Supplementary information

Formation of precisely composed cancer cell clusters using a cell assembly generator (CAGE) for studying paracrine signaling at single-cell resolution

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Supplementary Materials and Methods

Cell transfection and materials

The DAG biosensor mCherry-Ca1C1b has been previously described.[1] MRS 2179 was from Tocris Bioscience (Abingdon, United Kingdom). For transfection, ~2 × 10^5 MIN6 cells were suspended in 100 µl of Opti-MEM medium containing 0.5 µl of Lipofectamine 2000 (Invitrogen) and 0.2 µg of plasmid DNA (mCherry-C1aC1b) and plated on poly-L-lysine–coated coverslips. After cell attachment (3-5 hrs), the transfection was terminated by addition of complete cell culture medium in which cells were maintained for 18-24 hours. The calcium indicator Fluo-4-AM was from Life Technologies.

Total internal reflection fluorescence (TIRF) microscopy

Fluorescence in the sub–plasma membrane compartment was recorded with a TIRF microscopy setup based on an E600FN upright microscope (Nikon) contained in an acrylic glass box thermostated at 37°C by an air stream incubator as previously described.[2]

Supplementary Figure and Movie Legends

Fig. S1. Adhesion of MCF7 cells to the glass substrate. (A) MCF7 cells were seeded onto an untreated glass slide. After 15 minutes attempts to dislodge the cells by repeated pipetting were made, however the majority of cells remained firmly attached to the substrate. (B) MCF7 cells were seeded and clustered in the CAGE device where the bottom glass substrate had been treated with 0.1% gelatin. The cells adhered well and cell migration was monitored for 6 h: all cells moved relative to their starting position and one cell moved more than 50 µm.

Fig. S2. Defining MIN6 and MCF7 calcium dynamics in response to K^+ and ATP. (A) Fluo-4 fluorescence in MIN6 cells increases in response to 30 mM K^+ (means ± SEM for 40 cells from 3 independent experiments). (B) TIRF microscopy recordings of DAG biosensor fluorescence (mCherry-C1aC1b) in MIN6 cells, before and during exposure to 30 mM K^+. DAG signaling is temporarily silenced using the $P2Y_1$ (ATP-receptor) inhibitor MRS 2179 (MRS, 10 μM). (C) Fluo-4 fluorescence in MCF7 cells before, during and after exposure to 30 mM K^+ (means ± SEM for 39 cells from two independent experiments). (D) Fluo-4 fluorescence in MCF7 cells increases in response to ATP (10 μM and 100 μM). Resting Fluo-4 fluorescence initially increased in response to the SERCA-inhibitor Thapsigargin due to passive leak from the ER unmasked by SERCA inhibition. Thapsigargin treatment will ultimately lead to a depletion of ER-stored Ca^{2+}, which as seen here rendered the MCF cells insensitive to ATP (10 μM) (means ± SEM for 22 cells from one experiment). (E) Quantifications of the maximal Fluo-4 fluorescence increase in MCF7 cells in response to the indicated treatments (means ± SEM for 58 cells from 3 independent experiments, *** = P<0.001, paired 2-tailed Student's t-test).

Fig. S3. Categorization of cancer cells into high and low responders. By applying the average AFC of cancer cells in the absence of β-cells to the correlation models in Fig. 5D and E, distances could be identified to categorize cells into high and low responders (resp.).

