Supplemental Figures

Fig. S1

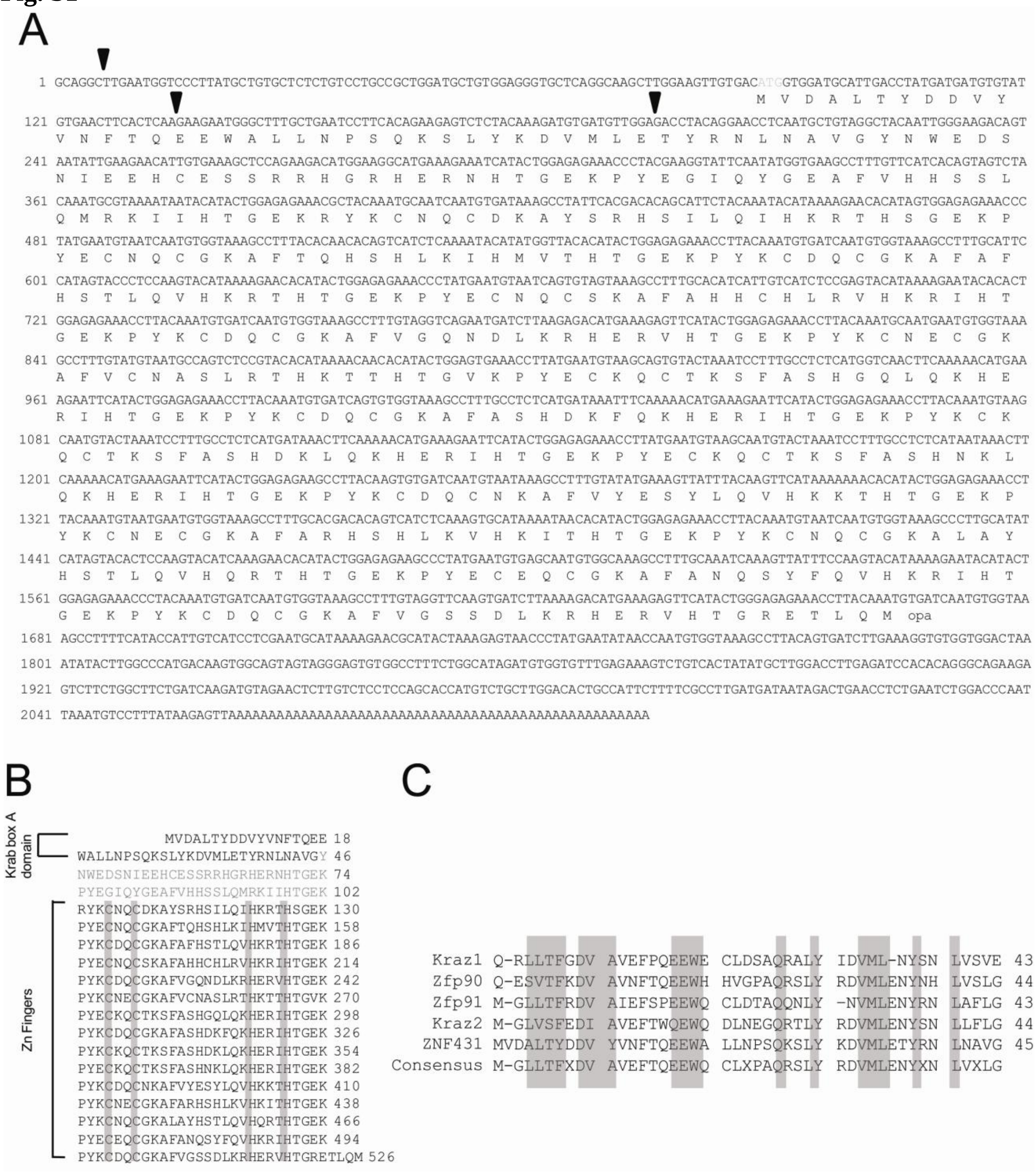

Fig. S1.Structure and amino acid sequence of ZNF431. A. cDNA and amino sequences of ZNF431. The coding sequence is from nucleotide 88 to 1668. Splice sites are indicated by triangles. B. Schematic representation of the structure of ZNF431. The KRAB-A domain and the zinc finger domains are highlighted in black and the Cystidines and Histidines of Zinc fingers are

shaded. C. Amino acid alignment of multiple KRAB domains. Conserved amino acids are shaded and consensus sequence is shown at the bottom.

