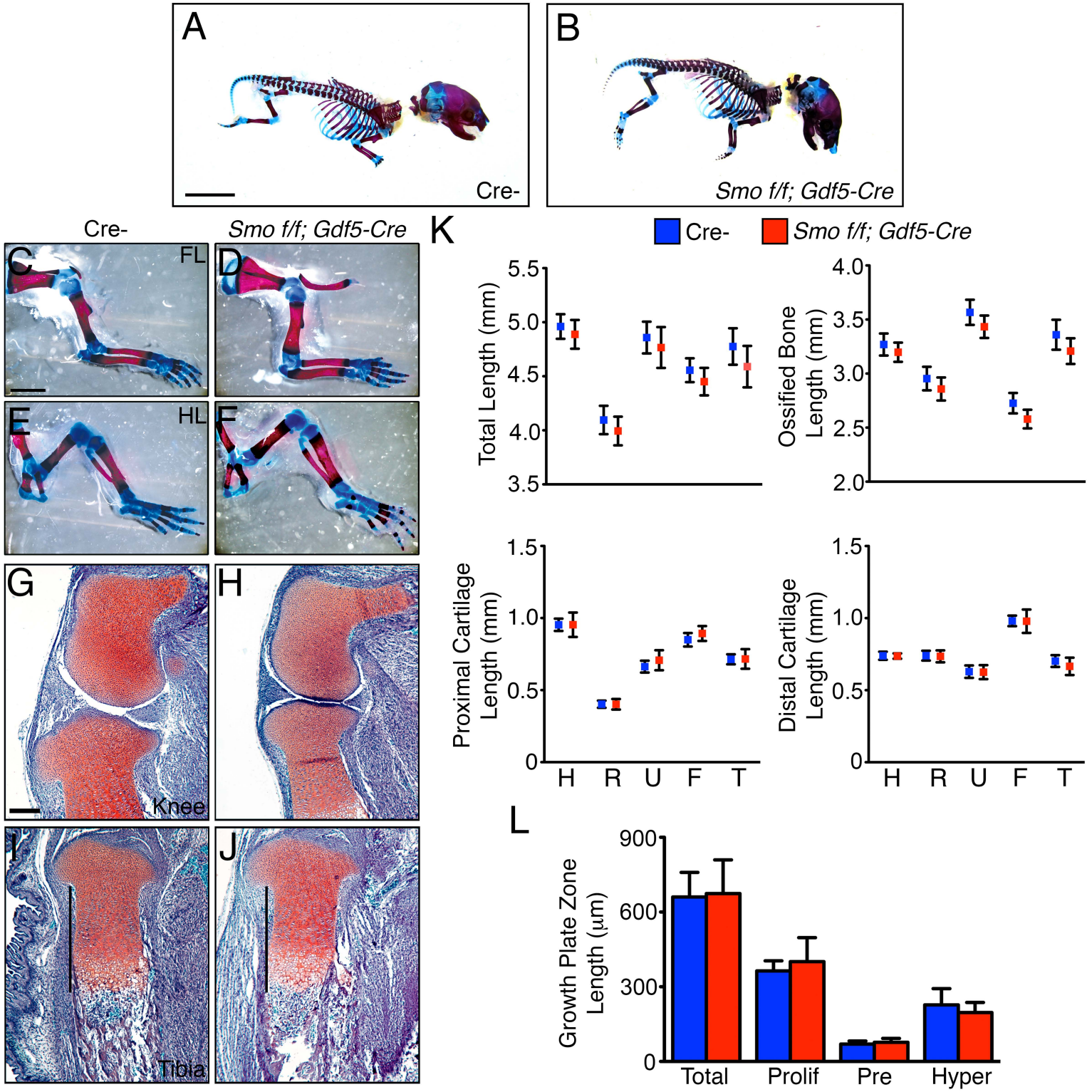
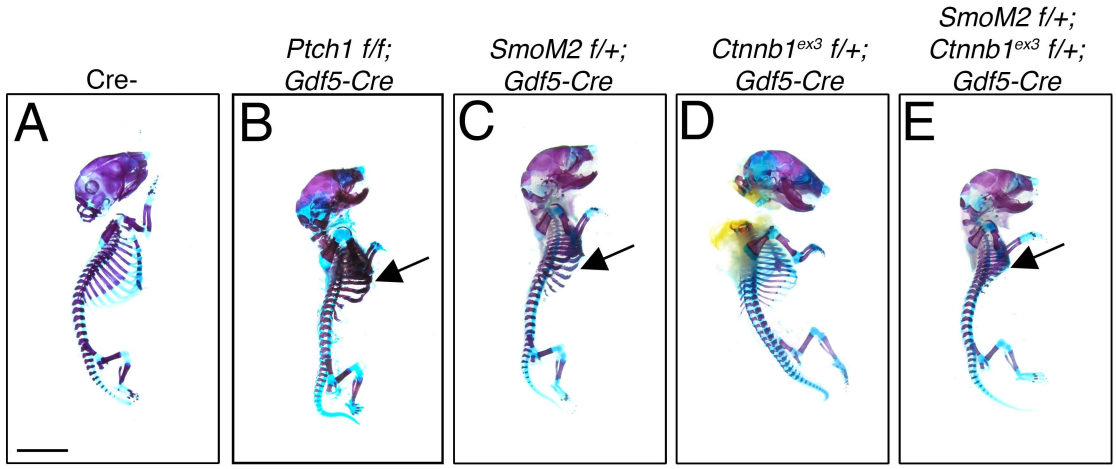
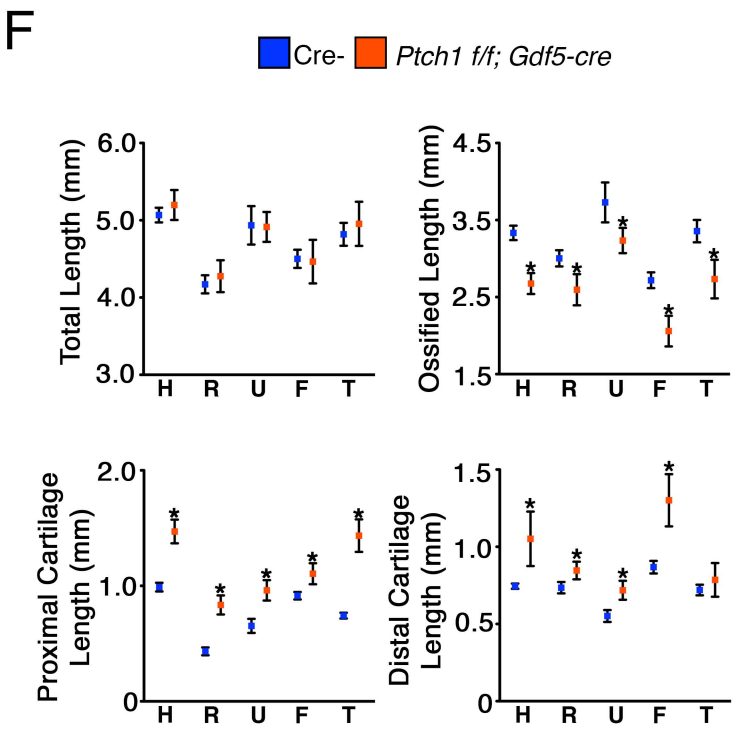
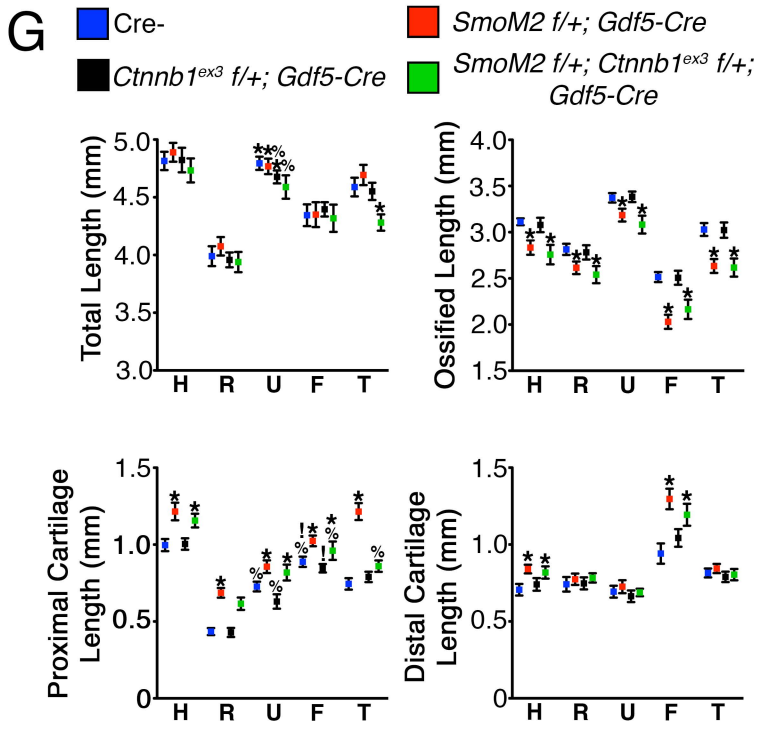
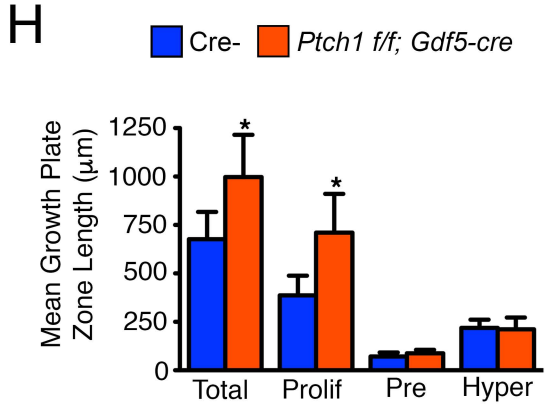


