Supplementary Information

PI3Kγ/δ and NOTCH1 cross-regulate pathways that define the T-cell acute lymphoblastic leukemia disease signature

Evgeni Efimenko,[1]* Utpal P. Davé,[2]* Irina V. Lebedeva,[1] Yao Shen,[3] Maria J. Sanchez-Quintero,[4] Daniel Diolaiti,[1] Andrew Kung,[1] Brian J. Lannutti,[5] Jianchung Chen,[1] Ronald Realubit,[3] Zoya Niatsetskiya,[1] Vadim Ten,[1] Charles Karan,[3] Xi Chen,[6] Andrea Califano,[3] and Thomas G. Diacovo[1,7]

Inventory of Supplementary Information

5 supplementary figures

Supplementary figure legends

3 supplementary tables

Supplementary methods

Supplementary references

Supplementary Figure S1

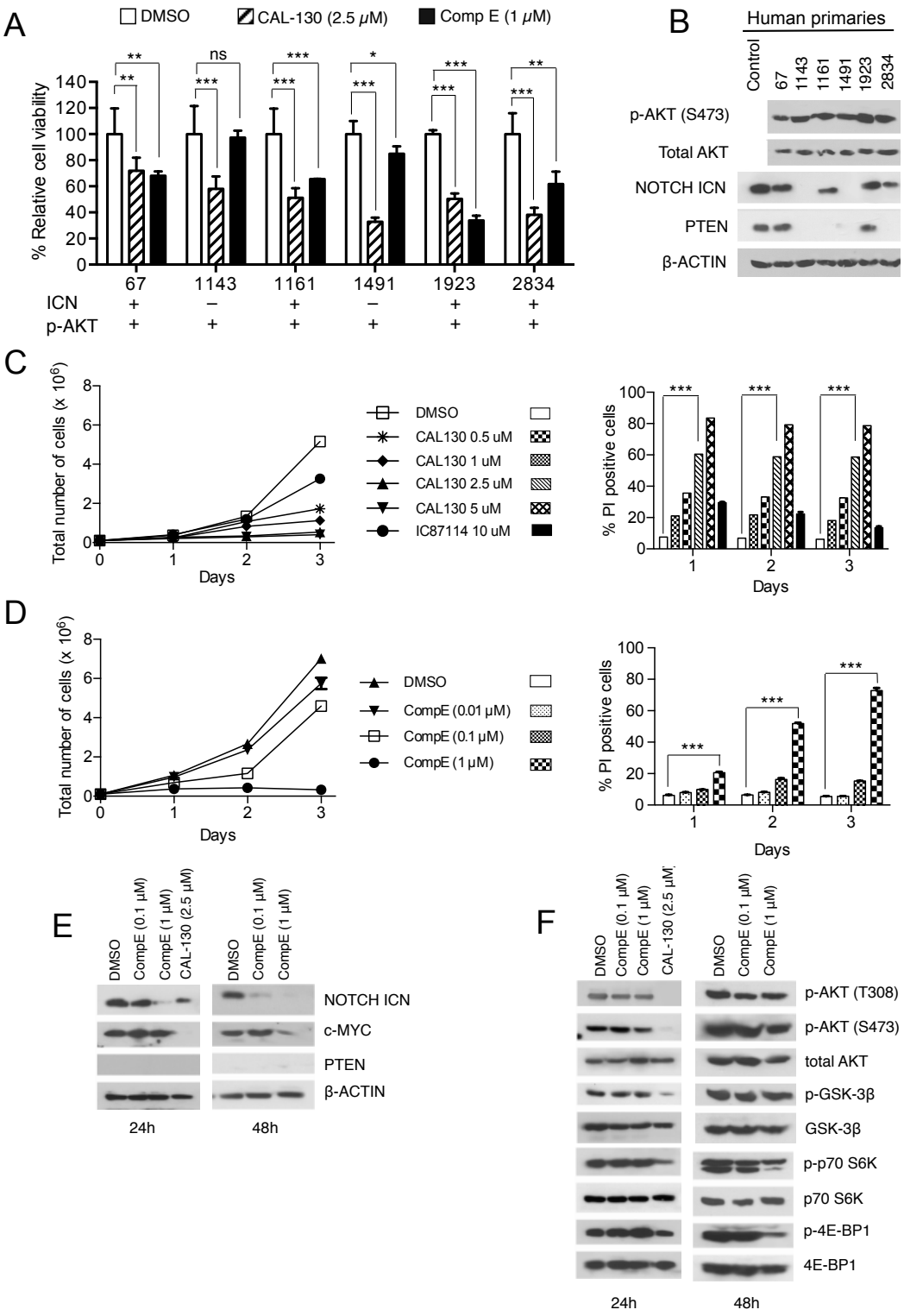

Figure S1 (Related to Figure 3). PI3Kγ/δ inhibition reduces the viability of primary human and mouse tumor cells. **A,** Percentage of primary human T-ALL tumor cells that remain viable after treatment with either CAL-130 or CompE for 72hrs (n = 6 performed in triplicate). **B**, Immunoblots of the identical cells depicting expression levels of PTEN as well as AKT and NOTCH1 activation state. Control sample used to demonstrate antibody detection of ICN and PTEN was the *CD2-Lmo2* T-ALL cell line 03020. **C** and **D,** Proliferation and survival analysis of the murine *CD2-Lmo2* T-ALL tumor derived cell line (03027) cultured in the presence of increasing concentrations of CAL-130 (**C**) or CompE (**D**) for 72 h. Data represent mean ± SEM (experiments performed in triplicate). ***, $P < 0.0001$ for drug treated cells relative to control (Student's *t* test). **E** and **F**, Immunoblots depicting expression of PTEN and cMYC as well as AKT and NOTCH1 activation states in the same cell line cultured in the presence of CAL-130 or CompE for 24 and 48 hours.

