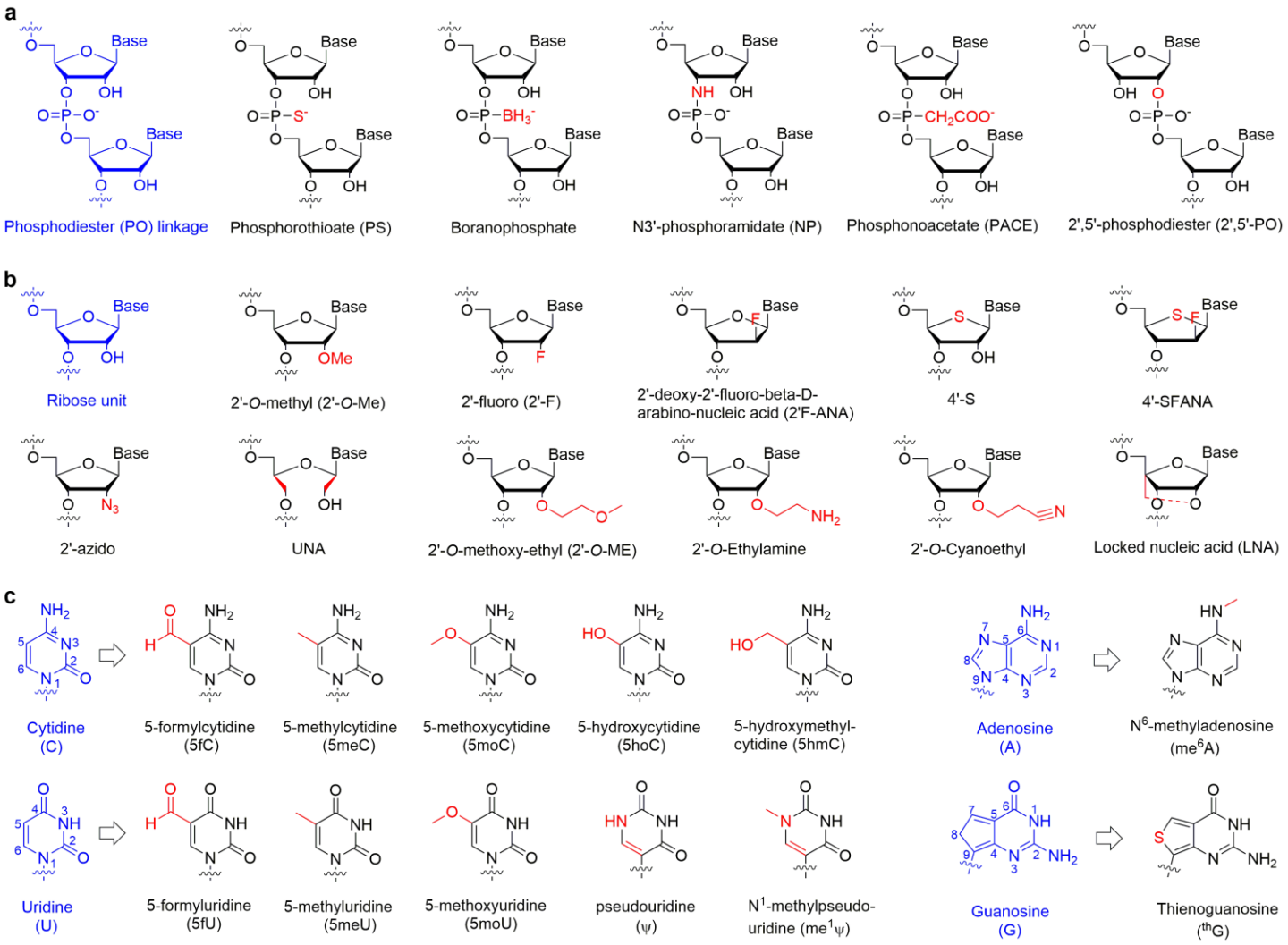

Supplementary Figure 1

Representative examples of chemically modified nucleotides.

(**a**) Linkage (backbone)-modified nucleotides. (**b**) Ribose-modified nucleotides. (**c**) Nitrogenous base (cytosine, uracil, adenine, and guanine)-modified nucleotides. Natural nucleotides are shown in blue. The modified groups are shown in red.

Supplementary Table 1 | Engineered crRNAs and their mass spectrometry data.

