Supporting Information for:

Target MicroRNA-Responsive DNA Hydrogel-Based SERS Sensor Arrays for MicroRNAs-Marked Cancer Screening

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Materials and Reagents.

N, N, N´, N´-tetramethylethylenediamine (TEMED), ammonium persulfate (APS) and acrylamide were purchased from J&K Scientific Ltd. (Beijing, China). Acrylic-DNA and all other oligonucleotides used in this work (Table S1-S2) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

Size Information for Special Quartz Sheet. The length, width and thickness of the quartz sheet were 76.2 mm, 26.4 mm, and 5.0 mm, respectively. The front side of the quartz sheet was uniformly distributed with 10 square sample grooves having a side length of 11.5 mm and a depth of 1.0 mm. Nine cylindrical detection grooves having a diameter of 2.5 mm and a depth of 2 mm were uniformly distributed in each square groove. The real photo of the special quartz sheet was shown in Figure S1.

Synthesis of the Polymer Strands (PS-I and PS-II). [1] An 8-μL volume of 25% acrylamide was first mixed with 24 μL of buffer solution, and then added into a 16-μL volume of 3 mM Acrydite-DNA (Strands-I or Strands-II in Table S1) solution. The mixture was kept in a vacuum drier for 10 min to remove the air. Then, 2 μL of 5% APS and 2 μL of 5% TEMED were added into the above solution. Next, the solution was immediately incubated in the vacuum drier for 18 min, and then the polymer strands of PS-I and PS-II were obtained and stored at 4 ℃ for further usage.

SERS Detection Parameters. For each SERS spectrum, the SERS signal was collected with a 633 nm laser, a 5× (NA 0.45) microscope objective with a long working distance, 2 mW laser output power, and 6 s acquisition time. For each SERS mapping image, the SERS signal was collected with a 633 nm laser, a 5× (NA 0.45) microscope objective with a long working distance, 2 mW laser output power, and 1 s acquisition time, Raman images are displayed in pseudocolor.

Table S1. Oligonucleotide sequences used in gel electrophoresis.

Substrate-link (Sub-L)	5’-CTACAACCACACATATCGAACTCACTATrAGGAAGAGAT GATGCACAACTACCAAACAG-3’
T21	5’-TAGCTTATCAGACTGATGTTGA-3’
T21-1	5’-AGCTTATCAGACTGATGTTG-3’
T21-2	5’-GCTTATCAGACTGATGTT-3’
T21-3	5’-CTTATCAGACTGATGT-3’
EA21	5’-TAGCTTCAACATCAGTCGGTCGAAATAGTGAGTTCGA-3’
EB21	5’-GCATCATCTCTTCTCCGAGCCTGATAAGCTA-3’
T221	5’-AGCTACATTGTCTGCTGGGTTTC-3’
T221-1	5’-GCTACATTGTCTGCTGGGTTT-3’
T221-2	5’-CTACATTGTCTGCTGGGTT-3’
T221-3	5’-TACATTGTCTGCTGGGT-3’
EA221	5’- AGCTA GAAACCCAGCA CGGTCGAA ATAGTGAGTTCGA-3’
EB221	5’-GCATCATCTCTTCTCCGAGC GACAATGTAGCT-3’
T141	5’-TAACACTGTCTGGTAAAGATGG -3’
T141-1	5’-AACACTGTCTGGTAAAGATG -3’
T141-2	5’-ACACTGTCTGGTAAAGAT -3’
T141-3	5’-CACTGTCTGGTAAAGA -3’
EA141	5’- TAACA CCAT CTTTACC CGGTCGAA ATAGTGAGTTCGA-3’
EB141	5’-GCATCATCTCTTCTCCGAGC AGACAGTGTTA-3’
T183	5’-TATGGCACTGGTAGAATTCACT-3’
T183-1	5’-ATGGCACTGGTAGAATTCAC-3’
T183-2	5’-TGGCACTGGTAGAATTCA-3’
T183-3	5’-GGCACTGGTAGAATTC-3’
EA183	5’- TATGG AGTGAATTCTA CGGTCGAA ATAGTGAGTTCGA-3’
EB183	5’-GCATCATCTCTTCTCCGAGCCCAGTGCCATA-3’
T25	5'-CATTGCACTTGTCTCGGTCTGA-3'