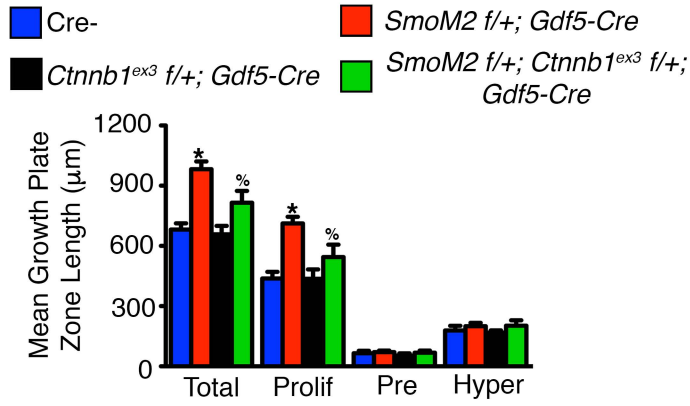
Figure S1. Conditional deletion of hedgehog signaling in interzone progeny does not affect joint or skeletal morphogenesis. (A-F) P0 Cre- or *Smo f/f; Gdf5-Cre* mice were stained by alcian blue/alizarin red. There were no overt differences in skeletal formation in whole skeletons (A & B), forelimbs (FL; C & D) or hindlimbs (HL; E & F). (G-J) E17.5 hindlimb sections were stained by SafO. Consistent with P0, there were no observable differences in knee joints (G & H) or growth plates (I & J) between Cre- (G, I) and *Smo f/f; Gdf5-Cre* (H, J) mice. (I, J) Black bars indicate length of growth plates. (K) Proximal and distal cartilage, and bone from the long bones of the forelimbs and hindlimbs of Cre- (blue) and *Smo f/f; Gdf5-cre* (red) mice at P0 were measured. H, humerus; R, radius; U, ulna; F, femur; T, tibia. Bars are mean ± 95% CI. No significant differences were observed in total bone length, proximal cartilage length, distal cartilage length, or ossified region as determined by Student's t-tests ($p > 0.05$). $n \geq 9$ per genotype. (L) Growth plate zone length was measured from Cre- (blue) and *Smo f/f; Gdf5-cre* (red) mice at E17.5. No significant differences were observed in total, proliferative (Prolif), prehypertrophic (Pre) or hypertrophic (Hyper) zone lengths as determined by Student's t-tests ($p > 0.05$). $n \geq 4$ per genotype. Scale bars: (A) 1 cm, (C) 2 mm, (G) 200 μm.

