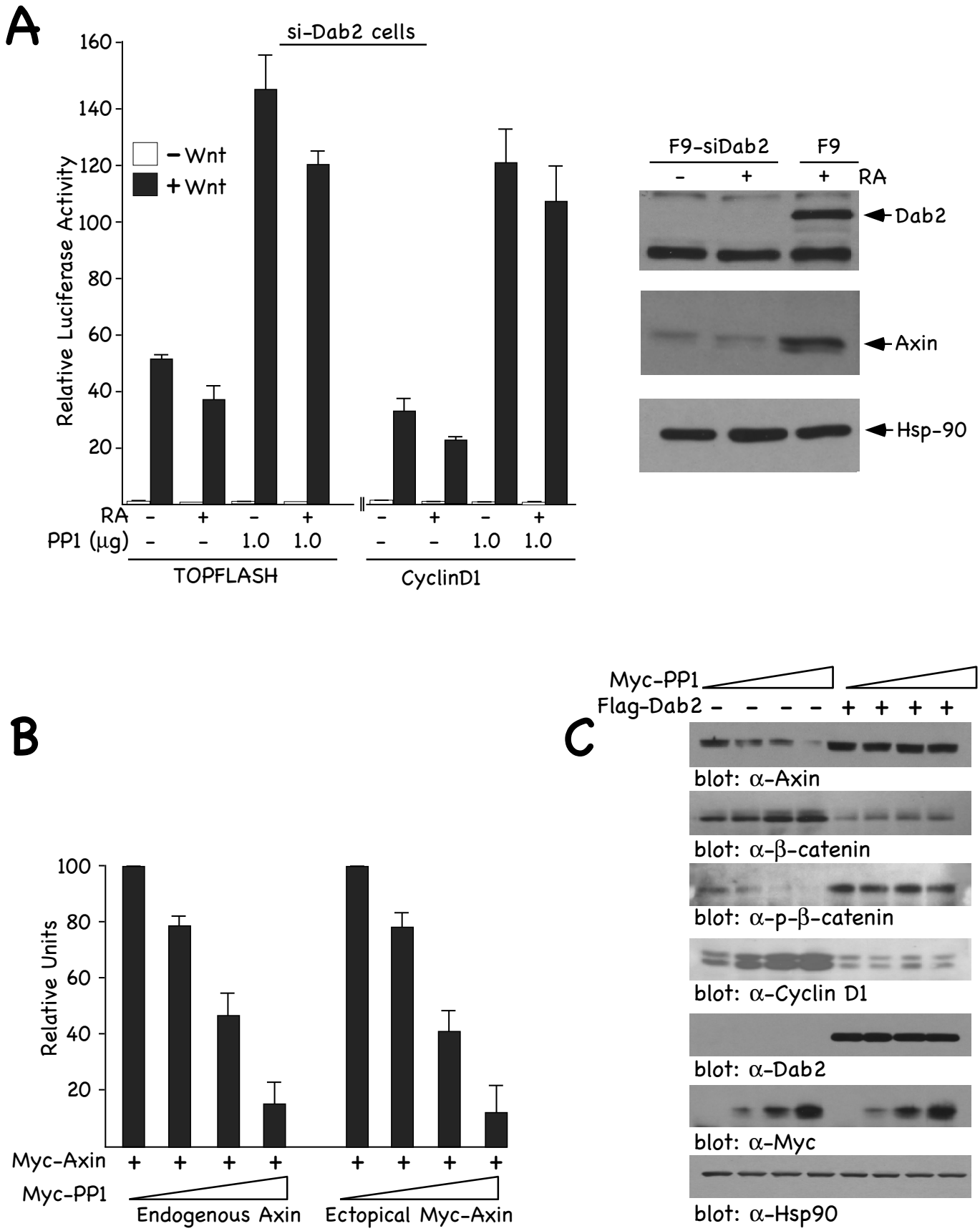
Figure S1


A
si-Dab2 cells
Relative Luciferase Activity
160
140
120
100
80
60
40
20
− Wnt
+ Wnt
RA − + − + − + − +
PP1 (μg) − − 1.0 1.0 − − 1.0 1.0
TOPFLASH
CyclinD1
F9-siDab2
F9
− + + RA
Dab2
Axin
Hsp-90
B
Relative Units
100
80
60
40
20
Myc-Axin + + + + + + + +
Myc-PP1
Endogenous Axin
Ectopical Myc-Axin
C
Myc-PP1
Flag-Dab2 − − − − + + + +
blot: α-Axin
blot: α-β-catenin
blot: α-p-β-catenin
blot: α-Cyclin D1
blot: α-Dab2
blot: α-Myc
blot: α-Hsp90

