Supporting Information for

Sensitive, general and portable detection of RNAs combining duplex-specific nuclease transduction with off-shelf signalling platform

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1. Chemicals and materials.

The Duplex-Specific Nuclease was purchased from Evrogen Joint Stock Company (Moscow, Russia). The Bayer Contour Next Blood Glucose Monitoring System and Bayer Contour Next Blood Glucose Test Strips were bought from Amazon.com and used for the tests in this work. Streptavidin-coated magnetic beads (MB, 1.5 μM in average diameter) were purchased from Bangs Laboratories Inc (Fishers, IN, USA) and the Amicon Ultra-2 mL 30 K was purchased from Millipore Inc (Billerica, MA, USA). Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and other chemicals and solvents were purchased from Sigma-Aldrich Inc. (St. Louis, MO,USA). 10×Isothermal Buffer (10×Iso) was obtained from New England Biolabs (Ipswich, MA, USA). Thermo-stable invertase (TmINV) derived from the hyperthermophilic bacteria was home-made according to our previous publication without modification. Zika template RNA used for NASBA reaction were synthetized from trigger plasmid (Addgene, plasmid number: 75008) using RNA Synthesis Kit and diluted to corresponding concentrations. NASBA Kits were obtained from Life

Sciences (Petersburg, FL, USA). SanPrep Column microRNA Extraction Kit was from Sangon Biotech Co., Ltd (Shanghai, China). RNase-Free Water, RNA simple Total RNA Kit were from Tiangen (Beijing, China). All the oligonucleotides used in this paper are listed in Table S1. The concentrations of the DNA and RNA suspensions were measured by UV spectrophotometry using the DeNovix DS-11+ FX spectrophotometer (DeNovix Inc., Wilmington, DE, USA). The fluorescence kinetic curves were monitored with the Coyote Mini-8 portable real-time PCR machine (Beijing, China) and a LightCycler® 96 Real-Time PCR System (Roche, Basel, Switzerland).

2. Experiments

2.1 Synthesis of TmINV-ssDNA conjugate.

The TmINV-ssDNA conjugate was synthesized according to our previously published protocol with slight modifications[1,2]. Invertase was conjugated to the linker by dissolving 2.5 mg of TmINV and 1 mg of the sulfo-SMCC linker in 1 mL PBS buffer (10 mM Sodium Phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4). This solution was shaken at 750 rpm for 2.5 hr at room temperature (RT, 25°C). Subsequently, unreacted reagents were removed by filtration through an Amicon-30 K filter and the TmINV-SMCC conjugate was subjected to six washes with PBS prior to resuspension in 850 μL PBS. Meanwhile, 54 μL of the thiol-labeled oligonucleotide SH-ssDNA (resuspended at a concentration of 180 μM in water) was activated for 1 hr at RT by continuously mixing with 15 μL of 100 mM TCEP. Excess TCEP and salts were subsequently removed by filtration through SephadexG-25. Activated SH-ssDNA and the TmINV-SMCC conjugate were then mixed and stirred overnight at 30°C. Unreacted SH-ssDNA was removed by filtration through an Amicon-30 K filter and the TmINV-ssDNA conjugate was subjected to at least six washes. The final TmINV-ssDNA conjugate was resuspended in PBS buffer at a concentration of 13.3 mg/mL (determined with a DeNovix DS-11+FX Spectrophotometer) and stored at 4°C until further use.

2.2 Preparation of the TmINV-ssDNA-MBs.

Some 200 μL of 1 mg/mL streptavidin coated Magnetic Beads (MBs) were transferred into a 1.5 mL centrifuge tube and washed with isothermal amplification buffer (1×Iso Buffer, 20

mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20, pH 8.8). The MBs were isolated by using an external magnetic rack and resuspended in 50 μL isothermal amplification buffer. 50 μL 13.3 mg/mL TmINV-ssDNA added to the above 50 μL isothermal amplification buffer contain MBs. Rotate and mix for 40 minutes on a vertical rotator at room temperature. After at least five washes with 100 μL isothermal amplification buffer to remove excess TmINV-ssDNA, the final TmINV-ssDNA-MBs were dispersed in 100 μL isothermal amplification buffer and stored at 4°C until further use.

