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Supplemental Information

Inducing CCR5Δ32/Δ32 Homozygotes in the Human Jurkat CD4+ Cell Line and Primary CD4+ Cells by CRISPR-Cas9 Genome-Editing Technology

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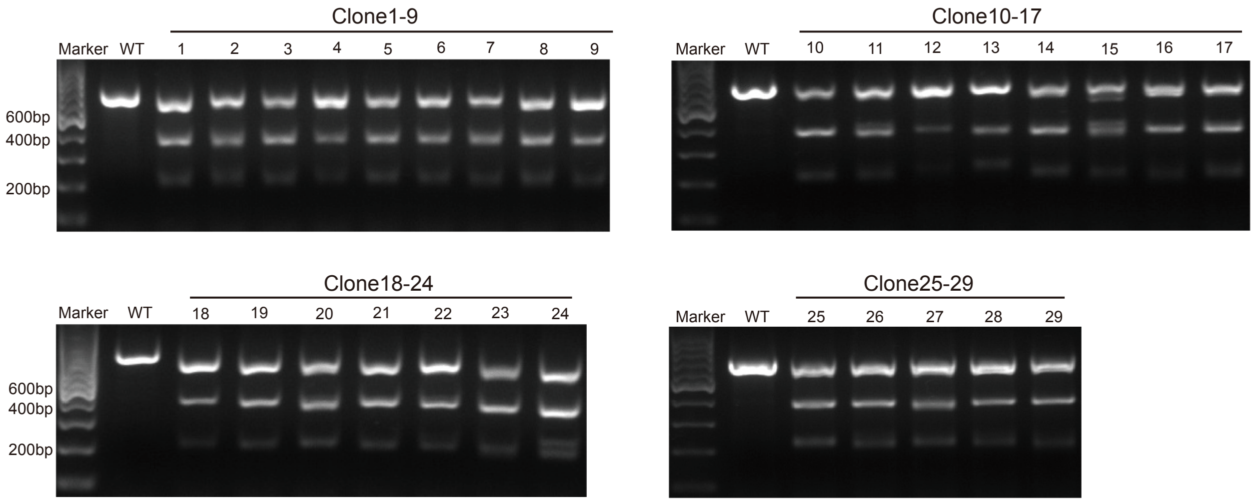

Figure S1. T7E1 digestion of CCR5 PCR products from CRISPR-Cas9 sgRNA1/2 treated puromycin-resistant monoclonal Jurkat cells.

Table S1. sgRNA for this study

sgRNA	Sequence (5' to 3')
sgRNA-1	CAGAAUUGAUACUGACUGUA
sgRNA-2	AGAUGACUAUCUUUAAUGUC

Table S2. Primers for amplifying CCR5 gene, constructing plamid and qPCR

Name	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product size (bp)	Application
CCR5	TCTTCTTCATCATCCTCCTG	GTTTGGCAATGTGCTTTT	609	Amplifying CCR5 gene across the target sites
sgRNA1-2	TTAAGAATTCGAGGGCCTATTTCCCATGAT	TAGCGAATTCAAAAAAGCACCGAC	372	Constructing lentiCRISPRv2 containing sgRNA-1 and sgRNA-2
real-time-CCR5	GGAAGAATTTCCAGACA	CTTCTCATTTCGACACC	116	Amplifying CCR5 gene for quantitative analysis
real-time-β-actin	CACCTTCTACAATGAGCTGCGTGTG	ATAGCACAGCCTGGATAGCAACGTAC	158	Amplifying β-actin gene for quantitative analysis

Table S3. Information of 15 potential off-target sites

sgRNA	Order	Chromosome	Strand	Mismatchs	Score	Sequence (5' to 3')
sgRNA-1	1	chr13	1	2	3.264103	TAGAATTGATACTGTCTGTATGG
	2	chr8	-1	3	2.392593	TATATTTGATACTGACTGTAAGG
	3	chr11	1	3	1.040778	CACAATTAATACTGACTGCAAAG
	4	chr8	-1	4	0.7171	AAAGATTGATAATGACTGTATGG
	5	chr12	-1	4	0.412429	CAAAATTCCTACTGACTGTGTGG
	6	che10	-1	3	0.195685	CAGAACTGATTCTGACTTTAGGG
	7	chr4	1	3	1.196118	CAAAATTAATACTGACAGTAAAG
	8	chr1	1	4	0.919204	GCAAATAGATACTGACTGTAGAG
sgRNA-2	1	chr3	-1	3	0.968125	AGATGATGTTCTTTAATGTCAAG
	2	chr4	1	3	0.929725	AAATGACTTTTTTTAATGTCCAG
	3	chr7	-1	3	0.869432	AGGTGACTCTCTTTAATGTGAGG
	4	chr3	1	3	0.854042	ACATGATTATCTTTAAAGTCAGG
	5	chrX	-1	4	0.770556	AGGCCACTATTTTTAATGTCTGG
	6	chr1	-1	4	0.485165	AAATTACTTTTTTTAATGTCTGG
	7	chr2	-1	4	0.328114	ATATGGATATTTTTAATGTCTGG

