

Supplementary information for:

**Mutations in mouse *Ift144* model the craniofacial, limb and rib defects in skeletal ciliopathies**

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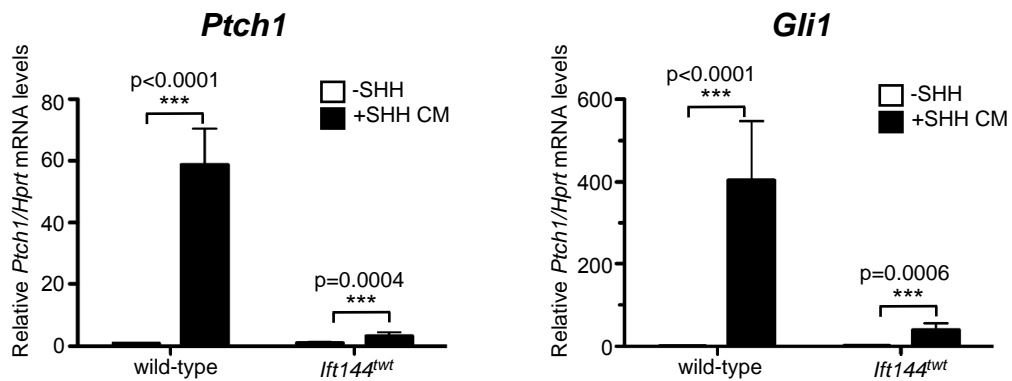
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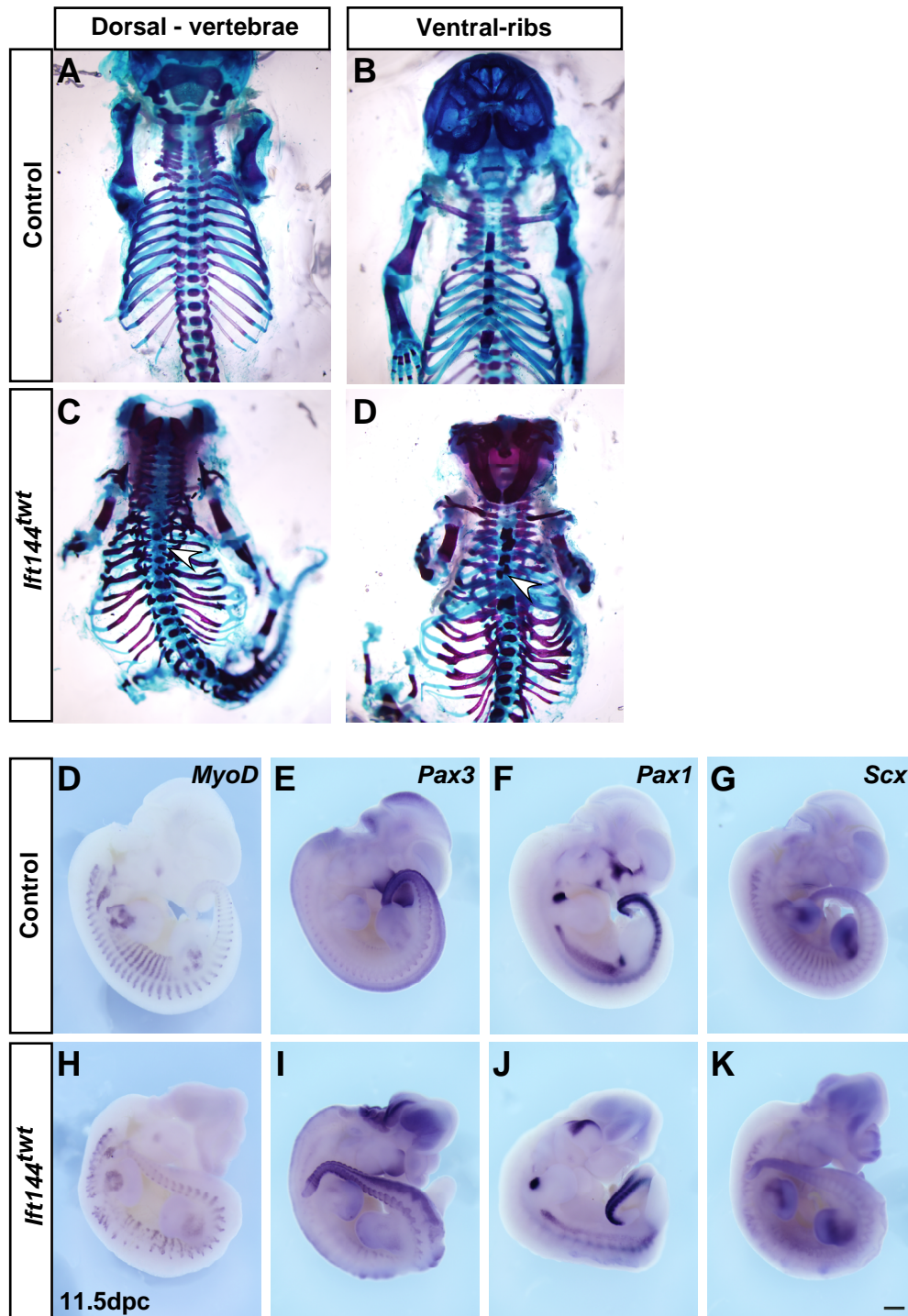
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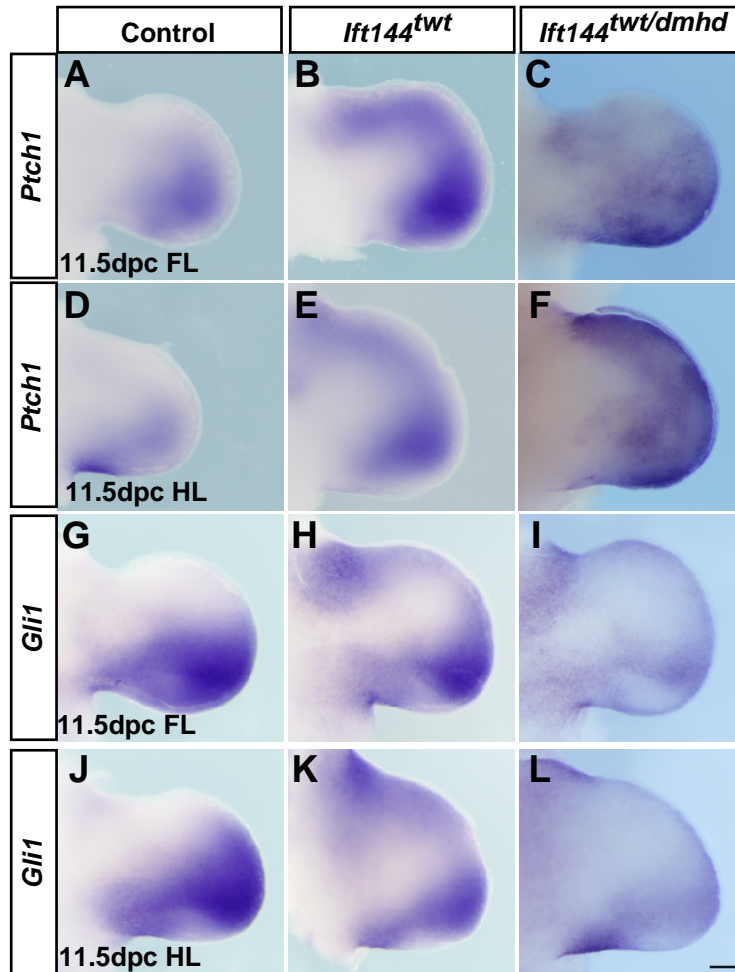
File contains : Supplementary Figures S1,2,3,4



