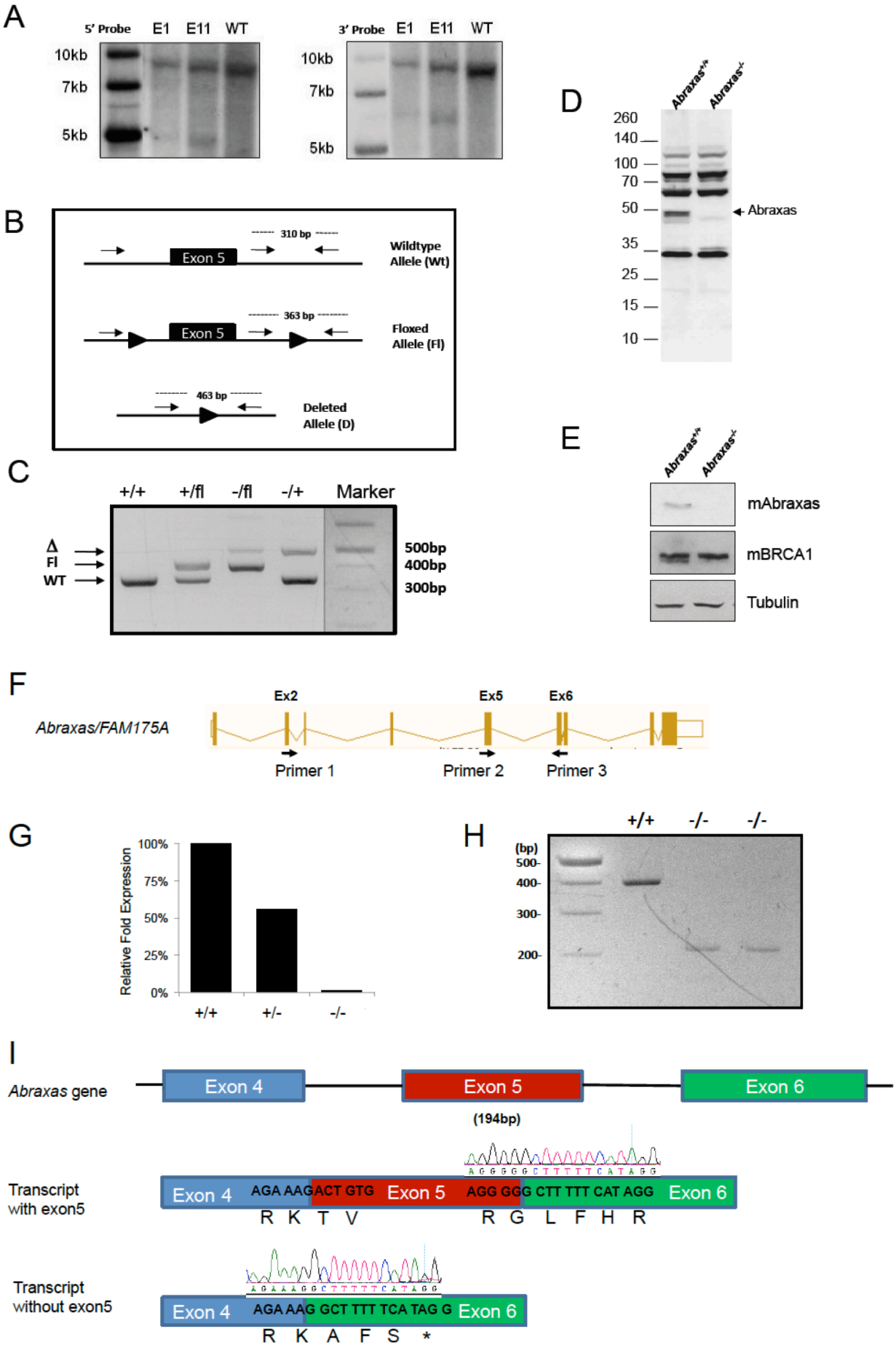
A
5' Probe
E1
E11
WT
10kb
7kb
5kb
3' Probe
E1
E11
WT
10kb
7kb
5kb
B
310 bp
Exon 5
Wildtype Allele (Wt)
363 bp
Exon 5
Floxed Allele (Fl)
463 bp
Deleted Allele (D)
C
+/+
+/fl
-/fl
-/+
Marker
Δ
Fl
WT
500bp
400bp
300bp
D
Abraxas+/+
Abraxas-/-
260
140
100
70
50
35
25
15
10
Abraxas
E
Abraxas+/+
Abraxas-/-
mAbraxas
mBRCA1
Tubulin
F
Abraxas/FAM175A
Ex2
Ex5
Ex6
Primer 1
Primer 2
Primer 3
G
Relative Fold Expression
100%
75%
50%
25%
0%
+/+
+/-
-/-
H
+/+
-/-
-/-
(bp)
500-
400-
300-
200-
I
Abraxas gene
Exon 4
Exon 5
Exon 6
(194bp)
Transcript with exon5
Exon 4
AGA AAG
ACT GTG
Exon 5
AGG GGG CTT TTT CAT AGG
Exon 6
R K T V
R G L F H R
Transcript without exon5
Exon 4
AGA AAG GCT TTT TCA TAG G
Exon 6
R K A F S *

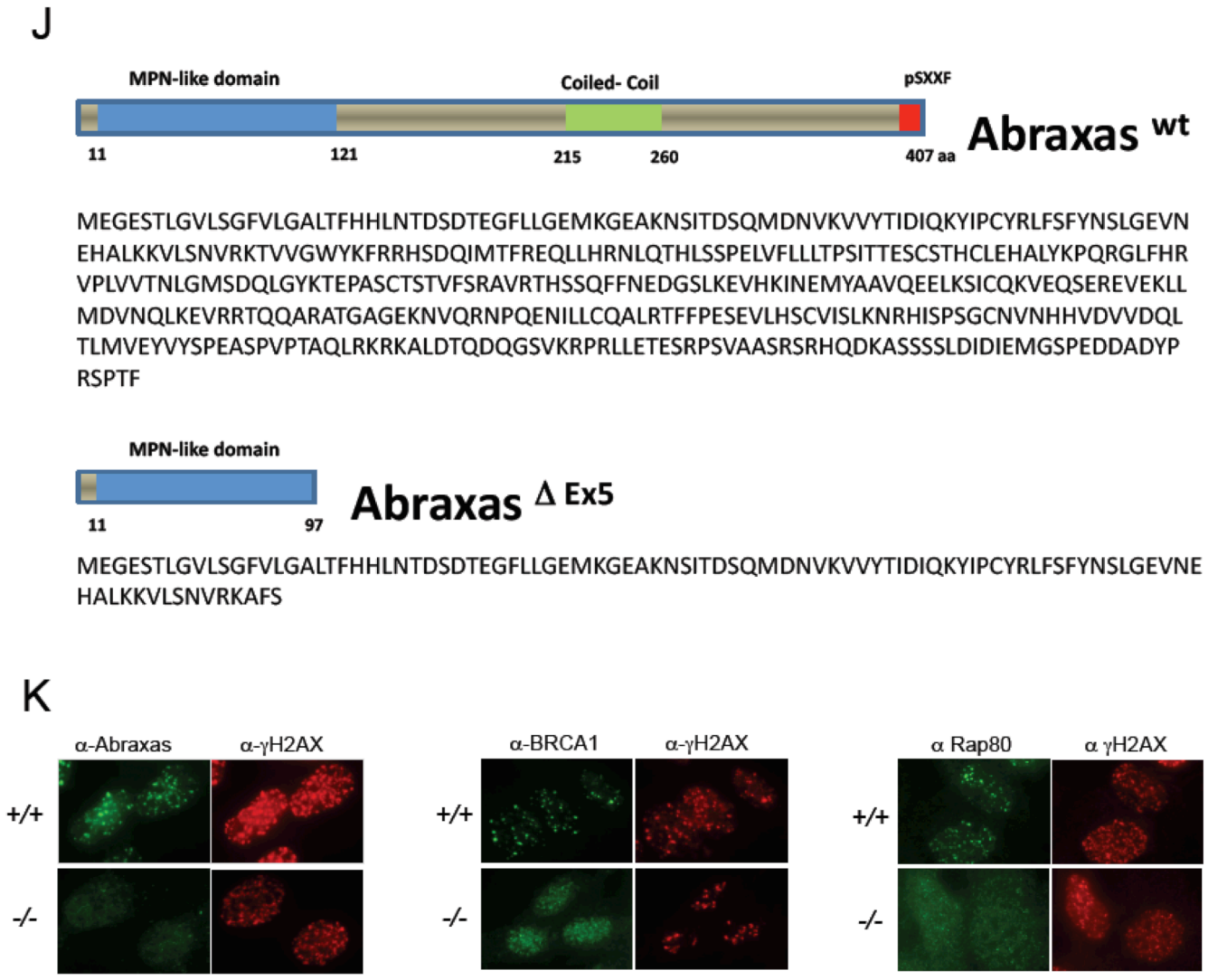

Figure S1 (related to Figure 1). Generation of Abraxas-deficient mice. (**A**) Southern blot analysis of two independent clones of targeted ES cells. Genomic DNA from ES cells