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SLC7A8 is a key amino acids supplier for the metabolic programs that sustain homeostasis and activation of type 2 innate lymphoid cells

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Group 2 innate lymphoid cells (ILC2) are innate counterparts of T helper 2 (Th2) cells that maintain tissue homeostasis and respond to injuries through rapid interleukin (IL)-5 and IL-13 secretion. ILC2s depend on availability of arginine and branched-chain amino acids for sustaining cellular fitness, proliferation, and cytokine secretion in both steady state and upon activation. However, the contribution of amino acid transporters to ILC2 functions is not known. Here, we found that ILC2s selectively express *Slc7a8*, encoding a transporter for arginine and large amino acids. *Slc7a8* was expressed in ILC2s in a tissue-specific manner in steady state and was further increased upon activation. Genetic ablation of *Slc7a8* in lymphocytes reduced the frequency of ILC2s, suppressed IL-5 and IL-13 production upon stimulation, and impaired type 2 immune responses to helminth infection. Consistent with this, *Slc7a8*-deficient ILC2s also failed to induce cytokine production and recruit eosinophils in a model of allergic lung inflammation. Mechanistically, reduced amino acid availability due to *Slc7a8* deficiency led to compromised mitochondrial oxidative phosphorylation, as well as impaired activation of mammalian target of rapamycin and c-Myc signaling pathways. These findings identify *Slc7a8* as a key supplier of amino acids for the metabolic programs underpinning fitness and activation of ILC2s.

innate lymphoid cells | amino acid | transporter | allergy | asthma

Significance

Metabolic adaptations enable immune responses. While much is known about how metabolism underlies T cell function, a metabolic framework for innate lymphoid cells (ILCs) is just developing. Group 2 ILCs (ILC2s), the innate counterparts of Th2, require arginine and branched-chain amino acids for proliferation and activation. How these amino acids reach the intracellular ILC2 compartment is unknown. Here, we show that SLC7A8 is the key amino acid transporter in ILC2s. *Slc7a8* deletion in lymphocytes reduced ILC2s and attenuated responses to helminth infection and house dust mite allergens. *Slc7a8* deficiency compromised mitochondrial OXPHOS and mammalian target of rapamycin and c-Myc pathways in ILC2s. Thus, SLC7A8 is a promising target for therapies aiming at attenuating immune inflammation in allergies and asthma.

Innate lymphoid cells (ILCs) are tissue sentinel lymphocytes that lack antigen receptors, which respond to pathogens and injuries by prompt secretion of cytokines (1). Classification of ILC subsets parallels the current paradigm for T helper (Th) cell differentiation with group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s) mirroring Th1, Th2, and Th17, respectively. ILC2s are marked by high GATA-3 expression, and secrete interleukin (IL)-5, IL-9, IL-13, and amphiregulin (Areg) in response to alarmins, such as IL-25 and IL-33, as well as neuropeptides released by neurons, such as Neuromedin U (NMU) (2). ILC2s can be further subcategorized into natural ILC2s (nILC2s) and inflammatory ILC2s (iILC2s) based on surface markers and cytokine production (3). nILC2s express low-to-intermediate levels of KLRG1, and are responsive to IL-33 via high expression of the IL-33 receptor ST2, whereas iILC2s are KLRG1^{high}ST2^{-} and are mainly responsive to IL-25 via expression of the IL-25 receptor, IL17Rb. ILC2s express divergent transcriptional profiles in peripheral tissues, such as small intestine, large intestine, lung, adipose tissue, and skin (4), where they contribute to immunity and metabolic homeostasis. ILC2s respond to helminth infection by secreting IL-5, IL-13, and Areg, facilitating worm expulsion and tissue repair (5). ILC2s also contribute to tissue restoration after lung epithelial damage due to influenza virus infection (6). Conversely, ILC2s aggravate airway hyperresponsiveness in mouse models of allergic lung disease (7, 8). ILC2s have been implicated in the control of lipid metabolism in the adipose tissue, by producing IL-13 to polarize macrophages toward an M2-like phenotype, and by promoting beiging of adipocytes to attenuate high fat diet-induced obesity and metabolic dysregulation (9, 10). In addition to mediating innate type 2 responses, ILC2s shape T cell immunity by presenting antigens through MHCII and expressing costimulatory molecules (11, 12). Recent investigations have also shown that ILC2s promote antitumor immune responses in models of melanoma, lung metastasis, and pancreatic cancer (13, 14), while facilitating tumor growth in other models of colorectal cancer (15) and lung metastasis (16).

Immune cell function is dependent on a capacity to adapt metabolic programs to the demands imposed by activation, expansion, and differentiation into various effector or memory states (17–19). While metabolic adaptations underpinning T cell responses have been extensively investigated, there is a growing appreciation that metabolic

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changes also underlie ILC functions. In response to helminth infection, ILC2s utilize fatty acid metabolism and mitochondrial oxidative phosphorylation (OXPHOS) for fueling their effector function (20). In contexts of hyperactivation, such as deficiency of the inhibitory checkpoint PD1, ILC2s switch their metabolism toward glycolysis (21). ILC2 proliferation and cytokine production also depend on arginine metabolism by arginase 1, which converts arginine into ornithine to promote wound healing and fibrosis by enhancing production of polyamines, L-proline, and type 2 cytokines (20, 22). Consistent with this, a recent report found that human ILC2s require arginine and branched-chain amino acids for mitochondrial OXPHOS, as demonstrated by reduced frequency of peripheral ILC2s in individuals with defective mitochondrial OXPHOS (23). ILC2 functions also depend on tryptophan metabolism. Tryptophan hydroxylase 1 (TPH1), the rate-limiting enzyme in the synthesis of serotonin, is required for ILC2 responses to helminth infection, particularly their ability to differentiate into inflammatory ILC2s (iILC2) that secrete IL-17 (24). Conversely, indole catabolites of tryptophan that activate the aryl hydrocarbon receptor (AHR) attenuate ILC2 activation (25). The importance of amino acids in ILC2 function is further supported by reports demonstrating that ILC2 activation depends on the mammalian target of rapamycin (mTOR) pathway (26, 27), which couples amino acid availability with initiation of translation and cell growth through the activation of S6 kinase (S6) and c-Myc (28–30).

