

Supplemental Data for

**TRPM2 mediates ischemic acute kidney injury and oxidant stress
through Rac1**

Guofeng Gao, Weiwei Wang, Raghu K. Tadagavadi, Nicole Briley, Michael Love, Barbara A. Miller, W. Brian Reeves

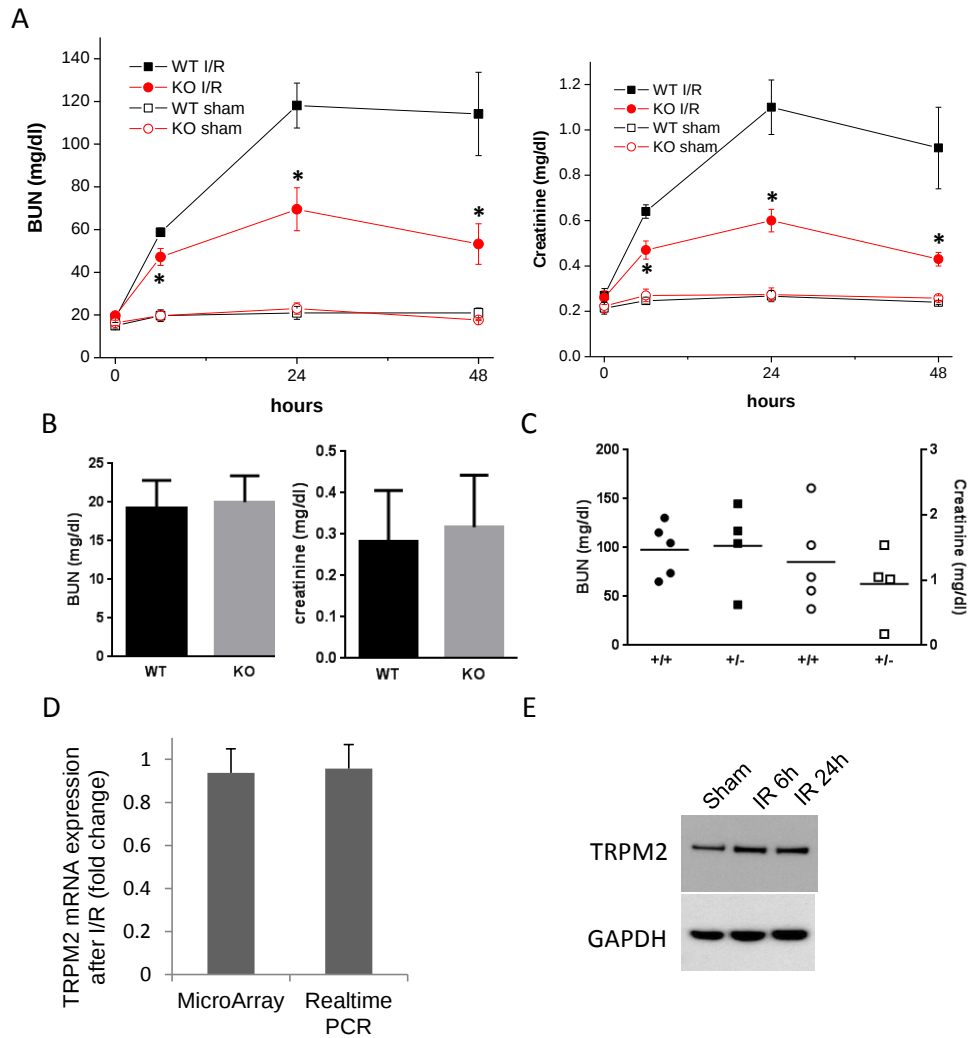


Figure 1. A. Time course of ischemic AKI in TRPM2 KO mice. TRPM2 KO and WT mice were subjected to 28 minutes of bilateral renal ischemia or sham surgery. Blood collected at various time points with respect to reperfusion was analyzed for BUN and serum creatinine as measures of renal function. Values are mean $\pm$ SEM, *, $P < 0.01$, $n = 3$ for sham and 12-20 for each I/R time point. B. Baseline levels of BUN and creatinine were not different between WT ($n=29$) and TRPM2 KO ($n=25$) mice. C. Heterozygous deficiency of TRPM2 did not