SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Tumor infiltrating T cells lose mitochondrial mass. Refers to Figure 1. (**A**) MitoTracker Deep Red FM, MitoTracker Green FM, and TMRE staining of $CD8^+$ T cells from spleen and LNs. The indicated histogram represents T cells pre-incubated with 100-111 μM CCCP, which collapses membrane potential. Results from CCCP titration are tabulated below. (**B**) MitoTracker Green FM and intracellular VDAC staining of LN and TIL from d14 B16 tumors as in Figure 1A. (**C**) MitoTracker Deep Red FM staining of naïve ($CD62L^{hi}CD44^{lo}$), effector memory ($CD62L^{lo}CD44^{hi}$), and central memory ($CD62L^{hi}CD44^{hi}$) $CD8^+$ T cells from LN and tumor-infiltrating compartments. (**D**) 2NBDG uptake and MitoTracker FM staining measurements from $CD8^+$ and $CD4^+$ T cells infiltrating day 18 MC38 tumors or (**E**) LLC tumors or from the nondraining or draining LN. Results represent 3 (A, B, D, E), or 6 (C) independent experiments. ** $p < 0.01$, **** $p < 0.001$ by unpaired (B) or paired (C) t test. Error bars indicate s.e.m.

Figure S2. Tumor infiltrating T cells lose mitochondrial mass and do not efficiently elaborate cytokines. Refers to Figure 2. . (**A**) VDAC staining of OT-I T cells cells injected as in Figure 2. (**B**) Cytokine production of OT-I T cells injected as in Figure 2. Some were rechallenged with cognate peptide overnight and others were stimulated with PMA/ionomycin, as tabulated below from TIL or spleen of VV^{OVA} infected miceError bars indicate s.e.m.

Figure S3. **OT-I T cells do not lose mitochondrial activity when activated by tumor cells *in vitro*. Refers to Figure 3.** (**A**) Flow cytogram and tabulated data of CTV-labeled OT-I splenocytes cocultured with either 25 ng/mL SIINFEKL peptide or in a 1:4 ratio with $B16^{OVA}$ cells, seeded 8 h prior to coculture, in the presence of 10 U/mL IL-2. (**B**) As in **A**, but using previously activated, purified effector OT-I T cells. Results represent the mean of 3 (of 8) independent experiments. Error bars indicate s.e.m.

Figure S4. Mitochondrial mass loss is a characteristic of exhausted T cells. Refers to Figure 4. (**A**) VDAC staining of LN or TIL $CD8^+$ T cells from B16-bearing mice expressing co-inhibitory molecules PD-1 and LAG-3. (**B**) Co-inhibitory molecule expression on $CD8^+$ and $CD4^+$ T cells that are LN-resident or infiltrating MC38 or (**C**) LLC tumors on day 18 post inoculation (7-10 mm diameter tumors). (**D**) $CD8^+$T cells were sorted flow cytometrically from B16-bearing animals based on MitoFM staining and washed extensively. Cells were then stimulated with anti-CD3/anti-CD28 for 16 h (final 4 h in the presence of a protein transport inhibitor), and then stained intracellularly for cytokines. Tabulated results for IFNγ staining are shown below. Results represent the mean of four independent experiments. Error bars indicate s.e.m. * < p 0.05, ** p < 0.01 by unpaired t test.

Figure S5. Regulatory T cells do not mediate mitochondrial dysfunction in tumor-infiltrating $CD8^+$ T cells. Refers to Figure 5. (**A)**, Flow cytogram of $CD4^+$ T cells from day 14 B16-bearing *Foxp3*$^{GFP.Cre.ERT2}$ or *Foxp3*$^{DTR.GFP}$ mice treated for 3 days with diphtheria toxin. (**B),** Representative flow cytogram and tabulated data of MitoTracker FM staining in $CD8^+$ T cells from mice in **A**. (**C**), Flow cytogram and tabulated data of CellTrace Violet labeled $CD8^+$ T cells stimulated with anti-CD3 and antigen presenting cells ($CD4^-CD8^-$ splenocytes) in the presence or absence of flow-cytometrically purified Treg cells ($CD4^+GFP^+$ cells from a *Foxp3*^{GRP} mouse). Results are representative of (**A**), or represent the mean of (**B, C**) two of three independent experiments. Error bars indicate s.e.m.

