

# Supporting Information

Kotova et al. 10.1073/pnas.0914152107

## SI Text

**Drosophila Strains and Genetics.** Genetic markers are described in Flybase (1), and stocks were obtained from the Bloomington Drosophila Stock Center, except as indicated. The *c03265* strain was generated in a single pBac-element mutagenesis screen (2). Precise excision of *c03256* was carried out using the source of pBac transposase on the CyO chromosome. *Parg*<sup>27.1</sup> was constructed by Hanai et al. (3). pP{w1, UAST::PARP1-DsRed}, called UAS::Parp1-DsRed, was described by Tulin et al. (4). The transgenic stock with pP{w1, UAST::PARG-EGFP}, called UAS::Parg-EGFP, was described by Tulin et al. (5). The following GAL4 driver strains were used: arm::GAL4 (no. 1560; Bloomington Drosophila Stock Center), da::GAL4 (gift from A. Veraksa, Boston, MA), 69B-GAL4 (6), and G1-GAL4 (gift from the laboratory of G. Cavalli, Montpellier, France). Balancer chromosomes carrying Kr::GFP (i.e., TM3, Sb, P{w<sup>+</sup>, Kr-GFP<sup>4</sup>}, FM7i, P{w1, Kr-GFP}) (7) were used to identify heterozygous and homozygous *c03256* and *Parg*<sup>27.1</sup>.

**Construction of Transgenic Drosophila.** To construct UAS::Parp ( $\Delta 300$ ,  $\Delta 740$ ,  $\Delta 1302$ )-EYFP, we generated corresponding ORFs using PCR. The following primers were used:

1. 5'-CACTGTCGACTTTAAAAACATTTTGAAATG-TACTTAGAAAATATACAAGAAGTTATAAGC-3', PARP $\Delta 300$  forward
2. 5'-CACTGTCGACCCTACTTCAGGTTTCGCGATGACA-3', PARP $\Delta 740$  forward
3. 5'-CACTGTCGACCACGATAGCAATAATATCAACA-GAATTAG-3', PARP $\Delta 1302$  forward
4. 5'-GATGGTACCATAAGAATACTTGAATTCCA-TACG-3', 33nt, PARP reverse

PCR products were cloned into pEYFP-N3 vector (Clontech) using Sall and KpnI restriction sites. Entire PARP-EYFP cDNA was subcloned into pUAST using BglII and NotI restriction sites. *y,w<sup>67c23(2)</sup>* was used as the host for transformation. Transformation was as described by Spradling and Rubin (8), with modifications (9).

**RT-PCR Assay and Southern Blot Analysis.** Total RNA was isolated using TRIzol reagent (Gibco BRL), precipitated twice with 3M LiCl, treated with Amplification Grade Dnase I (Gibco BRL), and polyA-containing RNA-purified using a MessageMarker kit (Gibco BRL). The SuperScript Preamplification System (Gibco BRL) was then used to synthesize cDNA and for RT-PCR analysis. The primer pairs that were used to test Parp expression in *parp*<sup>*c03256*</sup> mutants are shown below. The product of constitutively expressing gene H2Av was used as a loading control in the RT-PCR assay. To detect H2Av, the next primer pair used was 5'-GGCTGGCGGTAAAGCAGG-3' (forward), 5'-GTAG-GCCTGCGACAGAATG-3' (reverse). For Southern blot analysis, at least 2  $\mu$ g of total genomic DNA from third-instar larvae of *parp*<sup>*c03256*</sup> and WT flies was used for each lane. The PARP probe was from the DBD.

C03256 RT-PCR primer sequences were as follows:  
E3 (5'-AAATAATAAATGTCTTGAAATTG-3')  
E5 (5'-GTCTTGATTTTGTGTATACCG-3')  
E7 (5'-TTTTATGAAACCAATTCG-3')  
D1 (5'-GTGTCGTGGATGTGAAC-3')

