SUPPLEMENTAL FIGURES

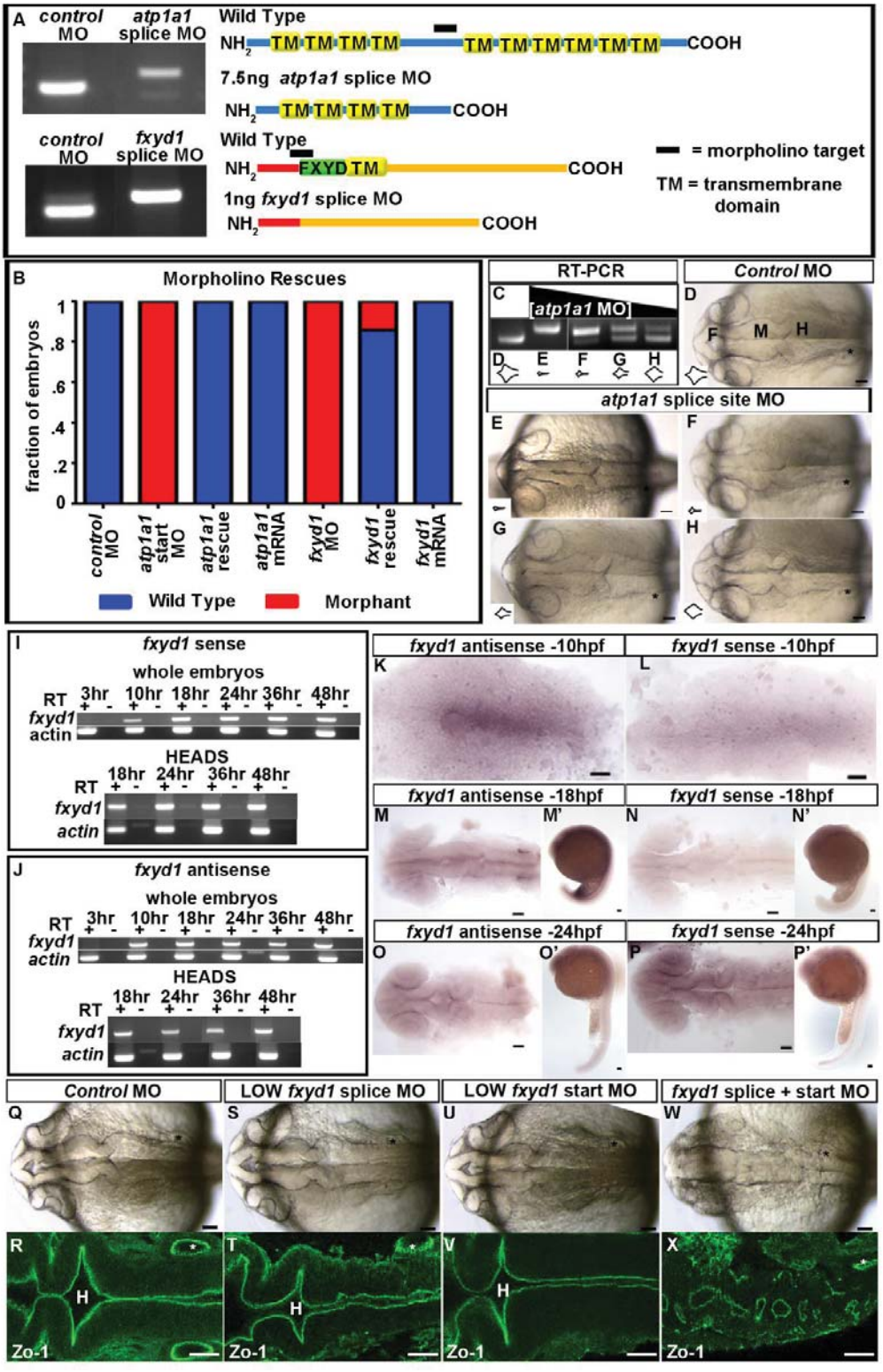


Fig. S1: Characterization of Na,K-ATPase phenotypes.

