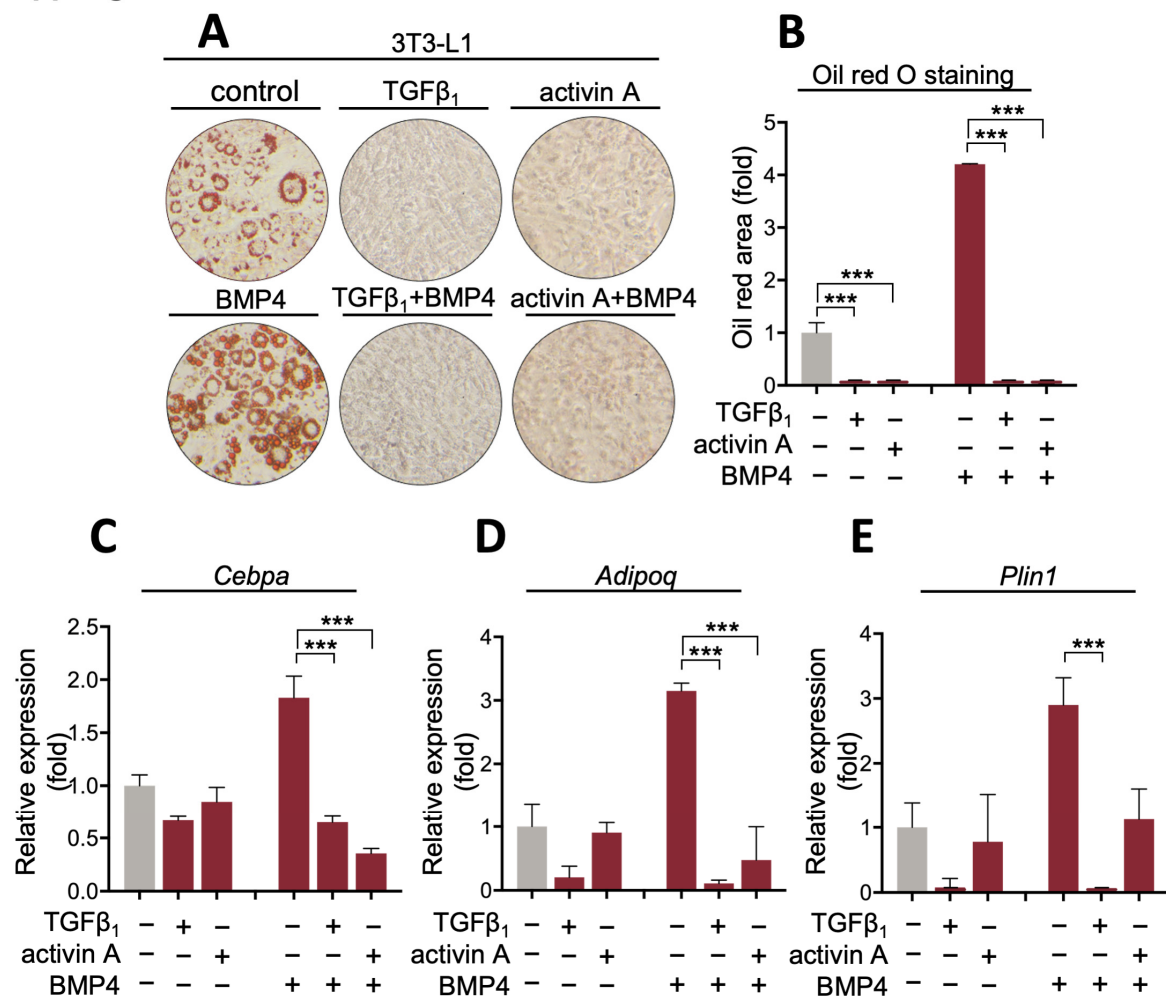


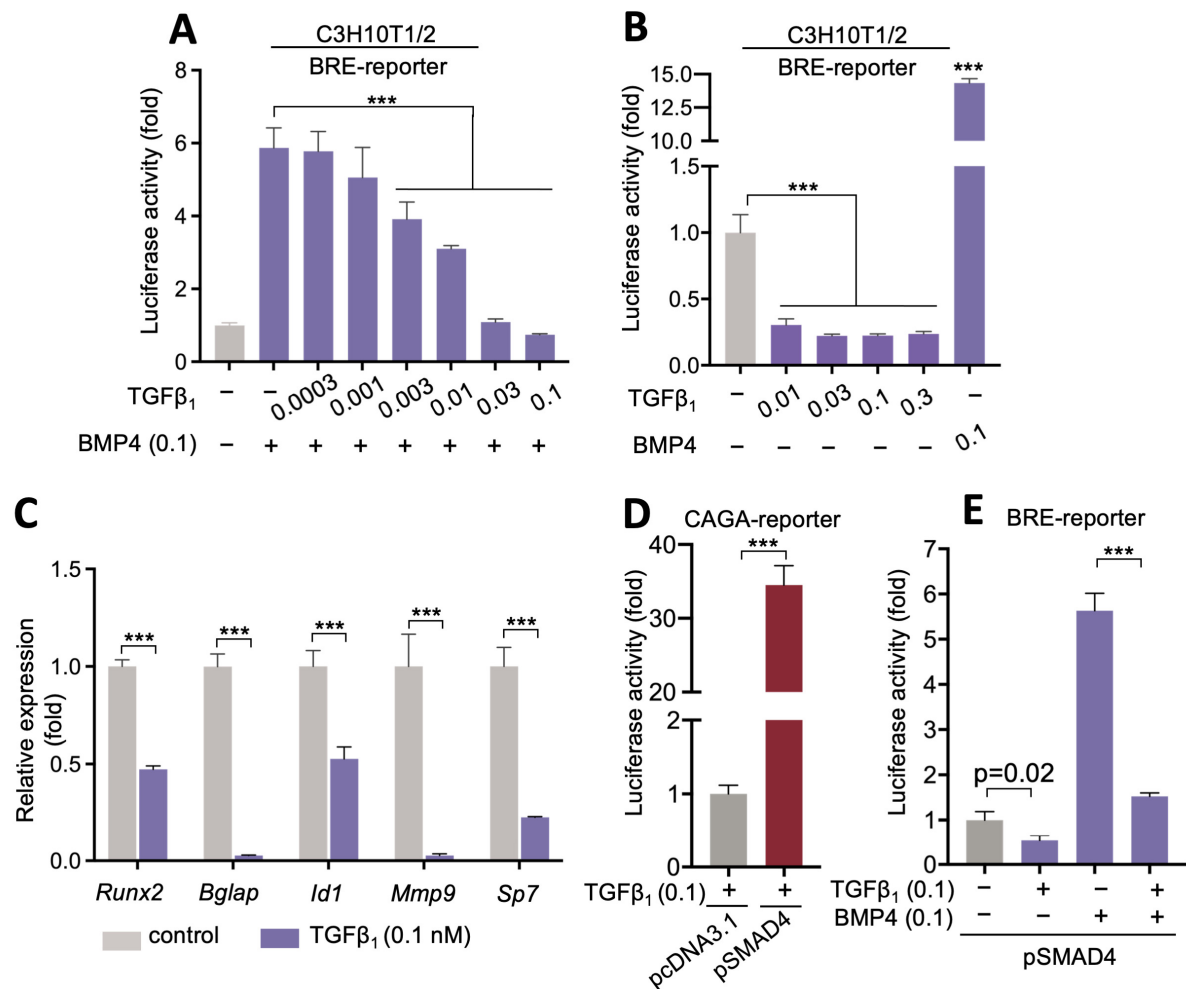
Supp Fig.1



Supplementary Fig. 1. Both TGF-β1 and activin A inhibit adipogenesis of 3T3-L1 cells.

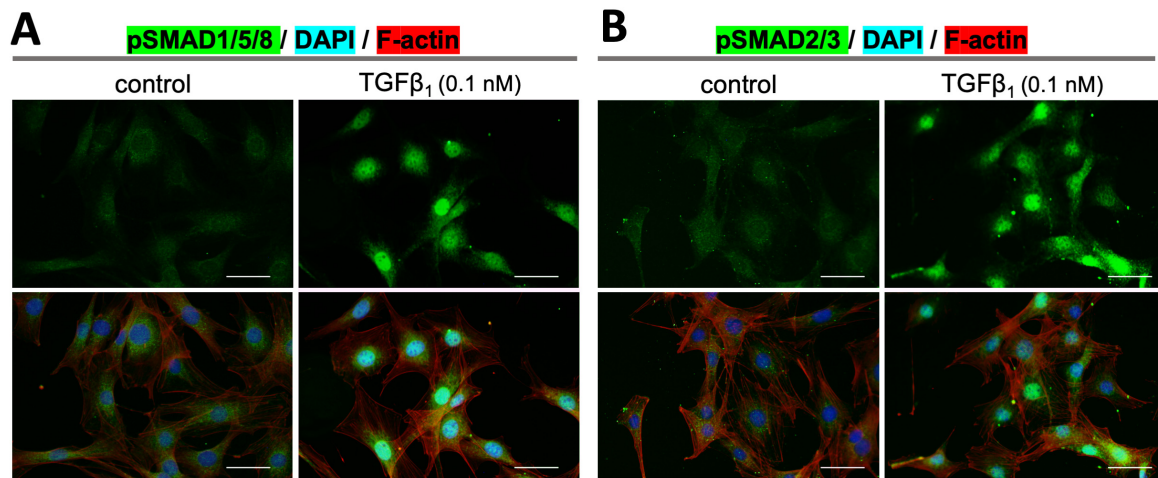
(A and B) Oil red O staining (A) and stained area quantification (B) of the lipid droplets in 3T3-L1 cells primed with TGF-β1 (1 nM), activin A (1 nM) and/or BMP4 (1 nM) during adipogenic induction. (C-E) Real-time PCR quantification of *Cebpa* (C), *Adipoq* (D) and *Plin1* (E) transcripts in differentiated cells. Data are means ± SD. ****P* < 0.001 compared with indicated control, one-way ANOVA followed by multiple comparison test. n=3 independent experiments.

Supp Fig.2



Supplementary Fig. 2. TGF-β1 suppresses BMP-induced transcriptional response in MSCs. (A and B) BRE-driven luciferase reporter activity in C3H10T1/2 cells treated