2.3 Optimization of the amount of DSN.

In order to find the best dosage of DSN enzyme on miRNA detection, we use 0.06U, 0.08U, 0.1U, 0.2U, 0.3U, 0.5U DSN enzyme in the mix reaction solution on fluorescence response. 10 U lyophilized DSN enzyme was diluted by adding 10 μL DSN storage buffer (50 mM Tris-HCl, pH 8.0), and mixed gently. Subsequently, the tube was spinned briefly and incubated at room temperature for 5 min. Following that, 10 μL glycerol (to 50% final glycerol concentration) was added to the tube. 1μL 100nM miRNA21 and 1μL 100 nM ssNDA probe were added to the mix solution (total volume 10 μL) contain of 1μL 10×DSN buffer (50 mM Tris-HCL, PH 8. 5 mM Mgcl2, and 1 mM DTT), 0.25μL 400U Rnase and 6.25μL RNase-Free Water. Consider both economics and signal generation 0.1U DSN enzyme is used in this paper shown in Figure S2.

2.4 Optimization of the amount of MBs.

The microliters of miR-21 was dissolved in RNase-Free Water and then added into the mixture solution (total volume 10 μL) consist of 1 μL 10×DSN buffer (50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, and 1 mM DTT), 0.25 μL 400U RNase inhibitor, and 0.1 μL 1U/μL DSN in 50% glycerol with final concentration of 10 nM. 6 μL, 8 μL, 10 μL, 12 μL, 14 μL MBs were placed close to the magnetic rack for 1 min. The clear solution was discarded and replaced by 10 μL above mixture solution. After rotate and mix for 30 minutes on a vertical rotator at 55°C and then perform simple magnetic separation, the clear solution was transferred to the same volume of 500 mM sucrose solution. React at 75°C the glucose value can be read with the personal glucometer. It has the highest signal-to-noise ratio when the MBs volume is 12 μL as shown in Figure S4.

2.5 Detection of miR-21 with the DSN-PGM sensing platform.

The microliters of miR-21 was dissolved in RNase-Free Water and then added into the mixture solution (total volume 10 μL) consist of 1 μL 10×DSN buffer (50 mM Tris-HCl, pH 8.0, 5 mM MgCl2, and 1 mM DTT), 0.25 μL 400U RNase inhibitor, and 0.1 μL 1U/μL DSN in 50% glycerol with various final concentrations of the targets of 1×10-14 to 2×10-8 M. A series of tubes containing 12 μL aliquots of the 3.68 mg/mL TmINV-ssDNA-MBs were placed close to the magnetic rack for 1 min. Such amount of TmINV-ssDNA-MBs has been selected after optimization (Figure S4). The clear solution was discarded and replaced by 10 μL above mixture solution. The reaction buffer was allowed to proceed for 30 min on a vertical rotator at 55°C. The TmINV-ssDNA-MBs were then separated using a magnetic rack and the clear solution containing released invertase were transferred into equal volumes of 500 mM sucrose. This mixture was incubated for 50 min at 75°C to allow invertase-mediated catalytic conversion of sucrose to glucose. Subsequently, 1.5 μL of the reaction solution was transferred to a PGM strip and the amount of glucose was measured by using a commercially available hand-held PGM.

2.6 Specificity test of the DSN-PGM sensing platform.

A series of tubes containing 12 μL aliquots of the 1.57 mg/mL TmINV-ssDNA-MBs were placed close to the magnetic rack for 1 min. The clear solution was discarded and replaced by 10 μL of the different miRNA target at 10 nM concentrations. The mixture solution is the same with mentioned above in 2.5. The DSN reactions were allowed to proceed for 30 min on a vertical rotator at 55°C. The TmINV-ssDNA-MBs were then separated using a magnetic rack and aliquots of the supernatant containing released TmINV were transferred into equal volumes of 500 mM sucrose. This mixture was incubated for 50 min at 75°C to allow invertase-mediated catalytic conversion of sucrose to glucose. Subsequently, 1.5 μL of the reaction solution was transferred to a PGM strip and the amount of glucose was measured by using a commercially available hand-held PGM.

