Supplemental Materials for

CRISPR RNAs trigger innate immune responses in human cells

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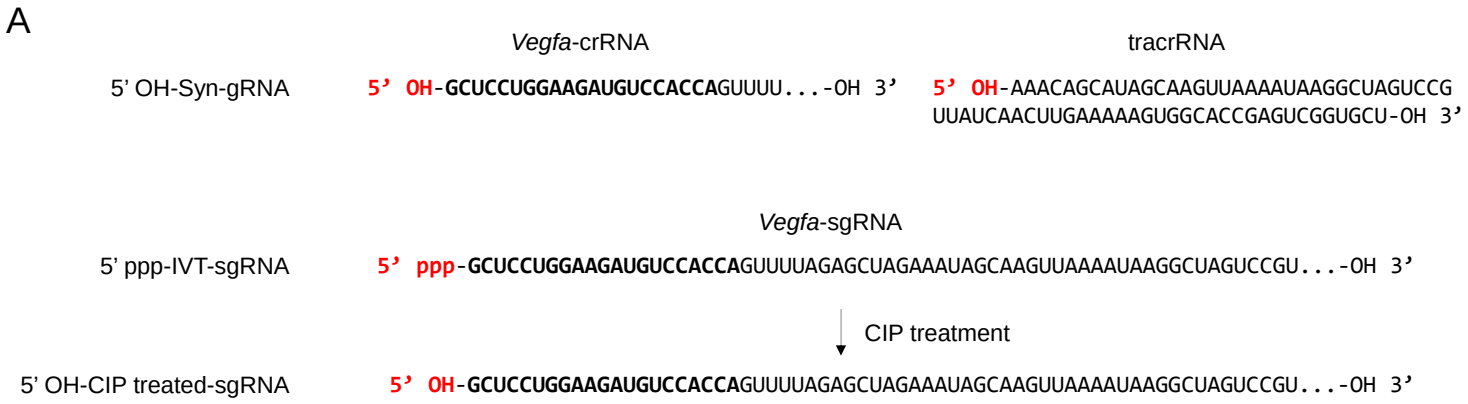
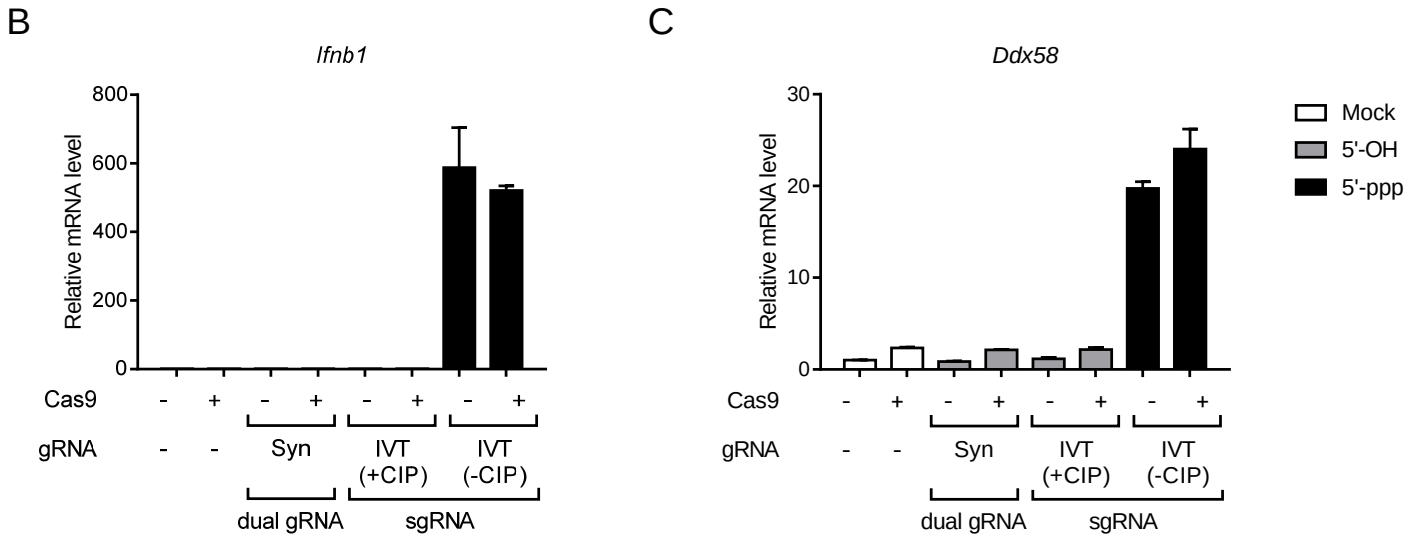
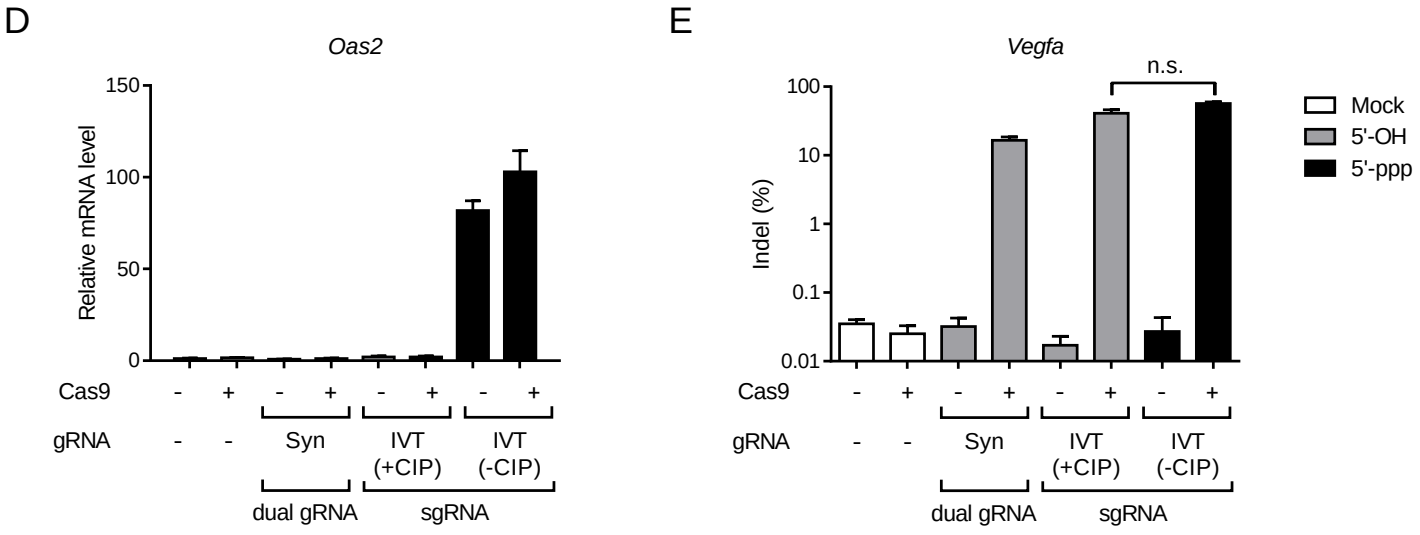
Supplemental Table S1. Oligonucleotides used in this study.

