Supplemental figures Legends, tables and figures

Xu et al.,

Maternal BCAS2 protects genomic integrity through RPA in mouse preimplantation embryos

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Figure S1

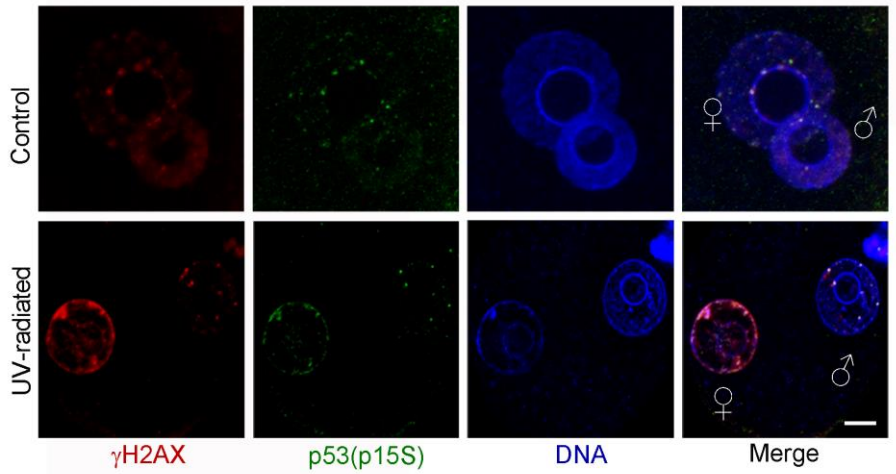

Fig. S1. BCAS2 responses to UV-induced DNA damage. MII oocytes were UV-irradiated and *in vitro* fertilized with normal sperms. Zygotes were cultured until PN4-5 and immunostained with phosphor-p53 (p15S) and γH2AX. Male- and female pronuclei are indicated with male and female symbols, respectively. Scale bar, 10 μm.

Figure S2

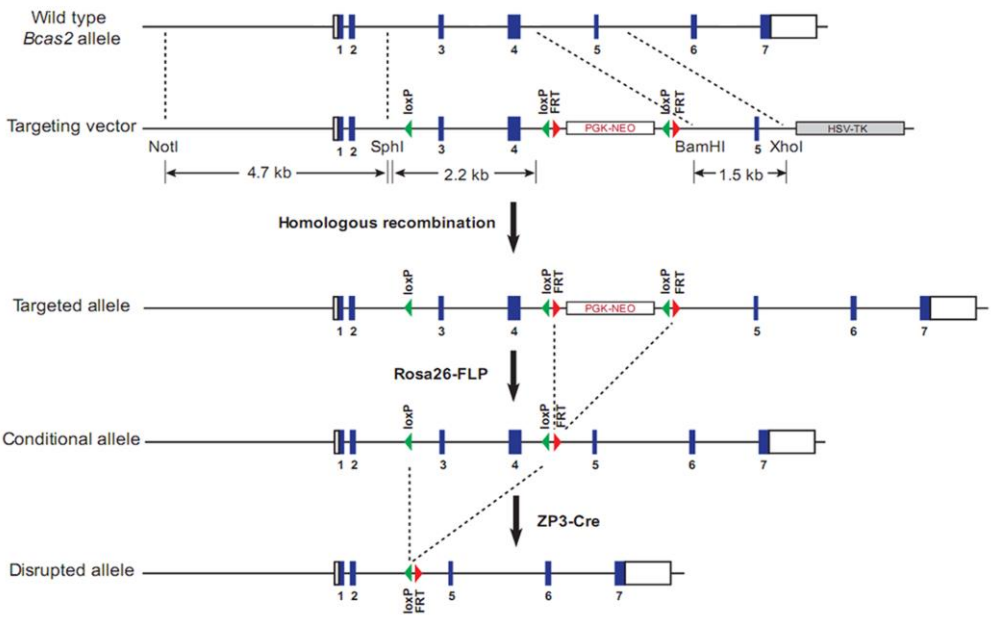

Fig. S2. *Bcas2* targeting strategy and analysis of F2 offspring. A targeting vector was constructed by anchoring LoxP sites around exon 3 and exon 4. HSV-TK expression was used for negative selection, and PGK-NEO expression was used for positive selection. Targeted ES clones were confirmed by sequencing and injected into blastocysts to produce chimeric mice.

