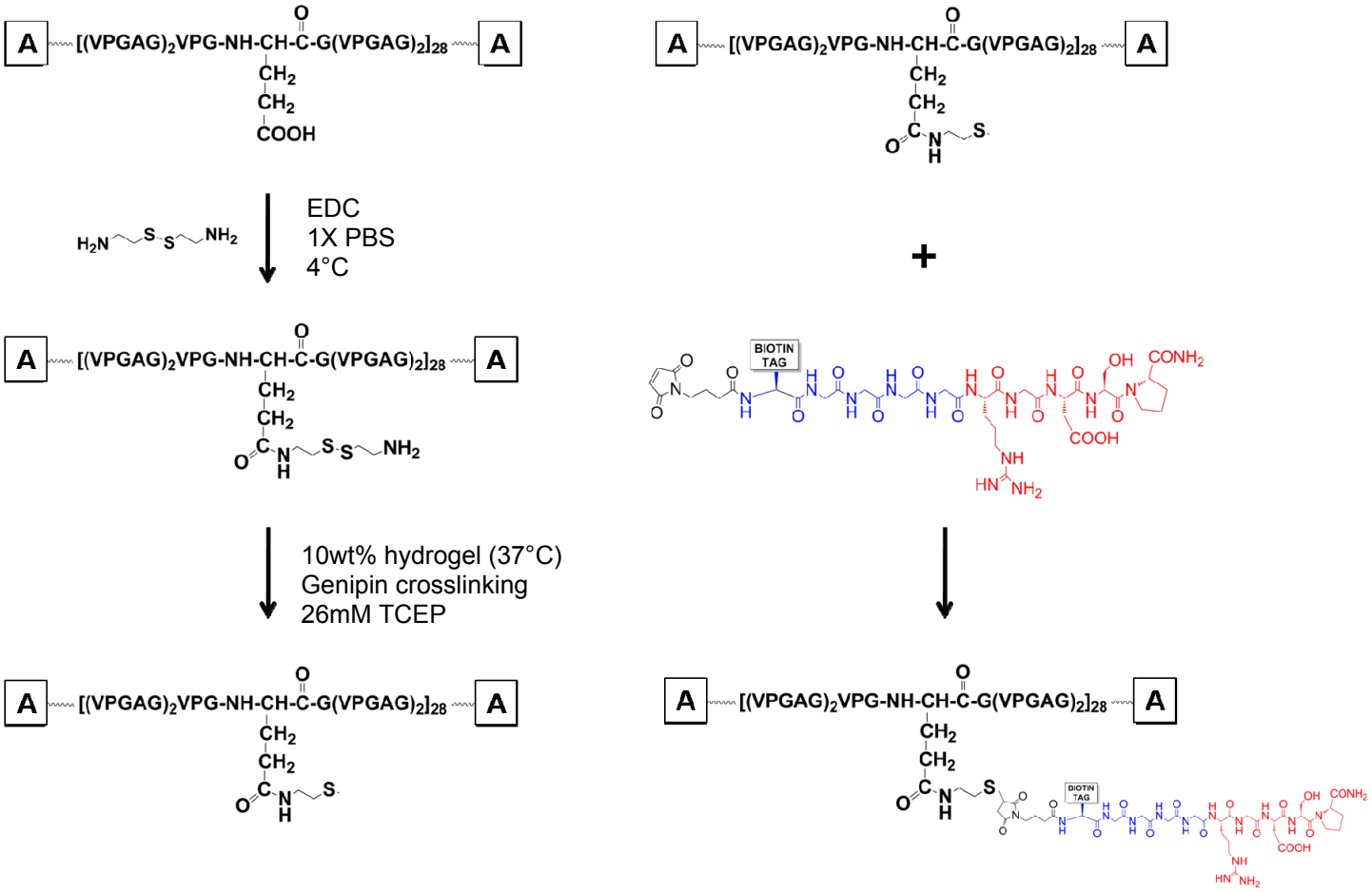

Scheme 1. Reaction scheme of *LysB10* modification and peptide coupling. Amide bond formation was mediated by the carbodiimide through the carboxylic group of the amino acid and the amine of cystamine, resulting in thiolated *LysB10***.** The plastic domains of *LysB10* are represented as **A** endblocks. Hydrogel formation was achieved by placing 10wt% thiol-*LysB10* solution at 37°C, well above the transition temperature of the protein polymer. Lysine residues of the protein polymer were crosslinked with a 6 mg/mL genipin solution for 24 hours, followed by stringent PBS rinsing to remove all genipin. The thiol groups were reduced with the addition of 26 mM Tris(2-carboxyethyl)phosphine (TCEP) to form free sulfhydryls. After rinsing the gels with three 20 minute PBS washes, thiol-reactive peptide linker was incubated for 2 hours at room