No.	AscrRNA	Modification pattern (5'- to -3')	M.W.	
Chemically modified crRNAs targeting *DNMT1*			**Calcd.**	**Found**
WT	crWT	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13552.5	13552.2
1	cr42PS	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	14227.0	14226.1
2	cr5'/3'3F2PS	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13628.7	13627.6
3	cr5'20M	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13832.5	13832.1
4	cr5'10M	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13692.5	13691.8
5	cr5'5M	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13622.5	13621.5
6	cr5'20F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13592.5	13590.6
7	cr5'10F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13572.5	13570.7
8	cr5'5F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13562.5	13560.9
9	crS3M3PS	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13642.7	13643.4
10	crS3F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13558.5	13557.9
11	crS2F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13556.5	13557.4
12	crS1F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13554.5	13553.6
13	cr3'10M	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13692.5	13691.2
14	cr3'5M	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13622.5	13621.2
15	cr3'10F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13572.5	13572.0
16	cr3'5F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13562.5	13561.4
17	cr3'5F4PS	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13626.7	13625.8
18	cr3'5U	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13626.7	13625.8
19	cr3'5L	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13626.7	13625.8
20	crI21F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13594.5	13594.2
21	crI16M5F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13786.5	13783.7
22	crI16M10PS5F	UAAUUUCUACUCUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13947.1	13944.2
Stem-engineered crRNAs targeting *DNMT1*				
23	crSplit-L	UAAUUUCUACUC	3678.5	3677.8
	crSplit-R	UUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	9812.1	9811.4

24	crDel2	UAAUU-CUACUCUUGUAG-UCUGAUGGUCCAUGUCUGUUACUC	12917.1	12916.2
25	crDel4	UAAUUUCU--UCUU--AGAUCUGAUGGUCCAUGUCUGUUACUC	12266.7	12266.4
26	crDel8	UAAUUU----UCUU----AUCUGAUGGUCCAUGUCUGUUACUC	10980.9	10980.9
27	crIns4	UAAUUUCUACACUCUUGUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	14838.3	14837.7
28	crIns4'	UAAUUUCUACUCUCUUGUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	14815.3	14813.5
29	crIns6'	UAAUUUCUACUGCUCUUGCUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	15465.6	15463.9
30	crIns8	UAAUUUCUACACACUCUUGUGUGUAGAUCUGAUGGUCCAUGUCUGUUAC UC	16124.1	16123.5
31	crIns12	UAAUUUCUACACACACUCUUGUGUGUGUAGAUCUGAUGGUCCAUGUCUG UUACUC	17409.8	17408.7

Loop-engineered crRNAs targeting *DNMT1*

32	FncrRNA	UAAUUUCUACUGUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13592.5	13593.0
33	Lb3crRNA	UAAUUUCUACGUUCUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13897.7	13898.2
34	BpcrRNA	UAAUUUCUACGUAAUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13944.7	13945.6
35	Pb/Pe/LicrRNA	UAAUUUCUACUUUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13553.5	13553.6
36	SscrRNA	UAAUUUCUACACGCGGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13958.7	13959.3
37	Lb2/Pc/PmcrRNA	UAAUUUCUACUAUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13576.5	13577.3
38	CMtcrRNA	UAAUUUCUACUCUUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13858.7	13859.1
39	EecrRNA	UAAUUUCUACU-UUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13247.3	13247.4
40	MbcrRNA	UAAUUUCUACUGUUUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13898.7	13898.6
41	PdcrRNA	UAAUUUCUACUUCGGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13591.5	13592.4
42	LbcrRNA	AAUUUCUACUAAGUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13638.5	13637.2

Chemically modified crRNAs targeting *AAVS1*

WT	crWT	UAAUUUCUACUCUUGUAGAUCUUACGAUGGAGCCAGAGAGGAU	13763.5	13763.2
1	cr3'5F	UAAUUUCUACUCUUGUAGAUCUUACGAUGGAGCCAGAGAGGAU	13773.5	13772.2

Chemically modified crRNAs targeting *FANCF*

WT	crWT	UAAUUUCUACUCUUGUAGAUGUCGGCAUGGCCCCAUUCGCACG	13627.4	13625.6
1	cr3'5F	UAAUUUCUACUCUUGUAGAUGUCGGCAUGGCCCCAUUCGCACG	13637.4	13635.6

No.	LbcrRNA	Modification pattern (5'- to -3')	M.W.	
Chemically modified crRNAs targeting *DNMT1*			**Calcd.**	**Found**
WT	crWT	AAUUUCUACUAAGUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13638.5	13637.2
1	cr3'5F	AAUUUCUACUAAGUGUAGAUCUGAUGGUCCAUGUCUGUUACUC	13648.5	13648.0

Unmodified nucleotides are shown in black. Full-length PS modifications are shown in pink. 2'-*O*-methyl modifications are shown in blue. 2'-F modifications are shown in red. 2'-*O*-methyl combined with PS modifications are shown in light blue. 2'-F combined with PS modifications are shown in green. Unlock nucleotides are shown in purple. Locked nucleotides are shown in yellow. The dashes in the stem-engineered crRNAs denote deleted nucleotides, and the orange letters denote inserted nucleotides. Red letters in the loop-engineered crRNAs denote nucleotides difference between AscrRNA and crRNAs from other 15 Cpf1 family orthologues. This table is adapted with permission from ref. 1, Nature Publishing Group.

Reference

1. Li, B. et al. Engineering CRISPR–Cpf1 crRNAs and mRNAs to maximize genome editing efficiency. *Nature Biomedical Engineering* **1**, 0066 (2017).