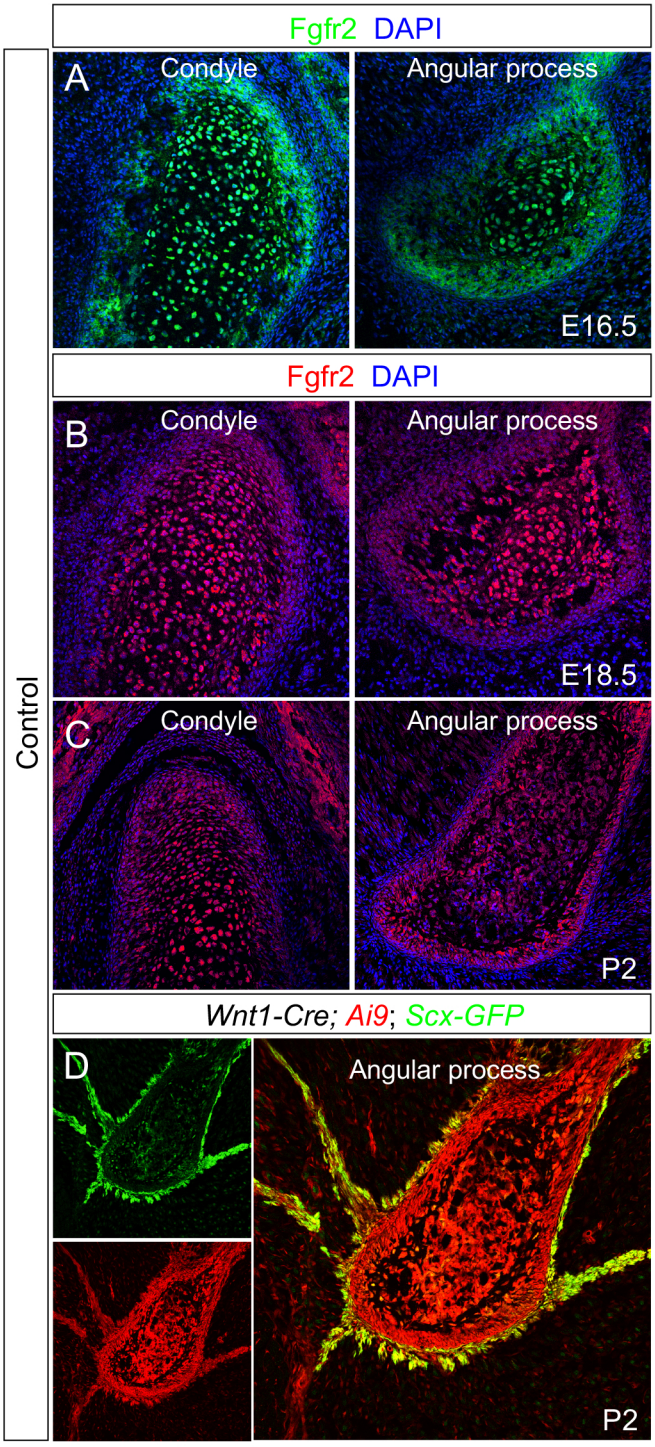

Figure S1. Mandibular entheses and tendons are NCC-derived and express Fgfr2. Immunofluorescence shows Fgfr2 expression in coronal sections of the control condyle and angular process at E16.5 **(A)**, E18.5 **(B)**, and P2 **(C)** (n=3 for each stage). (**D**) Coronal section of the *Wnt1-Cre; Ai9;Scx-GFP* angular process shows that the mandibular tendons and their entheses are derived from neural crest cells (n=3).

