Supplementary materials

Rapid design and development of CRISPR-Cas13a targeting SARS-CoV-2 spike protein

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Se	evere acute respiratory s	yndrome coronaviru	is 2 isolate SAR	RS-CoV-2/human/	USA/WA-U	W-4266	5/2020, complete gen	ome \$	56.0	100%	100%
Se	evere acute respiratory s	yndrome coronaviru	is 2 isolate SAR	RS-CoV-2/human/	USA/WA-U	W-4251	1/2020, complete gen	ome \$	56.0	100%	100%
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Figure S1. BLAST results of crRNA candidates. A. The crRNA-5 sequence was used as a representative sequence for alignment, revealing perfect pairing between crRNA and SARS-CoV-2 S RNA. B. Distribution of the top 100 BLAST hits on 100 subject sequences. C. BLAST tree view showing the score, query cover, and percent identity of crRNA sequence with hit sequences. D. Taxonomy indicating the specificity of the crRNA candidate targeting SARS-CoV-2.



Figure S2. Collateral cleavage induced by crRNA-6. A, B. qRT-PCR analysis showed that crRNAs 6, 10, 11, and 12 reduced GFP (A) and Cas13a (B) RNA expression in AT2 cells (P < 0.0001). C. RNA-denaturing gel electrophoresis showed that ribosomal RNA was cleaved by Cas13–crRNA-6 in AT2 cells expressing S, providing evidence of a collateral cleavage effect induced by crRNA-6. D. Western blots showing decreased levels of S, ACE2, and GAPDH in the Cas13+crRNA-6 group as compared with the Control group (P < 0.0001, P < 0.0001, P < 0.0001).



A. RNA-denaturing gel electrophoresis showing Cas13a-crRNA6 induced collateral cleavage in AT2-S cells. B. RNA-denaturing gel electrophoresis showing dCas13a-crRNA6 could not induce collateral cleavage effect in AT2-S cells. C. qRT-PCR analysis showing no significant difference in RNA levels of S and GFP between the dCas13a+crRNA6 group and Control group (P > 0.05, P > 0.05). D.

Immunofluorescence imaging showing the expression of S and ACE2 in AT2-S cells (Control group), AT2-dCas13-S cells (dCas13 group), and AT2-dCas13-S-crRNA6 cells (dCas13+crRNA6 group). E. Mean fluorescence intensity (MFI) data showing dCas13a+crRNA6 reduced S protein expression level (P < 0.001) without changing ACE2 expression (P > 0.05). F. CCK-8 analysis showing Cas13a-crRNA6 inhibited proliferation of AT2-S cells (48 h, P < 0.05; 72 h, P < 0.001; 96 h, P < 0.0001). G. No influence of dCas13a-crRNA6 on of AT2-S cell proliferation (P > 0.05).



Figure S4. The Cas13-crRNA6 neither influenced the viability of non-target cells nor induced the collateral cleavage effect in non-target cells. A. CCK-8 analysis showing no significant difference in OD values between AT2 and AT2+Cas13+crRNA6 (P > 0.05). B. CCK-8 analysis showing no significant difference in OD values between HepG2 and HepG2+Cas13+crRNA6 (P > 0.05). C. RNA-denaturing gel electrophoresis showing that Cas13-crRNA6 did not induce collateral cleavage in wild-type HepG2 and AT2 cells.



Figure S5. Cas13a-crRNA6 did not cleave RNA of SARS-CoV S. A. qRT-PCR analysis showing Cas13a-crRNA6 did not alter S' (SARS-CoV-2 S) expression level in AT2 cells expressing S' (P > 0.05). B. RNA-denaturing gel electrophoresis showing Cas13a-crRNA6 did not induce collateral cleavage effect in AT2 cells expressing S'.



Figure S6. Lentivirus vector maps. A. Vector used for S and GFP. B. Vector used for Cas13a.



Figure S7. Restriction digestion maps of 12 crRNAs.

