

Fig. S1. Bergmann glia and Purkinje neurons extend processes in *Gsk-3*^{DKO} cerebella. (A) Representative sagittal sections of cerebella from control and *Gsk-3*^{DKO} mice at P2 and P3 stained for the Purkinje cell marker, Calbindin, and the Bergmann glial marker, FABP7. Scale bars measure 500 μ m and 25 μ m in insets. Nuclei counterstained with DAPI are pseudocolored gray.

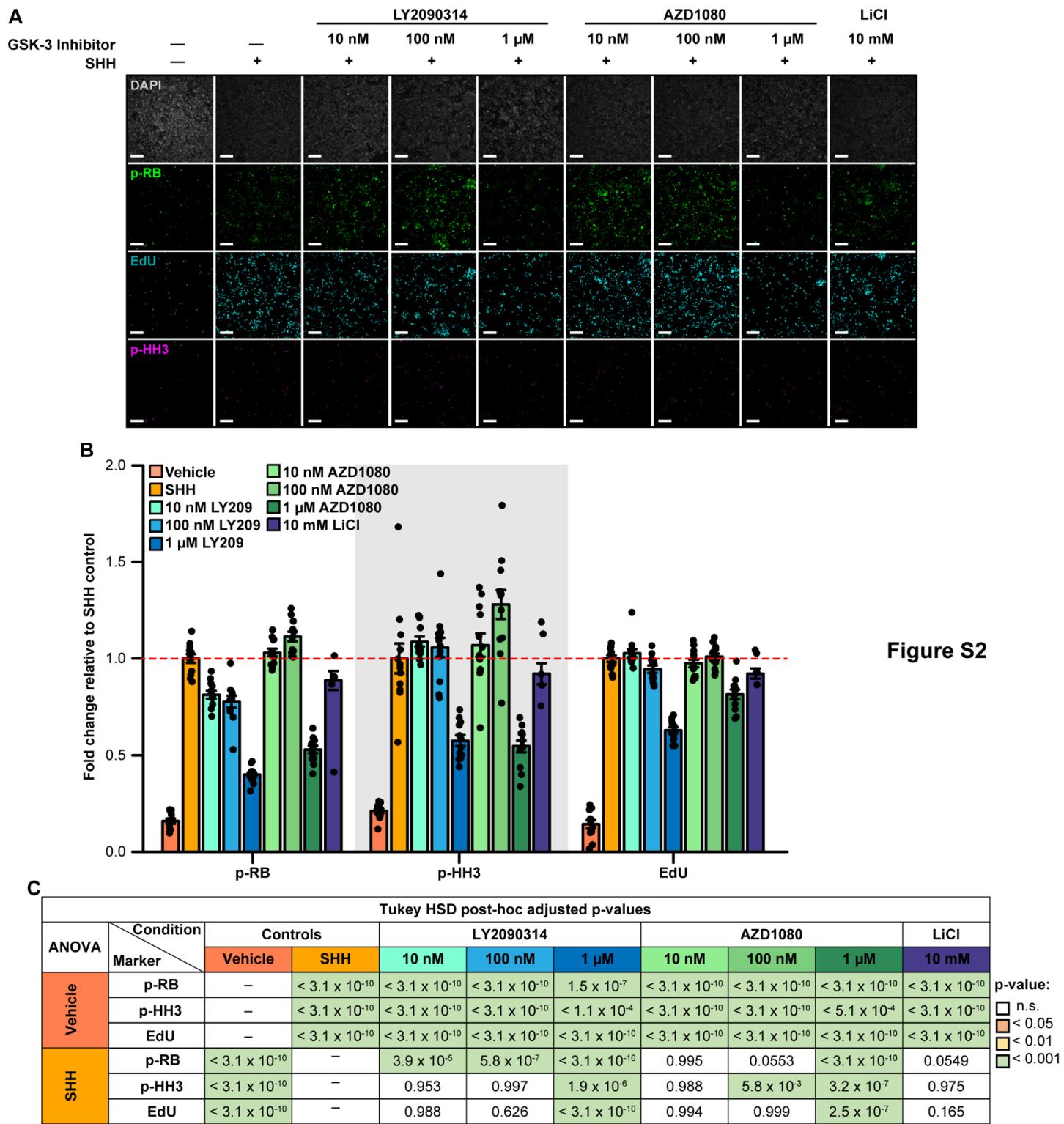


Fig. S2. GSK-3 inhibitors decrease proliferation in cultured CGNPs. (A) DAPI, EdU, p-RB and p-HH3 immunofluorescence images of cultured CGNPs treated with vehicle, SHH, or SHH with the indicated doses of the GSK-3 inhibitors LY2090314, AZD1080, or LiCl. CGNPs were pulse-labeled with EdU 1hr. before fixation. **(B)** Quantification of replicate cultures from **(A)** with the proportion of positive cells expressed as fold change relative to SHH controls (n=12 per condition). Error bars represent SEM. Dots represent replicate samples. **(C)**

