

FIGURE LEGENDS

Figure S1. Smad6 inhibits Tbx6-dependent transcriptional activity in a dose-dependent manner (A) Tbx6 mediates the activation of *Myf-5* in a dose-dependent manner. 50 ng *Myf-5* promoter reporter was co-transfected with increasing doses of Tbx6 construct (0, 25, 50, 100, 200, 300 and 400 ng) into TT-D6 cells. 2.5 ng pRL-SV40 plasmid expressing Renilla luciferase was used in each well as internal control. (B) Similar studies were carried out using the 4×Tbx-TK reporter construct. (C) Smad6 inhibits Tbx6-dependent transcriptional activity in a dose-dependent manner. *, $p < 0.05$; **, $p < 0.01$, using Student's *t*-test.

Figure S2. Smad6 induces Tbx6 proteasomal degradation. (A) Proteasome inhibitor enhances the interaction between Smad6 and Tbx6. TT-D6 cells were treated with 20 μ M MG132 or DMSO for 4 h before harvest. Cell extracts were subjected to immunoprecipitation with anti-Smad6 polyclonal antibody. The immunocomplexes were analyzed using anti-Tbx6 antibody. (B) Smurf1 promotes Smad6-induced Tbx6 degradation. 293T cells were transfected with expression constructs as indicated. Protein levels of Smad6, Smurf1 and Tbx6 were measured by WB, respectively.

Figure S3. Knockdown of Tbx6 diminishes Tbx6-mediated transcriptional activities. TT-D6 cells were transfected with control or Tbx6 siRNA and assayed by WB using anti-Tbx6 antibody (A) or subjected to RT-PCR analysis for the expression of Tbx6 target genes (B). **, $p < 0.01$.

Figure S4. Knockdown of endogenous Smurf2 has no obvious effect on Tbx6 protein expression and transcriptional activity. (A) TT-D6 cells were transfected with control or Smurf2 siRNA, and then assayed by WB using anti-Smurf2 and anti-Tbx6 antibodies. (B) 4×Tbx-TK reporter construct was co-transfected with Smurf1 and/or Smurf2 siRNA. n.s., not significant ($p > 0.05$).

Figure S5. The effects of BMP-2 on the osteoblastic differentiation of TT-D6 cells. Cells were cultured in the absence or presence of 500 ng/ml rhBMP-2 for 72 h. (A) ALP activity was determined by histochemical staining as visualized. (B) TT-D6 cells were stained with Alizarin Red S to detect calcified nodule formation. The bar represents 200 μ m for each section.

