Supporting Information

Wrighton *et al*. 10.1073/pnas.0800163105

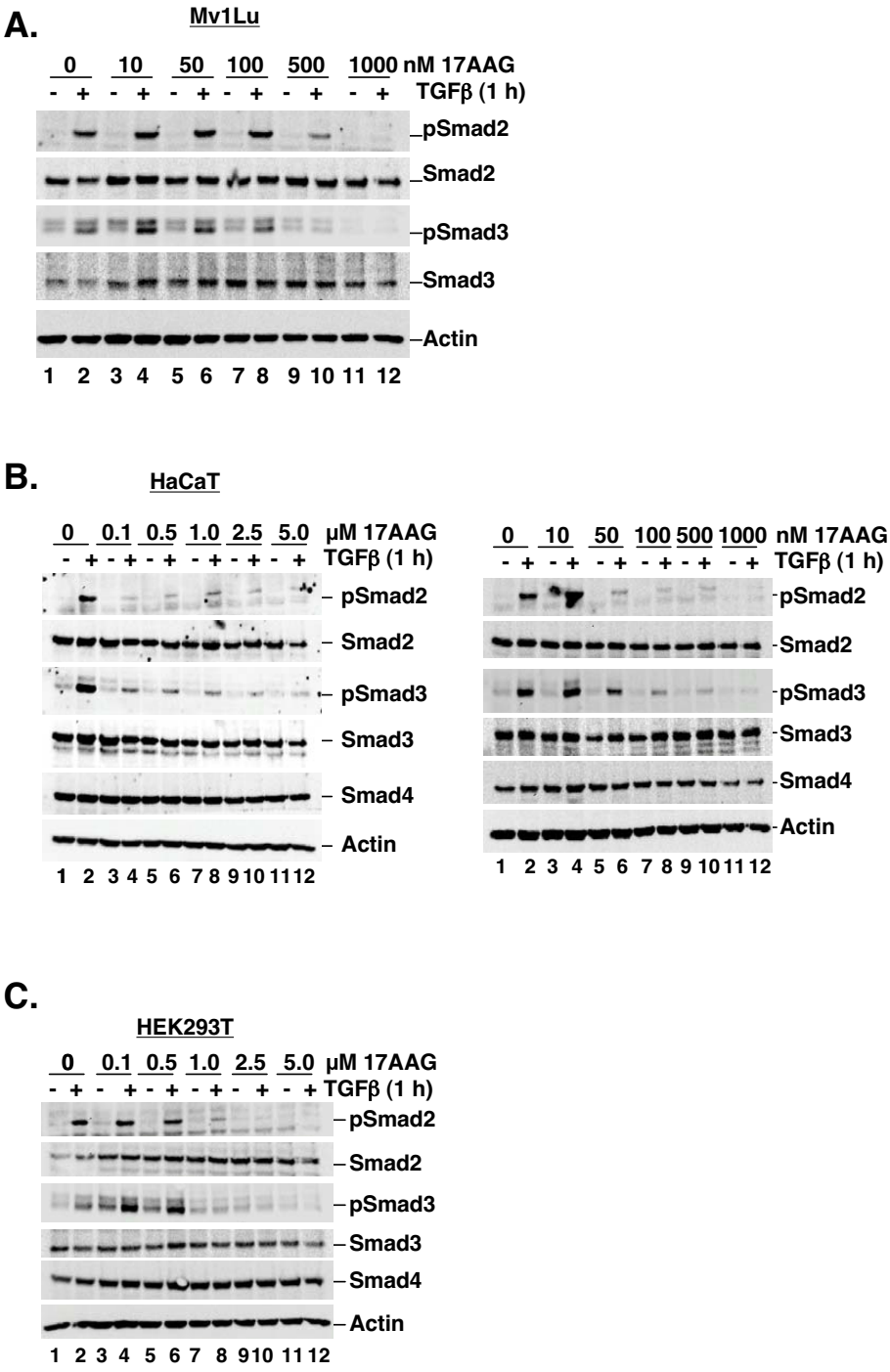

Fig. S1. 17AAG inhibits TGFβ-induced Smad2/3-phosphorylation in a concentration-dependent manner. Mv1Lu cells (*A*), HaCaT cells (*B*), or HEK293T cells (*C*) were treated with the indicated concentration of 17AAG for 6 h, with TGFβ added for the last 1 h. Cells were harvested for Western blotting with anti-Smad, anti-phospho-Smad, and anti-actin antibodies.

