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Supplemental information

CRISPR-Cas9-mediated gene disruption of HIV-1 co-receptors confers broad resistance to infection in human T cells and humanized mice

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Supplemental Figure 1.

Aligned_Sequence	n_deleted	n_inserted	n_mutated	#Reads	%Reads
GAAGCAAATCGCAGCCCGCCTCCTG**CCT**CCGCTCTACTCACTGGTGTTCATCTTT	0	0	0	137121	14.5
GAAGCAAATCGCAGCCCGCCTCCTGC-----CTCTACTCACTGGTGTTCATCTTT	5	0	0	144008	15.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTC--CTCTACTCACTGGTGTTCATCTTT	2	0	0	81880	8.6
GAAGCAAATCGCAGCCCGCCTCCTGCCTCC-CTCTACTCACTGGTGTTCATCTTT	1	0	0	45896	4.8
GAAGCAAATCGCAGCCCGCCTCCTGC----------CTCACTGGTGTTCATCTTT	10	0	0	36807	3.9
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG--CTACTCACTGGTGTTCATCTTT	2	0	0	34489	3.6
GAAGCAAATCGCAGCCCGC----------CTCTACTCACTGGTGTTCATCTTT	12	0	0	28678	3.0
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCCTCTACTCACTGGTGTTCATCTTT	0	1	0	26213	2.8
GAAGCAAATCGCAGCCCGCCTCCTGCCTC----CTACTCACTGGTGTTCATCTTT	4	0	0	21906	2.3
GAAGCAAATCGCAGCCCGCCTC----------CTACTCACTGGTGTTCATCTTT	11	0	0	15261	1.6
GAAGCAAATCGCAGCCCGCCTC---------CTCTACTCACTGGTGTTCATCTTT	9	0	0	13752	1.5
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCTCCTCTACTCACTGGTGTTCATCTTT	0	1	1	12572	1.3
GAAGCAAATCGCAGCCCGCCTC-----------------CTGGTGTTCATCTTT	18	0	0	11620	1.2
GAAGCAAATCGCAGCCCGCCTCCTGC-------CTACTCACTGGTGTTCATCTTT	7	0	0	11131	1.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTC----------CTGGTGTTCATCTTT	11	0	0	10887	1.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG-TCTACTCACTGGTGTTCATCTTT	1	0	0	6799	0.7
GAAGCAAATCGCAGCCCGC--------------CTACTCACTGGTGTTCATCTTT	14	0	0	6083	0.6
GAAGCAAATCGCAGCCCGCCTCCTGCCT---CTCTACTCACTGGTGTTCATCTTT	3	0	0	5768	0.6
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG-----CTCACTGGTGTTCATCTTT	5	0	0	5526	0.6
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG-------CACTGGTGTTCATCTTT	7	0	0	5184	0.5
GAAGCAAATCGCAGCCCGCCTCCTGCCTCC----------GGTGTTCATCTTT	12	0	0	4917	0.5
GAAGCAAATCGCAGCCCGCCTCCTGCCTCC----------GTGTTCATCTTT	13	0	0	4804	0.5
GAAGCAAATCGCAGCCCGC----------------CTCACTGGTGTTCATCTTT	17	0	0	4262	0.4
GAAGCAAATCGCAGCCCGCCTCCTGCCTC-------CTCACTGGTGTTCATCTTT	7	0	0	4213	0.4
GAAGCAAATCGCAGCCCGCCTCCTGCCTAC-----ACTCACTGGTGTTCATCTTT	5	0	1	3600	0.4
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCGTCTCTACTCACTGGTGTTCATCTTT	0	1	0	3540	0.4
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCTGCCTCCTCTACTCACTGGTGTTCATC	0	5	1	3486	0.4
GAAGCAAA----------TCACTGGTGTTCATCTTT	29	0	0	3303	0.3
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG---------CTGGTGTTCATCTTT	9	0	0	3253	0.3
GAAGCAAATCGCAGCCCGCCTCCTGC-------CTACTCGCTGGTGTTCATCTTT	7	0	1	3192	0.3
GAAGCAAATCGCA----------CACTCACTGGTGTTCATCTTT	21	0	1	3183	0.3
GAAG----------CTGGTGTTCATCTTT	36	0	0	2706	0.3
GAAGCAAATCGCAGCCCGCCTCC--------CTCTACTCACTGGTGTTCATCTTT	8	0	0	2373	0.3
GAAGCAAATCGCAGCCCGCCTCCTG----------CTGGTGTTCATCTTT	15	0	0	2364	0.2

