Supporting Information

Optical imaging of electrical and mechanical couplings between cells

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S1. Cell culture

Wild-type HEK293T and A549 cells were cultured in DMEM medium (Gibco) with 10% FBS (Gibco), according to the user's manual from ATCC. PC12A cells were cultured in F-12K medium (ATCC) with 2.5% FBS and 15% horse serum (Gibco), according to user's manual from ATCC. One day before the experiment, the cells were seeded on the collagen-coated glass coverslip dishes (MatTek) and incubated in 5% CO_2-humidified atmosphere at 37% over night.

S2. Procedure for imaging and modulation

The bright field microscopy was conducted on an inverted optical microscope (IX81, Olympus) with a 60× objective (NA = 0.65) and recorded with an sCMOS camera (Hamamatsu, ORCA-Flash 4.0) at 400 fps. Electrical modulation was implemented on with Axopatch 200B (Axon Instruments) in voltage-clamp mode. Glass micropipettes were pulled using a flaming puller (P-97, Sutter Instrument). Intracellular solution containing 10 mM NaCl, 135 mM K-gluconate, 10 mM HEPES, 2 mM $MgCl_2$, 2 mM Mg-ATP, and 1 mM EGTA (pH 7.4) was injected to the tip of micropipettes. The extracellular recording solution contains 135 mM NaCl, 5 mM KCl, 1.2 mM $MgCl_2$, 5 mM HEPES, 2.5 mM $CaCl_2$, and 10 mM glucose at pH 7.4. Micropipettes with typical resistance of 3 to 6 MΩ was used for patch. A seal resistance of >1 GΩ was obtained before the membrane breaks and after the membrane breaks, the membrane resistance >200 MΩ were used for modulation. The imaging and modulation were conducted at room temperature (~25 ºC). For mechanical modulation of cells in Figure S5, the same glass micropipettes were disturbed with a piezoelectric actuator (PAS005, Thorlab) driven by a function generator.

S3. Differential detection algorithm

A square ROI including cell edge was selected and split into 2 halves along the cell edge, one inside (red, figure S1A) and one outside (blue, figure S1B). The integrated intensities of the two halves were recorded as I_1 and I_2, respectively. The normalized differential intensity was defined as $(I_1-I_2)/(I_1+I_2)$, varying along with the electrical modulation. To calibrate the normalized differential intensity variation, the ROI was shifted pixel by pixel perpendicular to the cell edge from outside to inside (figure S1A), and the $(I_1-I_2)/(I_1+I_2)$ versus pixel shift was plotted (figure S1B). This pixel shift can be converted to displacement in nm by dividing pixel size of the camera (6.5 μm) with the optical zoom (60×) and digital interpolation factor (5×). As a result, a linear region near the center (shift pixels from -9 to 9) can be fitted (figure S1C), in which a calibration factor of 0.0003 a.u./nm can be obtained along this direction (arrow in figure S1A).

For mean displacements, the cell edge was selected manually. Along the edge, several equal spaced ROI (typically 36 ROI for closed edge) were selected. The normalized differential intensity calibration and displacement calculation were conducted for each ROI separately, and the mean displacements for whole cells were obtained by averaging the displacements in all ROI.

S4. Normalized displacement

Normalized displacement (ND) was used to compare the displacement on different cells. ND was defined as:

$$\mathrm{ND} = \frac{\mathrm{D@23Hz} - \text{mean of D(20-26Hz)}}{\text{standard deviation of D(20-26Hz)}}$$

where D@23Hz means the displacement at 23.0 Hz; D(20-26Hz) means the displacements from 20 to 26 Hz (excluding 23.0 Hz). The mean of D(20-26Hz) is the background displacements; and the standard deviation of D(20-26Hz) is regarded as the noise.

