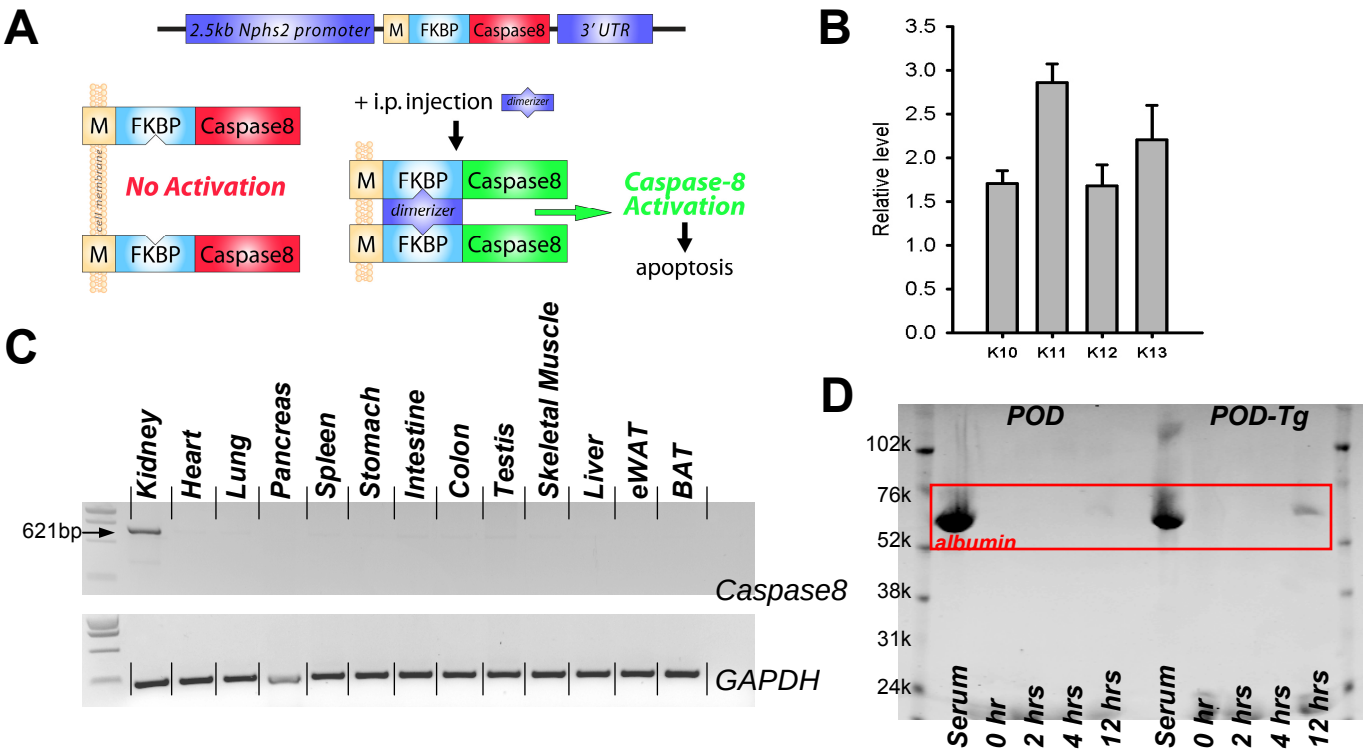
# Supplemental Material

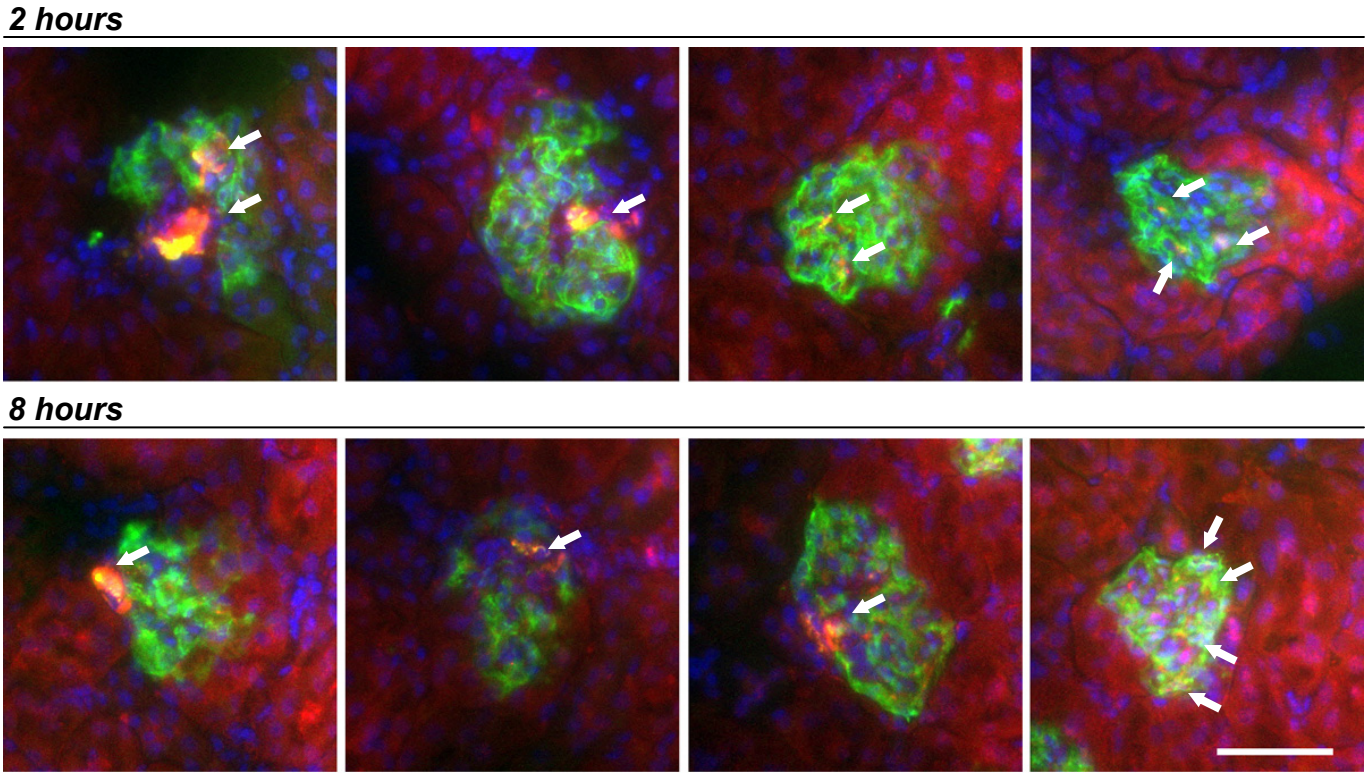
Rutkowski JM, et al., **Adiponectin modulates recovery of renal function in a novel model of podocyte ablation**

