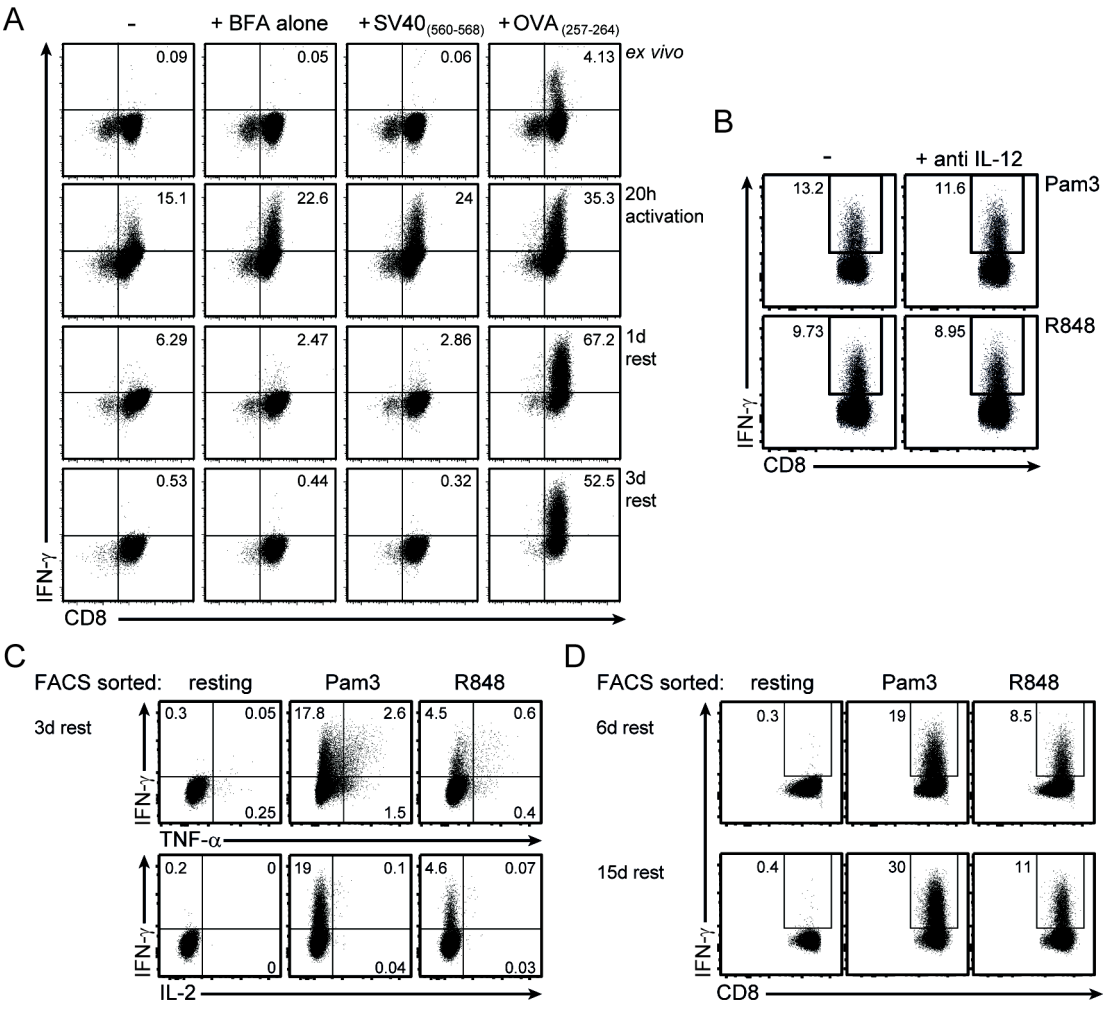
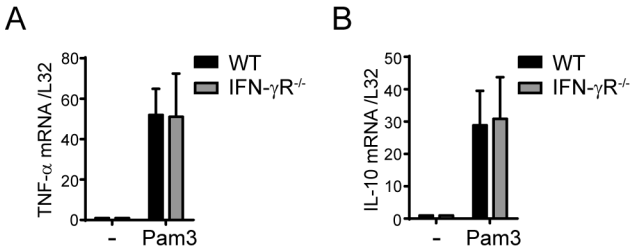
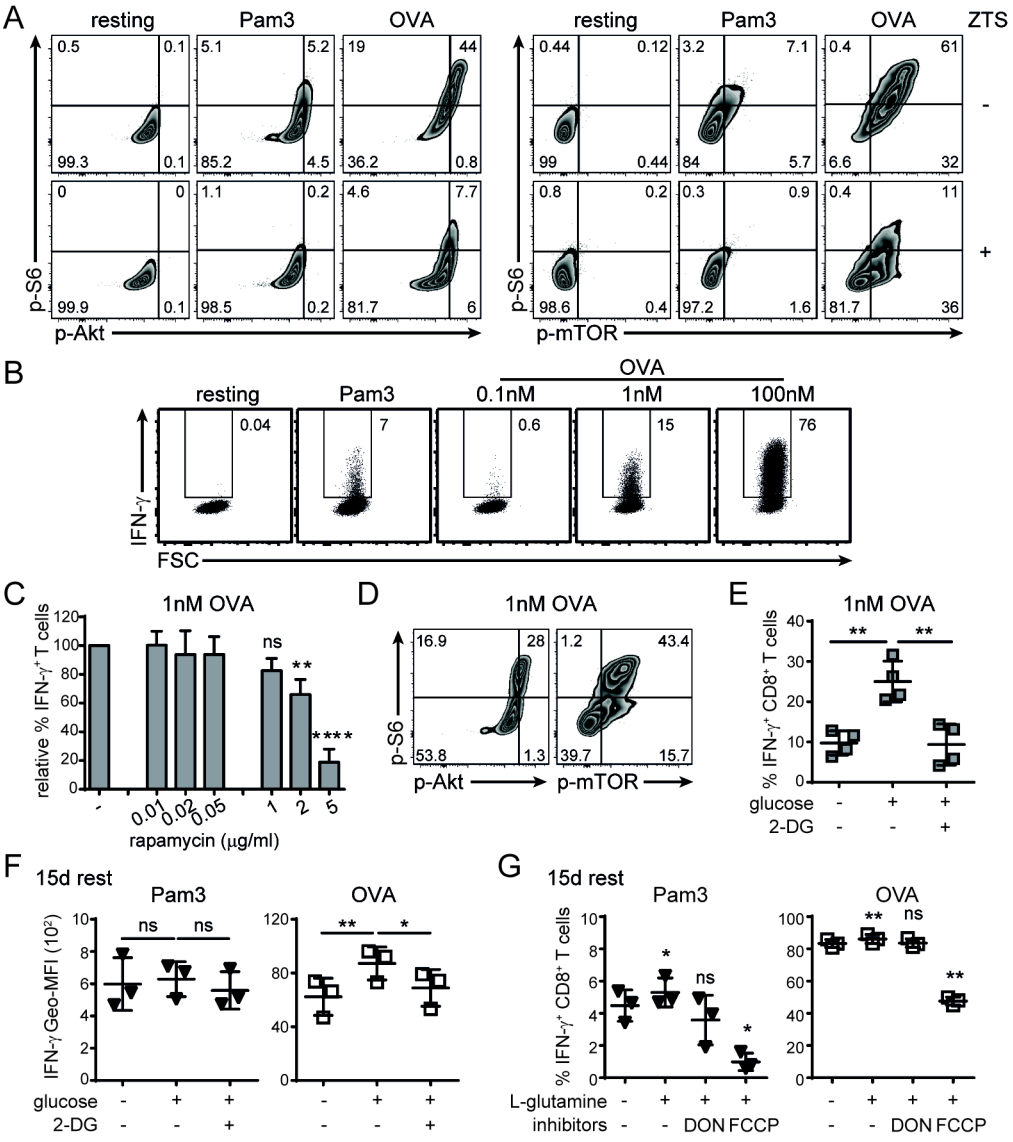


