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Supplemental Information

53BP1 Supports Immunoglobulin Class Switch Recombination Independently of Its DNA Double-Strand Break End Protection Function

Devakumar Sundaravinayagam, Ali Rahjouei, Matteo Andreani, Dagnija Tupiņa, Sandhya Balasubramanian, Tannishtha Saha, Verónica Delgado-Benito, Violeta Coralluzzo, Oliver Daumke, and Michela Di Virgilio

A

1098
DPVSPASQKMVIQGPSSPQGEAMVTDVLEDQKEGRSTNKENPSKALIERPSQNNIGIQTMECSLRVPETVSAATQTI

1175
KNVCEQGTSTVDQNFGKQDATVQTERGSGEKPVSAPGDDTESLHSQGEEEFDMPQPPH**GH**VLH**RH**MRTIREVRTLVT
----------EEE----------EEH--HHHEEHHHHHEE

1252
RVITDVYY**VDGT**EVERKVTE**ETEEP**IVECQEC**ETEVS**PSQTGGSSGDLGDISSFSSKASSLHRTSSGTSLSAMHSSG
EEEEEEEE----EEEEEEEE-----EEEEEEE----------

B

OD^{WT} (h53BP1 aa 1233-1288)
GHVLHRHMRTIREVRTLVTRVITDVYYVDGTEVERKVTEETEEPIVECQECETEVS
--EEH--HHHEEHHHHHEEEEEEEEEE----EEEEEEE-----EEEEEEE-----

OD^{D1256A}
GHVLHRHMRTIREVRTLVTRVIT**A**VYYVDGTEVERKVTEETEEPIVECQECETEVS

OD$^{\Delta Core}$
GHVLHRHMRTIREVRTLVTRV⟨⟩YVDGTEVERKVTEETEEPIVECQECETEVS

C

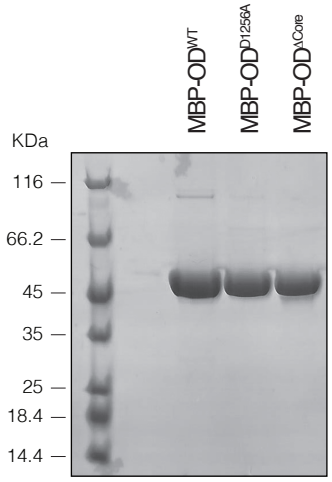


Figure S1

Figure S1. Secondary structure prediction for the oligomerization domain. Related to Figure 1. (A) JPRED secondary structure prediction (Drozdetskiy et al., 2015) of amino acids 1098-1328 of human 53BP1 (UniProt Q12888). Amino acids 1233-1288 are indicated in bold. H: alpha helix; E: beta sheet; "-": not H or E secondary structure. (B) Sequence of wild-type and mutated OD (amino acids 1233-1288 of human 53BP1) constructs fused to N-terminal MBP. The JPRED secondary structure prediction of the wild-type OD is indicated. (C) Coomassie-stained gel analysis of purified MBP-OD-fused protein constructs shown in panel B.