Supplementary Figure S2

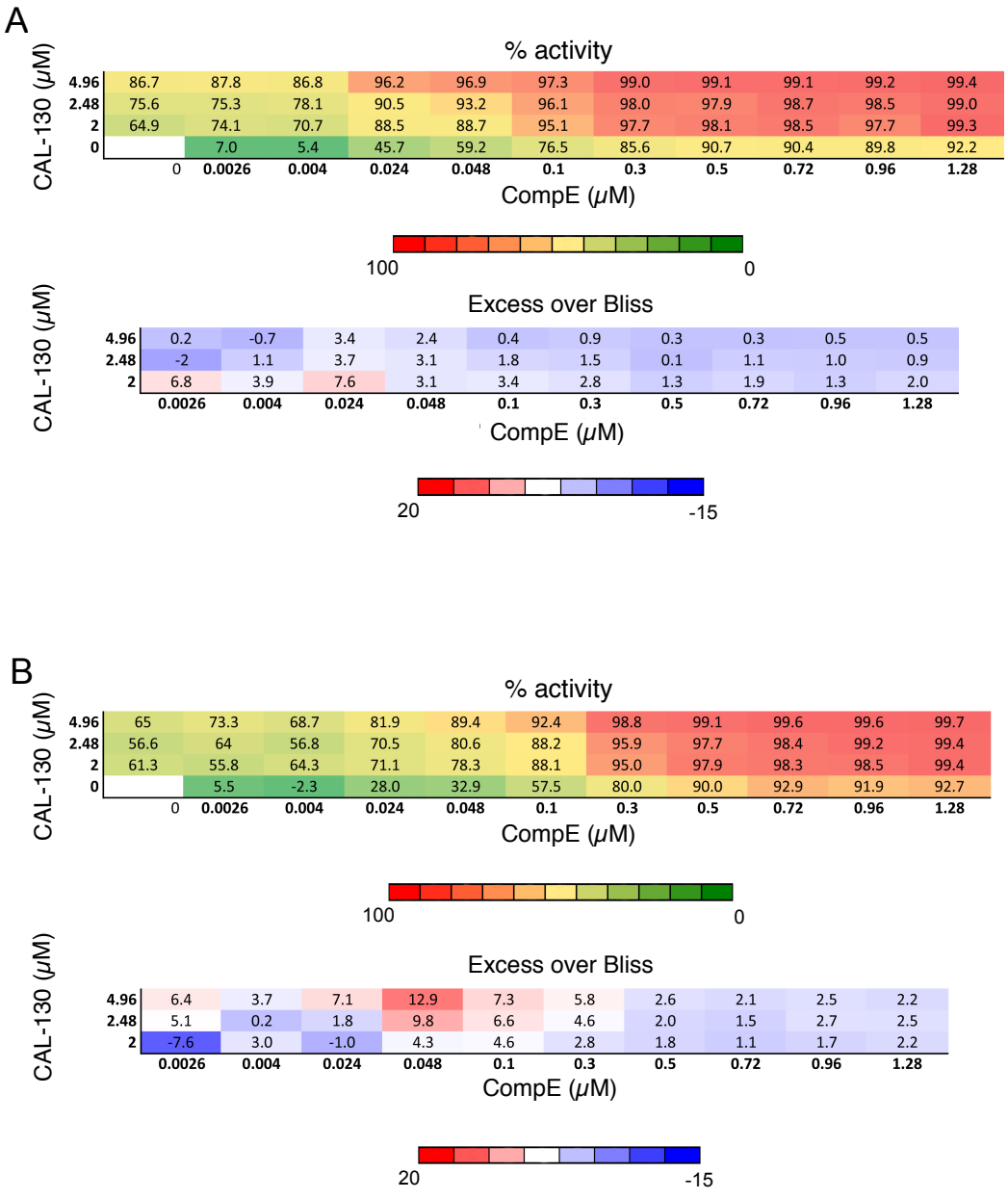

Figure S2 (Related to Figure 3). CAL-130 and CompE do not act synergistically in *Lmo2*-driven T-ALL. HTS assay was used to determine the combined *in vitro* effects of CAL-130 and CompE on the *CD2-Lmo2* T-ALL cell lines 03007 (**A**) and 03027 (**B**). CAL-130 was dosed in a range that targets both p110γ and p110δ isoforms (2 μM to 5 μM) (1); CompE was dosed in a range of 0.0026 μM to 1.28 μM. Heatmap showing percent of drug activity detected using Cell-Titer-Glo kit (Promega) (top table). Excess over Bliss (EoB) calculated as the difference between the observed effect and the predicted response (bottom table) are presented. Based on the Excess over Bliss model, a 5-10% EoB response was considered as weak, 10-20% as moderate, and values over 20% as strong synergism. Each value reported is the average of at least 3 independent experiments run in triplicate.

