Supplemental Material

Supplemental Methods

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Supplemental Methods

Origin of cell lines and cell culture conditions

Lenti-X 293T (Takara/Clontech), 293FT (ThermoFisher Scientific) and 293T/17 (ATCC) cells were cultured in DMEM supplemented with 10% heat-inactivated FCS (Sigma-Aldrich), 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml Streptomycin (Sigma-Aldrich or Thermo Fisher Scientific). The Burkitt lymphoma cell lines BL-2 and Daudi were purchased from the Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH in Braunschweig. Seraphine, BL-60, RAJI, Ramos were obtained from Thorsten Zenz (National Center for Tumor Diseases (NCT)/German CancerResearch Center (DKFZ), Heidelberg). The ABC-DLBCL cell lines U2932, HBL-1, TMD8, HLY-1, the GCB-DLBCL cell lines SU-DHL-4 and the BL cell lines BL30, BL70, Gumbus were provided by Louis Staudt (NIH, Bethesda). BL and DLBCL cell lines were cultured in RPMI and advanced RPMI supplemented with 5% to 20% heat-inactivated FCS, 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml Streptomycin. Cell lines were authenticated using Multiplex Cell Authentication by Multiplexion (Heidelberg, Germany) as described recently[50] or by profiling of highly-polymorphic short tandem repeat (STR) loci (Microsynth, Balgach, Switzerland or the Leibniz Institute DSMZ, Braunschweig, Germany). The SNP or STR profiles matched known profiles or were unique. Cell lines were regularly tested for mycoplasma according to a PCR protocol published by Uphoff and Drexler.[51]

For Cell titer glo experiments, stable cell lines were generated from primary tumors of Eμ:Myc and Cd79bcond.p.Y195H/wt;Myd88cond.p.L252P/wt;Rosa26LSL.BCL2.IRES.GFP/wt; Cd19Cre/wt mice. We have previously demonstrated that Myd88cond.p.L252P/wt;Rosa26LSL.BCL2.IRES.GFP/wt;Cd19Cre/wt mice develop clonal DLBCL-like lymphomas.[41] Moreover, DMBC mice also develop DLBCL-like disease with high penetrance (unpublished, detailed phenotyping of the Cd79b allele will be published elsewhere). Cell lines were cultured in DMEM (Gibco), containing 10% FCS, 1% penicillin/streptomycin, 2 mM L-glutamine (Gibco), 1 mM sodium pyruvate (Gibco), 10 mM HEPES, 1X non-essential amino acids (Gibco).

Mouse experiments

Xenograft experiments using BL60 cells were approved by the National Cancer Institute Animal Care and Use Committee (NCI-ACUC) and were performed in accordance with NCI-ACUC guidelines and under approved protocols. Female NSG (non-obese diabetic (NOD)/ severe combined immunodeficient (SCID)/Il2rg−/−) mice were obtained from NCI Fredrick Biological Testing Branch and used for the xenograft experiments between 6 and 8 weeks of age. BL60 tumors were established by subcutaneous injection of 5×10^6 cells in a 1:1 Matrigel/PBS suspension. Induction of shRNA by doxycycline injection was initiated when tumor volume reached a mean of 200 mm^3. Doxycycline was prepared with PBS and administered intraperitoneally once per day (2 mg/mouse/day).

Transplantation experiments using M2121 cells were approved by the local animal care committee and the relevant authorities (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, 84-02.04.2017.A131). Rag1-deficient animals were used (Jackson Laboratories (Cat# 002216)). 10^7 M2121 cells were transplanted by intraperitoneal injection at seven weeks of age. Animals were treated with either SHIN2 at a dose of 200 mg/kg (dissolved at 20 mg/ml in 20% hydroxypropyl-ß-cyclodextrin) or vehicle for five consecutive days twice daily by i.p. injection. SHIN2 was provided by J.D. Rabinowitz.[20] All animals were housed in a specific-pathogen-free facility and animal breedings.

Culture and treatment of primary BL cells

Irradiated YK6-CD40Lg-IL21 feeder cells[42] were cultivated in advanced RPMI supplemented with 20% heat-inactivated FCS, 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml Streptomycin. Cells were preplated at a density of 2,5x10^4/ml per well in 12 well plates one day prior to adding patient derived Burkitt Lymphoma (BL) sample from frozen stocks. Sample used in this study were provided by the University Cancer Center Frankfurt (UCT) in collaboration with the GMALL study group (German Multicenter Study Group on Adult Acute Lymphoblastic Leukemia). Written informed consent was obtained and the study was approved by the institutional Review Boards of the UCT and the Ethical Committee at the University Hospital Frankfurt (project-number: SHN-9-2017). Primary BL cells were added at a density of 1-2x10^6 cells in 1 ml advanced RPMI to one well of the preplated feeders. When proliferating, they were replated on fresh feeders at a density of 0,5-1x10^6 cells per well and treated with 5 µM SHIN1 (Shanghai Medicilon Inc.), 20 nM MTX (Pfizer) and a combination thereof for 96h.

CRISPR Cas9 screen – detailed method description

To generate Cas9 expressing cell lines, BL60, Ramos and RAJI were lentivirally transduced using the transfer plasmid lentiCas9-Blast which was a gift from Feng Zhang (Addgene plasmid # 52962 ; http://n2t.net/addgene:52962; RRID:Addgene_52962). After Blasticidin

selection, Cas9 expressing cell lines were tested for functional Cas9 cutting after transduction with an sgRNAs targeting the cell surface marker CD20 in the plentiGuide-Puro plasmid which was a gift from Feng Zhang (Addgene plasmid # 52963; http://n2t.net/addgene:52963; RRID:Addgene_52963). Enrichment of sgRNA transduced cells was achieved by Puromycin selection and CD20 expression was measured by flow cytometry upon staining for CD20 at day 7 post-transduction.

For genome wide screening, we utilized the human Brunello CRISPR knockout pooled library which was a gift from David Root and John Doench (Addgene #73178). Large scale virus production for the Brunello library was done by transfecting HEK293T cells with the packaging plasmids psPAX2 and p.MD2.G and the library in a 4:2:1.2 ratio using Lipofectamine 2000 and PLUS reagent (Cat# 11668-027 and 11514-015, both ThermoFisher Scientific). Virus-particle containing medium was collected after 32 h. Virus titration was performed on target cells which were Puromycin selected 48 h after transduction for 72 h. Cell viability was determined by flow cytometry on the basis of forward- and side-scatter (FSC/SSC).

For CRISPR screen, Cas9 expressing cells were seeded in T-225 cm cell culture flasks and infected with the Brunello lentiviral supernatant to achieve 261 cells / sgRNA upon 50 % transduction efficiency (the approximate coverage would be 250X). 48 h post-infection, cells were treated with puromycin and cultivated for another 72 h. Transduction efficiency was again determined on the basis of cell viability using flow cytometry. Cells were used for screening if 40-60% were viable. During cultivation, the cells were passaged every 3 days by keeping the minimum density of 5.4x10^7 cells in total (~ 706 cells / sgRNA) to maintain the library sgRNA representation. The screen was terminated at 22 days post-transduction and was performed in biological duplicates for each cell line. Genomic DNA extraction was performed from 1.5x10^8 cells per replicate using the Blood & Cell Culture DNA Maxi Kit (Qiagen) according to the manufacturer's recommendations.

A two-step nested PCR was used to amplify sgRNA sequences from genomic DNA and then to add Illumina NextSeq500 adapters and indices to each sample using ExTaq (Takara). A total of 396 µg of genomic DNA was amplified per sample to maintain ~700X coverage. Libraries were selected using an E-Gel SizeSelect II agarose gel, quantitated with Qubit dsDNA high-sensitivity assay kit (ThermoFisher Scientific) and sequenced with high-output single-read 75-cycle flow cells (Illumina). An average of 386X sequencing depth was achieved per sample. The primer sequences for both PCRs can be found in Table S5.

Sequencing quality measures and sample deconvolution were performed on Basespace.com. Previously published custom scripts[18] were used to extract sgRNA sequences from fastq files and to align extracted sequences to the Brunello reference library using Bowtie2 version 2.2.9 with the following parameters: -p 16 -f–local -k10–very-sensitive-local -L 9 -N 1. CRISPR screen scores (CSS) were generated by comparing the normalized read counts of each sgRNA from the day 21 sample to the reference plasmid control after filtering low abundance values. The log2 values of each sgRNA per gene were then averaged and a z-score was calculated for each gene versus the average gene for a given replicate.

Cloning of SHMT2res construct into lentiviral vector

pMXS-IRES-Blast-SHMT2res was a gift from Richard Possemato (Addgene plasmid # 106301; RRID:Addgene_106301). In order to clone SHMT2res into the lentiviral expression

vector pRRL.PPT.SF-MCS-IRES-EBFPnucmem_pre (kindly provided by Michael Rieger, Goethe University Hospital Frankfurt, Germany; vector based on Schambach et al.[52]), SHMT2res was PCR amplified using Q5 polymerase (NEB) and the following primers SHMT2 for 5´-ATACGCACTAGTGCCACCatgctgtacttctctttgttttg-3´ and SHMT2 rev 5´-ATACGCTACGTAtcaatgctcatcaaaaccaggc-3´. SH. The PCR product was inserted into the vector via the SpeI and SnaBI restriction sites.

Site-directed mutagenesis of SHMT2 to generate catalytically inactive K280A mutant

For site-directed mutagenesis of SHMT2res, overlap extension PCR was performed using Q5 polymerase (NEB) and SHMT2res was replaced by the mutant in the lentiviral expression vector pRRL.PPT.SF-SHMT2res-IRES-EBFPnucmem_pre using the SpeI and SnaBI restriction sites. Primer sequences are given in Table S5.

Cloning of shRNAs into viral vectors

The constitutive shRNA expression vector pRSI12-U6-(sh)-UbiC-TagRFP-2A-Puro was obtained from Cellecta. Annealed shRNA oligos were cloned into the vector using the BbsI restriction sites and standard cloning techniques. As a negative non-targeting control, an shRNA was used that had no sequence complementarity to any known human gene (shNT). Oligo sequences are given in Table S5. The guide sequence is underlined. The doxycycline-inducible lentiviral shRNA vector LT3-GECIR was provided by Johannes Zuber[33] (Research Institute of Molecular Pathology, Vienna, Austria). 97nt template oligos coding for the respective shRNAs (see Table S5; the guide sequence is underlined) served as templates for PCR amplification using the primer sequences for de novo generation of miR-E shRNAs.[22] Finally, the PCR products were cloned into the LT3-GECIR vector via the XhoI and EcoRI restriction sites. An LT3-GECIR vector containing the non-targeting GL2 shRNA against the Renilla Luciferase gene in the pGL2-basic cloning vector (GenBank X65323.2) was used as control.

Cloning of sgRNAs into CRISPR vectors

LentiCRISPR v2 (pLCv2) was a gift from Feng Zhang (Addgene plasmid # 52961; RRID:Addgene_52961). The puromycin resistance cassette in pLCv2 was replaced by green fluorescent protein (GFP) using standard cloning techniques to allow fluorescence activated cell sorting of transduced cells. Two effective sgRNA sequences against SHMT2 were selected from the Brunello library and a non-targeting sequence was used as sgControl. Annealed oligonucleotides were cloned into the BsmBI restriction sites of pLCv2-GFP according to the GoldenGate protocol using 150 ng plasmid, 3 µM of each sgRNA oligo (F and R), 10 units BsmBI, 400 units T4 DNA ligase and 1x T4 ligase buffer in a 20 µl reaction and the following thermal cycler program: 5 min at 37°C and 15 min at 16°C for 10 cycles followed by 30 min at 37°C (1 cycle) and heat inactivation for 15 min at 80°C. The pLKO.1-Puro-GFP vector was generated as described in Phelan et al. 2018[18] and sgRNA sequences for the non-targeting control (sgNT) and against ATG5 were taken from the Brunello library. sgRNA oligos carrying appropriate nucleotide overhangs were annealed and cloned into BfuAI digested pLKO.1-Puro-GFP using standard cloning techniques. Oligo sequences are given in Table S5.

Virus production and transduction of lymphoma cell lines

Viral particles were produced by transfecting HEK293T cells with viral packaging and transfer plasmids using calcium phosphate or the TransIT293 transfection reagent (Mirus, Madison, USA) according to the manufacturer´s protocol. For lentivirus production, pMD2.G (Addgene

plasmid # 12259; RRID:Addgene_12259) and psPAX2 (Addgene plasmid # 12260; RRID:Addgene_12260) were used as packaging plasmids. Both plasmids were a gift from Didier Trono. Retroviral particles were produced using an MLV gag/pol (M57-DAW) and a VSV-G (M5) expression plasmid. Viral particles were concentrated using ultracentrifugation (OPTIMA XPN-80, Beckman, 50,000xg, 2 hours, 4°C) or the Lenti-X Concentrator reagent (TakaRa Bio Europe, St Germaine n Laye, France) according to the manufacturer´s protocol. Lymphoma cell lines were transduced by adding concentrated virus to the cell culture medium. Transduced cells were enriched using fluorescence activated cell sorting (FACSAria III, BD, Heidelberg, Germany) or eukaryotic selection markers.

Cloning and transduction for tfLC3 flow cytometry experiments

tfLC3 (published by Shoemaker et al.[33]) was subcloned into pBMN-IRES-Lyt2 using standard cloning techniques. GALV pseudotyped virus was produced by transfecting the plasmids FL GALV and pHIT60 into HEK293T cells. BL60 and Ramos were transduced by spin infection (1000g for 90 min at RT) in 24-well plates using different amounts of virus supernatant and 10 µg/ml polybrene. Different cell batches were profiled with respect to their response to Torin 1 treatment and the cell batch with the best response was used for the experiments.

Immunhistochemistry in patient biopsies

Ethical approval for using the human tissue was obtained from the Ethics Committee of the University Medical Center Göttingen [#19-2-16]. Lymphoma and normal lymph node tissues were acquired from 102 patients from the University Medical Center Göttingen, Germany. Analysed cases included 18 cases of BL, 73 cases of DLBCL, 5 cases diagnosed grey zone lymphoma and 6 healthy tonsils. The tissue samples were fixed in 4% buffered formalin and embedded in paraffin. Immunohistochemical staining was performed as described previously.[53] Briefly, 2 µm tissue sections were incubated with EnVision Flex Target Retrieval Solution, pH low (Dako) followed by incubation of primary antibodies against SHMT2 (Cat# HPA020549, Atlas Antibodies, RRID:AB_1856834, 1:200) for 20 minutes at room temperature, HRPO peroxidase coupled to polymeric secondary antibodies (EnVision Flex+, Dako) and DAB (Dako, all Dako reagents are part of Cat# K801221-2). Stainings were analyzed by light microscopy (Olympus BX46) considering staining intensity as negative, weakly positive or strongly positive.

Immunoblotting

Cells were lysed with NP40-containing lysis buffer (150 mM NaCl, 50 mM Tris at pH 7.5–7.8, 5 mM NaF, 0.5% NP40, 1 mM sodium vanadate, complete protease inhibitor cocktail (Roche)) for 10 minutes on ice followed by centrifugation (21380xg, 4°C, 10 min) to remove cell debris. Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). For the pULK1/ULK1 blot, cells were lysed in an SDS lysis buffer (100 mM Tris-HCl pH8, 150 mM NaCl, 10 mM EDTA pH8, 10% SDS) and protein concentrations were measured using the Pierce 660nm Protein Assay Reagent containing the Ionic Detergent Compatibility Reagent (IDR) according to the manufacturer´s protocol (Thermo Fisher Scientific).

Mini Protean TGX Precast Gels (4-15%) or self-made SDS polyacrylamide gels with the desired percentage were used to separate the proteins in a Mini Protean electrophoresis chamber filled with 1x TGS running buffer (BioRad) at 120 V. Subsequently, separated proteins were transferred to nitrocellulose membranes or PVDF at 20-70 V for 1.5 – 16 h using a Mini Protean Wet Blot chamber and 1x TG buffer (BioRad) supplemented with 20% methanol. Membranes were blocked in 5% nonfat dry milk in TRIS-buffered saline with 0.1%

Tween-20 (TBS-T) at RT for 1 h and incubated overnight at 4°C in 5% BSA in TBS-T supplemented with protein specific antibody (dilutions according to the manufacturer´s recommendations). Blots were washed with TBS-T before and after incubation at RT for 1 h with secondary antibody (1:10000 in 5% nonfat dry milk in TBS-T) followed by detection of proteins employing the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific) and an Odyssee (LI-COR) or ChemiDoc (Bio-Rad) Imaging System. Chloroquine treatment was performed at a concentration of 100 µM for 4 h. Chloroquine was obtained from Bayer.

