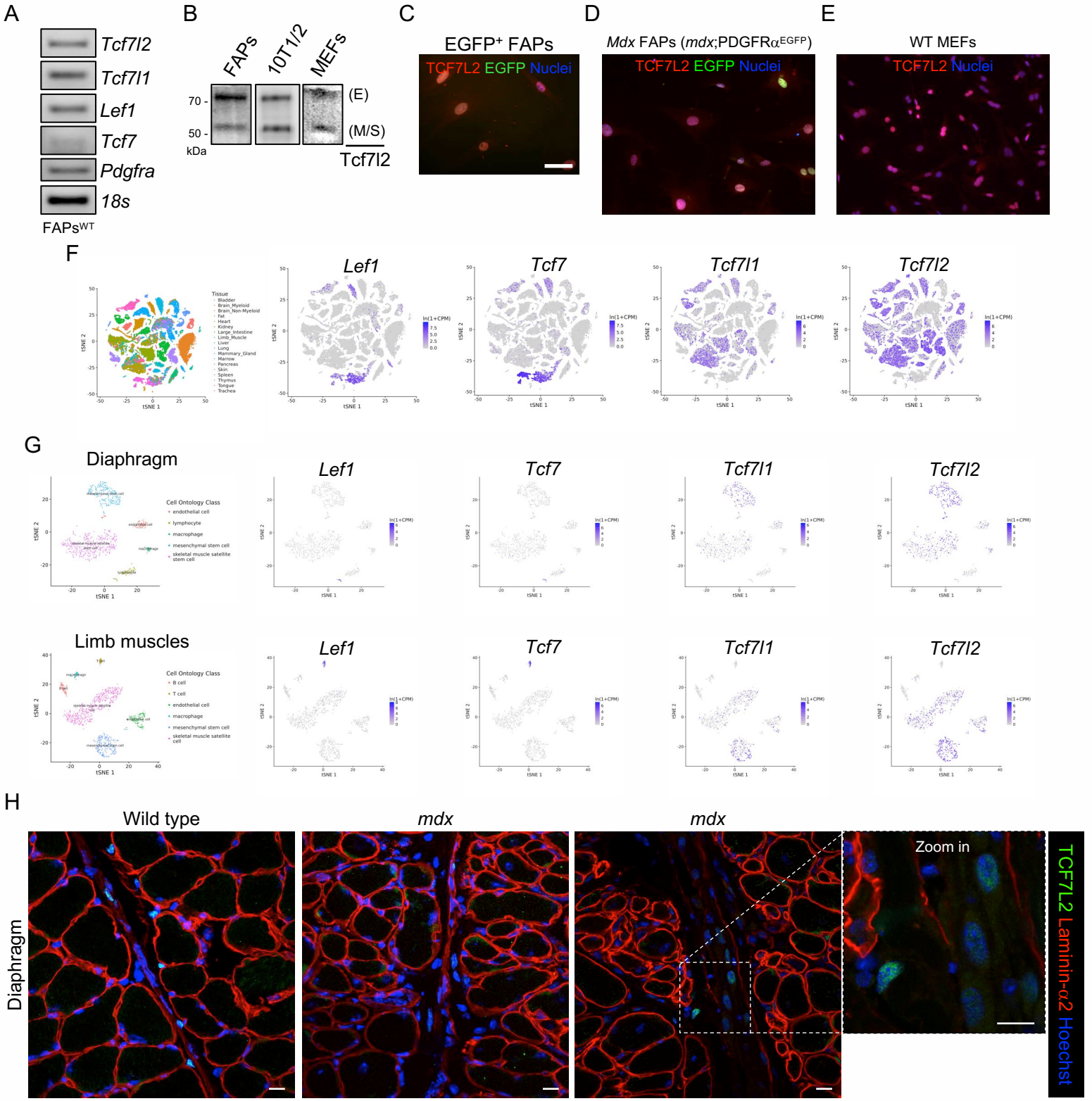

Fig. S1. Gene expression of Tcf/Lef family members in mesenchymal stromal cells.

