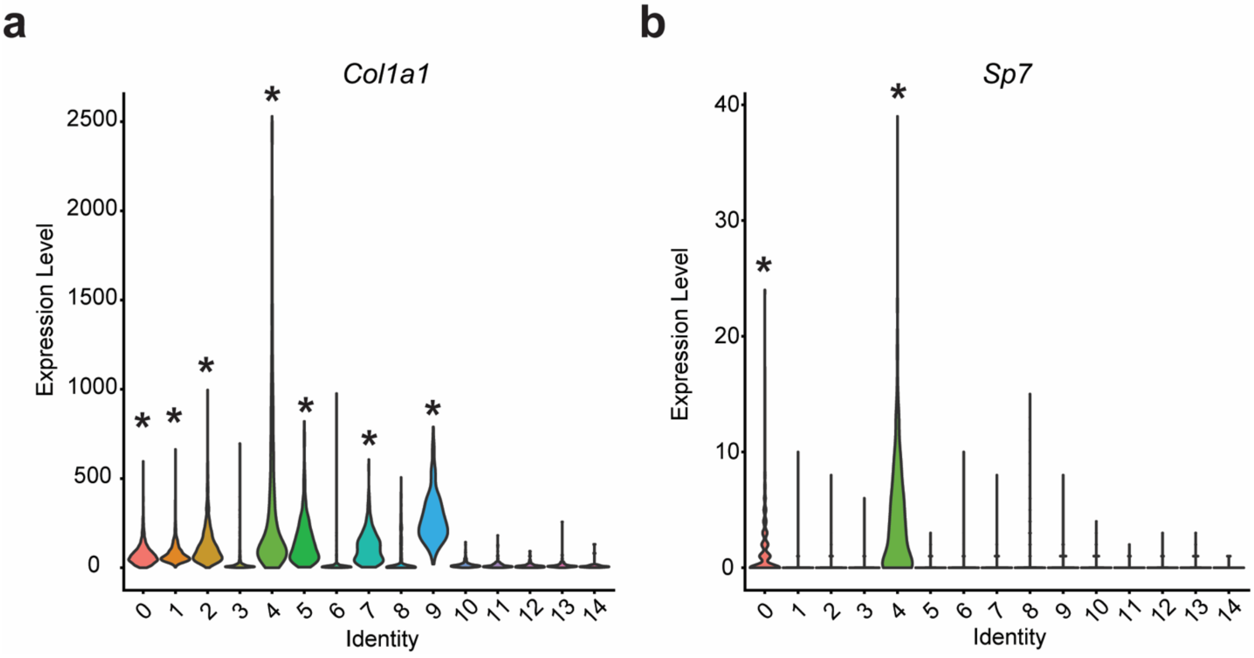
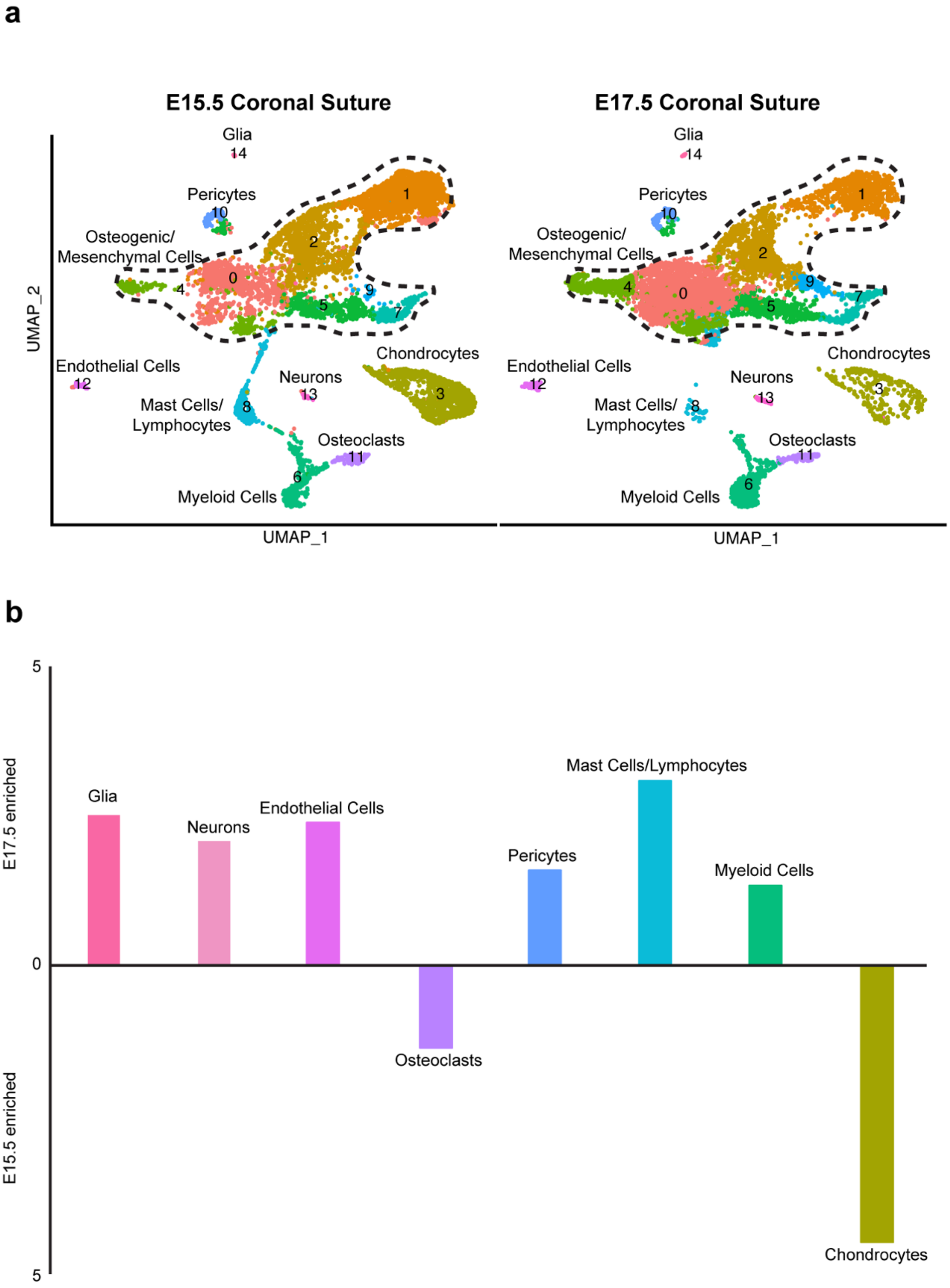
Supplementary Figure 1