- R2 (5'-TTGGAATTCTGGATTTTG-3')  
114. 5'-GTC GAT CGA CGT GTC GTG GAT GTG-3', 3ex. forward  
115. 5'-GAG CTT AGC AGA TGA TGA TCA AGC-3', 3ex. forward  
117. 5'-GGT TAG GCC AAC AAT ACC TCG TAT-3', 5ex. forward  
118. 5'-CTG TCC ACG TAA ACA TGG GCG ATG -3', 5ex. reverse  
120. 5'-GGT CAT CAT CAT ATT GAA TTT CGA-3', 6ex. reverse  
165. 5'-AAA GAA TGG CAA ACC AAG TCG-3', pBAC forward  
166. 5'-CAA TAT GAA TTC CGA GCT CTT CTG-3', 5ex. reverse  
167. 5'-CAC TAT GCA TGC CGA TCT TGT TGG -3', 4ex. reverse  
168. 5'-GTC ATC GCG AAA CCT GAA GTA GGC-3', 4ex. reverse  
169. 5'-CAG CGT CCC AGC TCA GTA GGA GAC-3', 2ex. forward  
202. 5'-CGA CTT TAA AAA CAT TTT GAA ATG TAC TTA-3', forward, intron  
205. 5'-CTC TTC GGA TTG CTG TCA TGG TTC-3', 1ex. forward

**Quantitative RT-PCR Assay.** Total RNA was isolated from third-instar larvae using an RNeasy Lipid Tissue Mini kit (Qiagen). DNA contamination was removed using TURBO DNA-free (Ambion). RNA concentration was measured using an Agilent 2100 BioAnalyzer (Agilent Technologies) in combination with an RNA 6000 Nano LabChip (Agilent Technologies). Real-time PCR assays were run using an ABI 7900 HT instrument (Applied Biosystems). The quantitative real-time PCR (qPCR) primer sequences for DM HSP70 were 5'-CAAGAACCTCAAGGGT-GAGC-3' (forward) and 5'-GCCGGTTGTCAAAGTCCTC-3' (reverse), and sequences for histone DmH4 were 5'-ATGACT-GGTCGTGGTAAAG-3' (forward) and 5'-ACCGCAAATC-CGTAGAG-3' (reverse). The difference in threshold cycle values ( $\Delta C_T$ ) between HSP70 and H4 was used to normalize the amount of DNA.

The qPCR primer sequences for the 5' end of the DM *copia* retrotransposon were 5'-TTATAAGTGAATTGTTGGCAGCT-G-3' (forward) and 5'-TGTCTCTATCGCTGTAATAATTCCATC-3' (reverse). For the 3' end of the DM *copia* retrotransposon, primers were 5'-GATTCTGATTGGGCTGGTAGTG-3' (forward) and 5'-TTGATGAGGCTGCTACTGAGTTC-3' (reverse). For the 5' end of the DRO GYP retrotransposon, primers were 5'-ACTAGACTGCAGTACTCGGACA-3' (forward) and 5'-GT-TAGCTTCTTTTCAACTTCATCGT-3' (reverse). For the 3' end of the DRO GYP retrotransposon, primers were 5'-CGCTC-ACTCCATGCTGGA-3' (forward) and 5'-TGCGGCTTCGTT-GATAATCA-3' (reverse). The sequences for DmRP49 were 5'-CCAAGGACTTCATCCGCCACC-3' (forward) and 5'-GCGG-GTGGCCTTGTTCGATCC-3' (reverse).

The difference in  $\Delta C_T$  between transposons and RP49 was used to normalize the amount of DNA.

**Western Blot Analysis.** The following antibodies were used for immunoblotting assays: anti-pADPr (rabbit 1:4,000, no. 528815;

Calbiochem), anti-pADPr (mouse monoclonal 1:500, no. 1020; Tulip), anti-B-actin (mouse monoclonal 1:5,000, no. A5441; Sigma), pAB H2A#618 (1:1,000; R. Glaser, Albany, NY), anti-lamin C (rabbit 1:200,000, P. Fisher, Stony Brook, NY), anti-GFP (rabbit 1:1,000, no. TP401; Torrey Pines Biolabs), and anti-GFP (mouse monoclonal 1:5,000, no. 632380; Clontech). Western blotting was done using a detection kit from Amersham/GE Healthcare (no. RPN2106), according to manufacturer's instructions.

**Immunostaining of Thick Sections.** For immunofluorescence, frozen samples prepared for transmission electron microscopy (TEM) were cut to sections 0.5  $\mu$ m in thickness and labeled with primary antibodies, followed by secondary antibodies conjugated to fluorochromes. Sections were washed in 200- $\mu$ L drops of 1 $\times$  PBS + 0.1% glycine solution five times for 1 min (coverslip was placed face down on a drop) and were then blocked in 1 $\times$  PBS + 1% BSA twice for 3 min and incubated with primary antibody dissolved in blocking solution in a humid chamber for 1 h. Sections were then washed with 1 $\times$  PBS (four times for 2 min) and incubated with secondary antibody in a humid chamber for 40 min. The mount was then washed with 1 $\times$  PBS (three times for 5 s and four times for 2 min) and mounted in Vectashield (Vector Laboratories). Samples were examined by confocal microscopy using a Leica TCS-NT microscope.