Aligned_Sequence	n_deleted	n_inserted	n_mutated	#Reads	%Reads
GAAGCAAATCGCAGCCCGCCTCCTGCCTC----------GGTGTTCATCTTT	13	0	0	2345	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCT----------CTGGTGTTCATCTTT	12	0	0	2221	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG----ACTCACTGGTGTTCATCTTT	4	0	0	2072	0.2
GAAGCAAATCGCAGCCCGCCTCCTGC----------CTGGTGTTCATCTTT	14	0	0	1896	0.2
GAAGCAAATCGCA----------GGTGTTCATCTTT	29	0	0	1867	0.2
GAAGCAAATCGCAGCCCAACTCCTGC-----CTCTACTCACTGGTGTTCATCTTT	5	0	2	1833	0.2
GAAGCAAATCGCAGCCCGC----------CTGGTGTTCATCTTT	21	0	0	1828	0.2
GAAGCAAATCGCAGCCCGCCTCCT------CCTCTACTCACTGGTGTTCATCTTT	6	0	1	1784	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG---TACTCACTGGTGTTCATCTTT	3	0	0	1746	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG-TCTACCCACTGGTGTTCATCTTT	1	0	1	1706	0.2
GAAGCAAATCGCAGCCCGCCTCCT------GCTCTACTCACTGGTGTTCATCTTT	6	0	0	1658	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCGGCTCTACTCACTGGTGTTCATCTTT	0	1	0	1605	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCC-----ACTCACTGGTGTTCATCTTT	5	0	0	1533	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG--------ACTGGTGTTCATCTTT	8	0	0	1501	0.2
GAAGCAAATCGCAGCCCGCCTCCT-----CCCTCTACTCACTGGTGTTCATCTTT	5	0	1	1500	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG------TCACTGGTGTTCATCTTT	6	0	0	1496	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCC----------TCATCTTT	20	0	0	1452	0.2
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCTCCTGCCTCTACTCACTGGTGTTCATC	0	5	1	1430	0.2
GAAGCAAATCGCAGCCCGC----------ATCTACTCACTGGTGTTCATCTTT	12	0	1	1351	0.1
GAAGCAAATCGCAGCCCGCCTCC----------TGTTCATCTTT	21	0	0	1332	0.1
GAAGCAAATCGCAGCCCGCAACAC----------CTCACTGGTGTTCATCTTI	14	2	1	1294	0.1
GAAGCAAATCTCTAGC----------AAATCTCTACTCACTGGTGTTCATC	16	5	1	1276	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCTCCTGC-CTACTCACTGGTGTTCATCI	1	4	0	1244	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCC----------GATGTTCATCTTT	12	0	1	1238	0.1
GAAGCAAATCGCAGCCCTCCTCCTGCCTC--CTCTACTCACTGGTGTTCATCTTT	2	0	1	1206	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG----------TGGTGTTCATCTTT	10	0	0	1200	0.1
GAAGCAAATCGCAGCCCG----------CTCACTGGTGTTCATCTTT	18	0	0	1200	0.1
GAAGCAAATCGTGG----------TGTTTTACTCACTGGTGTTCATCTTT	15	0	5	1196	0.1
GAAGCAAATCGCAGCCCG----------CTGGTGTTCATCTTT	22	0	0	1172	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCGCTTCTACTCACTGGTGTTCATCTTT	0	1	0	1156	0.1
GAAGCAAATCGCAGCCCGCCTCCCGCCTC--CTCTACTCACTGGTGTTCATCTTT	2	0	1	1135	0.1
GAAGCAAATCGCAGCCCGCCTC------CACCTCTACTCACTGGTGTTCATCTTT	6	0	2	1026	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCAG---TACTCACTGGTGTTCATCTTT	3	1	0	1022	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCGACTCTACTCACTGGTGTTCATCTTT	0	1	0	1021	0.1
GAAGCAAATCGCAGCCCGCCTCCTGCCTCCG-----ATCACTGGTGTTCATCTTT	5	0	1	1000	0.1

Figure S1. Deep sequencing analysis of CCR5 CRISPR generated indels in CCR5^{+}CD4^{+} CEM T cells. After transduction with the CRISPR/Cas9 vector targeting CCR5, cells were FACS sorted by gating of Tag-RFP expression. Genomic DNA was extracted and primers were designed to amplify about 150bp amplicons with the cutting site in the middle. PCR product was purified and sent for next generation sequencing. The first listed sequence is the reference sequence for the CCR5 target site. The bolded sequence (CCT, which is a reverse complement of NGG canonical PAM domain) in the reference sequence indicates the PAM domain for the target site. Indels that at least 1000 reads are listed. Red letters indicate nucleotides that were inserted or mutated, while red dashes indicate nucleotides that were deleted.

