Table S1. Related to materials and methods. Human and mouse primers for qRT-PCR.

Gene name	Forward primer	Reverse primer
human *IRF4*	GAGTCACCTGGAATCTTGGC	CCTGCAAGCTCTTTGACACA
human *SLC1A1* (GLUT1)	GGCATTGATGACTCCAGTGTT	ATGGAGCCCAGCAGCAA
human *HK2*	AGCCCTTTCTCCATCTCCTT	AACCATGACCAAGTGCAGAA
human *MYC*	CACCGAGTCGTAGTCGAGGT	TTTCGGGTAGTGGAAAACCA
human *PPRC1*	TGACAAAGCCAGAATCACCC	GTGGTTGGGGAAGTCGAAG
human *PGK1*	CTTGGGACAGCAGCCTTAAT	CAAGCTGGACGTTAAAGGGA
mouse *Slc2a1* (GLUT1)	GAGACCAAAGCGTGGTGAGT	GCAGTTCGGCTATAACACTGG
mouse *Slc2a3* (GLUT3)	ATCGTGGCATAGATCGGTTC	TCTCAGCAGCTCTCTGGGAT
mouse *Slc2a6* (GLUT6)	GCCCAGGAGGTCATTGAGTA	CCAGCACTACACCTGGACAA
mouse *Slc2a8* (GLUT8)	GGCATGTAGCACATGAGCAG	GGCATCCTCCTGGCCTAT
mouse *Irf4*	CAAAGCACAGAGTCACCTGG	TGCAAGCTCTTTGACACACA
mouse *Hif1a*	AAACTTCAGACTCTTTGCTTCG	CGGCGAGAACGAGAAGAA
mouse *Hk2*	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
mouse *Gpi*	TCAAGCTGCGCGAACTTTTTG	GGTTCTTGGAGTAGTCCACCAG
mouse *Pkfl*	GGAGGCGAGAACATCAAGCC	CGGCCTTCCCTCGTAGTGA
mouse *Aldoa*	CGTGTGAATCCCTGCATTGG	CAGCCCCTGGGTAGTTGTC
mouse *Pgk1*	ATGTCGCTTTCCAACAAGCTG	GCTCCATTGTCCAAGCAGAAT
mouse *Pgam1*	TCTGTGCAGAAGAGAGCAATCC	CTGTCAGACCGCCATAGTGT
mouse *Eno1*	TGCGTCCACTGGCATCTAC	CAGAGCAGGCGCAATAGTTTTA
mouse *Pkm2*	GCCGCCTGGACATTGACTC	CCATGAGAGAAATTCAGCCGAG
mouse *Ppargc1a* (PGC-1a)	TGAGGACCGCTAGCAAGTTT	TGTAGCGACCAATCGGAAAT
mouse *Ppargc1b* (PGC-1b)	GAGGTCAAGCTCTGGCAAGT	GCTCTCGTCCTTCTTCCTCA
mouse Pprc1	TCTGTCGAGTCTGTTGGAGCA	GGGAATGTCAATGCCTGAGTTT
mouse *Gapdh*	TTGATGGCAACAATCTCCAC	CGTCCCGTAGACAAAATGGT
mouse *18S*	CGGCGACGACCCATTCGAAC	GAATCGAACCCTGATTCCCCGT
mouse mtDNA region 1	TGAACGGCTAAACGAGGGTC	AGCTCCATAGGGTCTTCTCGT
mouse mtDNA region 2	CAGTCCCCTCCCTAGGACTT	ACCCTGGTCGGTTTGATGTT
mouse mtDNA region 3	TAATCGCACATGGCCTCACA	GAAGTCCTCGGGCCATGATT
mouse gDNA *B2m*	AGCAAAGAGGCCTAATTGAAGTC	GAAGTAGCCACAGGGTTGGG
mouse gDNA *Tuba1a*	TGAGGAGGTTGGTGTGGATTC	TGAGGAGGTTGGTGTGGATTC

