SUPPLEMENTARY INFORMATION

Two HEPN domains dictate CRISPR RNA maturation and target cleavage in Cas13d

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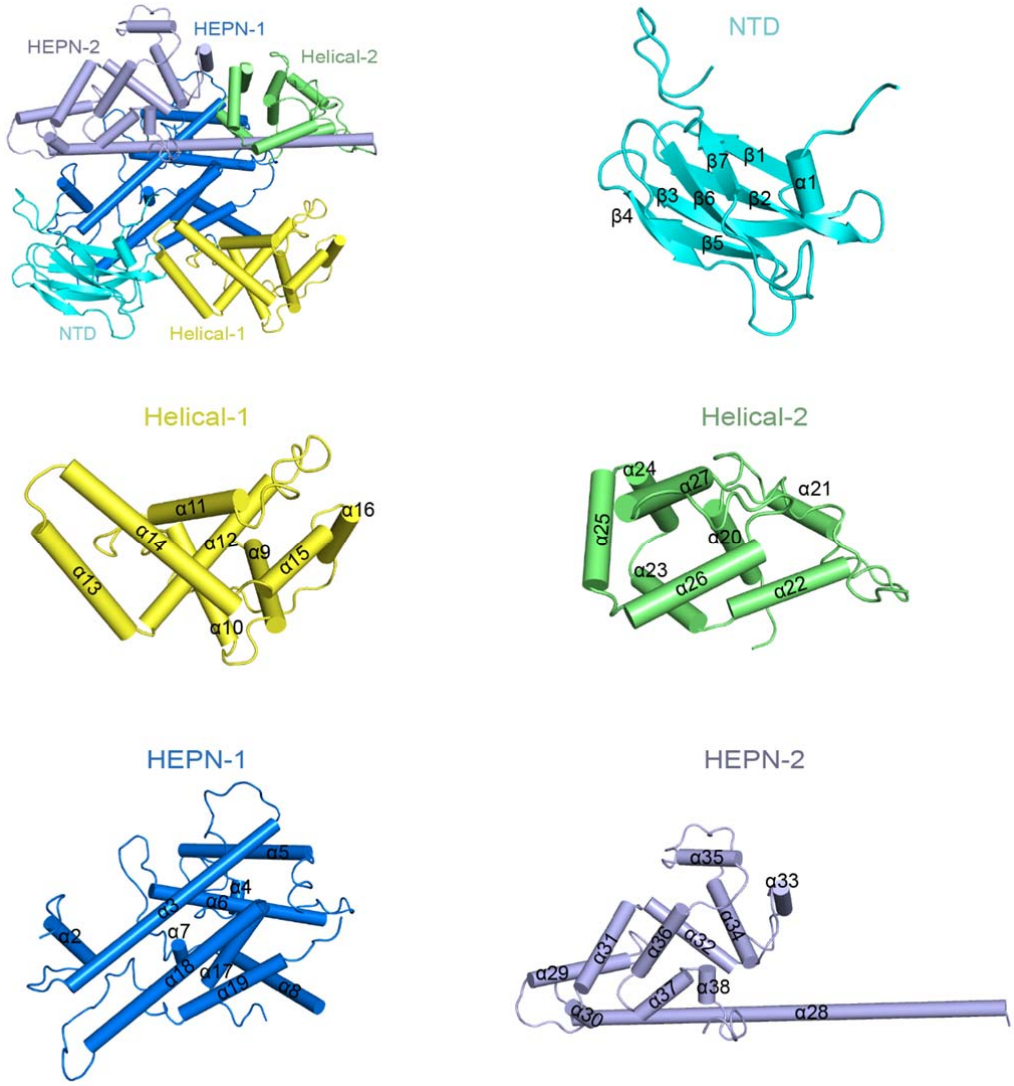
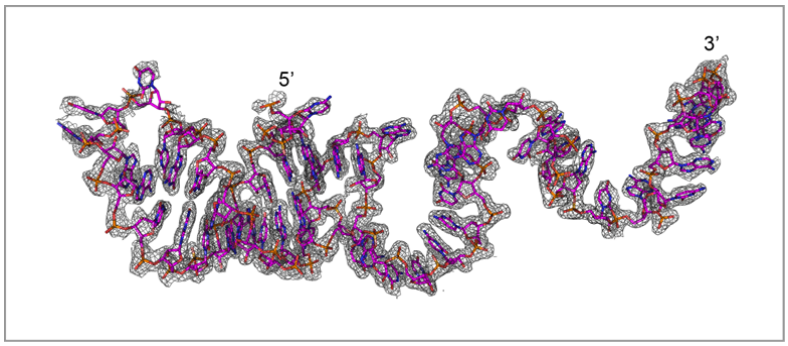


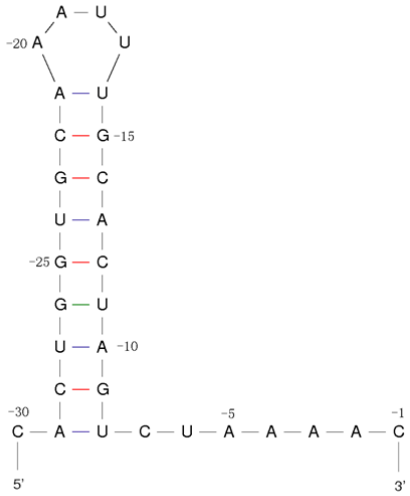
Supplementary Fig. 1 | Structures of individual domains of UrCas13d in the UrCas13d-crRNA binary complex.

Domains are colored according to Fig. 1.



