SUPPLEMENTARY MATERIAL

Generation and Genetic Engineering of hiPSCs Using Designed Zinc Finger Nucleases

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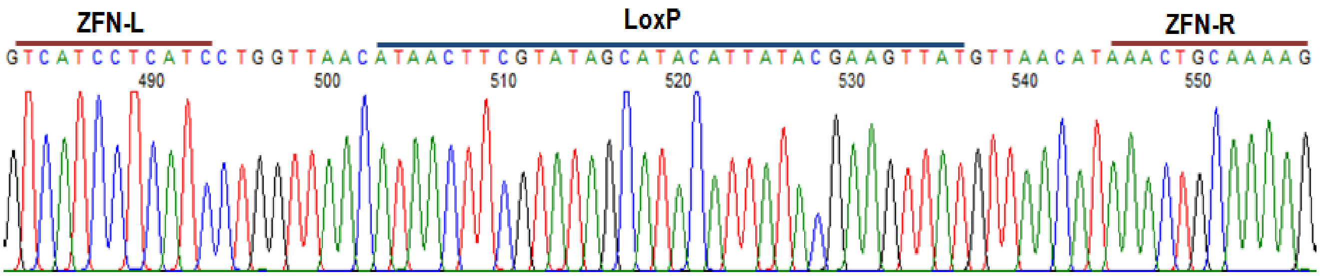
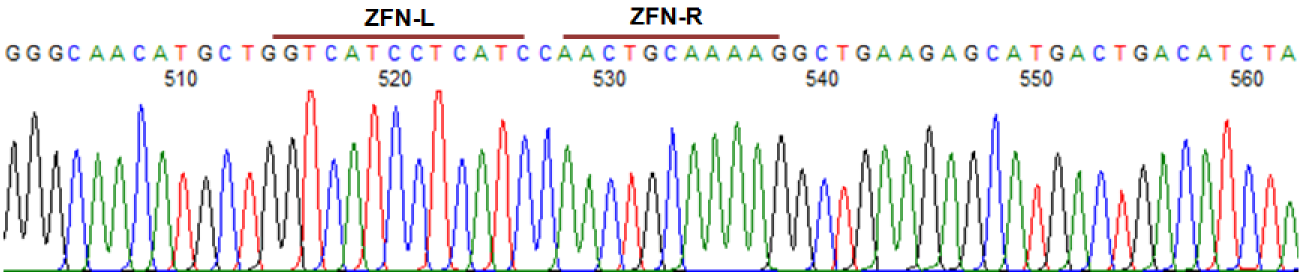
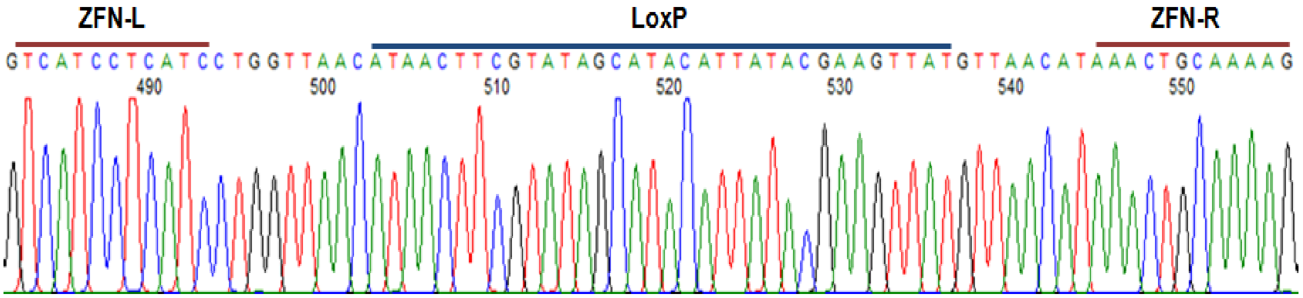
Figure S1a

