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Supplemental information

Clinically relevant T cell expansion media activate distinct metabolic programs uncoupled from cellular function

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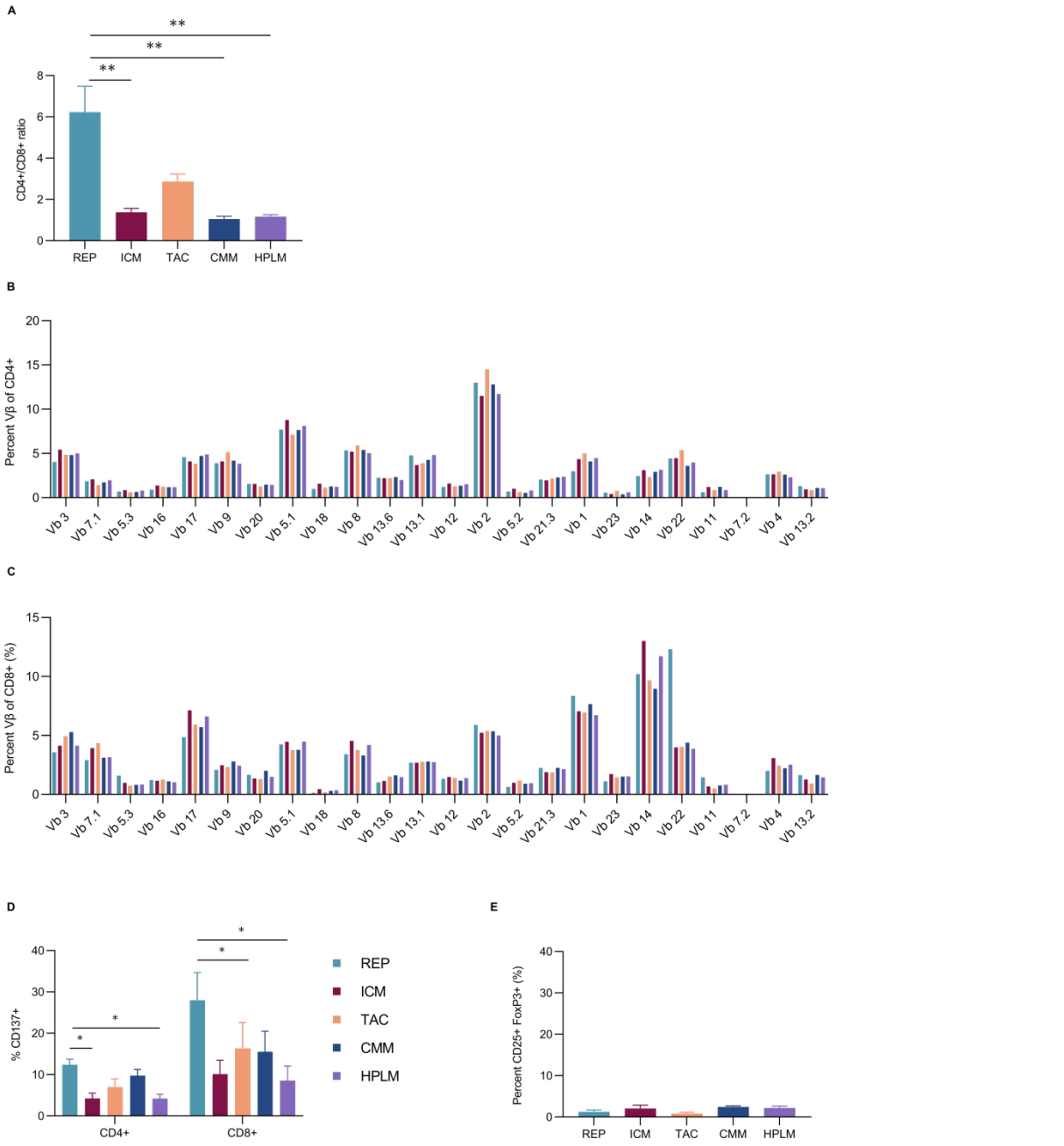
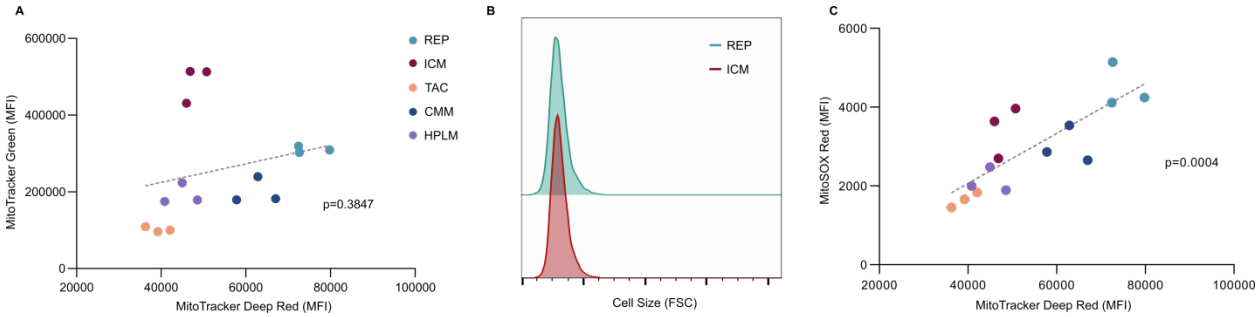
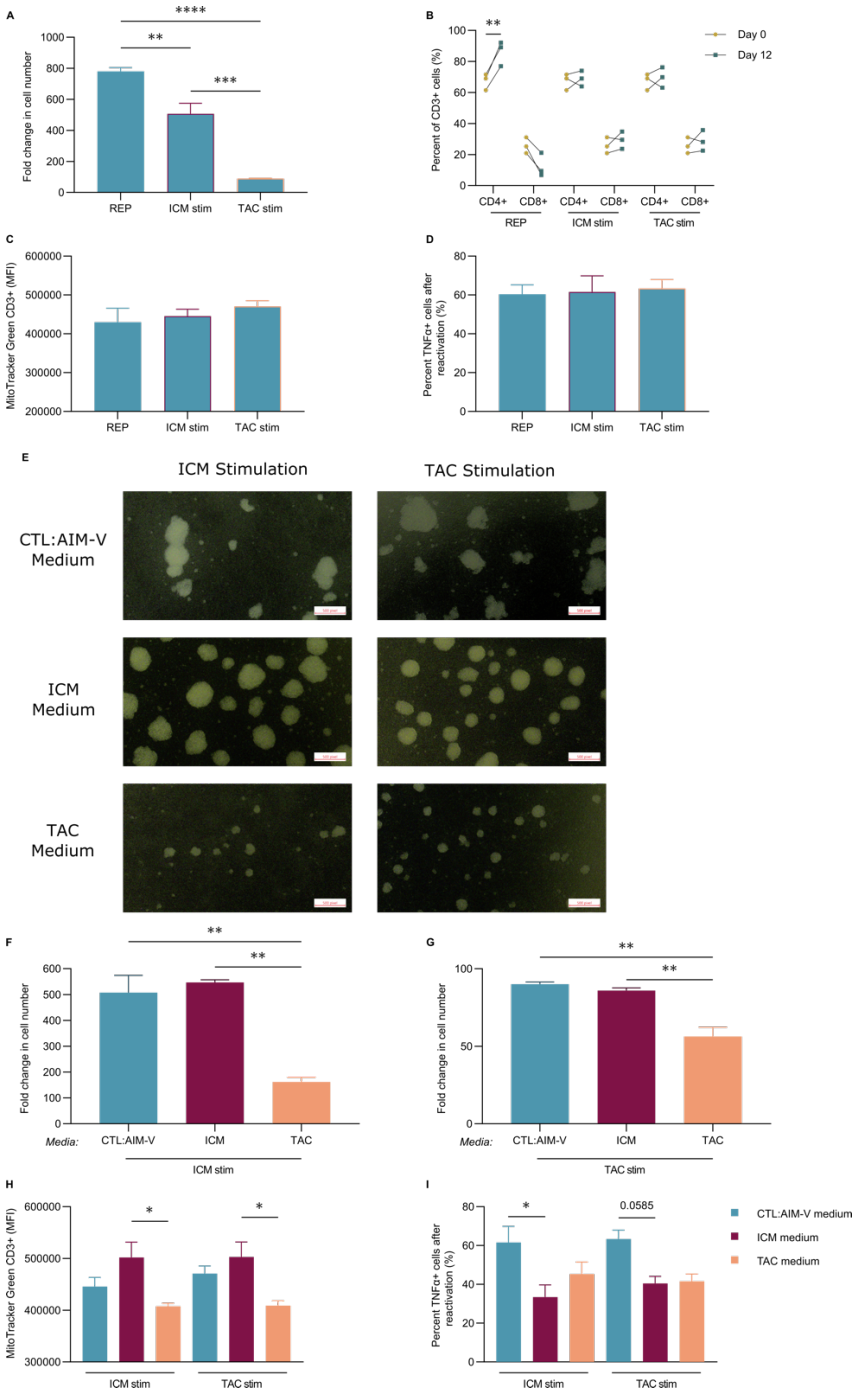


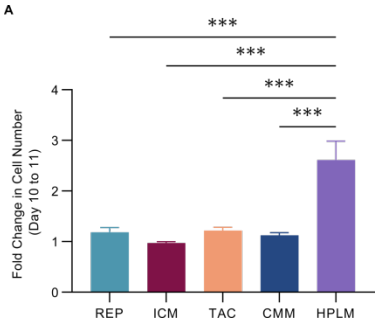
