Supplemental Information

Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins

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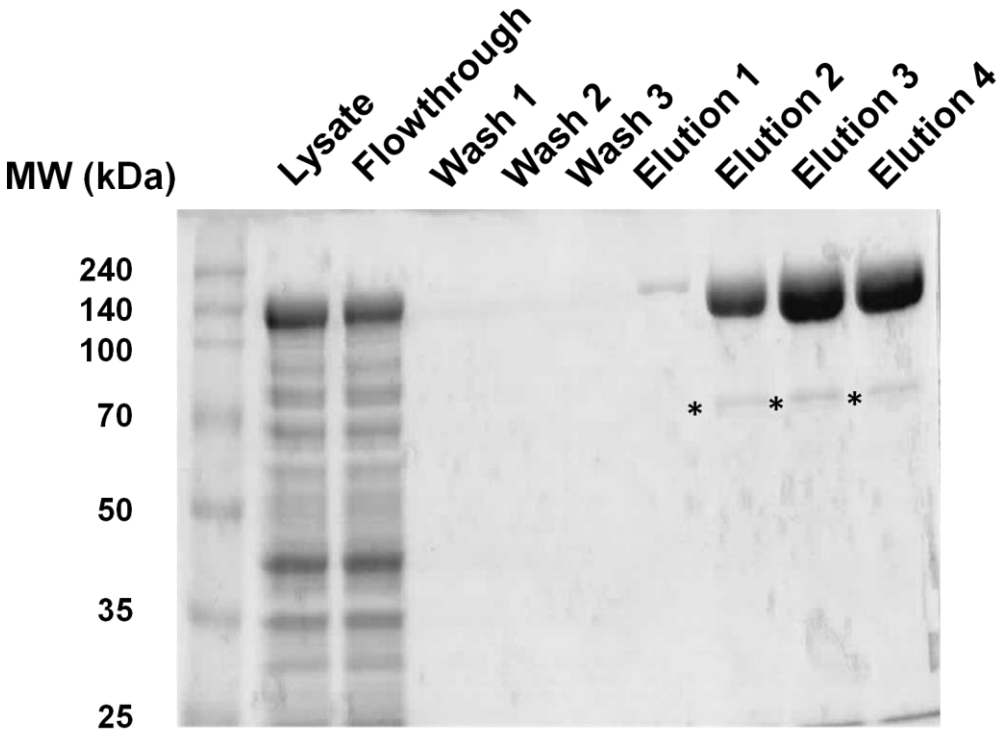
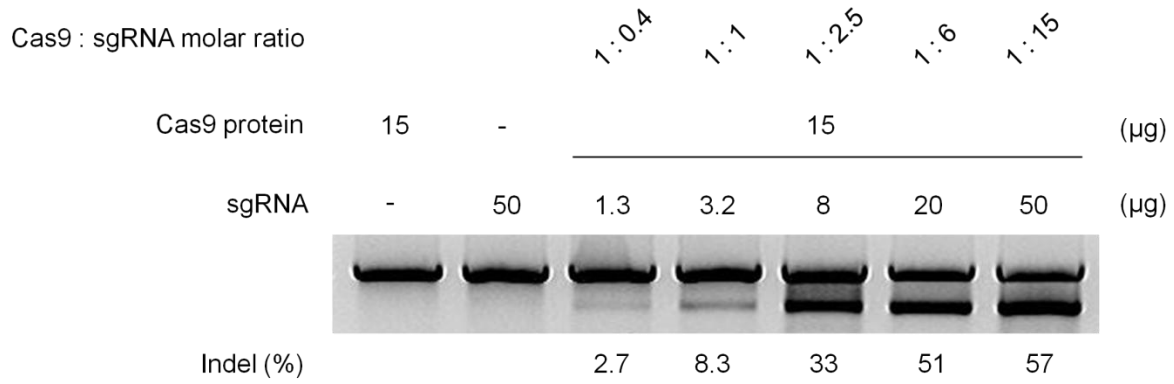
Supplemental Figures and Tables