Fig. S2

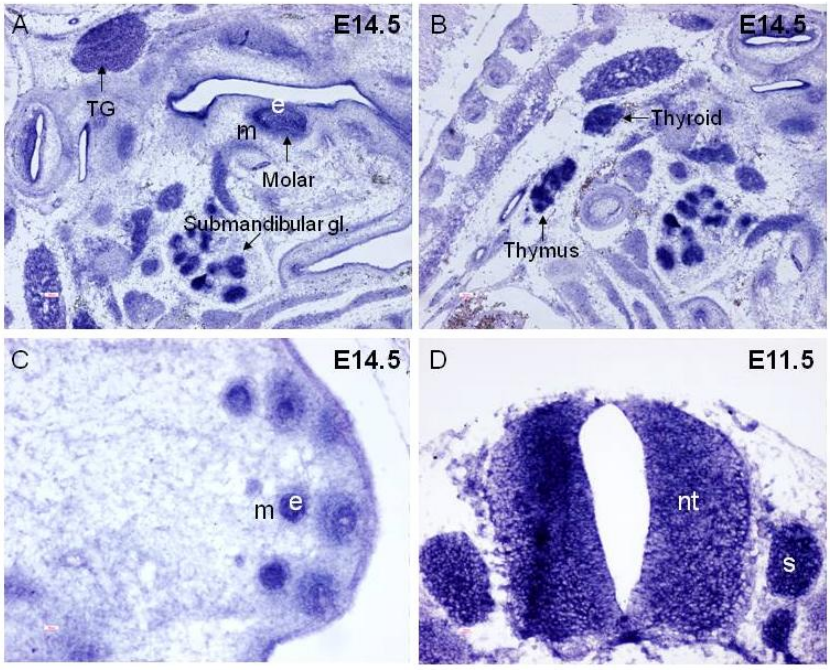

Fig. S2. In situ hybridization analysis of ZNF431 expression. ZNF431 transcript was detected in multiple organs in E14.5 embryos. ZNF431 is strongly expressed in the epithelial compartment of the tooth (A) and vibrissa follicles (C) whereas *Ptch1* was mainly expressed in the mesenchyme. e: epithelium, m: mesenchyme, TG: trigeminal ganglion, nt: neural tube, s: somite.

Fig. S3.

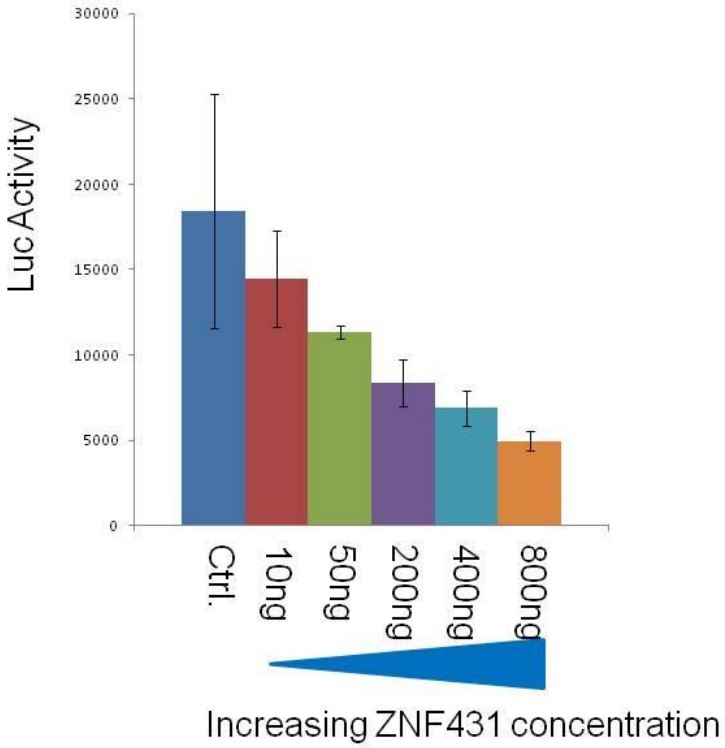

Fig. S3. Dose-response of ZNF431 repression. Increasing amount of GAL4DBDHAZNF431 expression plasmids was transfected into MPLB cells along with the GAL4-responsive Luc reporter. Reporter Luc activity decreases with increasing amount of ZNF431.

Fig. S4.