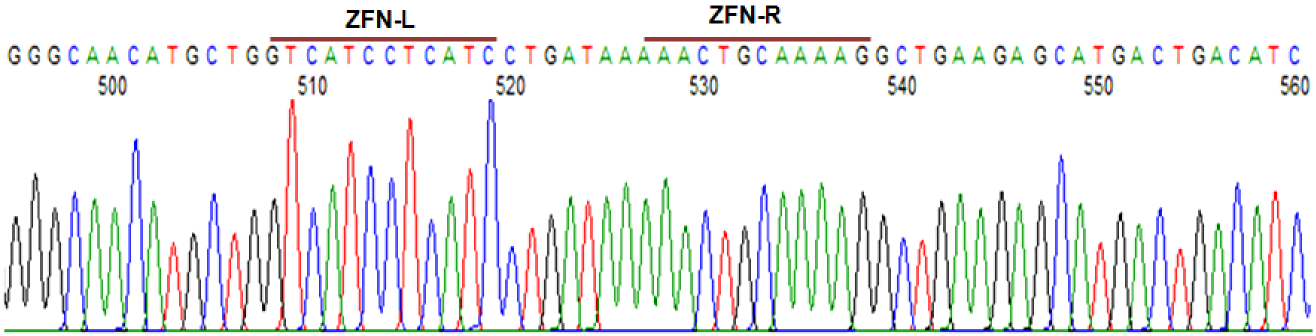
IMR90-CCR5-CRE-B-hiPSC1



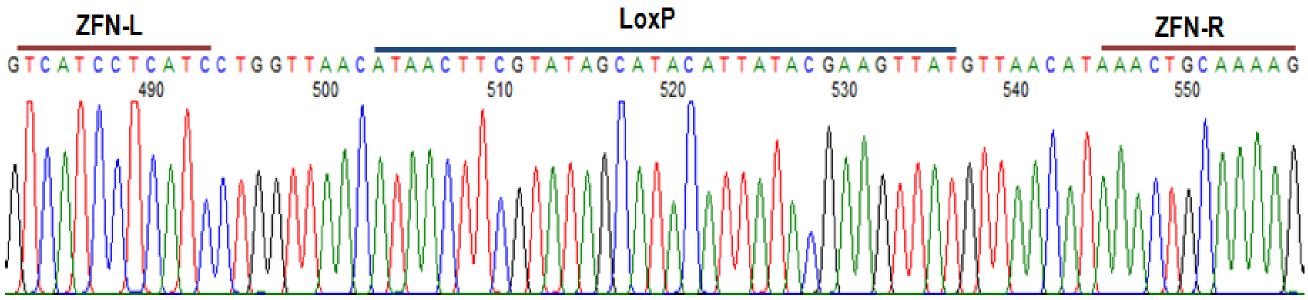


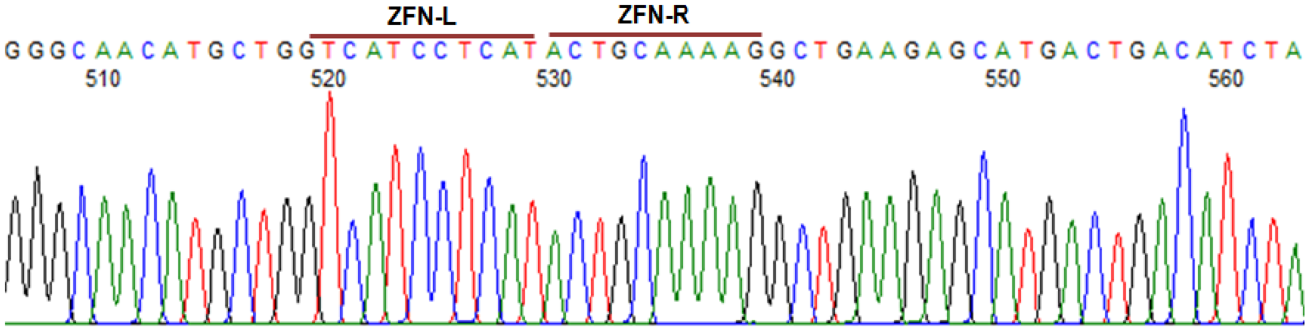
IMR90-CCR5-CRE-B-hiPSC2



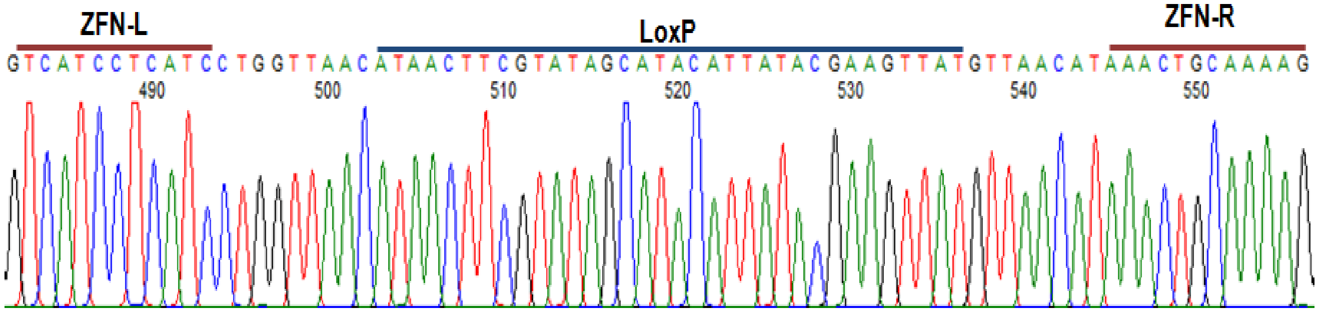


IMR90-CCR5-CRE-B-hiPSC3





IMR90-CCR5-CRE-B-hiPSC4



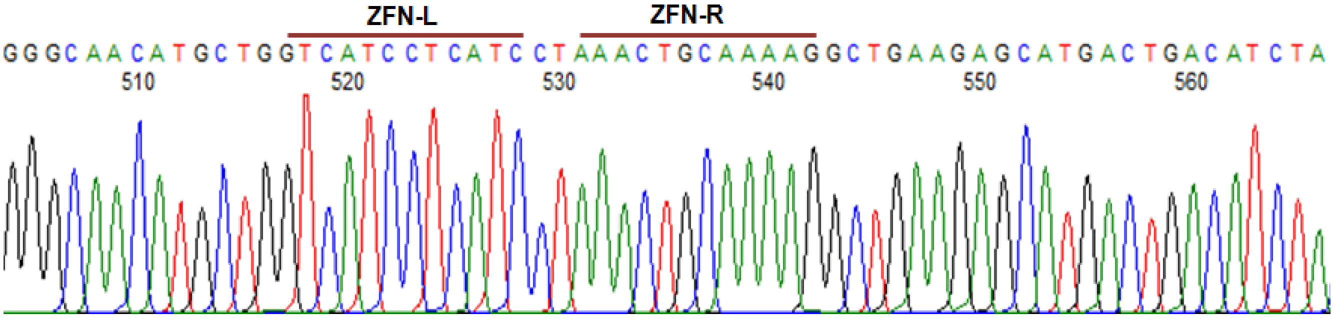
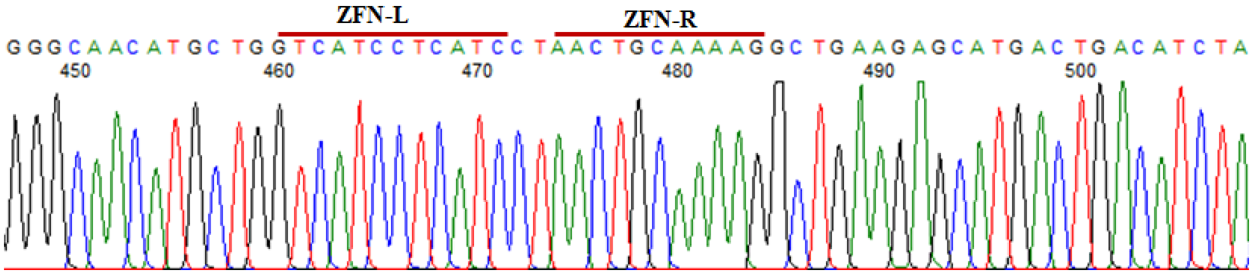


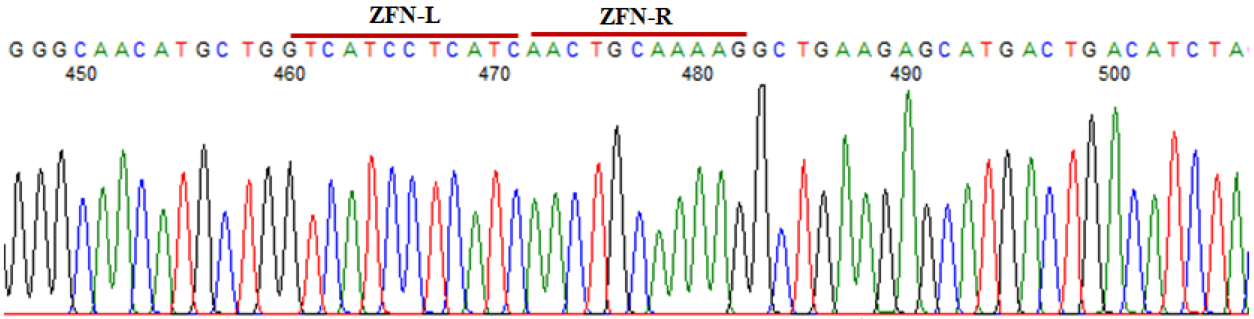
Figure S1a: Sequence traces of the CCR5 allele mutated by a loxP site and the other CCR5 allele mutated by NHEJ are shown for the four biallele CCR5-modified hiPSC lines (generated from IMR90 cells using ZFNs). The results of the sequence analysis are summarized in Table 1. B, denotes biallele CCR5-modified hiPSCs.

