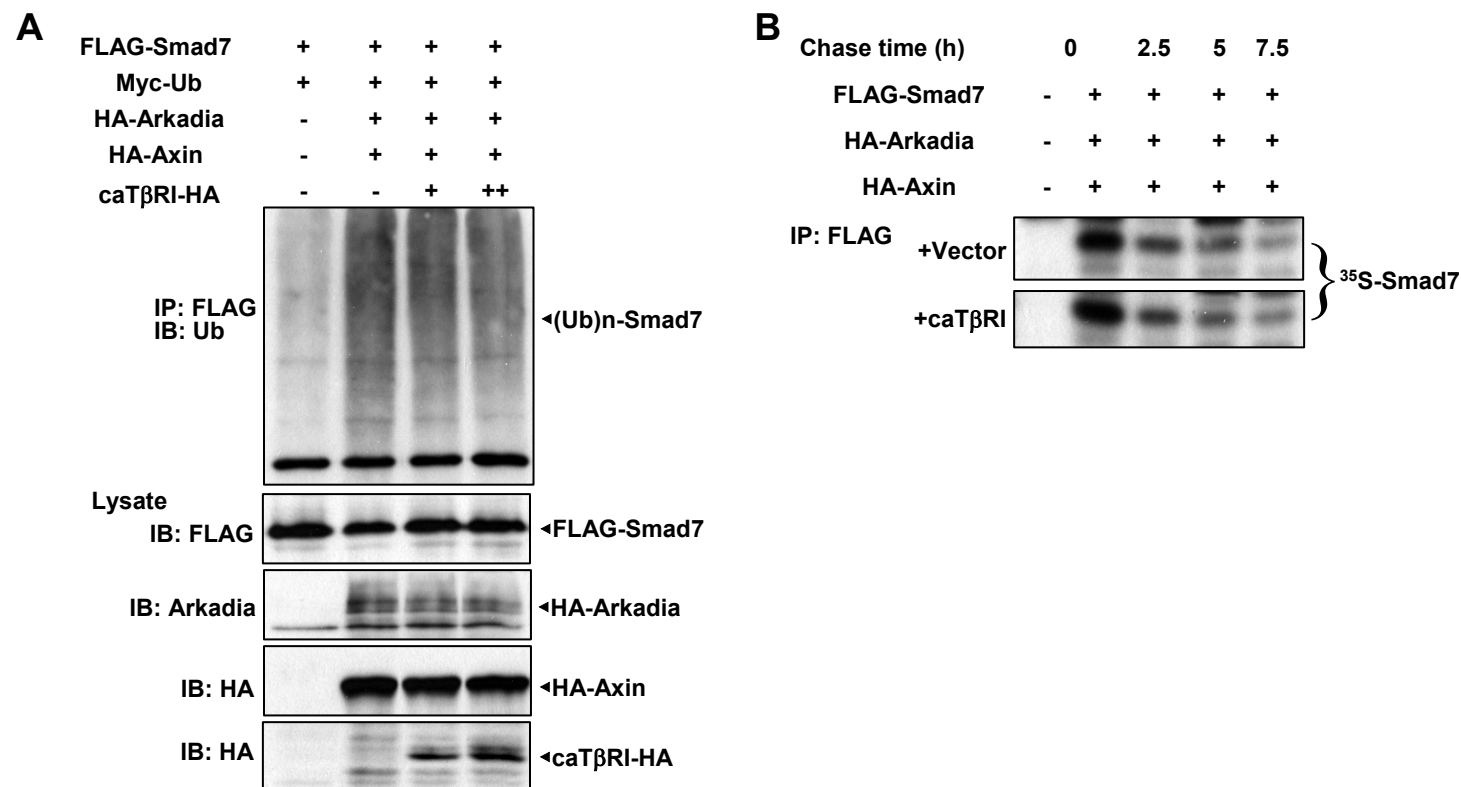


## Supplementary Figure 4



**Figure S4.** TGF- $\beta$  does not have an effect on Arkadia/Axin-induced poly-ubiquitination and degradation of Smad7. (A) FLAG-Smad7, Myc-Ub, with or without Arkadia, Axin were cotransfected with increasing amounts of caT $\beta$ RI. Cells were treated with 10  $\mu$ M MG132 for additional 4 h at 32 h post-transfection and were then lysed for immunoprecipitation with anti-FLAG, followed by Western blotting with different antibodies as indicated.

(B) HEK293T cells were transfected with FLAG-Smad7, Arkadia, and Axin in the presence or absence of CaT $\beta$ RI. At 36 h after transfection, cells were treated with 100  $\mu$ Ci/ml [ $^{35}$ S] methionine and cysteine in Methionine- and cysteine-free DMEM for 1 h at 37  $^{\circ}$ C, then washed with PBS and chased in DMEM supplemented with 10% FBS, 2 mM methionine and 0.5 mM cysteine for the time periods indicated. Cells were lysed with RIPA buffer and subjected to immunoprecipitation with anti-FLAG, followed by SDS-PAGE.