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Supplemental Information

Mitochondrial Pyruvate Carrier 1 Promotes Peripheral T Cell Homeostasis through Metabolic Regulation of Thymic Development

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Figure S1: Related to Figure 1

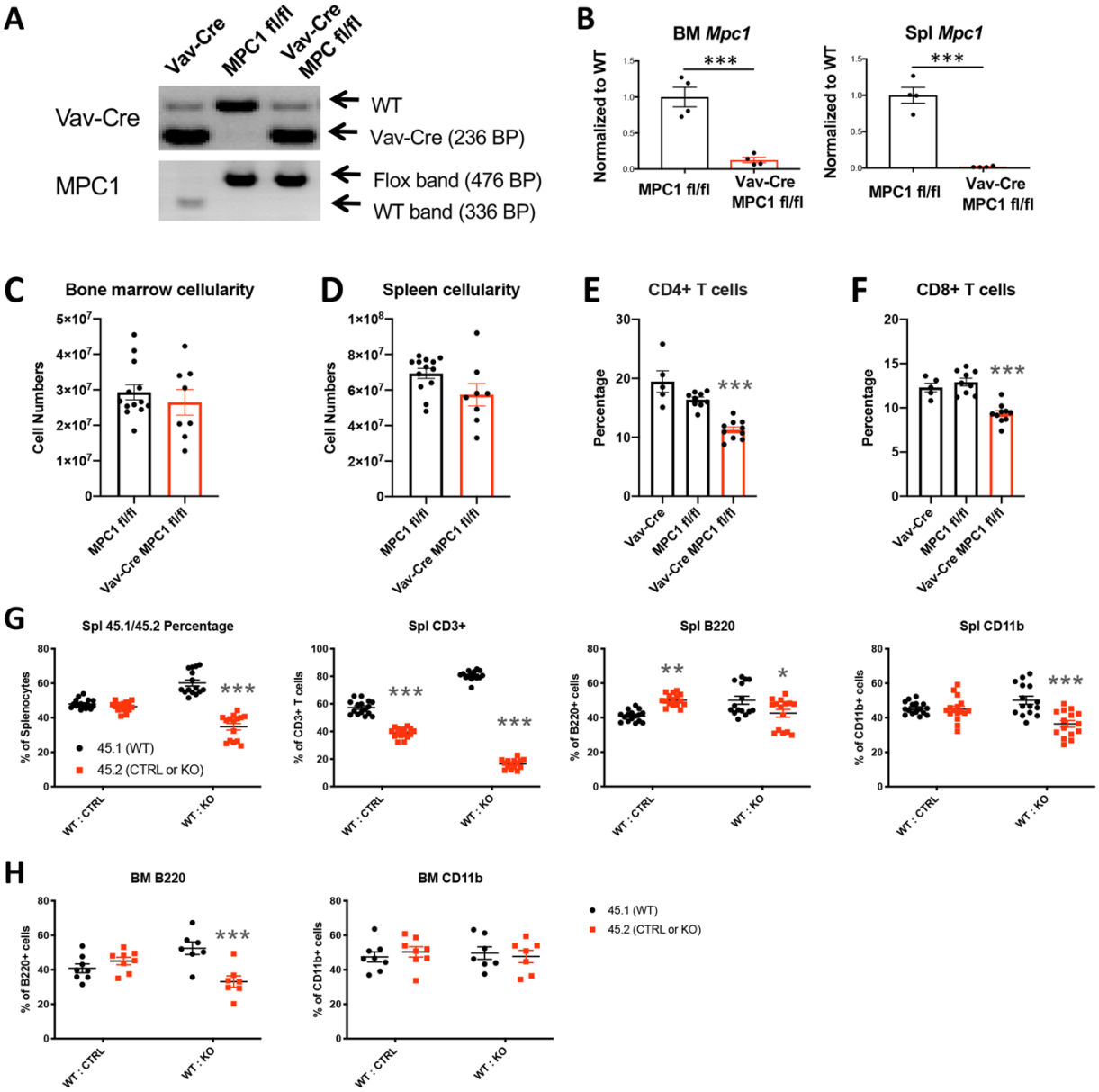

Figure S1 (Related to Figure 1). Deletion of MPC1 does not impact hematopoietic stem cells. A) Representative gel of Vav-Cre and MPC1 flox alleles by PCR. B) Mouse *Mpc1* gene expression in bone marrow and splenocytes measured by qPCR. C) Bone marrow cellularity. D) Spleen cellularity. E) Percent of splenocytes which are CD4+ T cells, with Vav-Cre only data added. F) Percent of splenocytes which are CD8+ T cells, with Vav-Cre only data added. G) Percentages of splenocytes, CD3+ T cells, B cells (B220+), and myeloid cells (CD11b+) in the spleen after mixed bone marrow chimera reconstitution. H) Percentages of B cells (B220+) and myeloid cells (CD11b+) in the bone marrow after reconstitution. All graphs contain combined data from multiple experiments and represent mean +/- SEM. Statistical significance was measured by Student's T test or Two-way ANOVA with Sidak post test. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Figure S2: Related to Figure 2

