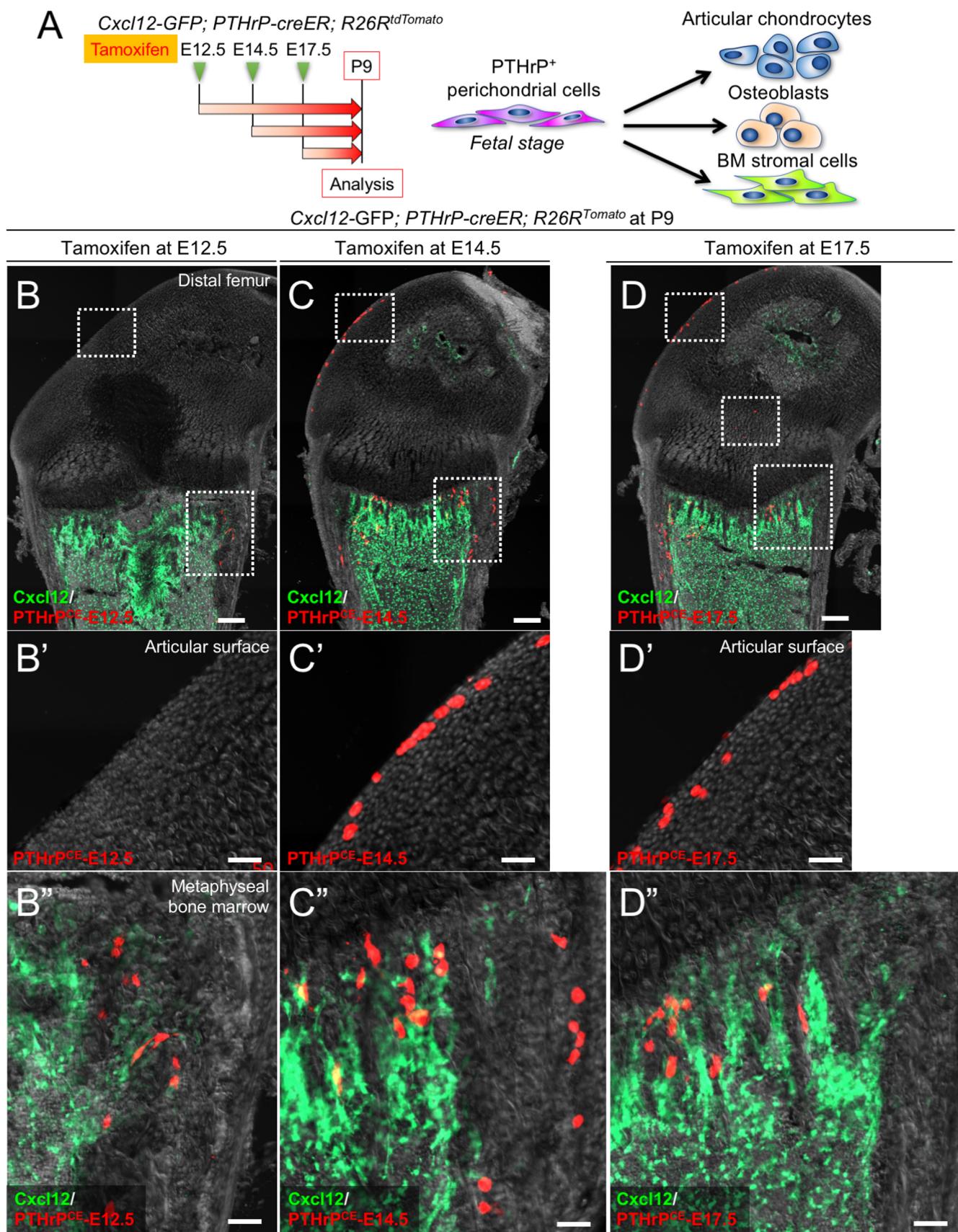


Supplemental Figure 1. Single cell RNA-seq characterization of neonatal growth plate chondrocytes.

