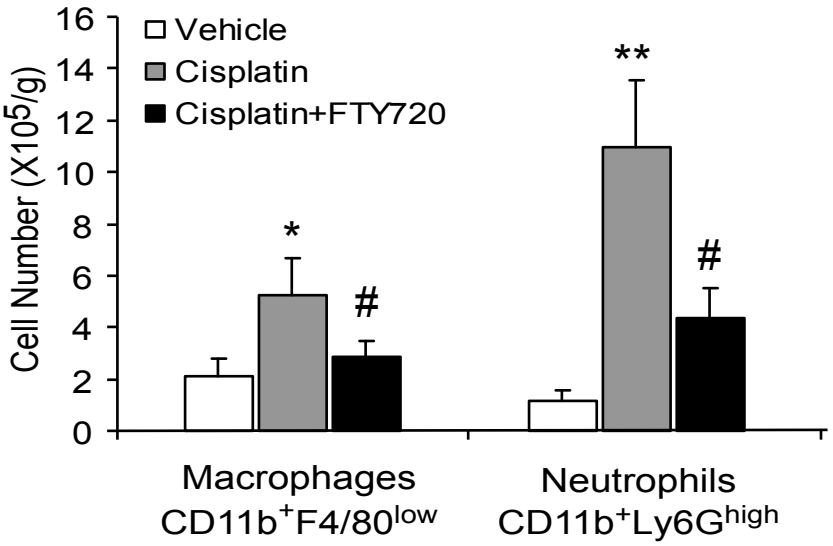
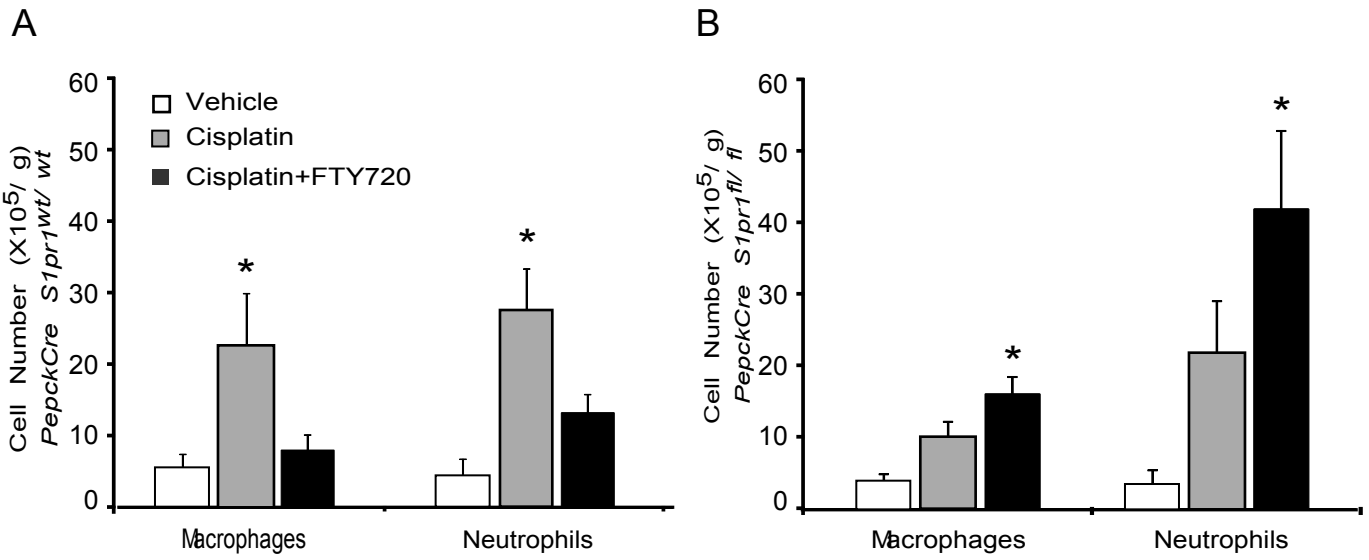
Supplemental Figure 1

