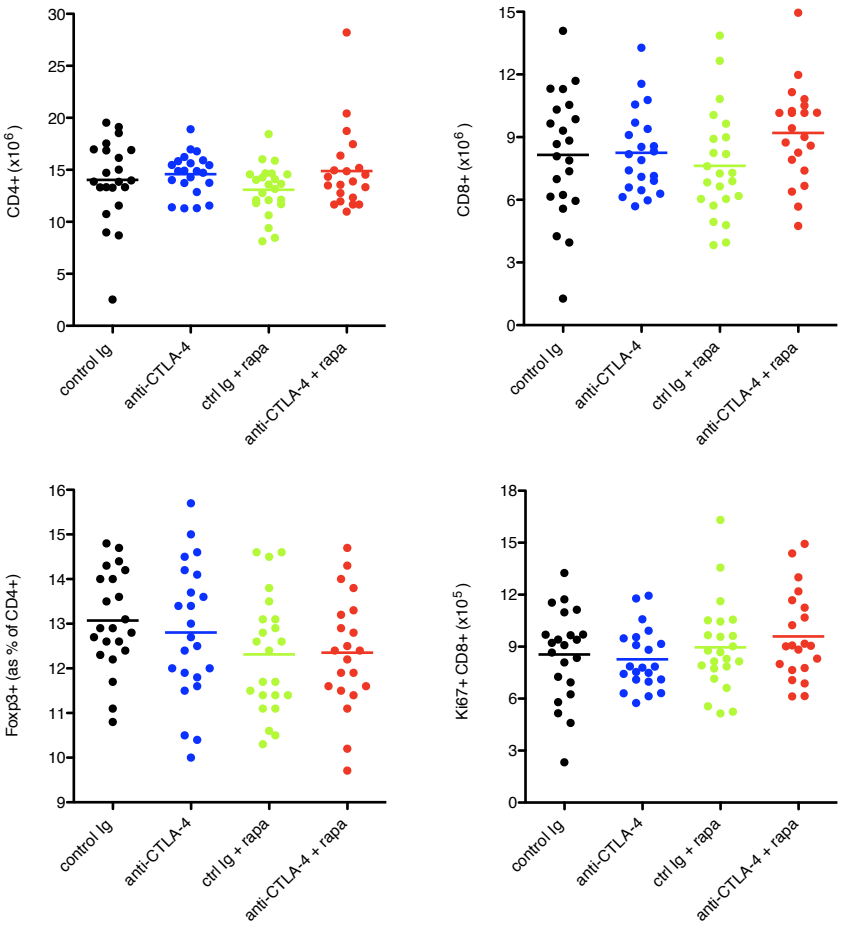

Figure S1. Immunization in the presence of CTLA-4 blockade and rapamycin does not adversely affect endogenous polyclonal T cell numbers or relative frequency. Wild-type C57BL/6 mice received either control Ig or anti-CTLA-4 with or without rapamycin just prior to i.v. inoculation with 5x10^3 cfu LM-OVA. Daily rapamycin injections were continued through day 8 post-immunization (p.i.). At day 45 p.i., lymphocytes were isolated from spleen and analyzed by flow cytometry. Absolute numbers were calculated based on flow cytometry frequency and total cell counts from each spleen. Data shown were pooled from 3 independent experiments with n=7-8 mice per group per experiment. All quantifications were analyzed by 1-way ANOVA but exhibited no significant differences ($p>0.05$).

