Supplementary information

Experimental details:

Materials

Precut wafers (P/Boron<100>, SI-MAT) were purchased from Litcon AB (Sweden) and cleaned in acetone, ethanol and MQ water (MilliQ gradient, Milipore) prior to use. PDDA (poly(diallyldimethylammonium chloride)), PSS (poly(sodium-4-styrenesulfonate)) and octadecylmercaptan were purchased from Sigma-Aldrich. PAX-XL60 (polyammonium chloride) was purchased from KemiraMiljø and polystyrene colloidal particles were purchased from Invitrogen. Buffers were made from MQ water, adjusted to pH 7.4 by adding hydrochlorid acid or sodium hydroxide and filtered prior to use. The used buffers were: 10mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and Ringers with 10 mM HEPES 154 mMNaCl and 7.2 mMKCl. PLL(20)-g[3.5]-PEG(2) from Surface Solution were dissolved in HEPES to 250µg/ml and filtered prior to use. Neutravidin(60 kDa) and biotinylated protein A(45kDa) were purchased from Fisher Scientific and diluted in Ringers to respectively 500 µg/ml and 20 µg/ml. Albumin from bovine serum(~66 kDa) from Sigma-Aldrich was diluted to 2% in Ringers. E-cad-Fc used was produced by recombinant expression in HEK293 cells and purified from cell culture media using a Protein ASephadex column (GE Healthcare). The extracellular domain of E-cadherin was fused at the C-terminus to the Fc domain of human IgG1.[1] (Drees et al 2005).E-cadherin:Fc was dissolved to 20 µg/ml in Ringers buffer. E-cadherin:Fc (E-cad:Fc) was fluorescent labelled with Cy5.5 monofunctional dye using AmershamFluorLink^{TM}labelling kit (GE Healthcare).

Sample preparation

The precut oxidized silicon wafers were coated by 4 nm Ti and 30 nm Au (RF magneton sputtering, $2 \cdot 10^{-3}$ mbar argon pressure, Ti deposition rate 1nm/s (6.45 W/cm^2), Au deposition rate 2.2nm/s (2.5 W/cm^2)). Au coated wafers were cleaned for 1h in UV/ozone followed by 1h in MQ water to reduce the oxidized Au back to Au(0). The nanostructured surfaces were made by sparse colloidal lithography by deposition of a triple layer of polyelectrolyte (2% PDDA in MQ, 2% PSS in MQ and 5% PAX-XL60) followed by self-assembly of charged polystyrene particles (0.2% in MQ for 100-200 nm and 0.5% in MQ for 300-800 nm). After deposition the samples were carefully rinsed. Before drying the substrates, samples with particles >100 nm were transferred to pressure chambers with MQ water and heated to 120°C for 1 h. After drying of the samples under a stream of nitrogen gas the samples were coated with 2 nm Ti and 11 nm SiO_2 by evaporation (3kW Multiple Crucible Linear e-gun, Port Townsend. Ti deposition rate 0.5-1 Å/s, SiO_2 deposition rate 1-10Å/s). The particles were removed by tape stripping and ultrasonication in ethanol and MQ water. The samples were characterized by Scanning Electron Microscopy (Magellan™ XHR SEM, FEI) to determine hole size and interhole distance. The diameter distribution of each patch size was measured using ImageJ.

The characteristic spacing of the holes on each sample type was determined by analysis of min. 4 SEM images from one sample of each type. The images were used to identify the center of each hole which was then used to calculate the hole radial distribution functions for each image and the average peak position marks the characteristic spacing, whereas the average full width at half maximum of the peak gives the error bars.

