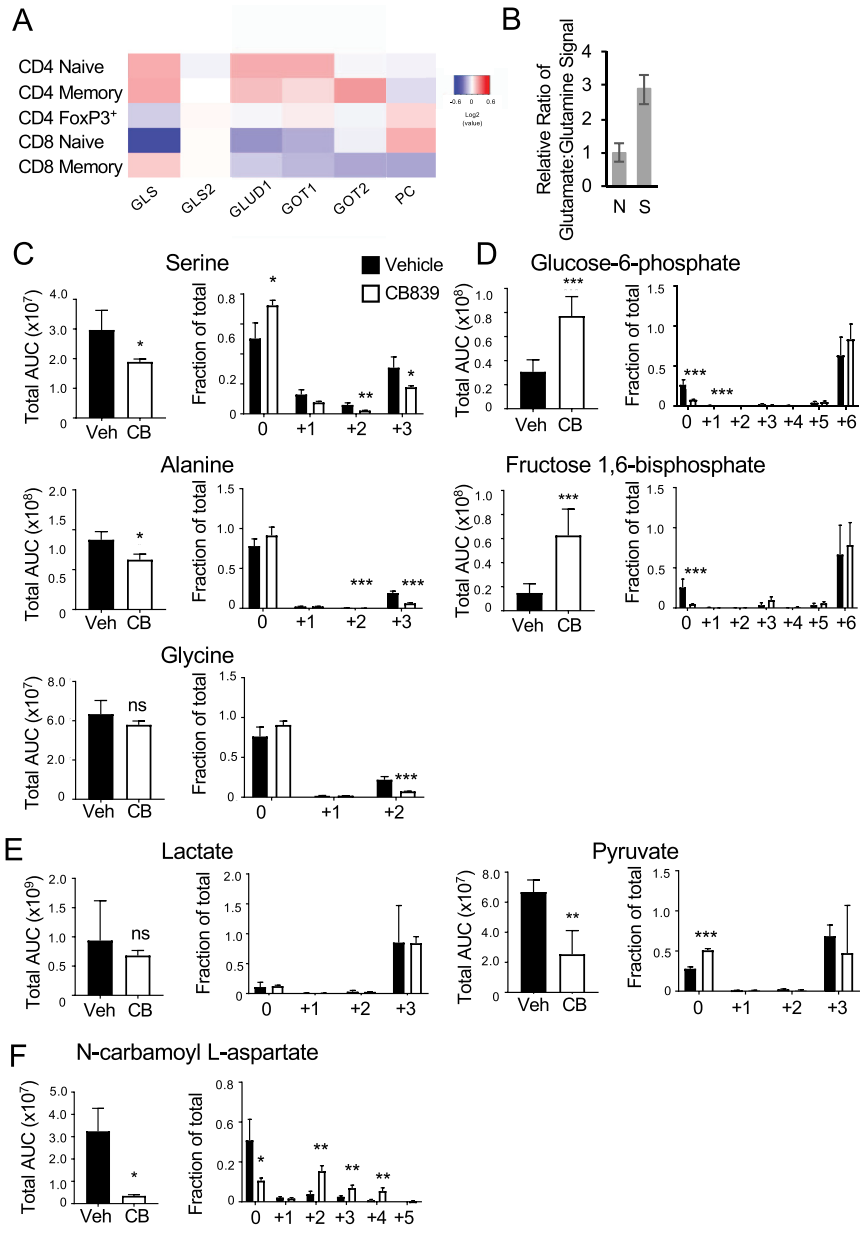

Figure S1. Glutamine Conversion to Glutamate Contributes to T Cell Metabolism, Related to Figure 1

(A) Relative expression of glutamine pathway genes, data from Immgen (immgen.org).

(B) Relative ratio of glutamate:glutamine metabolite levels normalized to IL-7 (naive, N) αCD3/CD28 (stimulated, S) normalized to naive in wild-type CD4$^+$ T cells.

(C–F) Additional intracellular metabolite abundance (left) and fraction labeled from ^{13}C-glucose (right). (C) Amino acids Serine, alanine, and glycine. (D) Glycolytic intermediates G6P, F16BP. (E) Lactate and Pyruvate. (F) Nucleotide precursor N-carbamoyl L-aspartate (average of n = 3 replicates/group). Means ± Std dev, (total abundance, left, ***p < 0.001, Student's t test; fractional labeling, right, ***p < 0.001, one way ANOVA).

