Figure S1. Control-Fc, TβRII-Fc and TβRI-TβRII-Fc chimeric receptors are expressed and secreted by 293T cells. 293T cells were transfected with plasmids expressing each Fc chimeric receptor (Control-Fc, TβRII-Fc, or TβRI-TβRII-Fc) as described in Materials and methods. The conditioned media of 293T cells expressing chimeric receptors were subjected to immunoblotting analysis using anti-human IgG-Fc antibody. TβRI, TGF-β type I receptor; TβRII, TGF-β type II receptor; IgG, immunoglobulin G.

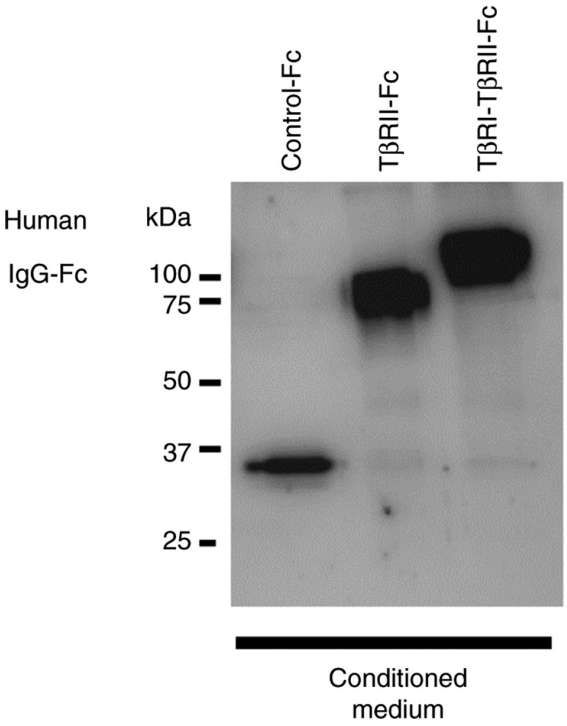

Figure S2. TβRI-TβRII-Fc chimeric receptor inhibits the expression of TGF-β direct target genes in Clone M3 melanoma cells. Clone M3 cells were incubated for 4 h in the absence (-) or presence of TGF-β isoforms [TGF-β1 (Tβ1), TGF-β2 (Tβ2), or TGF-β3 (Tβ3)] (3 ng/ml) in combination with vehicle (Control), 10 μM SB431542, or conditioned media from 293T cells containing Fc chimeric receptors (Control-Fc, TβRII-Fc or TβRI-βRII-Fc). The expression levels of (A) TMEPAI and (B) PAI-1 were evaluated by reverse transcription-quantitative PCR analysis. Each experiment was performed in triplicate and repeated twice. All data are normalized to the expression of β-actin. Error bars, SD. **P<0.01 and ***P<0.001. TβRI, TGF-β type I receptor; TβRII, TGF-β type II receptor; TGF-β, transforming growth factor-β; TMEPAI, transmembrane prostate androgen-induced protein; PAI-1, plasminogen activator inhibitor-1; NS, not significant.