Given the importance of amino acids in sustaining ILC2 functions, we hypothesized that amino acid transport contributes to ILC2 activation and maintenance. Here we found that ILC2s selectively express the solute carrier *(Slc)7a8* in a tissue-specific manner. Together with SLC3A2, SLC7A8 forms a heterodimeric amino acid transporter known as large amino acid transporter (LAT) 2, which transports various amino acids, including arginine and branched-chain amino acids across the plasma membrane (31). *Slc7a8* was highly expressed on small intestine lamina propria (siLP), lung and adipose tissue ILC2s in the steady-state, and its conditional deletion in lymphocytes reduced ILC2 numbers in these tissues. *Slc7a8* was further up-regulated upon IL-25 and IL-33 stimulation in response to increased amino acid demand; however, *Slc7a8* deficiency impaired production of IL-5 and IL-13 by ILC2s, resulting in attenuated type 2 immune responses to intestinal helminth infection and house dust mite (HDM) allergens. Mechanistically, *Slc7a8* deficiency impaired ILC2 functions via mitochondrial OXPHOS, mTOR signaling, and c-Myc expression. We conclude that SLC7A8 is a key supplier of amino acids for the metabolic programs that sustain fitness and activation of ILC2s.

Results

ILC2s Express *Slc7a8* in a Tissue-Specific Manner. We searched for amino acid transporters differentially expressed in ILCs using RNA-sequencing (RNA-seq) data from the Immgen database. We noted that *Slc3a2*, which encodes the common chain of the amino acid transporters LAT1 and LAT2, was highly expressed in siLP ILC2s compared to ILC3s. Among the binding partners of *Slc3a2*, *Slc7a8* (encoding LAT2) was also highly expressed in siLP ILC2s, whereas *Slc7a5* (encoding LAT1) was hardly detectable (Fig. 1 *A* and *B*). We further examined the expression of LAT1 and LAT2 transporters in ILC2s across different tissues in single-cell RNA-seq data of ILC2s in different organs (Fig. 1*C*) (4). *Slc7a8* was preferentially expressed in ILC2s from siLP, lung, fat, and bone marrow, whereas skin ILC2s showed low *Slc7a8* expression and high *Slc7a5* expression (Fig. 1*D* and *SI Appendix*, Fig. S1*A*), suggesting that most tissue resident ILC2s utilize LAT2, with the exception of skin ILC2s, which express mainly LAT1. Analysis of publicly available single-cell RNA-seq data (32) showed that *Slc7a8* expression was further up-regulated upon IL-25 and IL-33 stimulation (Fig. 1*E*). NMU, which costimulates ILC2s through NMU receptor 1 (32, 33), did not induce the expression of either *Slc7a5* or *Slc7a8* alone, but synergized with IL-25 to up-regulate *Slc7a5*. We confirmed these observations by assessing expression of *Slc7a8*, *Slc3a2*, and *Slc7a5* in ILC2s sort-purified from siLP of C57BL/6 mice. *Slc7a8* mRNA expression markedly increased after stimulation with either IL-25 or IL-33 in vitro; *Slc7a5* and *Slc3a2* expression also spiked after treatment with IL-25, but not IL-33 (Fig. 1 *F–H*). Thus, *Slc7a8* might play a role in ILC2 functions in both homeostasis and activation in most tissues, with the exception of skin in which *Slc7a5* may be more relevant.

