Supplemental Methods:

Cells: Cell lines were obtained as follows: SUDHL-6, Toledo, Farage and HT from ATCC; WSU-DLCL2, Karpas422, SUDHL-4, SUDHL-5, SUDHL-8, DB and DOHH2 from DSMZ; OCI-Ly1, OCI-Ly7, OCI-Ly8 and OCI-Ly19 used in collaboration with Melnick lab. Cell typing was confirmed by short tandem repeat profiling (Idex Bioresearch, Westbrook, Maine). Cells were used within 3 months of thawing. OCI-Ly19 was cultured in RPMI-1640 media (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum, 10mM HEPES buffer and gentamicin 50ug/mL. OCI-Ly1, OCI-Ly7 and OCI-LY8 were cultured in IMDM media (Caisson) supplemented with 20% heat inactivated fetal bovine serum and gentamicin 50ug/mL. All other cell lines were cultured in RPMI-1640 media (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum and gentamicin 50ug/mL (Sigma Aldrich). Cells were maintained at 37˚C and 5% CO_2.

Viability Assays: Cell viability was determined using an ATP-based luminescent assay (CellTiter-Glo, Promega) and the GloMax® Multi+ detection system (Promega). Drug dosing was opimtimized for each cell line based on IC50 values to approximate 50% killing. In combination and synergy experiments, cells were exposed to tazemetostat or vehicle for days 0-8 and to venetoclax or vehicle for days 6-8. Drug and vehicle were replaced with fresh media every 72 hours. For synergy experiments CompuSyn software (Biosoft) was used to plot dose effect curves and calculate CI value.

SUDHL-6 Xenografts: Six to eight-week-old NOD-SCID mice were injected subcutaneously in the flank with $1x10^7$ SUDHL-6 cells in PBS. Treatment began when

tumors reached 200-300 mm^3. Mice were randomized to treatment with vehicle, venetoclax 100mg/kg PO daily, tazemetostat 300mg/kg PO BID or the combination, with 5 mice in each cohort. Venetoclax was administered PO once daily at 100mg/kg in 5% DMSO, 50% PEG-300, 5% Tween-80 in dH_2O by oral gavage. Tazemetostat was administered PO BID at 300mg/kg in 0.5% NaCMC, 0.1% Tween-80 in dH_2O by oral gavage. Tumors were measured by caliper three times weekly. Mice were humanely sacrificed when tumors reached 2000mm^3. Blood for CBC and CMP were collected by terminal bleed. Mice were treated until sacrifice or for 28 days total. Surviving animals were monitored three times weekly for tumor recurrence. All procedures performed were accordance with Weill Cornell Medical College IACUC.

PDX xenografts: Six to eight-week-old NSG mice were implanted subcutaneously in the flank with tumor fragments from a previously characterized DLBCL PDX[1]. Tumor formation was monitored weekly by inspection and calipers. Treatment began when tumors reached 200-300 mm^3. Mice were randomized to vehicle, venetoclax, tazemetostat or combination groups with 5 mice in each cohort. Drug dosing and delivery was as described above. Mice were treated for 21 days total. Tumors were measured by caliper three times weekly. MRI was performed at Day 19, 34, 47, 68 and 92 to assess size of tumors immeasurable by caliper. Mice were humanely sacrificed when tumors reached 3000mm^3. Tumor confirmation of EZH2 mutation was performed by Genewiz (South Plainfield, NJ). All procedures performed were in accordance with Cornell University IACUC.

MRI imaging of mice: Animal subjects underwent magnetic resonance imaging (MRI) using a 1T M3 compact MRI from Aspect Imaging Ltd. Subjects were anesthetized using 2.5% isoflurane and placed onto the specimen arm, the coil was then slipped around the subject and fixed in place. Body temperature was maintained using heated water that is recirculated in the specimen arm. Body temperature and respiration was monitored during the imaging session. Subjects were scanned using a T2 weighted scan without contrast agent, slice thickness was set to 1mm, with an inter-slice gap of 0mm, a total of 20 slices were taken for a total scan time of six minutes. Total time under anesthesia was 10 minutes. Raw image DICOMS were exported from MR system and imported into VivoQuant image analysis software by Invicro, a Konica Minolta Company. A tumor region of interest layer was created and using automatic thresholding settings and a thickness of 5; the tumor was followed and highlighted throughout the image stack. Minor, manual modifications were made to the automatic tracing of the tumor when extraneous anatomy was included in the tumor region of interest. Once the tracing is complete a 3-dimensinal render is created and outputted into an animated GIF format.

