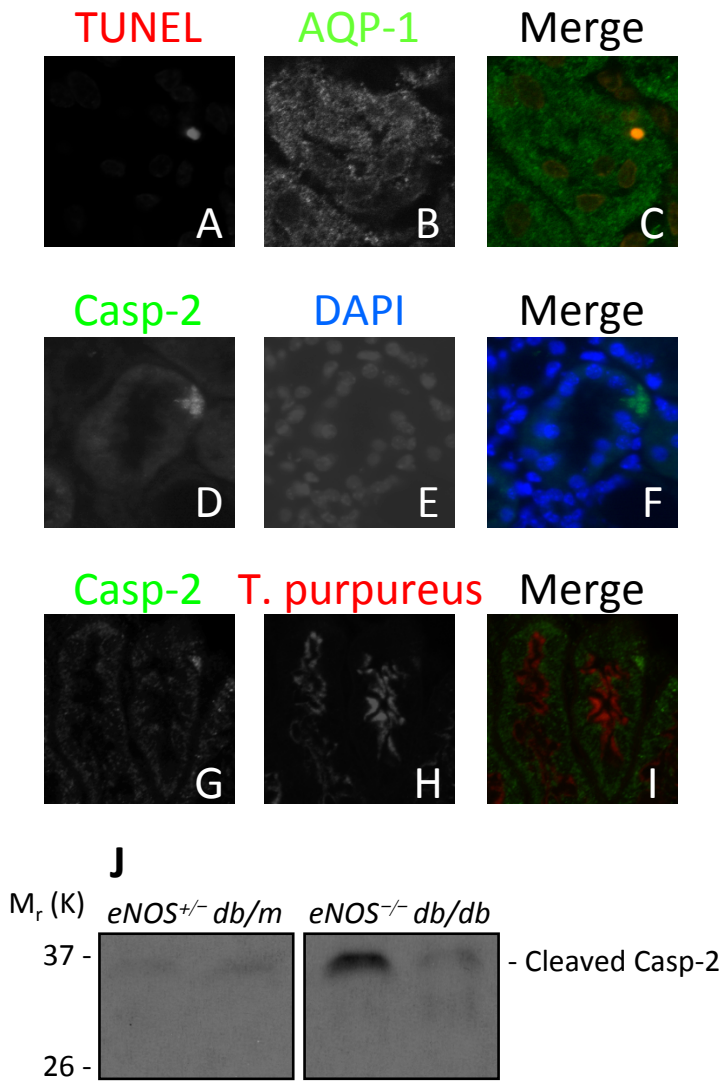
Supplementary text

Estimation of cellular LC-CoA concentration

The three LC-CoAs (palmitoyl, oleoyl, stearyl) from eNOS$^{-/-}$ *db/db* kidney cortex total 58 pmol/mg tissue (see Figure 2B for individual concentrations). If the cell is assumed to be entirely water, then: 58 pmol/mg x 10^{6} mg/L x 10^{-6} µmol/pmol = 58 µmole/L.

Strain	Age (wks)	N	U alb:creat	% apoptosis
$ROP^{+/+}$	26	13	232 ± 60	0.2 ± 0.1
	52	8	179 ± 41	0.3 ± 0.2
	96	3	845 ± 132	1.0 ± 0.2
$ROP^{Os/+}$	26	11	433 ± 95	0.6 ± 0.3
	52	12	559 ± 70	0.7 ± 0.2
	96	3	1986 ± 192	1.5 ± 0.4

Supplementary Table 1. *Renal phenotypes in the $ROP^{Os/+}$ model of FSGS*. Urine albumin was measured by ELISA, and urine creatinine by colorimetric assay, and the ratio was expressed in mg/g. Kidney frozen sections were assayed for apoptosis by TUNEL. All results are expressed as mean ± SEM.



Supplementary Figure 1. *Proximal tubule apoptosis in eNOS$^{-/-}$ db/db kidneys*. In frozen sections from *eNOS$^{-/-}$ db/db* kidneys; **A**, apoptotic cells were labeled by TUNEL; **B**, proximal tubules were counterstained with anti-aquaporin-1 (1:200, 14 hr, 4^{o} C) and FITC-conjugated IgG; **C**, merged image; **D** and **G**, mouse kidneys were labeled with rat anti-active (cleaved) caspase-2 IgG (1:100, 14 hr, 4^{o} C); **E**, DAPI nuclear counterstain; **F**, merged image **H**; TRITC-conjugated *Tetragonolobus purpureus* proximal tubule counterstain ; **I**, merged image. **J**, whole kidney lysates from *eNOS$^{+/-}$ db/m* and *eNOS$^{-/-}$ db/db* mice were immunoprecipitated with anti-active caspase-2 IgG, resolved by SDS-PAGE and re-probed with the same antibody. The data represent samples from one mouse per lane.