ILC2 Homeostasis and Cytokine Function Is Impaired in *Il7rα^{cre} Slc7a8$^{fl/fl}$* Mice. To investigate the role of SLC7A8 in ILC2s, we generated mice conditionally deficient of *Slc7a8* in lymphocytes using *Il7rα^{cre}* (*Il7rα^{cre}Slc7a8$^{fl/fl}$*). Since *Il7rα^{cre}* causes haploinsufficiency for *Il7r* that in itself impacts ILC2 numbers (*SI Appendix*, Fig. S1*B*), we always examined *Il7rα^{cre}Slc7a8$^{+/+}$* as controls. Both frequencies and numbers of ILC2s in the siLP, fat, and lung were reduced in *Il7rα^{cre}Slc7a8$^{fl/fl}$* mice compared to *Il7rα^{cre}Slc7a8$^{+/+}$* mice in steady state (Fig. 2 *A–G*), whereas there was no difference in ILC1, cNK, and different subsets of ILC3 numbers (*SI Appendix*, Fig. S1 *C–I*). Interestingly, numbers of skin ILC2s, which lack *Slc7a8* expression, were comparable between *Slc7a8*-deficient and control mice (*SI Appendix*, Fig. S1*J*). *Slc7a8*-deletion did not affect the expression of characteristic ILC2 markers (*SI Appendix*, Fig. S2 *A–D*). In addition, the frequency of splenic T cells and B cells was comparable between *Il7rα^{cre}Slc7a8$^{fl/fl}$* and *Il7rα^{cre}Slc7a8$^{+/+}$* mice (*SI Appendix*, Fig. S2 *E–I*). We then investigated the impact of SLC7A8 on ILC2 activation. IL-5 and IL-13 production were analyzed by intracellular staining of ILC2s isolated from siLP after in vitro stimulation with PMA/Ionomycin for 4 h. Frequencies of IL-5$^+$ and IL-13$^+$ ILC2s were reduced among *Il7rα^{cre}Slc7a8$^{fl/fl}$* ILC2s after stimulation, compared with *Il7rα^{cre}Slc7a8$^{+/+}$* control ILC2s (Fig. 2 *H–J*). We further examined whether SLC7A8 plays a differential role in nILC2 vs. iILC2 differentiation and activation. To test this, we intraperitoneally administered IL-25 to *Il7rα^{cre}Slc7a8$^{fl/fl}$* and *Il7rα^{cre}Slc7a8$^{+/+}$* mice for 3 d, then analyzed lung iILC2s and nILC2s by flow cytometry (*SI Appendix*, Fig. S1*K*). iILC2 and nILC2 numbers were comparable between *Il7rα^{cre}Slc7a8$^{+/+}$* and *Il7rα^{cre}Slc7a8$^{fl/fl}$* mice (Fig. 2 *K* and *L*), perhaps due to a compensatory increase of *Slc7a5* induced by IL-25 (Fig. 1*G*). Notwithstanding, IL-5 and IL-13 production by nILC2s, and IL-17 production by iILC2s were reduced in *Il7rα^{cre}Slc7a8$^{fl/fl}$* compared to *Il7rα^{cre}Slc7a8$^{+/+}$* mice (Fig. 2 *M–P*). Since basal ILC2 secretion of IL-5 is essential to maintain peripheral eosinophils (33), we compared the numbers of eosinophils in siLP and spleen of *Il7rα^{cre}Slc7a8$^{+/+}$* and *Il7rα^{cre}Slc7a8$^{fl/fl}$* mice. The numbers of eosinophils in both siLP and spleen were lower in *Il7rα^{cre}Slc7a8$^{fl/fl}$* mice than control mice (Fig. 2 *Q–S*), corroborating reduced systemic production of IL-5 by ILC2s. Altogether, these data suggested that SLC7A8 is required for ILC2 maintenance and activation.

ILC2 Responses to Helminth Infection and Allergic Lung Challenge Are Blunted in *Il7rα^{cre}Slc7a8$^{fl/fl}$* Mice. Since ILC2s are highly activated to produce type 2 cytokines during intestinal helminth

