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Supplemental information

Electrically synchronizing and modulating the dynamics of ERK activation to regulate cell fate

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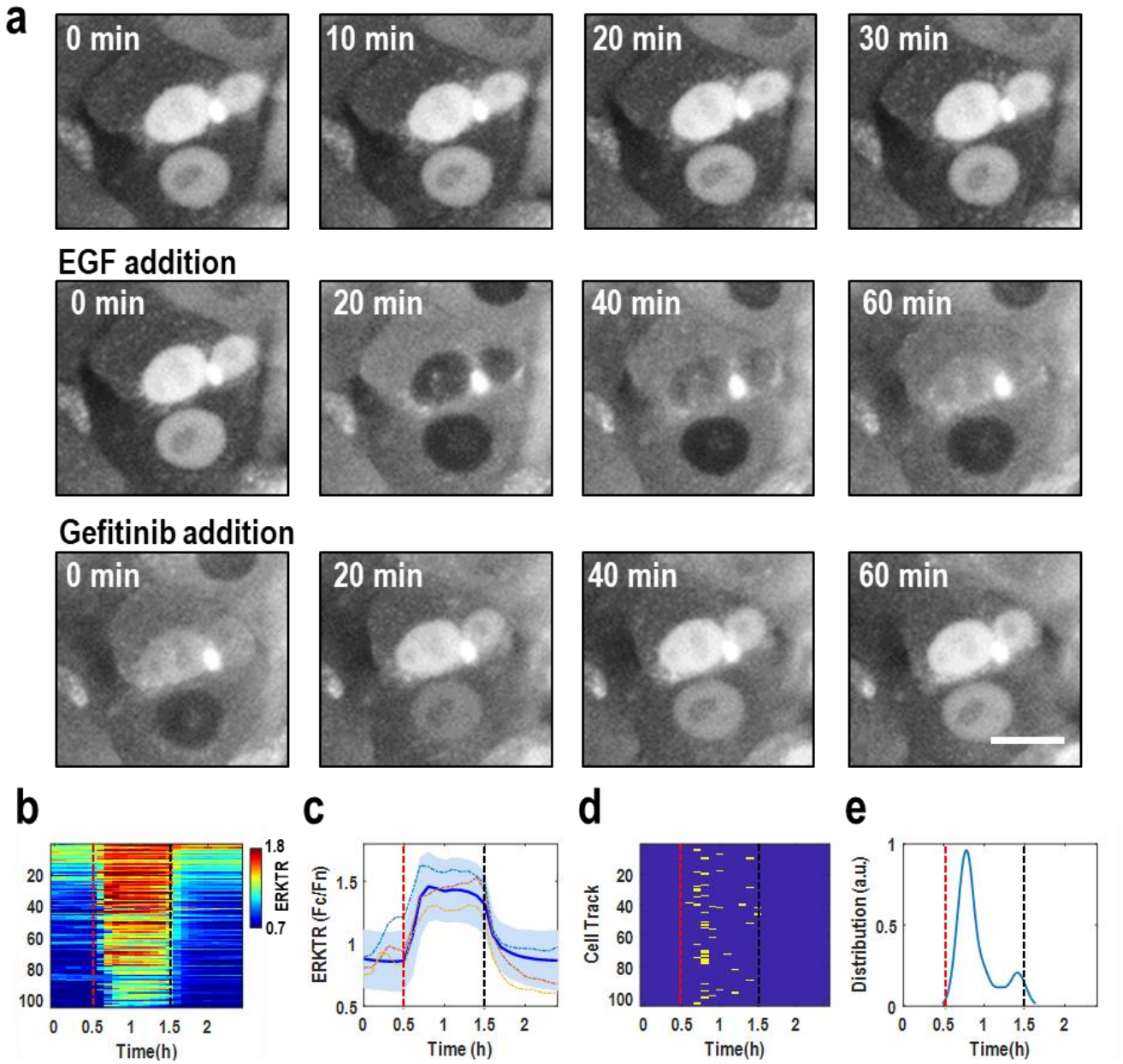

Figure S1. The response of ERK to EGF was abolished by EGFR/ERK inhibition. Related to Figure 2.

a. Representative time-lapse fluorescence images of MCF10A cell response to EGF (0.2ng/ml) stimulation followed by EGFR/ERK inhibition (Gefitinib, 1μM). Cells cultured in serum-free imaging medium and treated with EGF and Gefitinib successively.

b. A heat map shows ERK traces over 100 cells in which ERK activation was color-coded. Each row line indicates one single cell.

c. Time course of averaged ERK trace (solid thick line) and 3 individual ERK traces (dotted curve line) treated EGF (0.2ng/ml) and inhibitor (Gefitinib 1μM) at the indicated time.

d. Peak map showing ERK activation peaks identified automatically from the ERK traces of c. Each row represents one cell. Each yellow dot indicates one peak identified from each trace.

e. Peak distribution were quantified based on peak points from d.

Note: red and black vertical dotted lines indicate the addition of EGF and Gefitinib respectively. Scale bar,15 μm.