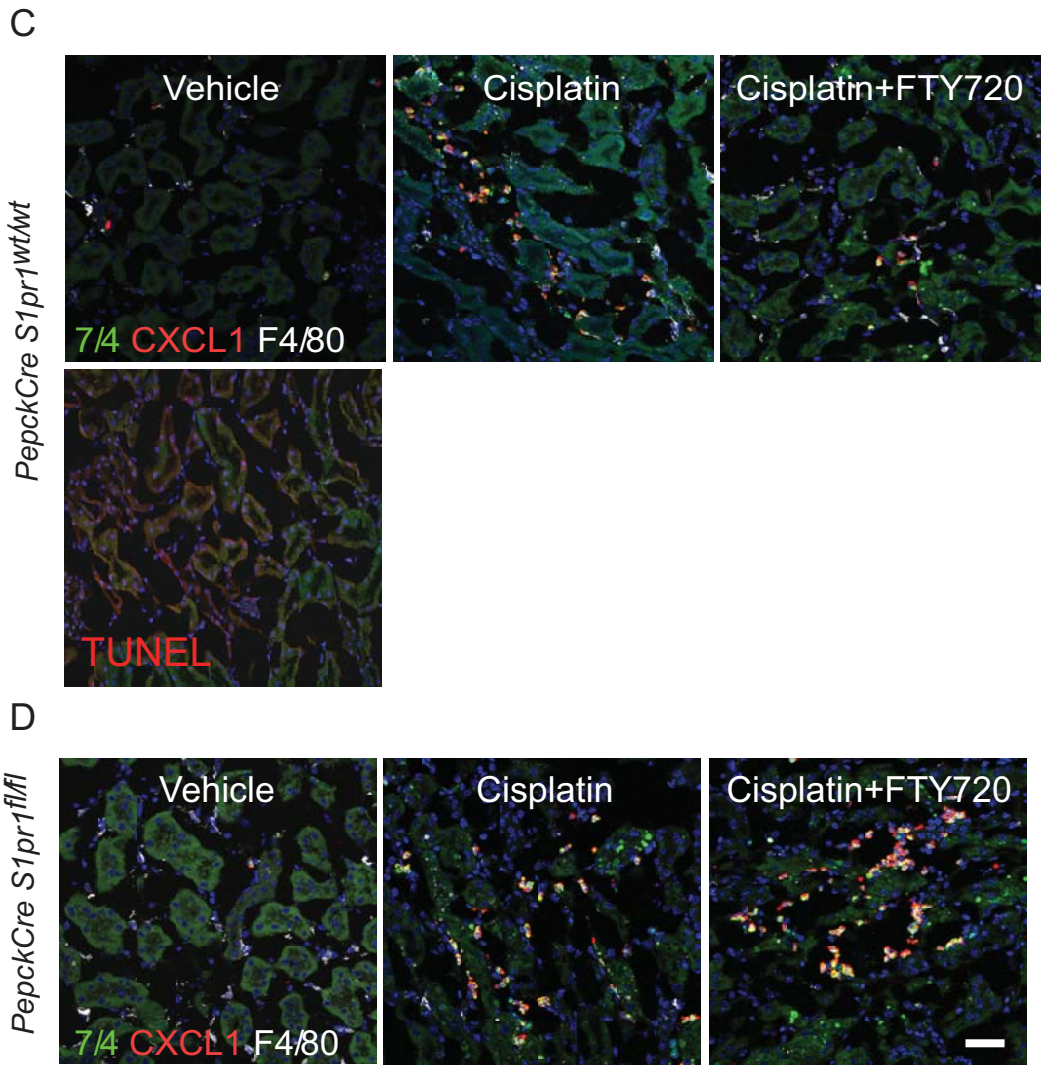


FTY720 treatment attenuates leukocyte infiltration into kidneys following cisplatin. WT mice were treated with FTY720 (240 μg/kg) 1 hr prior to a single dose of cisplatin (27 mg/kg) and once each day on the next 2 days. FACS analysis of subsets of total live (7AAD-) leukocytes ($CD45^+$) as determined by cell count of $CD45^+$ cells per gram of kidney weight. Values are mean ± SE; n=4-11, $^{**}p<0.01$ and $^{*}p<0.05$ compared to vehicle; # $p<0.05$ compared to cisplatin.

