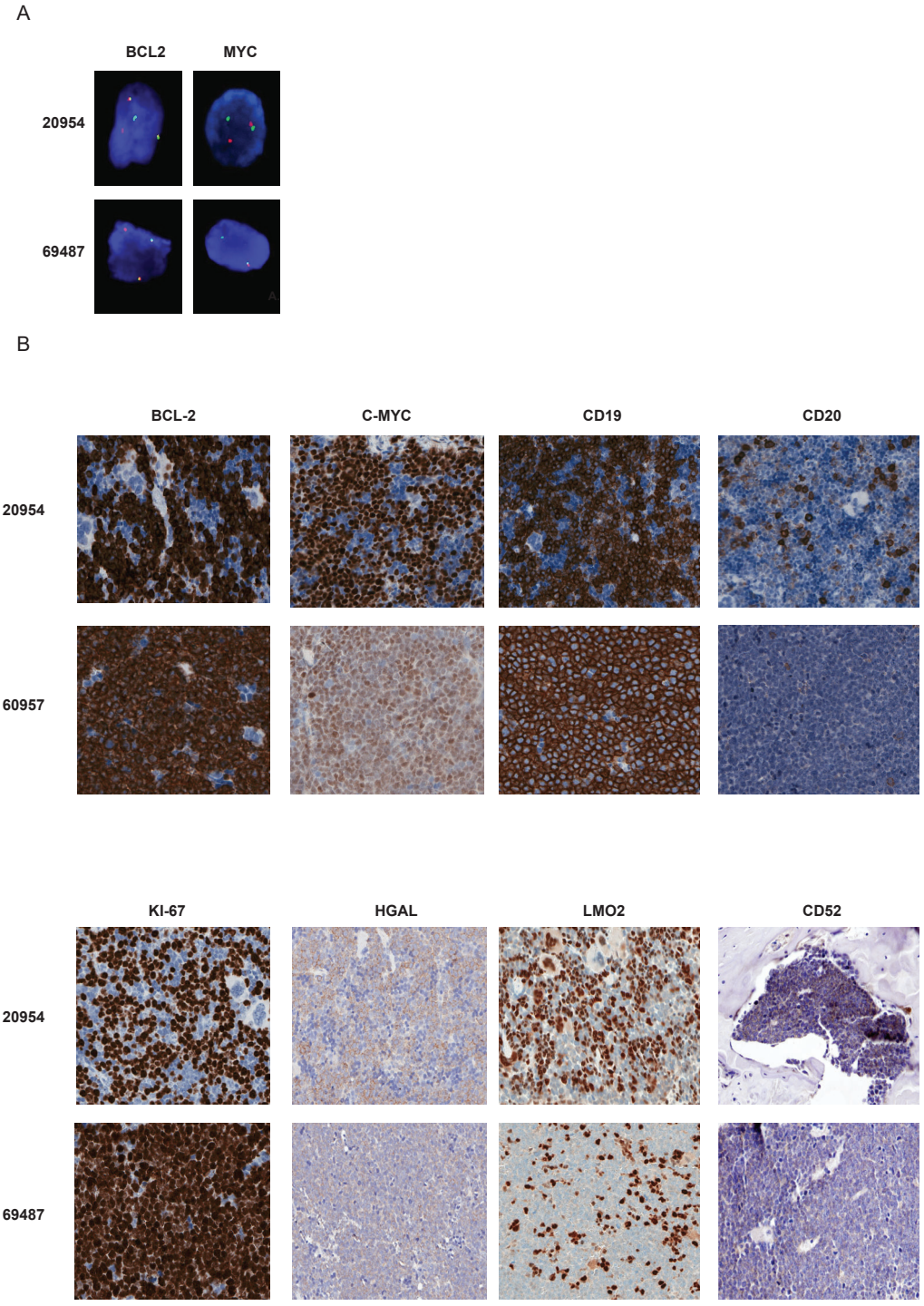
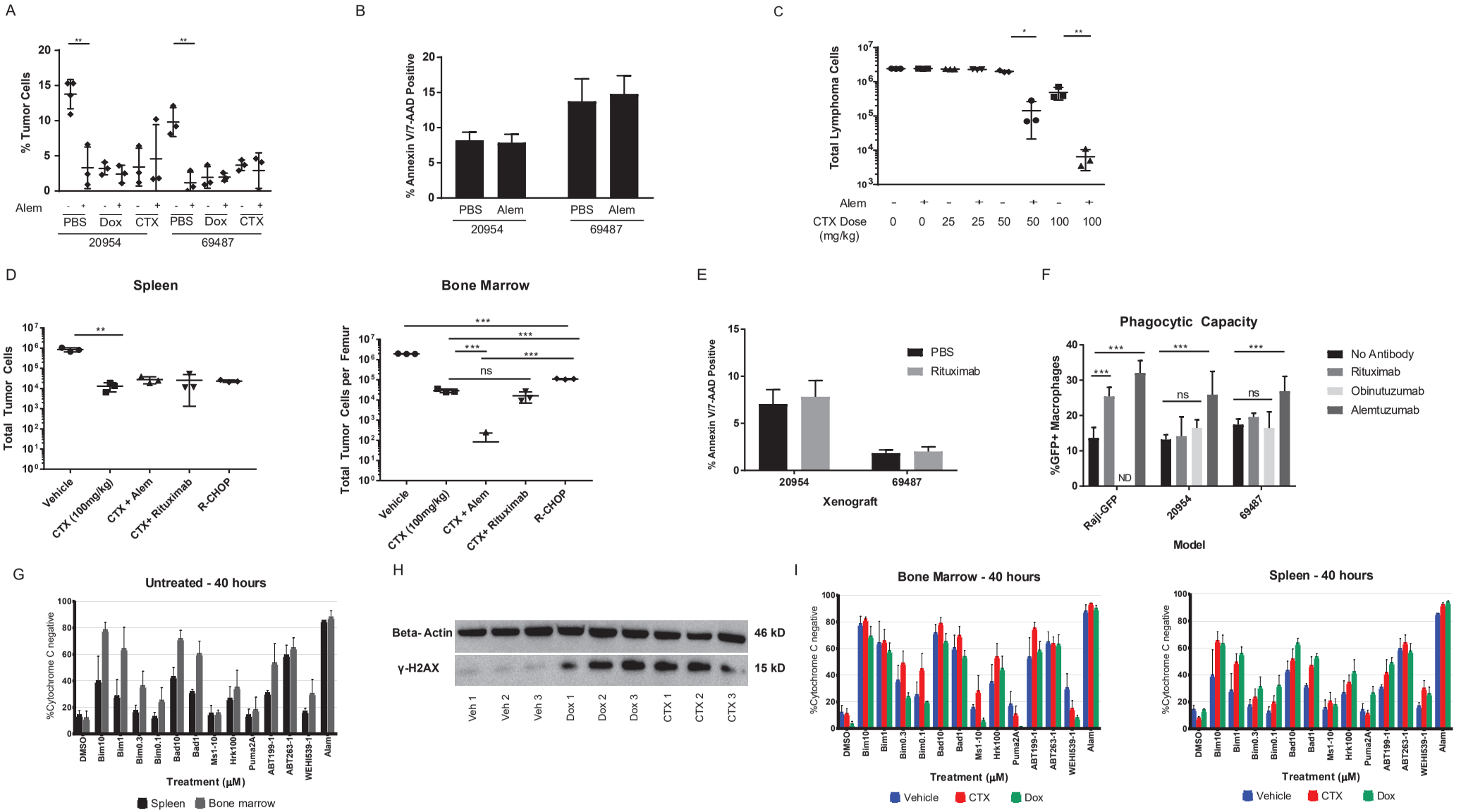

A
BCL2
MYC
20954
69487
B
BCL-2
C-MYC
CD19
CD20
20954
60957
KI-67
HGAL
LMO2
CD52
20954
69487

Supplementary Figure 1: Characterization of DHL Xenografts

(A) Fluorescence *in situ* hybridization (FISH) of DFBL-20954 and DFBL-69487 spleen cells from engrafted NSG mice demonstrating fusion between *IGH@* and *BCL2* (yellow signal; left-sided panels) and break-apart of *MYC* (right-sided panels). Break-apart of DFBL-20954 showed both red (centromeric; 5'MYC) and green (telomeric; 3'MYC) split signals, whereas only the telomeric probe was detected in DFBL-69487. IGH/BCL2 fusion was detected in 47/50 nuclei in DFBL-20954 and in 46/50 nuclei in DFBL-69487. *MYC* rearrangement was observed in 49/50 nuclei in DFBL-20954 and 40/50 nuclei in DFBL-69487.