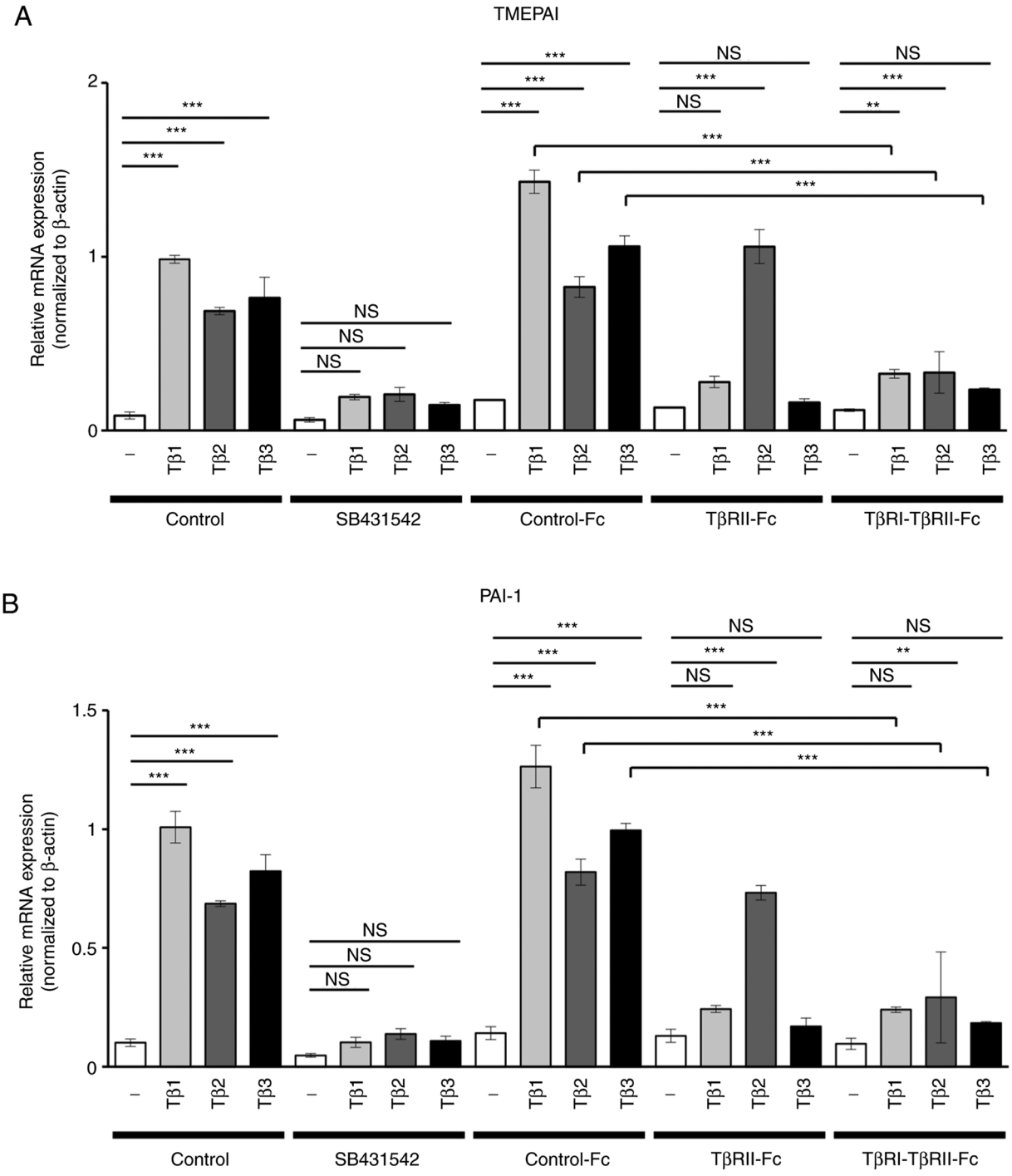

Figure S3. Expression of mesenchymal marker SM22α is induced by all TGF-β isoforms in Clone M3 melanoma cells. Clone M3 cells were cultured in the absence (Ctrl or Control) or presence of TGF-β1 (Tβ1), TGF-β2 (Tβ2), or TGF-β3 (Tβ3) (3 ng/ml) or the TGF-β signal inhibitor, SB431542 (SB; 10 μM) for 72 h, followed by (A) RT-qPCR, (B) immunoblotting, and (C) immunocytochemistry. Experiments were performed in triplicate and repeated twice. (A) The expression of mesenchymal marker SM22α was evaluated by RT-qPCR analysis. The data were normalized to the β-actin expression. Error bars, SD. (B) The immunoblotting analysis with antibodies specific to SM22α and α-tubulin (loading control). (C) Representative immunofluorescence images showing staining of SM22α (green) and nuclei (blue). Scale bar, 100 μm. ***P<0.001. SM22α, smooth muscle protein 22α; TGF-β, transforming growth factor-β; RT-qPCR, reverse transcription-quantitative PCR; NS, not significant.

