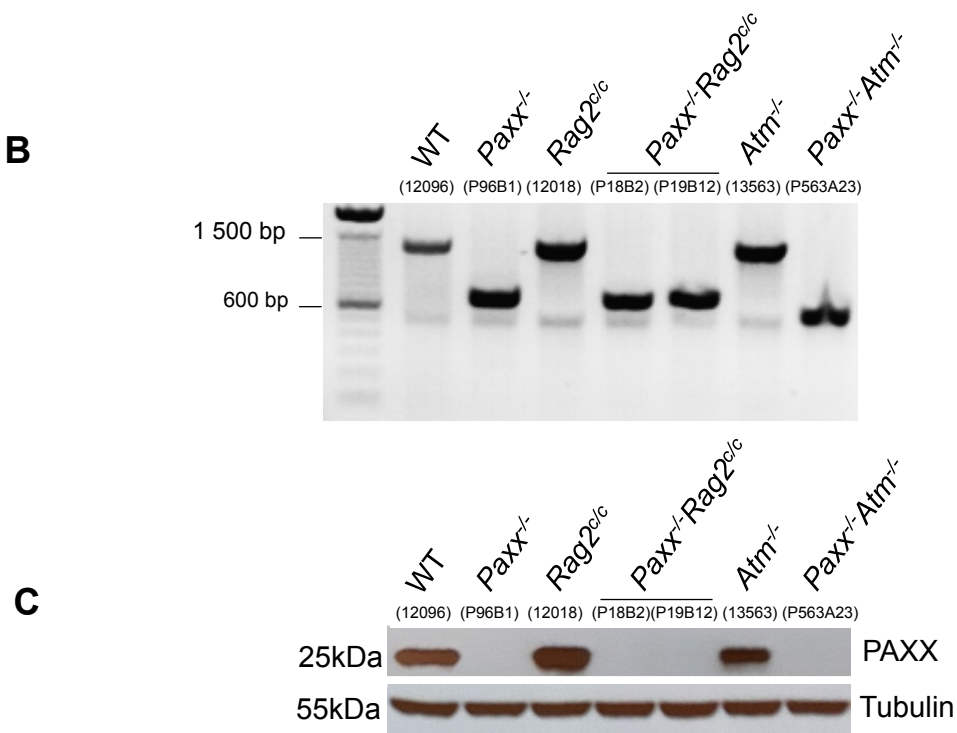
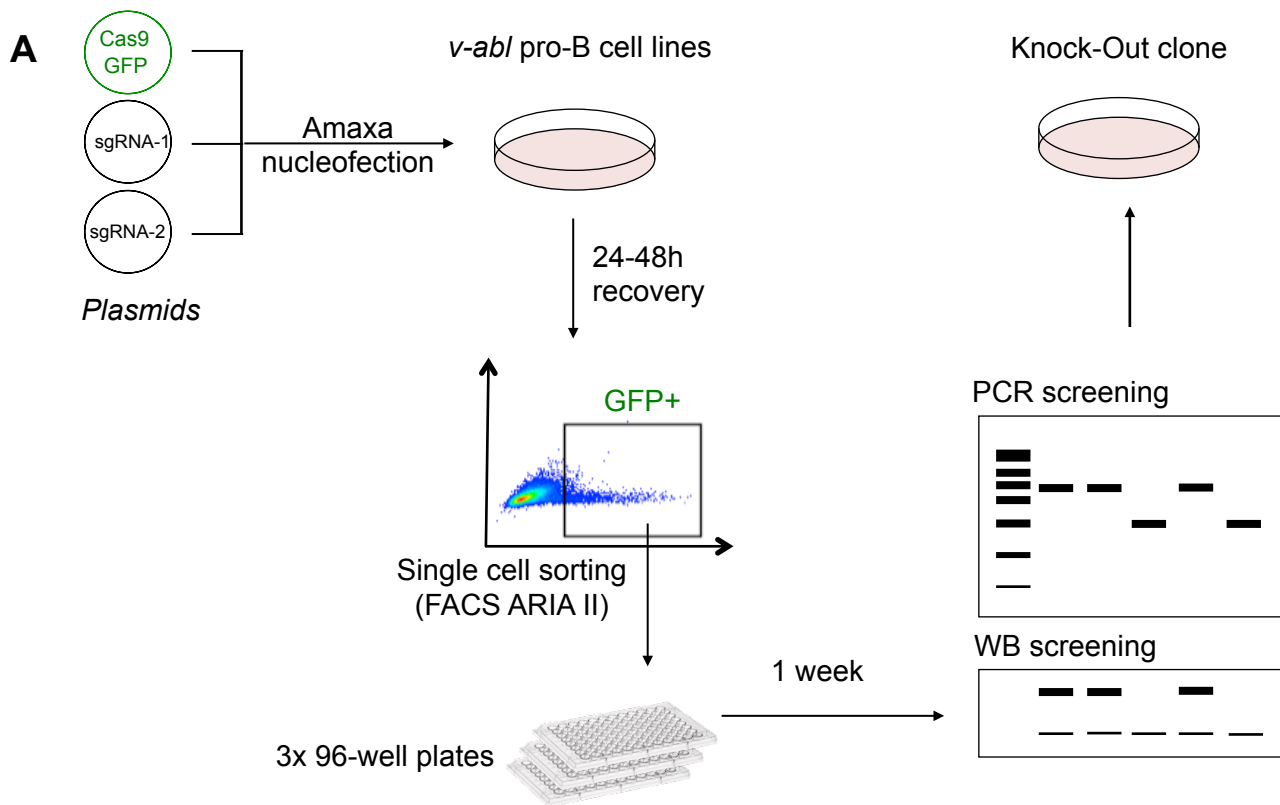


