

Supplementary Information for

CRISPR Anti-tag-Mediated Room-Temperature RNA Detection Using CRISPR/Cas13a

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Supplementary Table 1. RT-qPCR results of 30 clinical plasma samples. The raw data corresponds to the results presented in **Figure 6**.

Samples	Cq	
	Mean	SD
S1	-	-
S2	-	-
S3	-	-
S4	-	-
S5	-	-
S6	-	-
S7	-	-
S8	-	-
S9	-	-
S10	-	-
S11	-	-
S12	-	-
S13	-	-
S14	-	-
S15	-	-
S16	-	-
S17	-	-
S18	-	-
S19	34.904	0.241
S20	-	-
S21	38.48	1.185
S22	-	-
S23	38.943	0.427
S24	37.742	0.296
S25	30.361	0.321
S26	35.605	0.344
S27	37.185	1.082
S28	36.113	0.175
S29	-	-
S30	31.766	0.171

Supplementary Table 2. Cost comparison between CARRD assay and qPCR

CARRD assay:

Reagent	Price per kit	Volume per kit	Cost per reaction
LwCas13a (active)	\$170	100 μ L	\$0.044
crRNA	\$169	100 μ L	\$0.003
Fluorescence-quencher probe	\$704	750 μ L	\$0.141
CRISPR Anti-tag Hairpin Mediator	\$286	150 μ L	\$0.0038
Total			\$0.192

qPCR:

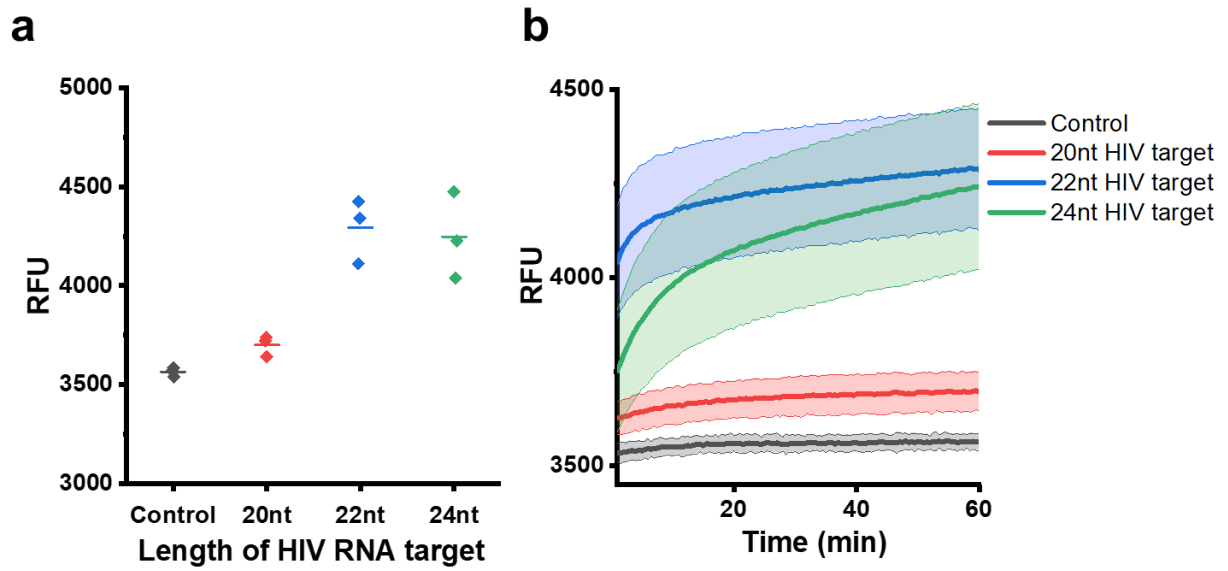
Reagent	Price per kit	Volume per kit	Cost per reaction
Reliance One-Step Supermix	\$549.00	1 mL (200 rxns)	\$2.75
Forward Primer (10 μ M)	\$25.00	250 μ L	\$0.05
Reverse Primer (10 μ M)	\$25.00	250 μ L	\$0.05
Probe (TaqMan, 10 μ M)	\$588.00	610 μ L	\$0.48
Total			\$3.33