2.7 MiRNA detection in real samples.

The three human cell lines included human breast cancer cell line (MCF-7), human embryonic kidney cell line (HEK293T) and cervical cancer cells (HELA) were cultured in

DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, and the medium of MCF-7 was further supplied with 0.01 mg/mL human recombinant insulin. All cells were incubated in a humidified incubator at 37°C with 5% CO_2. Total RNA samples were isolated from MCF-7, HEK293T and HeLa cell line by using RNA simple Total RNA Kit at their exponential growth stages. The total RNA was stored at -80°C for further use. 8 μL total RNA samples added into the mixture solution consist of 1 μL 10×DSN buffer (50 mM Tris-HCl, pH 8.0, 5 mM MgCl2, and 1 mM DTT), 0.25 μL 400U RNase inhibitor, and 0.1 μL 1U/μL DSN in 50% glycerol total volume 10 μL. A series of tubes containing 12 μL aliquots of the 3.68 mg/mL TmINV-ssDNA-MBs were placed close to the magnetic rack for 1 min. The clear solution was discarded and replaced by 10 μL above mixture solution. The reaction buffer was allowed to proceed for 30 min on a vertical rotator at 55°C. The TmINV-ssDNA-MBs were then separated using a magnetic rack and the clear solution containing released invertase were transferred into equal volumes of 500 mM sucrose. This mixture was incubated for 50 min at 75°C to allow invertase-mediated catalytic conversion of sucrose to glucose. Subsequently, 1.5 μL of the reaction solution was transferred to a PGM strip and the amount of glucose was measured by using a commercially available hand-held PGM.

2.8 Detection of NASBA amplicons with the DSN-PGM sensing platform (NASBA-DSN-PGM).

Zika virus is chosen as the model target because it is a flavivirus transmitted by mosquitoes and can cause severe fetal microcephaly and Guillain–Barre´ syndrome. Zika template RNAs used for NASBA reaction were synthetized from trigger plasmid (Addgene, plasmid number: 75008) using RNA Synthesis Kit and diluted to corresponding concentrations. NASBA reaction mixtures containing 3.35 μL Reaction buffer, 1.65 μL Nucleotide Mix, 0.4 μL primers mixtures including 12.5 μM of each primer, 0.6 μL nuclease free water, and 1 μL different concentrations of template RNAs were assembled on ice and heated at 65°C for 2 min, followed by a 10 min incubation at 41°C. Then, 2.5 μL Enzyme Mix was added to initiate the NASBA reaction (for a final volume of 10 μL), and the mixtures were incubated at 41°C for 2 hr. A series of tubes containing 12 μL aliquots of the 3.68 mg/mL

TmINV-ssDNA-MBs were placed close to the magnetic rack for 1 min. The clear solution was discarded and replaced by 8.9 μL above NASBA reaction buffer and 1 μL 10×DSN buffer (50 mM Tris-HCl, pH 8.0, 5 mM MgCl2, and 1 mM DTT) with 0.1 μL 1 U/μL DSN in 50% glycerol. The reaction buffer was allowed to proceed for 30 min on a vertical rotator at 55°C. The TmINV-ssDNA-MBs were then separated using a magnetic rack and the clear solution containing released invertase were transferred into equal volumes of 500 mM sucrose. This mixture was incubated for 50 min at 75°C to allow invertase-mediated catalytic conversion of sucrose to glucose. Subsequently, 1.5 μL of the reaction solution was transferred to a PGM strip and the amount of glucose was measured by using a commercially available hand-held PGM.

2.9 Fluorescence resonance energy transfer (FRET) experiments of DSN assisted NASBA product detection.

In addition to adding a single-stranded fluorescent probe with a final concentration of 10nM to the reaction system, NASBA reaction is the same as mentioned in above section 2.8. The fluorescence values was then been read by LightCycler® 96 Real-Time PCR System (Roche, Basel, Switzerland) (Figure 3B).

2.10 Standard dye experiments to confirm NASBA reaction.

1 μL eva green (for a final volume of 10 μL) was added to above mixture solution of NASBA described at section 2.8. The eva green values was then been read by the Coyote Mini-8 portable real-time PCR machine at 41°C for 1 hr (Figure S9).