Figure S6. **PGC1α is repressed progressively upon entry into the tumor microenvironment. Refers to Figure 6.** (**A**) qPCR analysis of *Ppargc1a* (encoding PGC1α) from $CD8^+$ T cells sorted from the indicated compartments based on co-inhibitory molecule expression from day 14 B16 tumors. Results are normalized to cyclophilin B expression and scaled to LN-resident $CD4^+$ T cells. (**B**) PGC1α MFI (per division) of congenically mismatched,

CTV-labeled OT-I T cells transferred into $B16^{OVA}$ bearing mice for 72 h under the cover of anti-PD1 treatment or its isotype control as in Figure 4. (**C**) Representative flow cytogram of MitoFM staining of $CD8^+$ T cells isolated from *Ppargc1a*$^{f/f}$*Cd4*^{Cre} mice or *Cd4*^{Cre} controls after *in vitro* expansion. (**D**) Metabolic flux measurements (Seahorse) from the cells in **C**. (**E**) Representative flow cytogram and tabulated results of PGC1α versus T-bet and (**F**) Ki67 staining. (**G**) Representative flow cytogram depicting phospho-Akt (S473) activation as a function of PD-1 status. Results are representative of three (**A, C, E, F**), five (**B, G**), or two (**D**) independent experiments. * $p < 0.05$, **** $p < 0.0001$ by unpaired (**C**) or paired (**E,F**) t test. Error bars indicate s.e.m.

Figure S7. **Enforced PGC1α expression results in general increases in mitochondrial function. Refers to Figure 7.** (**A**) Fuel usage test of PGC1α- or EV-expressing OT-I T cells. Cells were uncoupled with FCCP and then subjected to sequential inhibition of pyruvate oxidation (UK5099), fatty acid oxidation (etomoxir), and glutaminolysis (BPTES). Results are displayed as % of FCCP-uncoupled OCR sensitive to the appropriate inhibitor. (**B**) IFNγ and TNFα production of EV or PGC1α expressing T cells prior to adoptive transfer. (**C**) Expression of PD-1 and LAG-3 on LN or TIL-resident reprogrammed T cells. Results represent the mean of three (**A, B**) or are representative of five (**C**) independent experiments. Error bars indicate s.e.m.