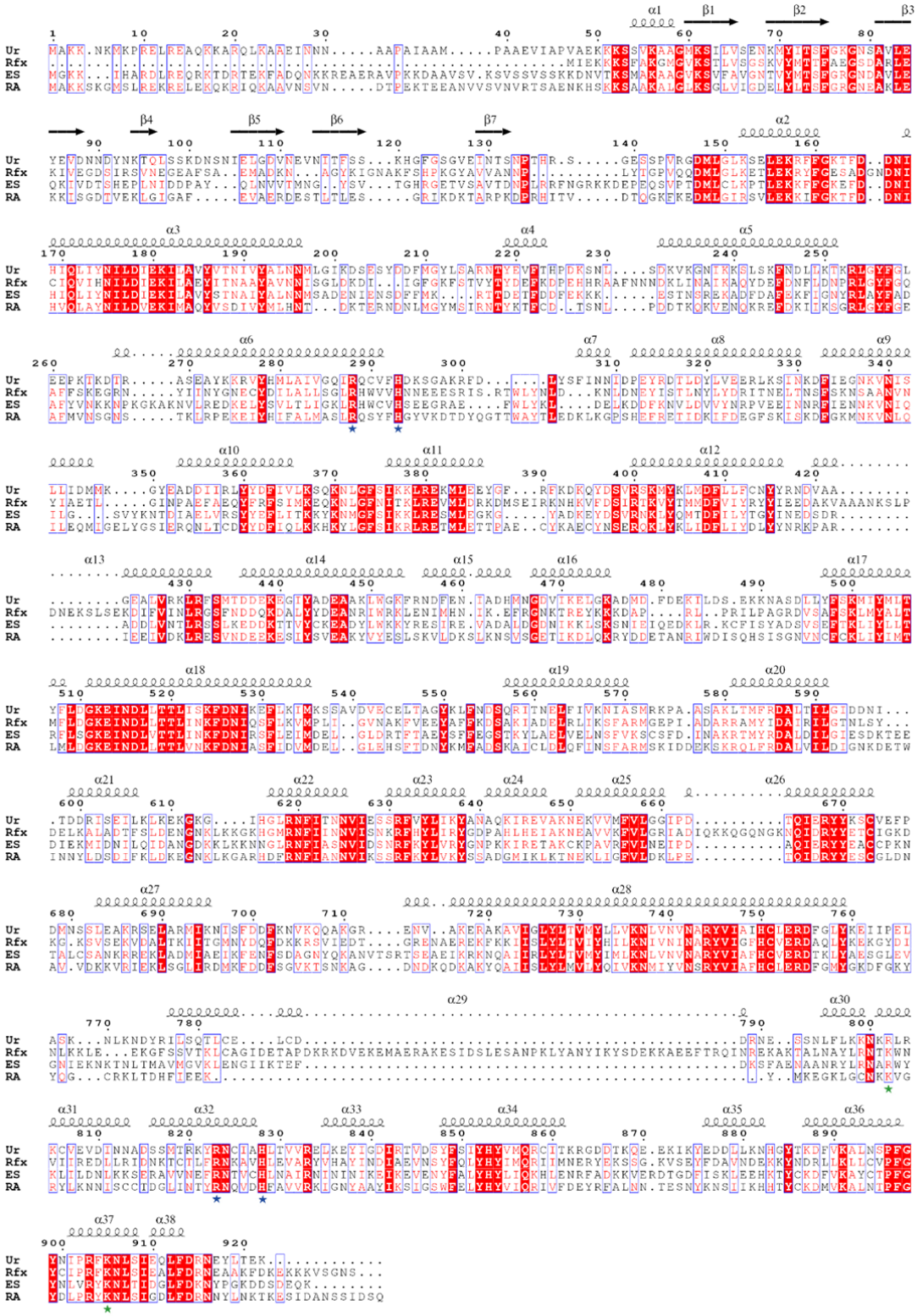
Supplementary Fig. 2 | Electron density maps of crRNAs in the UrCas13d binary complexes.

The electron density map of crRNA in the UrCas13d-crRNA binary complex. A 50-nt crRNA was determined in the binary complex. The 2Fo-Fc omit map was contoured at 1.0 σ level.



Supplementary Fig. 3 | Predicted secondary structure of the mature crRNA repeat region in the UrCas13d-crRNA binary complex.

Result predicted by the mfold web server (http://unafold.rna.albany.edu/?q=mfold).



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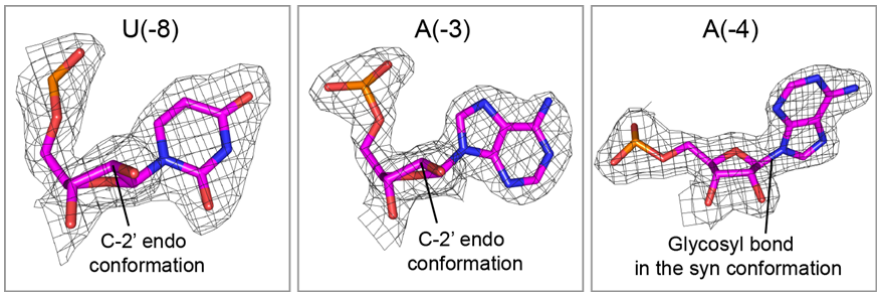
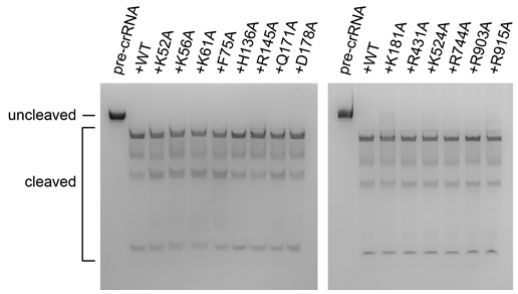
Supplementary Fig. 4 | Multiple sequence alignment of Cas13d proteins from different species.

Sequence alignment of UrCas13d (Uncultured *Ruminococcus sp.* Cas13d), RfxCas13d (*Ruminococcus flavefaciens* XPD3002 Cas13d), EsCas13d (*Eubacterium siraeum* Cas13d) and RaCas13d (*Ruminococcus albus* Cas13d) was generated using Clustal Omega and the figure was prepared using ESPript (http://espript.ibcp.fr). The secondary structure of UrCas13d is shown above the sequence. Identical and similar residues are highlighted in red and white boxes, respectively. Critical residues involved in the pre-crRNA cleavage are marked with green stars, and catalytic residues involved in the target RNA cleavage are marked with blue stars.

