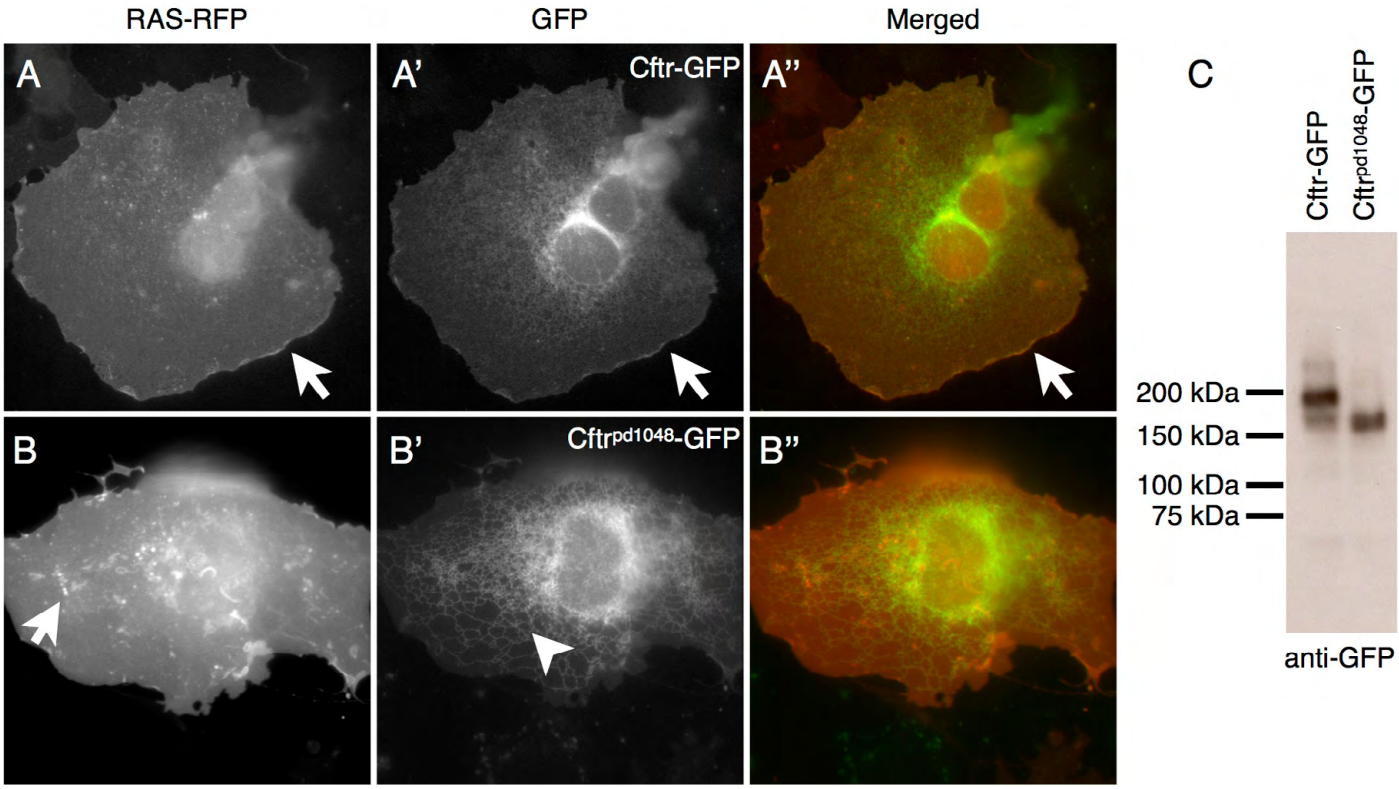

Fig. S1. Cftr^{pd1048}-GFP is mislocalized. (**A-A″**) Zebrafish Cftr-GFP expressed in Cos-7 cells is predominantly localized to the plasma membrane. (**B-B″**) The Cftr^{pd1048}-GFP fusion protein has a reduction in plasma membrane localization. Arrows point to membrane localization, arrowheads denote the internal pool. (**C**) A western blot for GFP detects Cftr-GFP and Cftr^{pd1048}-GFP expressed from HEK293 cells. Scale bars: 50 µm.