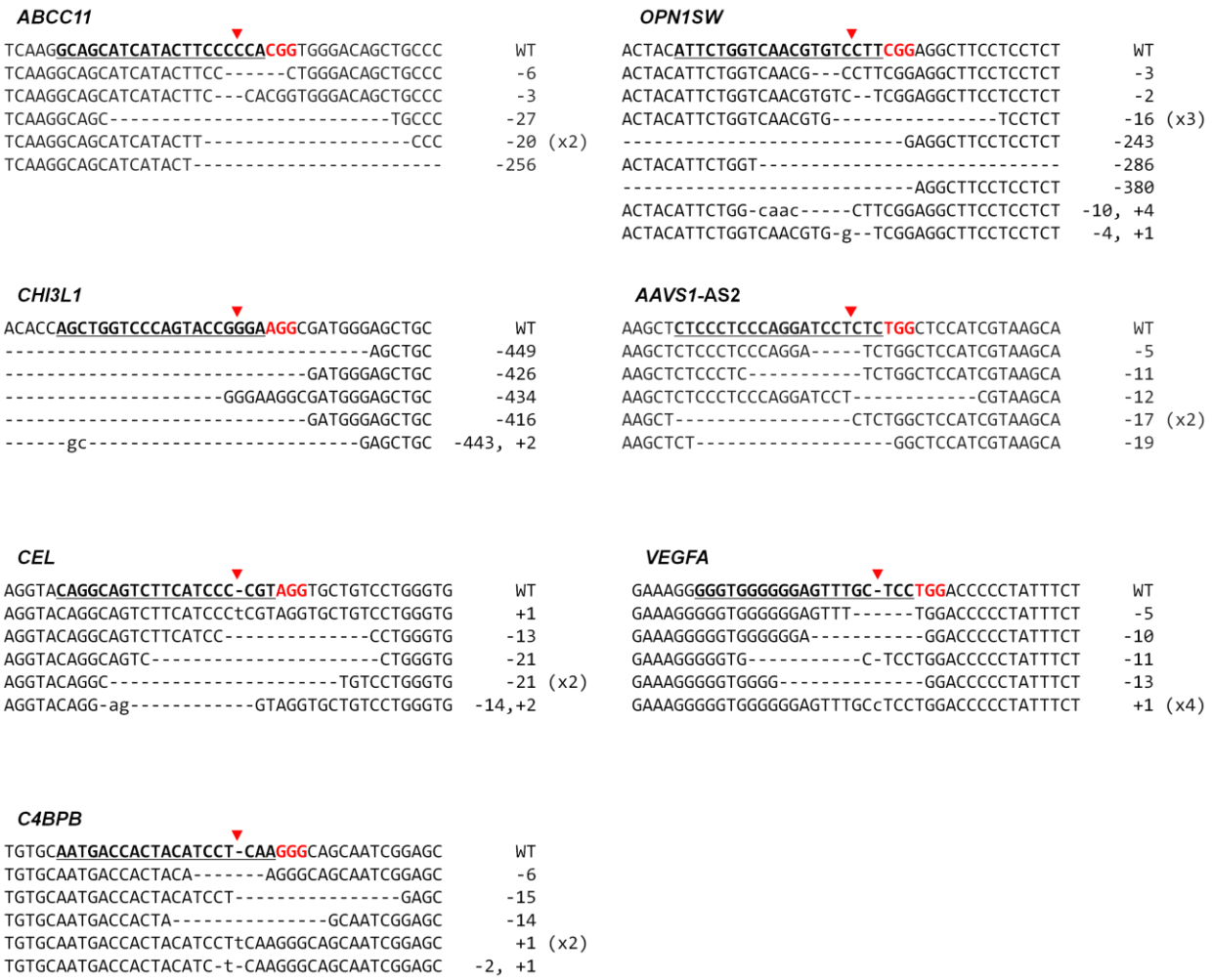
Supplemental Figure 1. SDS-PAGE image of purified Cas9 protein. His-tagged Cas9 protein was expressed in and purified from E. coli as described in the Method section. Asterisks indicate non-specific bands.



