

1 **Data Supplement**

2 **Manuscript Title: Ex vivo pretreatment with venetoclax boosts antileukemic efficacy of therapeutic**
3 **$\gamma\delta$ T cells in AML models**

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6 **Supplemental methods:**

7 **Human AML cell lines**

8 Human AML luciferase-expressing cell lines, including KG-1 α -luc, THP-1-luc, OCI-AML3-luc, and
9 MV4-11-luc cells, were kindly provided by UnicetBio Co., Ltd. KG-1 α -luc and THP-1-luc cells were
10 maintained in RPMI 1640 medium (Biosharp, Cat# BL303A), with 10% FBS (Sigma, Cat# F7524) and 1%
11 penicillin-streptomycin (Thermo Fisher Scientific, Cat# 15140122). OCI-AML3-luc cells were maintained
12 in RPMI 1640 supplemented with 20% FBS, 1 mM sodium pyruvate (Thermo Fisher Scientific, Cat#
13 11360070), 2 mM L-glutamine (Thermo Fisher Scientific, Cat# 25030081), 10 mM HEPES (Thermo Fisher
14 Scientific, Cat# 15630130) and 1% penicillin-streptomycin. MV4-11-luc cells were cultured in IMDM
15 (Thermo Fisher Scientific, Cat# 12440053) with 10% FBS and 1% penicillin-streptomycin. All cell lines
16 were maintained at 37°C, supplemented with 5% CO₂.

17 **Colony forming assays**

18 To assess clonogenic growth after co-culture with $\gamma\delta$ T cells or venetoclax-treated $\gamma\delta$ T cells (ven- $\gamma\delta$ T),
19 KG-1 α , OCI-AML3, MV4-11, or primary AML cells were pretreated for 12–14 h at an effector-to-target
20 (E:T) ratio of 1:1. Subsequently, 2×10^4 cells per well were plated in 6-well plates in 1 mL MethoCult
21 H4434 medium (StemCell Technologies, Cat# H4434). The plates were incubated at 37°C with 5% CO₂.
22 After 12–14 days, colonies were counted under an inverted microscope. Clusters containing ≥ 10 cells
23 were defined as a colony.

24 **Generation of CLL-1 CAR $\gamma\delta$ T cells**

CLL-1 CAR $\gamma\delta$ T cells were generated from healthy donor-derived V γ 9V δ 2 T cells using a lentiviral transduction protocol established in our laboratory and similar to that previously used for the generation of B7-H3 CAR-modified $\gamma\delta$ T cells (1). Briefly, CAR construct was cloned into an HIV-1-derived lentiviral vector. The CAR consisted of a mouse Ig kappa leader sequence, a CLL-1-specific single-chain variable fragment (scFv), a CD8 α hinge and transmembrane region, the intracellular costimulatory domain of 4-1BB, and the CD3 ζ signaling domain. The CLL-1-specific scFv was derived from the anti-CLL-1 monoclonal antibody clone 1075.7, as previously reported (2), with the heavy- and light-chain variable regions linked by a (GGGGS)₃ linker. A truncated epidermal growth factor receptor (EGFRt) was co-expressed downstream of the CAR sequence via a P2A peptide to enable monitoring of CAR expression. Expanded $\gamma\delta$ T cells were transduced with the CLL-1 CAR lentiviral vector on day 1 of culture, and CAR expression was confirmed by flow cytometry prior to cryopreservation and functional assays.

Flow cytometry

For phenotyping and counting, samples were acquired on a BD LSR II instrument and analyzed using FlowJo v10.x (BD). CountBright absolute counting beads (Thermo Fisher) were used for absolute quantification where indicated. The following anti-human antibodies were used for cell staining: CD3 (SK7)-APC Cy7 (Cat# 560176) or -FITC (Cat# 340542) were purchased from BD Biosciences. MCL-1 (LVUBKM)-PE (Cat# 12-9047-42) was purchased from Invitrogen. TCR V δ 2 (B6)-PE (Cat# 331408) or -APC (Cat# 331418), EGFR (AY13)-APC (Cat# 352906), CD4 (RPA-T4)-FITC (Cat# 300538) or -PE (Cat# 300508), CD8 (SK1)-FITC (Cat# 344704) or -PE (Cat# 344706), CD34 (581)-FITC (Cat# 343504) or -PE (Cat# 343506), CD33 (WM53)-APC (Cat# 303408) or -PE/Cyanine5 (Cat# 303406), CD62L (DREG-56)-

45 PE/Cyanine7 (Cat# 304822), CD45RA (HI100)-FITC (Cat# 304148), CD45 (HI30)-FITC (Cat# 304006),
46 NKG2D (1D11)-PE (Cat# 320806), DNAM-1 (11A8)-APC (Cat# 338312), CD25 (BC96)-FITC (Cat#
47 302604), CD69 (FN50)-PE (Cat# 310906), LAG3 (11C3C65)-FITC (Cat# 369308), TIGIT (A15153G)-
48 APC (Cat# 372706), TIM3 (F38-2E2)-PE (Cat# 345006), CD45RA (HI100)-APC (Cat# 304112), CD62L
49 (DREG-56)-Brilliant Violet 650 (Cat# 304832), CCR7 (G043H7)-PE/Cyanine7 (Cat# 353226), BCL-2
50 (100)-PE (Cat# 658708), Ki67-PE (Cat# 350504), Annexin V-FITC (Cat# 640906) or -Pacific Blue (Cat#
51 640918) and 7-AAD (Cat# 420404) were purchased from BioLegend. BCL-XL (54H6)-PE (Cat# 13835)
52 was purchased from Cell Signaling Technology. FACS data were analyzed using FlowJo software.

