Supplemental MATERIALS AND METHODS:

Experimental design

All experiments have been repeated and results reproduced. Where possible, error bars or p-values are shown to indicate statistical significance. In some Figures, error bars are not visible due to their short heights relative to the size of the symbols. $P < 0.05$ was considered statistically significant.

Cell Lines and Cell Culture

The ATLL cell lines were kindly provided by the following researchers: Michiyuki Maeda (Kyoto University; ED40515(-), ED41214C(-), ATL43Tb(-), and ATL55T(+)), Yasuaki Yamada and (Nagasaki University; ST1, KOB, KK1), Tomoko Hata (Nagasaki University; ST1), Naomichi Arima (Kagoshima University; Su9T01, S1T), Kazuo Sugamura (Tohoku University; TL-Om1). All lines were cultured with RPMI media containing 10% fetal calf serum (FCS), penicillin and streptomycin, with IL-2-dependent lines (ATL55T(+), KOB, KK1 and TL-Om1) cultured with the addition of human recombinant IL2 (100IU/mL, Hoffmann-La Roche) to this media, and were maintained in a humidified, 5% CO2 incubator at 37 °C. Mycoplasma contamination was eradicated by Mycoplasma Removal Agent. All cell lines were tested for unique profiles of polymorphic DNA copy number variants (CNV fingerprint; unpublished protocol from L. Bergsagel). All ATLL cell lines expresses HBZ but not Tax protein, which was confirmed by western blot in our previous study[1] and in Supplemental Figure 7. ATLL cell lines were engineered to express ecotropic retroviral receptors and TET repressor and the blasticidin resistance gene for transduction of lentiviral sgRNA vectors and retroviral expression vectors as previously described. For Cas9-mediated gene inactivation, KK1, ST1, Su9T01, ED40515(-) and TL-Om1 cell lines were engineered to express human codon-optimized S. pyogenes Cas9 using the pTO-Cas9-hygro vector (Cas9 from lentiCRISPR v2 ligated into pRCMV/TO-hygro vector) or lentiCas9-Blast. LentiCRISPR v2 and lentiCas9-Blast were gifts from Dr. Feng Zhang (Addgene plasmid # 52961 and # 52962, respectively).

Compounds and antibodies

Palbociclib was purchased from AdooQ BioScience. APR-246, everolimus, and

AZD8055 were purchased from Selleck Chemicals. The antibodies used in this study were purchased as following: p-Rb (D59B7, 8180), Rb (4H1, 9309), c-JUN (60A8, 9165), JUND (D17G2, 5000), p-STAT3 (D3A7, 9145), STAT3 (79D7, 4904), Cyclin D1 (92G2, 2978), Cyclin D2 (D52F9, 3741), Cyclin D3 (DCS22, 2936), CDK4 (DCS35, 1917), CDK6 (D4S8S, 13331), p21(12D1, 2947), p-S6 (2211), S6 (54D2, 2317), p-4EBP1 (9451), 4EBP1 (53H11, 9644), and mTOR (2972) from Cell Signaling Technology; JUNB (C-11, sc8051), p27 (C-19, sc528), Actin (AC-15, sc69879), GAPDH (V-18, sc20357), IRF4 (M-17, sc6059) and IRF4 (N-18, sc11450) from Santa Cruz Biotechnology; p53 (DO-7) from BioLegend); Rabbit-HBZ serum (Arnold et al., 2008); CDK2 (A18000) from ABclonal; BATF3 (3H1, H00055509-M04) from abnova. For flow cytometry, antibodies used in Supplemental Figure 6A were CD14 (clone: M5E2) and CD3 (clone: SP34-2) from BD biosciences, CD19 (clone: HIB19), CD25 (clone:M-A251), CD8 (clone: RPA-T8), CD4 (clone: RPA-T4) and CCR4 (clone: L291H4) from BioLegend, live/dead (eBioscience™ Fixable Viability Dye eFluor™ 780) from eBioscience. CD3 (558117) from BD Pharmingen, CD4 (300530) and CD8a (301048) from BioLegend, and FOXP3 (17-4776-42) from Invitrogen were used in Supplemental Figure 6C.

Whole-genome CRISPR library Screen

sgRNA library screening was performed as previously described[1]. Cell lines were transduced in duplicate with the Brunello CRISPR knockout pooled library (gift of David Root and John Doench; Addgene #73178). Following selection with puromycin (2 mg/mL) for 4 days, a cell aliquot was frozen as the day 0 sample. Cells were cultured for an additional 4 weeks, and an end point cell aliquot was harvested for genomic DNA extraction by QIAamp DNA blood Maxi kit (QIAGEN). sgRNA sequences were amplified by NEBNext High-Fidelity 2x PCR Master Mix (NEB) from the genomic DNA using the indexed PCR primers with next-generation sequencing adapters compatible with Illumina's NEXTSeq500. The primers are listed below. PCR products were size-selected using E-Gel, quantified by Qubit (Thermo Fisher Scientific) and sequenced using NEXTSeq500 (Illumina). Sequenced libraries were de-multiplexed using indexes compatible with the Illumina TrueSeq HT kit. The sgRNA read count and calculation of log2 fold change (day 28 / day 0) were performed by MAGeCK algorithm. The sgRNAs having > 50 read counts at day 0 were included in our analysis.

i5 indexed PCR primer:

D501,AATGATACGGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCC
TACACGACGCTCTTCCGATCTATGCATGCTCTTGTGGAAAGGACGAAACACC
G

D502,AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCC
TACACGACGCTCTTCCGATCTTGCATGCAGTCTTGTGGAAAGGACGAAACAC
CG

D503,AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCC
TACACGACGCTCTTCCGATCTGCATGCATCGTCTTGTGGAAAGGACGAAACA
CCG

D504,AATGATACGGCGACCACCGAGATCTACACGGCTCTGAACACTCTTTCCC
TACACGACGCTCTTCCGATCTCATGCATGACGTCTTGTGGAAAGGACGAAAC
ACCG

D505,AATGATACGGCGACCACCGAGATCTACACAGGCGAAGACACTCTTTCC
CTACACGACGCTCTTCCGATCTCGTACGTATACATCTTGTGGAAAGGACGAAA
CACCG

D506,AATGATACGGCGACCACCGAGATCTACACTAATCTTAACACTCTTTCCC
TACACGACGCTCTTCCGATCTACGTACGTGTATATCTTGTGGAAAGGACGAAA
CACCG

D507,AATGATACGGCGACCACCGAGATCTACACCAGGACGTACACTCTTTCCC
TACACGACGCTCTTCCGATCTTACGTACGCGTGTATCTTGTGGAAAGGACGA
AACACCG

D508,AATGATACGGCGACCACCGAGATCTACACGTACTGACACACTCTTTCCC
TACACGACGCTCTTCCGATCTGTACGTACACCCGTATCTTGTGGAAAGGACG
AAACACCG

i7 indexed PCR primer:

