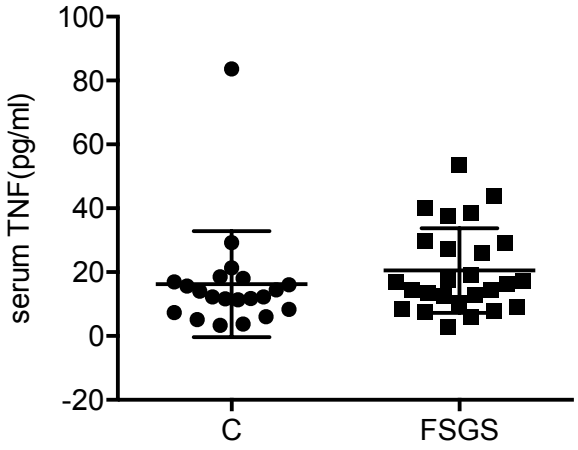
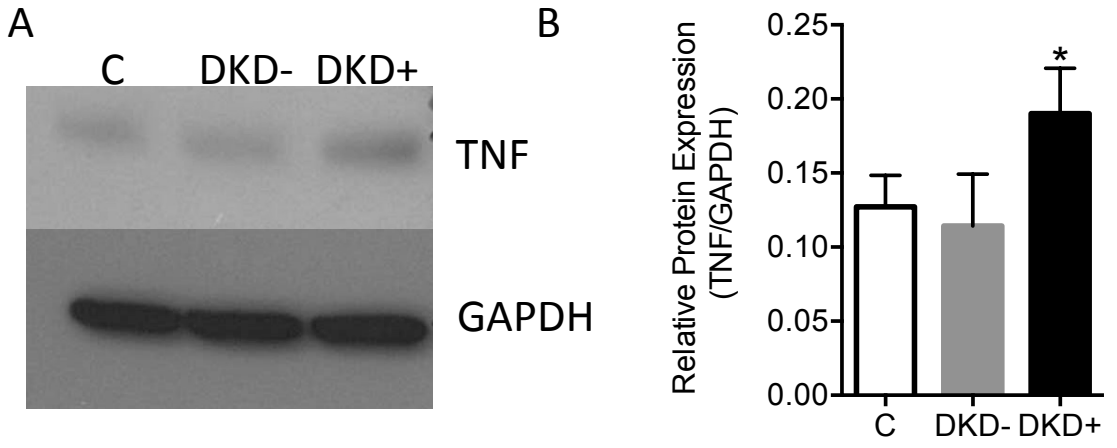
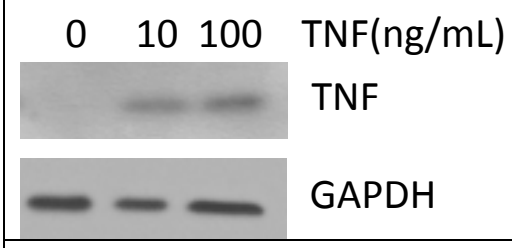
Supplemental Material



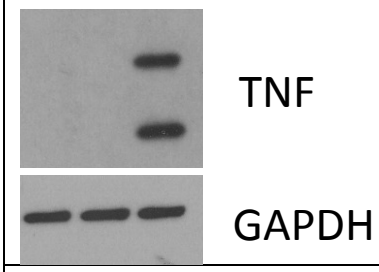
Supplemental Figure 1. Serum TNF levels in the NEPTUNE Cohort. Serum TNF levels are unaltered in patients with biopsy proven FSGS (FSGS, n=26) compared to healthy controls (C, n=21). Two-tailed Student's t-test, P=not significant.



