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BRIEF DEFINITIVE REPORT

SLC7A8 is essential for metabolic fitness and function of Th2 cells

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Amino acids are essential for the activation and function of CD4 T helper (Th) cells, which differentiate into Th1, Th2, Th17, and Treg subsets to coordinate immune responses. While specific amino acid transporters have been identified for Th1, Th17, and Tregs, a transporter regulating Th2 cells remains unknown. This study identifies SLC7A8 as a Th2-specific amino acid transporter in the Th compartment. We found that *Slc7a8* expression is upregulated in Th2 cells compared with other T helper subsets, and *Slc7a8* deficiency impairs Th2 cell proliferation and cytokine production. Furthermore, SLC7A8 was found to be crucial for an effective type 2 immune response to helminth infection and allergen-induced lung inflammation. Mechanistically, *Slc7a8* deficiency disrupted Th2 cell metabolism, leading to reduced mTOR activation and, consequently, diminished mitochondrial function along with an impaired c-Myc pathway; these defects cumulatively induced cellular stress that curtailed cell growth and survival. Collectively, these findings highlight a previously unknown role for SLC7A8 in Th2 cells, with potential implications for understanding and treating type 2 immune-related diseases.

Introduction

Naïve CD4 T cells are a central component of the adaptive immune system. They differentiate into effector Th1, Th2, T follicular helper cells (Tfh), or regulatory T cells (Tregs) and orchestrate the adaptive immune response against pathogens and autoantigens (Saravia et al., 2019; Zielinski, 2023). Th1 cells secrete IFN-γ and TNF-α and play a role in intracellular bacterial and viral infections. Th2 cells promote the immune response against helminths and allergens by secreting IL-5 and IL-13. Th17 cells secrete IL-17 and IL-22 to control extracellular bacterial and fungal infections (Luo et al., 2022; Sun et al., 2023; Walker and McKenzie, 2018). In contrast, Tregs regulate excessive activation of effector T cells and maintain tissue homeostasis (Campbell and Rudensky, 2020; Dikiy and Rudensky, 2023).

Distinct T helper cell (Th) differentiation pathways rely on coordinated action of transcription factors, the strength of TCR signaling, and co-stimulatory molecules, together with the cytokine milieu. For example, strong TCR signaling, IL-12, and T-bet induce Th1 differentiation, while weak TCR signaling, IL-4, and GATA3 induce Th2 differentiation (Afkarian et al., 2002; Gascoigne et al., 2016; van Panhuys et al., 2014; Zheng and Flavell, 1997). Similarly, RORγT and TGF-β induce Th17 differentiation, whereas FOXP3, along with TGF-β, regulates Treg differentiation (Ivanov et al., 2006; Zhou et al., 2008).

The differentiation and activation of Th subsets are metabolically demanding processes that require careful regulation of nutrient uptake through the controlled expression of nutrient transporters to meet their energy and biosynthetic needs (Ma et al., 2024). To meet their increased glucose demands, Th1, Th2, and Th17 cells heighten expression of glucose transporters (Macintyre et al., 2014). Similarly, amino acids are also essential; however, the upregulation of amino acid transporters is selective across different Th subsets. Accordingly, the depletion of specific amino acids or the genetic ablation of amino acid transporters specifically disrupts the activation and function of certain Th subsets (Wang and Zou, 2020). Glutamine unavailability, while limiting the differentiation of human Th1, potentiates that of Tregs in vitro (Metzler et al., 2016). Naive CD4 T cells deficient in the glutamine transporter SLC1A5 differentiate inefficiently into Th1 and Th17 (Nakaya et al., 2014). Conversely, SLC1A5 deficiency promotes Th2 differentiation, whereas Treg and cytotoxic T cells remain unaltered (Nakaya et al., 2014). Alanine depletion inhibits IL-17 and IFN-γ production by CD4 T cells; deficiency of the alanine transporter SLC38A1 impairs Th1 differentiation in a tissue-specific manner while being dispensable for Th17 (Ron-Harel et al., 2019; Sugiura et al., 2023, *Preprint*). Limitation of arginine suppresses human CD4 T cell proliferation and cytokine production (Werner et al., 2016). Furthermore, methionine availability regulates both Th1 and Treg function (Pandit et al., 2023; Saini et al., 2022). Large neutral amino acids, such as leucine, isoleucine, and valine, provide an important source of

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energy for Th cells. SLC7A5, a transporter of large neutral amino acids, is upregulated in activated T cells, and T cell–specific *Slc7a5* deletion impairs polarization of both Th1 and Th17 without affecting Treg (Sinclair et al., 2013). Additionally, this transporter contributes to cytotoxic T cell function. However, the role of large neutral amino acids and their transporters in Th2 differentiation and function is yet to be deciphered.

Recent studies reveal that SLC7A8, a transporter for large neutral amino acids and a homolog of SLC7A5, is selectively expressed in group 2 innate lymphoid cells (ILC2). This transporter plays a crucial role in the survival and function of ILC2 (Panda et al., 2022; Hodge et al., 2023). In this study, we further demonstrate that SLC7A8 serves as the Th2-specific transporter for large neutral amino acids. Both in vivo– and in vitro–differentiated Th2 cells expressed abundant transcripts of the *Slc7a8* gene. IL-7 receptor (*Il7r*)- and IL-5 (*R5*)-driven deletion of critical *Slc7a8* gene segments impaired Th2 function, which impacted both house dust mite (HDM)- and papain-induced allergy as well as immune response to infection by the intestinal parasite *Heligmosomoides polygyrus*. Mechanistically, FACS analysis showed that *Slc7a8* deficiency compromised the rapamycin (mTOR) signaling pathway and mitochondrial mass, ultimately attenuating c-Myc signaling. Collectively, these data identify a previously unrecognized role of SLC7A8 in Th2 cells.

Results and discussion

SLC7A8 is specifically expressed in Th2 cells

ILC2 and Th2 cells have similar transcriptional programs. Since SLC7A8-mediated amino acid transport is essential for ILC2 function, we asked whether Th2 cells also use this transporter. To do this, we first analyzed the expression of *Slc7a8* mRNA in Th cell subsets generated in vitro using publicly available RNA-sequencing (RNA-seq) data (Zhang et al., 2020). *Slc7a8* expression was specific to Th2 cells, similar to that of Th2 signature genes, such as *Gata3*, *Il4*, and *Il13*, compared with Th1, Th17, and Tregs (Fig. 1 A). Furthermore, *Slc7a8* expression was upregulated in Th cells induced by intestinal *H. polygyrus* infection in the intestinal lamina propria (Fig. 1 B) (Jarjour et al., 2020). To confirm these findings, we examined Th2 cells generated in vivo in a model of asthma induced by HDM. We intranasally instilled crude HDM into IFN-γ–, IL-13–, and IL-17–triple reporter mice (referred to as GGSSSS mice). We FACS-sorted Th1 (IFN-γ^+), Th2 (IL-13^+), and Th17 (IL-17^+) cells from the lungs and assessed the expression of *Slc7a8* and its homolog *Slc7a5* in each subset. Expression of *Slc7a8* was higher in Th2 cells, while *Slc7a5* expression was elevated in Th1 cells compared with naive CD4 T cells (Fig. 1 C). Previous studies have shown that TCR signaling upregulates amino acid transporter expression (Sinclair et al., 2013). To test whether this applies to SLC7A8, we cultured $CD4^+$ T cells under Th2-polarizing conditions with or without anti-CD3/CD28 stimulation. *Slc7a8lca* expression was clearly upregulated by TCR activation (Fig. S1 A). Together, these data suggested that SLC7A8 may play an important role in Th2 function.

To investigate this hypothesis, we examined $Il7r^{Cre}Slc7a8^{fl/fl}$ mice, which lack *Slc7a8* in the entire lymphocyte compartment (Panda et al., 2022). These mice exhibited normal thymocyte development and no defects in peripheral T cells (Fig. S1, B–I). We intranasally instilled HDM into $Il7r^{Cre}Slc7a8^{fl/fl}$ and $Il7r^{Cre}Slc7a8^{+/+}$ control mice and analyzed different Th subsets (Fig. S2 A). The frequency of $GATA3^+$ Th2 cells was reduced in *Slc7a8*-deficient mice compared with control mice, whereas other Th subsets, such as T-bet^+ Th1, $ROR\gamma T^+$ Th17, and $FOXP3^+$ Tregs, remained unaltered in the two genotypes (Fig. 1, D and E; and Fig. S2, B–D). In parallel, Th2-dependent eosinophil infiltration into the lungs was reduced in $Il7r^{Cre}Slc7a8^{fl/fl}$ mice compared with $Il7r^{Cre}Slc7a8^{+/+}$ mice (Fig. 1, F and G). Altogether, these data suggest that SLC7A8 is specifically expressed in Th2 cells and contributes to their differentiation and/or maintenance.

