AMERICAN SOCIETY FOR MICROBIOLOGY | Infection and Immunity®

HOST RESPONSE AND INFLAMMATION

Regulation of Pulmonary Bacterial Immunity by Follistatin-Like Protein 1

Matthew Henkel,[a,b,c] Justin A. Dutta,[a,c,d] Jessica Partyka,[a,b,c] Taylor Eddens,[a,b] Raphael Hirsch,[e,f] Jay K. Kolls,[b,c]* Brian T. Campfield[a,b,c]

[a]Division of Pediatric Infectious Diseases, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[b]UPMC Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA
[c]Richard K. Mellon Institute for Pediatric Research, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA
[d]Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
[e]Department of Pediatrics, University of California at San Francisco School of Medicine, San Francisco, California, USA
[f]Benioff Children's Hospitals, San Francisco, California, USA

Matthew Henkel and Justin A. Dutta contributed equally to this work; author order was determined by flipping a coin.

ABSTRACT *Klebsiella pneumoniae* is a common cause of antibiotic-resistant pneumonia. Follistatin-like protein 1 (FSTL-1) is highly expressed in the lung and is critical for lung homeostasis. The role of FSTL-1 in immunity to bacterial pneumonia is unknown. Wild-type (WT) and FSTL-1 hypomorphic (Hypo) mice were infected with *Klebsiella pneumoniae* to determine infectious burden, immune cell abundance, and cytokine production. FSTL-1 Hypo/TCR$\delta^{-/-}$ and FSTL-1 Hypo/IL17ra$^{-/-}$ were also generated to assess the role of $\gamma\delta$T17 cells in this model. FSTL-1 Hypo mice had reduced *K. pneumoniae* lung burden compared with that of WT controls. FSTL-1 Hypo mice had increased *Il17a*/interleukin-17A (IL-17A) and IL-17-dependent cytokine expression. FSTL-1 Hypo lungs also had increased IL-17A^{+} and TCR$\gamma\delta^{+}$ cells. FSTL-1 Hypo/TCR$\delta^{-/-}$ displayed a lung burden similar to that of FSTL-1 Hypo and reduced lung burden compared with the TCR$\delta^{-/-}$ controls. However, FSTL-1 Hypo/TCR$\delta^{-/-}$ mice had greater bacterial dissemination than FSTL-1 Hypo mice, suggesting that gamma delta T ($\gamma\delta$T) cells are dispensable for FSTL-1 Hypo control of pulmonary infection but are required for dissemination control. Confusing these observations, FSTL-1 Hypo/TCR$\delta^{-/-}$ lungs had an increased percentage of IL-17A-producing cells compared with that of TCR$\delta^{-/-}$ mice. Removal of IL-17A signaling in the FSTL-1 Hypo mouse resulted in an increased lung burden. These findings identify a novel role for FSTL-1 in innate lung immunity to bacterial infection, suggesting that FSTL-1 influences type-17 pulmonary bacterial immunity.

KEYWORDS pneumonia, follistatin-like 1, *Klebsiella pneumoniae*, interleukin-17

Klebsiella pneumoniae is a Gram-negative bacillus of the family *Enterobacteriaceae*. While *K. pneumoniae* commensally inhabits mucosal surfaces of healthy individuals, it is a common cause of pneumonia and bacteremia during nosocomial infection (1). Increasingly, *K. pneumoniae* infections are resistant to antibiotics, including carbapenems (carbapenem-resistant *Enterobacteriaceae* [CRE]) and extended-spectrum β-lactams (ESBLs), and thus constitute a major public health threat (2).

Interleukin-17A (IL-17A) and gamma delta T ($\gamma\delta$T) cells have been shown to be an integral component of innate host immunity to *K. pneumoniae* pulmonary infection (3, 4). While numerous IL-17A-associated inflammatory mediators (IL-23, G-CSF, CXCL1, CXCL2, CXCL5, and Lipocalin-2) have been well characterized in the clearance of *K. pneumoniae* from the lung, $\gamma\delta$T cells have been identified as an early, and critical,

Citation Henkel M, Dutta JA, Partyka J, Eddens T, Hirsch R, Kolls JK, Campfield BT. 2021. Regulation of pulmonary bacterial immunity by follistatin-like protein 1. Infect Immun 89:e00298-20. https://doi.org/10.1128/IAI.00298-20.

Editor Manuela Raffatellu, University of California San Diego School of Medicine

Address correspondence to Brian T. Campfield, brian.campfield@chp.edu.

* Present address: Jay K. Kolls, Center for Translational Research in Infection and Inflammation, Tulane University, New Orleans, Louisiana, USA.