Supplemental Figure 1: TLR-dependent production of IFN-γ by T cells (**A**) Intracellular IFN-γ staining was performed on MACS-enriched $CD8^+$ OT-I T cells directly post selection (*ex vivo*), after activation with MEC.B7.SigOVA cells for 20h or at indicated time points of rest in the absence of antigen. T cells were left untreated, or were incubated for 6h with 1μg/ml brefeldin A (BFA), in the presence or absence of 1μg/ml $OVA_{257-264}$ peptide or the irrelevant $SV40_{560-568}$ peptide. Numbers in the upper right corner indicate the percentage of IFN-γ^+ $CD8^+$ T cells. (**B**) MACS-selected T cells (rested for 3 days) were pre-incubated for 30 min with 10μg/ml IL-12 neutralizing antibody or left untreated (-), and then stimulated for 6h with Pam3 and R848 in the presence or absence of the IL-12 neutralizing antibody. IFN-γ production was measured by flow cytometry. (**C-D**) FACS-sorted naive $CD44^{low}$ $CD62L^{hi}$ $CD8^+$ OT-I T cells were activated with MEC.B7.SigOVA cells for 20h and removed from antigenic stimuli for indicated time points. (**C**) Dot plots represent IFN-γ and TNF-α (upper panel), and IFN-γ and IL-2 production (lower panel) of T cells (rested for 3 days) that were stimulated for 6h with 5μg/ml Pam3 or 10μg/ml R848. (**D**) Intracellular IFN-γ staining of T cells that were activated with Pam3 and R848 upon 6 days, or 15 days of rest.