Protein adsorption measurement

AT-cut quartz crystals with a fundamental frequency of 5MHz were purchased from Q-sense (Sweden) with gold electrodes and used as purchased or coated with 2 nm Ti and 11 nm SiO_2. The nanostructured surfaces were prepared on the QCM-crystals by sparse colloidal lithography and characterized by SEM. The measurement were performed with Q-sense E4 system (Q-sense AB) where the resonance frequency and the 3^{rd}-13^{th} overtone were recorded simultaneously with the dissipation. Prior the experiment the crystals were immersed in 2mM octadecylmercaptan in ethanol overnight. The self-assembled monolayer of alkanethiol was ultrasonicated in ethanol and MQ water. During rinsing and exchange of buffers, liquid was pumped through the sensors with a flow rate of 1ml/min. For protein adsorption 0.5 ml of the solution were pumped to the sensor with a flow rate of 0.1 ml/min. Thereafter the pumps were stopped and the protein adsorptions were allowed to continue under static condition. After a stable baseline was achieved PLL-g-PEG in HEPES was introduced and allowed to adsorb for 30 min. After rinsing and exchange of buffer to Ringers, Neutravidin was allowed to adsorb for 2h. Before and after blocking the surfaces with BSA for 30 min the sensors were rinsed with buffer. Hereafter protein A was introduced and allowed to adsorb for 45 min. Before the last protein adsorption the buffer was changed to Ringers and E-cadherin:Fc was adsorbed overnight. The data was modeled using Q-Tools software 3.0.11.512 and the signals were fitted to the viscoelastic model. Parameter used as fixed parameters were fluid density (1000 kg/m3), fluid viscosity (0.001 kg/ms) and layer density (1175 kg/m^3). The layer parameters were fitted within boundaries: viscosity (0.001-0.1 kg/ms), shear (10^4-10^8 Pa) and thickness (10^{-10}-10^{-6} m).

SPR measurements were performed with a Biacore X system from Biacore AB (Uppsala, Sweden). A flow rate of 10µL/min was used, and a stable baseline was attained before adsorption. The procedure followed that of a QCM-D experiment with the same buffers and chemicals. Maximum injections were 100ul giving a maximum injection time of 10 min. Neutravidinwas injected twice to obtain a longer

adsorption time. General the adsorption are finished faster for the SPR measurements than for the QCM-D experiments because of the flow versus static conditions.

Cell Adhesion Assay

Prepared samples (6· 6mm) went through an initial step of incubation with PLL-g-PEG (0.25mg/ml sterile filtered in HEPES buffer without salt, incubation for 30 minutes), buffer rinse and change to buffer with salt. The samples were incubated with neutravidin (0.5 mg/ml, incubated for 2 h at RT), blocked with 2% BSA, incubated with protein A (20µg/ml, 1h at RT) and subsequent in E-cad:Fc (20µg/ml, 2h at RT). Each adsorption step was followed by buffer rinse. The samples were transferred to 24-well low-adherent tissue culture plates and rinsed with Ringers.

Madin Darby canine kidney (MDCK) cells stably expressing E-cadherin tagged with enhanced green fluorescent protein[2] (Adams et al. 1998) were seeded onto Au samples in a volume of 100000 cells per well in a 24-well low adhesion plate. Cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 0.5 u/mL penicillin, 0.5 mg/mL streptomycin and 1 mg/mL kanamycin in a humidified atmosphere at 37˚C with 5% CO_2 for 16 hours. Cells were fixed by adding 12% paraformaldehyde directly to the growth medium so the final concentration in each well was 4%. Washing steps were omitted due to the very weak attachments of the cells on the smaller nanopatterns. After 10 minutes of fixing, the cells were washed twice in 1xPBS, permeabilized in 1xPBS + 0.5% Triton X-100 for 5 minutes and stained with Phalloidin-Rhodamine diluted 1:500 in 1xPBS + 3% BSA and 2 µg/mL Hoechst for 1 hour or 2 µg/mL Hoechst alone for 20 minutes. After three times of washing in 1xPBS, Au samples were mounted on microscope slides using Glycergel mounting medium (Dako).

Preparation of cells for SEM

A secondary fixation was done by immersing the fixed samples in osmium tetroxide (1 % in PBS, Sigma) for 20 min followed by washing in PBS. The cells were dehydrated in a graded series of ethanol (25%, 50%, 70%, 85%, 95% and 2 x 100%) for 5 min each. Dehydrated samples were incubated in hexamethyldisilazane (100%, Sigma) for 3 min and air-dried.