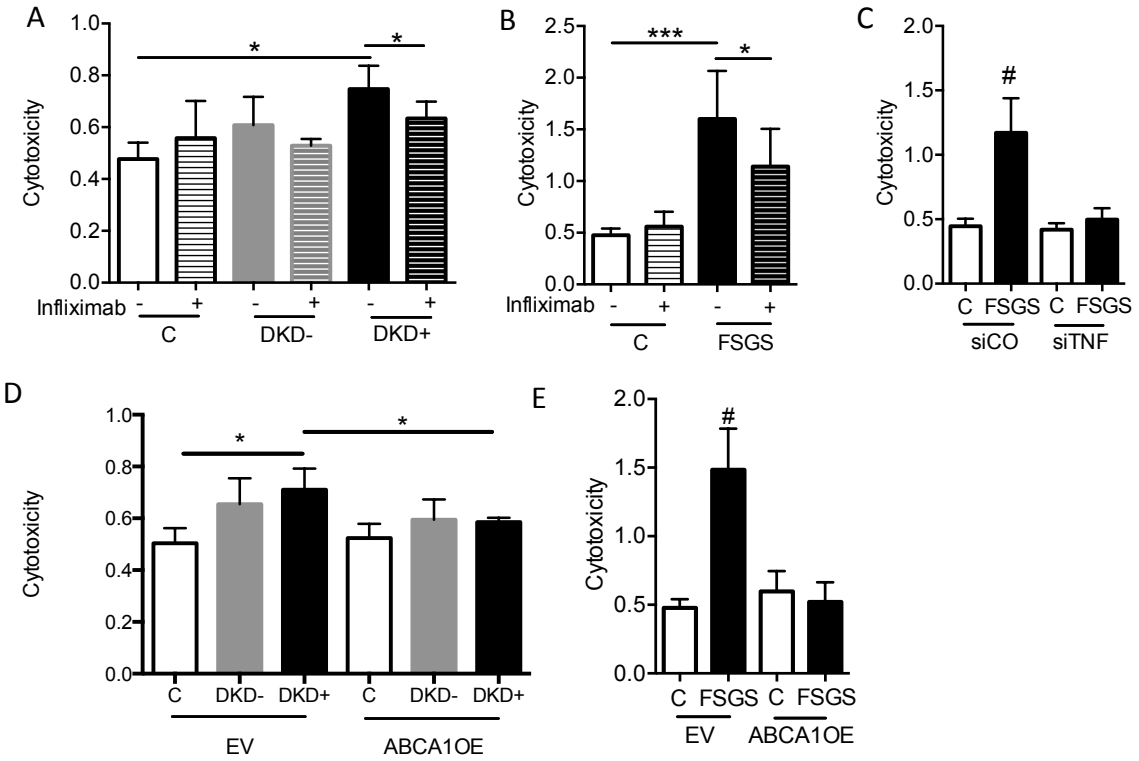
Supplemental Figure 2. Local TNF expression in DKD sera treated podocytes. **A.** Representative western blot analysis (n=3) of TNF levels in cultured human podocytes exposed to the sera from patients with diabetes and kidney disease (DKD+), patients with diabetes (DKD-) and healthy controls (C). **B.** Densitometric analysis of A (n=3). One-way ANOVA, *P<0.05.



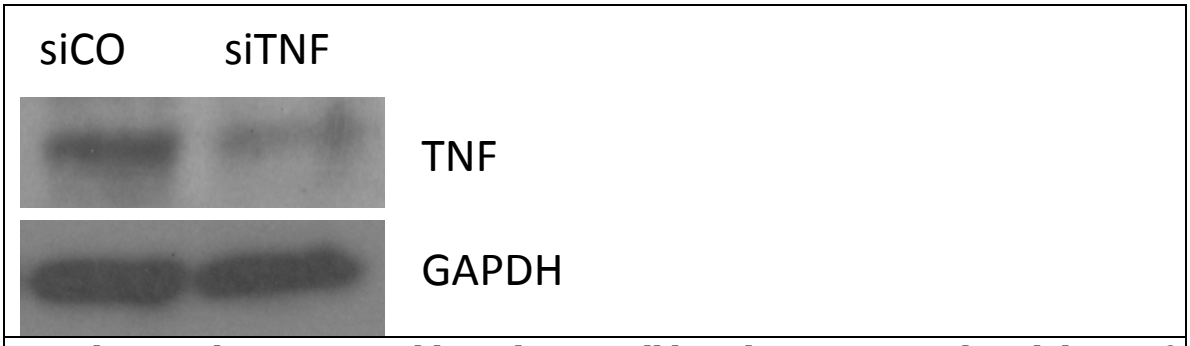
Supplemental Figure 3. TNF treatment increases local TNF expression. Representative western blot analysis of local TNF levels in cultured human podocytes exposed to increasing concentrations of recombinant human TNF (n=3).