(B) Immunohistochemical staining of tissues from mice engrafted with DFBL-20954 and DFBL-69487 cells for the indicated markers. CD52 expression was assayed on BM specimens and the remaining markers were assayed on spleen specimens. Images are 40x magnified.



Lossos et al. Supplementary Figure 2

Supplementary Figure 2: Cytotoxic Effects of Chemotherapies on DHL Cells

(A) Peripheral blood involvement for both PDX models. Tumor-bearing mice treated with PBS, Cyclophosphamide (CTX), Doxorubicin (Dox) Alem (Alem) or combinations, as indicated in Figure 1B. Mouse blood was RBC-lysed and viable ($7AAD^-$) cells were assessed for hCD19/hCD45 positivity. Two-sided Welch *t*-test, *p<0.05, **p<0.01

(B) Bone marrow cells from untreated mice (n=3 per condition) engrafted with DFBL-20954 and DFBL-69487 were co-cultured in the presence of Alem (Alem, 30 µg/ml). Apoptosis was assayed 48 hours after co-incubation.

(C) Bone marrow tumor burden of NSG mice engrafted with DFBL-20954 and treated with PBS, Alem, and/or CTX at the indicated dose. Total tumor cells present are represented as the product of total cells * percent $7AAD^-$hCD19+hCD45+ cells. BM cells represent tumor cells per mouse femur. Two-sided Welch *t*-test, *p<0.05, **p<0.01

(D) On day 8 of treatment, spleen was harvested and a single femur was flushed from mice treated as indicated. Total tumor cells present are represented as the product of total cells * percent $7AAD^-$hCD19+hCD45+ cells. BM cells represent tumor cells per mouse femur. All comparisons by two-sided Welch *t*-test, *p<0.05, **p<0.01, ***p<0.001.

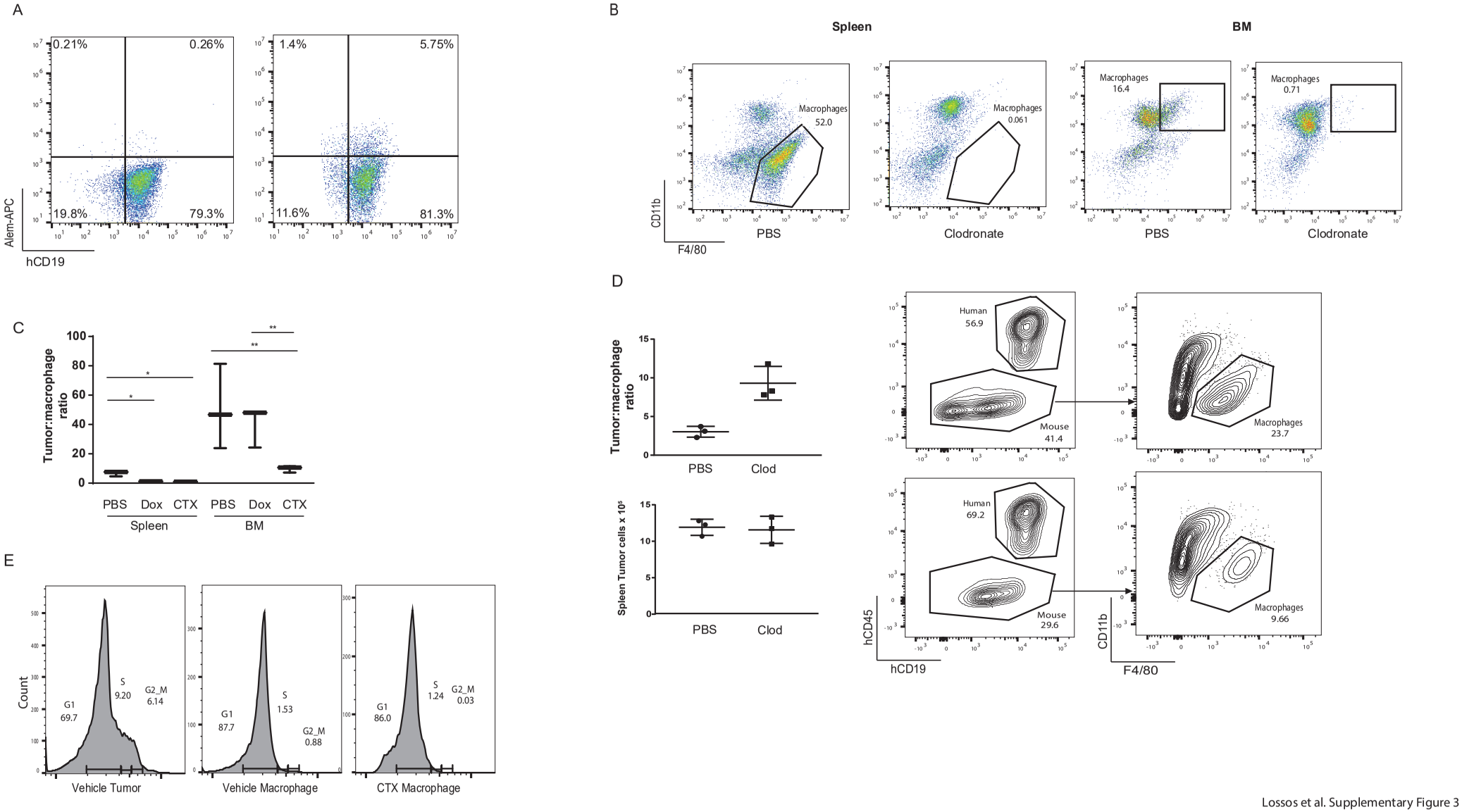
(E) Bone marrow cells from untreated mice (n=4 per condition) engrafted with DFBL-20954 or DFBL-69487 were co-cultured in the presence of rituximab (30 µg/ml). Apoptosis was assayed 48 hours after co-incubation.

(F) Raji-GFP cells (n=4) or bone marrow cells from untreated mice (n=5) engrafted with DFBL-20954 or DFBL-69487 were co-cultured with BMDMs with the indicated antibodies (20 µg/ml). Phagocytosis was quantified as the percent of CD11b+ cells that were CFSE+/GFP+. Two-sided Welch *t*-test, *p<0.05, **p<0.01, ***p<0.001.

(G) BH3 profiling of DFBL-20954 cells isolated from spleen and BM (n=3 per condition) at a time when disease burden was >2% in peripheral blood.

(H) Western blot of gamma-H2A.X levels in BM of mice bearing DFBL-20954 16 hours after the indicated treatment. Each lane represents an individual mouse

(I) BH3 profiling of DFBL-20954 cells isolated from the spleen and BM (n=3 per condition) 48 hours after treatment with PBS (vehicle), cyclophosphamide (CTX) or doxorubicin (Dox).