T25-1	5'-ATTGCACTTGTCTCGGTCTG-3'
T25-2	5'-TTGCACTTGTCTCGGTCT-3'
T25-3	5'-TGCACTTGTCTCGGTC-3'
EA25	5’- CATTG TCAGACCGAGA CGGTCGAA ATAGTGAGTTCGA-3’
EB25	5’-GCATCATCTCTTCTCCGAGCCAAGTGCAATG-3’
T18	5’-TAAGGTGCATCTAGTGCAGATAG-3’
T18-1	5’-AAGGTGCATCTAGTGCAGATA-3’
T18-2	5’-AGGTGCATCTAGTGCAGAT-3’
T18-3	5’-GGTGCATCTAGTGCAGA-3’
EA18	5’- TAAGG CTATCTGCACTA CGGTCGAA ATAGTGAGTTCGA-3’
EB18	5’-GCATCATCTCTTCTCCGAGCGATGCACCTTA-3’
T155-1	5’-TAATGCTAATCGTGATAGGGGT-3’
T155-2	5’-AATGCTAATCGTGATAGGGG-3’
T155-3	5’-ATGCTAATCGTGATAGGG-3’
T155-4	5’-TGCTAATCGTGATAGG-3’
EA155	5’- TTAAT AACCCC TATCAC CGGTCGAA ATAGTGAGTTCGA-3’
EB155	5’-GCATCATCTCTTCTCCGAGC GATTAGCATTAA-3’
T224-1	5’-CAAGTCACTAGTGGTTCCGTTTA-3’
T224-2	5’-AAGTCACTAGTGGTTCCGTTT-3’
T224-3	5’-AGTCACTAGTGGTTCCGTT-3’
T224-4	5’-GTCACTAGTGGTTCCGT-3’
EA224	5’- TCAAGCTAAACGGAACCA CGGTCGAA ATAGTGAGTTCGA-3’
EB224	5’-GCATCATCTCTTCTCCGAGC CTAGTGACTTGA-3’
T205	5’-TCCTTCATTCCACCGGAGTCTG-3’
T205-1	5‘-CCTTCATTCCACCGGAGTCT-3’
T205-2	5‘-CTTCATTCCACCGGAGTC-3’
T205-3	5‘-TTCATTCCACCGGAGT-3’
EA205	5’- TCCTT CAGACTCCGGT CGGTCGAA ATAGTGAGTTCGA-3’
EB205	5’-GCATCATCTCTTCTCCGAGC GGAATGAAGGA-3’

Table S2. Oligonucleotide sequences used in SERS sensing platform.

Substrate-link	5'-CTACAACCACACATATCGAACTCACTATrAGGAAGAGA TGATGCACAACTACCAAACAG-3'
Strands-I	5'- Acrydite- ATGTGTGGTTGTAG-3'
Strands-II	5'- Acrydite- CTGTTTGGTAGTTG-3'
miR-21	5'-UAGCUUAUCAGACUGAUGUUGA-3'
miR-155	5'-UUAAUGCUAAUCGUGAUAGGGGUU-3'
miR-221	5'-AGCUACAUUGUCUGCUGGGUUUC-3'
miR-205	5'-UCCUUCAUUCCACCGGAGUCUG-3'
miR-141	5'-UAACACUGUCUGGUAAAGAUGG -3'
miR-224	5'-UCAAGUCACUAGUGGUUCCGUUUAG-3'
miR-18	5'-UAAGGUGCAUCUAGUGCAGAUAG-3'
miR-25	5'-CAUUGCACUUGUCUCGGUCUGA-3'
miR-183	5'-UAUGGCACUGGUAGAAUUCACU-3'
miR-21DNA-1	5'-TAGCTTATCAGACTGA**A**GTTGA-3'
miR-21DNA-2	5'-TAGCTTATCAGACTGA**A**GTT**C**A-3'
miR-21DNA-3	5'-TAGCTTATCAGACTG**GA**GTT**C**A-3'
EA-21	5'-AAGCTACAACATCAGTCGGTCGAAATAGTGAGTTCGA-3'
EB-21	5'-GCATCATCTCTTCTCCGAGCCTGATAAGCTT-3'
EA-155	5'- AGTATGTACCCTATCACCGGTCGAAATAGTGAGTTCGA-3'
EB-155	5'-GCATCATCTCTTCTCCGAGC GATTAGCATACT-3'
EA-221	5'- TACTA ATAACCCAGCA CGGTCGAA ATAGTGAGTTCGA-3'
EB-221	5'-GCATCATCTCTTCTCCGAGC GACAATGTAGTA-3'
EA-205	5'- ACCTT TAGACTCCGGT CGGTCGAA ATAGTGAGTTCGA-3'
EB-205	5'-GCATCATCTCTTCTCCGAGC GGAATGAAGGT-3'
EA-141	5'- CAACA ACAT CTTTACC CGGTCGAA ATAGTGAGTTCGA-3'
EB-141	5'-GCATCATCTCTTCTCCGAGC AGACAGTGTTG-3'
EA-224	5'-GATAGTACAACGGAACCACGGTCGAA ATAGTGAGTTCGA-3'