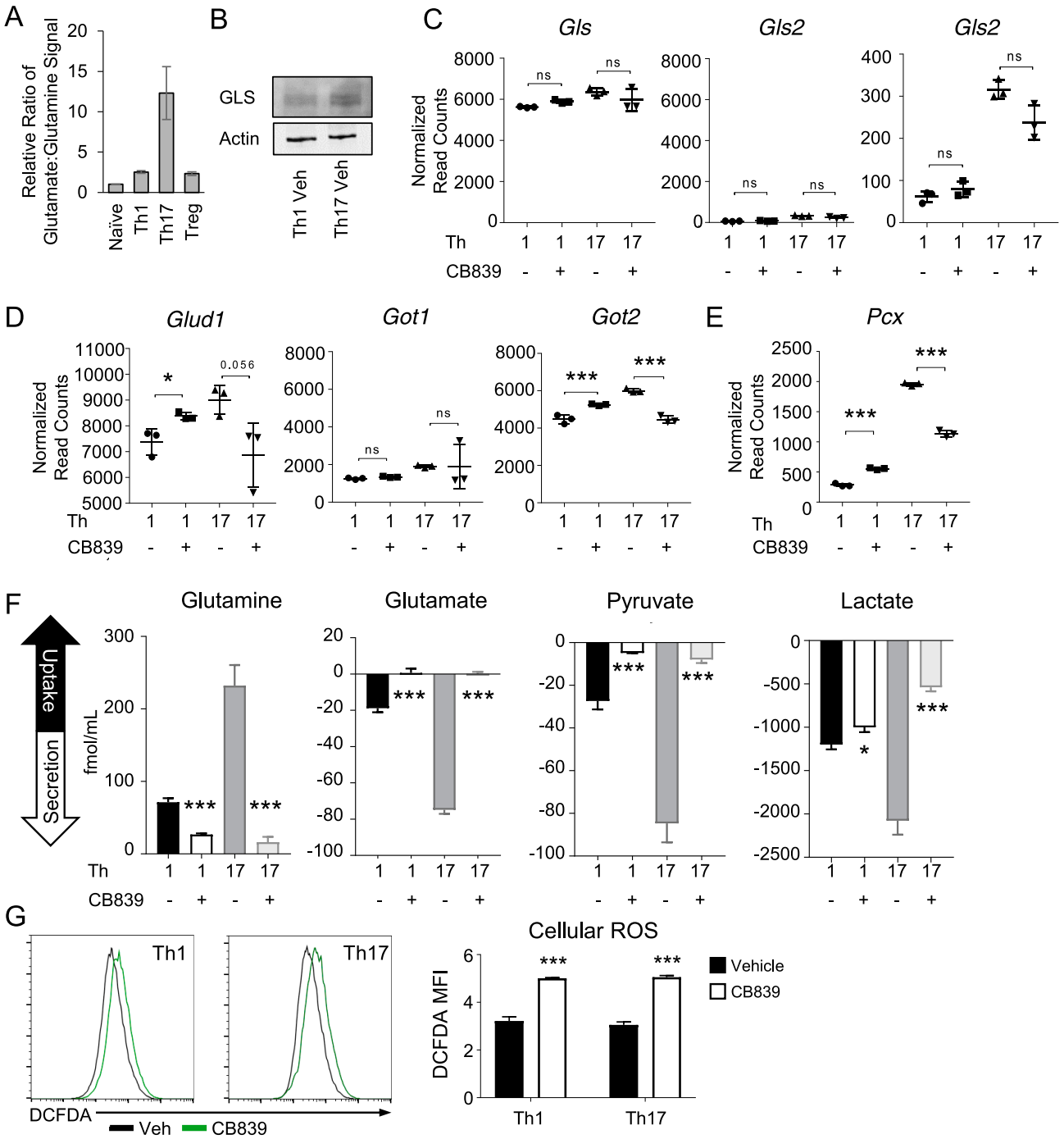

Figure S2. Glutamine and the Role of GLS in Th1 and Th17 Cell Metabolism, Related to Figure 2

(A) Relative ratio of intracellular metabolites glutamate:glutamine from CD4$^+$ T cells in Th1, Th17, and Treg skewing conditions normalized to naive (average n = 3 replicates/group).

(B) Immunoblot of GLS protein (top) and actin control (bottom) in T cells after five days in Th1 and Th17 skewing conditions.

(C–E) Normalized counts of message from RNA-Seq. (C) *Gls* enzyme RNA expression from RNA-Seq from Figure 2D. *Gls2* expression from RNA-Seq from Figure 2D on the same scale as *Gls* expression (left) and in smaller scale (right). For all RNA-Seq expression data, P values are determined from RNA-Seq analysis, all groups run in triplicate. (D) *Glud1*, *Got1*, and *Got2* expression as in (C). (E) *Pcx* RNA expression as in (C) (All p values from defSeq2 program, n = 3 replicates/group).