No.	Protospacer+PFS	crRNA complementa sequence	ary crRNA _start	crRNA _end	GC cont ent
1	ACUGAAAUCUAUCAGGCC	UGACUUUAGAUAGUC	CG 1	29	0.5
	GGUAGCACACC	GCCAUCGUGUG			
2	CUGAAAUCUAUCAGGCCG	GACUUUAGAUAGUCCO	GG 2	30	0.54
	GUAGCACACCU	CCAUCGUGUGG			
3	UGAAAUCUAUCAGGCCGG	ACUUUAGAUAGUCCG	GC 3	31	0.5
	UAGCACACCUU	CAUCGUGUGGA			
4	AAAUCUAUCAGGCCGGUA	UUUAGAUAGUCCGGC	CA 5	33	0.5
	GCACACCUUGU	UCGUGUGGAAC			
5	AAUCUAUCAGGCCGGUAG	UUAGAUAGUCCGGCCA	AU 6	34	0.5
	CACACCUUGUA	CGUGUGGAACA			
6	AUCUAUCAGGCCGGUAGC	UAGAUAGUCCGGCCAU	JC 7	35	0.5
	ACACCUUGUAA	GUGUGGAACAU			
7	UCUAUCAGGCCGGUAGCA	AGAUAGUCCGGCCAU	CG 8	36	0.5
	CACCUUGUAAU	UGUGGAACAUU			
8	AUCAGGCCGGUAGCACAC	UAGUCCGGCCAUCGUC	GU 11	39	0.54
	CUUGUAAUGGU	GGAACAUUACC			
9	CAGGCCGGUAGCACACCU	GUCCGGCCAUCGUGUC	GG 13	41	0.57
	UGUAAUGGUGU	AACAUUACCAC			
10	AGGCCGGUAGCACACCUU	UCCGGCCAUCGUGUGC	GA 14	42	0.54
	GUAAUGGUGUU	ACAUUACCACA			
11	GCCGGUAGCACACCUUGU	CGGCCAUCGUGUGGAA	AC 16	44	0.54
	AAUGGUGUUGA	AUUACCACAAC			
12	CCGGUAGCACACCUUGUA	GGCCAUCGUGUGGAAG	CA 17	45	0.5
	AUGGUGUUGAA	UUACCACAACU			
13	GUAGCACACCUUGUAAUG	CAUCGUGUGGAACAU	JA 20	48	0.46
	GUGUUGAAGGU	CCACAACUUCC			
14	UAGCACACCUUGUAAUGG	AUCGUGUGGAACAUUA	AC 21	49	0.43
	UGUUGAAGGUU	CACAACUUCCA			
15	AGCACACCUUGUAAUGGU	UCGUGUGGAACAUUA	CC 22	50	0.43
	GUUGAAGGUUU	ACAACUUCCAA			
16	GCACACCUUGUAAUGGUG	CGUGUGGAACAUUACO	CA 23	51	0.43
	UUGAAGGUUUU	CAACUUCCAAA			
17	CACACCUUGUAAUGGUGU	GUGUGGAACAUUACCA	AC 24	52	0.39
	UGAAGGUUUUA	AACUUCCAAAA			
18	ACACCUUGUAAUGGUGU	UGUGGAACAUUACCAG	CA 25	53	0.36
	UGAAGGUUUUAA	ACUUCCAAAAU			
19	CACCUUGUAAUGGUGUU	GUGGAACAUUACCACA	AA 26	54	0.36
	GAAGGUUUUAAU	CUUCCAAAAUU			
20	ACCUUGUAAUGGUGUUG	UGGAACAUUACCACAA	AC 27	55	0.32

Table S1. The crRNA library

	AAGGUUUUAAUU	UUCCAAAAUUA			
21	CUUGUAAUGGUGUUGAA	GAACAUUACCACAACUU	29	57	0.32
	GGUUUUAAUUGU	CCAAAAUUAAC			
22	UUGUAAUGGUGUUGAAG	AACAUUACCACAACUUC	30	58	0.29
	GUUUUAAUUGUU	CAAAAUUAACA			
23	UGUAAUGGUGUUGAAGG	ACAUUACCACAACUUCC	31	59	0.29
	UUUUAAUUGUUA	AAAAUUAACAA			
24	GUAAUGGUGUUGAAGGU	CAUUACCACAACUUCCA	32	60	0.29
	UUUAAUUGUUAC	AAAUUAACAAU			
25	UAAUGGUGUUGAAGGUU	AUUACCACAACUUCCAA	33	61	0.29
	UUAAUUGUUACU	AAUUAACAAUG			
26	AAUGGUGUUGAAGGUUU	UUACCACAACUUCCAAA	34	62	0.29
	UAAUUGUUACUU	AUUAACAAUGA			
27	AUGGUGUUGAAGGUUUU	UACCACAACUUCCAAAA	35	63	0.29
	AAUUGUUACUUU	UUAACAAUGAA			
28	UGGUGUUGAAGGUUUUA	ACCACAACUUCCAAAAU	36	64	0.29
	AUUGUUACUUUC	UAACAAUGAAA			
29	GGUGUUGAAGGUUUUAA	CCACAACUUCCAAAAUU	37	65	0.32
	UUGUUACUUUCC	AACAAUGAAAG			
30	GUGUUGAAGGUUUUAAU	CACAACUUCCAAAAUUA	38	66	0.32
	UGUUACUUUCCU	ACAAUGAAAGG			
31	UGUUGAAGGUUUUAAUU	ACAACUUCCAAAAUUAA	39	67	0.29
	GUUACUUUCCUU	CAAUGAAAGGA			
32	GUUGAAGGUUUUAAUUG	CAACUUCCAAAAUUAAC	40	68	0.29
	UUACUUUCCUUU	AAUGAAAGGAA			
33	UUGAAGGUUUUAAUUGU	AACUUCCAAAAUUAACA	41	69	0.25
	UACUUUCCUUUA	AUGAAAGGAAA			
34	UGAAGGUUUUAAUUGUU	ACUUCCAAAAUUAACAA	42	70	0.25
	ACUUUCCUUUAC	UGAAAGGAAAU			
35	GAAGGUUUUAAUUGUUA	CUUCCAAAAUUAACAAU	43	71	0.29
	CUUUCCUUUACA	GAAAGGAAAUG			
36	AAGGUUUUAAUUGUUAC	UUCCAAAAUUAACAAUG	44	72	0.25
	UUUCCUUUACAA	AAAGGAAAUGU			
37	AGGUUUUAAUUGUUACU	UCCAAAAUUAACAAUGA	45	73	0.25
	UUCCUUUACAAU	AAGGAAAUGUU			
38	GGUUUUAAUUGUUACUU	CCAAAAUUAACAAUGAA	46	74	0.25
	UCCUUUACAAUC	AGGAAAUGUUA			
39	GUUUUAAUUGUUACUUU	CAAAAUUAACAAUGAAA	47	75	0.25
	CCUUUACAAUCA	GGAAAUGUUAG			

