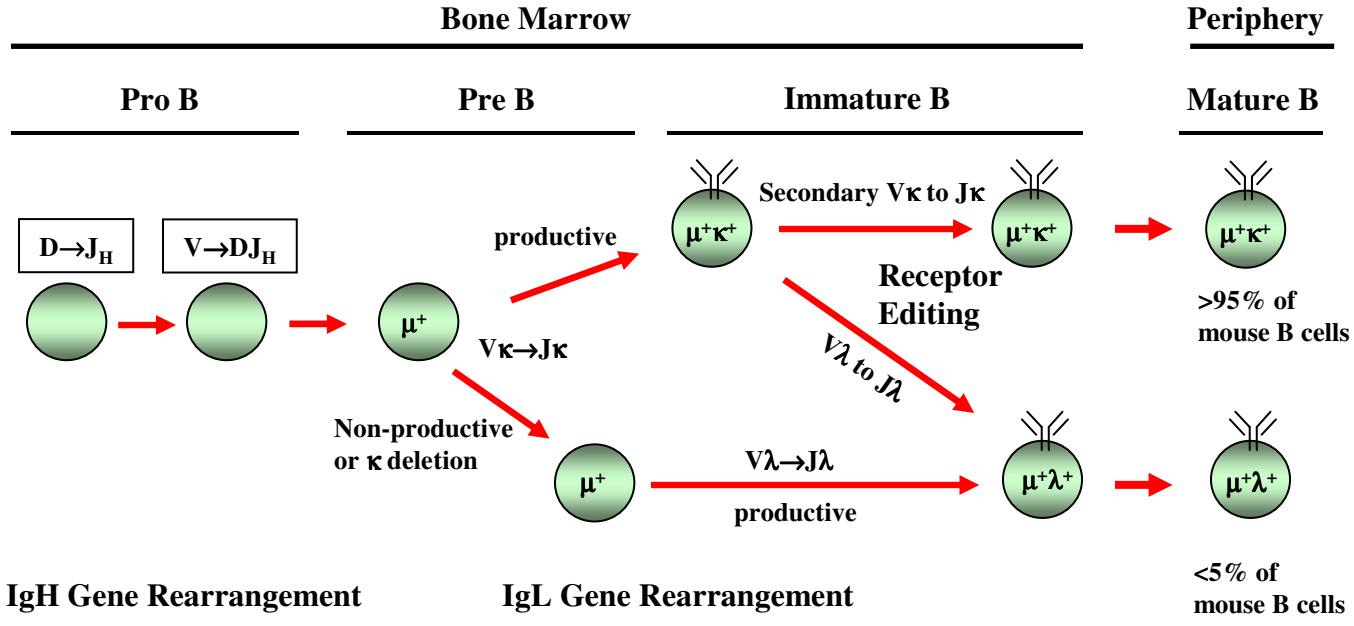
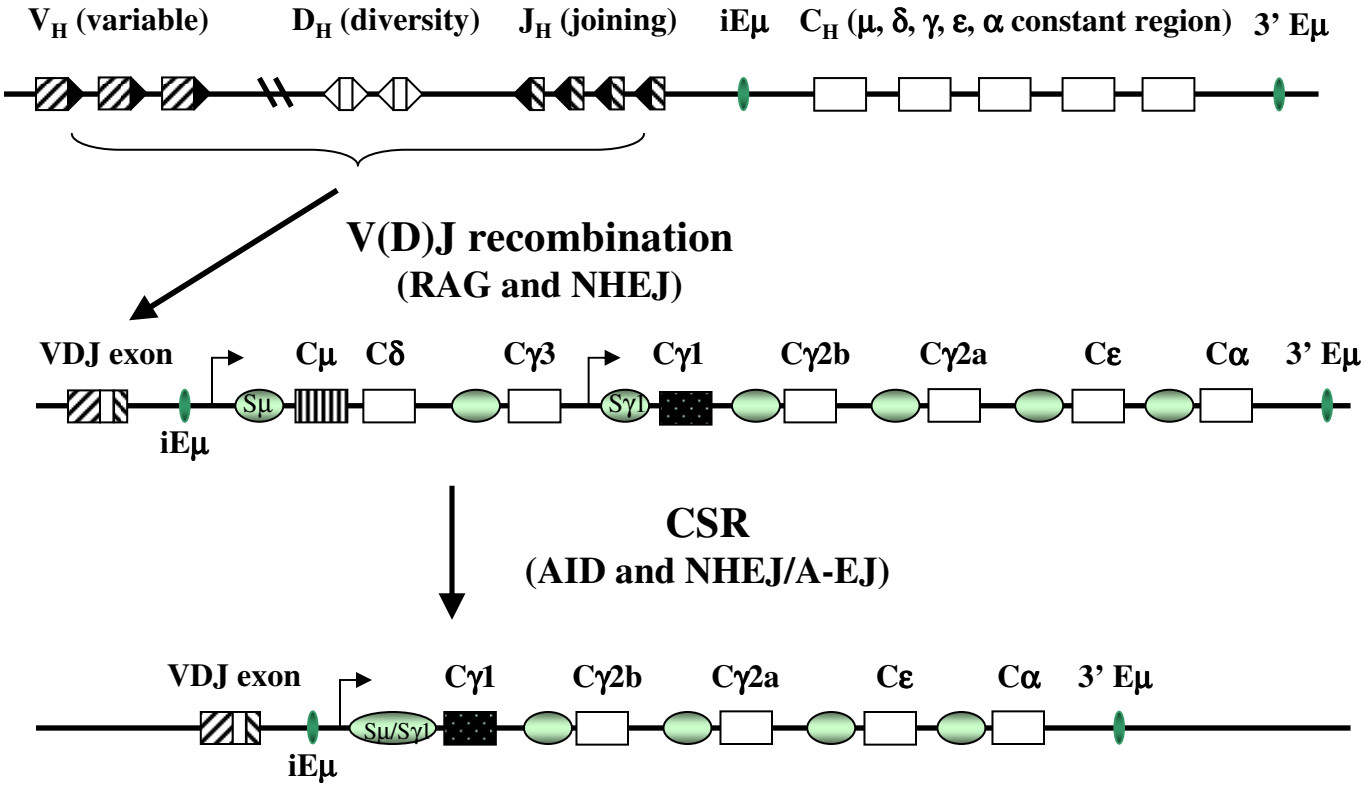
a

Primary and Secondary V(D)J Recombination during B Cell Development

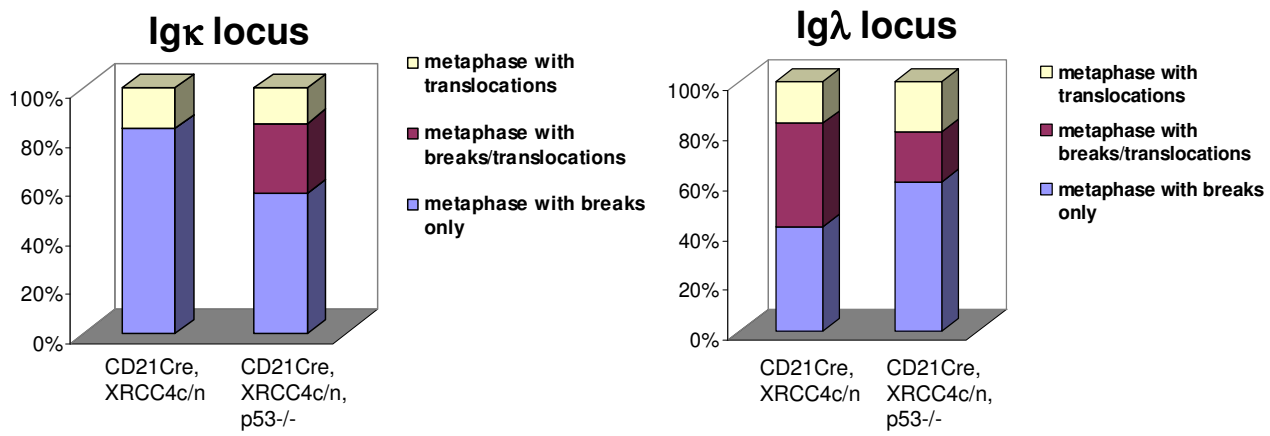


b

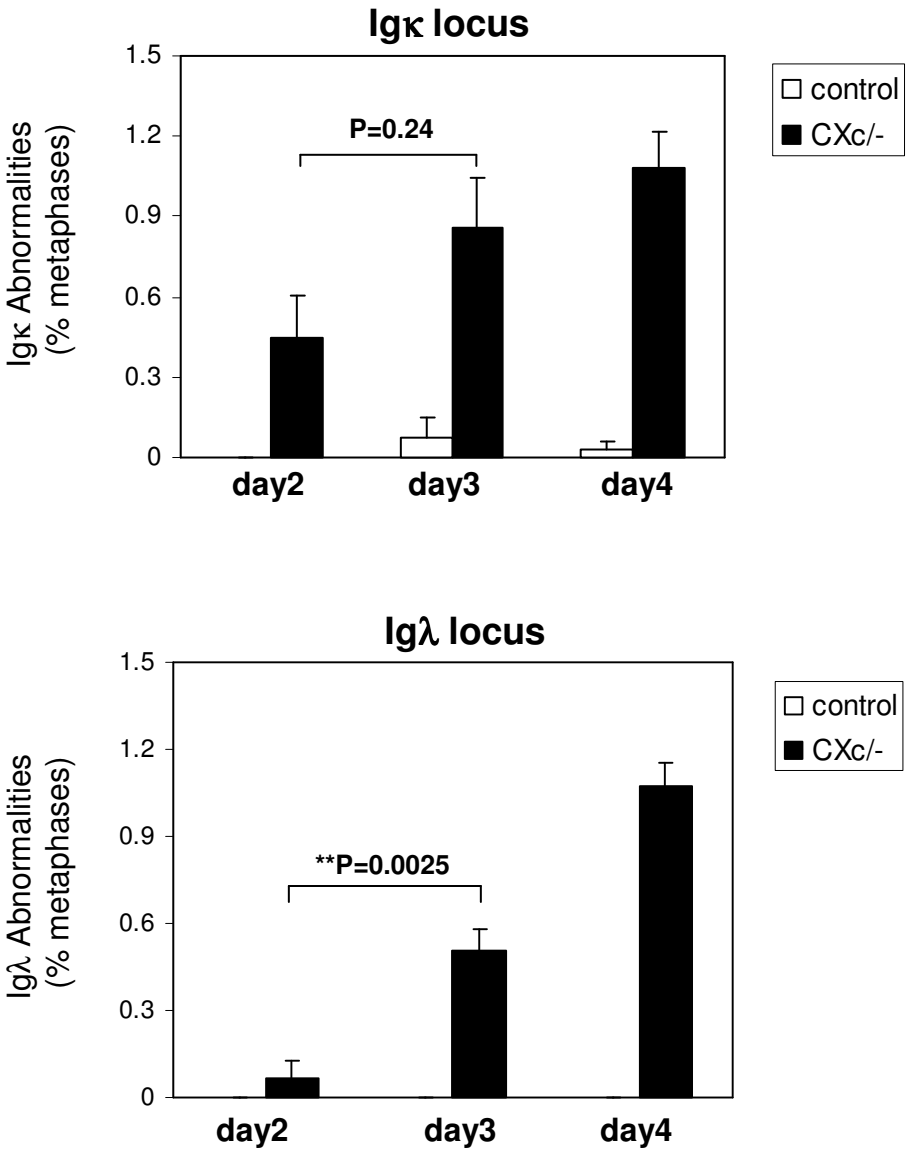
IgH V(D)J recombination and CSR



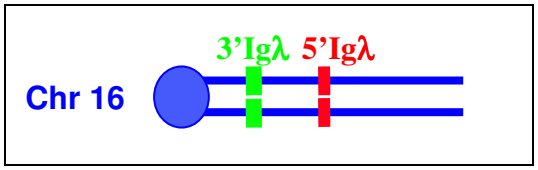
Supplementary Fig.1



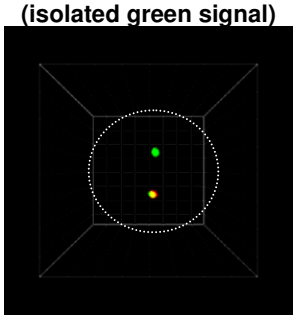
Supplementary Fig.2



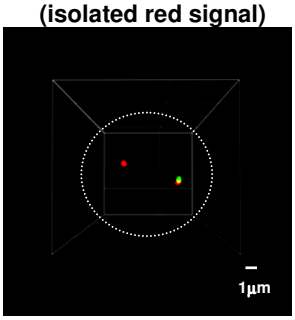
Supplementary Fig.3



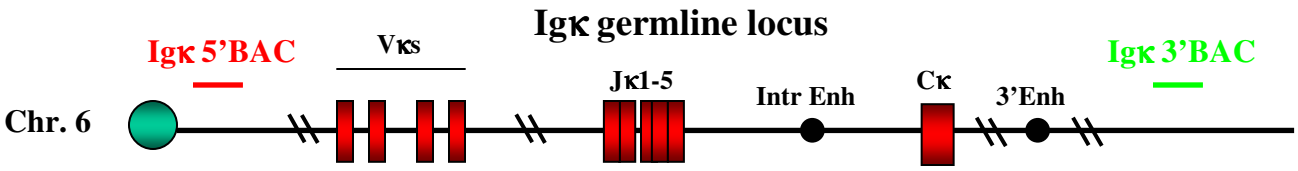
Missing 5'Igλ
(isolated green signal)