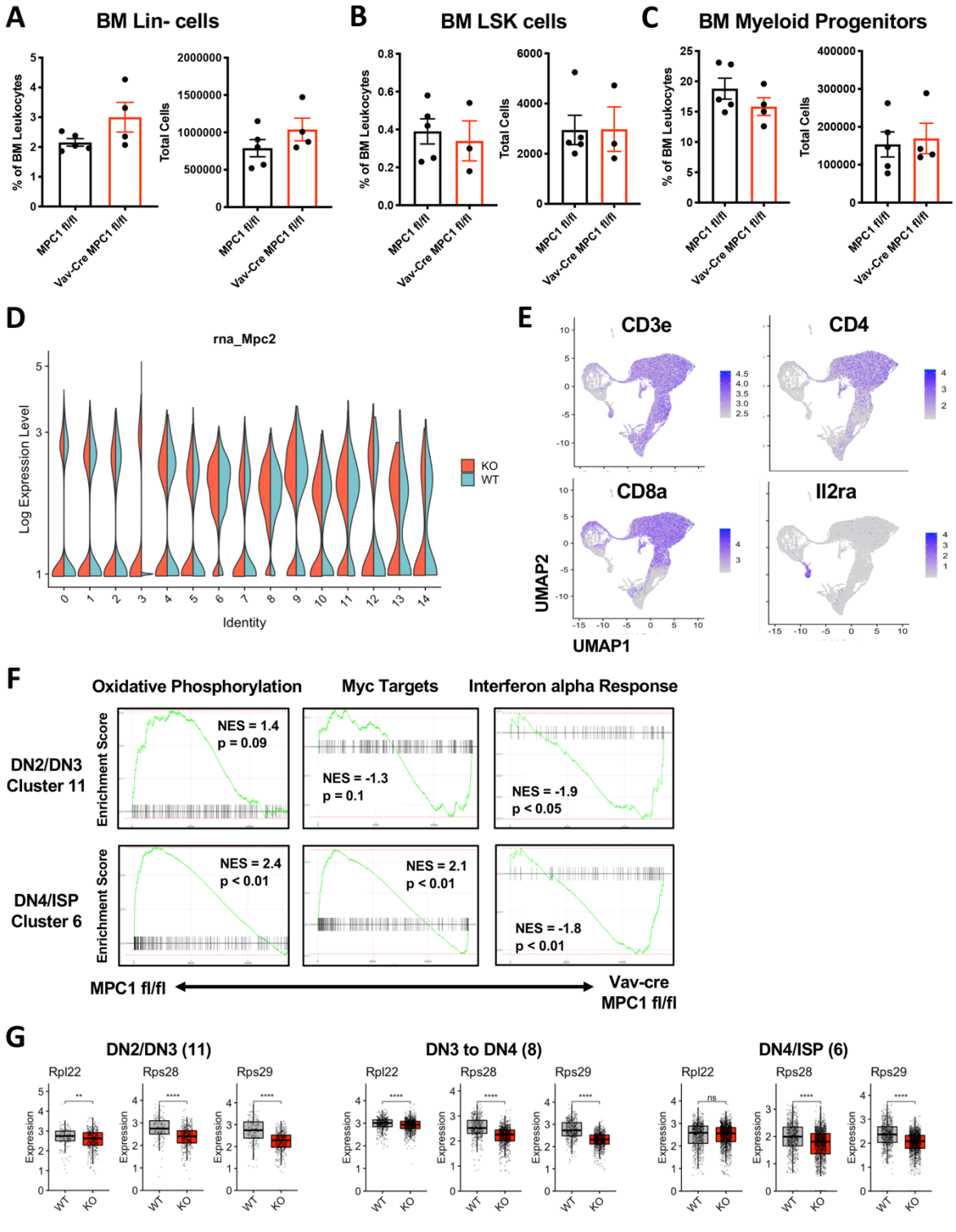

Figure S2 (Related to Figure 2). Additional single cell RNA seq data. A) Percentage and total numbers of lineage negative cells in the bone marrow. B) Percentage and total numbers of LSK cells in the bone marrow. C) Percentage and total numbers of Myeloid progenitor cells in the bone marrow. D) Gene expression of *Mpc2* in all scRNA-seq clusters. E) Representative graph of gene expression of *Cd3e*, *Cd4*, *Cd8*, and *Il2ra* in UMAP clusters. F) GSEA gene expression plots for clusters 11 and 6. G) Gene expression of additional ribosomal genes in clusters 11, 8, and 6. Graphs from A-C represent mean +/- SEM and statistical significance was measured by Student's T test. Graphs from G represents box-and-whisker plots and statistical significance was measured by Wilcoxon test with Holm correction for multiple testing. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Figure S3: Related to Figure 3

