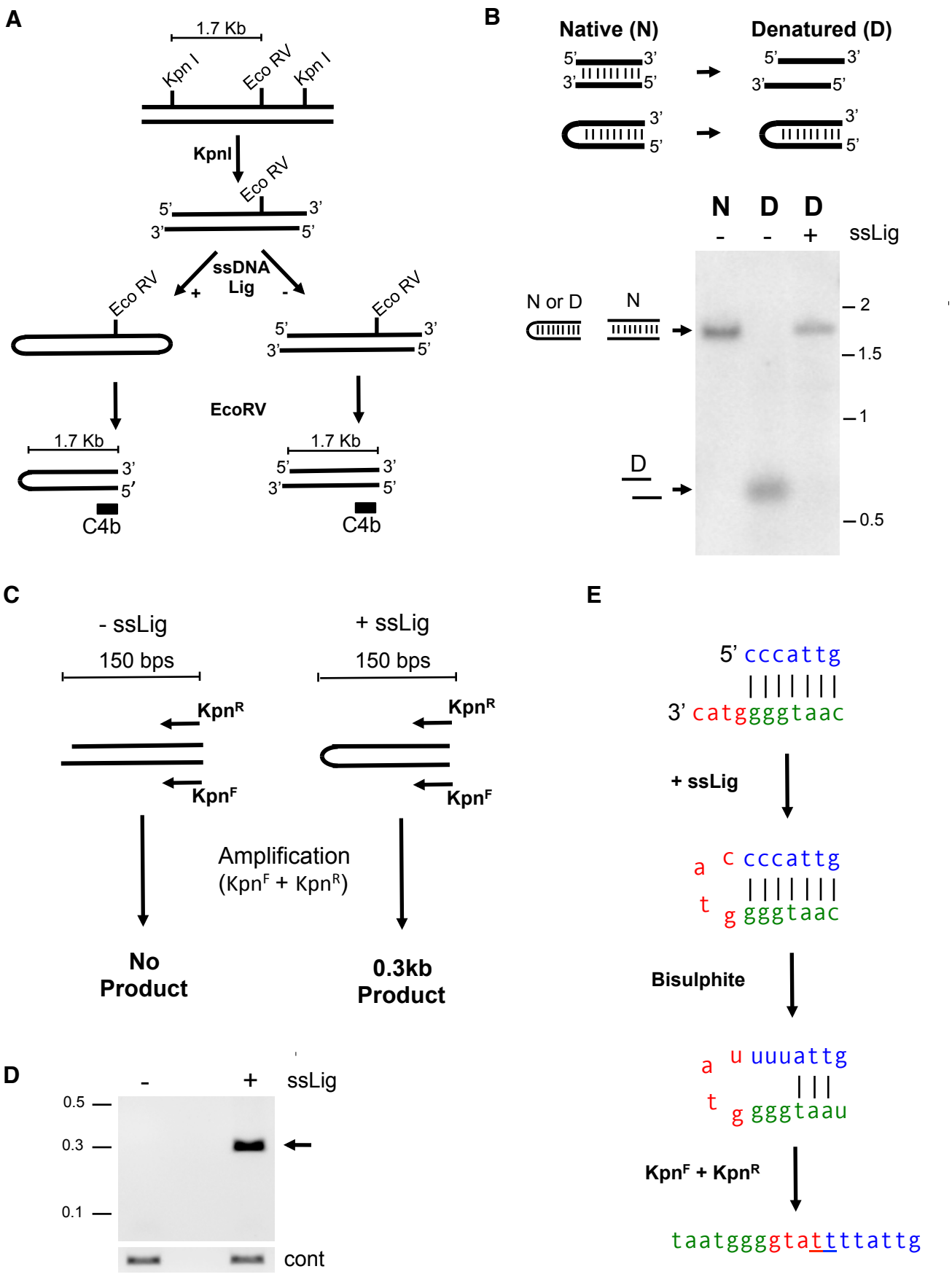
A
1.7 Kb
Kpn I
Eco RV
Kpn I
KpnI
Eco RV
5'
3'
3'
5'
ssDNA Lig
+
-
Eco RV
Eco RV
5'
3'
3'
5'
EcoRV
1.7 Kb
3'
5'
C4b
1.7 Kb
5'
3'
3'
5'
C4b
B
Native (N)
Denatured (D)
5'
3'
3'
5'
3'
5'
3'
5'
N
D
D
-
-
+
ssLig
N or D
N
D
2
1.5
1
0.5
C
- ssLig
150 bps
KpnR
KpnF
+ ssLig
150 bps
KpnR
KpnF
Amplification
(KpnF + KpnR)
No Product
0.3kb Product
D
-
+
ssLig
0.5
0.3
0.1
cont
E
5' cccattg
3' catggggtaac
+ ssLig
a c cccattg
t g gggtaac
Bisulphite
a u uuuattg
t g gggtaau
KpnF + KpnR
taatggggtattttattg

Figure S1.