EB-224	5’-GCATCATCTCTTCTCCGAGC CTAGTGACTATC-3’
EA-18	5’- AGAGG TAATCTGCACTA CGGTCGAA ATAGTGAGTTCGA-3’
EB-18	5’-GCATCATCTCTTCTCCGAGCGATGCACCTCT-3’
EA-25	5’- ACTTG CTAGACCGAGA CGGTCGAA ATAGTGAGTTCGA-3’
EB-25	5’-GCATCATCTCTTCTCCGAGCCAAGTGCAAGT-3’
EA-183	5’- AGTGG TATGAATTCTA CGGTCGAA ATAGTGAGTTCGA-3’
EB-183	5’-GCATCATCTCTTCTCCGAGCCCAGTGCCACT-3’

Figure S1. Photo of the special quartz sheet.

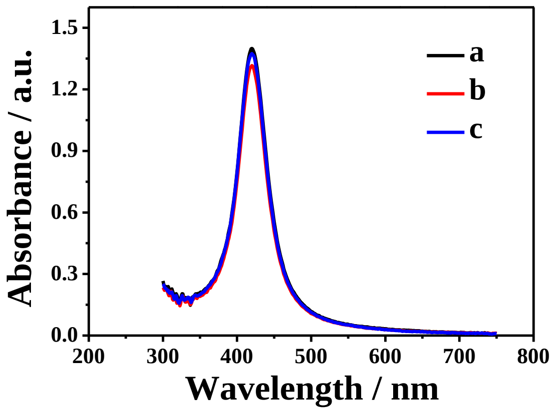

Figure S2. UV−Vis spectra of (a) AuAgNPs, (b) MPBN functionalized AuAgNPs, and (c) SERS tags.

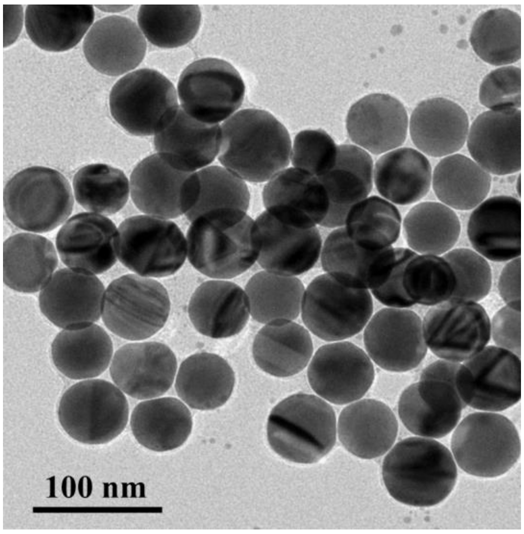

Figure S3. TEM image of AuAgNPs (~50 nm, Au/Ag=1/5).

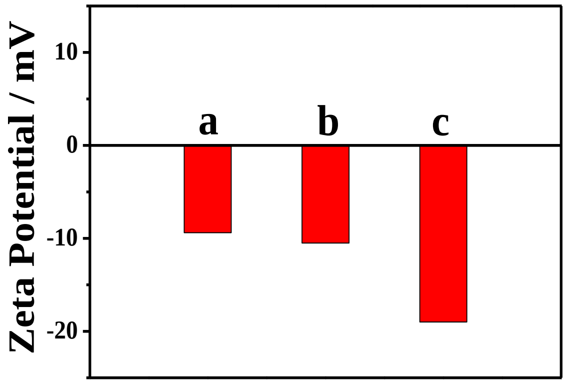

Figure S4. Zeta potential of (a) AuAgNPs, (b) AuAgNPs + MPBN, and (c) AuAgNPs + MPBN + Bio-PP.

