Supporting Information

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SI Materials and Methods

Generation of v-Abl–Transformed Pro-B Lines. The v-Abl kinase-transformed pro-B cells were generated from total bone marrow cells of Eμ-*Bcl2* transgenic *Xrcc4*$^{c/c}$*Xrcc1*$^{c/c}$ mice. Retroviral end-joining reporters were generated by cloning the I-SceI–containing region from the viral GEJ reporter (1) into the pMX retroviral backbone (2). End-joining reporters were transduced into v-Abl–transformed pro-B lines, and clones were screened by Southern blotting for single substrate integration. Three clones (nos. 26, 27, and 150) with independent single I-SceI substrate integrations were treated with Tat-Cre (3) and screened for *Xrcc4*$^{-/-}$ and *Xrcc4*$^{-/-}$*Xrcc1*$^{-/-}$ subclones. Two additional *Xrcc4*$^{-/-}$ clones (nos. 47 and 84) with single and independent I-SceI substrate integrations were also analyzed. I-SceI–GR fusion protein was introduced retrovirally, and nuclear translocation of I-SceI-GR was induced by treatment with 0.1 μM triamcinolone acetonide (TA) for 4 d. GFP expression after successful joining was recorded by flow cytometry. I-SceI junctions were amplified from TA-treated v-Abl–transformed pro-B lines with primers 5′-GCCTCGATCCTCCCTTTATC-3′ and 5′-GCAAGCTGACCCTGAAGTTC-3′, cloned, and sequenced.

CH12F3 Cell Culture and shRNA-Mediated Knockdown. CH12F3 cells were cultured in RPMI 1640 supplemented with 10% (vol/vol) FCS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 20 mM Hepes, 1% penicillin/streptomycin, 2 mM L-glutamine, and 5% (vol/vol) National Cancer Tissue Culture (NCTC)-109. Vesicular stomatitis virus glycoprotein G–pseudotyped lentiviral particles were generated by cotransfection of 293T cells with psPAX2 (Addgene), pMD2.G (Addgene), and pLKO.1-based shRNA vectors (The RNAi Consortium; Open Biosystems). CH12F3 cells were transduced in the presence of 8 μg/mL polybrene and selected with 2 μg/mL puromycin. For stimulation, cells were plated at 5×10^4 cells/mL in CH12F3 medium supplemented with 1 μg/mL αCD40, 1 ng/mL TGF-β, 20 ng/mL IL-4, and 1 μg/mL puromycin.

Sμ-Sα Junction Analysis in CH12F3 Cells. Sμ-Sα junctions were amplified from genomic DNA extracted from IL-4/αCD40/TGF-β–stimulated CH12F3 cells by nested PCR using previously established methods (4) and the following primers: PCR 1 (Sμ1, 5′-TAGTAAGCGAGGCTCTAAAAAGCAT-3′; Sα1, 5′-CAGCAGTGAGTTTAACAATCC-3′) and PCR 2 (Sμ2, 5′-ATCGAATTCGCTTGAGCCAAAATGAAGTAGACT-3′; Sα2, 5′-CCGGAATTCCTCAGTGCAACTCTATCTAGGTCT-3′).

***IgH/c-myc* Translocation Analysis in CH12F3 Cells.** *IgH/c-myc* translocation junctions (der15) were PCR-amplified (5) from genomic DNA of CH12F3 cells stimulated with IL-4/αCD40/TGF-β for 3 d. Multiple aliquots of DNA corresponding to 100,000 cells were analyzed in separate reactions. Reactions were performed using the Expand Long Template PCR System (Roche). For the first-round of PCR, the following primers were used: Sμ5′1a (5′-ACTATGCTATGGACTACTGGGGTCAAG-3′) and c-myc5′1a (5′-GTGAAAACCGACTGTGGCCCTGGAA-3′); conditions were 94 °C for 3 min, 94 °C for 15 s, 62 °C for 15 s, 68 °C for 7 min plus 20 s per cycle for 25 cycles, and 5 min at 68 °C for a final extension. For the second round of PCR, the following primers were used: Sμ5′1b (5′-CCTCAGTCACCGTCTCCTCAGGTA-3′) and c-myc5′1b (5′-GTGGAGGTGTATGGGGTGTAGAC-3′); conditions were 94 °C for 3 min, 94 °C for 15 s, 62 °C for 15 s, 68 °C for 7 min for 25 cycles, and 68 °C for 5 min for a final extension. PCR products were electrophoresed on agarose gels and subjected to Southern blot analysis with an internal *c-myc* locus oligonucleotide probe (T11, 5′-GGACTGCGCAGGGAGACCTACAGGGG-3′). For multiple comparisons, one-way ANOVA and a Tukey posttest were used to determine statistical significance.

