Supplemental Information

Supplemental Table 1: List of Genotypes

Figure 1	**Figure 7**
w;;*	*;cn, bw;*
w;;st^{1}/+*	*;cn, bw;Df (3L) ED218/+*
w;;Prp31P^{18}, st^{1}/+*	*GMR-w^{IR}; Rh1-Gal4/+; UAS Dicer-2/+*
w;;Prp31P^{17}, st^{1}/+*	*GMR-w^{IR}; Rh1-Gal4/+; UAS Dicer-2/UAS Prp RNAi (VDRC LineID: 35131)*
Figure 2	**Figure 8**
Oregon R	*w*;;*
w;;*	*w*;;st^{1}/+*
w;;st^{1}/+*	*w*;;Prp31P^{18}, st^{1}/+*
w;;Prp31P^{18}, st^{1}/+*	*w*,ey-FLP;;FRT82B crb^{11A22}/FRT82B P{w+}90E l(3)cl-R3*
w;;Prp31P^{17}, st^{1}/+*	**Figure 9**
w,ey-FLP;;FRT82B crb^{11A22}/FRT82B P{w+}90E l(3)cl-R3*	*;gstD-GFP/+;+*
w;;crb_C^{13A9}*	*;gstD-GFP/+; st^{1}/+*
Figure 3	*;gstD-GFP/+; Prp31P^{18,} st^{1}/+*
w;;*	*;cn, bw/ gstD-GFP;+*
w;;st^{1}/+*	*;cn, bw/ gstD-GFP; Df (3L) ED217/+*
w;;Prp31P^{18}, st^{1}/+*	**Supplemental Figures**
w,ey-FLP;;FRT82B crb^{11A22}/FRT82B P{w+}90E l(3)cl-R3*	*w*/w*;; (females)*
Figure 4	*w^{1118}/w^{1118};; (females)*
GMR-w^{IR}; Rh1-Gal4/+; UAS Dicer-2/+	*w*/w^{1118};; (females)*
GMR-w^{IR}; Rh1-Gal4/+; UAS Dicer-2/UAS Prp RNAi (VDRC LineID: 35131)	*w*;;Prp31P^{18}, st^{1}/ Prp31P^{18}, st^{1}*
Figure 5	*+;+;+*
;cn, bw;	*+;;st^{1}/+*
;cn, bw;Df (3L) Exel 6262/+	*w*;;*
;cn, bw;Df (3L) ED217/+	*w*;;st^{1}/+*
;cn, bw;Df (3L) ED218/+	*w*;;st^{1}/ st^{1}*
Figure 6	*w^{1118};;*
w;;*	*w^{1118};;st^{1}/+*
w;;st^{1}/+*	*;cn, bw;*
w;;Prp31P^{18}, st^{1}/+*	*; cn, bw; Df (3L) Exel 6262/+*
w;;Prp31P^{17}, st^{1}/+*	*;cn, bw;Df (3L) ED217/+*
w;;crb C^{13A9}*	*;cn, bw;Df (3L) ED217/ Prp31P^{18}, st^{1}/+*
;cn, bw;Df (3L) ED217/+	*;cn, bw;Df (3L) ED217/ Prp31P^{17}, st^{1}/+*
Figure 7	*w*;;*
w;;*	*w*;;Prp31P^{18}, st^{1}/+*
w;;st^{1}/+*	
w;;Prp31P^{18}, st^{1}/+*	

(all samples were age-matched males unless specified otherwise)

Supplemental Table 2: Characterization of *w** and w^{1118} with PCR using primers designated in Supplemental Table 3 and also schematized in Figure S1

allele	PCR 1: upst -> 5'UTR	PCR 2: 5'UTR -> ex 1	PCR 3: Doc -> int 1	PCR 4: 5'UTR -> int 1	PCR 5: ex 6	PCR6: 3'UTR -> out
+/+	+ (558 bp)	+	+ (1,2 kb)	+ (766 bp)	+	+
w^{1118}	-	-	-	-	+	+
w^{*}	-	-	-	-	+	+

(+) indicates PCR result as expected.
(-) indicates no PCR product
In contrast to published data for w^{1118} which report the partial absence of the *white* locus is located 5' of the P-element integration site in $w^{hd80k17}$(Flybase: FBrf0053691), (ENGELS *et al.* 1990; KURKULOS *et al.* 1991), exon 6 is present in w^{1118}.

