Supplementary methods

Patients and age-matched healthy donor materials

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque PLUS (VWR). Isolated PBMCs were cryopreserved under liquid nitrogen. CAR T cell infusion products were generated from R/R CLL patients as previously described[1]. In short, autologous T cells were obtained by leukapheresis and stimulated with CD3 and CD28 antibody coated paramagnetic polystyrene beads prior to transduction with a lentiviral vector encoding the anti-CD19 4-1BBz CAR (Novartis, Tisagenlecleucel). Subsequently transduced cells were expanded for 9-11 days.

Flow Cytometry

The following metabolic dyes (all from Thermo Fisher Scientific) were used to determine metabolic parameters: Mitochondrial reactive oxygen species (ROS) (MitoSOX), Mitochondrial mass (Mitotracker Green), Mitochondrial membrane potential (Mitotracker Orange), Glucose uptake (2-NBDG), and total cellular ROS (DCFDA). Cells were incubated with metabolic dyes in PBS for 15 minutes at 37° C at a final concentration of 3×10^6 cells per ml. For T cell proliferation assays, PBMCs were labeled with cell trace violet (CTV) according to manufacturer's instructions (Thermo Fisher Scientific). After labeling the cells with metabolic dyes or CTV, cells were washed with PBS supplemented with 0.5% BSA and 0.02% Sodium Azide and stained for cell surface markers using fluorescently conjugated antibodies for 20 minutes on ice. The following antibodies were used (also see supplementary table 1): CD3, CD4, CD8, CD25, CD27, CD28, CD38, CD45RA, CD45RO, CD71, CD95, CD107a, PD-1, CCR7, LAG-3, CAR[3], GLUT1, and GLUT1 RBD GFP (described previously[4-6]). The BD Biosciences fixation and permeabilization kit (Cat. No. 554714) was used to fix cells and subsequently stain intracellular proteins using conjugated or unconjugated antibodies. Intracellular proteins were either stained directly by using ERRα, HIF-1α, HO-1, NRF-2, IFN-γ, TNF-α, pAKT(T308), pAKT(S473), pS6(S240/244), p4E-BP1(T37/T46), pPI3K(Y458/Y199) antibodies, or incubated with an unconjugated primary antibody against SOD1, SOD2, GLUT1, or PGC1-α, and subsequently stained with anti-rabbit PE, with

exception of PGC1-α which was stained using anti-mouse AF488. To separate live cells from dead cells during FACS, Fixable Viability Dye 780, Fixable Viability Dye 506, Live/Dead Aqua or 7AAD (all Thermo Fisher Scientific) were used according to manufacturer's instructions. Following staining, cells were washed in a solution of 0.5% BSA, 0.02% sodium azide in PBS, and acquired on a BD Fortessa or FACS Canto II flow cytometer. Results were analyzed using FlowJo 10.4 (BD Biosciences).

Extracellular flux analysis

Seahorse XF96 or XFp extracellular flux analyzers (Agilent) were used to analyze sorted $CD8^+$ T cells, as previously described[7]. In short, cell plates were PDL-coated and assay medium was prepared (non-buffered RPMI-1640 (Sigma) containing glucose (25 mM), glutamine (2 mM, Thermo Fisher Scientific), and sodium pyruvate (1 mM, Thermo Fisher Scientific)). To analyze oxygen consumption rate (OCR), spare respiratory capacity (SRC) and basal extracellular acidification rate (ECAR), a mitochondrial stress test was performed using the following compounds (final concentrations between brackets): oligomycin (1 µM), FCCP (1.5 µM), Rotenone (100 nM) and Antimycin A (1 µM). The Seahorse injection protocol was performed as described previously[7]. Spare respiratory capacity (SRC) was calculated as the ratio between maximum and basal OCR. Results were analyzed using Seahorse Wave version 2.4.