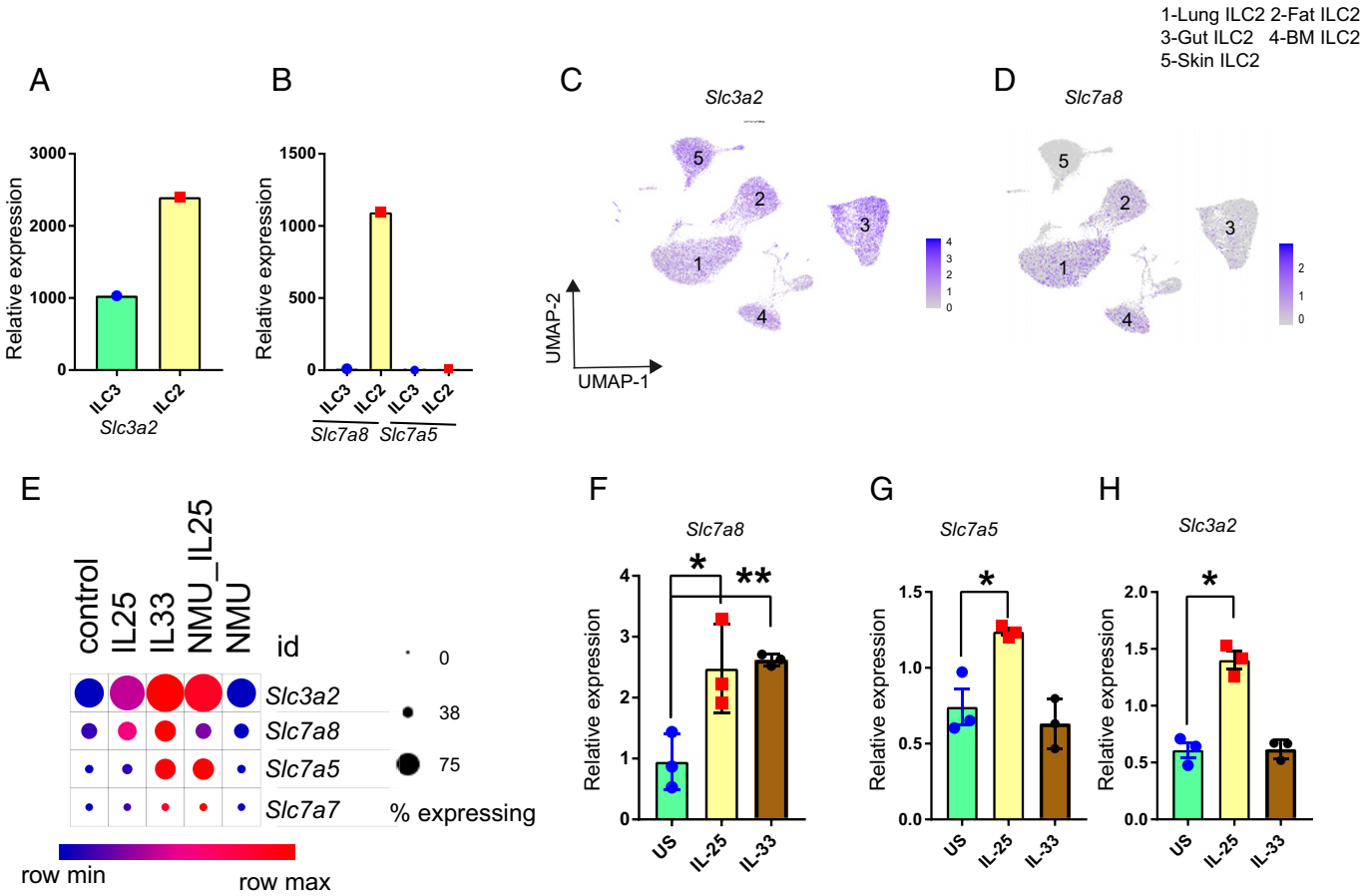

Fig. 1. ILC2s specifically express *Slc7a8*. (*A*) Expression of *Slc3a2* and its binding partners (*B*) *Slc7a5* and *Slc7a8* in siLP ILC2s (Immgen.org). (*C*) UMAP plots depicting the expression of *Slc3a2* and (*D*) *Slc7a8* in ILC2s from different organs (single-cell RNA-seq data obtained from the Gene Expression Omnibus under accession no. GSE117568). (*E*) Increased expression of *Slc7a8* in lung ILC2s upon IL-25 and IL-33 stimulation [data obtained from https://singlecell.broadinstitute.org/single_cell/study/SCP76/lung-ilcsunder-alarmin-and-neuropepide-activation (32)]. (*F–H*) Expression of *Slc7a8, Slc7a5*, and *Slc3a2* in ILC2s sort-purified from siLP upon stimulation with IL-25 and IL-33 in vitro. Data shown as mean ± SEM. Each dot represents an individual mouse. Statistical significance was determined by Students *t* test. $*P < 0.05$, $**P < 0.01$.

infection and in response to allergen challenge in the lungs, we investigated the impact of *Slc7a8* deletion in these settings. *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice were infected with L3 larvae of *Heligmosomoides polygyrus (H. polygyrus)* and parasite load was analyzed (Fig. 3*A*). Although *H. polygyrus* infection lasts 3 to 4 mo in C57BL/6 mice, we examined mice at early time points when infection is mainly impacted by innate ILC2 responses rather than adaptive Th2 responses. *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice had higher egg burden as compared to *Il7rα*^{cre}*Slc7a8*$^{+/+}$ mice 12 d postinfection (Fig. 3*B*). Consistent with an inability to mount robust ILC2 activation, the frequencies of IL-5$^+$ and IL-13$^+$ ILC2s were lower in small intestinal tissues from *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice compared to *Il7rα*^{cre}*Slc7a8*$^{+/+}$ control mice (Fig. 3 *C–E*). The frequency of Th2 in siLP and mesenteric lymph nodes was comparably low in *Slc7a8*-deficient and control mice at the early time point examined (*SI Appendix*, Fig. S3 *A* and *B*). We conclude that *Slc7a8*-deficiency impairs ILC2 responses to helminth infection.

Because lung ILC2s also produce type 2 cytokines in response to allergens (8), we tested whether deficiency of *Slc7a8* affected ILC2 activation and inflammation in response to allergic lung challenge. We intranasally instilled four doses of HDM extract to *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ and *Il7rα*^{cre}*Slc7a8*$^{+/+}$ control mice and analyzed ILC2s and airway inflammation 24 h after the final dose (Fig. 3*F*). We chose repeated challenges with HDM within a short time rather than presensitization followed by challenge to facilitate innate over Th2 responses. Frequencies and absolute numbers of lung ILC2s in the *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice were significantly lower than *Il7rα*^{cre}*Slc7a8*$^{+/+}$ control mice after HDM challenge (Fig. 3 *G–I*). Moreover, the percentage of IL-5$^+$ and IL-13$^+$ ILC2s were lower in the lungs of *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice as compared to *Il7rα*^{cre}*Slc7a8*$^{+/+}$ control mice (Fig. 3 *J–L*). HDM challenge also induced a significantly higher influx of eosinophils into the lungs of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ control mice as compared to *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice (Fig. 3 *M–O*), consistent with the ILC2 impairment. Only a minor population of CD4 T cells expressed IL-5 and IL-13 (*SI Appendix*, Fig. S3*C*), suggesting that ILC2 are the major contributors to IL-5 and eosinophilia in these settings. Taken together, these data demonstrate that SLC7A8 is a critical regulator of ILC2 functions in response to infectious and allergenic type 2 immune stimuli.