D701,CAAGCAGAAGACGGCATACGAGATCGAGTAATGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt

D702,CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt

D703,CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAG
ACGTGTGCTCTTCCGATCtctactattctttcccctgcactgt

D704,CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D705,CAAGCAGAAGACGGCATACGAGATTTCTGAATGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D706,CAAGCAGAAGACGGCATACGAGATACGAATTCGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D707,CAAGCAGAAGACGGCATACGAGATAGCTTCAGGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D708,CAAGCAGAAGACGGCATACGAGATGCGCATTAGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D709,CAAGCAGAAGACGGCATACGAGATCATAGCCGGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D710,CAAGCAGAAGACGGCATACGAGATTTCGCGGAGTGACTGGAGTTCAG
ACGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D711,CAAGCAGAAGACGGCATACGAGATGCGCGAGAGTGACTGGAGTTCAG
ACGTGTGCTCTTCCGATCtctactattctttcccctgcactgt
D712,CAAGCAGAAGACGGCATACGAGATCTATCGCTGTGACTGGAGTTCAGA
CGTGTGCTCTTCCGATCtctactattctttcccctgcactgt

sgRNA Toxicity Assay

Each sgRNA was cloned into pLenti-sg-pgk-PG (Puro-GFP fusion) vector[1] and lentivirally infected in Cas9-expressing cells. sgRNA-expressing cells were monitored by GFP reporter over time by flow cytometry. The sgRNA target sequences used in this study are listed in the supplemental Table 4.

Rescue Experiment for sgRNA-Mediated Toxicity

Cells were first transduced with a pRCMV/TO-puro vector[1] encording the cDNA of sgJUNB-resistant JUNB, cJUN, JUND, sgSTAT3-resistant STAT3-wild type, sgSTAT3-resistant STAT3-D556N, sgRNA-resistant STAT3-D661Y, sgSTAT3-resistant STAT3-Y640F, CCND1, sgCDK2-resistant CCND2, CCND3, CDK4, and sgCDK6-resistant CDK6. The sgRNA-resistant cDNAs had silent mutations where original sequences were targeted by sgRNA of interests. Following purification of transduced cells using puromycin, cells were infected with pLenti-sg-pgk-PG co-expressing GFP and the

sgRNAs of interest. The fraction of viable, GFP+ cells was monitored over time by flow cytometry.

Cell Cycle Analysis

Following inducible expression of specific sgRNA or after treatment with small molecule inhibitors, the cells were fixed with PBS/2% Paraformaldehyde, permeabilized with PBS/1% FBS/0.25% saponin and then stained with PBS/PI (propidium iodide, 50 μg/ml)/RNase A (500 μg/ml). DNA content was analyzed with a FACSCANTOII (BD Biosciences) excluding the sub G1 phase.

Apoptosis Analysis

To measure the induction of apoptosis following expression of specific sgRNA or after treatment with small molecule inhibitors, the cells were stained with the APC Annexin V (BD Pharmingen) and PI (Sigma) according the manufacturer's instructions, and analyzed by flow cytometric quantitation.

Immunoblot Analysis

Cells were washed and resuspended in 2x SDS sample buffer and boiled for 5 minutes. Samples were separated on Novex 4-12% Tris-Glycine gel (Invitrogen) and transferred to a PVDF membrane (Millipore, Immobilon-P). Membranes were incubated with primary antibody overnight at 4°C. After wash three times with PBS/tween, Membranes were incubated with HRP-conjugated secondary antibody for 1 hour. For detection, ECL reagent from GE Healthcare was used.

Immunoprecipitation

10^7 cells were lysed with 1 ml of a modified RIPA buffer (1.0% Triton X-100, 20 mM Tris-HCl pH 7.6, 150 mM NaCl, 1 mM EDTA and 0.1 mM PMSF) supplement with protease inhibitors (Roche) and incubated on ice for 10 min. The cell lysates were cleared by centrifugation at 14,000 g for 15 min at 4 °C, and they were used for immunoblot analysis or were incubated overnight at 4 °C with the indicated primary antibodies (BATF3 antibody (AF7437, R&D), JUNB antibody (HPA019149, Sigma-Aldrich)) followed by incubation for 6 h at 4 °C with protein G beads. Beads were washed three times with the lysis buffer, then boiled for 5 min at 98 °C in 2× SDS sample buffer.

Samples were separated on Novex 4-12% Tris-Glycine gel (Invitrogen) and transferred to a PVDF membrane (Millipore, Immobilon-P) for western blot analysis.

Immunohistochemical evaluation

Tissue microarray blocks constructed from formalin-fixed paraffin-embedded specimens of T-cell lymphoma were cut into 4-µm-thick sections. The immunohistochemical staining was performed using the EnVision FLEX system (Dako) according to the manufacturer instructions. Pretreatment for antigen retrieval were performed using High pH Target Retrieval Solution (TRS) for CDK6 and Low pH TRS for JUNB at 97°C for 20 min. Antibodies to CDK6 (clone EPR4515, 1:500; abcam) and JUNB (clone C37F9, 1:500; Cell Signaling Technology) were incubated for 30 min at room temperature. Sections were counterstained with hematoxylin. The expression levels for CDK6 and JUNB were independently evaluated by two experienced pathologists (KCH and YM). Cases showing nuclear and cytoplasmic staining for CDK6 and those showing nuclear staining for JUNB were considered positive if 50% or more of the tumor cells were immunostained moderately (1+) or strongly (2+).

In vitro cell proliferation measurements

A total of 5,000 cells per well was seeded in a final volume of 100 ul with the indicated amount of Palbociclib, everolimus or AZD8055 either alone or in combination per well in 96-well plates. Two days after, 100 ul of fresh media with the indicated amount of each drug was added. Metabolic activity was measured after 4 days of Palbociclib treatment by using CellTiter 96®AQueous One Solution Cell Proliferation (MTS) assay (Promega) according to the manufacturer's instructions. The absorbance was read at 490nm using Spectra Max Paradigm 96-well plate reader (Molecular Devices). The background was subtracted using a media only control. Values were normalized to those of their no treatment controls and analyzed in GraphPad Prism 7.0 by non-linear regression to obtain the IC50 values. Unpaired one-sided t-test was used in Figure 5F

Determination of the combination index

To quantify the synergistic activity of palbociclib with mTOR inhibitors, combination index (CI) was calculated to determine the synergy of the two drugs using Compusyn (Combosyn Inc, Paramus, NJ) according to the Chou-Talalay algorithm[2] by the formula:

CI = [D]1/[Dx]1 + [D]2/[Dx]2. [D]1 and [D]2 are the concentrations of drug 1 and drug 2 to show a certain effect when treated with two drugs together. [Dx]1 and [Dx]2 are the concentrations that show the same effect with a combination of drug 1 and drug 2 when treated with each drug alone. Synergism can be defined as follows: CI < 1 indicates a synergistic effect; CI = 1 indicates an additive effect; CI > 1 indicates an antagonistic effect.