(A) Gene expression of Tcf/Lef family members was analyzed by RT-qPCR in a 2% agarose gel. (B) Representative western blot analysis showing TCF7L2 protein levels in EGFP$^+$ FAPs, C3H 10T1/2 MSCs, and MEFs. TCF7L2 “E” and “M/S” isoforms are shown. (C-E) Immunofluorescence of TCF7L2 in isolated skeletal muscle PDGFRα-EGFP$^+$ FAPs (C), *mdx* FAPs (D), and MEFs (E). Scale bar: 50 μm. TCF7L2 immunofluorescence (*red*) in MEFs. (F) A t-SNE plot of all cells collected by the microfluidic-droplet method, colored by the predominant cell type that composes each cluster. Cells were colored by cell type for diaphragm and limb muscles and visualized with t-SNE. Cell types were determined by differential gene expression of known markers between clusters. t-SNE visualization of *Tcf/Lef* genes (from *grey*, low expression, to *blue*, high expression). (H) Z-stack confocal images showing the localization of TCF7L2$^+$ cells in diaphragm muscle sections of adult wild-type and from the dystrophic *mdx* mice. Scale bars: 10 μm.

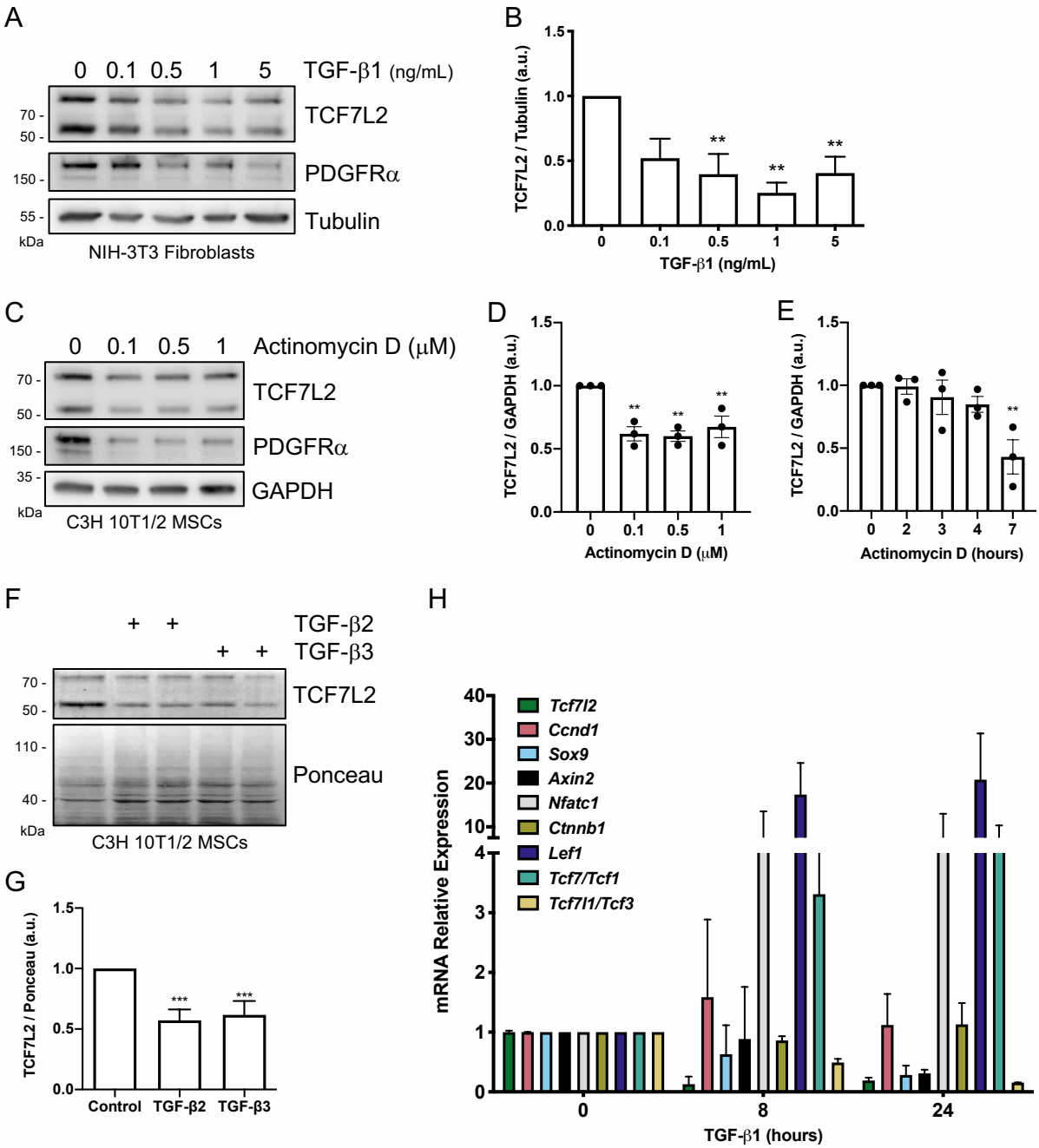

Fig. S2. Extracellular TGF-β ligands impair TCF7L2 expression and TCF7L2-mediated target gene expression.

