SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Expression of *Shh*, *Ptch1*, and *Gli1* in single and double mutants.

A) Transverse sections through the posterior neural tubes of E9.5 wild-type, *Tulp3*$^{-/-}$, *Smo*$^{-/-}$, *Kif3a*$^{-/-}$, *Smo*$^{-/-}$*Tulp3*$^{-/-}$ *and Kif3a*$^{-/-}$*Tulp3*$^{-/-}$ mutants processed for in situ hybridization. *Shh* expression in the neural tube of *Smo* and *Kif3a* mutants was disrupted. Neural tube expression of *Shh* was restored in *Smo/Tulp3* double mutants but not in *Kif3a/Tulp3* double mutants. The Shh target genes *Ptch1* and *Gli1* were ectopically expressed in dorsal regions of *Tulp3* and *Smo/Tulp3* mutants but *Kif3a* and *Kif3a/Tulp3* mutants showed only weak expression throughout the neural tube. B) Sections through E10.5 neural tubes of wild-type, *Tulp3*$^{-/-}$, *Shh*$^{-/-}$, *Gli2*$^{-/-}$, *Shh*$^{-/-}$ *and Gli2*$^{-/-}$*Tulp3*$^{-/-}$ mutants. Note that *Ptch1*, and *Gli1* were ectopically expressed outside of the normal domains in *Tulp3* single mutants. This pattern of expression was maintained in *Shh/Tulp3* double mutants but not in *Gli2/Tulp3* double mutants. The sequences contained within the *Shh* probe are do not fully overlap with those deleted by the *Shh* mutation, permitting detection of weak *Shh* epxression in *Tulp3/Shh* double mutants.

Figure S2. Limb patterning in Tulp3 mutants at E10.5

Expression of *Shh*, *Gli1*, *Ptch1*, *Hoxd12*, *Gli3*, and *dHand* in forelimbs and hindlimbs of E10.5 wild-type and *Tulp3* mutant embryos. The mutant forelimbs were smaller than wild-type, whereas the mutant hindlimbs were normal in size. *Gli1* and *Ptch1* showed ectopic anterior expression in the mutant forelimb and hindlimb buds at this stage (arrowheads), in a manner similar to that seen at E11.5 (Fig. 3). In contrast to our findings at E11.5, expression of *Shh* and *Hoxd12* appeared unaffected in *Tulp3* mutants at

E10.5. The normal expression pattern of *Gli3* and *dHand* in E10.5 in *Tulp3* mutant limb buds suggest that Shh-independent limb prepatterning occurs normally in these mutants.

Figure S3. Morphology of double and single mutants.
A) Whole-mount E10.5 embryos from *Shh/Tulp3* and *Gli2/Tulp3* epistasis experiments. Note that the size and brain morphological defects observed in *Shh* single mutants were largely suppressed in *Shh/Tulp3* double mutants. Although the exencephalic phenotype was not suppressed in *Gli2/Tulp3* double mutants, exencephaly was also frequently observed in *Gli2* single mutants (not shown) on the background we have studied (C3Heb/FeJ). B) Whole-mount E9.5 embryos from *Smo/Tulp3* and *Kif3a/Tulp3* epistasis experiments. Embryonic growth was partially restored in *Smo/Tulp3* double mutants in comparison to *Smo* single mutants, whereas *Kif3a* and *Kif3a/Tulp3* mutants were indistinguishable with respect to size and morphology. Scale bars are 0.5mm in A and 0.25mm in B.

Figure S4. Gene expression in *Shh/Tulp3* mutant limb buds. Expression of *Ptch1* and *Gli1* was maintained, although at greatly reduced levels, in E11.5 *Shh/Tulp3* double mutant limb buds and patchy *Hoxd12* expression could be detected in E12.5 *Shh/Tulp3* double mutant limb. *Shh* single mutant limbs do not express *Ptch1* or *Gli1* and exhibit only very weak expression of *Hoxd12* in hindlimb buds (1). The *Shh/Tulp3* double mutant limb buds were smaller than those of wild-type or *Tulp3* single mutants.

