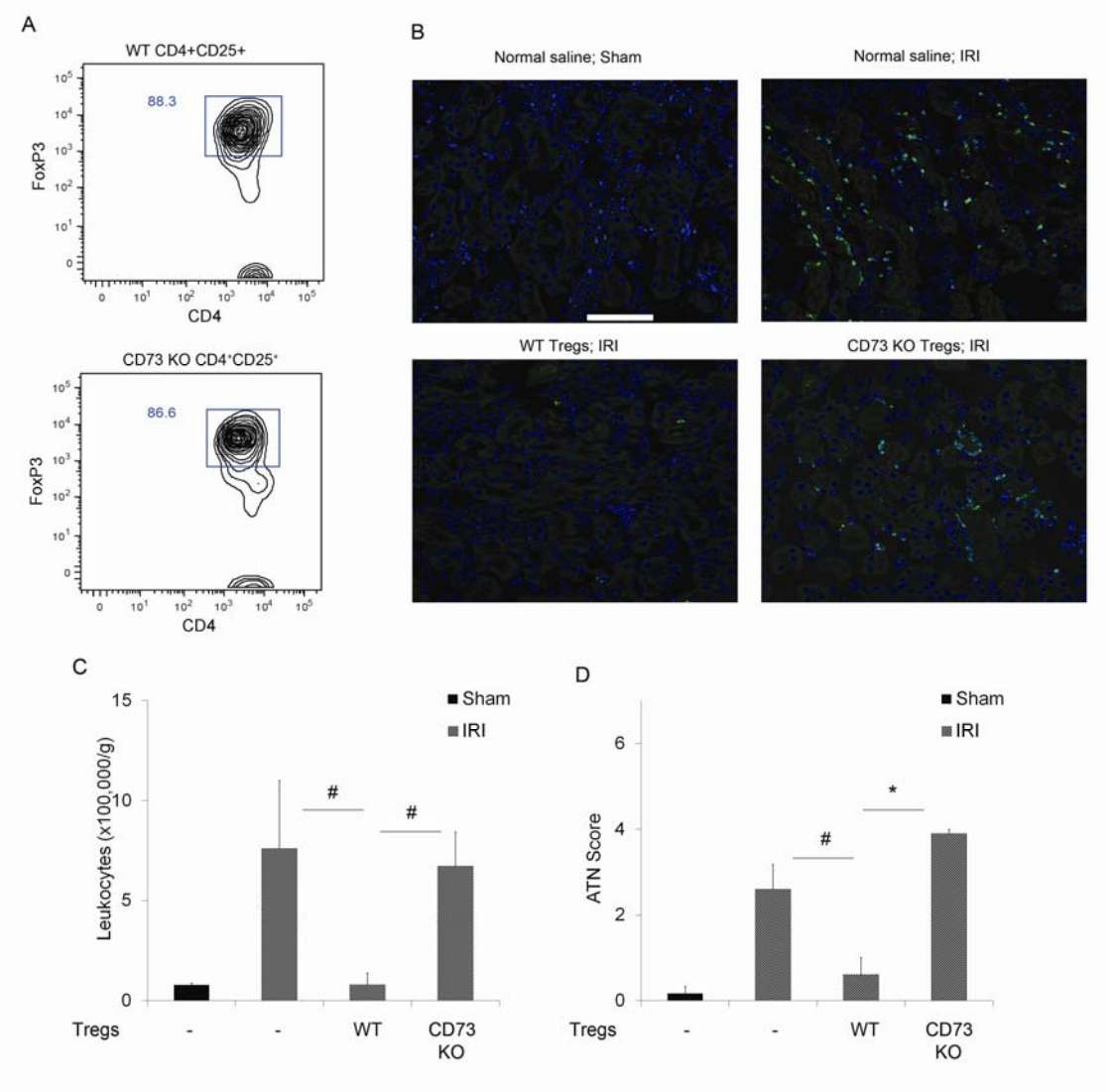
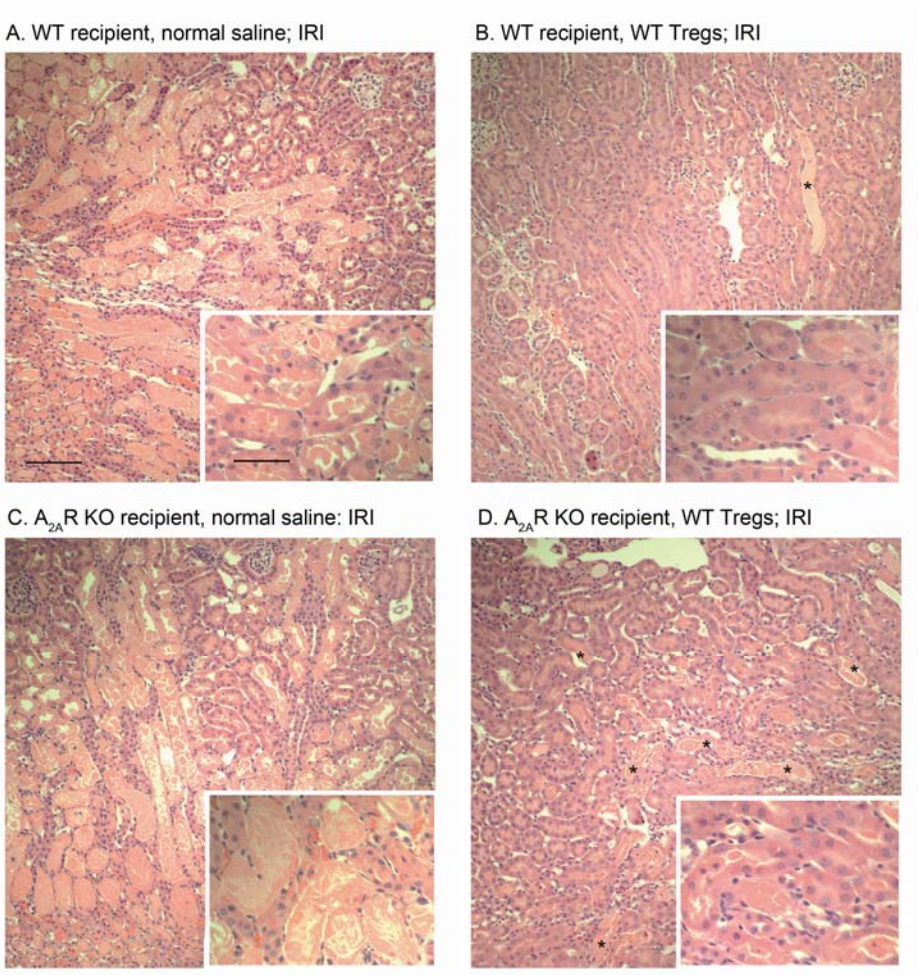
