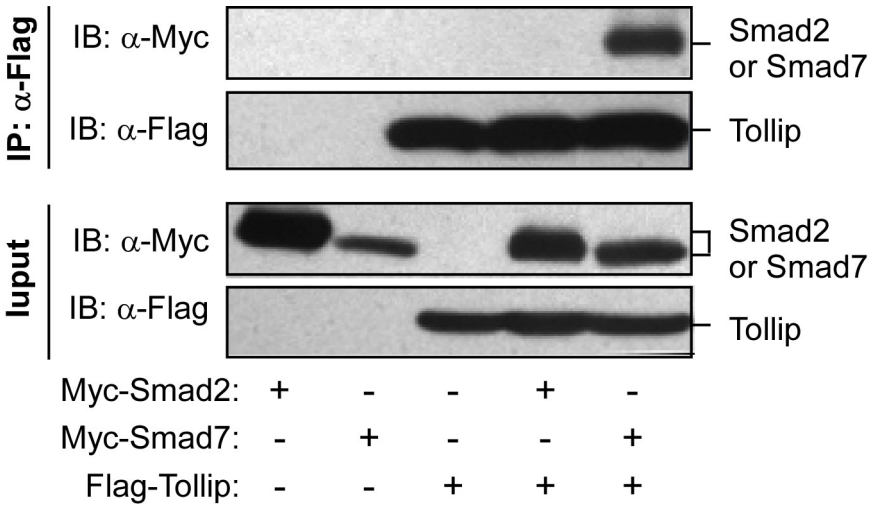
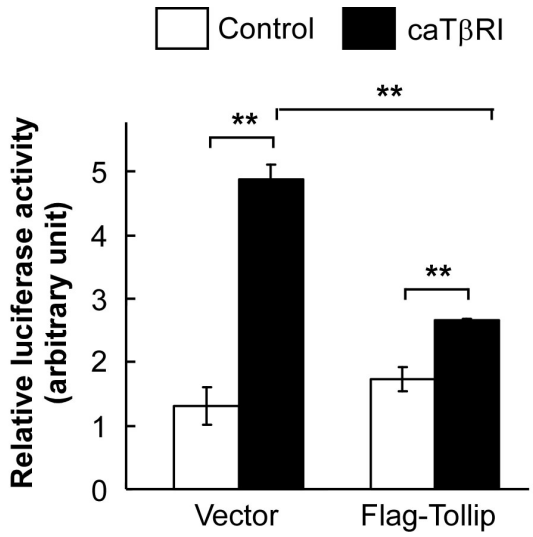
Supplemental Materials