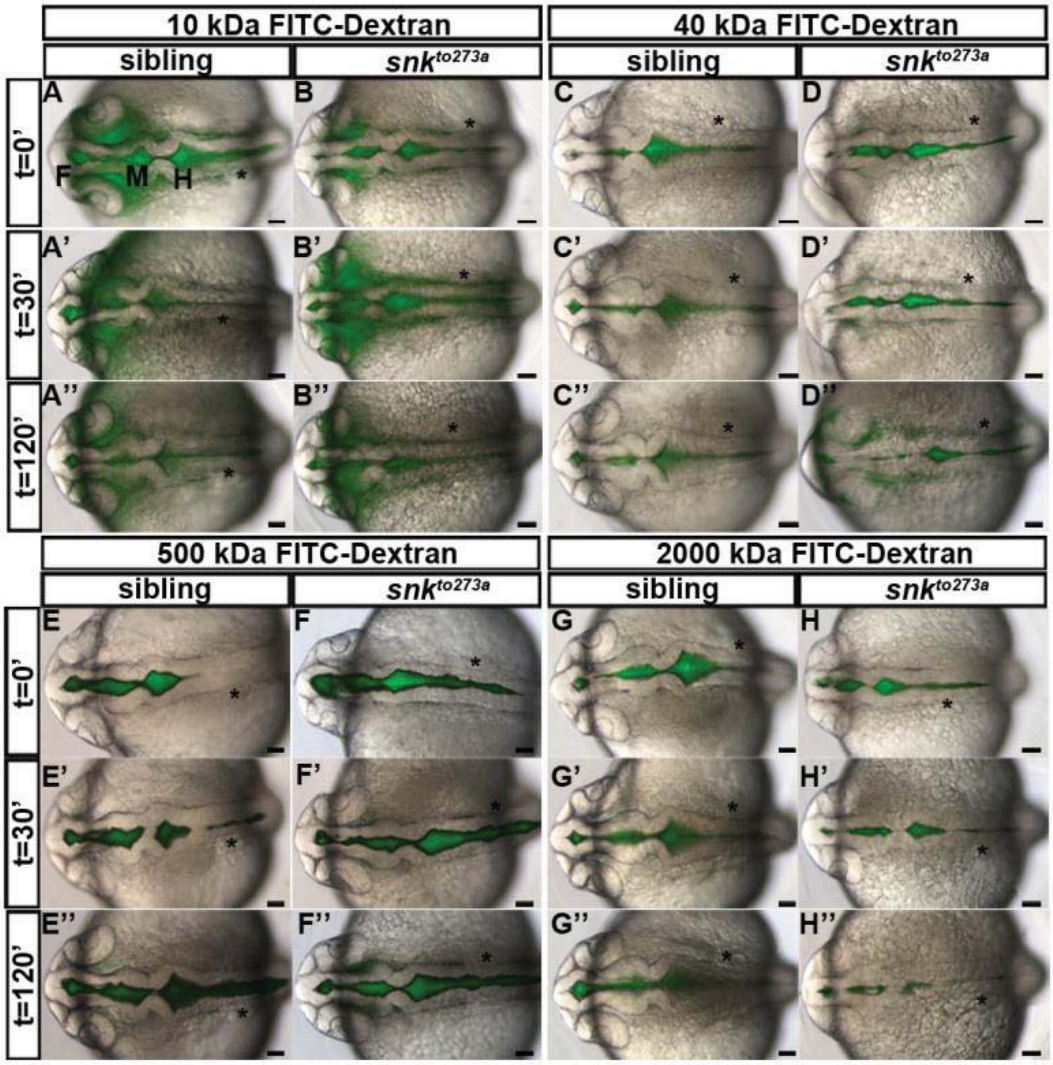


Fig. S2: Selective permeability of the neuroepithelium.