with TGF-β1 in combination with BMP4 (A), or individual ligands alone (B). Results are shown as means ± SD. *** $P < 0.001$ compared with BMP4-treated (A) or non-treated sample (B), one-way ANOVA followed by multiple comparison test. $n=3$ independent experiments. (C) Real-time PCR quantification of the BMP downstream gene transcripts in C3H10T1/2 cells without or with TGF-β1 treatment for 24 hours. (D and E) C3H10T1/2 cells without or with *SMAD4* overexpression were subjected to CAGA-driven (D) or BRE-driven (E) reporter activity assays with indicated ligand treatments. Data are means ± SD. *** $P < 0.001$ compared to the indicated group, students' t test. $n = 3$ independent experiments.

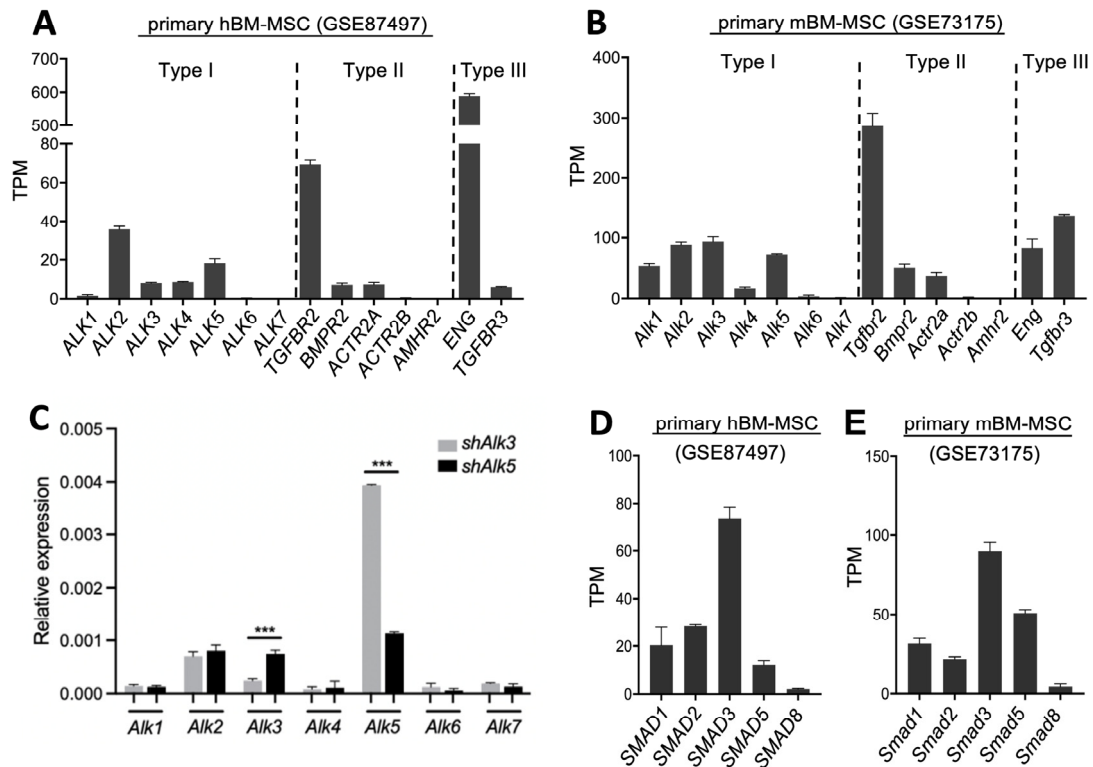
Supp Fig.3



Supplementary Fig. 3. Nuclear translocation of phosphorylated SMADs induced by TGF-β1 in C3H10T1/2 cells.

