Inventory of Supplemental Materials

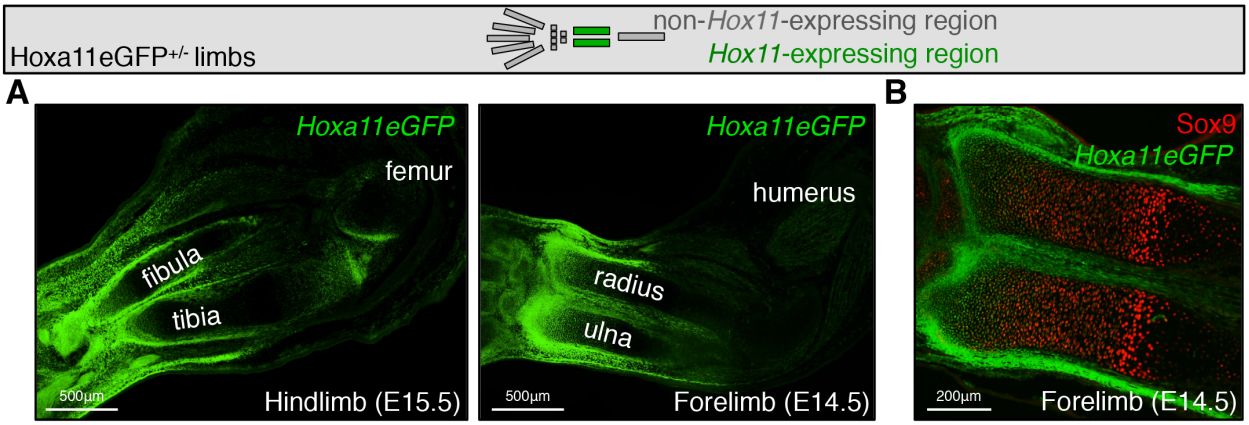

Figure S1 (related to Figure 1). Hoxa11eGFP surrounds the zeugopod skeletal elements of the
developing limbs. Limb schematic depicts Hoxa11eGFP regional expression (green). (A) Hoxa11eGFP expression is shown in embryonic hindlimbs (tibia/fibula) and forelimbs (radius/ulna). (B) Sox9 expression depicts the developing cartilage skeletal elements of the forelimb. Hoxa11eGFP surrounds the skeletal elements, largely excluded from the differentiating cartilage anlage.