Figure S1 – related to Figure 1

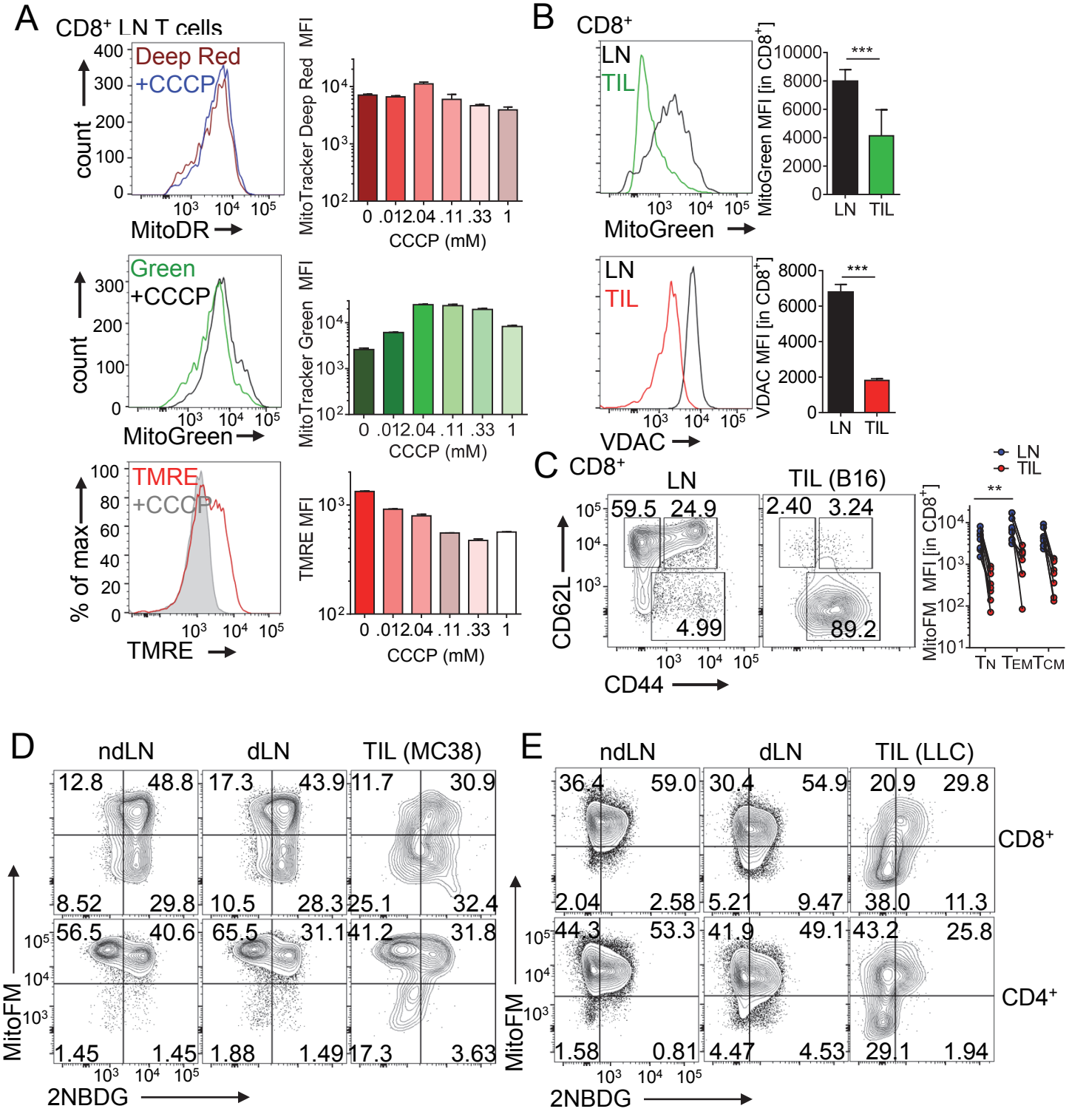


Figure S2 – related to Figure 2

