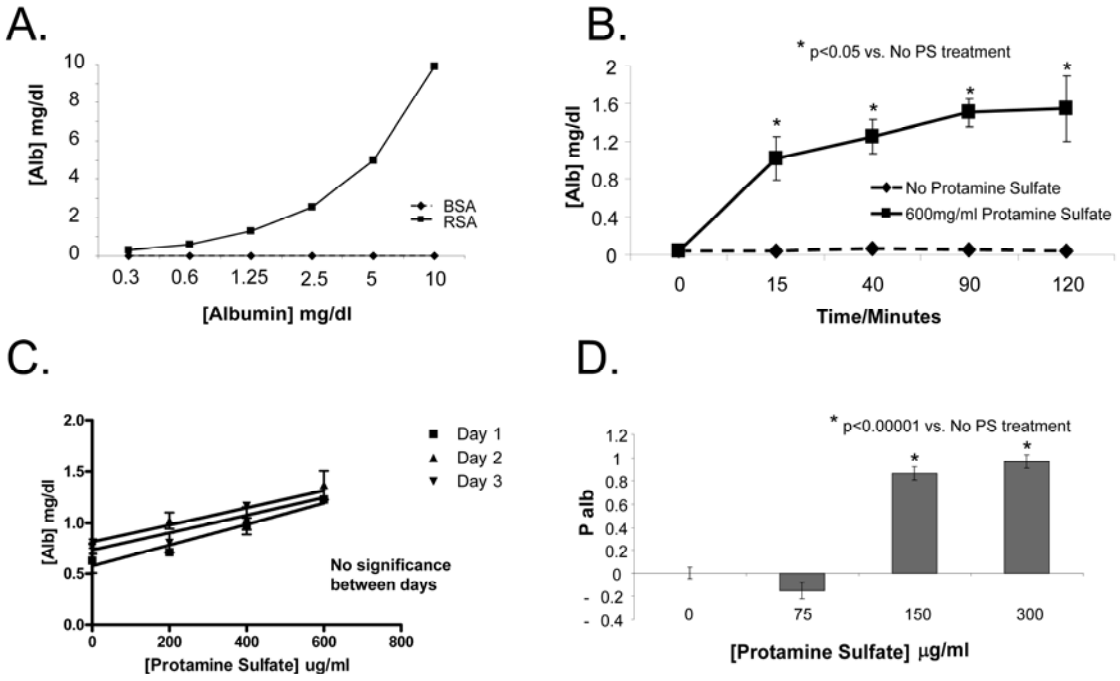
Supplemental Figure 1.



Supplemental Figure 1.

Reproducibility and controls for the glomerular permeability assay. (A) The Nephrat urinary albumin ELISA is highly specific for rat albumin. Standard curves for both bovine serum albumin (BSA) and rat serum albumin (RSA) were analyzed on a Nephrat plate. The RSA responded in a linear fashion while the BSA was undetectable, even at the highest concentration tested. (B) Glomerular albumin release correlates with the length of PS treatment. Albumin release from treated glomeruli (solid line) becomes statistically significant relative to untreated controls (dashed line) as quickly as 15 minutes after incubation with PS.

Subsequent experiments were performed with 40 minute PS incubation, as that time elicited a consistent response over multiple experiments. (C) Glomerular albumin release assays are reproducible in separate experiments. Three independent experiments measured glomerular permeability and dose-response to PS. Each experiment was performed using separate glomerular preps and ELISA plates. Variability between individual experiments was minimal indicating good assay reproducibility. (D) Before employing the ELISA based albumin leakage assay, we show that protamine sulfate (PS) can have effects on the volume of isolated glomeruli. Using the method developed by Savin et al,[26] glomeruli treated with PS for 40 minutes have a change in the volume which differs from the untreated. This is expressed as an arbitrary inverse value. Thus PS treated glomeruli have a higher pAlb value than do controls. Significant differences are noticeable at PS concentrations higher than 75 μg/ml. The ELISA based albumin leakage assay begins to respond at 100ug/ml PS. Although the Savin et al.[26] technique may show greater sensitivity at lower PS concentrations, the ELISA based method has a good linear range between 0-300 μg/ml PS. Thus there is good correlation between both methods.