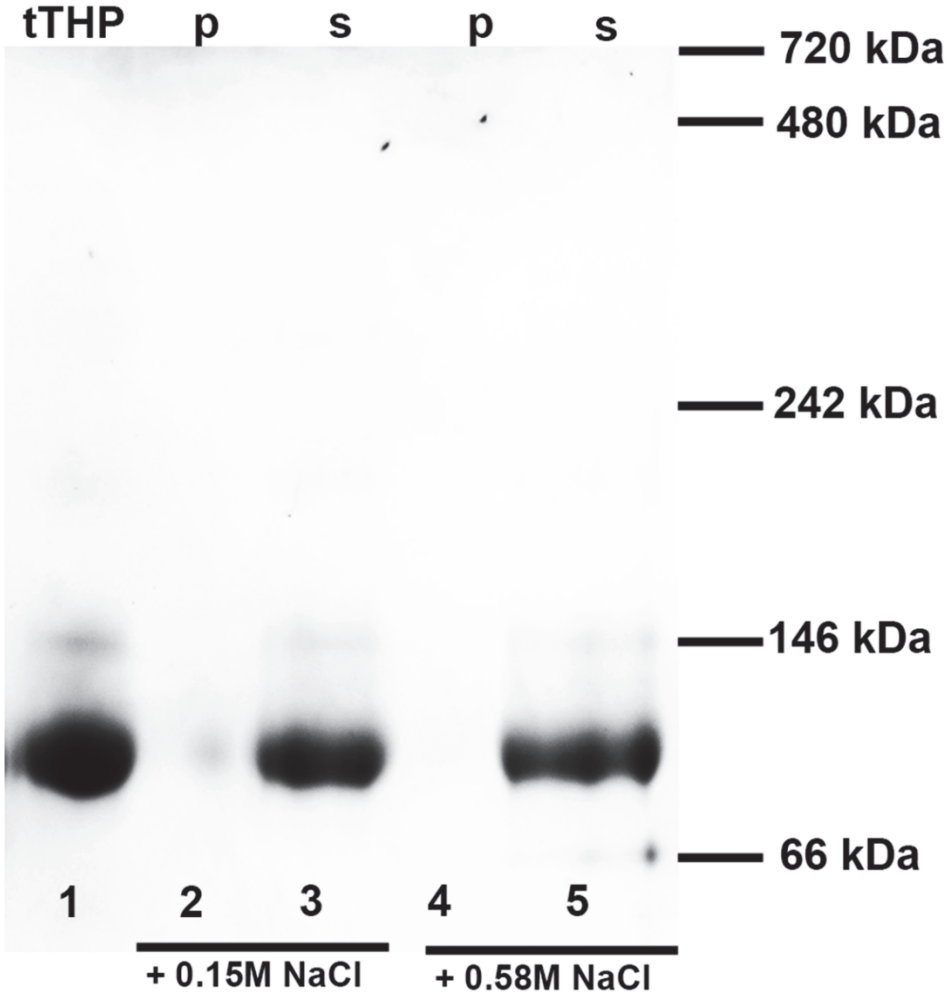
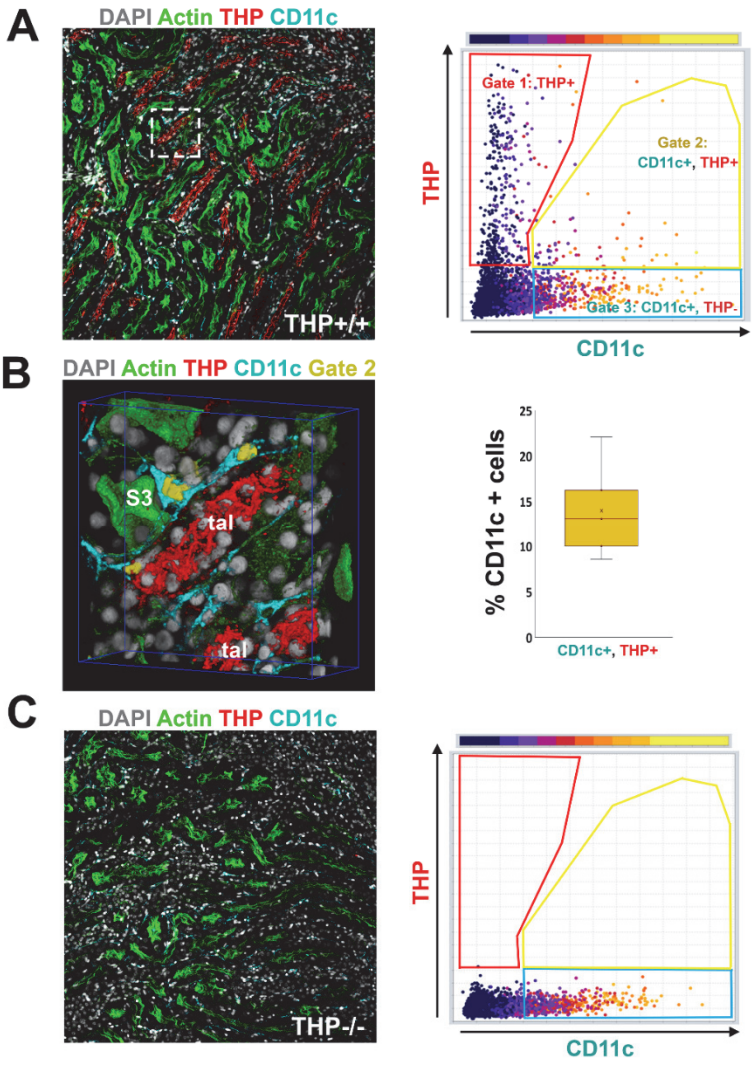
Supplemental Material



