Supplemental Methods:

Plasmids: For Axin-ΔEx4-5 one fragment was amplified to generate exons 2 to 3 and the second fragment was amplified to generate exons 6 to 11. For Axin-ΔEx4-9, exons 2 to 3 were generated and the second fragment was amplified to generate exons 10 to 11. For Axin-ΔEx8-9 one fragment was amplified to get exons 2 to 7 and the second fragment was amplified to get exons 10 to 11. N- and C- terminal fragments of Axin were directly cloned into the mammalian expression vector pDEST40 by Gateway cloning per manufacturers instructions. Internal deletion mutants of Axin1 generated using primers containing either BamHI or BglII restriction sites (underlined in primer sequence, Suppl. Table 1) were digested with BamHI or BglII and purified via gel extraction kit, QIAEX II (Qiagen). Pure, digested PCR products were ligated using T4 DNA ligase (Roche) and Gateway cloned into pDEST40 mammalian expression vector. Full-length Axin1 with a stop codon was cloned into pT-REX-Dest30 (Invitrogen) via Gateway cloning to be expressed in mammalian cells without a tag.

Cell lines: Stable 293tr-V5-Axin1 cells were generated by infecting 100mm dish of HEK-293 cells (ATCC) with ~10 MOI Lentivirus encoding the tet-repressor, pLenti6/TR (Invitrogen), in 5mL DMEM supplemented with 10% FBS, 1mM L-glutamine and 6mg/mL polybrene (Specialty Media) for 12 hours. Media was changed to DMEM supplemented with 10% FBS, 1mM L-glutamine and 1X penicillin/streptomycin and grown 36 hours. Media was changed and maintained in DMEM supplemented with 10% FBS, 1mM L-glutamine, 1X penicillin/streptomycin and 5μg/mL Blasticidin (Invitrogen) for 10 days until distinct colonies formed. Six colonies were picked, expanded and

screened for their ability to suppress the expression of CMV-driven HA-tagged c-Myc expressed from pT-REX-Dest30-HA-c-Myc by transient transfection as described above. The best suppressing colony was then infected with ~10 MOI Lentivirus expressing V5-Axin1, pLenti4/TO/V5-Dest-Axin1, as described for the tet-repressor infection, described above. Cells were selected in DMEM supplemented with 10% FBS, 1mM L-glutamine, 1X penicillin/streptomycin, 5μg/mL Blasticidin (Invitrogen) and 100μg/mL Zeocin (Invitrogen) for 10 days until distinct colonies formed. Six colonies were picked, expanded and screened for the ability to only express V5-Axin1 when treated with 1μg/mL Dox. The best clone was then used for further experiments and continually maintained in DMEM supplemented with 10% defined FBS, 2mM L-glutamine, 5μg/mL Blasticidin and 100μg/mL Zeocin.

RIPA lysis buffer: 10 mM Tris, pH 8; 150mM NaCl; 1mM EDTA; 1% Triton X-100; 1% Na-Deoxycholate; 0.1% SDS; 10mM NaF; 100μM NaVanadate, Protease and phosphatase inhibitors.

Ab lysis buffer: 20 mM Tris, pH 7.5; 50mM NaCl; 1mM EDTA; 0.5% Triton X-100; 0.5% Na-Deoxycholate; 0.5% SDS; 10mM NaF; 100μM NaVanadate, Protease and phosphatase Inhibitors.

Protease and phosphatase inhibitors: 10mM sodium fluoride, 100μM sodium vanadate, 10mM β-glycerolphosphate disodium pentahydrate, 1ug/ml aprotinin, 1ug/ml pepstatin, 0.5ug/ml leupeptin, 0.2mg/ml AEBSF, and 1.6mg/ml iodoacetamide.

Western blotting: Samples were separated by SDS-PAGE with sample load volumes adjusted to reflect transfection efficiencies and transferred to Immobilon-FL (Millipore, Billerica, MA). Odyssey Block Buffer was used to block membranes (LI-COR Biosciences, Lincoln, Nebraska), except when probed with α-P-T58-c-Myc antibody, which used 5% non-fat milk in PBS for blocking. Primary antibodies diluted in 1:1 Odyssey Block Buffer:PBS + 0.05% Tween or 2.5% milk in PBS + 0.05% Tween (P-T58 antibody) at indicated dilutions were detected by secondary anti-mouse and anti-rabbit near-infrared fluorescent dyes Alexa Fluor 680 (Molecular Probes, Eugene, OR) and IRDye800 (Rockland, Philadelphia, PA) used at 1:10K dilution in 1:1 Odyssey Block Buffer:1X PBS + 0.05% Tween or 2.5% non-fat milk in PBS + 0.05% Tween (P-T58 antibody). Immunoblots were visualized via LI-COR Odyssey Infrared Imager (Lincoln, Nebraska) that can simultaneously detect anti-rabbit and anti-mouse. Quantification of western blots was done using LI-COR Odyssey Infrared Imager software version 1.2. Error bars in graphs represent two standard deviations as calculated using Excel (Microsoft, Redmond, WA). T-test analysis (two-tailed distribution and two-sample unequal variance) was done using Excel to determine statistical differences as indicated on graphs.