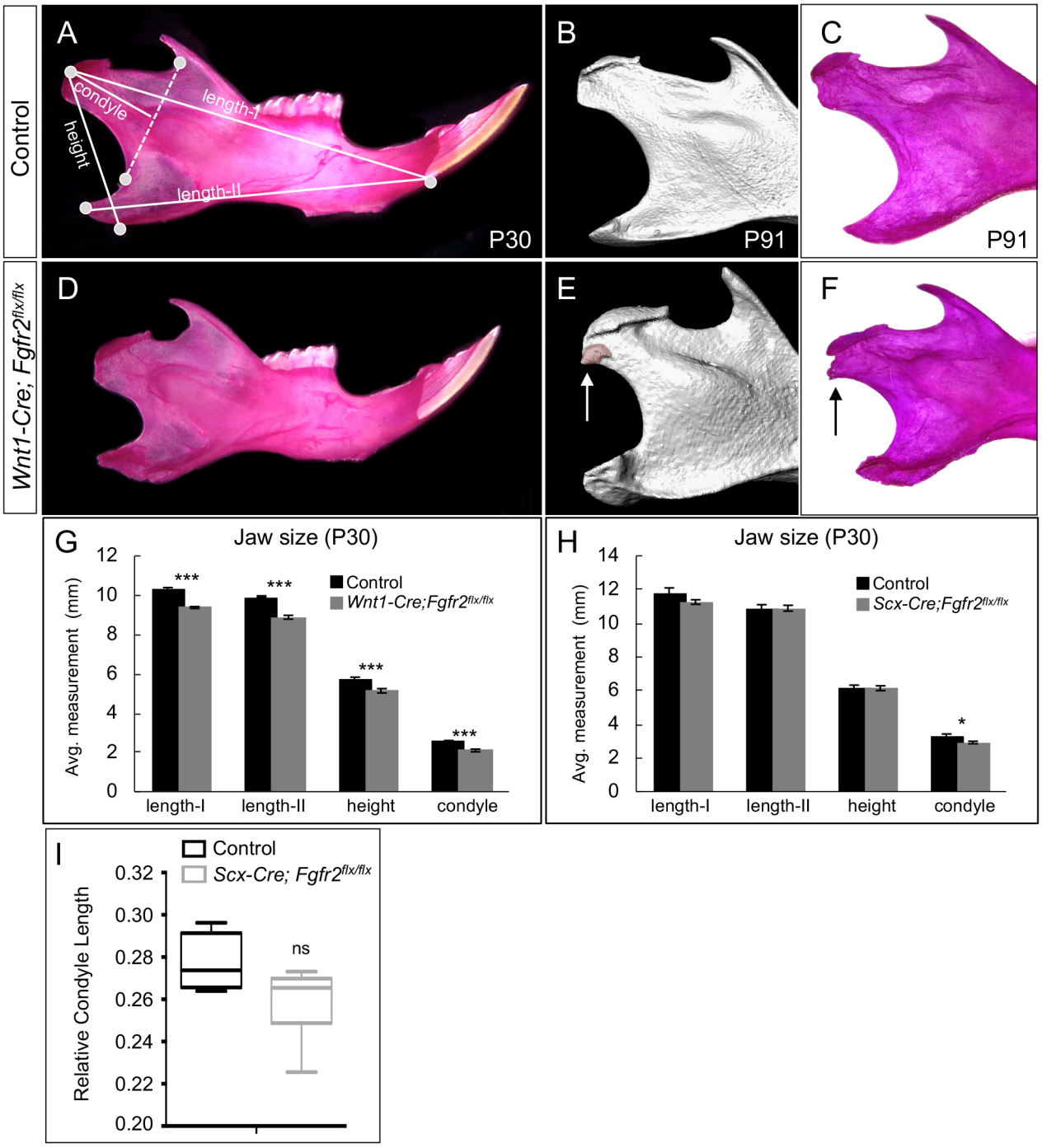

Figure S2. Loss of *Fgfr2* disrupts formation of the condyle and angular processes. (**A, D**) Whole mount with Alizarin red stained mandibles at P30 shows ectopic bone on the condyle and angular process in *Wnt1-Cre; Fgfr2*$^{flx/flx}$ mice as compared to controls. μCT analyses and whole mount skeletal preparation of P91 mandibles from control (**B-C**) and *Wnt1-Cre; Fgfr2*$^{flx/flx}$ (**E-F**)

littermates show that osteophyte-like structures develop on the condyle of the mutant (n=3 littermate pairs). Arrows in panels **E** and **F** indicate ectopic bone on the condyle (pseudo-colored). (**G-H**) Morphometric measurements, indicated by lines in panel **A**, were taken to assess jaw size at P30 (n=6, same sex littermate pairs) in *Wnt1-Cre; Fgfr2*$^{flx/flx}$ (**G**) and *Scx-Cre; Fgfr2*$^{flx/flx}$ (**H**) versus littermate controls. length-I = mandibular symphysis to condyle articular surface; length-II = mandibular symphysis to angular process; height = angular process to base of condyle articular surface; and condyle = condyle base to condyle articular surface. *p<0.05 and *** p<0.001, Error bars = SEM. (**I**) Morphometric analysis of relative condyle length in *Scx-Cre;Fgfr2*$^{flx/flx}$ mice compared to controls (p=0.12)(n=3 same-sex littermate pairs). (ns) not significant. Error bars=SEM

