Jain et al. – Supplementary Information

Figure Supplementary 1. Fabrication process for manufacturing electroporation-ready microwell arrays on ITO-coated glass substrates using two mask patterning steps. The first mask is used to pattern an electrode grid of titanium/gold on top of the ITO. A second mask is used to pattern SU-8, which insulates the titanium/gold grid and provides the physical structure for the microwells. After development and hard baking, the microwell arrays are silanized with APTES to create a positively charged ITO surface.

Figure Supplementary 2. Modification of the surface of microwell arrays to enhance siRNA binding. **A**) Surface modification of ITO-coated glass substrates using oxygen plasma and silanization with amino-propyly-triethoxy silane. **B**) Schematic showing methods used to confirm amine-functionalization with fluorescence reporter assays using amine reactive oregon-green-conjugated NHS ester (top) or electrostatic binding of negatively charged Alexa-fluor-488 conjugated siRNAs (bottom). **C**) Fluorescence images to verify existence of amine groups in control hydroxyl surfaces and ITO. Detection of fluorescence after silanization confirms the presence of amine head groups.

Figure Supplementary 3. Estimation of intial seeding and cell count in microwells. **A)** Estimation of initial cell plating numbers per microwell given cell seeding density (1800 cells μL^{-1}). Left image shows fabricated dimensions of the 9x9 microwell array. Right image shows fabricated dimensions of a single microwell. Thin red lines outline the SU-8 structure and thin blue lines delineate the underlying electrode grid. Based on open surface area, plating at this density should result in 80 cells per microwell given the spacing between anode and cathode at 300 μm. **B)** HeLa cell nuclei stained with Hoechst and imaged at 48 hr post-seeding. Cells in microwells were counted using the ImageJ cell counter plugin.

Figure Supplementary 4. Schematic and setup of apparatus used for electroporating individual

microwell arrays. **A**) A whole microscope slide comprising 16 fabricated microwell array devices is diced into eight individual pieces (two devices each). Four of these pieces are mounted in a custom fabricated electroporation holder with spacers and held down with copper clips. Copper clips holding down each piece are electrically connected and used as the cathode connection. Cells are added on top of the microwell arrays and an anode is immediately placed on top of the spacers, creating a pre-defined anode-cathode spacing for electroporation. After electroporation is complete, the pieces are transferred to a six-well culture dish and incubated. **B**) Top view and **C**) Side view of four devices housed in the apparatus with anodes placed on top. **D**) Close-up view of a single electroporation-ready microwell array clamped down with copper clips and covered with the anode plate at a specified distance implemented with a spacer.

Figure Supplementary 5. Image analysis and phenotypic assessment within microwell arrays. **A)** Images are acquired on an inverted fluorescence scope as described in Materials and Methods. User initiates the image-processing pipeline by selecting two opposite corner points on the opaque electrode grid bars of the microwell array (indicated by red arrows). Thereafter, the automatic pipeline estimates rotational errors in the image and identifies microwell regions (yellow outlines) using the electrode grid bars as a reference. Microwell regions of interest are then sequentially processed in a series of image manipulation steps, including median filtering (2 pixel neighborhood), black-white thresholding, conversion to binary, water-shedding (used as the segmenting algorithm) and analysis (such as particle count and mean intensity per microwell). Image processing was carried out in ImageJ using ImageJ's macro language. **B)** HEK293 cells stained with Hoechst (nuclei), Calcein AM (live), and Propidium Iodide (dead) and imaged at 72 hr post-electroporation. Representative microwells with cells electroporated with siRPS27a and siControl. Merge image shows lack of colocalization of Calcein (alive) and PI (dead) within the same cell. Viable cells are Hoescht and Calcein positive, but PI negative.

Figure Supplementary 6. Electroporation of functional nucleic acids suspended in buffer within the

microwell arrays. **A**) HeLa cells and **B**) HEK 293 cells electroporated (or not) with siRNAs (siRPS27a or siControl) and evaluated for phenotypes 48 hr later. The expected death phenotype is observed only in the electroporated siRPS27a condition. **C**) Primary mouse macrophages derived from a GFP transgenic mouse line electroporated with siRPS27a or siControl, and **D**) siGFP or siControl suspended in buffer and imaged for phenotype 7 days post-electroporation. Images indicate cell death phenotype with siRPS27a (C) and reduced GFP fluorescence with siGFP in comparison to control (D). **E**) Electroporation of plasmid GFP suspended in buffer (1 μg/μL) into HEK 293 cells and GFP expression 72 hr post-electroporation.

Figure Supplementary 7. Finite Element Modeling of electric field within microwells. **A)** Boundary conditions used for generating a simulated 2D model of electric field distribution within a single microwell (see Materials and Methods). **B)** Surface and contour plot showing electric field gradient inside a single microwell. Conditions: 30V, distance of 300μm between the electrodes. **C)** Electric field distribution along the X axis parallel to the ITO surface (E_x). Dashed region corresponds to the SU-8 wall with the embedded gold layer. **D)** Electric field distribution along the Y axis perpendicular to ITO surface (E_Y). Dashed region corresponds to SU-8 wall with the embedded gold layer.

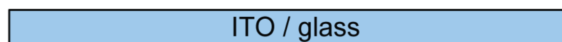
Figure Supplementary 8. Electroporation of siControl, siRPS27a, and siGFP loaded within microwells arrays in a desired pattern. Electroporation and assessment of phenotype using Design pattern 1 (quarter array) with HeLa-GFP cells within microwell arrays loaded with siControl (blue areas), siRPS27a (red area), and siGFP (green area). Only microwells loaded with siGFP display reduced fluorescence due to knockdown of GFP expression. Microwells loaded with siRPS27a display cells rounding up due to induction of death (the expected phenotype of RPS27a knock down).

Figure Supplementary 9. Electroporation of siControl and siGFP printed within microwells arrays. Electroporation and assessment of phenotype using Design pattern 1 (quarter array) with HeLa-GFP cells within microwell arrays printed with siControl (red areas) and siGFP (green areas). Quadrant

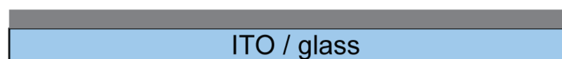
areas are depicted by Q1 & Q4 (for siControl) and Q2 & Q3 (for siGFP). In areas Q12 and Q34 siControl- and siGFP-printed microwells are adjacent. Fluorescence images of all areas were taken 72 hr post-electroporation. Only microwells printed with siGFP display reduced fluorescence due to knockdown of GFP expression. Graph indicates mean fluorescence intensity per microwell (n=9) in quadrants Q4 (siControl) and Q2 (siGFP).