Supplemental Figure 2.

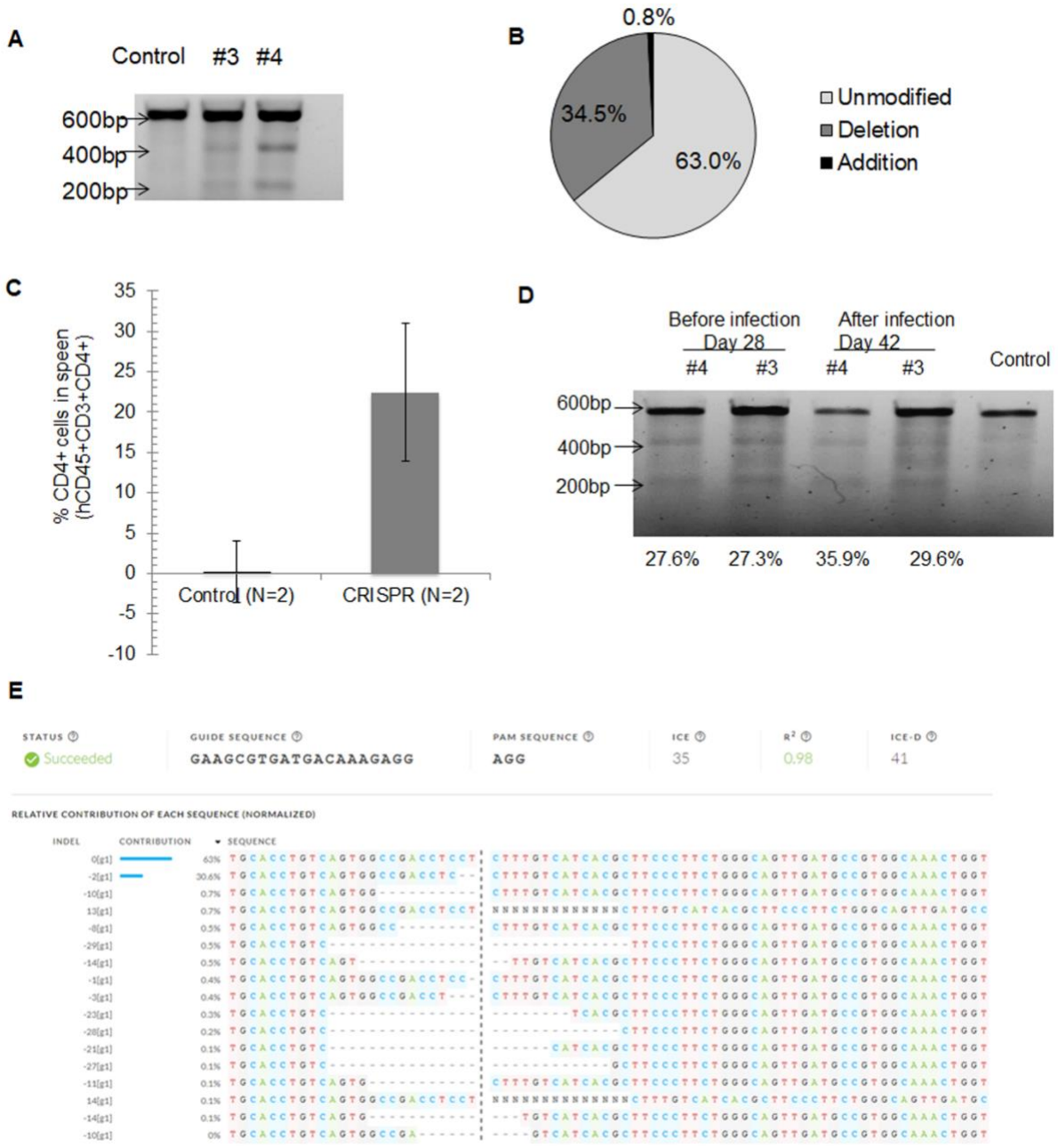

Figure S2. Surveyor assay, human CD4⁺ T cells detection and ICE analysis of indels generated by CRISPR CXCR4 modification in mice spleen cells. Mice spleens are harvested 12 weeks after transplantation which is 8 weeks after HIV-1 infection. **A.** Surveyor assay represent the allelic disruption of *cxcr4* gene in the spleen cells from humanized mice transplanted by using CXCR4 CRISPR modified or unmodified cells (control). **B.** Quantitative analysis of indels generated by CXCR4

