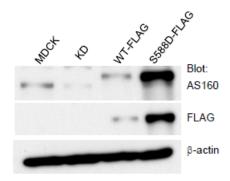
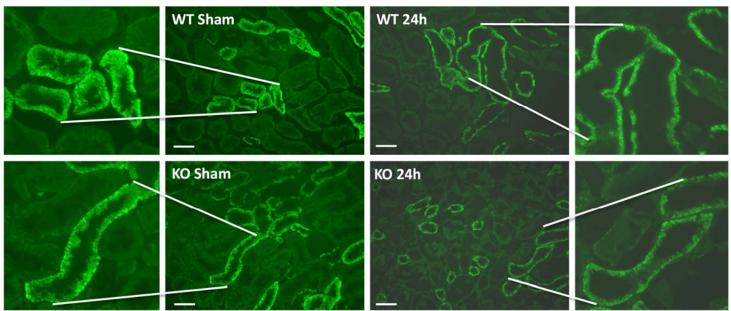
Supplemental data 1



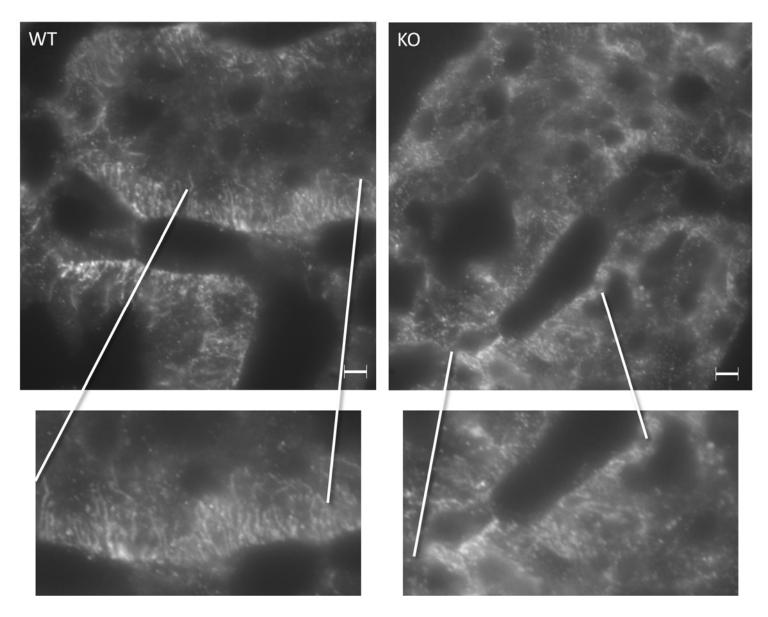
Supplemental data 1. AS160 knock down cell lines stably expressing AS160 WT-FLAG and the S588D-FLAG mutant form of AS160. Lysates from WT MDCK cells, AS160 knockdown MDCK cells (KD) and AS160 knockdown MDCK cells stably transfected with human AS160 WT-FLAG (WT-FLAG) or AS160 S588D-FLAG (S588D-FLAG) were immunoblotted with anti-FLAG and anti-AS160 antibodies to detect the levels of exogenous and endogenous AS160, respectively. The levels of endogenous AS160 were robustly decreased in the AS160 KD, WT-FLAG and S588D-FLAG cell lines. The β -actin immunoblot demonstrates that equal levels of total protein were loaded.

Supplemental Data 2



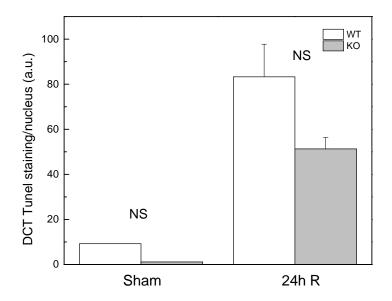
Supplemental Data 2. Renal ischemia-induced redistribution of Na,K-ATPase is reduced in the DCTs of AS160 knockout mice, low magnification images. Images depicting the distribution of the Na,K-ATPase in renal tissue derived from wild type (WT) and AS160 knockout (AS160 KO) mice subjected to sham surgery (Sham) or to ischemia followed by 24 hours of reperfusion (24h). The scale bars in lower left hand corners of each of the four central panels correspond to 50 μ. The insets correspond to higher magnifications of the indicated portions of the fields in each of the four central panels. Na,K-ATPase labeling associated with basolateral plasma membrane infoldings is present under sham conditions in tissue derived from both genotypes, as well as in the tissue derived from AS160 knockout mice subjected to ischemia and 24 hours of reperfusion. This pattern is lost in tissue derived from wild type mice subjected to ischemia and 24 hours of reperfusion, and is replaced by a largely intracellular pattern of staining.

Supplemental Data 3



Supplemental Data 3. Na,K-ATPase mis-localization after renal injury is similar in the proximal tubules of WT and AS160 knockout mice. Immunofluorescence images of kidney cortex stained with an antibody directed against Na,K-ATPase α -subunit (α 5). Wild type (WT) and AS160 KO (KO) mice were subjected to 30 min of bilateral renal pedicle clamping followed by 24 h of reperfusion (24h R). Na,K-ATPase appears to be redistributed

Supplemental Data 4



Supplemental Data 4. TUNEL analysis indicates that WT and AS160 KO mice exhibit similar levels of post-ischemic apoptosis. Cryostat sections from WT and AS160 KO animals subjected to sham or ischemia surgery followed by 24hs of reperfusion were analyzed by Tunel staining and the prevalence of positive-staining DCT cell nuclei was quantified in confocal images. Wild type and AS160 KO mice show similar levels of apoptosis in DCTs. NS: not significant.