Supplemental Material

Fluconazole increases osmotic water transport in renal collecting duct through effects on aquaporin 2 trafficking

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Running Title: Fluconazole causes antidiuresis

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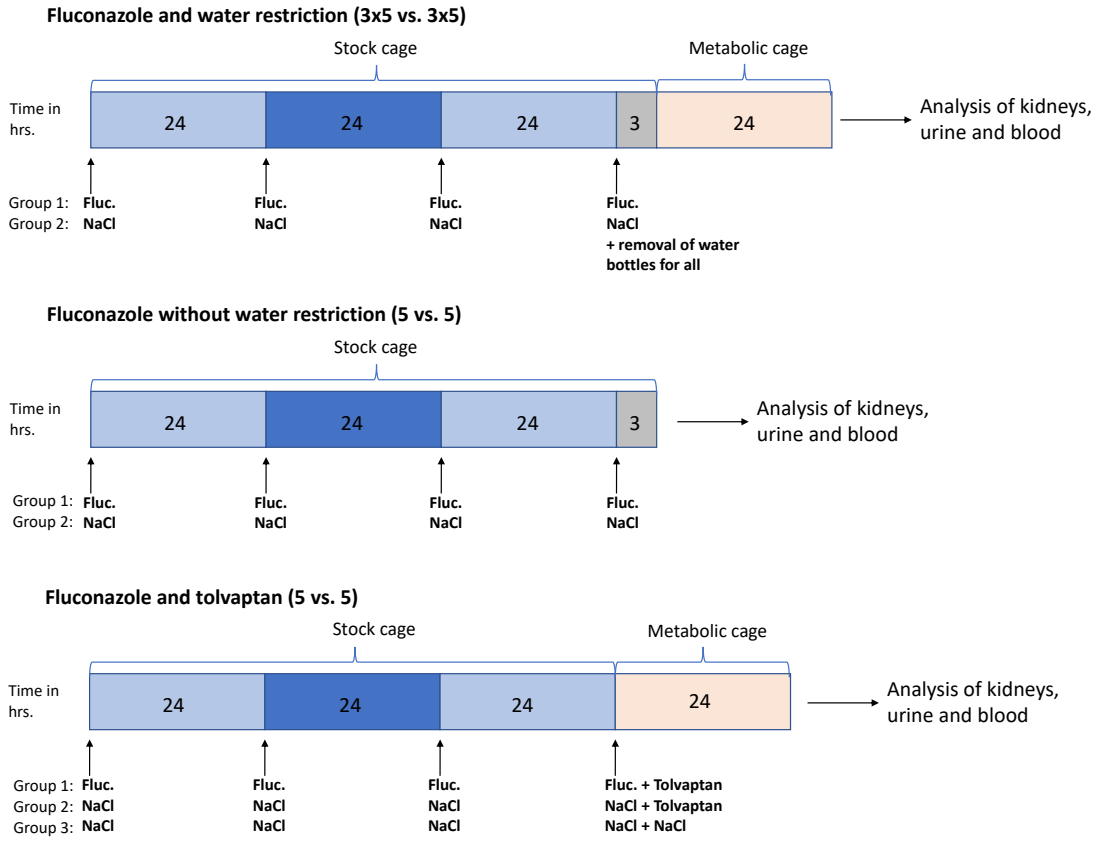
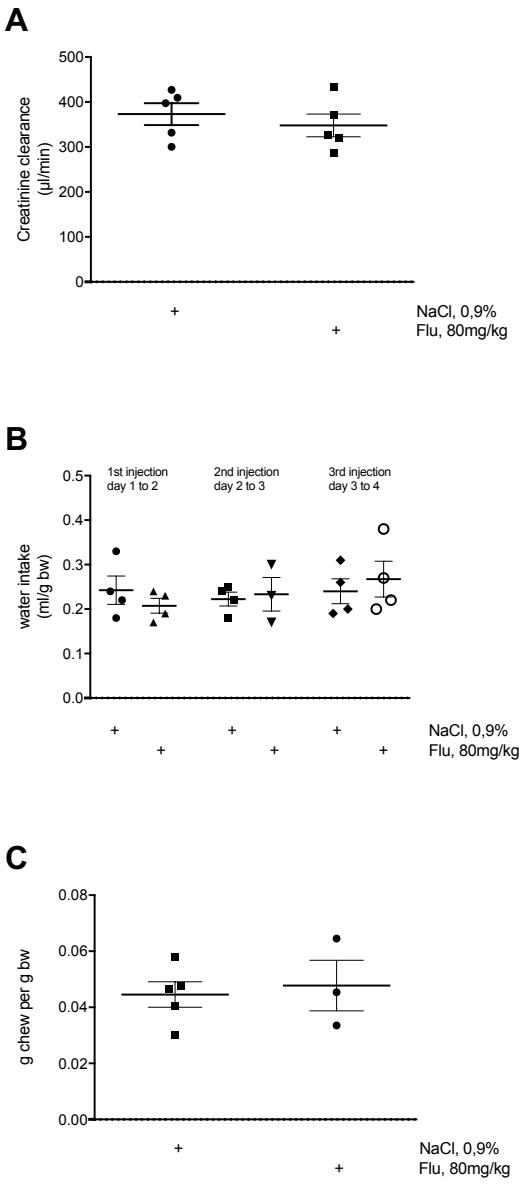
Content

