Supplementary Information

Supplemental Note 1. Reconstruction of the intracellular potential

As shown in Fig. 3B, the signals recorded by the chip are a distorted version of the actual signals (recorded by patch clamp). This can be attributed to the presence of a reference voltage compensation circuit (DC compensation loop) in the input amplifier (*10,18*), which eliminates electrode drift and offset effects. However, for low-frequency signals, this compensation acts as a high-pass filter and generates distortion. The amplifier transfer function can be modeled as:

$$H_{amp} = A_1 \frac{1+s \cdot C_f R_f}{A_2 R_f g_{mf} + s C_f R_f} \quad [1]$$

where A_1 is the amplifier gain, $A_2 R_f g_{mf}$ is the feedback gain, and $C_f R_f$ is the feedback loop time constant. All these parameters are determined by an automated calibration phase before the measurement. By applying the inverse of this transfer function to the measured signals, a reconstruction of the intracellular potential can be obtained:

$$V_{m,recon}(t) - V_{rest} \approx V_o(t) * \mathcal{L}^{-1}\left\{\frac{1}{A_1} \cdot \frac{A_2 R_f g_{mf} + s C_f R_f}{1+s \cdot C_f R_f}\right\} \quad [2]$$

where $\mathcal{L}^{-1}$ represents the inverse Laplace operator. The term V_{rest} represents the membrane resting potential. As the DC signal information was lost due to the capacitive coupling at the electrode, this term was subtracted from the reconstructed

signal. The inverse filtering was applied to the raw data using Matlab (The Mathworks).

Supplemental Note 2. Model derivations

Given the linear circuit model of Fig. 3A, the transfer function (in the Laplace domain) from the intracellular potential V_m to the electrode potential V_e can be expressed as:

$$\frac{V_e}{V_i}(s) = \frac{R_{seal}(1+s\cdot C_{m1}R_p)(1+s\cdot C_sR_s)}{R_p+R_{seal}+s\cdot[C_L(R_pR_s+R_pR_{seal}+R_sR_{seal})+C_{m1}R_pR_{seal}+C_sR_s(R_p+R_{seal})]+s^2\cdot R_pR_sR_{seal}[C_{m1}(C_L+C_s)+C_LC_s]} \quad [3]$$

where R_{seal} represents the ionic cleft formed between the plasma membrane and the chip surface and has typical values between 1-10 MΩ (*12,13*); C_{m1} represents the capacitance of the fraction of the cell membrane located on top of the electrode and can be approximated by $C_{m1} = A_s c_m \approx 100fF$, where A_s is the electrode area (i.e. 5 μm^2) and c_m is the characteristic capacitance of the cell membrane (i.e. ~20 fF/μm^2); R_p represents the resistance of the pore being generated by the electroporation pulses; C_s is the linearized model of the electrode double layer capacitance; R_s represents the Faradaic charge transfer at the electrode interface. C_L is the representation of the input capacitance of the amplifier located beneath the electrode which was approximately 300 fF. Impedance measurements of the electrode resulted in values of $C_s \approx 30\,pF$ for electrode areas of 5 μm^2 and $R_s > 10\,G\Omega$. Such high characteristic capacitance was obtained due to a 300 nm thick TiN layer on top of the electrodes. Given the

following assumptions: $C_s \gg C_L$, $R_s \gg R_{seal}$ and $C_s \gg C_{m1}$, we can approximate Eq. 3 as:

$$\frac{V_e}{V_i}(s) \approx \frac{\alpha \cdot (1+s \cdot C_{m1} R_p)(1+s \cdot C_s R_s)}{1+s \cdot C_s R_s + s^2 \cdot \alpha\, R_p R_s C_s (C_{m1}+C_L)} \quad [4]$$

where $\alpha = R_{seal}/(R_p + R_{seal})$. High values of α can be achieved for high values of R_{seal}, which can only be obtained if the cell fully covers the electrode (*5*,*6*).

