Supporting Information.

Highly sensitive *in Vitro* Diagnostic System of Pandemic Influenza A (H1N1) Virus Infection with Specific MicroRNA as a Biomarker

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Note	Sequence (5' to 3')
HA-1254	FAM/ GAA GCT GGA GCC TGC AAC ATA GTA CCT GGA AGT GCA GGC TCC AGC TTC CAG G /BHQ1
bHA-1254	FAM/ GAA GCT GGA GCC TGC AAC ATA GTA CC/Biotin-dT/ GGA AGT GCA GGC TCC AGC TTC CAG G /BHQ1
HB-1254	TGC AAC ATA GTA CCT GGA AGC TGG AGC CTG CAC TTC CAG GTA CTA TGT TGC AGG CT
has-miR-1254	AGC CUG GAA GCU GGA GCC UGC AGU
Synthetic Target-1254	AGC CTG GAA GCT GGA GCC TGC AGT
Synthetic Control-1254 (M1)	AGC GTG GAA GCT GGA GCC TGC AGT
Synthetic Control-1254 (M2)	AGC GTG GAA GCA GGA GCC TGC AGT
Synthetic Control-1254 (M3)	AGC GTG GAA GCA GGA GCC TCC AGT

HA-181c-5p	FAM/ TCA ACC TGT CGG TGA GGC GAC CGC ACA TTC AAC TCA CCG ACA GGT TGA ATG T/BHQ1
bHA-181c-5p	FAM/ TCA ACC TGT CGG TGA GGC GAC CGC ACA /Biotin-dT/TC AAC TCA CCG ACA GGT TGA ATG T/BHQ1
HB-181c-5p	TGA GGC GAC CGC ACA TTC AAC CTG TCG GTG AGT TGA ATG TGC GGT CGC CTC ACC GA
has-miR-181-5p	AAC AUU CAA CCU GUC GGU GAG U
Synthetic Target_181c-5p	AAC ATT CAA CCT GTC GGT GAG T
Synthetic Control-181c-5p (M1)	AAG ATT CAA CCT GTC GGT GAG T
Synthetic Control-181c-5p (M2)	AAG ATT CAA TCT GTC GGT GAG T
Synthetic Control-181c-5p (M3)	AAG ATT CAA TCT GTC GGT AAG T

Table S1. All DNA sequences used in the experiments.

