Supplementary figure legends

Figure S1. The role of the p53 isoform in the survival of flies exposed to paraquat**.** (**A**) Representation of the *p53* locus showing the alternative P1 and P2 promoters with the *p53* (*p53B*), ΔN*p53* (*p53A*) and *p53E* isoforms. The black arrows indicate the position of the primers used for detection of the *p53* isoforms by PCR. (**B**) Relative increase in ΔN*p53* (*p53A*) and *p53* (*p53B*) mRNA after paraquat treatment *as measured by semi-quantitative RT-PCR in whole flies (n = 2). (**C**) RT-PCR of p53 (p53B*) and ΔN*p53* (*p53A*) in control flies, w1118; $p53^{null}$ (homozygous $p53^{5A1-4}$ mutant); *p53*BAC, $p53^{null}$ mutant flies carrying a BAC sequence encompassing the wild-type p53 locus; *Ch-p53A STOP;* $p53^{null}$*,* $p53^{null}$ mutant flies carrying a p53 BAC sequence in which Δ*Np53* (p53A) is mutated; and *Ch-p53B STOP*, $p53^{null}$ mutant flies carrying a *p53* BAC sequence in which *p53* (*p53B*) is mutated. Representative gel of three independent experiments. (**D**) Survival curves of the indicated fly strains fed with normal or 20 mM paraquat (PQ)-containing media. Data are the mean ± SEM of *n* = 4. $p53^{null}$ flies are significantly more sensitive to PQ than control flies **** $P<0.0001$ (48 h) and *** $P<0.001$ (24 h) by ANOVA2+multiple comparison.

Figure S2. The role of ΔNp53 (p53A) isoform in the survival of flies exposed to paraquat. (**A**) RT-PCR of *p53* (*p53B*) and Δ*Np53* (*p53B*) transcripts in control flies (w1118); in CRISP-generated Δ*Np53* (*p53A*) mutant flies (homozygous $p53^{A39.4}$ and $p53^{A2.3}$); and $p53^{null}$ (homozygous $p53^{5A1-4}$ mutant). Representative gel of 2 independent experiments. (**B**) Western blot analysis of p53 CRISPR alleles. Protein extracts from adult flies homozygous

for the indicated *p53* alleles were analyzed by blotting with anti-human TP53/p53 antibody. $p53^{A39.4}$ and $p53^{A2.3}$are the new isoform-specific Δ*Np53 (p53A)* deletion alleles. The analysis of transcript levels by RT-PCR of Δ*Np53* (*p53A*) and *p53* (*p53B*) confirmed the absence of Δ*Np53* (*p53A*) but the presence of *p53* (*p53B*) transcripts in two *Drosophila* lines ($p53^{A2.3}$ and $p53^{A39.4}$). Western blot analysis, showed no p53 protein for $p53^{A2.3}$ but p53 protein was still detected for $p53^{A39.4}$, possibly due to cryptic splice site that puts first exon of Δ*Np53* (*p53A*) back in frame. (**C**) Survival curves of the indicated fly strains fed with normal or 20 mM PQ-containing media. Data are the mean ± SEM of $n = 3$. $p53^{null}$ flies are significantly more sensitive to PQ than control flies ***$P < 0.0001$ (24 h, 48 h and 96 h) by one-way ANOVA followed by the Bonferroni post hoc test. Note that in S2C there is a reduced toxicity of PQ compared to Fig 1 and Fig S1, which may be due to the different batches of PQ used in the different sets of experiments. The increased sensitivity to PQ of $p53^{null}$ flies serves as an internal control.

Figure S3. *p53* mutant flies exhibit an increased ref(2)P and increased caspase activation in response to paraquat. (**A**) Representative western blot of ref(2)P levels in whole control, $p53^{null}$, and $atg8^{-/-}$ flies treated or non-treated with PQ. (**B**) Quantification of immunoreactivity against cleaved human CASP3 from heads of control and $p53^{null}$ flies treated or non-treated with PQ. Data are the mean ± SEM of $n = 5$ western blots (not shown) and are expressed as the ratio of immunoreactivity against cleaved human CASP3:tubulin in PQ-treated compared with non-treated control flies. Controls are w^{1118} , $atg8^{-/-}$ are homozyogous $atg8a^{KG07569}$ and $p53^{null}$ are homozyogous $p53^{5A1-4}$ mutant flies.

