***SUPPLEMENT TO*: "BET bromodomain protein inhibition reverses chimeric antigen receptor extinction and reinvigorates exhausted T cells in CLL"**

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Supplemental Methods

Patient and Healthy Subject Samples

Peripheral blood mononuclear cells (PBMC) were collected for CAR T cell manufacturing via leukapheresis from CLL patients with persistent disease. These protocols were approved by the United States Food and Drug Administration (FDA), the Recombinant DNA Advisory Committee, and the Institutional Review Board (IRB) of the University of Pennsylvania. Healthy donor PBMC were also isolated from leukapheresis samples. Informed consent was obtained in accordance with the Declaration of Helsinki. Processing, cryopreservation and laboratory analysis of research samples were carried out under principles of Good Laboratory Practice with established standard operating procedures and/or protocols for sample receipt, processing, freezing, and analysis.

Flow Cytometry and other Correlative Assays

Cells were stained with antibodies against surface and intracellular antigens, according to our previously published methods (1, 2). Samples were then fixed and analyzed using an LSRFortessa (BD Biosciences), FlowJo software (FlowJo, LLC) and/or FCS Express (De Novo Software). Information pertaining to flow cytometry antibodies used in this study is listed below:

Specificity	**Clone**	**Conjugate**	**Supplier**
CD5	L17F12	Allophycocyanin (APC)	Becton Dickinson (BD)
CAR19	136.20.1	Alexa Fluor (AF) 647	Kind gift of B. Jena and L. Cooper (MD Anderson Cancer Center)

CCR7	150503	Fluorescein isothiocyanate (FITC), Phycoerythrin (PE) CF594	BD
CD10	HI10a	PECy7	BD
CD19	HIB19	Phycoerythrin PECy7	BioLegend
CD20	2H7	APCH7	BD
CD25	M-A251	PE-CF594	BD
CD3	OKT3, SK7	Brilliant Violet (BV) 605, APCH7	BioLegend
CD38	HIT2	BV785	BioLegend
CD4	OKT4	BV785	BioLegend
CD40	5C3	BV605	BioLegend
CD45RO	UCHL1	PE, BV570, BV605	BioLegend
CD69	FN50	BV605	BioLegend
CD70	113-16	APC	BioLegend
CD8	RPA-T8	BV650	BioLegend
CD80	L307.4	BV786	BD

CD86	IT2.2	BV785	BioLegend
CTLA-4	BNI3	PECy5	BD
HLA-DR	G46-6	APC-R700	BD
IFNγ	4S.B3	AF688, PE/Dazzle 594	BioLegend
Immunoglobulin Light Chain κ	MHK-49	PE	BioLegend
Immunoglobulin Light Chain λ	MHL-38	FITC	BioLegend
Ki-67	B56	AF700	BD
LAG-3	3DS223H	PECy7	eBioscience
PD-1	EH12.2H7	BV421	BioLegend
PD-L1	29E.2A3	BV421	BioLegend
Perforin	B-D48	BV421	BioLegend
tEGFR	BV711	AY13	BioLegend
TIM-3	F38-2E2	PE	BioLegend
TNFα	MAb11	PE, AF700	BioLegend

Additional correlative assays conducted on patients samples (e.g., qPCR for CAR T cell quantification over time) were carried out according to our published methods (1-3).

Cell Lines and Viral Vectors

Wild-type (parental) K562 and NALM-6 cells were originally purchased from the American Type Culture Collection. These cell lines were engineered to express CD19, PD-L1, or click beetle green luciferase and green fluorescent protein (CBG-GFP), as previously described (1, 2). Routine mycoplasma and cell line authentication testing were performed on banks of engineered cells according to our standard practices (2, 3). These cell lines were used as targets where indicated in the relevant experiments. T cells were transduced with retroviral or lentiviral vectors encoding the anti-CD19 CAR containing a 4-1BB intracellular signaling domain (4), the TET2-CS variant (1129-1936 Δ1481-1843), and/or a catalytically dead TET2 mutant (HxD) (3). The lentiviral TET2 vectors were engineered to achieve co-expression of a truncated epidermal growth factor receptor-like protein (tEGFR) as a selection marker.

Activation, Transduction and Expansion of T cells

Purified human T cells were stimulated with Human T-Activator CD3/CD28 Dynabeads (Life Technologies) at a 3:1 bead to cell ratio. For lentiviral transduction, appropriate amounts of virus were added 24 hours post-T cell activation according to our published methods (1). For γ-retroviral transduction, 1 ml of packaging supernatant was loaded onto Retronectin (Takara Bio) pre-coated, bovine serum albumin blocked non-treated 24-well plates and centrifuged for 2 hours at 2,000 rpm at 20°C. Supernatant was removed, wells were washed once with PBS, and 5×10^5 activated T cells were added and centrifuged for 1 hour at 1000 ×g, 20°C (spinoculation). Cells were then placed at 37°C for T cell expansion. A second round a spinoculation occurred two days later. T cell cultures were monitored via cell population doubling and size measurements using a Multisizer 3 Coulter

Counter (Beckman Coulter). CAR T cells were cryopreserved once the cell volume decreased to 300-350 fl. and the rate of population doublings decreased. Population doublings were calculated using the equation $A_t = A_0 2^n$, where *n* is the number of population doublings, A_0 is the input number of cells, and A_t is the total number of cells.

Analysis of Methylated Amplicons

Gel electrophoresis was used to confirm the presence and size of amplicons, as shown in Supplemental Figure 16. Optimized primer pairs that provided the greatest CpG coverage across the EF1α promoter (i.e., 50 total CpG sites) are as follows: F7R7 (amplicon size without adapter, 472 bp; 32 CpG sites) 5'-TATTATAGGGATAGTAGAGATTTAGTTTGGTTGTAT-3' (F7R7 forward), 5'-CCAAACCAAACCTCAACTCAAACA-3' (F7R7 reverse) and F2R4 (amplicon size without adapter, 395 bp, 18 CpG sites) 5'-TTTGTTGTAGGGAGTTTAAAATGGAGGA-3' (F2R4 forward), 5'-ATCACTAATAAAACCATAAATCCTCTAAATC-3' (F2R4 reverse). Targeted amplification of multiple samples was carried out via the Fluidigm 48.48 Access Array, according to the manufacturer's instructions. Following targeted amplification and library indexing, sequencing was performed on an Illumina MiSeq (5). Sequence data was demultiplexed and assessed for sample read coverage, bisulfite conversion rate, mapping efficiency and unique CpG coverage (5).

Chromatin Immunoprecipitation (ChIP) Assays

CLL patient CAR T cells were thawed and rested in RPMI 1640 media (ThermoFisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (Gemini Bio-Products; complete RPMI). Cells in media were subsequently mixed with molecular biology grade formaldehyde (Sigma-Aldrich) at a final concentration of 1% for 15 minutes at room temperature with periodic agitation. To quench fixation, 1/20th the volume of 2.5 M Glycine was added to the suspension, followed by further incubation at room temperature for 5 minutes. Cells were then

washed in cold 1× PBS, pelleted and then lysed with lysis buffer 1 (50 nM HEPES pH 7.3, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40 and 0.25% Triton X-100) and washed with lysis buffer 2 (10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA pH 8.0 and 0.5 mM EGTA pH 8.0). Cells were resuspended and sonicated in lysis buffer 3 (50 mM Tris-HCl pH 7.5, 140 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS) for 10 cycles at 30 seconds each with 60 seconds on ice between cycles. Sonicated lysates were cleared and incubated overnight at 4°C with magnetic beads bound with anti-BRD2 (Bethyl A302-583A) or anti-BRD4 (Bethyl A301-985A) antibodies to enrich for bound DNA fragments. Beads were washed three times with sonication buffer, one time with sonication buffer containing 500 mM NaCl, one time with LiCl wash buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate) and one time with TE buffer. DNA was eluted in elution buffer. Cross-links were reversed overnight. RNA and protein were digested using RNase A and Proteinase K, respectively and DNA was purified with phenol chloroform extraction and ethanol precipitation. BRD4 ChIP and input DNA were analyzed using SYBR Green real-time PCR analysis (Applied Biosystems). Enrichment data were calculated by determining the immunoprecipitated DNA percentage of input DNA for each sample. The ChIP primers are as follows: 5'-GGGACAGCAGAGATCCAGTT-3' (Vector EF1α 1 forward), 5'-TCTCTAGGCACCGGTTCAAT-3' (Vector EF1α 1 reverse), 5'-CAAAATTTTCGGGTTTATTACAGG-3' (Vector EF1α 2 forward), 5'-AACTTCTCGGGGACTGTGG -3' (Vector EF1α 2 reverse), 5'-TGGAATTTGCCCTTTTTGAG-3' (EF1α CAR 1 forward), 5'-AAGGCGGTCACTGGTAAGG-3' (EF1α CAR 1 reverse), 5'-TTTTTCTTCCATTTCAGGTGTC-3' (EF1α CAR 2 forward), 5'-GGCAGACAGGGAGGATGTAG-3' (EF1α CAR 2 reverse). Negative control primers for ChIP

qPCR were obtained from Active Motif (Human Negative Control Primer Set 1, #71001; and Set 2, #71002).

