Supporting Information

Mapping cell behavior across a wide range of vertical silicon nanocolumn densities

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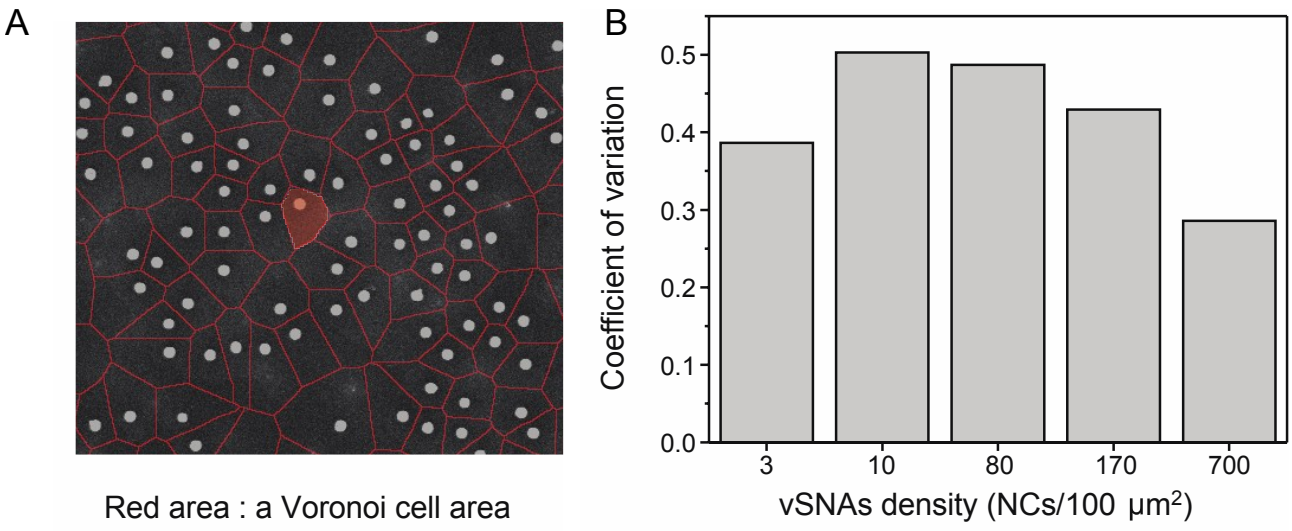


Figure S1: Quantifying the uniformity of the NC distribution during fabrication of vSNAs with different NC densities. **A** SEM top-view image of a vSNA with a Voronoi diagram drawn in red. **B** Measurements of Voronoi cell areas from SEM images as in **A** were performed using ImageJ (edge Voronoi cells not included) and were used to calculate a mean value and standard deviation for each vSNA density. The coefficient of variation was calculated as *mean value*/*standard deviation*.