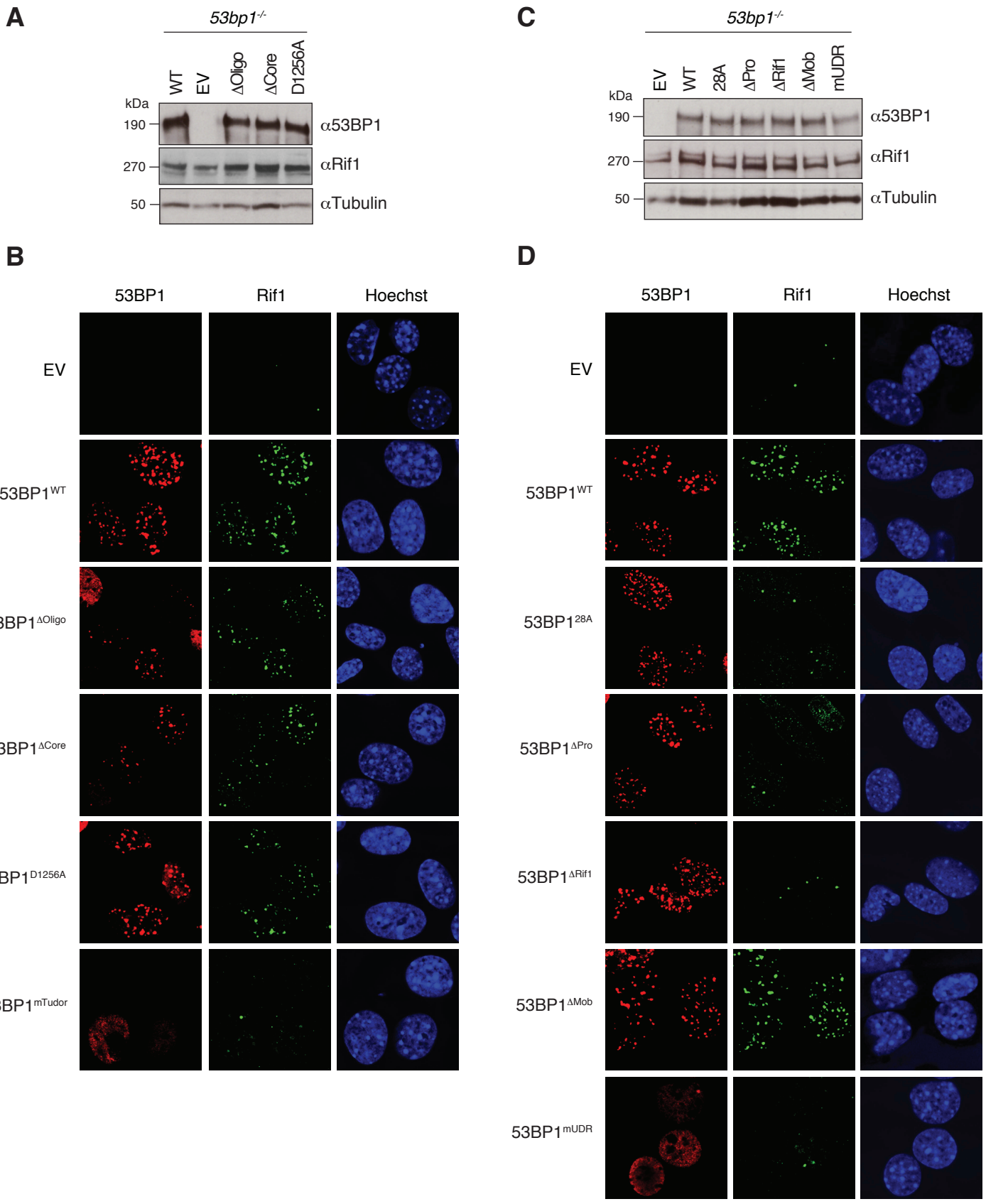

A
53bp1-/-
WT
EV
ΔOligo
ΔCore
D1256A
kDa
190
270
50
α53BP1
αRif1
αTubulin
C
53bp1-/-
EV
WT
28A
ΔPro
ΔRif1
ΔMob
mUDR
kDa
190
270
50
α53BP1
αRif1
αTubulin
B
53BP1
Rif1
Hoechst
EV
53BP1WT
53BP1ΔOligo
53BP1ΔCore
53BP1D1256A
53BP1mTudor
D
53BP1
Rif1
Hoechst
EV
53BP1WT
53BP128A
53BP1ΔPro
53BP1ΔRif1
53BP1ΔMob
53BP1mUDR

Figure S2

Figure S2. Rif1 IRIF formation is largely unaffected in cells expressing 53BP1 OD-mutants, but is abrogated in cells reconstituted with 53BP1 phospho-mutants. Related to Figures 1 and 2. (A and C) Representative WB analysis of *53bp1*$^{-/-}$ iMEF reconstituted with the indicated constructs. (B and D) Representative immunofluorescent staining images for 53BP1 and Rif1 in irradiated (10 Gy; 2 h recovery time) *53bp1*$^{-/-}$ iMEF reconstituted with the indicated constructs.

