Supporting Information

Gostissa et al. 10.1073/pnas.1324176111

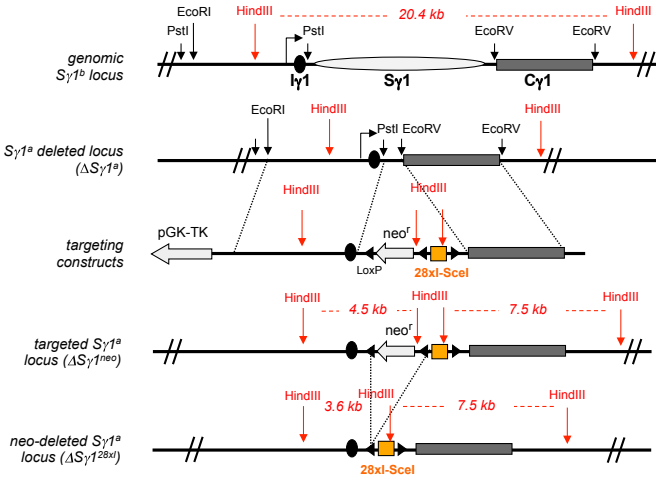

Fig. S1. Targeting strategy for replacement of Sγ1 with a 28× I-SceI site cassette. Targeting strategy for insertion of 28 I-SceI sites in place of Sγ1, as previously described (1). Position of HindIII-cut sites used for diagnostic digests is indicated in red. *LoxP* sites are indicated by black triangles, and I-SceI site cassettes, by orange boxes (not drawn to scale).

1. Zarrin AA, et al. (2007) Antibody class switching mediated by yeast endonuclease-generated DNA breaks. *Science* 315(5810):377–381.

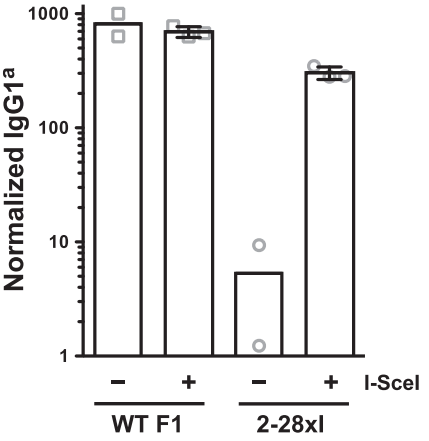

Fig. S2. Efficient I-SceI–mediated class switching in *IgH*$^{2\text{-}28\times I}$ B cells. Class switching to IgG1^{a} as measured by ELISA on supernatants from day 4 LPS/IL-4 B-cell cultures of the indicated genotypes, infected with either control (–) or I-SceI–expressing (+) retrovirus. Each IgG1^{a} data point was normalized against levels of Ig light chains in the same supernatant to control for cell number variation. Error bars represent SD.

A

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AGCATGGCTGAGCTGAGATGGGTGGGCTTCTCTGAGCGCTTCTAAAATGCGCTAAACTGAGGTGA   Sμ
AGCATGGCTGAGCTGAGATGGGTGGGCTTCTCTGAACAGGGTAATAAGATCCACTAGCAGATCTG   breakpoint
CCATAGGGATAaCAGGGTAATTTAATATAGGGATAACAGGGTAATAAGATCCaCTAGCAGATCTG   ΔSγ1-28xI

GATACCTCAGTGGTTTTTAATGGTGGGTTTAATATAGAAGGAATTTAAATTGGAAGCTAATTTAG   Sμ
GATACCTCAGTGGTTTTTAATGGTGGGTTTAATTTTAATATAGGGATAACAGGGTAATAAGATCC   MP67
AGGGTAATAAGATCCATAGGGATAaCAGGGTAATTTAATATAGGGATAACAGGGTAATAAGATCC   ΔSγ1-28xI

GAGCTGAGCTGGGCTGAGCTGGACTGAGCTGAGCTAGGGTGAGCTGAGCTGGGTGAGCTGAGCTA   Sμ
GAGCTGAGCTGGGCTGAGCTGGACTGAGCTGACAGGGTAATAAGATCCACTAGCAGATCTGCTAG   breakpoint
AGGGATAaCAGGGTAaTTTAATATAGGGATAACAGGGTAATAAGATCCaCTAGCAGATCTGCTAG   ΔSγ1-28xI