Slc7a8 deficiency impairs Th2 function in vivo

To further explore the specific role of SLC7A8 in Th2 differentiation and function, we generated $R5^{Cre}Slc7a8^{fl/fl}$ mice, which lack *Slc7a8* specifically in IL-5–producing cells, including ILC2s and Th2 cells. Similar to $Il7r^{Cre}Slc7a8^{fl/fl}$ mice, the frequency of T cells in thymus, spleen, and mesenteric lymph nodes (MLNs) was unaffected at steady state (data not shown), supporting that *Slc7a8* deficiency does not impact T cell development or peripheral T cell homeostasis.

Both $R5^{Cre}Slc7a8^{fl/fl}$ and $R5^{Cre}Slc7a8^{+/+}$ control mice were intranasally instilled with HDM or PBS, followed by analysis of the frequency of $GATA3^+$ Th2 cells in the lungs (Fig. 2 A). The frequency of $GATA3^+$ Th2 cells was significantly reduced in $R5^{Cre}Slc7a8^{fl/fl}$ mice compared with $R5^{Cre}Slc7a8^{+/+}$ controls upon HDM treatment (Fig. 2, B and C). Furthermore, the frequency and number of Th2 cells producing IL-5 were reduced in the lungs and mediastinal LNs of *Slc7a8*-deficient mice (Fig. 2, D–F). Conversely, IL-5^+ Th2 cell frequency was comparable in the lungs of $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice upon PBS instillation (Fig. S3 A). Notably, the frequency of Tfh cells, which were demonstrated to be precursors of Th2 cells upon HDM challenge (Ballesteros-Tato et al., 2016), was similar between $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice (Fig. S3, B and C). This suggests that SLC7A8 is necessary for proper Th2 function.

To further validate these findings, we utilized another model of lung allergy. Both $R5^{Cre}Slc7a8^{fl/fl}$ and $R5^{Cre}Slc7a8^{+/+}$ control mice were intranasally challenged with papain, after which we assessed the frequency of IL-5–producing Th2 cells as well as GATA3-expressing Th2 cells in the lungs (Fig. 2 G). Consistent with our previous observations, both the frequency and absolute number of IL-5^+ Th2 cells and $GATA3^+$ Th2 cells were markedly reduced in the lungs of *Slc7a8*-deficient mice compared with controls (Fig. 2, H–J).

We also examined the response of *Slc7a8*-deficient mice in an intestinal helminth infection, which is another model of type 2 immune response. Both $R5^{Cre}Slc7a8^{fl/fl}$ mice and $R5^{Cre}Slc7a8^{+/+}$ controls were orally gavaged with L3 *H. polygyrus* larvae (Fig. 2 K). $R5^{Cre}Slc7a8^{fl/fl}$ mice exhibited an increased egg burden compared with $R5^{Cre}Slc7a8^{+/+}$ mice (Fig. 2 L). The frequencies of $GATA3^+$ Th2 cells and IL-5–expressing CD4 T cells were

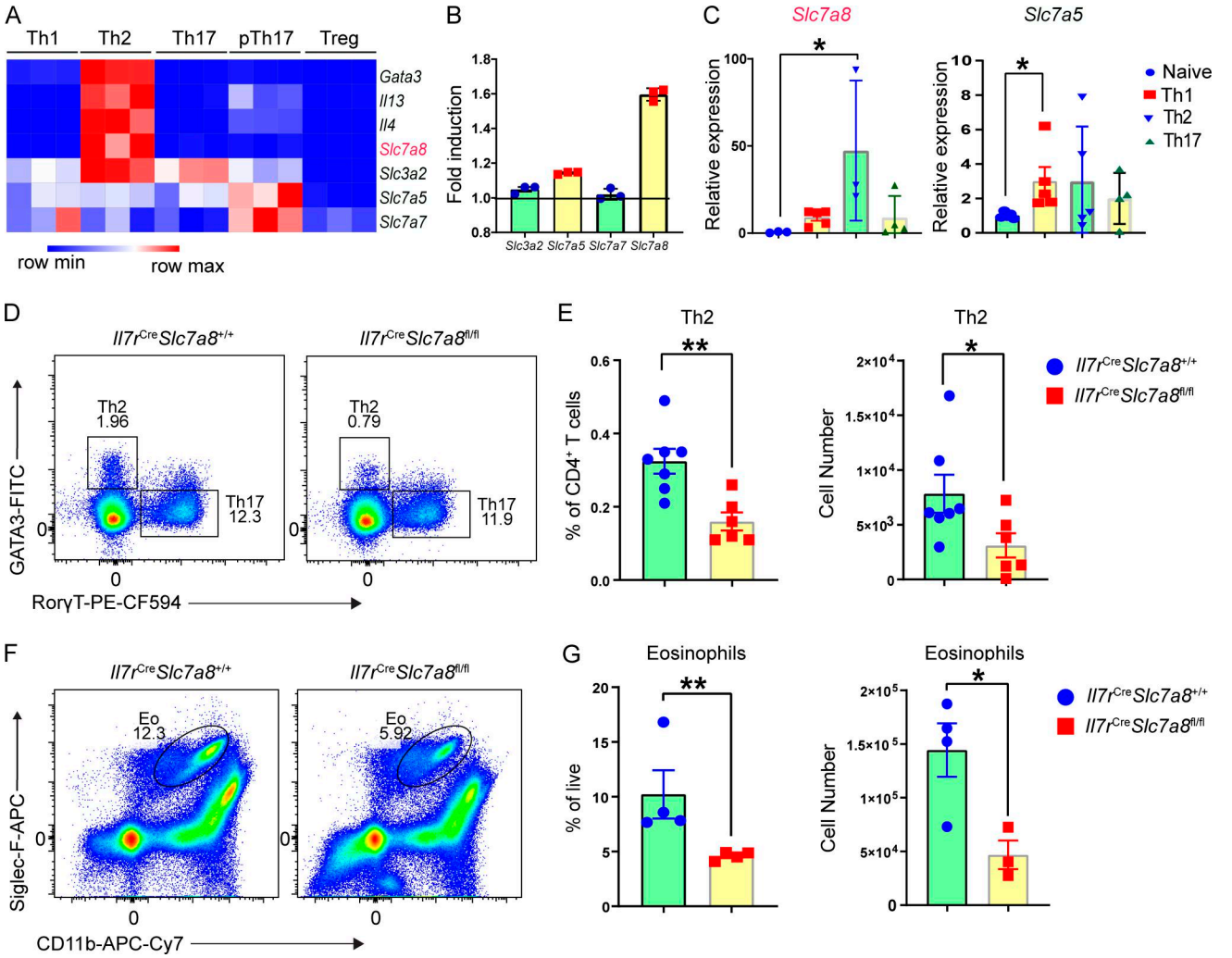

Figure 1. **SLC7A8 is specifically expressed in Th2 cells. (A)** Expression of *Slc7a8* in different subsets of CD4 T cells. **(B)** Fold induction of *Slc7a8* and other transporters in *H. polygyrus*–induced Th cells as compared with naïve Th cells. **(C)** qPCR data depicting the expression of *Slc7a8* and *Slc7a5* in different subset of Th cells. **(D–G)** *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice were intranasally instilled with HDM and different subsets of Th cells were scored. **(D)** Representative FACS plot showing the staining of GATA3 and RORγT in CD4$^+$ T cells (pre-gated on CD3$^+$, CD4$^+$, FOXP3$^-$, and Tbet$^-$) from the lungs of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(E)** Frequency and numbers of Th2 cells from the lungs of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(F)** Representative figure depicting the eosinophils in the lungs of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(G)** Frequency and number of eosinophils in the lungs of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. Data are pooled or representative of two individual experiments with n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by one-way ANOVA (C) and unpaired two-tailed Student's t test (E and G). *P < 0.05 and **P < 0.01.

markedly reduced in *Slc7a8*-deficient mice compared with controls (Fig. 2, M and N). Collectively, these results support that SLC7A8 plays an important role in Th2 function in vivo.