(A) Representative western blot analysis showing TCF7L2 and PDGFRα expression levels in NIH-3T3 fibroblasts after treatment with different concentrations of TGF-β1 for 24 h. GAPDH was used as the loading control. (B) Quantification of TCF7L2 protein expression. ***P*<0.005 by one-way ANOVA with Dunnett's post-test; *n*=4. (C) Representative western blot analysis showing TCF7L2 and PDGFRα expression levels in MSCs after treatment with different concentrations of actinomycin D for 7 h. GAPDH was used as the loading control. (D,E) Quantification of TCF7L2 protein expression. ***P*<0.005 by one-way ANOVA with Dunnett's post-test; *n*=3. (F) Representative western blot from three independent experiments, showing TCF7L2 protein levels after stimulation with TGF-β2 and TGF-β3 for 24 h (5 ng/ml) in MSCs. Ponceau was used as the loading control. (G) Quantification of TCF7L2 protein expression ****P*<0.001; One-Way ANOVA with Dunnett post-test; *n*=3. (H) Tcf4 (Tcf7l2), Ccnd1 (CyclinD1), Sox9, Axin2, Nfatc1, Ctnnb1 (β-catenin), Lef1, Tcf7 (Tcf1), and Tcf7l1 (Tcf3) mRNA expression levels were analyzed by quantitative PCR in TGF-β1-treated MSCs at different time points (0, 8, 24 h). *n*=3.

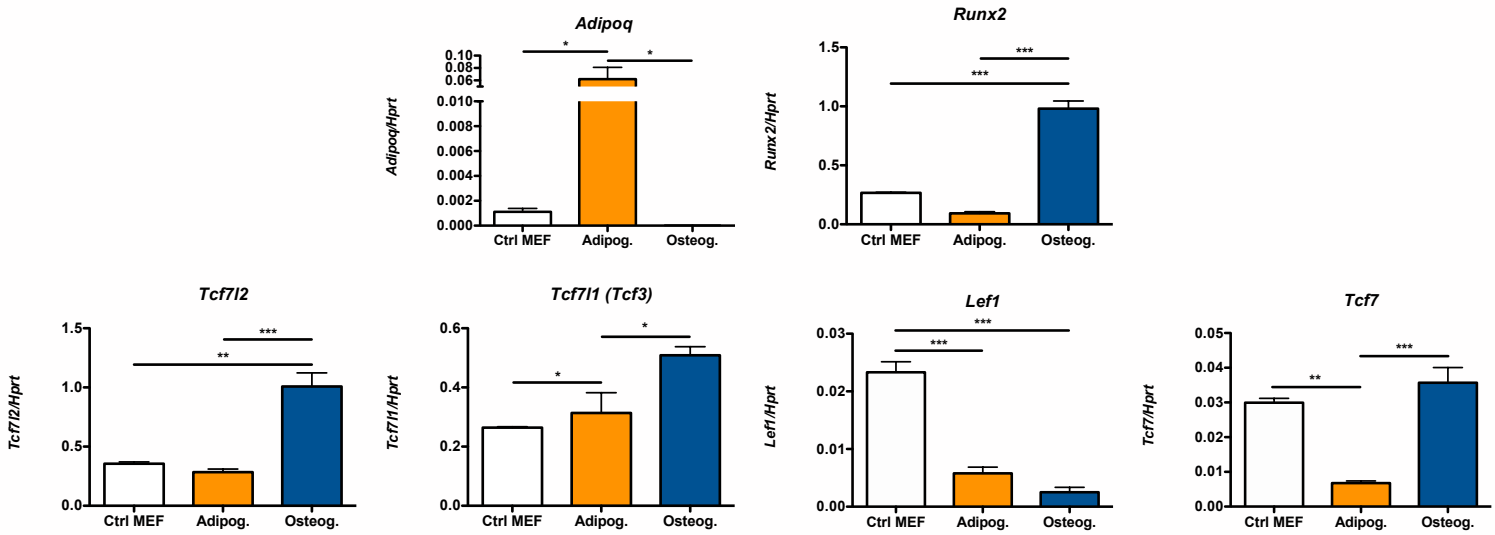

Fig. S3. Tcf/Lef members gene expression varies in *in vitro* adipocytes and osteocytes from MEFs.

