Supplementary information, Figure S2 Myc-TβRI labeling showed minor effect on TGF-β/Smad signaling transduction. (A) TβRI-deficient mink lung epithelial L17 cells were transfected with CAGA-luciferase reporter (200 ng) together with constructs encoding Renilla luciferase (20 ng) and Myc-TβRI (50 ng) or HA-TβRI (50 ng), and labeled with or without the anti-Myc antibody. Then the cells were treated with 10 ng/mL TGF-β1 for 20 h before harvested for luciferase activity measurement. Empty vectors were used to equalize the total amounts of plasmids in each sample. The labeling of Myc-TβRI by anti-myc antibodies showed minor effect on the luciferase activity. Each experiment was performed in triplicate, and the data are presented as means ± SD after normalization to Renilla activity. (B) HeLa cells expressing Myc-TβRI were sequentially incubated with anti-Myc antibodies and Alexa Flour 488-conjugated secondary antibodies at 4 °C. After wash, the cells were incubated at 37 °C for 30 min in the absence or presence of TGF-β1 (10 ng/ml). Then the cells were stained with anti-phosphorylated Smad2/3 antibodies (P-Smad2/3) and DAPI, and imaged by confocal microscopy. The Myc-TβRI and P-Smad2/3 images were merged and shown. The transfection of the cells with Myc-TβRI and labeling the cells with antibodies showed minor effect on the nuclear translocation of P-Smad3. Scale bar, 10 μm.