Figure S1b

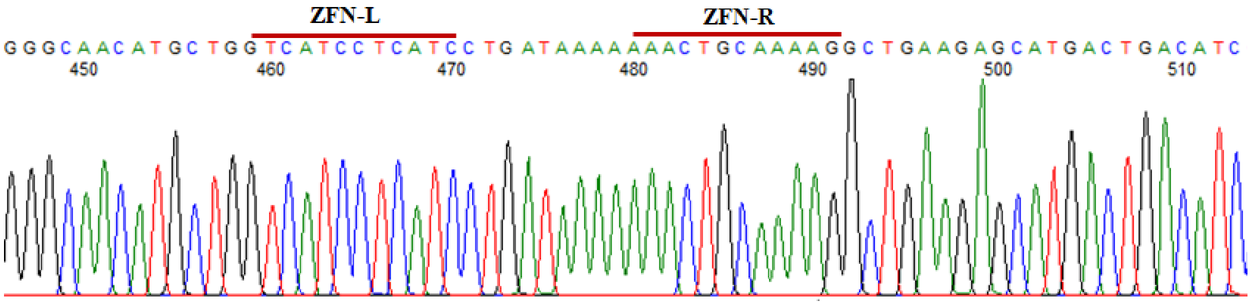
CBMNC-CCR5-B-hiPSC1 (before CRE treatment)



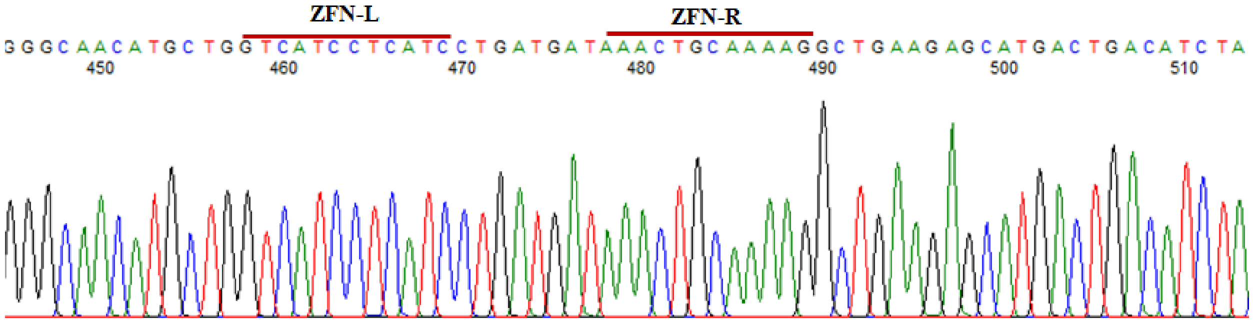
CBMNC-CCR5-B-hiPSC2 (before CRE treatment)



CBMNC-CCR5-B-hiPSC3 (before CRE treatment)



CBMNC-CCR5-B-hiPSC4 (before CRE treatment)



CBMNC-CCR5-B-hiPSC5 (before CRE treatment)

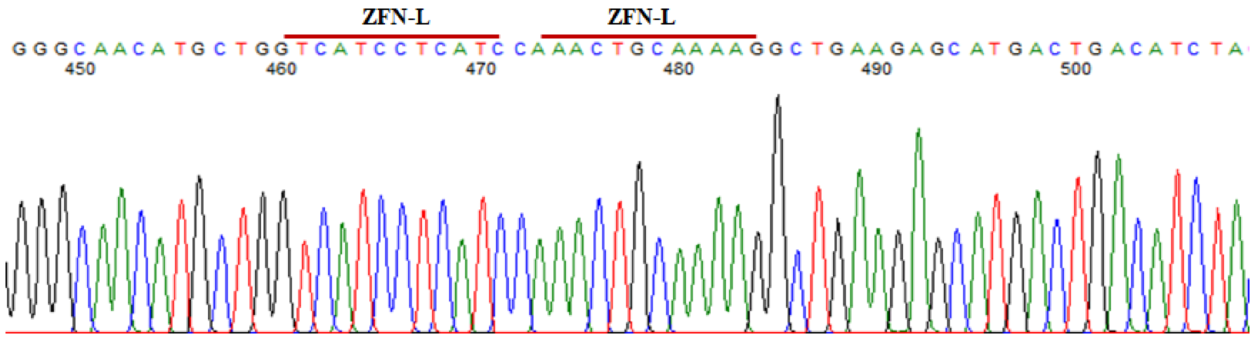
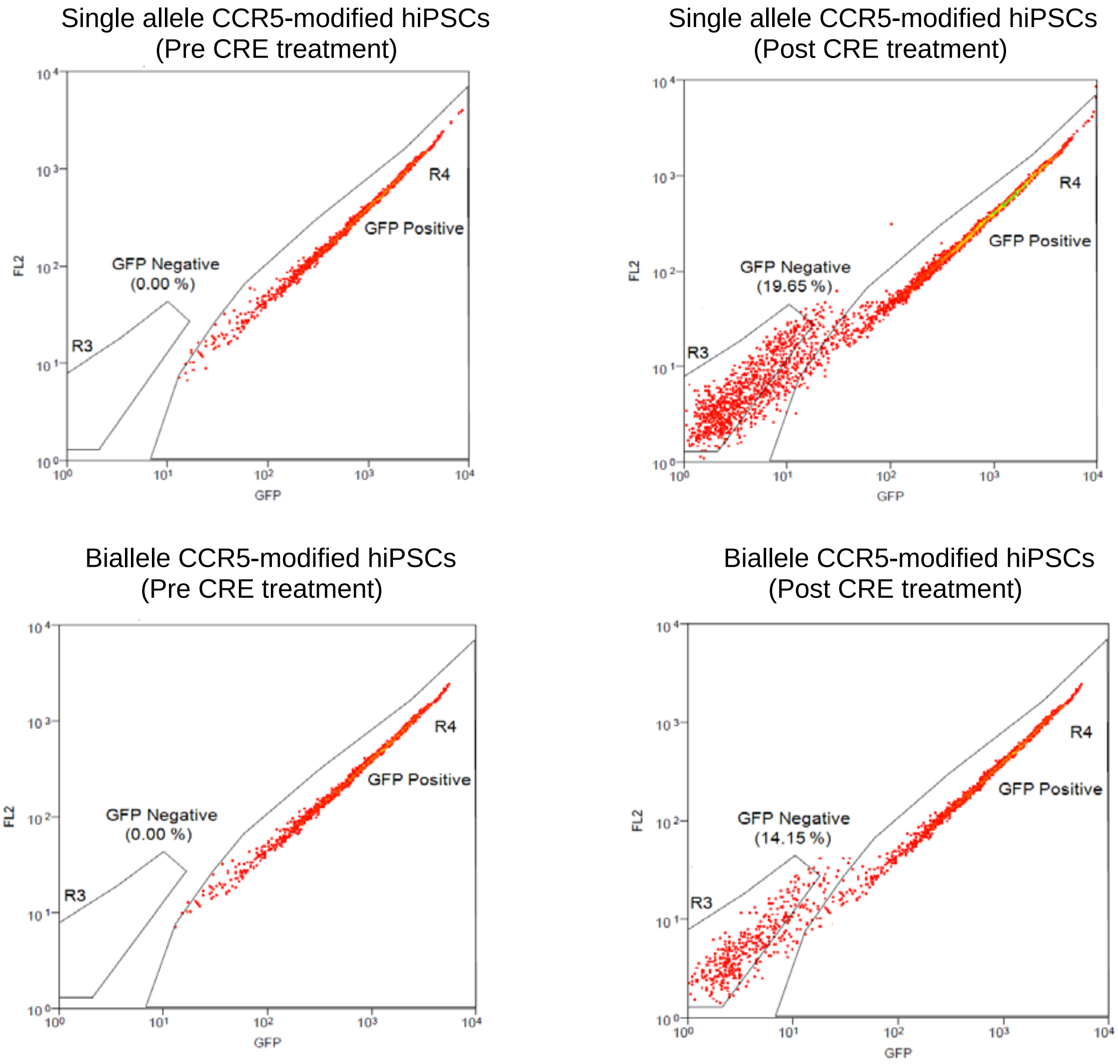