53 **RNA sequencing (RNA-seq) and data analysis**

54 For Figure 3A–B, $\gamma\delta$ T cells were drug-treated (venetoclax, 1 μ M, 14–18 h). Total RNA of $\gamma\delta$ T and ven-
55 $\gamma\delta$ T cells from three donors was isolated using TRIzol (Invitrogen, Cat# 15596018). mRNA enrichment
56 was performed on total RNA using oligo(dT)-attached magnetic beads. The enriched mRNA with poly(A)
57 tails was fragmented using a fragmentation buffer, followed by reverse transcription using random N6
58 primers to synthesize cDNA double strands. The synthesized double stranded DNA was then end-repaired
59 and 5'-phosphorylated, with a protruding 'A' at the 3' end forming a blunt end, followed by ligation of a
60 bubble-shaped adapter with a protruding 'T' at the 3' end. The ligation products were PCR amplified using
61 specific primers. The PCR products were denatured to single strands, and then single-stranded circular
62 DNA libraries were generated using a bridged primer. The constructed libraries were quality-checked and
63 sequenced after passing the quality control. Sequencing was performed on DNBSEQ platform with PE150
64 (read length). Raw reads were processed by fastp (version 0.20.1) to filter out low quality reads and remove

sequencing adapters. The R package DESeq2 (version 1.38.1) was used to perform differential expression analysis. The significant differentially expressed genes (DEGs) were indicated by fold change > 2 and P value < 0.05, GO, KEGG and GSEA analysis were carried out to explore the function and pathway. Raw and processed RNA-seq data are publicly available in GEO under accession number GSE306856.

Enzyme-Linked Immunosorbent Assay (ELISA)

For cytokine measurements, culture supernatants were clarified ($1,000 \times g$, 5 min, 4°C) and analyzed by ELISA (Cat# KHC4021, Thermo Fisher) according to the manufacturer's instructions. Briefly, 96-well plates pre-coated with capture antibody were brought to room temperature and washed 3 times with $1 \times$ wash buffer. Standards were prepared by serial dilution to generate a standard curve in duplicate. Samples were thawed on ice. One hundred microliters of standards, controls, and samples were added to the appropriate wells and incubated for 2 h at room temperature. After 3 washes, 100 μ L of HRP-conjugated detection antibody was added to each well and incubated for 1 h at room temperature. Plates were washed again 3 times, and 100 μ L of TMB substrate solution was added. Color was allowed to develop for 10–15 min protected from light, and the reaction was stopped with 50 μ L of 2 N H_2SO_4 . Absorbance was read at 450 nm (reference 570 nm) using a microplate reader. Cytokine concentrations were calculated from the standard curve.

Cell viability

For assessing viability of $\gamma\delta$ T cells after venetoclax treatment, cells were seeded into 96-well plates (10,000 cells/100 μ L/well) and treated with venetoclax for 14 h. Then the culture media were discarded and replaced by 100 μ L of PBS containing 0.1 μ L calcein AM (2 mM, Beyotime, Cat# C2012). After incubation, calcein

85 fluorescence was measured using a microplate reader (excitation, 490 nm; emission, 515 nm). After
86 staining with Hoechst 33342 for 15 min, images were obtained using fluorescence microscope. Moreover,
87 BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 488 (Beyotime, Cat# C0071S) was used to assess
88 the vitality of $\gamma\delta$ T cells according to manufacturer's instructions.

89 **Seahorse analysis**

90 Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) of $\gamma\delta$ T cells or ven- $\gamma\delta$ T
91 cells that had been co-cultured overnight with KG-1 α and isolated by positive selection with anti-TCR $\gamma\delta$
92 microbeads (Cat# 130-050-701, Miltenyi Biotec) were measured using Seahorse XFe96 extracellular flux
93 analyzer. These assays were performed according to the manufacturer's instructions with a Seahorse XF
94 Cell Mito Stress Test Kit (Agilent, Cat# 103015-100) and Seahorse XF Glycolysis Stress Test Kit (Agilent,
95 Cat# 103020-100). Briefly, the Seahorse XFe96 FluxPak cartridge was hydrated with distilled water
96 overnight at 37°C (no CO₂) and equilibrated for 1 hour on the day of the assay in Seahorse XF Calibrant
97 solution. Plates were pre-coated with 22.4 μ g/mL of Cell-tak (Corning, Cat# 354240) overnight at 4°C to
98 ensure continued adhesion of $\gamma\delta$ T cells on the plate during the assay. For OCR testing, cells were cultured
99 in XF DMEM medium supplemented with 10 mM glucose, 1 mM sodium pyruvate, and 2 mM L-glutamine,
100 with pH adjusted to 7.4. 1 μ M oligomycin, 2 μ M Carbonyl Cyanide 4-(Trifluoromethoxy) Phenylhydrazone
101 (FCCP), and 1 μ M rotenone/antimycin A were loaded at the indicated times. To test ECAR, cells were
102 cultured in XF DMEM medium supplemented with 2 mM L-glutamine, with pH adjusted to 7.4. 10 mM
103 glucose, 1 μ M oligomycin and 50 mM 2-DG were loaded at the indicated times. Seahorse data were
104 collected using Wave Controller 2.6 (Agilent).

105 **Mitochondrial DNA isolation and real-time polymerase chain reaction**

106 Genomic DNA was extracted using the HiPure Tissue DNA Mini Kit (MAGEN, Cat# D3121-02). Primers
107 for cytochrome c oxidase subunit I (CO1), a key gene for mitochondrial (mt) function and β -actin (Sangon
108 Biotech) were as follows: MT-CO1 (forward, 5'-ATACTACTAACAGACCGCAACCTC-3'; reverse, 5'-
109 GAGATTATTCCGAAGCCTGGT-3'). β -actin (forward, 5'- GAAGGATTCCTATGTGGGCGA-3';
110 reverse, 5'-CAGGGTGAGGATGCCTCTCTT-3'). Quantitative PCR (qPCR) was performed according to
111 PowerUp™ SYBR™ Green protocols (Thermo Fisher Scientific, Cat# A25741) on an Applied Biosystems
112 QuantStudio 5 sequence detection system. The expression of MT-CO1 was normalized to β -actin.

