Supplemental Methods

High-Throughput Screen in MEFs
The Biomek FX was used to dispense 5 uL of compounds (6X final concentration) to 384-well assay plates. 1,000 cells per well were plated in 25 uL of media to the pre-drugged assay plates with a Thermo Wellmate™ and a standard bore cassette head. The final volume in test wells was 30 uL, with a final compound concentration of 0.5 uM and final percentage of DMSO was 0.01% (0.5% maximum tolerance). Negative control was untreated cells. Positive control was 100 uM Hyamine.
Plates were incubated stacked two high for 72 hrs at 37 ^{0}C and 5% CO_2 in a humidified incubator. On the day of the read, 5-6 plates were taken out of the incubator at a time and equilibrated to room temperature. 30 uL/well of Cell-Titer Glo™ was added and incubated for 10 min at room temperature. Luminescence at 0.1s was read on the PE Envision™ plate reader.

Chemical Capture Mass Spectrometry
We synthesized a capture compound employing apilimod as selectivity function attached in a single orientation. Specific protein binders were identified using apilimod as a competitor ligand in whole cell lysate prepared from a cell line known to be sensitive to apilimod (H4). Proteins were subsequently detected and identified by LC-MS; those that were significantly diminished in competition control experiments were considered to be specific binders.

Kinase Screening and Dissociation Constant Analysis
A quantitative binding constant (K_d) of apilimod to PIKfyve was generated using the KdElect™ platform (DiscoveRX). An 11-point dose-response of apilimod (0.05 – 3000 nM) was used and the experiment was performed in duplicate. The KinomeScan™ platform (DiscoveRX) was used to conduct competition binding assays between apilimod (screening concentration was 1 μM) and 456 kinases, including disease-relevant kinases, as previously described [1]. A kinase interaction map was created with the TREEspot™ compound visualization tool (http://www.kinomescan.com/) to show the selectively of apilimod toward PIKfyve.

Hairpin Sequences
shNon-targeting:
F:CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT
R:AATTAAAAACAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTG

sh*PIKFYVE*#1 (sh*PIKFYVE*):
F:CCGGCGAGTTAAGGAGATCCTAATACTCGAGTATTAGGATCTCCTTAACTCGTTTTTTG
R:AATTCAAAAAACGAGTTAAGGAGATCCTAATACTCGAGTATTAGGATCTCCTTAACTCG

sh*PIKFYVE*#2:
F: CCGGCCTTGGATTGTAACAATGGAACTCGAGTTCCATTGTTACAATCCAAGGTTTTTTG
R: AATTCAAAAAACCTTGGATTGTAACAATGGAACTCGAGTTCCATTGTTACAATCCAAGG

Reverse Transcription and PCR
Equal amounts of total RNA was reverse transcribed using Superscript III™ and oligo(dT)20 (Invitrogen). Quantitative real time PCR was carried out on the cDNA using TaqMan Fast Advanced Master Mix™ (Life Technologies). PIKFYVE expression was normalized to *TBP* expression using TaqMan™ probes PIKFYVE FAM Hs01038899_m1 and TBP VIC Hs99999910_m1 or *GAPDH* using Fast SYBR® Green Master Mix (Life Technologies) and primers (GCCACATCGCTCAGACACC and GTACTCAGCGGCCAGCATCG)

Western Blotting
Following treatment, cells were lysed using 1 x RIPA supplemented with a protease inhibitor cocktail tablet (Thermofisher) and a PhosSTOPphosphatase inhibitor cocktail tablet (Sigma). Samples were centrifuged at 14000 rpm for 15 min at 4^{o}C and the resulting supernatants were used as total cell lysates. Where shown, cytoplasmic and nuclear lysates were generated using a USB nuclear extraction kit (Affymetrix), following the manufacturers protocol. For all extracts, 4 - 40 ug of protein was loaded per lane in a reducing gel. Protein was transferred to nitrocellulose or PVDF membrane at 4 ^{0}C for 1 hr at 100V and the membrane blocked with 3% BSA or 5% milk for 1 hr at room temperature followed by overnight incubation with the appropriate antibody at 4 ^{0}C. Membranes were washed (3 x 10 min) in TBS/Tween before addition of appropriate HRP-conjugated 2^{0} antibody for 1 hr at room temperature. Protein bands were detected using Western Lightning Plus-ECL™ (PerkinElmer) and visualized using ChemiDoc™ XRS+ with ImageLab™ software (BioRad).

List of antibodies used:

Antibody	Source	Dilution
TFEB	Cell Signaling Cat# 4240S	1:1000
PIKFYVE	R&D Cat# AF7885	1:500
Alpha-Tubulin	Sigma-Aldrich Cat# T9026	1:5000
Lamin A/C	Santa Cruz Cat# SC-376248	1:250
Cathepsin A	R&D Cat# AF1049	1:1000
Cathepsin D	R&D Cat# AF1014	1:1000
Beta actin	Cell Signaling #3700	1:1000
LC-3	Novus Cat# NB100-2331SS	1:500
p62	Cell Signaling Cat# 5114	1:1000
Vinculin	Cell Signaling Cat# 13901	1:1000

Cloning Strategies for overexpression constructs:
PIKFYVE was assembled from two PCR products amplified with primers (TCTCATCATTTTGGCAAAAACCATGGCCACAGATGATAAG and AAGAGAGATCGGGGCTTTTC) from cDNA clone HsCD00399349 (The ORFeome Collaboration) and (GAAAAGCCCCGATCTCTCTT and CCTGAGGAGTGAATTCATCAGCAATTCAGACCCAAG) from WSU-DLCL2 resistant clone cDNA.
TFEB was amplified from cDNA clone MGC:40490 with primers (TCTCATCATTTTGGCAAAAACCATGGCGTCACGCATAGG) and (CCTGAGGAGTGAATTCATCACAGCACATCGCCCTCCT).
Cathespin D (catD) was amplified from cDNA clone (Accession no.BC016320) with primers (TCTCATCATTTTGGCAAAAACCATGCAGCCCTCCAGCCTT) and (CCTGAGGAGTGAATCTAGAGGCGGGCAGCCTCGG)

Daudi Xenograft

The Daudi cell line was maintained in RPMI-1640 high glucose medium supplemented with 10% fetal bovine serum, 1% sodium pyruvate and 1% MEM non-essential amino acids at 37 °C in an atmosphere of 5% CO_2. The tumor cells were sub-cultured twice weekly and harvested during exponential growth for tumor inoculation. Seven to eight week old female SCID mice were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA). Each mouse was inoculated subcutaneously with Daudi tumor cells (5 x 10^6) in 0.1 ml of PBS with Matrigel (1:1). The tumors were then grown to a mean size of approximately 100-200 mm^3 and the mice were then split into 4 groups and treated as detailed in the table below. Tumor size was monitored for 19 days and measured in three dimensions using a caliper, and the volume is expressed in mm^3 using the formula: V = 0.5 L x W x T where L is the length, W is the width, and T is the thickness of the tumor, respectively.

Group	Treatment	Dose	Dosing schedule	Administration route	Number of mice
1	Vehicle (0.5% Methylcellulose)	NA	QD × 11	p.o.	8
2	Apilimod free base	50 mg/kg	QD × 11	p.o.	8
3	Apilimod free base	100 mg/kg	QD × 11	p.o.	8
4	Apilimod free base	150 mg/kg	QD × 11	p.o.	8

SU-DHL-6 Apilimod / Rituximab Combo Xenograft

The SU-DHL-6 cell line was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and L-glutamine (2 mM) at 37 °C in an atmosphere of 5% CO_2. The tumor cells were sub-cultured twice weekly and harvested during exponential growth for tumor inoculation. CB17 SCID mice obtained from Vital River Laboratory Animal Technology Co, Ltd. (VR, Beijing, China) were used for this study. Each mouse was inoculated subcutaneously in the right flank with SU-DHL-6 tumor cells (5 x 10^6) in 0.1 ml of PBS with Matrigel (1:1). The tumors were then grown to a mean size of approximately 80-120 mm^3 and the mice were then split into 4 groups and treated as detailed in the tables below. Tumor size was monitored for 26 days and measured twice a week in two dimensions using a caliper, and the volume is expressed in mm^3 using the formula: V = 0.5 a x b^2 where a and b are the long and short diameters of the tumor, respectively.