Lymphoma Organoids: OCI-Ly1 was cultured in IMDM media with 20% FBS and 1% Pen/Strep until ready for use. Live cells were counted based on trypan blue exclusion using a hemocytometer. $2.0x10^4$ DLBCL cells were encapsulated in polyethylene glycol-maleimide (PEG-MAL) organoids, as previously reported [2-5]. Organoid composition consisted of 7.5% w/v 4-arm PEG-MAL crosslinked by 2.4 mM dithiothreitol and 2.4 mM matrix metalloproteinase-degradable peptide sequence VPM (GCRD**VPM**SMRGGDRCG)[4,6]. Prior to gelation, PEG-MAL was functionalized with 3.2

mM adhesive peptide sequence RGD (GRGDSPC) by Michael-type addition chemistry[4,6] in HEPES buffer (1% v/v 1M HEPES in PBS++, pH 7.4). PDX organoids were generated from PDX tumors excised when tumors reached 2cm in size. Tumors were reduced to single cell suspensions by mechanical disruption of the tissues through a 70 mciron cell strainer in fresh RPMI media with 20% FBS, 1% Glutamax (Life Technologies), and 1% Pen/Strep. Live cells were counted based on trypan blue exclusion using a hemocytometer. 2.0x10^5 PDX cells were encapsulated with 5.0x10^4 CD40L expressing L stromal cells in polyethylene glycol-maleimide (PEG-MAL) organoids. Organoid composition consisted of 7.5% w/v 4-arm PEG-MAL crosslinked by 2.44mM dithiothreitol and 2.44mM matrix metalloproteinase-degradable peptide sequence VPM (GCRD**VPM**SMRGGDRCG). Prior to gelation, PEG-MAL was functionalized with 3.25mM adhesive peptide sequence RGD (GRGDSPC) by Michael-type addition chemistry in HEPES buffer (1% v/v 1M HEPES in PBS++, pH 7.4).

Both OCI-Ly1 and PDX organoids were cultured for 24 hours post-fabrication in media. After 24 hours of culture, media was removed and replaced with new media containing media for an untreated control, vehicle alone, or single active agents. 0.1% DMSO was added to vehicle-only and venetoclax-only organoids while tazemetostat-only and combination treatment groups received 5μM tazemetostat in 0.1% DMSO in media. Drug and vehicle were replaced every 48 hours. Combination group received 6 days of tazemetostat followed by treatment with 50nM venetoclax for the final 2 days. Venetoclax-only group received 50nM venetoclax for the final 2 days after 6 days in vehicle only conditions. Following completion of drug series, organoids were degraded with 125U/mL

Collagenase type I in serum-free media for 1 hour at 37˚C. Collagenase digestion was neutralized using equal volumes of FACS buffer (2% FBS in PBS++ with 0.25nM EDTA) then passed through a cell strainer to remove leftover hydrogel debris.

Flow cytometry: Cells from individual organoids were stained for live and dead populations. Briefly, cells were washed with PBS-/- and centrifuged at 300x G for 5 minutes. After washing, cells were stained for live and dead populations using far-red Live/Dead stain kit (Thermo cat. No. L34974) at 1:1000 dilution in PBS-/- for 1 hour on ice in the dark. Following incubation, cells were washed 2x in FACS buffer to remove staining solution. Cells were resuspended in FACS buffer and analyzed using a BD Accuri C6.

Immunofluorescence: Separate organoid samples that had undergone the drug treatment regimen were reserved for imaging. Organoids were washed in PBS-/- to remove cells escaped from organoid and stained in 4nM Calcein AM and 4nM Ethidium Homodimer in media for 45 minutes at 37˚C. After incubation, the staining solution was replaced with fresh media and directly imaged for live (green) and dead (red) populations, respectively, at 2x magnification on a Nikon TE200U microscope.