Adipoq, *Runx2*, *Tcf7l2*, *Tcf7l1*, *Lef1*, and *Tcf7* mRNA expression levels were analyzed by digital droplet RT-qPCR in MEFs control (Ctrl MEF), MEFs-derived adipocytes (Adipog.: adipogenic cell medium), and MEFs-derived osteocytes (Osteog.: osteogenic cell medium).

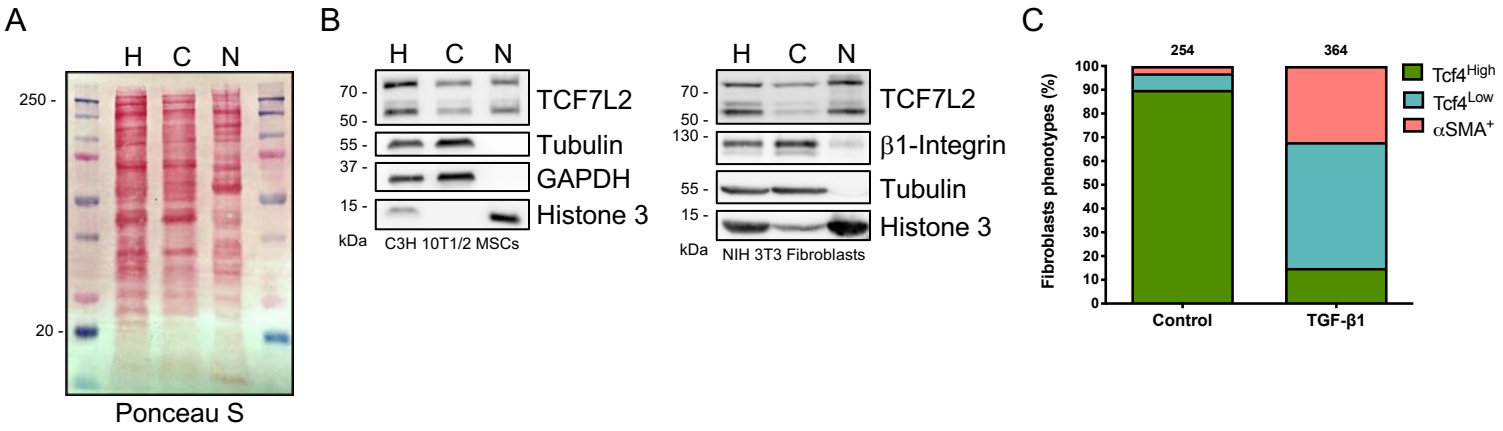

Fig. S4. Nuclear-cytoplasmic fractionation of MSCs and evaluation of TCF7L2 protein expression.

(A) Representative ponceau red staining of H: Whole cell lysate; C: Cytoplasmic lysate; N: Nuclei lysate. (B) Representative western blot analysis showing TCF7L2, Tubulin, GAPDH, Histone 3, and β1-Integrin protein levels in proliferating C3H/10T1/2 MSCs and NIH-3T3 fibroblasts. (C) Quantification of TCF7L2 fluorescence intensity in control- and TGF-β1-treated fibroblasts. TCF7L2^{Hi} (Tcf4^{Hi}), TCFL2^{low} (Tcf4^{low}), and αSMA^{+}-phenotypes were quantified in control and TGF-β1-stimulated C3H/10T1/2 MPCs at 36 h. The numbers above each graph show the total quantified number of cells. *n*=3.