There are a total of seven Supplemental Figures

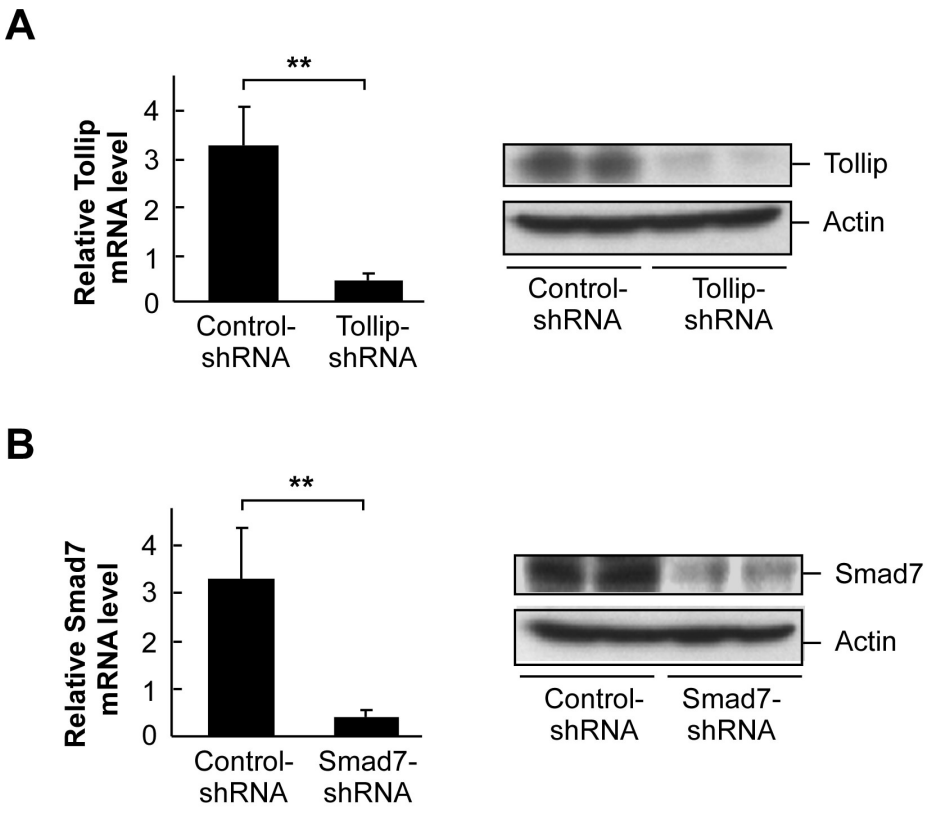


SFigure 1. Tollip does not associate with Smad2
HEK293T cells were transfected with the plasmids as indicated and the cell lysate was subjected to immunoprecipitation (IP) and immunoblotting (IB) using the antibodies as indicated.



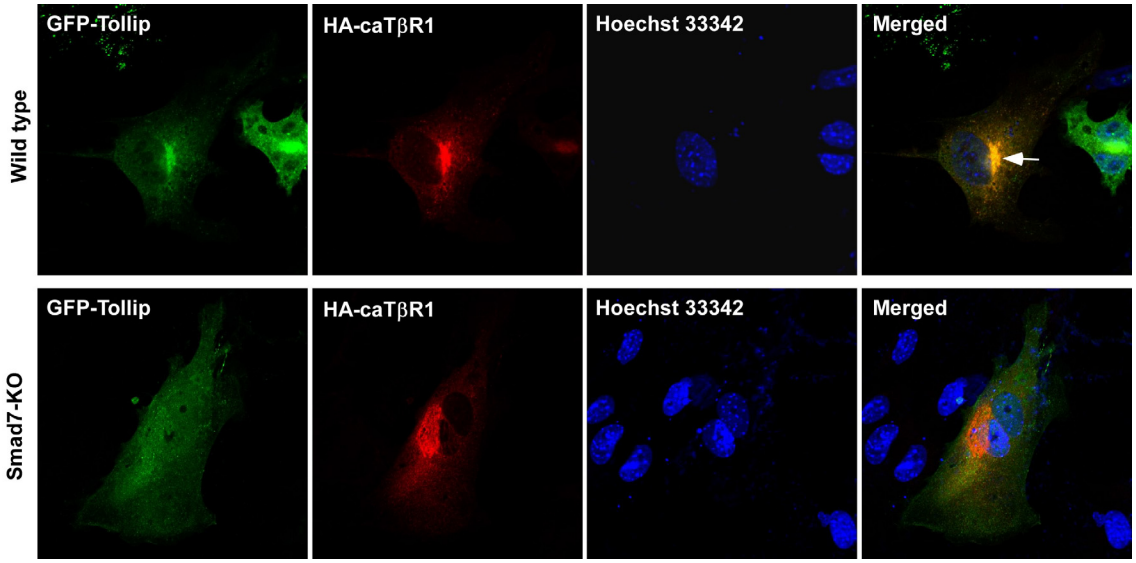
SFigure 2. Tollip suppresses TGF-β induced transcriptional response in HepG2 cells

HepG2 cells were transfected with a luciferase reporter containing 3 repeats of SBE, Renilla-luciferase, Tollip, and constitutively active TβRI (caTβRI) as indicated. Luciferase activity was measured 48 h after transfection. The data are presented as means ± SD after being normalized to Renilla activity. ** indicates $p<0.01$ as comparison between the groups as indicated by *Student's* t-test.