was digested with EcoRV and SpeI. Blots were probed with 5' and 3' DNA probes shown in Figure 1A. (**B**) PCR Genotyping strategy for identifying *Abraxas* wild type (+), floxed (fl), and deleted (-) allele. (**C**) PCR results of the genotyping of mouse tail DNA of different genotypes. (**D**) and (**E**) Abraxas protein is absent in *Abraxas*$^{-/-}$ MEFs. Cell lysates from *Abraxas*$^{+/+}$ and *Abraxas*$^{-/-}$ MEFs were separated in SDS-PAGE gel. Western blots were carried out with antibodies to Abraxas, BRCA1 and tubulin. (**F**) A map of the Exon arrangements of *Abraxas* gene and the RT-PCR primers used to amplify Abraxas transcripts. Primer 1 (located in exon 2), primer 2 (located in exon 5) and primer 3 (located in exon 6) are indicated. (**G**) Quantitative RT-PCR data using primers 2 and 3 detecting the expression levels of *Abraxas* full-length with intact exon 5 transcripts in *Abraxas* $^{+/+}$, *Abraxas*$^{+/-}$, and *Abraxas*$^{-/-}$ MEF cells. (**H**) Products of RT-PCR using primers 1 and 3 detecting transcripts spanning exon 2 through 6 revealed that exon 5 (194 bp in length) is deleted in the *Abraxas*$^{-/-}$ cells. (**I**) An illustration of Abraxas transcript without *exon 5* in *Abraxas*$^{-/-}$ cells contains a premature stop codon immediately after *exon 4*. RT-PCR product using primers 1 and 3 were sequenced and nucleotides sequence at the junction of exon 4 and exon 6 were shown. (**J**) Exon 5 deletion results in an inactive Abraxas truncated product, if exists, lacking all functional domains. (**K**) Abraxas is required for BRCA1 and Rap80 IRIF formation. Immortalized isogenic wild type (+/+) and *Abraxas* null (-/-) MEF cells were treated with 10 Gy IR and immunofluorescence staining was carried out 2 hours post-IR with antibodies to mouse Abraxas, BRCA1, Rap80 and γH2AX.

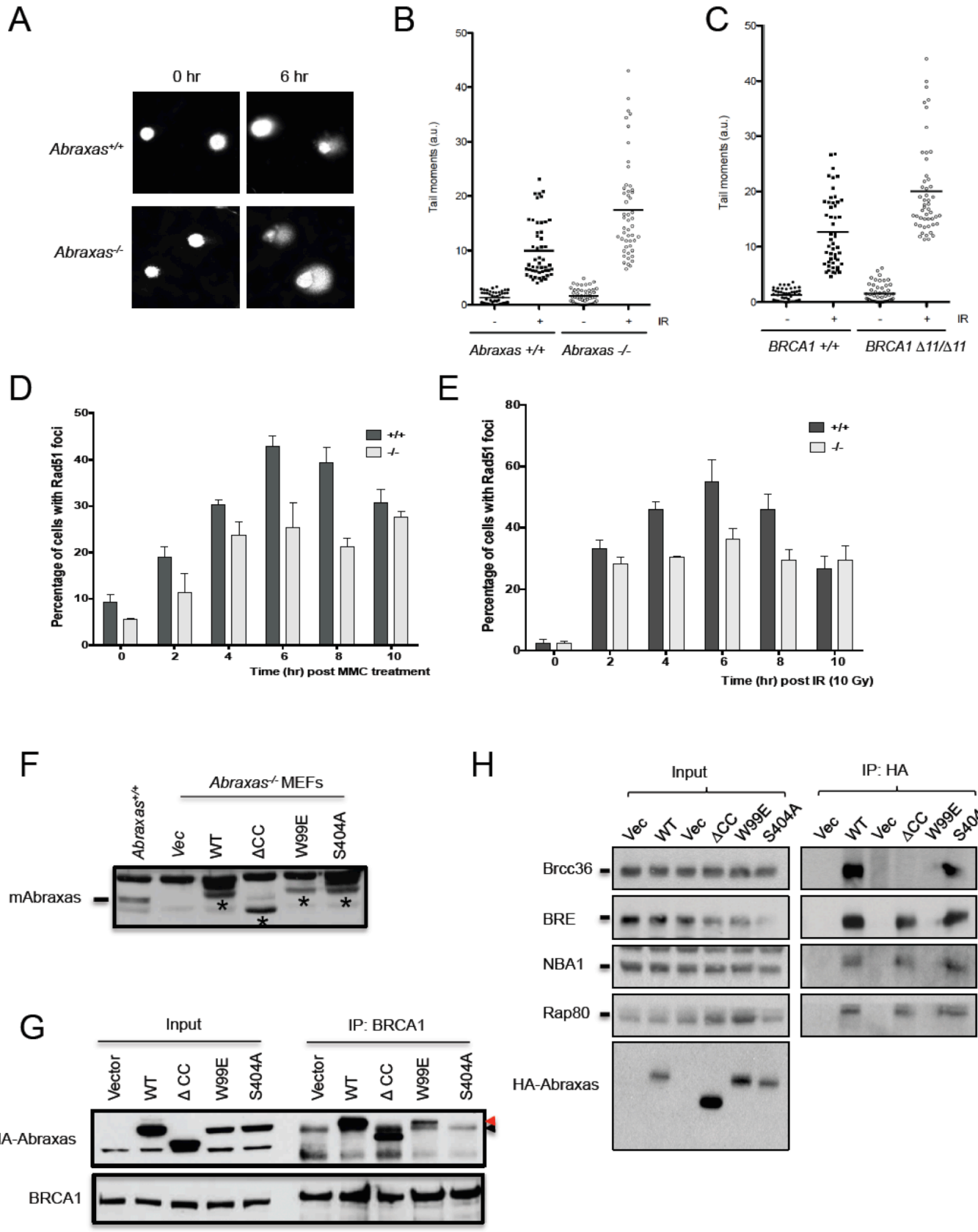
A
0 hr
6 hr
Abraxas+/+
Abraxas-/-
B
Tail moments (a.u.)