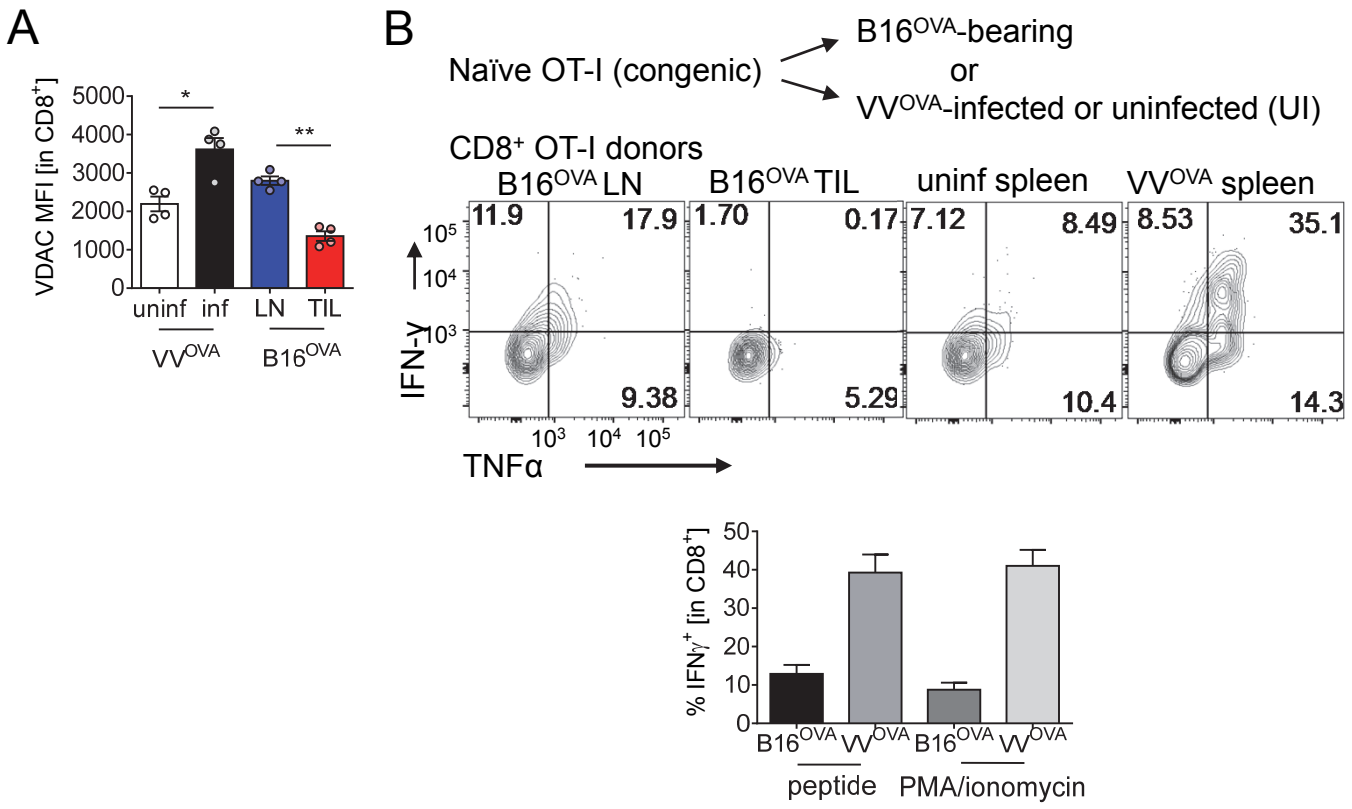


Figure S3 – related to Figure 3