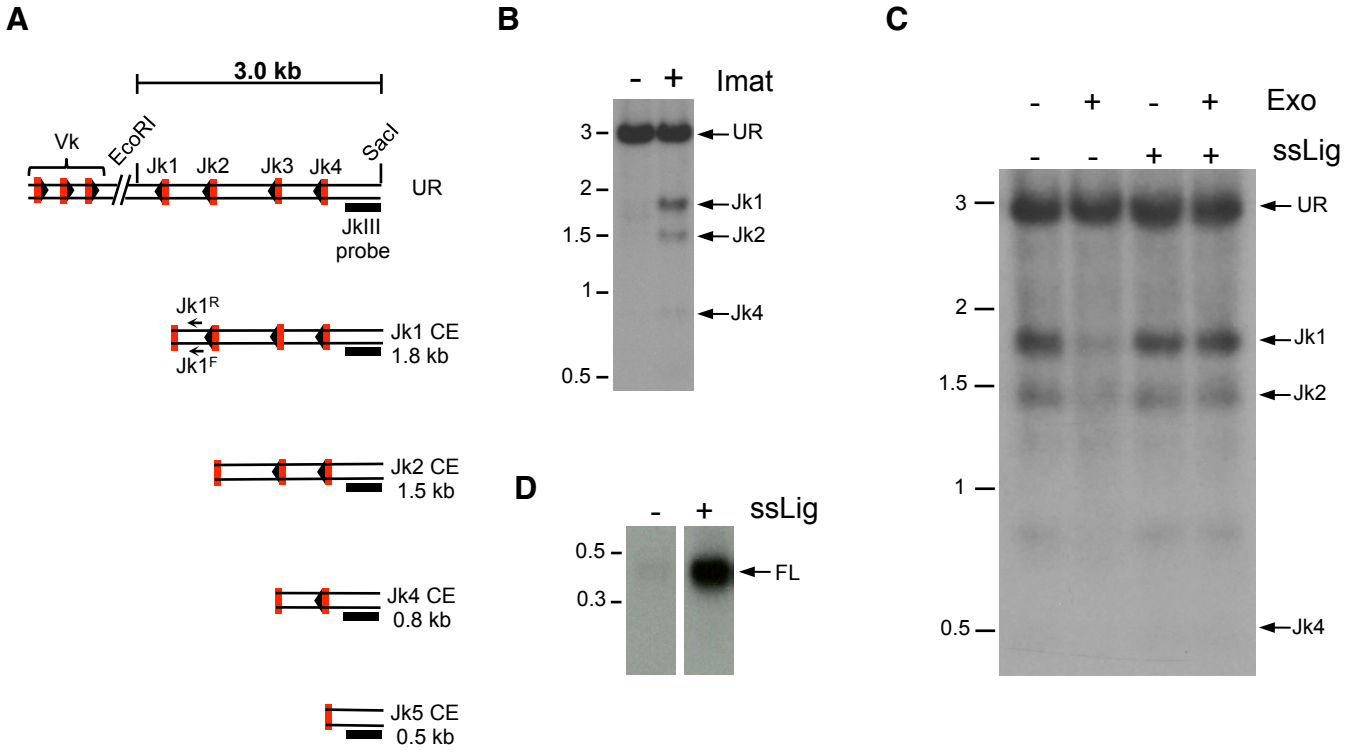
A
3.0 kb
Vk
EcoRI
Jk1
Jk2
Jk3
Jk4
SacI
UR
JkIII
probe
Jk1R
Jk1F
Jk1 CE
1.8 kb
Jk2 CE
1.5 kb
Jk4 CE
0.8 kb
Jk5 CE
0.5 kb
B
- + Imat
3
2
1.5
1
0.5
UR
Jk1
Jk2
Jk4
D
- + ssLig
0.5
0.3
FL
C
- + - + Exo
- - + + ssLig
3
2
1.5
1
0.5
UR
Jk1
Jk2
Jk4

Figure S2.

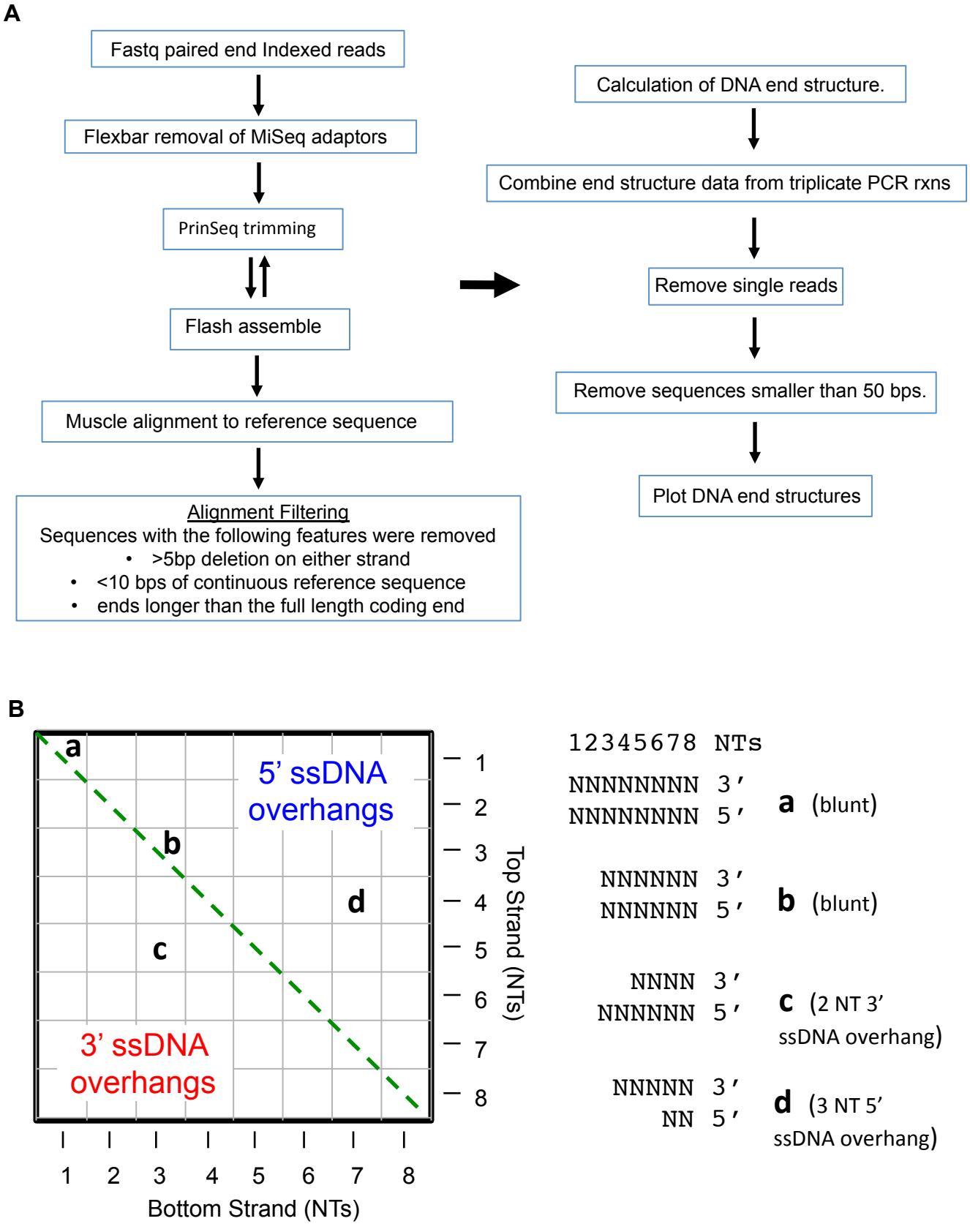
A
Fastq paired end Indexed reads
Flexbar removal of MiSeq adaptors
PrinSeq trimming
Flash assemble
Muscle alignment to reference sequence
Alignment Filtering
Sequences with the following features were removed
• >5bp deletion on either strand
• <10 bps of continuous reference sequence
• ends longer than the full length coding end
Calculation of DNA end structure.
Combine end structure data from triplicate PCR rxns
Remove single reads
Remove sequences smaller than 50 bps.
Plot DNA end structures
B
a
b
c
d
5' ssDNA overhangs
3' ssDNA overhangs
Top Strand (NTs)
Bottom Strand (NTs)
12345678 NTs
NNNNNNNN 3'
NNNNNNNN 5'
a (blunt)
NNNNNN 3'
NNNNNN 5'
b (blunt)
NNNN 3'
NNNNNN 5'
c (2 NT 3' ssDNA overhang)
NNNNN 3'
NN 5'
d (3 NT 5' ssDNA overhang)

