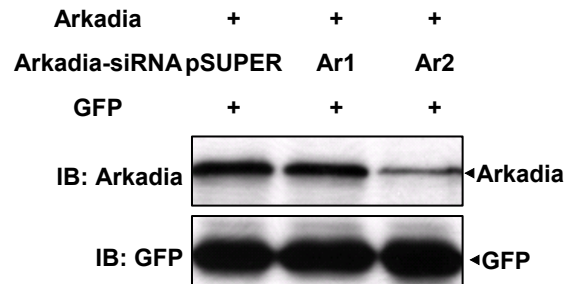
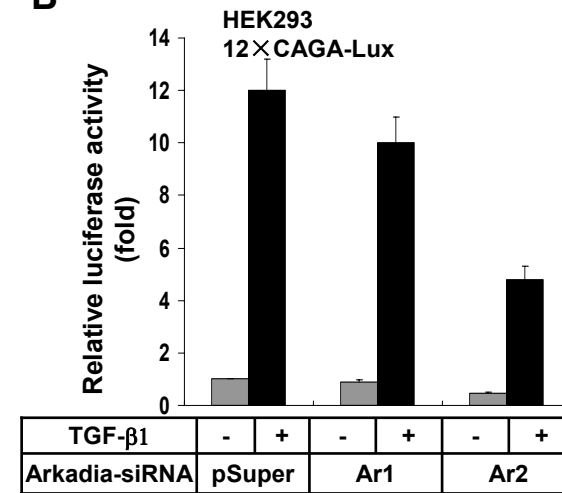


Supplementary Figure 3

A



B



C

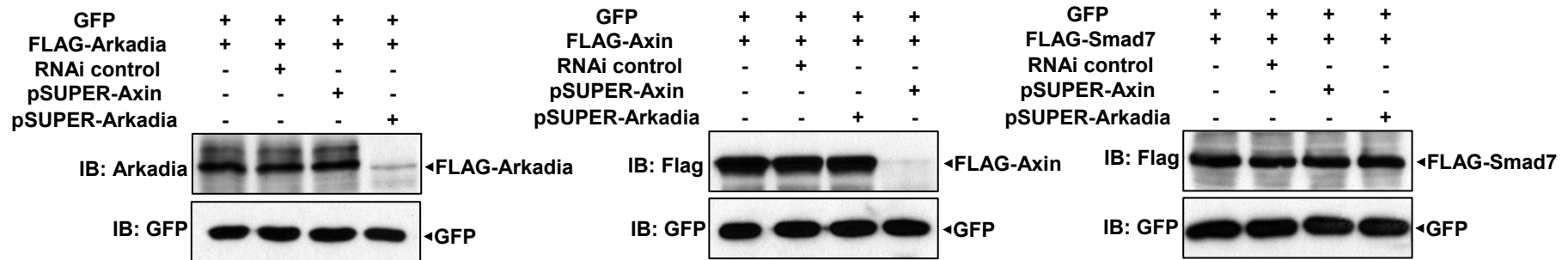


Figure S3. Characterization of the efficiency of siRNAs against Arkadia and Axin.

(A) The pSUPER constructs against Arkadia (Ar1 and Ar2) were separately cotransfected with Arkadia. pEGFPN1 was transfected as an internal control for similar transfection efficiency. Cell lysates were analyzed by western blotting and Ar2 was found to be more efficient than Ar1 and was hence used in the experiments shown in Figure 5C and Figure 6D.

(B) HEK293 cells transfected with 12×CAGA-Lux and pSUPER constructs indicated were treated with or without TGF-β. All transfections were performed in duplicate and the data are mean ± s.d. of three independent experiments after normalizing luciferase activity from the vector control to 1. Ar2, but not Ar1, can significantly inhibit the TGF-β signaling.

(C) The pSUPER-Arkadia and pSUPER-Axin were separately cotransfected with FLAG-ArC937A (left panel), FLAG-Axin (middle panel) and FLAG-Smad7 (right panel) into 293T cells, together with 0.5 μg of pEGFPN1 as an internal control. After 36 h, cells were harvested and blotted with antibodies indicated to examine the specificity of the respective siRNA.