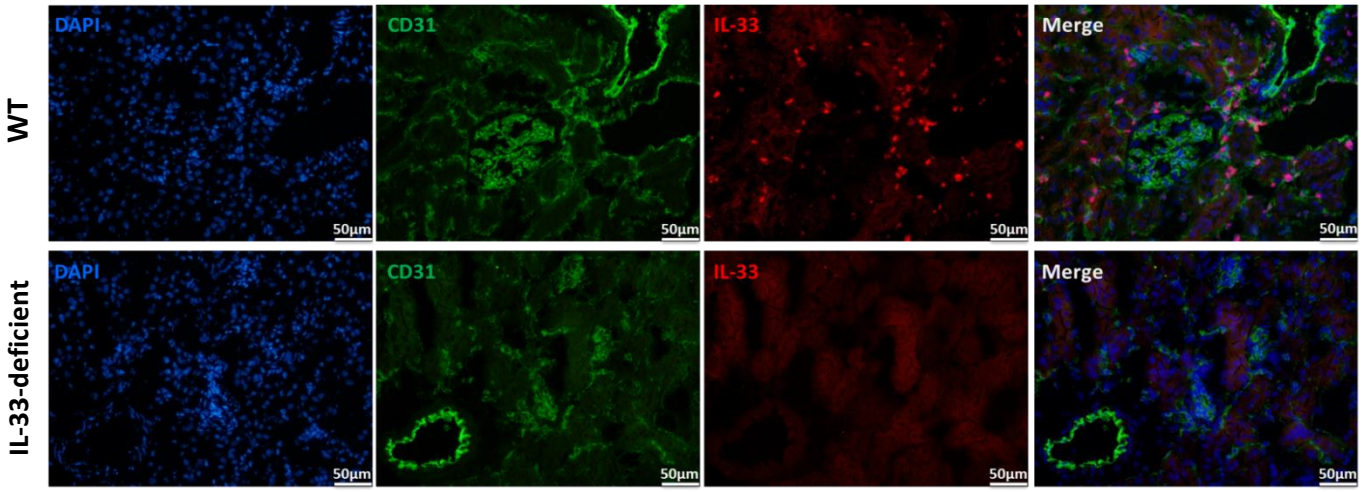
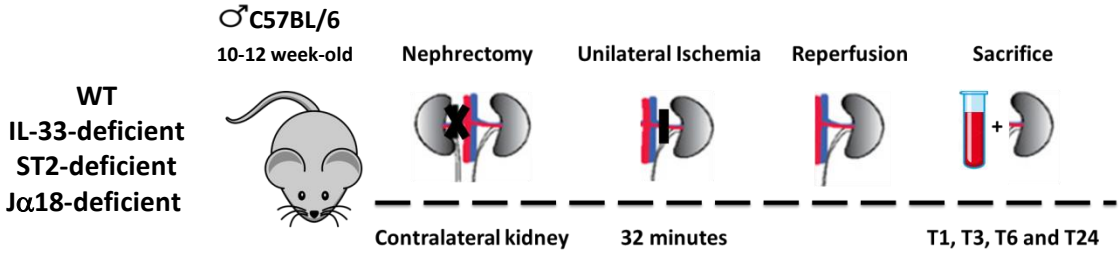
Supplemental Figures

Supplemental Figure 1



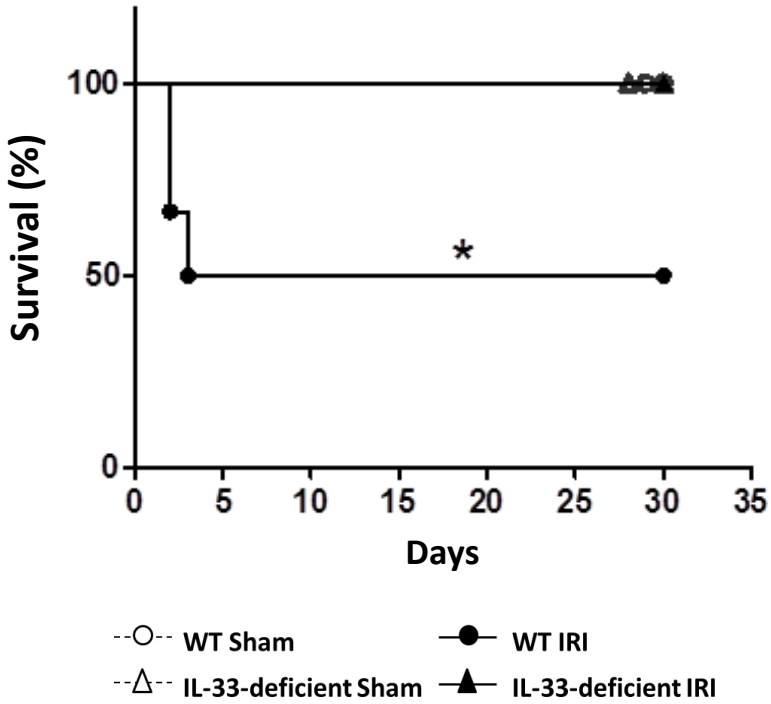
Supplemental Figure 1: Immunofluorescence staining for IL-33 on non-ischemic kidneys from wild-type and IL-33$^{Gt/Gt}$ mice. IL-33 (red), CD31 (green) and DAPI (blue) in non-ischemic kidneys from wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice. Note the absence of staining in healthy kidney tissues from IL-33-deficient mice used as controls.