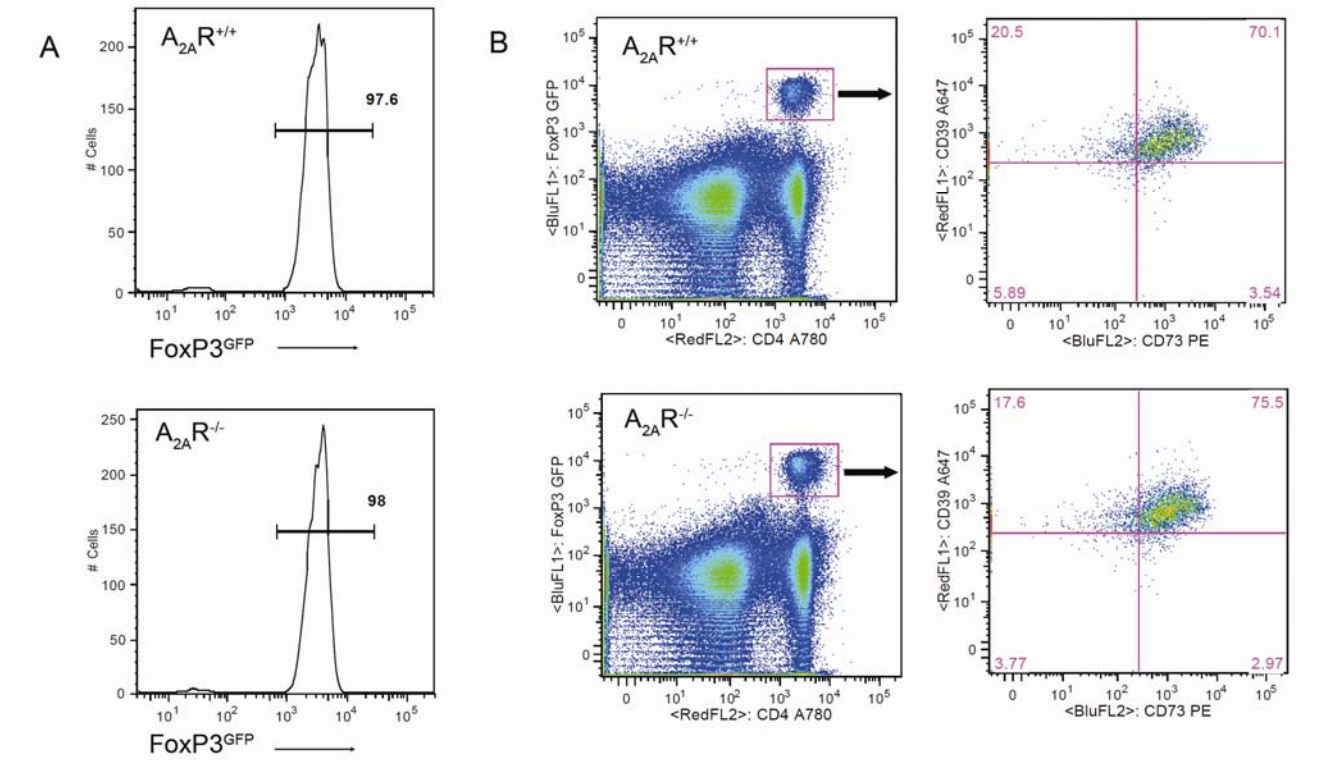


