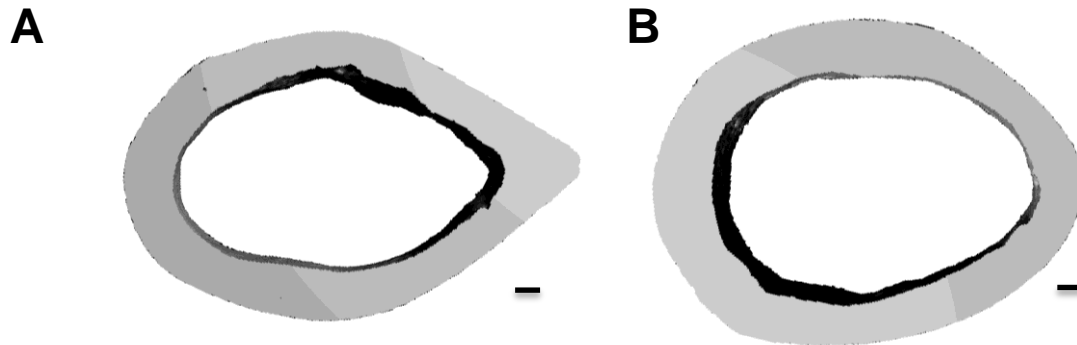


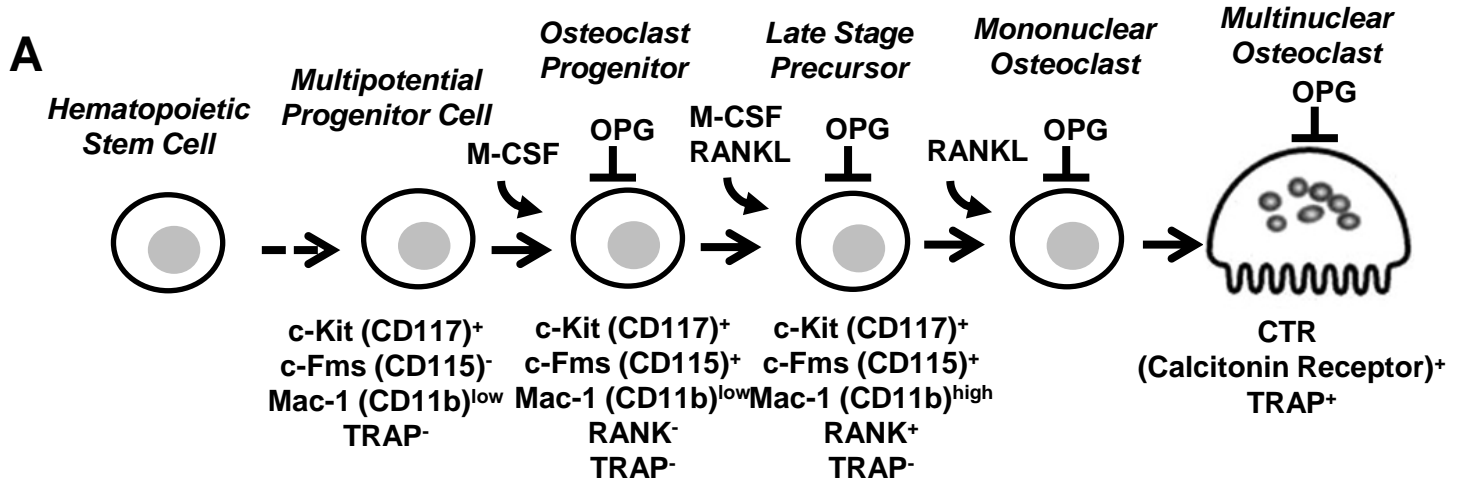
## Supplementary Figure 1.

**Micro-CT parameters for intact cortical bone in *WT* and *Sostdc1*<sup>-/-</sup> mice at 12 weeks of age.** (A) Reconstructed outline of *WT* cortical bone, and (B), *Sostdc1*<sup>-/-</sup> cortical bone. (C) No differences were observed in any parameters except Marrow Area (\* indicates p=0.029). Values shown are mean +/- standard deviation. Bar represents 100μm.