ICC staining was performed to visualize pSMAD1/5/8 (**A**) or pSMAD2/3 (**B**) in C3H10T1/2 cells without or with TGF-β1 treatment. The merged images (lower panel) further comprised F-actin (red) and DAPI (blue) staining. Scale bars represent 50 μm. n = 3 independent experiments.

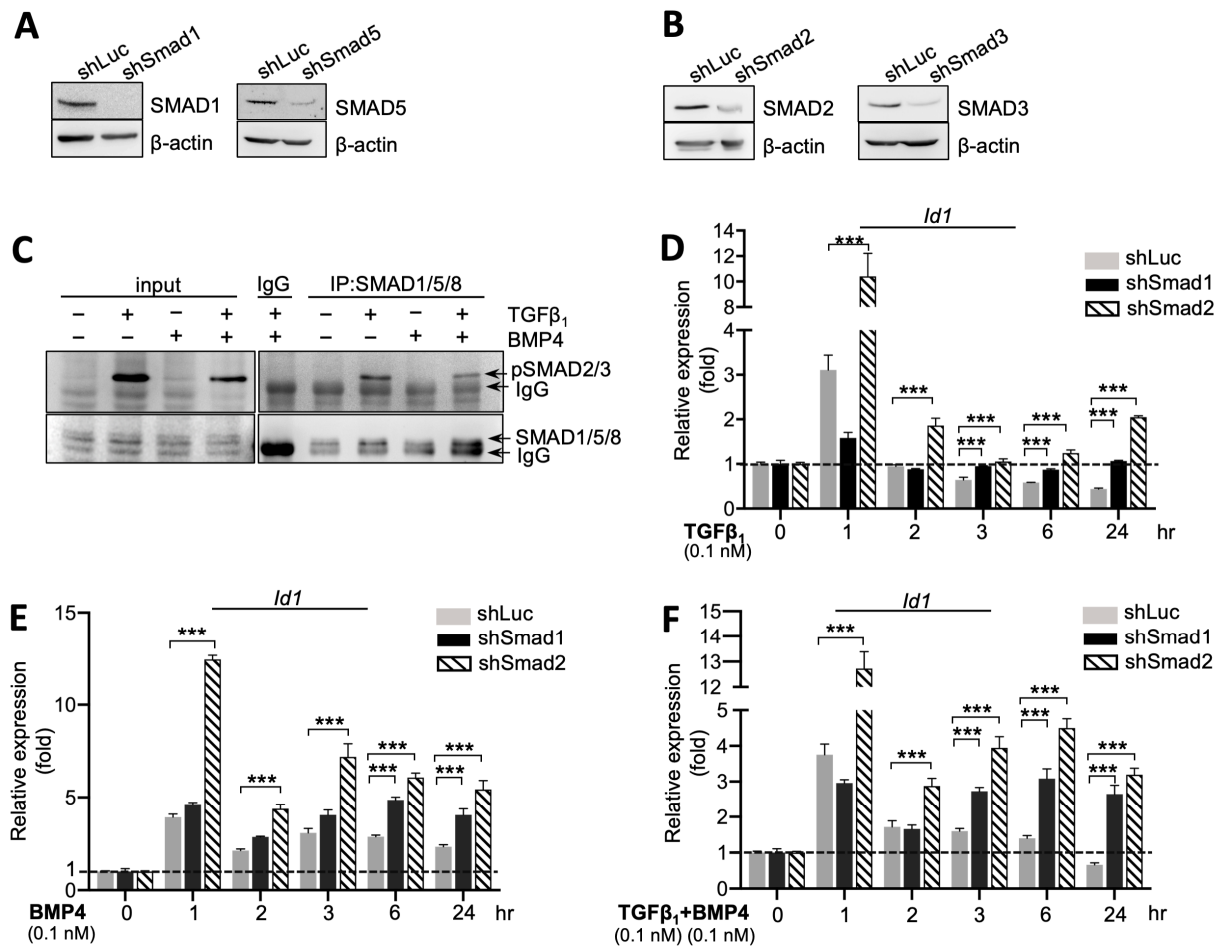
Supp Fig.4



Supplementary Fig. 4. Transcriptomic landscape of the genes related to TGF- β signaling receptors and R-SMADs in BM-MSCs.

GSE87497, a transcriptomic dataset from human normal BM-MSCs (n=3), and GSE73175, a transcriptomic dataset from 3 to 4-week-old C57BL/6 mice BM-MSCs (n=3) were used for data extraction. **(A and B)** The transcript levels of TGF- β signaling receptor genes were compared. Data are expressed as transcripts per million (TPM). **(C)** The transcript levels of the type-I receptor genes were quantified and compared in C3H10T1/2 cells with knockdown of *Alk3* or *Alk5*. Data are normalized with *Actb* and expressed as means $\pm$ SD. *** $P < 0.001$. **(D and E)** R-SMAD genes in the above transcriptomic datasets were compared.