IR
Abraxas +/+
Abraxas -/-
C
Tail moments (a.u.)
IR
BRCA1 +/+
BRCA1 Δ11/Δ11
D
Percentage of cells with Rad51 foci
+/+
-/-
Time (hr) post MMC treatment
E
Percentage of cells with Rad51 foci
+/+
-/-
Time (hr) post IR (10 Gy)
F
Abraxas-/- MEFs
Abraxas+/+
Vec
WT
ΔCC
W99E
S404A
mAbraxas
G
Input
IP: BRCA1
Vector
WT
Δ CC
W99E
S404A
HA-Abraxas
BRCA1
H
Input
IP: HA
Vec
WT
Vec
ΔCC
W99E
S404A
Brcc36
BRE
NBA1
Rap80
HA-Abraxas

Figure S2 (related to Figure 2). Abraxas-deficient cells are defective in DNA repair. (**A**) A representative image of comet assay results of *Abraxas* wild type and null cells. (**B**) Quantification of tail moments of an additional pair of *Abraxas* +/+ and -/- MEF cells treated with IR. (**C**) Quantification of tail moments of 5 Gy IR treated-wild type and *Brca1*$^{\Delta 11/\Delta 11}$ MEF cells. DNA damage was assessed in either untreated or 6 hr post 5Gy IR treatment. Tail moments were quantified from 50 cells and the mean value is calculated. The experiment has been repeated three times and a representative image and quantification is shown. (**D**) and (**E**) Rad 51 foci formation was decreased in Abraxas-/- cells treated with IR or MMC. Cells either treated with 10 Gy IR or 1ug/ml MMC for 1.5hr were incubated at 37°C for indicated hours followed by immunostaining with Rad51 antibody and appropriate fluorescent labeled secondary antibody. Percentage of cells containing more than 10 Rad51 foci was calculated. More than 300 cells were counted for the quantification. (**F**) Expression of Abraxas and mutants in *Abraxas*$^{-/-}$ MEFs. Lysates from *Abraxas*$^{+/+}$ and *Abraxas*$^{-/-}$ MEFs stably expressing HA-tagged Abraxas wild type and mutants were used in the western blot with antibodies to mouse Abraxas "*" marks the HA-tagged wild type and mutants of Abraxas. (**G**) Mouse Abraxas S404Amutants failed to bind BRCA1. DNA constructs containing HA-tagged Abraxas wild-type and mutants were transiently transfected into 293T cells. Cell lysates were used for immunoprecipitation with BRCA1 antibody and subsequent western blot analysis with antibodies against HA or BRCA1. The red arrow points to the position where S404A mutant did not appear in the BRCA1 antibodies IP. The black arrow marks a non-specific signal in the IP-western analysis. (**H**) Mouse Abraxas mutants ΔCC and W99E failed to form an intact BRCA1-A complex. Cell lysates from 293T cells transiently transfected with constructs expressing HA-tagged Abraxas wild type and mutants were used for immunoprecipitation and western blot analysis.