<b>C</b>	<b><i>WT</i></b>	<b><i>Sostdc1</i><sup>-/-</sup></b>
Bone Area (mm <sup>2</sup> )	0.889+/- 0.075	0.929+/-0.143
Marrow Area (mm <sup>2</sup> )	1.12+/-0.115	1.29+/-0.138*
Total Area (mm <sup>2</sup> )	2.013+/-0.160	2.220+/-0.269
Bone Area/Total Area (%)	44.18+/-2.42	41.65+/-2.41
Cortical Thickness (mm)	0.182+/-0.013	0.186+/-0.019
BMD (mg HA/cm <sup>3</sup> )	1221.25+/-25.77	1122.40+/-17.15

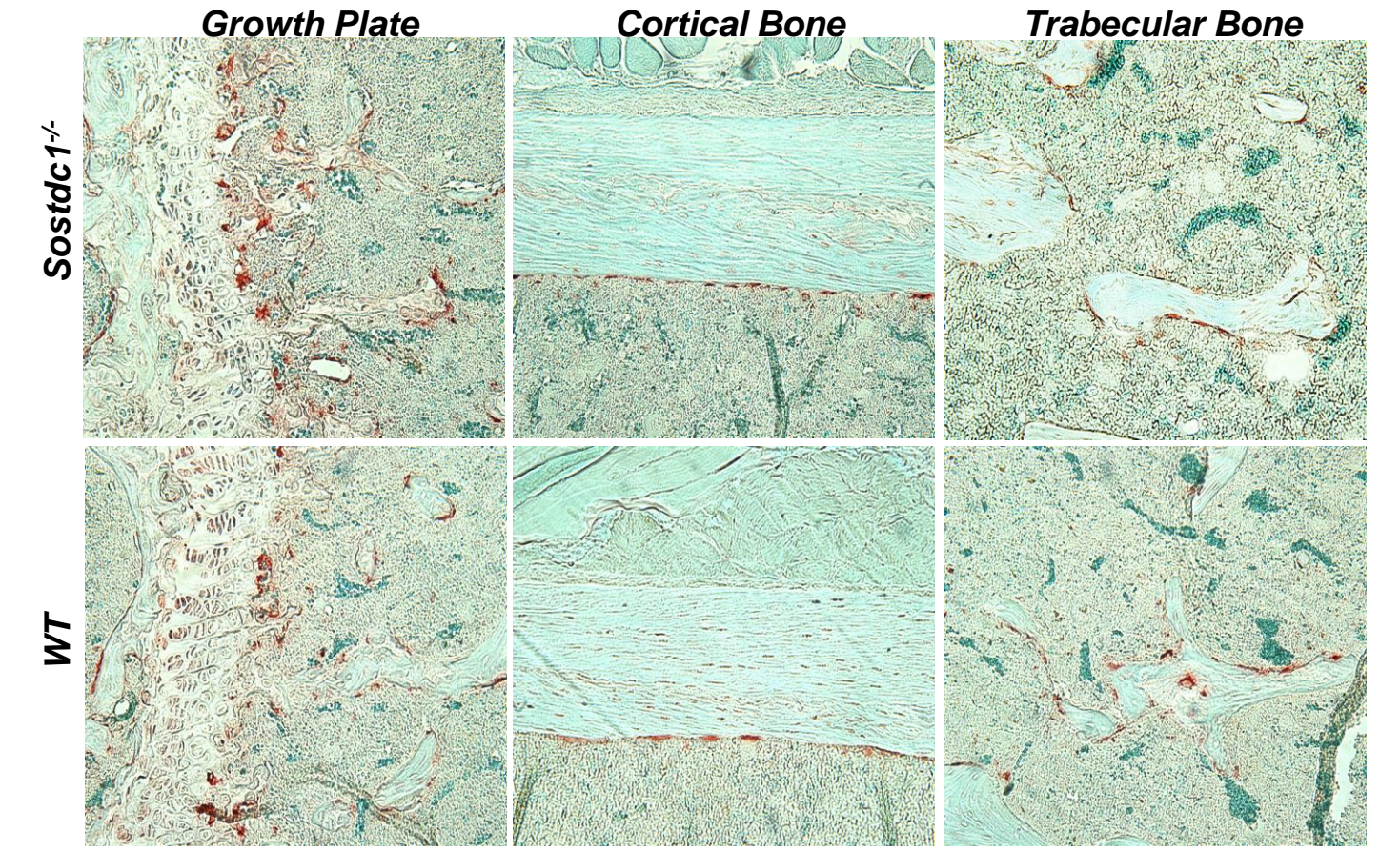
# Supplementary Figure 2.