Immunofluoresence: Cells were fixed in 4% paraformadehyde/HEPES buffer for 10 minutes on ice and then permeabilized with 0.5% triton in PBS for 10 minutes. Cells were blocked with 2% BSA in PBS (.05% Tween) for 2 hours on ice. α-V5 was added at 1:50 for 4 hours on ice followed by 4 washes in block media. FITC conjugated secondary

antibody (Molecular Probes) was added at 1:500 for 1 hour at room temperature followed by an incubation with 1:5,000 dilution of DAPI. Immunofluoresence was visualized on a Leica DMR microscope using de-convolution software.

Supplemental Table 1: Primer and shRNA target sequences.

Axin Primers	**Sequence:**
Axin1 Forward TOPO	5'- CACCATGGATATCCAAGAGCAGGGTTTCC-3' (TOPO sequence + Kozak change)
Axin1 Reverse	5'-TCAGTCTACCTTCTCCACTTTGCCG-3'
Axin1 Reverse minus stop	5'-GTCTACCTTCTCCACTTTGCCGATG-3'
Axin1 Exon 5 Forward	5'-CACCATGTACCGGGTGCCGAAGG-3' (TOPO sequence)
Axin1 Exon 6 Forward	5'-CACCGGATCCATGGAGGAGGAAGGTGA GGAC-3' (TOPO + BamHI sequence)
Axin1 Exon 9 Forward	5'-GGAGGATCCGAGACAAGATCGCAGAGGAAGGTG-3' (BamHI)
Axin1 Exon 3 Reverse	5'-GGCAGATCTCCATCCACGCTGCTGTCC-3' (BglII)
Axin1 Exon 6 Reverse	5'-GGCAGATCTACTGTGGGCGAGGCCATC-3' (BglII)
Axin1 Exon 7 Reverse	5'-GGCAGATCTCCCGTGGCCGGTCCT-3' (BglII)
Axin1 Exon 8 Reverse	5'-CCTCTGCTTGGAGGGTGCTC-3'
Axin1 Exon 9 Reverse	5'-CTCTGTCTCGGAGAGCTCCATG-3'
shRNA	**Target Sequence:**
Axin1	GAGGAAGAAAAGAGAGCCA
rtPCR	**Primer Sequence:**

E2F2 Forward	5'-TCACCCCTCTGCCATTAAAGG-3'
E2F2 Reverse	5'-AGCAGTGTATTCCCCAGGCC-3'
GAPDH For.	5'-GACTTCAACAGCGACACCCAC-3'
GAPDH Rev.	5'-ACCACCCTGTTGCTGTAGCC-3'

Supplemental Figure Legends:

Supplemental Figure 1. V5-Axin1 coimmunoprecipitates with HA-c-Myc. 293 cells were co-transfected with expression plasmids for V5-Axin1 and either HA-empty or HA-c-Myc. Cells were lysed in CoIP buffer and subject to α-HA immunoprecipitation. Input and immunoprecipitated proteins were detected by western blot analysis as indicated.