113 **Mitochondrial mass measurement**

114 Mitochondrial mass was measured using MitoTracker Green (Invitrogen, Cat# M7514) by flow cytometer
115 or fluorescence microscope. For flow cytometry, 2×10^5 $\gamma\delta$ T and ven- $\gamma\delta$ T cells were stained with 200 nM
116 MitoTracker Green at 37°C for 30 min. For mitochondrial imaging, after MitoTracker Green staining, cells
117 were counterstained with DAPI to visualize nuclei. Images were acquired using a Leica DMI8 inverted
118 microscope.

119 **Metabolite extraction and untargeted metabolomics analysis**

120 For sample preparation, $\gamma\delta$ T and ven- $\gamma\delta$ T cells from five donors were washed twice with PBS and pelleted
121 at $400 \times g$ for 5 min. Intracellular metabolites were extracted by adding 2 mL ice-cold 80% methanol to
122 the cell pellet, resuspending, and incubating for 2 h at -80°C to precipitate proteins. The methanol extracts
123 were centrifuged at $14,000 \times g$ for 20 min at 4°C, and supernatants were transferred to new tubes on dry
124 ice. Samples were evaporated in a vacuum concentrator for hydrophilic interaction liquid chromatography–

125 mass spectrometry (HILIC-LC-MS) analysis. Pooled quality control samples were prepared and tested in
126 the same manner as the analytic samples to monitor the stability of the analysis.

127 LC-MS analysis was performed by Metabolism and lipidomics platform, Tsinghua University (Beijing,
128 China). Briefly, in positive mode, a BEH Amide column (2.1×100 mm, 1.7 μ m, Waters, USA) was used for
129 separation with column temperature at 40°C. Separation is initiated at 1% mobile phase B with flow rate
130 at 300 μ L/min. The gradient is shown as follows: 0 min, 1% B; 2 min, 1% B; 3 min, 5% B; 11 min, 60% B;
131 14 min, 60% B; 15 min, 99% B; 17 min, 99% B; 17.1 min, 1% B; 20 min, 1% B. In negative mode, BEH
132 C18 column (2.1 × 100 mm ,1.7 μ m, Waters, USA) was used for separation with column temperature at
133 40°C. Gradient starts at 1% mobile phase B with flow rate at 220 μ L/min shown as follows: 0 min, 1% B;
134 2 min, 1% B; 12 min, 98% B; 17 min, 98% B; 17.1 min, 1% B; 20 min, 1% B.

135 For untargeted metabolites screening, Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific,
136 CA) was used. In positive mode, mobile phase A is prepared by dissolving 0.63 g of ammonium formate
137 in 50 mL of HPLC-grade water, then adding 950 mL of HPLC-grade acetonitrile and 1 μ L of formic acid.
138 Mobile phase B is prepared by dissolving 0.63 g of ammonium formate in 500 mL of HPLC-grade water,
139 followed by 500 mL of HPLC-grade acetonitrile and 1 mL formic acid. In negative mode, mobile phase A
140 was made by mixing 1 L of HPLC-grade water containing 0.3953 g of ammonium bicarbonate (pH ~8).
141 Mobile phase B contained 80% methanol & 20% acetonitrile.

142 Tracefinder 3.2 (Thermo, CA) was used for metabolite identification with in-house library containing
143 MS/MS spectra from over 1,500 endogenous metabolites. Mass tolerance of 10 ppm and 15 ppm were

applied for precursor and fragment search. Metabolites were assigned based on fragment matching with MS/MS spectra in the library. Two levels of identification were used: one with MS/MS confirmation and one based only on accurate precursor masses. Chromatographic peak area was used for relative quantitation. RT shift of 0.25 min was allowed for peak alignment. Statistical analysis of differential metabolites analysis and biochemical pathways through metabolic enrichment were performed using MetaboAnalyst 6.0. The complete metabolite concentration matrix from the non-targeted metabolomics profiling is provided in the Data Supplement (Supplemental Table 6).

RNA isolation and qPCR

RNA was isolated using an RNA Rapid Extraction kit (SparkJade, Cat# AC0201) according to the manufacturer's protocol. Complementary DNA was synthesized using RNA to cDNA kit (Takara, Cat# RR037) according to the manufacturer's instructions. Quantitative polymerase chain reaction (qPCR) was performed using SYBR Green supermix (Thermo Fisher, Cat# A25742) on an ABI Prism 7900HT sequence detection system (Applied Biosystems). Results were analysed using SDS 2.4 software (Applied Biosystems). Primer sequences are listed in Supplemental Table 8.

Immunoblotting

$\gamma\delta$ T and ven- $\gamma\delta$ T cells were lysed in RIPA buffer on ice for 30 min (Beyotime). Lysates were centrifuged at $12,000 \times g$ for 10 min, and supernatants were collected. Samples were separated on precast 4–20% bis-tris protein gels (Epizyme). Separated proteins were then transferred to PVDF membranes and blocked in 5% w/v milk overnight at 4°C. Membranes were then incubated with primary antibodies in 5% w/v BSA in TBS containing 0.1% Tween-20 for 2 h at room temperature, then with HRP-conjugated secondary

164 antibodies. Antibodies: CPT1A (D3B3) Rabbit Monoclonal Antibody (1:1000, Cat# 12252) and β -Actin
165 (D6A8) Rabbit Monoclonal Antibody (1:1000, Cat# 8457) were all Cell Signaling Technology. HRP-
166 Goat Anti-Rabbit Recombinant Secondary Antibody (H+L) (1:10000, Cat# RGAR001) was purchased
167 from Proteintech. Chemiluminescence detection was used to obtain protein bands.

