Supplemental Methods:

Cell culture, immunoblot, immunohistochemistry and reagents:

Kem I, Rael, and Mutu I were obtained from Wayne Tam in 2017. Kem III and Mutu III were obtained from Ben Gewurz in 2017. Daudi, Raji, and Ramos were purchased from American Type Culture Collection (ATCC) in 2013. Jiyoye was purchased from ATCC in 2014. LCL9001 was obtained by infection of peripheral blood lymphocytes with EBV. Cells were used within 3 months of thawing. Cell typing was confirmed by short tandem repeat profiling performed by Idex Bioresearch (Westbrook, Maine). Cells were cultured in RPMI-1640 media (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum and gentamicin 50ug/mL (Sigma Aldrich). Drugs were obtained from vendors as follows: Decitabine (Selleckchem), 5-azacytidine(Selleckchem), and EPZ-6438 (Selleckchem), BEZ-235 (Selleckchem), Cladribine (Selleckchem), Cytarabine (Selleckchem), EPZ-011989 (Epizyme), EPZ-6438 (Selleckchem), Ganetespib (gifted from Leandro Cerchietti), GDC-0032 (gifted from Lewis Cantley), NSCE (Cornell Chemistry Core), Obatoclax (Selleckchem), PU-H71 (gifted from Gabriella Chiosis), Venetoclax (Selleckchem), GSK-126 (GlaxoSmithKline), doxorubicin (Selleckchem), BYL-719 (provided by Lewis Cantley). Cell viability was determined using an ATP based luminescent assay (CellTiter-Glo, Promega) and the GloMax® Multi+ detection system (Promega). IC50 values were calculated using Prism 6 software.

Immunoblot was performed with the standard procedure using the following antibodies: β-actin (GeneTex), BZLF1 (Santa Cruz), EBNA1 (Santa Cruz), EBNA2 (AbCam), EBNA3C (gift from Benjamin Gewurz), GAPDH (GeneTex), and LMP1 (AbCam).

Cell blocks were generated from cell lines in suspension by fixation in 10% formalin. Immunohistochemistry on cell blocks and mouse tumors was performed with the following antibodies: EBNA2 (abcam #ab90543), LMP1 (Dako #M0897), BZLF1 (Santa Cruz #SC-53904), CD8 (Leica #PA0183), PD-1 (Dako #M3653), PD-L1 (Cell Marque #315M-98). The Halo® image analysis software program (Indica Labs) was used to quantify immunohistochemical stains.

qRT-PCR: RNA was extracted using the Direct-zol RNA kit (Zymo Research). DNase-treated total RNA was reverse transcribed with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Reactions were performed in triplicate and the change in threshold cycle number (ΔCt) was calculated for each sample, normalized to a housekeeping gene (*GAPDH*). The ΔCt in drug treated cells was normalized to the ΔCt in vehicle treated cells to obtain $\Delta\Delta$Ct. Fold change in mRNA levels was calculated as 2^($\Delta\Delta$Ct).

High throughput drug screen: Kem I cells were incubated with drug in 96-well format for 48 hours, then washed and resuspended in TRI Reagent (Zymo Research). RNA was extracted using the Direct-zol-96 RNA kit (Zymo Research). DNase-treated total RNA was reverse transcribed with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). qRT-PCR was performed as described above.

EBV-CTLs and Cr release assay:

EBV-CTLs are polyclonal lines generated from peripheral blood mononuclear cells separated by low density separation from peripheral blood of normal consented donors by stimulation with autologous B cells transformed with B95.8 Epstein Bar Virus as previously described [1,2] After 4 weeks of culture in Yssel's medium supplemented with 5% human AB serum in the presence of IL2 (50Un/ml) and weekly re-stimulations with autologous EBV BLCLs; the T cells were characterized for their EBV specificity and HLA restriction in a standard Cr51 release assay against both autologous and a panel of EBV-positive and EBV negative targets each matching one-two HLA alleles expressed by the CTL donor.