Figure S1. Related to Fig. 2

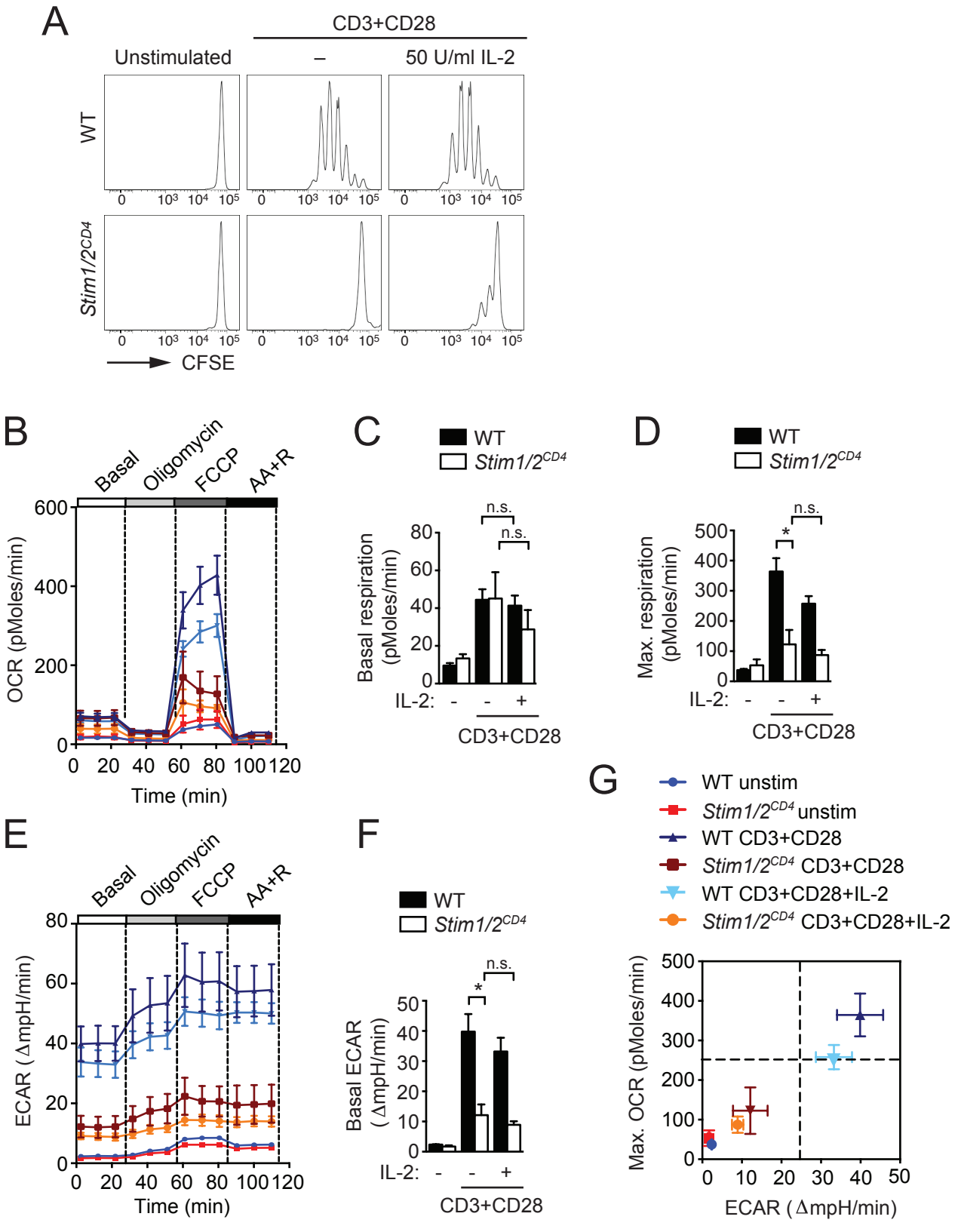

Figure S1. Related to Figure 2. Exogenous IL-2 does not rescue proliferation and metabolic reprogramming of *Stim1/2*-deficient CD4$^+$ T cells.

(A) Proliferation of CD4$^+$ T cells from WT and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4cre* (*Stim1/2*^{CD4}) mice 72 h after anti-CD3/CD28 stimulation with or without 50 U/ml IL-2 and measured by CFSE dilution; representative histograms of 2 repeat experiments.

(B-G) Exogenous IL-2 does not rescue impaired glycolysis and OXPHOS of *Stim1/2*^{CD4} CD4$^+$ T cells. Analysis of **(B)** oxygen consumption rate (OCR), **(C)** basal respiration, **(D)** maximal respiration after uncoupling of mitochondria with FCCP, **(E)** extracellular acidification rate (ECAR) and **(F)** basal ECAR of WT and *Stim1/2*^{CD4} CD4$^+$ T cells before and 24 h after anti-CD3/CD28 stimulation with or without exogenous IL-2 (50 U/ml) using an extracellular flux analyzer. Means ± SEM of n=3 mice. **(G)** Maximal OCR plotted against ECAR from data shown in (D and F).