**Cell Reports, Volume 16**

## **Supplemental Information**

### **Specific Roles of XRCC4 Paralogs PAXX and XLF during V(D)J Recombination**

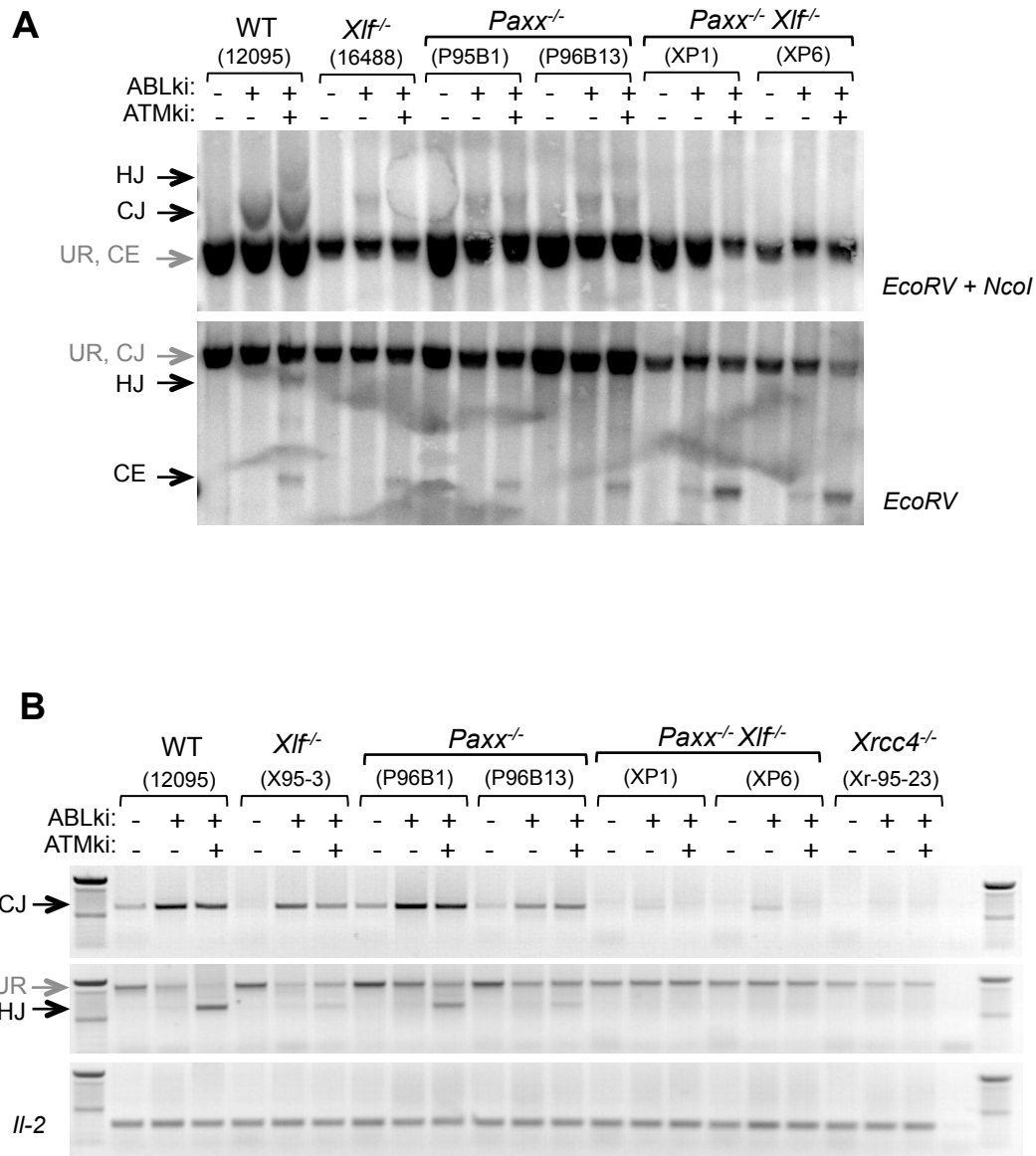
**Chloé Lescale, Hélène Lenden Hasse, Andrew N. Blackford, Gabriel Balmus, Joy J. Bianchi, Wei Yu, Léa Bacoccina, Angélique Jarade, Christophe Clouin, Rohan Sivapalan, Bernardo Reina-San-Martin, Stephen P. Jackson, and Ludovic Deriano**



**Figure S1. Experimental strategy, Related to Figure 1**

(A) Scheme depicting the experimental strategy for CRISPR/Cas9 genome editing of pro-B cell lines.  
 (B) PCR analysis showing the deletion of approximately 626bp in the *Paxx* gene (WT = 1299bp band; *Paxx* KO ≈ 673bp band).

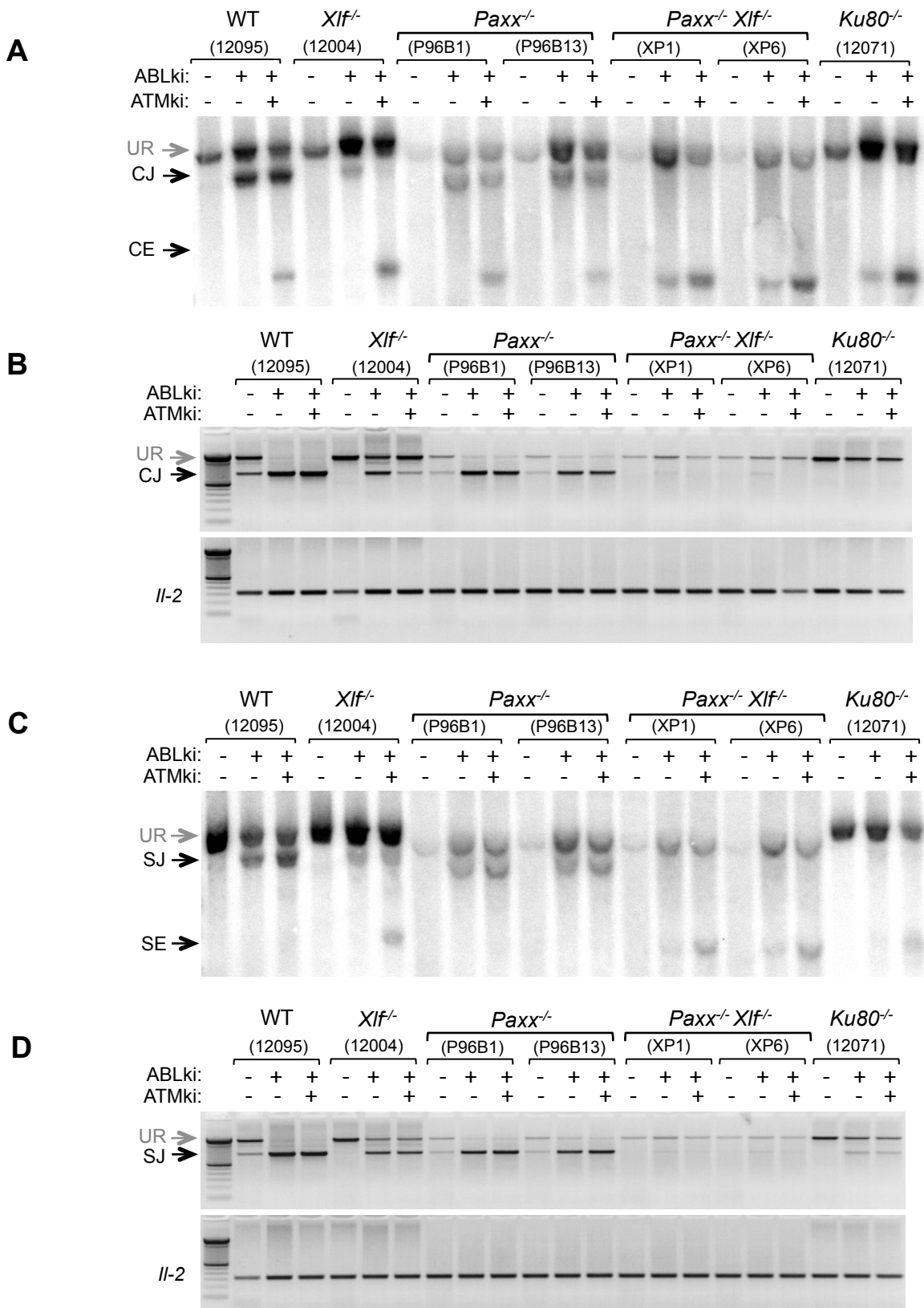
(C) Western blot showing the absence of the PAXX protein in *Paxx*<sup>-/-</sup> *Rag2*<sup>c/c</sup> and *Paxx*<sup>-/-</sup> *Atm*<sup>-/-</sup> pro-B cell clones.