EBV-CTLs were tested and found to be cytotoxic against autologous EBV-BLCLs but not autologous dendritic cells (DCs) or PHA blasts. Their restriction by HLA A0201 was established by their cytotoxic activity against allogeneic EBV-BLCLs sharing HLA A0201 but not against other EBV-BLCLs in the panel sharing other HLA alleles with the T-cell donor, nor with PHA blasts or DCs from the same allogeneic HLA A0201+ donor. These

characteristics indicate that the EBV-CTLs are EBV-specific and HLA A0201-restricted, and that they are depleted of reactivity against allogenic cells

The EBV proteins targeted by the EBV-CTL lines in Figures 7A and 7C were assessed by measuring their cytotoxicity against autologous DCs or PHA blasts loaded with pools of overlapping 15-mer peptides spanning the sequences of individual EBV proteins: LMP-1, LMP-2, (latency II) and EBNA 3A, EBNA 3B and EBNA 3C (latency III). The EBV-CTL line in Figure 7A was HLA A0201 restricted and was cytotoxic only against autologous DCs loaded with peptides of EBNA 3C. Those in Figure 7C were cytotoxic only against DCs loaded with peptides of LMP-1. The T-cell line evaluated in Figure 7B contained predominantly T-cells specific for EBNA3A, and bound tetramers of HLA A0201 in complex with the EBNA3A peptide SURDRLARL. T-cells responsive to LMP-1 or LMP-2 were not detected in this line, but responses to EBNA3B and 3C were not tested.

Cytotoxic activity of CTLs against each target was calculated as % of lysis = 100% x (Avg CTL induced release, cpm - Avg spontaneous release (SR), cpm)/(Avg Maximum release (MR), cpm - Avg spontaneous release (SR), cpm)., where the average is calculated for 3 replicates for the test wells and for 5-replicates for SR and MR wells. The HLA-A0201 restricted EBV CTLs were also characterized for the specificity to EBV antigens in Cr51 release assay against autologous EBV -negative antigen-presenting cells loaded with the A0201 EBV epitopes. The effect of hypomethylation on the susceptibility of the EBV+ Burkitt lymphoma cells (Mutu and Rael) to the EBV CTL mediated killing was tested in Cr51 assay after co-incubation of these cells with decitabine. In xenograft models EBV-CTLs were administered intravenously, either through the dorsal penile vein or the retroorbital sinus, a the dose of 1-2x10^7 T-cells/mouse. The animals were also treated with 2000Un of Inerleukin-2/mouse/dose injected i.p. twice/week.

Quantitative DNA methylation analysis using MassARRAY: DNA was isolated using the Qiagen DNeasy Blood and Tissue Kit (Qiagen) and 1 µg of DNA was converted with

sodium bisulfite using the EZ DNA methylation kit (Zymo Research). DNA methylation analysis of the EBV loci was carried out using the MassARRAY EpiTYPER assay (Agena Biosciences). In brief, DNA regions of interest were amplified with PCR primers specific for the EBV gene loci (primers and genome EBV positions listed in Supplemental Table 4) using the reference (B95-8 strain) genome (NCBI GenBank Accession: V01555.2) and annotation was obtained from O'Grady *et al*[3]. PCR products were *in-vitro* transcribed and fragmented with RNase A (Agena) and RNA oligonucleotide fragments were analyzed via Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-ToF) mass spectrometry. Ratios of unmethylated versus methylated mass peaks were used to calculate the percentage of DNA methylation for individual CpG dinucleotides.

We obtained an average of 1,046,254 reads in our untreated cells, 1,072,309 reads in the decitabine treated cells and 980,781 reads in cells treated with decitabine followed by a washout without drug (washout cells). Greater than 400,000 reads were uniquely mapped to the EBV genome in untreated, treated and washout cells. The average sequencing depth for CpGs covered in our library was 1181.