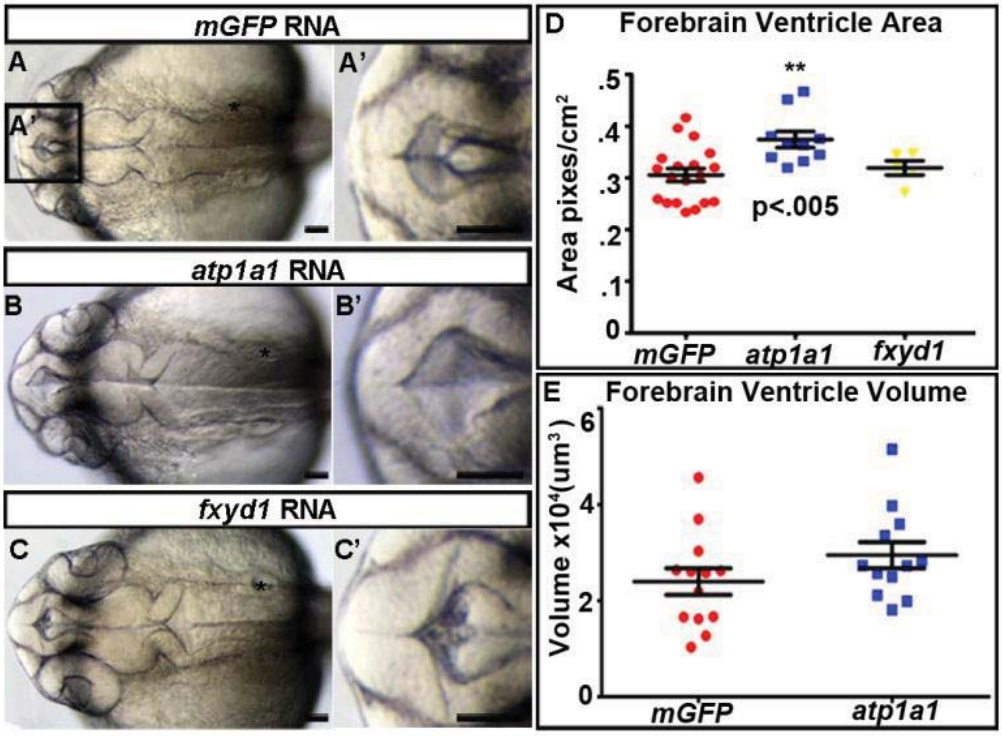


Fig. S3: Atp1a1 regulates brain ventricle size.

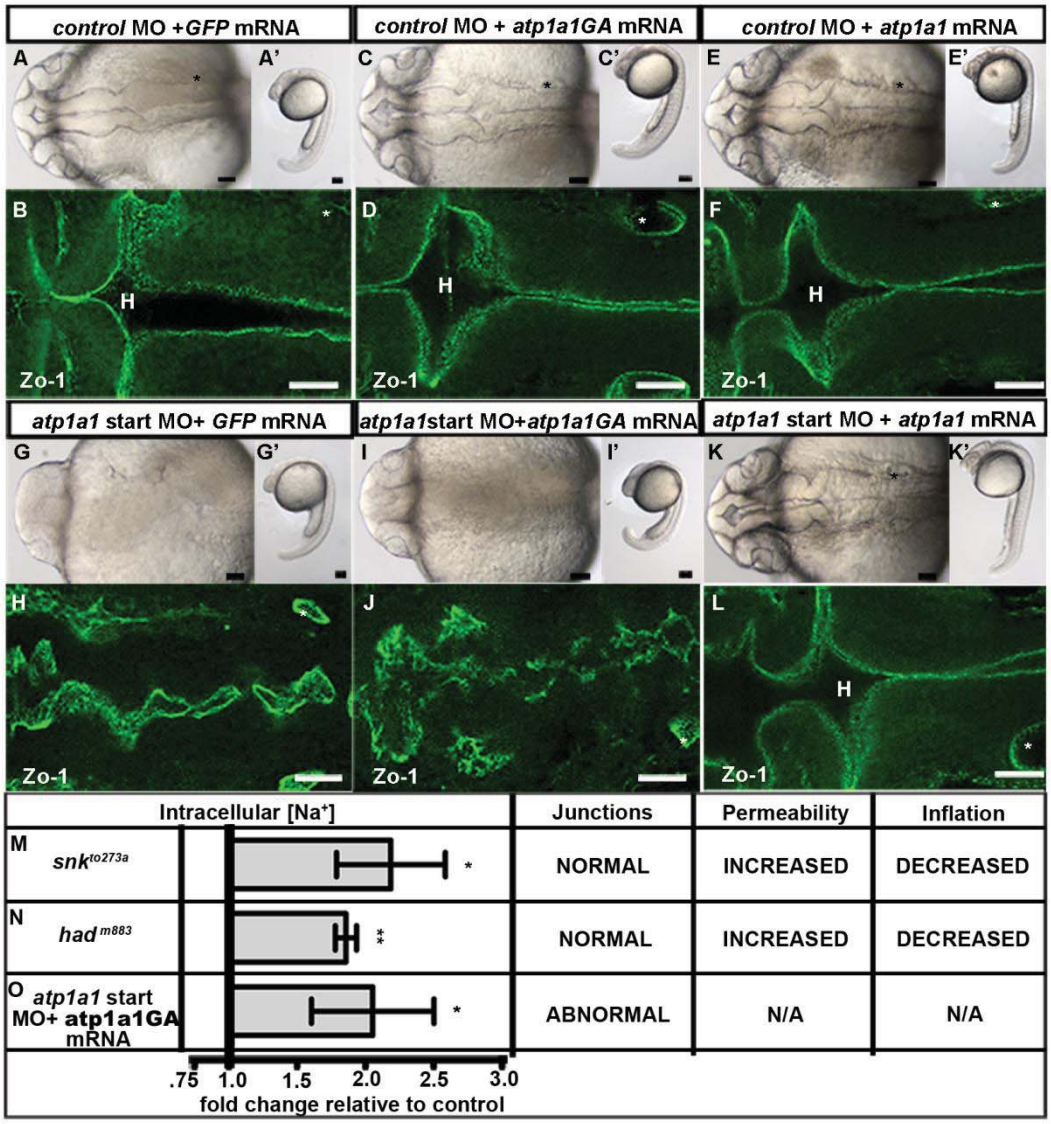


Fig. S4: Na,K-ATPase pumping is required for brain ventricle development.