## Supplemental Figure 1

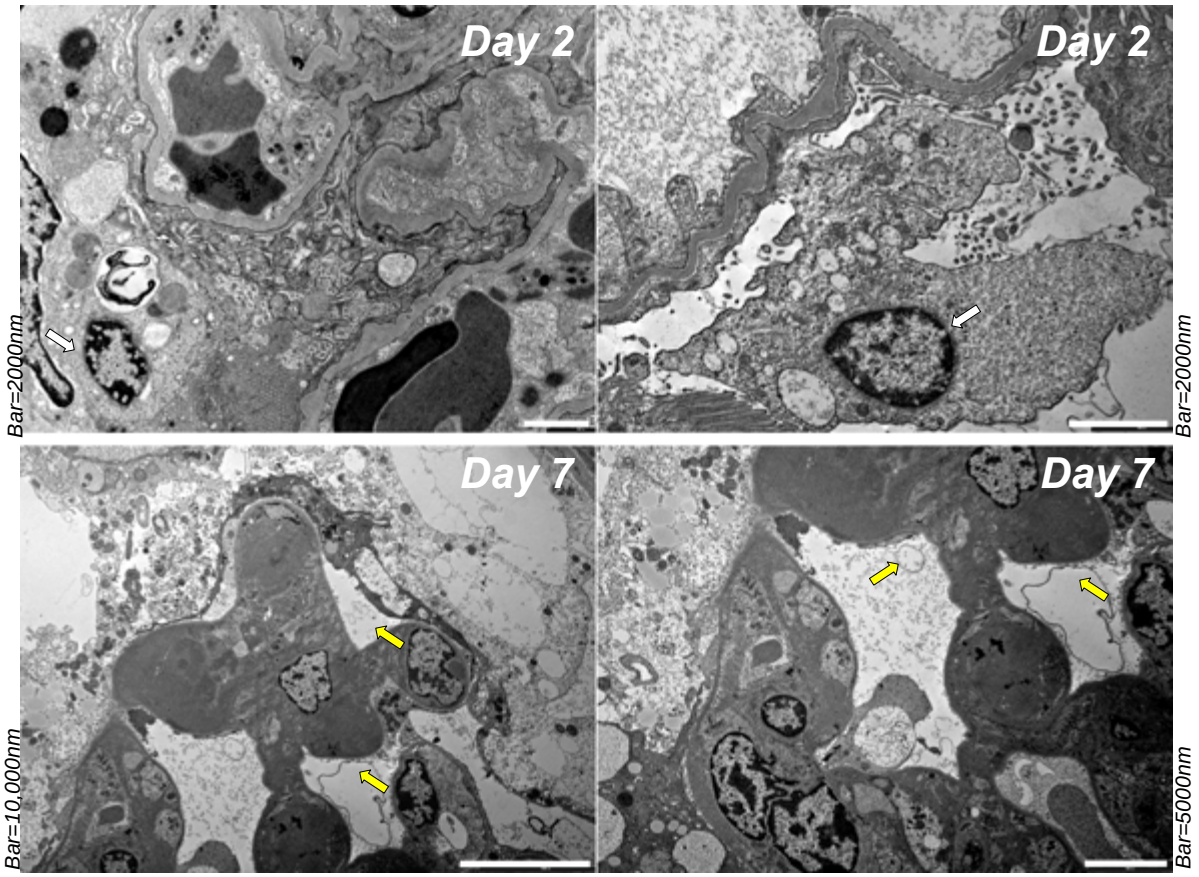



**Supplemental Figure 1:** Generation of the POD-ATTAC mouse. A) A construct consisting of regions for the podocin (Nphs2) promoter, a myristoylated mutant FKBP-Caspase-8 fusion protein, and a rabbit beta globin 3' untranslated region (UTR) was injected into FVB mouse embryos. Once expressed in podocytes, the mutant FKBP protein permits specific dimerization by AP20187 that then drives caspase-8 activation, leading to apoptosis. B) Four lines demonstrated germline transmission of the transgene. The highest-expressing K11 line was selected for further breeding and use in all experiments. C) RNA extracts were prepared from 13 tissues including epididymal (eWAT) and brown (BAT) adipose tissue. qPCR of the subsequent cDNA demonstrated expression of the caspase-8 transgene only in the kidney. D) Following dimerization, albuminuria is visible within 12 hours by SDS-PAGE separation of spot urine in POD and POD-Tg mice.

