SUPPLEMENTARY MATERIALS

Fig. S1. Increased MH usage in normalized Sμ-Sγ1 and Sμ-Sε CSR junctions from DSBR factors-deficient primary B cells

Libraries from three replicates of each genotype were selected and randomly normalized to the smallest number of Sμ-Sγ1 or Sμ-Sε junctions recovered among all libraries before calculating MH patterns. (**A and D**) MH usage from junctions with direct and up to 10 bp MH for 5'Sμ to Sγ1 (**A**) and to Sε (**D)** DSBs are plotted as percentage of total junctions. (**B-C and E-F**) Percentages of direct joins of 5'Sμ to Sγ1 (**B**) and Sε (**E**) and junctions with 4bp or longer MH in Sγ1 (**C**) or Sε (**F**) in different genetic backgrounds are compared. Unpaired two-tailed t-test was used to calculate *p* values for significant difference between samples (Quantitative data = average±s.e.m., * *p*≤ 0.05, ** *p*≤ 0.01, *** *p*≤ 0.001).

Fig. S2. Increased MH usage during S region DSB joining in H2AX- and Rif1-deficient activated primary B cells

    Primary spleen B cells from wild type, *H2AX$^{-/-}$* and *RIF1$^{flox/flox}$ CD19$^{Cre/+}$* were stimulated with αCD40/IL4 for 96 hours and assayed as described in Fig.1. Wild type, *H2AX$^{-/-}$* cells are in 129S background and *RIF1$^{flox/flox}$ CD19$^{Cre/+}$* cells in BL6 background that has very similar Sε sequences to that of 129S strains but different length for Sγ1 and thus only analyzed for Sε junctions. (**A, D and G**) MH pattern of junctions of 5'Sμ to Sγ1 (**A**) and 5'Sμ to Sε (**D, G**) breaks are plotted as percentage of total junctions. (**B-C, E-F and H-I**) Percentages of direct joins (**B, E, H**) and junctions with 4 bp and longer MH (**C, F, I**) of 5'Sμ to Sγ1 and Sε in different genetic backgrounds are compared. Unpaired two-tailed t-test was used to calculate *p* values for significant difference between samples (Quantitative data = average±s.e.m., ** *p*≤ 0.01, *** *p*≤ 0.001). At least 3 independent samples were used for each experiment and details are in SI Appendix, Table S2.

Fig. S3. Generation of ATM- and 53BP1-deficient CH12F3 cells and non-coding IgH allele Sμ-Sα region deleted CH12F3 cells with various genotypes.

Two independent clones of ATM- and 53BP1-deficient CH12F3 cells were made using CRISPR-Cas9 mediated gene deletion. (**A-D**) Southern blot and western blot verification of ATM$^{-/-}$ clones (**A, B**) and 53BP1$^{-/-}$ clones (**C, D**) are shown. (**E**) Non-productive allele- Sμ-Sα region deleted WT, ATM-, 53BP1- and Ligase4-deficient CH12F3 cells were made using CRISPR-Cas9 mediated gene deletion. Southern blot verification of noncoding allele- Sμ-Sα region deleted clones is shown. (**F**) CH12F3 cells with non-productive allele- Sμ-Sα region deletion in WT, ATM$^{-/-}$, 53BP1$^{-/-}$ and Ligase 4$^{-/-}$ backgrounds were stimulated with αCD40/IL4/TGF-β and assayed for IgA CSR 72 hrs post-stimulation. Each genotype has two independent clones and each clone has three experimental replicates. HTGTS libraries with genomic DNA harvested 72 hrs after stimulation were made with 5'Sμ primer as described (1).

Fig. S4. Distribution of junctions from Sμ to Sα in WT, ATM-, 53BP1-, Ligase 4-deficient clones with non-productive IgH allele Sμ-Sα region deletion

(**A**) Non-productive allele- Sμ-Sα region deleted WT, ATM-, 53BP1- and Ligase 4-deficient CH12F3 cells were stimulated with αCD40/IL4/TGF-β for 3 days. Each genotype has two independent clones and each clone has three experimental replicates for the HTGTS with 5'Sμ bait. All libraries were plotted linearly as percentage of junctions mapped to the 20 kb Sα region as described previously (1). (**B-C**) Inversion (INV):deletion (DEL) bias ratio for Sα junctions (**B**) and percentage of junctions with long resections (**C**) in various genotypes is shown. Unpaired two-tailed t-test was used to calculate *p* values for significant difference between samples which is shown in SI Appendix, Table S6-S7.

Fig. S5. MH usage pattern for junctions between *c-myc*-Cas9 bait and Sμ region

(**A**) Data shown here is derived from libraries shown in Fig. 3. MH usage in translocation junctions from *c-myc*-Cas9 break joining to Sμ breaks were plotted as percentage of junctions

with indicated length of MH over the total number of junctions in the region. (**B-C**) Percentages of direct joins (**B**) and joins with 4bp and longer MH (**C**) of *c-myc*-Cas9 break joining to Sµ breaks in different genetic backgrounds are shown. Statistical significance was calculated by two-tailed t test (Quantitative data = average ±s.e.m., *** $p \leq 0.001$). 3 independent samples were used for each experiment and details are SI Appendix, Table S9.

Fig. S6. Distribution of translocation junctions from *c-myc*-Cas9 DSB to Sµ and Sα regions in WT, ATM-, 53BP1-, and Ligase4-deficient CH12F3 cells

WT, ATM-, 53BP1- and Ligase4-deficient CH12F3 cells were stimulated with αCD40/IL4/TGF-β and *cmyc*-Cas9 bait DSB was introduced at 12 hrs after stimulation. HTGTS libraries were made with genomic DNA harvested 72 hrs after stimulation. Each clone has 3 experimental replicates. All libraries were plotted linearly as percentage of junctions mapped to 20 kb Sµ (**A**) or Sα (**B**) region.

Fig. S7. Comparison of translocation junctions to general DSBs in WT, ATM^{-/-}, p53^{-/-} and XRCC4^{-/-}p53^{-/-} NSPCs.

HTGTS libraries were made with *c-myc*-Cas9 DSB as bait in WT, ATM^{-/-}, p53^{-/-} and XRCC4^{-/-}p53^{-/-} NSPCs. (**A**) Translocation outcomes of c-myc-Cas9 DSB to genome-wide MH usage in junctions from *c-myc*-Cas9 break joining to genome-wide breaks (**A**) were plotted as percentage of junctions with indicated length of MH over the total number of junctions in the respective regions. (**B-C**) Percentages of direct joins from *c-myc*-Cas9 break joining to genome-wide breaks (**B**), and percentages of junctions with 4bp and longer MH from *c-myc*-Cas9 break to genome-wide breaks (**C**) in different genetic backgrounds are shown. Unpaired two-tailed t-test was used for statistical analysis (Quantitative data = average ±s.e.m., n.s. means $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$). Note that the WT, p53^{-/-} and XRCC4^{-/-}p53^{-/-} NSPCs are re-plotted from an earlier published study from our lab (2) for comparison with the new ATM^{-/-} data performed in the same cell type using a c-myc sgRNA described in (2).

Table S1. Percentage of junctions with insertions from WT, ATM- and 53BP1-deficient primary B cells

The percentage of Sµ-Sγ1 and Sµ-Sε junctions with various lengths of insertions among all junctions in the same region recovered from individual libraries made with αCD40/IL4 *in vitro* activated WT, ATM- and 53BP1-deficient primary B cells were listed.

Table S2. Summary for junctions analyzed in each experimental replicate in Sγ1 and Sε region from primary B cells with various genotypes

Summary of junctions recovered from each replicate library with αCD40/IL4-stimulated primary wild type, *ATM$^{-/-}$, H2AX$^{-/-}$, RIF1$^{flox/flox}$ CD19$^{Cre/+}$* and *53BP1$^{-/-}$* B cells, showing the Sγ1 and Sε junctions identified and percentage of direct joins in each region from each library.

Table S3. Summary of MH length in Sµ-Sγ1, Sµ-Sε junctions from primary B cells with various genotypes

Length of MH (average ±s.e.m.) for CSR junctions in various genotypes as described in Fig. 1 is listed. Numbers in parenthesis indicate total number of direct joins plus junctions with MH in the range of 1-10bp mapped to the S regions recovered from various replicates of indicated genotypes. Average length of MH for junctions joining in deletional orientation (-) and inversional orientation (+) are also shown.

Table S4. Summary for junctions analyzed in each experimental replicate in Sα region from CH12F3 cells with various genotypes

Summary of junctions recovered from each replicate library with αCD40/IL4/TGF-β-stimulated wild type, *ATM$^{-/-}$, 53BP1$^{-/-}$* and *Ligase4$^{-/-}$* CH12F3 cells with non-productive allele deletion, showing number of Sα junctions identified, percentage of direct joins, junctions with more than 4 bp MH and junctions with insertions from each library.

Table S5. Summary of MH length in Sμ-Sα junctions from CH12F3 cell lines with various genotypes

Length of MH (average ±s.e.m.) for CSR junctions in various genotypes as described in Fig. 2 is listed. Numbers in parenthesis indicate total number of direct joins plus junctions with MH in the range of 1-10bp mapped to the Sα regions recovered from various replicates of indicated genotypes. Average length of MH for junctions joining in deletional orientation (-) and inversional orientation (+) are also shown.

Table S6. Statistical comparison for orientation bias in Sα junctions from CH12F3 cells with various genotypes

p values calculated by unpaired two-tailed t-test for the degree of bias in the Sα junctions between wild-type and DSBR-deficient B cells described in Fig. S4B. Orientation bias was calculated as described in the methods for individual genotypes.

Table S7. Statistical comparison for resection in Sα junctions from CH12F3 cells with various genotypes

p values calculated by unpaired two-tailed t-test for the level of resections in the in the Sα junctions between wild-type and DSBR-deficient B cells described in Fig. S4C. Percentage of junctions with long resections was calculated as described in the methods.