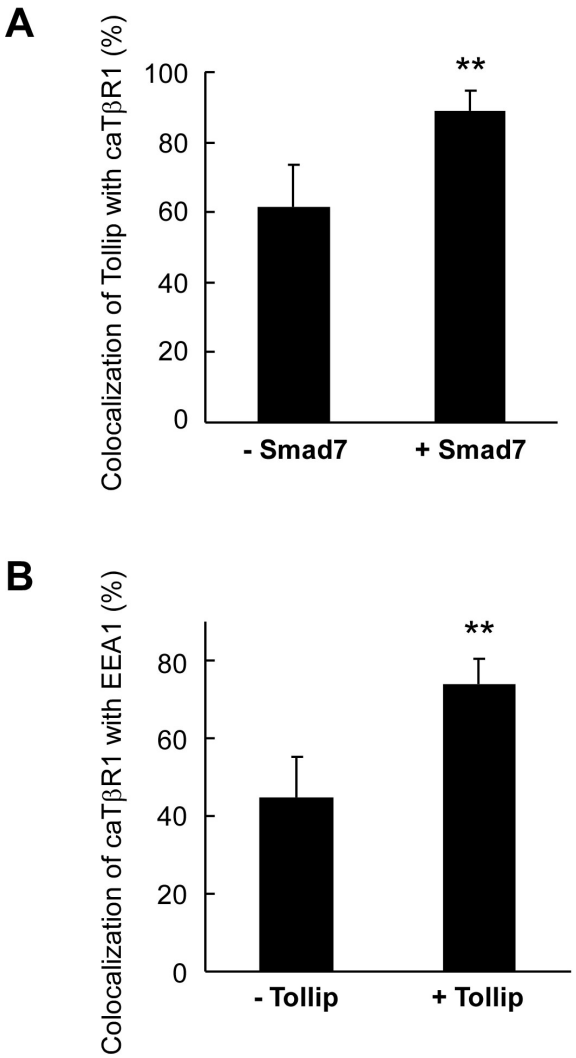


SFigure 3. The effectiveness of using shRNA to knock down Tollip and Smad7 expression

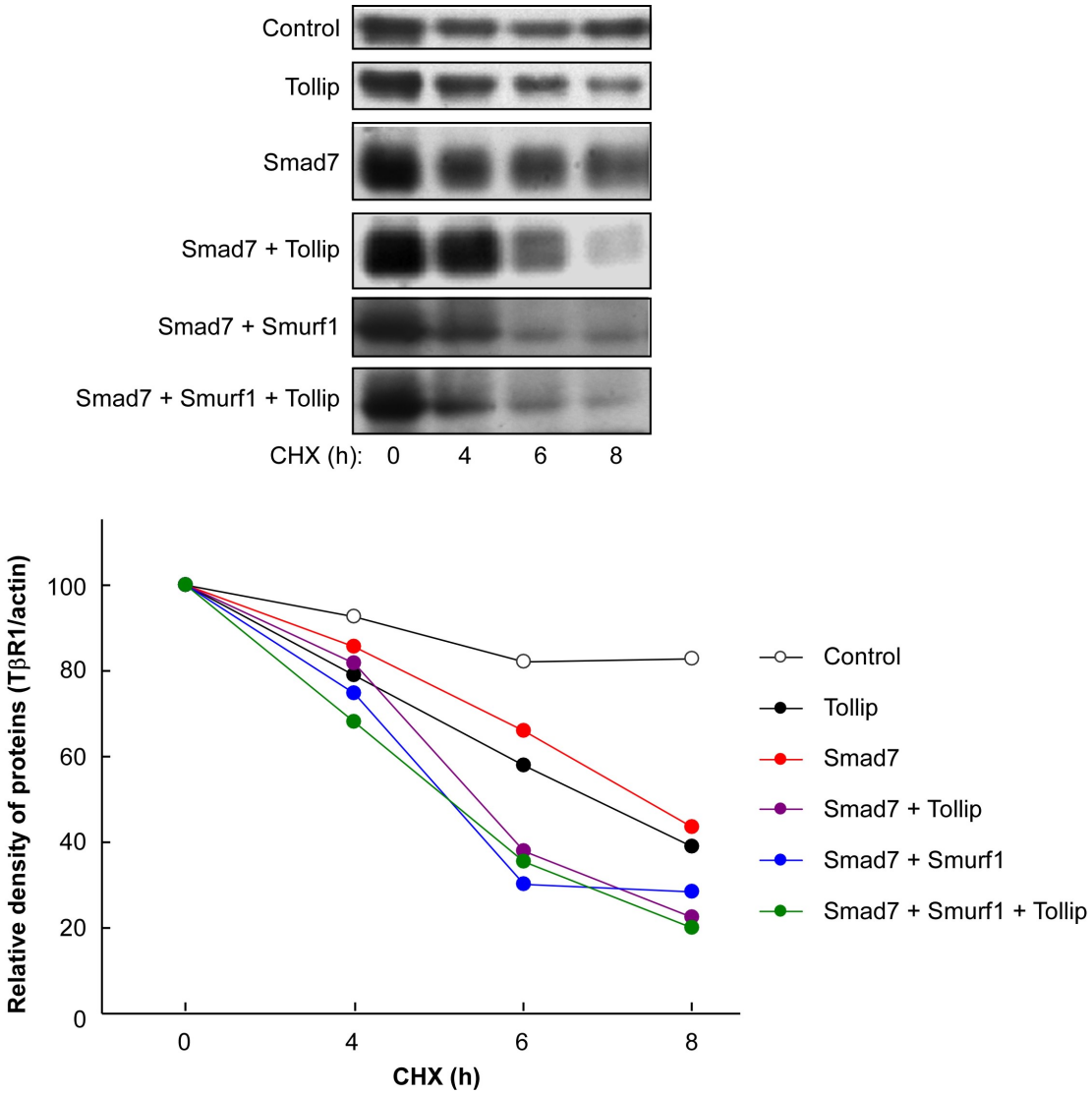
HepG2 cells transfected with control shRNA or shRNA specific for Tollip and Smad7 (as used in Figure 2D and 2E) were used in quantitative RT-PCR and immunoblotting. The RT-PCR data are presented as mean ± SD and ** indicates $p<0.01$ as comparison between the groups as indicated by *Student's* t-test.