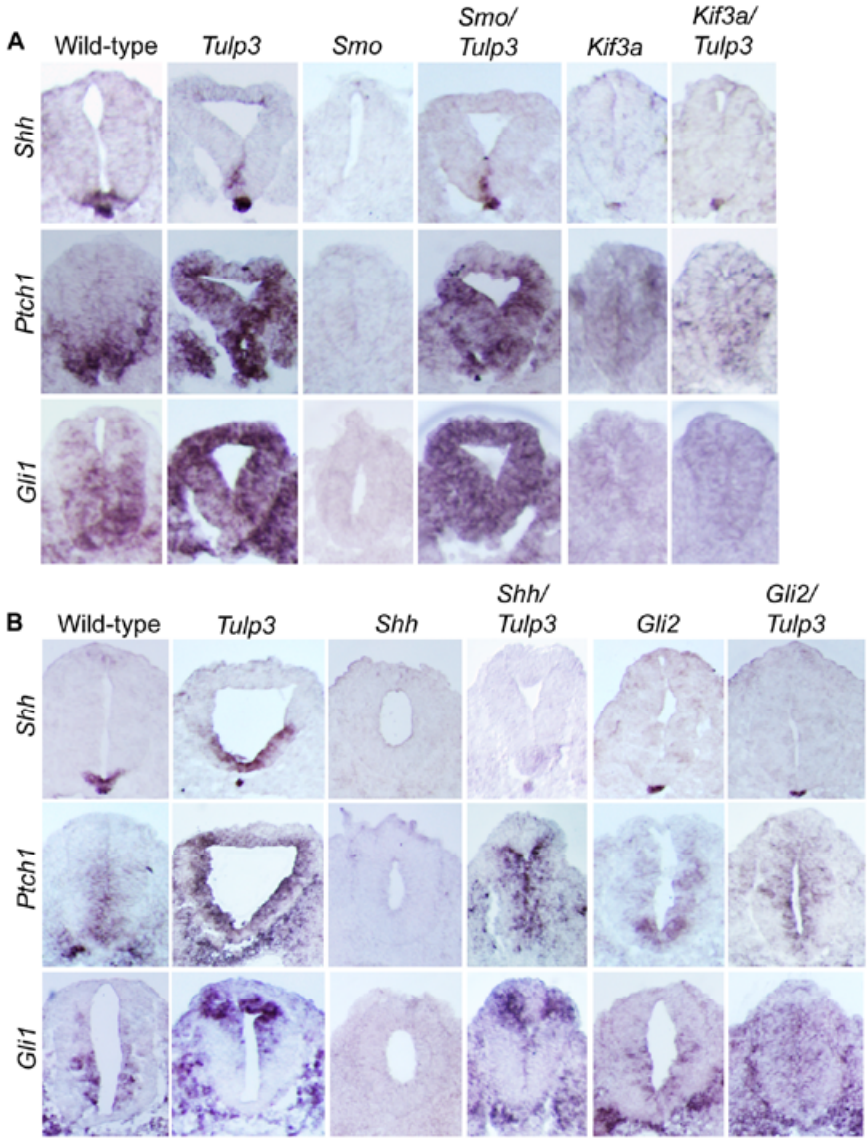
Figure S5. Gli proteins in *Tulp3* mutants. A) Levels of Gli2 in nuclear (n) and whole embryo (w) extracts from E9.5 embryos were not affected in *Tulp3* mutants. β-tubulin and HDAC2 served as loading controls. B) Levels of full-length Gli3 (Gli3-190) and processed Gli3 (Gli3-83) were similar in E9.5 wild-type and *Tulp3* mutants. β-actin was used as a loading control. C) Gli3 processing in forelimbs (FL) and hindlimbs (HL) from E11.5 wild-type, *Tulp3*, and *Gli3* mutant (negative control) embryos. Processed Gli3 (often seen as 83 and 75kDa species, ref. 2) appears somewhat reduced in the *Tulp3* mutant limb samples in this experiment but the effect was variable (not shown). D) Gli2 was not found in α-TULP3 immunoprecipitates from E9.5 embryos. p, pellet, s, supernatant. Mock precipitation was performed in the absence of antibodies. Some non-specific bands (*) were recognized by the TULP3 antiserum. E) Suppressor of fused (Sufu), but not TULP3, was found in Gli2 immunoprecipitates from wild-type extracts. F) Sufu co-immunoprecipitated with Gli2 similarly in E9.5 wild-type and *Tulp3* mutant extracts.

Figure S6. Cilia are generated normally in *Tulp3* mutants. *Tulp3* mutant pMEFs generated acetylated α-tubulin (green) positive cilia with normal localization of Ift88 (red). B) Ift88-labelled cilia (white arrowheads) were observed on the apical surfaces of E10.5 wild-type and *Tulp3* mutant neural progenitors. C) Gli2 (red) localizes normally to the tips of *Tulp3* mutant cilia in fibroblasts. D) Average lengths of wild-type and *Tulp3* mutant cilia (assayed by acetylated α-tubulin staining) did not differ between genotypes. Scale bars are 2μm in A and C and 5μm in B.