***Slc7a8*-Deficient ILC2s Have Lower Mitochondrial Mass and Impaired mTOR Signaling.** ILC2 metabolism relies on mitochondrial OXPHOS (20). Given the role of branched amino acids in supporting OXPHOS in ILC2s (23), we hypothesized that *Slc7a8*-deficiency may affect ILC2 OXPHOS metabolism. Assessment of mitochondrial mass and membrane potential of ILC2s by Mitotracker green and Mitotracker red staining, respectively, showed that *Slc7a8*-deficient ILC2s had lower mitochondrial mass and membrane potential compared to control ILC2s (Fig. 4 *A–D*), corroborating a defect in OXPHOS. Amino acid transport is also an important factor triggering the key amino acid sensor mTOR, which coordinates amino acid availability with protein synthesis, energy production, c-Myc

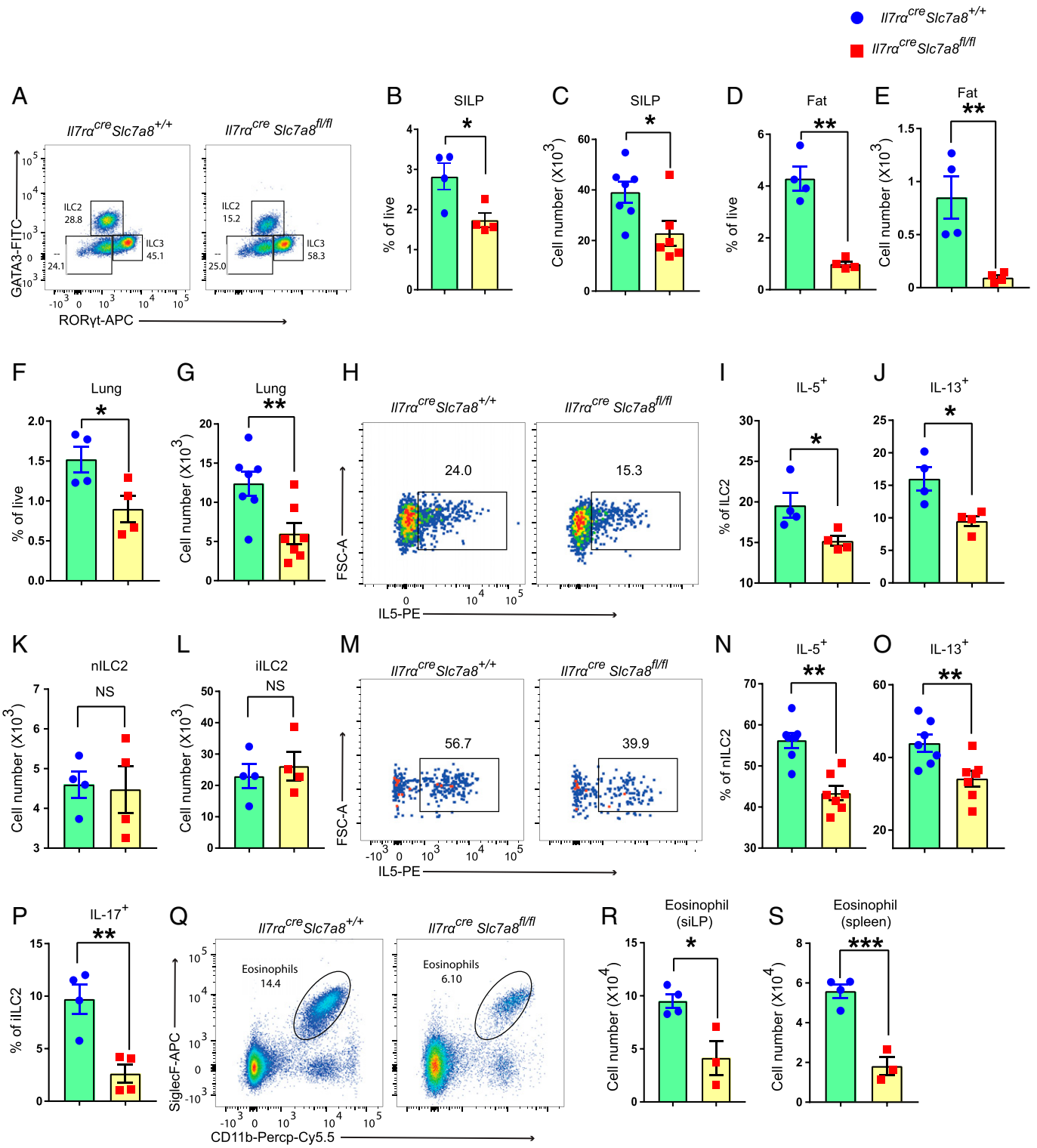

Fig. 2. *Slc7a8* deficiency affects ILC2 numbers and function. (*A*) Representative FACS plots showing the frequency of ILC2s and ILC3s in siLP of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. (*B–G*) Frequency and cell counts of ILC2s in siLP (*B* and *C*), fat (*D* and *E*), and lung (*F* and *G*) of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. (*H*) Representative FACS plot showing intracellular staining of IL-5 in siLP ILC2s. (*I* and *J*) Frequency of IL-5^{+} and IL-13^{+} ILC2s in SILP. (*K* and *L*) Cell numbers of nILC2s and iILC2s. (*M*) Representative FACS plot showing intracellular staining of IL-5 in lung ILC2s. (*N* and *O*) Frequency of IL-5^{+} and IL-13^{+} nILC2s in lungs of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. (*P*) Frequency of IL-17^{+} iILC2s in lungs of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. (*Q*) Representative FACS plots showing the frequency of eosinophils in siLP of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. (*R* and *S*) Eosinophil counts in siLP and spleen of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. Data shown as mean ± SEM. Each dot represents an individual mouse. Data are representative of two individual experiments. Statistical significance was determined by Students *t* test. *$P < 0.05$, **$P < 0.01$,***$P < 0.001$.