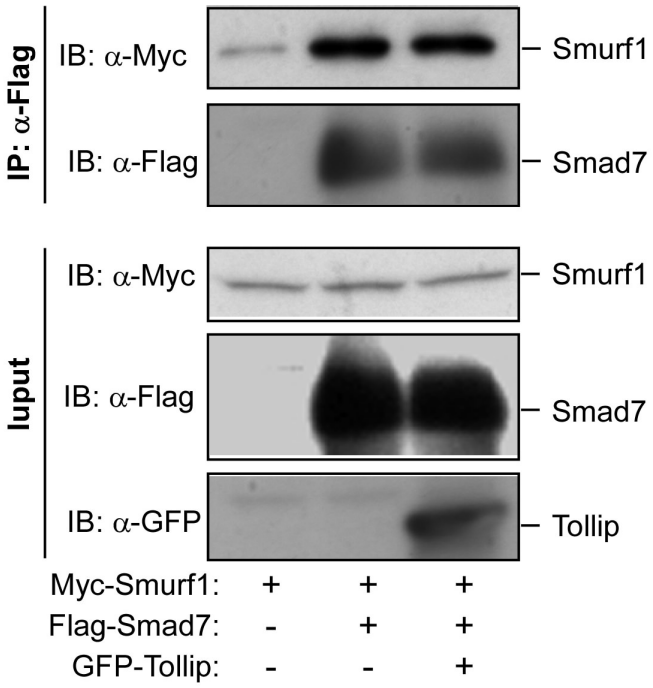
SFigure 4. Smad7 depletion reduces co-localization of Tollip with caTβRI
Wild type and Smad7-deleted MEFs were transfected with the plasmids as indicated, followed by the immunofluorescence staining and confocal analysis. The arrow indicates apparent colocalization of Tollip with caTβRI in wild type MEFs.



SFigure 5. Quantitation of immunostaining images for Figure 4F and Figure 6C
Quantification of colocalization of Tollip with caTβR1 (for Figure 4F) and caTβR1 with EEA1 (for Figure 6C) was performed using LSM images by Zeiss Confocal Microscopy Software and Physiology Software Rel. 3.2 as previously described (Tin et al. PAQR10 and PAQR11 mediate Ras signaling in the Golgi apparatus. Cell Res. 2012;22(4):661-76). We used the Weighted Colocalization Coeffecient of TβR1 signals *v.s.* Tollip signals (for A) or *v.s.* EEA1 signals (for B). The images presented in the figures were captured using standardized setting and exposure times. More than 100 cells were observed in three independent experiments, and at least 20 cells were randomly chosen and quantified for each experimental group. The data are presented as mean ± SD and ** indicates $p<0.01$ as comparison between the groups by *Student's* t-test.



SFigure 6. Tollip has little effect on Smurf1-induced degradation of caTβRI
HEK293T cells were transfected with constitutively active TβRI (HA tagged), Tollip (Flag tagged), Smad7 (Myc tagged) and Smurf1 (Myc tagged) as indicated. At 24 h after transfection, the cells were treated with 50 μg/ml cycloheximide (CHX) for various times, and then harvested for immunoblotting with an antibody against HA to detect caTβRI. The immunoblotting result was quantified by Bandscan software and shown in the lower panel. For simplicity, the statistic results by *Student's* t-test are now shown in picture. $P < 0.05$ is considered to be significantly different. Compared to the "Control" group, the "Tollip" group at 6 and 8 h is significantly different, the "Smad7" group at 8 h is significantly different, the other three groups at 4, 6 and 8 h are significantly different. Compared to the Tollip group, the "Smad7 + Tollip" group at 8 h is significantly different, the "Smad7 + Smurf1" group at 6 h is significantly different, the Smad7 + Smurf1 + Tollip" group at 6 and 8 h is significantly different. Compared to the "Smad7" group, the "Smad7 + Smurf1" group at 6 h is significantly different, the "Smad7 + Smurf1 + Tollip" group at 6 h is significantly different.



SFigure 7. Tollip does not affect association of Smurf1 with Smad7
HEK293T cells were co-transfected with Smurf1 (Myc tagged), Smad7 (Flag tagged) and Tollip (GFP fused) as indicated. The cell lysate was subjected to immunoprecipitation (IP) and immunoblotting (IB) using the antibodies as indicated.