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Supplemental Information

Complementary Genomic Screens Identify SERCA as a Therapeutic Target in *NOTCH1* Mutated Cancer

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Inventory of Supplemental Information

Supplemental Data

Figure S1, related to Figure 1

Table S1, related to Figure 1

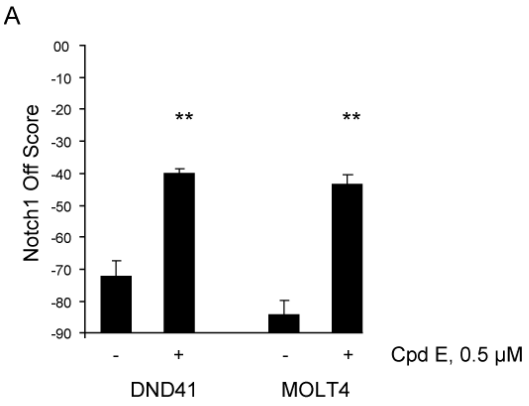
Figure S2, related to Figure 5

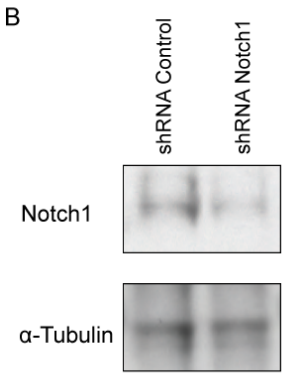
Figure S3, related to Figure 6

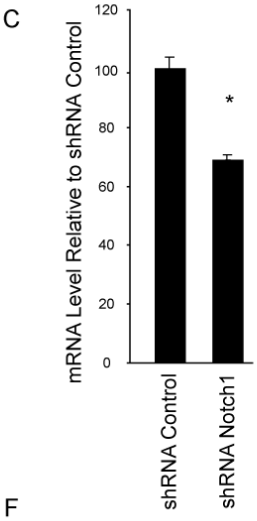
Supplemental Experimental Procedures

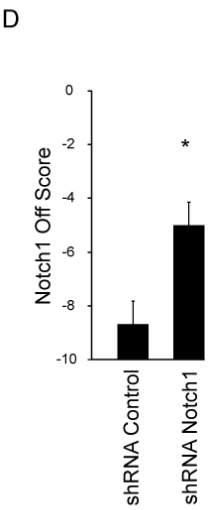
Supplemental References

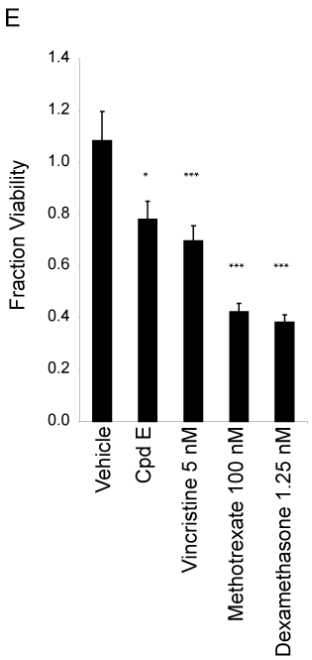
SUPPLEMENTAL DATA

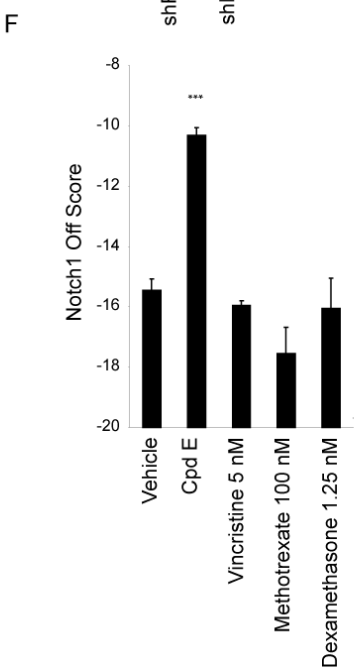












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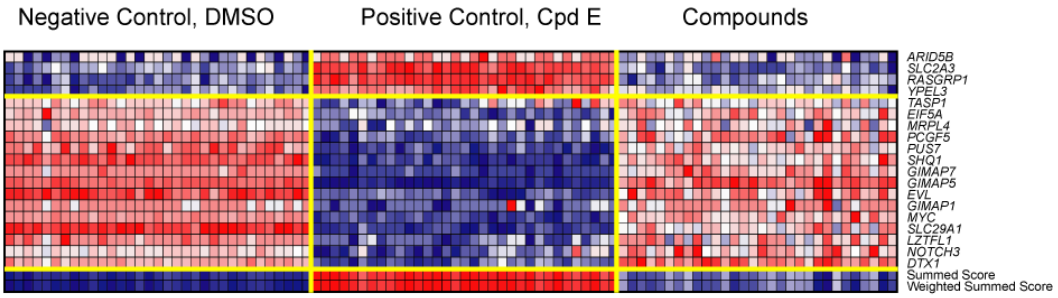
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Positive Control, Cpd E
Compounds
ARID5B
SLC2A3
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YPEL3
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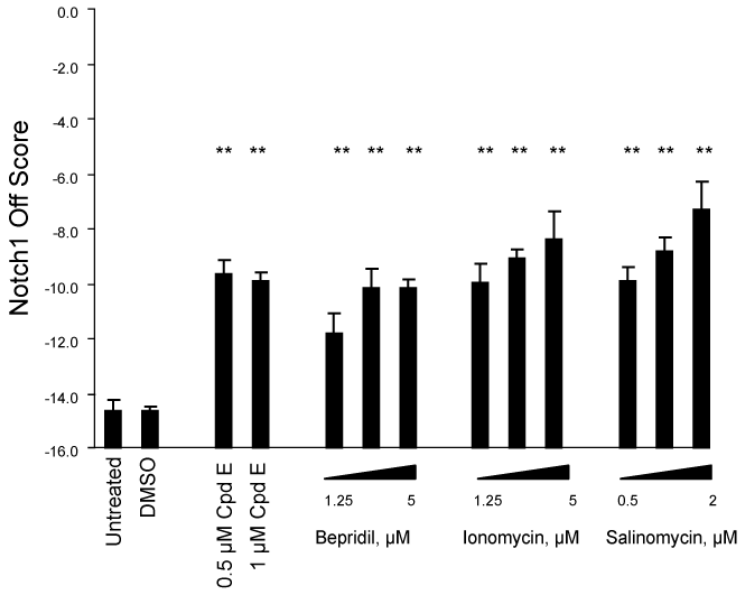
EIF5A
MRPL4
PCGF5
PUS7
SH01
GIMAP7
GIMAP5
EVL
GIMAP1
MYC
SLC29A1
LZTFL1
NOTCH3
DTX1
Summed Score
Weighted Summed Score