2.11 One-pot verification of the sensing platform.

The microliters of miRNA-21 was dissolved in RNase-Free Water and then added into the mixture solution (total volume 10 μL) consist of 1 μL 10×DSN buffer (50 mM Tris-HCL, pH 8.0, 5 mM $MgCl_2$, and 1 mM DTT), 0.25 μL 400U RNase inhibitor, and 0.1 μL 1U/μL DSN in 50% glycerol with various final concentrations of the targets of 10 nM. A series of tubes containing 12 μL aliquots of the 3.68 mg/mL TmINV-ssDNA-MBs were placed close to the magnetic rack for 1 min. The clear solution was discarded and replaced by 10 μL above mixture solution. The reaction buffer was allowed to proceed for 30 min on a vertical rotator at 55°C. The TmINV-ssDNA-MBs were then separated using a magnetic rack and the clear

solution containing released invertase were transferred into equal volumes of 500 mM sucrose. This mixture was incubated for 50 min at 55°C to allow invertase-mediated catalytic conversion of sucrose to glucose. Subsequently, 1.5 μL of the reaction solution was transferred to a PGM strip and the amount of glucose was measured by using a commercially available hand-held PGM.

3. Supporting Figures and Tables

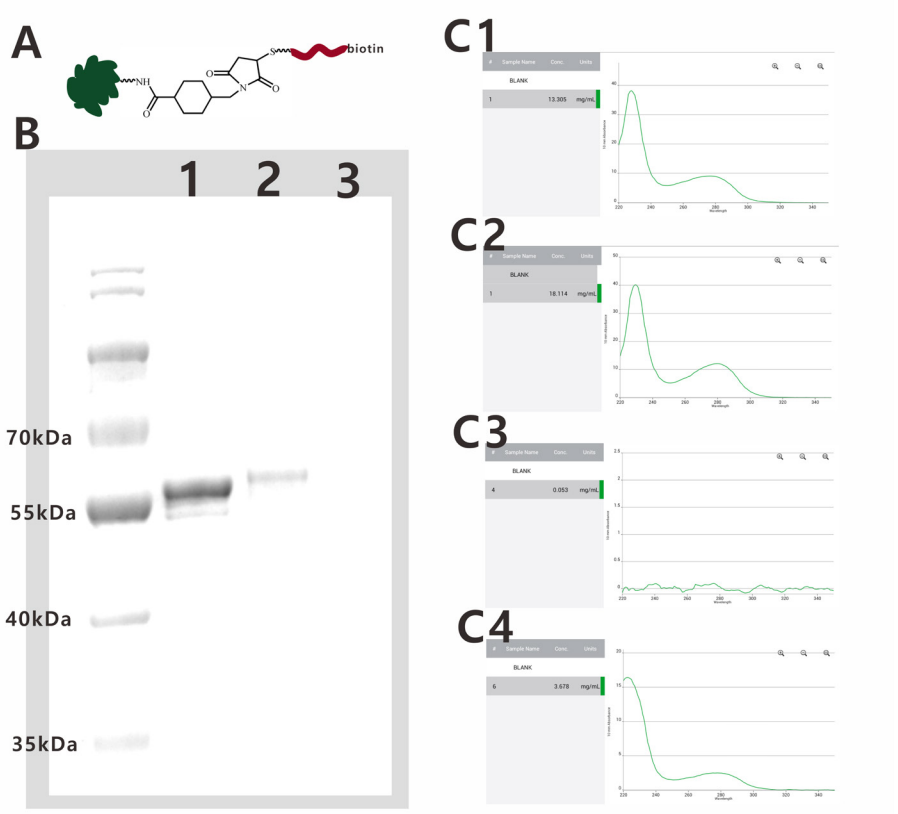

Figure S1. Conjugation verification of the TmINV-ssDNA and TmINV-ssDNA-MBs. (A) the conjugation of ssDNA and TmINV by the heterobifunctional linker (sulfo-SMCC). (B) SDS-PAGE (10% gradient gel) images of TmINV, TmINV-ssDNA and ssDNA. Lane1: TmINV; Lane 2: TmINV-ssDNA; Lane 3: ssDNA. Note: According to the bends of the markers, the lowest bend represents the species about 35 kD. The molecular weight of ssDNA is about 6.7 kD, which is much smaller than 35 kD, so it would have run out of the gel and can't been observed. (C) 1: the UV-visible spectrum of TmINV-ssDNA conjugation. A260/A280=0.79; 2: the UV-visible spectrum of TmINV only. A260/A280=0.45; 3: the UV-visible spectrum of MBs only; 4: the UV-visible spectrum of TmINV-ssDNA-MBs. A260/A280=0.72.

