Supporting Online Material

Materials and Methods

p53Rps transgenic transformation constructs: The transfomation vectors, pH-Stinger and pGreeen-H-Pelican (*1*), were obtained from Drosophila Genomics Resource Center (Bloomington, IN, USA). A 150bp enhancer (blue) containing a consensus p53 binding site (p53RE, red arrows, fig. 1A) drives two different eGFP constructs. One bears a nuclear localization signal (p53R-GFPnls) and one does not (p53R-GFPcyt). To obtain the 150bp fragment, two primers of 98 base pairs in length: CTA GAA TTC CGT CCG CTC GAC TTG TTC AAA CAT GTC AGG TTG GTT CTT CCA CTT TTA TTT GAG TAA TTT TCG CCC TTT TTC CAT AGA TTT TCA TAG AT, and AGA GGA TCC CTC GAA CAC GTC GAT GCA CGC TGA GTG AAG AAA TCT GAA AAC CCA TTC CGA AAA TTC GTT ATC TAT GAA AAT CTA TGG AAA AAG GGC GA, were synthesized and allowed to hybridize through 29bp overlapping sequence, then filled with Klenow and dNTPs. The fragment was further placed between EcoRI and BamHI sites. After standard transformation, obtained transgenic lines are named as GHP150/p53R-GFPcyt and STI150/p53R-GFPnls. Insertion site of each transgenic line, STI150 at 2R:52C and GHP150 at 3R:100C, were mapped using standard inverse PCR. Both transgenic lines are homozygous viable stocks.

Fly stocks and genetics: All fly stocks are maintained at 22-25°C on standard food media. We obtained *rad54* (*okr*^{AA}, *okr*^{RU}) and *chk2* (*mnk*^{p6}) from T. Schupbach (Princeton University); ATM (tefu^{wk}, *tefu*^{stg}) alleles from YS. Rong (National Cancer Institute). *spo11* (*mei-W68*^{1}) and ATR^{D3} strains were obtained from Bloomington Stock Center

(Indiana University, Bloomington, IN, USA). In meiotic recombination and *rad54* interaction studies, three p53 null alleles, ns, 1 and 2, were used in trans-combination to reduce genetic background influences in each individual stock. To generate *rad54* mutant flies, $rad54^{AA}$ (9 Q to ochre) and $rad54^{RU}$ (391 Q to amber) alleles were used in trans-heterzygous. Meiotic recombination frequency was measured by crossing wild-type (Canton S and *yw*) or p53 ($p53^{ns/1}$, $p53^{ns/2}$, $p53^{1/2}$) females, heterozygous for al^1 dp^{ov1} b^1 pr^1 Bl^1 cn^1 c^1 px^1 sp^1 to al^1 dp^{ov1} b^1 pr^1 c^1 px^1 sp^1 homozygous males. Segregations of three markers (*c, px, sp*) were scored from each progeny and the percentage of progeny with crossover events was plotted shown in Fig. 3A. Statistics analysis in table S1: Fraction was calculated using one haploid chromosome to determine the total number of crossover products and applied with the following formula: (2x number of progenies with observed crossovers)/ 2x(number of non-crossover + crossover). Symmetrical confidence interval at 95% CI was calculated using modified Wald method (http://graphpad.com/quickcalcs/ConfInterval1.cfm). Average of the two wild-type strains was used as a baseline to calculate decreased recombination frequency in p53 strains. Probability (p value) was calculated using CHITEST in Microsoft Excel 2008 for Mac Version 12.1.0. Nondisjunction assay was described in table S2.

Radiation response assay and microscopy: Staged embryos (4.5-7h AEL for early stage, or 9-12h AEL for late stage) were exposed to 4000 rads of ionizing radiation using a Cs-137 Mark 1-68A irradiator (J.L. Shepherd & Associates, San Ferando, CA, USA). To examine GFP expression, embryos were dechorionated in 50% bleach and immersed in halocarbon oil 700 (Sigma-Aldrich) for imaging. Time-lapse images were acquired on TCSSP Spectral Confocal Microscope (Leica Microsystems). Z-stacks of images from

each time point were projected using Image J software (NIH, Bethesda, MD, USA). Epifluorescence images were acquired on Axioplan 2E microscope (Carl Zeiss) attached with Hamamatsu monochrome digital camera. Figures were prepared using Adobe photoshop and Illustrator CS2 (Adobe Systems).

