



Dysregulated chondrocyte formation as a novel mechanism of lambdoid synostosis

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Introduction

Craniosynostosis is a common birth defect that affects 1 of every 2500 children worldwide. It presents with premature fusion of calvaria bones. This fusion prevents skull expansion coordinated with brain growth and results in increased intracranial pressure. Synostosis at the lambdoid sutures is rare, and its etiologies remains largely unknown. In this study, we reported that deregulated Platelet derived growth factor receptor alpha (Pdgfra) activity causes lambdoid synostosis. Our data indicates that excessive Pdgfra activity promotes expansion and transformation of calvarial chondrocytes, which further undergoes endochondral ossification and causes fusion of the lambdoid suture.

Results

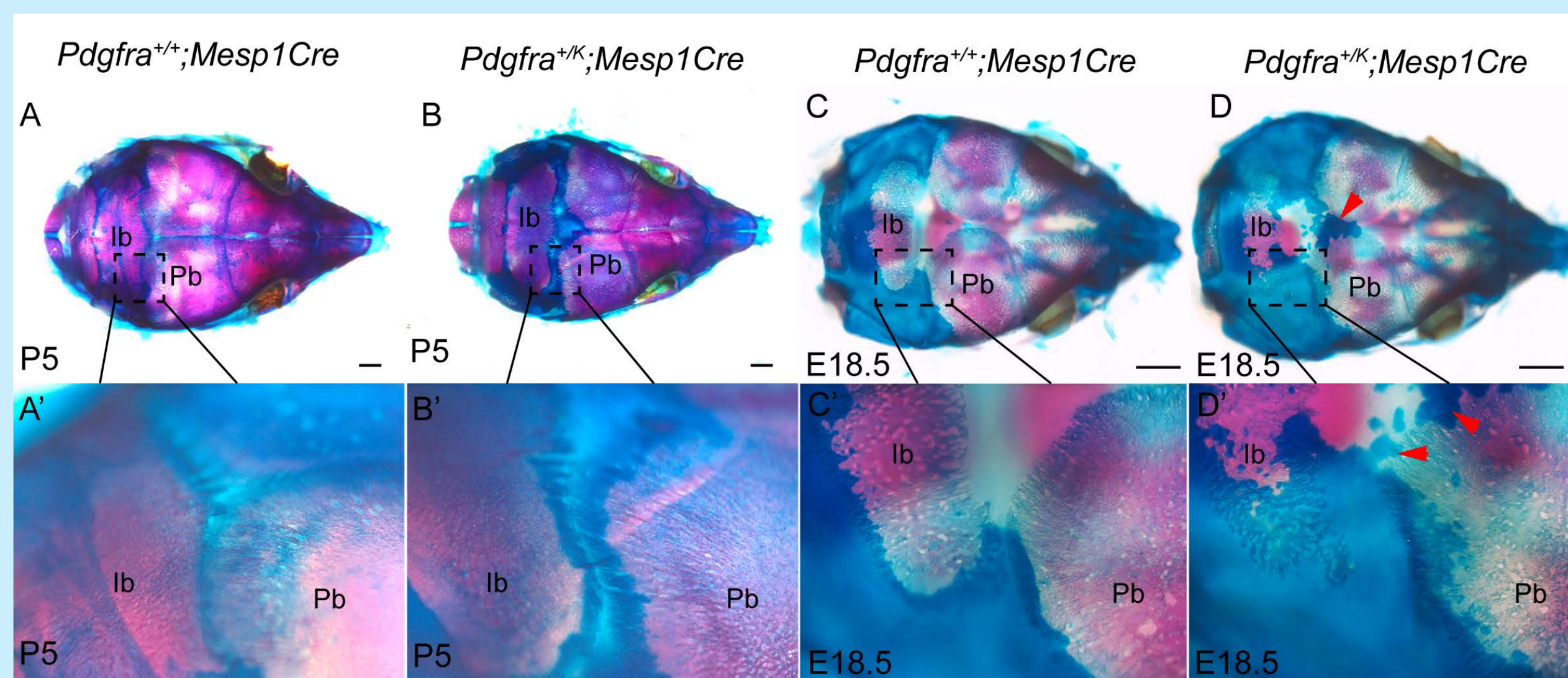
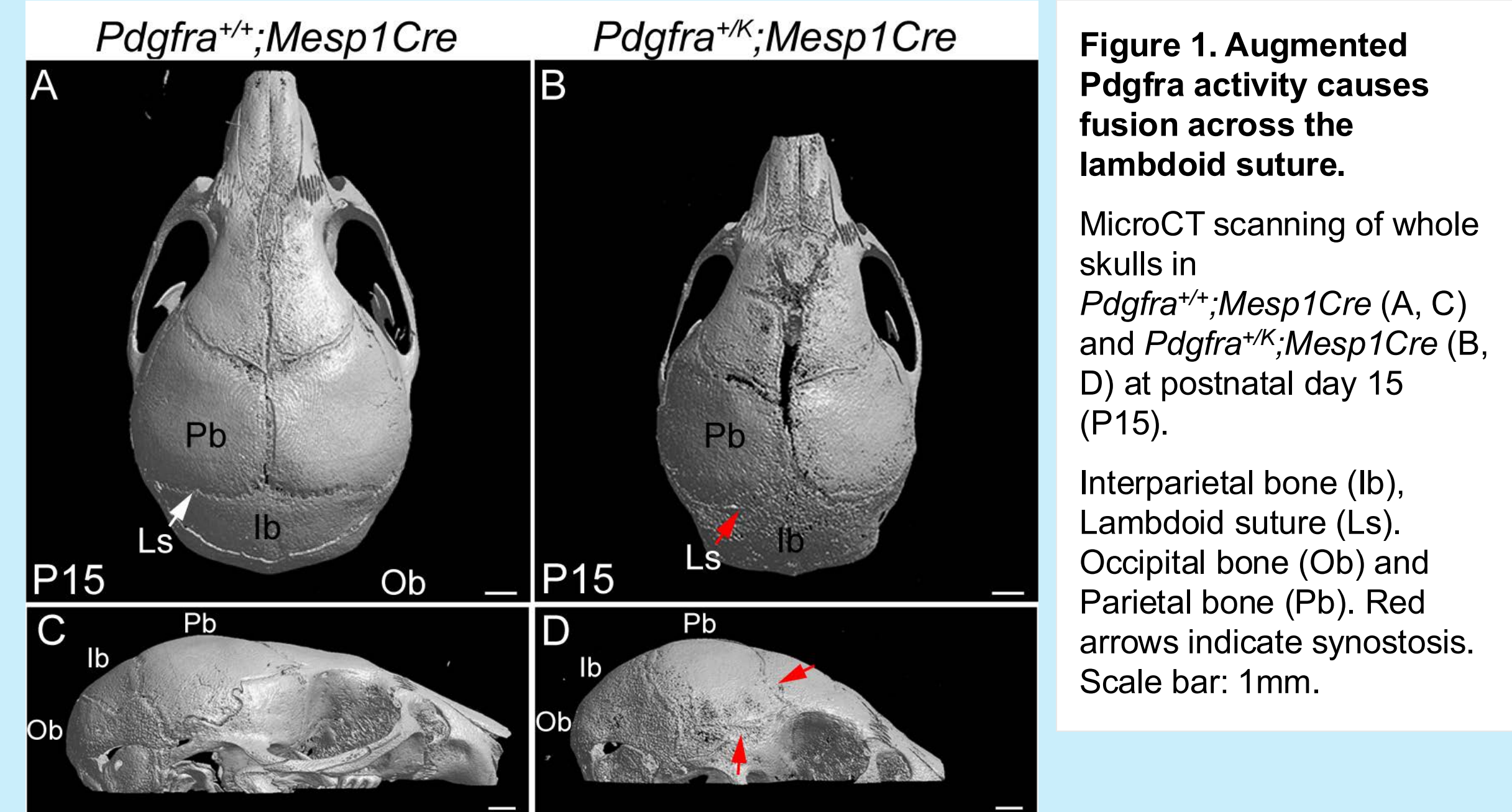