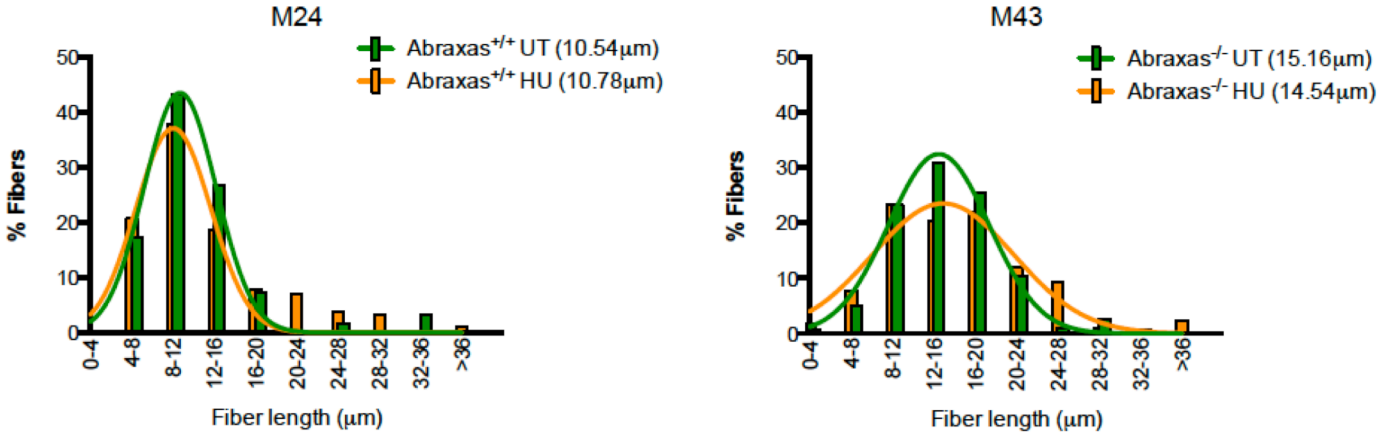

Figure S3 (related to Figure 3). Abraxas deficiency does not result in shortening of stalled replication fork. Fiber assay was performed with an additional pairs of *Abraxas*$^{+/+}$ or *Abraxas*$^{-/-}$ MEFs. The mean fiber lengths are calculated and indicated (*Abraxas-/-*, p=0.7050). The experiments were repeated three times with two pairs of *Abraxas* MEF cell lines and with reversing the analogs and the HU pulse with the same result. More than fifty fibers were measured for each condition.

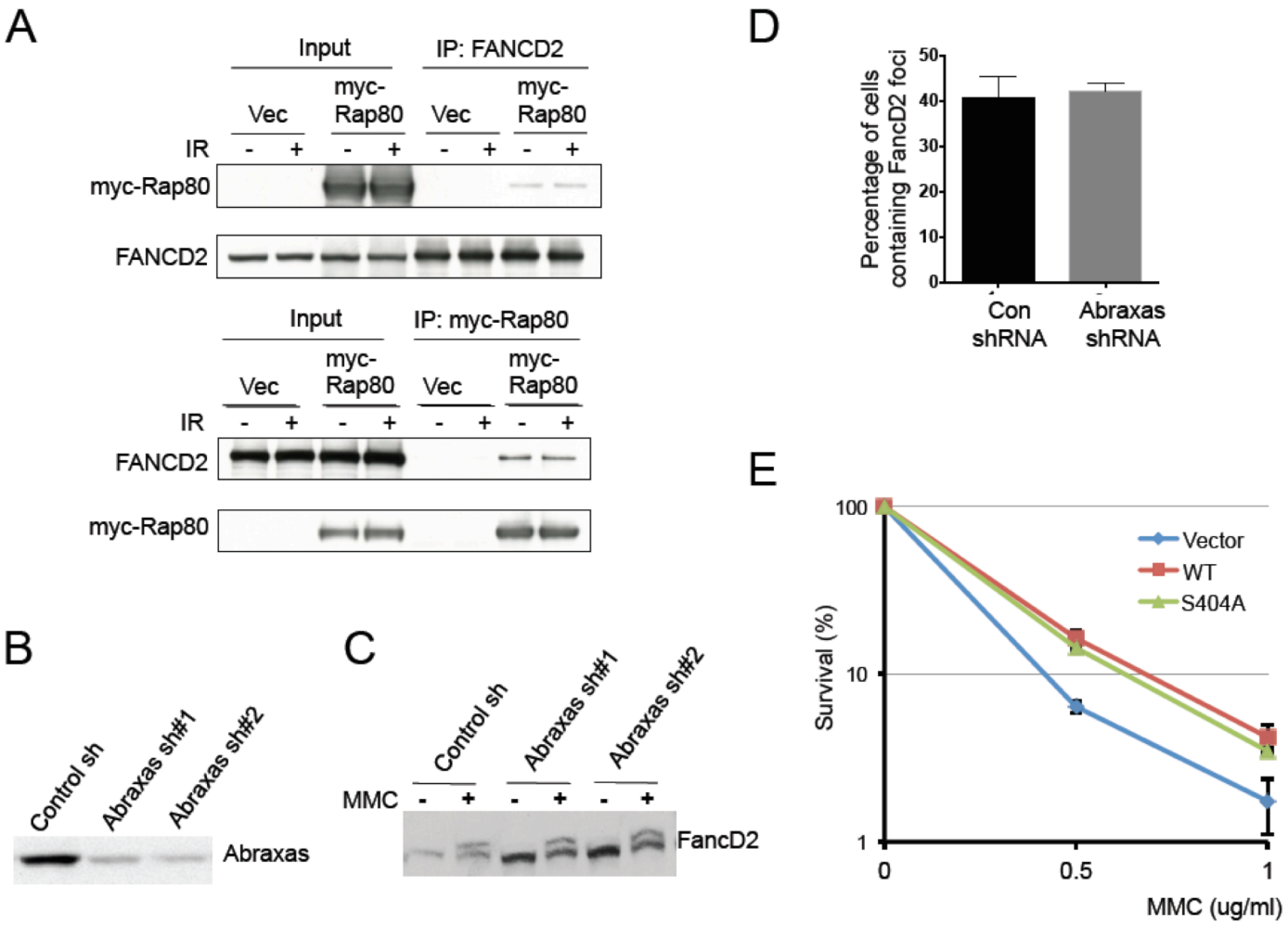

Figure S4 (related to Figure 4). Abraxas is not required for FancD2 ubiquitination and recruitment to crosslink DNA damage sites. (**A**) Rap80 interacts with FancD2. Co-immunoprecipitation of myc-tagged Rap80 and FancD2 were carried out using lysates of 293T human cells transiently transfected with myc-Rap80, untreated or treated with 10 Gy IR. Western blot was carried out with antibodies to myc or FancD2. (**B**) Generation of Abraxas knockdown cells using shRNAs against Abraxas. Western blot of Abraxas in cells expressing control shRNA or two different