Figure S1b: Sequence traces of the CCR5 allele mutated by NHEJ are shown for five biallele CCR5-modified hiPSC lines (generated from CBMNCs using ZFNs). In these lines, the other CCR5 allele is disrupted by insertion of the donor containing the five stem cell factor genes and eGFP. B, denotes biallele CCR5-modified hiPSCs.

Figure S2

(a)

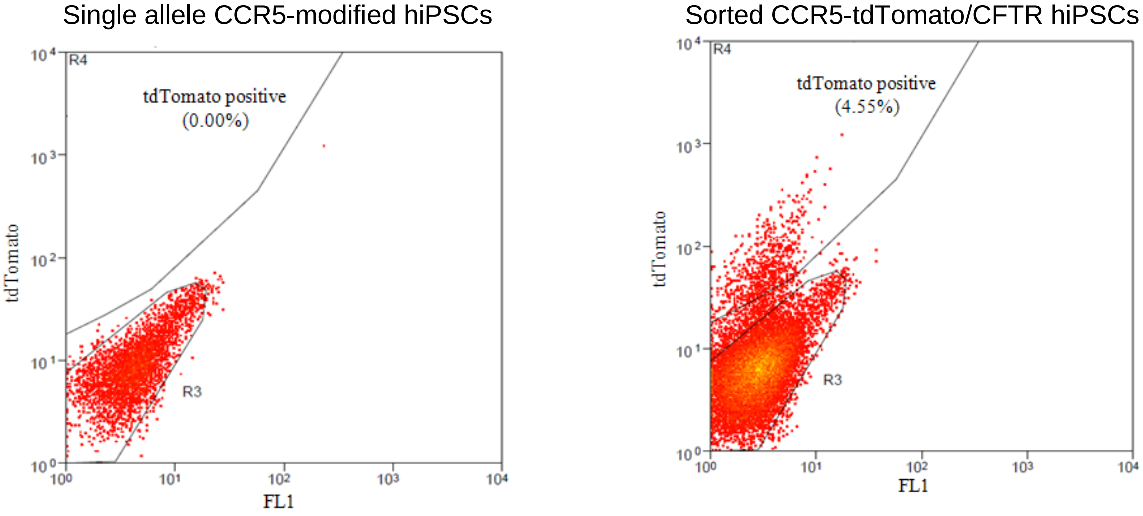

Single allele CCR5-modified hiPSCs (Pre CRE treatment)

Single allele CCR5-modified hiPSCs (Post CRE treatment)

Biallele CCR5-modified hiPSCs (Pre CRE treatment)

Biallele CCR5-modified hiPSCs (Post CRE treatment)

(b)


Single allele CCR5-modified hiPSCs

Sorted CCR5-tdTomato/CFTR hiPSCs

Figure S2: FACS analysis of CCR5-modified hiPSCs, generated from IMR90 cells using ZFNs. (A), FACS sorting profile of representative single allele and biallele CCR5-modified hiPSCs, pre- and post-treatment with Cre-recombinase. GFP negative cells were sorted and collected in matrigel coated 96-well plates. Percentage of GFP negative cells (19.65% and 14.15% respectively, for single allele and biallele CCR5-modified hiPSC lines) are bracketed in regions R3. Genomic DNAs were isolated from single cell colonies and transgene removal was confirmed by PCR and sequence analysis. (B), FACS sorting profile of single allele CCR5-modified hiPSCS after co-transfection with tdTomato/CFTR donor plasmid and CCR5-specific ZFNs. Three days post-transfection, tdTomato positive cells were FACS sorted and collected in matrigel coated 96-well plates. Percentage of tdTomato positive cells (4.55%) is bracketed in region R4. Genomic DNAs were isolated from single cell colonies and CFTR transgene addition was confirmed by PCR and sequence analysis.