Supplemental Figure 2



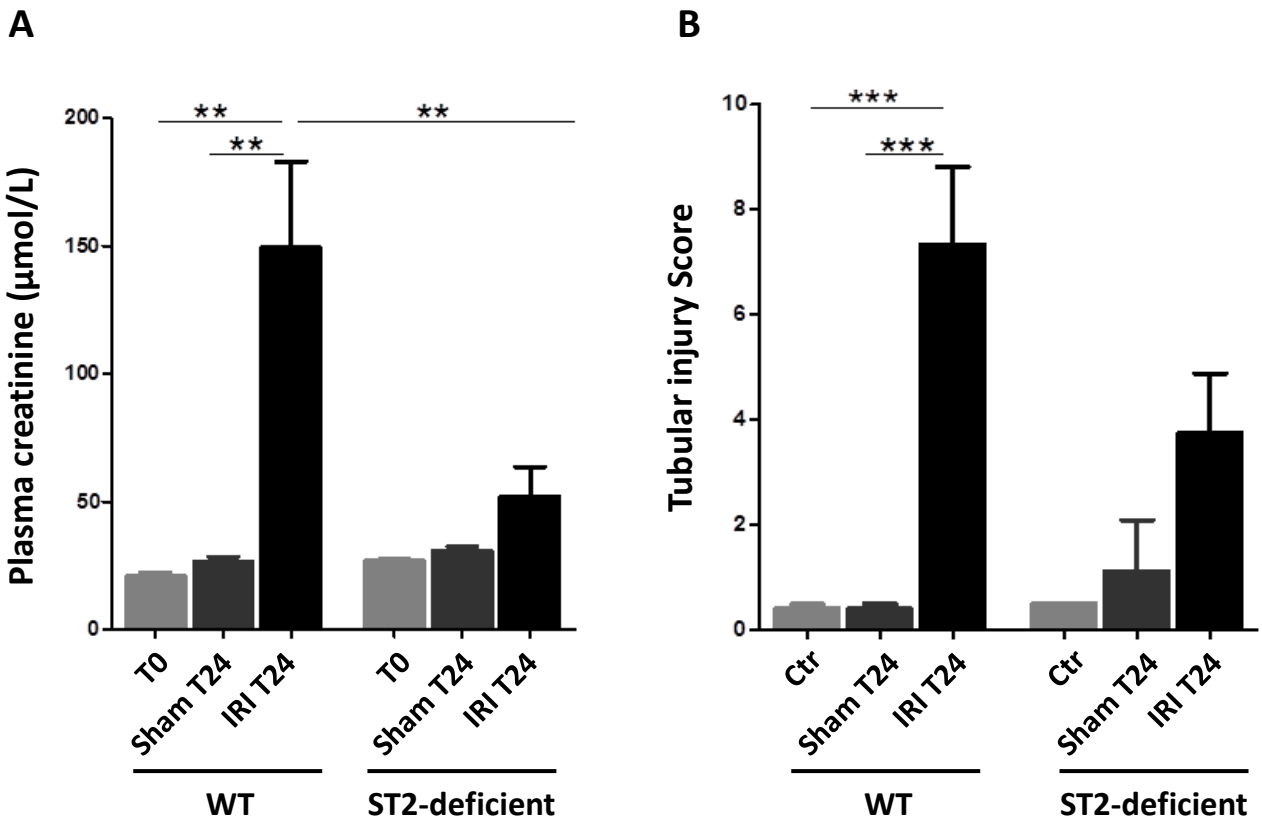
Supplemental Figure 2: Experimental procedure of renal IRI. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) male C57BL/6 mice were subjected to sham surgery (Sham) or 32 minutes of unilateral ischemia (IRI) following contralateral nephrectomy (Ctr). After 1, 3, 6 or 24 hours (T1, T3, T6, or T24) of reperfusion (from the end of the ischemic period), kidneys and peripheral blood were obtained. The healthy left Ctr kidney, ligated and removed before IRI induction, was used as for comparison with IRI and Sham kidneys. Control plasma (T0) was obtained from naive animals.

Supplemental Figure 3



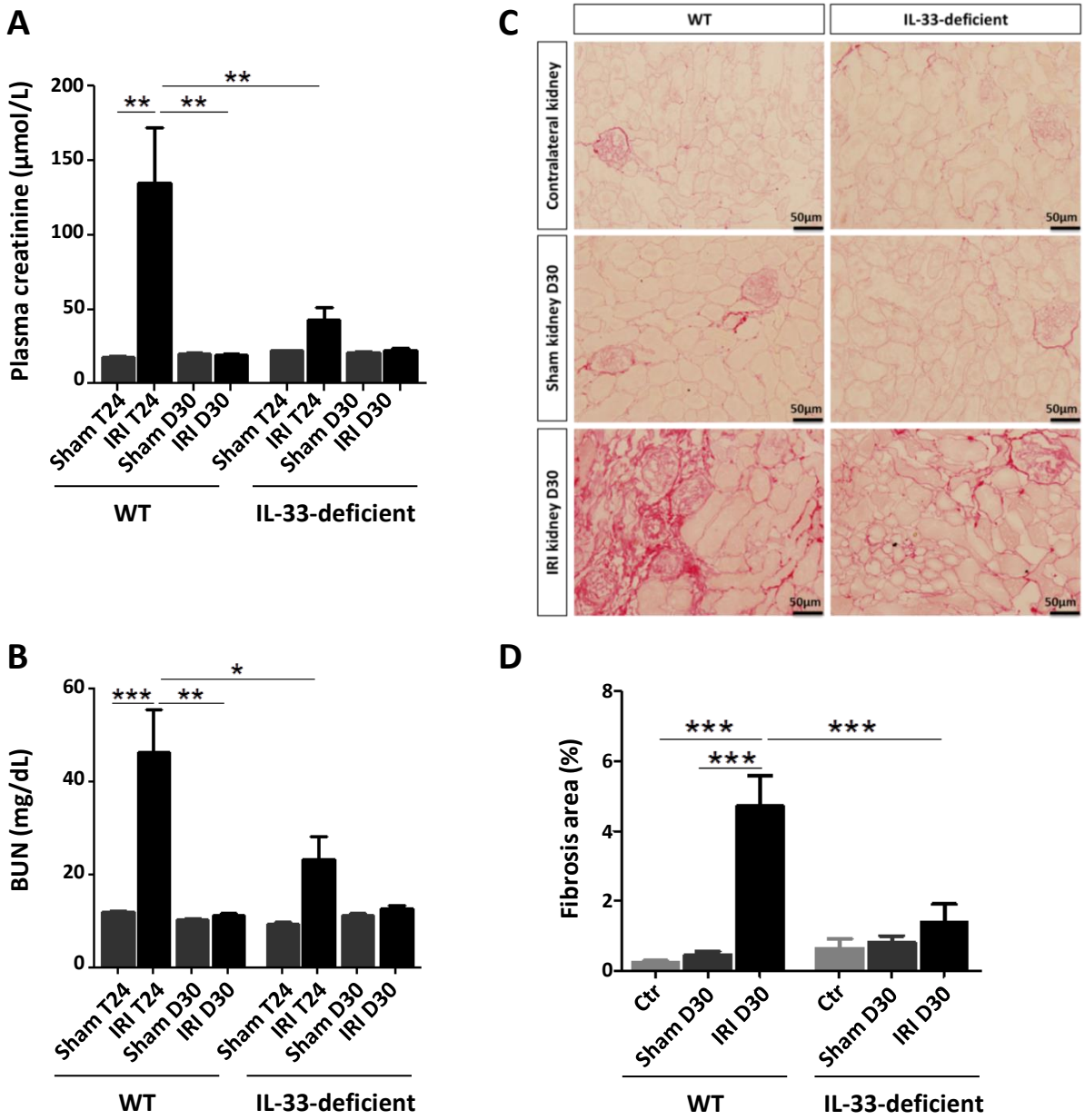
Supplemental Figure 3: Mortality is reduced in IL-33-deficient mice. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to sham surgery (Sham; 3 animals per group) or 32 minutes of unilateral ischemia (IRI; 6 animals per group) after contralateral nephrectomy. Kaplan-Meier survival curves for sham-operated and IRI WT and IL-33-deficient mice. Thirty days after IRI induction, a survival rate of 100% was observed in IL-33-deficient mice whereas 50% of WT mice succumbed within the first 3-4 days. Survival distribution of the WT IR group was significantly different from all other groups of mice. (Mantel-Cox, *P<0.05). IRI is presumably the cause of mortality because the vast majority of ischemia-reperfusion-induced WT mice were still anuric at 2-3 days post-IRI.