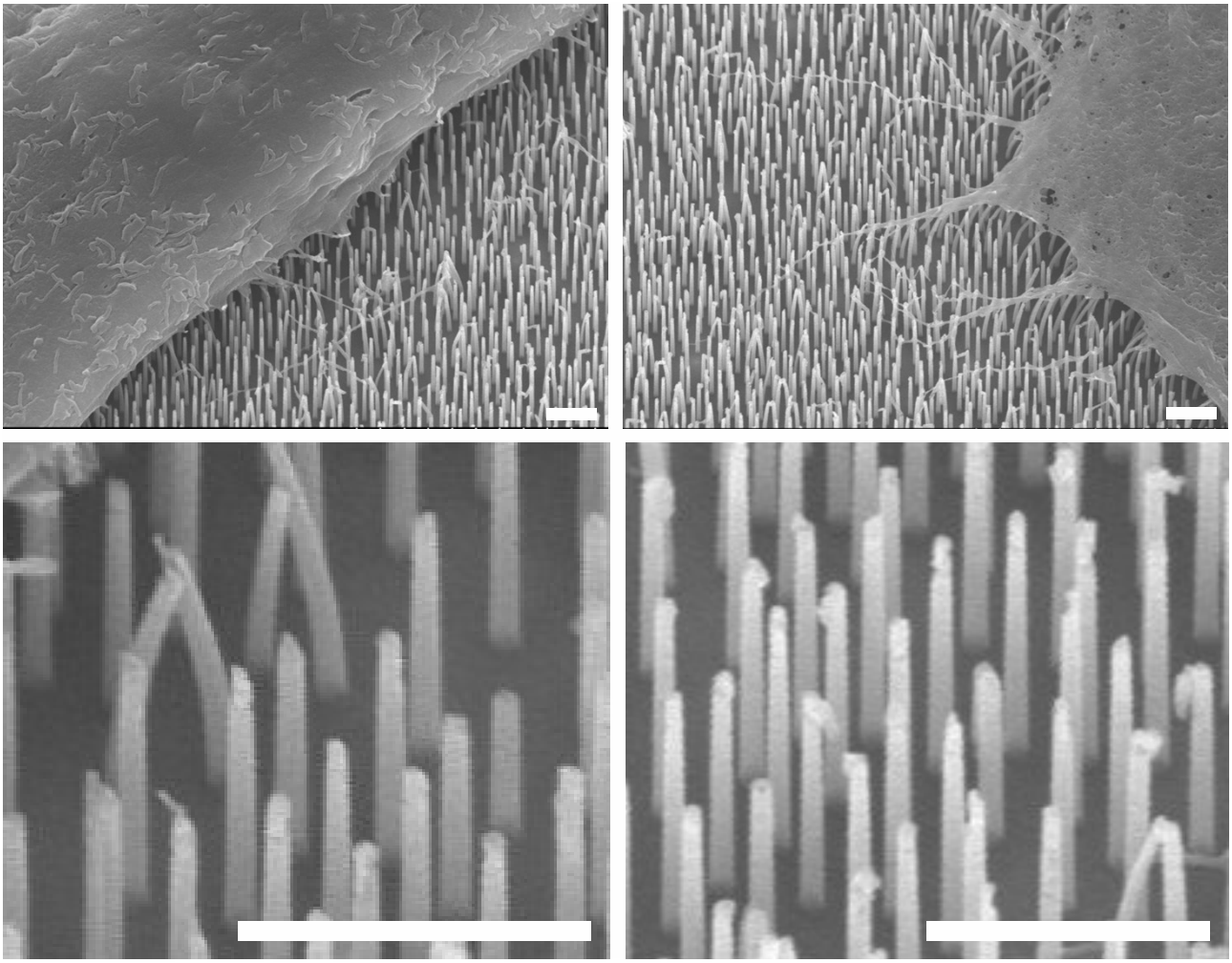


Figure S2: SEM images of NIH3T3 cells on vSNA_700. The upper images show cell filopodia adhering only to the very tips of the NCs. The lower SEM images show a magnified view of vertical silicon nanocolums with irregular tip morphology which could be attibuted to partial silicon erosion during the cell culture. All scale bars represent 1 μm.