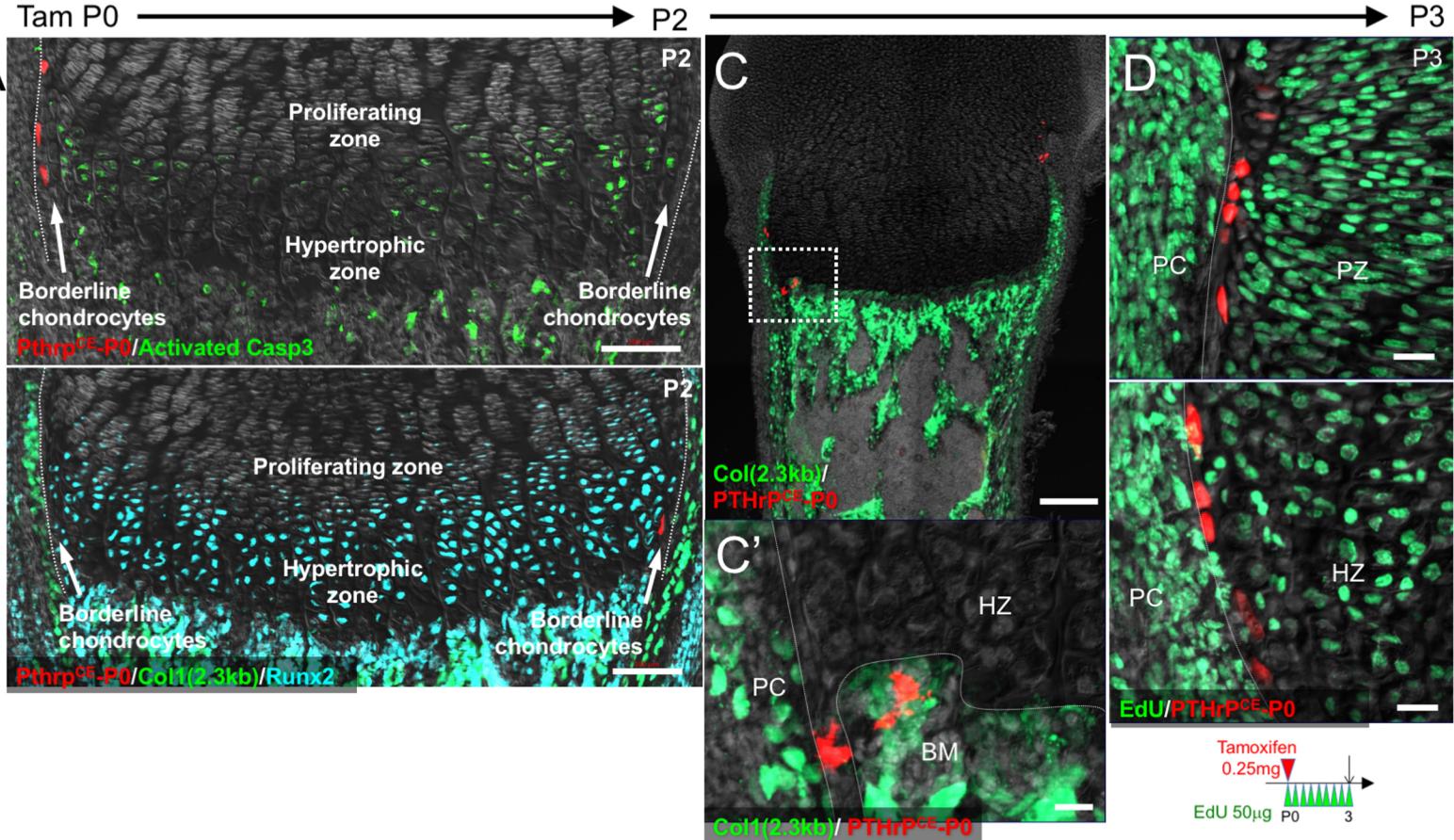
(A) t-SNE-based visualization of major classes of FACS-sorted *Col2a1-creER*⁺ neonatal growth plate chondrocytes at P2 (Cluster 0-10). Cluster 0,5,7: lower zone column-forming chondrocytes, Cluster 1,3: upper zone chondrocytes, Cluster 2,6: articular surface chondrocytes, Cluster 4: collagen-rich articular cells, Cluster 8: borderline chondrocytes, Cluster 9,10: cells in cell cycle. Feature plots: expression of the indicated gene. Blue: high expression, grey: no expression. n=8,486 cells. (B) Violin plots and feature plots for top 6 'borderline' markers most significantly enriched in Cluster 8 (*Srxn1*, *Ifrd1*, *Tnfrsf12a*, *Fosl1*, *Pmepa1*, *Myc*) and *tdTomato-WPRE*. Dots: individual cells, y-axis: expression level. (C) Average expression of *Pthlh* in each cluster. (D) Feature plots of hypertrophic markers. Green contour: Cluster 8.