Supplementary Table 3. Oligonucleotide sequences

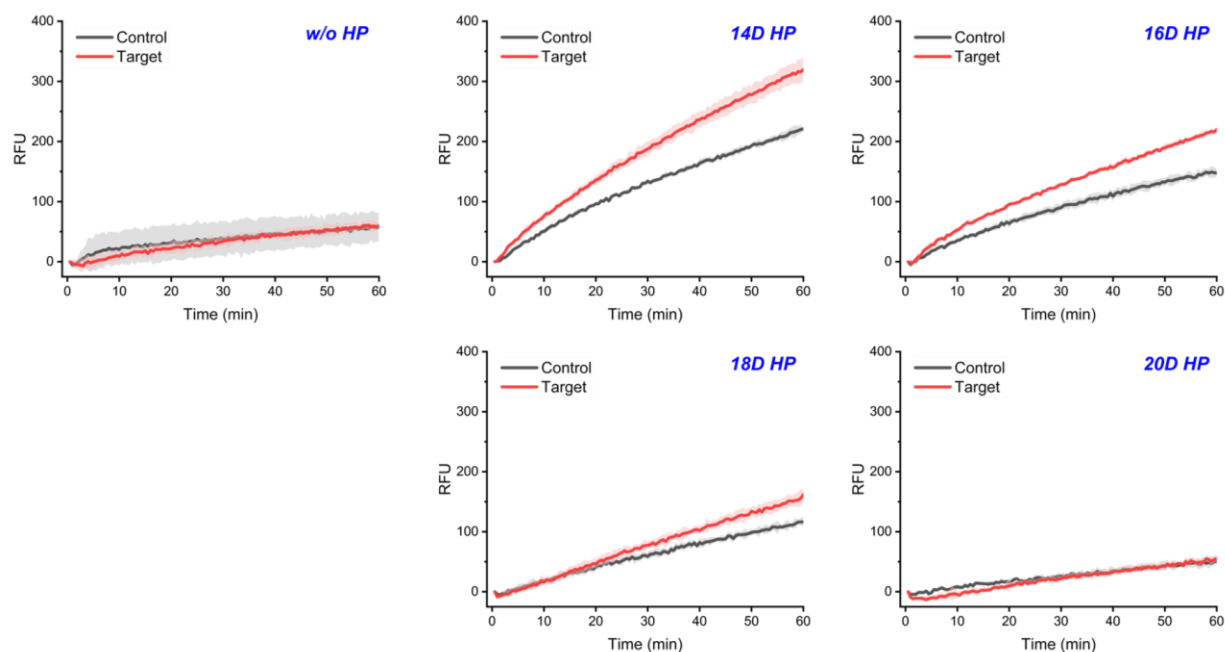
Name	Sequence (5'→3')	bp
HIV crRNA (#1)	rGrArU rUrUrA rGrArC rUrArC rCrCrC rArArA rArArC rGrArA rGrGrG rGrArC rUrArA rArArC rArUrC rCrCrA rUrUrC rUrGrC rArGrC rUrUrC rCrUrC rArUrU	60
24nt HIV RNA	rArArU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU	24
22nt HIV RNA	rU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU	22
20nt HIV RNA	rArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU	20
Complementary HIV DNA	ATC CCA TTC TGC AGC TTC CTC ATT	24
Anti-tag HIV RNA	rArArU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rGrUrU rUrUrA rGrU	32
5'-DNA tag-HP	ATC CCA TTC TGC AGC TTC CTC ATT rGrUrU rUrUrA rGrU rArArU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU	56
5'-RNA tag-HP	rArArU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rGrUrU rUrUrA rGrU ATC CCA TTC TGC AGC TTC CTC ATT	56
5'-RNA rU8-HP	rArArU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rUrUrU rUrUrU rUrU ATC CCA TTC TGC AGC TTC CTC ATT	56
HIV 20D HP	rU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rGrUrU rUrUrA rGrU ATC CCA TTC TGC AGC TTC CT	50
HIV 18D HP	rU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rGrUrU rUrUrA rGrU ATC CCA TTC TGC AGC TTC	48
HIV 16D HP	rU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rGrUrU rUrUrA rGrU ATC CCA TTC TGC AGC T	46
HIV 14D HP (#1)	rU rGrArG rGrArA rGrCrU rGrCrA rGrArA rUrGrG rGrArU rGrUrU rUrUrA rGrU ATC CCA TTC TGC AG	44

