Supplemental Information

Materials and Methods

rRNA gene copy number and rRNA expression

For rDNA qPCR, genomic DNA was extracted with phenol/chloroform/isoamyl alcohol (25:24:1) followed by ethanol precipitation. A total of 4ng of genomic DNA was used for each qPCR reaction of 10µl. For rRNA RT-qPCR, total RNAs were extracted with Trizol, purified by organic extraction followed by isopropanol precipitation and treated with DNase to remove DNA contamination. 500ng of RNAs were then used to produce cDNAs with oligo(dT) primers and random hexamers using SuperScript III Reverse Transcriptase (Life Technologies). Fluorescence-based quantitative real-time PCR (qPCR) was performed to assay levels of *18S* and *5S with tbp, gapdh* and *rpl32* as internal controls. cDNAs from SuperScript III Reverse Transcription were diluted 1:500 and 2ul aliquots of each cDNA sample were added to 5ul of 2x power SYBR Green PCR Master Mix (Applied Biosysterms part No.: 4367659, Lot No. :1305403), 0.5µl each of 10nm Forward & Reverse primer and 2ul of water in a 384-well plate. The resulting reactions were sealed, centrifuged, and cycled on an ABI 7900HT according to the instrument's standard protocol. Analysis of the fluorescence curves was done using ABI's SDS2.4 software. The Ct values were analyzed using the Biogazelle qBase Plus version 2.4 software to generate normalized relative quantities using assays for endogenous controls.

Primers for qPCRs	
Name	Sequence
18S-F	AGCCTGAGAAACGGCTACCA
18S-R	AGCTGGGAGTGGGTAATTTACG
5S set1-F	GACCATACCACGCTGAATA
5S set1-R	CCCGACGCTGCTTAAT
5S set2-F	CGCTGAATACATCGGTTCT
5S set2-R	CGCGGTGTTCCCAAG

Generation of the UASp-p35-Flag transgenic fly strain

The coding sequence of *p35* was PCR-amplified from the genomic DNA of the *UAS-p35* flies using the primers CACCATGTGTGTAATTTTTCCGGTAGAAATCG and TTTAATTGTGTTTAATATTACATTTTTGTTGAG, and was then cloned into the pENTR/D-TOPO vector (Invitrogen, K2400-20). The *p35-pENTR* was then recombined with the *pPWF* destination vector (Invitrogen, LR clonase II, 11791-020) to generate the *UASp-p35-Flag* plasmid.

Generation of the kinase-dead lok^{KD} mutant by CAS9/CRSPR

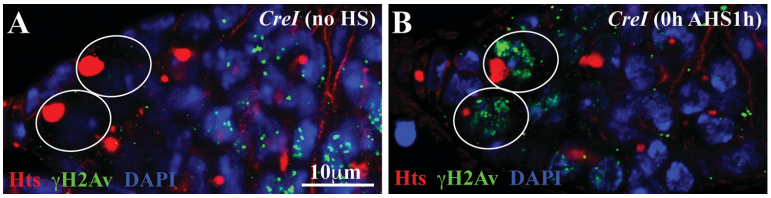
The 286th residue Asp in the transcript CHK2-PB, which is responsible for CHK2 kinase activity, was mutated into Ala to generate *lok^{KD}* by modifying the codon GAC to GCC. The sgRNA target site was "GTCAGGCTTAAGGTCACGAT**GGG**" (PAM in bold). To target this genomic site, we constructed the *lok^{KD}* donor and the U6B promoter-driven sgRNA plasmid. The *lok^{KD}* donor was generated by PCR on the *Drosophila* genomic DNA with AccuPrime™ Pfx DNA Polymerase (Invitrogen, 12344-024) using

primers, CTAGCTAGCTCAGAACCCACAAGAGCAG, GGAAGATCTCGGAATGG-TTTGCTGAAGA, CAACTACCTAGGTTCTACCTTTCAGGCATCACACATCGTGC-CCTTAAGCCTG and CAGGCTTAAGGGCACGATGTGTGATGCCTGAAAGGTAG-AACCTAGGTAGTTG, and was further cloned into the pBluescript plasmid cut by NheI and BglII. There is one AvrII site near the sgRNA target site in the lok^{KD} donor as a selection marker. The lok^{KD} donor and sgRNA plasmid were injected into *{nos-Cas9}attP2* embryos at the concentration of 300ng/µl and 100ng/µl, respectively. The lok^{KD} mutant lines were identified by sequencing PCR products using primers CTAGCTAGCTCAGAACCCACAAGAGCAG and GGAAGATCTCGGAATGGTTTGCTGAAGA.