Supplementary Table 1: Antibodies used for flow cytometry

Target antigen	**Fluorochrome**	**manufacturer**	**Cat#**
CD3	AF700	eBioscience	56-0038-82
CD3	V500	BD Biosciences	561416
CD4	PE-Cy7	BD Biosciences	348809
CD4	APC	BD Biosciences	555349
CD4	AF594	BioLegend	300544
CD4	BV605	BD Biosciences	562658
CD8	V450	BD Biosciences	580347
CD25	APC	BD Biosciences	340907
CD25	BV786	BD Biosciences	563700
CD27	BUV397	BD Biosciences	563816
CD27	PerCP-eFl710	eBioscience	46-0279-42
CD28	BUV737	BD Biosciences	564438

CD38	BUV395	BD Biosciences	563811
CD38	PE-Cy7	BD Biosciences	335825
CD45RA	BV650	BD Biosciences	563953
CD45RO	BV570	BioLegend	304225
CD71	BV786	BD Biosciences	563768
CD107a	PE-Cy7	BD Biosciences	561348
CD95	BV510	BioLegend	305639
PD-1	PE-Cy7	BD Biosciences	561272
CAR	AF647	See reference[3]	N/A
CCR7	BV711	BioLegend	353227
ERRα	AF647	BioTechne	NBP1-47254AF647
GLUT1	AF700	BioTechne	FAB1418N
GLUT1	PE	R&D Biosystems	FAB1418P
GLUT1	unconjugated	Abcam	ab652
GLUT1-RBD	GFP	Metafora Biosystems	Glut1-G100
HIF-1α	AF488	BioLegend	359708
HO-1	PE	Enzo LifeSciences	ADI-OSA-111PE-F
IFN-γ	BUV395	BD Biosciences	563563
LAG-3	Pe-Cy7	BioLegend	369310
Mouse IgG	AF488	Life Technologies	A21042
NRF-2	AF488	Abcam	ab194984
PD-1	V450	BD Biosciences	562516
PGC1α	unconjugated	Abcam	ab77210
Rabbit IgG	PE	Southern Biotechnology Associates	4010-09S
SOD1	unconjugated	Cell Signalling	ab20926
SOD2	unconjugated	Cell Signalling	13141S
TNF-α	AF700	BD Biosciences	557996
pAKT(T308)	Unconjugated	Cell Signalling	2965S
pAKT(S473)	Unconjugated	Cell Signalling	4060S
pS6(S240/244)	unconjugated	Cell Signalling	5364L
p4E-BP1(T37/T46)	unconjugated	Cell Signalling	2855S
pPI3K(Y458/Y199)	unconjugated	Thermo Fisher Scientific	MA5-28028

Supplementary Table 2: Primers used for qPCR

Primer	**Direction**	**Sequence (5'-3')**	**Species**
mtDNA (ND-1)	Forward	TCATATTATGGCCAAGGGTC	Human
mtDNA (ND-1)	Reverse	CTCCTTTAACCTCTCCACCC	Human
nDNA (beta globulin)	Forward	TTTTCCCACCCTTAGGCTG	Human
nDNA (beta globulin)	Reverse	CTCACTCAGTGTGGCAAAG	Human
18S	Forward	CGGCTACCACATCCAAGGAA	Human
18S	Reverse	GCTGGAATTACCGCGGCT	Human
GLUT1	Forward	AGGTGATCGAGGAGTTCTAC	Human
GLUT1	Reverse	TCAAAGGACTTGCCCAGTTT	Human

Supplementary References

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3. Jena B, Maiti S, Huls H, et al. Chimeric antigen receptor (CAR)-specific monoclonal antibody to detect CD19-specific T cells in clinical trials. *PLoS One*. 2013;8(3):e57838.
4. Manel N, Kim FJ, Kinet S, Taylor N, Sitbon M, Battini JL. The ubiquitous glucose transporter GLUT-1 is a receptor for HTLV. *Cell*. 2003;115(4):449-459.
5. Cretenet G, Clerc I, Matias M, et al. Cell surface Glut1 levels distinguish human CD4 and CD8 T lymphocyte subsets with distinct effector functions. *Sci Rep*. 2016;6:24129.
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7. van der Windt GJ, Chang CH, Pearce EL. Measuring Bioenergetics in T Cells Using a Seahorse Extracellular Flux Analyzer. *Curr Protoc Immunol*. 2016;113:3 16B 11-13 16B 14.

