SUPPLEMENTAL MATERIALS AND METHODS

Primary Antibodies

Pax6 was deposited to the DSHB by Kawakami, A. (DSHB Hybridoma Product PAX6). Nkx6.1 was deposited to the DSHB by Madsen, O.D. (DSHB Hybridoma Product F64A6B4). Nkx2.2 was deposited to the DSHB by Jessell, T.M. / Brenner-Morton, S. (DSHB Hybridoma Product 74.5A5) Pax6, Nkx6.1, and Nkx2.2 were used at 1:100. Anti-Olig2 was obtained from Millipore (catalog number MABN50) and used at 1:200. Anti-Acetylated α-tubulin was obtained from Sigma-Aldrich (catalog number T7451,) and used at 1:2000. Anti-γ-tubulin was obtained from Sigma-Aldrich (catalog number T5326) and used at 1:2000. Anti-IFT56 was obtained from Novus Biologicals (catalog number NBP1-84034) and used at 1:500. Anti-FoxA2 was obtained from Abcam (catalog number), Anti-ACIII was obtained from Santa Cruz (catalog number sc-588) and used at 1:200. Anti-Flag was obtained from Sigma-Aldrich (catalog number F7425) and used at 1:2000. Anti-PC2 (gift from Stefan Somlo) was used at 1:500, Anti-Arl13b (gift from Tamara Caspary) was used at 1:2000, Anti-Gli2 (gift from Jonathan Eggenschwiler) was used at 1:1000, Anti-Gli3 (gift from Suzie Scales) was used at 1:1000. Anti-IFT81 was obtained from Proteintech (catalog number 11744-1-AP) and used at 1:1000. Anti-IFT88 was obtained from Proteintech (catalog number 13967-1-AP) and used at 1:500. Anti-IFT27 (gift from Greg Pazour) was used at 1:1000, anti-IFT140 (gift from Greg Pazour) was used at 1:1000, and anti-IFT122 (gift from Jonathan Eggenschwiler) was used at 1:500.

Secondary Antibodies

Alexa Fluor 633 goat anti-mouse IgG was obtained from Molecular Probes Inc. (catalog number A21052) and used at 1:500. Alexa Fluor 533 goat anti-mouse IgG1 was obtained from Molecular Probes Inc. (catalog number A21126) and used at 1:500. Cy3-conjugated AffiniPure goat anti-mouse IgG was obtained from Jackson ImmunoResearch Laboratories

Inc. (catalog number 115-165-166) and used at 1:500. Alexa Fluor 568 goat anti-mouse IgG2b was obtained from Molecular Probes Inc. (catalog number A21144) and used at 1:500. Cy3-conjugated AffiniPure goat anti-mouse IgG2a was obtained from Jackson ImmunoResearch Laboratories Inc. (catalog number 115-165-206) and used at 1:500. Cy3-conjugated AffiniPure goat antimouse IgG2b was obtained from Jackson ImmunoResearch Laboratories Inc. (catalog number 115-165-207) and used at 1:500. Alexa Fluor 488 goat anti-mouse IgG1 was obtained from Jackson ImmunoResearch Laboratories Inc. (catalog number 115-545-205) and used at 1:500.

Riboprobes

The *Gli1* riboprobe plasmid (pBluescript) was a gift from Josh Catron. The *Shh* riboprobe plasmid (pBluescript II) was a gift from Andrew McMahon. The *Gremlin1* riboprobe plasmid (pBluescript) was a gift from Richard Harland.

Real-Time-PCR and analysis

Total RNA was extracted from e11 limb buds using standard protocol with TRIzol (Invitrogen). cDNA was reverse transcribed using the SuperScript III kit (Invitrogen). qRT-PCR was performed using *Power* SYBR Green PCR Master Mix (Applied Biosystems) on a ViiA7 Real-Time PCR system (Applied Biosystems). Relative transcript expression levels were determined by the standard comparative C_T method (Schmittgen and Livak, 2008).

