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Supplemental Information

Regulation of DNA End Joining, Resection, and Immunoglobulin Class Switch Recombination by 53BP1

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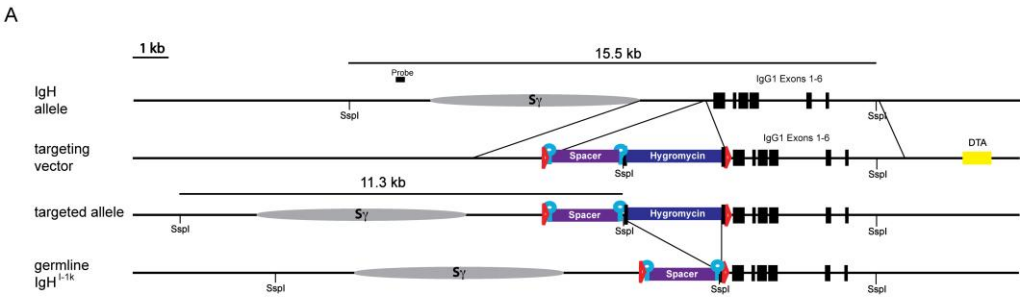
Supplemental Experimental Procedures

Joining and Resection Analysis. I-SceI infected cells were harvested on day 4 of culture and infection efficiency was determined by FACS analysis using the GFP reporter encoded by the retrovirus. DNA was phenol-chloroform extracted and precipitated with ethanol, before quantification. For the intrachromosomal recombination PCR assay, nested PCR reactions were performed with the Expand Long Template PCR System as described and validated previously (Bothmer et al., 2010). The first round of PCR for $IgH^{I\text{-}1k}$ joining was done with the following primers: 5'-CAGACCTGGGAATGTATGGTTGTG-3' and 5'-AGACAGGACAGGACAGGACCAAAC-3'. The first round of PCR for $IgH^{I\text{-}27M}$ joining was done with the following primers: 5'- CCAATACCCGAAGCATTTACAGT-3' and 5'- TAGGAAAATGCCCCACCTGC-3'. The following conditions were used for the first round of amplification: 10 cycles at 92C, 10s; 56C, 30 s; and 68C, 40s; followed by 25 cycles at 92 C, 15 s; 56C, 30 s; and 68C, 40s with 2s of additional extension time per cycle. The second round of PCR for both $IgH^{I\text{-}1k}$ and $IgH^{I\text{-}27M}$ was done with the following primers: 5' – ATGTATGGTTGTGGCTTCTGGG-3' and 5' - CCTGCTTTCCAGTATGGGTATCTG-3' using the following conditions: 10 cycles at 92C, 10s; 55C, 30 s; and 68C, 30s; followed by 15 cycles at 92C, 15s; 55C, 30 s; and 68C, 30s with 2s of additional extension time per cycle). For $Myc^{I/+}$ $IgH^{I/+}$ joining the primers and conditions were as previously described (Robbiani et al., 2008). The frequency of recombination was adjusted based on the rate of infection. For each experiment and genotype, serial dilutions were tested, and events were counted at the dilution that showed a maximum of two joining events per lane. PCR products were then isolated from gels and sequenced (Bothmer et al., 2010).

γ-H2AX chromatin immunoprecipitation (ChIP). Cells were fixed by adding 1% paraformaldehyde at 37°C for 10 min. Cells were then collected, washed once with cold PBS and lysed in RIPA buffer in the presence of Protease Inhibitor Cocktail (Roche), 0.5 mM PMSF and 5 mM NaF followed by sonication to 200-500 bp in length. Meanwhile, the γ-H2AX antibody (Millipore) was bound to Dynabeads Protein A (Invitrogen) following the manufacturer's instructions. The supernatants of sonicated samples were