Supplemental Figure 2. Fate of fetal *Pthrp-creER*⁺ perichondrial cells.

Cell fate analysis of fetal *Pthrp-creER*⁺ perichondrial cells. (A): Diagram of the pulse-chase experiment. Distal femurs were analyzed at P9 after being pulsed at preceding time points (E12.5, E14.5 and E17.5). (B-D) *Cxcl12-GFP; Pthrp-creER; R26R^{tdTomato}* distal femurs. (B'-D'): magnified views of articular surface, (B''-D''): magnified views of peripheral metaphyseal marrow space. Green: *Cxcl12-GFP* (A-D), red: *tdTomato*, Grey: DAPI and DIC. Scale bars: 200μm (B-D), 50μm (B'-D', B''-D'').

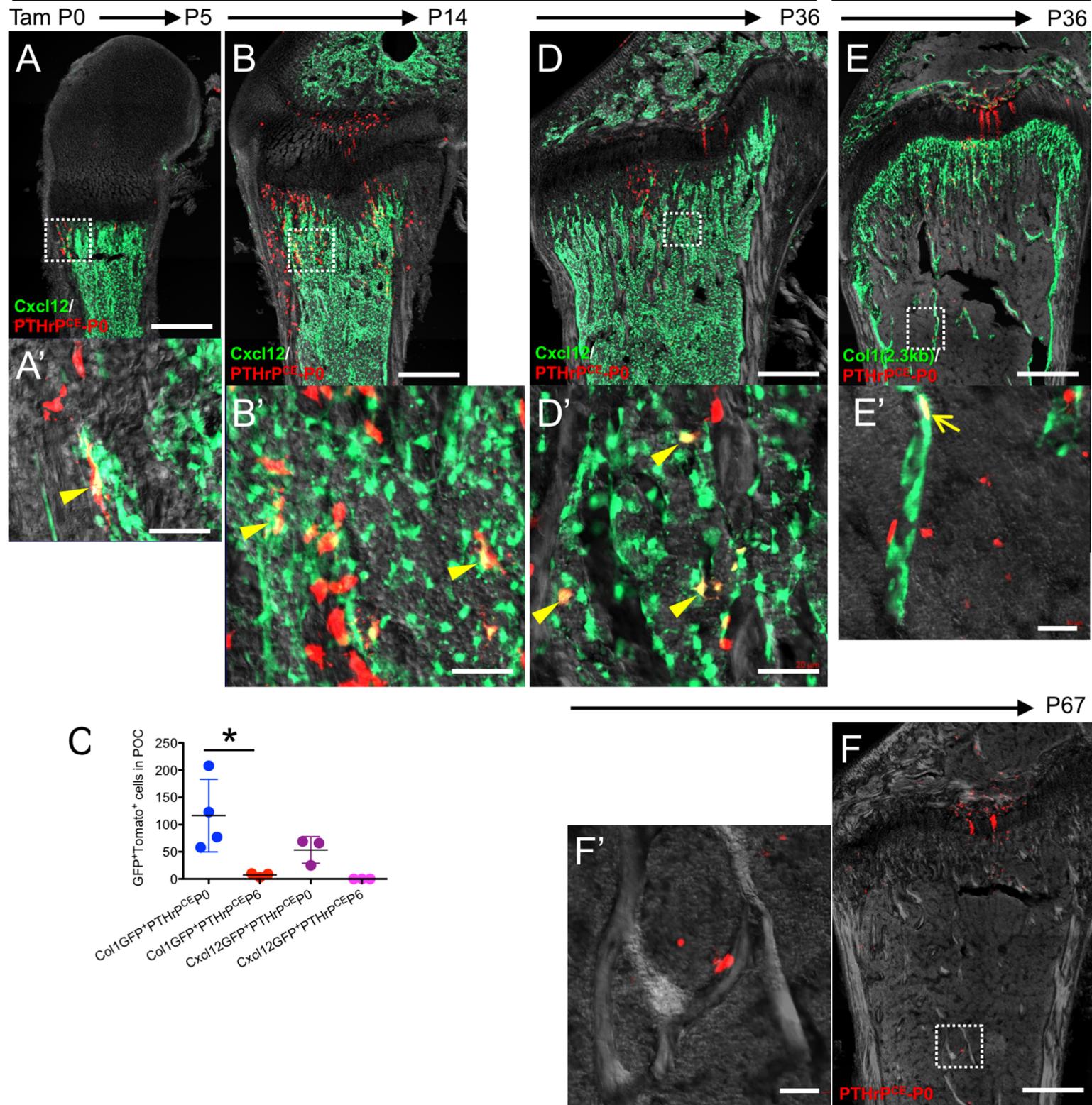
Col1(2.3kb)-GFP; PTHrP-creER; R26R^{Tomato}



