Legends to Supplementary Figures

Legend to Figure S1. Analysis of the genetic interaction between 53BP1 and ATM in organismal growth and development. A, body weight of 8 week-old *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ and control female mice. Similar data was obtained for males (not shown). B, representative microphotographs of mice in A. C, thymi of the indicated genotypes were disaggregated and the number of cells counted. D, for immunophenotyping, thymocytes were stained with antibodies to CD4 and CD8 and analyzed by flow cytometry; a representative experiment is shown. The percentage of double positive (CD4^{+}/CD8^{+}) thymocytes and more mature single-positive (CD4^{+} or CD8^{+}) thymocytes are indicated. E, spleens were disaggregated and the number of splenocytes counted after red cell lysis. To calculate the number of T cells, splenocytes were stained with antibodies to CD4 and CD8 and the number of CD4^{+} and CD8^{+} cells was added. F, representative example of staining as described in F. G, to calculate the number of B cells, splenocytes were counted and stained with antibodies to B220 and IgM to determine the percentage of B220^{+}/IgM^{+} cells (B cells) within the population. H, representative example of spleens stained as described in G. I, H/E staining of testis from 8 week-old *Atm*$^{-/-}$ and *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ mice reveals severe germ cell depletion. All bars in the figure represent the average and standard deviation of 3-5 mice per genotype.

Legend to Figure S2. Analysis of ploidy in *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ lymphomas. A, histograms indicate the distribution of the number of chromosomes per metaphase in two *Atm*$^{-/-}$ and six *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ thymic lymphomas. At least 20 metaphases per tumor were

analyzed. B, representative examples of euploid (top) or modestly aneuploidy (bottom) *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ tumor metaphases.

Legend to Figure S3. Analysis of *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ thymic lymphomas by TCRα/δ locus FISH. Representative metaphases from a control *Atm*$^{-/-}$ lymphoma and five *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ lymphomas hybridized with the indicated probes. "Split" BAC signals and amplification of sequences upstream to the TCRα/δ locus were found in all tumors.

Legend to Figure S4. SKY analysis of two *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ lymphomas. A, representative SKY hybridization showing two clonal translocations: t(12,14) and t(14,15).

Legend to Figure S5. Cell cycle analysis of resting lymphocytes after IR. Splenocytes were exposed to IR (2 Gy) and harvested at the indicated timepoints for staining with propidium iodide and analysis by flow cytometry. Data from two independent experiments is shown. The percentage of cells in subG1 (blue) is an indicator of cell death.

Legend to Figure S6. Aberrant *Vd2-Dd1*/*Dd2-Jd1* coding junctions are not detected in *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ mice. Coding junctions were PCR amplified from thymic DNA of 7-day old mice of the following genotypes: wt (n=3 mice), *Trp53bp1*$^{-/-}$ (n=4 mice), *Atm*$^{-/-}$ (n=3 mice) and *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ (n=4 mice). For reference, a rearranged coding junction

is given at the top of the figure. *Dd2* and *Dd1* gene segments are in red; nucleotide additions are in blue. A single large deletion is indicated in parentheses.

Legend to Figure S7. Aberrant *Vd5-Dd2* Recombination Signal Junctions are not detected in *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ mice. Signal junctions were PCR amplified from thymic DNA of 7-day old mice of the following genotypes: wt (n=2 mice), *Trp53bp1*$^{-/-}$ (n=2 mice), *Atm*$^{-/-}$ (n=2 mice) and *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ (n=2 mice). Top, *Vd5* and *Dd2* RSSs and flanking coding sequences. Heptamer and nonamer elements are indicated in red; coding sequences are in boldface. Below, sets of sequences from wild-type, *Trp53bp1*$^{-/-}$, *Atm*$^{-/-}$, and *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ joints. The number of occurrences of each sequence is indicated at right. N additions are indicated in blue; deletions are indicated by parentheses.

Legend to Figure S8. Histograms showing the frequency distribution of deletions from each coding end (A-D) and signal end (E-F) analyzed. No clear differences across genotypes are observed. No imprecise recombinant junction was counted more than once, even if it was represented more than once among the PCR products.

Legend to Figure S9. Cell cycle analysis of activated (cycling) lymphocytes after IR. Splenocytes were activated with α-CD40+IL-4 for two days, exposed to IR (2 Gy) and harvested at the indicated timepoints. Cells were stained with propidium iodide and analyzed by flow cytometry. Data is representative of five independent experiments.

Legend to Figure S10. Analysis of genomic stability in splenic B or T cells treated with olaparib. Cells of the indicated genotypes were activated with either α-CD40+IL-4 (B cells) or concanavalin A (T cells) and treated with 1 μM olaparib or vehicle for 24 hours prior to fixation. Colcemid was added in the last four hours. A, percentage of B cell metaphases containing chromosomal aberrations; B, number of aberrations per B cell metaphase. C, number of chromatid breaks per B cell metaphase. D, percentage of T cell metaphases containing chromosomal aberrations; E, number of aberrations per T cell metaphase. F, number of chromatid breaks per T cell metaphase. G, Representatie examples of olaparib-treated B cell metaphases. Yellow arrows point to chromosome breaks; white arrows point to chromatid breaks and fusions.

Legend to Figure S11. Analysis of the G2/M checkpoint. α-CD40/IL-4-activated B cells were harvested one hour after exposure to 2 Gy of IR, stained with a FITC-labeled antibody to phospho(P)-histone H3 (Ser10) and propidium iodide (PI) and analyzed by flow cytometry. Mock-irradiated controls were harvested in parallel. A, Percentage of P-H3(Ser10)$^{+}$ cells in irradiated cultures, normalized to mock-irradiated controls. Individual dotplots for the same cultures are shown in (B). The average and standard deviation of the number of P-H3(Ser10)$^{+}$ cells in the 3 *Atm*$^{-/-}$ and the 3 *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ mice in A-B is shown in C. Data was normalized to untreated cells.

Legend to Figure S12. A second primer set detects deletions at hybrid V(D)J recombination junctions in *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ thymic DNA from 7 day-old mice. A, assay

for *Dβ2-Vβ14* hybrid joints in *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ DNA using a primer set in which nested primer pairs are equidistant from the expected recombinant junction (see methods). Representative assays for two mice of each genotype are shown, representing 3 experiments. B-C, diagrams and sequences of aberrant hybrid joints, respectively, as described in Figure 5. N additions are shown in blue; potential microhomologies are underlined and size of deletions is shown in parenthesis.

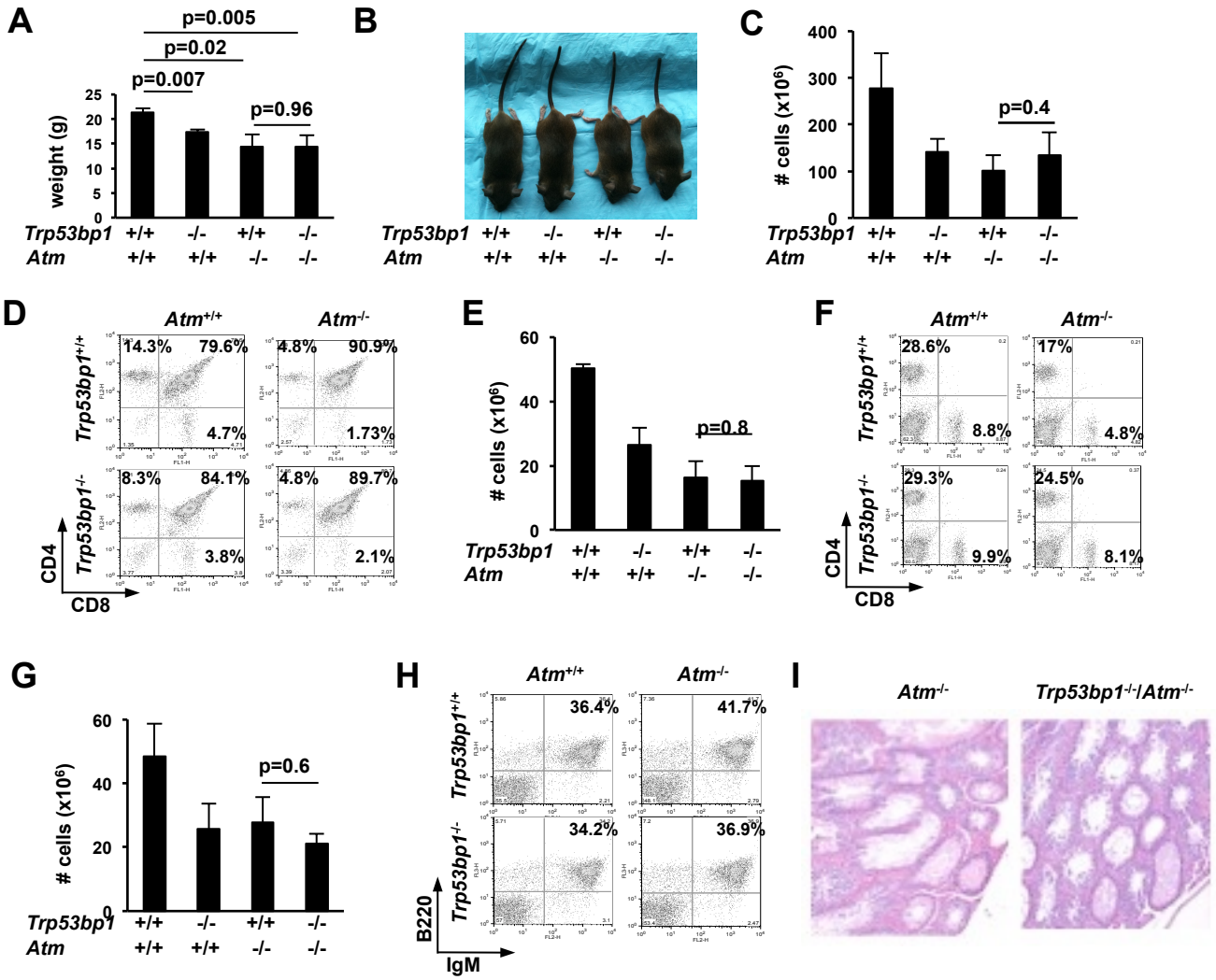

A
p=0.005
p=0.02
p=0.007
p=0.96
weight (g)
Trp53bp1 +/+ -/- +/+ -/-
Atm +/+ +/+ -/- -/-
B
Trp53bp1 +/+ -/- +/+ -/-
Atm +/+ +/+ -/- -/-
C
# cells (x10^6)
p=0.4
Trp53bp1 +/+ -/- +/+ -/-
Atm +/+ +/+ -/- -/-
D
Atm+/+
Atm-/-
Trp53bp1+/+
Trp53bp1-/-
14.3%
79.6%
4.7%
4.8%
90.9%
1.73%
8.3%
84.1%
3.8%
4.8%
89.7%
2.1%
CD4
CD8
E
# cells (x10^6)
p=0.8
Trp53bp1 +/+ -/- +/+ -/-
Atm +/+ +/+ -/- -/-
F
Atm+/+
Atm-/-
Trp53bp1+/+
Trp53bp1-/-
28.6%
8.8%
17%
4.8%
29.3%
9.9%
24.5%
8.1%
CD4
CD8
G
# cells (x10^6)
p=0.6
Trp53bp1 +/+ -/- +/+ -/-
Atm +/+ +/+ -/- -/-
H
Atm+/+
Atm-/-
Trp53bp1+/+
Trp53bp1-/-
36.4%
41.7%
34.2%
36.9%
B220
IgM
I
Atm-/-
Trp53bp1-/-/Atm-/-

