

## LEGENDS TO SUPPLEMENTAL FIGURES

### **Supplemental Figure 1. *Ptch1* and *Gli1* demonstrate increased expression in *hitchhiker***

(A,B) Quantitative RT-PCR analysis of *Ptch1* and *Gli1* expression in E9.5 caudal ends detects a significant increase in *Ptch1* (1.65-fold) in *hitchhiker* mutants, reflecting the increased expression seen by wholemount in situ hybridisation, and a significant increase in *Gli1* expression (1.5-fold).

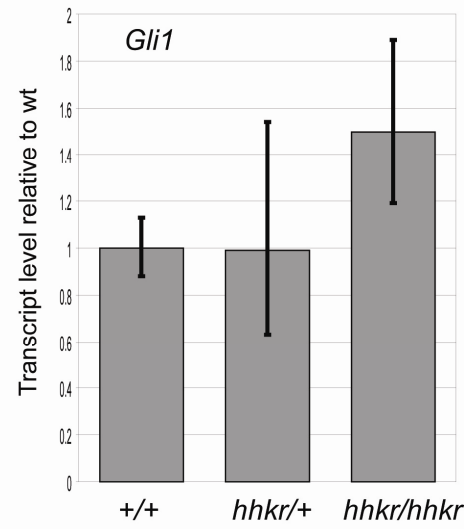
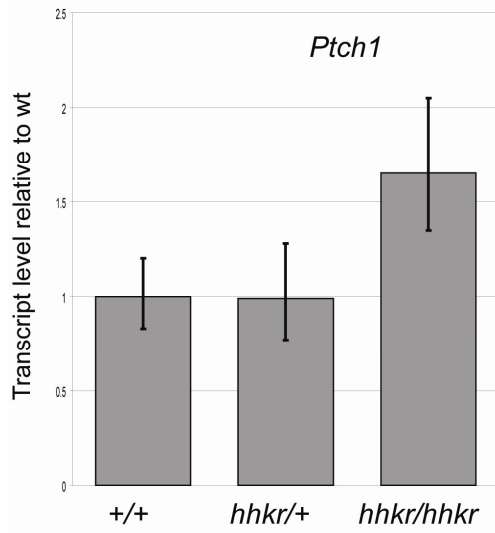
### **Supplemental Figure 2. Neural tube patterning at forelimb level looks normal**

Immunostaining of transverse sections through the neural tube at the level of the forelimb bud with antibodies against Nkx2.2 (A,B), Islet1 (C,D), Pax6 (E,F) and Msx (G,H), in wild-type (A,C,E,G) and *hitchhiker* (B,D,F,H) embryos at E10.5. Marker patterning looks similar in *hitchhiker* and wild-type embryos. Scale bar: 300  $\mu$ m.

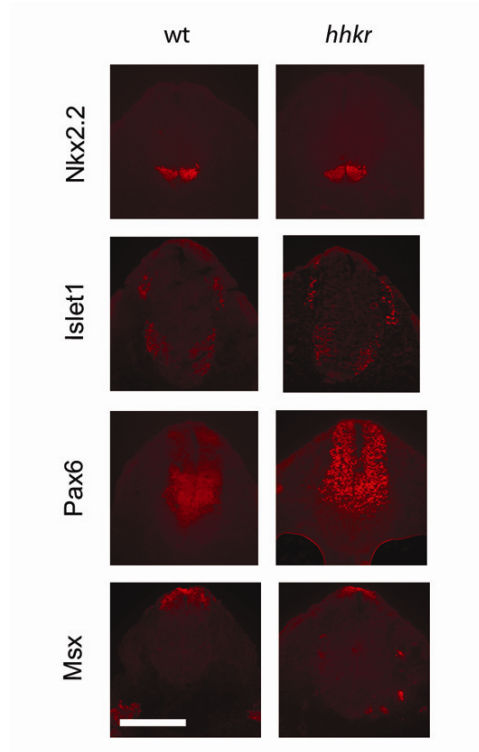
### **Supplemental Figure 3. *Shh/Shh;hhkr/hhkr* double mutants demonstrate upregulation of *Ptch1* and *Gli1* but absence of *Foxa2* expression**

(A-H) In situ hybridisation on transverse sections through the neural tube immediately anterior to the hindlimb bud with probes for *Ptch1* (A-D) and *Gli1* (E-F) in wild-type (A,E), *hitchhiker* (B,F), *Shh* (C,G) and *Shh/Shh;hhkr/hhkr* double mutant embryos (D,H) at E10.5. *Shh/Shh;hhkr/hhkr* double mutants exhibit increased *Ptch1* and *Gli1* expression similar to that seen in *hitchhiker*, and in contrast to the absence of expression in *Shh* mutants. (I-L) Immunostaining for *Foxa2* on transverse sections through the E10.5 caudal neural tube in wild-type (I), *hitchhiker* (J), *Shh* (K) and *Shh/Shh;hhkr/hhkr* double mutant embryos (L). *Foxa2* is expressed in an expanded domain in *hitchhiker* but not observed in *Shh/Shh;hhkr/hhkr* double mutant embryos. Scale bar: 300  $\mu$ m.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3

