

**Supplemental Material****Targeted chromosomal deletions in human cells using zinc finger nucleases**

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**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1.** ZFN-induced genome deletions at the *CCR2* and *CCR5* loci. PCR products corresponding to the 15-kbp genomic DNA deletions were cloned and sequenced. ZFN target sites are shown in bold letters. Microhomologies are underlined and inserted bases are shown in italics. Dashes indicate deleted bases. Non-conserved bases at the *CCR2* and *CCR5* loci are shown in small letters. In cases where a deletion sequence was detected more than once, the number of occurrences is shown in parentheses. WT, wild-type DNA sequence.

**Supplemental Figure 2.** Large nested deletions. PCR products corresponding to gross genomic DNA deletions were cloned and sequenced. Symbols are as in Supplemental Figure 1.

**Supplemental Figure 3.** Targeted genome deletions using two new ZFNs. PCR products corresponding to gross genomic DNA deletions were cloned and sequenced. Symbols are as in Supplemental Figure 1.

**Supplemental Table 1.** Zinc finger modules.

ZF No. <sup>1</sup>	ZF name	Amino acid sequence	Target subsite
N.A.	DSAR2	YSCGICGKSFSDDSSAKRRHCILH	GTC
ZF108	DSCR	YTCSDCGKAFRDKSCLNRHRRTH	GCC
N.A.	DSNR	YRCKYCDRSFSDSSNLQRHVNRNIH	GAC
ZF112	ISNR	YRCKYCDRSFSISSNLQRHVNRNIH	GAT
ZF113	KSNR	YGCHLCGKAFFSKSSNLRRHEMIH	GAG
N.A.	QNTQ	YTCSYCGKSFTQSNTLKQHTRIH	ATA
ZF117	QSHR2	YKCGQCCKFYQVSHLTRHQKIH	GGA
ZF120	QSHV	YECDHCGKSFSQSSHLNVHKRTH	YGA
ZF123	QSNR1	FECKDCGKAFFQKSNLIRHQRTTH	GAA
ZF126	QSSR1	YKCPDCGKSFSQSSSLIRHQRTTH	GYA
N.A.	RDER2	YHCDWDGCGWKFARSDELTRHYRKH	GYG
ZF132	RDHT	FQCKTCQRKFSRSDHLKTHTRTH	YGG
ZF134	RSHR	YKCMCEGKAFFNRRLSHLTRHQRIH	GGG
N.A.	VDYK	FHCGYCEKSFSVKDYLTKHIRTH	TAT
ZF137	VSNV	YECDHCGKAFFSVSSNLNVHRRRIH	AAT
ZF139	VSTR	YECNYCGKTFSVSSTLIRHQRIH	GCT
ZF140	WSNR	YRCEECGKAFFRWPSNLTRHKRIH	GGT

<sup>1</sup> ZF No. is based on the numbering scheme of the Zinc Finger Consortium Modular Assembly Kit 1.0

available from Addgene.

**Supplemental Table 2.** List of successful ZFNs.

ZFN name	F1	F2	F3	F4	Half-site sequence (5' to 3')
K33R	DSCR	QSHR2	QSHR2	QSHR2	GGA GGA GGA GCC
K33F	ISNR	VDYK	ISNR	RSHR	GGG GAT TAT GAT
K230R	QSHV	QSHR2	VSNV	QSSR1	GCA AAT GGA TGA
K230F	DSNR	QSHR2	VDYK	RSHR	GGG TAT GGA GAC
K243R	VDYK	RSHR	WSNR	QSHV	TGA GGT GGG TAT
K243F	RDER2	QSHR2	VSNV	DSAR2	GTC AAT GGA GTG
K276R	DSNR	RDHT	QSHV	RDHT	TGG TGA TGG GAC
K276F	RDHT	DSAR2	QNTQ	ISNR	GAT ATA GTC TGG
K781R	DSNR	QSSR1	VSTR	RDER2	GTG GCT GCA GAC
K781F	VSTR	QSSR1	ISNR	RDER2	GTG GAT GCA GCT
K835R	DSAR2	KSNR	WSNR	WSNR	GGT GGT GAG GTC
K835F	KSNR	KSNR	QSNR1	RDHT	TGG GAA GAG GAG
M15R	QSNR1	QNTQ	DSCR	VSTR	GCT GCC ATA GAA
M15F	DSNR	RDHT	QSNR1	QSHR2	GGA GAA TGG GAC

Zinc fingers are named using a four-letter code. F1 is the zinc finger at the N-terminus and F4 is the one at the C-terminus. F2 and F3 are those fingers positioned between F1 and F4. F1 interacts with the 3-bp sub-site at the 3' terminus, and F4 interacts with the 3-bp sub-site at the 5' terminus. Zinc fingers are linked to one another using the canonical "TGEKP" sequence to make ZFNs.

