Supplemental Information
Stress-Mediated Attenuation of Translation Undermines T Cell Tumor Control

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Running Title: Proteasome fueled metabolism supports tumor immunity

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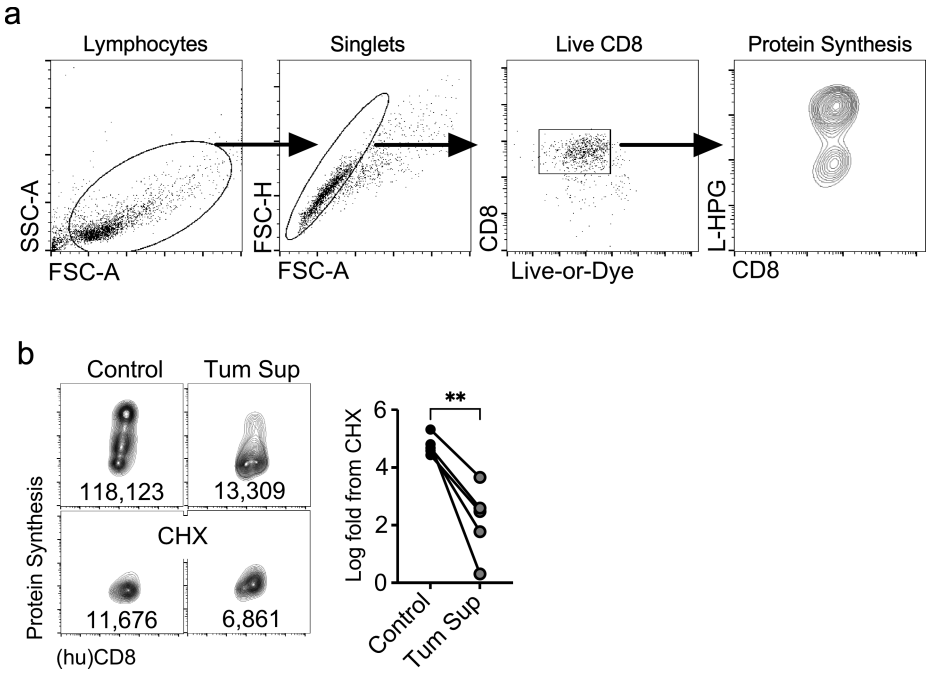
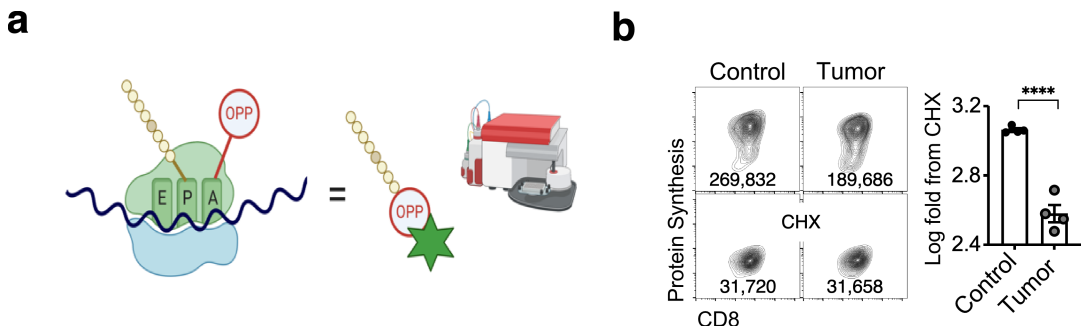
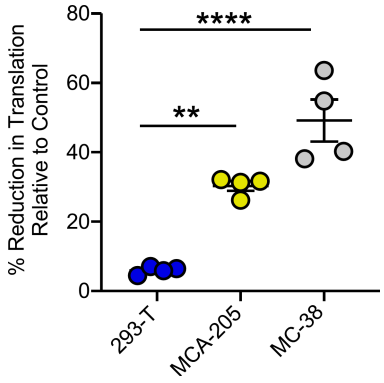