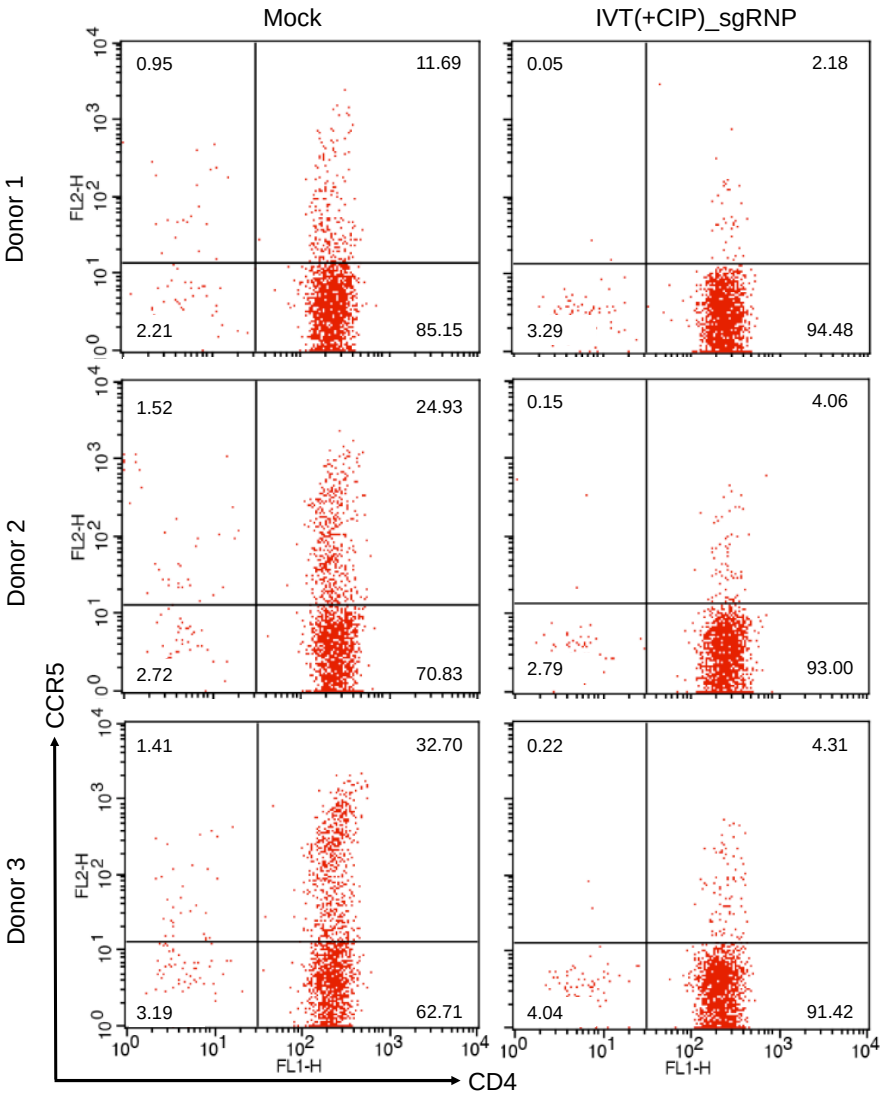
Supplemental Fig. S1. RNA-sensing immune responses activated by the Cas9 RNP containing a 5'-triphosphated gRNA in primary mouse embryonic fibroblast (MEF) cells. (A) Schematic illustration of *Vegfa*-targeting guide RNAs, which vary according to their preparation methods. (B, C and D) Relative *Ifnb1* (B), *Ddx58* (C) and *Oas2* (D) mRNA levels in MEF cells at 24hr post-transfection. Error bars, s.e.m.; n = 3. (E) Indel frequencies induced by the *Vegfa*-targeting Cas9 RNP were measured by targeted deep sequencing. Error bars, s.e.m.; n = 3. Statistical significances were calculated by t-test.