The higher A260/A280 value of TmINV-ssDNA over that of TmINV indicates the successful conjugation between DNA and TmINV. The appearance of UV-visible absorption at 260 nm and 280 nm of TmINV-ssDNA-MBs indicates the successful modification of TmINV-ssDNA onto MBs.

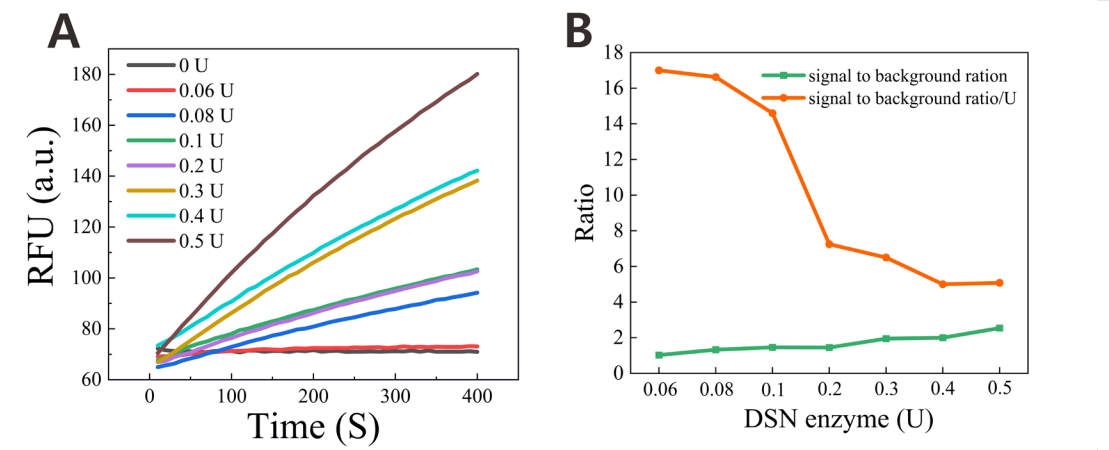

Figure S2. Optimization of DSN enzyme amount. (A) Kinetic curves of fluorescence signal triggered with different amount of DSN (B) Signal to background ratio of different amount of DSN and signal to background ratio of DSN in per unit.

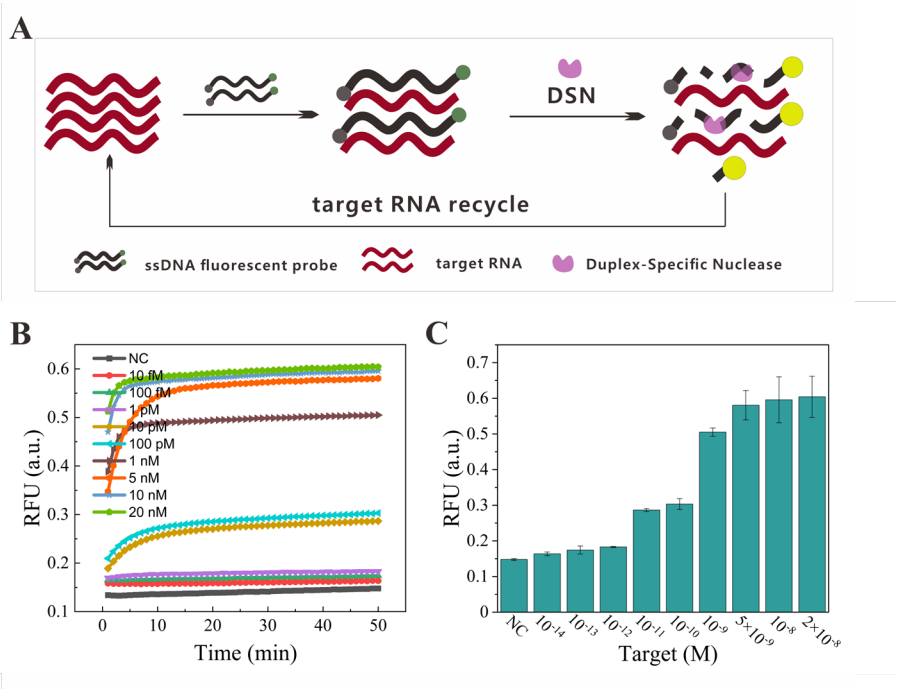

Figure S3. Fluorescence verification for miRNAs detection. (A) Schematic diagram of the fluorescence detection for miRNA. The two ends of ssDNA probe were modified with fluorophore and quencher, respectively. Once hybridizing to miR-21 and forming