Figure S3.

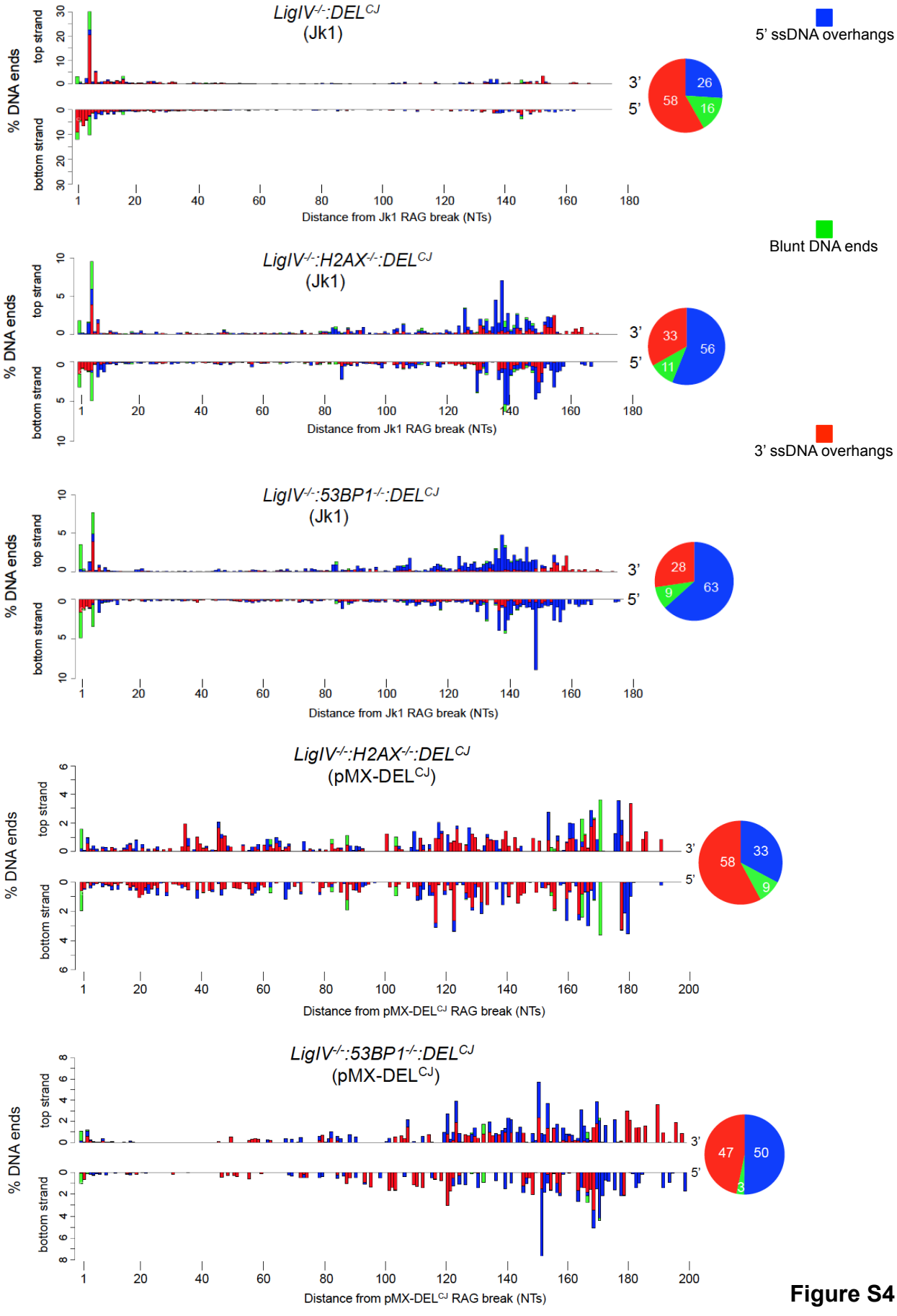
LigIV-/-:DELCJ
(Jk1)
% DNA ends
top strand
bottom strand
3'
5'
26
58
16
Distance from Jk1 RAG break (NTs)
LigIV-/-:H2AX-/-:DELCJ
(Jk1)
33
56
11
LigIV-/-:53BP1-/-:DELCJ
(Jk1)
28
63
9
LigIV-/-:H2AX-/-:DELCJ
(pMX-DELCJ)
58
33
9
Distance from pMX-DELCJ RAG break (NTs)
LigIV-/-:53BP1-/-:DELCJ
(pMX-DELCJ)
47
50
3
5' ssDNA overhangs
Blunt DNA ends
3' ssDNA overhangs