activation, and proliferation (28, 34). To determine the impact of *Slc7a8* deficiency in mTOR signaling, we stimulated ILC2s sort-purified from siLP with IL-25 and IL-33 and measured phosphorylation of S6 (pS6), a key enzyme downstream of mTOR signaling that controls translation initiation. Both flow cytometric and immunoblot analyses corroborated that pS6 was lower in *Slc7a8*-deficient ILC2s than control ILC2s (Fig. 4 *E–G*). Since reduction of amino acid availability and mTOR

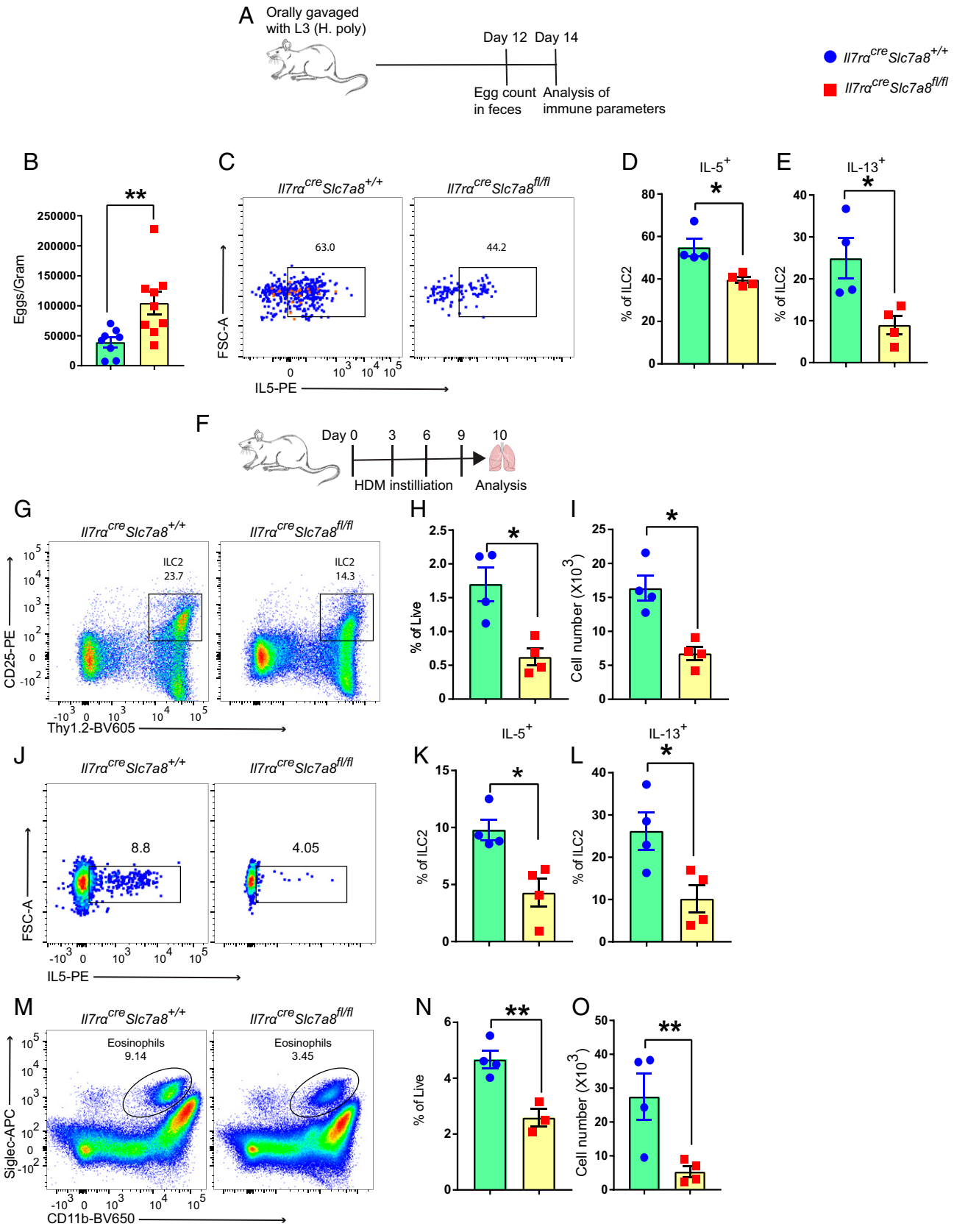

Fig. 3. *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice are susceptible to *H. polygyrus* infection but are resistant to HDM-induced airway inflammation. (*A*) *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice were orally gavaged with L3 larvae. Eggs were enumerated in the feces at day 12 postinfection and immune parameters were analyzed at day 14 postinfection. (*B*) Eggs per gram of feces. (*C*) Representative FACS plot showing intracellular staining of IL-5 in ILC2s of siLP of *H. polygyrus*-infected mice. (*D* and *E*) Frequency of IL-5$^+$ and IL-13$^+$ ILC2s of siLP. (*F*) Scheme depicting the HDM instillation experiment. (*G*) Representative FACS plot, (*H* and *I*) frequencies and numbers of ILC2s in the lungs of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice at day 10 of HDM instillation. (*J*) Representative FACS plot showing intracellular staining of IL-5 in lung ILC2s. (*K* and *L*) Frequency of IL-5$^+$ and IL-13$^+$ ILC2s of the lungs of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice at day 10 of HDM instillation. (*M*) Representative FACS plot, (*N* and *O*) frequency and numbers of eosinophils in the lungs of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. Data shown as mean ± SEM. Data are representative of two individual experiments. Each dot represents an individual mouse. Statistical significance was determined by Students *t* test. **P* < 0.05, ***P* < 0.01.

