Supplemental Information

Supplemental Figure Summary

Figure S1, related to Figure 1. Intrachromosomal repair of a single or two DSBs in LIG4-XRCC4 deficient HCT116 human cells
A. Indel formation: T7 assay at the p84 locus after a ZFN^{p84} DSB.
B. AseI-resistant junction sequences after a ZFN^{EWS} DSB on Chr22.
C. Junction sequences at a 3.2 kb deletion on Chr11 after ZFN^{FLI-A} and ZFN^{FLI-B} DSBs.

Figure S2, related to Figure 2. Translocation junction sequences from ZFN^{EWS} and ZFN^{p84} DSBs derived from wild-type and XRCC4 deficient HCT116 cells.

Figure S3, related to Figure 3. Translocations induced by ZFN^{EWS} and ZFN^{p84} DSBs in pre-B cells and patient-derived cells with LIG4 mutations.
A. Indel formation: T7 assay at the p84 locus after a ZFN^{p84} DSB.
B. Translocation junction sequences from pre-B cells.
C. Translocation junction sequences from patient-derived cells.

Figure S4, related to Figure 4. DSBs induced by TALENs, wild-type Cas9, and paired nCas.
A. Relative positions of nCas9 cleavage sites.
B. Indel formation at the ALK locus after a TAL^{ALK}, Cas9(ALK1) or nCas9(ALK1+ALK2) DSB.

Figure S5, related to Figure 5. NPM-ALK cancer translocation junctions induced by TALENs, wild-type Cas9, and paired nCas.

A. Nuclease expression is quantified after 48 h by Western blotting for the HA epitope for TALENs or GFP expression for Cas9 and nCas9.
B. Translocation junction sequences from TALENs.
C. Translocation junction sequences from wild-type Cas9.
D. Translocation junction sequences from paired nCas9.

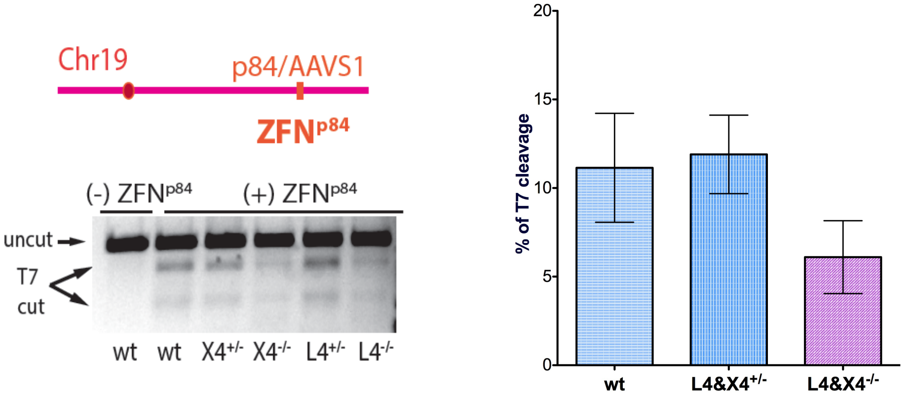
Figure S6, related to Figure 6. LIG3 is not required for translocation formation in human cells.
A. LIG3 null cells: Western blot, PCR genotyping, and ZFN expression, T7 assay at p84 and ALK loci.
B. Translocation junction sequences from ZFNs.
C. Translocation junction sequences from Cas9
D. Translocation junction sequences from paired nCas9.

Figure S7, related to Figure 7. Translocation junction sequences from ZFN^{EWS} and ZFN^{p84} expression after CtIP depletion.
A. Western blot of CtIP knock-down and T7 assay at p84 locus.
B. Translocation junction sequences from ZFNs in wild-type cells.
C. Translocation junction sequences from ZFNs in XRCC4-deficient cells.

Supplemental Figures

Figure S1, related to Figure 1. Intrachromosomal repair of single or two DSBs in LIG4/XRCC4-deficient human HCT116 cells.

A. Indel formation: Indel formation is reduced in both L4$^{-/-}$ and X4$^{-/-}$ cells at the ZFN^{p84} cleavage site, as monitored by the T7-endonuclease assay {n = 7 for wt, n = 10 for het (+/-) and mutant (-/-)}.



B. AseI-resistant junction sequences after a ZFN^{EWS} DSB on Chr22. Duplicate junction sequences were counted twice only if they arose in independent experiments, although results were similar if all sequences were counted. The ZFN recognition sequences at each DNA end are underlined; bps in italics represent the overhangs. Microhomologies (underlined), insertions (green) and lengths of deletions from each end are indicated.

HCT116 WT

Blue : AseI site

DNA ends:

AATAGCT**GCCTCCCCACTTTACATT***AAT* *GAC***TGATAGGGAGGCCAAA**AACGATGTT

29

AATAGCT**GCCTCCCCACTTTACATT**A-*1* **T** *TGAC**TGATAGGGAGGCCAAA**AACGATGTT*

42

AATAGCT**GCCTCCCCACTTTACATT**A-*1* *TGAC***TGATAGGGAGGCCAAA**AACGATGTT

11

AATAGCT**GCCTCCCCACTTTACATT**A*A*-*1* -*2*C**TGATAGGGAGGCCAAA**AACGATGTT

35

AATAGCT**GCCTCCCCACTTTACATT**A-*2* -1*A*C**TGATAGGGAGGCCAAA**AACGATGTT

22

AATAGCT**GCCTCCCCACTTTACA...**-5 *GA*C**TGATAGGGAGGCCAAA**AACGATGTT

9

AATAGCT**GCCTCCCCACTTTACA...**-5 -2C**TGATGGGGAGGCCAAA**AACGATGTT

3

AATAGCT**GCCTCCCCACTTTACATT.**-3 **G** -4**..GATAGGGAGGCCAAA**AACGATGTT

54

AATAGCT**GCCTCCCCACTTTACATT.**-3 **TT** -5**...ATAGGGAGGCCAAA**AACGATGTT

16

AATAGCT**GCCTCCCCAC.........**-9 *ATGA*C**TGATAGGGAGGCCAAA**AACGATGTT

36

AATAGCT**GCCTCCCCACTT.......**-9 **AT** *GA*C**TGATAGGGAGGCCAAA**AACGATGTT

53

AATAGCT**GCCTCCCCACTTTAC....**-6 -3**.TGATGGGGAGGCCAAA**AACGATGTT

26

AATAGCT**GCCTCCCCACT.......**-10 -3**.TGATAGGGAGGCCAAA**AACGATGTT

45

AATAGCT**GCCTCCCCACTTTA**.....-5		-8......**GGGAGGCCAAA**AACGATGTT
4		
AATAGCT**GCCTCCCCACT**.......-10		-4..**GATAGGGAGGCCAAA**AACGATGTT
52		
AATAGCT**GCCTCCCCA**.........-10	**A**	-13..........**GCCAAA**AACGATGTT
5		
AATAGCT**GCCTCCCCAC**........-11		-13..........**GCCAAA**AACGATGTT
6		
AATAGCT**GCC**...............-18		-16.............**AAA**AACGATGTT
47		
AATAGCT**GCCTCCCCA**.........-12		-15..............**AA**AACGATGTT
19		
AATAGCT**GCCTCCCCA**.........-12		-17..............**AA**AACGATGTT
41		
AATAGCT**GCCTCCC**...........-14		-16.............**AAA**AACGATGTT
46		
AATAGCT**GCCTCCC**...........-14		-16.............**AAA**AACGATGTT
25		
CTG......................-29		-5...**ATAGGGAGGCCAAA**AACGATGTT
43		
AATAGCT**GC**................-19		-16.............**AAA**AACGATGTT
48		
AATAG....................-24		-23....................ATGTT
39		
.........................-29	**+11**	-20.........................
GATAGCTGGC		
40		
.........................-49		*TGAC*T**GATAGGGAGGCCAAA**AACGATGTT
10		
AATAGCT**GCCTCCCCA**.........-12	**TG**	-44........................
37		
TG.......................-29		-30........................
1		
AATAGCT**GCCTCCCCA**.........-12		-59........................
15		
GC.......................-31		-42........................
49		
AATAGC...................-22		-72.......................
18		
CT.......................-30		-79........................
17		
AATAGCT**GCCTCC**............-15		-133.......................
34		
AATAGCT**GCCTCCCCACT**.......-10		-190.......................
24		
A........................-27		-208.......................
27		
AATAGCT**GC**................-19		-235.......................
51		
........................-251		-13...........**CAAA**AACGATGTT
50		
........................-393		-12..........**CCAAA**AACGATGTT
44		
........................-401		-11.........**GCCAAA**AACGATGTT

HCT116 X4+/-

AATAGCT**GCCTCCCCACTTTACATT***AAT*	*GAC***TGATAGGGAGGCCAAA**AACGATGTT

36	
AATAGCT**GCCTCCCCACTTTACATT**A-2	GAC**TGATAGGGAGGCCAAA**AACGATGTT
27	
AATAGCT**GCCTCCCCACTTTACATT**A*A-1*	-2C**TGATAGGGAGGCCAAA**AACGATGTT

32	AATAGCT**GCCTCCCCACTTTACAT**T.-3		*GA*C**TGATAGGGAGGCCAAA**AACGATGTT
11	AATAGCT**GCCTCCCCACTTTACATT**A-2		-2C**TGATAGGGAGGCCAAA**AACGATGTT
12	AATAGCT**GCCTCCCCACTTTACAT**..-4		*GA*C**TGATAGGGAGGCCAAA**AACGATGTT
3	AATAGCT**GCCTCCCCACTTT**......-8		*GA*C**TGATAGGGAGGCCAAA**AACGATGTT
39	AATAGCT**GCCTCCCCACTTT**......-8	**TAT**	*GA*C**TGATAGGGAGGCCAAA**AACGATGTT
25	AATAGCT**GCCTCCCCACT**.......-10		-4..**GATAGGGAGGCCAAA**AACGATGTT
17	AATAGCT**GCCTCCCCACTTTACA**...-5		-13..........**GCCAAA**AACGATGTT
24	AATAGCT**G**.................-20		*ATGA*C**TGATAGGGAGGCCAAA**AACGATGTT
21	AATAGCT**GCCTCCCC**..........-13		-18...............**A**AACGATGTT
33	AATAG....................-23		-9.......**GGAGGCCAAA**AACGATGTT
8	AATAGCT**GCCTCCCCACTTTACA**...-5		-18...............**A**AACGATGTT
38	AATAGCT**GCCTCC**............-15		-18...............**A**AACGATGTT
29	AATAG....................-23		-11........**AGGCCAAA**AACGATGTT
22	AATAGC...................-22		-15............**CAAA**AACGATGTT
23	..........................-34	**TTTTTGAT**	-23....................ATGTT
15	CA.......................-39		-75.........................
7	..........................-49	**CA**	-77.........................
40	C.......................-100		-73.........................
2	AATAGCT**GCCTCCCCACTTTACA**...-5		-226........................
10	AATAGCT**GCCTCC**............-15		-225........................
19	AATAGCT**GCCTCCCCAC**........-11		-229........................
31	CT......................-250		-35.........................
34	GA......................-284		-22.........................
30	CA.......................-90		-335........................

HCT116 L4+/-

	AATAGCT**GCCTCCCCACTTTAC****ATT**A*AT*	*GA*C**TGATAGGGAGGCCAAA**AACGATGTT
5	AATAGCT**GCCTCCCCACTTTACATT**A*A-1*	-2C**TGATAGGGAGGCCAAA**AACGATGTT
19	AATAGCT**GCCTCCCCACTTTACATT**A-2	-2C**TGATAGGGAGGCCAAA**AACGATGTT
13	AATAGCT**GCCTCCCCACTTT**......-8	*GA*C**TGATAGGGAGGCCAAA**AACGATGTT
24	AATAGCT**GCCTCCCCACTTT**......-*8*	*-4..*G**ATAGGGAGGCCAAA**AACGATGTT
3	AATAGCT**GCCTCCCCACTTT**......-8	*-5...***ATAGGGAGGCCAAA**AACGATGTT

18	AATAGCT**GCCTCCCCACTTTACATTA**-2		-12.........**GGCCAAA**AACGATGTT
34	AATAGCT**GCCTCCCCACTTTACATT.**-3		-12.........**GGCCAAA**AACGATGTT
11	AATAGCT**G**.................-20		*ATGAC***TGATAGGGAGGCCAAA**AACGATGTT
9	AATAGCT**GCCTCCCCA**.........-12		-18...............**A**AACGATGTT
1	AATAGCT**GCC**...............-18		-16.............**AAA**AACGATGTT
32	AATAGCT**GCC**...............-18		-16............C**AAA**AACGATGTT
10	AATAGCT**GCC**...............-18		-25......................GTT
12	AA.......................-26	**CATC**	-18...............**A**AACGATGTT
36	A........................-25	**T**	-20.................ACGATGTT
29	AA.......................-26		-21..................CGATGTT
31	.........................-30		-18...............**A**AACGATGTT
27	AATAGC...................-22	**CAGCTA**	-30.........................
17	CA.......................-38		-18...............**A**AACGATGTT
21	CTGCA....................-27		-39.........................
23	CAA......................-26		-18...............**A**AACGATGTT
33	AATA.....................-24		-44.........................
16	AA.......................-26		-20.................ACGATGTT
22	TTTACT...................-61		-31.........................
28	TGG......................-33		-144........................
30	AATAGCT**GCCTCC**............-15		-215........................
35	........................-370		-22...................GATGTT
25	TG......................-411		-24.....................TGTT
20	.........................-35	**+33**	-18...............**A**AACGATGTT

33bp : 11bp from Homo sapiens chr4, alternate assembly CHM1_1.1 and 22 bp from EWS inverted duplication downstream DSB
AAAATAGCTGCCTATTGCAGGCCACTATGATTT

HCT116 X4-/-

	AATAGCT**GCCTCCCCACTTTACATT***AAT*	*GAC***TGATAGGGAGGCCAAA**AACGATGTT
34	AATAGCT**GCCTC**.............-16	-18...............**A**AACGATGTT
17	AAT......................-25	-25......................GTT
26	AAT......................-25	-32.........................
7	AATAGCT..................-21	-37.........................
20		

AATA......................-24 -46.........................
4
..........................-37 **+11** -36.........................

11bp: TGCATACAAAA

1
CAA.......................-26 -49.........................
28
AG........................-36 -60.........................
29
CA........................-65 -39.........................
24
CATA......................-62 -46.........................
10
.........................-138 -20...............ACGATGTT
2
AA.......................-175 -20...............ACGATGTT
8
CC........................-30 -178.......................
6
..........................-198 **+27** -18..............**A**AACGATGTT

27pb : Duplication from chr22 419bp upstream DSB
CTGAGCTCCATAAATCAACACTACATC From Homo sapiens chromosome 22, GRCh37.p13

3
..........................-53 **A** -173.......................
33
ATGA.....................-180 -25.......................
9
AATAGCT**GCCTCCCCA**.........-12 -226.......................
36
.........................-403 -55.........................
35
TAAAAA...................-370 -136.......................
30
ATC......................-180 -363.......................
32
ATGTGT...................-334 -214.......................

HCT116 L4-/-

AATAGCT**GCCTCCCCACTTTACATT***AAT* *GAC***TGATAGGGAGGCCAAA**AACGATGTT

1
AATAGCT**GCCTCCCCACTTTACA**...-5 -16.............**AAA**AACGATGTT
4
AATAGCT**G**..................-20 -5...**ATAGGGAGGCCAAA**AACGATGTT
13
AATAGCT**G**..................-20 -10......**GAGGCCAAA**AACGATGTT
2
AATAGCT**GCC**................-18 -16.............**AAA**AACGATGTT
42
AATAGCT**GC**.................-19 -16.............**AAA**AACGATGTT
18
AAT.......................-25 **TC** -19................AACGATGTT
3
AAT.......................-25 -25......................GTT
20
AATAGCT**GC**.................-19 -43.........................
7
AAAA......................-42 -21..................CGATGTT
34
CA........................-42 -45.........................
22
CA........................-74 -41.........................

37	AC.......................-66		-38.........................
39	TGG......................-33		-278........................
41	........................-322	**CC**	-32.........................
8	........................-354		-10.............**AAA**AACGATGTT
36	AATAGCT**GCCTCCCCACTTT**......-8		-357........................
35	........................-411		-24.....................TGTT
44	TT......................-407		-109........................
43	........................-272	**ATT**	-278........................
40	TCT.....................-393		-162........................

C. Junction sequences at a 3.2 kb deletion on Chr11 after ZFN$^{\text{FLI-A}}$ and ZFN$^{\text{FLI-B}}$ DSBs.

Chr11

HCT116 WT

	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT(GC	GT)CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
15	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*G*.	.*T*CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
2	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT..	..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
5	GGAACC**CTAAGC**C**CTTTCCTTC**ATT-1.	..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
7	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*GC*	.-*1*CC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
4	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*GC*	..-*2*C**CGATGAAAAGCAGGTTA**GGTTGAGGGA
1	GGAACC**CTAAGC**C**CTTTCCTTC**AT-2..	..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
59	GGAACC**CTAAGC**C**CTTTCCTTC**ATT-1.	.-*1*CC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
28	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT..	..-3.CG**ATGAAAAGCAGGTTA**GGTTGAGGGA
14	GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*GC*	..-5...**ATGAAAAGCAGGTTA**GGTTGAGGGA
31	GGAACC**CTAAGC**C**CTTTCCTTC**..-4..	.-1CC**CGATGAAAAGCAGGTTA**GGTTGAGGGA
34	GGAACC**CTAAGC**C**CTTTCCTTC**AT-2..	..-5...**ATGAAAAGCAGGTTA**GGTTGAGGGA
17	GGAACC**CTAAGC**C**CTTTCC**.....-7..	..-2C**CGATGAAAAGCAGGTTA**GGTTGAGGGA
32	GGAACC**CTAAGC**C**CTTTCCTTC**ATT-1.	..-12.........**CAGGTTA**GGTTGAGGGA
8	GGAACC**C**................-19..	..-3.CG**ATGAAAAGCAGGTTA**GGTTGAGGGA

HCT116 X4+/-

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT(GC	GT)CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

5

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*G*.   .*T*CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

16

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT..   .TCCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

4

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT..   ..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

8

GGAACC**CTAAGC**C**CTTTCCTTC**ATT-1.   ..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

15

GGAACC**CTAAGC**C**CTTTCCTTC**AT-2..   ..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

21

GGAACC**CTAAGC**C**CTTTCCTTC**ATT-1.   .-*1*CC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

18

GGAACC**CTAAGC**C**CTTTCCTTC**A.-3..   ..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

19

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*G*.   ..—5...**ATGAAAAGCAGGTTA**GGTTGAGGGA

32

GGAACC**CTAAGC**C**CTTTCCTTC**..-4..   .-1CC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

41

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*G*.   ..-8......**AAAAGCAGGTTA**GGTTGAGGGA

20

GGAACC**CTAAGC**C**CTTTCC**.....-7..   ..-2C**CGATGAAAAGCAGGTTA**GGTTGAGGGA

1

GGAACC**CTAAGCCCT**........-11..   ..CCCCG**ATGAAAAGCAGGTTA**GGTTGAGGGA

1

GGAACC**CTAAGCC**..........-13..   ..-4..G**ATGAAAAGCAGGTTA**GGTTGAGGGA

32

GGAACC**CTAA**.............-15..   .*T*CCCCG**ATGAAAAGCAGGTTA**GGTTGAGGGA

12

GGAAC..................-20..   ..-4..G**ATGAAAAGCAGGTTA**GGTTGAGGGA

37

GGAACC**CTAAGC**C**CTTTCCTTC**ATT-1.   ..-25.....................AGGGA

27

GGAACC**CTAAGC**...........-14..   ..-39...........................