Figure S4

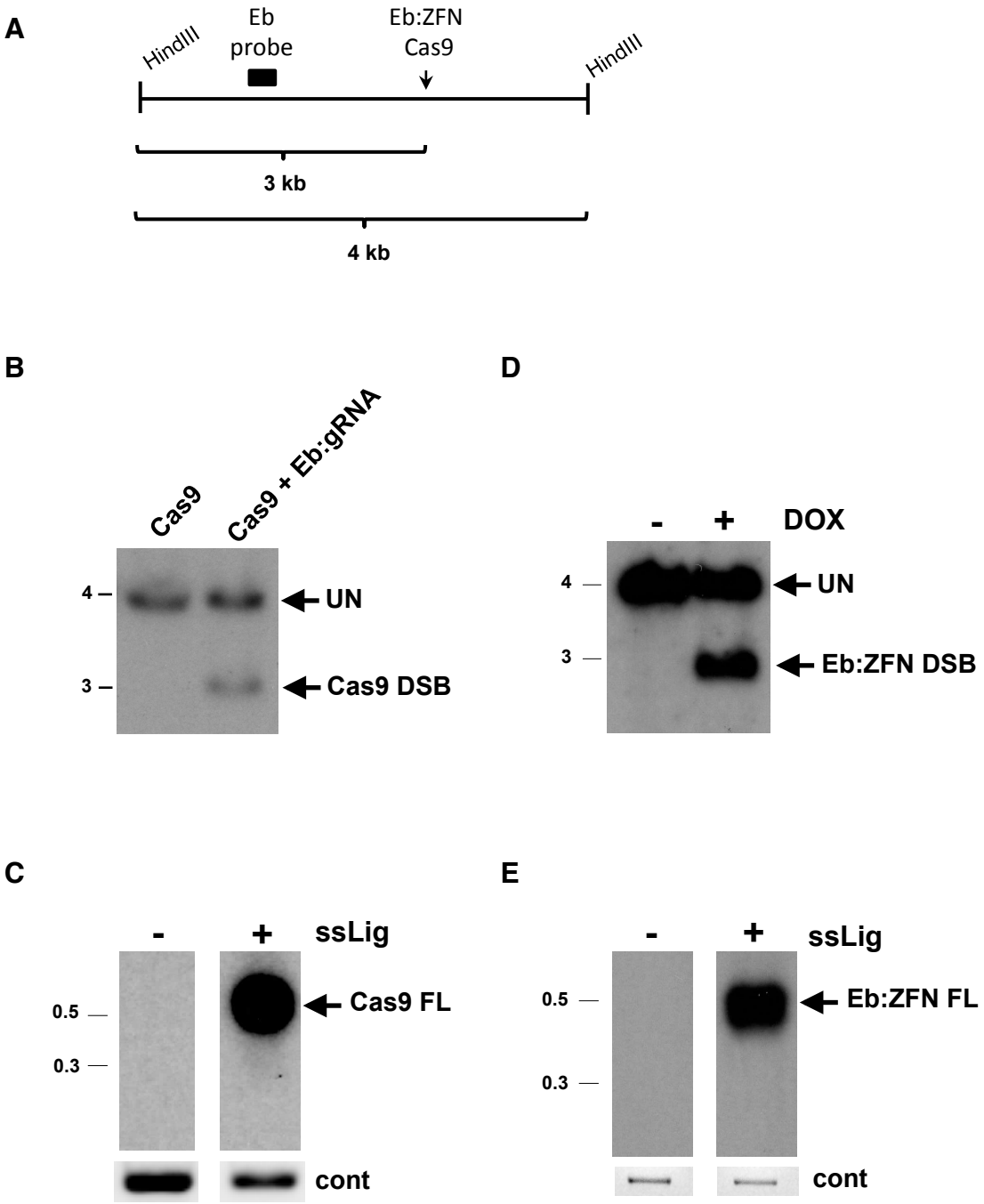
A
HindIII
Eb probe
Eb:ZFN Cas9
HindIII
3 kb
4 kb
B
Cas9
Cas9 + Eb:gRNA
4
3
UN
Cas9 DSB
D
-
+
DOX
4
3
UN
Eb:ZFN DSB
C
-
+
ssLig
0.5
0.3
Cas9 FL
cont
E
-
+
ssLig
0.5
0.3
Eb:ZFN FL
cont

Figure S5.

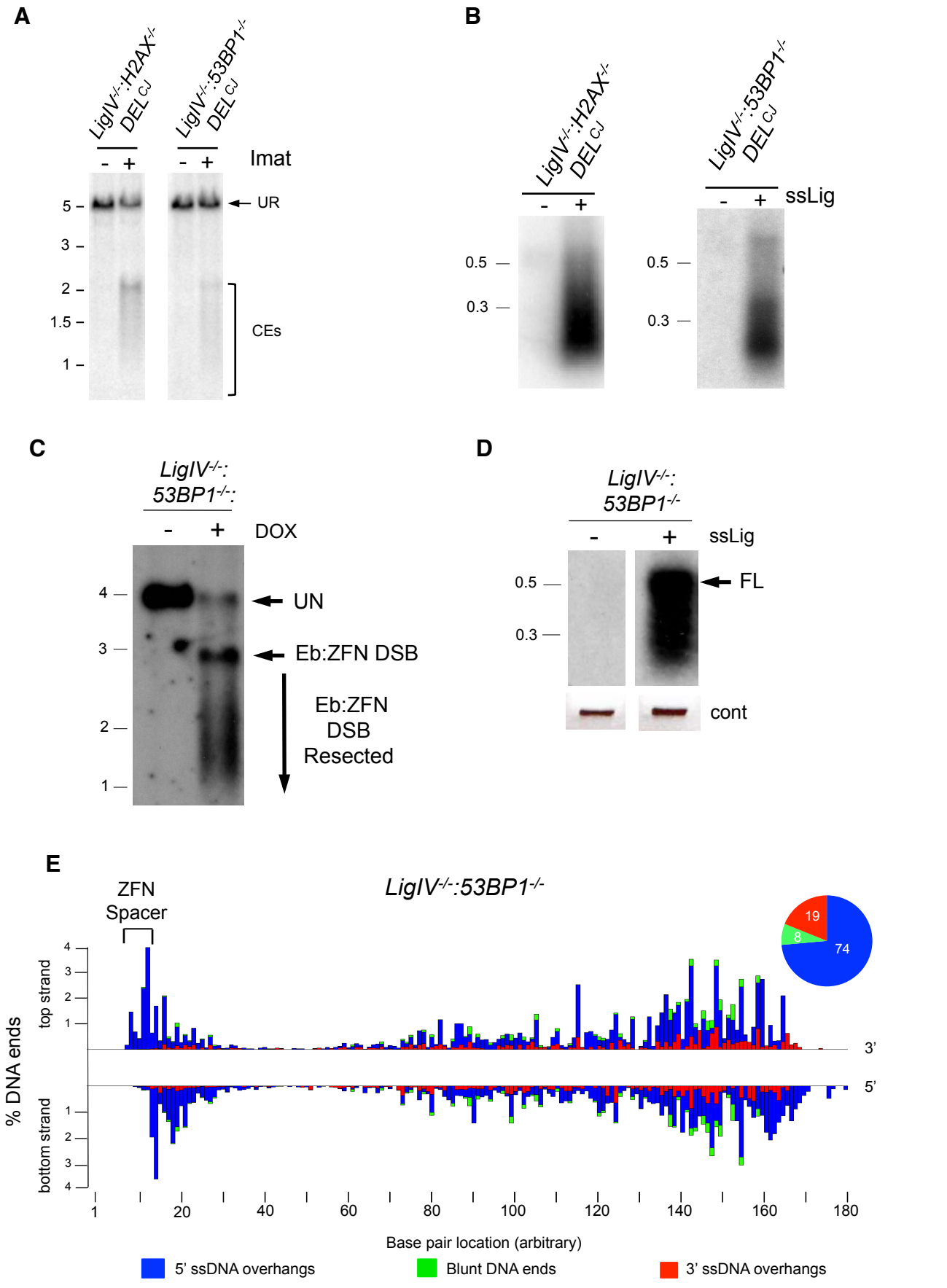
A
LigIV-/-:H2AX-/- DEL CJ
LigIV-/-:53BP1-/- DEL CJ
- + - +
Imat
5
3
2
1.5
1
UR
CEs
B
LigIV-/-:H2AX-/- DEL CJ
LigIV-/-:53BP1-/- DEL CJ
- + - +
ssLig
0.5
0.3
0.5
0.3
C
LigIV-/-: 53BP1-/-:
- +
DOX
4
3
2
1
UN
Eb:ZFN DSB
Eb:ZFN DSB Resected
D
LigIV-/-: 53BP1-/-
- +
ssLig
0.5
0.3
FL
cont
E
ZFN Spacer
LigIV-/-:53BP1-/-
19
8
74
% DNA ends
top strand
bottom strand
4 3 2 1
1 2 3 4
3'
5'
1 20 40 60 80 100 120 140 160 180
Base pair location (arbitrary)
5' ssDNA overhangs
Blunt DNA ends
3' ssDNA overhangs