1. Xie A, Kwok A, Scully R (2009) Role of mammalian Mre11 in classical and alternative nonhomologous end joining. *Nat Struct Mol Biol* 16:814–818.
2. Bredemeyer AL, et al. (2006) ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. *Nature* 442:466–470.
3. Zha S, et al. (2011) Ataxia telangiectasia-mutated protein and DNA-dependent protein kinase have complementary V(D)J recombination functions. *Proc Natl Acad Sci USA* 108:2028–2033.
4. Yan CT, et al. (2007) IgH class switching and translocations use a robust non-classical end-joining pathway. *Nature* 449:478–482.
5. Ramiro AR, et al. (2004) AID is required for c-myc/IgH chromosome translocations in vivo. *Cell* 118:431–438.

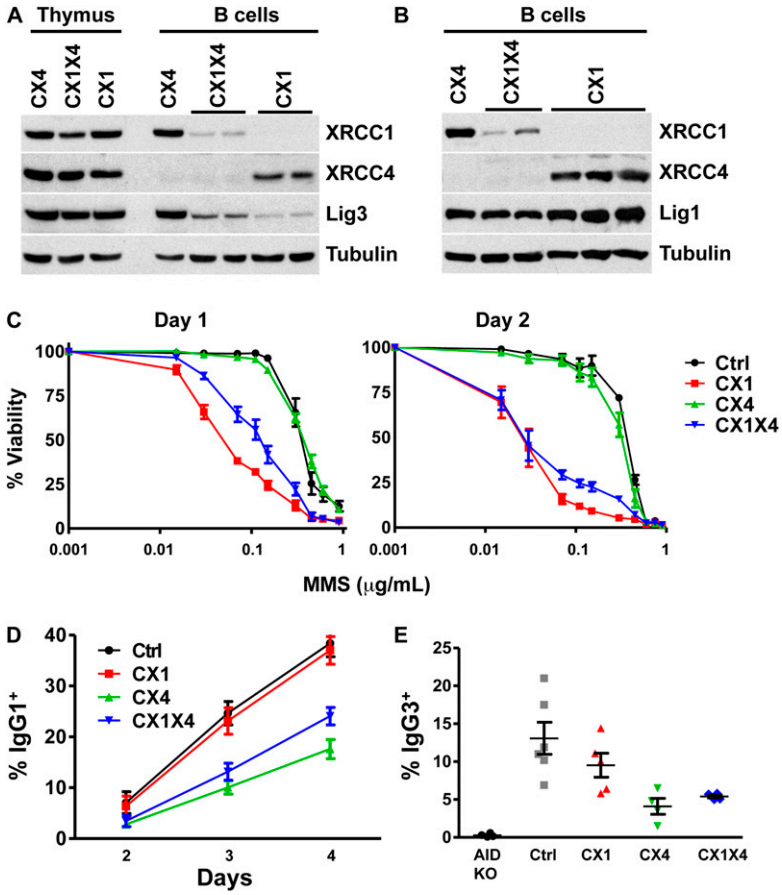

Fig. S1. Lig3 (*A*) and Lig1 (*B*) levels in *Xrcc1* and *Xrcc1/Xrcc4* conditionally deleted B cells. B cells were stimulated in culture with αCD40/IL-4 for 3 d. Cell lysates were analyzed using the indicated antibodies. Lig3 is reduced in XRCC1-deficient mature B cells. Lig1 levels are unaffected by *Xrcc1* and *Xrcc1/Xrcc4* deletion. (*C*) MMS survival curves of B cells of the indicated genotypes after 1 d (*Left*) or 2 d (*Right*) of culture with αCD40/IL-4, followed by MMS treatment for 6 h. At least three mice per genotype were used for each MMS concentration. (*D*) Time course for IgG1 CSR in XRCC1-deficient and