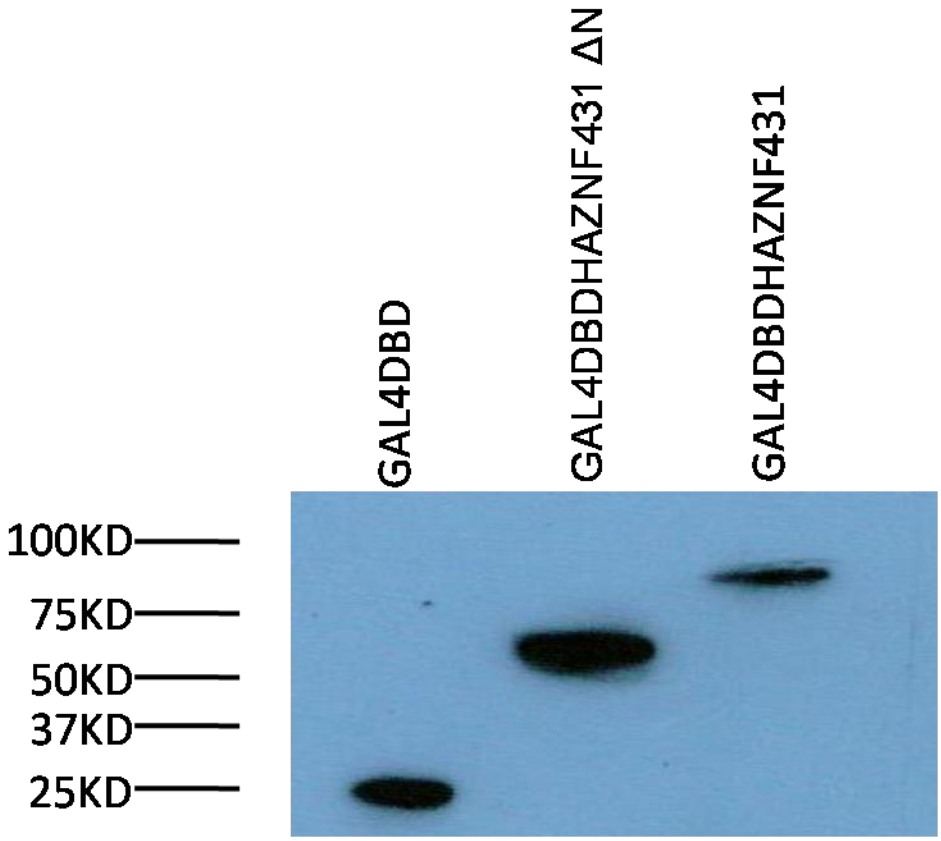

Fig. S4 GAL4HAZNF431 and GAL4HAZNF431ΔN expression in MPLB cells. Western blot with a Gal4 antibody showed that GAL4HAZNF431ΔN was expressed at a higher level compared to GAL4HAZNF431 in MPLB cells. Thus failure of GAL4HAZNF431ΔN to repress transcription is not due to a lower expression level.

Fig. S5.

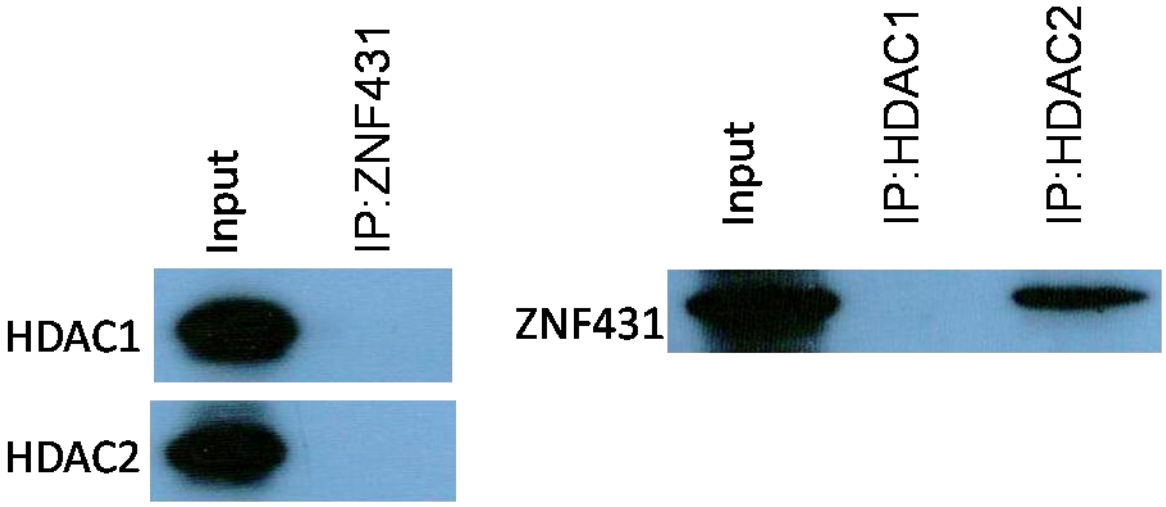

Fig. S5 Direct interaction between in vitro translated ZNF431 and HDAC2 proteins. Co-IP experiments showed that HDAC2 but not HDAC1 showed a direct interaction with ZNF431. HDAC1, HDAC2 and ZNF431 were synthesized as described. To each immunoprecipitations, 0.6ug antibody was added and incubated at room tempature for 1 hour. 5ul protein A agarose was added and incubated for 1 hour. Immunoprecipitates were collected by centrifugation at 7000g and washed 3 times. The final pellet was suspend in 20ul buffer and loaded to an SDS-PAGE.