Fig. S4. Early calcium dynamics in a cluster composed of a single MIN6 cell and three MCF7 cells during K^+ stimulation. (A) A 4-cell cluster was assembled in the CAGE consisting of a single Hoechst-stained MIN6 cell, serving as the ATP source (s), and three unstained MCF7 cancer cells, serving as the responders (r1-r3). (B) Before cluster assembly, cells were additionally labeled with Celltracker red and Fluo-4. Each cell was identified as a region of interest (ROI) and Fluo-4 and Celltracker red fluorescence was imaged during K+ perfusion. (B) Corrected Fluo-4 fluorescence for each cell in the cluster is presented. An expanded view of the first 30 s of corrected Fluo-4 fluorescence is presented in the upper plot, framed with a dashed line. The timing of the K^+ perfusion is indicated. Fluo-4 fluorescence is presented as the ratio Fr/Fr_0, where Fr was the ratio between Fluo-4 and Celltracker red fluorescence (which served to correct for fluorescence deviations due to focus adjustment) in an ROI at a specific time-point, and Fr_0 was the ratio between Fluo-4 and Celltracker red fluorescence at time-point zero.

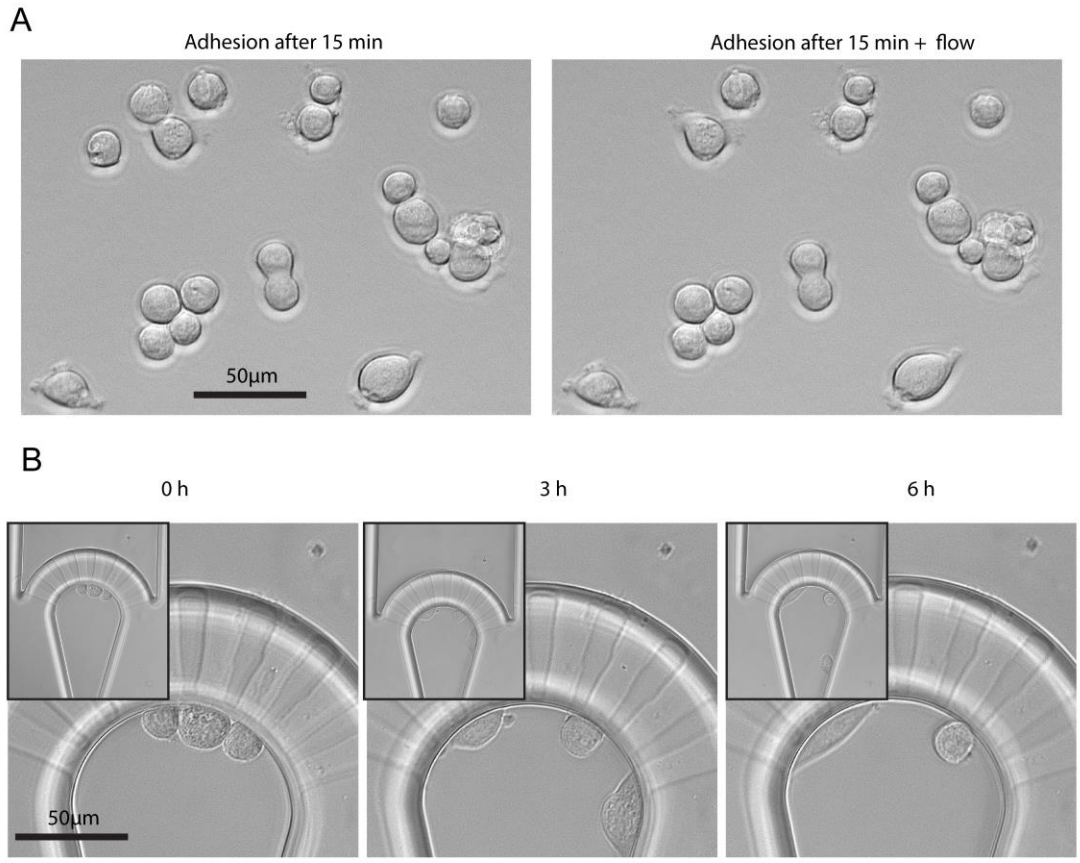
Fig. S5. Casting of functional CAGE PDMS chips from a 3D-printed mold. Images of structures coding for a single-cell trap (upper left panel) and a clustering chamber (upper right panel) in a 3D-printed mold for production of the CAGE chip. The mold was produced by the Dilase high-resolution 3D lithographic laser system (Kloé, Montpellier, France). Representative images of 4-cell clusters formed in CAGE chips cast from the 3D-printed molds (lower left and right panels).