The following primary antibodies and secondary antibodies were applied: SHMT2 (Cat# 12762, Cell Signaling, rabbit pAb, RRID:AB_2798018), TCF3/E2A (G-2, Cat# sc-133075, Santa Cruz, RRID:AB_2199145 or D2B1, Cat# 12258, rabbit mAb, Cell Signaling, RRID:AB_2797860), SHP-1 (C14H6, Cat# 3759, Cell Signalling, rabbit mAb, RRID:AB_2173694), cleaved PARP (Asp214, Cat# 9541, rabbit pAb, RRID:AB_331426 or Cat# 9546, mouse mAb, both Cell Signaling, RRID:AB_2160593), cleaved Caspase-3 (Asp175, Cat# 9661, rabbit pAb, RRID:AB_2341188 or Cat# 9664, rabbit mAb, RRID:AB_2070042, both Cell Signaling), β-Catenin (XP rabbit mAb, clone D10A8, Cat# 8480, Cell Signaling, RRID:AB_11127855), LC3B (rabbit pAb, Cat# NB100-2220, Novus Biologicals, RRID:AB_10003146), Phospho-ULK1 (Ser757, D7O6U, Cat# 14202, Cell Signaling, rabbit mAb, RRID:AB_2665508), ULK1 Antibody (Cat# F51035, NSJ Bioreagents, RRID:AB_2847854), ATG5 (Cat# 2630, Cell Signaling, rabbit pAb, RRID:AB_2062340), Phospho-Akt (Ser473, D9E, Cat#4060, Cell Signaling, XP® rabbit mAb, RRID: AB_2315049, Akt (pan, C67E7, Cat#4691, Cell Signaling, rabbit mAb, RRID: AB_915783), Phospho-SYK (Tyr525/526, clone C87C1, Cat# 2710, Cell Signaling, rabbit mAb, RRID:AB_2197222), SYK (clone D1I5Q, Cat# 12358, Cell Signaling, rabbit mAb, RRID:AB_2687923), GAPDH (XP rabbit mAb, clone D16H11, Cat# 5174, Cell Signaling, RRID:AB_10622025 and rabbit mAb, clone 14C10, Cat# 2118, Cell Signaling, RRID:AB_561053) and α/β-Tubulin (Cat# 2148, Cell Signaling, RRID:AB_2288042), β-Aktin (Cat# 4967, rabbit pAb, RRID:AB_330288 or rabbit mAb, clone D6A8, Cat# 8457, RRID:AB_10950489 or mouse mAb, clone 8H10D10, Cat# 3700, RRID:AB_2242334, all Cell Signaling), goat F(ab´)2 anti-rabbit IgG (H+L)-HRPO (Cat# 111-036-003, RRID:AB_2337942), goat F(ab´)2 anti-mouse IgG (H+L)-HRPO (Cat# 115-036-003, RRID:AB_2617176, both Jackson ImmunoResearch Labs).

Quantification of signal intensities was done using Image Studio Lite Version 5.2.5 or Image J.[54] For publishing purposes, images were edited with Adobe Photoshop CS6 Version 13.0 x64 and Adobe Illustrator CS6 Version 16.0.0 x 64. For LC3 Western blots (Figure 5 A, E), LC3-II band was quantified, then delta of LC3-II/Chloroquine treated and LC3-II/Chloroquine naïve was calculated, which was then in turn subjected to normalization. For statistical analysis, unpaired Student's t-test was used, unless indicated otherwise. Statistical analysis was performed using GraphPad Version 8.4.3.

SILAC labeling

For SILAC labeling,[55] cells were cultured in RPMI without lysine and arginine (Silantes) supplemented with 10% h.i. dialyzed FCS (Bio&SELL), 100 U/ml Penicillin / 100 mg/ml Streptomycin (Life Technologies) and heavy ($^{13}C_6{}^{15}N_4$ L-arginine and $^{13}C_6{}^{15}N_2$ L-lysine), medium-heavy ($^{13}C_6{}^{14}N_4$-L-arginine and 4,4,5,5-d4-L-lysine) (all Eurisotop) or regular (light) amino acids ($^{12}C_6{}^{14}N_4$ L-arginine and $^{12}C_6{}^{14}N_2$ L-lysine) (Sigma-Aldrich). Labeled BL60 cells carrying the doxycycline-inducible non-targeting shRNAs shGL2, shSHMT2.1266 or shCD79A were cultured for 3 or 5 days in SILAC medium containing 1 µg/ml doxycycline to induce shRNA expression.

Enrichment of phospho-peptides

For IP-based enrichment of tyrosine-phosphorylated peptides (pYome) the PTMScan Phospho-Tyrosine Rabbit mAb (P-Tyr-1000) Kit (Cat# 8803, Cell Signaling Technology) was applied according to Rush et al.[56] and following the instructions of the manufacturer. Briefly, after SILAC at least 1x10^8 cells were lysed in 10 ml urea lysis buffer (20 mM HEPES pH 8.0, 9 M urea, 1 mM sodium orthovanadate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate). Cell lysates from each SILAC condition were mixed in equal protein amounts followed by protein reduction with DTT and alkylation with iodoacetamide. After dilution with 20 mM HEPES, pH 8.0 to an urea concentration of 2 M protein digestion with trypsin was carried out overnight at 37 °C (enzyme-to-substrate-ratio 1:100). The peptide mixtures were purified with Sep-Pak C18 classic cartridges (Waters) and lyophilized. After sample solubilization in the kit buffer, peptides phosphorylated on tyrosine residues were immuno-precipitated with the anti-phospho-tyrosine-specific antibody P-Tyr-1000 (Cell Signaling Technology). Enriched phospho-peptides were eluted from the bead matrix under acidic conditions and purified using C18 microtips.

Mass spectrometry for phosphoproteomics and proteomics

For global proteome profiling, cells were lysed in NP40-containing lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.5, 5 mM NaF, 0.5% NP40, 1 mM sodium vanadate) supplemented with protease inhibitors (cOmplete cocktail, Roche) for 10 minutes on ice followed by centrifugation (21.380xg, 4°C, 10 min) to remove cell debris. Extracted proteins were subjected to gel-based proteome analysis as described previously.[29]

Phospho-peptide samples were analyzed on a Q Exactive HF orbitrap mass spectrometer (Thermo Fisher Scientifc) coupled to an Ultimate 3000 RSLCnano HPLC system (Dionex / Thermo Fisher Scientific). The peptides were first trapped on a precolumn (ReproSil-Pur 120 C18-AQ, 5 µm; Dr. Maisch GmbH; 100 µm x 5 cm) and separated on an analytical column (ReproSil-Pur 120 C18-AQ, 3 µm; Dr. Maisch GmbH; 350 × 0.075 mm) with a 90-min linear gradient of 2–40% solvent B [80% (vol/vol) ACN, 0.1% FA] and versus solvent A (0.1% FA in water) at a constant flow rate of 300 nL•min–1. Eluting peptides were analyzed by data-dependent acquisition using a top 20 MS/MS method with a survey scan resolution setting of 120,000 FWHM and an MS/MS resolution setting of 35,000 FWHM at 200 *m/z*. The 20 most abundant precursor peptide ions within the *m/z* range 350-1600 range were selected for HCD with an NCE setting of 28% and an isolation width of 1.4 *m/z*. AGC target values and maximum ion injection times for MS and MS/MS were set 1 ×10^6 in 40 ms and 1 × 10^5 in 64 ms, respectively. Selected precursor mass-to-charge ratio values were dynamically excluded from fragmentation for 45 s. Raw data files from LC-MS/MS measurements were analyzed with the MaxQuant software (version 1.6.0.1, MPI for Biochemistry).[57] Mass spectra were searched using the Andromeda search engine[58] against the UniProtKB human reference protein database (date: November 2016) supplemented with 245 frequently observed contaminants and setting trypsin as enzyme for protein digestion. After initial recalibration, precursor and fragment ion mass tolerances of 6 and 20 ppm were set, respectively. Oxidation of methionine and protein *N*-terminal acetylation were allowed as variable modifications, additionally including phosphorylation of serine, threonine and tyrosine for phosphorylation analysis. Carbamidomethylation of cysteine was defined as a fixed modification. Minimal peptide length was set to seven amino acids, with a maximum of two missed cleavages. On both the peptide and protein level the maximum false discovery rate (FDR) was set to 1% using a forward-and-reverse concatenated decoy database search strategy. SILAC multiplicity was set to triple labeling (Lys+0/Arg+0, Lys+4/Arg+6,

Lys+8/Arg+10) requiring at least two ratio counts for peptide quantitation and enabling the "re-quantify" option.

Subsequent evaluation of MaxQuant output data was conducted with the Perseus software (version 1.6.0.7, MPI for Biochemistry).[57] First, entries identified from the decoy database and potential contaminants were removed. For global proteome analysis also proteins identified only with modified peptides were discarded. For phosphorylation analysis, identified phospho-sites were filtered for a localization probability of at least 75%. The respective SILAC ratios were log-transformed, filtered for 2/3 valid values across replicate measurements and subjected to further statistical analyses. In particular, differentially expressed proteins were called using limma v3.38.0 at 1% FDR (Benjamini-Hochberg correction). Differentially phosphorylated sites were defined as phosphosites quantified in at least two replicates exhibiting an absolute log2 SILAC ratio > 0.5.

Metabolome analysis

1 million cells per condition were pelleted and washed with ice cold saline. Cell pellets were resuspended in 1 mL of 80% methanol solution containing internal standards. Samples were vortexed at 4 degrees, followed by centrifugation at 4°C for 10 minutes. Supernatant was transferred to a new tube, and samples dried using a Speedvac. Samples were collected in triplicate. For detailed description of the LC/MS, see the supplementary methods.

LC/MS for metabolome analysis

Dried cell extracts were resuspended in 100 µL HPLC grade water. LC-MS analysis was performed using a Q Exactive orbitrap mass spectrometer using an Ion Max source and heated electrospray ionization (HESI) probe coupled to a Dionex Ultimate 3000 UPLC system (Thermofisher). External mass calibration was performed every 7 days. Two runs with different instrument settings were performed to ensure all detected metabolites were within linear range. Samples were separated by chromatography by injecting 2 µL or 4 µL of sample on a SeQuant ZIC-pHILIC 2.1 mm x 150 mm (5 um particle size) column. Flow rate was set to 150 mL/min. and temperatures were set to 25°C for the column compartment and 4°C for the autosampler tray. Mobile phase A was 20 mM ammonium carbonate, 0.1% ammonium hydroxide. Mobile phase B was 100% acetonitrile. The chromatographic gradient was: 0–20 min.: linear gradient from 80% to 20% mobile phase B; 20–20.5 min.: linear gradient from 20% to 80% mobile phase B; 20.5 to 28 min.: hold at 80% mobile phase B. The mass spectrometer was operated in full scan, polarity-switching mode and the spray voltage was set to 3.0 kV, the heated capillary held at 275°C, and the HESI probe was held at 350°C. The sheath gas flow rate was 40 units, the auxiliary gas flow was 15 units and the sweep gas flow was one unit. The MS data acquisition was performed in full-scan, polarity switch mode, with a scan range of 70–1000 m/z. An additional narrow-range scan (220-700 m/z) was included in negative mode to enhance the detection of nucleotides. The resolution was set at 70,000, the AGC target at 1x10^6 , and the maximum injection time at 20 msec. Relative quantitation of polar metabolites was performed with TraceFinder 4.1 (Thermo Fisher Scientific) using a 5 ppm mass tolerance and referencing an in-house library of chemical standards. Peak areas were normalized to cell number and 13C-labled amino acids were used as internal standards (Cambridge Isotope Laboratories, Inc.).

Confocal fluorescence microscopy

All confocal fluorescence microscopy images were captured by using a Leica TCS SP5 (microscope type: DMI6000B-CS 11591084). Proximity ligation assays were performed as

previously described.[18,37] Detailed protocols for proximity ligation assays, LC3 staining and detailed microscope settings can be found below.

SHMT2 localization

3.33 x 10^5 cells were plated into a well of a tissue culture-treated 8 well µ-slide (ibidi). To allow attachment of the cells to the treated surface, cells were incubated at 37 °C and 5% CO_2 for 30 min. Thereafter, cells were fixed with 4% paraformaldehyde for 20 min, permeabilized with cold methanol at – 20 °C for 20 min and incubated for 30 min with signal enhancer (Invitrogen, Cat# I36933). Cells were blocked with 5% BSA for 1 h and stained with 0.77 µg/mL COX IV (4D11-B3-E8) Mouse mAb (Cell Signaling, Cat# 11967, RRID:AB_2797784) and 0.5 µg/ml SHMT2 polyclonal antibody (Invitrogen, Cat# PA5-54230, RRID:AB_2647281) overnight at 4°C. After staining, cells were washed twice in 1% BSA in PBS and incubated 1 h with secondary antibodies conjugated with Alexa Fluor 488 / 647 (Invitrogen, Cat# A-21202, RRID:AB_141607 and Cat# A-21244, RRID:AB_2535812) diluted 1:300 in 1% BSA in PBS. After washing, cells were mounted by adding mounting medium with DAPI (Invitrogen, Cat# P36935).

Equipment and settings: Confocal images were captured by using a Leica TCS SP5 (microscope type: DMI6000B-CS 11591084) with 63 X magnification (objective: HCX PI APO CS 63.0*1.40 OIL UV) and 1024x1024 pixel resolution. Leica Application Suite Advanced Fluorescence software was used to capture the images. Frame average was set to 3 and images were captured using 4 x zoom factor. Argon laser power was set to 30%. DAPI signal was detected by setting laser power of the 405 laser to 14%, setting smart gain to 834 and using a PMT detector and the Leica DAPI filter for detection. Signal of the Alexa Fluor 488 was detected by adjusting the 488 laser line to 50% and smart gain to 22. HyD detector and Leica 488 filter were used for detection of the signal. Leica 647 filter and HyD detector were used to observe the Alexa Fluor 647 signal by setting the 633 laser to 23 % and the smart gain to 76.

Image processing was done by using Images ImageJ version 1.39p. Images were cropped, pseudo-colorized (DAPI channel was colorized in blue, 488 channel was colorized in green and 647 channel was colorized in red), brightness and contrast was adjusted linear, covers the entire image and is equally adjusted to all images.

LC3 immunofluorescence

BL60 WT cells were treated for 24 h with 2.5 µM SHIN1 or equivalent volumes of DMSO. Moreover, cells were treated for 6 h with 50 nM Bafilomycin or equivalent volumes of DMSO. Following treatment 50 µl cell suspension of 1 x 10^6 cells/ml were plated in a well of a tissue culture treated 15 well angiogenesis µ-slide (ibidi). To allow attachment of the cells to the treated surface, cells were incubated at 37°C and 5% CO_2 for 30 min. Thereafter, cells were fixed with 4% paraformaldehyde for 20 min, washed in PBS, permeabilized with cold methanol for 20 min at – 20 °C and washed in PBS. Cells were blocked with Duolink blocking solution for 1 h and incubated with the primary antibody overnight at 4°C. Anti-LC3 pAb (MBL International, Cat# PM036, RRID:AB_2274121) was diluted 1:50 in Duolink Antibody Diluent. Thereafter, cells were washed twice in large volume of 1% BSA in PBS and incubated with Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Invitrogen, Cat# A-21244, RRID:AB_2535812), diluted 1:300 in Duolink Antibody Diluent, for 1 h at RT. Following the incubation, cells were washed twice in PBS for 5 min. Washing solution was aspirated and cells were mounted in Prolong Gold mounting media with DAPI (Invitrogen, Cat# P36935).

Equipment and settings: A Leica TCS SP5 (microscope type: DMI6000B-CS 11591084) with 63 x magnification (objective: HCX PI APO CS 63.0*1.40 OIL UV) and the Leica Application Suite Advanced Fluorescence software were used to capture confocal images.. Argon laser power was set to 29%. Images were captured using 512x512 pixel resolution with 8 bits per pixel. Per field of view 41 z-stacks were captured. Zoom factor was set to 2.5 x. DAPI signal was detected by setting laser power of the 405 laser to 15%, setting smart gain to 194, and using a HyD detector and the Leica DAPI filter (emission bandwidth 493 - 513nm) for detection. HyD detector and Leica ALEXA 647 filter (emission bandwidth 663 - 717nm) were used for detection of Alexa Fluor 647 signal, whereby 633 laser power was set to 31% and smart gain to 10. Image processing was performed by using ImageJ version 1.39p, representative images were pseudo-colorized (DAPI channel was colorized in blue and 647 channel in red), brightness and contrast was adjusted linear, covers the entire image and is equally adjusted to all images. Representative images were cropped and display the overlay max intensity of the 41 z-stacks of the 647 channel and the average intensity of the z-stacks of the DAPI signal.