Table S4. Primers for amplifying 15 potential off-target sites

sgRNA	Order	Forward primer (5' to 3')	Reverse primer (5' to 3')	Product size (bp)
sgRNA-1	1	TCTCGGTGCTAACTGGAT	AATAAATGGCCTGGCTTA	563
	2	CTATTCGGACAGGTTTAT	GATGGGAATGATTAGAAG	625
	3	CCTATCTCATAATGGGAACT	TCAGGGACCTTGTCTTTT	507
	4	TTCAGAAATCCCTCACAG	AAACTTCTACCCACTCCC	577
	5	ACCAGGAGTGAGACAGAT	AACGGAGGTCCATTTAGT	779
	6	GGTGGCGGAGTGATAAGC	AGCCTGGGAGGGAAGAGT	523
	7	CTCGTCCTTATTTGCTAC	TCAGTTTGTGGGCTCTTA	832
	8	ACAGGGTGGAGTTCTTTC	TGTTACCAGGGTCGTGAG	599
sgRNA-2	1	CACTATCTGATACAGGAGCCA	CTGGAGGAAGCAAGGTGA	644
	2	TCTTCTAAGCCATCCTAT	CATACCTTCGTGCATAAC	582
	3	TTATTAAAGGACCTGGGTG	TCTGCTGGGAATTATGCT	640
	4	TCACCACCTTGTCATAAT	TGGCACCTAATAAACACT	727
	5	AAGATGGTGCTTTGTTGC	AGAGCCAAGGTTGAGAAT	680
	6	CATTCAGAACCTGCCAATA	TGTTACCTGGAGCCAATC	784
	7	CGGTCTGATTTCTTAGGT	CAAGTAGGTTGGCTGTAT	551

Table S5. Information of 34 potential off-target sites

sgRNA	Order	Chromosome	Strand	Mismatchs	Score	Sequence (5' to 3')
sgRNA-1	1	chr5	1	4	0.899986	TAGATTAGAGACTGACTGTACAG
	2	chrX	-1	4	0.63923	CATAATTAACATTGACTGTATAG
	3	chr4	1	4	0.638056	CAGTCTTGAGAGTGACTGTATGG
	4	chr3	-1	4	0.556119	GATAAAAGATACTGACTGTATGG
	5	chr20	1	4	0.542337	CTGATTGGATTCTGACTGTAGAG
	6	chrY	1	4	0.497494	CAACACTGTTACTGACTGTACAG
	7	chr5	1	4	0.423293	CAGTATTTTTAATGACTGTATAG
	8	chr7	1	4	0.412612	CAGATATAATAGTGACTGTACAG
	9	chr2	1	4	0.409264	GAGAATTGCAAGTGACTGTAAAG
	10	chr17	-1	4	0.289109	CAGTATAGCTAGTGACTGTATAG
	11	chr19	-1	4	0.28003	CAGACCTGGTCCTGACTGTATAG
sgRNA-2	1	chr12	1	3	1.517516	AGAGTACTTTCTTTAATGTCAAG
	2	chr4	-1	4	1.296863	AAATTACAACCTTTAATGTCAAG
	3	chr7	-1	4	0.905329	ATGTTAATATCTTTAATGTCTAG
	4	chr7	-1	4	0.904531	AGAAAAACATCTTTAATGTCTAG
	5	chr8	1	3	0.81265	AGGTGACTTTCATTAATGTCAAG
	6	chr8	-1	4	0.807015	TTATGACAATTTTTAATGTCTAG
	7	chr2	-1	4	0.784602	AAATTTCTAGCTTTAATGTCCAG
	8	chr2	-1	4	0.782934	AATTAACTATATTTAATGTCCAG
	9	chr13	1	4	0.697697	AGATCACCAAATTTAATGTCAAG
	10	chr8	1	4	0.669827	TAATGACTAACATTAATGTCTAG
	11	chr5	1	4	0.578474	AAATTAATTTCTTTAATGTCTAG
	12	chr18	-1	4	0.488624	ATATTAATATCATTAATGTCAAG
	13	chr11	-1	4	0.480401	ACATTTCTATGTTTAATGTCTAG
	14	chr21	1	4	0.478373	ATGTGACTTTTTTTAATGTCTAG
	15	chr5	-1	4	0.442449	AAATGTCTACATTTAATGTCTGG
	16	chr8	-1	4	0.423293	AGAGTACTTTCCTTAATGTCAAG
	17	chr12	-1	4	0.254014	AGATGGCTTGATTTAATGTCTGG
	18	chr9	1	3	0.39575	AGATGACTCTGGTTAATGTCTAG
	19	chr8	1	4	0.35551	TGATGTTTTTCTTTAATGTCCAG
	20	chr5	1	4	0.329465	AGATGTAGTTCTTTAATGTCTAG
	21	chr2	-1	4	0.293525	AAATGGCTTTTTTTAATGTCAAG
	22	chr6	1	4	0.293525	ACATGTCTTTTTTTAATGTCAAG
	23	chr6	1	4	0.262611	AGAGGAATATGATTAATGTCCAG

