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Supplemental Information

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Electric-induced reversal of morphogenesis in *Hydra*

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Supporting Figures

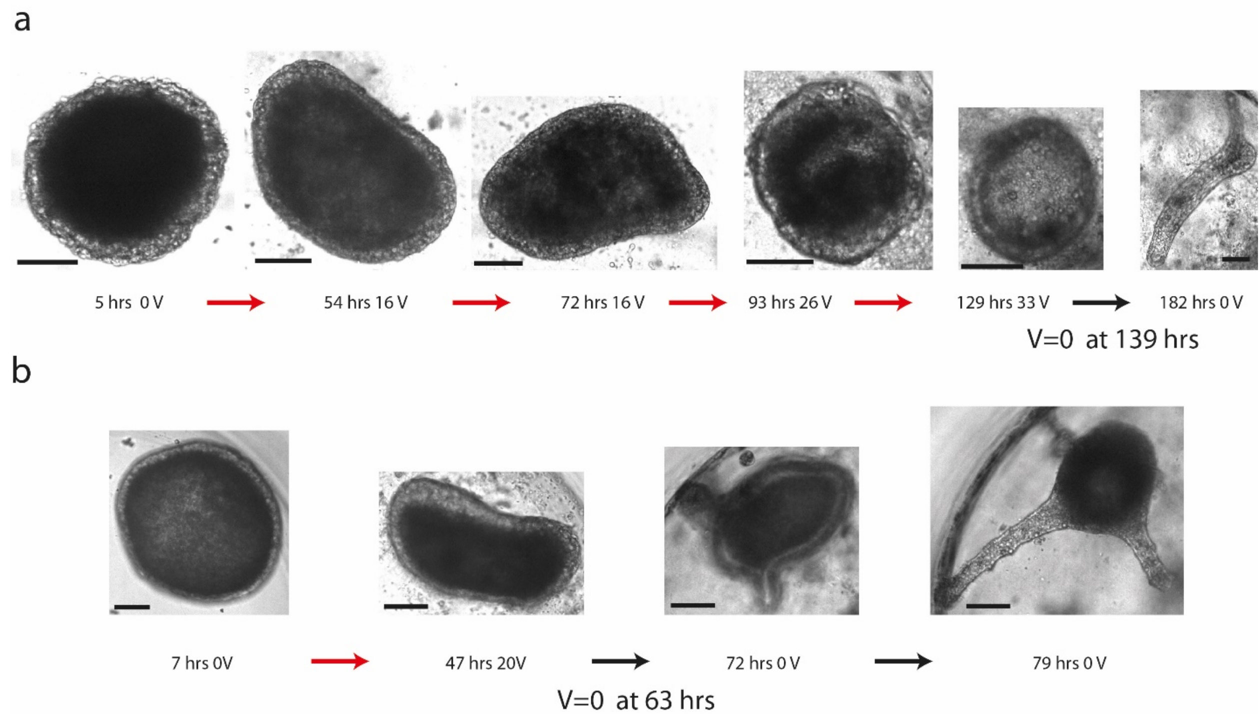


Fig. S1: Examples of halted regeneration by an external electric field. A series of images from two experiments (a, b) depicting the trajectory scheme in Fig. 1a top row of the main text. Time (hrs from tissue excising) and voltages (V_{pp}) are indicated for each image. The tissues do not develop under the electric field above its critical value (specific for each tissue; 1 kHz) until time points extending the maximal regeneration time observed in our experiments (~55 hrs) and they readily regenerate into a mature *Hydra* upon switching the voltage to zero at the indicated time. Red arrow – under voltage, black arrow – V=0. Bars 100 μm scale.

