# Supplementary information

## Repair of G1 induced DNA double-strand breaks in S-G2/M by alternative NHEJ

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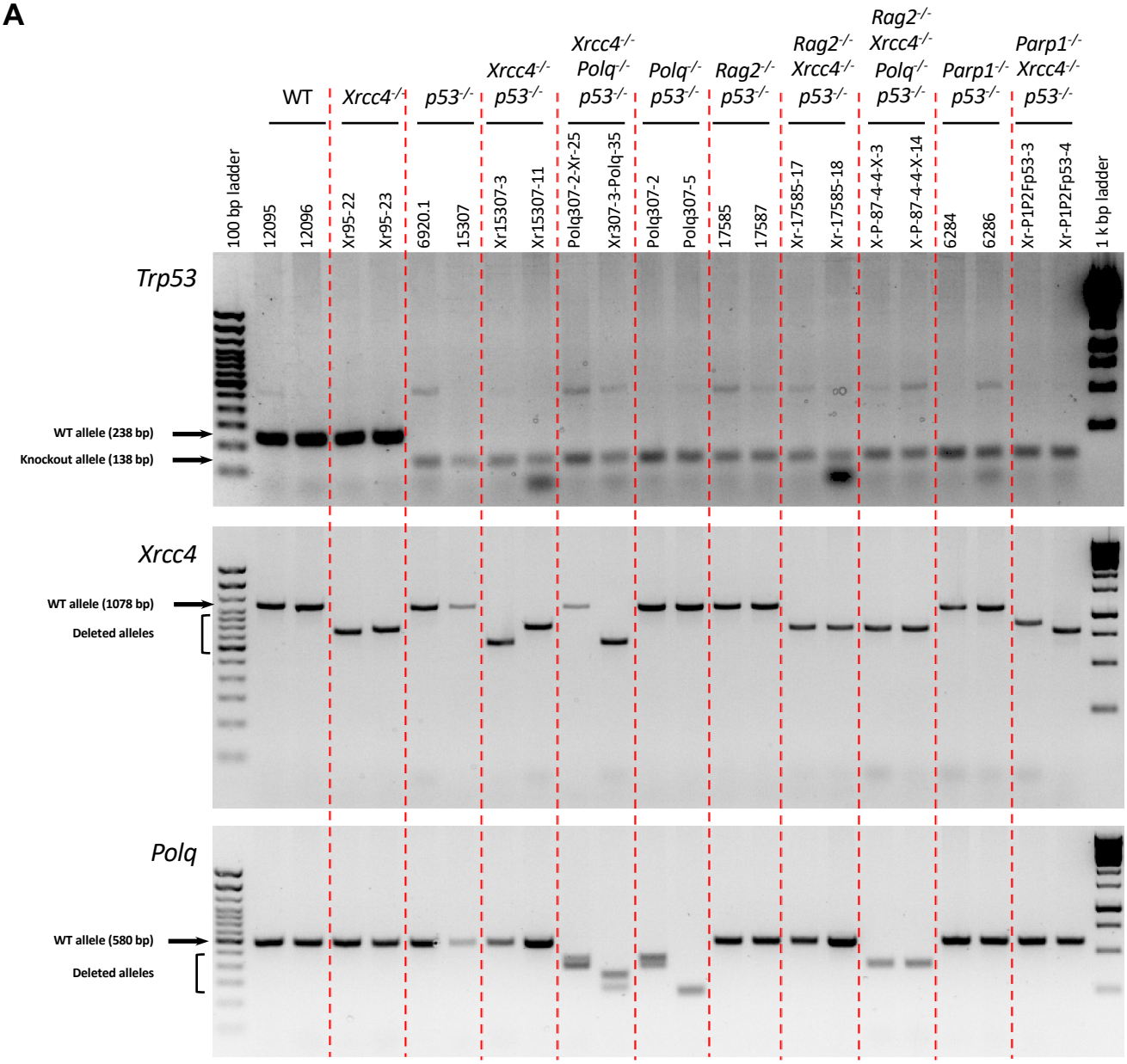
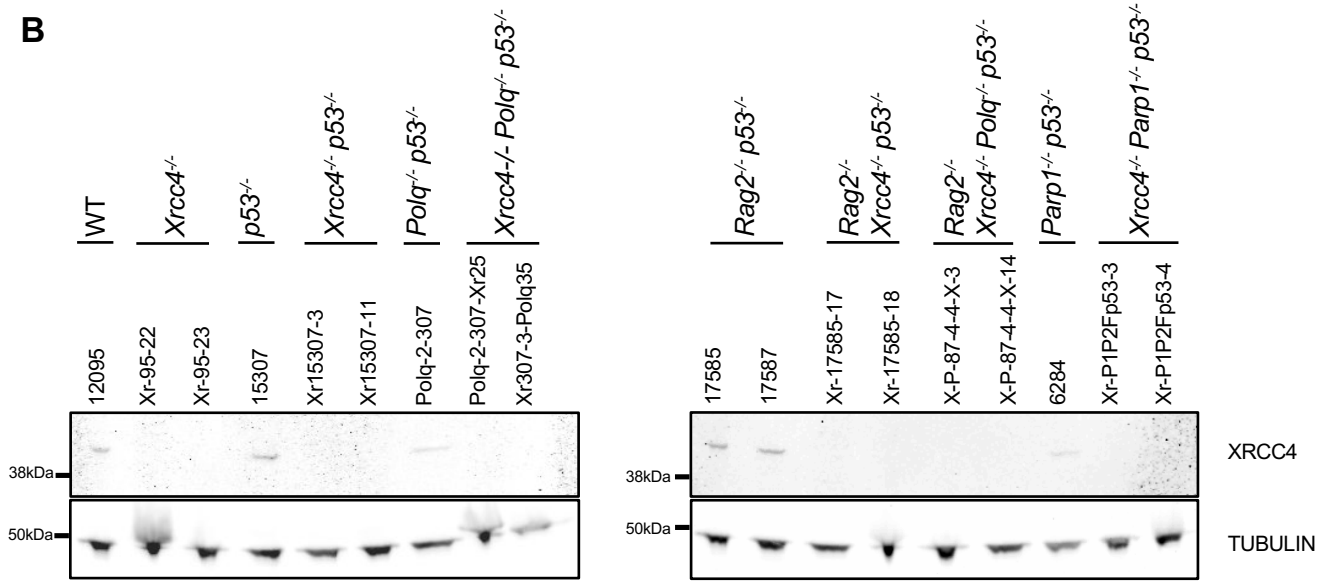
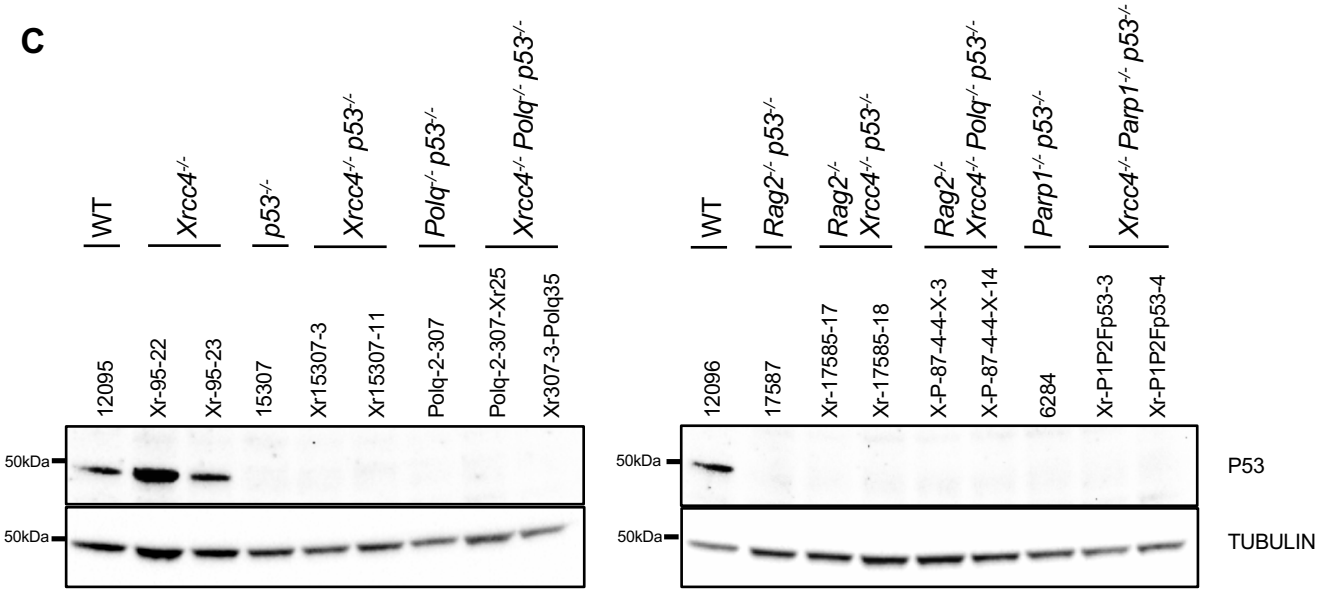
# Supplementary Figure 1

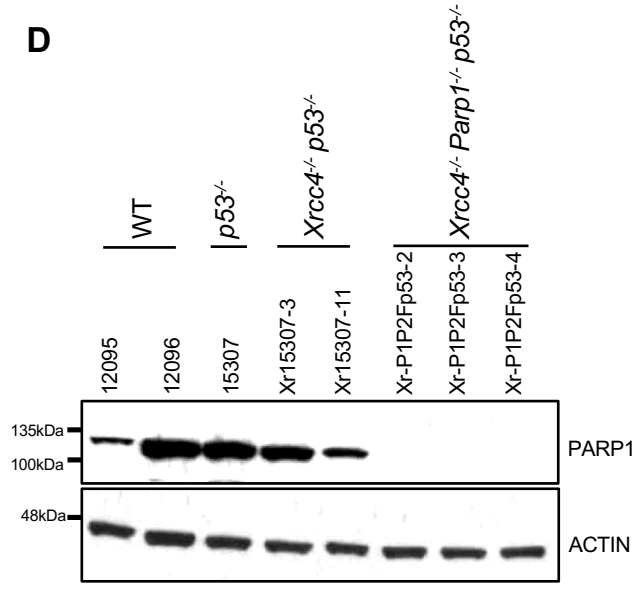
**A**



# Supplementary Figure 1

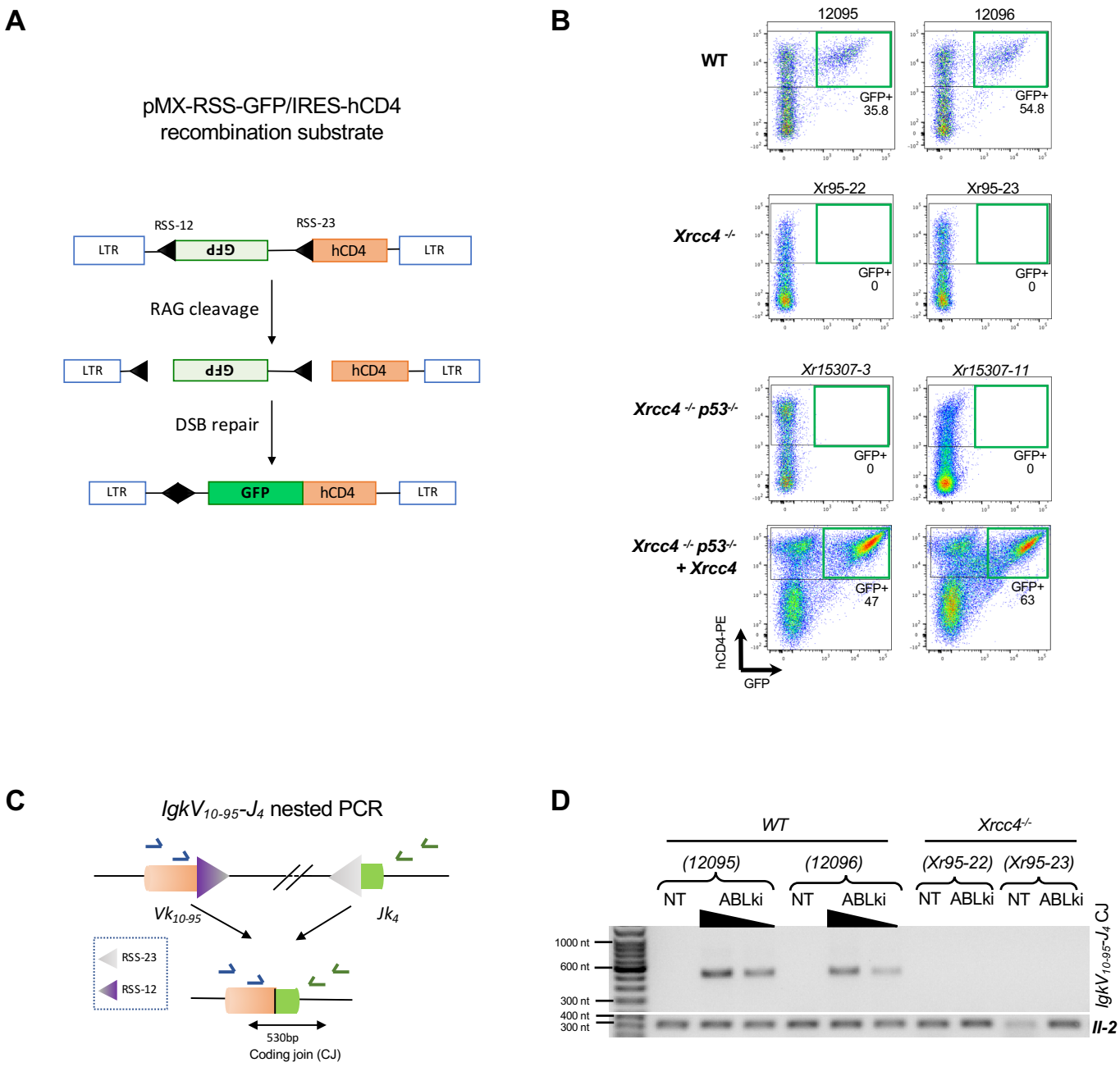


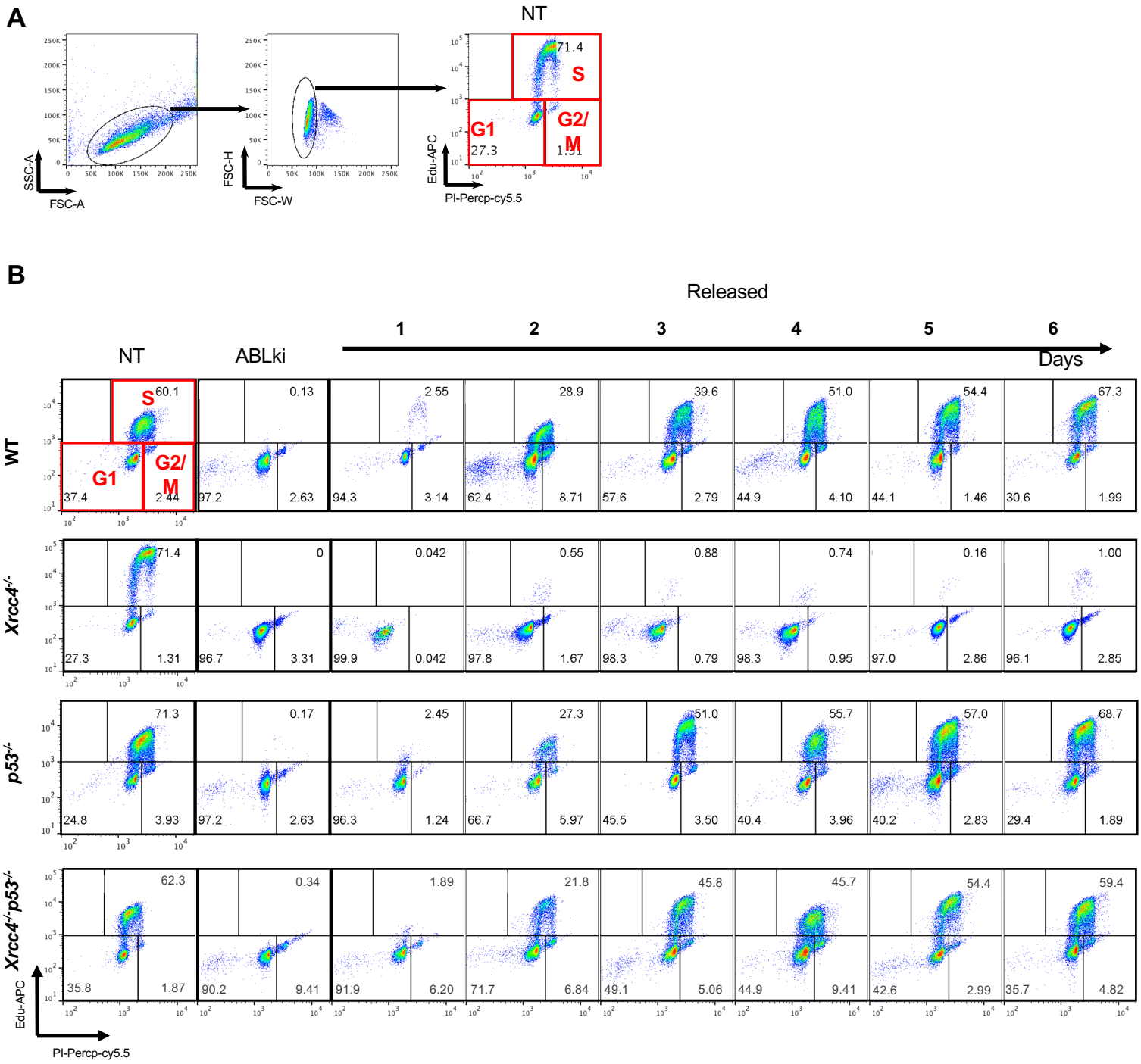

**Supplementary Figure 1.**
**(A)** PCR-based genotyping of indicated cell lines.
**(B)** XRCC4 Western blots for the indicated cell lines. γTubulin was used as a loading control.
**(C)** P53 Western blots for the indicated cell lines. γTubulin was used as a loading control.
**(D)** PARP1 Western blots for the indicated cell lines. ACTIN was used as a loading control. n=1 experiment.

# Supplementary Figure 2



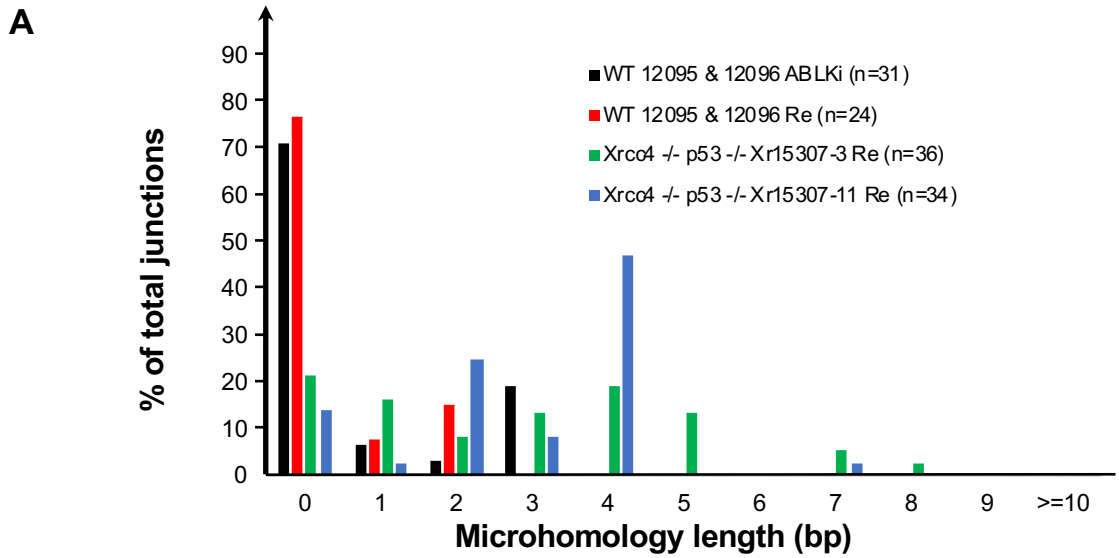

**Supplementary Figure 2.**
**Related to Figure 1.**
**(A)** Schematic representation of pMX-INV recombination substrate. The 12,23 -recombination signal sequences (black triangles), GFP cDNA, human CD4 cDNA (hCD4) and LTRs are indicated. **(B)** *v-Abl* pro–B cell lines treated for 72 hours with ABLki were assayed for PMX-INV rearrangement by flow cytometry. Representative plots are shown for 2 WT (*12095, 12096*), 2 *Xrcc4* $^{-/-}$ (*Xr95-22, Xr95-23*) and 2 *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ (*Xr15307-3, Xr15307-11*) independent cell lines. The percentage of GFP$^{+}$ cells among hCD4$^{+}$ cells is indicated. **(C)** Schematic representation of *IgkV*$_{10–95}$*-J*$_4$ coding join nested PCR strategy. **(D)** Semi-quantitative nested PCR analysis of *IgkV*$_{10–95}$*-J*$_4$ coding join from *v-Abl* pro-B cell lines in untreated conditions (NT) and after exposure to ABLki for 72 hours (ABLki). The PCR gel is representative of n=2 independent experiments.