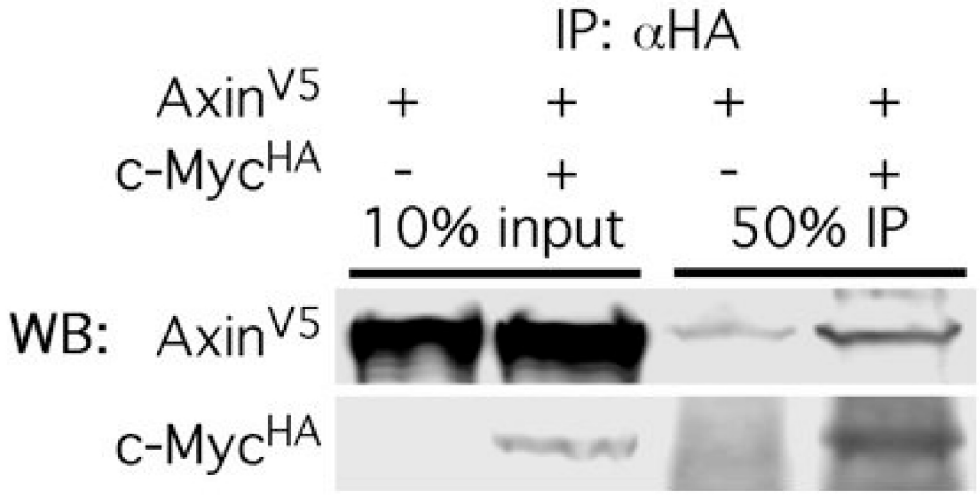
Supplemental Figure 2. The association of Axin1 with GSK3β and PP2A increases in serum-starved conditions when endogenous c-Myc protein expression is reduced. 293tr-V5-Axin cells were cultured in DMEM supplemented with 10%, 2%, or 0.2% FBS and either treated or untreated with 1mg/mL Dox for 4 hours. Cells were lysed in CoIP buffer and subjected to α-V5 immunoprecipitation. Input and immunoprecipitated proteins were detected by western blot analysis.

Supplemental Figure 3. Doxycycline treatment does not affect endogenous c-Myc expression in parental 293tr cells. Stable parental 293tr cells were treated with or

without Dox for the indicated times and cells were collected for western blot analysis as indicated.

Supplemental Figure 4. V5-Axin full-length and fragments are localized both to the cytoplasm and nucleus. 293 cells were transfected with expression plasmids for V5-Axin1v1, V5-Axin1v2, V5-Axin^{Ex2-6}, V5-Axin^{Ex2-7}, V5-Axin^{Ex2-8}, V5-Axin^{Ex2-9}, V5-Axin^{Ex6-11}, V5-Axin$^{\Delta Ex4-5}$, V5-Axin$^{\Delta Ex8-9}$, or V5-Axin^{mSupB15}. Cells were fixed and V5-Axin proteins were detected by α-V5 with FITC-labeled secondary (green). Nuclei were counterstained with DAPI (blue).

Arnold, Suppl. Fig. 1



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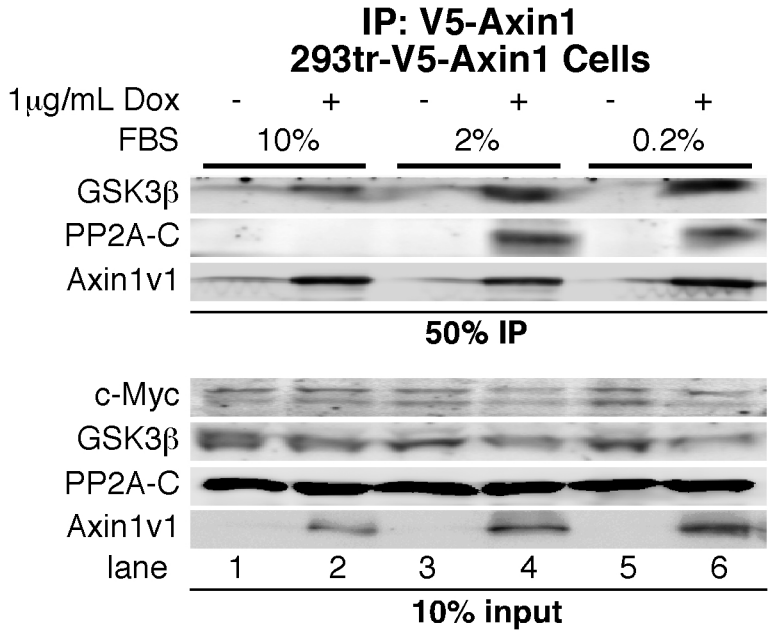
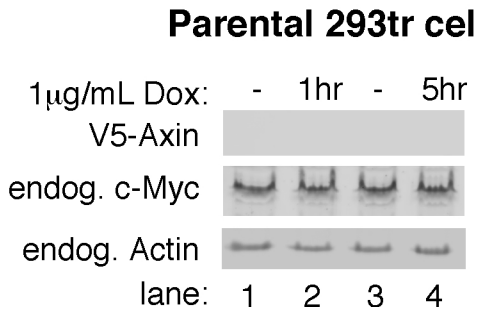


Arnold, Suppl. Fig. 3



Arnold, Suppl. Fig.4