XRCC1/XRCC4 double-deficient B cells. CX1, CX1X4, CX4, and Ctrl B cells were stimulated with αCD40/IL-4, and IgG1 CSR was analyzed on days 2, 3, and 4 of stimulation by flow cytometry. At least four mice per genotype were used in independent experiments for each time point. (*E*) IgG3 CSR. B cells were stimulated with LPS plus αIgD-dextran for 4 d. A total of six Ctrl, five CX1, four CX4, and four CX1X4 mice were analyzed in independent experiments. Data are means ± SEM.

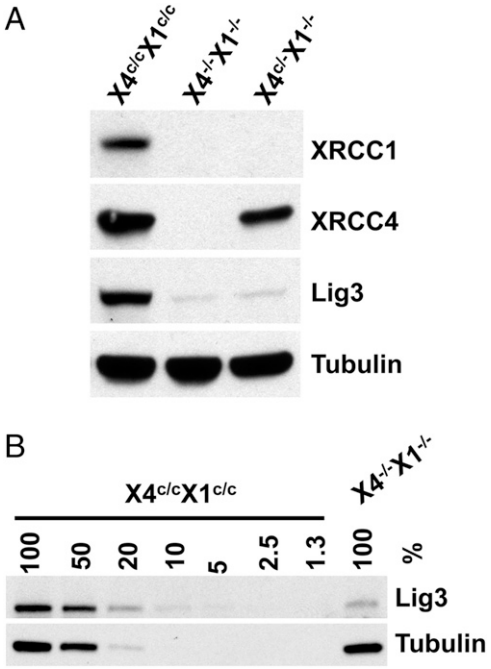

Fig. S2. (*A*) Levels of XRCC1, Lig3, XRCC4, and tubulin in v-Abl–transformed pro-B subclones of Tat-Cre–treated $X4^{c/c}X1^{c/c}$ clone number 150. (*B*) Estimation of residual Lig3 protein levels. Deletion of Xrcc1 reduces Lig3 levels to about 20% of $X4^{c/c}X1^{c/c}$. Fifty micrograms (100%) and the indicated dilutions of whole-cell extract from v-Abl–transformed $X4^{c/c}X1^{c/c}$ pro-B cells or 50 μg of whole-cell extract from v-Abl–transformed $X4^{-/-}X1^{-/-}$ pro-B cells were analyzed by immunoblotting with anti-Lig3 antibodies. Blots were stripped and reprobed for tubulin as a loading Ctrl.

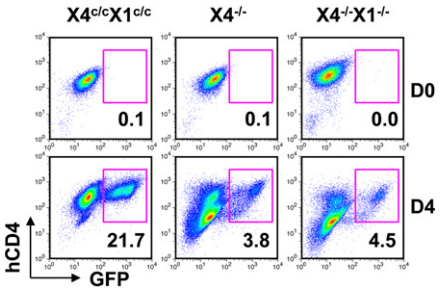

Fig. S3. Example of GFP expression in v-Abl–transformed pro-B cells after cutting and successful joining of single-copy chromosomal I-SceI reporters. D0, day 0; D4, day 4 after TA induction.