3

GGA....................-23..   ..-49...........................

29

GGAACC.................-20..   ..-49...........................

25

CAGCAG.................-35..   ..-44...........................

28

AA.....................-49..   ..-37...........................

HCT116 L4+/-

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT(GC   GT)CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

3

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*GC*   ..-4..**GATGAAAAGCAGGTTA**GGTTGAGGGA

4

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT..   ..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

1

GGAACC**CTAAGC**C**CTTTCCTTC**AT-2..   ..CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

2

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*GC*   ..-4..**GATGAAAAGCAGGTTA**GGTTGAGGGA

7

GGAACC**CTAAGC**C**CTTTCCTTC**..-4..   .-1CC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

8

GGAACC**CTAAGC**C**CTTTCC**.....-7..   ..-3.**CGATGAAAAGCAGGTTA**GGTTGAGGGA

11

CC.....................-47..   ..-3.**CGATGAAAAGCAGGTTA**GGTTGAGGGA

11

AACC...................-47..   ..-49...........................

HCT116 X4-/-

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT(GC GT)CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

1
GGAACC**CTAAGCCC**.........-12.. ..-4..G**ATGAAAAGCAGGTTA**GGTTGAGGGA
5
GGAACC**CTAAG**............-15.. ..-5...**ATGAAAAGCAGGTTA**GGTTGAGGGA
46
GGAACC**C**................-19.. ..-4..G**ATGAAAAGCAGGTTA**GGTTGAGGGA
19
GGAACC**CTAAGCC**..........-13.. ..-14...........**AGGTTA**GGTTGAGGGA
37
GGAACC**CTAAGC**C**CTTTCCTTC**ATTT*G*. ..-33...........................
24
GCAGG..................-24.. ..-17..............**TTA**GGTTGAGGGA
33
AGCAGG.................-34.. ..-17..............**TTA**GGTTGAGGGA
30
GGAA...................-22.. ..-31...........................
32
GGAACC.................-20.. ..-49...........................
2
CAGCAG.................-35.. ..-44...........................
14
AGGTTAG................-76.. ..-21..................GTTGAGGGA
29
TGAG...................-69.. ..-27........................GGA
39
TTC....................-41.. ..-65...........................
46
GGAACC**C**................-19.. ..-102..........................
7
GCAAAGC................-51. ..-77...........................
9
GGAACC**CTAAGC**C**CTTTCC**.....-7.. ..-169..........................
13
GGAACC**CTAAGCC**..........-13.. ..-198..........................
10
GGAACC**CTAAG**............-15.. **G** ..-201..........................
8
GGAACC**CTAAGC**C**CTTTCCT**....-6.. ..-207..........................
31
GGAACC**CTAAGCC**..........-13.. ..-219..........................
12
CAGC..................-168.. ..-133..........................

HCT116 L4-/-

GGAACC**CTAAGC**C**CTTTCCTTC**ATTT(GC GT)CCC**CGATGAAAAGCAGGTTA**GGTTGAGGGA

16
GGAACC**CTAAGC**C**CTTTCC**.....-7.. ..-3.**CGATGAAAAGCAGGTTA**GGTTGAGGGA
10
GGAACC**CTAAG**............-15.. ..-27........................GGA
23
GGA....................-23.. ..-23....................TGAGGGA
17
GGAA...................-22.. ..-31...........................
12

GCAG...................-25..		..-44...........................
31		
CAGCAG.................-35..		..-44...........................
46		
TGATT..................-42..		..-55...........................
11		
......................-117..		..-12.........**GCAGGTTA**GGTTGAGGGA
5		
......................-133..	**+13**	..-42...........................
13bp: TTATTTATTTTTA		
25		
......................-153..		..-24.....................GAGGGA
46		
CC....................-118..		..-69...........................
4		
......................-186..	**CTT**	..-191..........................
27		
TGATT..................-42..		..-55...........................

Figure S2, related to Figure 2.Translocation junction sequences from ZFN^{EWS} and ZFN^{p84} DSBs derived from wild-type and c-NHEJ mutant HCT116 cells.
Each junction was independently derived from small pool PCR. Deletions ≤ 800 bp could be identified. The ZFN recognition sequences at each DNA end are underlined; bps in italics represent the overhangs. The chromosome 19 end is in black and the chromosome 22 end is in red. Deletions from each end are indicated, as are microhomologies (underlined) and insertions (green). Insertions were observed in a fraction of breakpoint junctions from both control and mutant cells (wild-type, 18%; X4$^{+/-}$, 14%; X4$^{-/-}$, 24%).

Der19

HCT116 wt

DNA ends:		
TCTGTCACCAATCCTGTCCCTAGTGG*CC*		***GT*CATTAATGTAAAGTGGGGAGGCAGCT**
2		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*CC*		.*T*CATT**AATGTAAAGTGGGGAGGC**AGCT
24		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C.*		.*T*CATT**AATGTAAAGTGGGGAGGC**AGCT
26		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C.*		..CATT**AATGTAAAGTGGGGAGGC**AGCT
7,9,12,18,28		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*..*		..CATT**AATGTAAAGTGGGGAGGC**AGCT
8,20		
TCTGTCACCA**ATCCTGTCCCTA**GTG-1.		.*T*CATT**AATGTAAAGTGGGGAGGC**AGCT
22		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C.*		..-2TT**AATGTAAAGTGGGGAGGC**AGCT
5,21,31		
TCTGTCACCA**ATCCTGTCCCTA**GT-2..		..CATT**AATGTAAAGTGGGGAGGC**AGCT
32		
TCTGTCACCA**ATCCTGTCCCTA**GT-2..		.*T*CATT**AATGTAAAGTGGGGAGGC**AGCT
19		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C.*		..-3.T**AATGTAAAGTGGGGAGGC**AGCT
27		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*..*		..-3.T**AATGTAAAGTGGGGAGGC**AGCT
10		
TCTGTCACCA**ATCCTGTCCCTA**GTG-1.	**C**	..-3.T**AATGTAAAGTGGGGAGGC**AGCT
17		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**GGC**	..-3.T**AATGTAAAGTGGGGAGG**CAGCT
13		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C.*	**TTAAT**	..-6....**TGTAAAGTGGGGAGG**CAGCT
6		
TCTGTCACCA**ATCCTGTCC**.....-7..		..-26.....................T
1		
GCC.....................-29..		..-35......................
16		
GG......................-32..		..-34......................
23		
........................-63..	**CT**	..-35......................
5		
GCT...................-179..		..-26......................
14		
........................-29..		..-196.....................
25		
.......................-251..	**G**	..-27.....................

HCT116 X4+/-

```
 TCTGTCACCAATCCTGTCCCTAGTGGCC                                 GTCATTAATGTAAAGTGGGGAGGCAGCT

53
 TCTGTCACCAATCCTGTCCCTAGTGGC.                                 GTCATTAATGTAAAGTGGGGAGGCAGCT
36,42,51,56
 TCTGTCACCAATCCTGTCCCTAGTGGC.                                 .TCATTAATGTAAAGTGGGGAGGCAGCT
50,52,54
 TCTGTCACCAATCCTGTCCCTAGTGGC.                                 ..CATTAATGTAAAGTGGGGAGGCAGCT
35,38,41,46,49
 TCTGTCACCAATCCTGTCCCTAGTGG..                                 ..CATTAATGTAAAGTGGGGAGGCAGCT
34,39,47
 TCTGTCACCAATCCTGTCCCTAGTG-1.                                 .TCATTAATGTAAAGTGGGGAGGCAGCT
45
 TCTGTCACCAATCCTGTCCCTAGTGGC.                                 ..-2TTAATGTAAAGTGGGGAGGCAGCT
31
 TCTGTCACCAATCCTGTCCCTAGT-2..                                 ..CATTAATGTAAAGTGGGGAGGCAGCT
48
 TCTGTCACCAATCCTGTCCCTAGTG-1.              C                  ..-2TTAATGTAAAGTGGGGAGGCAGCT
62
 TCTGTCACCAATCCTGTCCCTAGTGGC.                                 ..-3.TAATGTAAAGTGGGGAGGCAGCT
64
 TCTGTCACCAATCCTGTCCCTAGTGGC.                                 ..-5...ATGTAAAGTGGGGAGGCAGCT
30
 GGC...................-29..                                  ..-35.......................
37
 AGG..................-148..                                  ..-21.................CAGCT
33
 TCTGTCACCAATCCTGTC......-8..              T                  ..-234.....................
40
  TCTGTCACCAATCCTGTCCCTAGTGGC.          +72                   GTCATTAATGTAAAGTGGGGAGGCAGCT
72bp directly from upstream of DSB on chr22
CTGCTAGCCCTGCTGTCTTTGGGGAAGTTGTATGCAGTGAGTAAATTCAACATCGTTTTTGGCCTCCCTATCA
```

HCT116 X4-/-

```
 TCTGTCACCAATCCTGTCCCTAGTGGCC                                 GTCATTAATGTAAAGTGGGGAGGCAGCT

83
 TCTGTCACCAATCCTGTCCCTAGTG-1..                                ..-9.......AAAGTGGGGAGGCAGCT
74
 TCTGTCACCAATCCTGTCCC.....-6..          ACTGTGGG              ..-9.......AAAGTGGGGAGGCAGCT
8bp directly from downstream of DSB on chr19
70
 TCTGTCACCAATCCTGTCCCTAGTGG..                                 ..-16.............GGAGGCAGCT
78
 TCTGTCACCAATCCTGT.......-9..                                 ..-9.......AAAGTGGGGAGGCAGCT
80
 TCTGTCACCAATCCTGTCCCTAGTGG..                                 ..-21.................CAGCT
84
 TCTGTCA................-21..                                 ..-2.TTATGTAAAGTGGGGAGGCAGCT
65
 TCTGTCACCAAT...........-14..                                 ..-11........AGTGGGGAGGCAGCT
93
 TCTGTCACCAATCCTGTCCCTAG.-3..                                 ..-24......................
69
 TCTGTCACCAATCCT........-11..                                 ..-26......................
64
 TCTGTCACCA.............-16..                                 ..-24....................CT
76
 ......................-29..                                  ..-13................GGCAGCT
92
```

TCTGTCACCA**ATCCTGTCC**.....-7.. ..-40.......................
66
.......................-35.. ..-13..........**TGGGGAGGC**AGCT
88
TCTGTCACCA**ATCCTGTCCCT**...-5.. **TTTT** ..-43.......................
81
TCTGTCACCA.............-16.. ..-37.......................
75
TCT....................-23.. ..-39.......................
86
GCT....................-28.. ..-26.......................
87
.......................-42.. **G** ..-23.......................
90
.......................-48.. ..-17..............**GGAGGC**AGCT
71
T......................-33.. ..-46.......................
73
TCTGTCACCA**ATCCTGTCCCTA**G.-3.. **+16** ..-144......................
+16bp :GCCAAAAACGATGGCC
67
......................-132.. **AAT** ..-34.......................
79
......................-134.. **G** ..-37.......................
68
.......................-32.. **T** ..-158......................
85
CT....................-256.. ..-26.......................
96
GCAGG.................-265.. ..-34.......................
89
TCTGTCACCA**ATC**..........-13.. ..-299......................
94
TCTGTCACC**AATCCTGTCCCTA**GT-2.. ..-311......................
91
......................-357.. ..-10.......**AAGTGGGGAGGC**AGCT

Der22

HCT116 WT

ATCGTTTTTGGCCTCCCTATCAGTCA*TT* ***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**

2,4,7,15,24,36,45
ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT* ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
6,18,34,46
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*. ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
19
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*. .-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
10,17,40
ATCGT**TTTTGGCCTCCCTATCA**GT-2.. ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
38
ATCGT**TTTTGGCCTCCCTATCA**..-4.. .-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
3
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*. ..-5...**CACTGTGGGGT**GGAGGGGACAGAT
26
ATCGT**TTTTGGCC**..........-13.. ..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
43
ATCGT**TTTTG**..............-16.. ..-12.........**GGGT**GGAGGGGACAGAT
25,27
ATCGT**TTTT**..............-17.. ..-12.........**GGGT**GGAGGGGACAGAT

35
.......................-47..   **C**   ..-22...................GACAGAT
11
ATCGT**TTTTGGCCT**.........-11..   ..-125.........................
39
ATCGT**TTTT**...............-17..   ..-196.........................
9
GCC...................-239..   ..-148.........................
44
ATCGT**TTTTGG**............-15..   **+119**   *GT*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
119bp from chr19
25bp from chr19 inverted duplication from 29bp downstream of DSB
GCTCTGGTTCTGGGTACTTTTATCT
94bp from chr19 directly upstream of DSB
GTCCTAACAGGAGGTGGGGGTTAGACCCAATATCAGGAGACTAGGAAGGAGGAGGCCTAAGGATGGGGCTTTTCTGTCACCAATCCTGTCCCTA

33
ATCGT**TTTTGGCCTCCCTATCA**GTC-1.   +134   ..-4..C**CACTGTGGGGT**GGAGGGGACAGAT
134bp from Homo sapiens chr16, alternate assembly HuRef
TGGCACGCGgCGGTACTGCCGGTGGTAGTAGTAATACCTGTTCTTTGCGTGCTGCCGCTCTATAAATTCTGCAAAGACAAAGCCACAGACTCAAACGCCTCATCGGTTTGGACAGGCTTTTAGCTTTCTTATTTC
1
ATCGT**TTTTGGCCTCCCTATCA**G.-3..   +233   ..-23...................ACAGAT
233bp from Human DNA sequence from clone RP11-335L15 on chromosome 9, complete sequence
TCAGACTCTTCAACATAGCAAGTCCTTCCCAGACAATTCCACTCCAATCATAAGGACCACTCCTTCCTCTAAGGATTCAAAGCACTTTGTACTTATAGGGCCTCTAGGGGTACTGCCCTCACTCTATTGTTAATGGTATATATTTCTATCTCCCTCACTAGGCTTCCAACTATTTAAGAAAAGGAtGCATATTTTTACTCCTCTtTGTGTCTTCAGCACCCTTCCACAGTGCCTGCAACA
32
ATCGT**TTTTGGCCTT**........-10..   **+221**   ..-63.........................
221bp:
-133bp from Homo sapiens chr7, alternate assembly HuRef:
TCAGGCCAGAGTGTGGGGAAAGGCTCAAGGAATTAGAATTCTCACCTGATTGTGCCCCCAGCTGTGGACCGTGGCCTCGGCTTCCTTTTCTCTATGATGACACAGTTGGGAAAGCTGCTCTCCAGGGTCCCCCG
-88bp from Homo sapiens chr10, alternate assembly HuRef
GCCACGGTGGGCCCTGTGACCCCTCTCTCTGCCTGCGCAGAACCTGGAGTACTGTATCATGGTCATTGGGGTCCCCAACGTGGGCAAGTCCT

HCT116 X4+/-

ATCGTTTTTGGCCTCCCTATCAGTCA*TT*   ***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**

34,43,49,58,61
ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT*   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
50,52,60,62
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
41,48
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.   .-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
51
ATCGT**TTTTGGCCTCCCTATCA**GT-2..   **A**   .-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
35
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.   ..-11........**GGGGT**GGAGGGGACAGAT
64
ATCGT**TTTTGGCCTCCC**.......-9..   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
45
ATCGT**TTT**................-18..   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
36
ATCGT**TTTTGGCCTCCCTAT**....-6..   ..-16.............GGAGGGGACAGAT
54
ATCGT**TTTTGGCCTCCCTAT**CAGTCA*TT*   **GAAGCAACCGA**   ..-41..........................
44

```
 ATCGTTTTTG.............-16..             A          ..-35..........................
55
 ATCGTTTTTGGCCTCCCTATC...-5..        ACATTTGCCCC     ..-62..........................
46
 .......................-40..             AG         ..-29..........................
57
 AGTA...................-34..                        ..-36..........................
47
 CAG....................-40                          ..-31..........................
37
 ATCGTTTTTGG............-15..                        ..-103.........................
56
 .......................-47..             +22        ..-115.........................
+22bp : 17bp from Homo sapiens chromosome 8, clone CTD-2517M22, complete sequence
A-CTGCGGAGCCCTGATGG-CGGG
63
 GT.....................-266..                       ..GGCCCCACTGTGGGGTGGAGGGGACAGAT
38
 .......................-311..                       ..-324.........................
40
 ATCGTTTTTGGCCTCCCTATCAGTCATT            +276        GTGGCCCCACTGTGGGGTGGAGGGGACAGAT
+276bp from Homo sapiens chromosome 17, clone RP11-216P6
TCTCAGTTCTGATTCAGAGCATATACTGCCTCCTGCAGAACTCTGGCCCAGCCCTGCAAGGCATCGCCACCCAGCTGGTGCTGTAG
TGGAGTCTTCAAAAGGCCATTCCACCATCTTATCAAACCAGCTGCTTCAAGATGGTGGGCAACATGGTCGTACCGGGGAATTCCAT
GAGCATGGGCTCGTTACCATACTTTCTTTGCTGTGAAGGGAGTTCCTCAGACAGAAGCAGTGCGGTGTGGCATACCGTGACAGTAG
GTACGGCATTCTGTGAGT
```