Proximity ligation assay

BL60 WT cells were treated for 18 h with 2.5 µM SHIN1 or equivalent volumes of DMSO. Ramos WT cells were treated for 17 h with 5 µM SHIN1 or equivalent volumes of DMSO. Glycine/formate supplementation was performed according to protocol of supplementation assay (concentrations 3.3 mM and 2 mM respectively). BL60 shGl2 and BL60 shTCF3 were induced with 1 µg/ml doxycycline four days prior to seeding. Following treatment 0.5 x 10^5 cells per well were plated in a tissue culture treated 15 well angiogenesis µ-slide (ibidi). For attachment of the cells to the treated surface, cells were incubated at 37°C and 5% CO_2 for 30 min. Thereafter, cells were fixed with 4% paraformaldehyde for 20 min, washed in PBS, permeabilized with cold methanol for 20 min at –20 °C and washed in PBS. PLA was performed as previously described.[18,37] Briefly, cells were blocked with Duolink blocking solution for 1 h and incubated with primary antibodies overnight at 4°C. Anti-LC3 pAb (MBL International, Cat# PM036, RRID:AB_2274121) was diluted 1:50 and anti-E2A (G-2) (Santa Cruz, Cat# sc-133075, RRID:AB_2199145) was diluted 1:100 in Duolink Antibody Diluent. Thereafter, cells were washed twice in large volumes of 5% BSA in PBS and incubated with Duolink secondary antibodies for 1 h at 37°C. Duolink™ In Situ PLA® Probe Anti-Rabbit PLUS and Duolink® In Situ PLA® Probe Anti-Mouse MINUS (Sigma-Aldrich, Cat# DUO92002, RRID:AB_2810940 and Cat# DUO92004, RRID:AB_2713942) were diluted 1:5 in Duolink Antibody Diluent. Following the incubation, cells were washed twice in TBST with 0.05% tween-20 for 5 min. Ligation and amplification steps were performed following the manufacturer's instructions using Duolink® In Situ Detection Reagents Red (Sigma-Aldrich, Cat# DUO92008). Afterwards cells were washed twice in 0.2 M Tris and 0.1 M NaCl solution for 10 min and once in 0.002 M Tris and 0.001 M NaCl. Washing solution was aspirated and cells were mounted in Prolong Gold mounting media with DAPI (Invitrogen, Cat# P36935).

Confocal images were captured by a Leica TCS SP5 (microscope type: DMI6000B-CS 11591084) with 63 x magnification (objective: HCX PI APO CS 63.0*1.40 OIL UV) by using Leica Application Suite Advanced Fluorescence software. Argon laser power was set to 29%.

For single cell analysis, images were captured using 512x512 pixel resolution with 8 bits per pixel. Frame average was set to 3, and images were captured using 2.5 x zoom factor. DAPI signal was detected by setting laser power of the 405 laser to 10%, setting smart gain to 160, and using a HyD detector and the Leica DAPI filter (emission bandwidth 414-504 nm) for detection. HyD detector and Leica Texas Red filter (emission bandwidth 599-694 nm) were

used for detection of the Texas Red signal, whereby 594 laser power was set to 50% and smart gain to 250.

For the representative Images 1024x1024 pixel resolution was used, with 12 bits per pixel. Frame average was set to 2, and images were captured using 17 x zoom factor. Leica DAPI filter (emission bandwidth 414-504 nm) and HyD detector were used to observe the DAPI signal by setting the 405 laser to 12 % and the smart gain to 194. Signal of Texas Red was detected by setting the laser power of the 594 laser to 70% and the smart gain to 235. HyD detector and Leica Texas Red filter (emission bandwidth 599-694 nm) were used for detection of the signal. Image processing of the representative images was performed by using ImageJ version 1.39p. Images were pseudo-colorized (DAPI channel was colorized in blue and Texas Red channel in red), brightness and contrast was adjusted linear, covers the entire image and is equally adjusted to all images in one experiment.

Cell viability assays

Drug sensitivity and synergy as well as cell viability upon metabolite supplementation and ectopic expression of the constitutively active catalytic PI3K subunit variant MP110*[10,30] were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay, the XTT cell proliferation kit II (Roche) or the CellTiter-Glo luminescent cell viability assay (Promega) according to the manufacturers´ protocols; detailed description of each assay can be found below.

Drug Sensitivity Assay

Cell viability in response to drug treatment was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay. MTX was purchased from medac. SHIN1 was provided by Raze Therapeutics or purchased from Shanghai Medicilon Inc. Cells were seeded in 96-well plates at a density of 0.2-0.4x10^6/ml in 50 µl growth medium. Subsequently, drugs diluted at the desired concentrations were added in 50 µl growth medium. Each concentration was assayed in triplicates. After 96 h, 25 µl MTT solution (2 mg/ml MTT (Cat# 20395.03, Serva) in PBS) was added to each well and cells were incubated for another 4 h at 37°C. Subsequently, the formazan was solubilized by addition of 100 µl SDS solution (20% w/v SDS in 50 % v/v DMF, pH 4.70) at 37°C. Absorbance was measured at 560 nm using 620 nm as reference wavelength on a Tecan infinite M200 plate reader. IC_{50} values were calculated using CalcuSyn from Biosoft.

Supplementation assay

For supplementation assays using the SHMT inhibitor SHIN1, 2.5x10^4 cells were seeded in 100 µl regular cell culture medium containing increasing concentrations of SHIN1 (0 µM, 0.1 µM, 0.5 µM, 1, 5 µM and 10 µM) or/and supplemented with glycine (1.33 mM (10x), 3.325 mM (25x); Cat# A3741, Sigma Aldrich), L-serine (2.86 mM (10x), 5.72 mM (20x); Cat# S4311, Sigma-Aldrich), sodium formate (0.1 mM, 1 mM, 2 mM; Cat# 67253, Sigma-Aldrich), EmbryoMax Nucleosides (Cat# ES-008-D, Sigma-Aldrich; containing 3 mM cytidine, guanosine, uridine, adenosine and 1 mM thymidine, diluted in the indicated percentages (1%, 2.5%, 5%). Cell viability was determined using MTT assay after 2 days as described above.

Drug synergy analysis

Pairwise drug interaction was measured using a typical checkerboard assay setup. Assay was performed in 96-well plate format with serial dilutions of two drugs.[59] Columns 3 to 9 contains 25 µl of 2-fold serial diluted drug A, rows A to G contain 25 µl 2-fold serial diluted

drug B. Column 10 contains a serial dilution of drug B alone, while row H contains a serial dilution of drug A alone. These controls are used to determine the IC_{50} for each drug, which was used to calculate the FIC value to assess synergism, additive/indifference or antagonism. Cells were seeded in all wells at a density of 0.2-0.4x10^6/ml in 50 µl growth medium. Cell viability was determined using MTT assay after 96 h as described above.

XTT

2x10^3 BL60 cells expressing MP110*[25] or empty vector (pABES-hygro) were seeded into the wells of a 96-well plate on day zero. The cells were cultured in the respective growth media described above and were treated with SHIN1 or DMSO. At given time points, the cells were treated using a mixture consisting of the XTT-labeling reagent and the electron coupling reagent according to the protocol of the cell proliferation kit II (Roche). Four hours later, the XTT-derived signals were monitored by an Elisa-Reader (Spectra Fluor Plus, specific absorbance filter: 475 nm, non-specific absorbance filter: 660 nm) (Tecan). Wells containing growth media served as background controls in all experiments.

CellTiterGlo assay

Cells were plated into opaque 384-well plates (Corning) at a density of 1x10^5 cells/ml in a total volume of 30 µl. Cells were then treated with varying doses of SHIN2 (highest concentration 66.7 µM, 1:2 dilution series) diluted in DMSO using a Tecan D330e digital dispenser. DMSO concentration was normalized and conditions were measured in triplicate and randomized across the plate. After 72 h of incubation, 30 µl of CellTiterGlo solution (Promega) was added to each well and luminescence was measured after 15 minutes on a plate reader (Tecan).

Flow cytometry

Competitive growth assay

Transduced and non-transduced cells were mixed in a 12-well plate and in case of inducible shRNA, shRNA expression was induced with 1 µg/ml doxycycline. Then, GFP, GFP/BFP, GFP/RFP or RFP expression was determined via flow cytometry over time at the indicated time points. Samples were analyzed on a BD LSRFortessa flow cytometer using BD FACSDiva software version 8.0.1. Data were analyzed using FlowJo v10.5.3.

Cell cycle analysis

Cell cycle analysis was performed using the BD Pharmingen APC BrdU Flow Kit (Cat# 552598) according to the manufacturer's protocol. 5x10^6 cells were labeled with 10 µM BrdU for 30 minutes (Ramos, BL2 and BL60) or 1.5 h (Seraphine) at 37°C, then washed with PBS to remove unincorporated BrdU and permeabilized/fixed with the Cytofix/Cytoperm and Cytoperm Plus solutions provided in the kit. Permebilization/Fixation solutions were removed by washing the cells with PermWash buffer according to the manufacturer´s instructions. Then DNA of the cells was digested with 30 µg DNase I (Sigma Aldrich) for 1 hour at 37°C. BrdU was stained with BrdU-APC Antibody (eBioscience, Cat# 17-5071-42, RRID:AB_11040534) 5 µl in 50 µl PermWash Buffer each for 20 minutes RT in the dark. After washing with PermWash DNA was stained using 5 µg/ml DAPI and then resuspended in 1 ml staining buffer without washing step. Samples were analyzed on a BD LSRFortessa flow cytometer. Data were analyzed using FlowJo v10.5.3.

Sequential transduction of BL60 for TCF3 rescue

5x10^5 BL60 cells in 500 µl growth medium were transduced with 10 µl concentrated lentivirus containing the constitutive shRNA vectors pRSI12.shNTC and shSHMT2.517. 4 days later, transduction efficiency was determined on the basis of RFP expression using flow cytometry and cells were transduced with retrovirus that was generated as described in the supplementary methods using pRetroCMV-TO-Puro-GFP-TCF3-E47 or the empty vector pRetroCMV-TO-Puro-GFP. Number of RFP and GFP positive cells was measured via flow cytometry at day 2, 5, 7 and 9 after the second transduction.

tfLC3 reporter assay

A modified tfLC3 reporter was used.[33] For cloning strategies and transduction, see supplementary methods. Cells were seeded at a concentration of 0.5x10^6 cells/ml in 96-well plates in glycine depleted (80% glycine, 107 µM glycine) RPMI medium and treated with SHIN1 (Medicilon) at a concentration of 5 µM, AZD2014 (Selleckchem, Cat#S2783) at a concentration of 200 nM and Torin 1 (Selleckchem, Cat#S2827) at a concentration of 500 nM for the indicated time. Bafilomycin A1 (Baf, Selleckchem, Cat#S1413) treatment at 50 nM was used to inhibit autophagy. At each time point, GFP and RFP MFI was analyzed on a Beckman Coulter CytoFlex LX flow cytometer and the GFP/RFP MFI ratio was normalized to the DMSO control of each time point.

Annexin V stain

Annexin-V apoptosis staining was performed according to the manufacturer's protocol (Annexin V-FITC Kit, Miltenyi Biotec, Cat# 130-092-052). 1µg/ml DAPI was used as viability dye and cells were costained with a CD19-PE antibody to detect BL cells (eBioscience, Cat# 302208). Samples were analyzed on a BD LSRFortessa flow cytometer. Data were analyzed using FlowJo v10.5.3.

Reactive oxygen species detection

Cellular ROS levels were determined using 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA, Thermo Fisher Scientific, Catalog number D399). 0.5-1x10^6 cells were washed once in prewarmed Dulbecco's phosphate-buffer saline and incubated in DPBS with 5 µM H_2DCFDA for up to 30 minutes at 37 °C. 1 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) was added to the sample to stain for viable/exclude apoptotic cells. 50 µM H_2O_2 (Fisher Scientific, Cat#10386643) was used as a positive control and added for 10 minutes during the H_2DCFDA incubation. N-acetyl-L-cysteine (NAC, Sigma-Aldrich, Cat# A7250) was used as a ROS scavenger. To this end, the cells were preincubated with 5 mM NAC added to the culture media for 1 hour prior to harvesting. The tubes were then transferred to ice and measured with the BD Fortessa. Data were analyzed using FlowJo v10.5.3.

Mitochondrial ROS levels were determined using MitoSOX™ Red Mitochondrial Superoxide Indicator (Thermo Fisher Scientific, Cat# M36008). 0.5-1x10^6 cells were transferred to phenol red-free full culture medium. 2.5 µM MitoSOX was added to the culture wells and cells were incubated for 30 minutes at 37 °C protected from light. Cells were washed twice with prewarmed Hank's balanced salt solution with calcium and magnesium (Thermo Fisher Scientific). 1 µg/ml 4′,6-diamidino-2-phenylindole (DAPI, Thermo Fisher Scientific, Cat# D1306) was added to the sample to assess viability. The tubes were then transferred to ice, protected from light and measured with the BD Fortessa. Data were analyzed using FlowJo v10.5.3.

IgM detection

2x10^6 cells were washed with PBS incubated in 200 µl staining solution (2 µl of αIgM antibody [BD, Cat. No. 550881] RRID:AB_393944 in PBS) for 30 min. Stained cells were washed again and incubated in propidium iodide solution (1 µg/ml) for 5 min. After washing, the cell suspensions were measured on a MACS Quant 16 (Miltenyi) flow cytometer. Data was analyzed with Kaluza software (Beckman Coulter).

Quantitative high-throughput screening with the MIPE 5.0 library

For high-throughput drug screening, BL2 cells were grown in advanced RMPI medium (Gibco) supplemented with 5% FBS, 2 mM Glutamine 100 U/ml Pen/Strep. Cells were maintained in a humidified CO_2 incubator at a density of 0.25/0.50 MLNs/ml before seeding. 500 cells/well were then seeded into 1536 well white polystyrene tissue culture treated plates (Greiner), in a final volume 5 µL of growth media containing either DMSO or sublethal doses of the racemic SHMT inhibitor SHIN1 (100 nM and 250 nM), by using a Multidrop Combi dispenser (Thermo Fisher). After cell addition, 23 nL of MIPE 5.0 compounds were transferred to individual wells (11 doses tested for each compound in separate wells) via a 1536 pin-tool. Bortezomib (final concentration 2.3 µM) was used as a positive control for cell cytotoxicity. Plates were covered by a stainless-steel gasketed lid to prevent evaporation and incubated for 48 hours in a humidified CO_2 incubator. At the 48 h time point, 3 µL of Cell Titer Glo (Promega) were added to each well and plates were incubated at room temperature for 15 minutes with the stainless-steel lid in place. Luminescence readings were taken using a Viewlux reader (PerkinElmer) with a 2 second exposure time per plate.

Formate analysis

Formate concentration was measured on a Tecan infinite M200 plate reader at 450 nm. For sample preparation, 2x10^6 cells were used and prepared with a Formate Assay Kit (Sigma Aldrich, Cat # MAK059) according to the manufacturer's instructions. Samples were measured in duplicates. For statistical analysis, unpaired Student's t-test was used. Statistical analysis was performed using GraphPad Version 8.4.3.

Cycloheximide treatment

Inducible SHMT2 knockdown cells were cultured for 5 days in doxycycline-containing medium at a concentration of 1 µg/ml. On d5, cells were treated with Cycloheximide (Sigma Aldrich, Cat# 01810-5G) at a concentration of 25 µM for 3 hours or with DMSO. Afterwards, 5x10^6 cells per condition were lysed using NP40 containing lysis buffer and analysed via Western blot (see chapter immunoblotting).

Immunohistochemistry in murine tissue

Formalin-fixed and paraffin-embedded tissues from mice were cut into 4 µm sections; sections were heated for 20 min at pH 6. For Ki67 staining, the antibody SP6, Cat# 275R, Cell Marque, RRID:AB_1158035 (diluted 1:50) and Histofine simple stain mouse (anti-rabbit, No. 414341F, Nichirei) were used.