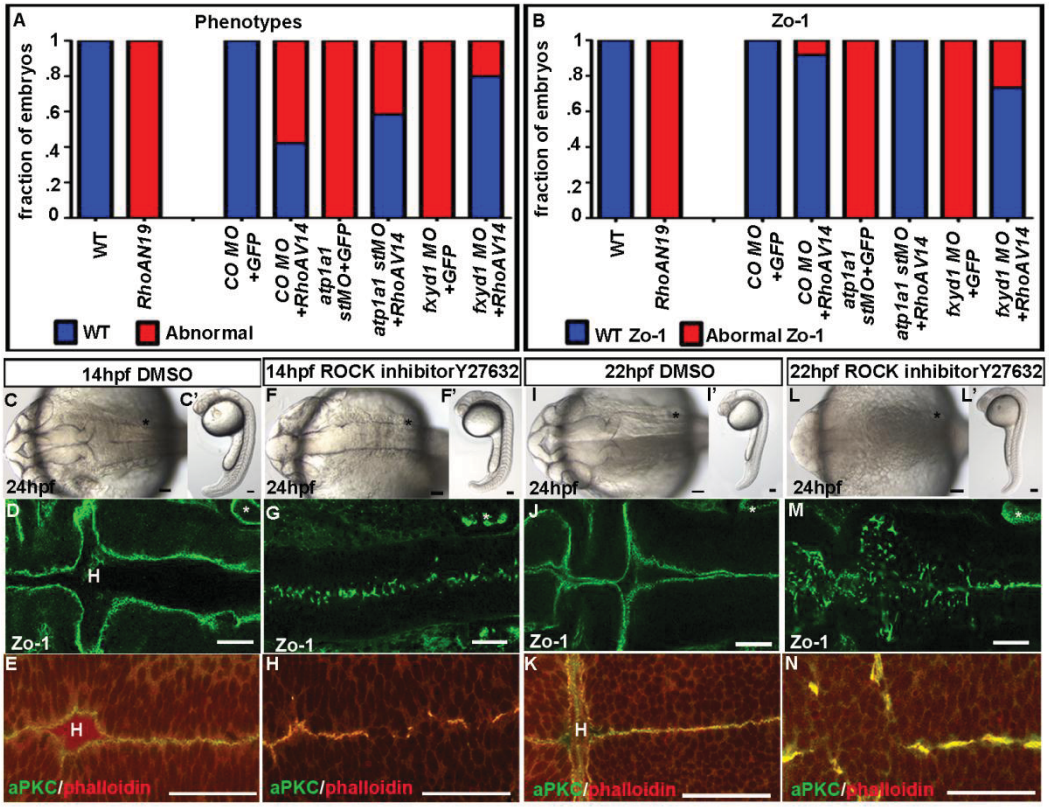


Fig. S5: RhoA and ROCK regulate neuroepithelial formation.

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1: Characterization of Na,K-ATPase phenotypes. (A) Na,K-ATPase morpholinos disrupt wild type splicing. TM = transmembrane domain. Black line = morpholino target. (B) Quantification for rescue of morphant phenotypes in control MO. (C) RT-PCR of differing levels of *atp1a1* splice site MO and the corresponding forebrain ventricle tracings of embryos in D-H. (D-H) Dose dependent regulation of brain ventricle inflation by *atp1a1* splice site MO. (I-J) RT-PCR analysis of *fxyd1* sense (non-coding) (I) and antisense (protein coding) (J) expression from 3 hpf – 48 hpf in whole embryos or dissected heads. (K-P) Dorsal (K-P) and lateral (M'-P') views of in situ hybridization of *fxyd1* antisense (K,M,O) and sense (L,N,P) expression from 10-24 hpf. (Q-X) *fxyd1* start and splice site MO synergy brightfield (Q,S,U,W) and Zo-1 (R,T,V,X) in control (Q-R), low levels of *fxyd1* splice (S-T) or start (U-V) site MO or a combination of low levels of *fxyd1* splice and low levels of *fxyd1* start site MO (W-X). All images taken at 24 hpf with anterior to left, asterisk = ear, F = forebrain, M = midbrain, H = hindbrain. Scale bar = 50μm.