Figure S3

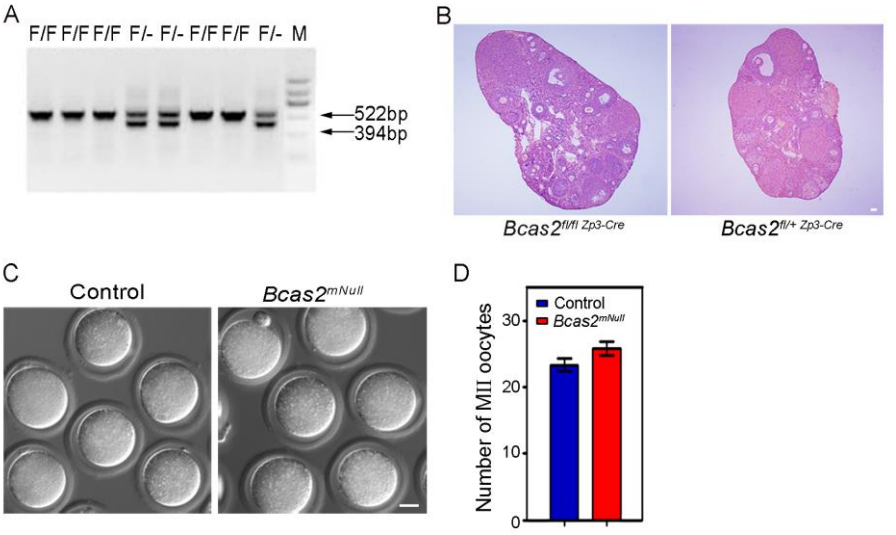

Fig. S3. Characterization of *Bcas2$^{fl/fl}$* and *Bcas2$^{fl/fl}$;Zp3-Cre* mice. (A) PCR analysis of genomic DNA derived from eight offspring. (B) Ovaries from two-month old *Bcas2$^{fl/+}$;Zp3-Cre* and *Bcas2$^{fl/fl}$;Zp3-Cre* females were fixed and stained with hematoxylin and eosin. Scale bar, 50 μm. (C and D) MII oocytes were obtained from *Bcas2$^{fl/+}$;Zp3-Cre* and *Bcas2$^{fl/fl}$;Zp3-Cre* females after 13 hours post-hCG. Scale bar, 20 μm.