**Figure S2. Defective inversional V(D)J recombination in *Paxx*<sup>-/-</sup> *Xif*<sup>-/-</sup> B cells, Related to Figure 3**

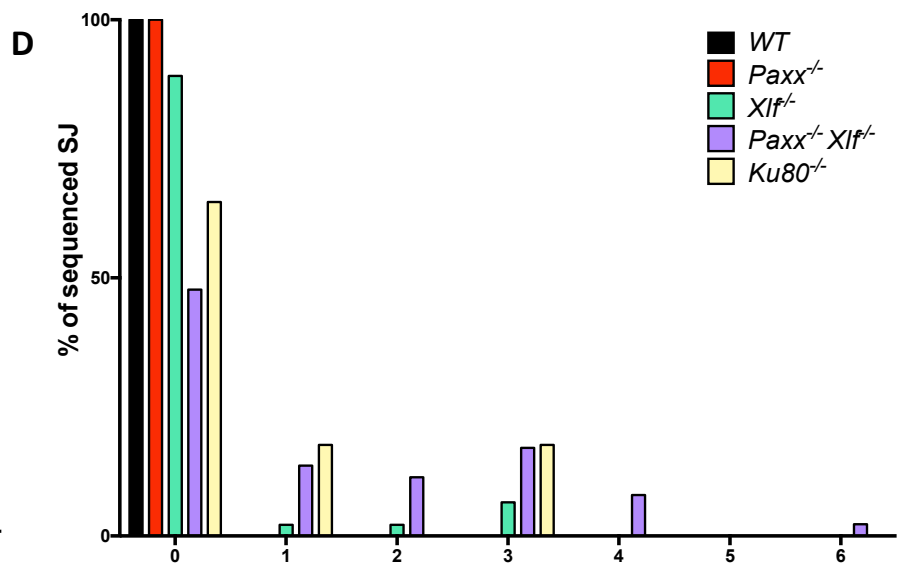
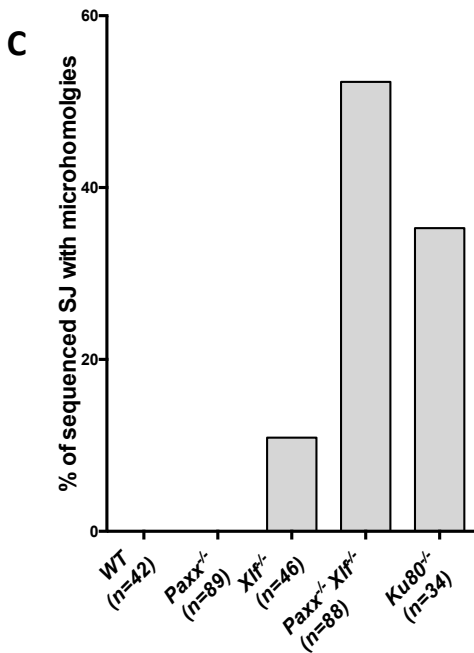
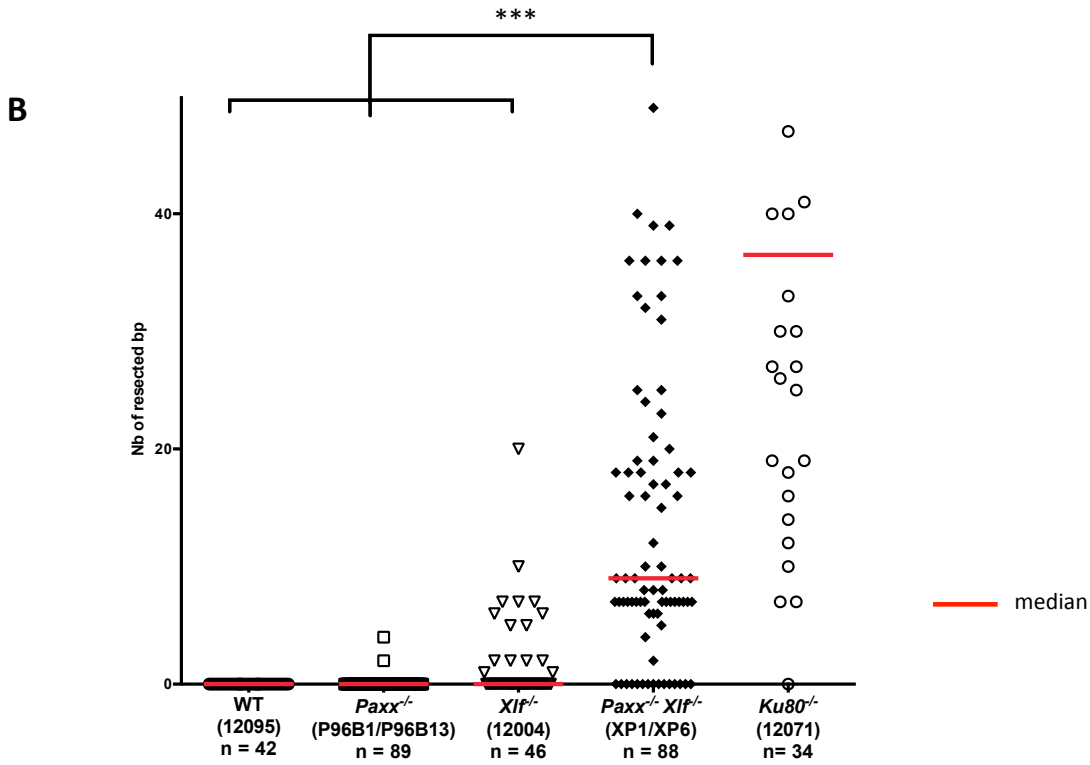
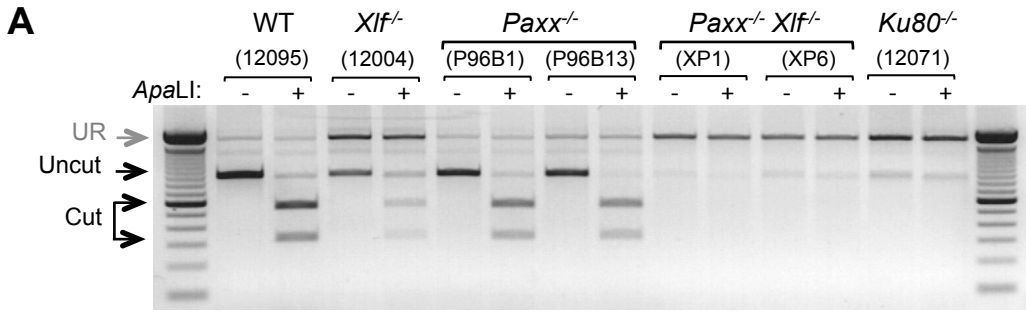
(A) The indicated *v-abl* pro-B cell lines containing the pMX-INV substrate were treated for 72h with ABLki with or without ATMki and assayed by Southern blotting; EcoRV/NcoI digest – C4 probe (top panel) and EcoRV digest – C4 probe (bottom panel).