H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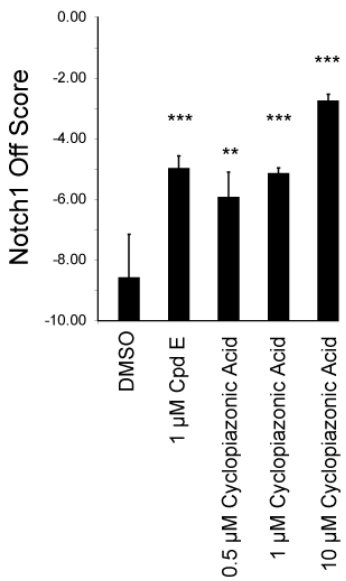
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
Untreated
DMSO
0.5 µM Cpd E
1 µM Cpd E
1.25
5
Bepridil, µM
1.25
5
Ionomycin, µM
0.5
2
Salinomycin, µM

I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-2.00
-4.00
-6.00
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-10.00
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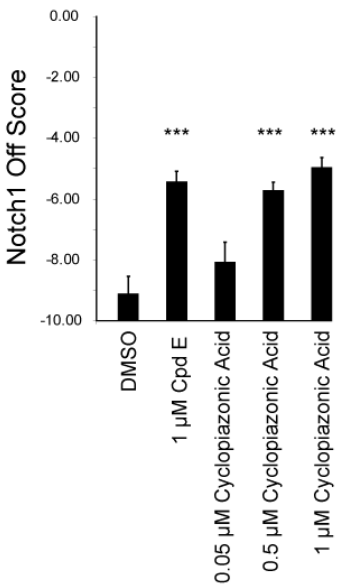
0.5 µM Cyclopiazonic Acid
1 µM Cyclopiazonic Acid
10 µM Cyclopiazonic Acid

J


Notch1 Off Score
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-2.00
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-6.00
-8.00
-10.00
DMSO
1 µM Cpd E
0.05 µM Cyclopiazonic Acid
0.5 µM Cyclopiazonic Acid
1 µM Cyclopiazonic Acid

Figure S1, related to Figure 1. GE-HTS development and validation.

(A) Weighted summed score for the Notch1 *off* signature after treatment with 0.5 μM of Cpd E or vehicle for 72 hr. Error bars indicate the mean ± SD of 10 replicates. Statistical significance was determined by Student's t-test (∗∗p<0.01).

(B) Immunoblot showing efficacy of Notch1-directed shRNA. Protein lysates were prepared 4 days post-infection (48 hr post-puromycin selection). The Notch1 protein was detected using an antibody that recognizes the C-terminus of Notch1.

(C) Effect of Notch1-directed shRNA on Notch1 gene expression. Transcript levels were measured by real-time PCR 4 days post-infection (48 hr post-puromycin selection). Error bars indicate the mean ± SD of 4 replicates. Statistical significance was determined by Student's t-test (∗p=0.05).

(D) Induction of the Notch1 *off* Score with Notch1-directed shRNA. Error bars indicate the mean ± SD of 8 replicates for the summed score. Statistical significance was determined by Student's t-test (∗p<0.05).

(E) Cytotoxic effects of vincristine, methotrexate and dexamethasone on DND41 cells treated for 72 hr. Cell numbers were estimated with an ATP-based assay and plotted as a ratio relative to vehicle-treated cells. Error bars indicate the mean ± SD of 6 replicates. A GSI control, Cpd E, was included. Statistical

significance (∗p<0.05; ∗∗∗p<0.001) was determined by one-way ANOVA using Bonferroni's correction for multiple comparison testing.

(F) Notch1 *off* score (weighted summed score) induced by treatment of DND41 with the indicated doses of chemotherapeutic agents or Cpd E. Error bars indicate the mean ± SD of 6 replicates. Statistical significance (∗∗∗p<0.001) was determined by one-way ANOVA using Bonferroni's correction for multiple comparison testing.

(G) Overall screen performance depicted in heatmap format. Dark red indicates high gene expression and dark blue indicates low gene expression. Each column represents the mean of the designated condition on individual plates; each row the signature genes for the top 19 performing genes. The bottom two rows indicate the summed score and the weighted summed score. Chemical plates were screened in triplicate, and for each plate a minimum of 32 negative controls (DMSO) and 32 positive controls (0.5 μM Cpd E) were included.

(H) Notch1 *off* score (weighted summed score) in DND41 cells treated with the indicated doses of hit compounds for 72 hr. Error bars indicate the mean ± SD of 8 replicates for untreated cells and 4 replicates for the 0.0125% DMSO, Cpd E, and hit compounds. Statistical significance (∗∗p<0.01) was determined by one-way ANOVA using Bonferroni's correction for multiple comparison testing.

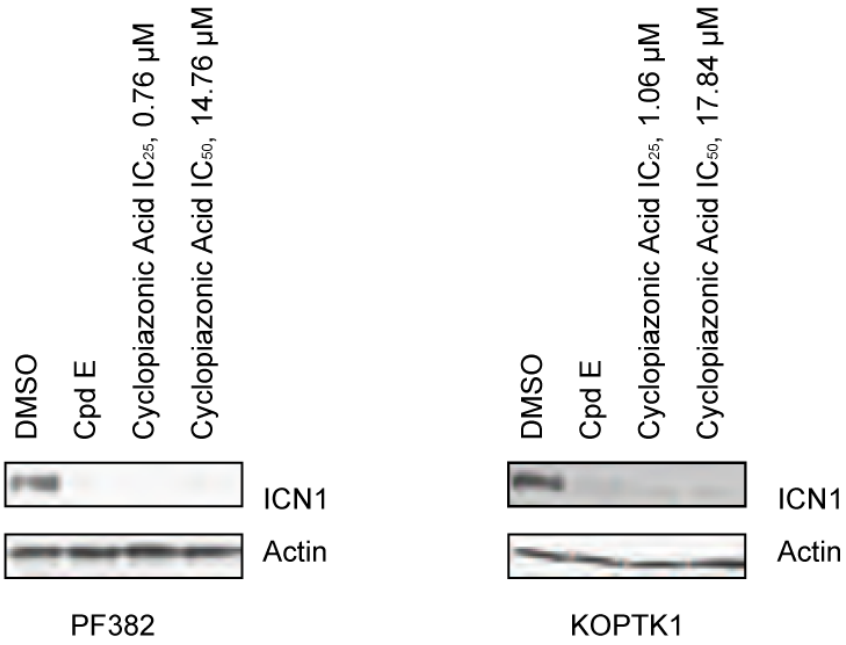
(I, J) Notch1 *off* score (weighted summed score) in DND41 cells (I) and MOLT4 (J) treated with the indicated doses of the SERCA inhibitor, cyclopiazonic acid, for 72 hr. Error bars indicate the mean ± SD of 6 replicates for untreated cells and 4 replicates for the 0.0125% DMSO, Cpd E, and hit compounds. Statistical significance (∗∗p<0.01; ∗∗∗p<0.001) was determined by one-way ANOVA using Bonferroni's correction for multiple comparison testing.