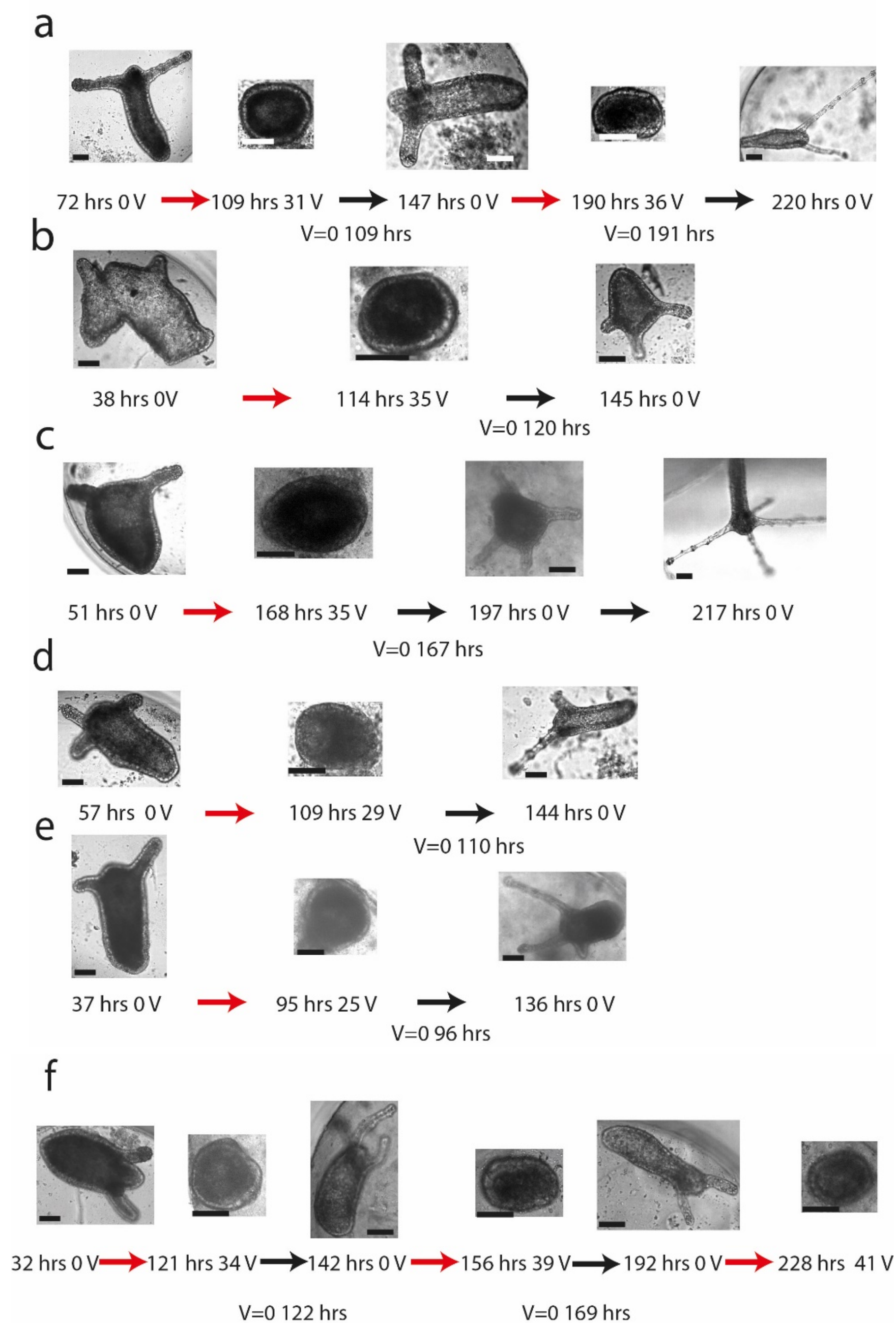


Fig. S2: Examples of reversal of morphogenesis under an external electric field and the emergence of new morphologies upon renewal of regeneration. A series of images depicting the trajectory scheme in Fig. 1a bottom row of the main text. Each row of images (a-f) is a separate experiment. Time (hrs from tissue excising) and voltages (V_{pp}) are depicted below the images (external AC field at 1 kHz). In all the experiments presented here, the spheroid tissue first regenerates into a mature *Hydra* and then folds back into a spheroid upon the increase of the externally applied voltage above the critical value (specific for each tissue). The reversed tissue regenerates again upon the reduction of the external voltage to zero at the indicated time. Note that the emerged renewal morphology is in some of the cases not similar to the initial morphology. The images in (a,f) demonstrate that this backward-forward cycle of morphogenesis can be repeated for the same tissue. Red arrow – under voltage, black arrow – V=0.Bars 100 μm scale.