Figure S4

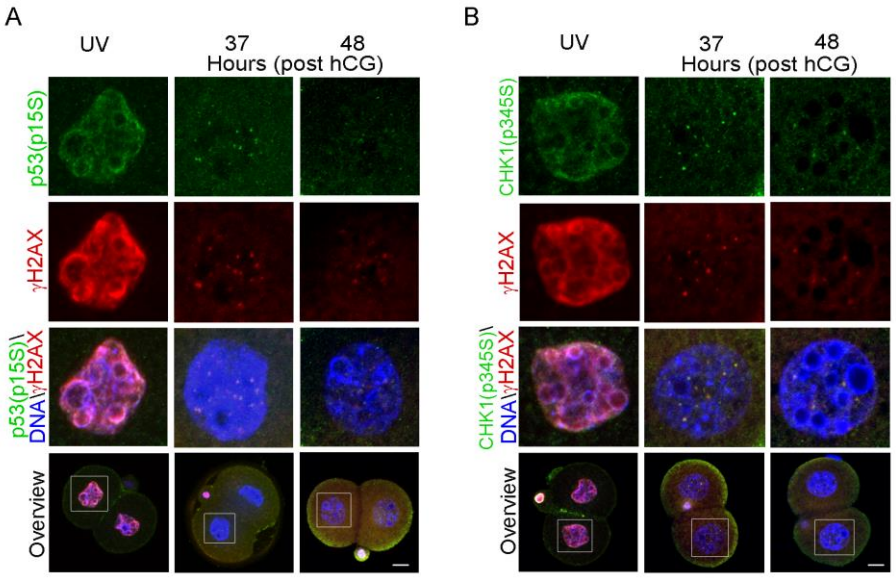

Fig. S4. Checkpoint activation during cell cycle progression in normal two-cell embryos. (A) Normal two-cell embryos at 37 and 48 hrs post-hCG were fixed and stained with γH2AX and phosphor-p53 (S15) antibodies under TP conditions. (B) Normal two-cell embryos at 37 and 48 hours post-hCG were fixed and stained with γH2AX and phosphor-pCHK1(S345) antibodies under TP conditions. Normal two-cell embryos irradiated by UV were positive control. Scale bar, 20 μm.

Figure S5

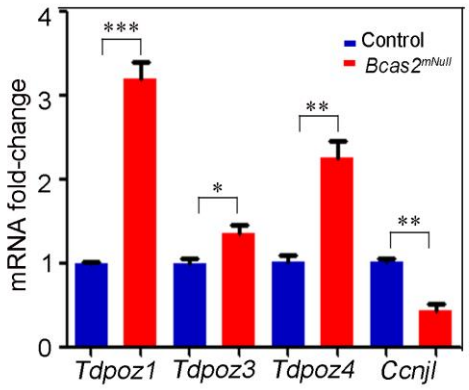

Fig. S5. Zygotic genome active is partially impaired in *Bcas2*^{mNull} two-cell embryos. Levels of *Tdpoz1, Tdpoz3, Tdpoz4, Ccnjl* and *Gm13043* were measured by qRT-PCR with specific primers (Table S 4) in two-cell embryos obtained at 48 hrs post-hCG. The error bars represent the SEM from three independent experiments. *, $P < 0.05$; **, $P < 0.01$; ***, $P< 0.001$.

Figure S6

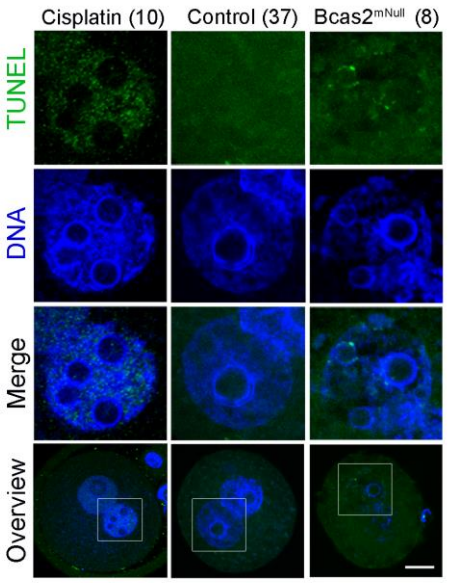

Fig. S6. BCAS2 is required for DNA repair in mouse zygotes. Control and BCAS2^{mNull} zygotes were obtained 30 hrs post-hCG and examined by TUNEL assay. Normal zygotes treated with Casplatin as the positive control.