Lossos et al. Supplementary Figure 3

Supplemental Figure 3: Clodronate and Chemotherapy effects on Murine Macrophages

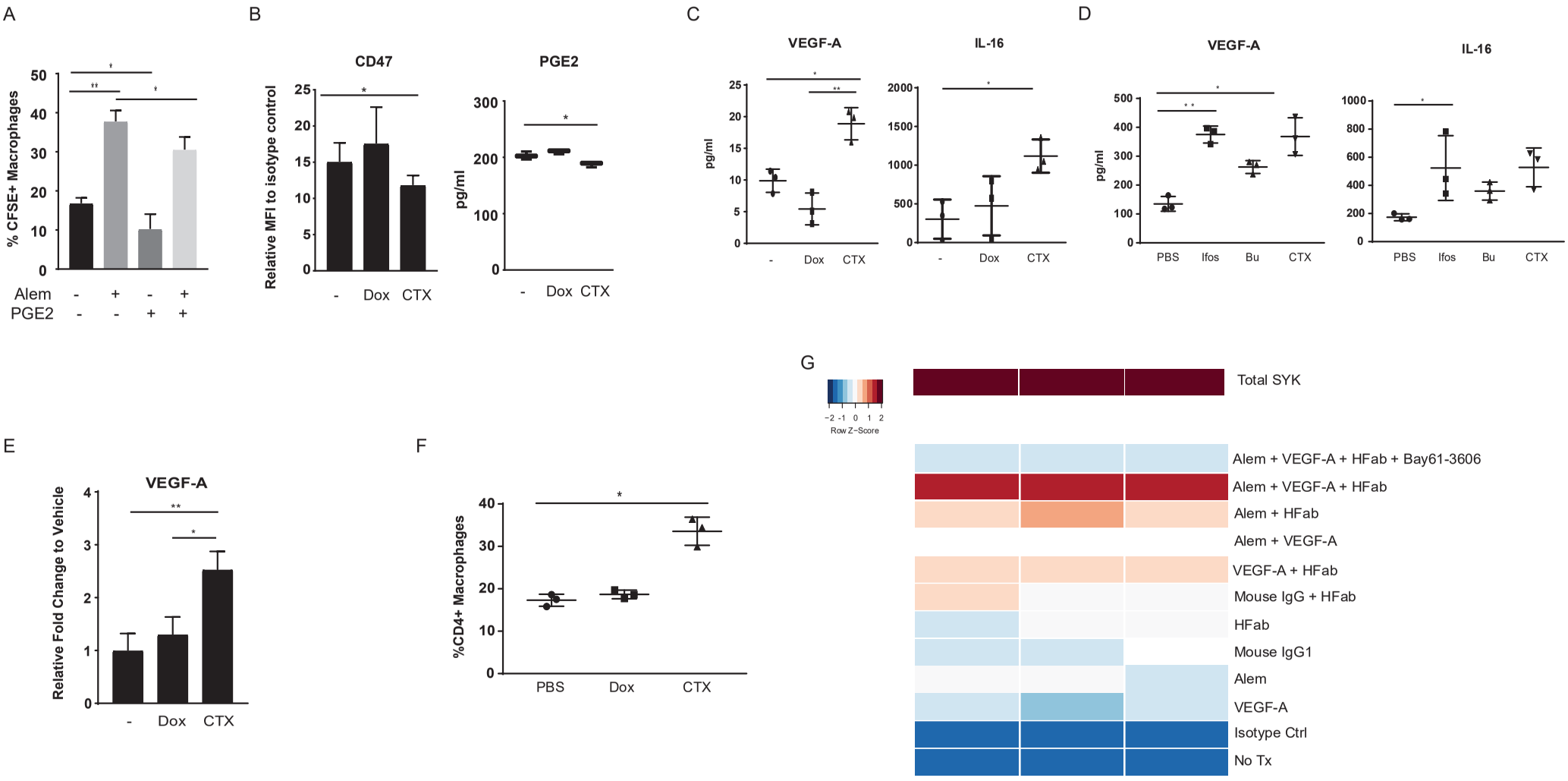
(A) Assessment of Alem-APC binding in DFBL-20954 cells from the bone marrow of engrafted mice. Mice were untreated (left) or treated 5 days prior to cell collection with Alem-APC *in vivo*.

(B) NSG mice were treated with clodronate liposomes (200 ul) and assessed for viable ($7AAD^-$) splenic ($CD11b^-F4/80^+$) and BM ($CD11b^+F4/80^+$) monocytes/macrophages. Representative flow cytometry plots are shown.

(C) Ratio of lymphoma ($hCD19^+hCD45^+$) to macrophage ($hCD19^-CD11b^-F4/80^+$) cells 48 hours after treatment of DFBL-69487-engrafted mice with the indicated agents. Unpaired two-sided *t*-test, *$p<0.05$, **$p<0.01$.

(D) Characterization of DFBL-20954 tumor burden and tumor: macrophage ratio upon incomplete macrophage depletion of the spleen with clodronate (25ul). Representative flow cytometry plots are shown.

(E) DFBL-20954-bearing NSG mice were treated with PBS or CTX (n=6 per condition) and BM macrophage cell cycle was assessed 16 hours following chemotherapy administration using Vybrant dye. Bone marrow DFBL-20954 cells from PBS-treated mice (Vehicle tumor) are shown as a control for cycling cells. Representative flow cytometry plots are shown.



Lossos et al. Supplementary Figure 4

Supplemental Figure 4: Cyclophosphamide Promotes An ER-Stress Mediated Secretory Phenotype in DHL Cells

(A) DFBL-69487 cells were isolated from the BM of untreated mice, labeled with CFSE and co-cultured with bone-marrow derived macrophages in the presence or absence of Alem and Prostaglandin E2 (PGE2, 2ng/ml). Phagocytosis was quantified as percent of $CD11b^+$ cells that were $CFSE^+$. The experiment was performed twice in triplicate. A representative experiment is depicted.

(B) Surface expression by mean fluorescence intensity (MFI) compared to isotype control or total protein level by ELISA for the indicated proteins in the BM of DFBL-69487-engrafted mice (n=3-6 per condition). CD47 was assessed 16 hours after treatment and PGE2 was assessed 48 hours after treatment. Two-sided Welch *t*-test, *p<0.05

(C) Levels of human VEGF-A and IL-16 from bone marrow of DFBL-69487 (n=3 mice per condition)-engrafted mice collected 40 hours after *in vivo* treatment with vehicle (-), doxorubicin (Dox) or cyclophosphamide (CTX). Representative experiment shown. Levels of murine VEGF-A using a murine-specific cytokine array were negligible (<1 pg/ml). Two-sided Welch *t*-test, *p<0.05, **p<0.01

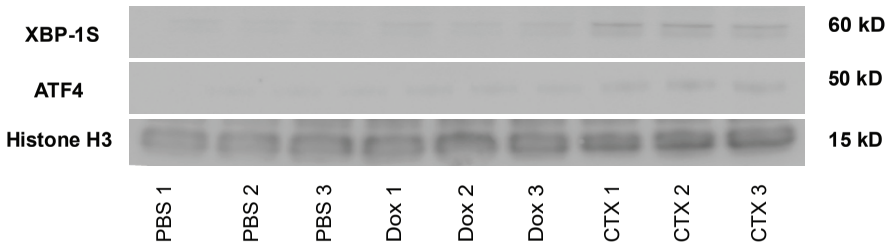
(D) Levels of human VEGF-A and IL-16 16 hours from BM of mice engrafted with DFBL-20954 after treatment with PBS (Veh), ifosfamide (ifos), busulfan (Bu) and CTX determined by cytokine array.

(E) DFBL-20954 BM cells were collected 6 hours after indicated treatments (n=3 per condition) and culture *in vitro* for 24 hours. Fold changes in human VEGF-A expression in the culture supernatants (i.e. conditioned media) are shown. Unpaired two-sided *t*-test.