Supplementary figures

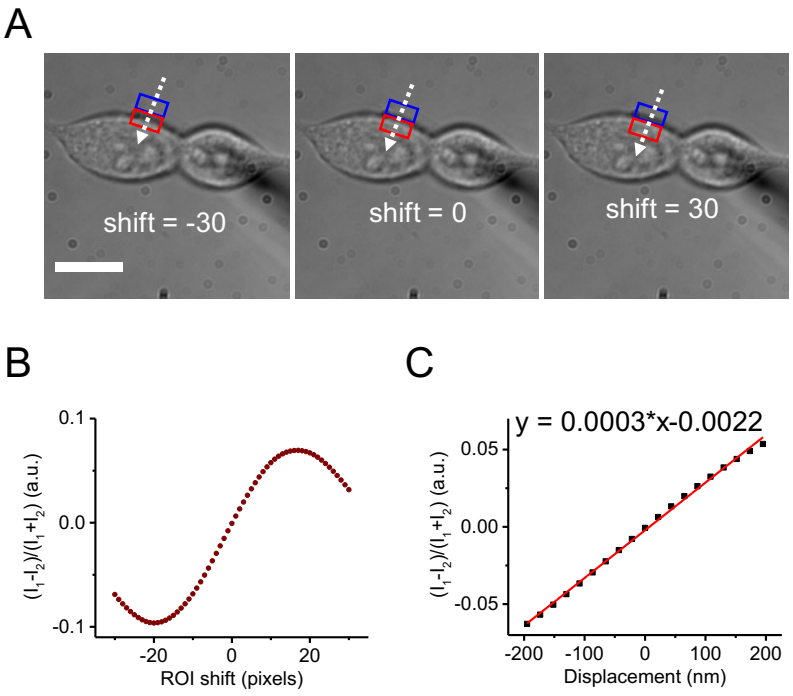

Figure S1. Scheme for differential detection algorithm. (A) An ROI at the cell edge shifts from outside to inside of the cell pixel by pixel. (B) Normalized differential intensity was plotted versus the relative ROI position. (C) Linear fitting of the ROI shift from -200 nm to 200 nm (pixel shifted from -9 to 9). Scale bar, 15 μm.

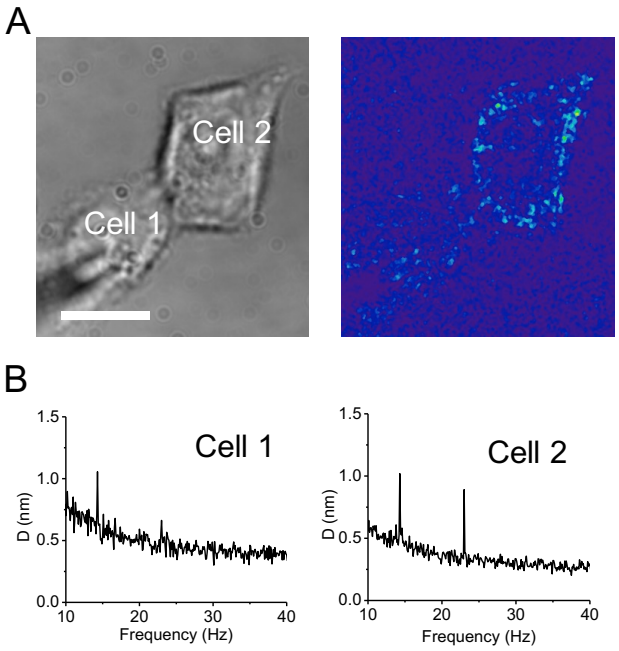

Figure S2. Electrical modulated mechanical motion in A549 cells. (A) Bright field image and difference image of two A549 cells. (B) The mean edge displacements of the cell 1 and cell 2 induced by electrical modulation. Scale bars, 15 μm.

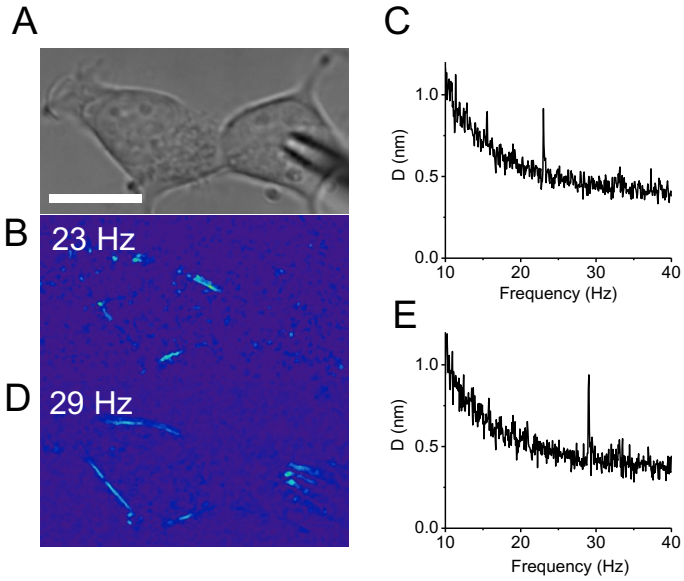

Figure S3. Mechanical motion in frequency domain is dependent on the electrical modulation frequency. (A) Bright field image of two cells. (B) Difference image at 23 Hz when modulated at 23 Hz. (C) The mean edge displacement of the cell shown in B. (D) Difference image at 29 Hz when modulated at 29 Hz. (E) The mean edge displacement of the cell shown in D. Scale bars, 15 μm.