HCT116 X4-/-

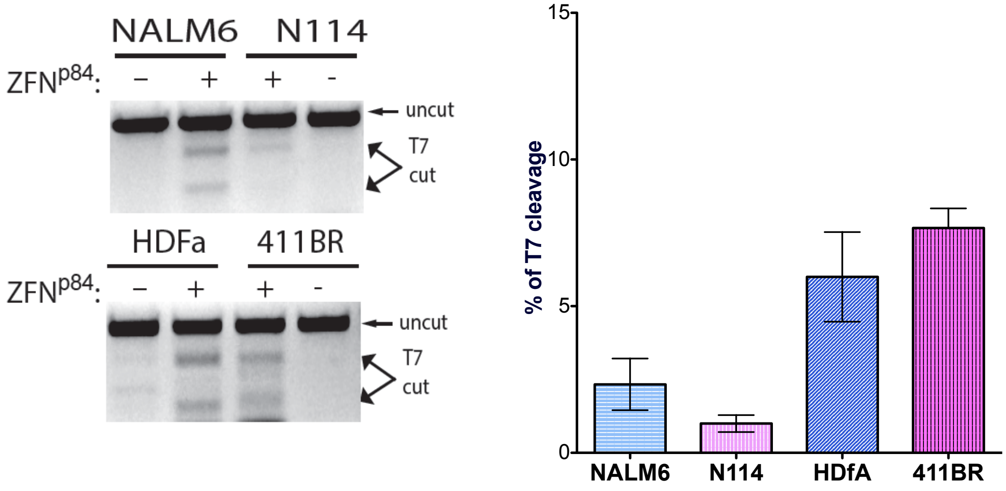
```
 ATCGTTTTTGGCCTCCCTATCAGTCATT                        GTGGCCCCACTGTGGGGTGGAGGGGACAGAT
```

```
94
 ATCGTTTTTGGCCTCCC.......-9..            A           ..-3.CCCACTGTGGGGTGGAGGGGACAGAT
70
 A......................-25..                        ..-2CCCCACTGTGGGGTGGAGGGGACAGAT
76,89
 CA.....................-25..                        ..-7.....CTGTGGGGTGGAGGGGACAGAT
80
 ACA....................-25..                        ..-26.........................
91
 ATCGTTTTTGGCCTCCCTAT....-6..                        ..-55.........................
95
 GT.....................-46..                        ..-16.............GGAGGGGACAGAT
79
 CA.....................-28..                        ..-40.........................
93
 .......................-41..           ACTT         ..-37..........................
66
 CAG....................-40..                        ..-41..........................
92
 .......................-48..                        ..-49..........................
87
 GG.....................-55..                        ..-62..........................
84
 GT.....................-83..                        ..-35..........................
85
 CAG....................-40..                        ..-87..........................
68
 GCCCTG.................-65..                        ..-67..........................
88
 CA.....................-41..                        ..-97..........................
```

```
82
 TAC...................-103..                        ..-37..........................
77
 ......................-115..          T             ..-68..........................
73
 ......................-221..          T             ..-8......TGTGGGGTGGAGGGGACAGAT
71
 ATCGTTTTTGGCCTCCCTATCAG.-3..                        ..-232.........................
83
 TCC...................-145..                        ..-129.........................
74
 GCC...................-277..                        ..-64..........................
65
 GAG....................-36..                        ..-337.........................
75
 GGA....................-52..                        ..-330.........................
81
 ATC....................-23..         +56            ..-276.........................
56bp: Inverted duplication from 2293bp upstream of DSB on chr19
CTGGACTTCGGCTTTTGTCCCCCCAAGTTTTGGACCCCTAAGGGAAGAATGAGAAAC
96
 ......................-220..         +37            ..-43..........................
37bp: AGATTCATTTATACAATGAATACATCATTGTCTTTGT
Part of the sequence may come from:
-18bp from Homo sapiens chr7, alternate assembly CHM1_1.1
ATTCATTTATACAATGAA
-16bp from Homo sapiens v-erb-b2 avian erythroblastic leukemia viral oncogene homolog
4 (ERBB4), RefSeqGene on chromosome 2
-ACATCATTGTCTTTGT
```

Figure S3, related to Figure 3. Translocations induced by ZFN^{EWS} and ZFN^{p84} DSBs in pre-B cells and patient-derived cells.

A. Indel formation: T7 assay on p84 locus after a ZFN^{p84} DSB. Indel formation at the ZFN^{p84} cleavage site, as monitored by the T7-endonuclease assay, in wild-type and LIG4 mutant pre-B cells and fibroblasts.



B. Translocation junction sequences from pre-B cells.
The ZFN recognition sequences at each DNA end are underlined; bps in italics represent the overhangs. The chromosome 19 end is in black and the chromosome 22 end is in red. Microhomologies (underlined), insertions (green) and lengths of deletions from each end are indicated.

Der19

NALM6

DNA ends:

TCTGTCACCAATCCTGTCCCTAGTGG*CC*		***GT*CATTAATGTAAAGTGGGGAGGCAGCT**
11		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C*.	**TGGCG**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
5 bp from chr19 duplication TGGC G		
14		
TCTGTCACCA**ATCCTGTCCCTA**GTGG..	**TT**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
23		
TCTGTCACCA**ATCCTGTCCCTA**GTGG*CC*	**C**	..-2TT**AATGTAAAGTGGGGAGGC**AGCT
22,39		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**G**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
37		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**GG**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
76		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**AG**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
3		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**AAG**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
10		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**GCG**	*GT*CATT**AATGTAAAGTGGGGAGGC**AGCT
8		
TCTGTCACCA**ATCCTGTCCCTA**G.-3..	**GCGC**	.*T*CATT**AATGTAAAGTGGGGAGGC**AGCT
59		

```
 TCTGTCACCAATCCTGTCCCTAG.-3..          GGA          .TCATTAATGTAAAGTGGGGAGGCAGCT
33
 TCTGTCACCAATCCTGTCCCTAG.-3..           GG          ..CATTAATGTAAAGTGGGGAGGCAGCT
20
 TCTGTCACCAATCCTGTCCCTAGTGGCC          TCG          ..-3.TAATGTAAAGTGGGGAGGCAGCT
83
 TCTGTCACCAATCCTGTCCCTAGTGGC.          GCC          ..-3.TAATGTAAAGTGGGGAGGCAGCT
26
 TCTGTCACCAATCCTGTCCCTAGTGGC.           GC          ..-3.TAATGTAAAGTGGGGAGGCAGCT
35
 TCTGTCACCAATCCTGTCCCTAGT-2..           TC          ..-2TTAATGTAAAGTGGGGAGGCAGCT
42
 TCTGTCACCAATCCTGTCCCT...-5..         GAGAG         GTCATTAATGTAAAGTGGGGAGGCAGCT
68
 TCTGTCACCAATCCTGTCCCTAGTGGC.        GCCCTGG        ..-6....TGTAAAGTGGGGAGGCAGCT
72
 TCTGTCACCAATCCTGTCCC....-6..         CGGGA         GTCATTAATGTAAAGTGGGGAGGCAGCT
2
 TCTGTCACCAATCCTGTCCC....-6..         CCCCA         ..-3.TAATGTAAAGTGGGGAGGCAGCT
24
 TCTGTCACCAATCCTGTCCCTA..-4..                       ..-5...ATGTAAAGTGGGGAGGCAGCT
12
 TCTGTCACCAATCCTG.......-10..        CCTGGGAC       .TCATTAATGTAAAGTGGGGAGGCAGCT
60
 TCTGTCACCAATCCTG.......-10..         AGGGGC        .TCATTAATGTAAAGTGGGGAGGCAGCT
81
 TCTGTCACCAATCCT........-11..        CTGAAGCC       .TCATTAATGTAAAGTGGGGAGGCAGCT
44
 TCTGTCACCAATC..........-13..         TAGGC         GTCATTAATGTAAAGTGGGGAGGCAGCT
13
 TCTGTCACCAATCCT........-11..         TTCC          ..-3.TAATGTAAAGTGGGGAGGCAGCT
18,32
 TCTGTCACCAATCCT........-11..                       ..-4..AATGTAAAGTGGGGAGGCAGCT
31
 TCTGTCACCAATCC.........-12..        CGTCC          ..-3.TAATGTAAAGTGGGGAGGCAGCT
21
 TCTGTCACCAATCCTG.......-10..          +52          ..-9.......AAAGTGGGGAGGCAGCT
52bp inverted sequence located 11bp downstream of DSB from chr19
GGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTTATCTGTCCCCTCCACCCCA
16
 TCTGTCAC...............-18..         TTTAC         ..-3.TAATGTAAAGTGGGGAGGCAGCT
54
 TCTG...................-22..          CGA          ..-3.TAATGTAAAGTGGGGAGGCAGCT
62
 TCTGTCACCAATCCTG.......-10..                       ..-20.................GCAGCT
67
 .......................-27..           G           ..-3.TAATGTAAAGTGGGGAGGCAGCT
34
 TCTGTCACCAA............-15..          CCT          ..-21..................CAGCT
43
 .......................-35..                       ..-13..........TGGGGAGGCAGCT
15
 .......................-32..                       ..-19................GGCAGCT
53
 .......................-34..                       ..-19................GGCAGCT
77
 .......................-31..          TC           ..-33.......................
71
 .......................-56..                       ..-18...............AGGCAGCT
38
 TG.....................-33..                       ..-48.......................
40
 ......................-118..                       ..-19................GGCAGCT
9
 ......................-139..                       ..-5...ATGTAAAGTGGGGAGGCAGCT
```

36
TCTG...................-22..   **CC**   ..-233......................
66
......................-289..   ..-12.........**GTGGGGAGGC**AGCT
6
......................-306..   **+51+8**   ..-23....................GCT
59bp: 51bp from chr22 17bp upstream of DSB
CTGCTGTCTTTGGGGAAGTTGTATGCAGTGAGTAAATTCAACATCGTTTTTCGTTTGTG
75
......................-205..   **C**   ..-3.TA**ATGTAAAGTGGGGAGGC**AGCT
41
.......................-57..   **AAT**   ..-283......................
89
......................-413..   ..-11........**AGTGGGGAGGC**AGCT
4
......................-256..   ..-119......................
79
TCTGTCACCA**ATCCTGTCCC**....-6..   **+229**   ..-7.....**GTAAAGTGGGGAGGC**AGCT
229bp from chr19 17bp directly downstream of DSB
CCCACTGTGGGGTGGAGGGGACAGATAAAAGTACCCAGAACCAGAgCACATTAACCGGCCCTGGGAATATAAGGTGGTcCCAGCTC
GGGGACACAGGATCCCTGGAGGCAGCAAACATGCTGTCCTGAAGTGGACATAgGGGCCCGGGTTGGAGGAAGAAGACTAGCTGAGC
TCTCGGACCCCTGGAAGATgcCATGACAGGGGGCTGGAAGAGCTAGCACAGACTAGAGAGGTAA

N114

TCTGTCACCAATCCTGTCCCTAGTGG*CC*   ***GT*CATTAATGTAAAGTGGGGAGGCAGCT**

83
TCTGTC.................-20..   **TG**   ..-8......**TAAAGTGGGGAGGC**AGCT
59
TCTGTCAC...............-18..   ..-11........**AGTGGGGAGGC**AGCT
64
TCTGTCACCA**ATCCT**........-11..   ..-26......................
58
TCTG...................-22..   ..-21..................**C**AGCT
71
.......................-23..   ..-24.....................**CT**
85
.......................-29..   ..-19................**GGC**AGCT
66
TT.....................-25..   ..-29.......................
61
.......................-36..   **GTGGG**   ..-23....................**GCT**
84
G......................-30..   ..-33.......................
62,70
GGC....................-29..   ..-35.......................
65
GG.....................-30..   ..-34.......................
88
GG.....................-31..   ..-34.......................
73
.......................-30..   ..-39.......................
76
CTA....................-39..   ..-40.......................
87
.......................-41..   ..-48.......................
77
.......................-29..   **AA**   ..-64.......................
80
AAG....................-37..   ..-57.......................
55
......................-123..   ..CATT**AATGTAAAGTGGGGAGGC**AGCT
81

```
 .......................-80..        6+27+4           ..-57.......................
37bp: 27 bp sequence from chr22 70bp upstream of DSB
CAGTCC ATCAGAAAAAATGTTTTAGACTGCTAG TTTC
75
 ......................-137..                         ..-12.........GTGGGGAGGCAGCT
74
 TC.....................-24..          +76            ..-126......................
76bp inverted sequence from chr22 20bp upstream of DSB
AACGATGTTGAATTTACTCACTGCATACAACTTCCCCAAAGACAGCAGGGCTAGCAGTCTAAAACATTTTTTCTGATCA
60
 A......................-60..                         ..-106......................
92
 GCAGG.................-243..                         ..-34.......................
57
 TCTGTCACCAATCCT........-11..                         ..-288......................
78
 AGA...................-107..                         ..-256......................
```

Der22

NALM6

```
 ATCGTTTTTGGCCTCCCTATCAGTCATT                       GTGGCCCCACTGTGGGGTGGAGGGGACAGAT

39
 ATCGTTTTTGGCCTCCCTATCAGTCATT                       ..GGCCCCACTGTGGGGTGGAGGGGACAGAT
22,41
 ATCGTTTTTGGCCTCCCTATCAGTCATT                       .-1GCCCCACTGTGGGGTGGAGGGGACAGAT
44
 ATCGTTTTTGGCCTCCCTATCAG.-3..         GGA           .TGGCCCCACTGTGGGGTGGAGGGGACAGAT
1
 ATCGTTTTTGGCCTCCCTATC...-5..        GGGGG          GTGGCCCCACTGTGGGGTGGAGGGGACAGAT
46
 ATCGTTTTTGGCCTCCCTA.....-7..         AA            GTGGCCCCACTGTGGGGTGGAGGGGACAGAT
42
 ATCGTTTTTGGCCTCCCTAT....-6..         TAA          ..-5...CACTGTGGGGTGGAGGGGACAGAT
12
 ATCGTTTTTGGCCTCC.......-10..         AT            ..-3.CCCACTGTGGGGTGGAGGGGACAGAT
34
 ATCGTTTTTGGCCTCC.......-10..          G            ..-3.CCCACTGTGGGGTGGAGGGGACAGAT
3
 ATCGTTTTTGGCCTCCC.......-9..                       ..-5...CACTGTGGGGTGGAGGGGACAGAT
7
 ATCGTTTTTGGCCTCCCTATCA..-4..        TGGCCA         ..-11........GGGGTGGAGGGGACAGAT
2
 ATCGTTTTTGGCC..........-13..          G            ..-5...CACTGTGGGGTGGAGGGGACAGAT
5
 ATCGTTTTTGGC...........-14..          T            ..-4..CCACTGTGGGGTGGAGGGGACAGAT
38
 ATCG...................-22..      CTATGTCCCTA      GTGGCCCCACTGTGGGGTGGAGGGGACAGAT
27
 .......................-26..       GTCGGCGAA       .TGGCCCCACTGTGGGGTGGAGGGGACAGAT
11
 ATC....................-23..          A             ..-5...CACTGTGGGGTGGAGGGGACAGAT
20,40
 ATCGTTTTTGGCCTCCCTATCAG.-3..                       ..-27..........................
29
 ATCGTT.................-20..                       ..-11........GGGGTGGAGGGGACAGAT
33
 .......................-29..          CCC          ..-9.......GTGGGGTGGAGGGGACAGAT
45
 .......................-47..        GATGGA         ..-16.............GGAGGGGACAGAT
37
```

........................-30..   **CA**   ..-11........**GGGGT**GGAGGGGACAGAT

8

ATCGT**T**.................-20..   ..-64........................

25

AGT....................-49..   ..-35........................

24

ATCGT**TTTTGGCC**..........-13..   ..-148........................

30

ATCGT**TTTTGGCCTCCCTATCA**G.-3..   ..-162........................

6

ATCGT**TTTTGGCCTCCCTATCA**G.-3..   ..-242........................

48

.......................-257..   **CCTGG**   ..-133........................

47

........................-46..   **+5**+31   ..-38........................

36bp: 31bp inverted sequence from 10bp downstream the DSB

GTCAC-CTGGGTACTTTTATCTGTCCCCTCCACCCCA

31

ATCGT**TTTTGGCCTCCCTATCA**..-4..   **+47**   ..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT

47bp: 44bp from Homo sapiens caspase 8, apoptosis-related cysteine peptidase (CASP8), RefSeqGene (LRG_34) on chromosome 2

CTGTGCCTGGCCATAATTCATTTATCAGTCATATATATATACAC-GTC

13

ATCGT**TTT**................-18..   **+539**   ..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT

539bp from ZFN^{EWS} coding vector

GCGTTTAAACTTAAGCTTATCCACTAGTCCAGTGTGGTGGAATTCGCCATGGACTACAAAGACCATGACGGTGATTATAAAAATCA
TGACATCGCTTACAAGGATGACGATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCATCCACGGGGTACCCGCCGCTATGG
CTGAGAGGCCCTTCCAGTGTCGAATCTGCATGCGTAAGTTTGCCCAGTCCAACCACCGCAAGACCCATACCAAGATACACACGGGC
GAGAAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTGACCGCTCCGACCTGTCCCGCCACATCCGCACCCACACCGGCGA
GAAGCCTTTTGCCTGTGACATTTGTGGGAGGAAATTTGCCCGCTCCGACAACCTGACCCGCCATACCAAGATACACACGGGCGGAG
GCGGATCTCAGAAGCCCTTCCAGTGTCGAATCTGCATGCGTAACTTCAGTCGCTCGACTACCTGTCCACCCACATCCGCACCCACA
CGGCGAGAAGCCTTTTGCCTGTG

N114

ATCGTTTTTGGCCTCCCTATCAGTCA*TT*   ***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**

79

ATCGT**TTTTGGCCTCCCTATC**...-5..   ..-5...**CACTGTGGGGT**GGAGGGGACAGAT

85

ATCGT**TTTTGGCCTCCCT**......-8..   ..-9.......**GTGGGGT**GGAGGGGACAGAT

65

ATCGT**TTTTGG**............-15..   **TGAACAT**   ..-6....**ACTGTGGGGT**GGAGGGGACAGAT

68

ATCGT**TTTTGG**............-15..   ..-13.........**GGT**GGAGGGGACAGAT

93

ATC....................-23..   **TGT**   ..-5...C**ACTGTGGGGT**GGAGGGGACAGAT

72

........................-28..   **TAGTG**   ..-5...C**ACTGTGGGGT**GGAGGGGACAGAT

74

........................-30..   ..-4..CC**ACTGTGGGGT**GGAGGGGACAGAT

87

ATCGT**TTTTGG**............-15..   ..-23...................ACAGAT

76

........................-41..   ..-9.......**GTGGGGT**GGAGGGGACAGAT

71

........................-53..   ..-10.......**TGGGGT**GGAGGGGACAGAT

73

AA.....................-33..   ..-33........................