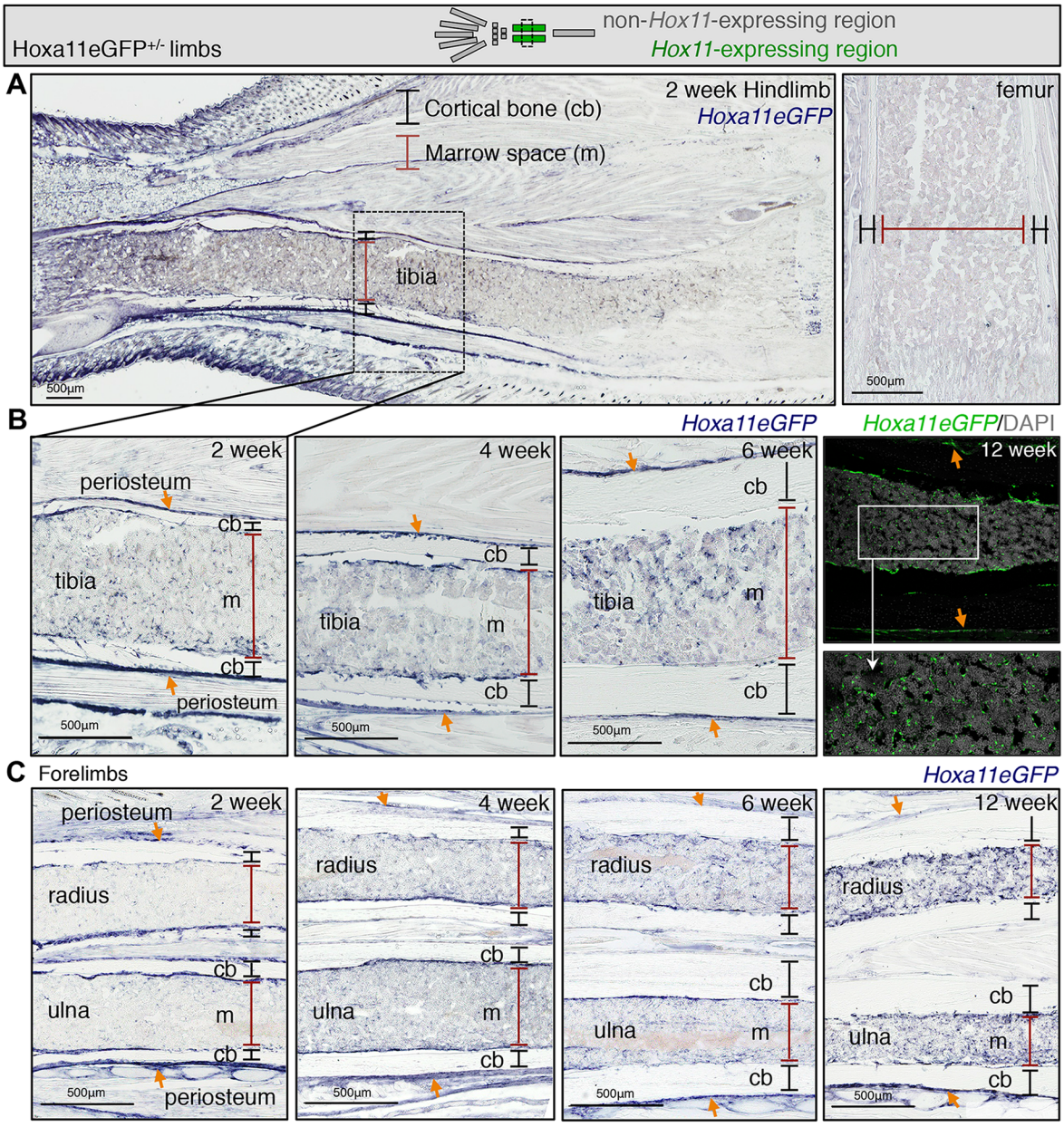

Figure S2 (related to Figure 1). Hoxa11eGFP is regionally expressed through postnatal and adult stages in hindlimbs and forelimbs. Limb schematic depicts Hoxa11eGFP regional expression (green). (A) Hoxa11eGFP is expressed in the hindlimb zeugopod (tibia/fibula) with the same pattern visualized in the forelimb. Lower magnification images at 2 weeks show that Hoxa11eGFP is restricted to the zeugopod and absent from the stylopod (femur) of the hindlimb. (B) Higher magnification images through 12 weeks of age show continued expression in the periosteum (orange arrows) and increasing in the bone marrow. (m and red bar) marrow; (cb and black bar) cortical bone. (C) Higher magnification forelimb images analogous to Figure 1 are shown developed with alkaline phosphatase. (m and red bar) marrow; (cb and black bar) cortical bone. See also Figure 1.