**B**

	% Osteoclast Precursors in the total bone marrow (OCPs): CD3 <sup>neg</sup> B220 <sup>neg</sup> Ter119 <sup>neg</sup> CD11b <sup>-/lo</sup> Ly66C <sup>high</sup>	% CD115+ CD117- OCPs	% CD115+ CD117+ OCPs
<b><i>Sostdc1</i><sup>-/-</sup></b>	3.18 ± 0.21	5.28 ± 1.29	1.02 ± 0.43
<b><i>WT</i></b>	3.15 ± 0.07	11.15 ± 0.03*	1.42 ± 0.47

**C**



**Supplementary Figure 2. Quantification of osteoclast development in *WT* and *Sostdc1*<sup>-/-</sup> mice.** Osteoclast progenitors and precursors indicating the progenitor pool were scored by flow cytometry using the indicated cell-surface markers (A). Percent of each type of progenitor cell were scored and statistically compared; no parameters were statistically different by genotype ( $p < 0.05$ ) (B). TRAP (Tartrate-Resistant Alkaline Phosphatase, red) and Fast Green counterstain (green) of growth plate/trabecular bone, cortical bone, and trabecular bone are indicated for both *Sostdc1*<sup>-/-</sup> mice and *WT* mice, in which no differences in osteoclast number are indicated (C).

### **Supplementary Material and Methods for Figure 2.**

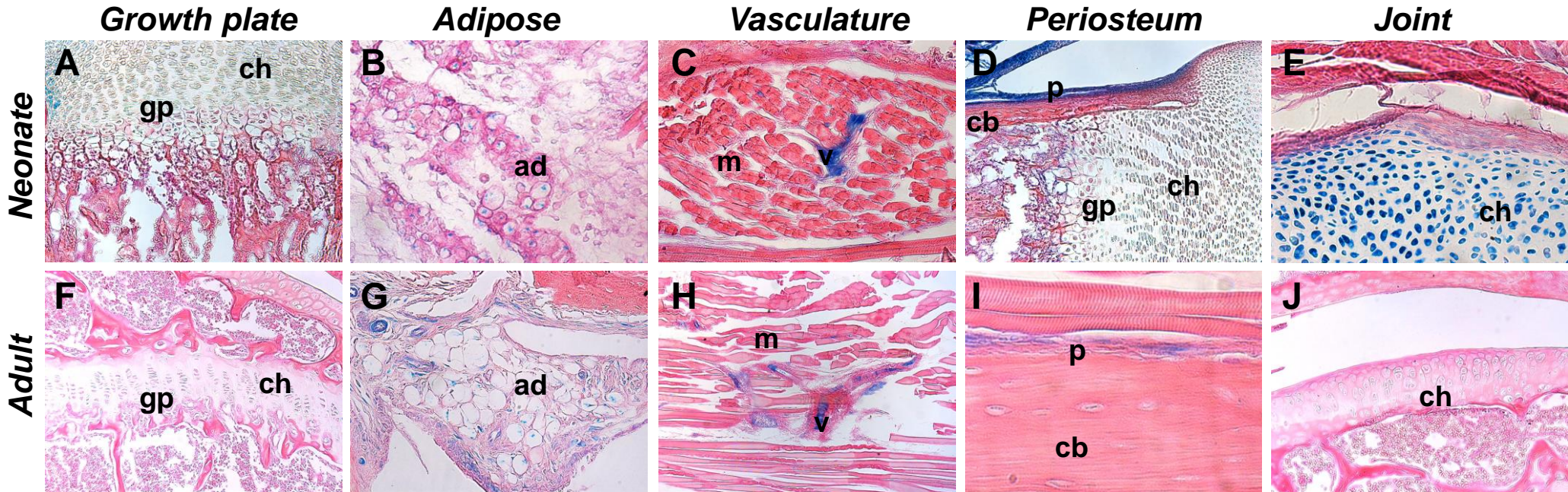
#### ***Quantification of osteoclast progenitors by flow cytometry***