The primary antibodies used were as follows: mouse mAb anti-H1 (1:1,000, sc-8030; Santa Cruz Biotechnology) and anti-pADPr (1:500, no. 1020; Tulip), rabbit polyclonal antibody (pAB) anti-GFP (1:1,000, TP401; Torrey Pines Biolabs), and anti-pADPr (1:5,000; Calbiochem). Mouse Alexa-488, rabbit Alexa-568, and rabbit Alexa-633 (all from Molecular Probes) were used as secondary antibodies (1:400). For DNA staining, propidium iodide was used at a final concentration of 0.05 mg/mL in Vectashield or Draq5 (no. BOS-889-001; Alexis Biochemicals); up to 2.5  $\mu$ M was added to a final wash with 1 $\times$  PBS for 2 min before mounting in Vectashield.

**Electron Microscopy.** For ultrastructural analysis, the salivary glands were dissected, fixed in 2% formaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), postfixed in 1% OsO<sub>4</sub>, dehydrated in ethanol and propyleneoxide, and embedded in EMBED-812 (EMS) in flat molds. After polymerization for 60 h at 65 °C, 70-nm sections were cut on a Leica Ultracut E microtome (Leica), placed on collodion/carbon-coated grids, and stained with 2% uranyl acetate/lead citrate. Sections were viewed on a Tecnai 12 transmission electron microscope (FEI).

For EM immunocytochemistry, samples were prepared according to Tokuyasu (10). In brief, the dissected salivary glands were fixed in 4% formaldehyde/0.2% glutaraldehyde in 0.1 M PHEM [60 mM Pipes, 25 mM HEPES, 2 mM MgCl<sub>2</sub>, 10 mM EGTA (pH 6.9)], cryoprotected in 2.3 M sucrose, mounted on aluminum pins, and frozen in liquid nitrogen. Thin-frozen sections were then cut on a Leica EM UC6/FC6 cryomicrotome (Leica), collected on a drop of sucrose/methylcellulose mixture, and placed on a formvar-carbon grid. The sections were labeled with primary antibody, and the label was subsequently visualized by colloidal gold conjugated to protein A. Sections were stained/embedded in 2% methylcellulose/0.2% uranyl acetate and observed under a Tecnai 12 transmission electron microscope.

**Polytene Chromosome Immunostaining.** Preparation and immunostaining of polytene chromosome squashes were performed exactly as described by Lavrov et al. (11). The primary antibody used was anti-GFP (rabbit 1:400, no. TP401; Torrey Pines Biolabs), and the secondary antibody used was goat anti-rabbit Alexa-

488 (1:400; Molecular Probes). Slides were mounted in Vectashield (Vector Laboratories) with propidium iodide at 0.05 mg/mL for DNA staining.

**Mononucleosome ChIP.** Nuclei were isolated from ~200 adult flies as described by Pinnola et al. (12), with some modifications. In one 1.5-mL Eppendorf tube, 50 larvae were homogenized with 300  $\mu$ L of A1 [60 mM KCl, 15 mM NaCl, 5 mM MgCl<sub>2</sub>, 15 mM Tris-HCl (pH 7.5)] 0.5% Triton X-100 (0.1 mM EGTA, 0.5 mM DTT, and 25 $\times$  complete protease inhibitors (Roche) EDTA-free)/0.3 M sucrose. The homogenates were passed over two layers of Miracloth tissue (Calbiochem) and centrifuged for 5 min at 8,000  $\times$  g at 4 °C. The pellet, resuspended in 300  $\mu$ L of A1 (without 0.5% Triton X-100)/0.3 M sucrose, was loaded into a sucrose gradient [800  $\mu$ L A1 (without 0.5% Triton X-100)/0.8 M sucrose + 150  $\mu$ L A1 (without 0.5% Triton X-100)/0.3 M sucrose] and further centrifuged for 6 min at 8,000  $\times$  g at 4 °C. The nuclei pellet resuspended in 100  $\mu$ L of micrococcal nuclease digestion buffer [60 mM KCl, 15 mM NaCl, 5 mM MgCl<sub>2</sub>, 15 mM Tris-HCl (pH 7.5), 1 mM CaCl<sub>2</sub>, 0.5 mM DTT, 0.34 M sucrose, and 25 $\times$  complete protease inhibitors (Roche)] was pooled together, and the chromatin DNA concentration was measured in 0.1% SDS buffer.