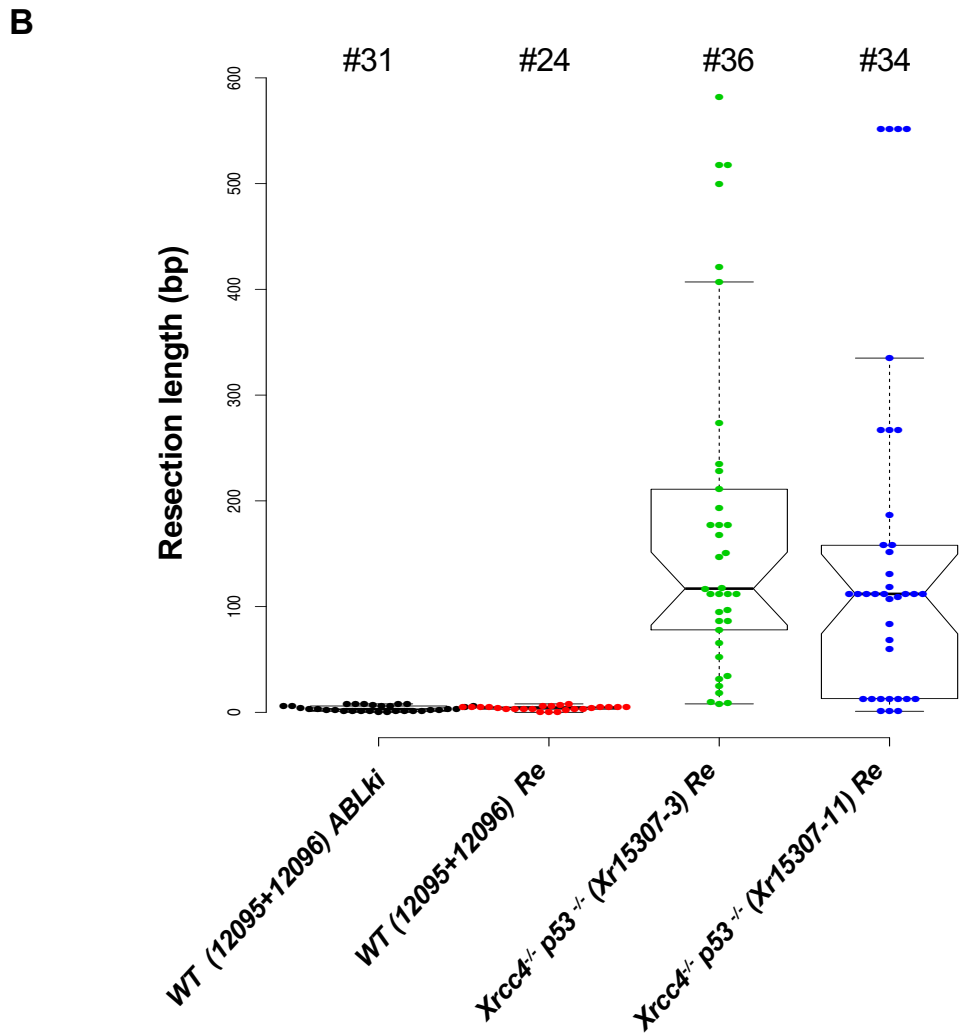
# Supplementary Figure 3




**Supplementary Figure 3.**
**Related to Figure 1.**
(A) Gating strategy to determine the percentage of cells in G1, S and G2/M phases.
(B) Cell cycle analysis of pro-B cells in untreated conditions (NT), after exposure to ABLki for 72 hours (ABLki) and one to six days after washing off ABLki (Released). For each condition, the percentages of G1 (lower left panel), S (upper right panel) and G2/M (lower right panel) cells among total live cells are indicated. Cell lines used: *WT (12095); Xrcc4$^{-/-}$ (Xr95-22); p53$^{-/-}$ (15307); Xrcc4$^{-/-}$ p53$^{-/-}$ (Xr15307-3).*

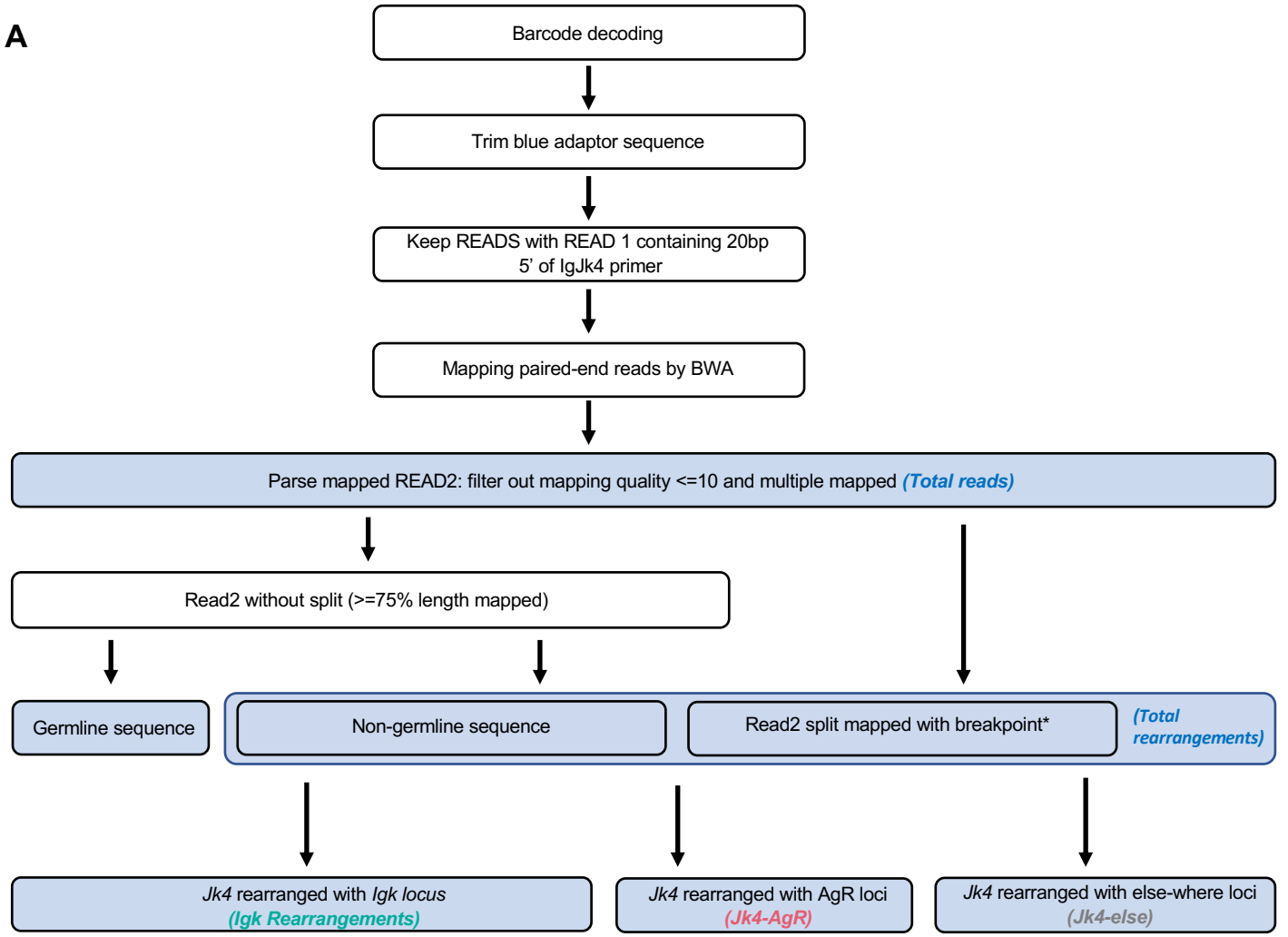
# Supplementary Figure 4

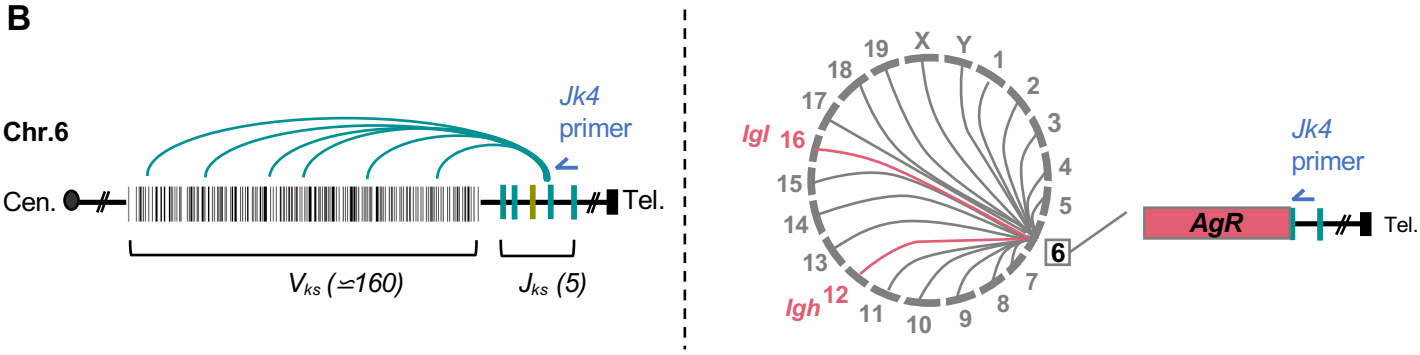



**Supplementary Figure 4.**
**Related to Figure 1.**
**(A)** Percentage of coding joins with variable length of microhomology. **(B)** Resection length of coding joins. Each dot represent one TOPO cloned sequence. The number of TOPO cloned sequences analysed is indicated above. Median values are in the center of box with bounds from 25 to 75 percentile, and the up and low whiskers are 1.5 times the range of the box bounds.

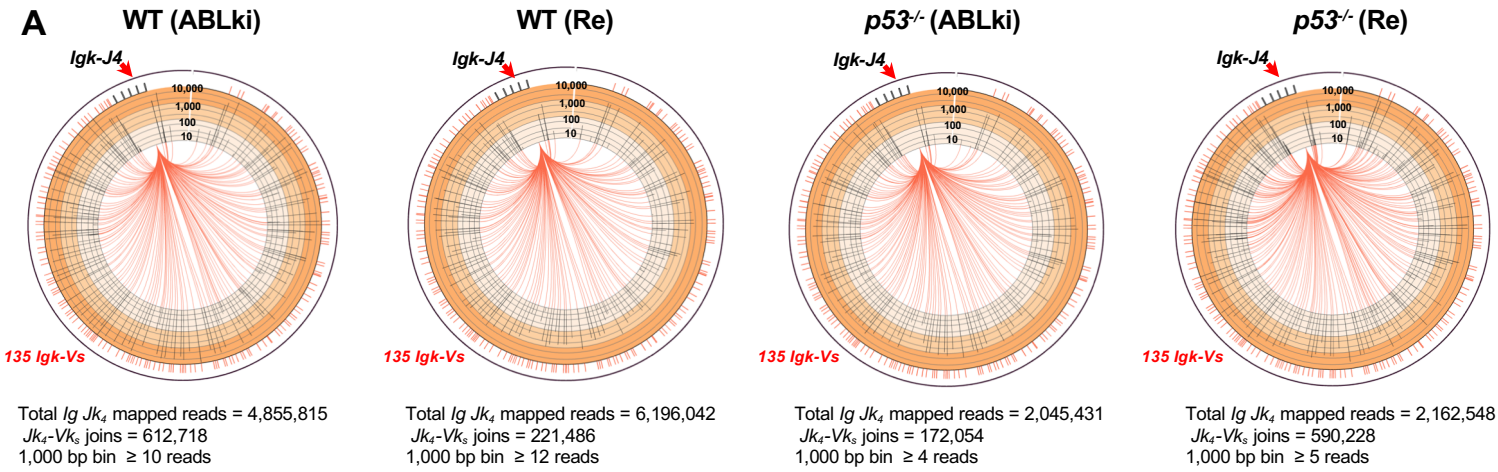
# Supplementary Figure 5

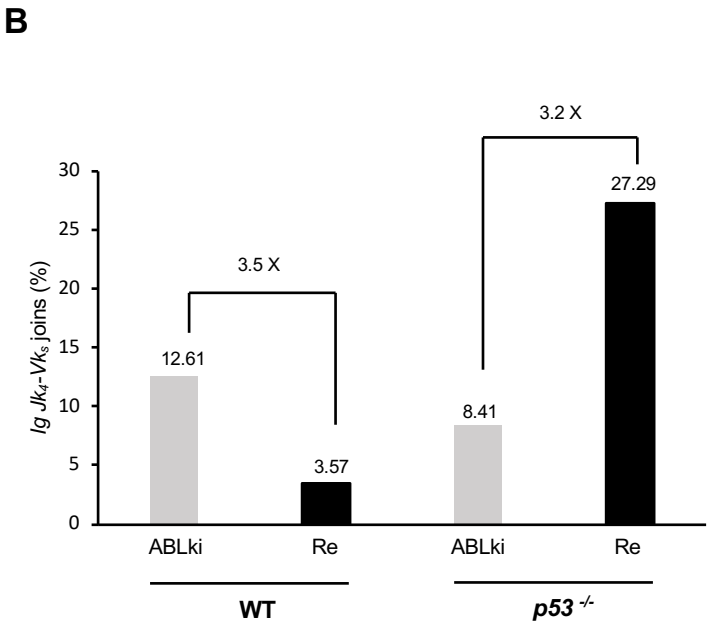



**Supplementary Figure 5.**
**Related to Figures 1,2 and 3.**
**(A)** LAM-HTGTS data analysis procedure. *Only reads containing read2 split mapped with breakpoint were used for the calculation of microhomology, insertion and resection. **(B)** Schematic representations of *IgkJ$_4$-V$_{region}$* junctions (left) and *IgkJ$_4$-AgR* translocations detected by LAM-HTGTS.

