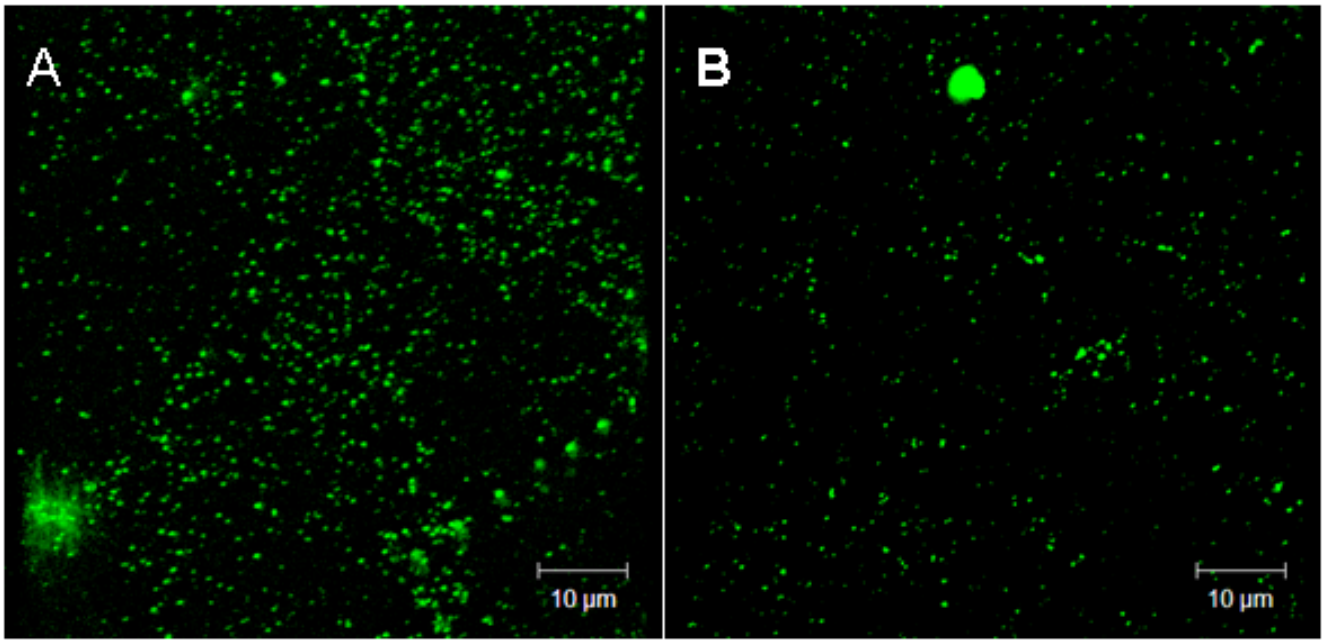
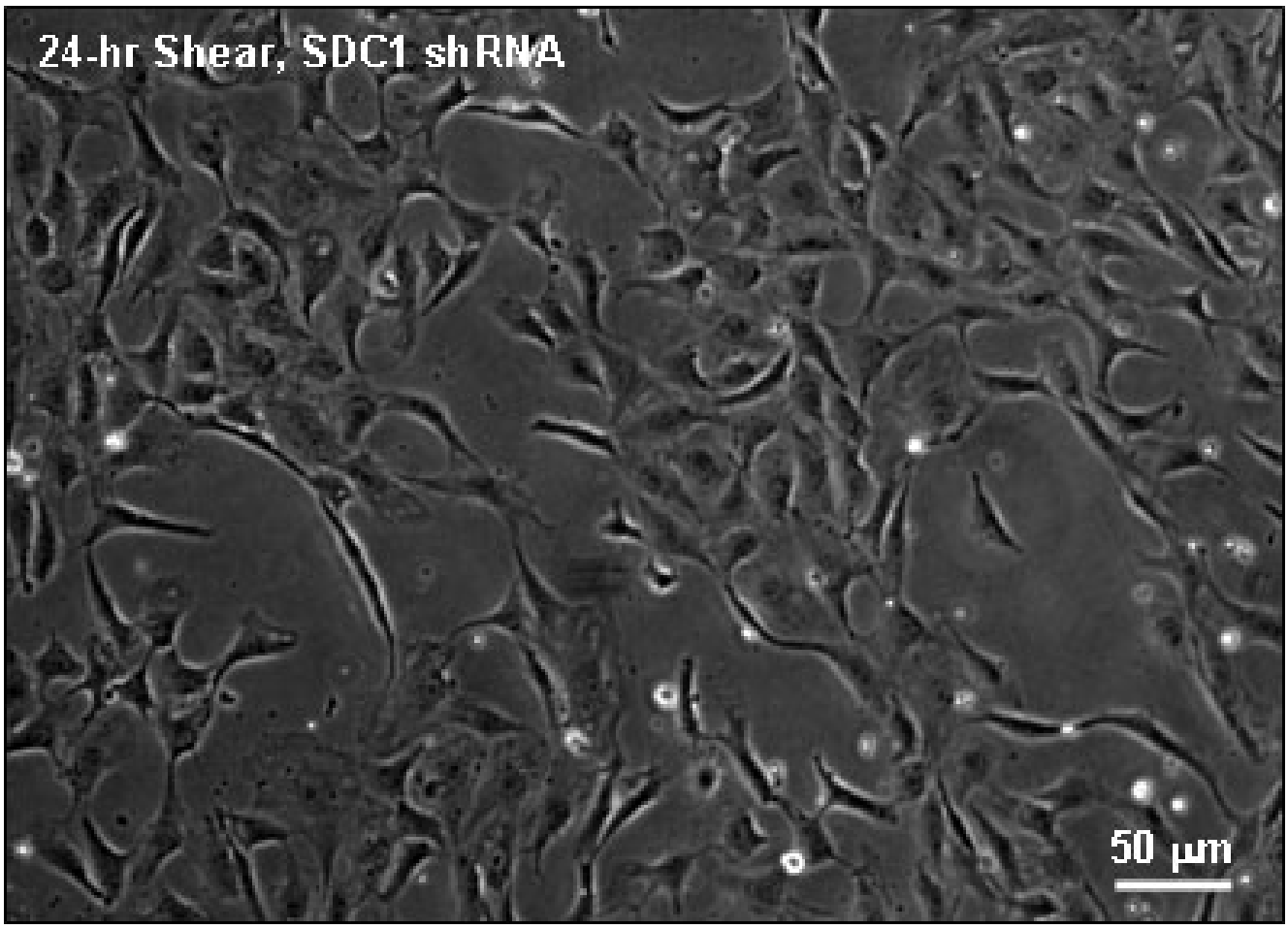
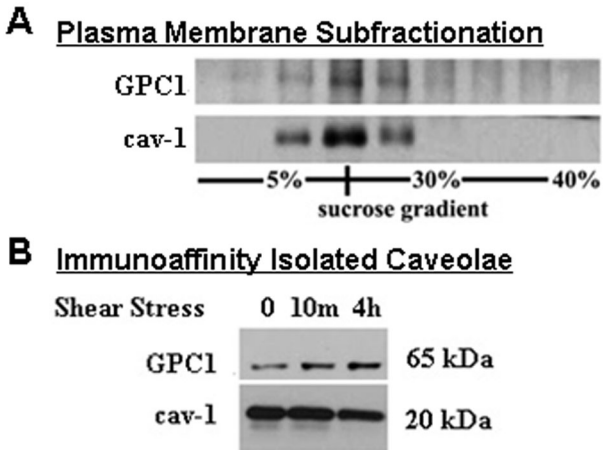


