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SUPPLEMENTAL DATA

Novel peptide inhibitors targeting the TGF-β receptor I/II complex modulate SMAD and ERK signaling *in vitro*

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Full article is available at the following link: Novel peptide inhibitors targeting the TGF-β receptor I/II complex modulate SMAD and ERK signaling *in vitro*

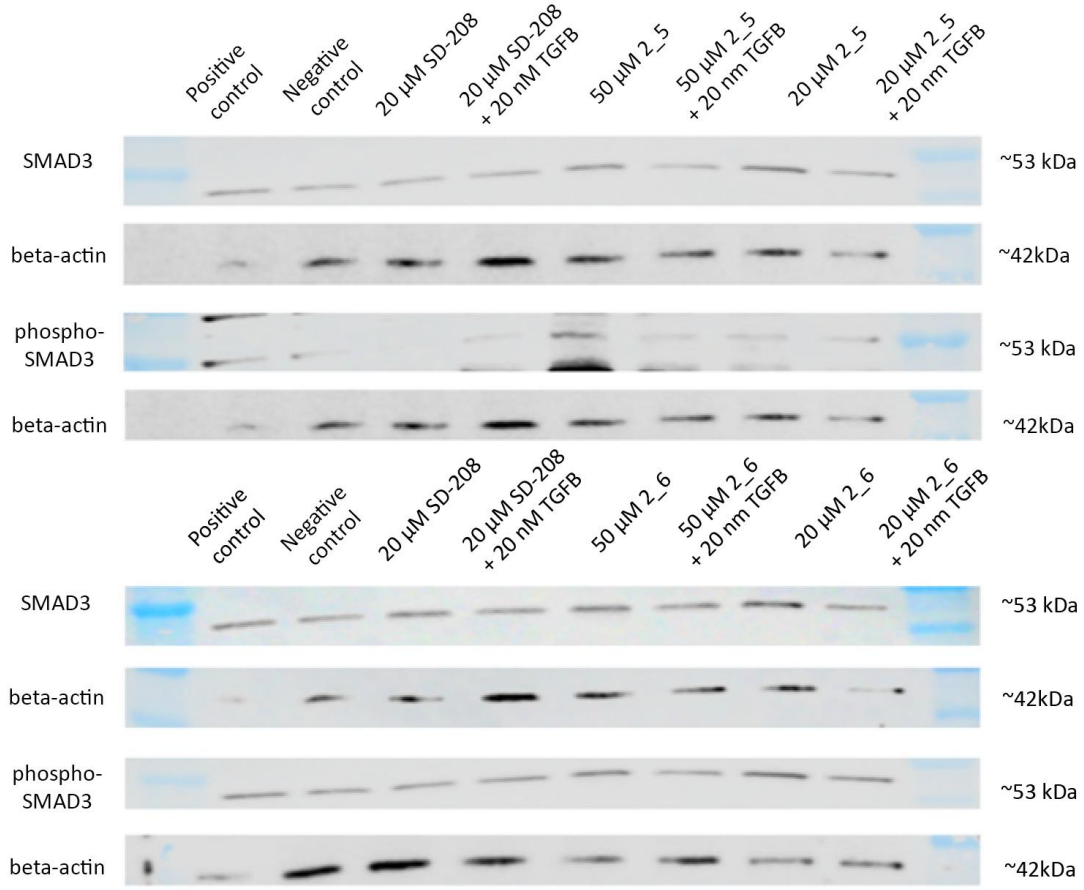


Figure S1. Effects of peptide inhibitors 2_5 and 2_6 on total and phosphorylated SMAD3 levels in HEK293T cells. Western blot analysis of SMAD3 and p-SMAD3 after pretreatment for 60 min with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_5 or 2_6 (20 or 50 µM), followed by 6 h of incubation with or without TGF-β (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. The upper blot series shows the peptide 2_5 treatment conditions, and the lower blot series shows the peptide 2_6 treatment conditions. β-actin was used as the loading control. Approximate molecular masses are 53 kDa for SMAD3 and p-SMAD3 and 42 kDa for β-actin. SMAD3 and p-SMAD3 signals were weak and insufficient for reliable interpretation of treatment-related changes. **Abbreviations**: HEK293T, human embryonic kidney 293T; p-SMAD3, phosphorylated SMAD family member 3; SMAD3, SMAD family member 3; TGF-β, transforming growth factor beta.