Fig. S1.

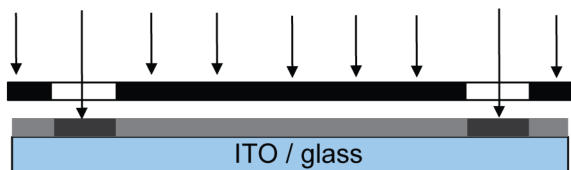
Step 1 Clean with solvents, bake and Oxygen plasma clean



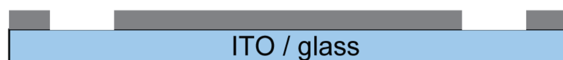
Step 2 Spin-coat positive photoresist and bake



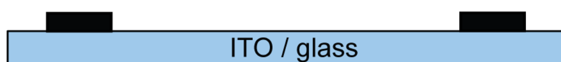
Step 3 Align mask to substrate edge and expose



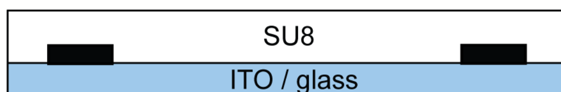
Step 4 Develop photoresist to expose areas for gold grid



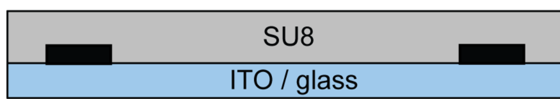
Step 5 Deposition of Titanium / Gold and lift-off



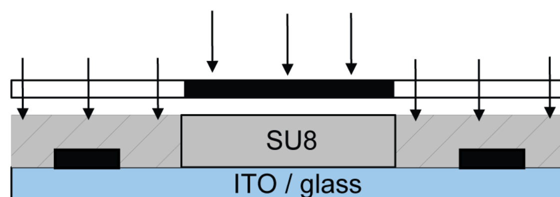
Step 6 Spin-coat SU8 (resin and solvent)



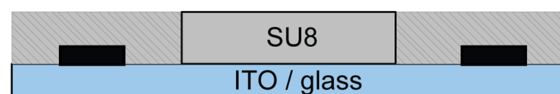
Step 7 Pre-exposure bake at 60-100 °C for 10 min



Step 8 Align mask and UV expose for 1 min

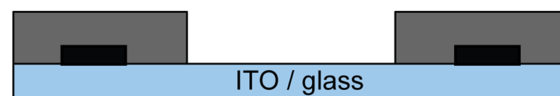


Step 9 Post-exposure bake at 60-100 C. for 10 min



Step 10 Development of SU8

Step 11 Ramped hard-bake at 160 °C.



Step 12 Oxygen plasma activation and Aminopropyltriexthoxy silane (APTES) treatment (2.5 % in toluene, RT for 6 hr)

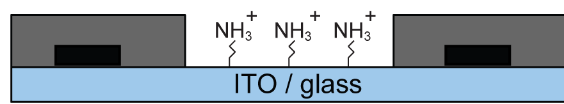


FIG. S2.

A. Surface functionalization of ITO-coated glass substrates

Solvent clean ITO coated glass slide

OH OH OH

Oxygen plasma increases surface hydroxyl groups

O - O - O - O -

OH OH OH

Amino-propyl triethoxy silane treatment in toluene

OH OH OH OH OH OH

NH_3^+

$Si(OCH_2CH_3)$

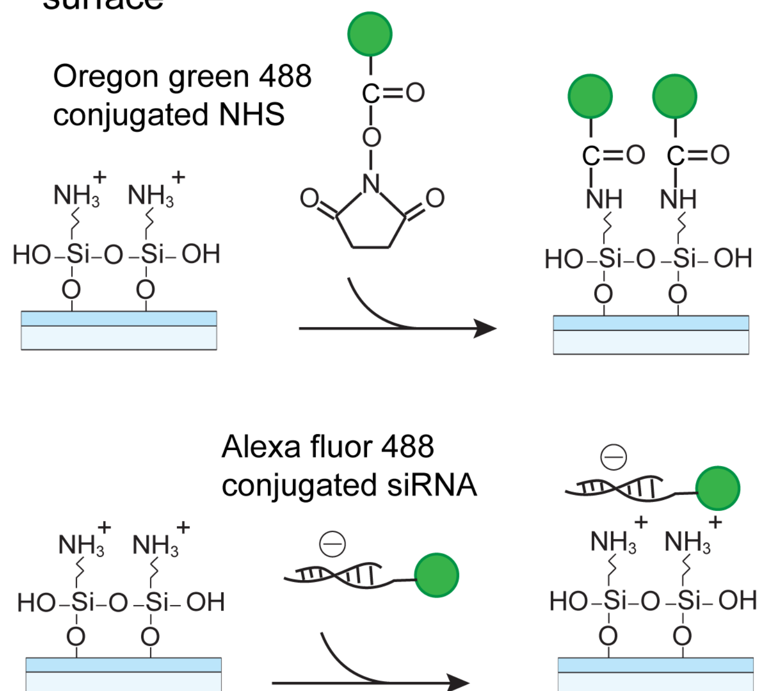
Self-assembled layer of functional amine groups

NH_3^+ NH_3^+ NH_3^+ NH_3^+ NH_3^+

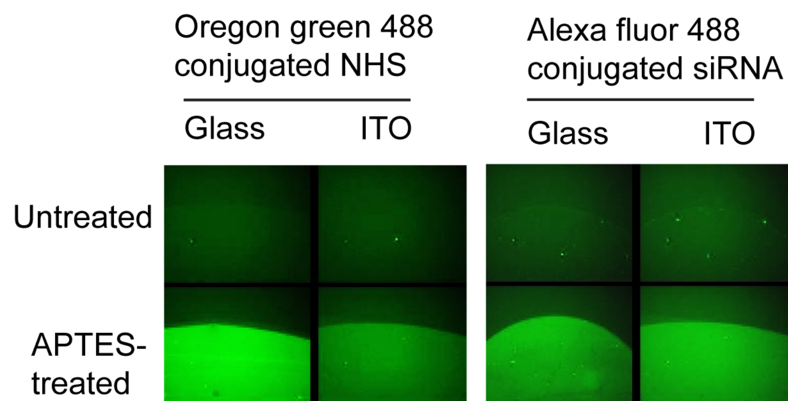
HO–Si–O–Si–O–Si–O–Si–O–Si–OH

O O O O O

B. Assays to verify amine groups on substrate surface



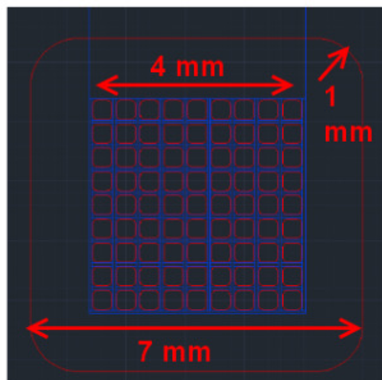
C. Images of treated and labeled ITO and glass surfaces



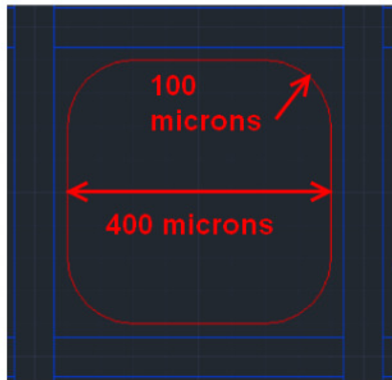
2mm

FIG. S3.