Wild-type NHE1

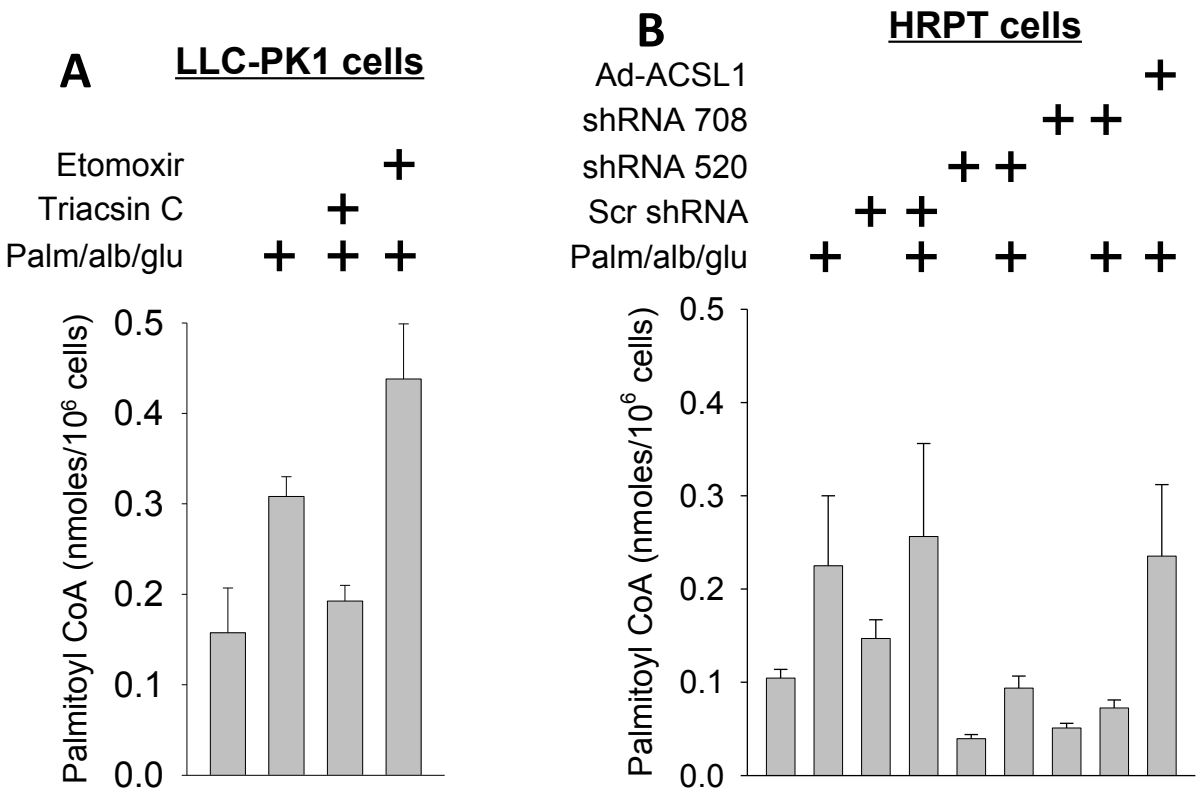
513-KKKQETKR---RFNKKYVKK-564---820

M1 + M2 KR/A mutant NHE1

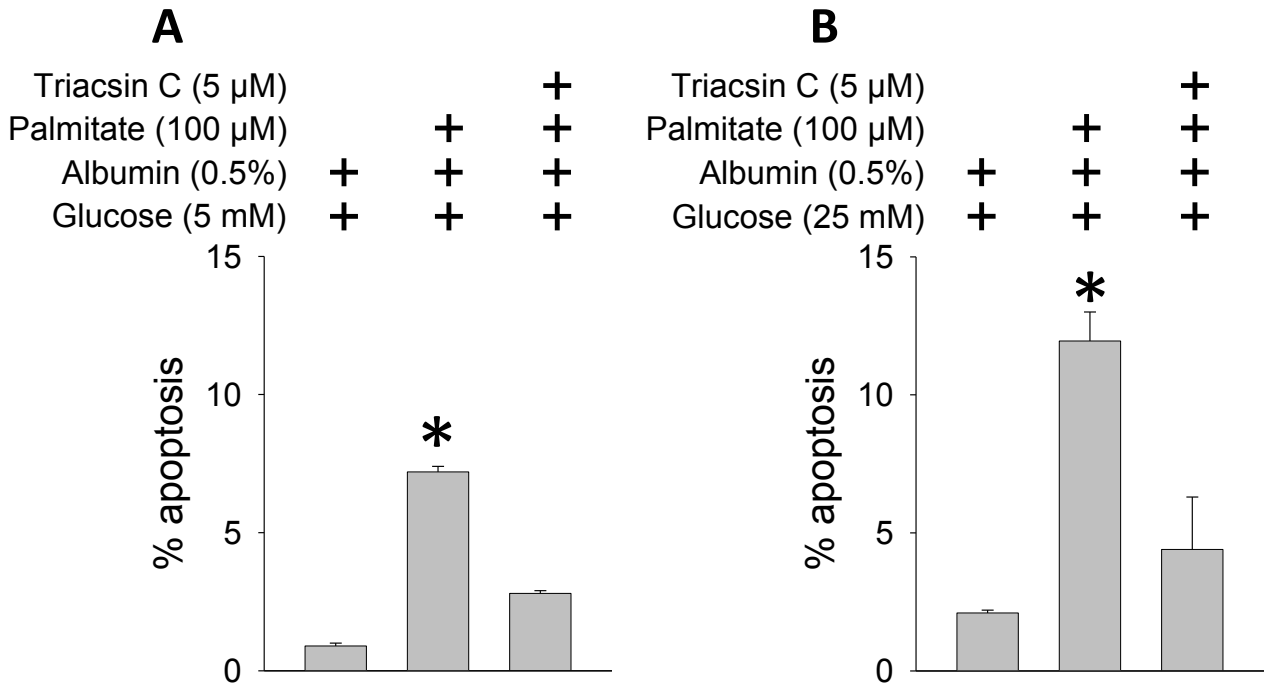
513-AAAQETAA---AFNAAYVAA-564---820