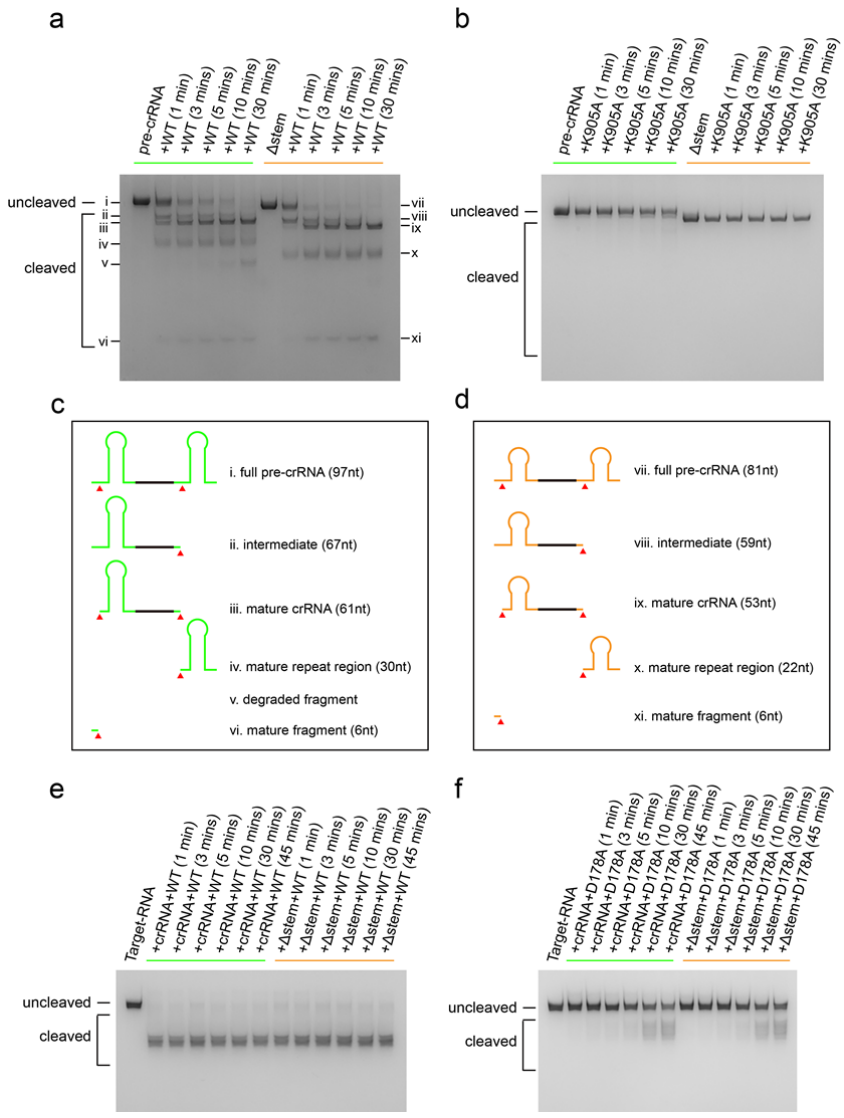


Supplementary Fig. 5 | Conformations of nucleotides U(-8), A(-3) and A(-4) within the U-shaped turn of the crRNA repeat region.

Compared to a standard A-form RNA, nucleotides U(-8), A(-4) and A(-3) within the U-shaped turn have unusual conformations with respect to their sugar pucker or glycosyl bond. The 2Fo-Fc omit map was contoured at 1.0 σ level.



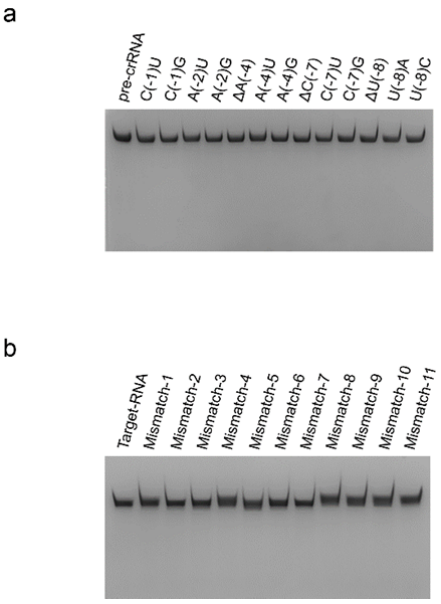
Supplementary Fig. 6 | Pre-crRNA processing by wild-type UrCas13d and its mutants. Denaturing gel demonstrating the cleavage of pre-crRNA by wild-type UrCas13d and its mutants.



Supplementary Fig. 7 | The time-course cleavage of pre-crRNA and target RNA by UrCas13d and its mutants.

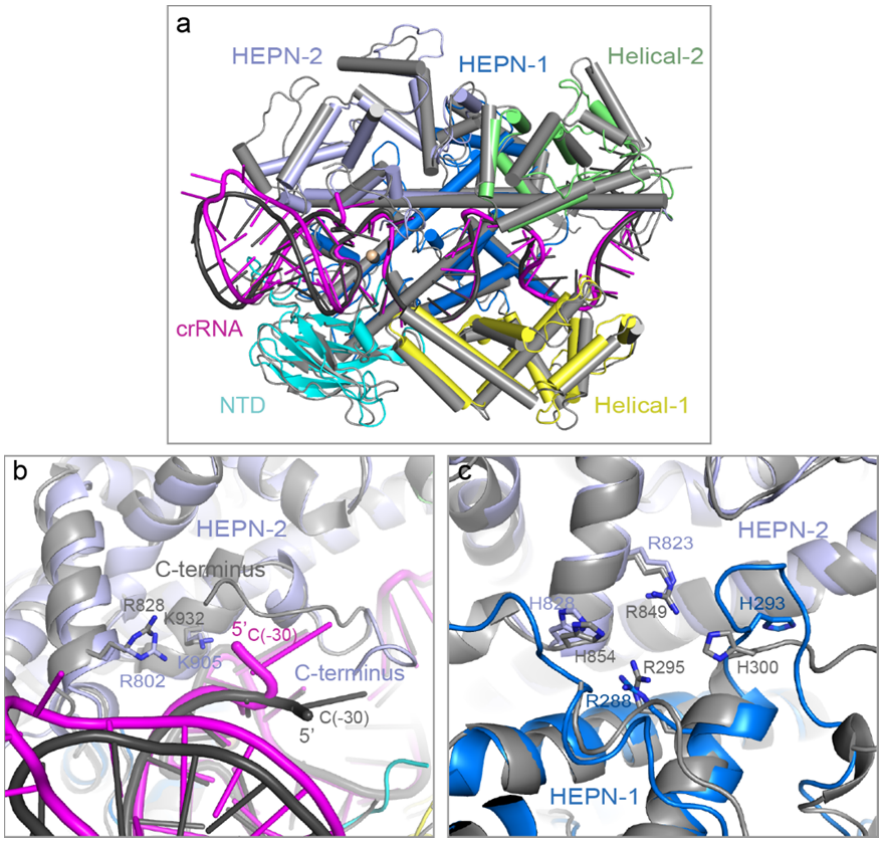
a-b, denaturing gel demonstrating the time-course cleavage of pre-crRNA or the Δstem mutant by the wild-type UrCas13d or the K905A mutant; **c-d**, the expected sizes of the pre-crRNA cleavage products were labeled as i-vi, and the expected sizes of the Δstem mutant cleavage products were labeled as vii-xi; **e-f**, denaturing gel demonstrating the time-course cleavage of target RNA by the wild-type UrCas13d or the D178A mutant in the presence of crRNA or the Δstem mutant. The rationale behind selecting these mutants in the

experiments is that the K905A mutant abolishes the pre-crRNA processing, while the D178A mutant greatly decreases the target RNA cleavage.



