Construct	Cas13d binary complex	Cas13d ternary complex	Cas13d apo form
Data collection and Image Processing			
Microscope	Arctica Talos	Titan Krios	Arctica Talos
Voltage (kV)	200	300	200
Camera	Gatan K2 Summit	Gatan K2 Summit	Gatan K2 Summit
Defocus range (μm)	0.5-2.0	1.0-3.0	0.8-2.5
Defocus mean ± std (μ m)	1.1±0.3	2.0±0.4	1.6±0.5
Exposure time (s)	12	12	12
Dose rate (e- /pixel/s)	2.5	3.0	2.5
Total dose (e- /Å2)	56.8	57.3	56.8
Pixel size (Å)	0.73	0.79	0.73
Exposure rate (ms)	100	200	100
Number of micrographs	1435	2205	1158
Number of particles (processed)	412591	684169	330986
Number of particles (3D classification & refinement)	207849	177910	154889
Number of particles (in final map)	43786	51885	15846
Symmetry	C1	C1	C1
Resolution (global) (Å)*	3.4	3.3	6.5
Resolution range (homogeneous portion) (Å)	3.0-5.0	3.0-5.0	6.0-8.0
Map sharpening	Spectral flattening between 8Å and 3.4Å	Spectral flattening between 8Å and 3.3Å	Spectral flattening between 10Å and 6.5Å
Sphericity @ threshold 0.75	0.98	0.89	0.97
Model refinement			
Number of atoms (modeled)	8163	8834	
Protein			
Favored rotamers (%)	97.4	94.6	
Poor rotamers (%)	0.26	0.0	
Ramachandran favored (%)	95.9	91.9	
Ramachandran outliers (%)	0.0	0.0	
Cβ deviations >0.25Å (%)	0.0	0.0	
Cis prolines (%)	0.0	0.0	
Cis prolines (%)	0.0	0.0	
Bad bonds (%)	0.0	0.0	
Bad angles (%)	0.0	0.0	
Nucleic acid			
Probably wrong sugar puckers (%)	0.0	0.0	
Bad backbone conformations (%)	11.8	12.7	
Bad bonds (%)	0.0	0.0	
Bad angles (%)	0.0	0.0	

*Resolution assessment based on frequency- limited refinement using the 0.143 threshold for resolution analysis

Supplementary Table 1. Cryo-EM data collection, image processing, and modeling summary.
Related to Figures 1 and 6

Name	Sequence (5' to 3')	Figure
ternary target	ACGUUUUGAUCUGAAAUAUUCAGGUCUAUA	1E-G, 2
ssRNA-1 target 1	UACGUACGCCAUCUCAUCCCUGCGUGUCUCCUACGAUUUGAUCUGAAAUUAAGAGGUCUCGUACAUCACCGACUGCCCAUAGAGAGGUUAUCCGCUCACAAUUCCACACAACAUACGAGCCGGAAGCAUAAAG	3F
ssRNA-2 target 2	UACGUACGCCAUCUCAUCCCUGCGUGUCUCCUACGAUUUGAUCUGAAAUAUUCAGGUCUCGUACAUCACCGACUGCCCAUAGAGAGGUUAUCCGCUCACAAUUCCACACAACAUACGAGCCGGAAGCAUAAAG	3G, S3G
competitor 1 - WT	UCCUUUUGAUCUGAAAUAUUCAGGUCUCCU	4D-E
competitor 1 - MM1	UCCUAAACAUCUGAAAUAUUCAGGUCUCCU	4D-E
competitor 1 - MM2	UCCUUUUGAAGACAAAUAUUCAGGUCUCCU	4D-E
competitor 1 - MM3	UCCUUUUGAUCUGUUUAAUUCAGGUCUCCU	4D-E
competitor 1 - MM4	UCCUUUUGAUCUGAAAUUAAGAGGUCUCCU	4D-E
competitor 1 - MM5	UCCUUUUGAUCUGAAAUAUUCACCAGUCCU	4D-E
competitor 2 - WT	UCCUGAGUUCAUCCAGUGUAUCCUUCUCCU	4D-E
competitor 2 - MM1	UCCUCUCAUCAUCCAGUGUAUCCUUCUCCU	4D-E
competitor 2 - MM2	UCCUGAGUUGUAGCAGUGUAUCCUUCUCCU	4D-E
competitor 2 - MM3	UCCUGAGUUCAUCGUCAGUAUCCUUCUCCU	4D-E
competitor 2 - MM4	UCCUGAGUUCAUCCAGUCAUACCUUCUCCU	4D-E
competitor 2 - MM5	UCCUGAGUUCAUCCAGUGUAUCGAAGUCCU	4D-E
competitor 3 - WT	UCCUCAAUGCGUGAGUGUGAAGAUAGUCCU	4D-E
competitor 3 - MM1	UCCUGUUAGCGUGAGUGUGAAGAUAGUCCU	4D-E
competitor 3 - MM2	UCCUCAAUGGCACAGUGUGAAGAUAGUCCU	4D-E
competitor 3 - MM3	UCCUCAAUGCGUGUCACUGAAGAUAGUCCU	4D-E
competitor 3 - MM4	UCCUCAAUGCGUGAGUGACUUGAUAGUCCU	4D-E
competitor 3 - MM5	UCCUCAAUGCGUGAGUGUGAAGUAUCUCCU	4D-E
competitor 4 - WT	UCCUCCAUAGAGAGGUUAUCCGCUCAUCCU	4D-E
competitor 4 - MM1	UCCUGGUAAGAGAGGUUAUCCGCUCAUCCU	4D-E
competitor 4 - MM2	UCCUCCAUACUCUGGUUAUCCGCUCAUCCU	4D-E
competitor 4 - MM3	UCCUCCAUAGAGACCAAAUCCGCUCAUCCU	4D-E
competitor 4 - MM4	UCCUCCAUAGAGAGGUUUAGGGCUCAUCCU	4D-E
competitor 4 - MM5	UCCUCCAUAGAGAGGUUAUCCGGAGUUCCU	4D-E

