Deletion (15 kbp)

```
WT      ..TGCTGGTCGTCCTCATCTTAATAAACTGCAAAAAG..15 kbp..GGTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..
DEL1A   ..TGCTGGTCGTCC----------------------------------------------(232 bp del)-ATGCTG.
DEL1B   ..TGCTGGTCGTCCT---------------------------------------------(77 bp del)-TCTTAC.
```

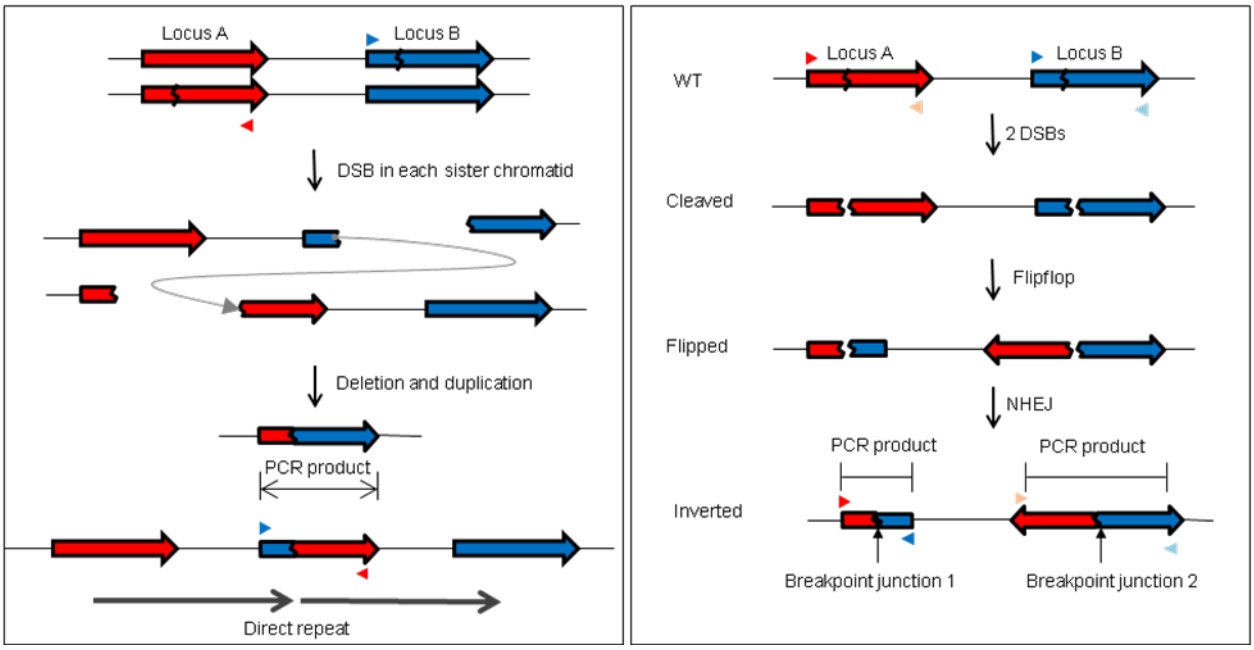
Inversion (15 kbp)

```
WT          ..TGCTGGTCGTCCTCATCTTAATAAACTGCAAAAAGCTGA..15 kbp..TGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..
            ..ACGACCAGCAGGAGTAGAATTATTTGACGTTTTTCGACT..15 kbp..ACGACCAGTAGGAGTAGGACTATTTGACGTTTTCCGACT..

Cleaved  ..TGCTGGTCGTCCTCATC    TTAATAAACTGCAAAAAGCTGA..15 kbp..TGCTGGTCATCCTCATC    CTGATAAACTGCAAAAGGCTGA..
         ..ACGACCAGCAGGAGTAGAATTA    TTTGACGTTTTTCGACT..15 kbp..ACGACCAGTAGGAGTAGGACTA    TTTGACGTTTTCCGACT..

Flipped  ..TGCTGGTCGTCCTCATC    ATCAGGATGAGGATGACCAGCA..15 kbp..TCAGCTTTTTGCAGTTT    CTGATAAACTGCAAAAGGCTGA..
         ..ACGACCAGCAGGAGTAGAATTA    CTACTCCTACTGGTCGT..15 kbp..AGTCGAAAAACGTCAAATAATT    TTTGACGTTTTCCGACT..

DEL1A    ..TGCTGGTCGTCCTCATCTTAAG--CAGGATGAGGATGACCAGCA..15 kbp..----------A..
DEL1B    ..TGCTGGTCGTCCTCATCTTAAG--CAGGATGAGGATGACCAGCA..15 kbp..----------A..
DEL2A    ..TGCTGGTCGTCCTCATC-----ATCAGGATGAGGATGACCAGCA..15 kbp..TCAGCTTTTTGCAGTTTATT----GATAAACTGCAAAAGGCTGA..
DEL2B    ..TGCTGGTCGTCCTCATC-----ATCAGGATGAGGATGACCAGCA..15 kbp..TCAGCTTTTTGCAGTTTATT----GATAAACTGCAAAAGGCTGA..
```