Figure S2

Adjusted p-values for comparisons of vehicle-treated and SHH-treated controls to CGNPs with the indicated doses of LY209, AZD1080, or LiCl. Significance is denoted by colored boxes indicated in the legend, as determined by ANOVA with Tukey HSD post-hoc test. Scale bars measure 50 μ m.

A Figure S3

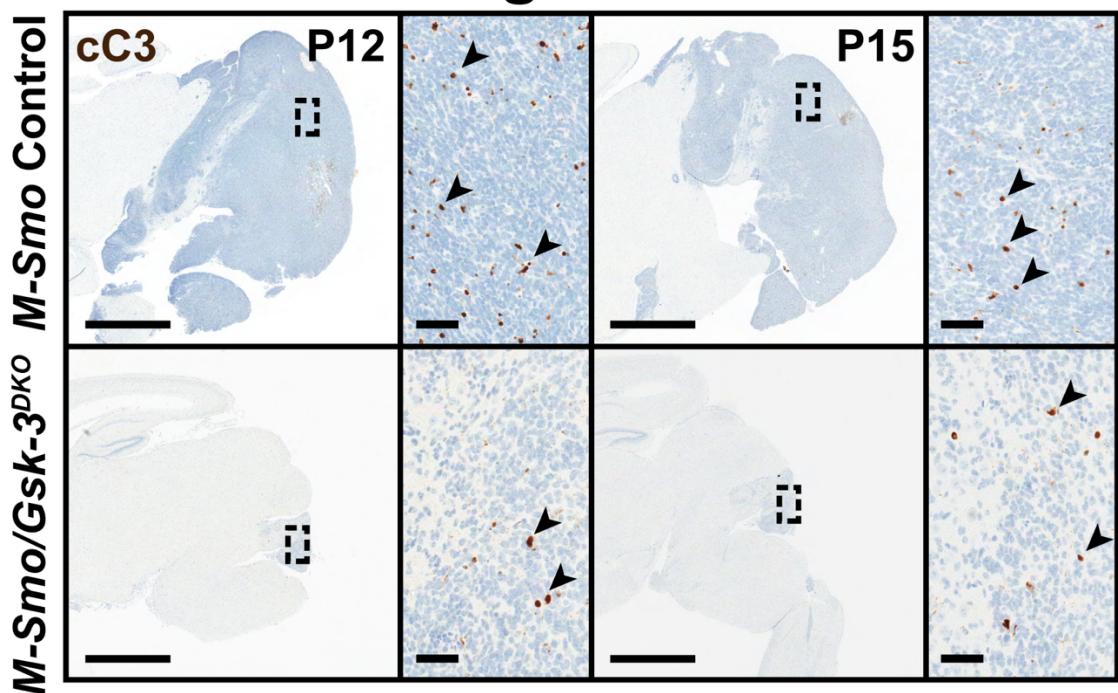


Fig. S3. Gsk-3 deletion does not induce apoptosis in medulloblastoma-prone mice. (A) Representative sagittal sections of *M-Smo* control and *M-Smo/Gsk-3*^{DKO} mice subjected to cC3 immunohistochemistry. Black arrowheads highlight cC3⁺ cells. Scale bars measure 2 mm for low magnification images and 50 μ m for higher magnification images. The regions of the higher magnification images are outlined by rectangles on the low magnification images.