Supplemental Figure 1



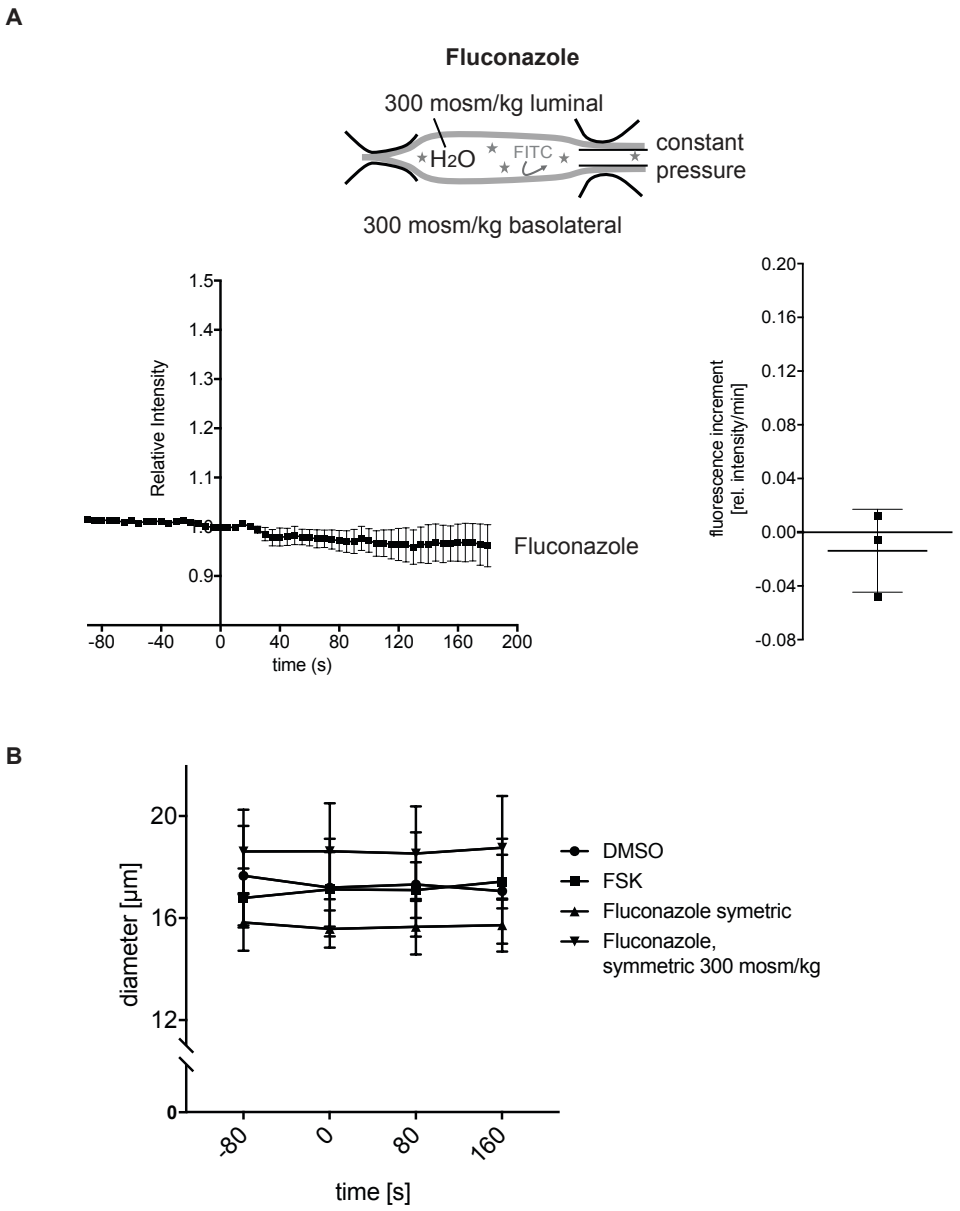
Supplemental Figure 1: Scheme depicting regimes of mouse treatments.