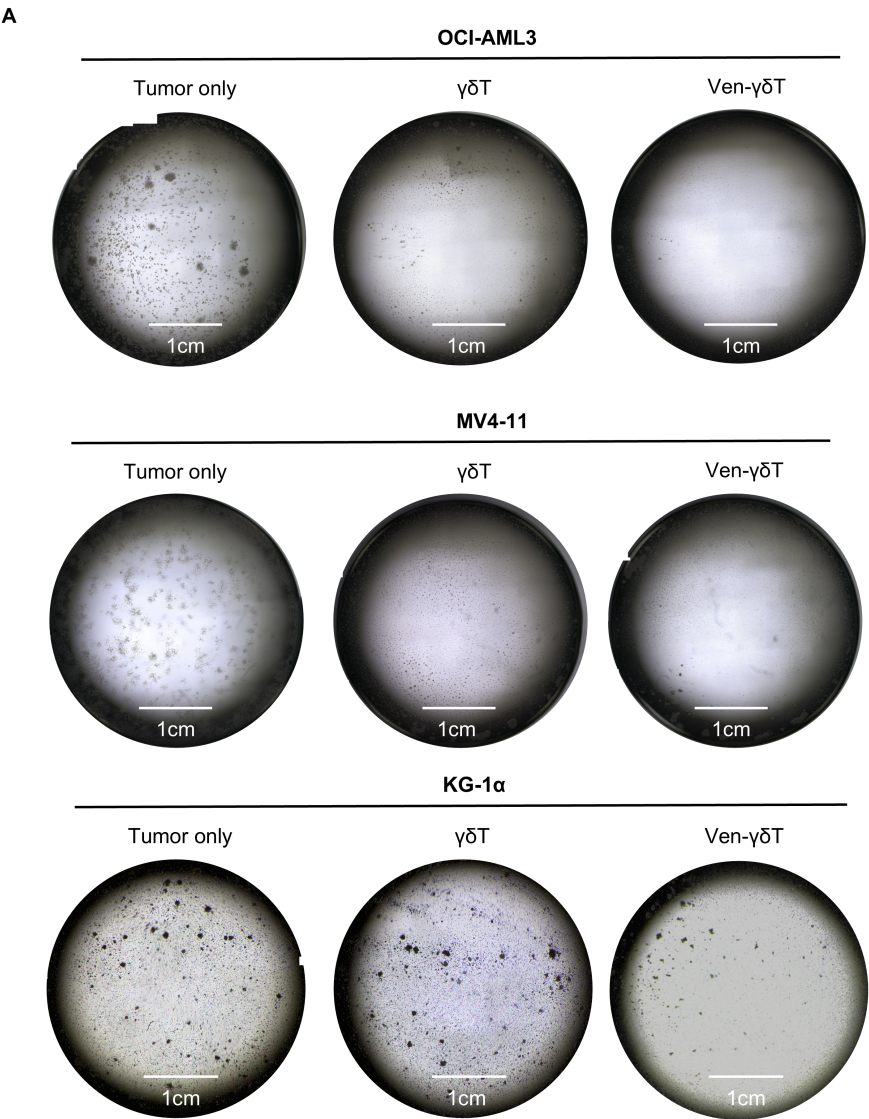
168 **Confocal imaging**

169 We used MitoTracker Green (Beyotime, Cat# C1048) to stain mitochondria, anti-human TCR V δ 2 antibody
170 (BV421, BioLegend, Cat# 331414) to stain cell membrane and DRAQ5 (Thermo Fisher, Cat# 62254) to
171 stain nuclei. Then live $\gamma\delta$ T and ven- $\gamma\delta$ T cells were imaged on a confocal laser-scanning microscope (Nikon
172 AX/AXT with nsparc inverted microscope). Images were captured with a 40 $\times$ /1.2-NA water objective.
173 Imaris Viewer 9.5.1 software was used to process the images.

174 **FAO pathway inhibition with etomoxir**

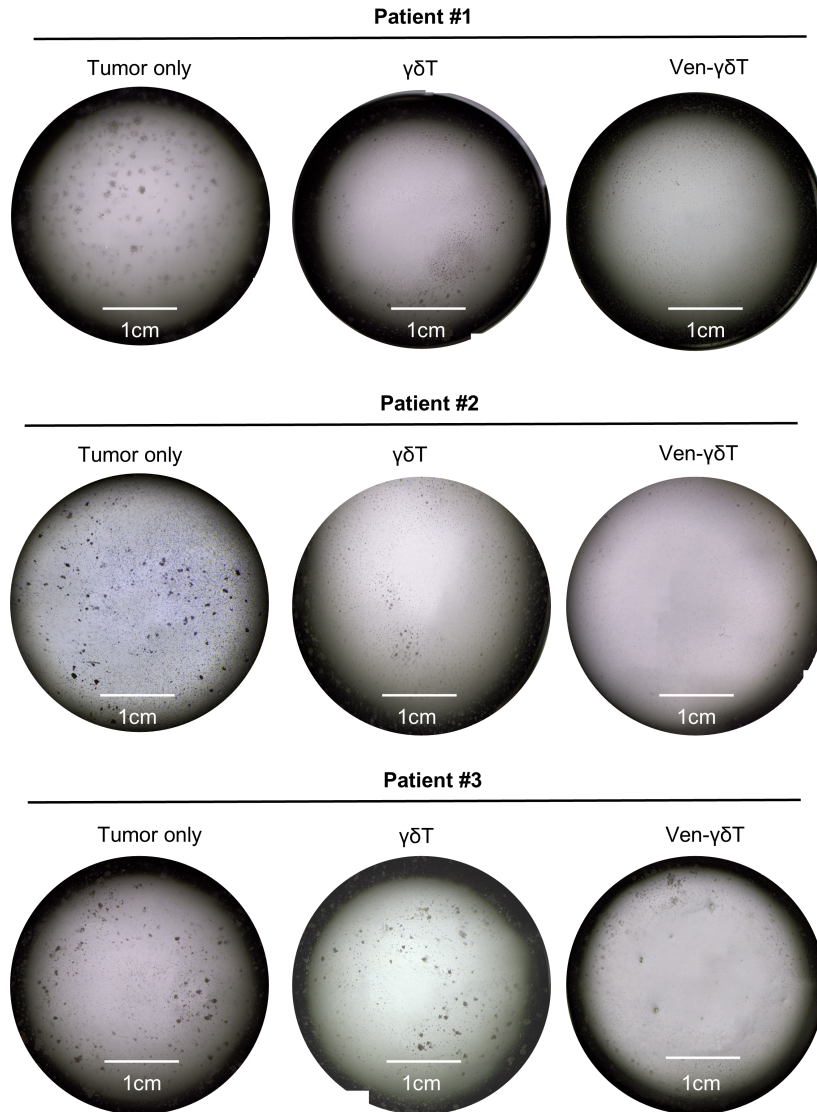
175 For experiments assessing the contribution of fatty acid oxidation, etomoxir was added to $\gamma\delta$ T-cell cultures
176 at a final concentration of 5 μ M for 18 h before OCR or cytotoxicity assays. Control cultures received
177 matched vehicle treatment.