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GCTGGGGTGAGCTGAGCTGAGCTGGGGTGAGCTTATTTTAATATAGGGATAACAGGGTAATAAGA   breakpoint
GATCCACTAGCAGATCCATAGGGATAACAGGGTAATTTTAATATAGGGATAACAGGGTAATAAGA   ΔSγ1-28xI

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ATTTTGAGGAAATCTTAGAAAACGTAACAGGGTAATAAGATCCACTAGCAGATCTGCTAGTTCTA   breakpoint
TAaCAGGGTAATTTAATATAGGGATAACAGGGTAATAAGATCCaCTAGCAGATCTGCTAGTTCTA   ΔSγ1-28xI

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TAACTTTCCTTGAAAAACTAGTAAAAGAAAAATGTTAACAGGGTAATTTTAATATAGGGATAACA   breakpoint
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GGGTGAGCTGATCTGAAATGAGCTACTCTGGAATAGCTGACAGGGTAATAAGATCCACTAAGCAT   breakpoint
GATCCATAGGGATAACAGGGTAATTTTAATATAGGGATAACAGGGTAATAAGATCCACTAGCAGA   ΔSγ1-28xI

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AATTTAGAATCAGTAAGGAGGGACCCAGGCTAAGAAGACCTCGAAGCGGCCTCGACCGCCGCTAAT   breakpoint
CCACTAGCAGATCTGCTAGTTCTAGAGCGGCCGCTCGACCTCGAAGCGGCCTCGACCGCCGCTAAT   ΔSγ1-28xI

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TGAGCTGGAGTGAGCTGAGCTGGGCTGAGCTGGGGTGTCCCTATATTAAAATTACCCTGAACAGGGT breakpoint
TTACCCTGTTATCCCTATGGATCTTATTACCCTGTTATCCCTATTAAAATTACCCTG-------- ΔSγ1-28xI

AGCTGGGGTGAGCTGGGCTGAGCTGGGGTGAGCTGGGCTGAGCTGAGCTGAGCTGGGGTGAG Sμ
AGCTGGGGTGAGCTGGGCTGAGCTGGGGTGACCTGTTATCCCTATGGATCTTATTACCCTGTTATAC breakpoint
TATTACCCTGTTATCCCTATATTAAAATTACCCTGTTATCCCTATGGATCTTATTACCCTGTTAT-- ΔSγ1-28xI
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B

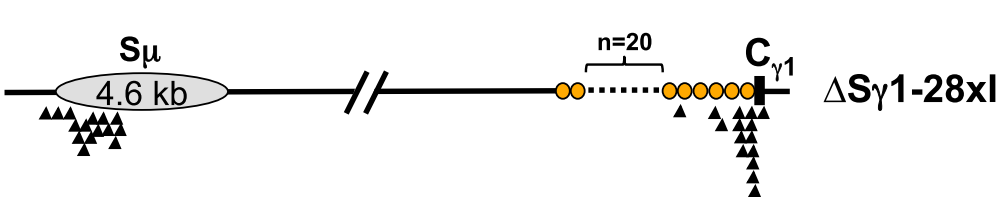
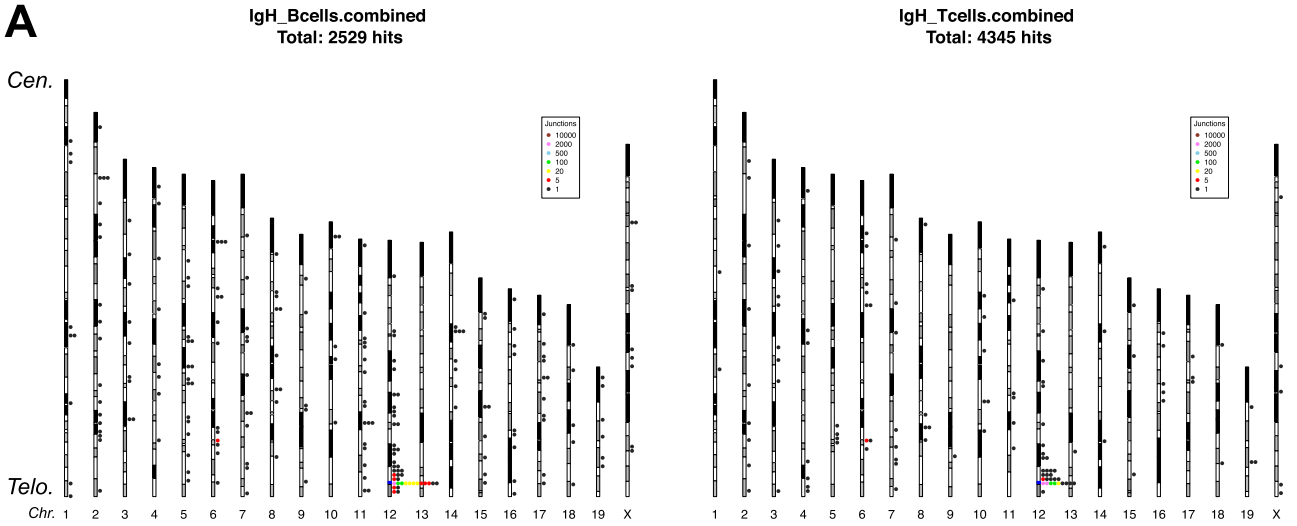