Supplemental Figure 1. Breakpoint junction sequences of various rearrangements in deletion clones. Each ZFN target site is shown in boldface letters. *CCR2* and *CCR5* target sites are shown in red and blue letters, respectively. Microhomologies are underlined and inserted bases are shown in italics. Dashes indicate deleted bases. WT, wild-type sequence.

A

B



Supplemental Figure 2. ZFN-mediated structural variations. *(A)* Schematic representation of ZFN-mediated duplications. *(B)* Schematic representation of ZFN-mediated inversions. Zigzag lines indicate ZFN target sites. Colored triangles indicate approximate positions of PCR primers.

A

Z360

CCR5 tgg..TtcTTctctGGAATC**TTCTTCATCATC**CTCCT **GACAATCGA**TAGgTACCTGGCTgTc..tgc
CCR2 aat..TatTTtggcGGAATC**TTCTTCATCATC**----------CTCCT**GACAATCGA**TAGaTACCTGGCTaTt..caa
→ tgg..TtcTTctctGGAATC**TTCTTCATCATC**CTCCT-(*154bp ins*)-CTCCT**GACAATCGA**TAGaTACCTGGCTaTt..caa
tgg..GGCAA(222bp del+*169bp ins*)----------**ACAATCGA**TAGaTACCTGGCTaTt..caa

Z430

CCR5 tgg..CTTTAAAAGCCAGGA**CGGTCACCT**TTGGGG**TGGTGACAAGTG**TGATCACtTGGgTGG..tgc
CCR2 aat..CTTTAAAAGCCAGGA**CGGTCACCT**TTGGGG**TGGTGACAAGTG**TGATCACcTGGtTGG..caa
→ tgg..CTTTAAAAGCCAGGA**CGGTCACCT**TTGGGG**TGGTGACAAGTG**TGATCACcTGGtTGG..caa

Z891

CCR5 tgg..CACTGCTGCATCAAc**CCCATCATCTAT**GCCTTt**GTcGGGGAGAAG**TTCAGAAacTAcCTC..tgc
CCR2 aat..CACTGCTGCATCAAt**CCCATCATCTAT**GCCTTc**GTtGGGGAGAAG**TTCAGAAggTAtCTC..caa
→ tgg..CACTGCTGCATCAAc**CCCATCATCTAT**GCCTTc**GTtGGGGAGAAG**TTCAGAAggTAtCTC..caa (×13)

B

K230+ZFN-224

Dup ..ACATGCTG**GTCATCCTCATC**CTGAT + CTCCTGGGTATGGAGACCCACCT..
..ACATGCTG**GTCATCCTCATC**CT----------CCTGGGTATGGAGACCCACCT.. (×2)
..ACATGCTG**GTC***G*----------(30bp)---ATTTT..