92

AGTA...................-34..   ..-36........................

83

ATT....................-30..   ..-56........................

77

```
 GAA....................-51..                       ..-43..........................
69
 ......................-144..                       ..-4..CCACTGTGGGGTGGAGGGGACAGAT
75
 ......................-210..                       ..-5...CACTGTGGGGTGGAGGGGACAGAT
90
 .......................-29..          CCAA         ..-217.........................
86
 ......................-163..          TGCC         ..-102.........................
70
 AGTGAG.................-36..                       ..-279.........................
88
 ATCGTTTTTGG............-15..           A           ..-361.........................
91
 ATCGTT.................-21..          +90          ..-24.....................CAGAT
90bp from TALEN expression vector
CGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTG
TCAA
67
 ATCGTTTTTGGCCTCCCTATCAG.-3..          +205          ..-35.........................
205bp from Homo sapiens chr13, alternate assembly CHM1_1.1
CAGGAACTCAAATACAATCCAGATGAAAAAATGAAGAGATGAACAAGGCCAAAGGCCACAGATGACATCCTCACAGAAAGACTGGC
TTTTGTGGGCCACCCCAGATATCTGGCTCAGCATGTAAGAGTCAGCAATGCTGAGGAAACTGGTGCCTCGGCCAGGGTTTTGCCAA
GAGCAGGCTCAAAGTGTGGGCAAGAGAAAGAGAGACACA
```

C. Translocation junction sequences from patient-derived cells.

Der19

HDFa

```
 TCTGTCACCAATCCTGTCCCTAGTGGCC                        GTCATTAATGTAAAGTGGGGAGGCAGCT

38,44
 TCTGTCACCAATCCTGTCCCTAGTGGC.                         .TCATTAATGTAAAGTGGGGAGGCAGCT
10
 TCTGTCACCAATCCTGTCCCTAGTG-1.                         .TCATTAATGTAAAGTGGGGAGGCAGCT
11,46
 TCTGTCACCAATCCTGTCCCTAGTGGC.                         .-1ATTAATGTAAAGTGGGGAGGCAGCT
6
 TCTGTCACCAATCCTGTCCCTAGT-2..                         .TCATTAATGTAAAGTGGGGAGGCAGCT
22,39,45,50
 TCTGTCACCAATCCTGTCCCTAGT-2..                         ..CATTAATGTAAAGTGGGGAGGCAGCT
15
 TCTGTCACCAATCCTGTCCCTAGTGGC.                         .-3..TAATGTAAAGTGGGGAGGCAGCT
27
 TCTGTCACCAATCCTGTCCCTAGT-2..                         .-1ATTAATGTAAAGTGGGGAGGCAGCT
9
 TCTGTCACCAATCCTGTCCCTAGTG-1.         CCGTC           ..-2TTAATGTAAAGTGGGGAGGCAGCT
23
 TCTGTCACCAATCCTGTCCCTAGTGGC.                         ..-3.TAATGTAAAGTGGGGAGGCAGCT
1
 TCTGTCACCAATCCTGTCCCTA..-4..                         ..CATTAATGTAAAGTGGGGAGGCAGCT
16
 TCTGTCACCAATCCTGTCCCTAGTGGC.                         ..-4..AATGTAAAGTGGGGAGGCAGCT
2
 TCTGTCACCAATCCTGTCCCTAGT-2..                         ..-3.TAATGTAAAGTGGGGAGGCAGCT
47
 TCTGTCACCAATCCTGTCCCTAGT-2..                         ..-3.TAATGTAAAGTGGGGAGGCAGCT
4
 TCTGTCACCAATCCTGTCCCTA..-4..                         ..-2TTAATGTAAAGTGGGGAGGCAGCT
```

17
TCTGTCACCA**ATCCTGTCCC**....-6..   ..CATT**AATGTAAAGTGGGGAGGC**AGCT
5
TCTGTCACCA**ATCCTGTCCCTA**..-4..   ..-3.T**AATGTAAAGTGGGGAGGC**AGCT
14,20
TCTGTCACCA**ATCCTGTCCCTA**..-4..   ..-5...**ATGTAAAGTGGGGAGGC**AGCT
33
TCTGTCACCA**ATCCTGTC**......-8..   .-*1*ATT**AATGTAAAGTGGGGAGGC**AGCT
12
TCTGTCACCA**ATCCTGTCCCTA**GTGG..   ..-16............**GGAGGC**AGCT
18
TCTGTCACCA**ATC**..........-13..   ..-5...**ATGTAAAGTGGGGAGGC**AGCT
41
......................-36..   ..-3.TA**ATGTAAAGTGGGGAGGC**AGCT

411BR

TCTGTCACCAATCCTGTCCCTAGTGG*CC*   ***GT*CATTAATGTAAAGTGGGGAGGCAGCT**

56
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C*.   .*T*CATT**AATGTAAAGTGGGGAGGC**AGCT
22,63
TCTGTCACCA**ATCCTGTCCCTA**GT-2..   ..CATT**AATGTAAAGTGGGGAGGC**AGCT
65
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C*.   ..-2TT**AATGTAAAGTGGGGAGGC**AGCT
10
TCTGTCACCA**ATCCTGTCCCTA**GTG-1.   **ATA**   ..-3.T**AATGTAAAGTGGGGAGGC**AGCT
84
TCTGTCACCA**ATCCTGTCCCTA**GT-2..   ..-2TT**AATGTAAAGTGGGGAGGC**AGCT
91
TCTGTCACCA**ATCCTGTCCC**....-6..   .-*1*ATT**AATGTAAAGTGGGGAGGC**AGCT
89
TCTGTCACCA**ATCCTGTCCCTA**..-4..   ..-5...**ATGTAAAGTGGGGAGGC**AGCT
27,70
TCTGTCACCA**ATCCTGTCCCTAG**T-2..   ..-9......**AAAGTGGGGAGGC**AGCT
12
TCTGTCACCA**ATCCTGTCCCTA**..-4..   ..-10......**AAGTGGGGAGGC**AGCT
75
TCTGTCACCA**ATCCTGTCCCTA**GTG-1.   **A**   ..-13..........**GGGGAGGC**AGCT
42,59
TCTGTCACCA**ATCCTGTCCCTA**GTGG..   ..-15...........**GGGAGGC**AGCT
1,24,47,53,76
TCTGTCACCA**ATCCTGTCCCTA**GTGG..   ..-16............**GGAGGC**AGCT
13
TCTGTCACC..............-17..   *GT*CAT**AATGTAAAGTGGGGAGGC**AGCT
17
TCTGTCACCA**ATCCTGTCCCTA**..-4..   ..-12.........**TGGGGAGGC**AGCT
13
TCTGTCACC..............-17..   *GT*CAT**AATGTAAAGTGGGGAGGC**AGCT
39
TCTGTCACCA**ATCCTGT**.......-9..   ..-8.....**TAAAGTGGGGAGGC**AGCT
54
TCTGTCACCA**ATCCTGT**.......-9..   **G**   ..-11.........**GTGGGGAGGC**AGCT
82
TCTGTCACCA**ATCCTGTC**.....-10..   ..-10......**AAGTGGGGAGGC**AGCT
11,73
TCTGTCACCA**AT**...........-14..   ..-7.....**GTAAAGTGGGGAGGC**AGCT
50
TCTGTCA................-19..   ..-2TT**AATGTAAAGTGGGGAGGC**AGCT
32
TCTGTCACCA**ATCCTGT**.......-9..   ..-13..........**GGGGAGGC**AGCT
92
TCTGTCACCA**ATCC**.........-12..   ..-11.......**AGTGGGGAGGC**AGCT

38
TCTGTCACCA**ATCCTG**.......-10..   ..-13...........**GGGGAGGC**AGCT
66
TCTGTCACCA**ATCC**.........-12..   **C**   ..-12..........**TGGGGAGGC**AGCT
31
TCTGTCAC...............-18..   ..-8......**TAAAGTGGGGAGGC**AGCT
3
TCTGTCACCA**ATCCTGTCCCTA**GTGG*C*.   **A**   ..-28.......................
33
TCTGTCACCA.............-16..   ..-14...........**GGGGAGGC**AGCT
81
TCTGTCACCA**AT**...........-14..   ..-16.............**GGAGGC**AGCT
88
TCTGTCACCA**ATC**..........-13..   ..-27.......................
7
.......................-30..   ..-2TT**AATGTAAAGTGGGGAGGC**AGCT
28
TCTGTCACCA.............-16..   ..-19................**GGC**AGCT
29
TC.....................-24..   ..-11........**AGTGGGGAGGC**AGCT
74
.......................-27..   ..-8......**TAAAGTGGGGAGGC**AGCT
86
T......................-25..   ..-10......**AAGTGGGGGAGGC**AGCT
23
TCTGTCACC..............-17..   **C**   ..-20................**GC**AGCT
6
T......................-25..   ..-14...........**GGGGAGGC**AGCT
67
TCTGTCACCA**ATCCTG**.......-10..   ..-29.......................
78
T......................-25..   ..-17..............**GAGGC**AGCT
51
.......................-37..   ..-22..................AGCT
46
TCTGT..................-21..   ..-167......................
61
.......................-72..   ..-176.....................

Der22

HDFa

ATCGTTTTTGGCCTCCCTATCAGTCA*TT*   ***GT*GGCCCCCACTGTGGGGTGGAGGGGACAGAT**

13
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.   *GT*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
83,7
ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT*   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
5
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
81,1,4
ATCGT**TTTTGGCCTCCCTATCA**GT-2..   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
2
ATCGT**TTTTGGCCTCCCTATCA**GTCA..   ..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
10
ATCGT**TTTTGGCCTCCCTATCA**G.-3..   .-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
82
ATCGT**TTTTGGCCTCCCTATCA**GTC-1.   ..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
86
ATCGT**TTTTGGCCTCCCTATCA**G.-3..   .-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
3
ATCGT**TTTTGGCCTCCCTATCA**GTC-1.   ..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
63

	ATCGT**TTTTGGCCTCCCTATCA**G.-3..		..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
62	ATCGT**TTTTGGCCTCCCTATC**...-5..		..-5...**CACTGTGGGGT**GGAGGGGACAGAT
69,77	ATCGT**TTTTGGCCTCCCTATCA**GT-2..		..-9......**GTGGGGT**GGAGGGGACAGAT
70	ATCGT**TTTTGGCCTCCCTAT**....-6..	**T**	..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
9	ATCGT**TTTTGGCCTCCCTA**.....-7..		..-7.....**CTGTGGGGT**GGAGGGGACAGAT
95	ATCGT**TTTTGGCCTCCCT**......-8..		..-10......**TGGGGT**GGAGGGGACAGAT
91	ATCGT**TTTTGGCCTCCC**.......-9..		..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
61	ATCGT**TTTTGGCCTCCCTATCA**G.-3..		..-21.................GGACAGAT
92	GC.....................-42..		..-171.........................

411BR

	ATCGTTTTTGGCCTCCCTATCAGTCA*TT*		***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**
46	ATCGT**TTTTGGCCTCCCTATCA**GTC-1.		..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
8	ATCGT**TTTTGGCCTCCCTATCA**G.-3..		.-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
49	ATCGT**TTTTGGCCTCCCTA**.....-7..		GTGGCC**CCACTGTGGGGT**GGAGGGGACAGAT
5,34	ATCGT**TTTTGGCCTCCCT**......-8..		..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
10,26,71	ATCGT**TTTTGGCCTCCCTATC**...-5..		..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
29	ATCGT**TTTTGGCCTCCCTA.....-7..**	**CC**	..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
4	ATCGT**TTTTGGCCTCC.......-10..**	**A**	.TGGCC**CCACTGTGGGGT**GGAGGGGACAGAT
32	ATCGT**TTTTGGCCTCCCTA**.....-7..	**G**	..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
54	ATCGT**TTTTGGCCTCCC.......-9..**	**AG**	..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
60	ATCGT**TTTTGGCCTCCC.......-9..**	**CCT**	..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
20	ATCGT**TTTTGGCCTCCCTA**.....-7..	**GTGC**	..-7.....**CTGTGGGGT**GGAGGGGACAGAT
48	ATCGT**TTTTGGCCTCC**.......-10..		..-6....**ACTGTGGGGT**GGAGGGGACAGAT
57	ATCGT**TTTTGGCCTCCCTATCA**GT-2..		..-16............GGAGGGGACAGAT
58	ATCGT**TTTTGGCCT**.........-12.		..-8.....**TGTGGGGT**GGAGGGGACAGAT
41	ATCG...................-21..	**A**	..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
70	ATCGT**TTTTGGCCT**.........-12.		..-9......**GTGGGGT**GGAGGGGACAGAT
1	ATCGT**TTTTGGCCTCCCTAT....-6..**	**A**	..-16............GGAGGGGACAGAT
17,52,62	ATCGT**TTTTGGCCT.........-12..**		..-11.......**GGGGT**GGAGGGGACAGAT
3	ATCGT**TTTTGGCCTCCC.......-9..**		..-15...........**T**GGAGGGGACAGAT
61	ATCGT**TTTTGGCCTCC.......-10..**		..-14..........**GT**GGAGGGGACAGAT

15

ATCGT**TTTTGG............-15..** ..-10.......**TGGGGT**GGAGGGGACAGAT

67

ATCGT**TTTTGGCCTCCC.......-9..** ..-16.............GGAGGGGACAGAT

51,53

ATCGT**TTTTGGCCTCCCTA.....-7..** ..-19................GGGGACAGAT

27

ATCGT**TTTTGGCCTCCC.......-9..** ..-21................GGGGACAGAT

38

ATCGT**TTTTGGCCT.........-12..** ..-18.............GGAGGGGACAGAT

25

ATCGT**TTTTGGCCT.........-12..** ..-20...............AGGGGACAGAT

47

ATCGT**TTTTGGCCTC**........-11.. ..-21................GGGGACAGAT

68

ATCGT**TTTTGGCCT.........-12..** **A** ..-20...............AGGGGACAGAT

21

ATCGT**TTTTGGCCT.........-12..** ..-25......................AGAT

69

ATCGT**TTTTGGCCTCCC.......-9..** **C** ..-31..........................

65

.......................-43.. ..-8......**TGTGGGGT**GGAGGGGACAGAT

36

ATCGT..................-21.. ..-31..........................

33

ATCG...................-21.. ..-47..........................

55

A......................-27.. ..-48..........................

50

.......................-74.. ..-11........**GGGGT**GGAGGGGACAGAT

12

G......................-47.. ..-41..........................

19

ATCGT..................-21.. ..-68..........................

37

.......................-49.. ..-45..........................

11

.......................-57.. A ..-44..........................

45

ATCGT**TTTTGG**............-15.. ..-358.........................

Figure S4, related to Figure 4. DSBs induced by TALENs, wild-type Cas9, and paired nCas.

A. Relative positions of nCas9 cleavage sites. nCas9 gives rise to DSBs with 5’ overhangs that are 41 bp (NPM1+NPM2) and 37 bp (ALK1+ALK2) apart. PAM sequences are underlined; bps in italics represent overhangs.

nCas9 : Sequence after nicking both strands:

CCTCGA *GTAACCCTAATCTGATCACGGTCGGTCCATTGCATAG***AGGAGG**
GGAGCT*TGACGATGACCCAAGTGGAGTCGGAGACCTTATCGATCTTG* **TCCTCC**

Wild-type Cas9 gives rise to DSBs with blunt ends or short overhangs. The TAL^{ALK} and TAL^{NPM} cleavage sites are between the positions of the two nicks and have short 5’ overhangs (Piganeau et al., 2013).

B. Indel formation at the ALK locus after a TAL^{ALK}, Cas9(ALK1) or nCas9(ALK1+ALK2) DSB. T7 endonuclease assay.
Number of experiments : n = 4 for TAL wt, n = 8 for het and mutant TAL (4 each for X4 and L4); n = 3 for Cas9 ; n = 4 for nCas9.

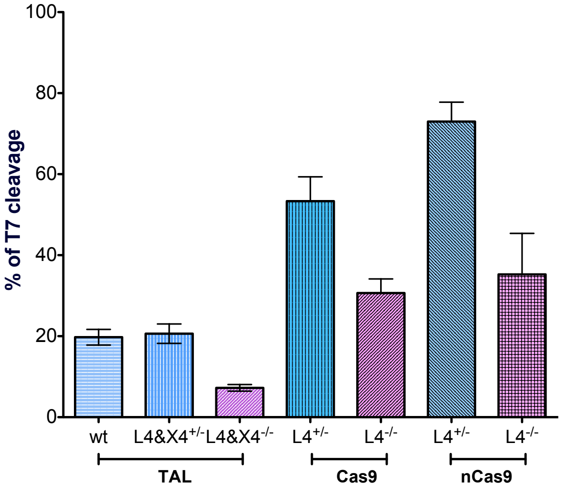
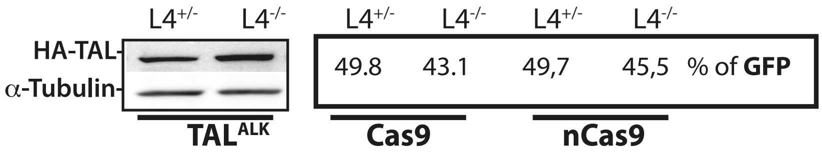


Figure S5, related to Figure 5. NPM-ALK cancer translocation junctions induced by TALENs, wild-type Cas9, and paired nCas.

A. Nuclease expression is quantified after 48 h by Western blotting for the HA epitope for TALEN or GFP expression for Cas9 and nCas9. The same blot was used to probe for both HA and tubulin.



B. Translocation junction sequences from TALENs. The TALEN recognition sequences at each DNA end are underlined; bps in italics represent the overhangs. The chromosome 5 end is in black and the chromosome 2 end is in red. Microhomologies (underlined), insertions (green) and lengths of deletions from each end are indicated.

