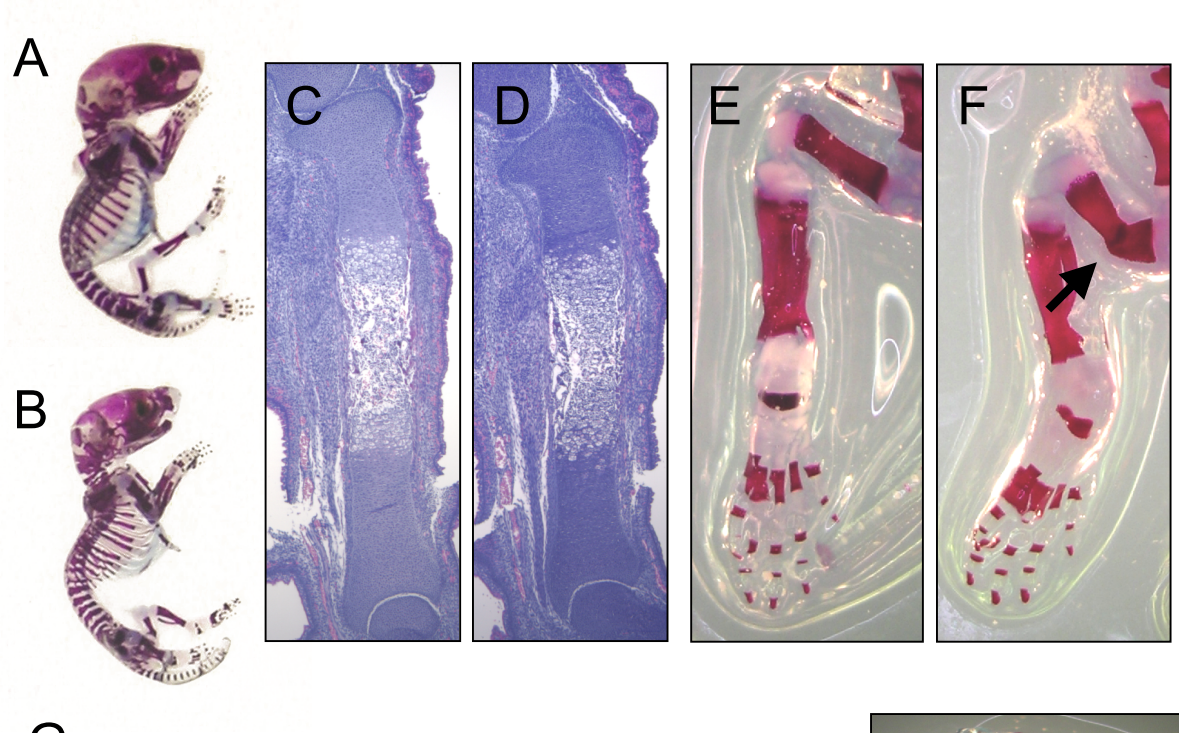
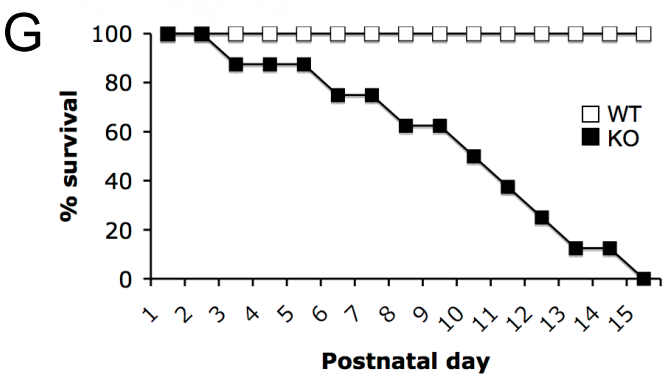
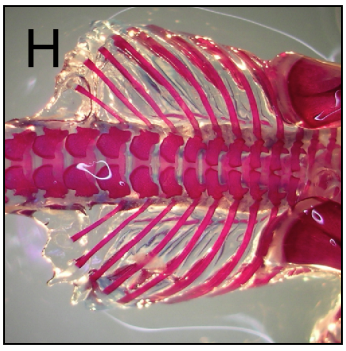
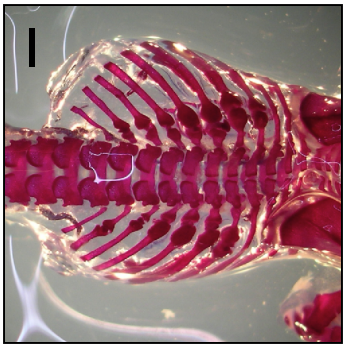
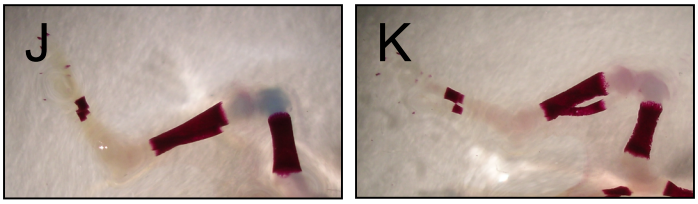
Supplementary Figure 1









