Supporting Information

Analysis of Cholesterol Induced Mechanical Modulation of Cell Membrane by Nanopipette-Generated Fluid Flow

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Experimental Section

Materials and Reagents

All chemicals and reagents were of analytical grade. Cholesterol, dehydroergosterol (DHE, Ex/Em = 324/375 nm), sodium chloride, chloroform, sodium hypochlorite solution, sodium dihydrogen phosphate, and disodium hydrogen phosphate were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). HeLa cells were obtained from Fuheng Cell Center (Shanghai, China). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA solution, penicillin-streptomycin (PS), and phosphate-buffered saline (PBS) were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The Calcein/PI Cell Viability/Cytotoxicity Assay Kit and 4% paraformaldehyde (PFA) solution were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). A silver wire (0.25 mm) used for preparing the Ag/AgCl electrode was obtained from Alfa Aesar. Quartz capillaries (O.D.: 1.0 mm, I.D.: 0.5 mm, 7.5 cm in length), the raw material for fabricating nanopipettes, were purchased from Sutter Instrument. All solutions were prepared using ultrapure water (18.2 MΩ·cm) obtained from a Milli-Q purification system (Millipore).

Apparatus

SEM characterization of nanopipettes was performed using a field-emission scanning electron microscope (ZEISS GeminiSEM 500). An inverted microscope (Eclipse TiU, Nikon, Japan) was used to obtain bright-field images of nanopipettes and cells. A laser scanning confocal microscope (Leica DMI 8, Leica, Germany) was used to obtain fluorescence images of cells. An MP-285 micromanipulator (Sutter Instrument, Novato, CA) was employed for precise positioning and control of nanoelectrodes. Electrochemical measurements and electrical signal acquisition were carried out using an Axopatch 200B low-noise amplifier and an Axon Digidata 1550A low-noise data acquisition system (Molecular Devices, Sunnyvale, CA) mounted on a holder (Axon Instruments, Union City, CA). A P-2000 laser puller (Sutter Instrument, Novato, CA) was used for fabrication of the nanopipettes. All reagents were weighed using an analytical balance (ME 104, METTLER TOLEDO).

Cell Culture

HeLa cells were cultured at 37℃ in a humidified atmosphere consisting of 5% CO_2/95% air using DMEM with 10% FBS, 100 μg mL^{-1} penicillin and 100 μg mL^{-1} streptomycin. When the cell density reached 80%, an appropriate number of cells were transferred into culture dishes and incubated overnight. After cell attachment, experiments were performed.

Preparation of Cholesterol Solutions

A cholesterol stock solution was prepared by dissolving 0.0077 g of cholesterol in 1 mL of chloroform to obtain a 20 mM solution. The stock solution was diluted with PBS to prepare a 200 μM working solution. For cell treatment, the working solution was further diluted with DMEM to achieve final cholesterol concentrations of 0, 2, 20, and 40 μM, respectively.

DHE Labeling and Imaging

A DHE stock solution was prepared by dissolving 1 mg of DHE in 1 mL of chloroform to obtain a 2.5 mM solution. The stock solution was diluted with PBS to prepare a 250 μM working solution. For cell treatment, the working solution was further diluted with DMEM to obtain final DHE concentrations of 0, 2, 20, and 40 μM, which were used to incubate HeLa cells for 20 min. The cells were then observed under a confocal fluorescence microscope.

Electrode Preparation

The electrodes described in the experiments were Ag/AgCl. Silver wires were cut to appropriate lengths and subsequently immersed in a sodium hypochlorite solution for 30 min. This treatment resulted in the formation of a uniform and compact silver chloride layer on the wire surface, exhibiting a characteristic dark gray appearance.

Preparation of Nanopipettes

Quartz nanopipettes were fabricated from quartz capillaries using a P-2000 laser puller (Sutter Instrument). After preheating the instrument, a two-step pulling protocol was applied with the following parameters: Line 1: Heat 680, Fil 3, Vel 30, Del 130, Pul 60; Line 2: Heat 700, Fil 4, Vel 30, Del 132, Pul 110. The capillary was positioned and aligned with the laser beam path at the center of the pulling chamber, and the program was executed to produce a pair of nanopipettes. The nanopipettes were backfilled with 2 mM PBS using a microloader. Briefly, about 10 μL of buffer was loaded into the microloader, which was gently inserted into the rear end of the nanopipette. The solution was dispensed slowly to minimize bubble formation, and the microloader was then withdrawn carefully. To ensure complete filling and remove any trapped air, the nanopipettes were centrifuged at 5000 rpm for 2 min prior to use.