A. SU8 layer including microwell array



Single microwell



Total area of SU8 layer including microwells = 48 sq. mm

Area of open microwell array region only *(Am)* = 12 sq. mm

Area of SU8 layer excluding microwells = 36 sq. mm

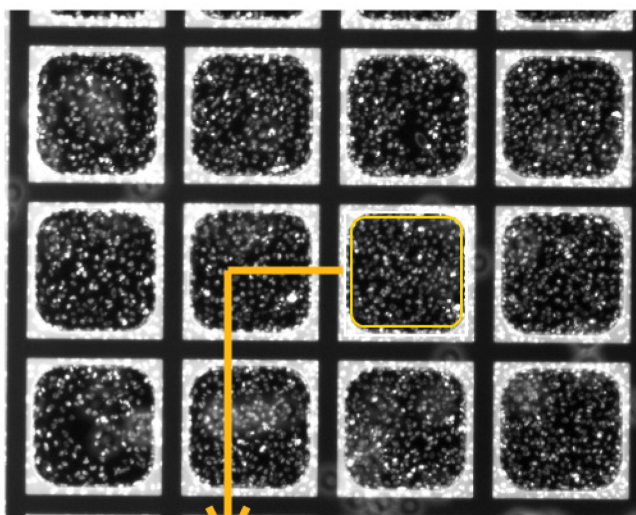
At cell seeding density *(Cd)* = *1800 cells* mm^{-3}

And anode to cathode (bottom of microwells) distance *(D)* = *0.3 mm*

Total cells within all (81) microwells = *(Cd x Am x D)* = *6480 cells*

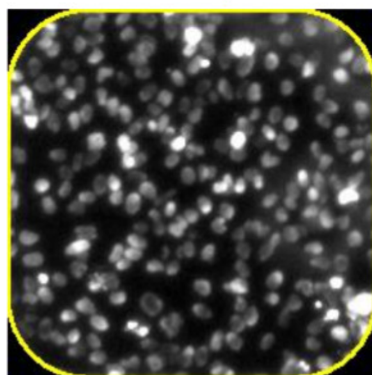
Therefore, initial cells plated per microwell = *80 cells*

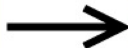
B.

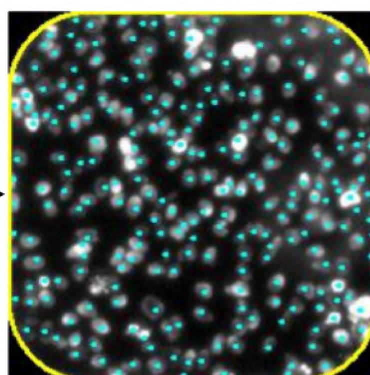


48 hrs post-seeding

Hoechst nuclei stain







255 nuclei counts

FIG. S4.

A.

16 microwell arrays (9x9 microwells)

Microwell array

ITO

Gold grid

Dice and assemble in setup

Cathode clips

1 2 3 4

1 2 3 4

Pipette cells on top of microwell arrays

Place anode plate on top of spacers

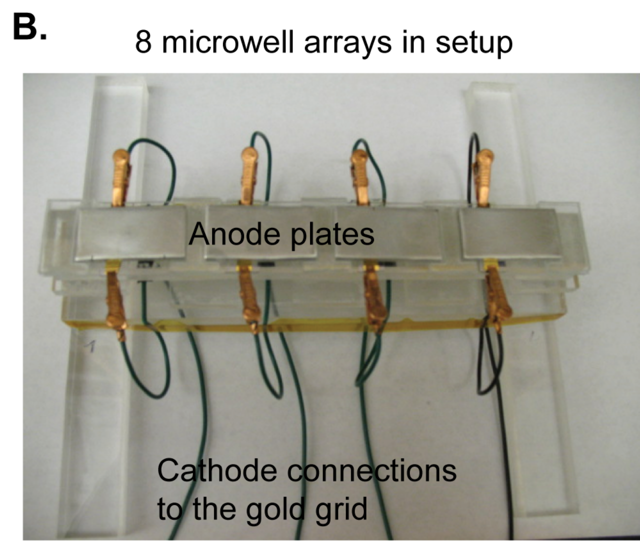
Electroporation pulse applied

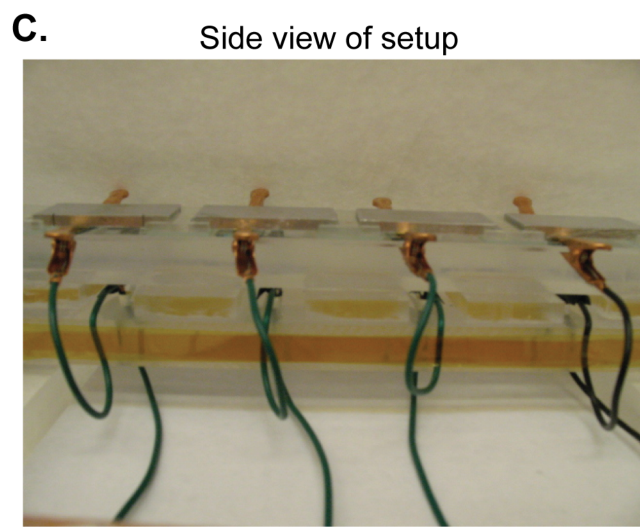
(-ve)