SUPPLEMENTARY REFERENCES

1. Chiang, C., Litingtung, Y., Harris, M.P., Simandl, B.K., Li, Y., Beachy, P.A. and Fallon, J.F. (2001) Manifestation of the limb prepattern: limb development in the absence of sonic hedgehog function. *Dev. Biol.,* 236, 421-435.
2. Wang, B., Fallon, J.F. and Beachy, P.A. (2000) Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell,* 100, 423-434.

Figure S1.



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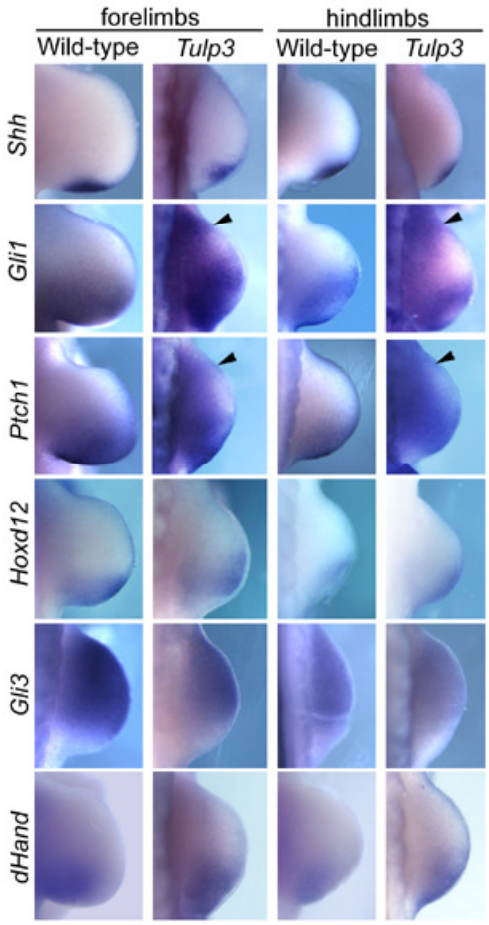
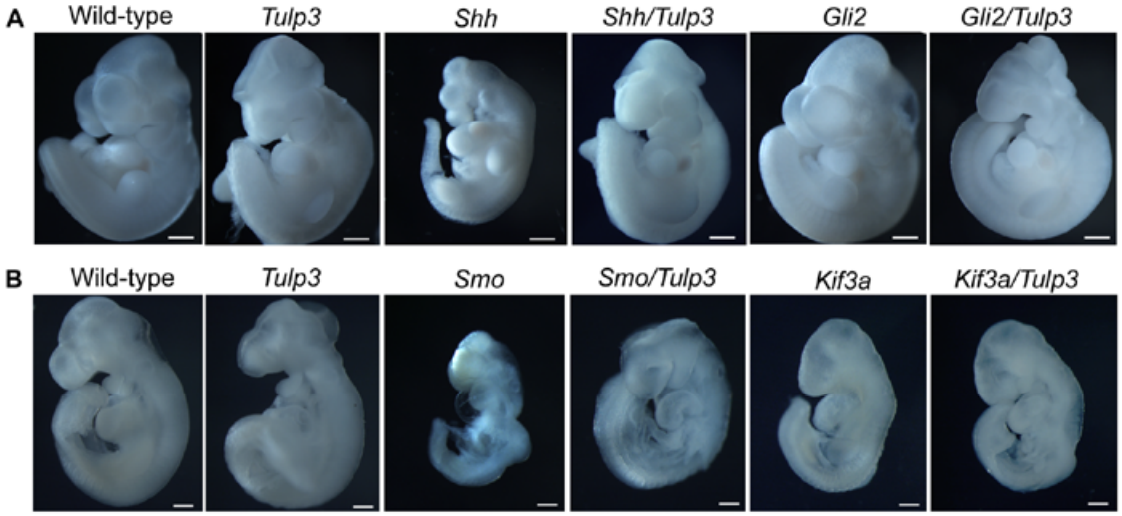

forelimbs
hindlimbs
Wild-type
Tulp3
Wild-type
Tulp3
Shh
Gli1
Ptch1
Hoxd12
Gli3
dHand

Figure S3.