Agilent SureSelect MethylomeCapture (custom panel): Library preparation for methylome capture, sequencing and post-processing of the raw data was performed at the Epigenomics Core at Weill Cornell Medical College as follows:

Libraries were made using SureSelect ^{XT} Methyl Reagent kit (G9651B), following manufacturer's recommendations (Agilent Technologies Inc. Santa Clara, CA). Briefly, 1000 ng from each DNA, were sonicated using a Covaris S220 sonicator (Covaris, Woburn, MA) to approximately 100-175 bp fragments, end-repaired, phosphorylated, A-tailed and ligated to SureSelect methylated adaptors to create pre-capture libraries. At each step, products were cleaned by the use of Agencourt AMPure XP beads following manufacturer's recommendation (Beckman Coulter, Indianapolis, IN). Pre-capture libraries were hybridization to an EBV custom capture library (SureDesign ID 3189341**)** for 16 hrs. at 65°C. Hybridized products were recovered by purification on Dynabeads MyOne Streptavidin T1 magnetic beads, and then subjected to bisulfite conversion (64°C

for 2.5hr) using the Zymo EZ DNA Methylation Gold kit (Cat # D5005, Zymo Research, Irvine CA). The post-capture bisulfite treated libraries were first PCR amplified for 8 cycles and Illumina indexes for multiplexed sequencing were added through 6 cycles of PCR amplification. Final yields were quantified in a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY), and quality of the library was assessed on a DNA1000 Bioanalyzer chip (Agilent Technologies, Santa Clara, CA). Libraries were normalized to 2nM, pooled and 10% phiX added before clustering at 10pM on a V2 pair end read flow cell and sequenced for 150 cycles on an Illumina MiSeq. Primary processing of sequencing images was done using Illumina's Real Time Analysis software (RTA) as suggested by Illumina. CASAVA 1.8.2 software was then used to demultiplex samples, generate raw reads and respective quality scores. Analysis of bisulfite treated sequence reads, was carried out as described in Garrett-Bakelman et al (1), except alignment was done to the EBV genome. https://www.ncbi.nlm.nih.gov/assembly/GCF_002402265.1. The percentage of bisulfite converted cytosines (representing unmethylated cytosines) and non-converted cytosines (representing methylated cytosines) were recorded for each cytosine position in CpG, CHG, and CHH contexts (with H corresponding to A, C, or T nucleotides).

1. Doubrovina E, Oflaz-Sozmen B, Prockop SE, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. *Blood*. 2012;119(11):2644-2656.
2. Roskrow MA, Suzuki N, Gan Y, et al. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the treatment of patients with EBV-positive relapsed Hodgkin's disease. *Blood*. 1998;91(8):2925-2934.
3. O'Grady T, Wang X, Honer Zu Bentrup K, Baddoo M, Concha M, Flemington EK. Global transcript structure resolution of high gene density genomes through multi-platform data integration. *Nucleic Acids Res*. 2016;44(18):e145.

Table S1: High throughput drug screen, pharmacologic agents and their targets (excel file attached)

Table S2: Pathways identified from drug screen hits (excel file attached)

Table S3: IC50 of hypomethylating agents in BL cells

	48 Hour IC50	
Cell Line	**5-Azacytidine**	**Decitabine**
Mutu I	4.0μM	>5μM
Kem I	>5.0μM	>5μM
Rael	2.2μM	>5μM

Table S4: Primers used and genomic locations for the MassARRAY EBV methylation assay.