Figure S2. Related to Fig. 2

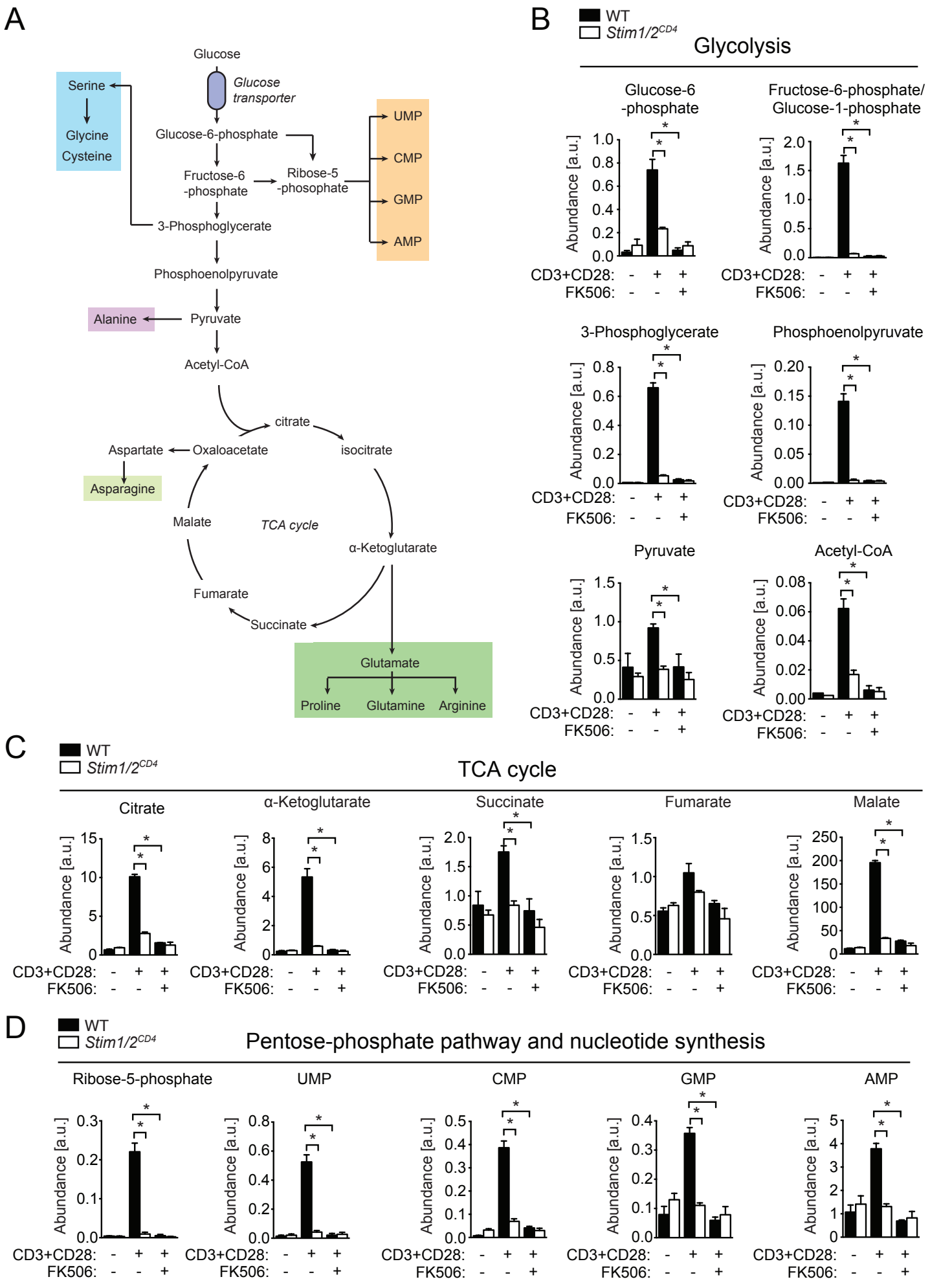

Figure S2. Related to Figure 2. SOCE and calcineurin control abundance of glycolytic and TCA cycle intermediates and nucleotides in activated T cells.

(A) Pathway diagram of glycolysis, the tricarboxylic acid (TCA) cycle and the synthesis of nucleotides and amino acids in T cells.

(B-D) Liquid chromatography/mass spectrometry (LC/MS) analysis of polar metabolites derived from glucose. Non-normalized abundance of metabolites of the **(B)** glycolytic pathway, **(C)** intermediates of the TCA cycle and **(D)** nucleotides in T cells from WT and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4cre* (*Stim1/2*^{CD4}) mice before and 36 h after anti-CD3/CD28 stimulation in the presence or absence of 1 μM FK506. Metabolite measurements of 3 WT and 4 *Stim1/2*^{CD4} mice per cohort; bar graphs are non-normalized mean ± SEM.