Fig. S6.

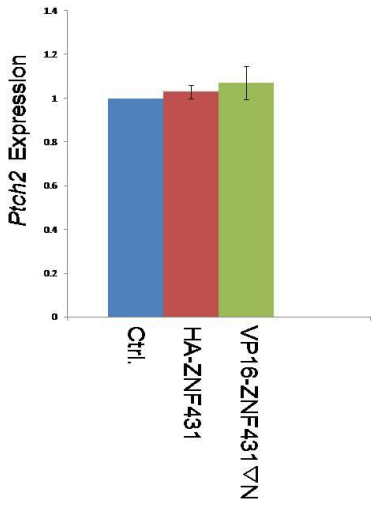

Fig. S6. Overexpression of HA-ZNF431 or VP16-ZNF431ΔN had no significant effect on *Ptch2* expression in MPLB cells.

Fig. S7.

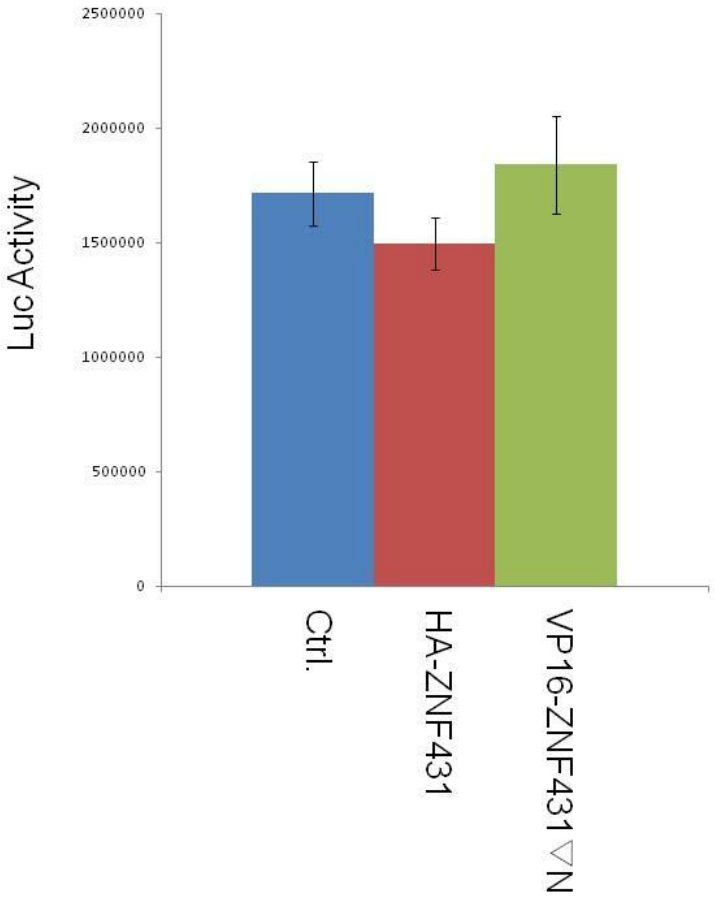

Fig. S7. Notch1 promoter is not regulated by ZNF431. A 4.7 kb Notch1 promoter driving reporter Luc was cotransfected with either HA-ANF431 or VP16-ZNF431ΔN. Neither construct significantly altered reporter activity.

Fig. S8.