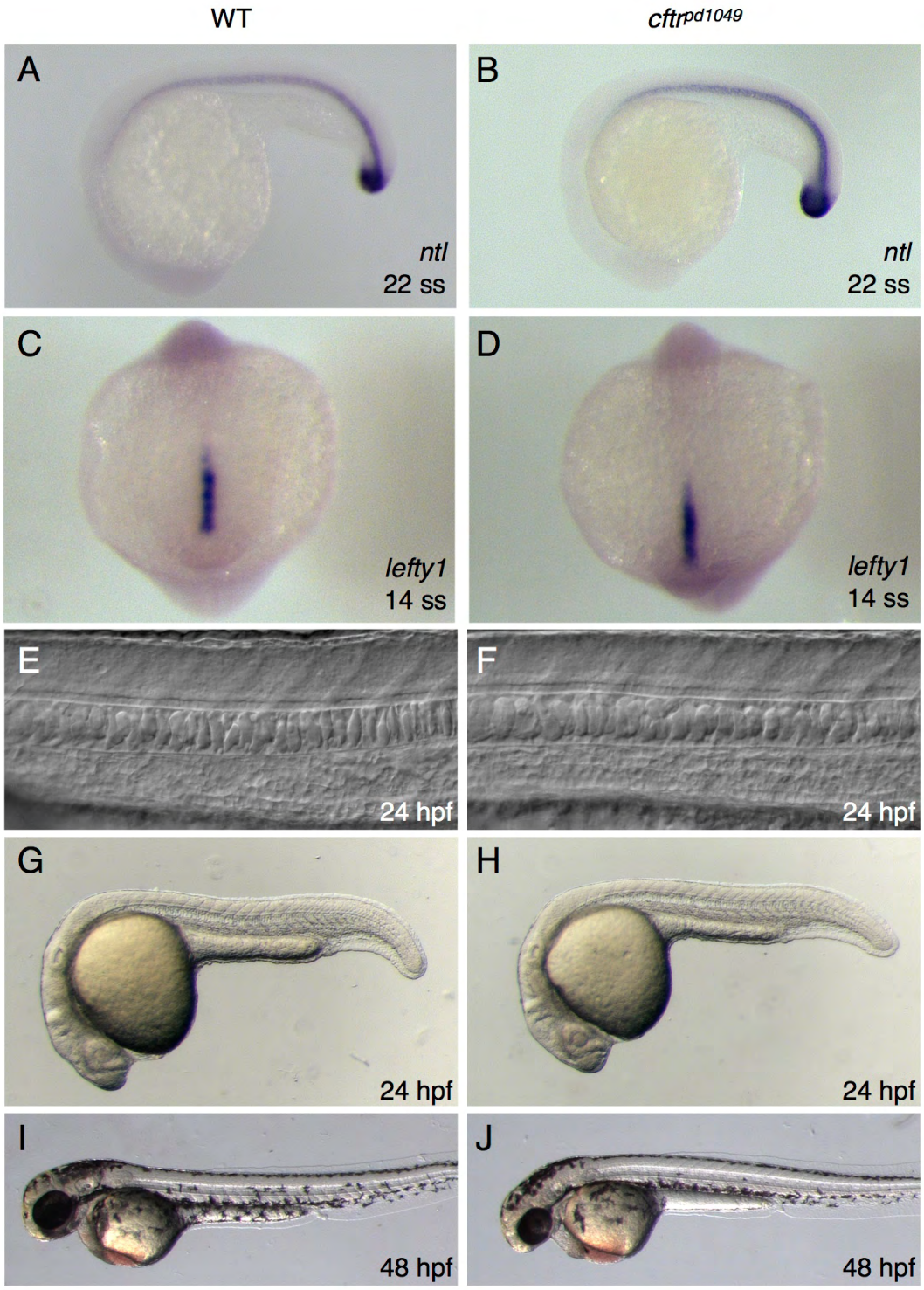

Fig. S2. *cftr*^{pd1049} mutants do not have midline defects. (**A**,**B**) *In situ* hybridization of WT and mutant embryos for *ntl*. (**C**,**D**) *In situ* hybridization of WT and mutant embryos for *lefty1*. (**E**,**F**) DIC images of WT and mutant showing normal notochord and floorplate morphology. (**G-J**) Whole-mount brightfield images of WT and mutant embryos at (G,H) 24 hpf and (I,J) 48 hpf.

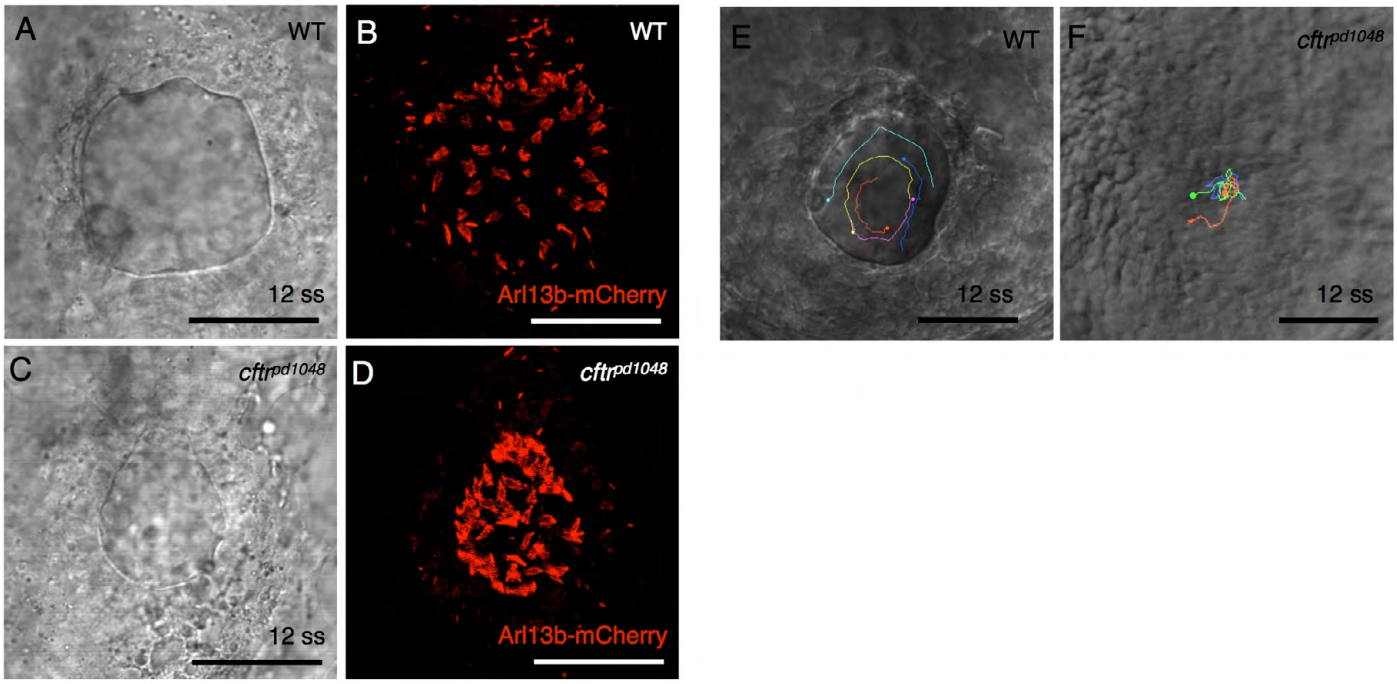

Fig. S3. Motile cilia are crowded in *cftr*^{pd1048} mutants. (**A**,**B**) Brightfield and confocal imaging of 12 ss embryos injected with RNA encoding Arl13b-mCherry show normal morphology of KV, which contains a large number of motile cilia. (**C**,**D**) In the hypomorphic *cftr*^{pd1048} allele, reduced lumen size leads to crowding of motile cilia. (**E**,**F**) Tracking of fluorescent beads in WT and *cftr*^{pd1048} KV. Scale bars: 50 μm.

Movie 1. Cftr-RFP expressed from the *TgBAC(cftr-RFP)* line is apically localized as multiple small lumens coalesce into one during single lumen formation in KV. *Tg(sox17:GFP)* expression marks the cytoplasm of KV epithelial cells.