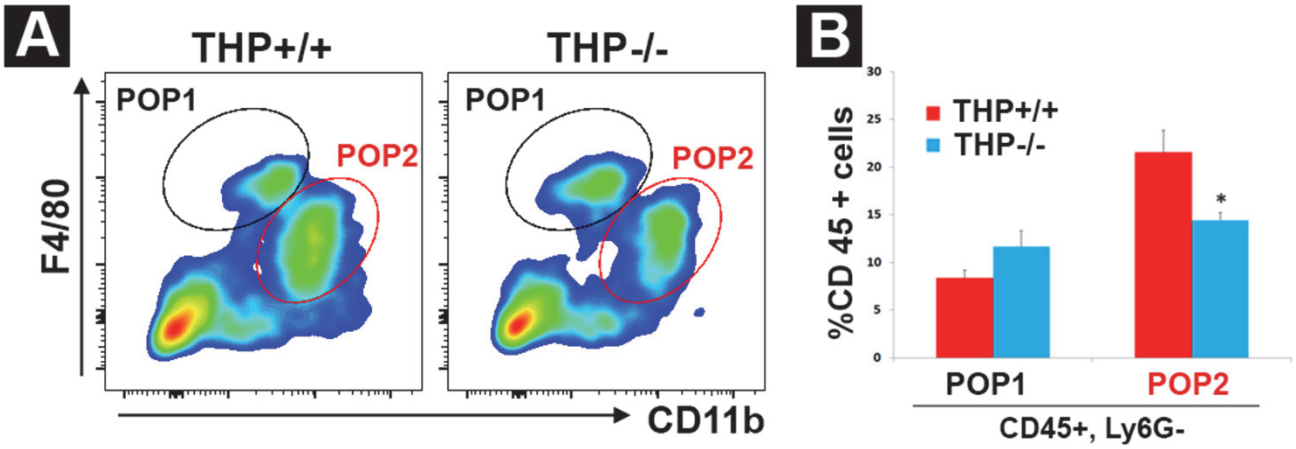
Supplemental Figure 1: Truncated THP isolated from urine does not polymerize.

Truncated THP (tTHP, lane 1) was treated with salt at physiological and salting-out concentrations overnight at 4^0C. After centrifugation at 20,000xg, and removal of the supernatants, the pellets were resuspended at equal starting volumes. The pellet (p) and supernatant (s) from each fractions were run on native PAGE. tTHP remained in solution (clean pellets, lane 2 and 4), and did not polymerize (lanes 3 and 4).