collected after centrifugation at 14000 rpm at 4C for 5 min and added to the γ-H2AX binding beads overnight. The next day, beads were washed 2X with RIPA, 2X with RIPA + 0.3 M NaCl, 2X with LiCl buffer (0.25 M LiCl, 0.5% NP-40, 0.5% NaDOC), 1X with TE+0.2% Triton X and 1X with TE. Beads were then resuspended in TE and reverse-cross linked using 3% SDS and Proteinase K (Sigma) at 65C for 4 hrs. After removal of beads DNA was extracted with Phenol/Chloroform and precipitated with EtOH and NaOAc in the presence of glycogen. The qPCR was performed using SybrGreen reagent (Agilent/Strategene). Primer sequences are available upon request. To calculate γ-H2AX enrichment, we normalized the γ-H2AX to the GAPDH signal (average of triplicates).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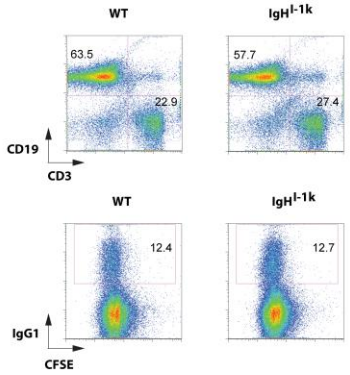
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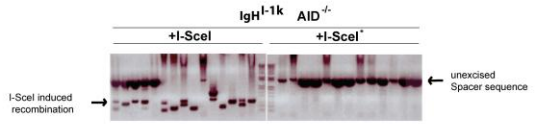
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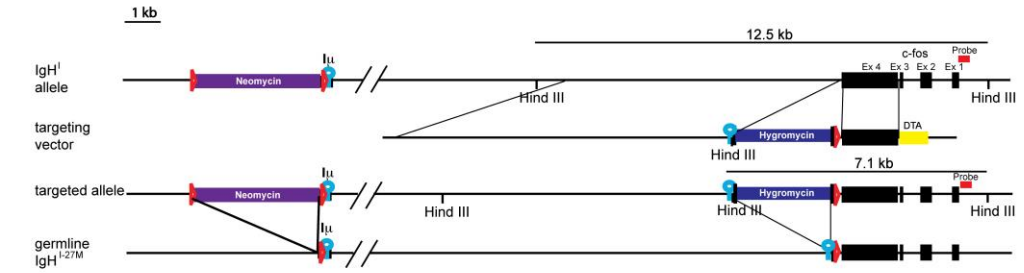
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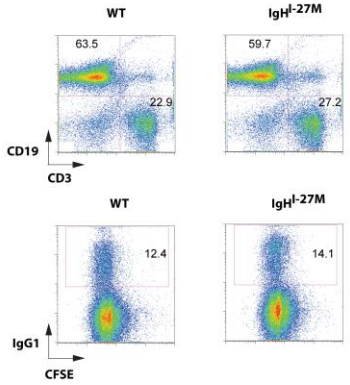
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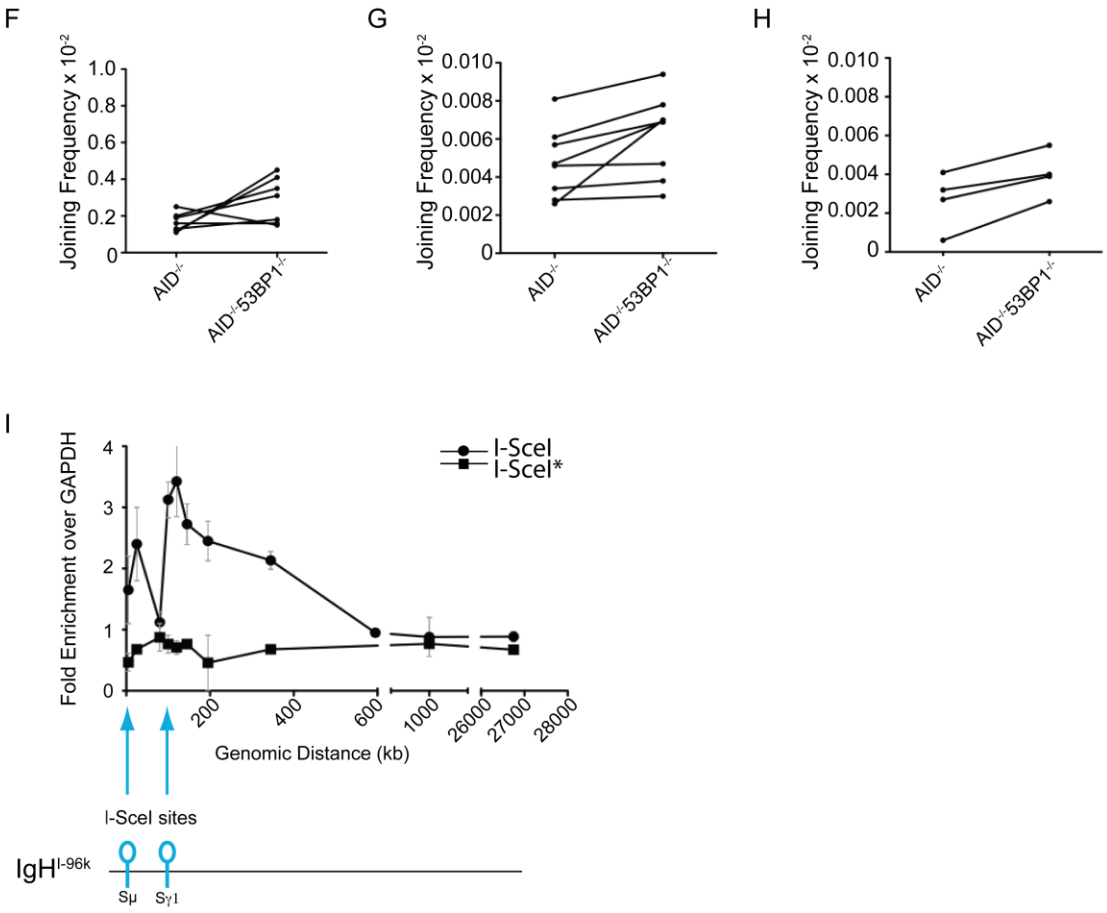