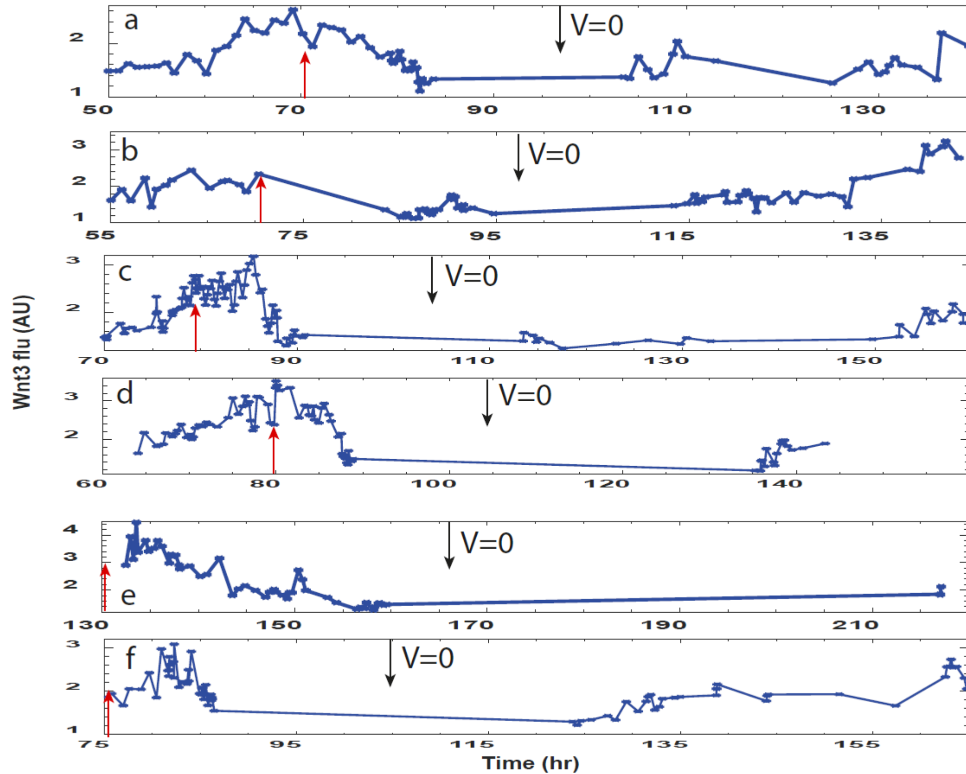
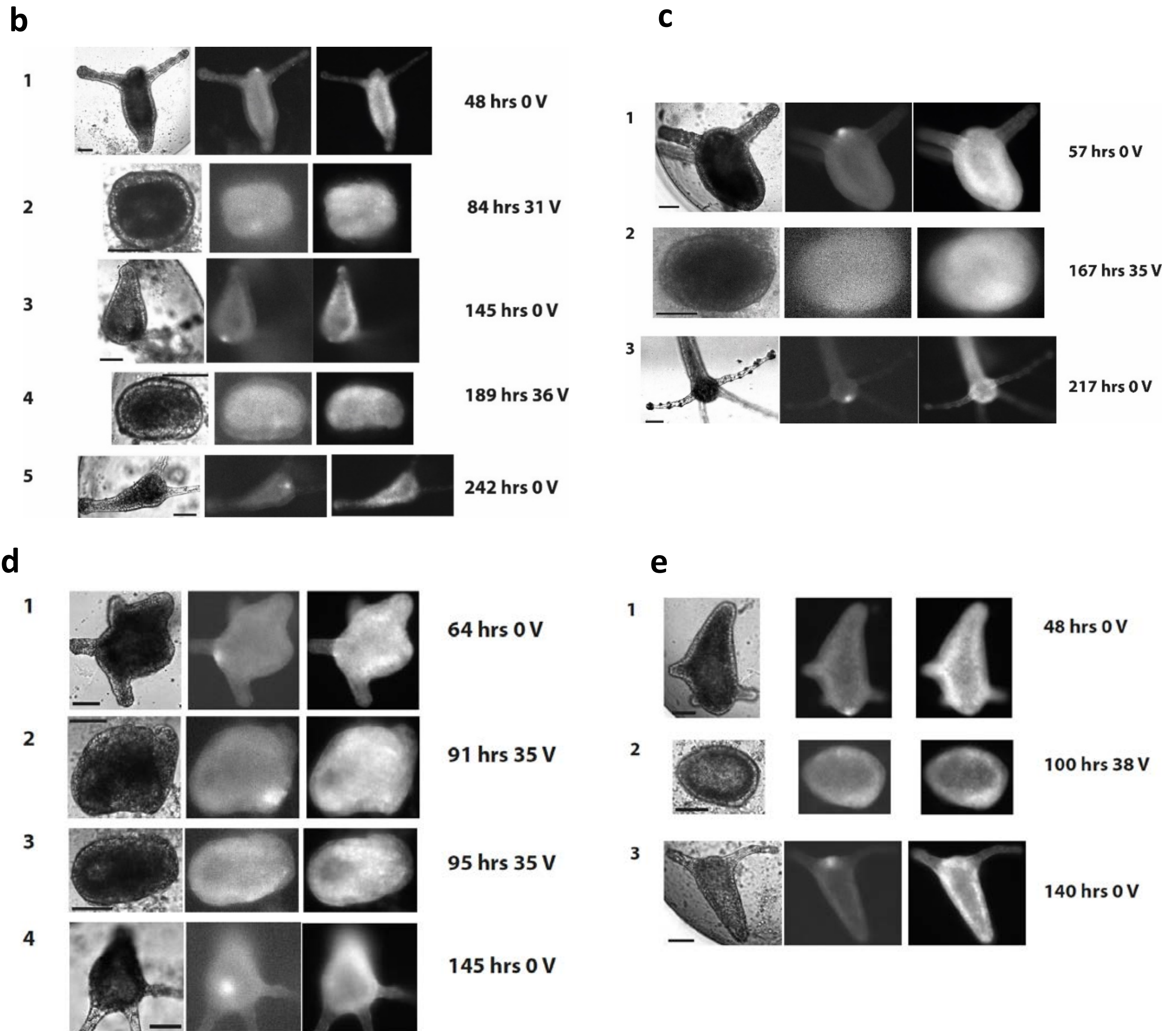