Fig. S3. Analysis of junctional sequences between Sμ and the 28×I cassette in place of Sγ1. (*A*) Sequences of break points between Sμ and I-SceI sites obtained from $IgH^{\Delta S\gamma 1\text{-}28\times I}$ hybridomas. I-SceI sites are underlined. (*B*) Schematic summary of the junctions listed in *A*. The black triangles indicate break points; the orange circles represent single I-SceI sites.

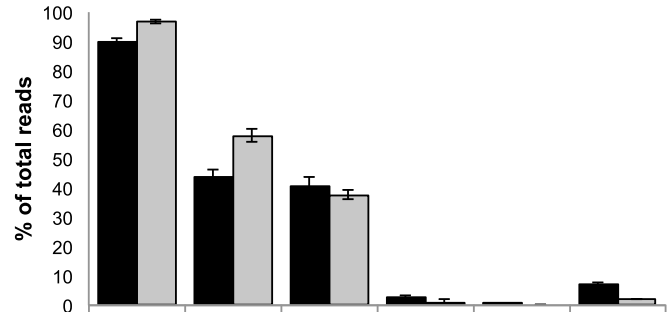
A



B

MOUSE N.	CELL TYPE	TOTAL JUNCT.	breaksite (300kb)	28x I (30kb)	2x I (30kb)
23	B	483	438	213	202
21	B	1115	1007	517	411
19	B	933	831	388	400
23	T	387	371	215	150
21	T	2707	2625	1623	974
19	T	1253	1219	727	478

C



	breaksite (300kb)	28x I (Sg1)	2x I (Sm)	other chr12	IgL/TCR	other chrs
B cells	90.02	44.02	40.52	2.54	0.07	7.38
T cells	96.71	57.85	37.63	1.15	0.00	2.15

D

	TOTAL JUNCTIONS	2xI (30kb)	2xI (220bp)	Perfect Joins
B cells	2529	1013	568 (56%)	10 (0.99%)
T cells	4345	1602	708 (44%)	4 (0.25%)