Supplemental Figure 4



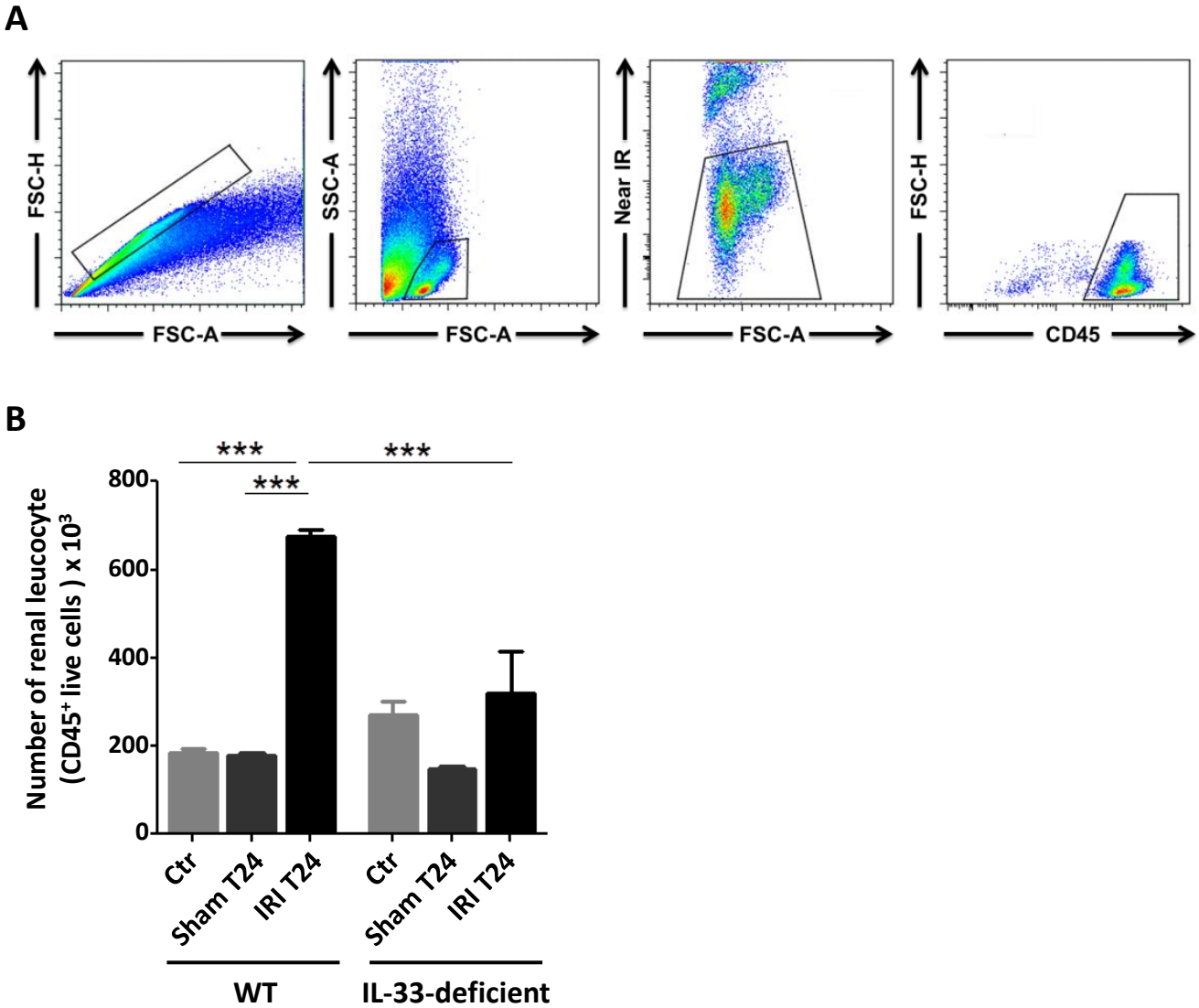
Supplemental Figure 4: ST2-deficient mice are protected against IRI. Wild-type (WT) and ST2KO (ST2-deficient) mice were subjected to sham surgery (Sham) or 32 minutes of unilateral ischemia (IRI) following contralateral nephrectomy (Ctr). After 24 hours (T24) of reperfusion, kidneys were obtained (4-6 animals per group). At T24 post-IRI, ST2-deficient mice demonstrated less elevated blood creatinine levels **(A)** and attenuated tubulointerstitial damage (**B**) as compared to their WT counterparts. Control plasma (T0) was obtained from naïve animals. Tubular injury was assessed with periodic acid-Shiff (PAS) staining. Sham and healthy Ctr kidneys from both WT and ST2-deficient mice exhibited normal tubular structure. Data are expressed as means±SEM. One-way ANOVA followed by Tukey post-test was used for three or more group comparisons. **P<0.01; ***P<0.001.

