Supplementary Information

Equilibrative nucleoside transporter ENT1 regulates post-ischemic blood-flow during acute kidney injury in mice

by

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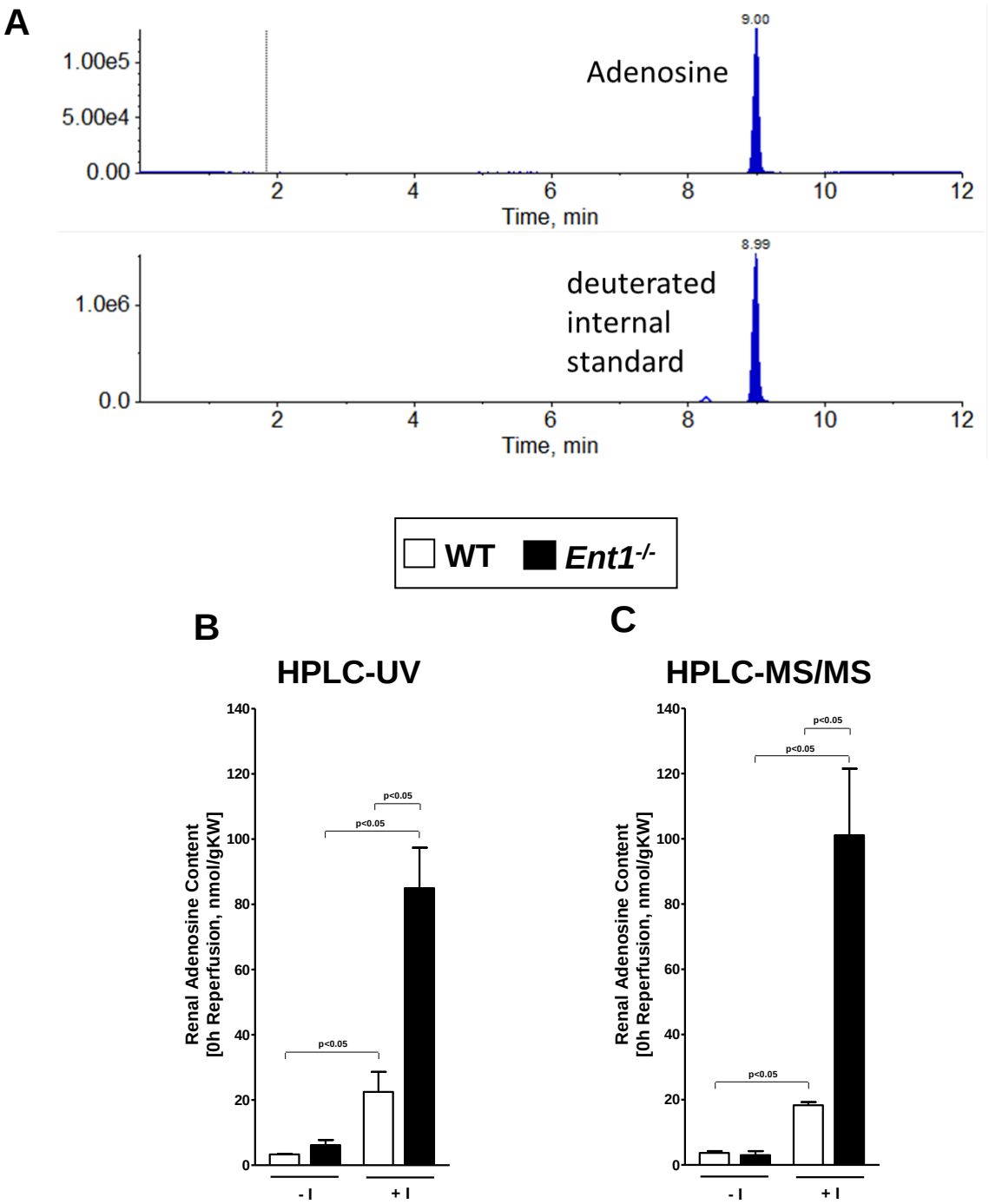
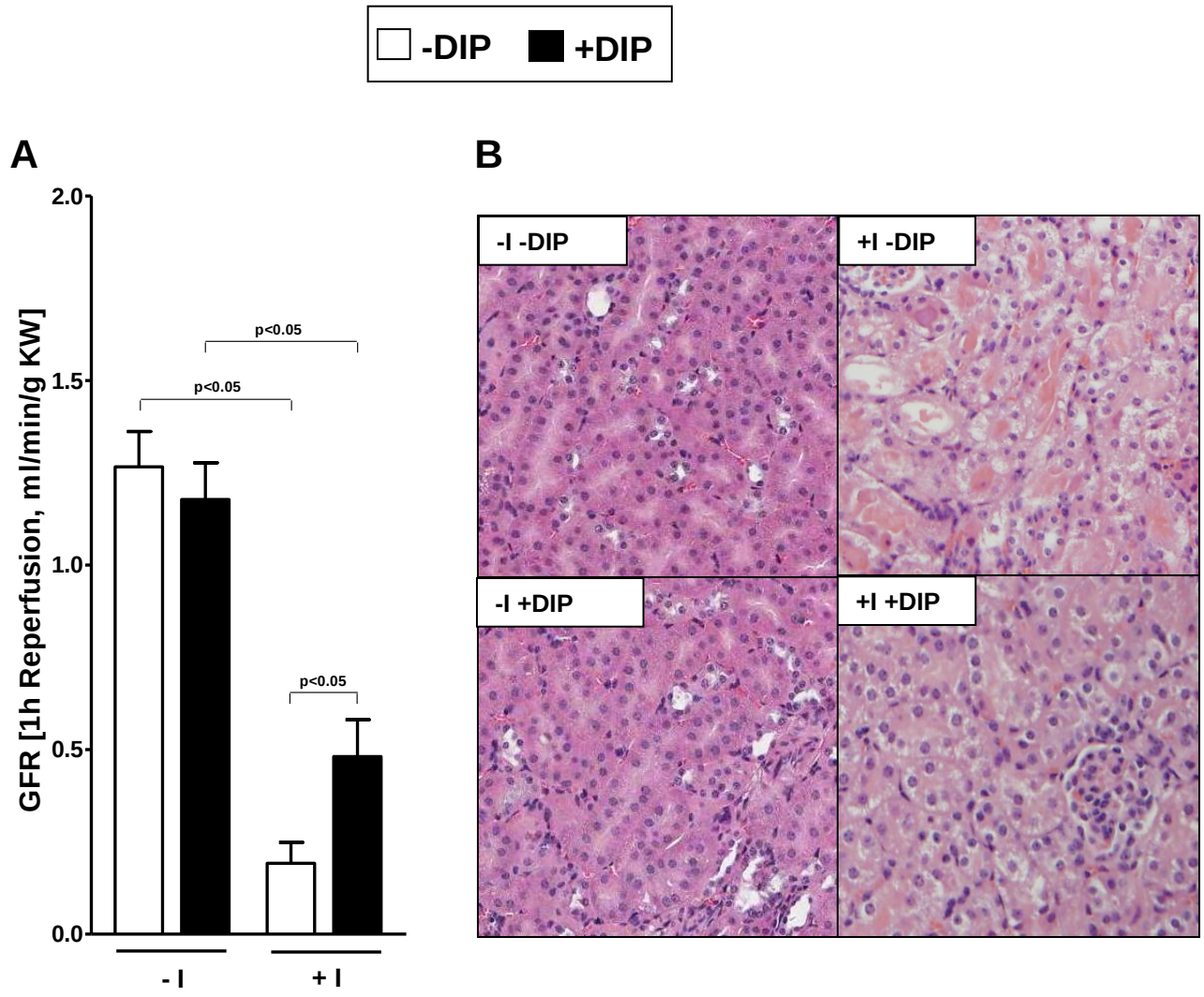
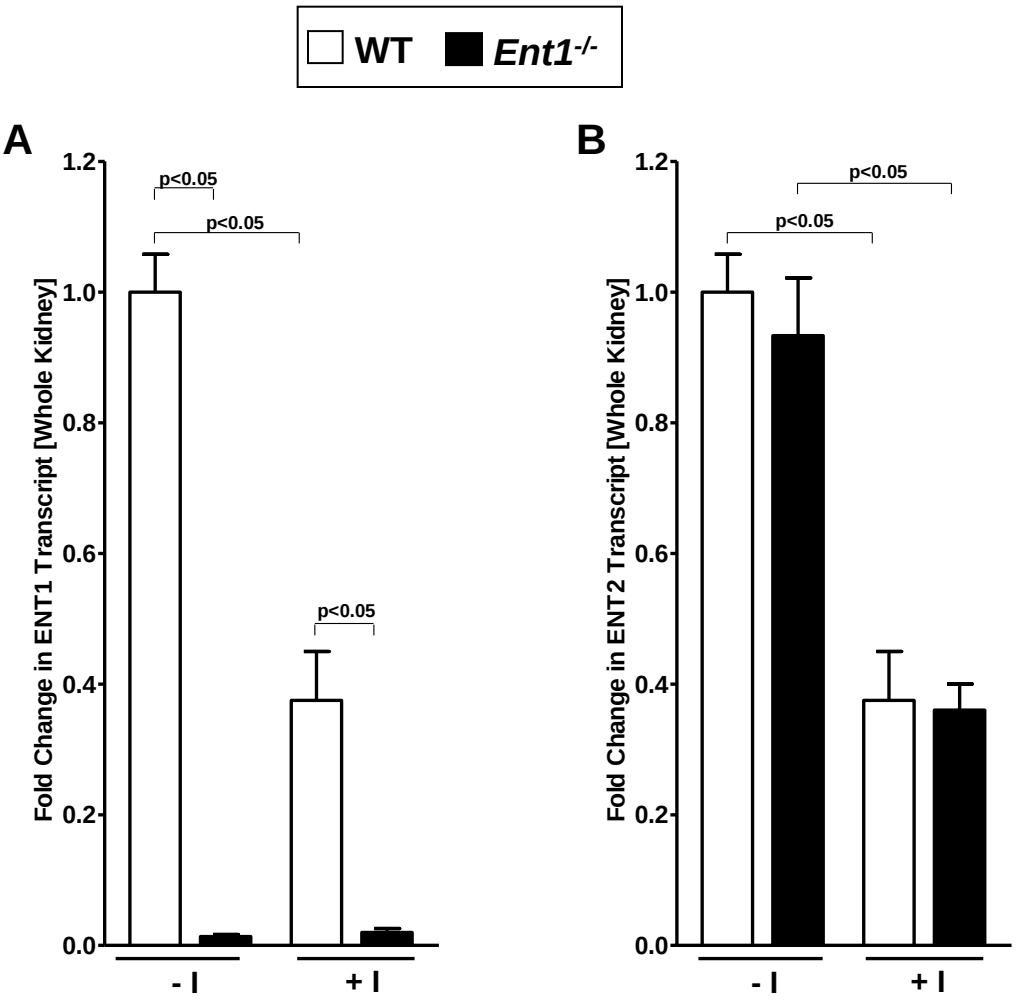