temperature to form a thioether bond with the protein polymer. Peptides were generated via solid phase synthesis, with key features incorporated in the design. The N-terminus of the molecule contains the thiol-reactive maleimide linker (black). Four glycine residues (blue) act as a spacer between the cell-binding RGD domain (red) and the remaining sequence to facilitate ligand-integrin presentation. A biotinyl-PEG_3 tag was incorporated into the peptide for detection of the molecule.

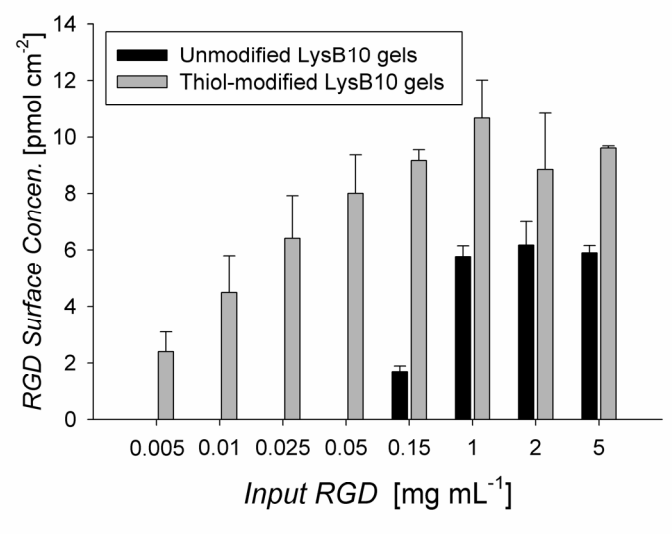

Figure 1. Coupled RGD peptide as a function of the amount of input peptide in surface modified *LysB10*. Data represent one of three similar experiments, with each condition run in quadruplicate. Peptide conjugation was assessed with the use of the biotin tag. In order to determine the moles of biotin conjugated to the *LysB10* hydrogel surface, the fluoreporter biotin quantitation assay kit was utilized.