Figure S4. Differential activation of caspase-dependent and caspase-independent cell death by *p53* (*p53B*) and ∆*Np53* (*p53A*). (**A** to **G**) PRs overexpressing control protein mCD8-RFP, *reaper (rpr)*, ∆Np53 *(p53A)*, *p53 (p53B)*, *p35, ∆Np53 (p53A)* and *p35,* and *p53 (p53B)* and *p35* under the control of *rh1-GAL4.* Representative staining of cleaved Dcp-1 in the adult retina. Actin and cell nuclei were visualized by staining with phalloidin (red) and DAPI (blue), respectively. Scale bars: 20 µm. (**H** and **I**) Quantification of degenerated photoreceptors (PR) expressing GFP (*Rh1-GFP*) by corneal neutralization using immersion microscopy. (**H**) The loss of PR induced by the overexpression of ∆*Np53 (p53A)* in photoreceptors (*Rh1-Gal4; UAS-*∆*Np53* (*p53A*)) is rescued by the pan-retinal expression of the caspase inhibitor *p35* (*GMR-p35*) in one day-old flies. (**I**) The loss of PR induced by the overexpression of *p53* (*p53B*) in photoreceptors (*Rh1-Gal4; UAS-p53* (*p53B*)) is not rescued by the pan-retinal expression of the caspase inhibitor *p35* (*GMR-p35*) in seven day-old flies. Note that the kinetic of PR degeneration is slower with *p53* (*p53B*) than with ∆*Np53* (*p53A*) overexpression. ***$P<0.001$ by the two-tail unpaired Student t test.

Figure S5. Induction of autophagy in photoreceptors expressing *p53* (*p53B*) or ∆*Np53* (*p53A*). (**A** to **F**) Representative fluorescence microscopy images of the retinas of adult flies expressing the autophagy reporter GFP-LC3 in photoreceptors (PRs). Flies also overexpressed *mCD8-RFP* (control protein), ∆*Np53* (*p53A*), *p53* (*p53B*), gug/*atro75QN*, ∆*Np53* (*p53A*) and *p35*, or *p53* (*p53B*) and *p35* in PRs under the control of *rh1-GAL4*. Actin was visualized by staining with phalloidin (red). Scale bars: 20 µm. (**G**) Quantification of

GFP-LC3 dots per retina area in the strains represented in (**A** to **F**) using the Find Maxima function of ImageJ software. Data are the mean ± SEM of $n = 10$ retinas. *$P < 0.05$, **$P < 0.01$ by the Student t test. **(H** and **I)** Representative transmission electronic microscopy pictures from photoreceptor overexpressing ∆*Np53* (*p53A*) (**H**) or *p53* (*p53B*) (**I**) under the control of *rh1-GAL4*. Autophagic vacuoles are shown with black arrowheads. Red stars mark photoreceptor pigment. m, mitochondria; R, rhabdomere. Scale bar: 0.2 μm. (**J** and **K**) Quantification of GFP-LC3 dots (**G**) and GFP-ref(2)P dots (**H**) per retina area in PRs from flies overexpressing control protein *mCD8-RFP* alone or with *p35.* Data are the mean ± SEM of $n = 8$.

Figure S6. Dcp-1 RNAi efficiently suppresses cleaved Dcp-1 labeling induced by ∆*Np53* (*p53A*) but does not alter basal GFP-Ref(2)P levels in PRs. (**A** to **D**) PRs overexpressing *mCD8-RFP* (control), *rpr*, ∆*Np53* (*p53A*), or ∆*Np53* (*p53A*) and *dcp-1* RNAi under the control of *rh1-GAL4*. Representative immunostaining of cleaved Dcp-1 (green), either alone or with phalloidin staining of actin (red). Cell nuclei are stained with DAPI (blue). Scale bars: 20 μm. (**E**) Quantification of GFP-ref(2)P dots per retina area in PRs overexpressing control protein *mCD8-RFP* alone or with either *dcp-1* RNAi or *drice* RNAi does not show statistical difference. Data are the mean ± SEM of $n = 8$.