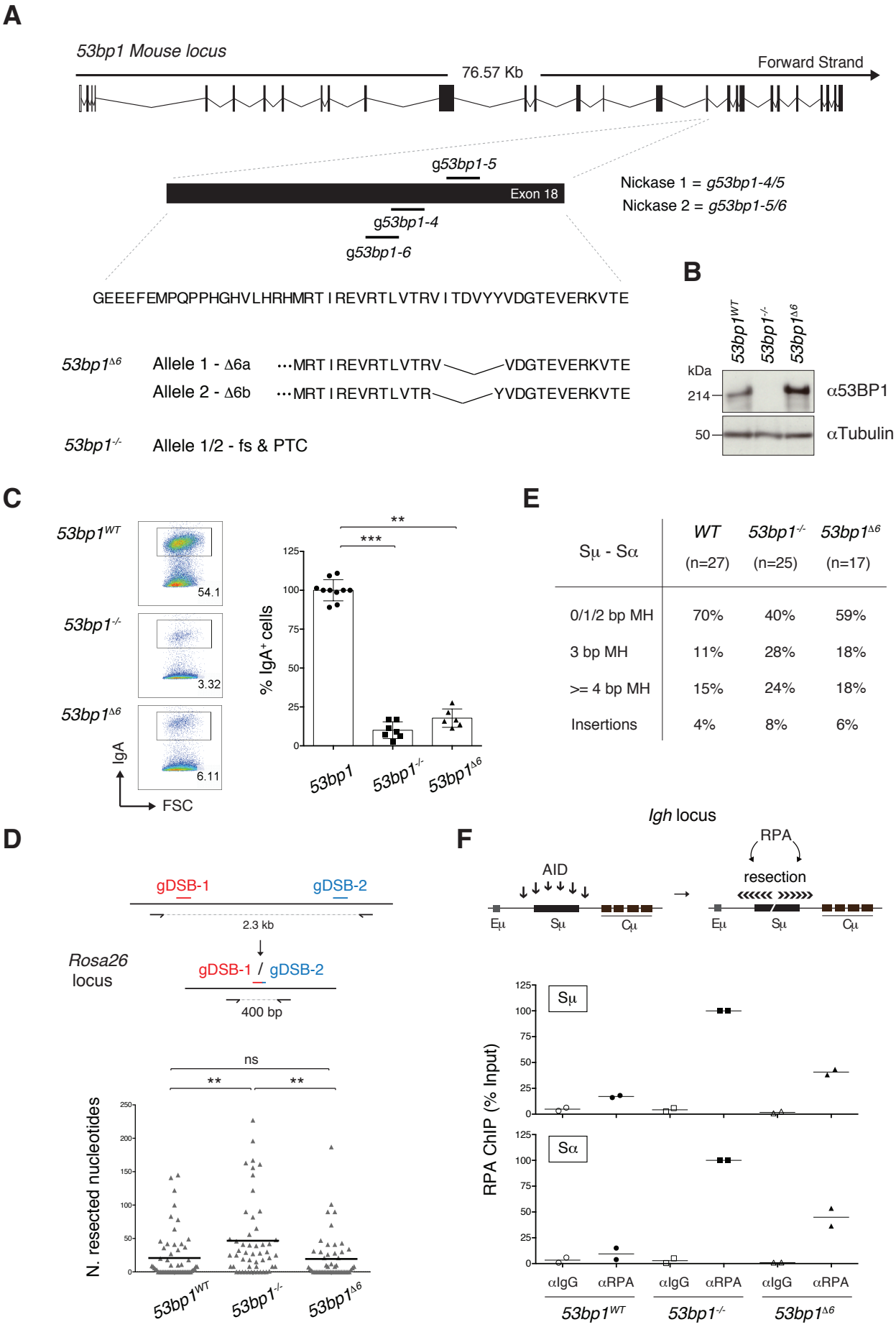
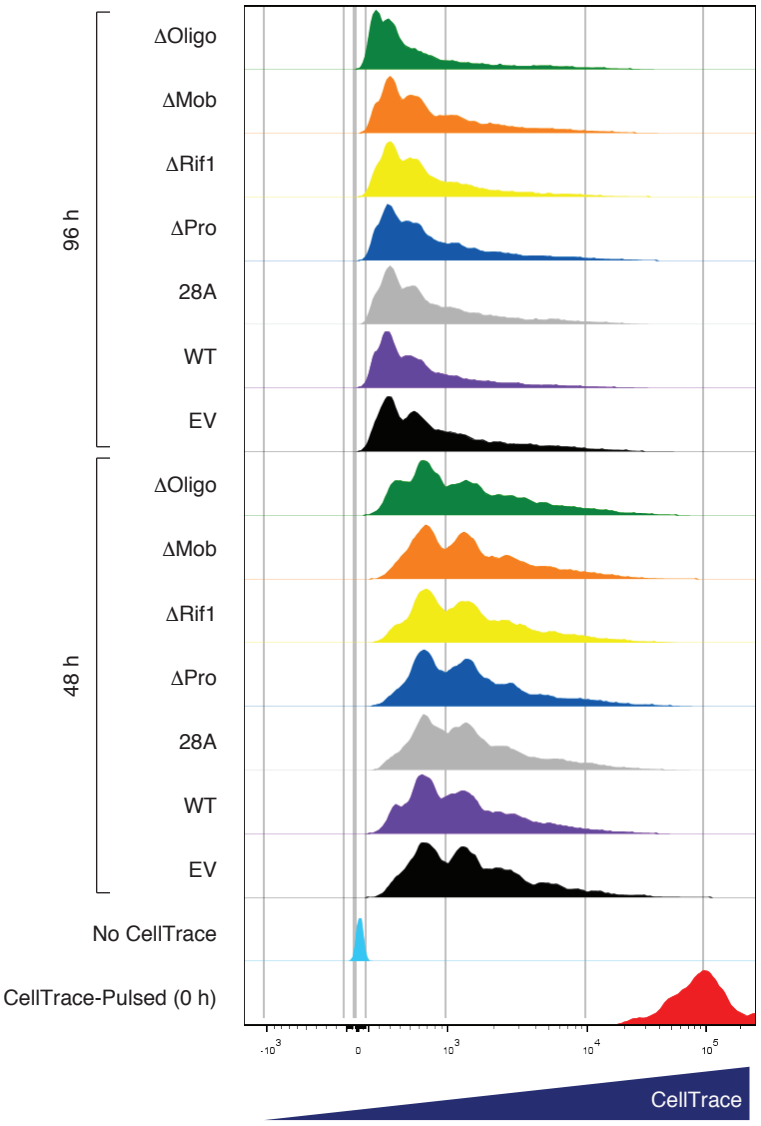