Movie M1. Movies showing (1) trapping of a cell in a hydrodynamic single-cell trap, (2) flow diversion around an occupied trap (here the concentration of the cell suspension used for loading was increased to better visualize the flow diversion), and finally (3) ejection of a cell from an occupied trap.

Supplementary references

1. A. Wuttke, O. Idevall-Hagren and A. Tengholm, *FASEB J*, 2013, **27**, 1610-1620.

2. O. Idevall-Hagren, S. Barg, E. Gylfe and A. Tengholm, *The Journal of biological chemistry*, 2010, **285**, 23007-23018.



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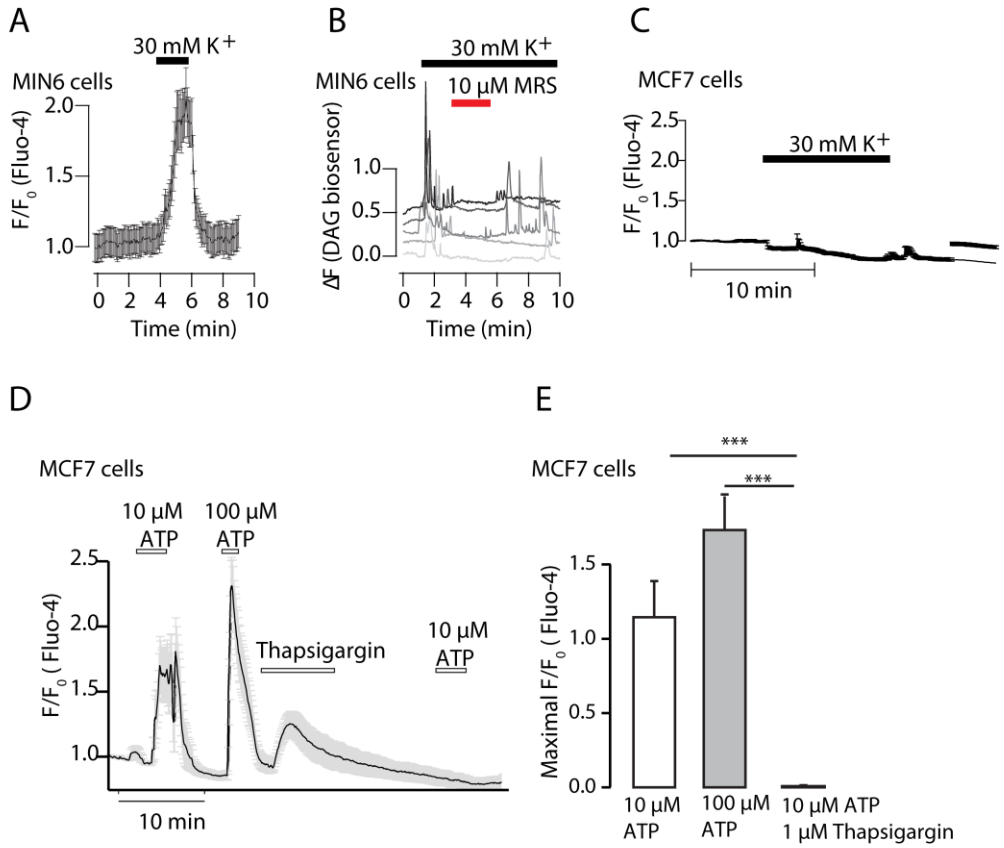