Fig. S3a: Traces of the *Wnt3* activity under a field. (a-f) The *Wnt3* activity is estimated in 6 samples (utilized together with the trace shown in Fig. 2 of the main text to produce the mean curve shown in the inset there). Transgenic *Hydra* expressing a GFP probe under the control of the *Wnt3* promoter is imaged under a fluorescence microscope with time-lapse images taken at 1 min intervals (as in Fig. 2 in the main text). The traces show the GFP fluorescence density in the region around the head organizer normalized by the average background GFP level of the tissue outside this region. Measurements at nearby time points show the fluctuation levels of the signal, due to the precise location of the head organizer relative to the surrounding tissue and fluctuations in the spatial organization of the cells comprising it. The red arrows in (a-d) mark the time point at which the external voltage is increased above 20 V_{pp} (this point is prior to the first measured point for traces (e-f)). The black arrows mark the time points at which the external voltage is switched to zero.



Figs. S3b-e: Example images of the *Wnt3* activity decay upon reversal of morphogenesis. The transgenic *Hydra* used to measure the *Wnt3* activity is also expressing dsRED (RFP) under the control of the ubiquitous actin promoter, serving as a fluorescence reference. Four experiments are shown as a sequence of 3 images (from left: bright field, GFP, RFP) over time. The running time (hrs from the point of tissue excision) and the applied external voltage (V_{pp}) are depicted to the right of the images. A clear fluorescence signal of GFP under the *Wnt3* promoter at the head of a transgenic *Hydra* emerges after the initial spheroid tissue regenerates (1) and decays upon the reversal of morphogenesis in (2;3 in d). The *Wnt3*-activated fluorescence reemerges upon renewal regeneration after the voltage is switched off in (3;4 in d). The experiment in (a) demonstrates a second cycle of reversal morphogenesis (4) and renewal of regeneration leading to re-emergence of the *Wnt3*-activity fluorescence signal. The images correspond to the following traces in (Fig. S3a): (b)-trace f, (c)-trace e, (d)-trace c, (e)-trace a. Bars 100 μm scale.

