Supplementary Information to Boettger et al. (*to be put on EMBO J. web site*)

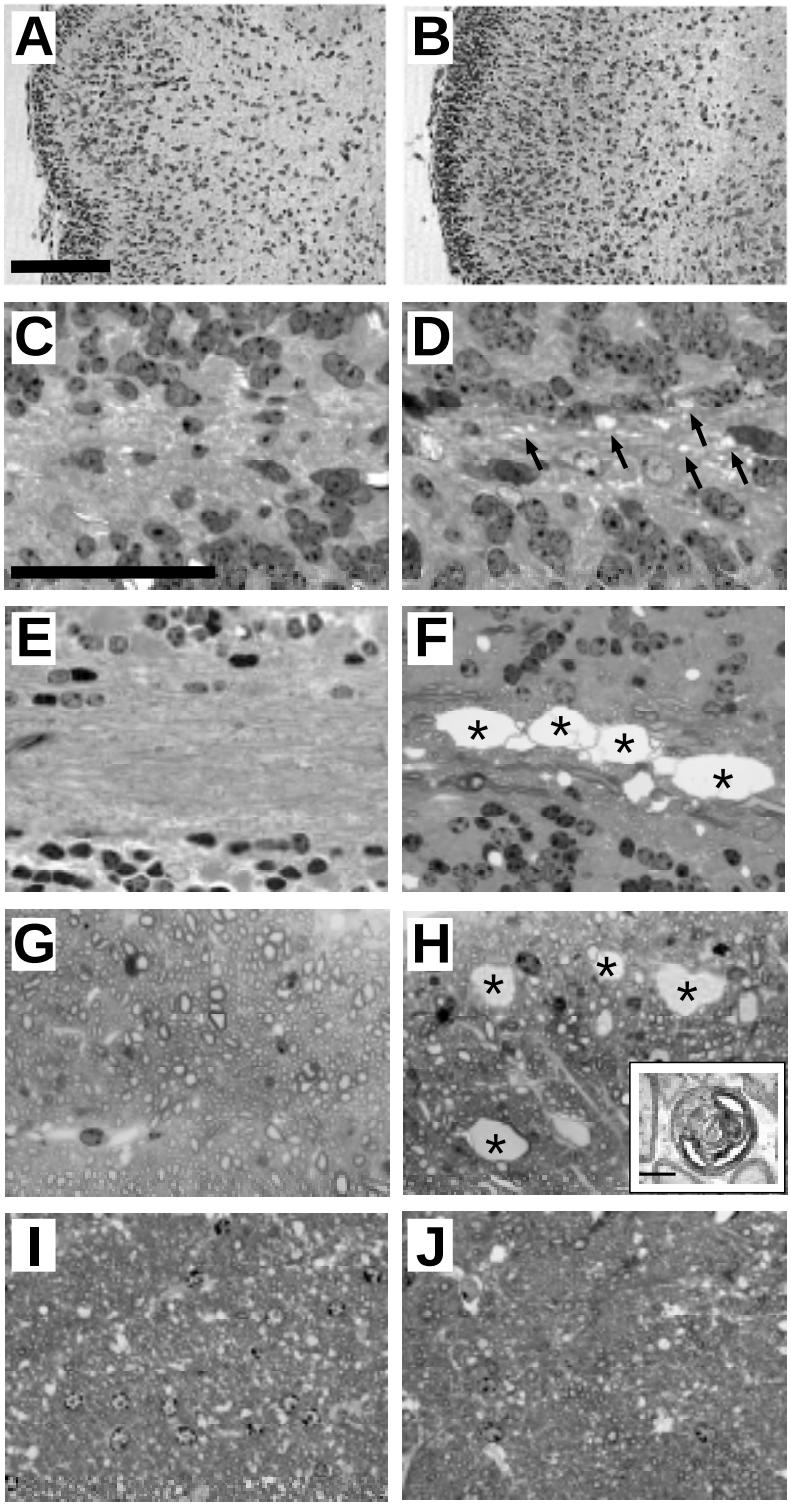

Figure S1. CNS degeneration in *Kcc3*$^{-/-}$ mice.
(**A**-**F**), cerebellum of WT (A,C,E) and KO (B,D,F) mice. Methylen blue stained semithin sections at P1 (A,B), P13 (C,D) and 16 months (E,F) old animals are shown. Note the ongoing degeneration and vacuolisation in the white matter in the knock-out animals. At P1, there are no signs of degeneration. At P13, swelling axons appear (arrows), whereas in adult animals the white matter is severely damaged (asterisk). (**G**, **H**) optic stalk of adult 16 months WT (G) and KO (H) mice. Many enlarged axons (asterisk) and brake down products as well as dense lamellar bodies (insert in H) were frequently found in the KO (H). (**I**, **J**), corpus callosum of 16 months WT (I) and KO (J) mice. No signs of degeneration could be detected. Scale bars A and B, 100µm; C-J, 50 µm; insert in H 10µm.

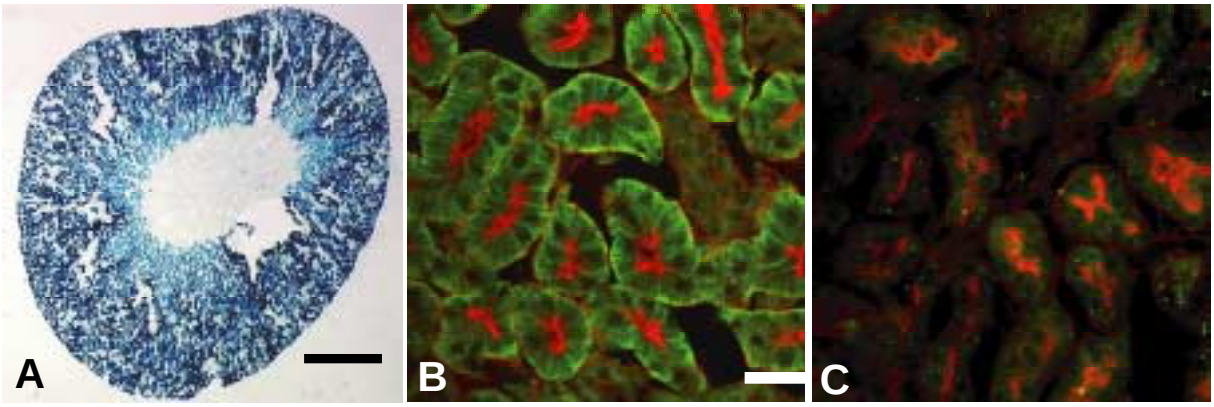

Figure S2. KCC3 expression in the kidney.
(**A**), lacZ staining from the of a kidney of an adult *KCC3*$^{+/-}$ kidney revealed a cortical expression pattern, compatible with predominant expression in proximal tubules.
(**B**), immunofluorescence analysis of the renal cortex. KCC3 (green) is present in the basolateral membranes of proximal tubular cells that were identified by staining their brush border with phalloidin in red.
(**C**), control staining of a *Kcc3*$^{-/-}$ kidney with the KCC3 antibody and phalloidin. It reveals that the proximal tubular staining in (B) was specific.
In other nephron segments, no specific staining for KCC3 could be detected (*data not shown*).
Scale bar in indicates 1mm in A and 19 µm in B, which also applies for C.