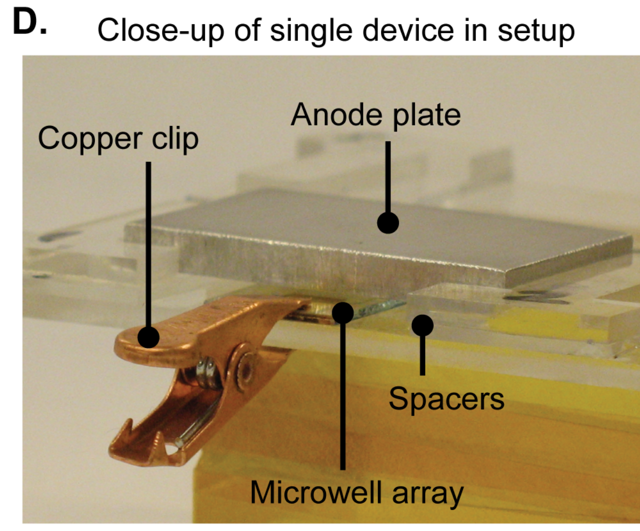
(+ve) (-ve)

Repeat for all four pairs

Incubate in culture media (6-well dish)







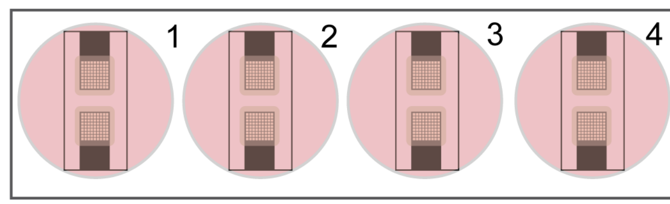
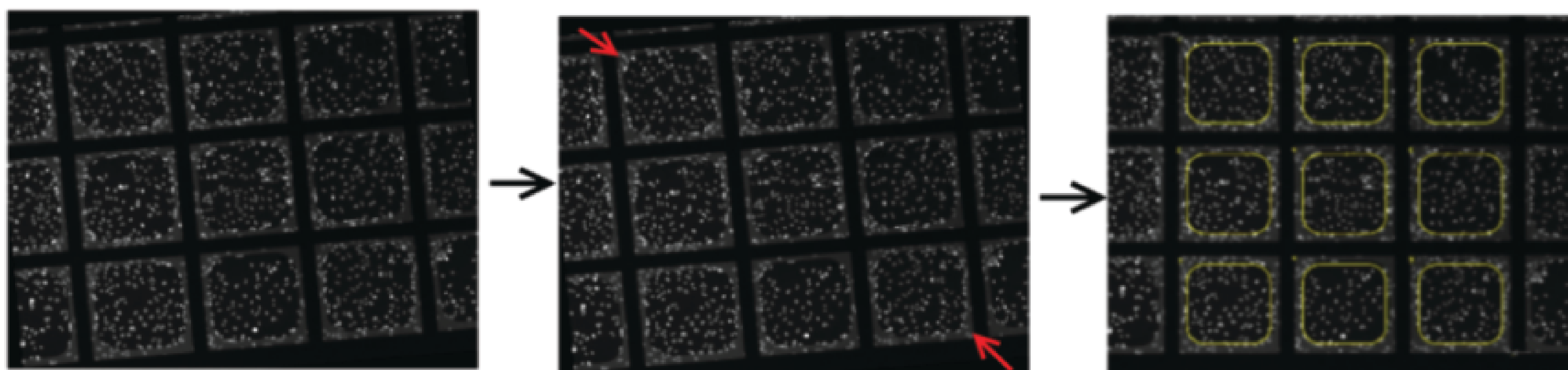


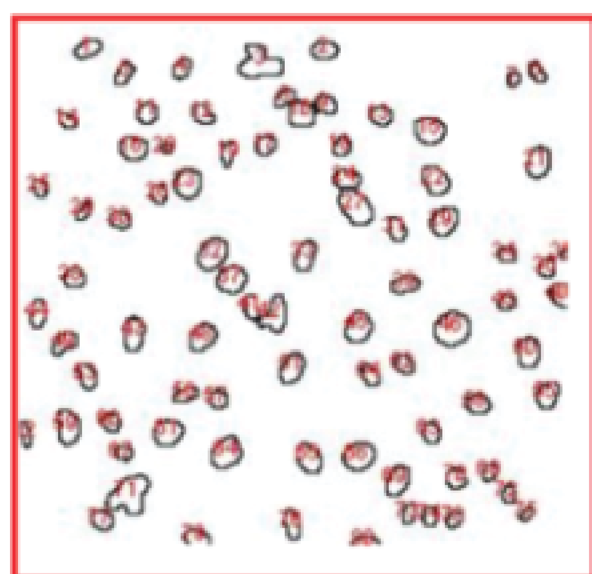
FIG. S5.

A. 4X image of microwell Hoechst stained nuclei → User identification of opposite corners → Image rotation and microwell region identification

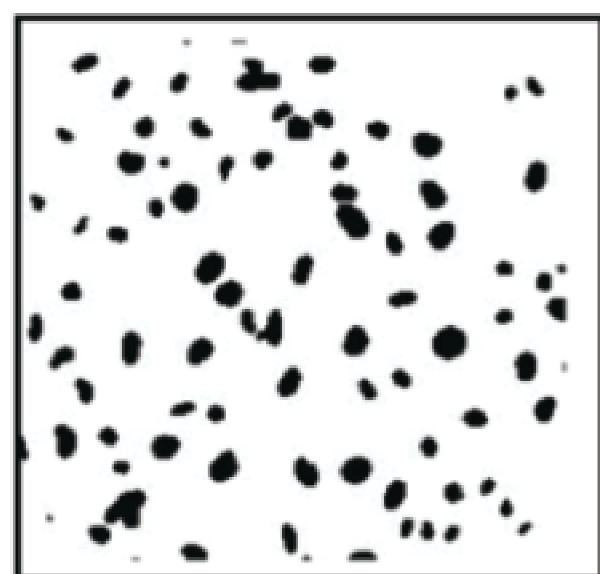


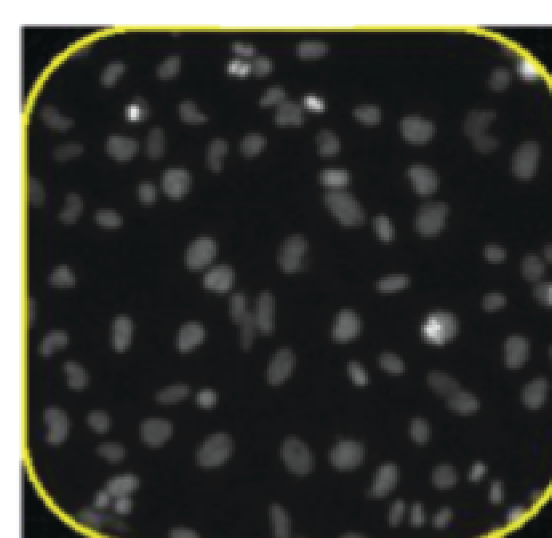
Microwell isolation

Analysis (nuclei count)



Segmented image





B.