Figure S1

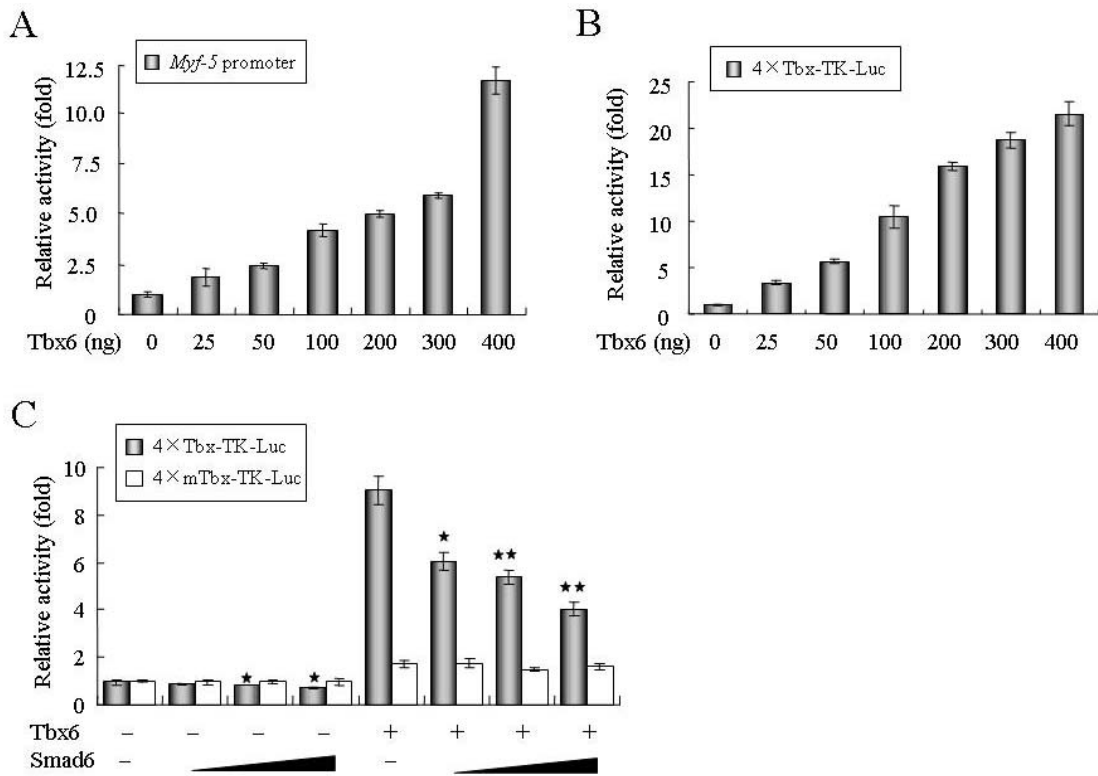


Figure S2

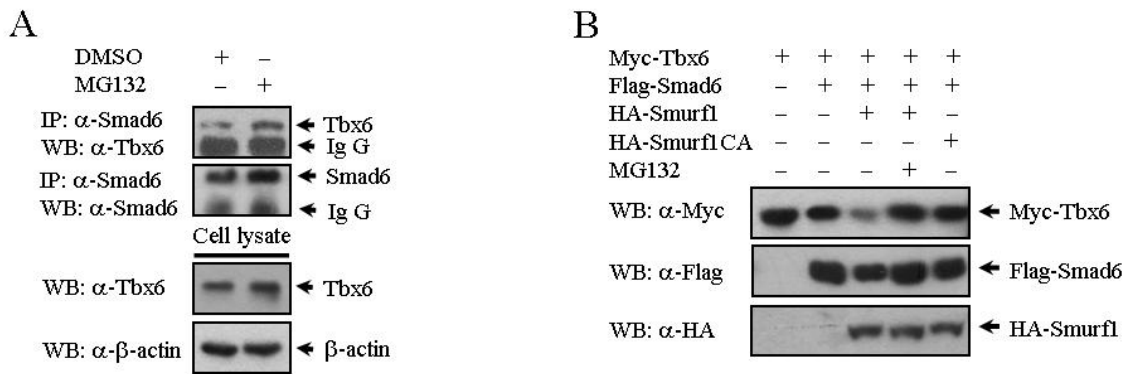


Figure S3

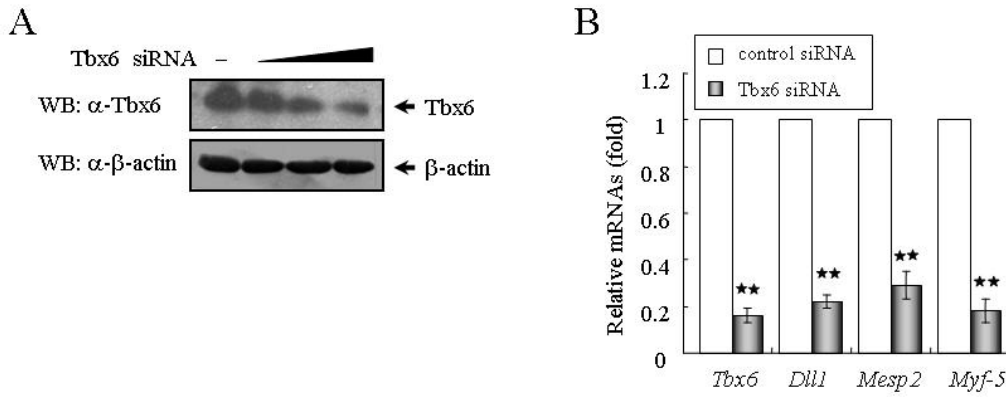


Figure S4

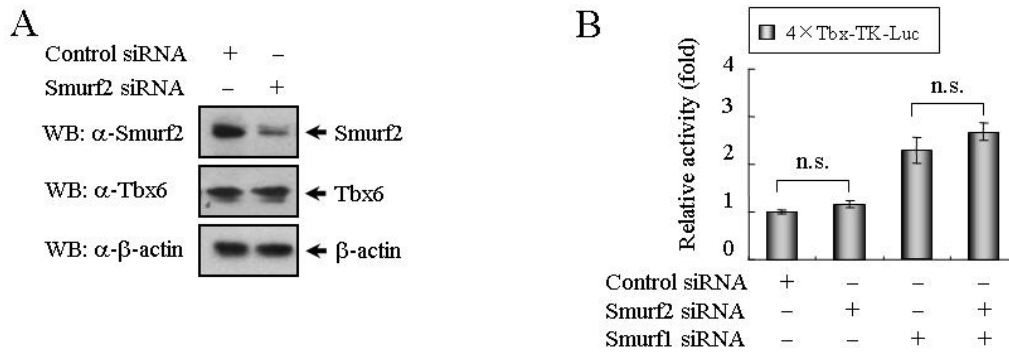


Figure S5