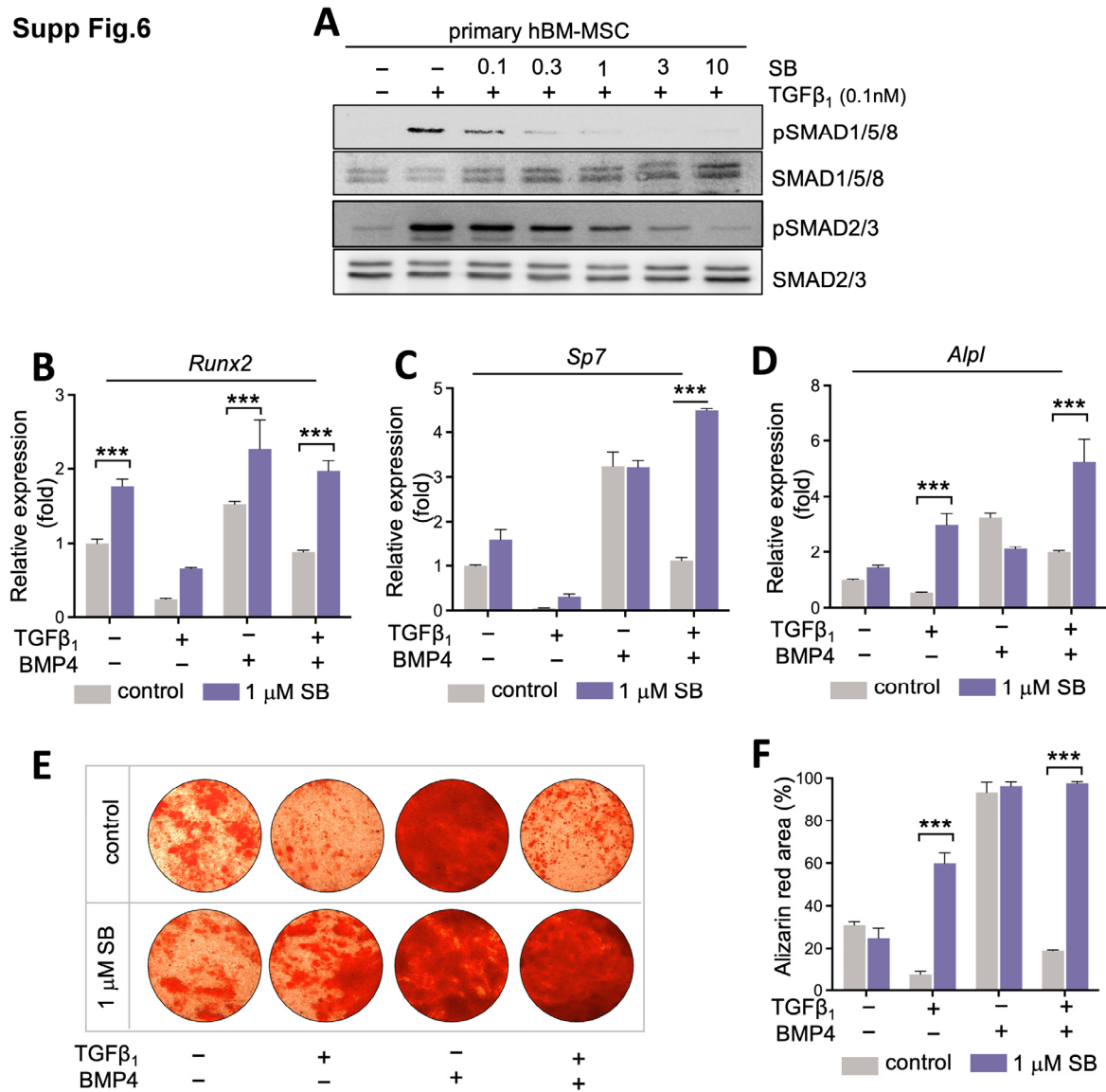
Supp Fig.5



Supplementary Fig. 5. The forming mixed SMAD1-SMAD2 complex mediates the TGF-β1 suppression of *Id1* expression.

(A and B) Knockdown efficiency of *Smad1* or *Smad5* (A), and *Smad2* or *Smad3* (B) was assessed by Western blotting in C3H10T1/2 cells infected with shRNA targeting to corresponding genes. (C) Detection of pSMAD2/3 that was co-immunoprecipitated by SMAD1/5/8 antibody in the lysates of C3H10T1/2 cells treated with TGF-β1 (0.1 nM) and/or BMP4 (0.1 nM) for 30 minutes. (D-F) C3H10T1/2 cells with knockdown of *Luc* control, *Smad1* or *Smad2* were treated with TGF-β1 (D), BMP4 (E), or both ligands (F) for different intervals. *Id1* transcripts were quantified by real-time PCR. Data are normalized with *Actb* and expressed as means ± SD. ****P* < 0.001 compared to the shLuc-infected control within each time point. Since no consideration for differences across time intervals, one-way ANOVA followed by a multiple comparison test was used. n = 3 independent experiments.