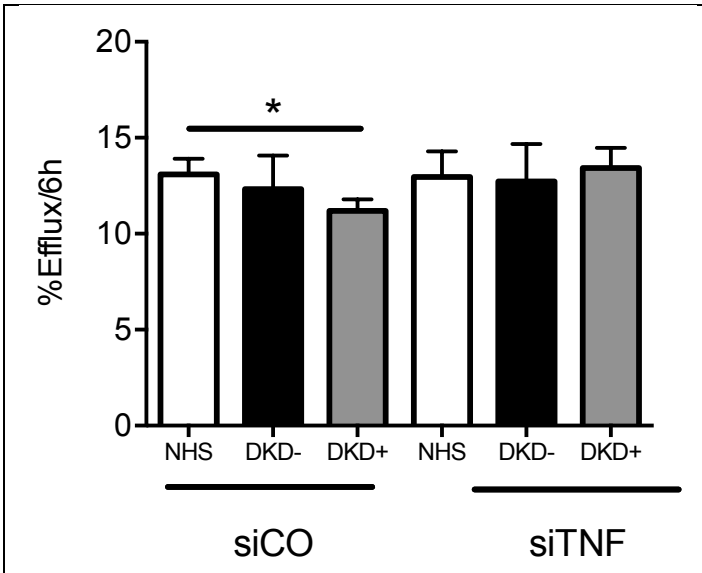
Supplemental Figure 4. Exposure of podocytes to TNFOE virus results in increased TNF. Representative western blot analysis (n=3) of local (top band) and soluble TNF (bottom band) expression in podocytes exposed to virus overexpressing TNF (TNFOE) compared to empty vector containing virus (EV) and untreated controls (C).



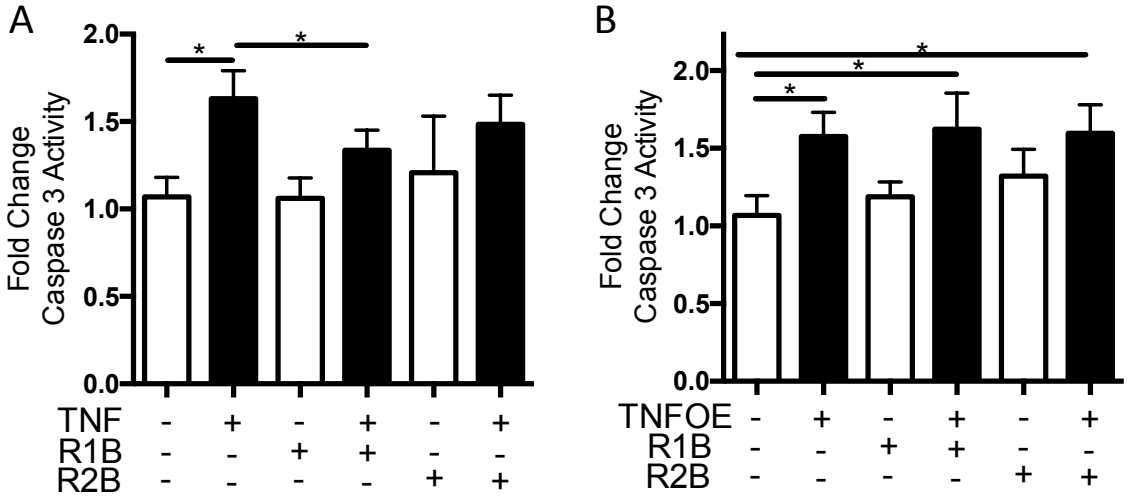
Supplemental Figure 5. Cytotoxicity in DKD and FSGS sera treated podocytes. **A.** Treatment of human podocytes with Infliximab prevents DKD+ sera induced cytotoxicity (n=4) compared to DKD+ sera treatment alone and untreated healthy controls (C). Two-tailed Student's t-test, *P<0.05 DKD+ versus C, DKD++Infl. **B.** Treatment of human podocytes with Infliximab prevents individual FSGS sera (n=6) induced cytotoxicity (n=4) compared to healthy controls (C). One-way ANOVA, ***P<0.01, *P<0.05. **C.** Treatment of TNF knockdown human podocytes (siTNF) protects from individual FSGS sera (n=6) induced cytotoxicity (n=4) compared to scramble sequence treated controls (siCO). One-way ANOVA, ##P<0.001 compared to all groups. **D.** Cytotoxicity analysis was performed (n=4) in ABCA1 overexpressing (ABCA1OE) and empty vector (EV) podocytes treated with individual pooled serum from patients with diabetes and kidney disease (DKD+), patients with diabetes (DKD-) and healthy controls (C). One-way ANOVA, *P<0.05. **E.** Cytotoxicity analysis was performed (n=4) in ABCA1 overexpressing (ABCA1OE) and empty vector (EV) podocytes treated with individual FSGS patient sera (n=6) and healthy controls (n=6). One-way ANOVA, #P<0.05 FSGS treated EV vs all conditions.