T cell–intrinsic SLC7A8 is required for Th2 maintenance and function

ILC2s induce Th2 responses in an MHC-II–dependent manner (Oliphant et al., 2014). Since both our Cre drivers (*R5*^{Cre} and *Il7r*^{Cre}) are expressed in ILC2s and Th2 cells, and previous research has shown that *Slc7a8* deficiency impairs ILC2 maintenance and function (Hodge et al., 2023; Panda et al., 2022), SLC7A8 may support Th2 function either cell intrinsically or indirectly through ILC2s. To distinguish between these possibilities, we cultured naïve CD4$^+$ T cells from the spleens of *R5*^{Cre}*Slc7a8*$^{fl/fl}$ and *R5*^{Cre}*Slc7a8*$^{+/+}$ control mice under Th2-polarizing conditions (Fig. 3 A). The generation of IL-5$^+$ and GATA3$^+$ Th2 cells was impaired in SLC7A8-deficient T cells (Fig. 3, B–E). SLC7A8 deficiency also reduced Th2 cell proliferation (Fig. 3 F) and prevented IL-13 production upon stimulation with PMA/ionomycin (Fig. 3, G and H). These findings indicate that SLC7A8 is intrinsically required for Th2 responses. However, because *Slc7a8* deletion is driven by IL-5 expression, the gene is lost only after cells have already initiated Th2 differentiation. This raised the question of whether the defect reflects impaired differentiation or impaired effector function. To address this, we examined IL-5 expression at day 4 of culture. At this stage, IL-5$^+$ CD4$^+$ T cells were already detectable at comparable frequencies in WT and SLC7A8-deficient cultures (Fig. S3, D and E), indicating that Th2 differentiation occurs normally in the absence of SLC7A8. At later stages, however, SLC7A8-

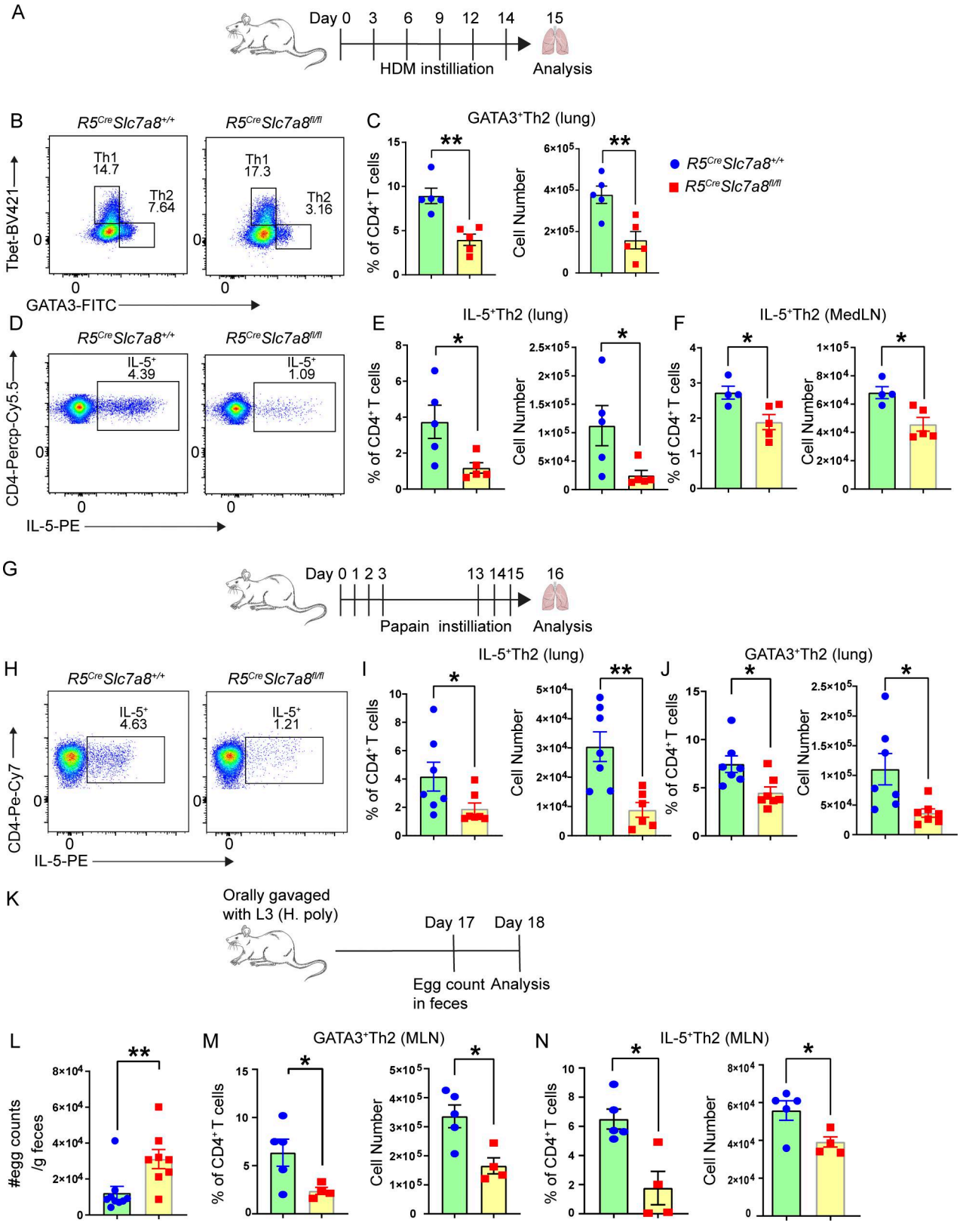

Figure 2. **Th2 response is impaired in *R5^{Cre}Slc7a8$^{fl/fl}$* mice. (A)** *R5^{Cre}Slc7a8$^{+/+}$* and *R5^{Cre}Slc7a8$^{fl/fl}$* mice were intranasally instilled with six doses of 50 µg HDM/mouse, and various subsets of Th cells were analyzed. **(B and C)** Representative FACS plot and quantification of GATA3$^+$ Th2 cells from the lungs of *R5^{Cre}Slc7a8$^{+/+}$* and *R5^{Cre}Slc7a8$^{fl/fl}$* mice. **(D–F)** Representative FACS plots and quantification of IL-5$^+$ Th2 cells from the lungs and mediastinal LNs (MedLN) of *R5^{Cre}Slc7a8$^{+/+}$* and *R5^{Cre}Slc7a8$^{fl/fl}$* mice. **(G)** *R5^{Cre}Slc7a8$^{+/+}$* and *R5^{Cre}Slc7a8$^{fl/fl}$* mice were intranasally instilled with seven doses of 40 µg papain/mouse on indicated days, and Th2 cells were analyzed. **(H–J)** Representative FACS plots and quantification of IL-5$^+$ Th2 cells and GATA3$^+$ CD4 T cells from the lungs of

$R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice. **(K)** $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice were orally gavaged with larvae of *H. polygyrus*. **(L–N)** Egg burden in the feces (L) and GATA3$^+$ (M) and IL-5$^+$ (N) Th2 cells in the MLN of *H. polygyrus*–infected $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice. Data are pooled or representative of two individual experiments n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test for all the panels and Mann–Whitney U test for panel I. *P < 0.05 and **P < 0.01.

deficient cells showed impaired proliferation and reduced cytokine production. Together, these findings suggest that SLC7A8 is dispensable for initial Th2 differentiation but is required for the maintenance and effector function of Th2 cells.

To further determine whether SLC7A8 is required in a cell-intrinsic manner for Th2 cells, independent of ILC2s, we performed both competitive bone marrow (BM) chimera and adoptive transfer experiments. In the BM chimera model, lethally irradiated C57BL/6 mice were reconstituted with a mixture of BM from $TCR\beta^{-/-}$ mice (providing an SLC7A8-sufficient ILC2 compartment) and BM from $R5^{Cre}Slc7a8^{fl/fl}$ mice (lacking SLC7A8 in both ILC2s and Th2 cells). As a control, irradiated mice were reconstituted with BM from $TCR\beta^{-/-}$ mice together with BM from $R5^{Cre}Slc7a8^{+/+}$ mice, which provide SLC7A8-