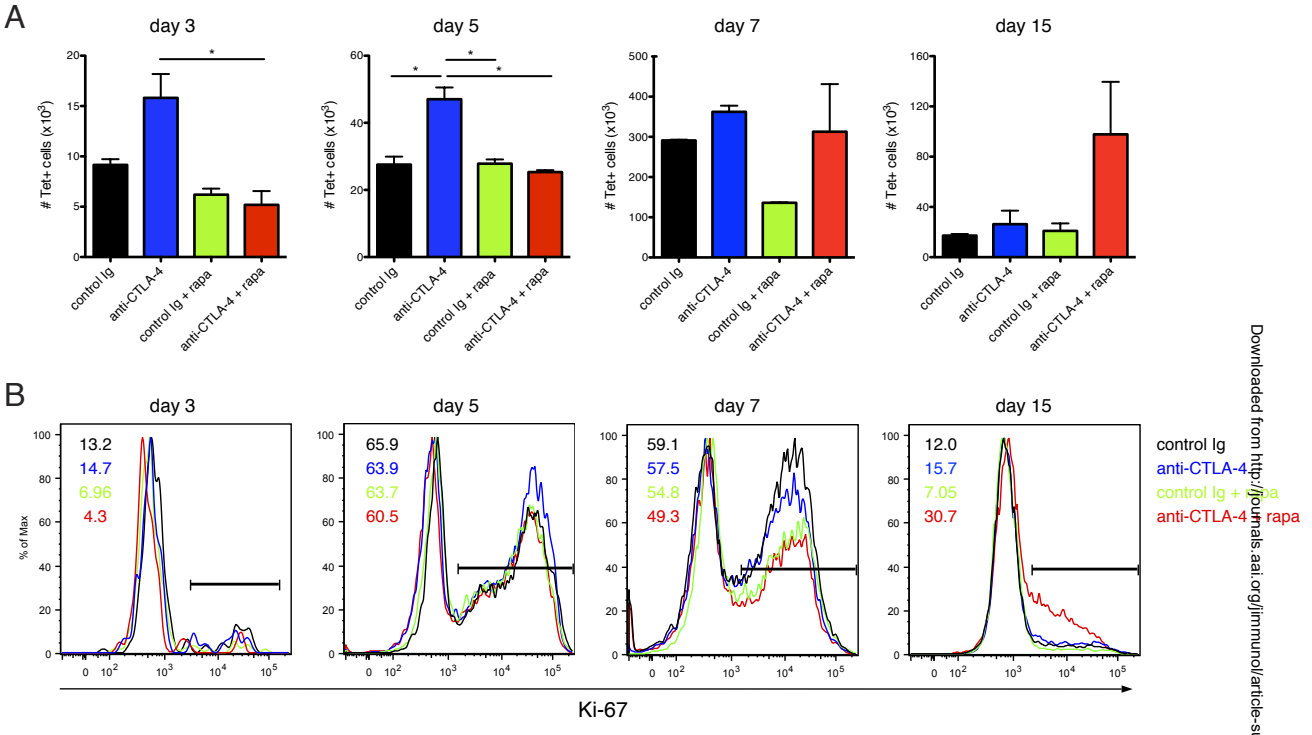

Figure S2. CTLA-4 blockade and rapamycin result in increased antigen-specific CD8+ T cell expansion. Lymphocytes were pooled from spleen and lymph nodes from mice immunized with LM-OVA in the presence of anti-CTLA-4 and/or rapamycin. Isolated cells were counted using an automatic cell counter and analyzed by flow cytometry to calculate absolute number of antigen-specific SIINFEKL tetramer+ CD8+ T cells at indicated time-points (A). Pooled lymphocytes from treated mice were also stained for Ki-67 to determine the frequency of proliferating cells, and representative histograms with corresponding frequencies among gated CD8+ cells are shown (B). Quantifications in (A) were pooled, and representative plots (B) were from 2 independent experiments with n=2-3 mice per group per time-point per experiment. All quantifications were analyzed by 1-way ANOVA. *, $p \leq 0.05$.