Human ATLL Samples

Written informed consent was obtained in accordance with the Declaration of Helsinki and was approved by the Investigational Review Board of the National Cancer Institute (NCI) and of Hokkaido University Faculty of Medicine. Peripheral blood mononuclear cells (PBMNCs) were isolated from ATLL patients by Ficoll-Hypaque.

Ex vivo cultures of PBMNCs from ATLL patients

Blood samples were obtained from ATLL patients under the care of the Clinical Trials Team, Lymphoid Malignancies Branch, NCI. This study protocol was approved by the Institutional Review Board of the NCI. Informed consent was obtained in writing in accordance with the Declaration of Helsinki. 10^5 of PBMNCs from chronic ATLL patients were cultured with PRMI/10%FBS/Penicillin-Streptomycin with the indicated concentration of drug in a well of 96 well plate for 6 days. The cells were pulsed with 1 μCi of 3H-thymidine for 6 hours, harvested and counted in a MicroBeta2 plate counter (Perkin Elmer).

TP53 mutation analysis

TP53 mutation analysis was performed in Leidos Biomedical Research, Inc. Briefly, 10 pairs of primers were designed to amplify the fragments containing all TP53 coding exons and splicing junctions. Amplicons were sequenced on illumina Miseq. Paired-end reads were analyzed used customized python scripts and CLC Genomics Workbench. Mutations with allele frequency greater than 4% were called.

Mice

All animal experiments were approved by the National Cancer Institute's Animal Care and Use Committee (NCI ACUC) and were performed in accordance with ACUC

guidelines. Female NSG mice (NOD scid gamma, NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ) were purchased from Jackson laboratory. $1x10^7$ ATL43Tb (-) cells were subcutaneously injected into the right flanks. When the average tumor volume reaches up to 50 mm^3, mice were divided into 4 groups; 6 mice in each group. Drugs were administrated intraperitoneally three days per week (12.5 mg/kg for Palbociclib and 2.5 mg/kg for Everolimus). $1x10^7$ Su9T01 (-) cells were subcutaneously injected into the right flanks. When the average tumor volume reaches up to 50 mm^3, mice were divided into 6 groups; 5 mice in each group. Drugs were administrated three days per week for 14 days (12.5 mg/kg intraperitoneally for Palbociclib, 15 mg/kg oral gavage for AZD8055 and 2.5 mg/kg intraperitoneally for Everolimus). The primary (1°) tumor size was assessed using caliper measurement to calculate tumor volumes (Vol.) plotted as mean tumor volume (mm^3 ± SEM) for each group. Quantification of bioluminescence flux (photons/second) was used as a surrogate measure of tumor burdens using a Xenogen IVIS imaging system (Caliper Life Science) after injecting 3 mg of D-luciferin/mouse intraperitoneally. The software Living Image version 4.1 was used to analyze bioluminescent signals as photons/second.

Data Availability

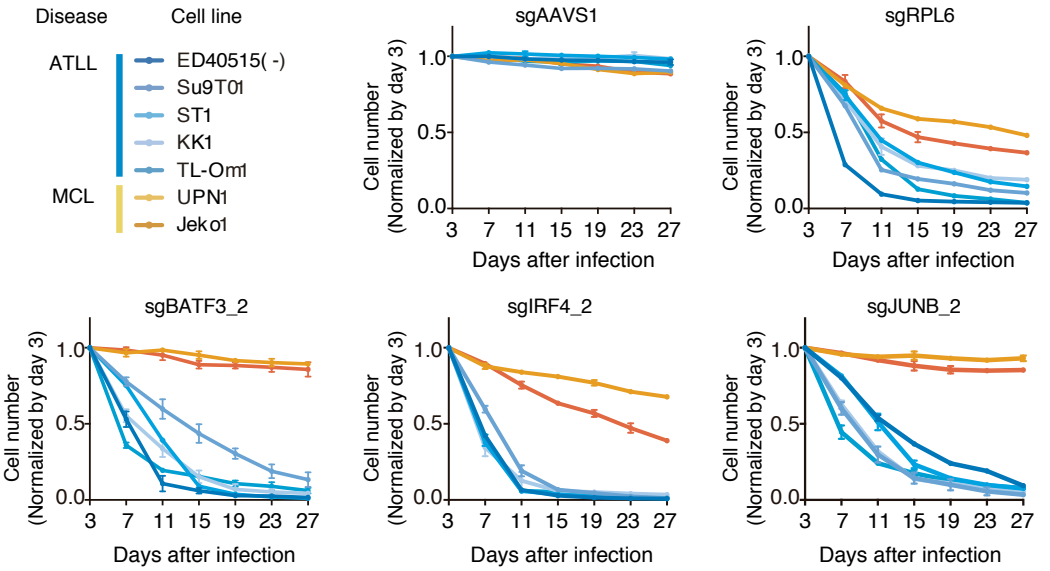
GSE94732. GSE6338, GSE14879, GSE19069 were used for analyzing microarray data of primary human T-cell lymphoma samples in Figure 4I.

1. Nakagawa M, Shaffer AL, 3rd, Ceribelli M, et al. Targeting the HTLV–I–Regulated BATF3/IRF4 Transcriptional Network in Adult T Cell Leukemia/Lymphoma. *Cancer Cell*. 2018;34(2):286–297 e210.

2. Chou TC. Drug combination studies and their synergy quantification using the Chou–Talalay method. *Cancer Res*. 2010;70(2):440–446.

