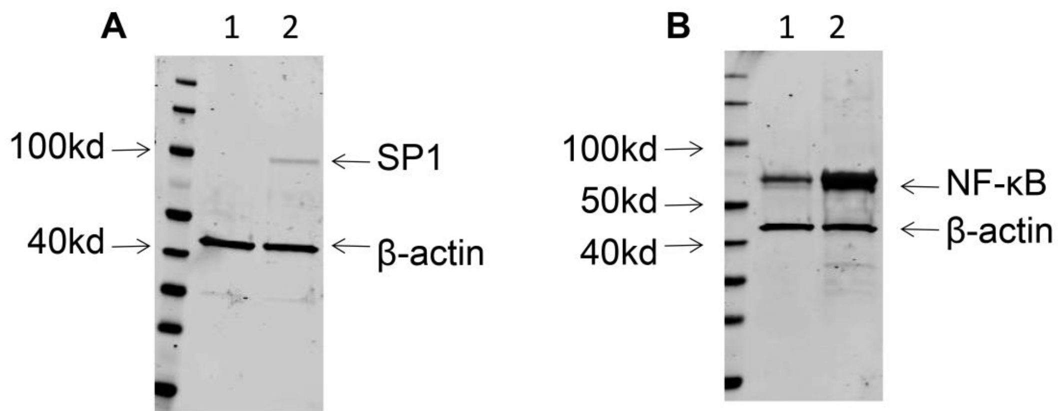
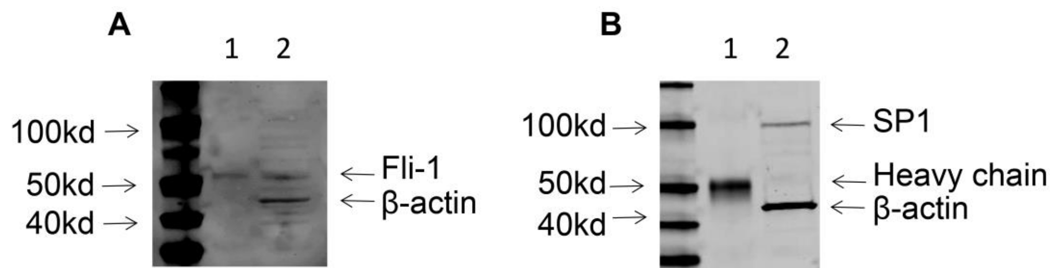
Supplemental Figure 1



Detection of Sp1 and NF-κB from NIH3T3 cells. **(A)** Western blot, representative of three experiments illustrating Sp1 protein concentrations from NIH3T3 cells after transfection into the NIH3T3 cells with 1 μg of empty plasmid (lane 1) or 1 μg of Sp1 plasmid (lane 2). Arrows are used to denote the Sp1 and β-actin loading control bands on the right. (**B**) Western blot, representative of three experiments illustrating NF-κB protein concentrations from NIH3T3 cells after transfection into the NIH3T3 cells with 1μg of empty plasmid (lane 1) 1 μg of NF-κB p65 plasmid (lane 2).

Supplemental Figure 2



Sp1 did not interact with Fli-1 by Co-immunoprecipitation. Human umbilical vein endothelial cell (HUVECs, 2X10^5 cells) were lysed by RIPA buffer containing Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO) for 30 min on ice. Lysates were spun at 10,000 x g for 15 min and supernatant was pre-cleared by incubation with 20 μL protein A/G Sepharose beads (ThermoFisher Scientific, Waltham, MA) for 30 min at 4ºC. After a brief centrifugation, the supernatant was incubated with 2 μg of anti-Fli-1 antibody for overnight at 4ºC. Immunoprecipitates were captured with 20 μL of protein A/G beads at 4ºC for one hour. Samples were centrifuged, washed three times with 1 mL of RIPA buffer, and eluted from the beads using 2x Laemli sample buffer as input (lane 1). HUVECs lysates was used as control (lane 2). Samples were subsequently analyzed by SDS-PAGE using 4-20% Criterion TGX gels (Bio-Rad, Hercules, CA) and transferred to PVDF membrane. Blots were probed with biotin-labeled anti-Fli-1 antibody and IRDye® 680RD Streptavidin (**A**) or using rabbit anti-Sp1 antibody and IRDye® 680RD donkey anti-rabbit antibody (**B**). The result was visualized using the Odyssey Imaging System (LI-COR, Lincoln, NE). Representative of three experiments.