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(F) Uptake (positive numbers) and secretion (negative numbers) of metabolites in CB839 treated wild-type $CD4^+$ T cells in Th1 and Th17 skewing conditions as measured by Nuclear Magnetic Resonance (NMR) (average of 3 replicates, ***$p < 0.001$, unpaired t test).
(G) Fluorescence of DCFDA dye by flow cytometry, representative histograms (left) and average of n = 3 replicates (right, ***$p < 0.001$, Student's t test) of vehicle or CB839-treated T cells in Th1 and Th17 skewing conditions.

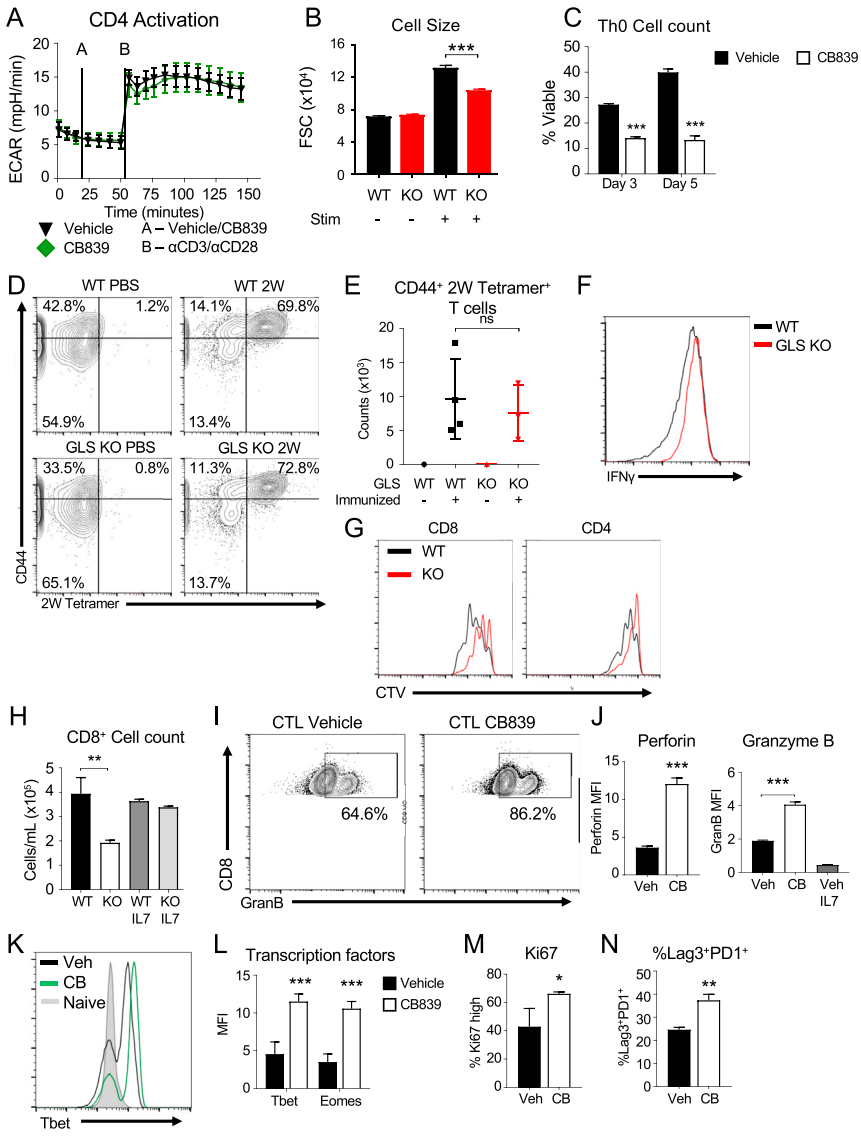

Figure S3. GLS Deficiency Does Not Alter Resting T Cell Phenotype but Enhances Th1 and CD8⁺ T Cell Differentiation and Cytokine Production, Related to Figure 3

(A) Extracellular Acidification Rate (ECAR) of naive CD4⁺ T cells treated with vehicle or CB839 as measured by Seahorse (n = 4 replicates/group).

(B) Average MFI of forward scatter (FSC) in activated CD8⁺ WT and GLS KO T cells (***p < 0.001, Student's t test, replicates of n = 3/group).

(C) Viability by propidium iodide staining at day 3 and day 5 of WT T cells in activation condition with no cytokines (***p < 0.001, Student's t test, average of n = 3 replicates).