Supplemental Figure 1

A



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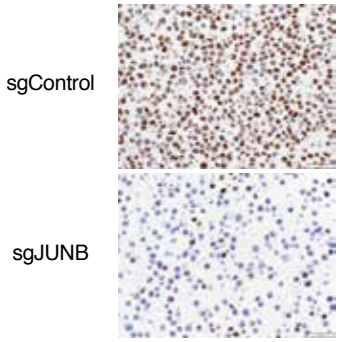

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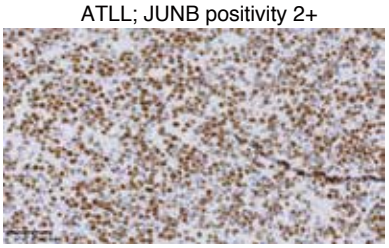
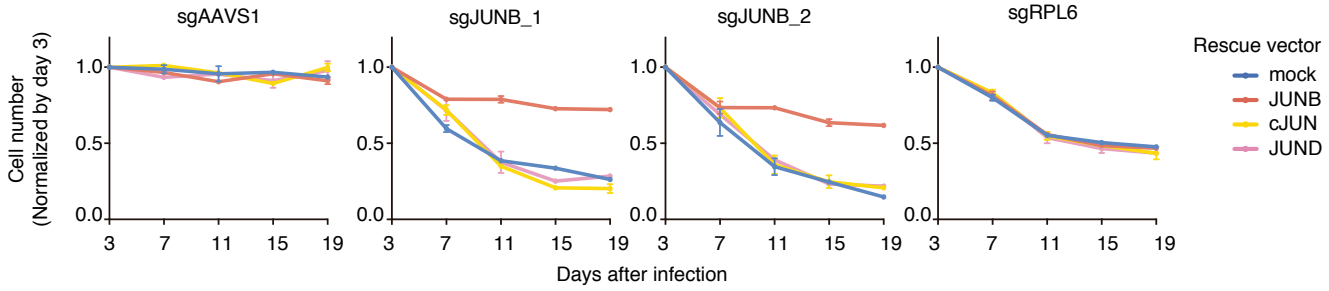


	JUNB positivity		
	0	1+	2+
ATLL (n=14)	0	5 (36%)	9 (64%)
PTCL-NOS (n=28)	13 (46%)	10 (36%)	5 (18%)

D

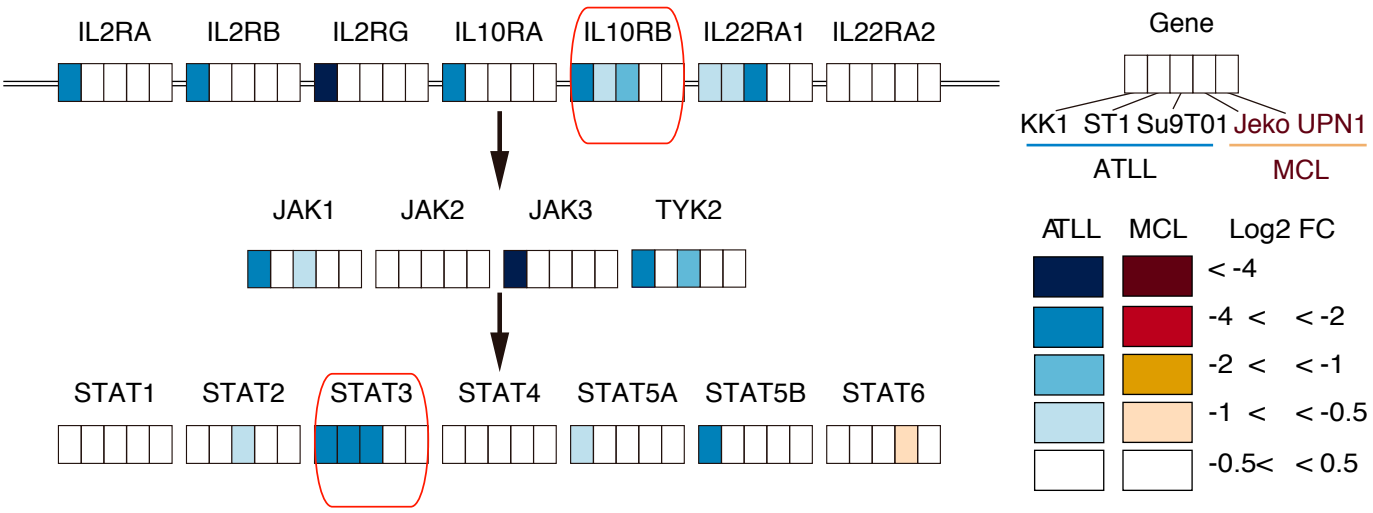


Supplemental Figure 1: BATF3 and JUNB are essential AP-1 factors in ATLL cells.

(A) The indicated cell lines were infected with a lentivirus expressing sgBATF3_2, sgIRF4_2 and sgJUNB_2 together with GFP. sgAAVS1 and sgRPL6 were used as a negative and positive control sgRNA, respectively. The GFP-positive cell fraction was monitored as in Figure 2A. (B) Immunohistochemical staining for JUNB in ED40515(-) cells transduced with sgControl or sgJUNB_1. (C) Representative images of lymph node biopsy samples immunohistochemically stained for JUNB. Upper panel shows a representative case from a cohort of 14 ATLL cases. Lower panel shows a summary of JUNB immunopositivities in biopsy samples in a cohort of ATLL (n = 14) and PTCL-NOS (n = 28) cases. (D) KK1 cells were transduced with retroviruses expressing an sgRNA-resistant JUNB, cJUN, JUND or with empty vector. After puromycin selection of transduced cells, cells were subsequently transduced with lentiviruses co-expressing GFP and either sgJUNB_1 or sgJUNB_2. sgAAVS1 and sgRPL6 were used as a negative and positive control sgRNA, respectively. The GFP-positive cell fraction was monitored as in Figure 2A. Error bars in (A) and (B) represent the SEM of replicates.

Supplemental Figure 2

A



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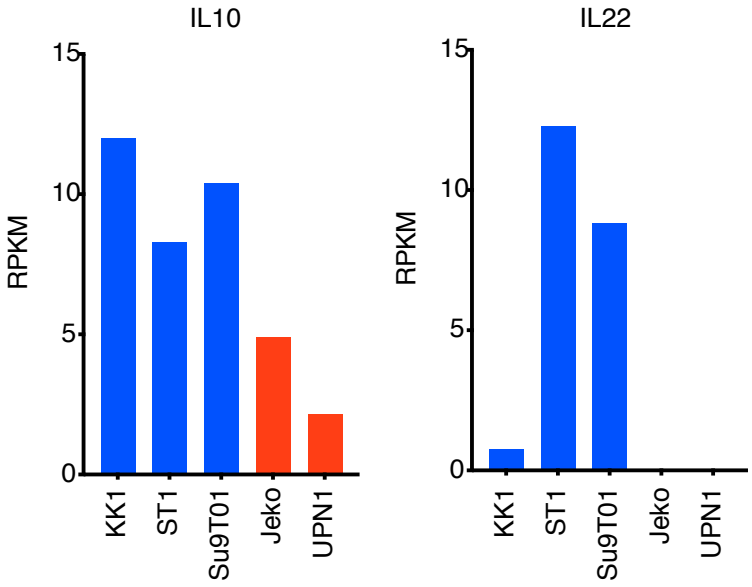