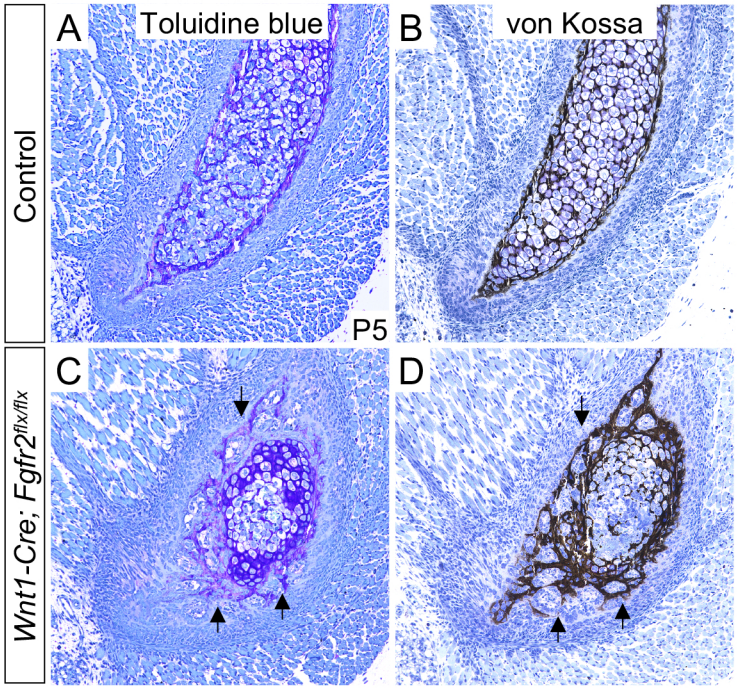

Figure S3. ***Wnt1-Cre;Fgfr2$^{flx/flx}$*** **mice develop ectopic endochondral bone in the perichondrium of the angular process.** Un-decalcified coronal sections of the angular process at P5 stained with Toluidine blue (**A,C**) to detect cartilage proteoglycans and von Kossa (**B,D**) to detect calcium deposits show dual labelling of the ectopic bone in *Wnt1-Cre;Fgfr2$^{flx/flx}$* mice (n=3 littermate pairs).