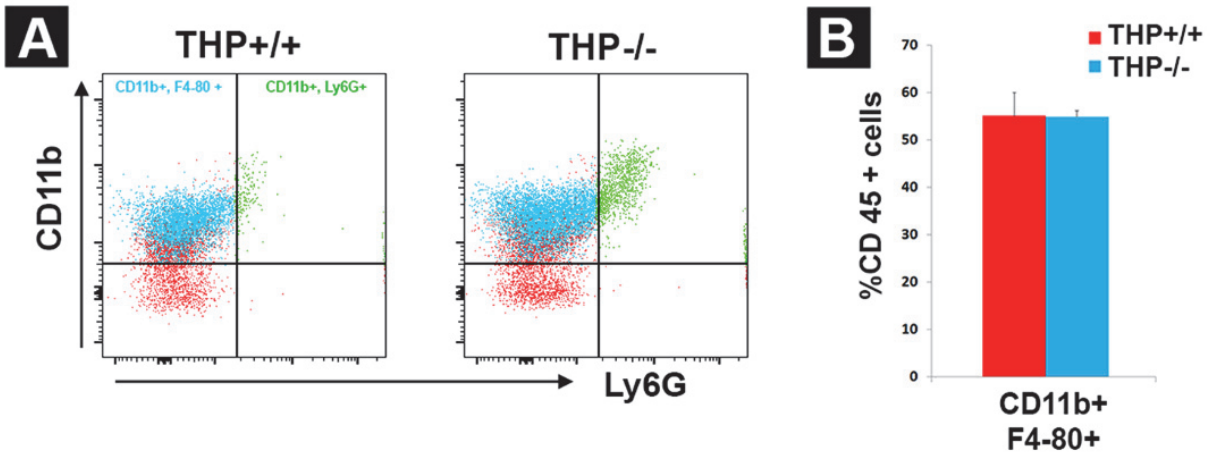
Supplemental Figure 2: 3-Dimensional Tissue Cytometry to quantitate CD11C+ cells with THP staining in the outer medulla

Figure in A is a representative maximum projection of a Z stack (50 µm), imaged using confocal microscopy, from THP+/+ kidney sections, labeled for THP, CD11c, F-Actin and DAPI. The representative scatter plot (right) shows the distribution of cells based on THP and CD11c staining using 3D tissue cytometry. Gate 2 depicts cells of interest (CD11c+, THP+). B is a rendered volume from the boxed area in A, showing the localizations of Gate 2 cells with a nuclear overlay, and confirming that these cells are interstitial CD11c+ with THP staining. The box plot in B shows quantification and distribution (mean marked by x) of CD11c+ THP+ cells as a percentage of total CD11c+ cell in the outer medulla of kidney sections from 4 different THP+/+ mice. C is a representative Z stack projection and 3D cytometry scatter plot from THP-/- tissue, serving as a negative control.



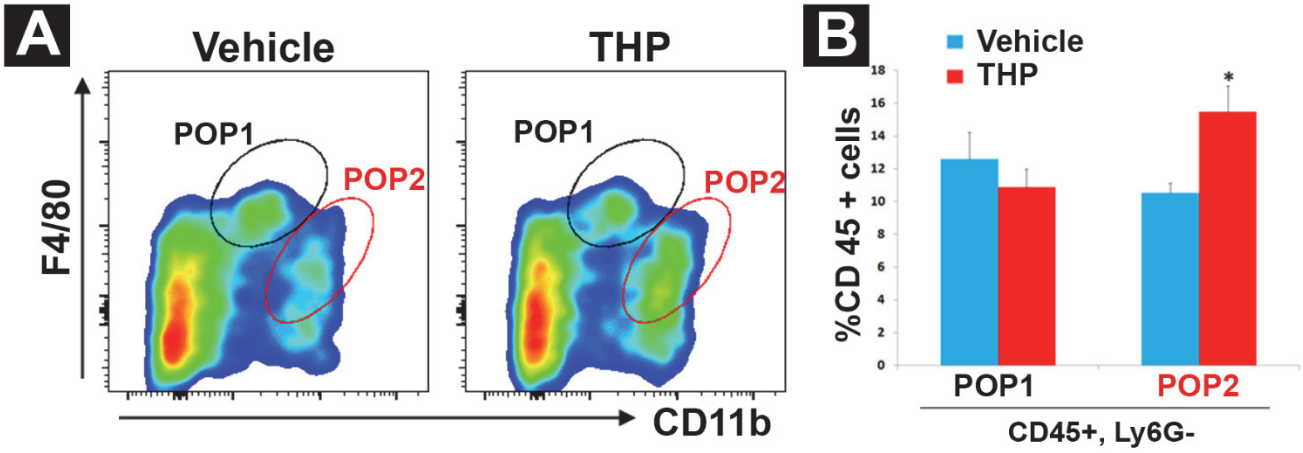
Supplemental Figure 3: Flow cytometry analysis for MPCs in THP+/+ and THP-/- kidneys using uniform nomenclature