alter susceptibility to ischemic AKI. WT (circles) and TRPM2 +/- mice (squares) were subjected to 28 minutes of ischemia. BUN (solid symbols) and creatinine (open symbols) were measured 24 h after reperfusion. $P > 0.05$. D. TRPM2 mRNA expression in WT kidneys was not changed 24 h after I/R as determined by microarray analysis ($n=4$) or quantitative RT-PCR ($n=4$). E. TRPM2 protein expression in WT kidney after sham surgery or 6 or 24 h after ischemia-reperfusion.

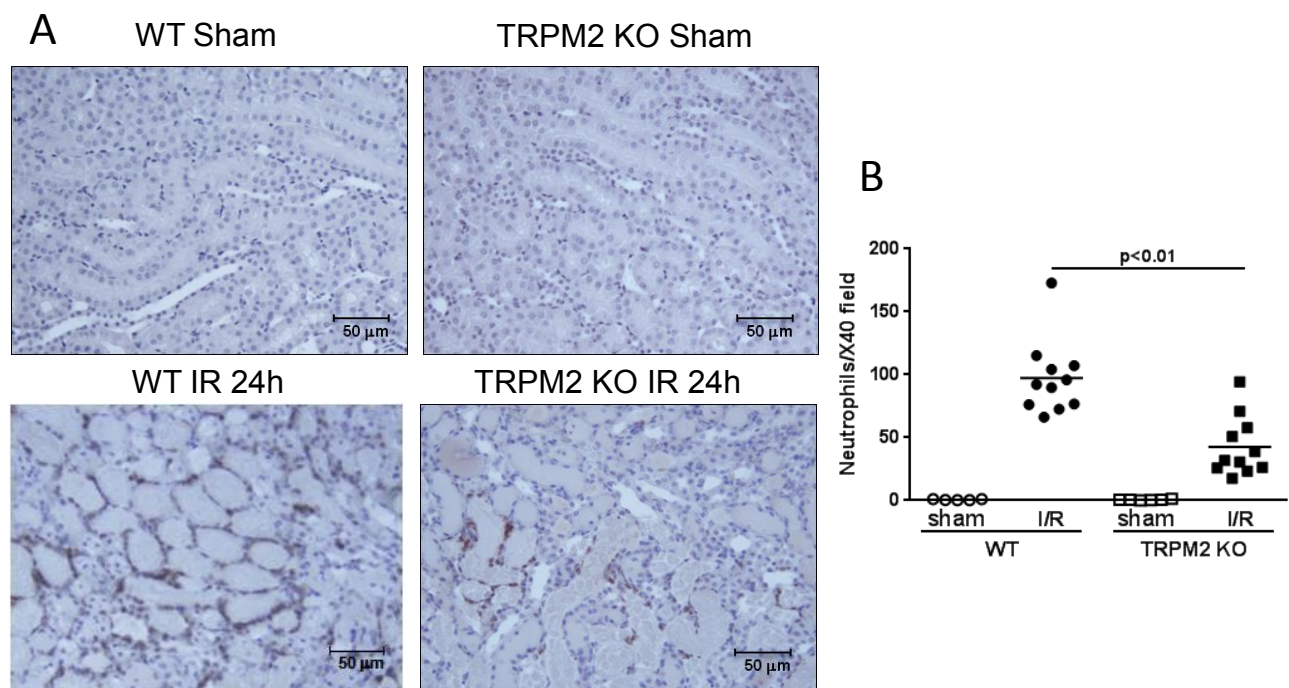


Figure 2. Immunohistochemical detection of neutrophils in the kidney. WT and TRPM2 KO mice were subjected to sham surgery or 28 minutes of bilateral renal ischemia followed by 24 h of reperfusion. A. Kidney sections were stained for neutrophils. B. Quantitation of neutrophil infiltration.