Supplementary Fig. 8 | Pre-crRNA, target RNA and their mutants.

a, Denaturing gel demonstrating pre-crRNA and its mutants. **b**, Denaturing gel demonstrating target RNA and the mismatched mutants.



Supplementary Fig. 9 | Comparison of the UrCas13d and EsCas13d binary complexes. **a**, Superimposition of the UrCas13d binary complex (PDB code: 6IV9) and the EsCas13d binary complex (PDB code: 6E9E). Domains and crRNA of the UrCas13d binary complex are colored and labeled according to Fig. 1. Domains and crRNA of the EsCas13d complex are colored in gray and black, respectively. **b**, Comparison of the pre-crRNA processing sites in two binary complexes. Two critical residues from HEPN-2 domain of the respective binary complexes are colored and labeled according to (**a**). **c**, Comparison of the target RNA-cleavage sites in two binary complexes. Four catalytic residues from HEPN-1 and HEPN-2 domains of the respective binary complexes are colored and labeled according to (**a**).

Supplementary Table 1 | X-ray crystallography data collection and refinement statistics.

Dataset	SeMet-UrCas13d-R288A/R823A-crRNA	UrCas13d-R288A/R823A-crRNA
Data collection		
Beamline	BL-17U1, SSRF	BL-17U1, SSRF
Wavelength (Å)	0.9792	0.9792
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, b, c (Å)	65.16, 145.73, 249.38	72.22, 136.48, 147.28
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution range (Å)*	62.91-2.15 (2.19-2.15)	100.11-1.86 (1.89-1.86)
Completeness (%)	99.70 (100.0)	98.90 (98.80)
$I/\sigma(I)$	18.1 (6.3)	21.0 (3.6)
R_{merge}	0.129 (0.842)	0.071 (0.882)
Multiplicity	13.3 (13.9)	13.5 (14.1)
Refinement		
Resolution (Å)	2.15	1.86
No. unique reflections	129,626	121,127
R_{work}/ R_{free} (%)	18.9/ 21.0	17.0/ 19.6
No. atoms		
Protein	14,076	7,126
Nucleic acid	2,196	1,054
Solvent	665	475
Mg^{2+}	2	2
B-factors (Å^2)		
Protein	48.36	38.91
Nucleic acid	60.94	42.76
Solvent	37.16	40.55
Mg^{2+}	26.88	44.18
R.m.s deviations		
Bond length (Å)	0.013	0.015
Bond angles (°)	1.78	1.74
Ramachandran plot (%)		
Favored region	98.37	98.39
Allowed region	1.63	1.61
Outliers region	0	0

*Highest resolution shell is shown in parentheses.

Supplementary Table 2 | DNA coding sequence of UrCas13d used in this study.

Description	DNA coding sequence*
UrCas13d	ATGGCAAAAAAAAATAAAATGAAACCGCGTGAACTGCGTGAAGCACAGAAAAAAGC
	ACGTCAGCTGAAAGCAGCAGAAATTAATAATAATGCAGCACCGGCAATTGCAGCAAT
	GCCGGCAGCAGAAGTTATTGCACCGGTTGCAGAAAAAAAAAAAAAGCAGCGTTAAAGC
	AGCAGGTATGAAAAGCATTCTGGTTAGCGAAAATAAAATGTATATTACCAGCTTTGGT
	AAAGGTAATAGCGCAGTTCTGGAATATGAAGTTGATAATAATGATTATAATAAAACC
	CAGCTGAGCAGCAAAGATAATAGCAATATTGAACTGGGTGATGTTAATGAAGTTAAT
	ATTACCTTTAGCAGCAAACATGGTTTTGGTAGCGGTGTTGAAATTAATACCAGCAATC
	CGACCCATCGTAGCGGTGAAAGCAGCCCGGTTCGTGGTGATATGCTGGGTCTGAAAA
	GCGAACTGGAAAAACGTTTTTTTGGTAAAACCTTTGATGATAATATTCATATTCAGCT
	GATTTATAATATTCTGGATATTGAAAAAATTCTGGCAGTTTATGTTACCAATATTGTTT
	ATGCACTGAATAATATGCTGGGTATTAAAGATAGCGAAAGCTATGATGATTTTATGGG
	TTATCTGAGCGCACGTAATACCTATGAAGTTTTTACCCATCCGGATAAAAGCAATCTG
	AGCGATAAAGTTAAAGGTAATATTAAAAAAAGCCTGAGCAAATTTAATGATCTGCTG
	AAAACCAAACGTCTGGGTTATTTTGGTCTGGAAGAACCGAAAACCAAAGATACCCGT
	GCAAGCGAAGCATATAAAAAACGTGTTTATCACATGCTGGCAATTGTTGGTCAGATTC
	GTCAGTGTGTTTTTCATGATAAAAGCGGTGCAAAACGTTTTGATCTGTATAGCTTTATT
	AATAATATTGATCCGGAATATCGTGATACCCTGGATTATCTGGTTGAAGAACGTCTGA
	AAAGCATTAATAAAGATTTTATTGAAGGTAATAAAGTTAATATTAGCCTGCTGATTGA
	TATGATGAAAGGTTATGAAGCAGATGATATTATTCGTCTGTATTATGATTTTATTGTTC
	TGAAAAGCCAGAAAAATCTGGGTTTTAGCATTAAAAAACTGCGTGAAAAAATGCTGG
	AAGAATATGGTTTTCGTTTTAAAGATAAACAGTATGATAGCGTTCGTAGCAAAATGTA
	TAAACTGATGGATTTTCTGCTGTTTTGTAATTATTATCGTAATGATGTTGCAGCAGGTG
	AAGCACTGGTTCGTAAACTGCGTTTTAGCATGACCGATGATGAAAAAGAAGGTATTTA
	TGCAGATGAAGCAGCAAAACTGTGGGGTAAATTTCGTAATGATTTTGAAAATATTGCA
	GATCACATGAATGGTGATGTTATTAAAGAACTGGGTAAAGCAGATATGGATTTTGATG
	AAAAAATTCTGGATAGCGAAAAAAAAAATGCAAGCGATCTGCTGTATTTTAGCAAAA
	TGATTTATATGCTGACCTATTTTCTGGATGGTAAAGAAATTAATGATCTGCTGACCACC
	CTGATTAGCAAATTTGATAATATTAAAGAATTTCTGAAAATTATGAAAAGCAGCGCAG
	TTGATGTTGAATGTGAACTGACCGCAGGTTATAAACTGTTTAATGATAGCCAGCGTAT
	TACCAATGAACTGTTTATTGTTAAAAATATTGCAAGCATGCGTAAACCGGCAGCAAGC
	GCAAAACTGACCATGTTTCGTGATGCACTGACCATTCTGGGTATTGATGATAATATTA
	CCGATGATCGTATTAGCGAAATTCTGAAACTGAAAGAAAAAGGTAAAGGTATTCATG
	GTCTGCGTAATTTTATTACCAATAATGTTATTGAAAGCAGCCGTTTTGTTTATCTGATT
	AAATATGCAAATGCACAGAAAATTCGTGAAGTTGCAAAAAATGAAAAAGTTGTTATG
	TTTGTTCTGGGTGGTATTCCGGATACCCAGATTGAACGTTATTATAAAAGCTGTGTTGA
	ATTTCCGGATATGAATAGCAGCCTGGAAGCAAAACGTAGCGAACTGGCACGTATGAT
	TAAAAATATTAGCTTTGATGATTTTAAAAATGTTAAACAGCAGGCAAAAGGTCGTGAA
	AATGTTGCAAAAGAACGTGCAAAAGCAGTTATTGGTCTGTATCTGACCGTTATGTATC
	TGCTGGTTAAAAATCTGGTTAATGTTAATGCACGTTATGTTATTGCAATTCATTGTCTG
	GAACGTGATTTTGGTCTGTATAAAGAAATTATTCCGGAACTGGCAAGCAAAAATCTGA