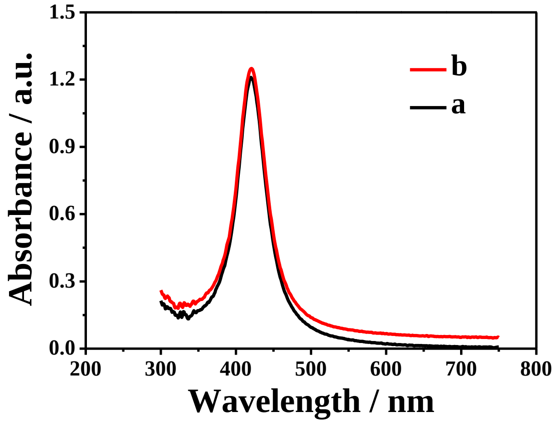

Figure S5. UV−vis spectra of (a) SERS tags, (b) SERS tags + Pb^{2+}.

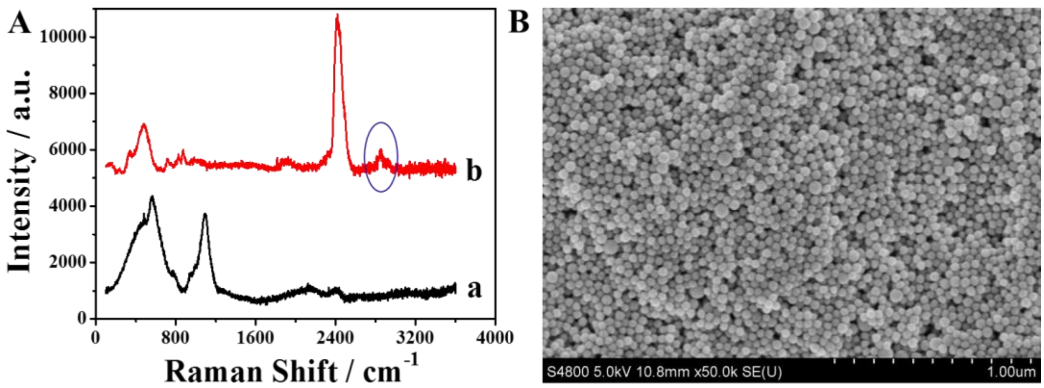

Figure S6. (A) Raman spectra of (a) the bare detection well's bottom surface, (b) APTES treated detection well's bottom surface. (B) SEM image of the detection well's bottom surface after aminosilylation and treated with the AuAgNPs.

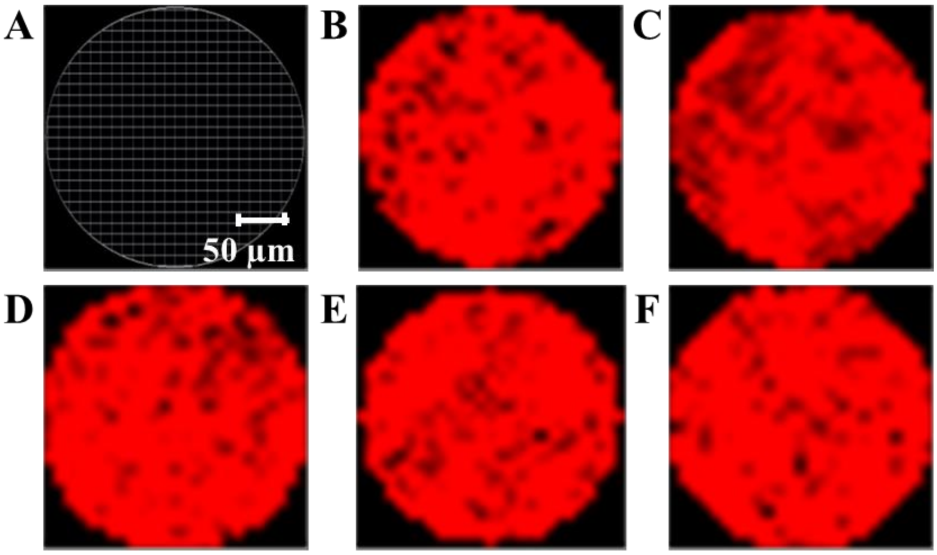

Figure S7. (A) The range of SERS mapping images. (B-F) SERS mapping images of five DNA hydrogel-based sensors (acquired at a peak intensity of 2214 cm^{-1}).