Figure 2. Expansion of cartilages precedes bony fusion of lambdoid suture in *Pdgfra*^{+/K};*Mesp1Cre* mice.
Skeletal preparation of *Pdgfra*^{+/+};*Mesp1Cre* at P5 (A, A'), E18.5 (C, C') and *Pdgfra*^{+/K};*Mesp1Cre* at P5 (B, B'), E18.5 (D, D'). Bone is stained by alizarin red and cartilage is stained by alcian blue. Red arrows point to ectopic cartilages. Scale bar: 1mm.

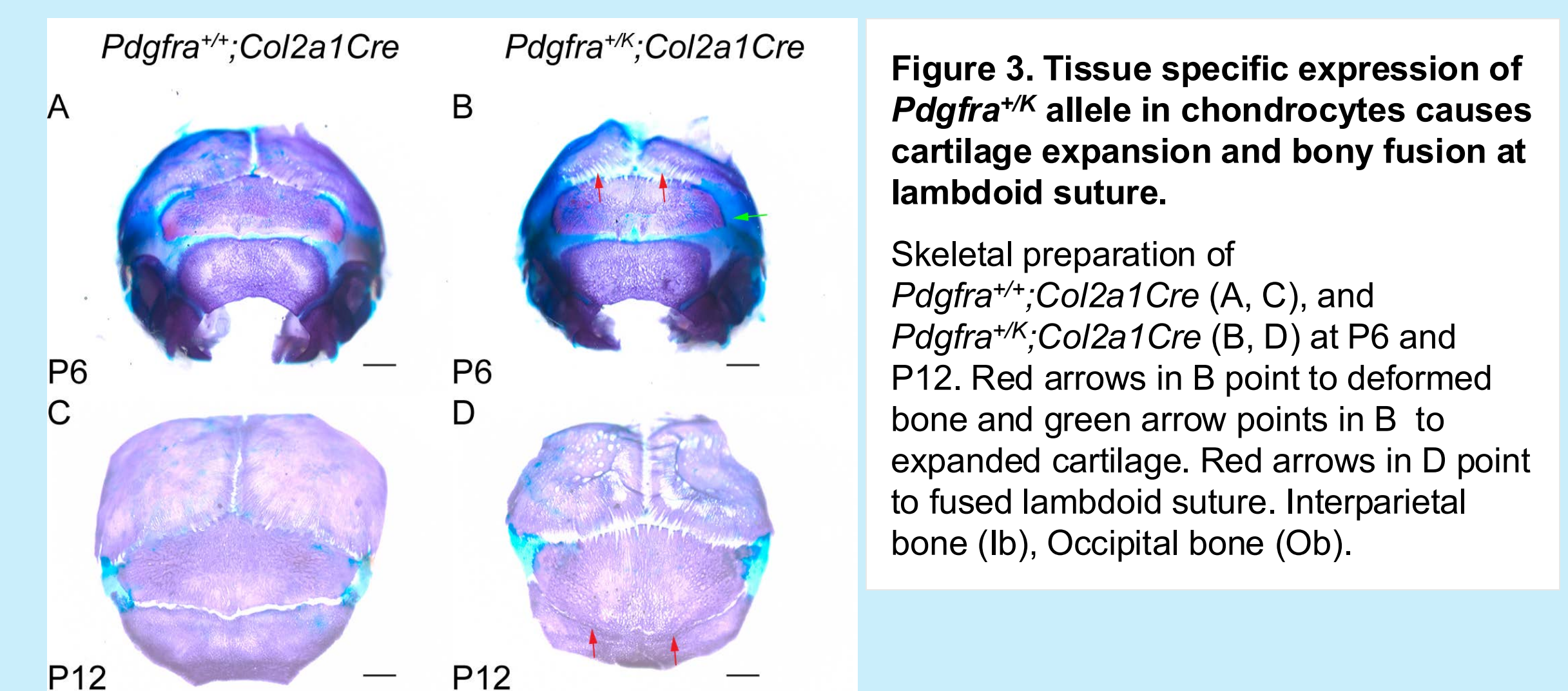


Figure 3. Tissue specific expression of *Pdgfra*^{+/K} allele in chondrocytes causes cartilage expansion and bony fusion at lambdoid suture.
Skeletal preparation of *Pdgfra*^{+/+};*Col2a1Cre* (A, C), and *Pdgfra*^{+/K};*Col2a1Cre* (B, D) at P6 and P12. Red arrows in B point to deformed bone and green arrow points in B to expanded cartilage. Red arrows in D point to fused lambdoid suture. Interparietal bone (Ib), Occipital bone (Ob).