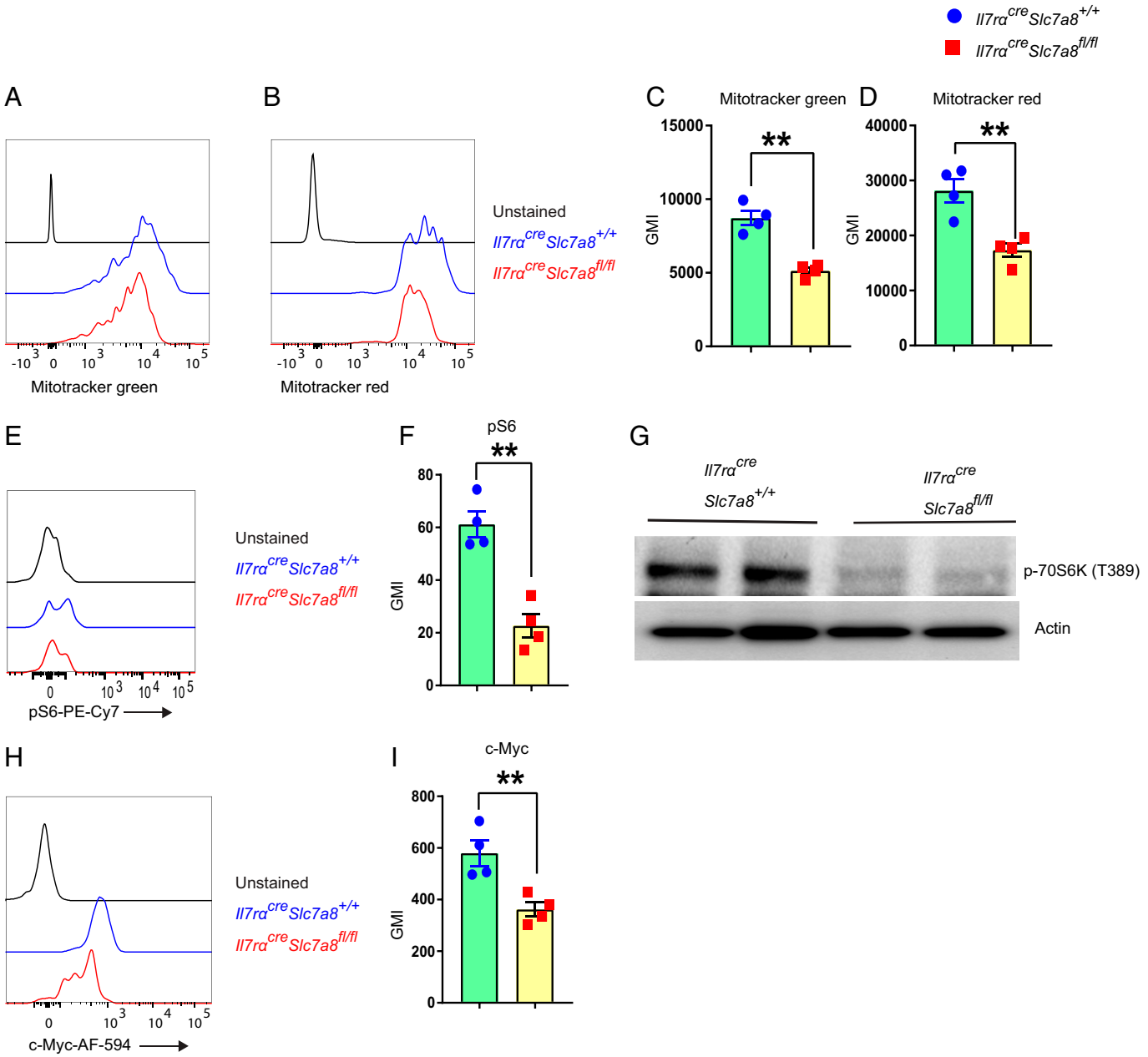

Fig. 4. *Slc7a8* deficiency impairs the metabolic fitness of ILC2. ILC2s from siLP were stained with Mitotracker green and Mitotracker red. (*A* and *B*) Representative histogram plot showing Mitotracker green and Mitotracker red staining in siLP ILC2s of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre}*Slc7a8*$^{fl/fl}$ mice. (*C* and *D*) Geometric mean intensity (GMI) of Mitotracker green and Mitotracker red of siLP ILC2s. (*E* and *F*) Representative histogram plots and GMI depicting pS6 levels in siLP ILC2s upon stimulation with IL-25 and IL-33. (*G*) Immunoblot showing pS6 and GAPDH in ILC2s sort purified from intestine and stimulated with IL-25 and IL-33 for 30 min. (*H* and *I*) Representative histogram plots and quantification of c-Myc expression in ILC2s of *Il7rα*^{cre}*Slc7a8*$^{+/+}$ and *Il7rα*^{cre} *Slc7a8*$^{fl/fl}$ mice upon IL-25 and IL-33 stimulation for 24 h. Data shown as mean ± SEM. Each dot represents an individual mouse. Statistical significance was determined by Students *t* test. *$P < 0.05$, **$P < 0.01$.

signaling also affect expression of c-Myc (30, 35) that is required for ILC2 activation (27), we examined *Slc7a8*-deficient and control siLP ILC2s for c-Myc expression by flow cytometry. Upon stimulation with IL-25 and IL-33, *Slc7a8*-deficient siLP ILC2s showed reduced c-Myc expression compared to control ILC2s (Fig. 4 *H* and *I*). Together these results demonstrated that *Slc7a8*-deficiency and resulting reduced availability of amino acids impair the OXPHOS, mTOR signaling, and c-Myc in ILC2s, compromising their maintenance and cytokine production.