A
53bp1 Mouse locus
76.57 Kb
Forward Strand
g53bp1-5
Exon 18
g53bp1-4
g53bp1-6
Nickase 1 = g53bp1-4/5
Nickase 2 = g53bp1-5/6
GEEEFEMPQPPHGHVLHRHMRTIREVRTLVTRVITDVYYVDGTEVERKVTE
53bp1Δ6
Allele 1 - Δ6a ···MRTIREVRTLVTRV VDGTEVERKVTE
Allele 2 - Δ6b ···MRTIREVRTLVTR YVDGTEVERKVTE
53bp1-/-
Allele 1/2 - fs & PTC
B
53bp1WT
53bp1-/-
53bp1Δ6
kDa
214
α53BP1
50
αTubulin
C
53bp1WT
54.1
53bp1-/-
3.32
53bp1Δ6
6.11
IgA
FSC
% IgA+ cells
53bp1
53bp1-/-
53bp1Δ6
E
Sμ - Sα
WT (n=27)
53bp1-/- (n=25)
53bp1Δ6 (n=17)
0/1/2 bp MH 70% 40% 59%
3 bp MH 11% 28% 18%
>= 4 bp MH 15% 24% 18%
Insertions 4% 8% 6%
D
gDSB-1
gDSB-2
2.3 kb
Rosa26 locus
gDSB-1 / gDSB-2
400 bp
ns
N. resected nucleotides
53bp1WT
53bp1-/-
53bp1Δ6
F
Igh locus
AID
RPA
resection
Eμ
Sμ
Cμ
RPA ChIP (% Input)
Sμ
Sα
αIgG
αRPA
53bp1WT
53bp1-/-
53bp1Δ6