CRISPR/Cas9-mediated Knock-out of PD-1 in CD19 CAR T cells

The genomic guide RNA (gRNA) *PDCD1* target sequence with protospacer adjacent motif (PAM) underlined was 5′-GGCGCCCTGGCCAGTCGTCTGGG-3′. A corresponding chemically-modified sgRNA was obtained from Integrated DNA Technologies, Inc. sgRNAs were complexed with TrueCut Cas9 protein (Thermo Fisher Scientific) and delivered into primary human T cells as ribonucleoprotein complexes, followed by lentiviral transduction with an anti-CD19 CAR containing a 4-1BB intracellular signaling domain as well as *in vitro* expansion, according to our previously published methods (6).

CAR T cell Functional Assays

CAR T cell *in vitro* proliferative "stress tests" were carried out as previously described (1, 2). Following determination of CAR and/or TET2 (via tEGFR expression) transduction efficiency, engineered T cells were first purified using anti-phycoerythrin (PE) and -allophycocyanine (APC) MultiSort Kits (Miltenyi Biotech). Cell purity was confirmed using flow cytometry and CAR T cells were serially restimulated with irradiated $K562_{CD19}$ or $K562_{CD19/PD\text{-}L1}$ cells at an effector to target cell ratio of 1:1. During the proliferation assay, absolute cell counts were obtained with a Luna automated cell counter (Logos Biosystems).

Twenty-four hours following stimulation with engineered K562 target cells, supernatants were collected from the above co-cultures for multiplex cytokine (Luminex) analysis, as described in our published work (1-3). For intracellular cytokine analysis, T cells were left unstimulated or incubated with phorbol myristate acetate (PMA)/ionomycin for 6 hours in the presence of monensin.

For analysis of cytotoxic potential, CLL patient CAR T cells were co-cultured with CBG/GFP-expressing NALM-6 or NALM-$6_{PD\text{-}L1}$ target cells at the indicated ratios for 16 hours. Cell extracts were generated using the Bright-Glo Luciferase Assay System (Promega Corporation) and luciferin substrate was added according to the manufacturer's directions. Luciferase measurements were taken on a SpectraMax luminescence microplate reader (Molecular Devices), and specific lysis was calculated as previously described (1). For "stressed" cytotoxicity assays, CLL patient CAR T cells were serially restimulated 3 times with irradiated NALM-6 or NALM-$6_{PD\text{-}L1}$ leukemia targets. Using the impedance-based xCELLigence system (ACEA Biosciences Inc.), the kinetics of leukemia cell lysis was then evaluated. For this portion of the assay, NALM-6 or NALM-$6_{PD\text{-}L1}$ were tethered/plated in a 96-well, resistor-bottomed plate in triplicate using the xCELLigence Liquid Tumor Killing Assay Kit according to the manufacturer's (ACEA Biosciences Inc.) instructions. "Stressed" CAR T cells were added to adjust the desired effector-to-target (E:T) ratios 24 hours later. The impedance was measured in 20 minute intervals over 4 days. Target cells lysed by 1% Triton X-100 were used as the positive control, while patient-matched untransduced T cells at the same E:T ratios listed above were assayed as negative controls. CAR T cell cytotoxic capacity was determined via continuous acquisition of impedance data for each well. Raw impedance data was analyzed using RTCA 2.1.0 software (ACEA Biosciences Inc.). The general equation for calculating the % Cytolysis for a sample at a time point *t* is: $\%\ \text{Cytolysis}_{st} = [1-(NCI_{st})/(\text{Avg}NCI_{Rt})]\times 100$, where, NCI_{st} is the Normalized Cell Index for the sample and NCI_{Rt} is the average of Normalize Cell Index for the matching reference wells.

RNA Sequencing

CLL patient $CD8^+$ CAR T cells were purified and libraries were prepared for sequencing as per our previously published methods (2). Briefly, sequencing was performed on an Illumina HiSeq 2500 platform to a target depth of 50 million paired-end reads per sample (2). Transcriptomic data was analyzed using our comprehensive pipeline of computational, bioinformatics and statistical tools successfully implemented in our group to analyze CAR T cell therapy samples (2). GSEA analysis (https://www.gsea-msigdb.org/gsea/index.jsp) was carried out using default parameter settings. Hallmark gene sets (MSigDB H), published gene sets (MSigDB C5) and curated gene sets of gene ontology from the biomedical literature (e.g., MSigDB C2, C7) were used for analysis.

Functional Analysis of TET2 Expression Constructs

Following denaturation, DNA samples were diluted and spotted on a polyvinylidene difluoride membrane. The blotted membrane was washed, gently vacuum-baked, blocked, and incubated at 4°C overnight with anti-5-mC (Abcam), anti-5-hmC or anti-5-caC (1:10,000) antibodies (Active Motif). Blots were subsequently washed and incubated with a 1:2,000 dilution of a secondary horse anti-mouse horseradish peroxidase (HRP; Cell Signaling Technology) or 1:5,000 goat anti-rabbit-HRP (Santa Cruz Biotechnology) for 2-hours. Imaging was performed using the Immobilon Western Chemiluminescent HRP Substrate (Millipore) and the Amersham Imager 600 (GE Healthcare Life Sciences). FLAG-tagged TET2 protein detection was carried out via Western blot analysis of whole cell lysates according to our published methods (3).

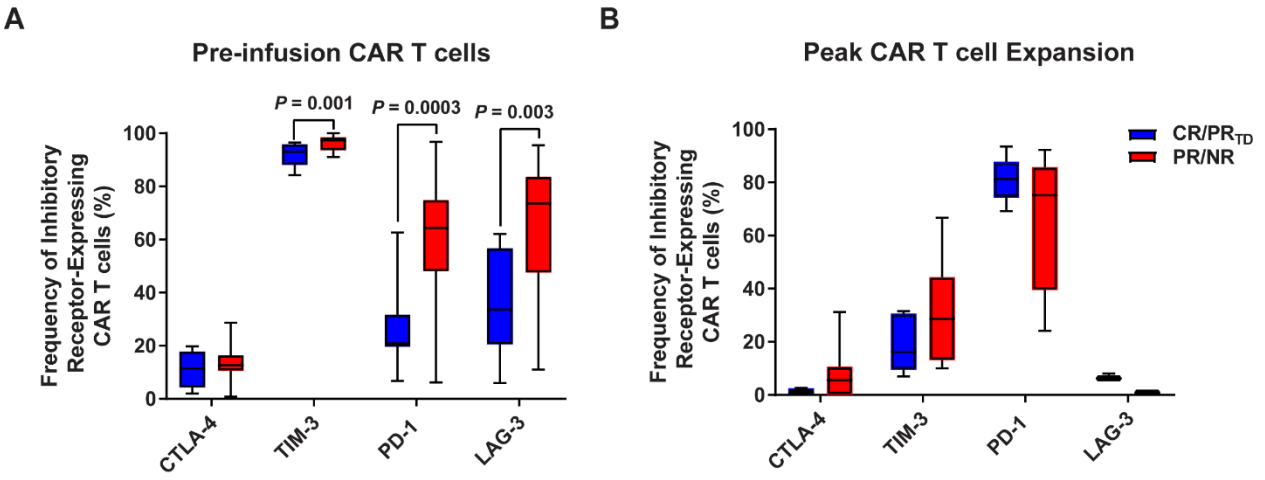
Statistical Analyses

Statistical analyses were performed using the D'Agostino-Pearson omnibus test for normality, the nonparametric Mann–Whitney U test, parametric Student's *t*-test or ANOVA as appropriate for paired or unpaired samples. When the sample size was too small to adequately examine normality,

nonparametric statistics were used. Spearman's rho or Pearson's statistics were used for measurements of correlation. Specific tests applied for different study variables are described in the figure legends. Statistical analyses were performed using SAS (SAS Institute Inc.), Stata 14.0 (StataCorp) or Prism 8-GraphPad (GraphPad Software). Where appropriate, unadjusted and adjusted *P* values are reported in figures and tables. *P* values < 0.05 were considered to be statistically significant.