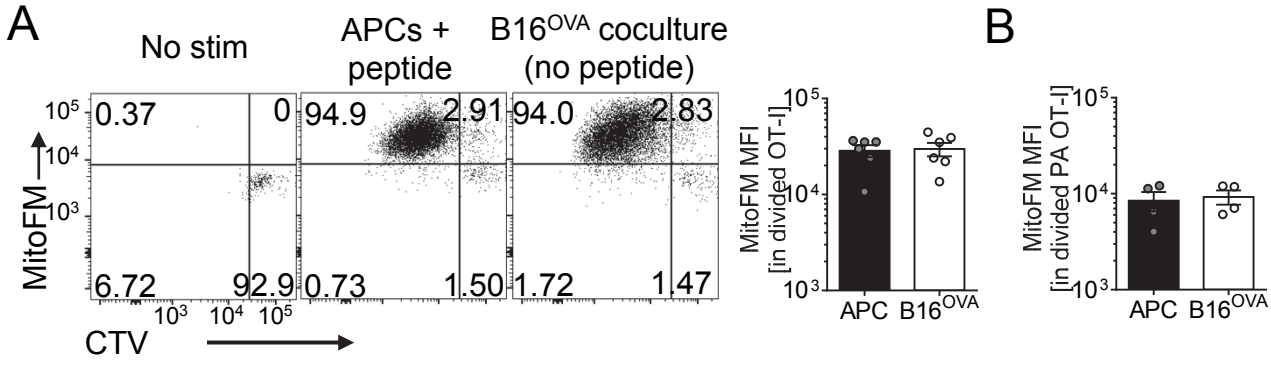


Figure S4 – related to Figure 4

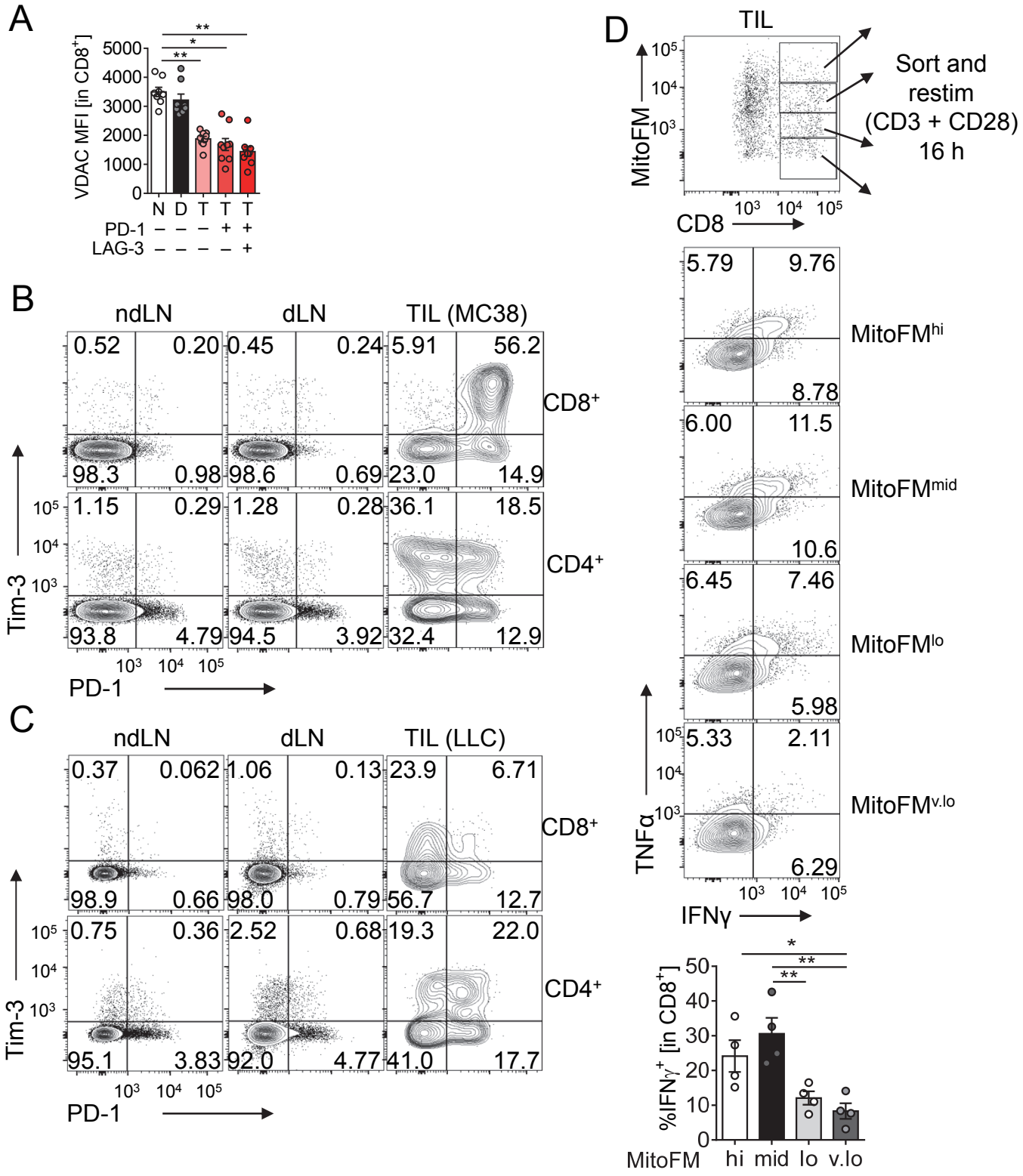