Figure S1. IgH$^{I\text{-}1k}$ and IgH$^{I\text{-}27M}$ Mice, Related to Figure 1

(A) Schematic representation of the gene targeting strategy to generate IgH$^{I\text{-}1k}$ mice. The targeting vector, targeted allele and targeted allele upon flp induced hygromycin excision (germline IgH$^{I\text{-}1k}$) are shown. LoxP sites are indicated as red triangles, I-SceI sites are blue circles and frt sites are black bars. DTA was used for negative selection. The targeting construct IgH$^{I\text{-}96k}$ (Bothmer et al., 2010) was used with modifications. The spacer sequence (human IgHG1 intron 4) was amplified using the following primers: 5' - CACACAAAGACTCTGGACCTCTCCTGCGAGACTGTGATGGTTCTTTC-3' and 5'- GGCCGGCCAATATTATTACCCTGTTATCCCTATGTGGCAGGACCCAGGATGTA G – 3'. The probe for Southern blotting was amplified using the following primers: 5'- TTCCTACCTTCTCCCCTGAGTCTC -3' and 5'- TTCCCAGAGTCACAGCCTTTGTCC-3'. The frt-flanked hygromycin cassette was removed *in vivo* by crossing to FLPer mice (Rodriguez et al., 2000). For genotyping, 38 cycles of PCR amplification (95° C, 45 s; 59° C, 45 s; and 72° C, 30 s) were performed with primers 5'- GCGGGTCCTGCTGAGGGCCAG-3' and 5'- TAGGAAAATGCCCCACCTGC'-3. The size of the IgH$^{I\text{-}1k}$ allele is 390 bp. **(B)** Flow cytometric analysis of spleen cells from age-matched wild type and mutant IgH$^{I\text{-}1k/+}$ mice reveals normal B cell development, CSR to IgG1 and proliferation. **(C)** Representative ethidium bromide stained argarose gel showing PCR products obtained after I-SceI and I-

SceI* induced recombination in $IgH^{I\text{-}1k/+}AID^{-/-}$ B cells.**(D)** Schematic representation of the gene targeting strategy to generate $IgH^{I\text{-}27M}$ mice starting from previously targeted IgH^{I} ES cells (Robbiani et al, 2008). The targeting vector, targeted allele and targeted allele upon flp induced hygromycin excision (germline $IgH^{I\text{-}27M}$) are shown. LoxP sites are indicated as red triangles, I-SceI sites are blue circles and frt sites are black bars. DTA was used for negative selection. The following primers were used to generate the construct: Long arm of homology: 5'- GGCCGGCCAAGCTT ATTACCCTGTTATCCCTA ACCAGTTTGTCAAGATGGGTGG-3' and 5'- GGCGCGCCAGATGTGCCAGATGTAGGTAGATGC-3'; Short arm of homology: 5'- TTAATTAACTGGATTTGACTGGAGGTCTGC-3' and 5'- CGATCGGCGGCCGC TCCTCAGAGGCCTTCCTGAAAC -3'. I-SceI site is underlined. LoxP site was present in hygromycin targeting backbone. Probe for Southern blotting was amplified using the following primers: 5'- GCGAGCAACTGAGAAGACTGGATAG -3' and 5'- AATGGTAGTAGGAAAGGCTGTCCC-3'. The frt-flanked hygromycin cassette was removed *in vivo* by crossing to FLPer mice (Rodriguez et al., 2000). For genotyping, 38 cycles of PCR amplification (95° C, 45 s; 55° C, 45 s; and 72° C, 30 s) were performed with primers 5'- ACCCATCTTGACAAACTGGTTAGG-3' and 5'- TAGGAAAATGCCCCACCTGC'-3. The size of the $IgH^{I\text{-}27M}$ allele is 231 bp. **(E)** Flow cytometric analysis of spleen cells from age-matched wild type and mutant $IgH^{I\text{-}27M/+}$ mice reveals normal B cell development, CSR to IgG1 and proliferation. **(F)** Dot plot showing I-SceI induced recombination frequency of $IgH^{I\text{-}1k/+}AID^{-/-}$ B cells in the presence or absence of 53BP1. Each matched pair corresponds to one measurement. **(G)** Dot plot showing I-SceI induced recombination frequency of $IgH^{I\text{-}27M/+}AID^{-/-}$ B cells in the presence or absence of 53BP1. Each matched pair corresponds to one measurement. **(H)** Dot plot showing I-SceI induced recombination frequency of $IgH^{I/+}Myc^{I/+}AID^{-/-}$ B cells in the presence or absence of 53BP1. Each matched pair corresponds to one measurement. **(I)** γ-H2AX density at the $IgH^{I\text{-}96k}$ allele upon infection of $IgH^{I\text{-}96k/+}AID^{-/-}$ B cells or $IgH^{I\text{-}96k/I\text{-}96k}AID^{-/-}$ B cells with I-SceI or I-SceI* control. Two independent experiments.