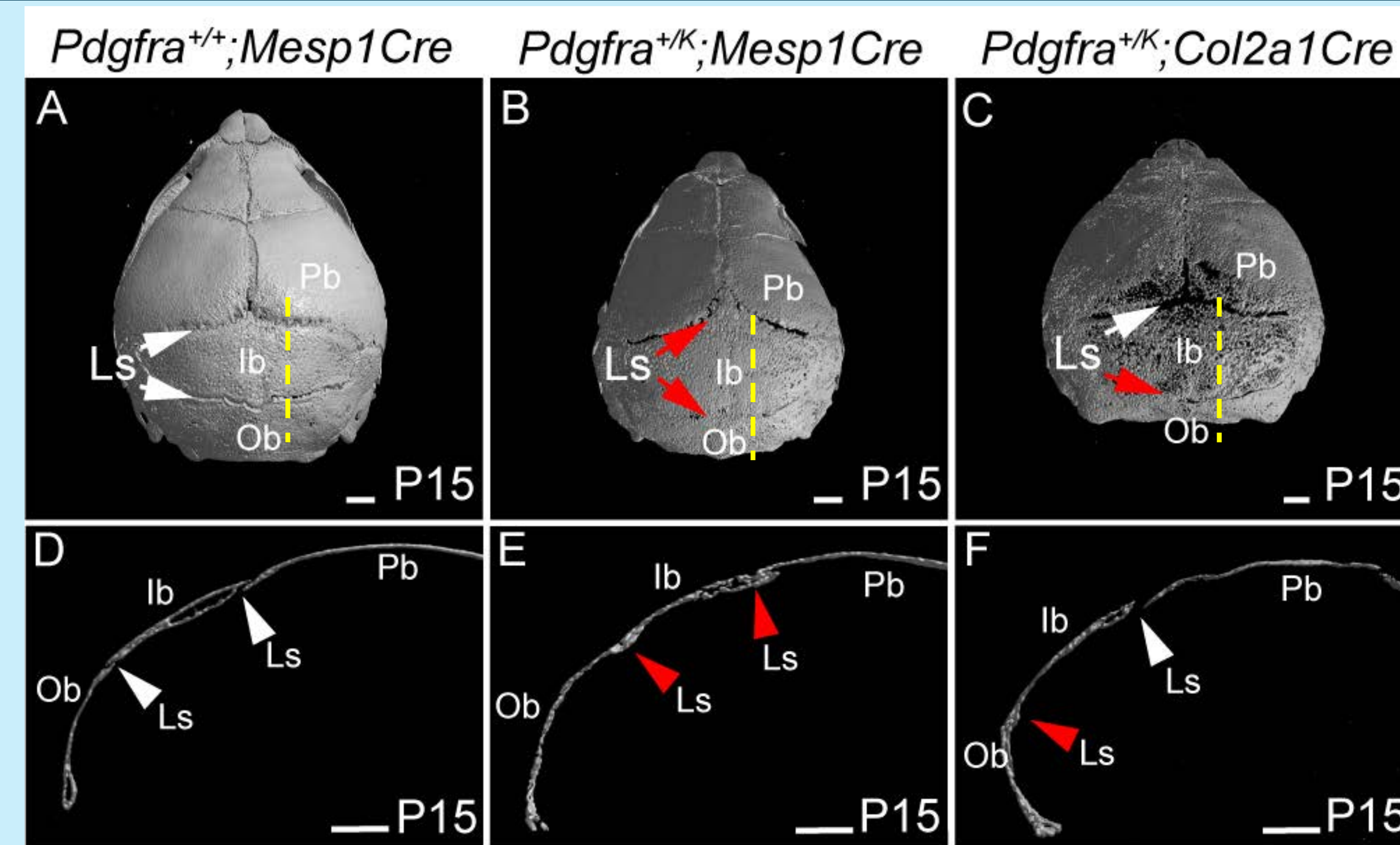


Figure 4. Tissue specific expression of autoactivated *Pdgfra* allele leads to lambdoid synostosis.
(A, B, C) MicroCT scanning of *Pdgfra*^{+/+};*Mesp1Cre* (A), *Pdgfra*^{+/K};*Mesp1Cre* (B) and *Pdgfra*^{+/K};*Col2a1Cre* (C) at P15. Red arrows point to bony fusion of lambdoid suture.
(D, E, F) Virtual section of above skulls at same level indicated by yellow dashed lines. Interparietal bone (Ib), Lambdoid suture (Ls). Occipital bone (Ob) and Parietal bone (Pb). Scale bar: 1mm.