Figure S3

Figure S3. 53BP1 ΔCore-mutant-expressing CH12 cells retain the ability to inhibit DNA end resection. Related to Figure 1. (A) Top: Scheme of murine *53bp1* genomic locus and location of gRNAs used for gene targeting (scheme adapted from Ensembl Trp53bp1-202 ENSMUST00000110648.7). Bottom: Amino acid sequences encoded by the targeted alleles in the selected *53bp1*$^{-/-}$ and 53BP1 ΔCore-mutant-expressing CH12 clonal derivatives. fs: frameshift; PTC: premature termination codon. (B) Representative WB analysis of *53bp1*^{WT}, *53bp1*$^{-/-}$, and *53bp1*$^{\Delta 6}$ CH12 cell lines. (C) Left: Representative flow cytometry plots measuring CSR to IgA in the indicated CH12 cell lines activated for 48 h with αCD40, IL-4, and TGFβ. Numbers in the plots refer to the percentage of switched cells (= IgA^{+} cells). Right: Summary dot plot for seven independent experiments. For the experiments including two different control cell lines (WT CH12 clonal derivatives generated by targeting with gRNAs against random sequences not present in the mouse genome), CSR efficiencies within each experiment were normalized to the average of the CSR value of the two WT cell lines, which was set to 100%. Significance was calculated with the Mann–Whitney U test, and error bars represent SD. ** = $p \leq 0.01$; *** = $p \leq 0.001$. (D) Top: Schematic representation of the Rosa26-ERA end resection assay. Bottom: Dot plot showing resection at the junction in ligation products of two CRISPR-Cas9-induced DSBs at the *Rosa26* locus in *53bp1*$^{-/-}$ and 53BP1 ΔCore-mutant-expressing CH12 cells. The graph is representative of two independent experiments. Each dot represents one junction product. Number of junctions analyzed per genotype is 59 for *53bp1*^{WT}, 52 for *53bp1*$^{-/-}$, and 52 for *53bp1*$^{\Delta 6}$ cell lines. Mean is indicated. p values were calculated with the Mann–Whitney U test. ** = $p \leq 0.01$; ns: not significant. (E) Sμ-Sα junction analysis in *WT*, *53bp1*$^{-/-}$ and *53bp1*$^{\Delta 6}$ CH12 cell lines. The number of junctions analyzed per genotype is indicated in the figure. See also Table S1 for sequences of analyzed junctions. MH: microhomology. (F) Top: Schematic representation of RPA loading at S regions following resection of AID-induced breaks. For simplicity, only the Sμ region is shown. Bottom: ChIP-qPCR for RPA occupancy at Sμ and Sα regions in activated CH12 cells of the indicated genotypes. ChIP-qPCR values within each experiment were normalized to *53bp1*$^{-/-}$ sample, which was set to 100%. The graph summarizes two independent experiments.

A


96 h
48 h
ΔOligo
ΔMob
ΔRif1
ΔPro
28A
WT
EV
ΔOligo
ΔMob
ΔRif1
ΔPro
28A
WT
EV
No CellTrace
CellTrace-Pulsed (0 h)
-10^3
0
10^3
10^4
10^5
CellTrace

B

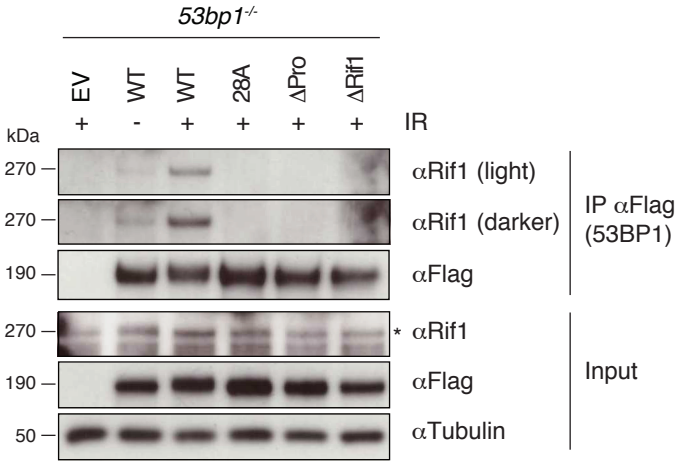

53bp1-/-
EV
WT
WT
28A
ΔPro
ΔRif1
+
-
+
+
+
+
IR
kDa
270
270
190
270
190
50
αRif1 (light)
αRif1 (darker)
αFlag
αRif1
αFlag
αTubulin
*
IP αFlag (53BP1)
Input

Figure S4

Figure S4. Cell proliferation and 53BP1-Rif1 co-immunoprecipitation analysis of 53BP1 mutant-reconstituted primary B cell cultures. Related to Figures 1, 3 and 4. (A) Proliferation analysis by CellTrace Violet dilution of *53bp1*$^{-/-}$ splenocytes transduced with the indicated constructs and stimulated with LPS and IL-4. The "No CellTrace" and "CellTrace-Pulsed (0 h)" histograms refer to the initial *53bp1*$^{-/-}$ splenocyte culture employed for the reconstitution experiment. Data are representative of two independent experiments. (B) WB analysis of anti-Flag(53BP1) immunoprecipitates from irradiated (20 Gy; 1 h recovery time) *53bp1*$^{-/-}$ splenocytes reconstituted with the indicated constructs. Light/darker: light/darker exposure of same anti-Rif1 blot. "*" indicates Rif1 band.

Table S1. Sμ-Sα junctions from *WT*, *53bp1*$^{-/-}$ and *53bp1*$^{\Delta 6}$ CH12 cells. Related to Figures 1 and S3.