Supplemental Figure 1: EC monolayer heparan sulfate fluorescence intensity. (A) Enface perspective of an untreated HS-stained BAEC monolayer viewed using a laser confocal microscope; Bar = 10 µm. (B) Enface confocal microscope perspective of GPC1-treated and HS-stained BAEC monolayer; Bar = 10 µm. The results shown in A and B confirmed the loss of HS coincident with reducing both GPC1 and SDC1 core protein expression using the GPC1 shRNA.



Supplemental Figure 2: Phase contrast image of 24-hour sheared BAEC that were SDC1 shRNA-treated. Bar = 50 µm.



Supplemental Figure 3: (A) Plasma Membrane Subfractionation: Plasma membranes derived from static-, 10 min, and 4 hr flow conditioned BAEC were subfractionated by sucrose gradient centrifugation to separate membranes rafts from other plasma membrane domains. Western blots indicated that both GPC1 and caveolin-1 (cav-1) are enriched in the light buoyant density raft fractions. (B) Immunoaffinity Isolated Caveolae: BAEC were subjected to flow for indicated time points. Plasma membranes were isolated by Percoll gradient centrifugation, sonicated, and processed for immunoaffinity isolation of intact caveolae vesicles. Caveolar proteins were resolved by SDS-PAGE and immunoblotted for GPC1 and cav-1. The data shows that a pool of GPC1 is present in caveolae. Exposure to 4 hrs of flow increases the content of GPC1 in caveolae, relative to static control. The protocols for generating membrane rafts and isolating caveolae are described in the *Supplemental Material and Methods*.

