Supplementary Information

Spatially Resolved Single-Molecule Profiling of MicroRNAs in Migrating Cells Driven by Microconfinement

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S1 Experimental Procedures

Synthesis of 15 nm stabilized AuNPs

AuNPs of diameter ~15 nm were synthesized according to the previously reported procedure.[1] In brief, 1% (w/v) of trisodium citrate solution (3.5 mL) was rapidly added to the heated $HAuCl_4$ (100 mL), stirring for 15 min. Then, the solution was naturally cooled to room temperature, purifying with a Millipore membrane filter. Finally, the obtained red solution was stored at 4 °C.

Fabrication of the confined microdevices for HeLa cells

The cell confiners used in this work have been reported in the previous literature, constructing a biomimetic microenvironment for cell migration. The confiner was composed of structured micropillars and suction cups. Briefly, the confining micropillars were made by pouring the polydimethylsiloxane (PDMS, RTV615, GE) mixture (PDMS A/crosslinker B, 8/1, w/w) into the silicon wafer mold with sunken cylinder arrays (height: 3 μm, diameter: 440 μm) and then activated for 3 minutes in the plasma chamber. Then, after baking it on a hot plate at 95 °C for 16 minutes to solidify, gently lifted away from the confining micropillars. Additionally, the soft suction cups were made in PDMS (PDMS A/crosslinker B, 35/1, w/w) in a custom-made mold. After baking at 80 °C on a hot plate for 1.5 h, which could be unmolded easily.

To realize parallel experiments under distinct conditions, a 6-well plate confiner was constructed. The six gel-film sheets (thickness: 100 mm) were positioned in the center of the lid on cover lid of the 6-well plate. The higher PDMS suctions were tightly attached to gel-film, and bonded below were the structured micropillars. This modified cover lid contributed to confining cells. Before the cells were confined, the substates of the 6-well plate were coated with PLL-PEG (0.5 $mg \cdot mL^{-1}$ in 10 mM HEPES, pH=7.4) and the micropillars were modified with PLL-PEG (0.5 $mg \cdot mL^{-1}$ in 10 mM HEPES, pH=8.5), putting them at room temperature for 3 hours to avoid cell adhesion. Then, the cells were immersed in the cell culture medium for 30 minutes to equilibrate the residual PDMS.

S2 Supplementary Methods

Quantification of the H1 probes on each AuNPs

The capture probes on each AuNPs were quantified according to the previous method.[2-4] Beer-Lambert Law ($A = \varepsilon cb$)[5] states that when a beam of parallel monochromatic light vertically passes through a uniform non-scattering light-absorbing substance solution, its absorbance (A) is proportional to the concentration (c) and optical path (b). Relying on the above description, the detection of the concentration of H1-AuNPs in solution obeys this rule, and the calculation formula is as follows.

$$c = \frac{A_{520}}{\varepsilon_{520} b}$$

The extinction coefficient is 2.7×10^8 L mol^{-1} cm^{-1} at λ_{520}. It can be seen in Figure S1C that the UV absorption peak at 520 nm is ~0.308, and the concentration of H1-AuNPs can be calculated to be ~1.14 nM.

Before measuring the density of H1 immobilized on AuNPs, 20mM mercaptoethanol was added to the nanoprobes solution, releasing the capture probes H1 bounded to AuNPs. After overnight shaking, we collected the supernatant containing excess H1 and then measured the fluorescence intensity using a fluorescence spectrometer. The fluorescence intensity is ~524 (Figure S2). According to the calibration equation: $F = 6.099C_{H1}（nM） - 4.509$, the concentration of H1 in the solution is 86.64 nM, so ~ 76 H1 probes were fixed on each AuNPs.

S3 Supplementary Tables

The sequences used in this work were shown in the table below

Table 1. The sequences used in this work

Strand	Sequences (5'-3')
H1	SH- TTTCCATCTTT(BHQ)ACCAGACAGTGTTAGGTGTGTGTGGTAACACTGTCTGGT-Cy5
H2	AGTGTTACCACACACACCTAACACTGTCTGGTAGGTGTGTGTGG
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-30a	CUUUCAGUCGGAUGUUUGCAGC
miR-155	UUAAUGCUAAUCGUGAUAGGGGU
miR-125b	UCCCUGAGACCCUAACUUGUGA
let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU
let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU
Anti-miR-141	CCATCTTTACCAGACAGTGTTA

S4 Supplementary Figures

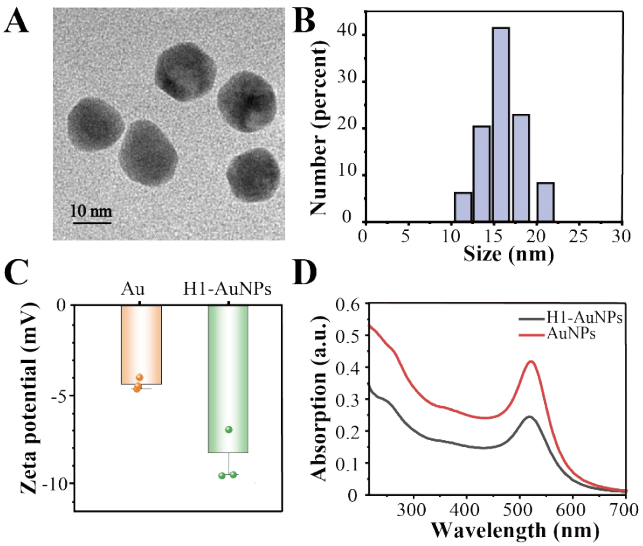

Figure S1. (A) TEM characterization of AuNPs. (B) DLS analysis of AuNPs. (C) Zeta potential analysis of AuNPs and H1-AuNPs. (D) UV absorption spectra of AuNPs and H1-AuNPs.