Supplementary Figure S3

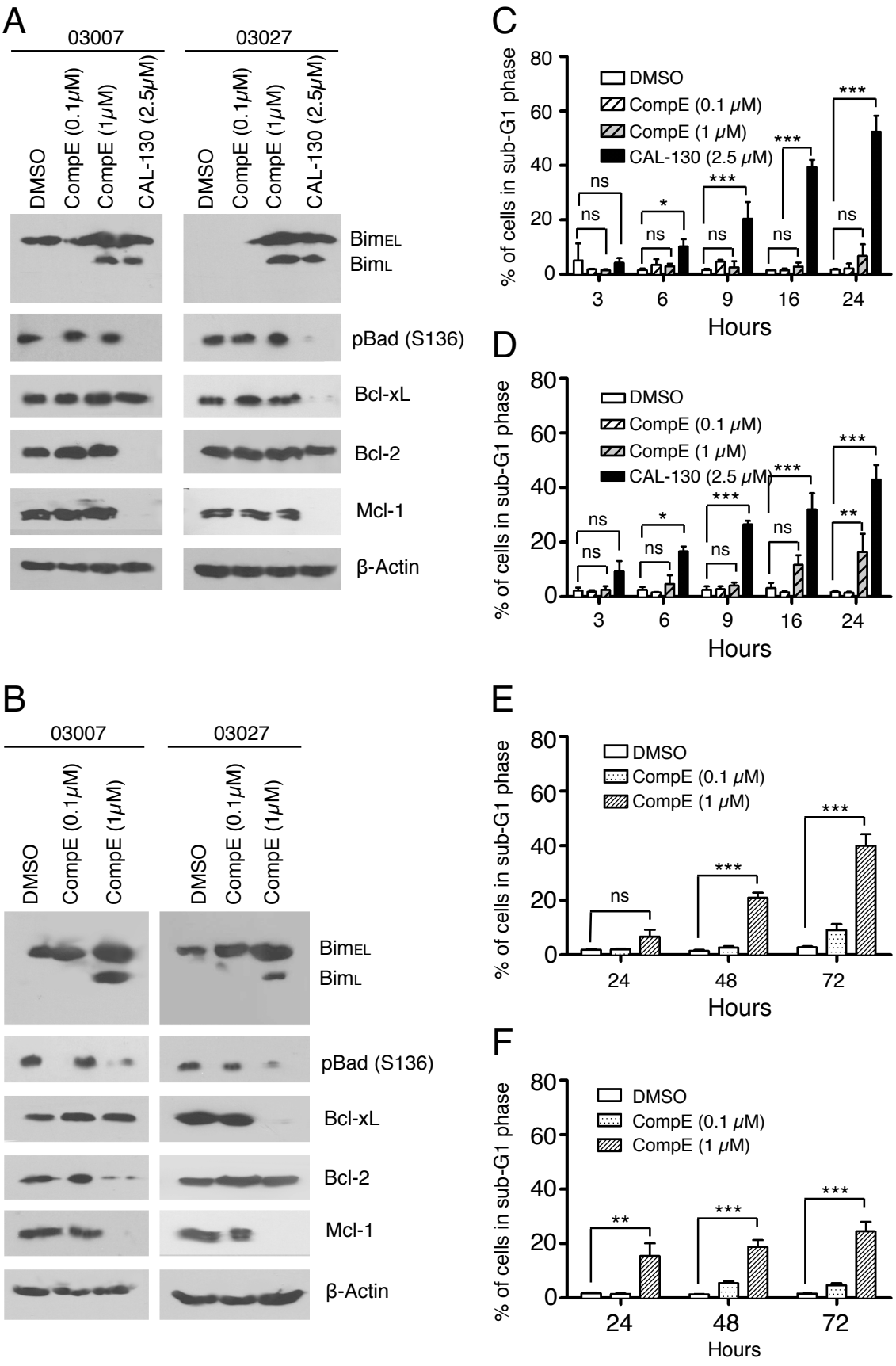

Figure S3 (Related to Figure 3). A and **B**, Immunoblots depicting changes in the levels of expression of pro- and anti-apoptotic proteins in the same cells treated with CAL-130, CompE or DMSO at 24 and 48 hours, respectively. **C–F**, Inhibition of either PI3Kγ/δ or NOTCH1 activity results in T-ALL cell death. CAL-130 treatment induces early (within 24 hours) apoptotic changes in the *CD2-Lmo2* T-ALL murine cell lines 03007 (**C**) and 03027 (**D**), while CompE effects were not evident until 48 to 72 hours (**E** and **F,** respectively). Data represent the mean ± SEM (2 independent experiments performed in triplicate). The fraction of apoptotic cells (sub-G_1) post-treatment was determined by flow cytometry. Statistical significance was determined by Student's t test for drug treated cells relative to control; *,P <0.01; **,P < 0.001, ***, P < 0.0001 for drug treated cells versus DMSO control.