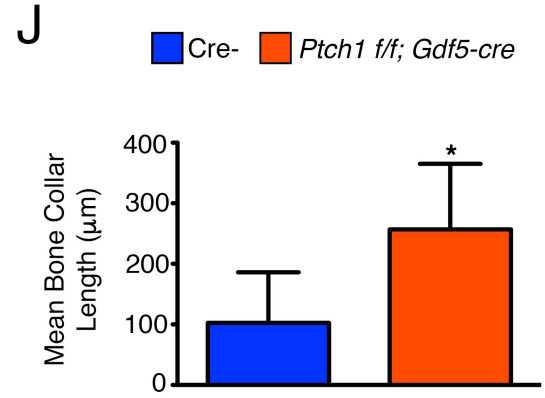

Cre-
Ptch1 f/f; Gdf5-Cre
SmoM2 f/+; Gdf5-Cre
Ctnnb1ex3 f/+; Gdf5-Cre
SmoM2 f/+; Ctnnb1ex3 f/+; Gdf5-Cre
A
B
C
D
E


F
Cre-
Ptch1 f/f; Gdf5-cre
Total Length (mm)
Ossified Length (mm)
Proximal Cartilage Length (mm)
Distal Cartilage Length (mm)
H R U F T


G
Cre-
Ctnnb1ex3 f/+; Gdf5-Cre
SmoM2 f/+; Gdf5-Cre
SmoM2 f/+; Ctnnb1ex3 f/+; Gdf5-Cre
Total Length (mm)
Ossified Length (mm)
Proximal Cartilage Length (mm)
Distal Cartilage Length (mm)
H R U F T


H
Cre-
Ptch1 f/f; Gdf5-cre
Mean Growth Plate Zone Length (µm)
Total
Prolif
Pre
Hyper


I
Cre-
Ctnnb1ex3 f/+; Gdf5-Cre
SmoM2 f/+; Gdf5-Cre
SmoM2 f/+; Ctnnb1ex3 f/+; Gdf5-Cre
Mean Growth Plate Zone Length (µm)
Total
Prolif
Pre
Hyper


J
Cre-
Ptch1 f/f; Gdf5-cre
Mean Bone Collar Length (µm)