Group	Treatment	Dose	Dosing schedule	Administration route	Number of mice
1	Vehicle (0.5% Methylcellulose) (PBS)	NA	BID x 11 Q4D x 3	p.o. i.p.	6
2	Apilimod dimesylate	60 mg/kg (41 mg/kg free base)	BID x 11	p.o.	6
3	Rituximab	7 mg/kg	Q4D x 3	i.p.	6

4	Apilimod dimesylate Rituximab	60 mg/kg (41 mg/kg free base) 7 mg/kg	BID x 11 Q4D x 3	p.o. i.p.	6

A20 Apilimod / anti-PD-L1 Combo Syngeneic Model

The A20 cell line was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and L-glutamine (2 mM) at 37 ºC in an atmosphere of 5% CO_2. The tumor cells were sub-cultured twice weekly and harvested during exponential growth for tumor inoculation. BALB/c mice obtained from Vital River Laboratories (Beijing, China) were used. Each mouse was inoculated subcutaneously in the right flank with A20 tumor cells (5 x 10^5) in 0.1 ml of PBS. The tumors were then grown to a mean size of approximately 80-100 mm^3 and the mice were then split into 4 groups and treated as detailed in the table below. Tumor size was monitored for 23 days and measured twice a week in two dimensions using a caliper, and the volume is expressed in mm^3 using the formula: V = 0.5 *a* x b^2 where *a* and *b* are the long and short diameters of the tumor, respectively.

Group	Treatment	Dose	Dosing schedule	Administration route	Number of mice
1	Vehicle (0.5% Methylcellulose) PBS	NA	BID x 12 BIW x 2	p.o. i.p.	8
2	Apilimod dimesylate	90 mg/kg (62 mg/kg free base) 80 mg/kg (55 mg/kg free base)	BID x 3 BID x 9	p.o.	8
3	Ant-PD-L1 (10F.9G2)	5 mg/kg	BIW x 2	i.p.	8
4	Apilimod dimesylate + Ant-PD-L1 (10F.9G2)	90 mg/kg (62 mg/kg free base) 80 mg/kg (55 mg/kg free base) 5 mg/kg	BID x 3 BID x 9 BIW x 2	p.o. i.p.	8

Inhibitor Treatment

Z-VAD-FMK combination treatment
B-NHL were seeded into 96 well plates and treated with apilimod (10 point dose response; 0.5 – 10000 nM) alone or in the presence of Z-VAD-FMK (25 uM) (Tocris). After 3 days, cell viability was determined using CellTiter-Glo (Promega).

Cathepsin combination treatment
B-NHL were seeded into 96 well plates and treated with apilimod (10 point dose response; 4 – 500 nM) alone or in combination with cathepsin inhibitor I (0.4 – 50 uM) (Calbiochem), cathepsin inhibitor III (0.4 – 50 uM) (Calbiochem), CA-074 Me (0.4 – 50 uM) (ApexBio Technology) or E64d (0.4 – 50 uM) (ApexBio Technology). After 3 days, cell viability was determined using CellTiter-Glo (Promega).

Necrostatin-1 combination treatment
B-NHL were seeded into 96 well plates and pretreated for 24 hours with 5 ug/mL necrostatin-1 (Sigma) and subsequently co-treated with 5 ug/mL necrostatin-1 and apilimod for 3 additional days.
Rapamycin combination treatment
B-NHL were treated with rapamycin (5 uM) (LC Labs) alone or in combination with apilimod (200 nM) for 24 hours. Parallel samples were additionally treated with bafilomycin A1 (500 nM) for the last 8 hours. Samples were harvested for western blotting as described.

Gene Set Enrichment Analysis (GSEA)
The autophagy and lysosomal signature used for the GSEA analysis was built by Perera R. et al, 2015[2] and contains genes associated with proteomics and interactome data corresponding to autophagy and lysosomes. It also includes genes directly associated with lysosomal diseases.

The GSEA analysis was performed with the software implemented by the Broad Institute (http://www.broadinstitute.org/gsea/index.jsp), using the expression profiles of apilimod- versus vehicle-treated lymphoma lines.

Statistical analysis
For comparison of one variable between 2 different groups, we used a parametric unpaired Student t-test to assess statistical significance. When more than two groups were involved in the comparison, we used one-way ANOVA followed by multiple comparison correction with Dunnett's post-test. The significance of the P-values are indicated in the figure legends, and represented by asterisks in each figure according to P-value < 0.05 (*), P-value < 0.01 (**) and P-value < 0.001 (***).
All experiments were independently repeated at least two times, unless otherwise specified.
The coefficient of drug interaction (CDI) was used to analyze the synergistically inhibitory effect of the drug combination *in vivo*. CDI was calculated as follows: CDI=AB/(A×B). AB is the ratio of the 2-drug combination group to the control group in tumor volume, and A or B is the ratio of the single drug group to the control group in tumor volume. CDI <1 indicates synergism, CDI <0.7 indicates a significantly synergistic effect, CDI=1 indicates additivity, and CDI >1 indicates antagonism.

Imaging
Images were acquired on a Zeiss Axiovert 25 CFL inverted microscope with an Axiocam MRM 1.4 Megapixel camera and Axiovision 3.1 software. An LDA-plan objective was used with a 0.5 Ph2 aperture.

CRISPR screen

The GeCKO V2 library consists of 123,411 sgRNAs that target 19,050 protein coding genes and 1,864 microRNAs with 6 targeting sgRNAs per gene [3]. It is divided into the A and B sublibraries, each containing 3 targeting sgRNAs per gene, and with the A library containing all microRNA targeting constructs. The GeCKO libraries were amplified according to the Addgene protocol available at (https://www.addgene.org/crispr/libraries/geckov2/) in Endura electrocompetent E. coli (Lucigen). Library diversity of DNA preps was confirmed by amplifying 100 ng of each prep with Herculase II enzyme (Agilent) in a single step PCR reaction to simultaneously amplify, barcode individual samples and add adaptors for next generation sequencing (Proton, Ion Torrent). Amplicons were size selected with Pippin Prep 2% gel (ThermoFisher), purified (Qiagen) and quantified by Bioanalyzer High Sensitivity DNA chip (Agilent) prior to Proton next generation sequencing.

PCR reaction conditions

5X Herculase Buffer	20 uL
25 mM dNTP	1 uL
25 mM $MgCl_2$	2 uL
10 uM Forward primer	5 uL
10 uM Reverse primer	5 uL
Herculase II polymerase	1 uL
Maxiprep DNA	100 ng
dH20	To 100 uL

Thermocycler conditions

Step	Temperature	Time
1	95^0C	2 min
2	95^0C	15 sec
3	60^0C	20 sec
4	72^0C	30 sec
5		Steps 2-4 x 30
6	72^0C	5 sec

Primers

Primer Name	Sequence
GECKOF	CCATCTCATCCCTGCGTGTCTCCGACTCAG [10mer Proton Barcode] GAT tggctttatatatcttgtggaaagg
GECKOR	CCACTACGCCTCCGCTTTCCTCTCTATGGGCAGTCGGTGATcggtgccactttttcaagtt

Library validation

To check library diversity, sequencing reads were demultiplexed in the Torrent Suit software (Life Technologies) and further processed to remove the primers and remaining lentiviral sequences using the cutadapt software [4], leaving only the sgRNA. Processed reads were aligned to the corresponding reference libraries using the bwa software [5], with default parameters. The final alignment was further filtered with samtools[5], to retain only the reads with high alignment quality, and calculate the number of uniquely mapped reads per sgRNA. The cumulative distribution of reads and the percentage of mapped reads per library were calculated in R (http://www.R-project.org/) (Figure S8).

Cell transduction

Once library prep diversity was validated, 1 x 10^7 SU-DHL-10 DLBCL cells were transduced with the lentiCas9-Blast vector at an MOI of 1. Cells were blasticidin selected for one week and subsequently expanded for transduction with the GeCKO library.