**Supplemental Table 3.** Primer sequences.

Primer Name	Sequence (5' To 3')
F2	CCACATCTCGTTCTCGGTTT
F2f	CCAGCAGACTCCCTTTTCAT
R2u	AGAACGAGATGTGGACAGCATGT
R2	GCACCTGCTTTACAGGTTTCT
F5	ATGGATTATCAAGTGTC AAG
F5f	GAGCCAAGCTCTCCATCTAGT
R5	TCACAAGCCCACAGATATTT
R-S162	GTATGGAAAATGAGAGCTG
R-S162n	CCAGAGAAGAAGCCTATAAAAATAG
F-K33	AGCATGGTTCAGAAGGCCAC
F-K33n	AGGAAGGCGAAGAGAAAGAA
F-K230	GGGAGCTGAAATACCTTCCTT
F-K230n	CATTGCTTGAAGCAAATCAC
R-K230	ATGTGGCATCACACATGGAG
F-K243	GCCGGGTTTGTACAAGGTAGA
F-K243n	GCCAGCTGACGTTAGACATC
R-K243	CCCTGTGTTCCCTTCTAAGC
F-K276	ATCCCTGCCTCACAGCTCAT
F-K276n	GAAGCCAGACAGCATTGTGT
F-K781	TGCAGGTACATGCCGAAGTG
F-K781n	TGTCAGTGCTGCCCTGAGT
R-K781	CCTACCATCCCCTTTCTCAG
F-K835	CCCCTGATGCTCTGATAGTTT
F-K835n	ACATGAGGCATAGCAGGGAT
F-M15	CGAGAAGGAAACCTAGCAAGG
F-M15n	GGAGGTCTTATGGCCTACAGT
R-M15	CAATTACTCCCCAGGTGTCC

## Supplemental Figure 1

### Z360

WT ..GAATC**TTCTTCATCATC**CTCCT**GACAATCGATAG**a..15kbp..**AATCTTCTTCATCATC**CTCCT**GACAATCGATAG**gT..  
..GAATC**TTCTTCATCATC**CTCCT-----**GACAATCGATAG**gT..(×2)  
..GAATC**TTCTTCATCATC**CTC-----gT..  
..GAATC**TTCTTCATCATC**C-----TCCT**GACAATCGATAG**gT..  
..GAATC**TTCTTCATCATC**CTCCTC-----CCT**GACAATCGATAG**gT..  
..TGGCC--(146bp del + 118bp insertion)-----**GACAATCGATAG**gT..

### Z426

WT ..TAAAAGCC**AGGACGGTCAC**CTTT**GGGGTGGTGACA**..15kbp..**GCCAGGACGGTCAC**CTTT**GGGGTGGTGACA**AGTGT..  
..TAAAAGCC**AGGACGGTCAC**CTT-----**TGGGGTGGTGACA**AGTGT..(×5)

### Z430

WT ..AGCCAGGAC**CGGTCAC**CTTT**GGGGTGGTGACA**AGT**G**..15kbp..**GGACGGTCAC**CTTT**GGGGTGGTGACA**AGT**G**TGATC..  
..AGCCAGGAC**CGGTCAC**CTTT**GGG**-----**GTGGTGACA**AGT**G**TGATC..(×4)  
..AGCCAGGAC**CGGTCAC**CTTT**G**-----**GTGGTGACA**AGT**G**TGATC..(×2)

### Z836

WT ..caacTGGAC**CAAGCcAc**GCAGGT**GACAGAGACT**CT..15kbp..**GGACCAAGCtAt**GCAGGT**GACAGAGACT**CTTGGGA..  
..caacTGGAC**CAAGCcAc**GCAGG-----**TGACAGAGACT**CTTGGGA..(×8)  
..caacTGGAC**CAAGCcAc**GCAGT----- (37bp del) -ATCAA.