SUPPLEMENTAL MATERIALS & METHODS

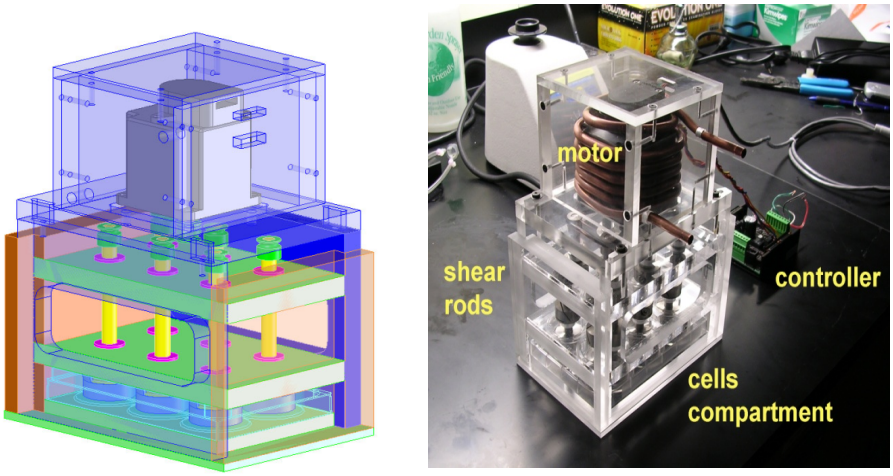
Cell Culture: BAEC were obtained from Lonza Walkersville, Inc. (Walkersville, MD). Cells were grown in tissue culture dishes according to Lonza instructions in EGM-MW growth medium supplemented with 5% fetal bovine serum (FBS), 0.04% hydrocortisone, 0.4% human fibroblast growth factor, 0.1% vascular endothelial growth factor, 0.1% R3-insulin-like growth factor-1, 0.1% ascorbic acid, 0.1% human recombinant epidermal growth factor, and 0.1% gentamicin and amphotericin-B (GA-1000), all purchased from Lonza. At 70% to 90% confluence, BAEC were sub-cultured with HEPES buffered saline solution (Lonza) followed by 0.25 mg/ml trypsin/EDTA solution (Lonza) to detach cells from their substrate. The cells were transferred to fibronectin-coated glass slides (30 μg/mL, Corning), seeded at a cell density of 5,000 cells/cm^2, and allowed to grow to confluency for 3-5 days.

HS Degradation: For short term shear stress experiments, HS was enzymatically degraded by adding F. heparinum heparinase III (HepIII) to the experimental medium during the two hour pre-flow period. For 24-hour shear stress experiments, HS was enzymatically degraded by HepIII treatment during the initial two hour pre-flow period and also throughout the duration of the flow experiment. The purpose of maintaining the presence of HepIII enzyme for the 24-hour flow experiment was to prevent the potential onset of HS restoration that occurs hours to days after enzyme removal [37]. As previously described, HepIII was used at a concentration of 15 mU/mL when obtained from Sigma. Occasionally, HepIII was obtained from IBEX Technologies Inc. and the concentration used was also 15 mU/mL, determined by converting IBEX international units (I.U.) to Sigma units (1 I.U. is equivalent to 600 Sigma units). HS degradation was confirmed by immunofluorescence microscopy of aldehyde-fixed BAEC stained with anti-mouse heparan sulfate (HepSS-1) monoclonal antibody (US Biological). HS fluorescent images of untreated and HepIII-treated monolayers were recorded using uniform camera settings. NIH ImageJ was employed to analyze the images, subtract background fluorescence, and measure fluorescence intensity by calculating the illuminated fraction (percent) of the total field of view.