Der5

HCT116 X4+/-

DNA ends:

GCTATATCCTCGAACTGCTACTGGGT*TCA(CCT C* **A)*ATCT*GATCACGGTCGGTCCATTGCATAGAG**

65

GCTATATCCTCGAACTGCTACTGGGT*TCA*... **AC** ..*TCT*GATCACGGTCGGTCCATTGCATAGAG

56

GCTATATCCTCGAACTGCTACTGGGT*TCA*... .....GATCACGGTCGGTCCATTGCATAGAG

57

GCTATATCCTCGAACTGCTACTGGGT*TC*.... **C** ..*TCT*GATCACGGTCGGTCCATTGCATAGAG

60

GCTATATCCTCGAACTGCTACTGGGT*T*..... **AG** .*ATCT*GATCACGGTCGGTCCATTGCATAGAG

55

GCTATATCCTCGAACTGCTACTGGGT*T*..... **GGA** .....GATCACGGTCGGTCCATTGCATAGAG

61

GCTATATCCTCGAACTGCTACTGGGT*TCA*... **+40** *AATCT*GATCACGGTCGGTCCATTGCATAGAG

40bp: from chr11 (Homo sapiens genomic DNA, chromosome 11q, clone:CMB9-3I4, complete sequence)

TTCCAGCGATCCTCCTACCTCGGCTTCCCAAAATGCTGAG

62

GCTATATCCTCGAACTGCTACTGGG-1..... **CTA** .....-8......TCGGTCCATTGCATAGAG

58

GCTATATCCTCGAAC........-11...... **CCT** ....-1ATCACGGTCGGTCCATTGCATAGAG

63

GCTATATCCTCGAACTGCTA....-6...... **+13** .....-11........GTCCATTGCATAGAG

13bp: ACGCCAGCAACGC

66

GCTATATC...............-18...... .....-6....GGTCGGTCCATTGCATAGAG

70

GCTATATCCTCGAACTGCTACTGGGT*T*..... .....-25.....................G

67

........................-27...... **A** ....*T*GATCACGGTCGGTCCATTGCATAGAG

71

```
 GCTATATCCTCGAACTGCTACTGGGTTCA...                     .....-27.......................
59
 GCTATA................-20......                      .....-25......................G
64
 ......................-47......                      .....-11........GTCCATTGCATAGAG
53
 G.....................-41......                      .....-49.......................
52
 G.....................-43......                      .....-85.......................
72
 ......................-102......        TGTT         .....-83......................
69
 ......................-203......        +14          .....GATCACGGTCGGTCCATTGCATAGAG
14bp: insertion of telomeric sequence:
TTAGGG-TTAGGG-TA
(Sequence upstream of insertion TAGAGAAGGG)
 50
 C.....................-56......                      .....-306.......................
```

HCT116 L4+/-

```
 GCTATATCCTCGAACTGCTACTGGGTTCA(CCT C                A)ATCTGATCACGGTCGGTCCATTGCATAGAG
```

```
27
 GCTATATCCTCGAACTGCTACTGGGTTCACCT                     .....GATCACGGTCGGTCCATTGCATAGAG
44
 GCTATATCCTCGAACTGCTACTGGGTTCA...                     .ATCTGATCACGGTCGGTCCATTGCATAGAG
28
 GCTATATCCTCGAACTGCTACTGGGTTCA...       TAATA         ....-1ATCACGGTCGGTCCATTGCATAGAG
26,32
 GCTATATCCTCGAACTGCTACTGGGT......                     .....-2TCACGGTCGGTCCATTGCATAGAG
39
 GCTATATCCTCGAACTGCTA....-6......        TGA          ....TGATCACGGTCGGTCCATTGCATAGAG
33
 GCTATATCCTCGAACTGCTA....-6......                     ....-1ATCACGGTCGGTCCATTGCATAGAG
35
 GCTATATCCTCGAACTGCTACTGGGTTC....                     .....-7.....GTCGGTCCATTGCATAGAG
47
 GCTATATCCTCGAACTGCTACTGG-2......                     .....-8......TCGGTCCATTGCATAGAG
48
 GCTATATCCTCGA..........-13......                     ....TGATCACGGTCGGTCCATTGCATAGAG
36
 GCTATATCCTCGAACTGCTACTGGGTTCACC.       CACCTC        .....-19................CATAGAG
25
 GCTATATCC.............-17......                      .....-15............ATTGCATAGAG
31
 GCTATATCCTCG..........-14......         T            .....-19................CATAGAG
30
 ......................-24......                      .....-16.............TTGCATAGAG
37
 GCTATAT...............-19......                      .....-43.......................
42
 CT....................-52......                      .....-54.......................
34
 ......................-56......         CCC          .....-70.......................
38
 GCTATATCCTCGAACTGCTACTGGGTTCACCTC     AGCCTCTGAA     .....-183......................
41
 GCTATATCCTCGAACTGCTACTGGGTTCACC.        +116         .....GATCACGGTCGGTCCATTGCATAGAG
116bp from TALEN expression vector
CAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGG
CGGGCCATTTACCGTAAGTTATGTAACGCG
```

HCT116 X4-/-

```
 GCTATATCCTCGAACTGCTACTGGGTTCA(CCT C               A)ATCTGATCACGGTCGGTCCATTGCATAGAG
80
 GCTATATCCTCGAACTGCTAC...-5......        AG          .....-4..ACGGTCGGTCCATTGCATAGAG
79
 GCTATATCCTCGAACTGCTACTG.-3......        C           .....-9.......CGGTCCATTGCATAGAG
74
 GCTATATCCTCGAACTGCTACTGGGTTCAC..        AC          .....-43.......................
76
 .......................-34......                    .....-19................CATAGAG
86
 .......................-50.....                     .....-5...CGGTCGGTCCATTGCATAGAG
84
 .......................-34......                    .....-32.......................
77
 AG.....................-43......                    .....-45.......................
90
 .......................-52......                    .....-52.......................
81
 TG.....................-41......                    .....-65.......................
75
 .......................-56......         AGT        .....-52.......................
95
 GCTATATCCTCGAA.........-12......    ATCCACTATAT     .....-106......................
83
 ......................-181......                    .....GATCACGGTCGGTCCATTGCATAGAG
94
 ......................-225......                    .....-2TCACGGTCGGTCCATTGCATAGAG
96
 GCTATATCCTCGAACT.......-10......                    .....-254......................
93
.......................-217.....         +28         .....-86.......................
28bp:
TTAGTAGC-TTTACACACAGTTTTCTGTA
20bp from Homo sapiens BAC clone RP11-402C9 from 4, complete sequence
TTTACACACAGTTTTCTGTA
78
 CC.....................-94......                    .....-338......................
87
 .......................-32......         CGTT       .....-475......................
```

HCT116 L4-/-

```
 GCTATATCCTCGAACTGCTACTGGGTTCA(CCT C               A)ATCTGATCACGGTCGGTCCATTGCATAGAG
81
 GCTATATCCTCGAACTGCTACTGGGTTCAC..                    .....-6....GGTCGGTCCATTGCATAGAG
56
 GCTATATCCTCGAACTGCTAC...-5......                    .....-4..ACGGTCGGTCCATTGCATAGAG
49,51
 GCTATATCCTCGAACTGCTACTGGGT......                    .....-9.......CGGTCCATTGCATAGAG
70
 GCTATATCCTCGAACTGCTACTG.-3......       CTACT        .....-6....GGTCGGTCCATTGCATAGAG
5 bp duplication from upstream of DSB on chr5
62
 GCTATATCCTCGAAC.........-11.....                    .....-6....GGTCGGTCCATTGCATAGAG
```

```
76
 GCT.....................-23.....         AA         ....TGATCACGGTCGGTCCATTGCATAGAG
71
 GCTATATCCTCG............-14.....                    .....-11........GTCCATTGCATAGAG
63
 GCTATATCCTCGAACTGCTA....-6......                    .....-43.......................
74
 GCTATATCCTCGAACTGCTACTGG-1......                    .....-48.......................
60
 ........................-36......       TAA         .....-15............ATTGCATAGAG
58
 ........................-58......                   .....-4..ACGGTCGGTCCATTGCATAGAG
54
 AGC.....................-28......                   .....-39.......................
50
 ........................-54......       GTCA        .....-54.......................
82
 G.......................-68......                   .....-50.......................
72
 GGCTGG..................-50......                   .....-85.......................
83
 TGCC....................-85......                   .....-61.......................
69
 .......................-171......                   .....-2TCACGGTCGGTCCATTGCATAGAG
65
 G......................-128......                   .....-48.......................
68
 ........................-41......                   .....-150......................
67
 GG......................-94......                   .....-107......................
59
 CCTGGCC................-163......                   .....-68.......................
64
 CTG....................-261......                   .....-35.......................
53
 GCTATATCCTC.............-15......                   .....-363......................
85
 .......................-100......        A          .....-309......................
77
 GCT....................-126......                   .....-373......................
80
 G.......................-36......                   .....-468......................
79
 .......................-212......       GTG         .....-352......................
84
 GCTATATCCTC.............-16......       +10         .....-506......................
10bp insertion
AAATCCACTA
61
 ........................-29.....        +19         .....-11.........TCCATTGCATAGAG
Insertion 19bp : 18bp from chromosome X clone RP11-366F6 map q28
AATGAATAGTTCTAGCTATT
73
 GCT.....................-23......       +69+2        .....-35.....................AG
Insertion 71bp
69bp insertion from 16 bp downstream of DSB on chr5
AGAACTACAGGCACACTCCACGCCTGGCTAATTTTTTTGTATATGTGCAGATGGGGTCTCAGTATGTTCT
66
 GCT.....................-32.....        1+49+1       .....-37........................
Insertion 51bp
49 bp insertion inverted from 37 bp downstream of DSB on chr5
CCAACATACTGAGACCCCATCTGCACATATACAAAAAAAATTAGCCAGGCT
78
 ........................-28......        +516        .....-18..............GCATAGAG
```

516bp insertion from 829bp upstream of DSB on chr2

CTGCCACTCTCGCTGATCCTCTCTGTGGTGACCTCTGCCCTCGTGGCCGCCCTGGTCCTGGCTTTCTCCGGCATCATGATTGGTGAGTGCACAGAGCCCCAGGGACTCCCAAGGGGGCAGGAAGGCAGGACTGAATAGTGTCTCAGGCTGTGCCACAGGTGCCAAGGTGTCACTTCGTTATGCTAGTCCCTGGAATTGGGTGGGGTGGTGATTAGGGCAGCCCAGGCCAAGCCAAAACGGAAGCTCCCAACCTTCCCCCCACCAGAGCAGCTGCAGTTCCCTGAGGAGCCCCTGATTCTGCACCTCAGCCCCGTGTGTATCCTCCTGGCTGATCAGGGGGTGGGGAGCTCCTTCAGTGTCCATCACGATGGTGAAAGCTCGCCCCCACCCCTAGACGTCACTTCTAGCTCCCACATGCTTCCACCGGCGCAGCTCCTGTTTGGCTCCCACCCTATGTAATGCACTAGCCCACTCTTCCCCAAACCAGCCCTCCACCACCATCCAGGCATAGAGGAG

C. Translocation junction sequences from wild-type Cas9. The PAM sequence is underlined; bp in italics represents a possible overhang.

Der5

HCT116 L4+/-

DNA ends: **GCAGTGATGTGATCATAGCTTGCTATATCCTCGA*A***		**GTAACCCTAATCTGATCACGGTCGGTCCATT**
1,10,12,14,17,23,38,39,41,45,48 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA*A*		GTAACCCTAATCTGATCACGGTCGGTCCATT
42 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA.		GTAACCCTAATCTGATCACGGTCGGTCCATT
2,3,9 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA*A*		-1TAACCCTAATCTGATCACGGTCGGTCCATT
37,43 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA.		-2.AACCCTAATCTGATCACGGTCGGTCCATT
31 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA*A*		-4...CCCTAATCTGATCACGGTCGGTCCATT
7 GCAGTGATGTGATCATAGCTTG.........-12.		GTAACCCTAATCTGATCACGGTCGGTCCATT
24 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA*A*		-14............ATCACGGTCGGTCCATT
25 GCAGTGATGTGATCATAG.............-16.		GTAACCCTAATCTGATCACGGTCGGTCCATT
44 GCAGTGATGTGATCATAGCTTGCTATATC...-5.	**A**	-11.........CTGATCACGGTCGGTCCATT
8 GCAGTGATGTGATCATAG.............-16.		-1TAACCCTAATCTGATCACGGTCGGTCCATT
20 GCAGTGATGTGATCA................-19.		-18................CGGTCGGTCCATT
28 GCAGTGATGTGATC.................-20.		-22.....................GGTCCATT
47 ....-48.		-20...................TCGGTCCATT
32 GC............................-151.		-215...........................
11 ....-248.	**38+107**	-126...........................

145bp insertion with 107bp inverted duplication from chr5 36bp upstream DSB

AGGTAGAAGGCTGGAG-TCCAGCCGTTATAATGAGACTGTCTTTATTAAAAAATAATTTTTAGGCCAGGCACAGTGGCTGACACGTGTAATACTAGCACTTTGAGAGGcCAGGGCGTGCAGATCACGTTAAGGCCAGGAGTTCAAG

HCT116 L4-/-

DNA ends: **GCAGTGATGTGATCATAGCTTGCTATATCCTCGA*A***		**GTAACCCTAATCTGATCACGGTCGGTCCATT**
5 GCAGTGATGTGATCA................-19.		-18...............CGGTCGGTCCATT
20 GCAGTGAT.......................-26.		-11........CTGATCACGGTCGGTCCATT

```
32
 GCAGTGATG......................-25.                -14...........ATCACGGTCGGTCCATT
45
 ...............................-45.                -17..............ACGGTCGGTCCATT
41
 GC.............................-32.                -80............................
19
 A..............................-81.                -36............................
15
 TAG...........................-111.                -37............................
33
 ..............................-154.                -5...CCTAATCTGATCACGGTCGGTCCATT
30
 ..............................-170.                -5...CCTAATCTGATCACGGTCGGTCCATT
40
 GCAGTGATGTGATCATAGCTTGCTATAT....-6.     +74        -195...........................
27bp inverted sequence on chr5 51bp downstream of DSB
GACCGGAATGGCTGGGCAACATACTGAGACCCCATCTGCACATATACAAAAAAAATTAGCCAGGCGTGGAGTGT
7
 GCAGTGATGTGATCATAGCTTGCTATATC...-5.      AC        -220...........................
35
 TCT...........................-122.                -117...........................
27
 GCAGTG.........................-28.                -253...........................
42
 GCAC..........................-231.                -54............................
10
 C..............................-81.                -230...........................
24
 TT............................-186.                -135...........................
11
 GC............................-279.                -80............................
29
 ...............................-57.                -317...........................
36
 GCAGTGATG......................-25.     +27        -369...........................
27bp inverted duplication on chr2 6bp downstream of DSB
CAATGGACCGACCGTGATCAGATTAG
21
 GC.............................-81.                -318...........................
12
 GCAG...........................-30.                -419...........................
48
 TC............................-304.                -148...........................
26
 TCACT.........................-301.                -201...........................
8
 GTT...........................-217.                -296...........................
46
 ..............................-277.     +17        -331...........................
17bp: CTGCCCTTCTTTAAAAT
1
 ...............................-90.     +352       -449...........................
352bp inverted duplication from chr2, 27bp downstream of DSB
CTGAGCCATGAGGACCAGGTCACAGGACCTCTTTGGACTGCAGTTTCCCTCTCTGTAGGCAGGGATGGTAACTCCTGCCCTGTTTC
CCTAACCGCTGCCACTCCCCACCCTCTAGGGTTGTCAATGAAATGAATTCACCAACATAAAATGGTTTTGAAAAATCCTAAAGAGC
TCTACCAATGTGAGTGACCATTATCACTCCTACATGTGAGGATGTTCTGGAAGGCAAACTCCATGGAAGCCAGAACAAAATTGTGA
TTCAGTGGGTAGATTCTGTGTGTAAAGCCCAGCCCCCCAACACATGGGCCAGGGCAAATGAgTCACCCGCTATGTGCTCAGTTCCC
TCCTCTATGCAATG
```

D. Translocation junction sequences from nCas9.

Der5

HCT116 L4+/-

```
DNA ends:
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    GTAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG

51
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCT...-5    -10.......TCTGATCACGGTCGGTCCATTGCATAGAGGAG
61
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCT...-5    -13..........GATCACGGTCGGTCCATTGCATAGAGGAG
76
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTG..........-13    -8......AATCTGATCACGGTCGGTCCATTGCATAGAGGAG
21
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    -24.....................GTCCATTGCATAGAGGAG
75
 CTCGAACTGCTAC..............................-33    -4..CCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG
9
 CTCGAACTGC.................................-36    -2AACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG
25
 CTCGAACTGCTACTGGGTTCACCTCAGCCTC............-15    -26.......................CCATTGCATAGAGGAG
31
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATA......-8    -26.......................CCATTGCATAGAGGAG
14,43
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAG.....-7    -37..................................AGGAG
34
 CTCGAACTGCTACTGGGTTCACCTCAGCC..............-17    -28.........................ATTGCATAGAGGAG
3
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    -47.................................-10...
45
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTA..-4+31 -43.................................-6....
31bp from chr2, from 5bp downstream of DSB
CCTAATCTGATCACGGTCGGTCCATTGCATA (followed by a 6 bp deletion)
66
 CTCGAACTGCTACTGGGT.........................-28 -7 -20..................TCGGTCCATTGCATAGAGGAG
6
 CTCGAACTGCTACTGGGTTCACCTCAG................-19    -37..................................AGGAG
54
 CTCGAACTGCTACTGGGTTC.......................-26    -31............................GCATAGAGGAG
7 bp: CGGTCCA
68
 CTCGAACTGCTACTGGGTTCACCTC..................-21    -39..................................-2GAG
16
 CTCGAACTGCTACTGGGTT........................-27    -31............................GCATAGAGGAG
23
 CTCGAACTGCTACTGGGTTCACCTCA.................-20 +9 -38.................................-1GGAG
TCAGCCTCT
60
 CTCGAACTGCTAC..............................-33    -29.........................ATTGCATAGAGGAG
33
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGG.........-12+18 -49..................................-12..
GTTCACCTCAGCCTCTGG
26
 C..-4......................................-45    -20.................GTCGGTCCATTGCATAGAGGAG
49
 CTC-2......................................-43    -27........................CATTGCATAGAGGAG
36
 CTCGAACTGCTACTGG...........................-30    -40..................................-3.AG
28
 CTCGAACTGCTA...............................-34    -41..................................-4..G
44
 CTCGAA.....................................-40    -37..................................AGGAG
72
 CTCGAACTGCTACTGGGT.........................-28    -63..................................-26..
8
 CTCGAACTGCTACTGGGTTCACCTCAGC...............-18 +8 -77..................................-40..
TGCTACTG
74
 CTCGAACTGCTAC..............................-33    -70..................................-33..
47
 CTCGAACTGCTA...............................-34    -70..................................-33..
48
 CTCGAACTG..................................-37 A  -76..................................-39..
```

```
30
 ..-15......................................-56 AC -58..................................-21..
13
 CATGC-15...................................-56    -59..................................-22..
53
 CTCGAACTGCTACTGGG..........................-29    -100.................................-63..
39
 GTGA-26....................................-67    -68..................................-31..
62
 TTG-12.....................................-53    -90..................................-53..
70
 CTCGAACTGCTACTGGGT.........................-28    -118.................................-81..
52
 G.-16......................................-57    -91..................................-54..
46
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAG.....-7 T  -155.................................-118.
18
 TCA-56.....................................-97    -69..................................-32..
11
 CTCGAACTGCTACTGGG..........................-29    -147.................................-117.
55
 CA..-19....................................-60    -122.................................-85..
2
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAG.....-7    -184.................................-147.
41
 GCT-10.....................................-51    -146.................................-109.
10
 A.-75.....................................-116    -130.................................-93..
15
 G.-12......................................-53    -212.................................-175.
19
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    -292.................................-255.
5
 CTCGAACTGCTA...............................-34    -264.................................-227.
20
 .-277.....................................-318    -31............................GCATAGAGGAG
42
 CTCA-120..................................-161    -203.................................-166.
5
 GCT-113..................................-154     -216.................................-179.
50
 CTCGAACTGC.................................-36+165-42..................................-5...
165bp from Homo sapiens chr17, clone RP11-670E13, complete sequence
TGCAGCAGGCCGGAAAGGCCCCTGTCCAAGGCCAAAACATCTTACCATTGTATTGCTGCTGCAGACAGGAAGGGGTGTATGCCTGTAGAAGTGCTGA
GGATATGTAAAGGGAAGCCAGGgCCTGCGGTGTTGACACGGGGCAAGTCAACTCACATTAGAAATGAACCA
29
 ..-31......................................-72+205-36.................................GAGGAG
205bp from Homo sapiens chr3, alternate assembly HuRef
CACAACTGGCACCATAGCCATGCCAGGTTGACAGGTGCAATTAACCATCACATTTGTCATCCAGAGAGAGTTTTTGCTATGTGATAAAAAGACATGC
TCTTTCTTTCCAGTTTTTTGTCTGTCTCCATGCAGACACAATTGACTTATTTAGAACTTATATGTATACTGTTTTTCCATTTTAAACACAAAGGTTA
TCTCATCCAATTT
27
 ..-51......................................-92+115-174.................................-137.
+115bp from pCASD10A vector
CGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGC
AGTGCTGCCATAACCATG
```

