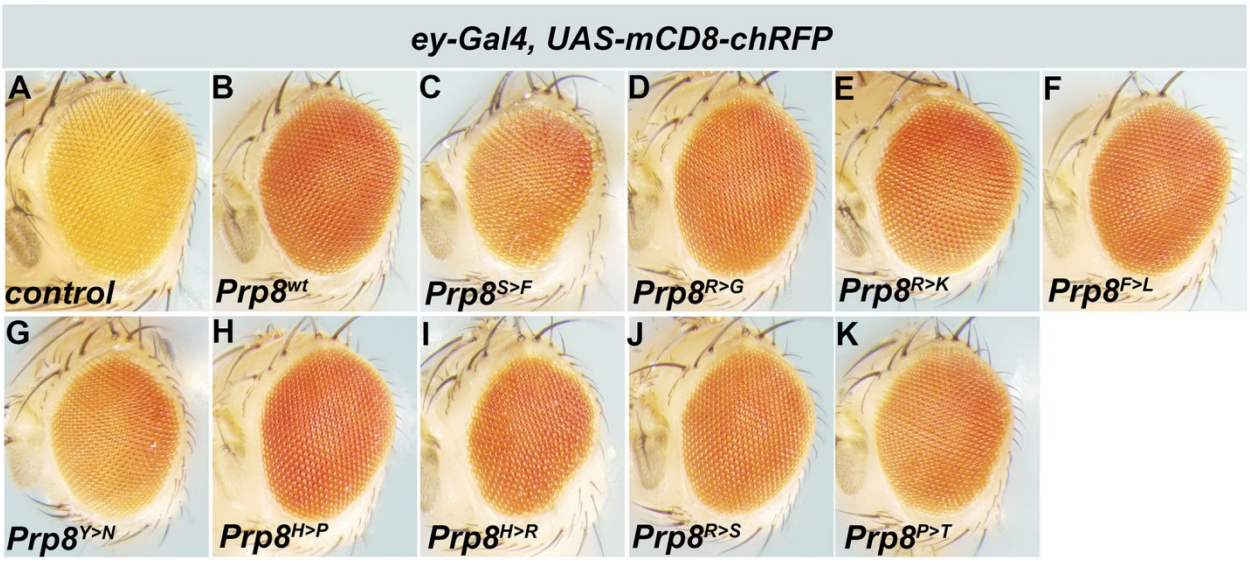

Fig. S4. Differential impact of early induction of RP-Prp8 mutations on the adult eye development.

(**A-K**) Expression of Prp8$^{S>F}$ (C) and Prp8$^{H>R}$ (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^{wt} (B) or other RP-Prp8 variants (D-H, J, K).