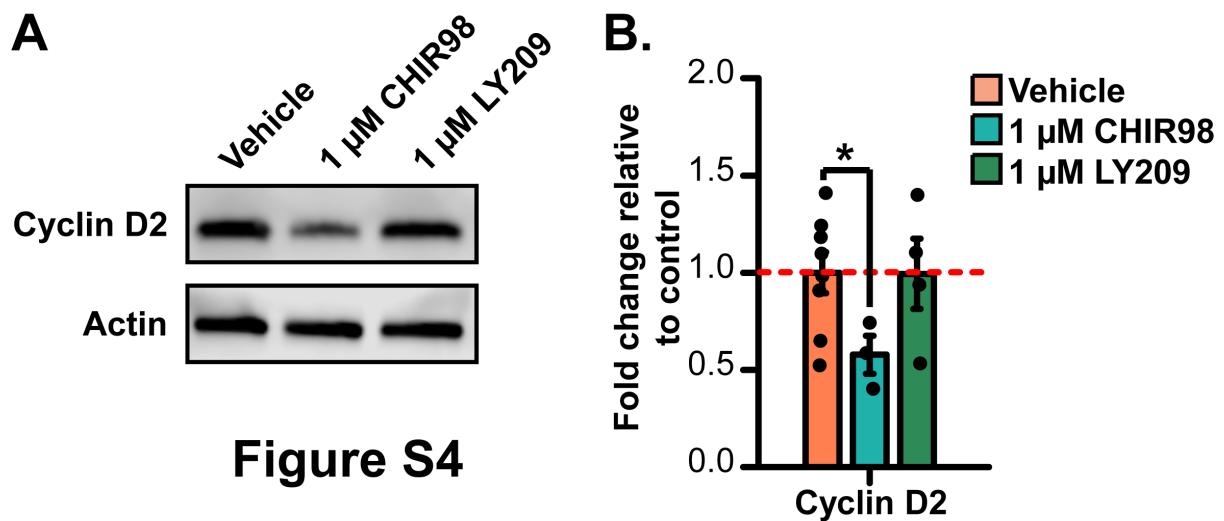


Fig. S4. CHIR98 decreases cyclin D2 levels in cultured medulloblastoma cells. (A) Representative western blot image comparing cyclin D2 and actin protein expression of medulloblastoma cells treated *in vitro* with vehicle, 1 μ M CHIR98, or 1 μ M LY209. (B) Quantification of replicate cultures from (A) with luminescence normalized to actin and expressed as fold change relative to control tumor cells (vehicle: n=8; CHIR98: n=3; LY209: n=4). Error bars indicate SEM. Dots represent replicate mice. Significance is denoted by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ as determined by student's t-test.

Table S1. The set of genes differentially expressed in whole cerebella of *Gsk-3^{DKO}* mice at P1, compared to two different, age-matched control groups, *M-Cre/Gsk-3α^{+/−}/Gsk-3β^{loxP/loxP}*, which retain a single *Gsk-3α* allele but have no *Gsk-3β* alleles in CGNPs, and *No Cre/Gsk-3α^{+/−}/Gsk-3β^{loxP/+}*, which retain a single *Gsk-3α* allele and retain two *Gsk-3β* alleles in CGNPs. Listed below are the gene names for 68 genes that were differentially expressed in the *Gsk-3^{DKO}* cerebella compared to both controls, with false discovery rate (FDR) adjusted p-value (q-value) less than 0.05 and absolute fold change (|FC|) greater than or equal to 2. The listed fold changes and p values are from the pairwise comparison of *Gsk-3^{DKO}* cerebella versus *M-Cre/Gsk-3α^{+/−}/Gsk-3β^{loxP/loxP}* controls.