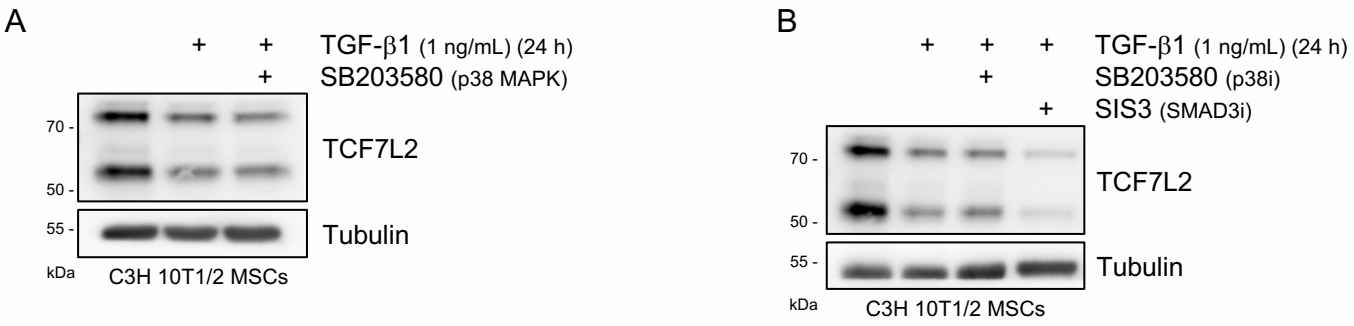

Fig. S5. Pharmacological Smad3 inhibition with SIS3 pronounces TGF-β-mediated downregulation of TCF7L2.

(A) Representative western blot analysis showing TCF7L2 expression levels in C3H 10T1/2 cells after TGF-β1 treatment (1 ng/ml) for 24 h. SB203580 (p38 MAPK inhibitor) was co-incubated with TGF-β1 for 24 h. Tubulin was used as the loading control. (B) Representative western blot analysis showing TCF7L2 expression levels in C3H 10T1/2 cells after TGF-β1 treatment (1 ng/ml) for 24 h. SB203580 (p38 MAPK inhibitor) or SIS3 (Smad3 inhibitor) were co-incubated with TGF-β1 for 24 h. Tubulin was used as the loading control.

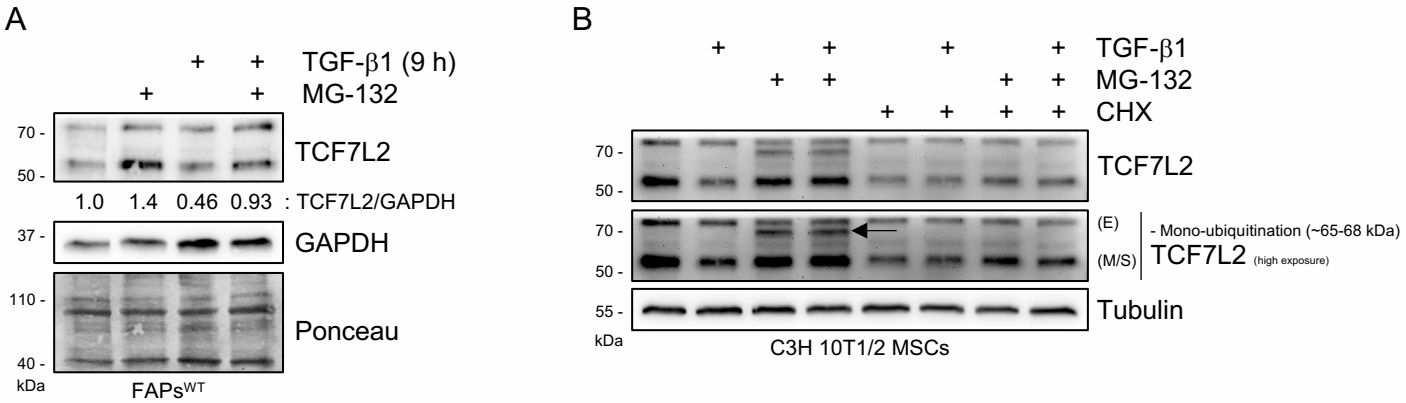

Fig. S6. Evaluation of the participation of the ubiquitin proteasome system via MG132 inhibition on the regulation of TCF7L2 protein expression.

(A) Representative western blot analysis showing TCF7L2 expression levels in C3H 10T1/2 cells after TGF-β1 treatment (5 ng/ml) for 9 h. MG132 (26S subunit proteasome inhibitor) was incubated alone or co-incubated with TGF-β1 for 9 h. GAPDH and ponceau red were used as the loading control. (B) Representative western blot analysis showing TCF7L2 expression levels in C3H 10T1/2 cells after TGF-β1 treatment (5 ng/ml) for 9 h. MG132 (26S subunit proteasome inhibitor) or cycloheximide (protein translation inhibitor) were incubated alone or co-incubated with TGF-β1 for 9 h. Tubulin was used as the loading control.