(B) PCR analysis of pMX-INV coding and hybrid joints from indicated *v-abl* *abl* pro-B cell lines treated for 72h with ABLki with or without ATMki. *Il-2* gene PCR was used as a loading control.



**Figure S3. Defective deletional coding and signal joint formation in *Paxx*<sup>-/-</sup> *Xif*<sup>-/-</sup> B cells, Related to Figure 4**

pMX-DEL<sup>CJ</sup> (A, B) and pMX-DEL<sup>SJ</sup> (C, D) *v-abl* pro-B cell lines were treated for 72h with ABLki with or without ATMki. Coding and signal joint formation were assessed by Southern blotting; EcoRV digest – C4 probe (A, C) and by PCR (B, D). *Il-2* gene PCR was used as a loading control (B, D).



**Figure S4. Analysis of signal joints sequences in *v-abl* pMX-DEL<sup>SJ</sup> pro-B cell lines, Related to Figure 4**

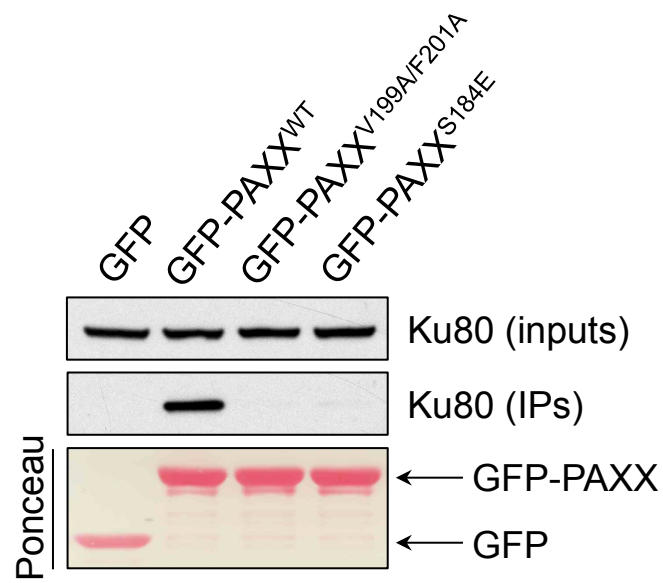
(A) PCR products of pMX-DEL<sup>SJ</sup> signal junctions were digested by *Apa*LI.

(B) Sanger sequencing of PCR amplified signal joints from *v-abl* pro-B cells (See also Table S4).

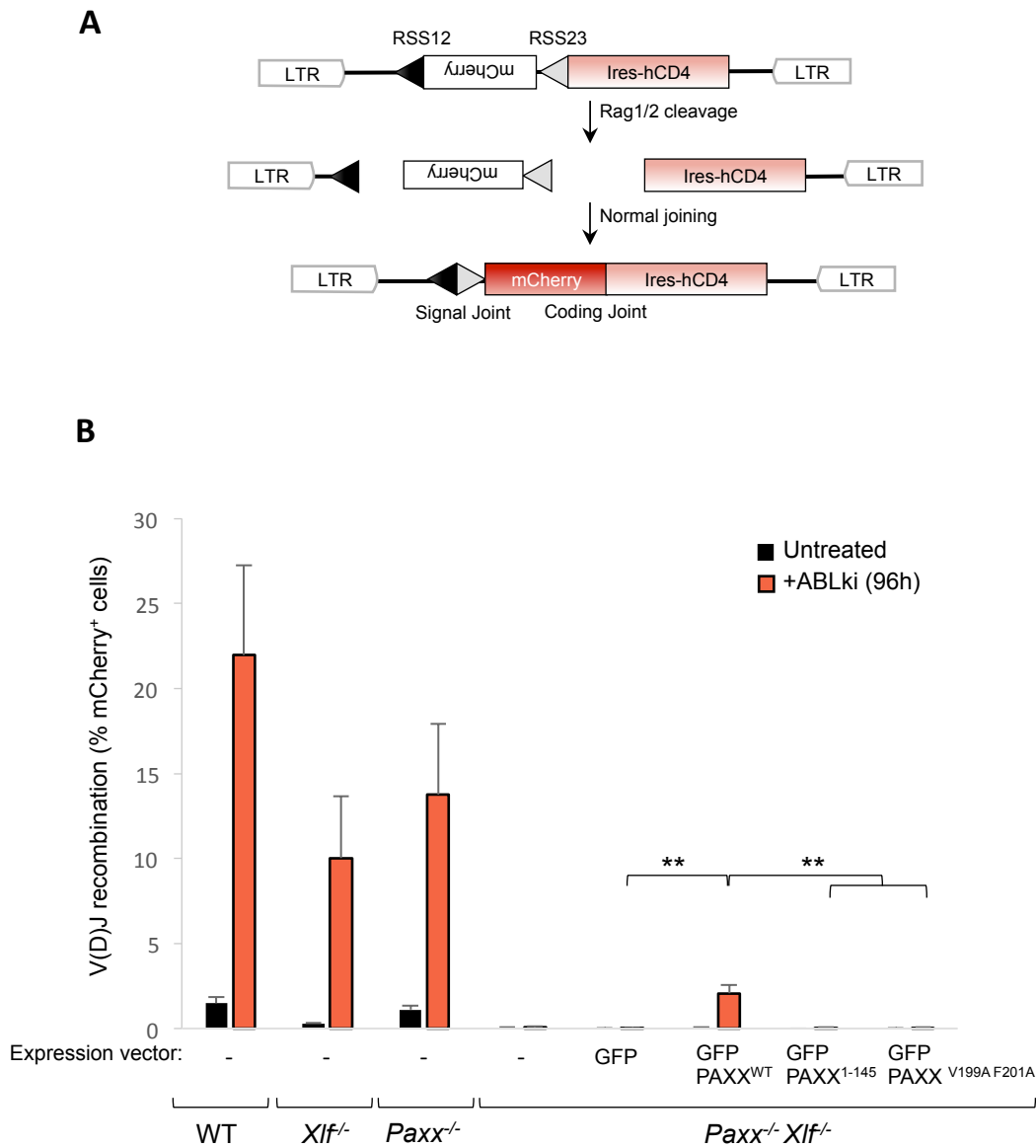
Number of base-pair deletions is indicated on the y-axis. The horizontal bar represents the median.