Flow cytometry of mononuclear phagocytic cells (MPCs) from THP+/+ and THP-/- kidneys, using gating and nomenclature adapted from George *et al.* (reference 38). All cells are gated on CD45+, Ly6G- cells. POP1 are CD11b lo, F4/80 hi cells, and POP2 as CD11b hi, F4/80 + (intermediate) cells. The difference in MPCs is noted in the POP2 cells, which is concurrent with the analysis in Figure 5. Asterisk denotes statistical significance between the two groups (n=5 per group, $p<0.01$).



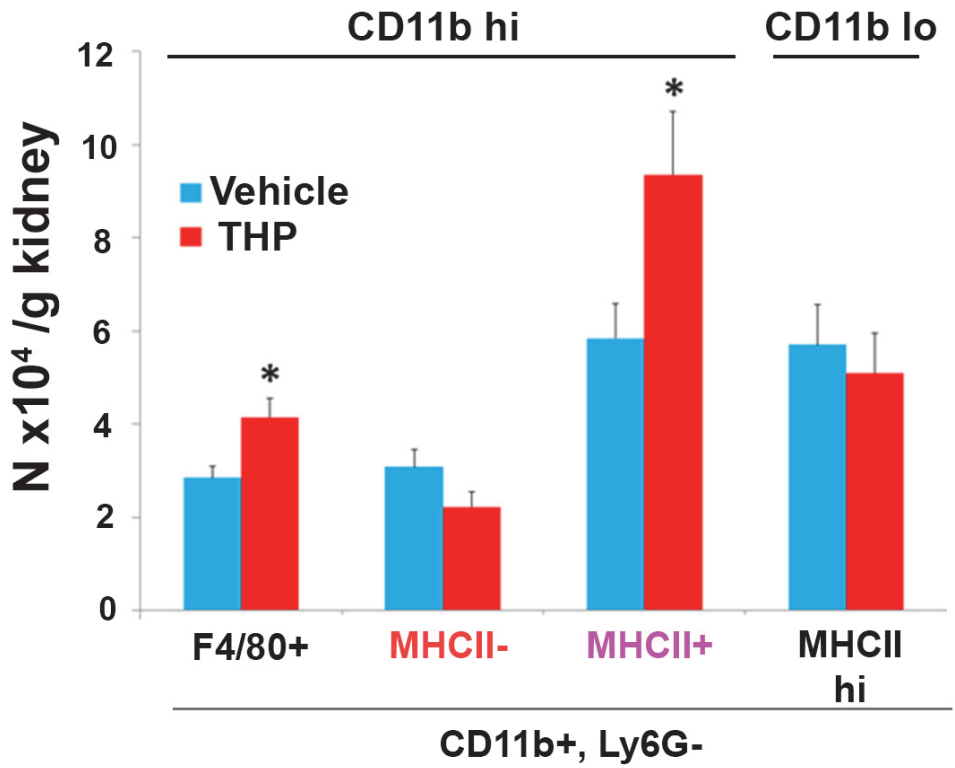
Supplemental Figure 4: Flow cytometry of the liver for myeloid cells

Scatter plots in A are representative of flow cytometric analysis on livers from THP+/+ and THP-/-. CD11b+/F4-80+ cells are back gated and shown in blue color within the plot. Quantitation of CD11b+/F4/80+ cells is shown in the bar graphs in B, as percentage of CD45+ cells. No difference between the two strains of mice is noted (n=5/group)