Supplemental Figure 2 - page 1

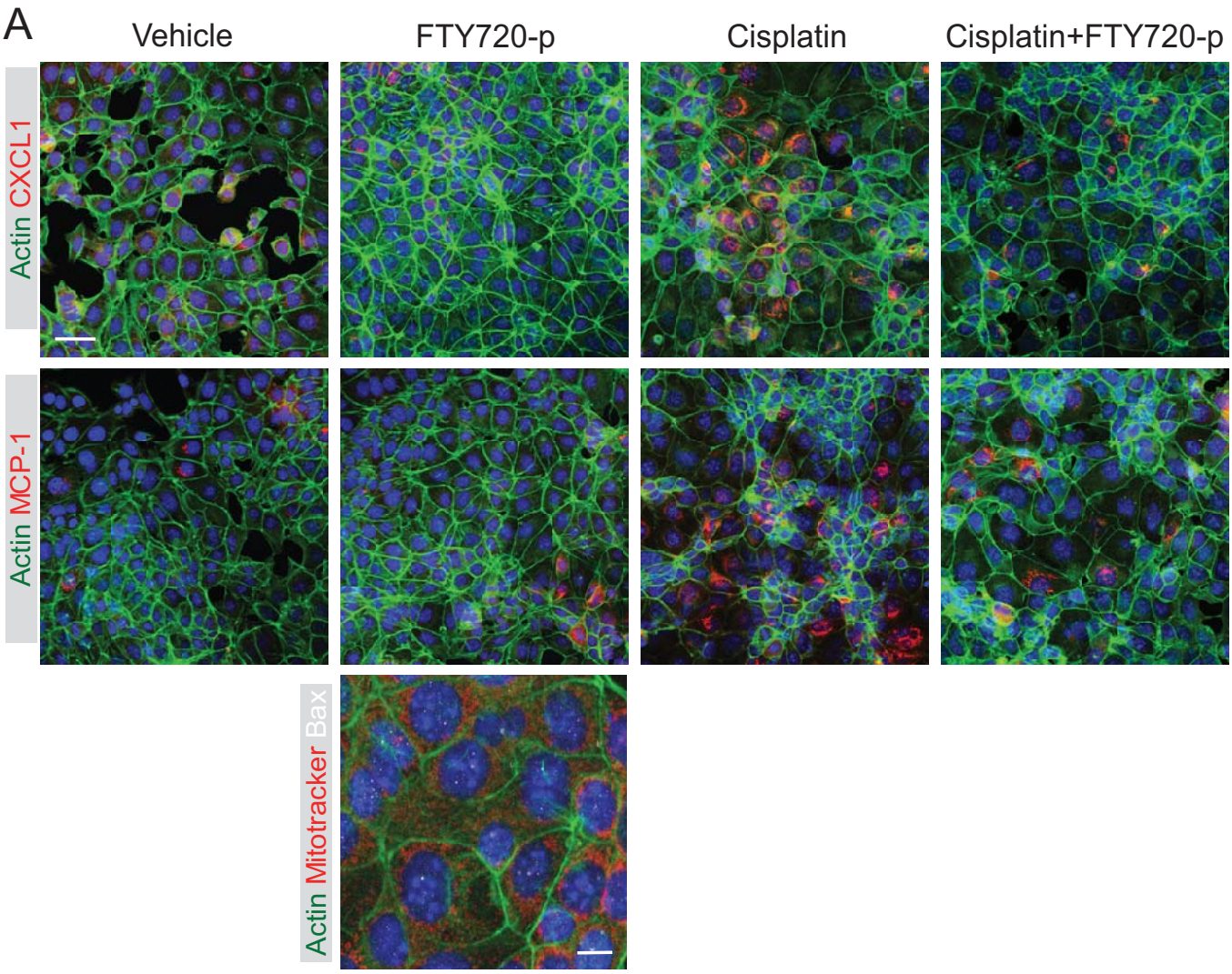


Supplemental Figure 2 - page 2

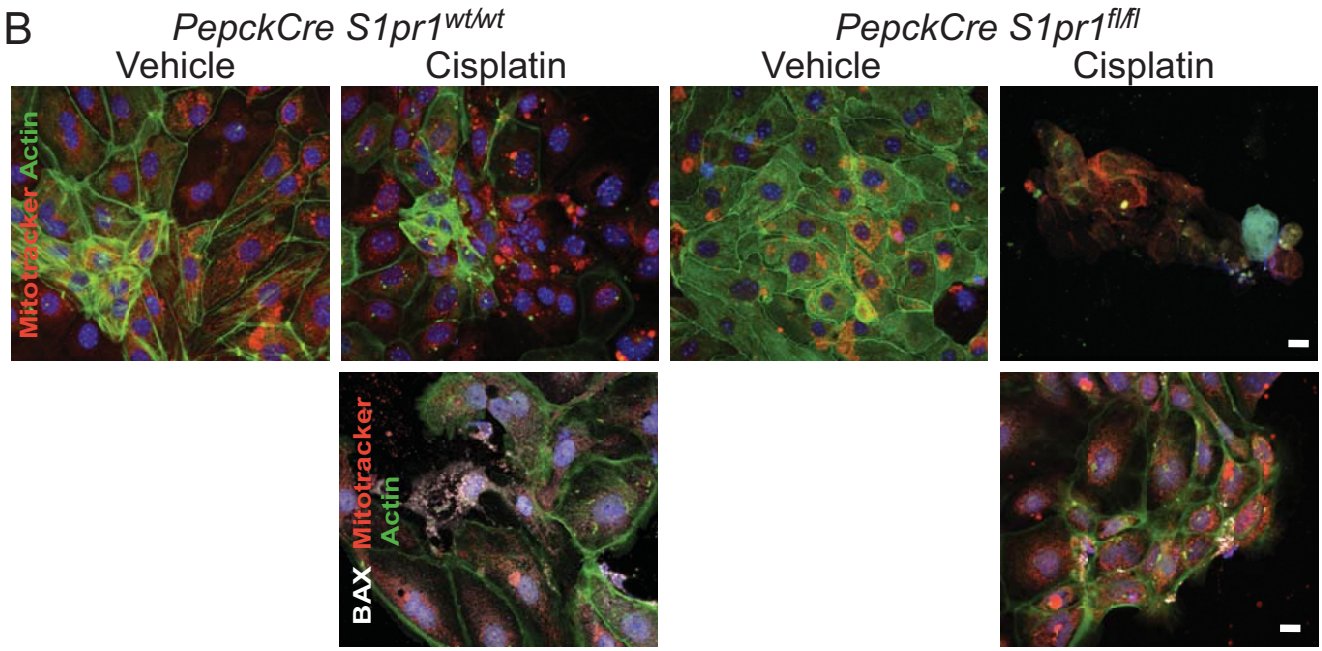


Deletion of proximal tubule sphingosine 1-phosphate 1 receptor (S1P1) exacerbates injury and its presence is necessary for FTY720-mediated protection from cisplatin-induced AKI: immune cell infiltration. Mice were treated with FTY720 1 hr prior to cisplatin and once each day on the next 2 days. (A, B) FACS analysis of subsets of total live (7AAD-) leukocytes (CD45+) as determined by cell count of CD45+ cells per gram of kidney weight from *PepckCreS1pr1*$^{wt/wt}$ (A) and *PepckCreS1pr1*$^{fl/fl}$ (B) mice. n=4-11, *p<0.05 compared to respective vehicle. (C, D) Immunofluorescent labeling of neutrophils ((7/4; green, or yellow if merged with CXCL1 labeling [red])), monocyte (F4/80; white), chemokine (CXCL1; red) and apoptosis (TUNEL, red; in vehicle-treated mice as control for panels in Figure 3C) in kidney sections from *PepckCreS1pr1*$^{wt/wt}$ (C) and *PepckCreS1pr1*$^{fl/fl}$ mice (D). TUNEL-staining (red) in DAPI-labeled (blue) nuclei appears magenta in color-merged image. Scale bar = 50 μm.