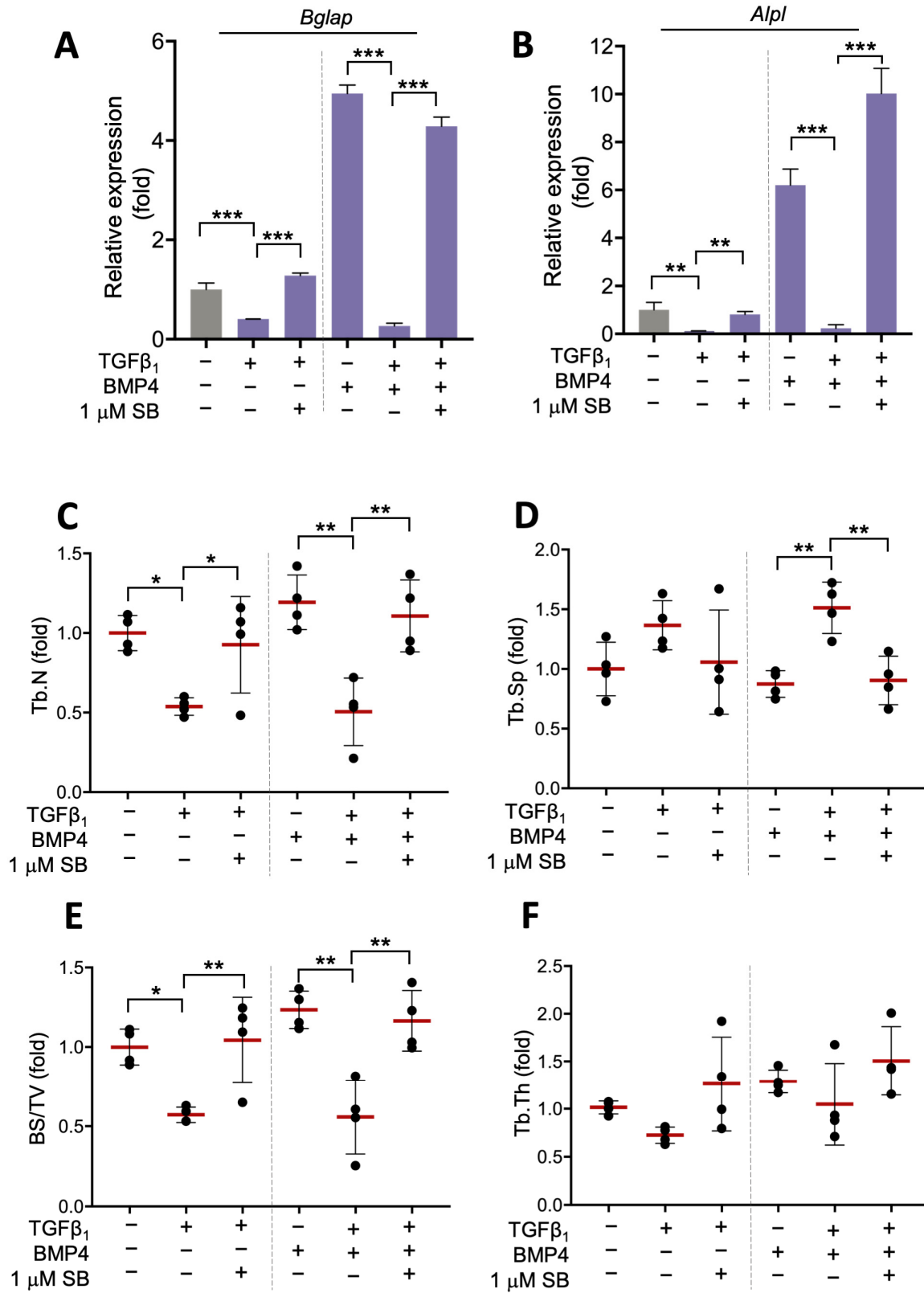
Supp Fig.6

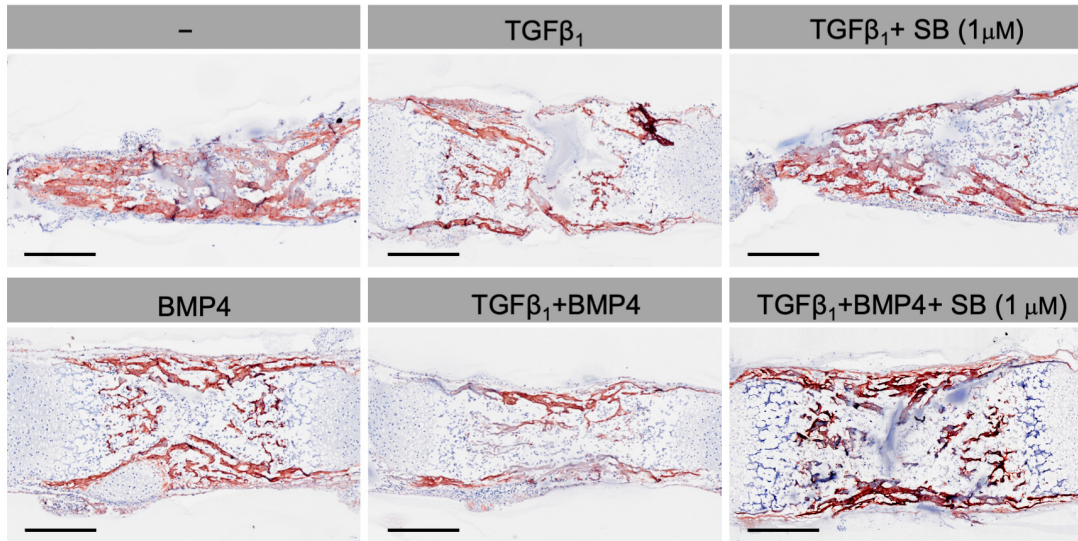


Supplementary Fig. 6. Blockage of the TGF-β1-induced SMAD1/5/8 signaling reverses the TGF-β1 suppression of osteogenesis in primary MSCs.

(A) Western blots to evaluate the dose effects of SB on TGF-β1-induced pSMAD1/5/8 and pSMAD2/3 in primary hBM-MSCs. Blots are representative of three independent experiments. (B-F) *In vitro* osteogenesis assay. Primary mBM-MSCs were primed without or with low-dose SB followed by treatment with TGF-β1 (1 nM) and/or BMP4 (1 nM) under osteogenic condition. The transcripts of *Runx2* (B), *Sp7* (C) and *Alpl* (D) were quantified at day 7. Alizarin Red S staining (E) was performed and the stained area (F) was quantified at day 14. Data are means ± SD. ****P* < 0.001 compared to the indicated control, two-way ANOVA followed by multiple comparison test. n = 3 independent experiments.

