Supporting Information

Ultrasensitive detection platform of disease biomarkers based on recombinase polymerase amplification with H-sandwich aptamer

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Table S1. K_d values of the selected aptamer candidates. (* used in this study)

Target	Length (bp)	K_d value (nM)
p24_apt1*	82	314 ± 101.2
p24_apt2	82	456 ± 157.2
Ebola_apt1	85	367 ± 119
Ebola_apt2	85	1249 ± 529.8
Ebola_apt3*	85	23 ± 14.2
SARS-CoV-2_apt1	76	0.39 ± 0.08
SARS-CoV-2_apt2*	76	0.57 ± 0.13
SARS-CoV-2_apt3	76	2.32 ± 0.48
SARS-CoV-2_apt4	76	0.8 ± 0.13
SARS-CoV-2_apt5	76	0.66 ± 0.16

Table S2. Comparison of detection efficiency of various immunoassays.

Detection method	Target	LOD	Multiplexed detection	Reference
PCR using short DNA aptamers	Thrombin	2 pM	No	39
Sandwich ELISA using DNA encapsulated liposomes	Protective antigen	4.1 $ng \cdot mL^{-1}$	Yes	40
Photoelectrochemical immunoassay using DNA labeling	HIV-1 p24	10 $ng \cdot mL^{-1}$	No	41
H-sandwich RPA using DNA aptamers	SARS-CoV-2 NP	1 $fg \cdot mL^{-1}$	Yes	This study
	HIV-1 p24	10 $fg \cdot mL^{-1}$		
	Ebola NP	10 $fg \cdot mL^{-1}$		
	Influenza A NP	1 $fg \cdot mL^{-1}$		
	Influenza B NP	10 $fg \cdot mL^{-1}$		