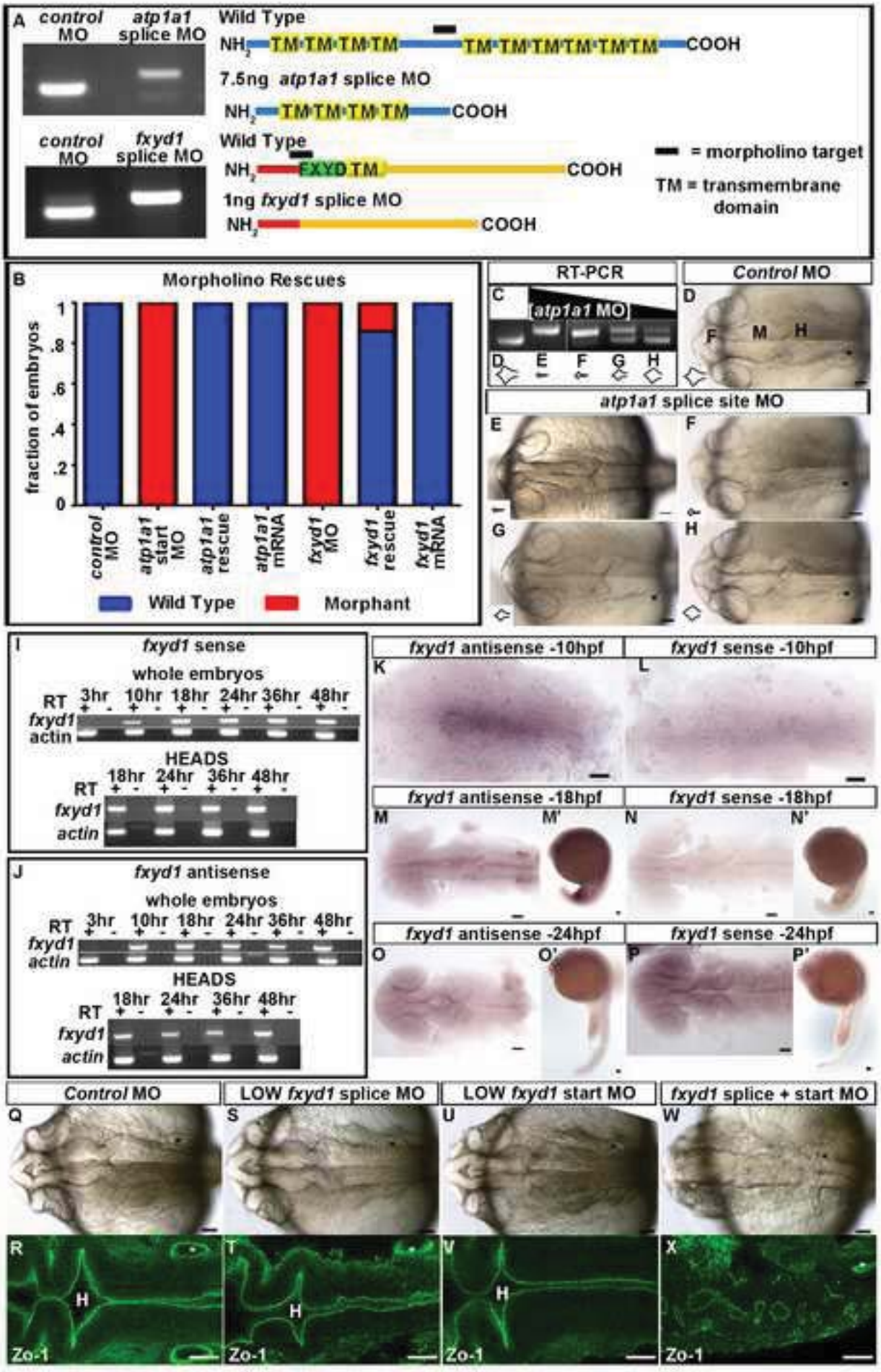
Fig. S2: Selective permeability of the neuroepithelium. (A-H) Neuroepithelial permeability in sibling (A,C,E,G) and snk^{to273} (B,D,F,H) embryos. Brain ventricle injection of FITC-Dextran dye of molecular weight 10 (A-B), 40 (C-D), 500 (E-F) or 2000 kDa (G-H) at t = 0 (A-H), 30 (A'-H') and 120 min (A''-H''). All images 22-24 hpf with anterior to left. Asterisk = ear. F = forebrain, M = midbrain, H = hindbrain. Scale bars = 50μm.