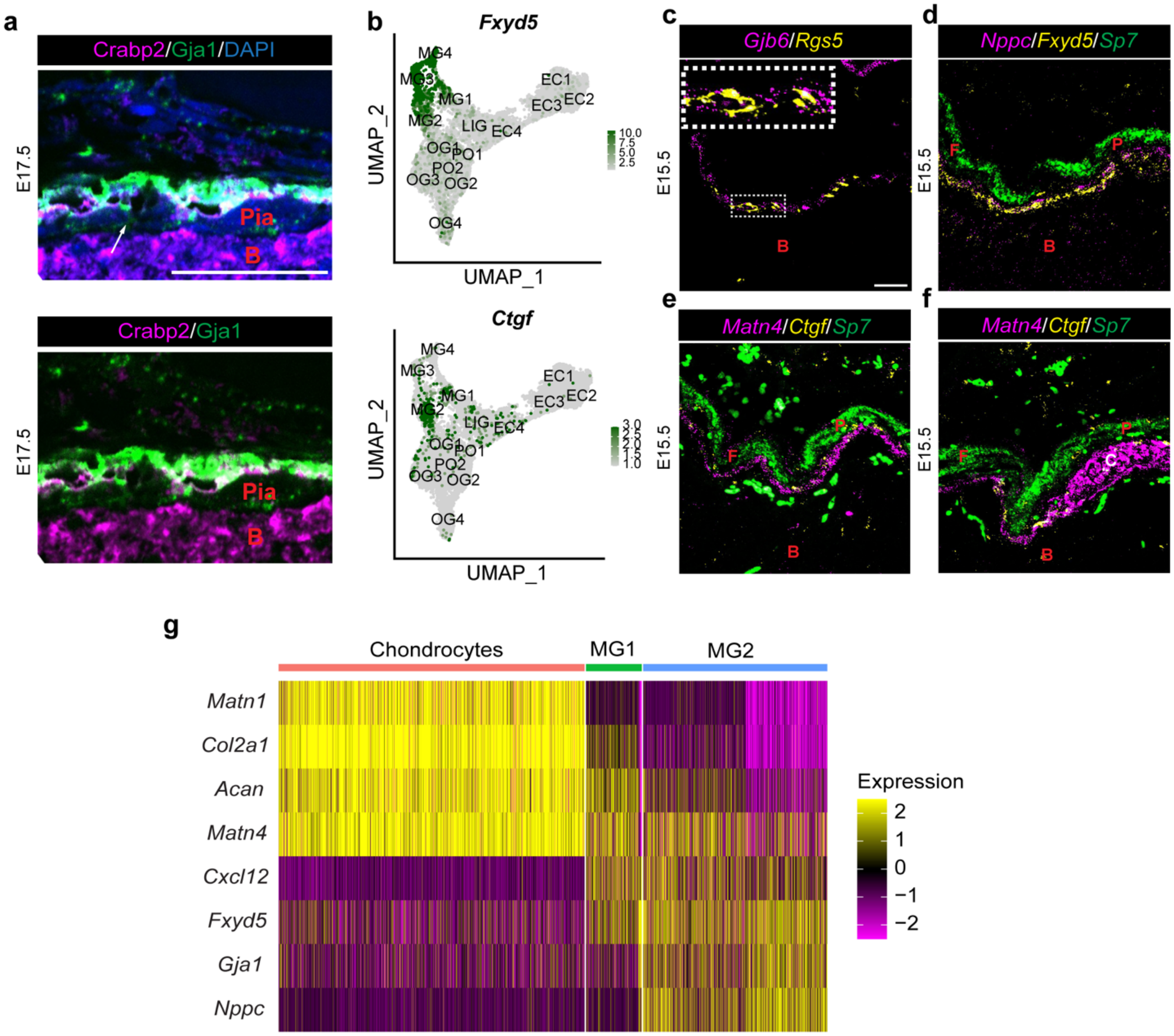
Supplementary Figure 1. Fibroblast and osteoblast markers label mesenchymal/osteogenic clusters. a Violin plot for *Col1a1*. **b** Violin plot for *Sp7*. Enriched clusters are marked by asterisks