Supplemental Figure 5



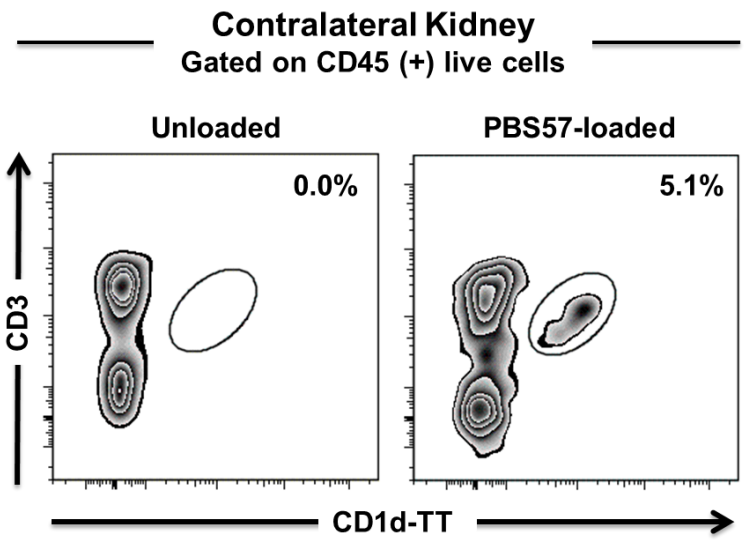
Supplemental Figure 5: Collagen deposition after IRI is reduced in IL-33-deficient mice. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to sham surgery (Sham; n=3) or 32 minutes of unilateral ischemia (IRI; n=6) after contralateral nephrectomy (Ctr). Blood was collected after 24 hours (T24) of reperfusion. At day 30 (D30) post-reperfusion, blood and kidneys were obtained from surviving mice. At D30 post-IRI, blood creatinine (**A**) and BUN (**B**) significantly returned to baseline values in both WT and IL-33-deficient mice. (**C-D**) Fibrosis was measured using Sirius red staining. Representative panels of collagen staining (C) and fibrosis quantification (total kidney collagen content) are shown (D). Significant collagen deposition in kidneys was found at D30 post-IRI in kidneys from WT (n=3) but not in IL-33-deficient (n=6) mice, as compared to their healthy Ctr (n=3) or Sham (n=3) counterparts. We attributed the lesser progression to fibrosis in IL-33-deficient mice to less initial AKI rather than to loss of IL-33. Data are expressed as means±SEM. Two-tailed Mann-Whitney U test was used for two group comparisons, and one-way ANOVA followed by Tukey post-test for three or more group comparisons. *P<0.05; **P<0.01; ***P<0.001.

Supplemental Figure 6



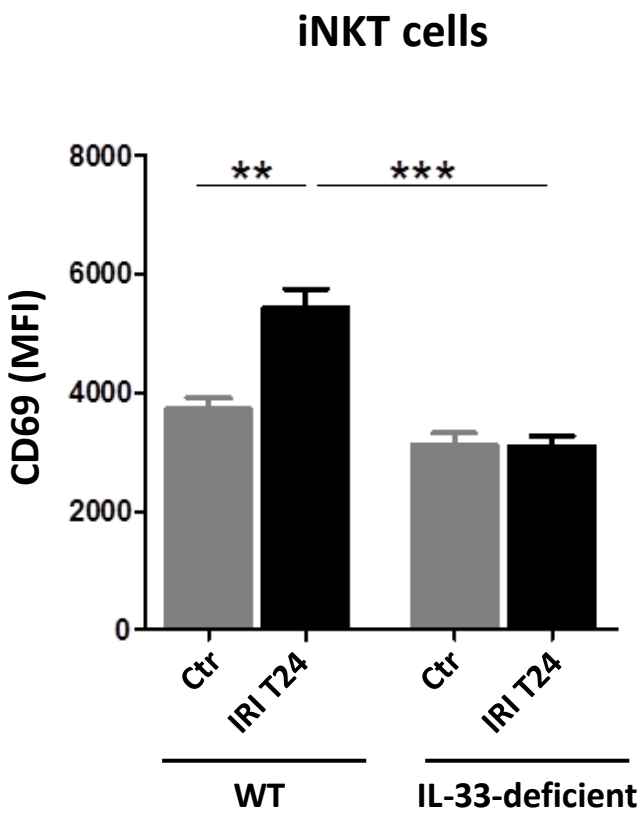
Supplemental Figure 6: Identification of kidney leukocytes by flow cytometry. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to sham surgery (Sham) or 32 minutes of unilateral ischemia (IRI) after contralateral nephrectomy (Ctr). After 24 hours, kidneys were obtained and leukocytes freshly isolated to examine the number of total leukocytes (CD45(+) cells). (**A**) Gating strategy. Kidney cell suspensions were prepared from ischemic WT mouse kidneys. Cells were first gated on size (FCS-A) and granularity (SSC-A) followed by Near IR exclusion to identify live cells for further analysis. Live cells were gated on CD45 expression to identify leukocytes. (**B**) Number (per kidney) of CD45(+) cells. Data are expressed as means±SEM (5-8 animals per group). One-way ANOVA followed by Tukey post-test was used for three or more group comparisons. ***P<0.001.