Figure S3. Related to Fig. 2

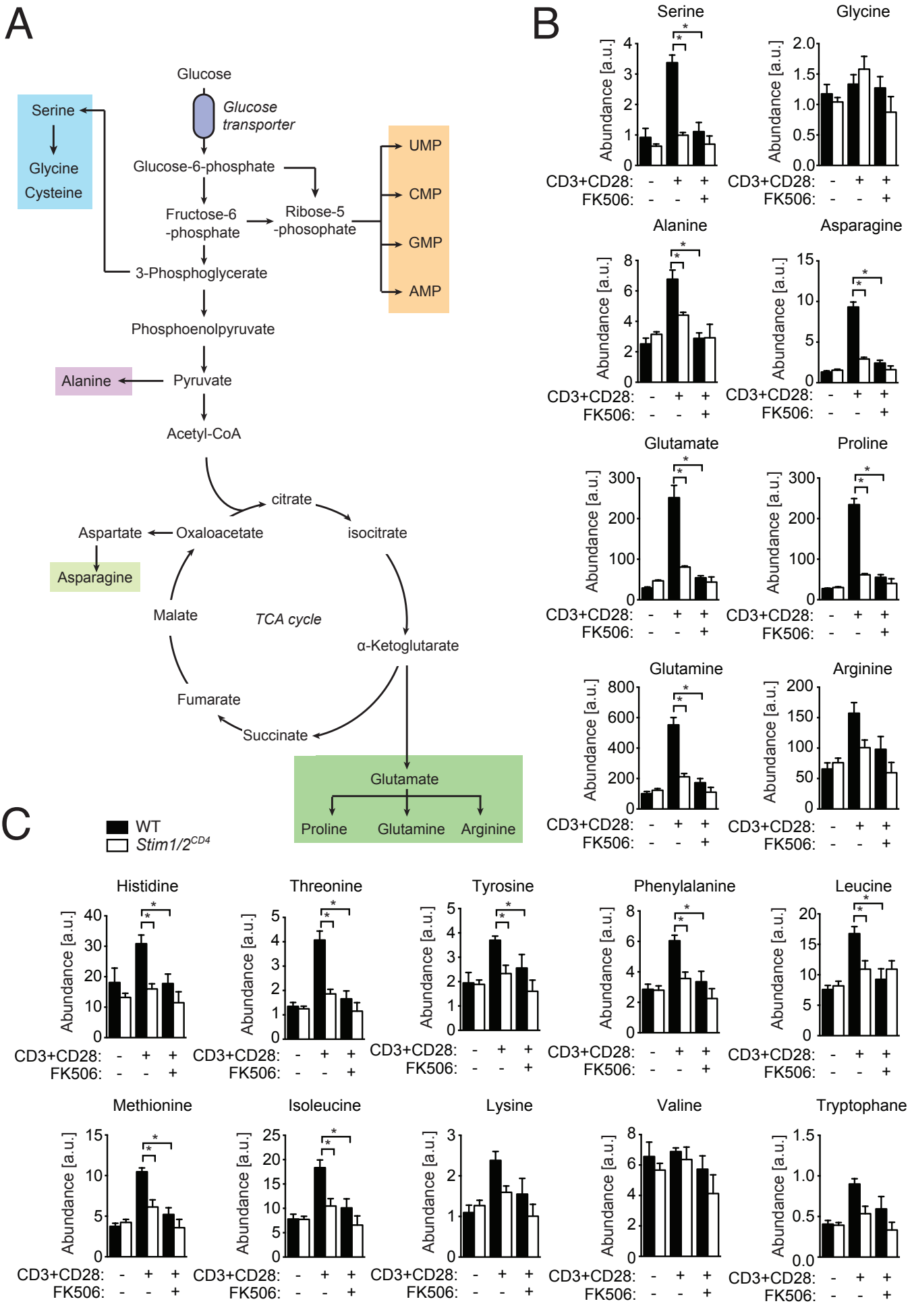

Figure S3. Related to Figure 2. SOCE and calcineurin control amino acid concentrations in activated T cells.

(A) Pathway diagram of glycolysis, the tricarboxylic acid (TCA) cycle and the synthesis of nucleotides and amino acids in T cells.

(B and C) Liquid chromatography/mass spectrometry (LC/MS) analysis of **(B)** non-essential and **(C)** essential amino acids in $CD4^+$ T cells from WT and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4cre* (*Stim1/2*^{CD4}) mice before and 36 h after anti-CD3/CD28 stimulation in the presence or absence of 1 μM FK506. Metabolite measurements of 3 WT and 4 *Stim1/2*^{CD4} individual mice per cohort; bar graphs are non-normalized mean ± SEM.