	Sμ-Sα junctions
WT n = 27	ACTTCATTAATCTAGGTTGAATAGAGCTAAAAAATGAGCTGAACTAGGATGGGATGGGAT GACTGTAATGAACTGGAATGAGCTGGGCC:TGAGATGGGATGAACTGACTGGGCTGGGCT TAGGGTGAGCTGAGCTGGTTGAGCTGAG:ACAATGAGCTAACATAAATTCAGCTGGCTGA TTCTGAGCTGAGATGAGCTGGGGTGAGGTCA:TGTCTGAGCTGGCCTAAGATGGACTTAG ATGAAGTAGACTGTAATGAACTGGAATGA:CCAGAATAGTCAGAGCTAGGCTGGAATTAG TTGAGAGCCCTAGTAAGCGAGGCTCTCAAA**A**ACTGACTGGGCTGGGCTCAGTTGACC AGAGCCCTAGTAAGCGAGGCTCTAAAAAAC**A**TGGGATGGGATGGGATGGGATGGGATGG GCTTGGCTGAACTAGGGTGAGCTGGGCTGACCT**G**CTGGGCTGAGTTAGTCTAGGCTGGA GCTAAAGTGAGGTGATTACTCTGA**G**CTAAGCTGGGATGGACTAGGATAAACTAAGCTGG AAACTAGGCTGGCTTAAACGAGATGA**G**GCTCAGTTGACCTTGCTCGTTTGAGCTGGTCT TAAAAAGCACAGCTGAGCTGAGATGGGTGG**G**GGATGGGATGAGATGGGATGAACTGACT GAAGTAGACTGTAATGAACTGGAATGAAC**T**TTGAGCTGGTCTAGATGGTCTAGTTGGGC AGCTGAGCTGGGTGAGCTGAGCTAAGCT**G**CTTGGCTGGTTACAATGAGCTAACATAAAT TGAGCTGAGATGAGCTGGGGTGAGCTCAA**C**AAATTTGACAGTGAGCTAGCCTGGGGTGA GGCTGGCTTAACCGAGATGAGCCA**A**TGGGATGGGATGGGATGGGATGGGATGGGATGAG AGGCTCTAAAAAGCACAGCTGATCT**GA**TGGGATGGGATGGGATGGGATGGGATGGGATGG TTGGAATGAACTTCATTAATCTAGGTTGA**AT**GGGATGGGATGGGATGGGATGGGATGGGA TGAACTGAGCTGAGCTTGGCTGAACTAGAG**TG**GTCTAGATGGTCTAGTTGGGCTGGCCA TGAGCTGAGATGGGTGGGCTTCTCTGAG**TG**GGATGGGATGGGATGAGATGGGATGAACT TTCTACTGCCTACACTGGACTGTTCTG**AG**AGCTAGGCTGGAATTAGGCGAAACTTGGCT ATTCTACTGCCTACACTGGACTGTT**CTG**GGCTCAGTTGACCTTGCTCGTTTGAGCTGGT GAGCTAGACTGAGCTGAACTAGGGTGAA**CTG**GCTGAACCAAACTTGACAGTGAGCTAGC GCTGATCTGAAATGAGATACTCT**GGA**TGGGATGAGATGGGATGAACTGACTGGGCTGG CTGAGCTAAGCTGGGGTGAACTGAGC**TGAG**ATGGGATGAACTGACTGGGCTGGGCTCAG GAGTAGCTGAGATGGGGTGAGATGGGG**TGAG**ATGGGATGAACTGACTGGGCTGGGCTCA TGAGCTGAGATGGGTGGGCTTCTC**TGAG**ATGGGATGAACTGACTGGGCTGGGCTCAGTT AATAGAGCTAAATTCTACTGCCTAC**ACTGG**GCTGGGCTCAGTTGACCTTGCTCGTTTGA
53bp1$^{-/-}$ n = 25	AAGTAGACTGTAATGAACTGGAATGAACTGGGATGGCCTAAGATGGACTTAGTTGAGGT CTGAGCTGAGTTAGGGTGAGCTGAGCTGAGCTGGGATGGGATGGGATGGGATGGGATGG GACTGTAATGAACTGGAATGAGCTGGGCCGC:GTTAGTCTGGACTAGGCTGAGTTAGTCT AAAAACACAGCTGAGCTGAGATGGGTGG:AAACTTGGCTAGGCTACAATGGATTGAGCTGA GCTCAGCTATGTTACGCTGTGTTGGGGTGA:CTAAAGTGAGCTGAACTAGGATGGGATGG GGTGAGCTCAGCTATGATACGC**T**CTAGTTGGGCTGGCCAGAATAGTCAGAGCTAGGCTG AAGTAGACTGTAATGAACTGGAATGAACTGG**G**ATGGCCTAAGATGGACTTAGTTGAGGT AGCTGGGGTGAGCTCAGCTATGTTACGCT**G**GATGGGATGGGATGGGATGGGATGGGATG