K243+ZFN-224

Dup ..ACATGCTG**GTCATCCTCATC**CTGAT + GTGTGGTCAATGGAGTGTACTGGT..
..ACATGCTG**GTCATCCTCATC**CTGAT*A*--------TGTGGTCAATGGAGTGTACAGGT.. (×2)
..ACATGCTG**GTCATCCTCATC**CTG----------TGGTCAATGGAGTGTACTGGT..

K276+ZFN-224

Dup ..ACATGCTG**GTCATCCTCATC**CTGAT + CGATGGATATAGTCTGGAGGGCCAG..
..ACATGCTG**GTCATCCTCATC**CCGAT----------GGATATAGTCTGGAGGGCCAG.. (×2)

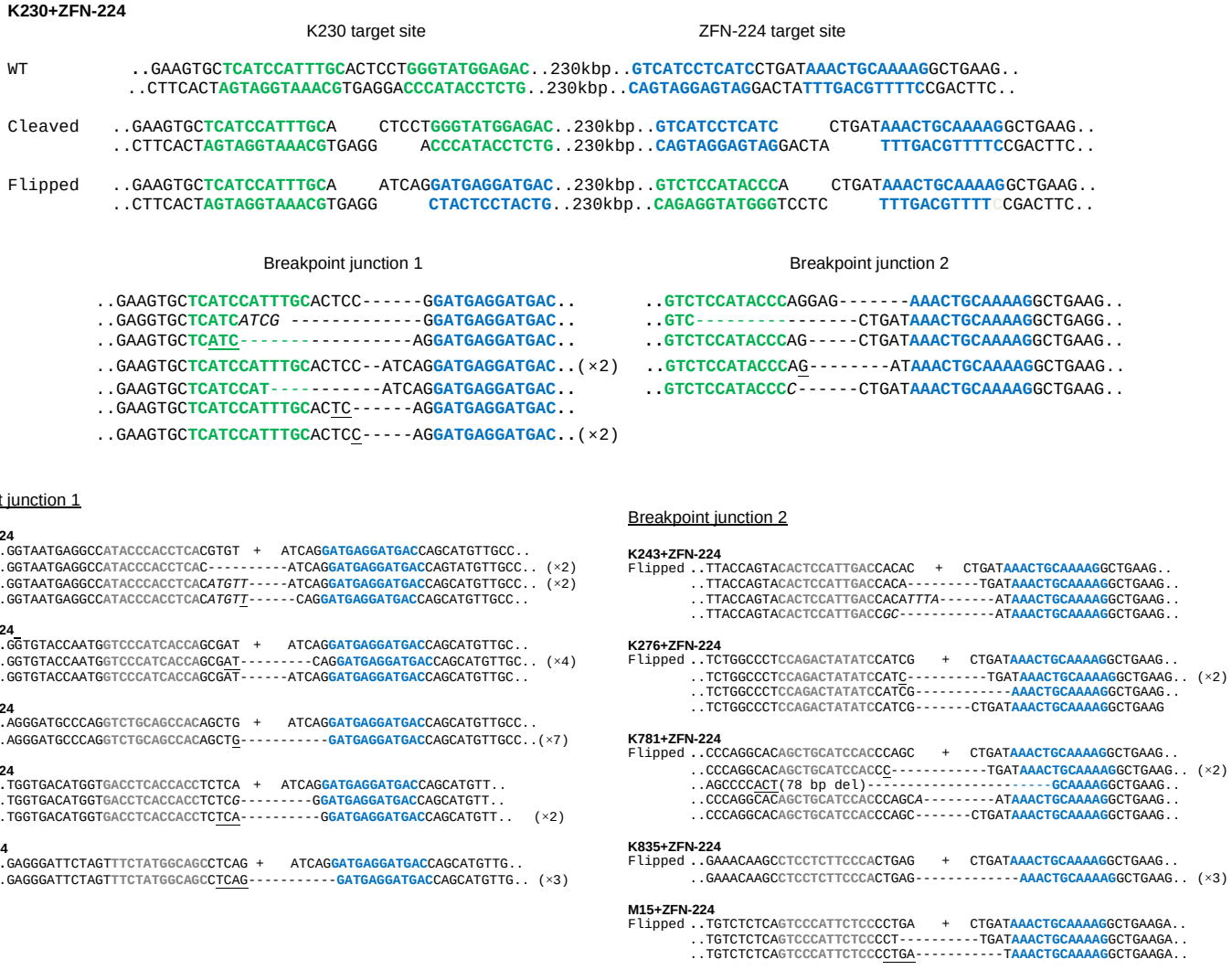
K781+ZFN-224

Dup ..ACATGCTG**GTCATCCTCATC**CTGAT + AGCTGGGTGGATGCAGCTGTGCCTG..
..ACATGCTG**GTCATCCTCATC**CTGAT*GCTGGGC*---------GGATGCAGCTGTGCCTG..
..ACATGCTG**GTCATCCTCATC**CTGAT----------GGGTGGATGCAGCTGTGCCTG..

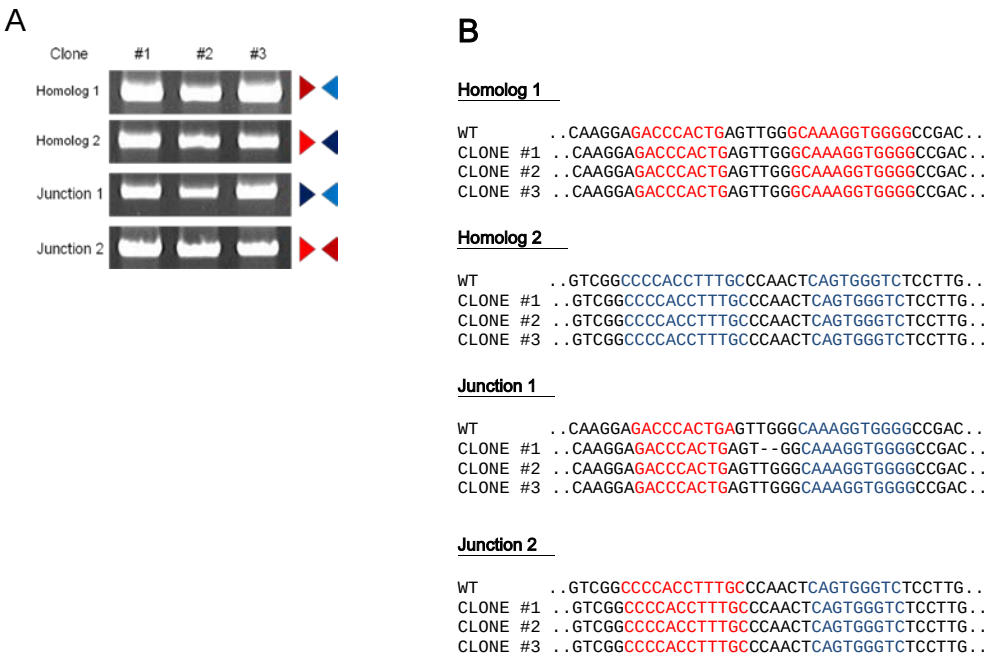
K835+ZFN-224

Dup ..ACATGCTG**GTCATCCTCATC**CTGAT + CTCAGTGGGAAGAGGAGGCTTGTT..
..ACATGCTG**GTCATCCTCATC**CTCA----------GTGGGAAGAGGAGGCTTGTT..
..ACATGCTG**GTCATCCTCATC**CTGAT--------CTCAGTGGGAAGAGGAGGCTTGTT..

Supplemental Figure 3. Breakpoint junction sequences of duplications induced by various ZFNs. (*A*) Duplications induced by ZFNs targeting *CCR2* and *CCR5*. (*B*) Duplications induced by various combinations of ZFNs. K230, K243, K276, K781, and K835 are ZFNs that target a site 230, 243, 276, 781, and 835 kbp, respectively, upstream of the *CCR5* locus. These ZFNs were described in Lee et al.(Lee et al. 2010) Each ZFN target site is shown in gray letters. Nucleotide sequences of *CCR5* and *CCR2* sites are shown in blue and red colors, respectively. Non-conserved bases at the *CCR2* and *CCR5* loci are shown in lowercase letters. Microhomologies are underlined and inserted bases are shown in italics. Dashes indicate deleted bases. In cases in which a sequence was detected more than once, the number of occurrences is shown in parentheses.