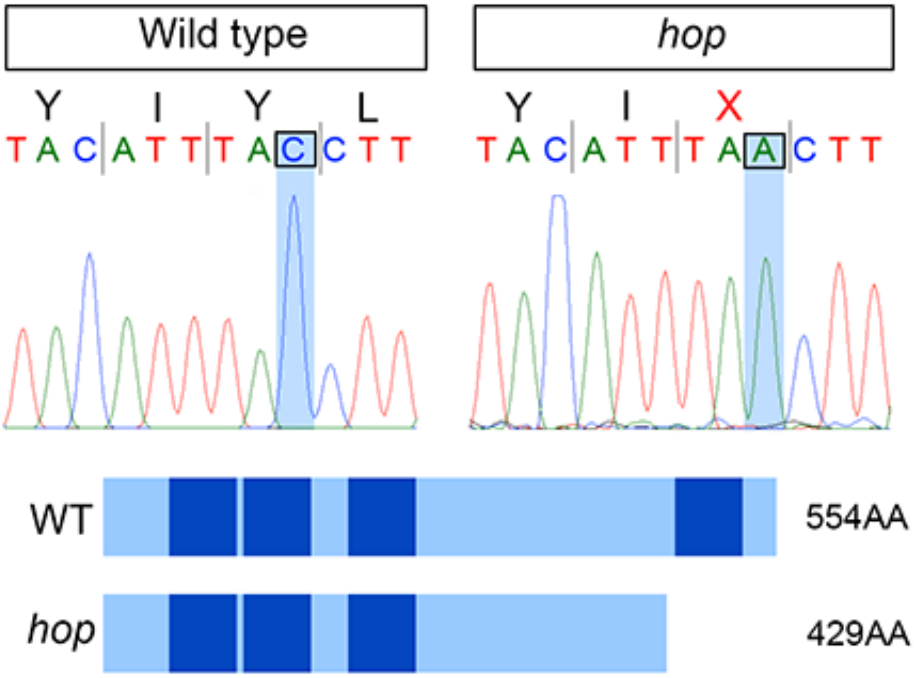

Figure S1. Sanger sequencing traces revealed that the *Ift56*^{hop} mutation is a cytosine-to-adenosine transversion (c.1290**C>A**) at mouse Chr6:38,362,071 within the coding region of *Ift56*, resulting in a truncation of the C-terminus (**Y430X**) and loss of the 4th TPR domain (dark blue shading) of the IFT56 protein product.

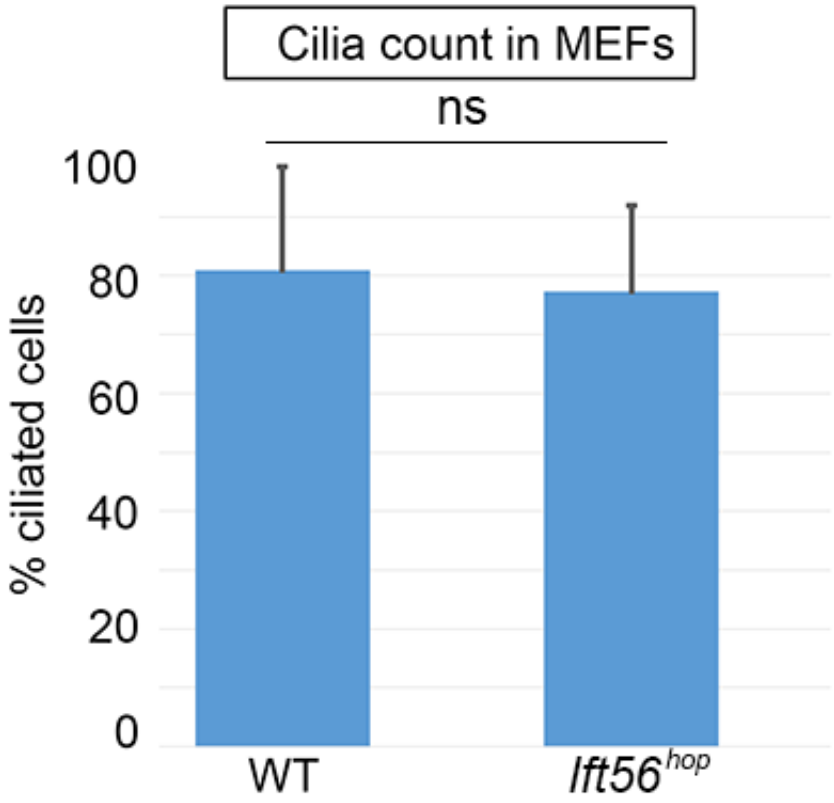

Figure S2. The percentage of ciliated MEFs was not significantly different between control and *Ift56*^{hop} cells. Cilia were marked by acetylated α-tubulin, and at least 3 slides were imaged for 3 or more control and mutants. At least 50 total cells were counted for ciliation per sample.