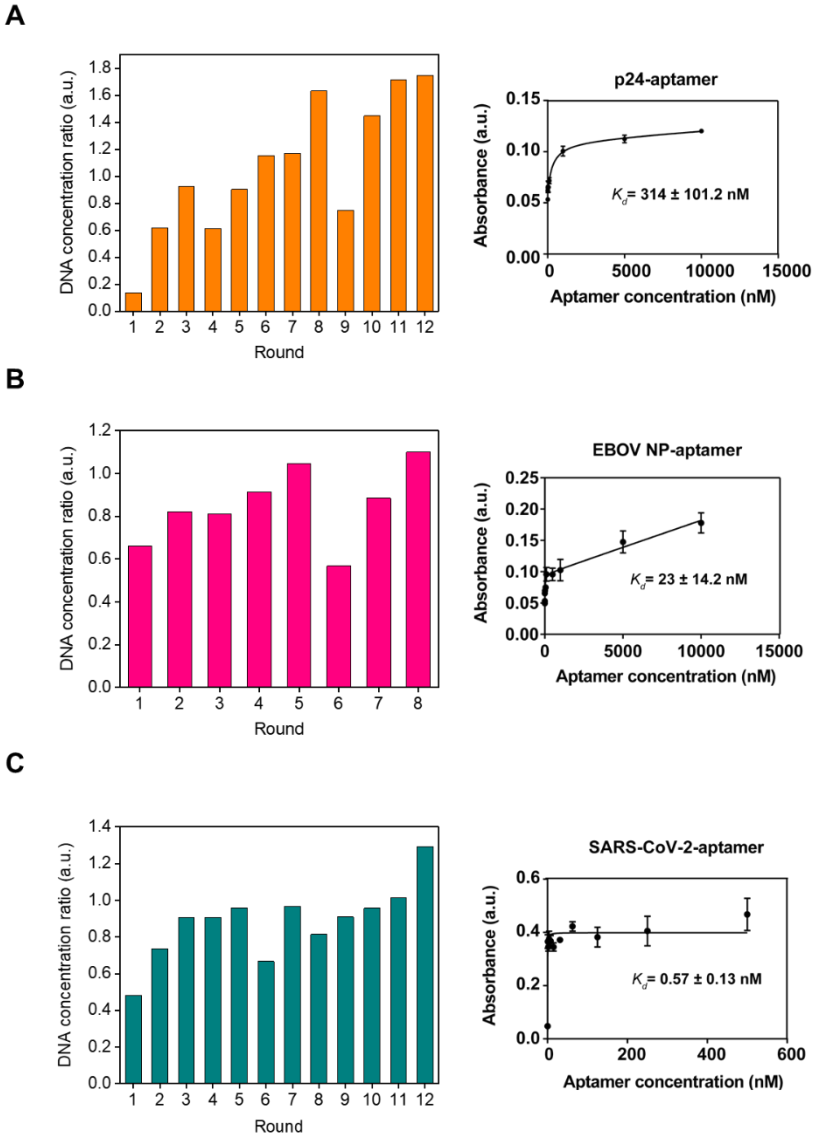

Figure S1. Elution profile according to SELEX round. The graphs and the K_d values (right) correspond to the aptamers used in the experiment from the aptamer candidates selected through each SELEX. (A) A total 12 round of selection was performed and Kd value of the used aptamer was 314 ± 101.2 nM (R^2 = 0.97). (B) A total 8 round of selection was performed and Kd value of the used aptamer was 23 ± 14.2 nM (R^2 = 0.93). (C) A total 12 round of selection was performed and Kd value of the used aptamer was 0.57 ± 0.13 nM (R^2 = 0.87). EBOV: Ebola virus

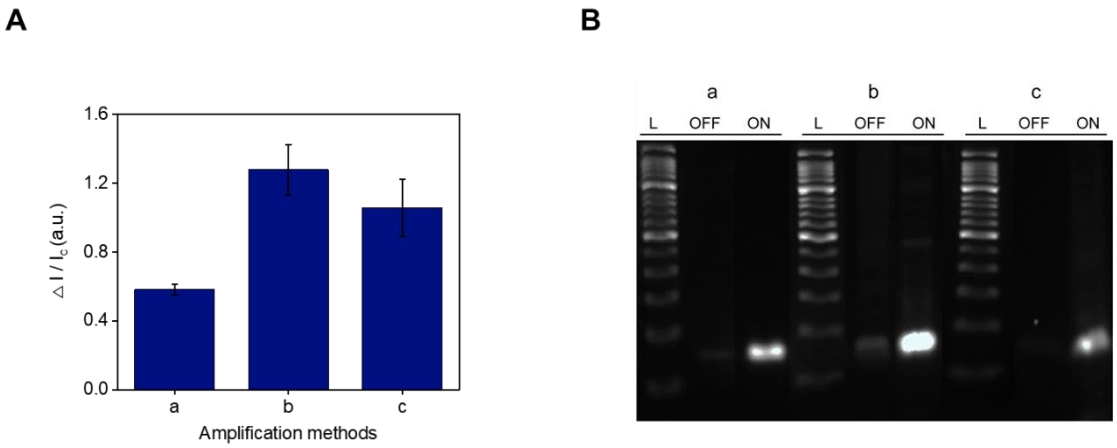

Figure S2. Intercalating dye efficiency under various amplification conditions. (A) Relative fluorescence intensity through amplification reactions (a: conventional PCR (30 cycles), b: RPA, c: H-sandwich RPA (NP and aptamer were used at 0.5 pg·mL^{-1} and 1 pM, respectively)). Error bars show the standard deviation of three experiments. (B) Gel shift image under each of the amplification reactions (OFF: without aptamer or NP, ON: with aptamer 1 pM, NP).

