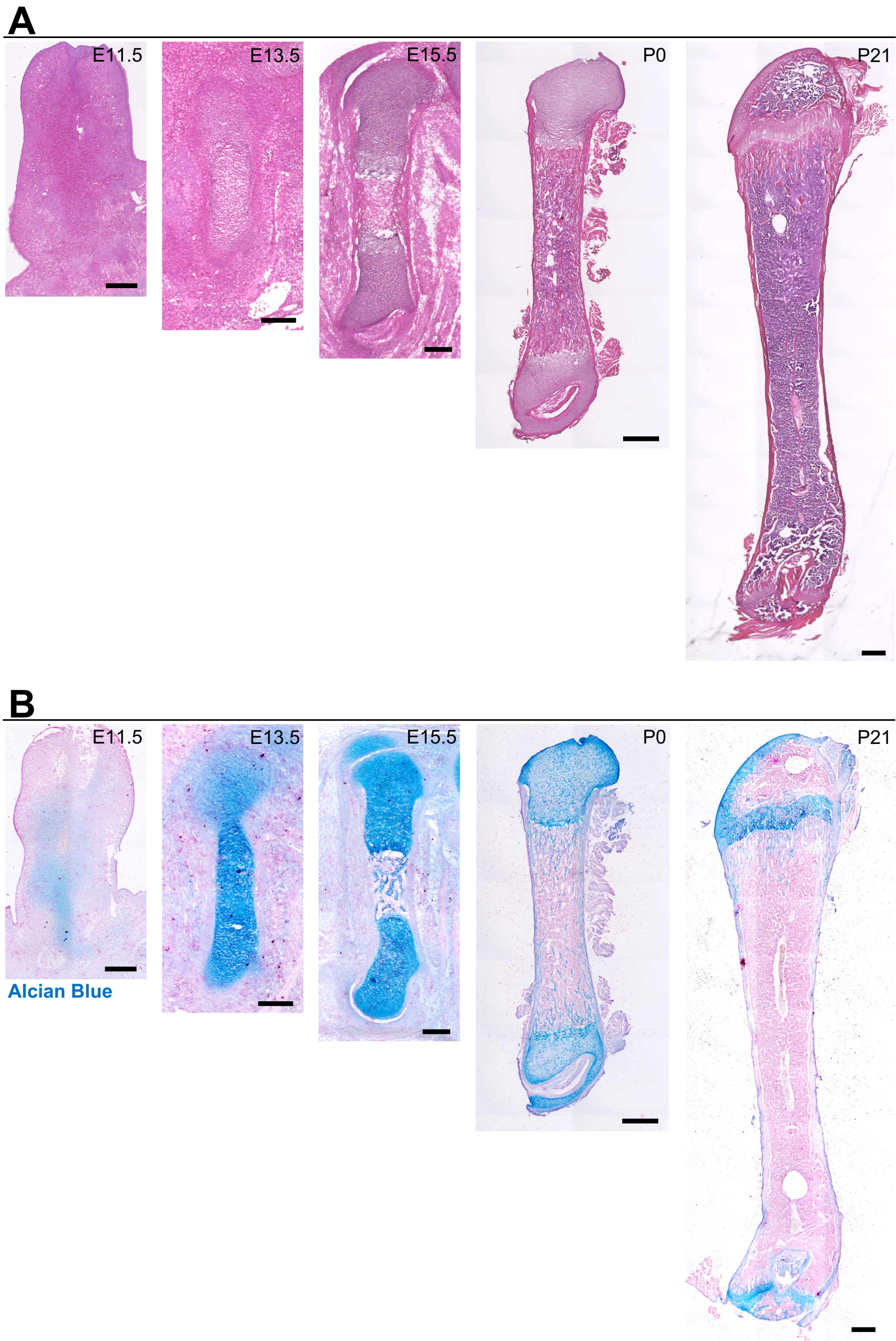


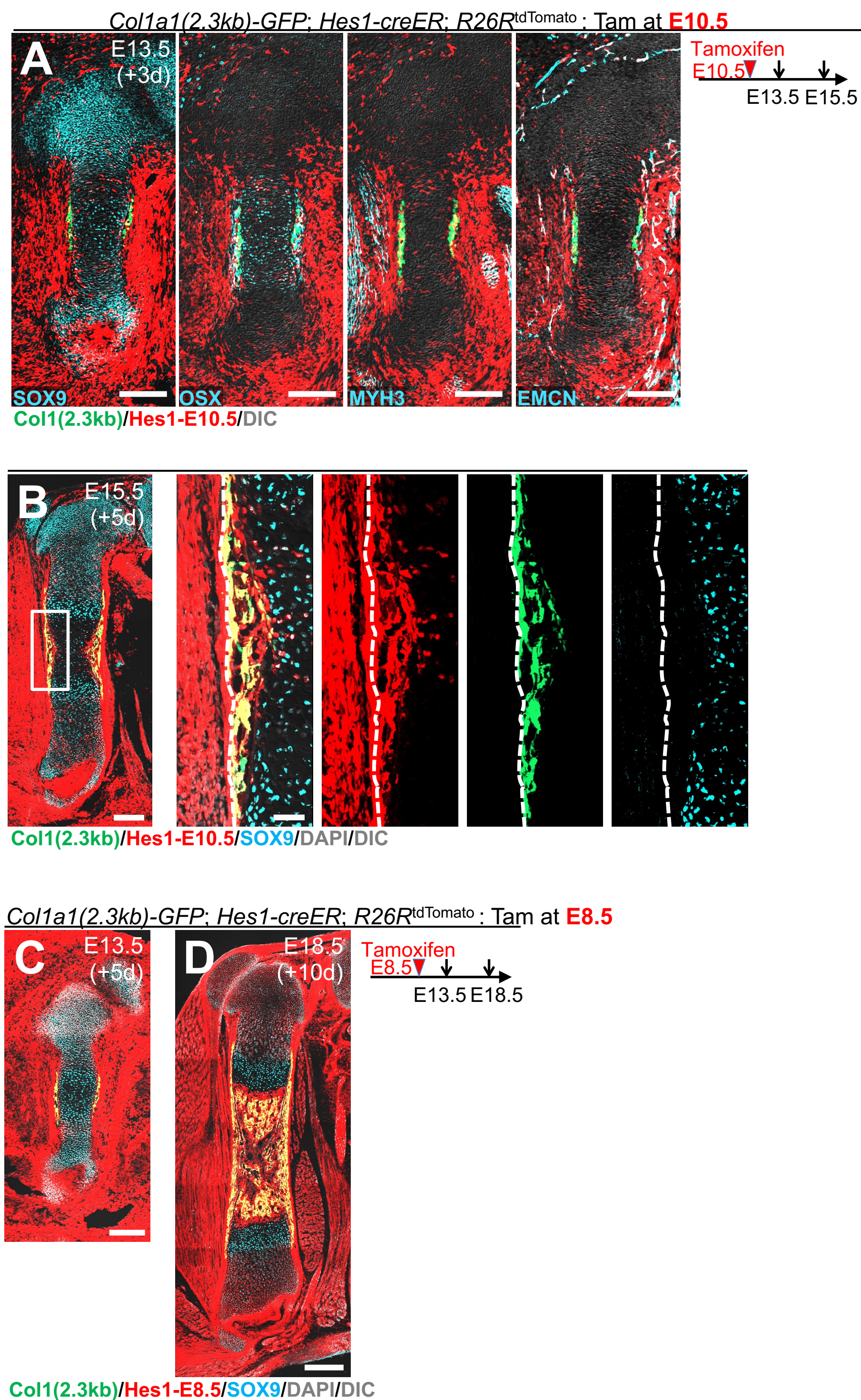
**Supplementary Figure 1. Single-cell RNA-seq identifies *Hes1* as a potential marker of peri-condensation mesenchymal cells.**

(A) Feature plots of genes enriched in each cluster from *Prrx1-cre*-marked limb bud mesenchymal cell-based single cell RNA-seq analysis at E11.5. Cluster 2,7: *Col2a1*<sup>+</sup>, Cluster 5: *Prrx1*<sup>+</sup>, Cluster 6: *Shh*<sup>+</sup>*Msx1*<sup>+</sup>, Cluster 5: *Lhx9*<sup>+</sup>*Msx1*<sup>+</sup>, Cluster 0: *Meox2*<sup>+</sup>*Osr1*<sup>+</sup>, Cluster 4: *Emx2*<sup>+</sup>, Cluster 3,7: *Irx3*<sup>+</sup>*Irx5*<sup>+</sup>. Red contour: featured clusters. (B) Notch reporter *CBF1:H2B-Venus* femur immunostained for SOX9 at E11.5. Scale bar: 200 $\mu$ m. *n*=3 mice.



