

UNC5B receptor deletion exacerbates tissue injury in response to acute kidney injury

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Table S1. Apoptotic pathways gene expression in WT and UNC5B knockout kidney epithelial cells.

Gene	Gene expression in fold over WT TKPTS control			
	WT TKPTS		UNC5B KO TKPTS	
	Control	10 μ M CIS	Control	10 μ M CIS
Apaf1	1	2.6	0.8	6.1
Atm	1	0.9	0.9	1.9
Bad	1	0.9	0.7	2.1
Bag1	1	0.6	0.4	1.0
Bag3	1	1.7	1.0	2.6
Bag4	1	1.3	0.8	1.8
Bak1	1	1.3	0.9	1.7
Bax	1	1.9	0.8	3.6
Bbc3	1	3.4	1.5	5.5
Bcl10	1	1.2	0.8	1.4
Bcl2	1	1.2	1.4	1.3
Bcl2a1	1	4.9	0.2	0.8
Bcl2l1	1	1.9	1.8	3.7
Bcl2l11	1	0.4	0.7	1.8
Bcl2l2	1	1.7	1.1	3.3
Bfar	1	1.9	1.0	3.1
Blk	1	2.4	1.4	5.5
Naip1	1	0.7	0.2	1.4
Birc2	1	1.7	1.0	2.6
Birc3	1	1.7	1.0	2.6
Xiap	1	1.7	1.0	2.6
Birc5	1	1.4	0.7	3.1
Birc6	1	1.4	1.2	2.2
Bnip3	1	1.3	0.2	0.7
Bre	1	0.3	0.6	0.4
Bok	1	0.9	1.0	2.1
Casp1	1	1.3	0.0	0.0
Casp4	1	2.0	0.0	0.1
Casp2	1	0.8	0.7	1.1
Casp3	1	2.3	0.9	2.7
Casp6	1	2.4	0.5	3.9
Casp7	1	1.0	0.4	1.3
Casp8	1	1.2	0.6	2.2
Casp8ap2	1	0.8	1.1	1.9
Casp9	1	1.4	0.8	2.1
Cflar	1	2.4	1.3	3.0
Chek1	1	0.7	0.7	0.7
Chek2	1	1.3	1.1	3.1
Cidea	1	16.7	1.1	13.8
Cideb	1	2.6	0.9	6.5
Cradd	1	1.0	0.9	1.4
Dapk1	1	1.1	0.3	1.0
Dapk2	1	1.1	0.4	1.1
Dffa	1	0.9	1.0	1.3
Dffb	1	7.2	1.1	13.0
Fadd	1	1.2	0.8	1.3
Gadd45a	1	0.6	1.4	0.5
Hrk	1	1.6	1.0	2.9
Ltbr	1	2.1	1.7	3.2
Mcl1	1	2.2	1.4	2.8
Myd88	1	2.0	0.8	3.6
Ripk1	1	2.8	1.6	5.8
Ripk2	1	0.9	0.3	0.5
Rpa3	1	1.3	1.1	1.6
Tank	1	0.7	0.8	0.9
Tnf	1	2.6	0.0	0.2
Tnfrsf10b	1	6.3	1.5	11.3
Tnfrsf11a	1	3.0	1.3	9.5
Tnfrsf11b	1	1.7	1.0	2.6
Tnfrsf14	1	6.1	0.1	7.6
Tnfrsf17	1	5.6	1.0	5.2
Tnfrsf1a	1	1.9	1.2	2.7
Tnfrsf21	1	2.3	1.7	3.6
Tnfrsf4	1	0.6	1.1	3.7
Cd40	1	3.8	0.0	1.0
Tnfrsf9	1	4.4	0.1	4.5
Tnfsf10	1	0.6	0.0	0.4
Tnfsf11	1	1.8	0.7	7.3
Tnfsf12	1	3.9	0.5	7.5
Tnfsf13b	1	2.5	3.2	5.9
Tnfsf14	1	2.4	1.1	12.0
Tnfsf15	1	1.7	0.2	1.0
Tnfsf18	1	4.9	1.9	10.8
Tnfsf4	1	63.7	1.3	160.7
Cd40lg	1	2.1	1.1	11.2
Fasf	1	2.2	0.8	10.6
Tnfsf8	1	2.0	2.3	7.8
Tnfsf9	1	6.8	1.7	13.2
Trp53	1	1.5	1.1	2.3
Trp73	1	29.7	3.3	29.7
Traf1	1	1.9	0.5	1.4
Traf2	1	1.1	1.3	3.7
Traf3	1	1.3	1.2	2.1
Traf4	1	2.3	1.3	3.6
Traf5	1	0.7	2.3	2.8
Traf6	1	1.4	1.7	3.0
Trip	1	1.4	1.0	2.2
Diablo	1	1.4	1.5	2.5

Figure S1.