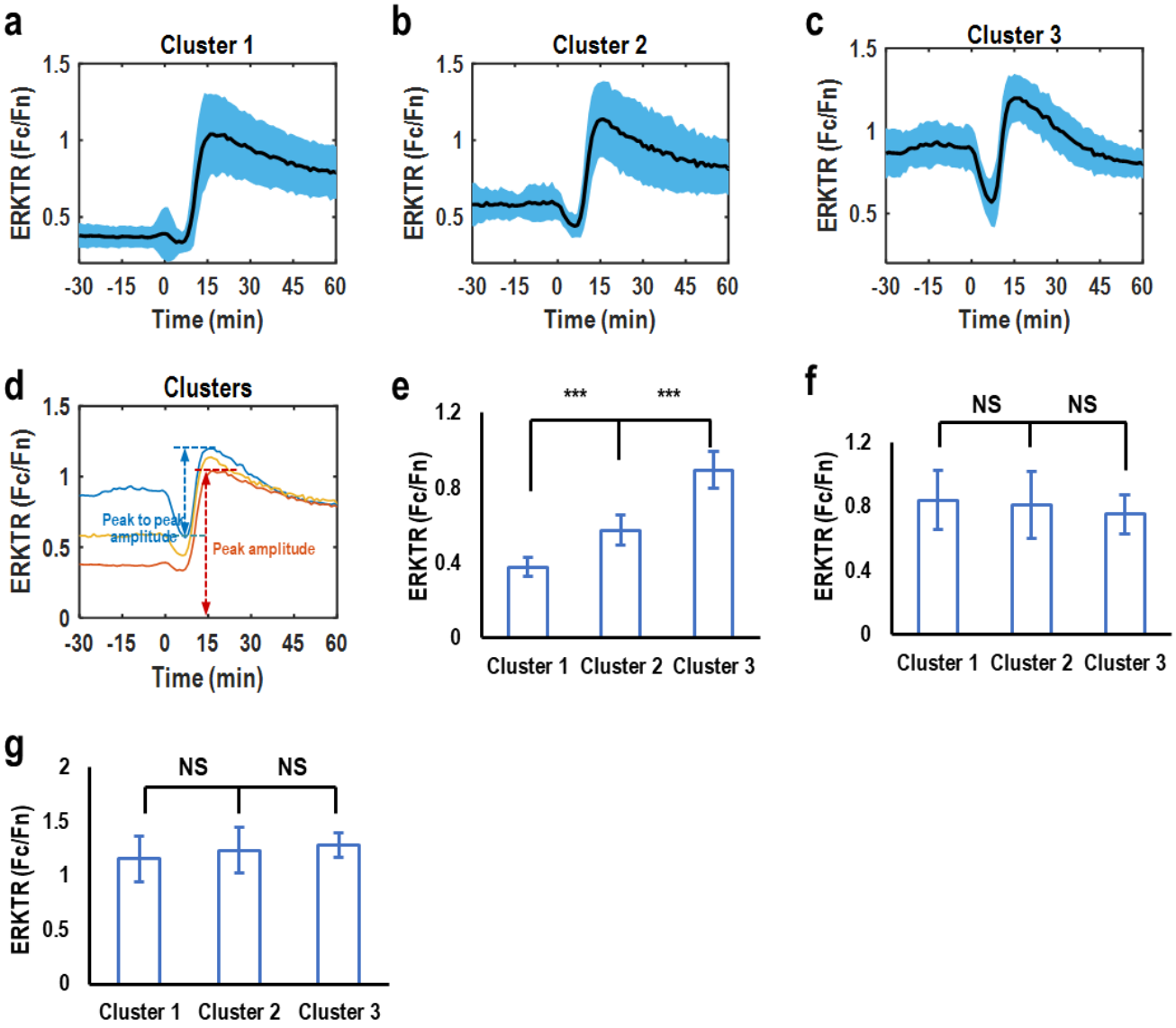

Figure S2. Three cell clusters separated by ERK activation levels before and after AC stimulation. Related to Figure 2.

a-c. Trajectories from all sustained AC stimulation experiments (n = 97 cells, as shown in Figure 1) were pooled and three clusters were identified using k-means clustering with squared Euclidean distance. Standard deviation range (blue shade) and mean trajectory of each cluster (black).

d. Overlaid mean trajectories of the three clusters. Dotted line with both arrows indicates the peak amplitude and peak to peak amplitude of ERK activation.

e. Accumulated ERK activation of 30 min before AC stimulation**.** Data are expressed as mean ± SD.

f. The peak-to-peak amplitudes of ERK activation of three clusters. Data are expressed as mean ± SD.

g. The peak amplitudes of ERK activation of three clusters. Data are expressed as mean ± SD.

Statistical significance was determined using an unpaired Student's t-test, NS: $p>0.05$, ***: $p<0.001$.

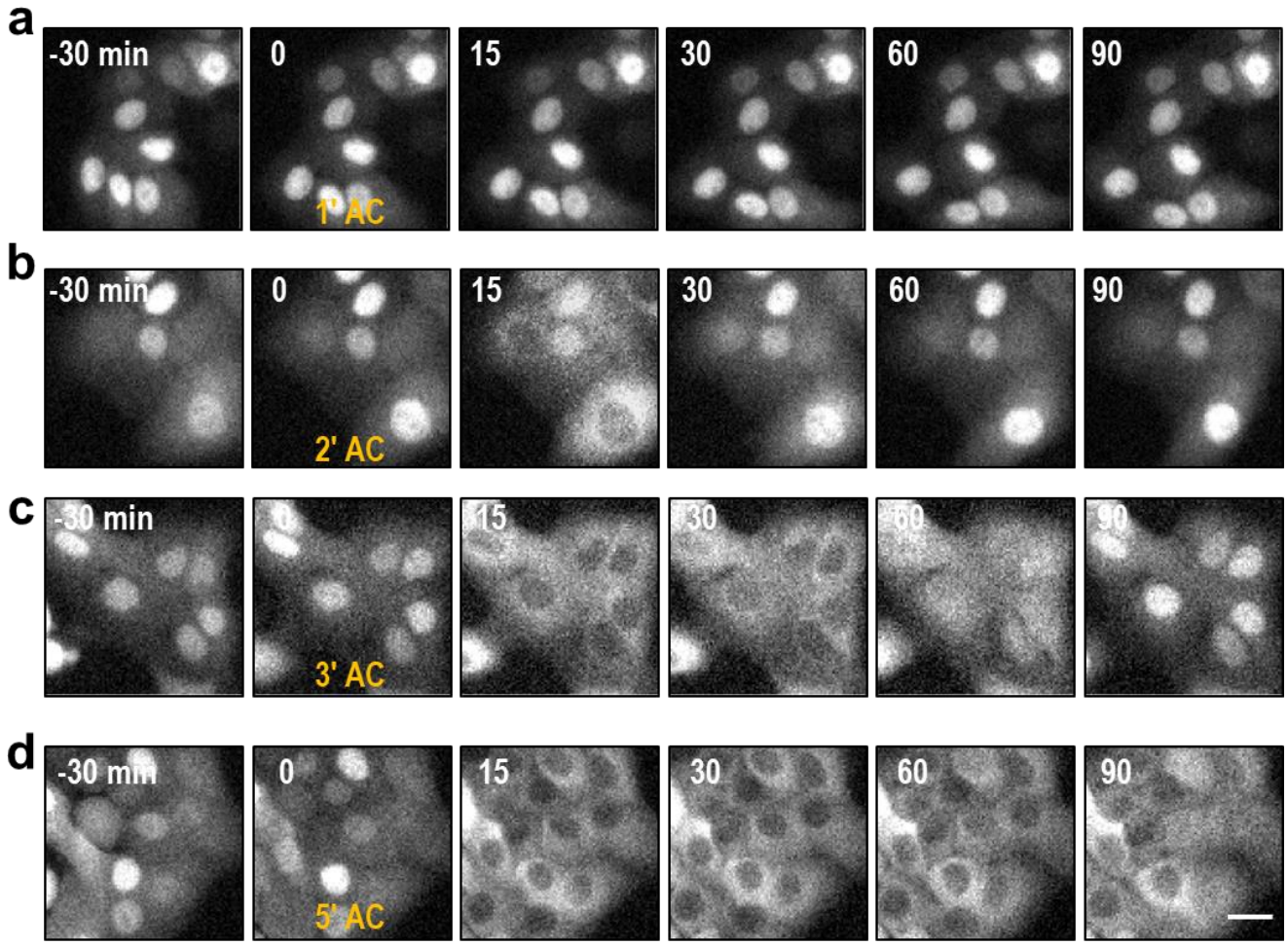

Figure S3. Short periods of AC stimulation induced ERK activation of different amplitude and duration.
Related to Figure 3. Time-lapse fluorescence images of MCF10A cells expressing ERKTR. Cells pre-incubated in serum-free imaging medium and exposed to AC stimulation at 0 min of the duration of 1′ (**a**), 2′ (**b**), 3′ (**c**), and 5′ (**d**). ERKTR translocates from the nucleus to the cytosol reporting activation of ERK. Scale bar=20μm.