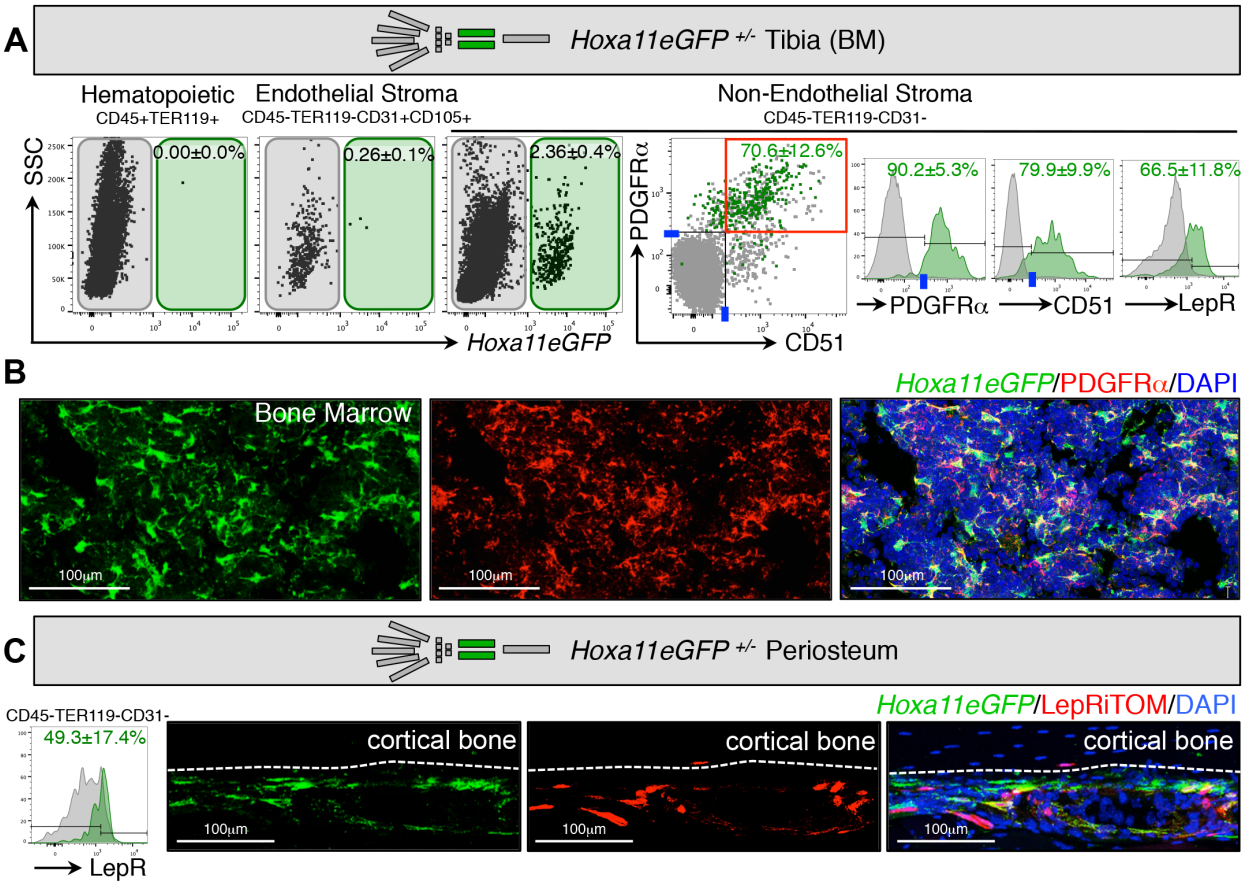

Figure S3 (related to Figure 3). Hoxa11eGFP-expressing cells from tibia bone marrow and from periosteum are identified as mesenchymal stem/stromal cells. (A) Limb schematic depicts Hoxa11eGFP regional expression (green). Live-cell FACS analysis of unfractured zeuogpod limbs from tibia bone marrow. Hoxa11eGFP is not expressed in CD45+/TER119+ hematopoietic cells or in CD105+/CD31+ endothelial cells. Hoxa11eGFP is expressed in CD45-/TER119-/CD31- non-endothelial stromal cells. Overlaid FACS plots or histograms display GFP+ (green) and GFP- (gray) cells from non-endothelial stroma. Hoxa11eGFP+ cells are predominantly PDGFRα+, CD51+, LepR+ cells. See also Figure 3. Data are represented as mean ± SEM.
(B) Immunohistochemsitry shows co-expression of Hoxa11eGFP+ cells and PDGFRα staining in the bone marrow. (C) In the periosteum, fewer Hoxa11eGFP+ cells, compared to bone marrow, are positive for LepR shown by FACS antibody and by mice carrying alleles for Hoxa11eGFP, LepRCre, and ROSA-tdTomato. Data are represented as mean ± SEM.