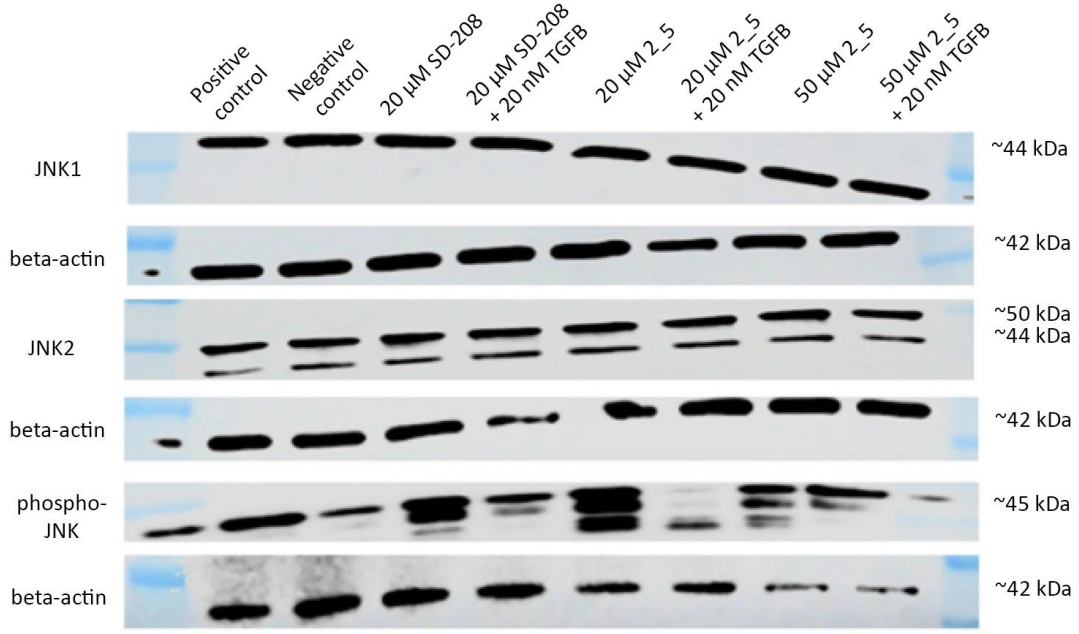


Figure S2. Effects of peptide inhibitor 2_5 on total and phosphorylated JNK levels in HEK293T cells. Western blot analysis of JNK1, JNK2, and p-JNK after pretreatment for 60 min with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_5 (20 or 50 µM), followed by 6 h of incubation with or without TGF-β (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. β-actin was used as the loading control for each blot. Approximate molecular masses are 44 kDa for JNK1, 50 and 44 kDa for the JNK2 doublet, 45 kDa for p-JNK, and 42 kDa for β-actin. No substantial treatment-related changes in JNK1, JNK2, or p-JNK levels were evident under the tested conditions. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK1, c-Jun N-terminal kinase 1; JNK2, c-Jun N-terminal kinase 2; p-JNK, phosphorylated c-Jun N-terminal kinase; TGF-β, transforming growth factor beta.