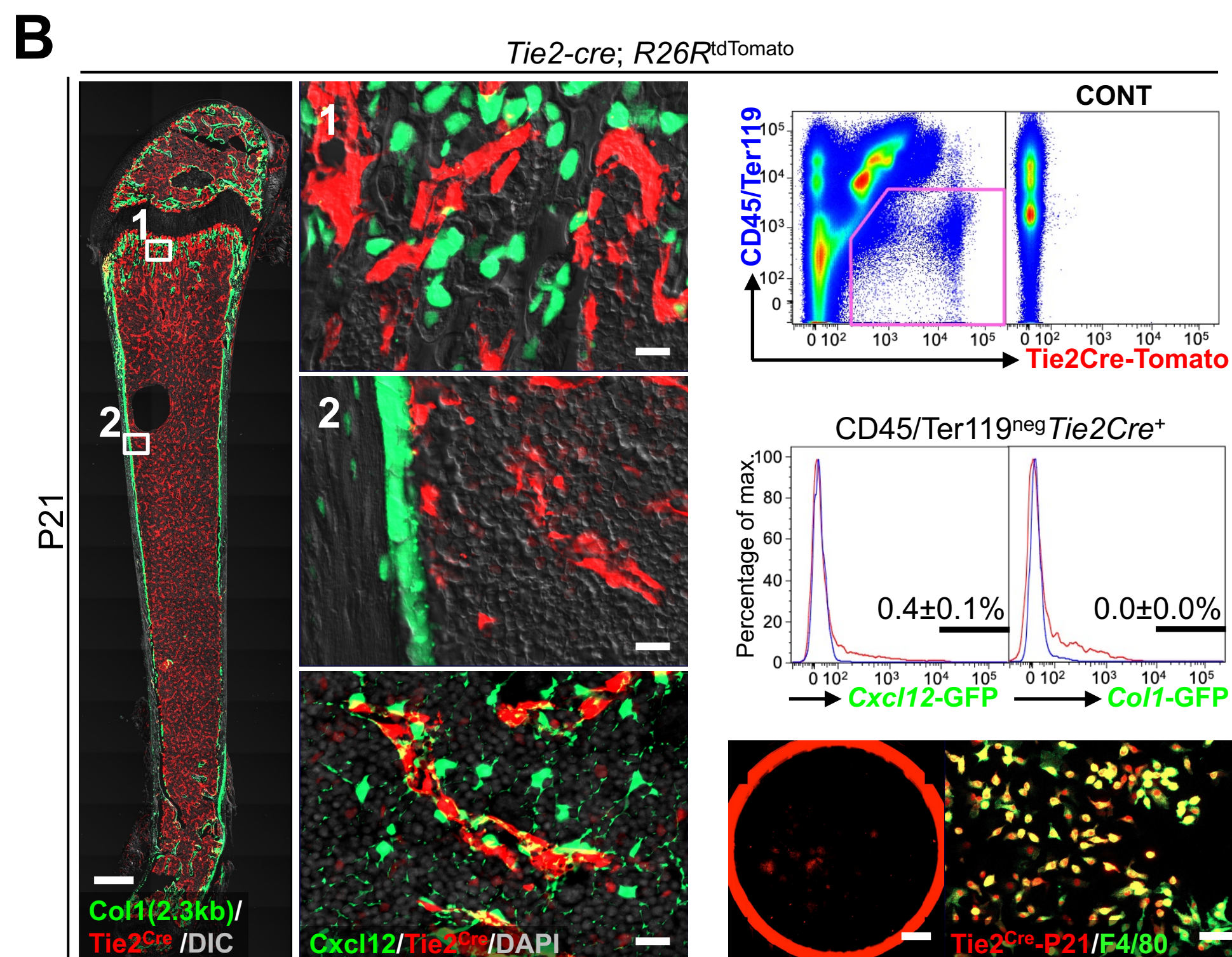
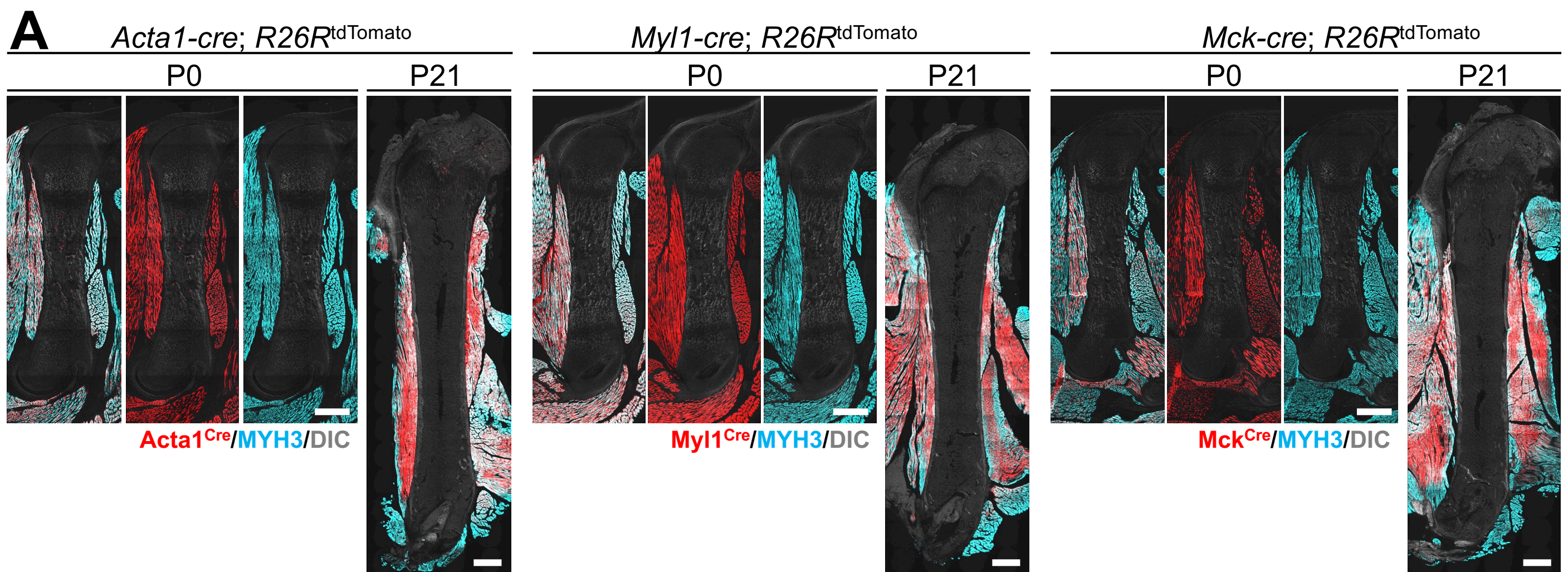
**Supplementary Figure 2. Stages of endochondral bone development.**

Sequential steps of endochondral bone development. (A): H&E staining, (B): Alcian Blue staining. E11.5: mesenchymal condensation. E13.5: cartilage template and perichondrium. E15.5: formation of the primary ossification center and incipient bone marrow. P0: at birth. P21: at weaning. Scale bar: 200 $\mu$ m (E11.5, E13.5, and E15.5). 500 $\mu$ m (P0 and P21).  $n=4$  mice.