Figure S5 – related to Figure 5

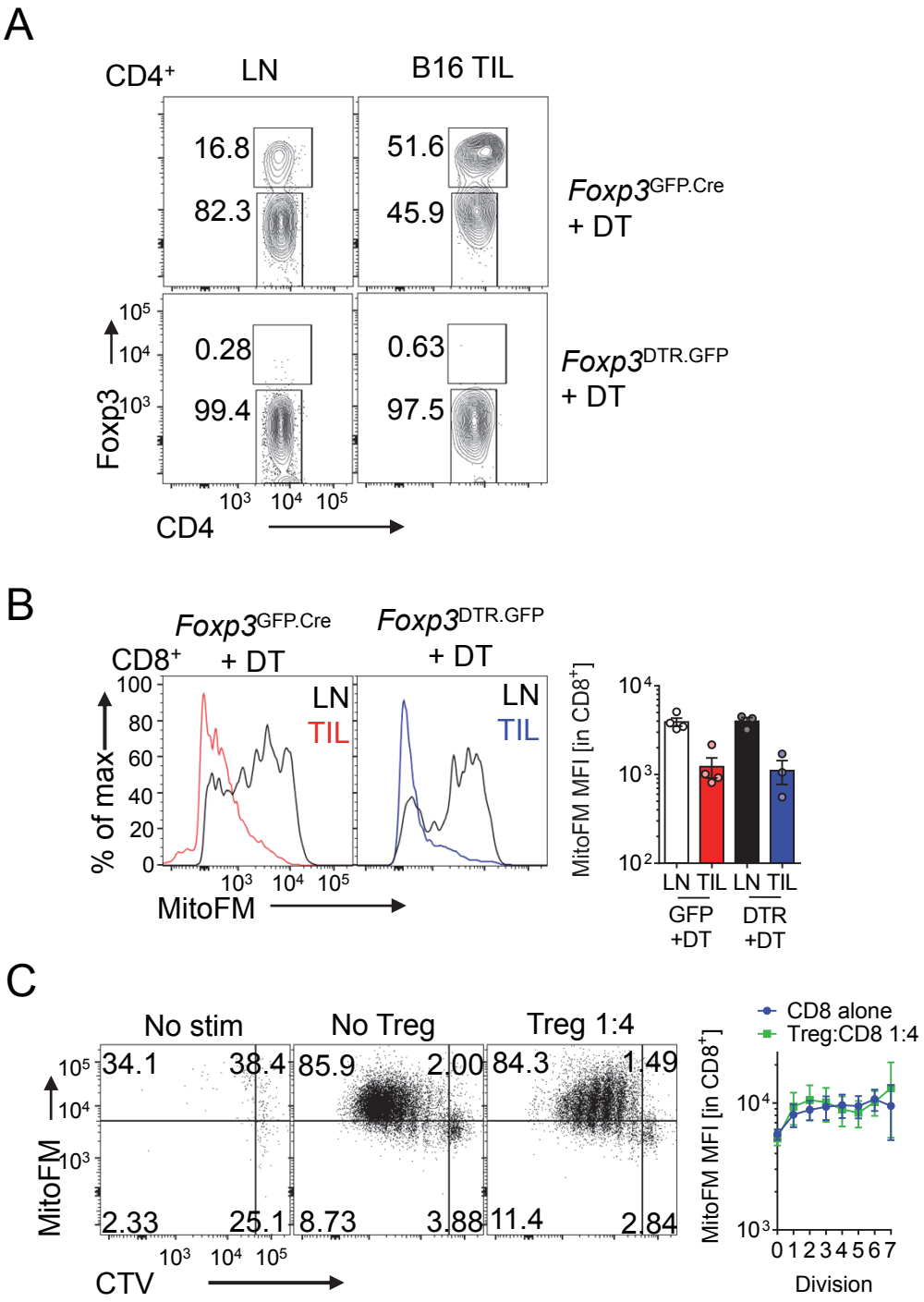


Figure S6 - related