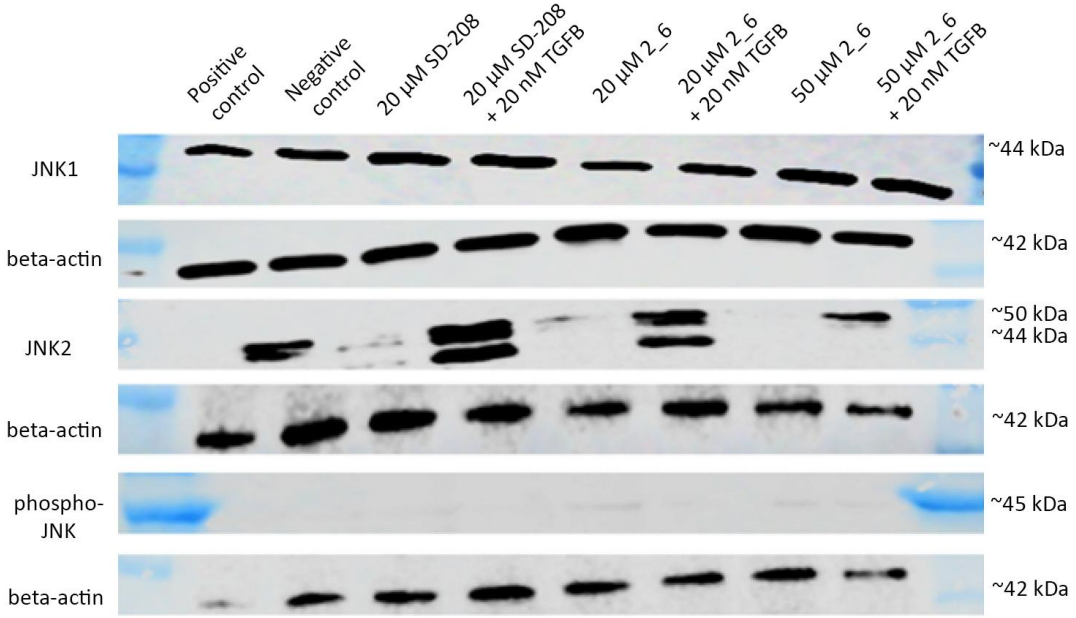


Figure S3. Effects of peptide inhibitor 2_6 on total and phosphorylated JNK levels in HEK293T cells. Western blot analysis of JNK1, JNK2, and p-JNK after pretreatment for 60 min with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_6 (20 or 50 µM), followed by 6 h of incubation with or without TGF-β (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. β-actin was used as the loading control for each blot. Approximate molecular masses are 44 kDa for JNK1, 50 and 44 kDa for the JNK2 doublet, 45 kDa for p-JNK, and 42 kDa for β-actin. No substantial treatment-related changes in JNK1, JNK2, or p-JNK levels were evident under the tested conditions. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK1, c-Jun N-terminal kinase 1; JNK2, c-Jun N-terminal kinase 2; p-JNK, phosphorylated c-Jun N-terminal kinase; TGF-β, transforming growth factor beta.

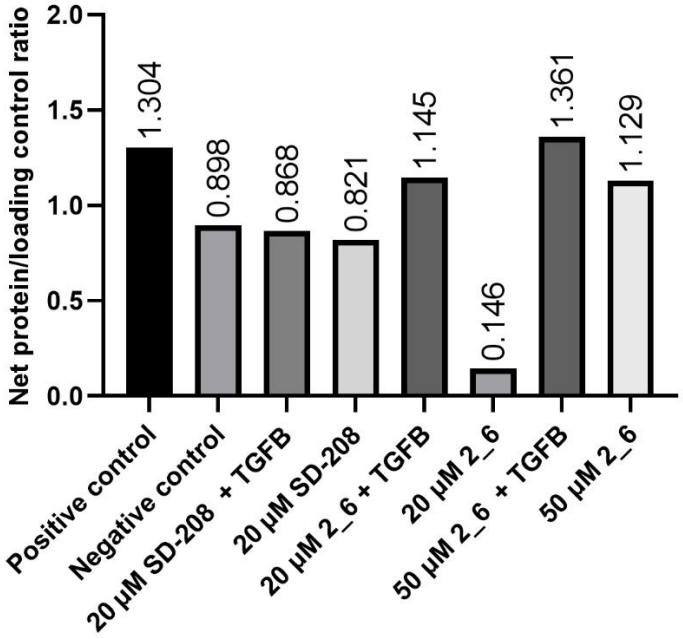


Figure S4. Effects of peptide inhibitor 2_6 on ERK1/2 levels in HEK293T cells. Densitometric quantification of total ERK1/2 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_6 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the ERK1/2 and corresponding β-actin Western blots shown in the lower blot series of Figure 2A. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: ERK1/2, extracellular signal-regulated kinase 1/2; HEK293T, human embryonic kidney 293T; TGF-β, transforming growth factor beta.

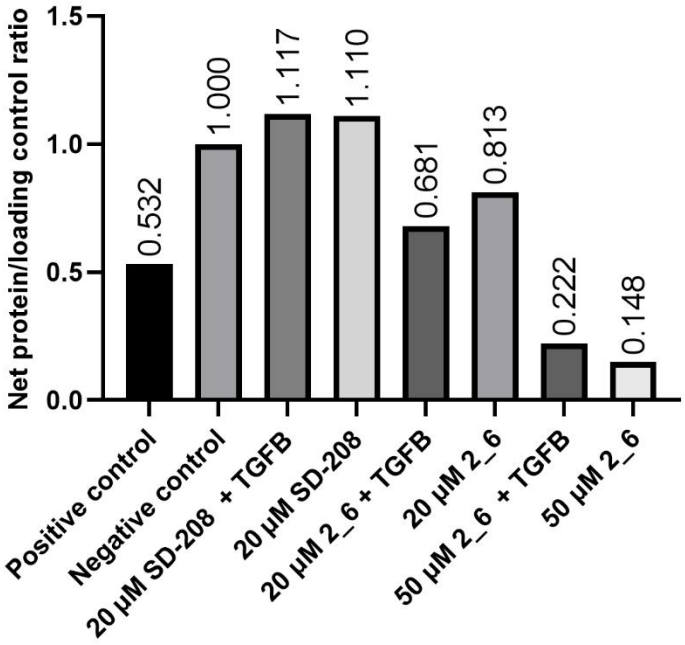


Figure S5. Effects of peptide inhibitor 2_6 on phosphorylated ERK1/2 levels in HEK293T cells. Densitometric quantification of p-ERK1/2 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_6 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the p-ERK1/2 and corresponding β-actin Western blots shown in the lower blot series of Figure 2A. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. The lower ratios observed with 50 μM peptide 2_6 should be interpreted cautiously because p-ERK1/2 signals in this blot series were weak and inconsistent. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: ERK1/2, extracellular signal-regulated kinase 1/2; HEK293T, human embryonic kidney 293T; p-ERK1/2, phosphorylated extracellular signal-regulated kinase 1/2; TGF-β, transforming growth factor beta.