Figure S3

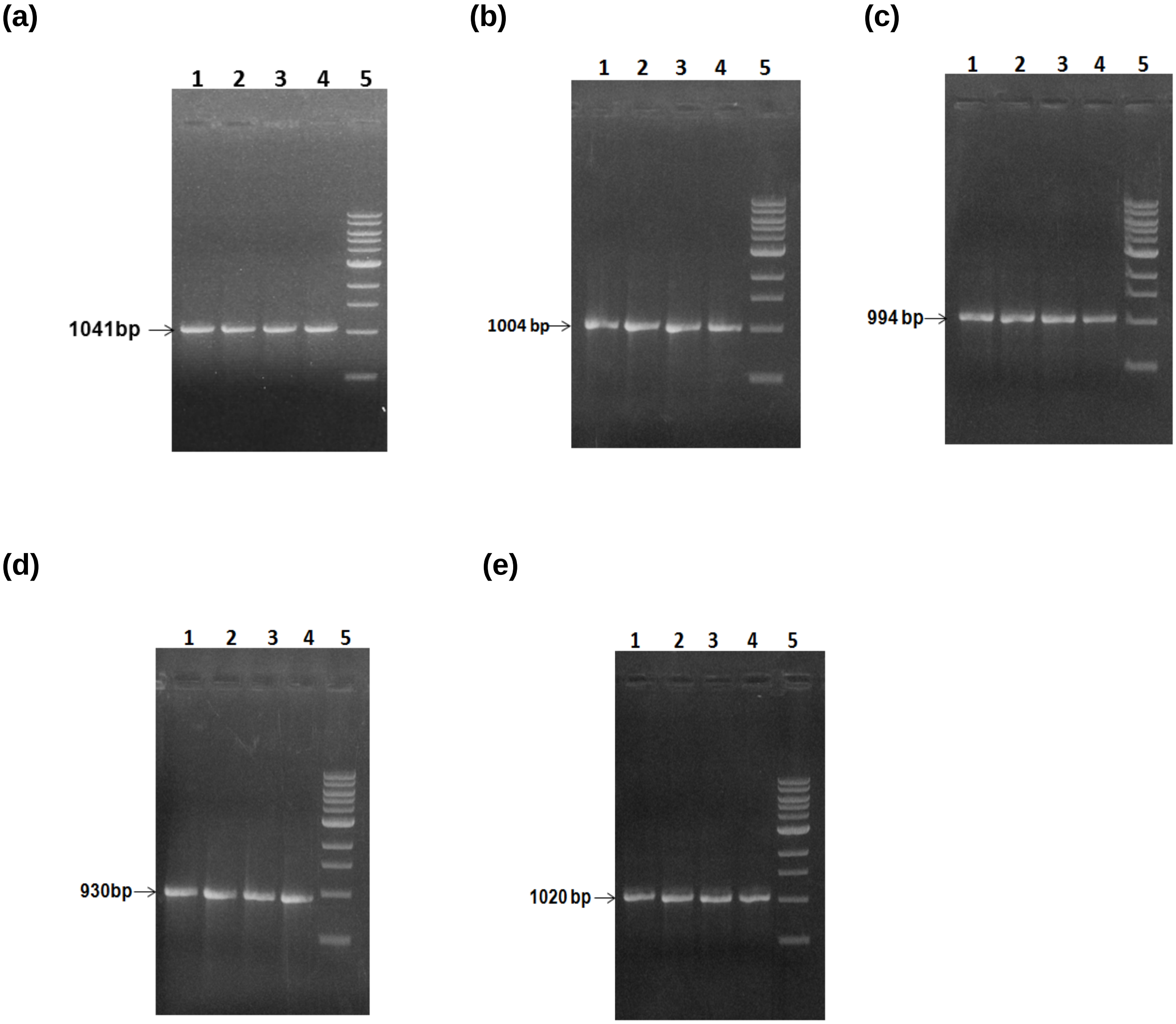
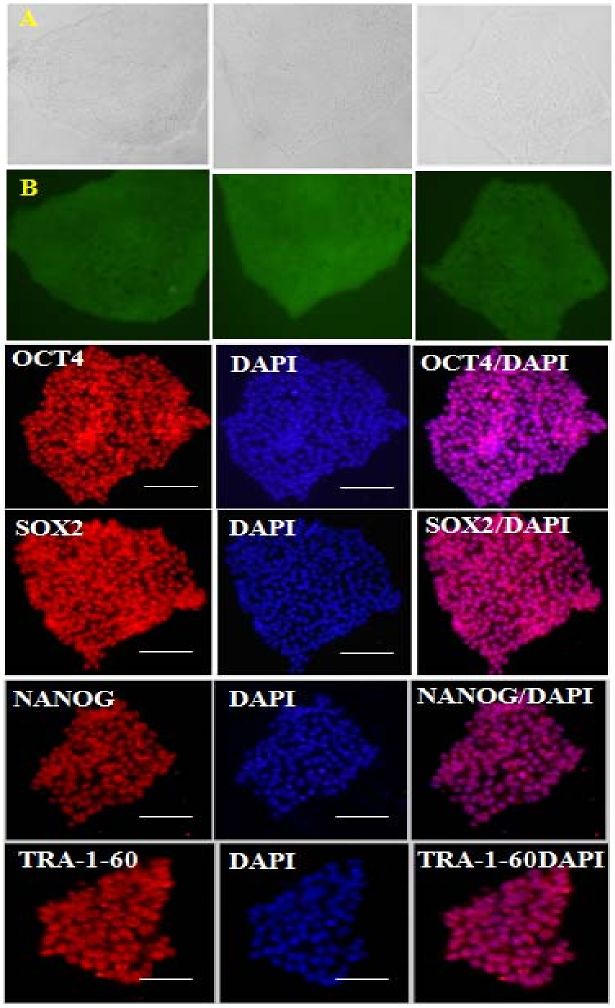


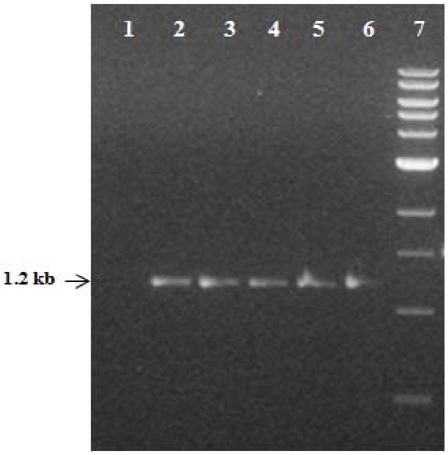
Figure S2 Assessment of off-target effects of CCR5-specific ZFNs. PCR amplification of the closely related CCR2 site (a) and four other previously reported susceptible sites *KRR1* (b), *ABLIM2*(c), *PKN2*(d) and *PGC* (e) using locus-specific primers (Table S1) from CCR5-modified CFTR expressing hiPSCs. The PCR products were subcloned and sequenced (Table S5). No mutations were observed at the CCR2 locus or any of the other four chromosomal loci.