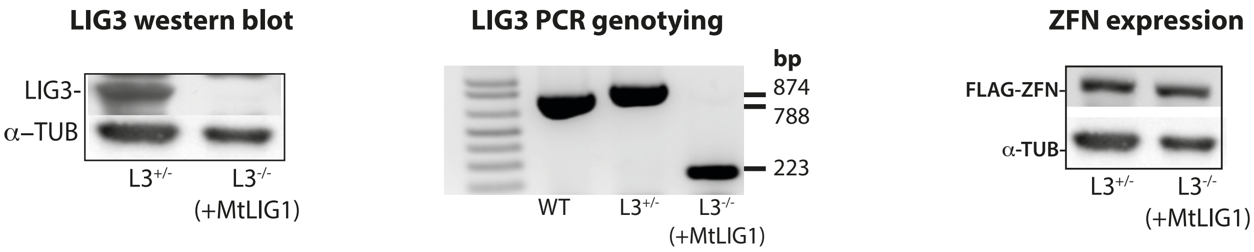
HCT116 L4-/-

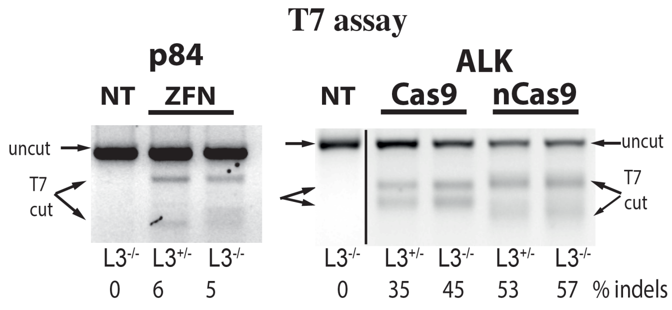
```
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    GTAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG

27,42
 CTCGAACTGCTACTG............................-31     -13..........ATCACGGTCGGTCCATTGCATAGAGGAG
30
 CTCGAACTG..................................-37     -49.................................-12..
32
 CTCGAACT...................................-38     -375................................-338.
22
 CTCGAACTGCTACTGGGTTCAC.....................-24 ACA -71.................................-34..
```

```
23
 CT.-3......................................-44     -71..................................-34..
36
 CATAGC-15..................................-56     -59..................................-22..
25
 C..-4......................................-45 ACA -494.................................-457.
28
 G..-176...................................-217     -44..................................-7...
40
 G..-35....................................-76      -500.................................-463.
21
 CT-10......................................-51     -48..................................-11..
41
 ATC-20.....................................-61     -116.................................-79..
39
 CT-39......................................-80     -272.................................-235.
14
 CTGA-22....................................-63     -50..................................-23..
24
 C...-32....................................-73     -324.................................-287.
5
 G.-31......................................-74     -400.................................-363.
4
 ATTTT-68..................................-109     -137.................................-100.
19
 GCCCTGGCC-125.............................-166     -81..................................-44..
6
 TGCC-86...................................-127     -75..................................-38..
10
 .-166.....................................-207     -174.................................-137. 2
 T-212.....................................-253     -375.................................-338.
9
 .-146.....................................-187+106 -53..................................-16..
106bp inverted insertion from chr5 117bp downstream of DSB
TCTCACTTTGGGGAGGCCAAGGCGGACCACTTGAGGCCAAGAGTCCAATCTGGGCAACATACTGAGACCCCATCTGCACATATACAAAAAAAATTAG
CCAGGCGTGTT
12
 CTCGAAC....................................-35+81 -335.................................-298..
81bp : 70bp from chr5 directly downstream of DSB
AGCCTCTGGAATAGCTAGAACTACAGGCACACTCCACGCCTGGCTAATTTTTTTTGTATATGTGCAGATG-ACACCAATTT
37
 .-8.....................................-49+389-413..................................-376..
389bp: NPM inverted sequence 310 bp downstream of DSB
GGACAAATTCCTTTTTTTGTTAAGATTCCGGAAGTATATCAAGTTTCTAAAATCATACTTACTCTTCATCATCATCCTCTTCATCATCATCGTCATC
ATCTTCATCAGCAGCAAGATTTACTTTTTTCTTTTAAAAGAAAAGATACGTACTCATGAATAAGAGCTGTTCTATCACCAAACTAAAAGCTATAATA
CAAAACCTATAATAAAATTGCCATCTCTACCTGTGGAACCTTGCTACCACCTCCAGGGGCAGACCGCTTTCCAGATATACTTAAGAGTTTCACATCC
TCCTCCTCTTCATCTTCTGACTCTGCATCTTCCTCCACAGCTAAATACAATTTATTAGACATTATAAAACTCAAGCAGCAAGAAGTCAGAAAAAAAC
T
29
 CTCGAACTGCTACTGGGTT........................-27+512 -29..........................TGCATAGAGGAG
512bp : 506bp from NPM 1077bp downstream of DSB
TCTTATGGTTTTATGTAGATATTTATTGACAAAAATAAGATTCTAAAAGGGATATTAAGATTTTCTTGGGATTTAAAATATGGTTGGAAACAATATT
TGATGACTTTATATTAAACTAGATCAAACTATTGTTACAAACAGTTAATACGCACACTGGTATAAAGTACTGTTTATAATTGGTCTTATGTGTGCCA
GTACCAGTAATGCATTGAATATGATTTGGCTCTCAGCTTTGTCCTTCAGTTCTGAGGTTGGTCCATATGCATTTATTGAAAACAAATATAAGAACAT
GCACTTTAAAAGAGAACCTGCATGAAAGATCAAATTGGGAGTTTAGGTTTTAAGCTGGTGGTTCTTCAAAATCTTTGAGCATGACGATGAAGGCAGA
AAACAGGAAAAAGGCCGAAAGAGCCGAAAGCTTAAAAATTCAAAGTATGACCAGGCGCAGTGGTTCACACCCGTAATCCTAACACCCAGCCAAGATG
AGTCTATGCAATGCAAATGAGTCTATG
```

Figure S6, related to Figure 6. LIG3 deficiency is not required for translocation formation in human cells.

A. LIG3 null cells: Western blot, PCR genotyping, ZFN expression, and indel formation. L3$^{-/-}$ HCT116 cells are viable due to MtLIG1 expression, as seen in mouse cells (Simsek et al., 2011a).
In the westerns, the same blot was used to probe for both epitopes.





Indel formation is not affected by LIG3 loss, as monitored by the T7-endonuclease assay in L3$^{+/-}$ and L3$^{-/-}$ cells expressing MtLIG1 (abbreviated L3$^{-/-}$). Two loci were examined, p84/AAVS1 using ZFN^{p84} and ALK using either wild-type Cas9 with gRNA ALK1 or nCas9 with gRNAs ALK1+ALK2. NT, not transfected (i.e., no nuclease).

B. Translocation junction sequences from ZFNs. The ZFN recognition sequences at each DNA end are underlined; bps in italics represent the overhangs. The chromosome 19 end is in black and the chromosome 22 end is in red. Microhomologies (underlined), insertions (green) and lengths of deletions from each end are indicated.

Der22

HCT116 Lig3+/-

ATCGTTTTTGGCCTCCCTATCAGTCA*TT*	***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**
6,47,52,69,75	
ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT*	..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
2,29,36,45,54,68,73	
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T.*	..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
62	
ATCGT**TTTTGGCCTCCCTATCA**GTCA*..*	..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
20	
ATCGT**TTTTGGCCTCCCTATCA**GTCA*T.*	.-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
64	
ATCGT**TTTTGGCCTCCCTATCA**GTCA*..*	.-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
11	

ATCGT**TTTTGGCCTCCCTATCA**GTC-1.   .TGGCC**CCACTGTGGGGT**GGAGGGGACAGAT

58

ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT*   CAT   ..-4..**CCACTGTGGGGT**GGAGGGGACAGAT

51

ATCGT**TTTTGGCCTCCCTATCA**GTCA*T.*   ..-5...**CACTGTGGGGT**GGAGGGGACAGAT

12

ATCGT**TTTTGGCCTCCCTATC**...-5..   .TGGCC**CCACTGTGGGGT**GGAGGGGACAGAT

39

ATCGT**TTTTGGCCTCCCT**......-8..   .TGGCC**CCACTGTGGGGT**GGAGGGGACAGAT

33

ATCGT**TTTTGGC**...........-14..   .TGGCC**CCACTGTGGGGT**GGAGGGGACAGAT

15

ATCGT**TTTTGGCC**..........-13..   ..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT

24

ATCGT**TTTTGGCC**..........-13..   ..-6....**ACTGTGGGGT**GGAGGGGACAGAT

8

ATCGT**TTTTGGCCTCCCTA**.....-7..   ..-11........**GGGGT**GGAGGGGACAGAT

76

ATCGT**TTTTG**.............-16..   **+17**   ..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT

17 bp : ACCCCCGGCATCCCCAC

4

ATCGT**TTT**...............-18..   ..-16.............GGAGGGGACAGAT

44

A......................-28..   ..-24..........................

21

A......................-28..   ..-33..........................

28

A......................-34..   ..-40..........................

27

.......................-43..   **T**   ..-41..........................

66

GTA....................-45..   ..-36..........................

22

CA.....................-41..   ..-52..........................

61

.......................-64..   **AG**   ..-60..........................

77

A.....................-100..   ..-110.........................

35

......................-108..   ..-115.........................

3

TA....................-205..   ..-36..........................

37

GAG....................-36..   ..-158.........................

32

.......................-37..   **+78**   .TGGCC**CCACTGTGGGGT**GGAGGGGACAGAT

78bp from ZFN plasmid

AGGTGATGGAGTTCTTCATGAAGGTGTACGGCTACAGGGGAAAGCACCTGGGCGGAAGCAGAAAGCCTGACGGCGCCA

19

ATCGT**TTTTGGCCTCCCTATCA**G.-3..   **+94**   ..-8......**TGTGGGGT**GGAGGGGACAGAT

94bp from Homo sapiens chromosome 10, alternate assembly HuRef

GGACAATGCCCTATGAGAAGATGGGAGAGAAATCAGAAAGAGGGCCCCTGGAAGACTGTAAGGGCCGGGACAGCCTGCAA
CCTGGACATTATTT

HCT116 Lig3-/-

ATCGTTTTTGGCCTCCCTATCAGTCA*TT*   ***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**

3,8,37,39,43

ATCGT**TTTTGGCCTCCCTATCA**GTCA*T.*   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT

9,31

ATCGT**TTTTGGCCTCCCTATCA**GT-2..   ..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT

48	ATCGT**TTTTGGCCTCCCTATCA**GTCA..		.-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
5	ATCGT**TTTTGGCCTCCCTATCA**GT-2..	G	*GT*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
23	ATCGT**TTTTGGCCTCCCTATCA**GTC-1.	TC	..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
4	ATCGT**TTTTGGCCTCCCTATCA**..-4..		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
45	ATCGT**TTTTGGCCTCCCTATCA**G.-3..		.-1GCC**CCACTGTGGGGT**GGAGGGGACAGAT
14	ATCGT**TTTTGGCCTCCCTATC**...-5..		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
10	ATCGT**TTTTGGCCTCCCTATCA**G.-3..		..-3.C**CCACTGTGGGGT**GGAGGGGACAGAT
30	ATCGT**TTTTGGCCTCCCTAT**....-6..		..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
20	ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.		..-5...**CACTGTGGGGT**GGAGGGGACAGAT
44	ATCGT**TTTTGGCCTCCCTATCA**GTCA*T*.		..-9......**GTGGGGT**GGAGGGGACAGAT
11	ATCGT**TTTTGGCCTCCCTA**.....-7..		..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
18	ATCGT**TTTTGGCCTCCCT**......-8..		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
17	ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT*		..-9......**GTGGGGT**GGAGGGGACAGAT
12	ATCGT**TTTTGGCC**..........-13..		..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
15	.......................-28..		..-23....................ACAGAT
36	ATC....................-23..		..-37..........................
1	TGGG...................-54..		..-69..........................
6	.......................-98..		..-19................GGGGACAGAT
38	.......................-52.		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
42	.......................-68.		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
19	......................-164..		..-29..........................
29	.......................-96..		..-83..........................
7	C.....................-172..		..-176.........................
26	CAT...................-199..		..-141.........................
16	.......................-30..	**+171**	..-35..........................

171bp insertion from chr22 45bp downstream of DSB
TTGATAGTCAAGTAAAAGCTATGTTTTTTTGTTGCTGTTTGTTTATATCCATTAAGGGGAAAAATGGCCAGGCATGGTGGCT
CACACCTGTAATCCCAGCACTTTGGGGGAGGCCAAGGCAGGAGGATCACTTGAGACCAGGAGTTTGAGACCACCCTGGGC
AACATAGTGAGACCC

C. Junction sequences from Cas9. The PAM sequence is underlined; bp in italics represents a possible overhang.

Der5

HCT116 Lig3+/-

```
DNA ends:
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                 GTAACCCTAATCTGATCACGGTCGGTCCATT

1,9,12,14,19,31,32,35,47
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                 GTAACCCTAATCTGATCACGGTCGGTCCATT
7,17,28,38,40
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGA.                 GTAACCCTAATCTGATCACGGTCGGTCCATT
10
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA        C       -1TAACCCTAATCTGATCACGGTCGGTCCATT
36
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                -8.......AATCTGATCACGGTCGGTCCATT
45
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                -19................GGTCGGTCCATT
5
 GCAGTGATGTGATCATAGCTTGCTATATCCT.-3.                 GTAACCCTAATCTGATCACGGTCGGTCCATT
42
 ..............................-48.                  GTAACCCTAATCTGATCACGGTCGGTCCATT
13
 ..............................-52.                  GTAACCCTAATCTGATCACGGTCGGTCCATT
26
 C.............................-32.                 -47............................
39
 TGTG..........................-88.                 -87............................
6
 C.............................-94.                 -59............................
43
 AAA...........................-76.                 -354...........................
41
 GCAGTGATGTGATCATAGCTTGCTATATCCT.-3.                -329...........................
18
 .............................-279.                 -13..........GATCACGGTCGGTCCATT
```

HCT116 Lig3-/-

```
DNA ends:
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                 GTAACCCTAATCTGATCACGGTCGGTCCATT

12,15,20,22,24,32,37,39
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                 GTAACCCTAATCTGATCACGGTCGGTCCATT
18
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA        C        GTAACCCTAATCTGATCACGGTCGGTCCATT
16,28
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA       CT        GTAACCCTAATCTGATCACGGTCGGTCCATT
11
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                -1TAACCCTAATCTGATCACGGTCGGTCCATT
26
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                -3..ACCCTAATCTGATCACGGTCGGTCCATT
38
 GCAGTGATGTGATCATAGCTTGCTATATCCTCG-1                -1TAACCCTAATCTGATCACGGTCGGTCCATT
29
 GCAGTGATG.....................-25.                 -20.................GTCGGTCCATT
23
 GCAGTGATGTGATCATAGCTTGCTATATCCTCGAA                -138...........................
15
 .............................-106.                 -21...................TCGGTCCATT
35
 ..............................-63.                 -42............................
25
 .............................-107.                 -74............................
36
 .............................-234.                GTAACCCTAATCTGATCACGGTCGGTCCATT
21
 A.............................-56.                 -113...........................
34
```

```
 CAG...........................-55.                    -337...........................
31
 GCAG..........................-32.                    -419...........................
```