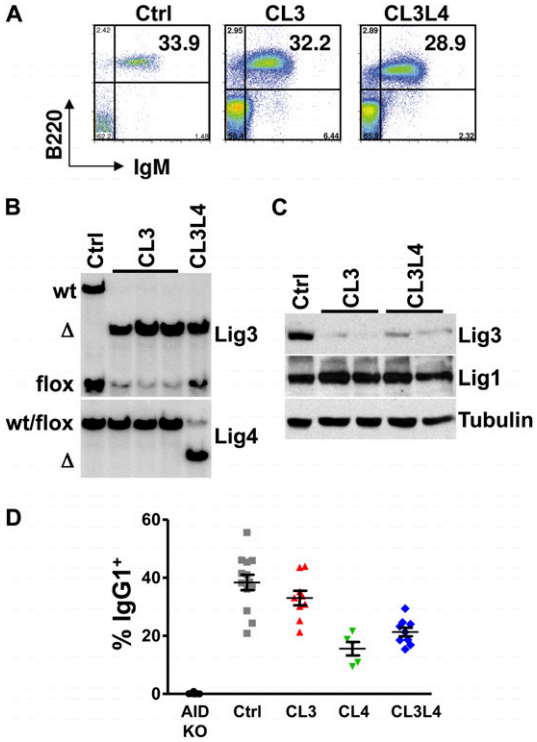

Fig. S4. (*A*) Mature B cells are present in the spleens of CL3 and CL3L4 mice in similar fractions to WT. Southern blot (*B*) and Western blot (*C*) analysis of mature splenic B cells from Ctrls [$Lig3^{c/+}Lig4^{c/+}$ and WT(wt)] vs. CL3 and CL3L4 mice stimulated in culture for 4 d with αCD40/IL-4. Each lane contains DNA or protein from an independent mouse. (*D*) CSR levels are not affected by *Lig3* deletion in Ctrl or *Lig4*-deleted B cells. IgG1 CSR was measured after 4 d of αCD40/IL-4 stimulation by flow cytometry.

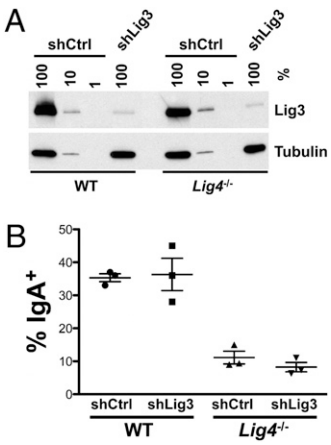

Fig. S5. (*A*) Lig3 shRNA reduces Lig3 protein levels by more than 90%. Sixty micrograms (100%) of whole-cell extract and dilutions were analyzed as described in Fig. S2*B*. (*B*) Summary of day 2 CSR experiments in CH12F3 B-cell lines. WT or $Lig4^{-/-}$ CH12F3 cells stably expressing either Ctrl or Lig3 shRNA were stimulated for 2 d with IL-4/αCD40/TGF-β, and surface IgA expression was analyzed by flow cytometry. Means ± SEM from three independent experiments are shown.

Table S1. Summary of switch region junctions in splenic B cells stimulated with αCD40/IL-4 for 4 d in culture