effective DNA-RNA hybrid, the DSN should cleave the ssDNA sequence and release the fluorophore far away from the quencher. As expected, the fluorescence intensity with different concentrations of miR-21 shows significant increasing whereas the negative control is always flat (Figure S2 (B) (C)). According to the kinetic curves, 20 min is obviously enough to finish the cleavage reaction which becomes a premise to implement miR-21 detection using DSN-PGM sensing platform. (B) Kinetic curves of fluorescence signal triggered with different concentrations of miRNA. (C) Fluorescence signal with different concentrations of miRNAs extracted plotted at 50 min. The error bar indicates the standard deviation of three replicate experiments.

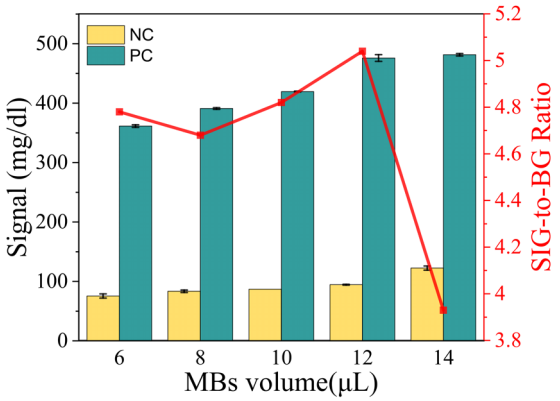

Figure S4. Optimization of magnetic beads dosage. Glucose signal with different volumes of magnetic beads plotted at 50 min glucose generation. The concentration of miRNAs used in this assay is 10 nM.

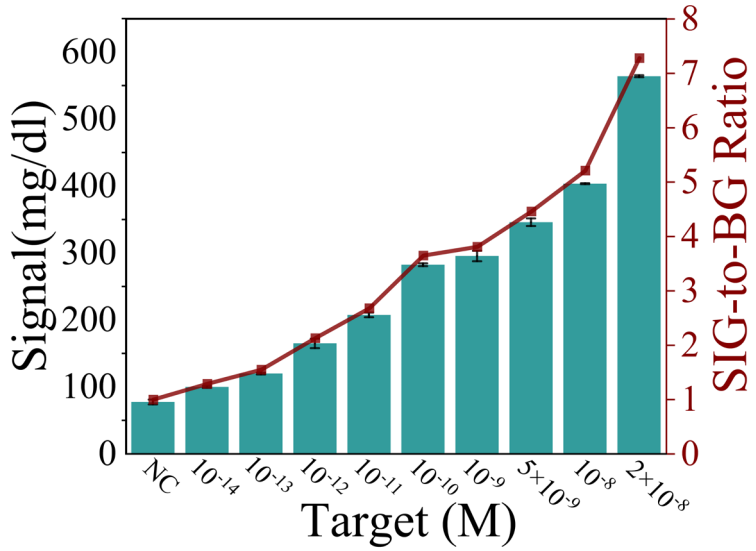

Figure S5. Glucose signal with different concentrations of miR-21 plotted at 50 min glucose generation.

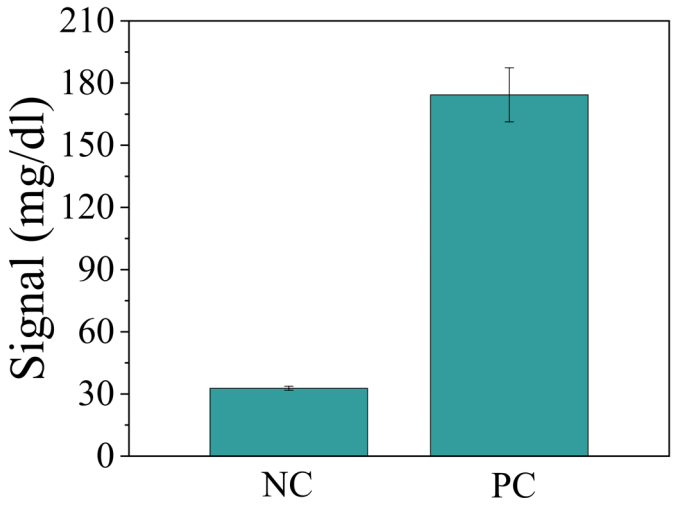

Figure S6. One-pot verification of the DSN-PGM sensing platform. Both DSN cleavage and glucose generation were carried out at 55°C. NC represents non-target in the reaction solution, while PC represents 10 nM targets (miR-21) in the reaction solution. The error bar indicates the standard deviation of three replicate experiments.