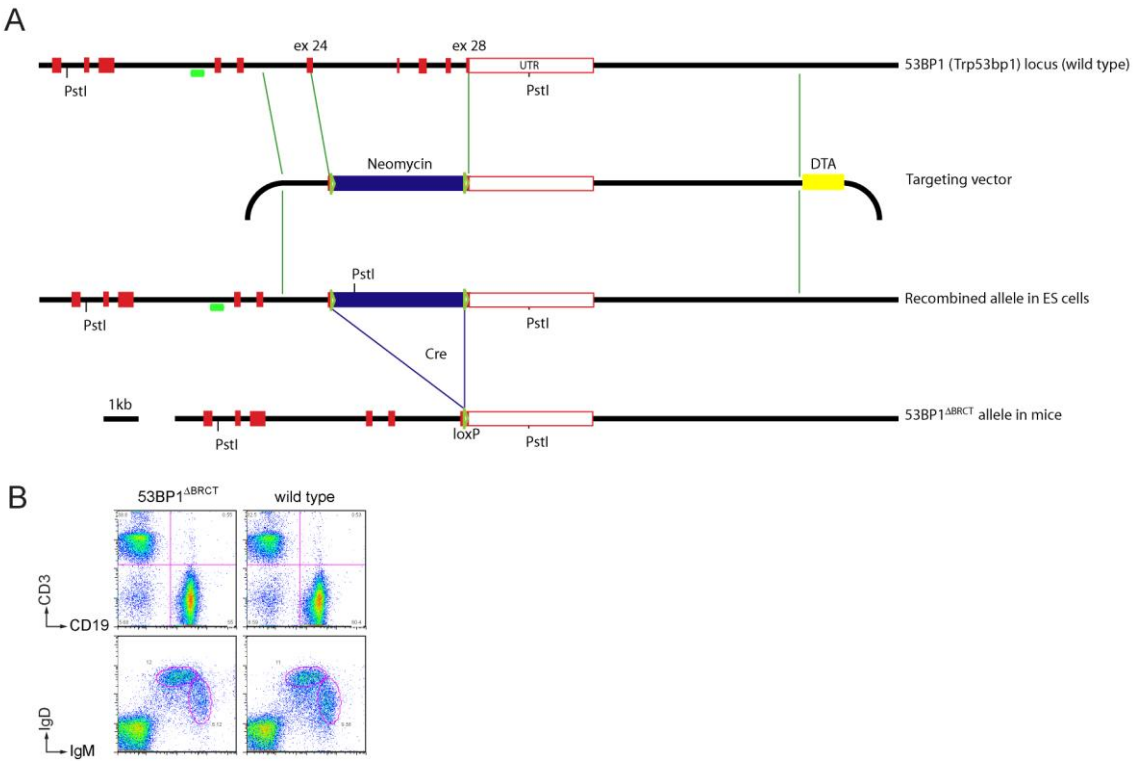

Figure S2. 53BP1$^{\Delta BRCT}$ Mice, Related to Figure 2

(A) Targeting strategy is shown along with the genomic structure of the wild type murine *Trp53bp1* locus (red boxes represent exons, UTR is 3' untranslated region), the targeting vector, the recombined 53BP1$^{\Delta BRCT}$ allele in ES cells and in mice after removal of the neomycin-based ACN cassette (Bunting et al., 1999). Southern blot analysis of ES cell genomic DNA digested with PstI revealed proper integration upon hybridization with a radiolabeled probe (PCR product of 5-GGTAAGAGCAGTGGGGTAGACAAG-3 and 5-TCTCCTGAAGTGAATGGCAAGG-3, green bar), yielding 12kb and 7kb fragments for germline and targeted alleles, respectively. Diphtheria toxin A (DTA) was used for negative selection. The following primers were used for generating the targeting vector: short arm of homology, 5-ggcgcgccTTTCCACTATCTTTCCCCCCAC-3 and 5-ggccggccTTATTAACCTATGTTGTCTCCAGTCTCAC-3; long arm of homology, 5-ttaattaaGTGCCTCATTGTTGGGGAGAG-3 and 5-gcggccgcTGTGTGGTTCACCTTCTCTCTATGG-3. For genotyping, 35 cycles of PCR (95° C, 45 s; 55° C, 45 s; and 72° C, 1 min) were performed with primers 5-TCAGAGACCTGGGTGGTGAATG-3, 5-CACCCTCCTCTTCTTCACTGCTTC-3 and 5- GGGATAACCTTGACAGTGATGCC-3. The size of the wild type allele is 0.6 kb, the 53BP1$^{\Delta BRCT}$ allele is 0.9 kb. **(B)** Flow cytometric analysis of splenocytes from age-matched wild type and 53BP1$^{\Delta BRCT}$ mutant mice reveals normal B lymphocyte development. Immuno-staining was performed with the indicated markers on total spleen cells. Representative of three independent experiments.