Figure S4. Related to Fig. 3 and Fig. 4

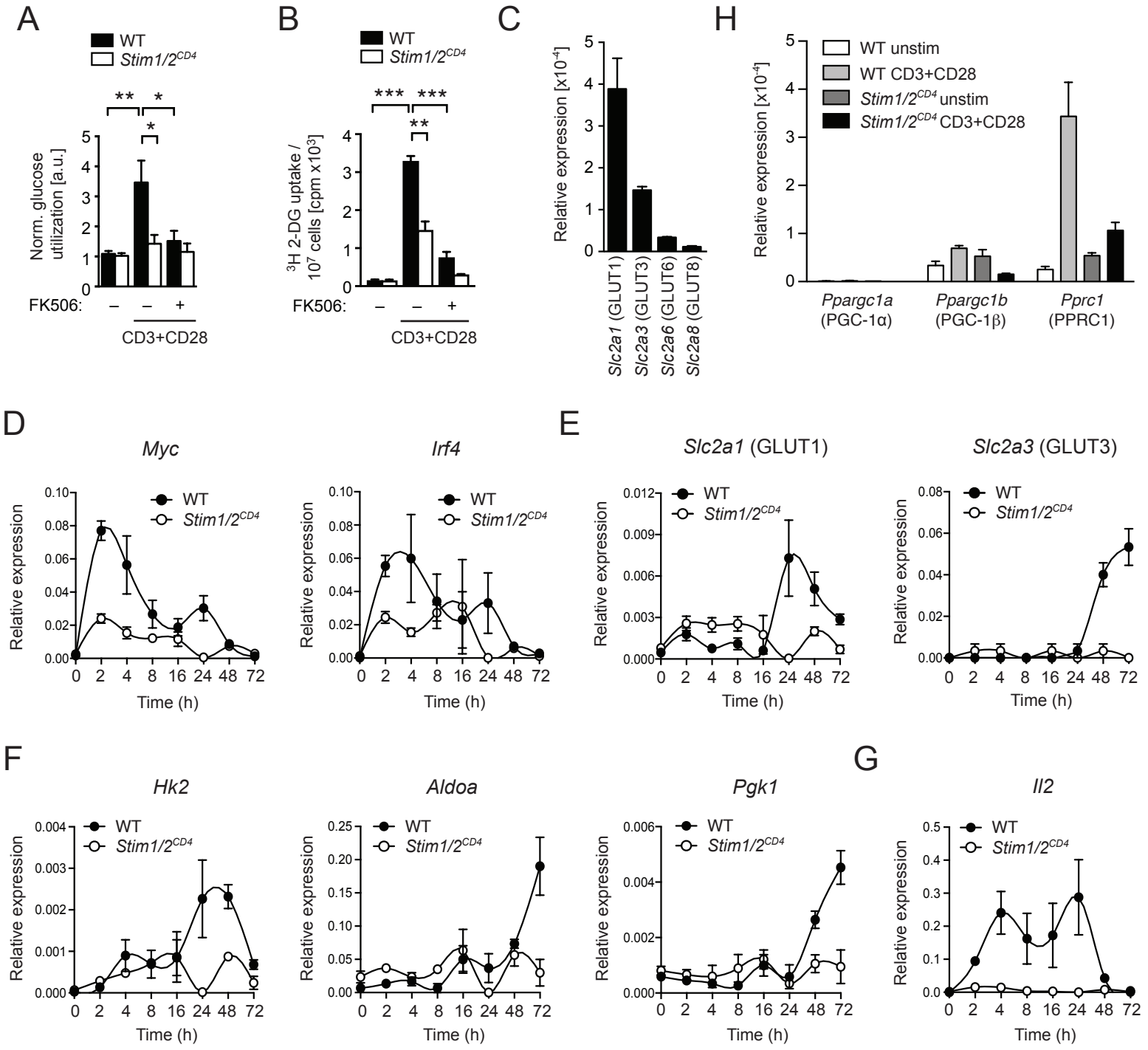

Figure S4. Related to Figure 3 and Figure 4. SOCE controls glucose utilization and expression of glucose transporters, glycolytic enzymes and regulatory factors in T cells.

(A) Glucose utilization from the extracellular medium by $CD4^+$ T cells from WT and *Stim1*$^{fl/fl}$*Stim2*$^{fl/fl}$*Cd4cre* (*Stim1/2*^{CD4}) mice before and 48 h after anti-CD3/CD28 stimulation in the presence or absence of 1 μM FK506. Means ± SEM of 9 mice per group.