Supplemental Figure 2. Optimization of conditions for RGEN RNP delivery. A T7E1 assay was performed after the transfection of Cas9 protein (15 μg) pre-mixed with variable amounts of the *CCR5*-targeting sgRNA into K562 cells.



Supplemental Figure 3. Targeted gene disruption via RGEN RNP delivery. RNP complex-driven mutations in the *ABCC11, OPN1SW, CHI3L1, AAVS1, CEL, VEGFA,* and *C4BPB* genes in K562 cells. A mixture of Cas9 protein (15 μg) and sgRNA (20 μg) was transfected into K562 cells. The 20-bp target sequence is underlined. The PAM sequence is shown in red.



Supplemental Figure 4. DNA sequences of mutant clones. *CCR5*-specific RGEN-induced mutant clones were isolated by limiting dilution from populations of K562 cells treated with RGEN RNPs. Cas9 protein (45 μg) mixed with crRNA (8 μg) and tracrRNA (16 μg) were transfected into $2x10^5$ K562 cells. The region of the target sequence complementary to the sgRNA is underlined. The PAM sequence recognized by Cas9 is shown in red.

12 % (= 5/42) mutated

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WT          ...CAATCTATGACATCAATTATTATACATCGGAGCCCTGC...

Clone 1     ...CAATCTATGA----------CATCGGAGCCCTGC...          -14

Clone 2a    ...CAATCTATGACATCAATTATTA--CATCGGAGCCCTGC...        -2
Clone 2b    ...CAATCTATGACATCA----------GAGCCCTGC...          -14

Clone 3     ...CAATCTATGAC--------------------------...       -27

Clone 4     ...CAATCTATGA----------CATCGGAGCCCTGC...          -14

Clone 5     ...CAATCTATGACATCAATTATTATA-----GAGCCCTGC...        -5
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Supplemental Table 1. Plasmid sequences inserted at RGEN on-target and off-target sites. We analyzed the mutant DNA sequences reported in Supplementary Figs. 3 and 4 in Fu et al. (2013) and those in Fig. 3 in Cradick et al. (2013) and found that 6 out of 26 insertions and an 216-bp insertion (shown in red), respectively, observed at RGEN off-target sites were derived from plasmids that encode Cas9 or sgRNA.