Table S1, related to Figure 1. GE-HTS secondary screen.

Chemical Name	SMILE Sequence
Oxyphenbutazone	CCCCC1C(=O)N(N(C1=O)c2ccc(O)cc2)c3ccccc3
Lanatoside C	CC1OC(CC(O)C1OC2CC(O)C(OC3CC(OC(=O)C)C(OC4OC(CO)C(O)C(O)C4O)C(C)O3)C(C)O2)OC5CCC6(C)C(CCC7C6CC(O)C8(C)C(CCC78O)C9=CC(=O)OC9)C5
Perhexiline	C(C(C1CCCCC1)C2CCCCC2)C3CCCCN3
Cyclopiazonic acid	C/C(=C/1\C(=O)C2C3C(Cc4cccc5[nH]cc3c45)C(C)(C)N2C1=O)/O
Neriifolin	CO[C@@H]1[C@@H](O)[C@H](C)O[C@@H](O[C@H]2CC[C@@]3(C)[C@H](CCC4C3CC[C@]5(C)C(CC[C@]45O)C6=CC(=O)OC6)C2)[C@H]1O
Nigericin sodium	CO[C@@H]1C[C@@H](C[C@H]2CC[C@H](C)[C@@H](O2)[C@@H](C)C(=O)O)O[C@]3(O[C@@](C)(C[C@H]3C)[C@H]4CC[C@](C)(O4)C5O[C@H](C[C@@H]5C)C6O[C@@](O)(CO)[C@H](C)C[C@@H]6C)[C@@H]1C
Peruvoside	CO[C@@H]1[C@@H](O)[C@H](C)O[C@H](O[C@H]2CC[C@@]3(C=O)[C@H](CCC4C3CC[C@]5(C)[C@H](CC[C@]45O)C6=CC(=O)OC6)C2)[C@H]1O
4'-hydroxychalcone	Oc1ccc(cc1)C(=O)/C=C/c2ccccc2
Monensin sodium	CC[C@]1(CC[C@@H](O1)[C@]2(C)CCC3(C[C@H](O)[C@@H](C)[C@H](O3)[C@H](C)[C@@H](OC)[C@H](C)C(=O)O)O2)C4O[C@H](C[C@@H]4C)C5O[C@@](O)(CC)[C@H](C)C[C@@H]5C
Homoharringtonine	COC(=O)CC(O)(CCCC(C)(C)O)C(=O)O[C@H]1[C@H]2c3cc4OCOc4cc3CCN5CCC[C@]25C=C1OC
Phorbol-12,13-didecanoate	CCCCCCCCCC(=O)O[C@@H]1[C@@H](C)[C@]2(O)[C@@H]3C=C(C)C(=O)[C@@]3(O)CC(=C[C@H]2[C@@H]4C(C)(C)[C@@]41OC(=O)CCCCCCCCC)CO
Bufalin	C[C@@]12CC[C@H]3[C@@H](CC[C@@H]4C[C@@H](O)CC[C@@]43C)[C@@]2(O)CC[C@@H]1c5ccc(=O)oc5
Sangivamycin	NC(=O)c1cn(C2OC(CO)[C@H](O)[C@@H]2O)c3ncnc(N)c13
Ionomycin	CC[C@@H](C)C[C@H](C)C[C@H](C)C(=O)/C=C(\O)/[C@H](C)C[C@H](C)C/C=C/[C@@H](C)[C@@H](O)[C@@H](C)[C@@H](O)C[C@@H]1CC[C@](C)(O1)[C@H]2CC[C@](C)(O2)[C@@H](C)O
Indirubin-3-monoxime, 5-Iodo-	O=C/1Nc2ccccc2\C1=C/3\Nc4ccccc4C3=O
Bepridil, Hydrochloride	CC(C)COCC(CN(Cc1ccccc1)c2ccccc2)N3CCCC3

Names and SMILES (Simplified Molecular Input Line Entry System) for compounds retested in the GE-HTS assay

A


DMSO
Cpd E
Cyclopiazonic Acid IC25, 0.76 µM
Cyclopiazonic Acid IC50, 14.76 µM
ICN1
Actin
PF382
DMSO
Cpd E
Cyclopiazonic Acid IC25, 1.06 µM
Cyclopiazonic Acid IC50, 17.84 µM
ICN1
Actin
KOPTK1

B

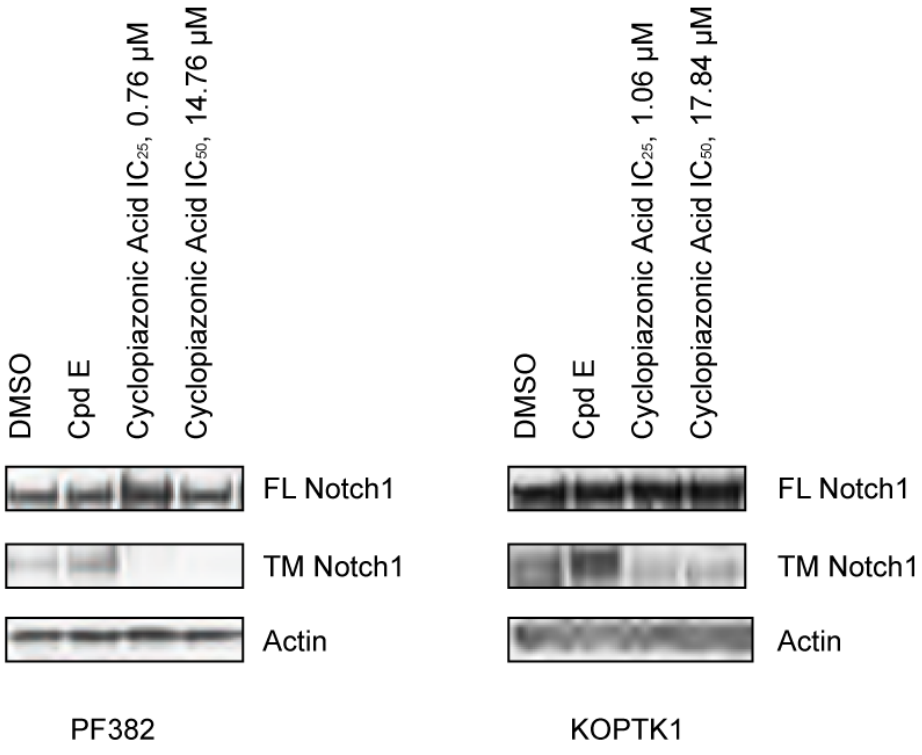
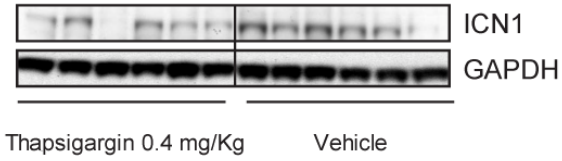