BH3 profiling: BH3 profiling was performed as described previously[7]. Cells were permeabilized with 0.001% digitonin suspended in MEB2 buffer (150 mM mannitol, 10 mM HEPES-KOH pH 7.5, 150 mM KCl, 1 mM EGTA, 1 mM EDTA, 0.1% BSA, 5 mM succinate) and treated with different BH3- only peptides in 384-well plate. After 60mins

incubation at 25°C, cells were fixed with 4% formaldehyde for 10 mins at RT followed by neutralization by adding N2 buffer (1.7 M Tris base, 1.25 M glycine, pH 9.1). Cells were stained overnight at 4°C with Hoechst 33342 (H3570, Invitrogen) and anti–cytochrome *c*–Alexa Fluor 488 (6H2.B4/612308). The rate of loss of cytochrome c was analyzed using Intellicyt iQue flow cytometer in response to each BH3 peptide. Assay were conducted in triplicates. DMSO and alamethicin were used as negative and positive control respectively for cytochrome c release.

Analysis of RNA-seq data: RNA-seq profiles of a panel of DLBCL cell lines (n=26) treated with vehicle vs. EZH2 inhibitor were obtained and log2 fold changes were calculated for each respective cell line. Heat map of log2 expression changes was generated using the gplots package in R (Gregory R. Warnes, Ben Bolker, Lodewijk Bonebakker, Robert Gentleman, Wolfgang Huber Andy Liaw, Thomas Lumley, Martin Maechler, Arni Magnusson, Steffen Moeller, Marc Schwartz and Bill Venables (2019). gplots: Various R Programming Tools for Plotting Data. R package version 3.0.1.1. https://CRAN.R-project.org/package=gplots). Significance was determined using a paired Wilcoxon signed-rank test.

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Supplemental Tables and Figure Legends