Supplementary Figure 2



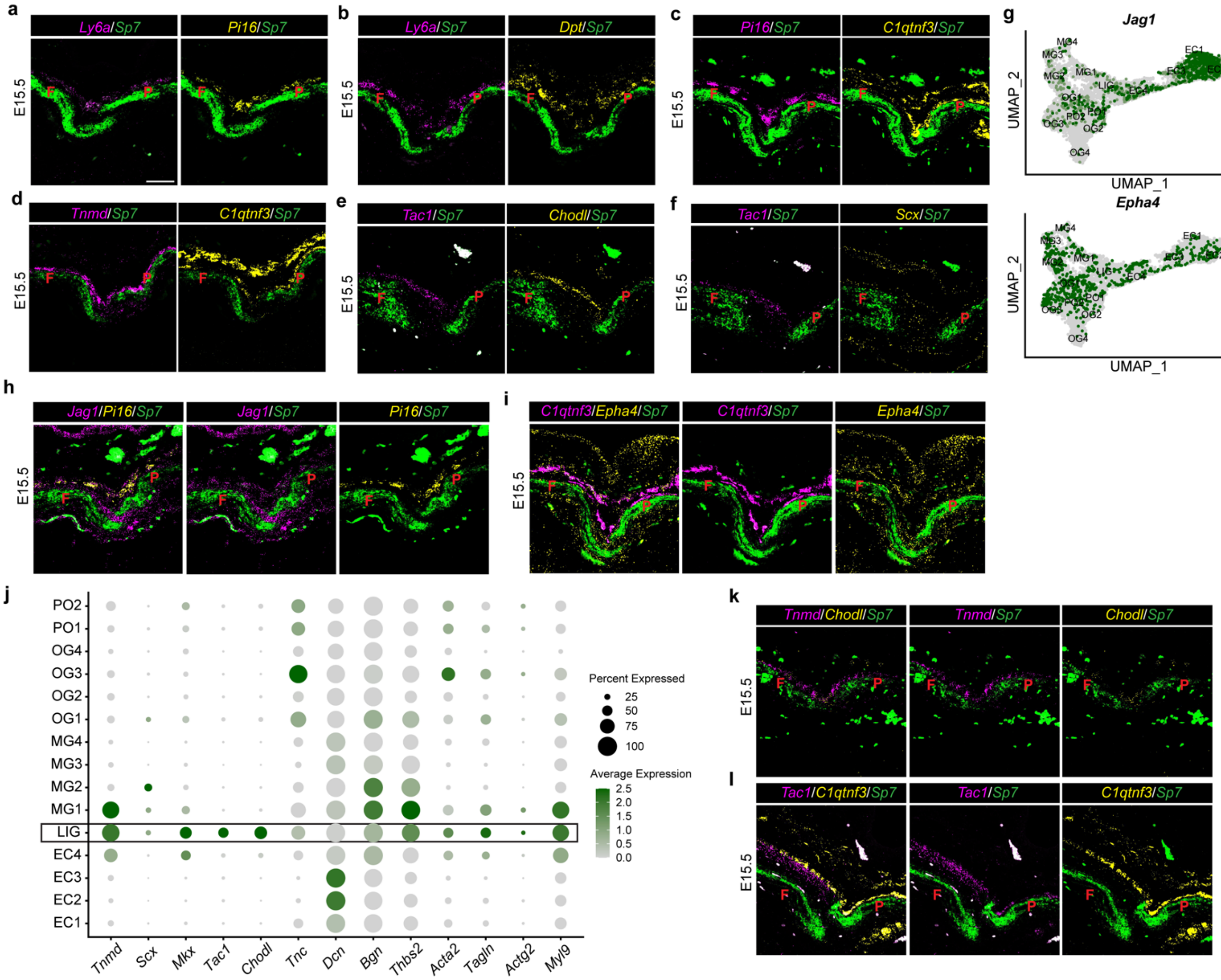
Supplementary Figure 2. Equivalent cell types are captured at E15.5 and E17.5. a UMAP plots separated by developmental stage. **b** Graphical depiction of the cluster proportions plotted as ratio between E15.5 and E17.5 cells within each cluster.