Supplemental Figure 3 - pg 1

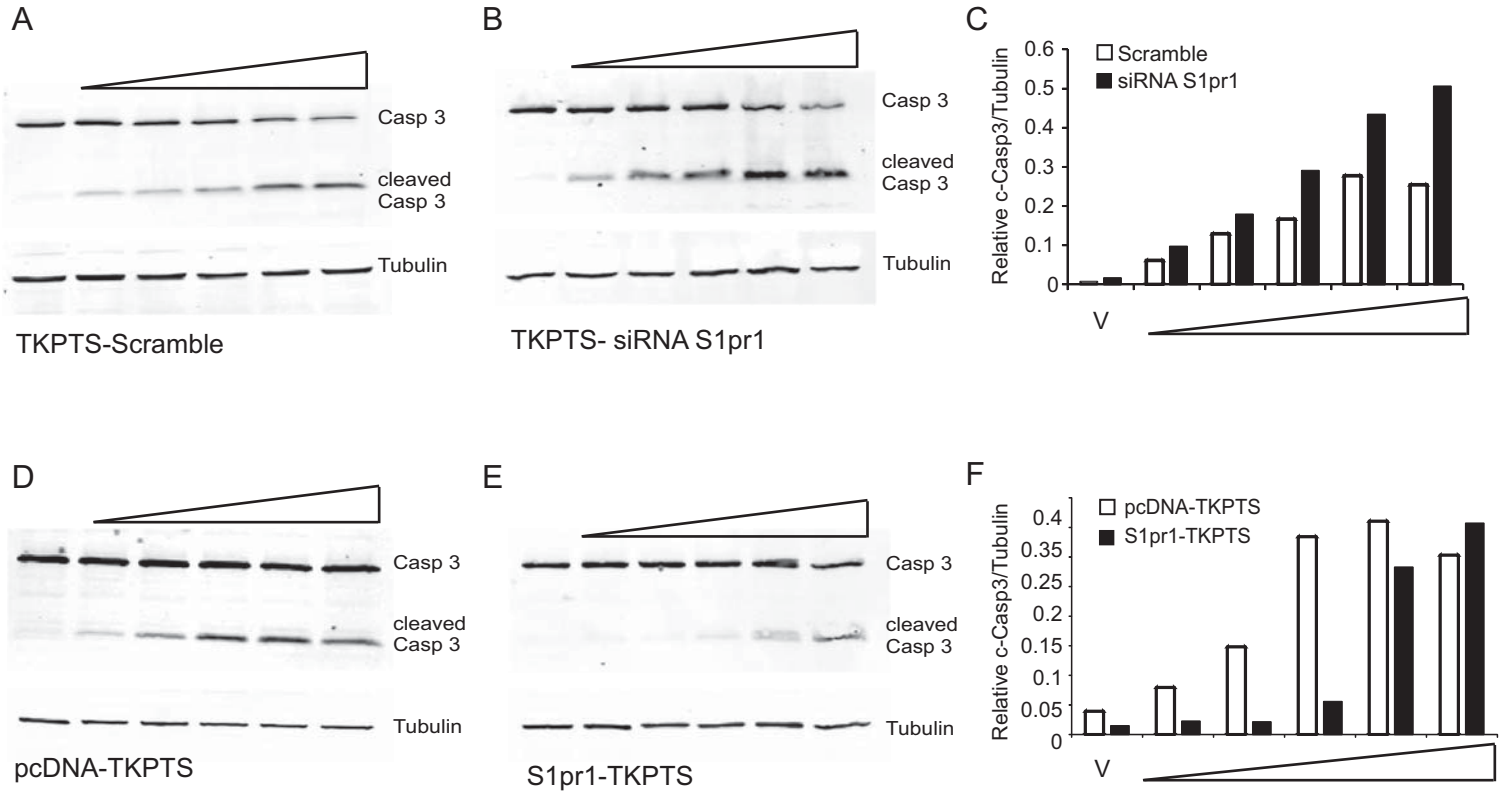


Supplemental Figure 3 - page 2



Phosphorylated FTY-720 (FTY720-p)-mediated protection of cisplatin-induced injury in TKPTS cells and primary cultures of tubule epithelial cells is attenuated in cultures from *PepckCreS1pr1$^{fl/f}$* mice. (A) Immunofluorescent labeling of actin cytoskeleton (FITC-phalloidin; Actin), cytokine (MCP-1), chemokine (CXCL1), apoptotic marker (Bcl-2-associated X protein; BAX, white), and mitochondria (MitoTracker, Red) in mouse kidney proximal tubule (TKPTS) cells treated with vehicle or cisplatin (20 μM for 24 hr). Blue, nuclei stained with DAPI, White and blue overlap is shown as pink. Scale bar = 50 μm. (B) Immunofluorescence labeling of mitochondria (MitoTracker, Red), actin cytoskeleton (FITC-phalloidin; Actin), BAX and nuclei (DAPI) in primary cell cultures of proximal tubule epithelial cells isolated from *PepckCreS1pr1$^{wt/wt}$* and *PepckCreS1pr1$^{fl/f}$* mice and treated with vehicle or cisplatin (20 μm for 24 hr). Scale bar = 50 μm. Images are from a representative experiment that was performed at least three times with n = 2-3 replicates of each treatment.

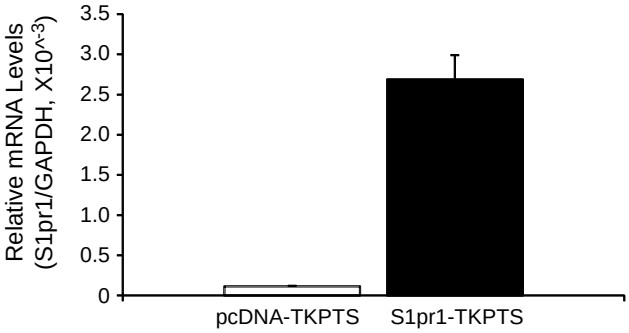
Supplemental Figure 4



Knockdown or over-expression of S1P1 alters the sensitivity of TKPTS cells to the apoptotic effects of cisplatin in a concentration-dependant manner. Changes in cleaved caspase 3 in response to incubations for 24 hr with vehicle (saline; V) or increasing concentrations of cisplatin in control TKPTS cells (siRNA scramble sequence (A), pcDNA-TKPTS (D)), after receptor knockdown in TKPTS cells with S1pr1 siRNA (B), and in S1P1 over-expressing TKPTS cells (E). Corresponding densitometric analyses of cleaved caspase 3 (c-Casp3)/tubulin) are shown in C and F. Concentrations of cisplatin (indicated on blot and bar graph by widening triangle): 20, 30, 40, 60, or 80 μM. n = 2 samples per treatment in each of 3 replicate experiments.

Supplemental Figure 5 - pg 1

A



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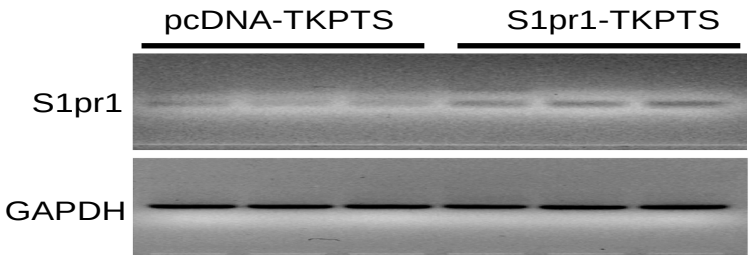
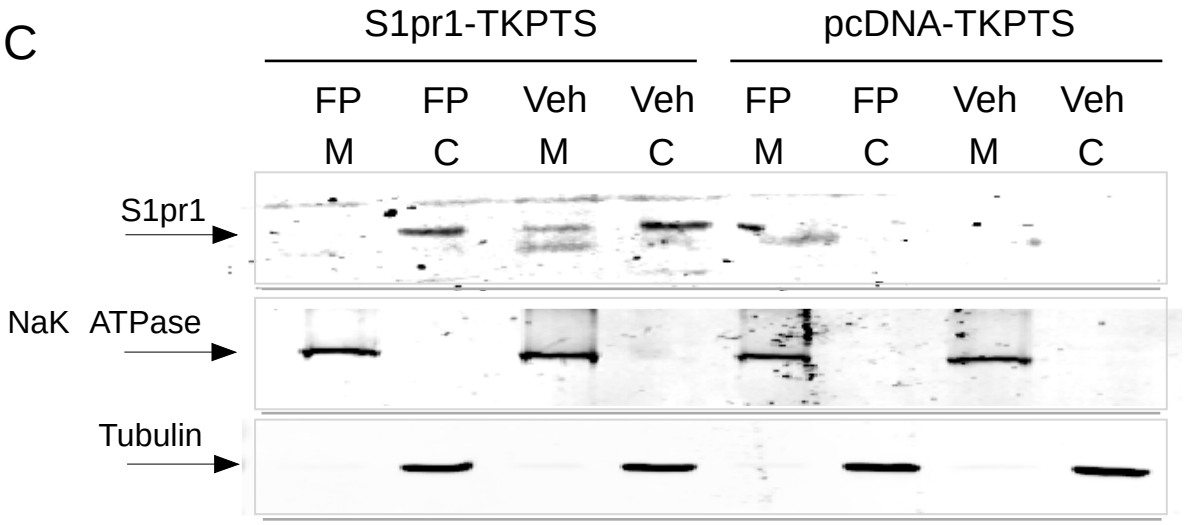
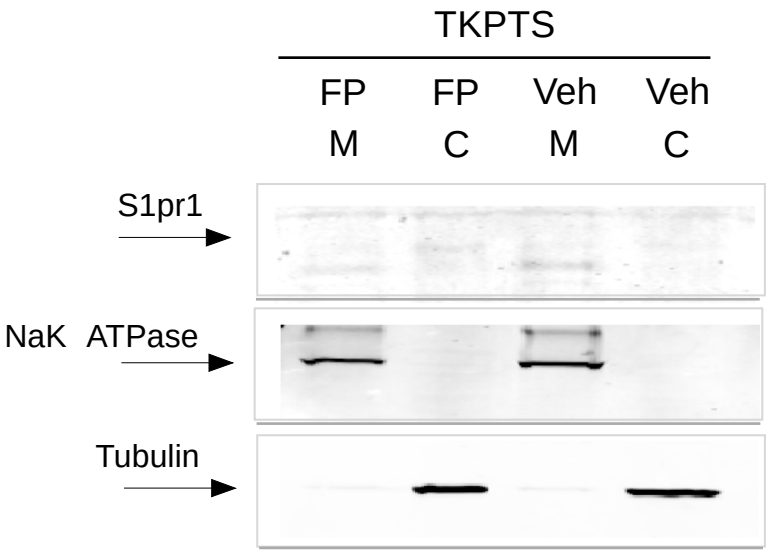