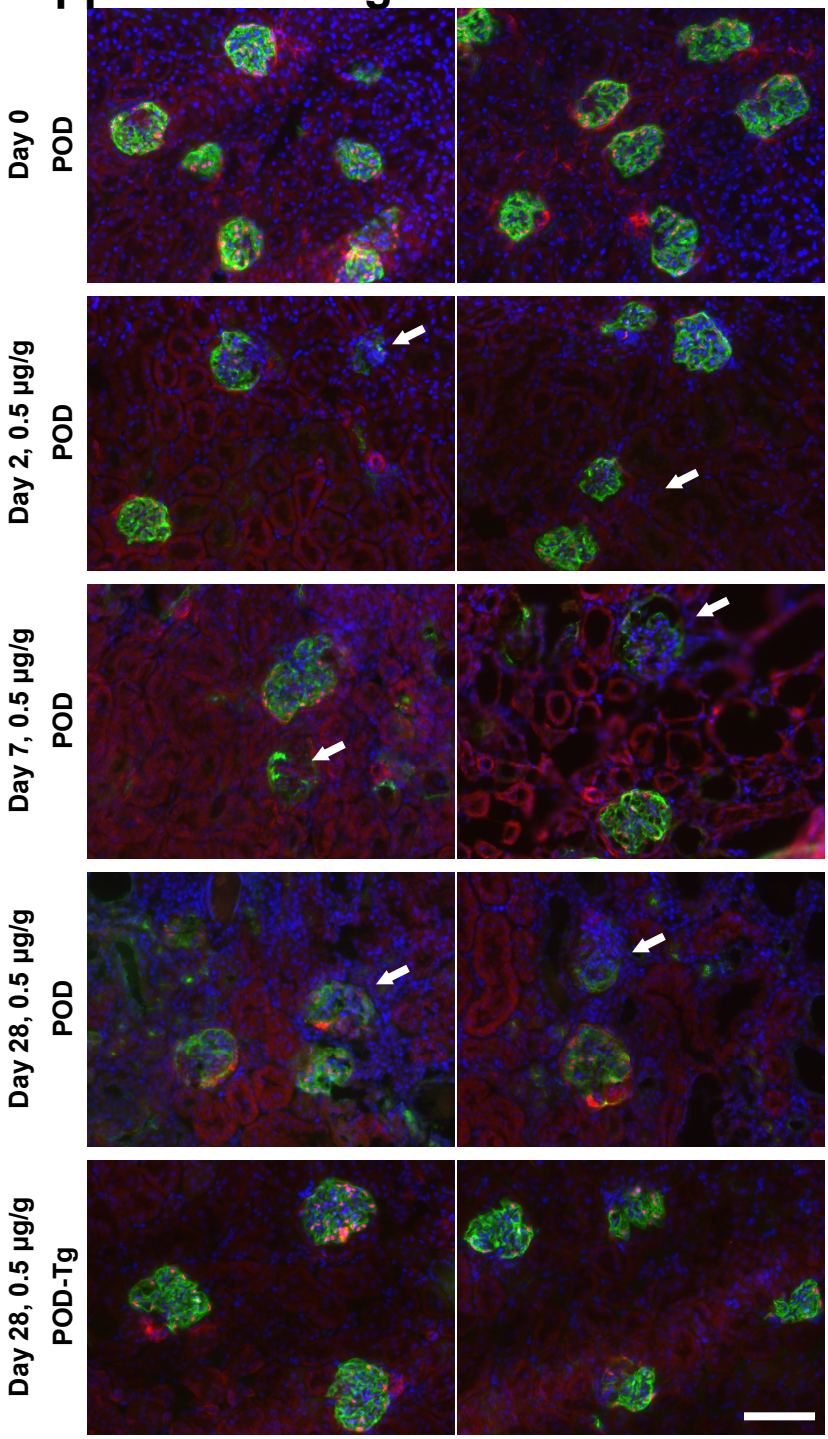
# Supplemental Figure 2A




# Supplemental Figure 2B

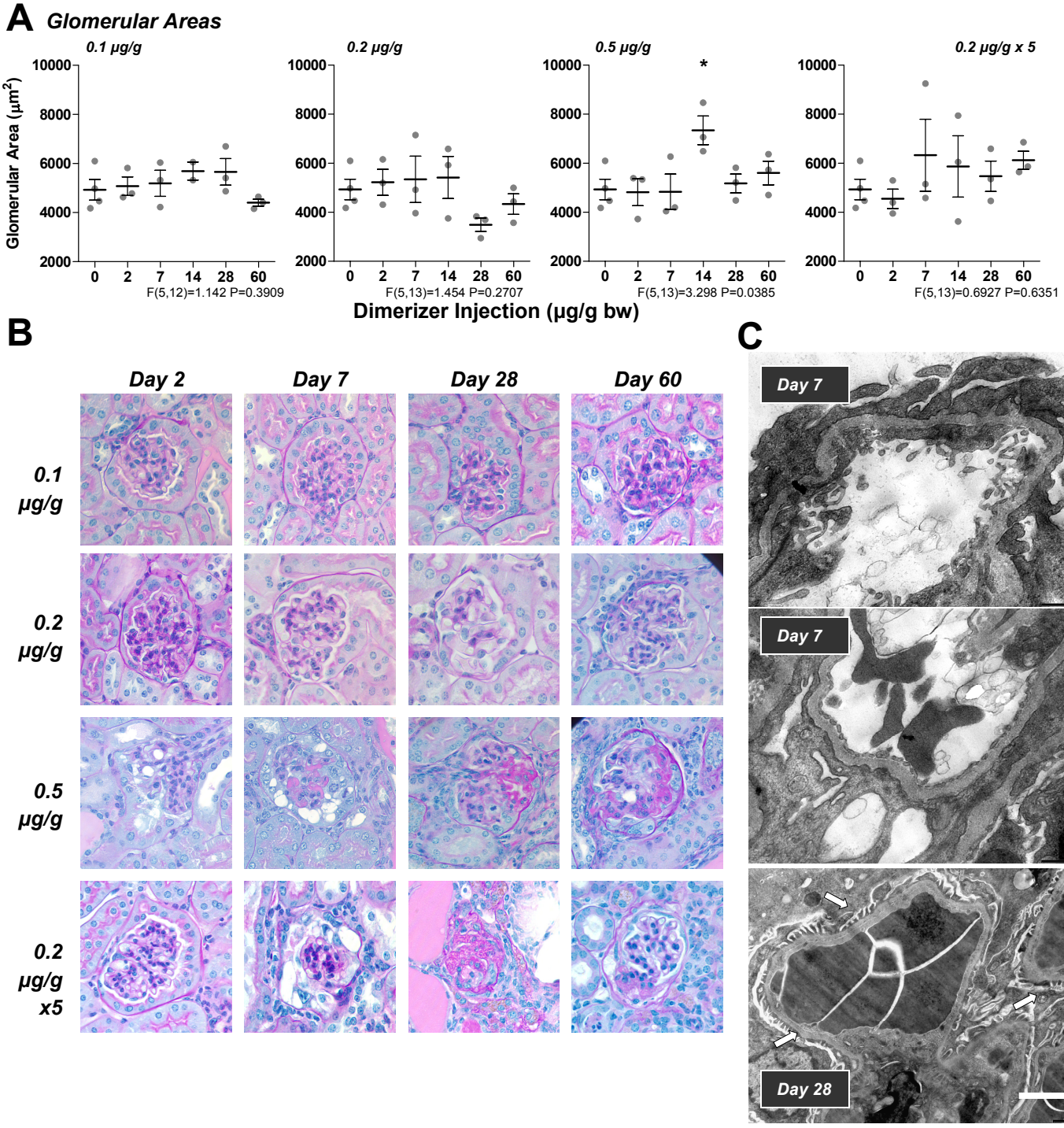


# Supplemental Figure 2C



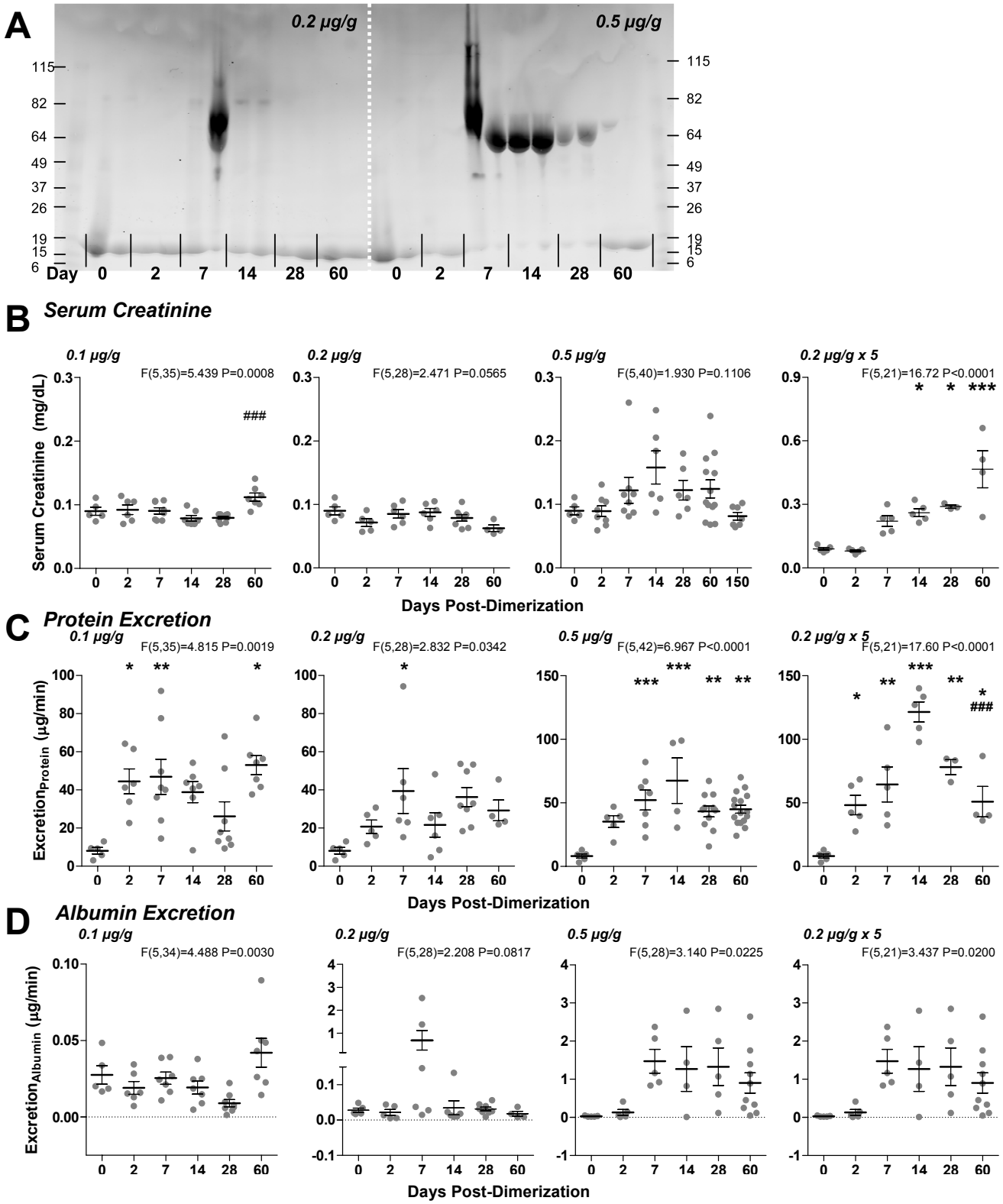

**Supplemental Figure 2:** Podocyte apoptosis and loss following the administration of dimerizer to POD mice. A) On fresh frozen kidney sections, cleaved caspase-3 labeling (red) in podocytes (green, podoplanin) (arrows) shortly following dimerizer administration demonstrating a window of apoptosis induction in podocytes. Bar=50µm. B) TEM images at d2 (top) and d7 (bottom) reveal podocyte apoptosis. Chromatin marginalization at d2 (white arrows) and GBMs lacking podocyte coverage at d7 (yellow arrows). C) WT1 labeling of podocyte nuclei on frozen tissue, following dimerization and apoptosis, demonstrating a loss of podocytes from POD glomeruli (arrows) (glomerular labeling: podoplanin, green, WT1, red) seen at days 2, 7, and even remaining at d28 in POD mice administered 0.5 µg/g AP20187. POD-Tg glomeruli appear to be improved, with restored WT1+ nuclei at day 28. Bar=100µm.