DMSO
Cpd E
Cyclopiazonic Acid IC25, 0.76 µM
Cyclopiazonic Acid IC50, 14.76 µM
FL Notch1
TM Notch1
Actin
PF382
DMSO
Cpd E
Cyclopiazonic Acid IC25, 1.06 µM
Cyclopiazonic Acid IC50, 17.84 µM
FL Notch1
TM Notch1
Actin
KOPTK1

Figure S2, related to Figure 5. Cyclopiazonic acid impairs Notch1 maturation.

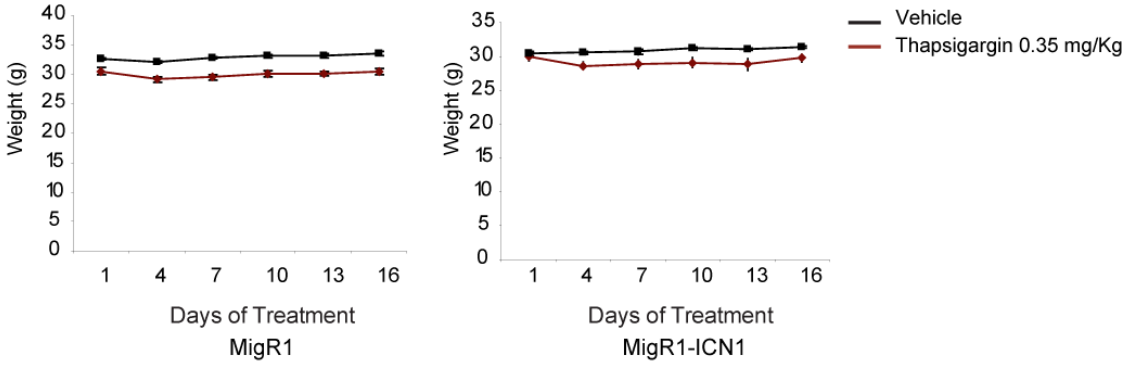
(A) Immunoblotting with anti-ICN1 (Val1744) antibody was used to evaluate effects of treatment with cyclopiazonic acid for 24 hr at the IC_{50} or IC_{25} for cell viability. Actin was used as a loading control.

(B) Immunoblotting with an antibody that recognizes the C-terminus of Notch1 was used to evaluate effects of treatment with cyclopiazonic acid for 24 hr at the IC_{50} or IC_{25}. Actin was used as a loading control.

A


ICN1
GAPDH
Thapsigargin 0.4 mg/Kg
Vehicle

B


Weight (g)
40
35
30
25
20
15
10
5
0
1
4
7
10
13
16
Days of Treatment
MigR1
Weight (g)
35
30
25
20
15
10
5
0
1
4
7
10
13
16
Days of Treatment
MigR1-ICN1
Vehicle
Thapsigargin 0.35 mg/Kg

C

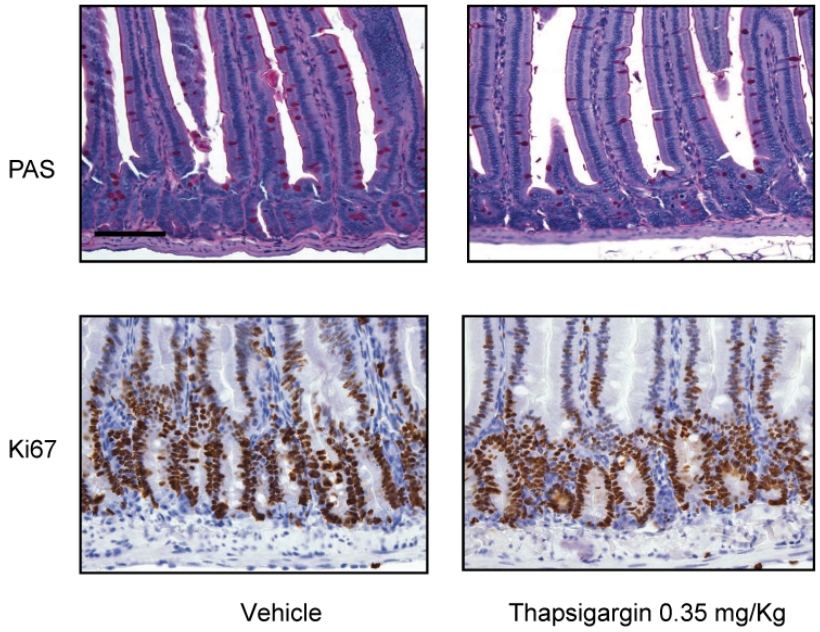

PAS
Ki67
Vehicle
Thapsigargin 0.35 mg/Kg

Figure S3, related to Figure 6. Effects of thapsigargin *in vivo*.

(A) Effect of thapsigargin treatment on ICN1 levels in xenografted MOLT4 tumors is shown. The immunoblot shown was stained with an ICN1-specific antibody. GAPDH was used as a loading control.

(B) Body weight analysis of DND41 xenografts treated with thapsigargin 0.35 mg/Kg or vehicle by intraperitoneal injection daily. Error bars indicate mean ± SD of replicates for each cohort. No statistical significance was observed among the two cohorts.