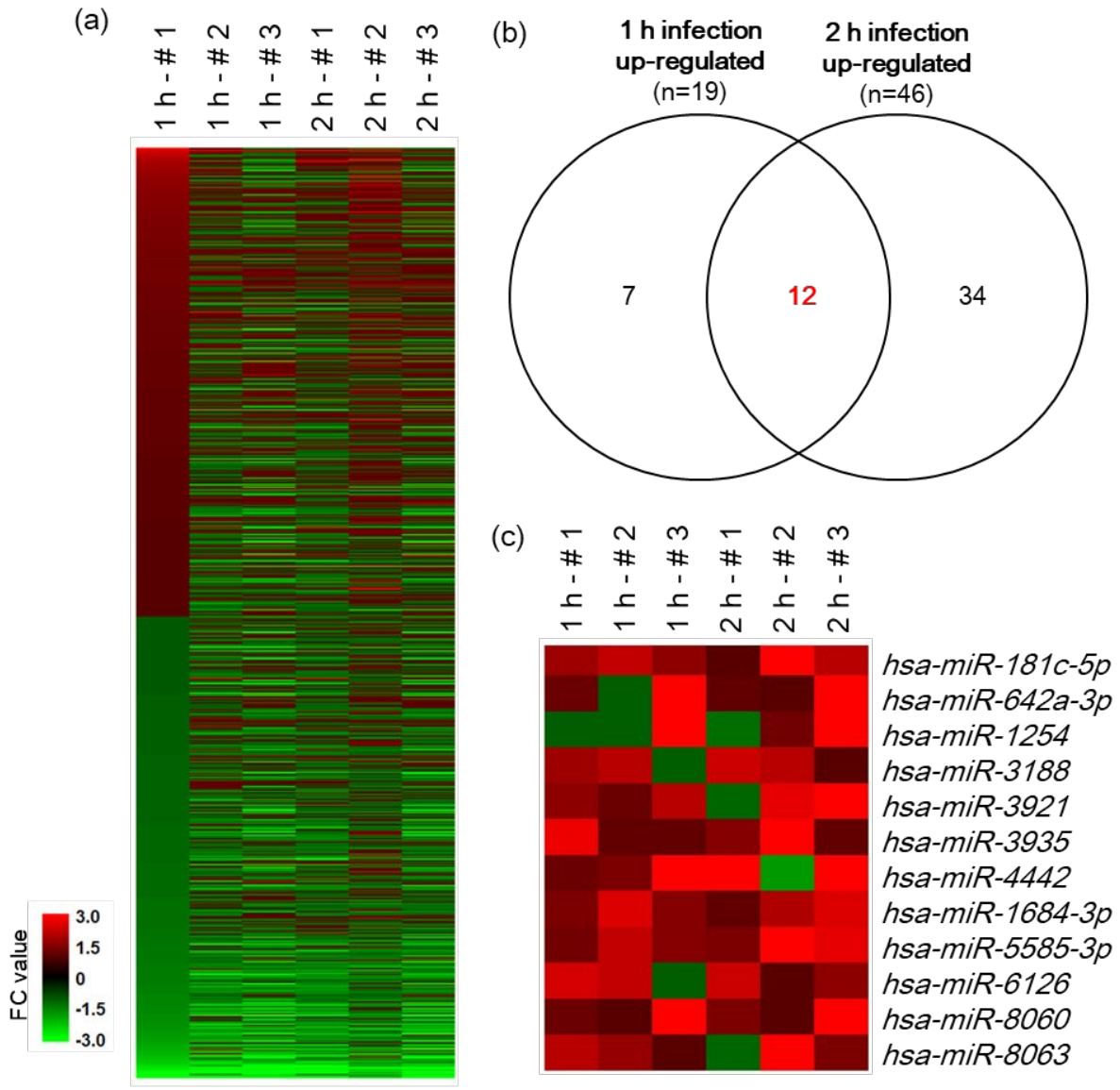

Figure S1. (a) Microarray analysis of 1,919 human miRNAs was performed with RNA extracts from influenza virus-infected A549 cells. The red color scale indicates the highest overexpression, and the green color indicates the highest underexpression of microRNA. (b) Venn diagram showing the number of upregulated miRNAs in response to pH1N1 virus infection (mock versus 1 h or 2 h infection conditions). (c) 12 miRNAs

upregulated under both infection conditions are listed (the intersection region of the Venn diagram).