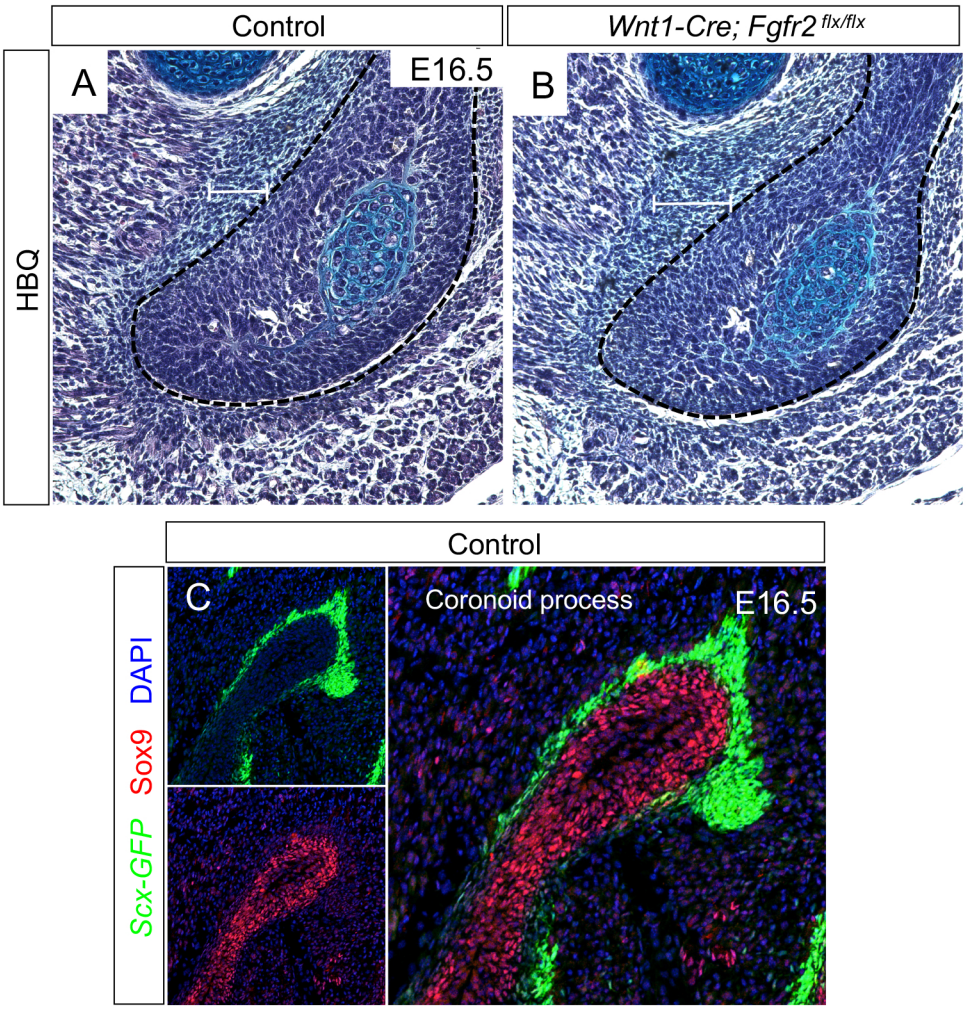

Figure S4. Histological sections of the control (**A**) and *Wnt1-Cre; Fgfr2*$^{flx/flx}$ (**B**) angular processes stained with HBQ (n=3 littermate pairs). The outer boundary of the core cartilage is indicated with dotted line and a bracketed line marks the perichondrium. (**C**) *Scx-GFP* expression and Sox9 immunofluorescence in the control coronoid process at E16.5.

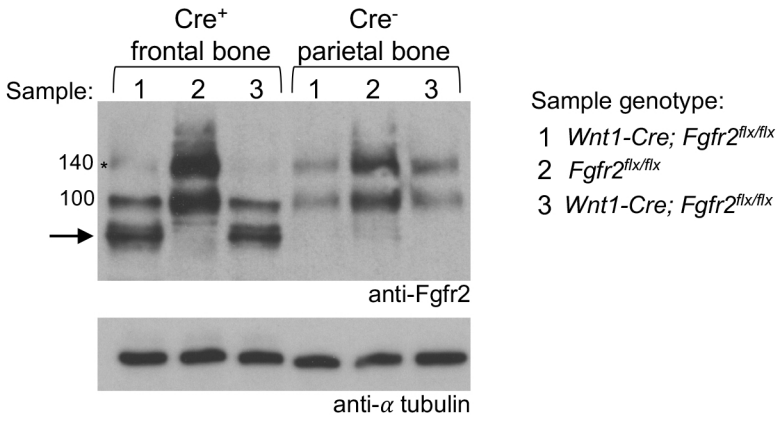

Figure S5. Anti-Fgfr2 antibody recognizes a truncated protein product. Western blot analysis of protein lysates extracted from the frontal and parietal bones of P5 mice. The frontal bones are NCC-derived and, therefore, targeted by the *Wnt1-Cre* driver. The parietal bones, on the other hand, are paraxial mesoderm-derived. In frontal bone protein lysates from *Wnt1-Cre; Fgfr2*$^{flx/flx}$ mice, the Fgfr2 antibody that recognizes amino acids 771–821, identified a new band (arrow) upon homologous recombination of the *Fgfr2*^{flx} allele, which excises exons 7-10 for the IIIb and IIIc isoforms, which include the ligand binding domains, as well as the transmembrane domain (Yu et al., 2003). This band was not present in the frontal and parietal bone lysates of *Fgfr2*$^{flx/flx}$ or in the parietal bone lysates of *Wnt1-Cre; Fgfr2*$^{flx/flx}$.