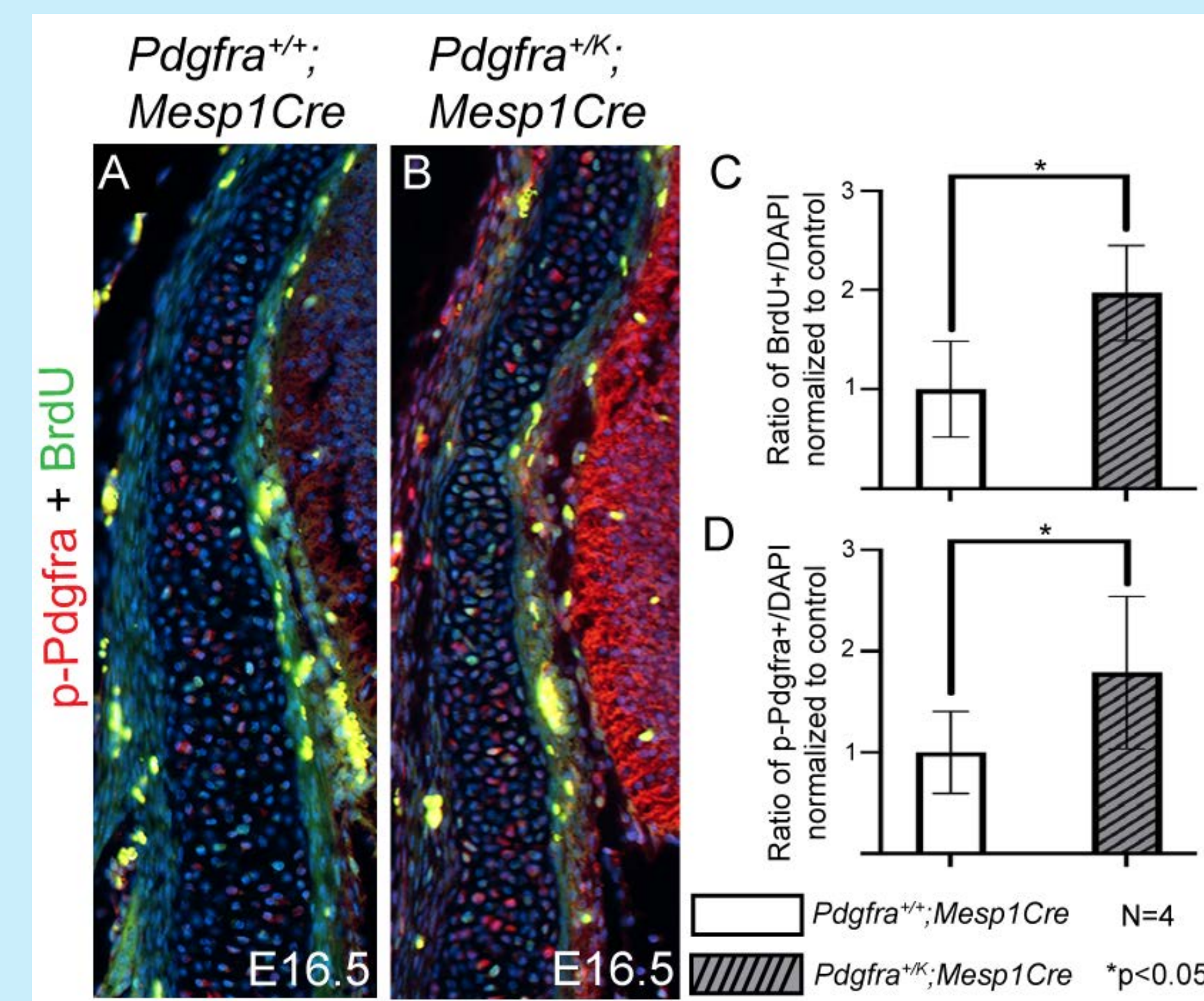


Figure 5. *Pdgfra* activation promotes chondrocyte proliferation.
(A, B) Double immunostaining with anti-phosphorylated *Pdgfra* (red) and anti-BrdU (green) on sagittal sections of *Pdgfra*^{+/+};*Mesp1Cre* (A) and *Pdgfra*^{+/K};*Mesp1Cre* (B) next to the lambdoid suture of E16.5 embryos.
(C, D) Quantification and statistical analysis of BrdU+ chondrocytes and *Pdgfra*+ chondrocytes in control and *Pdgfra*^{+/K};*Mesp1Cre*.