Figure S1

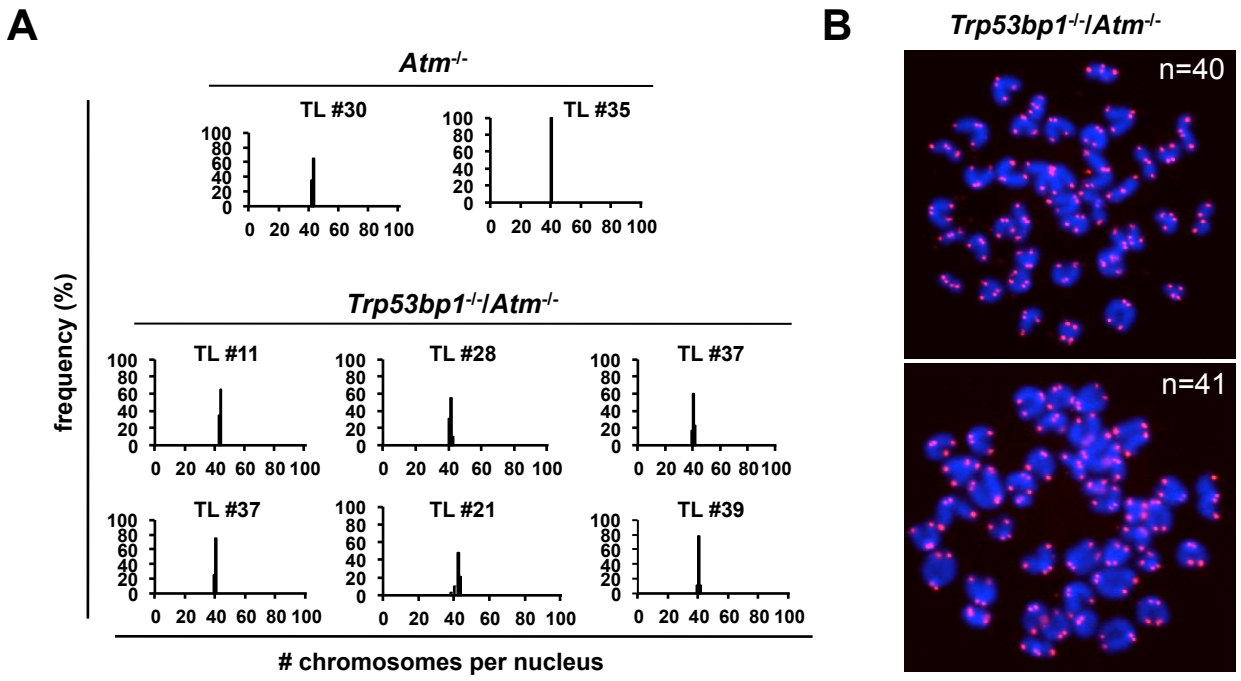

A
Atm-/-
TL #30
TL #35
Trp53bp1-/-/Atm-/-
TL #11
TL #28
TL #37
TL #37
TL #21
TL #39
frequency (%)
chromosomes per nucleus
B
Trp53bp1-/-/Atm-/-
n=40
n=41

Figure S2

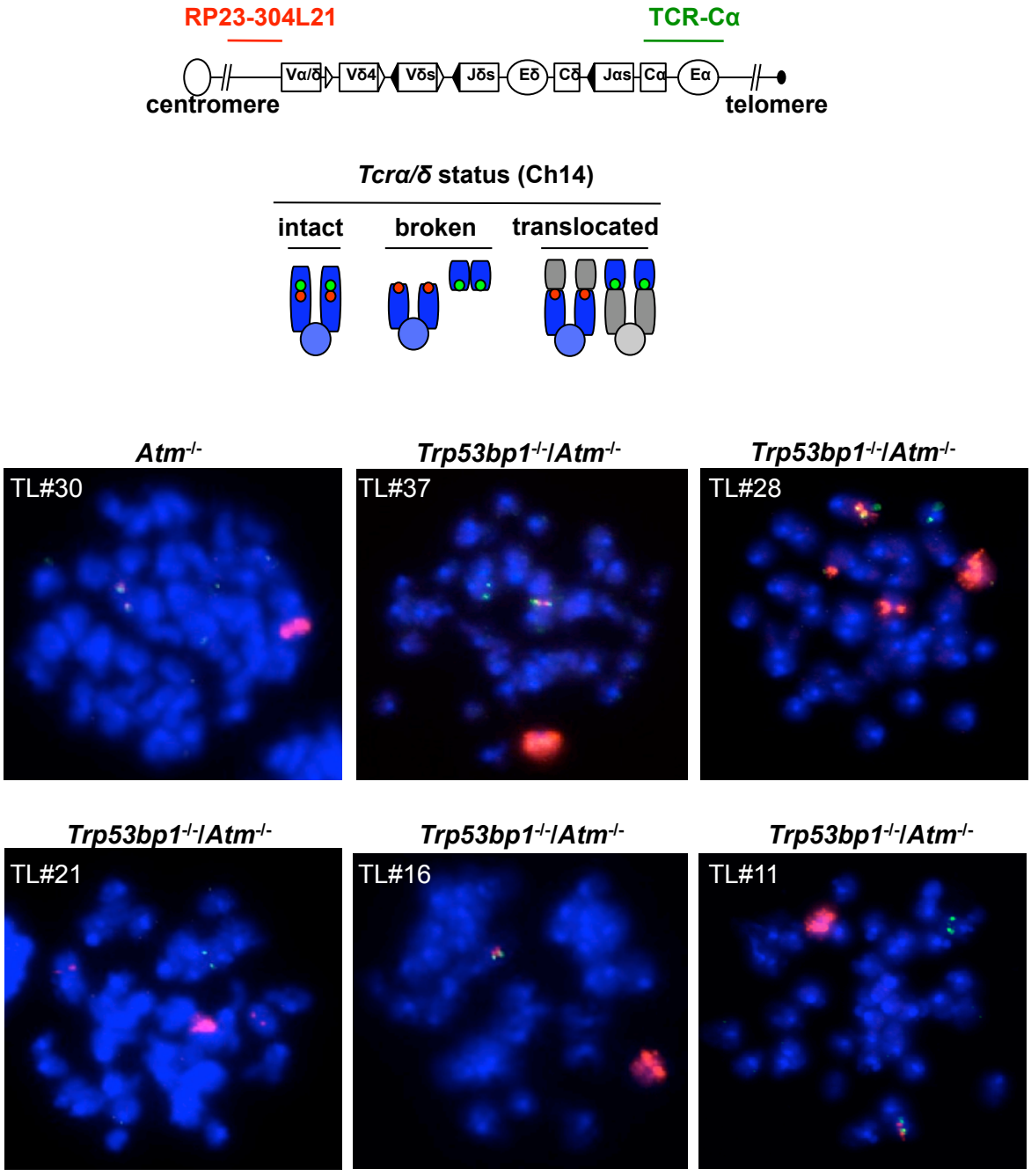
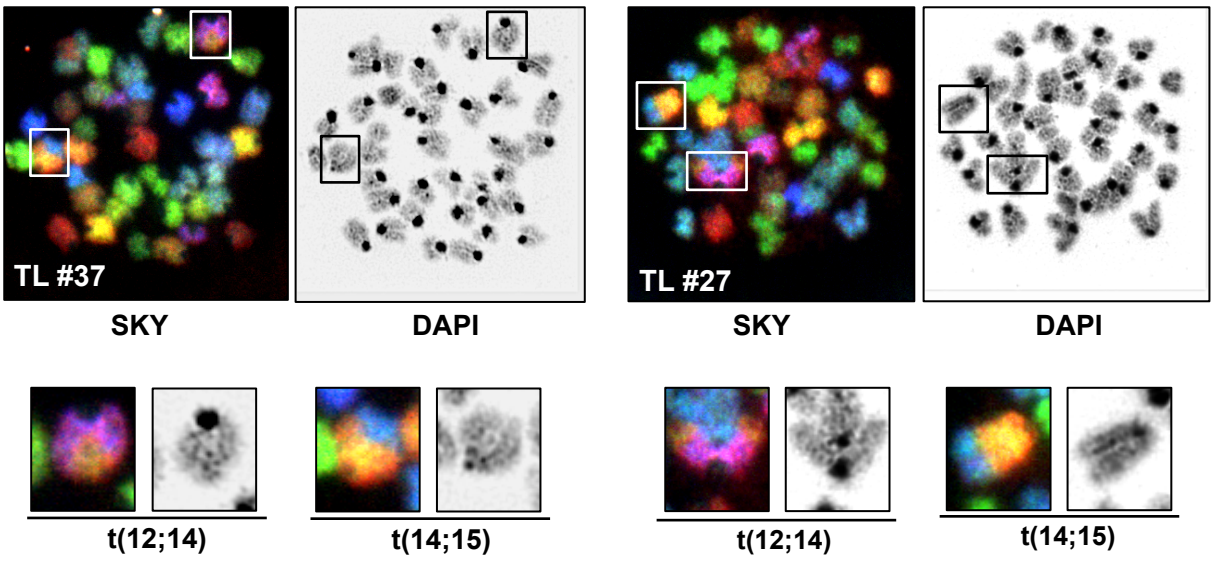