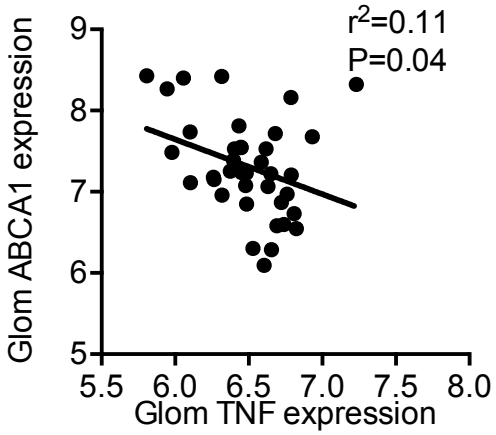
Supplemental Figure 6. Stable podocyte cell line demonstrating knockdown of TNF. Representative western blot analysis of TNF levels in scrambled control siRNA (siCO) or siRNA targeting TNF (siTNF) cultured human podocytes. (n=3).



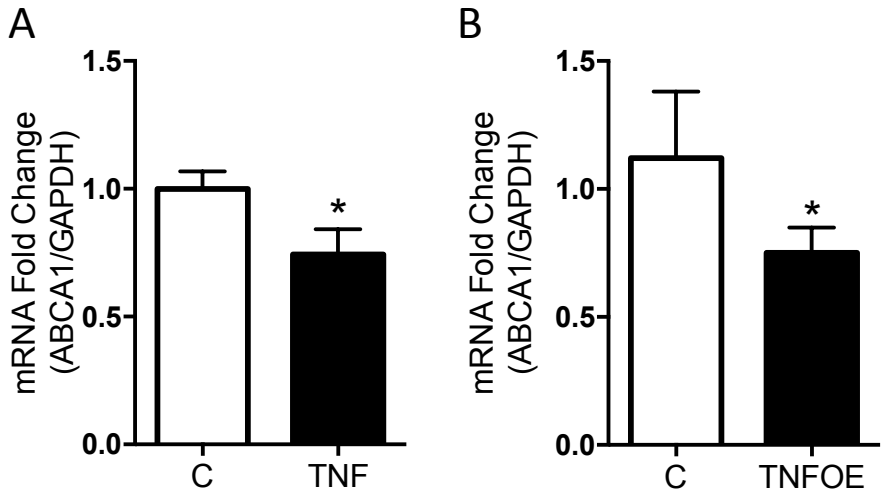
Supplemental Figure 7. Knockdown of TNF prevents reduced cholesterol efflux. DKD+ sera induced cholesterol efflux is reduced in scrambled control (siCO) treated podocytes but not podocytes with knockdown of TNF (siTNF) (n=4). Student's T-test, $P<0.05$.