Supplemental Figure 7



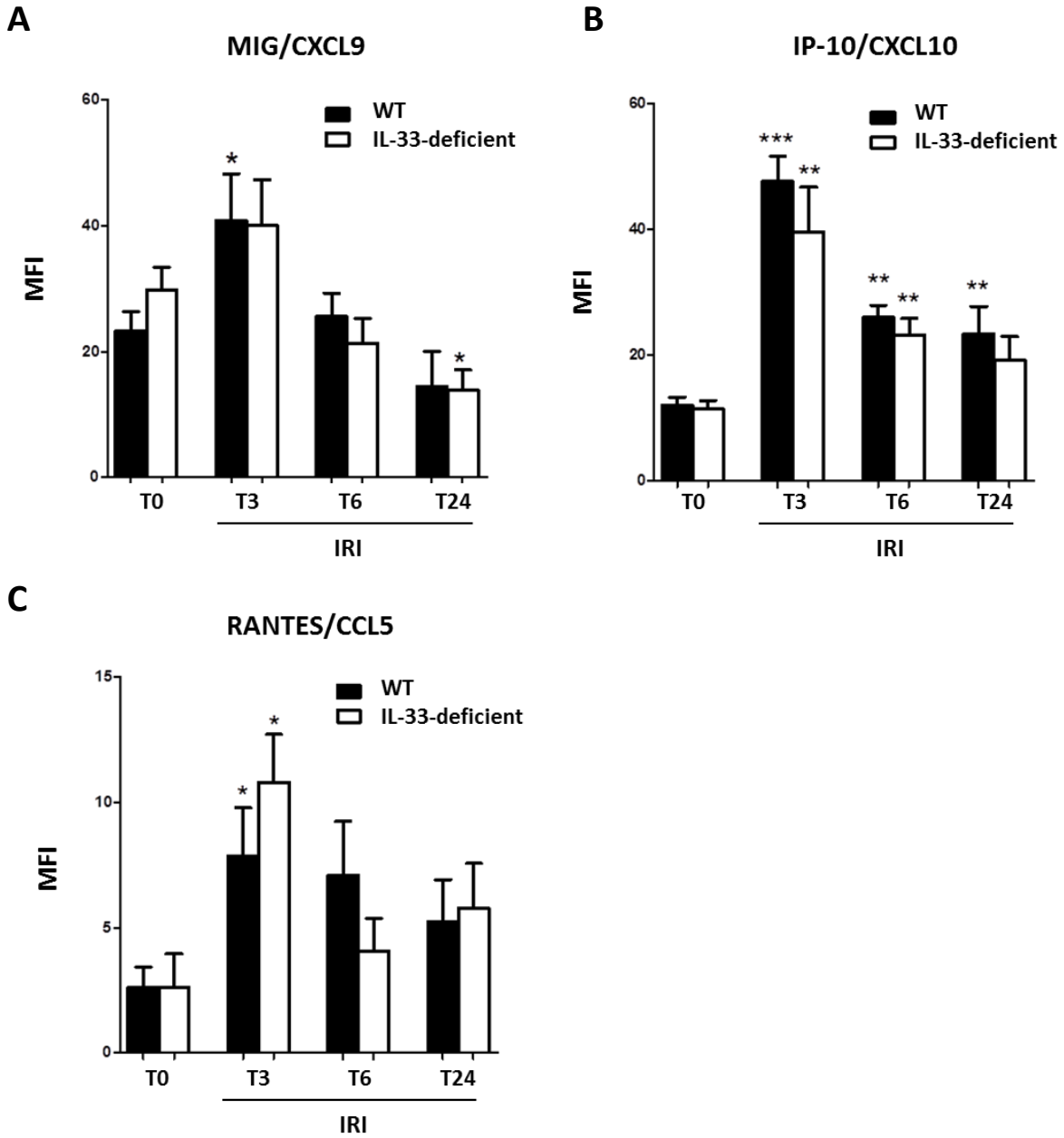
Supplemental Figure 7: Gating strategy to identify iNKT cells among leukocytes freshly isolated from kidneys. Leukocytes (CD45(+) cells) were membrane-labeled with anti-CD3 mAb and an empty CD1d TT (without PBS57) (**left panel**) or with anti-CD3 mAb and PBS57-loaded CD1d TT (**right panel**) to validate the staining of the PBS57-loaded CD1d TT. iNKT cells were defined as PBS57-loaded CD1d TT(+) cells with intermediate CD3 expression. A representative case of leukocytes isolated from a healthy contralateral kidney is shown. Numbers indicate the percentage of cells of interest among total leukocytes after gating on live CD45(+) cells.