Supplemental Table 3: Primer Details

fragment		Primer sequence	T[anneal]	Size (wt)
PCR 1	upstream-fwd	CAGCTTATGAGTACTGCCCA	68°C	558 bp
	5UTR-rev	TACCCACCCAAAACCAATCA		
PCR 2	5UTR-1-fwd	TGATTGGTTTTGGGTGGGTA	58°C	682 bp
	Intron1-rev	CGTGCAAACAACGAGGTATT		
PCR 3	upstream-fwd	CAGCTTATGAGTACTGCCCA	68°C	1219 bp
	Intron1-rev	CGTGCAAACAACGAGGTATT		
PCR 4	5UTR-2-fwd	CATCAATTAAACACAAAGTG	54°C	760 bp
	Intron1-rev	CGTGCAAACAACGAGGTATT		
PCR 5	Exon6-fwd	GCTGCCAGTTTTTATGAGGG	60°C	365 bp
	Exon6-rev	ACCATGAGAGGTACGACAAC		
PCR 6	3UTR-fwd	ACTGTTTATTGCCCCCTCAA	60°C	391 bp
	GC32795-rev	GAACCACTCGGAACCATTTG		

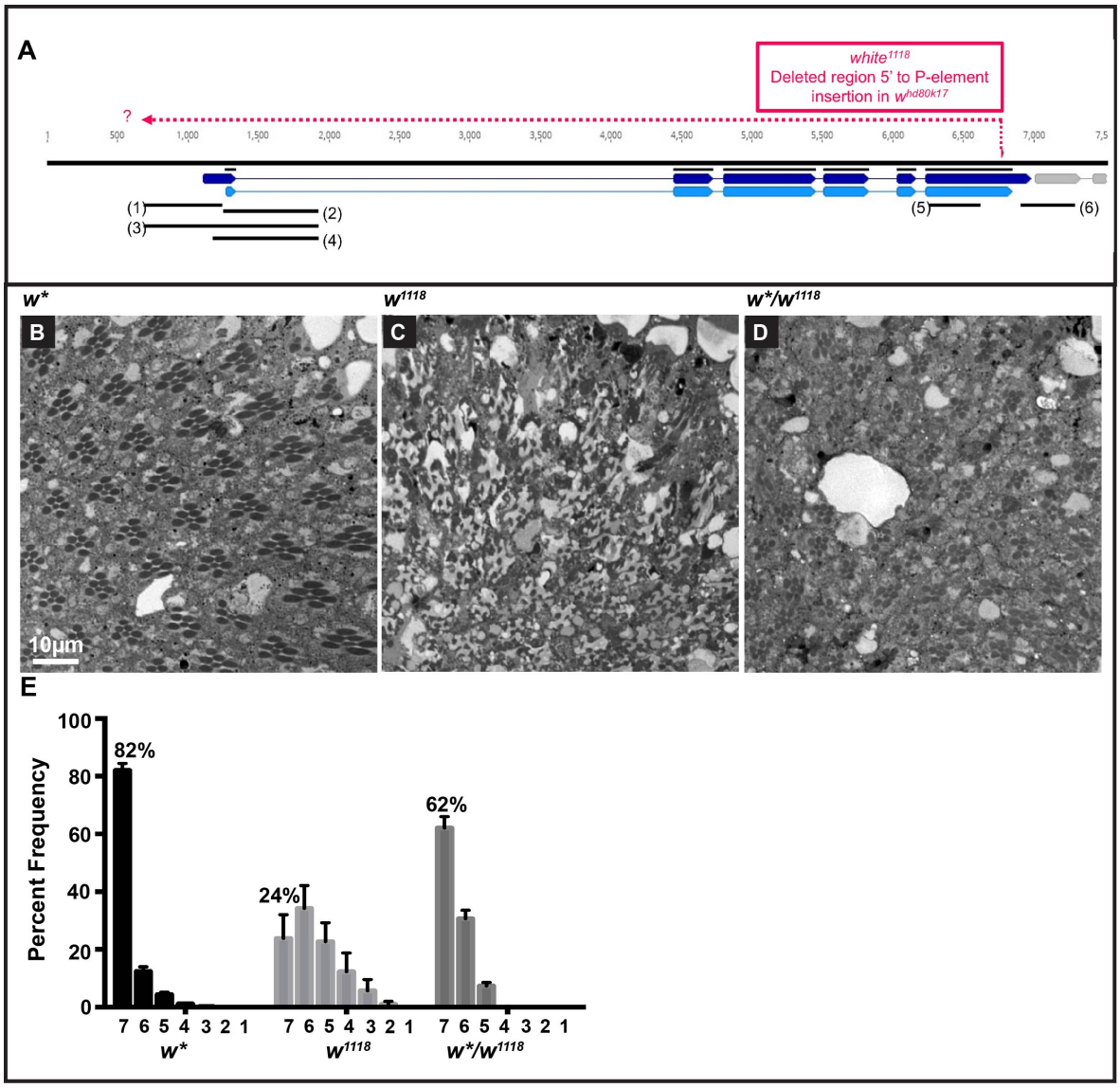

Figure S1

Figure S1: Comparison of *w and *w*^{1118}**

A: Schematic representation of the *white* locus on chromosome X: 2,790,599 to 2,796,466 (reverse complement plus 1,109 kb upstream and 600 kb downstream of the *white* locus / *Drosophila melanogaster*: Release 6 plus ISO1 MT (GCF_000001215.4)). The *white* mRNA is shown in light blue, the coding sequence in dark blue, in grey: mRNA of CG32795. The *w*^{1118} mutant is caused by a partial deletion of the *white* locus, which maps 5' to the P-element insertion in *w*^{hd80k17} and includes exon 1 (Flybase: FBrf0053691), (ENGELS *et al.* 1990; KURKULOS *et al.* 1991), the overall length of this deletion (red dotted line) has not been determined. PCR fragments (1-6) have been designed such that they cover the meaningful regions of the *white* locus (numbered black lines).

B-D: are representative bright-field images of Toluidine-blue stained, semi-thin sections of eyes of *w** / *w** (A), *w*^{1118}/ *w*^{1118} (B), and *w**/ *w*^{1118} (C). Upon eclosion, flies were kept for two days under regular light conditions and then subjected to a degeneration paradigm of 7 days of continuous, high intensity light exposure. It is evident that *w**(A) shows minimal damage in terms of surviving ommatidia with 7 rhabdomeres, as compared to *w*^{1118}. Scale bar = 10µm.

E: Quantification of retinal degeneration as indicated by the number of surviving rhabdomeres observed upon high intensity, continuous light exposure. Bars represent mean ± s.e.m. (a minimum of n=60 ommatidia from eyes of 3 biological replicates) of the percent frequency of ommatidia displaying 1-7 rhabdomeres (Y-axis).

