

Supplemental Tables:

Table S1. Percentage of cells expressing IgG1 as measured by FACS and by

metaphase-FISH analysis. Related to Figure 1 The increased frequency of switching identified by metaphases is likely related to the fact that some cells identified as switched might have only recombined the unrearranged V(D)J *Igh* allele. Cells with one or two C μ probes deleted were both classified as switched. Switching was measured after 72 hours of activation.

Genotype (n)	FACS (%)	Metaphases (%)
WT (9)	19.9 ± 3.8	21.5 ± 10.5
<i>Atm</i> ^{-/-} (2)	9.5 ± 2.1	14.0 ± 1.4
<i>Trp53bp1</i> ^{-/-} (5)	2.4 ± 0.6	6.5 ± 0.7
<i>H2afx</i> ^{-/-} (2)	6.5 ± 2.1	8.5 ± 0.7
WT+ATMi+PKi (2)	10.5 ± 4.95	14.5 ± 2.12
<i>Rif1</i> ^{-/-} (2)	5.0 ± 1.41	4.0 ± 1.41
<i>Aicda</i> ^{-/-} (2)	0%	0%

Table S2. Chromosomal abnormalities upon activation. Related to Figure 1.

Percentage represents the sum of chromosomal defects observed in each replicate divided by the total number of chromosomes 12 analyzed.

	WT			WT+ATMi			<i>Trp53bp1</i>^{-/-}			<i>Aicda</i>^{-/-}		
	rep1	rep2	%	rep1	rep2	%	rep1	rep2	%	rep1	rep2	%
<i>Igh</i> split ends	9	13	1.4	42	21	4.6	86	47	6.2	0	0	0
<i>Igh</i> Translocation / Dicentric	5	6	0.7	30	14	3.2	19	7	1.2	0	0	0
Chromosome 12 broken	2	1	0.2	7	5	0.9	13	19	1.5	2	1	1.1
Total Chromosomes 12	756	818		806	578		1194	956		124	154	

Table S3. Analysis of the location of the first break. Related to Figures 2 and 3.

Percentage of first break in S_{μ} or $S_{\gamma 1}$ is calculated by summing the occurrence for each category across all replicates and dividing by the sum of all alleles across replicates where the location of the first break can be determined (Total DSB origin). Unknown refers to *Igh* alleles where break location cannot be determined and its percentage is calculated in comparison to all *Igh* alleles identified with broken ends (Total Split *Igh*). The Total Split *Igh* category is the result of the sum of Total DSB Origin and Unknown categories. The Total *Igh* alleles category refers to the total number of chromosomes 12 analyzed for each replicate.

WT + DMSO	rep1	rep2	rep3	rep4	rep5	rep6	rep7	rep8	rep9	%	
Break S_{μ}	2	8	1	0	0	0	6	0	1	94.7	
Break $S_{\gamma 1}$	0	1	0	0	0	0	0	0	0	5.3	
Total DSB Origin	2	9	1	0	0	0	6	0	1		
Unknown	7	4	0	1	1	0	1	0	2	45.7	
Total Split <i>Igh</i>	9	13	1	1	1	0	7	0	3		
Total <i>Igh</i> alleles	756	818	908	372	424	271	1280	786	2		
<i>Atm</i>^{-/-}	rep1	rep2									%
Break S_{μ}	27	25									96.3
Break $S_{\gamma 1}$	2	0									3.7
Total DSB Origin	29	25									
Unknown	11	2									19.4
Total Split <i>Igh</i>	40	27									
Total <i>Igh</i> alleles	450	294									
WT + ATMi	rep1	rep2	rep3	rep4	rep5	rep6	rep7	rep8	%		
Break S_{μ}	18	11	24	7	9	10	8	0	94.6		
Break $S_{\gamma 1}$	0	0	0	2	1	2	0	0	5.4		
Total DSB Origin	18	11	24	9	10	12	8	0			
Unknown	24	10	17	2	2	1	2	0	38.7		
Total Split <i>Igh</i>	42	21	41	11	12	13	10	0			
Total <i>Igh</i> alleles	806	578	3274	494	728	418	732	220			

