Supplementary information

WEE1 inhibition induces glutamine addiction in T-cell acute lymphoblastic leukemia

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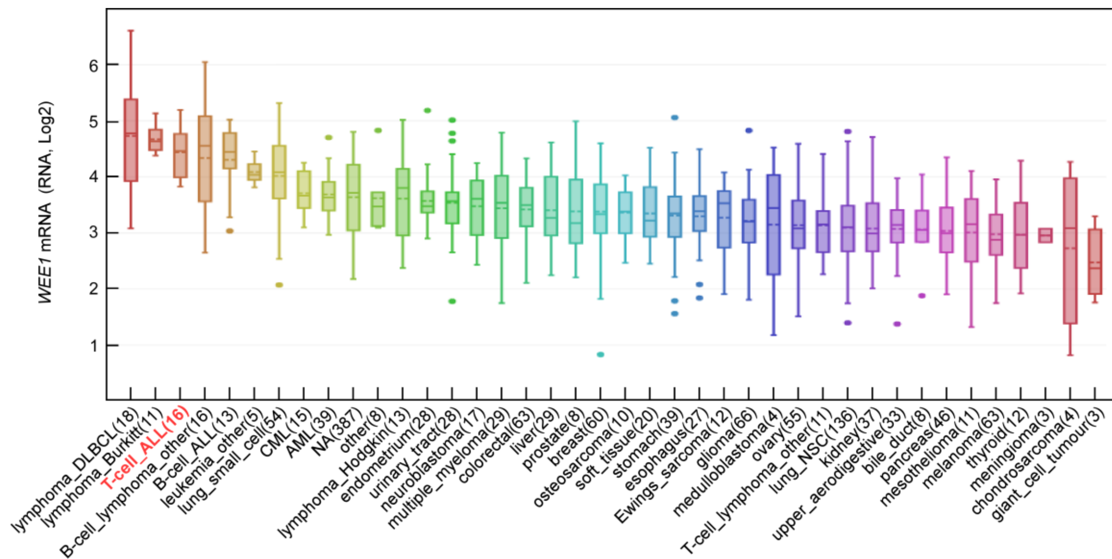
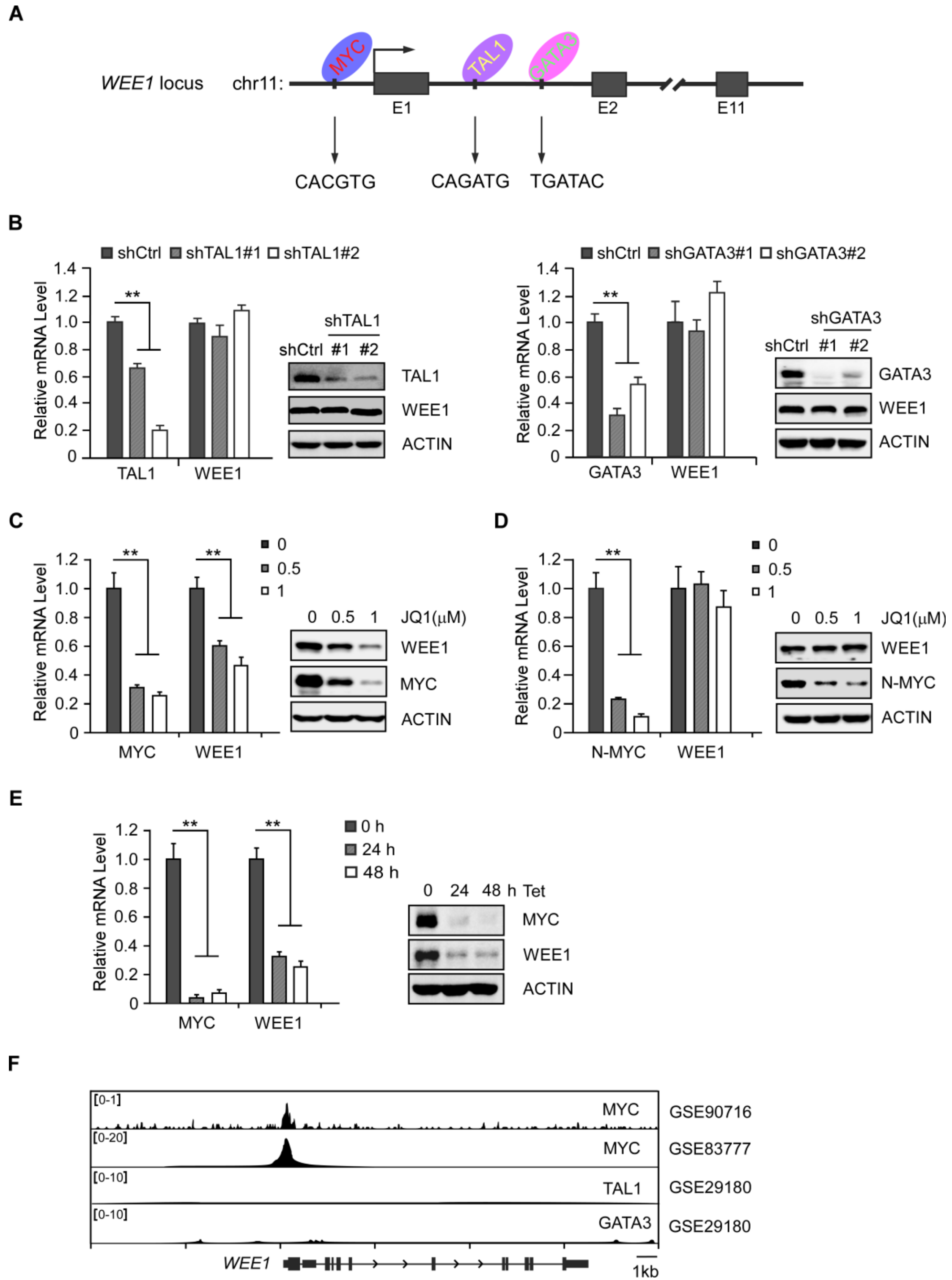
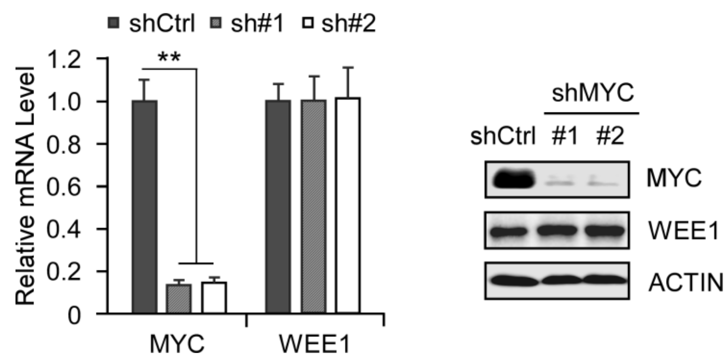