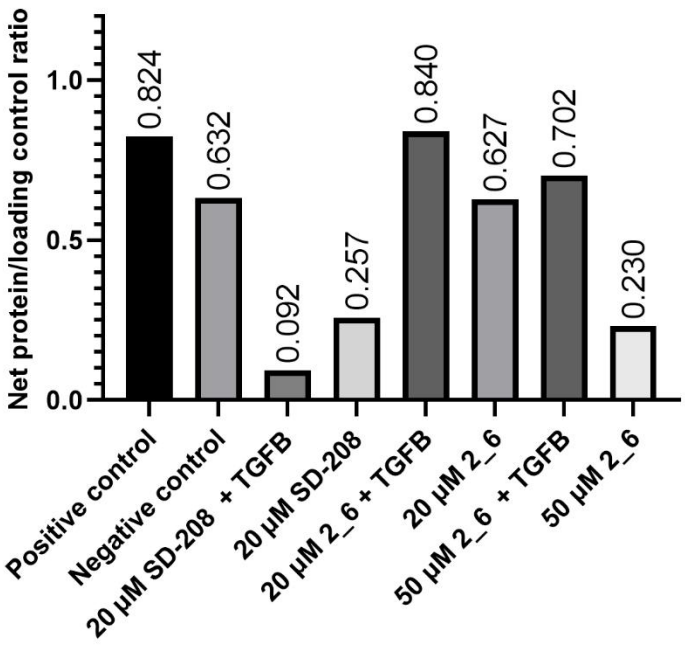


Figure S6. Effects of peptide inhibitor 2_6 on SMAD2 levels in HEK293T cells. Densitometric quantification of total SMAD2 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_6 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the SMAD2 and corresponding β-actin Western blots shown in the lower blot series of Figure 1A. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; SMAD2, SMAD family member 2; TGF-β, transforming growth factor beta.

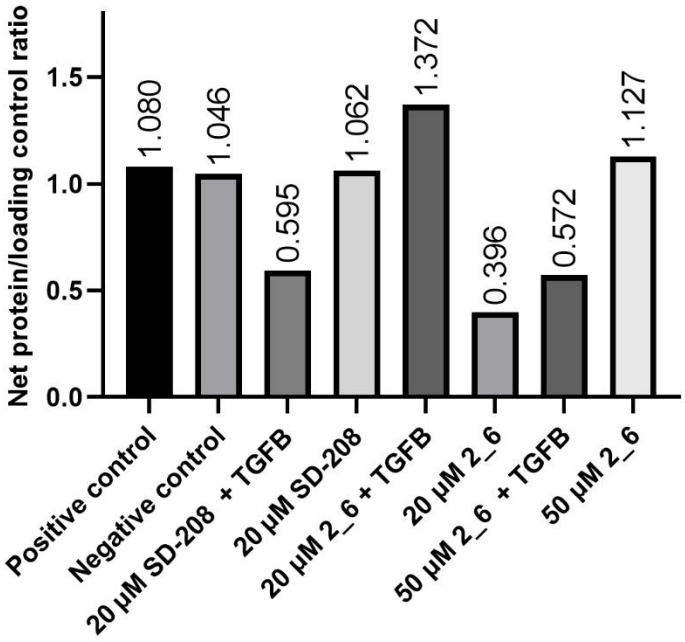


Figure S7. Effects of peptide inhibitor 2_6 on phosphorylated SMAD2 levels in HEK293T cells. Densitometric quantification of p-SMAD2 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_6 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the p-SMAD2 and corresponding β-actin Western blots shown in the lower blot series of Figure 1A. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. The effects of peptide 2_6 on p-SMAD2 were inconsistent across the tested conditions and should therefore be interpreted cautiously. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; p-SMAD2, phosphorylated SMAD family member 2; TGF-β, transforming growth factor beta.