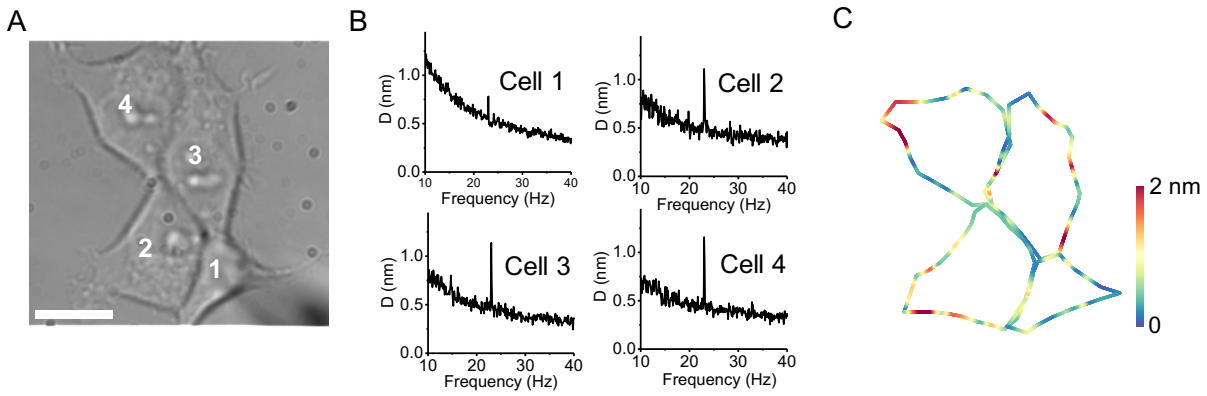

Figure S4. Electrical modulated mechanical motion in multiple connected cells. (A) Bright field image of 4 connected cells. (B) The mean edge displacements of the 4 cells. (C) Edge displacement map obtained from the differential algorithm. Scale bars, 15 μm.