The nuclei were digested with micrococcal nuclease (New England Biolabs) with 2 gel units per 1  $\mu$ g of chromatin for 6 min at 37 °C in MNase digestion buffer (NEB). To stop the reaction, 5 mM EDTA was added.

After MNase digestion, the nuclei reactions were centrifuged for 10 min at 10,000 g at 4 °C. The supernatant fraction containing about 40  $\mu$ g of mononucleosome chromatin DNA was precleared with 60  $\mu$ L of protein A agarose (Invitrogen) for 1 h at 4 °C and then incubated with 10  $\mu$ g of anti-GFP rabbit pAb (Torrey Pines Biolabs) overnight at 4 °C and further incubated with 30  $\mu$ L of protein A agarose (Invitrogen) for 2 h at 4 °C. The immunoprecipitated complexes were washed with buffer A once, buffer B twice, and buffer C twice, and they were then eluted with 500  $\mu$ L of elution buffer. The eluate and 10% input were digested with 5  $\mu$ L of proteinase K (20  $\mu$ g/ $\mu$ L; Invitrogen) for 4 h at 50 °C, and DNA was extracted using the phenol/chloroform/isoamylalcohol (25:24:1) method and precipitated by ethanol with 2  $\mu$ L of Paintpellet (Novagen) for better visualization. The DNA pellet was resuspended in 40  $\mu$ L of water and then treated with 1  $\mu$ L of RNase (2  $\mu$ g/ $\mu$ L; Invitrogen) for 20 min at room temperature.

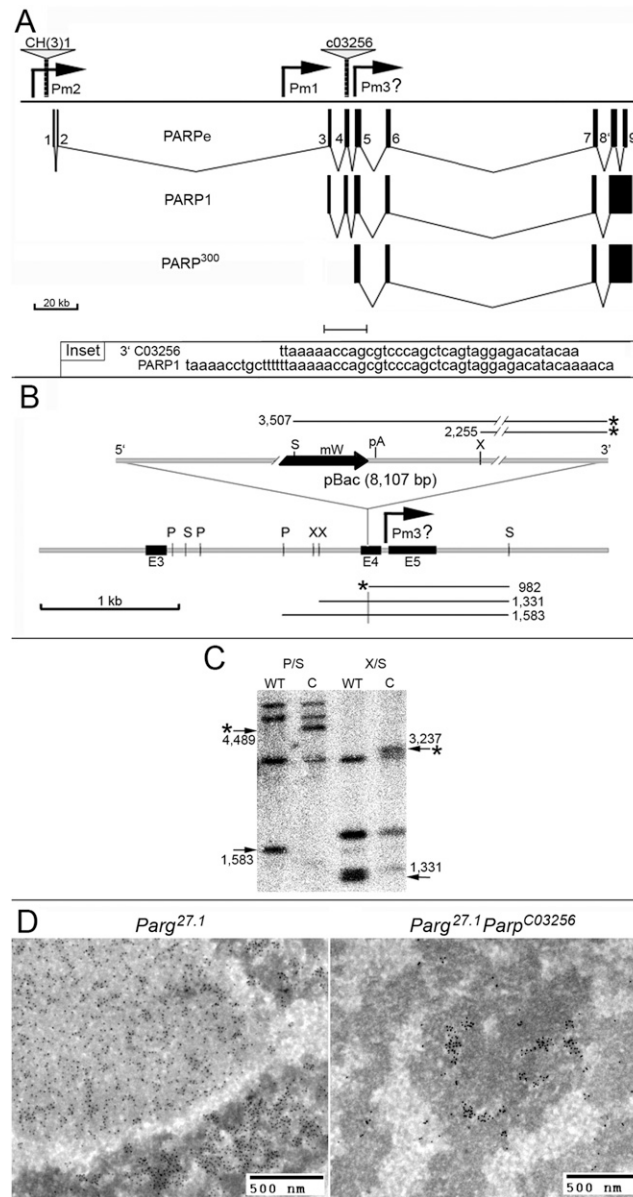
To detect hsp70 DNA in ChIP experiments, we used 24 pairs of primers (Table S3) distributed along 3 kb of the hsp70 genomic sequence (base pairs -500 to +2,500 relative to transcriptional start). Real-time PCR assays were performed using Power Sybr Green PCR master mix (Applied Biosystems) and the StepOne-Plus Real Time PCR System (Applied Biosystems). Cycling conditions were 95 °C for 10 min, followed by 40 (three-step) cycles (95 °C for 15 s; 63 °C for 30 s; 72 °C for 30 s). Reactions were done in triplicate. All the experiments were repeated twice, and the SD was calculated.

