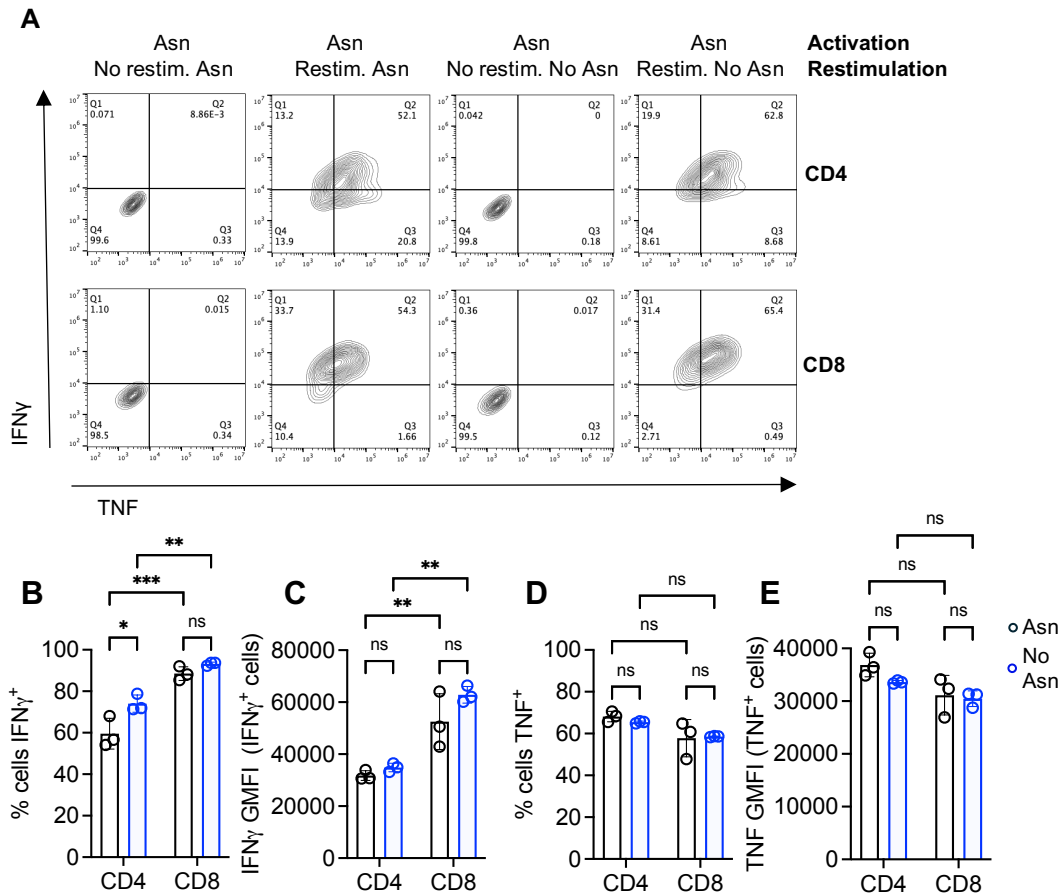


**Asparagine availability differentially regulates early vs late CD4⁺ and CD8⁺ T
cell activation, metabolism and autophagy**