Immunostaining of fly tissue: 3-5 days old well-fed females were dissected in PBS and fixed in 4% EM-grade formaldehyde (Polysciences) diluted in PBS-0.1% tween-20, with three times volume of heptane. After washing, tissues are blocked in 1.5% BSA, then incubated with primary antibodies at 4°C overnight. The following concentrations of primary antibodies were used: rabbit α-GFP, 1:1000 (Invitrogen); mouse α-HTS clone 1B1, 1:500 (obtained from D. McKearin). Alex-488, 568, 1:250-500 (Invitrogen) were used for fluorescence visualization. 0.1μg/ml of DAPI (Invitrogen) was used for DNA staining. Ovaries were further hand dissected and mounted in VECTASHIELD (Vector Laboratories).

Quantification of nurse cell nuclei number and egg length: Dissected egg chambers were stained with 0.1μg/ml of DAPI and imaged on Leica TCS SP5 confocal microscope. To facilitate visual counting, z stacks of each genotype were processed with the same scripts using Image J as follows: "Despeckle, Subtract Background, Gaussian Blur (sigma=2), 3D Project (projection=[Brightest Point] axis=Y-Axis slice='thickness of z-stack' initial=-30 total=180 rotation=15 lower=1 upper=255 opacity=0 surface=100 interior=50 interpolate), Make Montage (columns=5 rows=1 scale=1 first=1 last=5 in crement=1 border=2)". Sample sizes, n= 141 (spo11$^{-/-}$,*p53*$^{ns/1}$,*rad54*$^{AA/RU}$), 44 (*p53*$^{ns/1}$,*rad54*$^{AA/RU}$), 25 (*p53*$^{-/-}$), 24 (*rad54*$^{AA/RU}$), 21 (wild-type). For egg length measurements, eggs were collected on standard juice agar plates and manually orientated

horizontally for imaging. Images were taken on on the Zeiss SteREO Discovery V.12 and processed with Image J using the following script: “Enhance Contrast (saturated=0.5), RGB Color, Set Scale (distance=0 known=1 pixel=1 unit=pixel)”. Pixel unit was further converted to microns according to the scale of magnifications. Sample sizes, n= 936 (spo11$^{-/-}$, *p53*$^{ns/1}$,*rad54*$^{AA/RU}$), 1468 (*p53*$^{ns/1}$, *rad54*$^{AA/RU}$), 893 (*p53*$^{ns/2}$, *rad54*$^{AA/RU}$), 540 (*p53*$^{ns/1}$), 908(*rad54*$^{AA/RU}$), 1070 (wild-type). Prism 5 software (GraphPad) was used to perform statistics.

Immunohistochemistry of mouse testes: 10 week age wild-type male mice were initially fixed via whole-body transcardial perfusion with freshly-prepared, cold 4% PFA prior to organ dissection and further drop fixation. Sections were de-waxed in xylene and rehydrated in graded concentrations of alcohol. After rinsed in distilled water, antigen retrieval was performed in a modified citrate buffer, pH 6.1 (Target Retrieval Solution, S1700, DAKO) for 30min using a 95–99°C waterbath. After peroxidase block, slides were incubated with mouse anti-phospho-Ser15-p53 (16G8, Cell Signaling), 1:25 dilution in Antibody diluent (DAKO) in a humidified chamber at 4°C overnight. For negative controls, primary antibodies were omitted. To detect signals, EnVision HRP-polymer with DAB (DAKO) systems were used. Slides were then counterstained with hematoxylin and bluing agent (0.037 mol/L ammonia, Sigma) before standard dehydration and mounting procedures. Several modifications were made for Spo11-knockout and littermate controls: Sections from 3.5 month-age males were processed as described above, but additional antigen retrieval was performed in Retriever 2100 (PickCell). For signal amplification and detection, combinations of EnVision HRP-

polymer (DAKO) with TSA (Perkin Elmer) systems were used. Slides with fluorescent detection were extensively washed and mounted in mounting medium (DAKO) containing 0.1mg/ml of Hoechst 33342 (Invitrogen). Criteria used for staging of seminiferous tubules were based on nuclear morphology described here (*2*).