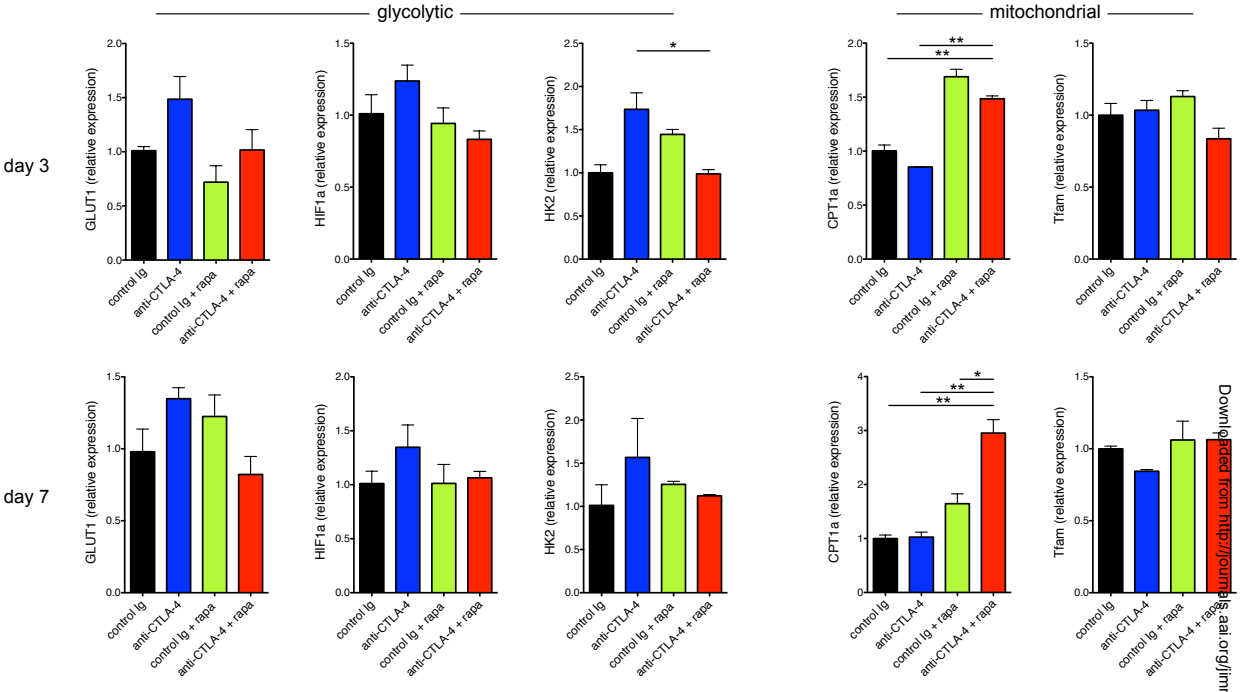

Figure S3. Altered metabolism-associated gene expression in antigen-specific CD8+ T cells early after immunization in the presence of CTLA-4 blockade and rapamycin. OVA-specific CD8+ T cells from pooled spleen and lymph nodes were sorted by FACS at 3 or 7 days after immunization in the presence of anti-CTLA-4 and rapamycin or an isotype control, and mRNA was isolated from sorted cells for analysis by real-time PCR. Relative expression of glycolysis-associated genes: glucose transporter 1 (GLUT1), hypoxia-inducible factor 1-alpha (HIF1a), and hexokinase 2 (HK2) along with relative expression of CPT1a and Tfam are shown in representative plots from one of 3 independent experiments with n=5 mice per group per experiment. All quantifications were analyzed by 1-way ANOVA. *, p≤0.05; **, p≤0.01.

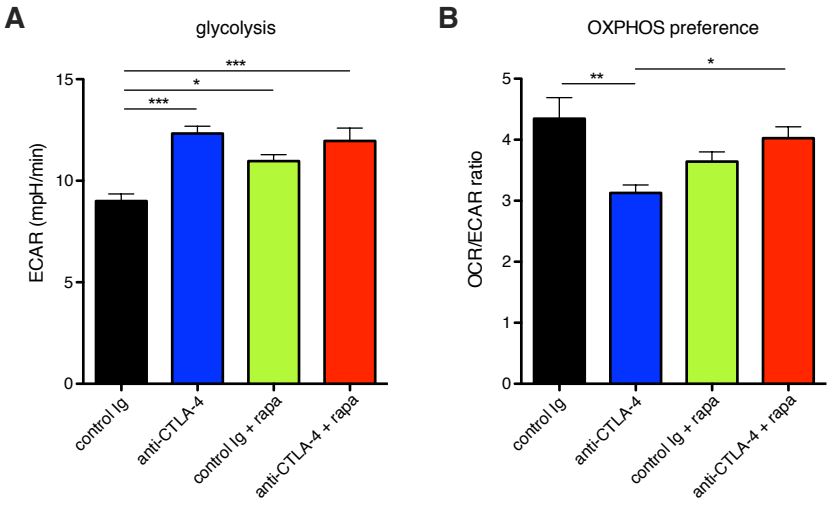

Figure S4. Differential preferences for glycolysis and OXPHOS in CD8+ T cells primed in the presence of rapamycin and anti-CTLA-4. OVA-specific OTI cells were adoptively transferred to naïve mice 1 day prior to inoculation with LM-OVA in the presence of anti-CTLA-4, rapamycin, and/or an isotype control. OTI cells from pooled spleen and lymph nodes were sorted by FACS at day 15 p.i. to measure extracellular acidification (ECAR) and oxygen consumption rates (OCR) in real time at 3 time-points under basal conditions. Representative data from one of 3 independent experiments with n=5 mice per group are shown. Data were analyzed by 1-way ANOVA. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.