Characterization of Nanopipettes

The glass nanopipettes were characterized by scanning electron microscopy (SEM). The nanopipette samples were mounted on aluminum stubs using conductive adhesive tape and coated with a thin layer of platinum to enhance surface conductivity. The rectification behavior of the nanopipette was characterized by recording its current-voltage (I-V) response. The voltage was applied to the nanopipette by uniformly increasing from -1000 mV to 1000 mV, and the corresponding current was recorded to plot the *I-V* curve. Both the nanopipette and the external bath were filled with 2 mM PBS. The *I-V* curves were measured for a total duration of 40 minutes.

Calcein-AM/PI Staining Assay

The culture medium was removed, and the cells were gently washed twice with PBS. They were then stained with 2 μM Calcein-AM (Ex/Em = 490/515 nm) for 20 min at 37℃, followed by incubation with 4.5 μM PI (Ex/Em = 535/617 nm) for 5 min at 37C. After staining, the samples were rinsed again with PBS and observed under a fluorescence microscope for 20 min.

Cell Fixation

Cells were rinsed with PBS and fixed with 4% (w/v) paraformaldehyde (PFA) in PBS for 15 min at room temperature. After fixation, the cells were washed three times with PBS to remove residual PFA.

Nanopipette-Based Electrochemical Measurement of Single Living Cells

An Ag/AgCl wire inserted into the back end of the PBS-filled nanopipette served as the working electrode, and a second Ag/AgCl wire immersed in the bath solution served as the reference electrode. The nanopipette was mounted on a micromanipulator and connected to an Axopatch 200B amplifier interfaced with a PC. Data acquisition and experimental control were performed using pCLAMP 10.7 (gap-free mode). Ionic current signals were low-pass filtered at 5 kHz and sampled at 100 kHz. For positioning, the nanopipette tip was first brought into the center of the microscope field of view by adjusting the x-, y-, and z-axes. The x- and y-axes were then fixed, and the z-axis was adjusted to set the tip at a visible reference height. A cell culture dish was placed on the microscope stage, and a single cell was selected as the target and centered in the field of view by moving

the stage. The nanopipette was subsequently lowered until the tip was immersed in the bath solution. The micromanipulator was switched to a low-torque mode, and a bias voltage of 50 mV was applied to minimize tip contamination. While monitoring the ionic current in real time, the pipette was advanced toward the cell membrane along the z-axis. A sudden drop in ionic current was taken as the electrical indicator of gentle tip-membrane contact. The z-motion was immediately stopped upon detecting this current decrease, and the current-time (I-t) traces associated with membrane oscillations were recorded. The same approach procedure and stopping criterion were applied for all measurements to ensure consistent tip-membrane positioning across experiments. All measurements were performed at room temperature. Data processing was carried out using Clampfit and MATLAB.

Supplementary Note 1.

The calculation formula of rectification ratio was defined as:

$$Rectification\ ratio = \frac{|I_{-1V}|}{I_{+1V}}$$

$|I_{-1\,V}|$ is the absolute value of the current under -1 V and $I_{+1\,V}$ is the current under +1 V.

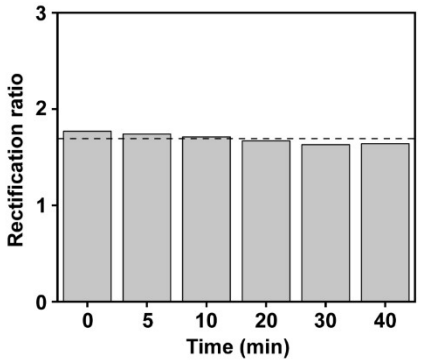

Fig. S1 Rectification ratios corresponding to the *I-V* curves in Figure 2c measured by a typical nanopipette placed using PBS (2 mM).

Supplementary Note 2.