C





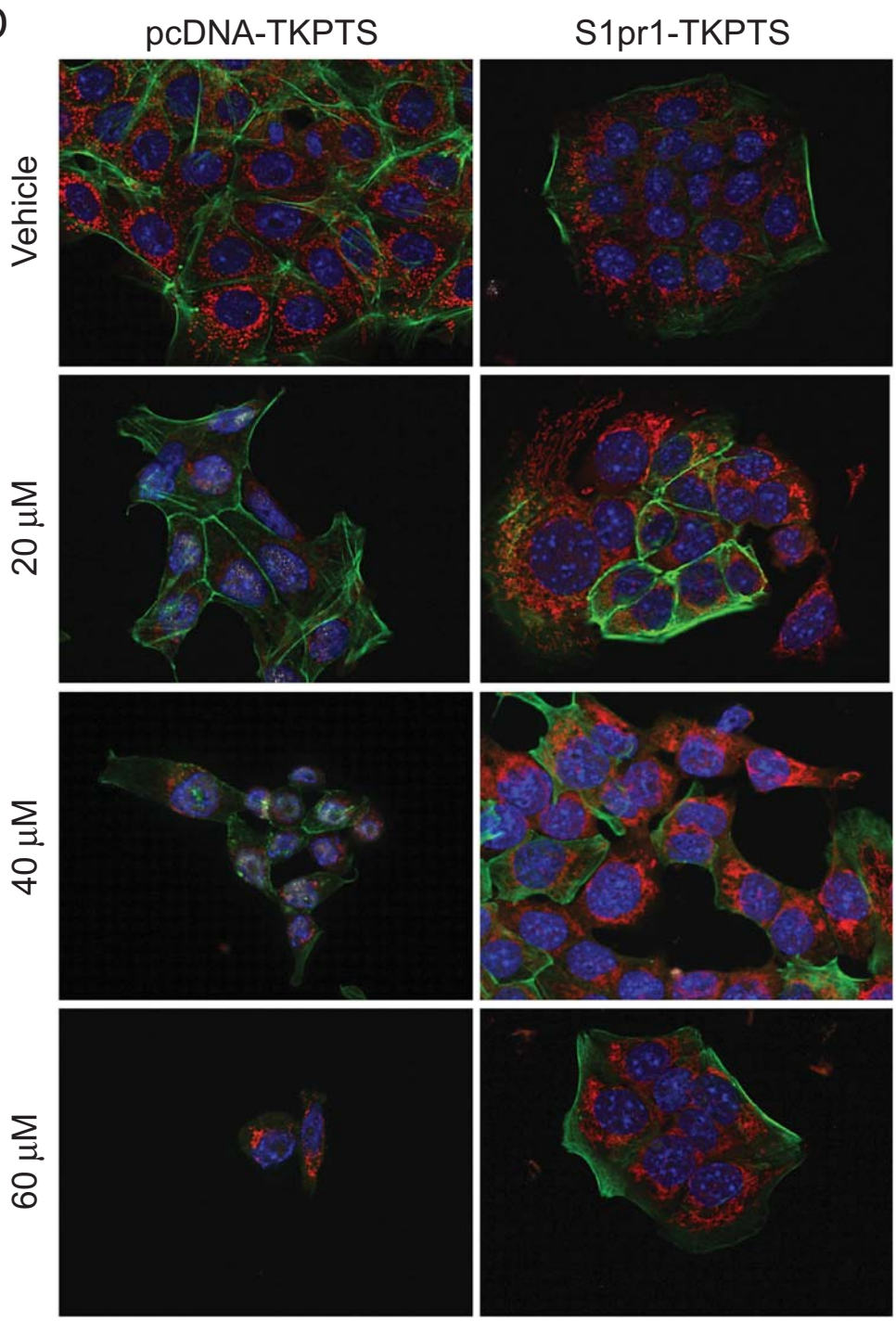
FP: FTY720-p

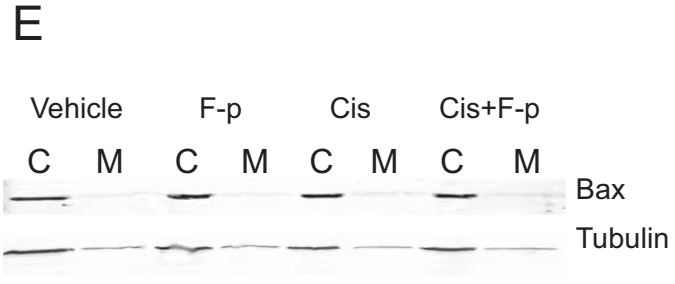
Veh: Vehicle

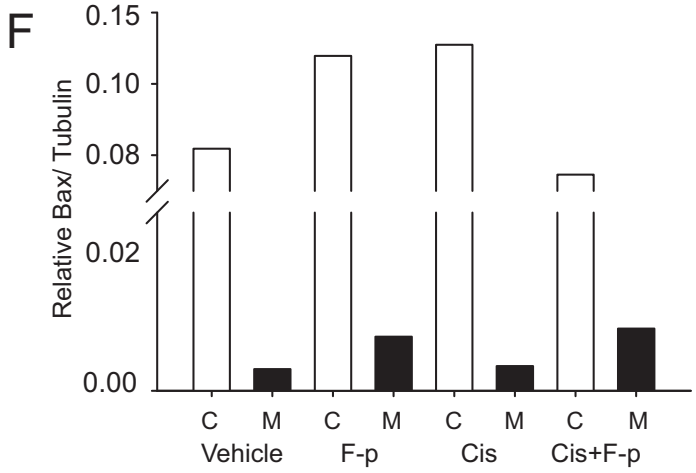
M: Membrane fraction

C : Cytoplasmic fraction

Supplemental Figure 5 - pg 2

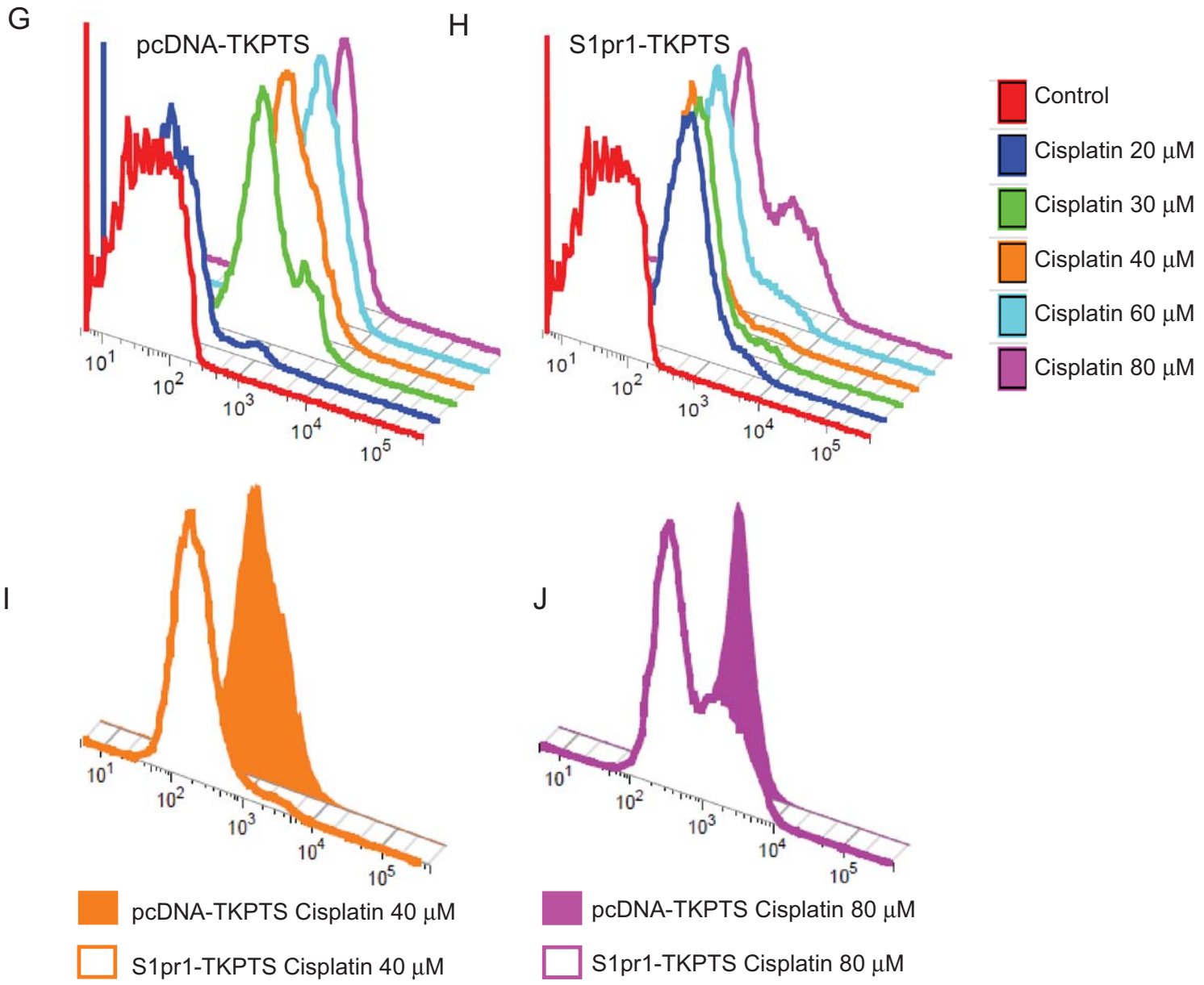






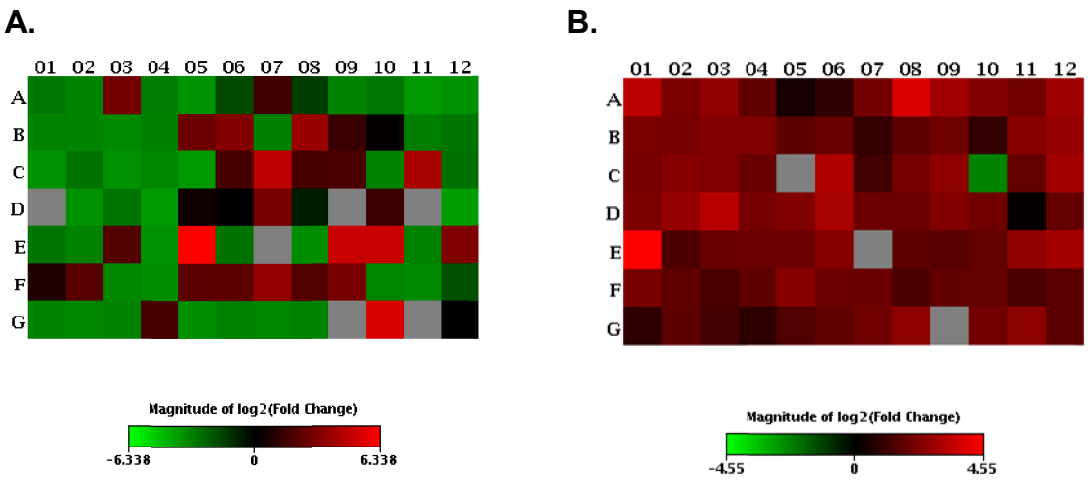
Supplemental Figure 5: Over-expression of S1P1 in TKPTS cells. TKPTS cells were stably transfected (and maintained under G418 selection) with empty pcDNA3.1/V5-His vector (pcDNA-TKPTS) or vector containing the sequence for S1p1r (S1pr1-TKPTS). A, B. Stable over-expression of S1p1r was confirmed by RT-PCR in 3 separate cultures. Relative mRNA levels (A; n = 3; mean ± SE) and representative agarose gel of PCR products from one experiment (B). **C. Cells were treated with vehicle or 10 nM FTY-720-p for 24 hr, cytoplasmic (C) and membrane (M) cell fractions were isolated, and western blotting for S1P1 was performed with NaK-ATPase or tubulin as controls for equal loading. S1P1 is evident in cytoplasmic and membrane fractions of vehicle-treated S1pr1-TKPTS cells but not in the membrane fraction after FTY720-p treatment. S1P1 is undetectable in untransfected TKPTS cells or pcDNA-TKPTS.** D. Cells were incubated with vehicle (saline) or cisplatin (20 μM) for 24 hr and then were labeled with FITC-phalloidin to label actin (green) or Mitotracker to label mitochondrai (red). E-F. S1pr1-TKPTS were incubated with vehicle (saline) or 20 μM cisplatin (Cis) for 24 hr or with 10 nM phosphorylated FTY720 (F-p) for 1 hr prior to vehicle or cisplatin. Cytoplasmic (C) and mitochondrial (M) cell fractions were isolated and western blotting was performed for BAX with tubulin as a control for equal loading.