to Figure 6

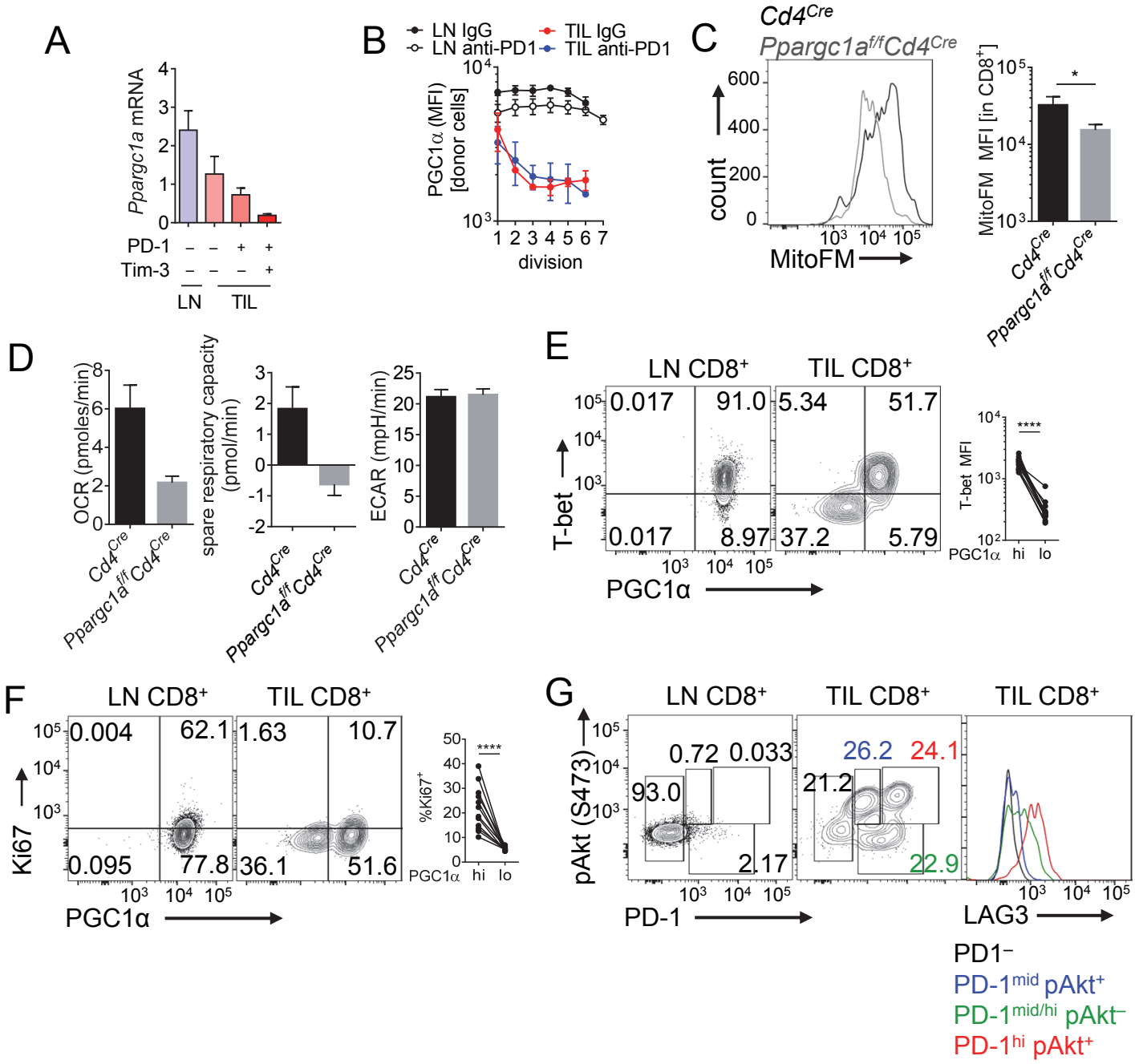
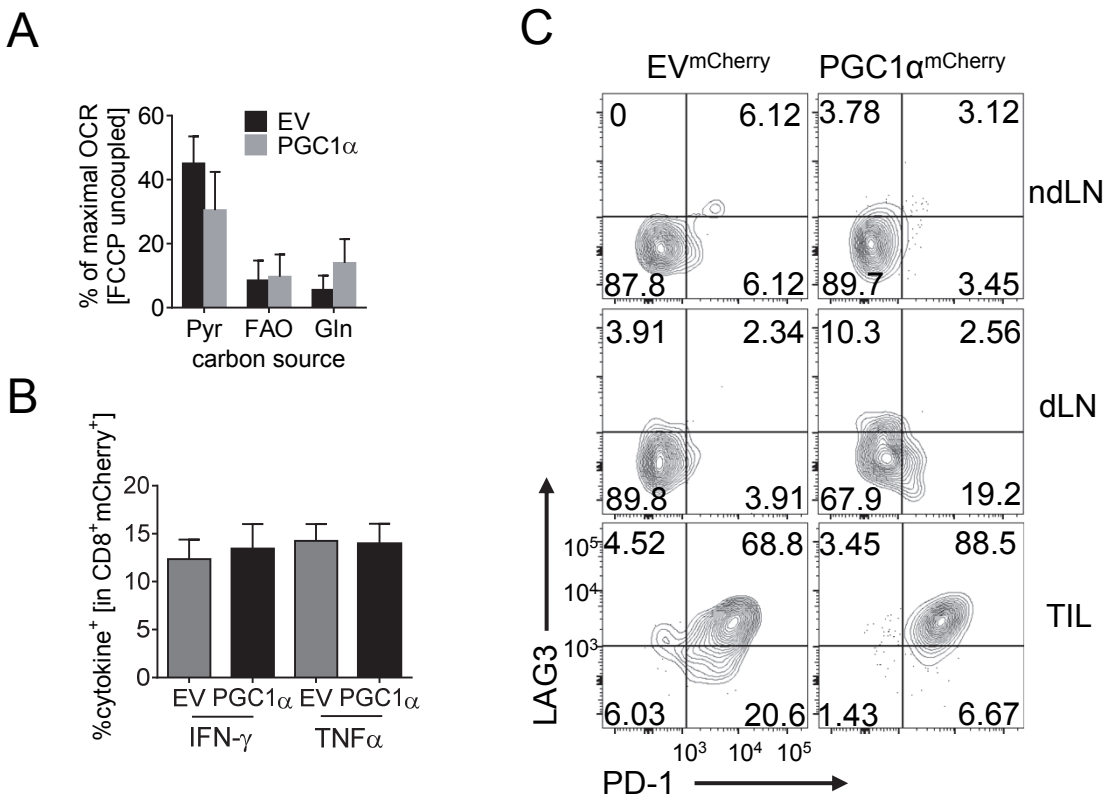