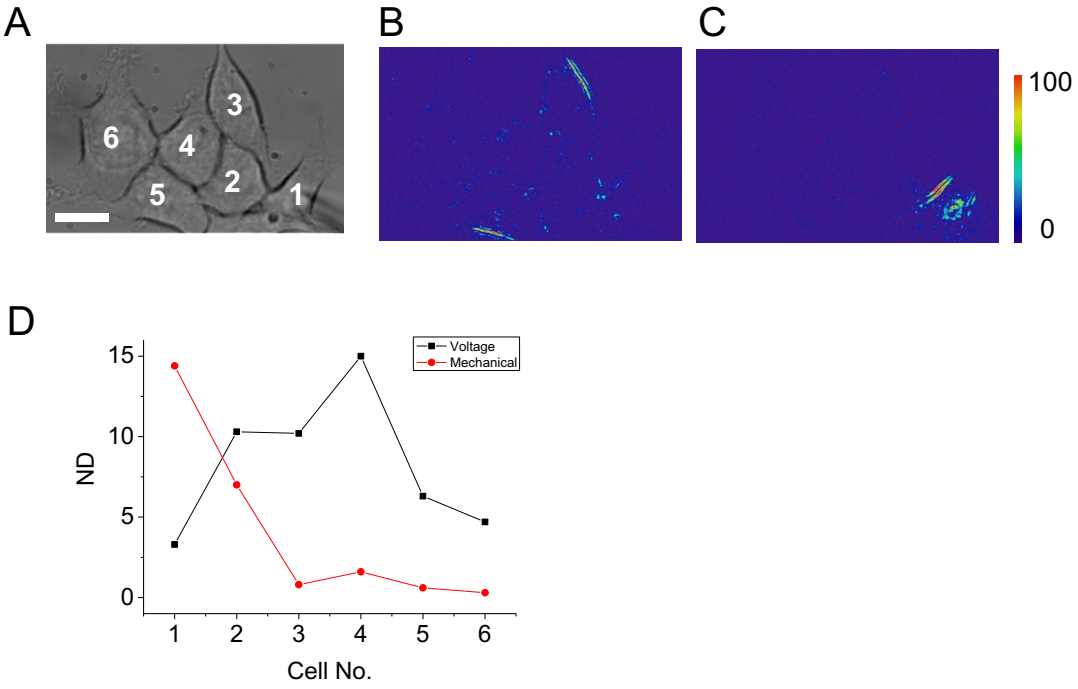

Figure S5. Spatial pattern of connected cells under the electrical and mechanical stimulus. (A) Bright field image of the cells. Cell 1 is patched. (B) and (C) Difference images of the cells at 23 Hz under the electrical and mechanical stimulus, respectively. Scale bar, 15 μm. (D) ND of each cell under the electrical and mechanical stimulus, respectively.

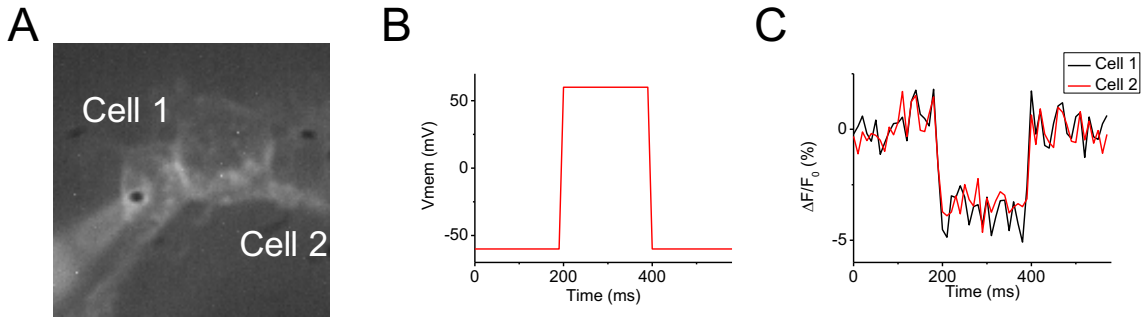

Figure S6. Intercellular electrical couple of connected cells revealed by membrane potential-sensitive probe (di-8-ANEPPS, Invitrogen) and fluorescent imaging. (A) Fluorescent image of connected cells. The fluorescence image was excited at 470 nm and the emission was collected with a long pass filter (>600 nm). (B) A 120 mV step (membrane potential from -60 mV to 60 mV, lasting for 200 ms) was used for triggering the electrical couple of cells. (C) The fluorescence intensity of each cell versus time, which shows that the membrane potential of the cell 2 varies along with the clamped cell 1 instantaneously.

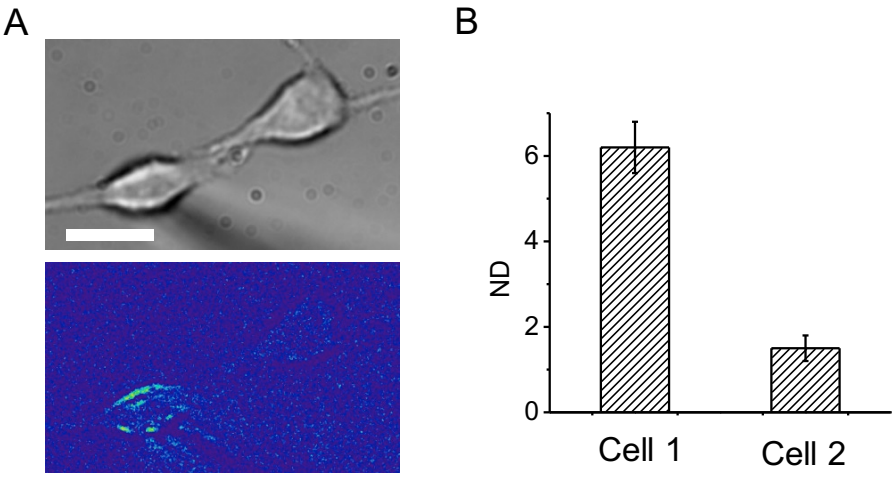

Figure S7. The coupled motion is not observed in PC12A cells. (A) Representative difference images at 23 Hz. (B) The normalized displacements of PC12A cell pairs. Cell pair, n = 6. Scale bar, 15 μm.