Bone marrow cells were harvested by gentle crushing of *WT* and *Sostdc1*<sup>-/-</sup> femurs and tibiae with a mortar and pestle into Medium 199 (M199) with 2% fetal bovine serum (M199+). Bone marrow cells were washed from the bone chips using a 5 ml pipette and transferred to a 15 ml conical. Cells were pelleted at 1500 rpm at 4C for 5 minutes, then gently resuspended by ratcheting or finger vortexing. Red blood cells (RBCs) were lysed by incubating the cell pellet in 1 ml ACK lysis buffer for 1 minute, and lysis was stopped with addition of M199+, and the cell suspension was centrifuged again. ACK-treated bone marrow cells were then counted using a hemacytometer with Trypan Blue.  $2 \times 10^6$  bone marrow cells were stained with the following antibody cocktail: anti-CD16/32 (clone 93), FITC-conjugated anti-Ly6C (HK1.4), PeCy7-conjugated anti-CD3 (145-2C11), B220 (RA3-6B2), biotinylated anti-Ter119 (TER119), APC-conjugated anti-CD115 (AF598), APCCy7-conjugated anti-CD11b (M1/70), and eFluor450 or Brilliant Violet 421-conjugated anti-CD117 (2B8). Antibodies were purchased from Biolegend or eBioscience. Cells were incubated with the antibody cocktail diluted in phosphate buffered saline (PBS) with 1% FCS for at least 15 minutes on ice, washed by centrifugation (2000 rpm for 3 minutes), and then incubated with streptavidin-PeCy7 for 5 minutes on ice, and washed again. Stained cells were then resuspended in 200 ml of staining buffer and run on the BD LSR II flow cytometric analyzer. To distinguish dead from viable cells during acquisition, propidium iodide solution was added to the cell suspension immediately before data acquisition to a final concentration of 0.5 mg/ml. Osteoclast progenitors (OCPs) were identified as CD3<sup>neg</sup> B220<sup>neg</sup> Ter119<sup>neg</sup> CD11b<sup>-/lo</sup> Ly6C<sup>high</sup>, and OCPs were then further analyzed for expression of CD115, CD135, and CD117, as described by Nakamura et al. 2012 (PMID: 23114597). Flow cytometric data was analyzed using FlowJo software (TreeStar, Ashland, OR).



# Supplementary Figure 3.

Histological characterization of *LacZ* expression [as a surrogate for *Sostdc1* expression] in *Sostdc1*<sup>+/*LacZ*</sup> mice . *Sostdc1* is expressed in neonatal cartilage (A, E), but not in the adult (F, J); adipocytes (B, G), vasculature (C, H) and periosteum (D, I) in both neonate and adults. m muscle; v blood vessel; p periosteum; pa patella; cb cortical bone; hc hypertrophic chondrocytes; bm bone marrow; b newly formed bone; ch chondrocytes.



## Supplementary Figure 4.

**Histological characterization of *LacZ* expression [as a surrogate for *Sostdc1* expression] in *cartilage of Sostdc1<sup>+/-LacZ</sup> mice*** . *Sostdc1* is not expressed in adult growth plate cartilage (A), but is expressed after injury in nearby fibrocartilage associated with the patella (B). During fracture healing, weak expression is seen in chondrocytes in the callus at D7 and D10 (C, D) but disappears shortly after this time point. gp growth plate; bm bone marrow; fc fibrocartilage; ch chondrocytes.