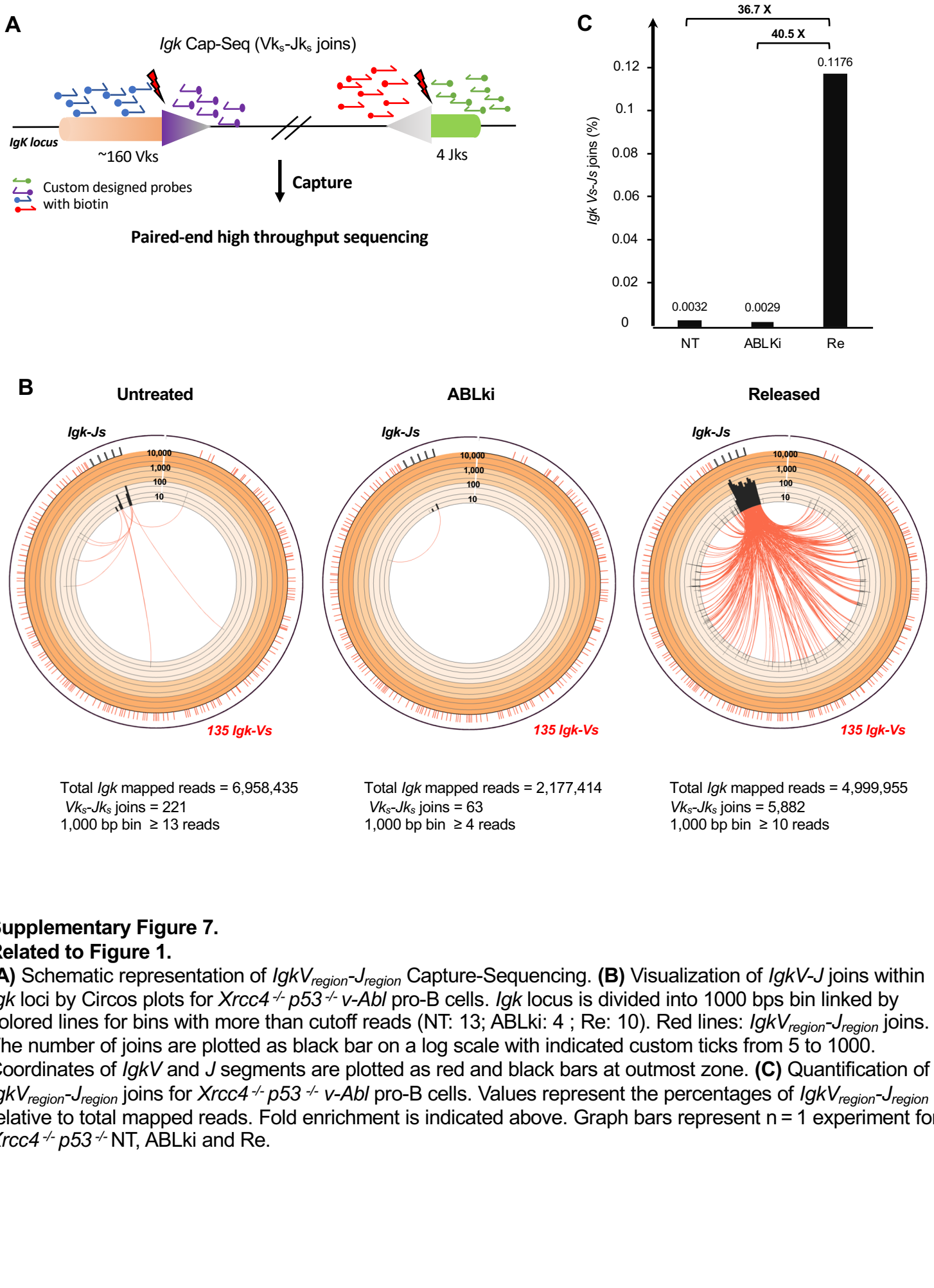
# Supplementary Figure 6



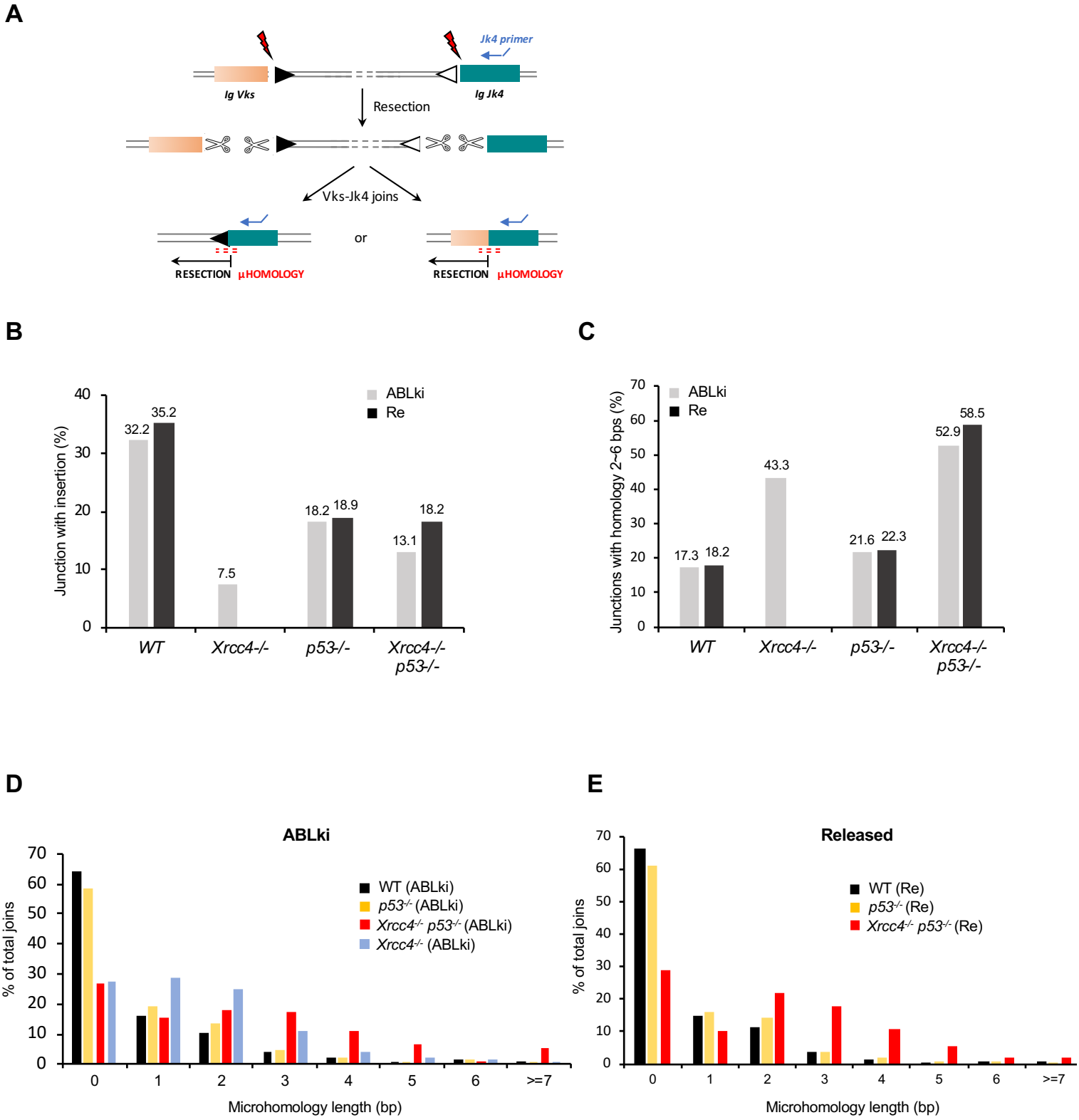

**Supplementary Figure 6.**
**Related to Figure 1.**
**(A)** Circos plots visualisation of *IgkJ*$_4$-*V*$_{region}$ junctions for G1 blocked (ABLki) and released (Re) *v-Abl* pro-B cells. *Igk* locus is divided into 1000 bps bins. Red lines indicate joins between bins and *Jk4* bait, with number of reads indicated below. The number of reads are plotted as black bar on a log scale with indicated custom ticks from 10 to 10000. Coordinates of *IgkV* and *J* segments are plotted as red and black bars at outmost zone. **(B)** Quantification of *IgkJ*$_4$-*V*$_{region}$ joins. Values are the percentages of translocations relative to total mapped reads. Fold enrichment in Released relative to ABLki is indicated above. Graph bars represent the pool of n = 4 independent experiments for WT G1 blocked cells, n=5 for WT released cells, n=3 for *p53* $^{-/-}$ G1 and released cells with two independent cell lines for each genotype.

# Supplementary Figure 7



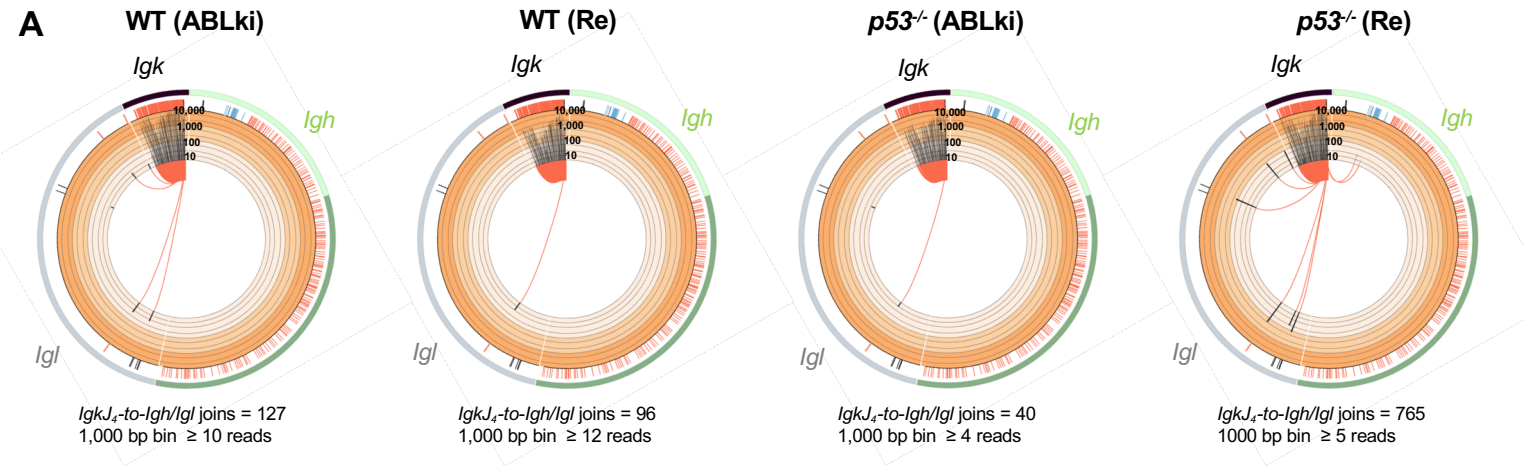

**Supplementary Figure 7.**
**Related to Figure 1.**
**(A)** Schematic representation of *IgkV*$_{region}$-*J*$_{region}$ Capture-Sequencing. **(B)** Visualization of *IgkV-J* joins within *Igk* loci by Circos plots for *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ *v-Abl* pro-B cells. *Igk* locus is divided into 1000 bps bin linked by colored lines for bins with more than cutoff reads (NT: 13; ABLki: 4 ; Re: 10). Red lines: *IgkV*$_{region}$-*J*$_{region}$ joins. The number of joins are plotted as black bar on a log scale with indicated custom ticks from 5 to 1000. Coordinates of *IgkV* and *J* segments are plotted as red and black bars at outmost zone. **(C)** Quantification of *IgkV*$_{region}$-*J*$_{region}$ joins for *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ *v-Abl* pro-B cells. Values represent the percentages of *IgkV*$_{region}$-*J*$_{region}$ relative to total mapped reads. Fold enrichment is indicated above. Graph bars represent n = 1 experiment for *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ NT, ABLki and Re.

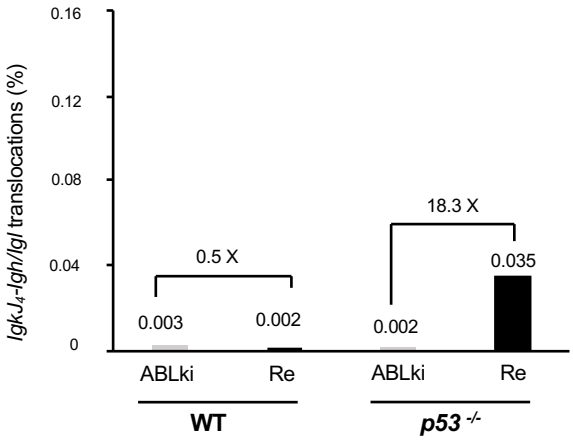
# Supplementary Figure 8




**Supplementary Figure 8.**
**Related to Figure 2.**
**(A)** Illustration of end resection and microhomology at $IgkJ_4$-$Vk_s$ junctions. **(B)** Percentage of $IgkJ_4$-$V_{region}$ joins with insertions. **(C)** Percentage of $IgkJ_4$-$V_{region}$ joins with 2-6 bps microhomology. **(D, E)** Microhomology length distribution of $IgkJ_4$-$V_{region}$ joins for ABLki treated (D) and released (E) *v-Abl* pro-B cells. Graph bars represent the pool of n = 4 independent experiments for WT G1 blocked cells, n=5 for WT released cells, n=3 for *p53*$^{-/-}$ G1 and released cells. n = 4 for *Xrcc4*$^{-/-}$ G1 blocked cells, n=3 for *Xrcc4*$^{-/-}$ *p53*$^{-/-}$ G1 blocked cells and n = 6 for *Xrcc4*$^{-/-}$ *p53*$^{-/-}$ released cells with two independent cell lines for each genotype.

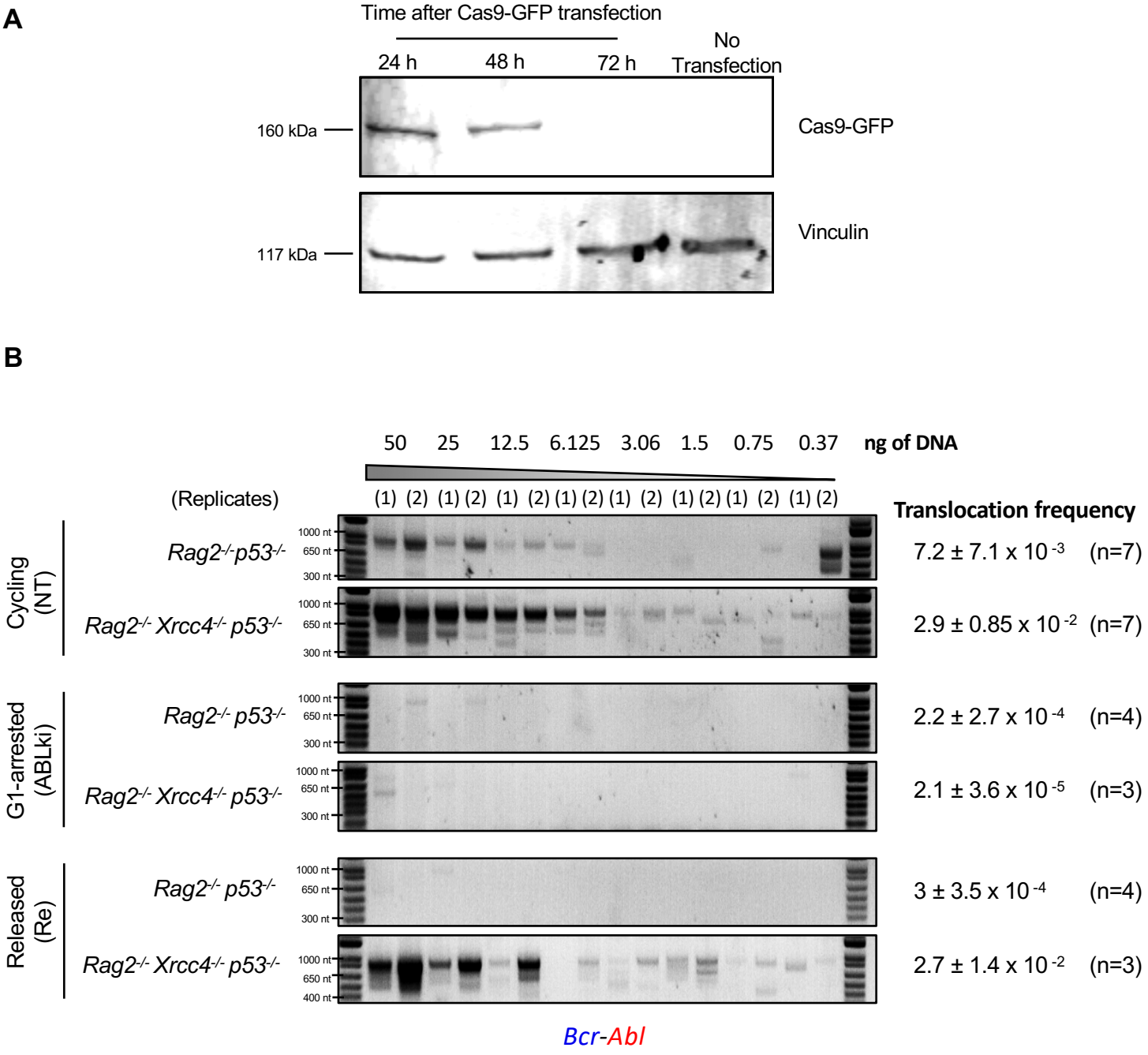
# Supplementary Figure 9




**Supplementary Figure 9.**
**Related to Figure 3.**
**(A)** Circos plots displaying *IgkJ*$_4$*-Igh/Igl* translocations. Translocations are represented as arcs originating from *IgkJ*$_4$ breaks with a minimum of indicated reads per 1,000bp bin for G1 blocked (ABLki) and released (Re) libraries. **(B)** Quantification of *IgkJ*$_4$*-Igh/Igl* translocations. Values are the percentages of translocations relative to total mapped reads. Fold enrichment in Released relative to ABLki is indicated above. Graph bars represent the pool of n = 4 independent experiments for WT G1 blocked cells, n=5 for WT released cells, n=3 for *p53* $^{-/-}$ G1 and released cells with two independent cell lines for each genotype.

# Supplementary Figure 10

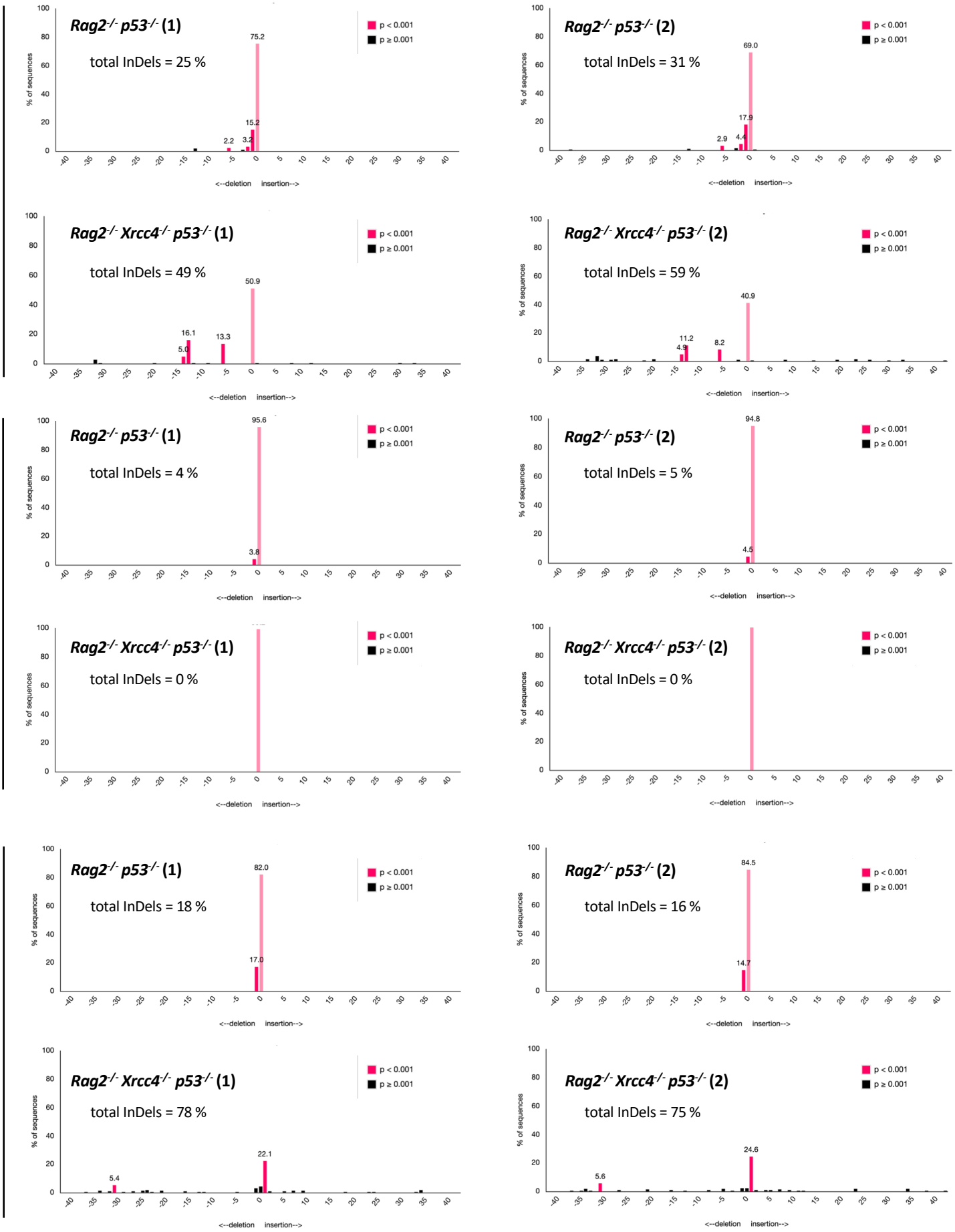



C

| | Number of clones with unique breakpoint sequence | % MH | % Insertion | Resection length (Mean/Median) |
|---|---|---|---|---|
| Cycling Rag2-/- p53-/- | 34 | 64.7 | 29.4 | 73.9/45 |
| Cycling Rag2 -/- Xrcc4 -/- p53 -/- | 68 | 61.7 | 30.8 | 79.9/29.5 |
| Released Rag2-/- Xrcc4-/- p53-/- | 56 | 69.6 | 23.2 | 107.5/46 |