RNA sequencing

BL60 cells carrying the Doxycycline inducible non-targeting shRNA shGL2 or the shRNA shSHMT2.1266 were cultured for 3 or 5 days in medium containing 1 µg/ml Doxycycline to induce shRNA expression. Total RNA was isolated from 5x10^6 cells per condition using the miRNeasy Mini kit (Cat# 217004) according to the manufacturer´s protocol including homogenization of cell lysates using QIAshredder homogenizers (Cat# 79654) and on-column DNase (Cat# 79254, all Qiagen) digests. RNA quality was assessed with an Agilent

Bioanalyzer 2100 and RNA 6000 Nano Chips (Cat# 5067-1511, both Agilent Technologies). RIN values ranged between 9.8 and 10. RNA concentrations were determined using the Qubit RNA HS Assay Kit (Cat# Q32855) and a Qubit 3 Fluorometer (both ThermoFisher Scientific). Library preparation and single read 50 bp RNAseq was done on a HiSeq 4000 system (Illumina) by the High Throughput Sequencing unit of the DKFZ Genomics and Proteomics Core Facility in Heidelberg, Germany. Data quality control was performed with FastQC v0.11.4. Reads were aligned to the human reference genome (Ensembl GRCh38 release 82) using STAR v2.4.2a. Gene count tables were generated while mapping, using Gencode v31 annotations. All downstream analyses were carried out using R v3.5.2[60] and BioConductor v3.8.[61] Exploratory analyses and differential gene expression analysis were carried out with DESeq2 v1.22.0.[62] For differential expression analysis, the Wald test was used for pairwise comparisons. In addition to an FDR cutoff of 1%, an absolute log fold change cutoff of 0.5 was used to call differentially expressed genes.

Pathway enrichment analysis

Pathway enrichment analysis was performed as described in Corso et al.,[29] using the BioC packages clusterProfiler v3.14.0 and ReactomePA v1.25.1.

Whole exome sequencing

Cell lines

For whole exome sequencing (WES), total DNA was isolated from the BL cell lines BL60 and Seraphine using the DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer´s protocol. DNA quality control, WES and bioinformatic analyses were done by Novogene. In brief, DNA degradation and contamination were monitored on a 1% agarose gel and DNA concentration was measured using the Qubit® DNA Assay Kit and a Qubit® 2.0 Flurometer (Life Technologies, CA, USA). For library preparation genomic DNA samples were randomly fragmented by sonication (Covaris, Massachusetts, USA) to the size of 180-280 bp fragments. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities and enzymes were removed. After adenylation of 3’ ends of DNA fragments, adapter oligonucleotides were ligated. DNA fragments with ligated adapter molecules on both ends were selectively enriched in a PCR reaction. After PCR reaction, the libraries were hybridized in buffer with biotin-labeled probes, then magnetic beads with streptomycin were used to capture the exons. Therefore, the SureSelect Human All Exome V6 kit (Agilent) was used. After washing beads and digesting probes, the captured libraries were enriched in a PCR reaction to add index tags. Products were purified with AMPure XP system (Beckman Coulter, Beverly, USA) and quantified by using the Agilent high sensitivity DNA assay on the Agilent Bioanalyzer 2100 system. Sequencing was done on an Illumina Novaseq 6000 with the read length 150PE. Burrows-Wheeler Aligner (BWA) was utilized to map the paired-end clean reads to the human reference genome (hg38, http://hgdownload.cse.ucsc.edu/goldenPath/hg38/bigZips/analysisSet/hg38.analysisSet.2bit). Following genomic variant detection, annotation of variants was performed with the tool ANNOVAR.[63]

Patient Sample

DNA was extracted using the AllPrep kit following the manufactures instructions (QIAGEN). Sequencing libraries were prepared using the Enzymatic Fragmentation and Human Core Exome Kit (Twist Bioscience). xGen UDI-UMI sequencing adapters were purchased from

IDT. Paired-end 150 bp read sequencing was performed on a Nextseq 550 using V2.5 chemistry (Illumina).

Paired-end reads were demultiplexed and unique molecular barcodes (UMI) extracted using picard (version 2.23.6-0) with default parameters. Resulting data was mapped to the human genome (NCBI build 37) using BWA-MEM version 0.7.17 with default parameters[64].

Reads that originate from the same DNA molecule are identified by unique molecular barcodes and genomic coordinates. Thus, these reads are collapsed into consensus read groups. To this end, reads were first sorted by genomic coordinates and then grouped by the coordinates and UMIs (GroupReadsByUmi from fgbio version 1.3.0 with strategy=adjacency min-map-q=20). Base qualities were adjusted and a consensus read was generated for each group (fgbio). A probability is calculated at each position of the reads for the 4 bases (A,T,C,G) and the base with the highest probability is then called. Consensus reads with high average consensus error rate are filtered out and all bases with low base quality and high consensus error rate are masked to N. Consensus reads with high proportion of N are filtered out (fgbio).

Variants were called with the mutect2 software using the following criteria[65]: Read count >20 and variant read frequency > 0.1 using only non-duplicated reads defined by genomic coordinates and one UMI. Variants that are identified as "clustered_events" and "haplotype" by mutect2 are included in the analysis. RefSeq annotation of variants was performed using snpEff, version 4.3.1 and ExAC data.

To identify candidate somatic mutations, only variants with population frequency >0.0001 in the ExAC Non-Finnish European (NFE) database were included. Variants were excluded that were identified by Exome sequencing analysis of in-house control DNA samples from normal peripheral blood mononuclear cell populations. Further validation of sequencing results were performed including variants that were identified from at least two consensus reads containing different UMI and different genomic coordinates, respectively. Amino acid changes were visualized within the functional domains of the respective protein using MutationMapper.[66]

Quantification and Statistical Analysis

GraphPad Prism Version 8.4.3 and R v3.5.2 software packages were used to perform the statistical analyses. Further details regarding quantification and statistical analysis are given in the description of individual methods. Statistical tests used are specified in the figure legends. Error bars, SEM, unless otherwise stated. The threshold for statistical significance is $P \leq 0.05$, unless otherwise specified.

In vivo experiments

For xenografts with BL60 cells, tumor growth was monitored every other day by measuring tumor size in two orthogonal dimensions and calculated as ½ x length × width x width. Statistical measure was performed in GraphPad Prism Version 8.4.3 using Two-Way ANOVA with Bonferroni posthoc correction. For transplantation experiments using M2121 cells, statistical analysis was performed using Log-rank test.

CRISPR Cas9 Screen

Replicate CSS values for each cell line were then averaged and compared to published CSS values generated from ABC DLBCL cell lines.[18] Downstream data analyses were performed as previously described.[18] Genes exhibiting an absolute CSS > 0.5 were considered hits.

Flow cytometry analyses

For competitive growth assays, two-way ANOVA was used for all experiments except Figure 2F and S2C, where unpaired Student's test was used at indicated time points. For BrdU analysis, unpaired Student's t-test was used. For flow cytometry analysis of cells transduced with tfLC3 reporter, Tukey's multiple comparison test was used. For ROS measurements, Student's t-test was used. Statistical analysis was performed using GraphPad Prism Version 8.4.3.

Cell viability assays

For MTT and XTT assays, two-way ANOVA with Boniferroni post correction was used. Statistical analysis was performed using GraphPad Prism Version 8.4.3. For CellTiterGlo, both Eμ:Myc lines were compared to both DMBC lines by Welch's t-test.

Confocal imaging analysis

For LC3 immunofluorescence, data were normalized to control and reported as percentage. Box plots represent the median and 25th–75th percentiles, whiskers display 10–90 percentiles, and outliers are displayed as dots. For data analysis, a Kruskal-Wallis test was performed, where $p<0.0001$. Statistical analysis was performed using GraphPad Prism Version 8.4.3.

For proximity ligation assays, images were processed by ImageJ version 1.47n5. PLA puncta were counted by Blobfinder version 3.2.[67] Data were normalized to control and reported as percentage. For each biological replicate, a minimum of 105 single cells were analyzed. Box plots represent the median and 25th–75th percentiles, whiskers display 10–90 percentiles, and outliers are displayed as dots. For BL60 WT data analysis, a Kruskal-Wallis test was performed. Analysis for BL60 shGL2 and BL60 shTCF3 and Ramos WT data consisted of an unpaired two tailed Mann–Whitney test. Statistical analysis was performed using GraphPad Prism Version 8.4.3.

Immunoblotting

For quantification of immunoblotting, please see immunoblotting section above. For immunoblotting, unpaired Student's t-test was used, unless indicated otherwise. Statistical analysis was performed using GraphPad Prism Version 8.4.3.

Phosphoproteomic and proteomic analysis

SILAC ratios were log-transformed, filtered for 2/3 valid values across replicate measurements and subjected to further statistical analyses. In particular, differentially expressed proteins were called using limma v3.38.0 at 1% FDR (Benjamini-Hochberg correction). Differentially phosphorylated sites were defined as phosphosites quantified in at least two replicates exhibiting an absolute log2 SILAC ratio > 0.5.

Metabolomic analysis

Metabolites and runs were subject to pre-defined quality control parameters: CV (standard deviation/ mean peak area across multiple injections of a representative (pooled) biological sample) below 0.25; R2 (linear correlation across a three-point dilution series of the

representative (pooled) biological sample) greater than 0.9. For statistical analysis, we performed a two-sided t-test to compare metabolite abundances between the two conditions. P-values were corrected for multiple hypothesis testing (Benjamini-Hochberg).

Highthroughput drug screen analysis

Viability of compound treated wells was normalized to DMSO and empty well controls present on each plate, and dose-response curve-fitting and curve-classification was automatically performed for each individual drug.

For each individual qHTS screening (DMSO, +100nM SHIN1 or +250nM SHIN1), Z-transformed area under the curve (Z-AUC) values were calculated for each compound and used to compare/contrast drug responses across experiments. Sensitization HITs were defined as agents with a good curve-class in the DMSO run (CCv2: -1.1, -1.2, -2.1 or -2.2. See https://tripod.nih.gov/curvefit/ for details) and with a SHIN1 250nM vs DMSO Z-AUC difference <= -0.60. Resistance HITs were defined as agents with a good curve-class in the DMSO run (CCv2: -1.1, -1.2, -2.1 or -2.2., See https://tripod.nih.gov/curvefit/ for details) and with SHIN1 250nM vs DMSO Z-AUC difference >= +0.60. All the screening data are publicly available and searchable via the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)

Data and Code Availability Statement

CRISPR Cas9 screen analysis raw data (Figure 1 and S1 A-F, Figure 4A, S4A) are reported in Table S1. RNAseq raw data (Figure S5A) have been deposited to the Sequence Read Archive (SRA)[68] and are available under SRA accession PRJNA623692. The mass spectrometry proteomics data (Figure 4A, I, J and S4 A,D,E) have been deposited to the ProteomeXchange Consortium via the PRIDE[69] partner repository with the dataset identifier PXD018961 (Username: reviewer24952@ebi.ac.uk, Password: eJhtSkGz). Results from the mass spectrometry phosphoproteomic data are also reported in Table S2. The metabolomic analysis (Figure 3 A,B and S3 A-C) has been made available at Metabolights (https://www.ebi.ac.uk/metabolights/MTBLS1702).[70] Code will be made available upon request. CRISPR screen analysis and RNAi screen analysis are publicly available on https://www.depmap.org/portal/ or McDonald et al. 2017.[71]

For original data, please contact thomas.oellerich@kgu.de.

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Figure S1

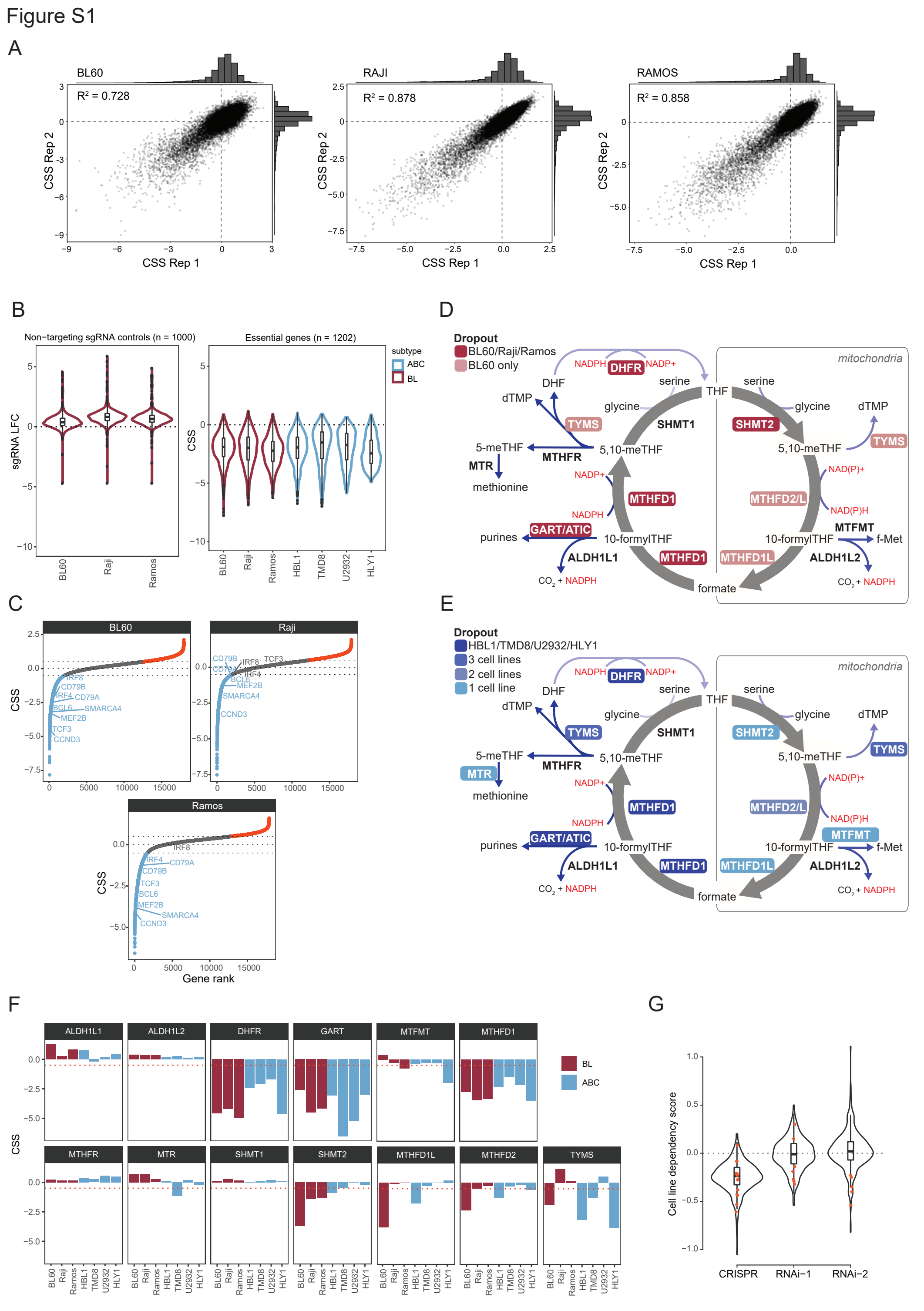
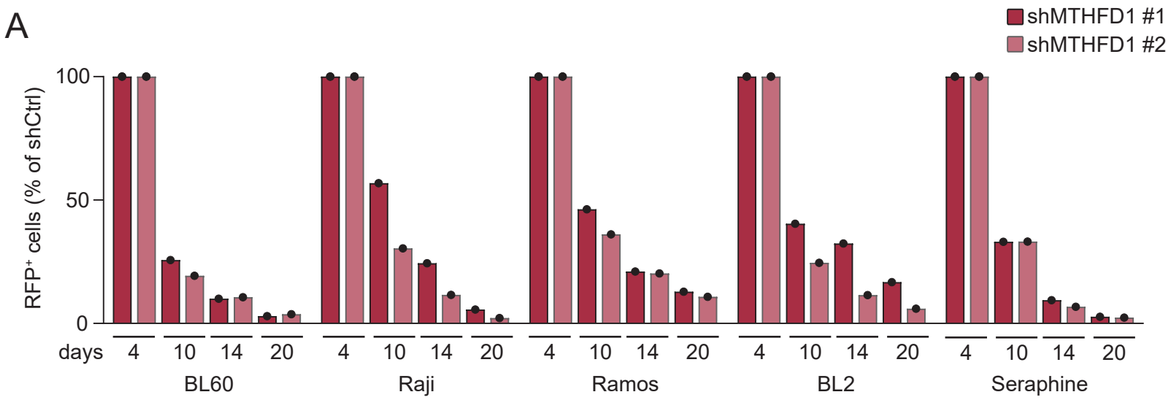
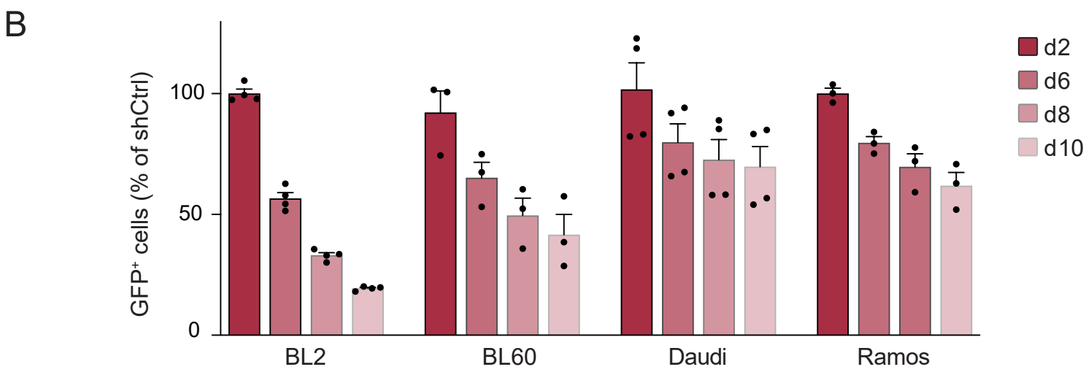
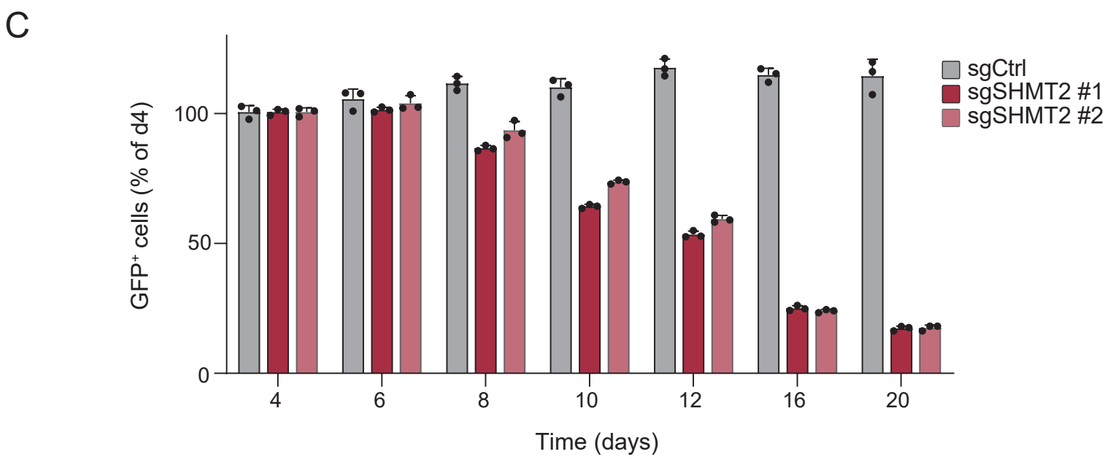
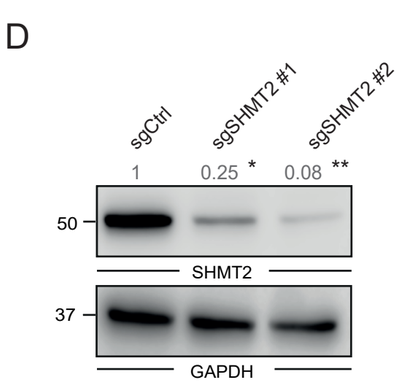