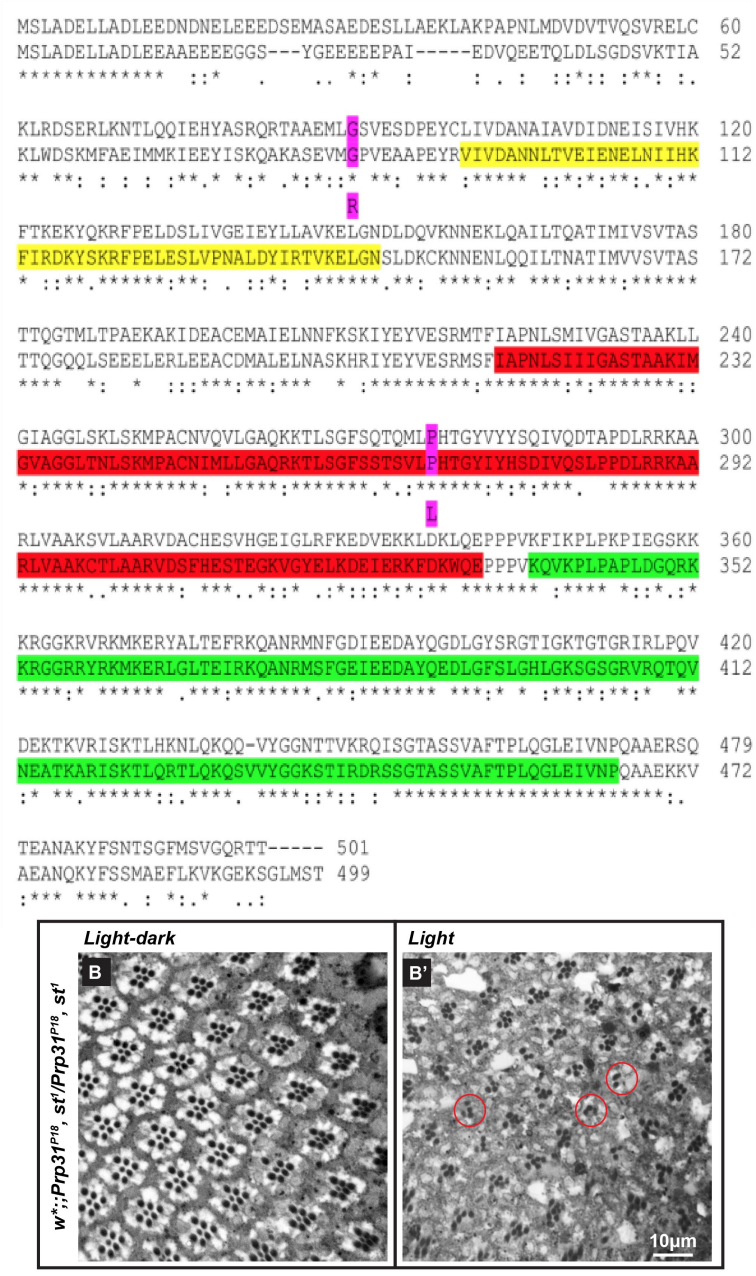

Figure S2: *Prp31* homozygous mutants also display light-induced retinal degeneration.

A. Amino acid sequence comparison of *Drosophila* Prp31 (upper) and human PRPF31 protein (lower). The NOSIC (yellow), Nop (red) and Prp31_C specific (green) domains are indicated as described on UniProt and Pfam websites. TILLING mutations are indicated in magenta. Asterisks (*) indicate fully conserved amino acids, colons (:) indicate groups of amino acids of strongly similar properties and periods (.) indicate amino acids with weakly similar properties. Alignment was made using ClustalO 1.2.3.

(B-B') Representative bright-field images of Toluidine-blue stained, semi-thin, sections of eyes of males of homozygotes $w^{*};;Prp31^{P18}, st^{1}/w^{*};;Prp31^{P18}, st^{1}$. Upon eclosion, flies were kept for two days under regular light conditions. They were split into two groups, processed for imaging (B), or, subjected to a degeneration paradigm of exposure to 7 days to continuous, high intensity light (B'). Scale bar=10 µm.