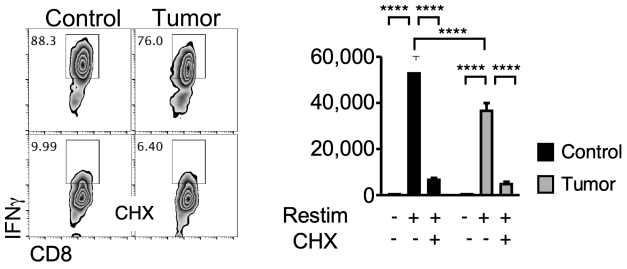
Supplemental Figure 1. Tumor conditioned media reduces T cell protein synthesis. a) Gating strategy for quantification of protein synthesis in live CD8 T cells harvested from the T cell-tumor cell transwell assay. **b**) Representative FACS plots and quantification of protein synthesis in human CD8 PBMC (n=5 donors) activated with CD3/CD28 in the presence or absence of supernatant from freshly isolated B16F1 melanoma tumors. Data presented as paired samples with n = 5 individual donors. ** $p < 0.01$, two tailed paired Student's *t* test.



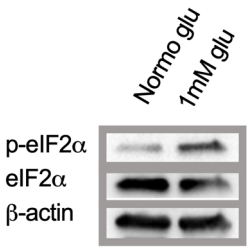
Supplemental Figure 2. Confirmation of tumor mediated translation attenuation in T cells. **a**) Schematic representation of the O-Propargyl-puromycin assay used to measure protein synthesis. **b**) Representative FACS plots and quantification of protein synthesis from OT-1 T cells harvested from the T cell-tumor cell transwell assay. Data presented as mean ± SEM, representative of two independent experiments. **** $p < 0.0001$, two tailed Student's *t* test.



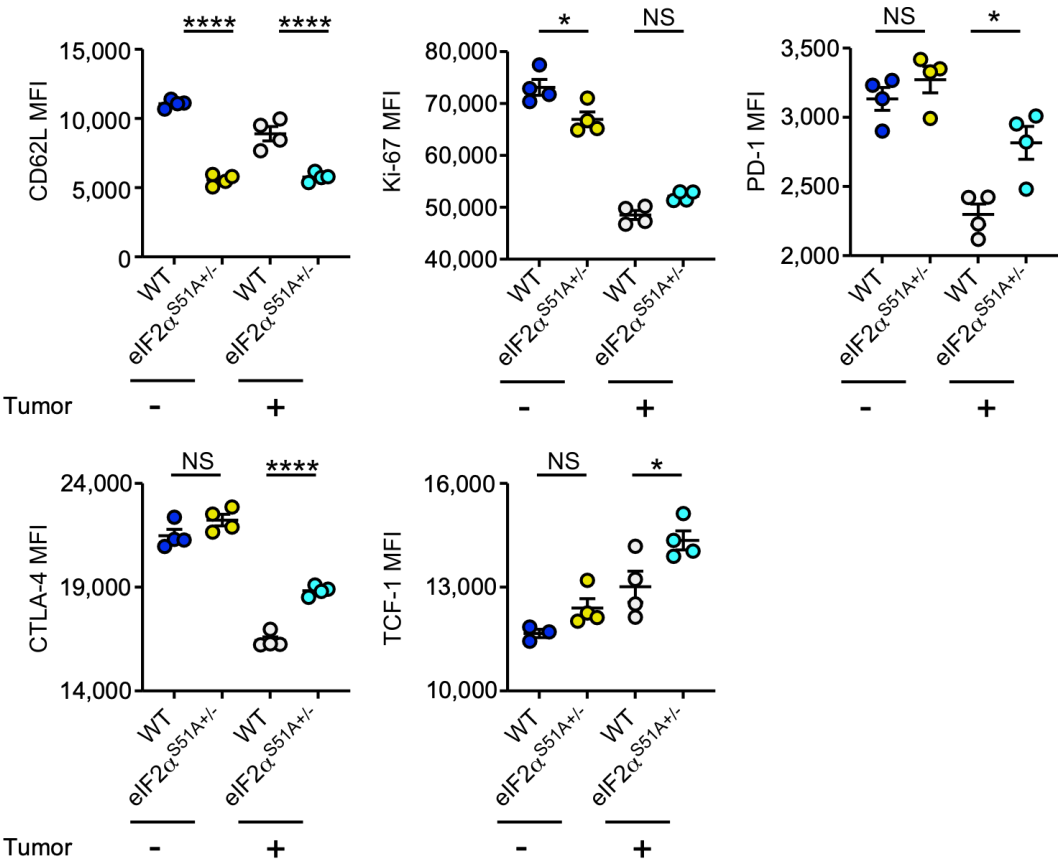
Supplemental Figure 3. Reduced protein synthesis is a cancer cell specific phenomenon
Percentage of reduction in translation rates relative to non-tumor controls of OT-1 T cells incubated for 36 hours in the presence of MCA-205 Fibrosacroma, MC-38 Adenocarcinoma, or 293-T Human Embryonic Kidney cells in the transwell coculture assay. Data are presented as mean ± SEM, representative of two independent experiments. $*$ $p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ by One Way ANOVA with Tukey's multiple comparison test.