Gene	fold_change	FDR adjusted p_value
Chrnb4	-3.56487	4.62E-07
Mfap4	-2.77489	5.34E-08
Chrna3	-2.54283	4.04E-07
Gsk3a	-2.53513	2.76E-10
Rnf148	-2.39022	9.31E-06
Nhlh1	-2.27388	1.64E-06
Cadps2	-2.21815	1.25E-06
Pisd-ps3	-2.15748	0.0263872
A930017K11Rik	-2.15248	2.42E-05
Gm10002	-2.12494	0.031835
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Slc22a6	-2.07768	0.0109233
Esd	-2.06687	0.0235768
Gm16169	-2.00995	8.85E-05
6430573F11Rik	2.00458	8.99E-08
LOC100503213	2.01892	0.0340935
Bmp4	2.06115	0.0127298
Gna14	2.08821	3.35E-08
Gm11744	2.09393	2.32E-05
Cbln4	2.12643	0.0210509
Hoxb9	2.12871	0.00380108
Nkd1	2.15054	2.14E-05
Mettl7a2	2.21692	1.65E-06
Lrrc26	2.22882	0.000573276
Lrrc55	2.24894	2.25E-05
Igk	2.26064	0.01754
Adamts19	2.29237	5.60E-07
Cdkn1a	2.32177	7.58E-09
Dock5	2.32746	6.89E-08
Glra1	2.33206	0.00218635
Gadd45g	2.34539	3.03E-06
Axin2	2.36502	1.23E-09
Dlx3	2.3702	7.96E-09
Gpr50	2.49813	0.00257124
Syk	2.49831	2.43E-05
2700046A07Rik	2.53502	7.68E-07
Wnt10a	2.53589	2.42E-05
Tcf7	2.54261	1.17E-08

Spo11	2.65145	4.61E-06
Gadd45a	2.67878	8.94E-05
Mylk	2.6964	1.71E-05
Mafb	2.73824	6.74E-05
Gm9926	2.82476	3.98E-05
Emilin3	2.87132	3.17E-06
Rasl11b	2.87278	1.35E-06
Zfp503	2.89595	2.29E-06
C030034L19Rik	3.00969	3.49E-07
Sp5	3.15039	3.47E-07
Fzd10	3.25743	1.74E-05
Sostdc1	3.3585	2.08E-05
Ascl4	3.48996	4.28E-08
Il6ra	3.52424	1.13E-05
Lef1	3.5281	1.51E-08
Onecut1	3.59348	8.25E-06
D630039A03Rik	3.65652	8.58E-08
Gabra6	3.72096	5.96E-07
Ism1	3.81828	1.31E-06
Myo1e	3.98353	1.58E-08
Lgr6	5.12478	9.71E-08
Esyt3	5.60377	6.02E-08
Fst	5.60408	1.51E-10
Adam18	5.75826	3.27E-06
Notum	6.26911	6.56E-11
Cryba2	7.95998	3.34E-07
Mybpc1	8.1496	1.17E-09
Pmaip1	9.39472	6.77E-09
Igk	11.0986	1.10E-05
Wif1	13.0107	1.91E-11

Table S2. Materials.

Listed below are all animals, reagents, kits and other materials used, with information on source and concentration, where appropriate.

Reagent	Concentration	Source	Identifier
Animal Studies			
C57BL/6 mice	N/A	The Jackson Laboratory	Stock #000664
<i>Math1-Cre</i> mice	N/A	The Jackson Laboratory	Stock #011104
<i>Gsk-3α</i> ^{-/-} mice	N/A	Donated by Dr. William Snider's lab	N/A
<i>Gsk-3β</i> ^{loxP/loxP} mice	N/A	The Jackson Laboratory	Stock #029592
<i>SmoM2-eYFP</i> ^{loxP/loxP} mice	N/A	The Jackson Laboratory	Stock #005130
<i>Trp53</i> ^{loxP/loxP} mice	N/A	The Jackson Laboratory	Stock #008462
<i>Ctnnb</i> ^{loxP/loxP} mice	N/A	The Jackson Laboratory	Stock #004152
<i>Cdkn1a</i> ^{-/-} mice	N/A	The Jackson Laboratory	Stock #016565
Isoflurane	Vapor	Piramal Critical Care, Inc.	NCD Code #66794-017-25
5-bromo-2-deoxyuridine (BrdU)	100 mg/kg in 25 µl of HBSS	ThermoFisher Scientific	Catalog #B23151
5-ethynyl-2'-deoxyuridine (EdU)	40 mg/kg in 25 µl of HBSS	Life Technologies	Catalog #A10044
PCR			
Tail lysis buffer	1X	Allele Biotechnology	Catalog #ABP-PP-MT01
Cre Forward Primer: GCG GTC TGG CAG TAA AAA CTA TC	200 µM	Invitrogen	JAX #oIMR1084
Cre Reverse Primer: GTG AAA CAG CAT TGC TGT CAC TT	200 µM	Invitrogen	JAX #oIMR1085
Gsk-3α Forward Primer: CCC CCA CCA AGT GAT TTC ACT GCT A	200 µM	Invitrogen	N/A
Gsk-3α Reverse Primer: AAC ATG AAA TTC CGG GCT CCA ACT CTA T	200 µM	Invitrogen	N/A
Gsk-3β Forward Primer: GCC ATC AAG AAA GTT CTA CAG GA	200 µM	Invitrogen	JAX #32390
Gsk-3β Reverse Primer: GCT GAA GTC CAG AGC AAG TCT	200 µM	Invitrogen	JAX #32391