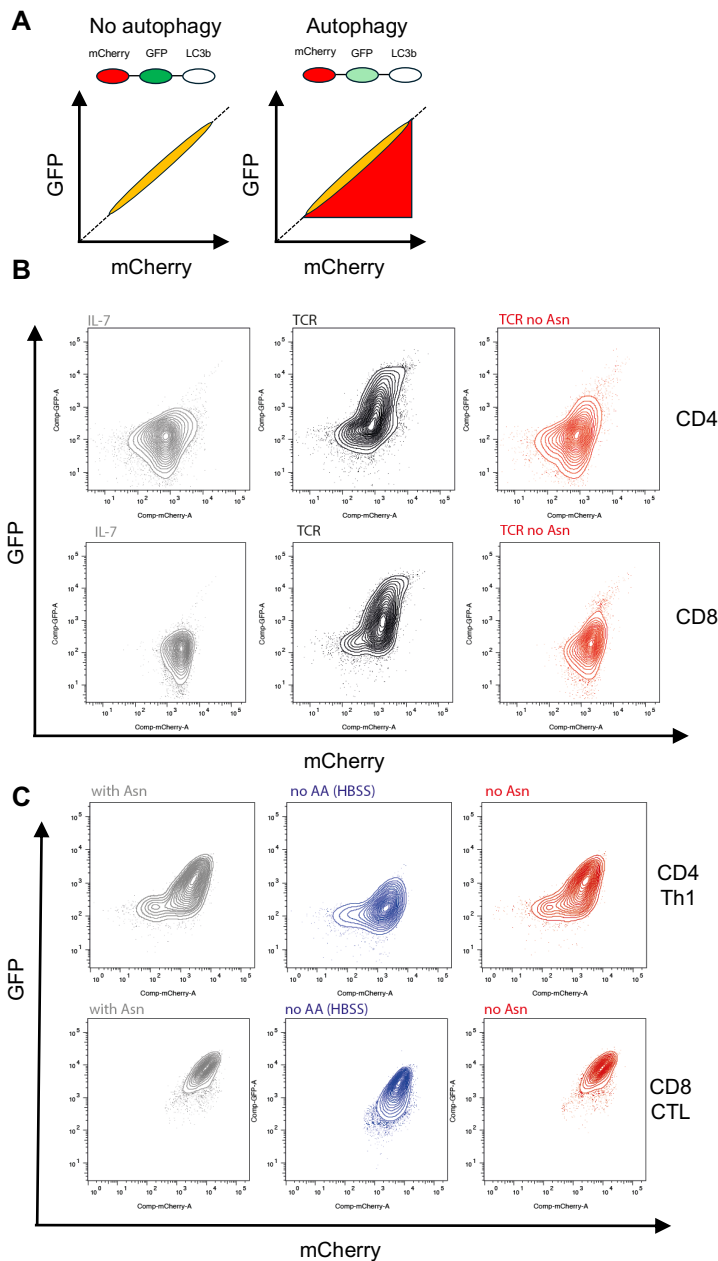
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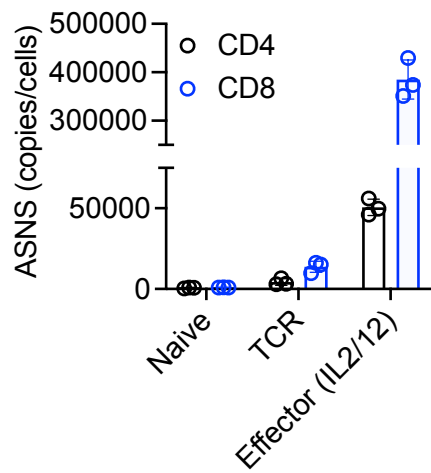
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Supplementary Figure 1. Asn availability is not required for cytokine production in effector T cells. To assess requirements for Asn in effector T cell cytokine production, lymph node T cells were differentiated to a Type 1 effector phenotype by stimulating for 72h with CD3 / CD28 antibodies and IL-12 in Asn-replete media, then restimulated with PDBU/Ionomycin in DMEM supplemented \pm Asn. Representative dotplots (A) show levels of IFN γ and TNF staining in gated CD4 $^+$ and CD8 $^+$ T cells, whilst barcharts show mean proportions of IFN γ $^+$ (B) and TNF $^+$ (D) cells or geometric mean fluorescence intensity (GMFI) or cytokine staining in gated cytokines-positive cells (C, E). Data are from 1 of 3 repeated experiments. In all cases, error bars represent SD. ns – not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ as determined by two-way ANOVA with Tukey's multiple comparisons test.



Supplementary Figure 2. Differential roles for Asn availability in regulation of autophagic flux in naïve vs effector T cells. (A) Levels of autophagic flux were assessed using mCherry-GFP-LC3b reporter mice by analysis of relative levels of GFP and mCherry signal: linear GFP-mCherry represent autophagy repression, quenching of GFP signal reflects recruitment of LC3b to autolysosomes and high autophagic flux. (B) Representative dotplots show levels of GFP and mCherry fluorescence in gated CD4⁺ and CD8⁺ T cells activated for 18h ± 300 μM Asn. (C) Effector T cells from autophagy reporter mice were generated by 5 days of stimulation *in vitro*. Cells were switched into DMEM ± Asn or amino acid-free conditions (HBSS) for 2h followed by assessment of autophagy.



Supplementary Figure 3. Increased expression of ASNS in effector CD8⁺ as compared to CD4⁺ T cells. Data are taken from the Immunological Proteome Resource (Ref. 25) and represent biological replicate values of ASNS protein copies / cell.