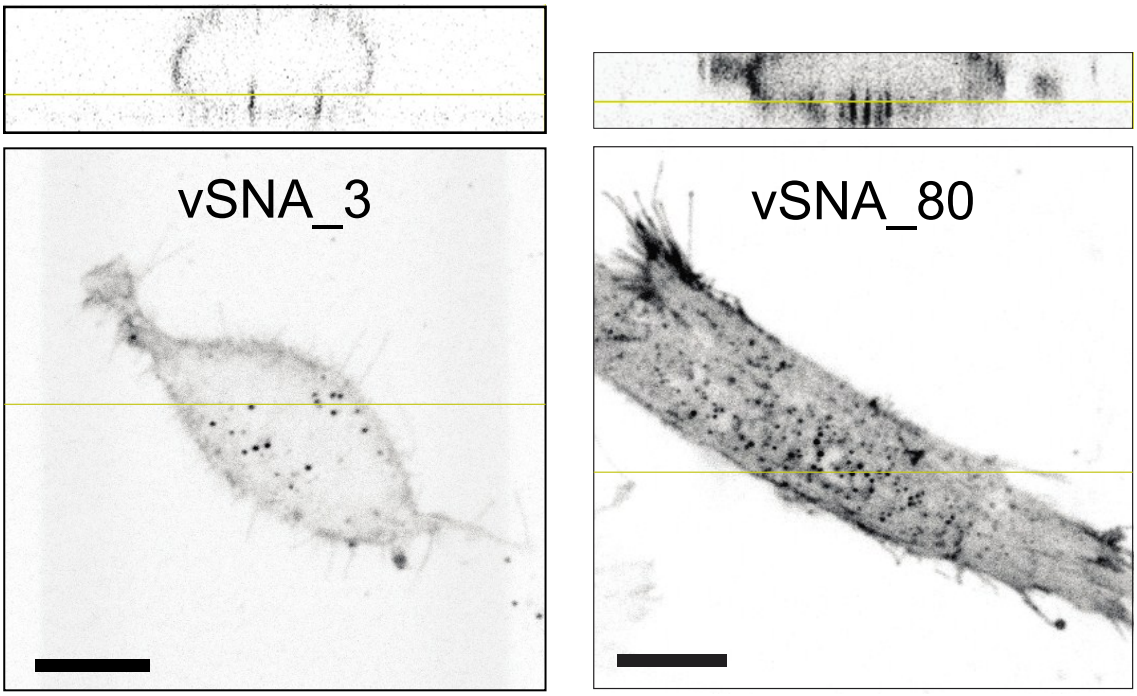


Figure S3: Summed confocal stacks (~2 µm) and orthogonal views through the fluorescently labeled membrane of NIH3T3 cells on the indicated vSNA densities (vSNA_3 = 3 NCs/100 µm^2, vSNA_80 = 80 NCs/100 µm^2). Scale bars denote 10 µm.