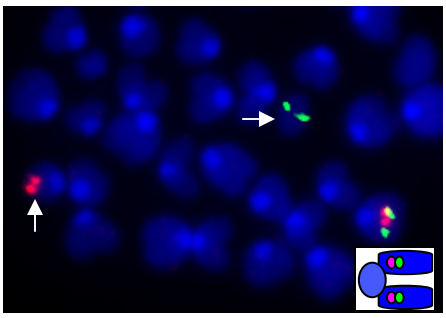
Missing 3'Igλ
(isolated red signal)



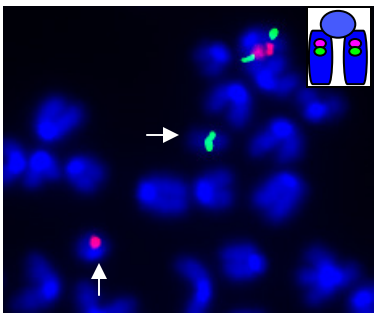
Supplementary Fig.4



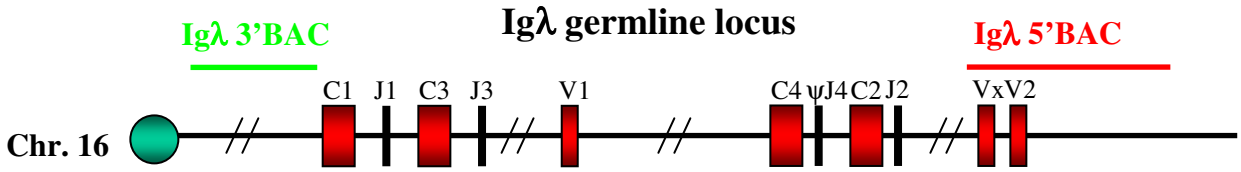
Igκ FISH in *CX$^{c/-}$AID$^{-/-}$* peripheral B cells



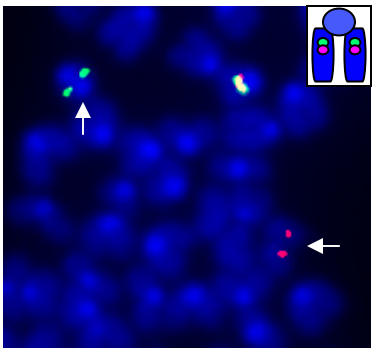
Chromosome break at Igκ



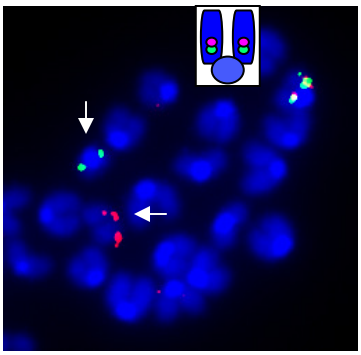
Chromosome break at Igκ



Igλ FISH in *CX$^{c/-}$AID$^{-/-}$* peripheral B cells

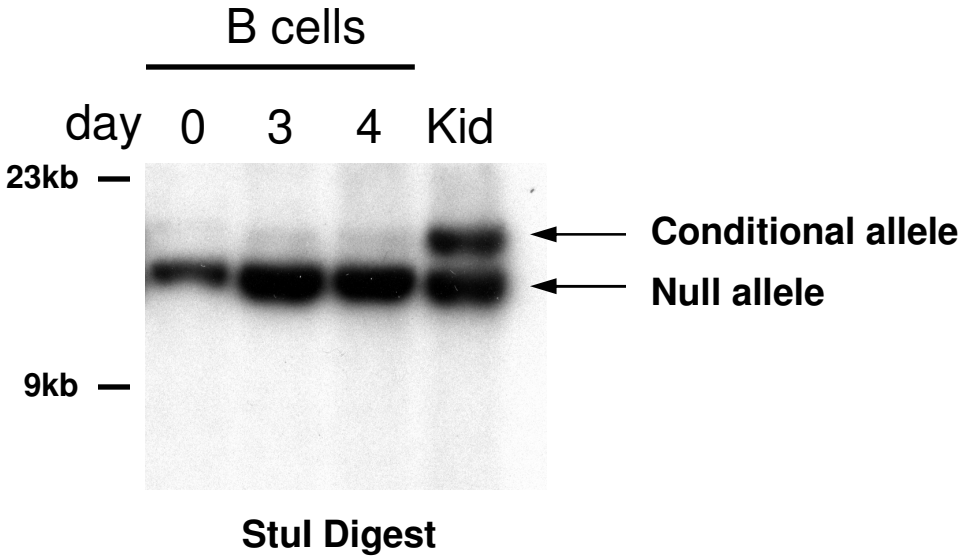


Chromosome break/translocation at Igλ



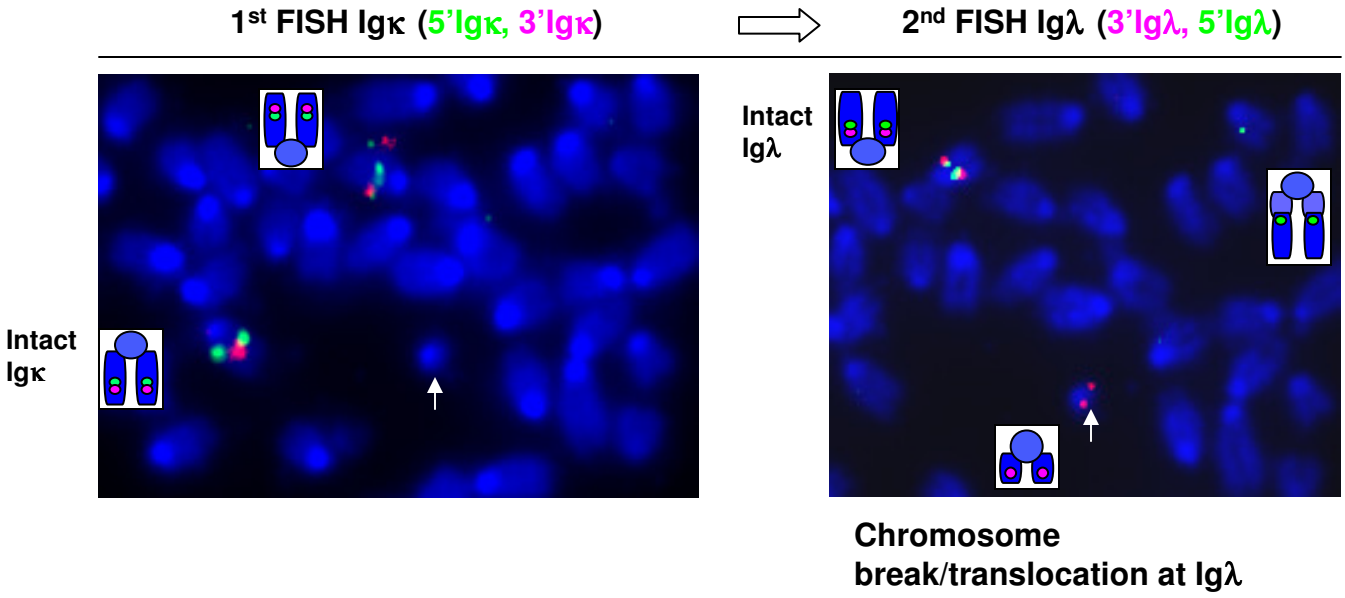
Chromosome break at Igλ

Supplementary Fig.5



Supplementary Fig.6

Sequential FISH on the same metaphase

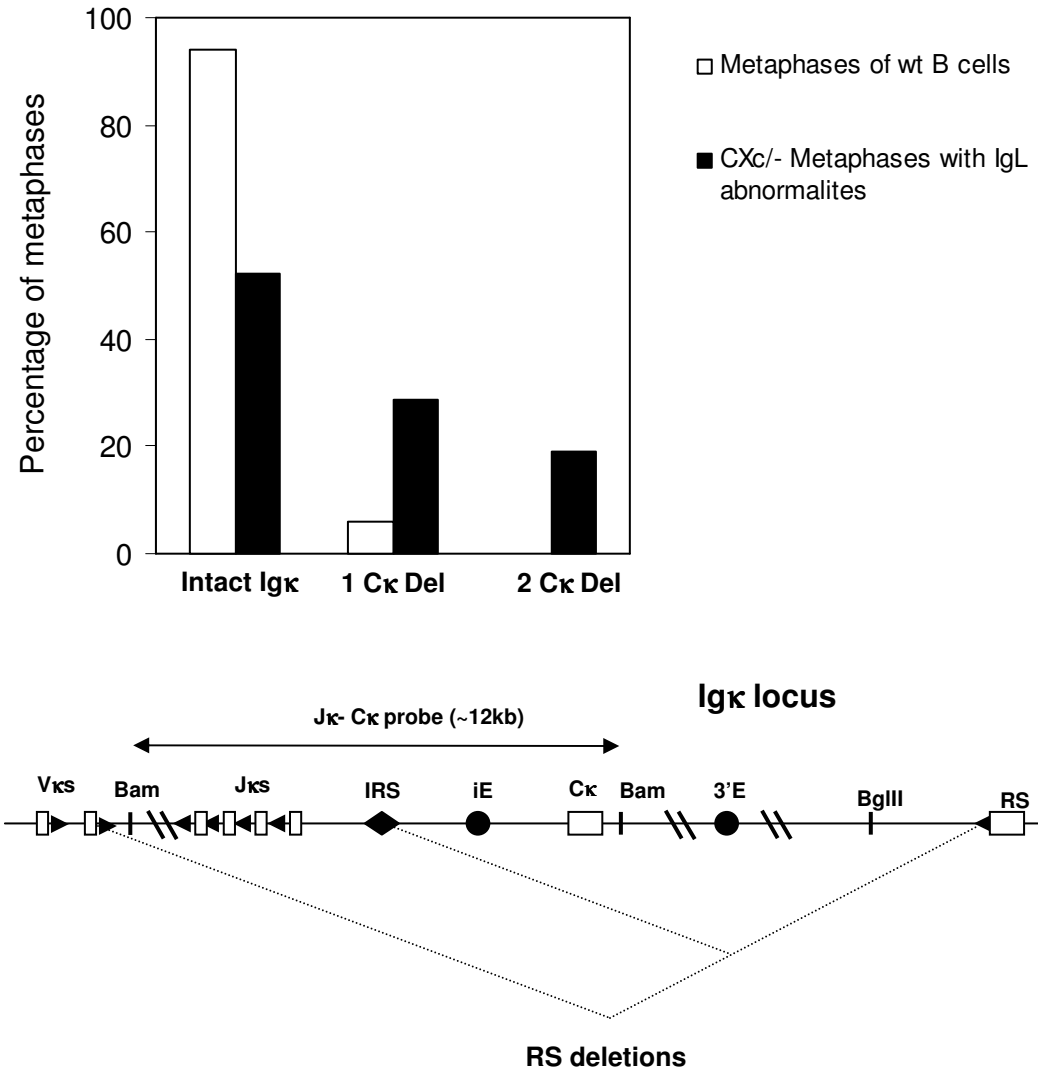


Absence of simultaneous Igκ and Igλ breaks but frequent simultaneous IgH and Igλ abnormalities in the same metaphases from Xrcc4-deficient B cells activated with αCD40/IL4 for 4 days

FISH	Locus	Number of mice	Number of Metaphases	Metaphases with Igκ abnormalities (%)	Metaphases with Igλ abnormalities (%)
1st	Igκ locus	6	1200	12 (1%)	0 out of 12 (0%)
2nd	Igλ locus	6	1200	0 out of 11 (0%)	11(0.92%)

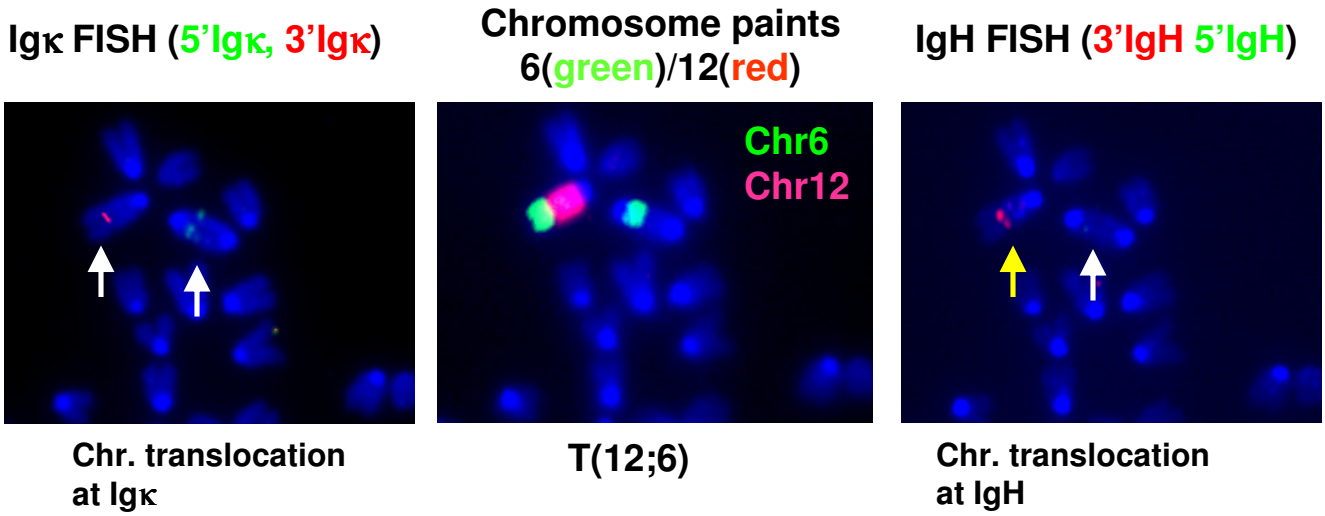
1st FISH	2nd FISH	Number of mice	Number of Metaphases	Metaphases with Igλ/IgH Abnormalities (%)	Metaphases with Igλ abnormalities only (%)
Igλ locus	IgH locus	3	2085	17 (0.82%)	11 (0.53%)