(B) Uptake of the tritiated (^{3}H) glucose analogue 2-DG in WT and *Stim1/2*^{CD4} T cells before and 48 h after anti-CD3/CD28 stimulation in the presence or absence of 1 μM FK506. ^{3}H c.p.m. values were normalized to cell numbers; means ± SEM of 3 mice per group.

(C) Analysis of *Slc2a1* (GLUT1), *Slc2a3* (GLUT3), *Slc2a6* (GLUT6) and *Slc2a8* (GLUT8) gene expression in WT $CD4^+$ T cells by qRT-PCR 24 h after anti-CD3/CD28 stimulation. Bar graphs are mean ± SEM of 3 individual mice.

(D-G) SOCE controls the expression of transcriptional regulators of glycolysis, glucose transporters and glycolytic enzymes in T cells. Analysis of **(D)** *Myc* and *Irf4*, **(E)** *Slc2a1* (GLUT1) and *Slc2a3* (GLUT3), **(F)** *Hk2, Aldoa* and *Pgk1,* and **(G)** *Il2* gene expression in WT and *Stim1/2*^{CD4} $CD4^+$ T cells over the course of 72 h after anti-CD3/CD28 stimulation. Means ± SEM of 3 mice.

(H) Analysis of *Ppargc1a* (PGC-1α), *Ppargc1b* (PGC-1β) and *Pprc1* (PPRC1) gene expression in WT and *Stim1/2*^{CD4} $CD4^+$ T cells by qRT-PCR 36 h after anti-CD3/CD28 stimulation. Bar graphs are mean ± SEM of 3 individual mice.