Figure S7

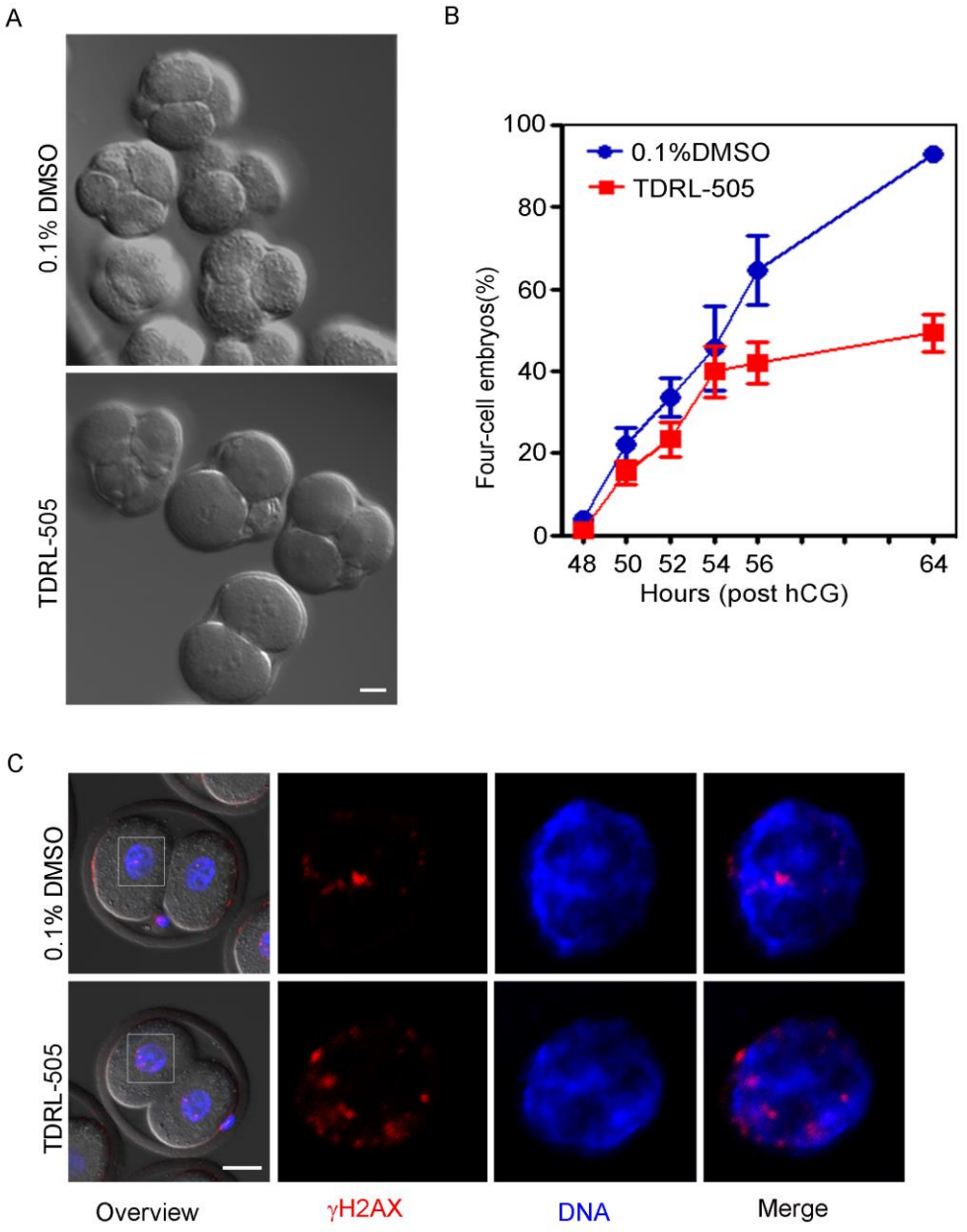

Fig. S7. RPA inhibition induces developmental arrest and increased γH2AX at two-cell stage. (A) Normal zygotes were recovered 23 hrs post-hCG. Zygotes were treated with either 0.1% DMSO as control (n=65) or 100 μM TDRL-505 (n=68) for 5 hrs and released. Zygotes were allowed to progress to the four-cell stage. (B) Quantification of (A) developmental rate

during the transition from the two- to four-cell stage. Developmental rates were calculated without the amount of arrested two-cell embryos. Error bars represented SEM. (C) Two-cell embryos from (B) were fixed after 48 hours post-hCG and immunostained with γH2AX. Scale bar, 20 μm.

Supplementary Table S1

PCR primers used for gene targeting at Bcas2 locus and genotyping.