# Supplemental Figure 3



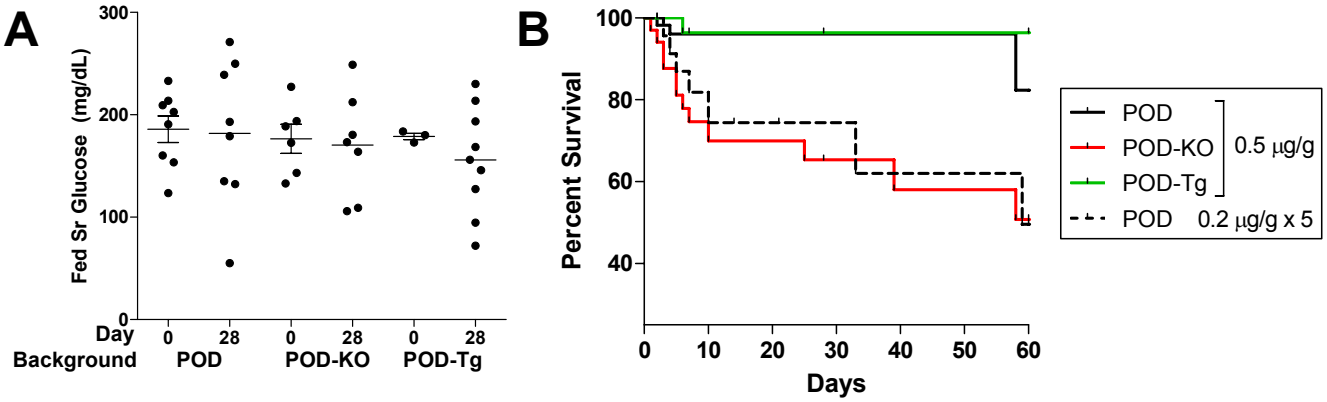

**Supplemental Figure 3:** Podocyte loss and pathology are dimerizer treatment dose-dependent in the POD-ATTAC mouse A) Glomerular areas in mice treated with 0.1 or 0.2 µg/g dimerizer were not significantly increased following podocyte injury. The 0.5 µg/g dimerization and repeat dosing regimens resulted in increasing glomerular hypertrophy, though this was largely not statistically significant. B) Periodic acid-Schiff staining of POD glomeruli demonstrates dose dependent glomerular changes characteristic of range of nephropathies. C) Additional TEM images show FPE at day 7 and restored foot processes at day 28 in 0.5 µg/g treated mice (arrows). Bar=2 µm