Fig. S4. High-throughput genome-wide translocation sequencing (HTGTS) libraries from $IgH^{2\text{-}28\times I}$ B and T cells. (*A*) Maps of translocations originating from the $IgH^{28\times I}$ cassette in α-CD40/IL-4–activated B cells (*Left*) or Con A/IL-2-activated T cells (*Right*). Single translocation junctions are represented by dots located at the corresponding chromosomal position. The bin size is 2 Mb. Clusters of translocations are indicated with color codes, as shown in the legend. *Cen.*, centromere; *Telo.*, telomere. Data are combined from three libraries per each condition. (*B* and *C*) Table and graph showing the numbers and distribution of junctions obtained in the three independent $IgH^{2\text{-}28\times I}$ B-cell and T-cell HTGTS libraries. The *IgH* break site is defined as a 300-kb region comprising the *IgH* locus and including both 2× and 28× I-SceI cassettes (*Chr12:114,450,000–114,750,000*). The 2× I-SceI break site in place of Sμ (2×I) is defined as a 30-kb region from position *114,645,000* to *114,675,000*, including the 2×I cassette and the whole Sμ sequence (present *in trans* on the nonmodified *IgH* allele). The 28× I-SceI break site in place of Sγ1 (28×I) is defined as a 30-kb region from position *114,552,000* to *114,582,000*, not including the 28×I cassette but including the whole Sγ1 sequence (present *in trans* on the nonmodified *IgH* allele). In *C*, error bars represent SD; numbers in the table indicate the average percentage from three independent libraries. "other chr12" refers to reads on chromosome 12 outside the 300-kb break site; "Ig/TCR," to reads on other *Ig* and *TCR* loci; and "others," to reads in all other chromosomal locations. (*D*) Table showing extent of end processing of 2×I break site junctions in combined B- and T-cell HTGTS libraries. Numbers in parentheses indicate percentages relative to the total number of junctions in the 30-kb 2×I break site.

