

Supplementary Information

SCHEEPDOG: programming electric cues to dynamically herd large-scale cell migration

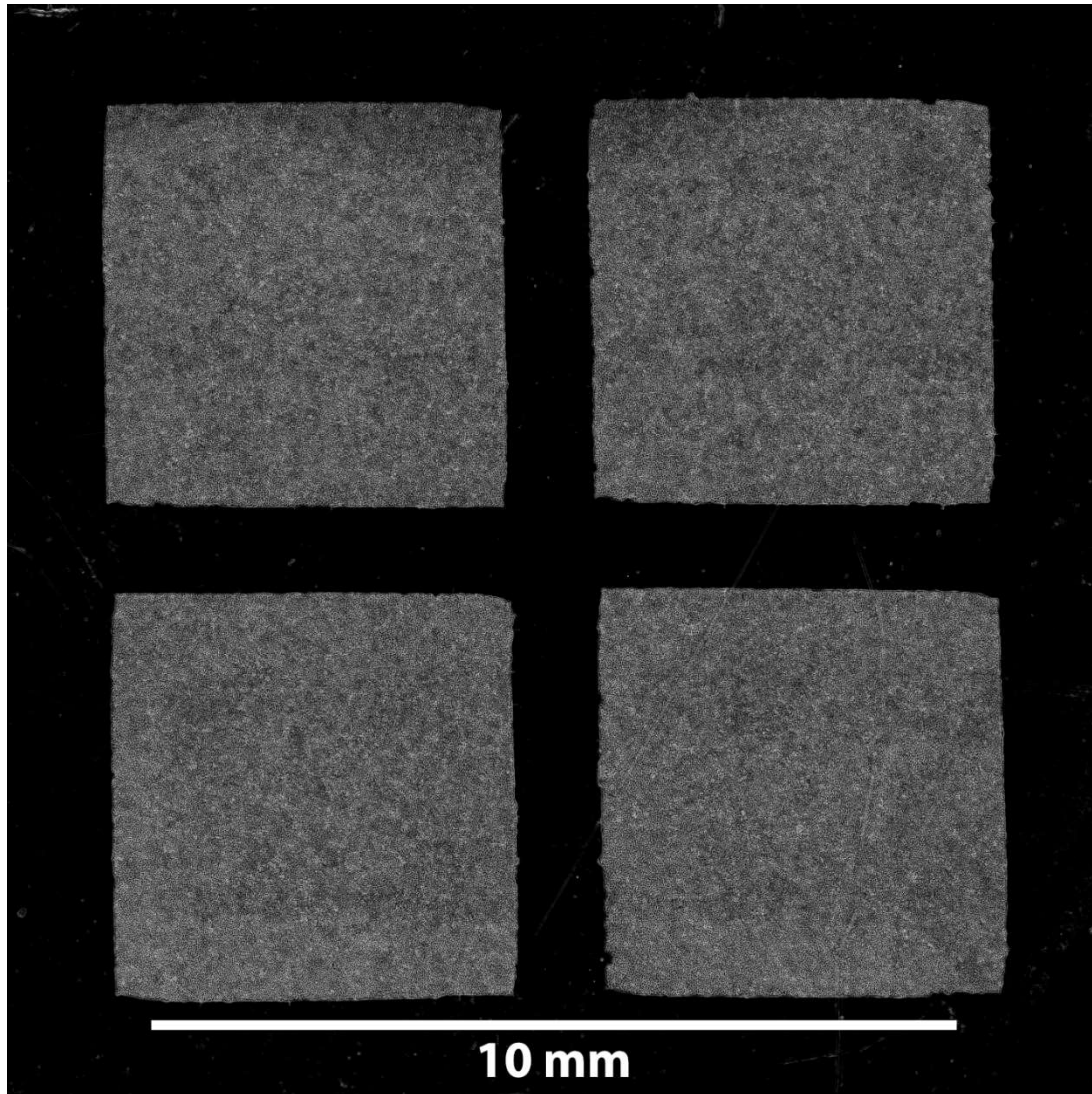
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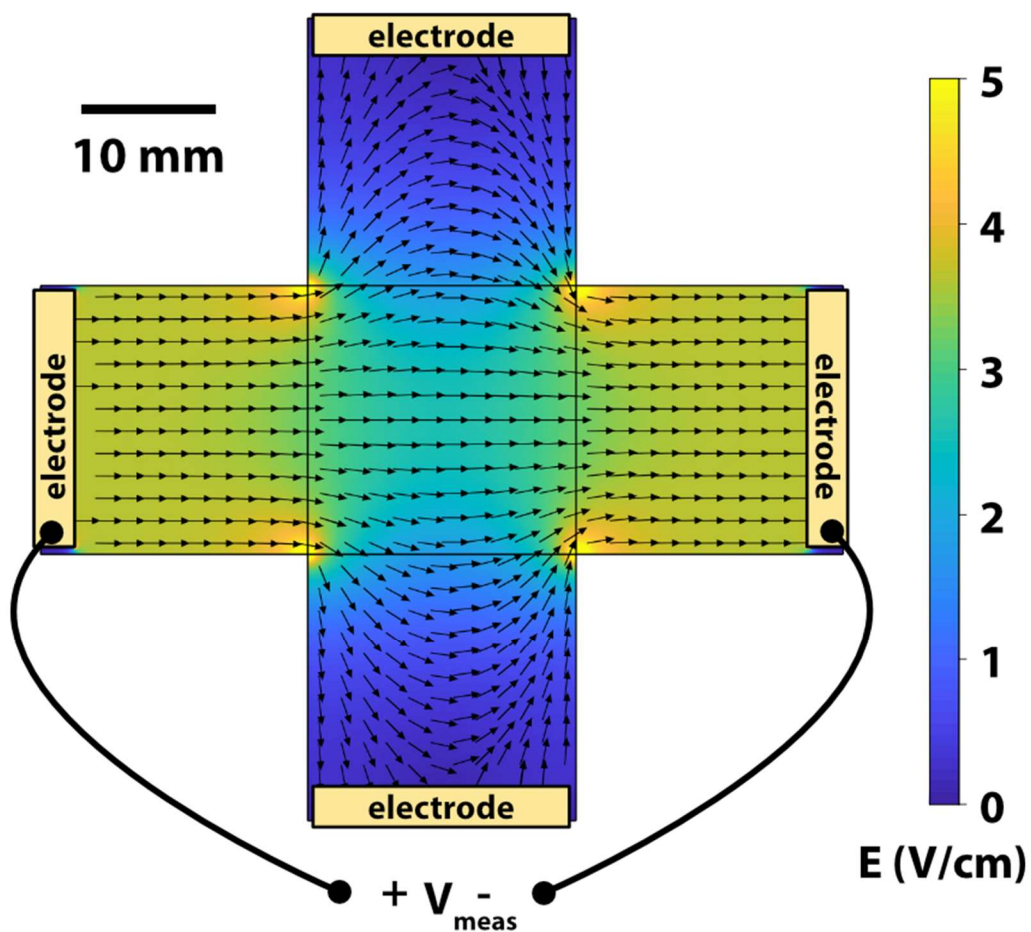