Supplementary Figure 3



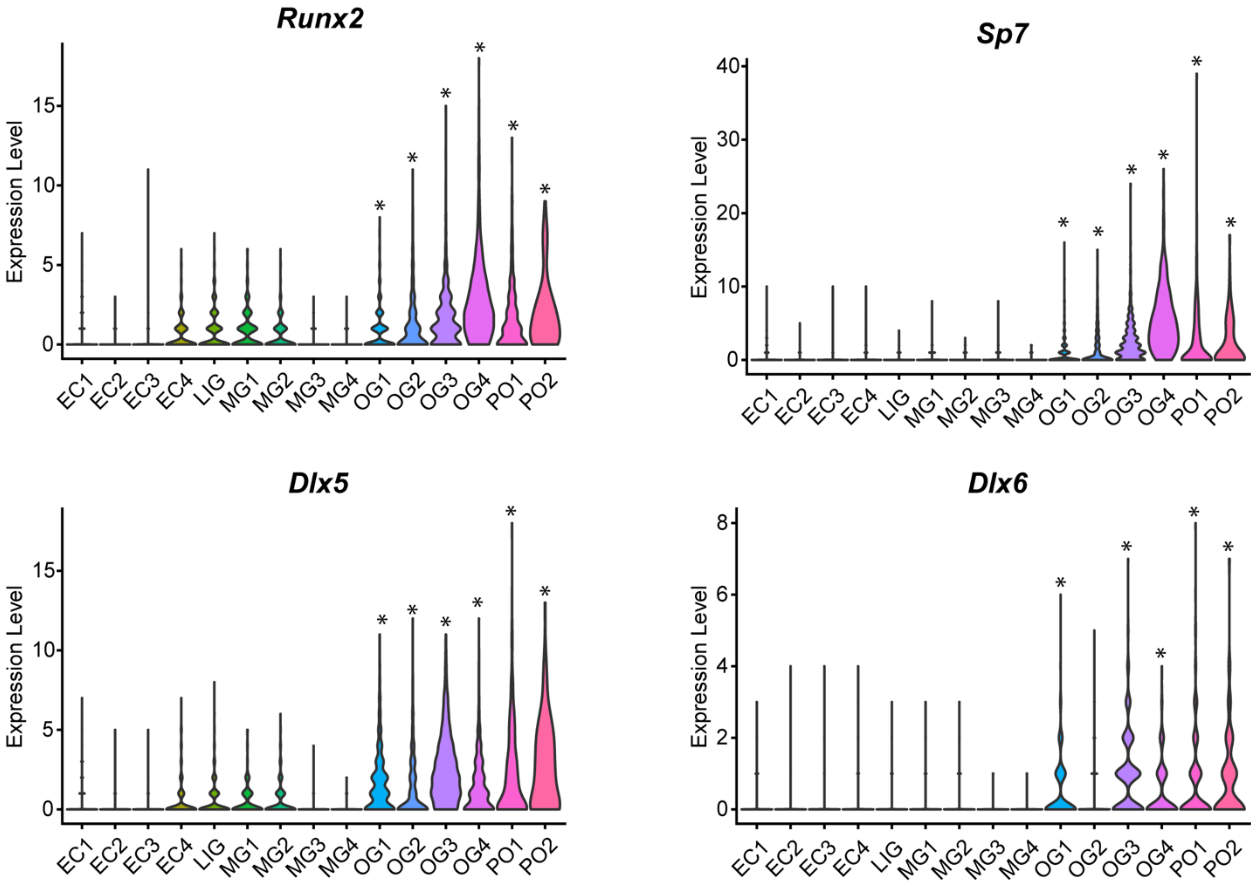
Supplementary Figure 3. Resolving meningeal subtypes. a Immunofluorescence of Crapb2 and Gja1 at E17.5. Scale bar = 25 µm. **b** Feature plots of genes validated by in situ experiments. **c-f** In situ analysis of coronal sutures for indicated markers at E15.5. **c** *Gjb6* and *Rgs5.* Dashed lines highlight magnified inset. **d** *Nppc and Fxyd5.* **e,f** *Matn4 and Ctgf. Sp7* marks the frontal (F) and parietal (P) bones in (d-f). **g** Heatmap of selected chondrocyte and dura mater markers. B, brain; C, cartilage. Scale bars = 50 µm.