Figure S5

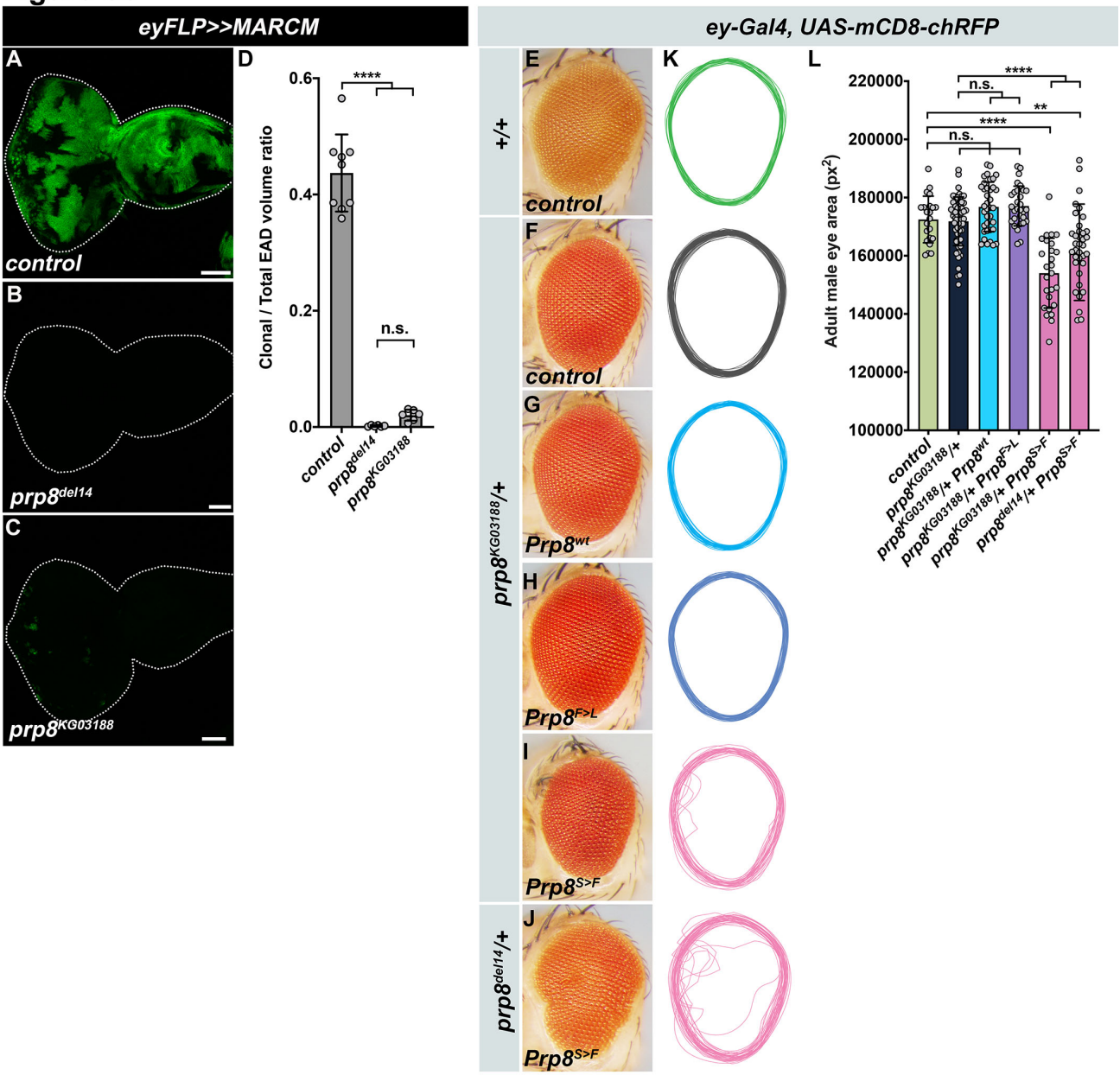

Fig. S5. Prp8 loss is incompatible with cell survival and halving the *prp8* gene dose enhances RP-Prp8-induced phenotypes.

(**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 $prp8^{del14}$ deletion allele (B) or the $prp8^{KG03188}$ 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 $prp8^{KG03188}$/+ heterozygous

background using the *ey-Gal4* driver. In contrast to controls (w^{1118} +/+, E; $prp8^{KG03188}$/+, F) and asymptomatic overexpression of $Prp8^{wt}$ (G) and $Prp8^{F>L}$ (H), $Prp8^{S>F}$ disrupts morphology and reduces size of the adult eyes (I, L) mimicking phenotypes observed when overexpressed in $prp8^{del14}$/+ 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.

Figure S6

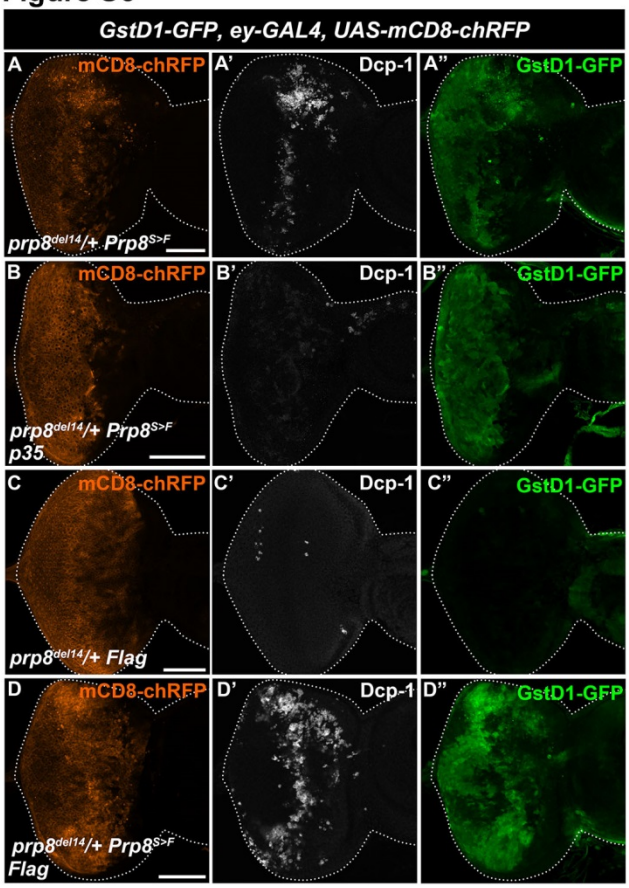

Fig. S6. Blocking apoptosis does not suppress Prp8$^{S>F}$-mediated GstD1 induction.