(F) Fraction of BM monocytes/macrophages ($hCD19^-CD11b^+F4/80^+$) that express surface CD4 following the indicated treatments. Macrophages were collected 48 hours after treatment initiation. Unpaired two-sided *t*-test.

(G) Heatmap of total SYK (top row) and phospho-SYK (all other rows) levels in BM-derived macrophages (BMDMs) following the indicated treatments as assayed by flow cytometry. Each column represents an individual mouse. Legend represents Z-scores of mean fluorescent intensities. HFab indicates cross-linking with anti-human Fab antibody.

A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ATF4
Histone H3
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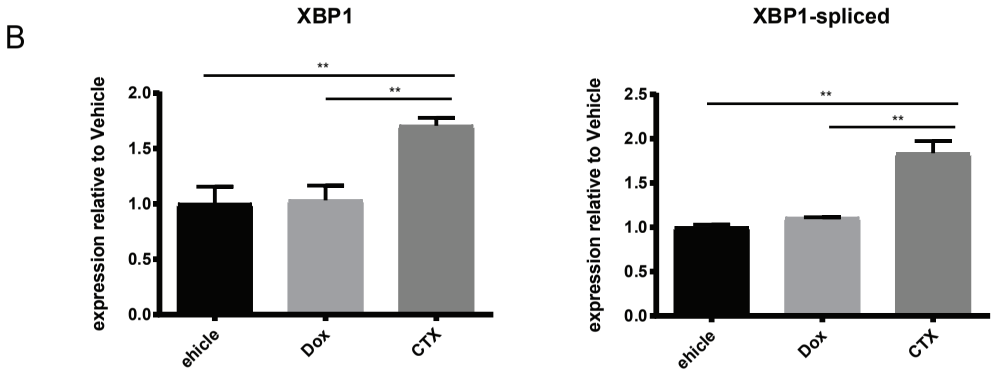
50 kD
15 kD
PBS 1
PBS 2
PBS 3
Dox 1
Dox 2
Dox 3
CTX 1
CTX 2
CTX 3

B


XBP1
XBP1-spliced
expression relative to Vehicle
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1.0
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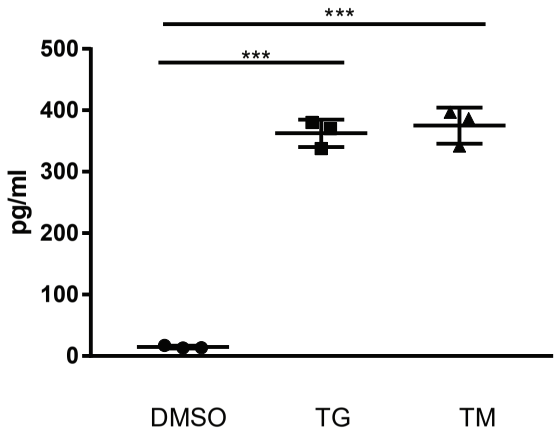
C

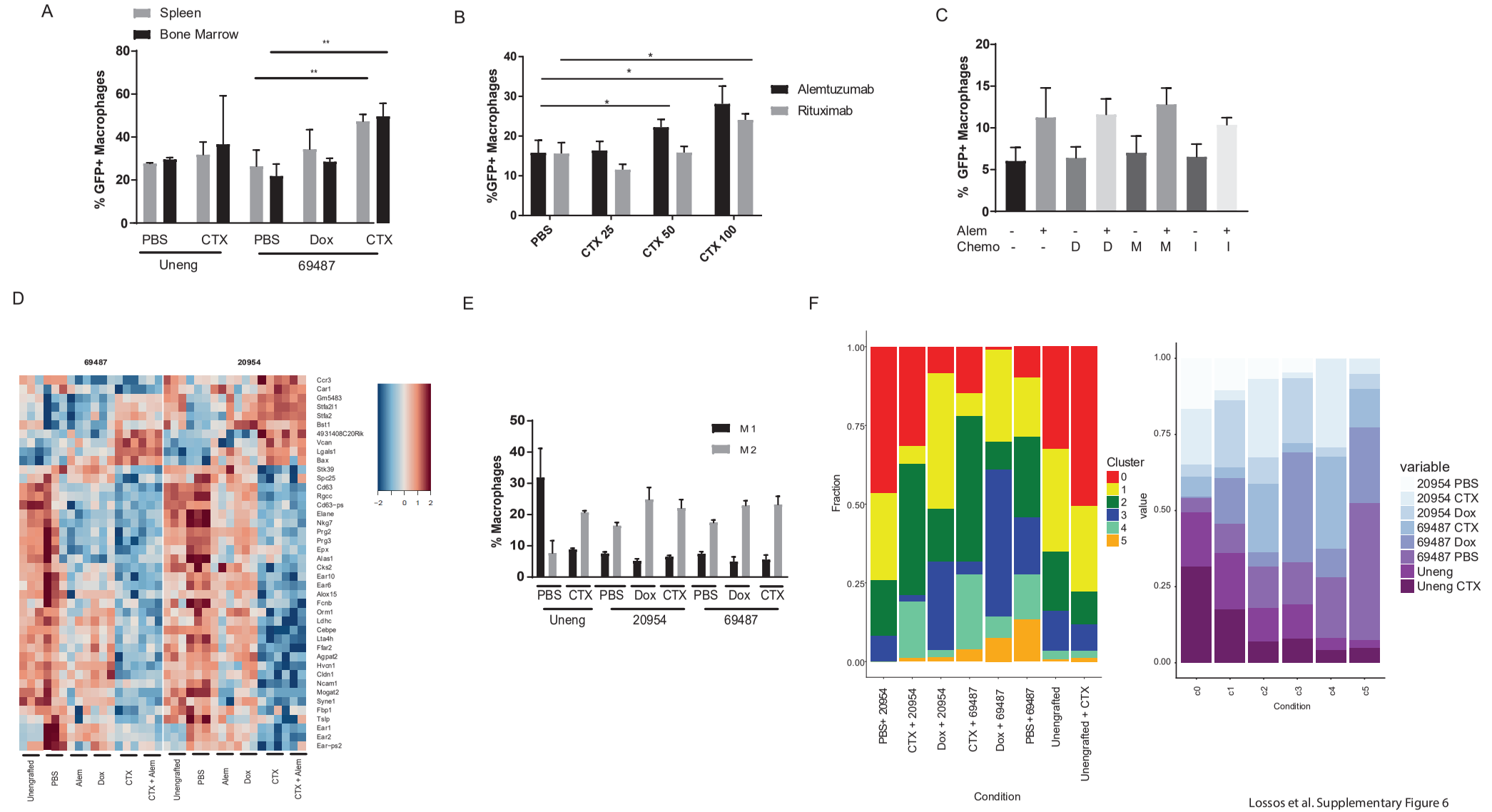

***
***
500
400
300
200
100
0
pg/ml
DMSO
TG
TM

Supplemental Figure 5: High-dose Cyclophosphamide Exerts Phenotypic Changes in Tumor Cells

(A) Immunoblotting for the indicated targets using lysates from purified, viable BM DFBL-69487 cells collected 48 hours after *in vivo* treatment with PBS (vehicle), doxorubicin (Dox) or CTX. Each lane represents an individual mouse.

(B) qPCR of XBP-1 and XBP-1s (spliced) using DFBL-20954 cells following treatment of engrafted mice with PBS (vehicle), doxorubicin (Dox) or cyclophosphamide (CTX). RNA was extracted 16 hours after treatment.

(C) Levels of human VEGF-A in DFBL-69487 cells isolated from BM and treated *ex vivo* for 24 hours with DMSO, thapsigargin (TG) or tunicamycin (TM). Two-sided Welch *t*-test * $p<0.05$, ** $p<0.01$, *** $p<0.001$. VEGF-A levels in conditioned media were also increased but were out of assay range following treatment.