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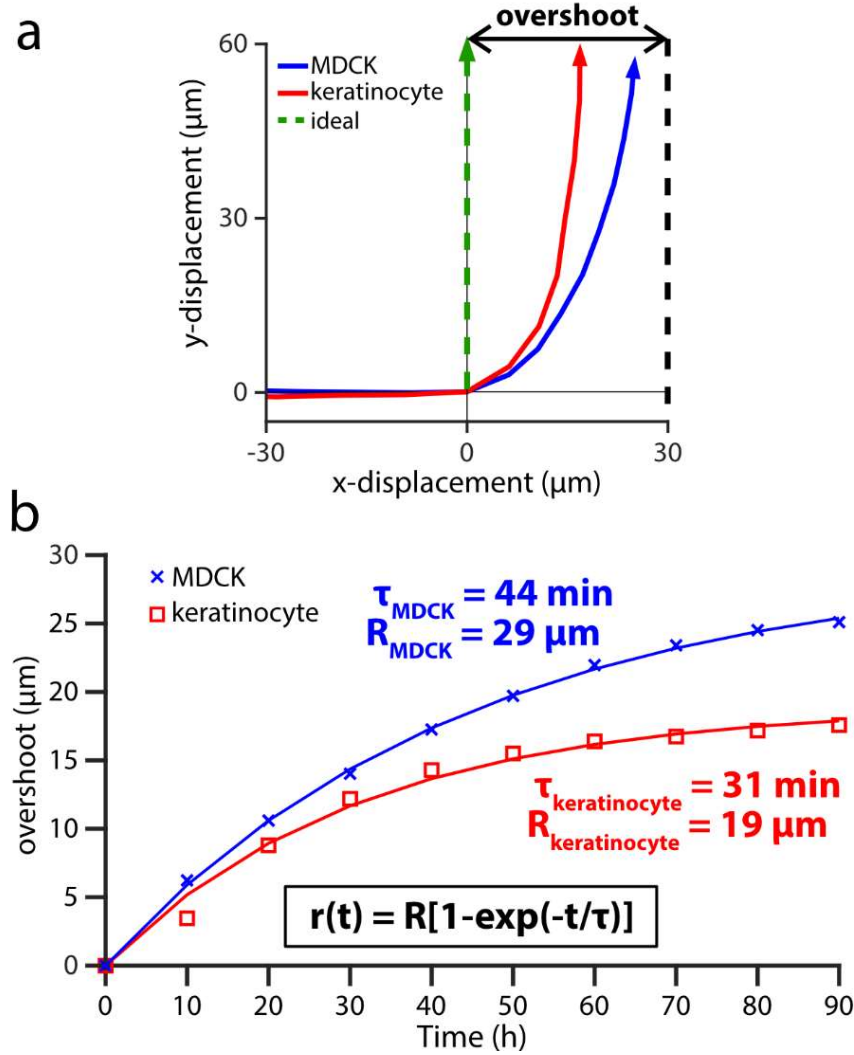
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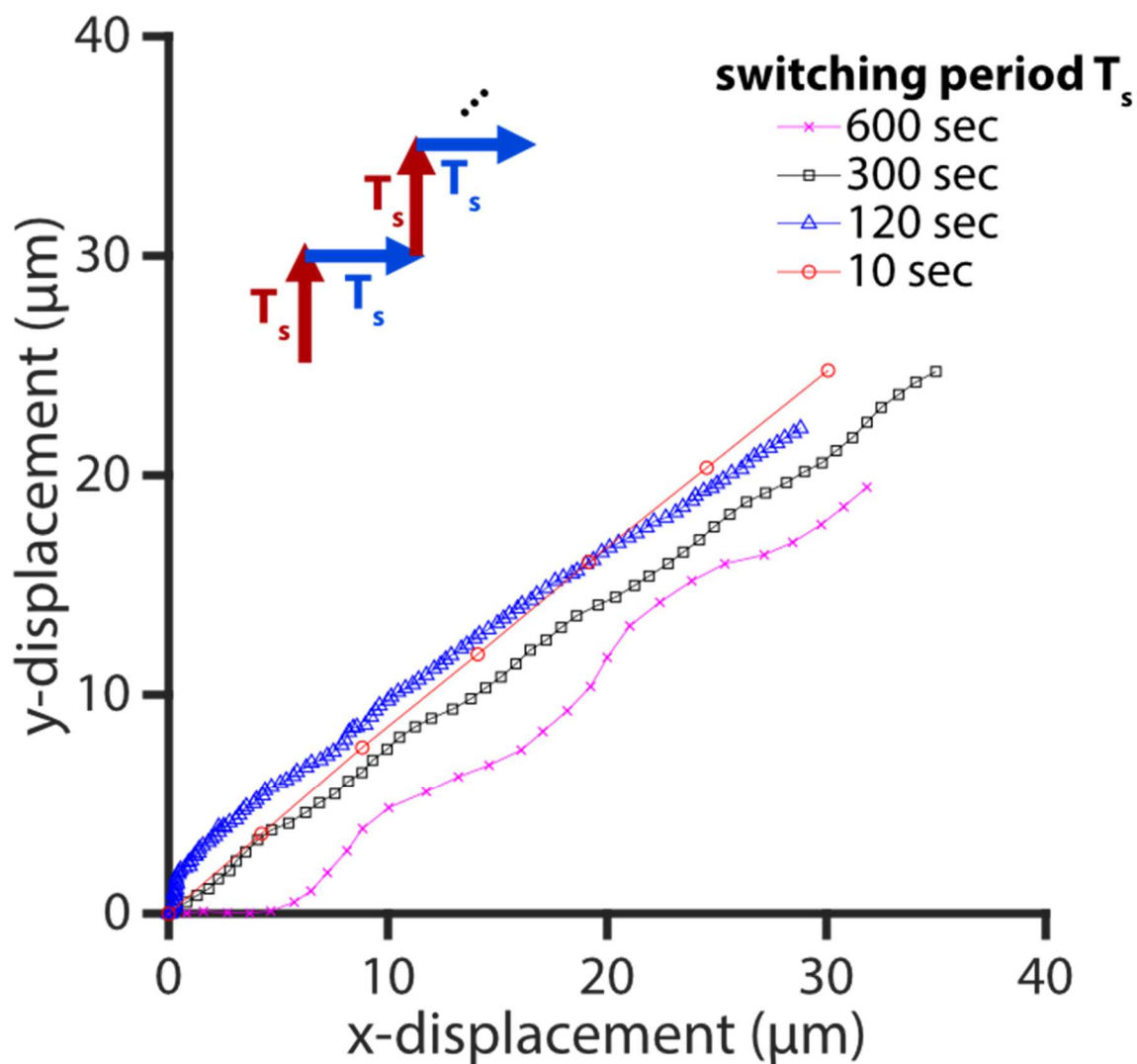
Supplementary Figure 1: Phase-contrast microscopy image of four 5×5 mm MDCK monolayers patterned inside the SCHEEPDOG device's stimulation zone. Image was background subtracted and its brightness was increased for visual clarity. Related to Figs. 1,2.



