**Supplementary Information**

Fig. S1. Optical images demonstrating the device assembly process.

Fig. S2. Optical image of a finished device.

Fig. S3. Equipment setup of the integrated microfluidic-nanoprobing platform for single-cell mRNA extraction.

Fig. S4. Standard curves used in RT-qPCR calibration.

Fig. S5. mRNA capturing efficiency at different probing voltages compared with the standard cell-lysing mRNA extraction method.

Movie S1. Trapping of 100 single HeLa cells in the ultra-thin membrane-sealed microfluidic array.

Movie S2. Extraction of mRNA molecules from a trapped single HeLa cell using the modified AFM nanoprobe via dielectrophoresis.
The optical images showing the assembly process of the ultra-thin PDMS membrane-sealed single-cell array (Fig. S1), and a finished device (Fig. S2).

**Fig. S1. Optical images demonstrating the device assembly process.** (A) The 1-μm-thick PDMS membrane was spin-coated on the 3-inch silicon wafer. (B) PDMS slab was bonded to the membrane. (C) The membrane-bonded PDMS device was peeled off from the silicon wafer.

**Fig. S2. Optical image of a finished device.** The microfluidic single-cell trapping array was sealed by a 1-μm-thick PDMS membrane, and bonded to a glass slide with a through-hole to facilitate the penetration of the DENT probe.
Mechanically cut the Au-coated tip-end to expose the inner Si core

Firstly, the Au-coated tip was placed in contact with the surface of a piece of Si₃N₄ wafer fixed on top of the AFM piezo scanner, with a contact force (set point) of 30 pN and a contact spot very close (≤ 90 µm) to the edge of the wafer. Secondly, the Si₃N₄ wafer was displaced away from its original position by controlling the AFM piezo scanner to move for 90 µm, so that the tip was no longer in contact with the wafer surface but was hanging in the air. This resulted in the relaxation of the AFM probe with no bending, thus the tip-end was slightly below the wafer surface. In the final step, the wafer was moved back to its original position, during which the tip-end hit the edge of the wafer and was chopped along the wafer surface, exposing the inner silicon core. As we could control the amount of the initial probe bending by controlling the contact force (set point), the relative height difference between the tip-end and the wafer surface after probe relaxation was also precisely controlled. We could, therefore, achieve relatively consistent exposure of the inner silicon core with a width ranging from 200 nm to 400 nm.

Details of the apparatus

The DENT probe was fixed on the probe holder with electrical connection during mRNA capturing (Fig. S3A). The inner Si core was connected to the silver-painted electrode, and the outer Au layer was connected to a thin piece of copper electrode via a spring contact. This copper electrode was connected with the AC power supply, and it also helped to fasten the DENT probe on the holder. The detailed setup of the integrated microfluidic-nanoprobing platform is shown in Fig. S3B. The apparatus was built upon an inverted microscope (Olympus IX71), equipped with an add-on upright imaging system consisted of a 40x lens tube with motorized zoom/focus function and a USB camera (1). The stepper motor (2) controlled the DENT probe fixed on the probe holder (3) to move along z direction for penetration. The microfluidic chip was placed on a 3D-printed
adapting stage (4) attached to the optically encoded x-y translational stage (5), which was controlled by the micromotor (6). The inverted image could be captured by the CCD camera (7). A LabVIEW controlled graphic user interface was also developed to facilitate operation.

**Fig. S3.** Equipment setup of the integrated microfluidic-nanoprobing platform for single-cell **mRNA extraction.** (A) The DENT probe fixed on the probe holder with electrical connection. (B) Microscopic setup during single-cell mRNA probing.
The absolute number of extracted mRNAs under an applied voltage from 1.1 to 1.9 V<sub>pp</sub> was calculated based on the Ct values of the RT-qPCR experiment and the standard curves (Fig. S4), and the mRNA capturing efficiency at different probing voltages was compared with the standard cell-lysing mRNA extraction method (Fig. S5).

**Fig. S4. Standard curves used in RT-qPCR calibration.** The standard curves used to calibrate the absolute number of captured mRNA molecules for ACTB (A), GAPDH (B), and HPRT (C) were made using synthetic oligomers with the sequences of target genes’ amplicons diluted in series as templates.
Fig. S5. mRNA capturing efficiency at different probing voltages compared with the standard cell-lysing mRNA extraction method. mRNAs extracted from bulk cell lysates using the cell-lysing RNA extraction kit (ISOLATE II RNA Mini Kit, Bioline) were diluted to a concentration of 10 cells’ mRNA molecules per 5 μL distilled water, and were quantified following the same RT-qPCR process to derive the single-cell Ct values. The calculated ΔCt values between the readings at different voltages and the averaged single-cell readings derived from cell lysates were used to calculate the capturing efficiency for the three housekeeping genes: ACTB (A), GAPDH (B), and HPRT (C).