A
BL60
RAJI
RAMOS
R² = 0.728
R² = 0.878
R² = 0.858
CSS Rep 1
CSS Rep 2
B
Non−targeting sgRNA controls (n = 1000)
Essential genes (n = 1202)
sgRNA LFC
CSS
subtype
ABC
BL
BL60
Raji
Ramos
HBL1
TMD8
U2932
HLY1
C
BL60
Raji
Ramos
CSS
Gene rank
IRF8
CD79B
CD79A
IRF4
BCL6
SMARCA4
MEF2B
TCF3
CCND3
D
Dropout
BL60/Raji/Ramos
BL60 only
mitochondria
NADPH
DHFR
NADP+
DHF
serine
THF
dTMP
glycine
TYMS
SHMT1
SHMT2
5-meTHF
MTHFR
5,10-meTHF
MTR
methionine
NADP+
MTHFD1
NAD(P)+
MTHFD2/L
NADPH
NAD(P)H
purines
GART/ATIC
10-formylTHF
MTFMT
f-Met
ALDH1L1
ALDH1L2
MTHFD1L
CO2 + NADPH
formate
E
Dropout
HBL1/TMD8/U2932/HLY1
3 cell lines
2 cell lines
1 cell line
F
ALDH1L1
ALDH1L2
DHFR
GART
MTFMT
MTHFD1
MTHFR
MTR
SHMT1
SHMT2
MTHFD1L
MTHFD2
TYMS
BL
ABC
CSS
G
Cell line dependency score
CRISPR
RNAi−1
RNAi−2

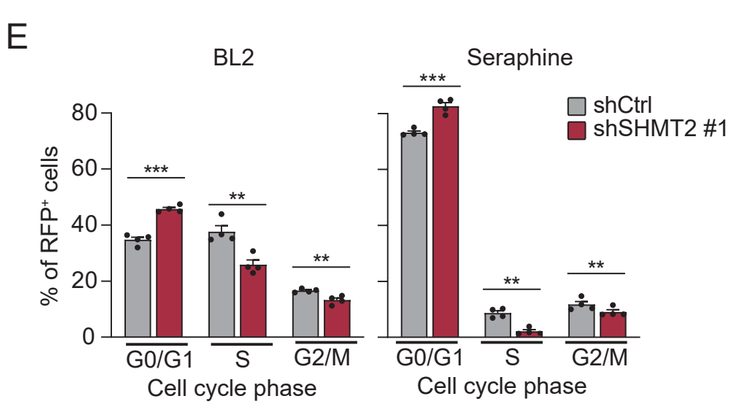
Figure S2

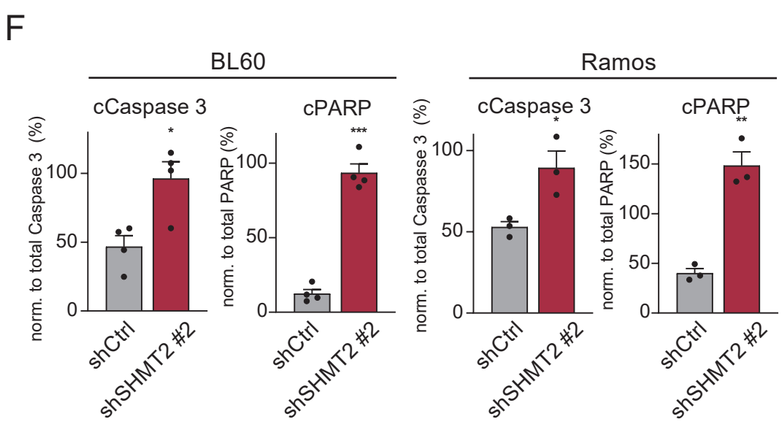

A
shMTHFD1 #1
shMTHFD1 #2
RFP⁺ cells (% of shCtrl)
100
50
0
days
4
10
14
20
BL60
Raji
Ramos
BL2
Seraphine

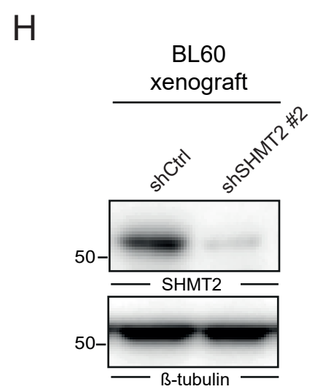

B
d2
d6
d8
d10
GFP⁺ cells (% of shCtrl)
100
50
0
BL2
BL60
Daudi
Ramos


C
sgCtrl
sgSHMT2 #1
sgSHMT2 #2
GFP⁺ cells (% of d4)
100
50
0
4
6
8
10
12
16
20
Time (days)


D
sgCtrl
sgSHMT2 #1
sgSHMT2 #2
1
0.25 *
0.08 **
50
SHMT2
37
GAPDH


E
BL2
Seraphine
shCtrl
shSHMT2 #1
% of RFP⁺ cells
80
60
40
20
0
***
**
G0/G1
S
G2/M
Cell cycle phase


F
BL60
Ramos
cCaspase 3
cPARP
norm. to total Caspase 3 (%)
norm. to total PARP (%)
norm. to total Caspasse 3 (%)
shCtrl
shSHMT2 #2


H
BL60
xenograft
shCtrl
shSHMT2 #2
50
SHMT2
50
ß-tubulin

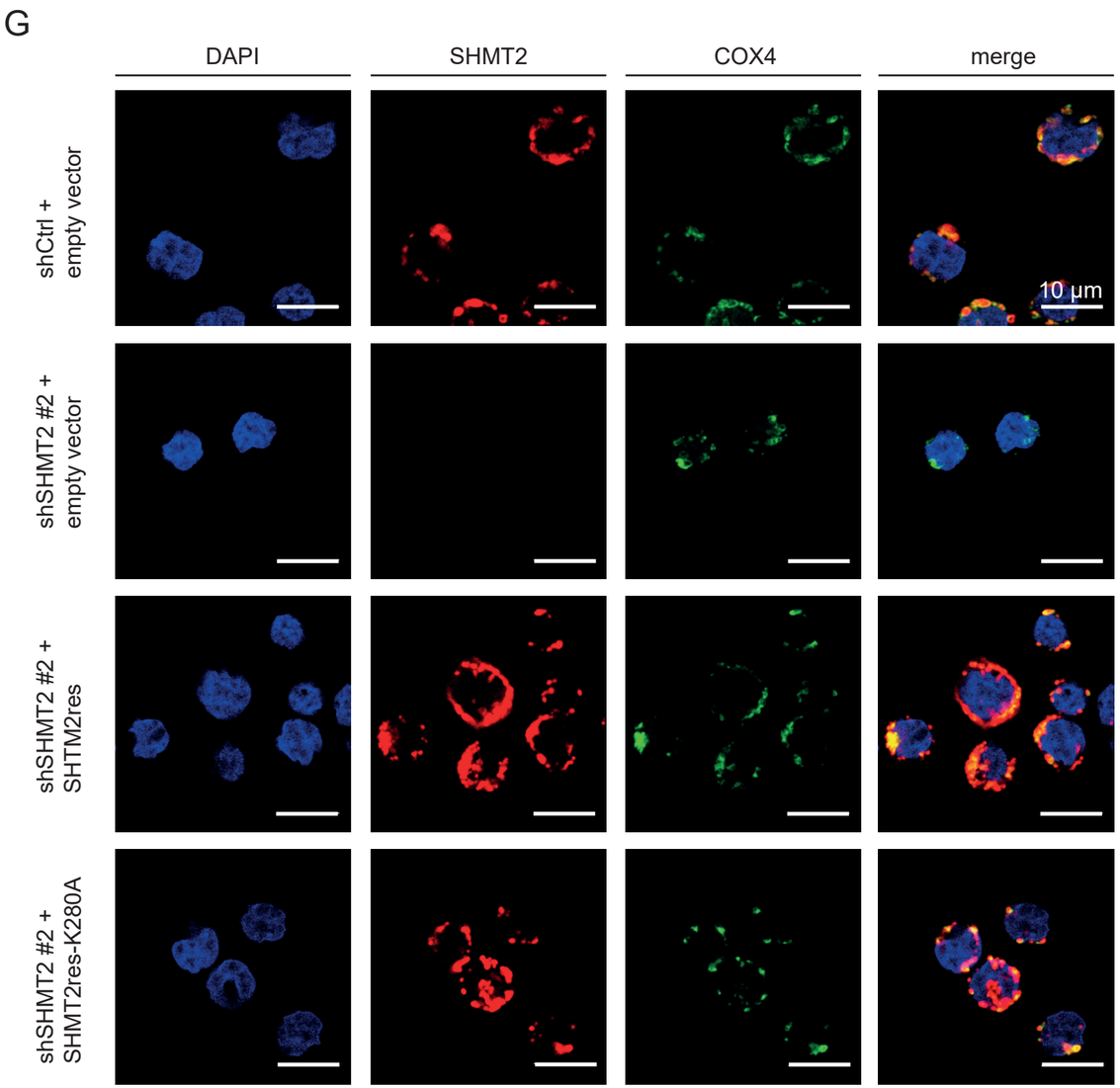
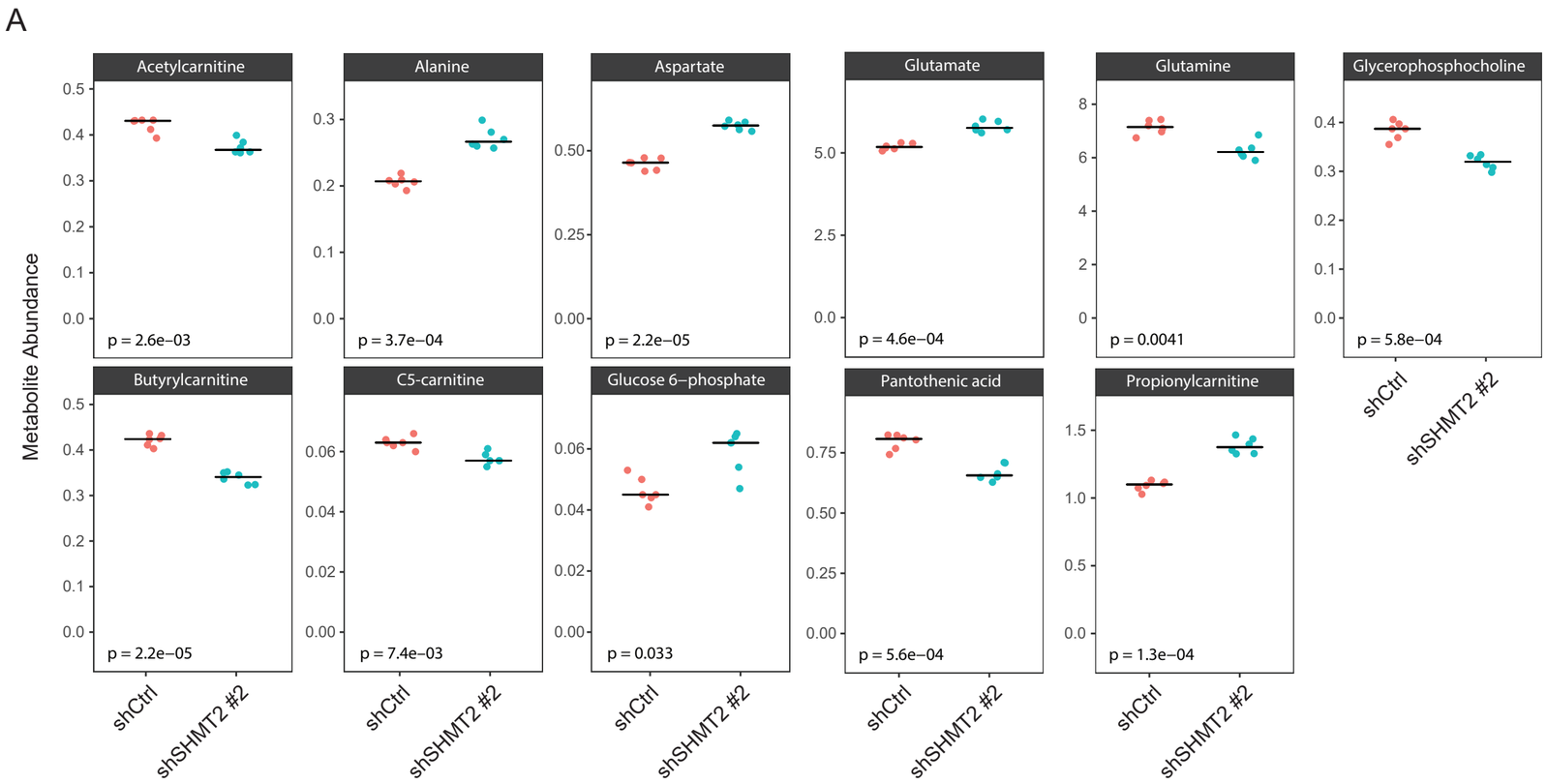
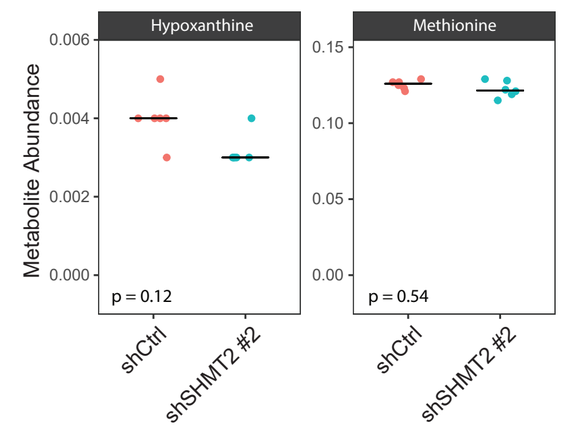
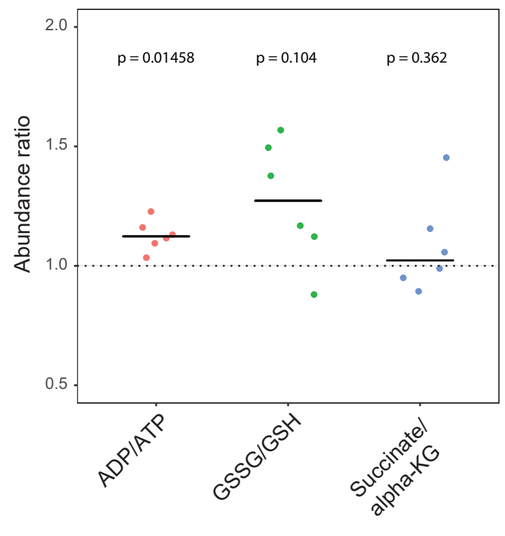
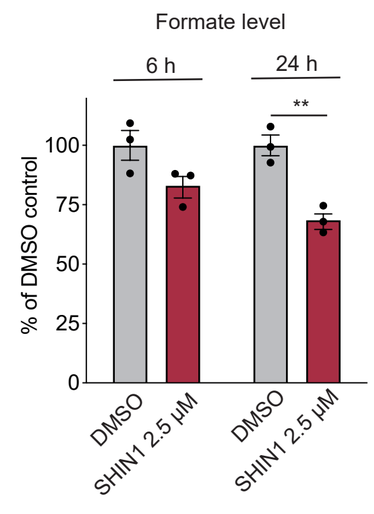