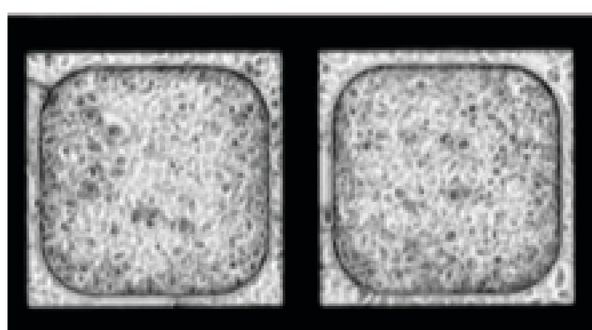
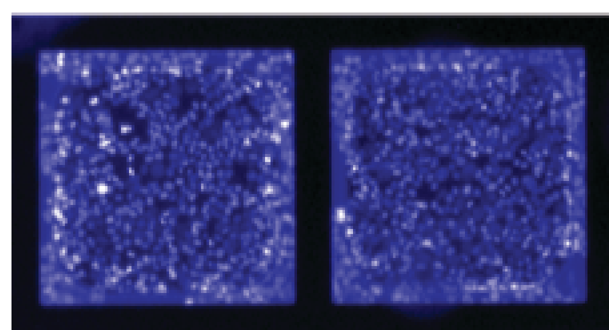
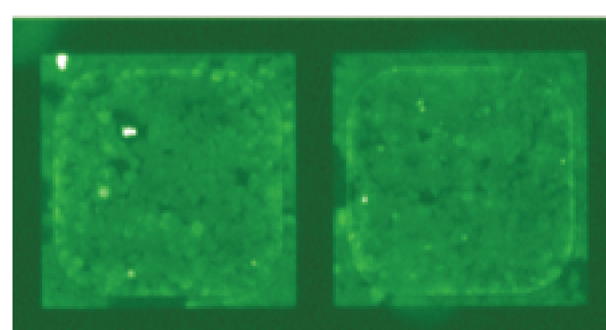
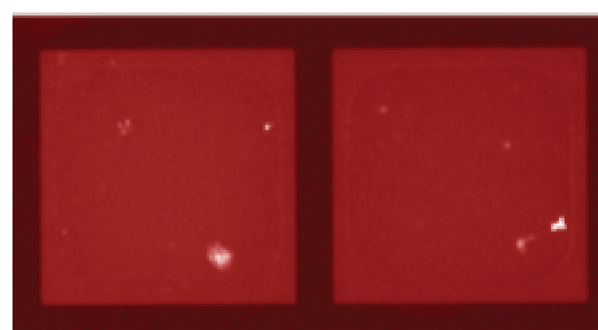
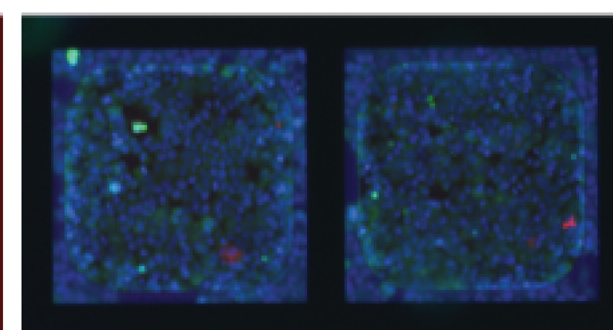
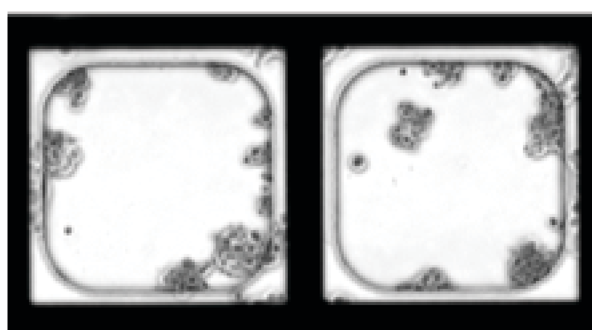
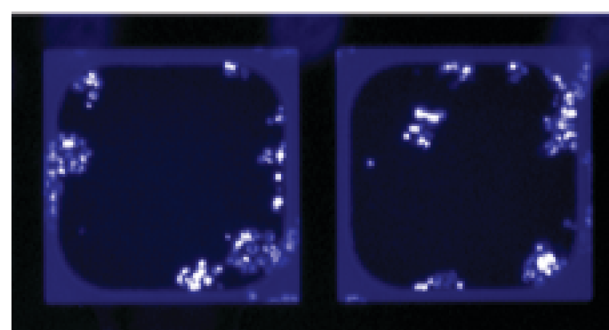
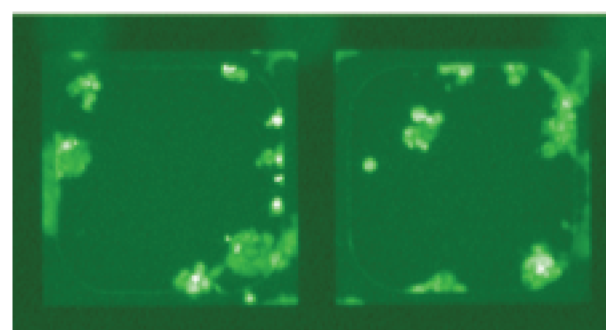