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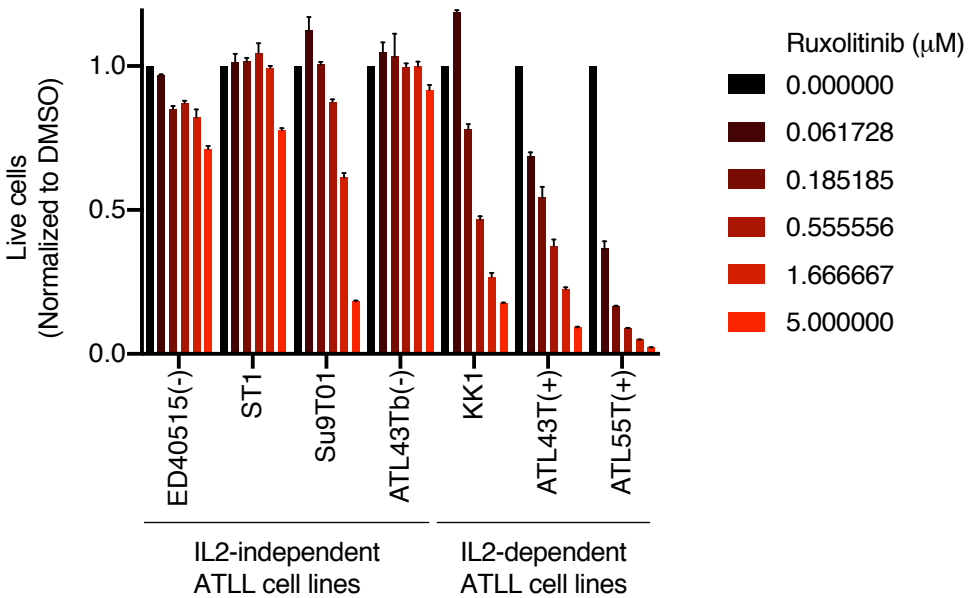
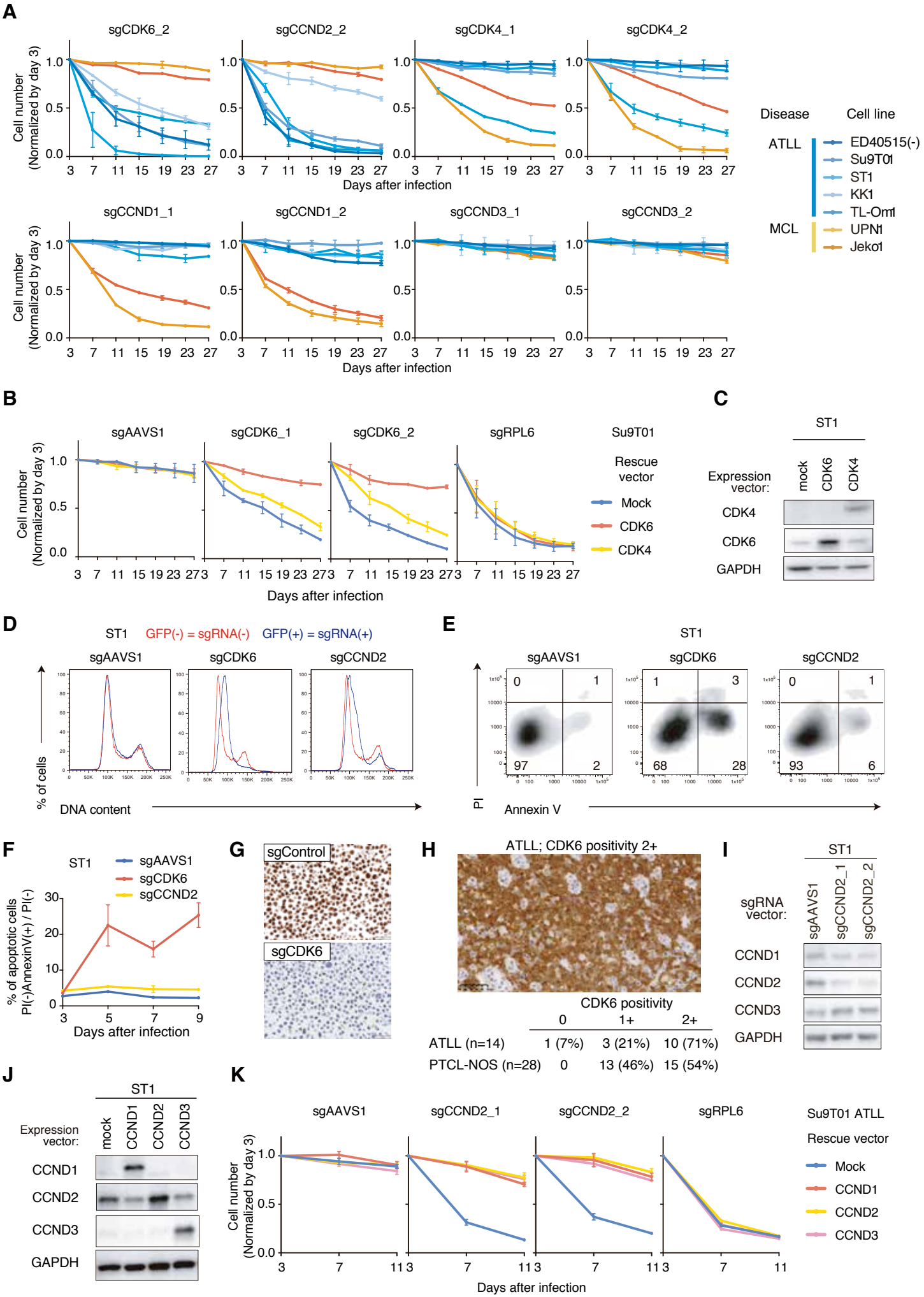


Supplemental Figure 2: STAT3 is an essential molecule in JAK/STAT signaling in ATLL.

(A) Shown were essentialities of genes in JAK/STAT pathway from whole-genome CRISPR library screening (Figure 1). (B) The amount of mRNA expression of IL10 and IL22 in ATLL and MCL cell lines. Y axis represents the values of RPKM from RNA-seq. (C) Viable cells were measured by the MTS assay for the indicated ATLL cell lines treated with FDA-approved JAK1/2 inhibitor ruxolitinib for 4 days. Error bars represent the SEM of replicates.