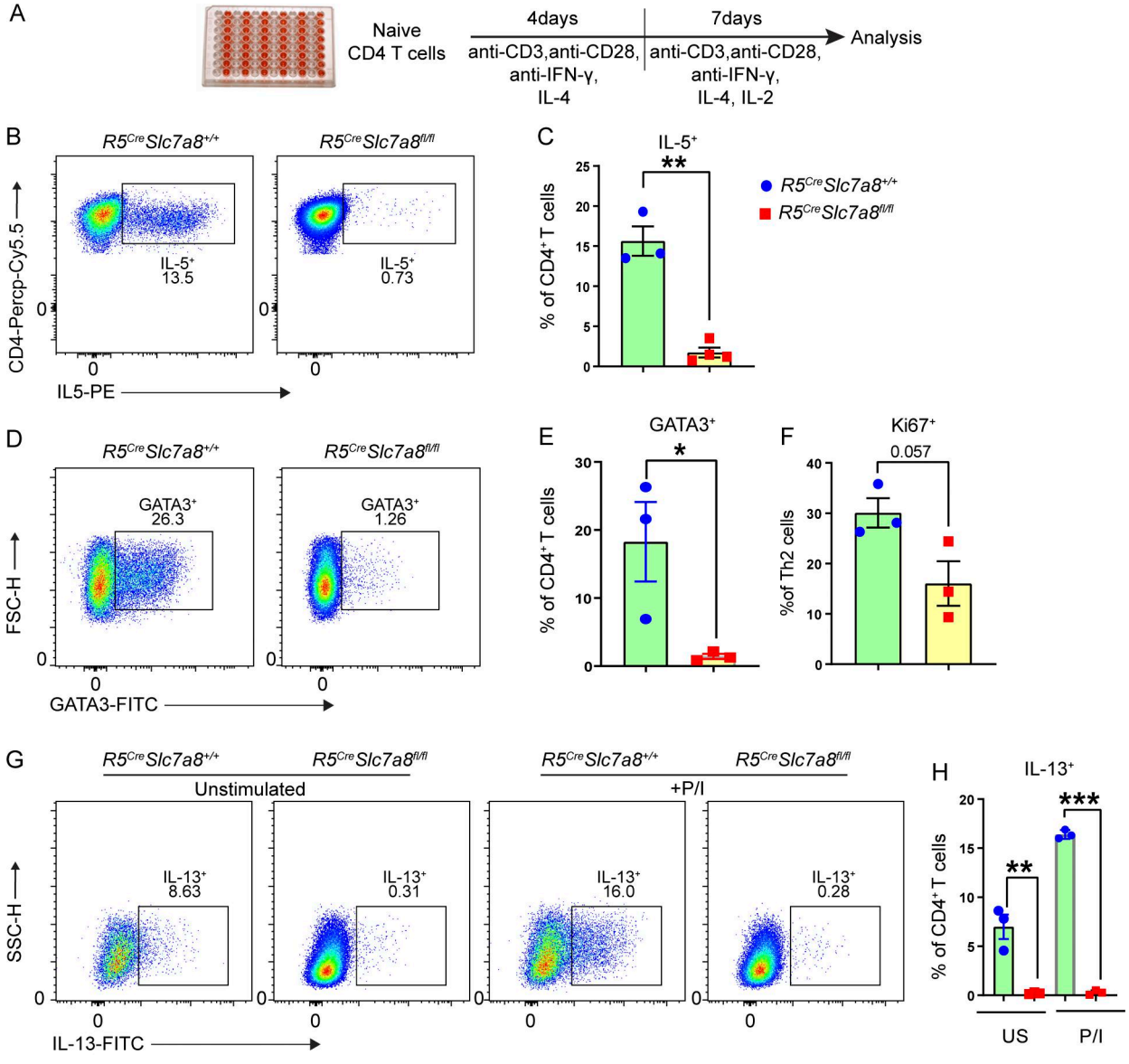

Figure 3. **SLC7A8 cell intrinsically regulate Th2 cell function. (A)** Naïve Th cells from spleens of $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice were cultured in Th2 conditions. **(B–E)** Representative FACS plot and quantification of IL-5$^+$ and Gata-3$^+$Th2 cells. **(F)** Frequency of Ki67$^+$ $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ Th2 cells. **(G and H)** IL-13 production by $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ Th2 cells upon stimulation with PMA/ionomycin. Data are pooled from two individual experiments with n = 1–2 per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test. *P < 0.05 and **P < 0.01, ***P < 0.001.

sufficient ILC2s and Th2 cells. 8 wk after reconstitution, mice were challenged intranasally with HDM, and lung Th2 cells were analyzed (Fig. 4 A). The frequencies of IL-5$^+$ and GATA3$^+$ Th2 cells were significantly reduced in recipients of *R5*^{Cre}*Slc7a8*$^{fl/fl}$ BM compared with controls (Fig. 4, B–D), indicating that SLC7A8 is intrinsically required for Th2 function.

To corroborate this result, we performed adoptive transfer experiments. Splenic CD4$^+$ T cells from *R5*^{Cre}*Slc7a8*$^{fl/fl}$ or *R5*^{Cre}*Slc7a8*$^{+/+}$ mice were transferred into *Rag2*$^{-/-}$ recipients, which lack endogenous T cells but retain an SLC7A8-sufficient ILC2 compartment (Fig. 4 E). 8 wk later, following intranasal HDM challenge, mice receiving *R5*^{Cre}*Slc7a8*$^{fl/fl}$ donor T cells showed reduced frequencies of IL-5$^+$ and GATA3$^+$ CD4$^+$ T cells compared with controls (Fig. 4, F–H), while ILC2 frequencies remained substantially unchanged (Fig. 4, I and J). Together, results from in vitro differentiation assays, BM chimera experiments, and adoptive transfer models consistently demonstrate that SLC7A8 is intrinsically required for the differentiation and function of Th2 cells.

SLC7A8 supports the metabolic fitness of Th2 cells

Large neutral amino acids activate the mTOR pathway, which integrates signals from nutrients, growth factors, and energy status to regulate anabolic and catabolic processes (Jewell and Guan, 2013; Simcox and Lamming, 2022). Moreover, mTORC1, a key complex in the mTOR pathway, is a primary regulator of mitochondrial biogenesis (Morita et al., 2013). Since SLC7A8 transports large neutral amino acids, we examined the impact of *Slc7a8* deficiency on mTOR signaling and mitochondria content of Th2 cells. Lymphocytes isolated from lungs of HDM-treated mice were stimulated with plate-bound anti-CD3 and anti-CD28 antibodies for 30 min, followed by measurement of phosphorylation of S6 (pS6), the characteristic downstream signaling molecule of mTOR. pS6 levels were lower in *Slc7a8*-deficient Th2 cells than in *R5*^{Cre}*Slc7a8*$^{+/+}$ Th2 cells (Fig. 5, A and B). Mitochondrial mass was reduced in Th2 cells isolated from lungs of *R5*^{Cre}*Slc7a8*$^{fl/fl}$ mice as well as in Th2 cells differentiated in vitro from *R5*^{Cre}*Slc7a8*$^{fl/fl}$ naive CD4 T cells in comparison to corresponding Th2 cells from *R5*^{Cre}*Slc7a8*$^{+/+}$ mice (Fig. 5, C–E). mTOR also augments protein levels of c-Myc in response to nutrients and growth factors, and in turn, c-Myc transcriptionally activates genes involved in mTOR signaling (Liu et al., 2017). Thus, we compared c-Myc expression in *R5*^{Cre}*Slc7a8*$^{fl/fl}$ and *R5*^{Cre}*Slc7a8*$^{+/+}$ Th2 cells isolated ex vivo or generated in vitro. c-Myc expression was clearly reduced in *Slc7a8*-deficient Th2 cells (Fig. 5, F–H). We conclude that by affecting mTOR, mitochondrial mass, and c-Myc, SLC7A8 deficiency impairs the metabolic processes that are necessary to meet the anabolic demands of expanding and cytokine-secreting Th2 cells.

A defect of *Slc7a8* induces cellular stress

To delve deeper into the impact of *Slc7a8* deficiency on the Th2 program, we performed RNA-seq analysis of IL-5$^+$ Th2 cells isolated from the lungs of HDM-treated *R5*^{Cre}*Slc7a8*$^{fl/fl}$ and *R5*^{Cre}*Slc7a8*$^{+/+}$ mice. We identified 40 genes that are differentially expressed (Fig. 5 I). Notably, *Slc7a8*-deficient Th2 cells exhibited increased expression of several genes associated with cellular response to metabolic and oxidative stress. These included the autophagy genes *Trim30D*, *Wdfy1*, *Trim34a*, and *Trim12a* (An et al., 2020; Teranishi et al., 2022), which may help Th2 cells cope with nutrient deprivation. Additional genes protecting from oxidative stress included *Ggt1*, which maintains glutathione homeostasis (Mitrić and Castellano, 2023), *Slc52a3*, a riboflavin transporter crucial for antioxidant defense (Boulet et al., 2018), and *Smox*, a regulator of oxidative damage (Murray Stewart et al., 2018). Other genes controlling cell survival in response to stress and damage included *Parp12* and *Xaf1* (Lee et al., 2014). *Slc7a8*-deficient Th2 cells also had elevated expression of IFN-stimulated genes, such as *Trim30D*, *Irgm1*, *Irf7*, *Oas1a*, and *Ifi209* (Schneider et al., 2014; Taylor et al., 2011). Genes downregulated in *Slc7a8*-deficient Th2 cells included genes controlling cell division and proliferation such as *Mis12*, *Cdin1*, and *Wdhd1* (Hsieh et al., 2011; Petrovic et al., 2016). Gene ontology (GO) analysis supported enhanced expression of pathways related to metabolic and oxidative stress responses and IFN signaling (Fig. 5 J). To confirm these observations, we scored C-11 BODIPY, a marker of oxidative stress in CD4 T cells from the lungs of HDM-instilled *R5*^{Cre}*Slc7a8*$^{fl/fl}$ and *R5*^{Cre}*Slc7a8*$^{+/+}$ mice. Interestingly, *Slc7a8*-deficient CD4 T cells exhibited increased ROS as compared with WT (*R5*^{Cre}*Slc7a8*$^{+/+}$) cells (Fig. 5, K and L). Taken together, these findings highlight that impaired transport of large neutral amino acids in *Slc7a8*-deficient Th2 cells impairs their proliferation and induces a marked cellular stress that affects their function.