Cas9-expressing SU-DHL-10 were subsequently transduced with each GeCKO half library with two independent infections per half library for a total of four independent screening pools: A1, B1, A2 and B2. In independent infections, 22 x 10^7 cells were transduced with the A library and 20 x 10^7 cells were transduced with the B library at an MOI of 1 in order to ensure an average of one sgRNA per cell and an average coverage of 1000x after selecting for cells containing library with puromycin. Library-transduced cells were selected with puromycin for one week and expanded for a second week prior to screening.

From each pool, genomic DNA (Qiagen) from 22 x 10^7 live cells was harvested from each condition and sgRNAs amplified from 400 ug gDNA per condition. PCR amplicons were subsequently size selected and quantified as previously described and sequenced on the Proton according to manufacturer instruction.

Identification of gene candidates, data processing and analysis

To determine the gene candidates conferring resistance to apilimod, we selected the most resistant pools (B1 600 nM reselected and A2 600 nM reselected) for analysis.

The raw bam files corresponding to these libraries were formatted into fastq using the bedtools software [6]. Reads were processed with cutadapt [4] to remove amplification primers and remaining lentiviral sequences, leaving the sgRNA sequences only. The bwa software was used to align the reads to the corresponding GeCKO reference libraries, with default parameters[5]. The aligned reads were further filtered keeping just the ones with high alignment quality and the number uniquely mapped reads was calculated using samtools [5].

Once sequencing quality was confirmed for these two samples, the number of uniquely mapped reads per sgRNAs was normalized according to the following equation:

$$normalized\ reads = 1 + 10^6\ x\ \frac{mapped\ reads\ per\ sgRNA}{total\ number\ of\ mapped\ reads}$$

After read normalization, the differential abundance between treated and untreated samples was calculated.

To identify genes whose deletion conferred resistance to apilimod, the log2 (treated/untreated) distribution of all sgRNAs targeting a gene was compared to the differential distribution of the non-targeting sgRNAs, using a one-sided Kolmogorov- Smirnov (K-S) test. A Z-score was calculated for all targeting sgRNAs taking into account the mean and standard deviation of the differential abundance of the non-targeting sgRNAs. Finally, the targeting sgRNAs with a Z-score > 1 were grouped by gene target, and the average Z-score was calculated. Genes with at least two targeting sgRNAs were ranked according to the average Z-score and selected as candidates conferring resistance to apilimod treatment when knocked-out (Table 8).

To determine the statistical significance of the differential abundance of sgRNA between treated and untreated samples, we used the library DESeq2 and calculated the false discovery rate (FDA). Our cutoff for significance was a FDR <= 0.01.

CRISPR Validation

Gene specific sgRNAs were cloned into the pLentiGuide-Puro construct and transduced into CAS9 expressing WSU-DLCL2 cells. The pools were then selected for one week in 1 ug/ml puromycin. 12.5 x 10^6 cells from the WSU-DLCL2 sgRNA pools were seeded at a density of 2.5 x 10^5 cells/mL in T150 flasks, and pulsed for 3 days with the 600 nM apilimod in order to enrich for edited cells. After 3 days, remaining live cells were recovered and allowed to expand in fresh media without apilimod. These cells were subsequently screened in 10-point apilimod dose response assays.

CRISPR Validation sgRNAs

sgRNA	Sequence
NT-A	ACGGAGGCTAAGCGTCGCAA
NT-B	CGCTTCCGCGGCCCGTTCAA
NT-C	ATCGTTTCCGCTTAACGGCG
OSTM1-A	CTGAAGGTTATGTTCAAAGC
OSTM1-B	TTGTGCCAGAAGTCTCTTAA
OSTM1-C	ATCTGCCATTAAGAGACTTC
CLCN7-A	AGGTGGGGTCCTGTTCAGCT
CLCN7-B	AACTTACGATCCTCCAGGTC
CLCN7-C	GACAGGAGCTTCTCGTTGTG

Supplemental References

1. Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol*. 2011;29(11):1046-1051.
2. Perera RM, Stoykova S, Nicolay BN, et al. Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature*. 2015;524(7565):361-365.
3. Sanjana NE, Shalem O, Zhang F. Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods*. 2014;11(8):783-784.
4. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. Vol. 17: EMBnet.journal 2011:10-12.
5. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760.
6. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*. 2010;26(6):841-842.

Supplemental Tables

Table S1 Apilimod IC_{50} values in human lymphoma cell lines.

Table S2 Apilimod IC_{50} values in human normal cell lines.

Table S1. Apilimod IC_{50} values in human lymphoma cell lines. Average 5-day IC_{50} of all human lymphoma cell lines tested. Double and triple hit lymphomas harboring *MYC* 8q24, *BCL2* and/or *BCL6* rearrangements sourced from Drexler et al.[1]

Cell Line	Cell Type	AVG 5-Day IC_{50} (nM)
ST486	Burkitt's lymphoma	7
EB2	Burkitt's lymphoma	53
P3HR1	Burkitt's lymphoma	77
BL-70	Burkitt's lymphoma	120
CA46	Burkitt's lymphoma	140
RAMOS	Burkitt's lymphoma	152
Daudi	Burkitt's lymphoma	187
Namalwa	Burkitt's lymphoma	305
BL-41	Burkitt's lymphoma	567
Jiyoye	Burkitt's lymphoma	826
Raji	Burkitt's lymphoma	3923
Ri-1	Double Hit Lymphoma	260
SU-DHL-10	Double Hit Lymphoma	14
SU-DHL-6	Double Hit Lymphoma	40
SU-DHL-5	Double Hit Lymphoma	45
DB	Double Hit Lymphoma	50
DOHH-2	Double Hit Lymphoma	144
NUDHL1	Double Hit Lymphoma	60
SC1	Triple Hit Lymphoma	185
VAL	Triple Hit Lymphoma	1843
WSU-DLCL2	Triple Hit Lymphoma	29
NUDUL1	Diffuse Large B Cell Lymphoma -ABC	25
SU-DHL-2	Diffuse Large B Cell Lymphoma -ABC	136
U2932	Diffuse Large B Cell Lymphoma -ABC	187
RC-K8	Diffuse Large B Cell Lymphoma -ABC	2546
OCI-LY-3	Diffuse Large B Cell Lymphoma -ABC	6813
SU-DHL-16	Diffuse Large B Cell Lymphoma -GCB	30
HT	Diffuse Large B Cell Lymphoma -GCB	97
SU-DHL-8	Diffuse Large B Cell Lymphoma -GCB	106
OCILY1	Diffuse Large B Cell Lymphoma -GCB	160
OCI-LY-19	Diffuse Large B Cell Lymphoma -GCB	160
Farage	Diffuse Large B Cell Lymphoma -GCB	164
Pfeiffer	Diffuse Large B Cell Lymphoma -GCB	242
OCI-LY-7	Diffuse Large B Cell Lymphoma -GCB	808
SU-DHL-4	Diffuse Large B Cell Lymphoma -GCB	14
CARNAVAL	Diffuse Large B Cell Lymphoma -GCB	33
Toledo	Diffuse Large B Cell Lymphoma -GCB	47

WSU-NHL	Follicular Lymphoma	15
WSU-FSCCL	Follicular Lymphoma	59
JeKo-1	Mantle Cell Lymphoma	60
Rec-1	Mantle Cell Lymphoma	127
Mino	Mantle Cell Lymphoma	163
GRANTA-519	Mantle Cell Lymphoma	856
IRM2	Mantle Cell Lymphoma	>5000
HBL2	Mantle Cell Lymphoma	55
UPN1	Mantle Cell Lymphoma	85
Z138	Mantle Cell Lymphoma	110
L128	Mantle Cell Lymphoma	562.5

Table S2. Apilimod IC_{50} values in human normal cell lines.
Average 5-day IC_{50} all human normal cell lines tested.

Cell Line	**Cell Type**	**AVG 5-Day IC_{50} (nM)**
CCD841CoN	Colon	16174
CCD18 Co	Colon	4521
HFF-1	Skin	31391
WI-38	Lung	16688
MRC-5	Lung	8478
PBMCs	Peripheral Blood Mononuclear Cells	5488
RWPE1	Prostate	15907
BUD-8	Skin	20897
Hs679sp	Spleen	>10000
Hs738.St/Int	Stomach/Intestine	14286
Hs67	Thymus	28826
Normal primary B cells	Peripheral blood B cells	7795

Reference

1. Drexler HG, Eberth S, Nagel S, MacLeod RAF. Malignant hematopoietic cell lines: in vitro models for double-hit B-cell lymphomas. *Leukemia & Lymphoma.* 2016; 57(5): 1015-1020.