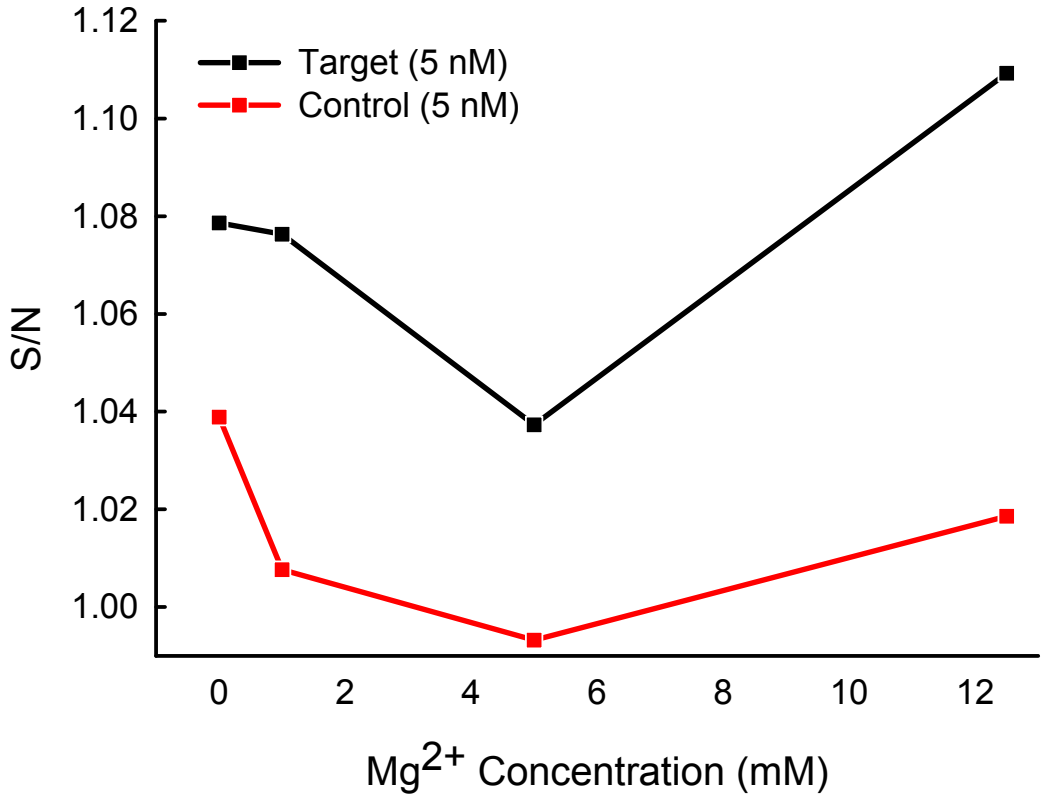

Figure S2. Optimization of the concentration of Mg^{2+} ions. The signal-to-noise ratio (S/N) evaluated the effect of Mg^{2+} concentration (n=3).

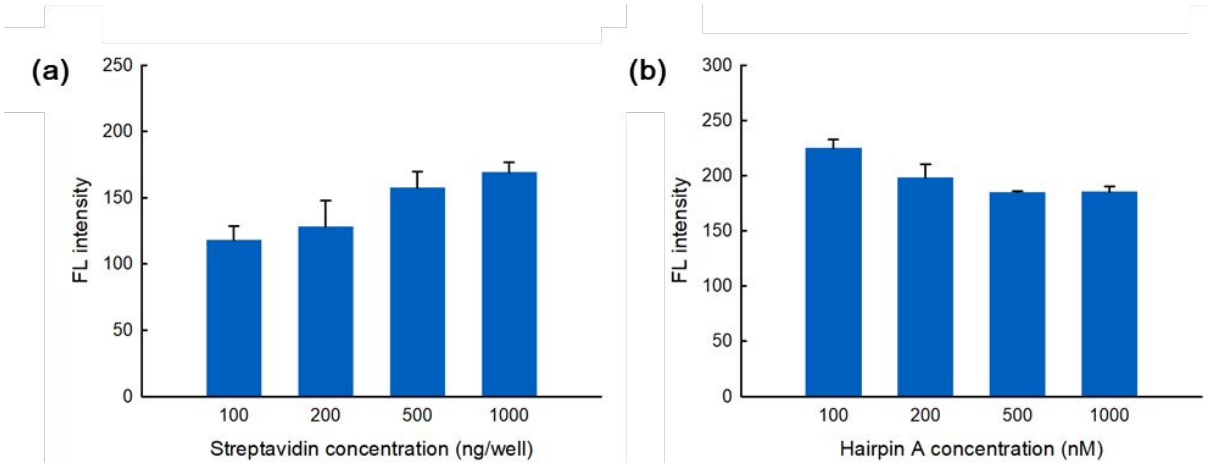

Figure S3. Optimization of experimental conditions. (a) Evaluation of the effect of the concentration of streptavidin. (b) Evaluation of the concentration of bHA.