(C) Histological analysis of the small intestines of DND41 xenografts treated with thapsigargin 0.35 mg/Kg or vehicle. The intestines of all xenografted mice were examined; representative results for one control animal and one thapsigargin-treated animal are shown. Formalin-fixed, paraffin-embedded tissue sections were stained using the Periodic Acid Schiff (PAS) method, which identifies goblet cells, and an immunohistochemical method that detects Ki-67, a marker of actively cycling cells. No goblet cell metaplasia or growth suppression of enterocytes was observed in any of the thapsigargin-treated animals. Scale bar represents 100 μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Culture

Human cell lines HPB-ALL, MOLT16, were purchased from Leibniz-Institut DSMZ-German collection of microorganisms and cell cultures; LOUCY and SUPT1 were purchased from American Type Culture Collection (ATCC); identity of DND41, KOPTK1, MOLT4, PF382, ALL/SIL, SUPT13 and K562 was confirmed by PCR sequencing for known *NOTCH1* mutations and short tandem repeat (STR) loci profiling. Cells were cultured in RPMI 1640 (Cellgro, Manassas, VA, USA) with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin-streptomycin and incubated at 37°C with 5% CO_2.

Compound Sources

Compounds were obtained from the following sources: compound E (Cpd E) (ENZO Life Sciences, ALX-270-415-M001), thapsigargin (ENZO Life Sciences, BML-PE180-0005), cyclopiazonic acid (ENZO Life Sciences, BML-CA415-0010), bepridil hydrochloride (Sigma-Aldrich, B5016), salinomycin (Sigma-Aldrich, S6201), ionomycin (Sigma-Aldrich, I3909), DAPT (N-[(3,5-Difluorophenyl)acetyl]-L-alanyl-2-phenyl]glycine-1,1-dimethylethyl ester) (Sigma-Aldrich, D5942), dexamethasone (Sigma-Aldrich, D4902), methotrexate (Sigma-Aldrich, M9929), and vincristine (Sigma-Aldrich, V8388).

Notch1 *Off* Signature Detection

Marker genes for the Notch1 *on* vs. *off* signature were chosen using publicly available Affymetrix microarray expression profiling data sets profiling 7 different *NOTCH1* mutant T-ALL cell lines treated in duplicate with vehicle versus compound E (500 nM) for 24 hours (GEO: GSE5716) (Palomero et al., 2006). Four control genes with stable expression across the two states were selected to control for well-to-well variability in total RNA: *GAPDH*, *NFX1*, *NISCH*, and *GTF.* Probes are detailed in the GE-HTS assay signature probes section below. The signature was adapted to an assay that uses ligation-mediated amplification (LMA) and fluorescence bead-based detection (FlexMap Technology, Luminex, Austin, TX, USA), as described (Peck et al., 2006). Signature performance was evaluated by calculating two scores that incorporate information about signature gene expression: the summed score and weighted summed score. The summed score metric combines expression ratios by summing them with a sign determined by the expected direction of regulation from the GSI-treated positive controls. The weighted summed score metric is a variant of the summed score metric that combines expression ratios by summing them with a weight and sign determined by the signal-to-noise ratio of GSI-treated positive controls and the DMSO-treated negative controls.

Small-molecule Library Screening

DND41 cells were plated in 384-well tissue culture plates in 50 μl of medium at 25,000 cells/well using an automated cell dispenser (Multidrop, Thermo

Scientific, Waltham, MA, USA). The following controls were included on each screen plate: medium only (32 wells), DND41 cells only (32 wells), and DND41 cells treated with 500 nM compound E (32 wells). Compounds were added at a final approximate concentration of 20 μM in DMSO by pin transfer and incubated for 72 hr at 37°C with 5% CO_2. We screened a total of 3,801 compounds in triplicate, including the BSPBio collection (Broad Institute Chemical Biology) that contains bioactive compounds from the Prestwick, Biomol, and Spectrum libraries and the HSCI1 collection (Broad Institute, Chemical Biology). The GE-HTS assay was performed as described (Peck et al., 2006; Stegmaier et al., 2004). Compounds that induce the Notch1 *off* signature were identified using 5 discrete analytic metrics: summed score, weighted summed score, K-nearest neighbor analysis, naïve Bayes classification, and support vector machine (SVM) as described (Hahn et al., 2009).

cDNA Library Screen and Validation

The cDNA screening strategy involved the use of three key components: 1) a pcDNA3 plasmid encoding a modestly strong *NOTCH1* gain-of-function mutant, L1601PΔP, driven from a CMV promoter (40 ng of cDNA/well), 2) a Notch firefly luciferase reporter (50 ng of cDNA/well), and 3) a pre-plated cDNA library cloned into the Sport6 plasmid (40 ng of cDNA/well). A *MAML1* cDNA was the positive control for each screen plate, while empty vector and a *DTX1* cDNA were negative controls (40 ng of cDNA/well). DNA spotting was performed using a Matrix Platemate (Thermo, Waltham, MA, USA) for the control wells. The Matrix

Wellmate (Thermo) was used to dispense the transfection mix (Fugene6) and following a 30 min incubation the cells were added to the wells (4000 U20S cells/well). Luminescence was measured 48 hr post-plating by the LJL Biosystems Analyst HT96:384.

For the validation studies, 4000 U2OS cells/well were seeded on Corning opaque 384-well plates with DMEM medium supplemented with 10% FBS (Gibco, Grand Island, NY, USA), penicillin-streptomycin and glutamine. FuGENE (Roche Diagnostics, Basel, Switzerland) transfection mix was prepared in OptiMEM (Gibco, Grand Island, NY, USA) serum-free medium with three plasmid components: 1) 50 ng/well of Notch firefly reporter plasmid; 2) 40 ng/well of pcDNA3-L1601PΔP plasmid; and 3) 40 ng/well of *ATP2A1*, *ATP2A2* or *ATP2A3* cDNAs cloned into pCMVSport6. pcDNA3-MAML1 and Flag-CMV-DTX1 plasmids (40 ng/well) served as positive and negative controls, respectively. Following 20 min at room temperature, 20 µl of the transfection reaction mix was added to the cells by multi-channel pipette. Twenty to 24 wells were analyzed for each individual transfection sample set. After 48 hr of incubation with the transfection mix, 35 µl/well of BriteLite-Plus (PerkinElmer, Waltham, MA, USA) luciferase reagent was added by multi-channel pipette; luminescence was measured by an LJL Biosystems Analyst HT96:384 (LJL BioSystems, Inc, Sunnyvale, CA, USA). Three individual repeats were performed for each experiment.