Supplementary Fig.7



Supplementary Fig.8

a

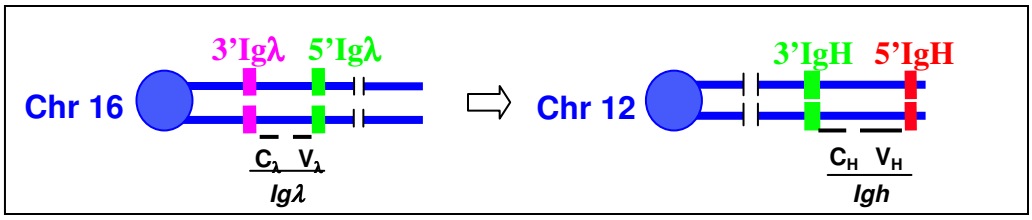


Sequential Igκ FISH, chr paints and IgH FISH on the same metaphases

→ : chrs with Igκ abnormalities; → : chrs with IgH abnormalities

Supplementary Fig.9a

b

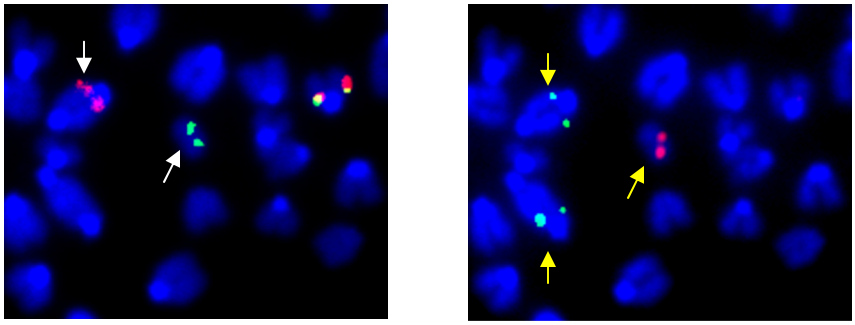


Sequential Igλ and IgH FISH on the same metaphases

→ : chrs with Igλ abnormalities; → : chrs with IgH abnormalities

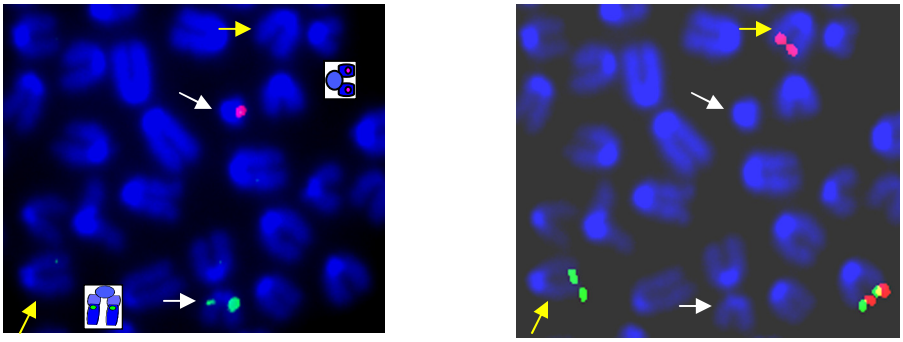
Metaphase 1

1st FISH Igλ (3'Igλ, 5'Igλ) ⇨ 2nd FISH IgH (3'IgH, 5'IgH)



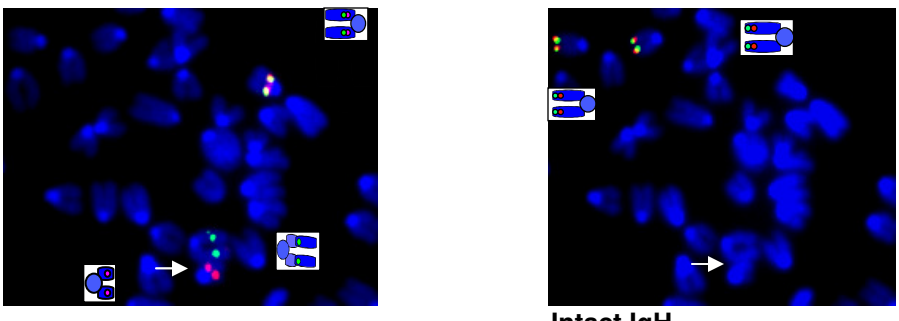
Metaphase 2

1st FISH Igλ (3'Igλ, 5'Igλ) ⇨ 2nd FISH IgH (3'IgH, 5'IgH)



Metaphase 3

1st FISH Igλ (3'Igλ, 5'Igλ) ⇨ 2nd FISH IgH (3'IgH, 5'IgH)



Chromosome break/translocation at Igλ

Intact IgH

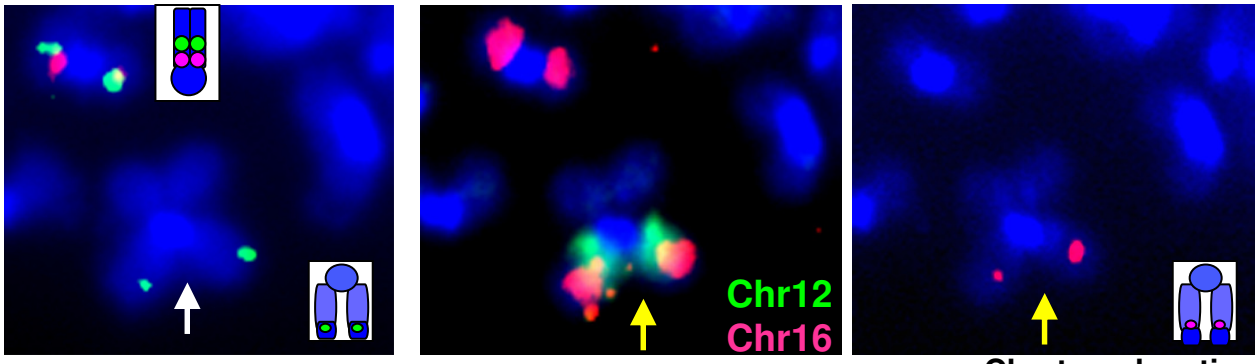
Supplementary Fig.9b

c

Igλ FISH (3'Igλ, 5'Igλ)

Chromosome paints 12(green)/16(red)

IgH FISH (3'IgH, 5'IgH)



Chr. translocation at Igλ

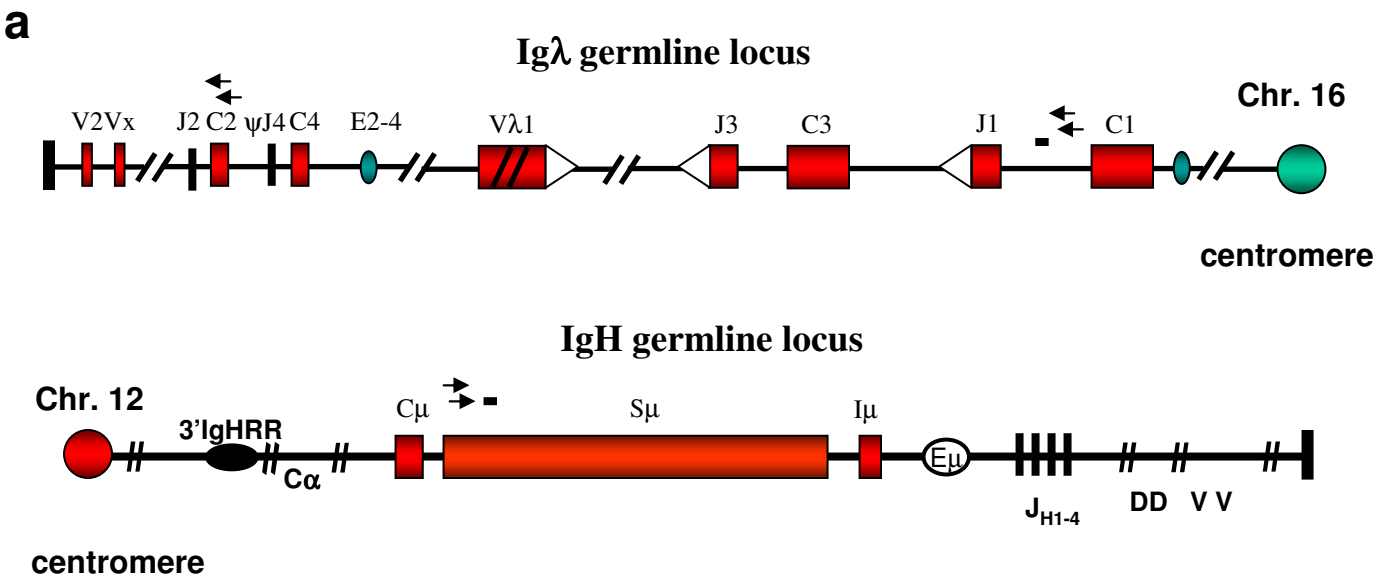
T(12;16)

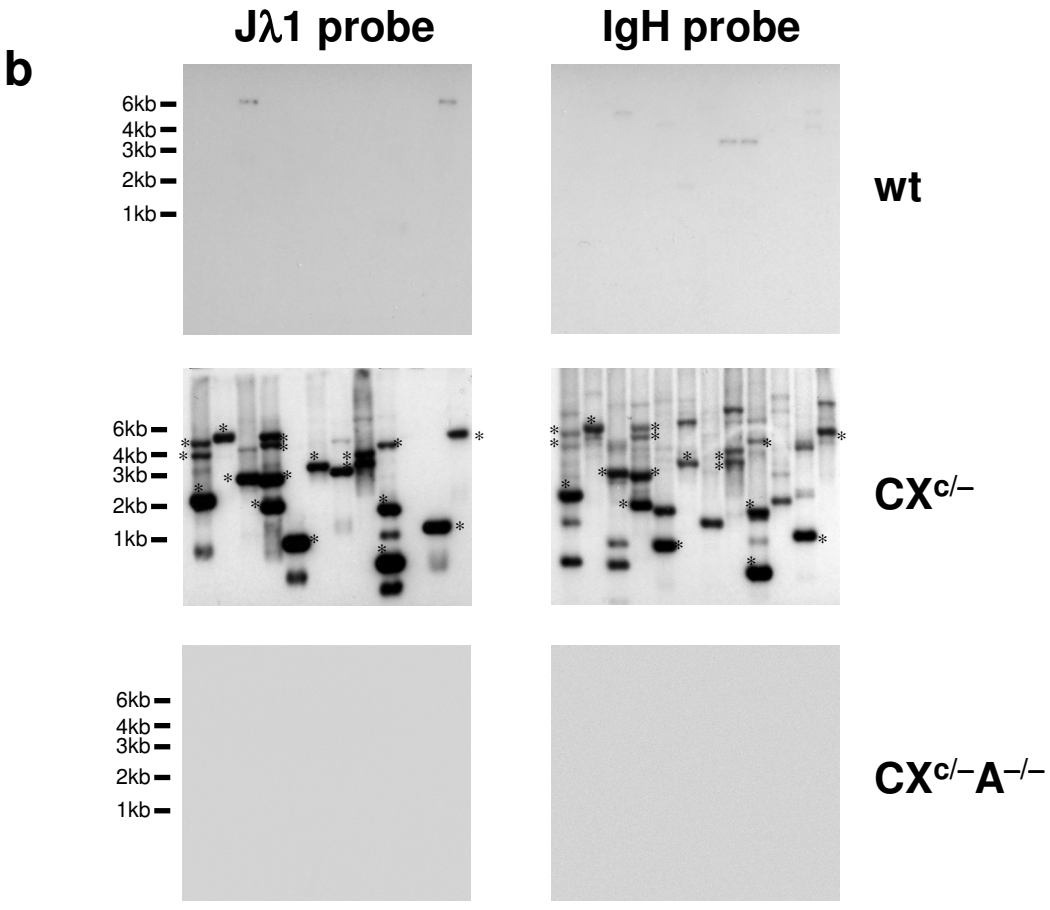
Chr. translocation at IgH

Sequential Igλ FISH, chr paints, and IgH FISH on the same metaphases

→ : chrs with Igλ abnormalities; → : chrs with IgH abnormalities

Supplementary Fig. 9c





Supplementary Fig. 10

Junction 1

Igλ locus (21bp downstream of Jλ1 heptamer)