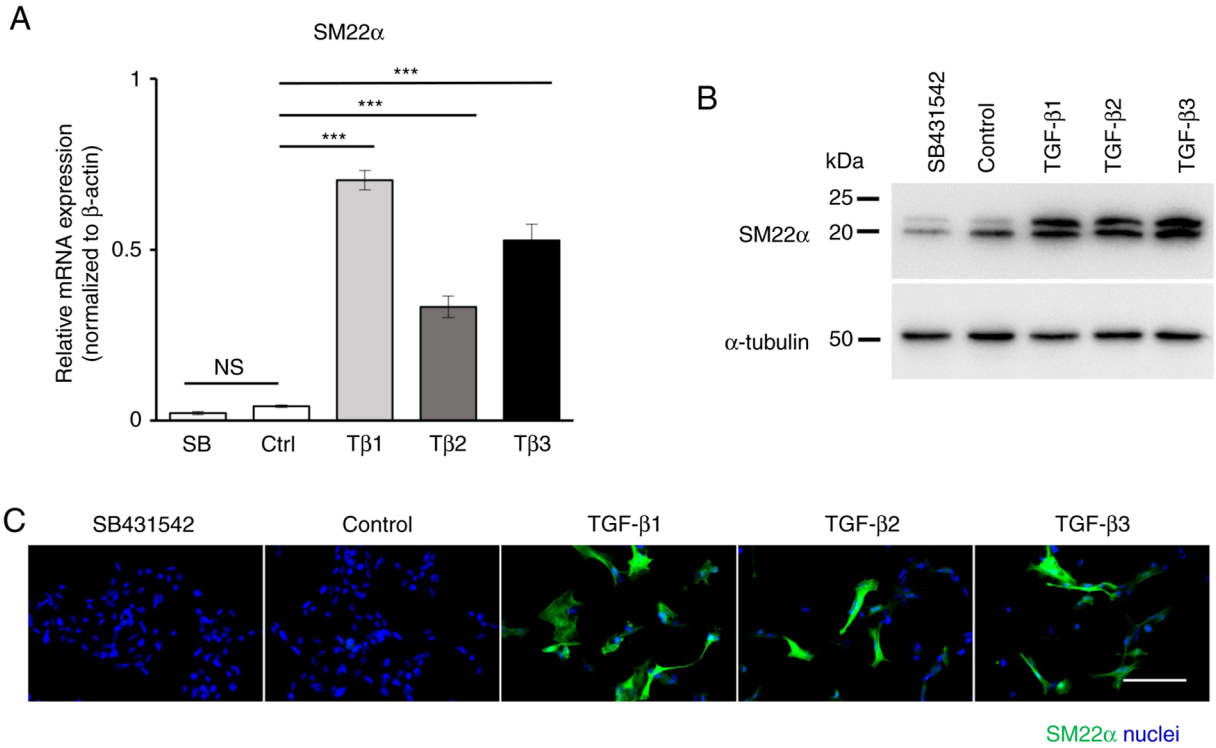

Figure S4. TβRI-TβRII-Fc chimeric receptor inhibits TGF-β-induced expression of mesenchymal marker SM22α in Clone M3 melanoma cells. Clone M3 cells were incubated in the absence (-) or presence of TGF-β isoforms [TGF-β1 (Tβ1), TGF-β2 (Tβ2), or TGF-β3 (Tβ3)] (3 ng/ml) in combination with conditioned media from 293T cells containing Fc chimeric receptors (Control-Fc, TβRII-Fc, or TβRI-TβRII-Fc). The effect of chimeric receptors on the expression of mesenchymal marker was evaluated by (A) RT-qPCR analysis, (B) immunoblotting and (C) immunocytochemistry. Each experiment was performed in triplicate and repeated twice. (A) The RT-qPCR analysis for the expression of mesenchymal marker SM22α. All data were normalized to the β-actin expression. Error bars, SD. (B) The immunoblotting analysis for the expression level of SM22α and α-tubulin (loading control). (C) Immunofluorescence staining of SM22α (green) and nuclei (blue). Scale bar, 100 μm. *P<0.05 and ***P<0.001. TβRI, TGF-β type I receptor; TβRII, TGF-β type II receptor; NS, not significant; SM22α, smooth muscle protein 22α; RT-qPCR, reverse transcription-quantitative PCR.