The total number of sequenced signal joints (n) is indicated. Outliers (> 49 bp deletion) are not shown but are included in calculation of the median. \*\*\*p<0.001.

(C and D) Microhomology utilization in pMX-DEL<sup>SJ</sup> SJs sequenced from indicated *v-abl* pro-B cell lines. (C) Percentage of sequences with microhomologies  $\geq$  1bp. (D) Microhomology length distribution among the same sequences.



**Figure S5. C-terminal PAXX mutations S184E or V199A/F201A similarly abolish the PAXX-Ku interaction, Related to Figure 6**  
 Pulldowns assays from 293FT cells transiently transfected with the indicated plasmids.



**Figure S6. PAXX function in the absence of XLF depends on its interaction with Ku, Related to Figure 6**

(A) Schematic representation of pMX-INV-Cherry recombination substrate.

(B) *Paxx*<sup>-/-</sup> *Xlf*<sup>-/-</sup> pro-B cell lines (XP1, XP6) were transfected with expression vectors encoding for GFP, GFP-PAXX<sup>WT</sup>, GFP-PAXX<sup>1-145</sup> or GFP-PAXX<sup>V199A/F201A</sup>. After 6h recovery, *Paxx*<sup>-/-</sup> *Xlf*<sup>-/-</sup> transfected cells, along with control untransfected cells, were treated for 96 hours with ABLki and assayed for pMX-INV-Cherry rearrangement by flow cytometry, with the percentage of mCherry expressing cells indicated. Data represent the means ± SEMs of three experiments with two independent cell lines for *Paxx*<sup>-/-</sup> *Xlf*<sup>-/-</sup> cell lines and one for the other cell lines.



<b>Genotype</b>	<b>Cell line #</b>	<b>Generation method</b>	<b>Reference</b>
<b>WT</b>	12096	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	Lescale et al., 2016
	12095	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	Lescale et al., 2016
<b><i>Paxx</i><sup>-/-</sup></b>	P96B1	CRISPR-Cas9 genome editing of 12096 cell line	This study
	P96B13	CRISPR-Cas9 genome editing of 12096 cell line	This study
	P95B1	CRISPR-Cas9 genome editing of 12095 cell line	This study
<b><i>Paxx</i><sup>-/-</sup> <i>Xlf</i><sup>-/-</sup></b>	XP1	CRISPR-Cas9 genome editing of P96B1 cell line	This study
	XP6	CRISPR-Cas9 genome editing of P96B1 cell line	This study
<b><i>Xlf</i><sup>-/-</sup></b>	X95-3	CRISPR-Cas9 genome editing of 12095 cell line	This study
	X95-4	CRISPR-Cas9 genome editing of 12095 cell line	This study
	16488	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	This study
	12004	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	Lescale et al., 2016
<b><i>Ku80</i><sup>-/-</sup></b>	12071	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	Lescale et al., 2016
<b><i>Xrcc4</i><sup>-/-</sup></b>	Xr-95-21	CRISPR-Cas9 genome editing of 12095 cell line	This study
	Xr-95-23	CRISPR-Cas9 genome editing of 12095 cell line	This study
<b><i>Rag2</i><sup>c/c</sup></b>	12018	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	Lescale et al., 2016
	12019	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	Lescale et al., 2016
<b><i>Paxx</i><sup>-/-</sup> <i>Rag2</i><sup>c/c</sup></b>	P18B2	CRISPR-Cas9 genome editing of 12018 cell line	This study
	P19B12	CRISPR-Cas9 genome editing of 12019 cell line	This study
<b><i>Atm</i><sup>-/-</sup></b>	13563	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	This study
	160	<i>v-Abl/Bcl2</i> immortalization of bone marrow pro-B cells	This study
<b><i>Paxx</i><sup>-/-</sup> <i>Atm</i><sup>-/-</sup></b>	P563A23	CRISPR-Cas9 genome editing of 13563 cell line	This study

**Table S1. List and origin of *v-abl* pro-B cell lines, Related to Figure 1**

**A**

EXPMT 1	Cell line #	NI	1 Gy	2.5 Gy	5 Gy
WT	12095	4.3 (100%)	4.12 (95.8%)	3.42 (79.5%)	1.49 (34.7%)
	12096	4.4 (100%)	4.31 (98.0%)	3.43 (78.0%)	1.11 (25.2%)
<i>Paxx</i> <sup>-/-</sup>	P96B1	4.3 (100%)	4.11 (95.6%)	2.27 (52.8%)	0.7 (16.3%)
	P96B13	4.5 (100%)	4.0 (88.9%)	2.78 (61.8%)	0.79 (17.6%)
<i>Xlf</i> <sup>-/-</sup>	16488	3.6 (100%)	2.17 (60.3%)	0.84 (23.3%)	0.37 (10.3%)
	X95-3	4.0 (100%)	3.58 (89.5%)	1.98 (49.5%)	0.57 (14.3%)
<i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	XP1	4.4 (100%)	1.8 (40.9%)	0.69 (15.7%)	0.38 (8.6%)
	XP6	4.7 (100%)	1.97 (41.9%)	0.76 (16.2%)	0.41 (8.7%)
<i>Xrcc4</i> <sup>-/-</sup>	Xr-95-21	3.8 (100%)	2.9 (76.3%)	0.81 (21.3%)	0.35 (9.2%)
	Xr-95-23	4.2 (100%)	3.79 (90.2%)	1.68 (40.0%)	0.51 (12.1%)