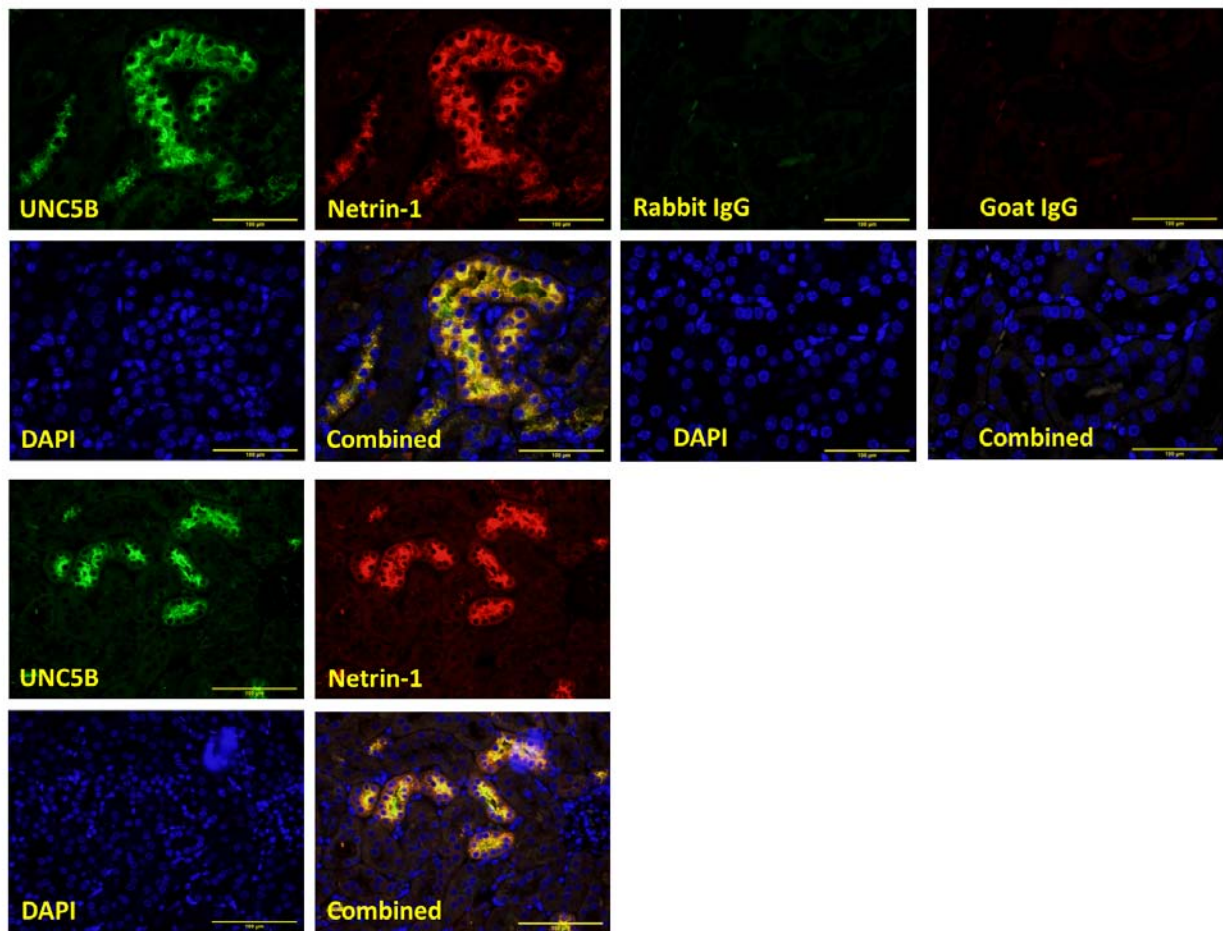


Figure S1. Colocalization of netrin-1 and UNC5B receptor in WT kidney that was subjected to 26 mins of ischemia followed by 24hr of reperfusion. Green indicate UNC5B expression, red indicate netrin-1 expression, blue indicate nuclear DNA stain and yellow indicate netrin-1 and UNC5B are colocalized. Isotype matched normal rabbit IgG (for UNC5B) and goat IgG (for netrin-1) was used as a primary antibody for staining control which does not show any staining. Scale bar: 100 μ M.