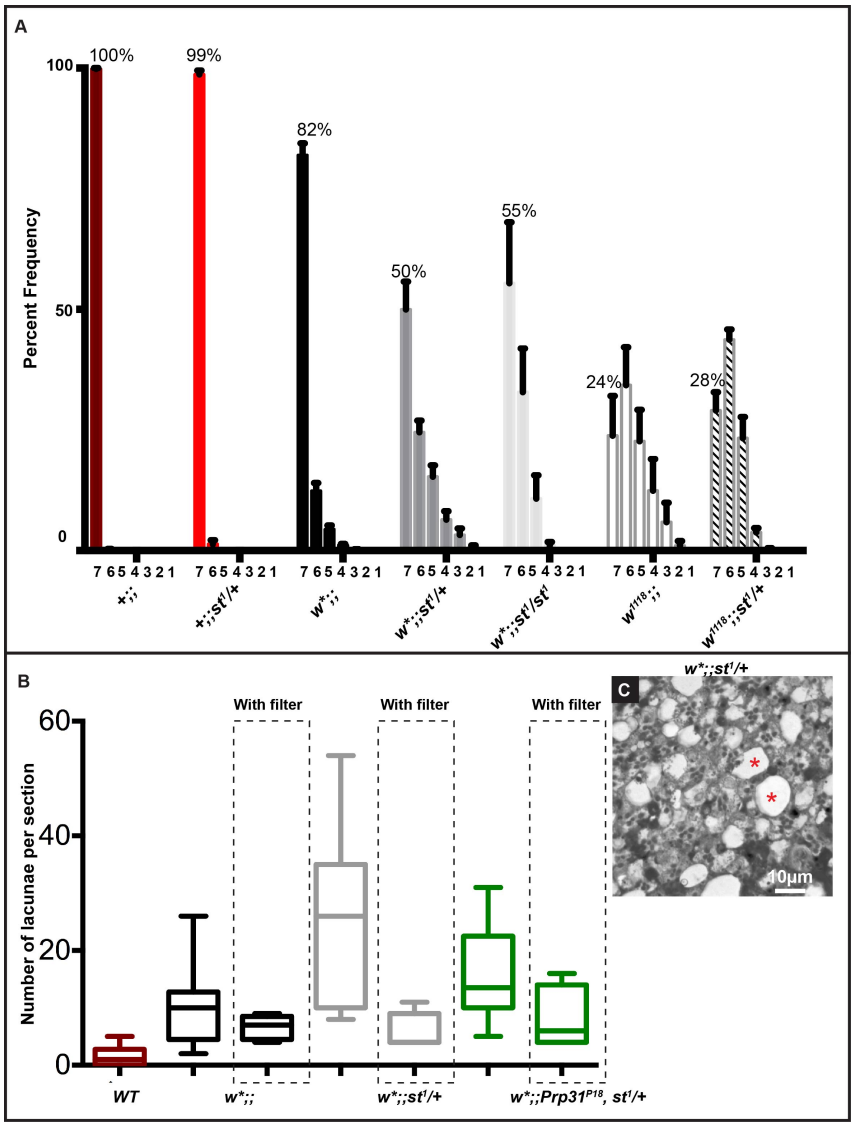

Figure S3: Genetic interaction between *w* and *st* manifests as exacerbation in light-induced damage upon combination of *w* and *st* mutants.

A. Quantification of retinal degeneration as indicated by the number of surviving rhabdomeres observed upon high intensity, continuous light exposure. Bars represent mean ± s.e.m. (a minimum of n=60 ommatidia from eyes of 3 biological replicates) of the percent frequency of ommatidia displaying 1-7 rhabdomeres (Y-axis). Genotypes are indicated below. Numbers on the graphs indicate the mean number of ommatidia displaying the full complement of 7 rhabdomeres.

B. Quantification of the damage to tissue in terms of the lacunae/holes following high intensity, continuous light exposure. Box plot represents the number of lacunae per section of the eye of the genotypes indicated; the whiskers on each plot represent the maximum and minimum number of lacunae, sample size =3 biological replicates. In case of WT (wild-type) flies, with pigmented eyes, the lacunae observed per section in minimal. For the other genotypes, in which there are substantially more lacunae observed, the left plot indicates surviving rhabdomeres following the routine light degeneration paradigm (solid line intensity profile in Fig. 3) whereas the right plot (outlined by a box) indicates surviving rhabdomeres following reduced light intensity exposure (dashed line intensity profile in Fig. 3).

C. Representative bright-field images of Toluidine-blue stained, semi-thin sections of eyes of males of *w*;; st^{1}/+* following high intensity, continuous light exposure. Asterisks indicate lacunae/holes in the section. Scale bar= 10 µm.