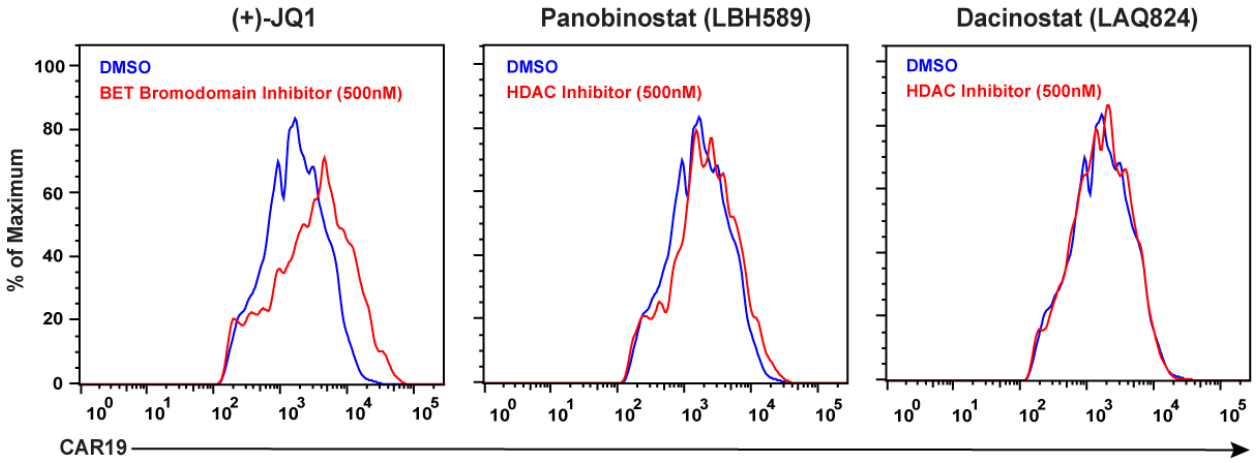
Supplemental Figures

Supplemental Figure 1



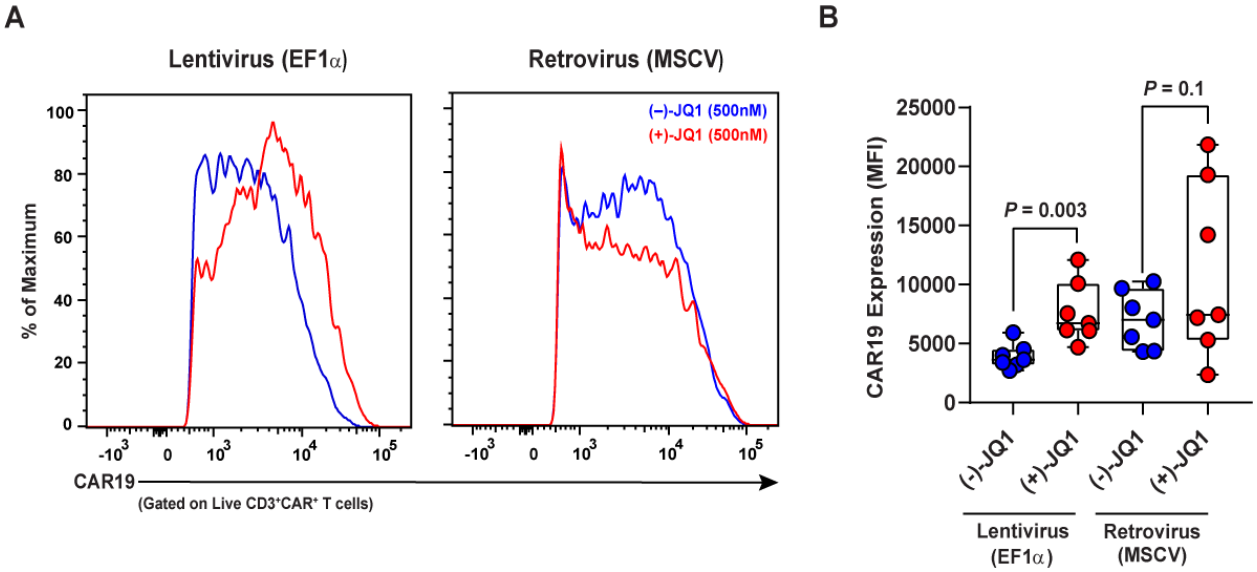
Supplemental Figure 1. Frequencies of pre- and post-infusion CAR T cells expressing inhibitory receptors. (**A**) Percentages of $CD8^+CAR^+$ T cells expressing CTLA-4, TIM-3, PD-1 and LAG-3 in infusion products and (**B**) post-infusion PBMCs at the time of peak expansion. Boxes extend from the twenty-fifth to seventy-fifth percentiles; middle line, median; whiskers, minimum and maximum. *P* values were calculated using a paired *t* test.

Supplemental Figure 2



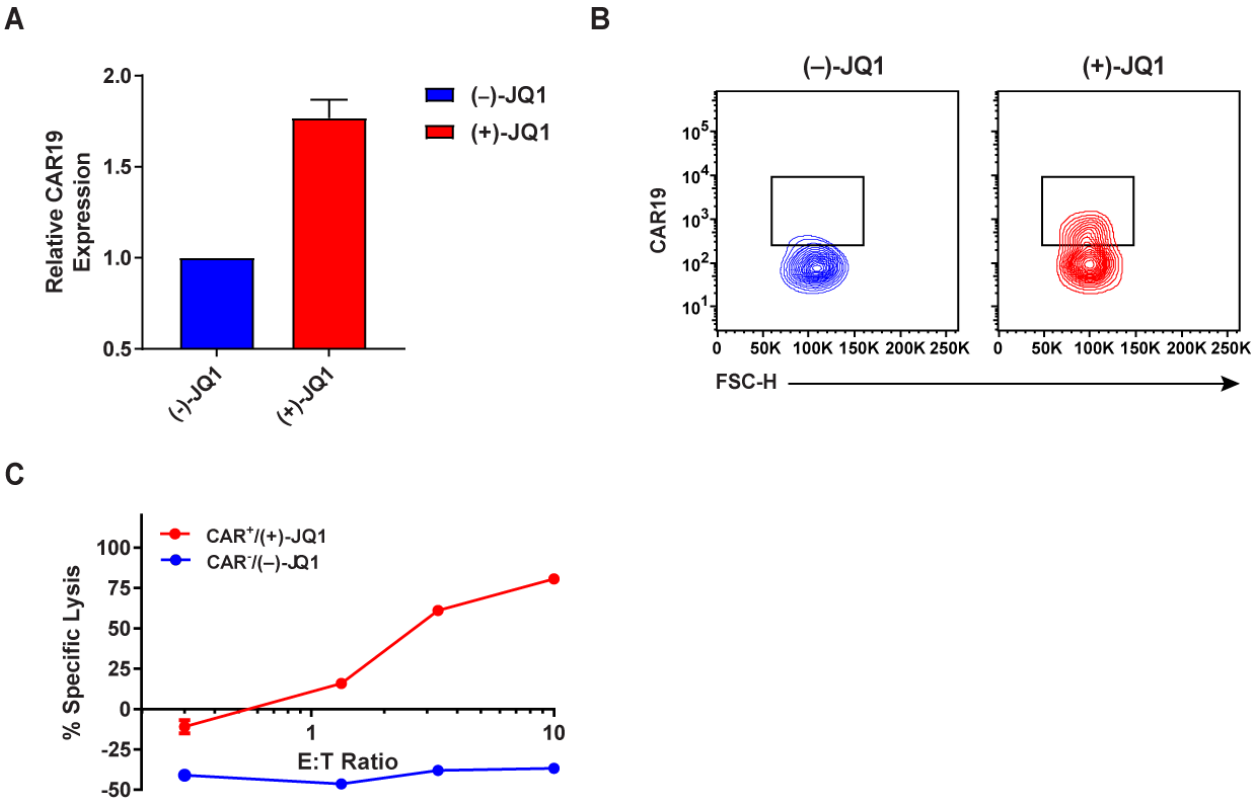
Supplemental Figure 2. Effects of epigenetic compounds on CAR expression in CLL patient T cells. CLL patient $CD8^+$ CAR T cells were treated with active JQ1, Panobinostat, Dacinostat (red histograms) or DMSO alone (blue histograms) for 4 days, followed by flow cytometric assessment of CAR19 expression. Results are representative of 3 independent experiments conducted with CAR T cells from 3 different patients.