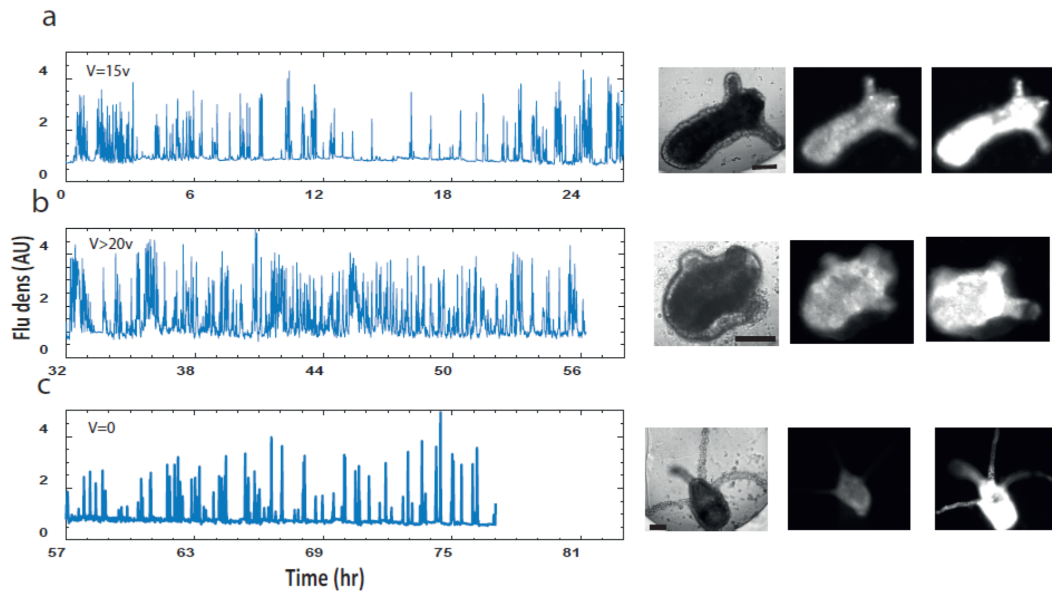


Fig. S4: Calcium dynamics. (a) Ca^{2+} dynamics as measured by the fluorescence density (fluorescence signal per unit area of the entire tissue normalized by the background signal) as in Fig. 3c in the main text. The measurement starts 3 hrs after the tissue excision at 15 V_{pp} which is below the critical value (a). The voltage is then increased to higher values (24 V_{pp}) in (b) and then switched off in (c). The images on the right show microscopy images of the sample at the end point of each trace (from left: bright field, low and high Ca^{2+} activity depicted by the fluorescence images). Bars 100 μm scale.

