# Supporting Information

## Zhang *et al.* 10.1073/pnas.0710831105

### SI Text

**Immunoprecipitation and Western Blot Analysis.** The cells were washed with PBS and 1 ml of ice-cold lysis buffer [50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 1 mM EGTA, 2 mM $Na_3VO_4$, 15 $\mu$g/ml aprotinin, 10 $\mu$g/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride] was added. The cells were lysed for 30 min at 4°C with occasional vortexing. The lysates were collected into 1.5-ml tubes and cleared of nuclei by centrifugation for 10 min at 15,000 $\times$ *g*. The supernatants were incubated with different antibodies for 16 h at 4°C, and protein A-agarose beads were added for the last hour. The beads were washed five times in TNEN buffer [20 mM Tris·HCl (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 10 mM $Na_3VO_4$, 1 mM phenylmethylsulfonyl fluoride, and 1 mM NaF]. Bound proteins were extracted with SDS/PAGE sample buffer, analyzed by SDS/PAGE, and subject to Western blot analysis with an ECL system used for protein detection.

***In Vitro* Protein Interaction Assay.** [$^{35}$S]Methionine-labeled protein was synthesized in a TNT T7 coupled *in vitro* transcription-translation system, using rabbit reticulocyte lysate (Promega) as described in ref. 1. Next, 10 $\mu$g of GST or GST-$\beta$-catenin was bound to 50% glutathione-agarose beads (Sigma) and equilibrated with 2$\times$ binding buffer GBB [20 mM Tris (pH 7.6), 50 mM NaCl, 1 mM DTT, 0.2% Nonidet P-40, and protease inhibitors 0.2 mM phenylmethylsulfonyl fluoride, 4.0 $\mu$g/ml aprotinin, 2.0 $\mu$g/ml leupeptin, and 1 $\mu$g/ml pepstatin A]. Equivalent volumes of [$^{35}$S]methionine-labeled proteins were incubated with the immobilized GST-$\beta$-catenin in 100 $\mu$l of 2$\times$ GBB for 2 h at 4°C with gentle rocking. The beads were washed twice with 0.3 ml of 1$\times$ GBB and once with 0.3 ml of 50 mM Tris·HCl buffer (pH 8.0). Bound proteins were eluted with 35 $\mu$l of 20 mM reduced glutathione in 50 mM Tris buffer. Eluted proteins were resolved by SDS/PAGE and visualized by autoradiography.

**Electrophoretic Mobility Shift Assay (EMSA).** DNA-binding reactions and EMSAs were performed as described in ref. 2. For Tcf1 studies, Osx, Osx mutants, and Tcf1 proteins were synthesized by TNT IVTT systems, and protein products were confirmed by Western blot analysis (data not shown). For Dkk1 studies, baculovirus-expressed Osx was used as Osx protein source.


1. Zhang C, *et al.* (2001) Ternary complexes and cooperative interplay between NCoA-62/Ski-interacting protein and steroid receptor coactivators in vitamin D receptor-mediated transcription. *J Biol Chem* 276:40614–40620.
2. Oosterwegel M, *et al.* (1991) Cloning of murine TCF-1, a T cell-specific transcription factor interacting with functional motifs in the CD3-epsilon and T cell receptor alpha enhancers. *J Exp Med* 173:1133–1142.

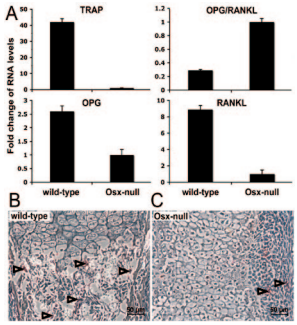


**Fig. S1.** Effect of Osx on OPG/RANKL and TRAP expression. (*A*) Fold change in RNA levels of specific RNAs from E18.5 Osx wild-type and *Osx*-null calvaria. RNA levels were measured by quantitative RT-PCR. Levels of each RNA from *Osx*-null calvaria were normalized to a value of 1. (*B*) TRAP staining of the humerus of E18.5 Osx wild-type embryo. (*C*) TRAP staining of the humerus of E18.5 Osx-null embryo.

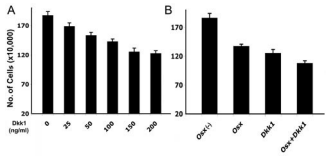


**Fig. S2.** Effect of Osx and Dkk1 on cell growth. C2C12 mesenchymal cells, in which Osx expression can be induced by removal of Tetracycline, were used. (*A*) Day 3 cell count of C2C12 cells to which increasing amount of Dkk1 were added. (*B*) Day 3 cell count of C2C12 cells in which either Osx expression was induced, or to which saturating amount of recombinant Dkk1 (150 ng/ml) were added, or C2C12 cells both undergoing induction of Osx and receiving saturating amount of Dkk1.