Supplemental Figure 3



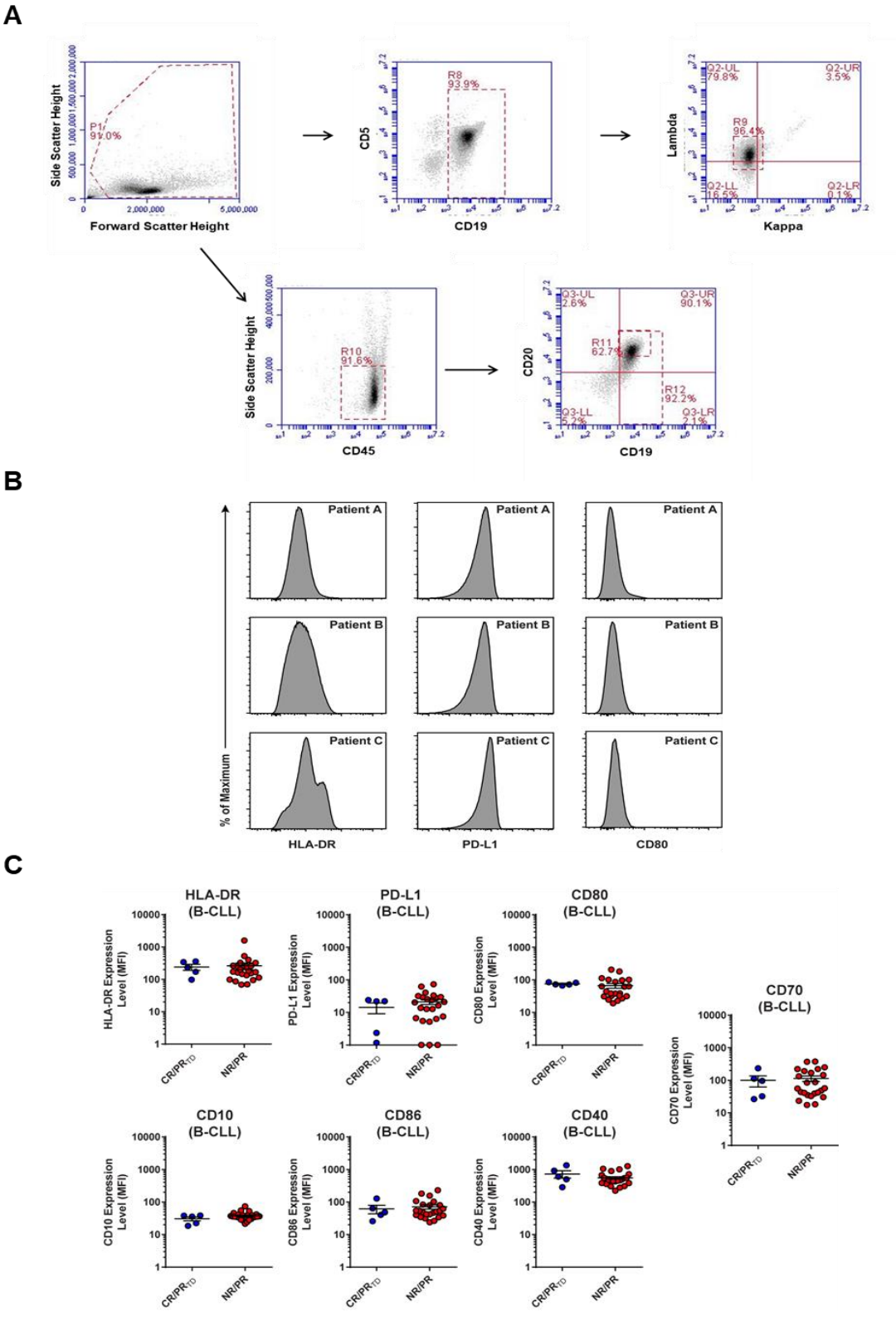
Supplemental Figure 3. Effect of JQ1 on CAR expression in T cells transduced with lentiviral versus retroviral vectors. Primary T cells (*n* = 7) were transduced with lentiviral or retroviral vectors encoding CAR19 and subsequently treated with inactive (–)-JQ1 or active (+)-JQ1 for 4 days, followed by flow cytometric assessment of CAR expression. (**A**) Representative flow cytometry depicting cell-surface CAR levels on T cells treated with (–)-JQ1 (blue histograms) or (+)-JQ1 (red histograms) are shown. (**B**) Summary of CAR expression levels as indicated by mean florescence intensity (MFI) of an anti-idiotypic antibody used to stain T cells transduced with lentiviral (EF1α promoter) or retroviral (MSCV promoter) vectors. Boxes extend from the twenty-fifth to seventy-fifth percentiles; middle line, median; whiskers, minimum and maximum. *P* values calculated using a paired Student's *t*-test.

Supplemental Figure 4



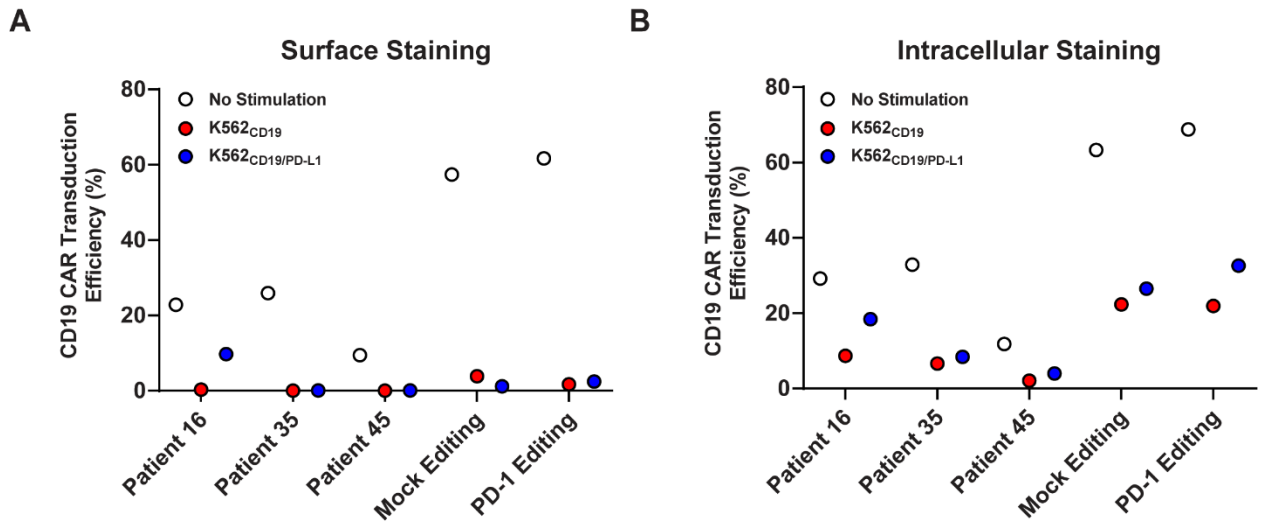
Supplemental Figure 4. Upregulation of CAR19 expression and anti-tumor function following active JQ1 treatment. Infusion product CAR T cells were treated with 500nM (–)-JQ1 or (+)-JQ1 for 4 days and CAR19 levels were assessed by (**A**) real-time RT-PCR and (**B**) flow cytometry. FSC-H stands for forward scatter height. (**C**) (–)-JQ1 or (+)-JQ1-treated CAR T cells were tested for cytolytic capacity following overnight co-culture with NALM-6 leukemia targets. E:T indicates the effector (CAR T cell) to target (NALM-6 cell) ratio.

Supplemental Figure 5



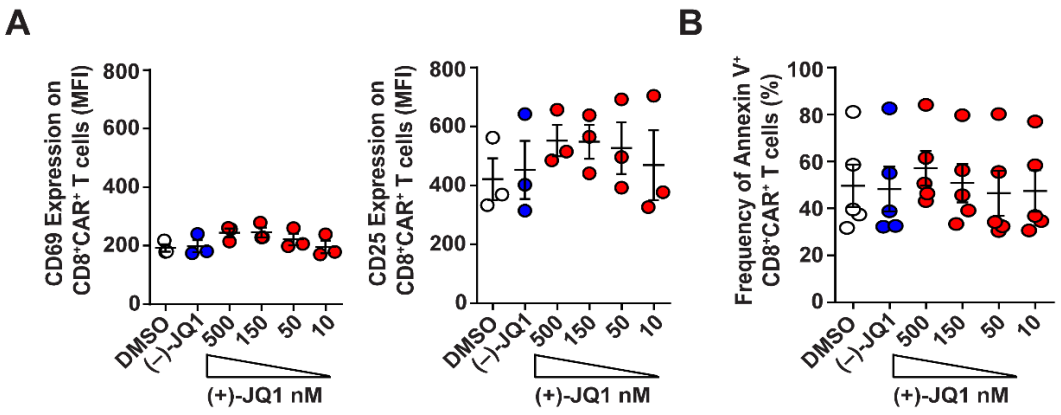
Supplemental Figure 5. Expression profiling of B cell chronic lymphocytic leukemia (B-CLL) cells in patients treated with CD19 CAR T cell therapy. (**A**) Gating strategy for B-CLL cells is shown. (**B**) Representative flow cytometric histograms showing expression levels of select markers (HLA-DR, PD-L1 and CD80) on B-CLL cells from 3 different patients are depicted. (**C**) The baseline levels of expression of HLA-DR, PD-L1, CD80, CD10, CD86, CD40 and CD70 on B-CLL cells at the time of leukapheresis in CR/PR_{TD} ($n = 5$) versus PR/NR ($n = 24$) patients are shown. The middle line depicts the mean and error bars indicate the SEM in each graph.