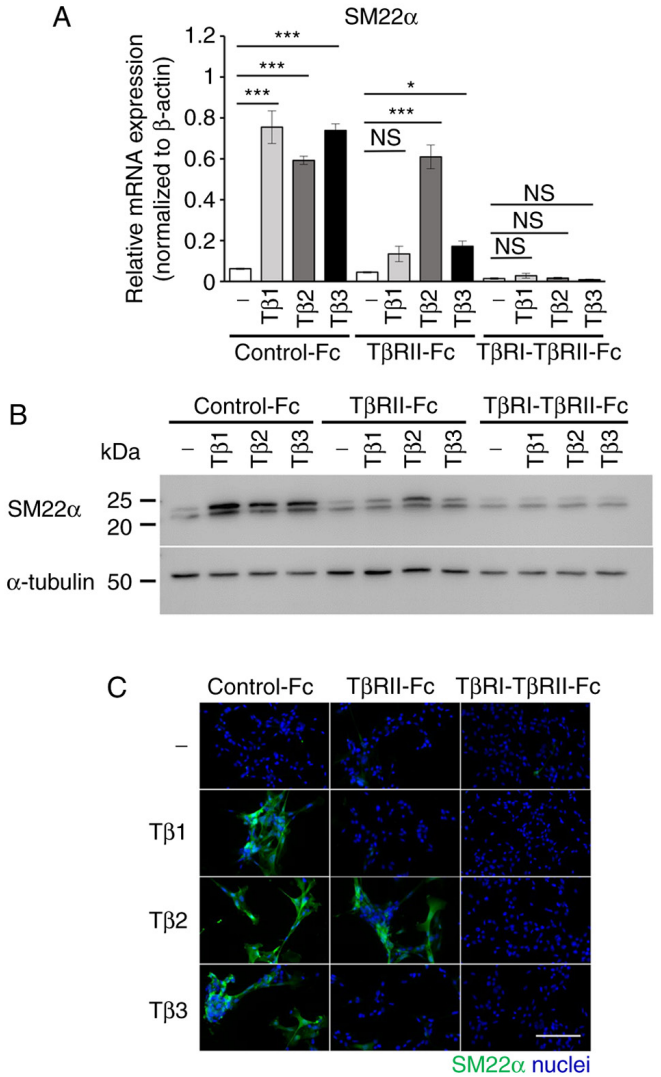

Figure S5. Lentiviral transduction of the Control-Fc, TβRII-Fc and TβRI-TβRII-Fc chimeric receptors to B16 melanoma cells. B16 cells were infected with lentiviruses expressing either GFP or each Fc chimeric receptor (GFP, Control-Fc, TβRII-Fc, or TβRI-TβRII-Fc). (A) Successful infection of lentivirus was confirmed by the observation of fluorescence in B16 cells transfected with lentivirus expressing GFP. Representative images of cells infected with lentiviruses expressing either GFP or each Fc chimeric receptor (GFP, Control-Fc, TβRII-Fc, or TβRI-TβRII-Fc). Fluorescence images (left panels), brightfield images (middle panels) and the merged images (right panels) are presented. Scale bar, 100 μm. (B) Expression of Fc chimeric receptors in transduced cells was confirmed by immunoblotting. The Fc chimeric receptors (black arrowhead; left, cell lysate; right, conditioned medium) were visualized with anti-human IgG-Fc antibody. α-tubulin was used as a loading control for the cell lysate. TβRI, TGF-β type I receptor; TβRII, TGF-β type II receptor; GFP, green fluorescent protein.