D. Junction sequences from nCas9. The bps in italics represent overhangs.

Der5

HCT116 Lig3+/-

```
DNA ends:
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    GTAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG
30
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    -28.........................ATTGCATAGAGGAG
7
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC GT -33..............................ATAGAGGAG
34
 CTCGAACTGCTACT.............................-32   -1TAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG
2
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGC....-6    -41..................................-4..G
29
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGA........-11    -36.................................GAGGAG
1
 CTCGAACTGCTACTGGGTTCACC....................-23 G  -26.......................CCATTGCATAGAGGAG
19
 CTCGAACTGCTACTGGGTTCACCTCAGC...............-18    -33..............................ATAGAGGAG
33
 CTCGAACTGCTACTGGG..........................-29    -22...................CGGTCCATTGCATAGAGGAG
13
 CTCGAACTGCT................................-35 CG -33..............................ATAGAGGAG
37
 CTCG-1.....................................-41    -27........................CATTGCATAGAGGAG
22.
 CTCGAAC....................................-39    -32.............................CATAGAGGAG
35
 CTCGA......................................-41    -34...............................TAGAGGAG
18
 CTCGAACTGCTACTG............................-31    -45..................................-8...
36
 CTCGAACTGCTACTG............................-31    -49..................................-12..
5
 CTCGAACTGCTACTGGGTTCACCTCAGCCT.............-16    -77..................................-40..
27
 ..-18.....................................-59    -37..................................AGGAG
32
 A..-9......................................-50    -50..................................-13..
31
 CATAGC-15..................................-56    -59..................................-22..
38
 CAT-18....................................-59    -84..................................-47..
6
 .-113....................................-154+26 -19................GGTCGGTCCATTGCATAGAGGAG
26bp : CGGCACTTTACAACCTAAATGCCCATCAGT
3
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGG.........-12GTTC-168.................................-131.
24
 ..-50......................................-91    -98..................................-61..
26
 GA-36......................................-77    -113..................................-76..
23
 .-129.....................................-170  C -35................................AGAGGAG
11
 CTCGAACTGCTACTGGG..........................-29    -185.................................-148.
10
 A-112.....................................-153    -150.................................-113.
8
 .-207.....................................-248    -64..................................-27..
21
TCGAACTGCTAC(TGGGTTCACCTCAGCCTCTGGAATAGCTAGA-2+304 GTAACCCTAATCTGATCACGGTCGGtCCATTGCATA)GAGGAG
```

+304 bp:

repeats of 36bp from ALK directly downstream of DSB + repeats of 31bp of NPM 2bp upstream of DSB:
(GTAACCCTAATCTGATCACGGTCGGTCCATTGCATA)(TGGGTTCACCTCAGCCTCTGGAATAGCTAGAGTAACCCTAATCTGATCACGGTCGGTCCATTGCATA)(TGGGTTCACCTCAGCCTCTGGAATAGCTAGAGTAACCCTAATCTGATCACGGTCGGtCCATTGCATA)(TGGGTTCACCTCAGCCTCTGGAATAGCTAGAGTAACCCTAATCTGATCACGGTCGGtCCATTGCATA)(TGGGTTCACCTCAGCCTCTGGAATAGCTAGA)

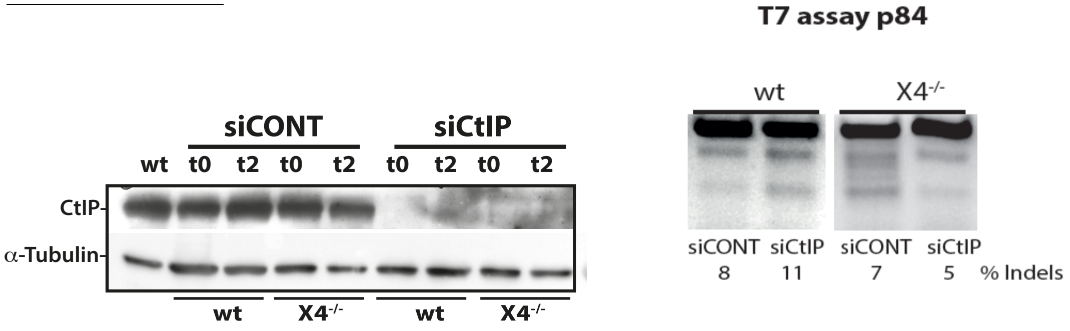
HCT116 Lig3-/-

```
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    GTAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG

35
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC    -32.............................CATAGAGGAG
49
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAG.3- T  -34...............................TAGAGGAG
26
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAG.....-7    -37..................................AGGAG
8
 CTCGAACTGCTACTGGGTTCACCTCAGCC..............-17    -28.........................ATTGCATAGAGGAG
46
 C..-4......................................-45+24-1TAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG
24bp repeat from chr2 directly downstream of DSB
CCTAATCTGATCACGGTCGGTCCA
41
 CTCGAACTGCTACTGGGTTCA.....................-25    -23....................GGTCCATTGCATAGAGGAG
4
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAG.....-7    -44..................................-7...
22
 CTCGAACTGCTACTGGGTTC......................-26    -27........................CATTGCATAGAGGAG
39
 CTCGAACTGCTACTGGG.........................-29    -24.....................GTCCATTGCATAGAGGAG
21
 CTCGAACTGCTACTGG..........................-30    -25......................TCCATTGCATAGAGGAG
32
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCT...........-14+24 -43..................................-8...
30
 CTCGA......................................-41 C  -21..................TCGGTCCATTGCATAGAGGAG
2
 CTCGAACTG..................................-37    -33..............................ATAGAGGAG
37
 CTCGAACTGC.................................-36    -33..............................ATAGAGGAG
48
 CTCGAACTGCTACTGG..........................-30    -40..................................-3.AG
42
 CTCGAACTGC.................................-36    -61..................................-24..
6
 CTCGAACT...................................-38 T  -69..................................-32..
9
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGG.........-12    -99..................................-62..
45
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGG.........-12    -99..................................-62..
43
 ..-81.....................................-122    GTAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGGAG
24
 CAT-18.....................................-59    -84..................................-47..
31
 CA-19......................................-60    -107.................................-70..
34
 CAC-92....................................-133    -54..................................-17..
17
 C..-4......................................-41    -159.................................-122.
40
 T-64......................................-104    -147.................................-110.
13
 CTCGAACTGCTACT.............................-32    -220.................................-183.
3
 CAC-103...................................-144    -123.................................-86..
18
 GTGGC-82..................................-123    -146.................................-108.
27
 TG.-90....................................-131    -159.................................-122.
```

```
7
 T..-73...................................-114     -180.................................-143.
38
 A-141.....................................-182    -169.................................-132.
28
 ..-187....................................-228    -90..................................-53..
33
 CTCGAA.....................................-40    -309.................................-272.
36
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAA.......-10 +80-33..............................ATAGAGGAG
80bp mixed repeats (chr5 and chr2) and insertions
CTGGGTTCACCTCAGCCTCTGGAA-CACGGTCGGTCCATTGC-TGGGTTCACCTCAGCCTCTGGAA-CCACGGTCGG-AACCAC
24bp GGACCGACCGCCTCTGGACCGTGA
1
 CTCGAACTG..................................-37+4+41-123................................-86..
45bp : 41bp inverted sequence 83bp downstream from DSB from ALK
GAATAGTGGGTAGATTCTGTGTGTAAAGCCCAGCCCCCCAACACA
44
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC+134-61..................................-24..
134bp
31bp from NPM directly downstream of the DSB
GCTACTGGGTTCACCTCAGCCTCTGGAATAG
103bp partly from CASD10A-coding plasmid
CTAGCCTTTTGCTCACATGTCATATGTAGAGAGGTACCTCGAGCGGCCCAAGCTTAAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGAT
AACGGACTA

14
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAAC+188-80..................................-43..
188 bp : patial repeat duplications of the overhang of NPM directly upstream of DSB
(CTGCTACTGGGTTCACCTCAGCCTCTGGA-CCTCAGCCTCTGGAATAGCTAGAAC)
(CTGCTACTGGGTTCACCTCAGC)
(CTGCTACTGGGTTCACCTCAGCCTCTGGA               ATAGCTAGAAC)
(CTGCTACTGGGTTCACCTCAGCCTCTGGA-CCTCAGCCTCTGGAATAGCTAGAAC)
(CTGCTACTGGGTTCACCT)

25
 CTCGAACTGCTACTGGGTTCACCTCAGCCTCTGGAAT.......-9+276-89..................................-52..
+276 bp from CAS9 coding plasmid
TACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCA
TTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGG
CAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGgcCCGCCTGGCATTATGCCCA
```

Figure S7, related to Figure 7. Translocation junction sequences from ZFN^{EWS} and ZFN^{p84} expression after CtIP depletion.

A. Indel formation at the ZFN^{p84} site is minimally affected by CtIP knockdown, as monitored by the T7-endonuclease assay in either wild-type or XRCC4-deficient HCT116 cells. Western blot of CtIP knock-down is shown on the left. The same blot was used to probe for both CtIP and tubulin.



B. Translocation junction sequences from ZFNs in wild-type cells. The ZFN recognition sequences at each DNA end are underlined; bps in italics represent the overhangs. The chromosome 19 end is in black and the chromosome 22 end is in red. Microhomologies (underlined), insertions (green) and lengths of deletions from each end are indicated.

Der22

HCT116 wt-SiCONT

```
DNA ends:
 ATCGTTTTTGGCCTCCCTATCAGTCATT                            GTGGCCCCACTGTGGGGTGGAGGGGACAGAT

3,5,25,27,31,40
 ATCGTTTTTGGCCTCCCTATCAGTCATT                            ..GGCCCCACTGTGGGGTGGAGGGGACAGAT
4,9,20,39
 ATCGTTTTTGGCCTCCCTATCAGTCAT.                            ..GGCCCCACTGTGGGGTGGAGGGGACAGAT
35
 ATCGTTTTTGGCCTCCCTATCAGTCAT.              AC            ..GGCCCCACTGTGGGGTGGAGGGGACAGAT
38
 ATCGTTTTTGGCCTCCCTATCAGTCAT.                            ..-3.CCCACTGTGGGGTGGAGGGGACAGAT
24
 ATCGTTTTTGGCCTCCCTATCAGTC-1.                            ..-4..CCACTGTGGGGTGGAGGGGACAGAT
23
 ATCGTTTTTGGCCTCCCTATCAG.-3..                            .-1GCCCCACTGTGGGGTGGAGGGGACAGAT
22
 ATCGTTTTTGGCCTCCCTATCAGTCAT.                            ..-5...CACTGTGGGGTGGAGGGGACAGAT
13
 ATCGTTTTTGGCCTCCCTATCAGTCAT.                            ..-11........GGGGTGGAGGGGACAGAT
34
 ATCGTTTTTG.............-16..                            ..-2CCCCACTGTGGGGTGGAGGGGACAGAT
33
 ATCGTTTTTGGCCTCCCTATCAG.-3..                            ..-12.........GGGTGGAGGGGACAGAT
42
 CA.....................-41..                            ..-52.........................
12
 GTA....................-45..                            ..-36.........................
29
 CAG....................-45..                            ..-47.........................
```

HCT116 wt-SiCtIP

	ATCGTTTTTGGCCTCCCTATCAGTCA*TT*		***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**
65,71,85	ATCGT**TTTTGGCCTCCCTATCA**GTCA*TT*		..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
81,84,88	ATCG**TTTTTGGCCTCCCTATCA**GTCA*T*.		..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
82	ATCG**TTTTTGGCCTCCCTATCA**GTCA*T*.	**ATAC**	*GT*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
63,66	ATCG**TTTTTGGCCTCCCTATCA**GTCA*T*.		.-*1*GCC**CCACTGTGGGGT**GGAGGGGACAGAT
72	ATCG**TTTTTGGCCTCCCTATCA**GTC-1.	**CTC**	..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
54	ATCG**TTTTTGGCCTCCCTATCA**GTCA*T*.	**C**	..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
59	ATCG**TTTTTGGCCTCCCTATCA**GTC-1.		..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
56	ATCG**TTTTTGGCCTCCCTATCA**GT-2..		..GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
78	ATCG**TTTTTGGCCTCCCTATCA**..-4..		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
55	ATCG**TTTTTGGCCTCCCTATCA**..-4..		..-2CC**CCACTGTGGGGT**GGAGGGGACAGAT
52	ATCG**TTTTTGGCCTCCCT**......-8..		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
51	ATCG**TTTTTGGCCTCCCTATCA**GTCA*T*.		..-11........**GGGGT**GGAGGGGACAGAT
80	.......................-32..		.*T*GGCC**CCACTGTGGGGT**GGAGGGGACAGAT
95	ATCG**TTTTTGGCCTCCCTATCA**GTCA*T*.		..-195........................

C. Translocation junction sequences from ZFNs in X4$^{-/-}$ cells.

HCT116 X4-/-siCTRL

	ATCGTTTTTGGCCTCCCTATCAGTCA*TT*		***GT*GGCCCCACTGTGGGGTGGAGGGGACAGAT**
15	ATCGT**TTTTGGCC**..........-13..		..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
33	......................-31..		..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
15	ATCGT**TTTTGGCC**..........-13..	**A**	..-25.....................AGAT
4	......................-33..	**CTAGAG**	..-4..**CCACTGTGGGGT**GGAGGGGACAGAT
6	ATCGT**T**.................-20..	**GTCAC**	..-24....................CAGAT
17	AT....................-24..		..-27........................
22	A.....................-25..	**GAA**	..-50........................
47	GA....................-36..		..-42........................
39	AT....................-24..	**TC**	..-41........................

```
37
 AGTA..................-34..                      ..-36..........................
19
 .......................-38..                      ..-24..........................
46
 .......................-36..         G            ..-31..........................
12
 AC.....................-26..                      ..-37..........................
13
 AG.....................-36..                      ..-41..........................
42
 .......................-44..                      ..-54..........................
11
 CAG....................-40..          G           ..-41..........................
35
 G......................-53..                      ..-49..........................
8
 .......................-50..                      ..-51..........................
7
 TGG....................-55..                      ..-81..........................
2
 GAG....................-36..                      ..-49..........................
10
 CAGA...................-68..                      ..-48..........................
3
 AGCC...................-68..                      ..-51..........................
38
 ......................-218..                      ..-4..CCACTGTGGGGTGGAGGGGACAGAT
20
 AA.....................-86..                      ..-164.........................
32
 GGGGA..................-52..                      ..-253.........................
27
 ......................-362..          T           ..-18..............AGGGGACAGAT
40
 ......................-315..                      ..-32..........................
5
 AAGA..................-162..                      ..-319.........................
16
 ......................-264..         ATCTG        ..-26..........................
26
 TTGT...................-46..                      ..-375.........................
29
 CC....................-126..                      ..-185.........................
21
 ATCGTTTTTGGCC..........-13..                      ..-432.........................
18
 AAG...................-109..                      ..-408.........................
41
 ......................-331..                      ..-194.........................
45
 CAA....................-27..                      ..-426.........................
31
 .......................-28..        +108          ..-393.........................
108 bp inverted insertion from 1321 bp upstream the DSB on chr19
GCATCGCCCCCCTGCTGTGGCTGTTCCCAAGTTCTTAGGGTACCCCACGTGGGTTTATCAACCACTTGGTGAGGCTGGTACCCTGC
CCCCATTCCTGCACTGCCATGG
```

HCT116 X4-/-siCtIP

```
 ATCGTTTTTGGCCTCCCTATCAGTCATT                      GTGGCCCCACTGTGGGGTGGAGGGGACAGAT
```

```
43
 ATCGTTTTTGGCCTCCCTATCA..-4..                       ..-7.....CTGTGGGGTGGAGGGGACAGAT
46
 ATCGTTTTTGGCCTCCC.......-9..                       ..-6....ACTGTGGGGTGGAGGGGACAGAT
38
 ATCGTTTTTGGCC..........-13..                       ..-4..CCACTGTGGGGTGGAGGGGACAGAT
4
 ATCGTTTTTGGCCTCCCTAT.....-6..         GA           ..-13..........GGTGGAGGGGACAGAT
1,56,73
 ATCGTTTTTGG.............-15..                       ..-13..........GGTGGAGGGGACAGAT
2
 AT.....................-24..          A            ..-5...CACTGTGGGGTGGAGGGGACAGAT
66
 ATCGTTTTTGGCCTCCCTAT.....-6..                       ..-24.....................CAGAT
85
 ATC....................-23..                       ..-7.....CTGTGGGGTGGAGGGGACAGAT
93
 ATCGTTTTTGG.............-15..                       ..-15............TGGAGGGGACAGAT
8
 CA.....................-25..                       ..-7.....CTGTGGGGTGGAGGGGACAGAT
29
 .......................-26..                       ..-6....ACTGTGGGGTGGAGGGGACAGAT
74
 ATCGTTTTTGGCCTCCCTATCAGT-2..                       ..-35..........................
20
 ATCGTTTTTGG.............-15..                       ..-22...................GACAGAT
82
 ATCGTTTTTGGCCTCCCTATCAG.-3..          AA           ..-37..........................
35
 ATCGTTTTTGG.............-15..                       ..-31..........................
18
 A......................-28..                       ..-24.....................CAGAT
59
 CA.....................-25..                       ..-40..........................
16
 .......................-34..        GTCAAGT        ..-31..........................
65
 .......................-43..                       ..-24.....................CAGAT
92
 ATCGTT.................-20..         GA            ..-47..........................
26
 CA.....................-58..                       ..-10.......TGGGGTGGAGGGGACAGAT
30
 AGTA...................-34..                       ..-36..........................
23
 ATC....................-24..        CTAGTAT        ..-48..........................
5
 ATCGTTTTTGGCCTCCCT......-8..                       ..-66..........................
55
 .......................-40..         +10           ..-37..........................
10bp:ACAAAACATT
70
 .......................-50..          AC           ..-29..........................
21
 CAG....................-40..                       ..-41..........................
52
 .......................-29..          G            ..-373.........................
85
 .......................-28..         +23           ..-61..........................
23bp:CATTAAGGATTGGTGACAGAAAA
7
 AG.....................-40..                       ..-49.........................
78
 C......................-60..                       ..-45..........................
87
```

```
 .......................-28..       GAGCTGT         ..-80.........................
13
 G.....................-84..                        ..-49.........................
33
 A.....................-28..                        ..-132........................
62
 ATCGTTTTT.............-17..                        ..-141........................
83
 ......................-169..                       .TGGCCCCACTGTGGGGTGGAGGGGACAGAT
31
 ......................-175..                       .-1GCCCCACTGTGGGGTGGAGGGGACAGAT
40
 ......................-183..                       ..-5...CACTGTGGGGTGGAGGGGACAGAT
61
 ......................-151..                       ..-38.........................
84
 GC....................-42..                        ..-147........................
42
 .......................-31..                       ..-173........................
19
 GAG...................-107..                       ..-111........................
44
 ......................-265..                       ..-5...CACTGTGGGGTGGAGGGGACAGAT
54
 ......................-323..                       ..-4..CCACTGTGGGGTGGAGGGGACAGAT
80
 ATCGTTTTTGGC..........-14..                        ..-323........................
50
 CAG...................-40..                        ..-47.........................
6
 CAA...................-27..                        ..-406........................
34
 ACATC.................-24..                        ..-436........................
58
 CAG...................-299..                       ..-312........................
51
 ATCGTTTTTGGCC.........-13..          +30           ..-50.........................
30bp duplication from 215 bp upstream of DSB on chr22
ACTGCGCCCAGCCACGTTTGGAGTTTTTGAACAGGGGGAATACTCTTTGCCATTGTTTGCTTTTGGAATCCAGGA
37
 .......................-44..         +52           ..-2CCCCACTGTGGGGTGGAGGGGACAGAT
52 bp from chr7 Homo sapiens BAC clone RP11-310H4 from 7, complete sequence
TCCAGTGTTTCTTGTGGTTTGTGCTGAAACCTTTGGTTCCCCTCCAGGTCCTCCCCA
24
ATCGTTTTT..............-17..          +46           ..-448........................
46bp inverted insertion from chr19, 333bp downstream of DSB
CTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCT
```

Supplemental Experimental Procedures

Nucleases

All nucleases target intronic sequences. ZFN$^{\text{EWS}}$, ZFN$^{\text{FLI1}}$, and ZFN$^{\text{P84}}$ have been described (Brunet et al., 2009; Piganeau et al., 2013). ZFN$^{\text{FLI-A}}$ and ZFN$^{\text{FLI-B}}$ were provided by Sangamo BioSciences, Inc. with an obligate heterodimer architecture through modification of the FokI nuclease domain (right ZFN FokI KK and left ZFN FokI EL) (Miller et al., 2007) and have the following recognition sites and helices. All ZFNs have FLAG epitope tags.