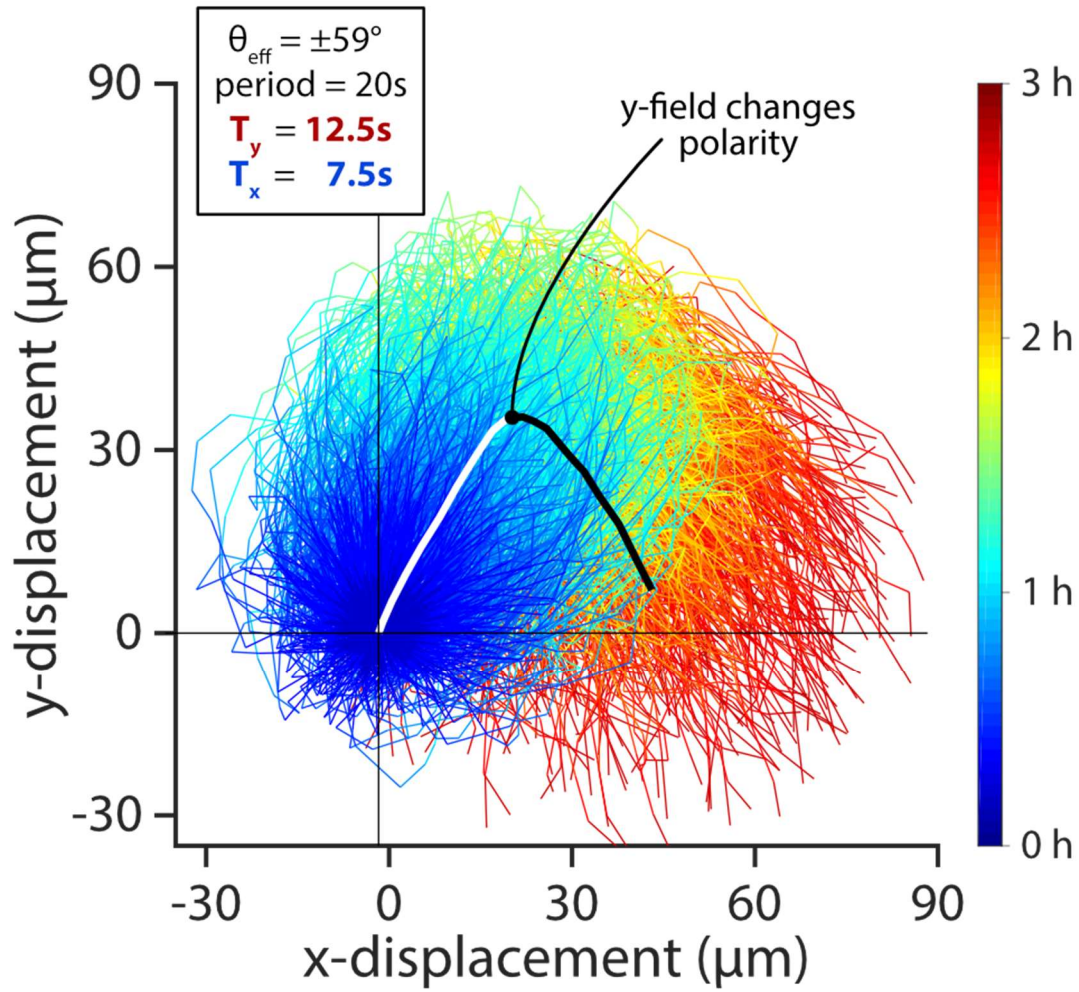
Supplementary Figure 2: Simple double-axis chamber design that results in non-uniform field. Care must be taken when adding a second pair of electrodes to an electrochemical stimulation system to avoid excessive current leak through the orthogonal axis. Otherwise, field uniformity in the center is adversely affected. Related to Fig. 1.