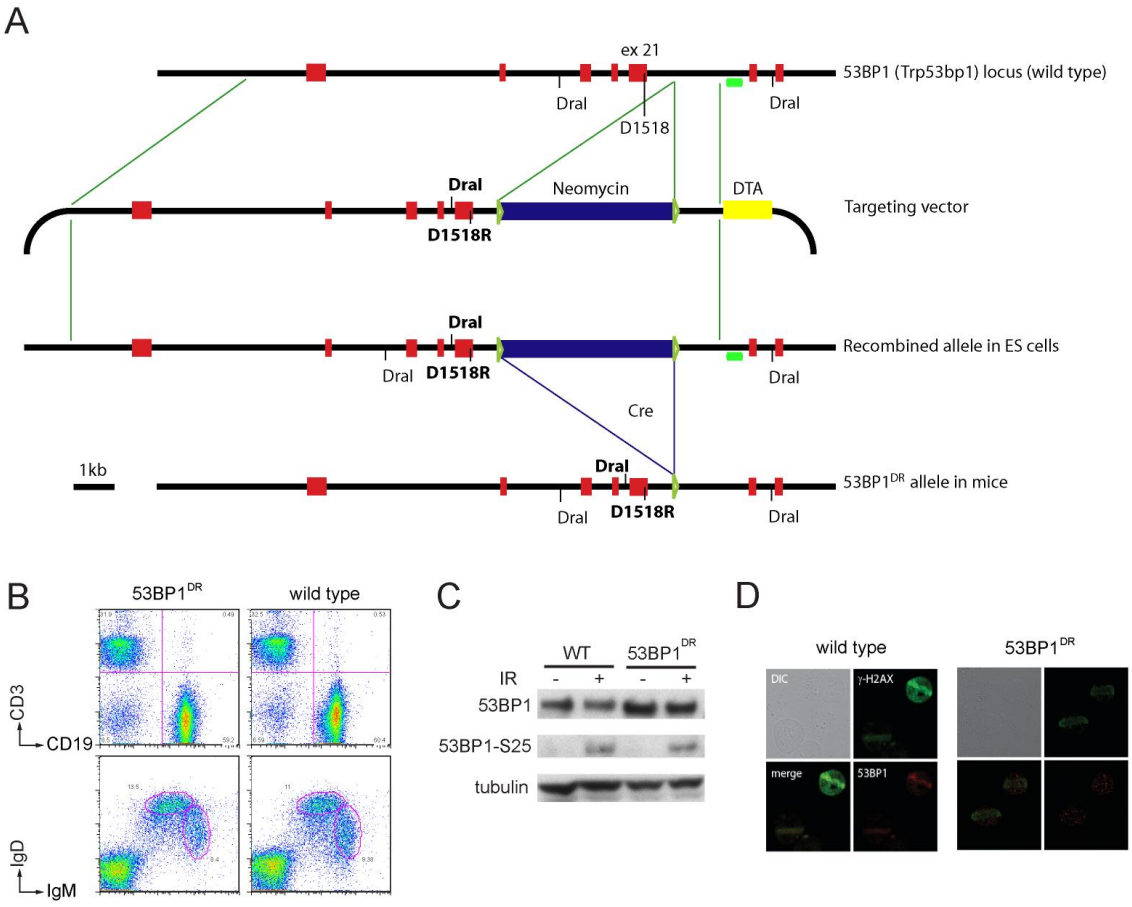

Figure S3. 53BP1^{DR} Mice, Related to Figure 3

(A) Targeting strategy is shown along with the genomic structure of the wild type murine *Trp53bp1* locus (red boxes represent exons), the targeting vector, the recombined 53BP1^{DR} allele in ES cells and in mice after removal of the neomycin-based ACN cassette (Bunting et al., 1999). Southern blot analysis of ES cell genomic DNA digested with DraI revealed proper integration upon hybridization with a radiolabeled probe (PCR product of 5- GGTAAGAGCAGTGGGGTAGACAAG-3 and 5- TCTCCTGAAGTGAATGGCAAGG-3, green bar), yielding 5kb and 8kb fragments for germline and targeted alleles, respectively. Diphtheria toxin A (DTA) was used for negative selection. The following primers were used for generating the targeting vector: short arm of homology, 5-ttaattaaGCAAGAAGCCAGTCTCAGTCCC-3 and 5- gcggccgcTTTACTCGGCTTGCTTGTCTC-3; long arm of homology, 5-ggcgcgccTCTGGAAGAGCAGTCAGTAACCG-3 and 5-ggccggccGGGACTGAGACTGGCTTCTTGCTT-3. The D1518R mutation was introduced with primers 5- TACAAGCTGCTCTTTGATcgcGGGTACGAATGTGACGTG-3 and 5-

