Supplementary Information for

Fever supports CD8+ effector T cell responses by promoting mitochondrial translation

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Figures S1 to S5
**Fig. S1.** Exposure to 39°C promotes metabolism of activated T cells.

(A) Number of proliferative divisions of 37°C T<sub>E</sub> or 39°C T<sub>E</sub> as assessed by Cell Trace Violet (CTV) 72h after activation. (B and C) Representative histogram and bar graphs of the MFI of surface expressed CD25 and CD98 and forward scatter cell size in CD69 negative cells, 24h post activation. (D) Representative extracellular acidification rate (ECAR) measured at baseline and following administration of oligomycin (oligo), FCCP, rotenone and antimycin (R/A), 24h post activation. n=>3 biological replicates/group as indicated by individual data points and shown as mean ± SEM.
Fig. S2. Exposure to 39°C enhances mitochondrial mass in T cells.
(A) Bar graphs showing the MFI of transcription factors, 24h post activation (B) Western blot of mitochondrial Tomm20, 24h post activation. Mitochondrial mass 24h post activation in cell cultured in (C) low glucose (1mM) conditions and (D) in the presence of 2DG (2mM). (E) Mitochondrial mass as 24h post activation in CD8+ OT-I T cells stimulated with dendritic cells (DC) loaded with SIINFEKL peptide. (F) Mitochondrial mass in CD8+ T cells 24h following activation, with phorbol myristate acetate and ionomycin (PMA/IONO). (G) Mitochondrial mass in CD8+ T cells 24h following activation ± ethanol (ETOH; 0.05%) to enhance membrane fluidity. (H) Naïve CD8+ T cells were exposed to 37°C or 39°C for 6 hours, prior to being activated at 37°C for 24 hours then assessed for mitochondrial mass. (I) Western blot of Uncoupling protein 1 and 2 (UCP1, UCP2), 24h post activation (J) Mitochondrial mass in CD8+ T cells 24h following activation ± NMSE973 (HSP90i; 200nM) (K) Representative plots of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) at baseline and following administration of oligomycin (oligo), FCCP, rotenone and antimycin (R/A) of T cells 3 days after activation (L and M) Basal oxygen consumption and spare respiratory capacity (SRC) 3 days after activation. (N) ECAR measured at baseline and at the maximal rate following addition of rotenone and antimycin. (O) Naïve CD8+ T cells were activated with anti-CD3/CD28 at 37°C or 39°C for 24h then subsequently cultured in IL-2 at 37°C until day 3; to induce a memory-like phenotype (T_M), cells were cultured from day 3-6 in IL-15 (P) Plots of OCR and ECAR at baseline and following ± PMA/IONO followed by oligomycin (O), FCCP (F) and rotenone and antimycin (R/A). Data shows mean ± SEM of three biological replicates. (Q) Representative histogram and bar graph of MFI of IFN-γ production and percentage IFN-γ+ assessed following overnight re-stimulation with anti-CD3/CD28. n=≥3 biological replicates/group as indicated by individual data points and shown as mean ± SEM. *p<0.05, **p<0.01, ***p<0.001.
Fig. S3. WBH augments OCR and ECAR of activated T cells.
(A) Average body temperature following whole body heating (WBH) or exposure to comparable conditions at ambient room temperature (CTRL) n>7 mice per group. (B) Activation state, measured as percent CD69+ of CD8+ T cells isolated from popliteal (pLN) and inguinal (iLN) lymph nodes 24 hours after in vivo activation with anti-CD3/CD28 ± WBH. Data from two independent experiments n=6 mice/group. (C and D) Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) at baseline and following administration of oligomycin (oligo) FCCP and rotenone and antimycin (RA) of CD8+ T cell from iLN 24h after activation (each group represents cells pooled from 3 mice). Data points shown as mean ± SEM.
Fig. S4. Febrile temperature promotes mitochondrial translation, but not the expression of PGC1α.

(A) MFI of PGC1α expression in CD69+ and CD69− CD8+ T cells, 24h after activation as measured by flow cytometry (B) Heatmap showing mRNA expression of mitochondrially expressed genes in naïve or activated CD8+ T cells. (C) Mitochondrial mass in CD8+ T cells 24h post-activation in control (CTRL) and rapamycin (RAPA) treated cells. (D) Western blot of MRPL39 expression in naïve or activated CD8+ T cells targeted with CRISPER/Cas9 guides against MRPL39 (gMRPL) or a non-targeting control (gCTRL), 72h post activation. (E) Mitochondrial mass in CD8+ T cells targeted with CRISPR/Cas9 guides against OXA1L (gOXA1L) or a non-targeting control (gCTRL), 72h post activation. (F) Mitochondrial mass, 24h post activation in 37°C treated with indicated concentrations of Tigecycline (TIG). (G) Protein synthesis, measured as MFI of O-propargyl-puromycin (OPP) incorporation by flow cytometry 24 hours after activation of 39°C T cells ± tigecycline (TIG) in cell exposed to vehicle (VEH) or cycloheximide (CH). n≥3 biological replicates/group as indicated by individual data points and shown as mean ± SEM (except Fig. S4D, n=1). *p<0.05, **p<0.01, ***p<0.001.
Fig. S5. Superior anti-tumor activity of 39°C primed T cells is dependent on enhanced mitochondrial translation.

(A) Representative histogram and bar graph showing percentage of donor cells isolated from the spleens of recipient mice. n=3 per group shown as mean ± SEM *p<0.05, ***p<0.001