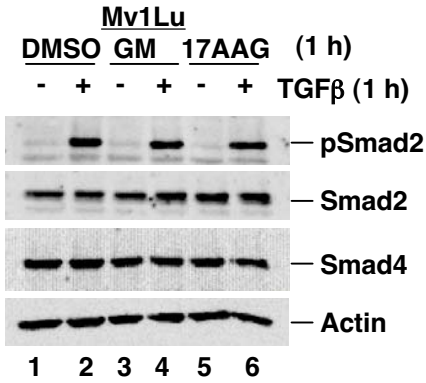

Fig. S2. 17AAG inhibits TGFβ-induced Smad2/3-phosphorylation in a time-dependent manner. Mv1Lu cells were treated with 17AAG or GM for 1 h in the absence or presence of TGFβ. Cells were harvested for Western blotting with anti-Smad, anti-phospho-Smad, and anti-actin antibodies.

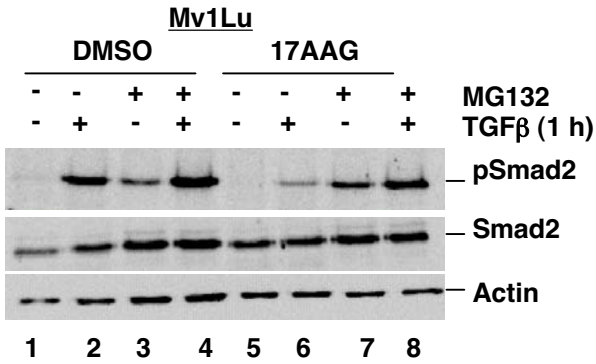

Fig. S3. 17AAG-induced loss of Smad-phosphorylation in Mv1Lu cells can be rescued by MG132. Mv1Lu cells were treated with 1 μM 17AAG for 6 h, with TGFβ added for the last 1 h. Cells were cotreated with MG132 in parallel with 17AAG as indicated. Cells were harvested for Western blotting with anti-Smad2, anti-phospho-Smad2, and anti-actin antibodies.

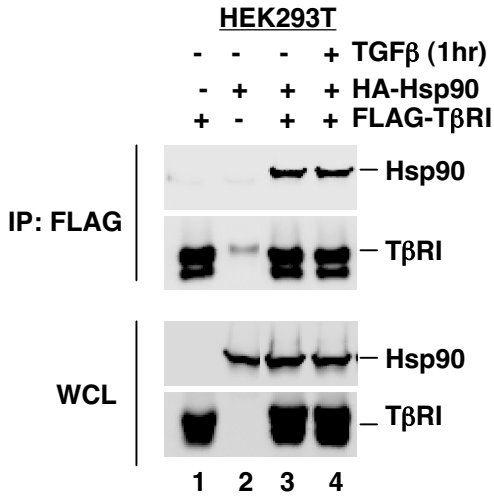

Fig. S4. TGFβ does not regulate TβRI-Hsp90 interactions *in vivo*. HEK293T cells were cotransfected with HA-Hsp90 and FLAG-TβRI, and cultured in the presence or absence of TGFβ for 1 h before harvest. FLAG-TβR-bound Hsp90 was identified by anti-FLAG immunoprecipitation (IP) and anti-HA Western blotting. WCL, whole-cell lysate.