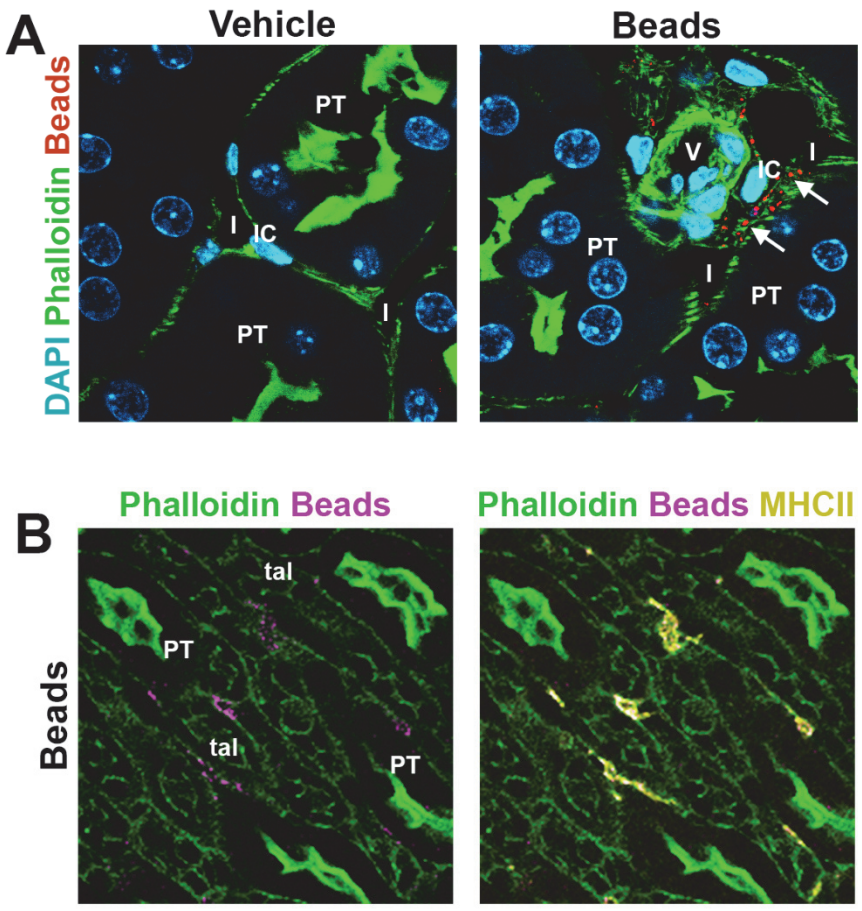
Supplemental Figure 5: Flow cytometry analysis for MPCs in THP-/- kidney after tTHP treatment using uniform nomenclature

Flow cytometry of mononuclear phagocytic cells (MPCs) from THP-/- kidneys after treatment with tTHP (as described in Figure 7E-G) , using gating and nomenclature adapted from George *et al.* (reference 38). All cells are gated on CD45+, Ly6G- cells. POP1 are CD11b lo, F4/80 hi cells, and POP2 as CD11b hi, F4/80 + (intermediate) cells. The difference in MPCs is noted in the POP2 cells, which is concurrent with the analysis in Figure 7E-G. Asterisk denotes statistical significance between the two groups (n=5 per group, $p<0.05$).



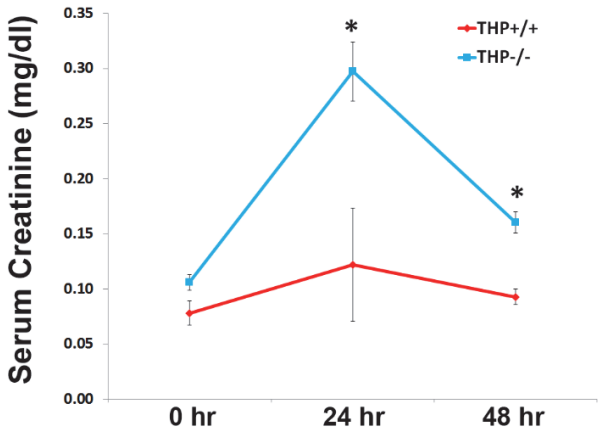
Supplemental Figure 6: Absolute quantitation of changes in MPCs in THP-/- kidneys after tTHP treatment

Quantitation of the gated population of cells in Figure 7 showing absolute cell counting normalized by kidney weight, using a dual platform approach as we described previously (Reference 41). This data concurs with what was shown in Figure 7 as percentage of CD45+ cells. Asterisk denotes statistical significance between the two groups ($p<0.05$).