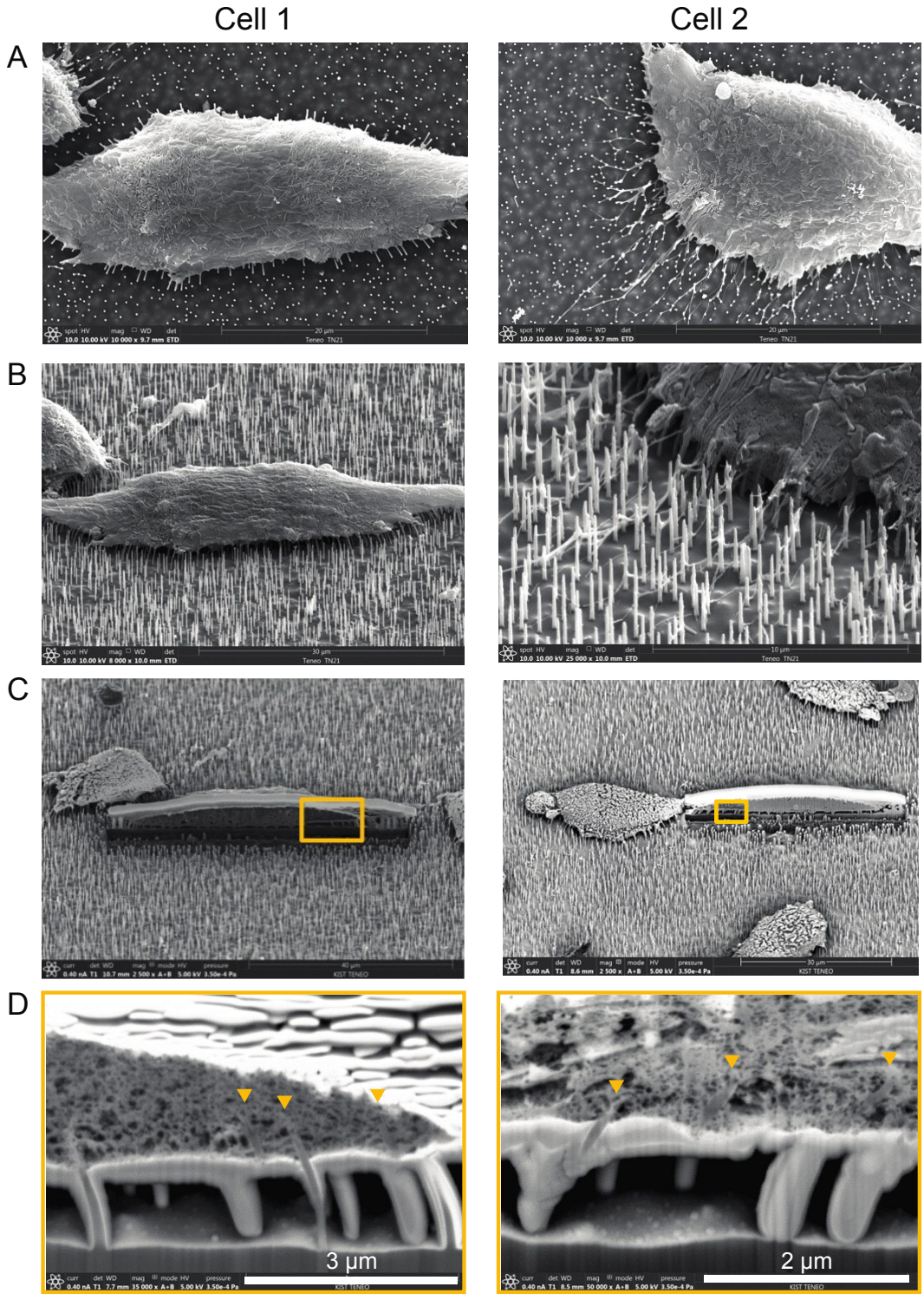


Figure S4: NIH3T3 cells on vSNA_80 imaged using focused ion beam (FIB-) SEM at 10 kV to reveal cell deformation around NCs. **A** Cells seen from above (0° tilt). **B** Same cells seen at 52° tilt. **C** Same cells seen at 52° tilt after focused ion beam milling. **D** Zoom-in on the position indicated with the yellow box in **C**. Clearly distinguished NCs are indicated with yellow triangles. Note, that the NCs appear thicker under the cell due to Pt deposition.