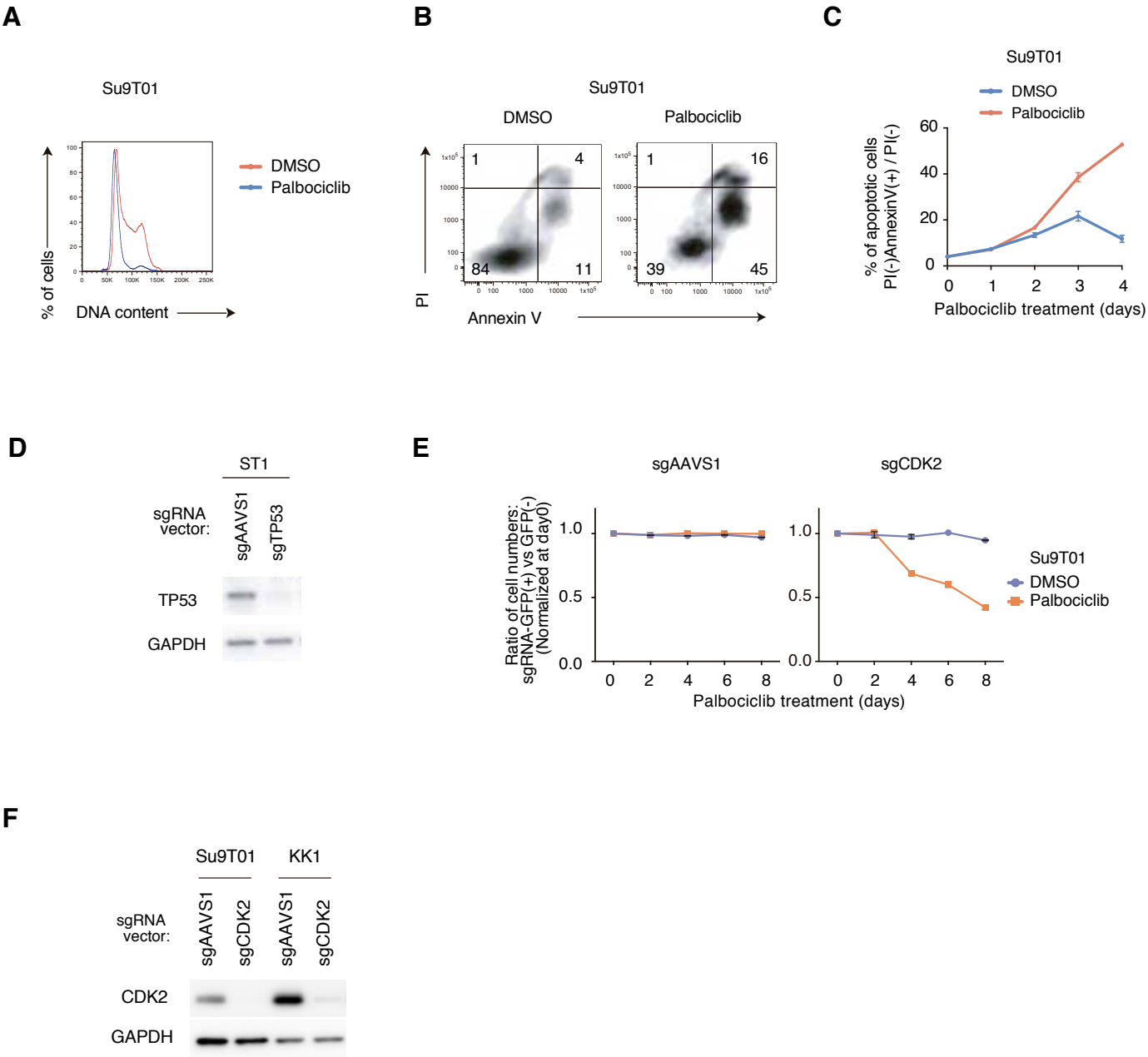
Supplemental Figure 3



Supplemental Figure 3: CDK6 and CCND2 are essential molecules in G1/S transition in ATLL cells.

(A) The indicated cell lines were infected with a lentivirus expressing sgCDK6_2, sgCCND2_2, sgCDK4_1, sgCDK4_2, sgCCND1_1,sgCCND1_2, sgCCND3_1 and sgCCND3_2 together with GFP. The GFP-positive cell fraction was monitored as in Figure 2A. (B) Su9T01 cells were transduced with retroviruses expressing an sgRNA-resistant CDK6, CDK4 or with empty vector. After puromycin selection of transduced cells, cells were subsequently transduced with lentiviruses co-expressing GFP and either sgCDK6_1 or sgCDK6_2. sgAAVS1 and sgRPL6 were used as a negative and positive control sgRNA, respectively. The GFP-positive cell fraction was monitored as in Figure 2A. (C) Immunoblot analysis of CDK6 and CDK4 protein in ST1 ATLL cells transduced with indicated cDNA-expressing vectors. (D) ST1 cells were transduced with vectors co-expressing GFP and either sgCDK6_1 and sgCCND2_1. DNA content was analyzed in the GFP-positive fraction (blue, the cells have sgRNA.) and GFP-negative cell fraction (red, the cells doesn't have sgRNA vector) on day 5 after sgRNA transduction. (E) The percents of apoptotic cells in ST1 were detected by analyzing annexin V and propidium iodide (PI) on flow cytometry on day 5 after sgRNA transduction. (F) The ratio of PI(-)Annexin V(+) cells/ PI(-) ST1 cells transduced with indicated sgRNAs were monitored over time. (G) Immunohistochemical staining for CDK6 in KK1 cells transduced with sgControl or sgCDK6_1. (H) Immunohistochemical staining for CDK6 expression in lymph node biopsy samples. Upper panel shows a representative case in a cohort of 14 ATLL cases. Lower panel shows a summary of CDK6 immunopositivities in biopsy samples in a cohort of patients with ATLL (n = 14) and PTCL-NOS (n = 28). (I) Immunoblot analysis of CCND1, CCND2 and CCND3 proteins in sgCCND2 -transduced ST1. (J) Immunoblot analysis of CCND1, CCND2 and CCND3 proteins in ST1 ATLL cells transduced with indicated cDNA-expressing vectors. (K) Su9T01 cells were transduced with lentiviruses expressing an sgRNA-resistant CCND2, CCND1, CCND3 or with empty vector. After puromycin selection of transduced cells, cells were subsequently transduced with lentiviruses co-expressing GFP and either sgCCND2_1 or sgCCND2_2. sgAAVS1 and sgRPL6 were used as a negative and positive control sgRNA, respectively. The GFP-positive cell fraction was monitored as in Figure 2A. Error bars in (A), (B), (F) and (K) represent the SEM of replicates.