Figure S6.

Supplementary Figure Legends

Figure S1, related to Figure 1. Analysis of KpnI-digested DNA. (A) Schematic of ligation of KpnI-digested genomic DNA and Southern blotting strategy for panel B. **(B)** Native (N) or denaturing (D) Southern blot analysis of KpnI-digested DNA that was ligated (+) or not (-) with the ssDNA ligase (ssLig), digested with EcoRV and probed with the C4b probe. Molecular weight markers are shown in kilobases. **(C)** Schematic for amplification of a ligated KpnI site in pMX-DEL^{CJ} using the Kpn^{R} and Kpn^{F} oligonucleotides. **(D)** PCR amplification of KpnI site after genomic DNA was treated (+) or not treated (-) with the ssDNA ligase (ssLig). Control (cont) PCR is shown as are MW markers in kilobases (kb). **(E)** Sequence schematic of the ligation, bisulfite treatment and amplification of a KpnI digested DNA end. Nucleotides derived from the top strand (blue), bottom strand (green) and 4 base 3' ssDNA overhang (red) are shown.

Figure S2, related to Figures 1 and 2. Ligation of Igk coding ends. (A) Schematic of the Igk locus with Vk and Jk gene segments. EcoRI and SacI restriction enzyme sites and JkIII probe used for Southern blotting are shown. Fragments generated by RAG cleavage at the four Jk gene segments are shown. The relative position of the Jk1^{R} and Jk1^{F} oligonucleotides used to amplify Jk1 coding ends are also shown. **(B)** Southern blot of EcoRI and SacI digested genomic DNA from *LigIV*$^{-/-}$*:DEL*^{CJ} abl pre-B cells not treated (-) or treated (+) with imatinib (imat). Hybridizing bands from the unrearranged (UR) and Jk coding ends (CE) are indicated. **(C)** Southern blot analysis as described in panel B of

genomic DNA from imatinib-treated *LigIV*$^{-/-}$*:DEL*^{CJ} abl pre-B cells treated (+) or not (-) with ssDNA ligase (ssLig) followed by Exonuclease I and Lambda Exonuclease I (Exo) treatment (+) or no treatment (-). **(D)** Southern blot using oligonucleotide Jk1^{P} as a probe for products of HCoDES PCR amplification of Jk1 coding ends using the Jk1^{R} and Jk1^{F} oligonucleotides for primary PCR and Miseq- Jk1^{R} and Miseq-Jk1^{F} oligonucleotides for secondary PCR. Control PCR for these DNA samples shown in figure 1E. Genomic DNA from *LigIV*$^{-/-}$*:DEL*^{CJ} abl pre-B cells treated (+) or not (-) with ssDNA ligase (ssLig) was analyzed.

Figure S3, related to Figures 2, 3, 4 and 6. Sequence analysis. (A) Schematic depicting the workflow of informatic processing of HCoDES data. **(B)** Heat maps show the frequency of broken DNA ends with specific 5' and 3' termini. The X-axis depicts nucleotide (NT) position on the bottom strand and the Y-axis shows the corresponding nucleotide on the top strand. The green dashed line marks boxes that correspond to blunt DNA ends. The boxes to the left are 3' ssDNA overhangs and those to the right are 5' ssDNA overhangs. Four different DNA ends structures (a-d) are shown as examples.

Figure S4, related to Figure 3. Jk1 and pMX-DEL^{CJ} coding end structures. Barplots of Jk1 coding ends in *LigIV*$^{-/-}$*:DEL*^{CJ} (BLig4CJ7, 305,805 sequences analyzed), *LigIV*$^{-/-}$*:H2AX*$^{-/-}$*:DEL*^{CJ} (LH8CJ148, 157,970 sequences analyzed) and *LigIV*$^{-/-}$*:53BP1*$^{-/-}$*:DEL*^{CJ} (B53BP1Lig4CJ6, 258,051 sequences analyzed) abl pre-B cells as described in legend to Figure 3. Also shown are barplots of pMX-

DEL^{CJ} coding ends in *LigIV$^{-/-}$:H2AX$^{-/-}$:DEL^{CJ}* (LH5CJ48, 76,560 sequences analyzed) and *LigIV$^{-/-}$:53BP1$^{-/-}$:DEL^{CJ}* (C5LCJ5, 416,959 sequences analyzed) as described in Figure 3.