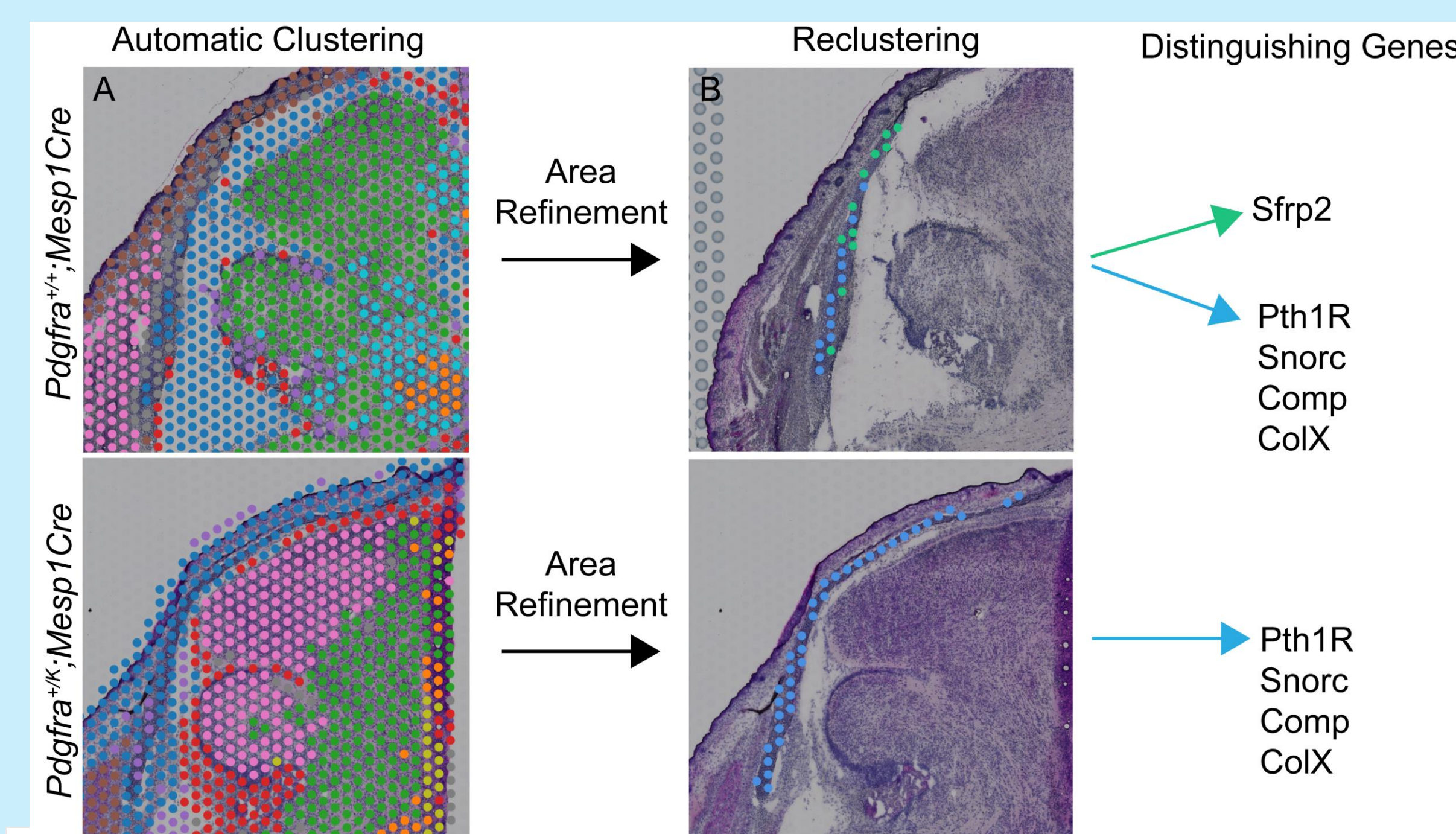


Figure 6. Spatial transcriptomics analysis reveals potential transformation of chondrocytes at the lambdoid suture.
Spatial clustering using the 10x Genomics platform Cloupe and gene expression analysis, with a focus on chondrocytes at the lambdoid suture of *Pdgfra*^{+/+};*Mesp1Cre* and *Pdgfra*^{+/K};*Mesp1Cre* at E16.5.