Received 18 May 2020
Returned for modification 7 July 2020
Accepted 5 October 2020

Accepted manuscript posted online 19 October 2020
Published 15 December 2020

IL-17A-producing cell type (5–10). Moreover, γδT cells are necessary for the prevention of bacterial systemic dissemination (3).

Follistatin-like protein 1 (FSTL-1) is a secreted glycoprotein with multiple functions in diverse physiological processes, including cardiovascular, respiratory, skeletal, muscular, nervous, and urinary development; signaling; homeostasis; and tissue remodeling; as well as mediating respiratory disease, cardiovascular disease, and cancer (11–23). FSTL-1 has also been shown to have important functions in immune-mediated diseases and immune signaling (24–30). Initially described as a novel mediator of inflammation in arthritis, FSTL-1 has been shown to enhance proinflammatory cytokine and chemokine production in a collagen-induced arthritis murine model (31–34), as well as promote metalloprotease gene transcription in human rheumatoid synoviocytes (35). We have previously shown that FSTL-1 is critical in lung homeostasis, as a decrease in FSTL-1 results in a spontaneous emphysema phenotype and is associated with altered Nur77/*Nr4a1*-dependent macrophage inflammatory signaling (25). However, the role *Nr4a1* in bacterial lung infection is poorly understood, and the impact of FSTL-1 on pulmonary immunity to infection has not been previously identified.

The present study examines the role of FSTL-1 in lung immunity in a model of *K. pneumoniae* pulmonary infection. The FSTL-1 hypomorphic (Hypo) mouse, a mouse strain with reduced levels of FSTL-1, demonstrated less bacterial lung burden than the wild type, suggesting that the FSTL-1 Hypo lung environment provides host protection against *K. pneumoniae*. These mice have an increased population of resident IL-17A-producing γδT cells compared with the wild type; however, crossing FSTL-1 Hypo with the TCRδ$^{-/-}$ mouse and generating the FSTL-1 Hypo/TCRδ$^{-/-}$ double knockout did not reverse the phenotype, likely due to compensatory IL-17A^{+} T cell populations. Attenuation or elimination of IL-17A signaling increased pulmonary burden in the FSTL-1 Hypo mouse. Cumulatively, these observations suggest that FSTL-1 mediates control of bacterial lung infection, in part, by influencing the development and function of IL-17A-producing cells.

RESULTS

FSTL-1 Hypo mice have reduced *Klebsiella pneumoniae* burden in the lung. To assess the innate immune impact of FSTL-1 in *K. pneumoniae* pulmonary infection, intratracheal (i.t.) *K. pneumoniae*-infected WT and FSTL-1 Hypo mice had lung burden determined by CFU at 24 and 48 hours postinfection. A difference in bacterial burden was not found at 24 hours postinfection (Fig. 1A). At 48 hours postinfection, FSTL-1 Hypo mice displayed a significant reduction in *K. pneumoniae* lung burden by CFU, compared with wild-type mice (Fig. 1B). To assess bacterial dissemination, spleens were harvested as well. At 24 and 48 hours postinfection, FSTL-1 Hypo mice displayed no significant difference in bacterial dissemination compared with wild-type mice by CFU (Fig. 1A and B). Surprisingly, despite the underlying spontaneous emphysema physiology (25), FSTL-1 Hypo mice were able to control *K. pneumoniae* from the lung, by the 48-hour time point, to a greater extent than wild-type mice, thereby suggesting that germ line *Fstl1* mutations resulting in reduced FSTL-1 expression conveyed a protective advantage against *K. pneumoniae* pulmonary infection.

FSTL-1 Hypo mice have increased IL-17A production following *Klebsiella pneumoniae* pulmonary infection. We next aimed to identify potential mechanisms underlying the reduced *K. pneumoniae* burden of the FSTL-1 Hypo lung, where we confirmed reduced *Fstl1* transcript levels and FSTL-1 protein abundance (Fig. 2A). At the 24-h time point, when burden was similar, we next identified that FSTL-1 Hypo mice had significantly increased *Il17a* gene expression and IL-17A protein (Fig. 2B), an integral host defense cytokine against *Klebsiella* lung infection. IL-17A-associated gene (*Csf3*, *Cxcl1*, *Cxcl2*, *Cxcl5*, *Il23a*, *Il23r*, and *Lcn2*) transcript levels were significantly elevated in the FSTL-1 Hypo mouse lung at 24 h postinfection (Fig. 2C), and there was an increased trend in the *Il22* transcript. Absolute numbers of CD3^{+}TCRγδ^{+} and CD11b^{+}Ly6G^{+} neutrophils present in the lung were also significantly higher in the FSTL-1 Hypo mouse than those in the wild type (Fig. 2D). These data suggest that a