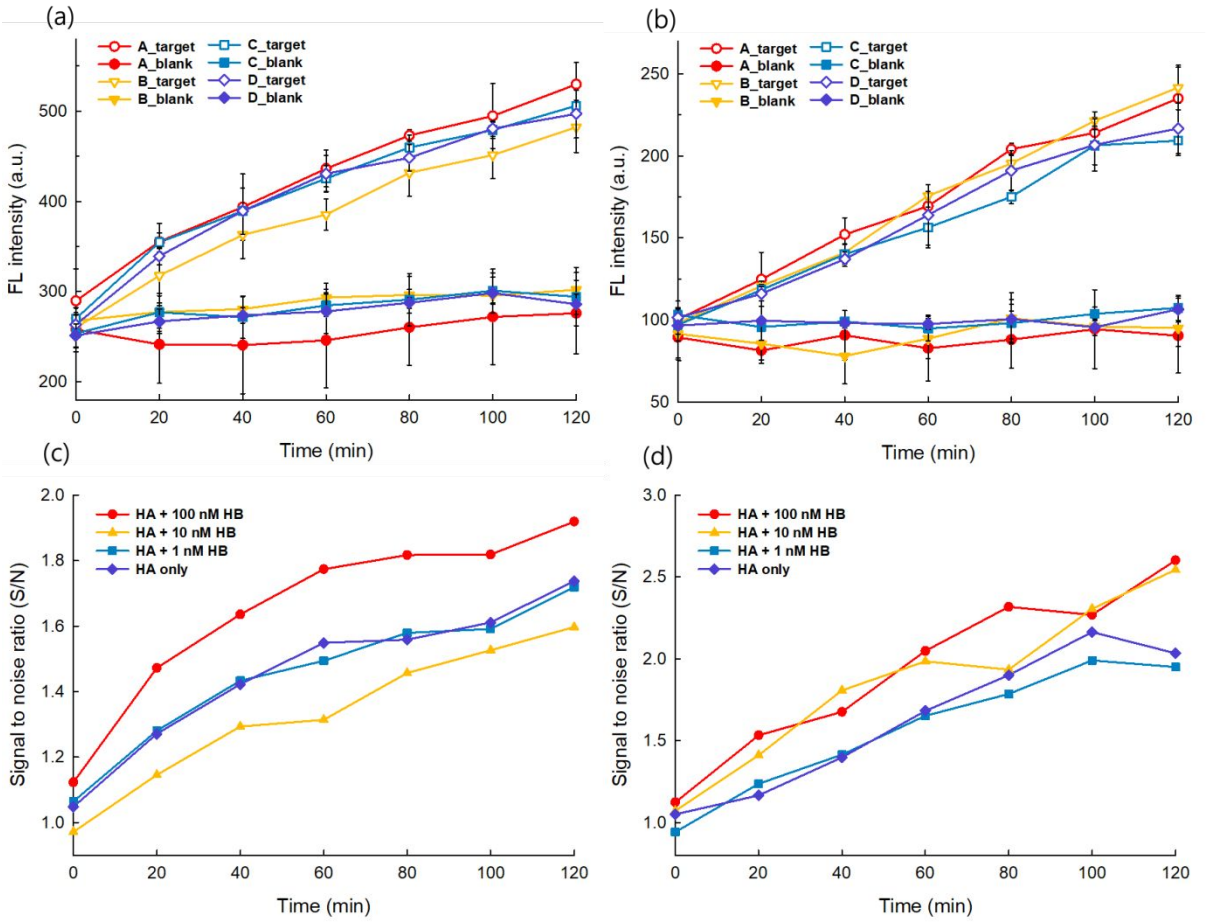

Figure S4. Fluorescence kinetics of (a) HA-181c-5p and (b) HA-1254 with each different HB concentration (A; HA + 100 nM HB, B; HA + 10 nM HB, C; HA + 1 nM HB, D; HA only) and their signal-to-noise ratios (S/N, $intensity_{target=n\ min}/intensity_{blank=n\ min}$) over 2 h (120 min) of detecting (c) 50 nM miR-181c-5p and (d) 50 nM miR-1254.