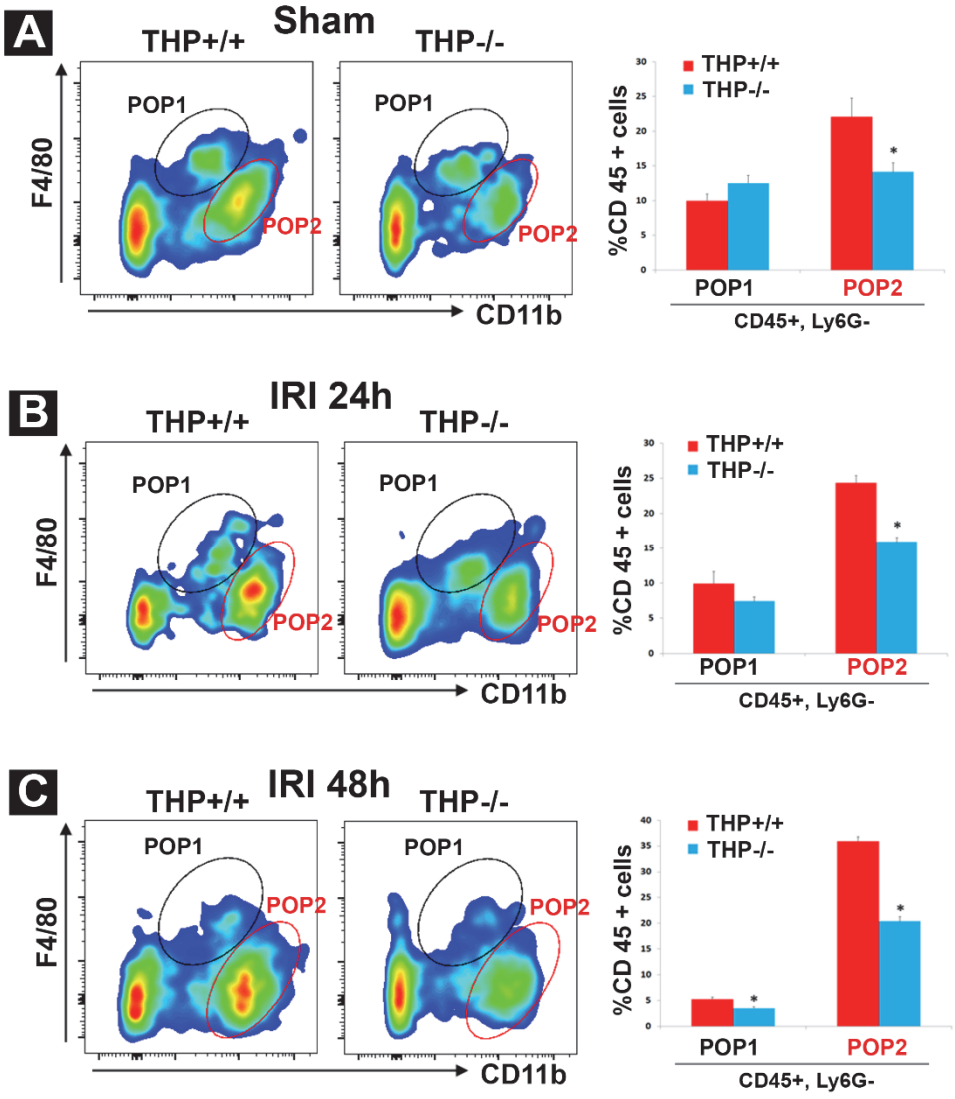
Supplemental Figure 7: : Fluorescent bead injection and phagocytosis by renal MPCs

Panels in A shows representative confocal microscopy images (40x objective) of kidney sections taken from mice injected with vehicle (left), or fluorescent beads (Right, 20 nm beads). Arrows point to the distinct localization of the beads within interstitial cells (IC). (The interstitial space is marked by I). No beads were observed in tubules (proximal tubules, PT) or blood vessels (V). Panels in B show 100 nm fluorescent beads within the kidney interstitium after systemic injection (20x objective). Beads signal co-localizes with staining for MHCII, which is expressed on antigen presenting MPCs. (Proximal tubules, PT; thick ascending limbs, tal).