Supplemental Figures

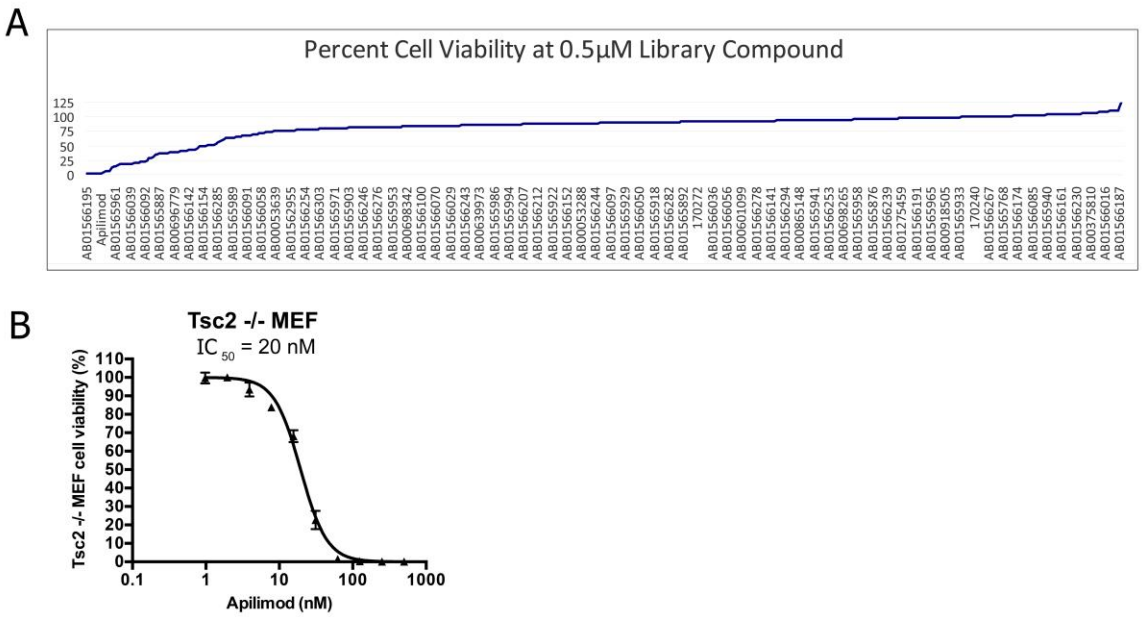


Figure S1. Apilimod potently induces cytotoxicity in *Tsc2* deficient MEFs. (A) Scatter plot depicting percentage of surviving MEFs relative to control after incubation with 0.5 uM of each library compound. Not all compounds listed on X axis due to space restriction. **(B)** Ten-point dose response of *Tsc2* null MEFs to 3-day treatment with apilimod using Cell-Titer Glo™ assay. Data are represented as mean ± SD.

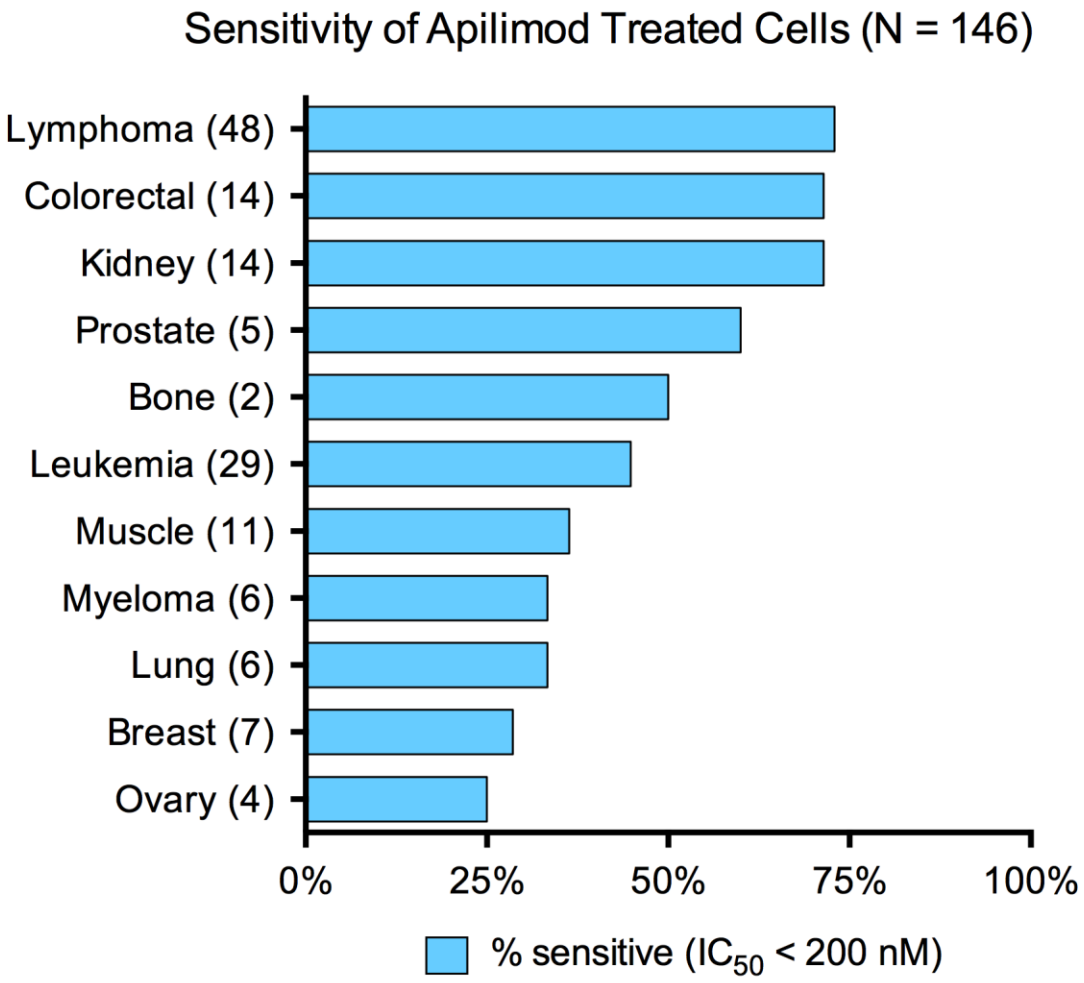


Figure S2. B-NHL cells display acute sensitivity to apilimod *in vitro*. Sensitivity of 146 human cancer cell lines to apilimod, with sensitivity defined as an IC_{50} less than 200 nM in a 5-day viability assay. The number of cell lines screened is indicated in parenthesis.