Microscopy and data analysis

Fluorescent images were captured using an Zeiss Axiovert 200M inverted fluorescence microscope (Zeiss, Germany) and high resolution images was obtained by using an apotome function.

5 pictures were taken for each magnification on each sample. The images with 20x magnification were randomly chosen on the sample, whereas the 63x magnification images were manually chosen as representative of the population on the surface. Images were analyzed using Image J software (available from http://rsbweb.nih.gov/ij/) and k-mean cluster analysis was performed using MatLab software.

Statistics

Every condition was run in quadruplicate and the whole assay was repeated twice. Quantitative data is displayed from one assay showing average and standard deviations.

Table S1: Characteristic properties of patterned substrates

Sample	Measured diameter (nm)	Area (nm^2)	Estimated #E-Cad-Fc immobilized per protein patch	Estimated # cellular cadherin above a protein patch	Characteristic center to center distance (nm)	Characteristic edge to edge distance (nm)
100	106 (±4)	8800 (±16)	40	6	264 (±54)	158
200	175 (±6)	24100 (±36)	110	17	397 (±91)	220
300	245 (±9)	47100 (±81)	220	33	542 (±125)	297
800	747 (±15)	43800 (±225)	2040	300	1170 (±170)	424

The characteristic spacing of the holes on each sample type was determined by analysis of min. 4 SEM images from one sample of each type. The images were used to characterize the diameter, area and center of each hole which was then used to calculate the hole radial distribution functions for each image and the average peak position marks the characteristic spacing, whereas the average full width at half maximum of the peak gives the spread of values. The produced patterns have coverage of gold patches ranging from 18% to 28% with the lowest coverage for the smallest patches. The distance between each pattern is at least 150 nm.

The estimate for the number of cadherin per protein patch is based on the areas of each patch and from a measured density of 4660 cadherin per square micron made from combined QCM-D and SPR measurements at homogeneous surfaces. The estimated number of cellular E-cadherins in AJs is based on a literature value estimate of 700 cadherins per micron within AJ's.[3] We estimate that the density of E-cadherin-Fc immobilised per patch is roughly 6 times higher than the density of cellular E-cadherin expected to found in AJs[3]. We believe that the density of surface immobilised ligands does not play a significant role in determining the cell binding properties.

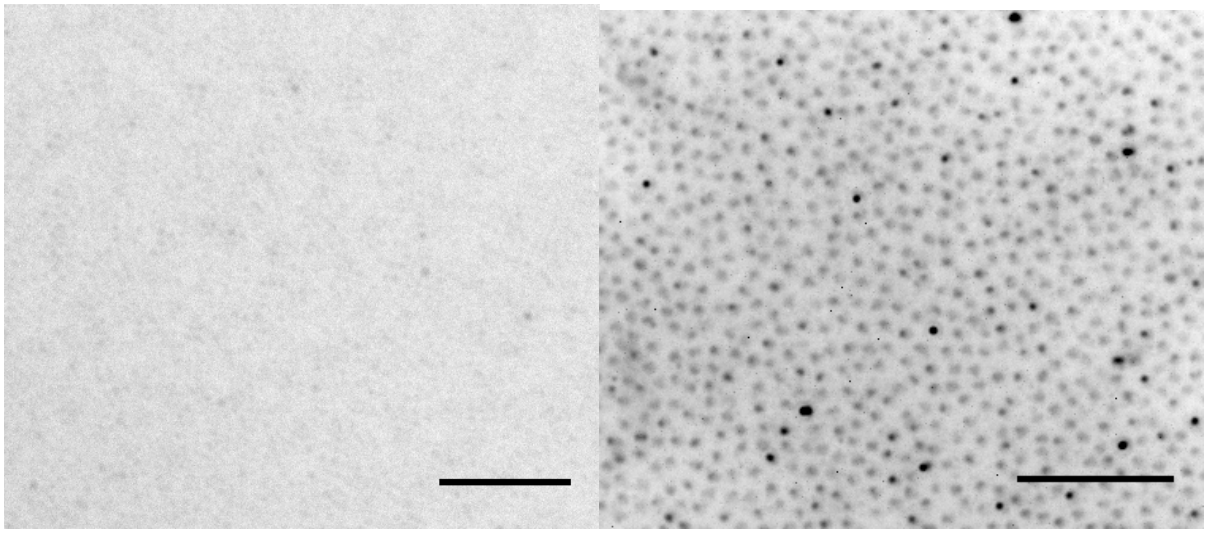

Figure S1: Fluorescence microscopy images of E-cadherin-Fc 300nm(left) and 800nm(right) pattern.Scalebar 10µm.