Lossos et al. Supplementary Figure 6

Supplemental Figure 6: Cyclophosphamide Uniquely Reprograms Macrophages in the Presence of DHL

(A) Primary viable (7AAD$^-$) splenic (hCD19$^-$CD11b$^-$F4/80$^+$) and BM (hCD19$^-$CD11b$^+$F4/80$^+$) monocytes/macrophages were sorted from unengrafted or DFBL-60487-engrafted mice treated with PBS (Veh), doxorubicin (Dox) or cyclophosphamide (CTX) and cultured in the presence of Alem-coated Raji cells. Phagocytosis was assessed as the percent of CD11b+ cells that were GFP+. Two-sided Welch *t*-test, *p<0.05, ** p<0.01

(B) Primary viable (Zombie-NIR$^-$) BM (hCD19$^-$CD11b$^+$F4/80$^+$) monocytes/macrophages were sorted from DFBL-20954-engrafted mice (n=3) treated with PBS or the indicated doses of CTX and were cultured in the presence of Alem- or rituximab-coated Raji cells. Phagocytosis was assessed as the percent of CD11b+ cells that were GFP+. Two-sided Welch *t*-test, *p<0.05

(C) Phagocytosis assays of CFSE-labeled, BM-derived DFBL-20954 cells with BMDMs pre-treated with PBS, doxorubicin (Dox, 200 ng), mafosfamide (M, 10 uM) or ifosfamide (I, 20 uM). Macrophages were treated with chemotherapy for 6 hours and then incubated in drug-free media for 12 hours prior to assay initiation.

(D) Heatmap of genes differentially expressed in bone marrow macrophages common to both lymphomas from CTX treatment compared to PBS, Alem and Dox. Macrophages were harvested 48 hours after treatment.

(E) Macrophage polarization of murine BM macrophages from unengrafted and tumor-bearing mice (n=3 per condition) treated with the indicated chemotherapies. M1 macrophages were defined as CD206-MHCII+ and M2 macrophages were defined as CD206+MHCII-. A representative experiment is depicted. The experiment was performed twice.

(F) Stacked bar-plots of cluster representation among BM macrophages subjected to scRNA-seq.

Supplementary Methods

Flow cytometry

NSG Mice were injected and cells isolated as described in the main text file. The following antibodies were used for flow cytometric analyses: human CD19 (clone HIB19, Biolegend), human CD20 (clone2H7, Biolegend), human CD47 (clone B6H12, Thermo Fisher Scientific), human CD52 (clone HI186, Biolegend), human calreticulin (clone FMC75, Abcam), human VISTA (clone 730804, R&D Systems), human CD32 (clone AT10, Abcam), murine CD4 (clone GK 1.5, Biolegend), murine F4/80 (clone BM8, BD Biosciences), Zombie-NIR (Biolegend), 7-AAD (Thermo-Fisher Scientific), murine CD64 (clone 290322, R&D systems), murine CD32B (clone AT130-2, eBioscience), murine CD16 (clone 275003, R&D Systems), murine CD16.2 (clone 9E9, Biolegend), murine CD36 (clone HM36, Biolegend), murine CD11b (clone M1/M70, Biolegend), murine CD206 (clone MMR, Biolegend) and mouse I-A/I-E (major histocompatibility complex class 2, clone M5/114.15.2, Biolegend). Human (Human TruStain FcX, Biolegend) and murine Fc-Block (BD Biosciences) were used in all instances except for when directly staining for Fc receptor expression. For all mean fluorescent intensity (MFI) stains 10^4 viable human/mouse cells were used with saturating amounts of antibody.

Immunoblotting

NSG mice were injected with 10^6 tumor cells intravenously and treated with PBS, Dox or CTX. 16 (DFBL-20954) or 48 (DFBL-69487) hours after treatment bone marrow or splenic cells were harvested as above and human cells purified using an EasySep Mouse/Human Chimera Isolation Kit (StemCell Technologies). Dead cells were removed by an EasySep Dead Cell Removal Kit (StemCell Technologies). Cells were lysed in RIPA buffer (Cell Signaling Technology)

supplemented with Halt protease and phosphatase inhibitors 100x (Life Technologies) and protein amounts quantified (Pierce BCA Protein Assay Kit, Life technologies). Lysates were incubated with NuPAGE LDS Sample Buffer (Invitrogen) supplemented with 2-ME at 95°C, run on an SDS gel and transferred to a PVDF membrane (iBlot2 Transfer Stacks, Life Sciences). Membranes were blocked for 1 hour with 5% dry milk and incubated with ATF4 (D4B8, Cell Signaling Technologies), XBP-1 Spliced (D2C1F, Cell Signaling Technologies), Phospho-Histone H2A.X (Ser 139, Cell Signaling Technology) and Beta-Actin (13E5, Cell Signaling Technologies) antibodies overnight. All antibodies were diluted 1:1000. Membranes were washed three times with TBS-T solution, incubated with HRP-conjugated Goat Anti-Rabbit IgG (Bio-Rad) and imaged with an ImageQuant LAS-4000 (GE Healthcare Life Sciences).

Chromatin Immunoprecipitation and quantitative PCR (ChIP-qPCR)

NSG mice were injected with 10^6 tumor cells intravenously and treated with PBS, Dox or CTX. 16 (DFBL-20954) or 48 (DFBL-69487) hours after treatment, bone marrow cells were harvested as above and human cells purified using an EasySep Mouse/Human Chimera Isolation Kit (StemCell Technologies). Dead cells were removed by an EasySep Dead Cell Removal Kit (StemCell Technologies). ChIP was performed using a SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling Technologies) per the manufacturer's instructions. Briefly, cells were cross-linked with 4% paraformaldehyde solution and quenched with glycine. Chromatin were digested with Micrococcal Nuclease and sonicated with a QSonica Q500 Sonicator with 8 (DFBL-20954) or 10 (DFBL-69487) cycles 20 seconds on 20 seconds off at 25% amplitude on ice. Input was removed and nuclei were incubated with rabbit anti-ATF4 (Abcam), Rabbit IgG polyclonal isotype control (Abcam) or Anti-Flag (D6W5B, Cell Signaling Technologies). Pulled DNA was

isolated with ChIP-grade Protein G Magnetic beads and subjected to washes in increased salt concentrations. Cross-linking was undone by incubation with 5M NaCl and Proteinase K and eluted using elution buffer. DNA was purified using the same kit per the manufacturer's instructions. DNA was amplified using a CFX96 Real Time System (Bio-Rad) with SYBR-green (PowerUp Master Mix, Life Technologies). Primers used were VEGF-A (Exon 1, Forward: 5'-CGTCGGGCCTCCGAAACCATGAACT-3', Reverse: 5'-GTGGAGGTAGAGCAGCAAGG-3'), ASNS (Exon 7, Forward: 5'-GCAGCTGAAAGAAGCCCAAGT-3', Reverse: 5'-TGTCTTCCATGCCAATTGCA-3').

RNA isolation and RNA-Sequencing

NSG mice were injected with 10^6 tumor cells intravenously and treated with PBS, Dox, Alem, CTX or combinations. 16 (tumor) or 48 (macrophages) hours after treatment bone marrow or splenic cells were harvested. For sequencing of human samples human cells were purified using an EasySep Mouse/Human Chimera Isolation Kit (StemCell Technologies) and total RNA was isolated with an RNEasy Plus Mini Kit (Qiagen). For sequencing of BM macrophages, cells were harvested and macrophages sorted as described in the text. Total RNA was isolated with an RNEasy Plus Micro Kit (Qiagen). Mapped read counts for each transcript were normalized using the Bioconductor package DESeq2 in R (1). Differential expression analysis was also performed using DESeq2. No additional variance stabilization transformation was performed on top of RLE. We mapped all 3pDGE data against a joint hg19/mm9 genome assembly and jointly quantitated human and murine genes based on refseq annotation using the ESAT package (2) (http://garberlab.umassmed.edu/software/esat/ and http://genome.cshlp.org/content/early/2016/09/15/gr.207902.116), with parameters -task score3p -alignments $sample_list -wLen 50 -wExt

5000 -wOlap 0 -sigTest 0.01 -multimap ignore. For GSEA genes were ranked with the test statistic from DESeq2. All RNA-seq data is publicly accessible at GEO (GSE:128728).