Figure S4

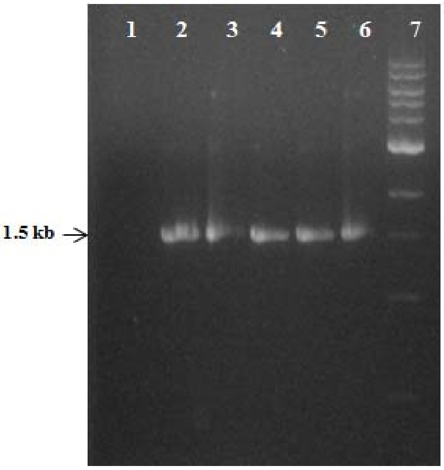
(i) Single allele CCR5-modfied CBMNC hiPSCs



(iii) PCR analysis of 5’ junction



(iv) PCR analysis of 3’ junction



(ii) Schematic representation of donor insertion site

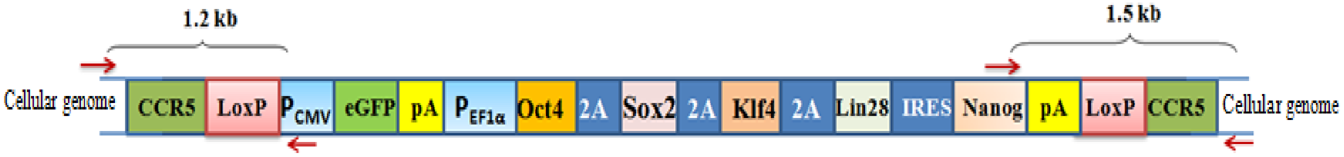


Figure S4. Characterization of single allele CCR5-modified hiPSC lines, generated from CBMNCs by targeted insertion of Oct4/Sox2/Klf4/Nanog/Lin28 transcription factors at the CCR5 locus, using ZFNs. (i) Morphology of precisely targeted single allele CCR5-modified hiPSCs before CRE treatment. Panel

A, bright field images of the morphology three representative single allele CCR5-modified hiPSC colonies; Panel B, GFP fluorescence images of hiPSC single cell colonies shown in A; Immuno-staining for Oct4/Sox2/Nanog/Tra1-60 and DAPI staining of the single allele CCR5-modified hiPSC lines are also shown. (ii) Schematic representation of donor (Oct4/Sox2/Klf4/Lin28/Nanog flanked by CCR5 homology arms) insertion site at the CCR5 locus of single allele CCR5-modified hiPSCs. PCR primers anchored outside the CCR5 homology arms and primers anchored inside the donor for the 5' and 3' junction sites are shown. pA denotes polyA sequence. (iii) Genotyping of single allele CCR5- modified hiPSCs from CBMNCs. PCR analysis of 5' junction of donor insertion site in five different single allele CCR5-modified hiPSC lines. Lanes: 1, Control CBMNCs; 2-6, single allele CCR5-modified hiPSC lines; and 7, 1 Kb ladder. PCR analysis yielded the expected band size (1.2 kb) confirming insertion of the donor at the CCR5 locus. (iv) PCR analysis of 3' junction of donor insertion site in five different single allele CCR5-modified hiPSC single cell colonies. Lanes: 1, Control CBMNCs; 2-6, single allele CCR5-modified hiPSC lines; and 7, 1 Kb ladder. PCR analysis yielded the expected band size (1.5 kb) confirming insertion of the donor at the CCR5 locus. Southern blot analysis for the single allele CCR5-modified hiPSC lines are shown in Figure 8(v) of the main article along with the biallele CCR5-modified hiPSC lines, which confirmed single donor insertion at the CCR5 locus.

Table S1: PCR primer sequences and amplification conditions

Gene locus	**Primer**	**Sequence (5' to 3')**	**Amplification conditions**
CCR5(5'end)	CCR5-F(5’end) CMV-R	CAACTCAAACTACAAACACAAACTTCACAG GGAAAGTCCCGTTGATTTTGGTGCC	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1 min, followed by extension at 72°C for 2 min
CCR5(3'end)	GFP-F(3’end) CCR5-R(3’end)	CAACGAGAAGCGCGATCACATGGT GAGTTTGATGCTTATTGAATGTGTAG	95°C for 5 min, then 35 cycles of 95°C for 30sec, 52°C for 2 min, followed by extension at 68°C for 3 min
CCR5(NHEJ)	CCR5(endo)-F CCR5(endo)-R	ATGGATTATCAAGTGTCAAGTCCA TCACAAGCCCACAGATATTTCC	95°C for 5 min, then 30 cycles of 95°C for 30sec, 58°C for 1min, followed by extension at 72°C for 5 min
CCR5-CFTR-tdtomato (5' end)	CCR5-F(CFTR) tdtomato-R	GTTGCCCTAAGGATTAAATGAATGAATG CTCCATGCGCACCTTGAAGCGCATGAAC	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1 min, followed by extension at 72°C for 2 min
CFTR-CCR5 (3' end)	CFTR-F CCR5-R	GCAGTACGATTCCATCCAGAAACTGCTG GGATGAATCTTAGACCCTCTATAACAG	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1 min, followed by extension at 72°C for 2 min
CCR5-CRE	CCR5-F CCR5-R	ACAAGATTTTATTTGGTGAGATGG AGAATTGATACTGACTGTATGG	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 30sec, followed by extension at 72°C for 1 min
CCR2	CCR2-F CCR2-R	ATGCTGTCCACATCTCGTTCT TGTAGTCACTTTCACATTCTT	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1min, followed by extension at 72°C for 5 min
KRR1	KRR1-F KRR1-R	TATTCTTTCACTGGCTTCTGTAAG GTTTATAAATACAATTCTATAATC	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1min, followed by extension at 72°C for 5 min
ABLIM2	ABLIM2-F ABLIM2-R	TAATCCCTCATGATAATGAAACC TCCCAGAGATCACGGAATGATGAG	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1min, followed by extension at 72°C for 5 min
PKN2	PKN2-F PKN2-R	TGACCCATCACACACGTACGCACA CTTTGGCAGAGGCTCTACCTTTCC	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1min, followed by extension at 72°C for 5 min
PGC	PGC-F PGC-R	CCTTAGCTGTGTCGCCTTGAGT CCGGCCAGATCTGCGTGTTTTAA	95°C for 5 min, then 30 cycles of 95°C for 30sec, 55°C for 1min, followed by extension at 72°C for 5 min

F, Forward primer and R, Reverse primer

Table S2a: Sequence analysis of 5' and 3' junctions of the donor insertion site in single allele and biallele CCR5-modified hiPSCs, before CRE treatment