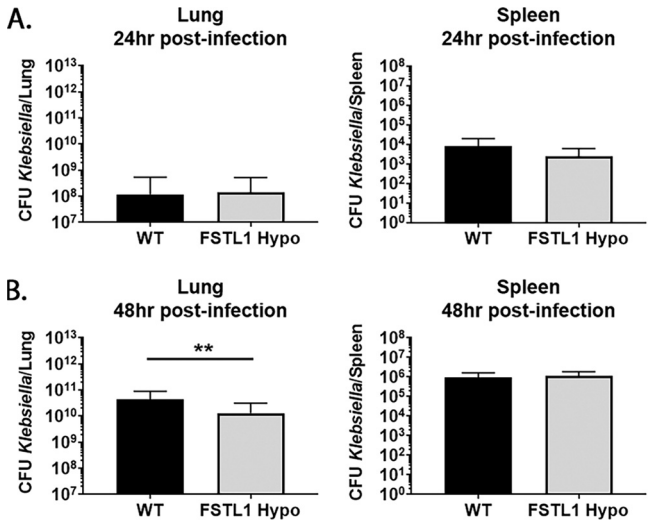

FIG 1 FSTL-1 Hypo mice have reduced *Klebsiella pneumoniae* burden in the lung. (A) *Klebsiella pneumoniae* infectious burden at 24 hours postinfection in the lung and spleen in WT and FSTL-1 Hypo mice. (B) *Klebsiella pneumoniae* infectious burden in the lung and spleen at 48 hours postinfection in WT and FSTL-1 Hypo mice. Data are representative of three combined experiments. **, $P = 0.0068$.

reduced burden in the lung may be mediated by an increase in IL-17A-mediated recruitment of neutrophils.

$\gamma\delta$T cells are the primary source of IL-17A during *K. pneumoniae* lung infection. We next sought to identify the cellular source of this increased IL-17A production. Using flow cytometry, we evaluated the abundance of cell types known to produce IL-17A ($CD3^-$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD3^+TCR\gamma\delta^+$) in the lung. There were no significant differences in the percentage of $CD3^+CD4^+$ or $CD3^+CD8^+$ cells 24 hours postinfection (see Fig. S1B and C in the supplemental material); however, $CD3^+$ $TCR\gamma\delta^+$ percentages were increased in the FSTL-1 Hypo lung compared to WT (Fig. 3B). Next, we assessed to what extent these cell types produced IL-17A during *K. pneumoniae* infection. Compared to the $CD3^+$ cells, $CD3^-$ cells produced negligible percentages of IL-17A regardless of genotype (Fig. 3A). FSTL-1 Hypo lung $CD3^+TCR\gamma\delta^+$ cells produced significantly higher percentages of IL-17A than those of wild-type lungs (Fig. 3C). Unlike $CD3^+TCR\gamma\delta^+$ cells, $CD3^+CD4^+$ and, to a lesser extent $CD3^+CD8^+$, cells produced similar amounts of IL-17A when comparing WT to FSTL-1 Hypo mice (Fig. 3C and D; see Fig. S2D in the supplemental material), suggesting that FSTL-1 Hypo mice have increased IL-17A-producing $\gamma\delta$T cells during *K. pneumoniae* lung infection compared with those of wild-type mice, potentially conferring protection against pulmonary infection.

FSTL-1 Hypo mice have greater lung-resident $TCR\gamma\delta^+$IL-17A$^+$ cell abundance. To determine whether the increase in lung IL-17A-producing $\gamma\delta$T cells in FSTL-1 Hypo mice was elicited by *K. pneumoniae* infection or was a preexisting developmental immune phenotype, we examined lung-resident cell percentages and IL-17A production by flow cytometry. The uninfected lungs of a FSTL-1 Hypo mouse displayed a similar dominant $CD3^+TCR\gamma\delta^+$IL-17A$^+$ lung phenotype compared with that of wild-type lungs (Fig. 4A to C). FSTL-1 Hypo lungs display a significantly greater percentage of $CD3^+$IL-17A$^+$ cells and $CD3^+TCR\gamma\delta^+$ cells than wild-type lungs, with no significant contribution of IL-17A from $CD3^-$, $CD3^+CD4^+$, or $CD3^+CD8^+$ cells (Fig. 4; see Fig. S3 in the supplemental material). These data suggest reduced FSTL-1 production in the FSTL-1 Hypo mouse may lead to the development of a lung environment with a greater type-17 immune potential, as manifested by increased lung-resident IL-17A-producing $\gamma\delta$T cells.