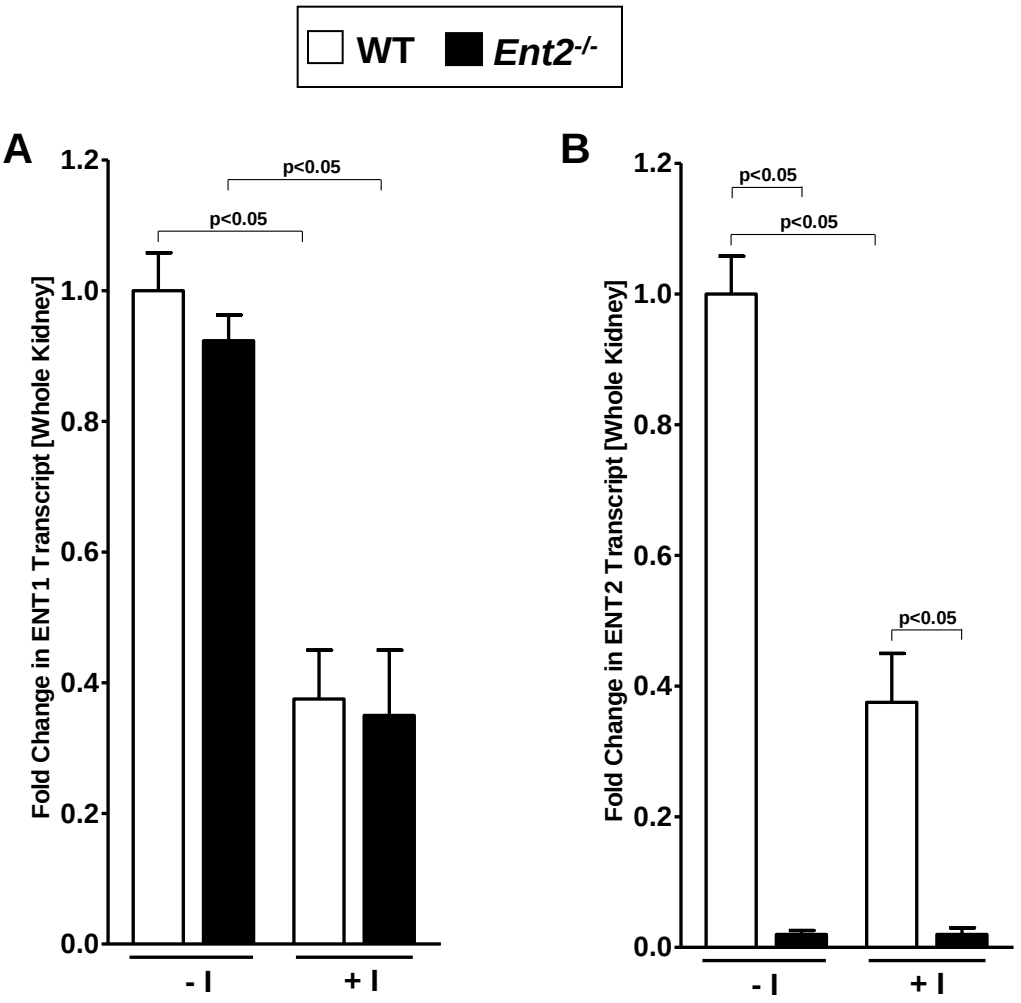
Supplementary Figure 1. Adenosine HPLC-MS/MS analysis. (**A**) An Agilent 1100 LC (Agilent Technologies) coupled to an API4000 tandem mass spectrometer with a Turbo V ion source (AB Sciex) was used for the measurement of adenosine. The graphs show representative ion chromatograms of adenosine from an extracted mouse kidney.
The adenosine signal is shown in the upper chromatogram and the deuterated adenosine internal standard in the lower chromatogram.
(**B**) Cross validation of adenosine measurements by using the same tissue samples with HPLC-UV or **(C)** with a selective highly sensitive HPLC tandem mass spectrometry assay. Renal adenosine content in *Ent1*$^{-/-}$ mice and wild-type control mice with (+I) and without ischemia (-I), (n=3-4 animals per condition, mean and s.d.).