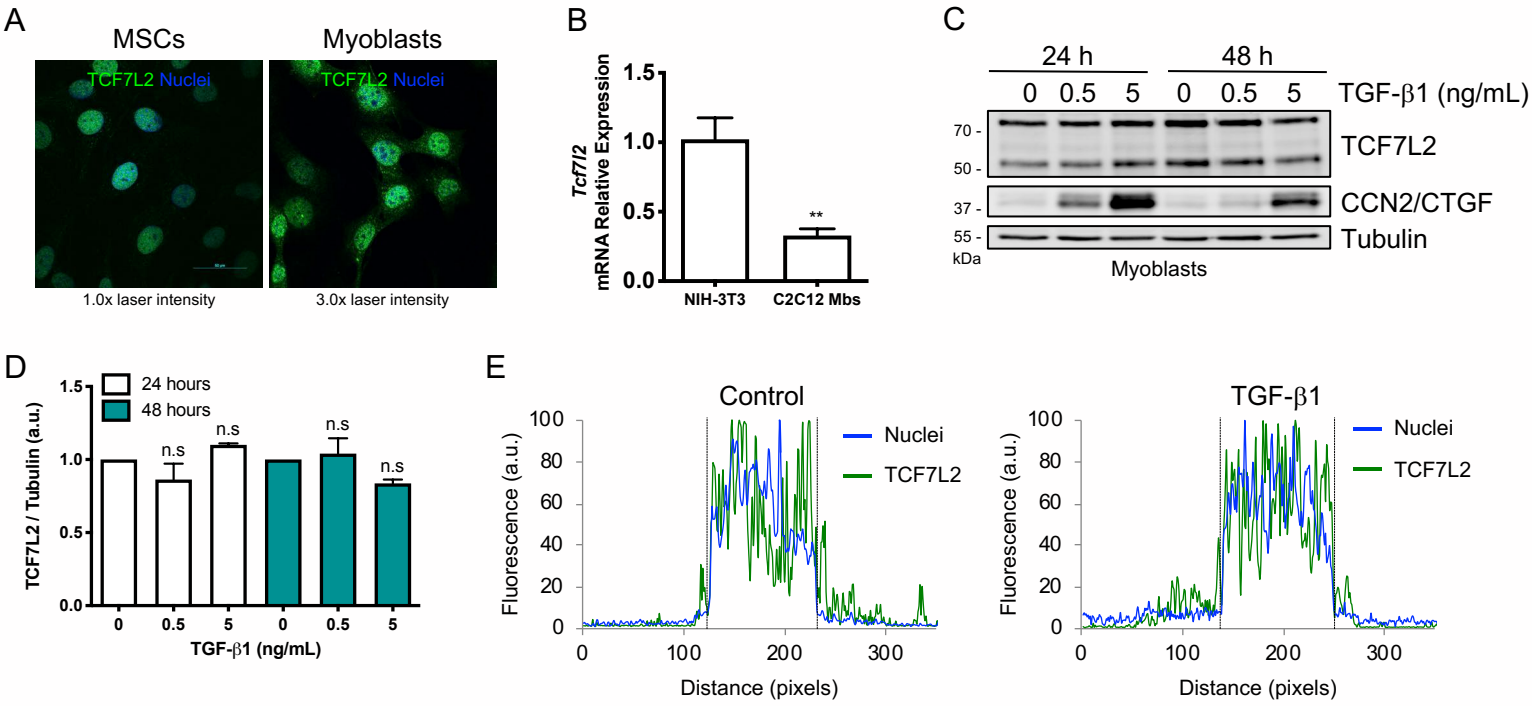

Fig. S7. The expression of TCF7L2 is not affected by TGF-β signaling in C2C12 myoblasts.

(A) Confocal images showing TCF7L2 localization in C3H/10T1/2 MSCs and C2C12 myoblasts cell types. Nuclei were stained with Hoechst (*blue*). Laser intensities (low vs high) were manually adjusted to show similar TCF7L2 fluorescence intensities in both cell types. (B) *Tcf7l2* mRNA expression levels were analyzed by quantitative PCR in proliferating NIH-3T3 fibroblasts and C2C12 myoblasts. ***P*<0.005 by two-tailed Student's t-test. *n*=3. (C) Representative western blot analysis of three independent experiments, showing TCF7L2 and CCN2/CTGF protein levels in TGF-β1-treated C2C12 myoblasts at different concentrations for 24 or 48 h. Tubulin was used as the loading control. (D) Quantification of TCF7L2 protein levels. n.s, not significant by one-way ANOVA with Dunnett's post-test; *n*=3. (E) Label-distribution graph showing the fluorescence intensity of TCF7L2 and Hoechst along the cell axis. Distance is shown in pixels. Dotted lines show the nucleus-cytoplasm boundary. (a.u.: arbitrary units).