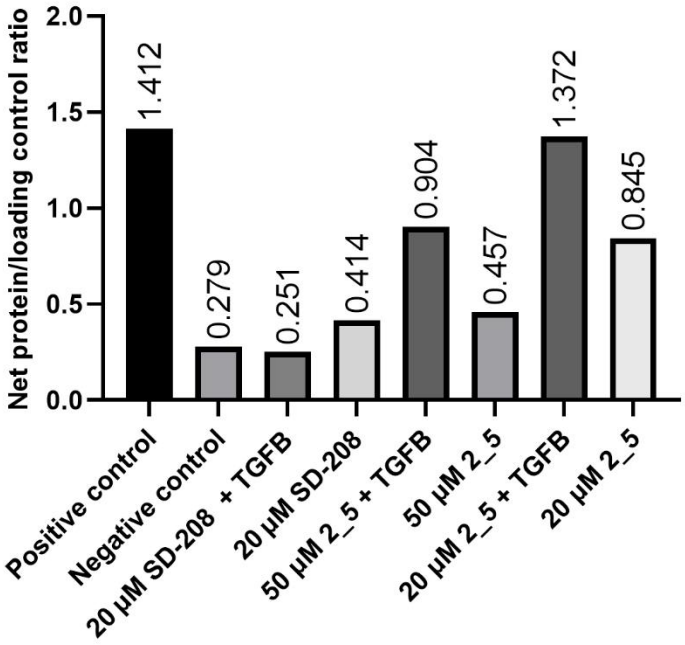


Figure S8. Effects of peptide inhibitor 2_5 on SMAD3 levels in HEK293T cells. Densitometric quantification of total SMAD3 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_5 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the SMAD3 and corresponding β-actin Western blots shown in the upper blot series of Figure S1. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Because SMAD3 signals were weak, the observed differences should be interpreted cautiously and not considered evidence of definitive treatment-related effects. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; SMAD3, SMAD family member 3; TGF-β, transforming growth factor beta.

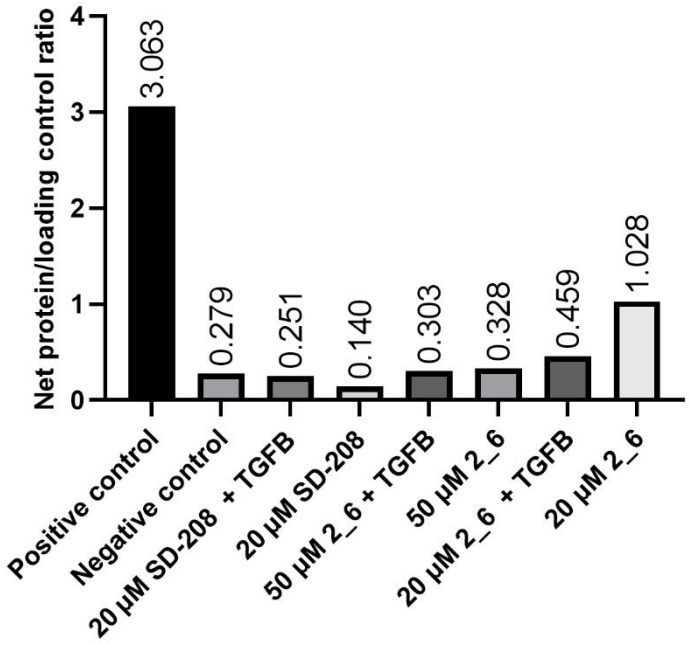


Figure S9. Effects of peptide inhibitor 2_6 on SMAD3 levels in HEK293T cells. Densitometric quantification of total SMAD3 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_6 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the SMAD3 and corresponding β-actin Western blots shown in the lower blot series of Figure S1. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Because SMAD3 signals were weak, the observed differences should be interpreted cautiously and not considered definitive evidence of treatment-related effects. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; SMAD3, SMAD family member 3; TGF-β, transforming growth factor beta.

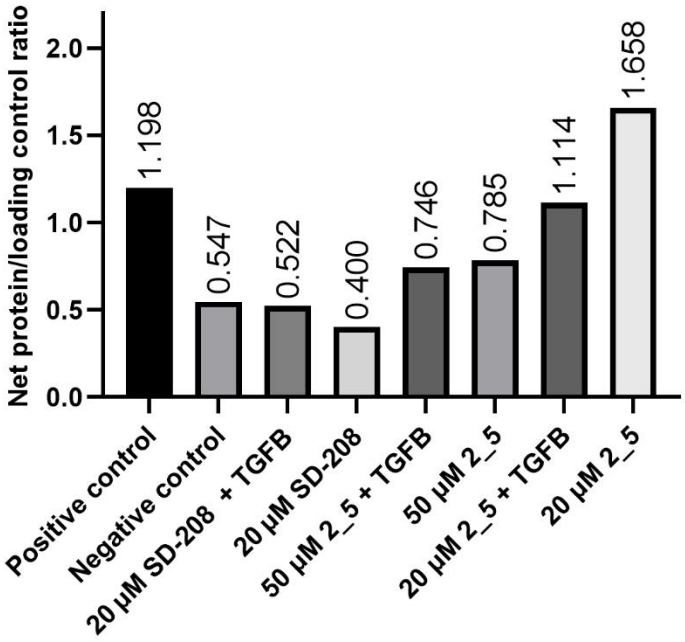


Figure S10. Effects of peptide inhibitor 2_5 on phosphorylated SMAD3 levels in HEK293T cells. Densitometric quantification of p-SMAD3 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_5 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the p-SMAD3 and corresponding β-actin Western blots shown in the upper blot series of Figure S1. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Because p-SMAD3 signals were weak, the observed differences should be interpreted cautiously and not considered definitive evidence of treatment-related effects. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; p-SMAD3, phosphorylated SMAD family member 3; TGF-β, transforming growth factor beta.