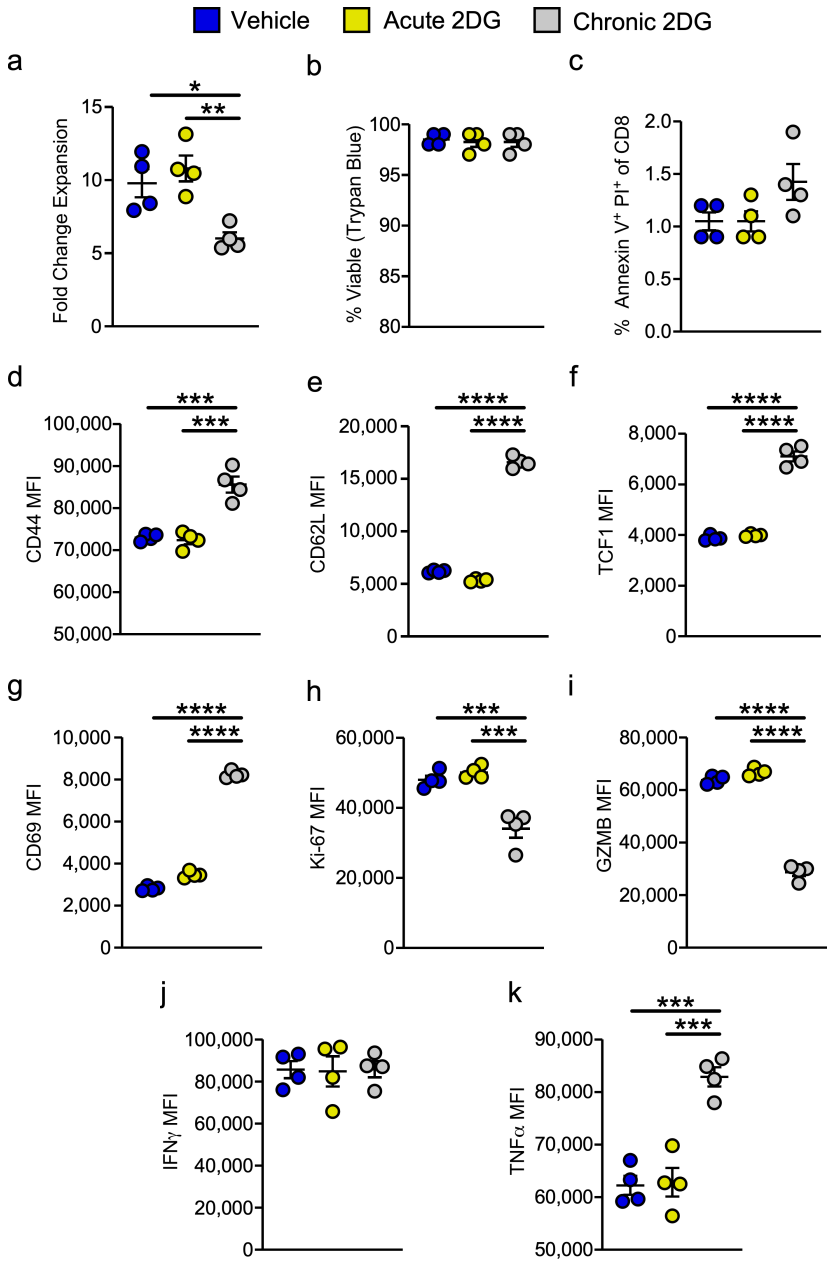
Supplemental Figure 4. **Tumor stress reduces T cell functionality.** Representative FACS plots and quantification of IFNγ production from OT-1 T cells harvested from the T cell-tumor cell transwell assay followed by a 4-hour stimulation with Cell Stimulation Cocktail and GolgiPlug Brefeldin A ± pretreatment with protein synthesis inhibitor cycloheximide (CHX). Data presented as mean ± SEM, representative of two independent experiments. **** $p < 0.0001$ by One-Way ANOVA.



