Supplemental Materials

Electron beam deposition was carried out with a PVD 75 electron beam evaporator (Kurt J. Lesker, Clairton, PA). Plasma oxidation was carried out in a Femto standard low pressure plasma system (Diener electronic GmbH+Co. KG, Nagold). Fluorescent and phase contrast images were obtained using a Nikon TE2000-PFS microscope running NIS-Elements imaging software and equipped with a Prior XY stage, EXFO X-Cite series 120PC UV illuminator, Photometrics CoolSNAP monochrome camera, and In Vivo Scientific incubation system.

**PDMS Stamp Preparation**

**Master Formation.** A silicon wafer (50 mm, Montco Silicon) was cleaned with acetone. AZ 9245 (1-2 mL, Mays Chemical Company, Indianapolis, IN) was applied to the wafer using a CEE 200CB spin/bake system (Brewer Science, Rolla, MO) and an even coating of resist (nominally 4.5 µm) was achieved using a two-cycle program (1000 rpm/500 rpm/s/5 s, 3800 rpm/3800 rpm/s/30 s). The wafer was soft baked at 110°C for 2 min. Photolithography was carried out using a LaserWriter system equipped with a 325 nm laser (Microtech, Palermo, Italy). The wafer was developed in 1:3 400K developer (Mays Chemical Company, Indianapolis, IN): deionized water for 2 min. The resulting master was used for stamp formation.

**PDMS Stamp Formation.** Sylgard 182 (Dow Corning, Midland, MI) was mixed 10:1 (resin:hardener) and poured over the patterned silicon master. The polymer was de-gassed using a vacuum dessicator and cured at 70°C for 2 h. The final stamp was separated from the master and cut to size.
**Patterning SAMs**

Glass coverslips (25mm, No. 1, VWR, Batavia, IL) were cleaned by oxygen plasma oxidation for 10 min at 100% power. Coverslips were then twice rinsed with water and ethanol, and dried under nitrogen. Deposition of 50Å titanium followed by 150Å gold onto the glass coverslips was carried out with a PVD 75 electron beam evaporator.

The stamp was coated with hexadecanethiol (Alfa Aesar, Ward Hill, MA) (10 mM in ethanol) by dropping the solution onto the stamp (5-6 drops) and drying with nitrogen. Slides were then stamped for 10 s. The bare regions of gold were allowed to react with 1 mM 12-mercaptododecanimide tetraethylene glycol (amide-linked glycol thiol) or 1 mM (1-mercaptoundec-11-yl)tetra(ethyleneglycol) (ether-linked glycol thiol) in ethanol for 12-14 h. After soaking, coverslips were twice rinsed with ethanol and dried under nitrogen.

**Patterned Cell Growth**

*Preparation of Fluorescently Labeled Fibronectin.* To 20 µL Human Plasma Fibronectin (1 mg/mL in 100 mM CAPS, 0.15 M NaCl, 1 mM calcium chloride, pH 11.5, Invitrogen, Carlsbad, CA) was added 1 µL of 1 M sodium bicarbonate in sterile water and 2 µL AlexaFluor 647 carboxylic acid, succinimidyl ester (5 mg/mL in DMF, Invitrogen). The reaction was mixed and allowed to proceed at room temperature for 1 h. The reaction was quenched by addition of 3 µL of 1.5
M hydroxylamine in 1 N sodium hydroxide and mixed with 20 µL unlabeled fibronectin.

Cell Culture. A patterned coverslip (stamped with 10 mM hexadecanethiol and incubated in 1 mM amide-linked glycol thiol or 1 mM ether-linked glycol thiol for 12-14 h) in either a Noryl or Teflon cell chamber was coated with fibronectin (prepared as described above) at 20 µg/mL in Dulbecco’s Phosphate Buffered Saline (DPBS) at 37°C for 1 h. Excess protein was removed by rinsing with DPBS (3x) and the coverslip was covered with fresh DPBS. CHO-K1 or NIH/3T3 cells (ATCC, Manassas, VA) were separated using TrypLE Express (Invitrogen), followed by resuspension in Dulbecco’s Modified Eagle Medium for CHO-K1 (DMEM, low glucose 1X, glutamax, 1 g/L D-glucose, 110 mg/L sodium pyruvate, 50 mL FBS, 5 mL penicillin/streptomycin (10,000 units/mL Penicillin G Sodium and 10,000 µg/mL Streptomycin Sulfate in 0.85% saline), Invitrogen) or Dulbecco's Modified Eagle Medium for NIH/3T3 (DMEM, high glucose 1X, glutamax, 1 g/L D-glucose, 110 mg/L sodium pyruvate, 50 mL NCS, 5 mL penicillin/streptomycin (10,000 units/mL Penicillin G Sodium and 10,000 µg/mL Streptomycin Sulfate in 0.85% saline), Invitrogen), and counted using a hemacytometer (Bright-Line, Hausser Scientific). After rinsing the patterned coverslip with DPBS, approximately 200,000 cells were applied in 1 mL of DMEM. Plated cells were grown at 37°C, 5% CO₂. Live cultures were visualized by inverted microscopy using phase contrast optics and labeled protein was visualized using a Cy5 filter cube (Semrock, Rochester, NY).
**Recycling with Detergent.** Cells were removed by soaking in 1% Triton X-100 in DPBS for 10-15 min. The substrate was rinsed 6-8x with sterile nanopure water followed by 4x with DPBS. The remaining fluorescent protein pattern was imaged after each washing (Figure S1). After visualization, substrates were reseeded with CHO-K1 or NIH/3T3 cells at a density of approximately 200,000 cells/dish.

Figure S1. AlexaFluor 647-labeled fibronectin after washing with 1% Triton X-100 and CHO-K1 or NIH/3T3 patterned cell growth. Scale bar is 100 µm.

**Recycling with TrypLE Express.** Cells were released from the patterned substrate with TrypLE Express. The slide was rinsed with DPBS then incubated with 1 mL TrypLE Express for 5 min at 37°C. The dish was rinsed 2x with fresh DMEM followed by seeding of CHO-K1 or NIH/3T3 cells at approximately 200,000 cells/dish.
Figure S2. Recycling patterned ether-linked glycol thiol monolayers six times.
a) The TrypLE Express mediated release method and b) the Triton X-100 washing method both allow for four full cycles before confinement is lost. Scale bar is 100 µm.