CATGCAAGGTTTTTTGCATGAGTCTATATCACAGTGCTGGGTGTTCGGTGGAGGAACCAAACTGACTGTCCTAGGTGAGTGACTCCTTCCTCCTTTGTTATTGT

GCCAACTCTAGGGGCATGAACACCCGTTCTCAGGGAGAGGCCTAGATCCTGGCTTCTCAAACTGACTGTCCTAGGTGAGTGACTCCTTCCTCCTTTGTTATTGT

GCCAACTCTAGGGGCATGAACACCCGTTCTCAGGGAGAGGCCTAGATCCTGGCTTCTCAAGTAGCCTTGGGCCTGTCCTGCTTGGCTTCCCTCTGAGACACTG

IgH locus (4731bp downstream of Iμ)

Junction 2

Igλ locus (~80bp downstream of Jλ1 heptamer)

GACTGTCCTAGGTGAGTGACTCCTTCCTCCTTTGTTATTGTTCTCTCCAAGACTTGAGGTGCTTTTTGTTGTATACTTTCCCTTTCTGTATTCTGCTTCATACCTATA

GCTCATTCAATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGAGGTGCTTTTTGTTGTATACTTTCCCTTTCTGTATTCTGCTTCATACCTATA

GCTCATTCAATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGATTTTTAACTCAGTTTAGACCTACTCAGCTCAGGCTGGTCCAGCTCAGGCT

IgH locus (4377bp downstream of Iμ)

Junction 3

Igλ locus (~414bp downstream of Jλ1 heptamer)

TTGCTATCTCATGGAGAAGGAAAACCAGAGTCATAGAGAGAGACACAGATGTTGAACTTGGAGAGCACAGAACATTCAGCACAGAGGCTGGGAAAGTACATGTC

ATTCTCATCCCAAAGTAATAGGTAGGTGGAGGTGGGGGAGGGGCTCAGGCTTGAACTTGGAGAGCACAGAACATTCAGCACAGAGGCTGGGAAAGTACATGTC

ATTCTCATCCCAAAGTAATAGGTAGGTGGAGGTGGGGGAGGGGCTCAGGCTTCTGTATCAGCACACAAAGCTGGGATTTACACACCAGACAGGCTTGTAGGGTA

IgH locus (4626bp downstream of Iμ)

Junction 4

Igλ locus (~3.2kb downstream of Jλ3 heptamer)

CTATCCTGTAACTATAACTTTAACAAAAATGTCATCCCTATATCATGTTAAGAAAGCTGATCCCAGAATTTATATCTTGTTAGCTAAAATTATACTTCTGCATTTTTTAA

CAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCACAGCTCAGCTCACCCTAGCTGATCCCAGAATTTATATCTTGTTAGCTAAAATTATACTTCTGCATTTTTTAA

CAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCCCAGCTCAC

IgH locus (~1.6 kb downstream of Iμ)

Junction5

Igλ locus (463bp downstream of Jλ2 heptamer)

TTACAGAGAGTGAGGTTATCTAAATTAGATCCACAGAAGCATAGTGACAGAGTAGTCTTGTAATAATGTTGAGATCTCTGTTCTTAAACTGGGCTGCTA

CCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTGAGCTGGGGGTGAGCTGGTAGTCTTGTAATAATGTTGAGATCTCTGTTCTTAAACTGGGCTGCTA

CCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCAGCTTACCCCAGCTCACCCCAGCTCAGCTCACCCCAGCTCACCCCAGCTCAGCCAAGCTCAC

IgH locus (2915bp downstream of Iμ)

Junction 6

Igλ locus (18bp downstream of Jλ1 heptamer)

TGCAAGGTTTTTTGCATGAGTCTATATCACAGTGCTGGGTGTTCGGTGGAGGAACCAAACTGACTGTCCTAGGTGAGTGACTCCTTCCTCCTTTGTTATTGTTCTC

ACCCAGCTCAGCTCACCCAGCTCAGCTCACCCCAGCTCACCCCAGACTAACTAACCAAACTGACTGTCCTAGGTGAGTGACTCCTTCCTCCTTTGTTATTGTTCTC

GATCACCCAGCTCAGCTCAGCTCAGCTCACCCCAGCTCACCCCAG--CTCAGCTCAGCTCACCCCAGCTCAGCTCAGCTTACCCCAGCTCACCCCAGCTCAGCTC

IgH locus (2958kb downstream of Iμ)

Junction7

Igλ locus (326bp downstream of Jλ1 heptamer)

TGTTAGGAAATGCAGCACTTCAATAAGAACTCCGTTGTGCTATTACCTTTTAATGTCTATTTTGCTGGTGAACTTTGTGAGGAATAAATTGAATTGCTATCTCATGG

CTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCACCCAGCTCAGCTCAGCTATTTTGCTGGTGAACTTTGTGAGGAATAAATTGAATTGCTATCTCATGG

CTCAGCTCACCCCAGCTCAGCTCACCC----------AGCTCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCACCCAG

IgH locus (3312bp downstream of Iμ)

Junction8

Igλ locus (1276bp downstream of Jλ3 heptamer)

GAGAGTATGCTTTGAAAAATTTAGTGAAAAAACTGTGTGTACTCAACTAGGCTCAATGGGGCAGGTGAGAAAAAAAGAAAAAGTGCCAGGTAAAAAGCATGGCCAA

AGGAACGTGACTTTGGAAGCCTTCACAGACAGGGAGAGATGCCTCTAGAGTAGGTGGGGCAGGTGAGAAAAAAAGAAAAAGTGCCAGGTAAAAAGCATGGCCAA

AGGAACGTGACTTTGGAAGCCTTCACAGACAGGGAGAGATGCCTCTAGAGTAGGTGGATCTGGCCCTTCAGCCAACTCTAGGGGCATGAACACCCGTTCTCAGG

IgH locus (4807bp downstream of Iμ)

Junction 9

Igλ locus (~252bp downstream of Jλ1 heptamer)

CTGTAGCCTTTCATGTCTAATCTCAAACACAGGGGGCTAAAAGAGATAAACCATCAATGTCTGTCTATAATTCTGTTAGGAAATGCAGCACTTCAATAAGAACT

TCAATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGATTTTTAATGTCTGTCTATAATTCTGTTAGGAAATGCAGCACTTCAATAAGAACT

TCAATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGATTTTTAATCAGTTTAGACCTACTCAGCTCAGGCTGGTCCAGCTCAGGCTAGGC

IgH locus (4366bp downstream of Iμ)

Supplementary Fig. 11

Junction 10

Igλ locus (~2010bp downstream of Jλ3 heptamer)

GTGGGGAGGGAGAATCATGGTTCTCAATGAGAAATTAATTTACATCTGAAACATACTGAGGGTACCACCCCAACAGTGACCTTTGCCCTGTTCTGTGCTGTTCTC
ACTGGTCCAGGCCTGGTGAAACTCATTCAGTTCATCCCAGCTCTGCTCATTCATACTGAGGGTACCACCCCAACAGTGACCTTTGCCCTGTTCTGTGCTGTTCTC
ACTGGTCCAGGCCTGGTGAAACTCATTCAGTTCATCCCAGCTCTGCTCATTCAATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGATTTT

IgH locus (~4422bp downstream of Iμ)

Junction 11

Igλ locus (~3079bp downstream of Jλ3 heptamer)

CAAGTTTGAACAGAAAAATTAAGAAACAATGAAAAAGTAATGTTCCCATATCTATGCAACACCAACACACATTTTACCTGGATTTTGATTCACAACTATAACTATTT
AGTTTGGCACAATTCAGGTAGTCTATTCTTAGCTCAACCCAGTTTATCCCAGCTGCAACACCAACACACATTTTACCTGGATTTTGATTCACAACTATAACTATTT
AGTTTGGCACAATTCAGGTAGTCTATTCTTAGCTCAACCCAGTTTATCCCAGCTGCACTCAGTTCAGCTCAATTCATGGTCTCCCAACCCAGCTCAGCCCAGTT

IgH locus (4095bp downstream of Iμ)

Junction 12

Igλ locus (~137bp downstream of Jλ1 heptamer)

GAGGTGCTTTTTGTTGTATACTTTCCCTTTCTGTATTCTGCTTCATACCTATACTTCACACTAGGTAAAGAATTTCTTTCTTCTCTAGATGCTTTGTCTCATTTCAGA
GCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCT
GCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCTCACCCCAGCTCAGCT

IgH locus (~1924bp downstream of Iμ)

Junction 13

Igλ locus (~183bp downstream of Jλ1 heptamer)

ATACCTATACTTCACACTAGGTAAAGAATTTCTTTCTTCTCTAGATGCTTTGTCTCATTTCAGACTGCTCCCTGTAGCCTTTCATGTCTAATCTCAAACACAGGGGGCT
CTCACCCCAGCTCAGCCCAGCTCACCCCAGCTCAGCTCAGCTCAGCTCAGCTCATTTCAGACTGCTCCCTGTAGCCTTTCATGTCTAATCTCAAACACAGGGGGCT
CTCACCCCAGCTCAGCCCAGCTCACCCCAGCTCAGCTCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCAGCTCAGCCCAGCTCACCCCAGCTCAGCTCAGCTCAG

IgH locus (3145bp downstream of Iμ)

Junction 14

Igλ locus (~96bp downstream of Jλ1 heptamer)

TGAGTGACTCCTTCCTCCTTTGTTATTGTTCTCTCCAAGACTTGAGGTGCTTTTTGTTGTATACTTTCCCTTTCTGTATTCTGCTTCATACCTATACTTCACACTAGGTAA
AGGCCAGTTCAACACATCCAACCAGTCCAGCTCAGCATAGTTTAGTCCGGCTCATCTGTATACTTTCCCTTTCTGTATTCTGCTTCATACCTATACTTCACACTAGGTAA
AGGCCAGTTCAACACATCCAACCAGTCCAGCTCAGCATAGTTTAGTCCGGCTCATCTAAATCCTGATAATTTCCAATTGATCCCAGCCCAGTTTGGCACAATTCAGGTA

IgH locus (~4181bp downstream of Iμ)

Junction 15

Igλ locus (~226bp downstream of Jλ1 heptamer)

AGATGCTTTGTCTCATTTCAGACTGCTCCCTGTAGCCTTTCATGTCTAATCTCAAACACAGGGGGCTAAAAGAGATAAACCATCAATGTCTGTCTATAATTCTGTTAG
AATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGATTTTTAACTCAGGGGGCTAAAAGAGATAAACCATCAATGTCTGTCTATAATTCTGTTAG
AATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCTGTTGATTTTTAACTCAGTTTAGACCTACTCAGCTCAGGCTGGTCCAGCTCAGGCTAGGCTAAA

IgH locus (~4364bp downstream of Iμ)

Junction 16

Igλ locus (~312bp downstream of Jλ1 heptamer)

TGTCTGTCTATAATTCTGTTAGGAAATGCAGCACTTCAATAAGAACTCCGTTGTGCTATTACCTTTTAATGTCTATTTTGCTGGTGAACTTTGTGAGGAATAAATTGAA
CACCTCAGCTCAGCTCACTCCAGCTCACCCAGCCCAGTTCACCCTAGCTCAGCTCACTTACCTTTTAATGTCTATTTTGCTGGTGAACTTTGTGAGGAATAAATTGAA
CACCTCAGCTCAGCTCACTCCAGCTCACCCAGCCCAGTTCACCCTAGCTCAGCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCAGCTCAGCTCACCCCAGCTCA

IgH locus (~3480bp downstream of Iμ)

Junction 17

Igλ locus (~2951bp downstream of Jλ3 heptamer)

CTGTCCCTAATAGTTGGAAGTGAAAATCACAGCAATGTTCCTTCTGGAGACAGCCAAAACTCATCCATACCCATTTTTTTTTTCAGAGACTCATAGTCTTGTTTCAAGTTT
AGGTCCTAGCCTAACTGGTCCAGGCCTGGTGAAACTCATTCAGTTCATCCCAGCCAAAACTCATCCATACCCATTTTTTTTTTCAGAGACTCATAGTCTTGTTTCAAGTTT
AGGTCCTA----------ACTGGTCCAGGCCTGGTGAAACTCATTCAGTTCATCCCAGCTCTGCTCATTCAATCTCAGACCTCAGGCTTGTTAAATTAGCTGTTAAATTATCCCT

IgH locus (~4434bp downstream of Iμ)