CRISPR in spleen cells in humanized mice. **C.** Flow cytometry analysis of $CD4^+$ T cell numbers in mice spleen 12 weeks after transplantation by using CXCR4 CRISPR modified or unmodified cells (control). **D.** Surveyor assay detection of the allelic disruption of *cxcr4* gene in CXCR4 CRISPR modified cells isolated from humanized NSG mice at 4 and 6 weeks after transplantation. Mice whole blood were collected by retro-orbital bleeding before HIV-1_{NL4-3} infection (4 weeks after transplantation) and 2 weeks after HIV-1_{NL4-3} infection (6 weeks after transplantation) **E.** Genomic DNA was extracted by using QiaAmp mini DNA kit and the sequences were PCR amplified by Phusion High-fidelity polymerase. PCR products are purified by using Qiagen PCR purification kit and then Sanger sequenced. The Sanger sequencing results were uploaded into SyntheGo ICE Sanger sequencing analysis system for analysis.

Supplemental Figure 3.

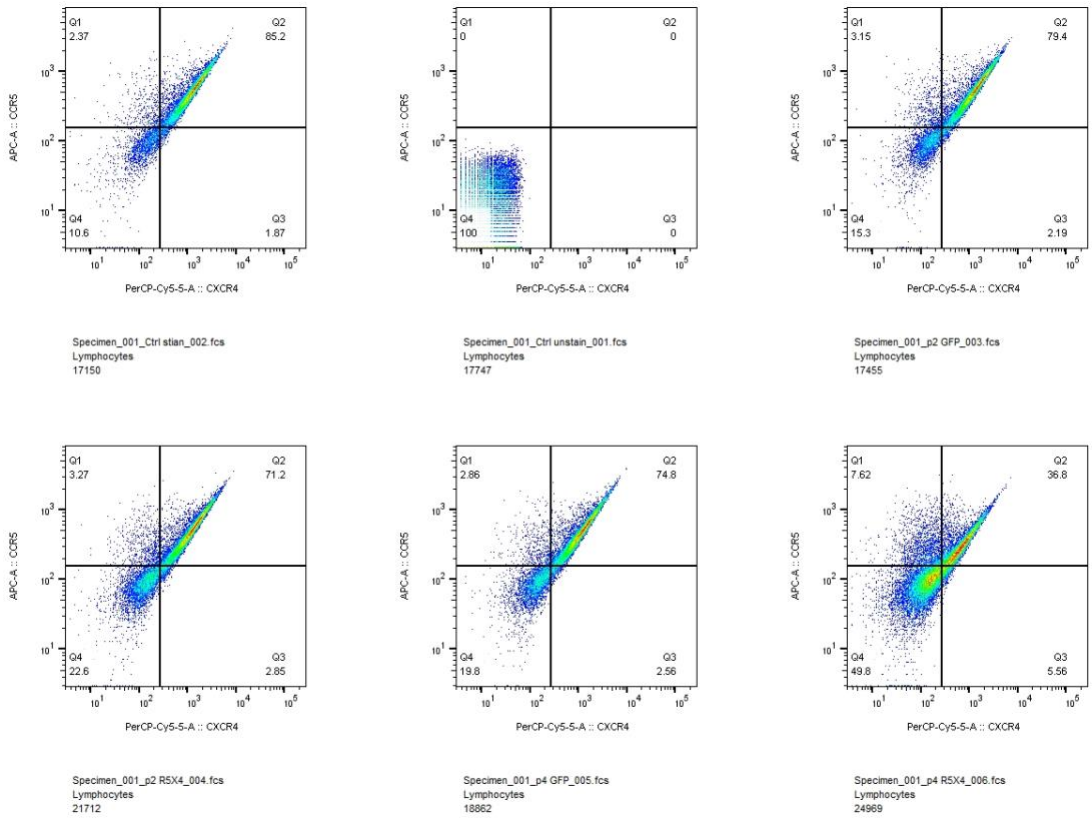

Figure S3. Double knockout population in CRISPR modified CD4$^+$ T cells by using MaxCyte electroporation. In the flow cytometry data matrix, the Q1 quadrant (upper left) indicates CCR5$^+$CXCR4$^-$ cells, Q2 (upper right) indicates CCR5$^+$CXCR4$^+$ cells, Q3 (lower right) indicates CCR5$^-$CXCR4$^-$ cells, and