Supplemental Figure 1:(A) Ratio of CD4+ and CD8+ T cells of live CD3+ cells 12 days post-expansion. **(B,C)** Percent of **(B)** CD4+ and **(C)** CD8+ T cells positive for 24 TCR Vβ types following 12 days of expansion. Figure is a representative from one of 3 donors. **(D)** Percentage of CD137+ CD4+ and CD8+ cells. **(E)** Percentage of T_{reg} cells (CD25+ FoxP3+; gated on live CD4+ cells). (**A,D,E**) Data are shown as mean of n=6 +SEM from healthy donors. Statistical significance was calculated by one-way ANOVA (* $p<0.05$, ** $p<0.01$).



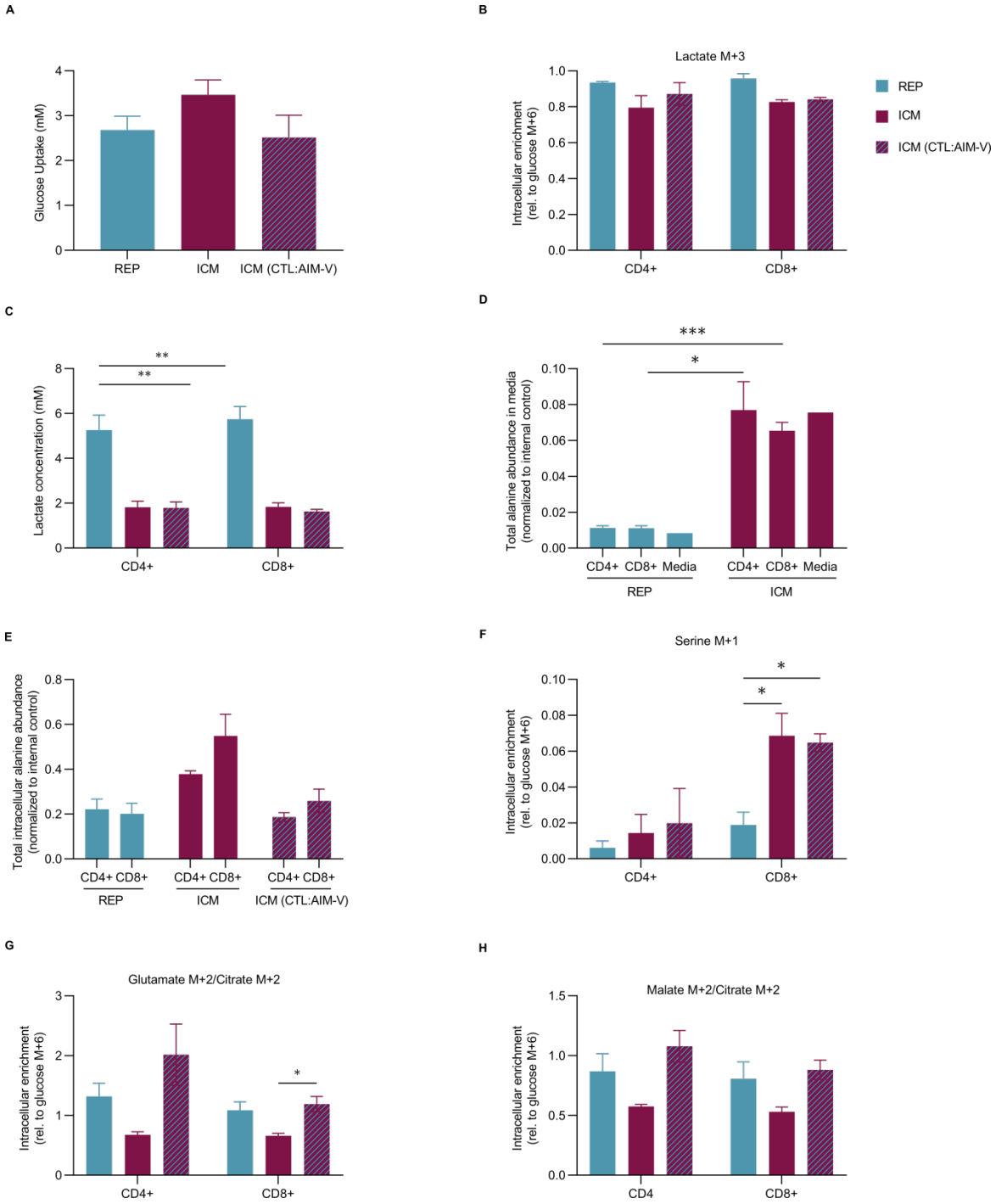
Supplemental Figure 2: (A-C) T cells from three healthy donors were expanded in 5 different conditions for 12 days. **(A)** Correlation between mitochondrial mass (MitoTracker Green) and mitochondrial activity (MitoTracker Deep Red) of live cells. **(B)** Representative plot of cell size of CD3+ T cells in REP (blue) and ICM (red) conditions on day 12. **(C)** Correlation between mitochondrial ROS (MitoSOX) and mitochondrial activity (MitoTracker Deep Red) of live cells. Dashed line represents simple linear regression, p-value determined by Pearson correlation.