When the term $(2\pi C_s R_s)^{-1}$ is lower than the minimum signal frequency of interest, a condition that is met with a large enough electrode capacitance, we can approximate Eq. 4 as:

$$\frac{v_e}{v_i}(s) \approx \frac{\alpha \cdot (1+s \cdot C_{m1} R_p)}{1+s \cdot \alpha R_p (C_{m1}+C_L)} \quad [5]$$

Similarly, if the maximum signal frequency of interest is lower than the term $\left(2\pi C_{m1} R_p\right)^{-1}$, the transfer function in Eq. 3 can be further reduced to:

$$\frac{v_e}{v_i}(s) \approx \alpha = \frac{R_{seal}}{R_p + R_{seal}} \quad [6]$$

Since most physiological signals are within a 2 kHz bandwidth, previous condition can be re-written as: $\pi^2 d_s^2 c_m \left(2 g_p\right)^{-1} > 2\ kHz$, where g_p is the pore conductivity and d_s is the electrode diameter.

Supplemental Table

Stim.Voltage (V_{p-p})	# Pulses	Pulse Width (ms)	ISI Before (s)	Stdev Before	ISI During (s)	Stdev During	ISI After (s)	Stdev After	p Value	# Trials
3.3	10	1	1.148	0.046	1.143	0.046	1.143	0.044	0.1956	19
2.9	10	1	1.121	0.026	1.112	0.021	1.118	0.035	0.097	12
2.5	10	1	1.128	0.045	1.126	0.042	1.132	0.044	0.0712	14

ISI is the interspike interval calculated using a peak detection algorithm. The p-values were calculated using the non-parametric Friedman's test for comparison of three different groups (Matlab, The Mathworks; GraphPad).

Supplemental Figure S3

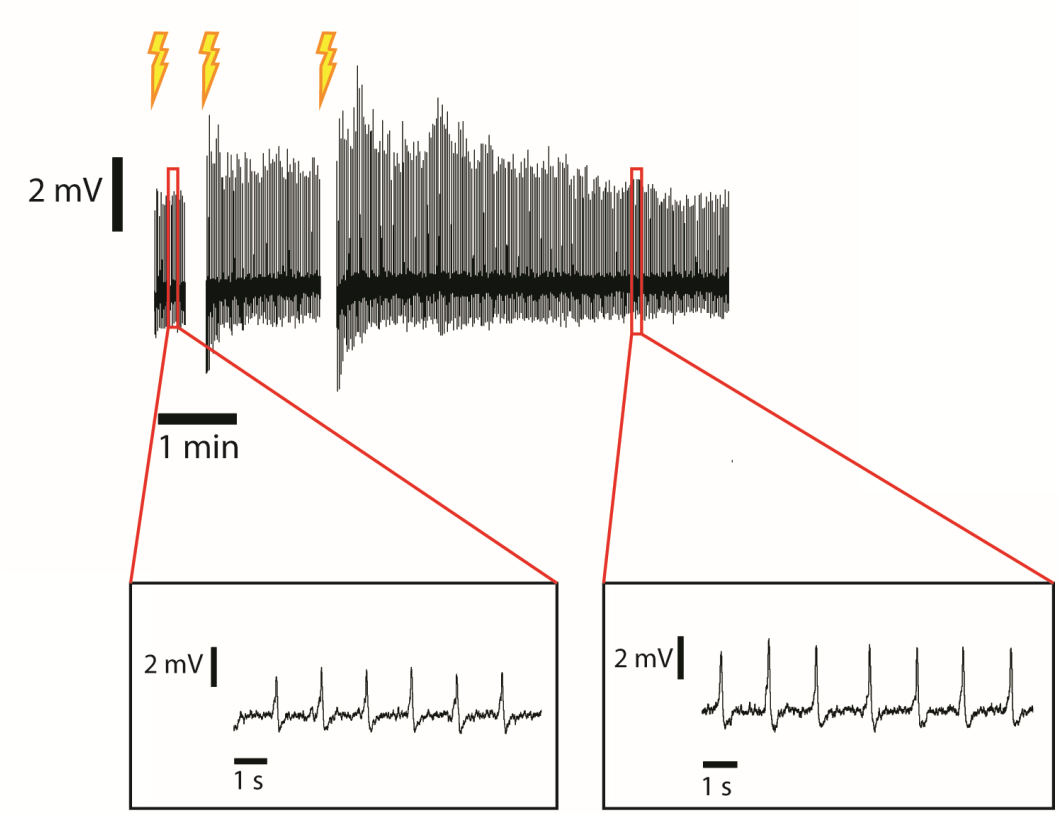

Fig. S3. Prolonged open-cell state by applying consecutive stimulation pulses on the same cell. Blank spaces between open-cell recordings mark electroporating stimuli.