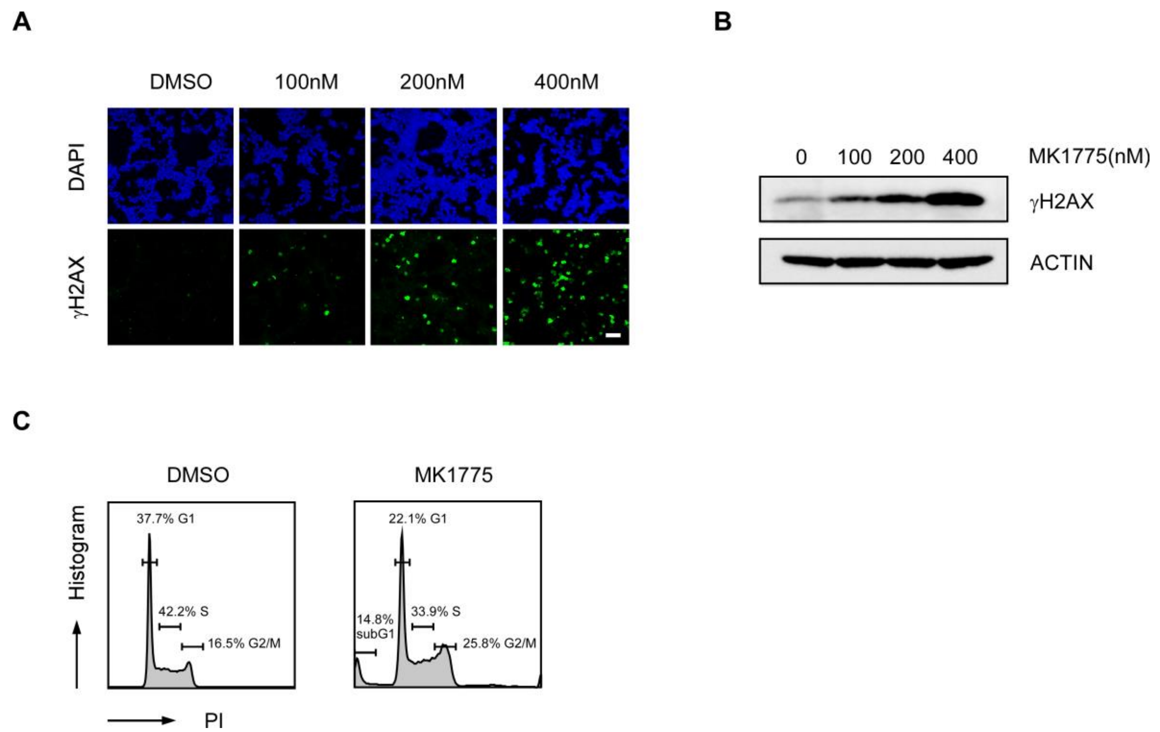
Supplementary Figure S1. ***WEE1*** **mRNA expression was analyzed among 1429 human cancer cell lines in CCLE database (https://portals.broadinstitute.org/ccle)**.