Discussion

Our study demonstrates that ILC2s require SLC7A8 for both maintenance during homeostasis and activation during immune responses. In the steady state, *Slc7a8* was highly expressed in most tissue ILC2s, with the exception of skin ILC2s; accordingly, *Slc7a8*-deficiency led to reduced ILC2 numbers in the siLP, lung, and adipose tissue, whereas skin ILC2 numbers remained unaffected. *Slc7a8* expression was further up-regulated in ILC2s stimulated by IL-25 and IL-33, indicating that activated ILC2s increase their demand for amino acid import via SLC7A8-mediated transport in concert with type 2 cytokine production. In agreement with this, *Slc7a8*-deficient siLP and lung ILC2s produced markedly less IL-5 and IL-13 in models of helminth infection and allergic lung disease. We noted that IL-25 induced up-regulation of *Slc7a5* in vitro; moreover, nILC2 and iILC2 numbers in lung were sustained in *Slc7a8*-deficient mice after intraperitoneal injection of IL-25 in vivo. Thus,

SLC7A5 may complement SLC7A8 in transporting amino acids in activated ILC2s, while being the major amino acid supplier for ILC2s in the skin.

Previous studies showed a role for arginine in proliferation and cytokine production of lung ILC2s, which express arginase 1, a key urea cycle enzyme that hydrolyzes arginase (22). Moreover, it was recently shown that arginine and branched-chain amino acids are required to sustain OXPHOS, cellular fitness, and maintenance of human peripheral ILC2s in the steady state (23). The mTOR pathway, which integrates intracellular amino acid sensing with anabolic and energy metabolism, was also shown to be required for ILC2 activation (26, 27), corroborating the importance of availability of intracellular amino acids. Our results connect these previous studies, demonstrating that SLC7A8 is the major transporter of arginine and other amino acids for the mTOR pathway in ILC2s residing in most tissues. Based on previous studies on CD4 T cells, it is possible that mTOR sustains ILC2 fatty acid metabolism and OXPHOS through the mTORC1–PPARγ and mTORC1–SREBP1 pathways, which are essential for fatty acid uptake and fatty acid synthesis respectively (36). Alternatively, amino acids supplied by SLC7A8 may be used for the synthesis of fatty acids that fuel ILC2 OXPHOS. Discriminating these possibilities is an important direction for future studies.

It is noteworthy that ILCs and T cells have distinct requirements for LATs in steady state versus activation. ILC2s have elevated expression of SLC7A8 in the steady state, which is further augmented upon activation along with the expression of SLC7A5; conversely, expression of SLC7A5 or SLC7A8 in T cells is induced upon activation but minimal in the steady state (30, 37). We envision that ILC2s, as all innate lymphocytes, are poised for cytokine secretion and therefore require metabolic support for OXPHOS in the steady state for their basal level of activation. In contrast, resting T cells proliferate and acquire effector or memory functions only after receiving strong extracellular signals that overcome a critical threshold, and hence require metabolic support only after activation. Accordingly, SLC7A8 and SLC7A5 are minimally expressed in resting T cells, but induced upon activation, for example in tumor-infiltrating CD8 T cells (37). Moreover, conditional deletion of *Slc3a2* in mouse T cells affects antigen-induced proliferation but not the number of peripheral T cells in the steady state (38). Similarly, conditional deletion of *Slc7a5* in T cells affects Th1 and Th17 differentiation as well as acquisition of CD8 T cell cytotoxicity without affecting T cell development (30). Remarkably, although Th2 produce IL-5 and IL-13 in response to allergens and parasites, such as *H. polygyrus* and HDM (39, 40), and have strong transcriptional similarities with ILC2s, the expression and functional impact of SLC7A8 and SLC7A5 in Th2 have not been investigated. In our study, we geared our analysis of type 2 immunity toward ILC2 responses by avoiding presensitization with HDM and examining all read-outs at early time points, such that potentially confounding Th2 responses were minimal. Future studies in different settings will be essential to evaluate LAT1 and LAT2 transporters in Th2 functions.

Unrestricted ILC2 activation has been reported in several allergic pathologies, including asthma (41). We demonstrated that *Slc7a8*-deficiency attenuates lung ILC2 responses to HDM allergen challenge, reducing airway inflammation and eosinophil infiltration in the lungs. Therapeutic targeting of ILC2s or ILC2-mediated cytokines is currently explored for these diseases (42). Since SLC7A8 acts as a key promoter of ILC2 maintenance and activation, SLC7A8 could be a potential therapeutic target for ILC2-mediated inflammatory disorders.

Materials and Methods

Experimental details on mice, reagents and cell lines, cell extraction from tissues, antibody staining for flow cytometry and sorting, infection and asthma models, and statistical analyses for this study are described in detail in *SI Appendix, Materials and Methods*. Animal studies were performed in accordance with the guidelines of the Washington University in Saint Louis Animal Studies Committee.

Data, Materials, and Software Availability. All study data are included in the main text and supporting information.

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