Sμ-Sγ1	Dir.	MH	Dir. + MH	Ins.	Total	Sμ-Sε	Dir.	MH	Dir. + MH	Ins.	Total
Ctrl 1	7 (35.0)	13 (65.0)	20 (100)	2	22	Ctrl 1	7 (29.2)	17 (70.8)	24 (100)	10	34
Ctrl 2	5 (41.7)	7 (58.3)	12 (100)	3	15	Ctrl 2	6 (19.4)	25 (80.6)	31 (100)	5	36
						Ctrl 3	11 (36.7)	19 (63.3)	30 (100)	3	33
Total	12	20	32	5	37	Total	24	61	85	18	103
Mean, %	38.3	61.7				Mean ± SEM, %	28.4 ± 5.0	71.6 ± 5.0			
CX1 1	3 (42.9)	4 (57.1)	7 (100)	5	12	CX1 1	8 (40.0)	12 (60.0)	20 (100)	6	26
CX1 2	7 (46.7)	8 (53.3)	15 (100)	4	19	CX1 2	9 (30.0)	21 (70.0)	30 (100)	12	42
CX1 3	4 (33.3)	8 (66.7)	12 (100)	3	15	CX1 3	8 (47.1)	9 (52.9)	17 (100)	4	21
CX1 4	4 (28.6)	10 (71.4)	14 (100)	1	15	CX1 4	7 (36.8)	12 (63.2)	19 (100)	5	24
Total	18	30	48	13	61	Total	32	54	86	27	113
Mean ± SEM, %	37.9 ± 4.2	62.1 ± 4.2				Mean ± SEM, %	38.5 ± 3.5	61.5 ± 3.5			
CX4 1	1 (3.8)	25 (96.2)	26 (100)	4	30	CX4 1	5 (18.5)	22 (81.5)	27 (100)	2	29
CX4 2	3 (12.5)	21 (87.5)	24 (100)	2	26	CX4 2	2 (11.1)	16 (88.9)	18 (100)	11	29
Total	4	46	50	6	56	Total	7	38	45	13	58
Mean, %	8.2	91.8				Mean, %	14.8	85.2			
CX1X4 1	7 (23.3)	23 (76.7)	30 (100)	8	38	CX1X4 1	7 (35.0)	13 (65.0)	20 (100)	1	21
CX1X4 2	1 (16.7)	5 (83.3)	6 (100)		6	CX1X4 2	7 (25.9)	20 (74.0)	27 (100)	9	36
Total	8	28	36	8	44	Total	14	33	47	10	57
Mean, %	20.0	80.0				Mean, %	30.5	69.5			
						CL3 1	10 (50.0)	10 (50.0)	20 (100)	9	29
						CL3 2	13 (48.1)	14 (51.9)	27 (100)	6	33
						CL3 3	7 (33.3)	14 (66.7)	21 (100)	3	24
						Total	30	38	68	18	86
						Mean ± SEM, %	43.8 ± 5.3	56.2 ± 5.3			

Percentages of direct (Dir.) and MH-mediated (MH) junctions are shown in parentheses. Percentages are calculated based on the total number of junctions, excluding insertions (Ins.).

Table S2. Summary of *IgH* genomic instability assessed on metaphases from B cells stimulated with αCD40/IL-4 for 4 d

Genotype	Metaphases analyzed	*IgH* chromosome breaks	*IgH* translocations
Ctrl 1	117	7 (6.0)	4 (3.4)
Ctrl 2	109	3 (2.8)	1 (0.9)
Ctrl 3	106	1 (0.9)	0 (0.0)
Ctrl 4	108	8 (7.4)	3 (2.8)
Ctrl 5	110	4 (3.6)	1 (0.9)
Ctrl 6	110	4 (3.6)	0 (0.0)
Ctrl 7	106	9 (8.5)	1 (0.9)
Ctrl 8	111	6 (5.4)	3 (2.7)
Ctrl 9	131	1 (0.8)	0 (0.0)
Total	1,008	43	13
Mean ± SEM, %		4.3 ± 0.9	1.3 ± 0.4
CX1 1	112	17 (15.2)	6 (5.4)
CX1 2	116	11 (9.5)	5 (4.3)
CX1 3	100	7 (7.0)	2 (2.0)
CX1 4	104	3 (2.9)	2 (1.9)
CX1 5	108	9 (8.3)	3 (2.8)
CX1 6	105	4 (3.8)	0 (0.0)
CX1 7	106	8 (7.5)	2 (1.9)
CX1 8	106	8 (7.5)	4 (3.8)
Total	772	67	24
Mean ± SEM, %		7.7 ± 1.3	2.8 ± 0.6
CX4 1	108	20 (18.5)	7 (6.5)
CX4 2	102	17 (16.7)	6 (5.9)
CX4 3	119	15 (12.6)	2 (1.7)
Total	329	45	15
Mean ± SEM, %		15.9 ± 1.7	4.7 ± 1.5

Number and percentage (in parentheses) of metaphases with chromosomal breaks or translocations are indicated.