Figure S2.

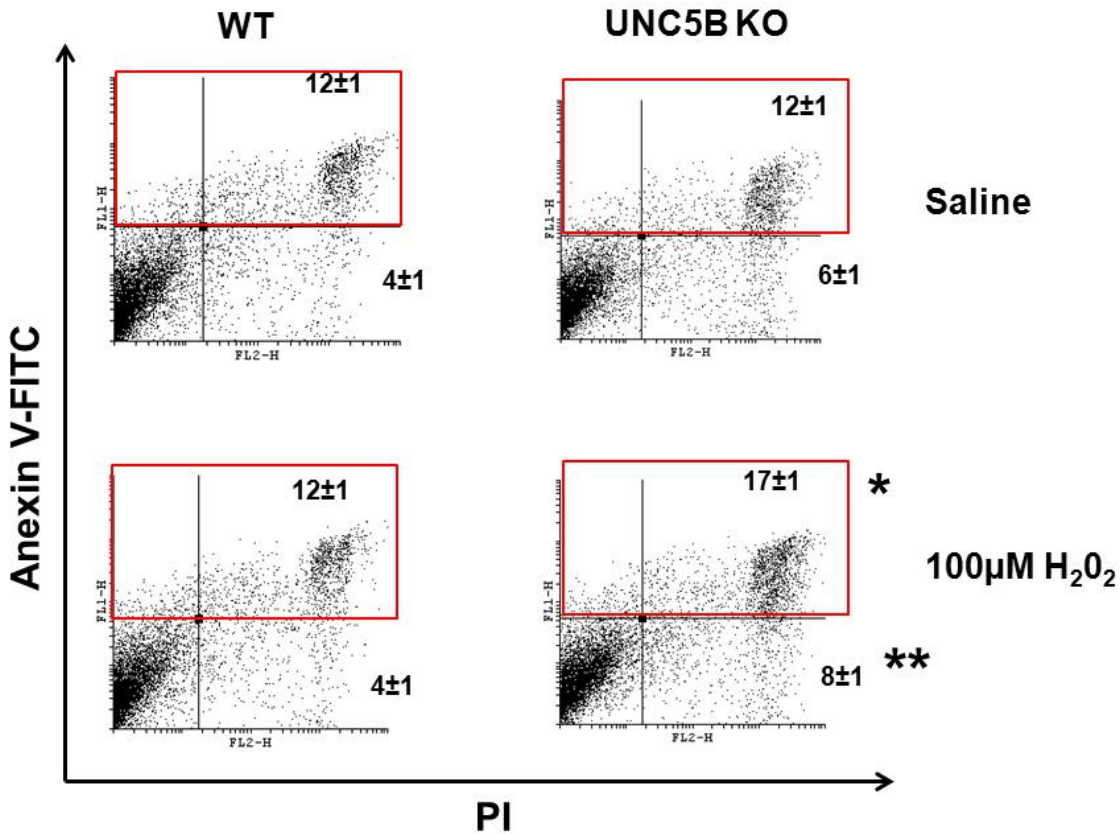


Figure S2. UNC5B depletion (UNC5B KO) in kidney epithelial cells (TKPTS) increases hydrogen peroxide (H₂O₂) induced apoptosis and necrosis. Flow cytometry analysis of H₂O₂ induced apoptosis in wild type TKPTS cells and UNC5B KO TKPTS cell. Low dose of H₂O₂ did not increase apoptosis (both propidium iodide (PI) and Annexin V positive) and necrosis (PI alone positive). However, depletion of UNC5B in TKPTS cells significantly increased both apoptosis and necrosis in response to H₂O₂. *, $p < 0.05$ vs. other groups for apoptosis. **, $p < 0.05$ vs. other groups for necrosis. N=4.

Figure S3.