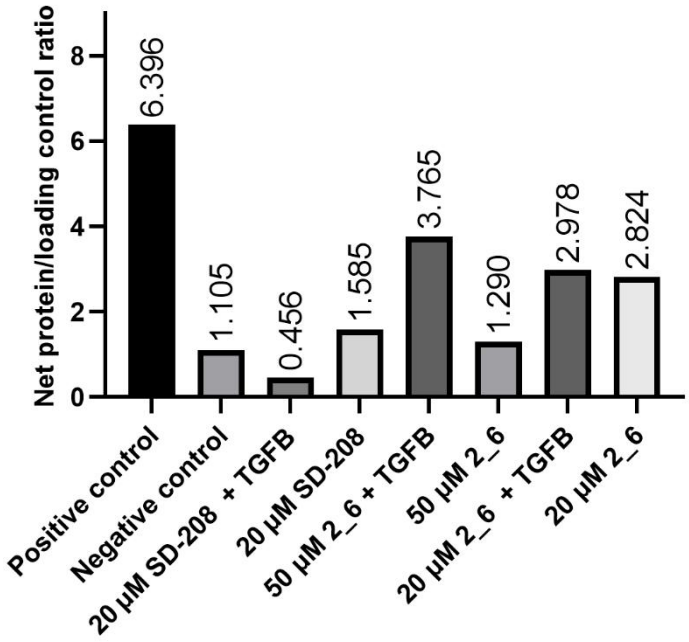


Figure S11. Effects of peptide inhibitor 2_6 on phosphorylated SMAD3 levels in HEK293T cells. Densitometric quantification of p-SMAD3 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_6 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the p-SMAD3 and corresponding β-actin Western blots shown in the lower blot series of Figure S1. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Because p-SMAD3 signals were weak, the observed differences should be interpreted cautiously and not considered definitive evidence of treatment-related effects. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; p-SMAD3, phosphorylated SMAD family member 3; TGF-β, transforming growth factor beta.

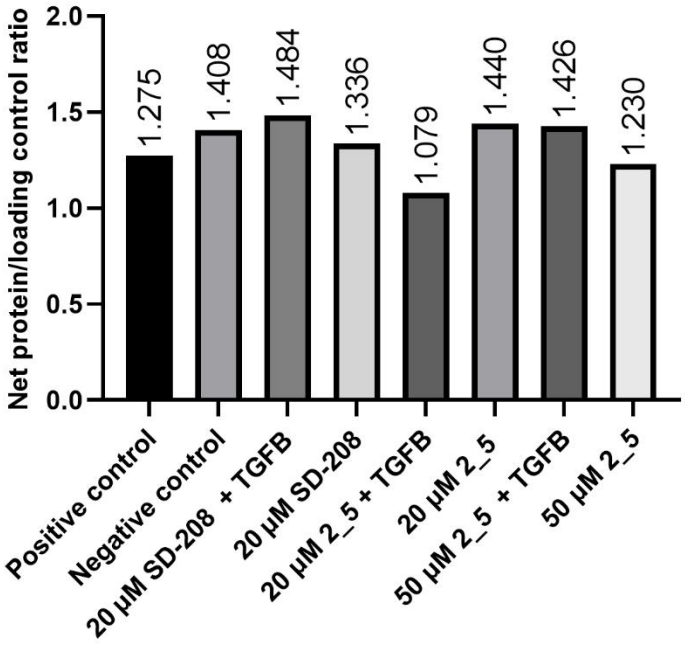


Figure S12. Effects of peptide inhibitor 2_5 on JNK1 levels in HEK293T cells. Densitometric quantification of JNK1 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_5 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the JNK1 and corresponding β-actin Western blots shown in Figure S2. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. No substantial treatment-related reduction in JNK1 levels was observed under the tested conditions. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK1, c-Jun N-terminal kinase 1; TGF-β, transforming growth factor beta.

