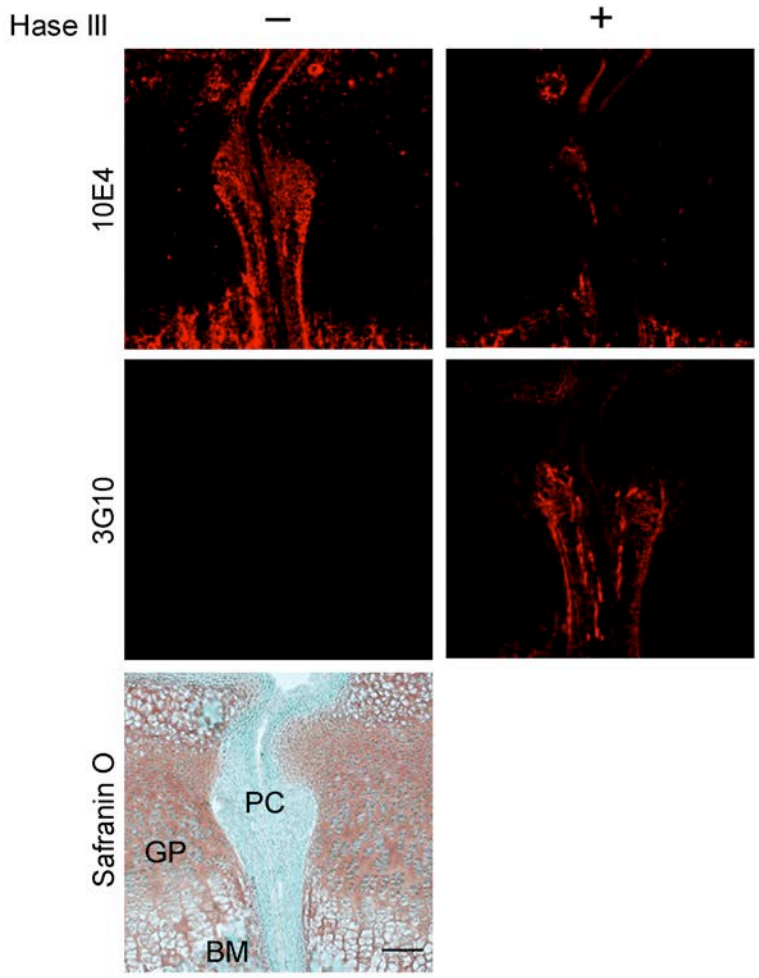
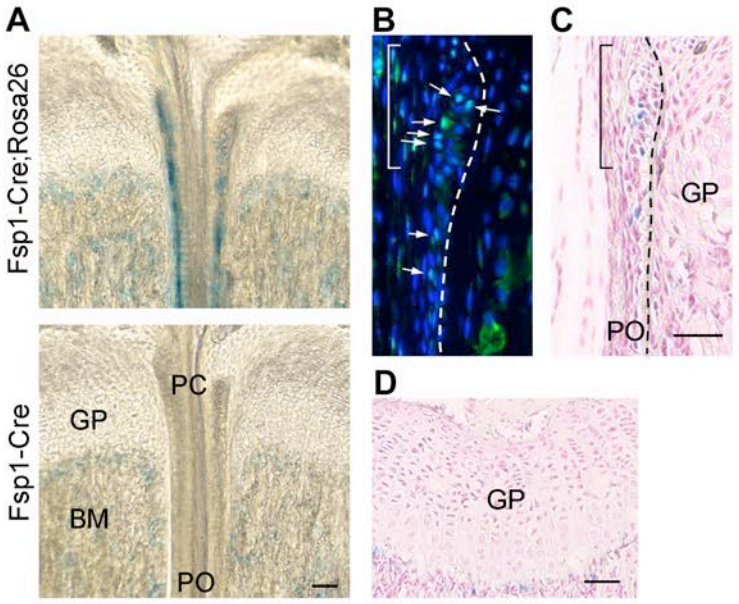
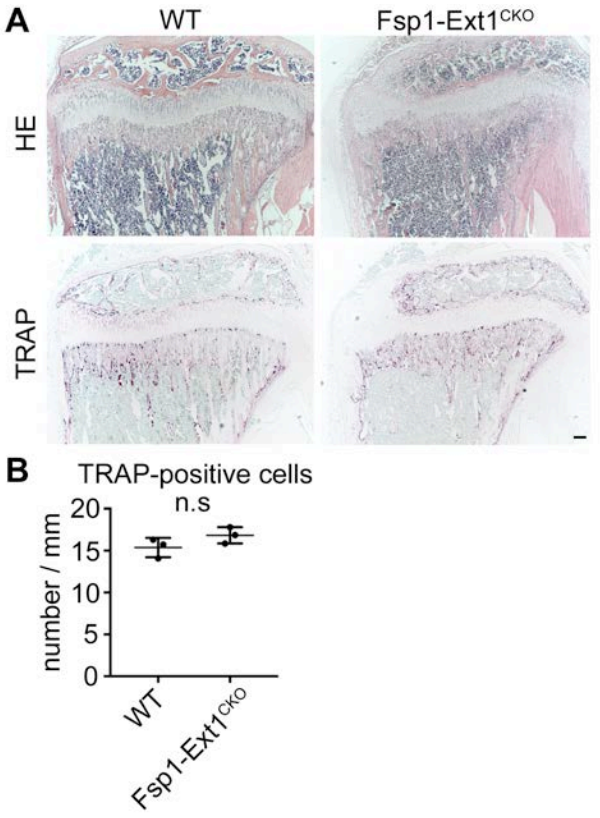


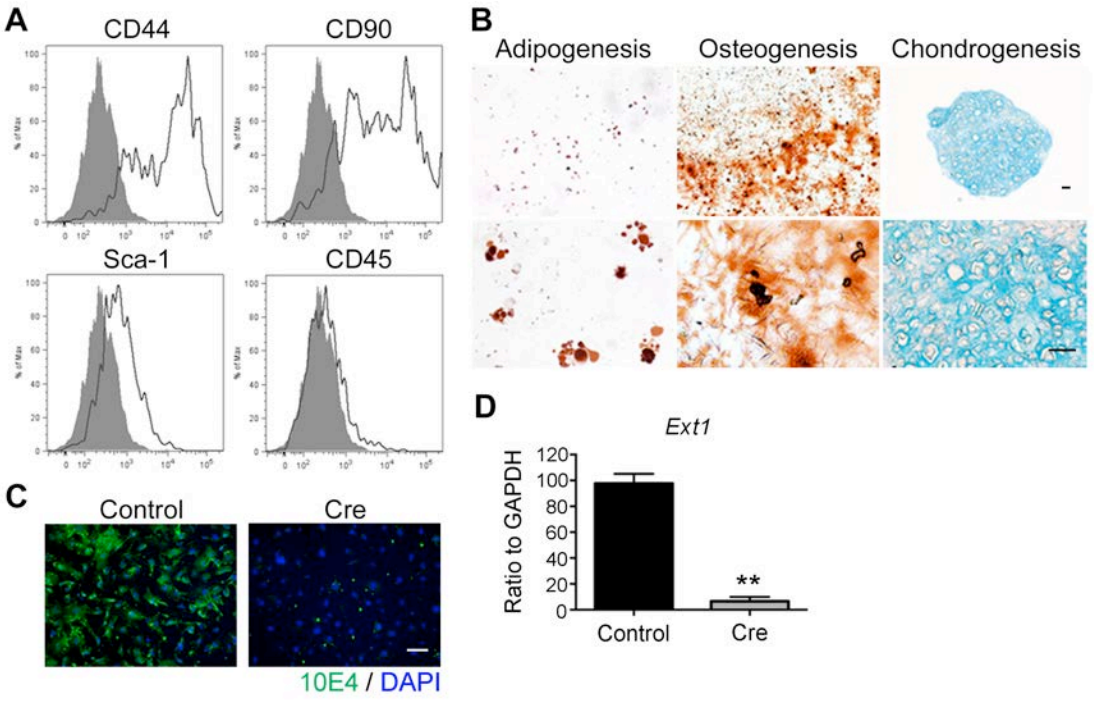
Supplementary Figure 1. HS expression in the perichondrial groove of Ranvier. Two anti-heparan sulfate (HS) monoclonal antibodies were used to analyze the expression of HS in the perichondrium of wild-type mice. The 10E4 antibody recognizes a sulfation-dependent epitope on HS chains; treatment with heparitinase III (Hase III) destroys the 10E4 immunoreactivity. The 3G10 antibody, on the other hand, recognizes a neo-epitope that is generated only after Hase III digestion; 3G10 does not react with intact HS chains. Sections through the distal end of the radius and ulna were stained with these two antibodies with or without prior Hase III treatment (0.02 U/ml, 60 min). An adjacent section was stained with Safranin O (*bottom panel*). Note that strong 10E4 immunoreactivity is seen in perichondrium including the groove of Ranvier (and in bone marrow), which is largely abolished after Hase III. Hase III-dependent 3G10 immunoreactivity is also localized to the perichondrium. Growth plate cartilage shows only weak reactivity to either antibody, indicating that the level of HS expression in growth plate chondrocytes is much lower than that in perichondrial cells. *PC*, perichondrium; *GP*, growth plate; *BM*, bone marrow. Scale bar, 0.1 mm.