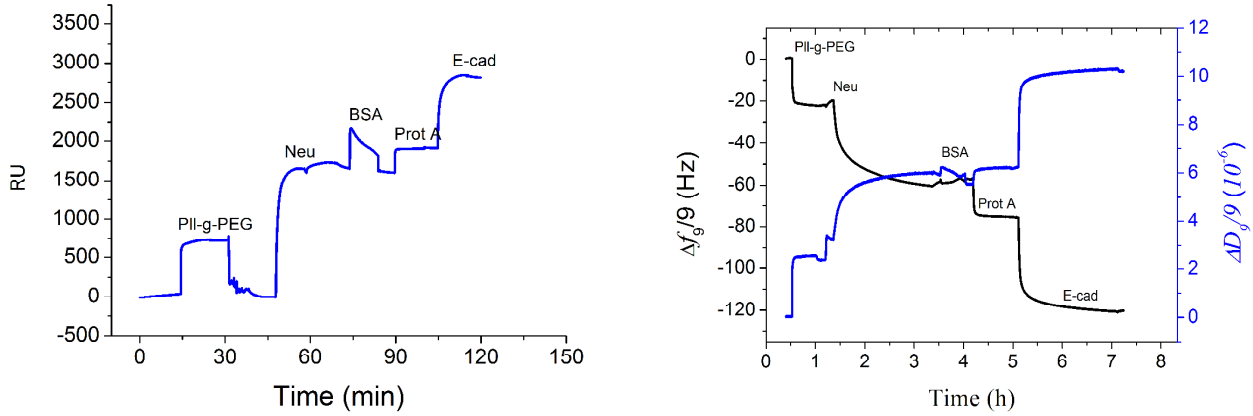

Figure S2: Representative SPR (left) and QCM-D (right) spectra of protein adsorption.

Table S2: Modeled parameters from SPR and QCM-D measurements after protein bindings

	ΔRU	m_{SPR}(ng/cm^2)	m_{QCM} (ng/cm^2)	d (nm)	ρ (kg/m^3)
Pll-g-PEG	520(68)	44.86(2)	451(16)	4.4(2)	1025(1)
Neutravidin	1696(117)	148.6(5)	949(105)	9.1(1)	1041(5)
Protein A	1958(116)	173.0(9)	1222(157)	12.8(2)	1037(5)
E-caderin	2925(115)	267.7(22)	2019(257)	19.5(3)	1035(5)

Adsorptions of the macromolecules onto alkanethiol modified homogenous surfaces were quantified

by SPR (Biacore X, Biacore AB, Sweden) and QCM-D (Q-sense E4, Q-sense AB, Sweden). Modeling of QCM-D data from viscoelastic layers gives a set of solutions where the density of the layer cannot be separated from the thickness. Combination of QCM-D and SPR allows for the determination of both the adsorbed mass and the effective density of the adsorbed layers. Modeling of QCM-D data gave a Voight mass for a given layer density guess (eq. 1). A surface plasmon resonance value of adsorbed dry mass (eq. 2) was then used to calculate a new layer density (eq. 3).[4]An iterative approach typically led to convergence after a few rounds.

$$m_{QCM} = d \cdot \rho_{effective} \quad (1)$$

$$m_{SPR} = \frac{l_{decay}}{2}\frac{dc}{dn}\frac{dn}{d\theta}\Delta\theta = C_{SPR}\Delta RU \quad (2)$$

$$\rho_{effective} = \frac{m_{QCM}}{\frac{m_{SPR}}{\rho_{prot}} + \frac{m_{SPR} - m_{QCM}}{\rho_{water}}} \quad (3)$$