### S162

WT ..TGCTG**GTCgTCCTCATC**tTaAT**AAACTGC**AAAA**aG**..15kbp..**GGTCaTCCTCATC**cTgAT**AAACTGC**AAAA**ag**GCTGA..  
..TGCTG**GTCgTCCTCATC**tTaAT-----cTgAT**AAACTGC**AAAA**ag**GCTGA..

## Supplemental Figure 2

### K33+S162

WT ..TACCAGGCTCCTCCTCCAACATTGGGGATTATGAT..33kbp..GGTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..  
..TACCAGGCTCCTCCTCCAACAT-----AAACTGCAAAAGGCTGA..(×4)  
..TACCAGGCTCCTCCTCCAA-----TAAACTGCAAAAGGCTGA..  
..TACCAGGCTCCTCCTCCAACA-----AAACTGCAAAAGGCTGA..

### K230+S162

WT ..AGTGCTCATCCATTTGCACTCCTGGGTATGGAGAC..230kbp..GGTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..  
..AGTGCTCATCCATTTGCACTCC-----CTGATAAACTGCAAAAGGCTGA..(×2)  
..AGTGCTCATCCATTTGCACTI-----GATAAACTGCAAAAGGCTGA..(×2)  
..AGTGCTCATCCATTTGCAC-----CTGATAAACTGCAAAAGGCTGA..  
..AGTGCTCATCCATTTGCACTCC-----AAACTGCAAAAGGCTGA..  
..AGTGCTCATCCATTTGCA-----AAAAGGCTGA..(×2)  
..AGTGCTCATCCATTTGCACATTCAT-----TAAACTGCAAAAGGCTGA..  
..AGTGCTCATCCATTTGCACTCCATGG-----CTGATAAACTGCAAAAGGCTGA..

### K243+S162

WT ..AGGCCATACCCACCTCACGTGTGGTCAATGGAGTG..243kbp..GGTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..  
..AGGCCATACCCACCTCACGTG-----ATAAACTGCAAAAGGCTGA..(×2)  
..AGGCCATACCCACCTCA----- (131bp del) -TCTTG..  
..AGGCCATACCCACCTCACATGTA-----ATAAACTGCAAAAGGCTGA..  
..AGGCCATACCCACCTCACA-----TGATAAACTGCAAAAGGCTGA..(×2)