**Supplementary Figure 3. Hes1-creER<sup>+</sup> peri-condensation mesenchymal cells can generate chondrocytes.**

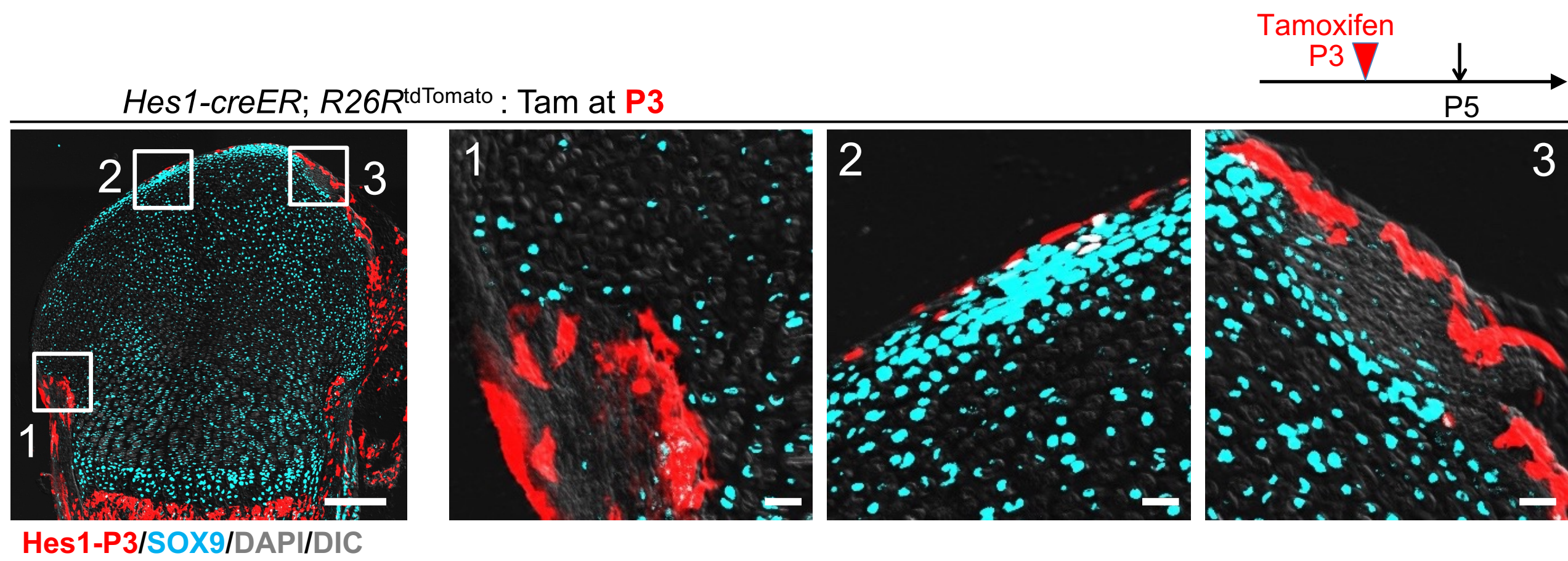
- (A) Cartilage template at E13.5, after 3 days of chase (pulsed at E10.5). Immunostaining for SOX9, OSX, MYH3 and EMCN. Scale bar: 200 $\mu$ m. *n*=4 mice.