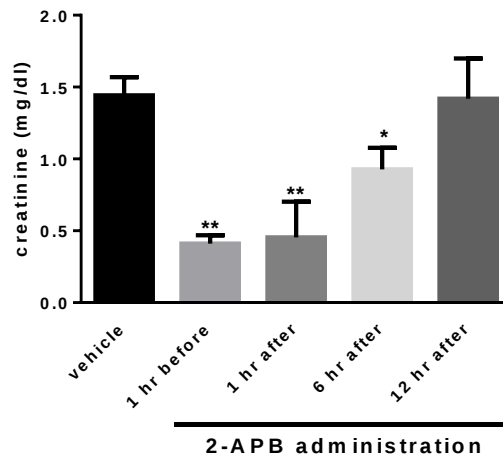


Figure 3. Inhibition of TRPM2 reduces ischemia-reperfusion injury. WT mice were treated with 2-APB (16 mg/kg) or vehicle (10%DMSO/90% saline) either 1 hour before or at varying times after reperfusion. Serum creatinine was measured 24 hours after reperfusion. **, $P < 0.01$ vs vehicle, * $P < 0.05$ vs vehicle. $N = 5-14$ for each 2-APB group.

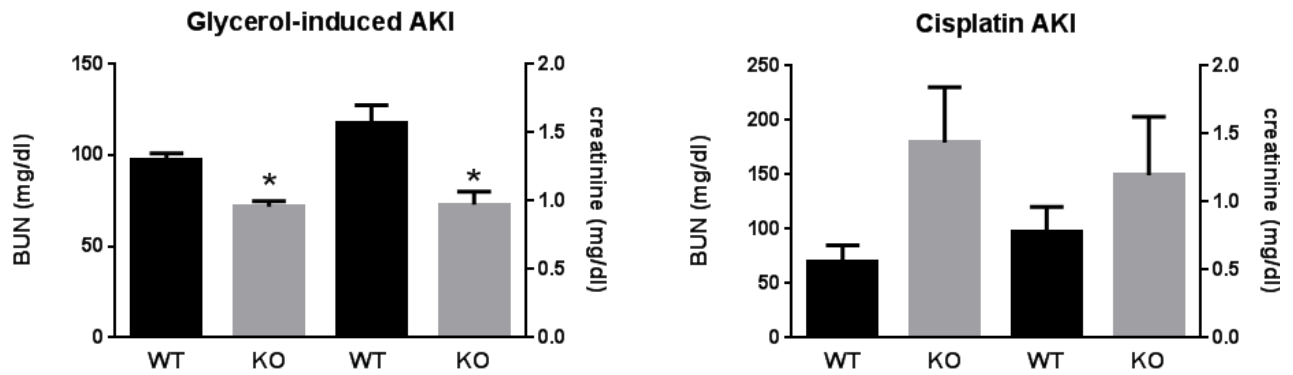


Figure 4. Effect of TRPM2 on AKI induced by glycerol-induced rhabdomyolysis and cisplatin. Left. Mice were injected with 7.5 ml/kg 50% glycerol into the hindlimbs. BUN and creatinine were measured 24 h after glycerol injection (n=4-5, * $P < 0.01$ vs WT). Right. Mice were treated with cisplatin (20 mg/kg IP). BUN and creatinine were measured 72 h after injection (n=7-8).