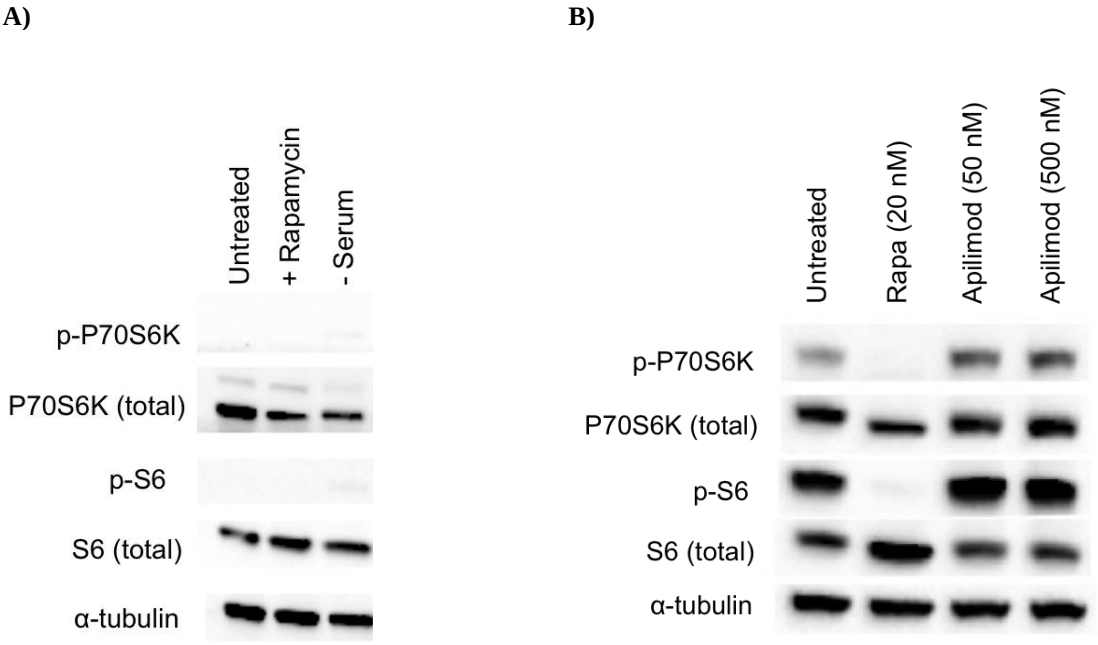


Figure S3. mTORC1 activation status in representative apilimod-sensitive B-NHL. (A) SU-DHL-4 cells were either treated with rapamycin (20 nM) or serum-starved for 24 hours, before being harvested for western-blotting. Lysates were probed with indicated antibodies. **(B)** SU-DHL-5 mTORC1 hyperactive B-NHL treated with indicated concentrations of rapamycin or apilimod for 24 hours. Lysates were probed with indicated antibodies. No effect of apilimod on mTORC1 activity was observed.

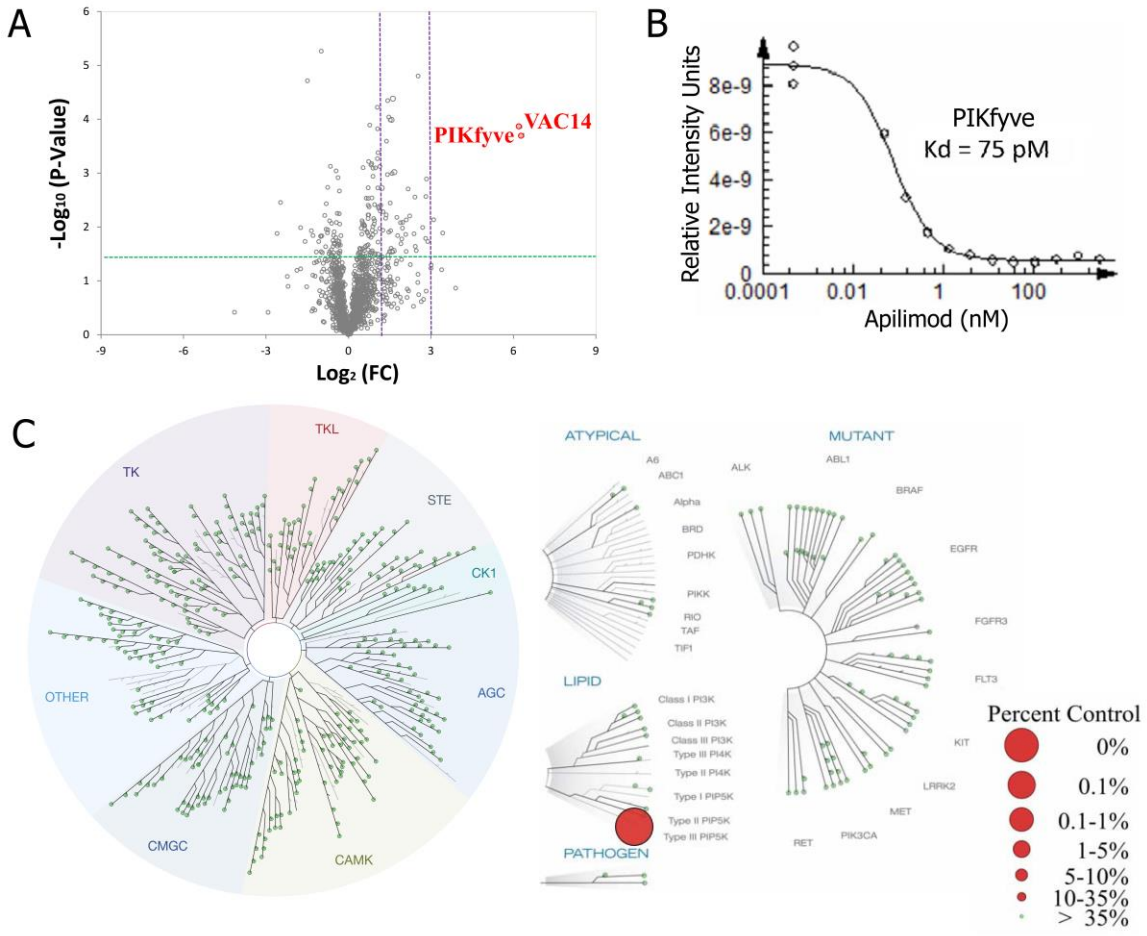


Figure S4. Apilimod potently and selectively targets the phosphoinositide kinase, PIKfyve. (A) Identification of binding partners of apilimod with chemical capture mass spectrometry (CCMS) performed with lysate of a cell line sensitive to apilimod (H4). Volcano plot of specific protein binders ranked according to their fold change (FC) values in the capture experiments. VAC14 and PIKfyve are highlighted in red. **(B)** Representative dissociation constant (Kd) curve of apilimod tested at increasing concentrations (0.05 – 3000 nM) against recombinant PIKfyve. **(C)** TREEspot™ data analysis of the binding affinity of apilimod to a panel of 456 kinases at a screening concentration of 1 uM. The red circle denotes kinases that are bound, expressed as the percent of control of all compound / kinase interactions. Larger circles (low percent control) indicate higher-affinity binding. The only kinase to which apilimod bound is the Type III PIP5K PIKfyve.

