



Chaya *et al.*, Figure S6

**Figure S6** Loss of ICK affects ciliary transport. (A–H)  $ICK^{+/+}$  and  $ICK^{-/-}$  MEFs were treated or not treated with Smo agonist (SAG) and immunostained with antibodies against Smo (green in A–D) and Gli2 (red in E–H). The cilia were immunostained with an antibody against acetylated  $\alpha$ -tubulin (green in E–H). Centrioles were immunostained with an antibody against  $\gamma$ -tubulin (red in A–D). Smo and Gli2 are aberrantly present in the  $ICK^{-/-}$  MEF cilia without stimulation with SAG. (I) Quantification of the cilia with Gli2 signals. The percentages of the cilia with Gli2 signals in  $ICK^{+/+}$  and  $ICK^{-/-}$  MEFs with or without treatment of SAG were quantified. (J) Shh signal-dependent expression of *Gli1* is defective in  $ICK^{-/-}$  MEFs.  $ICK^{+/+}$  and  $ICK^{-/-}$  MEFs were untreated or treated with SAG and *Gli1* mRNA expression level was measured by Q-PCR. (K–N) FLAG-tagged IFT57- (K, L) or IFT140- (M, N) expressing plasmids were transfected into  $ICK^{+/+}$  and  $ICK^{-/-}$  MEFs. Cells were stained with anti-acetylated  $\alpha$ -tubulin (red) and anti-FLAG (green) antibodies. Arrowheads indicate ciliary tips. (O–P')  $ICK^{+/+}$  (O, O') and  $ICK^{-/-}$  (P, P') neural tube cilia at E10.5 were immunostained with antibodies against IFT88 (green) and Arl13b (red). There was no obvious difference in ciliary localization of IFT88 between  $ICK^{+/+}$  and  $ICK^{-/-}$  neural tube cilia. Arrowheads indicate cilia. Nuclei were stained with DAPI (blue). Scale bars, 2  $\mu$ m (A–H, K–N) and 1  $\mu$ m (O–P'). Error bars show the SD. \*\* $p < 0.03$ , \* $p < 0.05$ .