Supplemental Figure 6



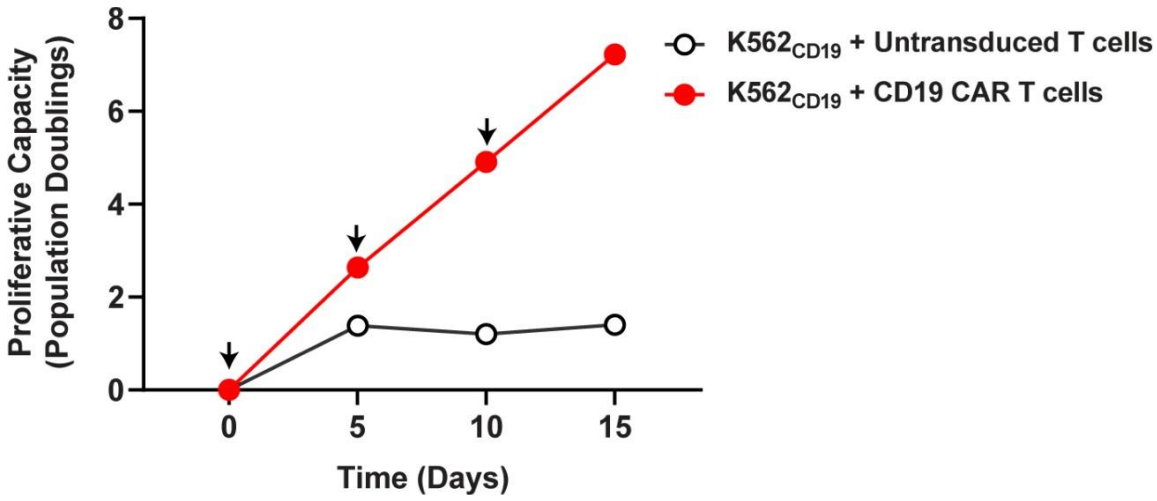
Supplemental Figure 6. Influence of PD-1 signaling on CD19 CAR transduction efficiency. (**A**) Percentages of CLL patient T cells expressing cell-surface and (**B**) intracellular CD19 CAR following stimulation with irradiated $K562_{CD19}$ or $K562_{CD19/PD\text{-}L1}$ targets. In a separate group, CAR T cells were mock-edited or PD-1 was knocked-out prior to co-culture with the indicated K562 cells.

Supplemental Figure 7



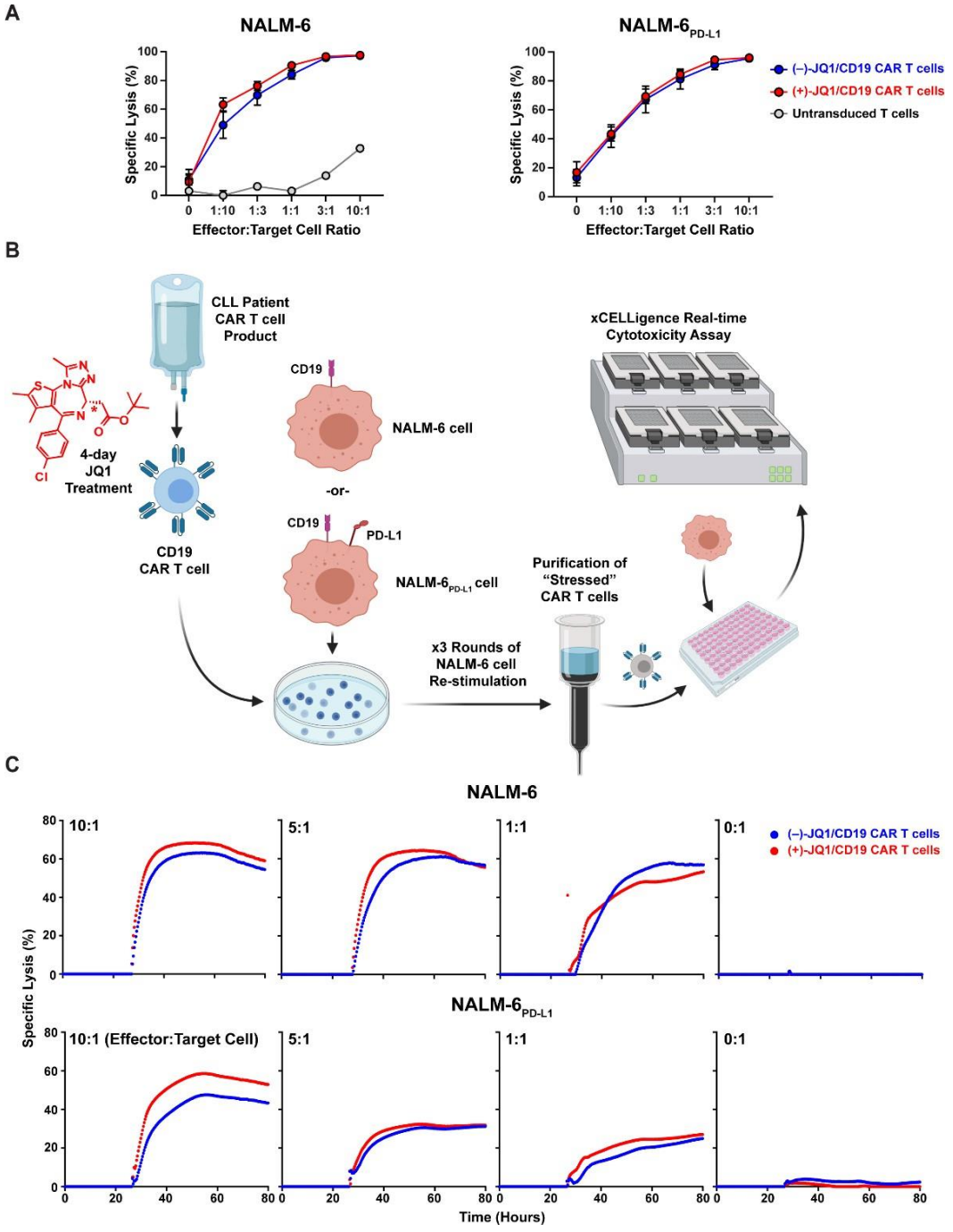
Supplemental Figure 7. Activation state and apoptosis sensitivity of JQ1-treated CLL patient CAR T cells. (**A**) Activation marker expression and (**B**) proportions of apoptotic $CD8^+CAR^+$ T cells from the above patients treated with 10-500nM (–)-JQ1 or (+)-JQ1 or DMSO alone.

Supplemental Figure 8



Supplemental Figure 8. Antigen-dependent proliferation of CD19 CAR T cells. Proliferative capacity of CD19 CAR T cells or donor-matched untransduced T cells serially re-stimulated with $K562_{CD19}$ targets. Black arrows indicate stimulation time points.