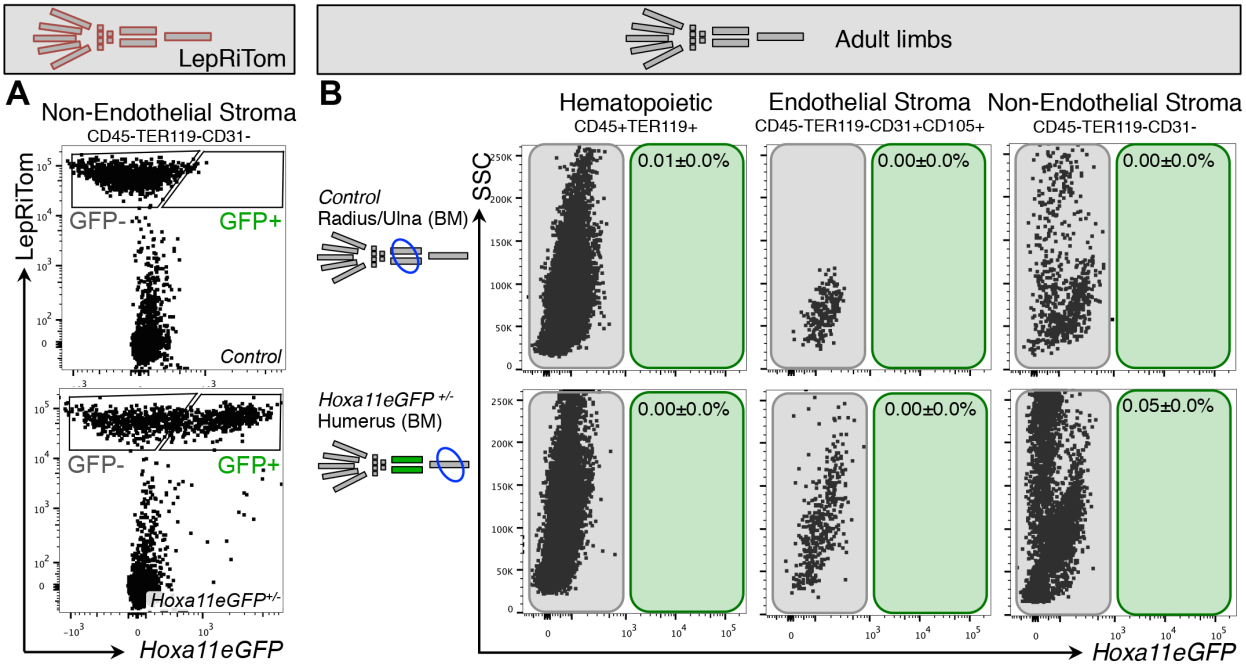

Figure S4 (related to Figure 3). FACS analysis controls for Hoxa11eGFP expression. (A) Limb schematic depicts tdTomato+ cells in the limb of LepRCre/tdTomato mice. Live-cell FACS analysis of bone marrow from control (LepRCre/tdTomato) and from LepRCre/tdTomato/Hoxa11eGFP mice confirms there are no GFP+ cells found in control LepR/tdTomato mice. GFP+ cells are only found in mice carrying the Hoxa11eGFP knock-in allele. (B) Live-cell FACS analysis of bone marrow from unfractured zeugopod control (non-GFP) limbs and the stylopod region of Hoxa11eGFP$^{+/-}$ animals shows no GFP+ cells. Data are represented as mean ± SEM.