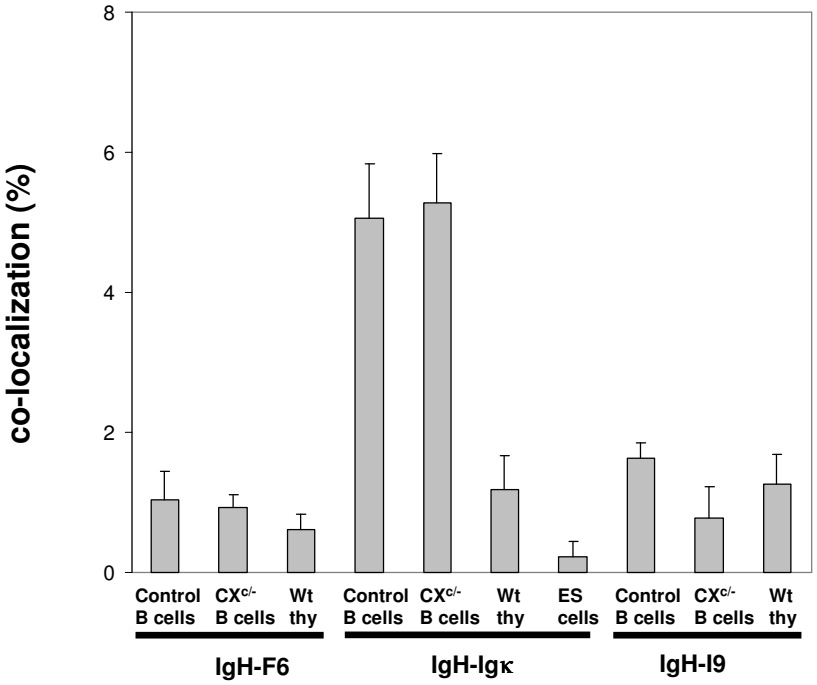
Junction 18

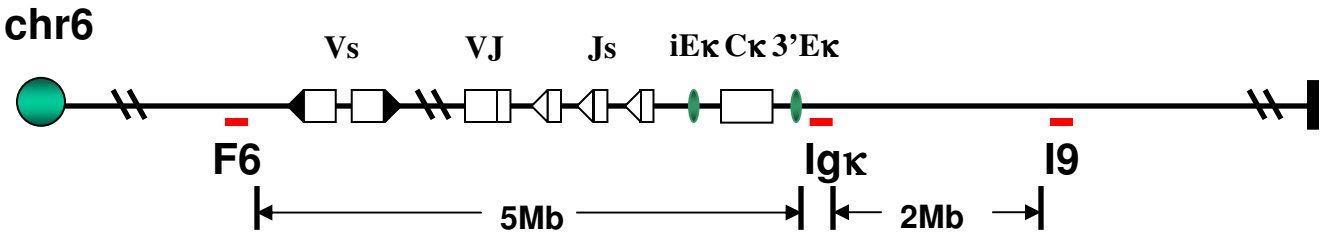
Igλ locus (~2508bp downstream of Jλ3 heptamer)

TCATGTCAGGTGGGGTCAAGGATGACTGAGTGTGACCTGTGAGCTAAATTCAAGCCATTTGCTTGGGTAAACTTAGGTGTAATATACCCCCATTTTACTGACCCATGT
AGTAGCCTTGGGCCTGTCCTGCTTGGCTTCCCTCTGAGACACTGGCTGGGAGAACTATTTGCTTGGGTAAACTTAGGTGTAATATACCCCCATTTTACTGACCCATGT
AGTAGCCTTGGGCCTGTCCTGCTTGGCTTCCCTCTGAGACACTGGCTGGGAGAACTATTCTCATCCCAAAGTAATAGGTAGGTGGAGGTGGGGGAGGGGCTCAGGC

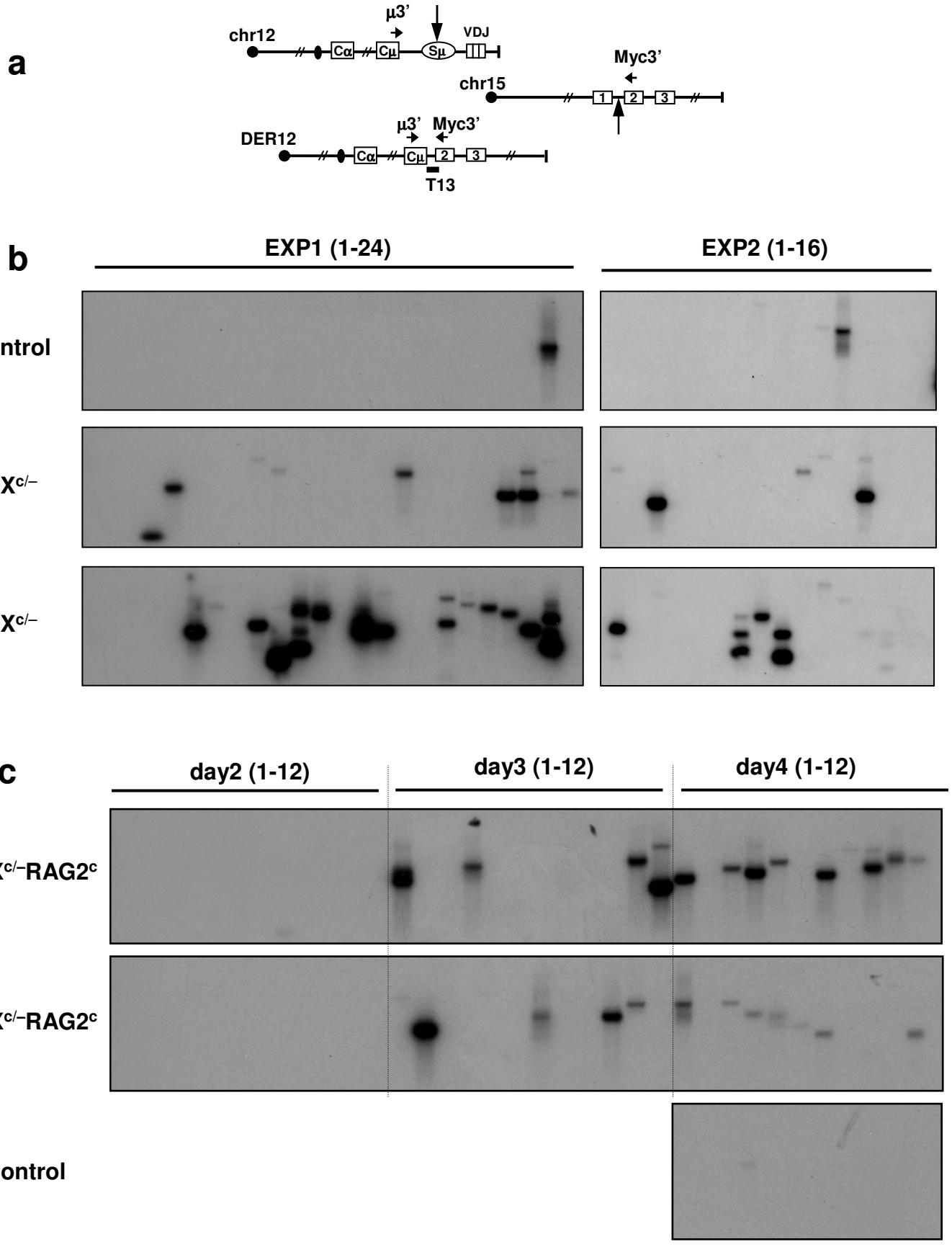
IgH locus (~4676bp downstream of Iμ)

Supplementary Fig. 11

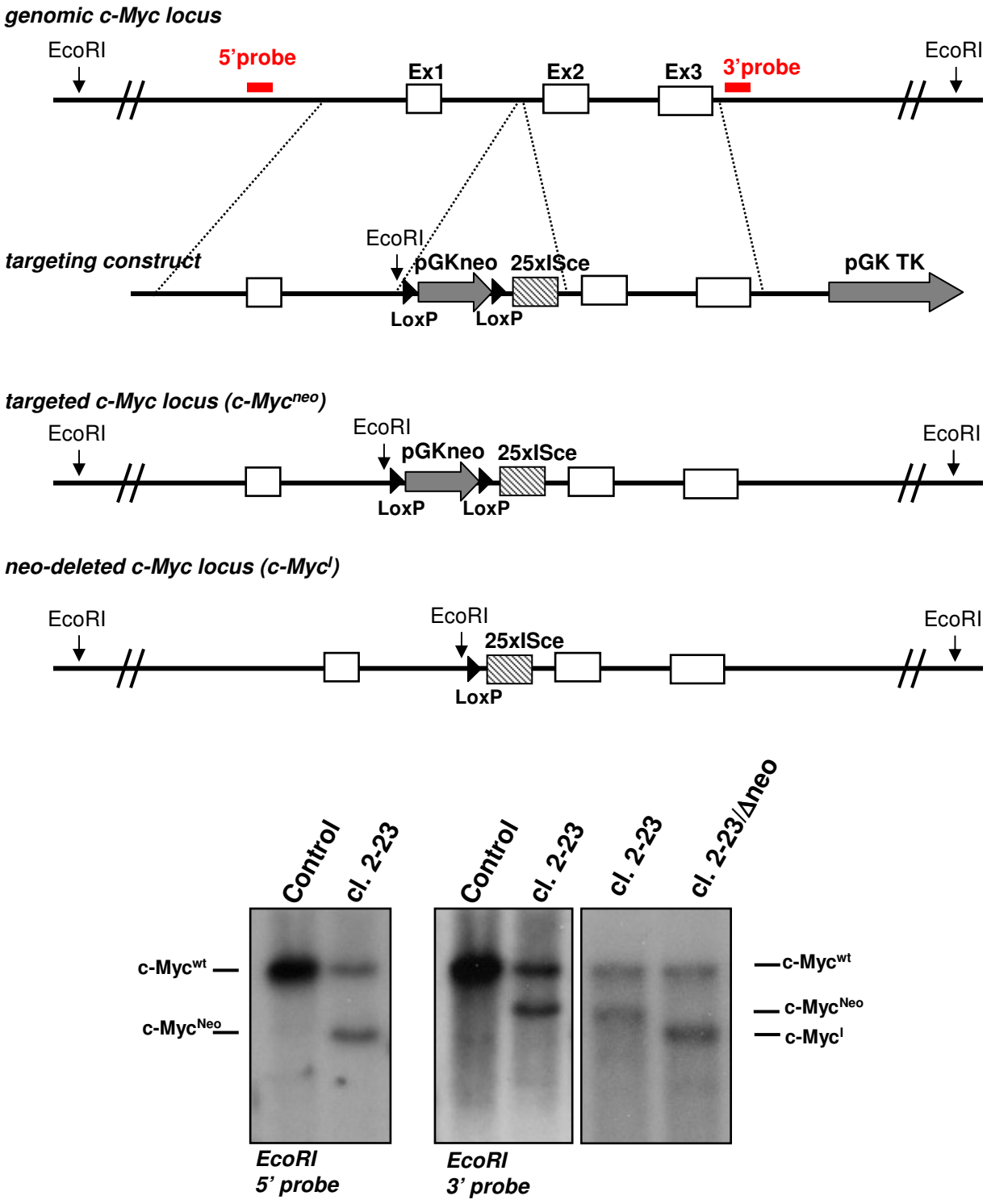




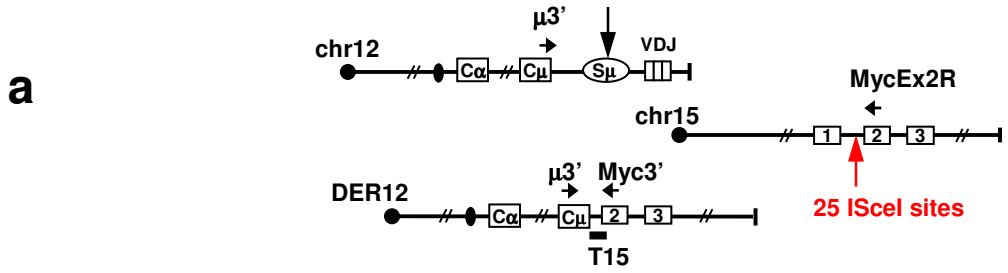
Supplementary Fig. 12

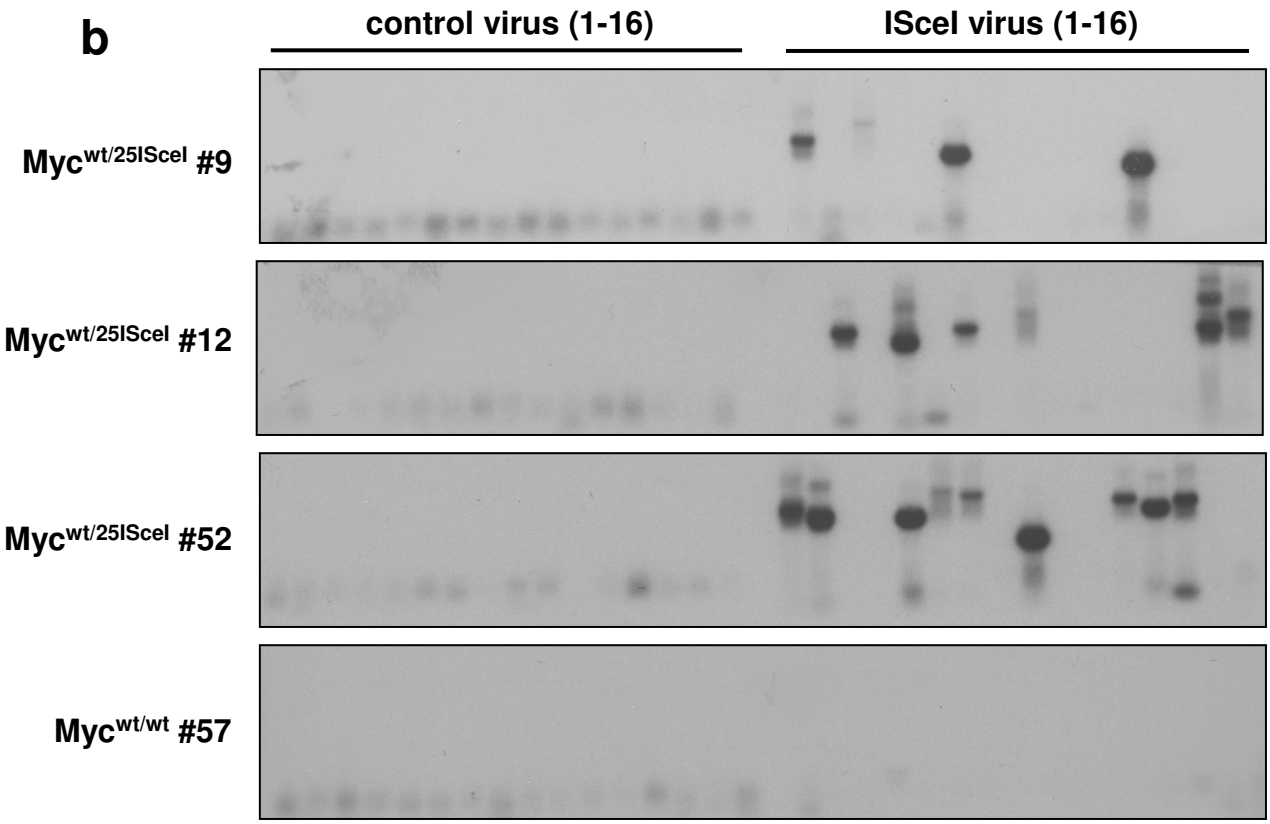


Supplementary Fig. 13a-c



Supplementary Fig. 14





Supplementary Fig. 15a-b

Supplementary Table 1. Frequency of IgH breaks in Xrcc4-deficient peripheral B cells activated with αCD40/IL4 for 4 days