A
WEE1 locus
chr11:
MYC
TAL1
GATA3
E1
E2
E11
CACGTG
CAGATG
TGATAC
B
shCtrl shTAL1#1 shTAL1#2
Relative mRNA Level
TAL1
WEE1
shTAL1
shCtrl #1 #2
TAL1
WEE1
ACTIN
shCtrl shGATA3#1 shGATA3#2
GATA3
WEE1
shGATA3
shCtrl #1 #2
GATA3
WEE1
ACTIN
C
0
0.5
1
MYC
WEE1
0 0.5 1 JQ1(μM)
WEE1
MYC
ACTIN
D
N-MYC
WEE1
0 0.5 1 JQ1(μM)
WEE1
N-MYC
ACTIN
E
0 h
24 h
48 h
MYC
WEE1
0 24 48 h Tet
MYC
WEE1
ACTIN
F
[0-1]
MYC
GSE90716
[0-20]
MYC
GSE83777
[0-10]
TAL1
GSE29180
[0-10]
GATA3
GSE29180
WEE1
1kb

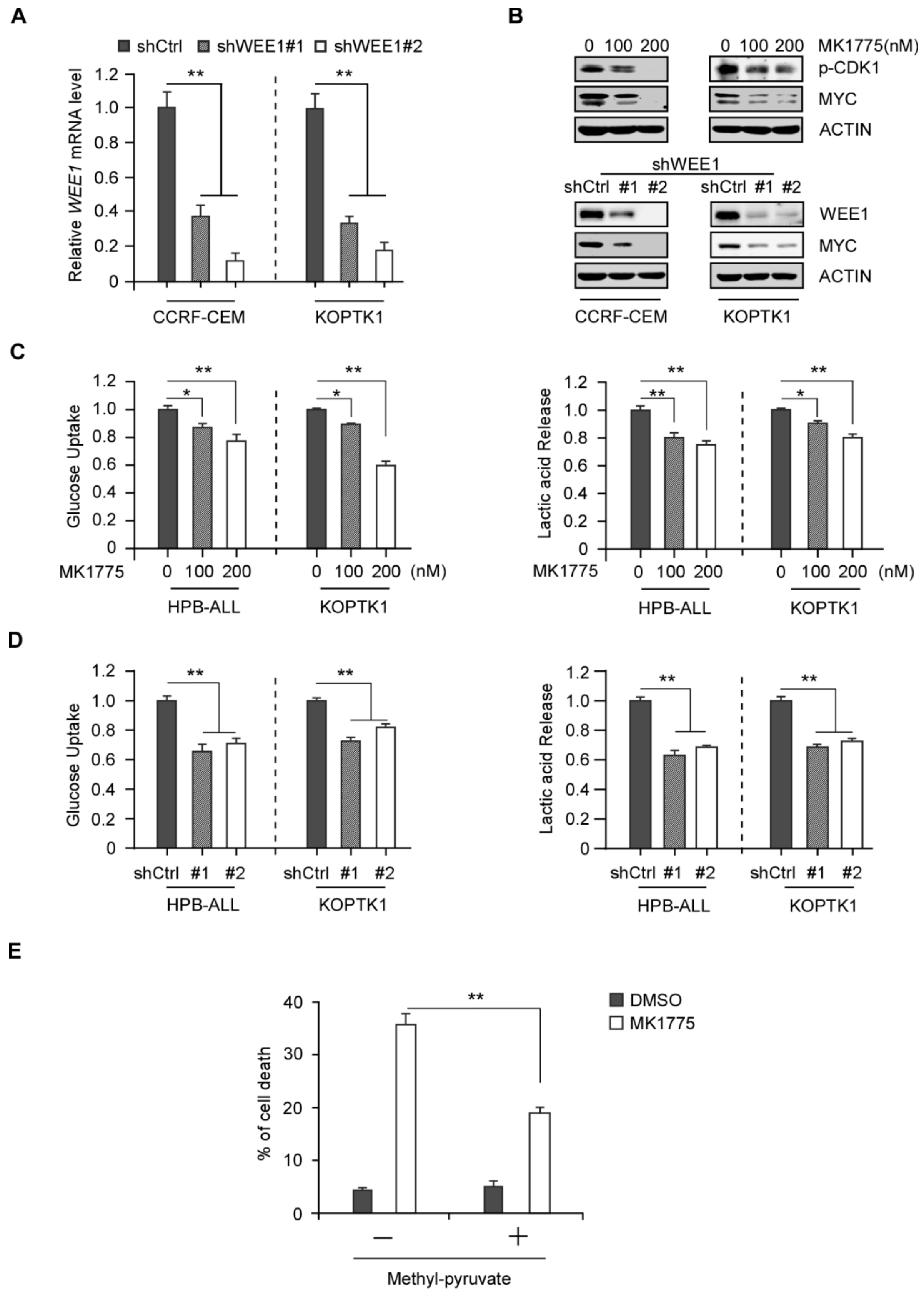
Supplementary Figure S2. MYC activates the *WEE1* transcription. (A) Schematic presentation of putative binding sites of MYC, TAL1 and GATA3 proximal to the *WEE1* transcription initiation site. Dark grey boxes denote the *WEE1* exons. Nucleic acid sequences of putative transcription factor binding sites are shown at the bottom. **(B)** CUTLL1 cells were infected with lentiviruses expressing control (shCtrl), *TAL1* shRNA (sh#1 or sh#2) or *GATA3* shRNA (sh#1 or sh#2), respectively. *WEE1* mRNA and protein levels were subsequently determined by RT-qPCR and immunoblots. ACTIN was used as a loading control. **(C)** CUTLL1 cells were treated with JQ1 for 24 h as indicated. *WEE1* mRNA was quantified by RT-qPCR, and WEE1 and MYC proteins were determined by immunoblots. **(D)** LOUCY cells were treated with JQ1 as shown in (C), followed by WEE1 and N-MYC expression analysis. **(E)** P493 cells, which bear a tetracycline (Tet) repressive *MYC* transgene, were treated with tetracycline (Tet, 100 ng/ml) for 24 and 48 h. *WEE1* mRNA and protein were analyzed by RT-qPCR and immunoblots. **(F)** ChIP-Seq data of MYC (GSE83777 and GSE90716), TAL1 (GSE29180) or GATA3 (GSE29180) were analyzed to reveal binding signals proximal to the *WEE1* locus. Above all, data shown represent the means (±SD) of biological triplicates. $^{**}p < 0.01$.