For heatmaps of gene expression, all normalized gene expression values were log2-transformed and scaled to have zero mean and unit standard deviation across samples. To generate a multidimensional scaling (MDS) plot of macrophage expression profiles, reference gene expression data for M0, M1 and M2 macrophages were downloaded from NCBI GEO (3), and all expression data were log2-transformed, scaled to have zero mean and unit variance, and then quantile normalized to remove batch effects (Figure R1) before performing MDS.

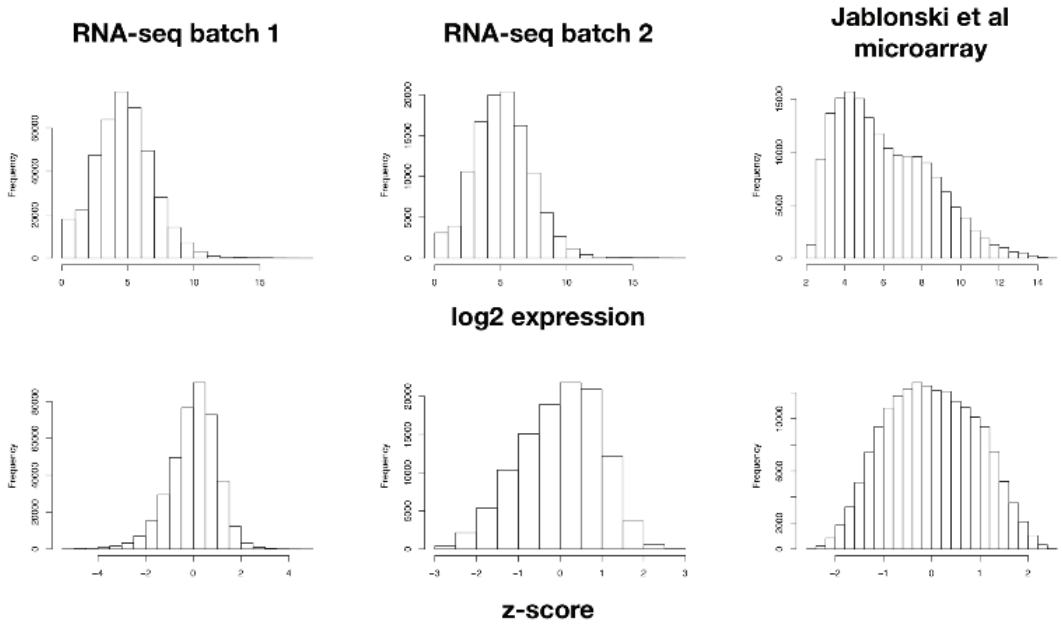


Figure R1 Comparison of distribution before and after z-score conversion for all bulk expression data sets used in this study. Upper panels: from left to right are histograms of RNA-seq (batch 1: macrophages harvested from mouse bone marrow in all treatment groups; batch 2: additional macrophage samples untreated or treated with cyclophosphamide) and reference macrophage microarray expression data values (from reference 3) in log2 space. Lower panels: histograms of scaled data (z-scales) in the same order.

MDS was implemented using the cmdscale function in R, and the number of dimensions retained was explicitly set to 2. To ensure that our analysis is robust to normalization methods and not biased by intrinsic differences in RNA-seq and microarray data, we employed multiple lines of analysis to position our macrophage samples within the M0-M1-M2 polarization paradigm. First we performed MDS on data normalized using feature-specific quantile normalization (FSQN), a method that has been shown to effectively remove cross-platform batch effects for classification problems (4). The resulting MDS plots show consistent relative positions of samples in the 2-D representation with our aforementioned normalization method (compare Figure R2 with Figure 6B).

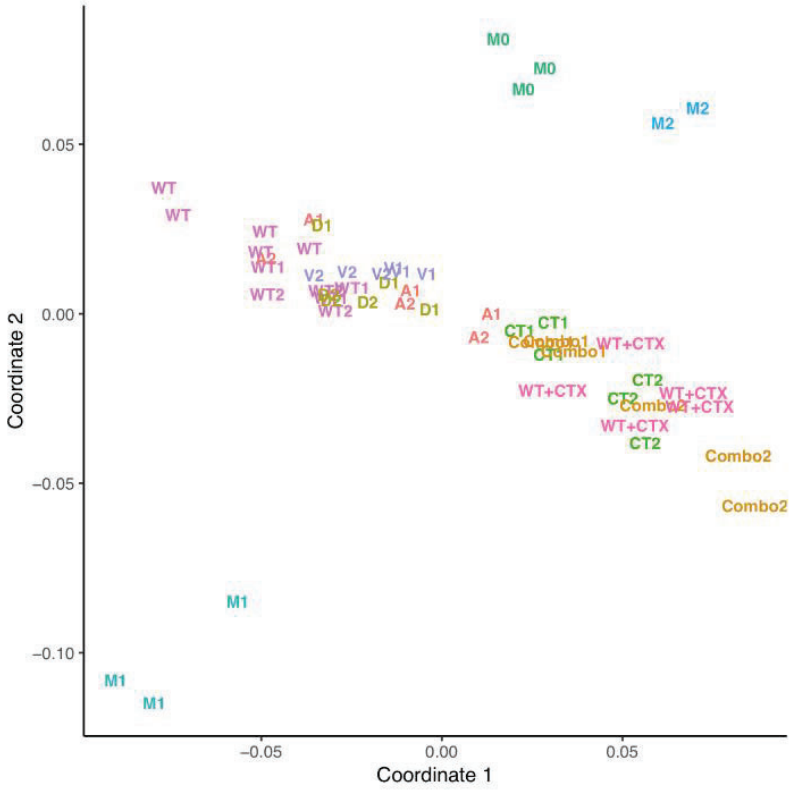


Figure R2 Normalized macrophage sample gene expression data in 2-D representation. Macrophage RNA-seq data from our study and microarray data from reference 3 were log2-transformed and normalized against each other to achieve comparable distributions using Feature-specific Quantile Normalization (FSQN) and projected onto a 2-D space using multidimensional scaling with number of dimensions set to 2.

Next, hierarchical clustering of normalized expression data from macrophage samples was performed to confirm proximity of CTX-treated macrophages to M2 macrophages in gene expression space (dendrogram in Figure R3).

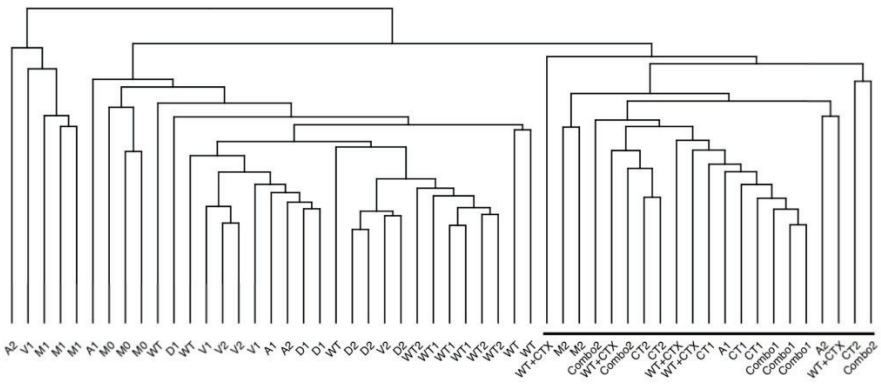


Figure R3 Dendrogram of samples generated from hierarchical clustering. FSQN-normalized log2 expression data and agglomerative clustering with complete linkage were used. A line is drawn under the cluster where all CTX treated samples belong.