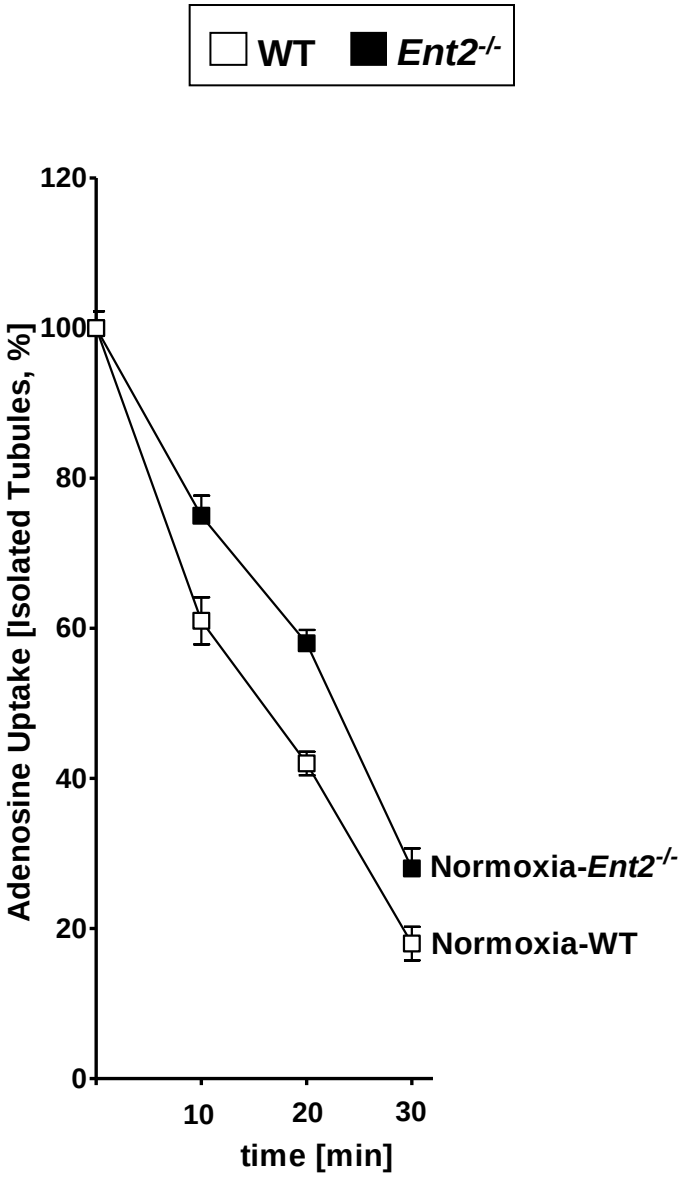
Supplementary Figure 2. Bilateral renal ischemia (20 minutes) in WT mice with dipyridamole (+DIP) and without dipyridamole (-DIP) treatment. **(A)** Glomerular filtration rate (GFR) one hour following renal ischemia. (**B**) histology (H&E, 400-fold magnification). Data are representative of four to six mice per condition; mean and s.d. or are from one experiment representative of three (images).