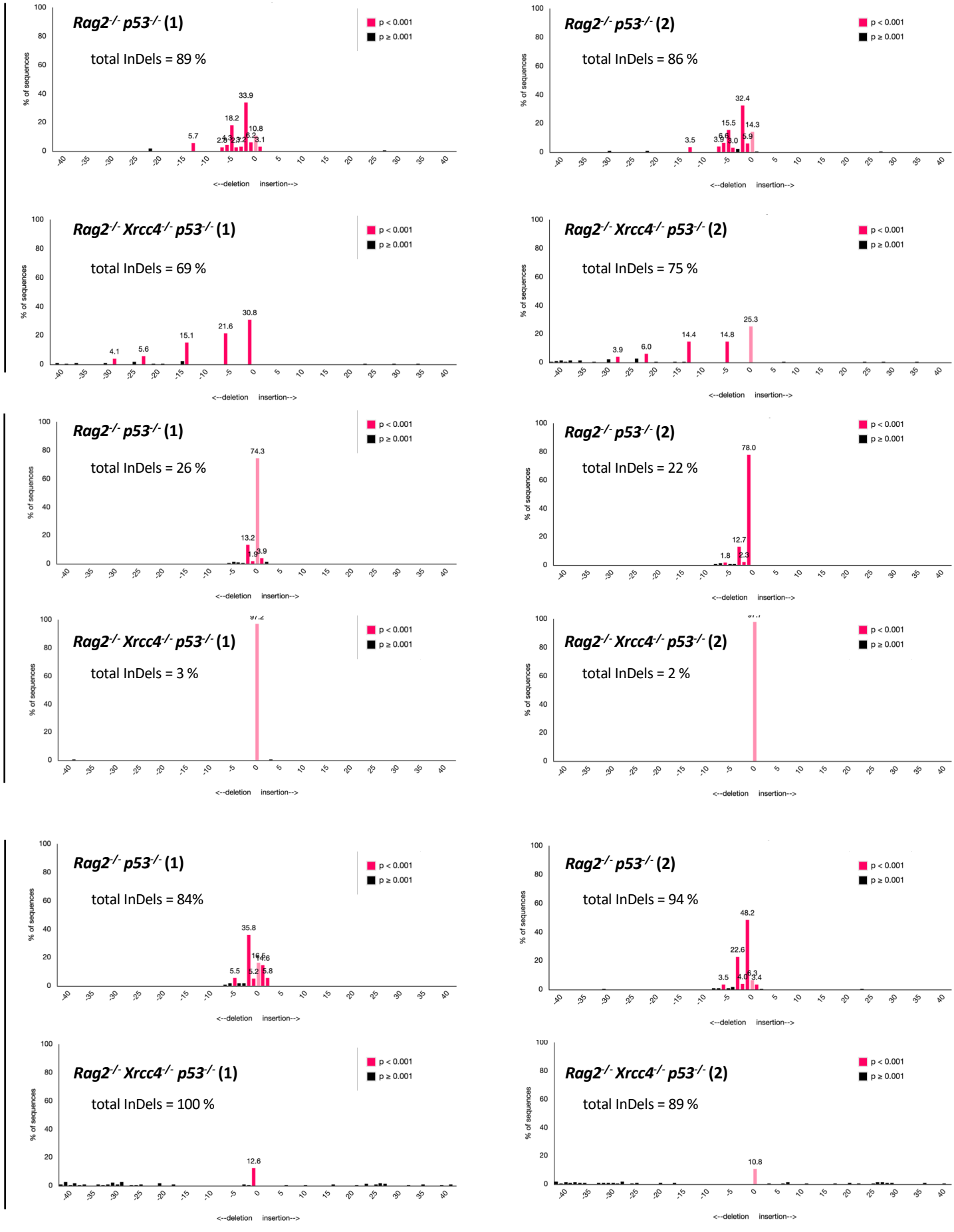
# Supplementary Figure 10

**D**



# Supplementary Figure 10

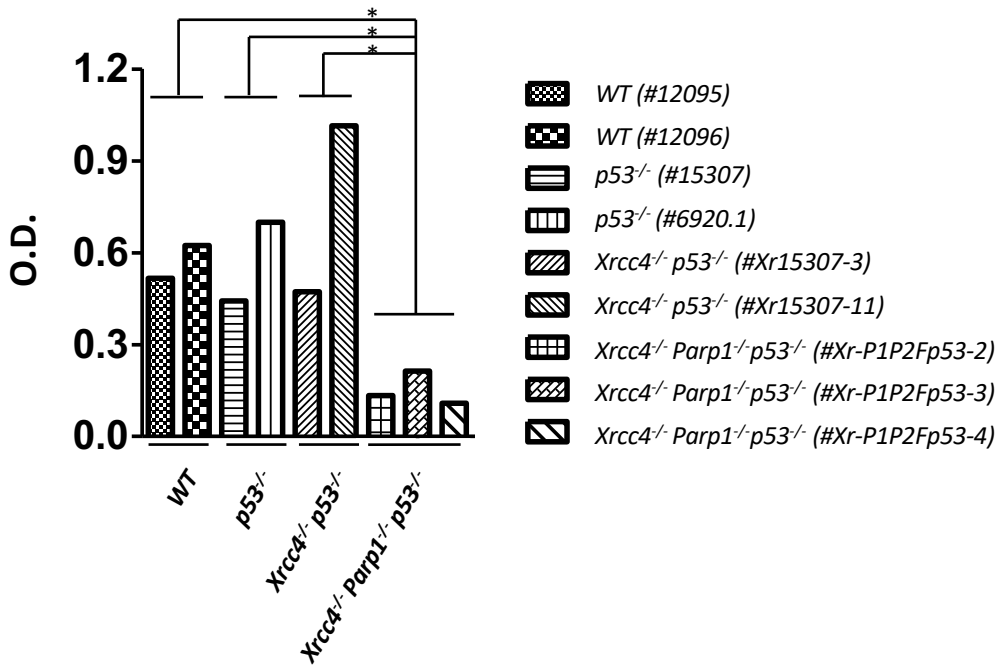
**E**



# Supplementary Figure 10

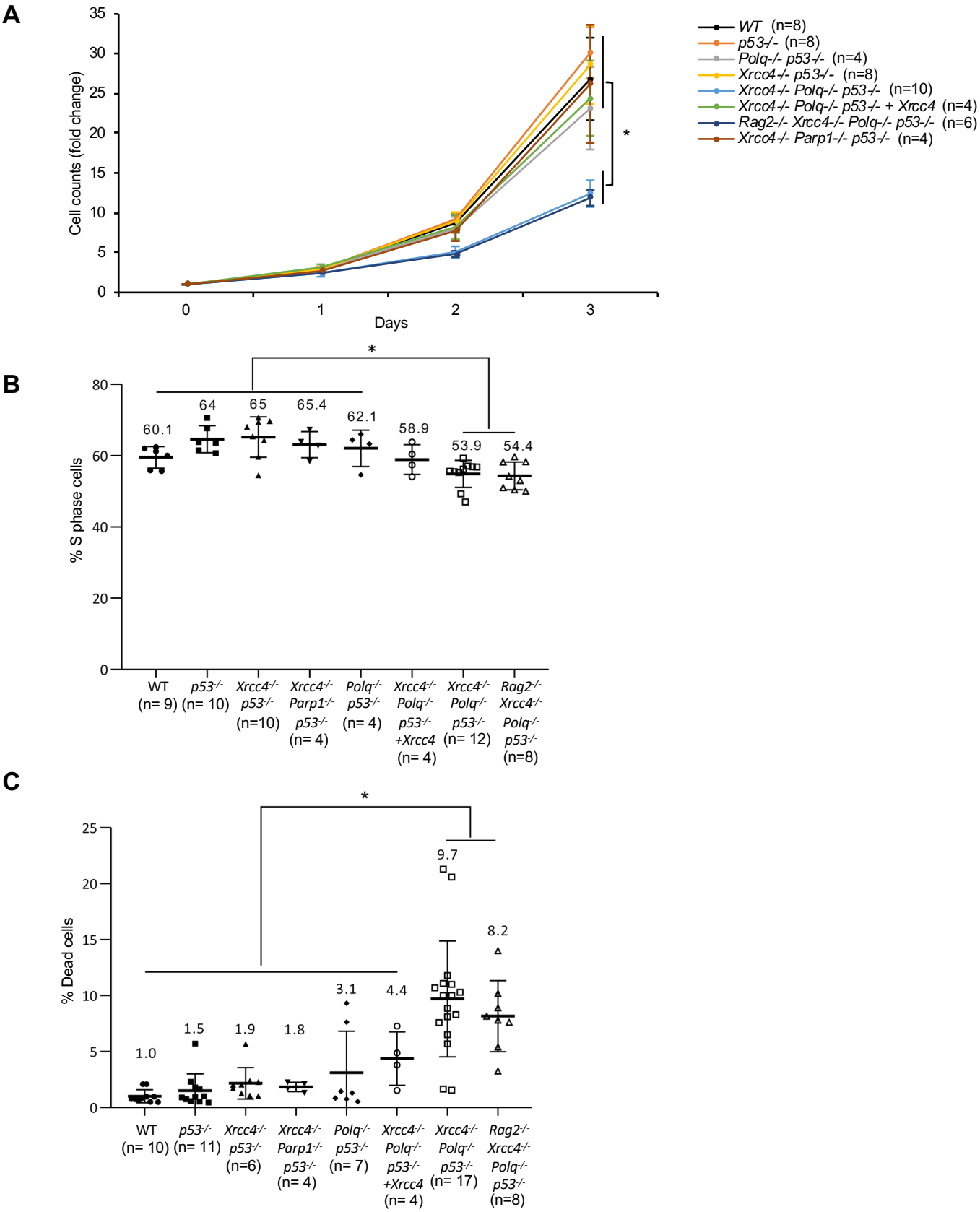
**Supplementary Figure 10.**
**Related to Figure 3.**
**(A)** Western blot showing Cas-GFP expression at 24 hours (h), 48h and 72h after transfection of ABLki-treated pro-B cells. Vinculin was used as loading control. The blot is representative of n=2 independent experiments.
**(B)** Nested PCR for *Bcr-Abl* translocation detection for each genotype on DNA serial dilutions (2 biological replicates for each dilution indicated as (1) and (2)). Cell lines used: *Rag2*$^{-/-}$ *p53*$^{-/-}$ (17585, 17587); *Rag2*$^{-/-}$ *Xrcc4*$^{-/-}$ *p53*$^{-/-}$ (Xr-17585-17, Xr-17585-18). Number of cells corresponding to each dilution is listed at the top of the gel (a diploid cell contains ~6 pg of DNA). Total amount of cells amplified for each genotype: $2x(50+25+12.5+6.125+3.06+1.5+0.75+0.37)/6x10^3=33101$ cells. Calculation of translocation frequency : f>= number of PCR amplicons / number of amplified cells as described in Renouf et al, Methods Enzymol., 2014. Indicated translocation frequencies are the mean ± standard deviation of all transfections for each genotype with the number (n) of experiments indicated.
**(C)** Topo cloning and sequencing analysis of *Bcr-Abl* PCR products with resection length, microhomologies (MH) and insertions at *Bcr-Abl* translocation breakpoint sites (see also Supplementary Table 5). **(D-E)** Graphical representation of indels frequency for each genotype generated with TIDE: Tracking of Indels by Decomposition (https://tide.deskgen.com/). n=2 independent experiments with two independent cell lines for each genotype **D.** *Abl* breakpoint junctions. **E.** *Bcr* breakpoint junctions. P-values were calculated using a Two-sided Pearson's chi-squared test.

# Supplementary Figure 11



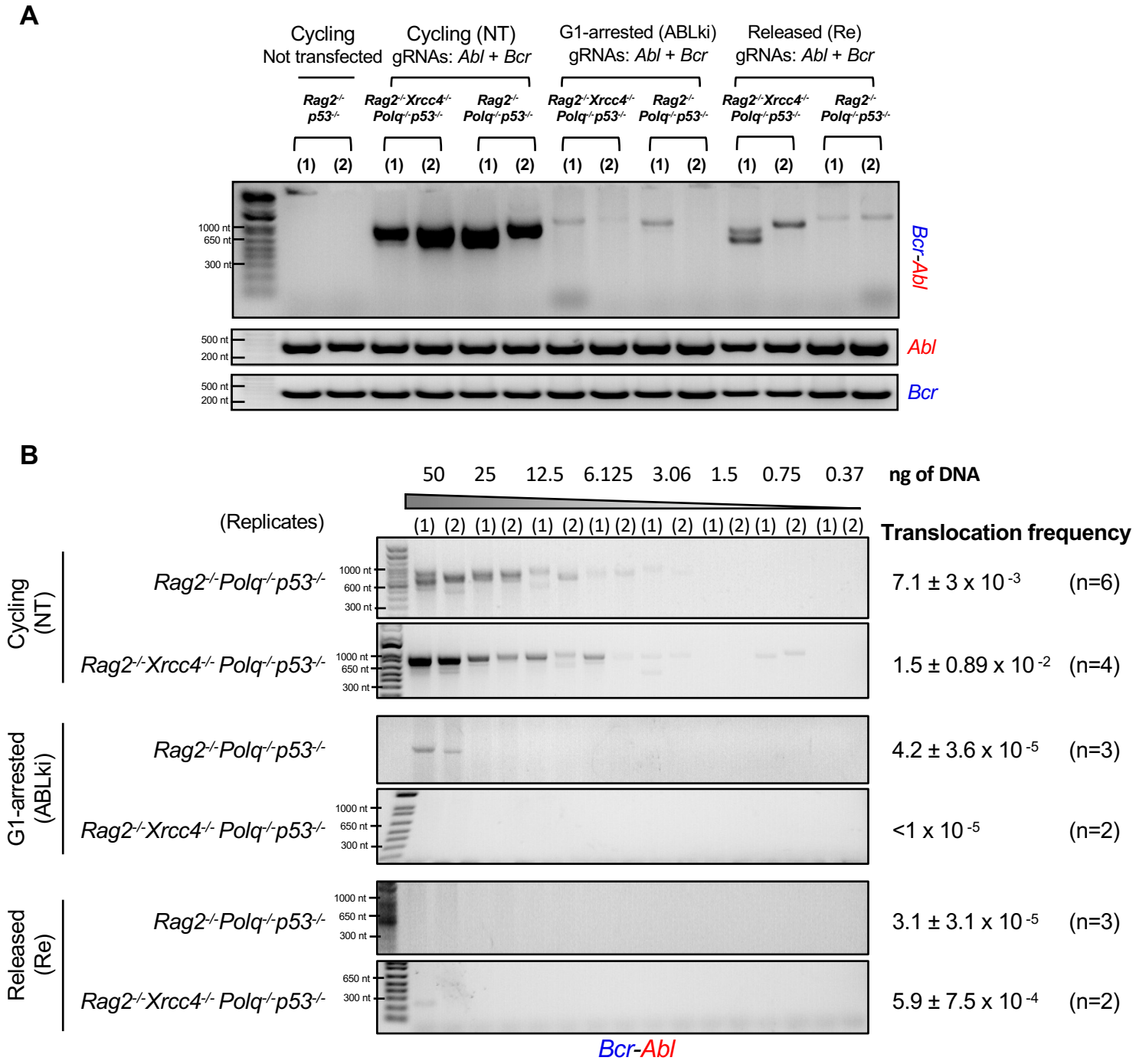

**Supplementary Figure 11.**
**Related to Figure 4.**
PARP activity in *v-Abl* pro-B cell lines. * p < 0.05 (Two-sided Student's t-test). WT vs *Xrcc4*$^{-/-}$ *Parp1*$^{-/-}$ *p53*$^{-/-}$ : *P*=0.0013; *p53*$^{-/-}$ vs *Xrcc4*$^{-/-}$ *Parp1*$^{-/-}$ *p53*$^{-/-}$ : *P*=0.0094; *Xrcc4*$^{-/-}$ *p53*$^{-/-}$ vs *Xrcc4*$^{-/-}$ *Parp1*$^{-/-}$ *p53*$^{-/-}$: *P*=0.037.

# Supplementary Figure 12

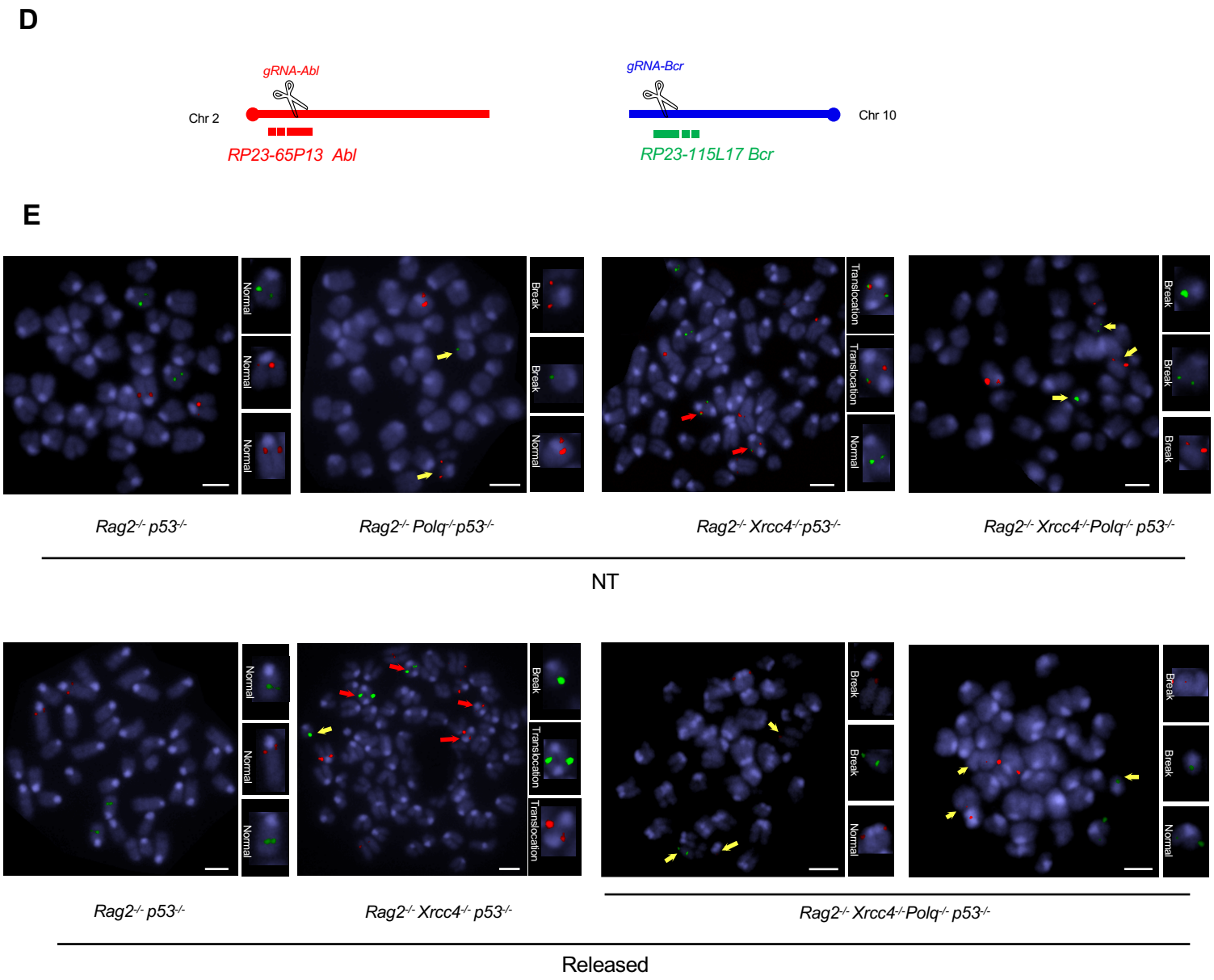


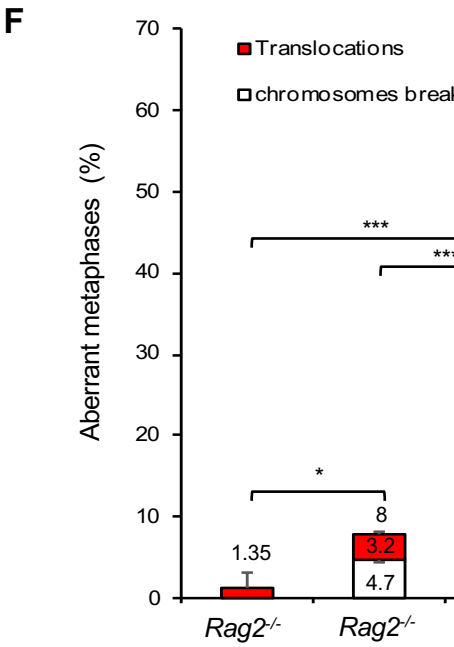

**Supplementary Figure 12.**
**Related to Figure 4.**
**(A)** Untreated cells were plated at 0.5 x $10^6$ cells/ml and then counted every 24hr. * $p < 0.05$ (Two-sided Student's t-test) at day 3. ). The number of independent experiments for each genotype is indicated into brackets. **(B)** Percentage of S phase cells (measured by Edu-PI cell cycle staining) in untreated cells. * $p < 0.05$ (Two-sided Student's t-test). ).The number of independent experiments for each genotype is indicated into brackets. **(C)** Percentages of dead cells in untreated cells. All data represent the mean ± standard deviation. * $p < 0.05$ (Two-sided Student's t-test). The number of independent experiments for each genotype is indicated into brackets. Two or three isogenetic clones were analysed for each genotype. Source data are provided as a Source Data file.