Table S8. Summary of MH length in c-myc-Sμ, Sα and genome-wide translocations from CH12F3 cells with various genotypes

Length of MH (average ±s.e.m.) for total (T) translocations in various genotypes as described in Fig. 3 and Fig. S5 is listed. Numbers in parenthesis indicate total number of direct joins plus junctions with MH in the range of 1-10bp mapped to the Sμ, Sα and genome-wide translocations from various replicates of indicated genotypes. Average length of MH for junctions joining in deletional orientation (-) and inversional orientation (+) are also shown.

Table S9. Summary for junctions analyzed in each experimental replicate in Sμ, Sα and genome-wide translocations from CH12F3 cells with various genotypes

 Summary of translocation junctions from *c-myc*-Cas9 break to S region breaks and genome-wide general DSBs recovered from each replicate library with αCD40/IL4/TGF-β-stimulated wild type, *ATM*^{-/-}, *53BP1*^{-/-} and *Ligase4* [-/-] CH12F3 cells, showing number of junctions identified in various regions and percentage of direct joins and junctions using 4 bp or longer MH from each library.

1. Dong J*, et al.* (2015) Orientation-specific joining of AID-initiated DNA breaks promotes antibody class switching. *Nature* 525(7567):134-139.
2. Wei PC*, et al.* (2016) Long Neural Genes Harbor Recurrent DNA Break Clusters in Neural Stem/Progenitor Cells. *Cell* 164(4):644-655.
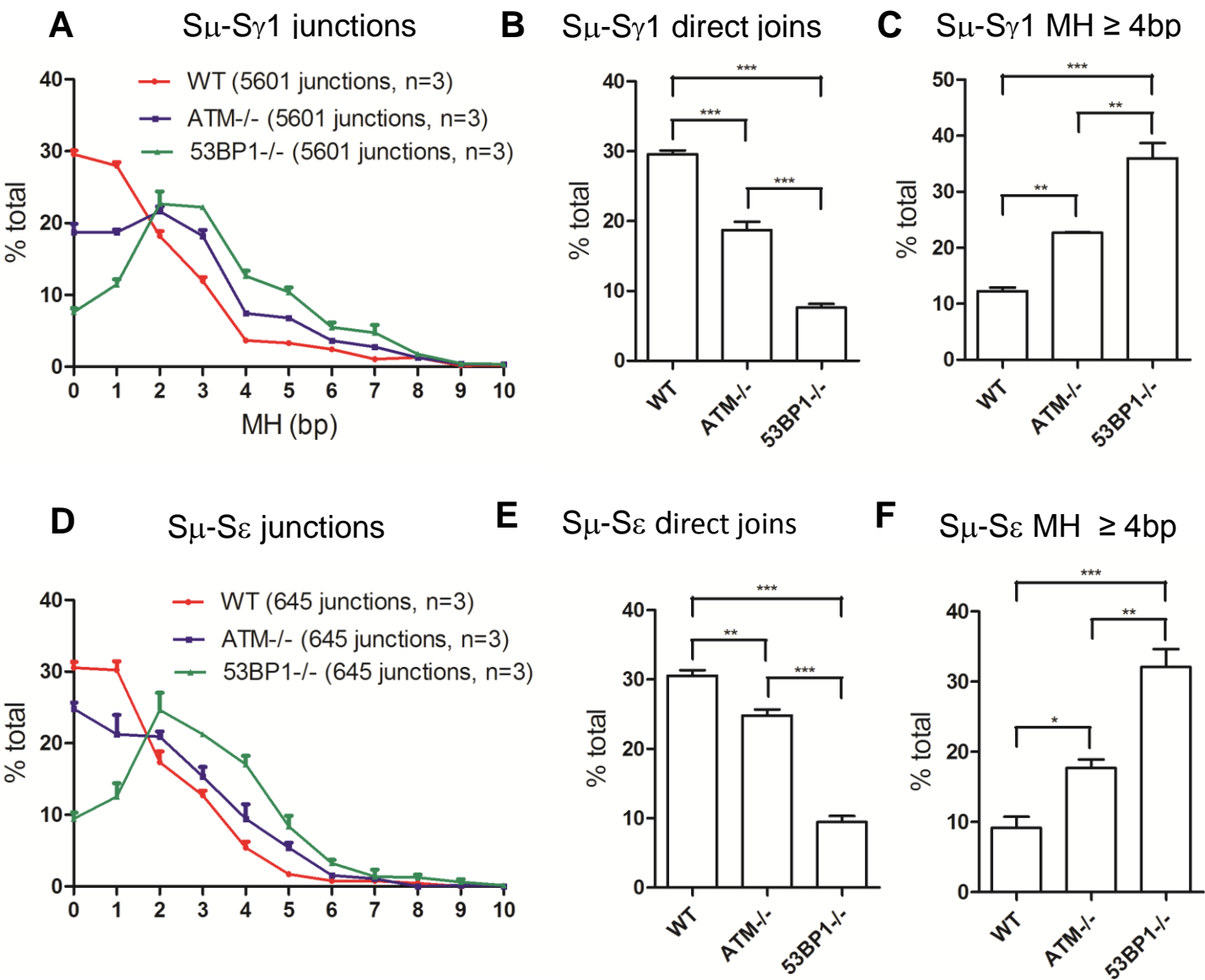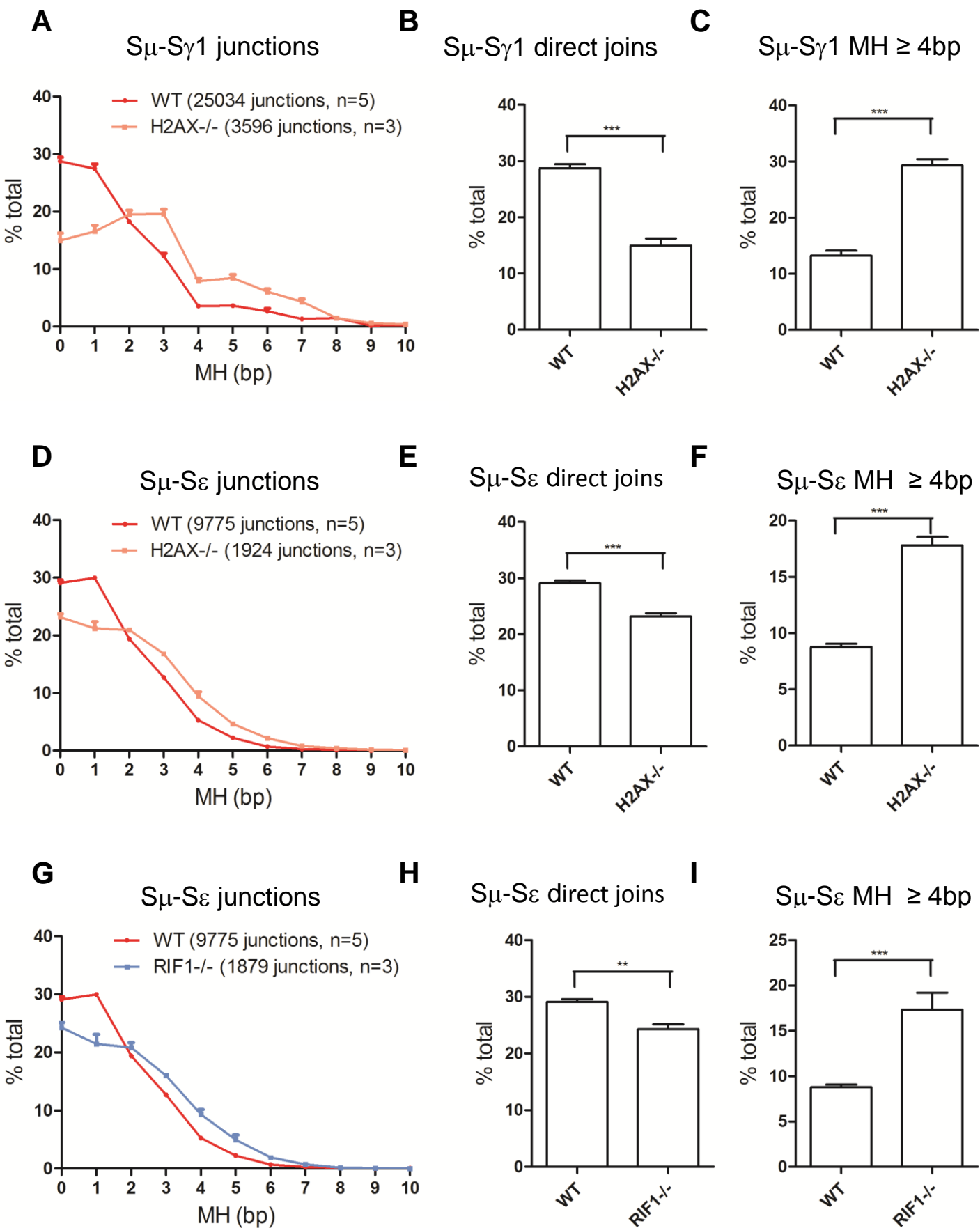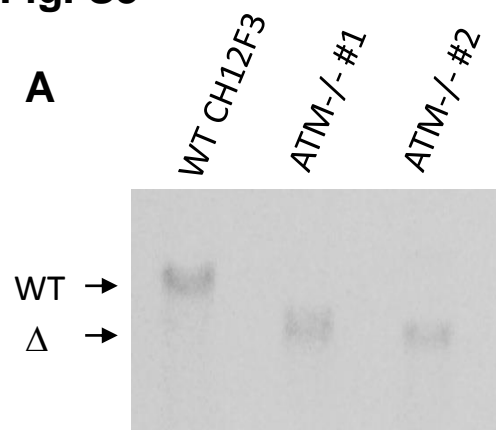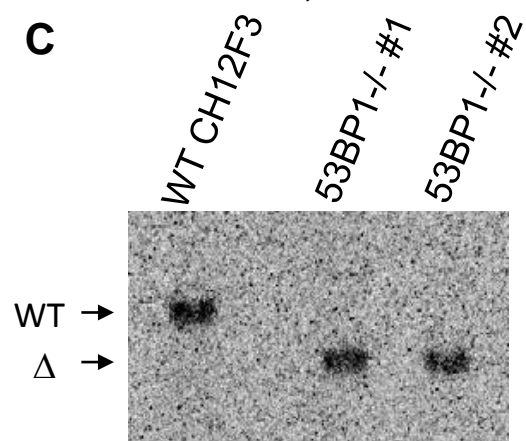
Fig. S1