Supp Fig.7

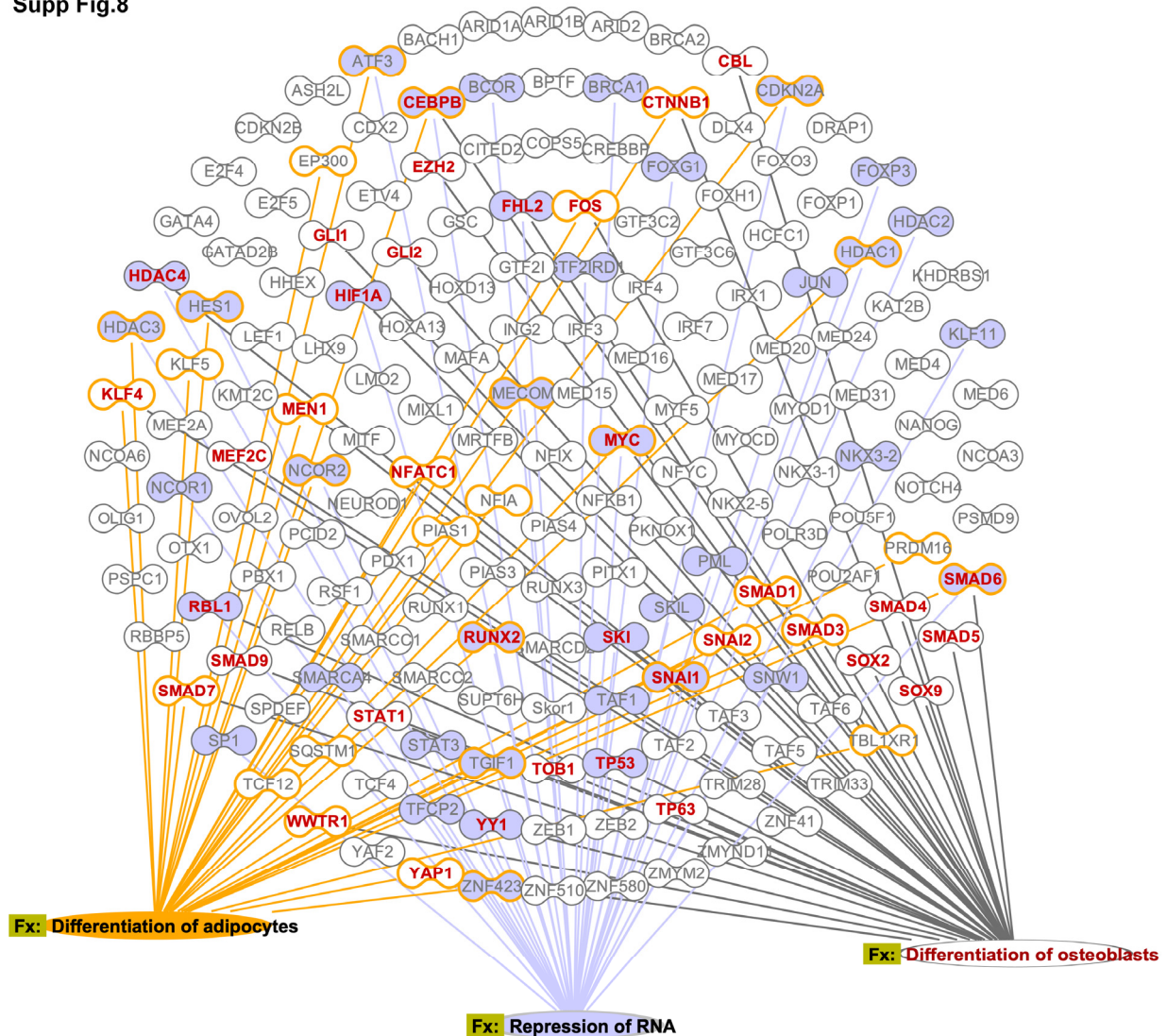


G

Supplementary Fig. 7. Blockage of the TGF- β 1-induced SMAD1/5/8 signaling reverses the TGF- β 1 suppression of osteogenesis in cultured bones.

(A and B) The foot bones from E16.5 mice were cultured in osteogenic medium supplemented with TGF- β 1 (1 nM), BMP4 (1 nM) and/or SB (1 μ M) as indicated for 9 days. The transcript levels of *Bglap* (A) and *Alpl* (B) in samples were then quantified by real-time PCR. **(C-F)** The cultured tibiae at day 13 were subjected to μ CT analysis to obtain the bone parameters: Tb.N: trabecular number (C), Tb.Sp: trabecular separation (D), BS/TV: bone surface /total volume (E), Tb.Th: trabecular thickness (F). Data are means $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001, one-way ANOVA followed by multiple comparison test. n=4 mice in each group. **(G)** Representative images of immunohistochemical staining of COL1A1 protein in the cultured tibiae at day 13. n = 3 independent experiments. Scale bars, 500 μ m.