Generation of Pnos-GFP-bam 3'UTR transgenic reporter flies

To construct *Pnos-eGFP-bam 3'UTR*, we amplified the *bam* 3'UTR from the *Drosophila* cDNA libraries (w^{1118}) using a pair of primers (one with a BamHI cutting site and the other with a SpeI cutting site). The amplified *bam* 3'UTR DNA was cut with BamHI and SpeI, and was then cloned into the BamHI-SpeI site of the *pVALIUM-Pnos-eGFP-nos 3'UTR* vector to replace the *nos* 3'UTR. The *pVALIUM-Pnos-eGFP-bam 3'UTR* construct was then introduced into the *attP* site in the *Drosophila* strain (BL#24482) using PhiC31 integrase-mediated transgenesis by Rainbow Company Inc.

Supplemental figures



C

treatment	No. of γH2Av foci	No. of GSCs examined
no HS	0	26
0h AHS1h	37.7±6.6	26

Figure S1. I-CreI expression can induce double-stranded DNA breaks. Ovals indicate GSCs. (**A**-**C**) GSCs in the *hs-I-CreI* germarium contain γ-H2Av-positive foci immediately after one-hour heatshock (0h AHS1h, **B**) in comparison with those GSCs in the control germarium (no HS, **A**). **C**: γ-H2Av foci quantification results. Scale bar: 10μm.