A   Sμ-Sγ1 junctions

Legend:
- WT (5601 junctions, n=3)
- ATM-/- (5601 junctions, n=3)
- 53BP1-/- (5601 junctions, n=3)

Y-axis: % total
X-axis: MH (bp)

B   Sμ-Sγ1 direct joins

Y-axis: % total
Categories: WT, ATM-/-, 53BP1-/-
*** *** ***

C   Sμ-Sγ1 MH ≥ 4bp

Y-axis: % total
Categories: WT, ATM-/-, 53BP1-/-
** *** **

D   Sμ-Sε junctions

Legend:
- WT (645 junctions, n=3)
- ATM-/- (645 junctions, n=3)
- 53BP1-/- (645 junctions, n=3)

Y-axis: % total

E   Sμ-Sε direct joins

Y-axis: % total
Categories: WT, ATM-/-, 53BP1-/-
** *** ***

F   Sμ-Sε MH ≥ 4bp

Y-axis: % total
Categories: WT, ATM-/-, 53BP1-/-
* *** **

Fig. S2

A

Sμ-Sγ1 junctions

- WT (25034 junctions, n=5)
- H2AX-/- (3596 junctions, n=3)

% total vs MH (bp)

B

Sμ-Sγ1 direct joins

***

WT, H2AX-/-

C

Sμ-Sγ1 MH ≥ 4bp

***

WT, H2AX-/-

D

Sμ-Sε junctions

- WT (9775 junctions, n=5)
- H2AX-/- (1924 junctions, n=3)

% total vs MH (bp)

E

Sμ-Sε direct joins

***

WT, H2AX-/-

F

Sμ-Sε MH ≥ 4bp

***

WT, H2AX-/-

G

Sμ-Sε junctions

- WT (9775 junctions, n=5)
- RIF1-/- (1879 junctions, n=3)

% total vs MH (bp)

H

Sμ-Sε direct joins

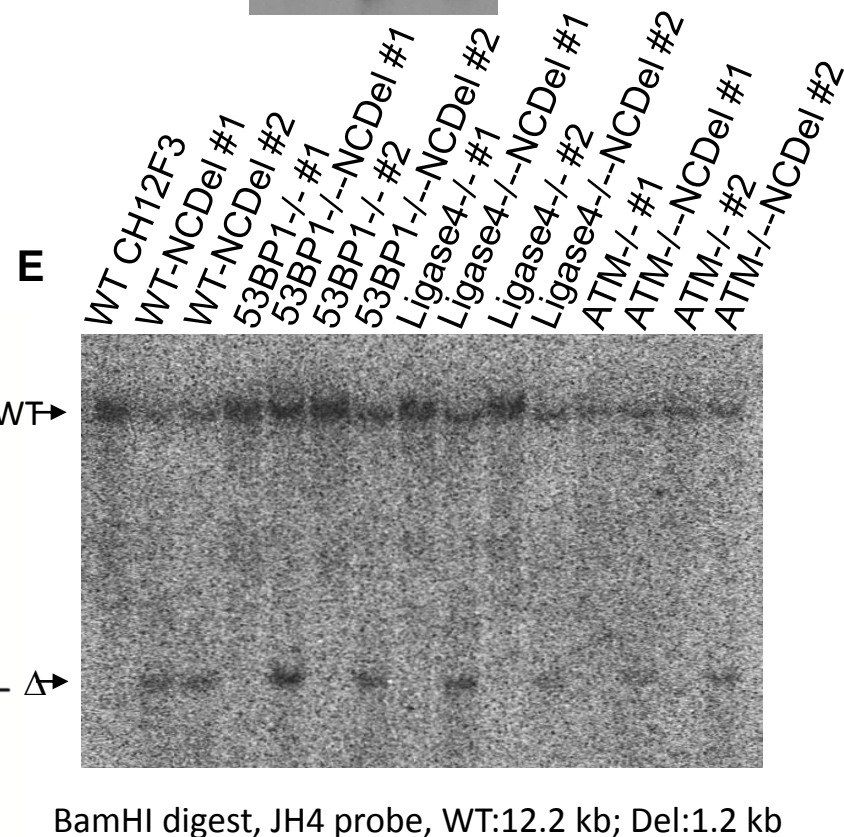

**

WT, RIF1-/-

I

Sμ-Sε MH ≥ 4bp

***

WT, RIF1-/-

Fig. S3

A

EcoRV+BamHI digest, exon-56 probe,
WT:7.5 kb; Del:6.0 kb

B

C

NdeI digest, 5' 53bp1 probe,
WT:4.32 kb; Del:3.46 kb

D

F

Day3 IgA CSR

E
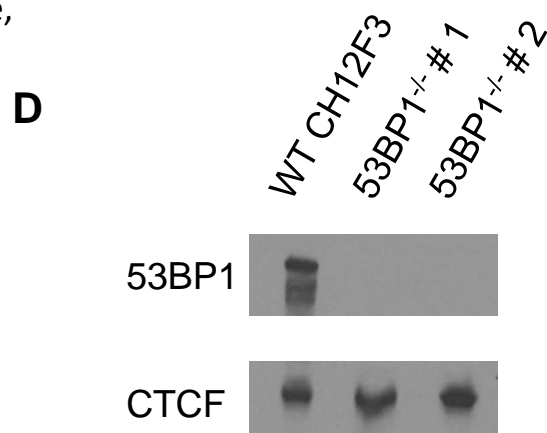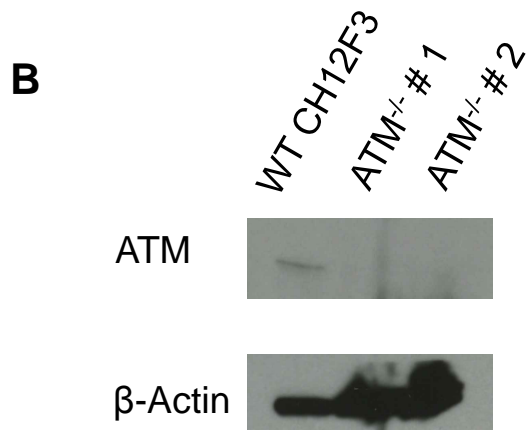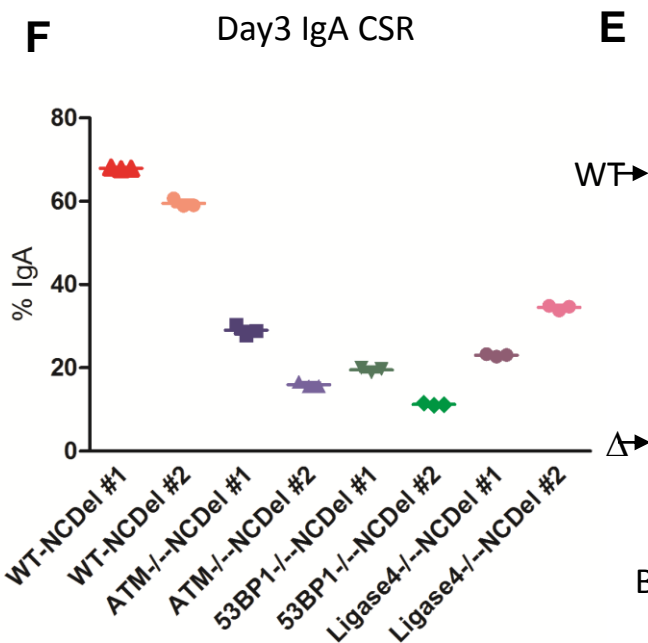
BamHI digest, JH4 probe, WT:12.2 kb; Del:1.2 kb