Supplemental Figure 4. Breakpoint junction sequences of inversions induced by various ZFNs. K230 and *CCR5* target sites are shown in green and blue letters, respectively. The other ZFN target site is shown in gray letters. M15 is the ZFN pair that targets a site 15 mega bp upstream of the *CCR5* locus. Microhomologies are underlined and inserted bases are shown in italics. Dashes indicate deleted bases. In cases in which a sequence was detected more than once, the number of occurrences is shown in parentheses.



Supplemental Figure 5. Analysis of clonal populations of cells in which the 140-kbp chromsomomal segment containing part of the *F8* gene was inverted by a ZFN. (*A*) PCR products validating inversion in three independent clones. Colored triangles represent PCR primers whose positions are shown in Fig. 4. (*B*) DNA sequences of PCR products that contain the Z10-target site amplified from each clone.

Supplemental Table 1. Frequencies of ZFN-induced genome rearrangements.

ZFN	Length (kbp)	Amount of genomic DNA (Copy number per half genome)										Frequency (%)		p value (Fit)
		100 ng (30000)	30 ng (10000)	10 ng (3000)	3 ng (1000)	1 ng (300)	300 pg (100)	100 pg (30)	30 pg (10)	10 pg (3)	3 pg (1)	Estimate	Upper and Lower Limits	
Duplication														
ZFN-224	15		1/1	1/1	1/1	10/21	6/21	2/21	2/21	0/1		0.3	(0.2-0.4)	0.67
Z836	15	4/4	10/17	6/17	5/17	2/17	0/17	0/1	0/1	0/1	0/1	0.01	(0.009-0.02)	0.06
Z891	15	1/1	1/1	1/1	17/17	14/17	5/17	1/17	0/1	0/1	0/1	0.4	(0.3-0.7)	0.15
K230+ZFN-224	230		1/1	1/1	21/21	14/21	9/21	1/21	0/1	0/1		0.4	(0.3-0.6)	0.53
K243+ZFN-224	243			9/9	10/17	11/17	0/17	0/17	0/9	0/1		0.1	(0.09-0.2)	0.90
Inversion														
ZFN-224	15	1/1	5/5	5/5	9/9	17/17	17/17	12/17	7/17			5	(3-7)	0.997
K230+ZFN-224	230		1/1	9/9	17/17	14/17	7/17	2/17	0/9	0/1		0.5	(0.4-0.8)	0.41
K243+ZFN-224	243		1/1	9/9	12/17	6/17	2/17	0/17	0/9	0/1		0.1	(0.08-0.2)	0.53
K276+ZFN-224	276		1/1	9/9	16/17	12/17	3/17	1/17	0/9	0/1		0.3	(0.2-0.4)	0.53
K781+ZFN-224	781		9/9	17/17	15/17	5/17	1/17	2/9	0/1	0/1		0.2	(0.1-0.3)	0.64
M15+ZFN-224	15000	2/2	7/7	15/17	7/17	1/7	0/3	0/1	0/1	0/1		0.06	(0.04-0.1)	0.44
Z10	150			16/16	16/16	6/16	4/16	0/1	0/1	0/1		0.25	(0.2-0.4)	0.25

Genomic DNA samples were serially diluted and subjected to digital PCR analysis. To account for stochastic amplifications of only a few template molecules in diluted samples, 16 to 21 reactions were performed in parallel at each critical dilution point. Results are shown as the number of reactions that yielded positive PCR products per number of total reactions. Frequencies are calculated using the Extreme Limiting Dilution Analysis software(Hu and Smyth 2009). Upper and Lower Limits indicate 95% confidence intervals. Fitting to a single-hit model is indicated by p values > 0.05.

Supplemental Table 2. Comparison of frequencies of ZFN-induced duplications and inversions with corresponding deletions.