AAAATGATTATCGTATTCTGAGCCAGACCCTGTGTGAACTGTGTGATGATCGTAATGA
AAGCAGCAATCTGTTTCTGAAAAAAAATAAACGTCTGCGTAAATGTGTTGAAGTTGAT
ATTAATAATGCAGATAGCAGCATGACCCGTAAATATCGTAATTGTATTGCACATCTGA
CCGTTGTTCGTGAACTGAAAGAATATATTGGTGATATTCGTACCGTTGATAGCTATTTT
AGCATTTATCATTATGTTATGCAGCGTTGTATTACCAAACGTGGTGATGATACCAAAC
AGGAAGAAAAAATTAAATATGAAGATGATCTGCTGAAAAATCATGGTTATACCAAAG
ATTTTGTTAAAGCACTGAATAGCCCGTTTGGTTATAATATTCCGCGTTTTAAAAATCTG
AGCATTGAACAGCTGTTTGATCGTAATGAATATCTGACCGAAAAATAA

*Codons have been optimized.

Supplementary Table 3 | RNA coding sequences used in the study.

Description	RNA coding sequences*
CRISPR RNA	CTACTACACTGGTGCAAATTTGCACTAGTCTAAAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTAAAACTCCTCGATTACATACACAAAGCAGCCTACTACACTG GTGCAAATTTGCACTAGTCTAAAACTCCTCGATTACATACACAA AGCAGCCTACTACACTGGTGCAAATTTGCACTAGTCTAAAACTC CTCGATTACATACACAAAGCAGCCTACTACACTGGTGCAAATTT GCACTAGTCTAAAACTCCTCGATTACATACACAAAGCAGCCTAC TACACTGGTGCAAATTTGCACTAGTCTAAAACTCCTCGATTACA TACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAGTCT AAAACTCCTCGATTACATACACAAAGCAGCCTACTACACTGGTG CAAATTTGCACTAGTCTAAAAC
Pre-crRNA	CTACTACACTGGTGCAAATTTGCACTAGTCTAAAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTAAAAC
The ΔU(-8) pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGCTAAAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG CTAAAAC
The U(-8)A pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGACTAAAACTCCTCGAT TACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTA GACTAAAAC
The U(-8)C pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGCCTAAAACTCCTCGAT TACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTA GCCTAAAAC
The ΔC(-7) pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTTAAAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TTAAAAC
The C(-7)U pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTTTAAAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TTTAAAAC
The C(-7)G pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTGTAAAACTCCTCGAT TACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTA GTGTAAAAC
The ΔA(-4) pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTAAACTCCTCGATTA CATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAGT CTAAAC
The A(-4)U pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTATAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTATAAC
The A(-4)G pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTAGAACTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTAGAAC
The A(-2)U pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTAAATCTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTAAATC
The A(-2)G pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTAAAGCTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTAAAGC
The C(-1)U pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTAAAATTCCTCGATT ACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTAG TCTAAAAT
The C(-1)G pre-crRNA mutant	CTACTACACTGGTGCAAATTTGCACTAGTCTAAAAGTCCTCGAT TACATACACAAAGCAGCCTACTACACTGGTGCAAATTTGCACTA GTCTAAAAG
The Δstem pre-crRNA mutant	CTACTACACTGGAATTCTAGTCTAAAACTCCTCGATTACATACA CAAAGCAGCCTACTACACTGGAATTCTAGTCTAAAAC
crRNA	CACTGGTGCAAATTTGCACTAGTCTAAAACTCCTCGATTACATA CACAAAGCA
The ΔU(-8) crRNA mutant	CACTGGTGCAAATTTGCACTAGCTAAAACTCCTCGATTACATAC ACAAAGCA
The U(-8)A crRNA mutant	CACTGGTGCAAATTTGCACTAGACTAAAACTCCTCGATTACATA