Primer	5'->3'
SphI-Loxp-F	ACAGCATGCATAACTTCGTATAGCATACATTATACGAAG TTATGTTTTTAAGTGGTTATTCTACAAGTGC
NotI-R	GAATTCGCGGCCGCTGTAGTTCTGAGACAATCCC
Not-F	GAATTCGCGGCCGCGTGCCAACGACAGTGTTTTC
SphI-R:	ACAGCATGCAGTTAGACATGCTTGTTCAGGC
BamHI-F:	TCGGATCCCAACGAAAGGCTACCTTGAG
XhoI-R:	TCCTCGAGCTAACTGTGAAGGTGTGTCAG
Neo-F:	TATCGCCTTCTTGACGAGTTC
screen-R	TTGGTCCTGCAGTCCAAATC
F-WT	ATTCCAGCAGTTGGTGTGGG
F2-KO	AGGTGTATGAATGCCTGAACAAG
R	CATTGCTGGACAGAAGGTGAG

Supplementary Table S2

Antibodies used in this study:

Name	Dilution	Application
anti-BCAS2 (Protein Tech Group, 10414)	IF	1: 200
	WB	1:600
anti-phosphor-p53 on Ser15 (Cell Signaling, 9284)	IF	1:100
	WB	1:1000
anti-γH2AX antibody (Cell signaling, 22551)	IF	1: 500
	WB	1:1000
phosphor-RPA2 (Bethyl Laboratories, A300-245A)	IF	1:100
anti-β-Actin monoclonal antibody (ZSGB-BIO, ZM0002)	WB	1: 5000
Alexa 488 donkey anti-rabbit (Jackson, 711545152)	IF	1:1000
Alexa 633 donkey anti-mouse (Jackson, A-21050)	IF	1:200
HRP-conjugated anti-mouse (ZSGB-BIO; 2304)	WB	1:5000
HRP-conjugated anti-rabbit secondary antibodies (ZSGB-BIO; 2301)	WB	1:5000

Abbreviation: IF, immunofluorescence staining; WB, western blotting.

SupplementaryTable S3

Primer sequences for *Bcas2* and *Rpa2* mutations:

Gene	Forward (5'->3')	Reverse (5'->3')
Bcas2	TAGAATTCGCCACCATGGGGGCACGGGCTTGGTAGCCGGAGAGG	TACCGCGGGAAGTCTTGGCGGATGTTTTCCTTGTTGGCCTCCC
ΔN	GAATTCGCCACCATGGCGGCACGGGCTTGGT	TTGACCGCGGTCATTCTCTCAGCTTAGATC
Rpa2	TGCGGATATCATGTGGAATAGCGGATTCGA	AGGAGGCGCGCCTCACTCTGCATCTGTAGACT
Rpa2(S4A/S8A)	AGGTGGAGCTGCTGAAGCCTTCGAATCCGCCATTCCACAT	ATGTGGAATGGCGGATTCGAAGGCTTCAGCAGCTCCACCT
Rpa2(S33A)	CTCGATTTCTTCTCCGCCTGCGCCGGTGTGGGCGACCCGA	TCGGGTCGCCCACACCGGCGCAGGCGGAGAAGAAATCGAG
Rpa2(Thr21A)	CCGAAGCCACCGGGGGACTGCGCGTAGCCGCCTGCTCCGCC	GGCGGAGCAGGCGGCTACGCGCAGTCCCCCGGTGGCTTCGG

Supplementary Table S4

Primers for qRT-PCR assay:

Gene	Forward (5'->3')	Reverse (5'->3')
Bcas2	5'TCGCTGCTCGACAACCGATTGAA3'	5'AGCTGCATGTTCTTTCGCTGCCA3'
P21	5'GCAGCCGAGAGGTGTGAGCC3'	5'CGGGACCGAAGAGACAACGGC3'
Gadd45a	CTGCAGAGCAGAAGACCGAA	TACACGCCGACCGTAATGG
Tdpoz1	TCAGAGAAGGATTACAAGCCCA	GGCTGAGCAAAACTAGGTAAACT
Tdpoz3	CCTGTCAGTTTATCTGGAGTTGC	CAGAAAGCATTGGACATTGGAGA
Tdpoz4	GCCCAAGTGCTAACACCAGA	TCCCACAGCTCCCCTACGT
Ccnjl	TGGCATATCGGGACTCGTTG	ATATCCAAGGGCTGGAGGGT
Gm13043	GCCCCACTCTATCTGTTTTGC	AACGAATCCTCCTCCTATTTACTG