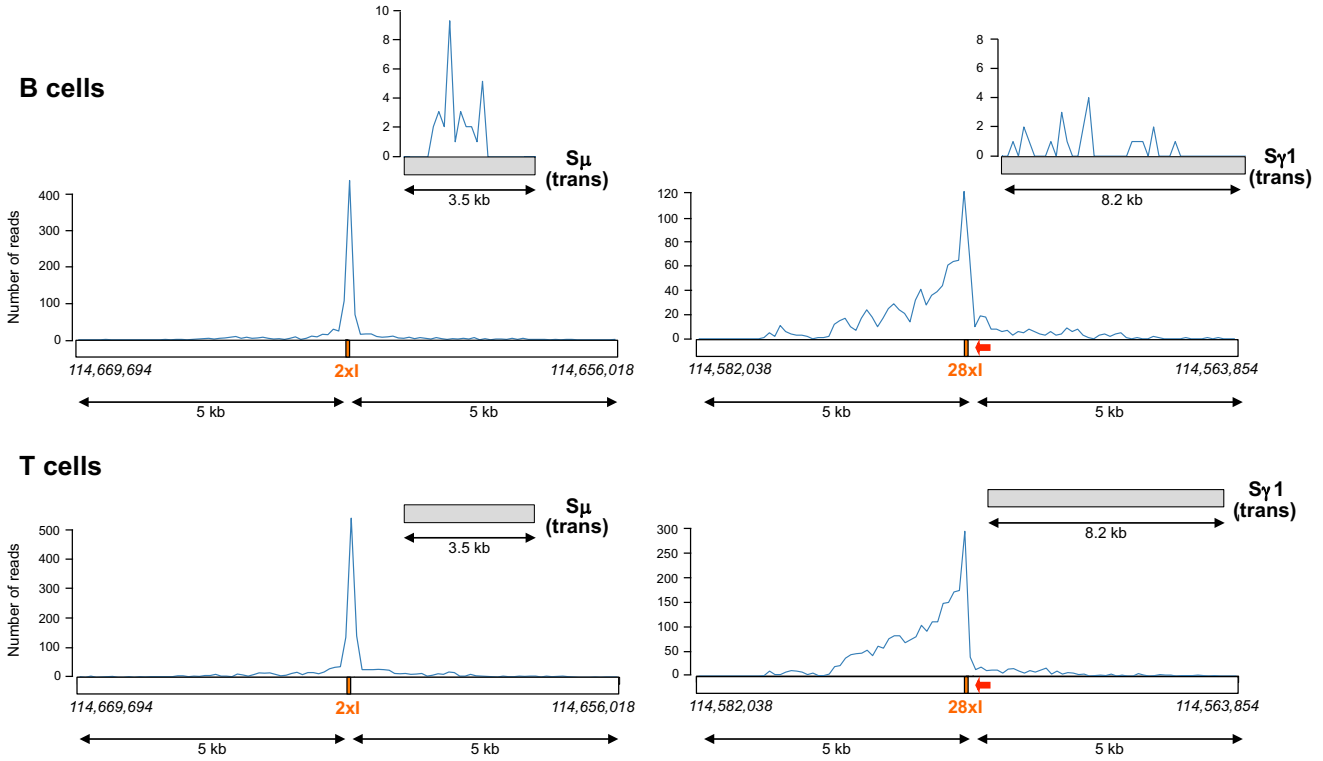


Fig. S5. Distribution of break site junctions in $IgH^{2\text{-}28\times I}$ HTGTS libraries. Details of junction distribution at the 2×I (*Left*) and 28×I (*Right*) break site in combined HTGTS libraries from B cells (*Upper*) and T cells (*Lower*). The regions shown extend 5 kb at each side of the break sites and include either the Sγ1 (8.2 kb) or the Sμ (3.5 kb) regions present *in trans* on the other *IgH* allele. Bin size is 100 bp. Red arrow, orientation of primers used for HTGTS.

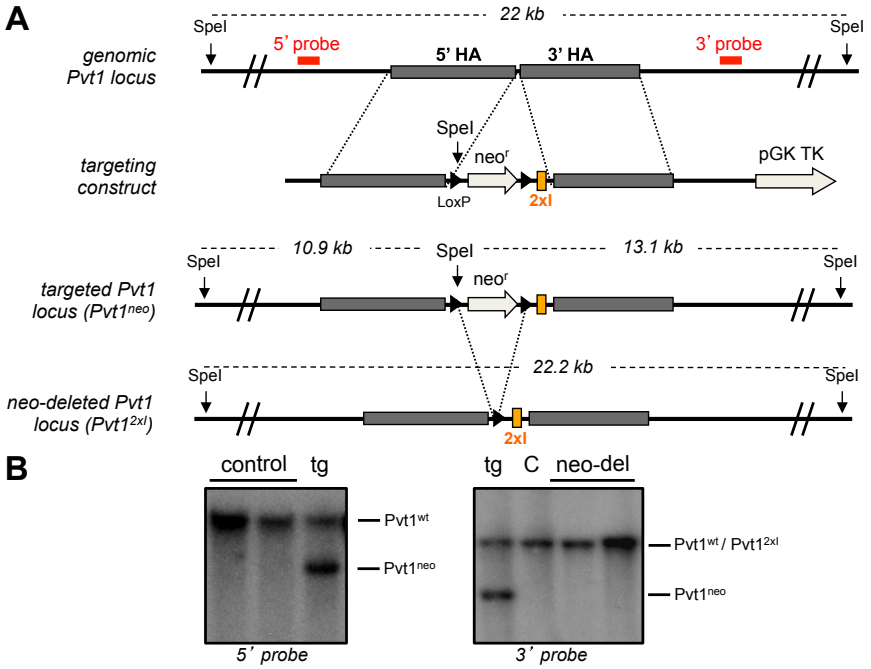
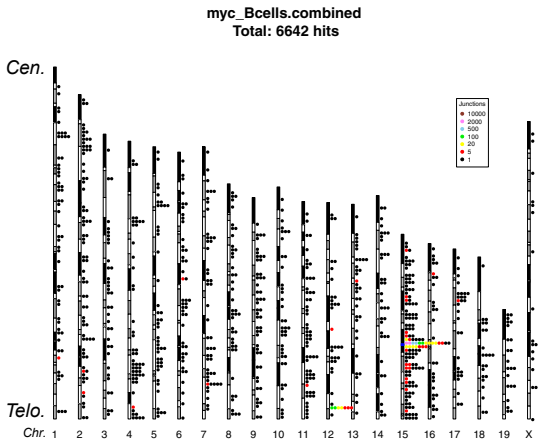


Fig. S6. Targeting strategy for insertion of a 2× I-SceI sites cassette in *Pvt1*. (*A*) Targeting strategy for insertion of two I-SceI sites (2×I) in the *Pvt1* locus at chromosomal position *Chr15:61,914,629*. Location of SpeI-cut sites used for Southern blot analysis with 5′- and 3′-probes is indicated. *LoxP* sites are indicated by black triangles; I-SceI site cassettes by orange boxes (not drawn to scale). (*B*) Southern blot analysis of DNA from control (C) and targeted (tg) ES clones before and after deletion of the Neomycin resistance (neo^r) cassette (neo-del). Genomic DNA was digested with SpeI; probes used are indicated at the bottom of each panel and shown in *A*.