Supplementary Figure S4

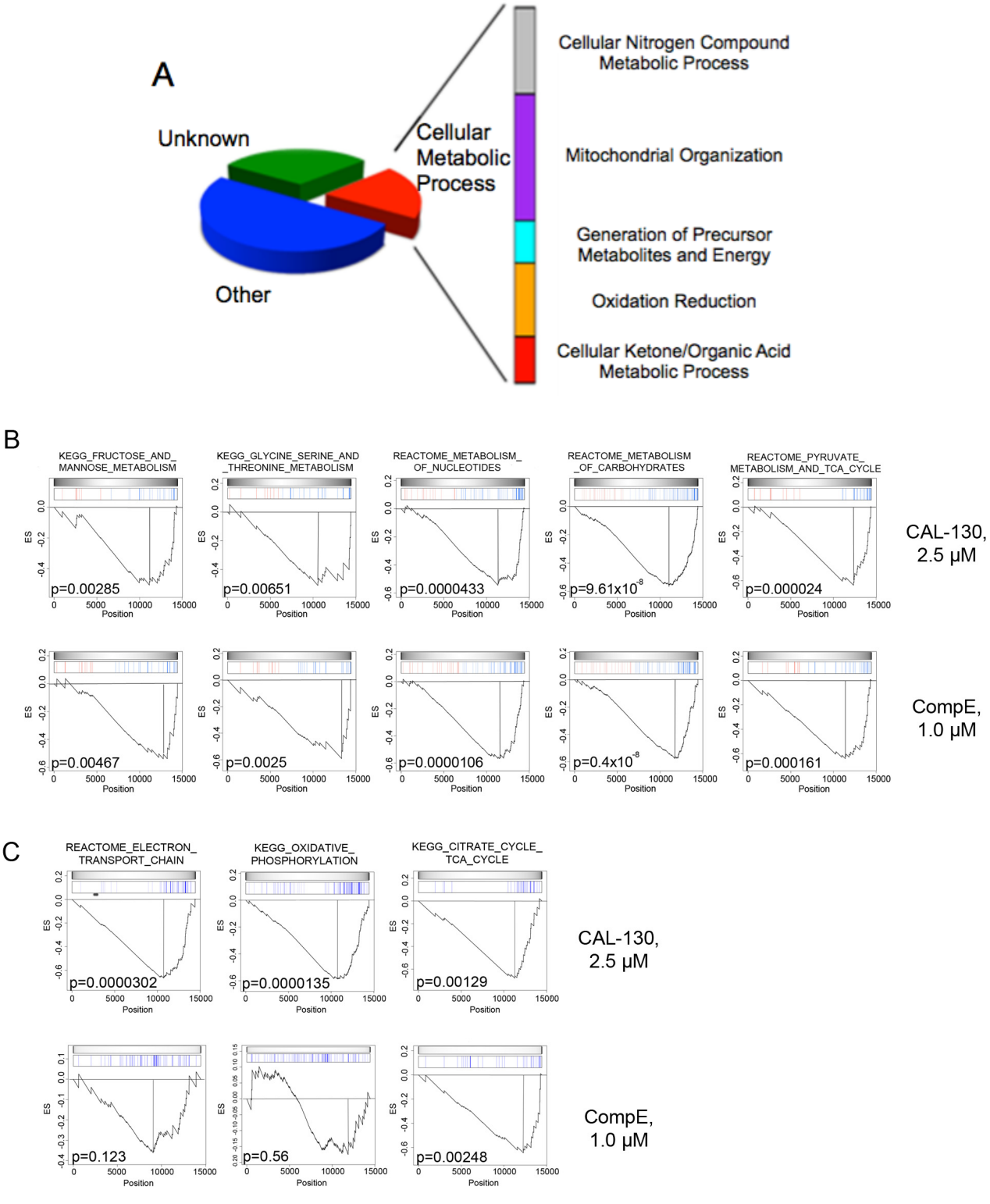

Figure S4 (Related to Figures 6 and 7). PI3Kγ/δ and NOTCH1 regulate similar metabolic pathways but differentially regulate mitochondrial pathways that contribute to the oncogenic programming of *Lmo2*-driven T-ALL. **A**, Functional annotation of top genes down regulated ($P < 0.0001$) in CompE (1μM, 48 hours) treated 03007 cells using DAVID. Pathways with Benjamini-Hochberg corrected P value < 0.05 are shown. Enrichment plots of selected top metabolic (**B**) and mitochondrial (**C**) gene sets significantly down-regulated by CAL-130 and CompE as determined by GSEA performed on the ranked genes according to the ratios of transcripts from DMSO and CAL-130 (2.5 μM, 10 hours) or CompE (1μM, 48 hours) treated cells.

Supplementary Figure S5.