Supplemental Information

Materials and Methods

Cell culture, transient transfection and luciferase assay: F9 and F9-siDab2 mouse embryonic carcinoma cells were cultured and differentiated with RA (100 nM) as described (Jiang *et al*., 2008). Transfections were performed using Lipofectamine reagent according to the manufacturer (Invitrogen). For luciferase reporter and transient expression assays, cells were transiently transfected with Lipofectamine 2000 (Invitrogen) as previously described (Hocevar et al., 2003). Relative luciferase activity is expressed as the ratio of TOP/FOPFLASH and cyclin D1 activity in control and Wnt-3A-treated cells. All assays were performed in at least triplicate and the data are presented as means +/- standard deviation. Wnt-3A conditioned media was prepared from mouse L cells overexpressing Wnt-3A as described previously (Shibamoto *et al*., 1998).

DNA constructs and antibodies: TOPFLASH, FOPFLASH luciferase reporter constructs were obtained from Millipore (Lake Placid, NY, USA). The cyclin D1 construct has been described previously (Hocevar *et al*., 2003). Expression constructs pcDNA3-myc-PP1 Dr. David M. Virshup (Luo *et al*., 2007), and pCMV5-HA-Axin WT, Axin NT and Axin CT were kindly provided by Dr. Sheng-Cai Lin. Myc-tagged Axin, Flag-tagged wild-type Dab2, Flag-tagged F166V mutant Dab2 and Flag-tagged truncation mutants 1-247, 1-322, 323-770, 534-770, and Flag-tagged LRP5 have been described previously (Jiang *et al*., 2008; Hocevar et al., 2001). α-Myc-tag, α-cyclin D1, α-HA-Tag, and α-p-β-catenin antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). α-p96 Dab2, α-GSK3β and α-β-catenin antibodies were purchased from Transduction Laboratories (San Jose, CA, USA). α-Flag antibody was from Sigma-Aldrich (St. Louis, MO, USA). α-Axin antibody was purchased from Millipore (Lake Placid, NY, USA), and α-p-serine from ZyMed (San Francisco, CA, USA).

Preparation of cell lysates, immunoprecipitation, and immunoblot analysis: For immunoprecipitation and immunoblot analyses, F9 cells were lysed in 'buffer D' and immunoprecipitation and immunoblotting analyses carried out as previously described (Hocevar *et al*., 2003). For zebrafish embryos, lysates were prepared by first removing the yolk sac from embryos with forceps, followed by Dounce homogenization and sonication in 'buffer D'.

Figure Legend

Figure S1. PP1 activates and Dab2 inhibits Wnt/β-catenin signaling.(A) F9-siDab2 cells were treated (+) or non-treated (-) with RA (100 nM) for 72h were co-transfected with TOP/FOPFLASH or cyclin D1 luciferase reporters +/- 1μg PP1. Luciferase activity was assayed and expressed in control and Wnt-3A-treated cells. The expression levels of Dab2 and Axin in these cells following RA treatment is shown in the right hand panel. (B) PP1 expression attenuates Axin expression levels. The experiment in Fig 1D was repeated three independent times and bands corresponding to either endogenous or ectopically expressed Axin were scanned and quantified using a STORM scanner and ImageQuant software from Molecular Dynamics (Fairfield, CT, USA). Levels are expressed as relative intensities at the various points. The data are indicated as means +/- s.d. (C) PP1 expression upregulates β-catenin and cyclin D1 expression levels and this upregulation is blocked by Dab2. F9 cells were transfected with increasing concentrations of PP1 (2, 4, 8 μg) +/- co-transfected Dab2 (5 μg) and WCLs were prepared and analyzed by immunoblotting using α-Axin, α-β-catenin, α-p-β-catenin, and α-cyclin D1 antibodies. Hsp-90 immunoblot analysis of lysates was used as loading controls.