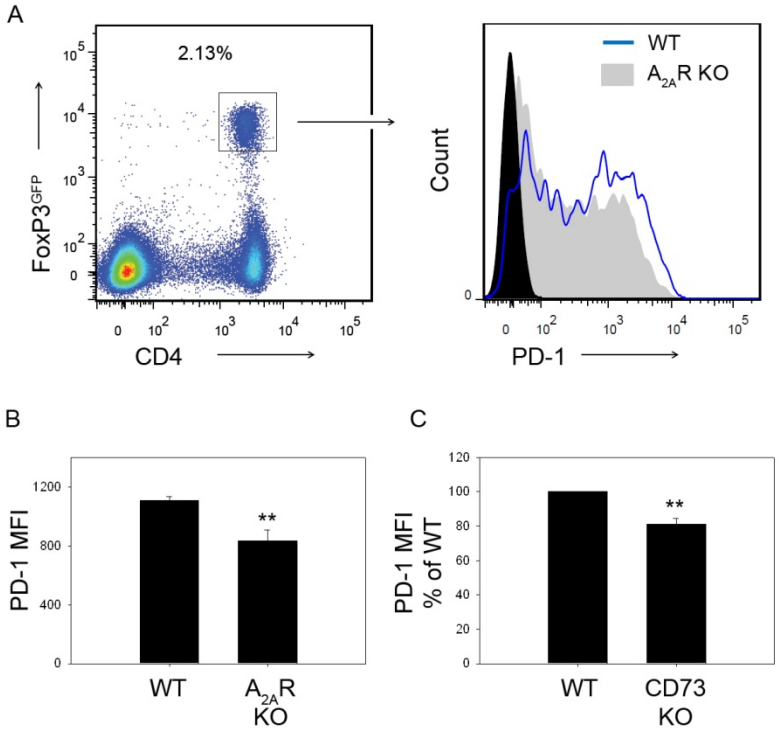
Supplemental Figure 1. CD73 KO Tregs express FoxP3 but cannot protect the kidney from IRI-induced inflammation and ATN. $CD4^+CD25^+$ Tregs isolated from WT and CD73 KO spleen were analyzed for expression of FoxP3 protein by flow cytometry (A). Flow cytometry results are typical of at least 3 separate experiments. Naïve C57Bl/6 (WT) mice were injected (via tail vein) with normal saline or freshly-isolated WT or CD73 KO Tregs (100,000 each) 18 hr prior to bilateral kidney ischemia or sham surgery. At 18 hr of reperfusion, frozen kidney sections were prepared and analyzed for the infiltration of innate inflammatory leukocytes with the antibody 7/4 (B). Bright green staining indicates 7/4 positive leukocytes in the outer medulla region of the kidney. Micrographs are representative of at least 3 separate mice per group from 2 independent experiments. Scale bar = 50 microns. At 48 hr of reperfusion renal neutrophil accumulation was measured by flow cytometry (C) and ATN was assessed in blinded fashion (D). Error bars in graphs represent the SEM, n=5 per group, pooled from 2 independent experiments. $^*P<0.001$, $^{\#}P<0.05$.