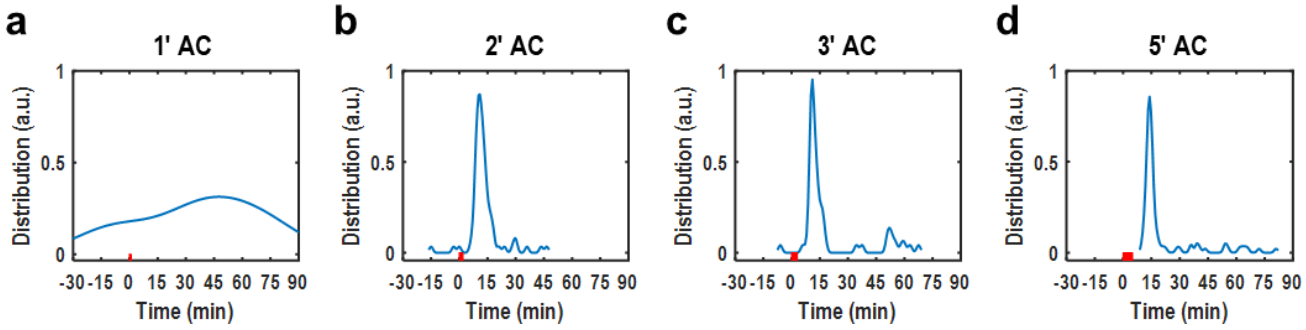

Figure S4. Peak distribution of ERK activation by short-duration AC stimulation. Related to Figure 3.
The peak distributions quantified from the peak points of the data shown in Figure 3, by 1′ (a), 2′ (b), 3′ (c), and 5′ AC stimulation (d). Red bars indicate the duration of AC stimulation.

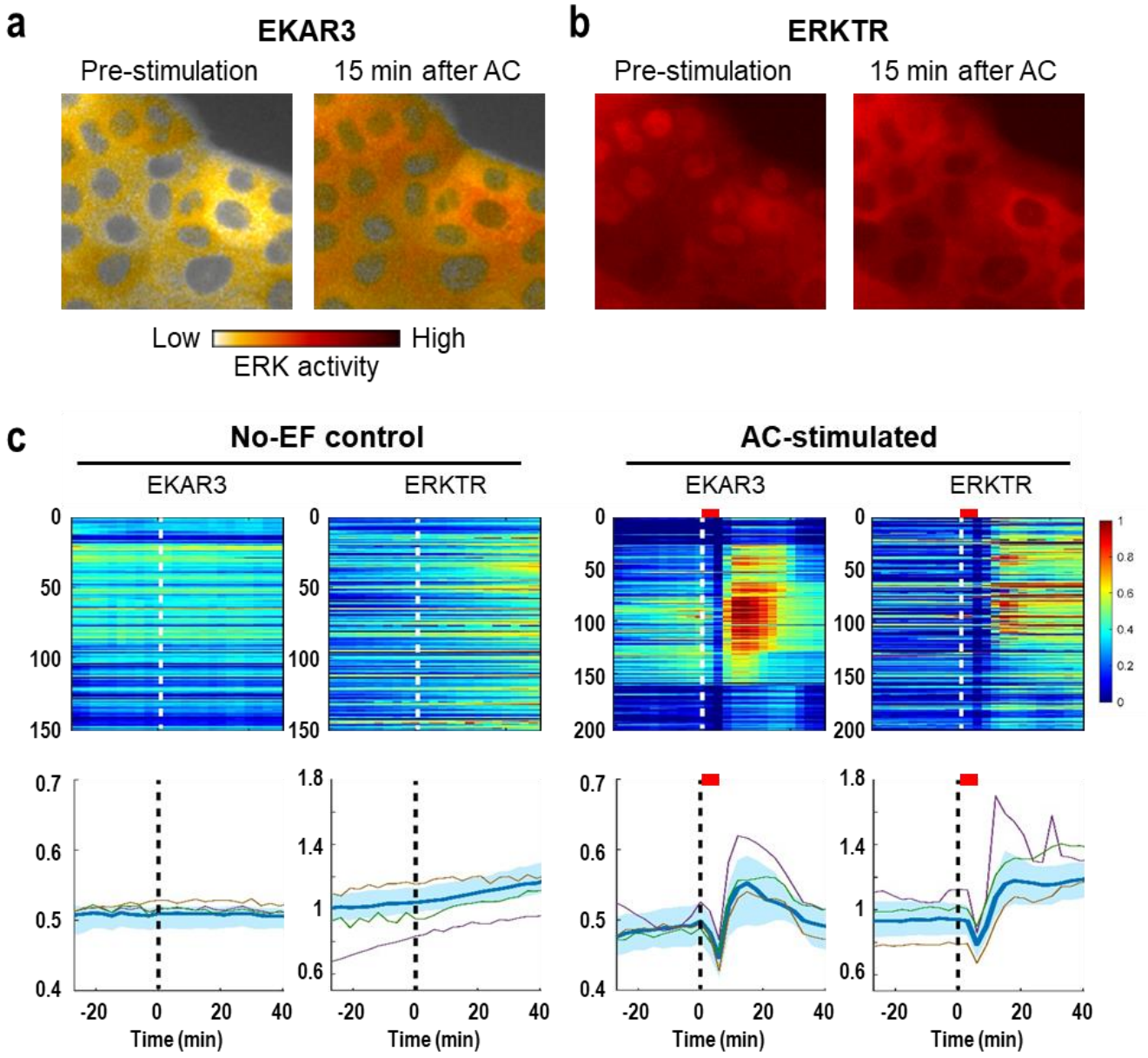

Figure S5. Electrical activation of ERK reported by ERK translocation and FRET biosensors. Related to Figure 3.

a. Ratiometric CFP/YFP images of MCF10A-EKAR3 cells before and after AC stimulation.

b. RFP images of MCF10A-ERKTR cells before and after AC stimulation.

c. Composite heatmaps and time courses of ERK activation dynamics evaluated by ERKTR and cytoEKAR3. AC stimulation (28V/cm, 500Hz) was applied at the vertical dotted line (t=0) for 5 min as indicated by the red bars.