<i>Trp53</i> Forward Primer: GGT TAA ACC CAG CTT GAC CA	200 µM	Invitrogen	JAX #oIMR8543
<i>Trp53</i> Reverse Primer: GGA GGC AGA GAC AGT TGG AG	200 µM	Invitrogen	JAX #oIMR8544
<i>Ctnnb</i> Forward Primer: AAG GTA GAG TGA TGA AAG TTG TT	200 µM	Invitrogen	JAX #oIMR1512
<i>Ctnnb</i> Reverse Primer: CAC CAT GTC CTC TGT CTA TTC	200 µM	Invitrogen	JAX #oIMR1513
<i>Cdkn1a</i> 1 Primer: GTT GTC CTC GCC CTC ATC TA	200 µM	Invitrogen	JAX #12427
<i>Cdkn1a</i> 2 Primer: GCC TAT GTT GGG AAA CCA GA	200 µM	Invitrogen	JAX #12428
<i>Cdkn1a</i> 3 Primer: CTG TCC ATC TGC ACG AGA CTA	200 µM	Invitrogen	JAX #12429
<i>SmoM2-eYFP</i> Forward primer: AAG TTC ATC TGC ACC ACC G	400 µM	Invitrogen	JAX #oIMR0872
<i>SmoM2-eYFP</i> Reverse primer: TCC TTG AAG AAG ATG GTG CG	400 µM	Invitrogen	JAX #oIMR1416
Apex Taq DNA Polymerase Master Mix	1X	Genessee Scientific	Catalog #42-138
Platinum Blue PCR SuperMix	1X	Invitrogen	Catalog # 12580015
Dissociation			
Papain Dissociation System	Per manufacturer's instructions	Worthington Biochemical Corporation	Catalog #LK003150
Heat inactivated FBS	100% for dissociation 10% in media	Gibco	Catalog #10437028
Hank's Balanced Salt Solution	1X	Gibco	Catalog #14175-095
D-(+)-Glucose	6g/L (33 mM)	Millipore Sigma	Catalog #G7021
Cell Culture			
Poly-L-Lysine	1X	Sigma-Aldrich	Catalog #P4832
DMEM/F12	1X	Gibco	Catalog #11330-032
N2 Supplement	1:10000 in media	BD Biosciences	Catalog #35-100
Penicillin-Streptomycin	1:10000 in media	Sigma-Aldrich	Catalog #P4333
Potassium Chloride (KCl)	2.5 µM	Mallinckrodt	Catalog #6858
Sonic hedgehog (SHH)	0.5 mg/mL	R&D Systems	Catalog #464SH
Paraformaldehyde (PFA)	4% fixative 0.1% in sheath fluid	Sigma-Aldrich	Catalog #P6148
Immunofluorescence and Western Blot			