HIV target #1, #2	rArGrA rGrArC rCrArU rCrArA rUrGrA rGrGrA rArGrC rUrGrC rArGrA rArUrG rGrGrA rUrArG rArGrU rGrCrA rU	43
M crRNA	rGrArU rUrUrA rGrArC rUrArC rCrCrC rArArA rArArC rGrArA rGrGrG rGrArC rUrArA rArArC rUrCrA rGrUrU rUrUrG rCrArU rArGrA rUrUrU rGrCrA rCrA	59
Target M	rUrGrU rGrCrA rArArU rCrUrA rUrGrC rArArA rArCrU rGrA	23
HIV crRNA #2	rGrArU rUrUrA rGrArC rUrArC rCrCrC rArArA rArArC rGrArA rGrGrG rGrArC rUrArA rArArC rArUrG rCrArC rUrCrU rArUrC rCrCrA rUrUrC rUrGrC rArGrC	60
HIV 14D HP #2	rU rGrCrA rGrArA rUrGrG rGrArU rArGrA rGrUrG rCrArU rGrUrU rUrUrA rGrU ATG CAC TCT ATC CC	44
HIV crRNA #3	rGrArU rUrUrA rGrArC rUrArC rCrCrC rArArA rArArC rGrArA rGrGrG rGrArC rUrArA rArArC rCrUrC rUrCrA rUrCrU rGrGrC rCrUrG rGrUrG rCrArA rUrArG	60
HIV 14D HP #3	rA rUrUrG rCrArC rCrArG rGrCrC rArGrA rUrGrA rGrArG rGrUrU rUrUrA rGrU CTC TCA TCT GGC CT	44
HIV target #3	rGrGrC rCrUrA rUrUrG rCrArC rCrArG rGrCrC rArGrA rUrGrA rGrArG rArArC	30
HCV crRNA	rGrArU rUrUrA rGrArC rUrArC rCrCrC rArArA rArArC rGrArA rGrGrG rGrArC rUrArA rArArC rCrArC rArGrG rGrGrA rGrUrG rArUrU rCrArU rGrGrU rGrG	59
HCV RNA	rArCrA rCrUrC rCrArC rCrArU rGrArA rUrCrA rCrUrC rCrCrC rUrGrU rGrArG rGrArA	33
HCV 14D HP	rC rArCrC rArUrG rArArU rCrArC rUrCrC rCrCrU rGrUrG rGrUrU rUrUrA rGrU CAC AGG GGA GTG AT	44
HCV 15D HP	rC rArCrC rArUrG rArArU rCrArC rUrCrC rCrCrU rGrUrG rGrUrU rUrUrA rGrU CAC AGG GGA GTG ATT	45
HCV 16D HP	rC rArCrC rArUrG rArArU rCrArC rUrCrC rCrCrU rGrUrG rGrUrU rUrUrA rGrU CAC AGG GGA GTG ATT C	46
HIV FW primer	CAA GCA GCC ATG CAA ATG TTA	21