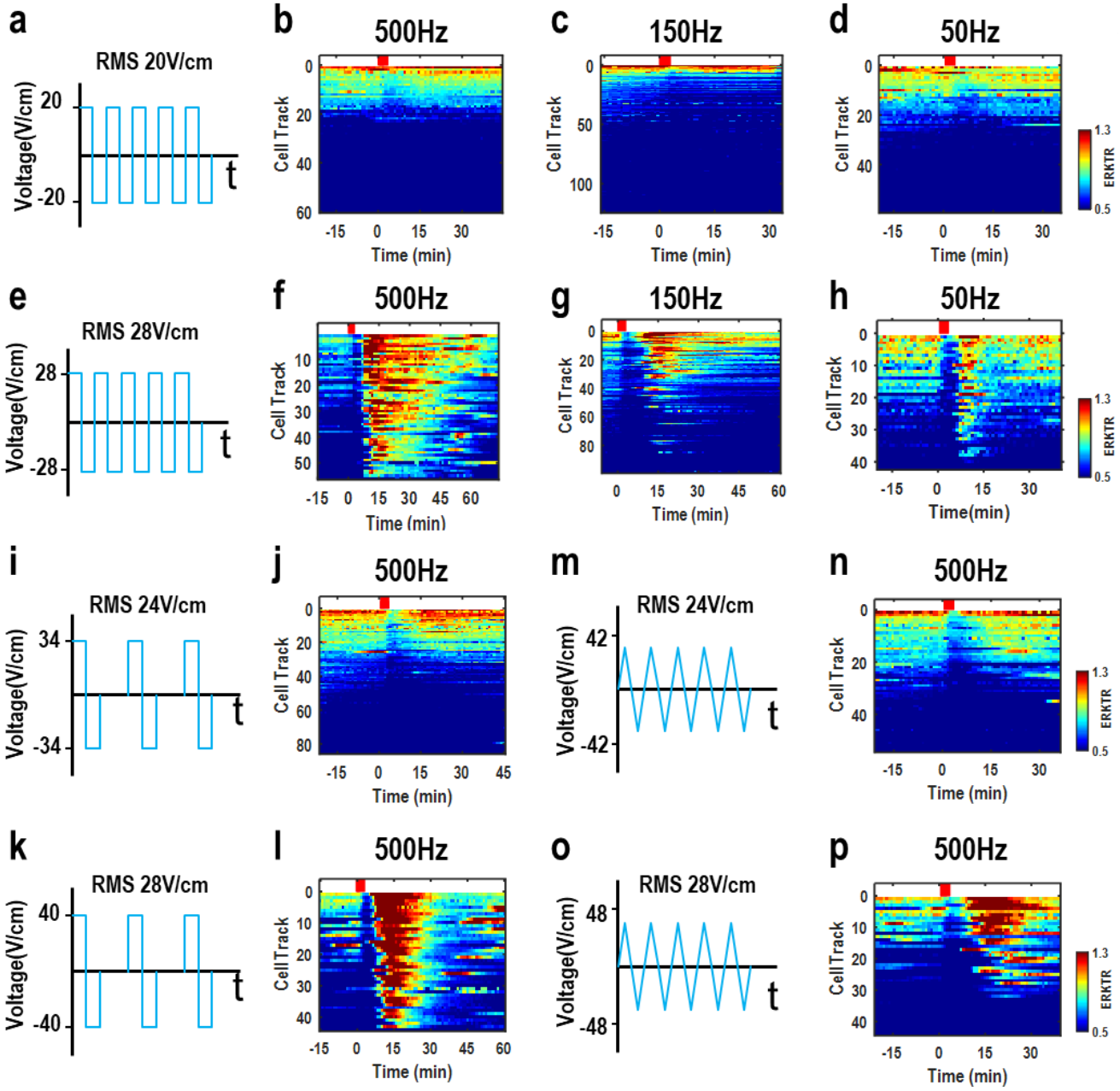

Figure S6. Voltage, frequency, duty cycle, and waveform dependence of ERK activation. Related to Figure 3.

a-h. AC-induced ERK activation is voltage-dependent and frequency-independent. 20 V_{RMS}/cm AC of a square wave with 100% duty cycle (**a**) did not induce ERK activation at a frequency of 500 Hz (**b**), 150 Hz (**c**), or 50 Hz (**d**), respectively, while 28 V_{RMS}/cm of the same waveform (**e**) activated ERK after 3' stimulation at all frequencies (**f, g,** and **h**).

i-l. Duty cycle-independence of AC-induced ERK activation. 24 V_{RMS}/cm square wave AC of 50% duty cycle (**i,** peak voltage = 34 V/cm) did not induce ERK activation at a frequency of 500Hz (**j**), while 28 V_{RMS}/cm (**k**, peak voltage = 40 V/cm) activated ERK (**l**).

m-p. Waveform-independence of AC-induced ERK activation. 24 V_{RMS}/cm AC of triangle wave (**m,** peak voltage = 42 V/cm) did not induce ERK activation at a frequency of 500Hz (**n**), while 28 V_{RMS}/cm (**o**, peak voltage = 48 V/cm) activated ERK (**p**). Note: Red bars indicate the duration of AC stimulation. n>40.

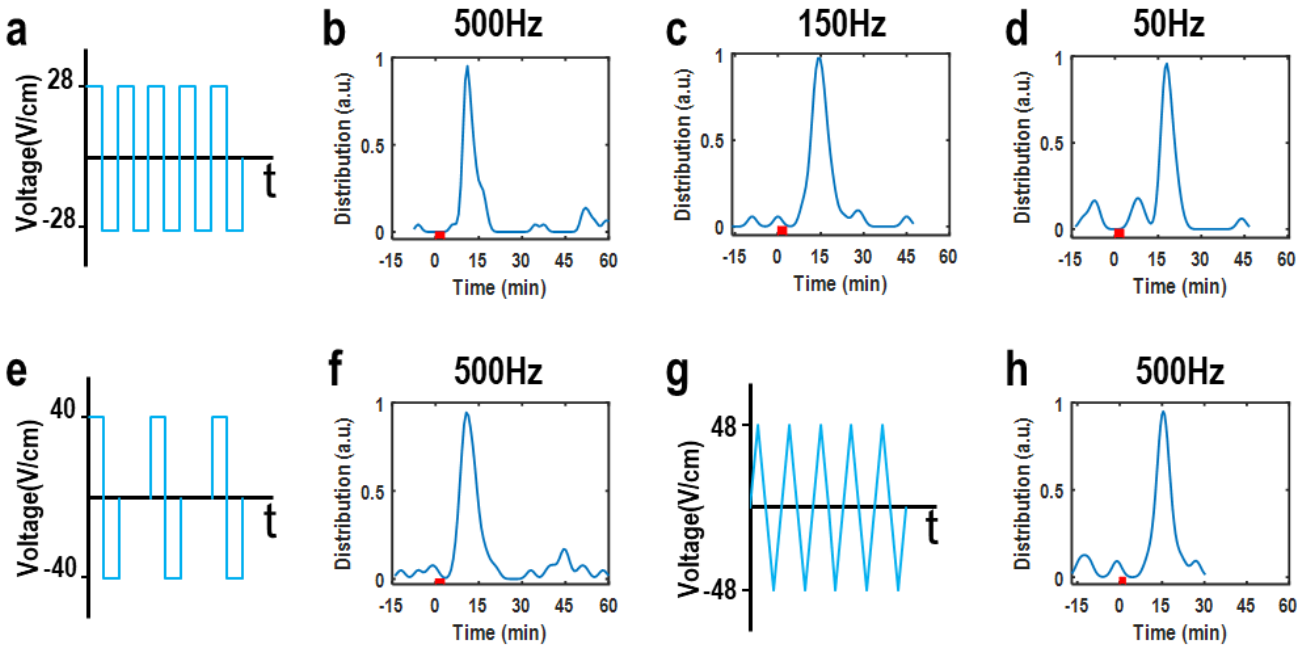

Figure S7. Peak distributions of ERK activation by 3' AC stimulation with different voltages, frequencies, duty cycles, and waveforms. Related to Figure 3.

a-d. Peak distribution of ERK activation induced by square wave AC of 100% duty cycle at different frequencies.

e and **f.** Peak distribution of ERK activation induced by square wave AC of 50% duty cycle at 500 Hz.

g and **h.** Peak distribution of ERK activation induced by AC of triangle wave at 500 Hz.

All peak distributions were quantified from the peak points of Figure S6. Red bars indicate the duration of AC stimulation.