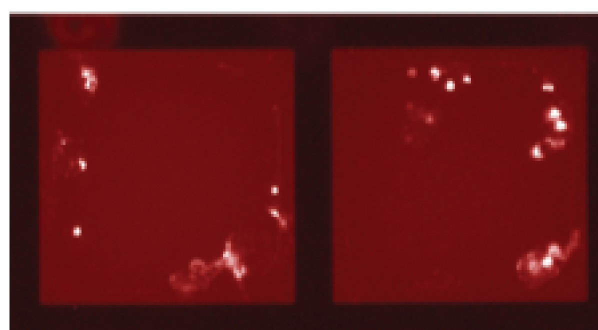
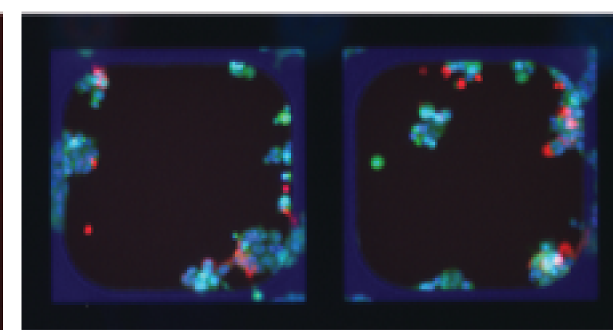
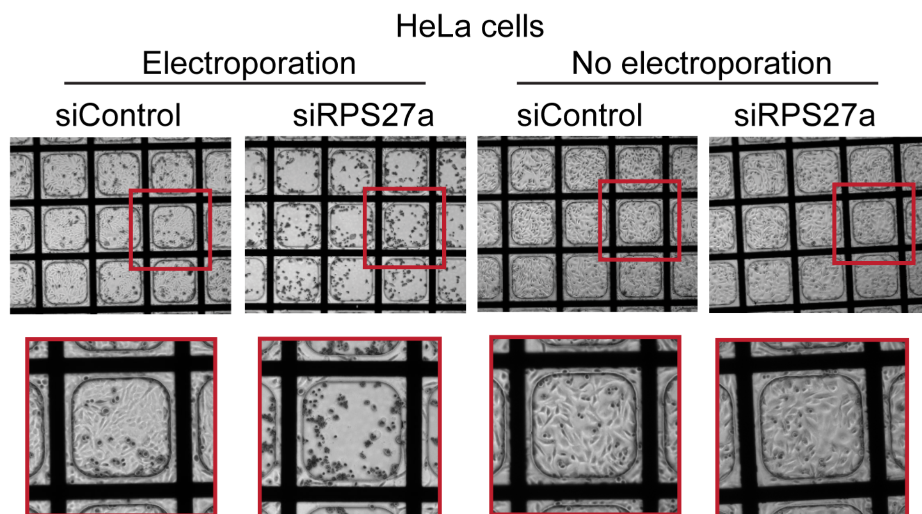
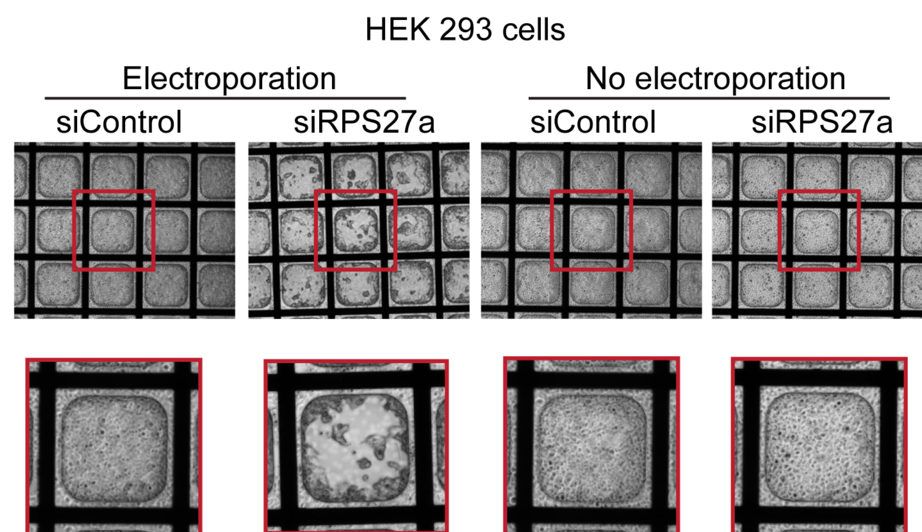
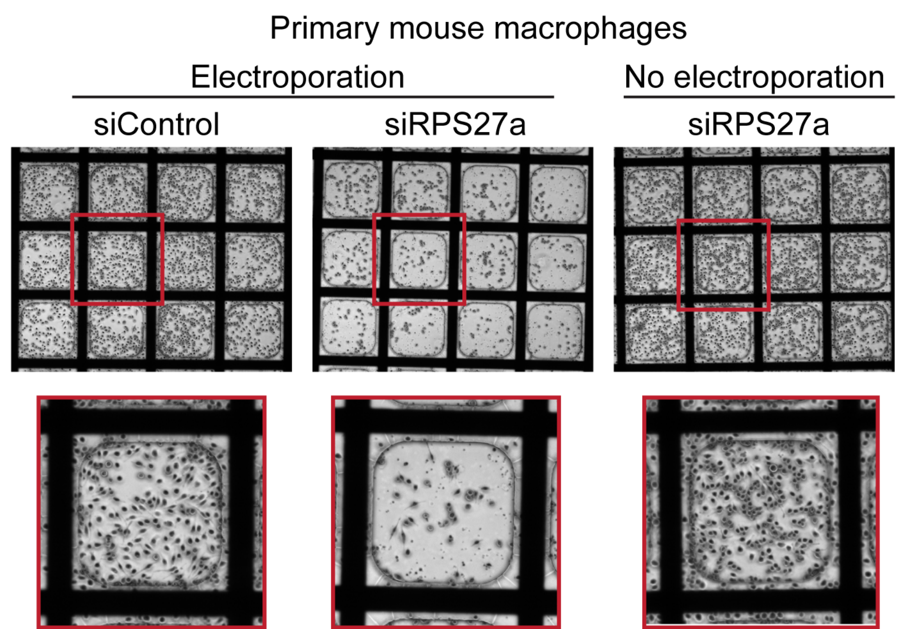
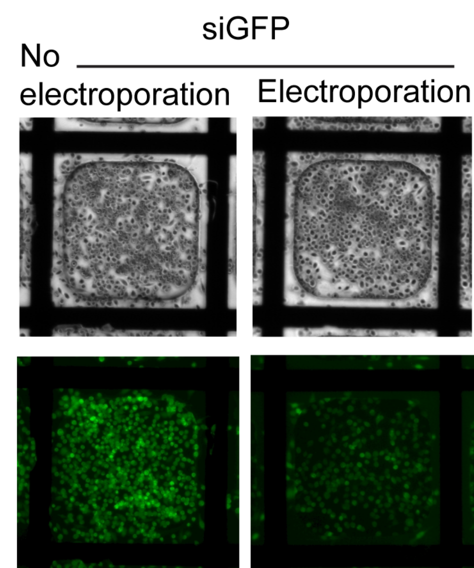
	Brightfield	Hoechst	Calcein AM	Propidium Iodide	Merge
siControl					
siRPS27a					