Fig. S4

A

WT-NCDel #1
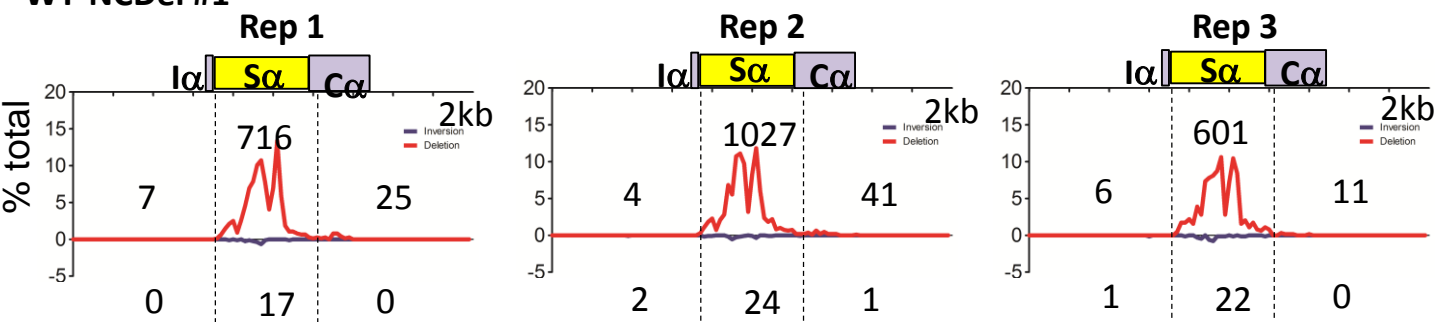
WT-NCDel #2
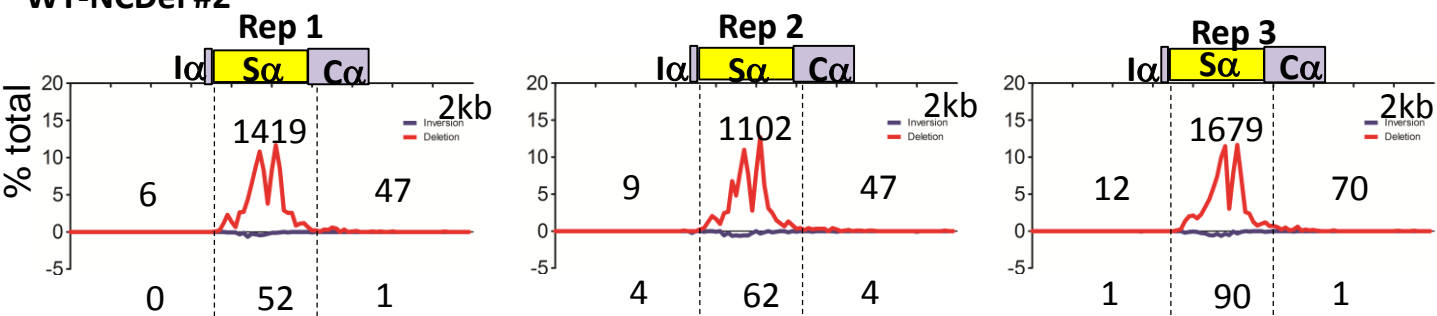
ATM-NCDel #1
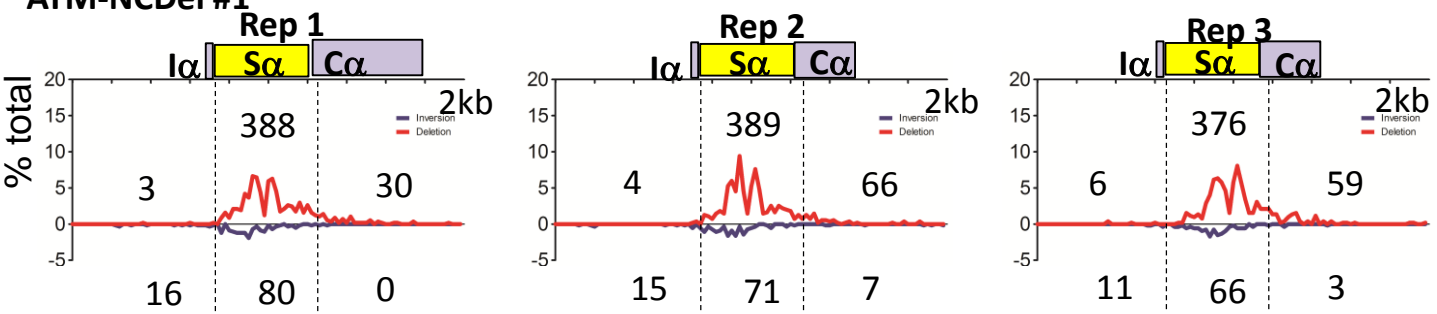
ATM-NCDel #2
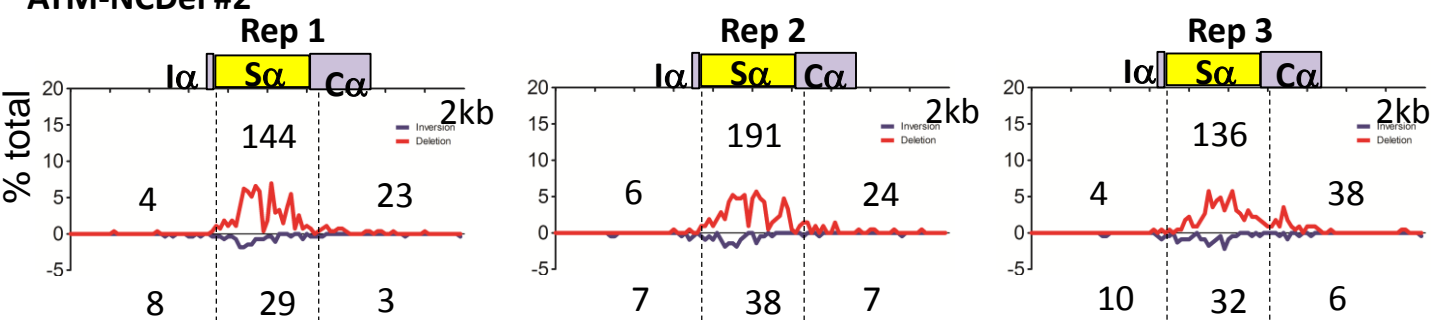
Fig. S4

A
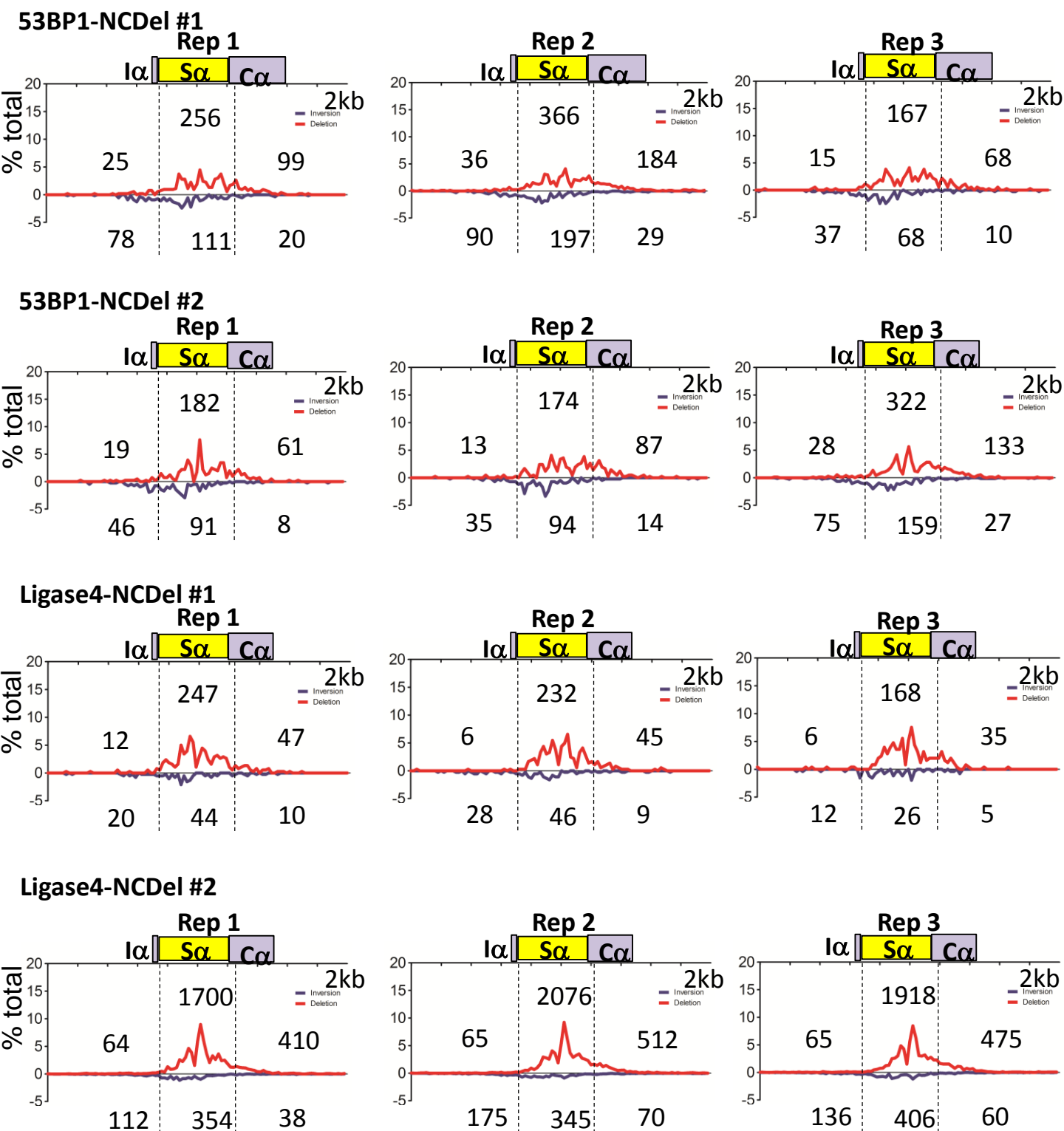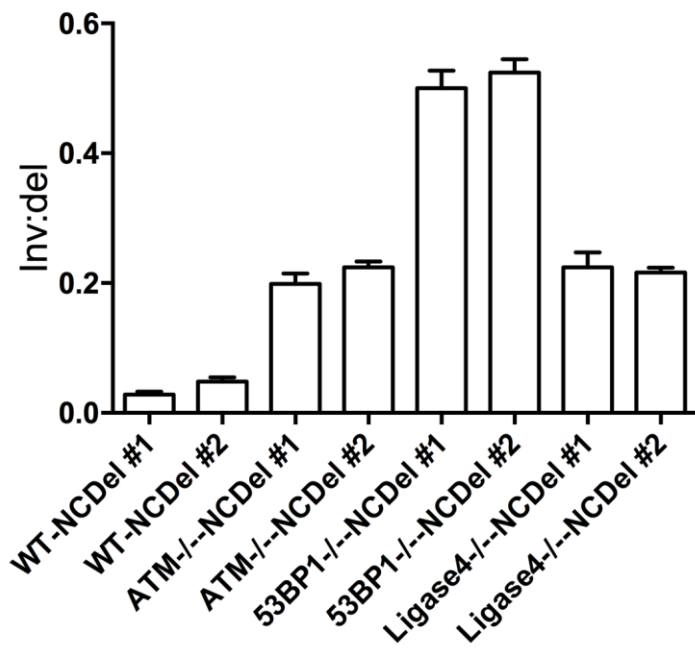
53BP1-NCDel #1

53BP1-NCDel #2

Ligase4-NCDel #1

Ligase4-NCDel #2

Fig. S4

B

Bias ratio Sα

C

Resection Sα

Fig. S5

A

c-myc to Sμ translocations

Legend:
- WT (1519 junctions, n=3)
- ATM-/- #1 (968 juctions, n=3)
- ATM-/- #2 (916 junctions, n=3)
- 53BP1-/- #1 (526 junctions, n=3)
- 53BP1-/- #2 (1754 junctions, n=3)
- Ligase4-/- #1 (1128 junctions, n=3)
- Ligase4-/- #2 (2965 junctions, n=3)

x-axis: MH (bp), y-axis: % total

B

c-myc-Sμ direct joins

*** significance bars. x-axis: WT, ATM-/- #1, ATM-/- #2, 53BP1-/- #1, 53BP1-/- #2, Lgase4-/- #1, Ligase4-/- #2. y-axis: % total