The availability of intracellular amino acids is essential for fueling T cell metabolism and epigenetic reprogramming during activation (Bian et al., 2020; Wang and Zou, 2020). To support amino acid transport, activated T cells significantly upregulate amino acid transporters, whereas their expression remains minimal in a steady state. Previous studies have shown that different T cell subsets rely on distinct transporters. In CD4$^+$ Th cells, SLC7A5 facilitates the transport of large neutral amino acids in Th1 and Th17 cells (Sinclair et al., 2013). In this study, we demonstrated that in Th2 cells, this function is mediated by SLC7A8, an isoform of SLC7A5. *Slc7a8* deficiency impaired Th2 function in the context of intestinal helminth infection and HDM-induced lung allergy. Mechanistically, the defective amino acid transport in *Slc7a8*-deficient T cells impaired metabolic fitness and tempered c-Myc signaling. Conversely, *Slc7a8* deficiency had no impact on Th1 or/and Th17. These findings underscore that amino acid transporters are essential components of the functional modules driving Th1, Th2, and Th17 functions, alongside secreted cytokines. Consequently, their expression likely regulated by the master transcription factors of these subsets. In line with this, mice lacking GATA3—the master transcription factor of Th2—exhibit significantly lower *Slc7a8* expression, and the promoter of the *Slc7a8* gene contains GATA3-binding sites (Furuya et al., 2024).

Given our previous work and that of others demonstrating a critical role for SLC7A8 in ILC2 metabolism and effector function (Panda et al., 2022; Hodge et al., 2023), it is not surprising that this transporter also influences CD4$^+$ Th2 cells, which share overlapping metabolic demands. However, there are important contextual differences in how SLC7A8 may regulate these cell

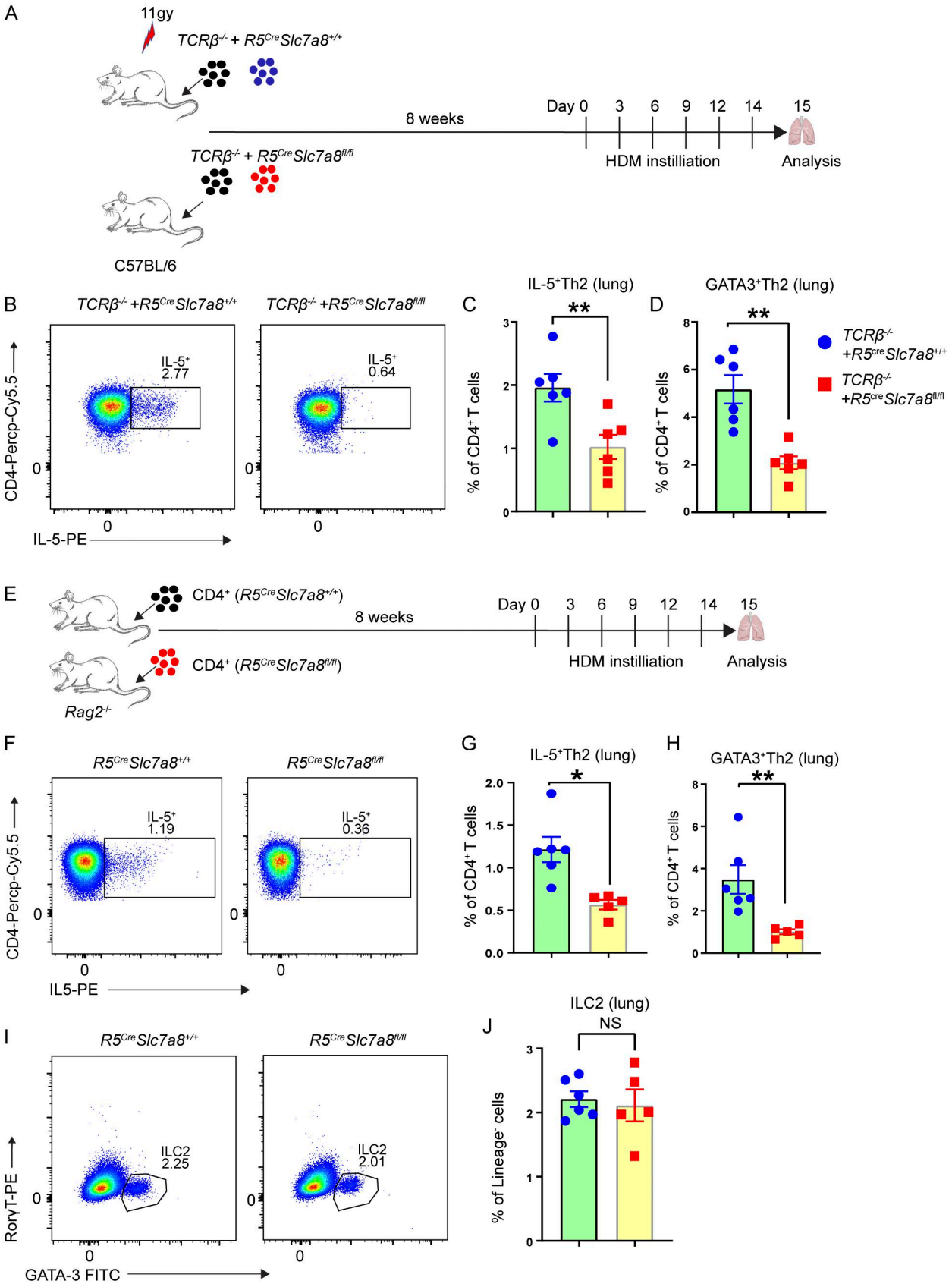

Figure 4. **SLC7A8 intrinsically regulates the effector functions of Th2 cells in vivo. (A)** Schematic of the competitive BM chimera experiment: lethally irradiated WT recipients with a 1:1 mixture of *Tcrb*$^{-/-}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ (or *R5*^{Cre}*Slc7a8*$^{+/+}$) BM. 8 wk after reconstitution, the chimeric mice were subjected to HDM-induced airway inflammation. **(B)** Representative FACS plots showing IL-5^{+}CD4^{+} T cells from the lungs of reconstituted mice. **(C and D)** Frequency of

IL-5$^+$CD4$^+$ T cells and GATA3$^+$CD4$^+$ T cells from the lungs of *Tcrb*$^{-/-}$+*R5*^{Cre}*Slc7a8*$^{+/+}$ and *Tcrb*$^{-/-}$+*R5*^{Cre}*Slc7a8*$^{fl/fl}$ constituted mice. **(E)** Experimental schematic: purified splenic CD4$^+$ T cells from *R5*^{Cre}*Slc7a8*$^{fl/fl}$ or *R5*^{Cre}*Slc7a8*$^{+/+}$ mice were transferred into *Rag2*$^{-/-}$ recipients. 8 wk later, mice were challenged intranasally with HDM, and Th2 responses were analyzed. **(F)** Representative FACS plots showing IL-5$^+$ CD4$^+$ T cells in the lungs of reconstituted mice. **(G and H)** Quantification of IL-5$^+$ CD4$^+$ T cells and GATA3$^+$ CD4$^+$ T cells in the lungs of mice receiving *R5*^{Cre}*Slc7a8*$^{fl/fl}$ versus *R5*^{Cre}*Slc7a8*$^{+/+}$ donor cells. **(I and J)** Frequency of ILC2s in *Rag2*$^{-/-}$ mice reconstituted with *R5*^{Cre}*Slc7a8*$^{+/+}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ CD4$^+$ T cells and subjected to HDM challenge. Data are pooled from two individual experiments with n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test. *P < 0.05 and **P < 0.01.

types. ILC2s are tissue resident that maintain rapid effector potential and rely heavily on amino acid transport to sustain basal metabolic activity and cytokine production in situ (Monticelli et al., 2016; Surace et al., 2021). In contrast, CD4$^+$ T cells are quiescent in the naïve state and undergo rapid proliferation and differentiation only upon antigen encounter, which likely imposes distinct temporal and quantitative requirements for SLC7A8-mediated amino acid uptake. Moreover, while SLC7A8 in ILC2s may primarily support steady-state metabolic fitness and readiness, in Th2 cells it may be more critical for sustaining proliferation, survival, and effector cytokine production during the expansion phase.