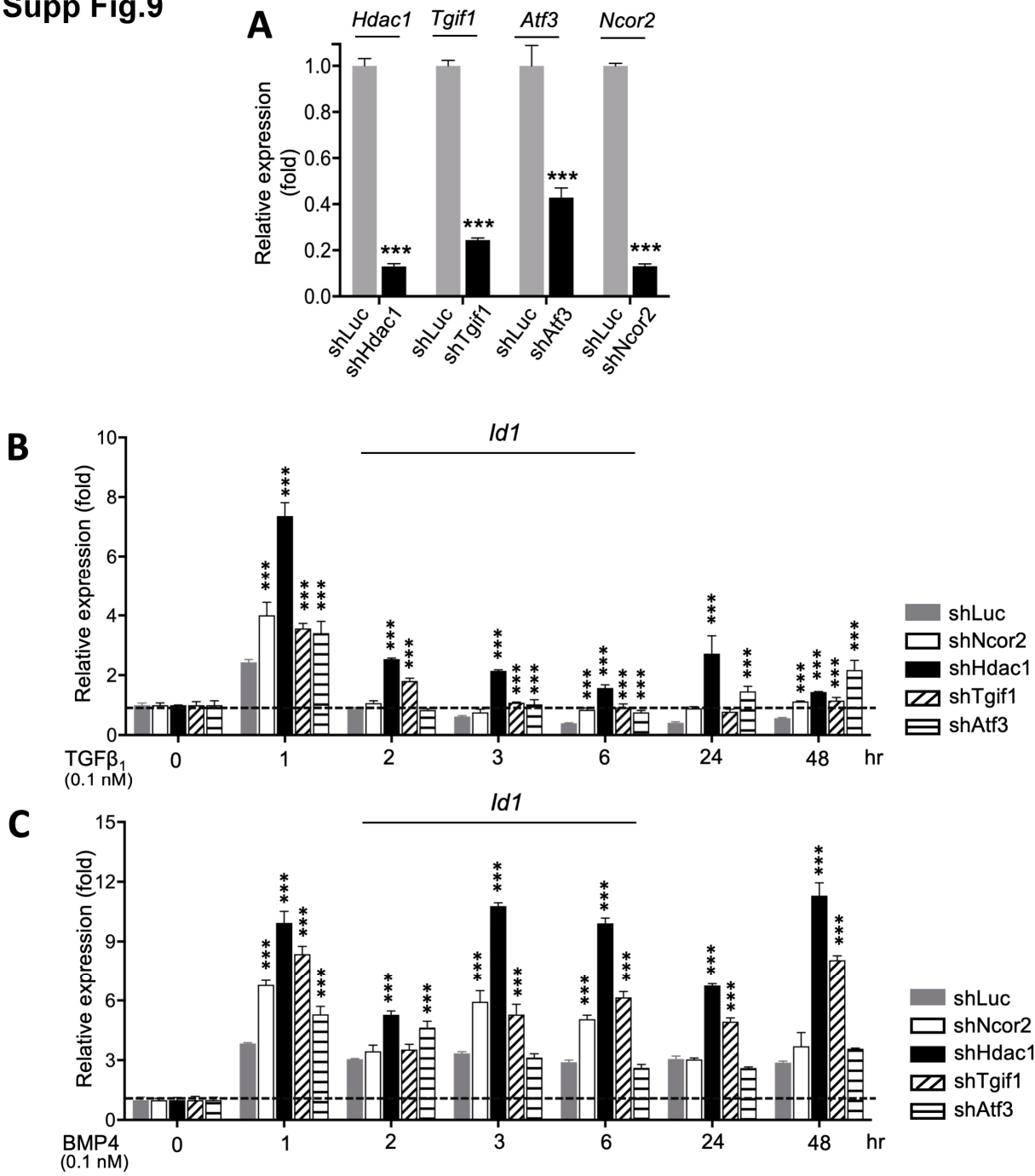
Supp Fig.8

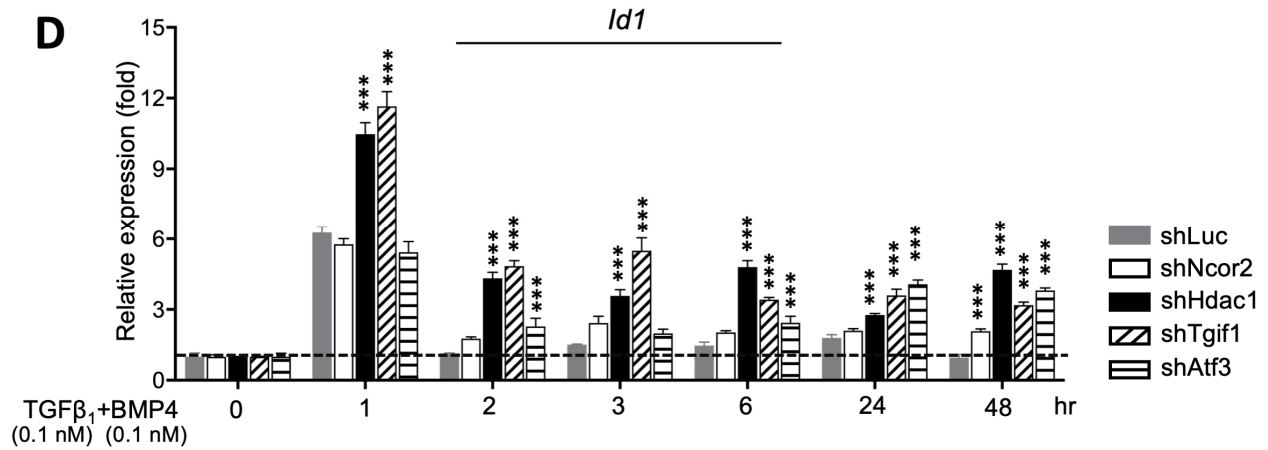


Supplementary Fig. 8. Functional correlation of SMAD2-interacting transcriptional regulators in the IPA database.

179 SMAD2-interacting transcriptional regulators were subjected to functional correlation prediction with the filters of [Repression of RNA], [Differentiation of osteogenesis] and [Differentiation of adipogenesis] in IPA analysis. Factors overlaid with [Repression of RNA], considered as co-repressors, were highlighted in purple. Factors associated with [Differentiation of osteogenesis] were written in red, while those associated with [Differentiation of adipogenesis] were framed in orange.

Supp Fig.9

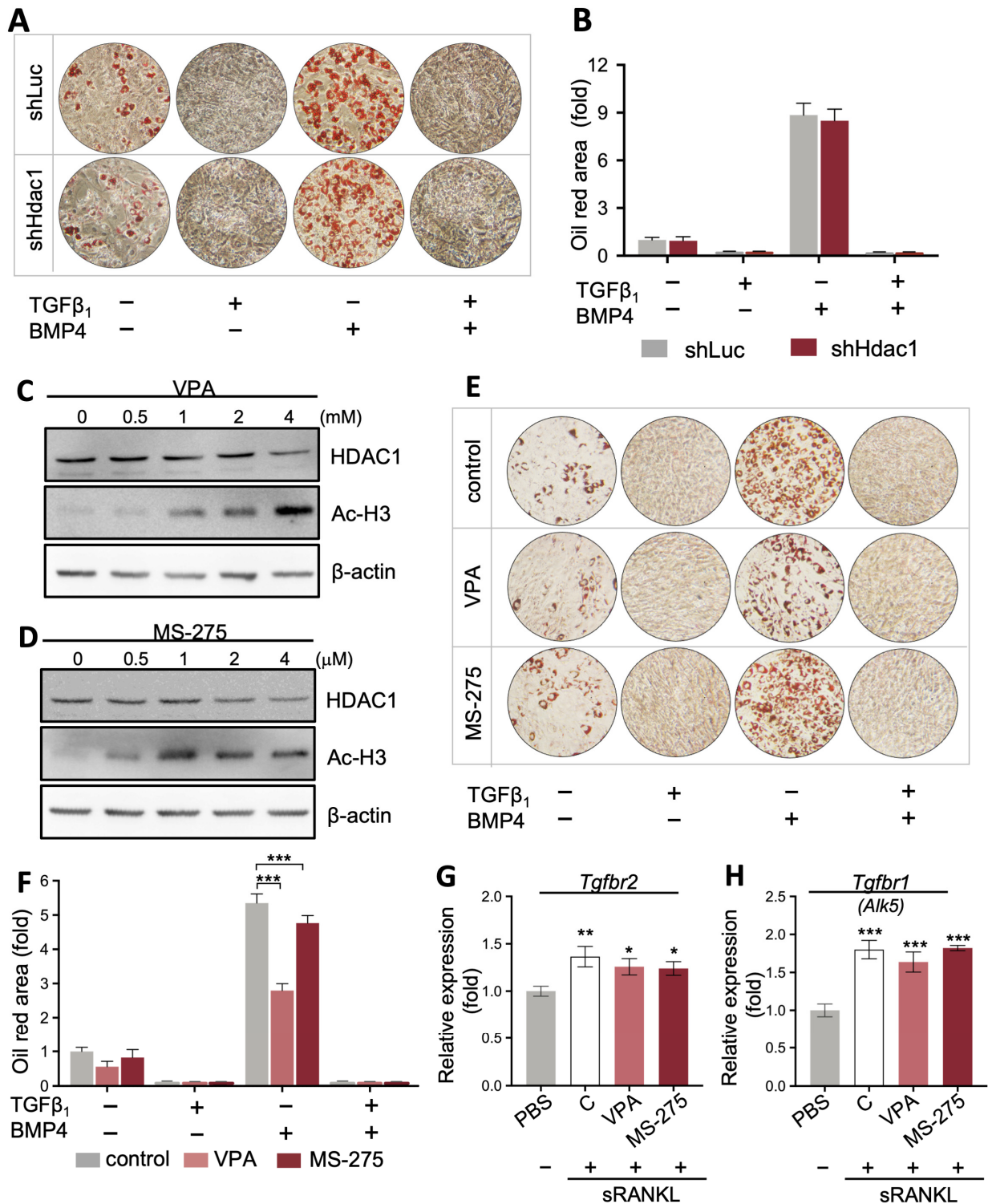




Supplementary Fig. 9. Knockdown of *Hdac1*, *Tgif1* or *Atf3* dampens the TGF-β1 suppression of *Id1* gene expression.