Supplemental Table 1: EZH2 and BCL2 molecular alterations in DLBCL cell lines

Cell Line	EZH2	BCL2 Translocation	BCL2 Mutations
SUDHL-6	Y646N	t(14;18)	T-755C, G-660A, C-571T, C-489T, T-441C, T-440C, A-432G, +G -425, A-416G, C-413G, C-411T, C-357T, A-355G, C-322T, G-291C, C-284G, D13bp -190-178, G-139A, A-77G, T-76C, T-16C, T113A, A145C, G191A, A290C, C328G, T354A, A361G, A372T, G377A, A379T, T406G, A433G, G449C, C457A, A489G, A518C, T533A, T618G, A712G, C734G, A735T, G738T, A856T, G927A, G930A, C1099T, C1343T, T1395C, DA stretch 1410-3, G1599T, C1600T, G1605A, C1609T, G2470A[1]
WSU-DLCL2	Y646S	t(14;18)	C-562T, A-17T, G6C, C185G, G678A, G679A, G724A, C1329G, A1801G[1]
OCI-Ly1	Y641N	t(14;18)	D7bp -460-454, C-445T, C-444T, G-436A, G-427A, '+C stretch -411-407, C-401T, T-397C, A-377T, T-356C, A-334G, G-330T, T-320C, C-311G, T-300C, C-290T, A-289T, T-286A, G-285A, C-239T, C-222G, G202A, G-180C, T-178G, G-161A, C-160A, A-137G, A125G, T-96A, T-46C, G-39C, A10G, G83A, C99T, A119T, G128T, A130T, A141C, G149C, G191A, A200C, T203A, A210G, G231C, A254G, G261A, T289C, A290T, T295G, T322A, T334C, T357C, C360G, G370A, A400T, T424C, G428C, T431C, C462G, T470C, A506T, A532G, T618A, G620A, G624C, C640T, T641A, G659A, G663A, G666C +/-, D14bp 685-98, A711G, G729A +/-, T744C, T774C, C848T +/-, T859C, C975T, C1106T, C1197T, C1274T, A1383T, A1384C, A1408G, C1442A, A1509G, G1513C, T1515C, G1517A, T1522A, G1524A, G1549C, A1559G +/-, G1570C, T1571A, T1575G, A1579T, G1588A, G1599C, C1634T +/-, T1669C, T1670C, C1727T, C1731T, A1759G, A2016G, T2377C, C2554T[1]
Karpas422	Y641N	t(14;18)	T618G, A712G, C734G, A735T, G738T, A856T, G927A, G930A, C1099T, C1343T, T1395C, DA stretch 1410-3, G1599T, C1600T, G1605A, C1609T, G2470A[1]
SUDHL-4	Y641S	t(14;18)	D31bp -466-436, A-435C, G-347A, C-307T, T-288C, C-219T, T-216A, C-207T, T88G, T100G, T153G, T195C, T203A, C272T, C309T, A335G, C336G, C447G, C457A, D4bp 469-472, C590T, C621G, C634T, G714T, A849G, C867G, C889A, C1065G, A1509T, A1535C, A1551T, A1579T, C1580G, C1581T, C1583T, A1642C, G1704C, A1828G[1]
DB	Y641N	t(14;18)	L119L, N172N, out of frame mutation[2]
DOHH2	WT	t(14;18)	out of frame mutation[2]
OCI-Ly19	WT	t(14;18)	E13D, L23L, V35M, A42V, L181L, N182, L185L, out of frame mutation[2]
Toledo	WT	t(14;18)	L181L[2]
OCI-Ly8	WT	t(14;18)	T-47C, C71G, C72T, C99G, A242G, A317C, G370A, C497T, C498T, G753A, G780A, G895A, G967A, C997G, G1071A, G1100A, G1224A, G1303A, G1328C, T1385A, G1391A, T1409G, C1461T, C1514T, G1543A, G1599C, G1646C, A1672G, T1684G, G1924A, G2178A, G2312T, G2428A, G2522A, G2643A, T3057C, C3078G[1]
Farage	WT	t(14;18)	WT[2]
OCI-Ly7	WT	WT	homozygous deletion[3]
SUDHL-5	WT	WT	WT[2]
SUDHL-8	WT	WT	WT[2]
HT	WT	WT	WT[2]

Supplemental Table 2: Tazemetostat and venetoclax doses used in combination experiments (Figure 1A)

Cell Line	Tazemetostat Dose (nM)	Venetoclax Dose (nM)
SUDHL-6	50	100
WSU-DLCL2	500	100
OCI-Ly1	5000	50
Karpas422	250	5000
SUDHL-4	4000	5000
DB	500	3000
DOHH2	5000	5
OCI-Ly19	5000	10
Toledo	5000	50
OCI-Ly8	5000	10
Farage	500	100
OCI-Ly7	3000	100
SUDHL-5	4000	100
SUDHL-8	5000	500
HT	1000	5000

Supplemental Table 3: Cell line specific fixed ratios and dose ranges used in synergy experiments (Figure 1B).

Cell Line	Fixed Ratio (Taz:Ven)	Tazemetostat Dose Range (nM)	Venetoclax Dose Range (nM)
SUDHL-6	1:2	25–800	50–1600
WSU-DLCL2	1:2	25–800	50–1600
OCI-Ly1	100:1	2560–7812.5	25.6–78.125
Karpas422	1:12.5	25–800	312.5–10000
SUDHL-4	1:1	3250–10000	3250–10000
DB	5:1	125–4000	25–800
DOHH2	1000:1	3250–10000	3.25–10
OCI-Ly19	400:1	3250–10000	8.125–25
Toledo	100:1	3250–10000	32.5–100
OCI-Ly8	400:1	3250–10000	8.125–25
Farage	5:1	62.5–2000	12.5–400
OCI-Ly7	30:1	888–6750	29.63–225
SUDHL-5	40:1	3250–10000	81.25–250
SUDHL-8	100:1	3250–10000	32.5–100
HT	1:2.5	125–4000	312.5–10000

Supplemental Figure Legends:

Figure S1: Molecular Characteristics of DLBCL PDX. A) Sanger sequencing of EZH2; B) Fluorescence in situ hybridization of PDX tumor using the spectrum green labeled IGH probe and spectrum orange labeled BCL2 probe (Vysis/Abbott Molecular Inc., Des Plaines, IL). Red arrows: fusion signals indicating the presence of IGH-BCL2 gene rearrangement; yellow arrows: normal alleles of IGH (green signal) and BCL2 (red signal). Image taken at 100X magnification on a Nikon-Eclipse E-800 microscope.