- (B) Embryonic femur at E15.5, after 5 days of chase (pulsed at E10.5). Immunostaining for SOX9. Scale bar: 200 $\mu$ m (left), 50 $\mu$ m (right 4 panels). *n*=4 mice.
- (C) Cartilage template at E13.5, after 5 days of chase (pulsed at E8.5). Immunostaining for SOX9. Scale bar: 200 $\mu$ m. *n*=4 mice.
- (D) Embryonic femur at E18.5, after 10 days of chase (pulsed at E8.5). Immunostaining for SOX9. Scale bar: 500 $\mu$ m. *n*=4 mice.



**Supplementary Figure 4. Skeletal muscle cells or endothelial cells do not contribute to chondrocytes, osteoblasts or bone marrow stromal cells.**

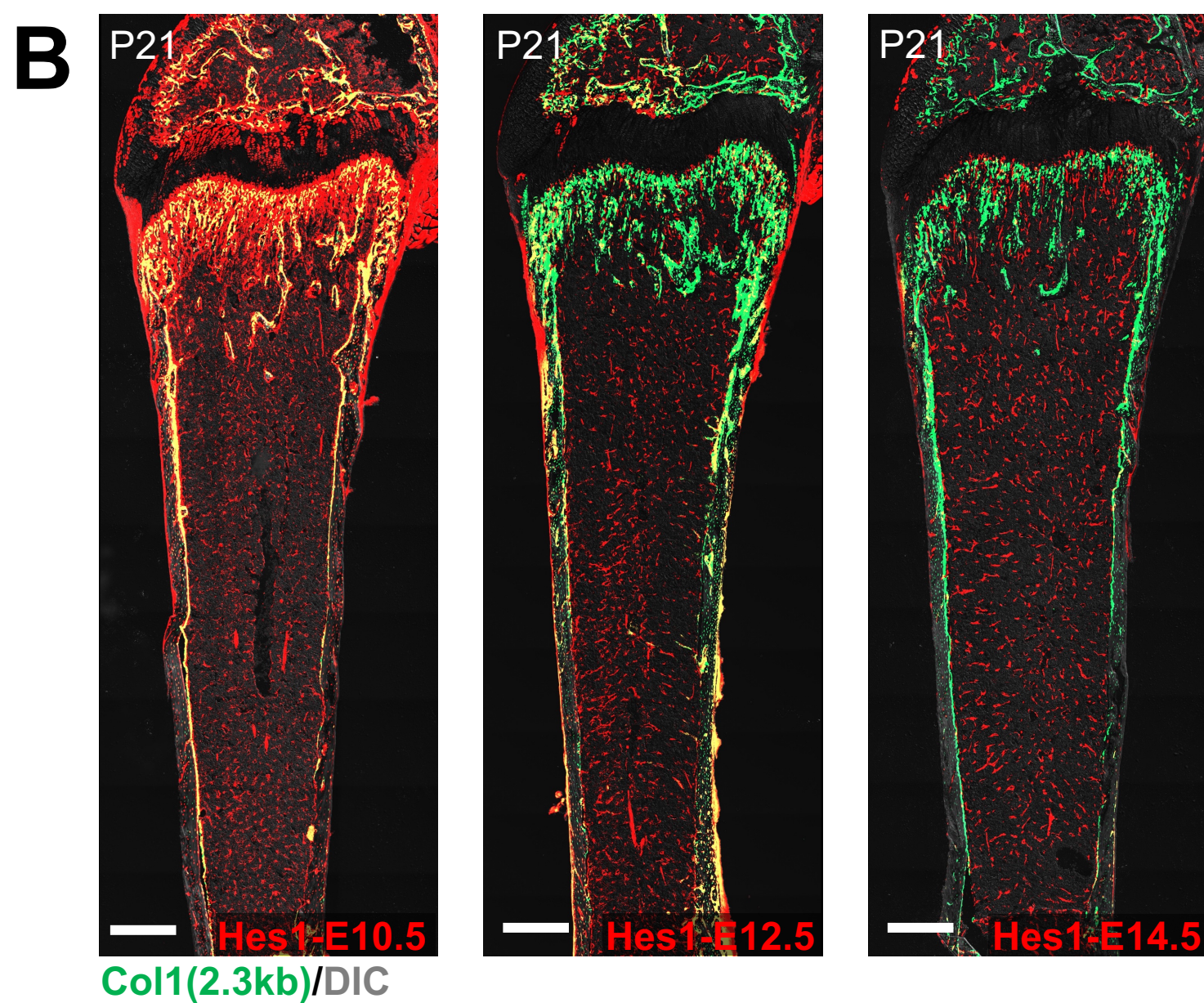
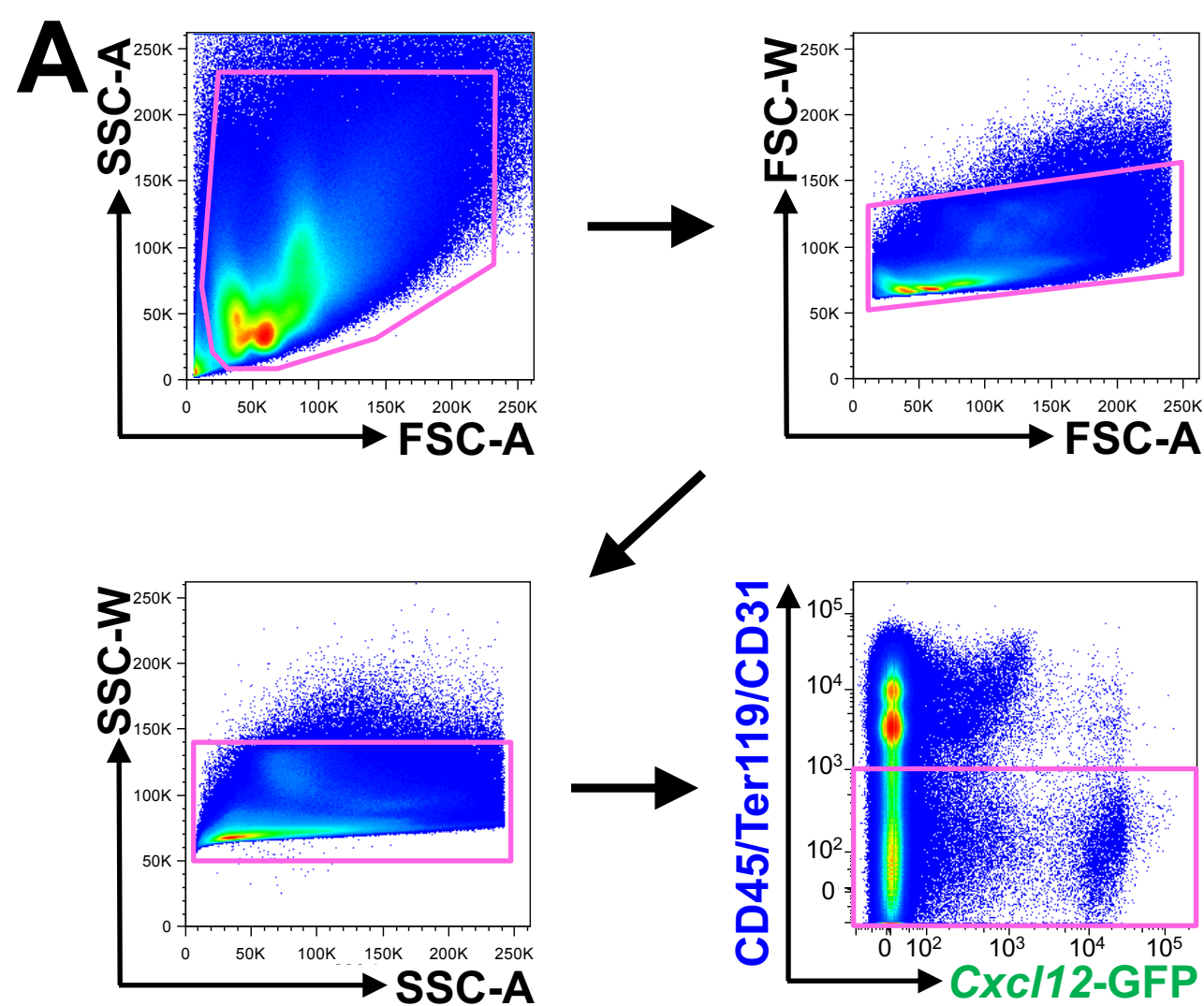
(A) Fate mapping analysis of *Acta1-cre*, *Myf1-cre* or *Mck-cre*-marked skeletal muscle cells at P0 (top) and P21 (bottom). *Acta1-cre; R26R<sup>tdTomato</sup>* (left), *Myf1-cre; R26R<sup>tdTomato</sup>* (center) and *Mck-cre; R26R<sup>tdTomato</sup>* (right) femurs. Immunostaining for MYH3. Grey: DIC. Scale bar: 500µm. *n*=3 mice per each group.