ZFN	Length (kbp)	Frequency (%)		
		Deletion	Duplication	Inversion
ZFN-224	15	10	0.3	5
K230+ZFN-224	230	1	0.4	0.5
K243+ ZFN-224	234	1	0.1	0.1
K276+ZFN-224	276	1	N.D.	0.3
K781+ZFN-224	781	1	N.D.	0.2
M15+ ZFN-224	150000	0.03	N.D.	0.06

Frequencies of ZFN-induced duplications and inversions are obtained from Supplemental Table 1 and frequencies of ZFN-induced deletions are obtained from Table 1 in Lee et al. (Genome Res. 2010). N.D. not determined.

Supplemental Table 3. Primer sequences.

Primer name	Sequence (5' to 3')	Used for the detection of
F2	CCACATCTCGTTCTCGGTTT	CCR2 WT, 15-kbp deletion and inversion
R2	GCACCTGCTTTACAGGTTTCT	CCR2 WT, 15-kbp duplication and inversion
F5	ATGGATTATCAAGTGTCAAG	CCR5 WT, 15-kbp, 230-kbp duplication and inversion
R5	TCACAAGCCCACAGATATTT	CCR5 WT, 15-kbp, 230-kbp deletion and inversion
R-S162	GTATGGAAAATGAGAGCTG	Inversion
R-S162n	CCAGAGAAGAAGCCTATAAAATAG	Inversion
F-K230	GGGAGCTGAAATACCTTCCTT	K230 WT, 230-kbp deletion and inversion
F-K230n	CATTTGCTTGAAGCAAATCAC	K230 WT, 230-kbp deletion and inversion
R-K230	ATGTGGCATCACACATGGAG	K230 WT, 230-kbp duplication and inversion
F-K243	GCCGGGTTTGTACAAGGTAGA	243-kbp inversion
F-K243n	GCCAGCTGACGTTAGACATC	243-kbp inversion
R-K243	CCCTGTGTTCCCTTCTAAGC	243-kbp duplication and inversion
F-K276	ATCCCTGCCTCACAGCTCAT	276-kbp inversion
F-K276n	GAAGCCAGACAGCATTGTGT	276-kbp inversion
R-K276	TTAGTTCCTGGTTTGGTGCC	276-kbp duplication and inversion
F-K781	TGCAGGTACATGCCGAACTG	781-kbp inversion
F-K781n	TGTCAGTGCTGCCCTGAGT	781-kbp inversion
R-K781	CCTACCATCCCCTTTCTCAG	781-kbp duplication and inversion
F-K835	CCCACTGATGCTCTGATAGTTT	835-kbp inversion
F-K835n	ACATGAGGCATAGCAGGGAT	835-kbp inversion
R-K835	TGGGAGATGAAAGGACCTTG	835-kbp duplication and inversion
F-M15	CGAGAAGGAAACCTAGCAAGG	15-Mbp inversion
F-M15n	GGAGGTCTTATGGCCTACAGT	15-Mbp inversion
R-M15	CAATTACTCCCCAGGTGTCC	15-Mbp inversion
F5-S1	TGACCCAGTCAGAGTTGTGC	Southern probe 1
R5-S1	TCTCCCAGGCCACAAGTCTC	Southern probe 1
F5-S2	AAGCCCGTAAATAAACCTTCAG	Southern probe 2
R5-S2	CCAAACTGTGACCCTTTCC	Southern probe 2
F-intron1	GAAGTCAGTCACCACATCCAAA	140kb inversion
F-intron1n	AAATCACCCAAGGAAGCACA	140kb inversion
R-intron1	CATTAATTCTCTCCCTGCTGCT	140kb inversion
R-intron1n	TGGCATTAACGTATTACTTGGAGA	140kb inversion
F-homo2	TAAGGGACATTTGCCAGGAG	140kb inversion
F-homo2n	GGCAGGGATCTTGTTGGTAAA	140kb inversion
R-homo2	TGCAGAAAAGAGGAGGCATT	140kb inversion
R-homo2n	TGCTGAGCTAGCAGGTTTAATG	140kb inversion