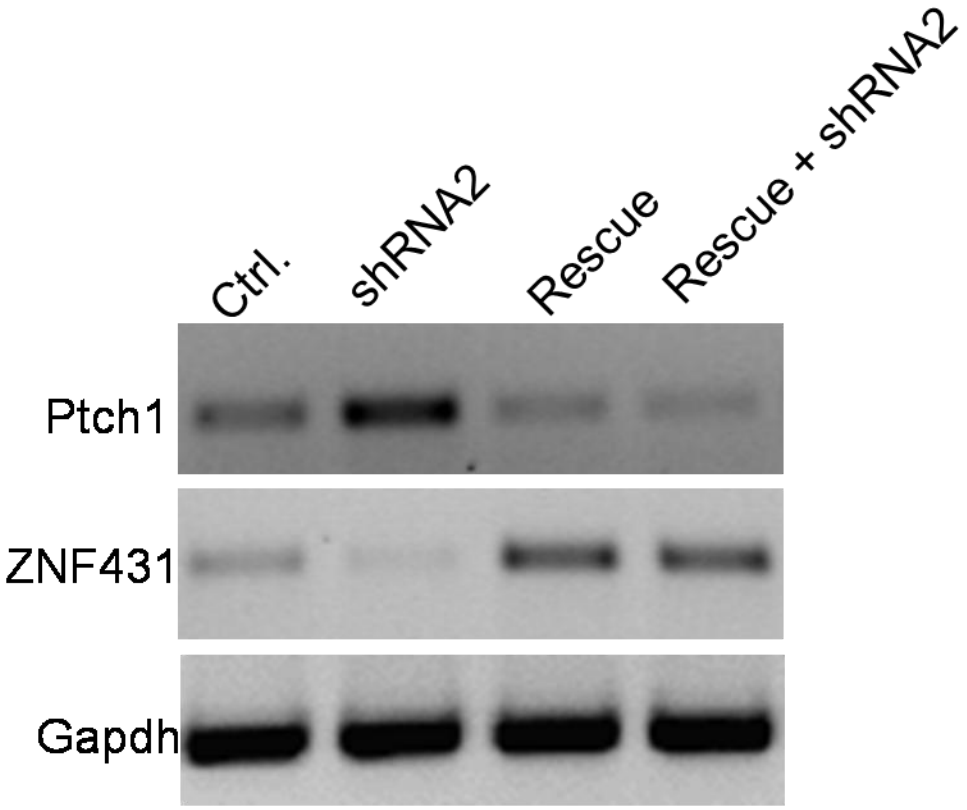

Fig. S8. Co-transfection of ZNF431 rescue construct with shRNA2 in MPLB cells. When co-transfected with a ZNF431 rescue construct which has the 3'UTR removed, ZNF431 expression was restored in MPLB cells and Ptch1 expression was reduced to about 50 percent of basal level due to ZNF431 overexpression.

Fig. S9

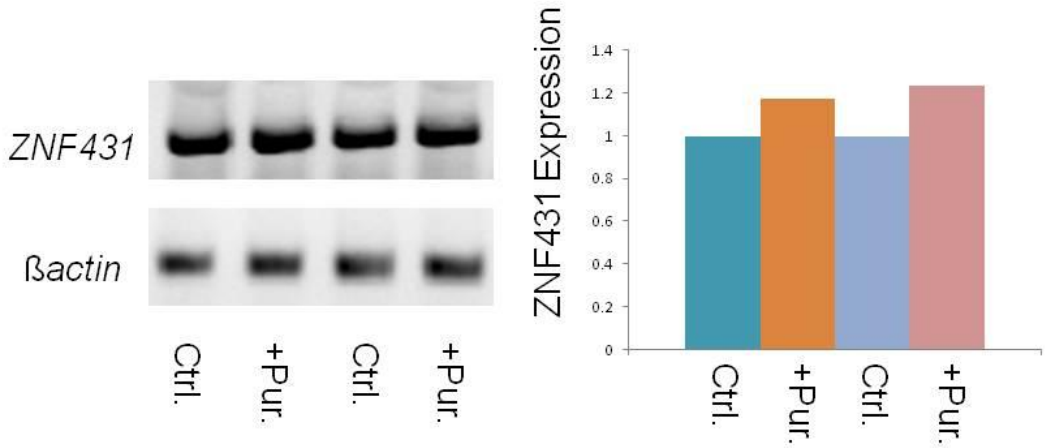

Fig. S9. Expression of ZNF431 is not regulated by HH signaling. MPLB cells were treated with either Purmorphamine (Pur.) or DMSO (control) and ZNF431 expression was examined by RT-PCR (left) and real-time RT-PCR (right).

Fig. S10.