Genotypes	Number of Metaphases Analyzed	IgH Abnormalities	% of IgH Abnormalities
control	51	1	2
control	100	0	0
control	100	0	0
control	128	4	3.125
control	120	1	0.833
control	118	3	2.542
$CX^{c/-}$	61	8	13.1
$CX^{c/-}$	72	11	15.3
$CX^{c/-}$	59	9	15.3
$CX^{c/-}$	62	9	14.5
$CX^{c/-}$	39	12	20.8
$CX^{c/-}$	90	10	11.1
$CX^{c/-}$	120	10	8.3
$CX^{c/-}$	102	17	16.7
$CX^{c/-}$	160	34	21.3
$CX^{c/-}AID^{-/-}$	1658	1	0.06
$CX^{c/-}AID^{-/-}$	1036	0	0
$CX^{c/-}AID^{-/-}$	1245	1	0.08
$CX^{c/-}AID^{-/-}$	1123	0	0
$CX^{c/-}AID^{-/-}$	60	0	0
$CX^{c/-}RAG2^{c}$	65	12	18.5
$CX^{c/-}RAG2^{c}$	68	6	8.8
$CX^{c/-}RAG2^{c}$	61	7	11.5
$CX^{c/-}RAG2^{c}$	70	15	21.4
$CX^{c/-}RAG2^{c}$	68	12	17.6
$CX^{c/-}RAG2^{c}$	71	14	19.7
$CX^{c/-}RAG2^{c}$	63	17	27.0
$CX^{c/-}RAG2^{c}$	62	13	21.0

Supplementary Table 2. General Genomic Instability Measured by Telomere-FISH

Genotype	Number of metaphase analyzed	# with aberrations (%)	total aberrations (%)
1st set of stimulations			
Xc/c	40	0 (0%)	0 (0%)
CXc/-	39	17 (43.6%)	24 (61.5%)
CXc/-p+/-AID-/-	34	9 (26.4%)	10 (29.4%)
2nd set of stimulations			
Xc/c	45	0 (0%)	0 (0%)
CXc/-	49	23 (46.9%)	26 (53.1%)
CXc/-p+/-AID-/-	50	17 (34.0%)	22 (44.0%)
3rd set of stimulations			
Xc/c	50	0 (0%)	0 (0%)
CXc/-	48	17 (35.4%)	19 (39.6%)
CXc/-p+/-AID-/-	50	12 (24.0%)	16 (32.0%)

Percent of aberrations are shown in parentheses (). Nomenclature: C=CD21cre; Xc=Xrcc4 floxed allele; P=p53. B Cells were stimulated with αCD40/IL4 in the culture for 4 days.

Supplementary Table 3. Frequency of Igλ breaks in Xrcc4-deficient peripheral B cells activated with αCD40/IL4 for 4 days

Genotypes	Number of Metaphases Analyzed	Igλ Abnormalities	% of Igλ Abnormalities
control	200	0	0
control	160	0	0
control	180	0	0
control	200	0	0
control	200	0	0
control	200	0	0
control	410	0	0
control	307	0	0
control	300	0	0
control	300	0	0
control	375	0	0
$CX^{c/-}$	400	3	0.75
$CX^{c/-}$	400	3	0.75
$CX^{c/-}$	400	5	1.25
$CX^{c/-}$	400	3	0.75
$CX^{c/-}$	400	6	1.5
$CX^{c/-}$	400	6	1.5
$CX^{c/-}$	300	3	1.0
$CX^{c/-}$	300	3	1.0
$CX^{c/-}$	353	4	1.13
$CX^{c/-}$	537	6	1.12
$CX^{c/-}$	585	6	1.03
$CX^{c/-}AID^{-/-}$	447	5	1.12
$CX^{c/-}AID^{-/-}$	356	3	0.84
$CX^{c/-}AID^{-/-}$	322	4	1.24
$CX^{c/-}RAG2^{c}$	1118	2	0.18
$CX^{c/-}RAG2^{c}$	450	5	1.11
$CX^{c/-}RAG2^{c}$	450	3	0.67
$CX^{c/-}RAG2^{c}$	1053	0	0.00
$CX^{c/-}RAG2^{c}$	420	0	0.00
$CX^{c/-}RAG2^{c}$	608	2	0.33
$CX^{c/-}RAG2^{c}$	687	3	0.44
$CX^{c/-}RAG2^{c}$	558	2	0.36
$CX^{c/-}p53^{-/-}$	200	1	0.50
$CX^{c/-}p53^{-/-}$	120	4	3.33
$CX^{c/-}p53^{-/-}$	200	3	1.5
$CX^{c/-}p53^{-/-}$	200	2	1.0

Supplementary Table 4. Frequency of Igκ breaks in Xrcc4-deficient peripheral B cells activated with αCD40/IL4 for 4 days

Genotypes	Number of Metaphases Analyzed	Igκ Abnormalities	% of Igκ Abnormalities
control	200	0	0
control	200	0	0
control	200	0	0
control	200	0	0
control	200	0	0
control	459	0	0
control	350	0	0
control	312	0	0
control	302	0	0
control	340	1	0.29
$CX^{c/-}$	200	2	1.0
$CX^{c/-}$	200	3	1.5
$CX^{c/-}$	200	1	0.5
$CX^{c/-}$	200	1	0.5
$CX^{c/-}$	200	4	2.0
$CX^{c/-}$	200	3	1.5
$CX^{c/-}$	432	5	1.16
$CX^{c/-}$	368	3	0.82
$CX^{c/-}$	310	3	0.97
$CX^{c/-}$	200	2	1.0
$CX^{c/-}$	211	2	0.95
$CX^{c/-}AID^{-/-}$	424	5	1.18
$CX^{c/-}AID^{-/-}$	398	4	1.01
$CX^{c/-}AID^{-/-}$	385	3	0.78
$CX^{c/-}RAG2^{c}$	525	8	1.52
$CX^{c/-}RAG2^{c}$	450	5	1.11
$CX^{c/-}RAG2^{c}$	477	6	1.26
$CX^{c/-}RAG2^{c}$	454	5	1.10
$CX^{c/-}RAG2^{c}$	373	2	0.54
$CX^{c/-}RAG2^{c}$	416	2	0.48
$CX^{c/-}RAG2^{c}$	450	1	0.22
$CX^{c/-}p53^{-/-}$	160	3	1.88
$CX^{c/-}p53^{-/-}$	200	3	1.5
$CX^{c/-}p53^{-/-}$	200	1	0.5

Supplementary Table 5. Absence of Igκ and Igλ breaks in Xrcc4-deficient ES cells			
Genotypes	**Number of Metaphases Analyzed**	**Igκ Abnormalities**	**Percentage(%)**
control	345	0	0
control	405	0	0
Xrcc4-/-	582	0	0
Xrcc4-/-	536	0	0
Xrcc4-/-	428	0	0
Genotypes	**Number of Metaphases Analyzed**	**Igλ Abnormalities**	**Percentage(%)**
control	247	0	0
control	420	0	0
Xrcc4-/-	500	0	0
Xrcc4-/-	306	0	0
Xrcc4-/-	400	0	0

Supplementary Table 6. Kinetics of Igλ and Igκ abnormalities in activated Xrcc4-deficient peripheral B cells			
Genotypes	**Number of Metaphases Analyzed**	**Igλ Abnormalities**	**% of Abnormalities**
day2			
control	333	0	0
control	300	0	0
control	318	0	0
$CX^{c/-}$	277	0	0
$CX^{c/-}$	340	0	0
$CX^{c/-}$	315	1	0.317
$CX^{c/-}$	400	0	0
$CX^{c/-}$	420	0	0
day3			
control	400	0	0
control	400	0	0
control	397	0	0
$CX^{c/-}$	400	3	0.75
$CX^{c/-}$	400	2	0.5
$CX^{c/-}$	279	1	0.358
$CX^{c/-}$	319	1	0.313
$CX^{c/-}$	335	2	0.597
Genotypes	**Number of Metaphases Analyzed**	**Igκ Abnormalities**	**% of Abnormalities**
day2			
control	389	0	0
control	360	0	0
control	428	0	0
$CX^{c/-}$	457	2	0.438
$CX^{c/-}$	427	0	0
$CX^{c/-}$	865	6	0.694
$CX^{c/-}$	462	3	0.649
day3			
control	379	0	0
control	310	0	0
control	446	1	0.224
$CX^{c/-}$	362	3	0.829
$CX^{c/-}$	208	2	0.962
$CX^{c/-}$	420	2	0.476
$CX^{c/-}$	358	1	0.279
$CX^{c/-}$	456	5	1.096
$CX^{c/-}$	460	7	1.522

Supplemental Table 7. Igλ Abnormalities in CXc/- B cells measured by 3D Interphase FISH

	Number of Interphase Nuclei	Interphase nuclei With Igλ abnormalities	Interphase nuclei with Igλ abnormalities (%)
day0			
control	476	0	0
control	577	0	0
control	550	0	0
$CX^{c/-}$	308	0	0
$CX^{c/-}$	697	1	0.14
$CX^{c/-}$	404	0	0
day4			
control	284	1	0.35
control	418	0	0
control	376	0	0
$CX^{c/-}$	250	6	2.4
$CX^{c/-}$	340	3	0.88
$CX^{c/-}$	322	5	1.55

Supplemental Table 8. IgH-Igλ translocations measured by two color IgH-Igλ FISH on control or CX$^{c/-}$ B cells activated with αCD40/IL4 for 4 days

Genotype	Total Metaphases analyzed	IgH-Igλ translocations	% of translocations
control	1515	0	0
control	1591	0	0
CX$^{c/-}$	800	1	0.125
CX$^{c/-}$	1775	3	0.169
CX$^{c/-}$	1477	4	0.271
CX$^{c/-}$	1634	3	0.184
CX$^{c/-}$AID$^{-/-}$	1658	0	0
CX$^{c/-}$AID$^{-/-}$	1036	0	0
CX$^{c/-}$AID$^{-/-}$	1245	0	0
CX$^{c/-}$AID$^{-/-}$	1123	0	0

Supplementary Table 9. Igλ-IgH co-localization in $X^{c/c}$ and $CX^{c/-}$ B cells and other cell types measured by 3D interphase FISH

Genotypes	Number of interphase nuclei analyzed	Nuclei with Igλ-IgH co-localized	Percentage of nuclei with co-localization
Day0-IgH-Igλ			
Control B cells	108	14	12.96%
$CX^{c/-}$ B cells	109	15	13.76%
Control B cells	122	19	15.6%
$CX^{c/-}$ B cells	127	17	13.4%
Control B cells	104	13	12.5%
$CX^{c/-}$ B cells	111	16	14.4%
15min-IgH-Igλ			
Wt B cells	105	10	9.52%
Wt B cells	112	11	9.82%
Wt B cells	126	14	11.11%
Wt thy	291	2	0.69%
Wt thy	139	0	0%
Wt thy	122	2	1.64%
ES cells	217	1	0.46%
ES cells	144	0	0%
ES cells	140	0	0%

Co-localization: IgH and Igλ signals (<0.5μm), only mono-allelic *IgH* and *Igλ* associations were observed and no nuclei contained co-localized signals for both *IgH* and *Igλ* alleles (not shown).