Figure S5. Related to Fig. 5

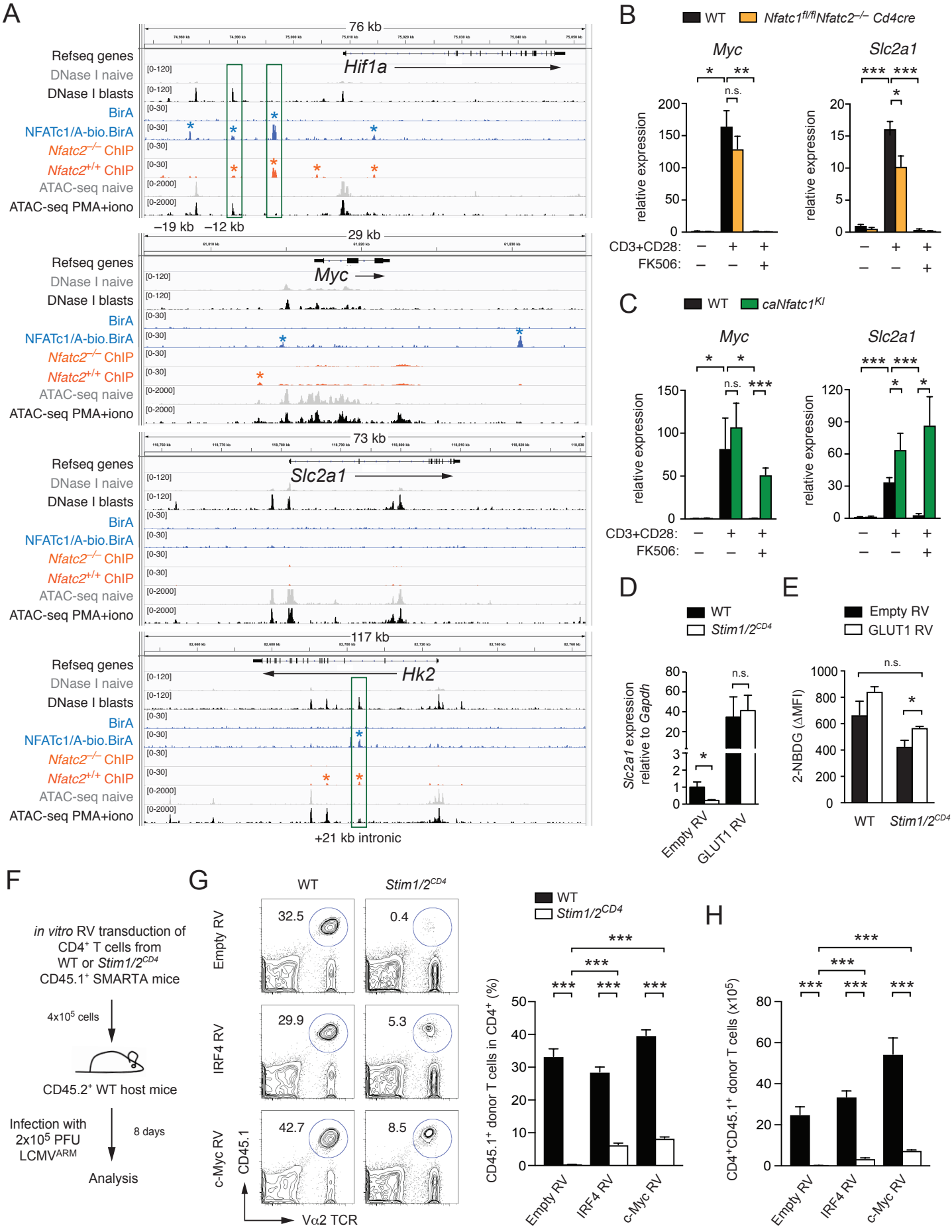

Figure S5. Related to Figure 5. NFAT, IRF4 and c-Myc contribute to the metabolic reprogramming of T cells.

(A) *In silico* analyses of NFATc1 and NFATc2 binding to *Hif1a, Myc, Slc2a1* (GLUT1) and *Hk2* genes in naive and activated $CD8^+$ T cells. Regulatory elements were identified by DNase I hypersensitivity site analysis (Bevington et al. 2016) and accessible chromatin regions were defined by ATAC sequencing (Mognol et al. 2017). NFATc1 (Klein-Hessling et al. 2017) and NFATc2 (Martinez et al. 2015) binding to promoters and regulatory elements was identified by the model-based analysis of ChIP-seq (MACS) method. NFATc1 and NFATc2 binding peaks considered significant by MACS are indicated by blue and orange asterisks, respectively.

(B) Analysis of *Myc* and *Slc2a1* (GLUT1) gene expression in $CD4^+$ T cells from WT and $Nfatc1^{fl/fl}Nfatc2^{-/-}$ *Cd4cre* mice before and 36 h after anti-CD3/CD28 stimulation in the presence or absence of 1 µM FK506 by qRT-PCR; means ± SEM of 3 mice.

(C) *Myc* and *Slc2a1* (GLUT1) gene expression in $CD4^+$ T cells from WT and $Rosa26^{LSL\text{-}caNfatc1}$ *dLckcre* ($caNfatc1^{KI}$) mice that express a constitutively active form of NFATc1/A (caNFATc1) as described in (B); means ± SEM of 4 mice.

(D) Analysis of *Slc2a1* (GLUT1) gene expression in $CD4^+$ T cells from WT SMARTA and $Stim1^{fl/fl}Stim2^{fl/fl}$*Cd4cre* ($Stim1/2^{CD4}$) SMARTA mice 2 days after retroviral transduction with GLUT1 (before transfer into congenic host mice); means ± SEM of 3-4 mice (see also Figure 5G-H).

(E) Analysis of glucose uptake using the fluorescent glucose analogue 2-NBDG in $CD4^+$ T cells from WT and $Stim1/2^{CD4}$ SMARTA mice after retroviral transduction with GLUT1, transfer into $CD45.2^+$ host mice and LCMV infection on day 8 post-infection; means ± SEM of 3-4 mice (see also Figure 5G-H).

(F-H) Retroviral transduction of $CD45.1^+CD4^+$ T cells from WT SMARTA or $Stim1/2^{CD4}$ SMARTA mice with IRF4 or c-Myc *in vitro* followed by adoptive transfer into congenic $CD45.2^+$ WT host mice. Host mice were infected with LCMV and donor T cell expansion was analyzed 8 d later.

(G) Representative flow cytometry plots of adoptively transferred donor $CD4^+$ $CD45.1^+$ Vα2 TCR^+ T cells from WT SMARTA and $Stim1/2^{CD4}$ SMARTA mice; bar graphs show frequencies of cells in spleen 8 days post-infection. Means ± SEM of 5 host mice.

(H) Absolute numbers of $CD4^+$ $CD45.1^+$ Vα2 TCR^+ donor T cells in spleen. Means ± SEM of 5 host mice.