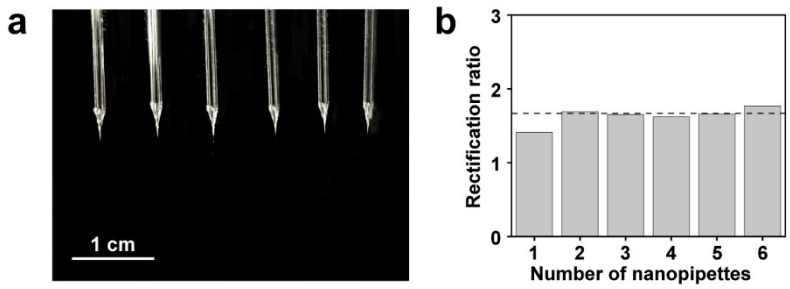

Fig. S2 (a) Photograph of fabricated nanopipettes. (b) Rectification ratio of six individual nanopipettes.

Supplementary Note 3.

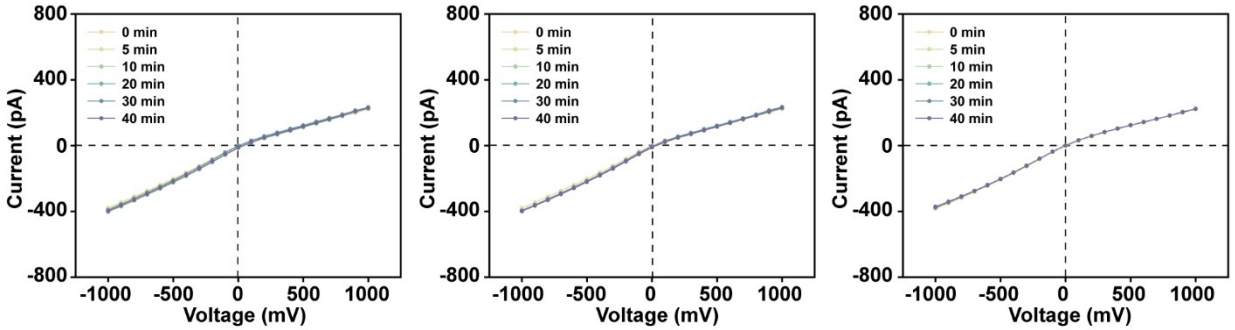

Fig. S3 *I-V* curves of three additional nanopipettes (measured in 2 mM PBS) recorded at 0, 5, 10, 20, 30, and 40 min.

Supplementary Note 4.

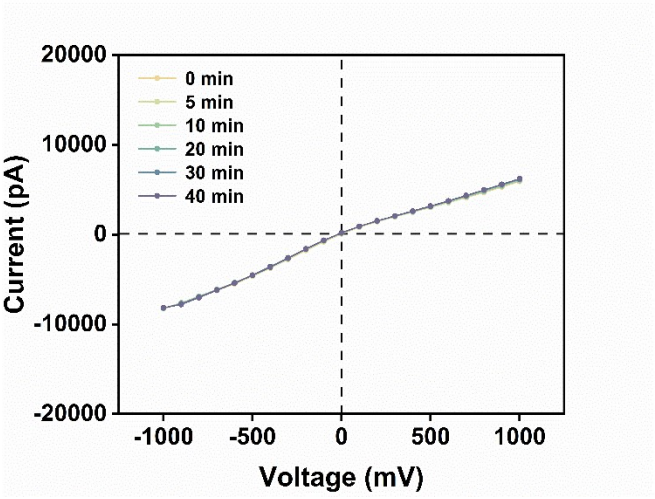

Fig.S4 I-V curves of a typical nanopipette placed in DMEM at different times (0 min, 5 min, 10 min, 20 min, 30 min, and 40 min).

Supplementary Note 5.