Supplementary Table 10. Igλ-IgH co-localization in activated control and CX$^{c/-}$ B cells measured by 3D interphase FISH

Genotypes	Number of interphase nuclei analyzed	Nuclei with Igλ-IgH co-localized	Percentage of nuclei with co-localization
Day3.5-IgH-Igλ			
Control B cells	113	11	9.73%
CX$^{c/-}$ B cells	120	14	11.67%
CX$^{c/-}$ B cells	112	9	8.04%
Control B cells	131	9	6.87%
CX$^{c/-}$ B cells	105	5	4.76%
Control B cells	201	10	4.98%
CX$^{c/-}$ B cells	114	6	5.26%
Day3.5-IgH-C2			
Control B cells	122	1	0.82%
CX$^{c/-}$ B cells	110	0	0%
Control B cells	107	1	0.93%
CX$^{c/-}$ B cells	132	2	1.52%
Control B cells	189	4	2.12%
CX$^{c/-}$ B cells	113	1	0.88%
Day3.5-IgH-K10			
Control B cells	120	2	1.67%
CX$^{c/-}$ B cells	111	1	0.9%
Control B cells	123	1	0.81%
CX$^{c/-}$ B cells	125	0	0%
Control B cells	103	1	0.97%
CX$^{c/-}$ B cells	123	2	1.63%

Supplementary Table 11. IgH-chr16 control loci co-localization in $X^{c/c}$ and $CX^{c/-}$ B cells and other cell types by Interphase FISH

Genotype	Number of interphase nuclei analyzed	Nuclei with IgH-control loci co-localized	Percentage of nuclei with co-localization
Day0-IgH-C2			
Control B cells	202	3	1.49%
CXc/- B cells	152	2	1.32%
Control B cells	141	3	2.13%
CXc/- B cells	104	1	0.96%
Control B cells	167	3	1.80%
CXc/- B cells	178	2	1.12%
Day0-IgH-K10			
Control B cells	144	6	4.17%
CXc/- B cells	154	4	2.6%
Control B cells	131	6	4.58%
CXc/- B cells	191	7	3.66%
Control B cells	103	3	2.91%
CXc/- B cells	162	10	6.17%
CXc/- B cells	143	5	3.50%
IgH-C2			
Wt thymocytes	153	1	0.65%
Wt thymocytes	136	2	1.47%
Wt thymocytes	146	3	2.05%
IgH-K10			
Wt thymocytes	130	5	3.85%
Wt thymocytes	141	8	5.67%
Wt thymocytes	108	3	2.78%

Co-localization: IgH and control BACs signals (<0.5μm), only one allele with associated signals, no nuclei has both alleles co-localized

Supplementary Table 12. Igκ-IgH co-localization in control and CX$^{c/-}$ B cells and other cell types by 3D Interphase FISH

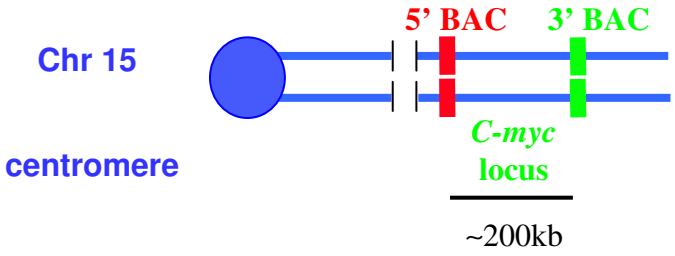
Genotype	Number of Interphase nuclei	Nuclei with Igκ-IgH co-localized	Percentage of nuclei with co-localization
Day0-B cells IgH-Igκ			
Control B cells	227	8	3.52%
CX$^{c/-}$ B cells	225	15	6.67%
Control B cells	216	13	6.02%
CX$^{c/-}$ B cells	248	12	4.84%
Control B cells	213	12	5.63%
CX$^{c/-}$ B cells	116	5	4.31%
Wt thymocytes	0	3	0.96%
Wt thymocytes	220	2	0.91%
Wt thymocytes	311	8	2.57%
Wt thymocytes	360	1	0.28%
ES cells	151	1	0.66%
ES cells	130	0	0%
ES cells	165	0	0%
Genotype	**Number of Interphase nuclei**	**Nuclei with IgH-F6 co-localized**	**Percentage of nuclei with co-localization**
Day0-B cells IgH-F6			
Control	223	4	1.79%
CXc/-	210	2	0.95%
Control	232	1	0.43%
CXc/-	162	2	1.23%
Control	113	1	0.88%
CXc/-	169	1	0.59%
Wt thymocyte	306	2	0.65%
Wt thymocyte	222	2	0.90%
Wt thymocyte	108	1	0.93%
Wt thymocyte	361	0	0%
Genotype	**Number of Interphase nuclei**	**Nuclei with IgH-I9 co-localized**	**Percentage of nuclei with co-localization**
Day0-B cells IgH-I9			
Control	240	5	2.08%
CXc/-	231	2	0.87%
Control	136	2	1.47%
CXc/-	202	3	1.49%
control	152	2	1.32%
CXc/-	176	0	0%
Wt thymocyte	323	3	0.93%
Wt thymocyte	181	4	2.21%
Wt thymocyte	121	2	1.65%
Wt thymocyte	458	1	0.22%

Supplementary Table 13. IgH-cmyc co-localization in B cells and other cell types by 3D Interphase FISH

	Number of Interphase nuclei	Nuclei with Signals co-localized	Percentage of nuclei with co-localization
Day0-B cells IgH-cmyc			
Control B cells	115	5	4.35%
$CX^{c/-}$ B cells	108	4	3.70%
Control B cells	143	9	6.29%
$CX^{c/-}$ B cells	137	11	8.03%
Control B cells	103	5	4.85%
$CX^{c/-}$ B cells	188	8	4.26%
Other cell types for IgH-cmyc			
ES cells	126	0	0%
ES cells	145	0	0%
ES cells	155	0	0%
15 mins activation			
Wt B cells	120	6	5.00%
Wt B cells	160	7	4.38%
Wt B cells	140	5	3.57%
Day3.5-IgH-cmyc			
Control B cells	112	5	4.46%
$CX^{c/-}$ B cells	108	4	3.70%
Control B cells	267	10	3.75%
$CX^{c/-}$ B cells	156	4	2.56%
Control B cells	174	7	4.02%
$CX^{c/-}$ B cells	128	6	4.69%

Supplementary Table 14. No c-myc abnormalities detected in activated Xrcc4-deficient B cells by two color FISH

Genotypes	Number of mice	Number of Metaphases Analyzed	Metaphase With c-myc abnormalities	% of c-myc Abnormalities
Control	2	1074	0	0
$CX^{c/-}$	3	1155	0	0



Supplementary Table 15. Kinetics of IgH-cmyc translocations in Xrcc4-deficient peripheral B cells

Genotype	Day	Exp1	Exp2	Exp3	Total IgH/c-myc translocations/cell#	Frequency of Translocations ($\times 10^{-6}$)
control	4	$0/8x10^5$	$0/8x10^5$	$0/8x10^5$	$0/2.4x10^6$	<0.4
control	4	$0/8x10^5$	$0/8x10^5$		$0/1.6x10^5$	<0.8
control	4	$0/6\times10^5$	$1/1.2x10^6$	$1/9x10^5$	$2/2.7x10^6$	0.7
control	4	$1/1.2x10^6$	$1/0.8x10^5$		$2/2x10^6$	1
$CX^{c/-}$	4	$6/1.6x10^6$	$7/8x10^5$		$13/2.4x10^6$	5.4
$CX^{c/-}$	4	$1/8x10^5$	$1/1.6x10^6$		$2/2.4x10^6$	0.8
$CX^{c/-}$	4	$6/1.2x10^6$	$3/8x10^5$		$9/2x10^6$	4.5
$CX^{c/-}$	4	$13/2.4x10^6$	$5/8x10^5$		$18/3.2x10^6$	5.6
Genotype	**Day**	**Exp1**	**Exp2**	**Exp3**	**Total IgH/c-myc translocations/cell#**	**Frequency of Translocations ($\times 10^{-6}$)**
$CX^{c/-}RAG2^c$	2	$0/6\times10^5$	$1/1\times10^6$	$0/6\times10^5$	$1/2.2\times10^6$	0.4
$CX^{c/-}RAG2^c$	3	$4/6\times10^5$	$5/1\times10^6$	$4/6\times10^5$	$13/2.2\times10^6$	5.9
$CX^{c/-}RAG2^c$	4	$2/6\times10^5$	$11/1\times10^6$	$9/6\times10^5$	$22/2.2\times10^6$	10
$CX^{c/-}RAG2^c$	2	$1/6\times10^5$	$1/1\times10^6$	$0/6\times10^5$	$2/2.2\times10^6$	0.9
$CX^{c/-}RAG2^c$	3	$1/6\times10^5$	$4/1\times10^6$	$4/6\times10^5$	$9/2.2\times10^6$	4.1
$CX^{c/-}RAG2^c$	4	$5/6\times10^5$	$9/1\times10^6$	$8/6\times10^5$	$22/2.2\times10^6$	10

Supplementary Table 16. c-myc breaks and IgH/c-myc translocation in ISceI targeted B cells

Genotype	Retroviral vector	C-myc breaks			IgH/c-myc translocations		
		# Cells	# breaks	% breaks	# Cells	# translocation	Transl/10^6 cells
c-myc$^{I/wt}$	control	107	0	0.0	$1.38x10^6$	0	0
	ISceI	96	9	9.4	$2.4x10^5$	12	50
c-myc$^{I/wt}$	control	110	0	0.0	$8.0x10^5$	0	0
	ISceI	105	6	5.7	$8.0x10^4$	5	62.5
c-myc$^{I/wt}$	control	112	0	0.0	$8.0x10^5$	0	0
	ISceI	107	14	13.1	$8.0x10^4$	9	112.5
c-myc$^{I/wt}$	control	108	1	0.9	ND	ND	ND
	ISceI	110	11	10.0	ND	ND	ND
c-myc$^{wt/wt}$	control	106	0	0.0	$8.0x10^5$	0	0
	ISceI	112	0	0.0	$8.0x10^4$	0	0

SUPPLEMENTARY FIGURE LEGENDS

Suppl. Figure 1. Overview of V(D)J recombination, receptor editing, and IgH CSR in B cells.

a, Diagram of Primary and Secondary V(D)J recombination during B cell development. See text for details. **b,** Diagram of IgH V(D)J recombination and CSR. See text for details.

Suppl. Figure 2. Distribution of different types of *Igλ and Igκ* abnormalities in activated *CX$^{c/-}$* or *CXP* splenic B cells.

The bar graphs show the distribution of the different types of *Igκ* and *Igλ* aberrations found in 4 day αCD40/IL4-activated *CX$^{c/-}$* or *CXP* splenic B cells categorized as the proportion of metaphases with either breaks alone, breaks plus translocations, or translocations alone.

Suppl. Figure 3. Kinetics of *Igκ* and *Igλ* breaks in activated *CX$^{c/-}$* splenic B cells.

Metaphases prepared from day 2, day 3 or day 4 αCD40/IL4-activated splenic B cells were analyzed via two-color FISH using *Igκ* or *Igλ* BAC probes as outlined in Figure 1. At least three mice were analyzed for each point (see Suppl. Table 6 for raw data). Data are presented as mean±s.e.m.

Suppl. Figure 4. Representative *Igλ* abnormalities visualized by 3D interphase FISH.

Interphase nuclei were isolated from day 4 αCD40/IL4-activated *CX$^{c/-}$* splenic B cells and analyzed by 3D FISH using 5' (red) and 3' (green) *Igλ* BAC probes as outlined in Figure 1d. Representative images with isolated centromeric or telomeric signals are shown. Among 14 *Igλ* locus breaks scored, 3 had split signals (the distance between green and red signals greater than 1.5μm), 3 had isolated red signals only, and 8 had isolated green signals only.

Suppl. Figure 5. *Igκ* and *Igλ* abnormalities in activated *CX$^{c/-}$* splenic B cells are not initiated by AID.

Metaphases from αCD40/IL4-activated splenic B cells (day 4) were analyzed by FISH for hybridization to *Igκ* or *Igλ* probes as outlined in Figure 1. Representative *Igκ* or *Igλ* breaks and translocations in *CX$^{c/-}$AID$^{-/-}$* splenic B cells are shown.

Suppl. Figure 6. Deletion of RAG conditional alleles in resting and activated splenic

***CX$^{c/-}$RAG^{c}* B cells.**

DNA samples were prepared from day 0, day 3, or day 4 αCD40/IL4-activated *CX$^{c/-}$RAG$^{c/-}$* B cells and from kidney (Kid) as a control. Genomic DNA was digested with StuI and hybridized with a 5' RAG2 probe.

Suppl. Figure 7. Lack of *Igκ* and *Igλ* breaks or translocations in the same *CX$^{c/-}$* metaphases.

Metaphases from αCD40/IL4-activated *CX$^{c/-}$* B cells (day 4) were analyzed by sequential FISH hybridization first with 5' and 3' *Igκ* probes and then 5' and 3' *Igλ* probes as indicated. **Upper:** Representative metaphases with an intact *Igκ* locus and a broken/translocated *Igλ* locus in *CX$^{c/-}$* splenic B cells are shown. **Bottom:** Quantification of these sequential FISH analyses.

Suppl. Figure 8. Increased *Jκ-Cκ* deletion in metaphases harboring *Igλ* abnormalities.

Metaphases prepared from day 4 aCD40/IL-4 activated wt (n=2) or *CX$^{c/-}$* (n=3) splenic B cells were first analyzed via two color FISH to identify those with *Igλ* breaks and translocations. Subsequently the *Igλ* signals were stripped and the metaphases were assayed by two color FISH using a 5' *Igκ* BAC probe (to identify chromosome 6 in the region of *Igκ*) and a *Jκ-Cκ* probe (indicated in the schematic map of *Igκ* locus) to assay for *Igκ* specific deletions. **Upper**: Bar graph showing that metaphases from wt B cells (n=207) have an intact *Igκ* locus in greater than 90% (open bars) while nearly 50% of metaphases from *CX$^{c/-}$* B cells with *Igλ* abnormalities (n=21) (black bars) had deletions of the *Cκ* region on either one or both alleles (scored as absence of *Jκ-Cκ* probe signal on the chromosome carrying the *5' Igκ* signal). Bottom: Map of *Igκ* locus showing position of *Jκ-Cκ* probe and indicating potential mechanisms for *Igκ* deletions via rearrangements to 3'RS.