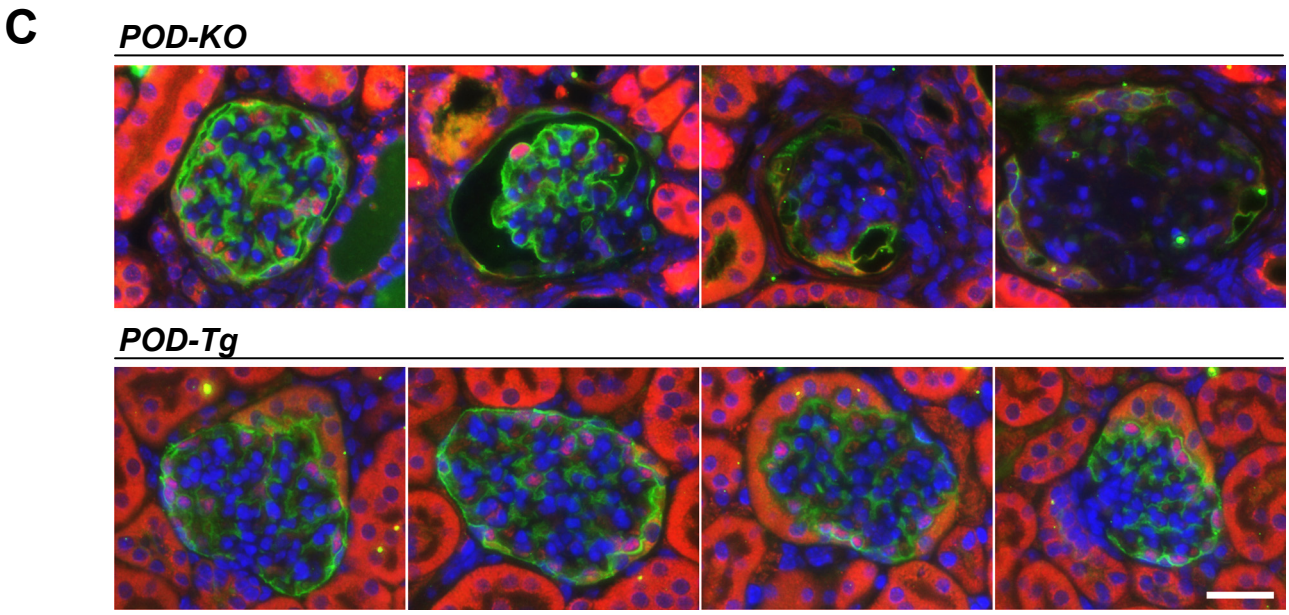
# Supplemental Figure 4

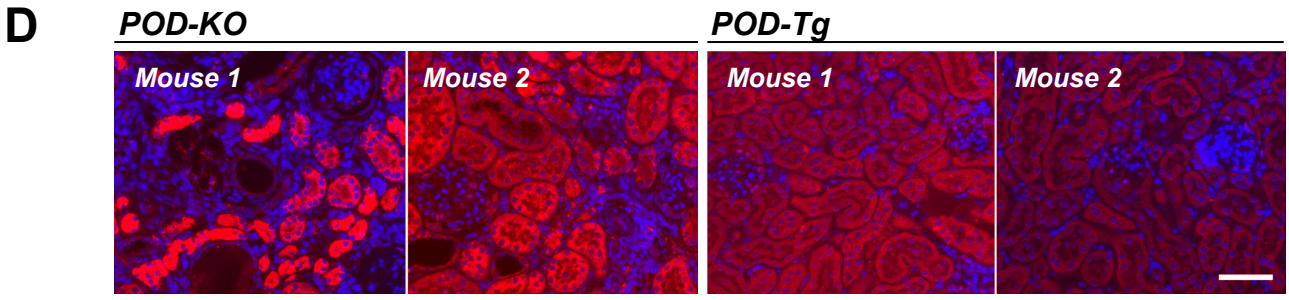


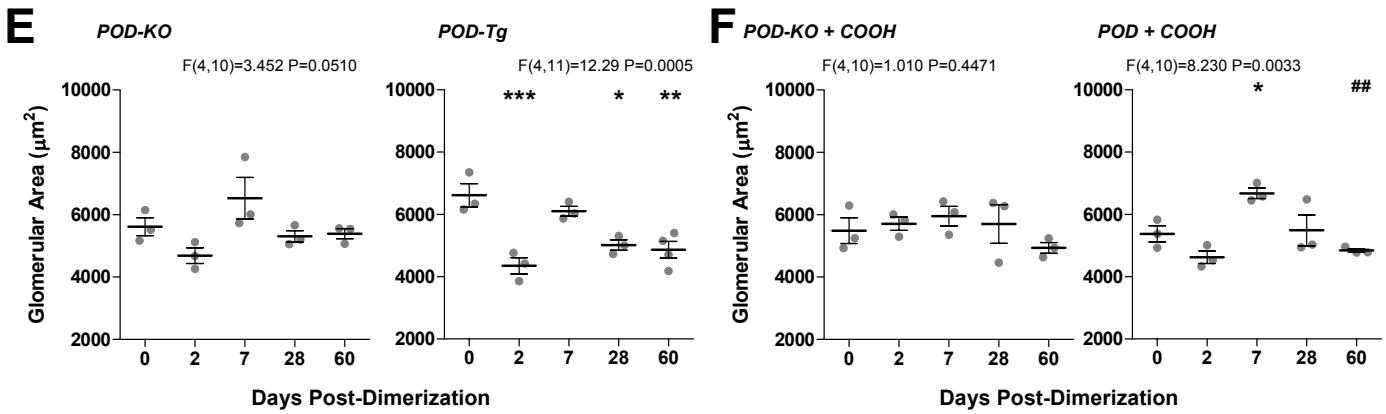

**Supplemental Figure 4:** Podocyte loss leads to albuminuria and impaired filtration function in POD-ATTAC mouse in a dose-dependent manner. A) Gel electrophoresis of 2 µL urine from 2 mice each at the indicated time points following 0.2 and 0.5 µg/g dimerizer treatment. Note the marked albuminuria present in some 0.2 µg/g samples (left) and most 0.5 µg/g (right) mice that resolves over time. B) Serum creatinine is increased over time in POD mice receiving higher doses of dimerizer, with minimal recover. C) Urinary protein excretion is increased with dimerizer does with some evidence of recovery. D) POD mice exhibit a 100x increase in albumin excretion.