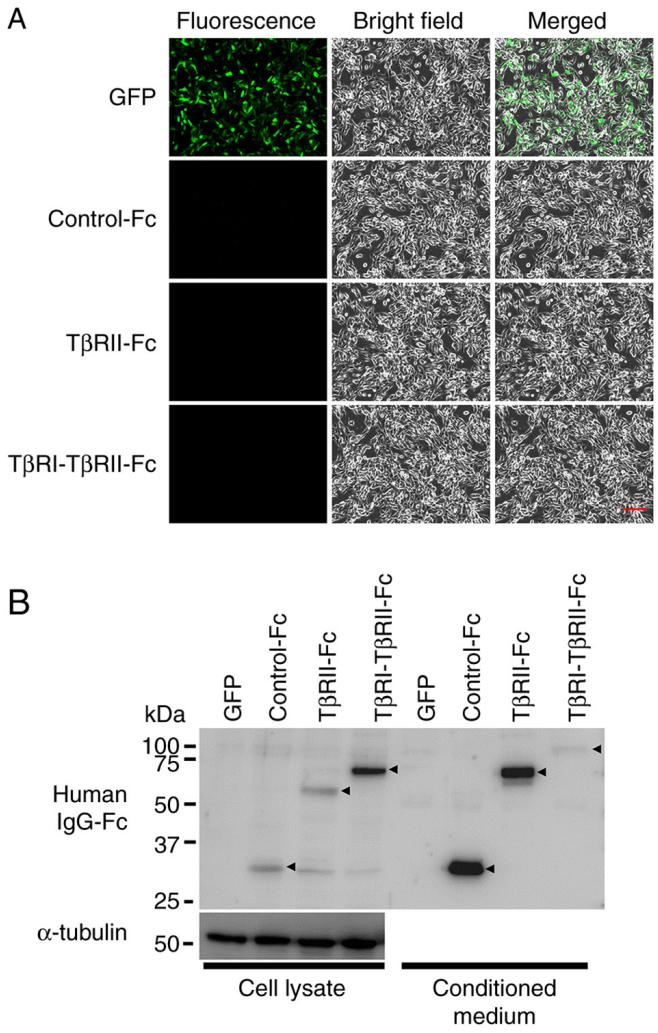

Figure S6. All TGF-β isoforms suppress B16 melanoma cell proliferation. B16 cells ($7.5x10^4$ cells/well) seeded into 6-well culture plates were treated with each TGF-β isoform or SB431542 for 72 h followed by direct cell counting with a hemocytometer. The experiment was performed in triplicate and repeated twice. Error bars, SD. ***P<0.001. TGF-β, transforming growth factor-β; NS, not significant.

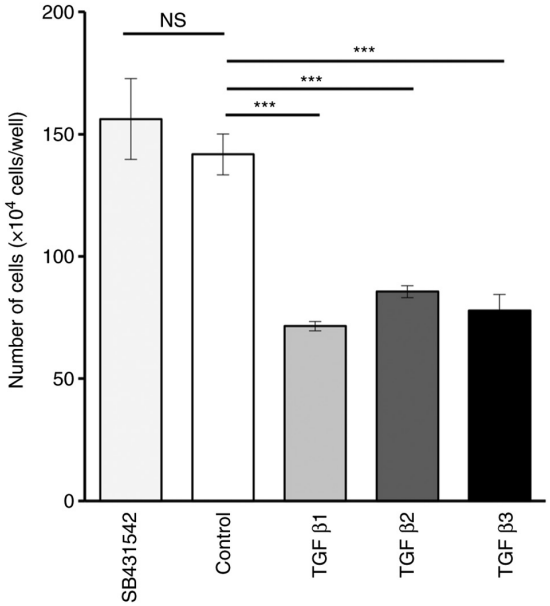

Table SI. Primers for reverse transcription-quantitative PCR.

Transcript	Primer	Sequence (5'→3')
Acta2	5'	AGCGTGAGATTGTCCGTGACAT
(αSMA)	3'	GCGTTCGTTTCCAATGGTGA
Actb	5'	TCACCCACACTGTGCCCATCTACGA
(β-actin)	3'	CAGCGGAACCGCTCATTGCCAATGG
Fn1	5'	GCCGTGGTCCTAACAAATCTCC
(Fibronectin)	3'	CGAGACCTGTTTTCTGCCTTCC
Pmepai	5'	TGGAGTTCGTGCAAATCGTG
(TMEPAI)	3'	TCCGAGGACAGTCCATCGTC
Serpine1	5'	CCACAAAGGTCTCATGGACCAT
(PAI-1)	3'	TGAAAGTGTTGTGCCCTCCAC
Tagln	5'	GTGTGGCTGAAGAATGGTGTGA
(SM22α)	3'	GCCACCTGTTCCATCTGCTTAA

αSMA, α-smooth muscle actin; TMEPAI, transmembrane prostate androgen-induced protein; PAI-1, plasminogen activator inhibitor-1; SM22α, smooth muscle protein 22α.