Supporting Information

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 Mouse 82
 FFEGFNATVFAYGQTGSGKTYTMGEASVASLHEDEQGIIPRAMAEAFK

 Fish 82
 FFHGFNATVSAYGQTGSGKTYTIGEASISAFRDDEQGIIPRAVAEIFKLLDENDLIDFSVRVSYMEVYKEVFRDLL

Fig. S1. Identification of the *Kif7^{maki}* mutation. (*A*) The crossing scheme of the genetic screen. An *HB9-eGFP* reporter line was generated in the FVB genetic background by using a previously described construct [Wichterle H, Lieberam I, Porter JA, Jessell TM (2002) *Cell* 110:385–397]. Third-generation embryos produced from crosses between G₂ females and the F₁ founder male were potentially homozygous for newly induced mutations and were scored for inappropriate expression of the HB9-eGFP reporter or for morphological abnormalities. (*B*) Position of the *maki* mutation relative to the predicted structure of Kif7. (*C*) Alignment of the region of the zebrafish and mouse Kif7 sequences in the region of the *maki* mutation, beginning at amino acid 82, the beginning of the P loop. Sequence analysis identified a T-to-C substitution in the *Kif7* ORF of *maki* mutants that would change a conserved leucine to proline (leucine highlighted in green) at amino acid 130, in a conserved region of the Kif7 motor domain between the P loop (red) and the M1 microtubule binding domain (blue).



Fig. S2. Dorsal-ventral neural patterning in the E10.5 *Kif7^{maki}* neural tube. FoxA2, a marker for the floor plate, is expressed in the same, or a slightly expanded domain, in *Kif7^{maki}* mutants. En1, which marks the V1 population of cells that is specified by low levels of Shh, is expressed at a more dorsal position in the *Kif7^{maki}* neural tube than in wild type. The domain of the progenitor marker Pax6 is shifted dorsally in the *Kif7^{maki}* neural tube.



Fig. S3. Localization of Kif7-eGFP in MDCK and IMCD3 cells. Confocal images (*A* and *B*) and apical-basal projections (*C*) of MDCK cells (*A*) and IMCD3 cells (*B* and *C*) expressing Kif7-eGFP. Wild-type Kif7-eGFP was enriched at the base of the primary cilia in MDCK cells and at both the base (arrows) and the tip (arrowhead) of cilia in IMCD3 cells (n = 40). Kif7^{L130P}-eGFP was only associated with the base (arrows) of the IMCD3 cilia (n = 35). Cell culture was performed as described previously [Follit JA, Tuft RA, Fogarty KE, Pazour GJ (2006) *Mol Biol Cell* 17:3781–3792]. Cilia (red) were visualized by using antibodies to acetylated α -tubulin (*A*) or Arl13b (*B*) [Caspary T, Larkins CE, Anderson KV (2007) *Dev Cell* 12:767–778]. (Scale bars: 5 μ m.)