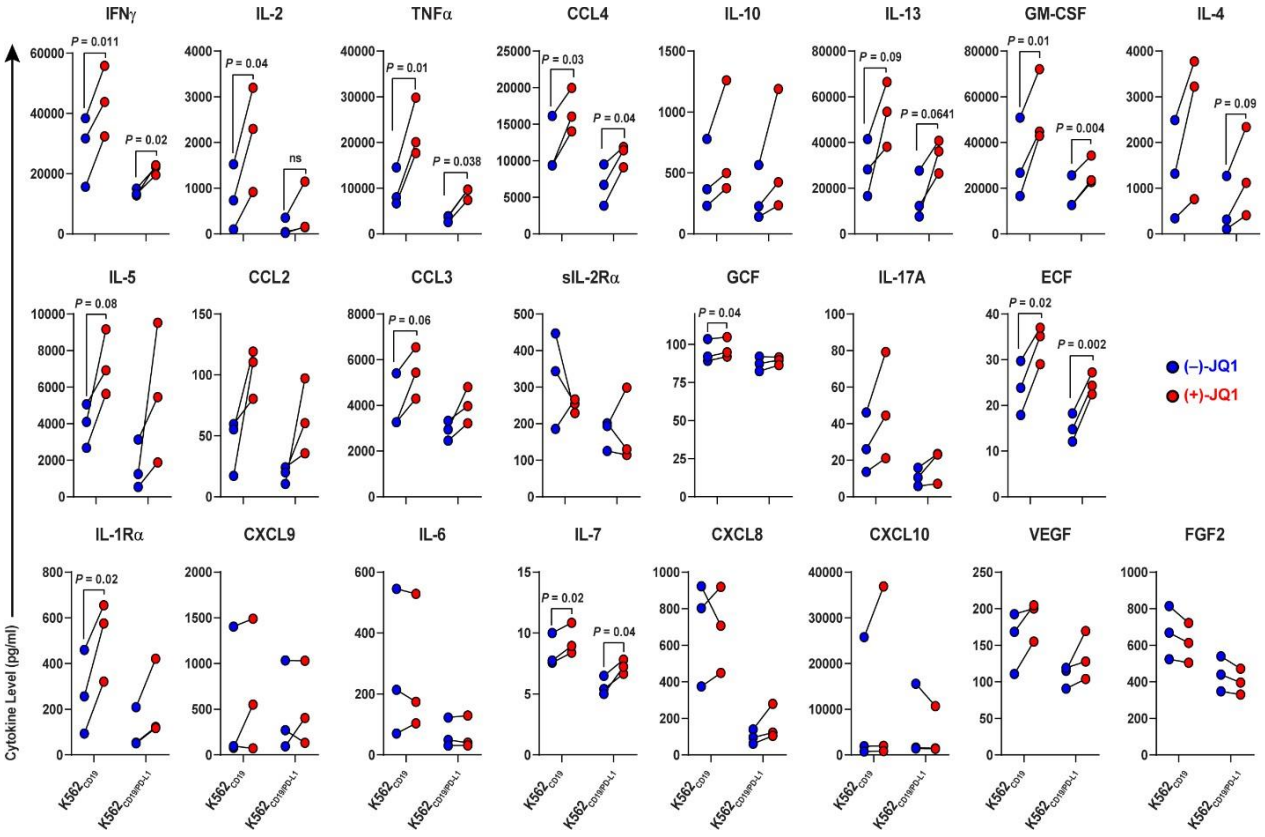
Supplemental Figure 9



Supplemental Figure 9. Cytotoxicity of CLL Patient CAR T cells treated with JQ1. (**A**) Cytotoxicity of CLL patient (n = 3) CD19 CAR T cells was tested after a 4-day treatment with 150nM (–)-JQ1 (blue) or (+)-JQ1 (red), followed by a 24-hour co-culture with luciferase

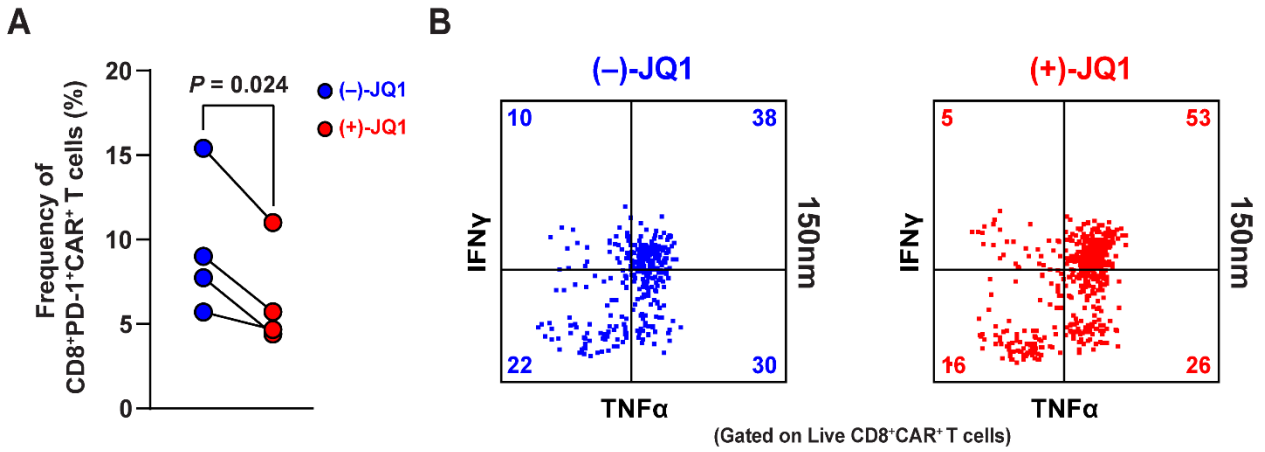
expressing NALM-6 or NALM-$6_{PD\text{-}L1}$ leukemia targets. Error bars denote SEM. (**B**) Schema depicting a "stressed" cytotoxicity assay in which the same JQ1-treated patient CAR T cells were serially re-stimulated prior to a real-time impendence-based longitudinal cytotoxicity assay. (**C**) Specific lysis measurements taken during the xCELLigence real-time cytotoxicity assay are shown over time.

Supplemental Figure 10



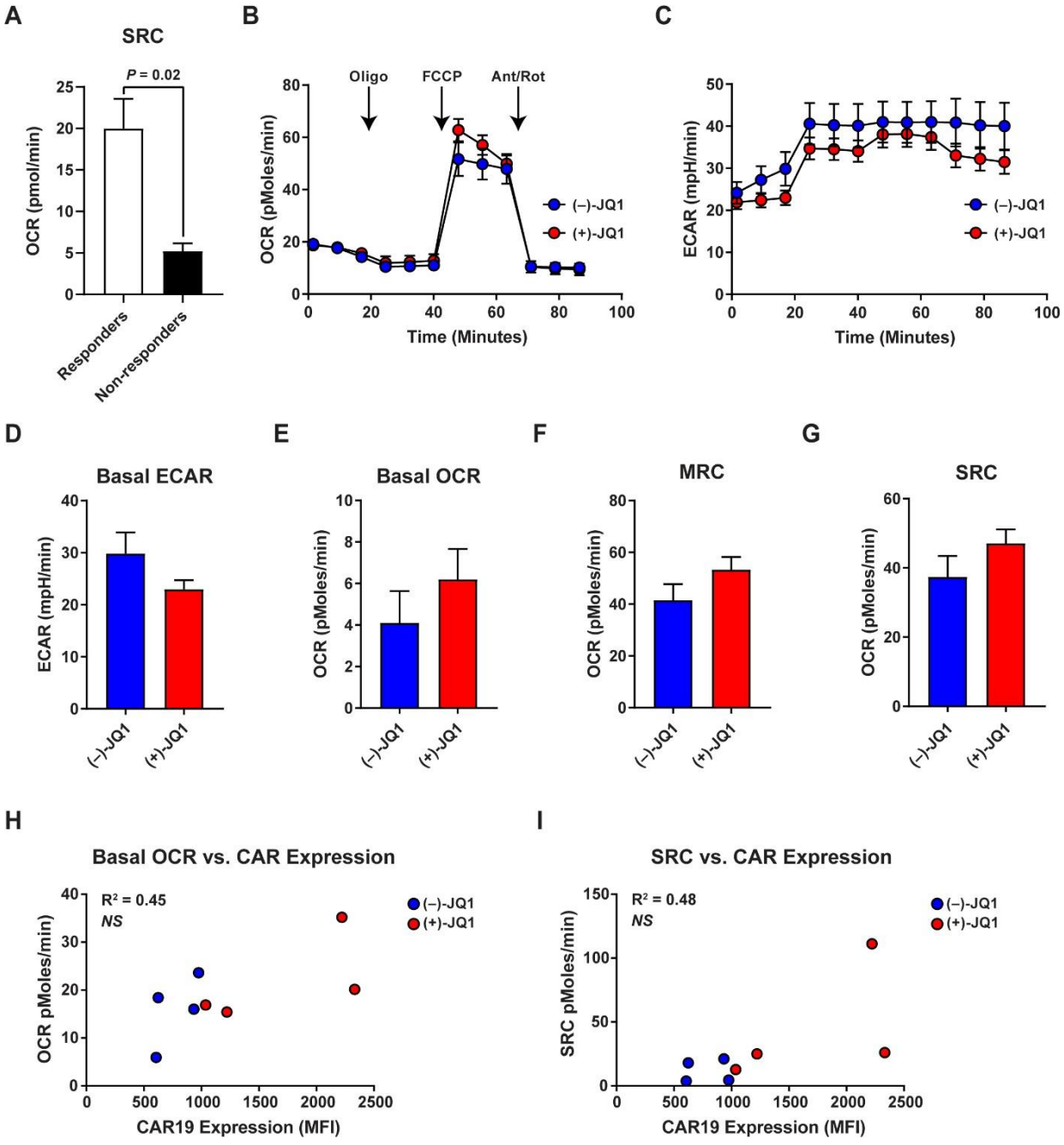
Supplemental Figure 10. Analysis of soluble factors produced by CLL Patient CAR T cells treated with JQ1. Elaboration of various cytokines, chemokines and growth factors by non-responding CLL patient (n = 3) anti-CD19 CAR T cells treated for 4 days with (–)-JQ1 or (+)-JQ1 and then stimulated with $K562_{CD19}$ or $K562_{CD19/PD\text{-}L1}$ targets. P values determined using a paired t-test.