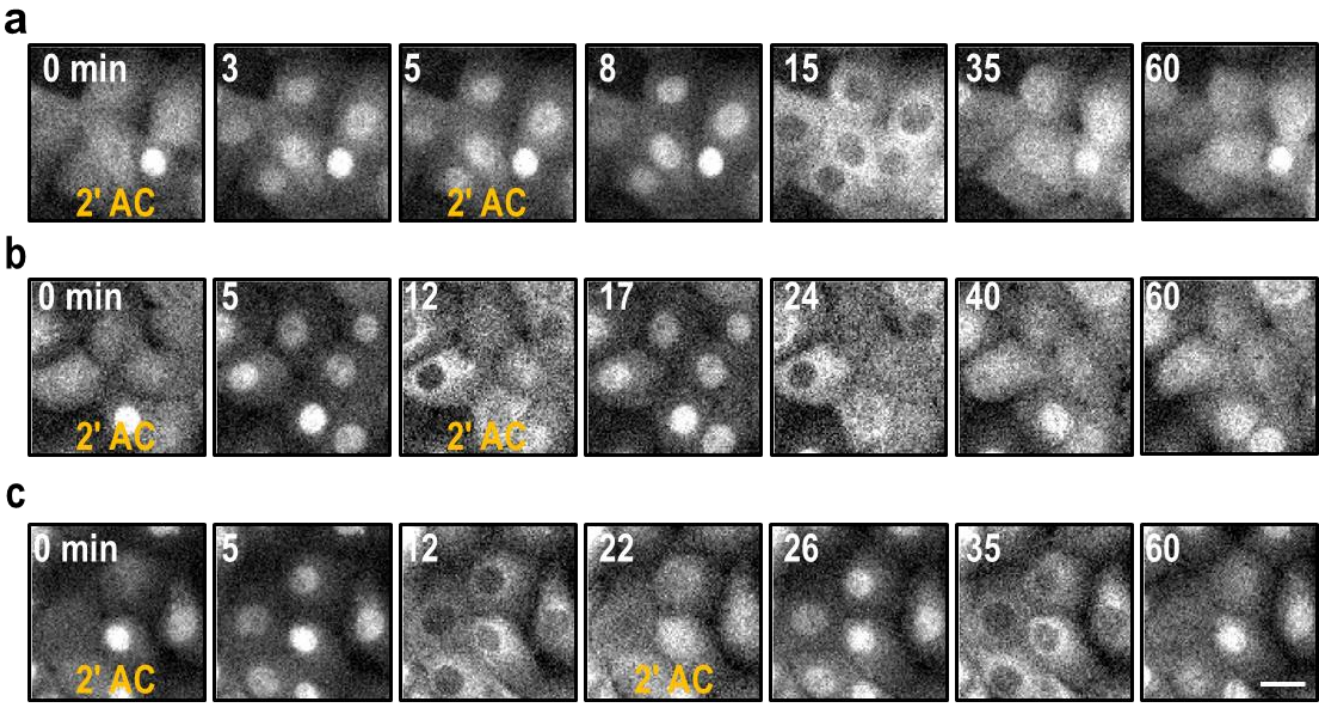

Figure S8. Fluorescence images of the interval-dependent responses of ERK activation induced by two consecutive AC stimulation of 2′ duration. Related to Figure 4.

a. Double 2′ stimulation with a 3′ interval only induced one ERK activation wave.

b. Double 2′ stimulation with a 10′ interval induced activations for twice.

c. Double 2′ stimulation with a 20′ interval induced two distinct activation waves.

Scale bar=20μm.

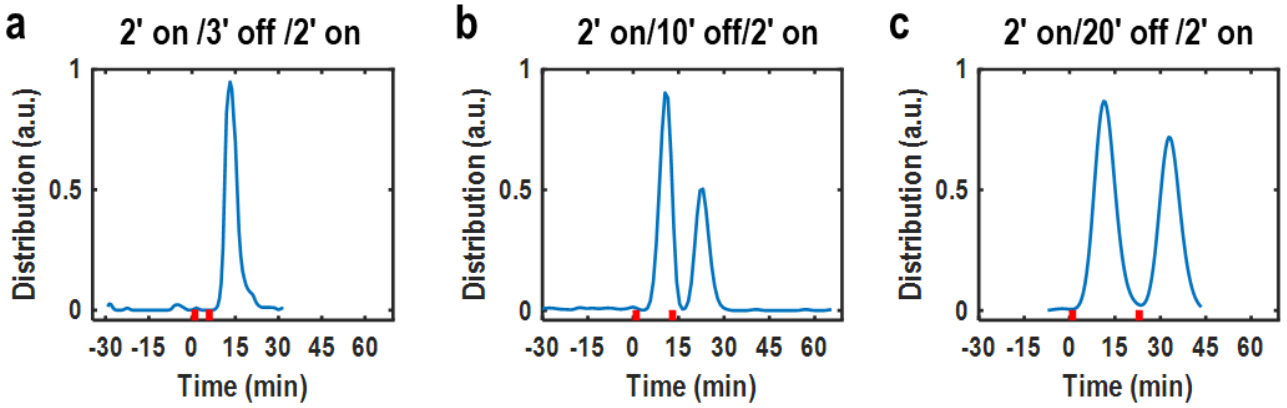

Figure S9. Peak distributions of ERK activation by double 2′ AC stimulation of different interval. Related to Figure 4.

The peak distributions were quantified from the peak points of Figure 4.

a. Peak distribution of ERK activation by the stimulation of 2′ on /3′ off /2′ on.

b. Peak distribution of ERK activation by the stimulation of 2′ on /10′ off /2′ on.

c. Peak distribution of ERK activation by the stimulation of 2′ on /3′ off /2′ on.