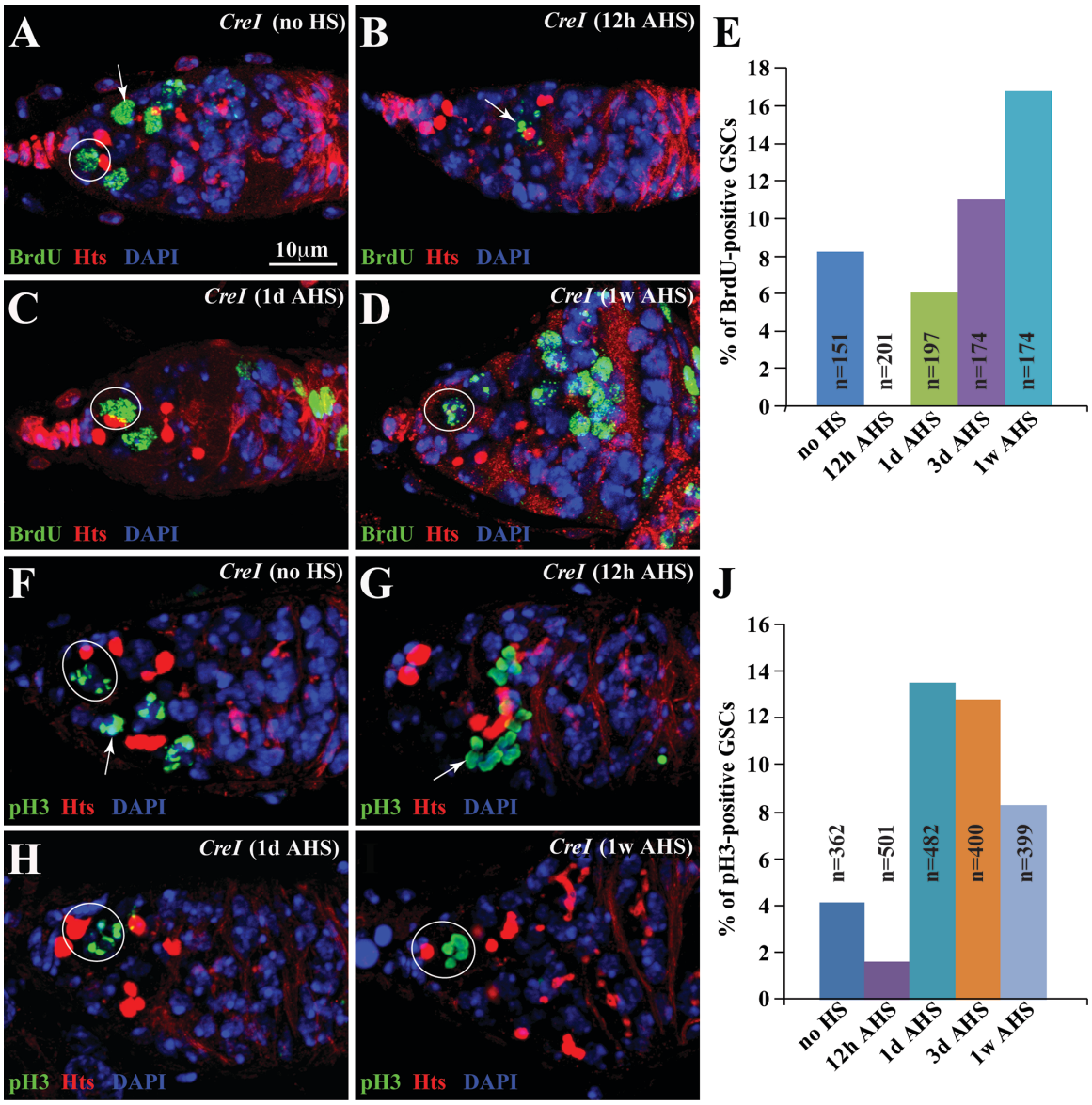


Figure S2. GSCs resume their proliferation one day after DNA damage. Ovals indicate GSCs, whereas arrows highlight mitotic cysts. (**A-E**) GSCs in the *hs-I-CreI* germarium are negative for BrdU labeling 12h AHS (**B**) and then become positive again 1d AHS (**C**) and 1w AHS (**D**) as in the control (no HS, **A**). **E**: BrdU-positive GSC quantification results. BrdU-positive GSCs undergo DNA replication. (**F-J**) GSCs in the *hs-I-CreI* germarium are negative for pH3 expression 12h AHS (**G**) and then become positive again 1d AHS (**H**) and 1w AHS (**I**) as in the control (no HS, **F**). **J**: pH3-positive GSC quantification results. pH3-positive GSCs are in late G2 phase of the cell cycle or in mitosis. Scale bar: 10μm.

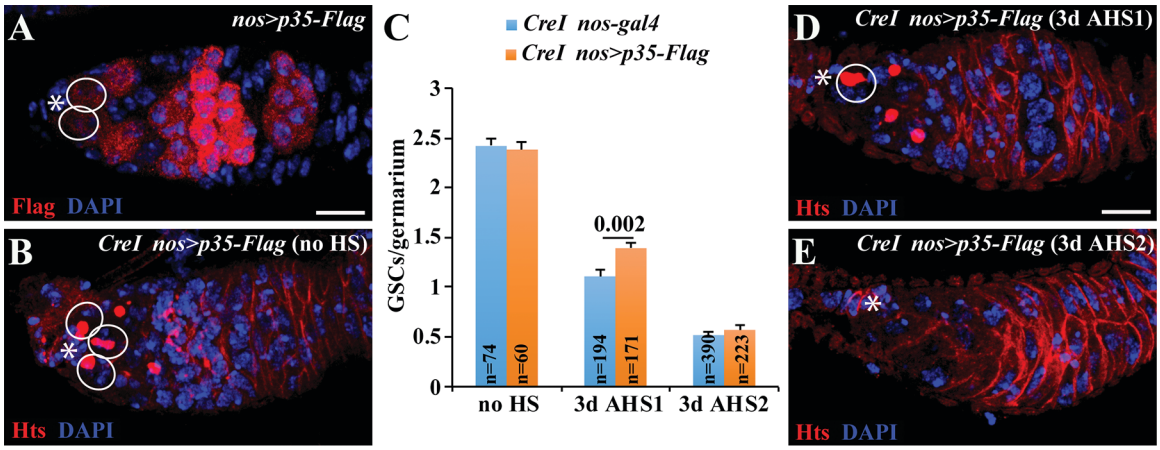


Figure S3. Germline-specific overexpression of the Baculovirus anti-apoptosis gene *p35* fails to rescue the DNA damage-induced GSC loss. Ovals and asterisks indicate GSCs and CPCs, respectively. (**A**) *nos-gal4*-driven expression of the C-terminal Flag-tagged *p35* (*nos>p35-Flag*) specifically in germ cells, including GSCs. (**B-E**) *nos>p35-Flag* germaria carry 3 GSCs in the absence of DNA damage (no HS, **B**), but contain 1 GSC 3 days after one-hour heatshock (3d AHS1, **D**) or 0 GSC 3 days after two-hour heatshock (3d AHS2, **E**). **C**: GSC quantification results. Scale bar: 10μm.