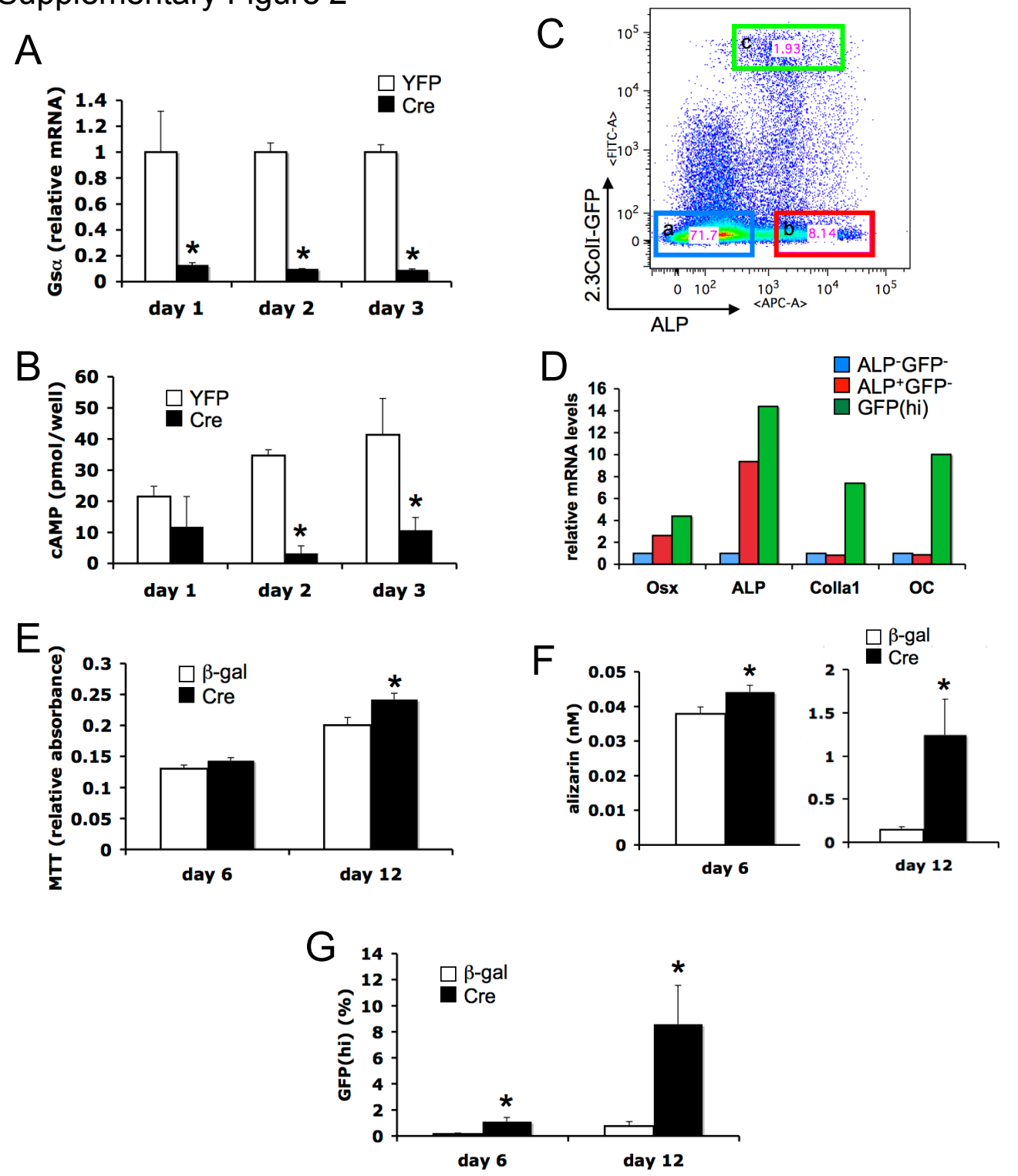


L

Fractures	E17.5	E18.5	P1	P7/8
WT	0/3	0/5	0/5	0/5
KO	0/3	0/5	3/6	5/5

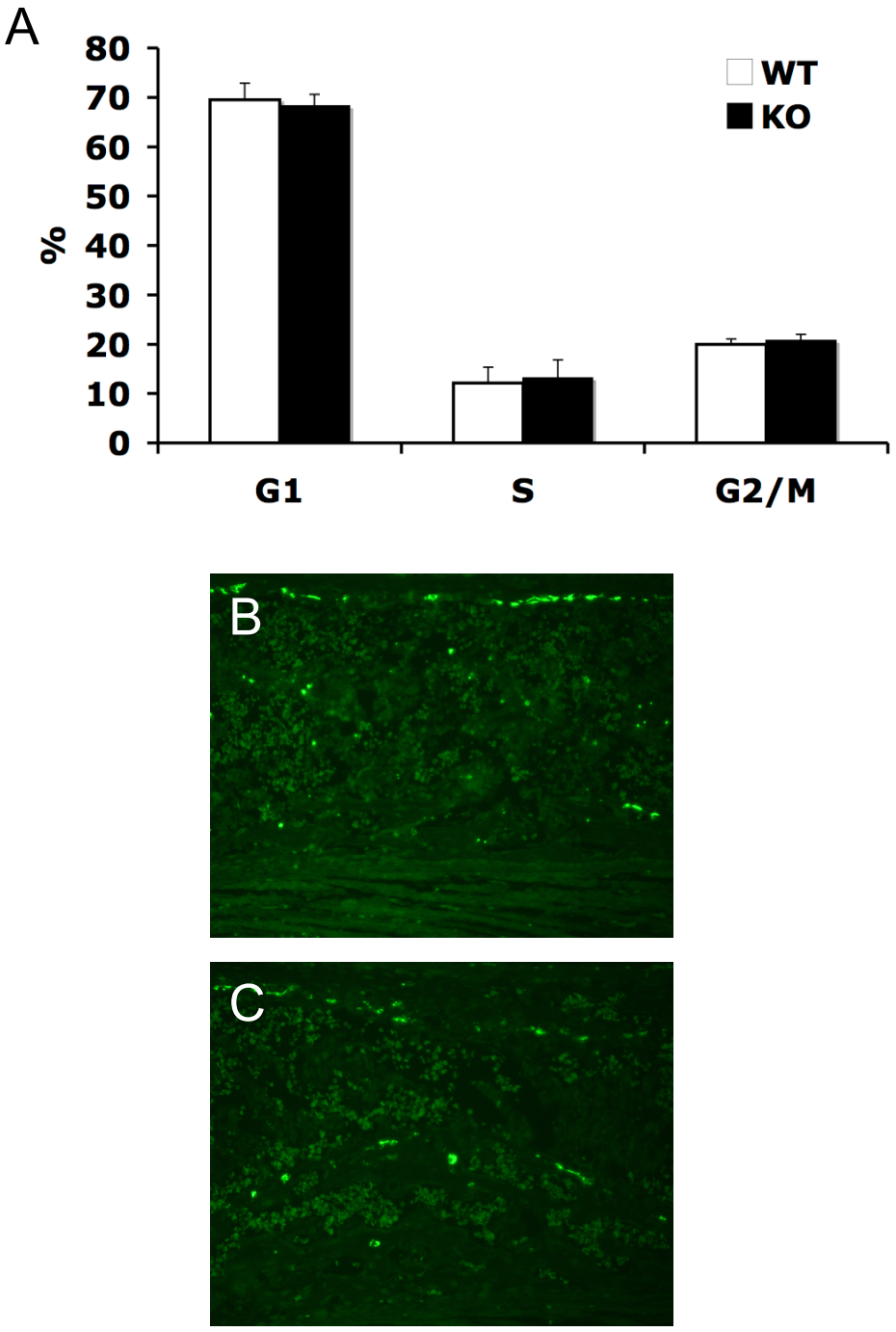
Supplementary Figure 1. $G_s\alpha^{OsxKO}$ mice have fractures at birth and early mortality. (A and B) Skeletal preparations of WT (A) and $G_s\alpha^{OsxKO}$ (KO) (B) mice at P1. (C and D) H&E-stained tibias of WT (C) and KO (D) mice at E15.5. (E and F) Skeletal preparations of WT (E) and KO (F and H) hindlimbs at P1 (E and F, arrow denotes fracture). (G) Survival frequency of early postnatal WT (n = 18) and KO (n = 8) mice. (H-K) Skeletal preparations of WT (H and J) and KO (I and K) ribs at 1 week (H and I) and hindlimbs at E17.5 (J and K). (L) Femur fractures in WT and KO mice from E17.5 to 1 week of age. Original magnification, x40 (C and D).