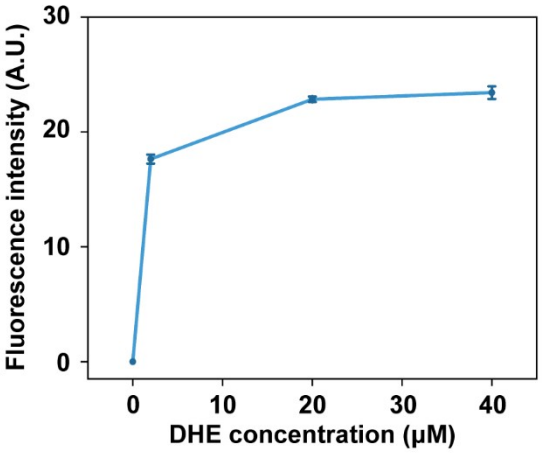

Fig. S5 Quantification of membrane-associated DHE fluorescence intensity. The error bars came from 3 parallel measurements.

Supplementary Note 6.

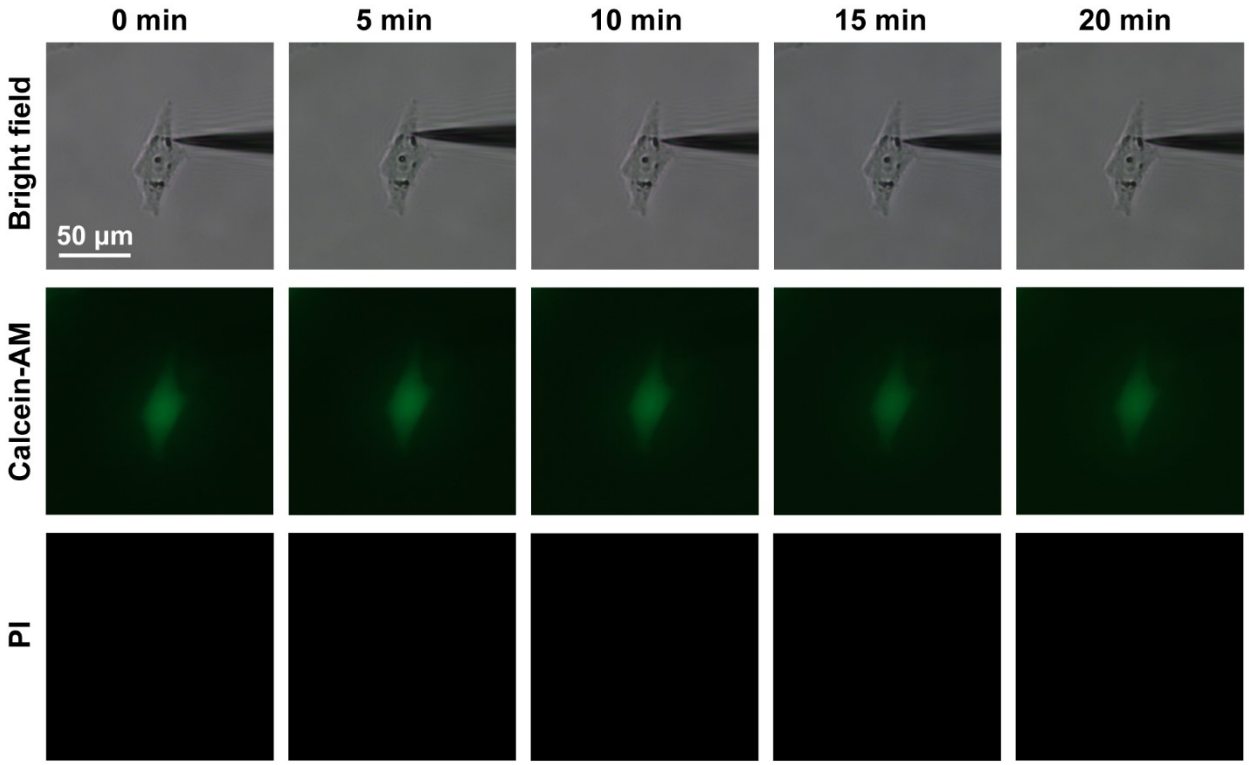

Fig. S6 Cell viability of a single HeLa cell after the nanopipette injection. From top to bottom: bright field images, corresponding fluorescent images of Calcein-AM and PI.

Supplementary Note 7.