Figure S7 – related to Figure 7



SUPPLEMENTAL EXPERIMENTAL PROCEDURES

$B16^{OVA}$ in vitro *stimulation*

Freshly isolated OT-I splenocytes or purified, previously activated OT-I $CD8^+$ T cells were CellTrace Violet labeled and plated at various ratios on B16 or $B16^{OVA}$ cells in 10% RPMI for 72 h. Proliferation and mitochondrial mass of the T cells was examined flow cytometrically.

Treg cell suppression assay

Lymph nodes and spleens from *Foxp3* reporter mice (*$Foxp3^{DTR.GFP}$*) were sorted based on expression of CD4 and GFP, then cocultured with CellTrace Violet-labeled $CD8^+$ T cells from WT mice at a 1:4 ratio (Treg:Teff cell) in the presence of antigen presenting cells ($CD4^-CD8^-$ splenic cells at a 2:1 APC:T cell ratio) and 1 µg/mL anti-CD3. After 72 h, proliferation and mitochondrial mass of the $CD8^+$ T cells were analyzed by flow cytometry.

Fuel usage test

100,000 previously activated, transduced T cells (Generated in Figure 7) were plated on CellTak-coated Seahorse plates in minimal, unbuffered Seahorse media containing glucose, and glutamine. Basal measurements were taken and then cells were uncoupled with FCCP and subjected to inhibition by UK5099, etomoxir, and BPTES. S7A depicts the percentage of the total FCCP uncoupled OCR inhibited by these agents.