# Supplementary Figure 13



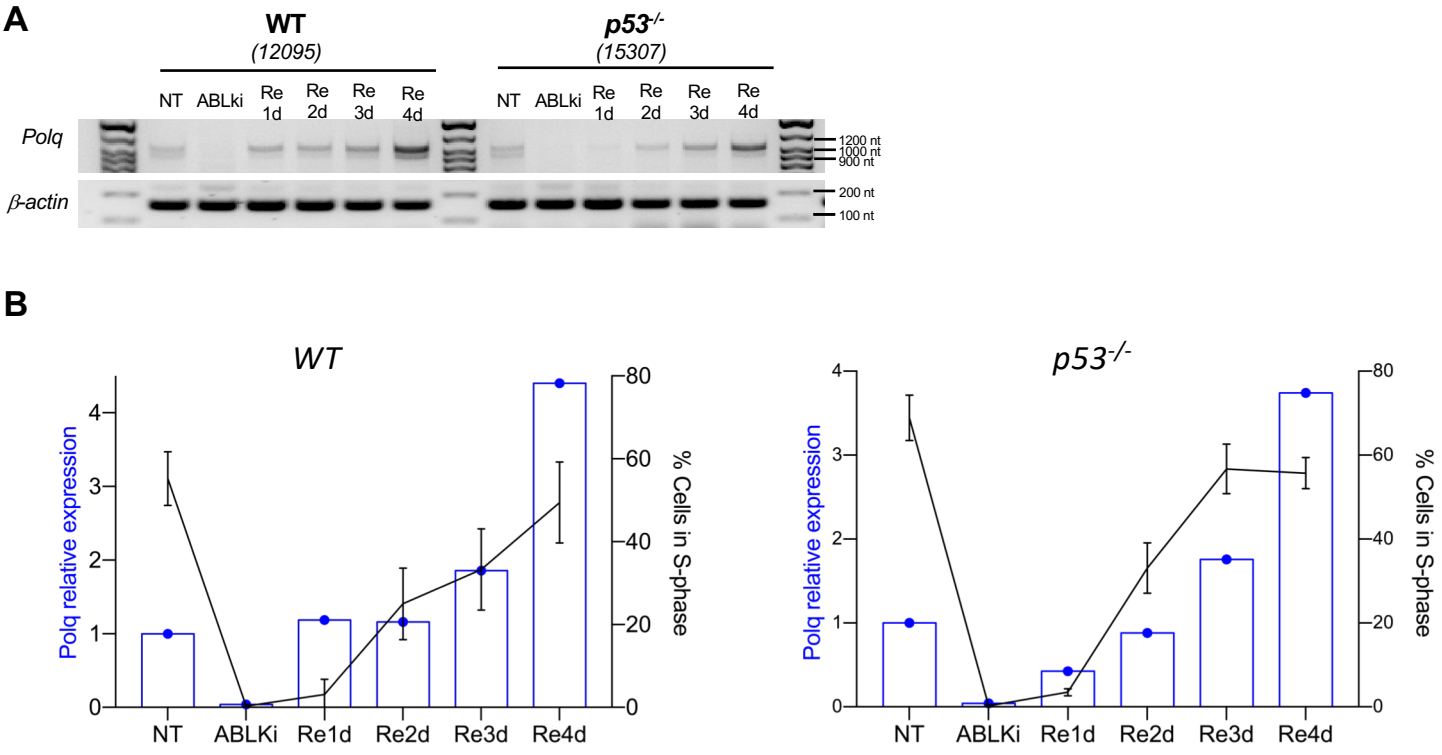
# Supplementary Figure 13



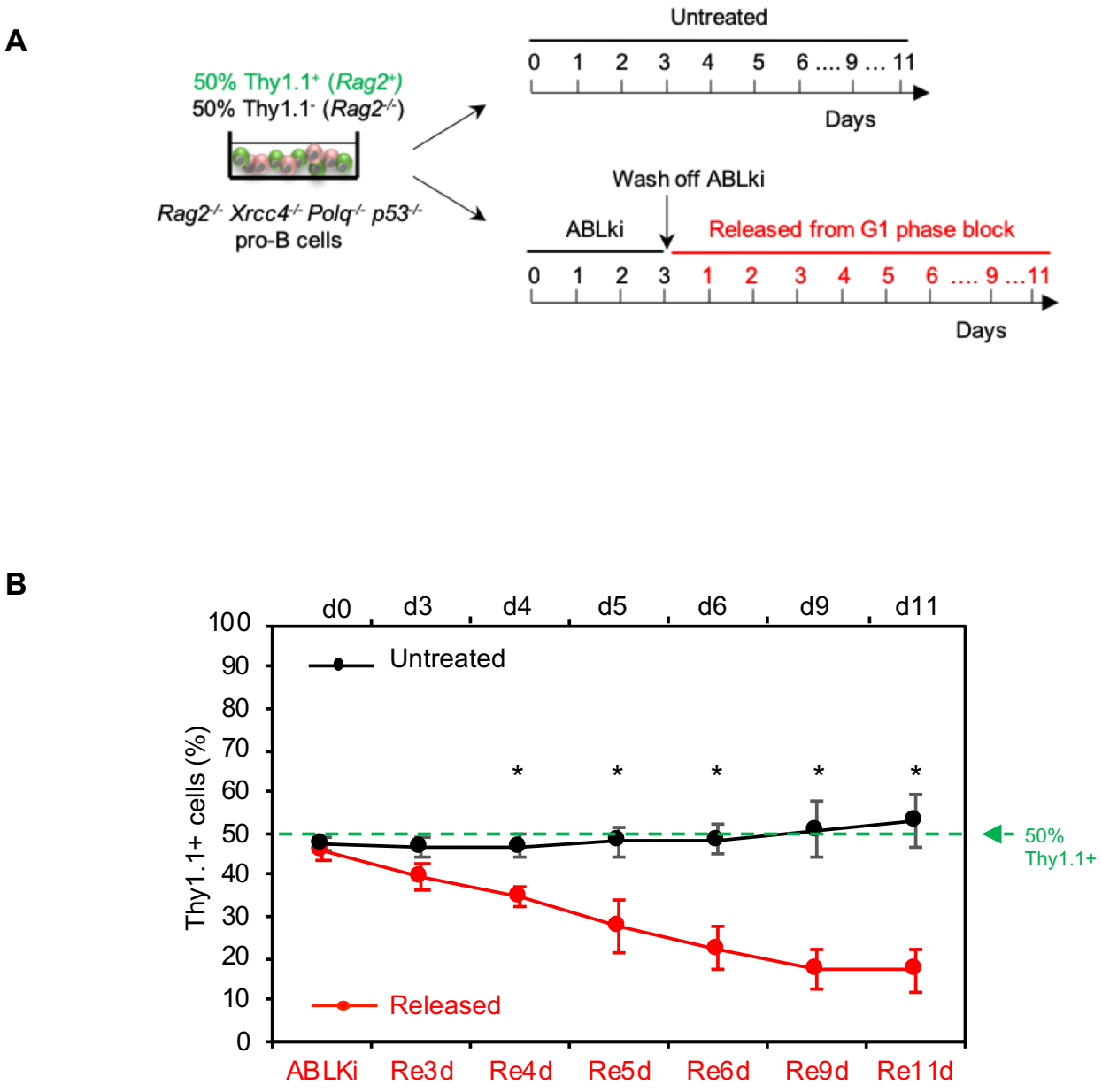
**Supplementary Figure 13.**
**(A)** Representative PCR amplifications of *Bcr-Abl* translocation breakpoints; in untreated cycling (NT) (transfected or not with Cas9 and gRNAs), G1-arrested (ABLki) and released/cycling (Re) *v-Abl* pro-B cells of the indicated genotype. PCR of *Abl* and *Bcr* loci were used as controls. Cell lines used: *Rag2$^{-/-}$ p53$^{-/-}$ (17585,17585); Rag2$^{-/-}$ Xrcc4$^{-/-}$ Polq$^{-/-}$ p53$^{-/-}$ (X-P-87-4-4-X-3, X-P-87-4-4-X-14); Rag2$^{-/-}$ Polq$^{-/-}$ p53$^{-/-}$ (Polq-17587-8, Polq-17587-13).* The number of independent experiments is indicated in Supplementary Figure 13C.
**(B)** Direct PCR for *Bcr-Abl* translocation detection for each genotype on DNA serial dilutions (Two biological replicates for each dilution indicated as (1) and (2)). Number of cells corresponding to each dilution is listed at the top of the gel (a diploid cell contains ~6 pg of DNA). Total amount of cells amplified for each genotype: 2x(50+25+12.5+6.125+3.06+1.5+0.75+0.37)/6x$10^3$=33101 cells. Indicated translocation frequencies are the mean ± standard deviation of all transfections for each genotype and the numbers of transfection are indicated in the right of panel.
**(C)** Dot plot of translocation frequencies after GFP-Cas9 transfections in untreated cycling (NT), G1-arrested (ABLki) and released/cycling (Re) *v-Abl* pro-B cells . Each dot indicates one transfection. Bars represent means ± s.d. from at least two independent experiments performed with a minimum of two independent B cell clones. The number of independent experiments is indicated into brackets.
**(D)** Schematic representation of the *Bcr* and *Abl* loci, with positions of the BACs used for generation of DNA FISH probes indicated.
**(E)** Representative metaphases from cycling (NT) and released *Rag2$^{-/-}$ p53$^{-/-}$* , *Rag2$^{-/-}$ Polq$^{-/-}$ p53$^{-/-}$, Rag2$^{-/-}$ Xrcc4$^{-/-}$ p53$^{-/-}$* and *Rag2$^{-/-}$ Xrcc4$^{-/-}$ Polq $^{-/-}$ p53$^{-/-}$ v-Abl* pro-B cells with *Abl* BAC probe (red), *Bcr* BAC probe (green). Yellow arrowheads point to broken and orange arrowheads for translocation. Scale bars = 3μm. The list of independent experiments is provided in Supplementary Table 6.
**(F)** Percentage of aberrant metaphases from cycling (NT) and release *v-Abl* pro-B cells of the indicated genotypes. Cycling cells were harvested 4 day after transfection. Released cells were harvested 5 days after washing off ABLki. Histograms represent means ± s.d. from at least two independent experiments performed with a minimum of two independent B cell clones. The mean percentage of total aberrant metaphases is indicated above. The mean percentages of metaphases with translocations (red) and chromosomes breaks (white) are indicated (See also Supplementary Table 6). The black lines are the statistic of aberrant cells vs normal cells. The dash lines are the statistic of cells with break vs other cells (normal metaphases + metaphases with translocations) and cells with translocation vs other cells (normal metaphases + metaphases with breaks). **P*<0.05; ****P*<0.001 (Two-sided Fisher exact test) (See also Supplementary Table 6). (**!**) A total of 12 metaphases were obtained for this condition (0 metaphase with *Bcr-Abl* chromosomal translocations and 8 metaphases with *Bcr* and/or *Abl* chromosome breaks) and thus statistical analysis was not performed (The list of independent experiments for each conditions is provided in Supplementary Table 6).

# Supplementary Figure 14



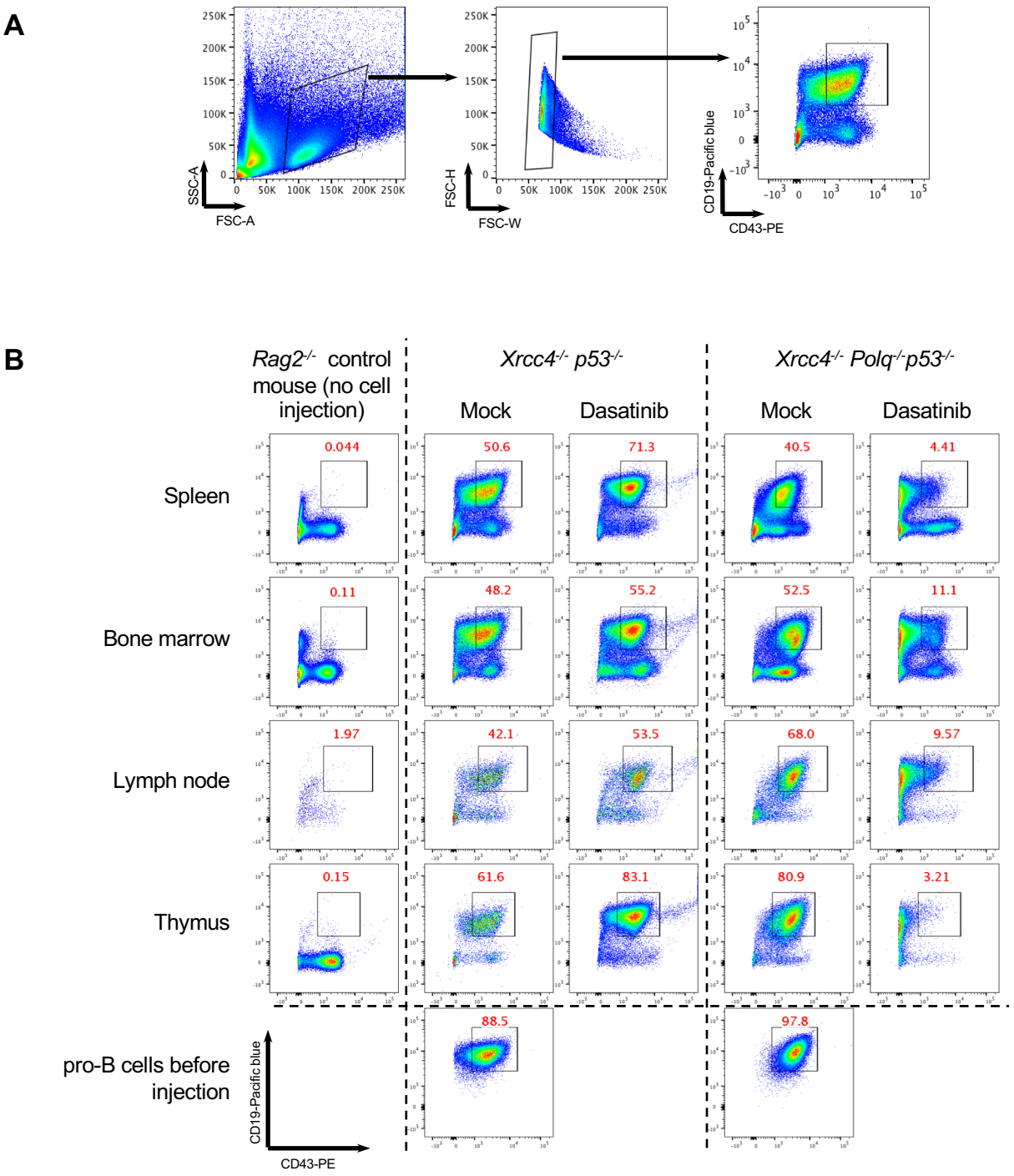

**Supplementary Figure 14.**
**Related to Figure 5.**
**(A)** *Polq* mRNA expression in untreated (NT), G1-blocked (ABLki) and released (Re1d to Re4d) WT *and p53*$^{-/-}$ *v-Abl* pro-B cells. *β-actin* mRNA was used as a control. Cell lines used: WT(*12095*); p53 $^{-/-}$ (*15307*). **(B)** Quantification of *Polq* mRNA expression (blue bar graphs, left y axis) and the percentage of S-phase cells (black curve graphs, right y axis, lines represent means ± S.D. from a minimum of two independent B cell clones) in WT and *p53*$^{-/-}$ *v-Abl* pro-B cells. NT : untreated; ABLki: treated for 72 hours with ABLki; Re1d to Re4d: released. The bar plots of Polq expression represent the quantification of n=1 independent experiment from panel A.

# Supplementary Figure 15




**Supplementary Figure 15.**
**Related to Figure 5.**
**(A)** RAG2 complemented Thy1.1+ and un-complemented Thy1.1- *Xrcc4 -/- Polq -/- p53 -/- v-Abl* pro-B cells were mixed at a ratio of 1:1 and the percentage of Thy1.1+ cells during normal cell culture conditions (Untreated) and after release from ABLki treatment (Released) was analyzed by FACS at different time points. **(B)** Quantification of live Thy1.1+ (Rag2+) and Thy1.1- (*Rag2-/-*) *Xrcc4 -/- Polq -/- p53 -/- v-Abl* pro-B cells at different time points during normal cell culture conditions (Untreated) and after release from ABLki treatment (Released). Dots represent mean ± SEM from two experiments performed with two independent cell lines. * p<0.05 (Two-sided Student's t-test). Cell lines used: *Rag2-/- Xrcc4-/- Polq-/- p53-/- (X-P-87-4-4-X-3, X-P-87-4-4-X-14).* Re4d vs d4: *P*=0.0043; Re5d vs d5: *P*=0.0096; Re6d vs d6: *P*=0.0018; Re9d vs d9: *P*=0.00067; Re11d vs d11: *P*=0.00093.

# Supplementary Figure 16




**Supplementary Figure 16.**
**Related to Figure 5.**
(A) Gating strategy to determine the percentage of CD19⁺ CD43⁺ leukemic cells.
(B) Representative plots of lymphoid organs obtained from sick mice injected with *v-Abl* pro-B leukemic cells. Numbers in red indicate the percentage of CD19⁺ CD43⁺ leukemic cells among live single cells.

# Supplementary Table 1

List of cell lines used in the study

| Genotype | Clone ID | Allele 1 | Allele 2 | Generation Method | Origin | Reference |
|---|---|---|---|---|---|---|
| WT | 12095 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Lescale C. et al., Nat Com. 2016 |
| | 12096 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Lescale C. et al., Nat Com. 2016 |
| p53 -/- | 6920.1 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Lescale C. et al., Nat Com. 2016 |
| | 6943.2 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Lescale C. et al., Nat Com. 2016 |
| | 15307 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Lescale C. et al., Nat Com. 2016; Jacks, T. et al, Curr. Biol. 1994 |
| Xrcc4-/- | Xr95-22 | -316bp | | CRISPR-CAS9 knockout | 12095 | Lescale C., Lenden Hasse H., et al. Cell Reports. 2016 |
| | Xr95-23 | -288bp | | CRISPR-CAS9 knockout | 12095 | Lescale C., Lenden Hasse H., et al. Cell Reports. 2016 |
| | Xr95-50 | -484bp | -514bp | CRISPR-CAS9 knockout | 12095 | Lescale C., Lenden Hasse H., et al. Cell Reports. 2016 |
| Xrcc4-/- p53-/- | Xr15307-3 | -422bp | | CRISPR-CAS9 knockout | 15307 | This study |
| | Xr15307-11 | -255bp | | CRISPR-CAS9 knockout | 15307 | This study |
| Polq-/- p53-/- | Polq-2-307 | -189bp | -183bp | CRISPR-CAS9 knockout | 15307 | This study |
| | Polq-5-307 | -323bp | | CRISPR-CAS9 knockout | 15307 | This study |
| Xrcc4-/- Polq-/- p53-/- | Polq-2-307-Xr25 | -4bp | | CRISPR-CAS9 knockout | Polq-2-307 | This study |
| | Polq-2-307-Xr211 | -324bp | | CRISPR-CAS9 knockout | Polq-2-307 | This study |
| | Xr307-3-Polq35 | -240bp | -265bp | CRISPR-CAS9 knockout | Xr15307-3 | This study |
| Rag2-/- p53-/- | 17585 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | This study |
| | 17587 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | This study |
| Rag2-/- Xrcc4-/- p53-/- | Xr-17585-17 | -288bp | | CRISPR-CAS9 knockout | 17585 | This study |
| | Xr-17585-18 | -288bp | | CRISPR-CAS9 knockout | 17585 | This study |
| Rag2-/- Polq-/- p53-/- | Polq-17587-8 | -183bp | | CRISPR-CAS9 knockout | 17587 | This study |
| | Polq-17587-13 | -202bp | | CRISPR-CAS9 knockout | 17587 | This study |
| | Polq-17587-22 | -183bp | | CRISPR-CAS9 knockout | 17587 | This study |
| Rag2-/- Xrcc4-/- Polq-/- p53-/- | X-P-87-4-4-X-3 | -288bp | | CRISPR-CAS9 knockout | Polq-17587-4 | This study |
| | X-P-87-4-4-X-14 | -288bp | | CRISPR-CAS9 knockout | Polq-17587-4 | This study |
| Parp1-/- p53-/- | 6284 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Galindo-Campos M.A., et al. Cell Death & Differentiation. 2019 |
| | 6286 | NA | NA | v-Abl/Bcl2 immortalization of bone marrow pro-B cells | Mouse | Galindo-Campos M.A., et al. Cell Death & Differentiation. 2019 |
| Parp1-/- Xrcc4-/- p53-/- | Xr-P1P2Fp53-2 | -764bp | | CRISPR-CAS9 knockout | 6284 | This study |
| | Xr-P1P2Fp53-3 | -214bp | | CRISPR-CAS9 knockout | 6284 | This study |
| | Xr-P1P2Fp53-4 | -299bp | | CRISPR-CAS9 knockout | 6284 | This study |