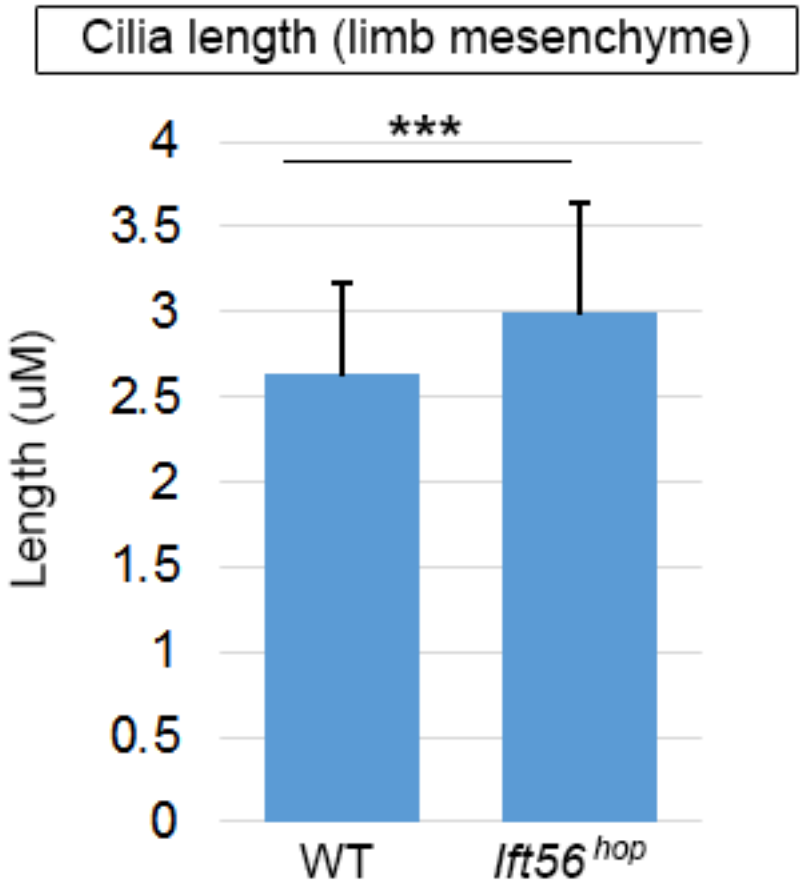

Figure S3. Cilia in the limb mesenchyme of *Ift56*^{hop} mutants are significantly longer compared to control animals. Cilia were marked by Arl13b, and at least 3 slides were imaged for 3 or more control and mutants. At least 30 total cells were counted for ciliation per sample.