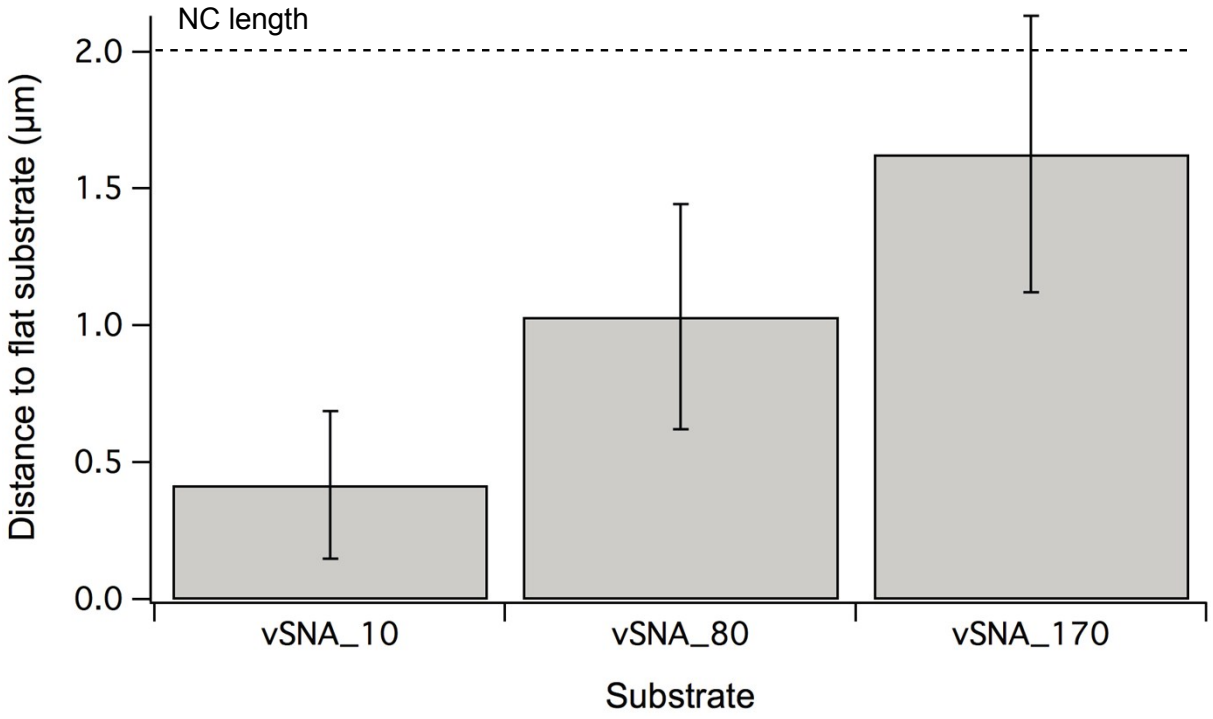


Figure S5: Average cell settling height measured from the flat substrate to the bottom of the cell in confocal sideviews after fluorescent staining of the extracellular medium as previously described.[1,2] The error bars represent standard deviations between single cells from one experiment. It was not technically possible to perform measurements on vSNA_700 due to the very high density of NCs and limited diffusion of the dye.

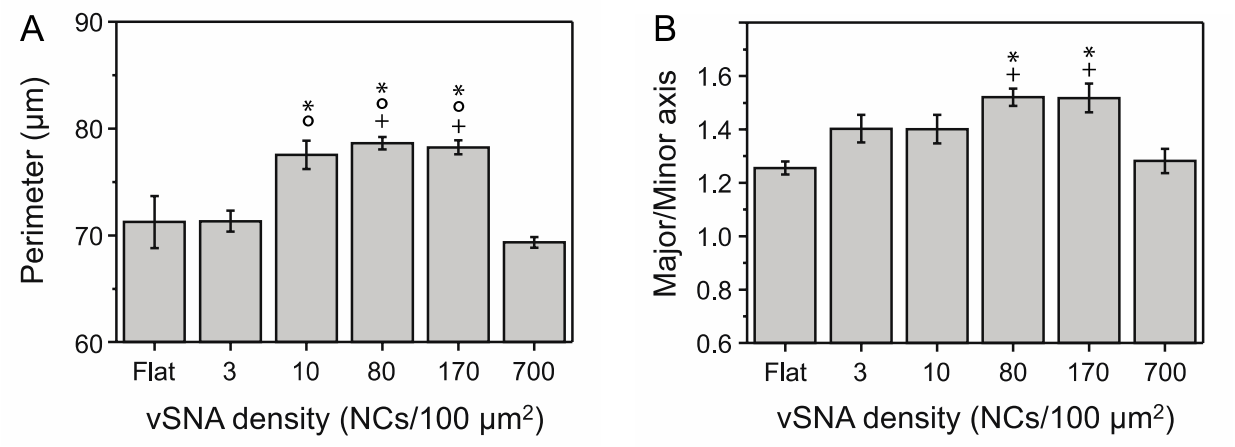


Figure S6: Average cell perimeter (**A**) and cell aspect ratio (**B**) extracted from fluorescence images of live NIH3T3 cells on the various vSNA densities and flat Si for reference. The error bars represent the standard error of the mean between independent experiments (n=3) and significant differences ($p<0.05$) are indicated as + (vs. Flat), ◦ (vs. 3 NCs/100 μm^2), and ∗ (vs. 700 NCs/100 μm^2).