Supplementary Figure 2. Spatial recombination pattern by *Fsp1-Cre* in the developing bone. (**A**) Thick vibratome sections (50 μm) through the distal end of the forearm from *Fsp1-Cre;R26R* and *Fsp1-Cre* (control) mice. X-gal staining reveals that *Fsp1-Cre*-mediated recombination occurs in the perichondrium and periosteum. Weak X-gal staining in bone marrow is mostly non-specific (compare with control mice). (**B**–**D**) Paraffin sections (5 μm) through the growth plate of *Fsp1-Cre;R26R* mice stained with anti-FSP1 and DAPI (*B*) or X-gal (*C, D*). Immunohistochemistry with anti-FSP1 antibody demonstrates that cells in the groove of Ranvier express FSP1 (*arrows* in *B*). X-gal staining shows that *Fsp1-Cre*-mediated recombination occurs in cells in the groove of Ranvier (*C*), but not in chondrocytes in growth plate cartilage (*D*). The approximate extent of the groove of Ranvier is shown by brackets in *B* and *C*. *PC*, perichondrium; *PO*, periosteum; *GP*, growth plate, *BM*, bone marrow. Scale bars, 0.1 mm.



Supplementary Figure 3. Osteoclastogenesis is not altered in *Fsp1-Ext1^{CKO}* mice. (**A**) Tartrate-resistant acid phosphatase (TRAP) staining of the epiphyseal region of the tibia of *Fsp1-Ext1^{CKO}* and control (*Ext1$^{flox/flox}$; WT*) littermates at 4 weeks of age. (**B**) The number of TRAP-positive cells along the trabecular bone surface in *Fsp1-Ext1^{CKO}* and control (*Ext1$^{flox/flox}$; WT*) normalized by the length of bone surface. Means ± SD (n = 3) are shown as horizontal bars. *n.s.*, not significant.



Supplementary Figure 4. Characterization of perichondrium-derived mesenchymal progenitor cells (PDPCs). (**A**) Flow cytometric characterization of PDPCs with mouse mesenchymal stem cell (MSC) markers. PDPCs are immunoreactive to the MSC markers CD44, CD90, and Sca-1, while negative for the hematopoietic cell marker CD45. (**B**) Differentiation capacity of PDPCs to three lineages. Differentiation of PDPCs into adipogenic, osteogenic, and chondrogenic lineages was induced as described in *Methods* and evaluated by staining with Oil Red O, Alizarin Red, and Alcian Blue, respectively, after 3 weeks in culture. (**C**) Lentivirus-mediated ablation of *Ext1* in PDPCs. *Ext1*-null and control PDPCs were prepared by infecting PDPCs with Cre-expressing lentivirus (*Cre*) or with insertless lentivirus (*Control*). Cells were stained with 10E4 anti-heparan sulfate (HS) antibody and DAPI. (**D**) Expression of *Ext1* mRNA and HS in *Ext1*-null and control PDPCs was analyzed by qRT-PCR and immunostaining with 10E4, respectively. *P* values were determined by Student's *t*-test. **p<0.01, n = 5. Scale bars, 0.1 mm (*B*); 50 μm (*C*).