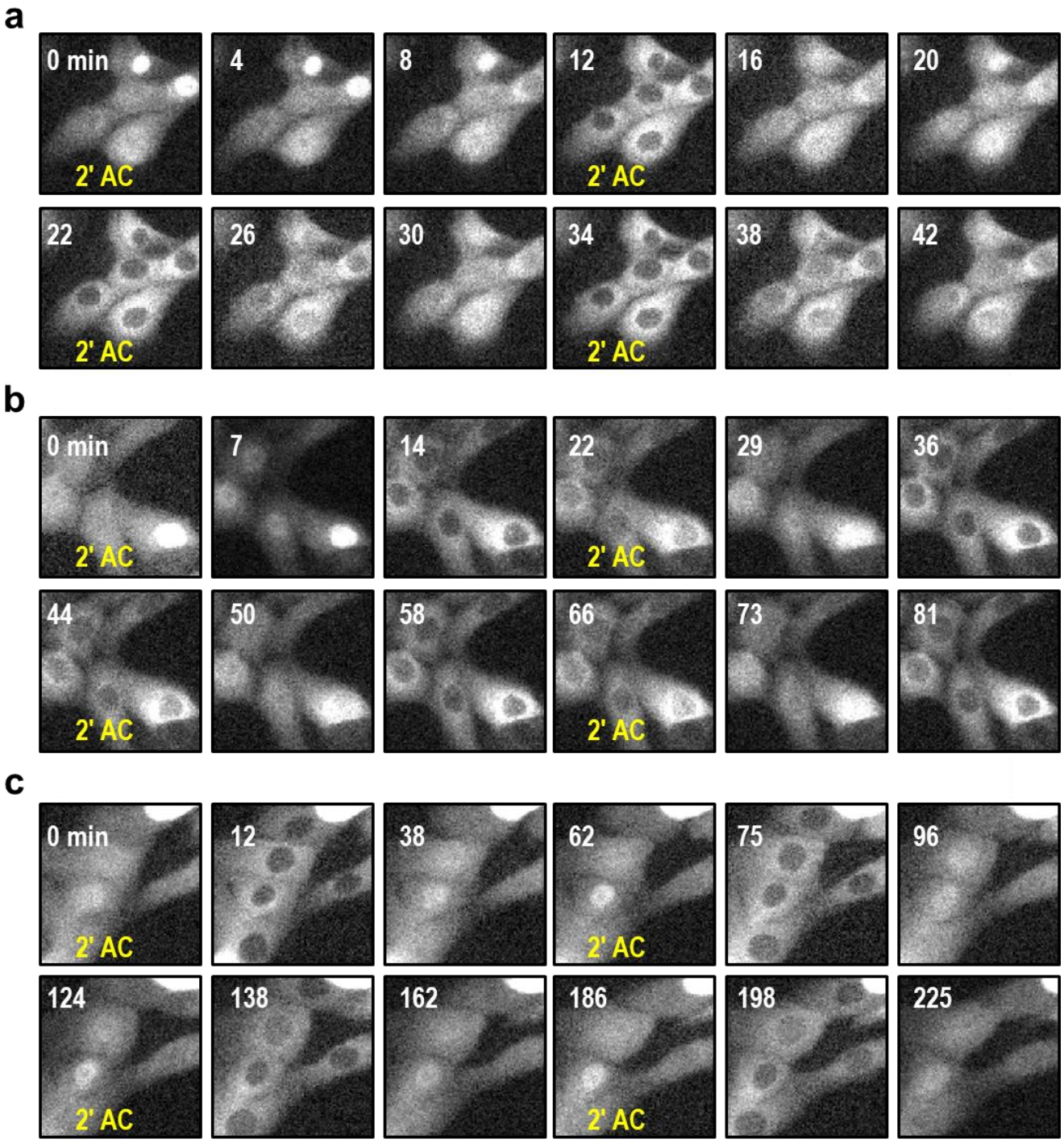

Figure S10. Representative fluorescence images of synchronized frequency modulation of ERK activation by trains of AC stimulation. Related to Figure 5.

a. 2′ stimulations with 10′ intervals induced ERK activation at 5 cph.

b. 2′ stimulations with 20′ intervals induced ERK activation at 3 cph.

c. 2′ stimulations with 60′ intervals induced ERK activation at 1 cph.

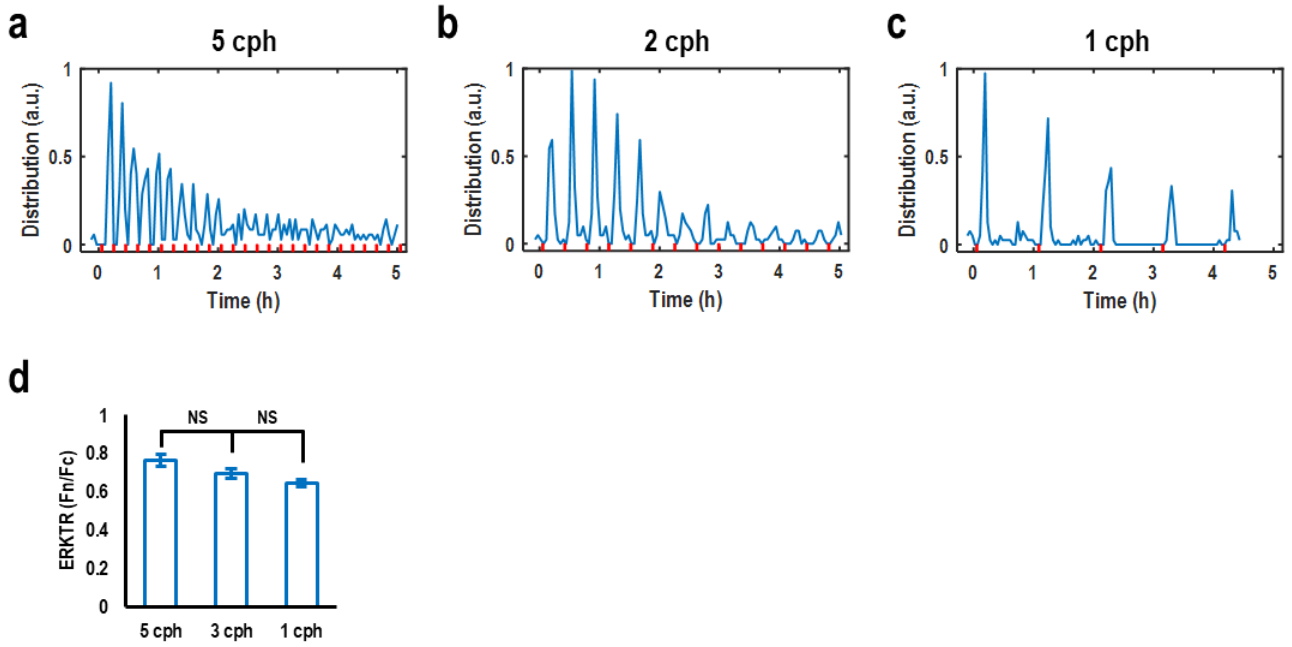

Figure S11. Train stimulation of AC induced robust activation and modulate the frequency of ERK. Related to Figure 5. MCF10A cells expressing ERKTR were exposed to multiple AC stimulations (28V/cm, 500Hz, and bipolar square wave) which indicated as red dots on the x-axes.

a, b, c. ERK peak distribution over time controlled by AC stimulation of 2' duration with 10', 20', or 60' interval. Synchronized ERK activation at frequencies are shown with 5 cph, 3 cph, and 1cph. Over 90% of ERK activation oscillation is induced with precisely controlled time points.

d. Accumulated ERK activation in the first 2 hours. Data are shown as mean ± SD. Statistical significance was determined using an unpaired Student's t-test, n>50, NS: p>0.05.