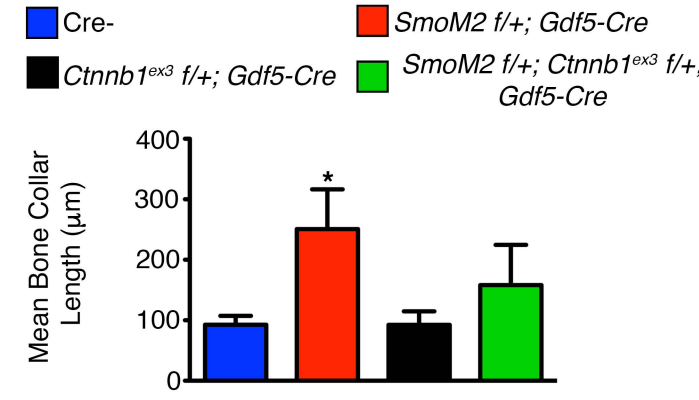

K
Cre-
Ctnnb1ex3 f/+; Gdf5-Cre
SmoM2 f/+; Gdf5-Cre
SmoM2 f/+; Ctnnb1ex3 f/+; Gdf5-Cre
Mean Bone Collar Length (µm)

Figure S2. Conditional deletion of *Ptch1* or expression of SmoM2 in interzone progeny induces cartilage and bone dysplasias, some of which can be rescued by activation of β-catenin. (A-E) Newborn (P0) mouse skeletons were stained with alcian blue/alizarin red for cartilage/bone. Mice with active hedgehog signaling in interzone progeny were perinatal lethal with increased ossification of the rib cage (B & C; arrows). (D) There were no overt skeletal phenotypes when β-catenin was activated in interzone cells alone. (E) Co-activation of β-catenin in interzone progeny could not rescue the hedgehog-induced perinatal lethality or the rib cage phenotype (arrow). (F-K) Length measurements of skeletal elements were made from forelimbs and hindlimbs of (F, H, J) Cre- (blue), or *Ptch1 f/f; Gdf5-Cre* (orange) mice and (G, I, K) Cre- (blue), *SmoM2 f/+; Gdf5-cre* (red), *Ctnnb^{ex3} f/+; Gdf5-cre* (black) or *SmoM2 f/+; Ctnnbex3 f/+; Gdf5-cre* (green) mice. (F & G) Total, ossified, proximal cartilage and distal cartilage length were measured from P0 alcian blue/alizarin red stained whole mount long bones. H, humerus; R, radius; U, ulna; F, femur; T, tibia. n ≥ 5 for each genotype. (H & I) Total, proliferative zone (Prolif), prehypertrophic zone (Pre) and hypertrophic zone (Hyper) lengths of the growth plate were measured from E17.5 mouse hindlimb sections stained with SafO. n ≥ 4 per genotype. (J & K) Mean bone collar length surrounding the dorsal and ventral sides of the tibial growth plate were measured from E17.5 hindlimb sections stained by Von Kossa. n ≥ 4 per genotype. (F-K) Data are mean ± 95% CI. (F, H,J) *, $p < 0.05$ by Student's t-test. (G, I, K) Data was analyzed by one way ANOVA and Tukey's post-hoc tests. Unlabeled bars or bars labeled with the same symbol are not significantly different within each limb element (G), within each zone (I) or between genotypes (K). Scale bar: (A) 1 cm.