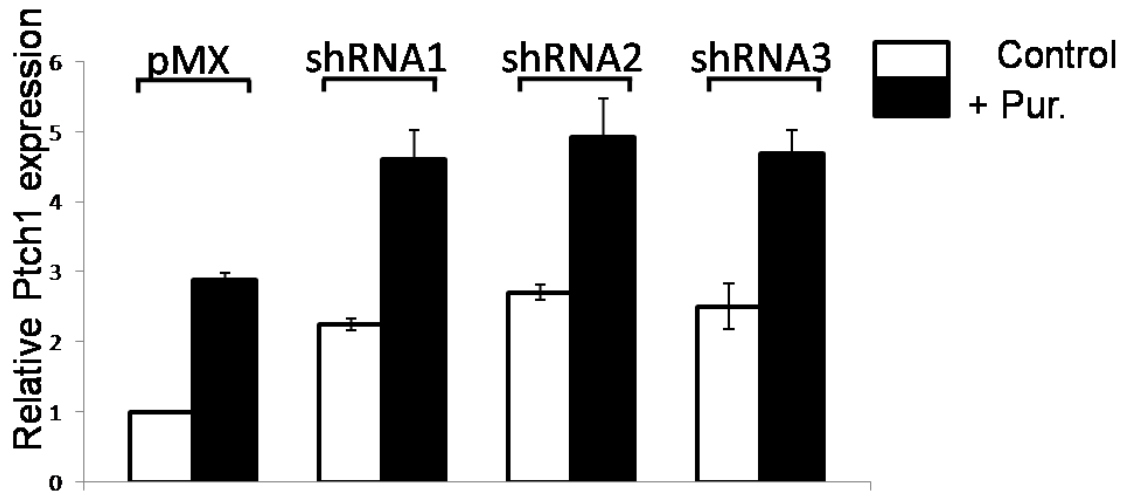

Fig. S10. ZNF431 level affected cellular responsiveness to HH signaling. Realtime RT PCR results showed that knocking down ZNF431 elevated Ptch1 expression in three shRNA constructs while cellular responsiveness to HH signaling was also reduced.

Fig. S11.

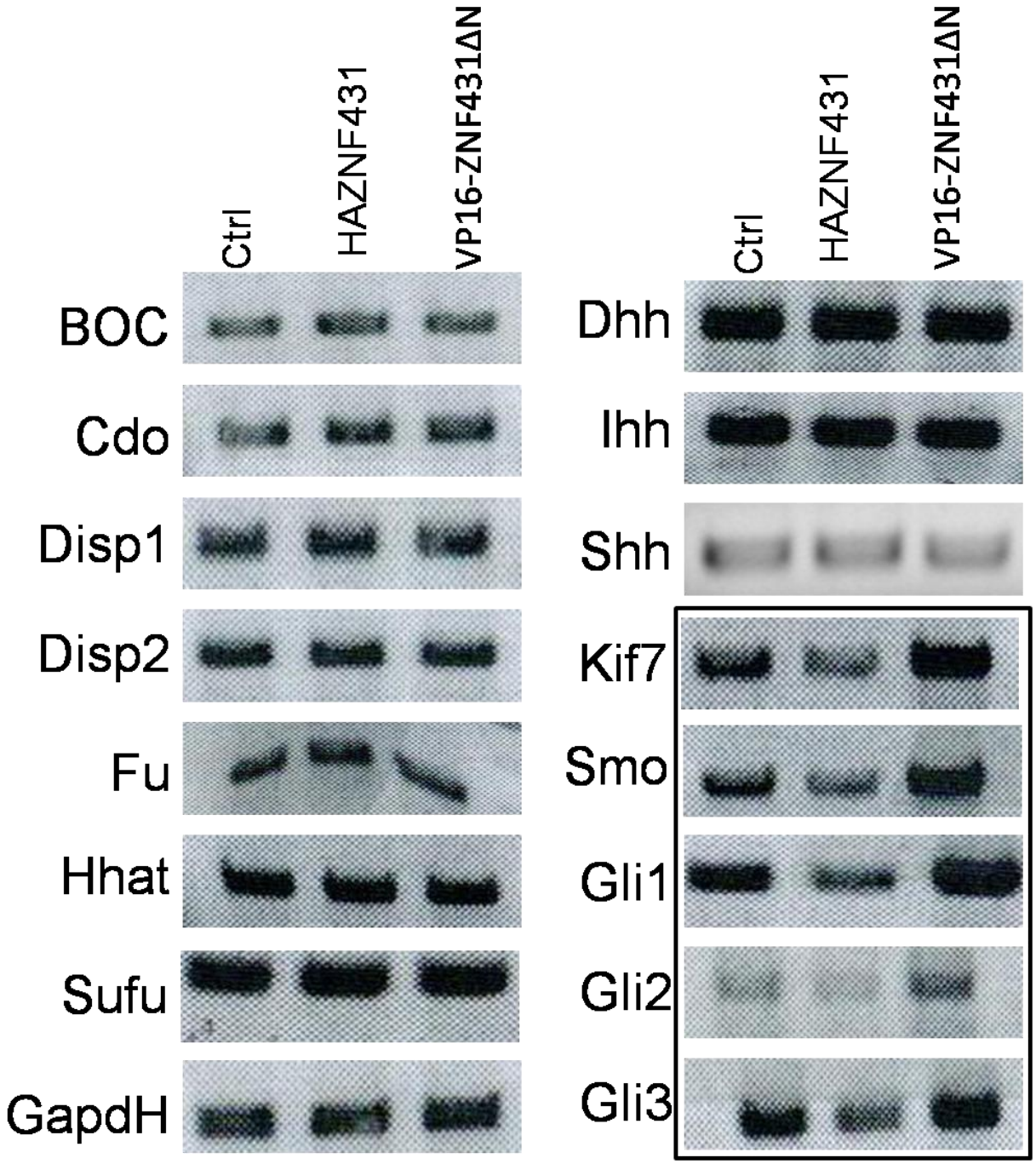

Fig. S 11. Regulation of Hh signal component expression in MPLB cells. Expression of 15 Hh signaling componentss was examined by RT-PCR in HAZNF431 (repressor) or VP16ZNF431ΔN (activator) overexpressing MPLB cells. Expression of 5 genes (Kif7, Smo, Gli1,2 and 3) were reduced in HAZNF431 overexpressing cells and elevated in VP16ZNF431ΔN overexpressing cells.