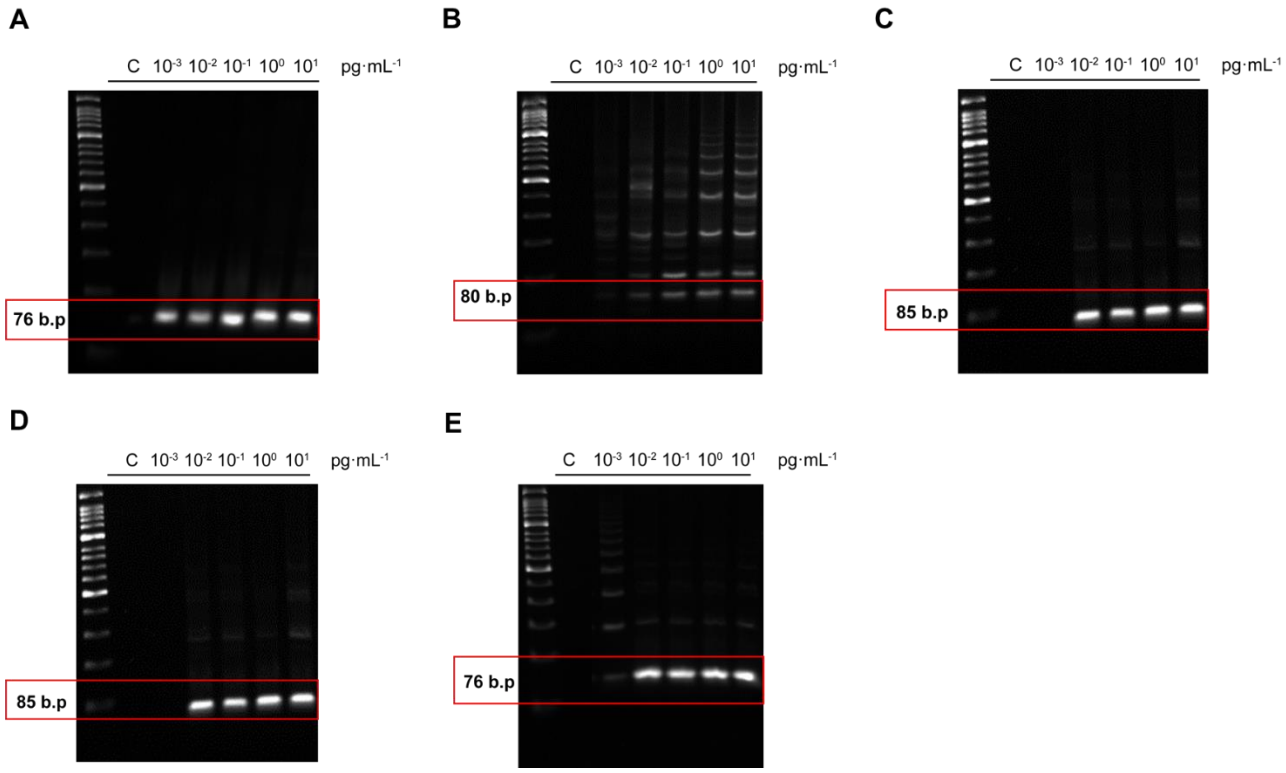

Figure S3. Gel shift images for the detection of amplified aptamers using the H-sandwich RPA. (A) Influenza A NP, aptamer length is 76 base pair. (B) Influenza B NP, aptamer length is 80 base pair. (C) HIV-1 p24, aptamer length is 82 base pair. (D) Ebola NP, aptamer length is 85 base pair. (E) SARS-CoV-2 NP, aptamer length is 76 base pair. Each antigen ranged in concentration from 0.001 to 10 $pg \cdot mL^{-1}$. b.p.: base pair

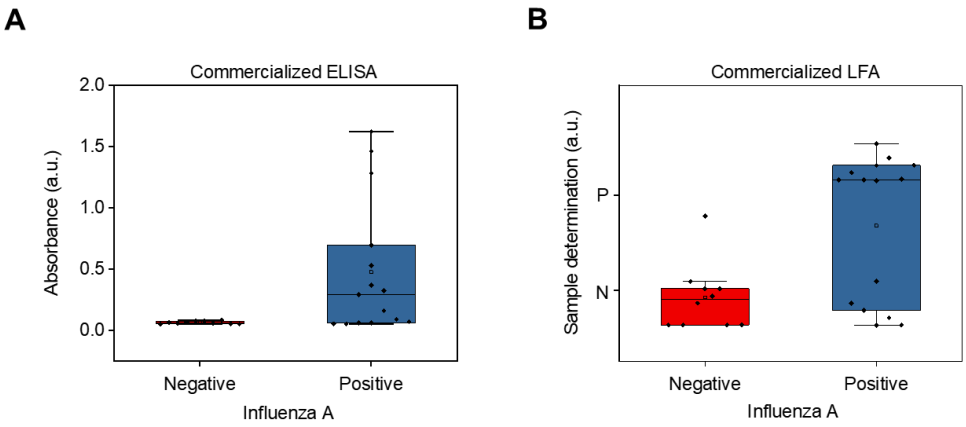

Figure S4. Detection efficiency in clinical samples according to commercialized kits. (A) Discrimination of negative and positive Influenza A patient samples using ELISA; negative n = 10, positive n = 15. (B) Discrimination of negative and positive Influenza A patient samples using LFA kit; negative n = 10, positive n = 15.