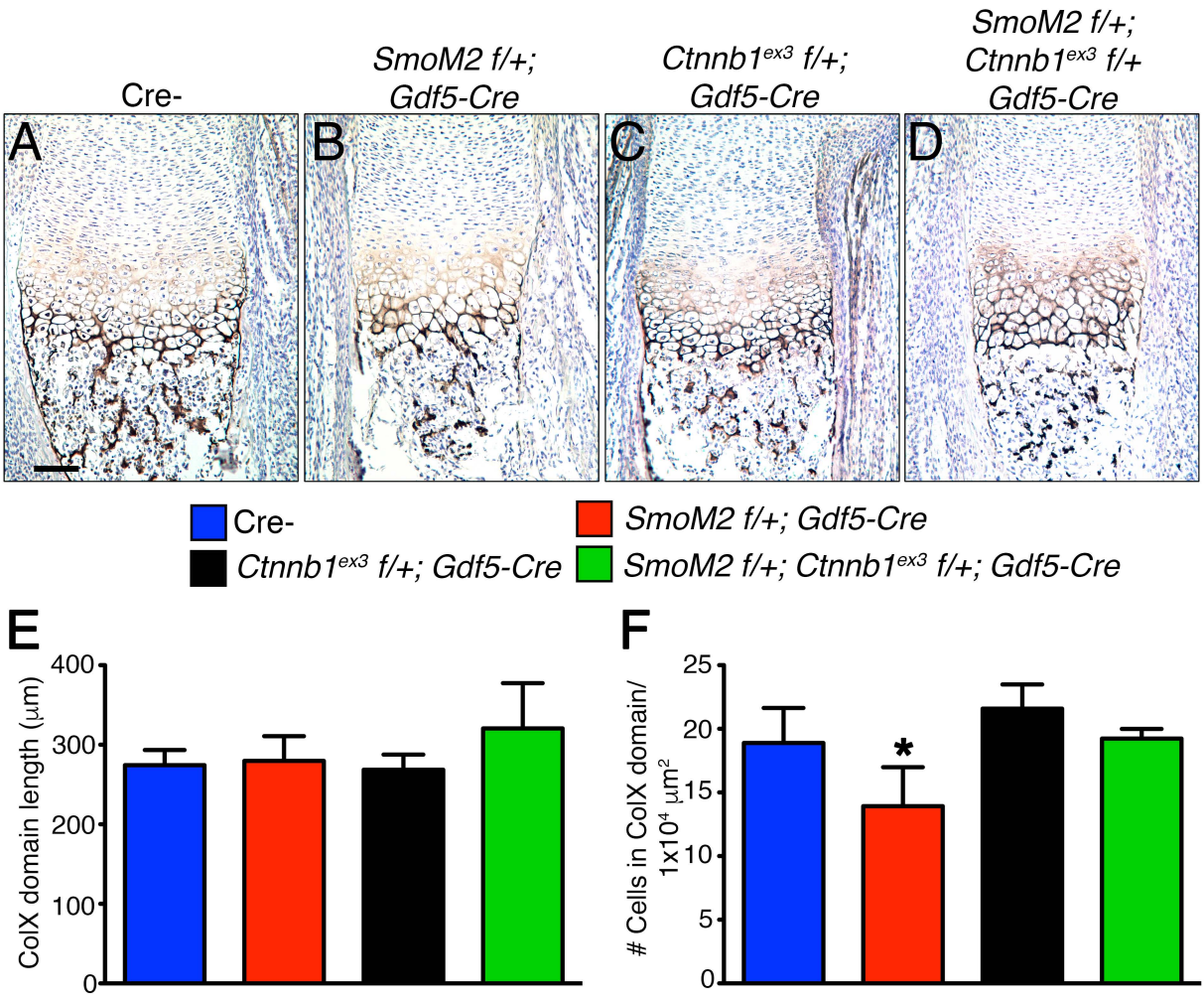


Figure S3. Activation of hedgehog signaling decreases cell density in the type X collagen domain. (A-D) Hindlimb sections from E17.5 mice were stained by immunohistochemistry for type X collagen. (E & F) The length of the type X collagen staining domain was measured (E) and the number of cells within the type X collagen staining domain/10^4 μm^2 (F) was determined in Cre- (blue), *SmoM2 f/+; Gdf5-Cre* (red), *Ctnnb1^{ex3} f/+; Gdf5-Cre* (black), and *SmoM2 f/+; Ctnnb1^{ex3} f/+; Gdf5-Cre* (green) mice. Bars are mean ± 95% CI. (E) There was no significant differences in the length of the type X collagen staining domain ($p > 0.05$), as determined by one-way ANOVA. $n \geq 6$ per genotype. (F) There was a significant reduction in the number of cells within the type X collagen staining domain per 10^4 μm in *SmoM2 f/+; Gdf5-Cre* mice as compared to all other genotypes as analyzed by one-way ANOVA followed by Tukey's post-hoc tests (*, $p < 0.01$ compared to all other bars). $n \geq 6$ per genotype. Scale bar: (A) 100 μm.