(D–F) 2W peptide immunization of WT and GLS KO. (D) Percent 2W-MHC II tetramer⁺ and CD44⁺ T cells by flow cytometry in both spleen and inguinal lymph nodes eight days after immunization with 2W antigen + CFA (right) or PBS control (left) in WT and GLS KO animals. (E) Average count of CD44⁺ Tetramer⁺ T cells as in (D) (p > 0.05, Student's t test). (F) IFNγ protein expression by flow from CD44⁺ MHC II tetramer⁺ T cells isolated from WT and GLS KO spleen and lymph nodes.

(G) Homeostatic proliferation of WT and GLS KO CD4/CD8⁺ T cells stained with cell trace violet (CTV) and injected into RAG1 KO recipient mice after five days (representative of n = 5 replicates/group).

(H) Cell counts of CD8⁺ T cells from WT and GLS KO animals activated on αCD3/CD28+IL2 for five days (**p < 0.01, Student's t test).

(I–N) CD8⁺ T cells activated αCD3/CD28+IL2 for five days in the presence of CB839 or vehicle. (I) Representative FACs plots of granzyme B producing cells, (J) Perforin MFI (left) or granzyme B MFI (right) (***p < 0.001, Student's t test). (K) Representative Tbet expression, (L) Average transcription factor expression (***p < 0.001, Student's t test, n = 3 replicates), (M) Ki67 expression, (N) Percent Lag3⁺ and PD1⁺ T cells as in (I). (*p < 0.01, **p < 0.01, Student's t test, average of n = 3 replicates,).