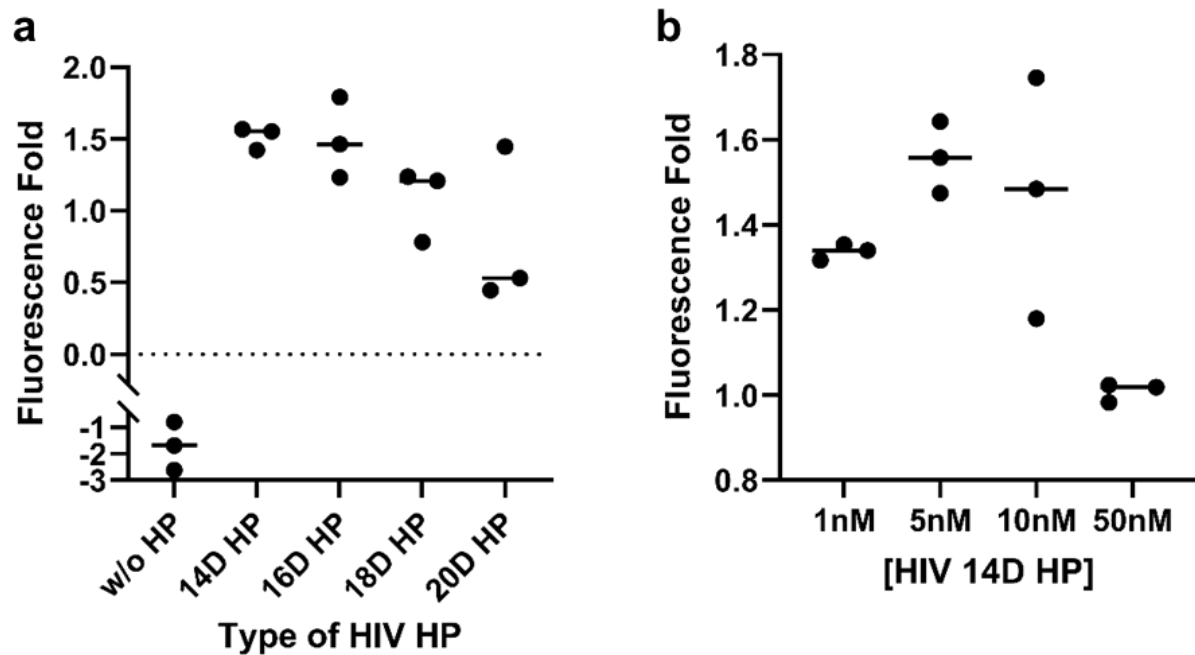
HIV RV primer	TGT CAT CCA TCC TAT TTG TTC CT	23
HIV PCR Probe	/56-FAM/TT GGA TCT C/ZEN/T TAT CTG GCC TGG TGC /3IABkFQ/	25
FAM reporter	/56-FAM/rUrU rUrUrU /3IABkFQ/	5