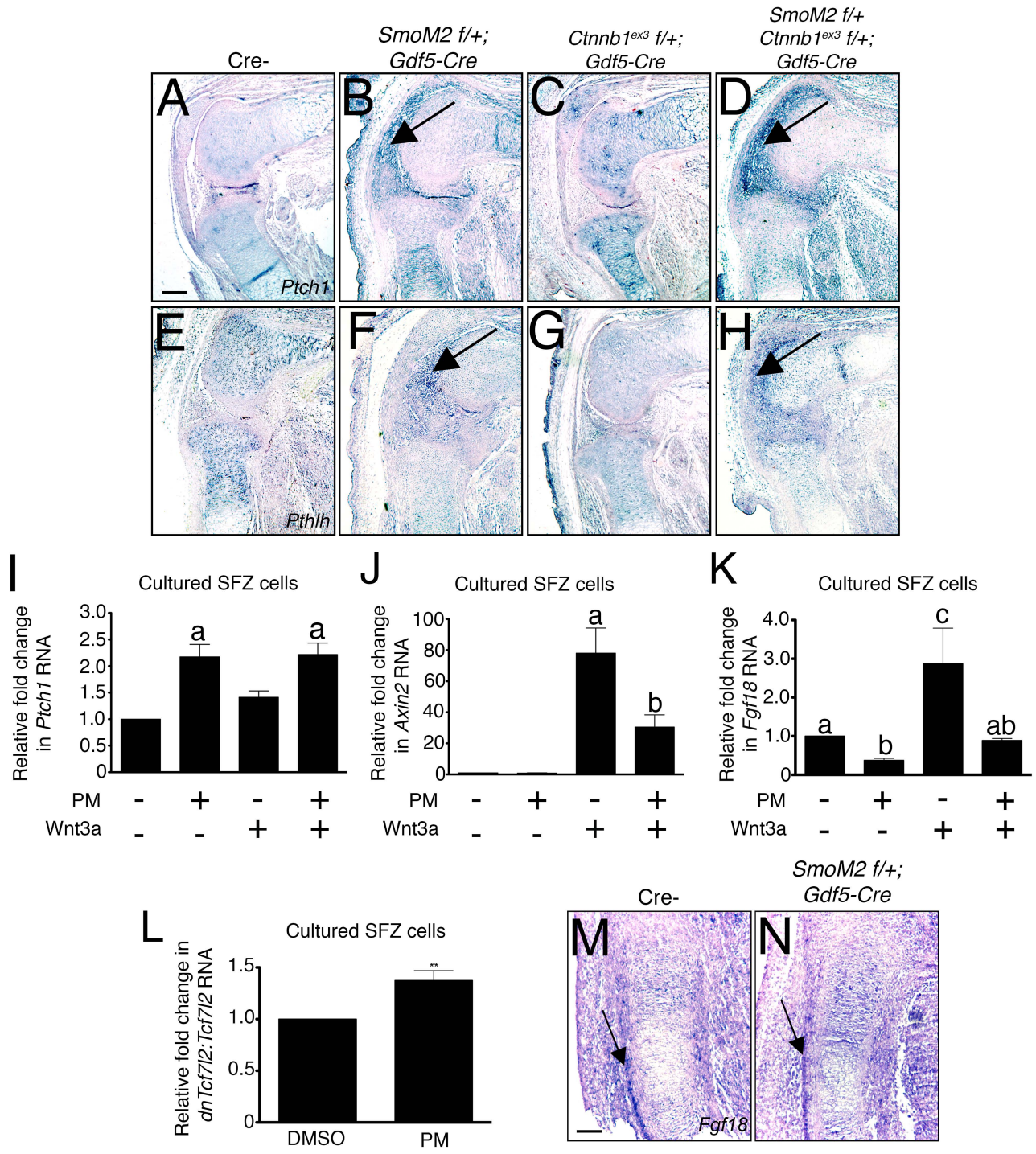


Figure S4. Hedgehog signaling in interzone progeny induces gene expression changes in *in vivo* and *in vitro*. (A-H) Hindlimb sections from E15.5 mice were stained for expression of *Ptch1* (A-D) or *Pthlh* (E-H) by in situ hybridization. *SmoM2 f/+; Gdf5-Cre* and *SmoM2 f/+; Ctnnb1^{ex3}; Gdf5-Cre* mice show increased *Ptch1* (B & D) and *Pthlh* (G & I) expression in interzone progeny (arrows) as compared to Cre- (A & E) or Ctnnb1^{ex3} f/+; Gdf5-cre (C & G) mice. n = 3 per genotype. (I-L) Superficial zone (SFZ) cells from P4-P5 mouse knees were treated with 0.1% DMSO, purmorphamine (PM, 5 μM) and/or Wnt3a (100 ng/ml) for 24 h. RNA from cultures was analyzed for levels of *Ptch1* (I), *Axin2* (J) and *Fgf18* (K) normalized to *18S* rRNA, or the ratio of *dnTcf7l2* to full length *Tcf7l2* (L), relative to control cultures (first bar). Bars are mean ± SE. Data was log transformed prior to analysis by one-way ANOVA and Tukey's post-hoc tests (I-K) or by Student's t-test (L). (I-K) Unlabeled bars or bars labeled with the same letter are not significantly different ($p > 0.05$). (L) **, $p < 0.01$. (I) n = 5. (J) n = 5. (K) n ≥ 3. (L) n = 5. (M, N) Hindlimb sections from E14.5 mice were stained for *Fgf18* by in situ hybridization. No difference in *Fgf18* expression in the perichondrium was observed between Cre- (A) and *SmoM2 f/+; Gdf5-Cre* mice (black arrows). n ≥ 5 per genotype. Scale bars: (A) 200 μm, (M) 100 μm.