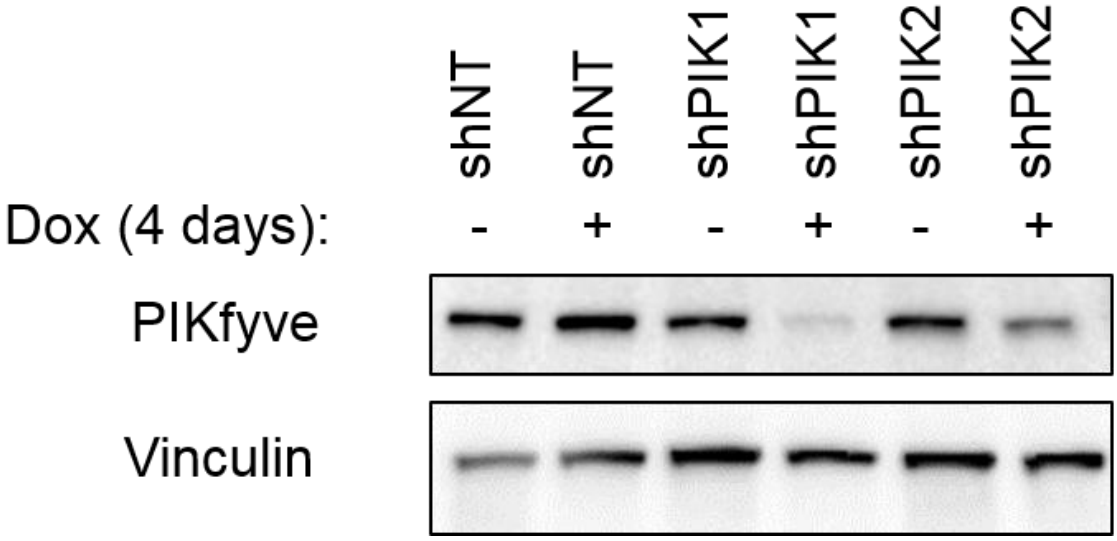
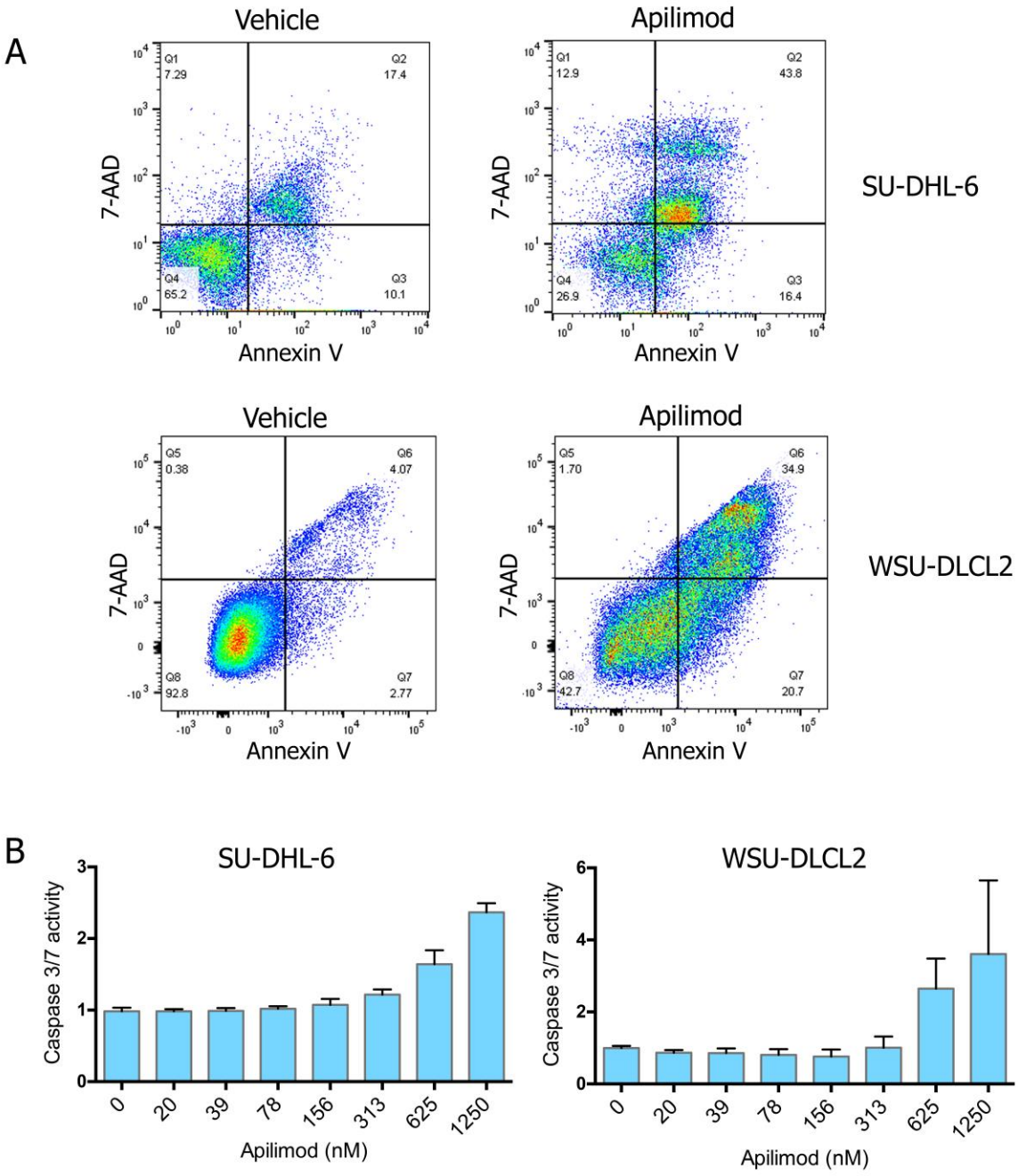


Figure S5. *PIKFYVE* knockdown in WSU-DLCL2 B-NHL cells. Validation of *PIKFYVE* knockdown by Western blot. Cells were treated with either vehicle or doxycycline for 4 days. Loading control vinculin is shown. NT indicates nontargeting shRNA and PIK1 and PIK2 indicate two different *PIKFYVE* shRNAs.


A
Vehicle
Apilimod
SU-DHL-6
WSU-DLCL2
7-AAD
Annexin V
Q1 7.29
Q2 17.4
Q4 65.2
Q3 10.1
Q1 12.9
Q2 43.8
Q4 26.9
Q3 16.4
Q5 0.38
Q6 4.07
Q8 92.8
Q7 2.77
Q5 1.70
Q6 34.9
Q8 42.7
Q7 20.7
B
SU-DHL-6
WSU-DLCL2
Caspase 3/7 activity
Apilimod (nM)
0
20
39
78
156
313
625
1250

