Mechanical stimulation

Ultrasound stimulation

For real-time imaging of samples a Leica TCS SP5 laser scanning microscope mounted on a Leica DMI 6000 CS inverted microscope (Leica Microsystems, Germany) with an HCX plan apo 40× oil immersion objective was used. For image analysis we used CaPTURE software, developed by our team.

- HEK293 cells were seeded (6x105 per well) on a 6-well glass-bottom plate (Cellvis) and upon reaching 60-70% confluency transfected with selected plasmids.
- Cells were loaded with fluorescent calcium indicators and incubated for 30 min at 37°C and 5% CO2.
- Medium was exchanged for fresh medium with 4 mM CaCl2 10 min before starting the stimulation.
- Cells were stimulated for 10 s and responses were analyzed in CaPTURE.

Stimulation of cells with touch

For detection of luminescence we used G:BOX (Syngene). For image analysis we used ImageJ (Image Processing and Analysis in Java) software (http://rsbweb.nih.gov/ij/).

- Cells were seeded at 2x106 in a petri dish (61 mm, TPP) and upon reaching 60-70 % confluency transfected with selected plasmids.
- 24 h after transfection the medium was exchanged for medium with 1 mM luciferin and 4 mM CaCl2.
- Cells were incubated for 30 min at 37°C, 5% CO2.
- After the incubation cells were transferred to G:BOX machine and stimulated by touching with a glass rod.
- Successive images were acquired for 30 s (four series) after stimulation.
- Images were exported in jpeg format and processed using ImageJ software.