(A) Knockdown efficiency of indicated co-repressor genes in C3H10T1/2 cells was evaluated by real-time qPCR. Data are means $\pm$ SD. *** $P < 0.001$ compared to the indicated sample, Student's t test. (B-D) C3H10T1/2 cells with knockdown of indicated gene were treated with TGF-β1 (B), BMP4 (C) or both ligands (D) for different intervals. *Id1* transcripts were then quantified by real-time qPCR. Data are means $\pm$ SD. *** $P < 0.001$ compared to the shLuc control within a group, one-way ANOVA followed by multiple comparison test.

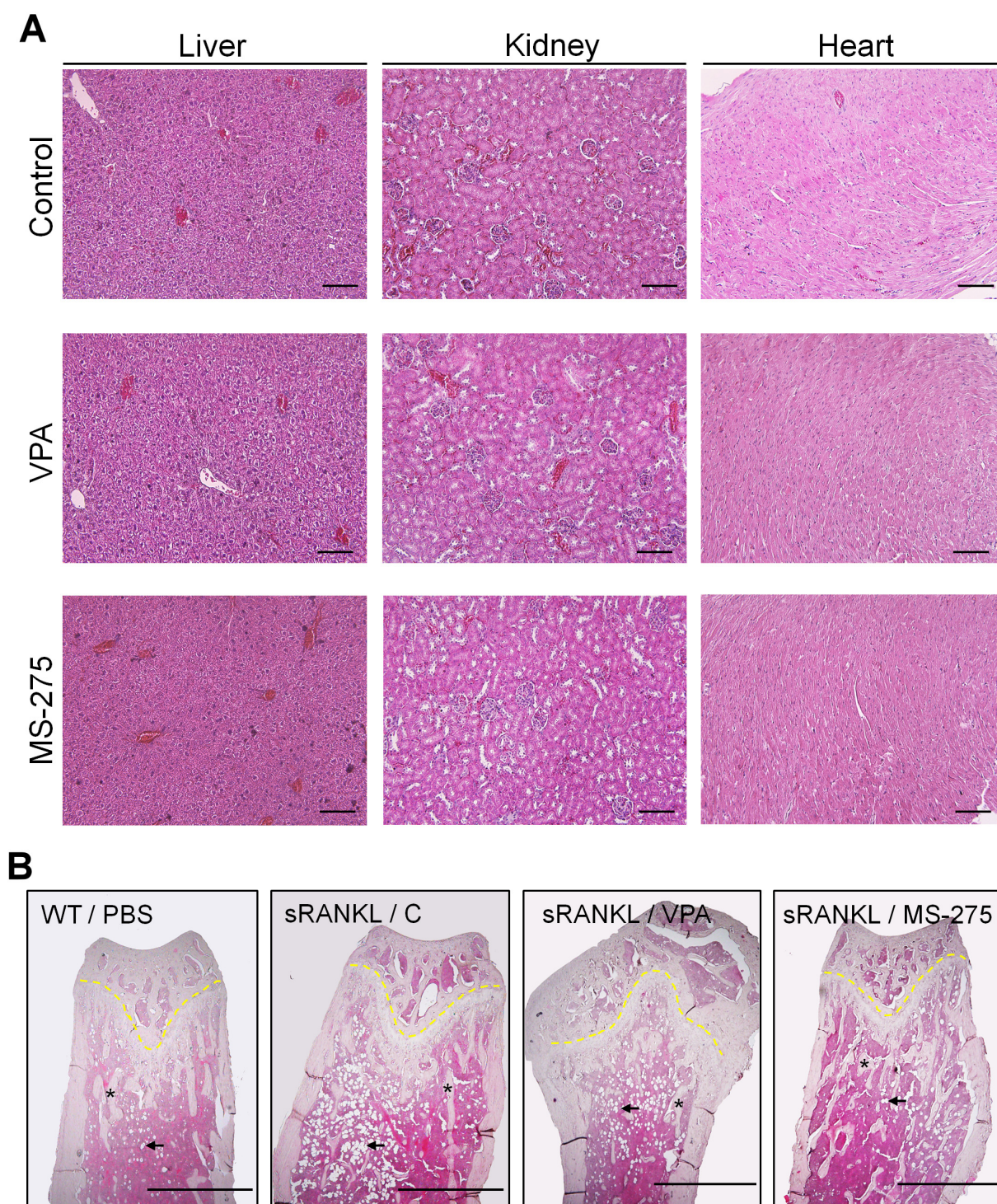
Supp Fig.10



Supplementary Fig. 10. Inhibition of HDAC1 does not affect the TGF- β 1 suppression of adipogenesis.

(A and B) C3H10T1/2 cells without or with *Hdac1* knockdown were treated with TGF- β 1 (1 nM) and/or BMP4 (1 nM) during adipogenic induction. Oil red O staining (A) and stained area quantification (B) were shown. **(C and D)** Dose tests of HDAC inhibitors. C3H10T1/2 cells treated with graded doses of VPA (C) or MS-275 (D) were subjected to Western blots to detect HDAC1 and acetylated histone H3 (Ac-H3). **(E and F)** C3H10T1/2 cells without or with HDAC inhibitor supplements were treated with TGF- β 1 (1 nM) and/or BMP4 (1 nM) during adipogenic induction. Oil red O staining (E) and the stained area quantification (F) were shown. $n = 3$ independent experiments. **(G and H)** Bone marrow MSCs were isolated from mice undergoing acute osteoporotic induction without or with HDAC1 inhibitor injection. Expressional changes in *Tgfbr2* (G) and *Tgfbr1* (Alk5) (H) were quantified by real-time qPCR. Data are means $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to indicated sample. One-way ANOVA followed by multiple comparison test.

Supp Fig. 11



Supplementary Fig. 11. Histological examination of the main organs in mice treated with HDAC inhibitors. (A) Mice were intraperitoneally injected with control vehicle or different HDAC inhibitors for 12 consecutive days. The main organs, including the liver, kidney and heart, were harvested for subsequent hematoxylin-eosin staining. Representative images were shown. Scale bar: 100 μ m. **(B)** Distal femurs from mice in the sRANKL-induced bone loss model were subjected to histological examination by hematoxylin-eosin staining. The yellow dashed line marks the growth plates; the asterisk indicates the trabecular bone; the arrow indicates adipocyte. Scale bar: 1mm.