Supplementary Information

for ‚*Disruption of erythroid KCl-cotransporters alters erythrocyte volume and partially rescues erythrocyte dehydration in SAD mice*' by Rust et al.

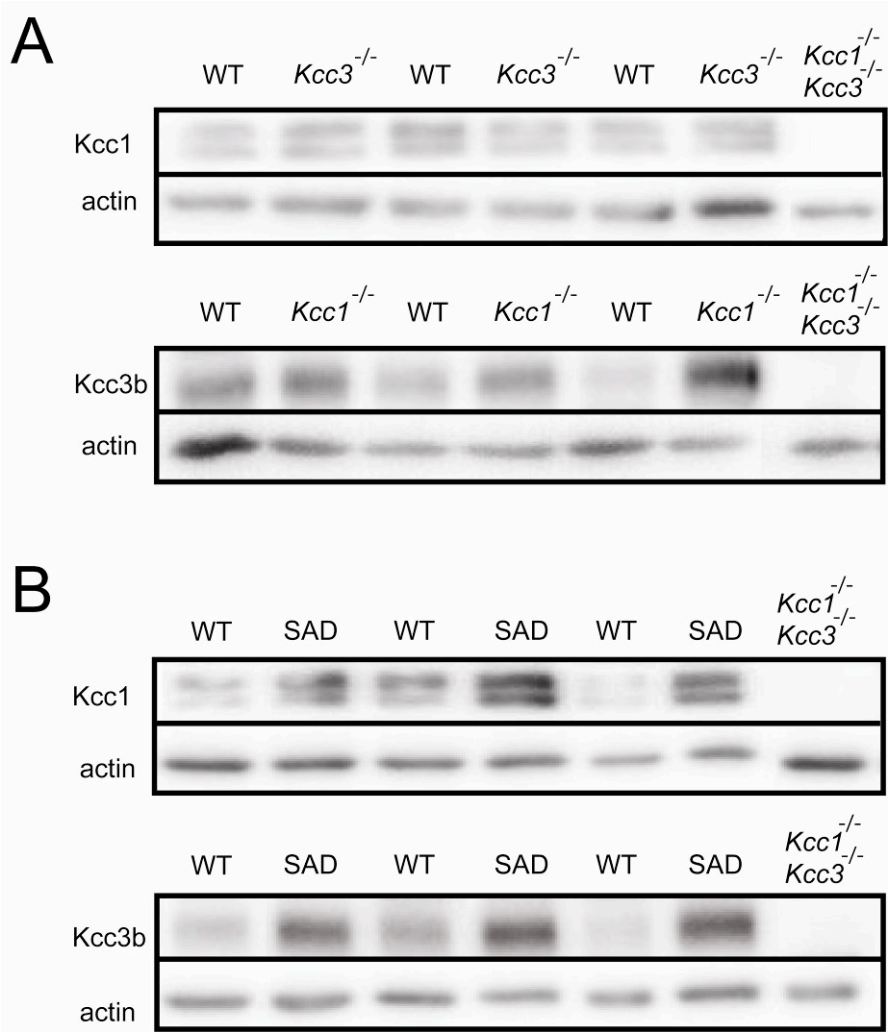

Figure S1. *Immunoblot analysis for KCC1 and KCC3 polypeptides of RBC ghosts* obtained from mice with different genotypes, with actin as a loading control. (A) KCC1 abundance is unchanged in *Kcc3*$^{-/-}$ ghost membranes, but KCC3 levels are higher in *Kcc1*$^{-/-}$ ghosts than in WT in all examined sample pairs. (B) Similar comparison of ghosts from WT and SAD mice show upregulation of both KCC1 and KCC3 polypeptides in SAD ghost membranes. Lanes represent samples from individual mice (i.e. 3 different pairs in each panel). These and additional experiments quantified with a CCD camera system were averaged to yield the data values presented in Figures 1F, G.

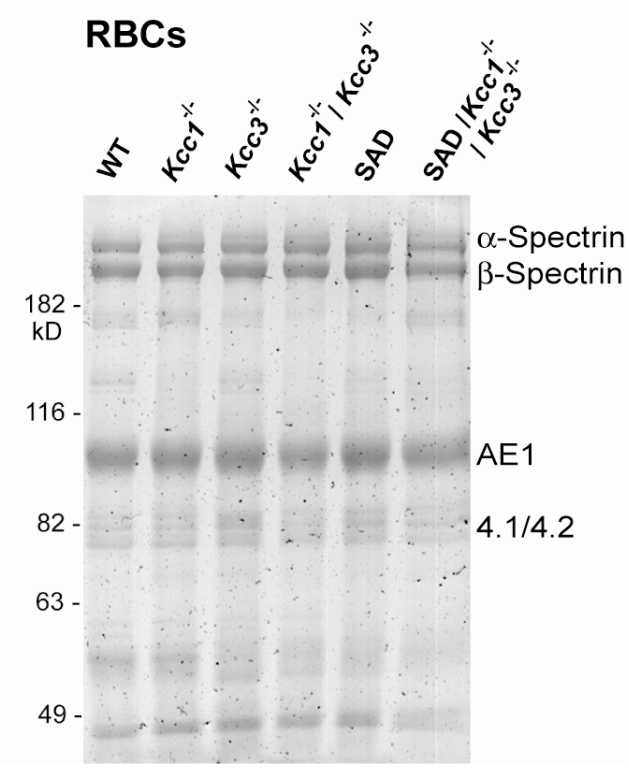

Figure S2. *PAGE analysis of RBC ghosts* from WT, *Kcc1*$^{-/-}$, *Kcc3*$^{-/-}$, *Kcc1*$^{-/-}$*/Kcc3*$^{-/-}$, SAD or SAD*Kcc1*$^{-/-}$*/Kcc3*$^{-/-}$ mice showed unaltered membrane protein composition as revealed by Coomassie Blue staining.

Genotype	n	Na^+ (mmol/kg Hb)	K^+ (mmol/kg Hb)
WT	9	33.7 ± 1.2	514 ± 11.2
Kcc1$^{-/-}$	8	30.6 ± 1.1	495 ± 21.8
Kcc3$^{-/-}$	7	32.1 ± 3.8	508 ± 15.3
Kcc1$^{-/-}$/*Kcc3*$^{-/-}$	8	24.9 ± 2.4	507 ± 11.4
SAD	6	24.6 ± 3.3	389 ± 6.7
SAD*Kcc1*$^{-/-}$	7	25.9 ± 1.2	388 ± 20.6
SAD*Kcc3*$^{-/-}$	9	22.5 ± 2.4	427 ± 14.2
SAD*Kcc1*$^{-/-}$/*Kcc3*$^{-/-}$	5	32.7 ± 1.4	475 ± 20.2

Table S1. *Cation concentrations in RBCs of various genotypes.* RBC Na^+ contents differ neither among WT and strains lacking one or both *Kcc* gene product(s), nor among SAD and SAD strains lacking one or both *Kcc* gene product(s), as judged by ANOVA corrected for multiple comparisons. RBC K^+ contents similarly did not differ among WT and strains lacking one or both *Kcc* gene product(s), but the reduced K^+ content of SAD RBC compared to WT ($P<0.00005$), though not rescued by isolated deficiency of either Kcc1 or Kcc3, was rescued by combined lack of Kcc1 and Kcc3 ($P<0.002$).