RUN	HBond Residue	BP of RNA	Distance	Angle DHA
1	LYS5	U38:crRNA	2.1	127.3
		U38:crRNA	2.3	124.4
		A28:RNA-SARS-Cov-2	1.9	153.2
2	ARG41	A28:RNA-SARS-Cov-2	2.7	131.4
		A28:RNA-SARS-Cov-2	1.9	158.6
3	LYS86	U27:RNA-SARS-Cov-2	1.8	102.2
4	GLN519	G13:RNA-SARS-Cov-2	1.8	106.2
5	ASN547	U47:crRNA	2.1	124.3
		U47:crRNA	1.6	161.2
		G46:crRNA	2.9	107.1
6	SER555	U45:crRNA	1.7	162.7
		U45:crRNA	2.6	140.3
7	THR557	U47:crRNA	2.8	147.1
8	LYS558	A16:RNA-SARS-Cov-2	1.7	157.8
		U15:RNA-SARS-Cov-2	2.8	99.5
		A16:RNA-SARS-Cov-2	2.3	115.2
9	LYS597	G14:RNA-SARS-Cov-2	1.8	107.2
10	TYR601	G46:crRNA	1.9	145.6
11	LYS652	U24:RNA-SARS-Cov-2	2.3	89
		C23:RNA-SARS-Cov-2	1.9	164.2
12	GLN659	A42:crRNA	2.4	143.8
13	HIS771	U50:crRNA	2.7	112.2
14	LYS778	U47:crRNA	1.6	164
15	GLU782	G46:crRNA	2	146.1
16	ASN808	U50:crRNA	1.9	119.9
		U50:crRNA	2	121.4
17	ARG809	C49:crRNA	2.3	113.1
18	ARG857	A51:crRNA	2.6	121.8
		A51:crRNA	1.5	146.2
		U50:crRNA	2	152.7
		U50:crRNA	2.6	134
		U50:crRNA	1.8	164
19	LYS902	C53:crRNA	2.3	111.9
20	HIS908	G17:RNA-SARS-Cov-2	2.9	158.2
21	LYS1124	C18:RNA-SARS-Cov-2	2	153.1
		C18:RNA-SARS-Cov-2	2.4	114.6
22	ARG1135	A19:RNA-SARS-Cov-2	2	112.6
		C18:RNA-SARS-Cov-2	2.8	109.5
23	GLN518	C12:RNA-SARS-Cov-2	2	139.1
		C12:RNA-SARS-Cov-2	2.1	141.4
24	SER522	C12:RNA-SARS-Cov-2	2.2	121.5

Table S2. Hydrogen-bonds between Cas13a and crRNA-6/viral-RNA

25	ARG527	C56:crRNA	2	129.7
		C56:crRNA	1.5	144
26	LYS718	G55:crRNA	1.8	126.4
		G55:crRNA	2.4	141.8
27	LYS723	C57:crRNA	1.5	146.1
		U58:crRNA	2.4	125.6
28	LYS727	U58:crRNA	1.7	114.8
29	GLN730	G59:crRNA	1.9	132.1
30	VAL810	C11:RNA-SARS-Cov-2	2.8	111
31	LYS845	C7:RNA-SARS-Cov-2	2.5	147.8
32	LYS894	A8:RNA-SARS-Cov-2	2.8	104.3