Supplemental Figure 11



Supplemental Figure 11. Proportions of post-infusion CAR T cells from non-responding CLL patients expressing PD-1 and producing effector cytokines. (**A**) Frequencies of post-infusion $CD8^+CAR^+PD\text{-}1^+$ T cells following a 4-day exposure to (–)-JQ1 or (+)-JQ1 (n = 3, paired t test). (**B**) Dot plots showing proportions of IFNγ and/or TNFα-producing post-infusion $CD8^+CAR^+$ T cells following treatment with (–)-JQ1 or (+)-JQ1 and stimulation with PMA/ionomycin (data representative of cells from n = 3 patients).

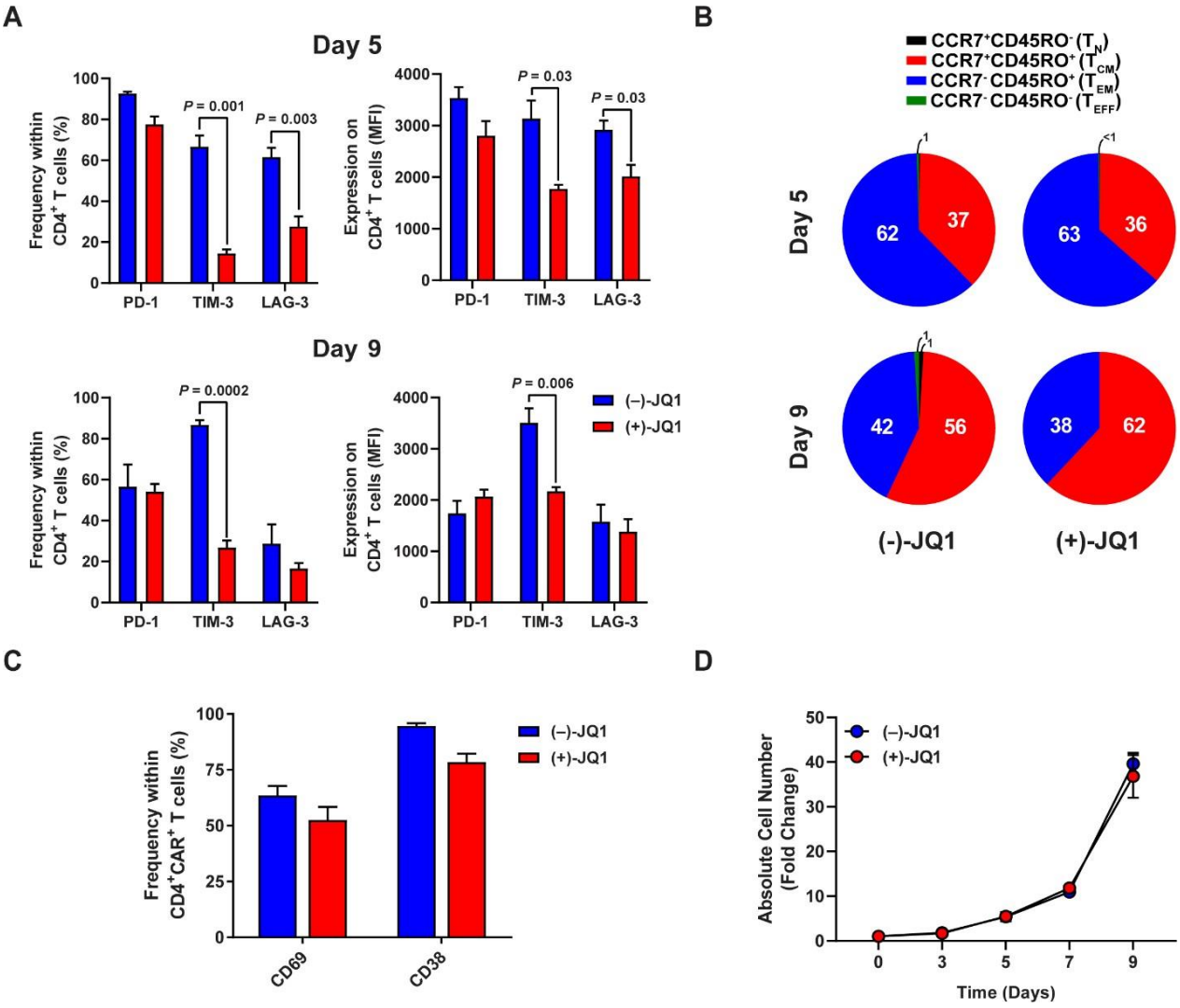
Supplemental Figure 12



Supplemental Figure 12. Metabolic profiles of CLL patient CAR T cells untreated or treated with JQ1. Metabolic flux profiling was performed on $CD8^+$ CAR T cells from (**A**) responder and non-responder CLL patients. Spare respiratory capacity (SRC) was used as a functional indicator

of metabolic fitness. *n* = 3 per group, Error bars depict SEM. (**B**) Longitudinal oxygen consumption rates (OCR) and (**C**) extracellular acidification rates (ECAR) were measured in purified $CD4^+$ T cells from CLL patient infusion products pre-treated for 4-days with 150nM (–)-JQ1 (blue) or (+)-JQ1 (red) during a mitochondrial stress test performed by injection of Oligomycin (Oligo), a mitochondrial decoupler (FCCP), followed by electron transport chain inhibitors, Antimycin A/Rotenone (Ant/Rot). (**D**) The basal ECAR and (**E**) OCR were measured prior to injection of inhibitors. (**F**) Maximal respiratory capacity (MRC) and (**G**) SRC of $CD4^+$ CAR T cells treated with (–)-JQ1 or (+)-JQ1 were determined following FCCP injection. (**B-G**) Same patients reported in Figure 5); error bars depict SEM. (**H**) Association of CAR19 expression levels and basal OCR or (**I**) SRC in CAR^+ T cells isolated from CLL patient infusion products treated with 150nM (–)-JQ1 (blue) or (+)-JQ1 (red). Results of Pearson's correlation shown in graph insets. *NS* = not statistically significant.