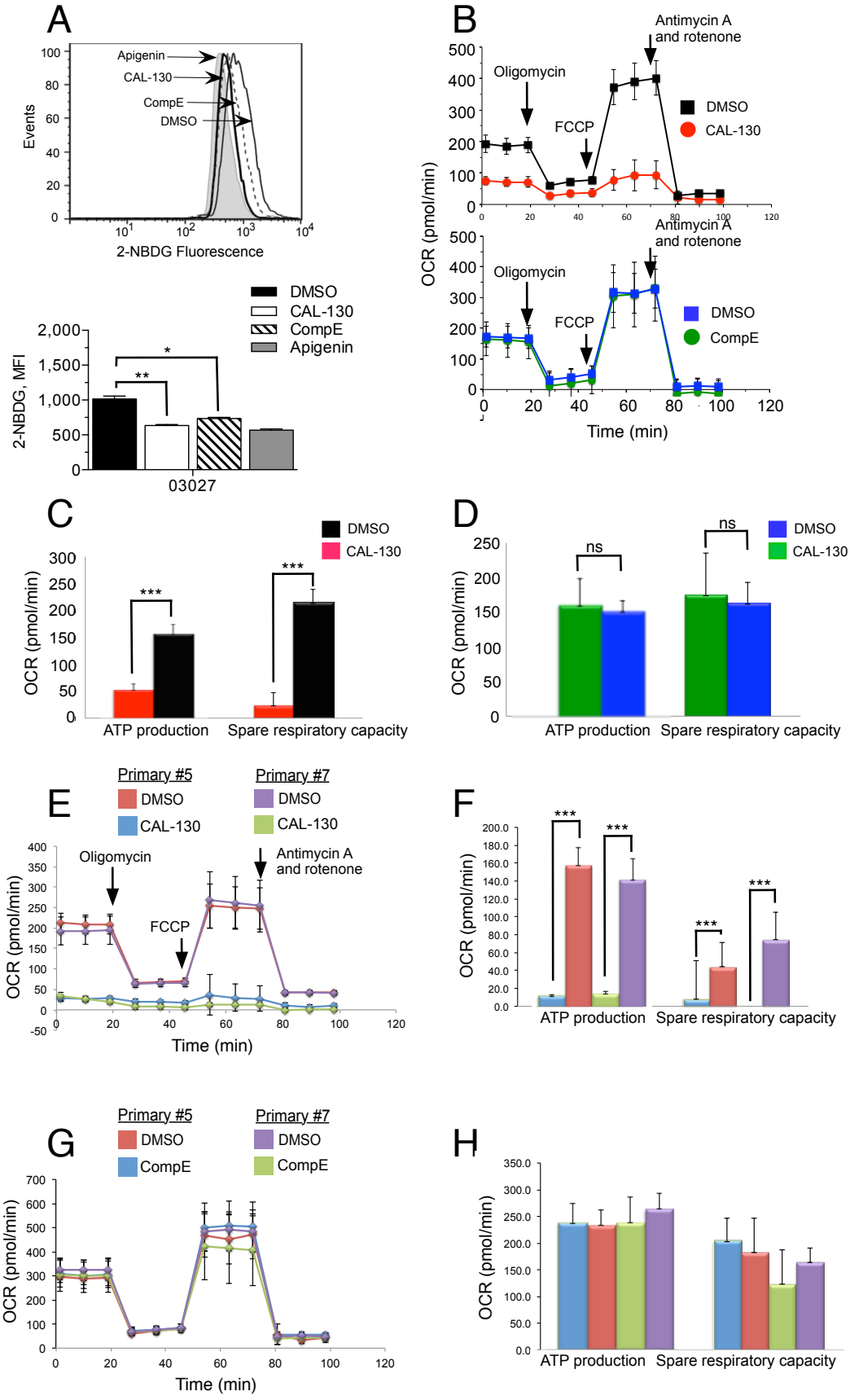

Figure S5 (Related to Figures 6 and 7). Regulation of bioenergetic pathways by PI3Kγ/δ versus NOTCH1 in *Lmo2*-driven T-ALL. **A**, Inhibition of glucose uptake by PI3Kγ/δ blockade or GSI. Representative histograms (**top**) and MFI (**bottom**) of 2-NBDG incorporation into 03027 cultured T-ALL cells treated with DMSO, CAL-130 (2.5 μM, 14 hours) or CompE (1μM, 72 hours) are shown. Apigenin (100μM, 30 min treatment), an inhibitor of Glut1 expression, was used as a positive control. *, $P < 0.01$; **, $P < 0.001$ relative to DMSO. **B**, Plots of oxygen consumption rates (OCR, oxidative metabolism) in 03027 murine T-ALL cells treated with DMSO, CAL-130 (2.5μM, 12hrs) or CompE (1μM, 48hrs) under basal conditions and following the addition of the mitochondrial (ATP-synthase) inhibitor oligomycin (1μM) or in the presence of the uncoupler FCCP (0.75μM), 1μM Rotenone and 1μM Antimycin A. **C** and **D**, ATP production and spare respiratory capacity in CAL-130 (**C**) or CompE (**D**) treated 03027 murine tumor cells. **E**–**H**, Plots of oxygen consumption rates (OCR, oxidative metabolism) and ATP production and spare respiratory capacity for 2 primary murine T-ALL tumors treated with DMSO, CAL-130 (2.5μM, 12hrs; **E** and **F**) or CompE (1μM, 48hrs; **G** and **H**) under basal conditions and following the addition of the mitochondrial (ATP-synthase) inhibitor oligomycin (1μM) or in the presence of the uncoupler FCCP (0.75μM), 1μM Rotenone and 1μM Antimycin A. Data represent mean ± SEM (***, $P < 0.0001$; n = 3, *t* test).

Supplemental Table 1. *Notch1* and *Pten* mutational status in primary mouse *CD2-Lmo2* T-ALL cells

Sample #	Mutations in *Notch1*	Mutations in *Pten*
Primary mouse T-ALL tumors		
1	None in PEST domain	p.R130W
2	p.P2420fs	None
3	p.R2361fs	p.F56_L60del
4	p.R2361fs	p.I253R; p.K254T; p.V255L; p.V275X
5	p.R2361fs	None
6	p.A2399fs	p.V166X
7	None in PEST domain	None
Primary human T-ALL tumors		
67	p.L1678P	None
1143	None	p.G209X
1161	p.Q2501X	p.P246fs
1491	None	p.R243fs
1923	p.I1675_V1676insTEGL	None
2834	p.Q2501X	p.R234fs

X = Stop codon; fs = frameshift; ins = insertion, del = deletion.

Supplemental Table 2. Pathways and transcription factors (TFs) significantly affected by treatment with CAL-130 (2.5μM) and CompE (1.0μM). Data are obtained by running ViPER on CAL-130 and CompE treatment gene signatures using the T-ALL interactome. The scores presented as normalized enrichment scores (NES) and considered to be significant at $|NES| > -1.96$, $P < 0.05$. Pathways or TF commonly affected by both drugs are shown in italic.

CAL-130 vs DMSO		**CompE vs DMSO**	
Pathway	**NES/p-value**	**Pathway**	**NES/p-value**
TFs	NES	TFs	NES
TP53	*-7.223*	*TP53*	*-4.687*
AATF	*-6.298*	*SNAPC5*	*-3.400*
SNAPC5	*-6.107*	*BPNT1*	*-3.255*
HNRNPAB	*-5.807*	*ZDHHC16*	*-3.180*
RFXANK	*-5.431*	*PA2G4*	*-2.844*
ZBTB8OS	*-4.950*	CREBL2	-2.588
ASH2L	*-4.650*	IFT57	-2.521
PA2G4	*-4.548*	*MSRB2*	*-2.257*
RBCK1	-4.472	NFXL1	-2.192
BPNT1	*-4.444*	*AATF*	*-2.164*
ZNHIT3	-4.257	*HNRNPAB*	*-2.123*
ZMAT2	-4.089	*ZBTB8OS*	*-2.091*
MSRB2	*-4.065*	*ZNF512*	*-2.084*
ZDHHC16	*-3.951*	*ZMYND19*	*-2.056*
PHF5A	-3.841	*ASH2L*	*-2.030*
HMGA1	-3.755	*ATF1*	*-2.028*
CARHSP1	-3.667	CBFB	-1.966
ZMYND19	*-3.651*		
ECSIT	-3.639		
EDF1	-3.578		
CNBP	-3.391		
MRPL28	-3.335		
THAP7	-3.232		
SUPT4H1	-3.223		
ZNF259	-2.998		
PFDN1	-2.937		
CREM	-2.928		
ZCCHC17	-2.893		
ZNHIT1	-2.889		
VPS72	-2.888		
CXXC1	-2.800		