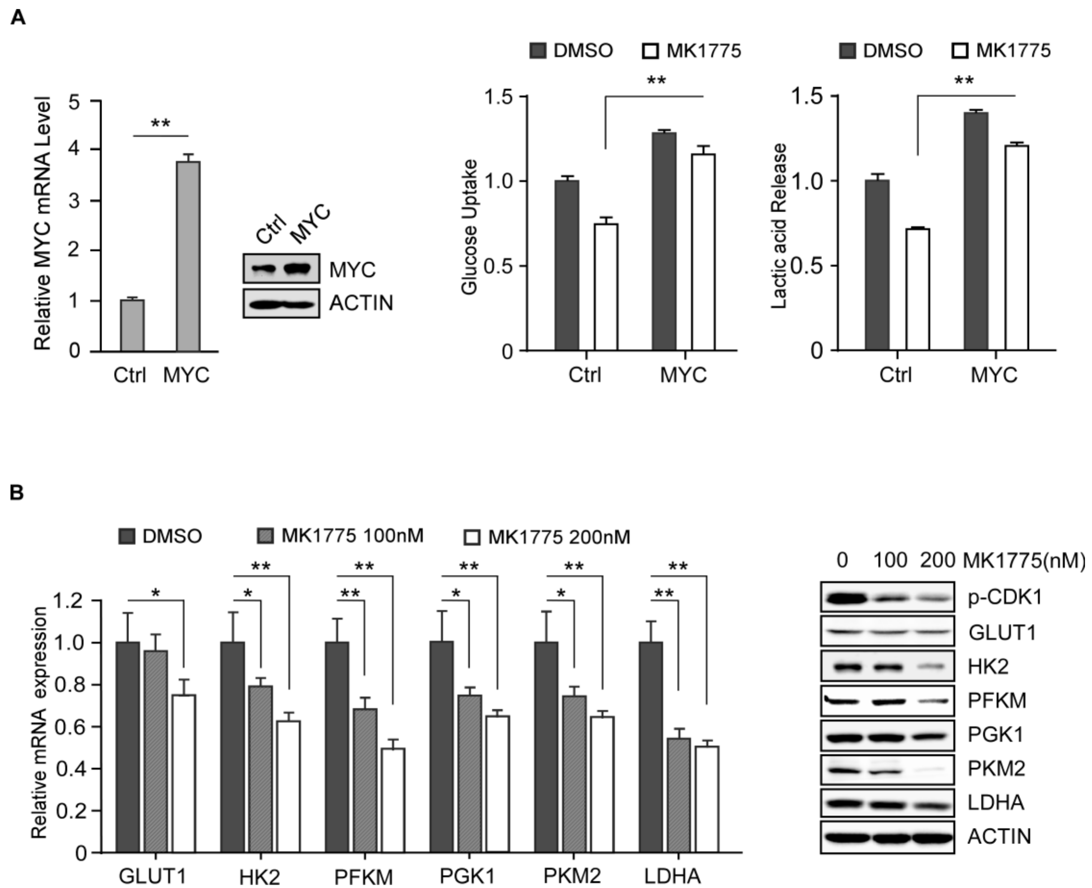
Supplementary Figure S3. *MYC* depletion barely decreases *WEE1* expression in *NOTCH1* WT T-ALL SUP-T1 cells. SUP-T1 cells were infected with lentiviruses expressing control (shCtrl) or *MYC* shRNA (sh#1 or sh#2). *WEE1* mRNA and protein levels were subsequently determined by RT-qPCR and immunoblots. ACTIN was used as a loading control. Data shown represent the means (±SD) of biological triplicates. **$p < 0.01$.