**Fly Radiation Sensitivity Assay.** One hundred early third-instar larvae of genotypes *parg*<sup>27.1/+</sup>; *parp*<sup>C03256/+</sup>, and *parg*<sup>27.1/FM7i</sup>; *parp*<sup>C03256/parp</sup><sup>C03256</sup> were  $\gamma$ -irradiated with 36 Gy at a dose rate of 1 Gy/min using a Cesium-137 panoramic gamma irradiator Shepherd Model 81-14R. Larvae were then incubated at 25 °C and allowed to continue development. Their ability to pupariate and develop into adult flies was measured. The fraction of flies that developed without  $\gamma$ -irradiation was set as 100%, and irradiated samples were compared with these values.

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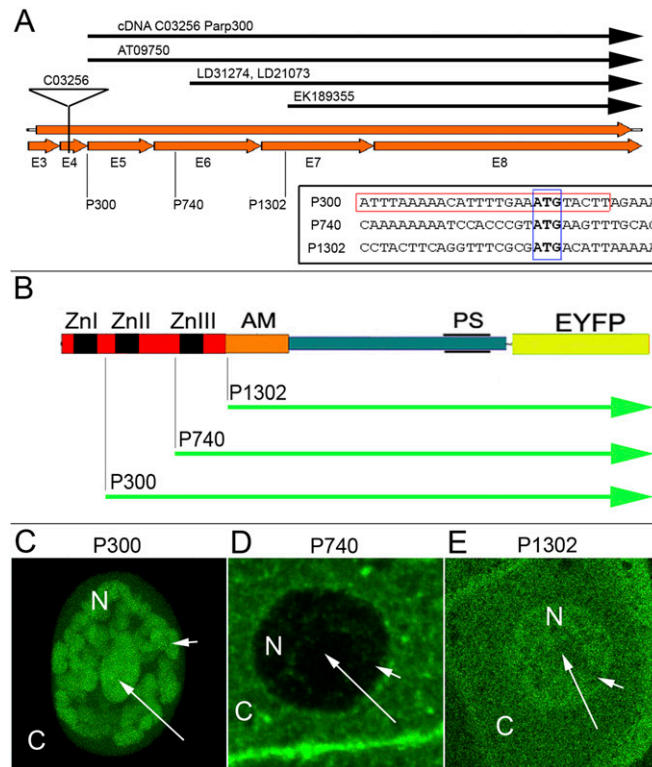
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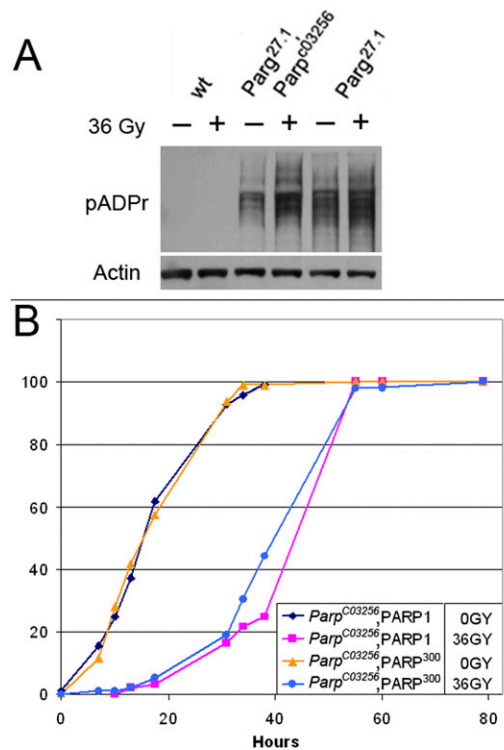