RP23-304L21
TCR-Cα
Vα/δ
Vδ4
Vδs
Jδs
Eδ
Cδ
Jαs
Cα
Eα
centromere
telomere
Tcrα/δ status (Ch14)
intact
broken
translocated
Atm-/-
Trp53bp1-/-/Atm-/-
Trp53bp1-/-/Atm-/-
TL#30
TL#37
TL#28
Trp53bp1-/-/Atm-/-
Trp53bp1-/-/Atm-/-
Trp53bp1-/-/Atm-/-
TL#21
TL#16
TL#11
RP23-304L21
Cα

Figure S3

A



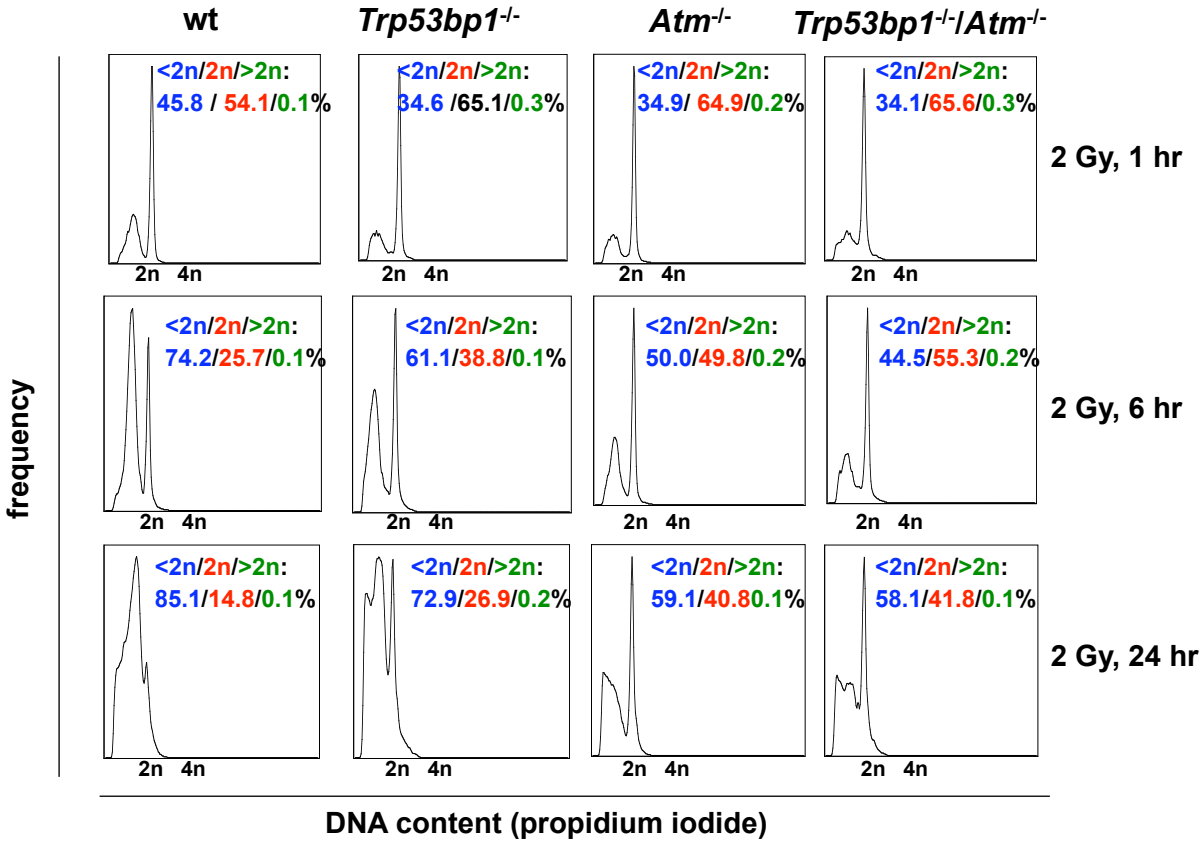
B

Karyotype of *Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$ lymphomas

Tumor ID	# metaphases	clonal translocations (# metaphases)
TL #37	7	t(12;14)$^{(7)}$; t(14;15)$^{(7)}$
TL #27	7	t(12;14)$^{(7)}$; t(14;15)$^{(7)}$

Figure S4

experiment#1



experiment#2

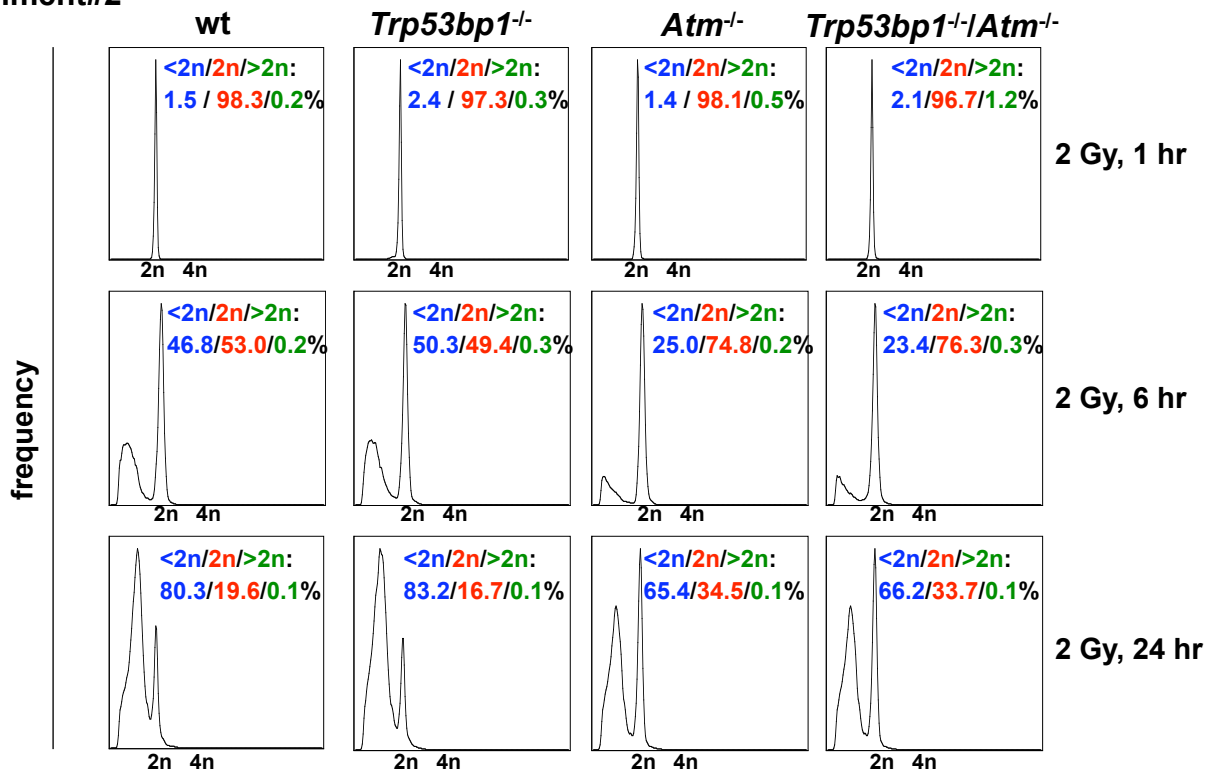


Figure S5

Vδ2–1-Dδ1/Dδ2-Jδ1
CTCAGGCACTTACCTCTGTGGAGGGAAAG----------GTGGCATAT----------------CTACCGACAAACTCGTCTTTGGACAAGGA
Dδ2 ATCGGAGGGATACGAG

wt (17)
CTCAGGCACTTACCTCTGTGGAGGGAAA-----C-----ATCGGAGGGATACGAG----CTC-----CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG--CTTACC----CGGAGGGATACGAG---C-------ACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGG------G--AT---ATCGGAGGG----------GGCGG-CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA----GCGAGG----CGGAGGGAT--------GG----CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG—TGGCATATCCCCAAATATCGGAGGGATACGAG--CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG—GC-GGCAT—ATCGGAGGGATAC----------------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG--CTCC-----TCGGAGGGATACGAG---TT-------CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG--CGA-------CGGAGGGATACGAG--CTA-------CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG--G----------GGAGGG-------CCCTCTA-----CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA--------------TCGGAGGGATACGAG----------------ACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGG------GGC--CTC--CGGAGGGATACGAG----CTA-----CCGACAAACTCGTCCTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA-----T-------ATCGGAGGGATACG-----G-------ACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA-----G------------ATAT-CGGGGGGGG-CGGA-C-ACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA---GTGGCAT--ATCGGAGGGATACGAG---------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA----C----GTGGCATAT-GG-GGGATACGAG-CTTGG-TACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---T----------GAG------------------ACCGACAAACTCGTCTTTGGACAAGGA

Atm-/- (18)
CTCAGGCACTTACCTCTGTGG--------GAG--------ATCGGAGGGAT-------CG----CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG—AG-GG-------CGGAGGGATA-------T--------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG—TTTCG-GGC-CAT-ATCGGAGGGATACGAG-T------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA-------G-AT-G-ATCGGAGGGATA-G----------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---CC--------CGGAGGGATACGAG--------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA--------------------GGGATAC--------------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGG--------C--------TG---------------------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGG-------T--------ATAT-CGGAGGGATACGA—AGGG----CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAA----A---------TCGGAGGGATACGAG-------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA-----G---GC-----CGGAGGGATACGAG-------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA------------CAT-CGGAGG--------------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---TTT--GG--A----AGGG-----CCCAG----CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAA---------------CGGAGGGATAC--------------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA----------------------GAT----------------GACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---CTCC--GG---G---GGGATACGAG-------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---GGA--CATAT-CGGAGGGATA---AG------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---AGG--GGC---CGGAGGGATA---GAGA--------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA----------------------GAT----------------GACAAACTCGTCTTTGGACAAGGA