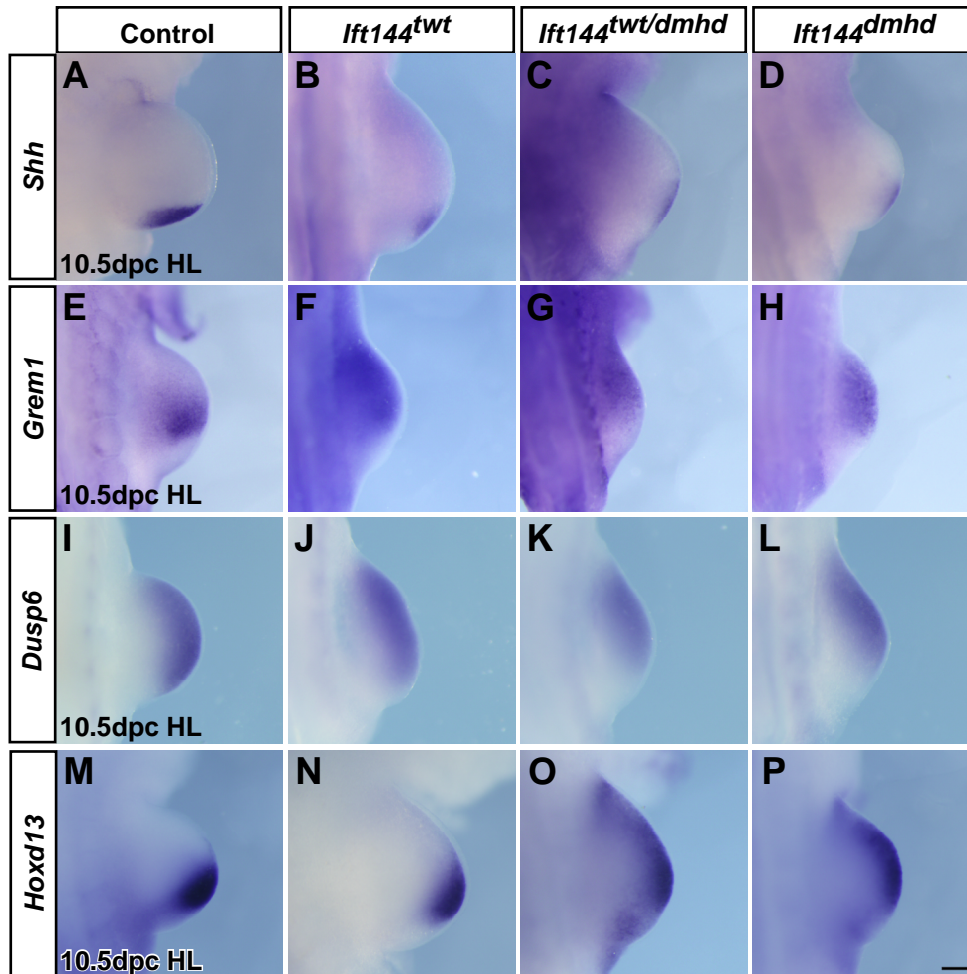
**Supplementary Figure S1. *Ift144<sup>tw</sup>* MEFs show an attenuated response to SHH conditioned media (CM).** *Ptch1* and *Gli1* levels were both significantly increased in wild-type and *Ift144<sup>tw</sup>* MEFs treated with SHH conditioned media, but in both cases the response was much lower in the mutant versus wild-type cells. The results shown were obtained from triplicate PCRs performed on each sample (N=3, comprised of 3 independently treated samples from one cell line per genotype). SHH CM was collected from HEK293 cells transfected with a SHH expression construct encoding the active N-terminal fragment of mouse SHH, and control media (-SHH) from cells transfected with vector only. Cells were treated for 48h with a 1:4 mix of CM media with serum free DMEM prior to RNA extraction. p-values are based on a Student's *t*-test. Error bars show standard error of the mean (s.e.m).