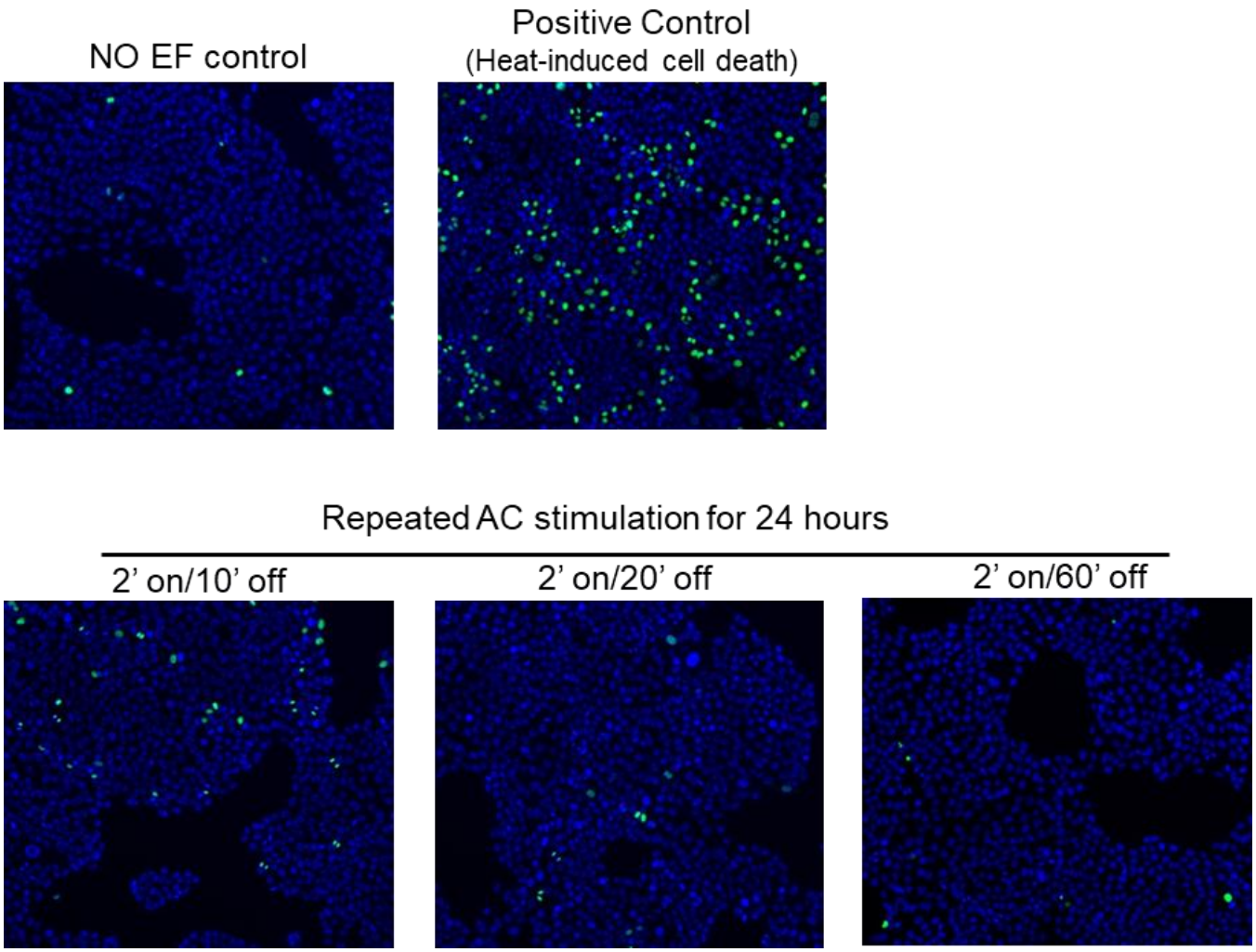

Figure S12. Viability of MCF10A cells after one day of AC stimulation. Related to results and Figure 5.
Blue and green show live and dead cells, respectively. Cell viability staining showed comparable % of healthy cells (> 95%) after 24h of AC exposure (28V/cm, square wave) with different stimulation schemes.

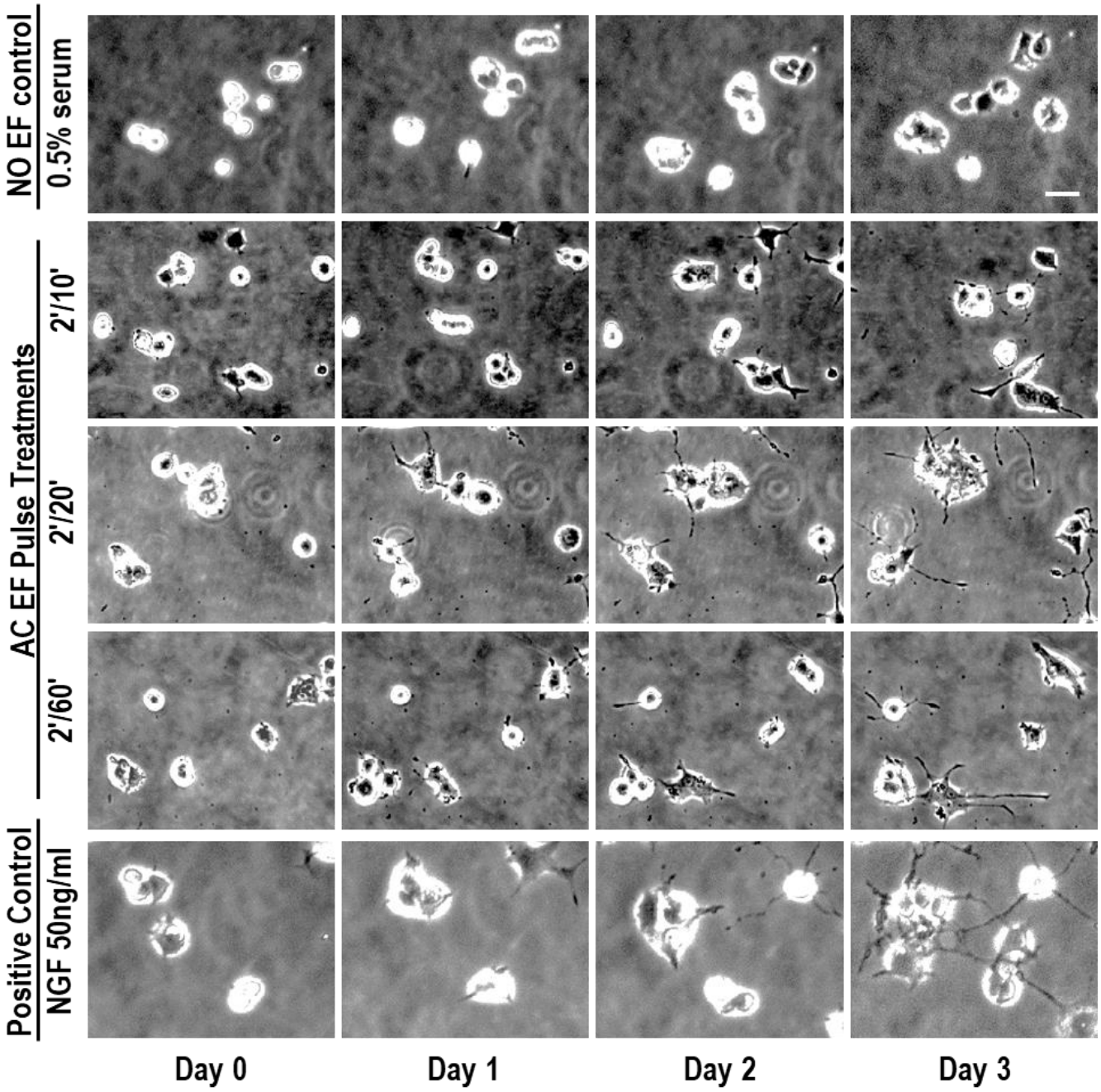

Figure S13. PC12 cell differentiation in time-lapse of 3 days. Related to Figure 7.

Representative phase-contrast images of PC12 cells of negative/positive control and under 2'/10', 2'/20', and 2'/60' AC stimulation regimes in 3 days time-lapse. Scale bar, 25 µm.

