

Supplementary information, Figure S2 Myc-TBRI labeling showed minor effect on TGF- β /Smad signaling transduction. (A) T β RI-deficient mink lung epithelial L17 cells were transfected with CAGA-luciferase reporter (200 ng) together with constructs encoding Renilla luciferase (20 ng) and Myc-TBRI (50 ng) or HA-TBRI (50 ng), and labeled with or without the anti-Myc antibody. Then the cells were treated with 10 ng/mL TGF- β 1 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-TBRI by anti-myc antibodies showed minor effect on the luciferase activity. Each experiment was performed in triplicate, and the data are presented as means \pm SD after normalization to Renilla activity. (B) HeLa cells expressing Myc-TßRI 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-B1 (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-TßRI and P-Smad2/3 images were merged and shown. The transfection of the cells with Myc-TBRI and labeling the cells with antibodies showed minor effect on the nuclear translocation of P-Smad3. Scale bar, 10 µm.