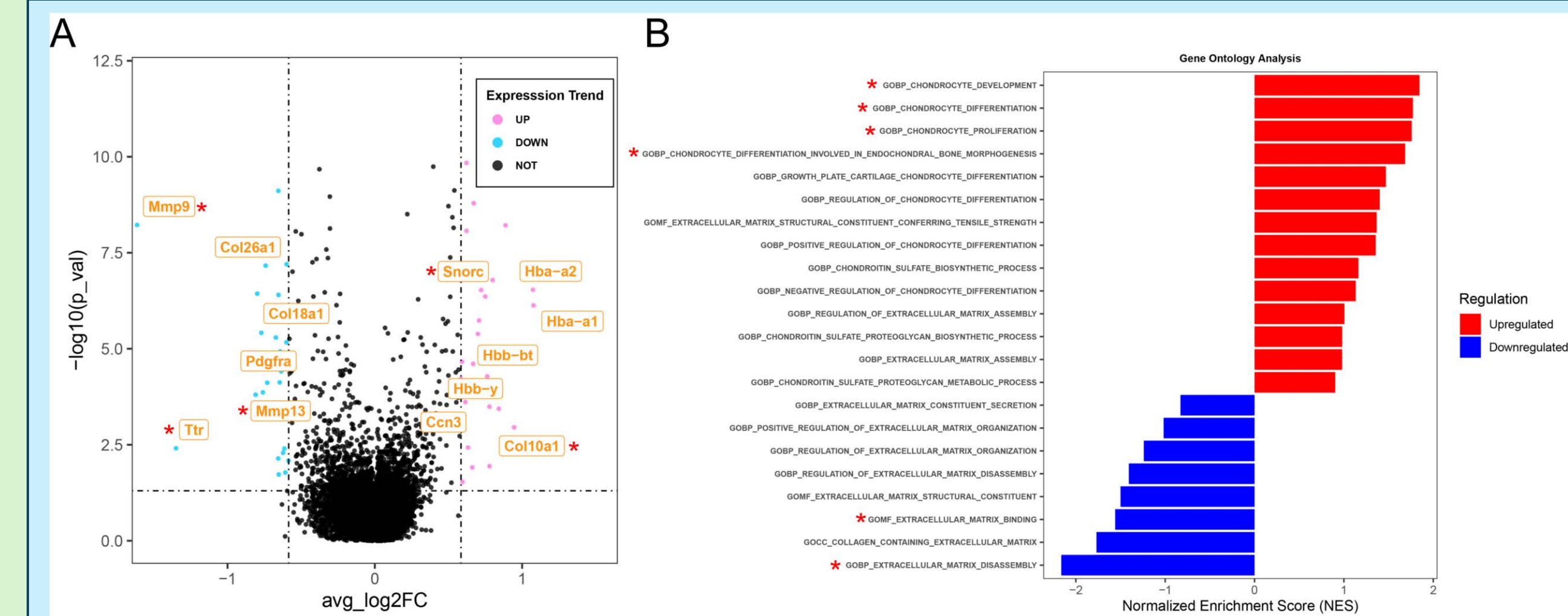


Figure 7. Bioinformatics analysis of 10X spatial transcriptomics data.
(A) Volcano plot of differentially expressed genes (DEGs) in *Pdgfra*^{+/+};*Mesp1Cre* and *Pdgfra*^{+/K};*Mesp1Cre*.
(B) Gene ontology analysis highlights genes regulating chondrocytes proliferation, differentiation and endochondral ossification are upregulated, and those implicated in extracellular matrix binding and disassembly are downregulated in *Pdgfra*^{+/K};*Mesp1Cre*.

