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# Supplementary Materials for

## Intercellular communication controls agonist-induced calcium oscillations independently of gap junctions in smooth muscle cells

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

(available at advances.sciencemag.org/cgi/content/full/6/32/eaba1149/DC1)

**Supplementary Materials**

**Materials and Methods**

**Fabrication of NuSil substrates at additional stiffnesses:** Equal parts of NuSil gel-8100 parts A and B (NuSil, Carpinteria, CA, USA) were mixed with various amounts of the crosslinking compound of Sylgard 184 (Dow Corning, Midland, MI, USA) to adjust substrate stiffness. Crosslinker volumes of 0.07% and 0.15% of the combined volumes of parts A and B were added to the 1:1 A:B mixture to create substrates with Young's modulus E= 0.6 kPa and 3 kPa, respectively. The spinning and curing protocol to finish the substrates can be found in the main manuscript.

**Histamine dose-response experiment:** Confluent SMCs cultured on soft (0.3 kPa) and stiff (13 kPa) substrates were incubated with FLIPR $Ca^{2+}$ 6 indicator and imaged following the protocol in the main manuscript. For each sample, histamine concentration was gradually increased and followed by 5 minutes of imaging. Stiff samples were exposed to concentrations increasing from $10^{-6}$ M, to $10^{-5}$ M, to $10^{-4}$ M, and soft samples were exposed to concentrations of $10^{-5}$ M, $10^{-4}$ M, and $10^{-3}$ M histamine.

## Figures

### Fig. S1.

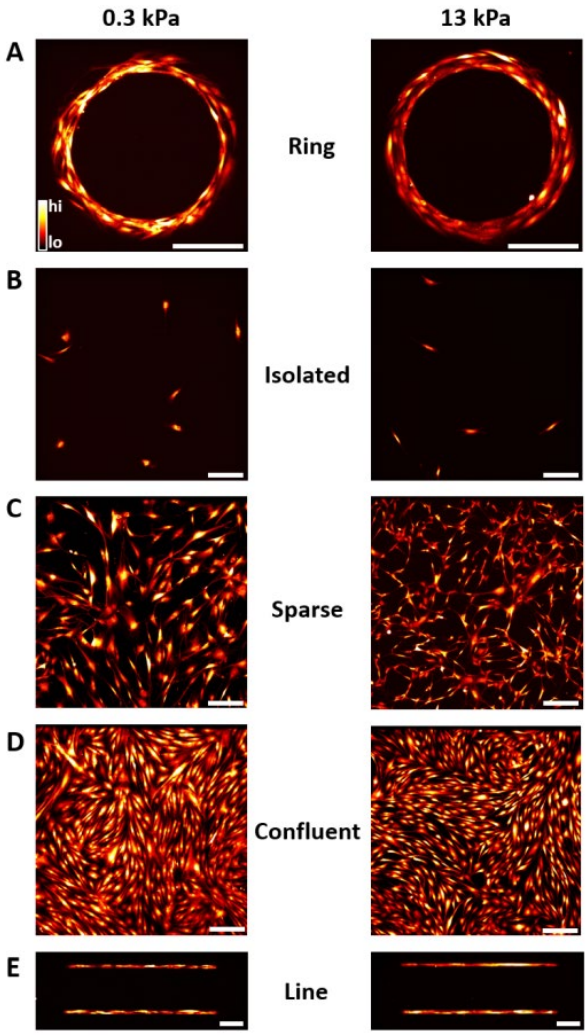


**Fig. S1. Morphology of SMCs on soft and stiff substrates.** Fluorescent images of cytosolic $Ca^{2+}$ in SMCs show similar appearance and density of cells in the different culture conditions used in this paper: (**A**) rings, (**B**) isolated, (**C**) sparse, (**D**) confluent, and (**E**) lines. The cytosolic [$Ca^{2+}$] is pseudo-colored following the color bar, as in Fig. 1. Scale bar = 200 μm.