CACGTCACATTCGTACCCgcgATCAAAGAGCAGCTTGTA-3; the diagnostic DraI site in intron 20 with 5-GGCTAATAAAGAATGGGGAAGAGAtttaaaGGGAAAAGAGTCAGAAG-3 and 5-CTTCTGACTCTTTTCCCtttaaaTCTCTTCCCCATTCTTTATTAGCC-3. For genotyping, 35 cycles of PCR (95° C, 45 s; 55° C, 45 s; and 72° C, 1 min) were performed with primers 5-CAGTTACCAGGACAGTCTCGTCTTC-3 and 5-ACAAAGGGGAAAACACTAATGCTG-3. The size of the wild type allele is 0.7 kb, the $53BP1^{DR}$ allele is 0.8 kb. **(B)** Flow cytometric analysis of splenocytes from age-matched wild type and $53BP1^{DR}$ mutant mice reveals normal B lymphocyte development. Immuno-staining was performed with the indicated markers on total spleen cells. Representative of three independent experiments. **(C)** Western blots showing 53BP1 expression levels and phosphorylation at Ser^{25} in response to 10 Gy of IR (90 min recovery) in WT and $53BP1^{DR}$ B cells. **(D)** Defective accumulation of $53BP1^{DR}$ in MEFs upon laser scissor damage.