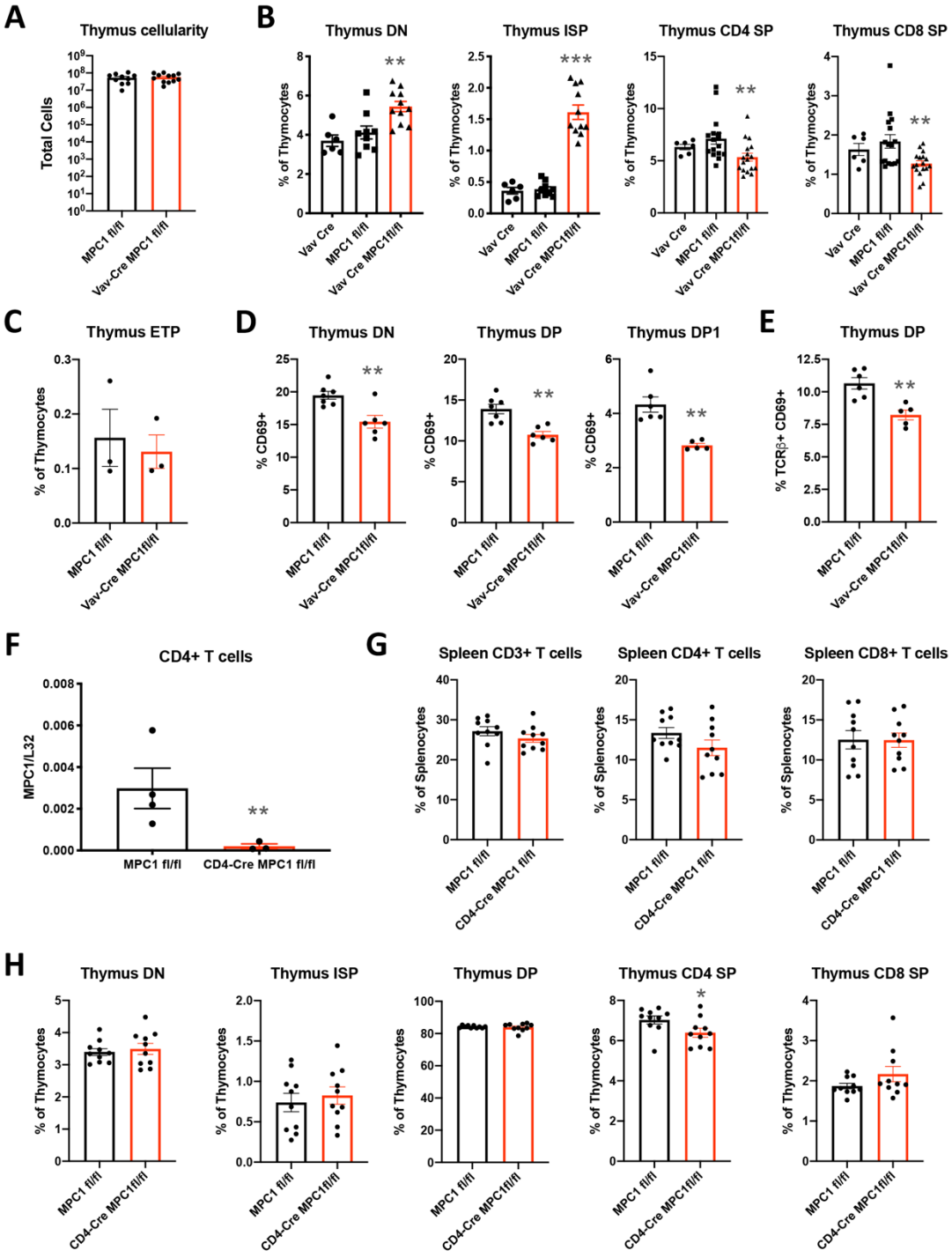

Figure S3 (Related to Figure 3). Additional thymocyte flow cytometry data. A) Thymus cellularity. B) Percent of total thymocytes for CD4- CD8- (DN), CD4- CD8+ TCR- (ISP), CD4+ CD8- TCRβ+ (CD4 SP), and CD4- CD8+ TCRβ+ (CD8 SP) populations, with Vav-Cre only data added. C) Percent of total thymocytes for early thymic progenitors (CD44+ CD25- cKit+ DN). D) Percent of DN, DP, and DP1 cells expressing CD69. E) Percent of DP thymocytes which are TCRβ+ CD69+. F) Gene expression of *Mpc1* in splenic CD4+ T cells from MPC1 fl/fl and CD4-Cre MPC1 fl/fl mice. G) Percent of CD3+, CD4+, and CD8+ T cells in the spleens of MPC1 fl/fl and CD4-Cre MPC1 fl/fl mice. H) Percent of total thymocytes for CD4- CD8- (DN), CD4- CD8+ TCRβ- (ISP), CD4+ CD8+ (DP), CD4+ CD8- TCRβ+ (CD4 SP), and CD4- CD8+ TCRβ+ (CD8 SP) populations in MPC1 fl/fl and CD4-Cre MPC1 fl/fl mice. Graphs represent mean +/- SEM with data from multiple experiments. Statistical significance was measured by Student's T test. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Figure S4: Related to Figure 4