shRNAs against Abraxas was shown. (**C**) Abraxas is not required for FancD2 ubiqutination. Cell lysates from control cells or Abraxas knockdown cells treated with 1.5 uM MMC were used in the western blot with anti-FancD2 antibody. (**D**) Control cells and Abraxas knockdown cells were treated with 1.5 uM MMC and collected at 6 hr post-treatment for immunofluorescence analysis. Percentage of cells containing more than five FancD2 foci was quantitated. More than 400 hundred cells were counted. (**E**) Rescue of the increased sensitivity to MMC of the *Abraxas*$^{-/-}$ MEF cells as measured by clonogenic survival assay. An additional clonogenic survival data was shown. Error bars represent standard deviation across triplicates.

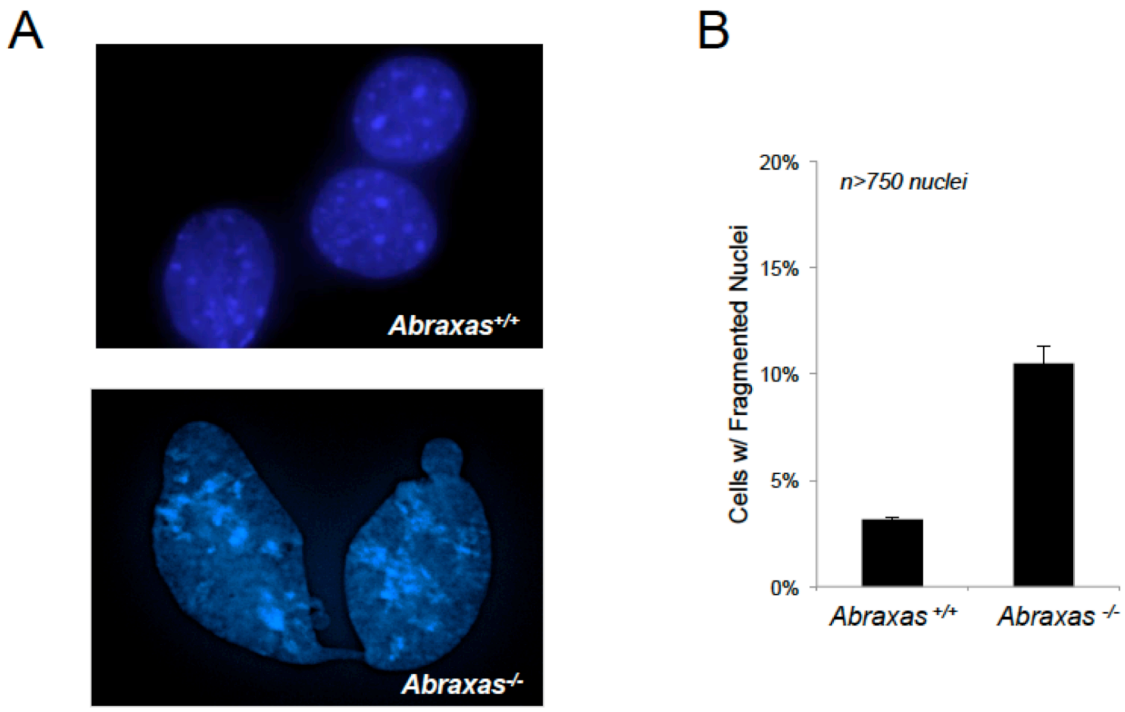

Figure S5 (related to Figure 5). Nuclear fragmentation in *Abraxas*$^{-/-}$ MEFs. (**A**) DAPI stained wild type and *Abraxas*$^{-/-}$ MEF cells. (**B**) Quantification of percentage of cells displaying abnormal nuclear morphology in wild type and *Abraxas*$^{-/-}$ MEF cells. Three independent counting were carried out. More than 750 nuclei were counted in total.