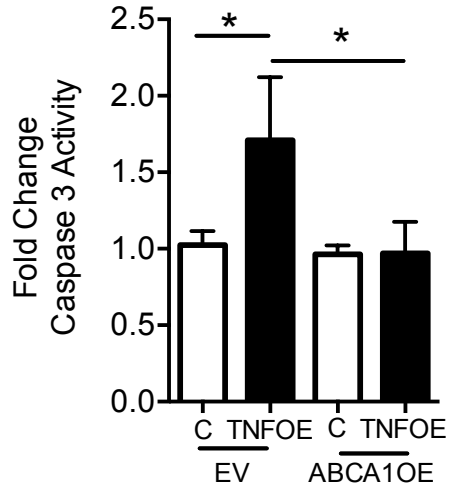
Supplemental Figure 8. Local TNF causes apoptosis independent of TNFR1 and TNFR2. A. TNF treated podocytes were protected from caspase 3 activity upon treatment with a TNFR1 blocking antibody (R1B) but not with a TNFR2 blocking antibody (R2B). One-way ANOVA, *P<0.05. **B.** TNF overexpressing (TNFOE) podocytes were not protected from caspase 3 activity with R1B or R2B treatment. One-way ANOVA, *P<0.05.



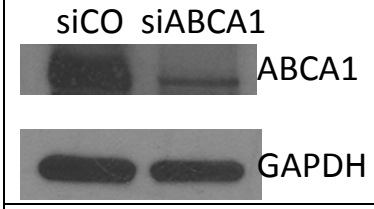
Supplemental Figure 9. Increased glomerular TNF expression correlated to reduced ABCA1 expression. Patients from the ERCB with higher glomerular *TNF* expression have reduced *ABCA1* expression (n=38). Correlation, P=0.04.



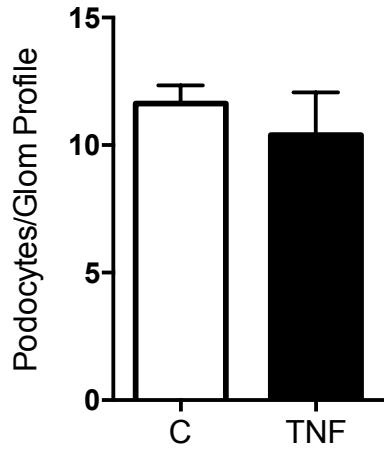
Supplemental Figure 10. TNF treatment or TNF overexpression reduces ABCA1 expression. A. Quantitative RT-PCR analysis of ABCA1 expression in normal human podocytes exposed to TNF (n=3) shows reduced ABCA1 expression when compared to untreated podocytes (C). Two-tailed Student's t-test, *P<0.05. **B.** Quantitative RT-PCR analysis of ABCA1 expression in TNF overexpressing podocytes compared to empty vector controls (C). Two-tailed Student's t-test, *P<0.05.



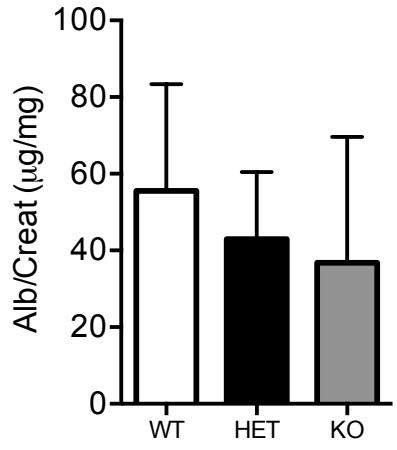
Supplemental Figure 11. ABCA1 overexpression prevents TNF overexpression mediated caspase 3 activity. A. TNFOE increases cleaved caspase 3 activity in empty vector (EV) containing podocytes compared to empty vector virus treated controls (C) *P<0.05. TNFOE does not increase cleaved caspase 3 activity in ABCA1 overexpressing podocytes (ABCA1OE) (n=3). One-way ANOVA, #P<0.05. need to change this



