

Fig. S1. Verification of *lacZ* expression in bone eminences of *Sox9-CreER* embryos. Prevalence of X-Gal-marked cells measured in eminences from E15.5 *Sox9-CreER*, *R26R-lacZ* heterozygous embryos, relative to their prevalence in the primary cartilage, defined as 1. Control staining (TM administration at E12.5-E13.5) shows the effectiveness of the system in marking bone eminence cells. Deltoid tuberosity: 0.01899 ± 0.008791 ; control deltoid tuberosity: 1.134 ± 0.06149 ; great tuberosity: 0.2003 ± 0.04917 ; control great tuberosity: 0.9819 ± 0.08007 ; calcaneal tuberosity: 0.109 ± 0.03516 ; control calcaneal tuberosity: 1.044 ± 0.05835 ; great trochanter: 0.1801 ± 0.02033 ; control great trochanter: 1.173 ± 0.0786 ; $P < 0.0001$. Error bars represent the s.e.m.

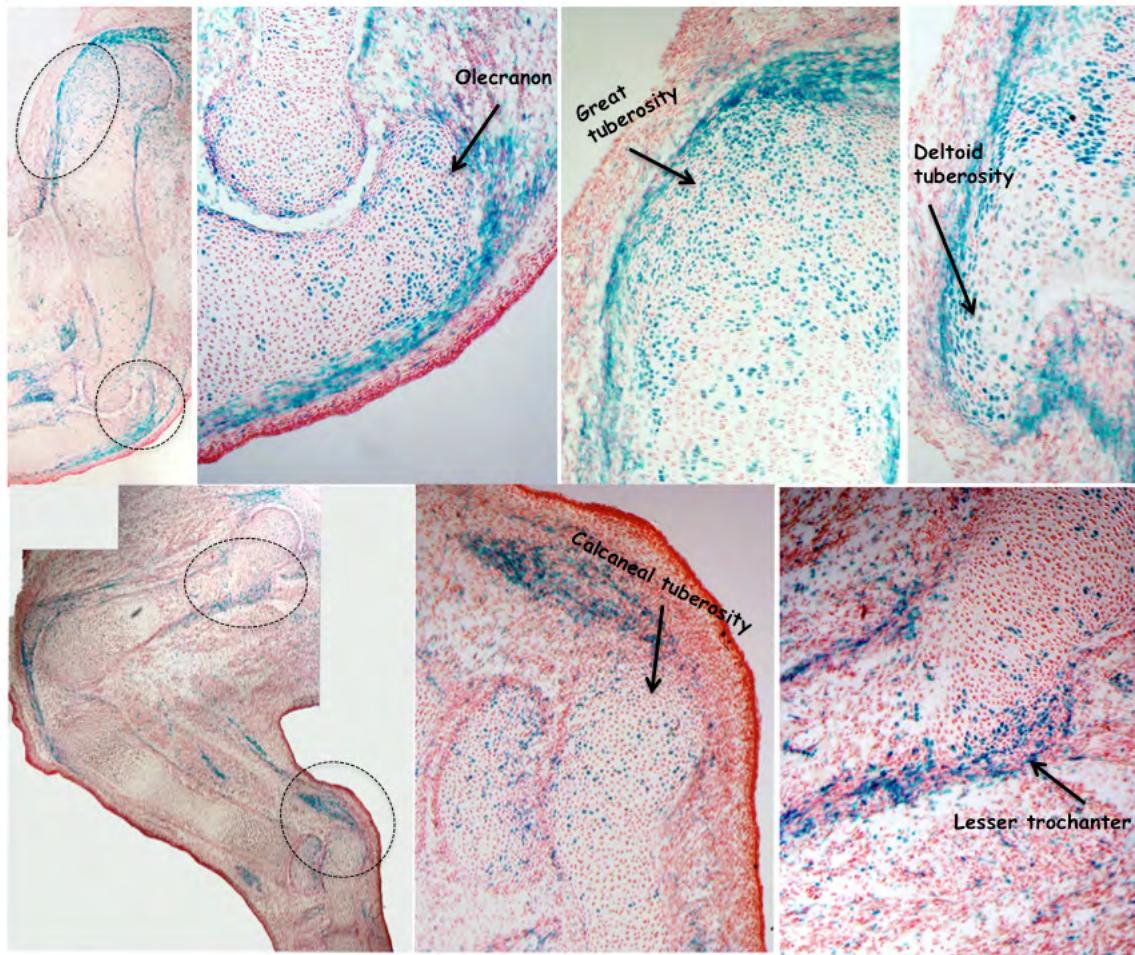


Fig. S2. Eminence progenitors express *Scx*. Cell lineage experiment using E16.5 *Scx-Cre R26R-lacZ* embryos demonstrates that *Scx*-positive cells contribute to various eminences in the forelimb and hindlimb, indicated by arrows. Dashed lines delineate eminence regions, which are magnified on the right.