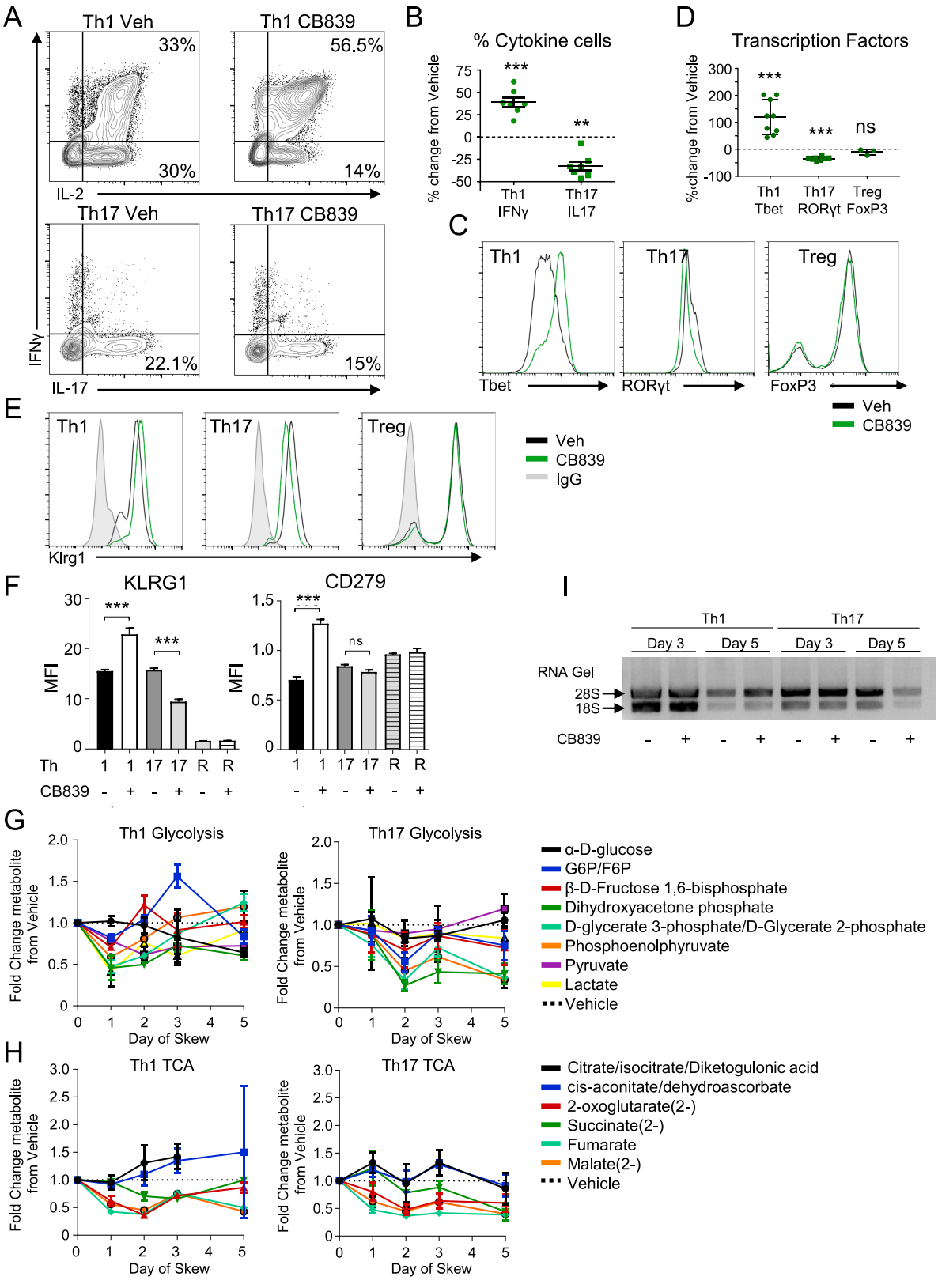

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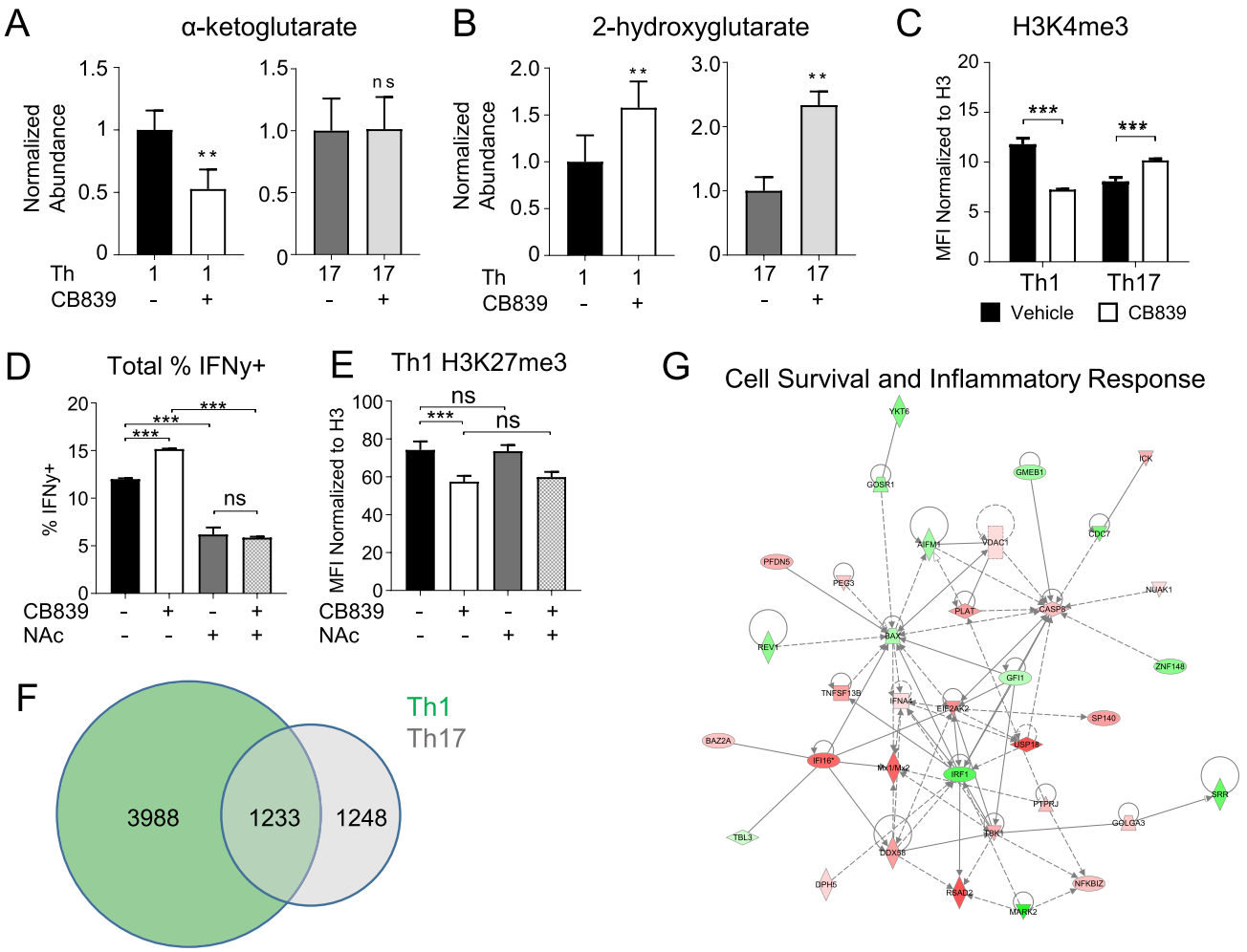
Figure S4. CB839 Inhibition Phenocopies GLS KO *In Vitro*, Related to Figure 4