**Fig. S2.**

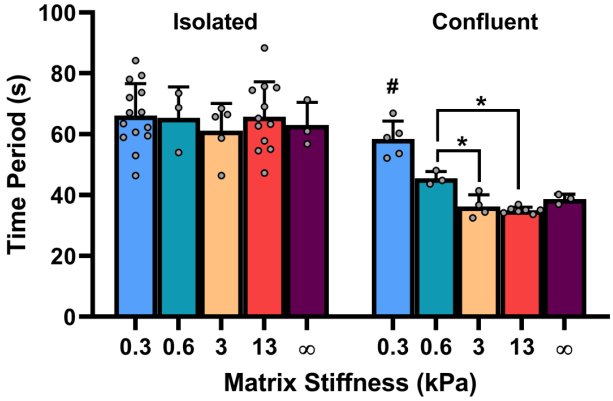


**Fig. S2. Effect of incremental matrix stiffening on agonist-induced $Ca^{2+}$ oscillations in SMCs.** PDMS substrate stiffness was tuned between our limits for soft (0.3 kPa) and stiff (13 kPa) matrix to create two intermediate stiffness matrices at 0.6 kPa and 3 kPa. Glass coverslips were used to represent matrix of infinite stiffness. In confluent cells, ECM stiffness had a significant impact on agonist-induced $Ca^{2+}$ oscillation time periods (One-Way ANOVA, $P<0.001$). Pairwise comparisons with a Holm-Sidak test are indicated by symbols. The time period at 0.3 kPa was significantly different from all other substrate stiffnesses (indicated by #, $P<0.001$). The time period at 0.6 kPa was also significantly different from that at 3 kPa and 13 kPa ($P=0.003$, $P<0.001$, respectively). There was no statistical difference between time periods at 3 kPa, 13 kPa and glass (3 kPa vs. 13 kPa $P=0.566$, 3 kPa vs. glass $P=0.379$, 13 kPa vs. glass $P=0.017$, respectively). Matrix stiffness had no effect on the agonist-induced $Ca^{2+}$ response in isolated cells (One-Way ANOVA, $P=0.907$).

**Fig. S3.**

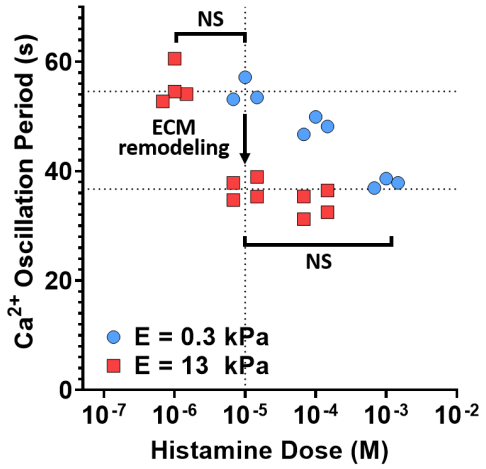


**Fig. S3. Effect of histamine dose on $Ca^{2+}$ oscillation time period for cells on soft and stiff ECM.** Confluent SMCs on soft and stiff matrix were exposed to multiple doses of histamine. Increasing concentration of histamine from $10^{-5}$ M to $10^{-3}$ M systematically decreased the mean $Ca^{2+}$ oscillation time periods of cells on soft matrix (One-Way ANOVA, $P<0.005$). On stiff matrix, increasing histamine dose from $10^{-6}$ M to $10^{-5}$ M caused a dramatic decrease in time period, but increasing the dose further had no effect (One-Way ANOVA, $P<0.001$, $P=0.174$, respectively). For SMCs on soft ($E=0.3$ kPa) substrate, the histamine dose needs to be increased from $10^{-5}$ M to $10^{-3}$ M (2 log scales higher) to match the $Ca^{2+}$ response of SMCs on the stiff substrate.