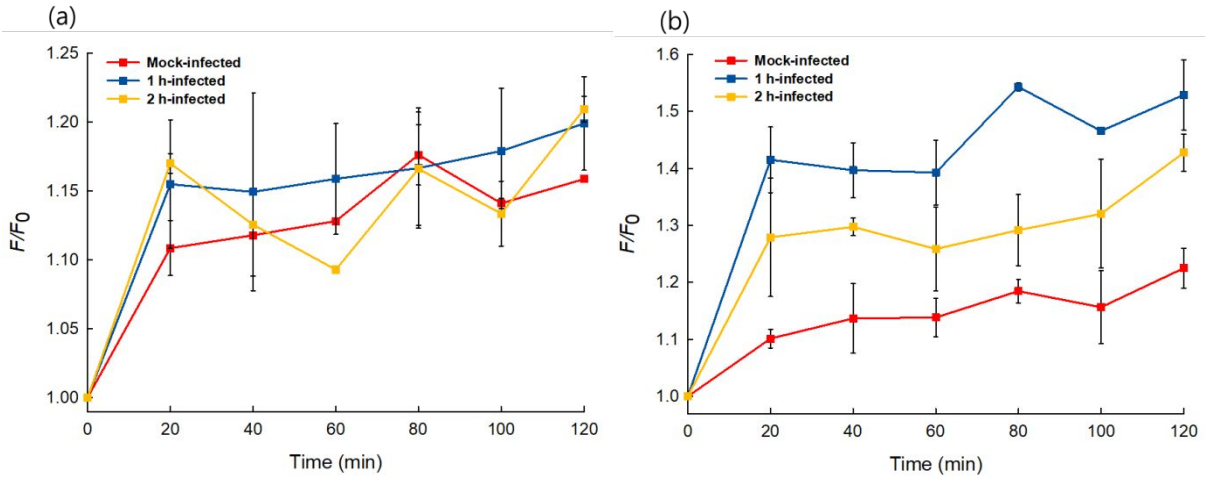

Figure S5. Observation of fluorescence kinetics with real RNA (1 μg per well). The fluorescence recovery ratio (F/F_0) was monitored by (a) the miR-181c-5p kit and (b) the miR-1254 kit for 2 h. Total RNA was isolated from mock-infected (no virus treated) cells and cells infected with the pH1N1 virus in the A549 cell line for 1 h and 2 h.

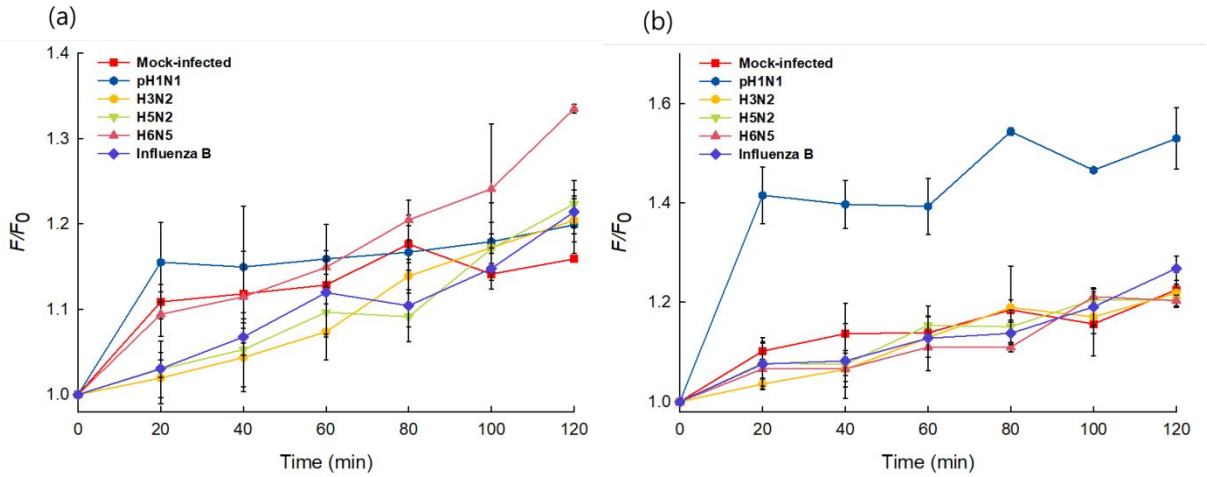

Figure S6. Fluorescence kinetics of A549 cells infected with various influenza viruses, including influenza A (pH1N1, H3N2, H5N2, H6N5) viruses and influenza B virus. The fluorescence recovery ratio (F/F_0) was monitored by (a) the miR-181c-5p kit and (b) miR-1254 kit for 2 h.