ZFN Binding Sequence (underlined)	ZFN	Finger 1	Finger 2	Finger 3	Finger 4	Finger 5
CCTAAGCCCTTTCCTTCATTTGCCAGGAGTAGAGAGGACA	FLI-A-R	DNPNLNR	RSDDLSR	QRTHLTQ	RSANLAR	RSDNLRE
GGATTCGGGAAAGGAAGTAAACGGTCCTCATCTCTCCTGT	FLI-A-L	RSDNLST	QSSDLRR	RSDSLSV	QNQHRIN	QSGNLAR
GCCCCCTGGCCCAGGTGTCCCCGATGAAAAGCAGGTTA	FLI-B-R	HRSSLRR	RSDNLSE	RNANRIT	QSGNLAR	TSGNLTR
CGGGGGACCGGGTCCACAGGGGCTACTTTTCGTCCAAT	FLI-B-L	RSDHLSR	RSDHLTQ	ERGTLAR	RSDHLTT	DQSTLRN

TALEN assembly and TAL$^{\text{ALK}}$ and TAL$^{\text{NPM}}$ have been described (Piganeau et al., 2013). The sequences of TAL$^{\text{p84}}$ and TAL$^{\text{LAM}}$ are shown below with the TAL DNA binding domains underlined. TAL$^{\text{ALK}}$ and TAL$^{\text{p84}}$ have HA epitope tags; TAL$^{\text{LAM}}$, and TAL$^{\text{NPM}}$ have FLAG epitope tags.

TAL$^{\text{p84}}$ subunits were assembled as described (Huang et al., 2011). For each TALEN subunit, the fragment containing the 16 RVD segment was obtained from single unit vectors: A (NI), T (NG), G (NN) and C (HD), kindly provided by the laboratory of Bo Zhang (Beijing) and subcloned in the pCS2 vector containing the wild-type FokI domain.

TAL$^{\text{p84}}$ recognition sequence (underlined):
TTTTCTGTCACCAATCCTGTCCCTAGTGGCCCCACTGTGGGGTGGAGGGGA

TAL$^{\text{p84-L}}$:
MAPKKKRKVYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAAHGTVDLRTLGYSQQQQEKIKPKVR
STVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARA
LEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASHD
GGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGL
TPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQ
AHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLP
VLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQ
RLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQAL
ETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNNGG
KQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGRPALESIVAQLSRPDPALAALTNDHLVALA
CLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA

TAL$^{\text{p84-R}}$:
MAPKKKRKVYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAAHGTVDLRTLGYSQQQQEKIKPKVR
STVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARA
LEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPEQVVAIASNG
GGKQALETVQRLLPVLCQAHGLTPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAI
ASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGL
TPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQ
AHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHDGGKQALETVQRLLP
VLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQ
RLLPVLCQAHGLTPDQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQAL
ETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGG
KQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGRPALESIVAQLSRPDPALAALTNDHLVALA
CLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA

TAL$^{\text{LAM}}$ cleaves the lamin locus on Chr1. Subunits were directly synthetized by Geneart as described (Miller et al., 2011) using the RVD: A (NI), T (NG), G (NK) and C (HD) and subcloned in the pVax vector containing the wild-type FokI domain.

TAL$^{\text{LAM}}$ recognition sequence (underlined):
TTGCTCCCGTTCTCTCTTCTTTTCCTCTTAAGCTCAGAGTAGCTA

TAL$^{\text{LAM-L}}$:
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPMVDLRTLGYSQQQQEKIKPKVRSTVA
QHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEAL
LTVAGELRGPPGTLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPDQVVAIASNGGGKQ
ALETVQRLLPVLCQDHGLTPEQVVAIASNKGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHD
GGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAI
ASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNKGGKQALETVQRLLPVLCQDHGL
TPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNGGGKQALETVQRLLPVLCQ
AHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNGGGKQALETVQRLLP
VLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGRPALESIV
AQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA

TAL$^{\text{LAM-R}}$:
MDYKDHDGDYKDHDIDYKDDDDKMAPKKKRKVGIHGVPMVDLRTLGYSQQQQEKIKPKVRSTVA
QHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDMIAALPEATHEAIVGVGKQWSGARALEAL
LTVAGELRGPPGTLDTGQLLKIAKRGGVTAVEAVHAWRNALTGAPLNLTPDQVVAIASNIGGKQ
ALETVQRLLPVLCQDHGLTPEQVVAIASNKGGKQALETVQRLLPVLCQAHGLTPDQVVAIASHD
GGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAI
ASNIGGKQALETVQRLLPVLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASNGGGKQALETVQRLLPVLCQDHGLTPDQVVAIASHDGGKQALETVQRLLPVLCQDHGL
TPEQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPDQVVAIASNKGGKQALETVQRLLPVLCQ
AHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQDHGLTPDQVVAIASNKGGKQALETVQRLLP
VLCQDHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQDHGLTPEQVVAIASNGGGRPALESIV
AQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA

pCas9 GFP expressing wild-type Cas9 (Addgene plasmid 44719) and pCas9D10A GFP expressing nCas9 (Addgene plasmid 44720) allow simultaneous expression of GFP (Ding et al., 2013; Mali et al., 2013b). The gRNA expression vector was derived from Addgene plasmid 43860 MLM3636. Specific target sequences are underlined and PAM sequences are in bold:

NPM: **CCT**CGAACTGCTACTGGGTTCACCTCAGCCTCTGGAATAGCTAGAACTAC**AGG**
gRNA(NPM1): 5'-GTGAACCCAGTAGCAGTTCG-3'
gRNA(NPM2): 5'-GCCTCTGGAATAGCTAGAACTAC-3'
ALK: **CCT**CAGGTAACCCTAATCTGATCACGGTCGGTCCATTGCATAGAGG**AGG**
gRNA(ALK1): 5'-GATCAGATTAGGGTTACCTG-3'
gRNA(ALK2): 5'-GTCGGTCCATTGCATAGAGG-3'

Cell lines

The human pre-B cell line NALM6 and its LIG4-defective derivative N114P2 (Grawunder et al., 1998) were maintained in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (FBS). Human Dermal Fibroblasts, adult, HDFa (Life Technologies) and 411BR primary skin fibroblasts (O'Driscoll et al., 2001) were cultured in DMEM supplemented with 15% FBS and 1% pyruvate sulfate. The human wild-type HCT116 cell line and its derivatives generated by gene targeting were cultured in McCoy's 5A medium containing 10% FCS. LIG4 mutant ($L4^{+/-}$, $L4^{-/-}$) cells have been described (Oh et al., 2013). A manuscript detailing the derivation of XRCC4 mutant ($X4^{+/-}$, $X4^{-/-}$) cells is in preparation (B.R. and E.A.H.). $X4^{-/-}$ cells were propagated in media containing G418 (1 mg/ml).

HCT116 $LIG3^{flox/-}$ cells containing a conditional LIG3 allele and a deletion allele (Oh et al., 2014), here termed $L3^{+/-}$, were further engineered to express human LIG1 with a mitochondrial leader sequence (MtLIG1) as follows: a cDNA encoding the mitochondrial localization signal of human LIG3 (residues 1- 85) fused to human LIG1 (residues 233-918, which contains the catalytic core but not the nuclear localization signal) was constructed by ligating two PCR products. The resultant DNA fragment was linked with a cDNA encoding EYFP from Aequrea Victoria (Addgene plasmid 11180:pCAG-YFP), such that YFP was fused to the LIG1 C terminus, and then subcloned into the mammalian expression vector pCAGGS that confers resistance to neomycin (Addgene plasmid 31264:pGCGFP-G418). The final expression vector (pCAG-MLig1-YFP-neo) was confirmed by DNA sequencing and then transfected into $L3^{+/-}$ cells and YFP-positive cells were isolated by flow cytometry. To delete the remaining conditional LIG3 allele, cells were infected with an adenovirus type 5 (dE1/E3) virus encoding the Cre recombinase, (Ad-CMV-Cre #1045, Vector Biolabs). After 24 h, cells were washed and then cultured in fresh medium containing 0.5 mg/ml G418. Single cells were isolated in 96-well plates using an SY3200 cell sorter. G418-resistant YFP-positive clones, i.e., expressing MtLIG1, that were also L3$^{-/-}$, were identified by Western blotting using antibodies to human LIG1 (Peng et al., 2012), GFP (Santa Cruz #8334) and LIG3 (GeneTEX #103172). The L3$^{-/-}$ genotype was confirmed using primers Lig3 Exon 5 F1: 5'-AAA GCA ACC CTC CTG TCT TCT CCT GCA AGT-3' and Lig3 Exon 5 R1: 5'-TGG TAC CAG GGA TAG AGT CAC GGA CAA ACC AA-3'.

Nucleofection

Cells were transfected by Amaxa technology (Lonza) using a cell line nucleofector. We used 10^6 HCT116 cells Kit V program D-032, 2 × 10^6 NALM6 and N114 cells Kit L program C-005, and 5 × 10^5 HDFa and 411BR cells Kit NHDF program P-022. For the AseI assay, HCT116 cells were nucleofected with 5 µg each ZFN^{EWS} plasmid (i.e., ZFN^{EWS-R} and ZFN^{EWS-L}). For inducing the 3.2-kb deletion at FLII intron 4, we used 5 µg each ZFN^{FLI1-A} and each ZFN^{FLI1-B} plasmid.

To induce t(19;22), cells were nucleofected with 3 to 5 µg each ZFN^{EWS} plasmid and 1 to 2.5 each µg ZFN^{p84} plasmid, depending on the cell line. To induce t(1;19), cells were nucleofected with 3 µg each TAL^{LAM} and 1 µg of TAL^{p84} plasmid. To induce t(2;5), cells were nucleofected with 2.5 µg each TAL^{NPM} and 1 µg each TAL^{ALK}, 3.5 µg pCas9 GFP mixed with 3.5 µg each gRNA plasmid (ALK1+NPM1), or 3.5 µg pnCas9D10A GFP mixed with 3.5 µg each gRNA plasmid (ALK1+ALK2+NPM1+NPM2).

Immunoblotting and knockdowns

Whole-cell extracts were prepared with protein lysis buffer (50 mM Tris-HCl at pH 7.4, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, and 1 mM DTT prepared from a 1 M DTT stock), with addition of cocktail protease inhibitor tablets (Complete, Roche). Typically 30 µg of protein extract from cells were electrophoresed on an 8% (w/v) Tris-HCl SDS PAGE gel, blotted, and then probed with antibody: anti-human LIG4 rabbit monoclonal antibody (Gene Tex), XRCC4 goat antibody clone C-20 (Santa Cruz Biotechnology), CtIP mouse monoclonal antibody 14-1 (Yu and Baer, 2000), ALK (31F12) mouse antibody (Cell Signal); anti-human LIG3 mouse monoclonal antibody (Becton Dickinson), FLAG mouse antibody (Sigma-Aldrich), HA rat antibody (Covance), alpha-tubulin (Sigma-Aldrich), and beta-actin (Sigma-Aldrich).

For CtIP knockdown experiments, cells were plated at a density of 2 × 10^5 cells per well in 6-well plates one day prior the transfection, and transfected using LipofectamineTM RNAiMax (Invitrogen) with 40 nmol siRNA: siControl 5’-UGUGACUUAUCGGUGUGAC-3’ or siCtIP 5'-

GCUAAAACAGGAACGAAUC-3', following the manufacturer's protocol. Cells were incubated with the siRNA for 24 h, and media was removed prior to ZFN nucleofection (t0), as described above. The level of knock down was evaluated using Western blotting. Translocation frequency was determined at 48 h (t2).

Repair assays

T7 endonuclease I assays, based on the Surveyor assay (Guschin et al., 2010), have been described (Piganeau et al., 2013). Genomic DNA was isolated 48 h after transfection. Quantifications were made with Image J software. For the p84/AAVS1 locus, the uncut band is 353 bp; T7 cleavage gives ~233 bp and ~120 bp cut bands; for the ALK locus, the uncut band is 401 bp; T7 cleavage gives ~231 bp and ~170 bp cut bands. For intrachromosomal repair involving a single DSB (AseI assay), genomic DNA was isolated from cells 48 h after ZFN^{EWS} nucleofection. A ~724 bp PCR amplicon including the ZFN^{EWS} target site was generated using primers surrounding the EWS site, and then digested in vitro with AseI (New England BioLabs). AseI-resistant PCR products were gel purified with a gel purification kit (Cycle Pure Kit, EZNA) and reamplified. Products were cloned with a TOPO TA cloning kit (Invitrogen) and sequenced (GATC Biotech).

For intrachromosomal distal repair involving two DSBs, genomic DNA was isolated from cells 48 h after ZFN$^{FLI\text{-}A}$ and ZFN$^{FLI\text{-}B}$ transfection. A fragment corresponding to the 3.2-kb deletion was PCR amplified using primers flanking the two DSBs, and products were cloned and sequenced. Serial dilutions were performed with the same primer set.

Translocation frequency was calculated from a 96-well screen using small pools of cells and nested PCR to amplify translocation junctions 48 h after transfection, as described (Brunet et al., 2009; Piganeau et al., 2013). Frequencies were normalized to the number of viable cells 24 h after transfection. Cells were also counted 48 h after transfection; overall, mutant cells were reduced in number ~15%. Primers are listed below. Statistical analyses used a t test for frequency comparisons and Mann-Whitney for deletion and microhomology distributions.

Primers

Intrachromosomal Assays		
gene		
EWS	AseI-F- GCCACGTTTGGAGTTTTTGA	AseI-R- GGGCTGAGCTCCATAAATCA
FLI1	FL1-NF- TCCACCCAGTTTTCCAGAGC	FL1-NR- TTTATTGAGGGCATTTTTGC
T7 Cleavage		
p84	p84-F-GTGTGTCACCAGATAAGGAATC	P84-R-TCTAGTCTGTGCTAGCTCTTCC
ALK	ALK-F-AGATGGGCAGAGGCTTGAAAAG	ALK-R-TGAGGATGTTCTGGAAGGCAAA
Translocations		
	PCR(1)	**Nested-PCR(2)**
t(1;19) Der1	DER1-F-CACCACGTGAGTGGTAGCC DER1-R-GGGTTCCCTTTTCCTTCTCC	DER1-NF- GCCTGGCCTTTCTTCTCTCT DER1-NR- CCTGTGCCATCTCTCGTTTC
t(19;22) Der19	DER19-F-CCTAGGACGCACCATTCTCA DER19-R-GGGGCTGAGCTCCATAAATC	DER19-NF-CAAAGGGAGTTTTCCACACG DER19-NR-GAAATCCCCGTGGATAGAATG
Der22	DER22-F-GCCTCCCGAGTAGCTGAGAT DER22-R-GGGTTCCCTTTTCCTTCTCC	DER22-NF-TGCCACTATGCCCAGCTATT DER22-NR-CCTGTGCCATCTCTCGTTTC
t(2;5) Der5	DER5-F-CAGTTGCTTGGTTCCCAGTT DER5-R-AGGAATTGGCCTGCCTTAGT	DER5-NF-GGGGAGAGGAAATCTTGCTG DER5-NR-GCAGCTTCAGTGCAATCACA

Supplemental References

Ding, Q., Lee, Y.K., Schaefer, E.A., Peters, D.T., Veres, A., Kim, K., Kuperwasser, N., Motola, D.L., Meissner, T.B., Hendriks, W.T., *et al.* (2013). A TALEN genome-editing system for generating human stem cell-based disease models. Cell Stem Cell *12*, 238-251.

Guschin, D.Y., Waite, A.J., Katibah, G.E., Miller, J.C., Holmes, M.C., and Rebar, E.J. (2010). A rapid and general assay for monitoring endogenous gene modification. Methods Mol Biol *649*, 247-256.

Huang, P., Xiao, A., Zhou, M., Zhu, Z., Lin, S., and Zhang, B. (2011). Heritable gene targeting in zebrafish using customized TALENs. Nat Biotechnol *29*, 699-700.

Miller, J.C., Holmes, M.C., Wang, J., Guschin, D.Y., Lee, Y.L., Rupniewski, I., Beausejour, C.M., Waite, A.J., Wang, N.S., Kim, K.A., *et al.* (2007). An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol *25*, 778-785.

Miller, J.C., Tan, S., Qiao, G., Barlow, K.A., Wang, J., Xia, D.F., Meng, X., Paschon, D.E., Leung, E., Hinkley, S.J., *et al.* (2011). A TALE nuclease architecture for efficient genome editing. Nat Biotechnol *29*, 143-148.

Peng, Z., Liao, Z., Dziegielewska, B., Matsumoto, Y., Thomas, S., Wan, Y., Yang, A., and Tomkinson, A.E. (2012). Phosphorylation of serine 51 regulates the interaction of human DNA ligase I with replication factor C and its participation in DNA replication and repair. J Biol Chem *287*, 36711-36719.

Simsek, D., Furda, A., Gao, Y., Artus, J., Brunet, E., Hadjantonakis, A.K., Van Houten, B., Shuman, S., McKinnon, P.J., and Jasin, M. (2011a). Crucial role for DNA ligase III in mitochondria but not in Xrcc1-dependent repair. Nature *471*, 245-248.

Yu, X., and Baer, R. (2000). Nuclear localization and cell cycle-specific expression of CtIP, a protein that associates with the BRCA1 tumor suppressor. J Biol Chem *275*, 18541-18549.