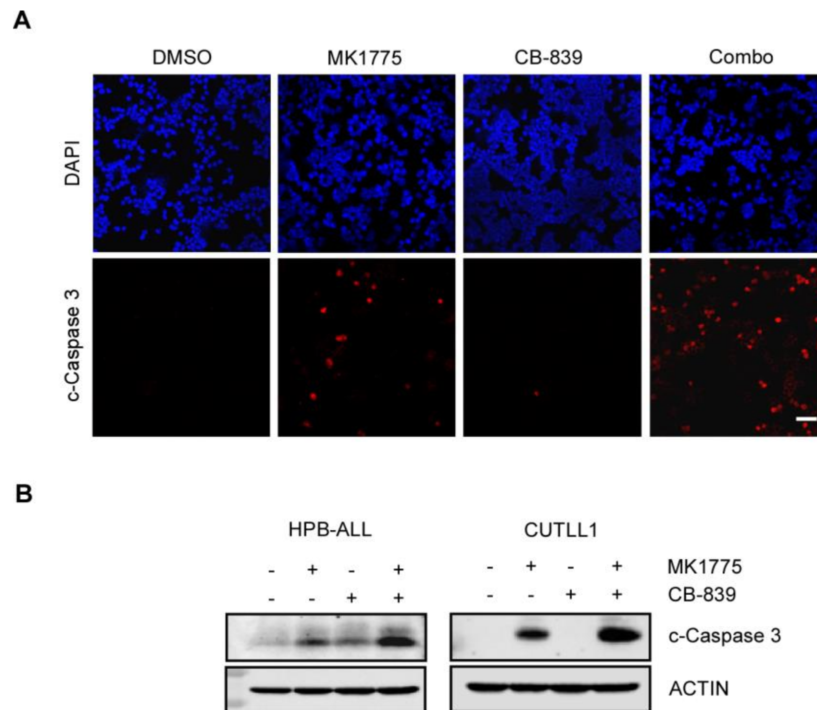
Supplementary Figure S4. MK1775 induces DNA damage and cell cycle change in CUTLL1 cells. **(A)** Immunofluorescence images of γH2AX (green) and DAPI (blue) in CUTLL1 cells undergoing DMSO or MK1775 treatments for 24 h. Scale bar, 50 µm. **(B)** Immunoblots of γH2AX with ACTIN shown as a loading control. **(C)** Representative cell cycle profiles of CUTLL1 cells treated with MK1775 (200 nM) or not for 24 h assessed by PI staining and flow cytometry analysis.


A
shCtrl
shWEE1#1
shWEE1#2
Relative WEE1 mRNA level
**
CCRF-CEM
KOPTK1
B
0 100 200
MK1775(nM)
p-CDK1
MYC
ACTIN
shWEE1
shCtrl #1 #2
WEE1
MYC
ACTIN
CCRF-CEM
KOPTK1
C
Glucose Uptake
Lactic acid Release
MK1775
0 100 200 (nM)
HPB-ALL
KOPTK1
D
Glucose Uptake
Lactic acid Release
shCtrl #1 #2
HPB-ALL
KOPTK1
E
% of cell death
DMSO
MK1775
Methyl-pyruvate

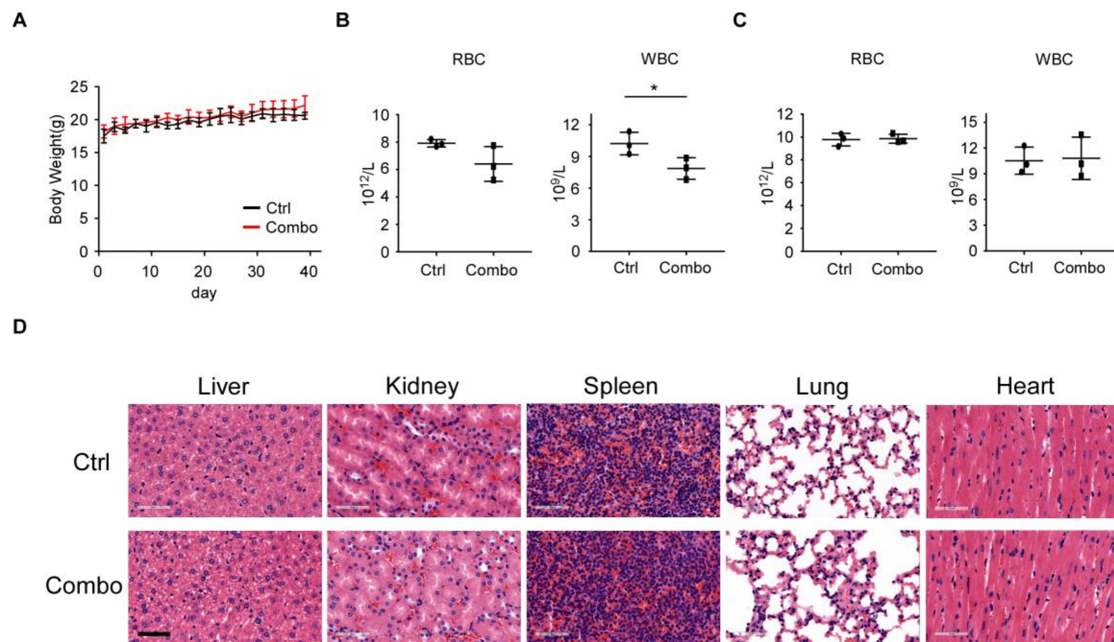
Supplementary Figure S5. Depletion of *WEE1* decreases MYC expression and impairs T-ALL cell glycolysis. **(A)** CCRF-CEM and KOPTK1 cells were infected with lentiviruses expressing control (shCtrl) or *WEE1* shRNA (sh#1 or sh#2). *WEE1* mRNA levels were analyzed by RT-qPCR. **(B)** Cell lysates from MK1775-treated or *WEE1* depleted cells were analyzed for p-CDK1, WEE1 and MYC protein expression by immunoblots. As a well-defined WEE1 substrate, CDK1 phosphorylation was used to reflect WEE1 activity. **(C)** HPB-ALL and KOPTK1 cells were treated with MK1775 for 24 h as indicated. Glucose consumption and lactate secretion were analyzed and normalized to the same live cell number. **(D)** HPB-ALL and KOPTK1 cells were infected with lentiviruses expressing control (shCtrl) or *WEE1* shRNA (sh#1 or sh#2) as indicated. Glucose consumption and lactate secretion were analyzed and normalized to the same live cell number. **(E)** HPB-ALL cells were treated with MK1775 (100 nM) in the presence or absence of methyl-pyruvate (2 mM) for 48 h. Apoptotic cell death was analyzed by Annexin V/PI staining and flow cytometry analysis. Above all, data shown represent the means (± SD) of biological triplicates. * $p < 0.05$, ** $p < 0.01$.