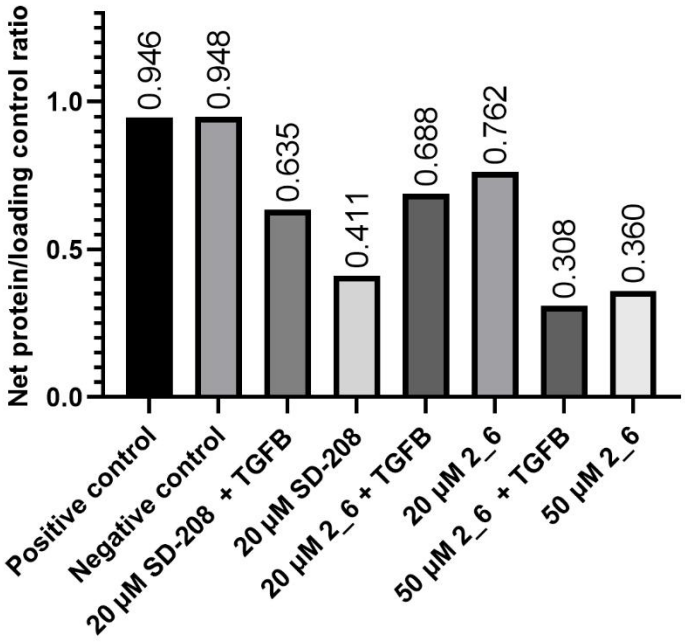


Figure S13. Effects of peptide inhibitor 2_6 on JNK1 levels in HEK293T cells. Densitometric quantification of JNK1 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_6 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the JNK1 and corresponding β-actin Western blots shown in Figure S3. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. No substantial treatment-related reduction in JNK1 levels was observed under the tested conditions. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK1, c-Jun N-terminal kinase 1; TGF-β, transforming growth factor beta.

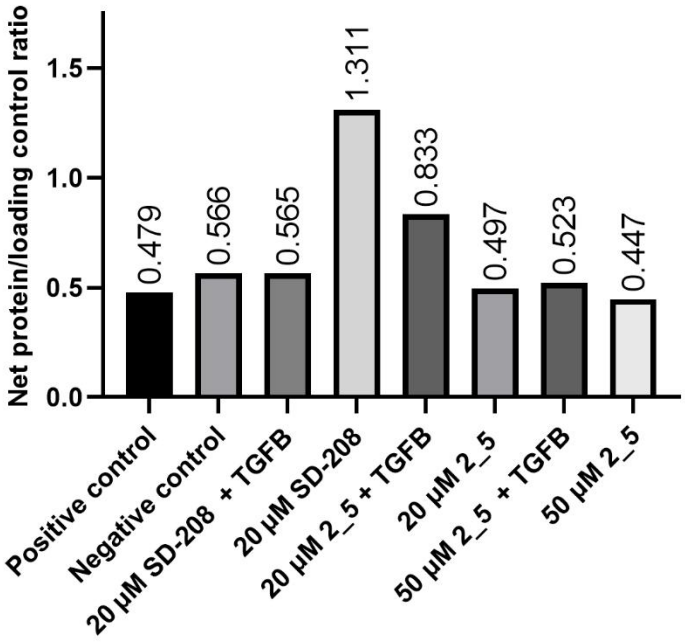


Figure S14. Effects of peptide inhibitor 2_5 on JNK2 levels in HEK293T cells. Densitometric quantification of JNK2 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 µM) or peptide 2_5 (20 or 50 µM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the JNK2 and corresponding β-actin Western blots shown in Figure S2. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. No substantial treatment-related reduction in JNK2 levels was evident under the tested conditions. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK2, c-Jun N-terminal kinase 2; TGF-β, transforming growth factor beta.

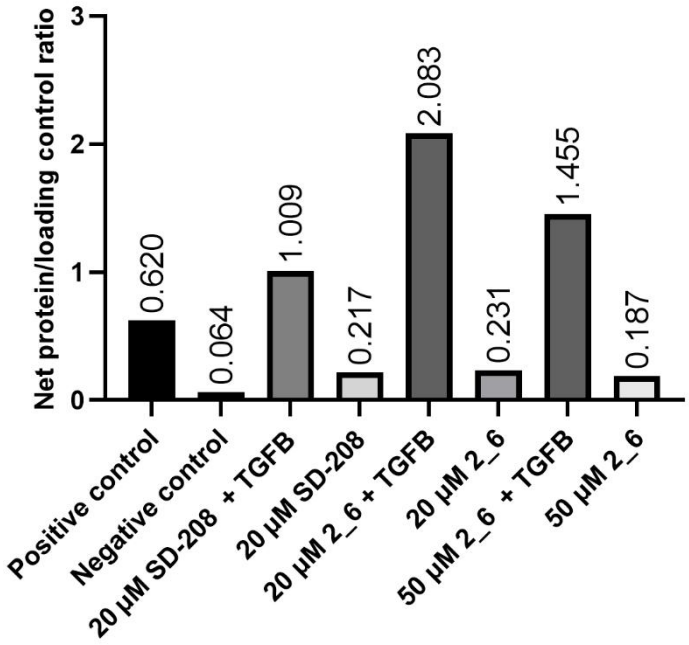


Figure S15. Effects of peptide inhibitor 2_6 on JNK2 levels in HEK293T cells. Densitometric quantification of JNK2 under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_6 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the JNK2 and corresponding β-actin Western blots shown in Figure S3. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. No consistent treatment-related reduction in JNK2 levels was observed under the tested conditions. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK2, c-Jun N-terminal kinase 2; TGF-β, transforming growth factor beta.