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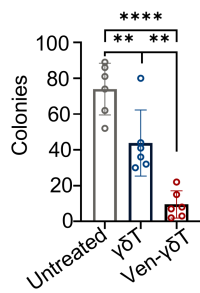


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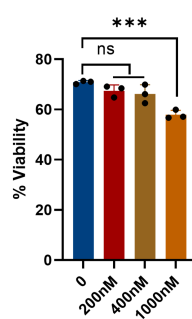
B



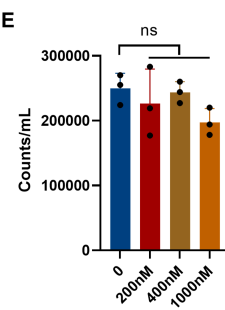
C



D



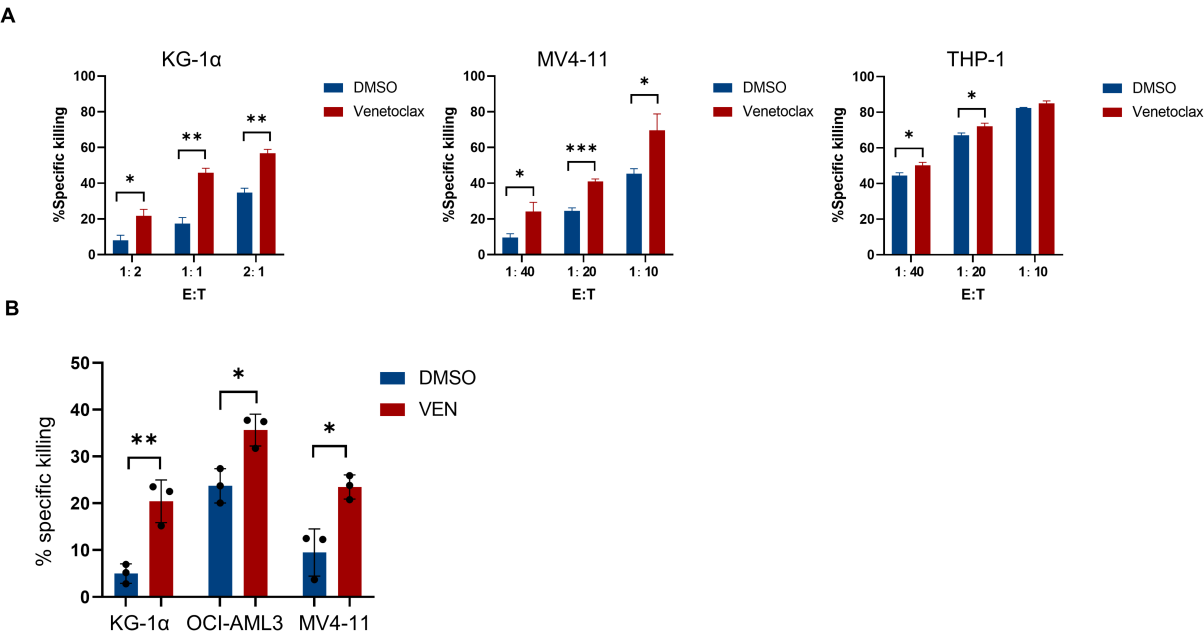
E



181 Supplemental Figure 1. Venetoclax enhances $\gamma\delta$ T cell-mediated killing of AML cells.

182 **(A-B)** Colony formation assay (CFU) assessing the survival of AML cell lines (OCI-AML3, MV4-11, KG-
183 1 α) **(A)** and primary AML patient cells **(B)** following co-culture with ven- $\gamma\delta$ T cells at a 1:1 E:T ratio for
184 12–14 h. AML cells (2×10^4 cells per dish) were then seeded and colonies were counted after 14 days.
185 **(C)** Colonies were counted after culture in methylcellulose medium, and total CFU numbers are shown.
186 Data are presented as mean $\pm$ standard deviation (SD). Data were analyzed using one-way ANOVA with
187 Tukey's comparison test. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.
188 **(D-E)** Viability and cell counts of patient-derived $\gamma\delta$ T cells after venetoclax treatment. $\gamma\delta$ T cells isolated
189 from patient PBMCs were treated with DMSO or venetoclax at the indicated concentrations (200 nM, 400
190 nM, and 1000 nM) for 18 h. Cell viability was measured by AO/PI staining (D), and cell numbers were
191 quantified by direct counting (E). Data are presented as mean $\pm$ SD. Data were analyzed using repeated-
192 measures one-way ANOVA with Dunnett's multiple-comparisons test. ***, $P < 0.001$.