(A–F) Naive $CD4^+$ T cells from WT differentiated in Th1, Th17, or Treg skewing media over five days in the presence of CB839 or vehicle as in Figure 4A. (A) IFNγ and IL2 production in Th1 skewing conditions (top) and IL-17 production in Th17 skewing conditions (bottom) (representative of n = 3 replicates/group). (B) Percent change cytokine producers in Th1 and Th17 cells from vehicle (Th1, Th17 n = 9 experiments, ***$p < 0.001$, Student's t test,). (C) Transcription factor expression in wild-type cells treated with Vehicle or CB839 (Tbet and RORyt, n = 9 experiments, Foxp3 n = 3 experiments). (D) Average percent change from WT of transcription factor expression (Th1, Th17 n = 7 experiments, Treg n = 3 experiments, ***$p < 0.001$, one-sample t test). (E) Representative Klrg1 protein expression and (F) average Klrg1 and CD279 expression (***$p < 0.001$, Student's t test).

(G and H) Metabolites in glycolysis (H) and Tricarboxylic Acid cycle (I) as in Figures 3I–3J (average of 3 replicates/group fold change from vehicle).

(I) Total RNA extracted from cells as in (A) at day 3 and day 5 (representative of n = 2 experiments).



H

Th1 more open	Trans. Factor	P value
	AP-1	1e-803
	ETS	1e-547
	IRF	1e-358
	CTCF	1e-234

Th17 more open	Trans. Factor	P value
	ETS	1e-74
	Runx	1e-52
	CTCF	1e-42
	AP-1	1e-19

Th1 more closed	Trans. Factor	P value
	ETS	1e-37
	CTCF	1e-37
	Runx	1e-26
	Nrf	1e-13

Th17 more closed	Trans. Factor	P value
	AP-1	1e-345
	CTCF	1e-153
	ETS	1e-109
	IRF	1e-38

Figure S5. GLS Deficiency Differentially Affects Th1 and Th17 T Cells and Modifies Epigenetic Landscape, Related to Figure 5

(A and B) Metabolite levels normalized to vehicle of each subset (A) Intracellular α-ketoglutarate metabolite levels and (B) 2-Hydroxyglutarate metabolite levels as in A (**$p < 0.01$, unpaired t test).

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(C) MFI of H3K4me3 in Th1 and Th17 cells (***p < 0.001, Two-way ANOVA, n = 3 replicates/group).
(D) Percent total IFNy+ producers in Th1 skewing conditions (***p < 0.001, one-way ANOVA).
(E) MFI of H3K27me3 in Th1 skewing conditions (***p < 0.001, one-way ANOVA).
(F) Venn diagram of ATAC-Seq total changed peaks (either open or closed).
(G) Ingenuity pathway analysis of altered ATACseq peaks from promoter regions in Th1 cells for Cell Survival and Inflammatory response (green – downregulated, red, upregulated, relative to vehicle treated).
(H) Motif analysis of the promoter regions with significantly changed peaks in Th1 and Th17 cells.