EBV genomic loci			
Primer Name	**Forward sequence**	**Reverse sequence**	**EBV genome location (NC_007605)**
Cp	GGGTTGGGTAAAGGGGTTTTA	CCATCTAATCTAAAATTTACAACAAAACA	11179-11353
BdRF1	TTTGTTGTTTAGGTTGGTTTGAAGT	CAAAAATATAACCAATATCCCAACC	136300-136543
BFRF1	TTTGGATAATTTTTTAGAAGTTGAGA	CAAAAACATCAAAAACAAAACCAC	47300-47608
BMRF1	AAGGGTTATTTGGATTTAGGAGTTG	AACCTAACCAATATCACCCAAAATA	67245-67404
BZLF1	GGGGATAATGGAGTTAATATTTAGG	CCAAATTTAAACAACTACTACAACACTACC	89971-90179
Zp	GGTTTGATTGGTTTTTTTATTAGGG	ACCCCTACCTACCTCTTTAACTCC	91358-91520
OriLyt	GGATTTTGGTGTTAGGTAGGGATT	ATATTACACAAAAACCCCAAAAAAA	40392-40562
OriLyt 2	TAATAGGGGAAGTAAGGTTTTTTGT	CCAAAACTAAATCCTAAAACCCAAA	140576-140771
Rp	GGTGTTGTGTTTTGTATGGTATTTTAT	TACCCCAACCAAATATTCAAAAAC	93866-94077
BBRF1	GGTTTTTATGAGGTGTTTAAATTGG	TAAACTCTCCCACCCAAACAAA	101042-101247
RPMS1	GTAAGTTTAAGTTTGGTGTTGGGGT	CCCTCTCTCTAAAAATTTACATTCCA	150463-150617
BARF0	TTGTAGAAGTTGTTGAAGGAGGTTTT	AAAATCTAACCAAACTACAATCCTACC	159134-159590
BORF1	GTTTATTTTTGTTAGGGGTGGTTG	TATATCAAAAAATCCCCAAAAACT	62881-63243
TR	GGTAGTGTAATTTGTATAAAGAGG	CACCTCATTCTAAAATTCCCATATC	169359-169564
Qp (EBNA)	TGTTTTTAATAGATAGAAATTGGGTG	ACCAAAATATAAAAATAACATATATTACCC	50030-50205
Qp (EBNA)	TTTGGTATTTTGGGTAGTTGGG	TATCAAAAACAAACAACAACAATCC	50200-50379
EBNA (intergenic)	GGTAATTTTTGGTAGTGATTTGGATT	AAAACTTATTCCTCTTTTCCCCTCT	13996-14208 17068-17280 20140-20352 23212-23424 26284-26496 29356-29568 32428-32640
Wp (EBNA)	ATAAGTTTTTAGATAGGGGAGTGGG	AAAAAATAAAAAACCCCCTCTTACA	14200-14441 17272-17513 20344-20585 23416-23657 26488-26729 29560-29801 32632-32873
BHLF1	GTGTATTTGGAAGGTAGGGGG	CCCTAAACCCCTAAACCTATACCAT	40195-40299
BVRF1	TGATGGTTTGGAGAGTATGTAGGTT	TTACCCCTCAACTACTTAAAAAACA	133411-133707
Fp	GGTTAGTTTGATTAAGGGTGAGGTTA	CCCTCAATAATCACCCAATTTCTAT	49898-50066
LMP1	GGTGGTTAAGTGTAATAGGAAATGG	ATTACCCCACAACCTTACCTCACCTA	169285-169471
OriP	GGGGTGTTAGAGATAATTAGTGGAGTT	AACAAAAACCAAAACAAATAAACCA	8413-8519
EBNA3C	GTGATGGATGTTGGTAAGGTTTAGT	TTAAAACCTTAAAATCCAAAAACCC	88234-88566
LMP2B	TTTTTAGGGAATGTTAGATTTTATTAAG	CCTTCTCTATCCACTTAAAACCCTT	168560-168792
EBER1	AAGAGGGGGTTATAAAGTTTAGGGT	CAATATCTACAAATCTACATCTCCTCAAA	6454-6626
LMP2A	GGATTATTTTAATTGGTAAGATTTGGG	CAACAACAATATATAAAAATTATCACAAC	165834-165982
BXLF1	GTTGTGTGATTGTGTTAATTTTTTT	TTTTAACCCAAAACTAAAAACTCTACCT	132930-133073
BGLF4	AGGGGGTTTTGGGGAGATATTTA	CCAAAAATCAACTACAAACAACTAAAA	111246-111429