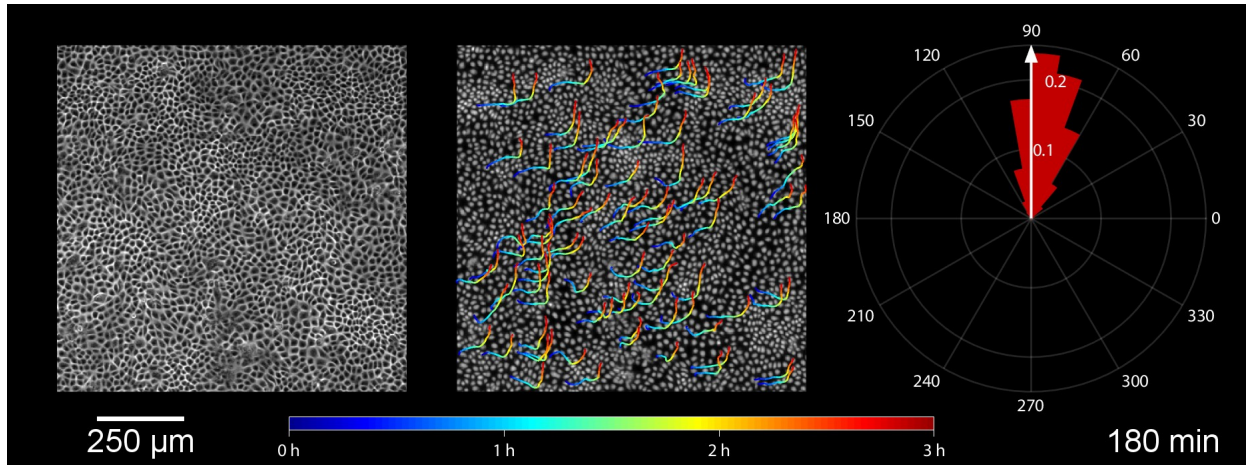
Supplementary Figure 3: Defining electrotactic performance metrics. Related to Fig. 3. We compared MDCK and keratinocyte tissue electrotaxis during programmed 90° turns. The electric field was directed ‘right’ for 1.5 h then ‘up’ for 1.5 h. Data were analyzed from the 2×2 mm central segments of 5×5 mm tissues. (a) Visualization of the mean trajectory overshoot from the ideal trajectory (green) of the MDCK (blue) and keratinocyte (red) tissues. Trajectories averaged across $n = 2$ tissues for each cell type ($n = 4086$ MDCK trajectories and $n = 2642$ keratinocyte trajectories). (b) Comparison of the overshoot, response time, relaxation time and turn time. Data plotted are the same as from panel (a), with least-squares fit lines overlaid. See also Supplementary Videos 1-3.



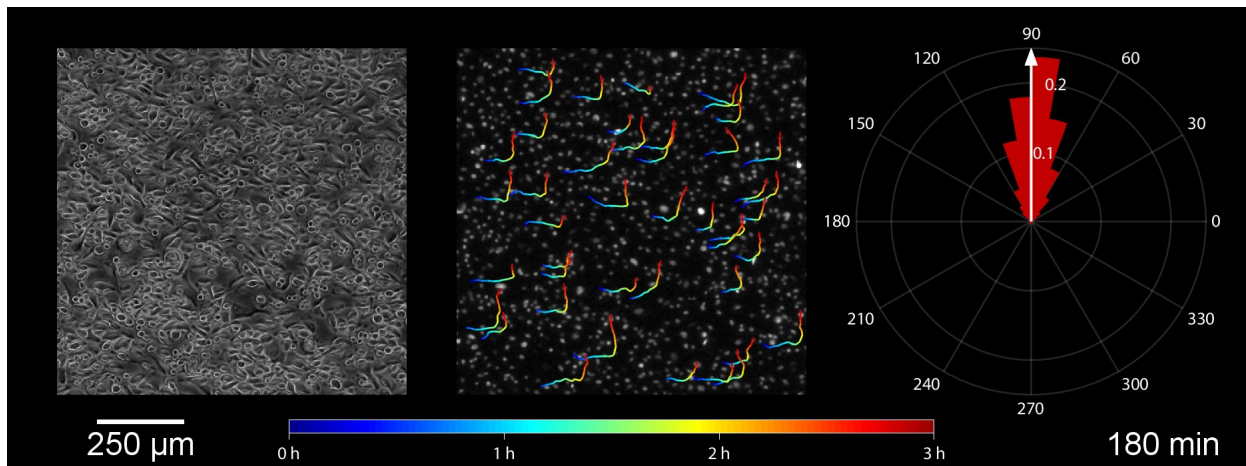
Supplementary Figure 4: Frequency sweep of programmed 45° command over keratinocyte monolayers. Related to Fig. 4. The switching period (T_s) between the horizontal and vertical command signals was varied from 10 seconds to 600 seconds, then mean trajectories for a stimulation period of 1 h were taken from cells within $n = 2$ monolayers for each condition and are plotted here (600 sec, pink x's; 300 sec, black squares; 120 sec, blue triangles; 10 sec, red circles). Meandering in the mean trajectory is apparent at 600 sec switching and 300 sec switching, suggesting that cells are responding to each command in sequence, while commands switching every 120 sec and faster are apparently averaged. Imaging rates were adjusted for $T_s = 600$ sec (every 120 seconds), $T_s = 300$ sec (every 60 seconds), and $T_s = 120$ sec (every 30 seconds). Data for $T_s = 10$ sec was taken from Fig. 4.