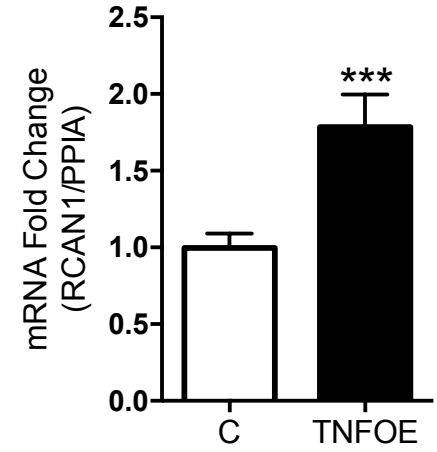
Supplemental Figure 12. Stable podocyte cell line demonstrating knockdown of ABCA1. Western blot of ABCA1 knock down (siABCA1) podocytes demonstrating reduced ABCA1 protein expression compared to scrambled controls (siCO) (n=3).



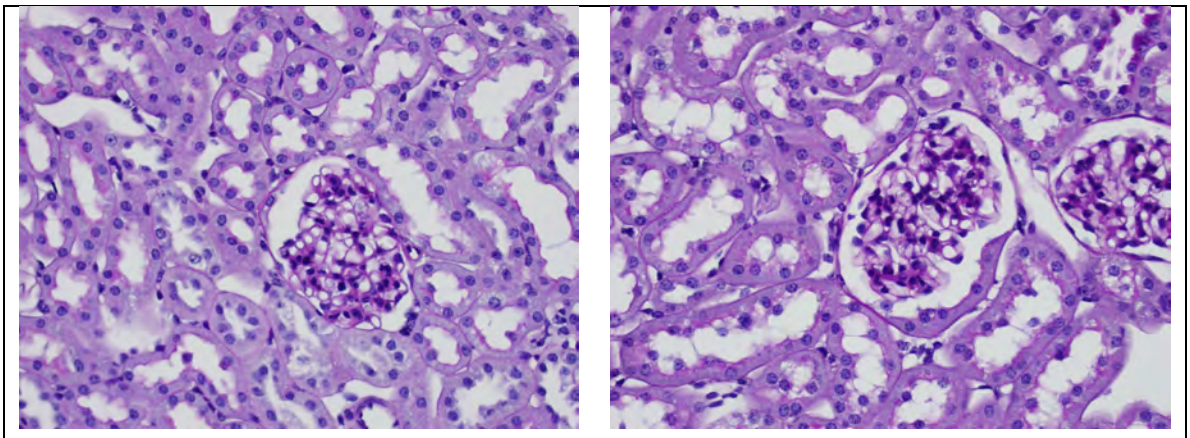
Supplemental Figure 13. TNF administration did not result in podocyte loss. Bar graph analysis of the number of podocytes per glomerular profiles of TNF injected mice and in vehicle treated controls (C) (n=5 per group). Two-tailed Student's t-test, P=Not Significant.



Supplemental Figure 14. Podocyte specific ABCA1 knockout mice do not develop albuminuria. The urinary albumin to creatinine ratio is unchanged in podocyte specific Abca1 knockout (KO), heterozygous floxed and cre positive mice (HET) or wild type littermates (WT) (n=5 per group). One-way ANOVA, P=Not Significant.



Supplemental Figure 15. TNF overexpression increases RCAN1 expression. A. Quantitative RT-PCR analysis of RCAN1 expression in TNF overexpressing podocytes (TNFOE) compared to empty vector control (C). Two-tailed Student's t-test, ***P<0.001.



Supplemental Figure 16. Podocyte specific constitutively active NFAT expressing mice do not develop lesions by light microscopy analysis after 4 days. Representative H&E staining reveals no changes in glomerular structure in double transgenic mice (DT) compared to single transgenic (ST) mice fed doxycycline for 4 days (n=5 mice per group).

	NS (n=14)
Age	10±5
Sex	M/F (10/4)
Serum Creatinine	0.5±0.2
Proteinuria (g/24h)	7.4±20
eGFR	135±25

Supplemental Table 1. Clinical characteristics of patients with nephrotic syndrome. Age, gender, serum creatinine, proteinuria and eGFR of patients with nephrotic syndrome.