Antigen Retrieval	1:100	Vector Laboratories	Catalog #H-3300
Phosphate Buffered Saline (PBS)	1X	Corning Inc.	Supplier #46-013-CM
Tween 20	0.3% in PBS	Sigma-Aldrich	Catalog #P9416
Donkey serum	2% in 0.3% PBST	Millipore Sigma	Catalog #D9663
Tris Buffered Saline with Tween (TBST)	1X	Cell Signaling Technology	Catalog #9997
Bovine Serum Albumin (BSA)	1% in 0.1% or 0.3% PBST	Fisher Scientific	Catalog #BP9700-100
Bicinchoninic acid (BCA) assay	Per manufacturer's instructions	Thermo Fisher Scientific	Catalog #23229
4-20% Mini-PROTEAN® TGX™ Precast Protein Gels	N/A	Bio Rad	Catalog #4561094
DAPI	1:2500 for IF	Invitrogen	Catalog #D1306
cleaved Caspase-3 (Asp175)	1:200 for IHC	Cell Signaling Technology	Catalog #9661
GSK-3α/β (D75D3)	1:100 for IF	Cell Signaling Technology	Catalog #5676
PCNA	1:2000 for IF	Abcam	Catalog #ab92552
CDKN1B/p27	1:1000	Cell Signaling Technology	Catalog #3686
phospho-RB (Ser807/811)	1:3000 for IF 1:500 for WB	Cell Signaling Technology	Catalog #8516
phospho-Histone H3 (Ser10) (6G3)	1:100 for IHC	Cell Signaling Technology	Catalog #9706
LEF1	1:200 for IF	Sigma	Catalog #HPA002087
TCF1 (C63D9)	1:100 for IF	Cell Signaling Technology	Catalog #2203
CDKN1A/p21	1:500 for WB 1:2000 for IHC	Abcam	Catalog #ab109199
phospho-CTNNB (Ser33/37/Thr41)	1:500 for WB	Cell Signaling Technology	Catalog #9561
CTNNB	1:500 for WB	Cell Signaling Technology	Catalog #8480
phospho-GSK-3β (Ser9) (D85E12)	1:500 for WB	Cell Signaling Technology	Catalog #5558
Cyclin D2 (D52F9)	1:500 for WB	Cell Signaling Technology	Catalog #3741
β-Actin (8H10D10)	1:5000 for WB	Cell Signaling Technology	Catalog #3700
Goat anti-rabbit Alexa Fluor 488	1:400 for IF	ThermoFisher Scientific	Catalog #A-11034
Goat anti-mouse Alexa Fluor 555	1:400 for IF	ThermoFisher Scientific	Catalog #A-21424

Novolink Polymer	Per manufacturer's instructions	Leica Biosystems	Catalog #RE7200-CE
ImmPRESS™ HRP Anti-Rabbit IgG	Per manufacturer's instructions	Vector Laboratories	Catalog #MP-7401
ImmPRESS™ HRP Anti-Mouse IgG	Per manufacturer's instructions	Vector Laboratories	Catalog #MP-7402
Anti-mouse IgG, HRP-linked Antibody	1.5:1000 for WB	Cell Signaling Technology	Catalog #7076
Anti-rabbit IgG, HRP-linked Antibody	1.5:1000 for WB	Cell Signaling Technology	Catalog #7074
Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 488	Per manufacturer's instructions	ThermoFisher Scientific	Catalog #C10337
SuperSignal West Femto Maximum Sensitivity Substrate	Per manufacturer's instructions	Thermo Fisher Scientific	Catalog #34095
Pharmacological Inhibition			
CHIR-98014	As indicated	Selleckchem	Catalog #S2745
LY2090314	As indicated	Selleckchem	Catalog #S7063
AZD1080	As indicated	Selleckchem	Catalog #S7145
Lithium Chloride (LiCl)	As indicated	Sigma Aldrich	Catalog #203637
Flow Cytometry			
FIX & PERM Cell Fixation & Cell Permeabilization Kit	Per manufacturer's instructions	ThermoFisher Scientific	Catalog #GAS003
Heat inactivated FBS	5% in FACS wash buffer	Gibco	Catalog #10437028
Sodium azide (NaN ₃)	0.1% in FACS wash buffer	Fisher Scientific	Catalog #S2271-25
FxCycle Violet	1:100	ThermoFisher Scientific	Catalog #F10347
phospho-RB Alexa Fluor 488	1:50	Cell Signaling Technology	Catalog #4277