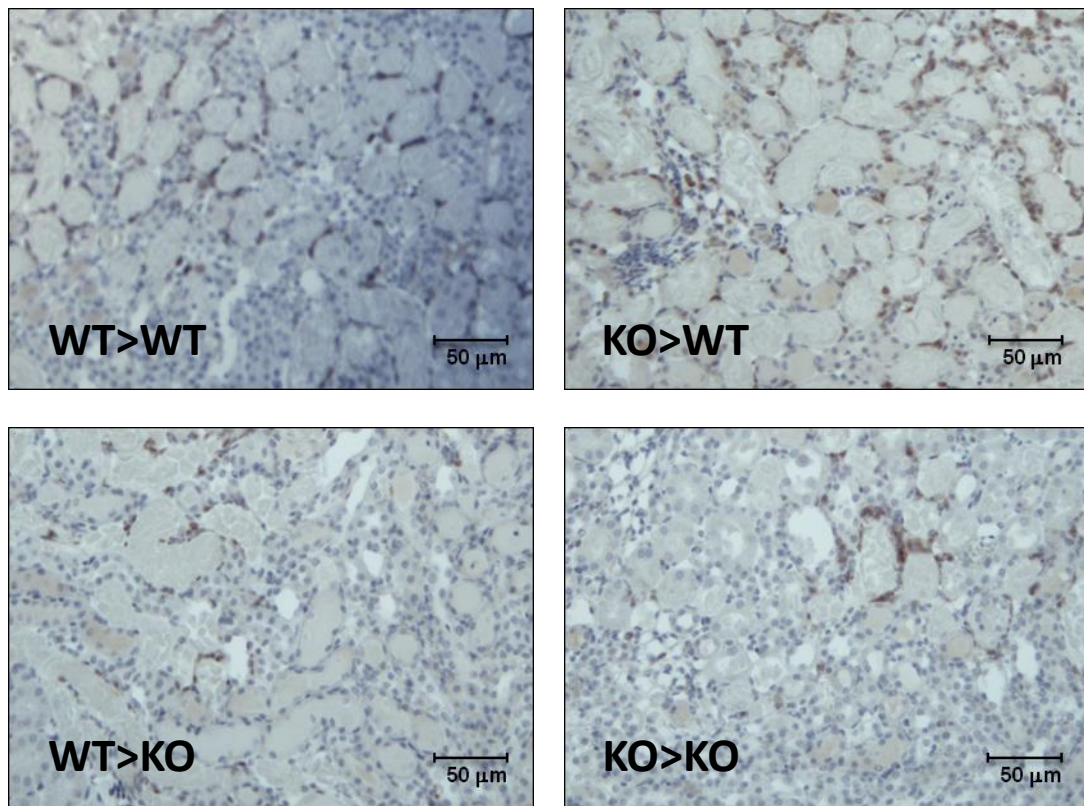


Figure 5. Decreased neutrophil infiltration in parenchymal TRPM2 deficient mice. Chimeric mice lacking TRPM2 in either bone marrow derived cells (KO-WT and KO-KO) or in parenchymal cells (WT-KO and KO-KO) were subjected to ischemia reperfusion. Kidneys were harvested 24 hrs after reperfusion and stained for neutrophils. WT-WT and KO-WT mice had robust infiltration of neutrophils while WT-KO and KO-KO mice had fewer neutrophils.

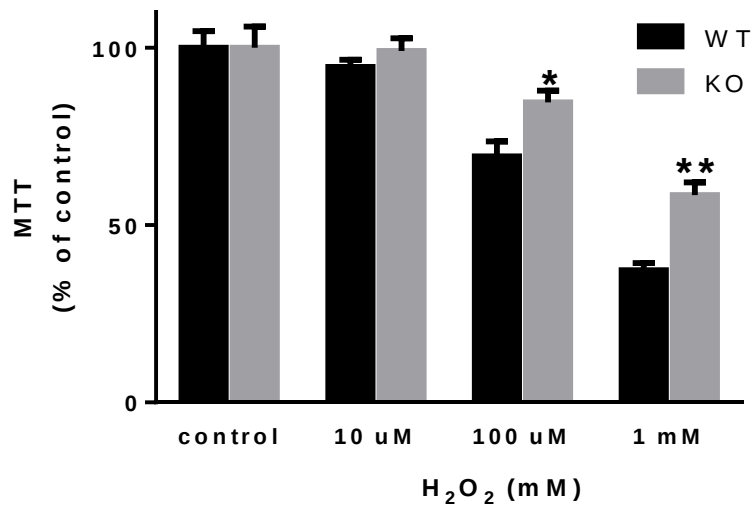


Figure 6. TRPM2 deficient proximal tubule cells are resistant to oxidant injury. Primary cultures of proximal tubule cells from WT and KO mice were treated with varying concentrations of H₂O₂ for 4 hours. Cell viability was determined using the MTT assay and normalized to untreated cells. * $p < 0.05$ vs WT, ** $P < 0.005$ vs WT, $n = 4$.