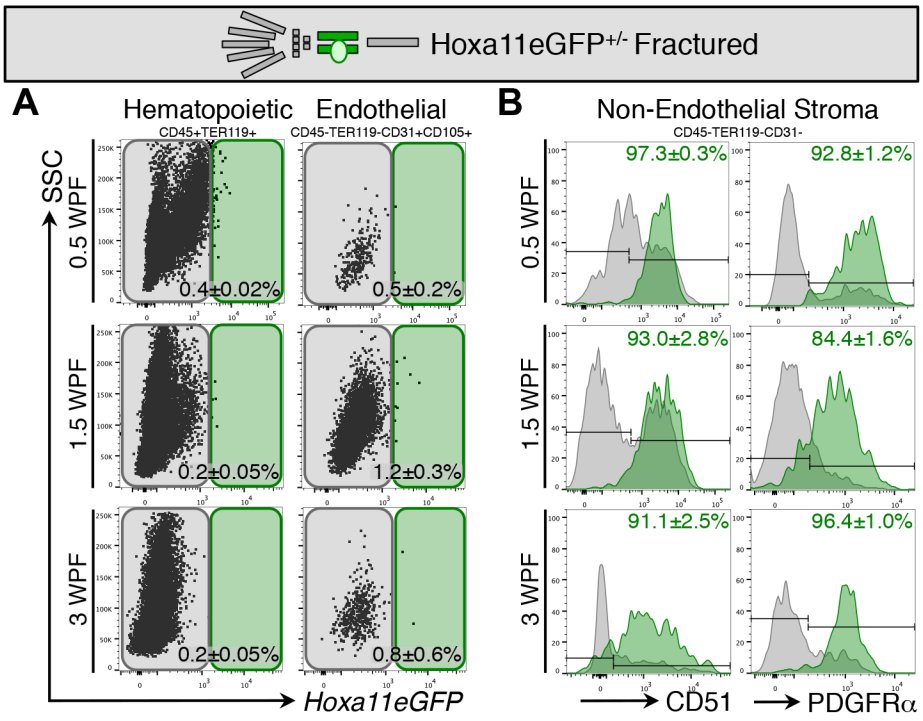

Figure S5 (related to Figure 5). Hoxa11eGFP-expressing cells throughout fracture healing maintain the FACS profile from uninjured bone. Limb schematic depicts Hoxa11eGFP regional expression (green) and the fracture callus in the zeugopod region (tibia or ulna). (A and B) Live cell FACS analysis of the fracture callus from Hoxa11eGFP+/- animals shows GFP+ cells excluded from CD45-TER119- (hematopoietic) cells and from CD105+CD13+ (endothelial) cells at 0.5WPF, 1.5WPF and 3WPF. GFP+ cells are largely CD45-TER119-CD31-PDGFR**α**+CD51+ cells. Data are represented as mean ± SEM. See also Figure 5C.

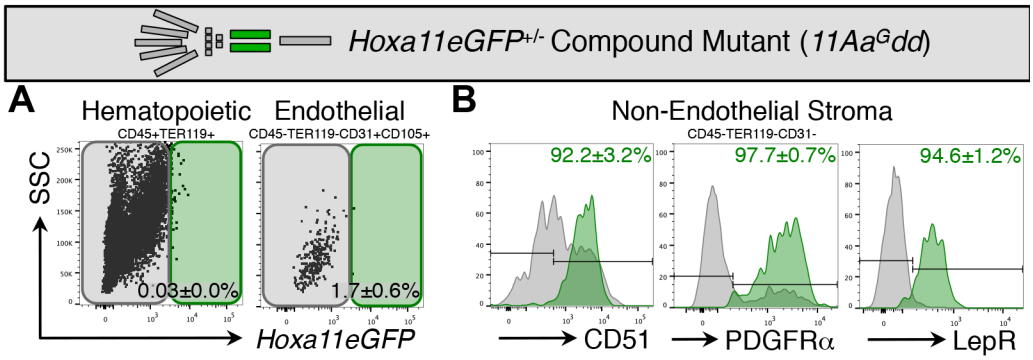

Figure S6 (related to Figure 6). Hoxa11eGFP-expressing cells in Hox11 compound mutant animals are non-hematopoietic and non-endothelial. Limb schematic depicts Hoxa11eGFP regional expression (green). (A and B) Live cell FACS analysis of the fracture callus from Hoxa11eGFP compound mutant (Hox11AaGdd) animals shows GFP+ cells excluded from CD45-TER119- (hematopoietic) cells and from CD105+CD13+ (endothelial) cells in the bone marrow. GFP+ cells are largely CD45-TER119-CD31-PDGFR**α**+CD51+LepR+ cells. Data are represented as mean ± SEM. See also Figure 6D.