Supplemental Figure 2. Recipient A_{2A}R expression is required for Tregs to fully protect the kidney from IRI. Isolated WT Tregs (100,000) or normal saline (-) was injected (via tail vein) to naïve C57Bl/6 (WT) or A_{2A}R KO mice 18 hr prior to bilateral renal ischemia or sham surgery. After 18 hr of reperfusion, kidney sections stained with H&E to assess the extent of tubular necrosis. In low power image, scale bar = 100 microns, in the inset scale bar = 50 microns. * - denotes tubules with evidence of ATN in panels B and D. Micrographs are representative of at least 4 separate mice per group from 2 independent experiments.



Supplemental Figure 3. Phenotype of $A_{2A}R^{-/-}FoxP3^{GFP}$ Tregs.
Representative purity of FACS sorted $A_{2A}R^{+/+}FoxP3^{GFP}$ and $A_{2A}R^{-/-}FoxP3^{GFP}$ Tregs used for adoptive transfer experiments (A). Age matched $A_{2A}R^{+/+}FoxP3^{GFP}$ and $A_{2A}R^{-/-}FoxP3^{GFP}$ mice splenocytes were isolated and $CD4^{+}FoxP3^{+}$ Treg were analyzed for the surface expression of CD39 and CD73 by flow cytometry, results are typical of 3 separate isolations (B).



Supplemental Figure 4. Lack of CD73 or the $A_{2A}R$ results in lower surface PD-1 expression on Tregs. $CD4^+FoxP3^+$ Tregs from age-matched $FoxP3^{GFP}$ WT or $FoxP3^{GFP}$ $A_{2A}R$ KO spleen were analyzed by flow cytometry to compare the baseline expression level (mean fluorescence intensity (MFI)) of surface PD-1 Brilliant Violet 421 on Tregs, n=5 (A,B), filled black histogram in A is isotype control fluorescence. Similarly, freshly isolated $CD4^+CD25^+$ Tregs from WT and CD73 KO mice were analyzed by flow cytometry to determine the expression level of surface PD-1, n=3 (C). Data are expressed as the mean + SEM, **P<0.01.

Supplemental Table. Real-time PCR primers

Gene	5’ to 3’ sequence
RPS29 f	GATCCGCAAATACGGGCTGAACAT
RPS29 r	GACTAGCATGATCGGTTCCACTTG
A1R f	CTCTGAAGAGATGCCATGGAACAG
A1R r	CTCAGAGAACAGCCAGGAATGATG
A2AR f	ACCTGCAGAACGTCACCAACTTCT
A2AR r	AGCCAAGAGGCTGAAGATGGAACT
A2BR f	CTGGGACACGAGCGAGAG
A2BR r	GCTGGTGGCACTGTCTTTAC
A3R f	GTGAGTTCTCTGGACTGTTGTGAC
A3R r	GATGTAGGTGATGTTCAGCCAGTC
PD-1 f	GAGATCTGAGTACAGCCTGCTTTG
PD-1 r	CCAGATCTTGGGATTGGTTCAGTC

f - forward primer, r – reverse primer