Experimental Procedures

Animal and genotype-Wild type and *Osx*-null mice are from C57BL genetic background. All mice were bred and maintained in a specific pathogen-free facility. Mice were genotyped using genomic DNA isolated from the tails. PCR genotyping was performed with two sets of primers: Osx5 and Osx3 for the wild-type allele and bpA and Osx3 for the mutant allele, producing 286 bp and 395 bp PCR fragments, respectively as previously described [2].

Baculovirus-mediated expression of Osx- Osx cDNA was subcloned into the pBac vector using a protocol previously described [18]. Recombinant baculovirus was isolated and plaque-purified by standard methods. A 500ml culture of Sf-9 cells (2×10^6 cells/ml) was infected for 48 h with the Osx-expressing recombinant baculovirus. Nuclear extracts were prepared, and recombinant Osx was purified by nickel affinity chromatography.

Chromatin Immunoprecipitation (ChIP) assay-ChIP assays were performed according to previously described protocol [19] with some modifications. Briefly, calvarial cells were isolated from wild-type newborn mice, and were cultured in DMEM supplemented with 10% FBS. Formaldehyde was used to cross link the cells for 10 min, and crosslinking was quenched with glycine. Cells were harvested, rinsed with PBS, and cell pellets were resuspended in 1 ml of lysis buffer. After sonication, 100 µl of sheared chromatin was diluted to 1 ml with IP dilution buffer for each immunoprecipitation. The chromatin solution was pre-cleared with 60 µl of protein G–Agarose beads at 4°C for 1hr. The pre-cleared chromatin was collected and incubated at 4°C overnight with 5 µg of anti-Osx antibody or Rabbit IgG (Sigma) as a negative control. The immune complexes were precipitated with 60 µl of protein G–Agarose beads at 4°C for 1 hr. After washes, the antibody-protein-DNA immunocomplexes were eluted twice with 100 µl of

elution buffer. Formaldehyde cross-linking was reversed by heating at 65°C overnight with the addition of 5 M NaCl. All the samples were digested with RNase A and proteinase K. The DNA was purified using spin columns, and analyzed by real time PCR. The primer sets used for amplification of Sost promoter regions were obtained from IDT, and the sequences were as follows; Primer set 1: Sost-1: 5'-TAC TGG GAG AGC TGG CTG TGT-3' and Sost-2: 5'-GTT TCC TCA CCC TCC TCC TCA-3'; Primer set 2: Sost-D-1: 5'-TAT CCA GGT GTG GTG GTG TG-3' and Sost-D-2: 5'-TCA GCG AGG CCG CTC ACT ATA-3'. Data were normalized by GAPDH.