Figure S2: BH3 profiling in DLBCL PDX cells: cytochrome release of PDX cells exposed to BH3 peptides at the doses listed. PDX tumor cells were separated from stromal cells by CD20 flow sorting. A) PDX with *EZH2* mutation and *BCL2* translocation PDX. B) PDX with WT *EZH2* and *BCL2* . *p<0.05, **p<0.01, ****p<0.0001. Veh, Vehicle; Taz, Tazemetostat.

Figure S3: Body weight (g) in SUDHL-6 (A) and PDX xenografts (B). Error bars represent SEM.

Figure S4: MRI Imaging in PDX mice. MRI 3D renderings of PDX mice taken the indicated timepoints. Images obtained using a 1T M3 compact MRI (Aspect Imaging Ltd) with T2 weighted scan without contrast. Red: tumor area of interest as determined by automatic thresholding settings.

Figure S5: Video of 3D MRI renderings from PDX mice. Representative images shown from reach cohort.

References:

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Figure S1

A

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ATC TCA GAA **A**AC TGT GGA GAG

CDS Position
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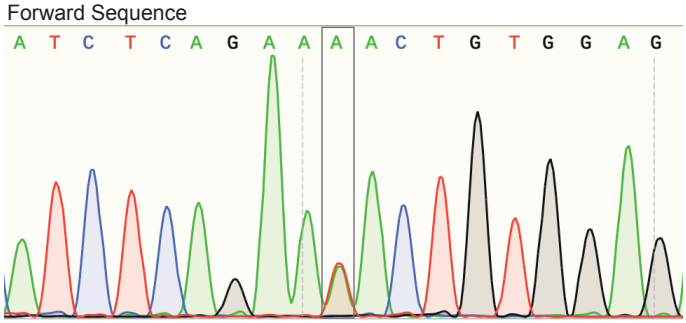
Forward Sequence



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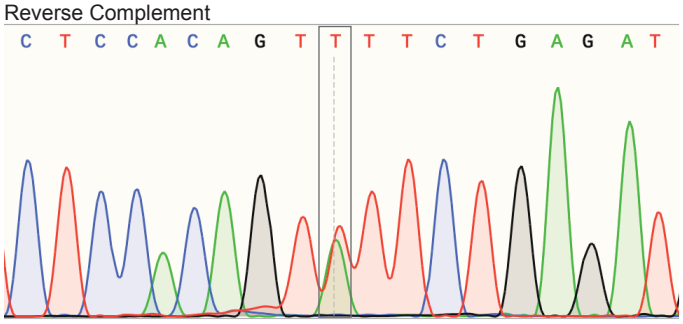