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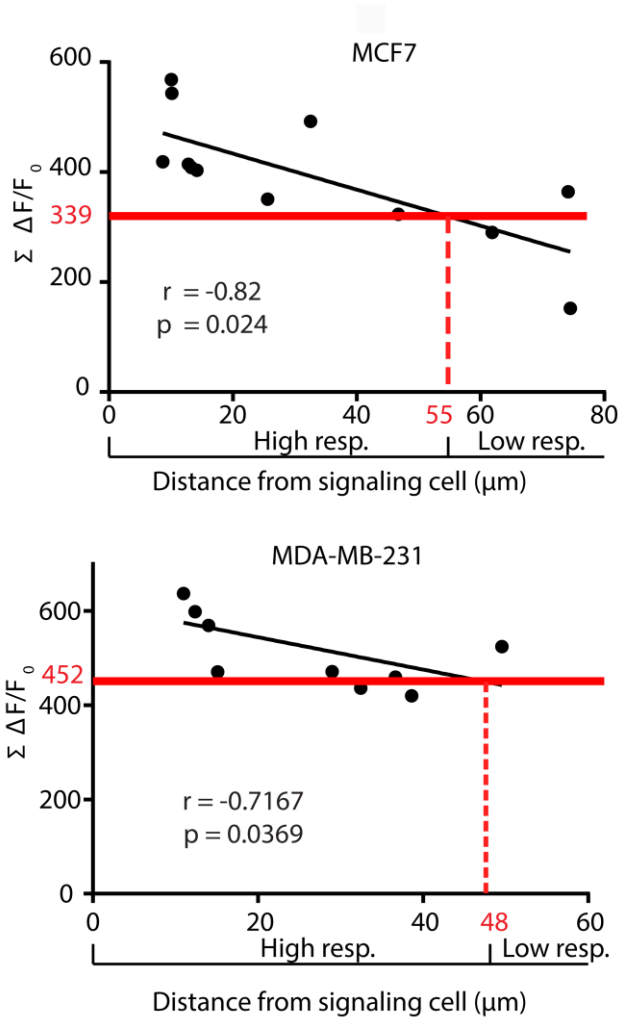

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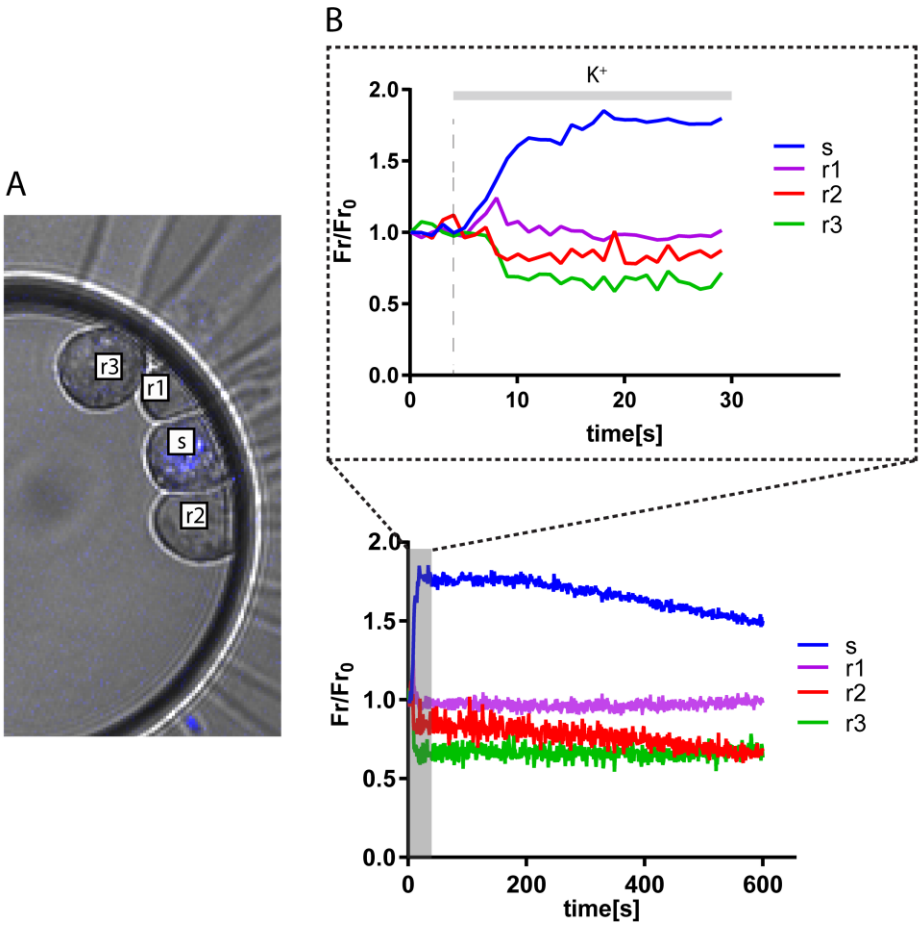