Clone ID	**5' junction sequence of the donor insertion site in single allele and biallele CCR5-modified hiPSC lines from IMR90 cells**
CCR5-S-hiPSC1*	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-S-hiPSC2*	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-S-hiPSC3*	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-S-hiPSC4*	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-B-hiPSC1	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-B-hiPSC2	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-B-hiPSC3	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
CCR5-B-hiPSC4	………**AGGCAGGA**……GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT………
Clone ID	**3' junction sequence of the donor insertion site in single allele and biallele CCR5-modified hiPSC lines from IMR90 cells**
CCR5--S-hiPSC1*	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-S-hiPSC2*	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-S-hiPSC3*	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-S-hiPSC4*	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC1	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC2	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC3	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC4	……GGCTCTATGG*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……

*The other CCR5 allele in the single allele CCR5-modified hiPSCs is wild type. Endogenous CCR5 sequence outside the donor homology arm is highlighted in blue, ZFN target site present in hCCR5 gene is highlighted in black. LoxP site is in italics. CMV promoter sequence is highlighted in green. polyA terminator sequence is highlighted in yellow. PCR fragments were amplified from each of the single allele mutant hiPSC clones using primers listed in Supplementary table, subcloned into *E. coli* and then sequenced. The endogenous CCR5 and transgene junction sequences are shown in the table. S, single allele CCR5-modified hiPSCs; B, biallele CCR5-modified hiPSCs.

Table S2b: Sequence analysis of 5' and 3' junctions of the donor insertion sites of single allele and biallele CCR5-modified CBMNC hiPSC lines.

Clone ID	5' junction sequence of the donor insertion site in single allele and biallele CCR5-modified CBMNC hiPSC lines
CCR5-S-hiPSC1	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-S-hiPSC2	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-S-hiPSC3	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-S-hiPSC4	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-S-hiPSC5	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-B-hiPSC1	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-B-hiPSC2	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-B-hiPSC3	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-B-hiPSC4	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........
CCR5-B-hiPSC5	**.........AGGCAGGA......**GTCATCCTCATCCTGGTTAAC*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GACATTGATTATT.........

Clone ID	3' junction sequence of the donor insertion site in single allele and biallele CCR5-modified CBMNC hiPSC lines
CCR5--S-hiPSC1	...AGTGACTT...*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG...GCCAAACG......
CCR5-S-hiPSC2	...AGTGACTT...*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG...GCCAAACG......
CCR5-S-hiPSC3	...AGTGACTT...*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG...GCCAAACG......
CCR5-S-hiPSC4	...AGTGACTT...*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG...GCCAAACG......
CCR5-S-hiPSC5	...AGTGACTT...*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG...GCCAAACG......
CCR5-B-hiPSC1	...AGTGACTT...*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG...GCCAAACG......

CCR5-B-hiPSC2	…AGTGACTT…*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC3	…AGTGACTT…*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC4	…AGTGACTT…*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……
CCR5-B-hiPSC5	…AGTGACTT…*ATAACTTCGTATAGCATACATTATACGAAGTTAT*GTTAACATAAACTGCAAAAG…GCCAAACG……

*The other CCR5 allele in these hiPSC lines is wild type. Endogenous CCR5 sequence outside the donor homology arm is highlighted in blue, ZFN target site present in hCCR5 gene is highlighted in black. LoxP site is in italics. CMV promoter sequence is highlighted in green. Nanog sequence is highlighted in yellow PCR fragments were amplified from each of the single allele mutant hiPSC clones using primers listed in Supplementary table were subcloned into *E. coli* and sequenced. The endogenous CCR5 and transgene junction sequence is shown in table. S, single allele CCR5-modified hiPSCs; B, biallele CCR5-modified hiPSCs.

Table S3: Analysis of the CCR5 locus repaired by NHEJ in the biallele CCR5-modified hiPSC lines, before CRE treatment

Clone ID	Nucleotide sequence of CCR5 locus of bi-allele CCR5-modified IMR90 hiPSC lines*
CCR5-B-hiPSC1	**GGGCAACATGCTGGTCATCCTCATCC.....AACTGCAAAAGGCTGAAGAGCATGACTGACATCTA (4)[#]**
CCR5-B-hiPSC2	**GGGCAACATGCTGGTCATCCTCATCCTGATaaAAACTGCAAAAGGCTGAAGAGCATGACTGACATC (4)[#]**
CCR5-B-hiPSC3	**GGGCAACATGCTGGTCATCCTCAT........ACTGCAAAAGGCTGAAGAGCATGACTGACATCTA (4)[#]**
CCR5-B-hiPSC4	**GGGCAACATGCTGGTCATCCTCATCCT...AAACTGCAAAAGGCTGAAGAGCATGACTGACATCTA (4)[#]**

Clone ID	Nucleotide sequence of CCR5 locus of bi-allele CCR5-modified CBMNC hiPSC lines*
CBMCs-CCR5-B-iPSC1	**GGGCAACATGCTGGTCATCCTCATCCT…AACTGCAAAAGGCTGAAGAGCATGACTGACATCTA(4)[#]**
CBMCs CCR5-B-hiPSC2	**GGGCAACATGCTGGTCATCCTCATC……AACTGCAAAAGGCTGAAGAGCATGACTGACATCTA(4)[#]**
CBMCs-CCR5-B-hiPSC3	**GGGCAACATGCTGGTCATCCTCATCCTGATaaaaAAACTGCAAAAGGCTGAAGAGCATGACTGACATC(4)[#]**
CBMCs-CCR5-B-hiPSC4	**GGGCAACATGCTGGTCATCCTCATCCTGATgatAAACTGCAAAAGGCTGAAGAGCATGACTGACATCTA(4)[#]**
CBMCs- CCR5-B-hiPSC5	**GGGCAACATGCTGGTCATCCTCATCC……AAACTGCAAAAGGCTGAAGAGCATGACTGACATCTA(4)[#]**

*The other CCR5 allele in these hiPSC lines is mutated by insertion of the stem cell factor genes (Tables 2A & 2B). ZFN target sites present in hCCR5 gene are highlighted in yellow. [#]PCR fragments amplified from each of the biallele hiPSC lines were subcloned into *E. coli*. Four recombinant clones from each experiment were sequenced. The number of times the same sequence appeared is shown in brackets. B, biallele CCR5-modified hiPSC lines.