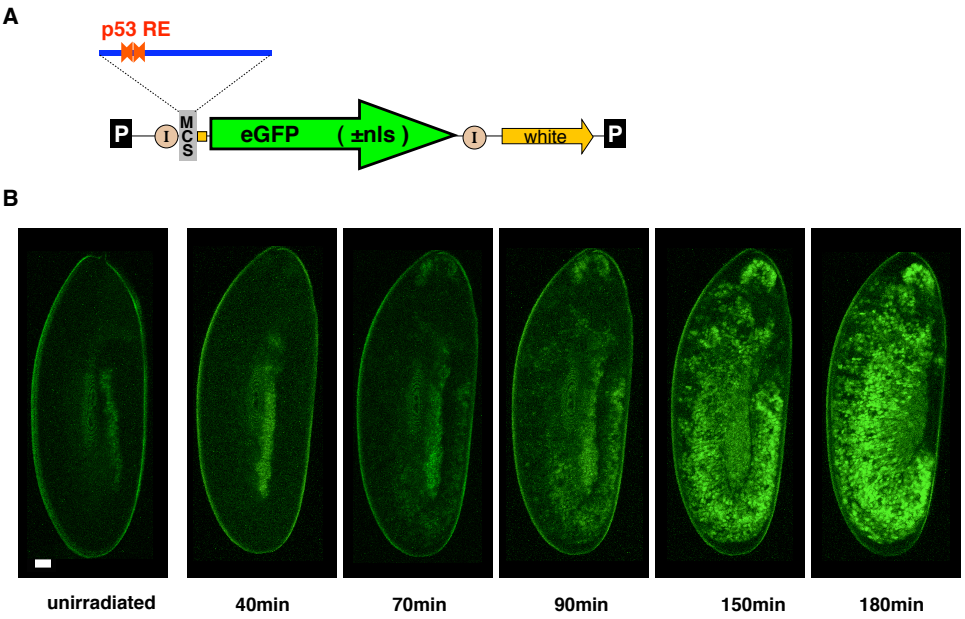

figure S1. p53Rps construct and its activation in Drosophila embryos

(A) Illustration of p53Rps transgenes. P, P element sequence; MCS, multiple cloning site; I, insulator; white, eye color for transformant selection; nls, nuclear localization signal; distances are not to scale. **(B)** Confocal images of p53R-GFPnls activation in *Drosophila* embryos at various time points after exposure to ionizing radiation followed by time-lapse live imaging. Scale bar, 10 microns.

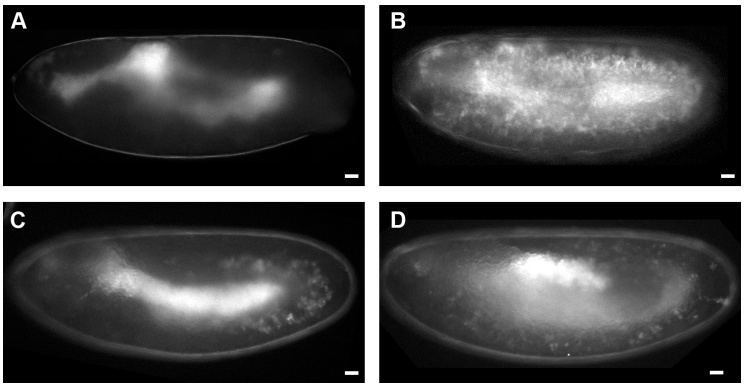

figure S2. Activation of the p53Rps by injected double-strand DNA and UV in Drosophila embryos

Epifluorescence images of p53R-GFPnls embryos. **(A)** Buffer-injected control embryos. **(B)** Embryos injected with ΦX174 HaeIII digested DNA fragments (0.5μg/μl). 0-30min after egg laying (AEL) embryos were collected, dechorionated and injected. After recovery at 25°C, embryos at ~8-9hr AEL of age were imaged. **(C)** Embryos irradiated with 100J/m^2 of UVB. **(D)** Embryos irradiated with 500J/m^2 of UVB. In these studies, both expressivity and penetrance of these responses were incomplete and appeared less robust than IR. Responses were not intended for quantitative comparison and were not kinetically measured. Scale bar, 10 microns.