EXPMT 2	Cell line #	NI	1 Gy	2.5 Gy	5 Gy
WT	12095	4.8 (100%)	4.23 (88.1%)	3.47 (72.3%)	2.0 (41.7%)
	12096	4.28 (100%)	3.78 (88.3%)	2.82 (65.9%)	1.17 (27.3%)
<i>Paxx</i> <sup>-/-</sup>	P96B1	4.43 (100%)	3.4 (76.7%)	2.78 (62.8%)	0.87 (19.6%)
	P96B13	4.54 (100%)	4.2 (92.5%)	2.6 (57.3%)	1.0 (22.0%)
<i>Xlf</i> <sup>-/-</sup>	16488	3.83 (100%)	3.2 (83.6%)	1.65 (43.1%)	0.63 (16.4%)
	X95-3	4.55 (100%)	3.48 (76.5%)	2.06 (45.3%)	0.72 (15.8%)
<i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	XP1	4.48 (100%)	1.52 (33.9%)	1.39 (31.0%)	0.51 (11.4%)
	XP6	4.86 (100%)	1.98 (40.7%)	1.2 (24.7%)	0.6 (12.3%)
<i>Xrcc4</i> <sup>-/-</sup>	Xr-95-21	4.29 (100%)	2.76 (64.3%)	1.29 (30.1%)	0.47 (11.0%)
	Xr-95-23	4.33 (100%)	3.62 (83.6%)	2.43 (56.1%)	1.0 (23.1%)

EXPMT 3	Cell line #	NI	1 Gy	2.5 Gy	5 Gy
WT	12095	4.28 (100%)	4.3 (100.5%)	3.7 (86.4%)	2.4 (56.1%)
	12096	4.32 (100%)	3.77 (87.3%)	3.43 (79.4%)	2.11 (48.8%)
<i>Paxx</i> <sup>-/-</sup>	P96B1	4.54 (100%)	3.8 (83.7%)	3.04 (67.0%)	1.68 (37.0%)
	P96B13	4.29 (100%)	3.91 (91.1%)	2.97 (69.2%)	1.34 (31.2%)
<i>Xlf</i> <sup>-/-</sup>	16488	4.28 (100%)	3.54 (82.7%)	2.82 (65.9%)	0.97 (22.7%)
	X95-3	4.3 (100%)	3.6 (83.7%)	2.53 (58.8%)	0.84 (19.5%)
<i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	XP1	4.2 (100%)	2.73 (65.0%)	1.23 (29.3%)	0.62 (14.8%)
	XP6	4.36 (100%)	2.88 (66.1%)	0.86 (19.7%)	0.5 (11.5%)
<i>Xrcc4</i> <sup>-/-</sup>	Xr-95-21	4.68 (100%)	3.02 (64.5%)	1.11 (23.7%)	0.59 (12.6%)
	Xr-95-23	4.57 (100%)	3.49 (76.4%)	2.38 (52.1%)	1.09 (23.9%)

**B**

		1 Gy		2.5 Gy	
		p value	Significant	p value	Significant
WT	vs <i>Paxx</i> <sup>-/-</sup>	0.39393	no	0.00865	yes
	vs <i>Xlf</i> <sup>-/-</sup>	0.01515	yes	0.00639	yes
	vs <i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	0.00216	yes	0.00216	yes
	vs <i>Xrcc4</i> <sup>-/-</sup>	0.01515	yes	0.00216	yes
<i>Paxx</i> <sup>-/-</sup>	vs <i>Xlf</i> <sup>-/-</sup>	0.07764	no	0.06493	no
	vs <i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	0.00216	yes	0.00216	yes
	vs <i>Xrcc4</i> <sup>-/-</sup>	0.02597	yes	0.00432	yes
<i>Xlf</i> <sup>-/-</sup>	vs <i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	0.00865	yes	0.01515	yes
	vs <i>Xrcc4</i> <sup>-/-</sup>	0.52110	no	0.30952	no
<i>Paxx</i> <sup>-/-</sup> <i>Xlf</i> <sup>-/-</sup>	vs <i>Xrcc4</i> <sup>-/-</sup>	0.02597	yes	0.09307	no

**Table S2. Irradiation sensitivity assay of pro-B cell lines, Related to Figure 1**

(A) Numbers indicate cell counts ( $10^6$  cells/ml) after 3 days irradiation at 1Gy, 2.5Gy and 5Gy. Percentages of viable cells compared to non irradiated cells are indicated in brackets.

(B) Statistical analysis was performed at 1 and 2.5 Gy using Mann Whitney test.

**A**

EXPMT 1	WT			<i>Paxx</i> <sup>-/-</sup>			<i>Xif</i> <sup>-/-</sup>			<i>Paxx</i> <sup>-/-</sup> <i>Xif</i> <sup>-/-</sup>		
	12095			P96B1			X95-3			XP1		
	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %
Cell line #1	0	3387	67.5	0	3167	63	0	1879	37.4	0	703	14.1
	1	1236	24.6	1	1663	33.1	1	2475	49.3	1	2784	55.9
	2	263	5.2	2	167	3.3	2	526	10.5	2	1209	24.3
	>2	130	2.6	>2	33	0.6	>2	144	2.8	>2	284	5.7
	TOTAL	5016	100	TOTAL	5030	100	TOTAL	5024	100	TOTAL	4980	100
Cell line #2	0	3204	63.8	0	2348	46.7	0	2885	56.7	0	721	14.4
	1	1488	29.6	1	2188	43.5	1	1727	33.9	1	3101	62
	2	248	4.9	2	400	8	2	360	7.1	2	994	19.9
	>2	81	1.5	>2	92	1.8	>2	117	2.2	>2	188	3.7
	TOTAL	5021	100	TOTAL	5028	100	TOTAL	5089	100	TOTAL	5004	100