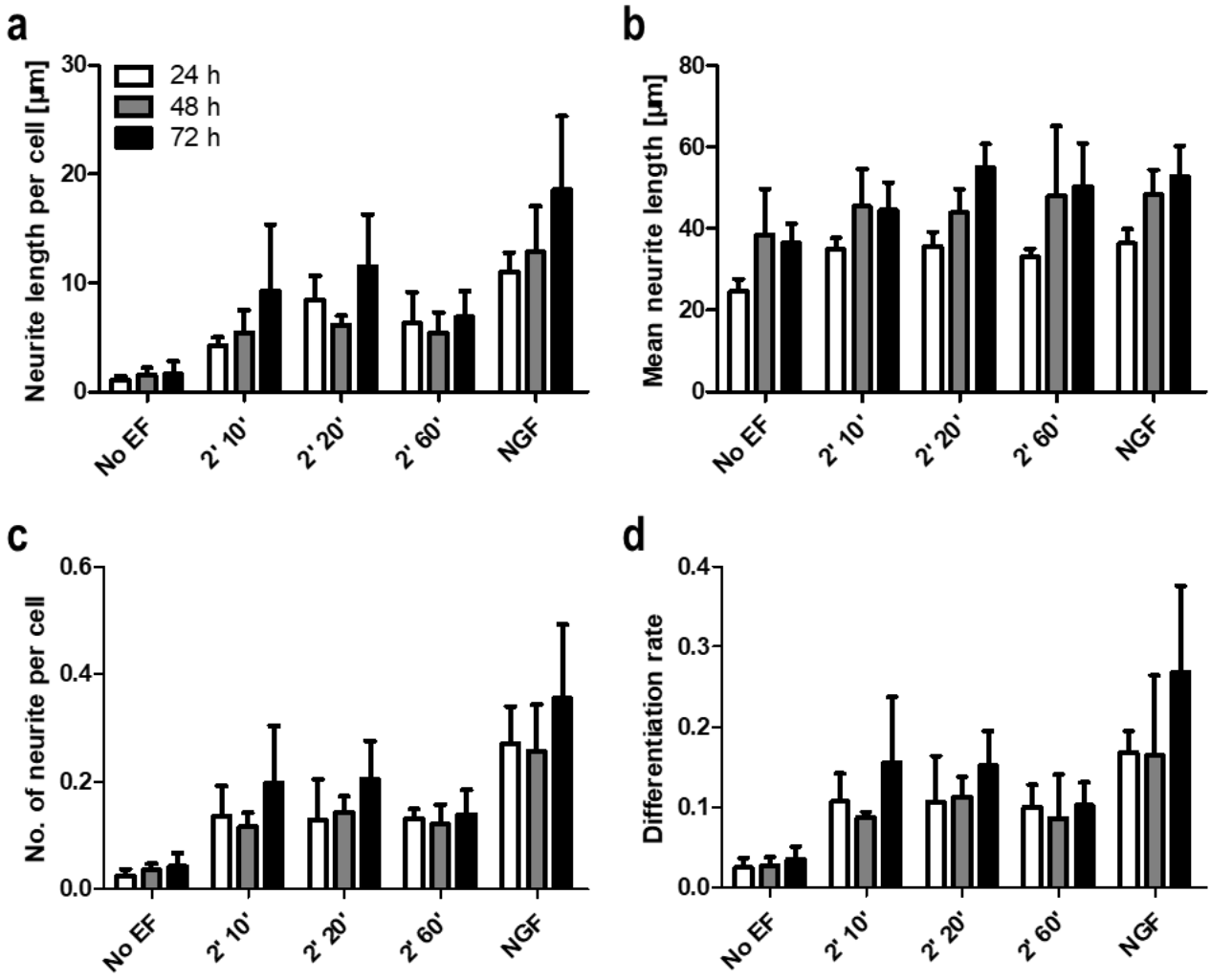

Figure S14. Differentiation of PC12 cells over 3 days. Related to Figure 7.
a. Neurite outgrowth length per cell.
b. Mean neurite outgrowth length.
c. Number of neurites per cell.
d. Differentiation rate of PC12 cells
At least 500 cells were quantified in 2 independent experiments for each condition. Only neurites with length greater than 20 µm were taken into account. Data are shown as mean ± SD.

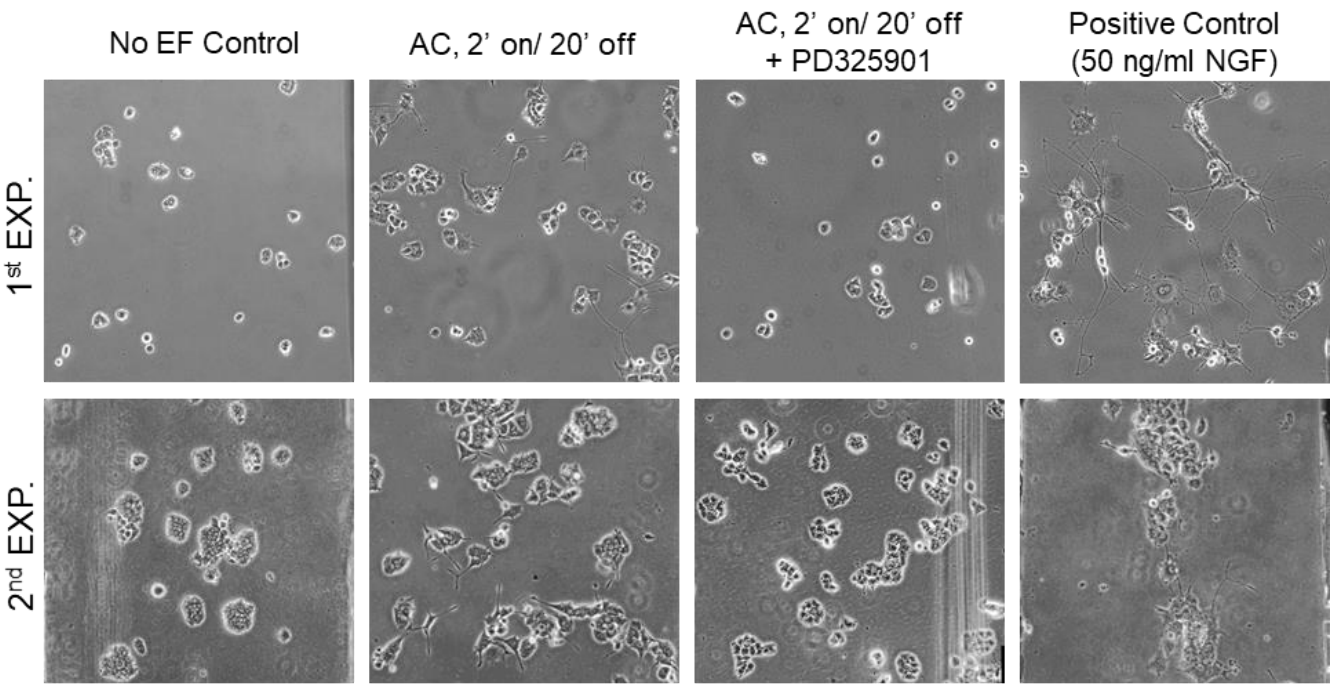

Figure S15. Inhibition of MEK/ERK signaling pathway abolished AC-induced PC12 differentiation. Related to Figure 7.

Representative phase contrast images of PC12 cells from 2 independent experiments. AC (28 V/cm, 500 Hz, square wave) with a selected stimulation scheme (2' on/ 20' off) was applied to cells for 3 days in the absence or presence of a MEK inhibitor, PD325901. Cells treated with NGF (50 ng/ml) was used as a positive control.