C

c-myc-Sμ MH ≥ 4bp

*** significance bars. x-axis: WT, ATM-/- #1, ATM-/- #2, 53BP1-/- #1, 53BP1-/- #2, Lgase4-/- #1, Ligase4-/- #2. y-axis: % total
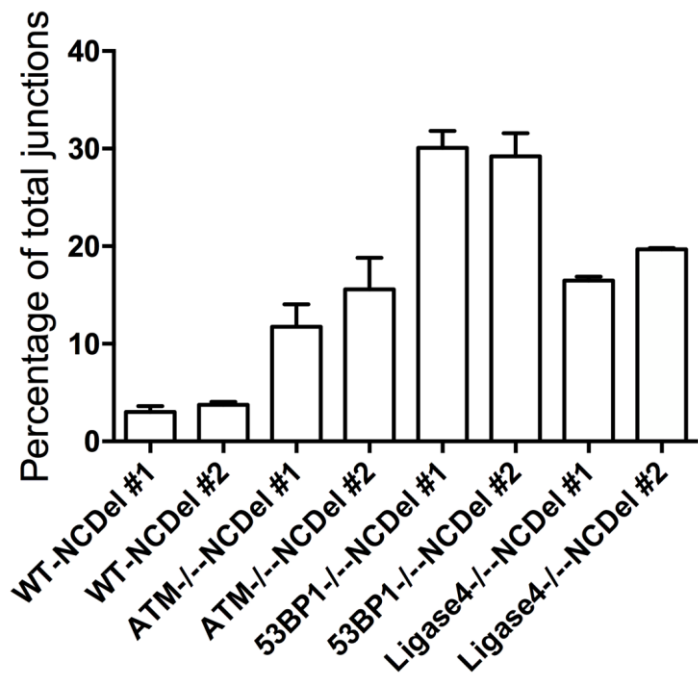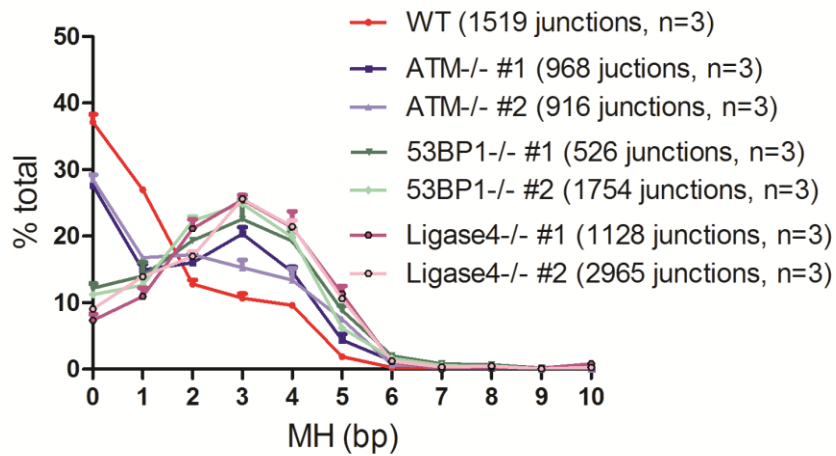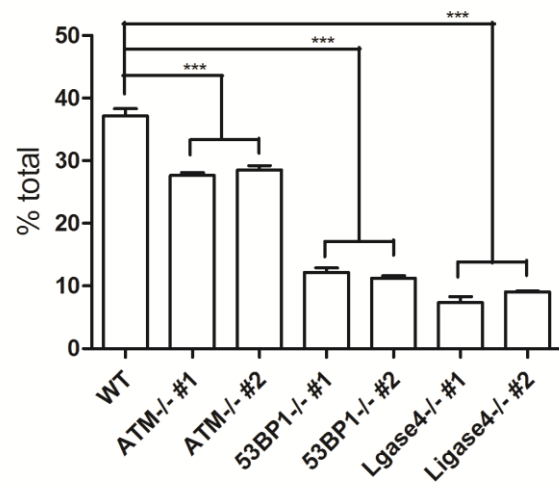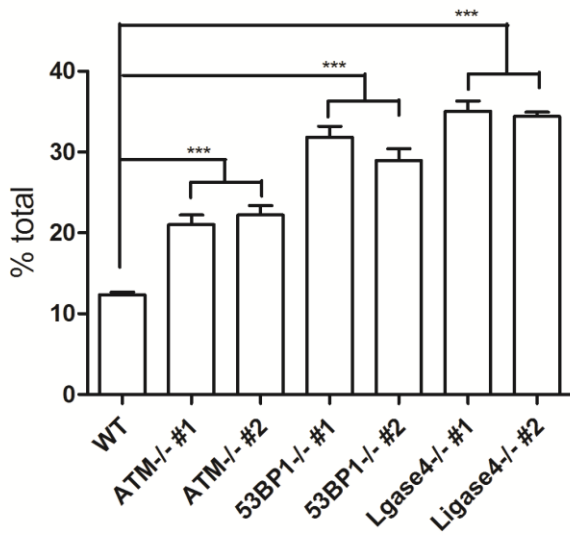
Fig. S6