EXPMT 2	WT			<i>Paxx</i> <sup>-/-</sup>			<i>Xif</i> <sup>-/-</sup>			<i>Paxx</i> <sup>-/-</sup> <i>Xif</i> <sup>-/-</sup>			<i>Xrcc4</i> <sup>-/-</sup>			<i>Ku80</i> <sup>-/-</sup>			<i>Rag2</i> <sup>-/-</sup>		
	12095			P96B1			X95-3			XP1			Xr-95-21			12071			129F-C1		
	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %
Cell line #1	0	3642	72.3	0	3407	67.9	0	3028	64.3	0	1373	27.4	0	1562	31.1	0	1703	33.6	0	3703	83
	1	958	19	1	1328	26.5	1	1292	27.4	1	2485	49.5	1	2394	47.6	1	2485	49	1	482	10.8
	2	292	5.8	2	200	4	2	292	6.2	2	937	18.7	2	864	17.2	2	716	14.1	2	166	3.7
	>2	148	2.9	>2	80	1.5	>2	98	2.1	>2	221	4.5	>2	207	4.1	>2	165	3.2	>2	109	2.4
	TOTAL	5004	100	TOTAL	5015	100	TOTAL	4710	100	TOTAL	5016	100	TOTAL	5027	100	TOTAL	5069	100	TOTAL	4460	100
Cell line #2	0	3124	74.1	0	2819	61.5	0	3161	63.2	0	1495	29.8	0	1592	31.5						
	1	926	22	1	1204	26.3	1	1568	31.3	1	2665	53	1	2533	50.1						
	2	132	3.1	2	330	7.2	2	237	4.7	2	700	13.9	2	779	15.4						
	>2	36	0.8	>2	232	5	>2	39	0.7	>2	164	3.2	>2	152	3						
	TOTAL	4218	100	TOTAL	4585	100	TOTAL	5005	100	TOTAL	5024	100	TOTAL	5056	100						

EXPMT 3	WT			<i>Paxx</i> <sup>-/-</sup>			<i>Xif</i> <sup>-/-</sup>			<i>Paxx</i> <sup>-/-</sup> <i>Xif</i> <sup>-/-</sup>			<i>Xrcc4</i> <sup>-/-</sup>			<i>Ku80</i> <sup>-/-</sup>			<i>Rag2</i> <sup>-/-</sup>		
	12095			P96B1			X95-3			XP1			Xr-95-21			12071			129F-C1		
	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %
Cell line #1	0	3389	67.7	0	3127	62.1	0	2146	42.8	0	946	18.9	0	1025	20.5	0	1891	37.7	0	4076	80.8
	1	1352	27	1	1686	33.5	1	2291	45.7	1	2672	53.3	1	2526	50.5	1	2217	44.3	1	720	14.3
	2	205	4.1	2	193	3.8	2	466	9.3	2	1163	23.2	2	1130	22.6	2	739	14.8	2	169	3.4
	>2	58	1.2	>2	29	0.6	>2	106	2.1	>2	235	4.7	>2	325	6.5	>2	163	3.3	>2	79	1.5
	TOTAL	5004	100	TOTAL	5035	100	TOTAL	5009	100	TOTAL	5016	100	TOTAL	5006	100	TOTAL	5010	100	TOTAL	5044	100
Cell line #2	0	3381	67.6	0	2106	41.9	0	2787	55.5	0	786	15.6	0	1156	23.1						
	1	1447	28.9	1	2354	46.8	1	1883	37.5	1	3073	61	1	2597	51.8						
	2	138	2.8	2	508	10.1	2	306	6.1	2	1022	20.3	2	941	18.8						
	>2	35	0.6	>2	59	1.1	>2	45	0.9	>2	154	3.1	>2	315	6.3						
	TOTAL	5001	100	TOTAL	5027	100	TOTAL	5021	100	TOTAL	5035	100	TOTAL	5009	100						

**B**

WT			<i>Paxx</i> <sup>-/-</sup>			<i>Xif</i> <sup>-/-</sup>			<i>Paxx</i> <sup>-/-</sup> <i>Xif</i> <sup>-/-</sup>			<i>Xrcc4</i> <sup>-/-</sup>			<i>Ku80</i> <sup>-/-</sup>			<i>Rag2</i> <sup>-/-</sup>		
Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %	Nb of 53BP1 foci/nuclei	Nuclei Nb	Nuclei %
0	20127	68.8	0	16974	57.2	0	15886	53.3	0	6024	20.0	0	5335	26.6	0	3594	35.7	0	7779	81.9
1	7407	25.2	1	10423	35.0	1	11236	37.5	1	16780	55.8	1	10050	50.0	1	4702	46.7	1	1202	12.6
2	1278	4.3	2	1798	6.1	2	2187	7.3	2	6025	20.1	2	3714	18.5	2	1455	14.5	2	335	3.6
>2	488	1.6	>2	525	1.8	>2	549	1.8	>2	1246	4.2	>2	999	5.0	>2	328	3.3	>2	188	2.0
Total nuclei	29300	100	TOTAL	29720	100	TOTAL	29858	100	TOTAL	30075	100	TOTAL	20098	100	TOTAL	10079	100	TOTAL	9504	100

**Table S3. *Paxx*<sup>-/-</sup> *Xif*<sup>-/-</sup> *v-abl* pro-B cell harbor increased 53BP1 DDR foci after RAG induction, Related to Figure 2**

(A) Table indicates the numbers and percentages of *v-abl* pro-B cells harboring 0, 1, 2 or >2 53BP1 foci 65h after ABLki treatment. Three independent experiments are shown.

(B) Table indicates total nuclei numbers and mean percentages of the experiments detailed in table A.

**WT**

Sequence #	5' SE	MH	N	3' SE	clones/total
	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	
1	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	38/42
2	GGTTTTGTCCAGTCTGTAGCACTGTG		GG	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/42
3	GGTTTTGTCCAGTCTGTAGCACTGTG		AG	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/42
4	GGTTTTGTCCAGTCTGTAGCACTGTG		AC	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/42
5	GGTTTTGTCCAGTCTGTAGCACTGTG		CC	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/42

**Paxx<sup>-/-</sup>**

Sequence #	5' SE	MH	N	3' SE	clones/total
	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	
1	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	83/89
2	GGTTTTGTCCAGTCTGTAGCACTGTG		AG	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/89
3	GGTTTTGTCCAGTCTGTAGCACTGTG		CC	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/89
4	GGTTTTGTCCAGTCTGTAGCACTGTG		GGGAT	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/89
5	GGTTTTGTCCAGTCTGTAGCACTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/89
6	GGTTTTGTCCAGTCTGTAGCACTG			CAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/89
7	GGTTTTGTCCAGTCTGTAGCACTGTG		409 bp	CCACGTGTATAAGAT (-384) ATAAGAT	1/89

