

*Supplementary Material*

**Human Naïve CD8<sup>+</sup> T-Cells Show Different Energetic Requirements Compared to Memory Cells and Simultaneously Engage mTOR and Autophagy Upon Activation**

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## 1.1 Supplementary Figures

**Figure S1.** Gating strategy for CD8<sup>+</sup> T-cell subsets. CD3<sup>+</sup> events were gated as CD8<sup>+</sup>. Differential expression of CD27, CD45RA, and CCR7 was used to identify subsets as naïve (N), central memory (CM), transitional memory (TM), effector memory (EM), or terminally differentiated effector memory (EMRA).

**Figure S2.** PD-1 expression in activated CD8<sup>+</sup> T-cell subsets. PBMCs were activated with plate-bound  $\alpha$ CD3. Expression of PD-1 was measured by flow cytometry after 24 hr. Left panel: one representative example is shown. Right panel: horizontal lines depict mean values. N = 15. Statistical significance was determined using a one-way paired ANOVA with Bonferroni's post-test. \*\* P < 0.01, \*\*\* P < 0.001.

**Figure S3.** Antigen-specific priming of naïve CD8<sup>+</sup> T-cells depends on autophagy and mTOR. (A–D) MelA-specific naïve CD8<sup>+</sup> T-cells were primed in the absence or presence of various metabolic inhibitors. (A and D) MelA-specific CD8<sup>+</sup> T-cells were quantified by flow cytometry after 10 days using cognate PE-conjugated ELA/HLA-A2 tetramers. Expression of granzyme B (B) and Tbet (C) in primed MelA-specific CD8<sup>+</sup> T-cells was measured by flow cytometry after 10 days. Horizontal lines represent mean values. Statistical significance was determined using the Wilcoxon signed rank test. N = 7 (A-C); N = 8 (D). \* P < 0.05.

## 1.2 Supplementary Tables

**Table S1.** Directly conjugated antibodies used for flow cytometry staining

**Table S2.** Gene expression in CD8<sup>+</sup> T-cell subsets.

**Table S3.** Basal metabolic properties of CD8<sup>+</sup> T-cell subsets.

**Table S4.** Response to activation of CD8<sup>+</sup> T-cell subsets.

**Table S5.** Metabolic switch after activation in CD8<sup>+</sup> T-cell subsets.

**Table S6.** Effect of metabolic inhibition on activation in CD8<sup>+</sup> T-cell subsets.