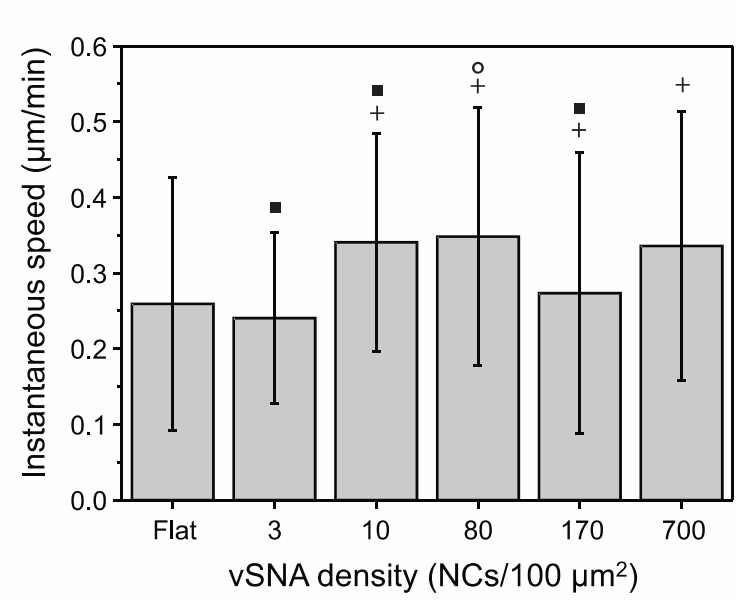


Figure S7: Instantaneous speed of cells on flat Si and vSNAs with different densities. The error bars represent the standard deviation between single cell trackings from one experiment and significant differences ($p<0.05$) are indicated as + (vs. Flat), ◦ (vs. 3 NCs/100 μm^2), and ■ (vs. 80 NCs/100 μm^2).