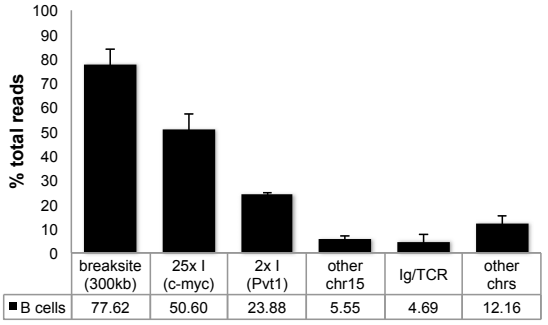
A



B

MOUSE N.	CELL TYPE	TOTAL JUNCT.	breaksite (300kb)	25x I (30kb)	2x I (30kb)	IgH
14	B	2918	2470	1696	672	49
36	B	2850	2085	1316	684	144
37	B	878	659	417	216	61

C



D

	TOTAL JUNCTIONS	2xI (30kb)	2xI (180bp)	Perfect Joins
B cells	6642	1572	775 (49%)	31 (1.97%)

Fig. S7. HTGTS libraries from c-*myc*$^{25\text{-}2\times I}$ B cells. (*A*) Map of translocations originating from the c-*myc*$^{25\times I}$ cassette in αCD40/IL-4–activated B cells. Single translocation junctions are represented by dots located at the corresponding chromosomal position. The bin size is 2 Mb. Clusters of translocations are indicated with color codes, as shown in the legend. *Cen*., centromere; *Telo*., telomere. Data are combined from three independent libraries. (*B* and *C*) Table and graph showing the numbers and distribution of junctions obtained in the three independent c-*myc*$^{25\text{-}2\times I}$–activated B-cell HTGTS libraries. The break site is defined as a 300-kb region encompassing the c-*myc* and *Pvt1* loci (from *Chr15:61,718,880–62,018,880*). The 25×I-SceI (25×I) break site at c-*myc* is defined as a 30-kb region from position *61,803,880* to *61,833,880*; the 2× I-SceI (2×I) break site at *Pvt1* is defined as a 30-kb region from position *61,898,880* to *61,928,880*. In *C*, error bars represent SD; numbers in the table indicate the average percentage from three independent libraries. "other chr15" refers to reads on chromosome 15 outside the 300-kb break site; "Ig/TCR," to reads on *Ig* and *TCR* loci (including *IgH*); and "others chrs," to reads to all other chromosomal locations. (*D*) Table showing extent of end processing of 2×I break site junctions in combined B-cell HTGTS libraries, as in Fig. S4.