FSTL-1 Hypo/$TCR\delta^{-/-}$ mice have reduced *Klebsiella pneumoniae* lung burden but are not protected against bacterial dissemination. Given the increase in lung-

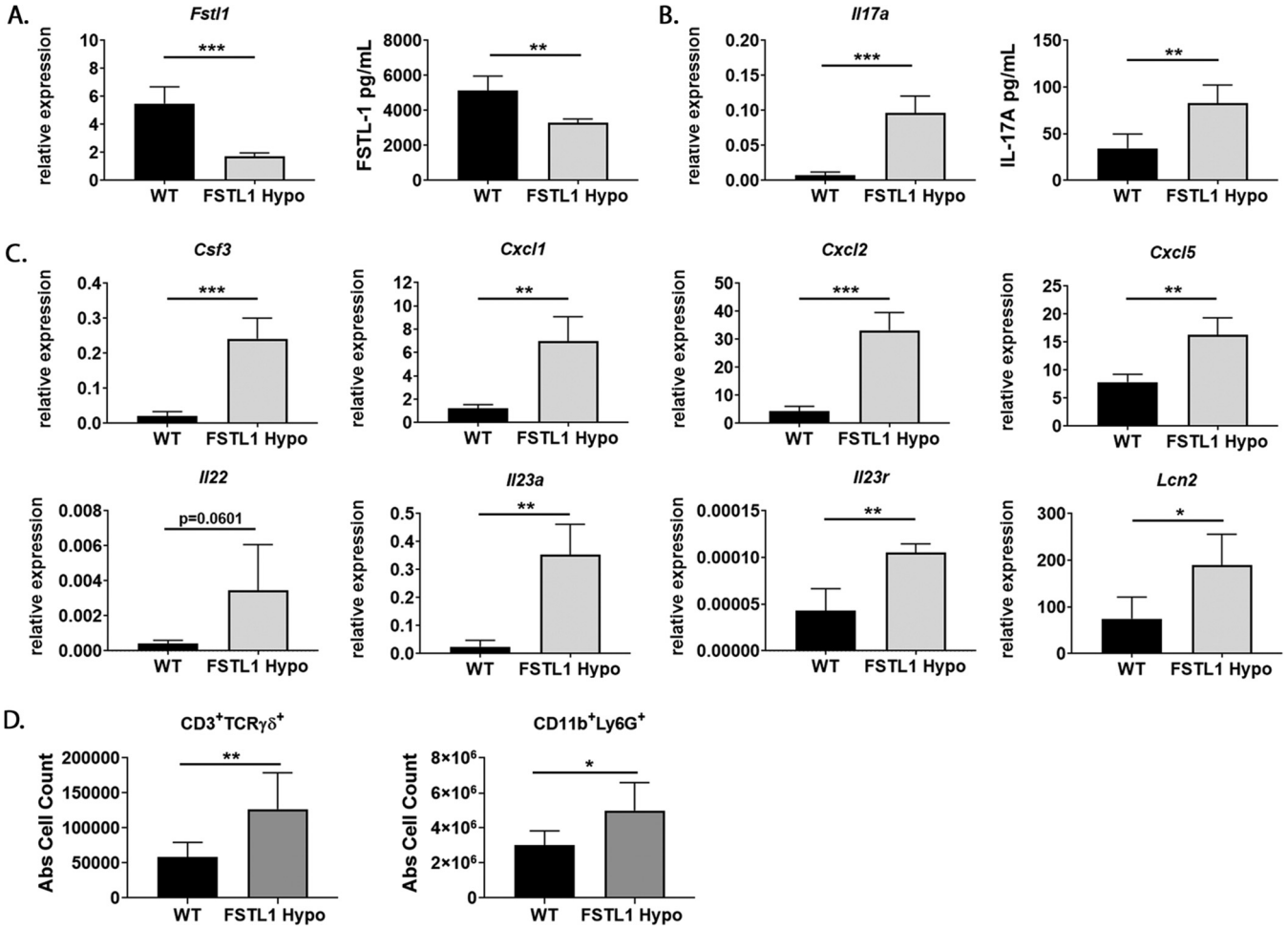

FIG 2 FSTL-1 Hypo mice have increased IL-17A production, and IL-17-associated pathways, following *Klebsiella pneumoniae* infection. WT and FSTL-1 Hypo mouse lung 24 hours postinfection. (A) *Fstl1*/FSTL-1 is reduced in FSTL-1 Hypo mice, as determined by gene expression and protein. (B) *Il17a*/IL-17A is elevated in FSTL-1 Hypo mice, as determined by gene expression and protein. (C) IL-17A-associated gene expression is elevated in FSTL-1 Hypo mice. All mRNA transcript levels were determined relative to reference gene *Hprt1*. (D) $CD11b^+Ly6G^+$ and $CD3^+$ $TCR\gamma\delta^+$ cell numbers are increased in FSTL-1 Hypo mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