Finally, we performed differential expression analysis between the microarray sample groups from reference 3 and defined four sets of genes – genes up- (down-) regulated only in M1 (M2) macrophages. We used the up and down genes in each type of macrophage profile as a signature and calculated the enrichment of that signature in our samples using the suggested sum(up gene z-scores) - sum(down gene z-scores) method. Here we show a plot of our samples along the two signature score axes (Figure R4). Again, we see that macrophages from CTX treated animals had higher M2 scores and lower M1 scores.

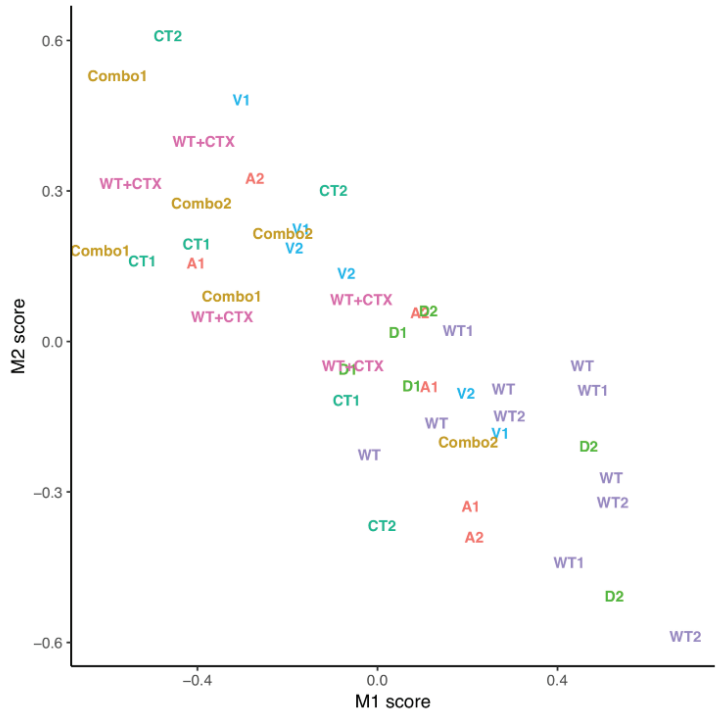


Figure R4 M1-M2 signature scores of all macrophage samples. Signatures were extracted from M0, M1 and M2 macrophage expression data from reference 3 by combining genes up- and down-regulated specifically in M1 and M2 macrophages, respectively. M1 and M2 scores were calculated as the difference between average expression of M1/M2 up-regulated genes and that of down-regulated genes in each sample.

BH3 Profiling

Tumor cells isolated from the spleen and bone marrow were subjected to flow cytometry-based BH3 profiling in accordance with previous methods (5). Single cells were resuspended in MEB buffer, permeabilized with 0.001% digitonin, and added to each peptide treatment well and incubated for 60 min at 28°C. Peptide exposure was terminated with 4% formaldehyde. Cells were stained overnight with antibody to Cytochrome C (Biolegend). Cytochrome C negativity was measured on a BD Biosciences LSR II flow cytometer.

Single-Cell Whole Transcriptome Amplification

Single-cell RNA-seq was performed using the Smart-Seq2 protocol as described in Trombetta et al. with minor modification (6). Briefly, following sorting, 96-well plates of lysed cell samples were cleaned with 2.2x volume AMPure XP SPRI beads (Beckman Coulter). Reverse transcription was performed using SuperScript iii reverse transcriptase (Thermo Fischer Scientific) at a volume of 0.5 µL per sample in place of the Maxima RT prescribed in the SmartSeq2 protocol. PCR was then performed on the samples as previously described. Following whole transcriptome amplification (WTA), PCR products were cleaned with 0.9x volume SPRI beads and eluted in water. The concentration of the amplified cDNA in the resulting solution was determined using a Qubit 3.0 Fluorimeter (ThermoFischer), and the amplified cDNA was analyzed using a D5000 high sensitivity DNA tape for Tapestation (Agilent).

Preparation of cDNA Libraries for RNA-seq

WTA products were diluted to a concentration of 0.2 ng/µL, or used as-is for samples less than 0.4 ng/µL, and tagmented and amplified using Nextera XT DNA Sample preparation reagents (Illumina). Tagmentation was performed according to manufacturer's instructions, modified to use ¼ the recommended volume of reagents, extending tagmentation time to 10 minutes and extending PCR time to 60s. PCR primers were ordered form Integrated DNA Technologies. Nextera products were then cleaned twice with a 0.9x volume of SPRI beads and eluted in water. The library was quantified using Qubit and analyzed using a high sensitivity DNA tape (Agilent). The library was diluted to 2.2 pM and sequenced on a NextSeq 500 (Illumina) to an average depth of 831390 reads using paired-end (PE) 30-base pair reads.

Single-Cell RNA-Seq Expression Quantification

RNA-seq reads were aligned to the UCSC genomeStudio mm10 mouse transcriptome using STAR aligner (7). The resulting transcriptome alignments were processed by RSEM to estimate the abundance (expected counts and TPM) of RefSeq transcripts (8).

Single-Cell Filtering and Gene Filtering

In sample filtering, we excluded libraries with poor values for number of aligned reads (<200,000) or percentage of aligned reads (<30%). Out of 1536 initial samples, we retained 1092 after filtering, or about 71% passing filter. We then removed any genes whose expression values summed to 0 following cell filtering, leaving 16,961 genes.

PCA and clustering

We used the Seurat R package (Seurat version 2.3.2, R version 3.5.0) to visualize and cluster our filtered data. We performed log-normalization, scaling and selected the 1169 top variable genes for principal components analysis (PCA). Running jackstraw, calculating percent variation captured by each PC, and plotting the standard deviation explained by each PC in an elbow plot suggested somewhere between the top 5 and 9 PCs were significant. After carefully exploring each of the top 9 PCs, we elected to use PC 1 through 5 in our downstream analysis. PCs 1 (17.9% variation) and 2 (7.34% variation) capture differences in cell cycle and separate the Lymphoma-engrafted CTX condition. PCs 3 (5.51% var) and 4 (2.53% var) further separate the lymphoma-engrafted CTX condition as well as the lymphoma-engrafted Dox condition. PC5 (1.52% var) captures some antigen presentation and phagocytosis related genes. PC6 (1.18% var, not included in further analysis) is defined by some lowly expressed genes present in only a few cells. Higher PCs had very low signal, both in terms of dynamic range of expression and number

of cells expressing top loaded genes. We created a tSNE plot using the top 5 PCs to visualize our dataset. We then performed
clustering using the shared nearest neighbors (SNN) algorithm (Waltman and van Eck (2013) The European Physical Journal B.) over the top 5 PCs.

Differential Expression and Pathway analysis

We applied the FindMarkers function from the Seurat package, calling the MAST package as the test under test.use to test for differential expression between the different clusters observed in the dataset. Genes differentially expressed with an adjusted p-value >0.1 (Bonferroni corrected), log-fold change >0.25 in either direction, and expressed in a minimum of 10% of the cells in either population were brought into IPA (Qiagen). We ran a core analysis over each DE gene list in IPA to identify pathways possibly activated/inhibited between the different groups.

scRNA-Seq analysis Statistics

Cluster enrichments by sample of origin were calculated by a Fischer's Exact Test with Bonferroni correction for multiple comparisons (Corrected for 25 comparisons, 5 clusters * 5 conditions). Contingency tables were made for each condition tested: number of cells in cluster tested, number of cells not in cluster tested by number of cells from treatment condition tested, and number of cells not from not treatment condition tested.