Cell line		Target	Sequence	Insertion size (bp)	Source
HEK 293 cells	Target 1 (VEGFA Site 1)	OT1-3	tcagacaggacattctgacacc<u>ccaggagcaaactccctccatcc</u>cacaaatccgtccttagatgtg	wild-type	
			tcagacaggacattctgacaccccagg**atgtttgtttgagtcagagtctctcttttgtcacccaggctgg agtgcagtggaaacctgtgccttttgtatatcctctttgaaggttaaagagtcatcatggatcancnnca taa**agcaaactccntccatcc	+116	pJDS246 (Cas9 expression vector)
			tcagacaggacattctgacaccccagga**taagcggcagggtcggaacaggagagcgcacgagggagcttc cagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctctaggggagttctgagctctg acgccgggcgtgttaggagatagcaggccgttaatgaccatcccagccgaattcctcactgtgcagatga ggaagtgagctcagggaggctgagtgtcccaggcctgttgccagatgaggccacgctgagactgt**gcaaa ctccctccatcccacaaatccgtccttagatgtg	+247	pJDS246 (Cas9 expression vector) or pMLM3636 (sgRNA expression vector)
	Target 2 (VEGFA Site 2)	OT2-24	gtccctctgggccccatcctccc<u>ctccccacccaccccgcctcagg</u>cttgaagaggaaagaagagca	wild-type	
			gtccctctgggccccatcctcccctccccacccaccccg**catcgtacgtgtccttactaagctgcaattt ggcatcttcagctaagtcgaagttcga**cctcaggcttgaagaggaaagaagagcg	+58	pJDS246 (Cas9 expression vector)
	Target 3 (VEGFA Site 3)	OT3-2	gagagcgagtgagtg<u>agtgagtgagtgtgtgtgtgggggg</u>gactcggcttgttgttgtcggtgactt	wild-type	
			gagagcgagtgagtgagtgagtgagtgtgngtntnnnn**atttcacacaacatacgagccggaagcataaa gtgtaaagcctagggtgcctaatgagtgagctaactcacattaattgcgttgcgctcactgcccgctttc cagtcgggaaacctgtcgtgccagctgcattaatgaatcggccaannnnngnnnagaggcggtttgcgta ttgggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatc agctcactcaaaggcggtaatacggttatccacanaan**gggactcggcttgttgttgtcggtgactt	+280	pJDS246 (Cas9 expression vector)
		OT3-18	caaagacagtagatcttaaatgt<u>cctcacgcacacactcacccaca</u>cataaaaggtggtaactgtgt	wild-type	
			caaagacagtagatcttaaatgtcctcac**gccgacnattgcatgaagaatctgcttagggttaggcgttt tgcgctgcttcgcgatgtacgggccagata**gcacacactcacccacacataaaaggtggtaactgtgt	+71	pJDS246 (Cas9 expression vector)
			caaagacagtagatcttaaatgtcctcac**gtggaaacatagtagatgaggtggcatatcatgaaaagtac ccaacgatttatcacctcanaaaaaagctagttgactcaactgataaagcggacctgaggttaatctact tggctcttgcccatatgataaagttccgtgggcactttctcattgagggtgatctaaatccggacaactc ggatgtcga**gcacacactcacccacacataaaaggtggtaactgtgt	+190	pJDS246 (Cas9 expression vector)
	human hemoglobin δ(HBD)	Off-target HDB	accaccaactgcatc<u>cacgttcactttgccccacaggg</u>cattgacagcagtcttctcctcaggag	wild-type	
			accaccaactgcatccacgttcactttgcccc**ggcgtcaatacgggataataccgcgccacatag**	+216	pX330 (Cas9 and sgRNA expression vector)

Supplemental Table 2. Oligonucleotides used in this study.

List of primers used for nested or hemi-nested PCR.