Viral Transduction

Oligonucleotides encoding shRNAs were cloned into pLKO.1 as described (Moffat and Sabatini, 2006). Sequences targeted by each shRNA are detailed in the NOTCH1-directed shRNA probes section below. For virus production, 500,000 293T cells plated in 10 cm plates were transfected with 1 µg of pLKO.1 and packaging plasmids (pCMVdeltaR8.91 and pCMV-VSVG expressing plasmid) according to the FuGENE 6 protocol (Roche Diagnostics, Basel, Switzerland). Medium was changed to RPMI 1640 24 hr post-transfection, and viral supernatant was harvested and filtered 48 hr post-transfection. Virus was concentrated 5 times using Peg-it^{TM} virus precipitation solution (System Bioscences, Mountain View, CA, USA). Cells were infected for 2 hr at 37°C with 2 ml lentivirus and 8 µg/ml polybrene (Sigma-Aldrich, St. Louis, MO, USA). Cells were selected 48 hr later with 1 µg/ml puromycin (Sigma-Aldrich, St. Louis, MO, USA). Viral supernatant production and retroviral infections were performed as described for MigR1 retroviral vectors (Aster et al., 2000). Transduction efficiency for MigR1 was monitored by assessing GFP expression with a FACScan flow cytometer (BD, Franklin Lakes, NJ, USA) (Aster et al., 2000). After viral infection, GFP-positive cells were sorted by flow cytometry with a FACSAria II (BD, Franklin Lakes, NJ, USA) and cultured under compound E (0.5 µM) negative selection for 10 days. Experiments were conducted 3 or more days after removal of compound E.

Cell Growth, Apoptosis and DNA Content Assays

Cell growth was assessed using the Promega Cell-Titer Glo ATP-based assay (Promega, Madison, WI, USA). Luminescence was measured using a Fluostar Omega instrument (BMG-labtech, Ortenberg, Germany). Values for IC_F were calculated using Prism 5 Software (version 5.03) using the model IC_F= {[(100-F)/F]^(1/H)}*IC_{50}, where H=Hill slope of the sigmoidal curve and F=the percentage of the response. Apoptosis was measured using a Caspase-Glo 3/7 assay (Promega, Madison, WI, USA), or by measuring Annexin V and propidium iodide (PI) staining by flow-cytometry (eBioscience, San Diego, CA, USA). Cellular DNA content was assessed by staining with propidium iodide (PI, 50 μg/ml). Cells were analyzed by flow cytometry with a FACScan flow cytometer (BD, Franklin Lakes, NJ, USA) and ModFit analytical software. At least 20,000 events were acquired and all determinations were replicated at least twice.

Reporter Gene Assays

Expression plasmids for L1601PΔP (Weng et al., 2004), L1601PΔP-GAL4 (Malecki et al., 2006), ΔEGFΔLNR (Chiang et al., 2006), and ICN1 (Aster et al., 2000) have been described. Expression plasmids were introduced into U2OS cells by transient transfection using FuGENE 6 according to the manufacturer's protocol (Roche Diagnostics, Basel, Switzerland). Co-transfection of U2OS cells with expression plasmids, a Notch firefly luciferase reporter gene, and an internal Renilla luciferase control gene, was performed as described (Aster et al., 2000). Dual luciferase reporter assays (Promega Madison, WI, USA) were performed

44-48 hr post-transfection. Normalized luciferase activities were measured in quadruplicate and fold induction expressed relative to the empty plasmid pcDNA3. A second approach used a Notch1-Gal4 receptor ligand stimulation assay as described (Malecki et al., 2006). Briefly, U2OS cells conditionally expressing a chimeric Notch1 or Notch2 receptor in the presence of tetracycline were co-transfected with 250 ng of a GAL4-sensitive firefly luciferase gene and 5 ng of pRL-TK, which expresses Renilla luciferase from a constitutively active thymidine kinase promoter element. Transfected cells were split into 24 well plates containing 3T3 cell feeders expressing the Notch ligand Jagged2. After 18 hr of co-culture, firefly and Renilla luciferase activity were measured using a dual luciferase reporter assay kit (Promega Madison, WI, USA).

Real-time RT-PCR

Primers and probes for real-time RT-PCR were obtained from Applied Biosystems (*GAPDH* #402869, *RPL13A* #Hs01926559_g1, *NOTCH1* #Hs01062014_m1, *MYC* #Hs00153401_m1, *HES1* #Hs00172878_m1, and *DTX1* #Hs00269995_m1). The data were analyzed using the $\Delta\Delta$CT method and plotted as percentage of transcript compared to vehicle.

Immunodetection of Notch1

Western blots were stained with antibodies specific for γ-secretase-cleaved Notch1 (Val1744, Cell Signaling, Beverly, MA, USA), the intracellular transcriptional activation domain (TAD) of Notch1 (Hasserjian et al., 1996), or the

C-terminus of Notch1 (SC-6014 (C-20) Santa Cruz Biotechnology, Santa Cruz, CA, USA). Control stains were carried out with antibodies specific for actin (ACTN05, Thermo Scientific, Waltham, MA, USA), vinculin (AbCAM 2907, Cambridge, MA, USA), or GAPDH (137179, Santa Cruz Biotechnology). Blots were developed with anti-rabbit-HRP (NA9340V, Amersham, Pittsburgh, PA, USA) or anti-mouse-HRP (NA9340V or NA9310V, Amersham). Staining was quantified using ImageQuant Tl V 7.0 (GE Health Care, Piscataway, NJ, USA).

Immunofluorescence staining was carried out on permeabilized cells using a murine monoclonal antibody against Notch1 [A6] (AbCAM 3294, Cambridge, MA, USA), rabbit polyclonal antibody to giantin (AbCAM 24586, Cambridge, MA, USA), and species-specific secondary antibodies linked to Alexa Fluor 488 or 568 (Invitrogen, Carlsbad, CA, USA). Slides were mounted with Prolong® Gold antifade reagents and counterstained with DAPI (Invitrogen, Carlsbad, CA, USA). Images were acquired using a Zeiss LSM510 confocal microscope at 63X power. Cell surface Notch1 was evaluated by staining non-permeabilized cells with monoclonal anti-human Notch1 antibody (R&D FAB5317P, Minneapolis, MN, USA).