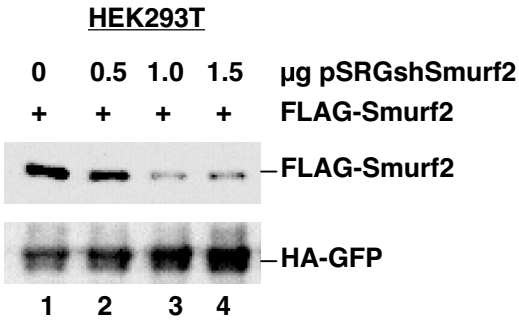

Fig. S5. pSRGshSmurf2 efficiently knocks down FLAG-Smurf2. HEK293T cells were cotransfected with FLAG-Smurf2, HA-GFP, and increasing concentrations of pSRGshSmurf2 as indicated. Cells were harvested for Western blotting with anti-FLAG and anti-HA antibodies.

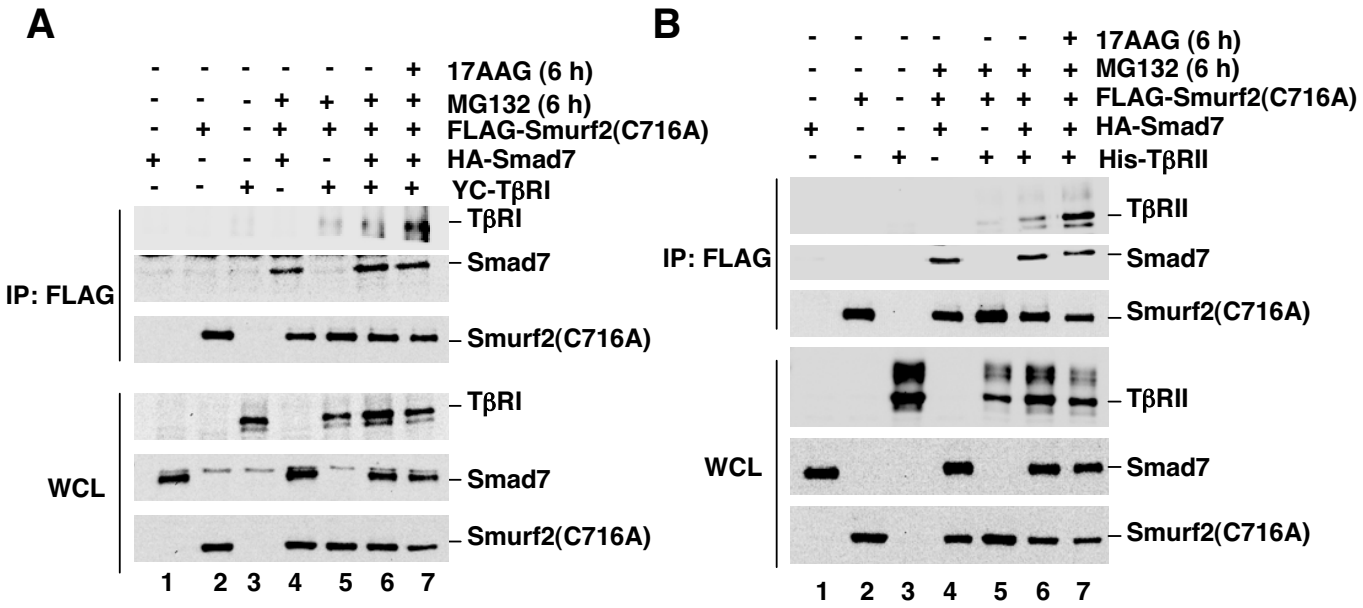

Fig. S6. Hsp90 inhibition by 17AAG promotes TβR-Smurf2 binding. (*A* and *B*) HEK293T cells were cotransfected with HA-Smad7, FLAG-Smurf2(C716A), and YC-TβRI (*A*) or His-TβRII (*B*). Cells were treated with 2 μM 17AAG and/or MG132 for 6 h as specified. FLAG-Smurf2(C716A)-bound TβR was identified by anti-FLAG IP and anti-GFP (*A*) or anti-His (*B*) Western blotting.