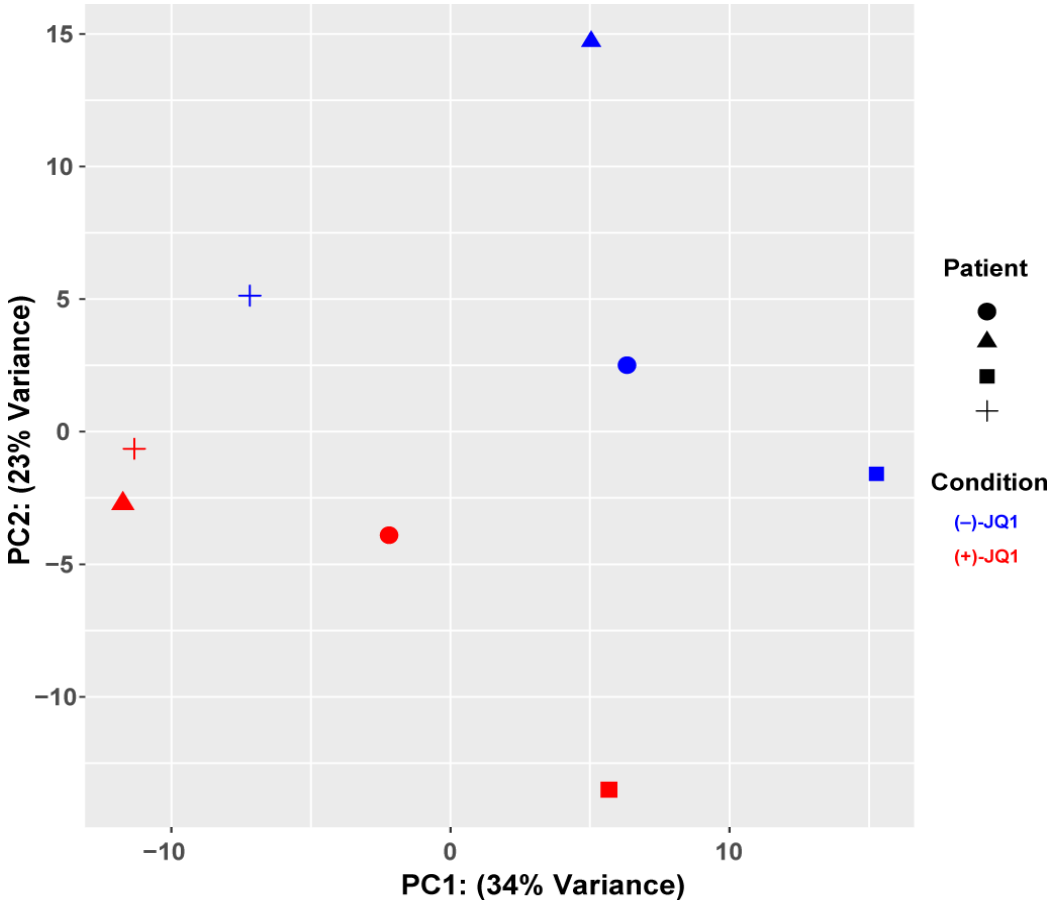
Supplemental Figure 13



Supplemental Figure 13. Effect of JQ1 treatment on CD4$^+$ T cell phenotype and bulk CAR T cell expansion. Bulk CD3$^+$ T cells were isolated from CLL patient PBMC and stimulated via CD3 and CD28 beads in the presence of 150nM (–)-JQ1 or (+)-JQ1. Early (Day 5) and late (Day 9) harvested CD4$^+$ T cells were analyzed for (**A**) the frequencies and mean fluorescent intensities (MFI) of inhibitory receptors (PD-1, TIM-3 and LAG-3) and (**B**) T cell differentiation status. T_N = naïve-like; T_{CM} = central memory; T_{EM} = effector memory; T_{EFF} = effector. (**C**) Frequencies

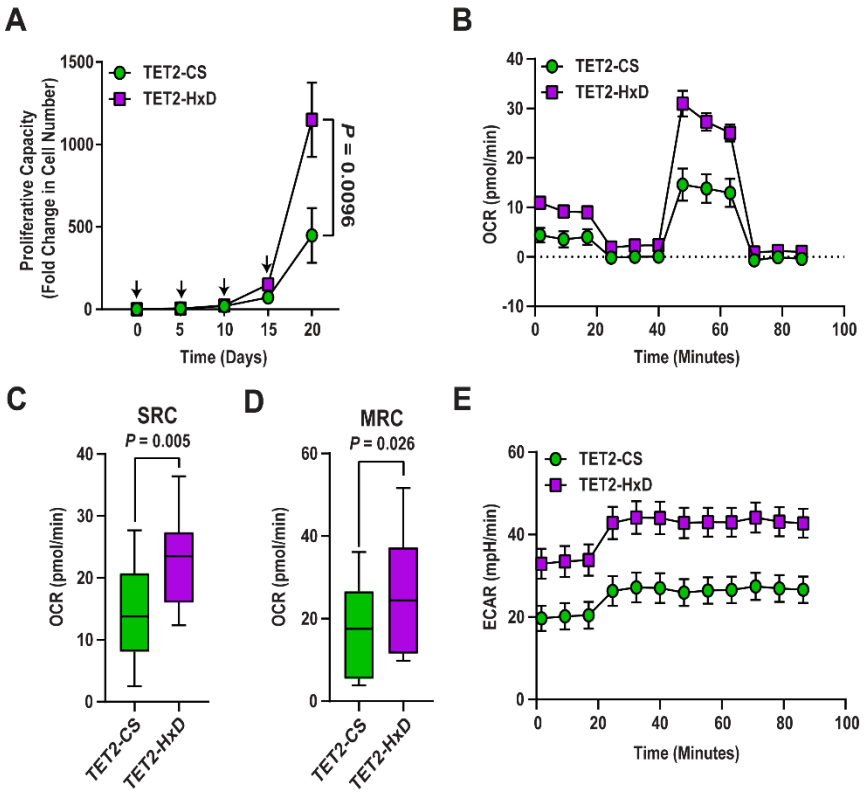
of $CD4^+CAR^+$ T cells expressing activations markers (CD69 and CD38) at the end of CART cell expansion are shown. (**D**) Changes in absolute numbers of T cells expanded in the presence of 150nM(–)-JQ1 or (+)-JQ1 are shown over the course of the initial CAR T cell expansion. Error bars depictthe SEM. *P* values were calculated using a paired *t* test.

Supplemental Figure 14



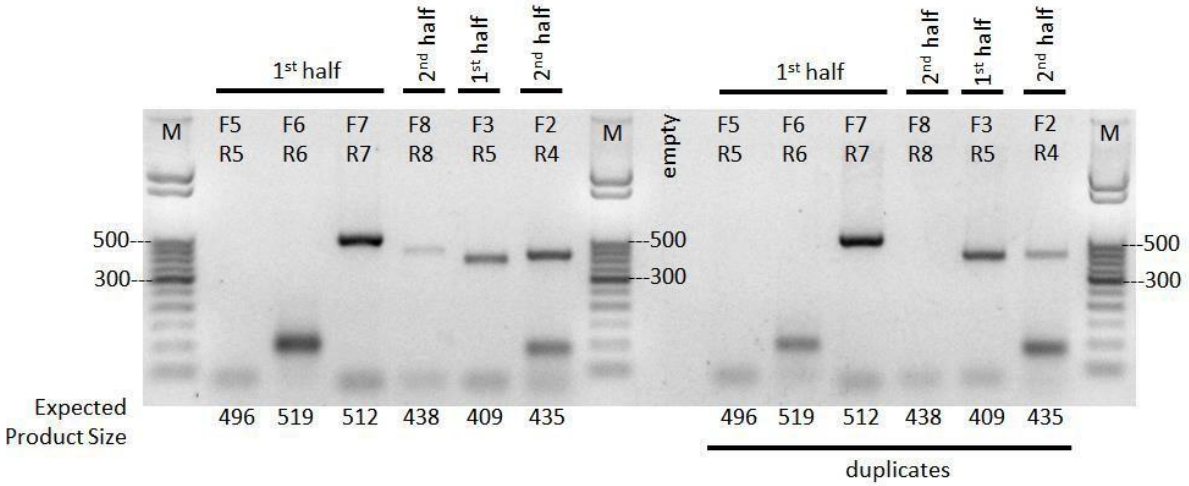
Supplemental Figure 14. Principal component analysis (PCA) of hypofunctional CLL patient CD8$^+$ CAR T cells treated with JQ1. Projection of RNA-sequencing data corresponding to relapsed/refractory CLL patient CD8$^+$ CAR T cells treated with (–)-JQ1 or (+)-JQ1 for 4-days (*n* = 4) onto the first two principal components. The percent variance explained by each component is provided in the axis labels. Each shape represents a different CLL patient. (–)-JQ1 or (+)-JQ1 treatments are differentially colored.

Supplemental Figure 15



Supplemental Figure 15. Proliferative capacity and metabolic fitness of CLL patient CAR T cells in the setting of TET2 overexpression. (**A**) CLL patient CAR^+ T cells were transduced with TET2-CS or TET2-HxD constructs and subsequently stimulated with irradiated $K562_{CD19}$ cells. Black arrows depict stimulation time points ($n = 4$, two-way ANOVA). (**B**) The OCR of CLL patient CAR T cells transduced with TET2-CS or TET2-HxD constructs was quantified over time during a mitochondrial stress test. (**C**) The SRC and (**D**) MRC of these engineered cells were measured after FCCP injection. (**E**) The ECAR of these cells was also longitudinally quantified during the same mitochondrial stress test ($n = 5$; paired *t* test).

Supplemental Figure 16



Supplemental Figure 16. PCR amplification of the targeted relevant regions of the EF1α promoter in bisulfite converted DNA originally isolated from CLL patient CAR T cells. Various primer combinations were used in duplicate PCR reactions to generate EF1α amplicons of the expected sizes. Two different primer combinations were employed for more complete coverage of CpG sites within the promoter sequence. From the above results, the best performing primer pair for the first half of the EF1α promoter was F7R7 and F2R4 for the second half. F = forward primer; R = reverse primer; M = DNA ladder. Expected product sizes (bp) are listed below each gel picture.

Supplemental References

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