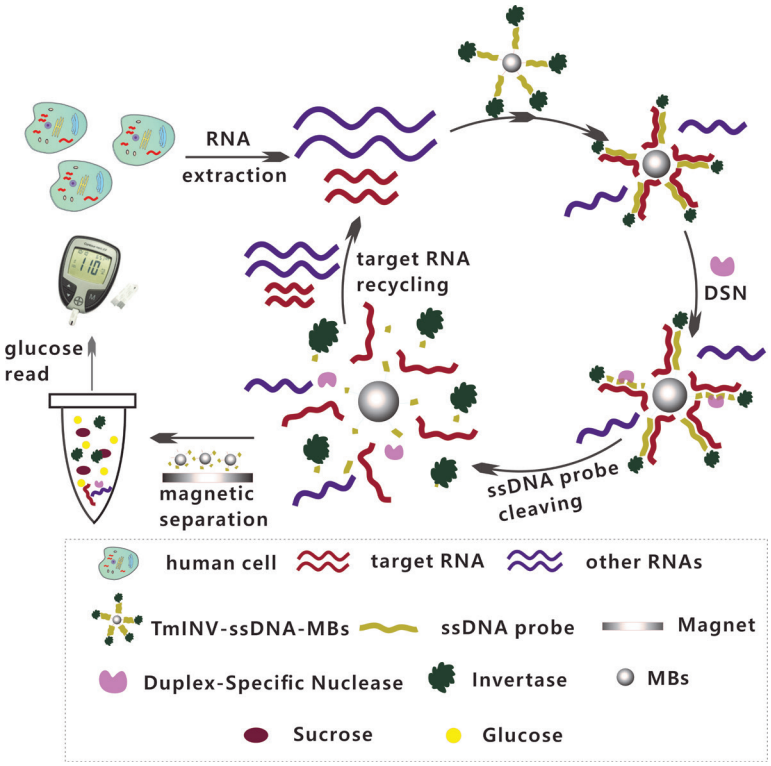

Figure S7. Schematic diagram of DSN-PGM sensing platform coupling with upstream total RNAs extraction from human cell lines.

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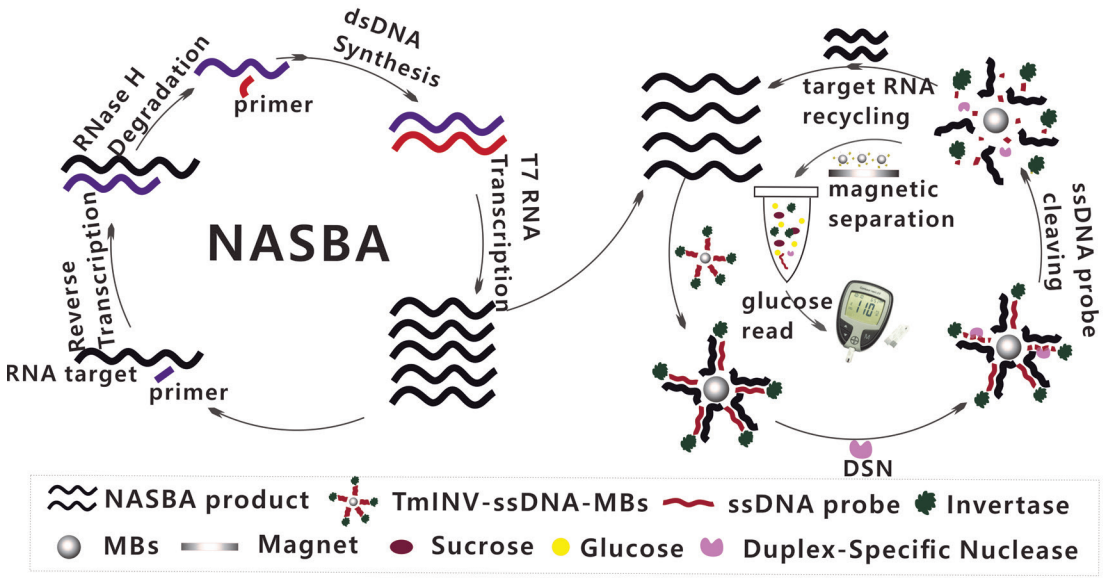

Figure S8. Scheme of NASBA-DSN-PGM sensing platform. The target RNA was amplified by NASBA amplification and then added into the standard DSN-PGM reaction system.