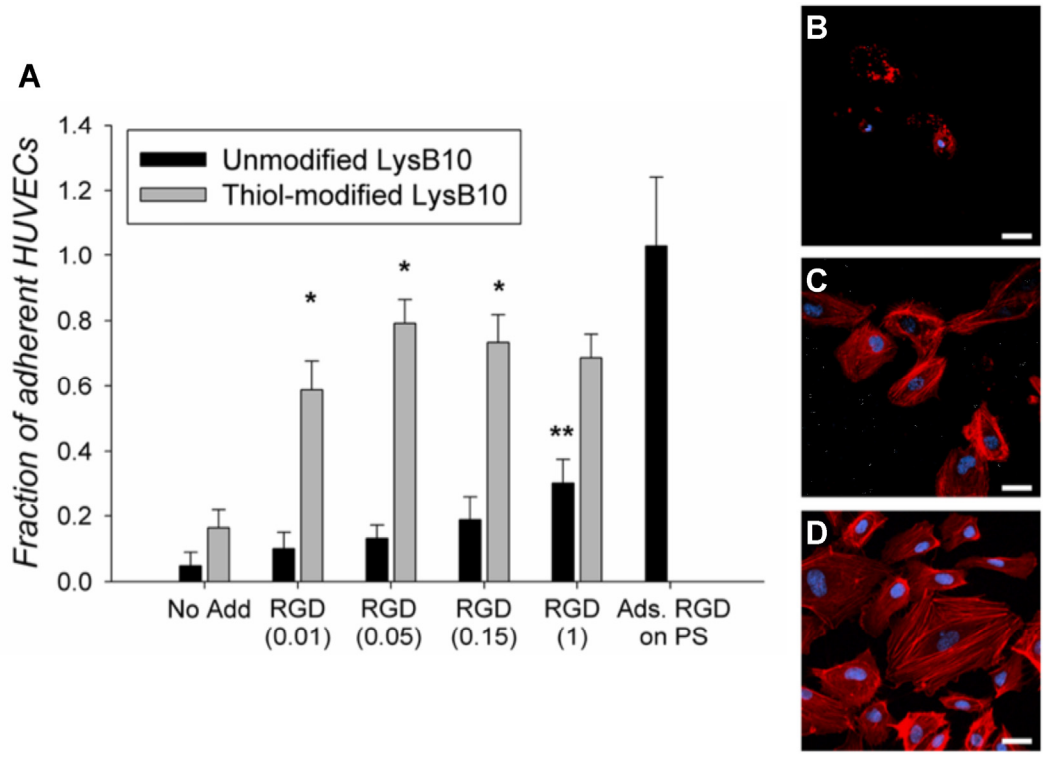

Figure 2. (A) HUVEC adhesion to varying *LysB10* hydrogel surfaces after 2 hours. RGD peptide concentrations ranging from 0.01 mg/mL to 1 mg/mL were added to unmodified and thiol-modified *LysB10* surfaces. 50 µg/mL fibronectin adsorbed to polystyrene served as a positive control, and all data was normalized to this control. Data represent one of three similar experiments, with each condition run in quadruplicate. *$p<0.01$ compared to unmodified *LysB10*-RGD at the same concentration. **$p<0.05$ compared to unmodified *LysB10*-no add control. Representative confocal images of HUVECs cultured on *LysB10* gels are shown, with white bars representing 20 microns. 10 wt% unmodified *LysB10* with adsorbed 50 µg/mL RGD linker **(B),** modified *LysB10* with conjugated 50 µg/mL RGD linker **(C),** and 50 µg/mL fibronectin coating **(D).** Fluorescently labeled actin is visualized in red.