Trp53BP1-/- (8)
CTCAGGCACTTACCTCTGTGGAGGGAAAG—GCA-A----ATCGGAGGGATACGAG---------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG--A---------CGGAGGGATACGAG---------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA----GTGGCATAT--CGGAGGGATACGAG----ACC----ACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG-AGGGAAAA---CGGAGGGATACGAG---CTGT-----CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAGCTTTCCCG-AT-ATCGGAGGGATACGAG--AA----TACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGG---CAT--------ATCGGAGGGATACGAG---------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAA-C-GTGGCATAT---AGGGGATACGAG---------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTG-------GGGGGAA--GTG------GGGATACGAG---------CTACCGACAAACTCGTCTTTGGACAAGGA

Atm-/-, Trp53bp1-/- (18)
CTCAGGCACTTACCTCTGTGGAGGGAAAG----C--CA---CGGAGGGATACGAG-----AGGG---CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG-------------GGCATAT--CGGAGGGATA--CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG--------ATAT--CGGAGGGATACG-CCTCTCG-CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA--------------TCGGAGGGATACG----------CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAA—TTGGGGAGG--GTGGCATAT—ATCGGAGGGATAC-----CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---A--GGC----CGGAGGGATACG---CCG----CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG---C--------TGGC---------------------CCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAA---AGAG-----ATCGGAGG-----------------ACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA---T---------ATCGGAGGGATACGA----------CAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTNCCTCTGTGGAGGGAAA----A----------AGGGATACG---C--------ANCGACAAACTCNNCTTNNNACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA----------TAT—CGGAGGGATACG----------ACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGA----G--------GTGG—T-ATCGGAGGGATA--AAGAAC----ACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGG-----CC--TAT--ATCGGAGGGATACGAG-----AA------CGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAA—GAACGGGG--GGCA--CCC---CGAG----GGT-------CGACAAACTCGTCTTTGGACAAGGA
CTCANGCACTTACCTCTGTGGAGGGAAAG—C-----TGGCATAT---CGGAGGGATACGAG---CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG—CCCCGCC----TAT--CGGAGGG---TTCCCG--CTACCGACAAACTCGTCTTTGGACAAGGA
CTCAGGCACTTACCTCTGTGGAGGGAAAG-----ATCG-ATTTGGCC--CATAT-ATCGGAGGGAT---GACAAACTCGTCTTTGGACAAGGA
GTGGGTTGATATAATGATGCTATCACTTGGGAAATATGGAGTAGGGGATTCACCATCT(-351)CTACCGACAAACTCGTCTTTGGACAAGGA

Fig. S6

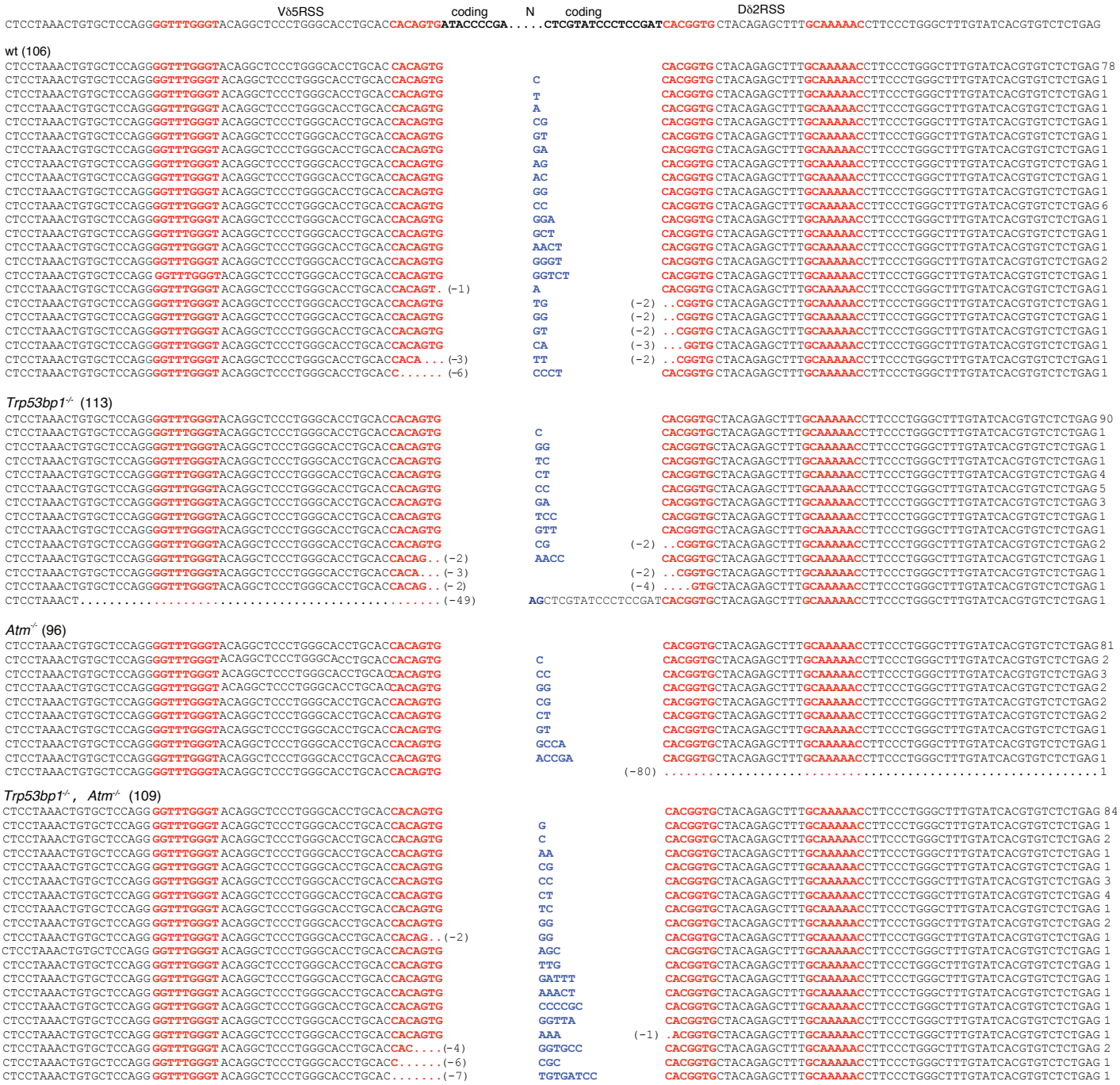

Vδ5RSS
coding
N
coding
Dδ2RSS
wt (106)
Trp53bp1-/- (113)
Atm-/- (96)
Trp53bp1-/-, Atm-/- (109)

Figure S7

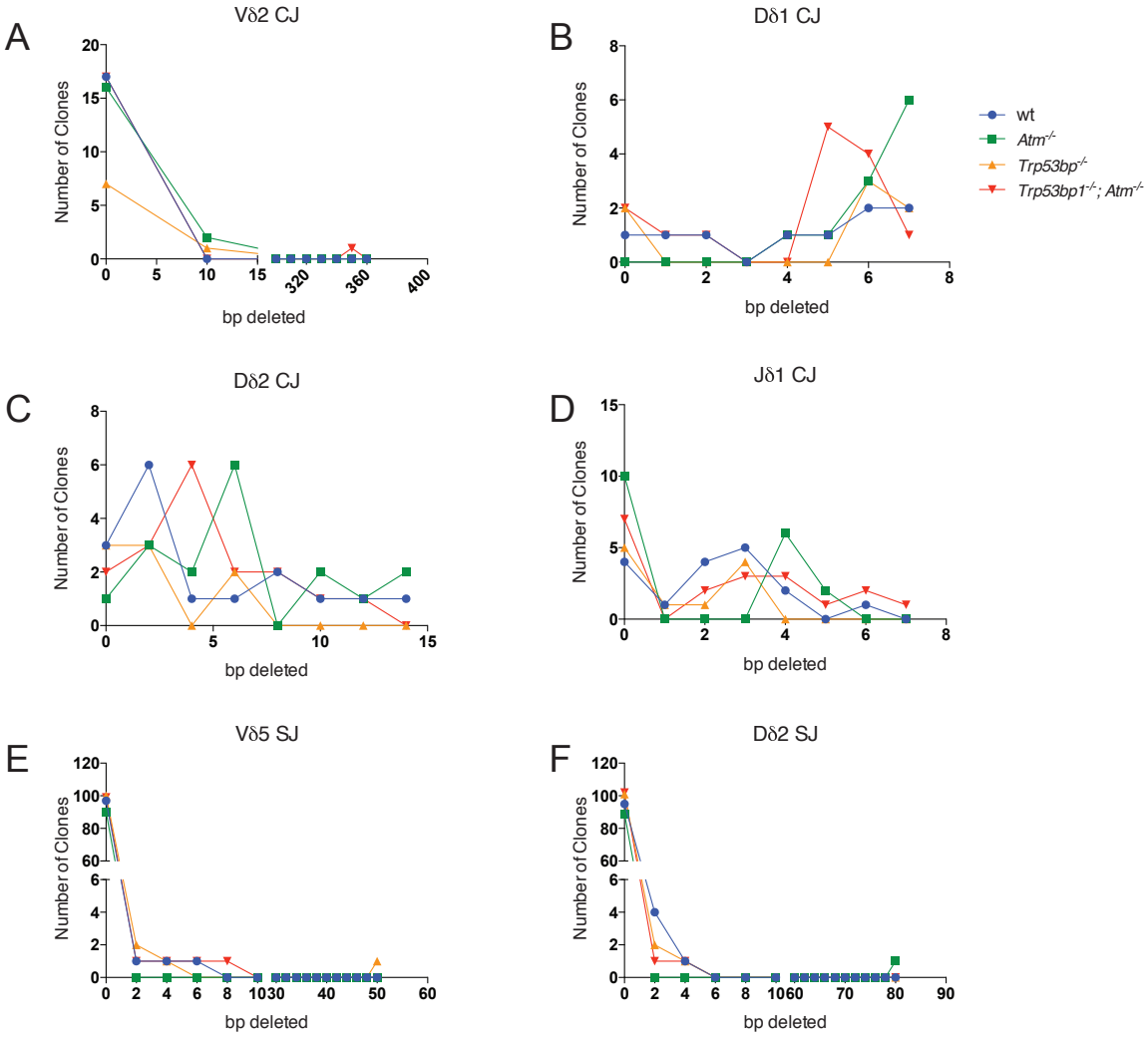

A
Vδ2 CJ
Number of Clones
bp deleted
B
Dδ1 CJ
Number of Clones
bp deleted
wt
Atm-/-
Trp53bp-/-
Trp53bp1-/-; Atm-/-
C
Dδ2 CJ
Number of Clones
bp deleted
D
Jδ1 CJ
Number of Clones
bp deleted
E
Vδ5 SJ
Number of Clones
bp deleted
F
Dδ2 SJ
Number of Clones
bp deleted