Supplemental Figure 8



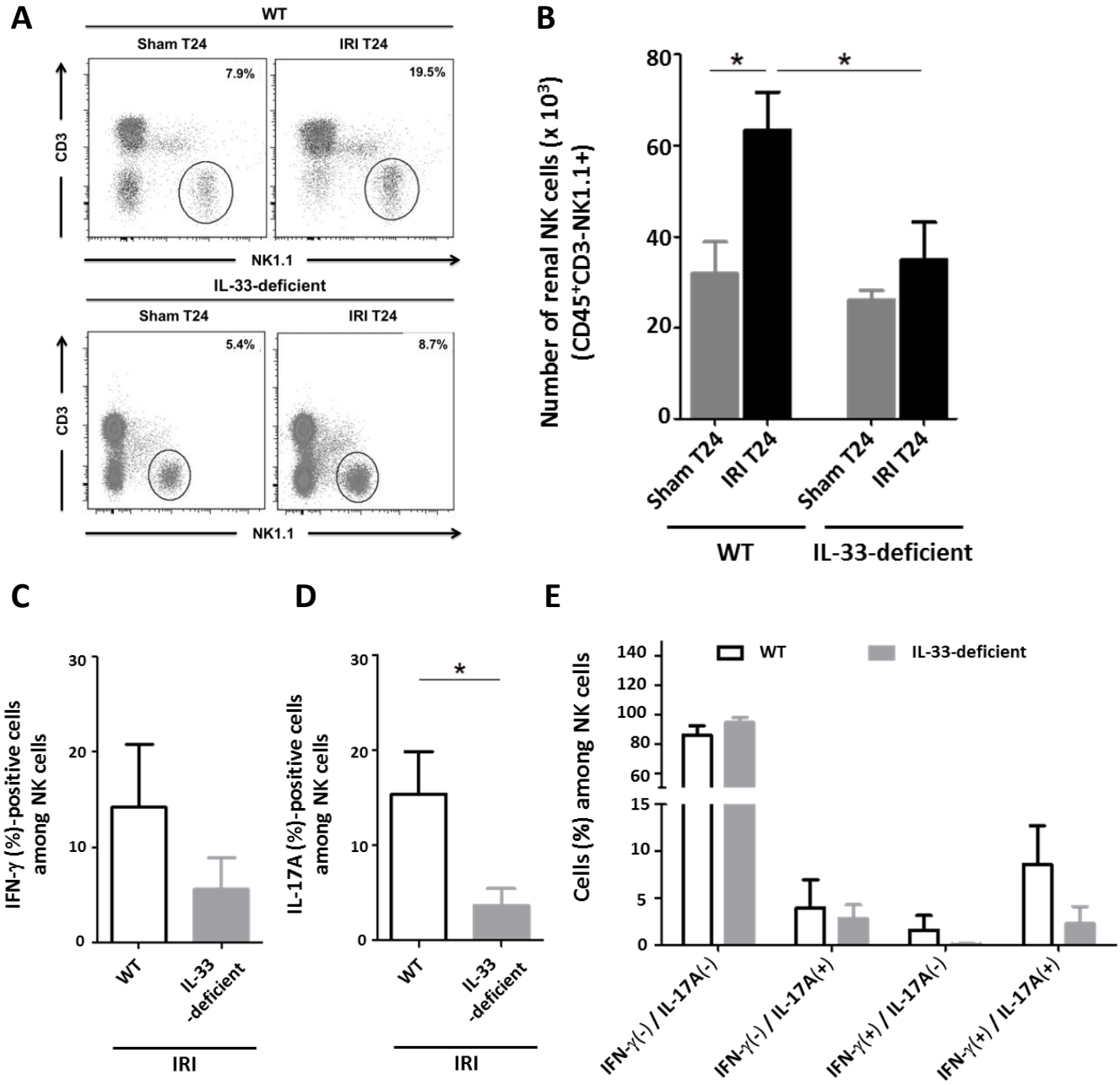
Supplemental Figure 8: Augmentation of surface CD69 expression on iNKT cells induced by ischemia-perperfusion is altered in the kidneys of IL-33-deficient mice relative to WT mice. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to 32 minutes of unilateral IRI after contralateral nephrectomy (Ctr). After 24 hours, kidneys were obtained and leukocytes freshly isolated to examine CD69 Mean Fluorescence Intensity (MFI) levels on iNKT (PBS57-loaded CD1d TT(+) CD3(+) cells after gating on CD45(+) cells. Data are expressed as means±SEM (3 animals per group). Two-tailed Mann-Whitney U test was used for two group comparisons. *P<0.05; **P<0.01; ***P<0.001.

Supplemental Figure 9



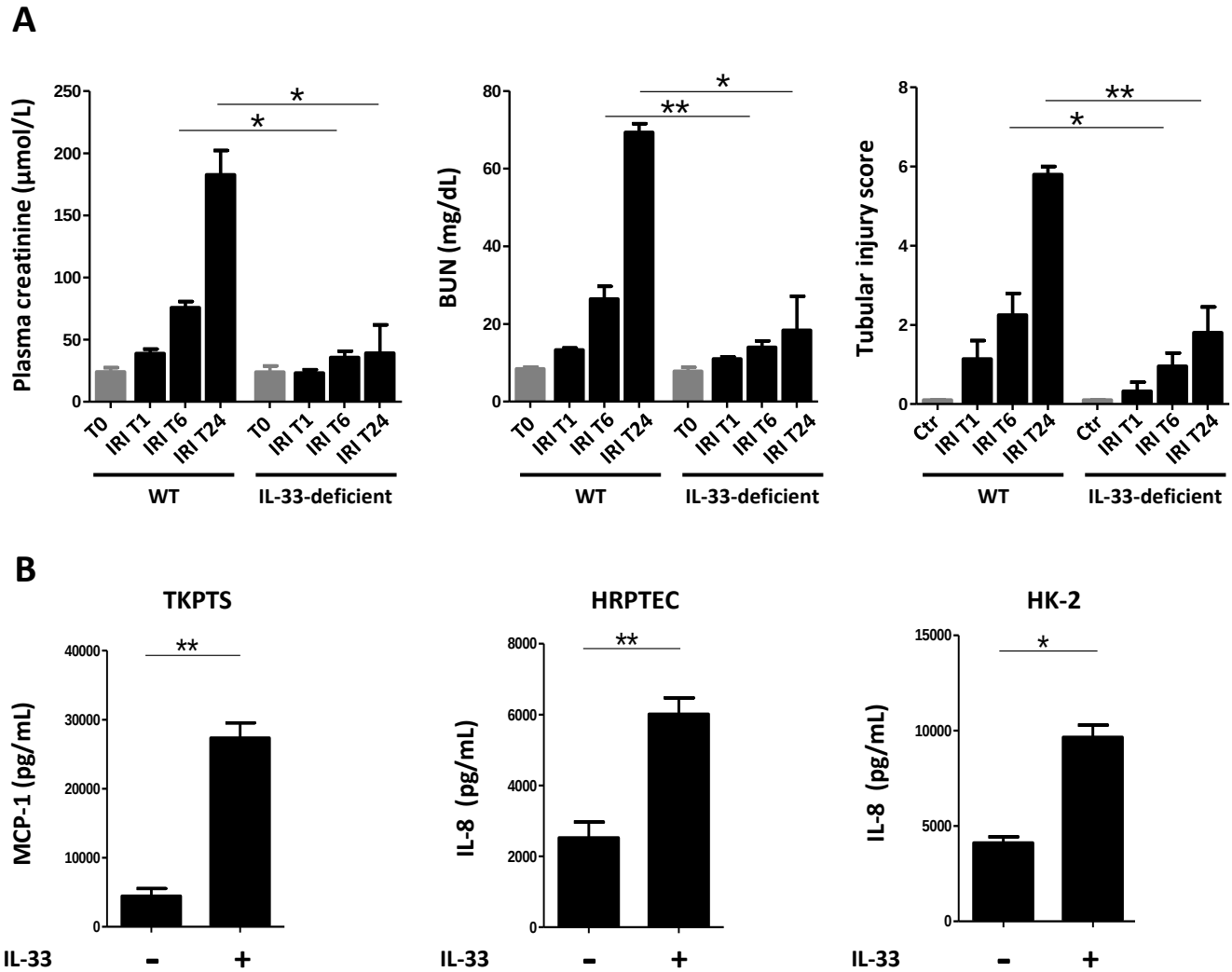
Supplemental Figure 9: Early increase in plasma levels of MIG, IP-10 and RANTES after IRI induction does not depend on IL-33. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to 32 minutes of unilateral ischemia (IRI) following contralateral nephrectomy. After 3, 6 or 24 hours (T3, T6, or T24) of reperfusion, peripheral blood was collected. Plasma levels (relative MFI) of MIG/CXCL9 (**A**), IP-10/CXCL10 (**B**) reperfusion in both WT and IL-33-deficient mice, compared to their basal levels in untreated (T0) mice. There was no significant difference in MIG, IP-10 and RANTES levels between WT and IL-33-deficient mice at all time points, which renders IL-33-induced enhancement of their production highly unlikely. Data are expressed as means±SEM (6 animals per group). Two-tailed Mann-Whitney U test was used for two group comparisons. *P<0.05; **P<0.01; ***P<0.001 *vs.* T0.