Supplemental Fig. 1:

PBMCs from CLL patients and healthy donors (HD) were analyzed directly after thawing (E), or stimulated using anti-CD3/CD28 antibodies for 2 (A-C, E-G, I, J) or 5 (D) days. (**A**) Gating strategy. (**B**) Relative increase of PD-1 expression. (**C**) IFN-γ and TNF-α expression by $CD8^+$ T cells (n=12). (**D**) $CD8^+$ T cells were analyzed for proliferation (n=8). (**E**) GLUT1 expression in $CD8^+$ T cells measured by a conjugated antibody (n=12). (**F**) Relative increase of GLUT-1 expression. (**G**) CLL derived $CD8^+$ T cell response to stimulation was determined to be "good" (% 2-NBDG hi > 40% of total $CD8^+$ T cells) or poor (% 2-NBDG hi < 40% of total $CD8^+$ T cells) based on data from Fig. 1E, and white blood cell count and %CLL were compared. (**H**) Expression of CD28 (n=4) and CD3 (CLL, n=21; HD, n=17) on $CD8^+$ cells. (**I**) Glucose uptake by $CD8^+$ T cells (CLL, n=23; HD, n=20; unstimulated control and anti-CD3/CD28 data are the same as in Fig. 1E). (**J**) Surface expression of GLUT1 by $CD8^+$ T cells using the GLUT1 RBD GFP construct (CLL, n=6; HD, n=4; unstimulated control and anti-CD3/CD28 data are the same as in Fig. 1D), and activated ($CD25^+$) $CD8^+$ T cells were analyzed for GLUT1 surface expression using the GLUT1 RBD GFP construct (CLL, n=6; HD, n=4). Normality was determined by a D'Agostino & Pearson normality test. The *p* value was calculated by a Mann-Whitney test (C, E-G, J), an unpaired T-test (D, H, I), a Welch's test (B, C). Data are presented as mean ± SEM, **p*<0.05; ***p*<0.005; ****p*<0.0005.

Supplemental fig. 2:

PBMCs from CLL patients and healthy donors (HD) were stimulated using anti-CD3/CD28 antibodies and analyzed after 2 days. CD8 T cells of HD and CLL were analyzed for the following intracellular proteins; (**A**) pAKT(S473), (**B**) pPI3K(Y458/Y199), (**C**) pS6(S240/S244), (**D**) pAKT(T308), (**E**) p4E-BP1(T37/T46), (**F**) HIF-1α. Normality was determined by a D'Agostino & Pearson normality test. The *p* value was calculated by a Mann-Whitney test (A-F). Data are presented as mean ± SEM, **p*<0.05; ***p*<0.005.

Supplemental fig. 3:

(**A**) Representative seahorse plot showing oxygen consumption rate (OCR) for 1 CLL patient and 1 heathy donor (sorted CD8 T cells). (**B**) Graphs showing proton leak, maximum respiration, and ATP production (CLL, n=6-7; HD, n=5-7). (**C**) Relative MFI of total cellular ROS sensor DCFDA measured directly after thawing and depleting $CD19^+$ cells (CLL, n=6; HD, n=6). (**D**) Gating strategy and representative plots for MitoSOX and Mitotracker Orange analyses. (**E**) Representative FACS plots showing the gating strategy (top plots showing general gating for 1 healthy donor). Relative values were obtained by normalizing to an unrelated control sample (C). Normality was determined by a D'Agostino & Pearson normality test. The *p* value was calculated by a Wilcoxon Signed Rank test (B), or an unpaired T test (C). Data are presented as mean ± SEM.

Supplemental Fig. 4:

