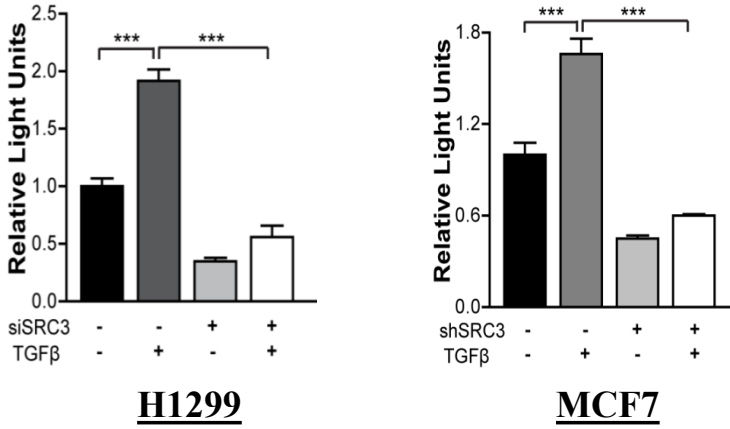
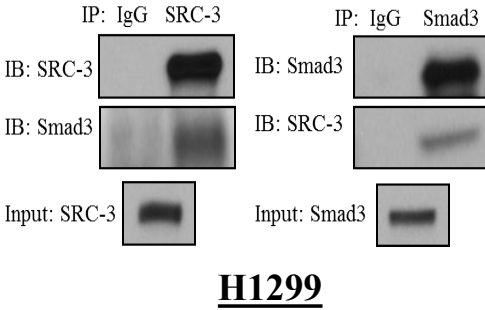


# Supplementary Figure 1

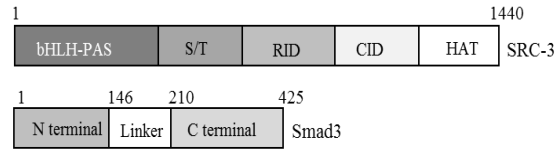
**A.**



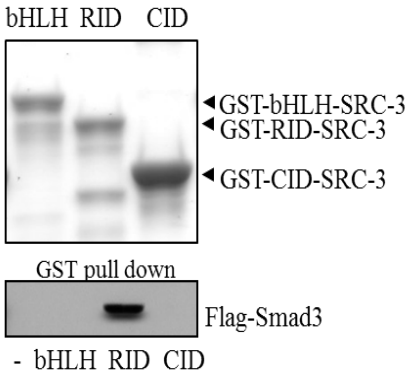
**B.**



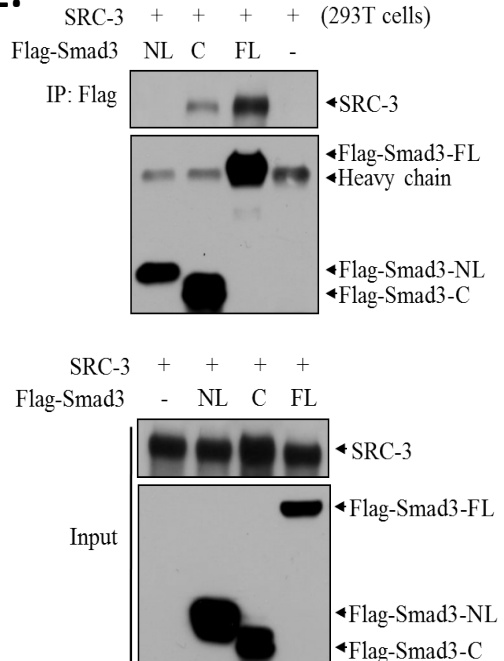
**C.**



**D.**



**E.**



### Supplementary Figure 1. SRC-3 coactivates TGF $\beta$ signaling through interaction with Smad3.

(A) Decreased expression of SRC-3 represses TGF $\beta$  mediated Smad transcriptional activation. *Left panel.* H1299 cells were transfected with either non-targeting (NT siRNA) or SRC-3 (siSRC-3) siRNA. 24 hours later cells were transfected with a Smad binding element promoter luciferase reporter (SBE4-luc) followed by treatment with either DPBS or 5 nM TGF $\beta$ . *Right panel.* Stable MCF7 cells expressing either NT shRNA or shSRC-3 were transfected with SBE4-luc reporter plasmid and placed in phenol red free DMEM with 5 % charcoal stripped serum. The next day transfected MCF7 cells were serum starved following which they were treated with either DPBS or 10 nM TGF $\beta$ . 24 hours post treatment both cell lines were lysed and total protein concentration and luciferase activity were measured. Luciferase activity was normalized to total protein. Fold change was calculated by setting the control to 1. Values are means  $\pm$  S.E.M. (n=3). One-way ANOVA and Tukey's multiple comparison tests were used for significance testing. \*\*\* =  $p < 0.005$  (B) SRC-3 and Smad3 are in a complex with each other. Total protein was isolated from H1299 cells and immunoprecipitated with antibodies to either SRC-3 or Smad3 and control IgG followed by immunoblotting. (C) Schematic for SRC-3 and Smad3 protein domains. SRC-3 protein domains - N-terminal basic helix-loop-helix (bHLH)/Per/Arnt/Sim (bHLH-PAS) domain, a nuclear receptor interaction domain (RID), CBP interaction domain (CID), histone acetyltransferase domain (HAT). Smad3 protein domains – N-terminal (N), linker (L), C-terminal (C). (D) SRC-3 RID interacts with Smad3. Total protein isolated from 293T cells transfected with a Flag-Smad3 expression vector was incubated with GST tagged domains of SRC-3 protein. Immunoblot analysis with an antibody to Flag (bottom panel) followed the GST pulldown. Top panel: Coomassie stained gel demonstrating GST-SRC-3 protein levels. (E) The C-terminal region of Smad3 is required for interaction with SRC-3. 293T cells were cotransfected with expression vectors for full length SRC-3 and either N terminal/Linker (NL), C terminal (C) or full length (FL) Smad3. Top panel: Protein lysates were immunoprecipitated with Flag antibody followed by immunoblotting with SRC-3 and Flag antibodies. Bottom panel: Protein lysates (input) were immunoblotted with antibodies to Flag and SRC-3.