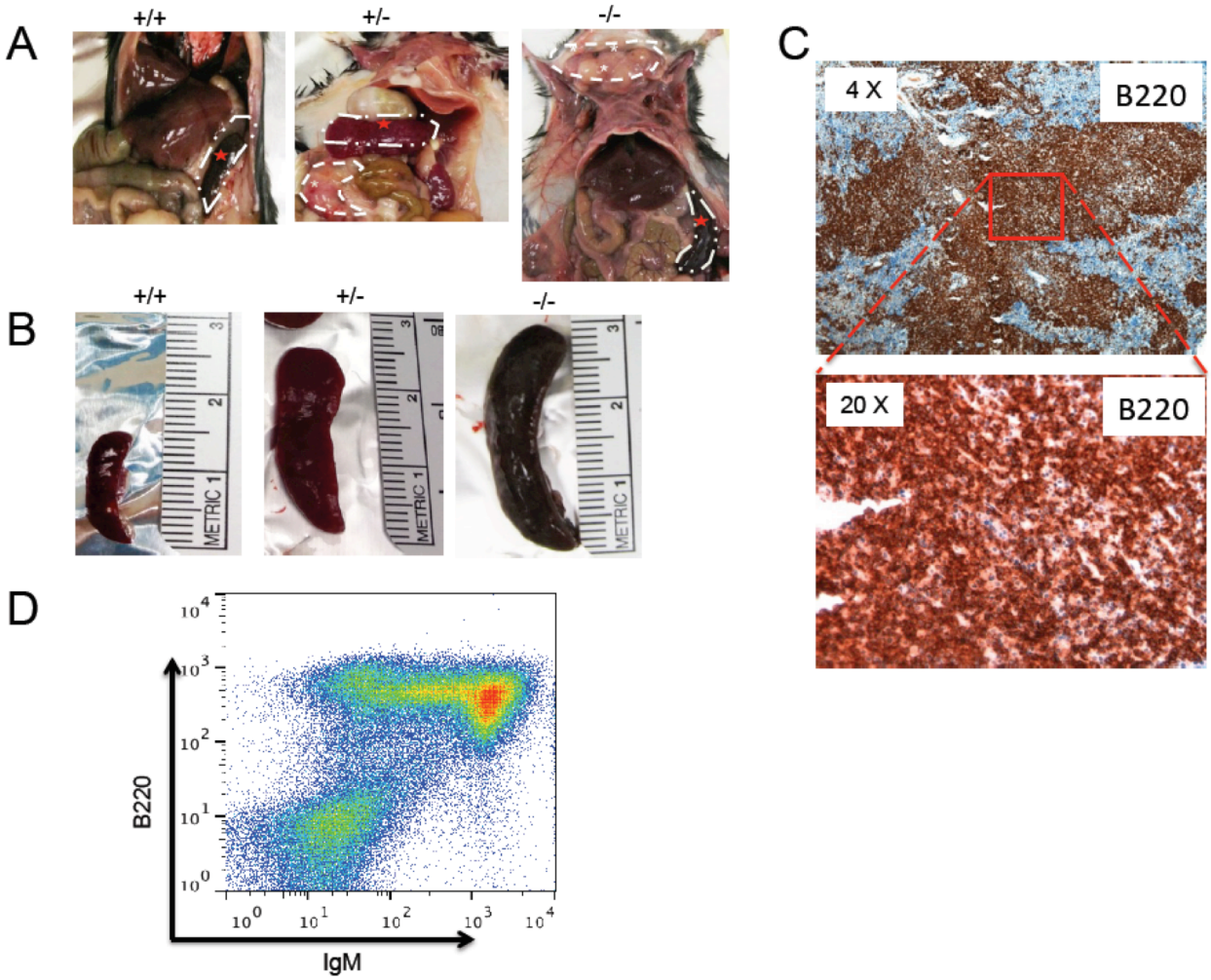

Figure S6. (related to Figure 6). *Abraxas*$^{+/-}$ and *Abraxas*$^{-/-}$ mice develop B-cell lymphoma. (**A**) Gross enlargement of the spleens (red star) and lymph nodes (white star) in *Abraxas*$^{+/-}$ and *Abraxas*$^{-/-}$ mice. (**B**) Representative samples of splenomegaly observed in *Abraxas*$^{+/-}$ and *Abraxas*$^{-/-}$ mice. (**C**) A representative image of *Abraxas*$^{-/-}$ lymphoma stained by a B-cell marker (B220^{+}) by Immunohistochemistry. (**D**) Flow cytometry analysis of tumor splenocytes from *Abraxas*$^{-/-}$ mouse using B220 and IgM marker. Three mice were analyzed and a representative image was shown.