DR mut crRNA WT	CACCCGUGCAAAAAUGCAGGGGUCUAAAACGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA G22A	CACCCGUGCAAAAAUGCAGGGCUCUAAAACGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA U23A	CACCCGUGCAAAAAUGCAGGGGACUAAAACGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA A26C	CACCCGUGCAAAAAUGCAGGGGUCUCAAACGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA A27C	CACCCGUGCAAAAAUGCAGGGGUCUACAACGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA A28C	CACCCGUGCAAAAAUGCAGGGGUCUAACACGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA A29C	CACCCGUGCAAAAAUGCAGGGGUCUAAACCGACCUCUUAA UUUCAGAUCAAA	3F
DR mut crRNA C30G	CACCCGUGCAAAAAUGCAGGGGUCUAAAAGGACCUCUUAA UUUCAGAUCAAA	3F
crRNA-1-Es	CACCCGUGCAAAAAUGCAGGGGUCUAAAACGACCUGAAUA UUUCAGAUCAAA	1, 2, 3G, 4D-E, S3D, S3G
crRNA-1-Ur	CACUGGUGCAAAUUAGCACUAGUCUAAAACGACCUGAAUA UUUCAGAUCAAA	3G
crRNA-1-Rff	UAGUAGUGUGAAUUUACACUACUCUAAAACGACCUGAAUA UUUCAGAUCAAA	3G
crRNA-1-Rfx	AACCCCUACCAACUGGUCGGGGUUUGAAACGACCUGAAUA UUUCAGAUCAAA	3G
crRNA-2	CACCCGUGCAAAAAUGCAGGGGUCUAAAACGAAGGAUACA CUGGAUGAACUC	4D-E
crRNA-3	CACCCGUGCAAAAAUGCAGGGGUCUAAAACCUAUCUUCAC ACUCACGCAUUG	4D-E
crRNA-4	CACCCGUGCAAAAAUGCAGGGGUCUAAAACUGAGCGGAUA ACCUCUCUAUGG	4D-E
pre-crRNA-1	GAACUACACCCGUGCAAAAAUGCAGGGGUCUAAAACGACC UGAAUAUUUCAGAUCAAAAUAGCAAUGAACUACACCCGUG CAAAAAUGCAGGGGUCUAAAACAUUCCUUAUCUAAAUAGC AUAUGCUUCUGU	S3A, S3C, S3G-H

Supplementary Table 2. Oligonucleotides used in this study. Related to Figures 1-4, and S3