A Mann-Whitney test calculated p values for expression of Fcgr genes between clusters. P values for one cluster vs all others pooled and Bonferroni corrected for 24 comparisons are reported in the text. Pair-wise comparisons for clusters of interest are also reported in Supplementary Table

5. For sc-RNA Seq expression of Fcgr genes, we also report effect sizes for the magnitude of the effect observed in addition to statistical significance (p-value). The calculation was performed as Cohen's d, where effect size d is the number of standard deviations equivalent to the difference in the mean for each population: d = (Mean1-Mean2)/(SD pooled).

XBP-1 Splicing Assay

To assay for *XBP-1* mRNA expression and splicing, qPCR primers for total and spliced form of *XBP-1* RNA were used to amplify cDNA generated from 500ng of each RNA sample, and each form of RNA was quantified relatively to beta-actin (*ACTB*) mRNA expression using the delta C_t method. Primer sequences for total *XBP-1* mRNA are as follows: forward 5'-CCTTGTAGTTGAGAACCAGG-3', reverse 5'-GGGGCTTGGTATATATGTGG -3'. Primer sequences for spliced form of *XBP-1* are as follows: forward 5'-TGCTGAGTCCGCAGCAGGTG-3', reverse 5'-GCTGGCAGGCTCTGGGGAAG-3'.

Primers for *ACTB* mRNA are as follows: forward 5'- CATGTACGTTGCTATCCAGGC-3', reverse 5'- CTCCTTAATGTCACGCACGAT-3'.

pSYK Phosphorylation Assay

BMDMs were generated as above. BMDMs were seeded at 10^5 cells per well in a 24 well plate and were treated with Alem (20 µg/mL) or mouse IgG1 isotype control (20µg/mL, sc-2025, Santa Cruz Biotechnology) in the presence or absence of human VEGF-A (10ng/ml, Peprotech) and SYK inhibitor BAY61-3606 (Sigma Aldrich). After 5 minutes Anti-Human Fab (I5260, Sigma-Aldrich) was added for 10 minutes (20 µg/mL). Cells were washed twice with PBS, detached with gentle scraping and fixed and permeabilized with appropriate buffers per

manufacturer's instructions (eBioscience IC Fixation and Permeabilization Buffers, Thermo Fisher Scientific). Macrophages were stained with 7-AAD for viability gating and pSYK (Tyr 348, clone moch1ct, Life Technologies), Total Syk (clone 5F5, Biolegend) or isotype control IgG.

Clodronate

Clodronate was purchased from clodronateliposomes.com. Mice were injected with 25 (partial depletion) or 200 (full depletion) µL of liposome solution stored at 4°C and warmed to room temperature i.v. as described in figure legends. Mice were assayed 48 hours post injection for depletion.

Immunohistochemistry

NSG mice were injected and treated as previously described. 16 and 48 hours after treatment femurs and spleens were excised and fixed in 4% paraformaldehyde for 48 hours then transferred to 70% ethanol. Immunohistochemistry for c-MYC, Bcl-2, CD20, CD19 and Ki-67 was performed on the Leica Bond III automated staining platform. Antibody c-Myc from Abcam catalogue # ab32072 clone Y69 was run at 1:100 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. Antibody Bcl-2 from Dako catalogue # M0887 clone 124 was run at 1:2500 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. Antibody CD20 from Dako catalogue # M0755 clone L26 was run at 1:500 dilution using the Leica Biosystems Refine Detection Kit with citrate antigen retrieval. Antibody CD19 from Cell Signaling Technology catalogue # 90176 clone D4V4B was run at 1:800 dilution using the Leica Biosystems Refine Detection Kit with citrate antigen retrieval. Antibody

Ki-67 from Biocare catalogue # CRM325 clone SP6 was run at 1:100 dilution using the Leica Biosystems Refine Detection Kit with EDTA antigen retrieval. For HGAL and LMO2 tissue sections were cut from formalin-fixed paraffin-embedded tissue blocks at 4 μm and mounted on positively charged slides. Immunohistochemistry was performed using a standard protocol as previously described (9). In brief, all tissue sections underwent heat-induced epitope retrieval in pH 6.0 citrate buffer (Dako, Carpinteria, CA, USA). Endogenous peroxidase was blocked by 3% H2O2 solution for 10 min. LMO2 staining was performed using LMO2 antibody (Lossos lab, Miami,FL, USA) using a 1:100 dilution and overnight antibody exposure time and HGAL (Lossos lab, Miami,FL, USA) using a 1:50 dilution and overnight antibody exposure time. CD52 staining was performed with clone YTH34.5 (Serotec, Oxford, United Kingdom) at a 1:2000 dilution as described previously (10). Immunohistochemistry for F4/80 was done by Applied Pathology with antibody CI:A3-1 from Abcam with Histroreveal antigen retrieval at a 1:1000 dilution.

BCL-2 and c-MYC FISH Analysis

Five-micron sections were cut from paraffin-embedded tissue onto charged slides and used for fluorescence in situ hybridization (FISH) studies. FISH evaluation for MYC rearrangement was performed on nuclei with the Vysis LSI MYC Dual Color, Break Apart Rearrangement Probe (Abbott Molecular) at 8q24. The normal range established for this probe in the performance laboratory was up to 3%. FISH evaluation for IGH-BCL2 rearrangement was performed on nuclei with the Vysis LSI IGH/BCL2 Dual Color, Dual Fusion Translocation Probe (Abbott Molecular) for IGH at 14q32 and BCL2 at 18q21. The normal range established for these probes in the performance laboratory was up to 1%. Evaluation for rearrangement was performed on 50

nuclei for each probe set; a result was considered abnormal when the percentage of rearranged nuclei exceeded the established normal range.

Small animal ^{18}F-FDG PET-CT imaging

2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) PET-CT imaging was carried out on a dedicated small animal scanner (Inveon Multimodality System, Siemens Medical Solutions USA, Inc., Hoffman Estates, IL). Prior to the imaging session, mice were placed on a high fat/ low protein/ low carbohydrate ketogenic diet overnight (~16 hours) while allowing free access to water. Ketogenic diet facilitates low blood glucose levels in mice and is used as an alternative to fasting. One hour prior to radiotracer administration, mice were warmed on Gaymar circulating warm water heating pads to reduce brown fat uptake and anesthetized through sevoflurane/air inhalation. The mice were injected with a bolus intravenous injection (through the lateral tail vein) of approximately 7.4 MBq of ^{18}F-FDG (PETNet Solutions, Woburn MA), and remained under anesthesia throughout the 60 min uptake period. The mice were subsequently placed into the scanner, and low dose CT scans were first acquired (80 kVp, 0.5 mA) for anatomical reference and to provide guidance for the delineation of selected tissue volume of interest (VOI). Static PET emission scans were then acquired in list-mode format over 10 min and corrected for decay and dead time. The acquired data were then sorted into 0.5 mm sinogram bins and 1-time frame for image reconstruction using FORE/3D-OSEM-MAP image reconstruction. The reconstructed PET-CT images were analyzed with the Siemens Inveon Research Workplace software. The radioactivity retention within the selected VOI was obtained from mean voxel intensity values within the VOI and then converted to megabecquerels per milliliter using the calibration factor determined for the Inveon PET System. These values were then divided by the

administered activity dose and body weight to obtain an image VOI-derived Standardized Uptake Value (SUV). We used the maximum SUV value (SUVmax) within a VOI as a quantitative imaging metric.

Supplementary References

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