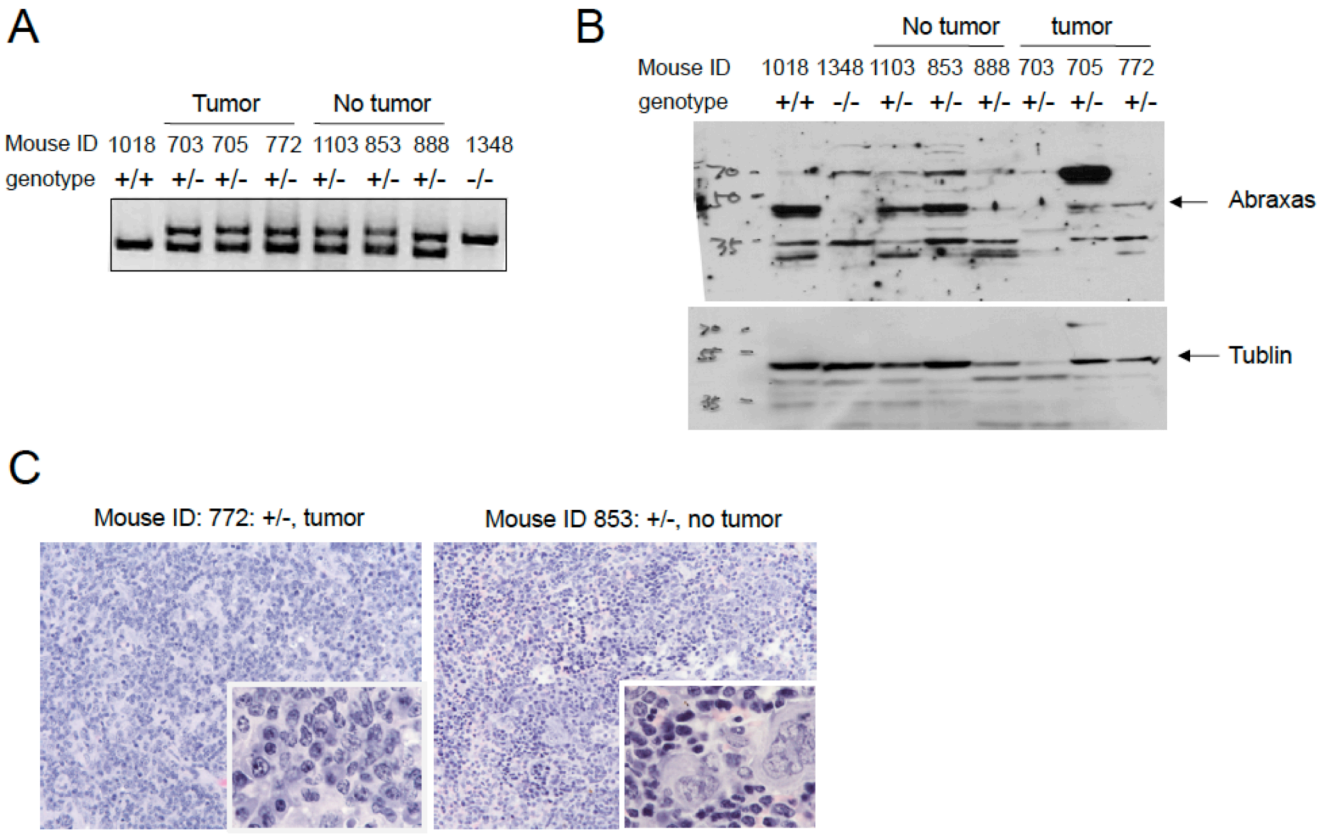

Figure S7 (related to Figure 6). Tumors derived from the *Abraxas*$^{+/-}$ mice retain wild type allele and expression of Abraxas. Tumors microdissected from spleens of *Abraxas*$^{+/-}$ mice were analyzed. Spleen tissues from *Abraxas*$^{+/-}$ mice not developing lymphoma, *Abraxas*$^{+/+}$ mice and *Abraxas*$^{-/-}$ mice were included as controls. (**A**) Wild type allele is retained in tumors derived from *Abraxas*$^{+/-}$ mice**.** PCR analysis was performed using genotype primers to detect wild-type and null allele of *Abraxas*. (**B**) Abraxas expression is detected in tumors derived from *Abraxas*$^{+/-}$ mice. Tumor samples as well as the control tissue were homogenized with a motor-driven tissue homogenizer (Polytron, Kinematica AG, Switzerland) in 8M urea buffer (8M urea, 50mM Tris and 150mM BME) followed by sonication. The homogenates were centrifuged at 15000rpm for 15 min and 50 ug of supernatant was analysed in the western blot. (**C**) Representative histological images (H&E staining) of tumors samples and the control sample without tumor were shown.