Table S5: HLA typing of BL cell lines

		Locus				
		A	**B**	**C**	**DRB1**	**DQB1**
Kem I	**Allele 1**	30:04:01G	15:10:01	03:04:02	01:01:01G	05:01:01
	Allele 2	32:01:01G	40:12	04:04:01	12:01:01G	05:01:01
Mutu I	**Allele 1**	01:01:01G	15:03:01G	06:02:01G	03:01:01G	02 (novel)
	Allele 2	02:01:01G	45:01:01G	08:02:01G	07:01:01G	02 (novel)
Rael	**Allele 1**	02:01:01G	15:03:01G	02:10:01	11:01:02	03:19:01
	Allele 2	02:01:01G	15:03:01G	02:10:01	11:01:02	03:19:01

Supplemental Data:

Figure S1: Cell viability after exposure to hypomethylating agents. BL cell lines were exposed to decitabine or 5-azacytadine for 48 hours at a range of doses as follows (from L to R): 0, 500nM, 1μM, 2μM, 4μM, and 9μM for 5-azacytidine and 0, 5nM, 50nM, 500nM, 5μM, 50μM for decitabine. Cell viability was measured using Cell Titer-Glo. Arrows indicate the dose at which maximal induction of *LMP1*/*Cp* transcripts were observed.

Figure S2: Evaluation of lytic induction after treatment with decitabine. A) Fold change in *BZLF1* for BL cells treated with decitabine for 72 hours and evaluated at the indicated timepoints. Experiment performed in triplicate. B) Upper panel: Immunohistochemistry for BZLF1 in BL xenografts treated at the indicated doses. Lower panel: HALO quantification of BZLF1 expression by IHC in xenograft tumors. Mice were treated with vehicle or decitabine at the indicated doses for 7 days. Tumors were harvested on day 7. Representative images were obtained on an Olympus BX 43 microscope. Camera: Jenoptik ProgResCF; software: ProgRes Mac Capture Pro, 2013.

Figure S3: Evaluation of decitabine followed by EBV-CTLs *in-vivo* in Rael xenografts. A, C) *In-vivo* imaging of Rael-luciferase xenograft mice; B) LMP1 and BZLF1 quantification in Rael xenograft tumors obtained after 7 days of treatment with vehicle or decitabine at 1mg/kg. DCB: decitabine. D) Immunohistochemistry for PD-1 in each of the 4 conditions as indicated. Representative images were obtained on an Olympus BX 43 microscope. Camera: Jenoptik ProgResCF; software: ProgRes Mac Capture Pro, 2013.

Figure S4: Decitabine induces T-cell homing in Mutu I xenografts. IHC in Mutu I xenograft tumors in the treatment cohorts listed. Microscope: Olympus BX 43 microscope. Camera: Jenoptik ProgResCF; software: ProgRes Mac Capture Pro, 2013. Original magnification x 600 with 60/0.80 objective lens.