Figure S5, related to Figure 2C. Cas9 and Eb:ZFN induced DSBs. (A) Schematic of the Southern blot strategy for analyzing Cas9 and Eb:ZFN DSBs on chromosome 6. Shown are the HindIII sites and the location of the Eb probe relative to the location of the Cas9 and Eb:ZFN DSBs. **(B)** *LigIV$^{-/-}$* (L4B25-3.41) abl pre-B cells were treated with imatinib followed by transient expression of Cas9 or Cas9 and Eb:gRNA. Genomic DNA was digested with *HindIII* and probed with Eb. **(C)** Southern blot of HCoDES PCR amplification of genomic DNA from *LigIV$^{-/-}$* abl pre-B cells after expression of Cas9 and Eb:gRNA (panel B) using the C6A^{F} and C6A^{R} oligonucleotides for primary PCR, Miseq-C6A^{F} and Miseq-C6A^{R} oligonucleotides for secondary PCR and the C6A^{P} oligonucleotide probe. Genomic DNA was ligated (+) or not ligated (-) with the ssDNA ligase (ssLig) prior to PCR. Control PCR is as described in figure 1E. **(D)** Southern blot of genomic DNA from *LigIV$^{-/-}$* (BLig4ZF11) abl pre-B cells not treated (-) or treated (+) with doxycycline (DOX) for induction of the Eb:ZFN as described in panel A. **(E)** HCoDES PCR of genomic DNA from *LigIV$^{-/-}$* abl pre-B cells after induction of Eb:ZFN as described in panel C.

Figure S6, related to Figure 3. pMX-DEL^{CJ} coding end and Eb:ZFN DNA ends in H2AX- and 53BP1-deficient cells. (A) Southern blot analysis of pMX-

DEL^{CJ} in *LigIV$^{-/-}$:H2AX$^{-/-}$:DEL^{CJ}* (LH5CJ48) and *LigIV$^{-/-}$:53BP1$^{-/-}$:DEL^{CJ}* (B53BP1Lig4CJ6) abl pre-B cells as described in Figure 1C. Bands due to unrearranged pMX-DEL^{CJ} (UR) and resected pMX-DEL^{CJ} coding ends are shown. **(B)** Southern blot of HCoDES amplification of pMX-DEL^{CJ} coding ends from panel A as described in Figure 1E. **(C)** Southern blot of genomic DNA from *LigIV$^{-/-}$:53BP1$^{-/-}$* (CL5ZFN12) abl pre-B cells after induction (+) or no induction (-) of Eb:ZFN with doxycycline as described in figure S7D. **(D)** HCoDES PCR of genomic DNA from *LigIV$^{-/-}$:53BP1$^{-/-}$* abl pre-B cells after induction of Eb:ZFN as described in figure S7E. **(E)** DNA end structures generated by DNA cleavage by Eb:ZFN in *LigIV$^{-/-}$:53BP1$^{-/-}$:DEL^{CJ}* abl pre-B cells as described in figure 3. Nucleotide numbering on the X axis is arbitrary. The position of the spacer region where the Eb:ZFN introduces DSBs is indicated.

Table S1. Oligonucleotides used for HCoDES. Bold text denotes complementary sequences downstream of the DSB. Red text are example index sequences. Non-bold text are MiSeq adaptor sequences. Numbers in parenthesis are the distances from the 3' end of the oligonucleotide to the non-resected DNA end.

pMX-DEL^{CJ} coding ends	
Primary PCR	
DCJ1^{R} (-214)	**CTTCAAAAAACTTCCAAAAAAACTACT**
DCJ1^{F} (-222)	**TTGTTTGTTTTTAAGAAGTTTTTAGAG**
Secondary PCR	
MiSeq-DCJ1^{R}	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**AAAACTTCCAAAAA AACTACTTCCTTCA**
MiSeq-DCJ1^{F}	CAAGCAGAAGACGGCATACGAGATATGATGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**GAAGTTT TTAGAGGAATTGTTTTTTTTA**
Probe	
DCJ1^{P} (-177)	**ACATTCAACAAACCTTACATTCCTTTA**
Jk1 coding ends	
Primary PCR	
Jk1^{R} (-187)	**TAACATTCATTCTCCAAAAAACATATCTAACC**
Jk1^{F} (-187)	**TTGGTATTTATTTTTTAGAGAATATGTTTAGTT**
Secondary PCR	
MiSeq-Jk1^{R}	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**ACATTCATTCTCCAA AAAACATATCTAACCTATTC**
MiSeq-Jk1^{F}	CAAGCAGAAGACGGCATACGAGATAACCTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**GGTATTT ATTTTTTAGAGAATATGTTTAGTTTAT**
Probe	
Jk1^{P} (-140)	**GAGTTATTTTGTATTTTTGTTTTGGAG**
***de novo* pMX-DEL^{CJ} DSBs**	
Primary PCR	
DCJ2^{F} (-110)	**ATATATAATTTTCCACCATATTACCATCTTTTAAC**
DCJ2^{R} (-121)	**TTTATTATATTGTCGTTTTTTGGTAATGTGAGGGT**
Secondary PCR	
MiSeq-DCJ2^{F}	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**ATATTACCATCTTTT AACAATATAAAAACC**
MiSeq-DCJ2^{R}	CAAGCAGAAGACGGCATACGAGATGGACTCCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**TTGTTGT TTTTTGGTAATGTGAGGGTTTGG**
Probe	
DCJ2^{P} (-154)	**ATGTTCGTTAAGAAGATAGGGTTAGGTTTT**

Table S1 continued.