**Fig. 51.** C03256 mutation disrupts the *Parp* locus. (A) Structure of *Drosophila Parp* locus. A diagram summarizes the organization of the *Parp* genomic region as determined from this and previous studies (1, 2). Gray triangles indicate mobile element insertions disrupting locus CH(3)1 (2) and C03256. Pm1 and Pm2 indicate promoters reported previously (1, 2), and Pm3 indicates presumptive promoter, as suggested from this study. The arrangement of the exons encoding PARPe (Top) and PARP1 (Middle) is shown. The bar at the bottom indicates the region that is magnified in Fig. 51B. (B) Diagram summarizing the sequence organization of the genomic region surrounding the C03256 insertion. The triangle indicates C03256 pBac element insertion in exon 4 (corresponding to the second exon of the Parp1 transcript). Black rectangles indicate exons. Black arrow corresponds to white minigene in the pBac element. Sites of restriction endonucleases are shown: S, Sall; P, PstI; X, XbaI. Thin lines (Upper) represent 3,507-bp and 2,255-bp fragments of pBac element fused to a 983-bp genomic fragment (Lower) generating 4,489-bp (S) and 3,237-bp (X/S) fragments, which were detected in the C03256 flies (Fig. 1C). Genomic 1,331-bp (XbaI/Sall) and 1,583-bp (PstI/Sall) fragments detected in WT flies (Fig. 1C) are shown below. (C) Southern blot analysis of genomic DNA from WT and C03256 (C) flies; hybridization with the Parp1 cDNA probe. Genomic DNA was digested with X/S and P/S enzymes. Insertion of the C03256 element in exon 4 results in elimination of 1,331-bp (X/S) and 1,583-bp (P/S) genomic fragments (WT) and appearance of synthetic 3,237-bp (X/S) and 4,489-bp (P/S) fragments (C03256). (D) Immunoelectron microscopy approach was employed to detect difference in pADPr accumulation in chromatin of *Parg*<sup>27.1</sup> mutant and *Parg*<sup>27.1</sup>; *Parp*<sup>C03256</sup> double-mutant animals.

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**Fig. S2.** C03256 mutation reveals putative previously undescribed PARP protein isoform. (A) Diagram shows structure of Parp1 cDNA with regard to C03256 insertion (triangle), exonic structure, starts of existing cDNA clones, and previously undescribed cDNA cloned from C03256 mutants (lines above) as well as deduced alternative starts of translation downstream from C03256. (Inset) Sites of putative starts of translation are shown. The P $\Delta$ 300 start is located between exons 4 and 5. The sequence of intron 4 is labeled with a red rectangle. (B) Structure of putative PARP isoforms in regard to PARP1 domain structure. The arrow shows the position of the nuclear localization signal. The localization of PARP $\Delta$ 300-EYFP (P300) (C), PARP $\Delta$ 740-EYFP (P740) (D), and PARP $\Delta$ 1302-EYFP (P1340) (E) recombinant proteins (green) in salivary gland cells is shown. C, cytoplasm; N, nucleus. The long arrow labels the nucleolus, and the short arrow labels chromatin. Only PARP $\Delta$ 300-EYFP protein localizes in the chromatin and nucleoli, whereas other recombinant proteins are excluded from the chromatin (PARP $\Delta$ 1302-EYFP) or completely excluded from the nuclei (PARP $\Delta$ 740-EYFP).



**Fig. S3.** PARP<sup>Δ300</sup> protein is sufficient for *Drosophila* viability during genotoxic stress response. (A) Western blot analysis shows the level of poly(ADP ribosyl)ation in WT (wt), *Parg*<sup>27.1</sup>; *Parp*<sup>C03256</sup> double-mutant, and *Parg*<sup>27.1</sup> mutant animals before and after irradiation. Mouse anti-pADPr antibody was used. An antiactin antibody was used as a loading control. (B) Timelines of pupariation of *Parp*<sup>C03256</sup> mutant rescued by PARP1-DsRed and PARP<sup>Δ300</sup>-EYFP expression with and without irradiation.