Figure S8

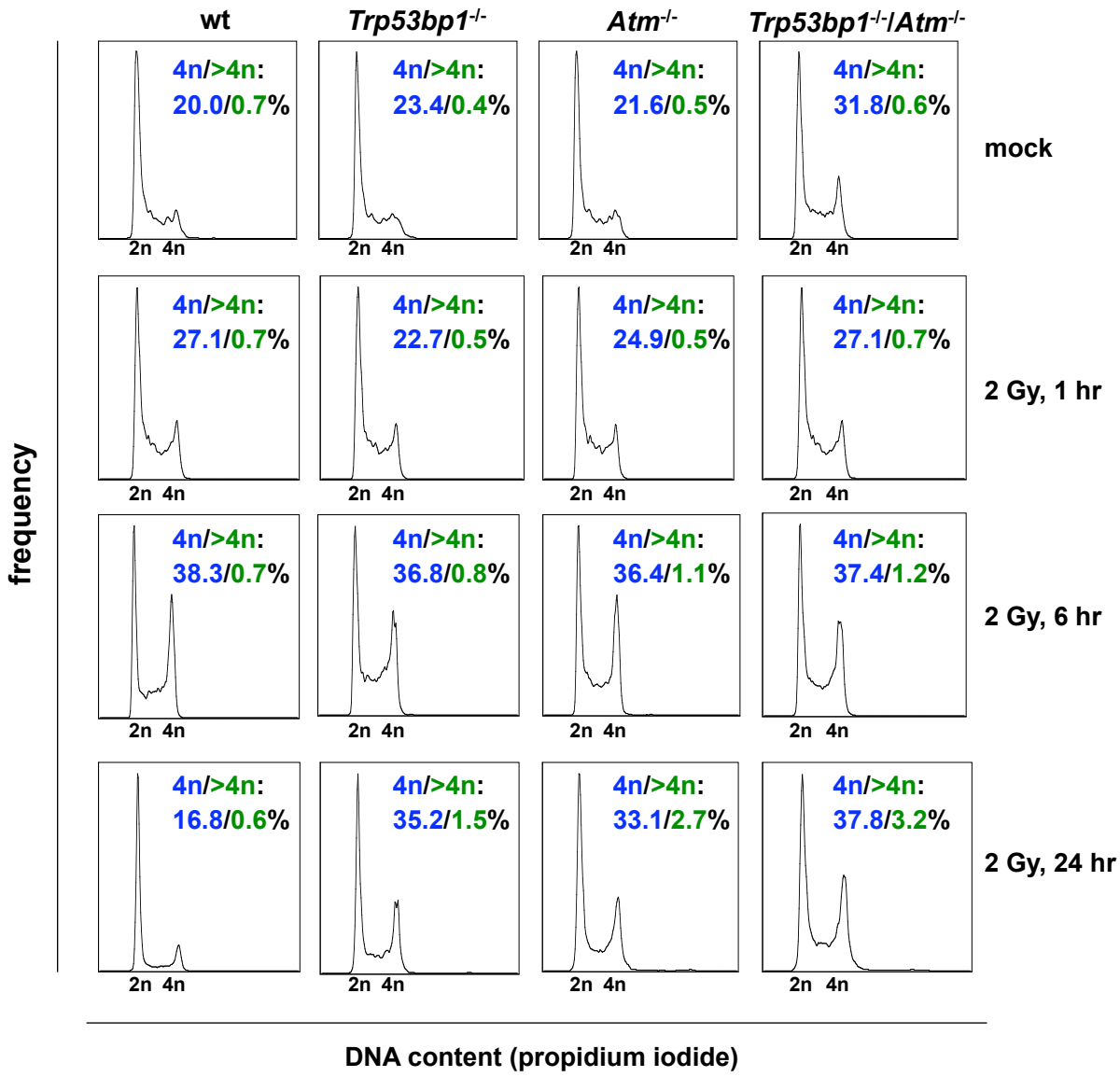

wt
Trp53bp1-/-
Atm-/-
Trp53bp1-/-/Atm-/-
4n/>4n:
20.0/0.7%
23.4/0.4%
21.6/0.5%
31.8/0.6%
mock
27.1/0.7%
22.7/0.5%
24.9/0.5%
27.1/0.7%
2 Gy, 1 hr
38.3/0.7%
36.8/0.8%
36.4/1.1%
37.4/1.2%
2 Gy, 6 hr
16.8/0.6%
35.2/1.5%
33.1/2.7%
37.8/3.2%
2 Gy, 24 hr
2n 4n
frequency
DNA content (propidium iodide)

Figure S9

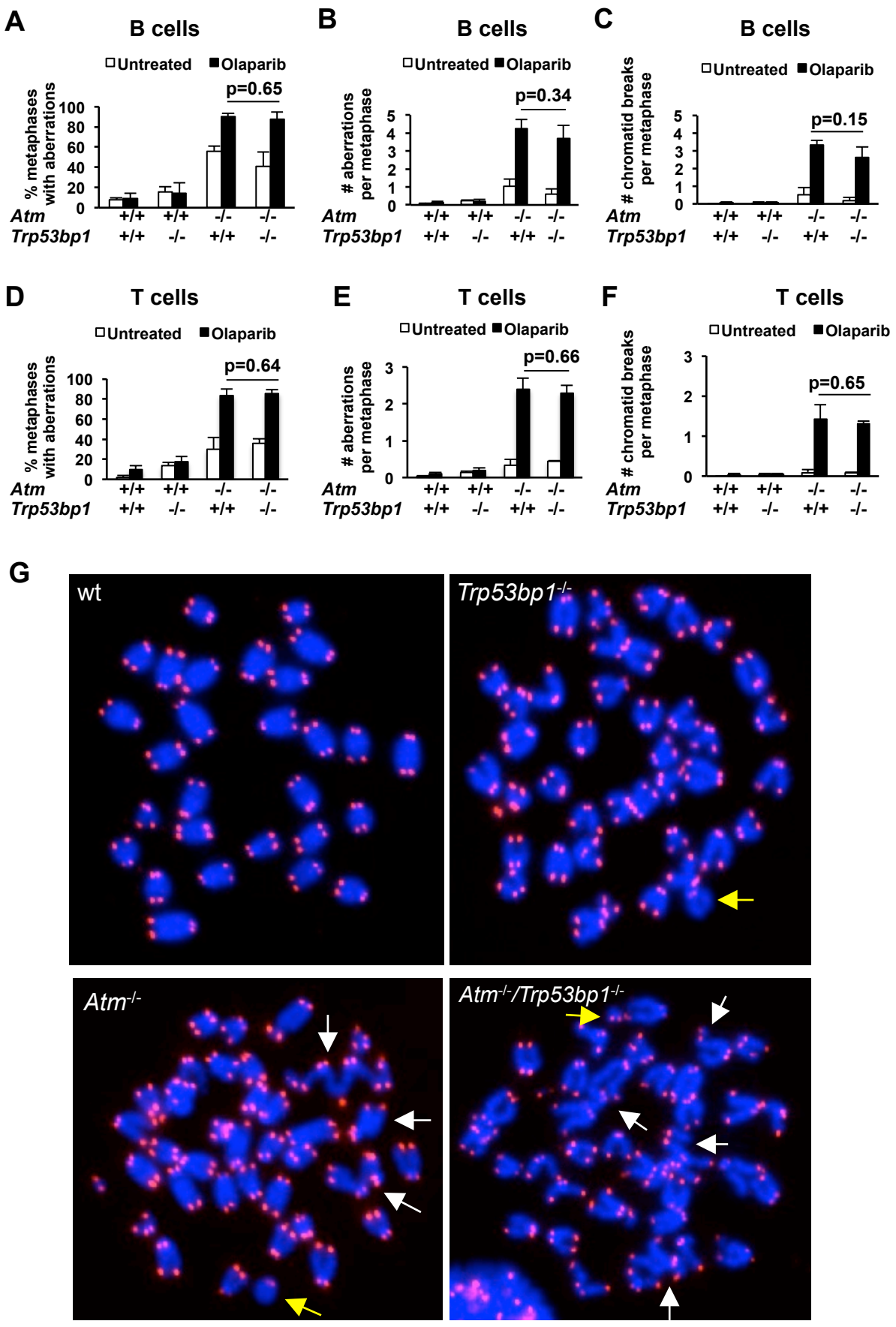

A
B cells
Untreated
Olaparib
p=0.65
% metaphases with aberrations
Atm
Trp53bp1
+/+ +/+ -/- -/-
+/+ -/- +/+ -/-
B
B cells
p=0.34
# aberrations per metaphase
C
B cells
p=0.15
# chromatid breaks per metaphase
D
T cells
p=0.64
E
T cells
p=0.66
F
T cells
p=0.65
G
wt
Trp53bp1-/-
Atm-/-
Atm-/-/Trp53bp1-/-
(TTAGGG)3
DAPI