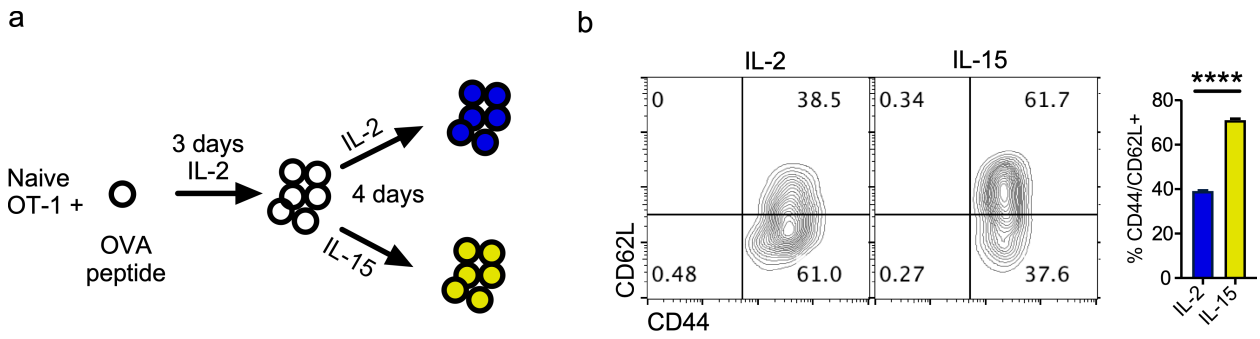
Supplemental Figure 5. **Glucose deprivation induces p-eIF2α in T cells.** Western blot analysis from OT-1 T cells cultured in normal or low glucose (1mM). Data are representative of two independent experiments.