Supplementary Figure 4



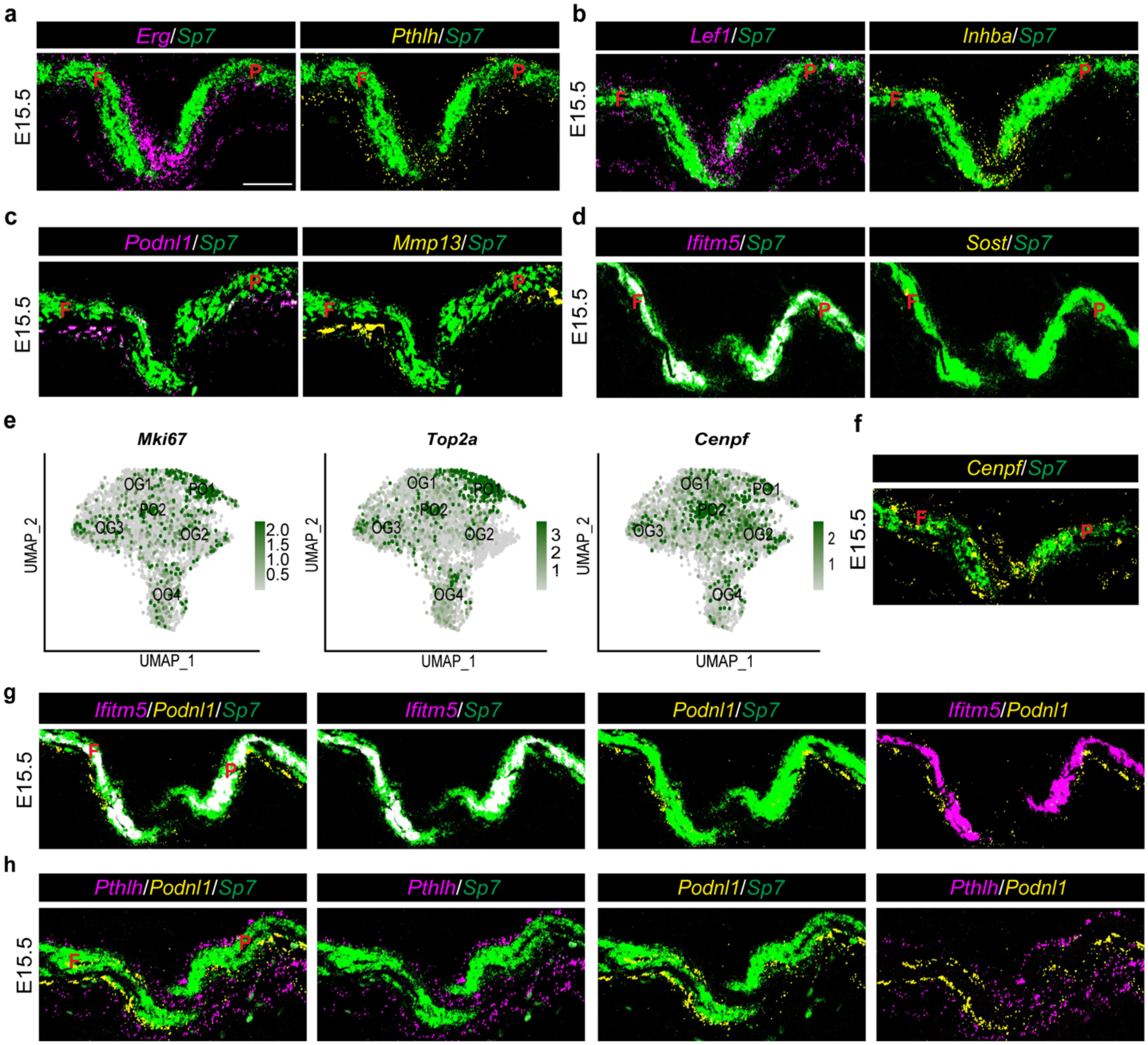
Supplementary Figure 4. A ligament-like layer overlaps the coronal suture. a-f Single channel images of genes evaluated in Figure 3. **g** Feature plots of genes validated by in situ experiments. **h, i** In situ analysis of coronal sutures for indicated markers at E15.5. **j** Dot plot of markers associated with tendon/ligament identity and cellular contractility. **k, l** In situ analysis for indicated markers at E15.5. *Sp7* marks the frontal (F) and parietal (P) bones in all in situs. Scale bar = 50 μm.

Supplementary Figure 5



Supplementary Figure 5. Markers for the osteogenic subset. Violin plots for *Runx2, Sp7, Dlx5*, and *Dlx6*. Enriched clusters are marked by asterisks.

Supplementary Figure 6



Supplementary Figure 6. In situ analysis of osteogenesis cluster markers. a-d Single channel images of genes evaluated in Figure 4. **e** Feature plots of genes marking clusters PO1 and PO2. **f-h** In situ analysis of coronal sutures for indicated markers at E15.5. *Sp7* marks the frontal (F) and parietal (P) bones in all in situs. Scale bar = 50 µm.