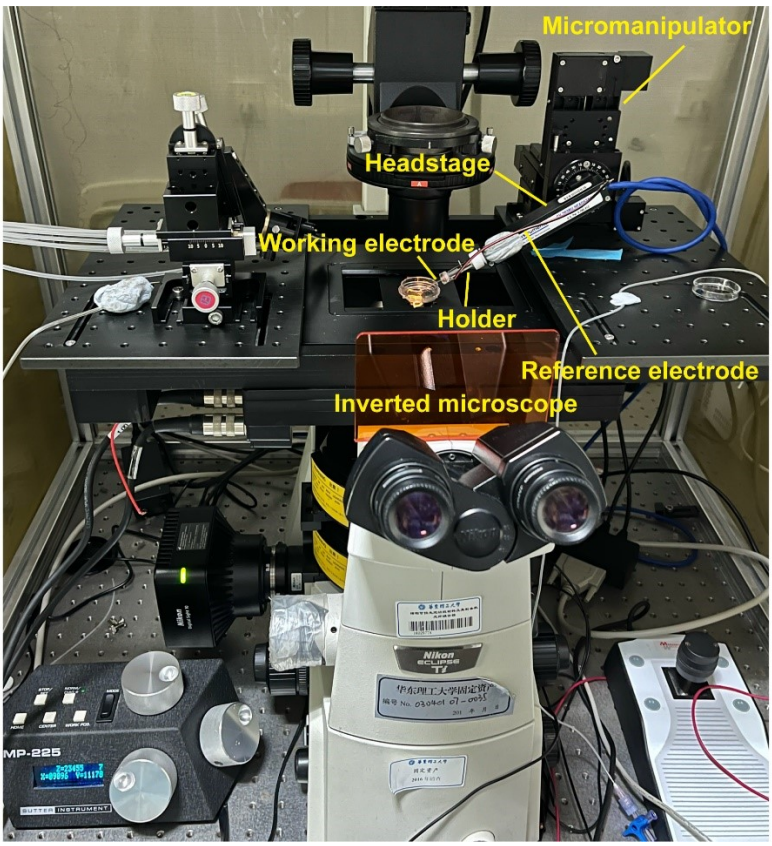

Fig. S7 Experimental equipment for electrochemical measurement of living cells.

Supplementary Note 8.

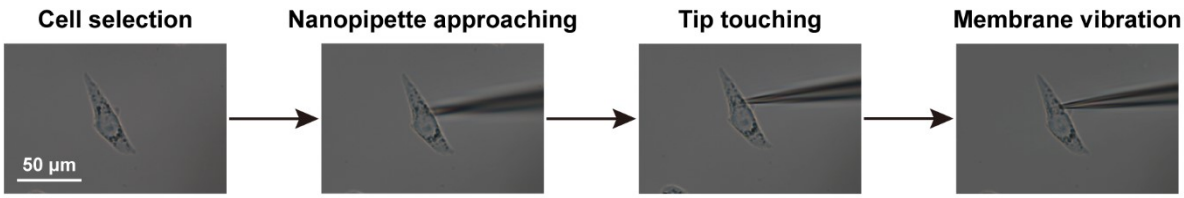

Fig. S8 Bright-field images of a living HeLa cell during the cell membrane oscillation measurement.

Supplementary Note 9.

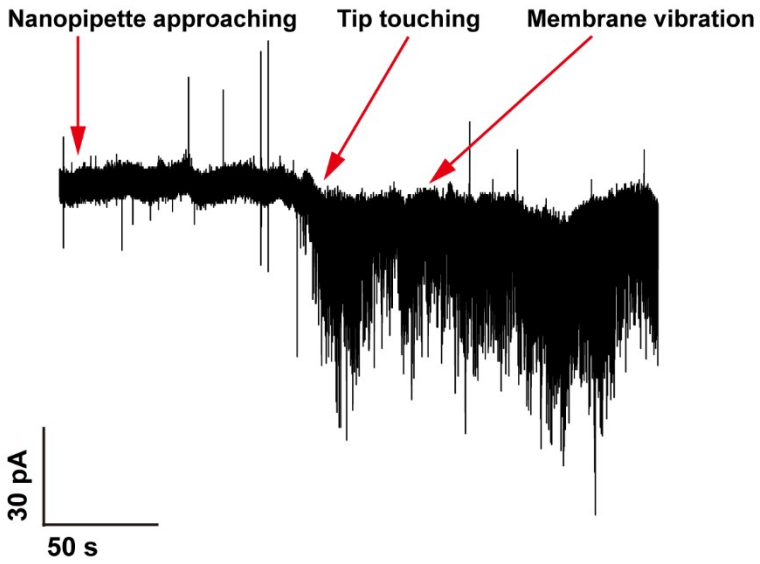

Fig. S9 Representative current-time (I-t) trace recorded upon initial contact of the nanopipette tip with the cell membrane, showing characteristic downward spikes.