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B

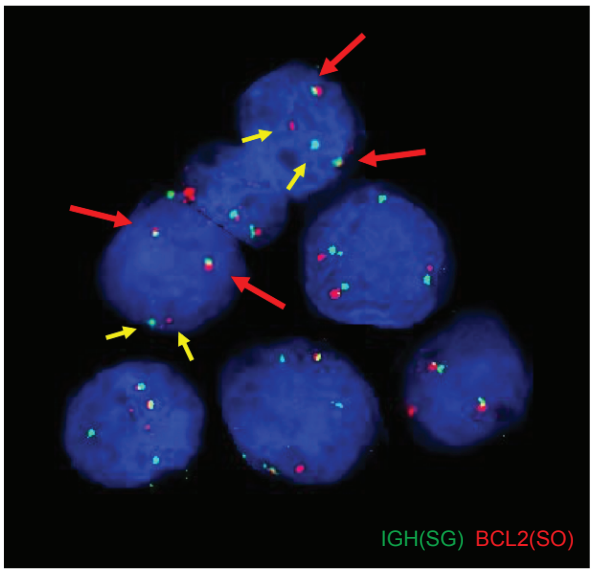
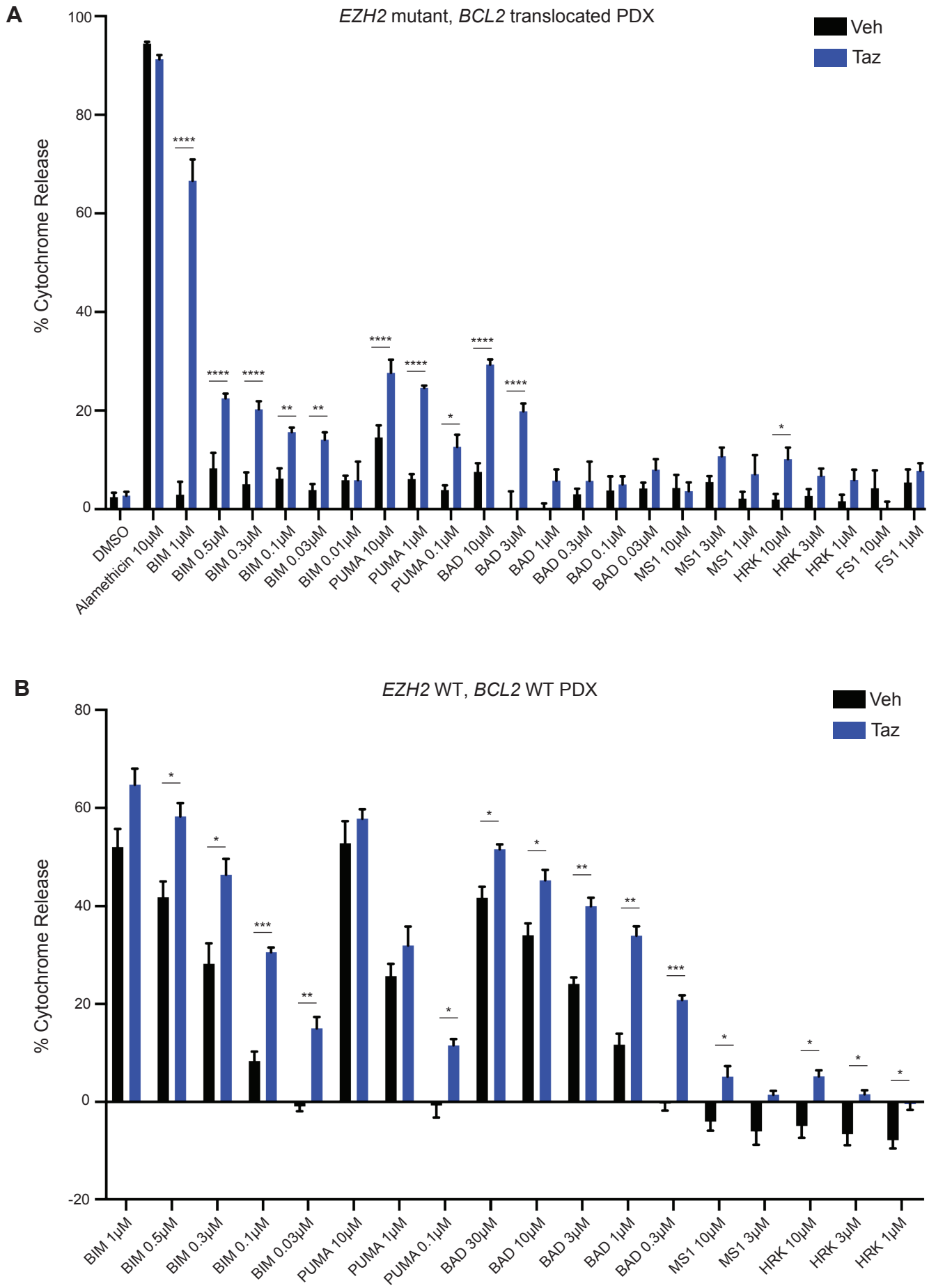