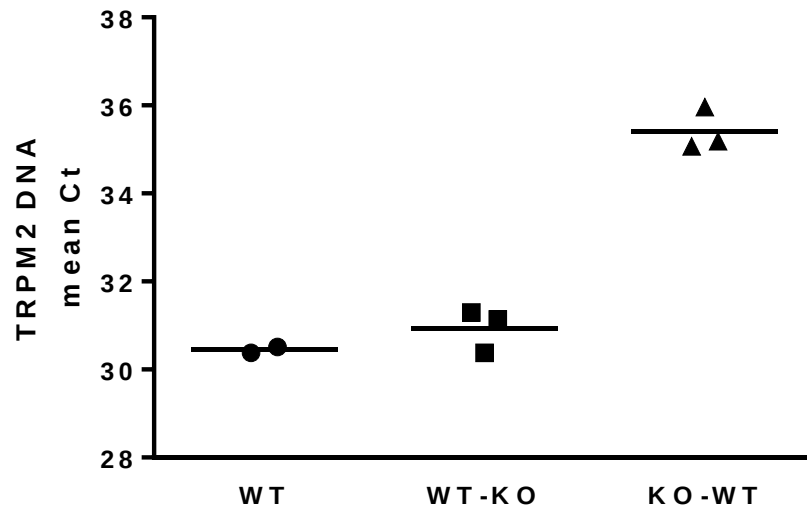


Figure 7. Confirmation of bone marrow chimerism. Genomic DNA was isolated from peripheral blood obtained 8 weeks after injection of donor bone marrow and amplified with primers which detect WT but not KO TRPM2. Ct values are shown. *TRPM2* DNA in WT-to-KO chimeras was similar to WT mice while KO-to-WT chimeras had <5% of *TRPM2* DNA levels seen in WT blood.

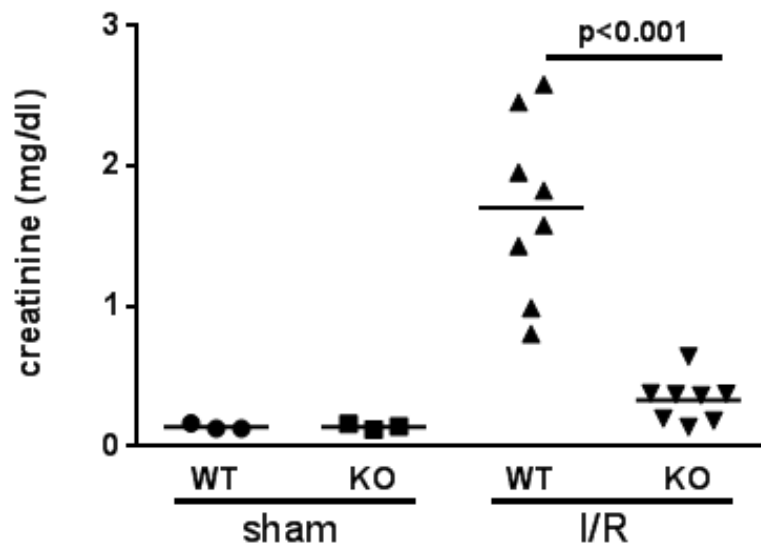


Figure 8. Measurement of creatinine by LC-MS/MS. Creatinine levels in blood samples collected 24 h after surgery were measured by LC-MS/MS rather than the enzymatic assay in routine use in this lab. LC-MS/MS measurements confirm that WT mice displayed significantly higher creatinine levels after ischemia compared to TRPM2 KO mice.