SLC7A8 transports various amino acids, including kynurenine, a metabolite of tryptophan produced by indoleamine 2,3-dioxygenase (IDO). Notably, IDO expression in antigen-presenting cells and eosinophils has been shown to enhance Th2 responses through an unknown mechanism (Xu et al., 2008). Our findings suggest that IDO-derived kynurenine may be transported into Th2 cells via SLC7A8, contributing to their function and maintenance.

Notably, bulk RNA-seq analysis revealed that *Slc7a8*-deficient Th2 cells exhibit gene expression signatures associated with oxidative stress and IFN responses. These signatures may indicate lack of functional plasticity of differentiated Th2 cells, leading to a maladaptive cellular response. Further studies are needed to determine whether similar maladaptive responses occur in humans and if they can be corrected through the administration of amino acid analogs.

Materials and methods

Mice

Slc7a8$^{fl/fl}$ mice were generated at the Washington University in St. Louis animal facility from ES cells (*Slc7a8*$^{tm2e(EUCOMM)Hmgu}$). *R5*^{Cre} and GGSSSS mice were obtained from Dr. Steven Vandyken's Laboratory at Washington University, St. Louis, MO, USA. Both *R5*^{Cre}*Slc7a8*$^{fl/fl}$ and *Il7r*^{Cre} *Slc7a8*$^{fl/fl}$ mice were generated in the animal facility of Washington University, St. Louis, MO, USA. The mouse strains were housed under specific pathogen–free conditions. All animal studies were conducted in strict accordance with the institutional (Washington University Animal Studies Committee) guidelines. Age match, sex match 8–10-wk-old mice were used for this study.

Helminth infection

R5^{Cre}*Slc7a8*$^{+/+}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ mice were orally gavaged with 200 infective larvae of *H. polygyrus* in 100 µl of PBS using a 20-gauge feeding tube. Fecal pellets were collected from individual mice on day 17, and eggs/gram were enumerated as previously described (Li et al., 2018). Briefly, two to three pellets were weighed and homogenized in 2 ml of distilled water, followed by the addition of 2 ml of saturated NaCl solution. After 5–10 min, eggs were counted under a light microscope using a McMaster 2-chamber. The egg burden was calculated as described previously. MLNs were harvested on day 18 and analyzed for Th2 cells.

HDM- or papain-induced allergy model

Anesthetized *R5*^{Cre}*Slc7a8*$^{+/+}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ mice, or *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre} *Slc7a8*$^{fl/fl}$ mice, were intranasally instilled with 50 µg of HDM in 50 µl of PBS for 14 days, having 3-day interval. On day 15, the lungs and mediastinal LNs were harvested and analyzed for different immune parameters. In the papain-induced allergy model, mice were intranasally primed with 40 µg papain on days 0–3, followed by restimulation on days 13–15. Lung tissues were harvested and analyzed on day 16.

Leukocyte isolation from different tissues

To isolate lung leukocytes, lungs were excised, cleared of surrounding fat and connective tissue, and finely chopped. Tissue digestion was performed using collagenase D and DNase I with the gentleMACS tissue dissociator. The suspension was filtered through a 100-µm mesh to remove undigested fragments. Red blood cells were lysed by using ACK lysing buffer, washed, and subjected to further analysis. MLNs and mediastinal LNs were ablated, and fats were removed and mechanically dissociated by a syringe plunger on a 100-µm mesh. The cell suspension was washed with PBS and analyzed.

FACS analysis

For surface staining, cells isolated from different tissues were stained with different fluorescently labeled antibodies in the presence of Fc block, followed by live/dead staining. Intracellular staining was performed according to the manufacturer's instructions. Briefly, for transcription factor staining, cells were fixed and permeabilized using FOXP3 staining kit, followed by staining with fluorescent conjugated antibodies.

Intracellular cytokine staining was performed as previously described (Panda et al., 2022). Briefly, cells isolated from different tissues were stimulated with PMA/ionomycin for 4 h with brefeldin for last 3 h. Afterward, cells were stained for surface markers, followed by fixation, permeabilization, and cytokine staining using BD fixation and permeabilization kit. For pS6 staining, cells were fixed and permeabilized with 70% methanol for intracellular staining.

In vitro T cell differentiation

Naïve splenic CD4$^+$ T cells were isolated by magnetic-activated cell sorting kit according to manufacturer's instructions. Sorted

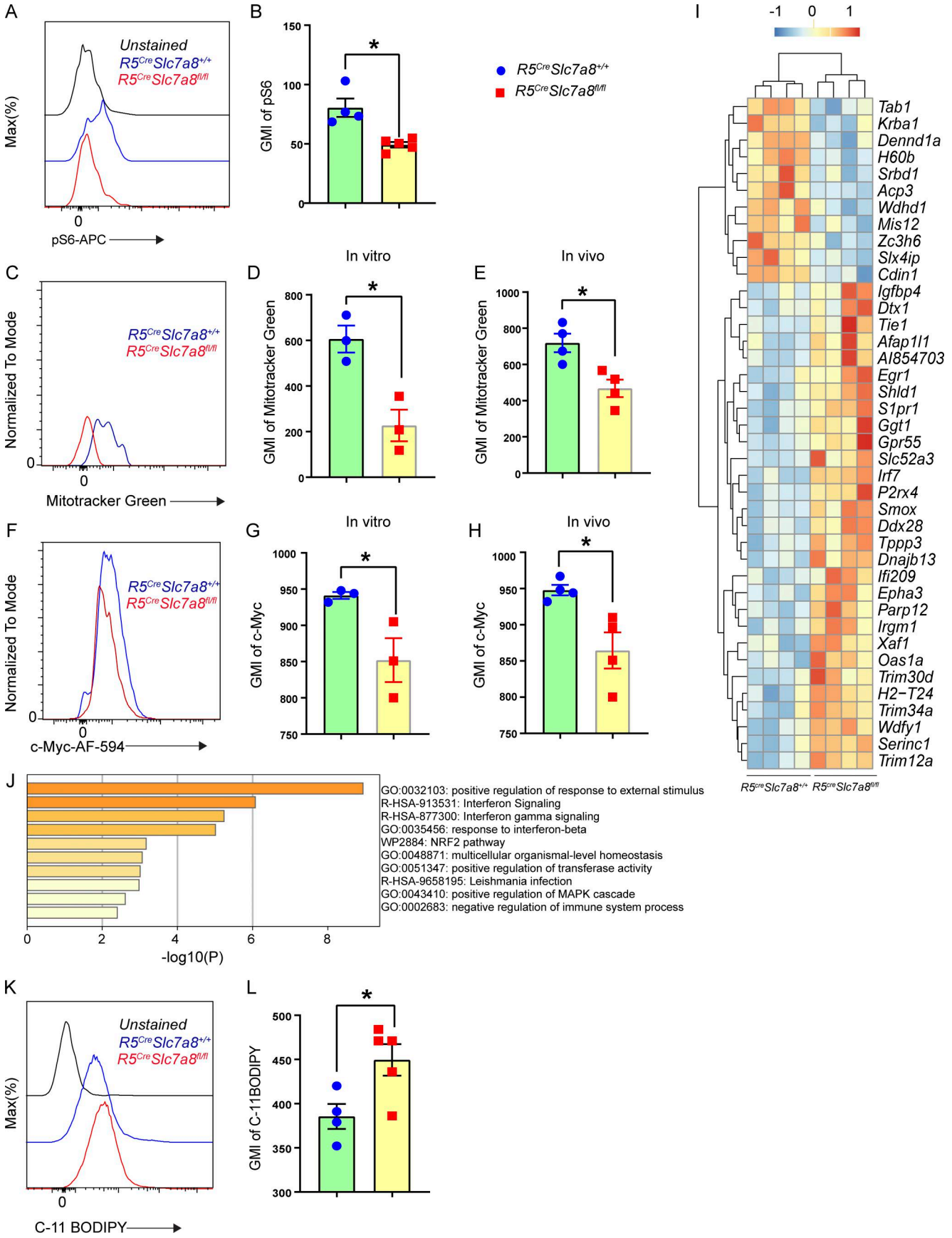

Figure 5. **SLC7A8 regulates the metabolic fitness of Th2 cells. (A and B)** Representative histogram and GMI (Geometric Mean Intensity) quantification of pS6 expression in HDM-instilled lung Th2 cells from $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice. **(C–H)** Representative histogram and GMI of MitoTracker Green and c-Myc expression in Th2 cells generated in vitro (C, D, F, and G) and in vivo (E and H) from $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice. **(I and J)** IL-5$^+$ Th2 cells were sort purified from the lungs of $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice following administration of HDM and subjected to RNA-seq analysis. Heatmap showing the differentially expressed genes (I) and GO analysis of pathways associated with differentially expressed genes (J). **(K and L)** Overlaid histograms and quantification showing C-11 BODIPY staining in HDM instilled lung CD4 T cells of from $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice. Data are pooled or representative of two individual experiments n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test. *P < 0.05.

cells were cultured in 96-well flat-bottom plates coated with anti-CD3 and anti-CD28 antibodies in RPMI complete media in the presence of anti-IFN-γ and IL-4. After 4 days, the media was replenished with fresh RPMI media containing anti-IFN-γ, IL-4, and IL-2. After 7 days, the cells were analyzed for different type 2 markers such as GATA-3, IL-5, and IL-13.

