Supplemental Figure Legends

Supplementary Figure S1: *Overexpression of Smad7 and SD-208 in SaOS2 cells blocks the TGF-β/Smad3 cascade.*

A) Left panels: Smad7 production was detected by Western Blot analysis in SaOS2 cells (parental (P), mock- (M) and Smad7-transfected cells (S7) (upper panel). Phospho-Smad3 levels were detected by Western Blot analysis of SaOS2 whole cell lysates treated or not with TGF-β (5 ng/ml, 15 min) in the presence or absence of SD-208 (10 μM). The specificity of the modulation was confirmed with an anti-Smad3 antibody (lower panel). Middle and right panels: SaOS2 cells were transfected with the Smad3/4-specific construct $(CAGA)_9$-luc. 24h after transfection, TGF-β (5 ng/ml) was added in the presence or absence of SD-208 as indicated and incubation was continued for another 48 h. Bars indicate mean ± S.D. of at least three independent experiments carried out in duplicate.

B) SaOS2 cells were treated with TGF-β1 (5 ng/ml) for 6h or 24h in the presence or absence of SD-208 (10 μM, as indicated). After incubations, mRNA steady-state levels of the specific TGF-β target genes *CTGF* (6h), *PAI-1* (24h*)* and *COL1A1* (24h) were determined by quantitative RT-PCR. Bars indicate means ± S.D. of at least three independent experiments, each performed in duplicate (***p<0.005, *p<0.05).

C) HOS cells were treated with BMP-6 (100 ng/ml) for 24h. After incubation, mRNA steady-state levels of the specific BMP target gene ID1 were determined by quantitative RT-PCR. Bars indicate means ± S.D. of at least three independent experiments, each performed in duplicate (***p<0.005, *p<0.05).

D) Parental (P), mock- (M) and Smad7-transfected (S7) HOS cells (upper panel) or parental HOS cells in the presence or absence of SD-208 (10 μM, lower panel) were treated with TGF-β1 (5 ng/ml) for 30 min. After incubation, Phospho-ERK1/2 levels were detected by Western Blot analysis of whole cell lysates. The specificity of the modulation was confirmed with an anti-ERK1/2 antibody.

Supplementary Figure S2: *Overexpression of Smad7 in SaOS2 cells or treatment of SaOS2 cells with SD-208 inhibits the ability of TGF-β to induce osteosarcoma cell migration and invasion*

A and **B**) Left panel: 30 000 parental, mock- or Smad7-transfected SaOS2 cells pre-treated during 24 h with 5 ng/ml TGF-β were seeded onto the upper surface of uncoated (A) or matrigel-coated (B) transwell inserts. 48 h after incubation in the presence or absence of TGF-β (5 ng/ml), the cells on the

underside of the membrane were fixed, stained with “cristal violet” and counted by bright-field microscopy in five random fields (magnification: X200). Bars indicate mean ± S.D. of at least three independent experiments carried out in duplicate (***p<0.005). Right panels: 30 000 parental SaOS2 cells pre-treated during 24 h with 5 ng/ml TGF-β in the presence or absence of SD-208 (as indicated) were seeded onto the upper surface of uncoated (A) or transwell coated with 2µg Matrigel (B) inserts. 48h after incubation in the presence or absence of TGF-β (5 ng/ml) and SD-208 (as indicated), the cells on the underside of the membrane were fixed, stained with “cristal violet” and counted by bright-field microscopy in five random fields. Bars indicate mean ± S.D. of at least three independent experiments carried out in duplicate (***p<0.005).

C) Zymography analysis of conditioned media from 48 h serum-free cultures of SaOS2-P, -M and -S7 cells treated with 5 ng/mL TGF-β or untreated. A Coomassie blue stained gel representative of three independent experiments is shown.

D) SaOS2-P, -M and -S7 cells were treated with TGF-β1 (5 ng/ml) for 24 h (left panel). SaOS2 cells were treated with TGF-β1 (5 ng/ml) in the presence or absence of SD-208 (as indicated) for 24 h (right panel). After incubation, *MMP-2* mRNA steady-state levels were determined by quantitative RT-PCR. Bars indicate mean ± S.D. of at least three independent experiments carried out in duplicate (*p<0.05, **p<0.01).