resident IL-17A-producing γδT cells of a FSTL-1 Hypo mouse, we next sought to examine the necessity of γδT cells in conferring FSTL-1 Hypo protection against *K. pneumoniae* by generation of a FSTL-1 Hypo/TCR$\delta^{-/-}$ double-knockout mouse (Fig. 5A and B). We hypothesized that if FSTL-1 Hypo mice were protected via lung-resident IL-17A-producing γδT cells, the absence of γδT cells in the FSTL-1 Hypo/TCR$\delta^{-/-}$ mouse would phenocopy the TCR$\delta^{-/-}$, as determined by *K. pneumoniae* infectious burden. Thus, lung and spleen *K. pneumoniae* burdens were determined 48 hours postinfection by CFU in FSTL-1 Hypo, TCR$\delta^{-/-}$, and FSTL-1 Hypo/TCR$\delta^{-/-}$. FSTL-1 Hypo mice had a significantly lower lung burden than that of TCR$\delta^{-/-}$ mice, with a similar burden compared with FSTL-1 Hypo/TCR$\delta^{-/-}$ mice (Fig. 5C). These findings suggest that FSTL-1 Hypo-mediated lung protection is independent of γδT cells.

When we examined bacterial dissemination, TCR$\delta^{-/-}$ demonstrated an increase in splenic bacterial burden relative to FSTL-1 Hypo mice (Fig. 5C). However, FSTL-1 Hypo/TCR$\delta^{-/-}$ mice also showed increased *K. pneumoniae* burden in spleen CFU compared with that of FSTL-1 Hypo spleen (Fig. 5C). This finding supports the necessity of γδT cells in protection from bacterial dissemination in the context of FSTL-1 Hypo, which is consistent with prior observations that γδT cells are required for the control of bacterial dissemination during pulmonary *K. pneumoniae* infection (3).

FSTL-1 Hypo/TCR$\delta^{-/-}$ mice have exaggerated, compensatory lung-resident IL-17A$^+$-producing cells. To understand the impact of IL-17 in the FSTL-1 Hypo/