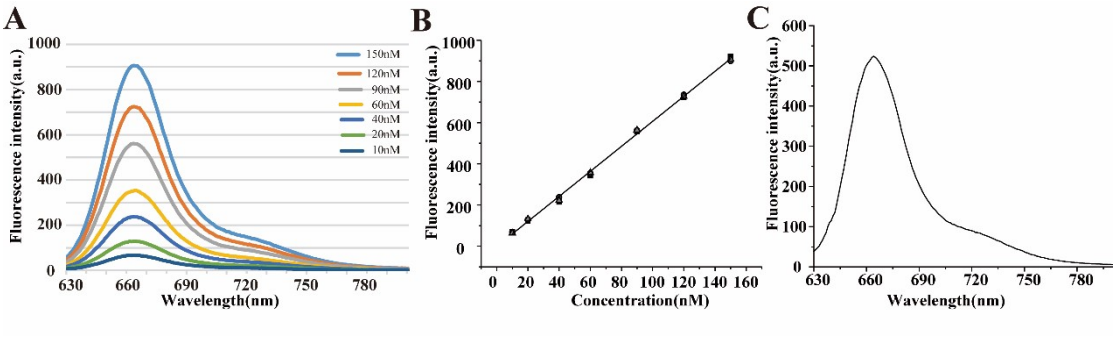

Figure S2. (A) Fluorescence spectra of different concentrations of capture probes H1 in standard solution. (B) Linear fitting diagram of fluorescence intensity and different concentrations of H1. (R^2=0.9983). (C) The fluorescence emission spectrum of the released H1.

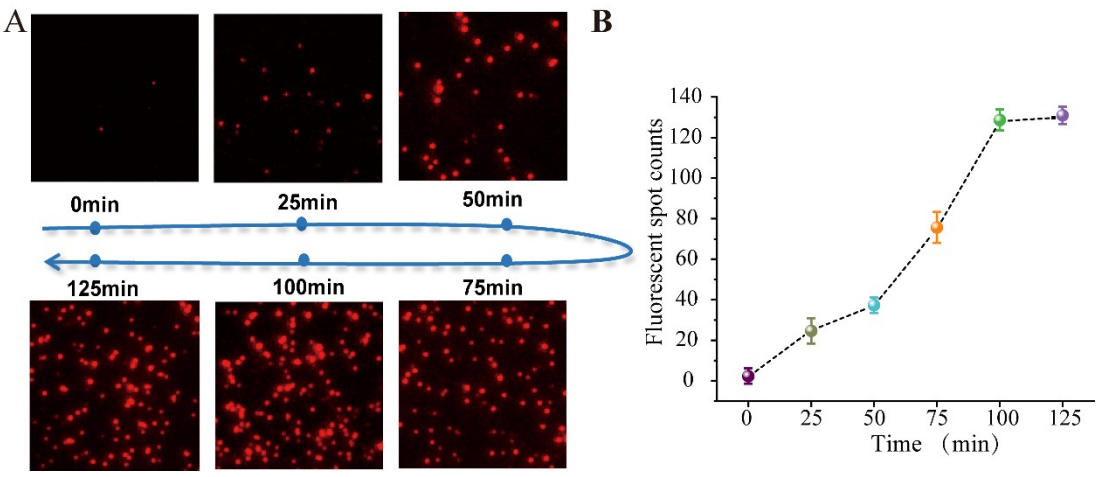

Figure S3. (A) Fluorescent spots triggered by miR-141 over time. (B) Reaction kinetics curves of miR-141 fluorescent spot counts.

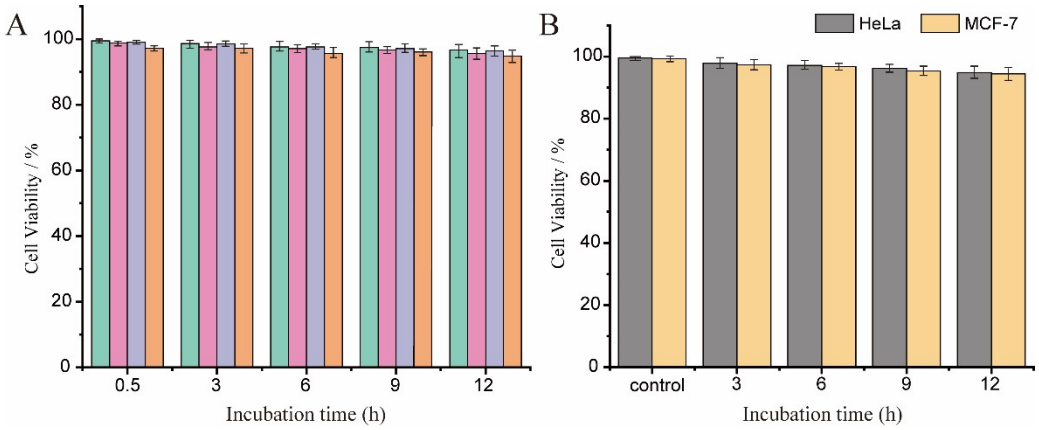

Figure S4. (A) Survival rate of HeLa cells under different conditions with various incubation time, culture medium (green), H1-AuNPs (pink), H2 (purple), H1-AuNPs and H2 (orange). (B) HeLa cells or MCF-7 cells were treated with H1-AuNPs and H2 nanoprobes at different incubation time in CCK-8.

References

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