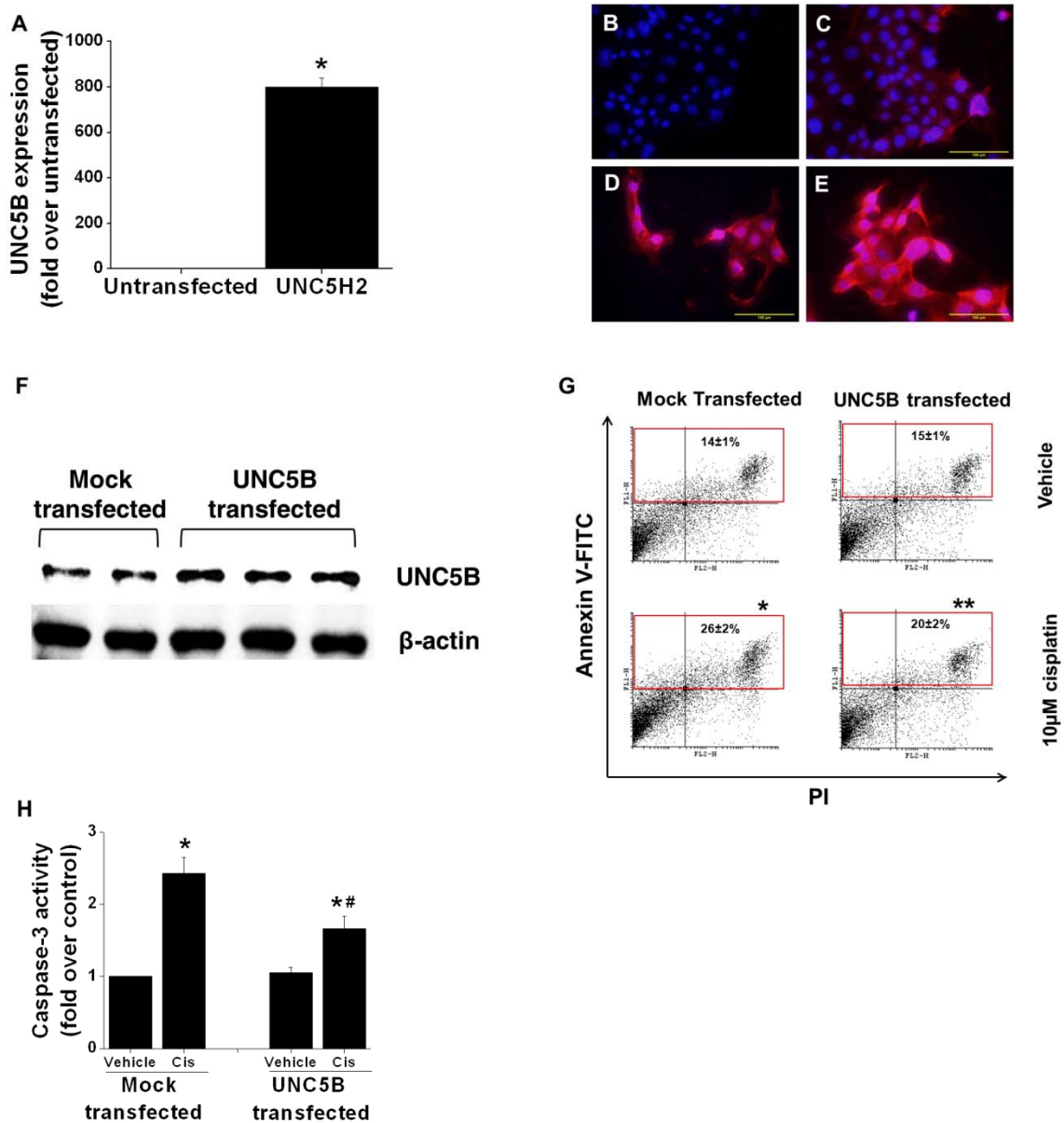


Figure S3. UNC5B overexpression in kidney epithelial cells (TKPTS) reduces cisplatin induced apoptosis. TKPTS cells was transfected with rat UNC5B expression construct. 48 hrs after transfection cells were treated with vehicle or 10μM cisplatin and cells were

harvested 24hrs after cisplatin addition. A. Real time PCR analysis showing overexpression of rat UNC5B. *, $p < 0.001$ vs. untransfected. N=4. B-E: immunohistochemical localization of UNC5B in mock transfected (C) and UNC5B plasmid transfected (D and E) kidney epithelial cells. 2ND antibody control does not show any staining (B). Red: UNC5B staining. Blue: DAPI nuclear stain. Scale bar: 100µM. F. Western blot analysis showing overexpression of UNC5B in transfected cells as compared to mock transfected TKPTS cells. G. Flow cytometry analysis of apoptosis in mock transfected and UNC5B transfected kidney epithelial cells. Cisplatin induced increase in apoptosis was significantly suppressed in UNC5B transfected cells. *, $p < 0.001$ vs. vehicle treated groups. #, $p < 0.05$ vs. mock transfected cisplatin treated cells. H. Caspase-3 activity was quantified in mock transfected and UNC5B transfected kidney epithelial cells treated with saline or 10µM cisplatin. *, $p < 0.05$ vs. vehicle. #, $p < 0.05$ vs. *. N=4.