G
DAPI
SHMT2
COX4
merge
shCtrl + empty vector
shSHMT2 #2 + empty vector
shSHMT2 #2 + SHTM2res
shSHMT2 #2 + SHMT2res-K280A
10 µm

Figure S3


A
Metabolite Abundance
Acetylcarnitine
p = 2.6e−03
Alanine
p = 3.7e−04
Aspartate
p = 2.2e−05
Glutamate
p = 4.6e−04
Glutamine
p = 0.0041
Glycerophosphocholine
p = 5.8e−04
Butyrylcarnitine
p = 2.2e−05
C5-carnitine
p = 7.4e−03
Glucose 6−phosphate
p = 0.033
Pantothenic acid
p = 5.6e−04
Propionylcarnitine
p = 1.3e−04
shCtrl
shSHMT2 #2


B
Metabolite Abundance
Hypoxanthine
p = 0.12
Methionine
p = 0.54
shCtrl
shSHMT2 #2


C
Abundance ratio
p = 0.01458
p = 0.104
p = 0.362
ADP/ATP
GSSG/GSH
Succinate/
alpha-KG


D
Formate level
6 h
24 h
**
% of DMSO control
DMSO
SHIN1 2.5 μM

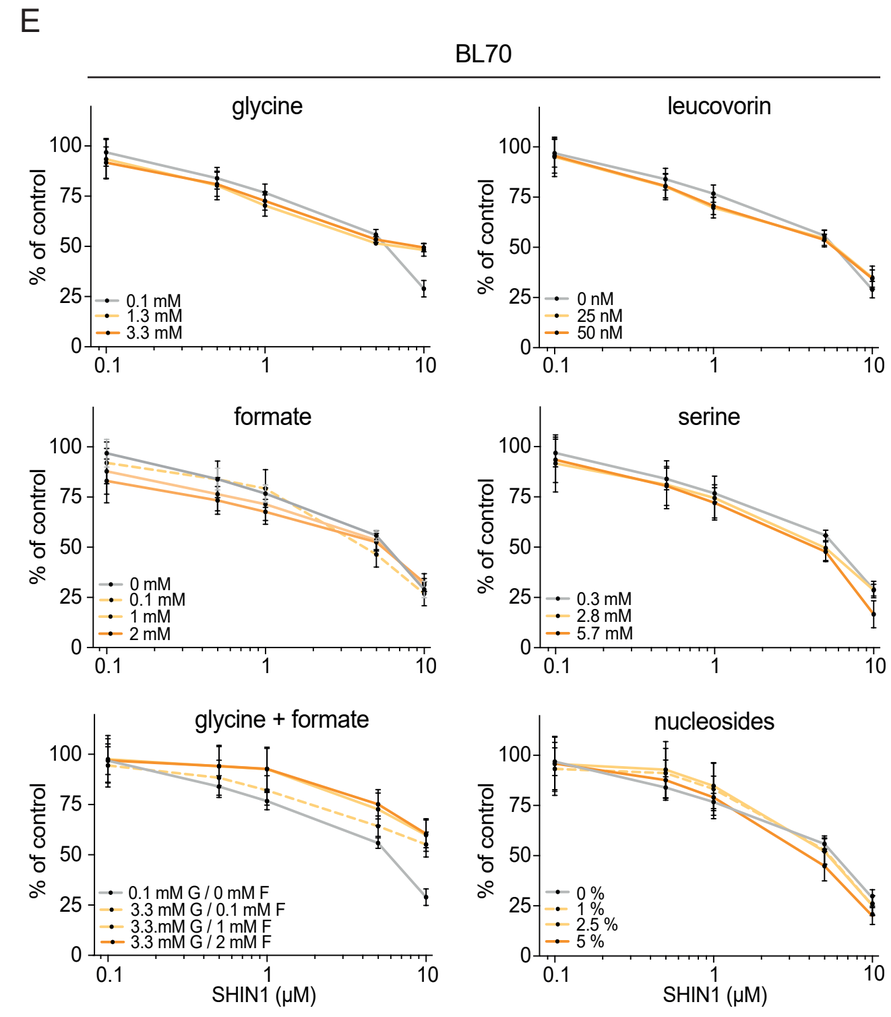

E
BL70
glycine
0.1 mM
1.3 mM
3.3 mM
leucovorin
0 nM
25 nM
50 nM
formate
0 mM
0.1 mM
1 mM
2 mM
serine
0.3 mM
2.8 mM
5.7 mM
glycine + formate
0.1 mM G / 0 mM F
3.3 mM G / 0.1 mM F
3.3.mM G / 1 mM F
3.3 mM G / 2 mM F
nucleosides
0 %
1 %
2.5 %
5 %
% of control
SHIN1 (μM)

Figure S4

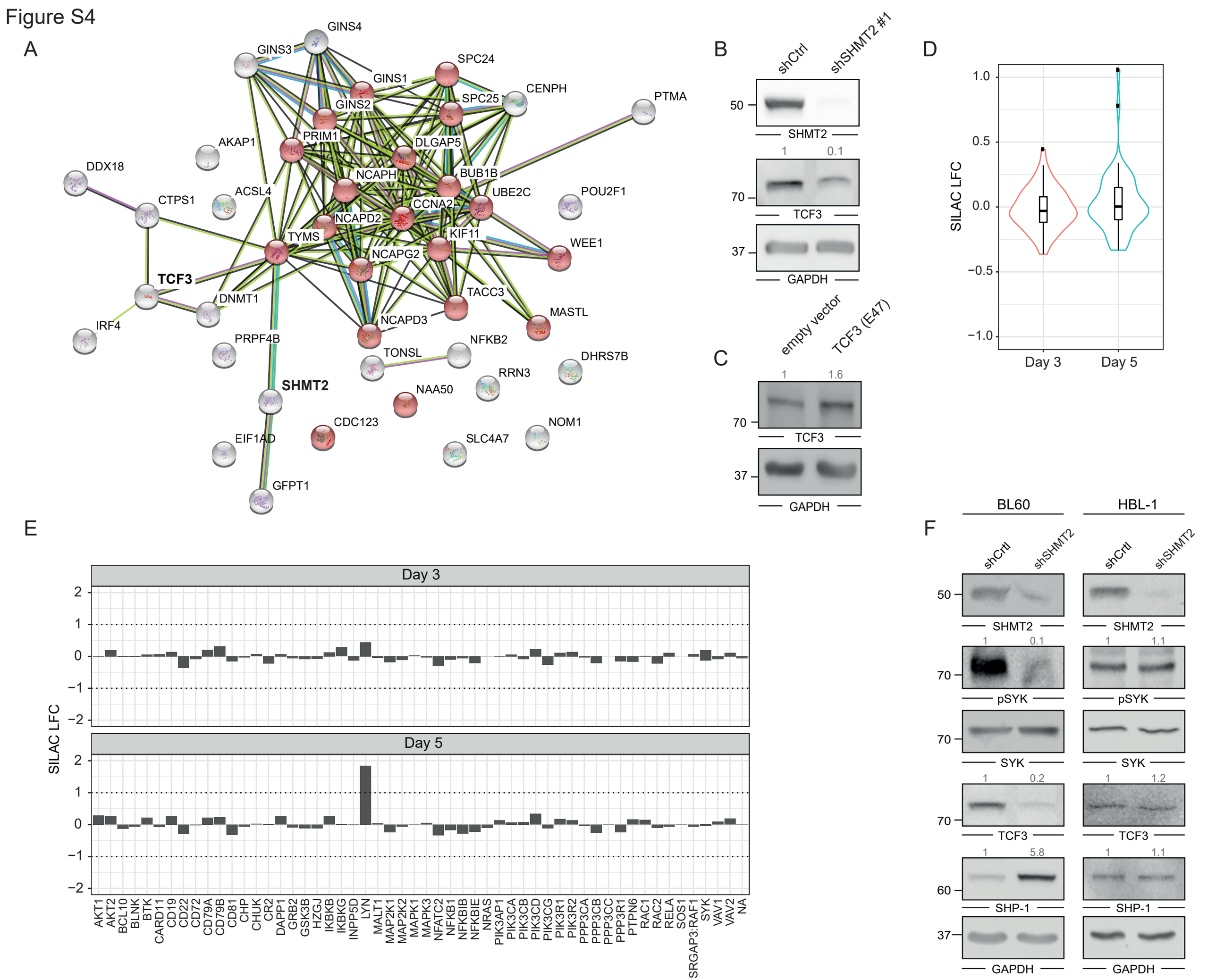

A
GINS4
GINS3
GINS1
GINS2
SPC24
SPC25
CENPH
PTMA
PRIM1
DLGAP5
AKAP1
DDX18
NCAPH
BUB1B
ACSL4
CTPS1
UBE2C
POU2F1
CCNA2
NCAPD2
TYMS
KIF11
WEE1
NCAPG2
TCF3
TACC3
DNMT1
MASTL
NCAPD3
IRF4
PRPF4B
NFKB2
TONSL
DHRS7B
RRN3
SHMT2
NAA50
CDC123
NOM1
EIF1AD
SLC4A7
GFPT1
B
shCtrl
shSHMT2 #1
50
SHMT2
1
0.1
70
TCF3
37
GAPDH
C
empty vector
TCF3 (E47)
1
1.6
70
TCF3
37
GAPDH
D
1.0
0.5
0.0
−0.5
−1.0
SILAC LFC
Day 3
Day 5
E
Day 3
Day 5
2
1
0
−1
−2
SILAC LFC
AKT1
AKT2
BCL10
BLNK
BTK
CARD11
CD19
CD22
CD72
CD79A
CD79B
CD81
CHP
CHUK
CR2
DAPP1
GRB2
GSK3B
HZGJ
IKBKB
IKBKG
INPP5D
LYN
MALT1
MAP2K1
MAP2K2
MAPK1
MAPK3
NFATC2
NFKB1
NFKBIB
NFKBIE
NRAS
PIK3AP1
PIK3CA
PIK3CB
PIK3CD
PIK3CG
PIK3R1
PIK3R2
PPP3CA
PPP3CB
PPP3CC
PPP3R1
PTPN6
RAC1
RAC2
RELA
SOS1
SRGAP3:RAF1
SYK
VAV1
VAV2
NA
F
BL60
HBL-1
shCrtl
shSHMT2
shCrtl
shSHMT2
50
SHMT2
SHMT2
1
0.1
1
1.1
70
pSYK
pSYK
70
SYK
SYK
1
0.2
1
1.2
70
TCF3
TCF3
1
5.8
1
1.1
60
SHP-1
SHP-1
37
GAPDH
GAPDH

Figure S5

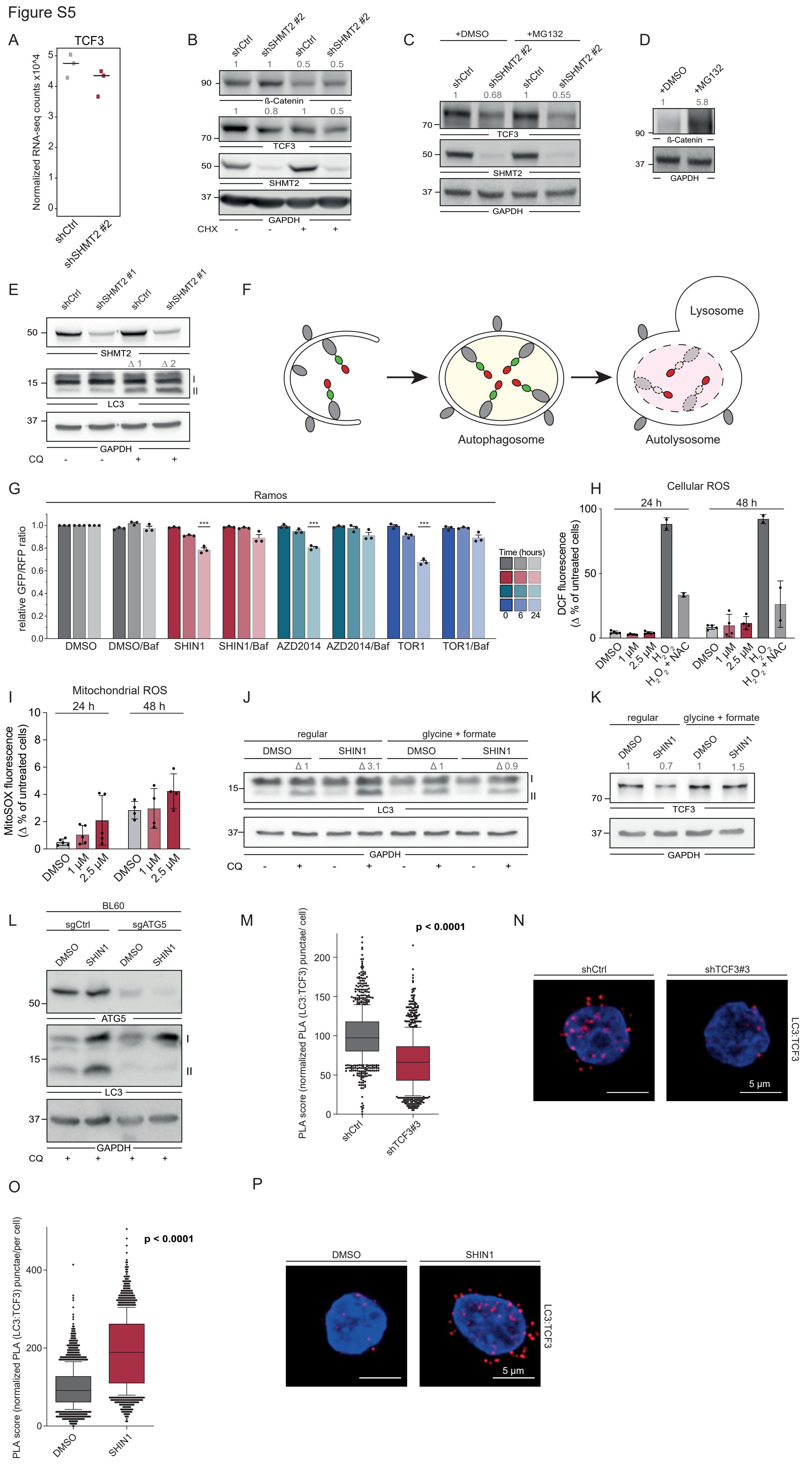

A
TCF3
Normalized RNA-seq counts x10^4
shCtrl
shSHMT2 #2
B
shCtrl
shSHMT2 #2
ß-Catenin
TCF3
SHMT2
GAPDH
CHX
C
+DMSO
+MG132
shCtrl
shSHMT2 #2
TCF3
SHMT2
GAPDH
D
+DMSO
+MG132
ß-Catenin
GAPDH
E
shCtrl
shSHMT2 #1
SHMT2
LC3
GAPDH
CQ
F
Lysosome
Autophagosome
Autolysosome
G
Ramos
relative GFP/RFP ratio
DMSO
DMSO/Baf
SHIN1
SHIN1/Baf
AZD2014
AZD2014/Baf
TOR1
TOR1/Baf
Time (hours)
H
Cellular ROS
24 h
48 h
DCF fluorescence (Δ % of untreated cells)
DMSO
1 µM
2.5 µM
H2O2
H2O2 + NAC
I
Mitochondrial ROS
MitoSOX fluorescence (Δ % of untreated cells)
J
regular
glycine + formate
DMSO
SHIN1
LC3
GAPDH
CQ
K
regular
glycine + formate
DMSO
SHIN1
TCF3
GAPDH
L
BL60
sgCtrl
sgATG5
DMSO
SHIN1
ATG5
LC3
GAPDH
CQ
M
p < 0.0001
PLA score (normalized PLA (LC3:TCF3) punctae/ cell)
shCtrl
shTCF3#3
N
shCtrl
shTCF3#3
LC3:TCF3
5 µm
O
p < 0.0001
PLA score (normalized PLA (LC3:TCF3) punctae/per cell)
DMSO
SHIN1
P
DMSO
SHIN1
LC3:TCF3
5 µm