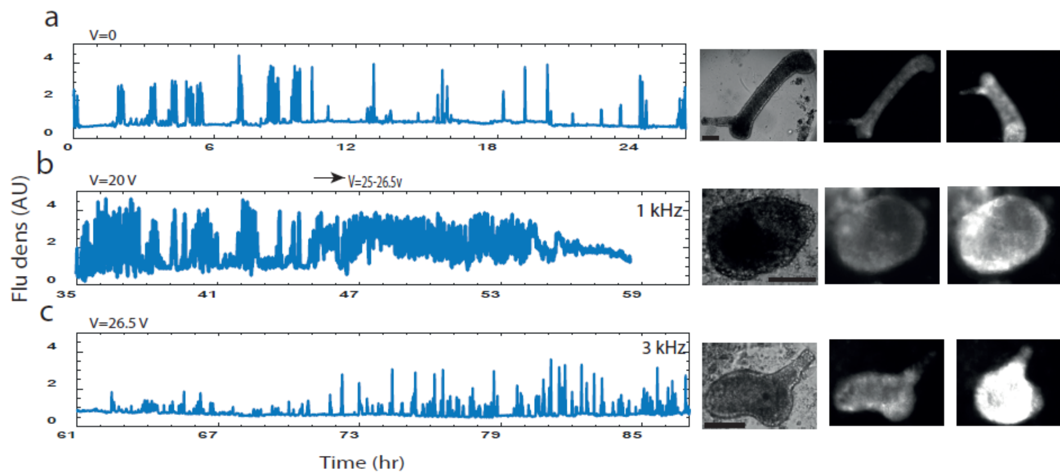


Fig. S5: Frequency cutoff. Ca^{2+} activity measured by the fluorescence density (fluorescence signal per unit area, measured over the entire tissue and normalized by the background signal) as in Fig. 4a in the main text, for two different frequencies of the external electric field. The measurement starts 3 hrs after the tissue excision at V=0 (a) and continues at high voltage (20-26.5 V_{pp}) at 1 kHz (b), showing enhanced Ca^{2+} activity. Switching the frequency of the external AC field to 3 kHz, while maintaining the voltage amplitude at 26.5 V_{pp}, shows reduction in Ca^{2+} activity (c). The images at the right show microscopy images of the sample at the end point of each trace (from left: bright field, low and high Ca^{2+} activity depicted by the fluorescence images). They show that the tissue fully regenerates for V=0 and then folds back into a spheroid morphology at high voltage, while resumption of a fully regenerated *Hydra* is observed upon the switch of the frequency to 3 kHz. Bars 100 μm scale.

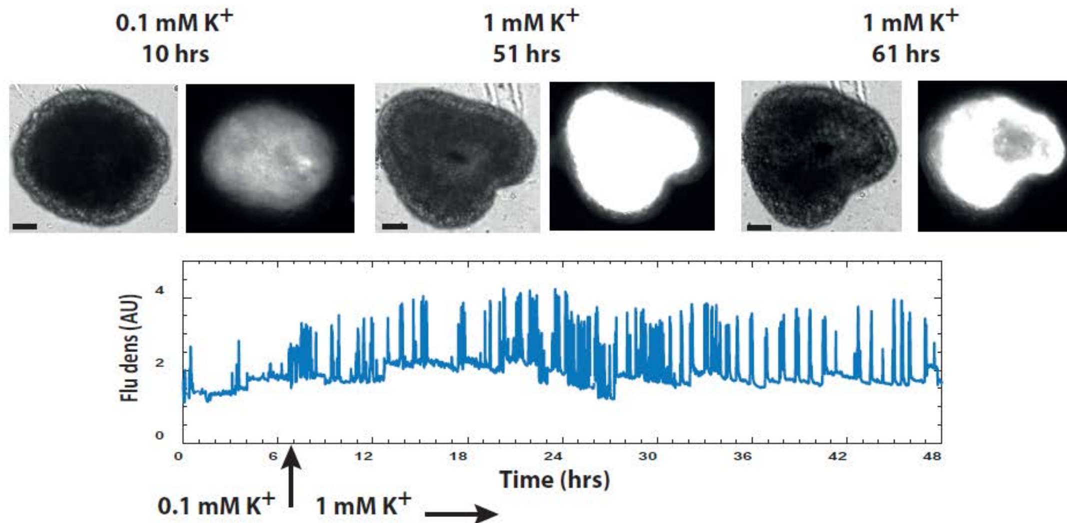


Fig. S6: Stimulation by elevated potassium. The trace shows enhanced Ca^{2+} activity measured by fluorescence density (fluorescence signal per unit area of the entire tissue normalized by the background signal) upon the increase of the potassium (K^+) in the medium from 0.1 mM (normal medium) to 1 mM at the time point marked by the arrow. The measurement starts 3 hrs after the tissue excision, in the absence of an external field. Microscopy images of the sample at the indicated time points (hrs from the excision point) and K^+ concentrations are shown above the trace in pairs (left: bright field; right Ca^{2+} activity depicted by the fluorescence images). The images show that after 61 hrs there are no signs of regeneration in 1 mM K^+ while all samples at the normal *Hydra* medium (0.1 mM K^+) regenerate within 55 hrs.