**Table S1.** Timelines of development of heterozygous and homozygous C03256 mutants

	First instar	Second instar	Third instar	Prepupae	Pupae	Adult
<i>Parp</i> <sup>C03256/+</sup> , n = 378	89%	81%	75%	71%	70%	69%
<i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup> , n = 291	91%	83%	59%	33%	27%	0%

**Table S2.** C03256 mutation disrupts *Parp* function, and C03256 complements *Parp*<sup>CH1</sup> mutation

	Viability	Fertility
<i>Parp</i> <sup>CH1/Parp</sup> <sup>CH1</sup>	Lethal, second-instar ecdysis II	NA
<i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Lethal, third-instar pupae	NA
<i>Parp</i> <sup>C03256/Parp</sup> <sup>CH1</sup>	Viable	Fertile
<i>Parg</i> <sup>27.1/Parg</sup> <sup>27.1</sup>	Lethal, third-instar pupae	NA
<i>Parg</i> <sup>27.1/Parg</sup> <sup>27.1</sup> ; <i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Lethal, third-instar early pupae	NA
<i>Parg</i> <sup>27.1/Parg</sup> <sup>27.1</sup> ; <i>Parp</i> <sup>C03256/TM6B</sup>	Lethal, third-instar early pupae	NA
<i>Parg</i> <sup>27.1/FM7i</sup> ; <i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Viable, 14%	Sterile
<i>Gal4</i> <sup>G1</sup> , UAS::Parp1-DsRed; <i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Viable	Fertile
<i>Gal4</i> <sup>G1</sup> , UAS::Parp1-EYFP; <i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Viable	Fertile
<i>Gal4</i> <sup>G1</sup> , UAS::Parpe-EGFP; <i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Lethal, third-instar pupae	NA
<i>Gal4</i> <sup>G1</sup> , UAS::Parp <sup>Δ300</sup> -EYFP; <i>Parp</i> <sup>C03256/Parp</sup> <sup>C03256</sup>	Viable, 6%	Sterile

C03256 lethality could be rescued by either *Parg* function suppression or *Parp* cDNA expression. NA, not applicable.