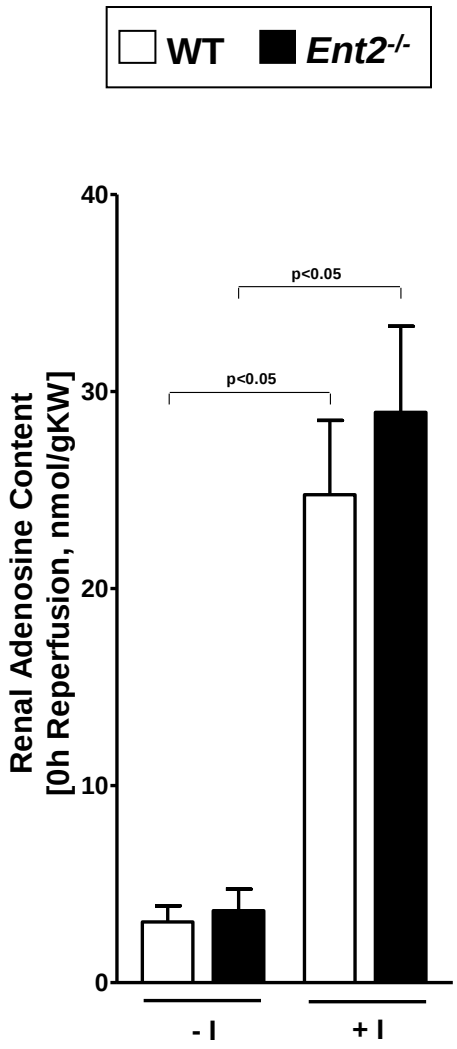
Supplementary Figure 3. Ent1 and Ent2 regulation in *Ent1*$^{-/-}$ mice and wildtype controls (WT). Mice were subjected to 30 minutes of ischemia prior to 2 hours of reperfusion.
(**A**) Renal Ent1 and (**B**) Ent2 transcript were measured by RT-PCR
(n=4-6 animals per condition, mean and s.d.).



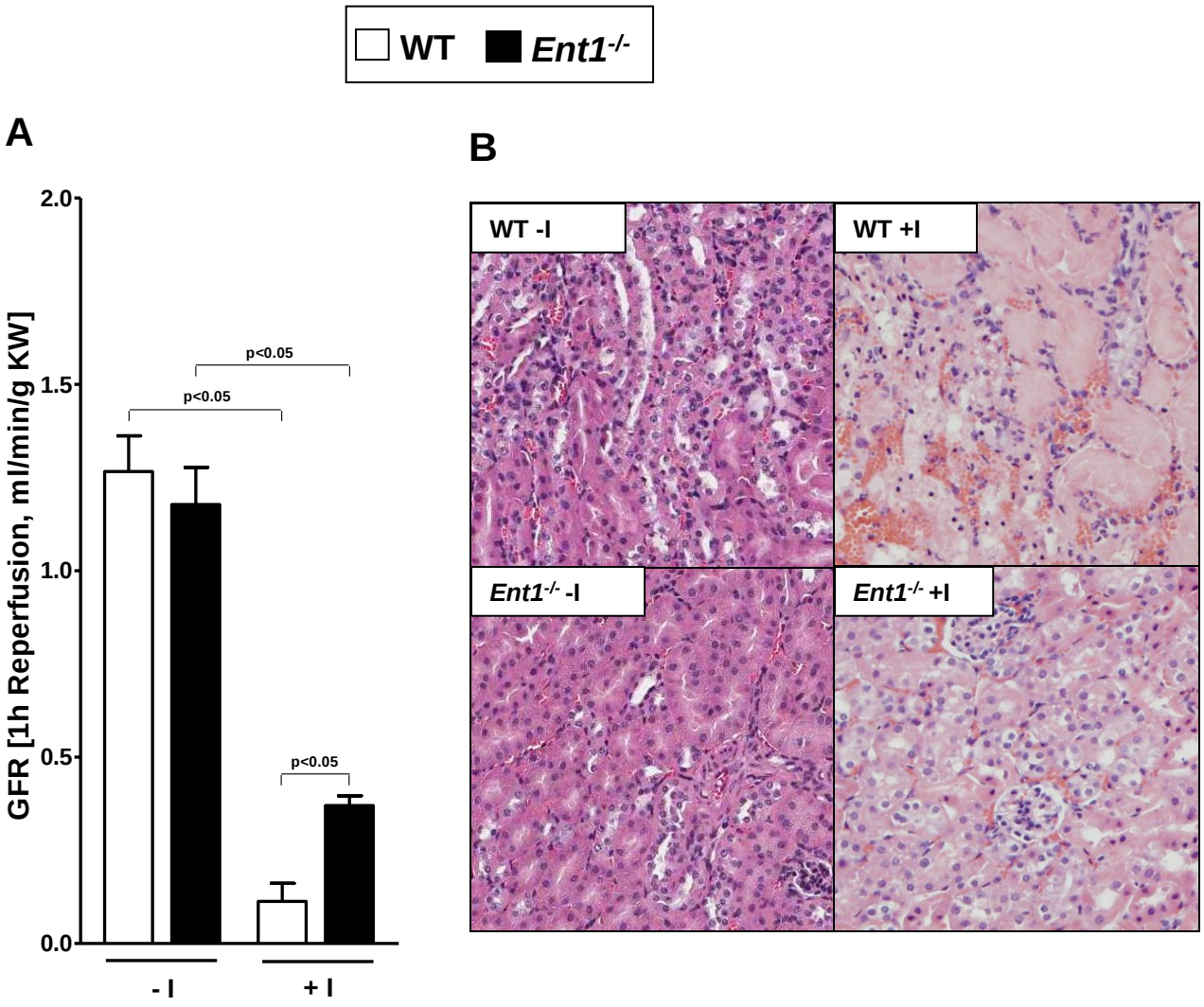
Supplementary Figure 4. Ent1 and Ent2 regulation in *Ent2*$^{-/-}$ mice and wildtype controls (WT). Mice were subjected to 30 minutes of ischemia prior to 2 hours of reperfusion. (**A**) Renal Ent1 and (**B**) Ent2 transcript were measured by RT-PCR (n=4-6 animals per condition, mean and s.d.).