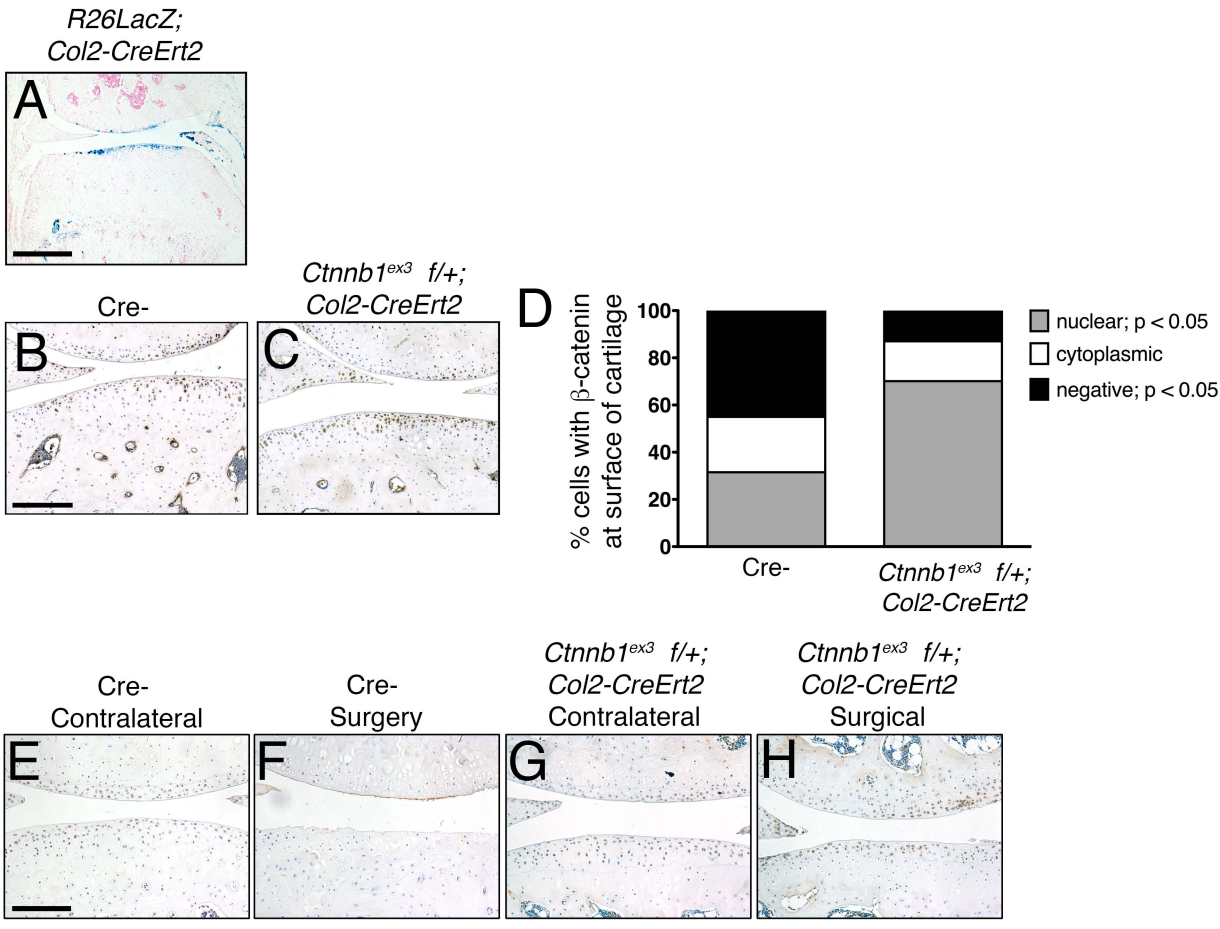


Figure S5. Col2-CreErt2 recombination efficiency and protein expression in transgenic mice and surgically-induced OA mice. (A) *R26LacZ f/+; Col2-CreErt2* mice were injected with tamoxifen at 8 weeks of age. Knees were collected 6 weeks after tamoxifen injection and stained with XGal. (B & C) Cre- or C*tnnb1*^{ex3} *f/+; Col2-CreErt2* were injected with tamoxifen at 8 weeks of age and collected 6 weeks after injection. Sections were stained for β-catenin by immunohistochemistry. (D) β-catenin localization was counted in 6 independent regions across the surface of the cartilage correlating to the zone of XGal staining identified in (A) in tamoxifen-treated Cre- and *Ctnnb1*^{ex3} *f/+; Col2-CreErt2* mice. A significant increases in the proportion of cells with nuclear localized β-catenin (grey) and s significant reduction in cells without β-catenin were observed in *Ctnnb1*^{ex3} *f/+; Col2-CreErt2* compared to Cre- mice ($p < 0.05$). Total cells counted: Cre- = 130, C*tnnb1*^{ex3} *f/+; Col2-CreErt2* = 214 from n = 3 independent mice per genotype. Proportional data was log transformed prior to analysis by multiple Student's t-tests. (E-H) Activation of β-catenin does not prevent the expression of ADAMTS5 in surgically-induced OA mice (H) compared to contralateral controls (G) as determined by immunohistochemistry. The absence of ADAMTS5 staining in Cre- mice with surgically-induced OA (F) is likely a result of loss of cartilage and cells at the surface as compared to contralateral limbs (E). Images are representative of n = 3 mice per genotype. Scale Bars (A) 400 μm, (B) 200 μm, (C) 100 μm.