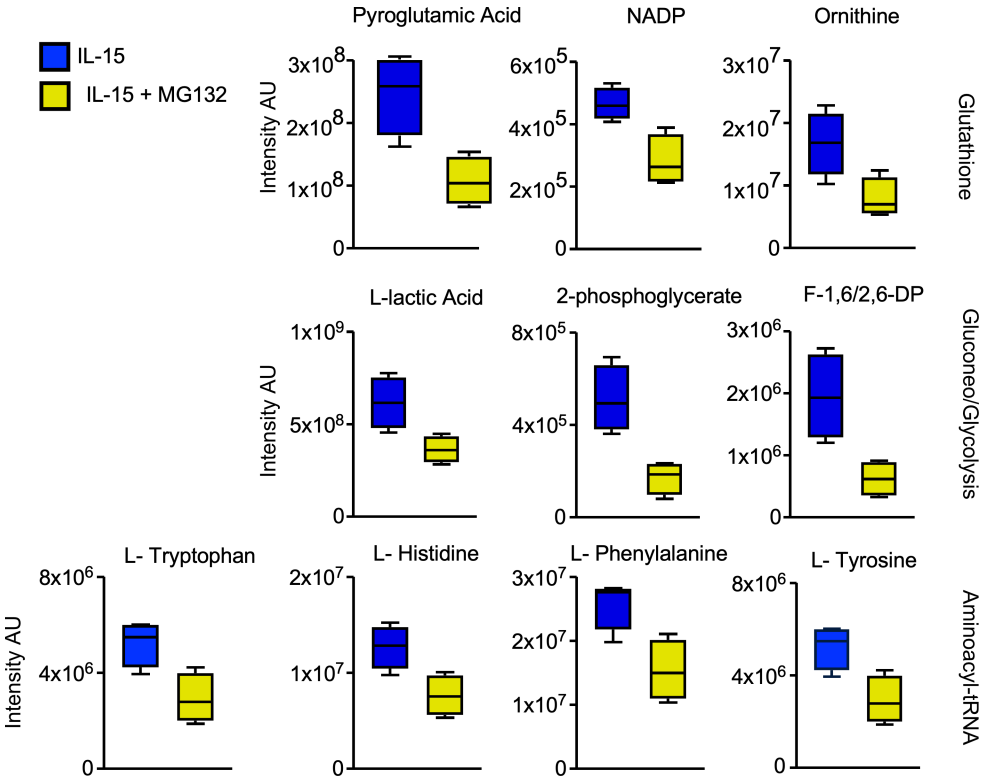
Supplemental Figure 6. Phenotypic analysis of p-eIF2a T cells. High dimensional flow cytometry analysis of WT or eIF2$\alpha^{S51A+/-}$ OT-1 T cells harvested from control or tumor-seeded transwell assay. Mean Fluorescent intensities of CD62L, Ki-67, PD-1**,** CTLA-4, and TCF-1. Data presented as mean ± SEM, representative of two independent experiments. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ by two tailed Student's *t* Test.



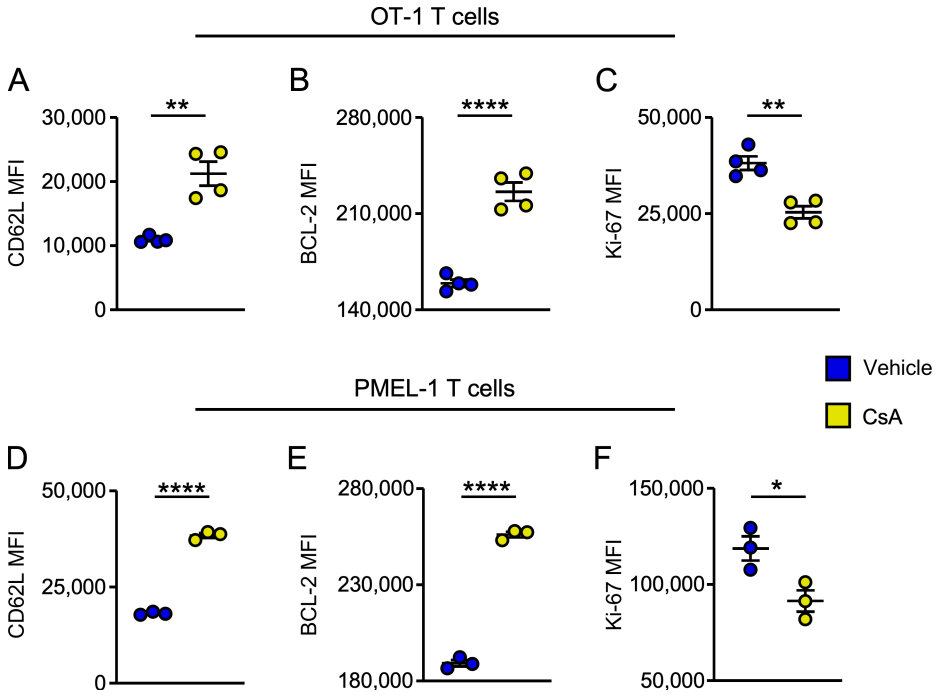
Supplemental Figure 7. Chronic 2DG drives a memory-like T cell phenotype. 3-day *ex vivo* expanded OT-1 T cells were treated with 2DG or vehicle for 2 (Acute) or 36 hours (chronic) and **a)** fold change expansion, **b)** trypan blue exclusion viability, and **c)** Annexin V and propidium iodide staining were assessed. **d-k)** Mean fluorescent intensities (MFI) from spectral flow analysis are depicted. For (**j-k**) cells were stimulated for 4 hours with Cell Stimulation Cocktail and GolgiPlug Brefeldin A. Data presented as mean ± SEM, representative of two independent experiments. $p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ by One Way-ANOVA with Tukey's multiple comparison test.