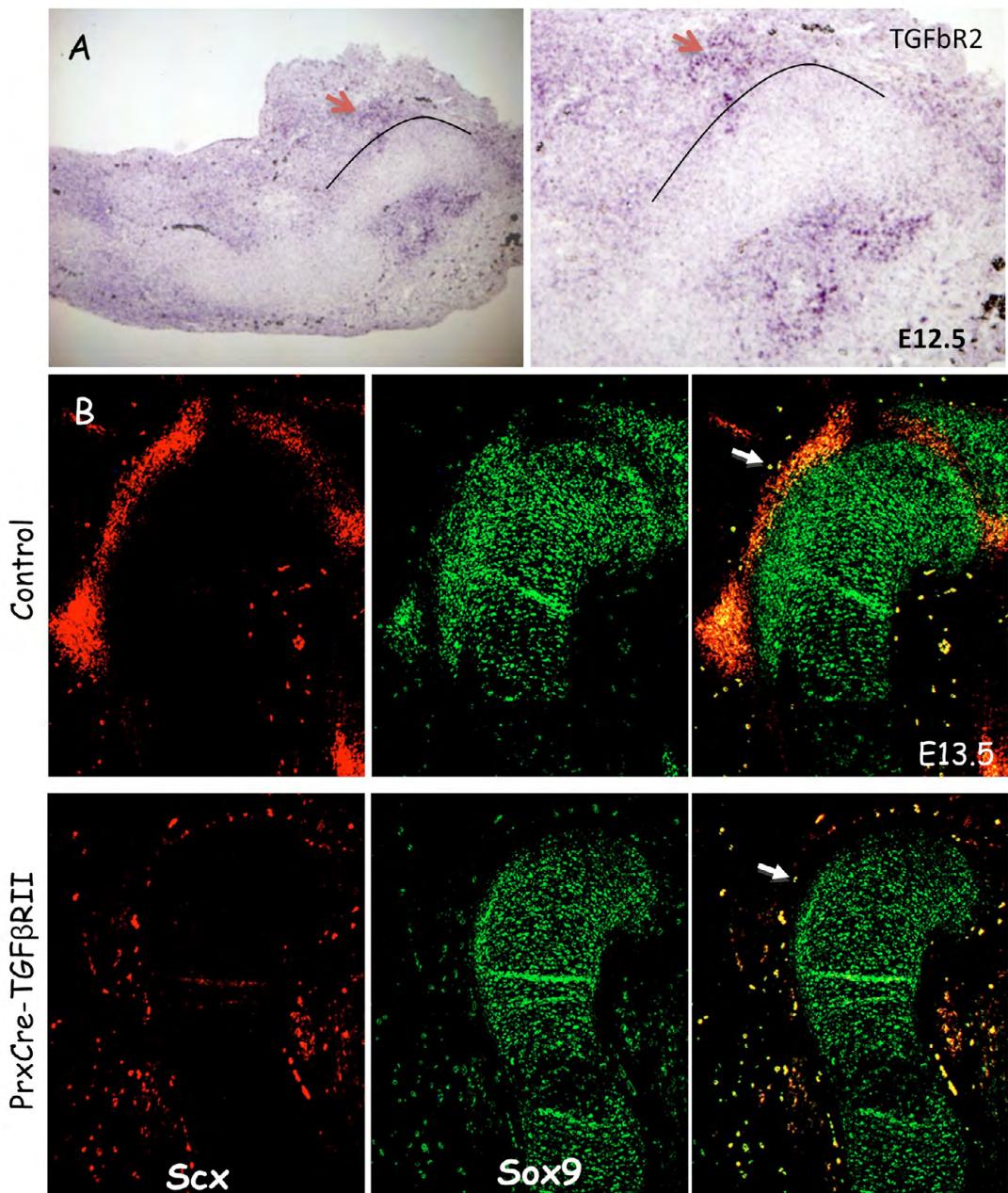


Fig. S3. *Tgfbr2* expression by bone eminence progenitors. (A) *In situ* hybridization analysis of longitudinal humeral sections from E12.5 wild-type mice using antisense complementary RNA probes for *Tgfbr2* mRNA. Red arrows indicate the expression of *Tgfbr2* in eminence progenitors. Black lines indicate the primary cartilage borders. Right panel is a magnification of the left panel. (B) Double fluorescence *In situ* hybridization of sagittal humeral sections from E13.5 control and *Prx1-Tgf- β RII* mutant mice using anti-sense complementary RNA probes for *Sox9* and *Scx* indicate the loss of *Scx* expression in the mutants. White arrowheads indicate tendon insertion into the humerus.

Table S1. Probe sequences for *in situ* hybridization