Supplementary Figure 7

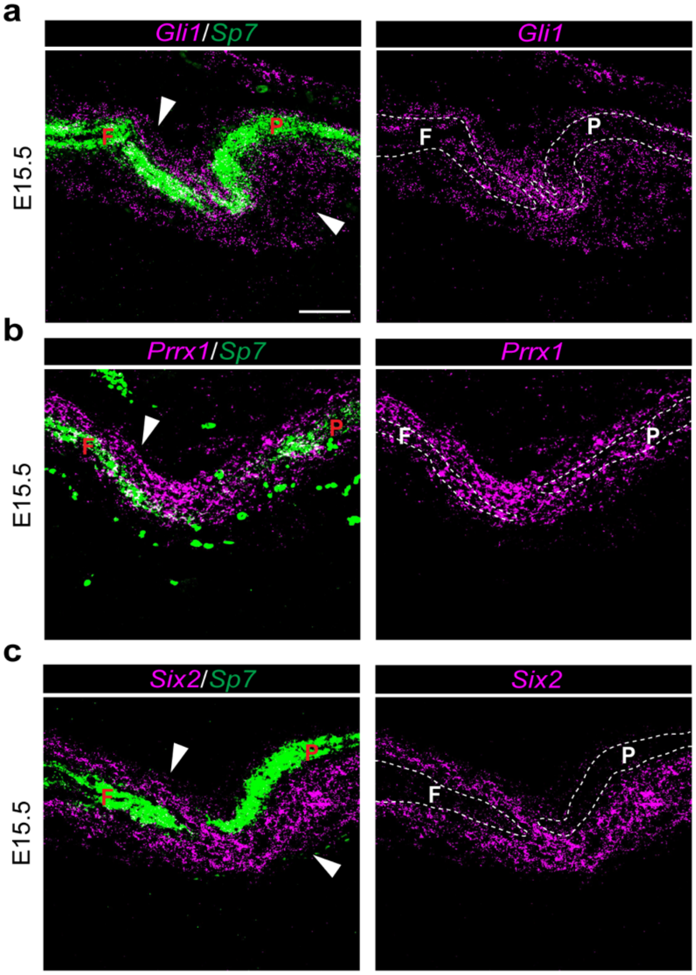
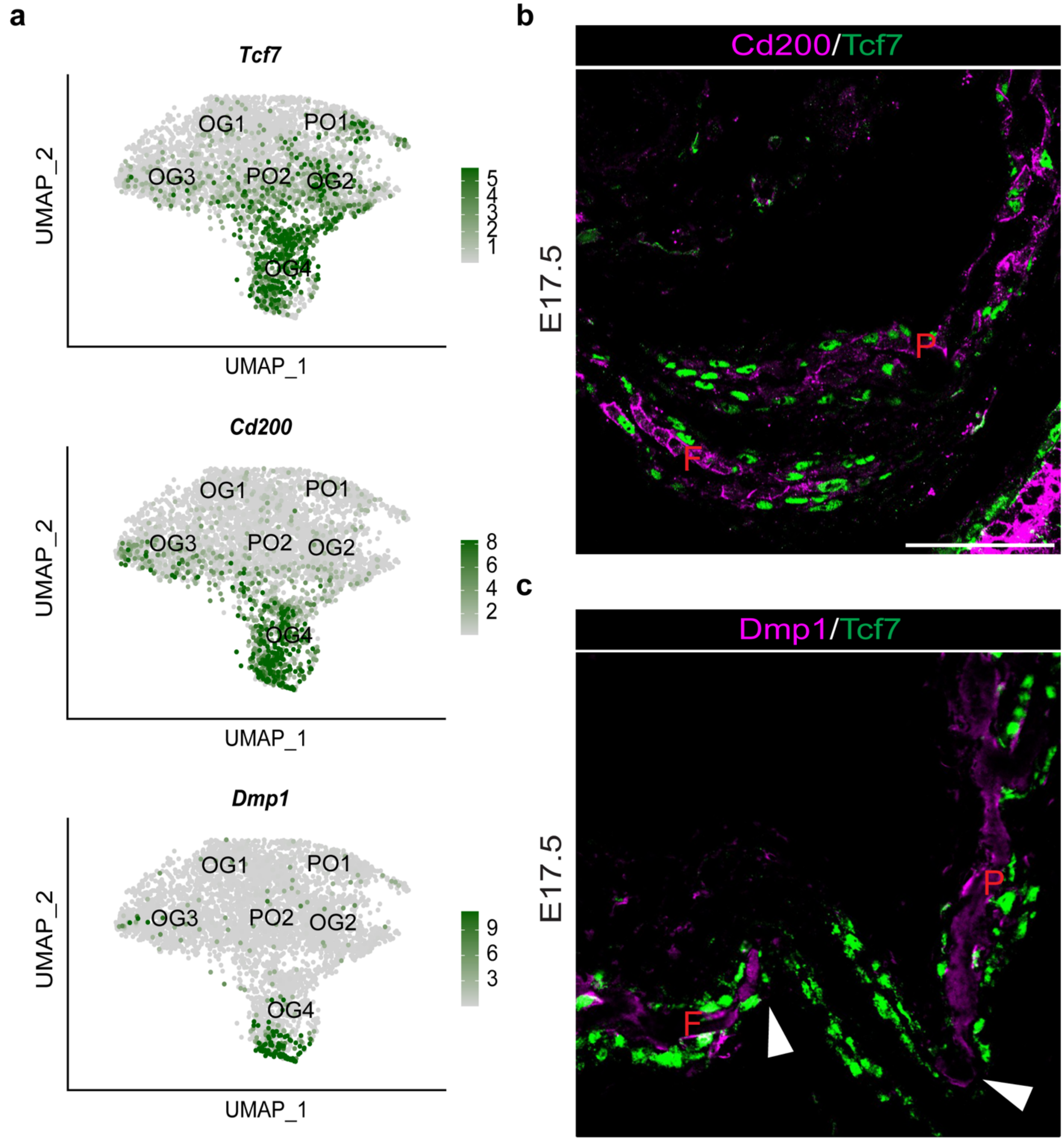