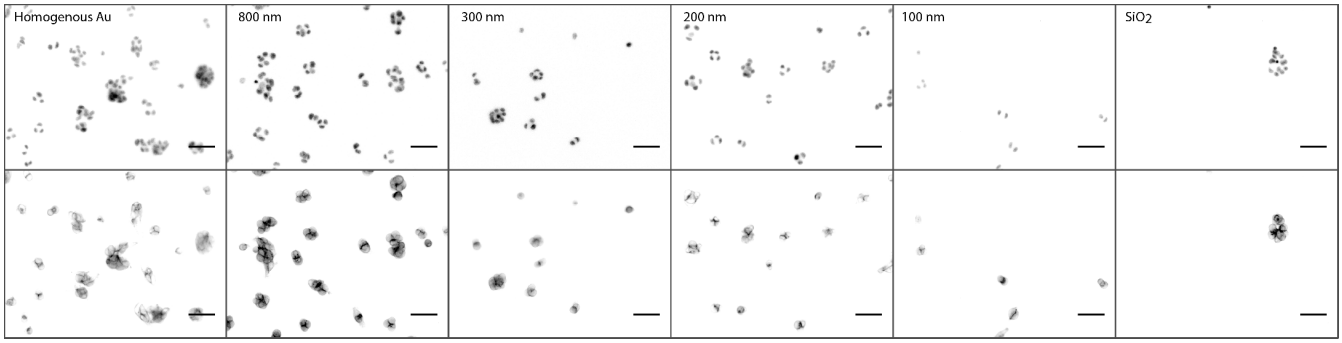

Figure S3: Fluorescent microscopy images using 20 x objectives. Top: nucleus staining, bottom: actin staining. The patch size is displayed for each sample type, scalebar 50µm.

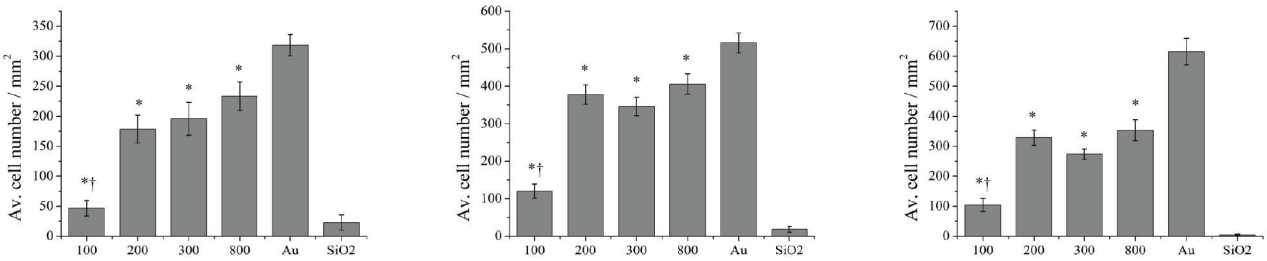

Figure S4: Quantitative data for cell adhesion to different nanopatterned surfaces from three full repeats showing cell number for each sample type. Standard error means are displayed. The data is

derived from 5 images per sample using a 20x objective, and 4 samples per condition.). * The means difference is significant different from homogenous Au at the 0.05 level. † The means difference is significant different from 200 nm at the 0.05 level.