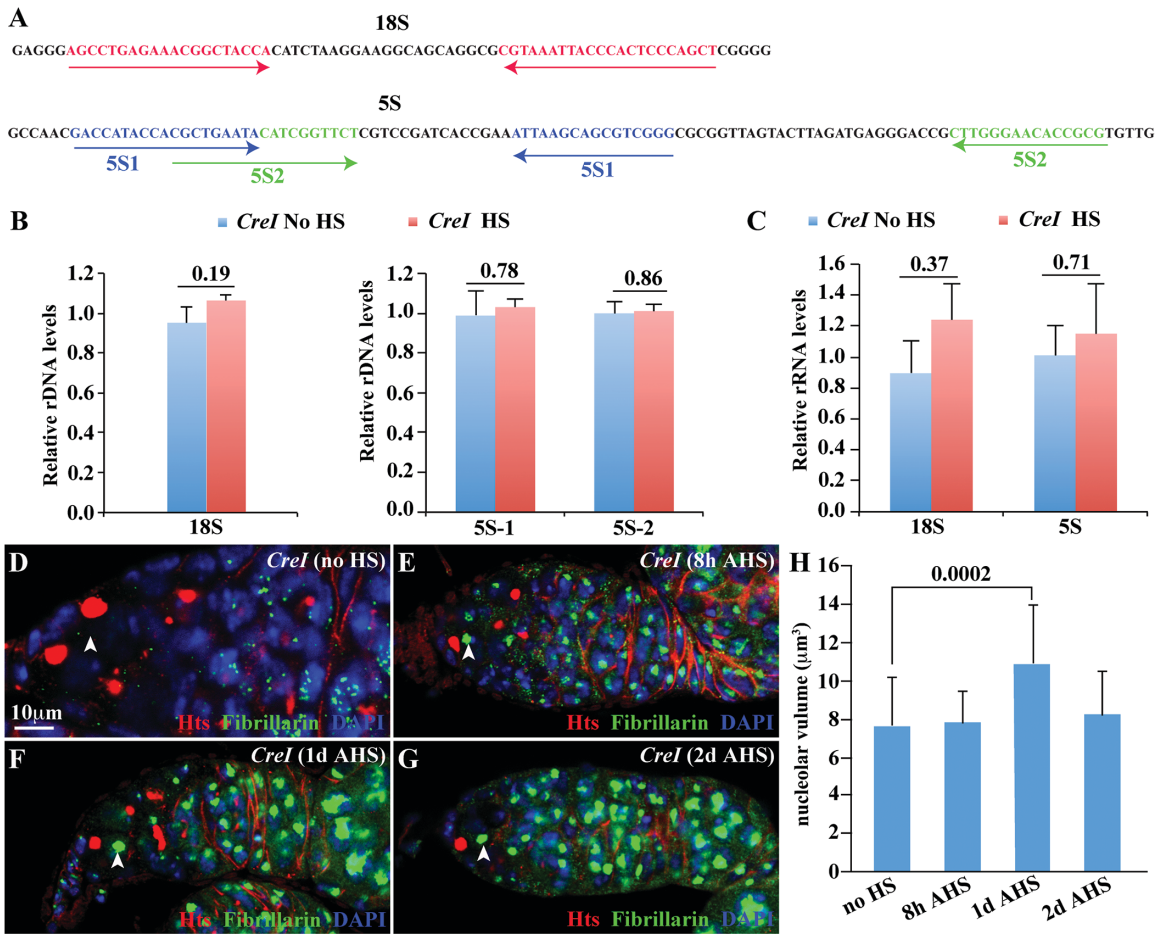


Figure S4. I-CreI-induced double-stranded DNA breaks do not affect *18S* rDNA repeats, 18S rRNA production and thus nucleolus volume. (**A**) Primer pairs for quantitative PCRs to detect the copy numbers of *18S* and *5S* genes. (**B**) Quantitative PCR results show that I-CreI-mediated double-stranded DNA breakage does not affect the copy numbers of *18S* and *5S* genes. (**C**) Quantitative RT-PCR results show that I-CreI-mediated double-stranded DNA breakage does not affect the production of *18S* and *5S* rRNAs. (**D-H**) The nucleolus size does not change in GSCs in the *hs-I-CreI* germaria 8h AHS (**E**), 1d AHS (**F**) and 2d AHS (**G**) in comparison with those in the control gernarium (no HS, **D**). **H**: nucleolus volume quantification results. Scale bar: 10μm.