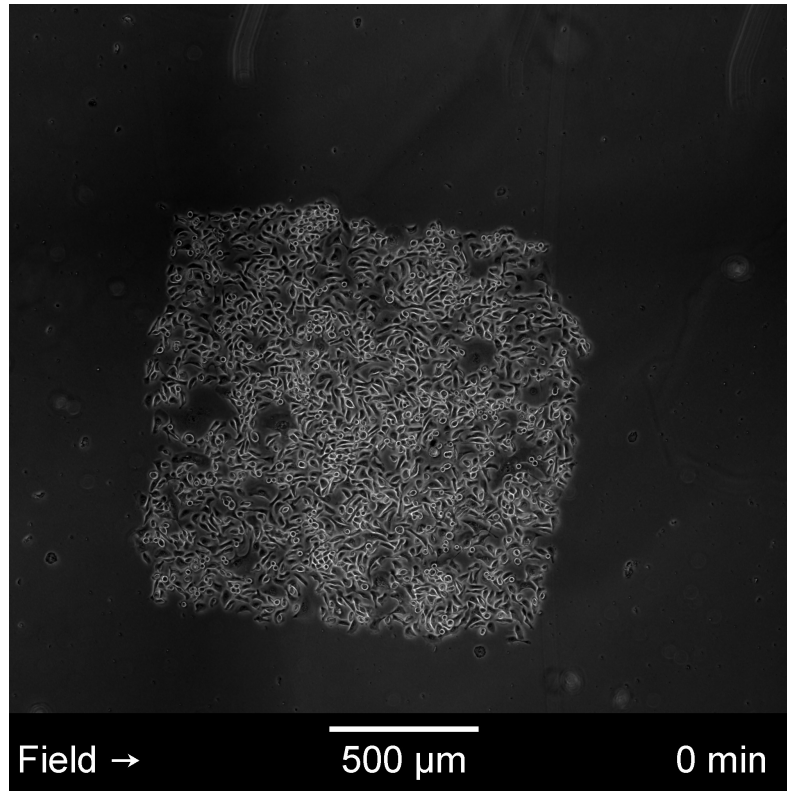
Supplementary Figure 5: Trajectories from programmed 60° then -60° diagonals in MDCK monolayer (trajectories measured from $n = 2$ tissues). Related to Fig. 4. Mean trajectory of individual cell trajectories (white/black). Individual trajectories were taken from 2×2 mm central segments of 5×5 mm tissues during induced migration. For visual clarity, tracks within one standard deviation of the mean cell trajectory are selected and plotted as time-coded multicolor lines ($n = 1337$ trajectories). See also Supplementary Video 6.



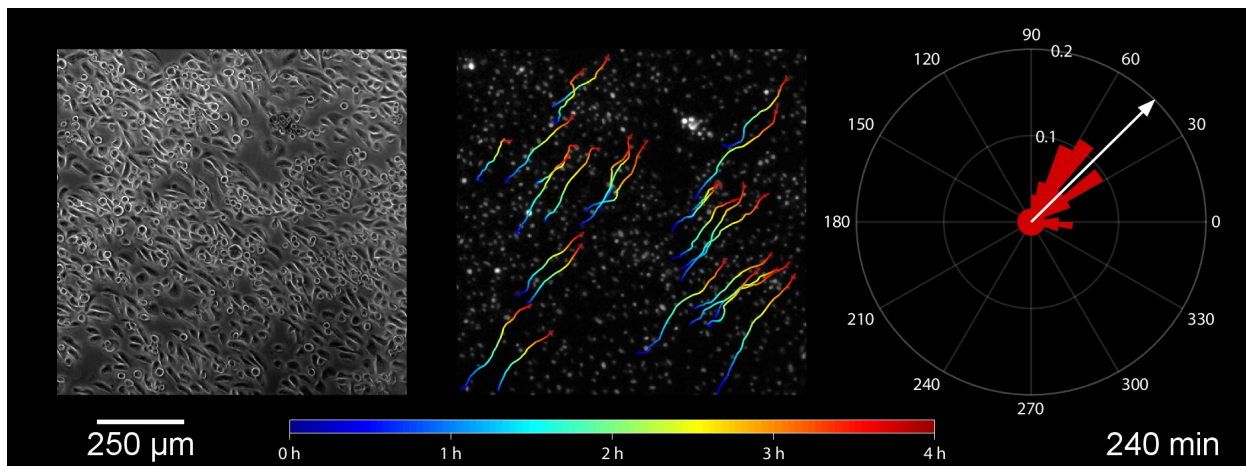
Supplementary Video 1: Time-lapse movie of programmed 90° turn in MDCK monolayer. Field was directed for 1.5 h ‘right,’ then 1.5 h ‘up.’ Left panel shows phase-contrast microscopy of 1 x 1 mm section in the center of the monolayer. Center panel shows selected trajectories overlaid onto nuclear data labeled by a machine-learning classifier. Right panel shows the field direction (white arrow) overlaid onto a polar histogram of cell displacement angle at each time point. See also Fig. 3.