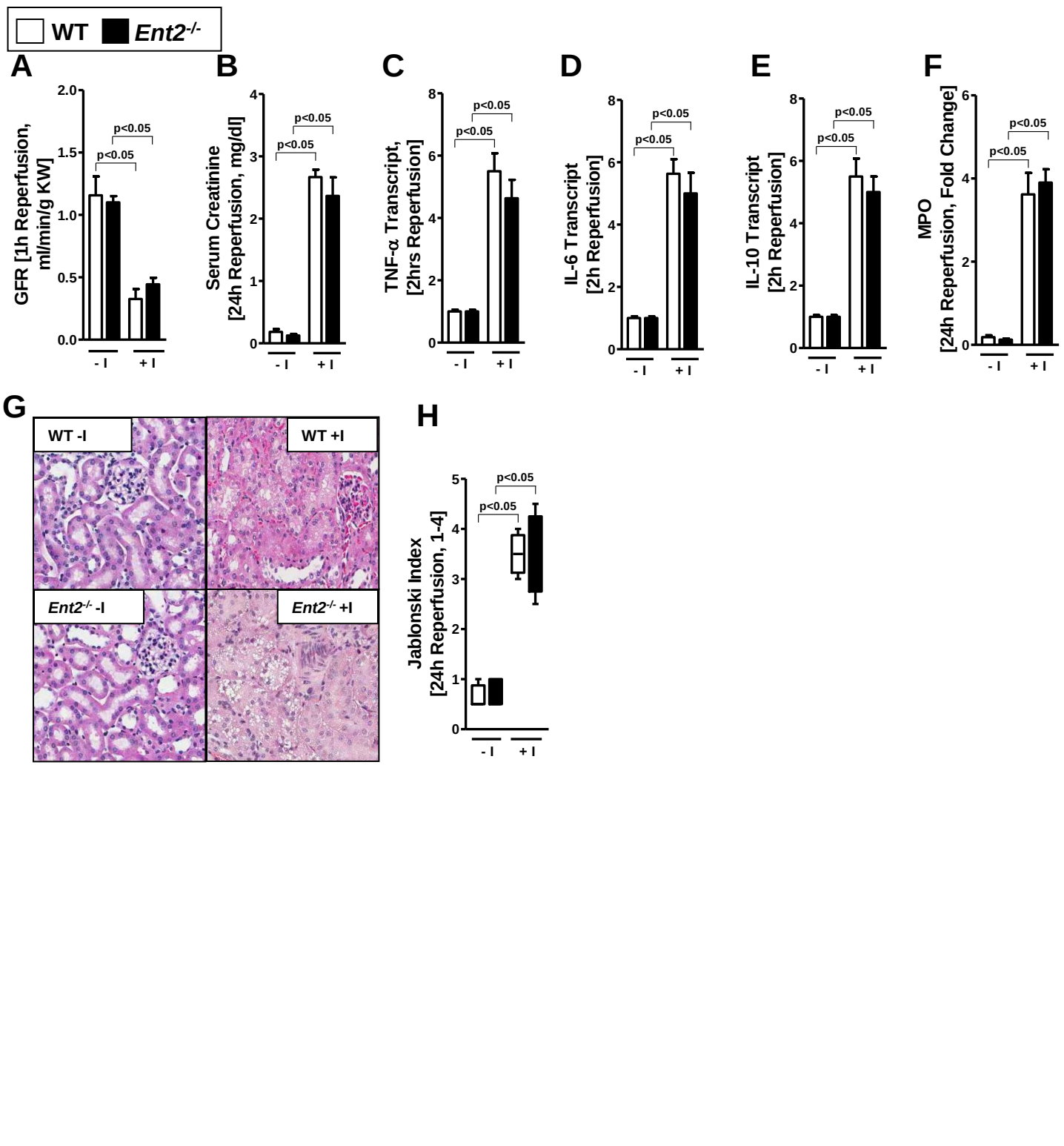
Supplementary Figure 5. Extracellular adenosine transport by isolated tubular cells from *Ent2*$^{-/-}$ and control wild-type mice (WT). Exogenous adenosine (5 µmol/l) was added to these cells and adenosine concentrations were measured at the indicated time points.
(n=4-6 animals per condition, mean and s.d.).