193 **Supplemental Figure 2**



194 **Supplemental Figure 2. Venetoclax enhances the antileukemic activity of Vδ1 and preserves the**
195 **cytotoxicity of venetoclax-expanded γδ T cells after cryopreservation against AML cell lines.**

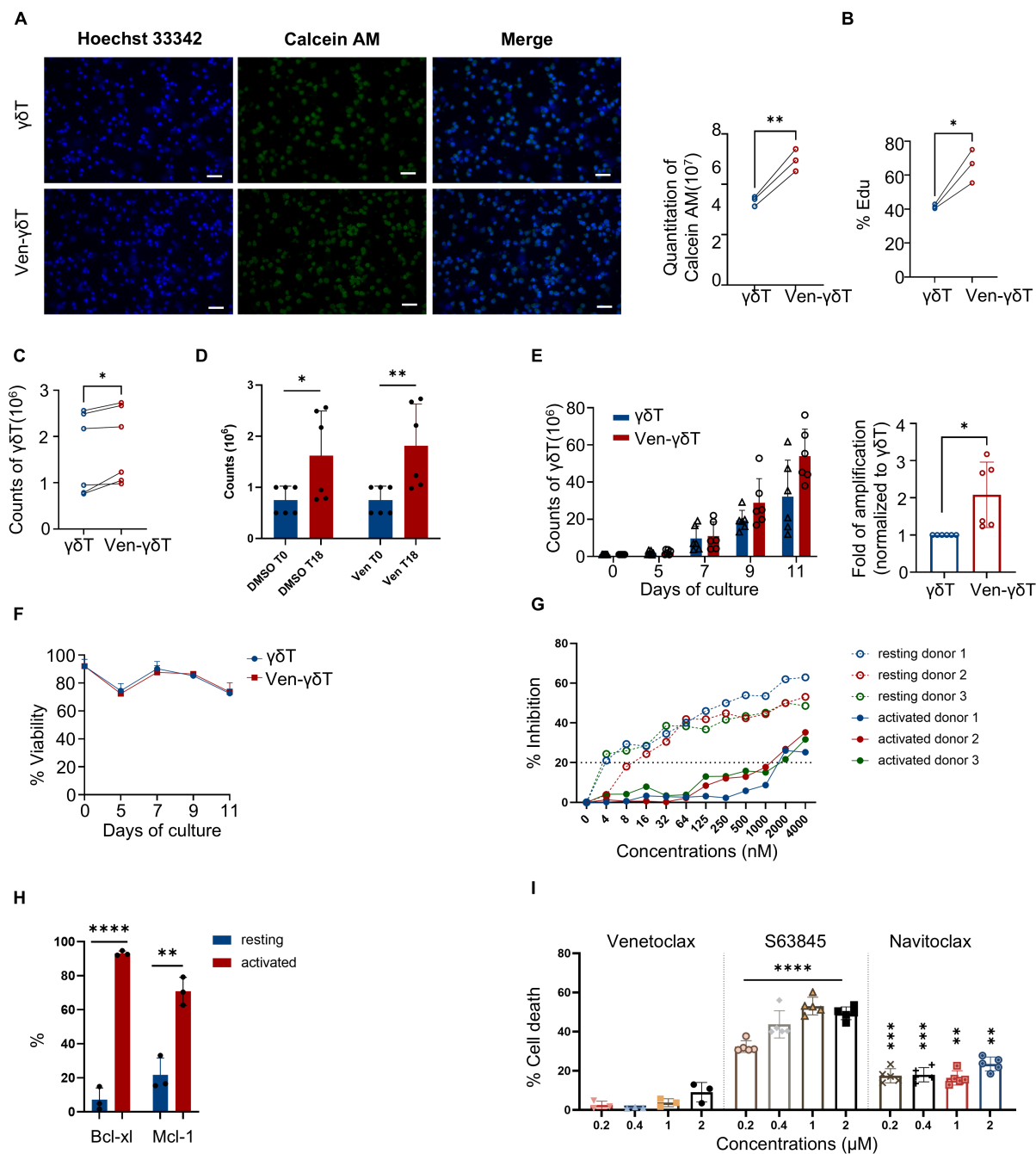
196 **(A)** Vδ1 T cells were obtained from UnicetBio, thawed as described in the Methods, and pretreated *ex vivo*
197 with 1 μM venetoclax or DMSO control before co-culture with AML cell lines. Cytotoxicity against KG-
198 1α, MV4-11, and THP-1 cells was evaluated at the indicated effector-to-target (E:T) ratios.

199 **(B)** Cryopreserved γδ T cells previously expanded under venetoclax-conditioned culture were thawed and
200 directly used for co-culture assays to evaluate cytotoxicity against the indicated AML cell lines after
201 recovery.

202 Data are presented as mean ± standard deviation (SD) and analyzed using two-tailed paired Student's t test.

203 *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

204



206

207 **Supplemental Figure 3. Venetoclax enhances proliferation of $\gamma\delta$ T cells without affecting their**

208 **viability.**

209 **(A)** Immunofluorescence staining of $\gamma\delta$ T cells using Calcein AM and Hoechst 33342, with corresponding
210 statistical plots of fluorescence intensity measured by microplate reader. Each point represents an
211 independent donor (n = 3).

212 **(B)** Quantification of EdU and Hoechst 33342 staining using flow cytometry. Each point represents an
213 independent donor (n = 3).

214 **(C)** Counts of $\gamma\delta$ T cells measured after 18-hour treatment with venetoclax (1 μ M) or DMSO control under
215 matched culture conditions. Each paired symbol represents $\gamma\delta$ T cells from an individual donor (n = 6).

216 **(D)** Absolute $\gamma\delta$ T-cell counts were measured before treatment and after 18 h of DMSO or venetoclax
217 exposure under matched culture conditions (n = 6).

218 **(E)** Fold expansion of $\gamma\delta$ T cells cultured with venetoclax (400 nM) over an extended period. Each point
219 represents an independent donor culture (n = 6).