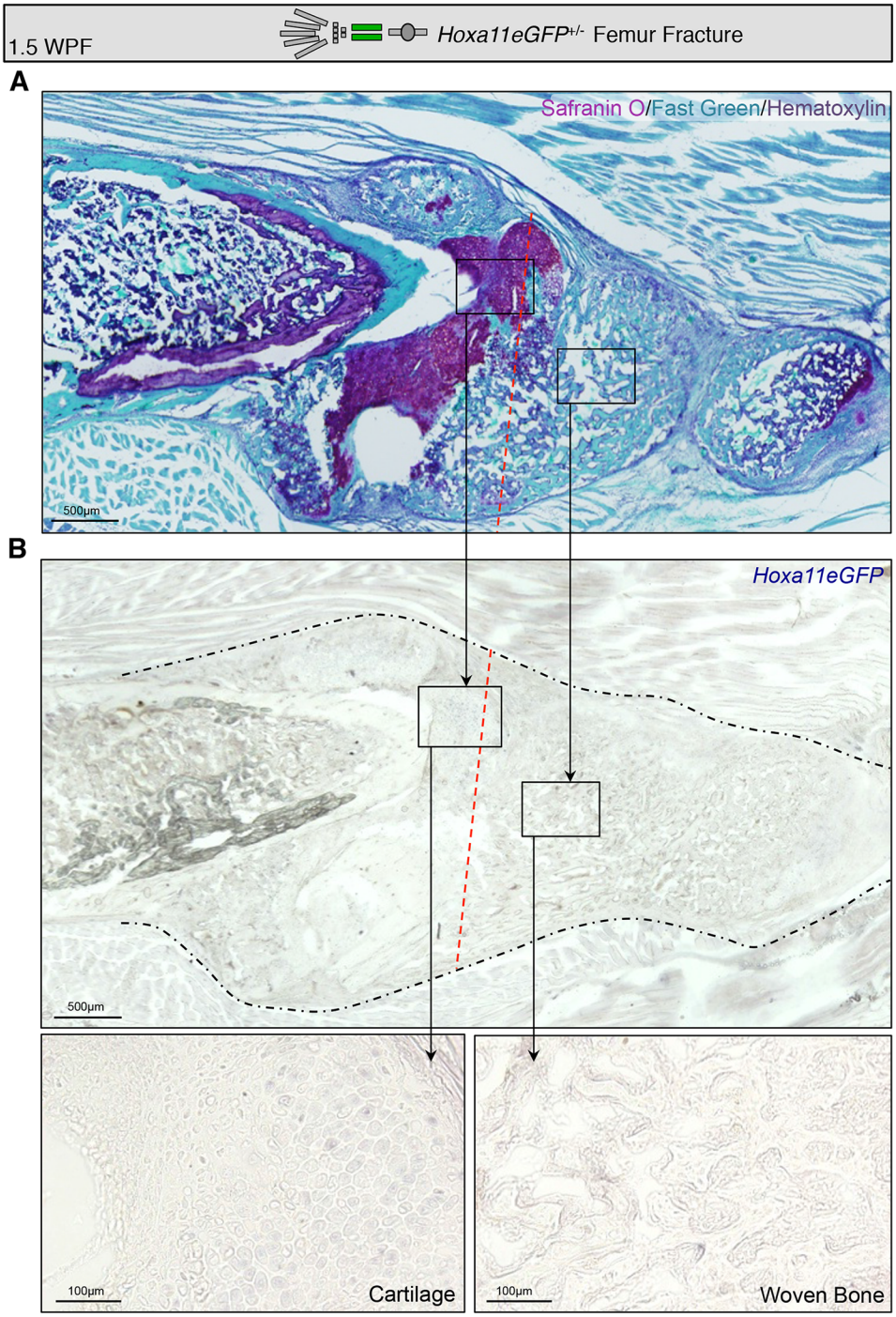

Figure S7 (related to Figure 7). Hoxa11eGFP expression maintains regional specificity in response to repair. (A-B) Limb schematic depicts Hoxa11eGFP regional expression (green) and the fracture callus in the stylopod region (Femur) of Hoxa11eGFP$^{+/-}$ mice. (A) Fractures collected at 1.5 WPF show endochondral ossification at the site of injury, consistent with tibial and ulnar fracture injuries as show by Safranin O staining. (B) Hoxa11eGFP is not expressed in any region of the femur fracture at 1.5 WPF. See also Figure 7.