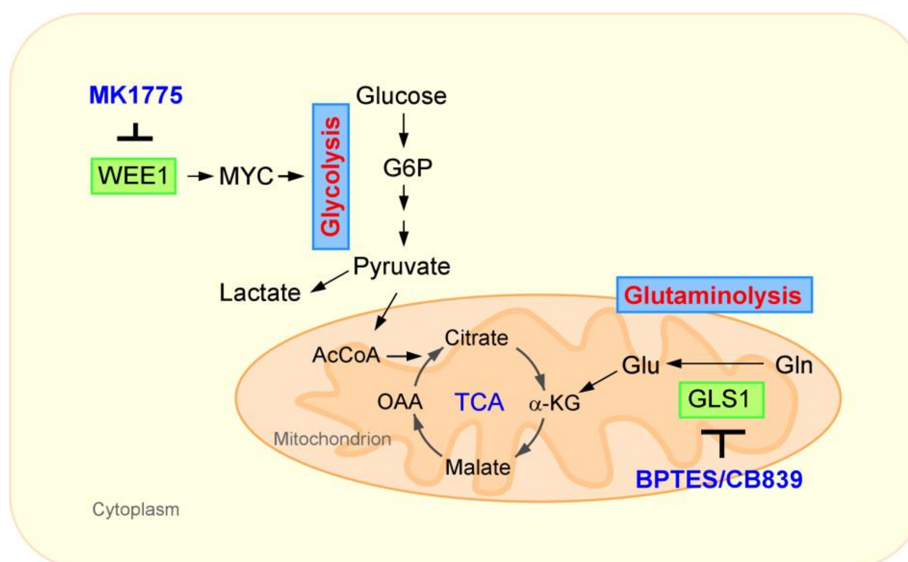
Supplementary Figure S6. MK1775 decreases glycolytic gene expression via MYC downregulation. (A) CUTLL1 cells were infected with lentiviruses expressing control (pCDH) or MYC (pCDH-MYC) and treated with or without MK1775 (200 nM) for 24 h. *MYC* mRNA and protein levels were subsequently determined by RT-qPCR and immunoblots. ACTIN was used as a loading control (left). Glucose consumption and lactate secretion were analyzed in these cells and normalized to the same live cell number (right). **(B)** KOPTK1 cells were treated with MK1775 for 24 h as indicated. Six representative genes involved in glycolysis pathway were analyzed by RT-qPCR (left). Immunoblots of indicated proteins are shown on the right. Above all, data shown represent the means (±SD) of biological triplicates. *$p < 0.05$, **$p < 0.01$.



Supplementary Figure S7. MK1775 and CB-839 induce synergistic lethality in CUTLL1 cells. **(A)** Immunofluorescence images of cleaved Caspase-3 (c-Caspase 3, red) and DAPI (blue) in CUTLL1 cells undergoing DMSO, MK1775 (200 nM), CB-839 (100 nM) or dual treatments for 24 h. Scale bar, 50 µm. **(B)** Immunoblots of cleaved Caspase-3 in HPB-ALL and CUTLL1 cells subjected to different treatments, with ACTIN shown as a loading control.