Supplemental Figure 2



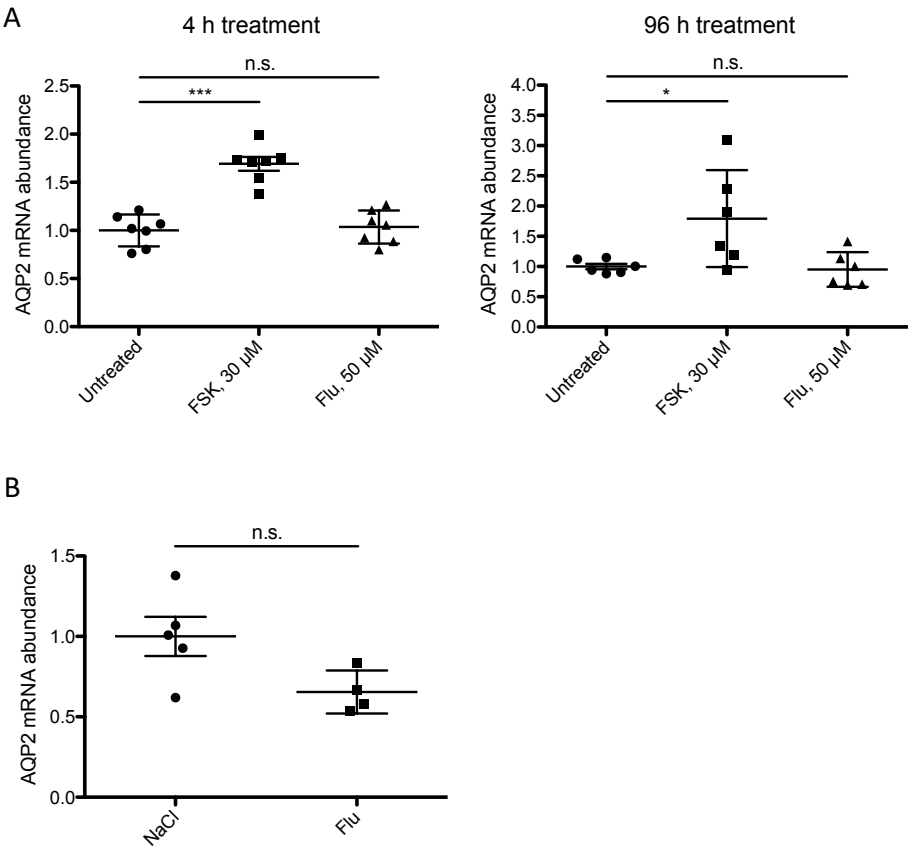
Supplemental Figure 2: Fluconazole does not affect (A) creatinine clearance, (B) water or (C) food intake of mice. The creatinine clearance in baseline male mice was calculated as a measure for the glomerular filtration rate (GFR) according to Dunn et al., 2004.[17]

Supplemental Figure 3



Supplemental Figure 3. (A) Without trans-epithelial osmotic gradient the application of 50 µM fluconazole does not induce trans-epithelial water flow across epithelia of isolated CCDs. The scheme depicts the experimental setting; control solution with 300 mosm/kg (145 mM NaCl, 0.4 mM KH_2PO4, 1.6 mM K_2HPO4, 1 mM $MgCl_2$, 1.3 mM Ca-gluconate, 5 mM Glucose, pH 7.4) on both sides, luminally supplemented with 50 µM 150 kDa FITC dextran. Change of relative fluorescence intensity over time, application of basolateral 50 µM fluconazole at time point 0s. Fluorescence increment values for n = 3 CCD of 3 C57Bl6J mice (8 weeks). **(B) Diameter of mouse CCDs during water flux measurements at a given osmotic gradient (300/150 mosm/kg) does not change in response to DMSO, fluconazole (50 µM) or forskolin (30 µM).** Luminal diameters were taken at time point -80s, 0 s, 80s and 160s. There was no systematic change in the diameter over time or after applications of the agents.

Supplemental Figure 4



Supplemental Figure 4. Fluconazole does not alter AQP2 mRNA abundance in primary IMCD cells and mouse kidney. Semiquantitative analysis of the effects of forskolin and fluconazole on AQP2 mRNA levels. (A) IMCD cells were left untreated or stimulated with forskolin (FSK, 30 µM) or fluconazole (Flu, 50 µM) for 4 h or every 24 h over a period of 96 h. Relative levels of AQP2 mRNA were determined by real-time PCR. Shown are means ± SEM of three independent experiments with duplicates each, *p=0.0169, ***p<0.0001. (B) Non water-deprived mice were treated every 24 h over a period of 96 h with fluconazole (i.p. injections; 80 mg/kg) or with 0.9% NaCl. Whole kidney lysates were prepared and relative levels of AQP2 mRNA were determined by real-time PCR. n = 5 animals per NaCl group and n = 4 animals per fluconazole group (mean ± SEM).