# Supplementary Table 2

List of primers and gRNAs used in the study

| **A. CRISPR oligonucleotide and PCR primer sequences. *Linker sequences for cloning into BsmBI-digested MLM3636 are underlined; **gRNA score and predicted off-targets are determined using the online CRISPR Design tool http://crispr.mit.edu/ website from the Zhang lab.** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Oligonucleotide name** | **Oligo sequence (5'>3')*** | **sgRNA target coordinate (mm10)** | **Score**** | **Deletion** | **Primer name** | **Primer sequence (5'-> 3')** | **Expected PCR size wild type allele** | **Expected PCR size CRISPR/Cas9 edited allele** | **Deletion size** |
| sgXrcc4_1_A | ACA CCG GAA TGT ATA ACA GGA GAC GGG | chr13: 90,062,346–90,062,365 | 79 | Part of the XRCC4 functional core region | pcr Xrcc4 1F | GGC TGA CAG TCT GAG GCT AT | 1078 bp | 790 bp | 288 bps |
| sgXrcc4_1_B | AAA ACC CGT CTC CTG TTA TAC ATT CCG | | | | pcr Xrcc4 1R | GCC TCC ATG TCA GTA CTG GT | | | |
| sgXrcc4_2_A | ACA CCG GCC GAG ACT CCT TAG AAA AGG | chr13: 90,062,044–90,062,063 | 65 | | pcr Xrcc4 2F | CCC TCA CAG AAA CAC AAC TCA | 1171 bp | 883 bp | |
| sgXrcc4_2_B | AAA ACC TTT TCT AAG GAG TCT CGG CCG | | | | pcr Xrcc4 2R | CAA GGA GGT GGC CAC TAG TT | | | |
| sgPolq_1_A | ACA CCG TTG GGG TTC TCC CGC GCG GG G | chr16: 37,011,828-37,011,847 | 96 | Polq first exon | pcr Polq 1F | CCG TCC CTC ACC ACT CTA TG | 580 bp | 398 bp | 182 bps |
| sgPolq_1_B | AAA ACC CCG CGC GGG AGA ACC CCA ACG | | | | pcr Polq 1R | AAC GCC CAT ATC TAC TGG GG | | | |
| sgPolq_2_A | ACA CCG CTG AGT CCA CCT CCG GGG CTG | chr16: 37,012,011-37,012,030 | 82 | | pcr Polq 2F | GAA GCC AGA TAT CAG CGC G | 572 bp | 390 bp | |
| sgPolq_2_B | AAA ACA GCC CCG GAG GTG GAC TCA GCG | | | | pcr Polq 2R | AAC ACC ATC AGC TGT GCA TG | | | |
| | | | | | | | | | |
| **B. TOPO TA cloning** | | | | | | | | | |
| T7 primer | TAA TAC GAC TCA CTA TAG GG | | | | | | | | |
| SP6 primer | ATT TAG GTG ACA CTA TAG | | | | | | | | |
| | | | | | | | | | |
| **C. Nested-PCR for *IgkJ4* and *IgkV10-95* coding join** | | | | | | | | | |
| JK4-pa | CGC TCA GCT TTC ACA CTG ACT | | | | | | | | |
| JK4-pb | CAG GTT GCC AGG AAT GGC TC | | | | | | | | |
| V10-95-pa | TTC CTT GGT CTC CTG TTG CT | | | | | | | | |
| V10-95-pb | TCT TGT TGA CTG GCG TTT TG | | | | | | | | |
| IMR42 | CTAGGCCACAG AATTGAAAGATCT | | | | | | | | |
| IMR43 | GTAGGTGGAAATTCTAGCATGA TGC | | | | | | | | |
| | | | | | | | | | |
| **D. LAM-HTGTS oligos** | | | | | | | | | |
| Biotin-*IgkJ4* | Biotin-CGC TCA GCT TTC ACA CTG ACT | | | | | | | | |
| Biotin-cmyc | Biotin-TGA TGT TGG GCT AGC GCA | | | | | | | | |
| Adapter-upper-6N | GCG ACT ATA GGG CAC GCG TGG NNNNNN -NH2 | | | | | | | | |
| Adapter-lower-NH2 | /5Phos/CCA CGC GTG CCC TAT AGT CGC-NH2 | | | | | | | | |
| P5-I5 | AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T | | | | | | | | |
| P7-I7 | CAA GCA GAA GAC GGC ATA CGA GAT CGG TCT CGG CAT TCC TGC TGA ACC GCT CTT C | | | | | | | | |
| I5-Red (IgkJ4) | TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T(6nts barcode)CAG GTT GCC AGG AAT GGC TC | | | | | | | | |
| I5-Red (c-myc) | TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T(6nts barcode)AGG GAT GTG ACC GAT TCG TTG | | | | | | | | |
| I5-Red (CMV3) | TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T(6nts barcode)GT ACG GTG GGA GGT CTA TA | | | | | | | | |
| I7-Blue | CTC GGC ATT CCT GCT GAA CCG CTC TTC CGA TCT GAC TAT AGG GCA CGC GTG G | | | | | | | | |
| | | | | | | | | | |
| **E. RT-PCR primers** | | | | | | | | | |
| Polq forward | TGGCTATATGGGCAGCACCT | | | | | | | | |
| Polq reverse | CAGAGCAATGCCCTTGGATTT | | | | | | | | |
| β-Actin forward | GGCTGTATTCCCCTCCATCG | | | | | | | | |
| β-Actin reverse | CCAGTTGGTAACAATGCCATGT | | | | | | | | |
| | | | | | | | | | |
| **F. sgRNA oligonucleotides and primers for Cas9-induced translocation assays** | | | | | | | | | |
| gRNA-Abl | GTTAACTTGAGGACCGGGAT | | | | | | | | |
| gRNA-Bcr | GTATCACCCTCCTTTAGCGT | | | | | | | | |
| Abl breakpoint PCR F | ATTCTGCAACTTTGGCCTGGTG | | | | | | | | |
| Abl breakpoint PCR R | ACACCTGGAGTAAGCAGGACAC | | | | | | | | |
| Bcr breakpoint PCR F | TGGGAGTCTTAAAGGCAGGAGC | | | | | | | | |
| Bcr breakpoint PCR R | GTCAGGAGAGGCAAGTCCAAGT | | | | | | | | |
| Bcr-Abl breakpoint direct PCR F | GCATTTCTAACCCGAAGTTCC | | | | | | | | |
| Bcr-Abl breakpoint direct PCR R | GCTCATTTGTTACCATGACCAG | | | | | | | | |

# Supplementary Table 3

## A ABLki WT pro-B cells (*12095+12096)*

| | *IgkV$_{10\text{-}95}$* coding end | IgkJ$_4$ coding end | # frequency |
|---|---|---|---|
| Ref: | TCAGCAGTATAGTAAGCTTCCTCC | ATTCACGTTCGGCTCGGGGACAAAG | |
| 1. | TCAGCAGTATAGTAAGC<u>TTC</u>**** | ****ACGTTCGGCTCGGGGACAAAG | 5/31 |
| 2. | TCAGCAGTATAGTAAGCT****** | ATTCACGTTCGGCTCGGGGACAAAG | 3/31 |
| 3. | TCAGCAGTATAGTAAGCTTCC*** | ATTCACGTTCGGCTCGGGGACAAAG | 3/31 |
| 4. | TCAGCAGTATAGTAAGCTTC<u>C</u>*** | ****ACGTTCGGCTCGGGGACAAAG | 2/31 |
| 5. | TCAGCAGTATAGTAAGCTTCCTC*TT* | TTCACGTTCGGCTCGGGGACAAAG | 2/31 |
| 6. | TCAGCAGTATAGTAAGCTTCCTCC | *TTCACGTTCGGCTCGGGGACAAAG | 2/31 |
| 7. | TCAGCAGTATAGTAAGCTTCCTC* | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 8. | TCAGCAGTATAGTAAGCTTCC<u>TC</u>* | ****ACGTTCGGCTCGGGGACAAAG | 1/31 |
| 9. | TCAGCAGTATAGTAAGCTT*AT* | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 10. | TCAGCAGTATAGTAAGCTTCC*** | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 11. | TCAGCAGTATAGTAAGCTTCC*AG* | TCACGTTCGGCTCGGGGACAAAG. | 1/31 |
| 12. | TATAGTAAGCTTCCTCC*GAAG* | TCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 13. | TCAGCAGTATAGTAAGCTTCCTCC*AG* | TTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 14. | TCAGCAGTATAGTAAGCTTC**** | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 15. | TCAGCAGTATAGTAAGCTTC<u>CCC</u> | *TTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 16. | TCAGCAGTATAGTAAGC*CCG* | ATTCACGTTCGGCTCGGGGACAAAG. | 1/31 |
| 17. | TCAGCAGTATAGTAAGCTTCCT** | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 18. | TCAGCAGTATAGTAAGCTTCCTCC | *TTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 19. | TCAGCAGTATAGTAAGCTTCCT** | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |
| 20. | TCAGCAGTATAGTAAGC******* | ATTCACGTTCGGCTCGGGGACAAAG | 1/31 |

## B Released WT pro-B cells (*12095+12096)*

| | *IgkV$_{10\text{-}95}$* coding end | *IgkJ$_4$* coding end | # frequency |
|---|---|---|---|
| Ref: | TCAGCAGTATAGTAAGCTTCCTCC | ATTCACGTTCGGCTCGGGGACAAA | |
| 1. | TCAGCAGTATAGTAAGCTTCC*** | ATTCACGTTCGGCTCGGGGACAAAG | 5/24 |
| 2. | TCAGCAGTATAGTAAGCTTCC<u>TC</u>* | ****ACGTTCGGCTCGGGGACAAAG | 4/24 |
| 3. | TCAGCAGTGTAGTAAGCTT***** | ATTCACGTTCGGCTCGGGGACAAAG | 2/24 |
| 4. | TCAGCAGTATAGTAAGCTTCCTC<u>C</u> | ****ACGTTCGGCTCGGGGACAAAG | 2/24 |
| 5. | TCAGCAGTATAGTAAGCTTCC<u>T</u>** | ***CACGTTCGGAGGGGGGACCAAG | 2/24 |
| 6. | TCAGCAGTATAGTAAGCTTCC*** | *****CGTTCGGCTCGGGGACAAAG | 1/24 |
| 7. | TCAGCAGTATAGTAAGCTTCCT*T* | ATTCACGTTCGGCTCGGGGACAAAG | 1/24 |
| 8. | TCAGCAGTATAGTAAGCTTCCTC* | **TCACGTTCGGCTCGGGGACAAAG | 1/24 |
| 9. | TCAGCAGTATAGTAAGCTT*AC* | ATTCACGTTCGGCTCGGGGACAAAG | 1/24 |
| 10. | TCAGCAGTATAGTAAGCTTC**** | **TCACGTTCGGCTCGGGGACAAAG | 1/24 |
| 11. | TCAGCAGTATAGTAAGCT****** | ATTCACGTTCGGCTCGGGGACAAAG | 1/24 |
| 12. | TCAGCAGTATAGTAAGCTTC**** | ***CATTCACGTTCGGCTCGGGGACA | 1/24 |
| 13. | TCAGCAGTATAGTAAGCTTCCTCC | **TCACGTTCGGCTCGGGGACAAAG | 1/24 |
| 14. | TCAGCAGTATAGTAAGCTTCC*AT* | ATTCACGTTCGGCTCGGGGACAAAG | 1/24 |

**<u>TTC</u>: Micro-homology**
***AT*: Insertion**
**** : Resection**

**C** **ABLki *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ pro-B cells** *(Xr15307-3)*

| ***IgkV*$_{10\text{-}95}$ coding end** | ***IgkJ*$_4$ coding end** | Frequency |
|---|---|---|
| CCTGAAGATATTGCCACTTACTATTGTCAGCAGTATAGTAAGCTTCCTCC | ATTCACGTTCGGCTCGGGGACAAAGTTGGAAATAAAACGTAAGTAGACTTTTGCTCATTTACTTGTG | Frequency |
| CCTGAAGATATTGCCACTTACT | ********** TTTGCTCATTTACTTGTG | 9/9 |

**D** **Released *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ pro-B cells** *(Xr15307-3)*

| ***IgkV*$_{10\text{-}95}$ coding end** | ***IgkJs* coding end** | Frequency |
|---|---|---|
| 1. AGGAGTCCCATCAAGGTTCAGTGGC | ** (95 bp)s ** ..... ** (112 bps *J4)* ** TAAATGAGCCATTCCTGGCAA | 4/36 |
| 2. AAGGTTCAGTGGCAGTGGGTCTGGG | ** (83 bps) **..... ** (177 bps *J1)* ** AATAGGCTAGACATGTTCTCTGG | 3/36 |
| 3. GGTTCAGTGGCAGTGGGTCTGGGG | ** (82 bps) ** ..... ** (86 bps *J4)* **ACGTTTTGGTTCTGTTTGGGTA | 2/36 |
| 4. GGTTCAGTGGCAGTGGG | ** (89 bps) **...*GTTTAACAGTTCCATCTGGTTTCG*... ** (117 bps *J1)* ** CTCTGAAACCAGATTCT | 2/36 |
| 5. CACCATCAGCAACCTGGAACCTGAAGAT | ** (41 bps) ** ..... ** (518 bps *J2)* ** TTGTGGGAGAAATGAGAAA | 2/36 |
| 6. CATCAAGGTTCAGTGGCAGTGGGTCT | ** (86 bps) ** ..... ** (34 bps *J4)* ** ACTTACGTTTTATTTCCAAC | 1/36 |
| 7. GATATGATATCCAGATGACACAGACT | ** (266 bps) ** ..... ** (168 bps *J2)* ** GTCCCACAAGAGGTTGGAATG | 1/36 |
| 8. AGTTTACACTCAGGAGTCCCATCAAT | ** (106 bps) ** ..... ** (235 bps *J2)* ** AACTAGGGGAAGAGGGATA | 1/36 |
| 9. GATATGATATCCAGATGACACAGACT | ** (266 bps) ** ..... ** (500 bps *J2* ** GACCTCATGTCAGATTTGTGG | 1/36 |
| 10. ATCAAGGTTCAGTGGCAGTGGGTC | ** (88 bps) ** ..... ** (9 bps *J4)* ** GGCTCGGGGACAAAGTTGGAAA | 1/36 |
| 11. ATTGTCAGCAGTATAGTAAGCTTC | ** (4 bps) ** ..... ** (151 bps *J1)* ** TGCTCATTTACTTGTGACGTT | 1/36 |
| 12. TTATTCTCTCACCATCAGCAACCTGGA | ** (51 bps) ** ..... ** (18 bps *J2)* ** CCAAGCTGGAAATAAAAC | 1/36 |
| 13. ACCATCAGCAACCTGGAAC | ** (49 bps) ** .....** (25 bps *J1)* ** AAACGTAAGTAGAATCCAAA | 1/36 |
| 14. ATCAGCAACCTGGAACCTGAA | ** (46 bps) **.....** (95 bps *J1)* ** AATGATGTATAAAATCTTACT | 1/36 |
| 15. AGTCACCATCAGTTGCAGGGCAAA | ** (211 bps) ** ..... ** (407 bps *J2)* ** ACTTAAATAGAAGAGAACCAAA | 1/36 |
| 16. CACCATCAGTGGCAGTGGGTCTGGGG | ** (83 bps) ** .....** (66 bps *J4)* ** ACGTTTTGGTTCTGTTTGGGTAA | 1/36 |
| 17. AGGTTCAGTGGCAGTGGGTCTGG | ** (84 bps) ** ...AACAGA... ** (421 bps *J2)* ** GATAAATGAACTATTC | 1/36 |
| 18. ACTATTGTCAGCAGTATAGTAAGCTTC | ** (4 bps) ** ..... ** (52 bps *J4)* ** TGCTCATTTACTTGTGACGTTTT | 1/36 |
| 19. GGATTACACTCAGGAGTCCCATCAAGG | ** (104 bps) **.....** (211 bps *J2)* ** CTTTCTAAACCAAAGTAACTAAA | 1/36 |
| 20. TCAGGAGTCCCATCAAGGTTCAGTGG | ** (96 bps) **.....** (10 bps *J4)* ** GCTCGGGGACAAAGTTGGAAACAA | 1/36 |
| 21. GTGGCAGTGGGTCT | ** (86 bps ** ...ACTTACGTTTTATTTCCAACTTTGTCCCCGAGCCG... ** (32 bps *J4)* ** AACGTAAGTAGACTT | 1/36 |
| 22. ACAGACTACTTCCTCCCTGTCTGCC | ** (248 bps) ** ..... ** (274 bps *J1)* ** CTAGACAAACCTTTACTCGGTGCT | 1/36 |
| 23. CCATCAAGGTTCAGTGGCAGTGGGTC | ** (87 bps) ** .....** (8 bps *J4)* ** GGCTCGGGGACAAAGTTGG | 1/36 |
| 24. TCTCACCATCAGCAACCTGGAACC | ** (48 bps) ** ...AGA... ** (97 bps *J4)* ** AATTTGTGACATTTGGATAAATGA | 1/36 |
| 25. ACAGACTACTTCCTCCCTGTCTGCCT | ** (246 bps) ** ..... ** (582 bps *J2)* ** CAGGCAGGTTTTTGTAAAGGGGGGC | 1/36 |
| 26. CAAGGTTCAGTGGCAGTGGGTCTGG | ** (84 bps) ** ...*AACAGA*... ** (423 bps *J2)* ** GATAAATGAACTATTCCTTGTAACC | 1/36 |
| 27. AACCTGGAACCTGAAGATATTGCCACTTACT | ** (28 bps) ** ...TTTGCCAACAGG... ** (228 bps *J2)* ** GTAACTAAACTAGGGGAAG | 1/36 |
| 28. GGGCAAGTGAGGACATTAGCACTTATTT | ** (189 bps) ** .....** (193 bps *J2)* ** TCAGGCTAAATTTTAGG | 1/36 |

**Released *Xrcc4* $^{-/-}$ *p53* $^{-/-}$ pro-B cells** *(Xr15307-11)*

| ***IgkV*$_{10\text{-}95}$ coding end** | ***IgkJs* coding end** | Frequency |
|---|---|---|
| 1. AGGAGTCCCATCAAGGTTCAGTGGC | ** (95 bps) **.....** (112 bps *J4)* ** TAAATGAGCCATTCCTGGCAA | 7/34 |
| 2. ACTCCTGATCTATTACACATC | ** (132 bps) **.....** (13 bps *J4)* **GGGGACAAAGTTGGAAATAAAACG | 6/34 |
| 3. GCAGTGGGTCTGGGGCAGATTATTCTCTC | ** (68 bps) **.....** (552 bps *J2)* ** TGAACTTAGCCTATCT | 4/34 |
| 4. GCGTTTTGAAGGGGTCCTTTCTTTTC | ** (339 bps) **..... **(1 bp *J4)* ** ACGTTCGGCTCGGGGACAA | 4/34 |
| 5. CTTGTTGACTGGCGTTTTGAAGGGGT | ** (350 bps) **.....** (267 bps *J1)* ** AGCCTGCCCTAGACAAACC | 3/34 |
| 6. TGAAGATATTGCCACTTACTATTGTC | ** (22 bps) **.....** (60 bps *J1)* ** TTCCGTTGTCTATGTCTGT | 1/34 |
| 7. GATGGAACTGTTAAACTCCTGATC | ** (143 bps) **.....** (107 bps *J4)* ** ATTTTGGCTAAATGAGCCATTCC | 1/34 |
| 8. CATCAAGGTTCAGTGGCAGTGGG | ** (89 bps) **.....** (335 bps *J2)* ** AGAAATGAGAAAGGAACAGTTTT | 1/34 |
| 9. CAAGGTTCAGTGGCAGTGGGTCTGG | ** (84 bps) **.....** (152 bps *J2)* ** TGTTAAGGAGGGAAAACTGTC | 1/34 |
| 10. TCTTGTTGACTGGCGTTTTG | ** (357 bps) **.....** (68 bps *J4)* ** GTTCTGTTTGGGTAACTTGTGTGA | 1/34 |
| 11. GATGGAACTGTTAAACTCCTGATC | ** (143 bps) ** ....*G*.... ** (109 bps *J4)* ** TTTTGGCTAAATGAGCCATTCC | 1/34 |
| 12. CAAGGTTCAGTGGCAGTGGGTCTGG | ** (84 bps) **.....** (131 bps *J1)* ** CACTCTCCAAGGCAAAGAT | 1/34 |
| 13. ACAGAGTCACCATCAGTTGCAGGG | ** (214 bps) **.....**(187 bps *J2)* **AATGATTTTCAGGCTAAATTTT | 1/34 |
| 14. GATTACACTCAGGAGTCCCATCAAGG | ** (104 bps) **.....** (158 bps *J2)* ****AGGGAAAACTGTCCCACAA | 1/34 |
| 15. CTCAGGAGTCCCATCAAGGTTCAGTGGC | ** (13 bps) **.....** (112 bps *J4)* **TAAATGAGCCATTCCTGGC | 1/34 |

**Supplementary Table 3.**
**Related to Figure 1.**
*IgkV*$_{10\text{–}95}$*-J*$_4$ coding joins sequences from ABLki WT (A), released WT (B), ABLki *Xrcc4*$^{-/-}$ *p53*$^{-/-}$ (C) and released *Xrcc4*$^{-/-}$ *p53*$^{-/-}$ (D) *v-Abl* proB *cells.* The reference sequence for 5' and 3' ends of *IgkV*$_{10\text{–}95}$*-J*$_4$ coding join is shown at the top (green for *IgkV*$_{10\text{-}95}$ and red for *IgkJs* coding end respectively). * indicate the number of resected base pairs. For long resection, the number of resected nucleotides is indicated in brackets. Microhomologies are underlined. Insertions are shown in purple.