Supplementary Figure S8. Toxicity evaluation of MK1775 and BPTES combination treatment in healthy C57/BL6 mice. (A) C57/BL6 mice were treated with MK1775 and BPTES (Combo) using the same strategy as shown in Figure 5A. Ctrl, mock treatment. Body weights of these mice were monitored right after treatment started. **(B-C)** Blood parameters were analyzed at the end of treatment (B) or two weeks after treatment termination (C). RBC, red blood cell counts; WBC, white blood cell counts. **(D)** Representative H&E staining images in the liver, kidney, spleen, lung and heart sections. Scale bar, 50 μm. Above all, data shown represent the means (±SD) of biological triplicates. *$p < 0.05$.



Supplementary Figure S9. Schematic presentation of anti-T-ALL mechanism underlying co-inhibition of WEE1 and GLS1. This drug combination strategy simultaneously blocks the glycolysis and glutaminolysis pathways in T-ALL cells. G6P, Glucose-6-phosphate; AcCoA, Acetyl-CoA; OAA, Oxaloacetate; α-KG, α-ketoglutarate; Gln, Glutamine; Glu, Glutamate.

Supplementary Table S1. Primer sequences detecting human genes are listed below.

Primers for real-time PCR	
ACTIN forward	5′-CACCATTGGCAATGAGCGGTTC-3′
ACTIN reverse	5′-AGGTCTTTGCGGATGTCCACGT-3′
WEE1 forward	5′-CGCACACGCCCAAGAGTTT-3′
WEE1 reverse	5′-ACTGGCTTCCATGTCTTCACC-3′
MYC forward	5′- TCGGATTCTCTGCTCTCCTC-3′
MYC reverse	5′- TGTTCCTCCTCAGAGTCGCT-3′
TAL1 forward	5′-CCCCTCAAACACCTAGGCAC-3′
TAL1 reverse	5′-GTCCTTTAGAGGTGCGTTTGC-3′
GATA3 forward	5′-GGGCAGATGACAGGTTCCAA-3′
GATA3 reverse	5′-CACATCGGATTGCTGCATGG-3′
PFKM forward	5′-TGGAGATGCCCAAGGTATGA-3′
PFKM reverse	5′-CGTCCTTCTCGTTCCCGAAA-3′
PGK1 forward	5′-TGGACGTTAAAGGGAAGCGG-3′
PGK1 reverse	5′-GCTCATAAGGACTACCGACTTGG-3′
GLUT1 forward	5′-GGCCAAGAGTGTGCTAAAGAA-3′
GLUT1 reverse	5′-ACAGCGTTGATGCCAGACAG-3′
LDHA forward	5′-ATGGCAACTCTAAAGGATCAGC-3′
LDHA reverse	5′-CCAACCCCAACAACTGTAATCT-3′
HK2 forward	5′-GAGCCACCACTCACCCTACT-3′
HK2 reverse	5′-CCAGGCATTCGGCAATGTG-3′
PKM2 forward	5′-CAGAGGCTGCCATCTACCAC-3′
PKM2 reverse	5′-CCAGACTTGGTGAGGACGAT-3′

Primers for ChIP assay	
ACTIN promoter forward	5'-GACTTCTAAGTGGCCGCAAG-3'
ACTIN promoter reverse	5'-TTGCCGACTTCAGAGCAAC-3'
WEE1 promoter forward	5'-AATCGCGTAGCTGGTCCTTCC-3'
WEE1 promoter reverse	5'-GTCTCCTCAGGTCCAGTCTCA-3'
NCL promoter forward	5'-CTCGGGGTGGAGAGATGAGA-3'
NCL promoter reverse	5'-GACTCCGACTAGGGCCGATA-3'
Primers for luciferase reporter constructs	
WEE1 promoter WT forward	5′-GGGGTACCTCATAGGCCCGGAACACCAC-3′
WEE1 promoter WT reverse	5′-CCGCTCGAGCAGGGCGAGAAGATCAGCTT-3′
WEE1 promoter mutant forward	5′-TGGACTGGGCACTAACTTCGTTAACGGTCGCGGGAAGCCG-3′
WEE1 promoter mutant reverse	5′-CGACCGTTAACGAAGTTAGTGCCCAGTCCA-3′
shRNA sequences	
WEE1_sh#1	5′-TTCTCATGTAGTTCGATATTT-3′
WEE1_sh#2	5′-ACAGACTCCTCAAGTGAATAT-3′
MYC_sh#1	5′-CAGTTGAAACACAAACTTGAA-3′
MYC_sh#2	5′-CCTGAGACAGATCAGCAACAA-3′
GATA3_sh#1	5′-CCTCTGCTTCATGGATCCCTA-3′
GATA3_sh#2	5′-CATCCAGACCAGAAACCGAAA-3′
TAL1_sh#1	5′-GCTCAGCAAGAATGAGATCCT-3′
TAL1_sh#2	5′-AAGAAGCTCAGCAAGAATGAG-3′

Supplementary Table S2. Antibodies used in the immunoblotting are listed below.