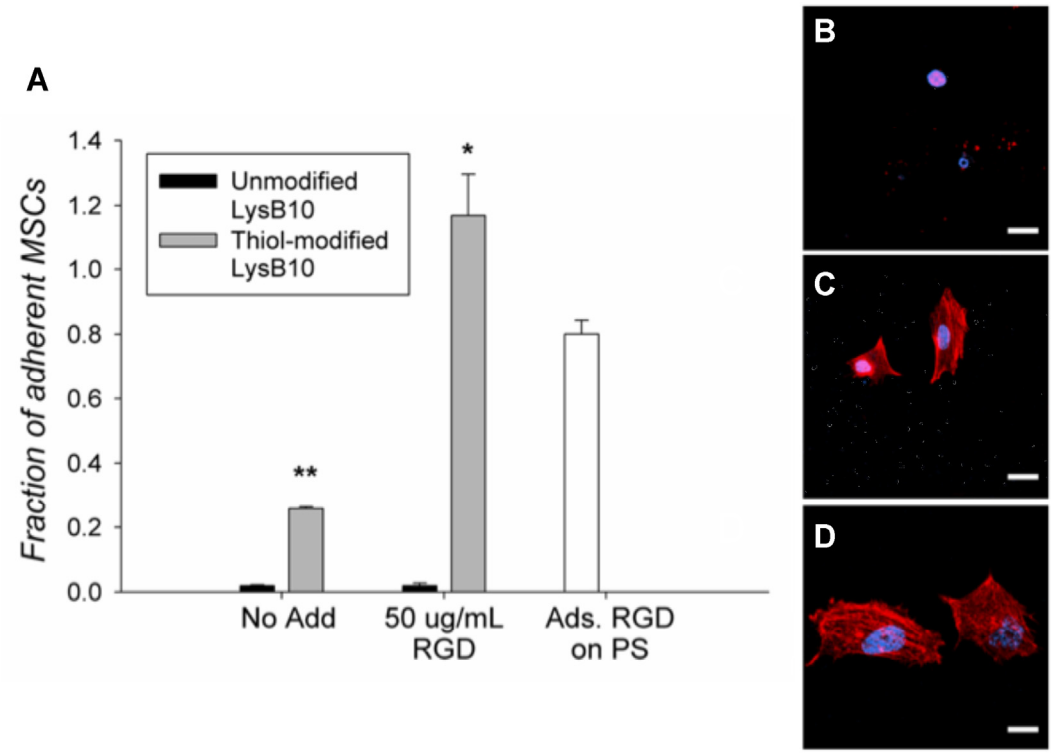

Figure 3. (A) MSC adhesion to varying LysB10 hydrogel surfaces after 2 hour assay. RGD peptide was added to the gels at a concentration of 50 μg/mL. 50 μg/mL fibronectin adsorbed to polystyrene served as a positive control, and all data was normalized to this control. Data represent one of three similar experiments, with each condition run in quadruplicate. *p<0.05 compared to thiol-modified *LysB10*-no add. **p<0.0 5 compared to unmodified *LysB10*-no add control. Representative confocal images of MSCs cultured on *LysB10* gels are shown, with white bars representing 20 microns. 10 wt% unmodified *LysB10* with adsorbed 50 μg/mL RGD linker **(B),** modified *LysB10* with conjugated 50 μg/mL RGD linker **(C),** and 50 μg/mL fibronectin coating **(D).** Fluorescently labeled actin is shown in red.

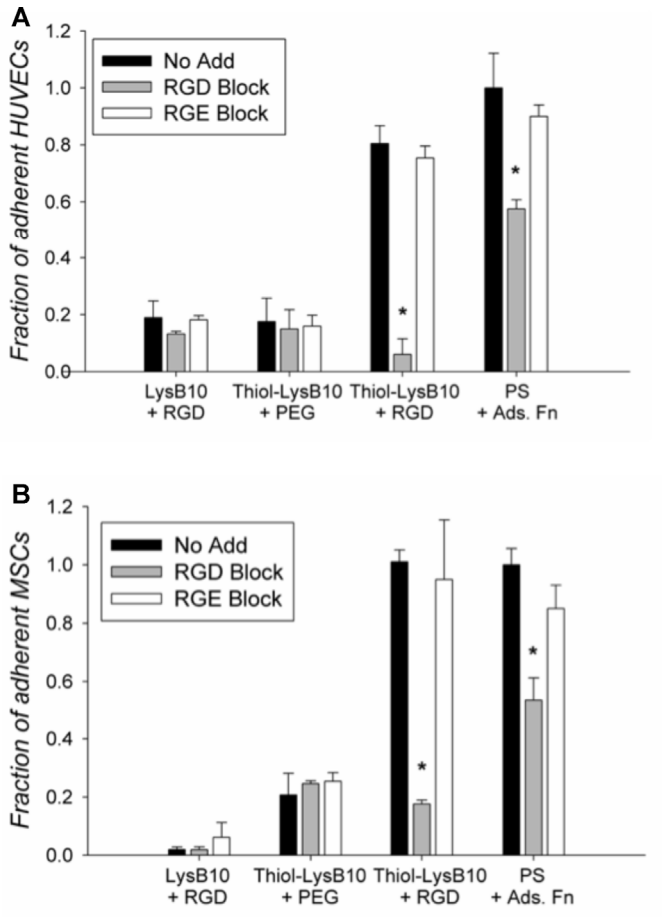

Figure 4. HUVEC (A) and pMSC (B) adhesion and specificity to treated *LysB10* hydrogel surfaces. Hydrogels were treated with either 50 μg/mL RGD linker or 50 μg/mL PEG linker (without RGD). Cells were treated with soluble GRGDSP (2 mM) and soluble GRGESP peptide (2 mM) for 30 minutes prior to plating. All data was normalized to the fibronectin, no add control. 50 μg/mL of fibronectin was adsorbed onto polystyrene. Data represent one of three similar experiments, with each condition run in quadruplicate. * $p<0.05$ compared to no-add treatment group.

