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 110ºC 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 70ºC 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^{o}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^{o}C, 5% CO_2. 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.

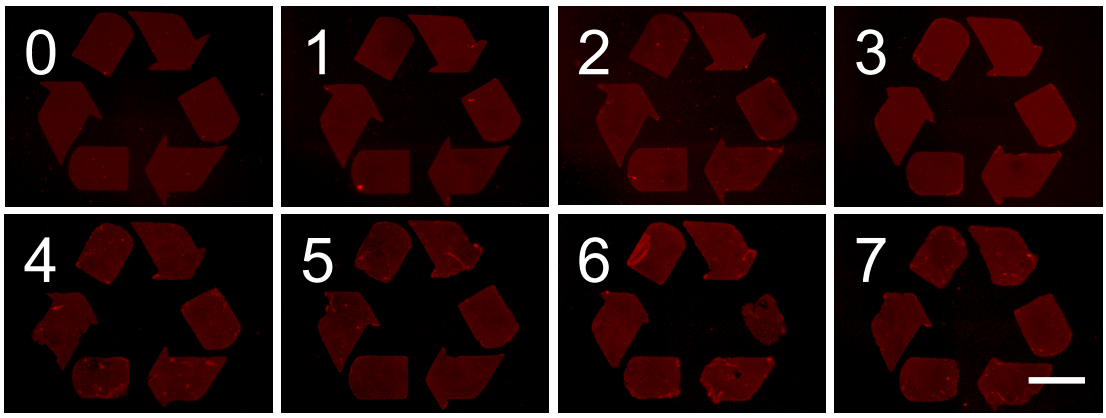

Figure S1. AlexaFluor 647-labeled fibronectin after washing with 1% Triton X-100 and CHO-K1 or NIH/3T3 patterned cell growth. Scale bar is 100 μm.

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^{o}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.

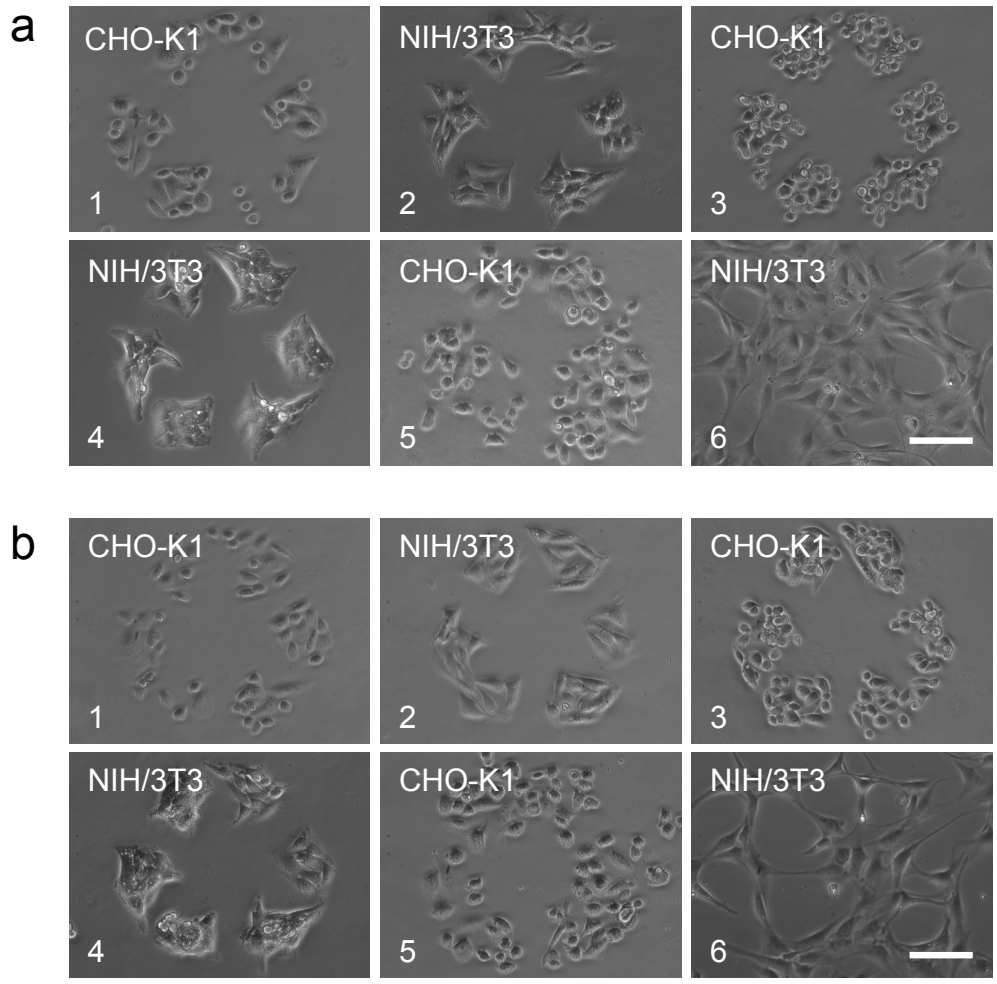

Figure S2. Recycling patterned ether-linked glycol thiol monolayers six times. a) The TrypLE Express mediated release method and b) the Triton X-100 washing method both allow for four full cycles before confinement is lost. Scale bar is 100 μm.