220 **(F)** Viability of $\gamma\delta$ T cells expanded with or without venetoclax assessed by Acridine Orange (AO) and
221 Propidium Iodide (PI) staining (n = 3).

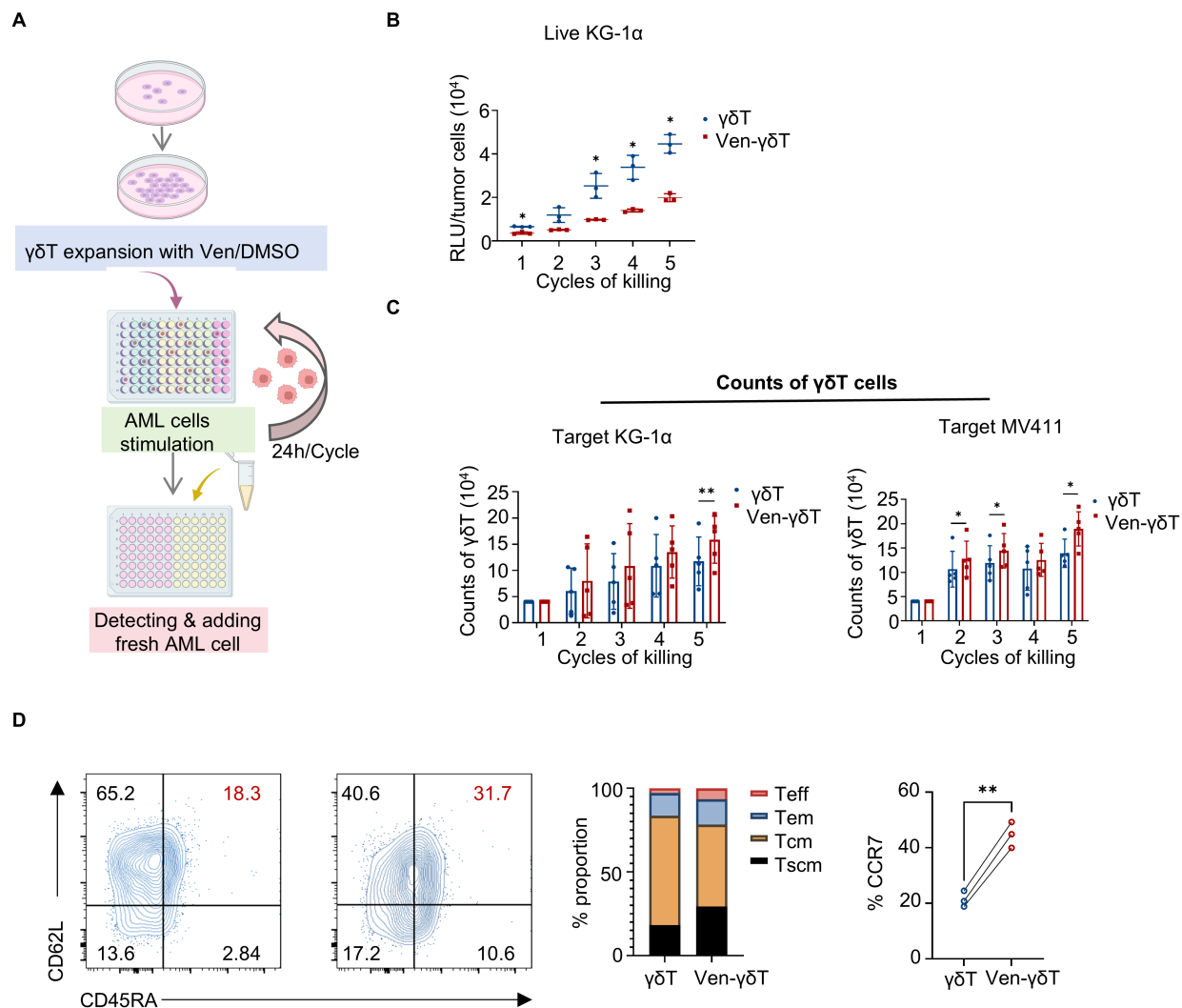
222 **(G-I)** Activated $\gamma\delta$ T cells exhibit reduced sensitivity to venetoclax-induced cell death and increased
223 expression of anti-apoptotic proteins. Resting $\gamma\delta$ T cells were obtained directly from healthy donor PBMCs,
224 whereas activated cells were $\gamma\delta$ T cells stimulated with zoledronic acid. In all flow cytometric analyses,
225 cells were first gated on the $\gamma\delta$ T-cell population, and apoptosis or protein expression was then evaluated
226 within the gated population. **(G)** Resting and activated $\gamma\delta$ T cells from three independent donors (n = 3)
227 were treated with venetoclax at the indicated concentrations (4–4000 nM), and cell death induction was
228 analyzed by flow cytometry. **(H)** Expression of the anti-apoptotic proteins BCL-XL and MCL-1 in resting

229 and activated $\gamma\delta$ T cells assessed by flow cytometry. **(I)** Activated $\gamma\delta$ T cells were treated with venetoclax,
230 the MCL-1 inhibitor S63845, or BCL-2/BCL-XL inhibitor navitoclax at the indicated concentrations, and
231 cell death was determined by flow cytometry.

232 Data are presented as mean $\pm$ standard deviation (SD). Data were analyzed using two-tailed paired
233 Student's *t* test (A-E). Data were analyzed using two-tailed unpaired Student's *t* test (G-I). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

235 **Supplemental Figure 4**

236



237

238 **Supplemental Figure 4. Venetoclax promotes long-term cytotoxic activity and memory phenotypic**
239 **changes of $\gamma\delta$ T cells.**

240 **(A)** Serial killing assay workflow chart.