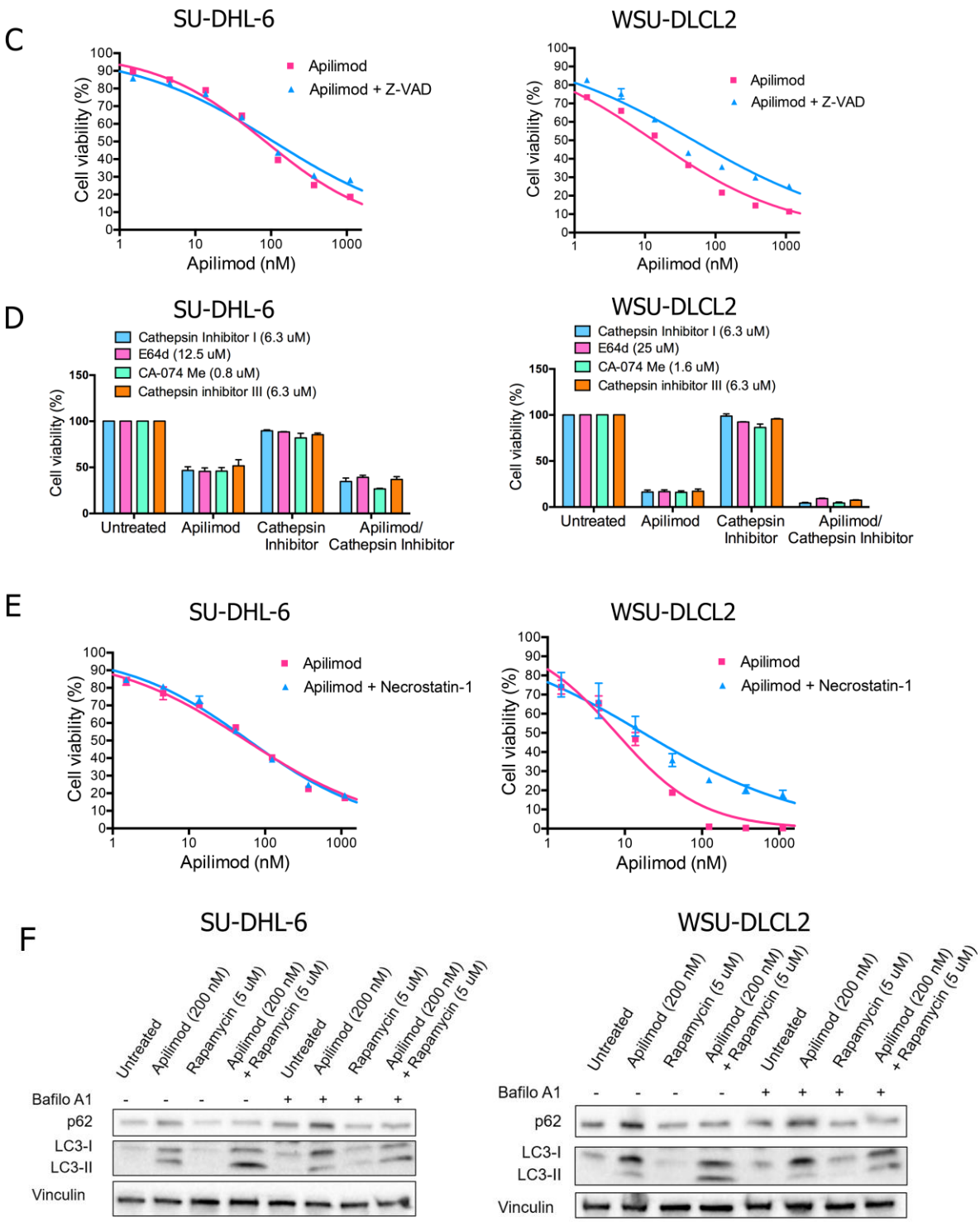

C
SU-DHL-6
Apilimod
Apilimod + Z-VAD
Cell viability (%)
Apilimod (nM)
WSU-DLCL2
Apilimod
Apilimod + Z-VAD
Cell viability (%)
Apilimod (nM)
D
SU-DHL-6
Cathepsin Inhibitor I (6.3 uM)
E64d (12.5 uM)
CA-074 Me (0.8 uM)
Cathepsin inhibitor III (6.3 uM)
Cell viability (%)
Untreated
Apilimod
Cathepsin Inhibitor
Apilimod/ Cathepsin Inhibitor
WSU-DLCL2
Cathepsin Inhibitor I (6.3 uM)
E64d (25 uM)
CA-074 Me (1.6 uM)
Cathepsin inhibitor III (6.3 uM)
Cell viability (%)
Untreated
Apilimod
Cathepsin Inhibitor
Apilimod/ Cathepsin Inhibitor
E
SU-DHL-6
Apilimod
Apilimod + Necrostatin-1
Cell viability (%)
Apilimod (nM)
WSU-DLCL2
Apilimod
Apilimod + Necrostatin-1
Cell viability (%)
Apilimod (nM)
F
SU-DHL-6
Untreated
Apilimod (200 nM)
Rapamycin (5 uM)
Apilimod (200 nM) + Rapamycin (5 uM)
Bafilo A1
p62
LC3-I
LC3-II
Vinculin
WSU-DLCL2
Untreated
Apilimod (200 nM)
Rapamycin (5 uM)
Apilimod (200 nM) + Rapamycin (5 uM)
Bafilo A1
p62
LC3-I
LC3-II
Vinculin

Figure S6. Apilimod induces non-canonical cytotoxicity in B-NHL cells. **(A)** Cytotoxicity of SU-DHL-6 (top) or WSU-DLCL2 (bottom) B-NHL treated with either DMSO or 200 nM apilimod for 3 days and stained with the viability dye 7-AAD and the apoptotic indicator Annexin V. Percentage of necrotic, early-apoptotic, and viable cells are respectively displayed in quadrants 2, 3, and 4. **(B)** Induction of DEVD-UltraGlo™ Luciferase cleavage activity in SU-DHL-6 (left) or WSU-DLCL2 (right) B-NHL after 2 day treatment with the indicated concentration of apilimod. **(C)** Viability of SU-DHL-6 (left) or WSU-DLCL2 (right) B-NHL measured under increasing concentrations of apilimod +/- 25 uM of the caspase inhibitor Z-VAD-FMK for 3 days. **(D)** Viability of SU-DHL-6 (left) or WSU-DLCL2 (right) B-NHL treated with indicated concentration of cathepsin inhibitor (cathepsin inhibitor I, E64d, CA-074 Me, or cathepsin inhibitor III) for 3 days. Apilimod was used at 156 nM as a single or combination agent. **(E)** Viability of SU-DHL-6 (left) or WSU-DLCL2 (right) B-NHL that were pretreated for 24 hours with 5 ug/mL of the necroptosis inhibitor necrostatin-1 and subsequently co-treated with 5 ug/mL necrostatin-1 and the indicated concentration of apilimod for 3 days. **(F)** Western blots of SU-DHL-6 and WSU-DLCL2 B-NHL that were treated with vehicle, apilimod (200 nM), the autophagy inducer rapamycin (5 uM) or the combination of apilimod and rapamycin in the absence or presence of the autophagy inhibitor bafilomycin A1 (500 nM added for the last 8 hours) for 24 hours. Cell lysates probed with LC3, p62 or Vinculin (loading control).

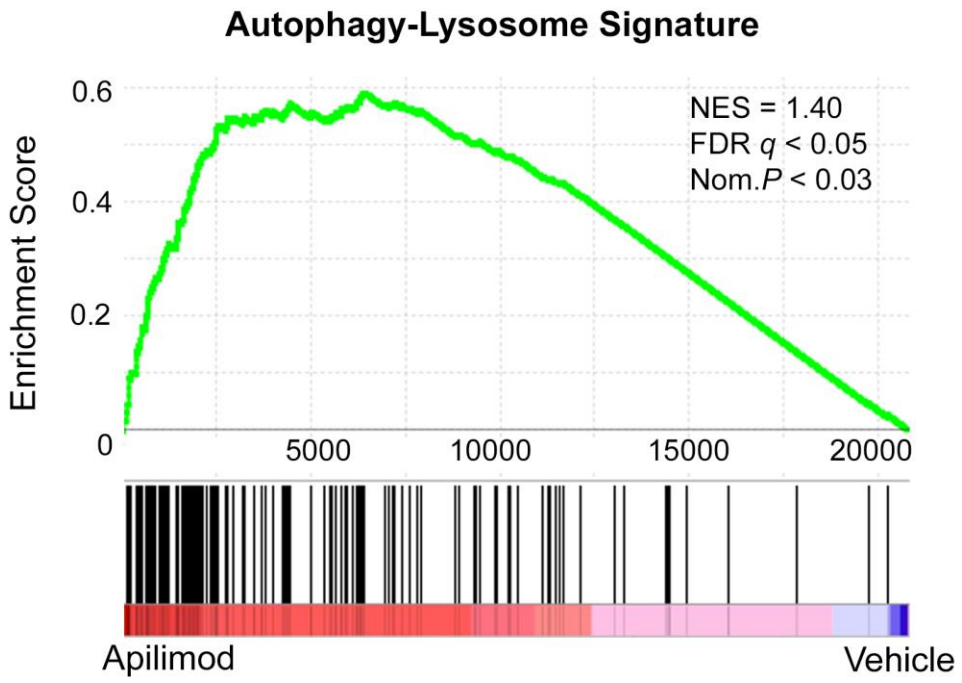


Figure S7. *PIKFYVE* induces autophagy-related gene expression in B-NHL cells. Gene set enrichment analysis of SU-DHL-10 and WSU-DLCL2 B-NHL lines treated with 300 nM apilimod for 24 hours shows a significant and positive enrichment of the autophagy-lysosome signature with apilimod-treated cells. The x-axis indicates the ranking of the genes from the most up-regulated to the most down-regulated upon apilimod treatment (NES: normalized enrichment score, FDR: false discovery rate, NomP: nominal P-value).