GTGAGCTGAACTGGGCTGAGCTAGACTGGGCTGAA**CT**GGGGTGAATTAGCATGACTGGA GCCAAATTGGAATGAACTTCATTAATCTAGGT**TG**GGATGGGATGGGATGGGATGGGATG AAAAAGCACAGCTGAGCTGAGATG**GG**CTAGGCTACAATGGATTGAGCTGAGCTAGAC AGCTGGGATGACCTGGGGTGAACTGAG**CT**TGGCTTGGCTGGTTACAATGAGCTAACATA CTACTGCCTACACTGGACTGTTCTGA**GCT**TGGCTGGTTACAATGAGCTAACATAAATTC GTTACGCTGTGTTGGGG**TGA**CTGGGCTGGGCTCAGTTGACCTTGCTCGTTTGAGCTGGT TCTACTGCCTACACTGGACTGTT**CTG**GTCTAGATGGTCTAGTTGGGCTGGCCAGAATA AAATGAGATACTCTGGAGTAGCTGAGATGGGG**TGA**ACTGACTGGGCTGGGCTCAGTTGA CTGGGCTTGAGCCAAAATGAAGTAGA**CTG**AGTTAGTCTGGGCTAGGCTGAGTTAGTCTG GAGCCAAAATGAAGTAGACTGTAA**TGA**CCTTGCTCGTTTGAGCTGGTCTAGATGGTCTA AAATTCTACTGCCTACACTGGACTGTTC**TGA**ACTGACTGGGCTGGGCTCAGTTGACCTT AAAAAGCACAGCTGAGCTGAGATGGG**TGGG**ATGGGATGGGATGGGATGAGATGGGATG GAGATGGGGTGAGATGGGGTGACCTGA**GCTGG**TTATAATGAGCTAACATAAATTCAGCT TGCGCTAAACTGAGGTGATTACT**CTGAG**CTGACCTAGGCAAGTTGTGCTGTCTGAGCTG CTGAGCTGGGTGAGCTGAGCTA**AGCTGG**TCTAGATGGTCTAGTTGGGCTGGCCAGAATA CTGTTCTGAGCTGAGATGAG**CTGGGGTGA**ATTAGCATGACTGGACTTATTCACAGTTCA TGAGCTGAACTAGGGTGAG**CTGAGCTGGG**CTGGACTGGGCTGGAATGAATTAGTCTGGG
53bp1$^{\Delta 6}$ n = 17	ACTGTTCTGAGCTGAGATGAGCTCAGTTGGGCTGGCCAGAATAGTCAGAGCTAGGCTGG TCTGAGCTGAGATGAACTGGGGTGAGCTCA:TCTAGTTGGGCTGGCCAGAATAGTCAGAG ACTGGACTGTTCTGAGCTGAGATGAACTG:AGTCTGGGGTGAATTAGCATGACTGGACTT CCAAACTGGAATGAACTTCATTAATCTAG:CCTTGCTCGTTTGAGCTGGTCTAGATGGTC TCATTAATCTAGGTTGAATAGAGCTAAACTCTAC:AATTCAGCTGGCTGAACCAAACTTGACAG GAGCTGAACTAGGGTGAGCTGAACTG:TAGTTGGGCTGGCCAGAATAGTCAGAGCTAGG AATGAACTTCATTAATCTAGGTTGA**A**ATGAGCTAACATAAATTCAGCTGGCTGAACCAAAC TGAAGTAGACTGTAATGAACTGGAATGAGT**TG**AGCTGGCCTAAGATGGACTTAGTTGAGGT GAGATACTCTGGAGTAGCTGAGATGGGGTGATAT**GG**ATGGGATGGGATGGGATGGGATGGG TGAAGTAGACTGTAATGAACTGGAAT**GA**TGGGATGAGATGGGATGAACTGACTGGGCTGGGC CTGGACTGTTCTGAGCTGAGATGAACTGGGG**TG**GGATGGGATGGGATGGGATGGGATGG TGAGATACTCTGGAGTAGCTGAGATGGGG**TGA**ACTGACTGGGCTGGGCTCAGTTGACCTTG GAAGTAGACTGTAATGAACTGGAATGA**GCT**ACAATGGATTGAGCTGAGCTAGACTTAGGGTG AGTAGACTGTAATGAACTGGAATGA**GCT**AAACTGAGCTGAACTAGGATGGGATGGGATGGGA TAAAAAGCACAGCTGAGCTGAGATGGG**TGGG**ATGGGATGAGATGGGATGAACTGACTGGGC CTGAAATGAGATACTCTGGAGTAGCTGAT**ATGGG**ATGGGATGGGATGGGATGGGATGGG GTAGACTGTAATGAA**CTGGAATGAGCTGGG**TTGAGCTGAACTAGTATAAACTTGGCTAGGCT