Q4 (lower left) indicates CCR5$^-$CXCR4$^-$ cells. MaxCyte GXT programs 2 and 4 were tested for optimal transfection efficiency. The top left panel is the untreated control condition with primary CD4$^+$ T cells showing 85.2% of cells expressing CCR5 and CXCR4. The top middle panel is the unstained control. The GFP control treated conditions were tested at the MaxCyte GXT program 2 setting (top right), the MaxCyte GXT program 4 setting (bottom center). The R5X4 CRISPR treated condition were tested at the MaxCyte GXT program 2 setting (bottom left) and the MaxCyte GXT program 4 setting (bottom right). Cells were fixed with 2% formaldehyde and stained using APC-conjugated anti-human CCR5 and PerCP-Cy5-conjugated anti-CXCR4 monoclonal antibodies.

Supplemental Figure 4.

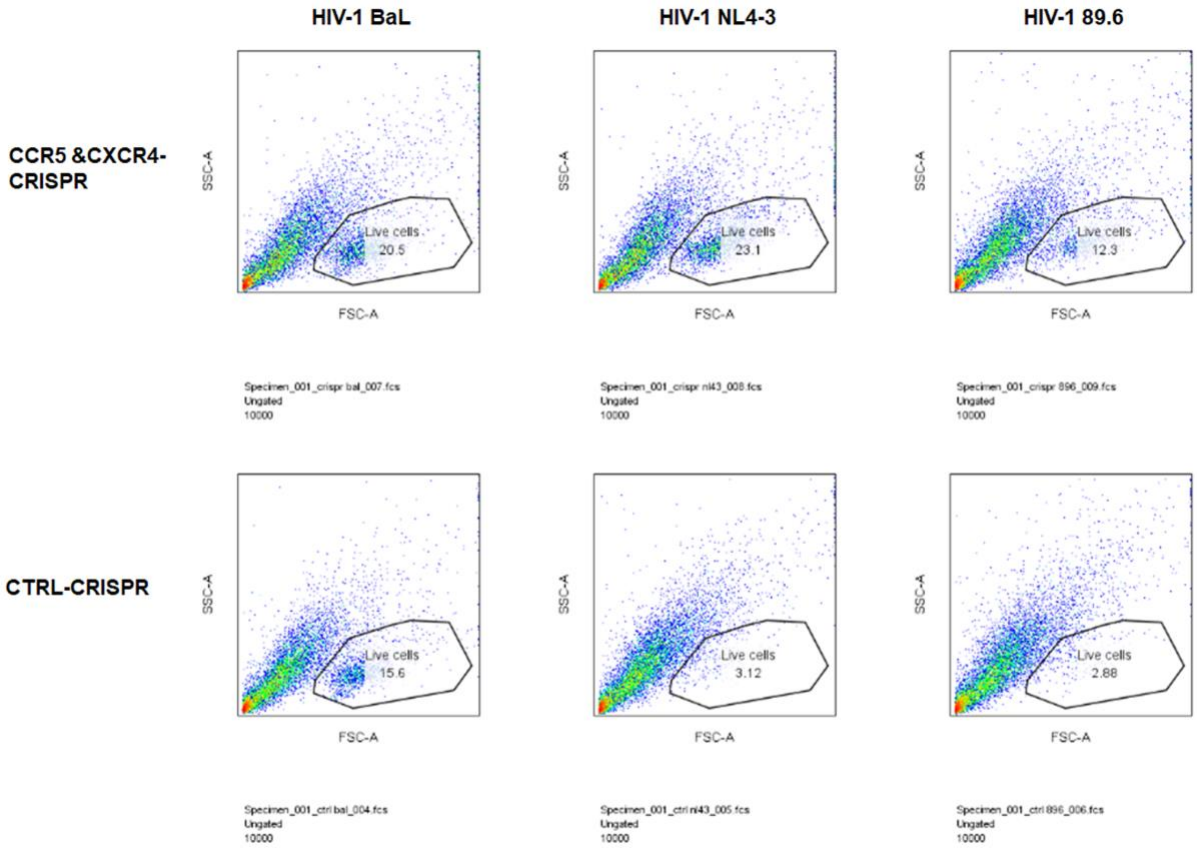

Figure S4. Flow cytometry of live cells for R5X4-CRISPR treated and control cells after challenge with each HIV-1 virus strain. Cells were analyzed by flow cytometry 5 weeks after infection with HIV-1_{BaL} (R5-tropic), HIV-1_{NL4-3} (X4-tropic), or HIV-$1_{89.6}$ (R5X4-tropic). Live cells were gated on forward scatter and sided scatter. These cells were further analyzed for CCR5 and CXCR4 surface expression (Figure 5C) or intracellular p24 antigen (Figure 5D).