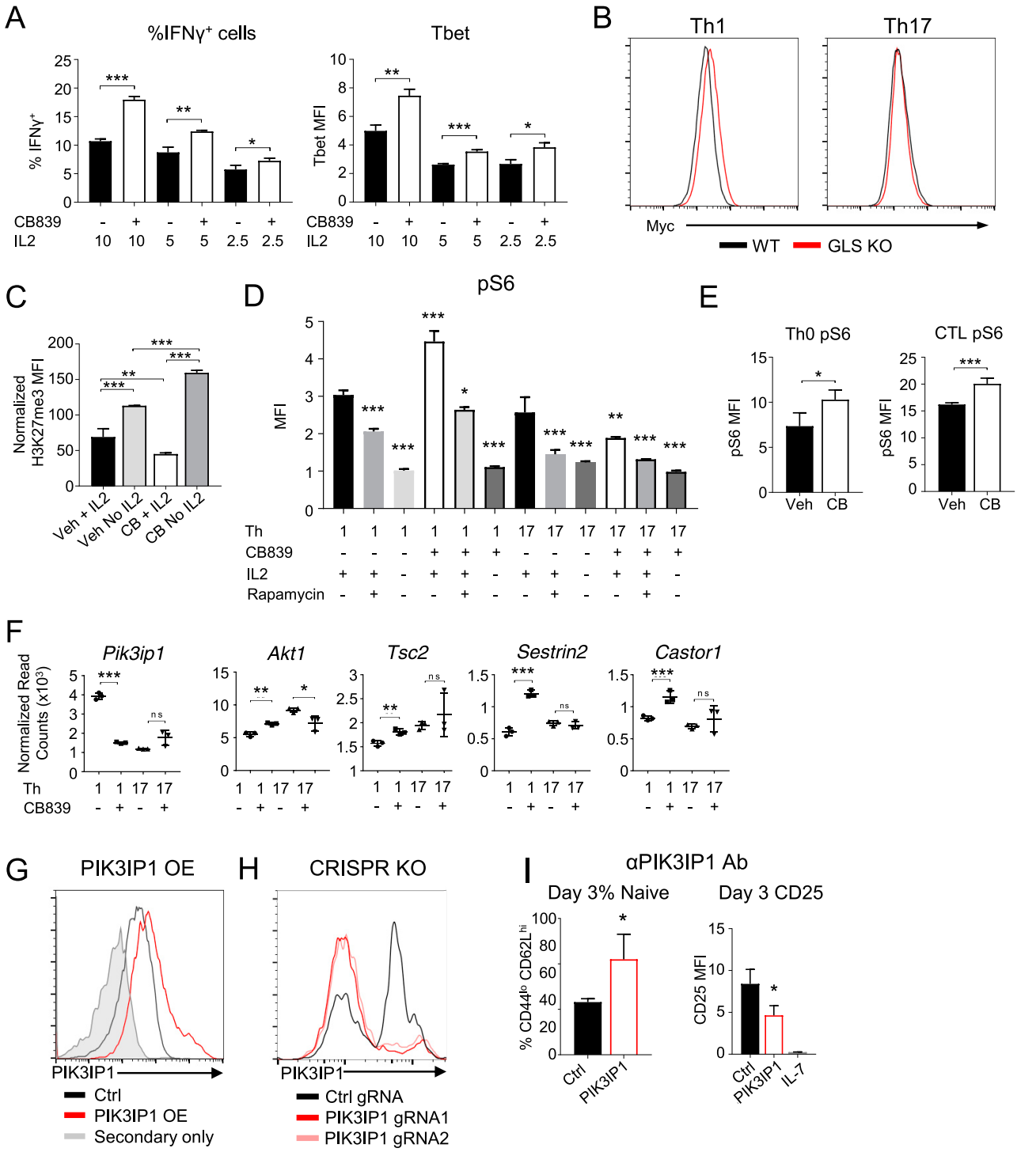

Figure S6. Th1 Cells Are Sensitive to mTOR Signaling in GLS Deficiency, Related to Figure 6

(A) Left: Percent IFNγ$^+$ producers in Th1 skewing conditions treated with or without CB839 and indicated levels of IL-2 (ng/mL). Right: Tbet protein expression as in left. (***p < 0.001, Student's t test).

(B) Myc protein expression in WT and GLS KO CD4$^+$ T cells in Th1 and Th17 skewing conditions (representative of n = 3 replicates).

(C) MFI of H3K27me3 normalized to total H3 of CD4+ T cells in Th1 skewing conditions with indicated IL2 with or without CB839 (***p < 0.001, one-way ANOVA, n = 3 replicates/group).

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(D and E) phospho-S6 protein expression measured by flow cytometry (D) in IL2 and IL2 depleted conditions with or without rapamycin (**$p < 0.01$, one-way ANOVA compared to vehicle of each group, n = 3 replicates/group) or (E) pS6 expression in Th0 (left) and CD8+ CTL cells (right) (***$p < 0.001$ Student's t test, n = 3 replicates/group).

(F) Normalized message counts from RNA-Seq described in Figure 6A, highlighting PI3K/Akt/mTOR pathway targets (***$p < 0.001$, p values obtained from defSeq2 program).

(G) PIK3IP1 protein expression in Wild-Type $CD4^+$ T cells in Th1 skewing conditions in the presence of CB839 infected with PIK3IP1 expression plasmid (representative of n = 3 replicates).

(H) PIK3IP1 protein expression in CAS9-expressing $CD4^+$ T cells in Th1 skewing conditions with guide RNAs targeting PIK3IP1 (CRISPR KO).

(I) Percent naive cells in control or PIK3IP1 antibody-treated activated T cells (left) and CD25 expression (right) (*$p < 0.05$, Student's t test, n = 3 replicates/group).