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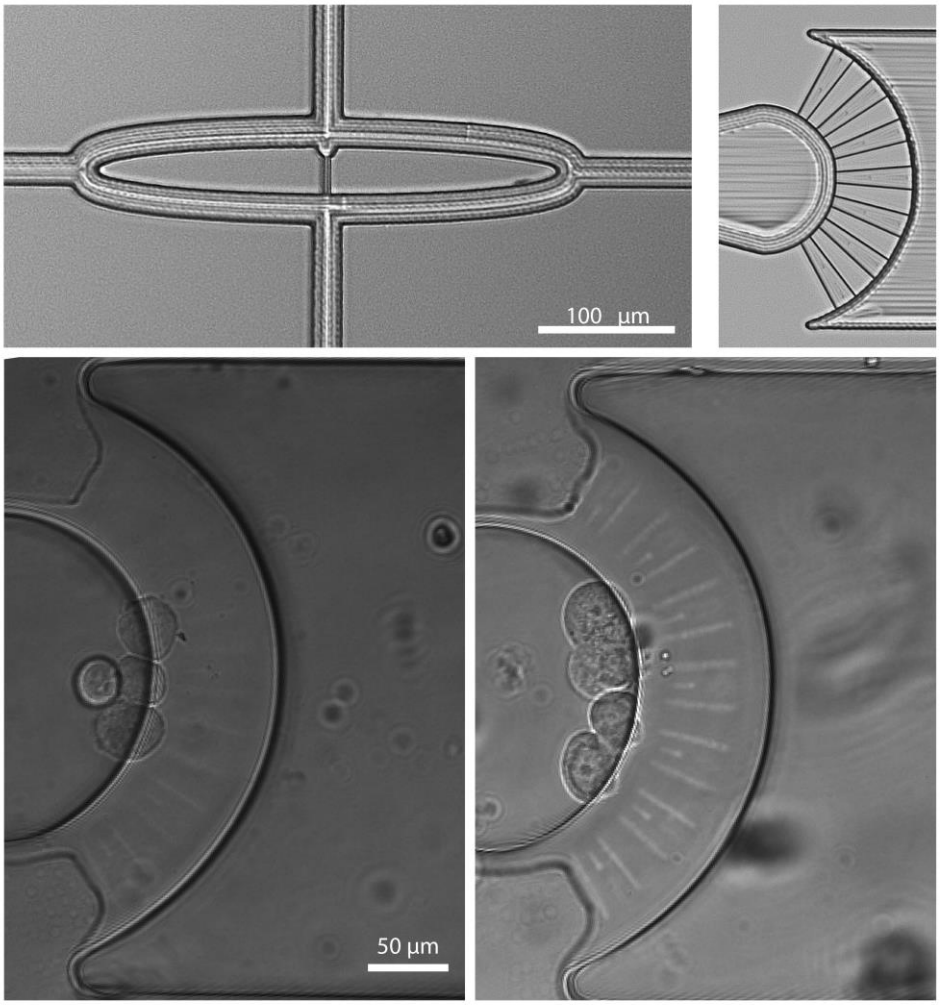

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