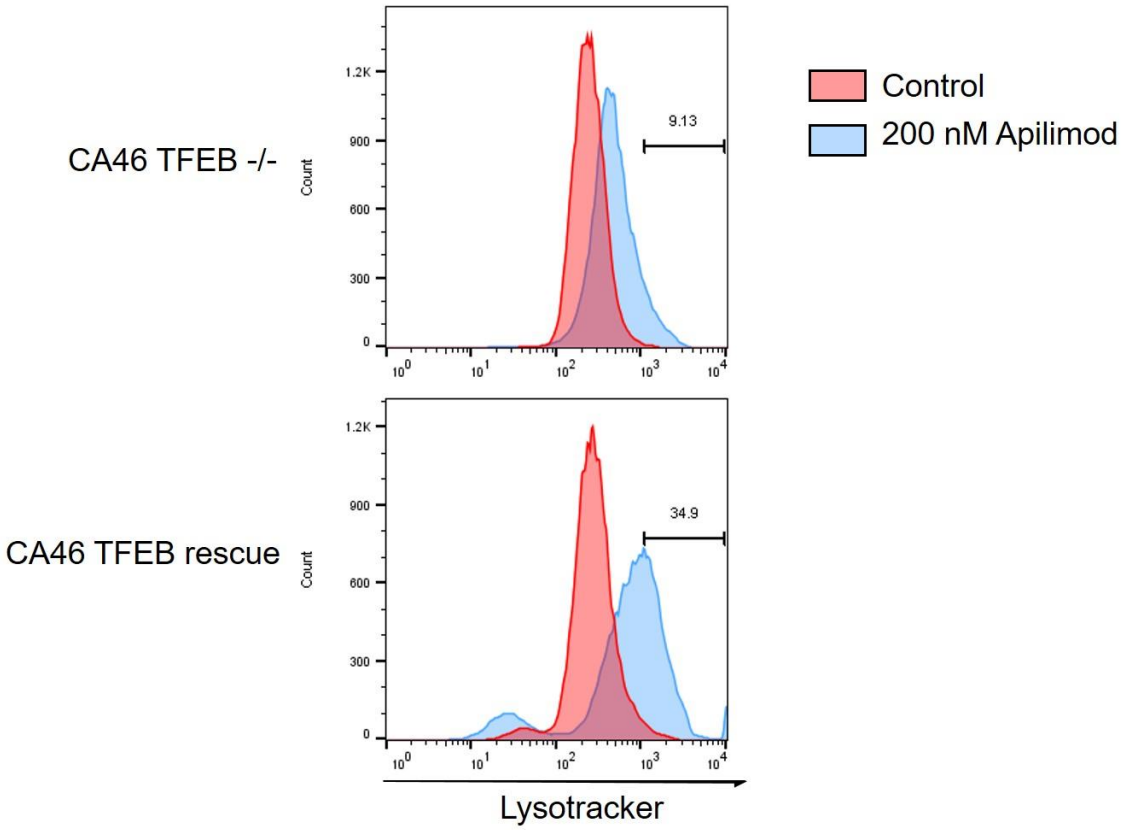


Figure S8. **Expansion of the acidified compartment in apilimod-treated CA46 TFEB-rescued B-NHL.** LysoTracker staining in CA46 [control (top), or TFEB rescue (bottom)] cells, 2 days after treatment with 200 nM (blue) apilimod compared to DMSO treated control (red). Percentage of cells with LysoTracker signal greater than the GFP control population is displayed.

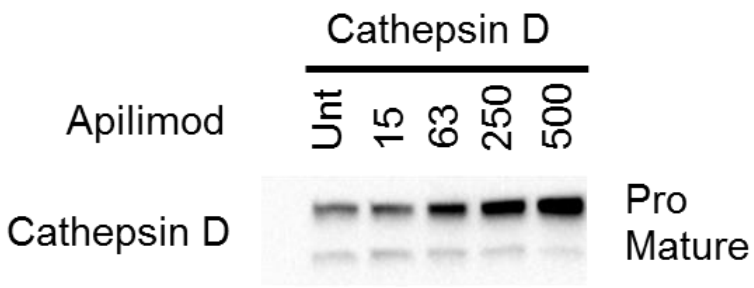


Figure S9. Overexpression of Cathepsin D in the TFEB-deficient cell line, CA46. Cathepsin D western blot analysis was performed on Cathepsin D CA46 stable clones (TFEB-deficient) treated with apilimod for 24 hours with the indicated doses in nanomolar (nM). Unt = untreated.

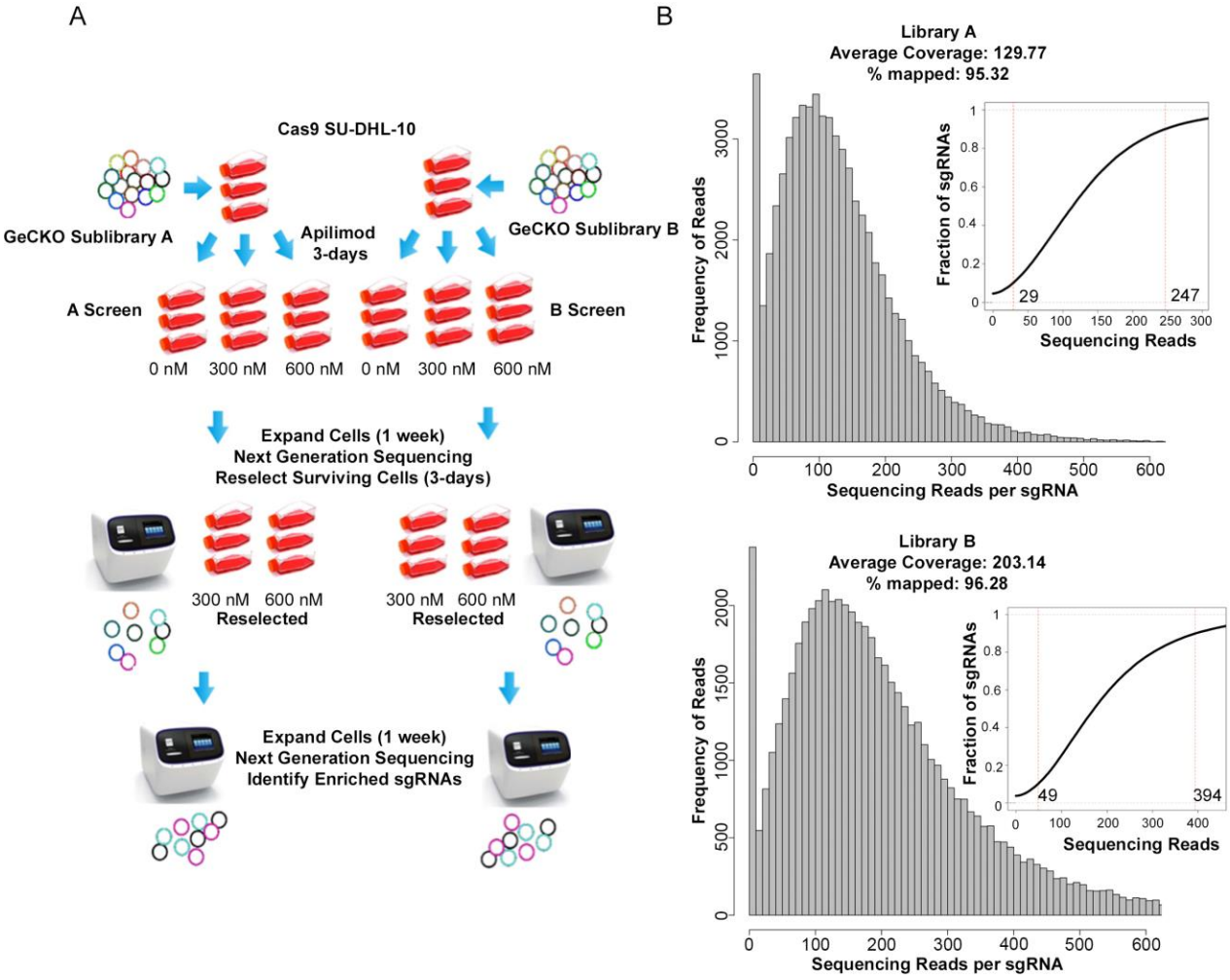


Figure S10. CRISPR resistance screen in SU-DHL-10 B-cell NHL using the GeCKO V2 library. (A) Schematic of CRISPR resistance screen in SU-DHL-10 B-cell NHL using the GeCKO V2 library. Each half library was independently transduced and screen in duplicate for a total of four independent screens: A1, B1, A2, and B2. A1 and B1 screens shown for simplicity. **(B)** Library diversity of each half library GeCKO A and GeCKO B. The inset represents the cumulative distribution of reads, and the 10^{th} and 90^{th} percentile are highlighted.

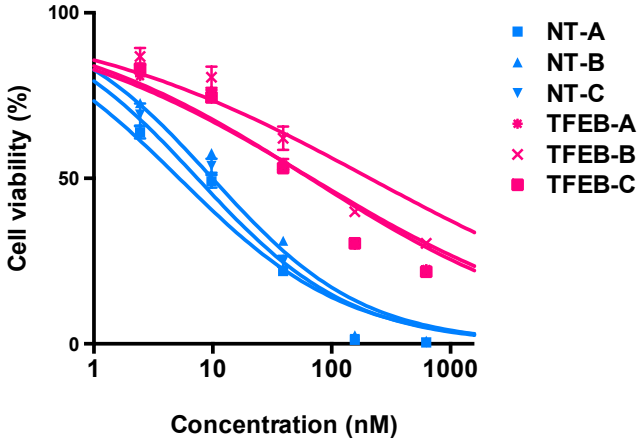


Figure S11. Response of TFEB knockout cells to apilimod treatment. WSU-DLCL2 B-NHL with the indicated *TFEB* or Non-Targeting (NT) sgRNA were treated with 10-point apilimod dose response for 3 days. Data are represented as mean ± SD.