Blunt junctions are indicated with "**:**", micro-homology in **bold**, and nucleotide additions are underlined. Micro-homology at the junction was determined by identifying the longest region at the switch junction of perfect uninterrupted donor/acceptor identity.

Table S2. List of oligonucleotides used in this study. Related to STAR Methods.

53BP1$^{\Delta BRCT}$ construct cloning

PCR primers	**Sequence (5'→3')**	**Reference**
53BP1$^{\Delta Pro}$- & 53BP1$^{\Delta Mob}$-3xFlag		
MDV_p187 (Fw)	GATCCGAATTCCACGCGGCCGCACGCG TACCATG	This paper
MDV_p240 (Rev) (SgrAI)	CAGATCGAGTCGCCGGTGACGGAACTG	This paper
MDV_p239 (Fw) (SgrAI)	CAGTTCCGTCACCGGCGACTCGATCTG	This paper
MDV_p188 (Rev)	ACTCCTGACACTCTACAATTGGCTCTTC AGTCTC	This paper
53BP1^{WT}-3xHA		
Forward - PCR 1 & 2	ACTGTTTCAGCAGCAACCCAGACTATAA AGAATGTG	This paper
Reverse - PCR 1	TCAGGAACGTCGTACGGGTAGCTACCT GCATAATCCGGCACATCATAAGGGTAT CCTCCACCGGTGTTGTCTC	This paper
Reverse - PCR 2	CGACTTAATTAATCACTAGGCGTAATC AGGAACATCGTAAGGATAGGATCCTGC GTAGTCAGGAACGTCGTACGGGTAG	This paper

CRISPR-Cas9 gene targeting

gRNAs	**Sequence (5'→3')**	**Reference**
g53bp1-4	TGACGCGGGTGACGAGTGTA	This paper
g53bp1-5	CAGATGTTTATTATGTGGAT	Delgado-Benito et al., 2018
g53bp1-6	GAGTGTACGGACTTCTCGAA	Delgado-Benito et al., 2018

RPA ChIP-qPCR

qPCR primers	**Sequence (5'→3')**	**Reference**
Sμ Fw	GTTGCCTGTTAACCAATAATCATAGAGC TCATGG	Wiedemann et al., 2016
Sμ Rev	GTATAACTGAAGTAGAGACAGCATCAGT ACCTCAAC	Wiedemann et al., 2016
Sα Fw	TGAAAAGACTTTGGATGAAATGTGAACC AA	Wiedemann et al., 2016
Sα Rev	GATACTAGGTTGCATGGCTCCATTCACA CA	Wiedemann et al., 2016

End resection assay

gRNAs	**Sequence (5'→3')**	**Reference**
gDSB-1	CATGGATTTCTCCGGTGAAT	Delgado-Benito et al., 2018
gDSB-2	AGTTGTCATTGCTGAATATC	Delgado-Benito et al., 2018
1st round of PCR		
MA_p45 (Fw)	CTGTTAGAGCATGCTTAAGGG	Delgado-Benito et al., 2018
MA_p42 (Rev)	TCACCATTAGGGCAAATGGC	Delgado-Benito et al., 2018
2nd round of PCR		
MA_p51 (Fw)	GTAGTTACTTGGCAGGCTCC	Delgado-Benito et al., 2018
MA_p48 (Rev)	AAAGTCATTCCACAGTTTGAC	Delgado-Benito et al., 2018

Switch junctional analysis

PCR primers	**Sequence (5'→3')**	**Reference**
MDV_p481	TTGAGAGCCCTAGTAAGCGAGGCTCTA	Lee-Theilen et al., 2011
MDV_p482	GAACTGTGAATAAGTCCAGTCATGCTAAT	Lee-Theilen et al., 2011