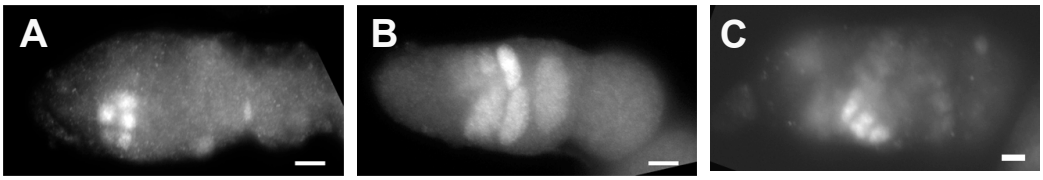

figure S3. Activation of the p53Rps in ATM and ATR mutants

Immunostaining of GFP from animals genotyped as **(A)** $ATM^{stg/wk}$ with p53R-GFPnls, ATR^{D3} with p53R-GFPcyt (B) or p53R-GFPnls (C). Note that stereotyped activation of p53Rps in regions 2 was unaffected in the intact germaria of those mutants. Scale bar, 10 microns.

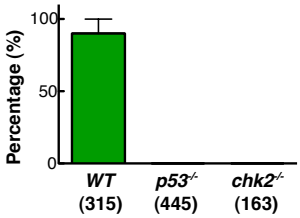

figure S4. Quantified activation of the p53Rps in *Drosophila* female germline

Percentage of germarium with p53R-GFPnls expression in regions 2a and 2b. Means of at least three independent trials ± standard deviations are plotted and sample size is denoted within parenthesis.