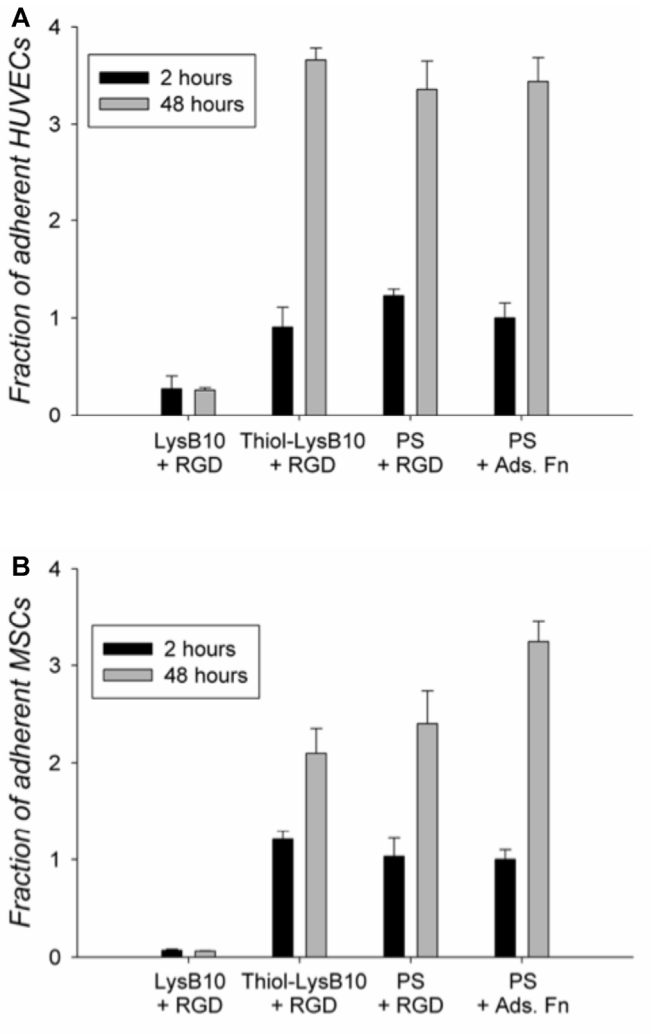

Figure 5. Proliferation rate of (A) HUVECs and (B) MSCs over a 48 hour period. Cells were seeded onto various *LysB10* gels at a density of 5,000 cells per well for 2 hours. Unbound cells were removed with media washes and substrate-bound cells were maintained in culture for another 48-hour period. All cell counts were normalized to the 2-hour adhesion value on fibronectin-coated polystyrene. Cell counts at 48 hours were compared to those at 2 hours for each treatment group.