M1 **M2**

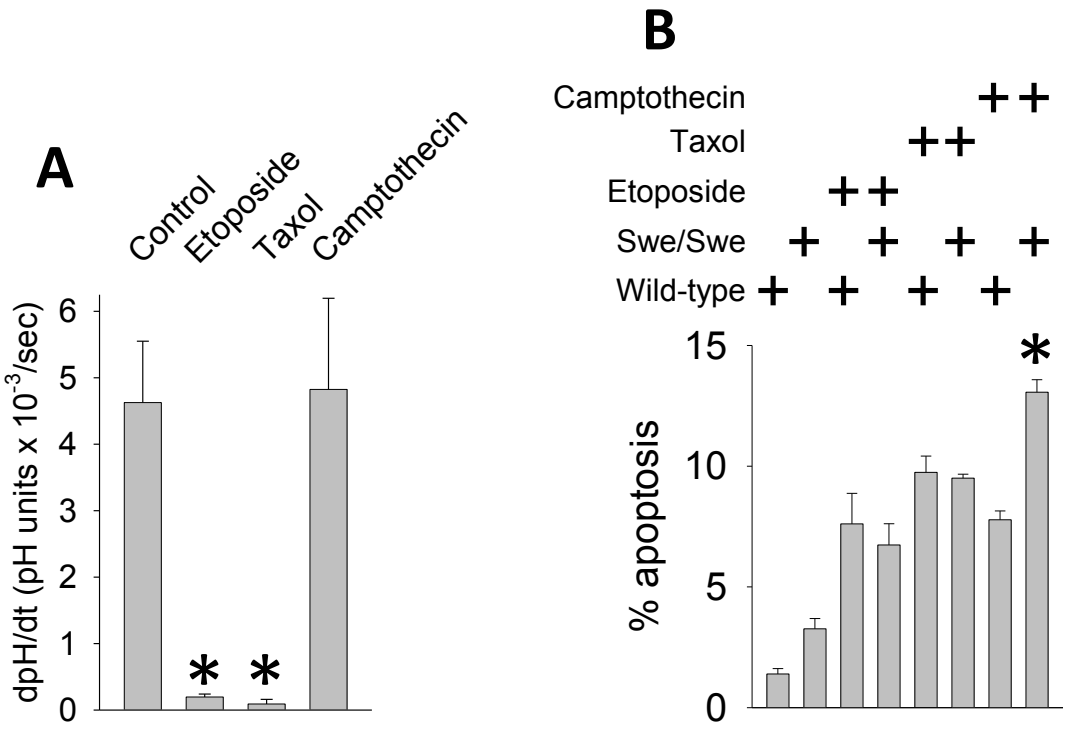
Supplementary Figure 2. Wild-type and mutant rat cNHE1 amino acid sequences. Residues not shown are identical between the two polypeptides.