Supplemental Figure 3. Characterization of *Pthrp-creER*⁺ borderline chondrocytes.

(A-C): *Pthrp-creER*; *R26R*^{tdTomato} (A), *Col1a1(2.3kb)-GFP*; *Pthrp-creER*; *R26R*^{tdTomato} (B,C) distal femurs staining for activated Caspase 3 (A) and Runx2 (B). Scale bars: 100µm.

(C,D): Short-chase analysis of *Pthrp-creER*⁺ borderline chondrocytes (P0-pulsed) at P3. (C'): magnified views of the dotted areas. (D): EdU (50µg) was serially injected 9 times at an 8-hour interval between P0 and P3. PC: perichondrium, PZ: proliferating zone, HZ: hypertrophic zone, BM: bone marrow. Scale bars: 200µm (C), 20µm (C',D).



Supplemental Figure 4. *Pthrp-creER*⁺ borderline chondrocytes behave as precursors for CXCL12⁺ bone marrow stromal cells.

Cell fate analysis of *Pthrp-creER*⁺ borderline chondrocytes (P0-pulsed) at P5 (A), P14 (B,C), P36 (D,E) and P67 (F). (A,B,D): Cxcl12-GFP; *Pthrp-creER*; R26R^{TdTomato} distal femurs, (C): Quantification of Col1a1(2.3kb)-GFP⁺tdTomato⁺ cells and Cxcl12-GFP⁺tdTomato⁺ cells at P14, after being pulsed at P0 or P6. *p<0.05, One-way ANOVA followed by Tukey's multiple comparison test. (E): Col1a1(2.3kb)-GFP; *Pthrp-creER*; R26R^{TdTomato} distal femur, (F): *Pthrp-creER*; R26R^{TdTomato} distal femur. (A'-F'): magnified views of the dotted areas. Arrowheads: Cxcl12-GFP⁺tdTomato⁺ cells in marrow space, arrow: Col1a1(2.3kb)-GFP⁺tdTomato⁺ Green: Cxcl12-GFP, red: tdTomato, Grey: DAPI and DIC. Scale bars: 500μm, 50μm.