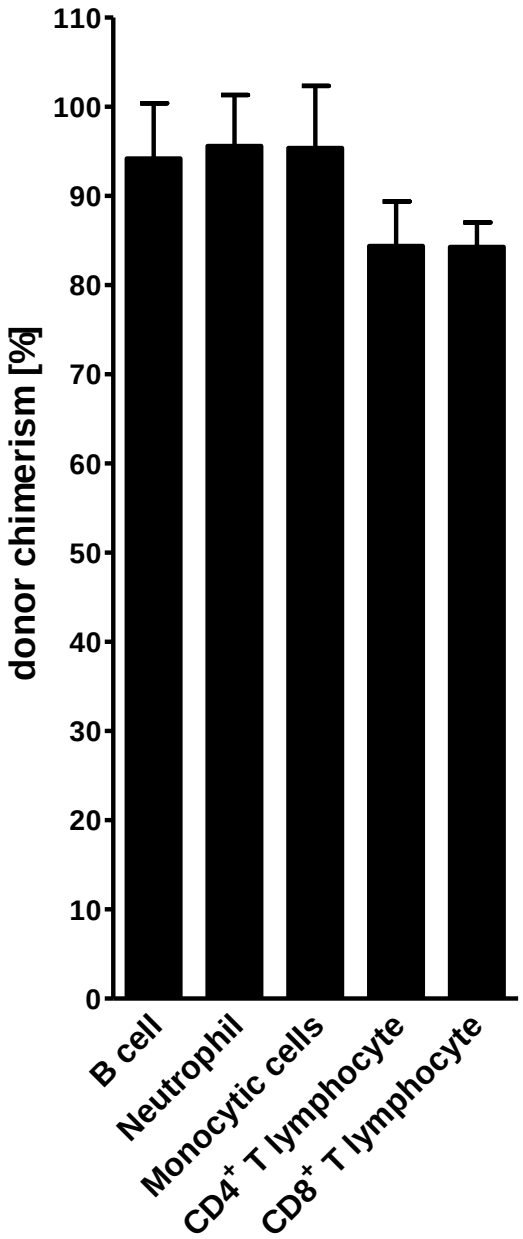
Supplementary Figure 6. Renal adenosine content in *Ent2*$^{-/-}$ mice and wild-type control mice with (+I) and without ischemia (-I), (n=4-6 animals per condition, mean and s.d.).



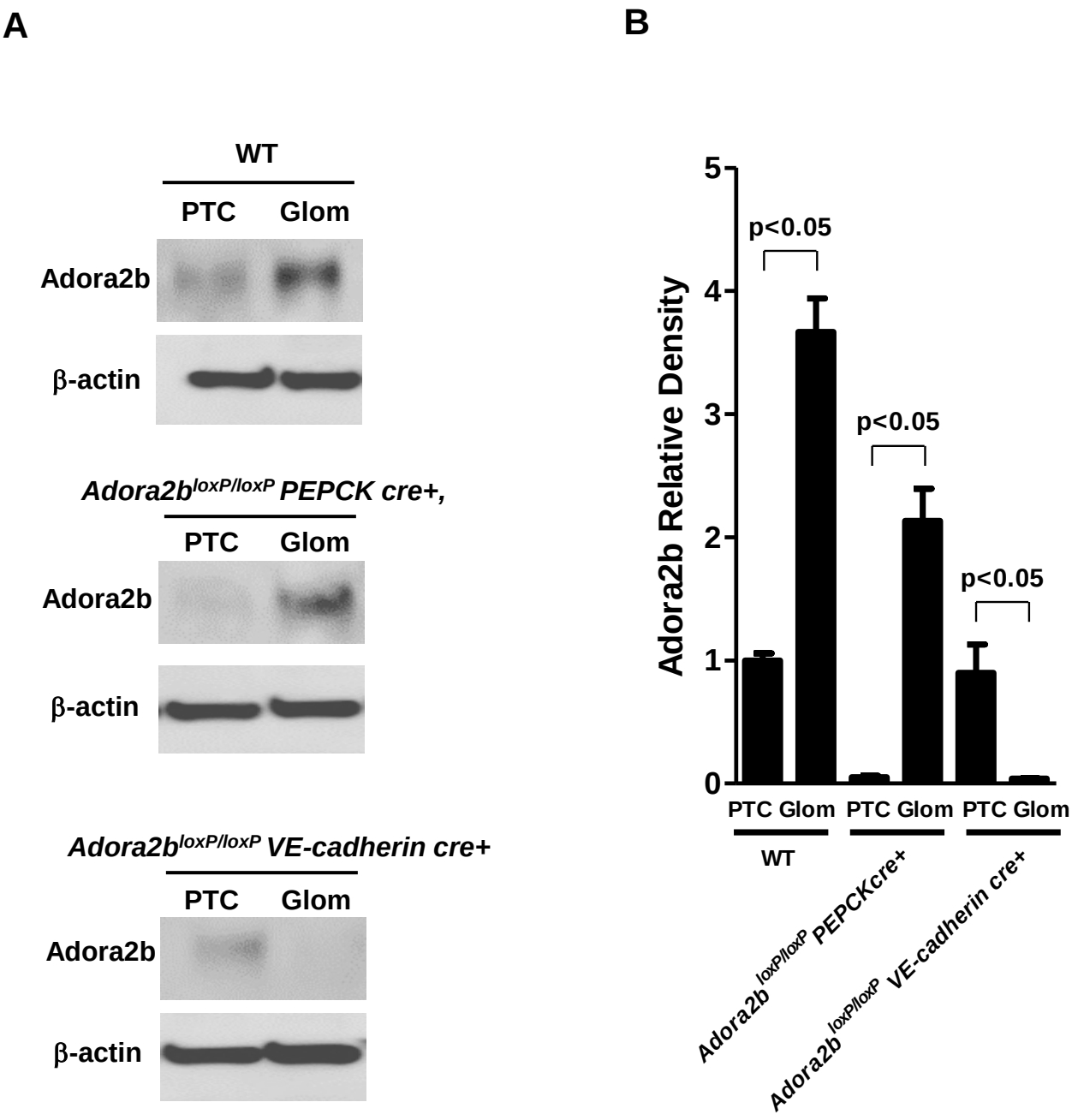
Supplementary Figure 7. Bilateral renal ischemia (20 minutes) in *Ent1*$^{-/-}$ mice and wild-type control mice. **(A)** Glomerular filtration rate (GFR) one hour following renal ischemia, (**B**) histology (H&E, 400-fold magnification). Data are representative of four to six mice per condition; mean and s.d. or are from one experiment representative of three (images).