A
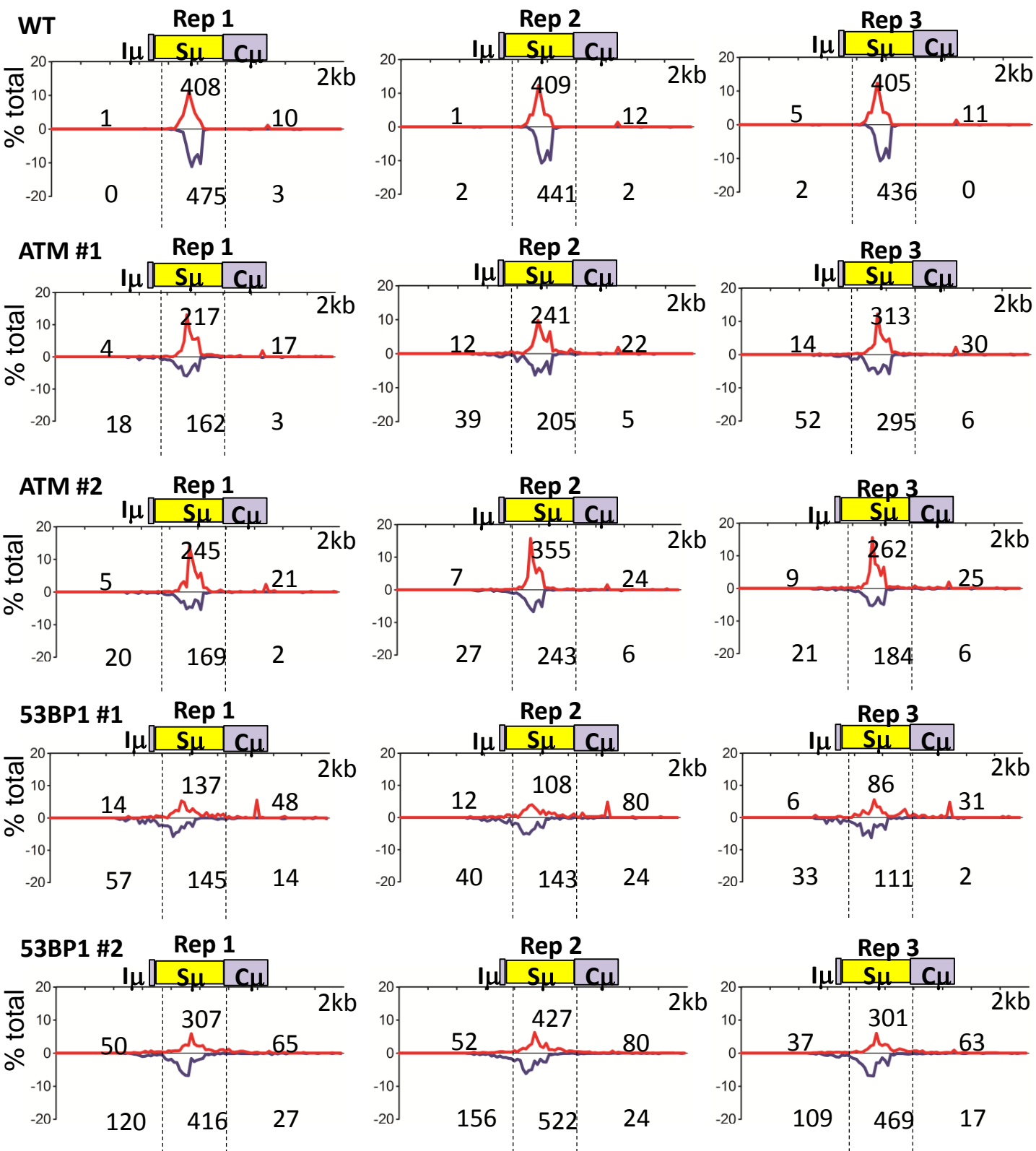
Fig. S6

A
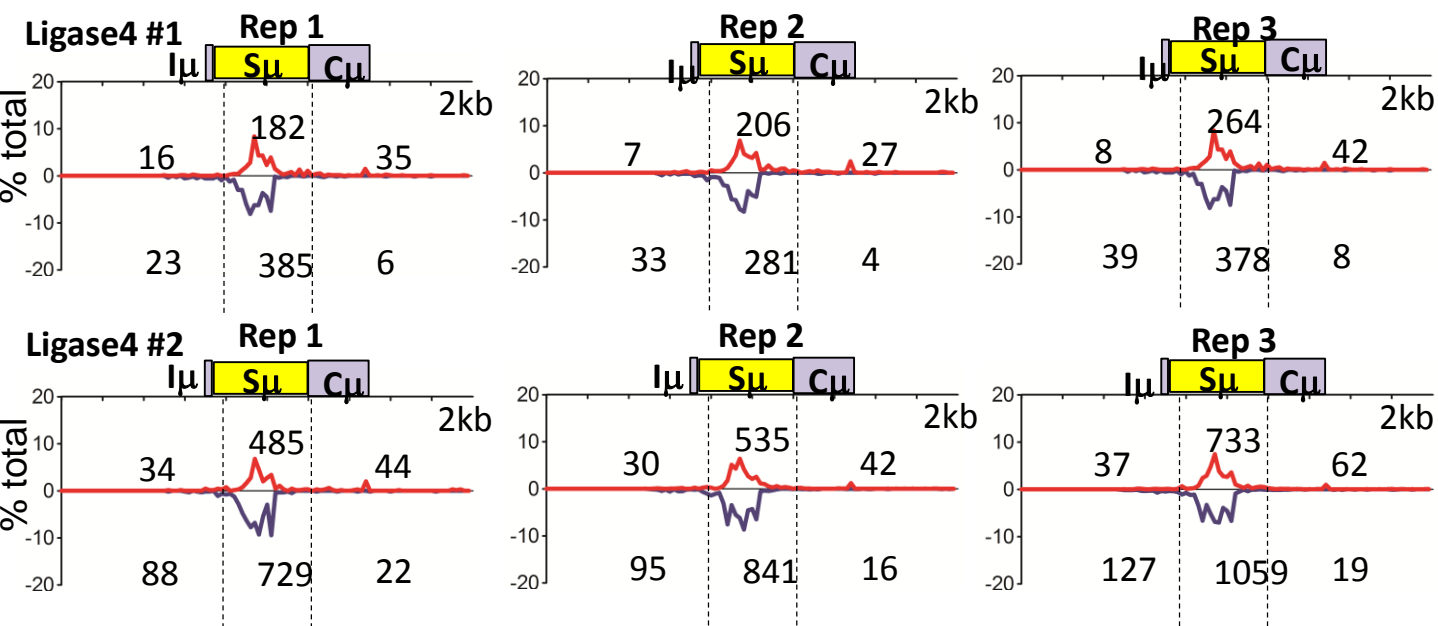
Fig. S6

B
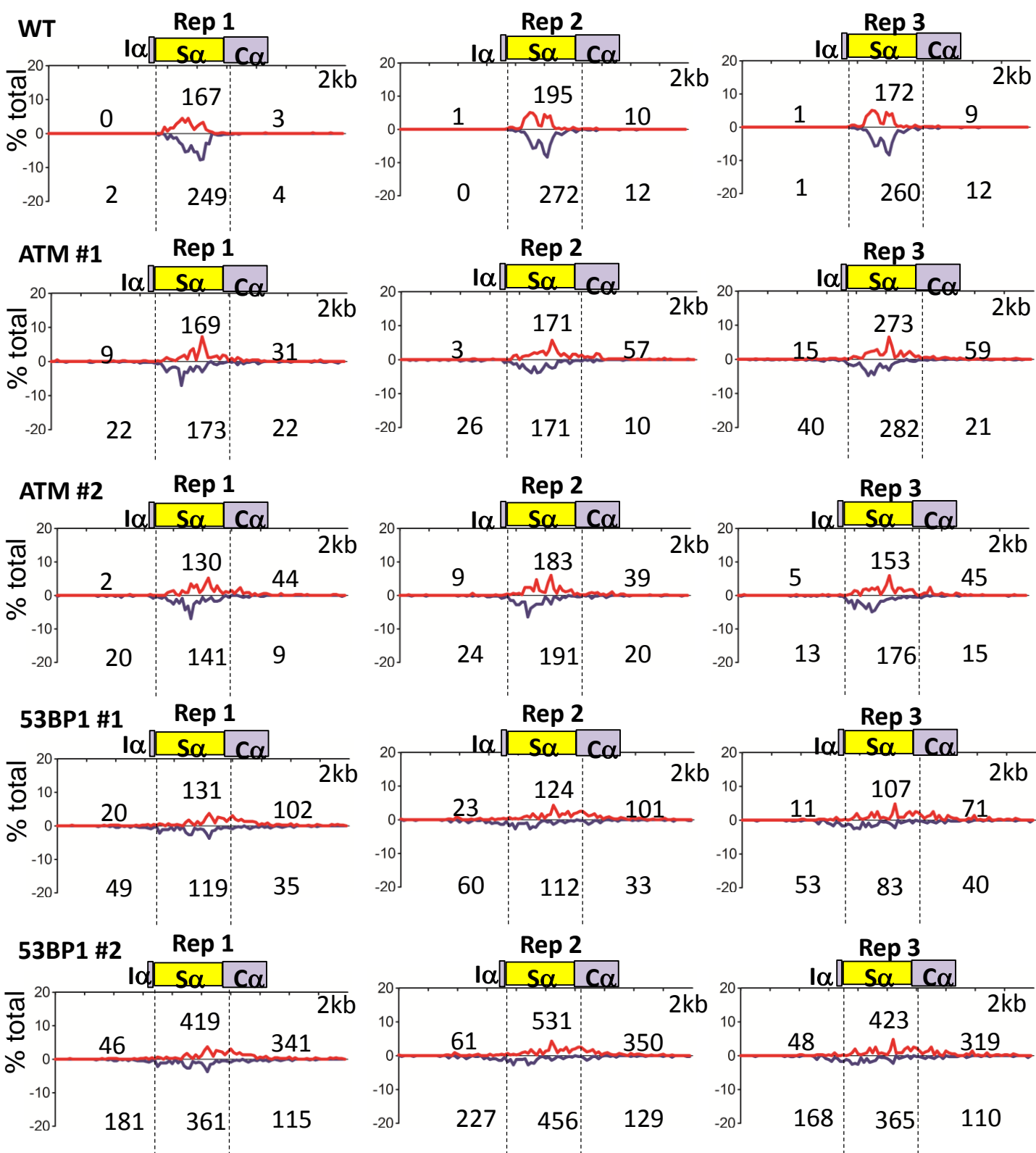
Fig. S6

B
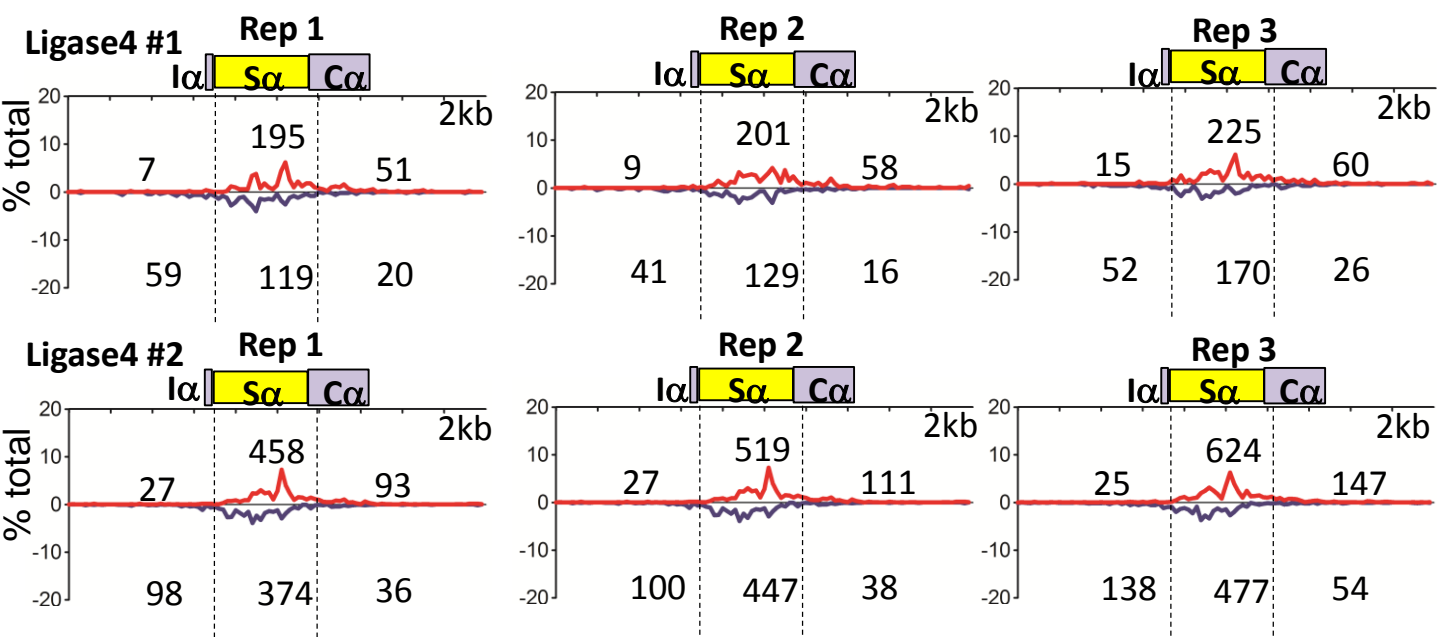
Fig. S7

A

c-myc to genome-wide
translocations

- ● WT (8095 junctions, n=3)
- ▼ ATM-/- (11283 junctions, n=3)
- ▲ p53-/- (12818 junctions, n=3)
- ✦ XRCC4-/-p53-/- (5217 junctions, n=3)
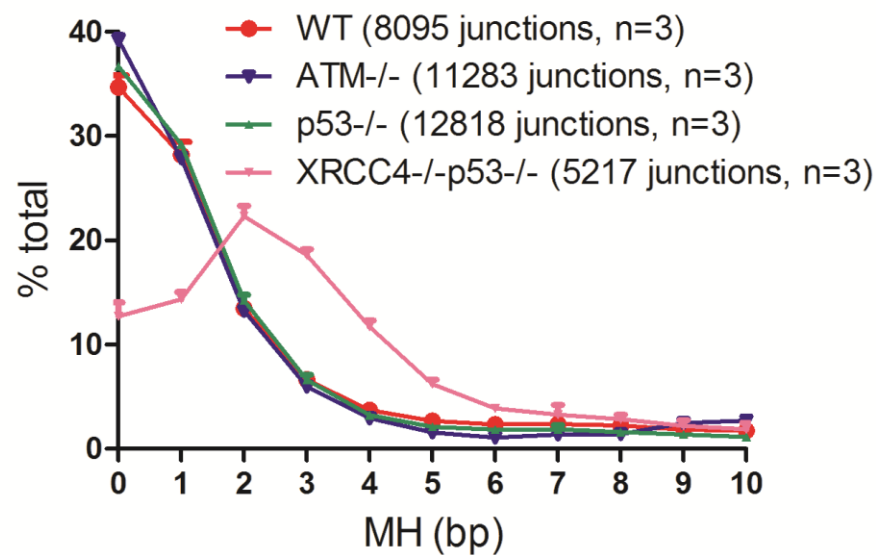
B