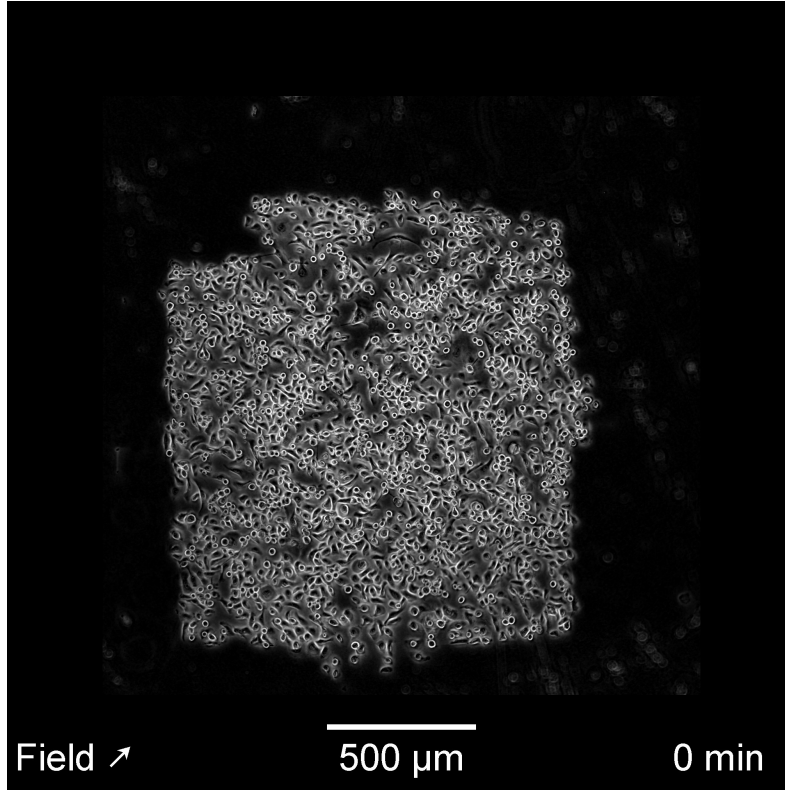
Supplementary Video 2: Time-lapse movie of programmed 90° turn in keratinocyte monolayer. Field was directed for 1.5 h ‘right,’ then 1.5 h ‘up.’ Left panel shows phase-contrast microscopy of 1 x 1 mm section in the center of the monolayer. Center panel shows selected trajectories overlaid onto nuclear data collected by Hoechst 33342 labeling and fluorescence microscopy. Right panel shows the field direction (white arrow) overlaid onto a polar histogram of cell displacement angle at each time point. See also Fig. 3.



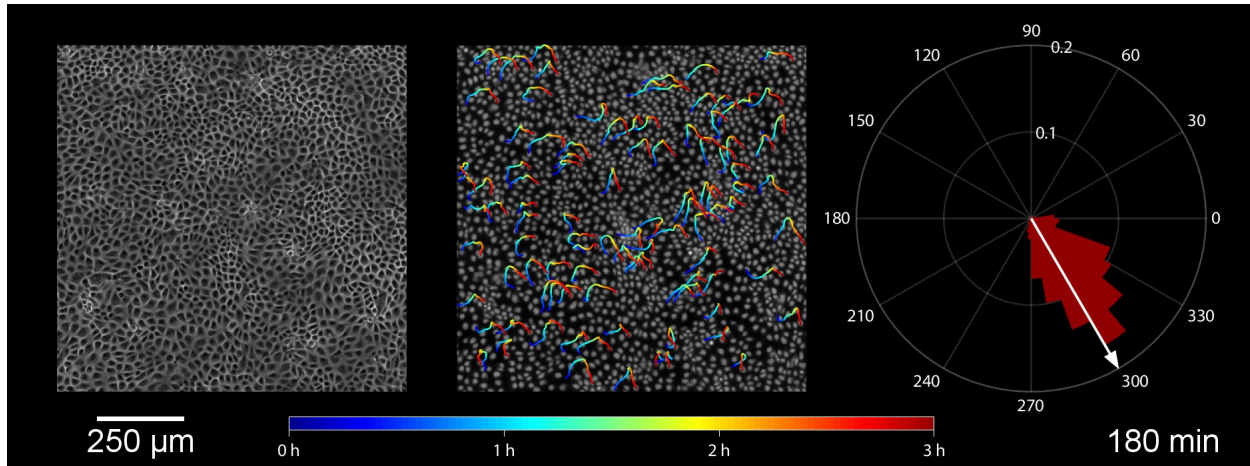
Supplementary Video 3: Time-lapse movie of extended 90° turn in keratinocyte monolayer, showing whole monolayer translation. Field was directed for 4 h ‘right,’ then 4 h ‘up.’ See also Fig. 3.