# Supplemental Figure 5



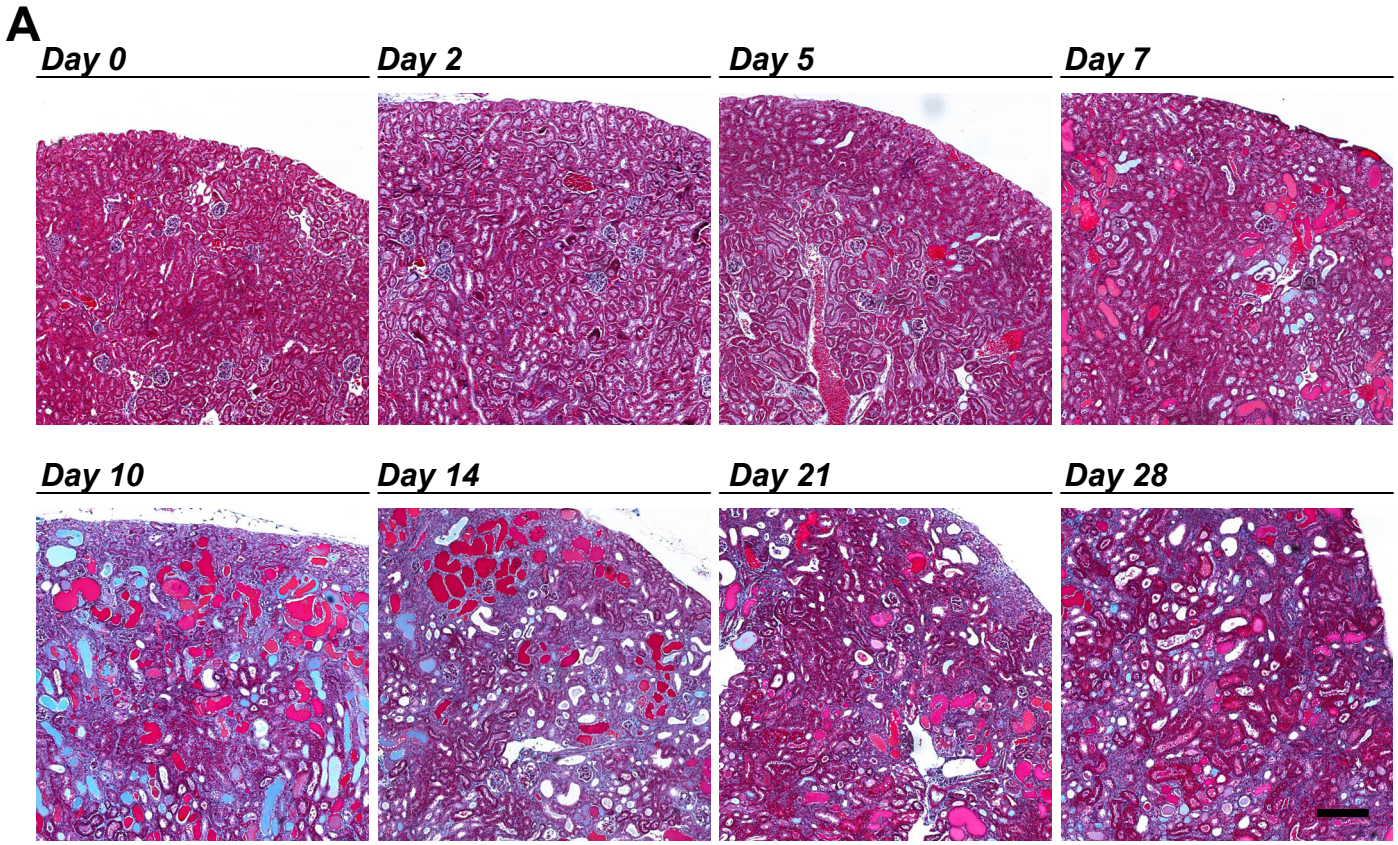



**Supplemental Figure 5:** Podocyte loss led to acute renal failure, chronic kidney disease and death in POD mice treated with multiple doses of dimerizer and POD-KO mice. A) POD, POD-KO, and POD-Tg mice have equivalent serum glucose levels before and after dimerization. B) Nearly all (>80%) POD mice treated with a single 0.5 μg/g dose (black solid line) survived through 60 days. Approximately 30% of POD-KO mice died within 7 days of a single 0.5 μg/g dimerizer injection. Surviving POD-KO mice were lost over time, reducing populations to approximately 50% by day 60. Conversely, only 3% of POD-Tg mice died acutely, and no more through 60 days. Multiple dose POD mice (0.2 μg/g x 5) exhibited a similar survival curve to POD-KO mice. C) Day 60 glomeruli appear normal (podoplanin, green, WT1, red) in POD-Tg mice, but POD-KO mice exhibit failed glomeruli (4 pictured from 1 mouse of each; Bar=50μm). D) Increased WT1+ tubules at day 60 in POD-KO mice demonstrating effects beyond podocyte loss (red, WT1; Bar=100 μm). E) Glomerular areas ($\mu m^2$) were unchanged over time in POD-KO mice, but significantly reduced in POD-Tg mice as compared to their increased starting areas. F) Glomerular areas (μm2) were unchanged in dimerized POD-KO mice treated with the PPARγ agonist, but slightly increased wildtype POD mice with treatment at the height of albuminuria. The hypertrophy was transient, however, with a significant reduction by day 60.

# Supplemental Figure 6




**Supplemental Figure 6:** Progressive renal fibrosis following induced podocyte-specific apoptosis. A) Trichrome staining in kidney sections from 0.2 µg/g x 5 dimerizer-treated mice demonstrates increased fibrosis throughout the cortex from day 14 onward. By day 5/7, protein casts can be seen. Bar = 200µm. B) TUNEL labeling of POD versus POD-Tg at day 2 suggests downstream cell apoptosis following podocyte loss that may lead to developing interstitial fibrosis.