Drosophila **Experiments**

To generate RAF(gof) tumors in the adult *Drosophila* midgut, we created a stock containing the X-linked UAS-RAF(gof) transgene (Brand and Perrimon, 1994) and the second chromosome-linked esg-Gal4, UAS-GFP, Tubulin, Gal80(ts) transgenes (Micchelli and Perrimon, 2006). The y v: Ca-P60A RNAi (JF01948)

and y v: attP2 control stocks were gifts from the Harvard TRiP. Drugs were prepared in DMSO and mixed with fly food 1:100 and fed to flies for 7 days. Flies were given freshly prepared drug every 2-3 days. The final concentrations were: compound E (100 μM), DAPT (400 μM), cyclopiazonic acid (1 mM) and thapsigargin (100 μM),

Drug effects were evaluated by immunofluorescence microscopy. Primary antibodies (mouse monoclonal anti-Delta 1:50 and anti-Prospero 1:100) were obtained from the Developmental Studies Hybridoma Bank (Iowa City, Iowa, USA) and the secondary antibody Alexa 555-conjugated donkey anti-mouse from Molecular Probes (Invitrogen, Carlsbad, CA, USA). Adult fly guts were dissected in PBS and fixed for 20 minutes as described (Ohlstein and Spradling, 2007). Samples were incubated in 5% NDS blocking solution (1.5 hr), primary antibodies (overnight), secondary antibody (1.5 hr) and DAPI (6 min). Samples were mounted in Vectashield mounting medium and imaged with a Leica TCS SP2 confocal microscope under 400X power. Histology studies were carried out by the Dana-Farber/ Harvard Cancer Center specialized histopathology services core.

T-ALL Xenograft Studies

MOLT4 xenografts were established by injecting $1.7x10^6$ MOLT4 cells subcutaneously into 6-week-old female SCID-beige mice (Charles River Labs, Wilmington, MA, USA). Tumor volume was determined by caliper measurements and calculated using the equation $Vol=0.5 \times L \times W^2$. When tumors reached a

mean volume of 75 mm^3, mice were divided into groups that were treated with thapsigargin 0.4 mg/Kg or vehicle by intraperitoneal injection daily. Three mice that died prematurely due to drug toxicity were excluded from the study, leaving 6 evaluable mice in the thapsigargin-treated arm and 9 mice in the vehicle control arm. DND41 MigR1 and DND41 MigR1-ICN1 xenografts were established by injecting $10x10^6$ DND41 cells subcutaneously into NSG mice. Mice were injected with either DND41 or DND41-ICN1 cells (n=20 per line). When tumor volume reached over 50 mm^3 mice were divided into two treatment group: thapsigargin 0.35 mg/Kg or vehicle 10mL/Kg by intraperitoneal injection daily. Mice that were not ready at start of treatment were subsequently added to treatment groups when their tumors reached appropriate sizes. Mice were treated daily through the course of the study and tumors were measured every three days. Five thapsigargin-treated mice were found dead during the course of the study with no prior weight loss or clinical signs of illness.

All animal studies were performed under a Dana-Farber Cancer Institute IACUC-approved protocol.

NOTCH1-directed shRNA probes

NCBI ID	Name	Target Sequence	Oligo Sequence
4851	*NOTCH1*	GATGCCAAATGCCTGCCAGAA	CCGGGATGCCAAATGCCTGCCAGAACTCGAGTTCTGGCAGGCATTTGGCATCTTTTT
4851	*NOTCH1*	CTTTGTTTCAGGTTCAGTATT	CCGGCTTTGTTTCAGGTTCAGTATTCTCGAGAATACTGAACCTGAAACAAAGTTTTT
4851	*NOTCH1*	CGCTGCCTGGACAAGATCAAT	CCGGCGCTGCCTGGACAAGATCAATCTCGAGATTGATCTTGTCCAGGCAGCGTTTTT
4851	*NOTCH1*	GCCGAACCAATACAACCCTCT	CCGGGCCGAACCAATACAACCCTCTCTCGAGAGAGGGTTGTATTGGTTCGGCTTTTT
4851	*NOTCH1*	CAAAGACATGACCAGTGGCTA	CCGGCAAAGACATGACCAGTGGCTACTCGAGTAGCCACTGGTCATGTCTTTGTTTTT

