Supplementary Materials and Methods:

RNA Interference

The pSUPER vector was used as siRNA expression vector (gift of Dr. Agami) to knock down the expression of Arkadia. Oligonucleotides harboring 19-mer siRNA sequences for human Arkadia (Ar1: 5'-ccaggttactgccaatgaa-3'; Ar2: 5'-agctatcggtctcgttcaa-3') were separately inserted into the pSUPER vector, according to the instructions (Brummelkamp *et al*, 2002). To test for the efficiency of the siRNA expression vectors, cells were cotransfected with 4 μg of pSUPER-Ar1 or pSUPER-Ar2 and 2 μg of Flag-Arkadia, together with 0.3 μg of pEGFPN1 in each transfection as a control for transfection efficiency. After 36 h, cells were lysed, and the expression of Arkadia was analyzed by immunoblotting.

Supplementary References

Brummelkamp TR, Bernards R, and Agami R (2002). A system for stable expression of short interfering RNAs in mammalian cells. *Science* **296:** 550-553

Supplementary figure legends

Supplementary figure 1 Characterization of the anti-Arkadia rabbit polyclonal antibody that was raised against a bacterially expressed GST-fusion protein containing aa1-132 of mouse Arkadia.

(**A**) 293T cells were transfected with Flag-Axin, Flag-Smad7, Flag-ArC937A and Flag-ArΔ1-240, respectively. Equal volumes of cell lysates were analyzed by western blotting using anti-Flag to indicate the expression of the transfected proteins (lower panel). When the same samples were probed with the anti-Arkadia polyclonal antibody, only Flag-tagged full-length Arkadia was detected (upper panel).

(**B**) 293T cells were transfected as indicated. Cell lysates were subjected to immunoprecipitation with anti-Arkadia, and Flag-tagged protein was detected only in the immunoprecipitates from cells overexpressing Arkadia.

Supplementary figure 2 Interaction of Axin2/Conductin with Arkadia and Smad7.

(**A**) HA-Conductin and Flag-ArC937A were separately transfected into 293T cells and immunoprecipitation was performed with anti-Flag antibody. HA-Conductin was detected in the Arkadia immunoprecipitate by western blotting with anti-HA antibody.

(**B**) 293T cells were transfected as indicated and subjected to immunoprecipitation with anti-HA antibody. Immunoblotting of the precipitates with anti-Myc antibody shows that Smad7 can be detected in the Conductin immunoprecipitate.

Supplementary figure 3 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 Ar1and 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 mg 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.

Supplementary figure 4 TGF-β 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 caTβRI. Cells were treated with 10 mM 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 CaTbRI. At 36 h after transfection, cells were treated with 100 mCi/ml [^{35}S] methionine and cysteine in Methionine- and cysteine-free DMEM for 1 h at 37 oC, 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.