Suppl. Figure 9. Frequent *IgH* and *Igλ* abnormalities in activated *CX$^{c/-}$* peripheral B cells.

a, Shown is an example of a metaphase with an *Igκ* break joined to an *IgH* break. **Left:** *Igκ* FISH shows a chromosomal translocation involving *Igκ*. Sequential hybridizations of the same metaphase with chromosome 12 and chromosome 6 paints (**center**) followed by *IgH* FISH (**right**) revealed a translocation involving chromosome 12 (red) and chromosome 6 (green) with

3'*IgH* and 3'*Igκ* sequences at the breakpoint. **b**, **Upper**: Diagram of *Igλ* and *IgH* probes. **Lower:** Examples of metaphases with simultaneous *Igλ* and *IgH* breaks, as revealed by sequential *Igλ* FISH followed by *IgH* FISH. **Metaphase 1**: The broken *Igλ* locus (*3'Igλ* probe, red signal, left panel) is joined to a broken *IgH* locus (*3'IgH* probe, green signal, right panel) to form a dicentric chromosome, while the telomeric portion of chromosome 16 (*5'Igλ* probe, green signal, left panel) is fused with the telomeric portion of chromosome 12 (*5'IgH* probe, red signal, right panel) in an acentric fragment. The other chromosome 12 in the same metaphase is also involved in formation of a dicentric chromosome, which is only positive for the *3'IgH* probe (green signal, right panel). **Metaphase 2**: Both the *IgH* and the *Igλ* loci are broken; and the chromosomal fragments containing the *5'Igλ* probe (green, left panel) and the *5'IgH* probe (red, right panel) are involved in translocations with different chromosomes. **Metaphase 3**: The *Igλ* locus is broken, with the *5'Igλ* probe (green) joined to another chromosome to form a translocation (left panel), whereas the *IgH* locus is intact (right panel). **c,** Example is shown of a metaphase with an *Igλ* break joined to *IgH* break. Sequential hybridization of the same metaphase with *Igλ* probes, chromosome 12 and chromosome 16 paints and *IgH* probes revealed the telomeric portion of chromosome 16, containing the *5'Igλ* probe (green, left panel) joined to the centromeric portion of chromosome 12, containing the *3'IgH* probe (red, right panel).

Suppl. Figure 10. PCR assay to detect *IgH/Igλ* translocations in day 4 activated control and $CX^{c/-}$ splenic B cells.

a, Schematic representation of the PCR assay used for *IgH/Igλ* translocations. Primers used to detect chromosome 12/16 translocations are represented as horizontal black arrows. The internal oligonucleotide probe used in Southern blot experiments is shown as a horizontal black bar. **b,** Representative Southern blots with the *IgH* or *Jλ1* probe are shown for activated control, $CX^{c/-}$ and $CX^{c/-}A^{-/-}$ peripheral B cells (day 4 of αCD40/IL4 activation). No *IgH/Igλ* translocation was detected in 1.5×10^6 cells from $CX^{c/-}A^{-/-}$ mice. In each reaction DNA from ~20000 cells was used.

Suppl. Figure 11. Sequence analyses of junctions *of IgH/Igλ* translocations.

IgH/Igλ translocations were cloned from 4 day αCD40/IL4-activated $CX^{c/-}$ (n=3) B cells by PCR. Sequences are aligned with genomic *IgH* (AJ851868) or *Igλ* (NG_004051) locus sequences. Mutations, insertions, or deletion were found in the Sμ regions (underlined). Micro-homology at the junctions is underlined.

Suppl. Figure 12. Spatial proximity of *IgH* and *Igκ*.

The *3' IgH* probe and one of the three indicated *Igκ* region probes (F6, Igκ, I9) (diagrammed in lower panel) were used for 3D interphase FISH on control or $CX^{c/-}$ peripheral B cell nuclei. The bar graph in the upper panel shows the percentage of nuclei in which the *3'IgH* was found co-localized (closer than 0.5μm) with each of the *Igκ* regions as indicated. At least three mice were analyzed for each point (see Suppl. Table 12). The percentage of co-localization is presented as mean ± s.e.m based on results of at least 3 different experiments per sample.

Suppl. Figure 13. PCR assay to detect *IgH/c-myc* translocations in day 4 activated control and $CX^{c/-}$ splenic B cells.

a, Schematic representation of the PCR assay used for *IgH/c-myc* translocations. Primers used to detect derivative chromosome 12 (der12) translocations are represented as horizontal black arrows. The internal oligonucleotide probe used in Southern blot experiments is shown as a horizontal black bar (T13). **b,** Representative Southern blots with the T13 probe are shown for activated control and $CX^{c/-}$ peripheral B cells (day 4 of αCD40/IL4). In each reaction DNA from 100000 or 50000 cells was used (see Suppl. Table 15). **c,** Representative Southern blots with T13 probe are shown for activated control and $CX^{c/-}RAG2^{c}$ splenic B cells at day 2 and day 3 of activation with αCD40/IL4. Each reaction used DNA from 50000 cells.

Suppl. Figure 14. Gene targeting strategy and Southern blot analyses of *c-myc*^{25ISceI} allele.

Top Diagram: Schematic maps of the targeting strategy for insertion of 25 ISceI sites into the 1st intron of *c-myc* locus. The position of the 5' and 3' probes used for ES cell screening are shown in red. **Bottom Panels:** Southern blot analysis of a targeted clone before and after Neo cassette deletion.

Suppl. Figure 15. PCR assay to detect *IgH/c-myc* translocations in day 4-activated *c-*

***myc*$^{25IsceI/wt}$ splenic B cells infected with control or ISceI virus.**

a, Schematic representation of the PCR assay used for *IgH/c-myc* translocations. Primers used to detect derivative chromosome 12 (der12) translocations are represented as horizontal black arrows. The internal oligonucleotide probe used in Southern blot experiments is shown as a horizontal black bar (T15). **b,** Representative Southern blots with the T15 probe are shown for activated *c-myc*$^{25IsceI/wt}$ and *c-myc*$^{wt/wt}$ peripheral B cells (day 4 of αCD40/IL4) infected with either control or ISceI virus. In each reaction, DNA from 50000 (control-infected samples) or 5000 (ISceI-infected samples) cells was used (see Suppl. Table 16).

ONLINE METHODS

Gene targeting of *c-myc*^{25ISceI} allele.

To generate the targeting construct, the ISceI site cassette was inserted into the pLNTK targeting vector and flanked with a 4.6-kb SphI-SphI 5' homology arm encompassing *c-myc* exon1 and a 2.6-kb SphI-SphI 3' homology arm containing *c-myc* exon 2 and 3. We electroporated the targeting construct into TC1 (129/Sv) ES cells and screened EcoRI-digested DNA to identify potentially targeted ES cell clones via Southern blotting with a 5' probe consisting of the 1.5kb XbaI-XbaI fragment upstream of *c-myc* exon1 and a 3' probe consisting of the 1.5kb XhoI-KpnI fragment downstream of *c-myc* exon3. We deleted the Neo cassette from targeted ES clones by infection with adenovirus expressing Cre-recombinase and used those to inject for germline transmission to generate c-myc$^{25IsceI/wt}$ mice.

Retroviral Infection and CSR Assays

Empty pMX or pMX-IsceI vectors were transfected into Phoenix packaging cells. Viral supernatants were collected at 48 and 72 hours post-transfection and used for infecting αCD40/IL4-stimulated B cells at day 1 and day 2 of stimulation. Cells were processed at day 4 of stimulation. Infection efficiency ranged from 70% to 90% as measured by IRES-GFP expression from the retroviral vector by flow cytometry. High titer retroviral infection of activated wt B cells leads to inhibition of end joining as evidenced by increased accumulation of AID-initiated *IgH* chromosome breaks (MG and FWA, unpublished data). This effect facilitates visualization of ISceI-induced *c-myc* breaks in c-myc$^{25ISceI/wt}$ wt B cells.

Cytogenetic analysis: Two color FISH and FISH probes.

The FISH probes were as follows: a BAC that covered the 3' region of the *IgH* locus, encompassing the *3'IgH* enhancer and 100kb of downstream sequences (*3'IgH* BAC) and a BAC just upstream of the *IgH* V_H region (*5'IgH* BAC) as described previously[10]. All BACs outlined below were obtained from the BACPAC CHORI database. BACs for *Igλ* regions are RP23-382P9 (*5'Igλ*) and RP23-374P12 (*3'Igλ*), and BACs for *Igκ* regions are RP23-84F6 (*5'Igκ*), RP24-279F16 (*Igκ*) and RP23-64I9 (*3'Igκ*). BACs for *c-myc* regions are RP23-307D14 that

contains *c-myc* gene for interphase FISH, RP24-434C10 (*5' c-myc*) and RP23-113O21 (*3' c-myc*). In all FISH experiments, intact loci show co-localization of the red and green probes, while split red and green signals are scored as broken loci. Broken loci can be free, with the centromeric and telomeric portion of the locus either present in the metaphases or lost. Alternatively, broken loci (both the centromeric and telomeric parts or only one of them) can be involved in translocations with other chromosomes. For *IgH/Igλ* translocation FISH, representative *IgH/Igλ* translocation shows green and red signals juxtaposed on a dicentric chromosome. Also visible are a normal chromosome 12 with a red signal at the telomeric end and a normal chromosome 16 with a green signal close to the centromere. Whole chromosome paint specific for mouse chromosome 6, 12 and 16 were used according to the manufacturer instructions (Applied Spectral Imaging). Images of metaphases were captured with BX61 Microscope (Olympus) equipped with a motorized automatic stage, a cooled-CCD camera and an interferometer (Applied Spectral Imaging). 60× objective len was used. Analysis was performed with the ScanView software (Applied Spectral Imaging).

3D interphase FISH.

B cells, thymocytes or ES cells were transferred to a glass slide coated with Cell Tak (Becton Dickinson), fixed for 10 min with 4% paraformaldehyde, permeabilized in 0.1% saponin/0.1% Triton X-100 in PBS for 10 min, and immersed in liquid nitrogen for three freeze/thaw cycles. Slides were incubated in 0.1 M HCl, blocked in 3% BSA/100 mg/ml RNAase A, and re-permeabilized in 0.5% saponin/0.1% Triton X-100 in PBS. Genomic DNA was denatured at 73°C and hybridized with FISH probes labeled with either biotin or digoxigenin (Roche). Detecting antibodies were Streptavidin-Alexa 488 (Molecular Probes) and mouse anti-Dig-cy3 (Jackson Immuno Research laboratory) used at 1:200 dilution. We could not perform *Igκ* locus assays since the large size of *Igκ* (greater than 3Mb) obviates chromosomal break measurements via interphase FISH.

Primers and PCR conditions for *IgH/Igλ* translocations.

Primers for *Jλ1* regions: 1st round primer, 5'-ATTGAATTTCTGTGGCCATAACTCC-3', 2nd

round primer, 5'-ACTCCCCTCTGTTTTTACTCCTTATTA-3'

Primers for *Jλ2* regions: 1st round primer, 5'- AGTTATTTCCTCTTTGCTCCTGTGGTGAT-3', 2nd round primer, 5'-CTACTCTGGGGTTGGTGCCTACTG-3'

Primers for *IgH Sμ* regions: 1st round primer, 5'-GATCCCTCTACACCCTGCTATTTC-3', 2nd round primer, 5'-CACCCTGCTATTTCCTTGTTGCTACTCTTA-3'

Probes: for *Jλ1* 5'-GCCTCTGTGCTGAATGTTCTGTGCTC-3', for Jλ2 5'-TCAGGAGGTAGAGCACAGCAGCCACTT-3', for Igh 5'-CCTGGTATACAGGACGAAACTGCAGCAG-3'

PCR condition: 1st round PCR, 94°C 3min, 94°C 15 sec, 58.2°C 15 sec, 68°C 7min + 20sec per cycle, 25 cycles, 68°C 5min for final extension. 2nd round PCR, 94°C 3min, 94°C 15 sec, 58.2°C 15 sec for Igh/Jλ1 or 60.3°C,15sec for Igh/Jλ2, 68°C 7min, 25 cycles, 68°C 5min for final extension.

Primers and PCR conditions for Igh/c-myc translocations.

Primers for *c-myc^{wt}*: 1st round primer, 5'- GGGGAGGGGGTGTCAAATAATAAGA -3', 2nd round primer, 5'- GACACCTCCCTTCTACACTCTAAACCG -3'

Primers for *c-myc^{25ISce}*: 1st round primer, 5'-GCTCTGCTGTTGCTGGTGATAGAA -3', 2nd round primer, 5'- ATAGGGCTGTACGGAGTCGTAGTC -3'

Primers for *IgH Sμ* region: 1st round primer, 5'- TGAGGACCAGAGAGGGATAAAAGAGAA -3', 2nd round primer, 5'- CACCCTGCTATTTCCTTGTTGCTAC -3'.

PCR conditions: 1st round PCR, 94°C 3min, 94°C 15 sec, 62 °C 15 sec, 68°C 7min + 20sec per cycle, 25 cycles, 68°C 5min for final extension. 2nd round PCR, 94°C 3min, 94°C 15 sec, 62°C 15 sec, 68°C 7min, 25 cycles, 68°C 5min for final extension.