FIG. S6.









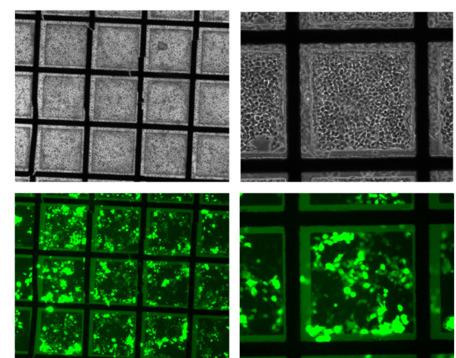
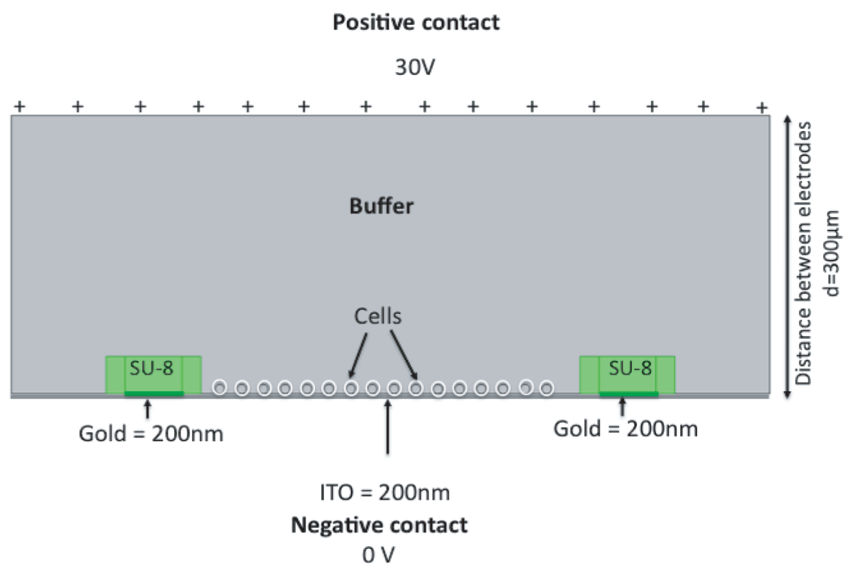
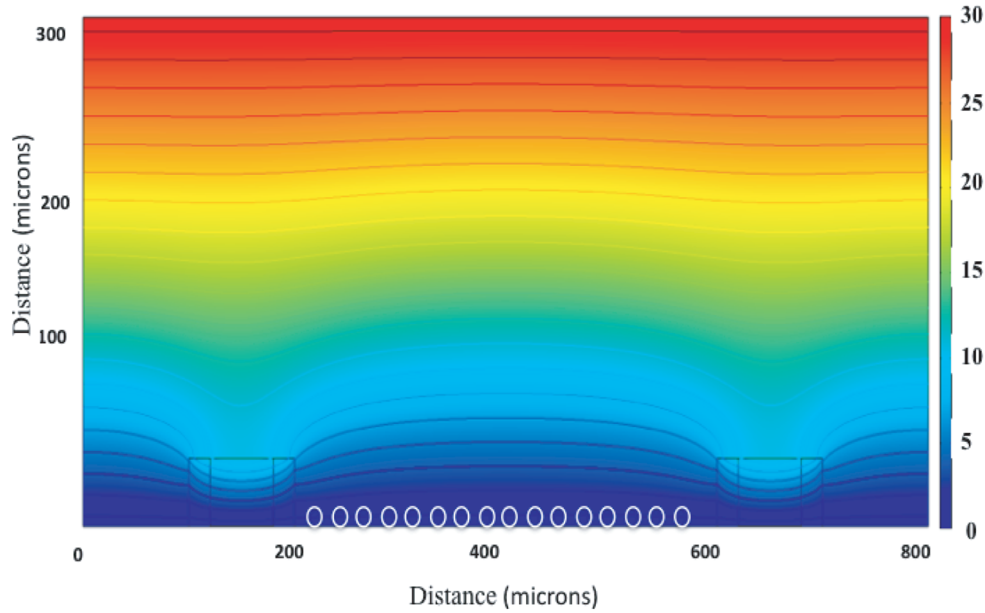
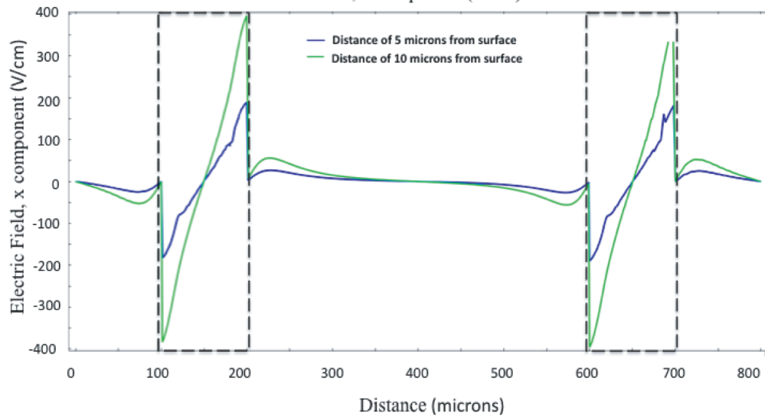


FIG. S7.







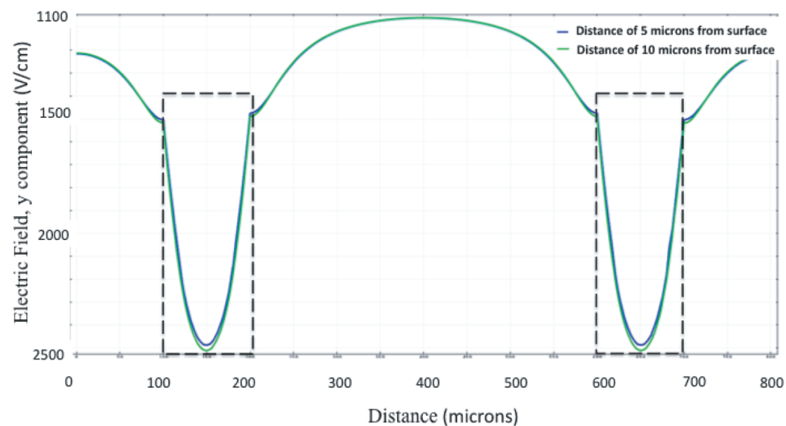
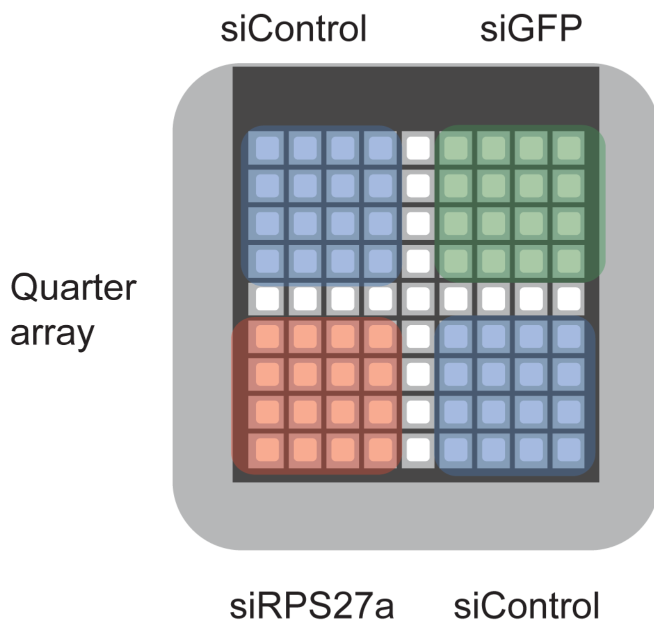