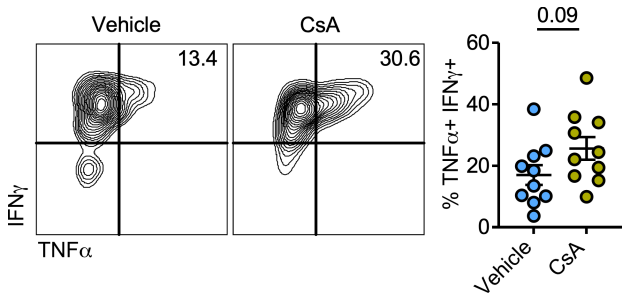
Supplemental Figure 8. Conditioning with IL-15 generates a memory-like T Cell phenotype. **a**) Graphical representation of the memory or effector like cytokine conditioning scheme. **b)** Representative FACS plots and quantification of CD44 and CD62L expression on IL-2 or IL-15 conditioned OT-1 T cells as demonstrated in (**a**). Data presented as mean ± SEM, representative of two independent experiments. ∗ $p < 0.05$, ∗∗$p < 0.01$, ∗∗∗$p < 0.001$, ∗∗∗∗$p < 0.0001$ by two tailed student's *t* test.



Supplemental Figure 9. Metabolites responsible for driving pathway enrichment. Individual intensities of select metabolites identified as differentially expressed between groups organized by associated enriched pathways (across rows). Data generated from global metabolomics analysis of OT-1 conditioned T cells harvested from the tumor-transwell coculture system conditioned with IL-15 ± MG132 for 4 hours prior to harvest. Data presented as mean ± the min max (whisker) with 1st and 3rd quartile, representative of two independent experiments.



Supplemental Figure 10. Phenotypic analysis of CsA conditioned OT1 and pmel T cells. OT-1 or PMEL T cells were activated for 3 days with their respective cognate antigens and differentiated in the presence of IL-2 and Vehicle or CsA (2.5μM) for 4 days followed by High dimensional spectral flow cytometry analysis. Mean fluorescent intensities of CD62L, BCL-2, and Ki-67 in OT-1 (**A-C**) or PMEL (**D-F**) T cells. Data presented as mean ± SEM, representative of two independent experiments. $p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ by two tailed Student's *t* Test.



Supplemental Figure 11. Polyfunctional cytokine synthesis of CsA-infused OT1 T cells. 7-day expanded OT1 T cells ($CD45.2^+$) treated with vehicle or CsA were infused to $CD45.1^+$ C57BL/6 mice bearing 7-day established B16-F1-OVA melanomas and cytokine synthesis from transferred CD8 TILs was assessed 5 days post transfer. Data are representative of two independent experiments. p values are noted in each panel based on statistical analysis by two tailed Student's t-test. Data points represent individual mice, all error bars indicate the SEM.