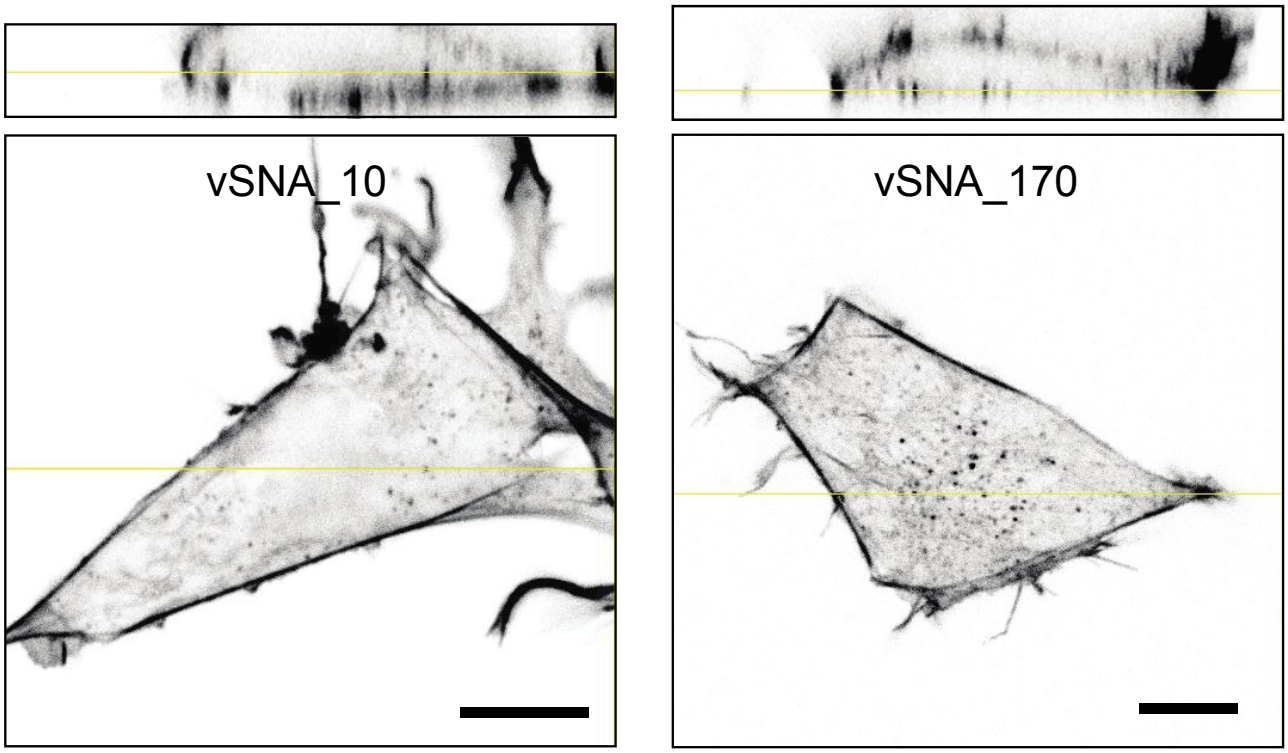


Figure S8: Confocal slices and orthogonal views through the actin signal of fixed and immunostained NIH3T3 on the indicated vSNA densities (vSNA_10 = 10 NCs/100 μm^2, vSNA_170 = 170 NCs/100 μm^2). Scale bars denote 10 μm.

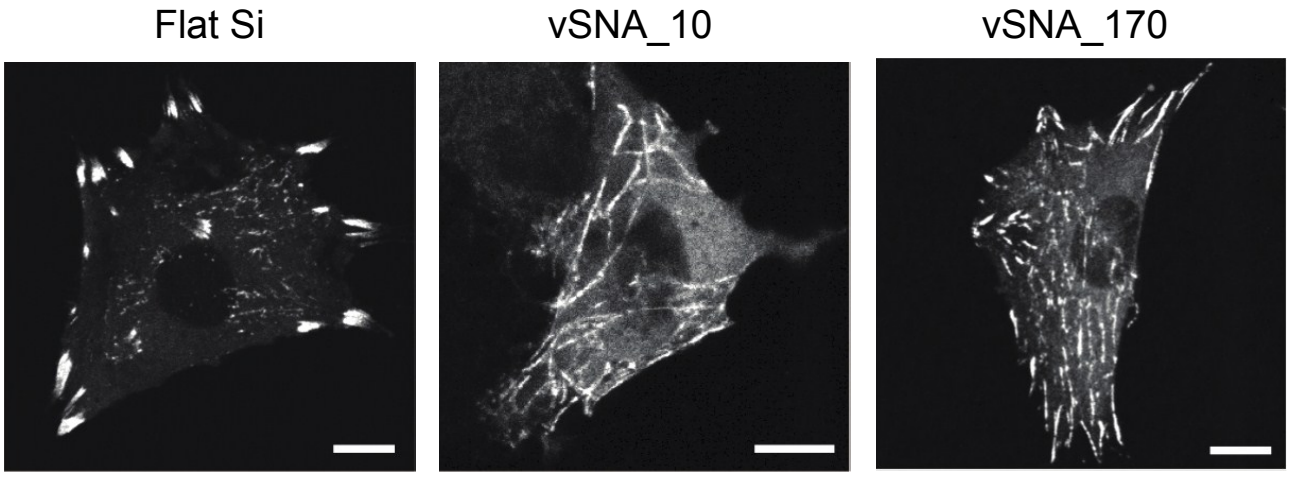
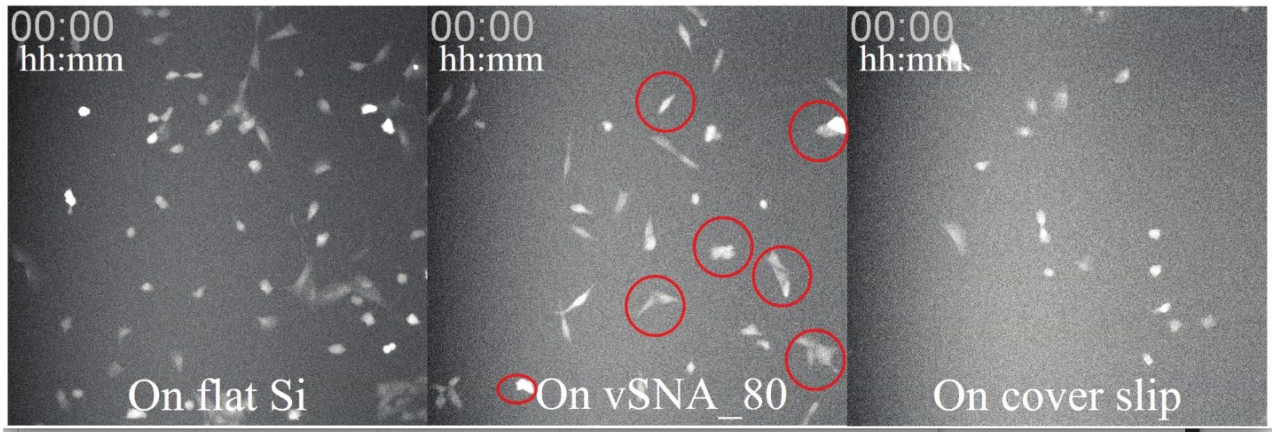
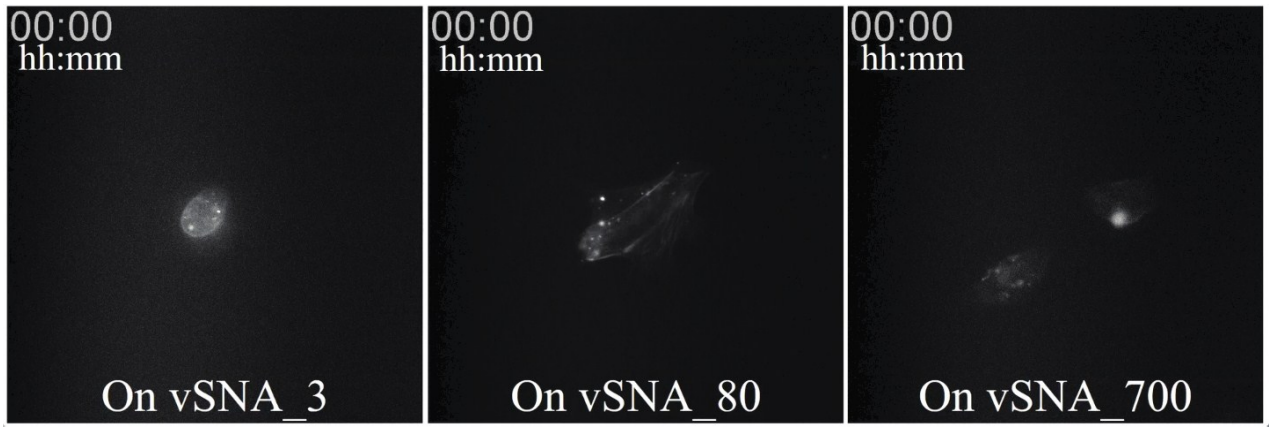