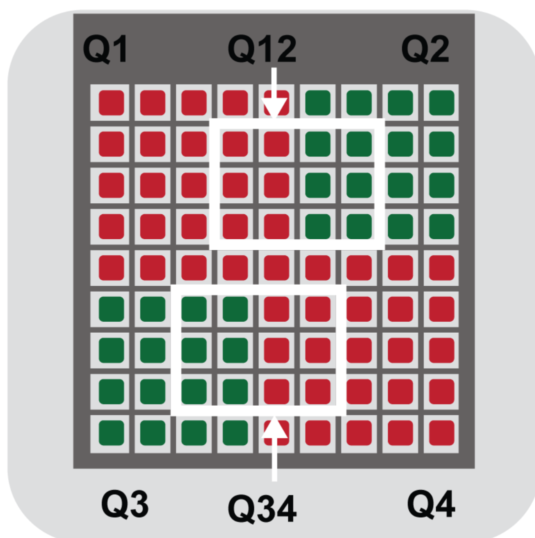
FIG. S8.



siControl siGFP

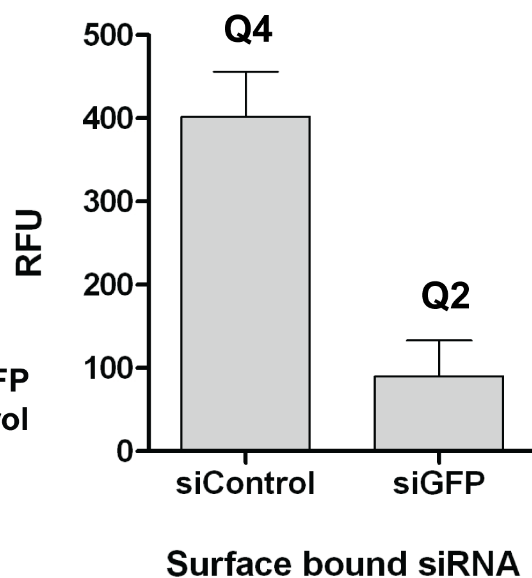
siRPS27a siControl

FIG. S9.



Q1 - siControl
Q2 - siGFP
Q3 - siGFP
Q4 - siControl

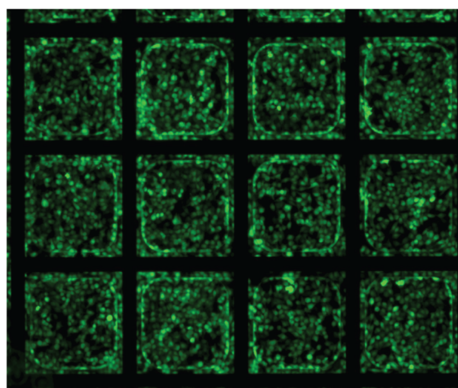
Q12 - siControl / siGFP
Q34 - siGFP / siControl

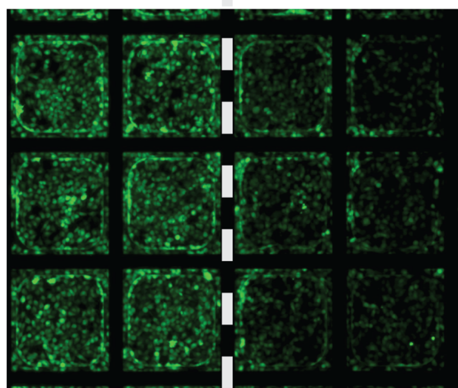


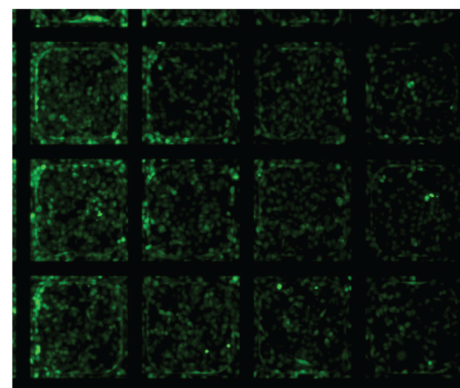
Q1 siControl

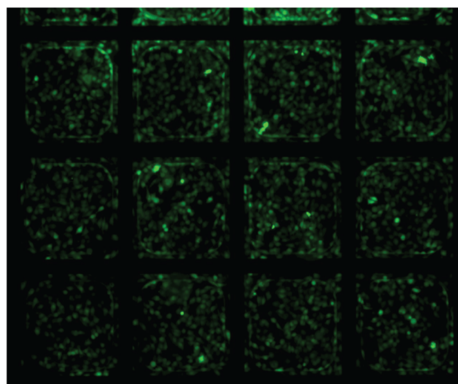
Q12 siControl siGFP

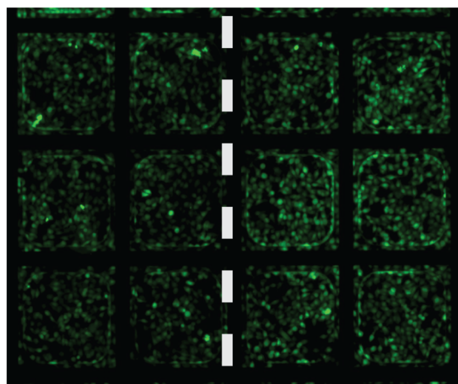
Q2 siGFP

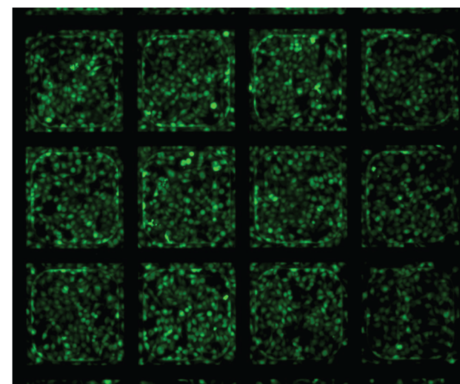












siGFP Q3

siGFP siControl Q34

siControl Q4