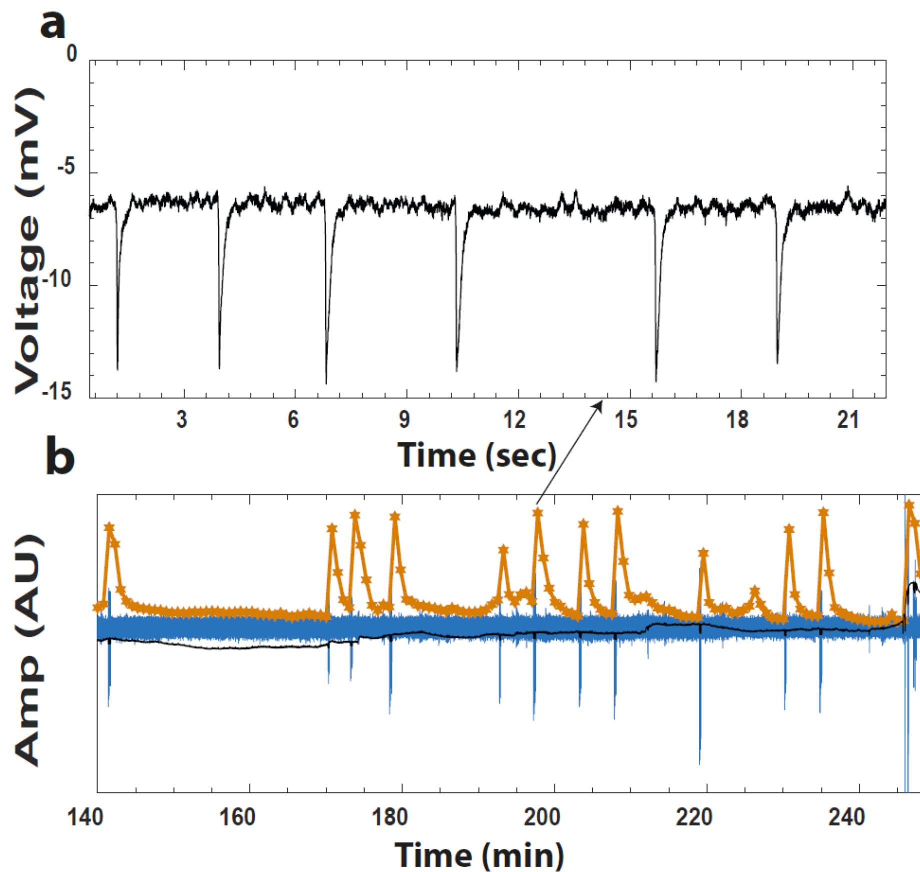


Fig. S7a: Electrical measurement in a tissue fragment. (a) Voltage measurement of a spike burst by a silver-chloride electrode inserted into a tissue fragment embedded in a low-melting 2% agarose gel. (b) Simultaneous recording of spontaneous Ca^{2+} activity by fluorescence microscopy (yellow) and the voltage by an electrode (black). The blue trace shows the voltage spikes extracted from the original voltage measurement by subtracting a smoothed trace (0.1 sec window) from the original one and amplifying the resulting trace (distorting the uniphase spikes, but allowing easy identification of them). Each Ca^{2+} spike follows a burst of electrical spikes. The burst in (a) is a zoom over a burst near 197 min in (b) - arrow. Note the seconds time scale in (a) and minutes in (b). Measurements are done in the absence of an external field.