Table S6. Distribution of 6 and 4 Indels in the wide-type and sgRNA1/2 group

	Target sites			Indels					Group	
sgRNA	Chr	Position	Sequence (5' to 3')	Chr	Position	Reference	Mutation	Gene	WT	sgRNA1/2
sgRNA-1	chr7	116721734	CAGATATAATAGTGACTGTACAG	chr7	116721946	T	TGAGAGA	het	1	1
sgRNA-2	chr12	101090256	AGAGTACTTTCTTTAATGTCAAG	chr12	101090121	G	GA	het	1	1
	chr4	173334767	AAATTACAACCTTTAATGTCAAG	chr4	173334806	GT	G	hom	1	1
	chr2	191608841	AAATGGCTTTTTTTAATGTCAAG	chr2	191608793	A	ACT	het	1	1
	chr6	7818465	ACATGTCTTTTTTTAATGTCAAG	chr6	7818565	G	GA	het	1	1
	chr13	32346002	AGATCACCAAATTTAATGTCAAG	chr13	32346144	T	TAA	het	1	1
					32345928	T	TAA	het	1	1

The red part indicates the four Indels when the whole genome sequencing were covered by at least 20× repeats in the wide-type and sgRNA1/2 group.

Table S7. Distribution of 17 SNVs in the wide-type and sgRNA1/2 group

	Target sites			SNVs					Group	
sgRNA	Chr	Position	Sequence (5' to 3')	Chr	Position	Reference	Mutation	Gene	WT	sgRNA1/2
sgRNA-1	chr13	54263119	TAGAATTGATACTGTCTGTATGG	chr13	54262910	G	T	het	1	1
					54263249	C	T	het	1	1
	chr8	139024558	TATATTTGATACTGACTGTAAGG	chr8	139024326	A	G	hom	1	1
	chr12	67433224	CAAAATTCCTACTGACTGTGTGG	chr12	67433136	A	C	hom	1	1
	chr4	179505551	CAAAATTAATACTGACAGTAAAG	chr4	179505530	G	C	hom	1	1
	chr17	9510334	CAGTATAGCTAGTGACTGTATAG	chr17	9510599	A	G	hom	1	1
	chr19	3152159	CAGACCTGGTCCTGACTGTATAG	chr19	3152298	C	T	hom	1	1
sgRNA-2	chr4	27944663	AAATGACTTTTTTTAATGTCCAG	chr4	27944440	A	T	het	1	1
					27944883	T	A	hom	1	1
	chr12	101090256	AGAGTACTTTCTTTAATGTCAAG	chr12	101090545	G	T	hom	1	1
	chr2	159448454	AAATTTCTAGCTTTAATGTCCAG	chr2	159448238	G	A	het	1	1
					159448306	G	C	het	1	1
	chr13	32346002	AGATCACCAAATTTAATGTCAAG	chr13	32345752	T	C	het	1	1
	chr5	18818030	AAATTAATTTCTTTAATGTCTAG	chr5	18817870	A	G	het	1	1
	chr18	41236144	ATATTAATATCATTAATGTCAAG	chr18	41236042	G	A	hom	1	1
					41236054	G	A	het	1	1
	chr8	63481472	AGAGTACTTTCCTTAATGTCAAG	chr8	63481760	G	A	hom	1	1
	chr9	132110847	AGATGACTCTGGTTAATGTCTAG	chr9	132110836	C	T	hom	1	1
	chr8	3892234	TGATGTTTTTCTTTAATGTCCAG	chr8	3892017	C	A	het	1	1
	chr5	72612523	AGATGTAGTTCTTTAATGTCTAG	chr5	72612727	A	G	hom	1	1
	chr2	191608841	AAATGGCTTTTTTTAATGTCAAG	chr2	191608752	A	G	het	1	1