Supplemental Figure 10



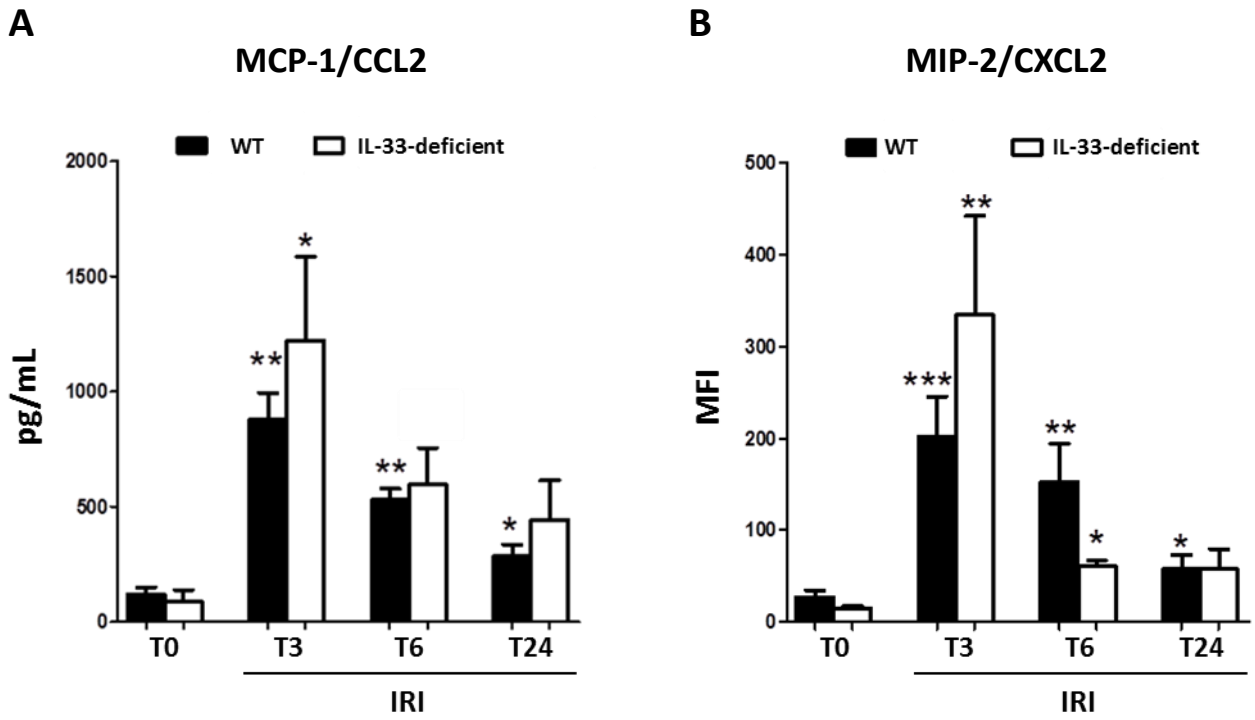
Supplemental Figure 10: NK cell recruitment and function are impaired following IRI in IL-33-deficient mice. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to sham surgery (Sham) or 32 minutes of unilateral IRI following contralateral nephrectomy (Ctr). After 24 hours of reperfusion, kidneys were obtained and leukocytes freshly isolated to examine NK cell recruitment and cytokine production by flow cytometry analysis. (**A**) Representative dot plots are shown for CD3(-) NK1.1(+) cells that defines NK cells after gating on live CD45(+) cells. Numbers indicate the percentages of NK cells (**B**) Absolute number of NK cells (5-8 animals for each group). Percentages of IFN-γ (**C**), IL-17A-expressing NK cells **(D)** and frequency of IFN-γ and IL-17A-expressing NK cells (**E**) (3 animals for each column). Data are expressed as means±SEM. Two-tailed Mann-Whitney U test was used for two group comparisons, and one-way ANOVA followed by Tukey post-test for three or more group comparisons. *P<0.05.