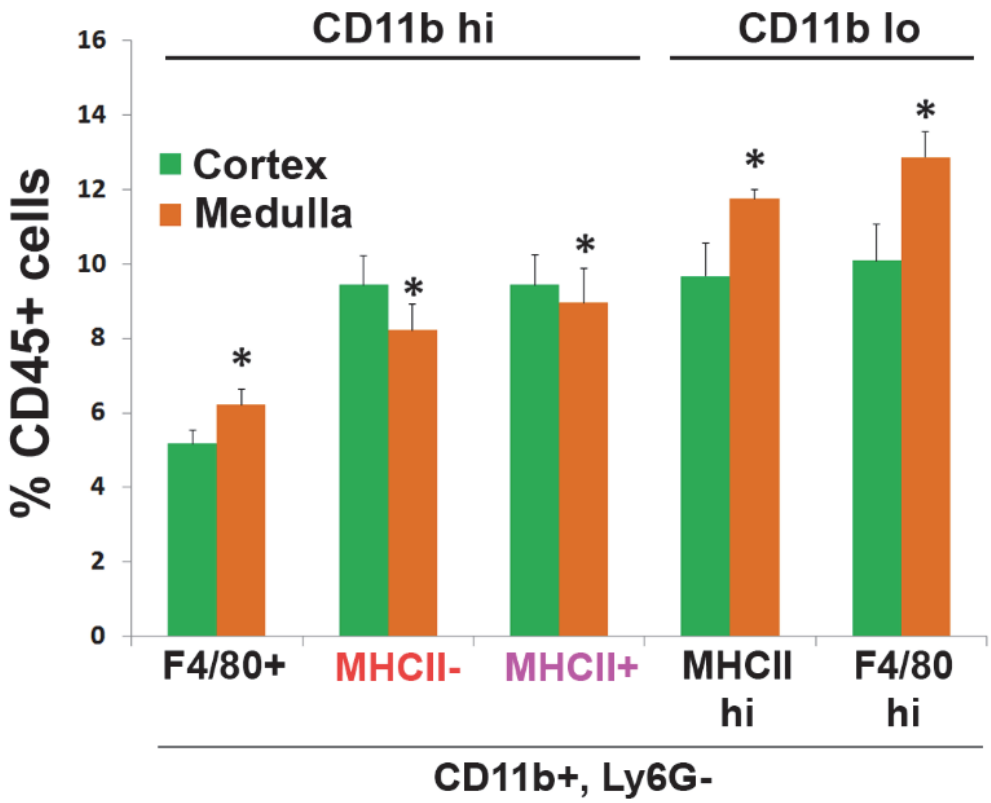
Supplemental Figure 8: Serum Creatinine during AKI in THP+/+ and THP-/-

Mean± standard error for THP+/+ and THP-/- mice at various time point after IRI are shown (n=5 each group). Serum creatinine was determined using capillary electrophoresis, as described previously. Asterisk * denotes statistical significance between the strains ($p<0.05$).



Supplemental Figure 9: Flow cytometry analysis for MPCs in THP+/+ and THP-/- kidneys after ischemia-reperfusion injury (IRI), using a uniform nomenclature

Flow cytometry of mononuclear phagocytic cells (MPCs) from THP+/+ and THP-/- kidneys in sham, 24 and 48 hours after IRI (IRI24h and IRI 48h, respectively), using gating and nomenclature adapted from George *et al*. (reference 38). All cells are gated on CD45+, Ly6G- cells. POP1 are CD11b lo, F4/80 hi cells, and POP2 as CD11b hi, F4/80 + (intermediate) cells. The difference in MPCs is again noted in the POP2 cells, in sham and IRI24. 48hours after IRI, both POP1 and POP2 cells are decreased in THP-/- compared to THP+/+. Asterisk denotes statistical significance between the two groups (n=5 per group, $p<0.05$).



Supplemental Figure 10: Flow cytometry analysis of the cortex and medulla from THP+/+ kidneys

Bars are mean +/- standard error of the percentage of sub populations of MPCs in the cortex and medulla from THP+/+kidneys. A paired analysis was used to compare the cortex to the corresponding medulla from each kidney (n=5, asterisk denotes statistical significance between cortex and medulla, $p<0.05$).

Supplemental Video 1: Time series showing Intravital imaging of the kidney from CX_3CR1-EGFP mouse after intravenous injection of Alexa 568- labeled THP (red). Mononuclear phagocytic cells (MPCs, both dendritic cells and macrophages) display green fluorescence. Both sessile and mobile MPCs in the interstitium show uptake of THP. In the center of the field, a macrophage with THP labeling appears to travel and communicate with other MPCs