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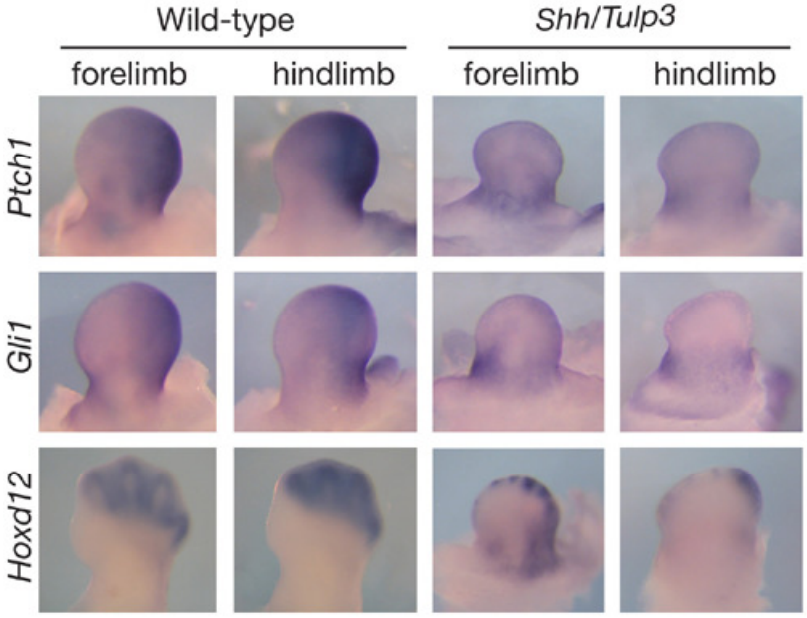

Figure S5.

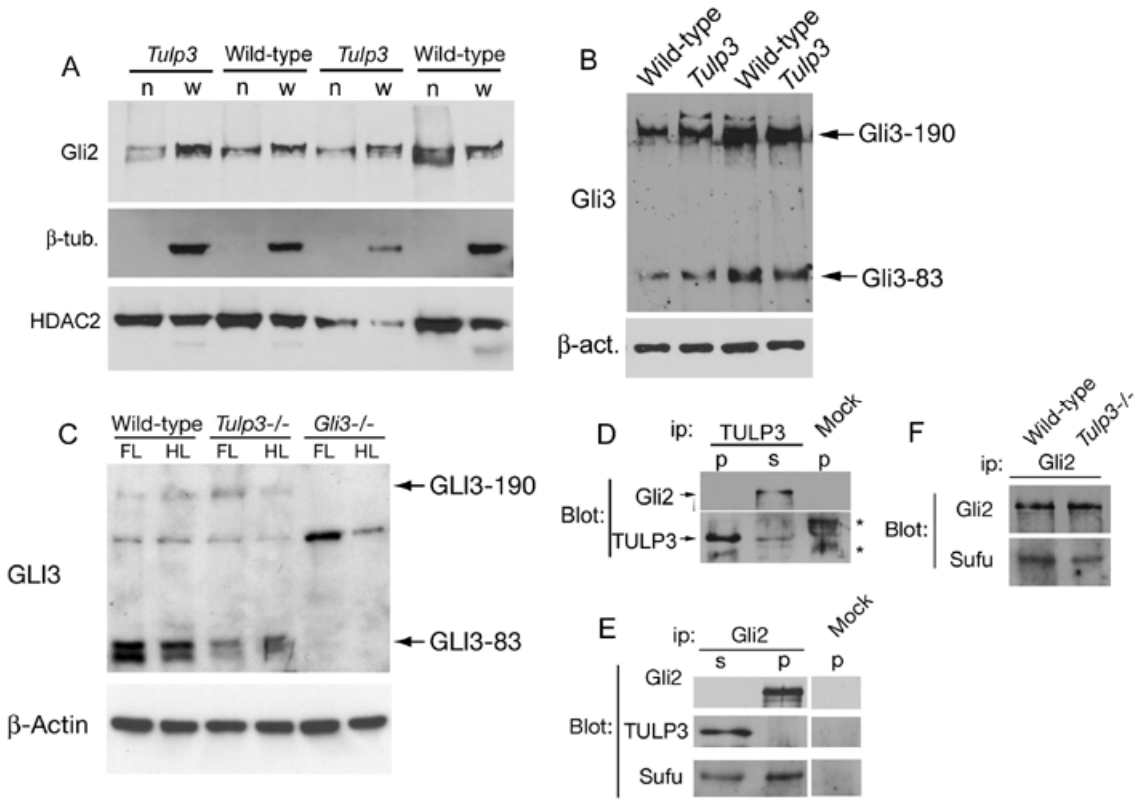

Figure S6.