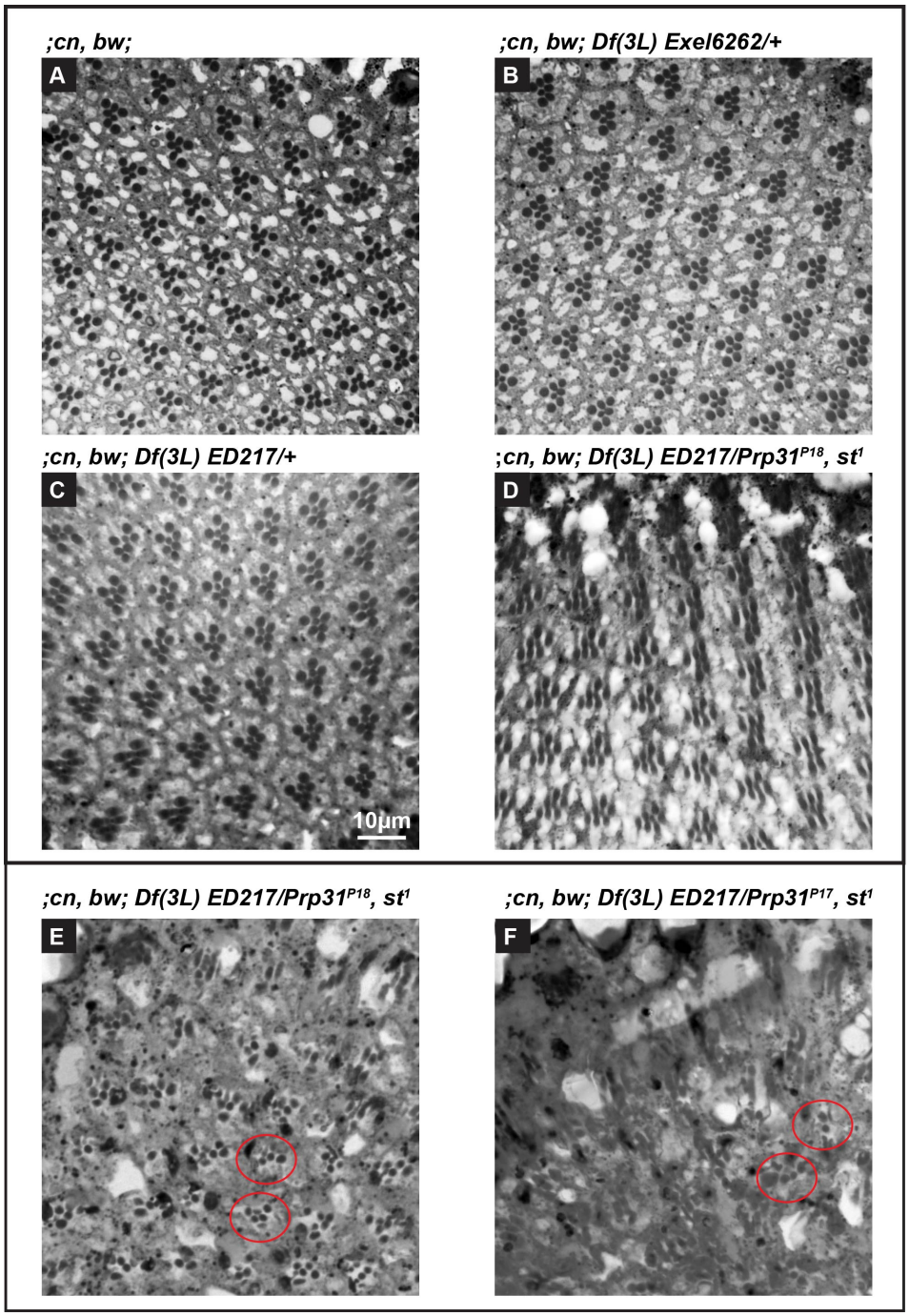

Figure S4: Eye of *Prp31* hemizygous animals develop normally.

(A-D) are representative bright-field images of Toluidine-blue stained, semi-thin sections of eyes of males of *;cn, bw;* (A), *;cn, bw; Df (3L) Exel 6262/+ (*B), and *;cn, bw; Df (3L) ED217/+* (C) and *;cn, bw; Df (3L) ED217/Prp31^{P18}, st^{1}* (D) that are kept for 2 days under regular light conditions.