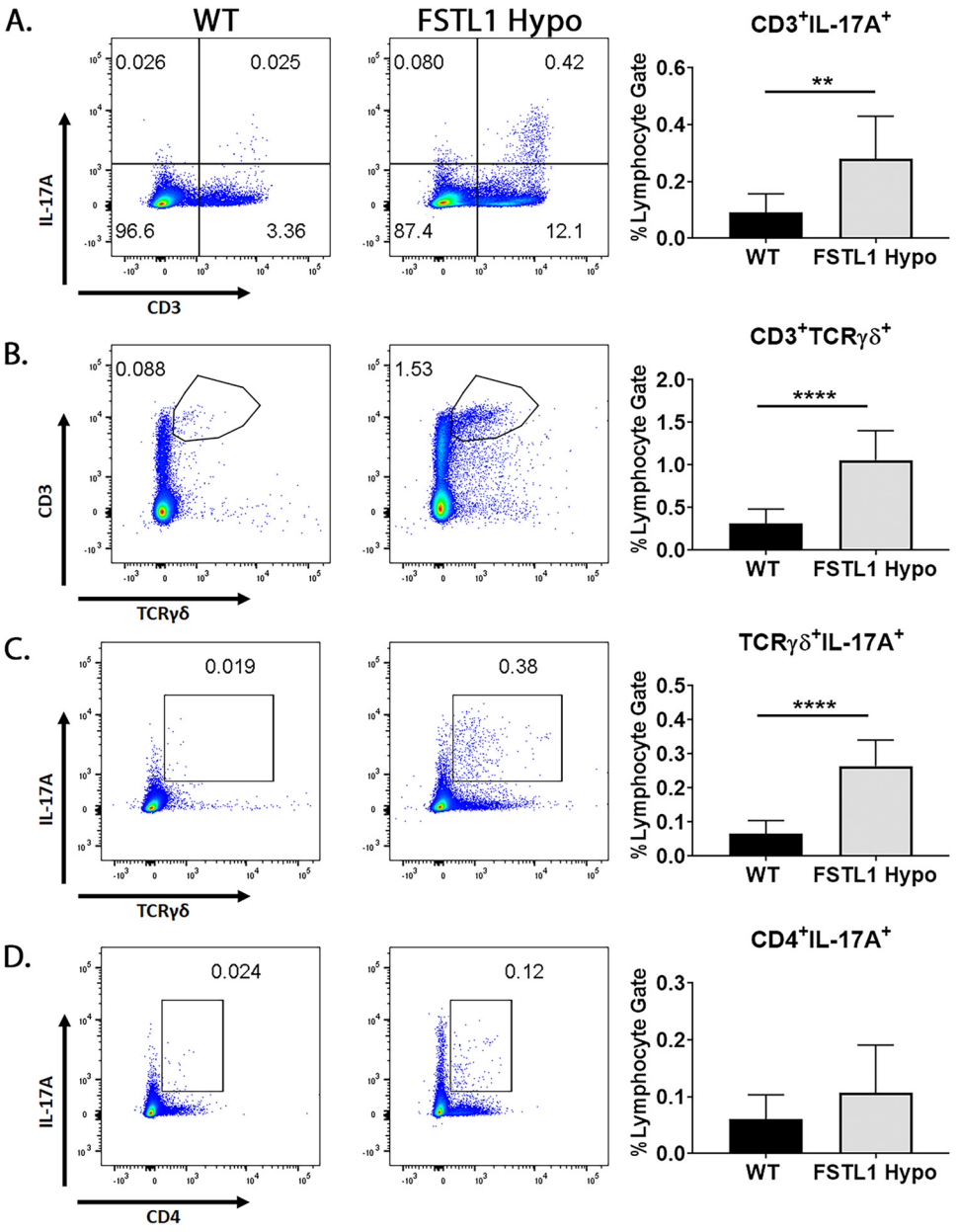

FIG 3 FSTL-1 Hypo mice have increased $\gamma\delta^+$IL-17A$^+$ cells following *Klebsiella pneumoniae* infection. WT and FSTL-1 Hypo mouse lung 24 hours postinfection. CD3$^+$IL-17A$^+$ (A), CD3$^+$TCR$\gamma\delta^+$ (B), and TCR$\gamma\delta^+$IL-17A$^+$ (C) cells are elevated in FSTL-1 Hypo mice by flow cytometric analysis. (D) CD4$^+$IL-17A$^+$ cells are not significantly different between FSTL-1 Hypo and WT mice by flow cytometric analysis. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

TCR$\delta^{-/-}$ mouse, in the setting of *K. pneumoniae* lung infection, we sought to determine the immune phenotype. Knowing that FSTL-1 Hypo mice have an increase in lung-resident IL-17A-producing $\gamma\delta$T cells, we next sought to phenotype the type-17 immune response in the FSTL-1 Hypo/TCR$\delta^{-/-}$ mouse. Uninfected FSTL-1 Hypo/TCR$\delta^{-/-}$ mice exhibited a significant increase in the percentage of lung-resident CD3$^+$IL-17A$^+$ cells compared with that of both FSTL-1 Hypo and TCR$\delta^{-/-}$ mice, with little to no contribution of IL-17A from the CD3$^-$ population (Fig. 6A). In the FSTL-1 Hypo lung, the dominant IL-17A-producing cells are the TCR$\gamma\delta^+$ cells (Fig. 6B to D). However, TCR$\delta^{-/-}$ and FSTL-1 Hypo/TCR$\delta^{-/-}$ lung-resident IL-17A-producing cells are significantly dominated by CD3$^+$CD4$^+$ and CD3$^+$CD4$^-$CD8$^-$ cell populations (Fig. 6D). CD8$^+$ cells were not a significant contributor of IL-17A (see Fig. S4D in the supplemental material). These