Antibodies	Vendor	Catalogue #	Dilution
WEE1	Cell Signaling Technology	13084S	1:1000
MYC	Santa Cruz Biotechnology	SC-764	1:1000
TAL1	Santa Cruz Biotechnology	SC-393287	1:10000
GATA3	ABclonal	A19636	1:1000
N-MYC	Santa Cruz Biotechnology	SC-53993	1:1000
p-CDK1	Cell Signaling Technology	4539S	1:1000
γH2AX	Millipore	#05-636	1:1000
GLUT1	Abcam	ab652	1:1000
HK2	Cell Signaling Technology	#2106	1:1000
PFKM	ABclonal	A5477	1:1000
PGK1	Santa Cruz Biotechnology	SC-17943	1:500
PKM2	Abcam	ab38237	1:500
LDHA	Cell Signaling Technology	#2012	1:1000
cleaved Caspase-3	Cell Signaling Technology	9664S	1:1000
β-ACTIN	Santa Cruz Biotechnology	SC-47778	1:1000

Supplementary Table S3. Primary T-ALL samples used in this study. Sample #1 was applied in the PDX model.

Sample ID	Age	Gender	Immunophenotype (positive)	Subtype
#1	50	Female	CD7,CD2,CD5, CD10,cytoCD3,CD4,TdT	Late cortical (LMO2)
#2	31	Male	CD2,CD3,CD4,CD5,CD7,CD8,CD38, cytoCD3,TdT	NA
#3	22	Male	CD7, CD3,CD5	NA
#4	15	Male	CD2,CD3,CD4,CD5,CD7,CD8,CD38, TdT	NA
#5	32	Male	CD3,CD4,CD8	Late cortical
#6	14	Male	CD3,CD4,CD8	Late cortical
#7	19	Female	CD4,CD5,CD99,CD7,TdT,CD1a,CD8, CD2,cytoCD3	Early cortical
#8	16	Male	CD3,CD7,CD5,TdT,CD38	NA
#9	12	Male	CD7,CD4,CD10,CD38,TdT,CD2,	NA
#10	11	Male	CD7,cytoCD3,CD38	NA

Online Methods

Lentiviral transduction

For viral production, lentiviral vectors (pLKO.1 for shRNA and pCDH for overexpression) were used for plasmid construction and transfected into 293T cells simultaneously with helper plasmids (pMD2.G and psPAX2). Viral supernatants were generally collected 48 h post-transfection. Transduction of T-ALL cells was carried out as follows. Briefly, 1×10^6 cells were incubated with 0.5 mL viral supernatant and 8 μg/mL polybrene (Sigma, St. Louis, MO, USA) in culture medium with a final volume of 2 mL for 0.5 h, then subjected to centrifugation at $1000\times g$ for 90 min at room temperature, followed by additional 48 h cell culture.

In silico analysis predicting transcription factors that regulates *WEE1* expression

Potential transcription factors that regulate *WEE1* were identified in the UCSC genome browser gateway (http://genome.ucsc.edu/) from the human genomic information of GRCh38/hg38. Tentative transcription factors and binding regions were obtained in ChIP-seq clusters and peaks (340 factors in 129 cell types) generated from ENCODE 3.

Chromatin immunoprecipitation (ChIP) assay

CUTLL1 cells were fixed with 1% paraformaldehyde at room temperature for 10 min. Cells were subjected to a Bioruptor Pico Sonifier to shear chromatin DNA to a size range of 500-1000 bp. Precleared chromatin was immunoprecipitated with anti-MYC

or rabbit IgG (Santa Cruz Biotechnology, Dallas, TX, USA) over night at 4 ℃. Antibody-chromatin complexes were pulled down with protein G agarose/salmon sperm DNA beads (Roche, Mannheim, Germany) (1 h, 4 ℃). The eluted material was reverse-cross-linked and treated with proteinase K. Immunoprecipitated DNA was purified and quantified by qPCR using specific primers listed in Supplementary Table S1.

Luciferase reporter assay

Firefly luciferase reporter expressing the wild type (WT) or mutant (MUT) *WEE1* promoter were constructed in the pGL3-basic vector using specific primers listed in Supplementary Table S1. To detect luciferase reporter activity, 0.8 μg reporter construct and 0.2 μg pcDNA3-MYC plasmid, along with 50 ng pRL-TK renilla luciferase reporter construct, were co-transfected into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Luciferase activities were measured 24 h later using Dual Luciferase Reporter Assay System (Promega, Madison, WI, USA). Firefly luciferase activities were normalized with renilla luciferase control, and relative to values from the empty vector.

Flow cytometry analysis

Cells stained with indicated antibodies were resuspended in phosphate-buffered saline. Acquisition was performed on an Accuri C6 (BD Biosciences, Franklin Lakes, NJ, USA) and live cells were gated based on FSC-A and SSC-A characteristics. Data were

analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

Immunohistochemistry

The immunohistological analysis was carried out using Histostain-Plus IHC Kit (Thermo Fisher Scientific). Paraffin-embedded tissue sections were incubated with the antibodies against cleaved Caspase-3 (Cell Signaling Technology, Danvers, MA, USA) or PCNA (Santa Cruz Biotechnology) overnight at 4 ℃. These slides were then subjected to horseradish peroxidase-linked secondary antibodies for 1 h at room temperature. Staining was visualized by the DAB substrate kit (Vector Laboratories, Burlingame, CA, USA).