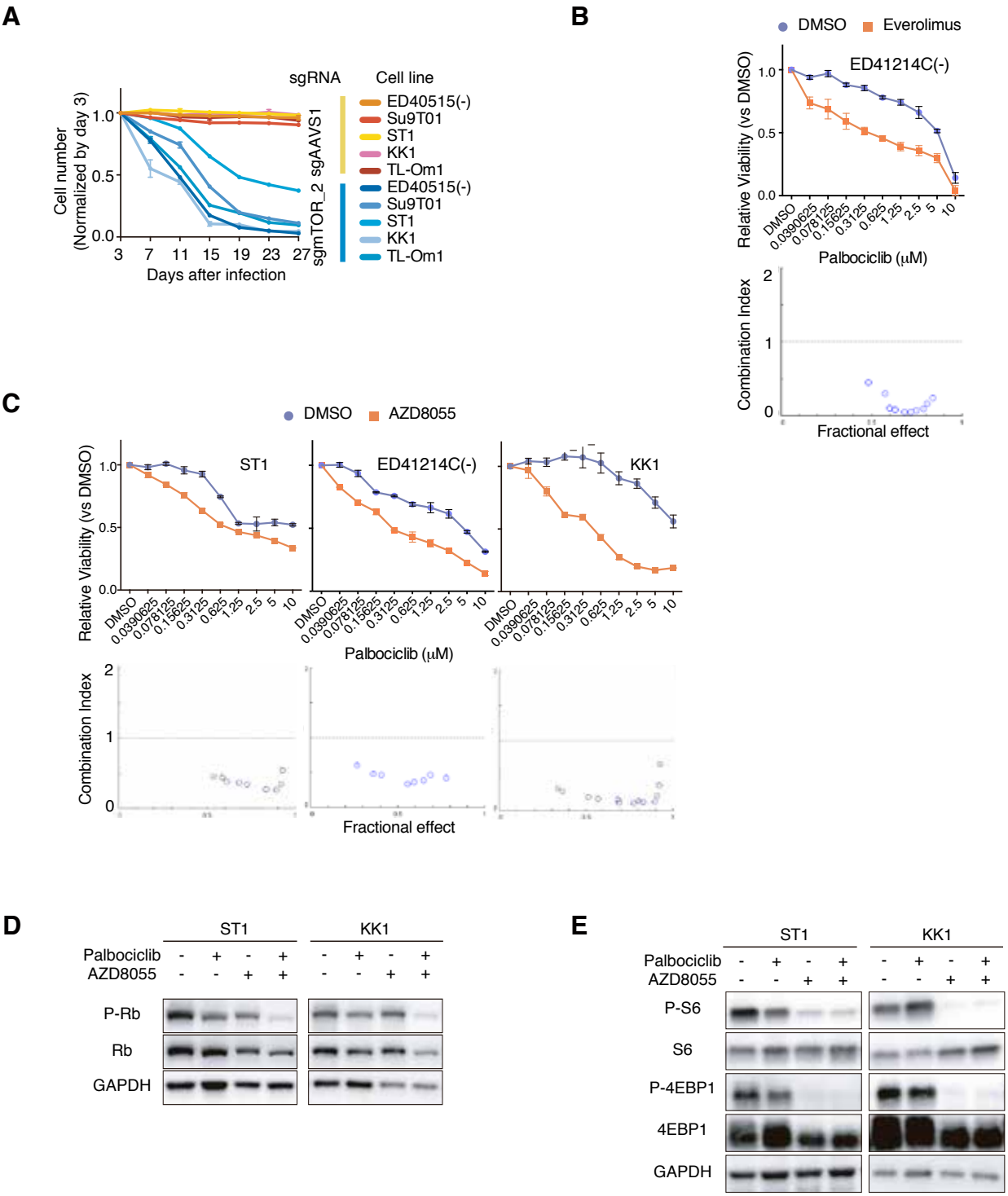
Supplemental Figure 4



Supplemental Figure 4: CDK4/6 inhibitor palbociclib inhibits ATLL cell proliferation and survival.

(A) DNA content was analyzed in Su9T01 ATLL cells treated with 1 μM of palbociclib for 24 hours. (B) Apoptotic Su9T01cells were detected by analyzing annexin V and PI on flow cytometry on day 4 after palbociclib treatment with 1 μM. (C) The ratio of PI(-)Annexin V(+) cells/ PI(-) Su9T01 cells were monitored over time. (D) Immunoblot analysis of TP53 protein in sgTP53-transduced ST1 cells. (E) Su9T01 cells were infected with a lentivirus that expresses sgCDK2 or control sgAAVS1 together with GFP, followed by treatment with palbociclib (0.25 μM). Shown is the fraction of GFP-positive cells over time relative to the GFP-positive fraction on day 0. (F) Immunoblot analysis of CDK2 protein in sgCDK2-transduced KK1 and Su9T01 cells. Error bars in (C) and (E) represent the SEM of replicates.