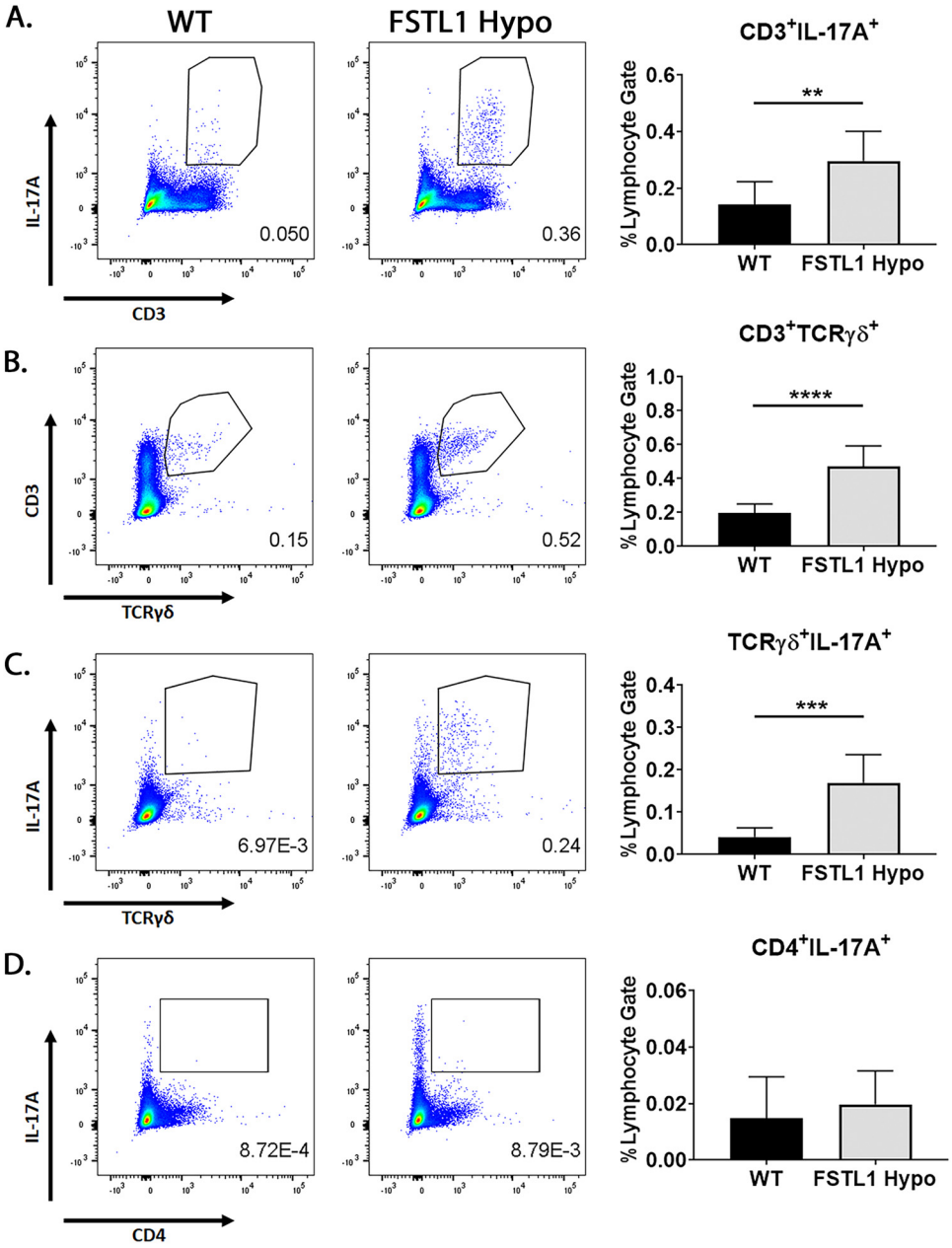

FIG 4 FSTL-1 Hypo mice have greater lung-resident $\gamma\delta^+$IL-17A$^+$ cell abundance. CD3$^+$IL-17A$^+$ (A), CD3$^+$TCR$\gamma\delta^+$ (B), and TCR$\gamma\delta^+$IL-17A+ (C) cells are elevated in uninfected FSTL-1 Hypo lungs by flow cytometric analysis. (D) CD4$^+$IL-17A$^+$ cells are not significantly different between FSTL-1 Hypo and WT uninfected lungs by flow cytometric analysis. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

data suggest that, within the milieu of a FSTL-1 Hypo mouse, elimination of $\gamma\delta$T cells promotes a compensatory development of IL-17A production via CD3$^+$CD4$^+$ cells and CD3$^+$CD4$^-$CD8$^-$ cells.

Attenuation of IL-17A signaling in FSTL-1 Hypo mice increased *Klebsiella pneumoniae* lung burden. We next aimed to determine the importance of increased IL-17A production in FSTL-1 Hypo for protection against *K. pneumoniae* pulmonary infection using two independent techniques. First, we employed the anti-IL-17A neutralization antibody in the FSTL-1 Hypo mouse and assessed bacterial lung and spleen burden by CFU at 48 hours postinfection. Compared with the IgG control antibody group, the anti-IL-17A-treated group had significantly increased lung CFU and no difference in spleen CFU (Fig. 7A and B). Next, a FSTL-1 Hypo/Il17ra$^{-/-}$ double-