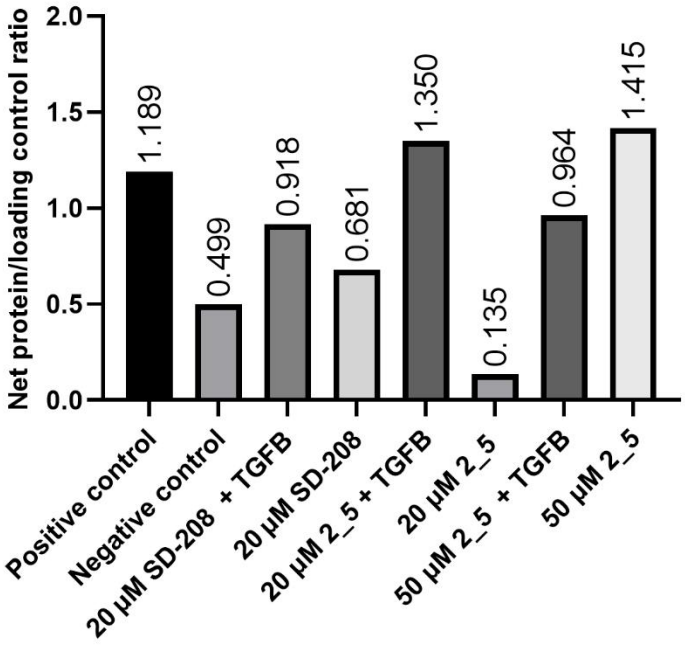


Figure S16. Effects of peptide inhibitor 2_5 on phosphorylated JNK levels in HEK293T cells. Densitometric quantification of p-JNK under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_5 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the p-JNK and corresponding β-actin Western blots shown in Figure S2. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. No consistent treatment-related reduction in p-JNK levels was observed under the tested conditions. Each bar represents a single densitometric measurement from one experiment (n = 1); no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK, c-Jun N-terminal kinase; p-JNK, phosphorylated c-Jun N-terminal kinase; TGF-β, transforming growth factor beta.

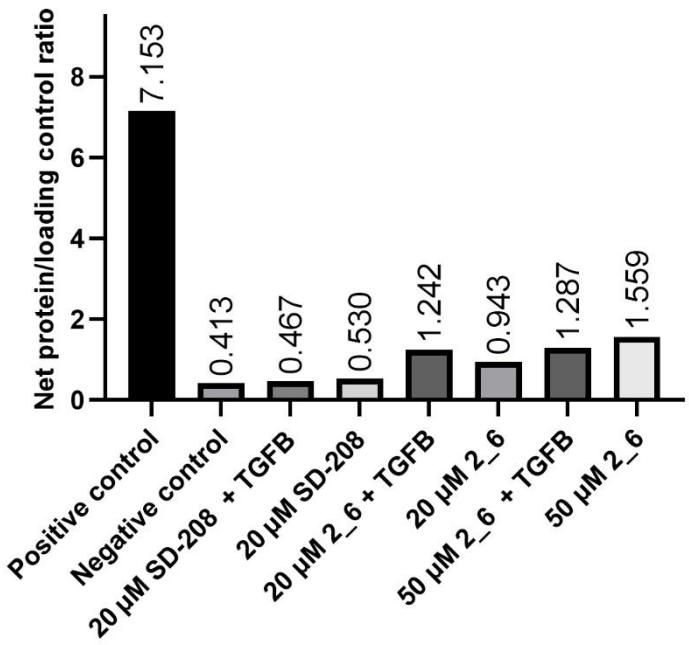


Figure S17. Effects of peptide inhibitor 2_6 on phosphorylated JNK levels in HEK293T cells. Densitometric quantification of p-JNK under the indicated treatment conditions following pretreatment with the small-molecule TGF-β receptor I inhibitor SD-208 (20 μM) or peptide 2_6 (20 or 50 μM), with or without subsequent TGF-β stimulation (20 nM). Positive controls were treated with TGF-β alone, whereas negative controls were untreated. Quantification was based on the p-JNK and corresponding β-actin Western blots shown in Figure S3. Band intensities were normalized to the corresponding β-actin signals and expressed as net protein/loading-control ratios. Each bar represents a single densitometric measurement from one experiment (n = 1); therefore, the observed differences should be interpreted descriptively, and no inferential statistical analysis was performed. **Abbreviations**: HEK293T, human embryonic kidney 293T; JNK, c-Jun N-terminal kinase; p-JNK, phosphorylated c-Jun N-terminal kinase; TGF-β, transforming growth factor beta.