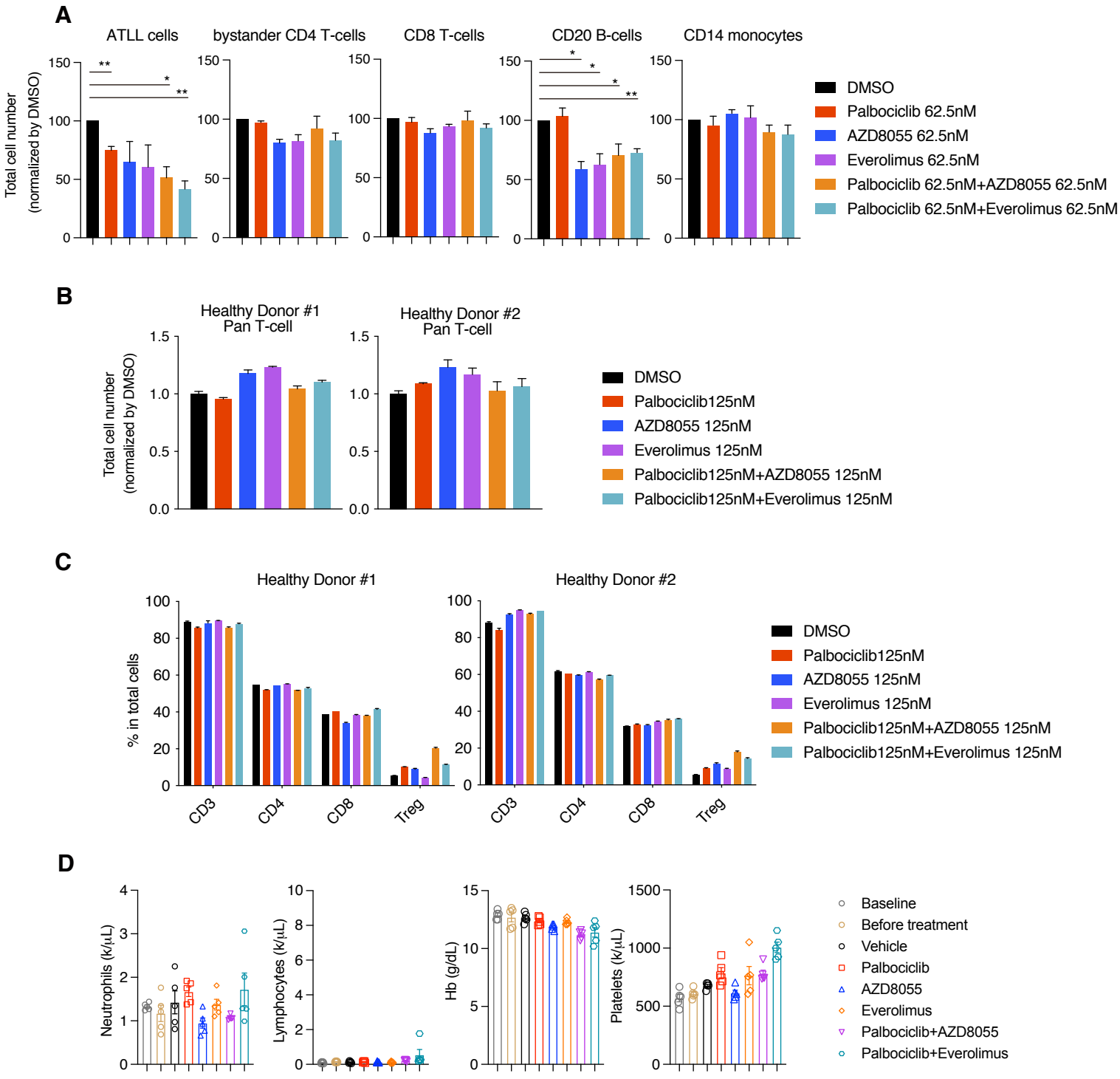
Supplemental Figure 5



Supplemental Figure 5: MTOR is a targetable vulnerability in ATLL and the combination with palbociclib and mTORC inhibitor is synergistically toxic for ATLL cells

(A) The indicated cell lines were infected with a lentivirus expressing sgMTOR_2 or control sgAAVS1 together with GFP. The GFP-positive cell fraction was monitored as in Figure 2A. Error bars represent the SEM of replicates. (B) Upper panel; Viable cells were measured by the MTS assay for the ED41214C(-) ATLL cell lines treated with the indicated concentrations of palbociclib and everolimus for 4 days. Lower panel; A combination index is shown. Lower than 1.0 indicates a synergistic effect. (C) Upper panel; Viable cells were measured by the MTS assay for the indicated ATLL cell lines treated with the indicated concentrations of palbociclib and AZD8055 for 4 days. Lower panel; A combination index is shown. Lower than 1.0 indicates a synergistic effect. (D-E) Immunoblot analysis of phosphorylated Rb (D), phosphorylated S6 (E) and phosphorylated 4EBP1 proteins (E) in ST1 and KK1 cells treated with palbociclib (0.5 μM and 1.25 μM, respectively) and AZD8055 (0.05 μM) for 24 hours. Error bars in (A) - (C) represent the SEM of replicates.

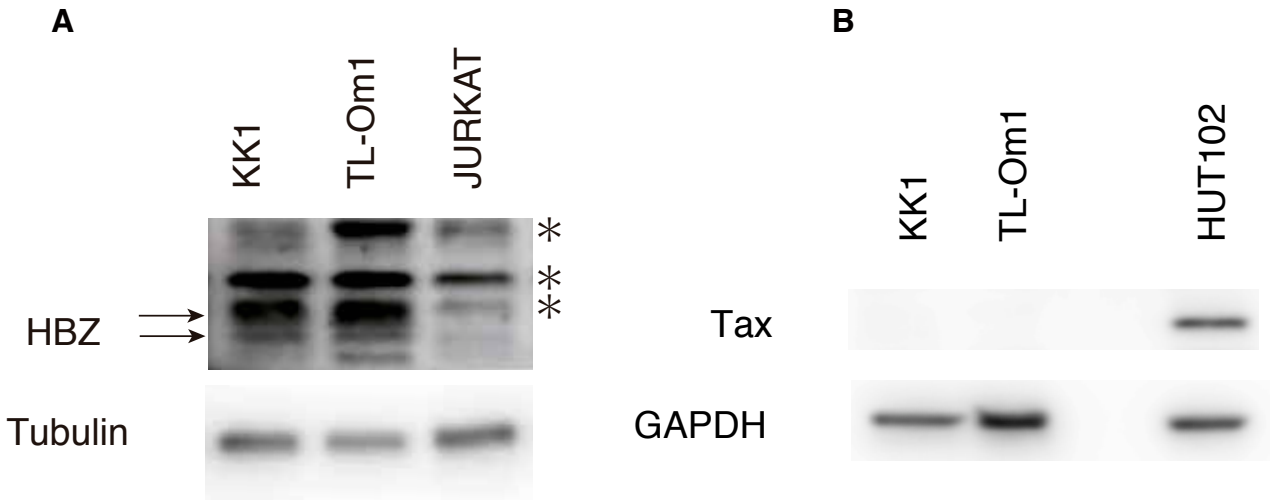
Supplemental Figure 6



Supplemental Figure 6: Combination with Palbociclib and MTOR inhibitors is effective in patient samples in vitro and xenograft in vivo

(A) Percentages of primary ATLL cells, bystander $CD4^+$ T cells, $CD8^+$ Tcells, $CD20^+$ B cells, and $CD14^+$ monocytes after five days of treatment with palbociclib, everolimus, and AZD8055, alone or in indicated combinations. PBMNC from patients with ATLL (#1–3) were treated with indicated drugs as Figure 7A and B and were analyzed by flow cytometry. The averages from different treatment conditions were normalized to that of the DMSO treatment. (B) The MTS assay was used to measure cellular viability after six days of treatment with indicated drugs in normal T cells isolated from two healthy donors. (C) Percentages of specific T-cell subpopulations in cultures shown in (B) treated with indicated drugs for five days, determined using flow cytometry. (D) Blood cell counts of the Su9T01-xenografted mice treated with palbociclib, everolimus and AZD8055 were shown. "Baseline" indicates the mice without xenograft and drugs. "Before treatment" indicates the mice with xenograft before treatment. Other data indicates the xenografted mice after 19 days treatment. Error bars represent the SEM of replicates.

Supplemental Figure 7



Supplemental Figure 7: HBZ and Tax protein expression in ATLL cells.
(A) Immunoblot analysis of HBZ protein expression in KK and TL-Om1 cells. JURKAT cells, a T-ALL cell line, is used as a negative control. (B) Immunoblot analysis of Tax protein expression in KK1 and TL-Om1 cells. HUT102, an HTLV-I-infected cell line without a fully established ATLL, is used as a positive control. * Non-specific bands.