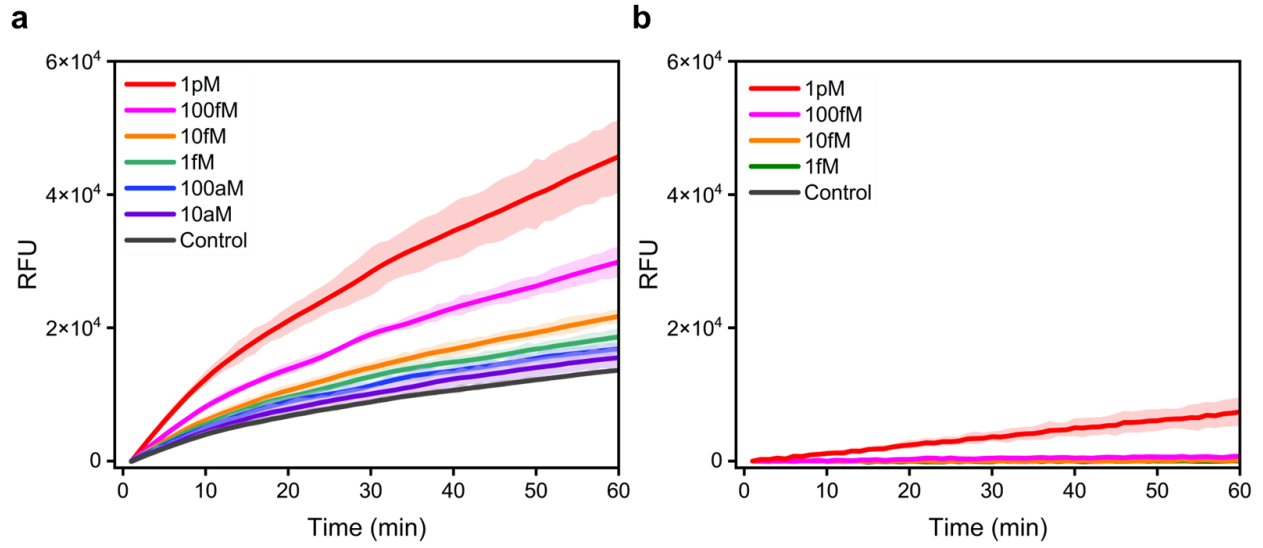
Supplementary Figure 1. Cas13a/HIV crRNA *trans*-cleavage reaction depending on the length of the HIV RNA target (20, 22, and 24 nt). **(a)** Final fluorescence intensity at 60 min and **(b)** real-time fluorescence signal. (n = 3 and data represent mean $\pm$ s.d. of three technical replicates)



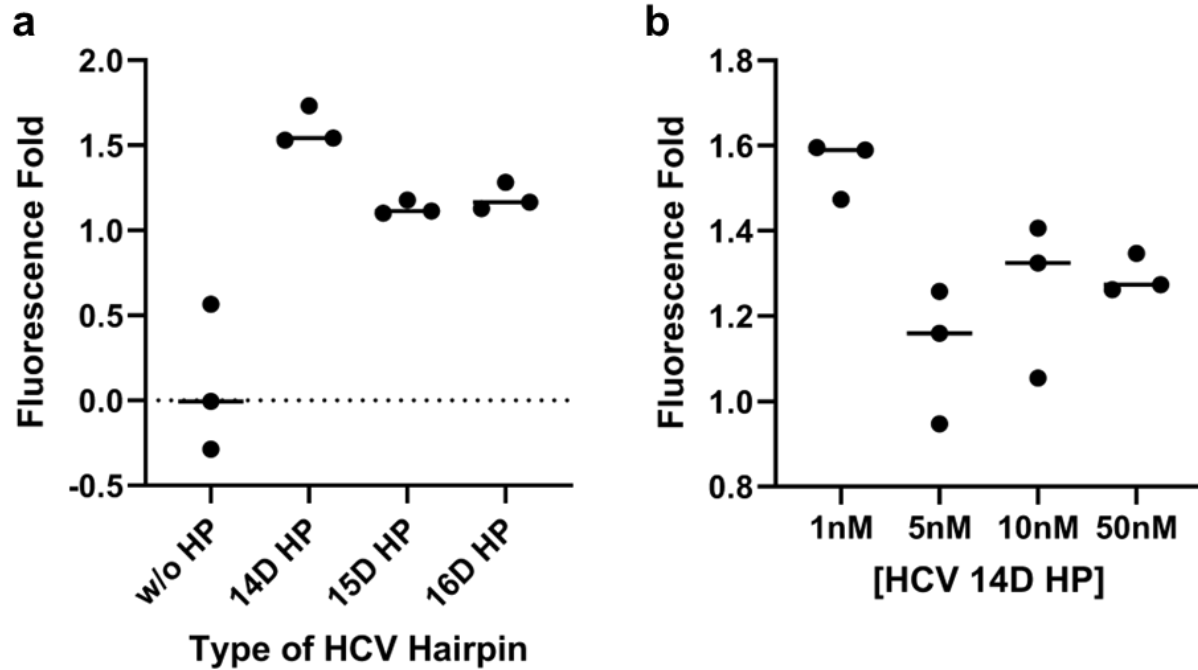
Supplementary Figure 2. Real-time fluorescence signals of sequential Cas13a reactions with various types of CRISPR anti-tag hairpins (HPs) in **Figure 3c**. The fluorescence signals were measured to compare the effectiveness of different HP constructs (14D-, 16D-, 18D-, and 20D-HP) in the presence and absence of target M. (n = 3 and data represent mean $\pm$ s.d. of three technical replicates)