c-myc-genome-wide
direct joins
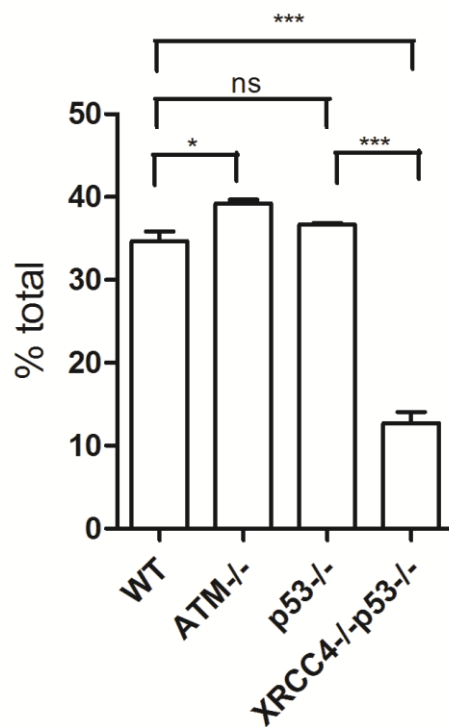
C

c-myc-genome-wide
MH ≥ 4bp
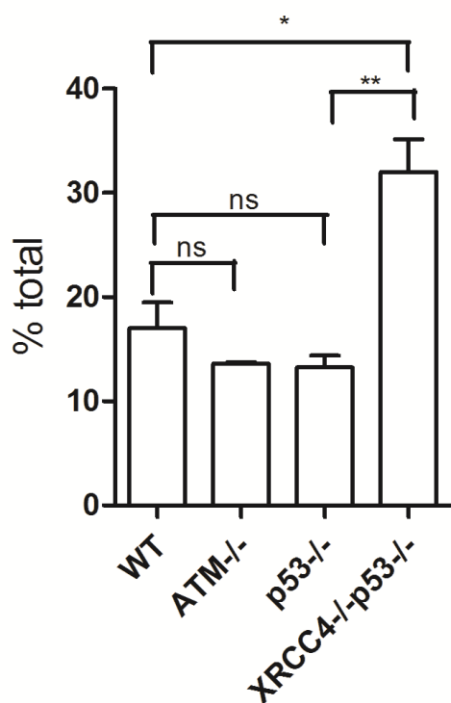
Table S1 Percentage of junctions with insertions from WT, ATM- and 53BP1-deficient primary B cells

Genotype	% insertion in Sγ1 junctions	% insertion in Sε junctions
WT-1	19.0%	17.5%
WT-2	17.4%	12.7%
WT-3	18.5%	17.2%
WT-4	25.6%	23.8%
WT-5	22.5%	20.4%
ATM-1	16.7%	15.8%
ATM-2	15.4%	16.4%
ATM-3	15.9%	17.0%
53BP1-1	12.7%	16.1%
53BP1-2	14.0%	17.2%
53BP1-3	15.3%	19.4%
53BP1-4	16.3%	17.6%
53BP1-5	16.4%	19.6%
53BP1-6	14.4%	19.8%
53BP1-7	17.9%	18.1%
53BP1-8	14.2%	15.9%

Table S2 Summary for junctions analyzed in each experiment replicate in Sγ1 and Sε region from primary B cells with various genotypes

Genotype	Sγ1 jxns (% IgH)	Sε jxns (% IgH)	IgH total	Sγ1 jxns % direct	Sε jxns % direct
WT-1	1465 (58%)	479 (19%)	2522	26.0%	28.5%
WT-2	2764 (62%)	732 (16%)	4460	29.2%	30.4%
WT-3	17135 (56%)	7913 (25%)	30368	29.9%	29.7%
WT-4	9124 (64%)	2560 (18%)	14123	29.2%	28.1%
WT-5	2398 (56%)	988 (23%)	4285	29.3%	29.6%
ATM-1	7453 (42%)	2002 (11%)	17712	17.0%	18.2%
ATM-2	6246 (46%)	1467 (11%)	13503	18.6%	22.8%
ATM-3	6761 (44%)	1827 (12%)	15376	19.5%	24.1%
53BP1-1	566 (12%)	199 (4.2%)	4690	8.5%	13.1%
53BP1-2	726 (14%)	256 (4.9%)	5191	7.1%	9.9%
53BP1-3	1475 (20%)	119 (1.6%)	7256	6.9%	11.7%
53BP1-4	2311 (21%)	272 (2.5%)	10851	7.0%	8.0%
53BP1-5	1733 (20%)	155 (1.8%)	8552	7.7%	3.0%
53BP1-6	817 (14%)	119 (1.6%)	5919	8.2%	11.5%
53BP1-7	3090 (22%)	381 (2.8%)	13506	7.4%	11.7%
53BP1-8	3325 (21%)	372(2.5%)	14876	6.4%	8.7%
H2AX-1	671 (22%)	393 (13%)	3080	12.6%	23.2%
H2AX-2	1639 (19%)	1205 (14%)	8471	14.7%	25.4%
H2AX-3	2146 (25%)	1015 (12%)	8750	17.2%	23.7%
RIF1-1	-	691 (14%)	5047	-	27.0%
RIF1-2	-	1320 (13%)	10489	-	24.4%
RIF1-3	-	609 (11%)	5623	-	24.0%

Table S3 Summary of MH length in Sμ-Sγ1, Sμ-Sε junctions from primary B cells with various genotypes

Genotype	Sγ1	Sε
WT	1.67±0.01 (n=25034)	1.48±0.01 (n=9775)
	Sγ1(-): 1.68±0.01 (n=23236)	*Sε(-): 1.46±0.01 (n=9305)*
	Sγ1(+): 1.55±0.04 (n=1798)	*Sε(+): 1.71±0.08 (n=470)*
ATM$^{-/-}$	2.40±0.02 (n=16730)	2.04±0.03 (n=4422)
	Sγ1(-): 2.45±0.02 (n=13963)	*Sε(-): 2.00±0.03 (n=3931)*
	Sγ1(+): 2.15±0.03 (n=2767)	*Sε(+): 2.38±0.08 (n=491)*
H2AX$^{-/-}$	2.84±0.04 (n=3596)	1.96±0.04 (n=1924)
	Sγ1(-): 2.87±0.04 (n=3278)	*Sε(-): 1.89±0.04 (n=1771)*
	Sγ1(+): 2.55±0.13 (n=318)	*Sε(+): 2.74±0.14 (n=153)*
Rif1$^{-/-}$	N.A.	1.89±0.04 (n=1879)
		Sε(-): 1.77±0.04 (n=1637)
		Sε(+): 2.28±0.12 (n=242)
53BP1$^{-/-}$	3.17±0.02 (n=8578)	2.80±0.04(n=1828)
	Sγ1(-): 3.35±0.03 (n=5970)	*Sε(-): 2.76±0.05 (n=1142)*
	Sγ1(+): 2.76±0.03(n=2608)	*Sε(+): 2.84±0.07 (n=686)*

Table S4 Summary for junctions analyzed in each experiment replicate in Sα region from CH12F3 cells with various genotypes

Genotype	core Sa	% direct joins/MH $\geq$ 4bp	% insertion joins
WT-NCDel #1			
Exp 1	733	24.2/13.2	25.5
Exp 2	1051	25.4/12.4	20.8
Exp 3	623	25.7/15.2	24.7
WT-NCDel #2			
Exp 1	1471	24.5/14.0	26.1
Exp 2	1164	26.4/14.2	27.1
Exp 3	1770	26.0/13.7	27.9
ATM$^{-/-}$-NCDel #1			
Exp 1	468	15.4/28.2	12.6
Exp 2	460	17.6/24.8	15.9
Exp 3	442	18.7/22.5	12.7
ATM$^{-/-}$-NCDel #2			
Exp 1	173	13.5/28.4	10.4
Exp 2	229	15.3/28.2	8.7
Exp 3	168	12.2/38.1	12.5
53BP1$^{-/-}$-NCDel #1			
Exp 1	367	5.6/32.5	17.5
Exp 2	563	9.3/32.3	16.0
Exp 3	235	8.2/28.9	17.4
53BP1$^{-/-}$-NCDel #2			
Exp 1	273	10.6/31.6	17.6
Exp 2	268	5.5/39.6	19.0
Exp 3	482	8.7/30.3	18.5
Ligase4$^{-/-}$-NCDel #1			
Exp 1	291	5.8/27.1	11.3
Exp 2	278	5.6/29.3	10.4
Exp 3	194	5.6/32.5	17.5
Ligase4$^{-/-}$-NCDel #2			
Exp 1	2055	8.0/28.9	21.1
Exp 2	2421	7.2/27.2	23.5
Exp 3	2324	7.3/27.7	22.8

Table S5 Summary of MH length in Sμ-Sα junctions from CH12F3 cell lines with various genotypes