	CACAAAGCA
The U(-8)C crRNA mutant	CACTGGTGCAAATTTGCACTAGCCTAAAACTCCTCGATTACATA CACAAAGCA
The ∆C(-7) crRNA mutant	CACTGGTGCAAATTTGCACTAGTTAAAACTCCTCGATTACATAC ACAAAGCA
The C(-7)U crRNA mutant	CACTGGTGCAAATTTGCACTAGTTTAAAACTCCTCGATTACATA CACAAAGCA
The C(-7)G crRNA mutant	CACTGGTGCAAATTTGCACTAGTGTAAAACTCCTCGATTACATA CACAAAGCA
The ∆A(-4) crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTAAACTCCTCGATTACATAC ACAAAGCA
The A(-4)U crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTATAACTCCTCGATTACATAC ACAAAGCA
The A(-4)G crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTAGAACTCCTCGATTACATA CACAAAGCA
The A(-2)U crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTAAATCTCCTCGATTACATAC ACAAAGCA
The A(-2)G crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTAAAGCTCCTCGATTACATA CACAAAGCA
The C(-1)U crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTAAAATTCCTCGATTACATA CACAAAGCA
The C(-1)G crRNA mutant	CACTGGTGCAAATTTGCACTAGTCTAAAAGTCCTCGATTACATA CACAAAGCA
The ∆stem crRNA mutant	CACTGGAATTCTAGTCTAAAACTCCTCGATTACATACACAAAGC A
Target RNA	TATATAGCTTTGTGTATGTAATCGAGGAACAAGCT
The mismatched Target RNA-1	TATATAGCTTTGTGTATGTAATCGAGCTACAAGCT
The mismatched Target RNA-2	TATATAGCTTTGTGTATGTAATCGTCGAACAAGCT
The mismatched Target RNA-3	TATATAGCTTTGTGTATGTAATGCAGGAACAAGCT
The mismatched Target RNA-4	TATATAGCTTTGTGTATGTATACGAGGAACAAGCT
The mismatched Target RNA-5	TATATAGCTTTGTGTATGATATCGAGGAACAAGCT
The mismatched Target RNA-6	TATATAGCTTTGTGTAACTAATCGAGGAACAAGCT
The mismatched Target RNA-7	TATATAGCTTTGTGATTGTAATCGAGGAACAAGCT
The mismatched Target RNA-8	TATATAGCTTTGACTATGTAATCGAGGAACAAGCT
The mismatched Target RNA-9	TATATAGCTTACTGTATGTAATCGAGGAACAAGCT
The mismatched Target RNA-10	TATATAGCAATGTGTATGTAATCGAGGAACAAGCT
The mismatched Target RNA-11	TATATACGTTTGTGTATGTAATCGAGGAACAAGCT

*The mutational nucleotides are colored in red.

Supplementary Table 4 | Primers used for mutagenesis of UrCas13d.

Description	Primers used for mutagenesis*
UrCas13d_K52A_F	CCGGTTGCAGAAAAAAAAGCAAGCAGCGTTAAAGCAGCA
UrCas13d_K52A_R	TGCTGCTTTAACGCTGCTTGCTTTTTTTTCTGCAACCGG
UrCas13d_K56A_F	AAAAAAAAAAGCAGCGTTGCAGCAGCAGGTATGAAAAGC
UrCas13d_K56A_R	GCTTTTCATACCTGCTGCTGCAACGCTGCTTTTTTTTTT
UrCas13d_K61A_F	GTTAAAGCAGCAGGTATGGCAAGCATTCTGGTTAGCGAA
UrCas13d_K61A_R	TTCGCTAACCAGAATGCTTGCCATACCTGCTGCTTTAAC
UrCas13d_F75A_F	AAAATGTATATTACCAGCGCAGGTAAAGGTAATAGCGCAG
UrCas13d_F75A_R	CTGCGCTATTACCTTTACCTGCGCTGGTAATATACATTTT
UrCas13d_H136A_F	AATACCAGCAATCCGACCGCACGTAGCGGTGAAAGCAGC
UrCas13d_H136A_R	GCTGCTTTCACCGCTACGTGCGGTCGGATTGCTGGTATT
UrCas13d_R145A_F	GGTGAAAGCAGCCCGGTTGCAGGTGATATGCTGGGTCT
UrCas13d_R145A_R	AGACCCAGCATATCACCTGCAACCGGGCTGCTTTCACC
UrCas13d_Q171A_F	TGATGATAATATTCATATTGCACTGATTTATAATATTCTG
UrCas13d_Q171A_R	CAGAATATTATAAATCAGTGCAATATGAATATTATCATCA
UrCas13d_D178A_F	CTGATTTATAATATTCTGGCAATTGAAAAAATTCTGGCA
UrCas13d_D178A_R	TGCCAGAATTTTTTCAATTGCCAGAATATTATAAATCAG
UrCas13d_K181A_F	AATATTCTGGATATTGAAGCAATTCTGGCAGTTTATGTTAC
UrCas13d_K181A_R	GTAACATAAACTGCCAGAATTGCTTCAATATCCAGAATATT
UrCas13d_R288A_F	GCAATTGTTGGTCAGATTGCACAGTGTGTTTTTCATGAT
UrCas13d_R288A_R	ATCATGAAAAACACACTGTGCAATCTGACCAACAATTGC
UrCas13d_H293A_F	ATTCGTCAGTGTGTTTTTGCAGATAAAAGCGGTGCAAAAC
UrCas13d_H293A_R	GTTTTGCACCGCTTTTATCTGCAAAAACACACTGACGAAT
UrCas13d_K367A_F	TATGATTTTATTGTTCTGGCAAGCCAGAAAAATCTGGG
UrCas13d_K367A_R	CCCAGATTTTTCTGGCTTGCCAGAACAATAAAATCATA
UrCas13d_K370A_F	ATTGTTCTGAAAAGCCAGGCAAATCTGGGTTTTAGCATTA
UrCas13d_K370A_R	TAATGCTAAAACCCAGATTTGCCTGGCTTTTCAGAACAAT
UrCas13d_K402A_F	GTATGATAGCGTTCGTAGCGCAATGTATAAACTGATGGA
UrCas13d_K402A_R	TCCATCAGTTTATACATTGCGCTACGAACGCTATCATAC
UrCas13d_K405A_F	GTTCGTAGCAAAATGTATGCACTGATGGATTTTCTGCTGT
UrCas13d_K405A_R	ACAGCAGAAAATCCATCAGTGCATACATTTTGCTACGAAC
UrCas13d_R431A_F	GCACTGGTTCGTAAACTGGCATTTAGCATGACCGATGAT
UrCas13d_R431A_R	ATCATCGGTCATGCTAAATGCCAGTTTACGAACCAGTGC
UrCas13d_K439A_F	AGCATGACCGATGATGAAGCAGAAGGTATTTATGCAGATG
UrCas13d_K439A_R	CATCTGCATAAATACCTTCTGCTTCATCATCGGTCATGCT
UrCas13d_Y443A_F	GATGAAAAAGAAGGTATTGCAGCAGATGAAGCAGCAAAA
UrCas13d_Y443A_R	TTTTGCTGCTTCATCTGCTGCAATACCTTCTTTTTCATC
UrCas13d_K512A_F	ACCTATTTTCTGGATGGTGCAGAAATTAATGATCTGCTG
UrCas13d_K512A_R	CAGCAGATCATTAATTTCTGCACCATCCAGAAAATAGGT
UrCas13d_N515A_F	CTGGATGGTAAAGAAATTGCAGATCTGCTGACCACCCTGA
UrCas13d_N515A_R	TCAGGGTGGTCAGCAGATCTGCAATTTCTTTACCATCCAG
UrCas13d_K524A_F	CTGACCACCCTGATTAGCGCATTTGATAATATTAAAGAA
UrCas13d_K524A_R	TTCTTTAATATTATCAAATGCGCTAATCAGGGTGGTCAG
UrCas13d_K567A_F	AATGAACTGTTTATTGTTGCAAATATTGCAAGCATGCG
UrCas13d_K567A_R	CGCATGCTTGCAATATTTGCAACAATAAACAGTTCATT
UrCas13d_R619A_F	AAAGGTATTCATGGTCTGGCAAATTTTATTACCAATAATG
UrCas13d_R619A_R	CATTATTGGTAATAAAATTTGCCAGACCATGAATACCTTT
UrCas13d_N620A_F	GGTATTCATGGTCTGCGTGCATTTATTACCAATAATGTTA
UrCas13d_N620A_R	TAACATTATTGGTAATAAATGCACGCAGACCATGAATACC
UrCas13d_R631A_F	AATGTTATTGAAAGCAGCGCATTTGTTTATCTGATTAAA
UrCas13d_R631A_R	TTTAATCAGATAAACAAATGCGCTGCTTTCAATAACATT
UrCas13d_K717A_F	GGTCGTGAAAATGTTGCAGCAGAACGTGCAAAAGCAGTTA
UrCas13d_K717A_R	TAACTGCTTTTGCACGTTCTGCTGCAACATTTTCACGACC
UrCas13d_K736A_F	GTTATGTATCTGCTGGTTGCAAATCTGGTTAATGTTAAT
UrCas13d_K736A_R	ATTAACATTAACCAGATTTGCAACCAGCAGATACATAAC
UrCas13d_N737A_F	ATGTATCTGCTGGTTAAAGCACTGGTTAATGTTAATGCAC
UrCas13d_N737A_R	GTGCATTAACATTAACCAGTGCTTTAACCAGCAGATACAT
UrCas13d_R744A_F	CTGGTTAATGTTAATGCAGCATATGTTATTGCAATTCAT
UrCas13d_R744A_R	ATGAATTGCAATAACATATGCTGCATTAACATTAACCAG
UrCas13d_R802A_F	GTTTCTGAAAAAAAATAAAGCACTGCGTAAATGTGTTGAA
UrCas13d_R802A_R	TTCAACACATTTACGCAGTGCTTTATTTTTTTTCAGAAAC