Fig. S12.

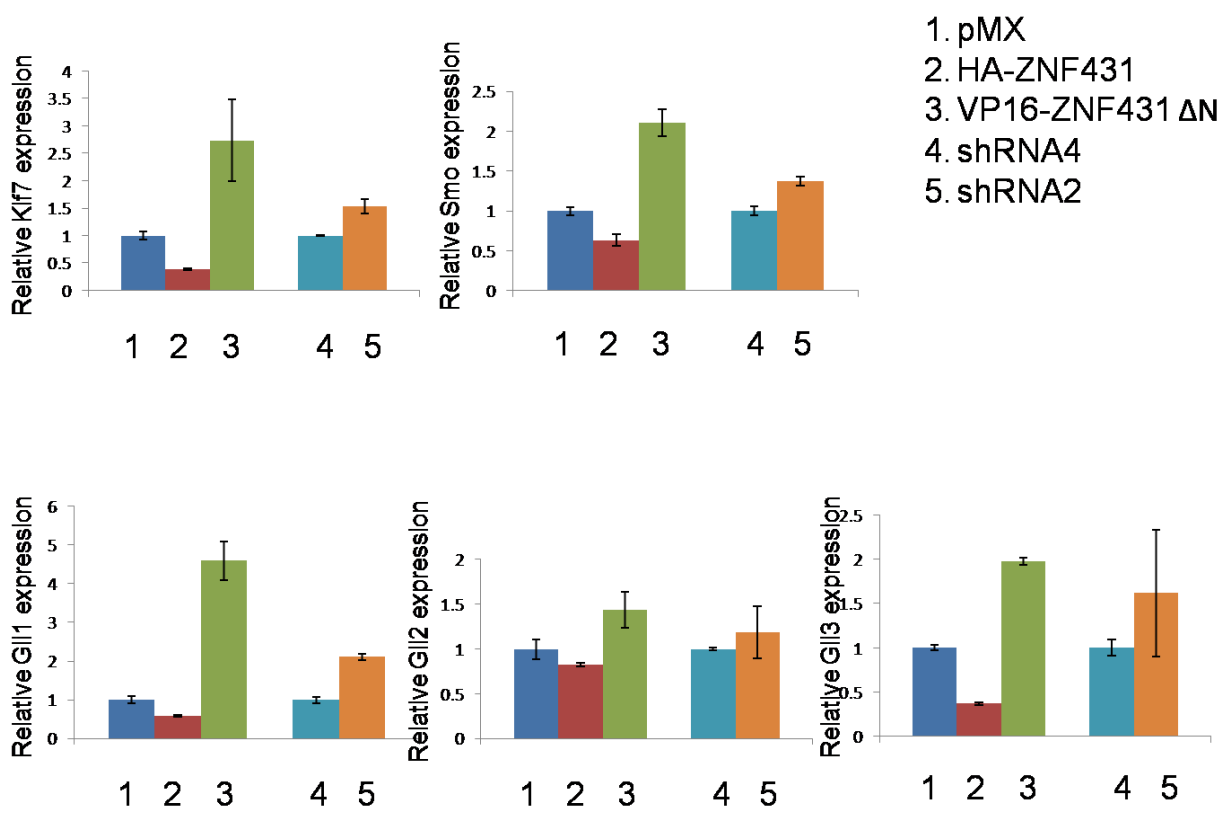

Fig. S12. Expression of Kif7, Smo, Gli1,2 and 3 in MPLB cells. Realtime RT PCR was performed to deterimine expression of Kif7, Smo, Gli1, 2 and 3 in MPLB cells. Expression of these 5 genes were reduced in HAZNF431 overexpressing cells and elevated in VP16ZNF431ΔN overexpressing cells. shRNA2 but not shRNA4 (no effect on ZNF431 expression) was able to elevate the expression of these genes to various degrees.

Fig. S13.