**Xlf<sup>-/-</sup>**

Sequence #	5' SE	MH	N	3' SE	clones/total
	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	
1	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	29/46
2	GGTTTTGTCCAGTCTGTAGCACTGTG		CC	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	2/46
3	GGTTTTGTCCAGTCTGTAGCACTGT			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
4	GGTTTTGTCCAGTCTGTAGCACTGT		C	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
5	GGTTTTGTCCAGTCTGTAGCACTGTG		CG	CAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
6	GGTTTTGTCCAGTCTGTAGCACTG		CG	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
7	GGTTTTGTCCAGTCTGTAGCACTG		CCC	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
8	GGTTTTGTCCAGTCTGTAGCACTGTG			CAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
9	GGTTTTGTCCAGTCTGTAGCACTGT	G		TGGTAGTACTCCACTGTCCGGCTGTACAAAAACC	1/46
10	GGTTTTGTCCAGTCTGTAGCACTG		CG	AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
11	GGTTTTGTCCAGTCTGTAGCACTG		AG	GTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
12	GGTTTTGTCCAGTCTGTAGCAC			CAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
13	GGTTTTGTCCAGTCTGTAG	CAC		AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	3/46
14	GGTTTTGTCCAGTCTGTAGCACT	GT		AGTACTCCACTGTCTGGCTGTACAAAAACC	1/46
15	GGTTTTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/46

*Paxx*<sup>-/-</sup> *Xlf*<sup>-/-</sup>

Sequence #	5' SE	MH	N	3' SE	clones/total
	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	
1	GGTTTTGTCCAGTCTGTAGCACTGTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	14/88
2	GGTTTTGTCCAGTCTGTAGCACTGTG		804	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
3	GGTTTTGTCCAGTCTGTAGCACTG			CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
4	GGTTTTGTCCAGTCTGTAGCACTG			CAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
5	GGTTTTGTCCAGTCTGTAGCA	C		ACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
6	GGTTTTGTCCAGTCTGTAGCACTGTG			GGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
7	GGTTTTGTCCAGTCTGTAGCACT		A	AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
8	GGTTTTGTCCAGTCTGTAGCACT			AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
9	GGTTTTGTCCAGTCTGTAGCA	C		AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	2/88
10	GGTTTTGTCCAGTCTGTAG	CAC		AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	12/88
11	GGTTTTGTCCAGTCTGTAGCACT	GTG		GTAGTACTCCACTGTCTGGCTGTACAAAAACC	2/88
12	GGTTTTGTCCAGTCTGTAGCAC			GTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
13	GGTTTTGTCCAGTCTGTAGCACTG			GGTAGTACTCCACTGTCTGGCTGTACAAAAACC	2/88
14	GGTTTTGTCCAGTCTGTAG	CA		GTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	6/88
15	GGTTTTGTCCAGTCTGTAGCACT	GT		AGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
16	GGTTTTGTCCAGTCTG		C	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
17	GGTTTTGTCCAGTCTGTAG		A	TGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
18	GGTTTTGTCCAGTCT	GT		GGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
19	GGTTTTGTCCAGTCTG			GGTAGTACTCCACTGTCTGGCTGTACAAAAACC	2/88
20	GGTTTTGTCCAGTCTG		C	GGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
21	GGTTTTGTCCAG		G	AGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	2/88
22	GGTTTTGTCCAGTCT	GTAG		TACTCCACTGTCTGGCTGTACAAAAACC	4/88
23	GGTTTTGTT	C		ACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
24	GGTTTTGTT	CAGT		GGTAGTACTCCACTGTCTGGCTGTACAAAAGACC	2/88
25	GGTTTTGTCCAGTCTGTAGCACTG			CTGTCTGGCTGTACAAAAACC	1/88
26	GGTTTTGTCCAGTCT	GTA		CTCCACTGTCTGGCTGTACAAAAACC	1/88
27	GGTTTTGTCCAGTCTGTAG	CACTGT		CTGGCTGTACAAAAACC	1/88
28	GGTTTTGTCCAGTCTG		G	TCCACTGTCTGGCTGTACAAAAACC	1/88
29	GGTTTTGTCCAGTCTGTAG	C		TGTCTGGCTGTACAAAAACC	2/88
30	GGTTTTGTT	C		TCCACTGTCTGGCTGTACAAAAACC	1/88
31	GGTTTTGTCCAGTCTGTA	G		GCTGTACAAAAACC	1/88
32	GGTTTTGTTCCAG	T		GTCTGGCTGTACAAAAACC	1/88
33	G	G		GTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
34	GG			GTA TCCACTGTCTGGCTGTACAAAAACC	4/88
35	GGTTTTGTT	C		TGGCTGTACAAAAACC	2/88
36	GGTTTTGT	TC		TGGCTGTACAAAAACC	1/88
37	GGTTTTGTCCAGTCTGTA	GCAC		(-45) GCACCCGGGTA CCGGGTA	1/88
38	<i>GGATCTCGCGG</i> GGATCT (-53)		T	TGTCTGGCTGTACAAAAACC	1/88
39	<i>GCCGACCCCGG</i> GCCGACC (-87)		162 bp	CACAGTGGTAGTACTCCACTGTCTGGCTGTACAAAAACC	1/88
40	GGTTTTGTCCAGTCTGTAGCACTGTG		221 bp	(-199) CCTGTCTTCTGA TTCITGA	1/88
41	GGTTTTGTCCAGTCTGTAGCACTG	TG		(-327) CCTTTGCAGGCA CAGGCA	1/88
42	GGTTTTGTCCAGTCTGTAGCACTGTG		192 bp	(-347) CACTGGCGACA CTGGCGACA	1/88
43	GGTTTTGTCCAGTCTGTAGCACTG		223 bp	(-397) ACCTGCAAAGGC CTGCAAAGGC	1/88
44	<i>AGACCTCTGGCGG</i> AGACCT (-298)	CTGGCG		(-347) CCTGGCGACAGG ACAGG	1/88
45	<i>GTCCGCCGACCC</i> GTCCGCC (-216)			(-440) GATAGTTGTGGA TTGTGGA	1/88

**Table S4. Signal Joints sequences from WT:DEL<sup>SJ</sup>, *Paxx*<sup>-/-</sup>:DEL<sup>SJ</sup>, *Xlf*<sup>-/-</sup>:DEL<sup>SJ</sup> and *Paxx*<sup>-/-</sup> *Xlf*<sup>-/-</sup> DEL<sup>SJ</sup> *v-abl* pro-B cells, Related to Figure 4**

Reference sequences for 5' and 3' signal ends generated from pMX-DEL<sup>SJ</sup> substrate are indicated on top. Microhomologies (MH) and N-nucleotides additions are indicated. For sequences with additions greater than 5 nucleotides, the numbers of additional bases pairs are indicated instead. When signal ends are resected further than the reference sequence, the numbers of resected nucleotides are shown in brackets along with the reference sequence flanking the resected end in italics. Frequency of unique sequence is indicated (last column). *Paxx*<sup>-/-</sup> *Xlf*<sup>-/-</sup> sequences were obtained from two independent cell lines (XP1, XP6).