Genotype	Sα
WT-NCDel #1	1.70±0.03 (n=1855)
	Sα(-): 1.69±0.04 (n=1808)
	Sα(+): 2.02±0.27 (n=47)
WT-NCDel #2	1.74±0.03 (n=3212)
	Sα(-): 1.73±0.03 (n=3068)
	Sα(+): 1.90±0.13 (n=144)
ATM-NCDel #1	2.40±0.05 (n=1182)
	Sα(-): 2.34±0.06 (n=991)
	Sα(+): 2.69±0.12 (n=191)
ATM-NCDel #2	2.61±0.08 (n=511)
	Sα(-): 2.55±0.08 (n=421)
	Sα(+): 2.86±0.19 (n=90)
53BP1$^{-/-}$-NCDel #1	2.80±0.05 (n=976)
	Sα(-): 2.70±0.07 (n=645)
	Sα(+): 2.99±0.09 (n=331)
53BP1$^{-/-}$-NCDel #2	2.81±0.05 (n=835)
	Sα(-): 2.77±0.07 (n=535)
	Sα(+): 2.87±0.09 (n=300)
Ligase4$^{-/-}$-NCDel #1	2.81±0.06 (n=667)
	Sα(-): 2.81±0.06 (n=559)
	Sα(+): 2.83±0.14 (n=108)
Ligase4$^{-/-}$-NCDel #2	2.69±0.02 (n=5272)
	Sα(-): 2.65±0.02 (n=4356)
	Sα(+): 2.89±0.05 (n=916)

Table S6 Statistical comparison for orientation bias in Sα junctions from CH12F3 cells with various genotypes

	WT #1	WT #2	ATM #1	ATM #2	53BP1 #1	53BP1 #2	Ligase4 #1	Ligase4 #2
WT#1								
WT #2	N/A							
ATM #1	0.0005	0.001						
ATM #2	<0.0001	0.0001	N/A					
53BP1 #1	<0.0001	<0.0001	0.0006	0.0006				
53BP1 #2	<0.0001	<0.0001	0.0002	0.0002	N/A			
Ligase4 #1	0.0012	0.0019	N/A	N/A	0.0015	0.0006		
Ligase4 #2	<0.0001	<0.0001	N/A	N/A	0.0005	0.0002	N/A	

Table S7 Statistical comparison for resection in Sα junctions from CH12F3 cells with various genotypes

	WT #1	WT #2	ATM #1	ATM l#2	53BP1 #1	53BP1 #2	Ligase4 #1	Ligase4 #2
WT #1								
WT #2	N/A							
ATM #1	0.0214	0.0262						
ATM #2	0.0184	0.0215	N/A					
53BP1 #1	0.0001	0.0001	0.0031	0.0163				
53BP1 #2	<0.0001	<0.0001	0.0062	0.0270	N/A			
Ligase4 #1	<0.0001	<0.0001	N/A	N/A	0.0015	0.0061		
Ligase4 #2	<0.0001	<0.0001	0.0262	N/A	0.0037	0.016	0.0014	

Table S8 Summary of MH length in Sμ, Sα and genome-wide translocations with c-myc bait from CH12F3 cells with various genotypes

Genotype	genome-wide translocations	core Sμ translocations	core Sα translocations
WT (T)	1.70±0.02 (n=12576)	1.38±0.04 (n=1519)	1.32±0.04 (n=1135)
(-)	*1.68±0.02 (n=6464)*	*1.66±0.06 (n=768)*	*1.51±0.07 (n=452)*
(+)	*1.71±0.03 (n=6212)*	*1.09±0.05 (n=751)*	*1.17±0.05 (n=683)*
ATM$^{-/-}$ #1 (T)	1.83±0.02 (n=23520)	2.02±0.05 (n=968)	1.80±0.03 (n=1070)
(-)	*1.84±0.02 (n=11996)*	*2.02±0.08 (n=515)*	*2.24±0.07 (n=537)*
(+)	*1.82±0.02 (n=11524)*	*2.01±0.07 (n=453)*	*1.88±0.07 (n=533)*
ATM$^{-/-}$ #2 (T)	1.90±0.01 (n=28291)	1.98±0.06 (n=916)	2.02±0.06 (n=820)
(-)	*1.90±0.02 (n=14527)*	*1.84±0.08 (n=522)*	*2.24±0.09 (n=397)*
(+)	*1.90±0.02 (n=13764)*	*2.15±0.09 (n=394)*	*1.82±0.07 (n=423)*
53BP1$^{-/-}$ #1 (T)	2.28±0.02 (n=15136)	2.68±0.07 (n=526)	2.85±0.06 (n=585)
(-)	*2. 28±0.02 (n=7628)*	*2.68±0.11 (n=247)*	*2.98±0.09 (n=318)*
(+)	*2. 28±0.02 (n=7508)*	*2.68±0.10 (n=279)*	*2.69±0.09 (n=267)*
53BP1$^{-/-}$ #2 (T)	2.43±0.01 (n=27103)	2.62±0.04 (n=1754)	2.68±0.03 (n=2146)
(-)	*2. 42±0.02 (n=13845)*	*2.75±0.06 (n=738)*	*2.82±0.05 (n=1159)*
(+)	*2. 43±0.02 (n=13258)*	*2.53±0.05 (n=1016)*	*2.52±0.05 (n=987)*
Ligase4$^{-/-}$ #1 (T)	2.36±0.01 (n=19016)	2.91±0.05 (n=1128)	2.80±0.05 (n=908)
(-)	*2.36±0.02 (n=9705)*	*3.02±0.07 (n=439)*	*2.95±0.07 (n=541)*
(+)	*2.36±0.02 (n=9311)*	*2.83±0.06 (n=689)*	*2.55±0.07 (n=367)*
Ligase4$^{-/-}$ #2 (T)	2.56±0.01 (n=31505)	2.80±0.03 (n=2965)	2.77±0.04 (n=2384)
(-)	*2.56±0.01 (n=16274)*	*2.97±0.05 (n=1186)*	*2.94±0.04 (n=1346)*
(+)	*2.57±0.01 (n=15231)*	*2.69±0.04 (n=1779)*	*2.49±0.04 (n=1037)*

Table S9 Summary for junctions analyzed in each experiment replicate in Sμ, Sα and genome-wide translocations from CH12F3 cells with various genotypes

Genotype	genome-wide translocations		core Sμ translocations		core Sα translocations	
	#	% direct joins/ MH ≥ 4bp	#	% direct joins/MH ≥ 4bp	#	% direct joins/MH ≥ 4bp
WT						
Exp 1	4381	27.6/12.2	528	41.5/11.4	349	37.0/7.7
Exp 2	4233	28.1/11.7	503	34.4/11.7	409	35.2/7.8
Exp 3	3962	31.4/10.7	488	34.0/12.3	377	31.8/7.2
ATM$^{-/-}$ #1						
Exp 1	5979	28.7/14.2	273	28.6/23.1	303	25.1/19.5
Exp 2	7101	30.0/14.3	301	27.2/18.9	295	24.4/19.3
Exp 3	10440	30.0/13.4	394	27.2/21.1	472	23.5/18.6
ATM$^{-/-}$ #2						
Exp 1	8687	28.0/16.0	265	27.5/24.5	231	22.5/20.8
Exp 2	11056	28.1/15.5	370	28.1/20.8	311	27.3/18.3
Exp 3	8548	29.7/13.6	281	29.9/21.4	278	26.3/18.7
53BP1$^{-/-}$ #1						
Exp 1	6352	17.8/19.3	198	13.1/29.3	220	7.3/31.4
Exp 2	4398	17.7/18.9	186	10.8/33.9	194	7.7/30.4
Exp 3	4386	18.9/19.1	142	12.7/32.4	171	6.4/29.8
53BP1$^{-/-}$ #2						
Exp 1	8631	14.7/20.9	532	10.5/26.3	640	5.3/28.75
Exp 2	10356	14.1/21.2	660	12.0/31.4	830	8.2/24.0
Exp 3	8116	14.3/20.3	562	11.2/29.2	676	7.0/22.9
Ligase4$^{-/-}$ #1						
Exp 1	6160	12.5/19.4	387	7.5/36.4	310	6.5/32.6
Exp 2	5913	14.3/18.5	335	9.0/32.5	271	4.8/31.4
Exp 3	6943	12.8/20.1	406	5.7/36.2	327	6.4/27.5
Ligase4$^{-/-}$ #2						
Exp 1	9273	10.6/22.3	828	9.3/35.4	701	6.0/29.2
Exp 2	9783	10.7/23.1	935	9.1/33.6	780	6.7/30.9
Exp 3	12449	10.6/22.6	1202	8.7/34.3	903	6.3/27.4

Table S10 DNA oligos used in this study

Name	Sequences	purpose
Adapter-upper	GCGACTATAGGGCACGCGTGGNNNNNN /3AmMO/	Adaptor oligo
Adapter-lower	/5Phos/CCACGCGTGCCCTATAGTCGC /3AmMO/	Adaptor oligo
5'-Bio-Iμ	/5BiosG/CAGACCTGGGAATGTATGGT	Bio-primer for Iμ bait
5'-RED-Iμ	CACACAAAGACTCTGGACCTC	Red-primer for Iμ bait
c-Myc-Bio-Iμ	/5BiosG/GCCTCGGCTCTTAGCAGACTG	Bio-primer for c-Myc bait
c-Myc-RED-Iμ	CCTCTGAAGCCAAGGCCGATG	Red-primer for c-Myc bait
53BP1-Cas9-1	GTTCCTTCCCAATTCCCACC	gRNA for generating 53BP1-/- CH12F3 cells
53BP1-Cas9-2	GCCTTACCCAGTTCCCGAGG	gRNA for generating 53BP1-/- CH12F3 cells
53BP1-Cas9-3	GGCACTGTTTCTCCAGCTCA	gRNA for generating 53BP1-/- CH12F3 cells
ATM-Cas9-1	GTCCTCAGTCGATTATCACT	gRNA for generating ATM-/- CH12F3 cells
ATM-Cas9-2	TATCTTGATAAACGAGCAGT	gRNA for generating ATM-/- CH12F3 cells
3'-Cα_Cas9	GGAACCTAGGACTGCTGAGT	gRNA for non-productive Sμ-Sα deletion in CH12F3 cells
JH4_Cas9	GGAGCCGGCTGAGAGAAGTT	gRNA for non-productive Sμ-Sα deletion in CH12F3 cells
c-Myc-Cas9	GACGAGCGTCACTGATAGTA	gRNA for producing c-myc bait for HTGTS