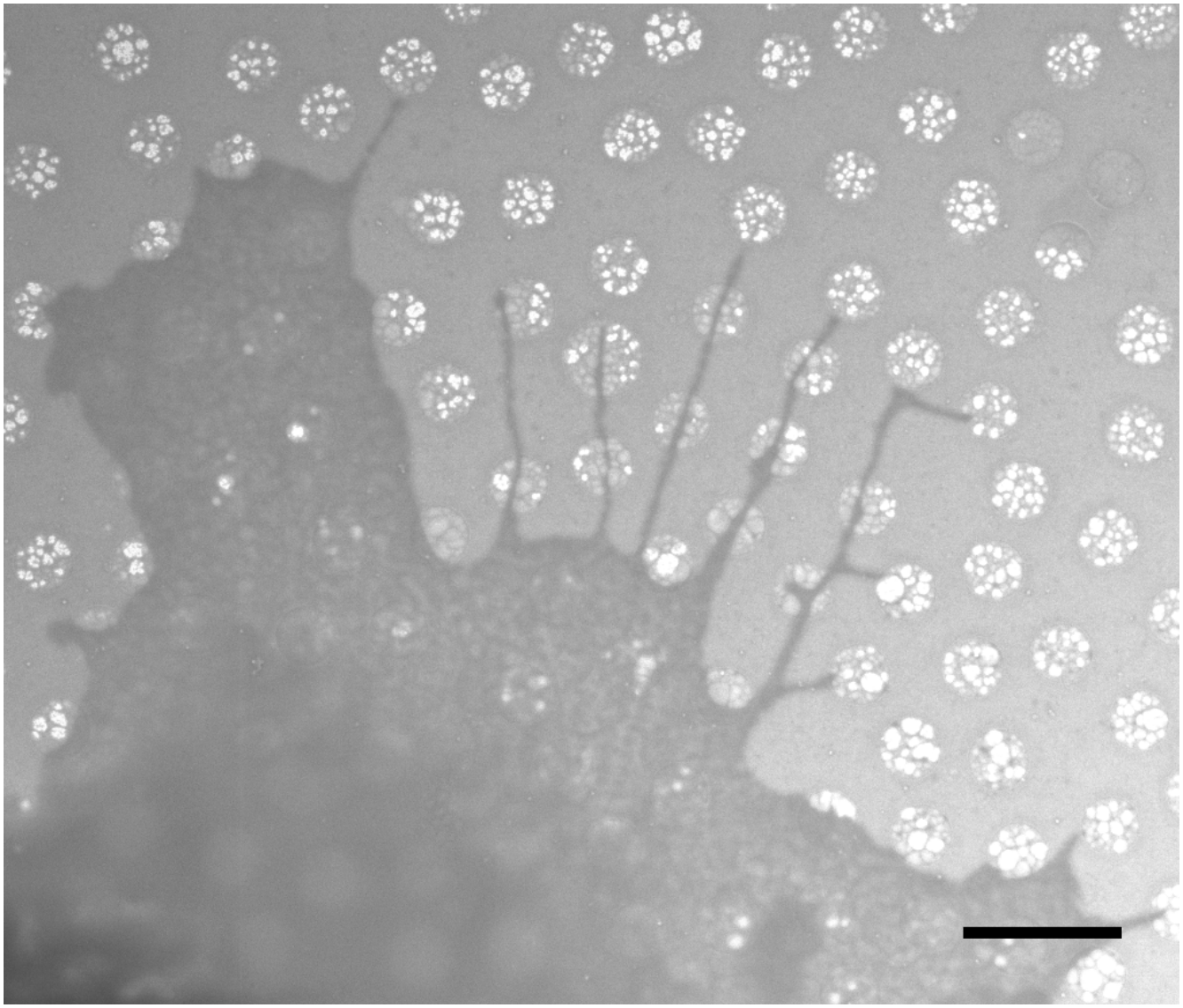

Figure S5: Scanning electron microscopy images of an MDCK cell interacting with 800nm E-cad:Fc protein patches. Long refractions fibers located on top of the protein patches can be seen. Scale bar 2µm.