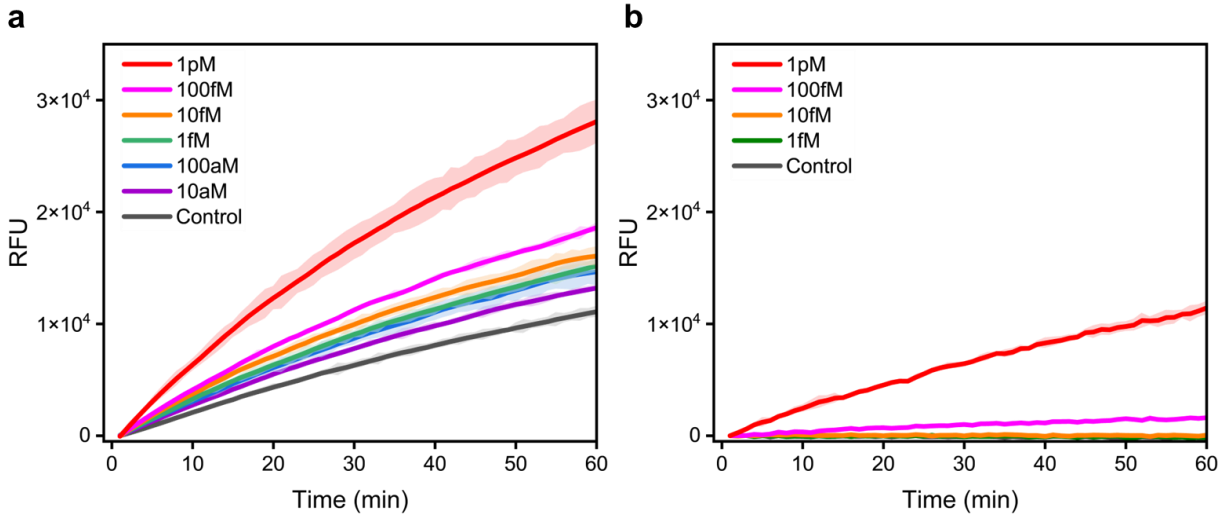
Supplementary Figure 3. Optimization of HIV detection based on **(a)** the type of CRISPR anti-tag hairpin (HP) containing the HIV target sequence and **(b)** the concentration of HIV 14D HP. (n = 3 and data represent mean $\pm$ s.d. of three technical replicates)



Supplementary Figure 4. Real-time HIV detection **(a)** with HIV 14D HP (CARRD assay) and **(b)** without HIV 14D HP (i.e., the conventional Cas13a/HIV crRNA reaction) ($n = 3$ and data represent mean $\pm$ s.d. of three technical replicates)



Supplementary Figure 5. Optimization of HCV detection based on (a) the type of CRISPR anti-tag hairpin (HP) containing the HCV target sequence and (b) the concentration of HCV 14D HP. (n = 3 and data represent mean $\pm$ s.d. of three technical replicates)



Supplementary Figure 6. Real-time HCV detection **(a)** with HCV 14D HP (CARRD assay) and **(b)** without HCV 14D HP (i.e., the conventional Cas13a/HCV crRNA reaction) ($n = 3$ and data represent mean $\pm$ s.d. of three technical replicates)