GE-HTS assay signature probes

Name	Sequence Reference	Probe Sequence
ARID5B	NM_032199.2	TAATACGACTCACTATAGGG CTTTAATCTCAATCAATACAAATCCACATTTGGTGGTGGTCAGC
		/5-PHOS/CAAGTCGCATCTGGTCTAGTTCCCTTTAGTGAGGGTTAAT
SLC2A3	NM_006931.1	TAATACGACTCACTATAGGG TACACTTTATCAAATCTTACAATCTCTGGCTCCTCAAACAGTAG
		/5-PHOS/GTTGGCAGTAAGGCAGGGTCTCCCTTTAGTGAGGGTTAAT
RASGRP1	NM_005739.2	TAATACGACTCACTATAGGG CAATTCAAATCACAATAATCAATCATCAGTTCCCATTTGAGTTC
		/5-PHOS/CTCCTTGCACTGAGTTTTAGTCCCTTTAGTGAGGGTTAAT
YPEL3	NM_031477	TAATACGACTCACTATAGGG CAATTCATTTACCAATTTACCAATGCAGCCAGAAGTACAAAGAG
		/5-PHOS/GGGAAGTACATCATTGAACTTCCCTTTAGTGAGGGTTAAT
TASP1	NM_017714	TAATACGACTCACTATAGGG TAATCTTCTATATCAACATCTTACGAGTGTGAAGCGTCTCAGAG
		/5-PHOS/GCATTTCAGAACCTGAGCTT TCCCTTTAGTGAGGGTTAAT
EIF5A	NM_001970.1	TAATACGACTCACTATAGGG TACAAATCATCAATCACTTTAATC CAATCTGGAATCAGAAAGCG
		/5-PHOS/GTGGATTCTGGCAAATGGTCTCCCTTTAGTGAGGGTTAAT
MRPL4	NM_015956.1	TAATACGACTCACTATAGGG CAATAAACTATACTTCTTCACTAA CTGGAGGACAAGCTGCTCTG
		/5-PHOS/GCAGGACTCACGTTACAGACTCCCTTTAGTGAGGGTTAAT
IFRD2	NM_006764	TAATACGACTCACTATAGGG ATACTTCATTCATTCATCAATTCAGGCGAGATCCAGGACAGATG
		/5-PHOS/GAAGCCTTTCTACACCTGTGTCCCTTTAGTGAGGGTTAAT
PCGF5	NM_032373	TAATACGACTCACTATAGGG CTTTAATCCTTTATCACTTTATCAGCTTGTTGGGGGAGTACCAG
		/5-PHOS/GGTTCTGCTGCTTTCCTGCATCCCTTTAGTGAGGGTTAAT
HDAC4	NM_006037.2	TAATACGACTCACTATAGGGTCAATCAATTACTTACTCAAATACTAAGTTTCCTACACCTGGAG
		/5Phos/GAGGTTGCATGGACTGTACGTCCCTTTAGTGAGGGTTAAT
GAPDH	NM_002046	TAATACGACTCACTATAGGGATTATTCACTTCAAACTAATCTACCCCCTTGAAGAGGGGAGGGG
		/5PHOS/CCTAGGGAGCCGCACCTTGTTCCCTTTAGTGAGGGTTAAT
PUS7	NM_019042.1	TAATACGACTCACTATAGGG AATCCTTTCTTTAATCTCAAATCA CTTCTATCAGTAGCGTTTAC
		/5-PHOS/CCTTTGTCACCAGTGGCTTTTCCCTTTAGTGAGGGTTAAT
SHQ1	NM_018130.1	TAATACGACTCACTATAGGG TTCAATCATTCAAATCTCAACTTTAGTTCTACTGCCCGTGTCTC
		/5-PHOS/CAGAATGTAGAGCCCATCAATCCCTTTAGTGAGGGTTAAT
GIMAP7	NM_153236	TAATACGACTCACTATAGGG CTTTTCAATTACTTCAAATCTTCAGAGGAAAGGCTGAAACAACG
		/5-PHOS/GGAAGAGGTTTTGAGGAAAATCCCTTTAGTGAGGGTTAAT
GIMAP5	NM_018384.1	TAATACGACTCACTATAGGG CTTTTCAAATCAATACTCAACTTTGCCTGCTGTAAACACTATTC
		/5-PHOS/CACTCTGTCTGCCAACAACTTCCCTTTAGTGAGGGTTAAT
EVL	NM_016337.1	TAATACGACTCACTATAGGG AATCTTACTACAAATCCTTTCTTT CTGCTTCATTAGACGGTTTC
		/5-PHOS/CAGGTTTTCTCCCAGGTGACTCCCTTTAGTGAGGGTTAAT
DHX37	NM_032656	TAATACGACTCACTATAGGG TTCACTTTTCAATCAACTTTAATCCCAGCAAGAAAGAGGGACAG
		/5-PHOS/GAGCACCCCAGGCAGGACCATCCCTTTAGTGAGGGTTAAT
P2RX5	NM_002561	TAATACGACTCACTATAGGG TCAATTACTTCACTTTAATCCTTTGTTCCTAGATTACCTCACTG
		/5-PHOS/GGAATAGCATTGTGCGTGTCTCCCTTTAGTGAGGGTTAAT
GIMAP1	NM_130759.2	TAATACGACTCACTATAGGG CAATTTCATCATTCATTCATTTCAGCGCCCATTACTCCAACGAG
		/5-PHOS/GTGTATGAGCTGGCGCAGGT TCCCTTTAGTGAGGGTTAAT
MYC	NM_002467.1	TAATACGACTCACTATAGGG CTTTTCATCTTTTCATCTTTCAAT AAAAAGCCACAGCATACATC
		/5-PHOS/CTGTCCGTCCAAGCAGAGGATCCCTTTAGTGAGGGTTAAT
HES1	NM_005524.2	TAATACGACTCACTATAGGG TACACAATCTTTTCATTACATCATAACAGGAACTTGAATACTGG
		/5-PHOS/GAGAGAAGAGGACTTTTTTGTCCCTTTAGTGAGGGTTAAT
MYO7B	NM_001080527	TAATACGACTCACTATAGGG TTACTACACAATATACTCATCAAT TCAACATATCTCCAGGGAAG
		/5-PHOS/GATGTGAATGCAGACACCAT TCCCTTTAGTGAGGGTTAAT
SLC29A1	NM_004955.1	TAATACGACTCACTATAGGG CTTTCAATTACAATACTCATTACA TACTCCATTCTCCCCTGCGC
		/5-PHOS/CTCCTCCTCTGTGTTCTCTCTCCCTTTAGTGAGGGTTAAT
RHOU	NM_021205	TAATACGACTCACTATAGGG TCATTTCACAATTCAATTACTCAA TCTCTCCAGACTATCGTAAC
		/5-PHOS/CTGGTGCCTTACCAAGTTGTTCCCTTTAGTGAGGGTTAAT
IGHG1	NG_001019	TAATACGACTCACTATAGGG CTTCTCATTAACTTACTTCATAAT ACTCCGACGGCTCCTTCTTC
		/5-PHOS/CTCTACAGCAAGCTCACCGTTCCCTTTAGTGAGGGTTAAT
PCBP3	NM_020528.1	TAATACGACTCACTATAGGG TCATCAATCTTTCAATTTACTTACAGAACTGTTGCCTGAGACCC
		/5-PHOS/CTCCTCTCTCACACAGCCCTTCCCTTTAGTGAGGGTTAAT
LZTFL1	NM_020347.1	TAATACGACTCACTATAGGG TCATTTCAATCAATCATCAACAATTTTTGGAGCTTTTGTTGAGG
		/5-PHOS/GCTTCATTCTCACCCTGTAT TCCCTTTAGTGAGGGTTAAT
NOTCH3	NM_000435.1	TAATACGACTCACTATAGGG TAATTATACATCTCATCTTCTACACCAAGCTGGATTCTGTGTAC
		/5-PHOS/CTAGTACCAGGCATGACTGGTCCCTTTAGTGAGGGTTAAT
DTX1	NM_004416.2	TAATACGACTCACTATAGGG TATATACACTTCTCAATAACTAACTGCTGCGGCTGCTCATCACG
		/5-PHOS/GCCTGGGAGAGAAGACTCATTCCCTTTAGTGAGGGTTAAT
NFX1	NM_002504.4	TAATACGACTCACTATAGGG CAATATCATCATCTTTATCATTACTGATTGCTCTGAGAGTTGAG
		/5-PHOS/GGACTATTGGGCTTTATTTGTCCCTTTAGTGAGGGTTAAT
NISCH	NM_007184	TAATACGACTCACTATAGGG TCATCAATCAATCTTTTTCACTTTTGTGTCCACTACCCACTGCC
		/5-PHOS/CGAGTTTGCCAAAGAGCCGC TCCCTTTAGTGAGGGTTAAT
GTF3A	NM_002097	TAATACGACTCACTATAGGGAAACAAACTTCACATCTCAATAATGAGAGTCACCCAACTGTGTG
		\5PHOS\GAAGACAAGATGCTCTCGACTCCCTTTAGTGAGGGTTAAT

SUPPLEMENTAL REFERENCES

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