ROS analysis

Surface staining for different markers, such as CD3 and CD4, was performed to identify Th2 cells from both $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice, followed by live/dead staining. Then the cells were incubated with C-11 BODIPY for 30 min at 37°C, followed by washing two times with PBS and analyzed by flow cytometry.

Adoptive transfer of splenic T cells

Splenic CD4$^+$ T cells were isolated from $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice using the Pan CD4$^+$ T Cell Isolation Kit (Miltenyi Biotec) according to the manufacturer's instructions. 2 × 10^6 cells were intravenously injected into $Rag2^{-/-}$-recipient mice. 8 wk after transfer, the mice were subjected to HDM-induced airway inflammation, and Th2 cell responses were evaluated.

BM chimera

C57BL/6 mice were lethally irradiated with two doses of 550 rad, followed by intravenous injection of 2 × 10^6 BM cells from $TCR\beta^{-/-}$ mice mixed 1:1 with BM cells from either $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ donors. The mice were challenged intranasally with HDM after 8 wk of reconstitution.

Quantitative RT-PCR

Th1, Th2, and Th17 cells were FACS sorted from the lungs of intranasally HDM-instilled GGSSSS mice. RNA was isolated using Qiagen RNA easy micro RNA Kit, followed by cDNA synthesis using Quanta bio cDNA synthesis Kit. Quantitative PCR (qPCR) was performed using iTaq Universal SYBR Green Supermix and appropriate primer sets. Gene expression levels were analyzed by Ct values by normalizing with GAPDH.

RNA-seq analysis

Th2 (CD3$^+$, CD4$^+$, and IL-5$^+$) cells were sort purified from lungs of intranasally HDM-instilled $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice; total RNA was isolated and subjected to RNA-seq analysis as previously described. Briefly, RNA-seq reads were aligned to the Ensembl release 76 primary assembly with Spliced Transcripts Alignment to a Reference version 2.5.1a, and then aligned gene counts were processed using DESeq2 package with R. DEGs were defined as protein-coding genes with an average expression >100 counts and false discovery rate <0.05. Data are deposited in the Sequence Read Archive (SRA) database (PRJNA1337183). GO analysis was performed using Metascape with default settings.

Publicly available datasets from the Gene Expression Omnibus were analyzed using Phantasus (https://artyomovlab.wustl.edu/phantasus; Kleverov et al., 2024).

Statistical analysis

Two experimental groups were compared by Student's t test or Mann–Whitney U test. Multiple groups were compared by one-way ANOVA followed by Dunnett's test for multiple comparison. Statistical analysis was performed using GraphPad prism software (version 8). P < 0.05 was considered statistically significant. *P < 0.05, **P < 0.01, and ***P < 0.001.

Online supplemental material

We are including three supplemental figures that further support and extend the findings presented in the manuscript. Fig. S1 shows *Slc7a8* expression upon TCR signaling and examines the effect of *Slc7a8* deficiency on T cell development and peripheral homeostasis. Fig. S2 outlines the gating strategy for different Th cell subsets and demonstrates the impact of *Slc7a8* deficiency on Th1, Th17, and Treg differentiation in the lungs of HDM-induced allergy. Fig. S3 presents the frequencies of Th2 and Tfh cells in the lungs and mediastinal LNs of HDM-treated $R5^{Cre}Slc7a8^{+/+}$ and $R5^{Cre}Slc7a8^{fl/fl}$ mice. It also includes the in vitro differentiation of Th2 cells after 4 days of culture.

Data availability

Sequencing data of RNA-seq generated in this study are deposited in the SRA database (PRJNA1337183).

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Supplemental material

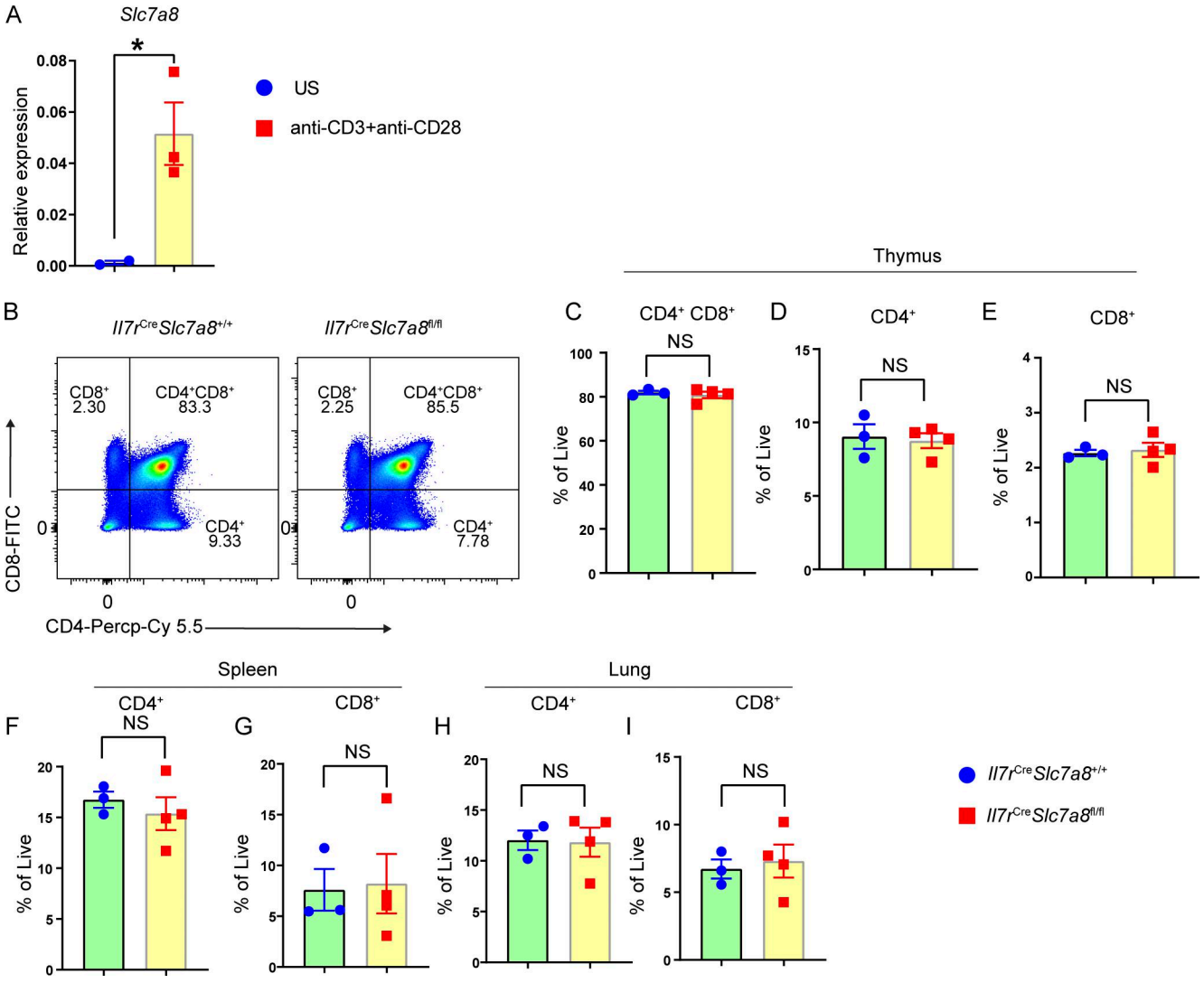

Figure S1. ***Slc7a8* is dispensable for T cell development and peripheral homeostasis. (A)** Expression of *Slc7a8* in splenic T cells upon anti-CD3 and anti-CD28 stimulation in vitro. Frequencies of both CD4⁺ and CD8⁺ T cells were analyzed in the thymus, spleen, and lungs of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(B)** Representative FACS plot showing the staining of CD4 and CD8 in thymus of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r* ^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(C–E)** Percentage of double-positive (CD4⁺ CD8⁺) (C), CD4⁺ (D), and CD8⁺ (E) T cells in the thymus of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(F–I)** Frequency of CD4⁺ (F and H) and CD8⁺ (G and I) T cells of spleen and lungs of *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. Data are pooled or representative of two individual experiments with n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test. NS, not significant. *P < 0.05.