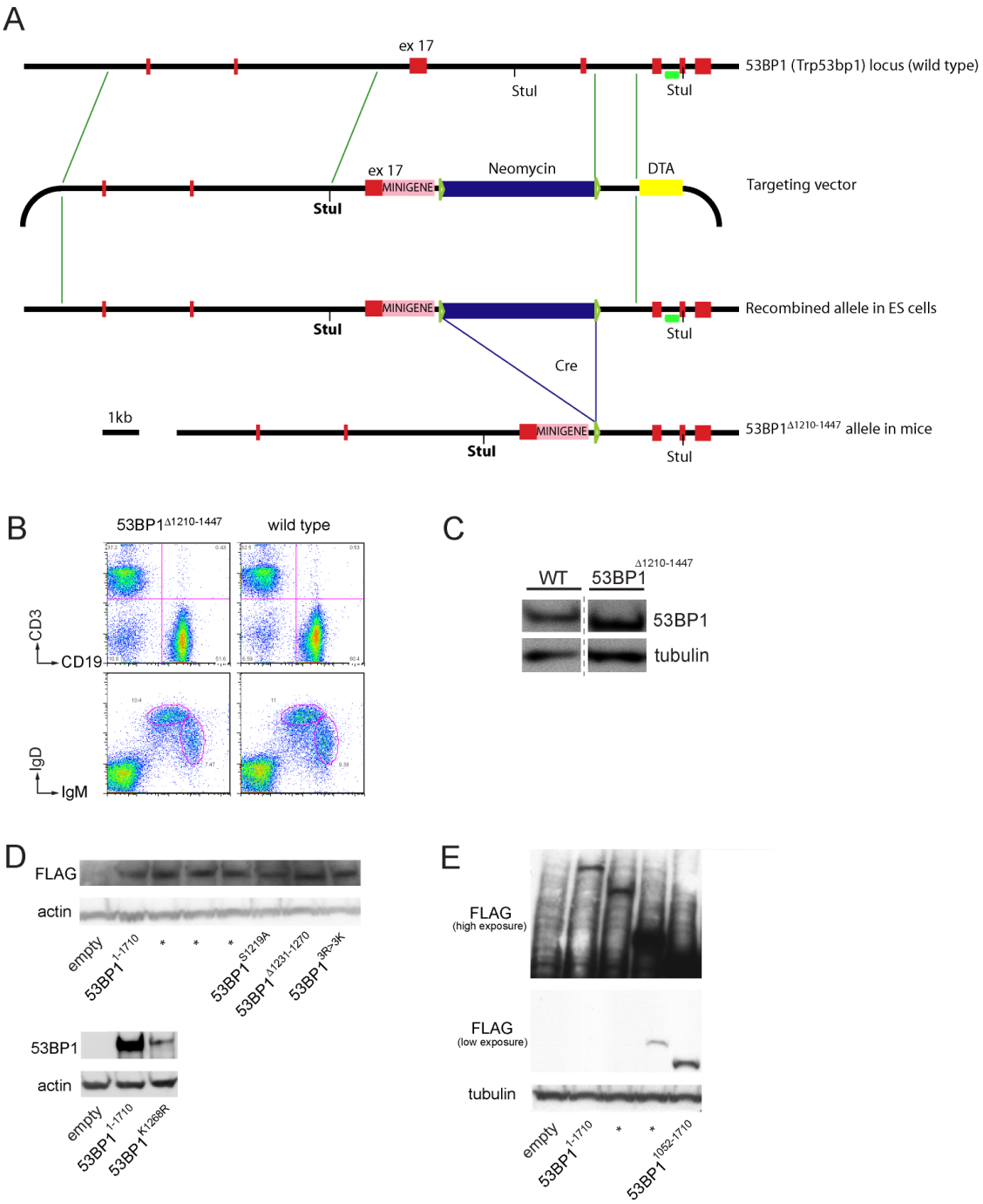

Figure S4. 53BP1$^{\Delta 1210\text{-}1447}$ Mie , Related to Figure 5

(A) Targeting strategy is shown along with the genomic structure of the wild type murine *Trp53bp1* locus (red boxes represent exons), the targeting vector, the recombined 53BP1$^{\Delta 1210\text{-}1447}$ allele in ES cells and in mice after removal of the neomycin-based ACN cassette (Bunting et al., 1999). Southern blot analysis of ES cell genomic DNA digested with StuI revealed proper integration upon hybridization with a radiolabeled probe (PCR product of 5-GCTCTATGTCCTTGGTTTTTGGTC-3 and

5-AGGGCAACAGAACTTGGCTCAC-3, green bar), yielding 5kb and 10kb fragments for germline and targeted alleles, respectively. Diphtheria toxin A (DTA) was used for negative selection. The following primers were used for generating the targeting vector: short arm of homology,
5-ttaattaaGGATGGGACAGAAGTGGAA-3 and
5-gcggccgcAGAAAGAACCCTTTCTATGG-3; distal portion of long arm of homology, 5-ggcgcgccTTGGGTAAAGCCTTGGGTGC-3 and 5-ggccggccTCTAACAAGGTCACTGGGGTAGGG-3; proximal portion of long arm of homology (introducing the diagnostic StuI site in intron 16 and the minigene), 5-GGCCGGCCaggcctTCTTAATTACTTCCCAAAGGCAAC-3 and 5-ggccggccAGATACATTGATGAGTTTGGA-3. The minigene (pink box) was in frame with exon 17 and followed by the polyadenylation signal of SV40 (not shown). It was designed to encode for the deletion of residues 1210-1447. For genotyping, 35 cycles of PCR (95° C, 45 s; 59° C, 45 s; and 72° C, 30 s) were performed with primers 5-GATGGAAACAGACCTGCTGGAAG-3,
5-AGAATCACTCCGCCGCAAAG-3 and 5-TTCACACACAAGCCCCAAGTG-3. The size of the wild type allele is 0.4 kb, the 53BP1$^{\Delta 1210\text{-}1447}$ allele is 0.3 kb. **(B)** Flow cytometric analysis of splenocytes from age-matched wild type and 53BP1$^{\Delta 1210\text{-}1447}$ mutant mice reveals normal B lymphocyte development. Immuno-staining was performed with the indicated markers on total spleen cells. Representative of three independent experiments. **(C)** Western blot showing 53BP1 expression levels in WT and 53BP1$^{\Delta 1210\text{-}1447}$ B cells. **(D and E)** Control western blot analysis on whole cell lysates from a representative experiment in Figures 5D and 5G respectively, to confirm that a protein of the expected size is produced upon retroviral infection. Asterisks denote lanes with unrelated samples, to be disregarded.