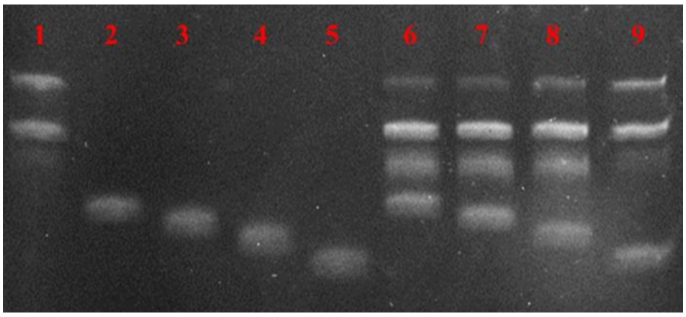

Figure S8. Gel image for investigating the effect of the recognition sequence length (miR-21 sensor) on the cleavage reaction. 1: Sub-L+EA21+EB21; 2: T21; 3: T21-1; 4: T21-2; 5: T21-3; 6: Sub-L+EA21+EB21+T21; 7: Sub-L+EA21+EB21+T21-1; 8: Sub-L+EA21+EB21+T21-2; 9: Sub-L+EA21+EB21+T21-3.

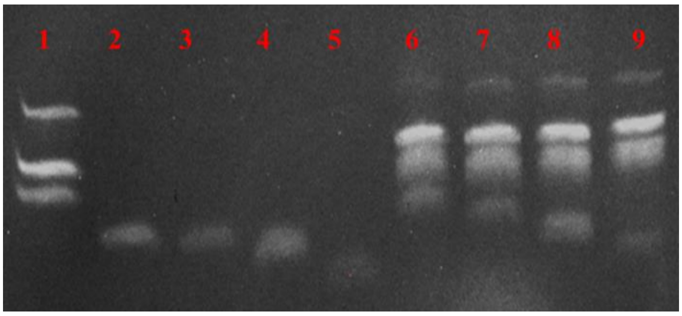

Figure S9. Gel image for investigating the effect of the recognition sequence length (miR-221 sensor) on the cleavage reaction. 1: Sub-L+EA221+EB221; 2: T221; 3: T221-1; 4: T221-2; 5: T221-3; 6: Sub-L+EA221+EB221+T221; 7: Sub-L+EA221+EB221+T221-1; 8: Sub-L+EA221+ EB221+T221-2; 9: Sub-L+EA221+EB221+T221-3.

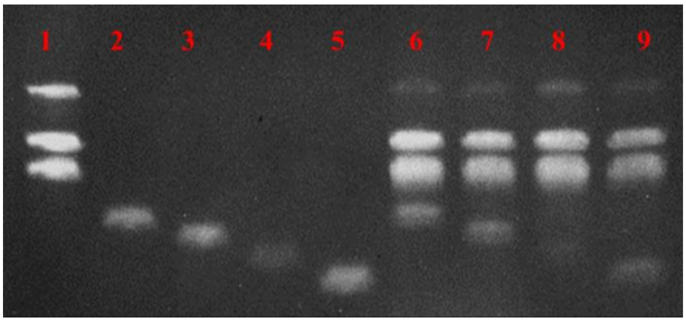

Figure S10. Gel image for investigating the effect of the recognition sequence length (miR-141 sensor) on the cleavage reaction. 1: Sub-L+EA141+EB141; 2: T141; 3: T141-1; 4: T141-2; 5: T141-3; 6: Sub-L+EA141+ EB141+T141; 7: Sub+EA141+EB141+T141-1; 8: Sub-L+EA141+ EB141+T141-2; 9: Sub-L+EA141+EB141+T141-3.