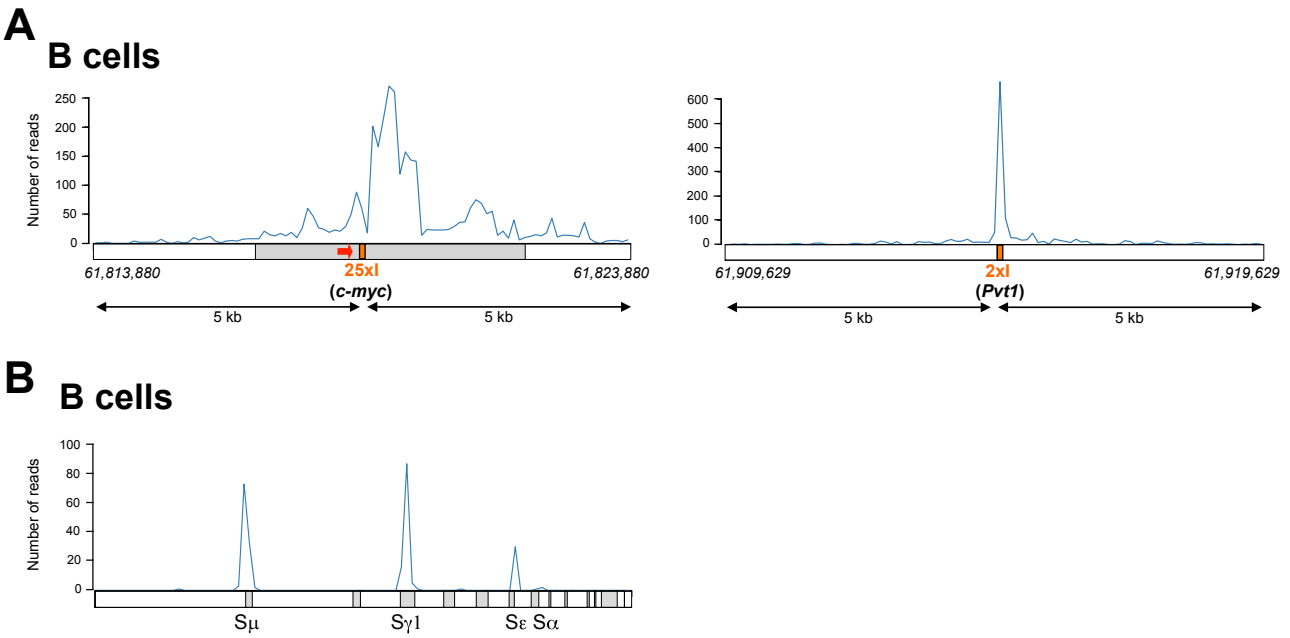
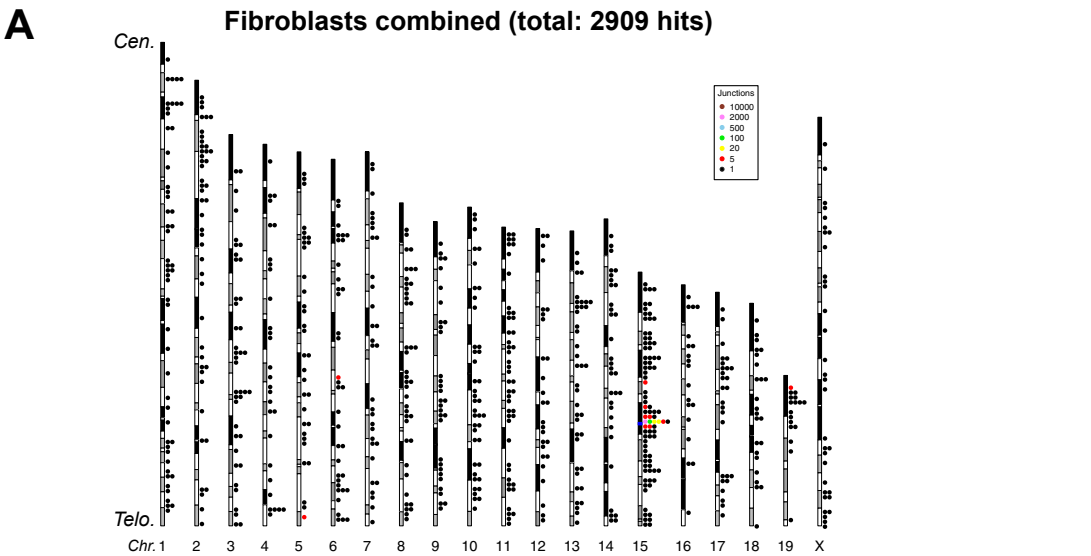
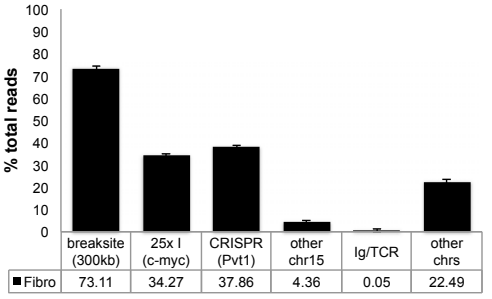