Supplemental Figure 2: Pam3 stimulation of IFN-γR$^{-/-}$ macrophages. BM derived-macrophages from Balb-c WT and IFN-γR$^{-/-}$ mice were cultured for 5h with 5µg/ml Pam3 or left untreated. (**A**) TNF-α and (**B**) IL-10 mRNA expression was analyzed by RT-PCR. Data are pooled from 2 independently performed experiments.



Supplemental Figure 3: TCR-dependent IFN-γ production is controlled by both mTORC1 and mTORC2 pathways, and is supported by glycolysis (A) Dot plots represent Akt, mTOR and S6 phosphorylation 30 minutes after T cell reactivation with 5μg/ml Pam3 or 100nM $OVA_{257–264}$ peptide. When indicated, cells were pretreated for 30 minutes with ZTS inhibitor. (**B**) Intracellular IFN-γ staining of $CD8^+$ T cells that were activated for 6h with 5μg/ml Pam3, 0.1nM , 1nM or 100nM $OVA_{257–264}$ peptide or were left unstimulated. (**C**) Resting T cells were incubated for 30 min with indicated concentrations of rapamycin or left untreated prior to reactivation with 1nM $OVA_{27–264}$ peptide. Graph indicates percentage of IFN-γ producing cells relative to cells that were reactivated in the absence of rapamycin. Data are pooled from 4 independent experiments ± SD [one-way ANOVA with Dunnett's multiple comparison to untreated control; **p<0.005; ***p<0.0001]. (**D**) Akt, mTOR and S6 phosphorylation of T cells reactivated for 30 minutes with low amount (1nM) of $OVA_{257–264}$ peptide. (**E**) T cells were activated for 6h with 1nM $OVA_{257–264}$ peptide in FCS-free, glucose-free RPMI that was supplemented with 25mM glucose, or with 25mM glucose plus 10mM 2-DG, or left unsupplemented. [one-way ANOVA with Dunnett's multiple comparison to glucose condition; **p<0.005]. (**F**) Glucose-dependency, (**G**) glutamine-dependency and mitochondrial respiration-dependency assays were performed when T cells were rested for 15 day and then stimulated for 6h with 5μg/ml Pam3 or 100nM $OVA_{257–264}$ peptide. [one-way ANOVA with Dunnett's multiple comparison to negative condition; *p<0.05; **p<0.005].

Supplemental Table I: Primers used for RT-PCR

Gene (mouse)	**Forward sequences**	**Reverse sequences**
L32	5’-GAAACTGGCGGAAACCCA-3’	5’-GGATCTGGCCCTTGAACCTT-3’
IFN-γ	5’-GGATGCATTCATGAGTATTGC-3’	5’-CCTTTTCCGCTTCCTGAGG-3’
TNF-α	5'-ACAGAAAGCATGATCCGCG-3'	5'-GCCCCCCATCTTTTGGG-3'
IL-10	5'-GGTTGCCAAGCCTTATCGGA-3'	5'-ACCTGCTCCACTGCCTTGCT-3'
TLR2	5'-AAGAGGAAGCCCAAGAAAGC-3'	5'-CGATGGAATCGATGATGTTG-3'
TLR3	5'-GGTGGTCCCGTTAATTTCCT-3'	5'-CCCGAAAACATCCTTCTCAA-3'
TLR4	5'-TCAGAACTTCAGTGGCTGGATTT-3'	5'-AACTCTGGATAGGGTTTCCTGTC-3'
TLR5	5'-AAGTTCCGGGGAATCTGTTT-3'	5'-GCATAGCCTGAGCCTGTTTC-3'
TLR7	5'-GGCTGAACCATCTGGAAGAA-3'	5'-TAAGCTGGATGGCAGATCCT-3'
TLR9	5'-AGCTGAACATGAACGGCATCT-3'	5'-CCAGCCATCTGAGCGTGTACT-3'