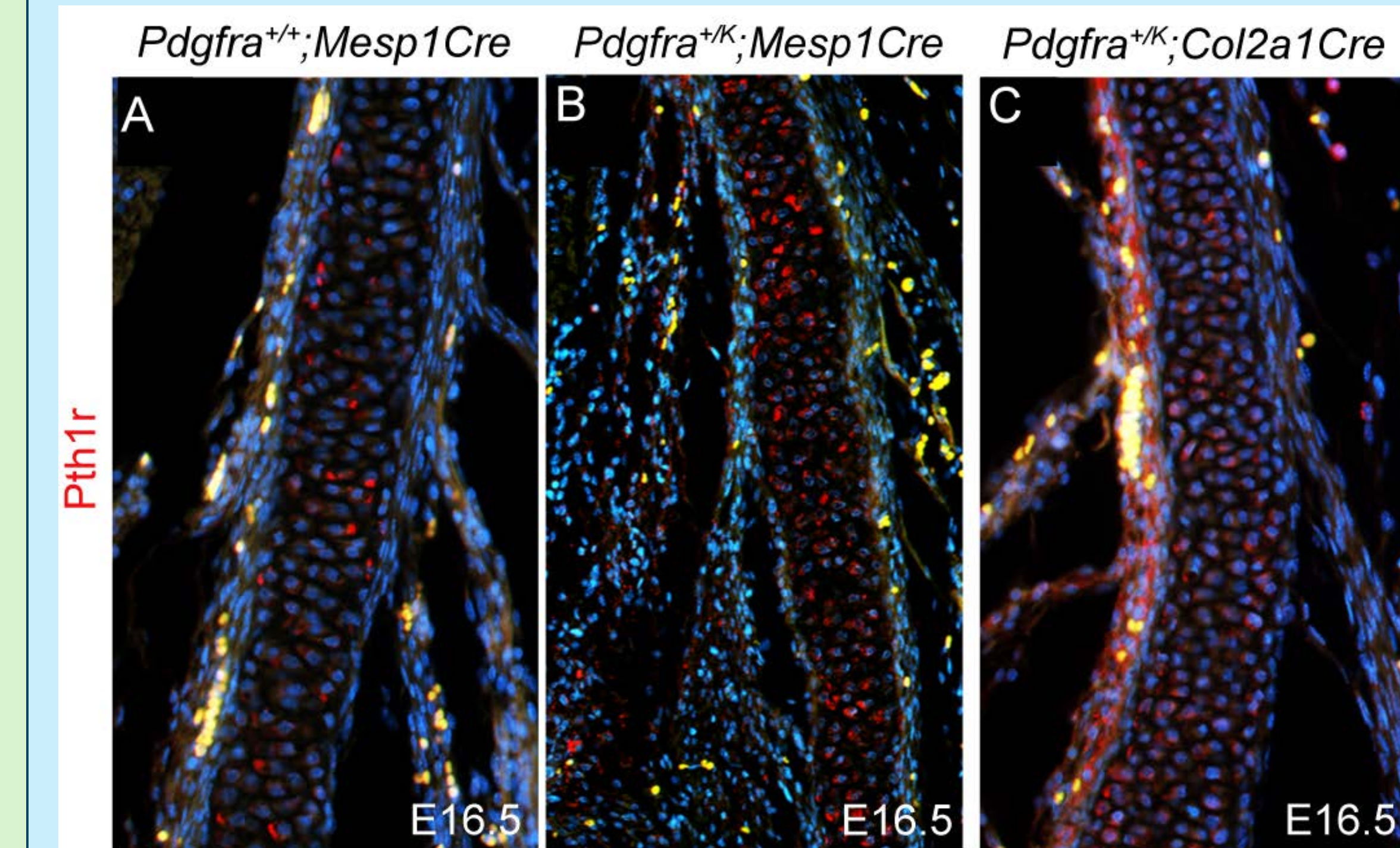


Figure 8. Altered *Pth1r* expression indicates *Pdgfra* activation promotes chondrocytes differentiation towards endochondral ossification.
(A, B, C) Immunostaining with anti-*Pth1r* on sagittal sections of *Pdgfra*^{+/+};*Mesp1Cre* (A), *Pdgfra*^{+/K};*Mesp1Cre* (B) and *Pdgfra*^{+/K};*Col2a1Cre* (C) at E16.5.