**Fig. S4.**

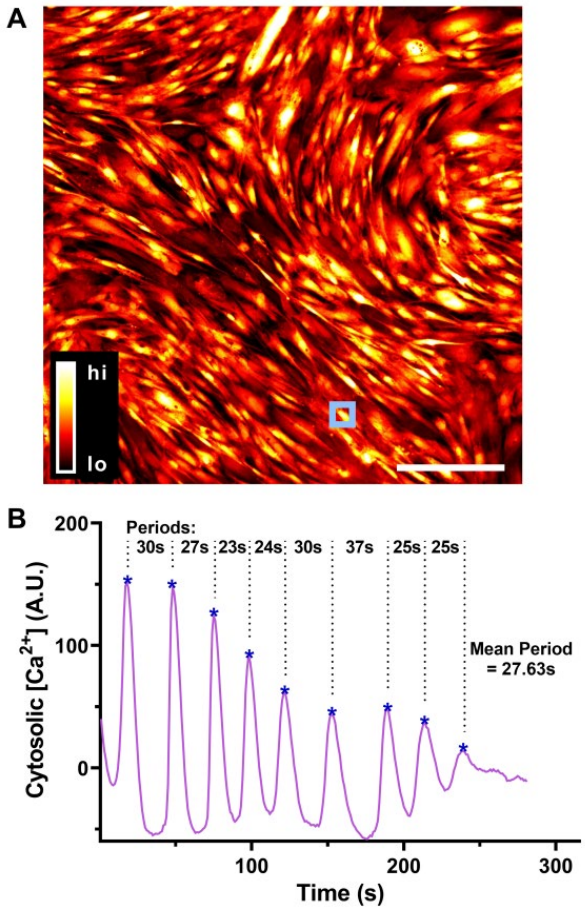


**Fig. S4. Method of calculating $Ca^{2+}$ oscillation periods.** (**A**) Images for each experiment were loaded into Image J, and 5x5 pixel regions of interest (ROIs) were hand-selected in the cytosol of the smooth muscle cell. The mean gray intensity of each ROI for each frame was imported into MATLAB and plotted to show changes over time for each cell. The $Ca^{2+}$ indicator used in our experiments indicates cytosolic [$Ca^{2+}$], therefore changes in grayscale intensity correspond with changes in cytosolic [$Ca^{2+}$]. Scale bar = 200 μm. (**B**) The portion of data after the initial spike in $Ca^{2+}$ due to histamine addition was selected for further processing. First, the code subtracted the mean [$Ca^{2+}$] value of each time series from the time series. Next, local peaks above a threshold value were identified (indicated by *), and the time between sequential peaks were recorded as

oscillation periods. Time periods are measured for a minimum of 40 cells/trial in confluent cells and for every SMC in isolated cells. The average time period of all measured cells is reported for each independent trial.

**Movies S1 to S4 Captions**

**Movie S1. Cytosolic $Ca^{2+}$ oscillations in confluent smooth muscle cells on stiff matrix.** Confluent SMCs on stiff matrix (E=13 kPa) loaded with a fluorescent $Ca^{2+}$ indicator were imaged before and during exposure to a $10^{-5}$ M dose of the contractile agonist histamine, added at the time indicated. This movie shows the first 2.5 minutes of the experiment. The movie is compressed to 8 bit and 0.25 frames/second to match journal data guidelines. The full 5 minutes of $Ca^{2+}$ oscillations recorded at 1 frame/second, 16-bit, 2048 x 2048 images used to make measurements is available from the authors on reasonable request. Scale bar = 200 μm.

**Movie S2. Cytosolic $Ca^{2+}$ oscillations in confluent smooth muscle cells on soft matrix.** Confluent SMCs on soft matrix (E=0.3 kPa) loaded with a fluorescent $Ca^{2+}$ indicator were imaged before and during exposure to a $10^{-5}$ M dose of the contractile agonist histamine, added at the time indicated. This movie shows the first 2.5 minutes of the experiment. The movie is compressed to 8 bit and 0.25 frames/second to match journal data guidelines. The full 5 minutes of $Ca^{2+}$ oscillations recorded at 1 frame/second, 16-bit, 2048 x 2048 images used to make measurements is available from the authors on reasonable request. Scale bar = 200 μm.

**Movie S3. Cytosolic $Ca^{2+}$ oscillations in isolated smooth muscle cells on stiff matrix.** Isolated SMCs on stiff matrix (E=13 kPa) loaded with a fluorescent $Ca^{2+}$ indicator were imaged before and during exposure to a $10^{-5}$ M dose of the contractile agonist histamine, added at the time indicated. This movie shows the first 2.5 minutes of the experiment. The movie is compressed to 8 bit and 0.25 frames/second to match journal data guidelines. The full 5 minutes of $Ca^{2+}$ oscillations

recorded at 1 frame/second, 16-bit, 2048 x 2048 images used to make measurements is available from the authors on reasonable request. Scale bar = 200 μm.

**Movie S4. Cytosolic $Ca^{2+}$ oscillations in isolated smooth muscle cells on soft matrix.** Isolated SMCs on soft matrix (E=0.3 kPa) loaded with a fluorescent $Ca^{2+}$ indicator were imaged before and during exposure to a $10^{-5}$ M dose of the contractile agonist histamine, added at the time indicated. This movie shows the first 2.5 minutes of the experiment. The movie is compressed to 8 bit and 0.25 frames/second to match journal data guidelines. The full 5 minutes of $Ca^{2+}$ oscillations recorded at 1 frame/second, 16-bit, 2048 x 2048 images used to make measurements is available from the authors on reasonable request. Scale bar = 200 μm.