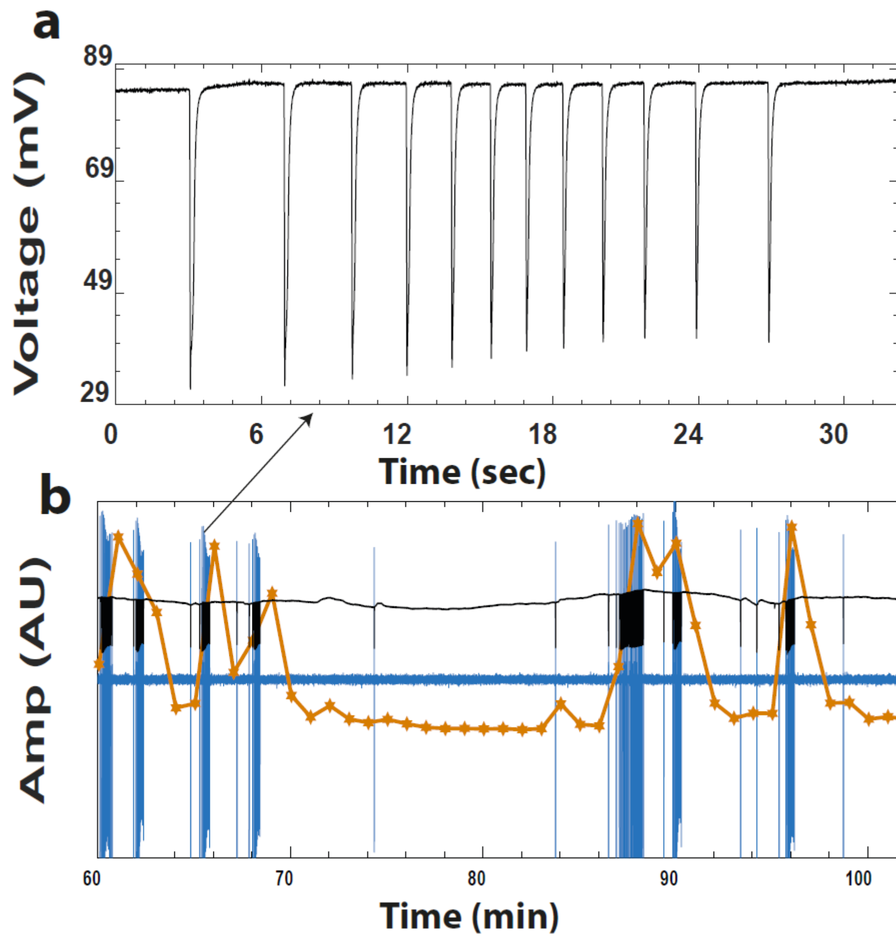


Fig. S7b: Electrical measurement in a spheroid. (a) Voltage measurement of a spike burst by a silver-chloride electrode inserted into a tissue spheroid (around 3 hrs after excision and folding in solution) embedded in a low-melting 2% agarose gel. (b) The simultaneously recording of the spontaneous Ca^{2+} activity by fluorescence microscopy (yellow) and the voltage by an electrode (black). The blue trace shows the voltage spikes extracted from the original voltage measurement as in Fig. S7a. Each Ca^{2+} spike follows a burst of electrical spikes. The burst in (a) is a zoom over a burst near 65 min in (b) - arrow. Note the seconds time scale in (a) and minutes in (b). Measurements are done in the absence of an external field.

Supplementary Videos:

Movie S1: Reversal of morphogenesis. Bright-field microscopy movie of a tissue spheroid regenerating into a mature *Hydra* and then folding back its morphology under an electric field. The reversed tissue regenerates again upon the reduction of the external voltage to zero. A second round of reversal of morphogenesis then follows. Note the differences in the critical voltages required for reversal of morphogenesis for the first and second rounds; the voltage range in the first round is 20-30 V while in the second round is 30-40 V. The scale of the image is ∼1.1 mm and the running time is from the point of tissue excision.

Movie S2: Decay of *Wnt3* during reversal of morphogenesis. The same sample as in Movie S1 is observed under a fluorescence microscope for GFP (left) and RFP (right). The GFP is under the *Wnt3* promoter and the RFP is under the control of the ubiquitous *Hydra* actin promoter and serves as a fluorescence reference. The movie shows the two rounds of reversal of morphogenesis as in Movie 1. The scale of the image is ∼1.1 mm and the running time is from the point of tissue excision.

Movie S3: Dynamics of Ca^{2+} in reversal of morphogenesis under an electric field. Time lapse fluorescence microscopy of a strain expressing the GCaMP6s probe reporting Ca^{2+} activity in the epithelial cells. The tissue spheroid regenerates under a voltage below the critical value and then folds back as the voltage is increased. The tissue regenerates again upon switching the voltage to zero. The scale of the image is ∼1.1 mm and the running time is from the point of tissue excision.

Movie S4: Frequency cutoff of the external field. Bright-field microscopy of a tissue under an external AC electric field at two different frequencies. At 1 kHz and voltage above criticality, the *Hydra* exhibits reversal of morphogenesis and folds back into a spheroid while it regenerates again at 3 kHz under the same voltage. The movie shows two rounds of the backward-forward morphogenesis cycle under the frequency switch. The scale of the image is ∼1.1 mm and the running time is from the point of tissue excision.