241 **(B)** $\gamma\delta$ T cells were expanded with DMSO or venetoclax (400 nM). Then $\gamma\delta$ T cells or ven- $\gamma\delta$ T cells were
242 stimulated with KG-1α-luc, MV4-11-luc cells at initial E:T ratio of 4:1 at 24-h intervals. AML viability

243 was determined by luminescence. Data are mean $\pm$ SD from triplicate wells. Representative results of n =
244 3 donors.

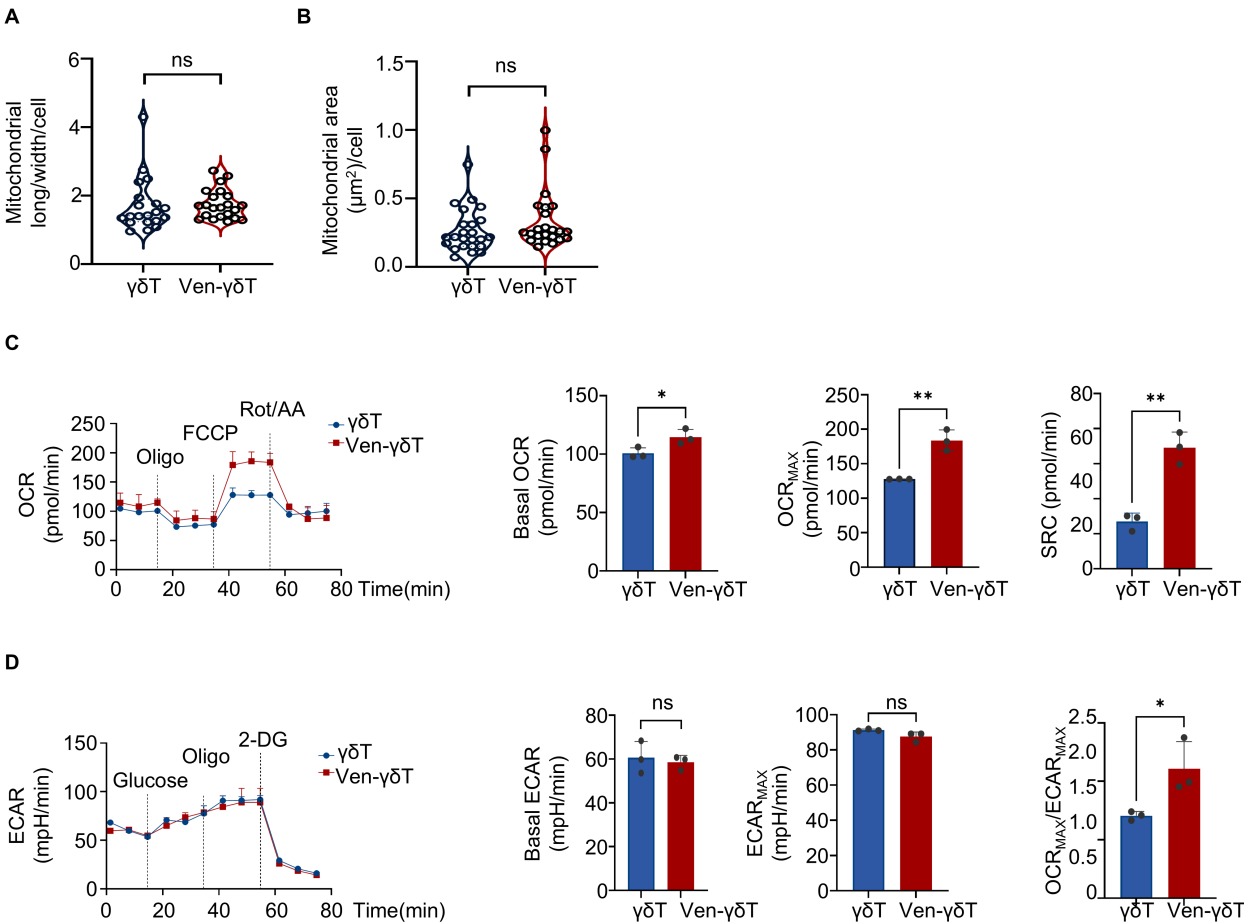
245 **(C)** Absolute counts of $\gamma\delta$ T cells were quantified every cycle. Data are mean $\pm$ SD from triplicate wells.
246 Representative results of n = 5 donors.

247 **(D)** Flow cytometric analysis and quantification of CD45RA⁺CD62L⁺ $\gamma\delta$ T cells (left), CCR7⁺ $\gamma\delta$ T cells
248 (middle), and proportion of T cell memory subsets (right) after venetoclax treatment.

249 Data are presented as mean $\pm$ standard deviation (SD). Data were analyzed using two-tailed paired
250 Student's *t* test. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

251 **Supplemental Figure 5**

252



253

254 **Supplemental Figure 5. Venetoclax promotes oxidative metabolism in γδ T cells.**

255 **(A-B)** Quantification of length-to-width ratio (A), and area (B) in DMSO- and ven-γδ T cells of
256 transmission electron microscopic (TEM). Data are mean ± SD of over 5 randomly chosen cells per group
257 (n = 3 donors).

258 **(C)** Oxygen consumption rate (OCR) traces for DMSO- and ven-γδ T cells with quantification of basal,
259 maximal respiration and spare respiratory capacity (SRC). Data are represented as mean ± SD and are

260 representative of 3 independent experiments. Oligo, oligomycin; Rot/AA, rotenone and antimycin A; FCCP,
261 Carbonyl Cyanide 4-(Trifluoromethoxy) Phenylhydrazone.

262 **(D)** Extracellular acidification rate (ECAR) traces with basal and maximal ECAR quantification. Basal and
263 maximal ECAR values are shown on the right. 2-DG, 2-deoxy-D-glucose.

264 Data are presented as mean $\pm$ SD and analyzed using two-tailed paired Student's t test. *, $P < 0.05$; **, P
265 < 0.01 ; ***, $P < 0.001$.

266 **References**

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