BAZ1B	-2.799		
HSF1	-2.685		
HCFC1	-2.680		
REXO4	-2.676		
ZNF599	-2.645		
PREB	-2.582		
UHRF1	-2.502		
TRIM28	-2.501		
SMARCA4	-2.470		
ZNF747	-2.461		
ZSWIM7	-2.460		
YBX1	-2.435		
ZC3H4	-2.407		
ZNF512	-2.348		
DBP	-2.302		
KDM1A	-2.283		
NFYB	-2.241		
ZC4H2	-2.225		
MTA1	-2.204		
ZDHHC12	-2.178		
CAMTA1	-2.172		
RFX5	-2.159		
YEATS4	-2.097		
RB1	-2.093		
ATF1	-2.091		
AEBP1	-2.059		
ILF2	-2.038		
ZNF263	-1.992		

Supplemental Table 3. Ranking positions of the 53 MYC-regulated genes contained within the CAL-130 or CompE treated gene expression signature. Differentially expressed genes for either treatment group (compared to DMSO) were ranked from lowest to highest. For CAL-130, 39 of the 53 genes regulated by cMYC were found within the top 1,000 ranked genes, as compared to 22 of the 53 genes regulated by cMYC for CompE.

Gene name (CAL-130 treated)	Rank (cMyc regulated)	Gene name (CompE treated)	Rank (cMyc regulated)
Rexo2	7	Nsun2	90
Aimp2	29	Rrp1b	115
Apex1	32	Gsto1	221
Bop1	37	Eif1a	232
Ifrd2	40	Pa2g4	244
Gar1	46	Bag2	292
Eif1a	49	Srm	294
Tsr1	56	Nop16	302
Srm	63	Ipo5	361
Rcl1	95	Gar1	431
Gsto1	143	Gart	514
Gnl3	156	Shmt1	542
Pprc1	182	Rexo2	685
Shmt1	222	Cct3	688
Nop56	225	Aimp2	697
Gart	226	Ipo4	884
Rpf2	230	Rcl1	893
Ppan	241	Ppan	900
Nsun2	248	Gusb	914
Smyd2	264	Smyd2	925
Hspa9	291	Ifrd2	972
Rrp1b	350	Bcap29	1000
Bcap29	389	2610201A13Rik	1079
Ppa1	414	Tsr1	1087
Nop16	422	Hspa9	1143
Nme1	437	Rrs1	1179
Cacybp	442	Apex1	1192
Nhp2	490	Odc1	1193
Adsl	504	Bop1	1442
Ipo5	514	Adk	1484

Bag2	721	Nhp2	1503
Gusb	756	Ppa1	1722
Tpi1	763	Adsl	1846
Rrs1	781	Pprc1	1855
Adk	859	Ebna1bp2	1879
Cct3	871	Tpi1	2015
Psme3	914	Dtd1	2283
Pa2g4	932	Tmem97	2295
Ebna1bp2	987	Nop56	2430
Nop58	1097	Mt2	2622
Tmem97	1145	Psme3	2651
Ddx21	1336	Ttc27	2692
Odc1	1464	Mtm1	2821
Ipo4	1559	Nop58	2831
Mtm1	1562	Gnl3	3146
Polr2e	1609	Lamb2	3217
Mat2a	2248	Cacybp	3366
Ttc27	2331	Rpf2	3583
2610201A13Rik	2777	Ddx21	3591
Mt2	7007	Nme1	3615
Dtd1	11009	Mt1	6795
Lamb2	12785	Polr2e	8938
Mt1	14554	Mat2a	12375

Supplemental Methods

Quantitative Real-Time PCR.

qRT-PCR was performed as previously described (1). RNA from cells was isolated using the Qiagen RNeasy Mini Kit (cat#74104) according to the manufacturer's protocol. The isolated total RNA was reverse transcribed using a high capacity cDNA synthesis kit (SuperScript FirstStrand Synthesis System, Invitrogen, Cat#11904-018) according to the manufacturer's protocol. Predesigned labeled primer and probe sets for mouse *Notch1* (Mm00435249_ml), mouse *Deltex* (Mm00492297_ml), and mouse *GAPDH* (Mm99999915_g1) were from Applied Biosystems. The PCR reactions were set up following the protocol of USB (hotStart-IT Probe qPCR system Cat# 75764). Real time relative quantitative PCR were run on ABI7500 with cycling conditions of 50°C 2 min, 95°C 10 min, 40 cycles of 95°C 15 sec and 60°C 1 min. Data exported from ABI7500 machine were processed and analyzed using Excel spread sheet. Briefly, target genes were normalized to the housekeeping gene GAPDH to obtain a ΔCT value. Relative quantitative expression was calculated with equation (2^- ΔΔCT) where the ΔΔCT is the deference between the ΔCT of tumor samples and control samples (ΔΔCT=ΔCT tumor - ΔCT control). A Student's t test was used to determine statistical difference in expression levels with $P < 0.05$ considered significant.