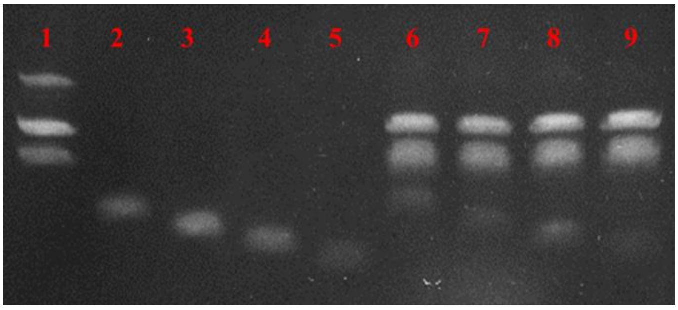

Figure S11. Gel image for investigating the effect of the recognition sequence length (miR-183 sensor) on the cleavage reaction. 1: Sub-L+EA183+ EB183; 2: T183; 3: T183-1; 4: T183-2; 5: T183-3; 6: Sub-L+EA183+EB183+T183; 7: Sub-L+EA183+EB183+T183-1; 8: Sub-L+EA183+ EB183+T183-2; 9: Sub-L+EA183+EB183+T183-3。

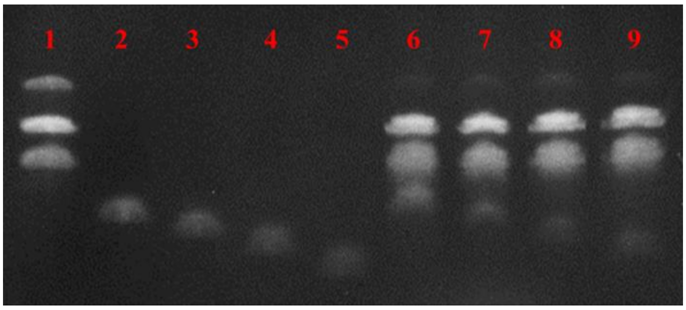

Figure S12. Gel image for investigating the effect of the recognition sequence length (miR-25 sensor) on the cleavage reaction. 1: Sub-L+EA25+ EB25; 2: T25; 3: T25-1; 4: T25-2; 5: T25-3; 6: Sub-L+EA25+ EB25+T25; 7: Sub-L+EA25+EB25+T25-1; 8: Sub-L+EA25+EB25+ T25-2; 9: Sub-L+EA25+EB25+ T25-3.

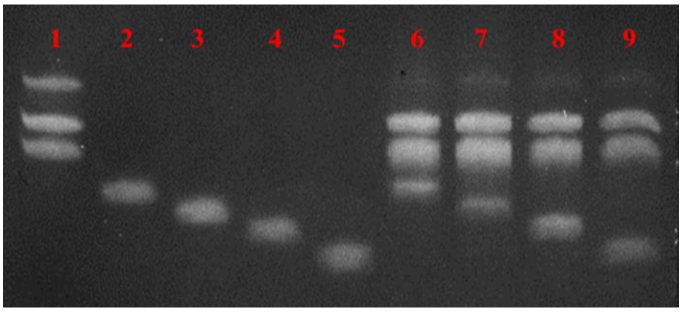

Figure S13. Gel image for investigating the effect of the recognition sequence length (miR-18 sensor) on the cleavage reaction. 1: Sub-L+EA18+ EB18; 2: T18; 3: T18-1; 4: T18-2; 5: T18-3; 6: Sub-L+EA18+EB18+T18; 7: Sub-L+EA18+EB18+T18-1; 8: Sub-L+EA18+EB18+T18-2; 9: Sub-L+EA18+EB18+T18-3.

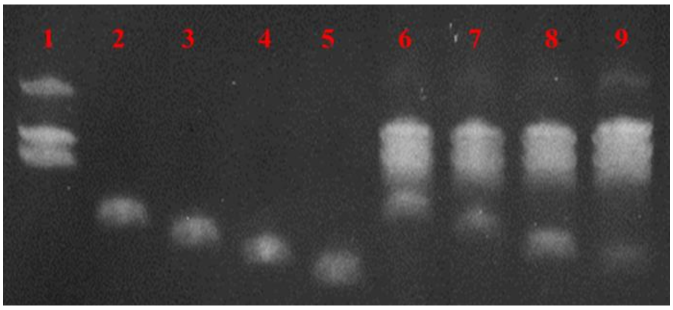

Figure S14. Gel image for investigating the effect of the recognition sequence length (miR-155 sensor) on the cleavage reaction. 1: Sub-L+EA155+EB155; 2: T155-1; 3: T155-2; 4: T155-3; 5: T155-4; 6: Sub-L+EA155+EB155+T155-1; 7: Sub-L+EA155+EB155+T155-2; 8: Sub-L+EA155 +EB155+T155-3; 9: Sub-L+EA155+EB155+T155-4.

