

Supplementary Figures 1 Legend

Panc-1 cells were transfected with siRNA against Smad2 and Smad3 (A) or Smad2 alone (B) and treated with TGF- β 10ng/ul for 18, 24 or 48h. Total cell lysates were extracted for immunoblot analyses to determine successful depletion of Smad molecules and to analyse the expression levels of NFATc1 and c-Myc. (C) MEF-2 Smad3 wt or MEF-2 Smad3^{-/-} were transfected with control vector or TGF- β RI ligand before cell lysates were extracted for western blot analysis. (D) Reporter gene assays were performed in 8988t cells following transfection of pTARE promoter along with control vector or Smad3 and Smad4 expression vector. Firefly luciferase reporter gene activities of the pTARE promoters were normalized to Renilla luciferase activity and expressed as RLA. Reporter gene activities were expressed as mean fold induction compared to control that was arbitrarily set to 1. (E) Calcineurin phosphatase activity was determined in 8988t cells through measurement of the phosphate released from the RII phosphopeptide after treating the cells with TGF- β 10ng/ul for 6hr, 12hr, 24hrs, 48hrs. Mean values were calculated from three independent experiments and are shown as mean \pm SD. (F) DNA pull-down experiment (top blot) demonstrating inverse binding of NFAT factors and Smad3 on the c-Myc/TIE upon TGF- β . Nuclear extracts (bottom blot) from 8988t cells were prepared and incubated with the wild-type c-Myc/TIE oligonucleotide sequence. DNA-protein complexes were precipitated with streptavidin-agarose beads, and NFAT/Smad3 binding was analyzed by western blotting using anti-NFATc2 and anti-Smad3 antibodies, respectively.