Figure S6. Related to Fig. 6 and Fig. 7

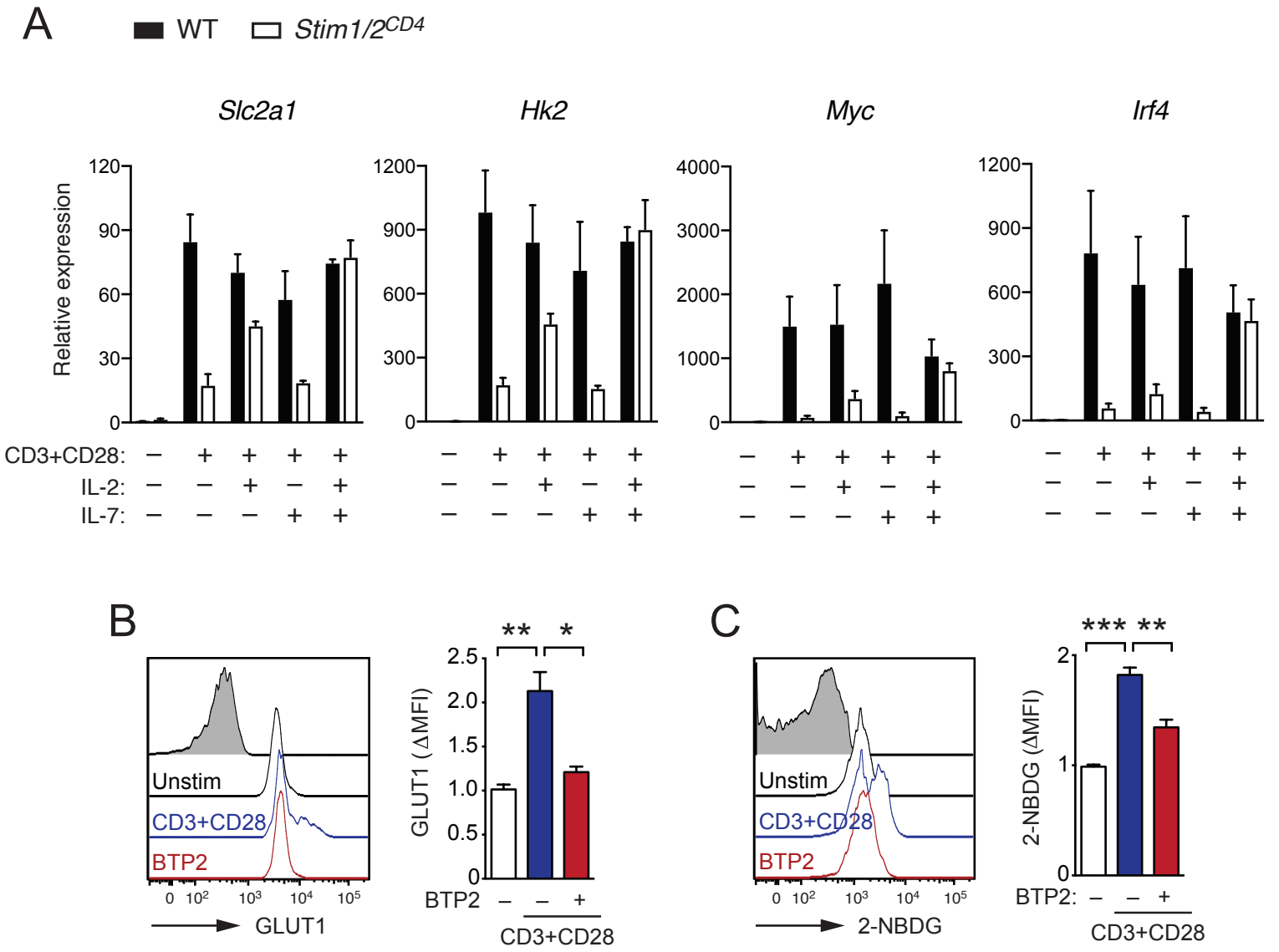

Figure S6. Related to Figures 6 and Figure 7. SOCE-dependent and -independent regulation of glycolysis by TCR stimulation and cytokines in T cells.

(A) IL-2 and IL-7 together, but not individually, restore expression of glycolysis-related genes in *Stim1/2*-deficient T cells. qRT-PCR analysis of *Slc2a1* (GLUT1), *Hk2, Myc* and *Irf4* gene expression in $CD4^+$ T cells from WT and $Stim1^{fl/fl}Stim2^{fl/fl}Cd4cre$ ($Stim1/2^{CD4}$) mice before and 36 h after stimulation in the presence or absence of 50 U/ml IL-2, 5 ng/ml IL-7 or both; means ± SEM of 2 mice.

(B and C) Inhibition of SOCE impairs GLUT1 expression and glucose uptake in human T cells. **(B)** GLUT1 protein expression measured by flow cytometry in $CD4^+$ T cells of healthy donors (HD) 16 h after anti-CD3/CD28 stimulation in the presence or absence of 500 nM BTP-2. Representative histogram plots (left panel) and summary of 3 HD (right panel); bar graphs are means ± SEM. **(C)** Analysis of glucose uptake by HD $CD4^+$ T cells 16 h after anti-CD3/CD28 stimulation in the presence or absence 500 nM BTP-2 using the fluorescent glucose analogue 2-NBDG. Representative histogram plots (left panel) and summary of 3 HD (right panel); bar graphs are means ± SEM.