(**A-D**) Eye-specific expression of Prp8$^{S>F}$ in the *prp8^{del14}*/+ 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$^{S>F}$-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$^{S>F}$ (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, UAS-p35, 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>	*y1 w67c23; P{w+mC=dpp-GAL4.PS}6A/ TM3, Ser1*	Bloomington Drosophila Stock Center	RRID:BDSC_7007
dpp>mCD8.ChRFP	*w; ey-Gal4, UAS-mCD8.ChRFP/ CyO*	this study	N/A
phm>	*y1 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-myr-mRFP	*w1118; P{w w+mC =UAS-myr-mRFP}1*	Bloomington Drosophila Stock Center	RRID:BDSC_7118
GMR>myr-mRFP	*w; GMR-Gal4, UAS-myr-mRFP/ CyO*	this study	N/A
ey MARCM>>FRT82B Green	*eyFLP; act>y+>Gal4, UAS-GFP; P{ry+t7.2=neoFRT}82B tub-Gal80*	Pagliarini and Xu (2003)	N/A
ey MARCM>>FRT42D Green	*eyFLP; P{ry+t7.2=neoFRT }42D tub-Gal80/ T(2;3)B3, CyO:TM6 Tb1/ act>y+>Gal4, UAS-GFP*	this study	N/A
FRT42D	*P{ry+t7.2=neoFRT}42D; ry605*	Bloomington Drosophila Stock Center	RRID:BDSC_1802

FRT82B	*P{ry$^{+t7.2}$=neoFRT}82B ry^{605}*	Bloomington Drosophila Stock Center	RRID:BDSC_2035
Act5C-cas9, Lig4[169]	*y^{1}, M(Act5C-Cas9)ZH-2A, w^{1118}, 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$^{+t7.7}$=nos-phiC31\int.NLS}X; P{y$^{+t7.7}$=CaryP}attP2*	Bloomington Drosophila Stock Center	RRID:BDSC_25710
Prp8^{wt}	*w;; pUAST-attB-Prp8^{wt}* attP-9A / *TM6B*	this study	N/A
Prp8$^{H>R}$	*w;; pUAST-attB-prp8^{H2369R}* attP-9A/ *TM6B*	this study	N/A
Prp8$^{H>P}$	*w;; pUAST-attB-prp8^{H2369P}* attP-9A / *TM6B*	this study	N/A
Prp8$^{Y>N}$	*w;; pUAST-attB-prp8^{Y2395N}* attP-9A / *TM6B*	this study	N/A
Prp8$^{F>L}$	*w;; pUAST-attB-prp8^{F2374L}* attP-9A / *TM6B*	this study	N/A
Prp8$^{R>G}$	*w;; pUAST-attB-prp8^{H2370G}* attP-9A / *TM6B*	this study	N/A
Prp8$^{S>F}$	*w;; pUAST-attB-prp8^{S2178F}* attP-9A / *TM6B*	this study	N/A
Prp8$^{R>K}$	*w;; pUAST-attB-prp8^{R2370K}* attP-9A / *TM6B*	this study	N/A
Prp8$^{R>S}$	*w;; pUAST-attB-prp8^{R2370S}/ TM6B*	this study	N/A
Prp8$^{P>T}$	*w;; pUAST-attB-prp8^{P2361T}* attP-9A / *TM6B*	this study	N/A
Flag::Prp8^{wt}	*w;; pUAST-attB-Flag::Prp8^{wt}* attP2/ *TM6B*	this study	N/A
Flag::Prp8$^{H>R}$	*w;; pUAST-attB-Flag::Prp8^{H2369R}* attP2/ *TM6B*	this study	N/A
Flag::Prp8$^{H>P}$	*w;; pUAST-attB-Flag::Prp8^{H2369P}* attP2/ *TM3*	this study	N/A
Flag::Prp8$^{F>L}$	*w;; pUAST-attB-Flag::Prp8^{F2374L}* attP2/ *TM6B*	this study	N/A
Flag::Prp8$^{R>K}$	*w;; pUAST-attB-Flag::Prp8^{R2370K}* attP2/ *TM6B*	this study	N/A
Flag::Prp8$^{P>T}$	*w;; pUAST-attB-Flag::Prp8^{P2361T}* attP2/ *TM6B*	this study	N/A
Flag::Prp8$^{S>F}$	*w;; pUAST-attB-Flag::Prp8^{S2178F}* attP2/ *TM6B*	this study	N/A
Flag::Prp8$^{R>S}$	*w;; pUAST-attB-Flag::Prp8^{R2370S}* attP2/ *TM6B*	this study	N/A
Flag	*w; pTFW*	this study	N/A