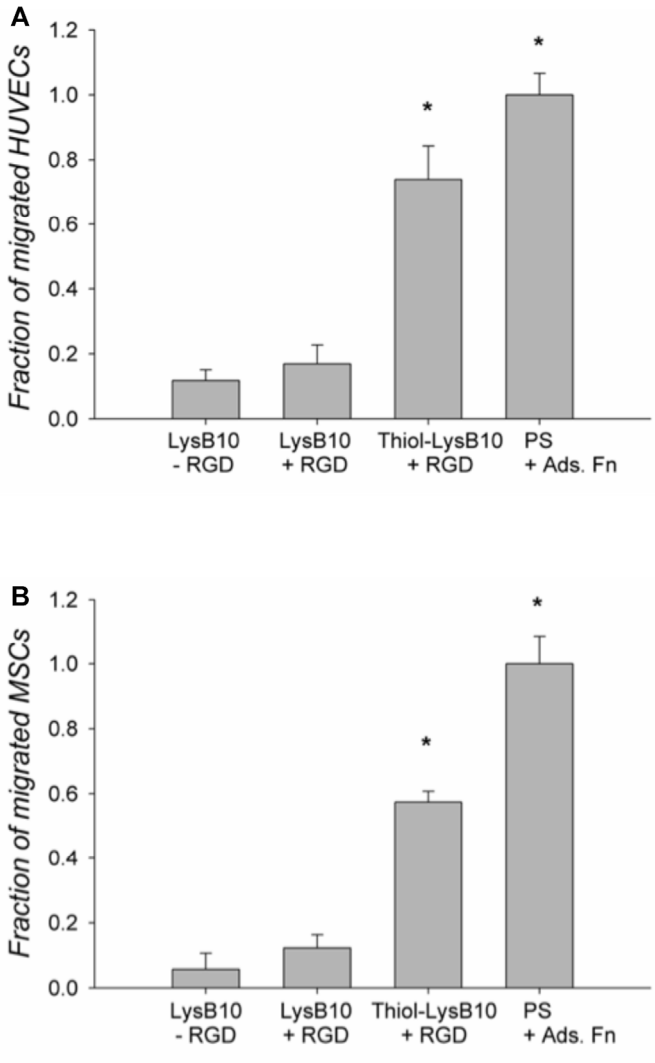

Figure 6. Radial migration assay of (A) HUVECs and (B) MSCs on modified surfaces. Cells were seeded onto an outer annulus area and monitored for motility into an inner radial zone over a 36-hour period. Quantification was achieved with fluorescent measurement of the number of migrated cells into the detectable inner zone, which was normalized against the number of migrated cells on fibronectin-coated polystyrene .*$p<0.05$ compared to non-RGD treated, unmodified *LysB10*.

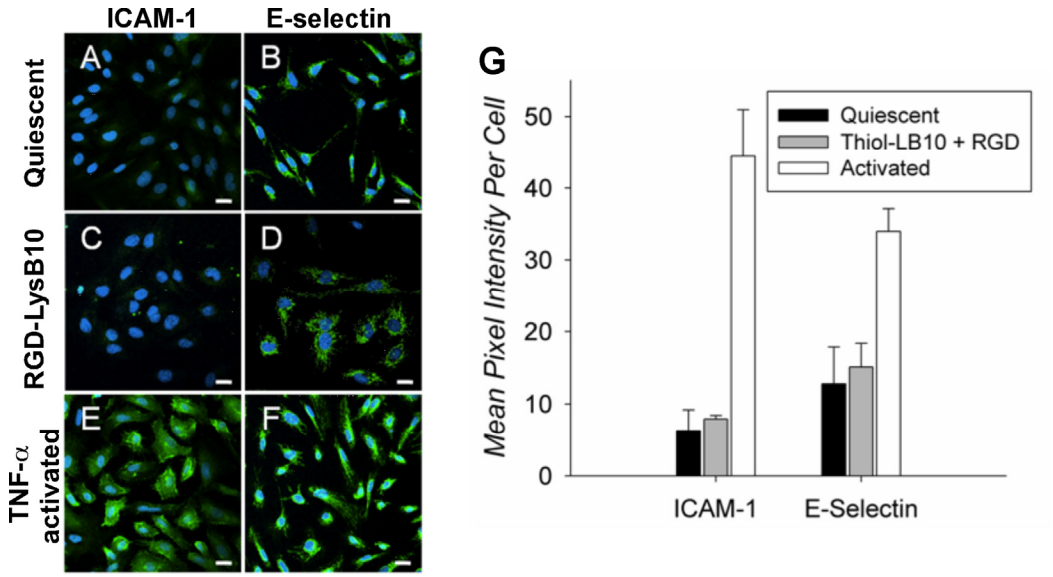

Figure 7. Representative confocal images of HUVECs cultured on various substrates. Cells that were cultured on fibronectin-coated slides without TNF-α stimulation (**A & B**) maintained a quiescent phenotype. Activation was achieved with the addition of TNF-α to the culture medium (**E&F**). HUVEC activation or quiescence was compared to that on RGD-conjugated LysB10 films (**C & D**). Markers of HUVEC activation were ICAM-1 (**A,C, and E**) and E-selectin (**B,D, and F**). Quantification demonstrated that RGD-LysB10 similar to quiescent controls (**G**). White bars signify 20 microns.