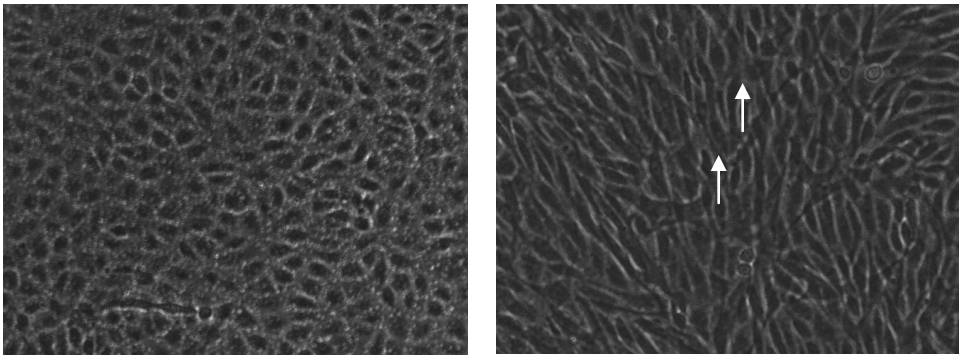
Shear Stress Experiments: BAEC were sheared in a parallel plate flow chamber (C&L Instruments) for 3 or 6 hours, or BAEC were sheared in a rotating disk flow apparatus (Supplemental Materials & Methods) for 24 hours. In the parallel plate flow chamber, with a cell contact surface area of 6.6 cm^2 and a channel height of 250 μm, confluent BAEC monolayers were sheared at 15 $dyne/cm^2$. Since all cells were exposed to the same magnitude of shear stress, the entire monolayer was suitable for protein quantification. Fluid was driven through the parallel plate chamber from a multi-head peristaltic pump (Rainin) then via a vibration damper, followed by the flow chamber, and finally a receiving reservoir before being re-circulated. The rotating disk apparatus was custom designed to shear BAEC grown in a 6-well plate, in order to maximize the number of experiments that could be performed in a 24-hour time period (Supplemental Materials & Methods). In each well, the cell contact surface was 2.7 cm^2 on a 3.1-cm diameter round glass coverslip fixed to the bottom of the well using sterile silicone grease (supplemental information). 6 cylindrical disks were suspended at 500 μm above 6 cell layers. Using a motor drive, the disks were simultaneously rotated at the number of rotations per minute required to generate an average shear stress of 14 $dyne/cm^2$ (supplemental information). The parallel plate and rotating disk flow systems were both maintained in an incubator at 37 °C with a supply of 5% CO_2. At the end of the experiments, samples were processed as described below.

Six well apparatus: To apply shear stress to endothelial cell monolayers with high-throughput, we custom designed and built a 6-well apparatus in our laboratory and in the machine shop of the Department of Mechanical Engineering at the City College of New York. The dimensions of the device were selected to support commercially available 6-well culture trays (Becton Dickenson). All components were SolidWorks drafted (supplemental fig 3). We used a mill to machine the main body of the apparatus. A lathe was used to machine stainless steel shear rods. With the appropriate bearings and collars (Mc. MasterCarr, Princeton, NJ), and at the appropriate height above the EC monolayer, the shear rods can perform the revolutions per minute needed to apply the 14 dyn/cm^2 of average shear stress. The device was assembled (supplemental fig 3) and powered with a motor and controller (Anaheim Automation, Anaheim, CA), which could produce the rotational speed needed to generate

more than 14 dyn/cm^2 of average shear stress. The motor was optionally refrigerated by running ice cold water through a copper tube coiled around the motor. A 6-well culture tray seeded with BAEC was placed inside a built-in compartment of the apparatus. The UV-sterilized and fully assembled (Figure 1) device, with cultured EC inside, was operated in a humidified, 37^{o}C, and 5% CO_2 air mixture incubator. After running the system for 24 hours, we observed no contamination and EC elongation in the direction of the shear as compared to cobblestone-shaped static controls (supplemental fig 4).



Supplemental Figure 3: Schematic (left) of the 6 well rotating shaft apparatus (right) designed and fabricated in order to apply shear stress to cultured EC monolayers.



Supplemental Figure 4: Control EC layer is shown on the left. On the right is the EC layer sheared in the 6-well apparatus. Arrows: flow direction.

Caveolar Protein Extraction and Western Blotting: Membrane rafts were fractionated from BAEC as previously described (Yang B et al., Faseb J, 2006, 20: 1501-1503). Briefly, BAEC were scraped into ice-cold, detergent-free Tricene buffer (250mM sucrose, 1mM EDTA, 20mM Tricene, pH 7.4) and centrifuged to precipitate nuclear material. The resulting supernatant was mixed with 30%

Percoll in Tricene buffer and subjected to ultracentifugation for 25 minutes (Beckman MLS50 rotor, 77,000xg, 4^{o}C). The separated plasma membranes were collected, sonicated (3 x 30 second bursts) and mixed with 60% sucrose (to a final concentration of 40%) prior to being overlaid with a 35-5% step sucrose gradient and subjected to overnight ultracentrifugation (Beckman MLS50 rotor, 87,400xg, 4^{o}C). Membrane raft fractions were collected every 400μL from the top sucrose layer and proteins were precipitated using a solution of 0.1% w/v deoxycholic acid in 100% w/v tricholoroacetic acid. Isolation of caveolar organelles was performed according to the method published by Oh and Schnitzer (J Biol Chem, 1999, 274: 23144-23154). Briefly, goat anti-mouse IgG-coated magnetic beads (Dynal Biotech) were pre-incubated with anti-caveolin-1 for two to four hours at room temperature. Sonicated plasma membrane rafts, prepared as described above, were added to beads and incubated for 1 hour at 4^{o}C. Bound material, representative of caveolae vesicles, was separated magnetically from unbound, non-caveolar membranes, subjected to SDS-PAGE and immunoblotted using antibodies against glypican-1 and caveolin-1.