Figure S6

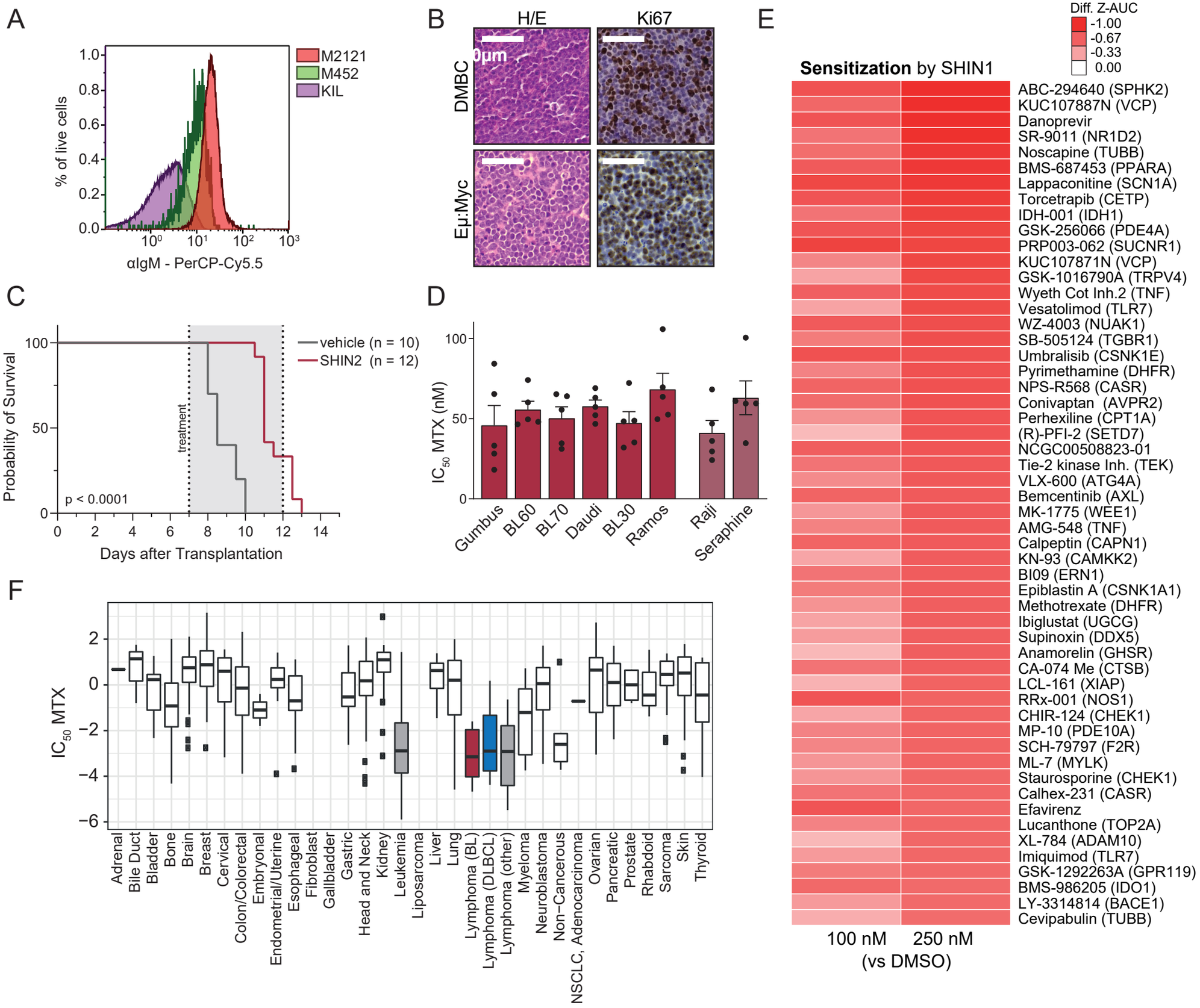

A
M2121
M452
KIL
% of live cells
αIgM - PerCP-Cy5.5
B
H/E
Ki67
DMBC
Eμ:Myc
C
vehicle (n = 10)
SHIN2 (n = 12)
treatment
p < 0.0001
Probability of Survival
Days after Transplantation
D
IC50 MTX (nM)
Gumbus
BL60
BL70
Daudi
BL30
Ramos
Raji
Seraphine
E
Diff. Z-AUC
-1.00
-0.67
-0.33
0.00
Sensitization by SHIN1
ABC-294640 (SPHK2)
KUC107887N (VCP)
Danoprevir
SR-9011 (NR1D2)
Noscapine (TUBB)
BMS-687453 (PPARA)
Lappaconitine (SCN1A)
Torcetrapib (CETP)
IDH-001 (IDH1)
GSK-256066 (PDE4A)
PRP003-062 (SUCNR1)
KUC107871N (VCP)
GSK-1016790A (TRPV4)
Wyeth Cot Inh.2 (TNF)
Vesatolimod (TLR7)
WZ-4003 (NUAK1)
SB-505124 (TGBR1)
Umbralisib (CSNK1E)
Pyrimethamine (DHFR)
NPS-R568 (CASR)
Conivaptan (AVPR2)
Perhexiline (CPT1A)
(R)-PFI-2 (SETD7)
NCGC00508823-01
Tie-2 kinase Inh. (TEK)
VLX-600 (ATG4A)
Bemcentinib (AXL)
MK-1775 (WEE1)
AMG-548 (TNF)
Calpeptin (CAPN1)
KN-93 (CAMKK2)
BI09 (ERN1)
Epiblastin A (CSNK1A1)
Methotrexate (DHFR)
Ibiglustat (UGCG)
Supinoxin (DDX5)
Anamorelin (GHSR)
CA-074 Me (CTSB)
LCL-161 (XIAP)
RRx-001 (NOS1)
CHIR-124 (CHEK1)
MP-10 (PDE10A)
SCH-79797 (F2R)
ML-7 (MYLK)
Staurosporine (CHEK1)
Calhex-231 (CASR)
Efavirenz
Lucanthone (TOP2A)
XL-784 (ADAM10)
Imiquimod (TLR7)
GSK-1292263A (GPR119)
BMS-986205 (IDO1)
LY-3314814 (BACE1)
Cevipabulin (TUBB)
100 nM
250 nM
(vs DMSO)
F
IC50 MTX
Adrenal
Bile Duct
Bladder
Bone
Brain
Breast
Cervical
Colon/Colorectal
Embryonal
Endometrial/Uterine
Esophageal
Fibroblast
Gallbladder
Gastric
Head and Neck
Kidney
Leukemia
Liposarcoma
Liver
Lung
Lymphoma (BL)
Lymphoma (DLBCL)
Lymphoma (other)
Myeloma
Neuroblastoma
Non−Cancerous
NSCLC, Adenocarcinoma
Ovarian
Pancreatic
Prostate
Rhabdoid
Sarcoma
Skin
Thyroid

Figure S7

A

ARID1A
R1722*
ARID
BAF250_C
0
400
800
1200
1600
2000
2285aa
CCND3
Q276*
Cyclin_N
Cyclin_C
0
100
200
292aa
ID3
V55fs
L64F
HLH
0
119aa
MYC
P17A
E54D
Myc_N
HLH
Myc-LZ
0
100
200
300
400
454aa

B

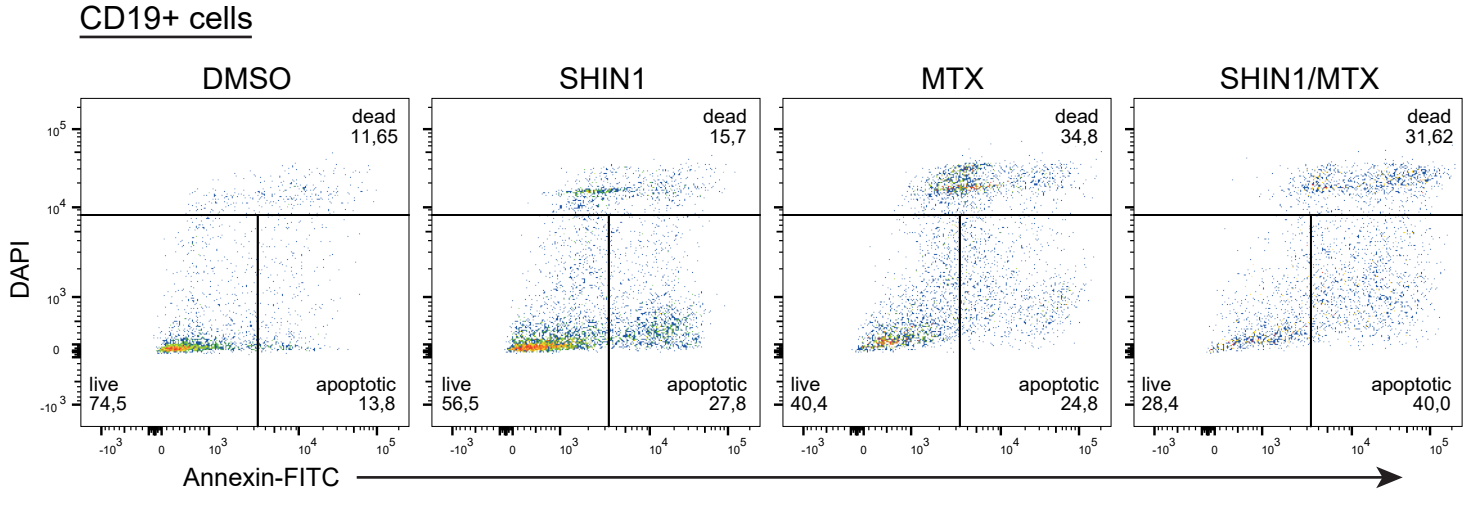
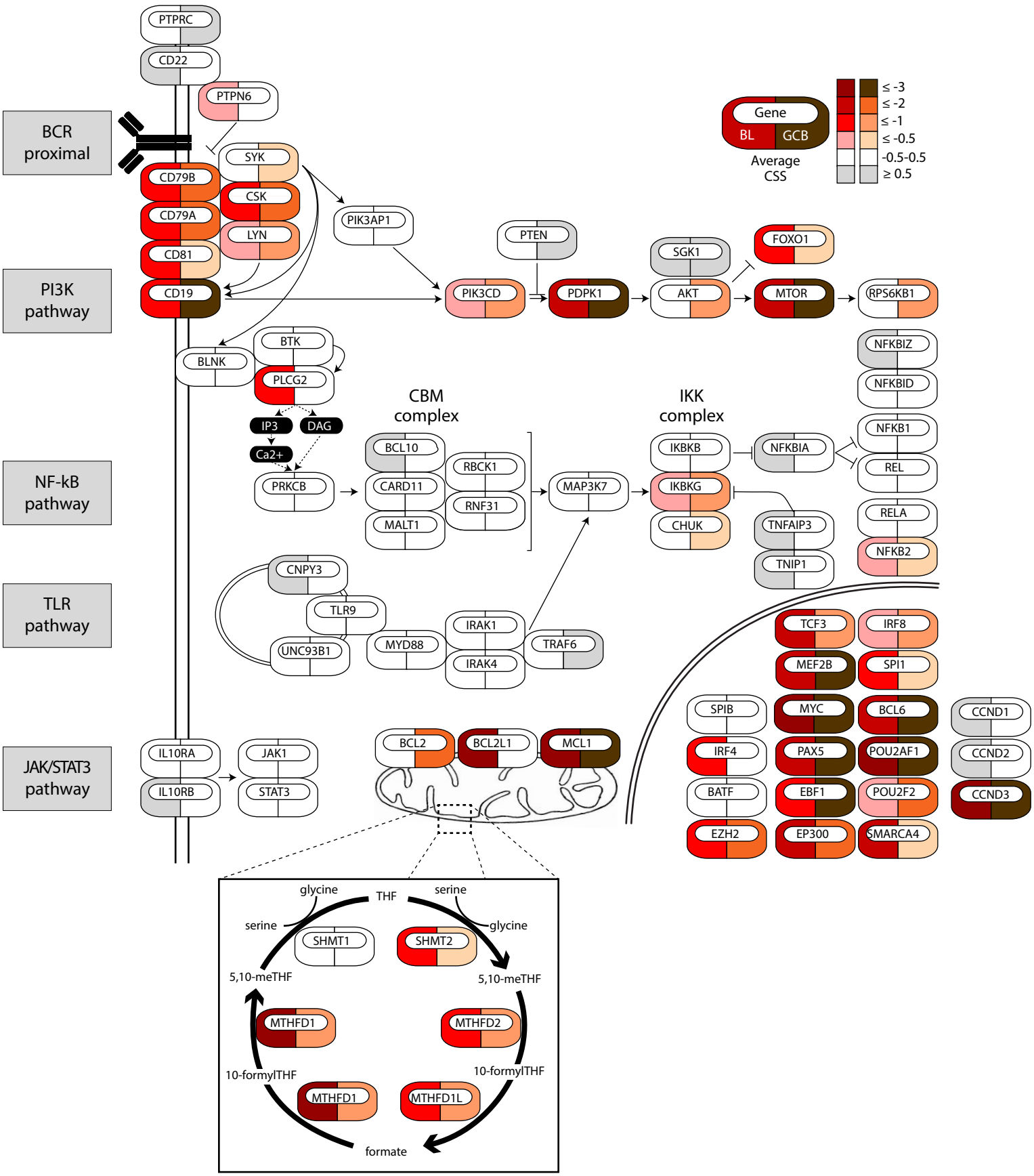

CD19+ cells
DMSO
SHIN1
MTX
SHIN1/MTX
DAPI
Annexin-FITC
dead 11,65
live 74,5
apoptotic 13,8
dead 15,7
live 56,5
apoptotic 27,8
dead 34,8
live 40,4
apoptotic 24,8
dead 31,62
live 28,4
apoptotic 40,0

Figure S8


BCR proximal
PI3K pathway
NF-kB pathway
TLR pathway
JAK/STAT3 pathway
PTPRC
CD22
PTPN6
SYK
CSK
LYN
CD79B
CD79A
CD81
CD19
PIK3AP1
PTEN
PIK3CD
PDPK1
SGK1
AKT
FOXO1
MTOR
RPS6KB1
BLNK
BTK
PLCG2
IP3
DAG
Ca2+
PRKCB
CBM complex
BCL10
CARD11
MALT1
RBCK1
RNF31
MAP3K7
IKK complex
IKBKB
IKBKG
CHUK
NFKBIA
TNFAIP3
TNIP1
NFKBIZ
NFKBID
NFKB1
REL
RELA
NFKB2
CNPY3
TLR9
UNC93B1
MYD88
IRAK1
IRAK4
TRAF6
IL10RA
IL10RB
JAK1
STAT3
BCL2
BCL2L1
MCL1
TCF3
IRF8
MEF2B
SPI1
SPIB
MYC
BCL6
IRF4
PAX5
POU2AF1
BATF
EBF1
POU2F2
EZH2
EP300
SMARCA4
CCND1
CCND2
CCND3
Gene
BL
GCB
Average CSS
≤ -3
≤ -2
≤ -1
≤ -0.5
-0.5-0.5
≥ 0.5
glycine
THF
serine
serine
glycine
SHMT1
SHMT2
5,10-meTHF
5,10-meTHF
MTHFD1
MTHFD2
10-formylTHF
10-formylTHF
MTHFD1
MTHFD1L
formate

Supplemental Figures: Titles and Legends

Figure S1: Essential genes in BL and ABC-DLBCL (supplemental information)

(A) Comparison of CRISPR screen scores (CSS) from two biological replicates for the indicated BL cell lines. R^2 values are shown. (B) (left) log2 fold change distribution of 1000 non-targeting sgRNAs (negative control) (right) CSS distribution for 1202 essential genes in 3 Burkitt lymphoma (BL) and 4 activated B-cell-like (ABC) diffuse large B-cell lymphoma cell lines. Data for HBL-1 and TMD8 were derived from CRISPR-Cas9 screen published by Phelan et al. Nature 2018 (C) Gene ranking based on CSS for the indicated BL cell line. Dashed lines correspond to CSS values of -0.5, 0 and 0.5. Gene dropouts (CSS values < -0.5) are shown in blue, while genes with CSS > 0.5 are shown in red. Selected genes are labeled. (D) Lymphoma cell line dependencies on one-carbon metabolism revealed by CRISPR-Cas9 screens. (E) Same as D, for the indicated ABC cell lines. (Data for HBL-1 and TMD8 derived from CRISPR-Cas9 screen published by Phelan et al. Nature 2018). (F) CSS values for one-carbon metabolism components for the indicated BL and ABC cell lines. Dashed lines correspond to CSS < -0.5. (G) Distribution of cell line dependency scores for SHMT2 in CRISPR/Cas9 screen in 789 cell lines (CERES scores, data from https://www.depmap.org/portal/) and two different RNAi screens in 712 cell lines (Demeter2 scores, combined screening data from Broad/Novartis/Marcotte, data from https://www.depmap.org/portal) and 397 cell lines (Demeter2 scores, DRIVE version 12 Novartis, McDonald et al., 2017)[31] respectively. Red dots denote B cell lines.