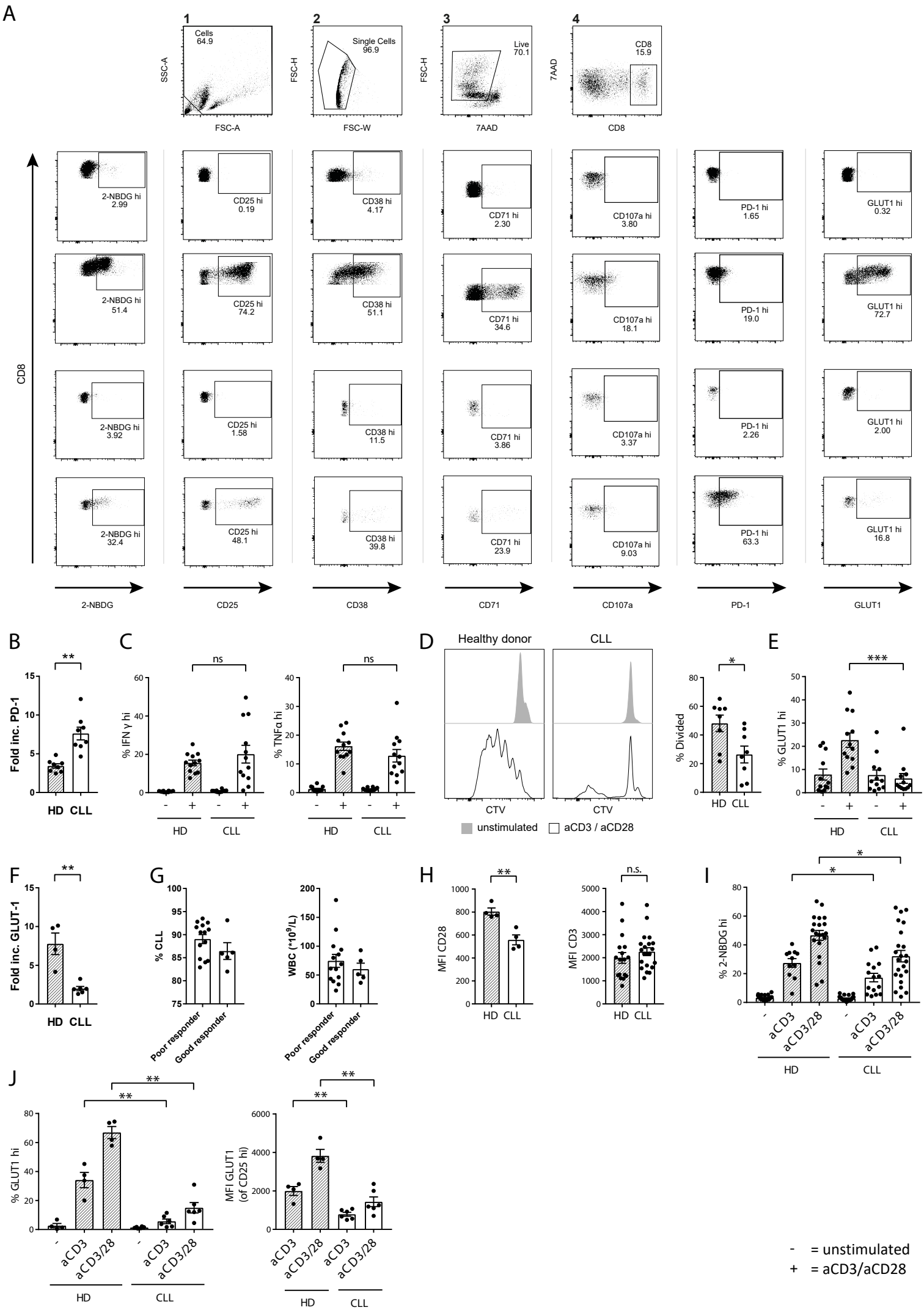
(**A**) Representative dot plots showing the gating strategy to separate $CD3^+$ $CD8^+$ naïve cells ($CD27^+CD45RA^+CCR7^+CD95^-$), memory stem cells ($CD27^+CD45RA^+CCR7^+CD95^+$), memory cells ($CD27^+CD45RA^-$), and effector cells ($CD27^-CD45RA^{-/+}$). (**B**) Induction of mitochondrial mass calculated by dividing the MFI of Mitotracker green of stimulated cells by MFI of unstimulated cells, similar experiment as in Fig 5C, but with larger cohort (CLL, n=16; HD, n=17). Normality was determined by a

D'Agostino & Pearson normality test. The *p* value was calculated by a Mann-Whitney test (B). Data are presented as mean ± SEM.

Supplemental Fig. 5:

Representative dot plots showing the gating strategy to separate $CD3^+CD8^+CAR^+$ effector ($CD27^-$ $CD45RO^+$), and memory T cells ($CD27^+$ $CD45RO^+$).