Figure S2


A
EZH2 mutant, BCL2 translocated PDX
Veh
Taz
% Cytochrome Release
100
80
60
40
20
0
DMSO
Alamethicin 10μM
BIM 1μM
BIM 0.5μM
BIM 0.3μM
BIM 0.1μM
BIM 0.03μM
BIM 0.01μM
PUMA 10μM
PUMA 1μM
PUMA 0.1μM
BAD 10μM
BAD 3μM
BAD 1μM
BAD 0.3μM
BAD 0.1μM
BAD 0.03μM
MS1 10μM
MS1 3μM
MS1 1μM
HRK 10μM
HRK 3μM
HRK 1μM
FS1 10μM
FS1 1μM
B
EZH2 WT, BCL2 WT PDX
Veh
Taz
% Cytochrome Release
80
60
40
20
0
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BIM 0.3μM
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BIM 0.03μM
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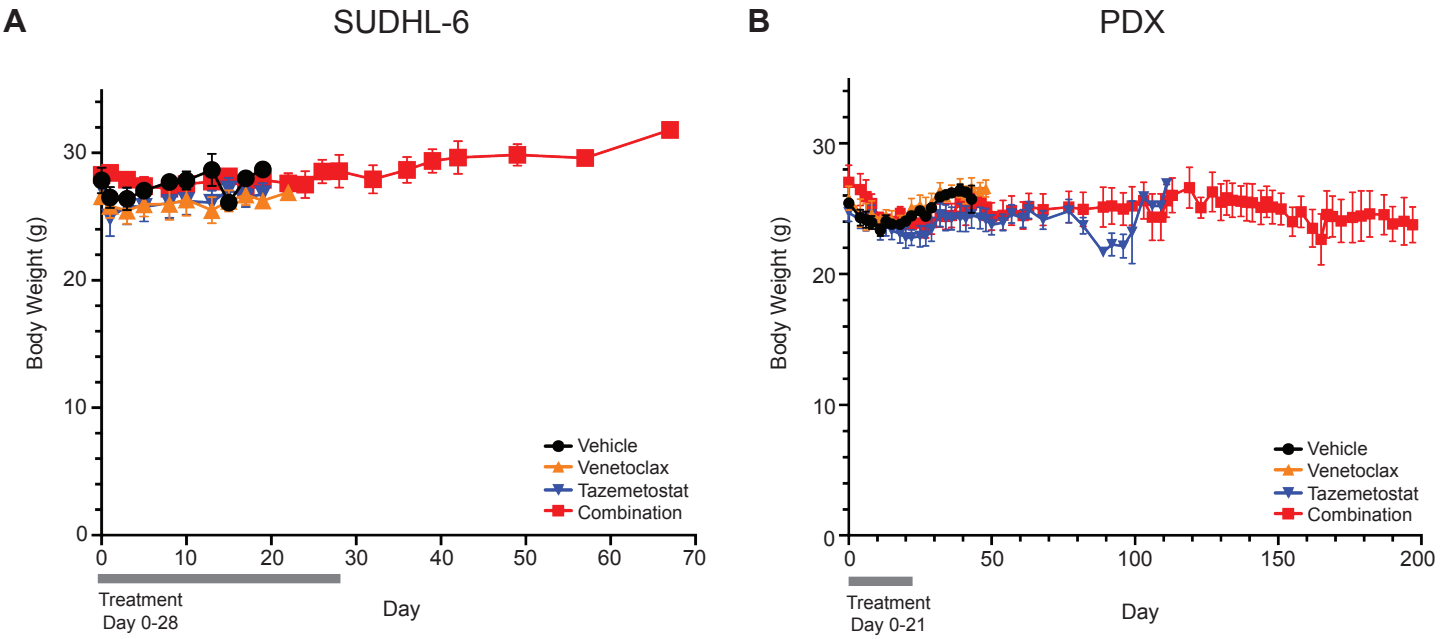
Figure S3