Table S3. Primer pairs used to detect hsp70 DNA in ChIP experiments

No.	Position	5'–start location	Forward	5'–start location	Reverse
1	–259	4,350	CTAGAATCCAAAACAACACTGGTTG	4,504	CGAGAACAGTGCGCCGTTTACTGTG
2	–231	4,378	CGGTAGGTCATTTGTTGGCAGA	4,534	GAGGCGCGCTCTCTCGAAGC
3	–189	4,420	CTCTGGCCGTTATTCTCTATTCTG	4,571	CCTCTATTTATACTCCGGCGCTCT
4	–162	4,447	GTGACTCTCCCTCTTTGTACTATTGCTC	4,592	GACTCTCCGTCGACGAAGCTC
5	–134	4,475	TCTACTCTGTCCACAGTAAACGG	4,625	CGATGTGTTCACTTTGCTTGTGTTG
6	–103	4,506	GTTCTCGTTGCTTCGAGAGAGCG	4,642	GCTTAGCTTTCGCTTAGCGATG
7	–74	4,535	GAATGTTCCGCGAAAAGAGCG	4,681	TGCAGATTGTTAGCTTGTTCAGCT
8	–31	4,578	CGTCGACGGAGAGCTCAATTCTATTC	4,729	CTTGGTTGTTGGTTACTTTAATTGATTC
9	0	4,609	GCAAAGTGAACACATCGCTAAGC	4,770	TCTTGGTTGATTTCAAGTAGTTGCAGT
10	37	4,646	CAAAACAAGCGCAGCTGAACAAG	4,800	CAGAGTTCCTTCTGTCTTCAATAATTAC
11	69	4,678	TGCAATAAAGTGCAAGTTAAAGTGAATC	4,836	GTTCTTCTCCTCGGTAACGACTTG
12	105	4,714	AGTAACCAACAACCAAGTAATTAACATAAA	4,867	GATCGATTCCAATAGCAGGCATT
13	136	4,745	CTGCAACTACTGAAATCAACCAAGAA	4,903	GTTGGTAGACACCCACGCAGG
14	171	4,780	GAAGACAAGAGAGAAGCTCTGAATACTTTC	4,935	CTGGTCGTTGGCGATAATCTCC
15	206	4,815	GTCGTTACCGAGGAAGAAGAACTCA	4,963	CCACGTAGGACGGCGTGGTG
16	263	4,872	CACCACCTACTCCTGCGTGG	5,022	GTTTCATGGCCACTGTTCTTAG
17	329	4,938	CAACCGCACCCGCTCCT	5,097	CATGTCCTCTGCGATCTTGGG
18	391	5,000	CTAAGAACCAGGTGCCATGAAC	5,145	GATCTTGGGCTTCCGCCGT
19	459	5,068	TACGACGACCCCAAGATCGC	5,219	TCTTGGTCAGTACCATCGAGCTG
20	530	5,139	CAAGATCGGGGTGGAGTATAAGG	5,290	CGTTGAAGTAGGCTGGAAGTGTG
21	594	5,203	GTAAGTACCAAGATGAAGGAGACGG	5,355	GATGATGCGGAGCACATTTCAG
22	663	5,272	GTTCCAGCCTACTTCAACGACTCC	5,423	GCACATTGCGCTCACCTTG
23	732	5,341	GTGCTCCGCATCATCAATGAG	5,490	GAACAGTGATCCCTCGTCGATG
24	803	5,412	GCGCAATGTGCTTATCTTCGAC	5,562	CAGATGAGTACTAGCCGGTTGTC
25	867	5,476	GAGGGATCACTGTTTCGAGGTGC	5,630	CTGAGGCGTCTGAGGGCGCG
26	930	5,539	GACAACCGGCTAGTCACTCATCTG	5,688	CTCGATGGTGGCTCCGTTGC
27	992	5,601	CCAACCTCGCGCCCTACGA	5,756	GCACAGCTCCTCAACCTGGC
28	1,059	5,668	CACGGAGGCCACCATCGAGA	5,823	CTTATCCATCTTGGCATCGTTGAG
29	1,128	5,737	GCCAGGTTTGAGGAGCTGTGC	5,892	CTGCAGCAGACTTTGCACCTTG
30	1,191	5,800	CTCAACGATGCCAAGATGGATAAG	5,956	CTCCGATGCAACTGCCTCGT
31	1,256	5,865	CATTCCTCAAGGTGCAAAGTCTG	6,015	CAGCACGTCTGGATCTTGC
32	1,326	5,935	GACGAGGCAAGTTGCATACGGA	6,084	CTCGATCAGCTTGGTCAATACACC
33	1,391	6,000	GATCCAGGACGTGTCTGCTGGT	6,146	GCTGGTTGTCGCGTATGTG
34	1,460	6,069	GACCAAGCTGATCGAGCGCA	6,224	GACAGATCGAAGGTGCCAAT
35	1,533	6,142	CAGCCCCGAGTCTCCATTCA	6,294	GATTCCATTGGCGTCCAAGTC
36	1,604	6,213	CTTCGATCTGTCGGCATTCC	6,364	GTCCTTGTGCTTCTGATCGTG
37	1,671	6,280	GACGCCAATGGAATCCTGAAC	6,437	GATGCTTCTGTCCTCGTCCG
38	1,739	6,348	CAAGAACGACAAGGGACGGCT	6,504	CTGTTCCACGGCTGCTTCA
39	1,808	6,417	CGACGAGGACGAGAAGCATCG	6,564	GTCGTTGCACTTGCCAAGACG
40	1,875	6,484	GTGAAGCAGGCCGTGGAACA	6,635	GTGAGCTCTCCAGCTTGTGG
41	1,941	6,550	GACAAGTGCAACGACACTATCCG	6,700	GTGCGGGAGCTGGAGCTGGT
42	2,006	6,615	CCACAAGCTGGAGGAGCTCAC	6,765	CTCCTCGACCTGGGTCCAG
43	2,068	6,677	ACCAGCTCCAGCTCCCGCAC	6,822	ACCCATATGTTATAACCCATTGATGAAC
44	2,135	6,744	CTCTGGACCCACGGTCGAGG	6,897	CTATTGTTGTTCTATGGAATATTCGATCG
45	2,189	6,798	CATCAATGGGTTATAACATATGGGTTATAT	6,960	AATGTTATTGCTTATAGAAAAATAAATTA
46	2,252	6,861	GAATGTTTCGATCGAATATTCATAGA	7,015	AGTCTACAAAACATTAATGACAAGTTGAC
47	2,317	6,926	GCAAAAATGTTATTGCTTATAGAAAAATA	7,076	CATCACTAATATAGAATGTAGAATGAACCA
48	2,377	6,986	GTCATTTAATGTTTTGTAGACTTTTGAAAGT	7,129	CAGAAAATCATCAAAGCCGAATATAAT