KpnI digestion	
Primary PCR	
KpnI^{R} (-123)	**CCTTACCAAACATAATTATAACCATATTATC**
Kpn^{F} (-127)	**GTTTTGTTGAGTATGGTTGTGGTTATATTATT**
Secondary PCR	
MiSeq-KpnI^{R}	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**CTTACCAAACATAATTA TAACCATATTATCAT**
MiSeq-KpnI^{F}	CAAGCAGAAGACGGCATACGAGATTCAGTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**TTTGTTGAG TATGGTTGTGGTTATATTATTAT**
Control PCR	
Primary PCR	
C^{F}	**GATTAGTAAATAGTTTTAAGTTTTTTA**
C^{R}	**AACCACCGTTTTAAAACCAACTAAAAC**
Probe	
C^{P}	**TTGTATTTTATTGTGATAATTATTTTTT**
Cas9 and Eb:ZFN DSBs	
Primary PCR	
C6A^{F} (-182)	**ATAGTTTTGATTTTATTAGGGTTTTTATGG**
C6A^{R} (-186)	**AATAACAACTCTAACCTCATTAAAATTCCC**
Secondary PCR	
MiSeq-C6A^{R}	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT**CAACTCTAACCTCATTA AAATTCCCATAAC**
MiSeq-C6A^{FZ}	CAAGCAGAAGACGGCATACGAGATAACCTCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**TTGATTTTAT TAGGGTTTTTATGGTTTTTA**
MiSeq-C6A^{FC}	CAAGCAGAAGACGGCATACGAGATCTTGTTAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT**GTTTTGATTT TATTAGGGTTTTTATGGTTT**
Probe	
C6A^{P_CG}	**TCCCACATCACCGCTCTTCCCACTTCTCTT**
C6A^{P_TG}	**TCCCACATCACCACTCTTCCCACTTCTCTT**

Table S2. Primers used for RPA ChIP. Numbers in position columns indicate distance from coding end. F: Forward; R: Reverse; NA: Not applicable.

Location	Position (base pairs)	Position indicated in Figure 7 (Kilobases)	Orientation	Sequence
c-myc	upstream_A	c-myc	F	TGCCAGGTTTCAGGAAGTTGG
c-myc	upstream_A	c-myc	R	GAGGAACATCTTTTGGGTGTATGC
c-myc	upstream_B	NA	F	TTCCTTTGACGCACATTGGAG
c-myc	upstream_B	NA	R	TGTCCCCAGTTACTTTCTTCCTCAG
pMX-DEL^{CJ}	92	0.1	F	CCGTCTTTTGGCAATGTGAGG
pMX-DEL^{CJ}	92	0.1	R	CCTTTGGCGAGAGGGGAAAG
pMX-DEL^{CJ}	183	0.2	F	AAGGTCTGTTGAATGTCGTGAAGG
pMX-DEL^{CJ}	183	0.2	R	TGCCTGCAAAGGGTCGCTAC
pMX-DEL^{CJ}	350	0.3	F	CAGTGCCACGTTGTGAGTTGG
pMX-DEL^{CJ}	350	0.3	R	CTTCAGCCCCTTGTTGAATACG
pMX-DEL^{CJ}	448	0.4	F	TTGTATGGGATCTGATCTGGGG
pMX-DEL^{CJ}	448	0.4	R	GCCTAGACGTTTTTTTAACCTCGAC
pMX-DEL^{CJ}	612	0.6	F	GGAGTCCCTTTTAGGCACTTGC
pMX-DEL^{CJ}	612	0.6	R	CAGCACCACTTTCTTTCCCTGAG
pMX-DEL^{CJ}	784	0.8	F	TTCTGGGAAATCAGGGCTCC
pMX-DEL^{CJ}	784	0.8	R	AAAGGCTTCTTCTTGAGTCAGCG
pMX-DEL^{CJ}	927	0.9	F	TGTGAAGTGGAGGACCAGAAGG
pMX-DEL^{CJ}	927	0.9	R	AGCAGGTGGGTGTCAGAGTTGG
pMX-DEL^{CJ}	1012	1	F	AGAGCCTGACCCTGACCTTGGAGAG
pMX-DEL^{CJ}	1012	1	R	CCCTGTATGTTTTTACCCCTTGGAC
pMX-DEL^{CJ}	1178	1.2	F	GGTGGAGTTCAAAATAGACATCGTG
pMX-DEL^{CJ}	1178	1.2	R	CACCTGTTCCCCCTCTTTCTTATAG
pMX-DEL^{CJ}	1374	1.4	F	AAGTCTTGGATCACCTTTGACCTG
pMX-DEL^{CJ}	1374	1.4	R	CATCTGGAGCTTAGGGTCCTGG
pMX-DEL^{CJ}	1559	1.6	F	GGAAGTGAACCTGGTGGTGATG
pMX-DEL^{CJ}	1559	1.6	R	ATCAGCTTAGGGGAGGTGGGTC

Supplemental Methods:

Cell culture: Abl pre-B cells were cultured as previously described (Bredemeyer et al., 2006). Culture of these cells with imatinib and the ATM kinase inhibitor, KU55933 was carried out as previously described (Bredemeyer et al., 2006).

Southern blotting: Native and denaturing genomic Southern blot analyses for RAG cleavage at pMX-DEL^{CJ} and the Igk locus using the C4b and JkIII probes, respectively, were carried out as previously described (Bredemeyer et al., 2006; Helmink et al., 2011). The Eb probe has been previously described (Tubbs et al., 2014). Southern blotting of PCR reactions was carried out as previously described (Bredemeyer et al., 2006). The DCJ1^{P} oligonucleotide was used to probe products amplified with oligonucleotides MiSeq-DCJ1^{F} and MiSeq-DCJ1^{R}; the DCJ2^{P} oligonucleotide was used to probe products amplified with oligonucleotides MiSeq-DCJ2^{F} and MiSeq-DCJ2^{R}; the Jk1^{P} oligonucleotide was used to probe products amplified with oligonucleotides MiSeq-Jk1^{F} and MiSeq-Jk1^{R}; the C^{P} oligonucleotide was used to probe products amplified with primers C^{F} and C^{R}. The C6A^{P_CG} and C6A^{P_TG} oligonucleotides were mixed in a 1:1 ratio and used to probe products amplified with MiSeq-C6A^{F} and MiSeq-C6A^{R}. See Table S1 for oligonucleotide sequences.