(B) Fate-mapping analysis of *Tie2-cre*-marked endothelial cells at P21. *Col1a1(2.3kb)-GFP; Tie2-cre; R26R<sup>tdTomato</sup>* and *Cxcl12<sup>GFP/+</sup>; Tie2-cre; R26R<sup>tdTomato</sup>* femurs. Left panel: whole bone. Scale bar: 500µm. Upper center panels: magnified views of boxed areas (1: trabecular bone, 2: endosteal marrow space). Scale bar: 20µm. Lower center panel: magnified view of diaphyseal bone marrow of *Cxcl12<sup>GFP/+</sup>; Tie2-cre; R26R<sup>tdTomato</sup>* femur. Scale bar: 20µm. *n*=3 mice per each group. Upper right panels: flow cytometry analysis of bone marrow cells. Right center panel: histogram showing GFP expression. Blue lines: control cells. *n*=5 (*Cxcl12<sup>GFP/+</sup>*), *n*=4 (*Col1a1(2.3kb)-GFP*) mice. Lower right panels: CFU-F assay of *Tie2-cre; R26R<sup>tdTomato</sup>* bone marrow cells. Left: tdTomato epifluorescence. Scale bar: 5mm. Right: F4/80 immunostaining. Scale bar: 50µm. *n*=3 mice.