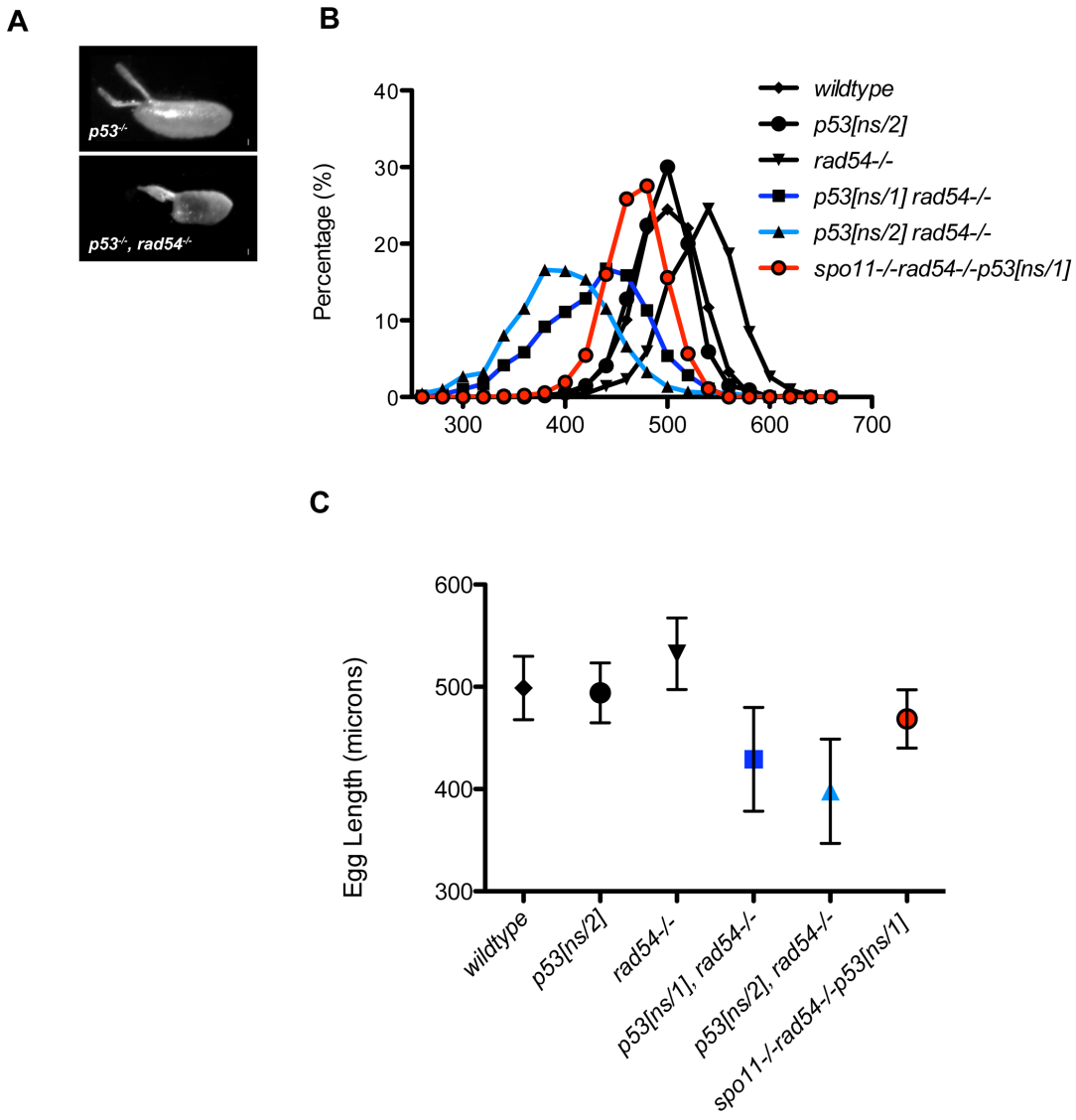

figure S5. Egg length measurements for genetic interactions of p53 with *rad54*

(A) Image of the eggs laid by $p53^{-/-}$ or $p53^{-/-}$,$rad54^{AA/RU}$ females. **(B)** Frequency distribution of the measured egg length. Scale bar, 10 microns. **(C)** Average egg length ± S.D. from at least three independent trials. The reduction in egg length, shown by the left-shift of distribution in (B, blue lines) and lower average in (C, blue symbols), is largely rescued in eggs laid by s$po11^{-/-}p53^{-/-}rad54^{-/-}$ females (red symbol).

Text of Movie

movie S1. Time-lapse live imaging of p53Rps activation in Drosophila embryos

A control unirradiated embryo (left) was imaged side by side with an irradiated embryo (right). Both embryos are wild-type and homozygotes for the p53R-GFPnls transgene.

Table S1 Meiotic recombination frequency in p53 mutants

Interval	Genotype		Frequency	95% CI (low, high)	Relative Difference (%)	p value†
c-px	WT-Average		0.245			
		CS	0.211	(0.1866, 0.2368)		
		yw	0.279	(0.2496, 0.3097)		
	p53	*ns/1*	0.174	(0.1486, 0.2036)	-28.7	0.00001 ***
		ns/2	0.206	(0.1810, 0.2341)	-15.7	0.00766 **
		1/2	0.185	(0.1419, 0.2365)	-24.5	0.02431 *
px-sp	WT-Average		0.098			
		CS	0.095	(0.0772, 0.1168)		
		yw	0.101	(0.0807, 0.1260)		
	p53	*ns/1*	0.046	(0.0319, 0.0646)	-53.5	0.00001 ***
		ns/2	0.066	(0.0504, 0.0854)	-32.9	0.00226 **
		1/2	0.060	(0.0352, 0.0987)	-39.0	0.04884 *
c-sp	WT-Average		0.319			
		CS	0.287	(0.2604, 0.3160)		
		yw	0.351	(0.3200, 0.3839)		
	p53	*ns/1*	0.207	(0.1793, 0.2379)	-35.2	0.00000 ***
		ns/2	0.265	(0.2367, 0.2945)	-17.2	0.00045 ***
		1/2	0.238	(0.1906, 0.2940)	-25.3	0.00515 **

† Significance degrees were denoted with ***, p<0.001; **, p=0.001 to 0.01; *, p=0.01 to 0.05

Table S2 Meiotic nondisjunction rate in p53 mutants

Maternal genotype	N	XO	X non-disjunction rate (%)
Canton S/yw	2,323	1	0.086
p53[ns]/[1]	2,270	1	0.088

Chromosome segregation defects were scored from a cross of yvf/Y males to wild type (CS/yw) or w/yw; p53[ns]/[1] females from 0~2weeks old. Normal disjunction of the X chromosome in females gives rise to +/Y (XY) males; nondisjunction gives rise to exceptional yvf/O (XO) males that can be distinguished from their wild-type siblings. Male progeny were counted, and the number of XO males was multiplied by 2, to account for the YO products. Nondisjunction was calculated as (2 × [XO males]/N) × 100.

References

1. S. Barolo, B. Castro, J. W. Posakony, *Biotechniques* **36**, 436 (Mar, 2004).

2. E. A. Ahmed, D. G. de Rooij, *Methods Mol Biol* **558**, 263 (2009).