(E-F) are representative bright-field images of Toluidine-blue stained, semi-thin sections of eyes of males of *;cn, bw; Df (3L) ED217/Prp31^{P18}, st^{1}*(E) *;cn, bw; Df (3L) ED217/Prp31^{P18}, st^{1}*(F) that are subjected to a degeneration paradigm of exposure to 7 days to continuous, high intensity light. Scale bar= 10 µm.

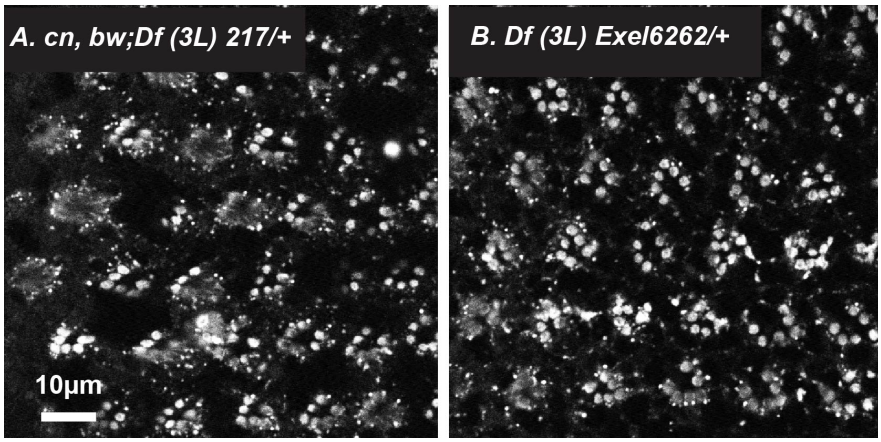

Figure S5: Rh1 immunostaining pattern in deficiency lines
A-B are confocal images of 1µm optical sections from 12µm cross-sections of eyes of adults (2 days old) reared under 12h light/12h dark. These are examples of two deficiencies covering the *Prp31* locus, which exhibit increased Rh1 staining as compared to their control (shown in Fig. 7). Scale bar as indicated.