Supplemental Figure 5 - pg 3



Susceptibility of control and S1P1 over-expressing TKPTS cells to increasing concentrations of cisplatin. Cells were incubated with vehicle (saline) or cisplatin (20-60 μM) for 24 hr and then with FITC-phalloidin to label actin (green) or Mitotracker to label mitochondria (red). S1P1 over-expressing cells were less susceptible than control cells to the apoptotic effects of increasing concentrations of cisplatin. (G-J) Stable over-expression of S1P1 protects TKPTS cells from cisplatin-induced increases in mitochondrial superoxide. Cells were incubated with vehicle (saline) or cisplatin (20-80 μM) for 24 hr then were labeled with Mitosox (5 μM for 10 min) and analyzed by flow cytometry (BBRC 358:203-208, 2007). (G) Histograms for cells incubated with saline (control) or 20-80 μM cisplatin are shown for pcDNA- (H) and S1pr1-TKPTS cells (I). Mitosox fluorescence intensity increased in a concentration-dependent manner with increasing concentrations of cisplatin in control but not over-expressing cells (mean values of replicate experiments shown in Figure 4). To more clearly illustrate the increase in Mitosox fluorescence in control but not in over-expressing cells after cisplatin, examples are illustrated for two concentrations of cisplatin: 40 μM (J) and 80 μM (J).

SUPPLEMENTAL FIGURE 6



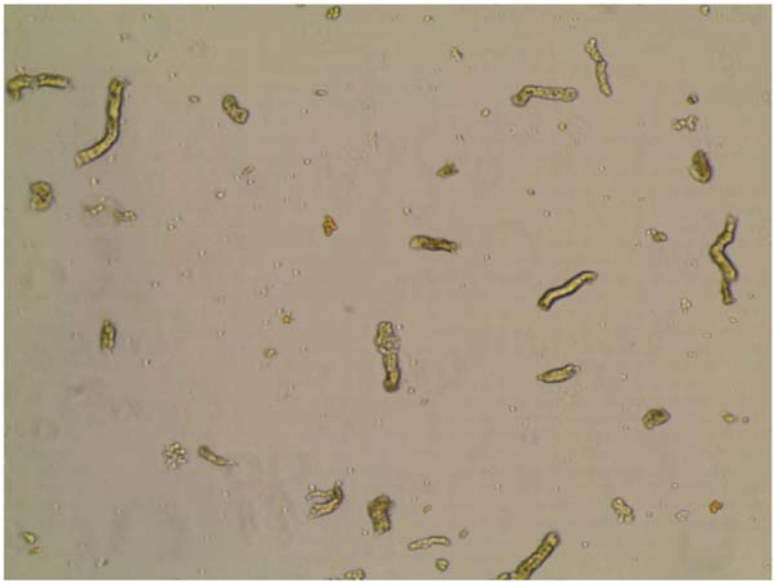
A. Changes in gene expression in *PepckCreS1pr1*$^{fl/fl}$ mice compared to *PepckCreS1pr1*$^{wt/wt}$

B. Changes in gene expression in S1pr1-TKPTS cells compared to pcDNA-TKPTS

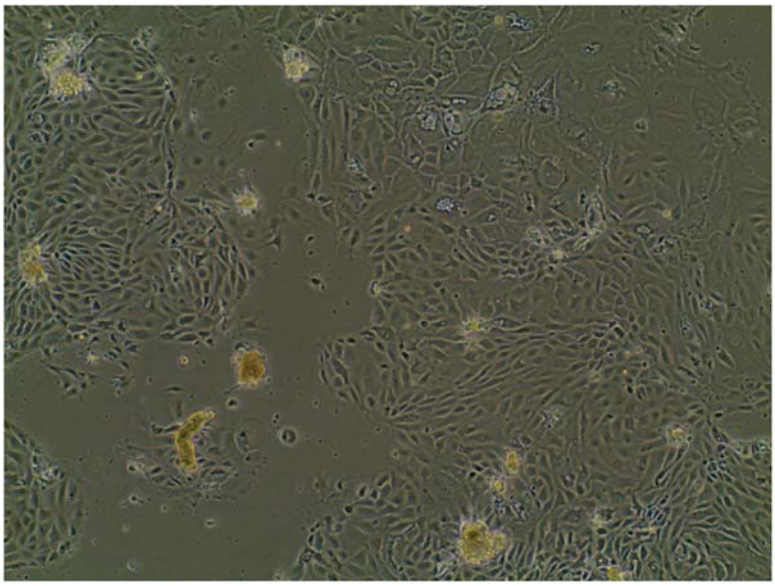
Expression analysis by PCR array demonstrates mitochondrial genes that are regulated in mouse proximal tubule cells deficient in or over-expressing S1P1. Heat map comparisons generated from PCR array and reflecting relative gene expression values in (A) primary tubule cell cultures from *PepckCreS1pr1*$^{fl/fl}$ mice compared to *PepckCreS1pr1*$^{wt/wt}$ mice and (B) S1P1 over-expressing compared to control (V5-transfected) TKPTS cells. Details of specific genes are shown in Table 4.