A
SUDHL-6
B
PDX
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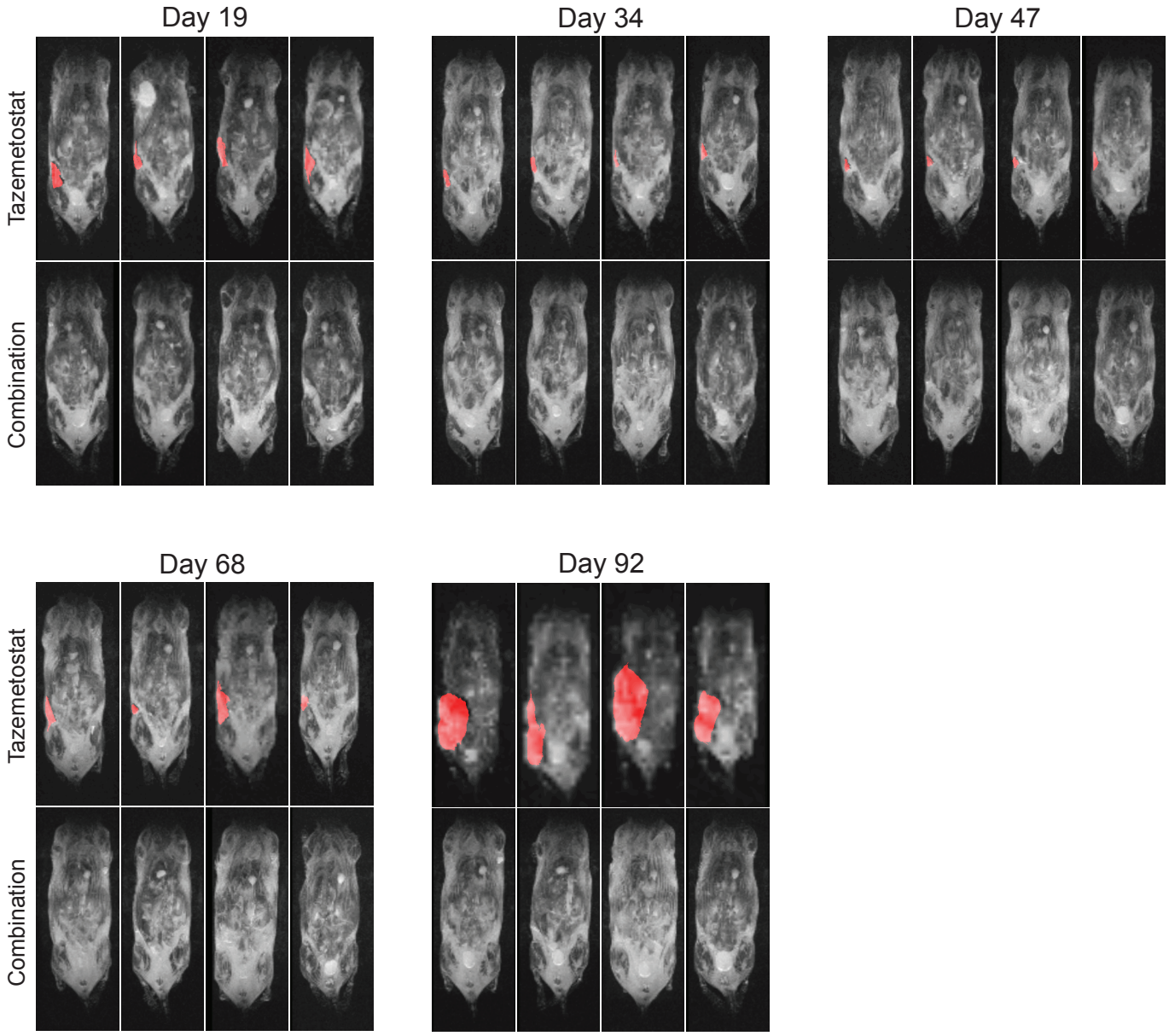
Venetoclax
Tazemetostat
Combination
Treatment
Day 0-28
Treatment
Day 0-21
Day

Figure S4


Day 19
Day 34
Day 47
Day 68
Day 92
Tazemetostat
Combination
Tazemetostat
Combination