Figure S2: SHMT2 enzymatic activity is essential for BL cell growth and viability (supplemental information)

(A) Competitive growth assay of BL cell lines that were lentivirally transduced with either a constitutive shRNA vector targeting MTHFD1 or a non-targeting control shRNA (shCtrl) both expressed in combination with RFP as fluorescent reporter in co-culture with non-transduced wildtype cells. Percentages of RFP positive cells were measured by flow cytometry and values of MTHFD1 knockdown cells were normalized to the ones of the control shRNA expressing cells, n = 1. (B) Competitive growth assay of different BL wildtype cells and cells inducibly expressing either SHMT2-specific shRNA (shSHMT2#2) or non-targeting shRNA (shCtrl), n = 3-4, mean ± SEM is shown. The fluorescent marker GFP was co-expressed in transduced cells and monitored by flow cytometry. Data is shown as a ratio to control cells normalized to d1. (C) Competitive growth assay of BL60 wildtype cells and BL60 cells expressing either SHMT2-specific sgRNA or non-targeting sgRNA (sgCtrl). Data was normalized to d4 after transduction, n = 3, mean ± SEM is shown. Statistical comparison of d20 is significant in Student's t-test for both sgRNA compared to non-targeting sgRNA, $p < 0.001$. (D) Representative Western blot validation of CRISPR/Cas9-based SHMT2 knockout in BL60 cells. GAPDH served as loading control. A cropped blot of a representative experiment with quantification is shown. n = 5, for sgSHMT2#1 $p = 0.018$, for sgSHMT2#2 $p = 0.004$ in Student's t-test. (E) BrdU-based cell cycle analyses of the BL cell lines BL2 and Seraphine constitutively expressing an shRNA against SHMT2 (SHMT2 KD) or a non-targeting control shRNA (shCtrl). Analysis was performed on d5 after transduction. Mean ± SEM, n = 4, ** indicates $p < 0.01$ in Student's t-test, *** indicates $p < 0.001$ in Student's t-test. (F) Related to Figure 2E: Fold change of cleaved Caspase-3 and cleaved PARP levels normalized to total Caspase-3 and total PARP respectively in Western blot experiments, mean ± SEM, n = 4, * indicates $p < 0.05$ in Student's t-test, ** indicates $p < 0.01$ in Student's t-test, *** indicates $p < 0.001$ in Student's t-test. (G) Confocal

images showing the colocalization of SHMT2 and the mitochondrial marker COX4 in BL60 cells. SHMT2 was stained with Alexa Fluor 647 (red), COX4 was stained with Alexa Fluor 488 (green) and Nuclei were counterstained with DAPI (blue). The merge channel represents the composite image. Ectopically expressed SHMT2res and SHMT2res-K208A in the cells where the endogenous SHMT2 was knocked down show similar localization patterns as endogenous SHMT2 in the control cells (shCtrl + empty vector). n = 1. Scale bar: 10 µm. (H) Representative Western blot analysis of lysates derived from the subcutaneous BL60 cell tumors expressing doxycycline inducible shRNA against SHMT2 (shSHMT2#2) or a non-targeting control shRNA (shCtrl). Cells were transplanted into NOD/SCID mice and harvested as well as sorted for GFP expression on d10. β-Tubulin served as control. A cropped blot of a representative xenograft is shown. n = 3, p = 0.002 in Student's t-test.

Figure S3: The metabolic effect of SHMT2 inhibition (supplemental information)

(A) Different metabolites detected by LC/MS-based analysis in BL60 cells expressing doxycyclin inducible shCtrl versus shSHMT2, up to n = 6, median is shown. (B) Abundance of hypoxanthine and methionine detected by LC/MS-based analysis in BL60 cells expressing doxycyclin inducible shCtrl versus shSHMT2, up to n = 6, median is shown. (C) Abundance ratio of ADP/ATP, GSSG/GSH, Succinate/alpha-Ketoglutarate detected by LC/MS-based analysis in BL60 cells expressing doxycyclin inducible shCtrl versus shSHMT2, up to n = 6, median is shown. (D) Intracellular formate levels in BL60 cells upon SHIN1 treatment with 2.5 µM in comparison to DMSO treatment in enzyme-based colorimetric assay. n = 3, mean ± SEM is shown. ** indicates p < 0.01 in Student's t-test. (E) Cell viability assay (MTT assay) after 48 h of SHIN1 treatment and supplementation with indicated metabolites. 0.1 mM glycine and 0.3 mM serine corresponds to regular medium concentration. Nucleoside stock

solution contains 3 mM cytidine, guanosine, uridine, adenosine and 1 mM thymidine, diluted in the indicated percentages (1%, 2.5%, 5%). The final concentrations of the nucleosides are for 1%: 30 µM cytidine, guanosine, uridine and adenosine, and 10 µM thymidine; for 2.5% 75 µM cytidine, guanosine, uridine and adenosine, and 25 µM thymidine; for 5% 150 µM cytidine, guanosine, uridine and adenosine, and 50 µM thymidine. Data were normalized to medium control. n = 5, mean ± SEM is shown. Supplementation with 3.3 mM glycine and 2 mM formate lead to a significant rescue in Bonferroni posthoc test of cell viability at 10 µM SHIN1 in BL70.

Figure S4: TCF3 expression is dependent on SHMT2 function (supplemental information)

(A) STRING data-base derived interaction network of genes/proteins in BL60 cells that showed reduced protein expression levels after SHMT2 knockdown in the proteome analysis and that were identified as dependencies in the CRISPR-Cas9 screen (https://string-db.org/). shSHMT2#2 was used for the proteome analysis. Red labeled genes/proteins belong to the GO category ‘cell cycle’. (B) Representative Western blot analysis in BL70 cell line at d8 upon SHMT2 knockdown. GAPDH served as loading control. n = 3, a cropped blot of a representative experiment with quantification is shown. TCF3 $p < 0.001$ in Student’s t-test. (C) TCF3 levels shown in representative Western blot analysis in BL60 cell line transduced with a vector containing a constitutively expressed E47 sequence compared to the empty vector control. GAPDH served as loading control. A cropped blot of a representative experiment with quantification is shown. n = 4, $p = 0.043$ in paired t-test (D/E) Expression levels of BCR signaling proteins detected by LC/MS analysis at d3 and d5 upon inducible expression of SHMT2-specific shRNA (versus non-targeting shRNA). shSHMT2#2 was used for the proteome analysis. (F) Western blot analysis

of BL60 and HBL-1 cells expressing an shRNA against SHMT2 (shSHMT2#1 in BL60, #2 in HBL-1) or a non-targeting control shRNA (shCtrl) for TCF3, SHP-1 and the phosphorylation of the BCR effector SYK (Tyr525/526). pSYK and SYK were probed on different membranes, but samples were derived from the same experiment and blots were processed in parallel. GAPDH served as loading control. n = 4 for BL60, n = 3 for HBL-1, cropped blots of representative experiments are shown. $p < 0.001$ for pSYK in BL60 in paired t-test.

Figure S5: SHMT2 inhibition induces autophagic degradation of TCF3 (supplemental information)

(A) TCF3 mRNA levels detected by RNA-seq in inducible SHMT2 knockdown BL60 cells at d5 after induction of knockdown. shSHMT2#2 was used. Median is shown, n = 3. (B) Representative Western blot analysis of Cycloheximide (CHX) treatment of inducible SHMT2 knockdown in BL60 cells (shSHMT2#2) at d5 after induction. CHX treatment was performed for 3 h at a concentration of 25 µM. GAPDH served as loading control. A cropped blot of a representative experiment with quantification is shown. n = 3; p = 0.014 in Student's t-test. (C) Representative Western blots analysis in BL60 cells with inducible SHMT2 knockdown (versus control) (d5 upon induction of knockdown). Cells were treated for 5 h with either MG132 (5 µM) or DMSO. GAPDH served as loading control. A cropped blot of a representative experiment with quantification is shown. n = 4, for DMSO/shCtrl vs shSHMT2: p = 0.041, for MG-132/shCtrl vs shSHMT2 p = 0.025 in Student's t-test. (D) Representative Western blot analysis of BL60 wildtype cells showing accumulation of ß-Catenin upon MG132 treatment. A cropped blot of a representative experiment with quantification is shown. n = 4, for DMSO vs. MG-132 $p < 0.001$ in Student's t-test. (E) Representative Western blot analysis in Ramos cell line with constitutive knockdown of SHMT2 on

day 7 after transduction. LC3-I and LC3-II levels are shown, LC3-II has been normalized to LC3-I and difference between Chloroquine (CQ) treated and untreated samples are indicated to show autophagic flux (CQ treatment was performed for 4 h at 100 µM). GAPDH is shown as loading control. n = 3, $p < 0.01$ in Student's t-test. (F) Mode of functioning of the tfLC3 reporter to measure autophagic flux (compare also to Shoemaker et al., 2019). (G) GFP/RFP ratio of Ramos cells transduced with GFP-LC3-RFP-encoding vector upon treatment with SHIN1 at a concentration of 5 µM, AZD2014 at a concentration of 200 nM and Torin 1 (TOR1) at a concentration of 500 nM at different time points as indicated, a reduced ratio reflecting an increased level of autophagy. Bafilomycin A1 (Baf) treatment at 50 nM was used to inhibit autophagy. n = 3, mean ± SEM, $p < 0.0001$ in Tukey's multiple comparison test for TOR1, for AZD2014 and SHIN1 after 24 h. p = ns in comparison to DMSO control for rescue with Baf after 6 h and 24 h. (H) Levels of intracellular reactive oxygen species (ROS) determined by flow cytometry measurements upon staining with H_2DCFDA. Cells were treated with SHIN1 at indicated concentration for 24 h and 48 h and compared to DMSO control. H_2O_2 treatment with 50 µM served as positive control, rescue was performed with N-acetylcysteine pretreatment (5 mM for 1 h). n = 4, mean ± SEM is shown. (I) Levels of mitochondrial reactive oxygen species (ROS), determined by flow cytometry upon staining with MitoSOX™. Cells were treated with SHIN1 at indicated concentration for 24 h and 48 h and compared to DMSO control. n = 4, mean ± SEM is shown. Differences between SHIN1 treated and control (DMSO) cells were not statistically significant according to a Student´s t-test. (J) Western blot analysis in Ramos cell line treated with SHIN1 at a concentration of 5 µM for 48 h in regular medium and glycine and formate supplemented medium (+G/F). CQ treatment was performed for 5 ± 1 h at 100 µM. LC3-II has been normalized to GAPDH and difference between Chloroquine treated and untreated

samples are indicated to show autophagic flux. GAPDH is shown as loading control. n = 5. All samples were normalized to DMSO regular medium. $p < 0.01$ in paired t-test for DMSO vs. SHIN1 in regular medium, for SHIN1/ glycine+formate vs. DMSO/regular medium and vs. DMSO/ glycine+formate $p > 0.05$ (ns). (K) Western blot analysis in Ramos cells showing TCF3 levels after treatment with 5 µM SHIN1 for 48 h in regular medium and upon supplementation with glycine and formate (3.3 mM and 2 mM respectively). GAPDH served as loading control. n = 4, $p < 0.01$ in Student's t-test for TCF3 levels normalized to DMSO control in regular medium vs. glycine/formate supplementation. (L) Representative Western blot showing LC3 levels in ATG5 KO (sgATG5) compared to control sgRNA (sgCtrl) in BL60 cell line upon induction of knockout with 250 ng/ml doxycycline and 48 h of SHIN1 treatment at a concentration of 2.5 µM in regular medium vs DMSO control. Moreover, cells were treated with 100 µM chloroquine (CQ) for the last 4 h. GAPDH served as loading control. Cropped blots of a representative experiment are shown. n = 4; LC3-II level < 10% (mean 5.4% upon SHIN1/CQ treatment) after ATG5 KO, $p < 0.001$ in Student's test. (M) PLA score is shown for PLA of TCF3 and LC3 in TCF3 knockdown cells of BL60 cell line compared to non-target control shRNA 72 h after induction of shRNA expression. n = 3, box plots represent the median and 25th–75th percentiles, whiskers display 10–90 percentiles, and outliers are displayed as dots, $p < 0.0001$ in unpaired two-tailed Mann-Whitney test. (N) Representative confocal images from PLA for TCF3 and LC3 in TCF3 knockdown in BL60 cell line, as described in Figure S5M. Merged images represent the composite images of the PLA of TCF3 and LC3 (red) and the DAPI signal (blue). (O) PLA score is shown for PLA of TCF3 and LC3 in SHIN1 treated Ramos cells at a concentration of 5 µM for 17 h compared to DMSO control. n = 4, with n ≥ 359 single cells per condition. Box plots represent the median and 25th–75th percentiles whiskers display 10–90 percentiles,

and outliers are displayed as dots. p <0.0001 in two-tailed Mann-Whitney-test. (P) Representative confocal images from PLA for TCF3 and LC3 in Ramos cell line, as described in Figure S5O. Merged images represent the composite images of the PLA of TCF3 and LC3 (red) and the DAPI signal (blue).

Figure S6: Identification of drugs acting synergistically with an SHMT inhibitor (supplemental information)

(A) M2121 and M452 were stained for IgM without prior cell permeabilization to measure surface-IgM levels. The murine NK cell line KIL served as a negative control. (B) Exemplary hematoxylin/eosin and Ki67 stainings of Eμ:Myc and DMBC tumors. Scale bars represent 50 μm. (C) Rag1-deficient recipient mice were transplanted with 107 M2121 cells. Treatment with SHIN2 (200 mg/kg, i.p., twice daily) was initiated at day 7 and continued for 5 consecutive days (treatment period is marked grey). P-value was calculated by log-rank test. (D) IC_{50} of MTX in different BL cell lines measured by MTT assay. Cells were treated for 4 days. n = 4, mean ± SEM is shown. (E) Drugs that synergized with SHIN1 treatment in BL60 cells revealed by the “spiked in”, quantitative high-throughput drug screening (qHTS). (F) IC_{50} of MTX in 827 different cell lines (data derived from http://www.depmap.org/portal). Median is shown, whiskers show 1.5*IQR (interquartile range). Burkitt lymphoma (BL) are highlighted in dark red, diffuse-large B cell lymphoma in blue, other lymphoma and leukemia in grey.

Figure S7: Genetics and response to treatment of primary BL sample

(A) Mutation charts (lollipop plots) of non-synonymous mutations in BL sample for genes recurrently mutated in BL. Amino acid changes are visualized within the functional domains of the respective protein using MutationMapper (Cerami et al.

Cancer Discov 2012).[66] Sample corresponds to Fig. 6G and S7B. (B) Flow cytometry results from primary BL sample from a 27-year-old patient with Burkitt Lymphoma in bone marrow with ID3 mutations (L64F, V55fs), corresponding to bar diagram in Fig. 6G. CD19 positive cells are shown.

Figure S8: Essential genes in BL and GCB-DLBCL.

Icons indicate essential genes from CRISPR screens colored by the average CSS in BL (red) or GCB-DLBCL (orange/brown) lines. BL cell lines: BL60, RAJI, Ramos, GCB-DLBCL cell lines: DOHH2, SUDHL4, SUDHL5, WSU-DLCL2. Results for GCB DLBCL cell lines were previously published in Phelan et al. Nature 2018.[18]

Table S4: Candidate somatic mutations determined by Exome sequencing of primary BL Sample.

Shown are variants with population frequency > 0.01 % in the ExAC Non-Finnish European (NFE) database, > 10% variant allele frequency, and non-duplicated read count >20. Validation by consensus calling includes variants that were identified from at least two consensus reads containing different UMI and different genomic coordinates (see Suppl. Methods). Data correspond to sample shown in Fig. 6G, Fig. S7A and B.