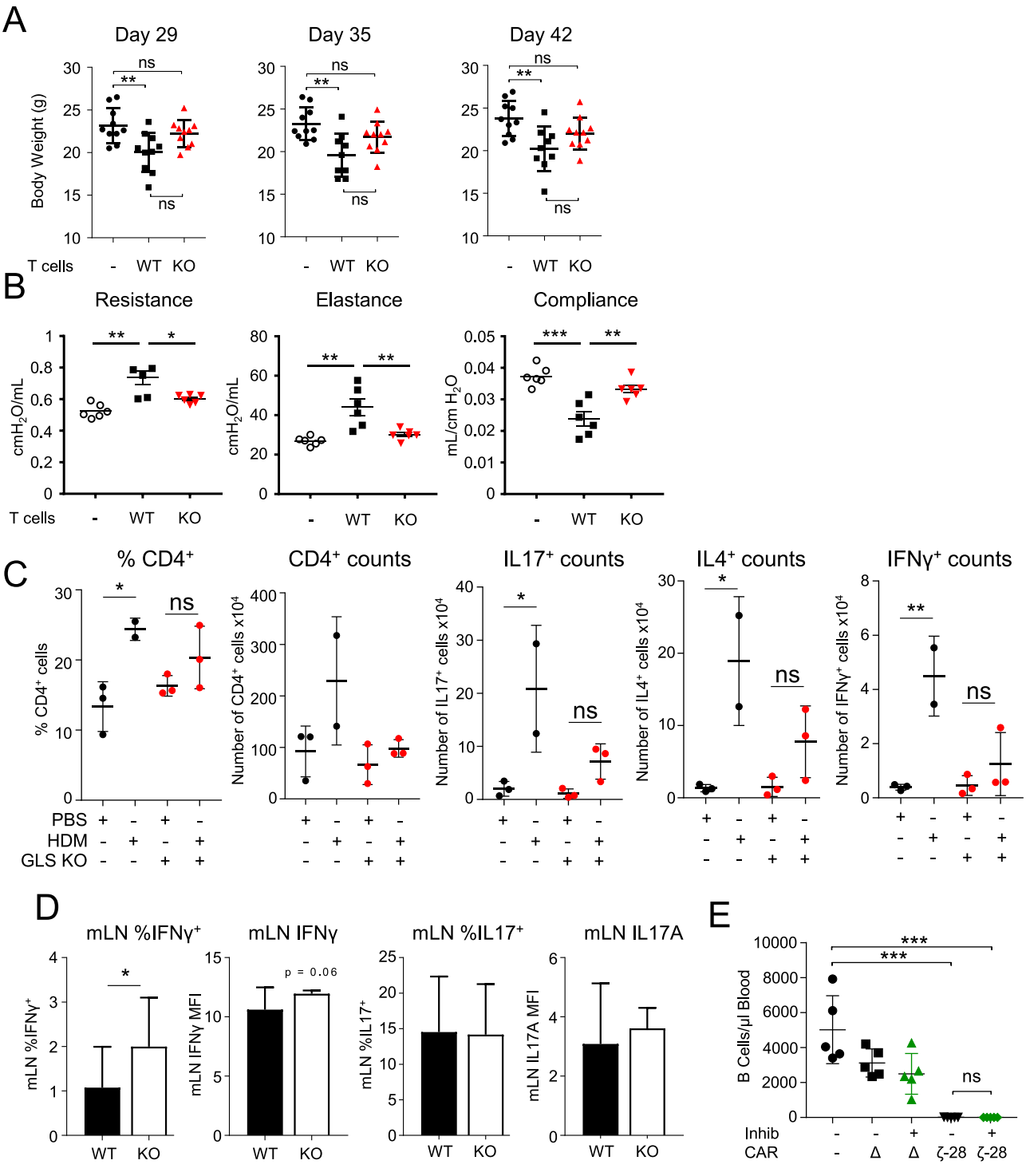

Figure S7. GLS Is Essential *In Vivo* for Inflammation, but Transient GLS Inhibition Does Not Prevent CAR T Cell-Mediated Responses, Related to Figure 7

(A and B) cGVHD in C57BL6 animals as in Figure 7A. (A) Bodyweights of recipient mice injected with T cell depleted bone marrow and either WT $CD4^+$ or GLS KO $CD4^+$ T cells from spleen. n = 9 animals/group (**p < 0.01, one-way ANOVA). (B) Lung physiology measurements (read out of Bronchiole Obliterans) from (A) (***p < 0.001, one-way ANOVA).

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(C) Percent of $CD4^+$ T cells (left), $CD4^+$ counts, $IL17^+$ counts, $IL4^+$, and $IFN\gamma^+$ counts in WT and GLS KO mice immunized with PBS or house dust mite antigen (HDM) over 14 days (*$p < 0.05$, Student's t test).

(D) Percent $IFN\gamma^+$, IFNy MFI, or percent $IL17^+$, and IL17A MFI in mesenteric lymph nodes collected from RAG1 KO mice injected with wild-type or GLS KO naive CD4 T cells in IBD (*$p < 0.05$, Student's t test).

(E) Frequency of $CD19^+$ B cells in blood 4 weeks after injection of T cells activated and infected with CAR T cell construct 28-ζ or control delta-ζ with (green) or without (black) GLS inhibitor (***$p < 0.001$, one-way ANOVA).