Supplemental figure 1



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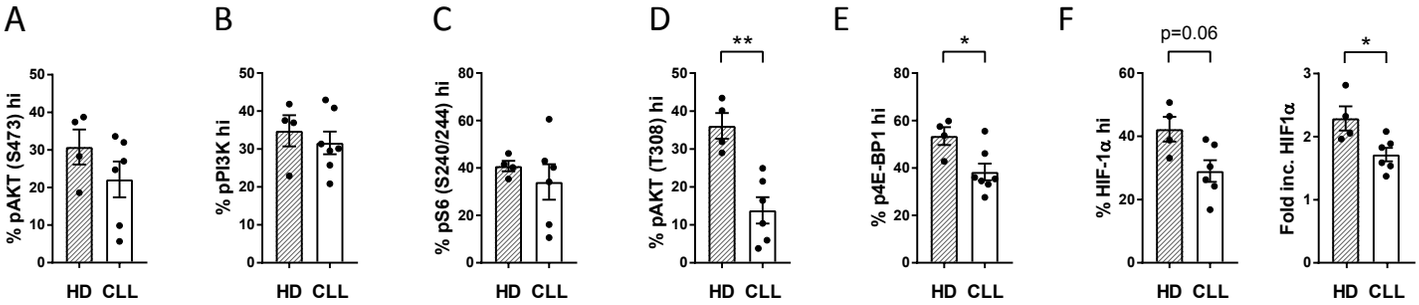