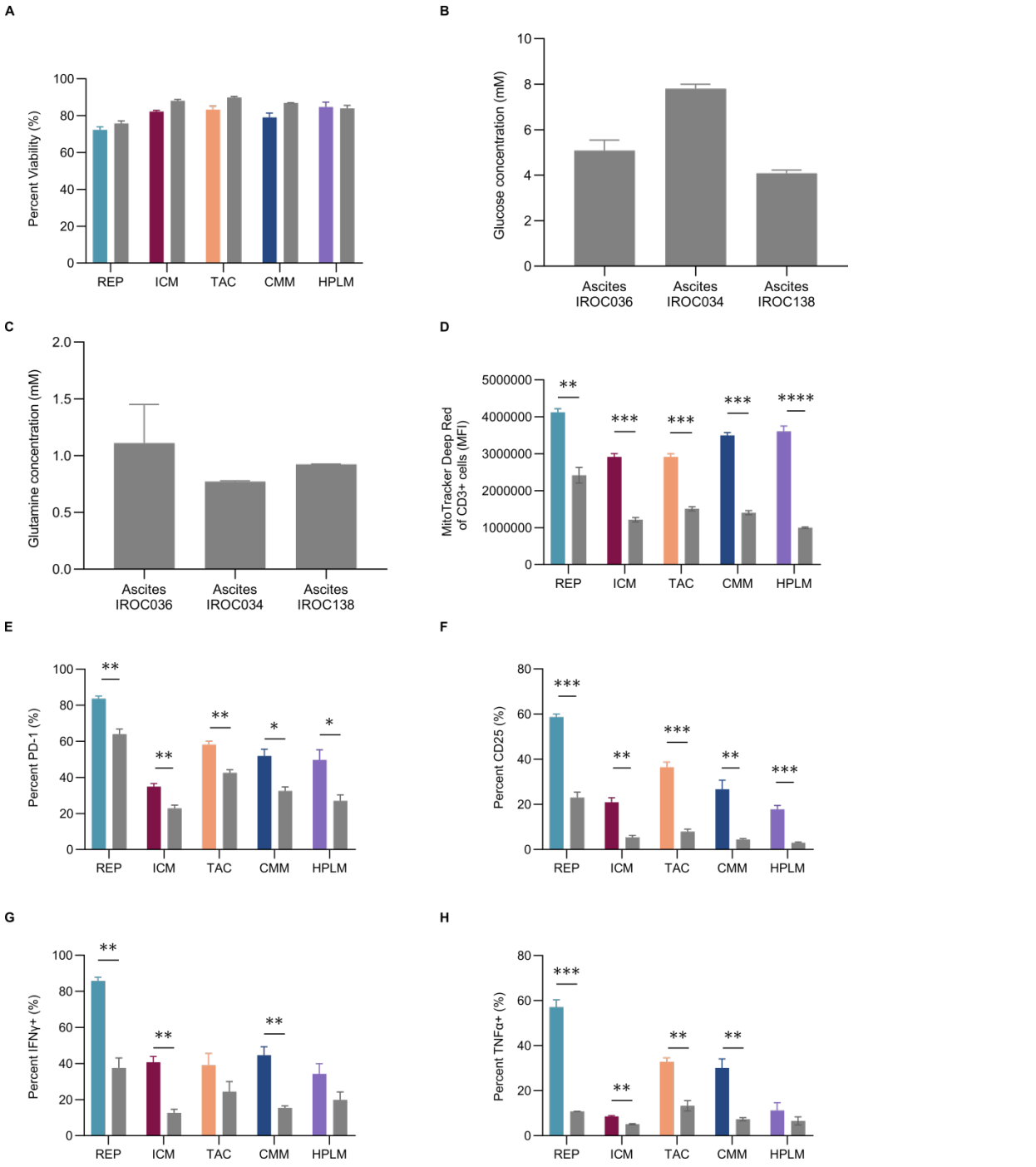
Supplemental Figure 3: (A-D) T cells from 3 healthy donors were expanded with 3 different stimulation protocols (REP, ICM, TAC) within the same medium (CTL:AIM-V) for 12 days. **(A)** Fold change in cell number from day 0 to 11. **(B)** Proportion of CD4+ and CD8+ T cells of live CD3+ cells, pre- and post-expansion. **(C)** Median fluorescence intensity (MFI) of mitochondrial mass (MitoTracker Green). **(D)** Percentage of TNFα positive cells after CD3/CD28 reactivation following expansion. **(E-I)** T cells from 3 healthy donors were expanded in 3 different medias (CTL:AIM-V, ICM and TAC) and activated by either ICM or TAC methods for 12 days. **(E)** Microscope images of T cells 3 days after culture. Stimulation condition (column) and culture media (row) are indicated. Fold change in cell number from day 0 to 11 in ICM **(F)** or TAC **(G)** stimulated cells within different media. **(H)** Median fluorescence intensity (MFI) of mitochondrial mass (MitoTracker Green) **(I)** Percent TNFα positive of live cells after CD3/CD28 reactivation following expansion.