UrCas13d_L803A_F	CTGAAAAAAAATAAACGTGCACGTAAATGTGTTGAAGTT
UrCas13d_L803A_R	AACTTCAACACATTTACGTGCACGTTTATTTTTTTTCAG
UrCas13d_C806A_F	AATAAACGTCTGCGTAAAGCAGTTGAAGTTGATATTAATAATG
UrCas13d_C806A_R	CATTATTAATATCAACTTCAACTGCTTTACGCAGACGTTTATT
UrCas13d_R823A_F	AGCATGACCCGTAAATATGCAAATTGTATTGCACATCTG
UrCas13d_R823A_R	CAGATGTGCAATACAATTTGCATATTTACGGGTCATGCT
UrCas13d_H828A_F	ATCGTAATTGTATTGCAGCACTGACCGTTGTTCGTGAAC
UrCas13d_H828A_R	GTTCACGAACAACGGTCAGTGCTGCAATACAATTACGAT
UrCas13d_K887A_F	GAAAAATCATGGTTATACCGCAGATTTTGTTAAAGCACTGA
UrCas13d_K887A_R	TCAGTGCTTTAACAAAATCTGCGGTATAACCATGATTTTTC
UrCas13d_K891A_F	TATACCAAAGATTTTGTTGCAGCACTGAATAGCCCGTTTG
UrCas13d_K891A_R	CAAACGGGCTATTCAGTGCTGCAACAAAATCTTTGGTATA
UrCas13d_R903A_F	GTTTGGTTATAATATTCCGGCATTTAAAAATCTGAGCATT
UrCas13d_R903A_R	AATGCTCAGATTTTTAAATGCCGGAATATTATAACCAAAC
UrCas13d_F904A_F	GGTTATAATATTCCGCGTGCAAAAAATCTGAGCATTGAA
UrCas13d_F904A_R	TTCAATGCTCAGATTTTTTGCACGCGGAATATTATAACC
UrCas13d_K905A_F	TATAATATTCCGCGTTTTGCAAATCTGAGCATTGAACAG
UrCas13d_K905A_R	CTGTTCAATGCTCAGATTTGCAAAACGCGGAATATTATA
UrCas13d_I909A_F	CGTTTTAAAAATCTGAGCGCAGAACAGCTGTTTGATCGT
UrCas13d_I909A_R	ACGATCAAACAGCTGTTCTGCGCTCAGATTTTTAAAACG
UrCas13d_Q911A_F	TAAAAATCTGAGCATTGAAGCACTGTTTGATCGTAATGAAT
UrCas13d_Q911A_R	ATTCATTACGATCAAACAGTGCTTCAATGCTCAGATTTTTA
UrCas13d_R915A_F	CATTGAACAGCTGTTTGATGCAAATGAATATCTGACCGAA
UrCas13d_R915A_R	TTCGGTCAGATATTCATTTGCATCAAACAGCTGTTCAATG

*Forward and reverse primers are indicated by “F” and “R”, respectively.