Figure S10

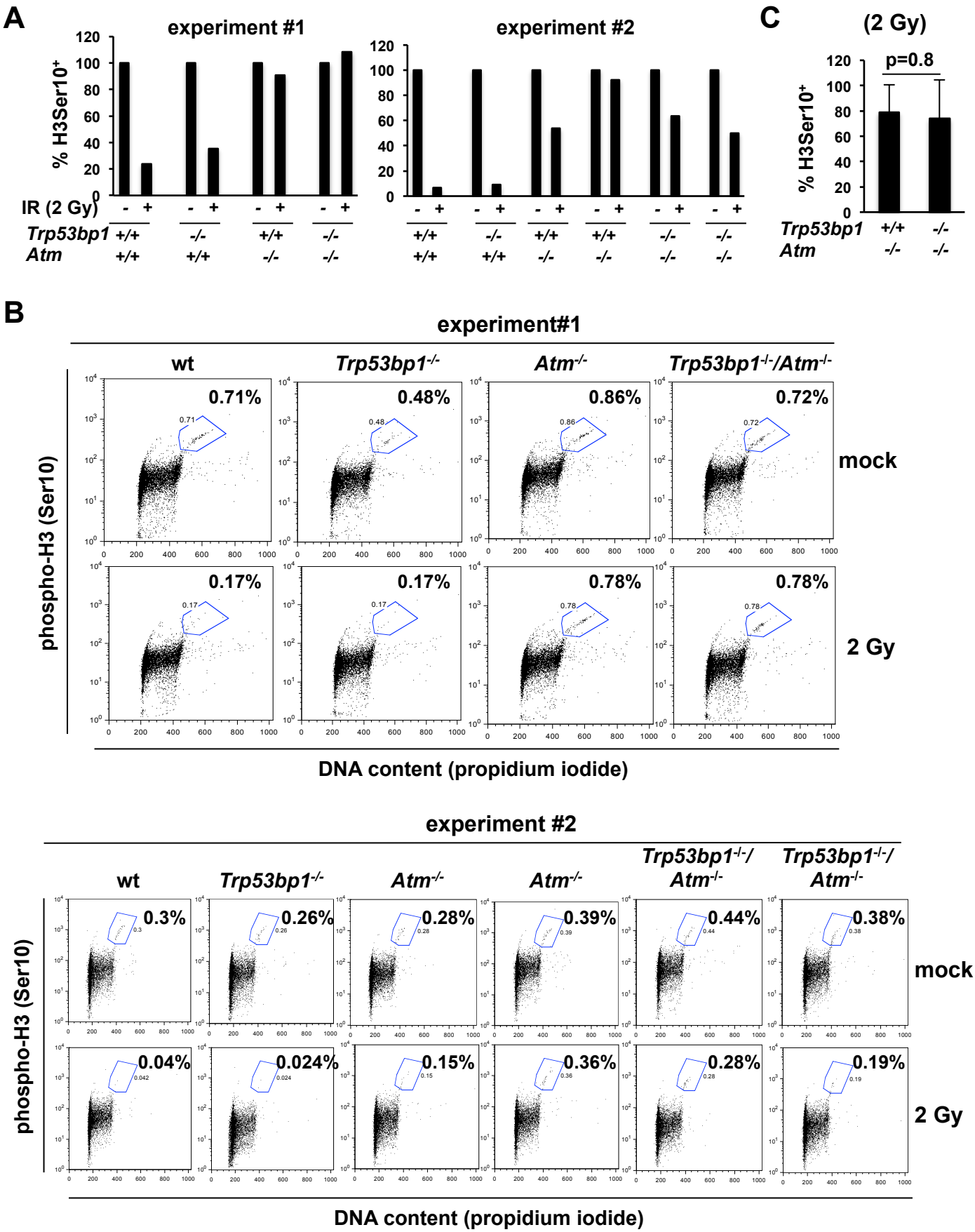

A
experiment #1
experiment #2
% H3Ser10+
IR (2 Gy)
Trp53bp1
Atm
C
(2 Gy)
p=0.8
B
experiment#1
wt
Trp53bp1-/-
Atm-/-
Trp53bp1-/-/Atm-/-
0.71%
0.48%
0.86%
0.72%
0.17%
0.17%
0.78%
0.78%
mock
2 Gy
phospho-H3 (Ser10)
DNA content (propidium iodide)
experiment #2
Trp53bp1-/-/ Atm-/-
0.3%
0.26%
0.28%
0.39%
0.44%
0.38%
0.04%
0.024%
0.15%
0.36%
0.28%
0.19%

Figure S11

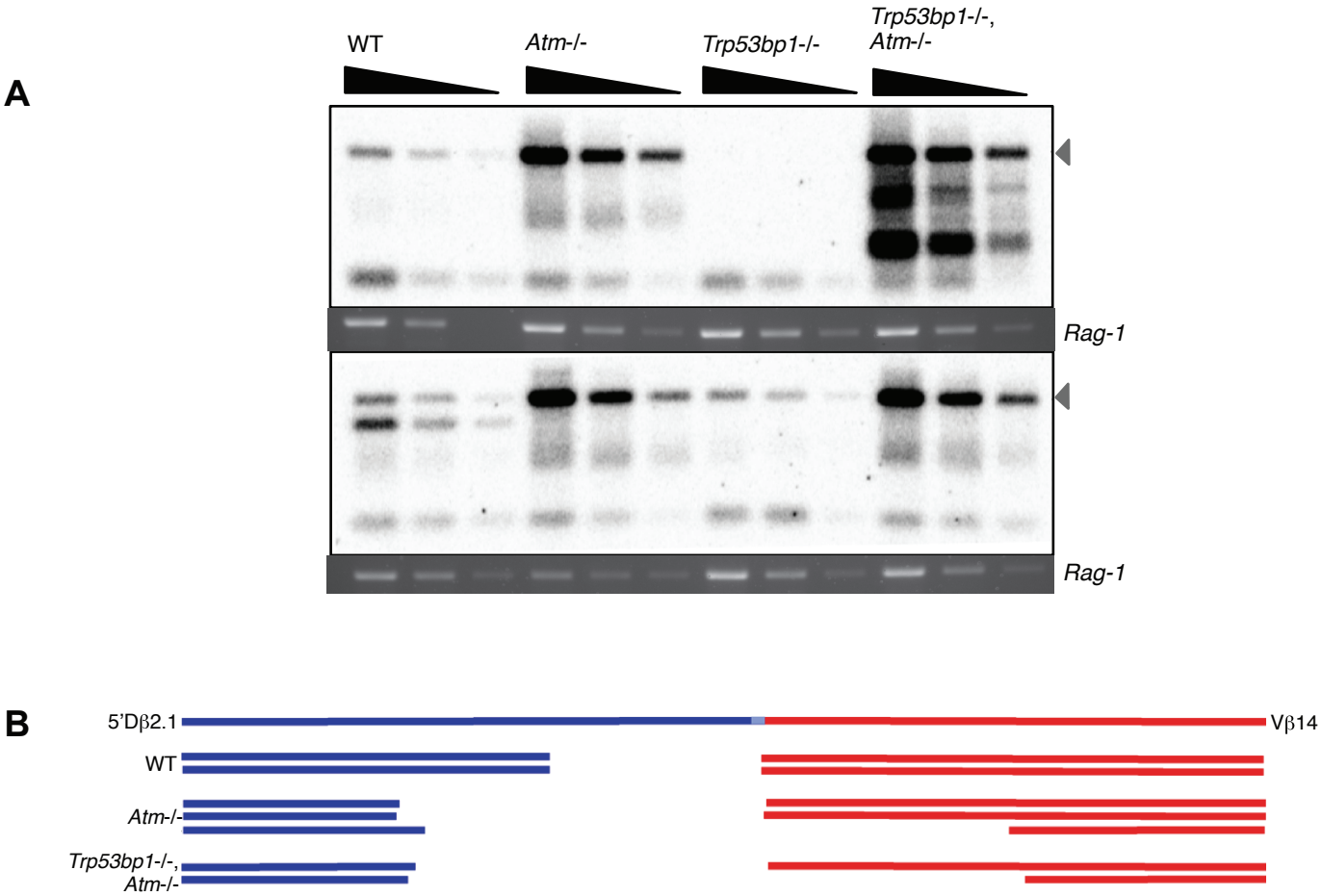

A
WT
Atm-/-
Trp53bp1-/-
Trp53bp1-/-,
Atm-/-
Rag-1
Rag-1
B
5'Dβ2.1
Vβ14
WT
Atm-/-
Trp53bp1-/-,
Atm-/-
C
5'Dβ2.1 CCTCCCACCCATTTCTCTATAAATGCCAGTCTAAGCAGG (-289) GT AGACTCCAGGCACAGAGGTAGAAGCCAGAGTGGCTGA Vβ14
WT CCTCCCACCCATTTCTCTATAAATGCCAGTCTAAGCAGGTGG (-286) CCCT AGACTCCAGGCACAGAGGTAGAAGCCAGAGTGGCTGA
Atm-/-
CTGGCAACCAGCTCAGAAGCTAACACATAGAGCCTAATGCA (-484) CCCTTC..ACTCCAGGCACAGAGGTAGAAGCCAGAGTGGCTGA
TTCCAGCTCAGAAGCTAACACATAGAGCCTAATGCATAGCC (-479) GGCGC..... CCAGGCACAGAGGTAGAAGCCAGAGTGGCTGA
GGCCCACTGCATACTACGGACTAAGTAAAAGAGAAAGCTGA (-442) TTCC (-324) CACACACCTCTACACATACACACACACCAGCTT
GGCCCACTGCATACTACGGACTAAGTAAAAGAGAAAGCTGA (-442) TCC (-324) CACACACCTCTACACATACACACACACCAGCTT
AGCCCACTGCATACTACGGACTAAGTAAAAGAGAAAGCTGA (-442) TTCT (-324) CACACACCTCTACACATACACACACACCAGCTT
Trp53bp1-/-, ATAGAGCCTAATGCATAGCCCACTGCATACTACGGACTAAGTA (-456) GTCCCGGGG (-4) TCCAGGCACAGAGGTAGAAGCCAGAGTGGC
Atm-/- ATAGAGCCTAATGCATAGCCCACTGCATAC (-469) C (-348) CACCAGCTTGCTTGCCTTCCCTCTGAAGGGGTTAC

Figure S12

Table S1. Mendelian ratios in liveborn mice from $53BP1^{+/-}$ /$ATM^{+/-}$ intercrosses (n=19 litters)