Supplementary Note 10.

The electroosmotic flow (EOF) at the nanopipette tip was characterized by calculating the following quantities:

Electroosmotic velocity:

$$V_0 = -\frac{E\zeta\varepsilon_0\varepsilon_r}{\eta}$$

Electroosmotic mobility：

$$\mu_{eo} = -\frac{\varepsilon_0\varepsilon_r\zeta}{\eta}$$

Total volumetric flow rate：

$$Q_{\Delta\Psi} = \mu_{eo}\,\pi R_0 \tan(\theta)\,\Delta\Psi$$

Hydrodynamic impact force：

$$F = \rho\pi R_0^2 V_0^2$$

Where η is the viscosity of the bulk solution inside the nanopipette, E is the magnitude of the electric field inside the nanopipette, ζ is the zeta potential of the nanopipette wall, ε_r is the relative permittivity of the solution, ε_0 is the permittivity of vacuum, R_0 is the radius of the nanopipette tip, θ is the half-cone angle of the nanopipette, and ρ is the density of water.

Tab. S1 Electroosmotic parameters at the nanopipette tip.

$\Delta\Psi$ (mV)	50
v_0 (mm / s)	1.34
$Q_{\Delta\Psi}$ (fL / s)	17.8
F (N, $\times 10^{-17}$)	2.4

Supplementary Note 11.

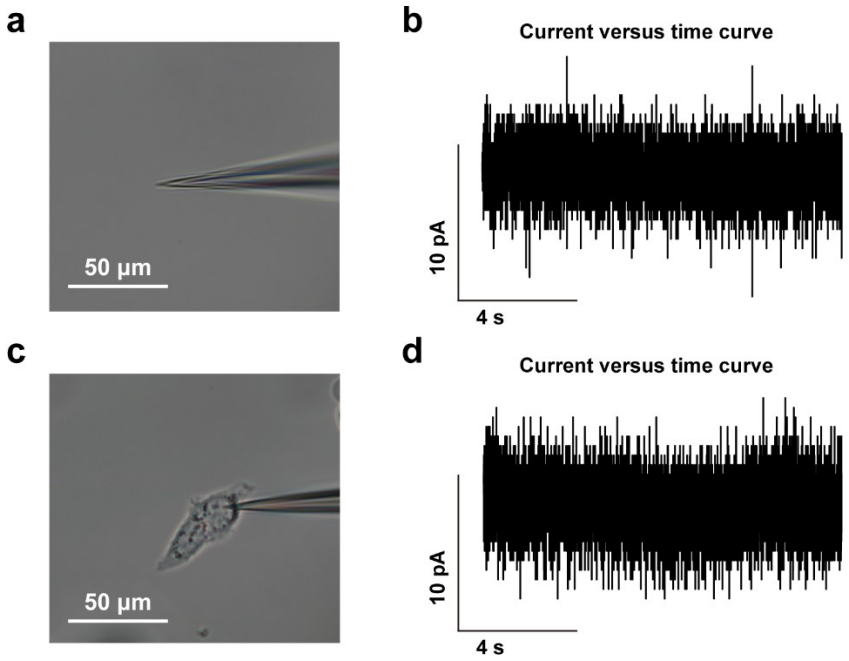

Fig. S10 (a) Bright-field image of a nanopipette positioned in PBS solution without cells. (b) Representative current-time (I-t) recording acquired corresponding to (a). (c) Bright-field image of a chemically fixed HeLa cell measured using the same nanopipette configuration and approach protocol. (d) Representative I-t recording acquired corresponding to (c). Scale bar: 50 μm.

Supplementary Note 12.