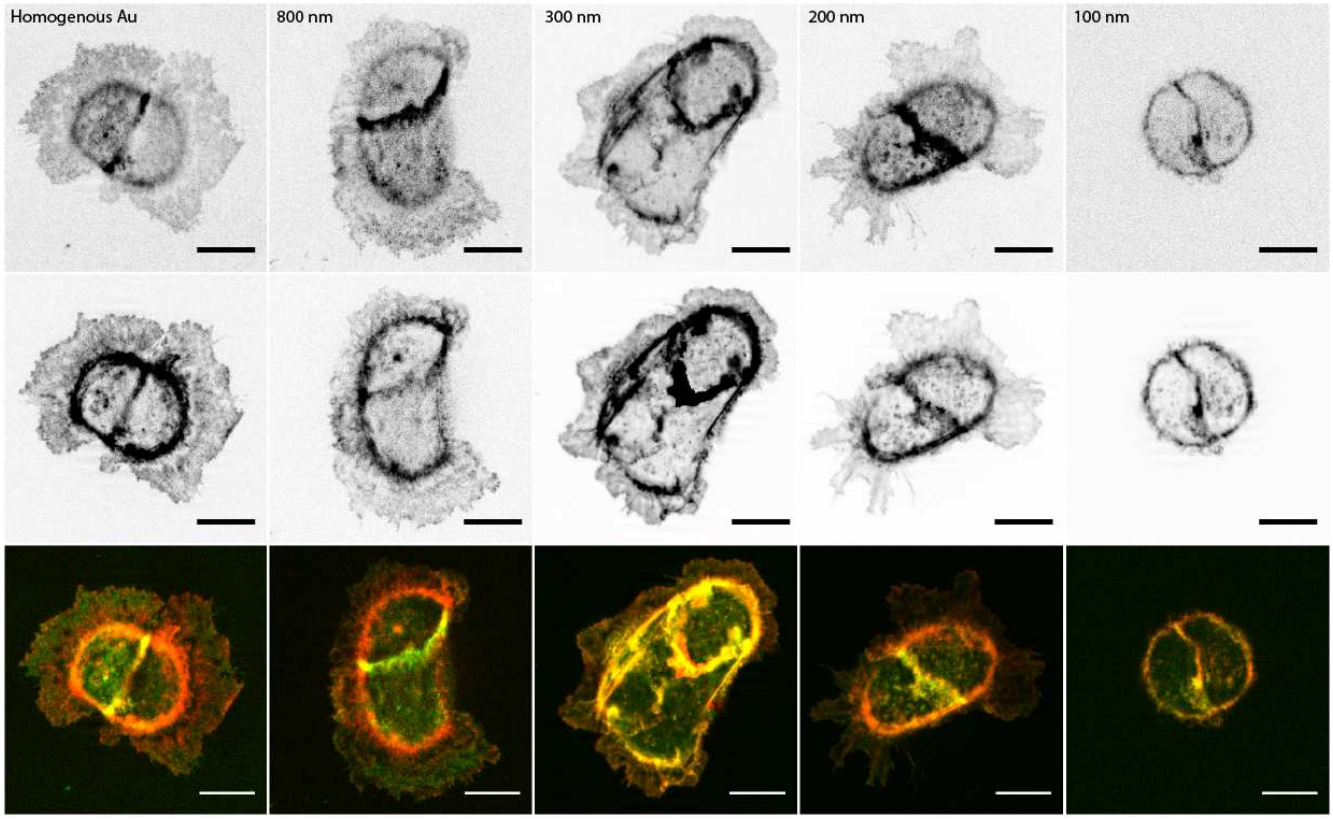

Figure S6: Fluorescent microscopy images of double or triple cells staining cellular E-cadherin (top), actin (middle) and merged (bottom). The patch size is displayed for each image. Scale bar 10 µm

1. Drees, F., et al., *alpha-catenin is a molecular switch that binds E-cadherin-beta-catenin and regulates actin-filament assembly.* Cell, 2005. **123**(5): p. 903-915.
2. Adams, C.L., et al., *Mechanisms of epithelial cell-cell adhesion and cell compaction revealed by high-resolution tracking of E-cadherin-green fluorescent protein.* Journal of Cell Biology, 1998. **142**(4): p. 1105-1119.
3. Katsuyuki, M., *Ultrastructure of the Zonula Adherens Revealed by Rapid-Freeze Deep-Etching.* Journal of Structural Biology, 2000. **132**(3): p. 169-178.
4. Reimhult, E., et al., *Simultaneous surface plasmon resonance and quartz crystal microbalance with dissipation monitoring measurements of biomolecular adsorption events involving structural transformations and variations in coupled water.* Analytical Chemistry, 2004. **76**(24): p. 7211-7220.