Genotype		Observed (%)	Expected (%)	Ratio
$ATM^{+/+}$	$53BP1^{+/+}$	5 (4.9%)	6.4 (6.25%)	1/16
	$53BP1^{+/-}$	15 (14.7%)	12.8 (12.5%)	1/8
	$53BP1^{-/-}$	4 (3.9%)	6.4 (6.25%)	1/16
$ATM^{+/-}$	$53BP1^{+/+}$	7 (6.9%)	12.8 (12.5%)	1/8
	$53BP1^{+/-}$	30 (29.4%)	25.5 (25%)	1/4
	$53BP1^{-/-}$	13 (12.7%)	12.8 (12.5%)	1/8
$ATM^{-/-}$	$53BP1^{+/+}$	5 (4.9%)	6.4 (6.25%)	1/16
	$53BP1^{+/-}$	13 (12.7%)	12.8 (12.5%)	1/8
	$53BP1^{-/-}$	10 (9.8%)	6.4 (6.25%)	1/16
	Total	**102**		

Table S2. Analysis of genomic stability in HU-treated B lymphocytes deficient for 53BP1 and/or ATM. $CD43^-$ splenocytes were activated with α-CD40+IL-4 and treated with HU for 16 hours prior to fixation. Metaphase spreads were stained with a telomere probe prior to quantification of aberrations

Mouse ID	Genotype	HU dose (mM)	# metaphases analyzed	# metaphases with aberrations (%)	total # aberrations (aberrations per metaphase)	types of aberrations[a]
Stimulation#1						
M#1	wt	-	30	0 (0.0%)	0 (0.0)	-
M#2	*Atm*$^{-/-}$	-	30	19 (63.3%)	36 (1.2)	19 CB, 17 cb
M#1	wt	0.25 mM	30	5 (16.7%)	6 (0.2)	5 CB, 1 cb
M#2	*Atm*$^{-/-}$	0.25 mM	30	21 (70.0%)	76 (2.5)	24 CB, 52 cb
Stimulation#2						
M#32	wt	-	30	0 (0.0%)	0 (0.0)	-
M#28	*Trp53bp1*$^{-/-}$	-	30	6 (20.0%)	6 (0.2)	6 CB
M#30	*Atm*$^{-/-}$	-	30	16 (53.3 %)	20 (0.7)	12 CB, 7 cb, 1 CR
M#34	*Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$	-	30	21 (70.0%)	43 (1.4)	27 CB, 16 cb
M#32	wt	0.25 mM	30	10 (33.3%)	14 (0.5)	10 CB, 4 cb
M#28	*Trp53bp1*$^{-/-}$	0.25 mM	30	17 (56.7%)	29 (1.0)	15 CB, 13 cb, 1 CR
M#30	*Atm*$^{-/-}$	0.25 mM	30	19 (63.3%)	49 (1.6)	20 CB, 29 cb
M#34	*Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$	0.25 mM	30	18 (60.0%)	42 (1.4)	15 CB, 27 cb
Stimulation#3						
M#1	wt	-	30	2 (6.7%)	2 (0.0)	1 CB, 1 CR
M#52	*Trp53bp1*$^{-/-}$	-	30	4 (13.3%)	4 (0.1)	3 CB, 1 cb
M#54	*Atm*$^{-/-}$	-	30	16 (53.3%)	21 (0.7)	17 CB, 4 cb
M#37	*Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$	-	30	13 (43.3%)	20 (0.7)	12 CB, 8 cb
M#1	wt	0.25 mM	30	8 (26.7%)	13 (0.4)	6 CB, 7 cb
M#52	*Trp53bp1*$^{-/-}$	0.25 mM	30	12 (40.0%)	17 (0.6)	12 CB, 5 cb
M#54	*Atm*$^{-/-}$	0.25 mM	30	17 (56.7%)	27 (0.9)	15 CB, 12 cb
M#37	*Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$	0.25 mM	30	22 (73.3%)	61 (2.0)	18 CB, 43 cb
Stimulation#4						
M#1	wt	-	30	0 (0.0%)	0 (0.0)	-
M#83	*Trp53bp1*$^{-/-}$	-	30	5 (16.7%)	5 (0.2)	4 CB, 1 cb
M#82	*Atm*$^{-/-}$	-	30	10 (33.3%)	11 (0.4)	6 CB, 2 cb, 3 CR
M#72	*Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$	-	30	15 (50.0%)	24 (0.8)	19 CB, 4 cb, 1 CR
M#1	wt	0.25 mM	30	4 (13.3%)	5 (0.2)	1 CB, 1 cb
M#83	*Trp53bp1*$^{-/-}$	0.25 mM	30	5 (16.7%)	5 (0.2)	4 CB, 1 cb
M#82	*Atm*$^{-/-}$	0.25 mM	30	19 (63.3%)	67 (2.2)	10 CB, 57 cb
M#72	*Trp53bp1*$^{-/-}$/*Atm*$^{-/-}$	0.25 mM	30	19 (63.3%)	45 (1.5)	16 CB, 29 cb
Summary (n=4 experiments)						
	wt	-	90	2 (2.2%)	6 (0.1)	3 CT, 2 ACT, 1 cb
	Trp53bp1$^{-/-}$	-	90	15 (16.7%)	17 (0.2)	10 CT, 5 ACT, 2 cb
	Atm$^{-/-}$	-	90	42 (46.7%)	62 (0.7)	33 CT, 18 ACT, 11 cb
	Trp53bp1$^{-/-}$/*Atm*$^{-/-}$	-	90	49 (54.4%)	95 (1.1)	43 CT, 24 ACT, 28 cb
	wt	0.25 mM	120	27 (22.5%)	38 (0.32)	22 CB, 16 cb
	Trp53bp1$^{-/-}$	0.25 mM	90	34 (37.8%)	52 (0.58)	33 CB, 19 cb
	Atm$^{-/-}$	0.25 mM	120	76 (63.3%)	219 (1.83)	69 CB, 150 cb
	Trp53bp1$^{-/-}$/*Atm*$^{-/-}$	0.25 mM	90	59 (65.6%)	148 (1.64)	49 CB, 99 cb

[a]CB, chromosome break; cb, chromatid break; CR, chromosomal rearrangement

Table S3. Analysis of genomic stability in α-CD40+IL-4activated B lymphocytes deficient for 53BP1 and/or ATM. Metaphases were obtained 24 hr after IR and stained with a telomere probe. For data on indivual experiments, see Table S .

Genotype	IR dose (Gy)	# mice	# metaphases	% metaphases with aberrations	total # aberrations (aberrations per metaphase)	p value (# aberrations per metaphase)
wt	mock	6	180	2.8 ± 3.3	0.0±0.0	
Trp53bp1$^{-/-}$	mock	6	180	16.1 ± 3.3	0.2±0.1	p= 0.004 (vs wt)
Atm$^{-/-}$	mock	6	180	45 ± 10.7	0.8±0.3	p=0.02 (vs wt)
Trp53bp1$^{-/-}$Atm$^{-/-}$	mock	6	180	46.1 ± 14.4	0.9±0.4	p=0.0019 (vs wt); p=0.005 (vs *Trp53bp1*$^{-/-}$); p=0.74 (vs *Atm*$^{-/-}$)
wt	2 Gy	6	180	39.4 ± 8.5	0.9±0.2	
Trp53bp1$^{-/-}$	2 Gy	6	180	75.6 ± 8.6	3.6±1.5	p= 0.006 (vs wt)
Atm$^{-/-}$	2 Gy	6	172	93.3 ± 6.3	4.7±1.1	p=0.0002 (vs wt)
Trp53bp1$^{-/-}$Atm$^{-/-}$	2 Gy	6	180	96.1 ± 3.8	6.4±1.8	p=0.0006 (vs wt); p=0.01 (vs *Trp53bp1*$^{-/-}$); p=0.08 (vs *Atm*$^{-/-}$)

Table S4. Analysis of genomic stability in α-CD40/IL-4 activated B cells deficient for 53BP1 and/or ATM. Metaphase spreads were obtained 24 hr after IR and stained with a telomere probe.