Table S4a: ZFN-mediated reprogramming efficiency of human lung fibroblasts (IMR90 cells) and cord blood mononuclear cells (CBMNCs)

No. of cells seeded	Cell source	Plasmids used for transfection	Number of Transcription factors used	Number of hiPSC colonies obtained	Number of transfections
$3x10^5$	Human lung fibroblast (IMR90)	CCR5-specific ZFNs + Oct4/Sox2/eGFP donor	2 (Oct4/Sox2)	120	2
$3x10^5$	Human lung fibroblast (IMR90)	CCR5-specific ZFNs + Oct4/Sox2/eGFP/puroR donor	2 (Oct4/Sox2)	70	2
$2x10^6$	Corld blood Mononuclear cells (CBMNCs)	CCR5-specific ZFNs + Oct4/Sox2/Klf4/Lin28/Nanog/eGFP donor	5 (Oct4/Sox2/Klf4/ Lin28/Nanog)	732	1

Table S4b: Ratio of single allele versus biallele CCR5-modified colonies

Cell source	Number of colonies analyzed	Number of single allele CCR5-modified colonies	Number of biallele CCR5-modified colonies	Ratio of single allele versus biallele CCR5-modified colonies
Human lung fibroblast (IMR90)	20	16	4	4:1
Cord blood mononuclear cells (CBMNCs)	20	15	5	3:1

Table S5: Nucleotide sequences of *CCR2, KRR1, ABLIM2, PKN2 and PGC* loci in tdTomato/CFTR expressing hiPSC lines

Clone ID	**Nucleotide sequence at the endogenous CCR2 locus**
Ref sequence (WT-CCR5)	**ZFN-L** **ZFN-R** ............TGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGAC......
CFTR-hiPSC1	............TGGGCAACATGCTGGTCGTCCTCATCTTAATAAACTGCAAAAAGCTGAAGTGCTTGACTGAC......(2)
CFTR-hiPSC2	............TGGGCAACATGCTGGTCGTCCTCATCTTAATAAACTGCAAAAAGCTGAAGTGCTTGACTGAC......(2)
CFTR-hiPSC3	............TGGGCAACATGCTGGTCGTCCTCATCTTAATAAACTGCAAAAAGCTGAAGTGCTTGACTGAC......(2)
CFTR-hiPSC4	............TGGGCAACATGCTGGTCGTCCTCATCTTAATAAACTGCAAAAAGCTGAAGTGCTTGACTGAC......(2)
Clone ID	**Nucleotide sequence at the endogenous *KRR1* locus**
Ref sequence (WT-CCR5)	**ZFN-L** **ZFN-R** ............TGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGAC...
CFTR-hiPSC1	............GAGCCACCGCGCCTGGCCTCCTCATCTCTTTAAACTGGAAATGCTACCTATCTTTTTGTTTT...(2)
CFTR-hiPSC2	.........GAGCCACCGCGCCTGGCCTCCTCATCTCTTTAAACTGGAAATGCTACCTATCTTTTTGTTTT......(2)
CFTR-hiPSC3	............GAGCCACCGCGCCTGGCCTCCTCATCTCTTTAAACTGGAAATGCTACCTATCTTTTTGTTTT...(2)
CFTR-hiPSC4	............GAGCCACCGCGCCTGGCCTCCTCATCTCTTTAAACTGGAAATGCTACCTATCTTTTTGTTTT...(2)
Clone ID	**Nucleotide sequence at the endogenous *ABLIM2* locus**
Ref sequence (WT-CCR5)	**ZFN-L** **ZFN-R** ............TGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGAC...
CFTR-hiPSC1	............CACAGGAATGGTCAGTCATCCTCATCTCACGGATGAGGATGCCAAGGCTAGGAGATGGTCACA...(2)
CFTR-hiPSC2	............CACAGGAATGGTCAGTCATCCTCATCTCACGGATGAGGATGCCAAGGCTAGGAGATGGTCACA...(2)
CFTR-hiPSC3	............CACAGGAATGGTCAGTCATCCTCATCTCACGGATGAGGATGCCAAGGCTAGGAGATGGTCACA...(2)
CFTR-hiPSC4	............CACAGGAATGGTCAGTCATCCTCATCTCACGGATGAGGATGCCAAGGCTAGGAGATGGTCACA...(2)

Clone ID	Nucleotide sequence at the endogenous *PKN2* locus
Ref sequence (WT-CCR5)	ZFN-L ZFN-R ............TGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGAC......
CFTR-hiPSC1	............TGATCTTTTGAGGTTTATCATTTCTTTATATAAACTGTAAAAGGGAGCTTTGTGAAGAACAA...(2)
CFTR-hiPSC2	............TGATCTTTTGAGGTTTATCATTTCTTTATATAAACTGTAAAAGGGAGCTTTGTGAAGAACAA...(2)
CFTR-hiPSC3	............TGATCTTTTGAGGTTTATCATTTCTTTATATAAACTGTAAAAGGGAGCTTTGTGAAGAACAA...(2)
CFTR-hiPSC4	............TGATCTTTTGAGGTTTATCATTTCTTTATATAAACTGTAAAAGGGAGCTTTGTGAAGAACAA...(2)

Clone ID	Nucleotide sequence at the endogenous *PGC* locus
Ref sequence (WT-CCR5)	ZFN-L ZFN-R ............TGGGCAACATGCTGGTCATCCTCATCCTGATAAACTGCAAAAGGCTGAAGAGCATGACTGAC......
CFTR-hiPSC1	............GCAGATTCTGAATGCTGTTACAGTTCACGAGAAACTGCAAGAGGAATAGGTCCCTGGTTAAC...(2)
CFTR-hiPSC2	............GCAGATTCTGAATGCTGTTACAGTTCACGAGAAACTGCAAGAGGAATAGGTCCCTGGTTAAC...(2)
CFTR-hiPSC3	............GCAGATTCTGAATGCTGTTACAGTTCACGAGAAACTGCAAGAGGAATAGGTCCCTGGTTAAC...(2)
CFTR-hiPSC4	............GCAGATTCTGAATGCTGTTACAGTTCACGAGAAACTGCAAGAGGAATAGGTCCCTGGTTAAC...(2)

The CCR2, KRR1, ABLIM2, PKN2 and PGC loci of the tdTomato/CFTR expressing hiPSC lines were amplified by PCR using corresponding gene-specific primers (see Table S1), subcloned in *E. coli* and two clones from each were sequenced. The reference CCR5 ZFN binding sites and the related sequences in other target genes are highlighted in yellow and the base differences between the two are underlined. The sequences at each of the above gene loci were wild type in all of the tdTomato/CFTR expressing hiPSC lines that were examined. The number of times the same wild type sequence appeared is shown in brackets.