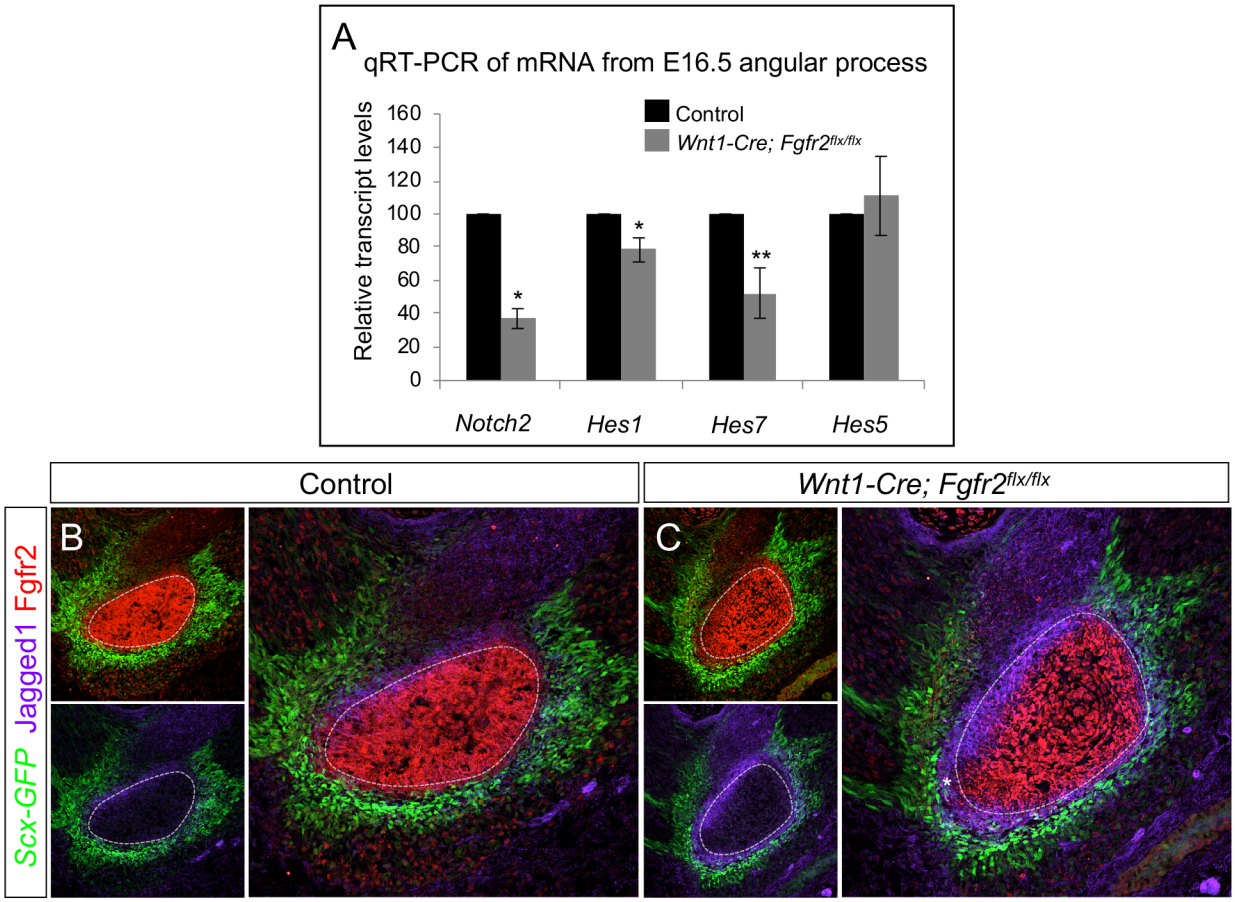

Figure S6. Expression of Notch pathway members are altered in the angular process of *Wnt1-Cre;Fgfr2 flx/flx* mice (**A**) RT-qPCR analysis of cDNA from E16.5 angular processes show reduced expression of *Notch2* and its targets *Hes1* and *Hes7*, but not *Hes5* in the mutant (n=3 littermate pairs). *p<0.05, **p<0.01, Error bars=SEM. (**B**) At E16.5, Jagged1 is in the perichondrial region along with Fgfr2. (**C**) In the *Wnt1-Cre;Fgfr2*$^{flx/flx}$ angular process, Jagged1 in the tendon-bone interface is expanded in a region occupied by *Fgfr2* knockout cells (asterisk).