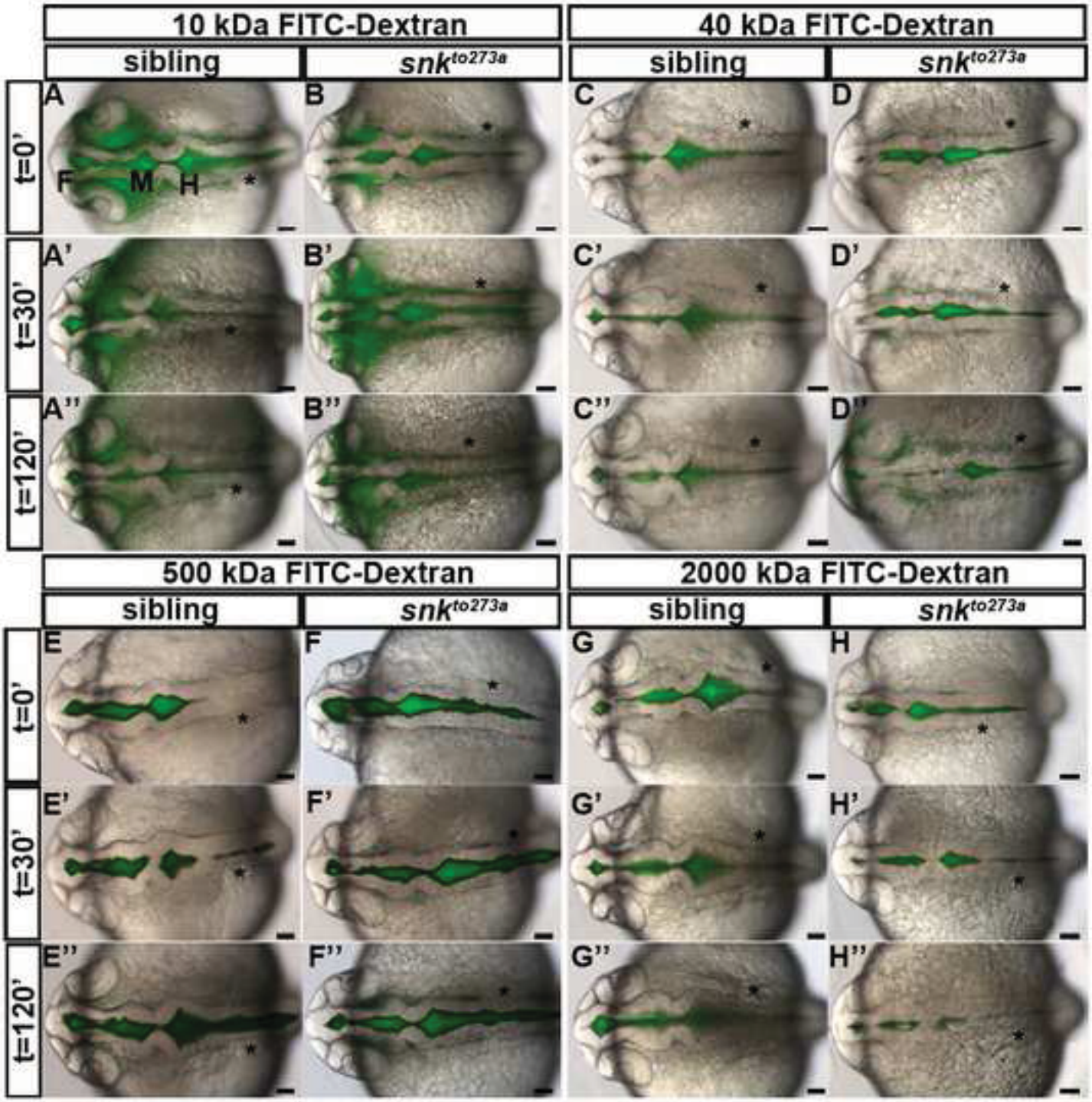
Fig. S3: Atp1a1 regulates brain ventricle size. (A-C) Dorsal brightfield views (A-C) and magnified forebrain ventricles (A'-C') of control mRNA (*mGFP*) (A) *atp1a1* mRNA (B) and *fxyd1* mRNA (C). (D-E) Quantification of forebrain ventricle area (D) and volume (E). Taken from 5-7 independent experiments and represented as mean +/- SEM. **= p<.005 compared to control (mGP). All images taken at 24 hpf with anterior to left, asterisk= ear. Scale bar = 50μm

Fig. S4: Na,K-ATPase pumping is required for brain ventricle development. (A-L) *atp1a1GA* mutant mRNA does not rescue *atp1a1* start site morphants. Control MO plus control (*GFP)* (A-B), *atp1a1GA* (C-D) or wild type *atp1a1* (E-F) *mRNA* or *atp1a1* start site MO plus *GFP* (G-H) *atp1a1GA* (I-J) or wild type *atp1a1* (K-L) *mRNA*. Brightfield dorsal (A-K) and lateral (A'-K') images or Zo-1 staining (B,D,F,H,J,L). (M-O) Quantification of fold changes in $[Na^+]_i$ and