**Supplementary Figure S2. Skeletal phenotype of *Ift144<sup>tw</sup>* embryos.** (A-D) Dorsal (A,C) and ventral (B,D) views of skeletal preparations of 18.5dpc embryos showing vertebrae and ribs respectively. Vertebrae are disordered in *Ift144<sup>tw</sup>* embryos (arrowhead in C), and sternbrae are misaligned resulting in a partially split sternum (arrowhead in D). (D-K) Expression of indicated markers in control (D-G), and *Ift144<sup>tw</sup>* (H-K) embryos at 11.5dpc. Scale bar: 500 $\mu$ m.



**Supplementary Figure S3. Hedgehog signalling is expanded in *Ift144<sup>tw</sup>* and *Ift144<sup>dmhd/tw</sup>* limbs.** (A-F) Expression of *Ptch1* in 11.5dpc forelimbs (FL; A-C) and hindlimbs (HL; D-F) of indicated genotypes. (G-L) Expression of *Gli1* in forelimbs (G-I) and hindlimbs (J-L). Scale bar: 200 $\mu$ m.



**Supplementary Figure S4. Hindlimbs of all three *Ift144* genotypes are similar at the morphological and expression level.** Expression of *Shh* (A-D), *Grem1* (E-H) *Dusp6* (I-L) and *Hoxd13* (M-P) in 10.5dpc hindlimbs (HL). Scale bar: 200 $\mu$ m.