Table S3. Summary of I-SceI joining experiments in v-Abl cell lines

Experiment	$X4^{c/c}X1^{c/c}$	$X4^{-/-}X1^{-/-}$	$X4^{-/-}$	$X4^{-/-}X1^{c/-}$		$X4^{c/-}X1^{-/-}$
Clone 26						
1	18.8	2.6	2.5	3.9		
2	11.9	6.9	2.2	3.4		
3	27.9	3.6	5.0	3.4		
4	26.3	5.0	4.2			
5	23.9	4.0				
6	19.9	3.9				
7	15.2	7.6				
Mean ± SEM, %	20.6 ± 2.2	4.8 ± 0.7	3.5 ± 0.7	3.6 ± 0.2		
Clone 27						
1	45.4	7.2		4.9	23.7	
2	38.4	10.5		5.4	30.4	
3	35.2	4.3		3.8	23.7	
4	18.0	3.4		3.3	31.3	
5	41.8	4.8			32.3	
6	37.0	4.3				
7	42.3	3.0				
8	39.7	7.7				
9	34.3	3.0				
10	42.6	2.5				
11	35.3	3.8				
12		3.4				
Mean ± SEM, %	37.3 ± 2.2	4.8 ± 0.5		4.4 ± 0.5	28.3 ± 0.9	
Clone 150						
1	37.0	5.0				15.4
2	16.0	6.3				15.9
3	22.2	4.3				20.3
4	28.3	4.6				21.6
5	15.7	4.2				9.7
6	16.7	4.4				
7	31.5	5.2				
8	21.8	3.0				
9	35.1	2.8				
10	25.7	3.8				
11	11.4	3.6				
12	13.4	4.5				
13	21.8	5.1				
14	29.0	5.3				
15	11.7	3.1				
16	22.7	2.5				
17	15.1	4.2				
18		4.2				
19		4.3				
20		3.2				
Mean ± SEM, %	22.1 ± 1.9	4.2 ± 0.2				16.6 ± 2.1

End-joining is indicated by the percentage of GFP-positive cells after 4 d of TA induction. All subclones of clones no. 26, 27, and 150 contain a single, unique copy of the I-SceI–joining substrate. Additional *Xrcc4*$^{-/-}$ clones (nos. 47 and 84) with different single-copy substrate integration were also analyzed (Table S4).

Table S4. Summary of additional $X4^{-/-}$ clones with single and distinct I-SceI substrate integration

Experiment	No. 47	No. 84
1	6.3	4.8
2	6.0	3.5
3	3.0	3.4
4	3.2	
Mean ± SEM, %	4.6 ± 1.8	3.9 ± 0.5

End-joining is indicated by the percentage of GFP-positive cells after 4 d of TA induction.

Table S5. Summary of switch region junctions in stimulated CH12F3 B cells

	Dir.	MH	Dir. + MH	Ins.	Total
Lig4$^{-/-}$ shCtrl					
Exp. 1	0 (0.0)	10 (100.0)	10 (100)	4	14
Exp. 2	1 (9.1)	10 (90.9)	11 (100)	2	13
Exp. 3	0 (0.0)	9 (100.0)	9 (100)	5	14
Mean ± SEM, %	3.0 ± 3.0	97.0 ± 3.0			
Lig4$^{-/-}$ shLig3					
Exp. 1	0 (0.0)	6 (100.0)	6 (100)	2	8
Exp. 2	0 (0.0)	2 (100.0)	2 (100)	2	4
Exp. 3	0 (0.0)	9 (100.0)	9 (100)	6	15
Mean ± SEM, %	0.0 ± 0.0	100.0 ± 0.0			

Percentages of direct (Dir.) and MH-mediated (MH) junctions are shown in parentheses. Percentages are calculated based on the total number of junctions, excluding insertions (Ins.). Exp., experiment.