Mouse ID	Genotype	IR dose (Gy)	# metaphases analyzed	# metaphases with aberrations (%)	total # aberrations (aberrations per metaphase)	types of aberrations[a]
Stimulation#1						
M#32	wt	mock	30	0 (0.0%)	0 (0.0)	-
M#28	*Trp53bp1*$^{-/-}$	mock	30	6 (20.0%)	8 (0.3)	6 CT, 2 ACT
M#30	*Atm*$^{-/-}$	mock	30	16 (53.3%)	21 (0.7)	11 CT, 5 ACT, 5 cb
M#34	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	21 (70.0%)	44 (1.5)	16 CT, 12 ACT, 16 cb
M#32	wt	2 Gy	30	15 (50%)	24 (0.8)	15 CT, 9 ACT
M#28	*Trp53bp1*$^{-/-}$	2 Gy	30	23 (76.7%)	73 (2.4)	40 CT, 27 ACT, 6 cb
M#30	*Atm*$^{-/-}$	2 Gy	22	22 (100%)	117 (5.3)	62 CT, 43 ACT, 12 cb
M#34	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	30 (100%)	1890 (6.0)	76 CT, 67 ACT, 37 cb
Stimulation#2						
M#1	wt	mock	30	2 (6.7%)	6 (0.2)	3 CT, 2 ACT, 1 cb
M#52	*Trp53bp1*$^{-/-}$	mock	30	4 (13.3%)	4 (0.1)	1 CT, 2 ACT, 1 cb
M#54	*Atm*$^{-/-}$	mock	30	16 (53.3%)	24 (0.8)	15 CT, 5 ACT, 4 cb
M#37	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	13 (43.3%)	23 (0.8)	9 CT, 6 ACT, 8 cb
M#1	wt	2 Gy	30	11 (36.7%)	32 (1.1)	11 CT, 17 ACT, 4 cb
M#52	*Trp53bp1*$^{-/-}$	2 Gy	30	25 (83.3%)	188 (6.3)	92 CT, 68 ACT, 28 cb
M#54	*Atm*$^{-/-}$	2 Gy	30	27 (90.0%)	126 (4.2)	64 CT, 41 ACT, 21 cb
M#37	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	27 (90.0%)	159 (5.3)	72 CT, 53 ACT, 34 cb
Stimulation#3						
M#1	wt	mock	30	0 (0.0%)	0 (0.0)	-
M#83	*Trp53bp1*$^{-/-}$	mock	30	5 (16.7%)	5 (0.2)	3 CT, 1 ACT, 1 cb
M#82	*Atm*$^{-/-}$	mock	30	10 (33.3%)	17 (0.6)	7 CT, 8 ACT, 2 cb
M#72	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	15 (50.0%)	28 (0.9)	18 CT, 6 ACT, 4 cb
M#1	wt	2 Gy	30	10 (33.3%)	15 (0.5)	11 CT, 3 ACT, 1 cb
M#83	*Trp53bp1*$^{-/-}$	2 Gy	30	25 (83.3%)	86 (2.9)	52 CT, 30 ACT, 4 cb
M#82	*Atm*$^{-/-}$	2 Gy	30	28 (93.3%)	94 (3.1)	48 CT, 28 ACT, 18 cb
M#72	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	29 (96.7%)	108 (3.6)	52 CT, 44 ACT, 12 cb
Stimulation#4						
M#3	wt	mock	30	0 (0.0%)	0 (0.0)	-
M#7	*Trp53bp1*$^{-/-}$	mock	30	4 (13.3%)	5 (0.2)	2 CT, 2 ACT, 1 cb
M#8	*Atm*$^{-/-}$	mock	30	14 (46.7%)	37 (1.2)	18 CT, 8 ACT, 11 cb
M#5	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	10 (33.3%)	14 (0.5)	6 CT, 4 ACT, 4 cb
M#3	wt	2 Gy	30	11 (36.7%)	34 (1.1)	17 CT, 9 ACT, 8 cb
M#7	*Trp53bp1*$^{-/-}$	2 Gy	30	19 (63.3%)	84 (2.8)	49 CT, 32 ACT, 3 cb
M#8	*Atm*$^{-/-}$	2 Gy	30	30 (100%)	188 (6.3)	87 CT, 79 ACT, 22 cb
M#5	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	29 (96.7%)	254 (8.5)	136 CT, 92 ACT, 26 cb
Stimulation#5						
M#20	wt	mock	30	1 (3.3%)	1 (0.03)	1 CT
M#10	*Trp53bp1*$^{-/-}$	mock	30	4 (13.3%)	8 (0.3)	6 CT, 2 ACT
M#24	*Atm*$^{-/-}$	mock	30	16 (53.3%)	32 (1.1)	15 CT, 6 ACT, 11 cb
M#27	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	15 (50.0%)	31 (1.0)	22 CT, 7 ACT, 2 cb
M#20	wt	2 Gy	30	15 (50.0%)	30 (1.0)	19 CT, 11 ACT
M#10	*Trp53bp1*$^{-/-}$	2 Gy	30	24 (80.0%)	134 (4.5)	78 CT, 51 ACT, 5 cb
M#24	*Atm*$^{-/-}$	2 Gy	30	25 (83.3%)	145 (4.8)	84 CT, 58 ACT, 3 cb
M#27	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	28 (93.3%)	241 (8.0)	131 CT, 86 ACT, 24 cb
Stimulation #6						
M#1	wt	mock	30	2 (6.7%)	2 (0.06)	2 CT
M#44	*Trp53bp1*$^{-/-}$	mock	30	6 (20.0%)	11 (0.4)	7 CT, 4 ACT
M#30	*Atm*$^{-/-}$	mock	30	9 (30.0%)	16 (0.5)	12 CT, 2 ACT, 2 cb
M#39	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	9 (30.0%)	18 (0.6)	10 CT, 5 ACT, 3 cb
M#1	wt	2 Gy	30	9 (30.0%)	27 (0.9)	10 CT, 9 ACT, 8 cb
M#44	*Trp53bp1*$^{-/-}$	2 Gy	30	20 (66.7%)	83 (2.8)	36 CT, 32 ACT, 15 cb
M#30	*Atm*$^{-/-}$	2 Gy	30	28 (93.3%)	138 (4.6)	74 CT, 61 ACT, 3 cb
M#39	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	30 (100.0%)	205 (6.8)	104 CT, 98ACT, 3 cb

[a]CT, centric; ACT, acentric; cb, chromatid break

Table S5. Analysis of genomic stability in LPS-activated B cells deficient for 53BP1 and/or ATM. Metaphases were obtained 24 hr after exposure to IR and stained with a telomere probe

Mouse ID	Genotype	IR dose (Gy)	# metaphases analyzed	# metaphases with aberrations (%)	total # aberrations (aberrations per metaphase)	types of aberrations[a]
Stimulation #1						
M#3	wt	mock	30	0 (0.0%)	0 (0.0)	-
M#7	*Trp53bp1*$^{-/-}$	mock	30	3 (10%)	3 (0.1)	2CT, 1 ACT
M#8	*Atm*$^{-/-}$	mock	30	13 (43.3%)	23 (0.8)	13 CT, 7 ACT, 3 cb
M#5	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	14 (46.7%)	23 (0.8)	11 CT, 3 ACT, 9 cb
M#3	wt	2 Gy	30	14 (46.7%)	38 (1.3)	18 CT, 19 ACT, 1 cb
M#7	*Trp53bp1*$^{-/-}$	2 Gy	30	18 (60.0%)	56 (1.9)	22 CT, 34 ACT
M#8	*Atm*$^{-/-}$	2 Gy	30	27 (90%)	100 (3.3)	54 CT, 42 ACT, 4 cb
M#5	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	30 (100%)	129 (4.3)	69 CT, 49 ACT, 11 cb
Stimulation #2						
M#20	wt	mock	30	1 (3.3%)	1 (0.03)	1 CT
M#10	*Trp53bp1*$^{-/-}$	mock	30	2 (6.7%)	3 (0.1)	2 CT, 1 ACT
M#24	*Atm*$^{-/-}$	mock	30	12 (40.0%)	17 (0.6)	12 CT, 4 ACT, 1 cb
M#27	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	14 (46.7%)	24 (0.8)	17 CT, 4 ACT, 3 cb
M#20	wt	2 Gy	30	12 (40.0%)	29 (1.0)	21 CT, 8 ACT
M#10	*Trp53bp1*$^{-/-}$	2 Gy	30	19 (63.3%)	110 (3.7)	59 CT, 38 ACT, 13 cb
M#24	*Atm*$^{-/-}$	2 Gy	30	27 (90.0%)	110 (3.7)	58 CT, 42 ACT, 10 cb
M#27	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	24 (80%)	118 (3.9)	8 CT, 41 ACT, 9 cb
Stimulation #3						
M#1	wt	mock	30	3 (10%)	3 (0.1)	3 CT
M#44	*Trp53bp1*$^{-/-}$	mock	30	4 (13.3%)	8 (0.3)	7 CT,1 ACT
M#30	*Atm*$^{-/-}$	mock	30	11 (36.7%)	16 (0.5)	10CT, 6 ACT
M#39	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	mock	30	14 (46.7%)	18 (0.6)	12 CT, 6 ACT
M#1	wt	2 Gy	30	13 (43.3%)	15 (0.5)	15 CT
M#44	*Trp53bp1*$^{-/-}$	2 Gy	30	14 (46.7%)	41 (1.4)	22 CT, 19 ACT
M#30	*Atm*$^{-/-}$	2 Gy	30	28 (93.3%)	122 (4.1)	71 CT, 46 ACT, 5 cb
M#39	*Trp53bp1*$^{-/-}$Atm$^{-/-}$	2 Gy	30	29 (96.7%)	145 (4.8)	87 CT, 57 ACT, 1 cb
Summary (n=3 experiments)						
	wt	mock	90	4 (4.4%)	4 (0.04)	4 CT
	Trp53bp1$^{-/-}$	mock	90	9 (10.0%)	14 (0.16)	11 CT, 3 ACT
	Atm$^{-/-}$	mock	90	36 (40%)	56 (0.6)	35 CT, 17 ACT, 4 cb
	Trp53bp1$^{-/-}$/*Atm*$^{-/-}$	mock	90	42 (46.7%)	65 (0.7)	40 CT, 13 ACT, 12 cb
	wt	2 Gy	90	39 (43.3%)	82 (0.91)	54 CT, 27 ACT, 1 cb
	Trp53bp1$^{-/-}$	2 Gy	90	51 (56.7%)	207 (2.3)	103 CT, 91 ACT, 13 cb
	Atm$^{-/-}$	2 Gy	90	82 (91.1%)	332 (3.7)	183 CT, 130 ACT, 19 cb
	Trp53bp1$^{-/-}$/*Atm*$^{-/-}$	2 Gy	90	83 (92.2%)	392 (4.4)	224 CT, 147 ACT, 21 cb

[a]CT, centric; ACT, acentric; cb, chromatid break

Table S6. Analysis of genomic stability in T lymphocytes deficient for 53BP1 and/or ATM. $CD43^+$ splenocytes were activated with concanavalin A and, 24 hours after irradiation, fixed and analyzed by telomere FISH

Genotype	**# mice**	**# metaphases analyzed**	**IR dose**	**# metaphases with aberrations (%)**	**total # aberrations (aberrations per metaphase)**	**types of aberrations[a]**
wt	3	90	mock	2 (2.2%)	2 (0.02)	2 CT
Trp53bp1$^{-/-}$	3	80	mock	5 (6.3%)	5 (0.06)	3 CT, 1 ACT, 1cb
Atm$^{-/-}$	3	90	mock	30 (33.3%)	52 (0.5)	28 CT, 18 ACT, 6 cb
Trp53bp1$^{-/-}$/ *Atm*$^{-/-}$	3	80	mock	23 (28.7%)	28 (0.3)	20 CT, 4 CT, 4 cb
wt	3	80	2 Gy	26 (32.5%)	36 (0.4)	28 CT, 8 ACT
Trp53bp1$^{-/-}$	3	90	2 Gy	46 (51.1%)	189 (2.1)	100 CT, 75 ACT, 14 cb
Atm$^{-/-}$	3	83	2 Gy	64 (77.1%)	257 (3.0)	123 CT, 107 ACT, 27 cb
Trp53bp1$^{-/-}$/ *Atm*$^{-/-}$	3	80	2 Gy	72 (90.0%)	277 (3.4)	146 CT, 84 ACT, 47 cb

[a]CT, centric, ACT, acentric, cb, chromatid break