Exonuclease I and Lambda Exonuclease treatment of genomic DNA: 20 µg of ligated and non-ligated genomic DNA was treated with 30 units Exonuclease I (NEB) and 7.5 units Lambda exonuclease (NEB) in a 200 µL volume using Exonuclease I reaction buffer (NEB) at 37° for 1 hour. The reactions were then placed at 80ºC for 20 minutes to heat inactivate the exonucleases. DNA was precipitated with 2 volumes ETOH and 1/10th volume 3M NaAc and then re-suspended in TE. Re-suspended DNA was digested with EcoRI (NEB) and SacI (NEB) and run out for genomic Southerns as previously described (Helmink et al., 2011).

Chromatin preparation: Cells were fixed for 10 min at room temperature with 1% formaldehyde at a cell concentration of 2 x 10^6 cells per ml of growth medium. Fixation was stopped by addition of glycine to a final concentration of 0.125 M. Fixed cells were collected by centrifugation at 1000 X g for 5 min at 4ºC, washed twice with cold phosphate-buffered saline (PBS), and the cell pellet was frozen on dry ice and stored at -80ºC. To isolate chromatin, 20 x 10^6 fixed cells were suspended in 300 µL of sonication buffer [10 mM Tris·HCl pH 7.6, 0.4% sodium dodecyl sulfate (SDS)] containing freshly-added protease inhibitors (Roche cOmplete, EDTA-free) and incubated on ice for 20 min. The cell suspension was sonicated for 16 to 18 cycles (30 sec on, 30 sec off) at 4ºC to shear the chromatin to an average DNA length of approximately 500 bp (determined by gel electrophoresis after reversing the crosslinks). A total of 900 µL of adjustment buffer [10 mM Tris.HCl pH 7.6, 1.33 mM EDTA pH 8.0, 0.133% sodium

deoxycholate, 1.33% Triton X-100] was added per 300 µL of sonicated cells and the mixture incubated on ice for 20 min. The chromatin was clarified by centrifugation at 14,000 rpm in an Eppendorf centrifuge for 10 min at 4ºC. Glycerol was added to the supernatant to a final concentration of 5% and the chromatin was snap-frozen and stored in aliquots at -80ºC.

RPA Chromatin Immunoprecipitation: To perform ChIP, 600 µL of chromatin (10 X 10^6 cells) was precleared by incubating with 40 µL of protein G dynabeads (Life Technologies, prewashed with PBS) for 1 hour at 4ºC. After bead removal, the precleared chromatin was incubated with protein G dynabeads bound to anti-RPA mouse monoclonal antibody (Millipore NA19L). These anti-RPA beads were prepared prior to use by incubating 40 µL of protein G dynabeads [prewashed in PBS containing 0.5% bovine serum albumin (BSA)] with 10 mg of antibody in 60 µL of PBS at room temperature for 90 min, and then washing the beads twice with PBS without BSA. The chromatin was incubated with anti-RPA beads overnight at 4ºC with gentle rotation. The beads were washed at 4ºC for 10 min per wash, twice with RIPA (10 mM Tris·HCl pH 7.6, 1 mM EDTA pH 8.0, 0.1% sodium deoxycholate, 1% Triton X-100), twice with RIPA containing 0.3 M NaCl, twice with LiCl buffer (0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate), once with TET (10 mM Tris·HCl pH 7.6, 1 mM EDTA pH 8.0, 0.2% Triton X-100), and twice with TE (10 mM Tris·HCl pH 7.6, 1 mM EDTA pH 8.0). To reverse the crosslinks, the washed beads were suspended in 150 µL of TE, and incubated at 65ºC for 5 hours with gentle mixing after addition of 5 µL of 10% SDS and 7 µL of

proteinase K (20 mg per mL). The supernatant containing eluted DNA was collected, the beads washed once with 150 µL of TE containing 0.5 M NaCl, and the washed supernatant pooled with the original supernatant. The DNA was extracted with phenol-chloroform, precipitated with a few micrograms of glycogen and two volumes of ethanol. Precipitated DNA was dissolved in 30 µL of 3 mM Tris·HCl pH 8.0. Enrichment of specific sequences in the eluted DNA was determined by quantitative real-time PCR using iTAQ Universal SYBR Green. Enrichment of RPA at pMX-DEL^{CJ} was quantified by calculating the fold enrichment of RPA at this locus relative to a region upstream of c-myc (upstream_B primers, Table S2), as previously described (Fay et al., 2011; Pfaffl, 2001). c-myc upstream location A (upstream_A primers, Table S2) served as a negative control for RPA binding. 0.25 µL of the eluted ChIP was used per real time PCR reaction. Real time PCR cycling parameters were 95ºC 30 sec and then 30 cycles of 95ºC 5 sec followed by 57ºC 10 sec. Sequences of all oligonucleotides used for ChIP analyses can be found in Table S2.

Construction of Eb:gRNA expressing plasmid: The Eb:gRNA expression vector was generated using the cloning protocol described by Addgene for the gRNA Empty Vector (Catalogue # 41824). Briefly, the forward oligonucleotide 5'-TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCGAAAGCCAGCCAATGAATGC-3' and reverse oligonucleotide 5'-GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAACGCATTCATTGGCTGGCTTTC-3', were designed for the target site sequence 5'-

GAAAGCCAGCCAATGAATGC-3' on mouse chromosome 6. The oligos were annealed and amplified with NEB Phusion Polymerase (NEB) to generate the Eb:gRNA cassette. The gRNA Empty Vector was linearized by AflII and assembled with the Eb:gRNA cassette using the NEB Gibson Assembly Master Mix (NEB) to generate the Eb:gRNA expressing plasmid.

Induction of Cas9 and Eb:ZFN DSBs: Eb:ZFN DSBs were generated as previously described (Tubbs et al., 2014). Cas9 DSBs were generated by nucleofection of Cas9 and Eb:gRNA expressing plasmids. Briefly, abl pre-B cells that had been treated with imatinib for 1 day were nucleofected using the Amaxa Human B Cell Nucleofector Kit (Lonza) and the Amaxa Nucleofector II (Lonza) following the manufacturer's instructions. Abl pre-B cells were transfected with 750 ng of Topo-Cas9-HA plasmid alone or 750 ng of Topo-Cas9-HA plasmid and 750 ng of Eb:gRNA plasmid. Cells were harvested 1 day after nucleofection and viable cells were isolated using Lympolyte-M (Cedarlane Labs) according to the manufacturer's instructions.

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