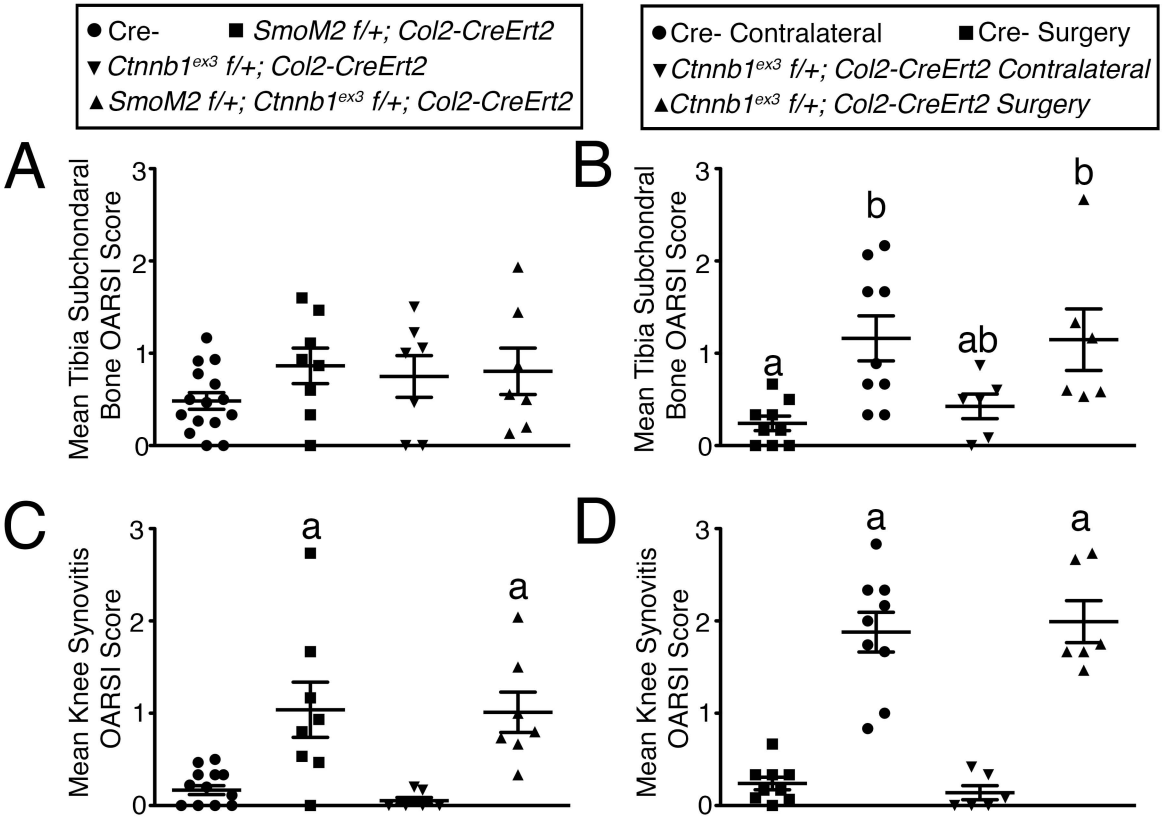


Figure S6. OARSI scoring of subchondral bone thickening and synovitis from transgenic and surgically-induced OA mice. Average OARSI score of subchondral bone (A & B) or knee synovitis (C & D) was determined from transgenic alone (A & C) or transgenic and surgically-induced OA (B & D) mice. Individual points are an average of 3-8 sections from two-four slides separated by a minimum of 60 μm and graded by OARSI scoring recommended for the mouse by 3 blinded, independent reviewers. Bars are mean ± SE. Data was analyzed by one-way ANOVA followed by Tukey's post-hoc tests. Unlabeled bars or bars labeled with the same letter are not significantly different ($p > 0.05$). $n \geq 6$ per group.

Table S1. Summary of characterization of cellular phenotypes from histological sections of mouse tibias.

Genotype / Phenotype	Cre-	*SmoM2 f/+; Col2-CreErt2*	*Ctnnb1^{ex3} f/+; Col2-CreErt2*	*SmoM2 f/+; Ctnnb1^{ex3} f/+; Col2-CreErt2*	Cre- Contralateral	Cre- Surgery	*Ctnnb1^{ex3} f/+; Col2-CreErt2* Contralateral	*Ctnnb1^{ex3} f/+; Col2-CreErt2* Surgery
Superficial Zone Loss	–	++	–	–/+	–	++/+++	–	+
Empty Lacunae	–	–/+	–	–	–	+/++	–	–/+
Decreased Cellularity	–	++	–	–	–	++	–	+
Chondrocyte Clusters	–	–/+	–	–	–	+	–	–/+

–, minimal (< 10%); +, moderate (10-30%); ++, strong (30-50%) +++; very strong (> 50%).