## Supplemental data

## Supplemental Figure 1. Characterization of opossum kidney cell line stably transfected with rat

 AQP1 (AQP1-OKC). (A) AQP1-OKC form cilia and microvilli as demonstrated by staining of microtubules (red) and actin filaments (green). Magnification: scale bar $=10 \mu \mathrm{~m}$. (B) Western blot of AQP1-OKC (lane 1), untransfected OK cells (OKC; lane 2), and mouse kidney BBM fraction (lane 3). Typical AQP1 signal is observed in lane 1 and 3 at 28 kDa (unglycosylated AQP1) and 35-50 kDa (glycosylated AQP1).Supplemental Figure 2. Calcein fluorescence quenching. (Left) Calcein-loaded AQP1-OKC were stimulated for 1 hour with FSS, 8-bromo-cGMP or 8-bromo-cAMP, and calcein fluorescence was monitored. Representative curves show the time-course of changes in calcein fluorescence in response to a switch in solution osmolality from 500 to 150 mOsmol. (Right) AQP1-OKC grown in flow chambers were calcein-loaded and various rates of fluid-shear stress were applied for 1 h . Representative curves showing the time-course of changes in calcein fluorescence in response to a switch in solution osmolality from 500 to 150 mOsmol .

Supplemental Table 1. AQP1 mRNA abundance. AQP1 mRNA was analyzed by real time PCR. $\Delta \Delta C T$ values of AQP1 and the house keeping gene, GAPDH, of $\operatorname{Lrp} 2^{\mathrm{fl/fl}} ; \mathrm{apoE}^{\mathrm{Cre}}, \mathrm{Clcn}^{\mathrm{fl} / \mathrm{y}} ; \mathrm{villin}^{\mathrm{Cre}}$, Clcn5 ${ }^{+/ y}$, and Cclcn5 ${ }^{-1 y}$ mice are shown. Values are presented as means $\pm$ SD; $n=6$. AQP1 mRNA is not different between groups.

## Supplemental Figure 1



## Supplemental Figure 2



## Supplemental Table 1

|  | Calculations of AQP1 mRNA $\triangle \triangle C T$ |
| :---: | :---: |
| $\operatorname{Lrp2}^{\text {f//fl }}$ vs. Lrp2 $^{\text {t//fl }} ; \mathrm{apoE}^{\text {Cre }}$ | $0 \pm 1.2 \%$ vs. $-0.02 \pm 0.8 \%$ |
| Clcn5 ${ }^{\text {t/y }}$ vs. Clcn $^{\text {fl/y }}$; villin ${ }^{\text {Cre }}$ | $0 \pm 0.3 \%$ vs. $-0.04 \pm 0.26 \%$ |
| Clan5 ${ }^{\text {+/y }}$ vs. Clan5 $^{-1 / y}$ | $0 \pm 0.2 \%$ vs. $0 \pm 0.26 \%$ |

