Supplementary materials

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

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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).
Figure S3. dCas13a-crRNA6 reduced protein expression of S without inducing collateral cleavage effect.

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 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.
Table S1. The crRNA library

| No. | Protospacer+PFS | crRNA sequence | complementary crRNA sequence | crRNA_start | crRNA_end | GC content |
|-----|-----------------|----------------|--------------------------------|-------------|-----------|------------|
| 1   | ACUGAAAUCAUCAGGCCGUAAGCACACC | UGACUUUAGAUAGUCCG | GCCAUCGUGUG | 1 | 29 | 0.5 |
| 2   | CUGAAAUCAUCAGGCCGUAAGCACACC | GACUUUAGAUAGUCCG | CCAUCGUGUGG | 2 | 30 | 0.54 |
| 3   | UGAAAUCAUCAGGCCGUAAGCACACC | ACUUUAGAUAGUCCGCC | CAUCGUGUGGA | 3 | 31 | 0.5 |
| 4   | AAUCUAUCAGGCCGUAAGCACACC | UUUAGAUAGUCCGCCA | UCGUGUGGAAC | 5 | 33 | 0.5 |
| 5   | AAUCUAUCAGGCCGUAAGCACACC | UUAGAUAGUCCGCCAU | CGUGUGGAACA | 6 | 34 | 0.5 |
| 6   | AAUCUAUCAGGCCGUAAGCACACC | UAGAUAGUCCGCCAUC | GUGUGGAACA | 7 | 35 | 0.5 |
| 7   | UCUAUCAUCAGGCCGUAAGCACACC | UGAGUAGUCCGCCAUCG | UGUGGAACA | 8 | 36 | 0.5 |
| 8   | AUCAGGCGGCUAGCACACC | UAGUCCGCCAACUGUGU | GGAACAUUACC | 11 | 39 | 0.54 |
| 9   | CAGGCGGCUAGCACACC | GUCCGCGCAUCGUGUGG | ACAUUACCAC | 13 | 41 | 0.57 |
| 10  | AGGCUGCUGACACACC | UCGCCGCAUGUGUGGA | ACAUUAACCAC | 14 | 42 | 0.54 |
| 11  | GCRCUGCUGACACACC | CGGCCAUCGUGUGGAAC | AAUAACCACAC | 16 | 44 | 0.54 |
| 12  | CCGGUAGCACCCUUGGA | GGCRCGUGUGGGAACA | AAUAACCACAC | 17 | 45 | 0.5 |
| 13  | GUAGCACCCUUGGAUUG | CAUCGUGUGGAACAUUA | CCACACUUC | 20 | 48 | 0.46 |
| 14  | UAGCAUCAUCUUGUAAUG | AUCGUGUGGAACAUUAC | CAACACUUC | 21 | 49 | 0.43 |
| 15  | AGCAUCAUCAUCUUGUAAUG | UCGUGUGGAACAUUACC | ACAACUACCAA | 22 | 50 | 0.43 |
| 16  | GCACCAUUCUUGUAAUGG | CGUGUGGAACAUUACCA | ACAAACUACCAA | 23 | 51 | 0.43 |
| 17  | CAACCAUUCUUGUAAUGG | GUGUGGAACAUUACCA | AACAACUCCAAA | 24 | 52 | 0.39 |
| 18  | ACACCAUUCUUGUAAUGG | UGUGGAACAUUACCA | AACACUCCAAA | 25 | 53 | 0.36 |
| 19  | CACACCAUUCUUGUAAUGG | UGUGGAACAUUACCACCA | ACACUCCAAA | 26 | 54 | 0.36 |
| 20  | ACCACCAUUCUUGUAAUGG | UGUGGAACAUUACCACCA | ACACUCCAAA | 27 | 55 | 0.32 |
|   | Sequence                                           | Length | Identity | Similarity |
|---|----------------------------------------------------|--------|----------|------------|
| 21| AAGGUUUAUUAGGUUGUUGAA UUCCAAAAUUA                 | 29     | 57       | 0.32       |
| 22| CUUGUAAUUGGUGUUGAAG GGUUUUAAUUGUU                | 30     | 58       | 0.29       |
| 23| UUGUAAUGGUGUUGAAGG UUUUUAUGUUGUU                 | 31     | 59       | 0.29       |
| 24| GUAAGGUGUUGAAGGGU GUUAAUUGUACU                   | 32     | 60       | 0.29       |
| 25| UAAUGGUGUUGAAGGGU UUAAUGUUCUU                   | 33     | 61       | 0.29       |
| 26| AAUGGUGUUGAAGGGU UUAAUGUUCUU                   | 34     | 62       | 0.29       |
| 27| AUGGUGUUGAAGGGU UUAAUGUUCUU                   | 35     | 63       | 0.29       |
| 28| UUGGUGUUGAAGGGU UUAAUGUUCUU                   | 36     | 64       | 0.29       |
| 29| GGUGUUGAAGGGU UUAAUGUUCUU                   | 37     | 65       | 0.32       |
| 30| GGUUGUAGGUGUU UUAAUGUUCUU                   | 38     | 66       | 0.32       |
| 31| UGUUGAAGGUGUU UUAAUGUUCUU                   | 39     | 67       | 0.29       |
| 32| GUUGAAGGUGUU UUAAUGUUCUU                   | 40     | 68       | 0.29       |
| 33| UUGAAGGUGUU UUAAUGUUCUU                   | 41     | 69       | 0.25       |
| 34| UGAAGGUGUU UUAAUGUUCUU                   | 42     | 70       | 0.25       |
| 35| GAAGGUGUU UUAAUGUUCUU                   | 43     | 71       | 0.29       |
| 36| AAGGUGUU UUAAUGUUCUU                   | 44     | 72       | 0.25       |
| 37| AGGUUUAUUGGUGUUGUACU UUCCUUCAAAU              | 45     | 73       | 0.25       |
| 38| GGUUUUAUUGGUGUUGUACU UUCCUUCAAAU              | 46     | 74       | 0.25       |
| 39| GUUUUAUUGGUGUUGUACU UUCCUUCAAAU              | 47     | 75       | 0.25       |
| 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     |
|    |       |                  |     |      |
|----|-------|------------------|-----|------|
| 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|