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Supplemental Material



Supplemental Figure 1. Angiotensin (Ang) II induces KLF4 mRNA expression in M1activated macrophages. ("Macro KO" = peritoneal macrophages isolated from mice lacking type 1 angiotensin receptor in myeloid cells)



0

ŵT

KLF4 MKO

Supplemental Figure 2. Normal renal architecture and function in KLF4 MKO sham controls. (A) Representative kidney histology in sham control WT and KLF4 MKO kidneys. (B) BUN and (C) urine albumin/creatinine ratios (ACR) in sham control WT and KLF4 MKO mice (n=3 for WT and KLF4 MKO). Scale bar = 50 µm. Data represent the mean±SEM (ns, not significant).

0

wт

KLF4 MKO



Supplemental Figure 3. mRNA expression for M1 and M2 markers in obstructed kidneys from WT and KLF4 MKO mice. (A-D) mRNA expression of (A) IFN γ , (B) CCL5, (C) Egr2, (D) Retnla in WT and KLF4 MKO kidneys at day 7 of UUO (n=10 in each group). Data represent the mean±SEM (* *P*<0.05).



Supplemental Figure 4. KLF4 deficiency in macrophages permits induction of M1 markers in macrophages infiltrating the NTN kidney. (A-F) mRNA expression for (A) KLF4, (B) TNF α , (C) CCL2, (D) CCL5, (E) TGF β , and (F) Retnla in CD11b⁺Ly6C^{hi} macrophages sorted from NTN kidneys of WT and KLF4 MKO mice (n=6 for WT and n=5 for KLF4 MKO). Data represent the mean±SEM (* *P*<0.05).



Supplemental Figure 5. Myeloid cell characterization in WT and KLF4 MKO kidneys during NTN. (A) Representative flow cytometry gating strategy for single cell suspensions from NTN kidneys. (B) Number of CD64⁺ macrophages in kidneys from WT and KLF4 MKO mice at day 14 of NTN (n=8 for WT and n=6 for KLF4 MKO in NTN). (C) Number of CD11b⁺ TNF α^+ myeloid cells in kidneys from WT and KLF4 MKO mice at day 14 of NTN (n=5 for WT and n=6 for KLF4 MKO mice at day 14 of NTN (n=5 for WT and n=6 for KLF4 MKO in NTN). (D-E) Number of (D) neutrophils and dendritic cells in kidneys from WT and KLF4 MKO mice at day 14 of NTN (n=8 for WT and n=6 for KLF4 MKO). Data represent the mean±SEM (* *P*<0.05).



Supplemental Figure 6. Characterization of macrophages from WT and TNF MKO mice during NTN. (A) mRNA expression for TNF in isolated T cells, B cells, CD11b+ splenic macrophages (Macro), and in whole kidney and heart from wildtype (WT) and LysM-Cre⁺ TNF^{fl/fl} (TNF MKO) mice (n≥6 for each group). (B) Number of CD11b⁺Ly6C^{hi} macrophages in kidneys from WT and TNF MKO mice at day 14 of NTN. (C-G) mRNA expression levels for (C) TNF α , (D) CCL2, (E) CCL5, (F) TGF β , and (G) Retnla in CD11b⁺Ly6C^{hi} macrophages sorted from NTN kidneys of WT and TNF MKO mice (n=5 for each group). Data represent the mean±SEM (* *P*<0.05).



Supplemental Figure 7. Normal renal architecture and function in TNF MKO sham controls. (A) Renal pathology in sham control WT and TNF MKO kidneys. (B) BUN and (C) urine albumin/creatinine ratios (ACR) in sham control WT and TNF MKO mice (n=3 for WT and TNF MKO). Data represent the mean±SEM (ns, not significant).



Supplemental Figure 8. Renal RIPK3 and MLKL protein levels in WT and KLF4 MKO sham controls. Western blots for whole and phosphorylated RIPK3 and MLKL protein in WT and KLF4 MKO sham control kidneys.