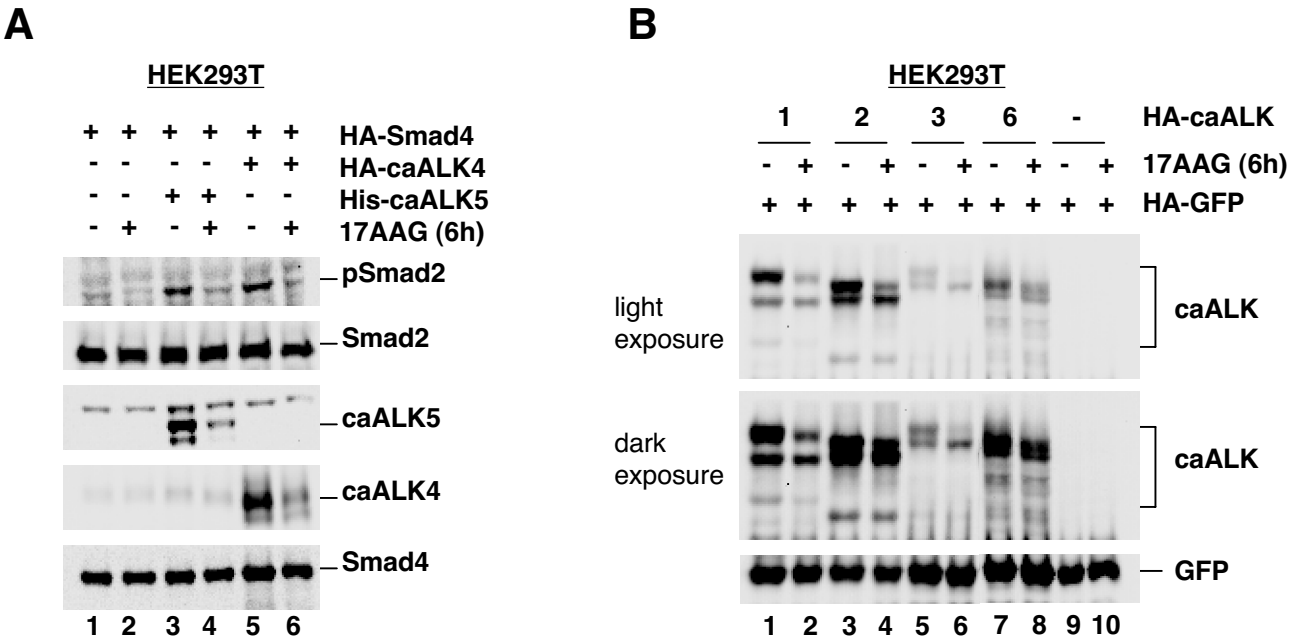

Fig. S7. Hsp90 inhibition by 17AAG decreases the protein level of all six type I TGFβ superfamily receptors assessed. (*A*) 17AAG blocks endogenous Smad2 activation by constitutively active (ca) TβRI and ActRIB. HEK293T cells were transfected with HA-Smad4 and His-caALK5 (constitutively active mutant of TβRI) or His-caALK4 (constitutively active mutant of ActRIB). Cells were treated for 6 h with 2 μM 17AAG or DMSO, before harvest for Western blotting with anti-Smad2, anti-phospho-Smad2, anti-HA, and anti-His antibodies. (*B*) 17AAG reduces the protein level of caALK1 (ACTVRL1), caALK2 (ActRIA/ACVR1), caALK3 (BMPR1A), and caALK6 (BMPR1B). HEK293T cells were transfected with HA-GFP and HA-caALK1, -2, -3, or -6. Cells were treated for 6 h with 2 μM 17AAG or DMSO before harvest for Western blotting with anti-HA antibody.