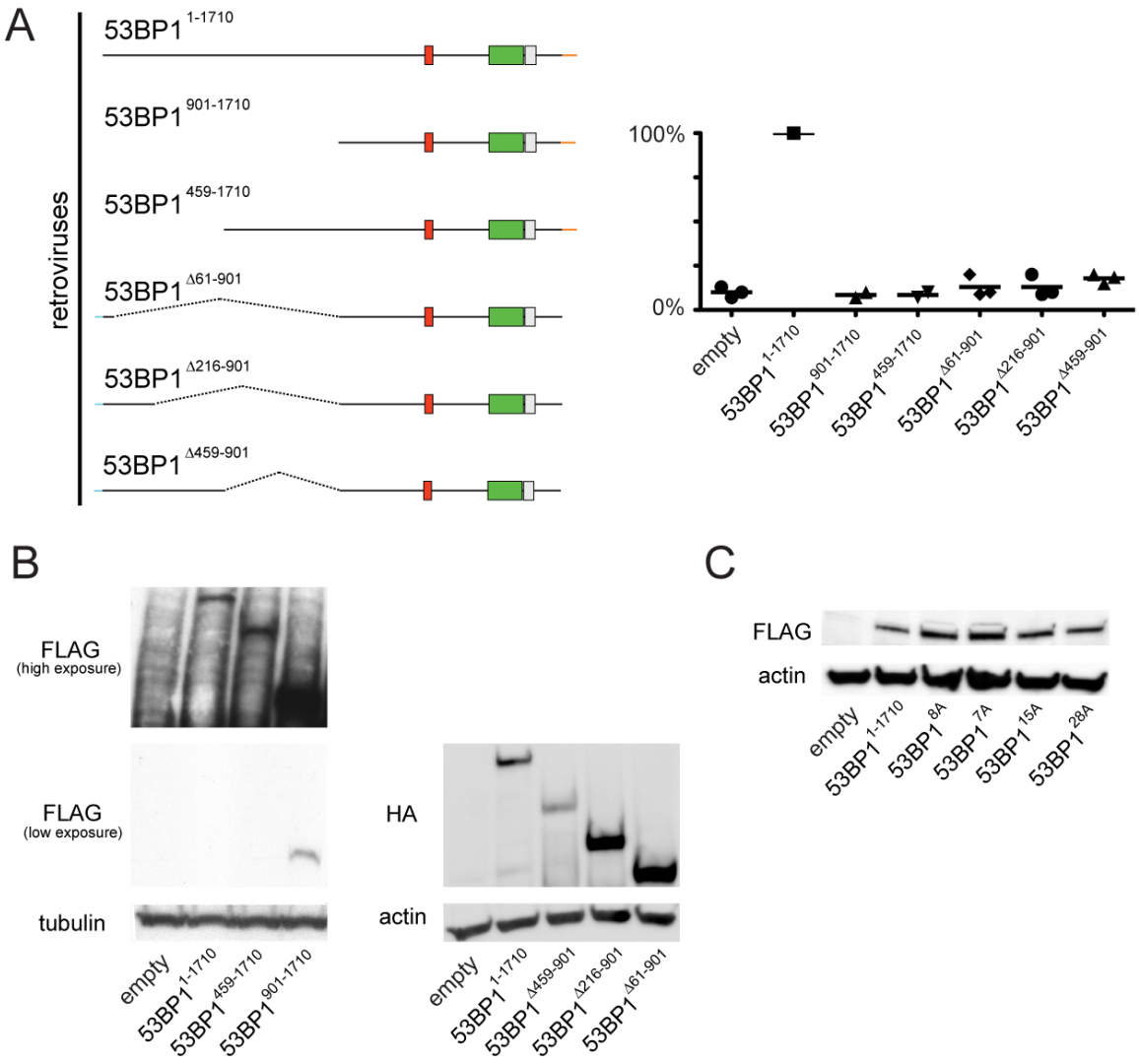

Figure S5. 53BP1's N-Terminal Mutant Retroviruses, Related to Figure 6
(A) Cartoon diagram of 53BP1 N-terminal deletion mutants (left) and percentage of $53BP1^{1\text{-}1710}$ class switching for each mutant (right). $53BP1^{\Delta 61\text{-}901}$, $53BP1^{\Delta 216\text{-}901}$, and $53BP1^{\Delta 459\text{-}901}$ are N-terminally HA-tagged (blue). Each dot represents an independent experiment; the bar indicates the mean value. **(B and C)** Control western blot analysis on whole cell lysates from a representative experiment in Figures S6A and 6B, respectively, to confirm that a protein of the expected size is produced upon retroviral infection.

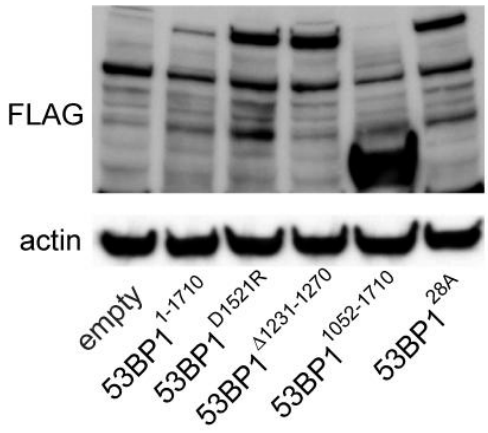

Figure S6. Domains of 53BP1 Required for Preventing DNA End Resection, Related to Figure 7

Control western blot analysis on whole cell lysates from a representative experiment in Figure 7A, to confirm that a protein of the expected size is produced upon retroviral infection.

Supplemental References

Bunting, M., Bernstein, K.E., Greer, J.M., Capecchi, M.R., and Thomas, K.R. (1999). Targeting genes for self-excision in the germ line. Genes Dev *13*, 1524-1528.

Rodriguez, C.I., Buchholz, F., Galloway, J., Sequerra, R., Kasper, J., Ayala, R., Stewart, A.F., and Dymecki, S.M. (2000). High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Nat Genet *25*, 139-140.