Supplementary Figure 2



Supplementary figure 2. Deletion of $G_s\alpha$ in vitro results in enhanced osteogenic differentiation. (A) $G_s\alpha$ mRNA levels and (B) basal cyclic AMP (cAMP) levels in $G_s\alpha$(fl/fl) calvarial osteoblasts infected with adenoviral Cre recombinase (black) or control (YFP, white). *P<0.05 (n = 3). (C) Col2.3::GFP and alkaline phosphatase expression distinguishes ALP^-GFP^- cells (a), ALP^+GFP^- osteoprogenitors (b) and GFP(hi) (c) mature osteoblasts. (D) mRNA levels of osteogenic markers osterix (Osx), alkaline phosphatase (ALP), collagen 1α1 (Col1a1) and osteocalcin (OC) in sorted fractions a, b, and c from (C). (E) MTT assay in $G_s\alpha$(fl/fl) calvarial osteoblasts infected with adeno-Cre (black) or control (β-gal, white). (F) Alizarin staining and (G) frequency of Col2.3::GFP(hi) mature osteoblasts with osteogenic differentiation. *P<0.05 (n = 3).

Supplementary Figure 3



Supplementary figure 3. Proliferation and apoptosis are not altered in the osteoblast lineage in $G_s\alpha^{OsxKO}$ mice. (A) Cell cycle distribution of Osx1-GFP::Cre^+ cells isolated from WT (white) and KO (black) mice (n = 7). (B and C) TUNEL staining of WT (B) and KO (C) tibia at E18.5. Original magnification, x200 (B and C).