Supplemental Figure 7 - pg 1

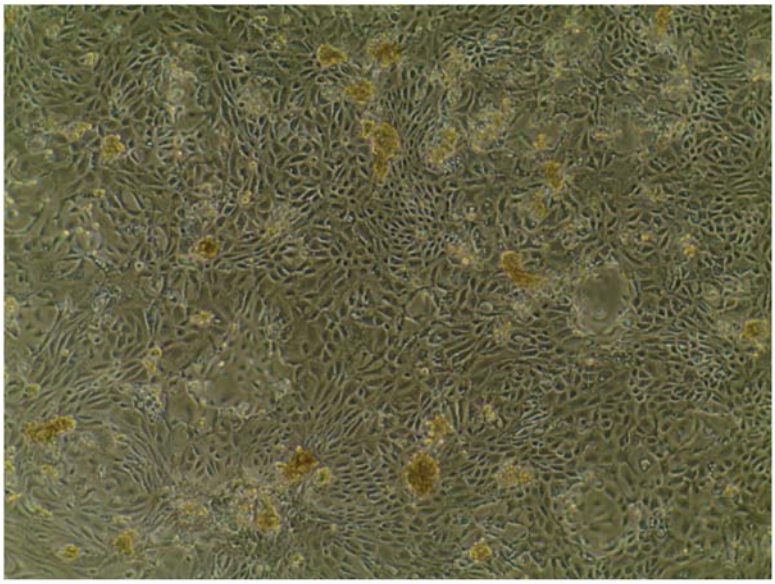
A. Primary culture of mouse tubular epithelial cells



Day 0 - just after plating



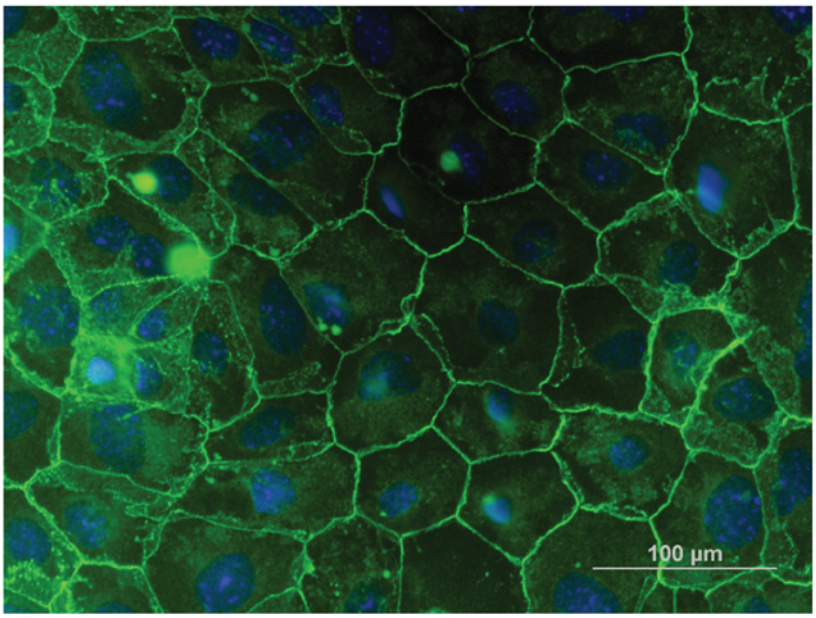
Day 5



Day 7-10

Supplemental Figure 7 - pg 2

B



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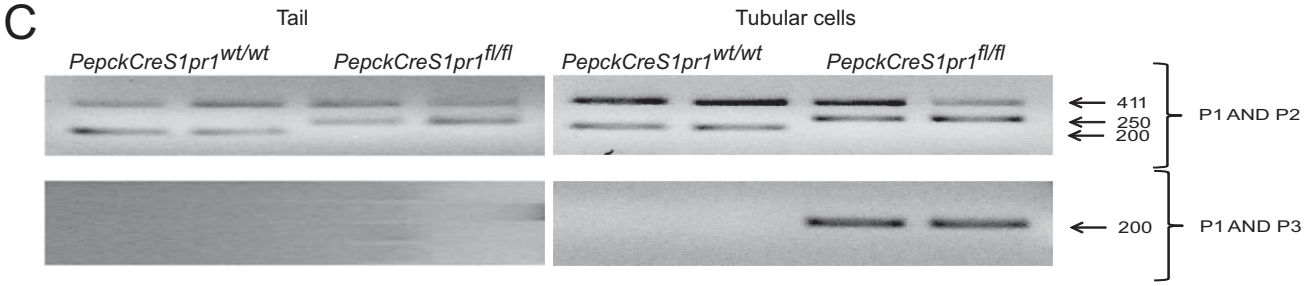

Isolation and primary culture of renal tubular epithelial cells (TECs) from PEPCK-Cre-S1P1$^{wt/wt}$ and PEPCK-Cre-S1P1$^{fl/fl}$ mice. (A) Phase contrast photomicrographs of live primary cell cultures on day 0, 5 and 7-10 after plating. For experiments, cells were used between day 7 and 10 of culture when a confluent monolayer is evident. (B) Immunofluorescent labeling of E-cadherin (green) in 7 day cultures (20 µg/ml Alexa 488-labeled ms x E-cadherin, clone DECMA-1, eBioscience, San Diego, CA). Blue, nuclei stained with DAPI. (C) Agarose gel of PCR products resulting from genotyping of samples of tail and isolated tubular epithelial cells from *PepckCreS1pr1$^{wt/wt}$* and *PepckCreS1pr1$^{fl/f}$* mice using primers (P1-3) specific for wt and floxed alleles demonstrates selective deletion of *S1pr1* from kidney tubules. Molecular weight of bands indicated at right.

Supplemental Table 1. Morphometric analysis of mitochondria in TKPTS cells: contour measurements[d]

	Feret Maximum[a]	Compactness[b]	Shape Factor[b]	n (mitochondria)	n (cells)
Vehicle	0.50 ± 0.01	0.61 ± 0.01	4.06 ± 0.02	385	22
Scramble	0.52 ± 0.01	0.62 ± 0.01	3.98 ± 0.02	326	22
siRNA #1	0.52 ± 0.01	0.62 ± 0.01	4.00 ± 0.02	374	20
siRNA #4	0.55 ± 0.01	0.58 ± 0.01	4.16 ± 0.03	421	24
FTY720	0.55 ± 0.01	0.61 ± 0.01	4.03 ± 0.02	394	20

[a] the longest projection of the minimal bounding box, i.e. parallel tangents apposing opposite sides of the profile; μm

[b] values are 0-1; a circle has a value of 1

[c] defines a relationship of perimeter to area; a circle has a value of 3.54

[d] TKPTS cells were 1) transiently transfected with one of two *S1pr1* siRNAs or a scrambled sequence and harvested after 48 hr or 2) were incubated with 10 nM FTY720 on day 0, 3 and 6 and harvested 24 hr after the last treatment. Cells were prepared for electron microscopy. 20-25 cells were photographed at 6000X from each treatment, and mitochondrial morphometrics were analyzed by using MBF Bioscience StereoInvestigator software (Williston, VT).