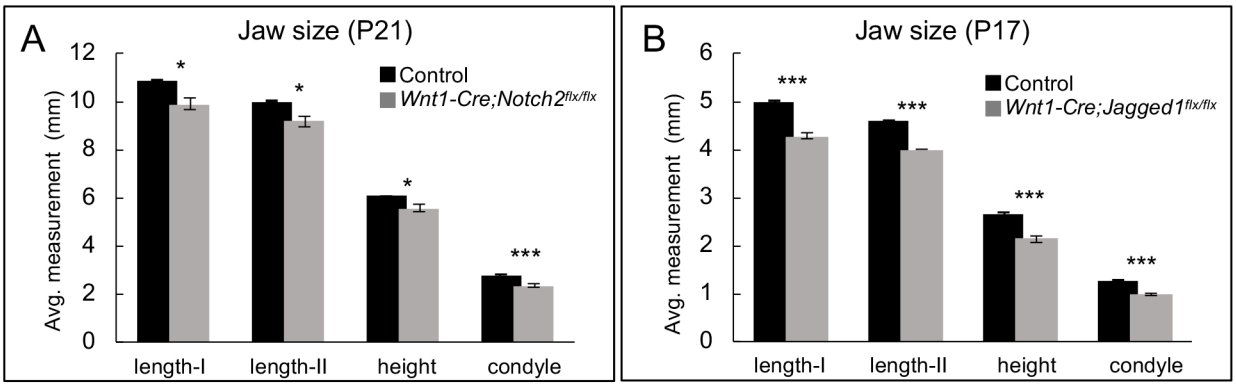

Figure S7. Morphometric analysis of *Notch2* and *Jagged1* conditional knockout mandibles. Morphometric measurements, indicated by lines in panel Supplemental Figure 2A, were taken to assess jaw size at in (**A**) Wnt1-Cre; *Notch2*$^{flx/flx}$ mice at P21 (n=4, same sex littermate pairs) and (**B**) *Wnt1-Cre; Jagged1*$^{flx/flx}$ mice at P17 (n=10, same sex littermate pairs). *p<0.05 and *** p<0.001, Error bars = SEM

Table S1. Antibodies for immunofluorescence.

Antigen	Species	Source	Catalog number	Dilution
Bek (Fgfr2)	rabbit	Santa Cruz Biotechnology	sc-122	1:200
Collagen I	rabbit	ABCAM	ab34710	1:500
Collagen II	mouse	Developmental Hybridoma Bank	CIIC1	1:100
Delta like 1	rabbit	ABCAM	ab10554	1:100
Jagged1	goat	Santa Cruz Biotechnology	sc-6011	1:800
Notch2	rabbit	Santa Cruz Biotechnology	sc-5545	1:100
Sox9	rabbit	Novus Biologicals	NBP1-85551	1:1000

Table S2. Primer for qPCR.

Gene	Primers (5'-3')	Tm	Exon	Product size (bp)
Hes1	F: ATCATGGAGAAGAGGCGAAGG	59.6	2	119
	R: ATGTCTGCCTTCTCTAGCTTGG	59.8	3	
Hes5	F: AGAAGGCCGACATCCTGGAG	61.3	2	100
	R: TCGCTGTAGTCCTGGTGCAG	61.9	3	
Hes7	F: AAAGCGGAGATACTGGAGTTCG	60.2	3	127
	R: GGAAGCCGGACAAGTAGCAG	60.7	4	
Notch2	F: GATGGTGCATACTGTGATGTGC	60.0	20	82
	R: GGCACAAGTGTTCAACAGGTAC	60.0	21	

Table S3. Secondary antibodies for immunofluorescence.

Source	Catalog number	Secondary antibody description
Thermo Fisher Scientific	A-11008	Goat anti-rabbit IgG Alexa Fluor 488 conjugate
Thermo Fisher Scientific	A-11004	Goat anti-mouse IgG Alexa Fluor 568 conjugate
Thermo Fisher Scientific	A-11055	Donkey anti-goat IgG Alexa Fluor 488 conjugate
Thermo Fisher Scientific	A-11057	Donkey anti-goat IgG Alexa Fluor 568 conjugate
Thermo Fisher Scientific	A-21206	Donkey anti-rabbit IgG Alexa Fluor 488 conjugate
Thermo Fisher Scientific	A-21447	Donkey anti-goat IgG Alexa Fluor 647 conjugate
Thermo Fisher Scientific	A-31573	Donkey anti-rabbit IgG Alexa Fluor 647 conjugate