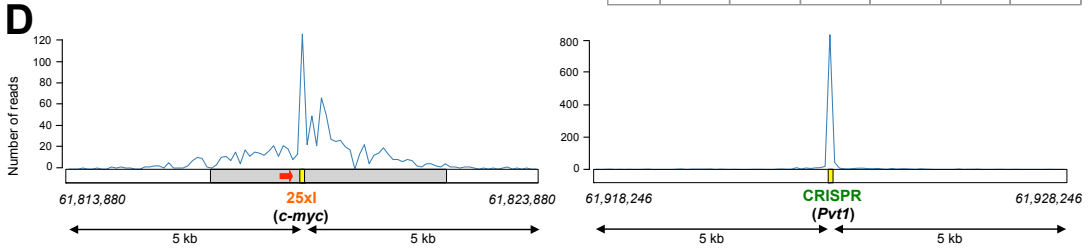
Fig. S8. Distribution of break site junctions in c-*myc*$^{25\text{-}2\times I}$ HTGTS libraries. (*A*) Junction distribution at the c-*myc* 25× I-SceI (25×I) break site (*Left*) and *Pvt1* 2× I-SceI (2×I) break site (*Right*) from three combined HTGTS B-cell libraries. The region shown extends 5 kb at each side of the break site. Bin size is 100 bp. Red arrow, orientation of primers used for HTGTS. (*B*) Distribution of junctions at *IgH* in c-*myc*$^{25\text{-}2\times I}$ B-cell libraries. Bin size is 3 kb.



B

LIBRARY	CELL TYPE	TOTAL JUNCT.	breaksite (300kb)	25x I (30kb)	CRISPR (30kb)	Ig/TCR
BS029	Fibro	769	553	282	264	0
BS030	Fibro	620	469	215	246	0
BS031	Fibro	562	406	186	215	1
AC016	Fibro	980	712	320	384	0





E

	TOTAL JUNCTIONS	CRISPR (30kb)	CRISPR (220bp)	20bp proto-spacer
fibro.	2909	1109	884 (80%)	791 (71%)

Fig. S9. HTGTS libraries from c-*myc*$^{25\times I}$;*ROSA*$^{I\text{-}SceI\text{-}GR}$ fibroblasts. (*A*) Map of translocations originating from the c-*myc*$^{25\times I}$ cassette in the c-*myc*$^{25\times I}$;*ROSA*$^{I\text{-}SceI\text{-}GR}$ fibroblast cell line, upon induction of I-SceI-GR and expression of a *Pvt1*-specific CRISPR/Cas9. Single translocation junctions are represented by dots located at the corresponding chromosomal position. The bin size is 2 Mb. Clusters of translocations are indicated with color codes, as shown in the legend (*Inset*). *Cen*., centromere; *Telo*., telomere. Data are combined from four independent libraries. (*B and C*) Table and graph showing the numbers and distribution of junctions obtained in the four c-*myc*$^{25\times I}$;*ROSA*$^{I\text{-}SceI\text{-}GR}$ fibroblast HTGTS libraries. The break site is defined as a 300-kb region encompassing the *c-myc* and *Pvt1* loci (*Chr15:61,718,880–62,018,880*). The 25× I-SceI (25×I) break site at c-*myc* is defined as a 30-kb region from position *61,803,880* to *61,833,880*; the CRISPR/Cas9 break site in *Pvt1* is defined as a 30-kb region from position *61,908,246* to *61,938,246*. In *C*, error bars represent SD, and numbers in the table indicate the average percentage from three independent libraries. "other chr15" refers to reads on chromosome 15 outside the 300-kb break site; "Ig/TCR," to reads on *Ig* and *TCR* loci (including *IgH*); and "others chrs," to reads to all other chromosomal locations. (*D*) Details of junction distribution in 10-kb regions surrounding the c-*myc* 25×I break site (*Left*) and *Pvt1* CRISPR/Cas9 break site (*Right*) in combined fibroblast HTGTS libraries. Bin size is 100 bp. Red arrow, orientation of primers used for HTGTS. (*E*) Table showing extent of end processing of CRISPR/Cas9 junctions in combined fibroblast HTGTS libraries, as in Fig. S4.