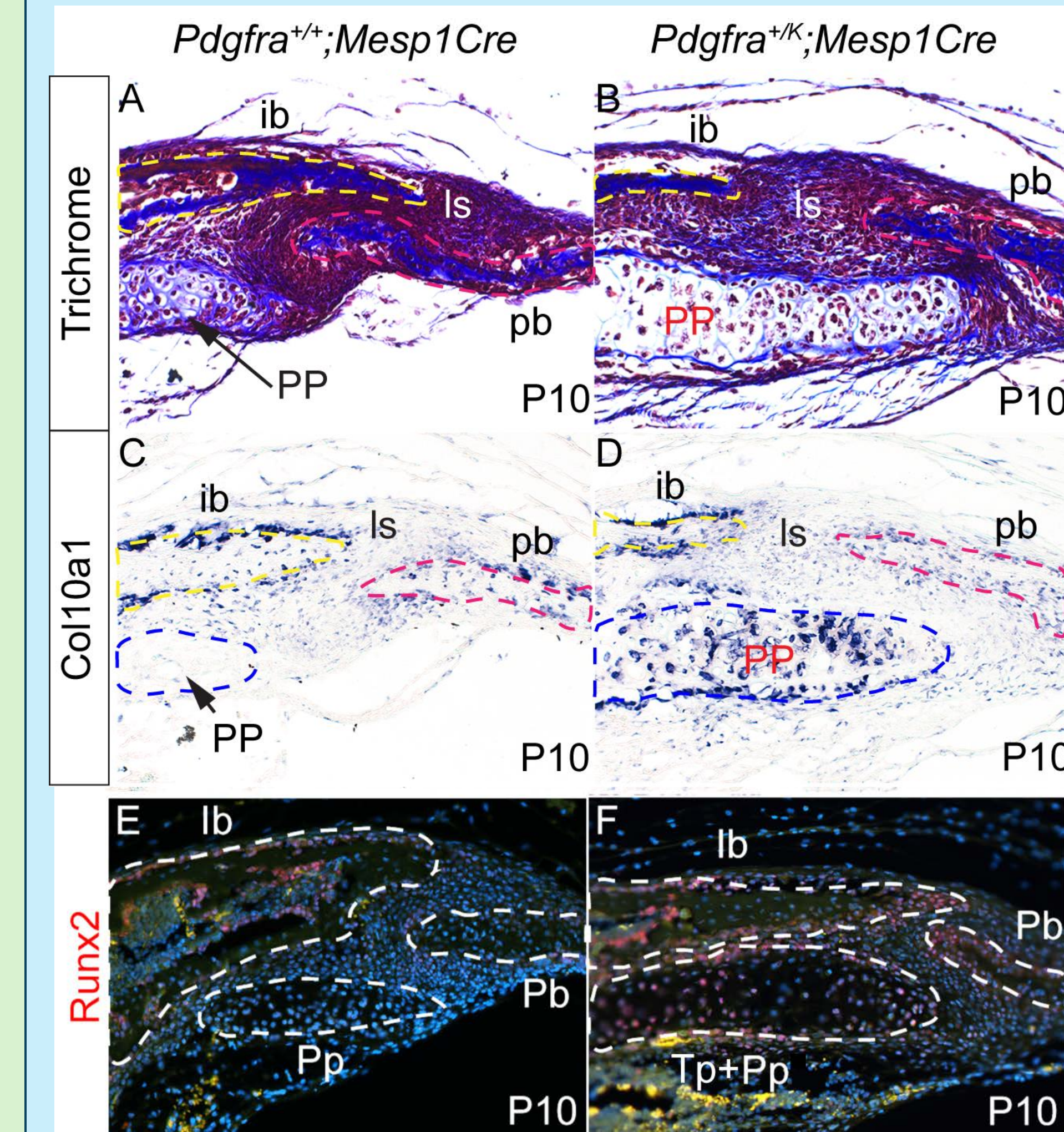


Figure 9. Constitutive activation of *Pdgfra* promotes hypertrophic chondrocytes and subsequent ossification at lambdoid suture.
(A, B) Trichrome staining, (C, D) In situ hybridization with antisense probe of *Col10a1*, and (E, F) Immunostaining with anti-Runx2 antibody (red) on sagittal sections across lambdoid suture of *Pdgfra*^{+/+};*Mesp1Cre* and *Pdgfra*^{+/K};*Mesp1Cre* at P10.

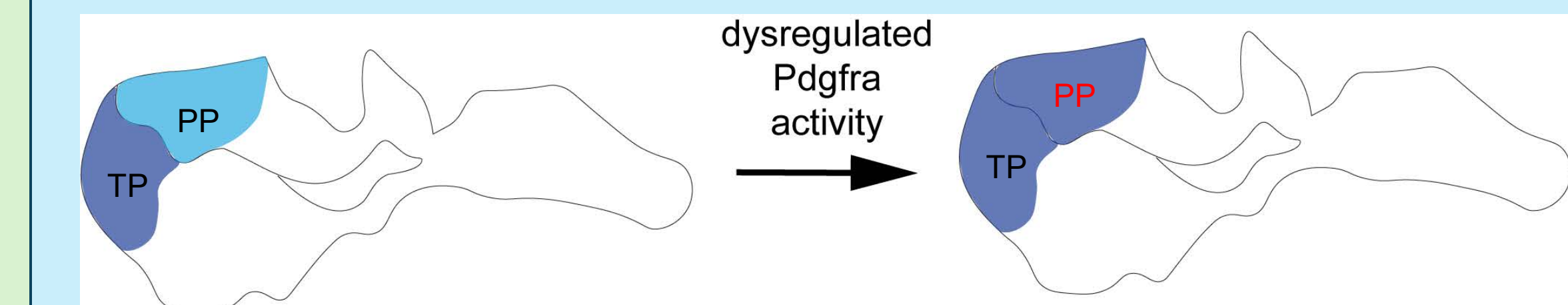


Figure 10. A proposed model of lambdoid synostosis caused by dysregulated *Pdgfra* activity.
Two types of cartilages are documented in mouse calvaria. One is resorbed (light blue, such as parietal plate, PP) and the other one undergo endochondral ossification (dark blue, such as tectum posterius, TP). Our data indicate activation of *Pdgfra* directed PP chondrocytes shifting towards endochondral ossification represented by TP.

Conclusion

- Constitutive activation of *Pdgfra* causes cartilage expansion and lambdoid synostosis in mice.
- Tissue specific activation of *Pdgfra* in chondrocytes leads to bony fusion of lambdoid suture.
- Pdgfra* activation promotes chondrocyte proliferation and differentiation.
- Chondrocyte malformation plays a potential role in lambdoid synostosis formation.

Acknowledgements

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