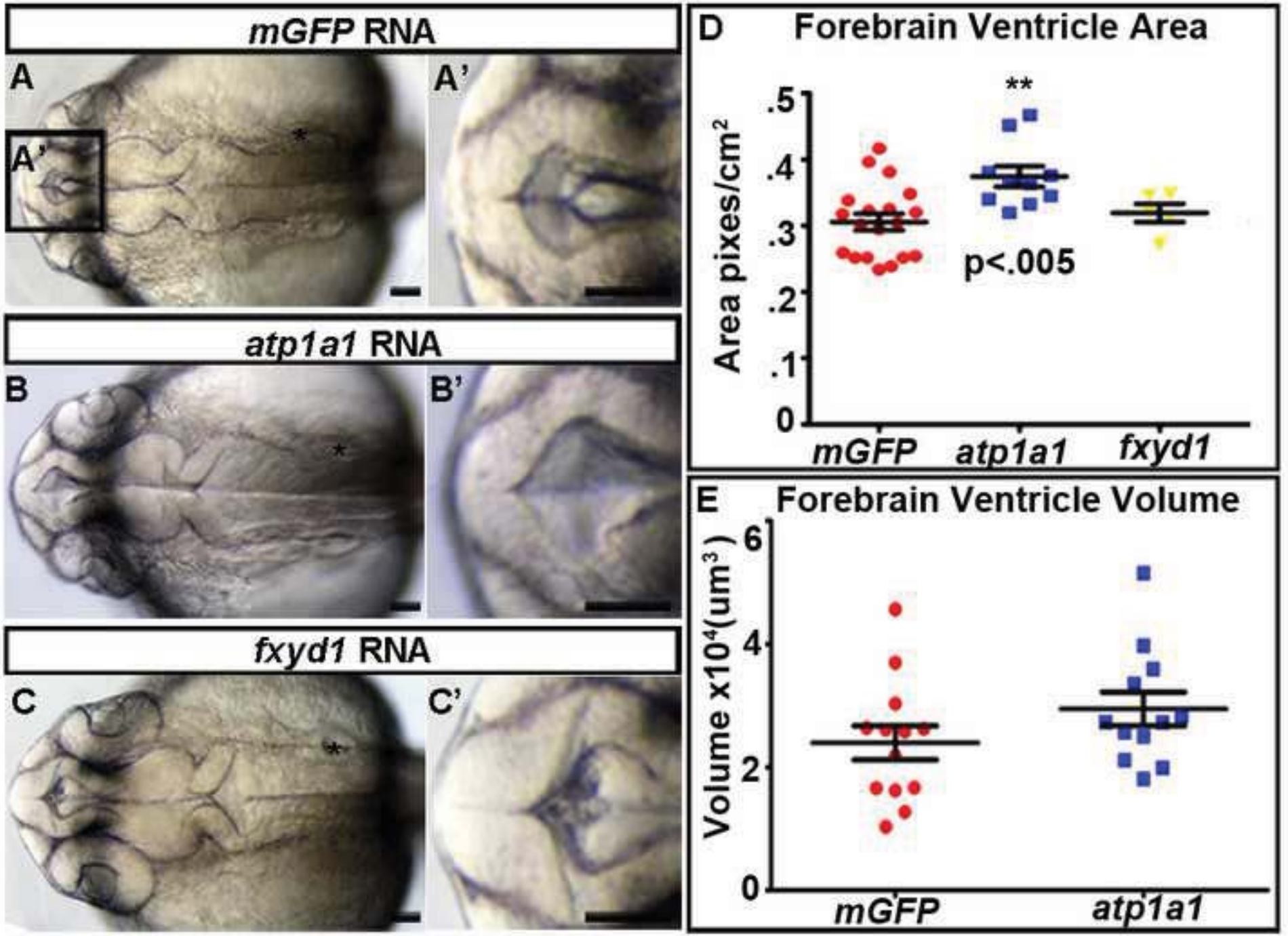
corresponding brain ventricle phenotype. Data from 3-8 independent experiments represented as fold change compared to control MO (equal to 1) plotted as a mean +/- SEM. *= P<.05, **= p<.005 compared to control. All embryos at 24 hpf, anterior to left; Asterisk = ear. Scale bars = 50μm.