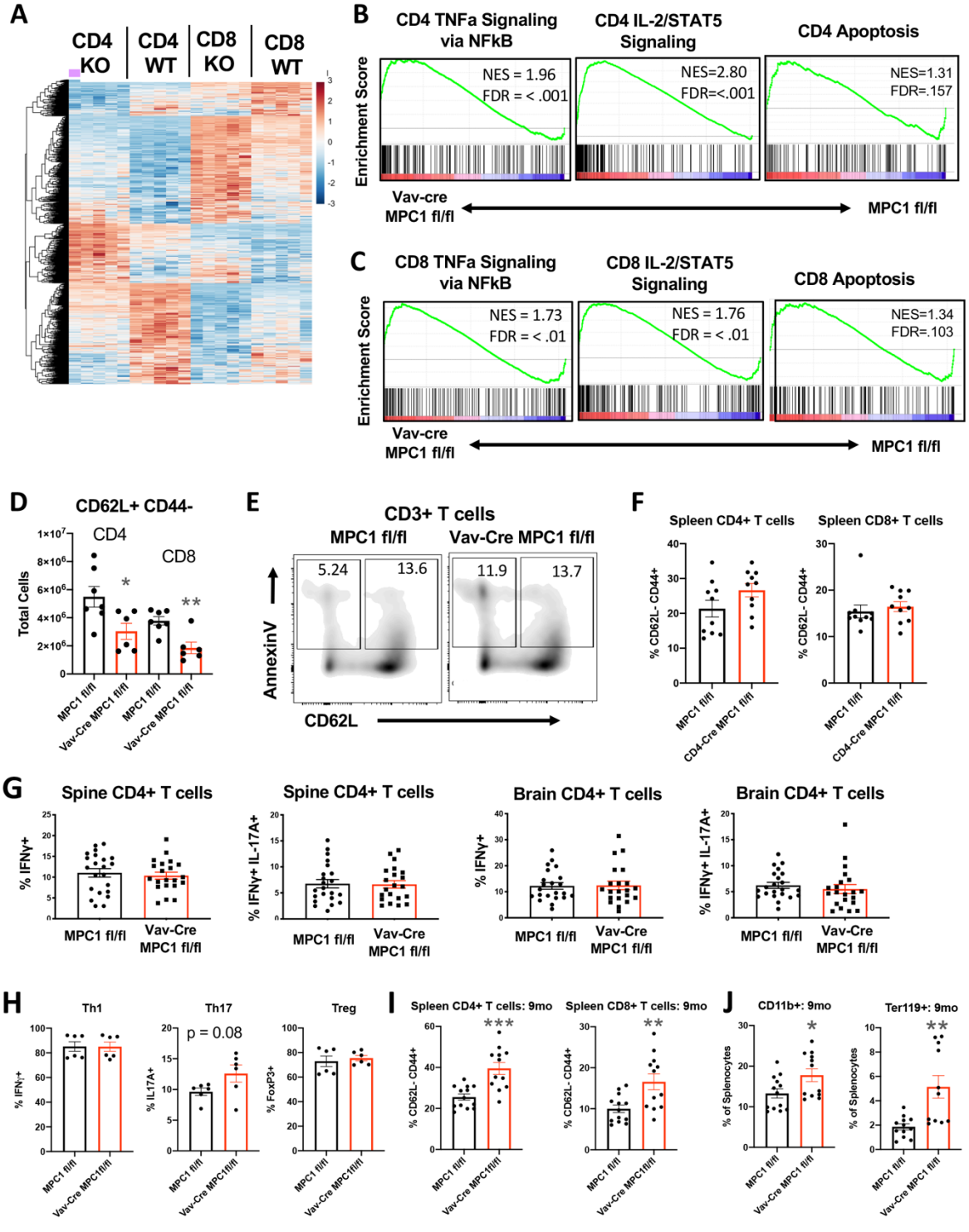

Figure S4 (Related to Figure 4). Additional data for homeostatic activation of peripheral T cells. A) Heat map of RNA seq results from MPC1 fl/fl and Vav-Cre MPC1 fl/fl CD4+ and CD8+ T cells with n = 5/group. B) GSEA of TNFα Signaling via NFκB, IL-2/STAT5 Signaling, and Apoptosis pathways in splenic CD4+ T cells. C) GSEA of TNFα Signaling via NFκB, IL-2/STAT5 Signaling, and Apoptosis pathways in splenic CD8+ T cells. D) Total cell numbers of naïve (CD62L+ CD44-) CD4+ and CD8+ T cells in the spleen. E) Representative plot of Annexin V staining on CD62L+ and CD62L- CD3+ T cells. F) Percent of splenic CD4+ and CD8+ T cells which are CD62L- CD44+ in MPC1 fl/fl and CD4-Cre MPC1 fl/fl mice. G) Percent of CD4+ T cells in spines and brains of EAE mice which are IFNγ+ or IL-17A+ IFNγ+ as measured by flow cytometry. H) Percent of splenic CD4+ T cells expressing IFNγ, IL-17A, or FoxP3 after Th skewing *in vitro*. Graphs represent combined data from two separate experiments. I) Percent of splenic CD4+ and CD8+ T cells from 9-month-old mice expressing CD62L- CD44+. J) Percent of CD11b+ and Ter119+ cells in 9-month-old mouse spleens. All graphs represent mean +/- SEM from multiple experiments. Statistical significance was measured by Student's T test. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Figure S5: Related to Figure 5