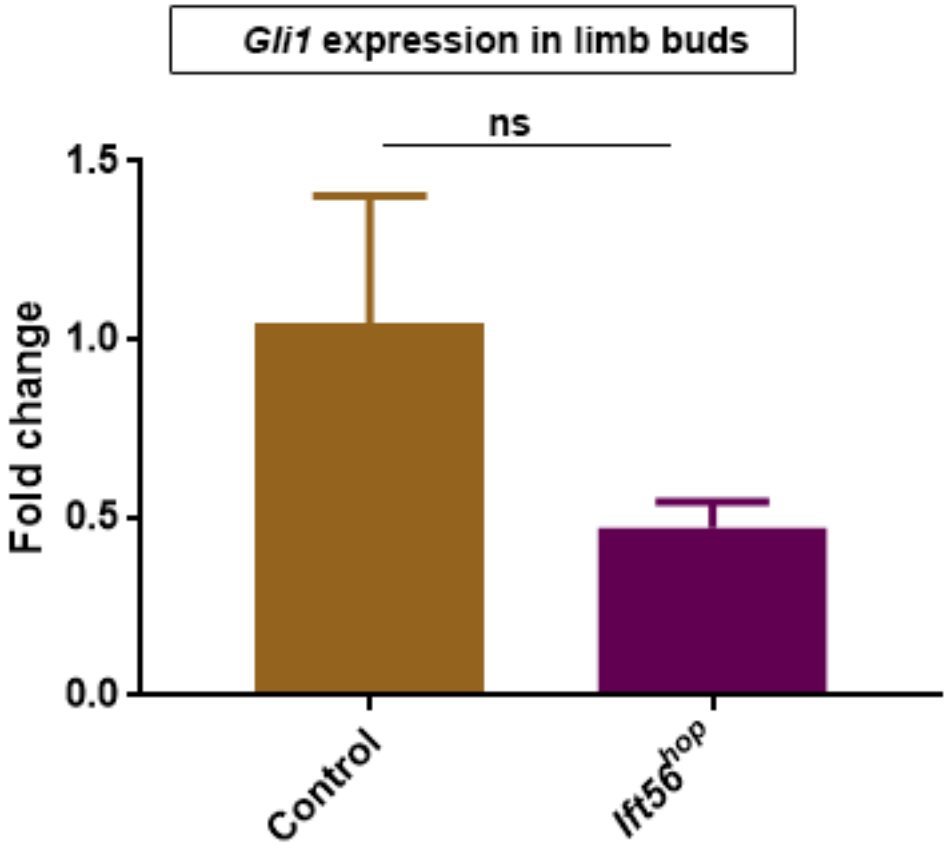

Figure S4. *Gli1* transcript levels in the *Ift56*^{hop} limb buds are reduced but not quite significant (p=0.0531 by unpaired two-tailed student's t-test with equal variances, data is calculated as mean±s.d). 3 technical replicates were performed per sample for 3 controls and 3 mutants.

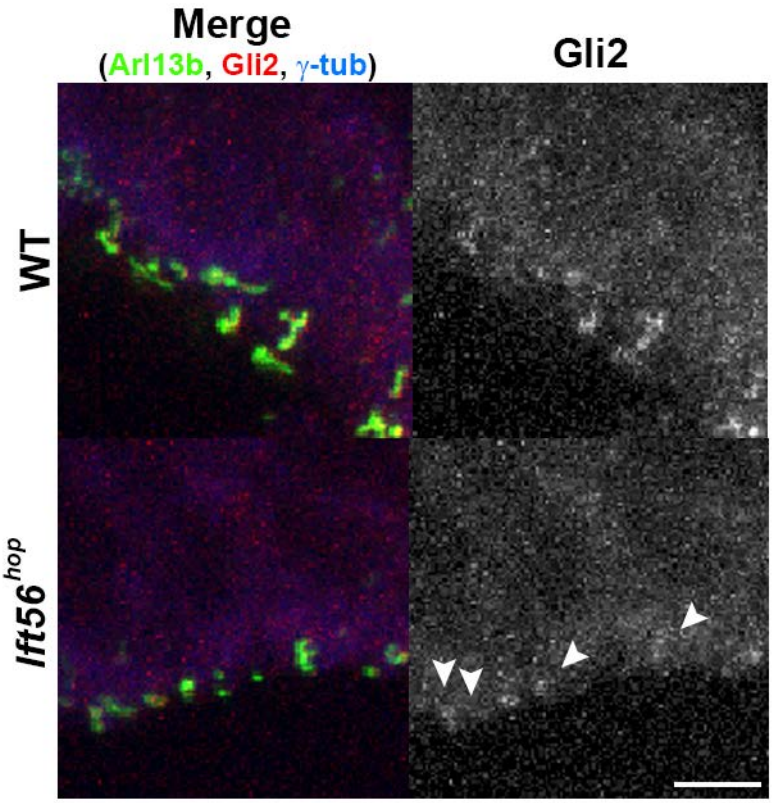

Figure S5. Neural tube cilia were assayed for with Gli2 (red) and Arl13b (green) to mark ciliary axonemes. Gli2-only channel (right) shows fainter Gli2 in *Ift56*^{hop} cilia (arrowheads). Representative images were taken along the ventral wall of caudal (hindlimb) regions. At least 3 sections were imaged per sample of 4 or more controls and mutants.