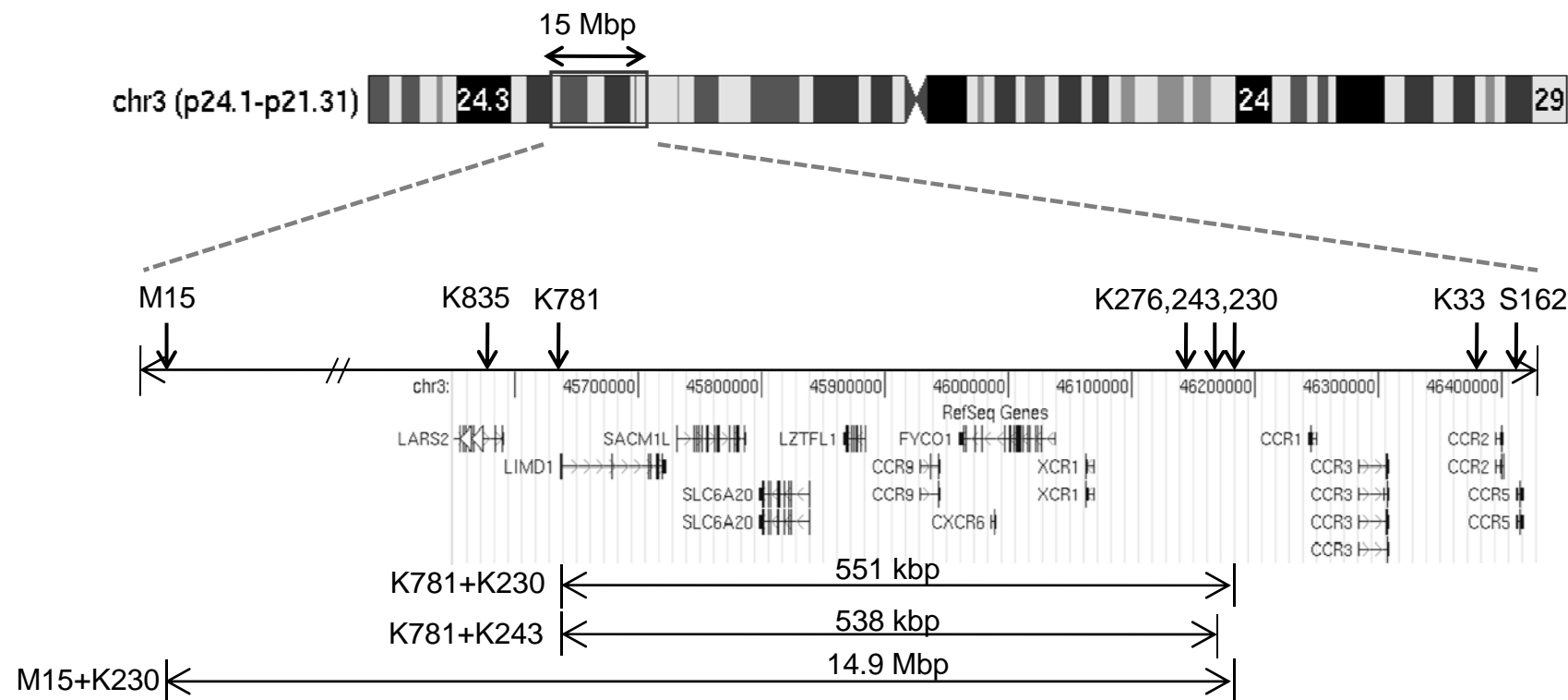
### K835+S162

WT ..ATGGTGACCTCACCACCTCTCAGTGGGAAGAGGAG..835kbp..GGTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..  
..ATGGTGACCTCACCACCTCTI-----GATAAACTGCAAAAGGCTGA..(×3)  
..ATGGTGACCTCACCACCTCTCA-----AAACTGCAAAAGGCTGA..(×2)  
..ATGGTGACCTCACCACCTCTCA-----GATAAACTGCAAAAGGCTGA..  
..ATGGTGACCTCACCACCTCTCA-----TAAACTGCAAAAGGCTGA..(×3)

### M15+S162

WT ..CTAGTTTCTATGGCAGCCTCAGGGGAGAATGGGAC..15.1Mbp..GTCATCCTCATCCTGATAAACTGCAAAAGGCTGA..  
..CTAGTTTCTATGGCAGCCTCAG-----AAAAGGCTGA..(×2)  
..CTAGTTTCTATGGCAGCCTCA-----ATAAACTGCAAAAGGCTGA..(×3)  
..CTAGTTTCTATGGCAGCCTCAG-----ATAAACTGCAAAAGGCTGA..(×3)  
..CTAGTTTCTATGGCAGCCTCI-----TGATAAACTGCAAAAGGCTGA..

Supplemental Figure 3



K781+K230

WT ..CCCAGG**TCTGCAGCCAC**AGCTGGG**TGGATGCAGCT**..551kbp..**TCATCCATT**TGCACTCCTGGG**TATGGAGACCCACC**..  
..CCCAGG**TCTGCAGCCACAGCTG**-----GG**TATGGAGACCCACC**..(×2)  
..CCCAGG**TCTGCAGCCACAGCT**-----CCTGGG**TATGGAGACCCACC**..  
..ATG-(23bp del)-----CTCCTGGG**TATGGAGACCCACC**..

K781+K243

WT ..CCCAGG**TCTGCAGCCAC**AGCTGGG**TGGATGCAGCT**..538kbp..**ATACCCACCTC**ACGTGTGG**TCAATGGAGTG**TACTG..  
..CCCAGG**TCTGCAGCCACAGCTG**-----TGG**TCAATGGAGTG**TACTG..(×2)

M15+K230

WT ..CTAG**TTTCTATGGCAGCCTC**AGGGG**GAGAATGGGAC**..14.9Mbp..**TCATCCATT**TGCACTCCTGGG**TATGGAGACCCAC**..  
..CTAG**TTTCTATGGCAGCCTC**-----TCCTGGG**TATGGAGACCCAC**..  
..CTAG**TTTCTATGGCAGCCTC**-----CTGGG**TATGGAGACCCAC**..(×3)