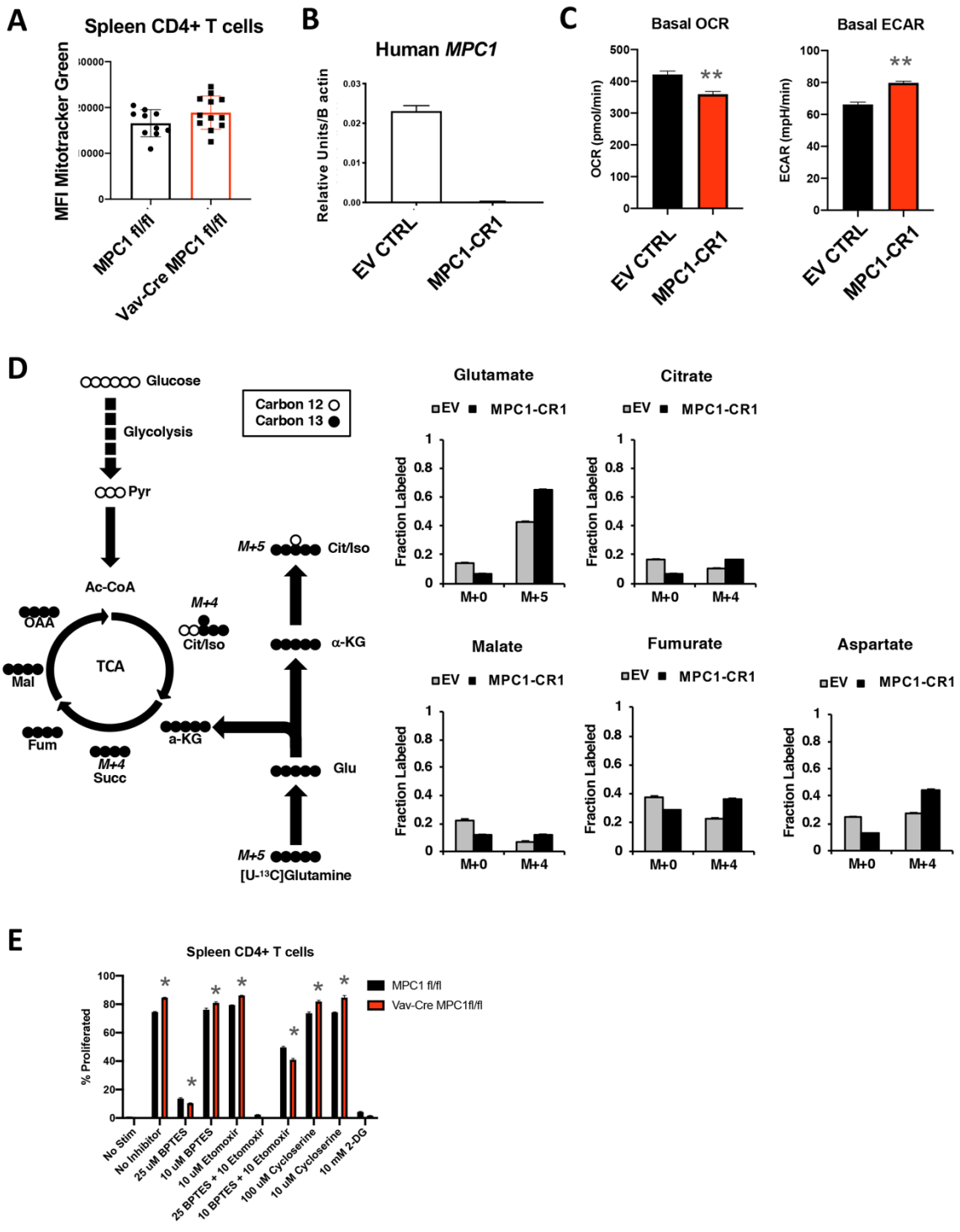

Figure S5 (Related to Figure 5). Additional data for T cell metabolism. A) Mean fluorescence of Mitotracker green on splenic CD4+ T cells. B) Human *MPC1* gene expression in developed Jurkat T cell lines measured by qPCR in duplicate. C) Basal OCR and ECAR measurements of Jurkat T cells by Seahorse analysis with n = 3/group. D) ^{13}C-glutamine tracing after 4 hr culture of empty vector (EV) or MPC1-deficient (MPC1-CR1) Jurkat T cells measured by mass spectrometry in triplicate. E) Percent of proliferated CD4+ T cells after activation with αCD3/αCD28 and IL-2 in the presence or absence of several metabolic inhibitors measured by reduced Cell Trace Violet staining by flow cytometry in duplicate. Statistical significance was measured by Two-way ANOVA with Sidak post test and compares MPC1 fl/fl to Vav-Cre MPC1 fl/fl samples for each treatment group. All graphs represent mean +/- SEM. Statistical significance was measured by Student's T test unless otherwise noted. *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.