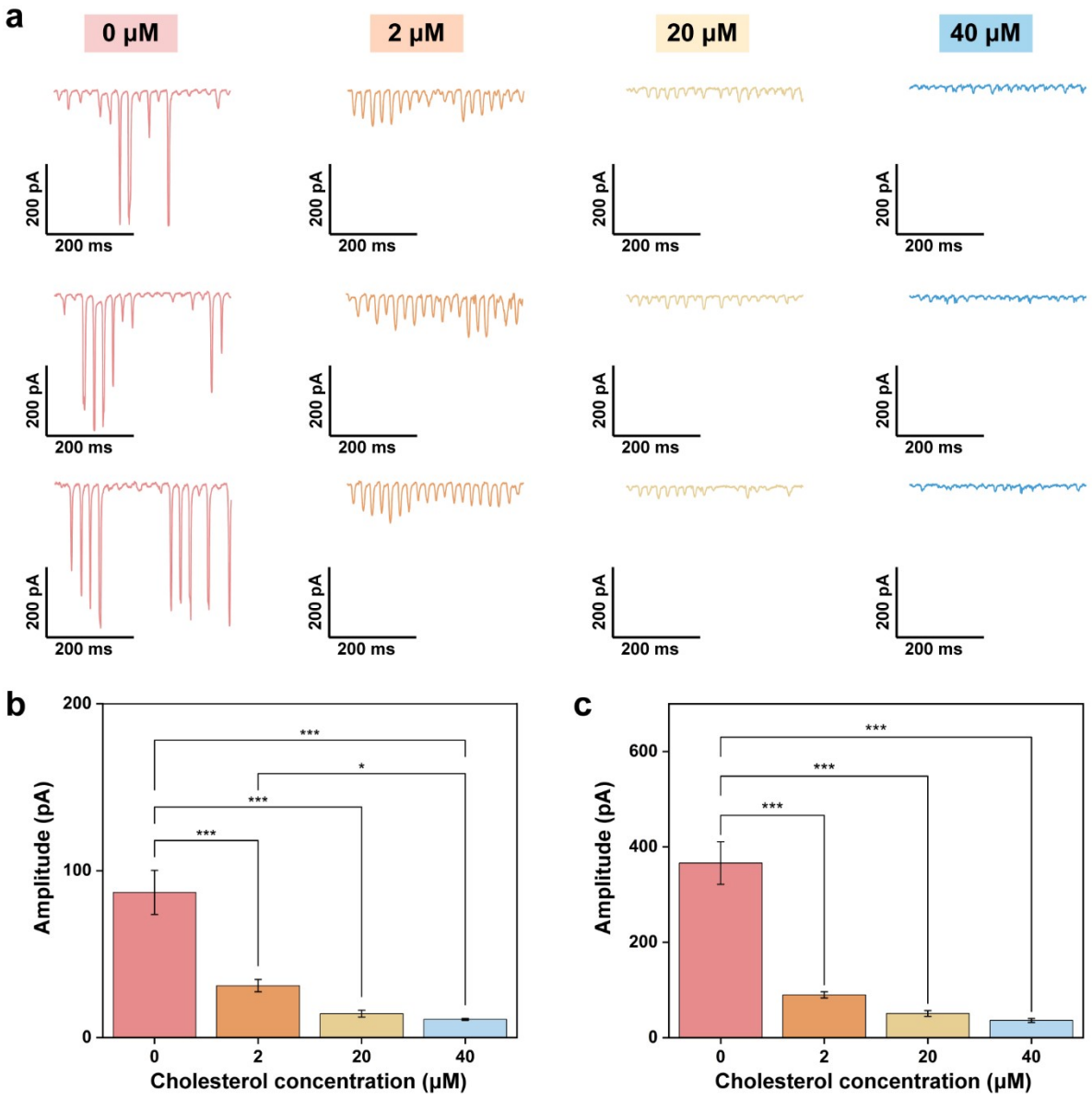

Fig. S11 (a) Additional representative ionic-current time traces of independent HeLa cells under different cholesterol concentrations recorded at a constant bias. (b) Statistical distribution of the average oscillation amplitudes and (c) maximum amplitudes for each concentration. Data are presented as mean ± SD (n = 3 per group). Significant differences are indicated as *p ≤ 0.05, **p ≤ 0.01, ***p ≤0.001.