**Supplementary Figure 5. *Hes1-creER*<sup>+</sup> cells at an early postnatal stage.**

Cell-fate analysis of *Hes1-creER*<sup>+</sup> cells. Distal area of femur at P5 (pulsed at P3). Immunostaining for SOX9.  
 1: Groove of Ranvier. 2 and 3: Articular cartilage. Scale bar: 500 $\mu$ m (left), 50 $\mu$ m (right 3 panels).  $n=4$  mice.



**Supplementary Figure 6. Contribution of *Hes1*-creER<sup>+</sup> cells to the postnatal stromal compartment**

(A) Flow cytometry analysis of CD45/Ter119/ CD31<sup>neg</sup> cells at P21. Gating strategy for bone marrow cells isolated from *Cxcl12*<sup>GFP/+</sup>; *Hes1*-creER; *R26R*<sup>tdTomato</sup> femurs (pulsed at E10.5, E12.5 or E14.5). (B) Contribution of fetal *Hes1*-creER<sup>+</sup> cells (pulsed at E10.5, E12.5 or E14.5) to skeleton at P21. *Colla1*(2.3kb)-GFP; *Hes1*-creER; *R26R*<sup>tdTomato</sup> femurs with growth plates on top. Scale bar: 500 $\mu$ m (left panels). *n*=4 mice per each group.