Figure S9: Summed confocal stacks showing FAs (seen via fluorecent paxillin) in live NIH3T3 on flat Si or the indicated vSNA densities (vSNA_10 = 10 NCs/100 μm^2, vSNA_170 = 170 NCs/100 μm^2). Scale bars denote 10 μm.



Movie S1: Time-lapse imaging of RFP-NIH3T3 on vSNA_80 (80 NCs/100 μm^2), flat Si or a coverslip. Images were collected every 2 min for ~24 h with an upright fluorescence microscope, while keeping the cells at 37°C and 5% CO_2 in a live cell chamber. Stationary cells on vSNA_80 are indicated with red circles.



Movie S2: Time-lapse imaging of fluorescently stained F-actin (SiR-actin) in NIH3T3 on vSNA_80 (80 NCs/100 μm^2), flat Si or a coverslip. Images were collected every 2 min for ~8 h with an upright fluorescence microscope, while keeping the cells at 37°C and 5% CO_2 in a live cell chamber.

References

[1] S. Bonde, T. Berthing, M. H. Madsen, T. K. Andersen, N. Buch-Månson, L. Guo, X. Li, F. Badique, K. Anselme, J. Nygård, K. L. Martinez. *ACS Appl. Mater. Interfaces* **2013**, *5*, 10510.

[2] N. Buch-Månson, S. Bonde, J. Bolinsson, T. Berthing, J. Nygård, K. L. Martinez. *Adv. Funct. Mater.* **2015**, *25*, 3246.