<i>Trp53bp1</i>^{-/-}	rep1	rep2	rep3	rep4	rep5	%
Break S _μ	18	20	7	4	39	46.6
Break S _{γ1}	41	13	9	4	34	53.4
Total DSB Origin	59	33	16	8	73	
Unknown	27	14	10	6	69	40.0
Total Split <i>Igh</i>	86	47	26	14	142	
Total <i>Igh</i> alleles	1194	956	946	44	3972	

WT+ATMi (LPS)	rep1	rep2	%
Break S _μ	16	26	97.7
Break S _{γ1}	1	0	2.3
Total DSB Origin	17	26	
Unknown	4	10	24.6
Total Split <i>Igh</i>	21	36	
Total <i>Igh</i> alleles	352	1690	

<i>Trp53bp1</i>^{-/-} (LPS)	rep1	rep2	%
Break S _μ	13	13	59.1
Break S _{γ1}	10	8	40.9
Total DSB Origin	23	21	
Unknown	7	5	21.4
Total Split <i>Igh</i>	30	26	
Total <i>Igh</i> alleles	1456	1480	

CH12 +ATMi	rep1	%
Break S _μ	36	97.3
Break S _{γ1}	1	2.7
Total DSB Origin	37	
Unknown	5	11.9
Total Split <i>Igh</i>	42	
Total <i>Igh</i> alleles	788	

<i>Nbs1</i>^{hypo}	rep1	rep2	%
Break S _μ	15	4	82.6
Break S _{γ1}	4	0	17.4
Total DSB Origin	19	4	
Unknown	23	14	61.7
Total Split <i>Igh</i>	42	18	
Total <i>Igh</i> alleles	2468	1330	

<i>H2afx</i>^{-/-}	rep1	rep2	%
Break S _μ	36	13	92.5
Break S _{γ1}	4	0	7.5
Total DSB Origin	40	13	
Unknown	19	2	28.4
Total Split <i>Igh</i>	59	15	
Total <i>Igh</i> alleles	1422	311	
DNA PKi +ATMi	rep1	rep2	%
Break S _μ	14	13	90.0
Break S _{γ1}	1	2	10.0
Total DSB Origin	15	15	
Unknown	7	17	44.4
Total Split <i>Igh</i>	22	32	
Total <i>Igh</i> alleles	648	394	
<i>Rif1</i>^{-/-}	rep1	rep2	%
Break S _μ	43	51	83.9
Break S _{γ1}	7	11	16.1
Total DSB Origin	50	62	
Unknown	14	19	22.8
Total Split <i>Igh</i>	64	81	
Total <i>Igh</i> alleles	3482	2966	
<i>Trp53bp1</i>^{-/-} + ATMi	rep1	rep2	%
Break S _μ	10	11	33.9
Break S _{γ1}	32	9	66.1
Total DSB Origin	42	20	
Unknown	19	7	29.5
Total Split <i>Igh</i>	61	27	
Total <i>Igh</i> alleles	746	542	
<i>53BP1</i>^{DR}	rep1	rep2	%
Break S _μ	8	13	53.8
Break S _{γ1}	6	12	46.2
Total DSB Origin	14	25	
Unknown	11	19	42.9
Total Split <i>Igh</i>	25	45	
Total <i>Igh</i> alleles	2146	1316	

<i>Suv4-20h^{dn}</i>	rep1	rep2	%
Break S μ	8	10	100.0
Break S γ 1	0	0	0.0
Total DSB Origin	8	10	
Unknown	2	1	14.3
Total Split <i>Igh</i>	10	11	
Total <i>Igh</i> alleles	605	417	
<i>Suv4-20h1^{ff}/h2^{+/-}</i> +ATMi	rep1	rep2	%
Break S μ	5	7	100.0
Break S γ 1	0	0	0.0
Total DSB Origin	5	7	
Unknown	1	1	14.3
Total Split <i>Igh</i>	6	8	
Total <i>Igh</i> alleles	806	600	