Supplementary Table 5 | Primers used for mutagenesis of RNA coding sequences.

Description	Primers used for mutagenesis*
The ΔU(-8) crRNA mutant_F	GGTGCAAATTTGCACTAGCTAAAACTCCTCGATTACA
The ΔU(-8) crRNA mutant_R	TGTAATCGAGGAGTTTTAGCTAGTGCAAATTTGCACC
The U(-8)A crRNA mutant_F	GGTGCAAATTTGCACTAGACTAAAACTCCTCGATTACA
The U(-8)A crRNA mutant_R	TGTAATCGAGGAGTTTTAGTCTAGTGCAAATTTGCACC
The U(-8)C crRNA mutant_F	GGTGCAAATTTGCACTAGCCTAAAACTCCTCGATTACA
The U(-8)C crRNA mutant_R	TGTAATCGAGGAGTTTTAGGCTAGTGCAAATTTGCACC
The ΔC(-7) crRNA mutant_F	GTGCAAATTTGCACTAGTTAAAACTCCTCGATTACA
The ΔC(-7) crRNA mutant_R	TGTAATCGAGGAGTTTTAACTAGTGCAAATTTGCAC
The C(-7)U crRNA mutant_F	GTGCAAATTTGCACTAGTTTAAAACTCCTCGATTACA
The C(-7)U crRNA mutant_R	TGTAATCGAGGAGTTTTAAACTAGTGCAAATTTGCAC
The C(-7)G crRNA mutant_F	GTGCAAATTTGCACTAGTGTAAAACTCCTCGATTACA
The C(-7)G crRNA mutant_R	TGTAATCGAGGAGTTTTACACTAGTGCAAATTTGCAC
The ΔA(-4) crRNA mutant_F	GCAAATTTGCACTAGTCTAAACTCCTCGATTACATACA
The ΔA(-4) crRNA mutant_R	TGTATGTAATCGAGGAGTTTAGACTAGTGCAAATTTGC
The A(-4)U crRNA mutant_F	GCAAATTTGCACTAGTCTATAACTCCTCGATTACATACA
The A(-4)U crRNA mutant_R	TGTATGTAATCGAGGAGTTATAGACTAGTGCAAATTTGC
The A(-4)G crRNA mutant_F	GCAAATTTGCACTAGTCTAGAACTCCTCGATTACATACA
The A(-4)G crRNA mutant_R	TGTATGTAATCGAGGAGTTCTAGACTAGTGCAAATTTGC
The A(-2)U crRNA mutant_F	AATTTGCACTAGTCTAAATCTCCTCGATTACATACAC
The A(-2)U crRNA mutant_R	GTGTATGTAATCGAGGAGATTTAGACTAGTGCAAATT
The A(-2)G crRNA mutant_F	AATTTGCACTAGTCTAAAGCTCCTCGATTACATACAC
The A(-2)G crRNA mutant_R	GTGTATGTAATCGAGGAGCTTTAGACTAGTGCAAATT
The C(-1)U crRNA mutant_F	AATTTGCACTAGTCTAAAATTCCTCGATTACATACAC
The C(-1)U crRNA mutant_R	GTGTATGTAATCGAGGAATTTTAGACTAGTGCAAATT
The C(-1)G crRNA mutant_F	AATTTGCACTAGTCTAAAAGTCCTCGATTACATACAC
The C(-1)G crRNA mutant_R	GTGTATGTAATCGAGGACTTTTAGACTAGTGCAAATT
The mismatched Target RNA-1_F	AGCTTTGTGTATGTAATCGAGCTACAAGCTTGGCGTAATCATG
The mismatched Target RNA-1_R	CATGATTACGCCAAGCTTGTAGCTCGATTACATACACAAAGCT
The mismatched Target RNA-2_F	TAGCTTTGTGTATGTAATCGTCGAACAAGCTTGGCGTAATC
The mismatched Target RNA-2_R	GATTACGCCAAGCTTGTTCGACGATTACATACACAAAGCTA
The mismatched Target RNA-3_F	TATAGCTTTGTGTATGTAATGCAGGAACAAGCTTGGCGTAATC
The mismatched Target RNA-3_R	GATTACGCCAAGCTTGTTCCTGCATTACATACACAAAGCTATA
The mismatched Target RNA-4_F	GTATATAGCTTTGTGTATGTATACGAGGAACAAGCTTGGCGTA
The mismatched Target RNA-4_R	TACGCCAAGCTTGTTCCTCGTATACATACACAAAGCTATATAC
The mismatched Target RNA-5_F	GGTATATAGCTTTGTGTATGATATCGAGGAACAAGCTTGGCGT
The mismatched Target RNA-5_R	ACGCCAAGCTTGTTCCTCGATATCATACACAAAGCTATATACC
The mismatched Target RNA-6_F	TAGGTATATAGCTTTGTGTAACTAATCGAGGAACAAGCTTGG
The mismatched Target RNA-6_R	CCAAGCTTGTTCCTCGATTAGTTACACAAAGCTATATACCTA
The mismatched Target RNA-7_F	TATAGGTATATAGCTTTGTGATTGTAATCGAGGAACAAGCTTG
The mismatched Target RNA-7_R	CAAGCTTGTTCCTCGATTACAATCACAAAGCTATATACCTATA
The mismatched Target RNA-8_F	ACTATAGGTATATAGCTTTGACTATGTAATCGAGGAACAAGC
The mismatched Target RNA-8_R	GCTTGTTCCTCGATTACATAGTCAAAGCTATATACCTATAGT
The mismatched Target RNA-9_F	CACTATAGGTATATAGCTTACTGTATGTAATCGAGGAACAA
The mismatched Target RNA-9_R	TTGTTCCTCGATTACATACAGTAAGCTATATACCTATAGTG
The mismatched Target RNA-10_F	ACTCACTATAGGTATATAGCAATGTGTATGTAATCGAGGAAC
The mismatched Target RNA-10_R	GTTCCTCGATTACATACACATTGCTATATACCTATAGTGAGT
The mismatched Target RNA-11_F	CGACTCACTATAGGTATATACGTTTGTGTATGTAATCGAGGA
The mismatched Target RNA-11_R	TCCTCGATTACATACACAAACGTATATACCTATAGTGAGTCG

*Forward and reverse primers are indicated by “F” and “R”, respectively.