Supplemental Figure 4: (A) T cells from three healthy donors were expanded in 5 different conditions for 11 days. Fold change in cell number from day 10-11 in the respective conditions. Data are shown as mean of n=3 +SEM from healthy donors. Statistical significance was calculated one-way ANOVA (** $p<0.01$, *** $p<0.001$).



Supplemental Figure 5: (A-H) T cells from three healthy donors were expanded in REP and ICM for 11 days. On day 11, CD4+ and CD8+ cells were separated and incubated in [U-^{13}C]glucose media for 24 hours: REP cells in CTL:AIM-V medium (blue bar), ICM cells in ICM medium (red bar) and ICM cells in CTL:AIM-V medium (red and blue dashes). **(A)** Glucose uptake from day 11-12 in CD4+ and CD8+ T cells. **(B)** Intracellular lactate M+3 relative to intracellular glucose M+6 enrichment. **(C)** Lactate concentration in media from day 11-12 in CD4+ and CD8+ T cells. **(D-E)** Alanine abundance calculated by totaling peak area of all alanine isotopologues and normalizing to internal control norvaline peak area. **(F)** Intracellular serine M+1 relative to intracellular glucose M+6 enrichment. **(G)** Glutamate M+2 and **(H)** malate M+2 relative to citrate M+2. Data are shown as mean of n=3 +SEM from healthy donors. Statistical significance was calculated by Student's t-tests (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).



Supplemental Figure 6: (A, D-H) T cells from three healthy donors were expanded in 5 different conditions over 12 days. On day 12 T cell products were reactivated (CD3/CD28) in media (coloured bars) or ascites (grey bars), T cell metabolism and function was assessed after 2 days. **(A)** Percent viability of CD3+ T cells in media and ascites. **(B-C)** Glucose **(B)** and Glutamine **(C)** concentrations measured in primary ovarian cancer ascites fluid from three patients. **(D)** Median fluorescence intensity (MFI) of mitochondrial activity (MitoTracker Deep Red). Percentage of PD-1 **(E)**, CD25 **(F)**, IFNγ **(G)**, and TNFα **(H)** positive cells of live CD3+ cells in the media and ascites. Data is shown as mean of n=3 +SEM from 3 healthy donors. Statistical significance was calculated by Student's t-tests (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$).

Supplementary Table 1: Flow Cytometry Antibodies

Fluorochrome	Marker	Expression	Clone	Company	Catalogue number
FITC	TNFα	Function	MAb11	BD Biosciences	554512
PE	IFNγ	Function	4S.B3	BD Biosciences	554552
PE CF594	FoxP3	Tregs	236A/E7	BD Biosciences	563955
PerCP	CD8	Effector T cells	RPA-T8	BioLegend	301030
PerCP-eFluor710	CD25	Activation	4E3	Thermo	46-0257-41
PE-Cy7	CD45RO	Phenotype	UCHL1	Thermo	25-0457-42
---	MitoTracker Deep Red	Mitochondrial activity	---	Thermo	M22426
AF700	CD4	Helper T cells	RPA-T4	BioLegend	300526
APC/Fire750	CCR7	Phenotype	G043H7	BioLegend	353246
BV605	CD137	Activation	4B4-1	BioLegend	309822
BV650	PD1	Activation/Exhaustion	EH12.2H7	BioLegend	329950
BV750	CD3	T cells	SK7	BioLegend	344845
---	MitoTracker Green	Mitochondrial Mass	---	Thermo	M7514
---	MitoSOX Red	Mitochondrial ROS	---	Thermo	M36008
eFlour506	Viability	Live/dead cells	---	Thermo	65-0866-14
FITC	Vβ	TCR	---	Beckman	IM3497
PE	Vβ	TCR	---	Beckman	IM3497
ZombieNIR	Viability	Live/dead cells	---	BioLegend	423105