Supplementary Figure 8. Renal ischemia in *Ent2*$^{-/-}$ mice and wild-type control mice. (**A**) Glomerular filtration rate (GFR) one hour following renal ischemia, (**B**) Serum creatinine 24 hours following renal ischemia, (**C**) renal TNF-alpha transcript, (**D**) renal IL-6 transcript, (**E**) renal Il-10 transcript 2 hours following renal ischemia, (**F)** renal myeloperoxidase content 24 hours following renal ischemia, (**G**) histology (H&E, 400-fold magnification), (**H**) Quantification of renal injury due to ischemia (Jablonski Index) 24 hours following renal ischemia. Data are representative of four to six mice per condition; mean and s.d. or are from one experiment representative of three (images).



Supplementary Figure 9. Determination of repopulation efficiency of bone marrow-derived cells in chimeric mice. Irradiated mice lacking the CD45.1 epitope received bone marrow cells from CD45.1-positive mice (B6.SJL-Ptprca Pep3b/BoyJ). Eight weeks after transplantation the percentage of CD45.1 positive cells in the different blood cell populations was examined by immunofluorescent cell analysis. Data are presented as mean ± SD (n = 3 to 5).



Supplementary Figure 10. (**A**) Adora2b protein expression in isolated proximale tubules (PTC) and glomeruli (Glom) of *Adora2b$^{loxP/loxP}$ PEPCK cre+, Adora2b$^{loxP/loxP}$ VE-cadherin cre+ and wild type control* mice. Adora2b protein levels assessed by Western blotting (β-actin to control for loading conditions; one representative blot of three is shown). (**B**) Quantification by densitometry (n=3).

Mouse primer sequences	Forward Primer	Reverse Primer
Ent1	CTTGGGATTCAGGGTCAGAA	ATCAGGTCACACGACACCAA
Ent2	CATGGAAACTGAGGGGAAGA	GTTCCAAAGGCCTCACAGAG
TNF-α	ACTCCAGGCGGTGCCTATGT	TCCAGCTGCTCCTCCACTTG
IL-6	ACCGCTATGAAGTTCCTCTC	CTCTCCGGACTTGTGAAGTA
IL-10	CTTACTGACTGGCATGAGGA	GCATTAAGGAGTCGGTTAGC
Beta Actin	CTAGGCACCAGGGTGTGAT	TGCCAGATCTTCTCCATGTC

Supplementary Table 1. Murine RT PCR primer sets.

Human primer sequences	Forward Primer	Reverse Primer
ENT1	CTTGGGCTTGGAGAACAC	AAGGCACCTGGTTTCTGTC
ENT2	CTTCCATACCCACTCTCTCACC	GAGAGAGAGGGGATTGGGTC
Beta Actin	GGAGAAAATCTGGCACCACA	AGAGGCGTACAGGGATAGCA

Supplementary Table 2. Human RT PCR primer sets.