Figure S1

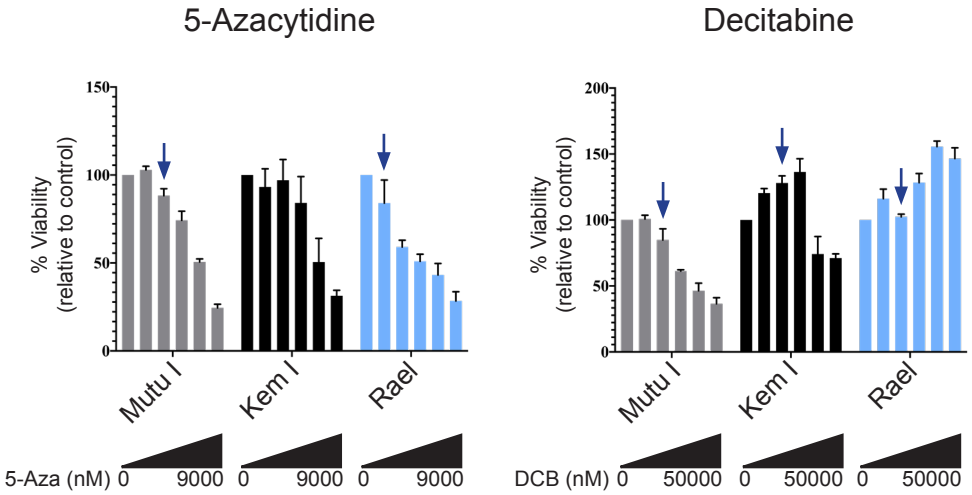
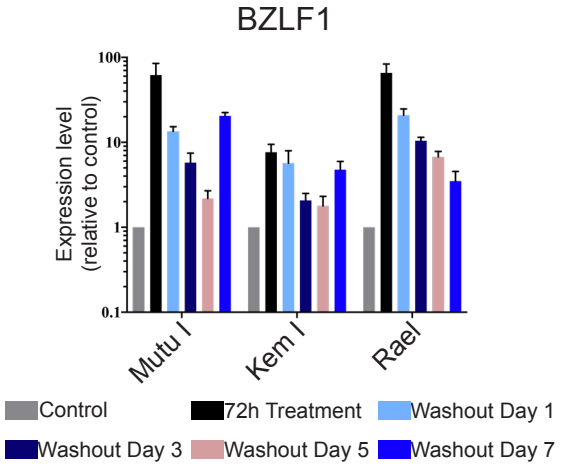

5-Azacytidine
Decitabine
% Viability
(relative to control)
150
100
50
0
200
150
100
50
0
Mutu I
Kem I
Rael
5-Aza (nM) 0 9000 0 9000 0 9000
DCB (nM) 0 50000 0 50000 0 50000

Figure S2

A


BZLF1
Expression level (relative to control)
100
10
1
0.1
Mutu I
Kem I
Rael
Control
72h Treatment
Washout Day 1
Washout Day 3
Washout Day 5
Washout Day 7

B

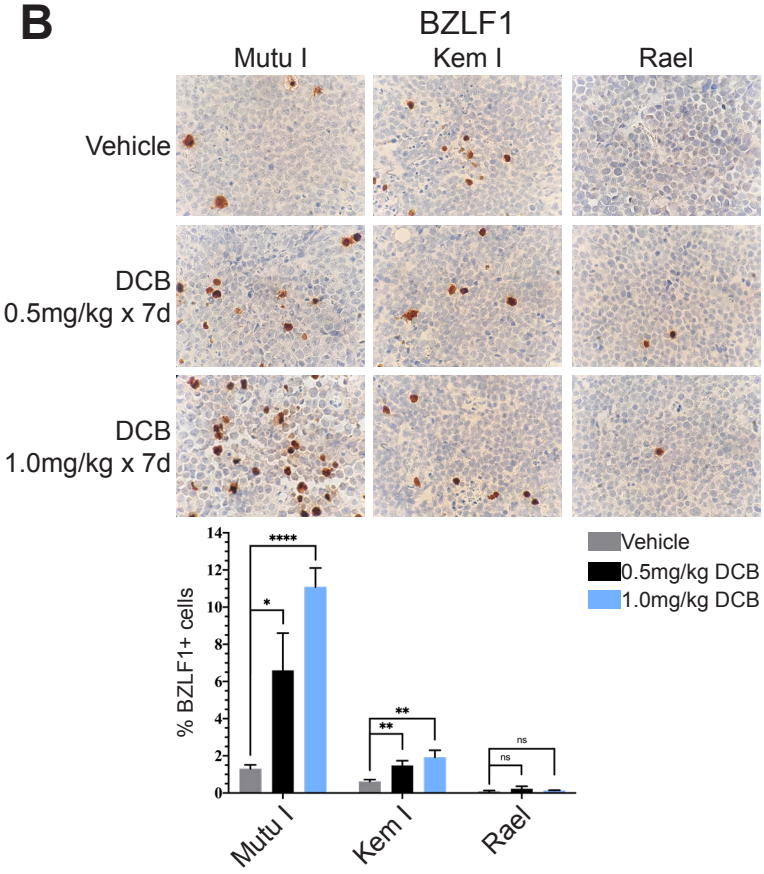
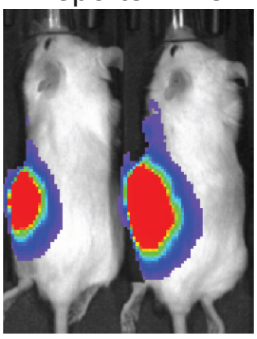