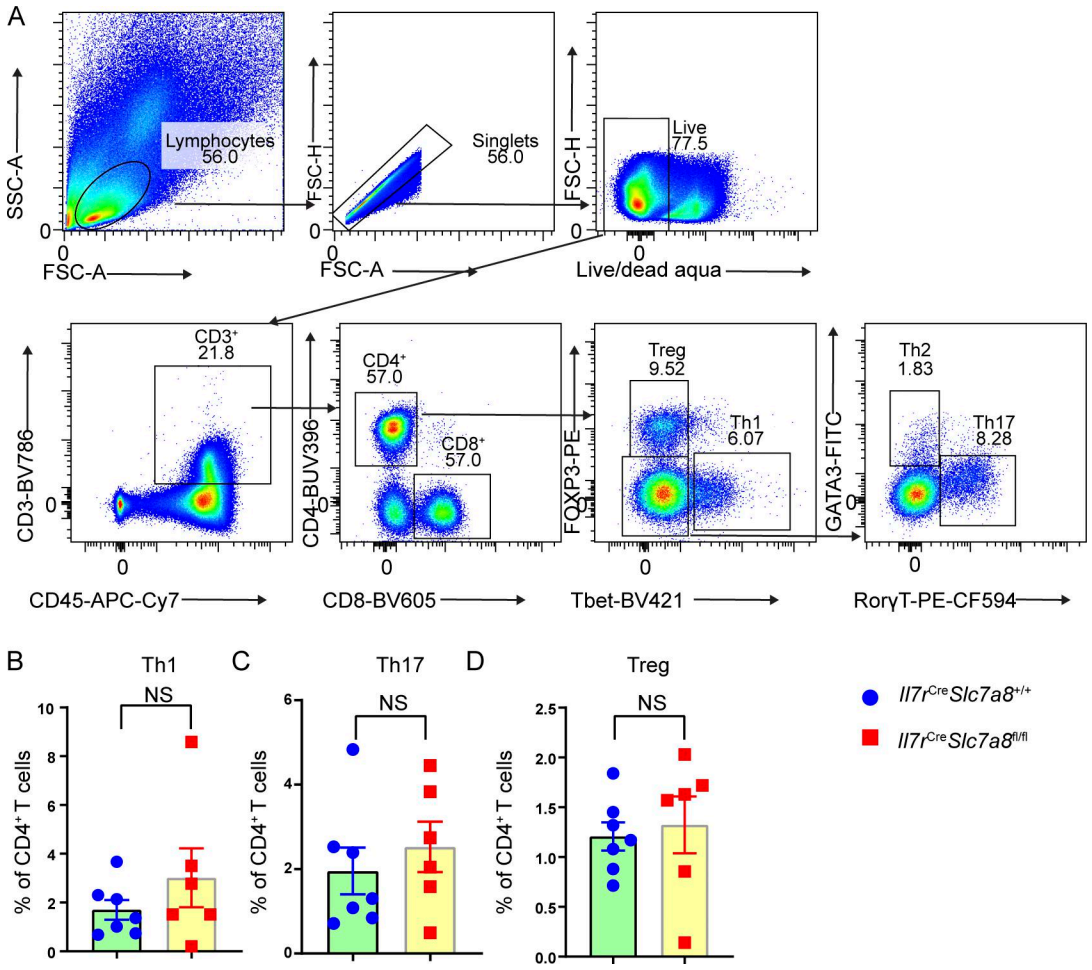

Figure S2. ***Slc7a8* deficiency does not affect Th1, Treg, and Th17 cells in the lung upon HDM-induced allergy. (A)** Gating strategy for different Th cells such as Th1, Th2, Th17, and Tregs. **(B–D)** Frequency of Th1 (B), Th17 (C), and Treg (D) in the lungs of HDM-treated *Il7r*^{Cre}*Slc7a8*$^{+/+}$ and *Il7r*^{Cre}*Slc7a8*$^{fl/fl}$ mice. Data are pooled or representative of two individual experiments with n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test. NS, not significant.

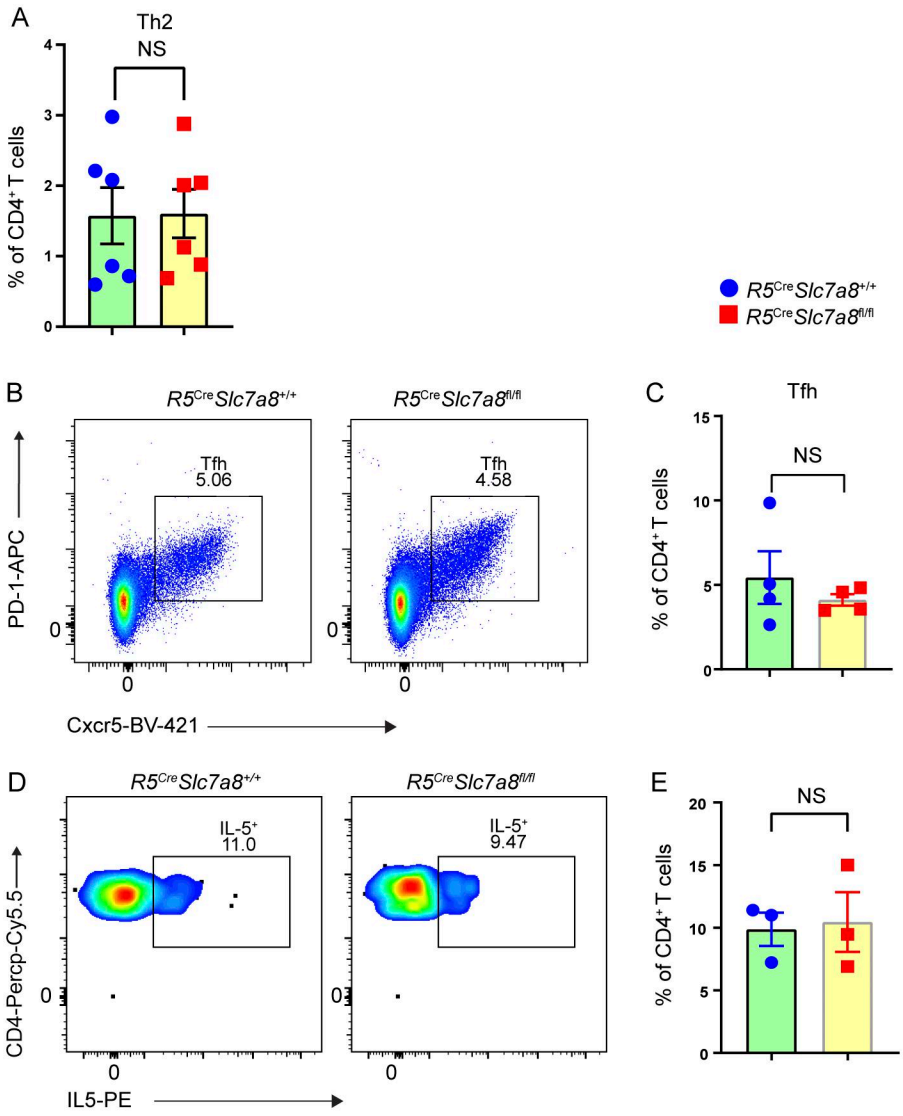

Figure S3. **Tfh cells are comparable between *R5*^{Cre}*Slc7a8*$^{+/+}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ mice in the lungs upon HDM-induced allergy. (A)** Frequency of IL-5$^+$ Th2 cells in the lungs of PBS-instilled *R5*^{Cre}*Slc7a8*$^{+/+}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(B and C)** Representative FACS plot showing the staining of Tfh (B) and (C) frequency of Tfh in the mediastinal LNs of *R5*^{Cre}*Slc7a8*$^{+/+}$ and *R5*^{Cre}*Slc7a8*$^{fl/fl}$ mice. **(D and E)** Representative FACS plot and quantification of IL-5$^+$ CD4 T cells after 4 days of culture in Th2 condition. Data are pooled or representative of two individual experiments with n = 3–5 mice per group. Each dot represents an individual mouse. Data are plotted as means ± SEM. Statistics were calculated by unpaired two-tailed Student's t test. NS, not significant.