# Supplementary Table 4

**A**

| % All | WT ABLKi (#4) | WT Re (#5) | Xrcc4-/- ABLKi (#4) | p53-/- ABLKi (#3) | p53-/- Re (#3) | Xrcc4-/- p53-/- ABLKi (#3) | Xrcc4-/- p53-/- Re (#6) |
|---|---|---|---|---|---|---|---|
| Total # | 4855815 | 6196042 | 2664685 | 2045431 | 2162548 | 1692118 | 4770380 |
| Germline # (%) | 4 242 318 (87.36) | 5 973 012 (96.4) | 2 664 148 (99.99) | 1 872 047 (91.52) | 1 568 666 (72.53) | 1 691 427 (99.95) | 4 589 255 (96.20) |
| Total re-arrangements | 613 497 (12.63) | 223 030 (3.6) | 515 (0.02) | 173 384 (8.47) | 593 882 (12.63) | 691 (0.041) | 181 125 (3.797) |
| IgK re-arrangement # (%) | 612 718 (12.61) | 221 486 (3.57) | 431 (0.016) | 172 054 (8.41) | 590 228 (27.29) | 484 (0.029) | 162 649 (3.409) |
| JK4-AgR # (%) | 129 (0.003) | 96 (0.002) | 3 (0.0000) | 40 (0.002) | 776 (0.036) | 4 (0.0002) | 6 439 (0.134) |
| JK4-IgH/IgL # (%) | 127 (0.003) | 96 (0.002) | 3 (0.0000) | 40 (0.002) | 765 (0.035) | 4 (0.0002) | 6 311 (0.132) |
| # JK4-else # (%) | 650 (0.013) | 1 449(0.023) | 104 (0.004) | 1 290(0.063) | 2 878 (0.133) | 205 (0.012) | 11 985 (0.251) |

**B**

| % All | WT NT (#2) |
|---|---|
| Total # | 501850 |
| Germline # (%) | 501 721 (99.97) |
| Total re-arrangements # (%) | 129 (0.0257) |
| IgK re-arrangement # (%) | 39 (0.007) |
| JK4-AgR # (%) | 76 (0.0151) |
| JK4-IgH/IgL # (%) | 76 (0.0151) |
| # JK4-else # (%) | 14 (0.0027) |

**C**

| | NT | ABLKi | Re |
|---|---|---|---|
| Total # | 11 352 700 | 7 386 173 | 14 580 394 |
| Reads with *IgK* | 6 958 435 | 2 177 414 | 4 999 955 |
| *Vk-Jk* joins # (%) | 221 (0.003) | 63 (0.002) | 5 882 (0.117) |

**Supplementary Table 4**.
**Related to Figure 1.**
**(A)** Summary of LAM-HTGTS sequencing data analysis using *IgkJ4* bait in *v-Abl* pro-B cell lines. Upper table: number (#) of germline and rearrangement reads for ABLki and released (Re) cell lines. Sequenced cell lines: *WT (12095, 12096); Xrcc4$^{-/-}$ (Xr95-22, Xr95-23, Xr95-50); p53$^{-/-}$ (15307, 6920.1); Xrcc4$^{-/-}$ p53$^{-/-}$ (Xr15307-3, Xr15307-11).*
**(B)** Number (#) of germline and rearrangement reads for untreated cell lines. Analysis steps are explained in Figure S5. **(C)** Summary of Agilent captured sequencing data analysis in *Xrcc4 $^{-/-}$ p53 $^{-/-}$* pro-B cell line. Percentages correspond to the number of reads relative to total number of reads with *Igk* locus sequence. Sequenced cell lines: *Xrcc4$^{-/-}$ p53$^{-/-}$ (Xr15307-3).*

# Supplementary Table 5

Sequence analysis of *Bcr-Abl* chromosomal translocations

| Sample | | | Resection Length (bps) | | microhomology | | Insertion | |
|---|---|---|---|---|---|---|---|---|
| | # Sequence | Frequency | BCR end | ABL end | Length (bps) | Sequence | Length (bps) | Sequence |
| Cycling Rag2-/- p53-/- | 1 | 5/60 | 67 | 484 | 4 | AGTG | | |
| | 2 | 5/60 | 318 | 170 | 2 | TC | | |
| | 3 | 4/60 | 67 | 444 | 2 | TG | | |
| | 4 | 3/60 | 91 | 1 | 4 | ATTG | | |
| | 5 | 3/60 | 1 | 4 | | | 14 | TATCCTTTGAAATG |
| | 6 | 3/60 | 16 | 4 | 1 | G | | |
| | 7 | 3/60 | 324 | 68 | 3 | GGA | | |
| | 8 | 3/60 | 69 | 22 | 5 | GAGAG | | |
| | 9 | 2/60 | 0 | 0 | | | | |
| | 10 | 2/60 | 44 | 47 | 4 | TGCT | | |
| | 11 | 2/60 | 16 | 7 | 2 | GG | | |
| | 12 | 2/60 | 24 | 49 | 3 | CTG | | |
| | 13 | 2/60 | 0 | 0 | | | 1 | C |
| | 14 | 1/60 | 21 | 20 | | | 5 | GAAGG |
| | 15 | 1/60 | 285 | 23 | 1 | T | | |
| | 16 | 1/60 | 2 | 0 | | | 2 | CG |
| | 17 | 1/60 | 73 | 47 | 4 | TGCT | | |
| | 18 | 1/60 | 15 | 18 | 3 | GGT | | |
| | 19 | 1/60 | 290 | 23 | | | 4 | GTGC |
| | 20 | 1/60 | 2 | 2 | 2 | TT | | |
| | 21 | 1/60 | 0 | 1 | | | | |
| | 22 | 1/60 | 102 | 127 | | | 1 | C |
| | 23 | 1/60 | 46 | 50 | 2 | TG | | |
| | 24 | 1/60 | 324 | 76 | | | 5 | CGGTG |
| | 25 | 1/60 | 64 | 37 | 4 | GAGG | | |
| | 26 | 1/60 | 105 | 121 | 4 | CAGC | | |
| | 27 | 1/60 | 39 | 92 | 3 | CTC | | |
| | 28 | 1/60 | 3 | 2 | | | 3 | AAC |
| | 29 | 1/60 | 0 | 56 | | | 12 | GTCTTTGCATCT |
| | 30 | 1/60 | 79 | 72 | 2 | GG | | |
| | 31 | 1/60 | 116 | 57 | 6 | AGGCAG | | |
| | 32 | 1/60 | 72 | 25 | | | 1 | A |
| | 33 | 1/60 | 24 | 46 | 3 | CTG | | |
| | 34 | 1/60 | 22 | 114 | 6 | TCTGTC | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cycling Rag2 -/- Xrcc4 -/- p53 -/- | 1 | 3/83 | 131 | 7 | 2 | GG | | |
| | 2 | 2/83 | 2 | 317 | | | | |
| | 3 | 2/83 | 44 | 47 | 4 | TGCT | | |
| | 4 | 2/83 | 91 | 1 | 4 | ATTG | | |
| | 5 | 2/83 | 335 | 17 | | | 4 | GAAA |
| | 6 | 2/83 | 7 | 475 | | | 3 | TTG |
| | 7 | 2/83 | 211 | 20 | | | | |
| | 8 | 2/83 | 16 | 17 | 2 | GG | | |
| | 9 | 2/83 | 15 | 18 | 3 | ACC | | |
| | 10 | 2/83 | 21 | 309 | 3 | TCA | | |
| | 11 | 2/83 | 61 | 80 | 2 | TG | | |
| | 12 | 2/83 | 16 | 29 | 3 | AGG | | |
| | 13 | 2/83 | 312 | 66 | 5 | TCCTG | | |
| | 14 | 2/83 | 16 | 31 | 2 | CC | | |
| | 15 | 1/83 | 16 | 38 | 3 | AGG | | |
| | 16 | 1/83 | 3 | 31 | | | 5 | GAGTT |
| | 17 | 1/83 | 24 | 17 | 1 | G | | |
| | 18 | 1/83 | 58 | 92 | 2 | CT | | |
| | 19 | 1/83 | 4 | 0 | | | 4 | GGGT |
| | 20 | 1/83 | 12 | 114 | 2 | TC | | |
| | 21 | 1/83 | 0 | 17 | | | 2 | GA |
| | 22 | 1/83 | 2 | 2 | 2 | TT | | |
| | 23 | 1/83 | 10 | 2 | | | 3 | ATC |
| | 24 | 1/83 | 16 | 16 | 2 | GG | | |
| | 25 | 1/83 | 3 | 112 | 3 | CTT | | |
| | 26 | 1/83 | 259 | 9 | | | | |
| | 27 | 1/83 | 338 | 95 | 4 | CCTA | | |
| | 28 | 1/83 | 273 | 10 | | | 4 | GATG |
| | 29 | 1/83 | 18 | 25 | 1 | A | | |
| | 30 | 1/83 | 243 | 10 | 2 | CA | | |
| | 31 | 1/83 | 38 | 226 | | | 8 | CTTTAGTC |
| | 32 | 1/83 | 26 | 226 | 4 | GATT | | |
| | 33 | 1/83 | 26 | 0 | | | 2 | CC |
| | 34 | 1/83 | 7 | 45 | 3 | AGG | | |
| | 35 | 1/83 | 26 | 226 | 4 | AATC | | |
| | 36 | 1/83 | 22 | 54 | 2 | TC | | |
| | 37 | 1/83 | 313 | 84 | 3 | CCT | | |
| | 38 | 1/83 | 18 | 29 | | | 2 | TT |
| | 39 | 1/83 | 24 | 31 | 1 | G | | |
| | 40 | 1/83 | 61 | 80 | 2 | TG | | |
| | 41 | 1/83 | 22 | 31 | | | 230 | TTGAGCAACAAAAATTTAAGTACATTCT GCAACTTTGGCCTGGTGAGCTGTGATC ATTCTTACAGTTGATGAGTATGGCTGAC TATGAAATGAAGAAAGTAACTTGGCAC TGATGCACTGTGAGTCAGTTTTGACATC AGCCTGGACTGGGAATTGGCTGGAAG GTTTTGTTGCTACACTCCATGTCTCCATC CAGAGGCACACATGAGCAATACTTCCCT TTGGGCTTT |
| | 42 | 1/83 | 32 | 21 | | | 26 | CTCTCACCTTGGGCCCTGGGAGACAC |
| | 43 | 1/83 | 79 | 83 | 4 | AAGG | | |
| | 44 | 1/83 | 72 | 171 | | | | |
| | 45 | 1/83 | 16 | 4 | 2 | GG | | |
| | 46 | 1/83 | 157 | 107 | 1 | G | | |
| | 47 | 1/83 | 17 | 8 | 1 | C | | |
| | 48 | 1/83 | 64 | 57 | 3 | AGG | | |
| | 49 | 1/83 | 15 | 10 | 1 | T | | |
| | 50 | 1/83 | 4 | 18 | | | 182 | CAAGTTAACAGCCAAAGCCCAAAGGGA AGTATTGCTCATGTGTGCCTCTGGATGG AGACATGGAGTGTAGCAACAAAACCTT CCAGCCAATTCCCAGTCCAGGCTGATGT CAAAACTGACTCACAGTGCATCAGTGCC AAGTTACTTTCTTCATTTCATAGTCAGCC ATACTCATCAACTGC |
| | 51 | 1/83 | 7 | 22 | | | 204 | TCTTACAGTTGATGAGTATGGCTGACTA TGAAATGAAGAAAGTAACTTGGCACTG |
| | 52 | 1/83 | 50 | 235 | 1 | G | | |
| | 53 | 1/83 | 43 | 100 | | | 10 | CACTCCCTAT |
| | 54 | 1/83 | 49 | 235 | 2 | GC | | |
| | 55 | 1/83 | 10 | 30 | | | 378 | TGATGGCTGTGGTGTTCTTACTTCTCCA CTCCAGTTCCTCTACACCCTGTGACTTGT TGTTTCGGGGTGCTGGGAAAATTGAGT TCTGGATGTGAGGTAATACAAAGGCTG TATTTAGTTAAATGCGGTTTTGTTATTTT AATAATCTGAACTGTGGGTGAGGGTCT GAGTGATAAGAGGGATGCAGTGGCGT TCAGCAGGTTTACATTGCGGGAAATAA GACTTTTCCTCCAAGCTCACATTATGAG GAACTCACGAAACAGTTACAGACTGACT TGAGAATATTCTGAGCATGACCTCATCA GCTGGAACAGAAAGGAGCTAAAACATG AAGTCCTCTCCTTTGCAAAGGGTCTGTT TTACAGATCTGCGCATTGC |
| | 56 | 1/83 | 303 | 56 | 2 | CA | | |
| | 57 | 1/83 | 29 | 11 | | | 11 | GTCTGTCAAAA |
| | 58 | 1/83 | 49 | 40 | 2 | GC | | |
| | 59 | 1/83 | 324 | 17 | | | 1 | A |
| | 60 | 1/83 | 12 | 412 | | | 5 | GCCCT |
| | 61 | 1/83 | 49 | 235 | 2 | GC | | |
| | 62 | 1/83 | 4 | 412 | 3 | CCT | | |
| | 63 | 1/83 | 76 | 8 | 1 | A | | |
| | 64 | 1/83 | 4 | 314 | 4 | TCCT | | |
| | 65 | 1/83 | 256 | 24 | | | 30 | CACCCTACCCTCTCTCTCAACCCCACCCCA |
| | 66 | 1/83 | 3 | 15 | | | 1 | A |
| | 67 | 1/83 | 48 | 48 | 3 | GCT | | |
| | 68 | 1/83 | 191 | 25 | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| G1 Rag2-/- p53-/- | 1 | 3/3 | 381 | 517 | 2 | CC | | |
| | | | | | | | | |
| G1 Rag2-/- Xrcc4-/- p53-/- | 1 | 3/8 | 313 | 84 | 3 | AGG | | |
| | 2 | 3/8 | 13 | 9 | 2 | AT | | |
| | 3 | 2/8 | 62 | 67 | 5 | GTCCT | | |
| | | | | | | | | |
| Released Rag2-/- p53-/- | 1 | 6/9 | 0 | 0 | | | | |
| | 2 | 3/9 | 1 | 0 | 1 | A | | |
| | | | | | | | | |
| Released Rag2-/- Xrcc4-/- p53-/- | 1 | 4/84 | 156 | 255 | 1 | A | | |
| | 2 | 4/84 | 62 | 286 | | | 1 | C |
| | 3 | 4/84 | 57 | 32 | 4 | GCTT | | |
| | 4 | 4/84 | 373 | 20 | 2 | TT | | |
| | 5 | 4/84 | 381 | 412 | 2 | CC | | |
| | 6 | 3/84 | 44 | 47 | 4 | TGCT | | |
| | 7 | 2/84 | 180 | 42 | 5 | GGATG | | |
| | 8 | 2/84 | 58 | 163 | | | 1 | G |
| | 9 | 2/84 | 45 | 369 | 3 | TGC | | |
| | 10 | 2/84 | 52 | 49 | 2 | CT | | |
| | 11 | 2/84 | 46 | 273 | | | | |
| | 12 | 2/84 | 48 | 221 | | | 1 | A |
| | 13 | 2/84 | 35 | 463 | | | 4 | AAGT |
| | 14 | 2/84 | 38 | 442 | 3 | TCT | | |
| | 15 | 2/84 | 99 | 48 | 5 | GCTGG | | |
| | 16 | 2/84 | 29 | 143 | 3 | TCA | | |
| | 17 | 2/84 | 39 | 412 | 1 | C | | |
| | 18 | 1/84 | 64 | 17 | 2 | GG | | |
| | 19 | 1/84 | 15 | 72 | 3 | ACC | | |
| | 20 | 1/84 | 48 | 46 | 2 | CT | | |
| | 21 | 1/84 | 46 | 52 | 1 | C | | |
| | 22 | 1/84 | 24 | 15 | 2 | TG | | |
| | 23 | 1/84 | 11 | 48 | | | 4 | TTTG |
| | 24 | 1/84 | 15 | 7 | | | | |
| | 25 | 1/84 | 58 | 48 | 3 | GCT | | |
| | 26 | 1/84 | 12 | 6 | 1 | C | | |
| | 27 | 1/84 | 15 | 18 | 3 | GGT | | |
| | 28 | 1/84 | 49 | 52 | 2 | GA | | |
| | 29 | 1/84 | 78 | 32 | | | 65 | TAATATATTTAAAATGTCATTTAGAAGGACACAGAAGAAAGTGCTTGTGGCAGAGAAAGAGAGAGA |
| | 30 | 1/84 | 31 | 19 | 2 | GT | | |
| | 31 | 1/84 | 13 | 504 | 2 | AT | | |
| | 32 | 1/84 | 49 | 26 | | | 4 | CCTA |
| | 33 | 1/84 | 15 | 13 | 3 | ACC | | |
| | 34 | 1/84 | 18 | 335 | 5 | CAAAA | | |
| | 35 | 1/84 | 46 | 439 | 3 | TTG | | |
| | 36 | 1/84 | 15 | 25 | | | 4 | GATA |
| | 37 | 1/84 | 15 | 17 | | | 5 | GGAGA |
| | 38 | 1/84 | 46 | 32 | 5 | GCTTG | | |
| | 39 | 1/84 | 16 | 4 | 2 | GG | | |
| | 40 | 1/84 | 41 | 142 | 3 | TTC | | |
| | 41 | 1/84 | 56 | 477 | 3 | TTA | | |
| | 42 | 1/84 | 151 | 12 | 4 | GGGT | | |
| | 43 | 1/84 | 48 | 48 | 3 | GCT | | |
| | 44 | 1/84 | 49 | 25 | | | 3 | CCT |
| | 45 | 1/84 | 41 | 332 | 3 | TTC | | |
| | 46 | 1/84 | 16 | 7 | 2 | GG | | |
| | 47 | 1/84 | 293 | 455 | 2 | CA | | |
| | 48 | 1/84 | 158 | 8 | 2 | GA | | |
| | 49 | 1/84 | 7 | 256 | | | | |
| | 50 | 1/84 | 323 | 11 | | | 6 | AGTATA |
| | 51 | 1/84 | 15 | 34 | | | | |
| | 52 | 1/84 | 43 | 32 | 4 | AAGC | | |
| | 53 | 1/84 | 15 | 18 | 3 | GGT | | |
| | 54 | 1/84 | 57 | 31 | 5 | AAGCT | | |
| | 55 | 1/84 | 161 | 255 | | | 4 | CTCT |
| | 56 | 1/84 | 21 | 456 | | | 745 | CTGAACTGTGGGTGAGGGTCTGAGTGATAAGAGGGATGCAGTGGCGTTCAGCAGGTTTACATTGTGGGAAATAAGACTTTTCCTCCAAGCTCACATTATGAGGAACTCACGAAACAGTTACAGACTGACTTGAGAATATTCTGAGCATGACCTCATCAGCTGGAACAGAAAGGAGCTAAAACATGAAGTCCTCTCCTTTGCAAAGGGTCTGTTTTACAGATCTGCGCATTGCGGAGATTCCTCTAGCCAGAGGAAATCTCAAAGGGGCCACCAAGGAAAGAAAGTTACTTTGGCAGGGCTTAAGTCCTTGAAATGAGCACAGGAAATGGGACTGTGTGTTTAGGGACATACCCTTTCTGTTCCTCCCCAACGAGGAAGTGTCTCCCAGGGCCCAAGGTGAGGACTGTGGGAAGTGCTGTGCAGCTGGCTGCCCCACACTCCACGGGGACCTGGGGACCAGTGCCAACAGGAAGTCAGGAGCTTCCTGGGGATGTTTTTCCTGGAGGCAAATTCTACCGTCTCCCTGAGACAGTGAAGTCTCCTTCCCCTGGGTCTCAGGGCAGCCACTGGCTCCCCTCATTCCTCACAGCGATCTAGCCCAGATATGCCCCATCCAAGGATCTTCTGTAAACAGTCTTCAACAGTGACTCACAACCTTGAAAAGGGATTTCCTTTTATTTTAATAAAAAGGACCTTTAACATACTCAAACAGTTTTCCT |

**Supplementary Table 5**.
**Related to Figure 3.**
TOPO cloning sequence analysis for PCR products of *Bcr-Abl* translocation breakpoint junctions including resection length from *Bcr* and *Abl* breakpoint sites and microhomology and insertion at breakpoint junctions. Each genotype represents two independent cell clones.