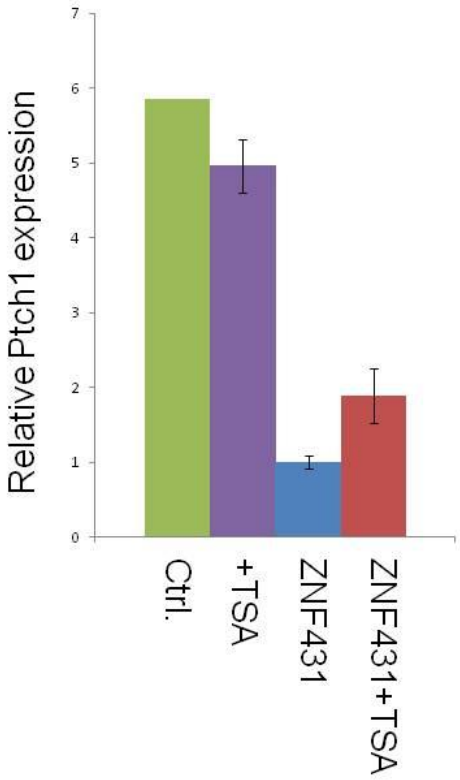

Fig. S13. Inhibition of HDAC activity partially rescues Ptch1 repression by ZNF431. TSA-treatment (50ng/ml) partially rescued ZNF431-mediated transcriptional repression on *Ptch1* expression but did not affected *Ptch1* expression in the absence of ZNF431 overexpression as assayed by realtime PCR.

Fig. S14.

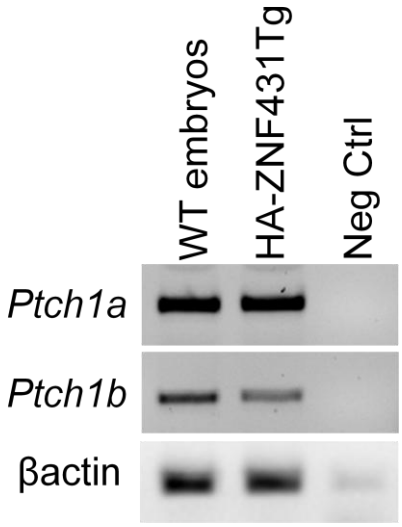

Fig. S14. Overexpression of ZNF431 downregulated *Ptch1* 1b but not the 1a isoform in mouse embryos as assayed by RT-PCR.

Fig. S15.

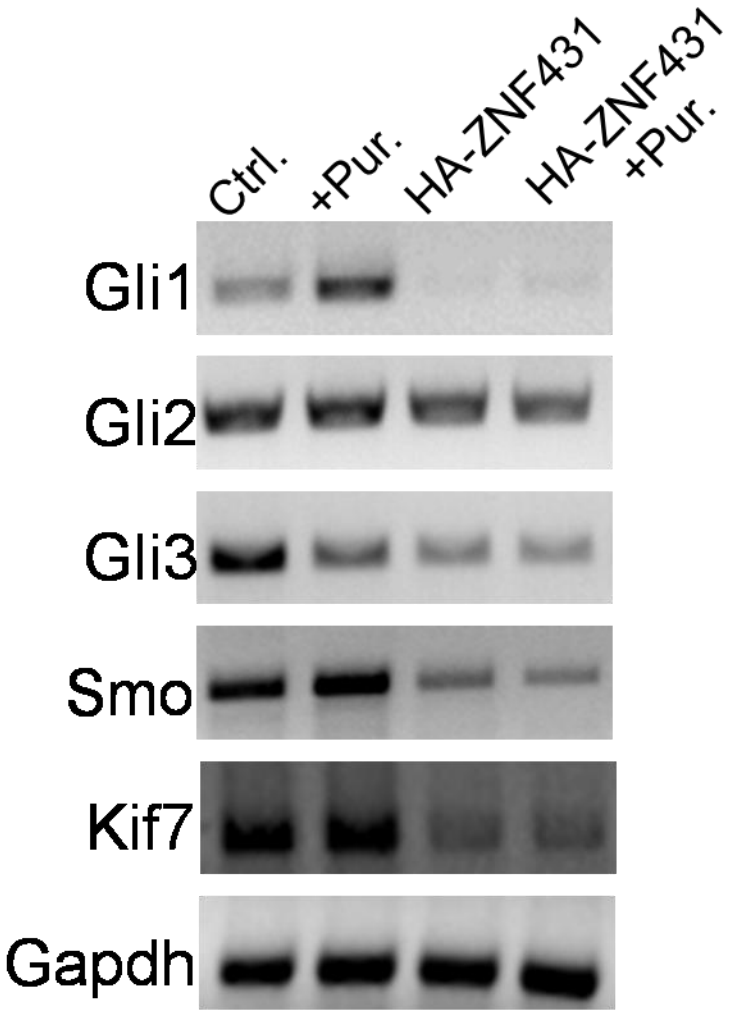

Fig. S15. Smo, Kif7, Gli1,2 and 3 do not respond to Hh stimulus when ZNF431 is overexpressed in MPLB cells as assayed by RT-PCR.