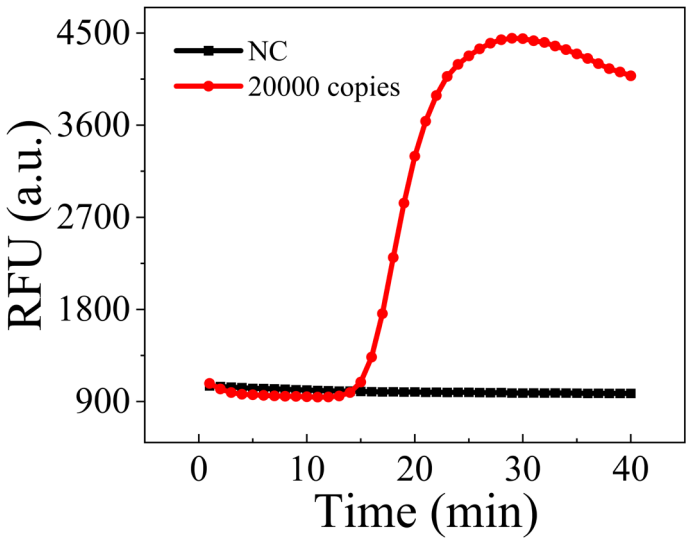

Figure S9. NASBA reaction verification with Eva Green, with (20000 copies) and without (NC) Zika RNA.

Name	Sequence (form 5'to 3')
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-429	UAAUACUGUCUGGUAAAACCGU
miR-141	UAACACUGUCUGGUAAAGAUGG
Let-7d	AGAGGUAGUAGGUUGCAUAGUU
miR-200b	UAAUACUGCCUGGUAAUGAUGA
Zika-forward primer [3.]	AATTCTAATACGACTCACTATAGGGAGAAGGGCACAGTGGGATGATCGTTA
Zika-reverse primer [3]	CCTGTCCTCGGTTCACAATCAA
Zika target RNA[3]	GGGCCAGCACAGUGGGAUGAUCGUUAAUGACACAGGACAUGAAACUGAUGA GAAUAGAGCGAAAGUUGAGAUAACGCCCAAUUCACCAAGAGCCGAAGCCACC CUGGGGGGGUUUGGAAGCCUAGGACUUGAUUGUGAACCGAGGACAGG

Name	Sequence (form 5'to 3')	Label and notes
Zika-NASBA-Tm INV-ssDNA probe	AATTAGCTTCGGCTCTTGGTGAATT	5'Biotin 3'HS C6
Zika-NASBA-FQ -ssDNA probe	TAGCTTCGGCTCTTGGTGAA	5'FAM 3'BHQ1
miR-21-FQ-ssDN A probe	TCAACATCAGTCTGATAAGCTA	5'FAM 3'BHQ1
miR-21-TmINV-s sDNA probe	TAAAATCAACATCAGTCTGATAAGCTAAAAA	5'Biotin 3'HS C6

Table S1: Oligonucleotides used in this paper. All labeled sequences were purified with high-pressure liquid chromatography. All unlabeled sequences were polyacrylamide gel electrophoresis self-purified.

Reference:

1. Y. Du., R. A. Hughes., S. Bhadra., Y. S. Jiang., A. D. Ellington., B. Li., *Sci. Rep*. 2015. **5**, 11039.
2. L. L. Guo., B. Y. Lu., Q. Dong., Y. D. Tang., Y. Du., B. Li., *Anal Chim Acta*. 2020. **1106**, 191-198.
3. K. Pardee., A. A. Green., M. K. Takahashi., D. Braff., G. Lambert., J. W. Lee., J. J. Collins., *Cell*. 2016. **165**(5), 1255–1266.