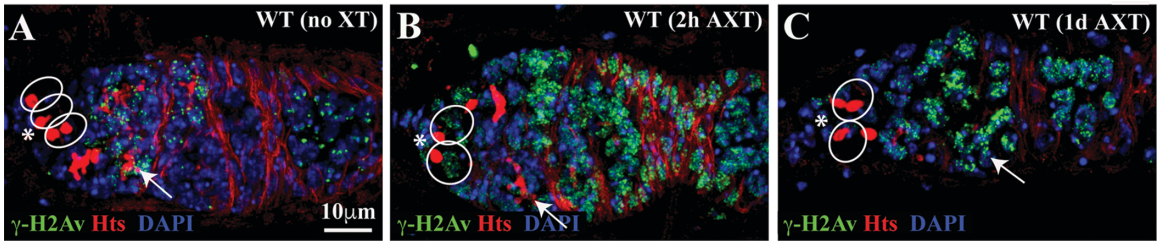


Figure S5. X-ray irradiation can efficiently introduce DNA damage into GSCs and their progeny. Ovals and asterisks indicate GSCs and CPCs, respectively. (**A**) Wild-type (WT) germarium contains γ-H2Av-negaitve GSCs and γ-H2Av-positive meiotic germ cells (arrow) without X-ray treatment (no XT). (**B**) Germarium contains γ-H2Av-positive germ cells, including 2 GSCs and differentiated germ cells (arrow) 2 hours after 20000rad X-ray treatment (2h AXT). (**C**) Germarium contains γ-H2Av-negaitve GSCs and γ-H2Av-positive differentiated germ cells (arrow) 1d AXT, indicating that DNA damage has been successfully repaired in GSCs. Scale bar: 10μm.

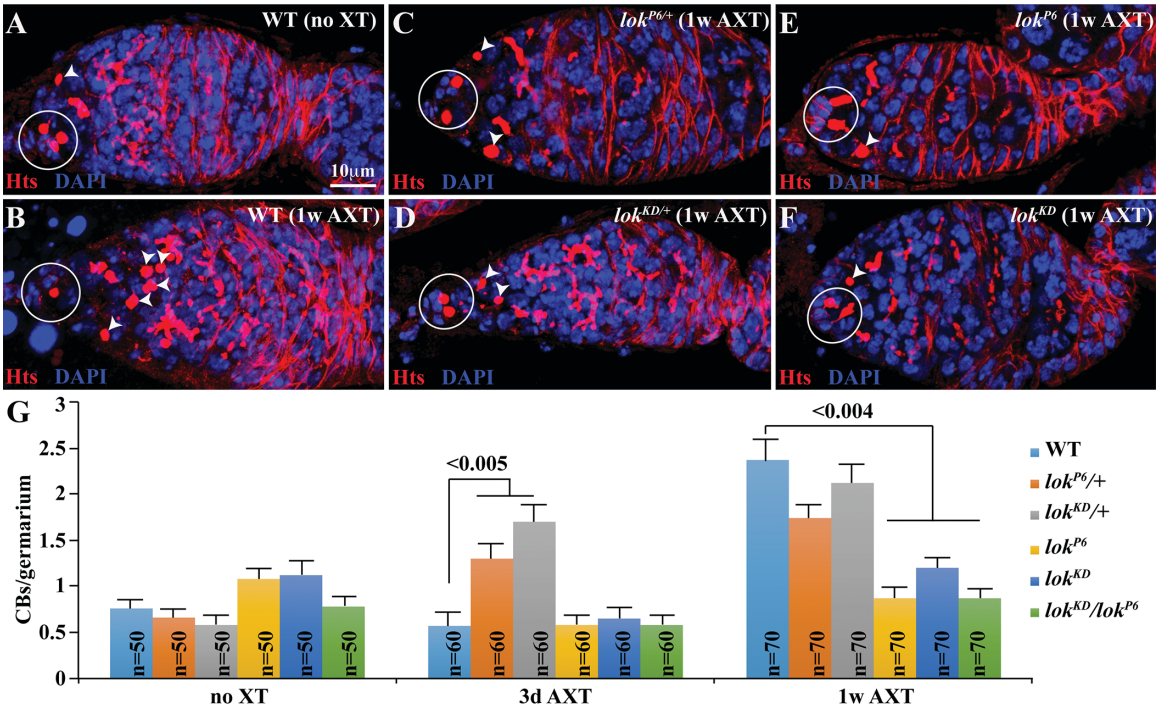


Figure S6. CHK2 inactivation can rescue the germ cell differentiation defect caused by X-ray-induced DNA damage. Circles and arrowheads indicate CPCs/GSCs and CBs, respectively. (**A**, **B**) X-ray-treated germarium (1w AXT, **B**) contains excess CBs in comparison with the control untreated germarium containing 1 CB (no XT, **A**). (**C**, **D**) X-ray-treated *lok*^{P6} (1w AXT, **C**) and *lok*^{KD} (1w AXT, **D**) heterozygous germaria contain 2 CBs. (**E**, **F**) X-ray-treated *lok*^{P6} (1w AXT, **E**) and *lok*^{KD} (1w AXT, **F**) homozygous germaria contain 1 CB. **G**: CB quantification results. Please note that both *lok*^{P6} and *lok*^{KD} heterozygous mutations actually promote the germ cell differentiation defect 3d AXT. Scale bar: 10μm.