FRT42D, prp8^{del14}	*w; P{ry$^{+t7.2}$=neoFRT }42D, prp8^{del14}/ CyO, P{ActGFP}JMR1*	this study	N/A
FRT42D, prp8^{del14}; Prp8$^{S>F}$	*w; P{ry$^{+t7.2}$=neoFRT }42D, prp8^{del14}/ T(2;3)B3, CyO:TM6 Tb^{1}/ pUAST-attB-Prp8^{S2178F}* attP-9A	this study	N/A
FRT42D, prp8^{del14}; Prp8^{wt}	*w; P{ry$^{+t7.2}$=neoFRT}42D, prp8^{del14}/ T(2;3)B3, CyO:TM6 Tb^{1}/ pUAST-attB-Prp8^{wt}* attP-9A	this study	N/A
FRT42D, prp8^{del14}; Prp8$^{F>L}$	*w; P{ry$^{+t7.2}$=neoFRT}42D, prp8^{del14}/ T(2;3)B3, CyO:TM6 Tb^{1}/ pUAST-attB-Prp8^{F2374L}* attP-9A	this study	N/A
FRT42D, prp8^{del14}; Prp8$^{H>R}$	*w; P{ry$^{+t7.2}$=neoFRT}42D, prp8^{del14}/ T(2;3)B3, CyO:TM6 Tb^{1}/ pUAST-attB-Prp8^{H2369R}* attP-9A	this study	N/A
prp8^{RNAi}	*w; UAS-prp8$^{RNAi\ [GD18565]}$*	Vienna Drosophila Resource Center	N/A
prp8^{RNAi}, FRT82B	*w; UAS-prp8$^{RNAi\ [GD18565]}$; P{ry$^{+t7.2}$=neoFRT}82B*	this study	N/A
FRT42D; Prp8^{wt}	*w; P{ry$^{+t7.2}$=neoFRT}42D; pUAST-attB-Prp8^{wt}* attP-9A/ *TM6B*	this study	N/A
p35	*w; UAS-p35*	Bloomington Drosophila Stock Center	RRID: BDSC_5072
FRT42D prp8^{KG03188}	*w; P{ry$^{+t7.2}$=neoFRT}42D P{y$^{+mDint2}$ w$^{BR.E.BR}$=SUPor-P}Prp8^{KG03188}/ CyO, y^{+}*	Kyoto stock Center 111506	
prp8^{KG03188}	*P{SUPor-P}Prp8 Prp8^{KG03188}/ CyO, P{ActGFP}JMR1*	Bloomington Drosophila Stock Center	RRID:BDSC_13006
FRT42D prp8^{KG03188}; Prp8$^{F>L}$	*w; P{ry$^{+t7.2}$=neoFRT}42D P{y$^{+mDint2}$ w$^{BR.E.BR}$=SUPor-P}Prp8^{KG03188}/ T(2;3)B3, CyO:TM6 Tb^{1}/pUAST-attB-Prp8^{F2374L}* attP-9A	Kyoto stock Center 111506	
FRT42D prp8^{KG03188}; Prp8^{wt}	*w; P{ry$^{+t7.2}$=neoFRT}42D P{y$^{+mDint2}$ w$^{BR.E.BR}$=SUPor-P}Prp8^{KG03188}/ T(2;3)B3, CyO:TM6 Tb^{1}/ pUAST-attB-Prp8^{wt}* attP-9A	Bloomington Drosophila Stock Center	RRID:BDSC_13006
FRT42D, prp8^{KG03188}; Prp8$^{S>F}$	*w; P{ry$^{+t7.2}$=neoFRT}42D, prp8^{del14}/ T(2;3)B3, CyO:TM6 Tb^{1}/ pUAST-attB-Prp8^{S2178F}* attP-9A	this study	N/A

Table S2. List of vectors and plasmids.