Drug synergy experiments

The density of each *CD2-Lmo2* T-ALL derived cell line was first optimized to ensure log phase cell growth in the tissue culture treated 384 well plates (Greiner Bio-One 781080)

for the duration of the experiment. Starting with 16,000 cells per well, the cells were serially diluted in 2-fold increments to test 10 different cell densities in the microplate. Cell-Titer-Glo (Promega) was used to measure total ATP levels of the wells every 24 hours for 96 hours. Plating of each cell line was done employing the Cell::Explorer automation system at the optimal density into white tissue culture treated 384 well plates on a Perkin Elmer Janus Automated Workstation. The plates were incubated in the Liconic STX-500 ICSA for 24 hours prior to drug addition. All cell lines were first treated with CAL-130 or CompE as a single agent to determine the range of activity. The HP D300 Digital Dispenser was used to dispense specific amounts of the drugs for a titration curve (in triplicate). The DMSO concentration was normalized across all the wells and Thimerosal was used as the positive control, with DMSO treated wells serving as the negative control. For drug combination experiments, CompE was added 24 hours after the initial cell seeding and CAL-130 subsequently added 48 hours later due to its more rapid onset of action. Assay well viability was measured 12 hours later. Using Pipeline Pilot and the Plate Data Analytics collection, the viability of the cells were calculated as a percentage of the difference measured between the positive and negative controls. Then a concentration response curve was generated using a sigmoidal equation for graphing the viability versus the concentration of the compound. The HP D300 Digital Dispenser software was used to design a matrix of 100 wells for combining two drugs. Titration curves of both drugs alone as a single agent were also included in the plate. The fractional inhibition of each individual compound D (expressed as a value between 0 to 1) were used to calculate the Bliss values with this equation:

$$C = D_1 + D_2 - D_1 * D_2$$

where C is the predicted combined response of two drugs in the combination with single drug effects D1 and D (2, 3). Excess over Bliss (EoB) was then calculated as the difference between the observed effect and the predicted response. A synergistic drug pair would have a greater observed effect than the predicted response and if the observed effect is less than the predicted response then the drug pair is antagonistic. An additive drug combination would have observed values equal to the predicted response.

Apoptosis assay

Apoptosis (percentage of the cells exhibiting DNA fragmentation) was assessed in ethanol-fixed cells by staining with PI as described (4). Briefly, CD2-Lmo2 T-ALL cell lines, treated with different agents as described in the Results, were collected by centrifugation, washed twice with cold PBS and fixed overnight at -20°C in 70% ethanol. After washing twice in PBS, cells were re-suspended in 1 ml of PBS containing 1 mg/ml of RNase A and 0.5 mg/ml of PI. After 30 min incubation, cells were analyzed by flow cytometry.

Western blotting

Cell lysates (from cell lines or mice thymocytes) were prepared on ice in M-PER Mammalian Protein Extraction reagent (Pierce) containing a cocktail of protease and phosphatase inhibitors as previously described (5). Equal amounts of total protein from lysates were subjected to SDS-PAGE, transferred to PVDF membrane (Immobilon-P; Millipore), and membranes were probed by overnight incubation with appropriate primary antibodies. Bound antibodies were visualized with HRP-conjugated secondary

antibodies and ECL chemistry (SuperPico West; Pierce). Antibodies for AKT (cat. 9272), pAKT Ser 473 (cat. 4058), pAKT Thr308 (cat. 9275), 4E-BP1 (cat. 9644), p4E-BP1 Thr37/46 (cat. 2855), GSK-3β (cat. 9315), pGSK-3β Ser9 (cat. 9323), p70 S6K (cat. 9202), pp70 S6K Thr389 (cat. 9205), cleaved NOTCH1 Val1744 (cat.2421), PI3K p110α (cat.4255), Bim (cat. 2933), Bcl-xL (cat.2764), pBad Ser136 (cat. 4366), Bcl-2 (cat. 2870), Mcl-1 (cat.5453), SHMT2 (cat. 12762), β-ACTIN (cat. 4967) were purchased from Cell Signaling Technology. Antibodies for PTEN (cat. sc7974), c-MYC (N-262) (cat. sc764) and PI3K p110δ (cat. sc7176) were from Santa Cruz Biotechnology. COX IV antibody (cat. 14744) was obtained from Abcam, PI3K p110β (cat. 04-40) was from Millipore, PI3K p110γ antibody (ABD-026S) was from Jena Bioscience, and α-Tubulin (cat. T6074) was from Sigma.

Flow Cytometry for cell surface and intracellular staining of mouse whole blood.

Whole blood collected in EDTA, incubated with desired antibodies and blood processed using the BD Bioscience BD FACS Lysing Solution according to the manufacturer's instructions (1). For intracellular staining of Ki67, immediately after RBC lysis with the BD FACS Lysing solution, cells were permeabilized without washing with 0.025% Tween 20 in lysing solution for an additional 15 min, washed, and then incubated with Ki67 antibodies. Samples were analyzed using a FACS Calibur flow cytometer (Becton Dickinson). The obtained data were analyzed using FlowJo software (Tree Star). Fluorescently labeled antibodies CD3ϵ-Alexa 488 (cat. 557666), CD4-APC (cat. 553051), CD8-PerCP-Cy5.5 (cat. 551162), CD90.2-APC (Thy1.1) (cat. 553007), Ki67-FITC (cat.

556026), CD25-FITC (cat. 553071), CD44-APC (cat. 559250) and CD45-APC (cat.555485) were obtained from BD Biosciences.

Mitochondria isolation and respiration studies

Mitochondria were isolated using differential centrifugation and percoll gradient method as described (6). Mitochondrial respiration was tested in TRIS-MOPS buffer at 32°C using Clark's type electrode (Hansatech, UK) as described (7). Two substrates were used, glutamate-malate (complex-I linked substrate) and N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride – ascorbate (TMPD-ascorbate) which supplies electrons directly to the complex IV. Phosphorylating respiration was initiated by supplementation of 100 nmoles of ADP, and uncoupled respiration was induced by adding of 35 mM of 2',3' Dinitrophenole (DNP). The respiration rates were expressed in nmoles of O_2 consumption / mg mitochondrial protein / min.

