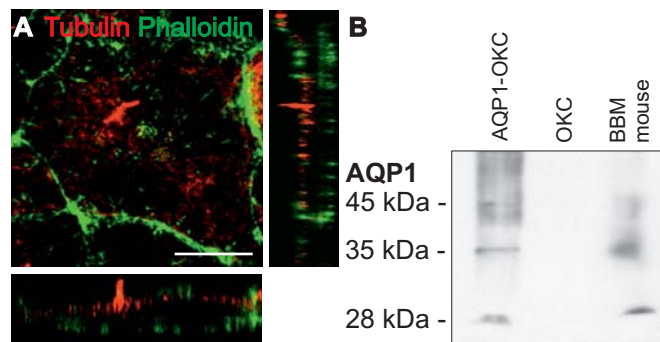## Supplemental data

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 μm. (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 1h. 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. $\Delta\Delta$CT values of AQP1 and the house keeping gene, GAPDH, of Lrp2$^{fl/fl}$;apoE^{Cre}, Clcn5$^{fl/y}$;villin^{Cre}, Clcn5$^{+/y}$, and Cclcn5$^{-/y}$ mice are shown. Values are presented as means ± SD; n = 6. AQP1 mRNA is not different between groups.

Supplemental Figure 1

Supplemental Figure 2
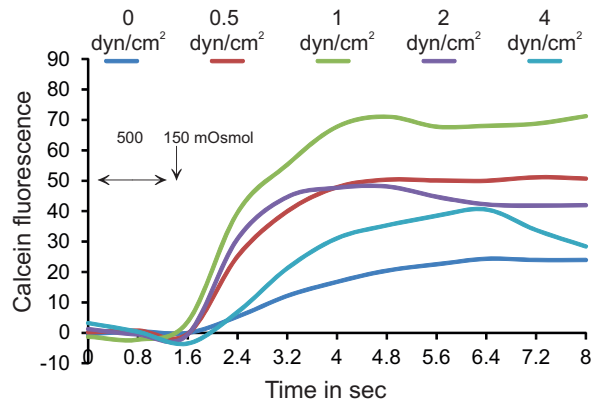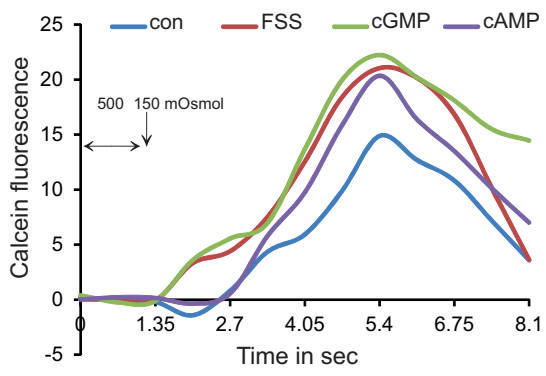
Supplemental Table 1

	Calculations of AQP1 mRNA ΔΔCT
Lrp2$^{fl/fl}$ vs. Lrp2$^{fl/fl}$;apoE^{Cre}	0 ± 1.2% vs. −0.02 ± 0.8%
Clcn5$^{fl/y}$ vs. Clcn5$^{fl/y}$;villin^{Cre}	0 ± 0.3% vs. −0.04 ± 0.26%
Clcn5$^{+/y}$ vs. Clcn5$^{-/y}$	0 ± 0.2% vs. 0 ± 0.26%