Supplemental Figure 11



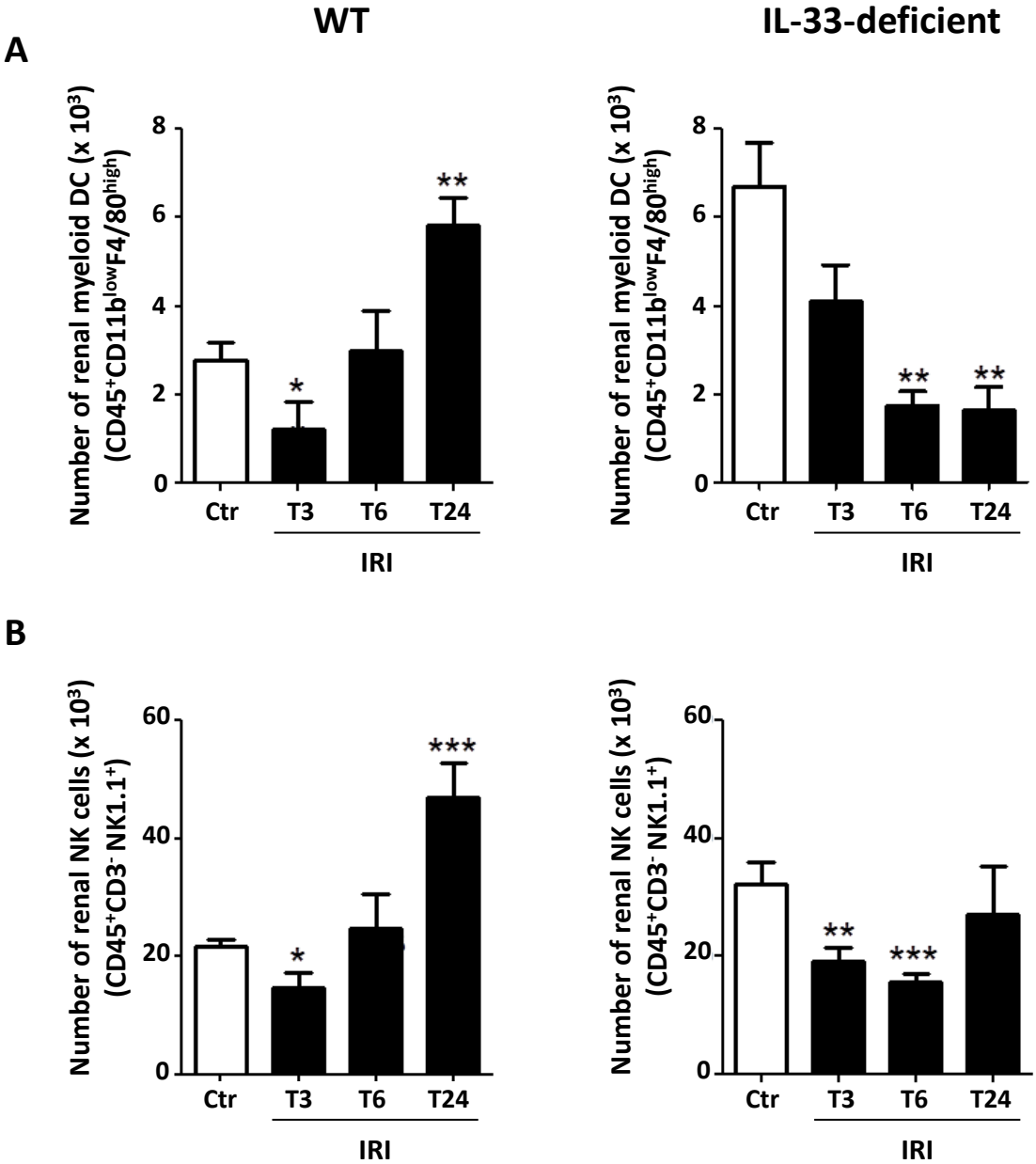
Supplemental Figure 11: IL-33 contributes to initial IRI-induced kidney lesions (A) and targets *in vitro* renal proximal tubule epithelial cells (B). (A) Wild-type (WT) mice and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to 32 min of unilateral ischemia after contralateral nephrectomy (Ctr). After 1, 6 or 24 hours (T1, T6, or T24) of reperfusion, blood was collected and kidneys were harvested to assess blood creatinine, urea nitrogen (BUN) and tissue damage scores. As soon as 6 hours after reperfusion, IL-33-deficient mice exhibited decreased blood creatinine (left panel), BUN levels (middle panel) and attenuated histological changes (right panel) when compared to WT mice (3-4 animals per group). **(B)** Proximal tubule epithelial (mouse TKPTS; human HK-2 and HRPTEC) cells were incubated (see Concise Methods section) with or without recombinant IL-33 (10-20 ng/mL). MCP-1 or IL-8 were quantified in supernatants by standard ELISA. (A-B) Two-tailed Mann-Whitney U test was used for two group comparisons. *P<0.05 and **P<0.01.

Supplemental Figure 12



Supplemental Figure 12: Early increase of plasma levels of MCP-1 and MIP-2 after IRI induction does not depend on IL-33. Wild-type (WT) and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to 32 minutes of unilateral ischemia (IRI) following contralateral nephrectomy. After 3, 6 or 24 hours (T3, T6, or T24) of reperfusion, peripheral blood was collected. Plasma levels of MCP-1/CCL2 (pg/mL) (**A**) and MIP-2/CXCL2 (relative MFI) (**B**) were significantly increased following IRI, as soon as 3 hours after reperfusion in both WT and IL-33-deficient mice, compared to their basal levels in untreated (T0) mice. There was no significant difference in MCP-1 and MIP-2 levels between WT and IL-33-deficient mice at all time points. Data are expressed as means±SEM (6 animals per group). Two-tailed Mann-Whitney U test was used for two group comparisons. For each WT or IL-33-deficient group of mice: *P<0.05 and **P<0.01 *vs.* T0.

Supplemental Figure 13



Supplemental Figure 13: Myeloid DC and NK cell infiltration induced by IRI require IL-33. Wild-type (WT) mice and IL-33$^{Gt/Gt}$ (IL-33-deficient) mice were subjected to 32 min of unilateral ischemia after contralateral nephrectomy (Ctr). After 3, 6 or 24 hours (T3, T6, or T24) of reperfusion, kidneys were obtained and leukocytes freshly isolated to examine the number of CD45(+) CD11b^{low} F4/80^{high} cells (myeloid DC) and CD45(+) CD3(-) NK1.1(+) cells (NK cells). Absolute number (per kidney) of CD45(+) CD11b^{low} F4/80^{high} cells (**A**) and CD45(+) CD3(-) NK1.1(+) cells (**B**). WT mice (left panels). IL-33-deficient mice (right panels). Data are expressed as the means±SEM of 6 animals per group for each time. Two-tailed Mann-Whitney U test was used to compare IRI groups to the Ctr group. *P<0.05; **P<0.01; ***P<0.001.