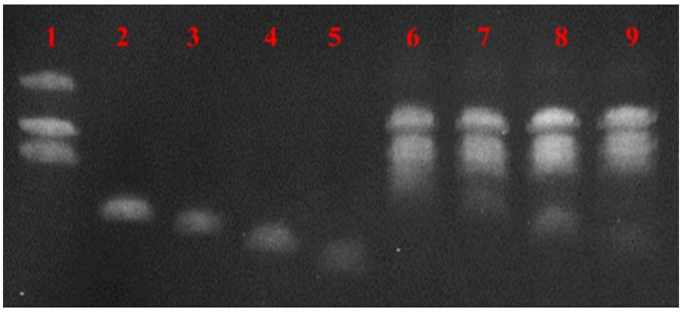

Figure S15. Gel image for investigating the effect of the recognition sequence length (miR-224 sensor) on the cleavage reaction. 1: Sub-L+EA224+ EB224; 2: T224-1; 3: T224-2; 4: T224-3; 5: T224-4; 6: Sub-L+EA224+EB224+T224-1; 7: Sub-L+EA224+EB224+T224-2; 8: Sub-L+EA224 +EB224+T224-3; 9: Sub-L +EA224+EB224+T224-4.

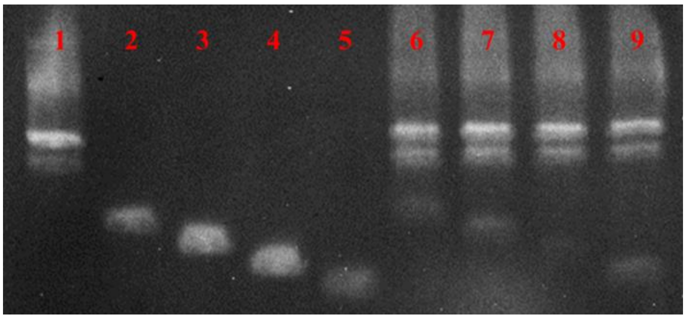

Figure S16. Gel image for investigating the effect of the recognition sequence length (miR-205 sensor) on the cleavage reaction. 1: Sub-L+EA205+ EB205; 2: T205; 3: T205-1; 4: T205-2; 5: T205-3; 6: Sub-L+EA205+EB205+T205; 7: Sub-L+EA205+EB205+T205-1; 8: Sub-L+EA205 +EB205+T205-2; 9: Sub-L+EA205+EB205+T205-3.

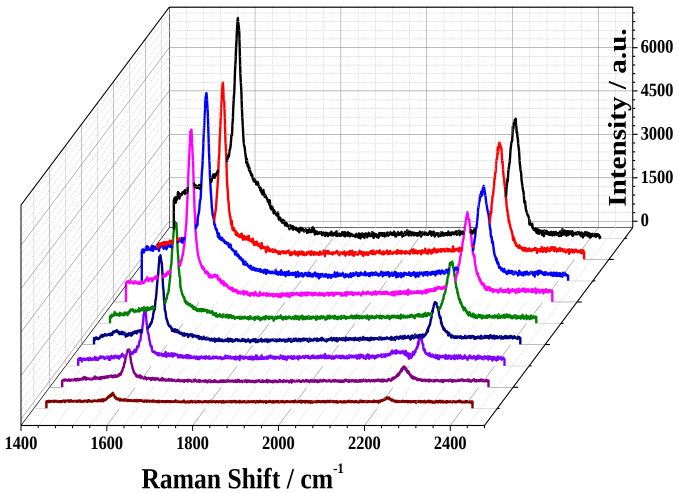

Figure S17. SERS spectra of the miR-21 sensor unit under varying concentrations of miR-21 (From brown curve to black curve: 4, 8, 15, 30, 50, 100, 300, 1200, 1600 nM).

Reference

(1) Si, Y.; Li, L.; Wang, N.; Zheng, J.; Yang, R.; Li, J. Oligonucleotide cross-linked hydrogel for recognition and quantitation of microRNAs based on a portable glucometer readout. *ACS Appl. Mater. Interface* **2019**, *11*, 7792-7799.