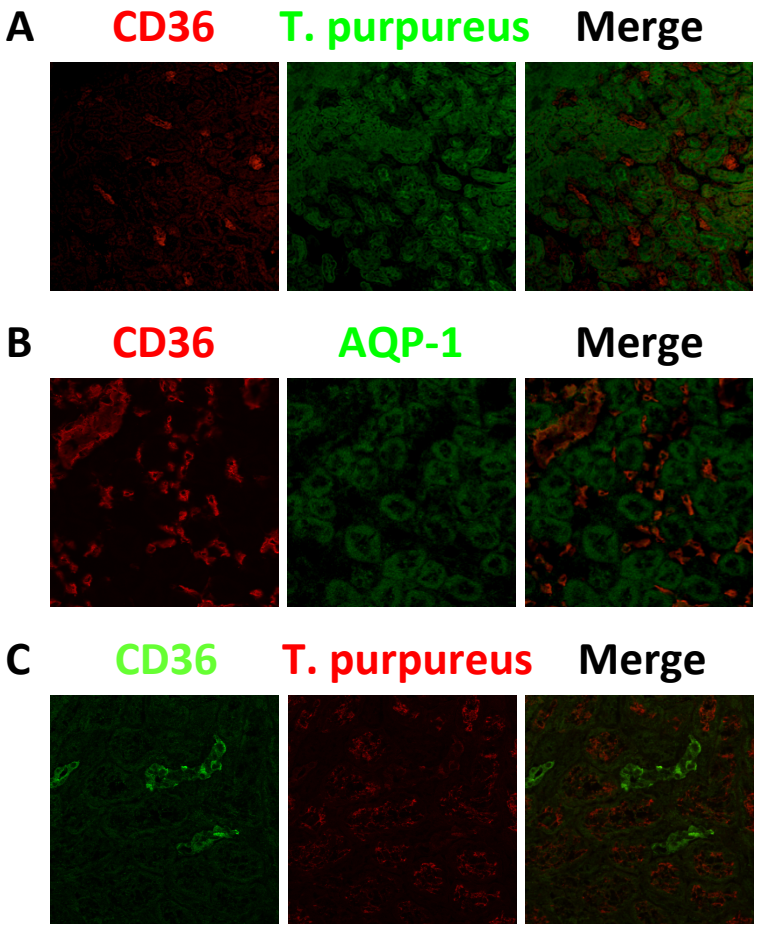
Supplementary Figure 3. *Manipulation of intracellular LC-CoAs in LLC-PK1 and HRPT cells*. Palmitoyl-CoA concentrations were measured by LC-MS/MS methods, from two, pooled 10 cm plates per condition of LLC-PK1 (**A**) or HRPT (**B)** cells. LLC-PK1 conditions included incubation with glucose (25 mM, 24 hr), delipidated BSA (0.5%, 24 hr), palmitate (100 µM, 24 hr) ± etomoxir (100 µM, 24 hr), ± triacsin C (5 µM, 12 hr). HRPT conditions included cells transfected with viral vectors for ACSL1 shRNAs or ACSL1 cDNA, followed by incubation with glucose (25 mM, 24 hr), delipidated BSA (0.5%, 24 hr), and palmitate (100 µM, 24 hr). Results are expressed as mean ± SEM from three to five separate experiments.