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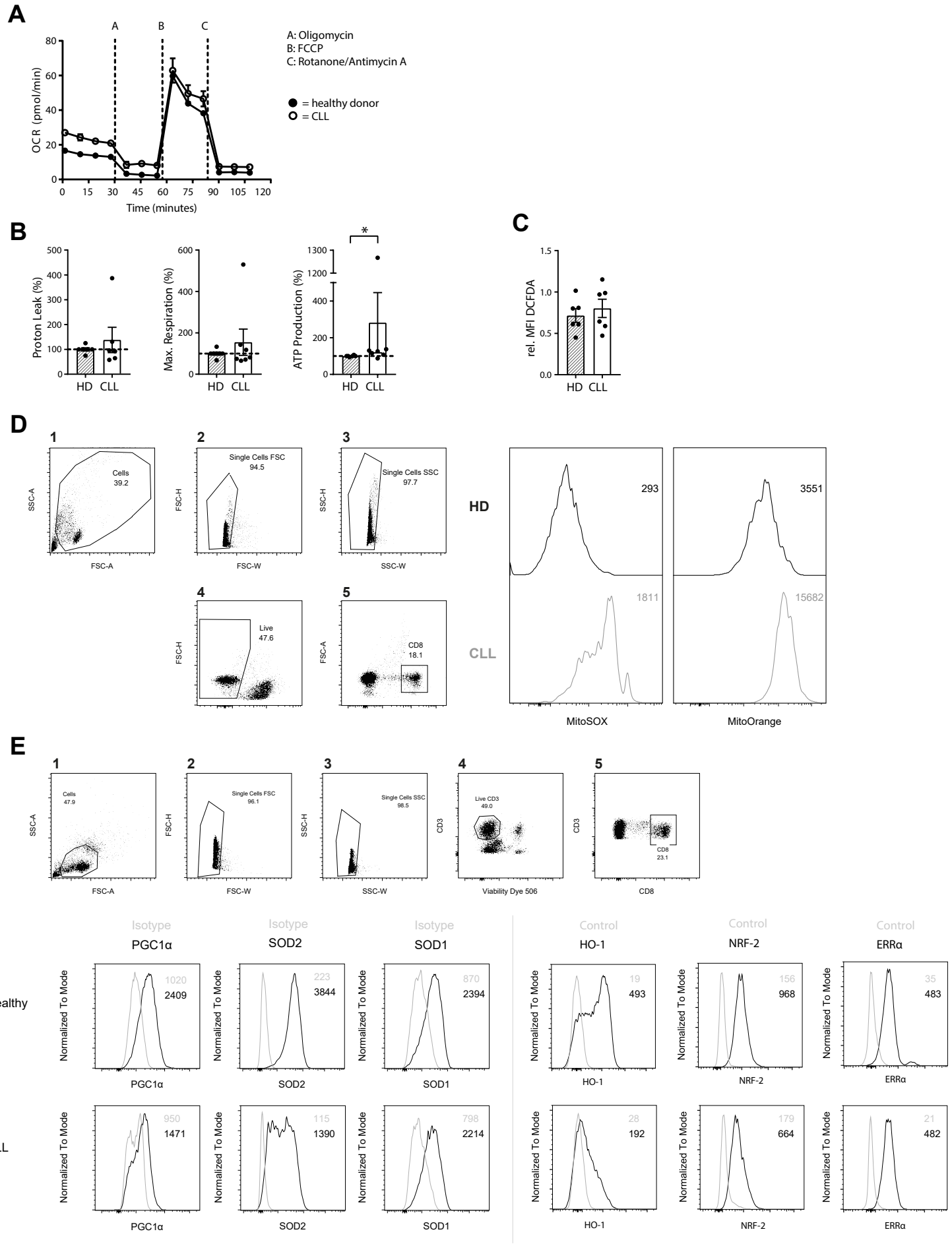
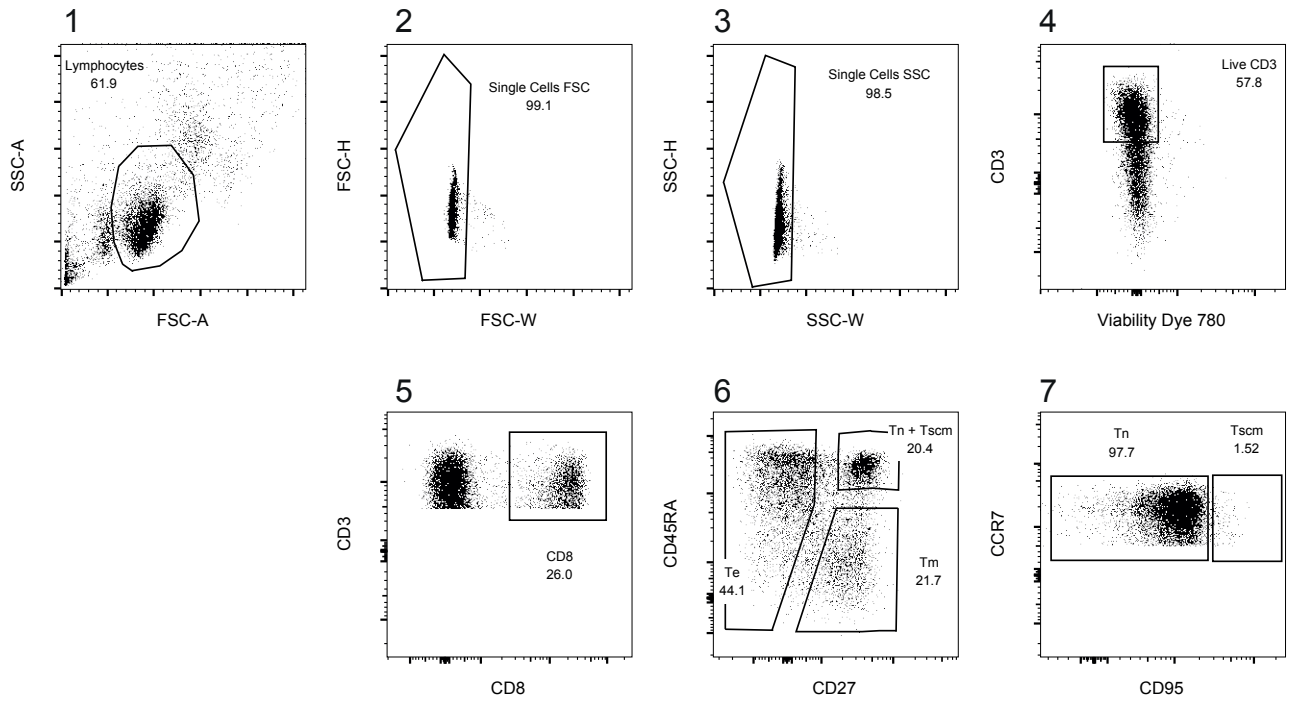


Supplemental figure 4

A



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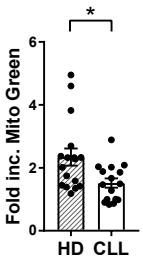

Supplemental figure 5