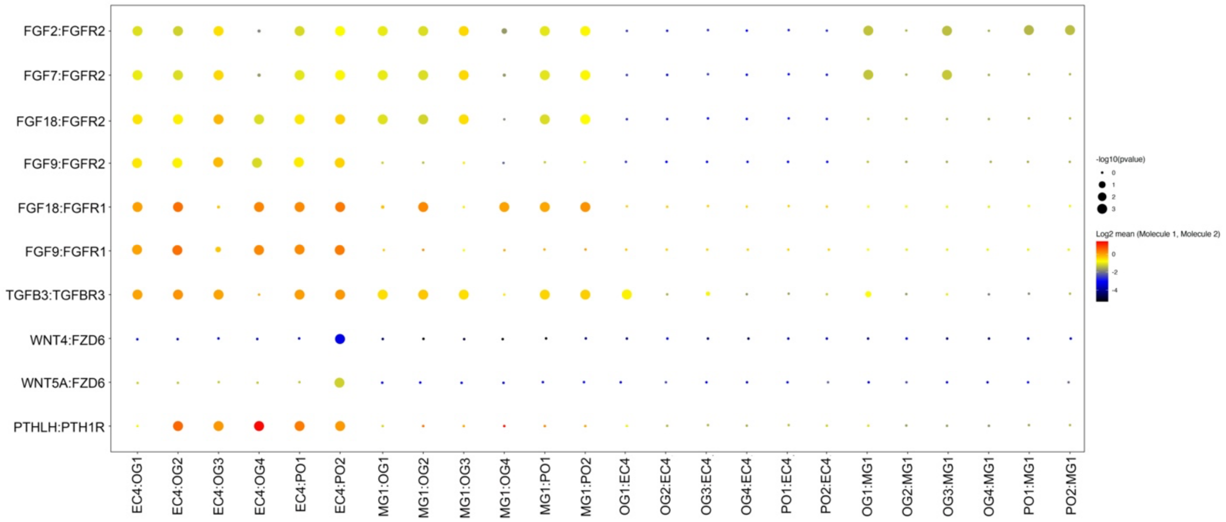
Figure 7. Asymmetric distribution of mesenchyme across the frontal and parietal bones. a-f In situ analysis of coronal sutures for indicated markers at E15.5. *Sp7* marks the frontal (F) and parietal (P) bones. Arrowheads indicate surfaces of bones where markers are over-enriched. Scale bar = 50 μm.

Supplementary Figure 8



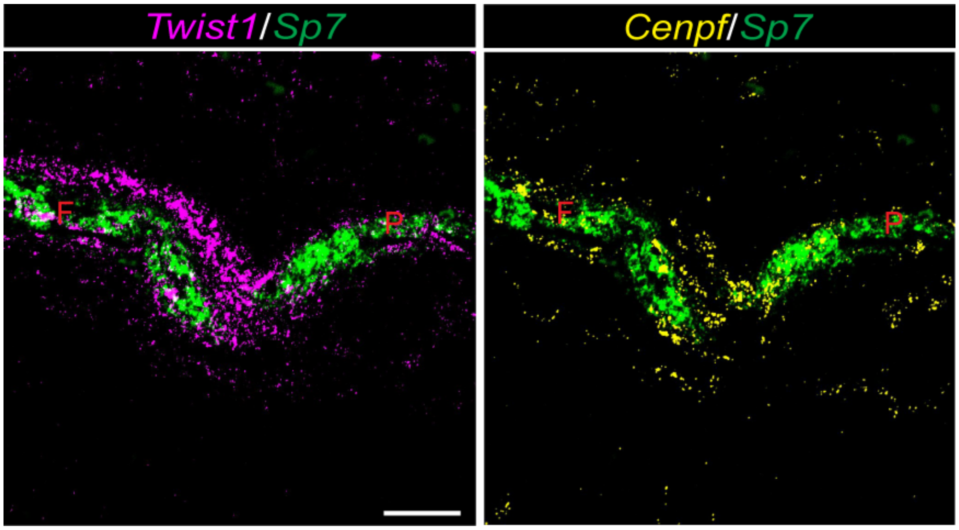
Supplementary Figure 8. Immunofluorescent analysis resolves osteogenic subtypes within the coronal suture. a Feature plots of genes validated by antibody staining. **b-c** Combinatorial antibody staining of coronal sutures for indicated markers at E17.5. Arrowheads mark beginning of Dmp1 signal away from bone fronts. F, Frontal bone; P, Parietal bone. Scale bars = 50 μm.