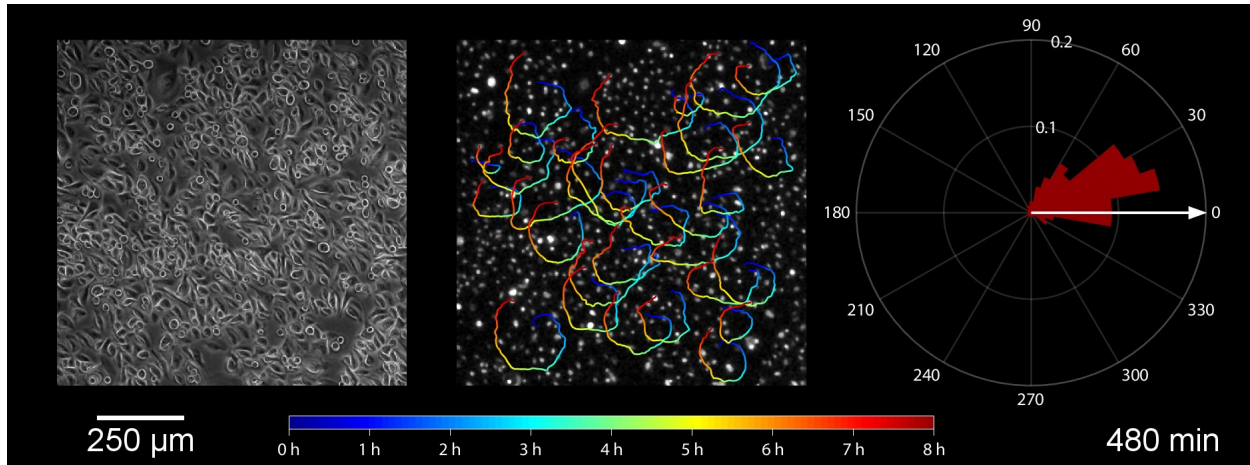
Supplementary Video 4: Time-lapse movie of programmed 45° diagonal in keratinocyte monolayer. Field was rapidly alternated between axes for effective stimulation angle of $\theta_{\text{eff}} = 45^\circ$ for 4 h. Left panel shows phase-contrast microscopy of 1 x 1 mm section in the center of the monolayer. Center panel shows selected trajectories overlaid onto nuclear data collected by Hoechst 33342 labeling and fluorescence microscopy. Right panel shows the field direction (white arrow) overlaid onto a polar histogram of cell displacement angle at each time point. See also Fig. 4.



Supplementary Video 5: Time-lapse movie of whole tissue translation at 45° angle in keratinocyte monolayer. Field was rapidly alternated between axes for effective stimulation angle of $\theta_{\text{eff}} = 45^\circ$ for 4 h. See also Fig. 4.



Supplementary Video 6: Time-lapse movie of programmed 60° , then -60° diagonals in MDCK monolayer. Field was rapidly alternated between axes for effective stimulation angle of $\theta_{\text{eff}} = 60^\circ$ for 1.5 h, then $\theta_{\text{eff}} = -60^\circ$ for 1.5 h. Left panel shows phase-contrast microscopy of 1×1 mm section in the center of the monolayer. Center panel shows selected trajectories overlaid onto nuclear data labeled by a machine-learning classifier. Right panel shows the field direction (white arrow) overlaid onto a polar histogram of cell displacement angle at each time point. See also Supplementary Fig. 5.



Supplementary Video 7: Time-lapse movie of programmed circle maneuver in keratinocyte monolayer. Field was rapidly alternated between axes to rotate the field clockwise at a rate of 45° h^{-1} over 8 h. Left panel shows phase-contrast microscopy of $1 \times 1 \text{ mm}$ section in the center of the monolayer. Center panel shows selected trajectories overlaid onto nuclear data collected by Hoechst 33342 labeling and fluorescence microscopy. Right panel shows the field direction (white arrow) overlaid onto a polar histogram of cell displacement angle at each time point. See also Fig. 5.