		1st PCR		2nd PCR	
	Target	Forward (5' to 3')	Reverse (5' to 3')	Forward (5' to 3')	Reverse (5' to 3')
T7E1	*CCR5*	CTCCATGGTGCTATAGAGCA	GCCCTGTCAAGAGTTGACAC	GAGCCAAGCTCTCCATCTAGT	
	ABCC11	TCTGTCAATCCTTGCTGTGC	CACAGCACCTTCCTCAAACA	GGTCCCTCATTCTGCAGGTA	
	OPN1SW	GCAGACTTAAGATGGGCACC	GACAGGGCTGGACTGACATT	GGGCCACAAAAGGTTTAGGT	
	AAVS1-AS2	GGAGTTTTCCACACGGACAC	CCCCTATGTCCACTTCAGGA	TGCTTCTCCTCTTGGGAAGT	CGGTTAATGTGGCTCTGGTT
	CHI3L1	ACTAAAGGTGCCAGCCTGAA	GTCACAGGCTTCATGGGAGT		CTCCCTGGAGAGCACGTTAG
	VEGFA	TTGGTGCCAAATTCTTCTCC	CCAAGGTTCACAGCCTGAAA	TCCAGATGGCACATTGTCAG	AGGGAGCAGGAAAGTGAGGT
	CEL	GAGGATCGGAGTCAAAGCTG	CAGATCATAACGGGCAGGTC	GCTTTGAAGCTGTCTTTGCC	
	C4BPB	TATTTGGCTGGTTGAAAGGG	AAAGTCATGAAATAAACACACCCA	CTGCATTGATATGGTAGTACCATG	GCTGTTCATTGCAATGGAATG
Off-target (*AAVS1*-S3)	On-target	GGAGTTTTCCACACGGACAC	CCCCTATGTCCACTTCAGGA	TGCTTCTCCTCTTGGGAAGT	CGGTTAATGTGGCTCTGGTT
	S3 Off-1	CAGCTTCCTGGAAAGACCTG	GGTTTTGGCCTGACTGTGTT	TATCTCCTCTCCCCCTGCTT	GAGCTGCCTGACTTCCCTAA
	S3 Off-2	AGCTTCTGCAACTGGTGGTT	AGTTGCTGGTAGGAGGCTGA	GGATGAGAGAGCTGGTTTGC	AGGCTTCTCTTCCCTCGTTC
Off-target (AAVS1-AS2)	On-target	GGAGTTTTCCACACGGACAC	CCCCTATGTCCACTTCAGGA	TGCTTCTCCTCTTGGGAAGT	CGGTTAATGTGGCTCTGGTT
	AS2 Off-1	CAGGCTCCTTGTTCTTCTGG	AAGCCATGGTCTGCTTCACT	TGCAGGAAAAAGTTGCAGTG	GGCCAAACTGGAGAGACAGA
Off-target (*EMX1*)	On-target	CCATCCCCTTCTGTGAATGT	AATCTACCACCCCAGGCTCT	GGAGCAGCTGGTCAGAGGGG	
	OT4-1	TTGAGATGGCTGTTCAGAGG	TTGAGACATGGGGATAGAATCA	GTGGGGAGATTTGCATCTGT	CAATGGGAAGGACAGCTTCT
HR RFLP	*AAVS1*	ATAAGCTGGTCACCCCACAC	TTGCTCTCTGCTGTGTTGCT	GTGAGTTTGCCAAGCAGTCA	ACCACGTGATGTCCTCTGAG
Large deletion	L1_10kb deletion	CTCCATGGTGCTATAGAGCA	TGGAGTCTACATCTGACCAG	GAGCCAAGCTCTCCATCTAGT	TTGGTAGCAGGTTAATCCACC
	L2_30kb deletion	CTCCATGGTGCTATAGAGCA	ACTGGGTATACACCCAGAGG	GAGCCAAGCTCTCCATCTAGT	CATGAAGACACACGCATGTG
	L3_100kb deletion	CTCCATGGTGCTATAGAGCA	GTACACATTGCCAGAGTAGAG	GAGCCAAGCTCTCCATCTAGT	GTTCTTCAGGAGCAGATTATGG

Single-stranded oligonucleotide used in HR

		Sequence (5' to 3')
HR ssODN	*AAVS1*	TCCCTCCCACCCCCTGCCAAGCTCTCCCTCCCAGGATCCTTCTAGACTCTGGCTCCATCGTAAGCAAACCTTAGAGGTTCTGGCAA

***In vitro* transcription templates**

	Target	Sequence (5' to 3')
sgRNA_F	*CCR5*	GAAATTAATACGACTCACTATAGG**TGACATCAATTATTATACAT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	ABCC11	GAAATTAATACGACTCACTATAGG**GCAGCATCATACTTCCCCCA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	OPN1SW	GAAATTAATACGACTCACTATAGG**ATTCTGGTCAACGTGTCCTT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	CHI3L1	GAAATTAATACGACTCACTATAGG**AGCTGGTCCCAGTACCGGGA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	VEGFA	GAAATTAATACGACTCACTATA**GGGTGGGGGGAGTTTGCTCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	CEL	GAAATTAATACGACTCACTATAGG**CAGGCAGTCTTCATCCCCGT**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	C4BPB	GAAATTAATACGACTCACTATAGG**AATGACCACTACATCCTCAA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	AAVS1-S3	GAAATTAATACGACTCACTATAGG**GGGAGGGAGAGCTTGGCAGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	AAVS1-AS2	GAAATTAATACGACTCACTATAGG**CTCCCTCCCAGGATCCTCTC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	EMX1	GAAATTAATACGACTCACTATA**GTCACCTCCAATGACTAGGG**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	*CCR5*_L1	GAAATTAATACGACTCACTATAGG**TGAGGGCTTAACACAGTGCC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	*CCR5*_L2	GAAATTAATACGACTCACTATAGG**GAACACATGAACTCAAAGAA**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
	*CCR5*_L3	GAAATTAATACGACTCACTATAGG**ACCTAGAGACATGGGGAGTC**GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCG
sgRNA_R		AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGC