Fig. S1. Cftr<sup>ΔΔ48</sup>-GFP is mislocalized. (A-A″) Zebrafish Cftr-GFP expressed in Cos-7 cells is predominantly localized to the plasma membrane. (B-B″) The Cftr<sup>ΔΔ48</sup>-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<sup>ΔΔ48</sup>-GFP expressed from HEK293 cells. Scale bars: 50 μm.
Fig. S2. *cftr*<sup>Δ1849</sup> 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<sup>pol1048</sup> 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<sup>pol1048</sup> allele, reduced lumen size leads to crowding of motile cilia. (E,F) Tracking of fluorescent beads in WT and cftr<sup>pol1048</sup> 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.