Supplemental Figure 5.

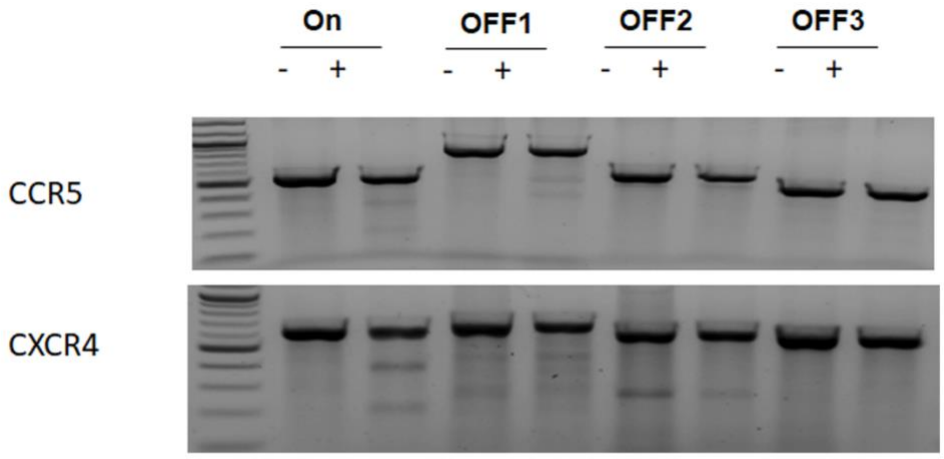

Figure S5. Off target sites predicted by Cas-OFFinder. Top three off target gene were analyzed by Surveyor assay in the CCR5 and CXCR4 CRISPR treated cells, as indicated in Tables S1-S2. The respective CCR5 or CXCR4 on-target sites are indicated on the left lanes.

Table S1. *CCR5* target sequences, off target sequences and PCR primer sequences used for Surveyor assay after MaxCyte electroporation

Target name	Target sequence	NCBI Reference Sequences	PCR primers 5'-3'
R5	**AACACCAGTGAGTAGAGCGGAGG**	NC_000003.12 46372628-46373208	For: AGCACAAGATTTTATTTGGT Rev: AATAGAGCCCTGTCAAGAGT
R5OFF1	**AACACCAGcGAGTAGAGCGGAGG**	NC_000003.12 46357081-46358102	For: GGTTCTCTTGTGTCTGTCTTA Rev: TCCATCCTCGTGGAAAATAAG
R5OFF2	**AACACCAGgGAGTAcAGCGGGGG**	NC_000003.12 46264879-46265526	For: ATCTGTATCCCCATTCTTCACCA Rev: TCGATTGTCAGCAGGATTATGA
R5OFF3	**AACtCCAGTGAGgAGAGgGGTGG**	NC_000003.12 13075002-13075572	For: GTGAGGCATCGAACAGAACT Rev: GATGTGCAGATGCATGTCAT

Table S2. *CXCR4* target sequences, off target sequences and PCR primer sequences used for Surveyor assay after MaxCyte electroporation

Target name	Target sequence	Chromosome/ NCBI Reference Sequence	PCR primers 5'-3'
X4	**GAAGCGTGATGACAAAGAGGAGG**	NC_000002.12 136115905-136115270	For: CTTCAGATAACTACACCGAG Rev: AGCTTGGAGATGATAATGCA
X4OFF1	**GAgGCCTcATGACAAAGAGGGGG**	NC_000001.11 167302688-167303358	For: TGAGCACTGAGTATGGATCT Rev: AGACTATCCATCAGGACACA
X4OFF2	**GAAGgGaGATGACAcAGAGGGGG**	NC_000002.12 116027317-116027921	For: AACAGGTCTTGAAAGGTGAA Rev: AACCACCACTAACAACAAAG
X4OFF3	**GAAaCgTGATGtCAcAGAGGAGG**	NC_000002.12 233427337-233426746	For: GCAGGGCAATAAACTGAACT Rev: ATGCGTTTGCATTTCTGTGG