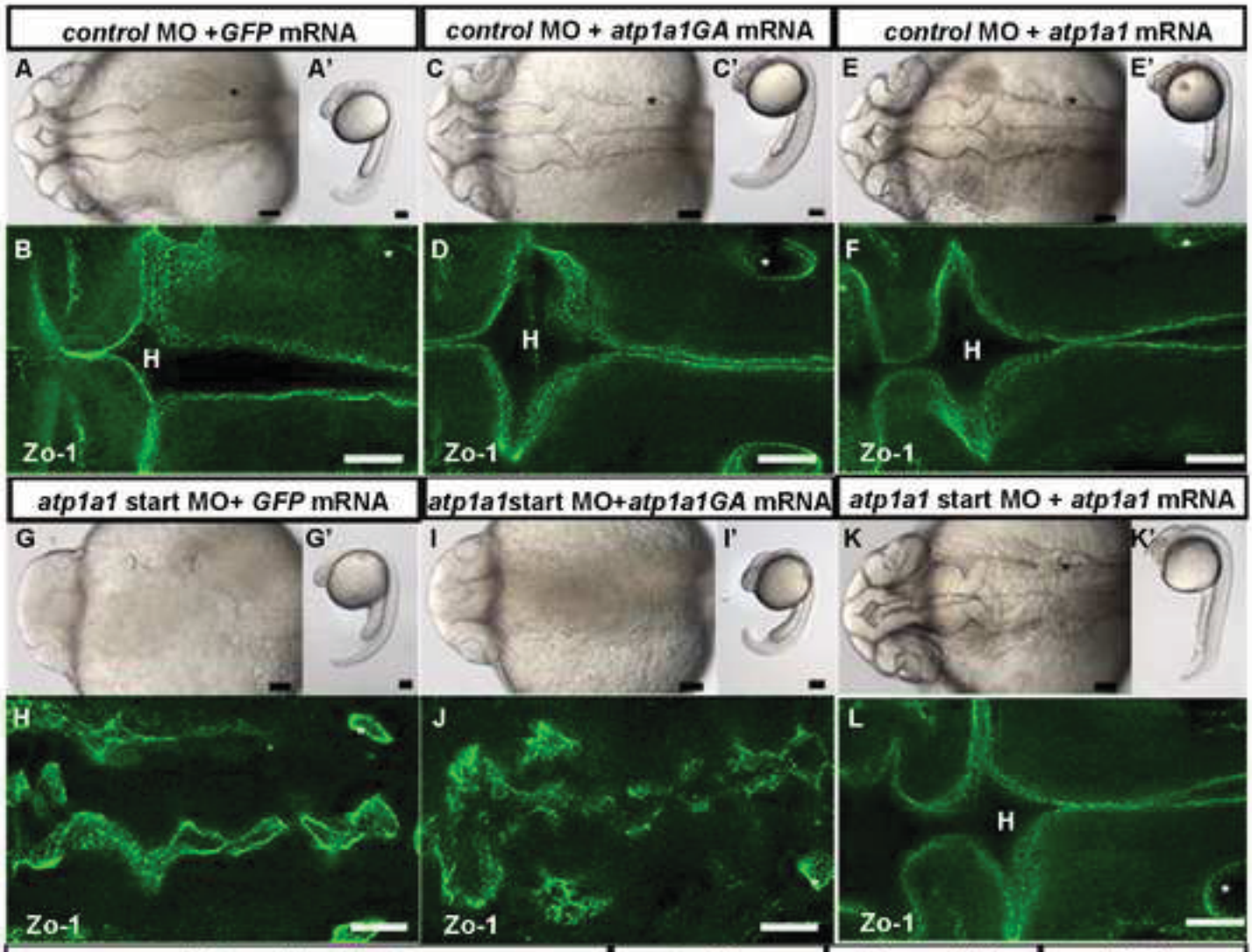
Fig. S5: RhoA and ROCK regulate neuroepithelial formation. (A-B) Quantification of wild type vs. abnormal embryos for brain ventricle inflation phenotype (A) and neuroepithelial formation (Zo-1) (B) in *RhoAN19* injected embryos and *RhoAV14* rescue of *atp1a1* start site and *fxyd1* morphants. *GFP, RhoAN19, and RhoAV14* all mRNA. **(C-N)** Inhibition of ROCK, using the drug Y27632, disrupts neuroepithelial formation. Brightfield dorsal (C,F,I,L) and lateral (C',F'I',L') views, Zo-1 staining (D,G,J,M) and aPKC (green)/phalloidin (actin, red) staining (E,H,K,N) in embryos treated with DMSO or ROCK inhibitor at 14 (C-H) or 22 hpf (I-N). All images taken at 24 hpf with anterior to left. Asterisk = ear, H = Hindbrain. Scale bars = 50μm.





Supplemental Figure 3





Supplemental Figure 5