Name	Source	Catalog Number	Identifier
pDest17	Thermo Scientific	Cat. #11803012	N/A
pDest17 Prp8-CTD	this study		N/A
pCFD4-U6:1_U6:3tandemgRNAs	Port et al. (2014), Addgene	Cat. #49411	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	Cat. #1419	N/A
pENTR4 dual selection	Thermo Scientific	Cat. #A10465	N/A
pTFW	Drosophila Genomics Resource Center	Cat. #1115	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
pUAST-attB-Flag::Prp8[F2374L]	this study		N/A
pENTR4-Prp8[R2370K]	this study		N/A
pENTR4-Prp8[R2370S]	this study		N/A
pENTR4-Prp8[H2369R]	this study		N/A

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	TATATAGGAAAGATATCCGGGTGAACTTCGTACTAGTACAT ATGCTAAGTGTTTTAGAGCTAGAAATAGCAAG	cloning
sgRNA_pCFD4_Prp 8_3'UTR_1_Rev	ATTTTAACTTGCTATTTCTAGCTCTAAAACTAAGACTCCATA TGCGCACCGACGTTAAATTGAAAATAGGTC	cloning
Spok iQ For	GCTCTTTGGCGGTGATCGAAACAA	qPCR
Spok iQ Rev	CGCCGAGCTAAATTTCTCCGCTTT	qPCR
Spok iQ For intron	CGAGAATTTGGTTGCCAGCGAAACA	qPCR
Spok iQ Rev intron	GCCATCCTCTTAAGGAGTGTGGTCAT	qPCR
RpL49 For	TCCTACCAGCTTCAAGATGAC	qPCR
RpL49 Rev	CACGTTGTGCACCAGGAACT	qPCR
Prp8_ H2369R_For	ATCACGAGTTGCGTCGCACCTCGCA	mutagenesis
Prp8_ H2369R_Rev	TGCGAGGTGCGACGCAACTCGTGAT	mutagenesis
Prp8_H2309P_For	ATCACGAGTTGCCTCGCACCTC	mutagenesis
Prp8_H2309P_Rev	GAGGTGCGAGGCAACTCGTGAT	mutagenesis
Prp8_Y2334N_for	CGGGAGGATGTGAACGCGTAAGCGC	mutagenesis
Prp8_Y2334N_rev	GCGCTTACGCGTTCACATCCTCCCG	mutagenesis
Prp8_F2374L_For	ACCTCGCATTTACTGCTCTTCTCG	mutagenesis
Prp8_F2374L_Rev	CGAGAAGAGCAGTAAATGCGAGGT	mutagenesis
Prp8_R2310G_For	CACGAGTTGCATGGCACCTCGCATT	mutagenesis
Prp8_R2310G_Rev	AATGCGAGGTGCCATGCAACTCGTG	mutagenesis
Prp8_S2118F_For	GTCACGATCTTTGATCTGCGGGCC	mutagenesis
Prp8_S2118F_Rev	GGCCCGCAGATCAAAGATCGTGAC	mutagenesis
Prp8_R2310K_For	ACGAGTTGCATAAGACCTCGCATTT	mutagenesis
Prp8_R2310K_Rev	AAATGCGAGGTCTTATGCAACTCGT	mutagenesis
Prp8_R2310S_For	ACGAGTTGCATTCCACCTCGCATTT	mutagenesis
Prp8_R2310S_Rev	AAATGCGAGGTGGAATGCAACTCGT	mutagenesis
Prp8_P2361T_For	AGCTGGCCAACACGAAGGAGTTCTA	mutagenesis
Prp8_P2361T_Rev	TAGAACTCCTTCGTGTTGGCCAGCT	mutagenesis
PRP8 cDNA Bcl1 Forward	AAATGATCACGATGTCCATTCCGCCGTACATG	cloning
PRP8 cDNA Not1 Rev	AAAGCGGCCGCGCTTACGCGTACACATCCTCCC	cloning
PRP8 CTD EcoRI For	AAAGAATTCCCATGCAGACCAAGGAGCAAAACCAAC	cloning
ets21c iQ For	ATTAATGCCATGCATCAGGATGTCCG	qPCR
ets21c iQ Rev	GTGGGAACTTCCGTCTCCTTCG	qPCR
GstE6 iQ For	CCAAGGAGCGATACGATGCCA	qPCR
GstE6 iQ Rev	CCACGAAGGCCTCAAGGGAG	qPCR

dilp8 iQ For	GCACAACAAGCATCACTACATCA	qPCR
dilp8 iQ Rev	GTTGTAGGACCTGCTCGAGTG	qPCR
arc1 iQ For	GCTGAACATCAAGTACCGCAAGCA	qPCR
arc1 iQ Rev	GTGTTCTTTGCTGTGGCAAGCTGT	qPCR
CG42260 iQ For	TACTCAATATGGGCACCGC	qPCR
CG42260 iQ Rev	CTTCAGCACGTCCCACATG	qPCR

Supplementary Dataset 1

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Supplementary Dataset 2

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