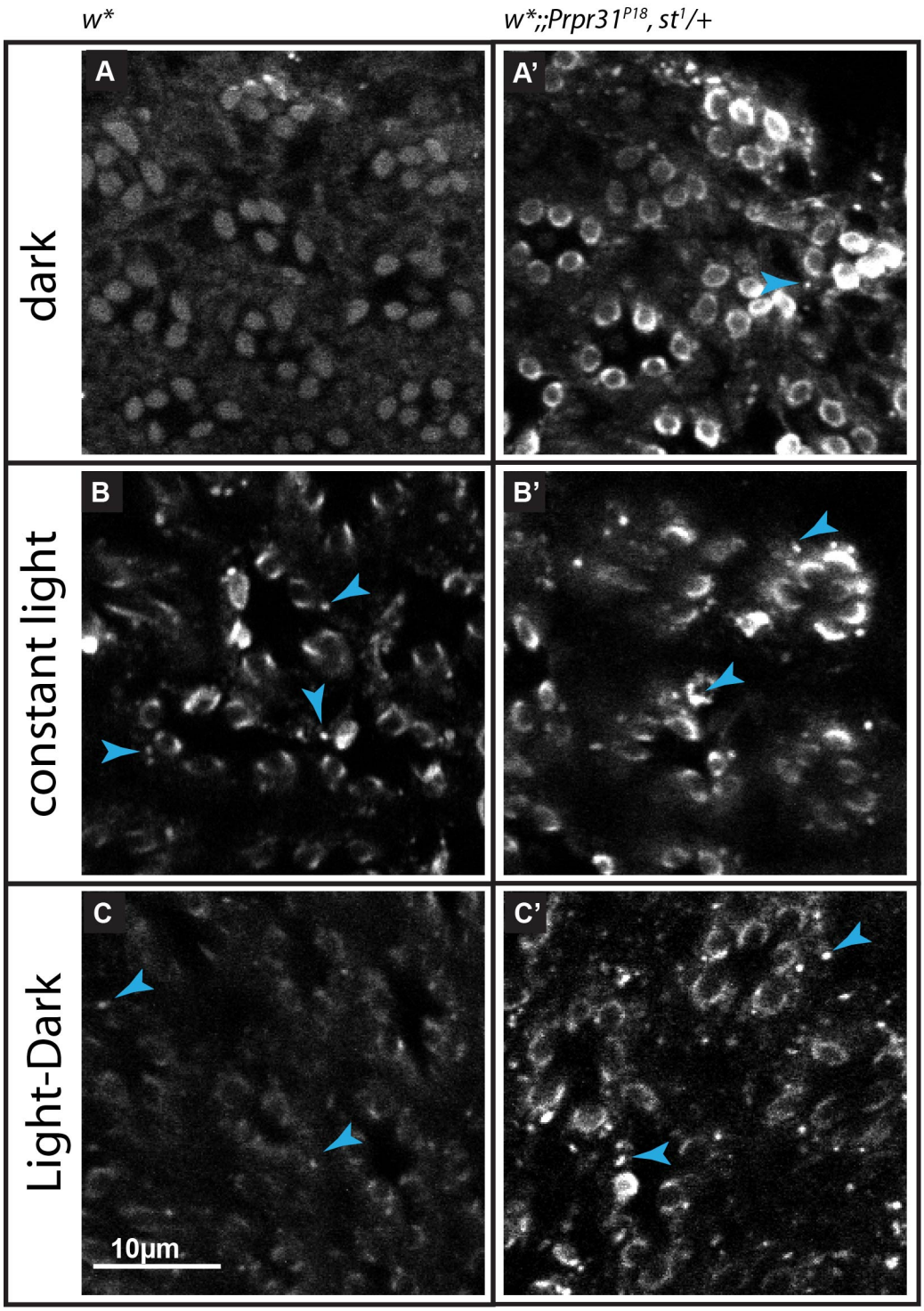

Figure S6: Rh1 immunostaining pattern in eyes of flies reared under different light conditions

A-C' are confocal images of of 1µm optical sections from 12µm cross-sections of eyes of adults of 2 days age reared under constant darkness (A-A'), constant light (B-B') and 12h light/12h darkness (C-C') for w* (A-C) and for *w*;;Prp31^{P18}, st^{1}/+* (A'-C') labelled with anti-Rh1. Blue arrowheads indicate intracellular Rh1 labelling. Scale bar as indicated.

Supplemental Methods

Extraction of genomic DNA of single flies

Single flies (+/+ (Oregon R), *w*, w*^{1118}) were frozen at -80°C. Single flies were lysed in a high salt buffer and RNA removed by Lithium chloride precipitation, genomic DNA was precipitated by Isopropanol, washed with 70% Ethanol, and eluted in TE (WINKLER *et al.* 2005).

DNA amplification by PCR

PCR fragments 2, 4, 5, and 6 have been amplified from 10 ng genomic DNA of a single fly making use of the Phusion Flash High fidelity PCR mastermix (Thermo Fisher) following the recommended setup of the manufacturer. PCR cycling was done as follows: initial denaturation at 98°C for 2 min, cycling at 98°C for 15 sec, annealing was optimized for each primer set (see Supplemental Table 3) and done for 20 sec, and extension at 72°C for 30 sec for a total of 35 cycles, followed by a final extension step at 72°C for 8 min. PCR fragment 1 and 3 have been performed with LA Taq DNA polymerase (TAKARA, Clontech) that is optimized for long-range PCR on 10 ng of genomic DNA of a single fly. PCR set up was done as recommend by the manufacturer. Cycling conditions are: initial denaturation at 95°C for 1 min, cycling at 98°C for 10 sec, primer binding and extension at 68°C for 8 min and a total of 30 cycles, followed by a final extension step at 72°C for 10 min. Visualization of PCR fragments has been done on 0.7 and 1% agarose gels.

References

Engels, W. R., D. M. Johnson-Schlitz, W. B. Eggleston and J. Sved, 1990 High-frequency P element loss in *Drosophila* is homolog dependent. Cell 62**:** 515-525.

Kurkulos, M., J. M. Weinberg, M. E. Pepling and S. M. Mount, 1991 Polyadenylylation in copia requires unusually distant upstream sequences. Proc Natl Acad Sci U S A 88**:** 3038-3042.

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