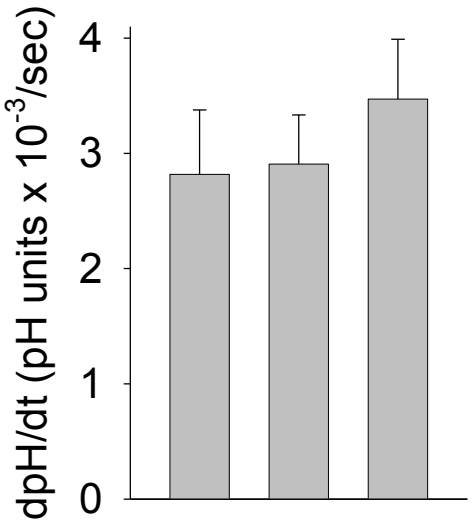
Supplementary Figure 4. LLC-PK1 cells were grown to near confluence and maintained for 24 hr in serum-free media and 5 mM (**A**) versus 25 mM (**B**) glucose, which was supplemented with palmitate-complexed albumin or triacsin C, as indicated above. Apoptosis was quantitated by TUNEL assay.



Supplementary Figure 5. *Differences in apoptosis are dependent upon NHE1 activity*. **A**, Proximal tubule cell lines derived from C57BL/6 wild type mice were assayed for cariporide-inhibitable NHE1 activity following incubation with etoposide (200 µM, 14 hrs, 37° C), taxol (50 µM,14 hrs, 37° C) or camptothecin (50 µM,14 hrs, 37° C). * P <0.05 compared to control and camptothecin groups. **B**, Proximal tubule cell lines derived from C57BL/6 wild type and NHE1-null Swe/Swe mice were incubated with etoposide (200 µM, 14 hrs, 37° C), taxol (50 µM,14 hrs, 37° C) or camptothecin (50 µM,14 hrs, 37° C) and then assayed for apoptosis by DAPI-labeled nuclear morphology, by previously described methods (Wu KL *et al. Am J Physiol Renal Physiol* 284:F829-F839, 2003). * P <0.05 compared to camptothecin-treated wild type cells.



Supplementary Figure 6. *CD36 is not expressed in mouse proximal tubule*. Frozen sections of cortex from C57BL/6 (**A-B**) or *eNOS*$^{-/-}$ *db/db* mouse kidneys (**C**) were fixed in 4% paraformaldehyde, cryopreserved in sucrose, and then labeled as follows: **A**, anti-CD36 (1:100, 14 hrs, 4^{o} C) + Texas red-conjugated IgG, and FITC-conjugated *Tetragonolobus purpureus* lectin (1:100, 30-60 min, room temp) for proximal tubule identification; **B**, CD36 as in **A**, plus anti-aquaporin-1 (AQPI, 1:200, 14 hrs, 4^{o} C) for proximal tubule identification; **C**, anti-CD36 (1:100, 14 hrs, 4^{o} C) + FITC-conjugated IgG, and TRITC-conjugated *Tetragonolobus purpureus* (1:100, 30-60 min, room temp).



Supplementary Figure 7. *Cariporide-inhibitable NHE1 activity in mouse proximal tubule suspensions.* Because there is some EIPA overlap for NHE1 and NHE3 [in cultured cells overexpressing NHEs, IC_{50} = 1.5 x 10^{-8} M for NHE1; 2.4 x 10^{-6} M for NHE3 (Orlowski J *et al. J Biol Chem* 268:16369-16377, 1993), experiments were repeated with cariporide, which is more specific for NHE1. Mouse proximal tubule cell lines, which express NHE1, but not NHE3, demonstrated complete inhibition of Na^+/H^+ exchange with cariporide 1-10 µM, an intermediate concentration between the IC_{50} for NHE1 (0.8-8.0 x 10^{-8} M) and NHE3 (0.9-1.0 x 10^{-3} M) (Scholz W *et al. Cardiovasc Res* 29:260-268, 1995; Schwark JR *et al. Pflugers Arch* 436:797-800, 1998; Fischer H *et al. J Membr Biol* 168:39-45, 1999). Cariporide-inhibitable dpH/dt in the cell line (middle bar in histogram) was virtually identical to the value for EIPA-inhibitable proximal tubules in suspension (left bar). Cariporide (1 µM) preincubation resulted in partial inhibition of Na^+/H^+ exchange in mouse proximal tubule suspensions, and cariporide-inhibitable dpH/dt, shown in the right bar, is not significantly different compared to the EIPA-inhibitable dpH/dt value (left bar).