Supplementary Figure 9



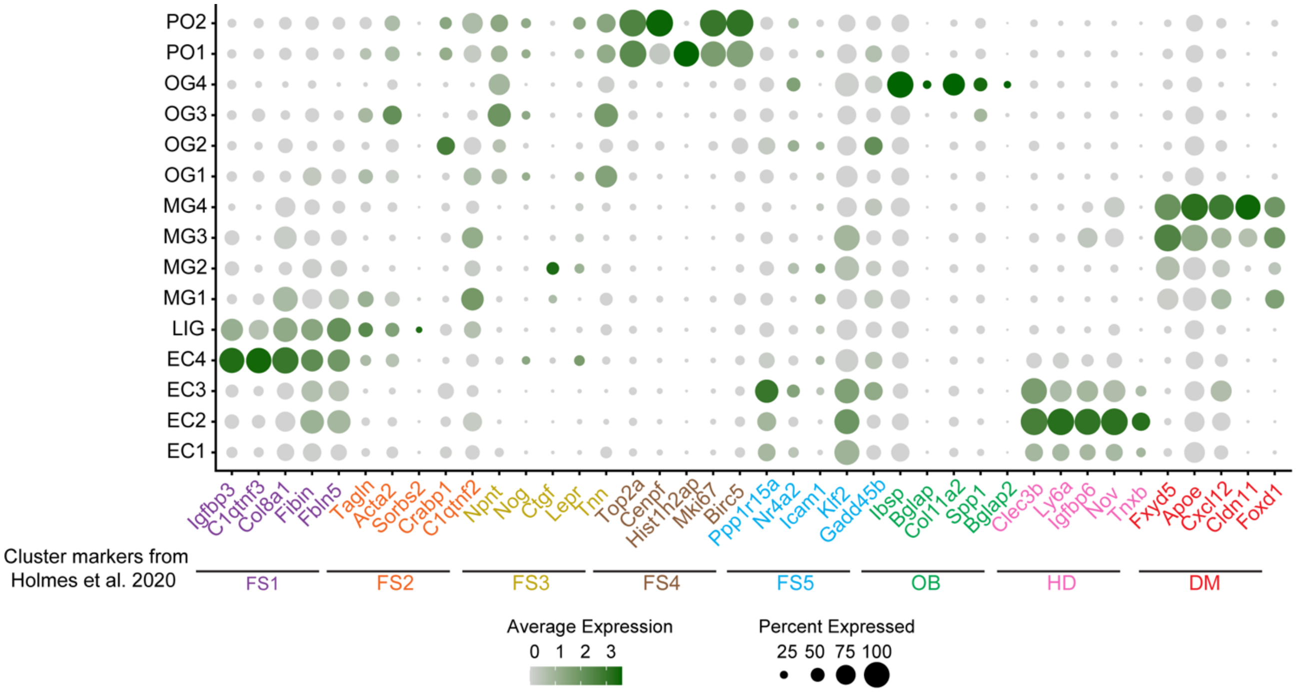
Supplementary Figure 9. Putative signaling interactions between osteogenic and non-osteogenic populations. Dot plot representing selected examples of ligand-receptor interactions between osteogenic and non- osteogenic clusters captured from CellPhone DB analysis.

Supplementary Figure 10



Supplementary Figure 10. *Twist1* and *Cenpf* are enriched with proliferative cells at osteogenic fronts. Single channel images of genes evaluated in Figure 6. F, Frontal bone; P, Parietal bone. Scale bars = 50 µm.

Supplementary Figure 11



Supplementary Figure 11. Comparison of frontal and coronal datasets. Dot plot showing the intersection of cluster markers from Holmes et al.[30] with coronal suture clusters.

Supplementary Table 1. Enriched gene list by cluster for integrated E15.5 and E17.5 datasets. Table of enriched genes for each cluster in the E15.5/E17.5 integrated dataset using the RNA assay.

Supplementary Table 2. Enriched gene list by cluster for integrated E15.5 and E17.5 osteogenic/mesenchymal subset. Table of enriched genes for each cluster in the osteogenic/mesenchymal subset using the RNA assay.