Supplemental Fig. S2. Percentages of cells positive for both surface CCR5 and CD4 protein in mock- and *CCR5*-targeting RNP-treated cell populations from 3 healthy donors were determined by flow cytometry.



Supplemental Table S1. Oligonucleotides used in this study.

List of primers used in RT-qPCR

Target	Forward (5' to 3')	Reverse (5' to 3')
ACTB	CCCAGCCATGTACGTTGCTA	TCACCGGAGTCCATCACGAT
IFNB1	TGCTTCTCCACTACAGCTCTT	GCAGTATTCAAGCCTCCCAT
DDX58	GGACGTGGCAAAACAAATCAG	GCAATGTCAATGCCTTCATCA
OAS2	TCAGAAGAGAAGCCAACGTGA	CGGAGACAGCGAGGGTAAAT
Actb	CACCAGTTCGCCATGGAT	CCTCGTCACCCACATAGGAG
Ifnb1	CACAGCCCTCTCCATCAACT	CTTTCCATTCAGCTGCTCCA
Ddx58	CCACCTACATCCTCAGCTACATGA	TGGGCCCTTGTTGTTCTTCT
Oas2	GAATCCCAGCTCCTGTGTCT	AAGCAGTCTGAGAACTCCCC

List of primers used in targeted deep sequencing

	1st PCR		2nd PCR	
Target	Forward (5' to 3')	Reverse (5' to 3')	Forward (5' to 3')	Reverse (5' to 3')
HBB	GAGCCAGGCCATCACTAAAG	TGGTATGGGGCCAAGAGATA	ACACTCTTTCCCTACACGACGCTCTTCCGATCT TGCCTATCAGAAACCCAAGAG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC TGCAACCTCAAACAGACACCA
Vegfa	TCGTCTTCTCACCCTCAACC	GTGGAGCTGTAAGGAGTGGT	ACACTCTTTCCCTACACGACGCTCTTCCGATCT GGGCTTCATCGTTACAGCAG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC TCACAAATCTGGGTGGCGATA
DNMT1	CCAGAAGTCCCGTGCAAATC	ATCTTTCTCAAGGGGCTGCT	ACACTCTTTCCCTACACGACGCTCTTCCGATCT CAGTGCATGTTGGGGATTCC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC TTGAACGTTCCCTTAGCACTCT
CCR5	GAGCCAAGCTCTCCATCTAGT	GCCCTGTCAAGAGTTGACAC	ACACTCTTTCCCTACACGACGCTCTTCCGATCT GCCTCCTGCCTCCGCTCTACTCAC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC TCCAAAGTCCCACTGGGCGGC

Oligonucleotides for *in vitro* transcription templates

	Target	Sequence (5' to 3')
sgRNA_F	*HBB*	GAAATTAATACGACTCACTATAGGGGAGGGGAAGTTTGCTCCGTTTTAGAGCTAGAAATAGCAAG
	Vegfa	GAAATTAATACGACTCACTATAGGAGGAGGGGAGTCTGCTCCGTTTTAGAGCTAGAAATAGCAAG
	CCR5	GAAATTAATACGACTCACTATAGCAGCATAGTGAGCCCAGAAGTTTTAGAGCTAGAAATAGCAAG
sgRNA_R		AAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTA GCTCTAAAAC
crRNA_F	*HBB*	GAAATTAATACGACTCACTATAgTTGCCCCACAGGGCAGTAAGTTTTAGAGCTATGCTGTTTTG
crRNA_R		CAAAACAGCATAGCTCTAAAACTTACTGCCCTGTGGGGCAAcTATAGTGAGTCGTATTAATTTC
tracrRNA_F		GAAATTAATACGACTCACTATAGGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCG
tracrRNA_R		AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATG
crRNA_F	*DNMT1*	GAAATTAATACGACTCACTATAGGG
crRNA_R		GAGTAACAGACATGGACCATCAGATCTACAAGAGTAGAAATTACCCTATAGTGAGTCGTATTAATTTC