Gene Expression Analysis. Microarray data are available through the Gene Expression Omnibus repository (accession no. to be supplied).

Gene signatures for CAL-130 versus CompE treated 03007 murine *CD2-Lmo2* T-ALL cell line were generated by differential gene expression analysis of same drug treatment samples and matching DMSO controls, using Student's t-test. To represent the changes for each gene upon drug treatment, $\log_{10}$ (P) with the sign from Student's *t*-test was used. GSEA analysis (8) was used to assess similarity between CAL-130 and CompE effects tumor cells by measuring enrichment of the top 350 most over and under-expressed genes

following treatment with one drug in genes differentially expressed following treatment with the other drug.

For comparison of mouse CompE treatment signature with a human GSI treatment signature, a consensus GSI perturbational signature was obtained from the expression profiles (GSE5827) of seven human T-ALL cell lines treated with DMSO and CompE (9). CompE treatment signature was then compared to GSI treatment signature by computing the enrichment of the 200 most over and under-expressed genes following GSI perturbation of human T-ALL cell lines in genes differentially expressed following CompE treatment of mouse cells. See below for human to mouse gene mapping details.

The T-ALL interactome was obtained by ARACNe-based reverse engineering analysis of HG-U133a Plus.2 Affymetrix microarray samples from 223 T-ALL patients using GC-RMA normalization (10). Then ARACNe algorithm with 100 bootstrap steps (11) was applied against 1060 genes annotated as transcription factors (TFs) in Gene Ontology Database. The mouse genes were then mapped to human genes to perform ViPER analysis on human T-ALL interactome that contains 1060 TFs, 11270 target genes and 124909 total interactions between TFs and target genes (11). TF activity signatures upon drugs treatment were obtained by running ViPER algorithm on CAL-130 and Compound E treatment gene signatures using T-ALL interactome (12). Pathway analysis of drug treatment signatures was performed on the top down-regulated genes (P<0.0001 for CompE and P<0.00001 for CAL-130) using DAVID Functional Annotation tool (13, 14).

For Gene Set Enrichment Analysis GSEA, the metabolic pathways were downloaded from the MSigDB database (8). Then, GSEA was performed to compute the enrichment

of each metabolic pathway gene set over the D1 or D2 drug pertrubational signature, sorted from the highest to the lowest expression. *P* values were calculated from 1000 permutations of the gene list. *P* values were then FDR-corrected to adjust for multiple testing.

To obtain a list of genes regulated by c-MYC, we analyzed microarrays of T cell lymphoblasts expressing MYC gene (E-MEXP-169) (15). We then compared gene expression profiles in the same cell lines, including 1415T, 4347T, 4502T, and 7755T, treated for 12 hours with doxycycline to abrogate MYC expression. Genes are considered as MYC-regulated if they are differentially expressed in the same cell line with or without doxycycline treatment (fold change > 2) in at least two out of the four cell lines.

Supplemental references

1. Subramaniam PS, Whye DW, Efimenko E, Chen J, Tosello V, De Keersmaecker K, et al. Targeting nonclassical oncogenes for therapy in T-ALL. Cancer Cell 2012;21:459-72.
2. Berenbaum MC. Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 1981;35:269-335.
3. Borisy AA, Elliott PJ, Hurst NW, Lee MS, Lehar J, Price ER, et al. Systematic discovery of multicomponent therapeutics. Proc Natl Acad Sci U S A 2003;100: 7977-82.
4. Riccardi C and Nicoletti I. Analysis of apoptosis by propidium iodide staining and flow cytometry. Nat Protoc 2006;1:1458-61.
5. Swat W, Montgrain V, Doggett TA, Douangpanya J, Puri K, Vermi W, et al. Essential role of PI3Kdelta and PI3Kgamma in thymocyte survival. Blood 2006; 107:2415-22.
6. Niatsetskaya ZV, Sosunov SA, Matsiukevich D, Utkina-Sosunova IV, Ratner VI, Starkov AA, et al. The oxygen free radicals originating from mitochondrial complex I contribute to oxidative brain injury following hypoxia-ischemia in neonatal mice. J Neurosci 2012;32:3235-44.
7. Sosunov SA, Ameer X, Niatsetskaya ZV, Utkina-Sosunova I, Ratner VI, and Ten VS. Isoflurane anesthesia initiated at the onset of reperfusion attenuates oxidative and hypoxic-ischemic brain injury. PLoS One 2015;10:e0120456.

8. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102:15545-50.

9. Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, Margolin A, et al. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci U S A 2006;103: 18261-6.

10. Piovan E, Yu J, Tosello V, Herranz D, Ambesi-Impiombato A, Da Silva AC, et al. Direct reversal of glucocorticoid resistance by AKT inhibition in acute lymphoblastic leukemia. Cancer Cell 2013;24:766-76.

11. Margolin AA, Nemenman I, Basso K, Wiggins C, Stolovitzky G, Dalla Favera R, et al. ARACNE: an algorithm for the reconstruction of gene regulatory networks in a mammalian cellular context. BMC Bioinformatics 2006;7 Suppl 1:S7.

12. Aytes A, Mitrofanova A, Lefebvre C, Alvarez MJ, Castillo-Martin M, Zheng T, et al. Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. Cancer Cell 2014;25:638-51.

13. Huang da W, Sherman BT, and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4: 44-57.

14. Huang da W, Sherman BT, and Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1-13.

15. Palomero T, Barnes KC, Real PJ, Glade Bender JL, Sulis ML, Murty VV, et al. CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to gamma-secretase inhibitors. Leukemia 2006;20:1279-87.