BZLF1
Mutu I
Kem I
Rael
Vehicle
DCB 0.5mg/kg x 7d
DCB 1.0mg/kg x 7d
% BZLF1+ cells
14
12
10
8
6
4
2
0
****
*
**
**
ns
ns
Vehicle
0.5mg/kg DCB
1.0mg/kg DCB
Mutu I
Kem I
Rael

Figure S3

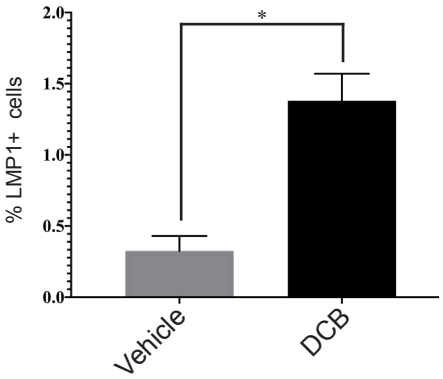
A

Rael-Luciferase
Reporter Line

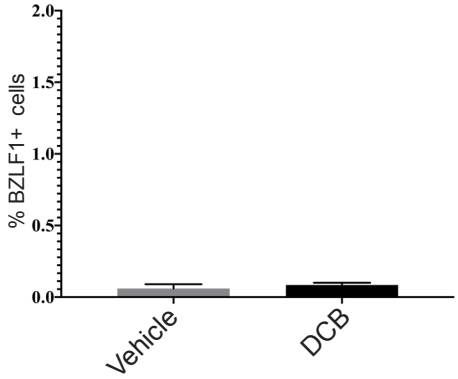


B

Rael-Luciferase
LMP1

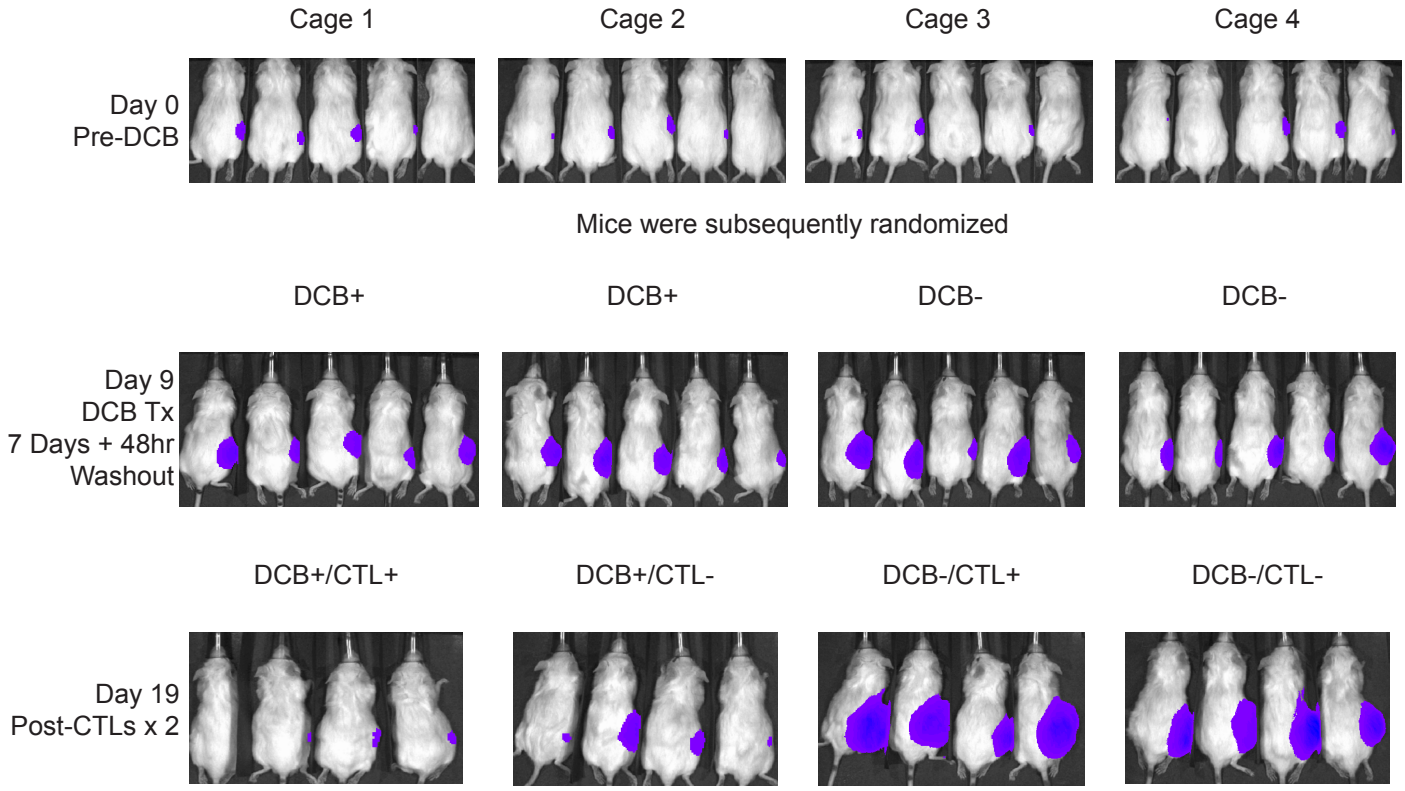


Rael-Luciferase
BZLF1



C

Rael-Luciferase



D

PD-L1

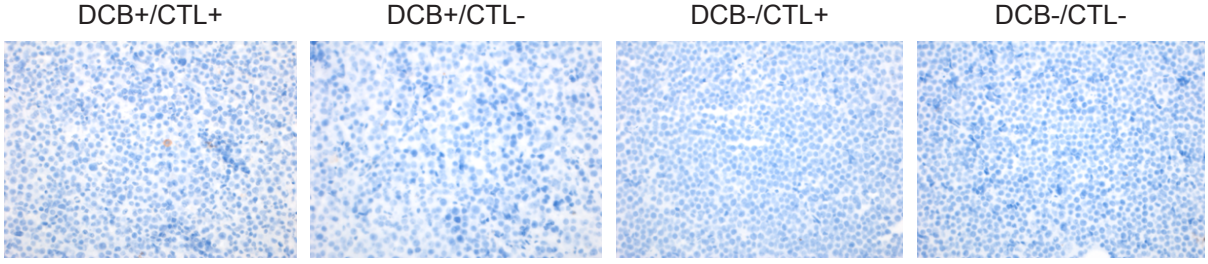


Figure S4