# Supplementary Table 6

## A

| Genotype | Cell line + Treatment | Total metaphases analyzed | Total normal metaphases | Metaphases with chromosome breaks | Metaphases with translocations | aberrant metaphases | % Metaphases with chromosome breaks | % Metaphases with translocations | % aberrant metaphases |
|---|---|---|---|---|---|---|---|---|---|
| *p53-/-* | 15307 NT * | 130 | 122 | 4 | 4 | 8 | 3.1 | 3.1 | 6.2 |
| | 6920.1 NT | 146 | 143 | 1 | 2 | 3 | 0.7 | 1.4 | 2.1 |
| | **Total** | **276** | **265** | **5** | **6** | **11** | **1.8** | **2.2** | **4.0** |
| | **Mean (±SD)** | | | | | | **1.9 (±1.7)** | **2.2 (±1.2)** | **4.1 (±2.9)** |
| | 6920.1 Re * | 250 | 241 | 7 | 2 | 9 | 2.8 | 0.8 | 3.6 |
| | 6943.2 Re * | 251 | 235 | 8 | 8 | 16 | 3.2 | 3.2 | 6.4 |
| | 15307 Re * | 244 | 224 | 7 | 13 | 20 | 2.9 | 5.3 | 8.2 |
| | **Total** | **745** | **700** | **22** | **23** | **45** | **3.0** | **3.1** | **6.0** |
| | **Mean (±SD)** | | | | | | **3.0 (±0.2)** | **3.3 (±2.3)** | **6.1 (±2.3)** |
| *Xrcc4-/- p53-/-* | Xr15307-3 NT | 218 | 217 | 0 | 1 | 1 | 0.0 | 0.5 | 0.5 |
| | Xr15307-11 NT | 155 | 154 | 0 | 1 | 1 | 0.0 | 0.6 | 0.6 |
| | **Total** | **373** | **371** | **0** | **2** | **2** | **0.0** | **0.5** | **0.5** |
| | **Mean (±SD)** | | | | | | **0 (±0)** | **0.55 (±0.1)** | **0.55 (±0.1)** |
| | Xr15307-3 Re (1) | 257 | 162 | 48 | 50 | 98 | 18.7 | 19.5 | 38.1 |
| | Xr15307-3 Re (2) | 178 | 116 | 32 | 30 | 62 | 18.0 | 16.9 | 34.8 |
| | Xr15307-3 Re (3) | 61 | 41 | 7 | 13 | 20 | 11.5 | 21.3 | 32.8 |
| | Xr15307-11 Re (1) | 226 | 159 | 35 | 36 | 71 | 15.5 | 15.9 | 31.4 |
| | Xr15307-11 Re (2) | 91 | 54 | 18 | 15 | 33 | 19.8 | 16.5 | 36.3 |
| | **Total** | **813** | **532** | **140** | **144** | **284** | **17.2** | **17.7** | **34.9** |
| | **Mean (±SD)** | | | | | | **16.7 (±3.3)** | **18 (±2.3)** | **34.7 (±2.7)** |
| *Xrcc4-/- p53-/- + Xrcc4* | Xr15307-3 + Xrcc4 NT | 95 | 91 | 4 | 0 | 4 | 4.2 | 0.0 | 4.2 |
| | Xr15307-11 + Xrcc4 NT | 110 | 109 | 0 | 1 | 1 | 0 | 0.9 | 0.9 |
| | **Total** | **205** | **200** | **4** | **1** | **5** | **2.0** | **0.5** | **2.4** |
| | **Mean (±SD)** | | | | | | **2.1 (±3)** | **0.5 (±0.6)** | **2.6 (±2.3)** |
| | Xr15307-3 + Xrcc4 Re | 218 | 196 | 13 | 9 | 22 | 6 | 4.2 | 10.2 |
| | Xr15307-11 + Xrcc4 Re | 279 | 266 | 9 | 4 | 13 | 3.2 | 1.4 | 4.6 |
| | **Total** | **497** | **462** | **22** | **13** | **35** | **4.4** | **2.6** | **7.0** |
| | **Mean (±SD)** | | | | | | **4.6 (±2)** | **2.8 (±2)** | **7.4 (±4)** |
| *Polq-/- p53-/-* | Polq-2-307 NT (1) | 53 | 53 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| | Polq-2-307 NT (2) | 108 | 107 | 0 | 1 | 1 | 0.0 | 0.9 | 0.9 |
| | Polq-5-307 NT | 115 | 114 | 1 | 0 | 1 | 0.9 | 0.0 | 0.9 |
| | **Total** | **276** | **274** | **1** | **1** | **2** | **0.4** | **0.4** | **0.7** |
| | **Mean (±SD)** | | | | | | **0.3 (±0.5)** | **0.3 (±0.5)** | **0.6 (±0.5)** |
| | Polq-2-307 Re (1) | 41 | 39 | 1 | 2 | 3 | 2.4 | 4.9 | 7.3 |
| | Polq-2-307 Re (2) | 120 | 117 | 1 | 2 | 3 | 0.8 | 1.7 | 2.5 |
| | Polq-5-307 Re | 111 | 110 | 0 | 1 | 1 | 0.0 | 0.9 | 0.9 |
| | **Total** | **272** | **266** | **2** | **5** | **7** | **0.7** | **1.8** | **2.6** |
| | **Mean (±SD)** | | | | | | **1.1 (±1.2)** | **2.5 (±2.1)** | **3.6 (±3.3)** |
| *Xrcc4-/- Polq-/- p53-/-* | Polq-2-307-Xr25 NT | 92 | 86 | 4 | 2 | 6 | 4.3 | 2.2 | 6.5 |
| | Xr307-3-Polq35 NT | 214 | 210 | 3 | 1 | 4 | 1.4 | 0.5 | 1.9 |
| | **Total** | **306** | **296** | **7** | **3** | **10** | **2.3** | **1.0** | **3.3** |
| | **Mean (±SD)** | | | | | | **2.9 (±2.1)** | **1.3 (±1.2)** | **4.2 (±3.3)** |
| | Polq-2-307-Xr25 Re (1) | 58 | 19 | 38 | 2 | 40 | 65.5 | 3.4 | 69.0 |
| | Polq-2-307-Xr25 Re (2) | 99 | 23 | 75 | 1 | 76 | 75.8 | 1.0 | 76.8 |
| | Xr307-3-Polq35 Re (1) | 101 | 34 | 62 | 5 | 67 | 61.4 | 5.0 | 66.3 |
| | Xr307-3-Polq35 Re (2) | 115 | 23 | 91 | 1 | 92 | 79.1 | 0.9 | 80.0 |
| | **Total** | **373** | **99** | **266** | **9** | **275** | **71.3** | **2.4** | **73.7** |
| | **Mean (±SD)** | | | | | | **70.5 (±8.4)** | **2.6 (±2.0)** | **73 (±6.4)** |
| *Xrcc4-/- Polq-/- p53-/- + Xrcc4* | Polq-2-307-Xr25 + Xrcc4 NT | 187 | 187 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| | Xr307-3-Polq35 + Xrcc4 NT | 166 | 166 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| | **Total** | **353** | **353** | **0** | **0** | **0** | **0.0** | **0.0** | **0.0** |
| | **Mean (±SD)** | | | | | | **0 (±0)** | **0 (±0)** | **0 (±0)** |
| | Polq-2-307-Xr25 + Xrcc4 Re | 192 | 190 | 0 | 2 | 2 | 0.0 | 1.0 | 1.0 |
| | Xr307-3-Polq35 + Xrcc4 Re | 125 | 123 | 1 | 1 | 2 | 0.8 | 0.8 | 1.6 |
| | **Total** | **317** | **313** | **1** | **3** | **4** | **0.3** | **0.9** | **1.3** |
| | **Mean (±SD)** | | | | | | **0.4 (±0.6)** | **0.9 (±0.2)** | **1.3 (±0.4)** |
| *Rag2-/- Xrcc4-/- Polq-/- p53-/-* | X-P-87-4-4-X-3 NT | 254 | 254 | 0 | 0 | 0 | 0 | 0 | 0 |
| | X-P-87-4-4-X-14 NT | 266 | 265 | 0 | 1 | 1 | 0 | 0.4 | 0.4 |
| | X-P-87-4-4-X-16 NT | 180 | 180 | 1 | 0 | 1 | 0.6 | 0 | 0.6 |
| | **Total** | **700** | **699** | **1** | **1** | **2** | **0.1** | **0.1** | **0.3** |
| | **Mean (±SD)** | | | | | | **0.2 (±0.3)** | **0.1 (±0.2)** | **0.3 (±0.3)** |
| | X-P-87-4-4-X-3 Re | 330 | 327 | 1 | 2 | 3 | 0.3 | 0.6 | 0.9 |
| | X-P-87-4-4-X-14 Re | 172 | 170 | 2 | 0 | 2 | 1.2 | 0 | 1.2 |
| | X-P-87-4-4-X-16 Re | 290 | 289 | 1 | 0 | 1 | 0.3 | 0 | 0.3 |
| | **Total** | **792** | **786** | **4** | **2** | **6** | **0.5** | **0.3** | **0.8** |
| | **Mean (±SD)** | | | | | | **0.6 (±0.5)** | **0.2 (±0.3)** | **0.8 (±0.5)** |
| *Xrcc4-/- Parp1-/- p53-/-* | Xr-P1P2Fp53-2 NT | 204 | 201 | 2 | 1 | 3 | 1.0 | 0.5 | 1.5 |
| | Xr-P1P2Fp53-3 NT | 90 | 90 | 0 | 0 | 0 | 0.0 | 0.0 | 0.0 |
| | **Total** | **294** | **291** | **2** | **1** | **3** | **0.7** | **0.3** | **1.0** |
| | **Mean (±SD)** | | | | | | **0.5 (±0.7)** | **0.3 (±0.3)** | **0.8 (±1)** |
| | Xr-P1P2Fp53-2 Re | 111 | 69 | 25 | 17 | 42 | 22.5 | 15.3 | 37.8 |
| | Xr-P1P2Fp53-3 Re | 146 | 95 | 28 | 23 | 51 | 19.2 | 15.8 | 34.9 |
| | **Total** | **257** | **164** | **53** | **40** | **93** | **20.6** | **15.6** | **36.2** |
| | **Mean (±SD)** | | | | | | **20.9 (±2.4)** | **15.5 (±0.3)** | **36.4 (±2.1)** |

## B

| Statistical analysis (two-sided Fisher exact test) | | Normal vs Aberrant (p-value) | Level |
|---|---|---|---|
| Xrcc4-/- p53-/- Re *vs* | Xrcc4-/- p53-/- NT | 3.8E-51 | *** |
| | p53-/- Re | 4.9E-47 | *** |
| | Xrcc4-/- p53-/- + Xrcc4 Re | 7.9E-34 | *** |
| | Xrcc4-/- Parp1-/- p53-/- Re | 0.708 | NS |
| Xrcc4-/- Polq-/- p53-/- Re *vs* | Xrcc4-/- Polq-/- p53-/- NT | 3.17E-89 | *** |
| | Polq-/- p53-/- Re | 1.02E-85 | *** |
| | Xrcc4-/- p53-/- Re | 4.79E-35 | *** |
| | Rag2-/- Xrcc4-/- Polq-/- p53-/ | 9.8E-172 | *** |
| | Xrcc4-/- Polq-/- p53-/- + Xrcc4 | 2.60E-100 | *** |

* $p < 0.05$

*** $p < 0.01$

**C**

| Genotype | Cell line + Treatment | Total metaphases analyzed | Total normal metaphases | Metaphases with chromosome breaks | Metaphases with translocations | Total aberrant metaphases | % Metaphases with chromosome breaks | % Metaphases with translocations | % aberrant metaphases |
|---|---|---|---|---|---|---|---|---|---|
| *Rag2-/- p53-/-* | 17585 NT | 37 | 36 | 0 | 1 | 1 | 0 | 2.7 | 2.7 |
| | 17587 NT | 37 | 37 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Total** | **74** | **73** | **0** | **1** | **1** | **0** | **1.4** | **1.4** |
| | **Mean (±SD)** | | | | | | **0 (±0)** | **1.4 (±1.9)** | **1.35 (±1.9)** |
| | 17585 Re | 126 | 126 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 17587 Re | 65 | 65 | 0 | 0 | 0 | 0 | 0 | 0 |
| | **Total** | **191** | **191** | **0** | **0** | **0** | **0** | **0** | **0** |
| | **Mean (±SD)** | | | | | | **0 (±0)** | **0 (±0)** | **0 (±0)** |
| *Rag2-/- Polq-/- p53-/-* | Polq-17587-8 NT | 98 | 90 | 5 | 3 | 8 | 5.1 | 3.1 | 8.2 |
| | Polq-17587-22 NT | 90 | 83 | 4 | 3 | 7 | 4.4 | 3.3 | 7.8 |
| | **Total** | **188** | **173** | **9** | **6** | **15** | **4.8** | **3.2** | **8.0** |
| | **Mean (±SD)** | | | | | | **4.8 (±0.5)** | **3.2 (±0.2)** | **8 (±0.3)** |
| *Rag2-/- Xrcc4-/- p53-/-* | Xr17585-17 (1) NT | 32 | 20 | 6 | 6 | 12 | 18.8 | 18.8 | 37.5 |
| | Xr17585-17 (2) NT | 64 | 49 | 7 | 8 | 15 | 10.9 | 12.5 | 23.4 |
| | Xr17585-18 NT | 92 | 64 | 10 | 18 | 28 | 10.9 | 19.6 | 30.4 |
| | **Total** | **188** | **133** | **23** | **32** | **55** | **12.2** | **17.0** | **29.3** |
| | **Mean (±SD)** | | | | | | **13.5 (±4.5)** | **16.9 (±3.9)** | **30.4 (±7)** |
| | Xr17585-17 Re | 45 | 30 | 5 | 9 | 14 | 11.1 | 20.0 | 31.1 |
| | Xr17585-18 Re | 53 | 33 | 9 | 11 | 20 | 17.0 | 20.8 | 37.7 |
| | **Total** | **98** | **63** | **14** | **20** | **34** | **14.3** | **20.4** | **34.7** |
| | **Mean (±SD)** | | | | | | **14 (±4.2)** | **20.4 (±0.5)** | **34.4 (±4.7)** |
| *Rag2-/- Xrcc4-/- Polq-/- p53-/-* | X-P-87-4-4-X-14 NT | 42 | 27 | 13 | 2 | 15 | 31.0 | 4.8 | 35.7 |
| | X-P-87-4-4-X-3 NT | 101 | 59 | 33 | 9 | 42 | 32.7 | 8.9 | 41.6 |
| | **Total** | **143** | **86** | **46** | **11** | **57** | **32.2** | **7.7** | **39.9** |
| | **Mean (±SD)** | | | | | | **31.8 (±1.2)** | **6.8 (±2.9)** | **38.6 (±4.2)** |
| | X-P-87-4-4-X-3 Re | 12 | 4 | 8 | 0 | 8 | 66.7 | 0 | 66.7 |

**D**

| Statistical analysis (two-sided Fisher exact test) | | Normal *vs* Aberrant | Break *vs* Others | Translocation *vs* Others |
|---|---|---|---|---|
| Rag2-/- Xrcc4-/- p53-/- NT *vs* | Rag2-/- p53-/- NT | 6.10E-05 | | |
| | Rag2-/- Polq-/- p53-/- NT | 1.24E-07 | | |
| | Rag2-/- Xrcc4-/- Polq-/- p53-/- | 0.046 | 1.74E-05 | 0.013 |
| Rag2-/- Polq-/- p53-/- NT *vs* | Rag2-/- p53-/- NT | 0.046 | | |
| | Rag2-/- Xrcc4-/- Polq-/- p53-/- | 2.98E-12 | | |
| Rag2-/- Xrcc4-/- p53-/- Re *vs* | Rag2-/- p53-/- Re | 5.68E-12 | | |

**Supplementary Table 6. Genomic instability at the *Igk and Bcr/Abl* loci in pro-B cells. Related to Figure 4.**

**(A)** Number and percentage of aberrant metaphases harboring chromosomes breaks and/or translocations involving the *Igk* locus from pro-B cell lines of the indicated genotype and respective treatment. DNA FISH experiments were performed using probes centromeric (*Igk V*) and telomeric (*Igk C*) to the *Igk* locus plus specific paint for chromosome 6. NT = untreated cycling conditions; Re = ABLki treatment followed by release of the cells into the cell cycle. * Data from Lescale C. et al. Nature Communications 2016. **(B)** Statistical analysis using two-sided Fisher exact test. **(C)** Number and percentage of aberrant metaphases harboring chromosomes breaks and/or translocations involving the *Bcr* and *Abl* loci from pro-B cell lines of the indicated genotype and respective treatment. NT = untreated cycling conditions + Cas9/gRNA nucleofection; Re = ABLki treatment + Cas9/gRNA nuclofection in blocked cells followed by release of the cells into the cell cycle. DNA FISH experiments were performed using probes to the *Bcr* and *Abl* loci. **(D)** Statistical analysis using two-sided Fisher exact test.