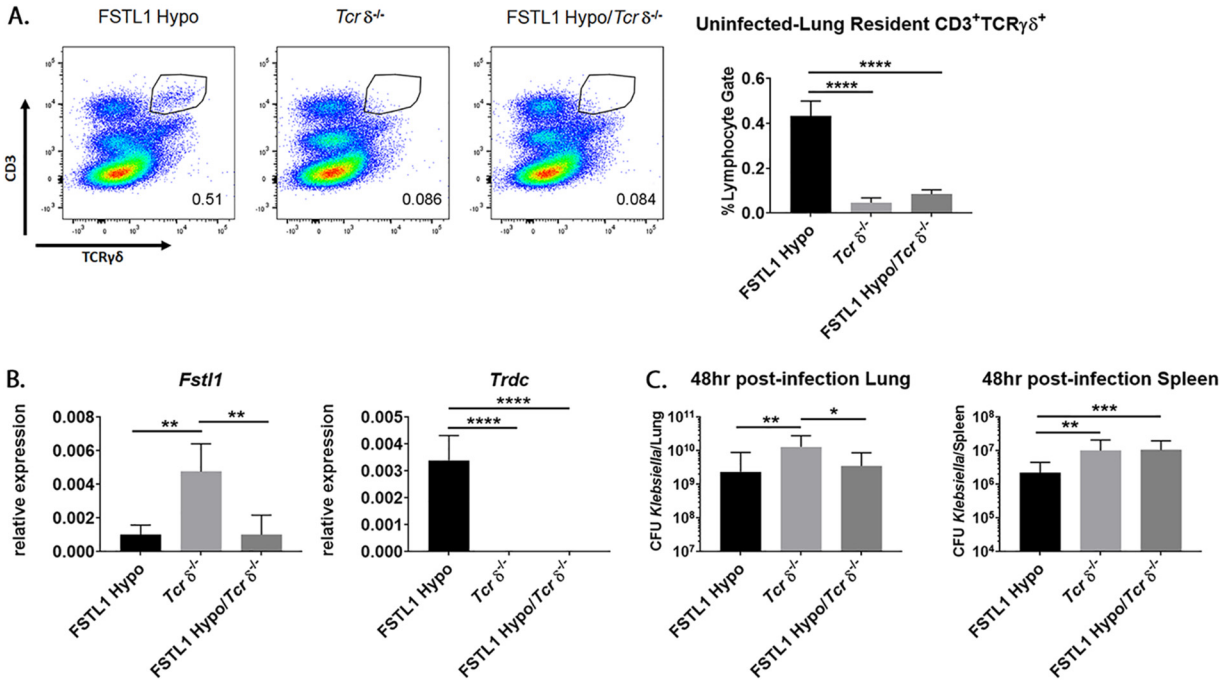

FIG 5 FSTL-1 Hypo-TCR$\delta^{-/-}$ mice have reduced *Klebsiella pneumoniae* lung burden, but are not protected against bacterial dissemination. (A) TCRδ^{+} cells are absent in naive lung of Tcr$\delta^{-/-}$ and FSTL-1 Hypo/Tcr$\delta^{-/-}$ mice, as determined with a fluorescence-activated cell sorter (FACS). (B) *Fstl1* and *Trdc* gene expression in naive lung of FSTL-1 Hypo, Tcr$\delta^{-/-}$, and FSTL-1 Hypo/Tcr$\delta^{-/-}$ mice. All mRNA transcript levels were determined relative to reference gene *Hprt1*. (C) *Klebsiella pneumoniae* infectious burden in lung and spleen 48 hours postinfection in FSTL-1 Hypo, Tcr$\delta^{-/-}$, and FSTL-1 Hypo/Tcr$\delta^{-/-}$ mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

transgenic mouse was assessed for infectious burden 48 hours following *K. pneumoniae* pulmonary infection. Compared with the FSTL-1 Hypo mouse, the FSTL-1 Hypo/Il17ra$^{-/-}$ mouse had significantly increased lung burden and yet no difference in bacterial dissemination (Fig. 7C and D), consistent with the impact of IL-17A neutralization. Together, these findings suggest that the FSTL-1 Hypo mouse lung environment protects against pulmonary *K. pneumoniae* infection in an IL-17A-dependent manner.

DISCUSSION

Antibiotic-resistant infections are a worldwide public health crisis, specifically carbapenem-resistant *Enterobacteriaceae* (CRE), including *Klebsiella pneumoniae* (2). *K. pneumoniae* is also a common cause of hospital-acquired infection and pneumonia. Development of new approaches to treat and prevent infection and colonization with antibiotic-resistant organisms, such as *K. pneumoniae*, is increasingly urgently needed. Modulating host immunity to aid in the clearance of infection is an understudied approach, and a deeper understanding of host-pathogen interactions that control *K. pneumoniae* lung infection is required. Several aspects of this pulmonary host defense pathway have been characterized (36); T cell immune responses are essential for orchestrating effective bacterial clearance, including IL-17-mediated signaling (5, 10, 37–39). Moore et al. established that interferon gamma (IFN-γ) is required for effective *K. pneumoniae* responses in the lung (40). Alpha beta T ($\alpha\beta$T) cell responses are dispensable for controlling acute infection, although Th17 cells confer antibody-independent protection against subsequent heterologous infections, including *K. pneumoniae* (37). During acute infection, $\gamma\delta$T cell responses are required to control infection, including *K. pneumoniae* dissemination, cumulatively suggesting that innate type-17 immunity is critical for controlling *K. pneumoniae* lung infection and dissemination (41).

Having observed that the FSTL-1 Hypo mouse controls *K. pneumoniae* lung burden, we then determined cellular and molecular constituents of this phenotype, specifically

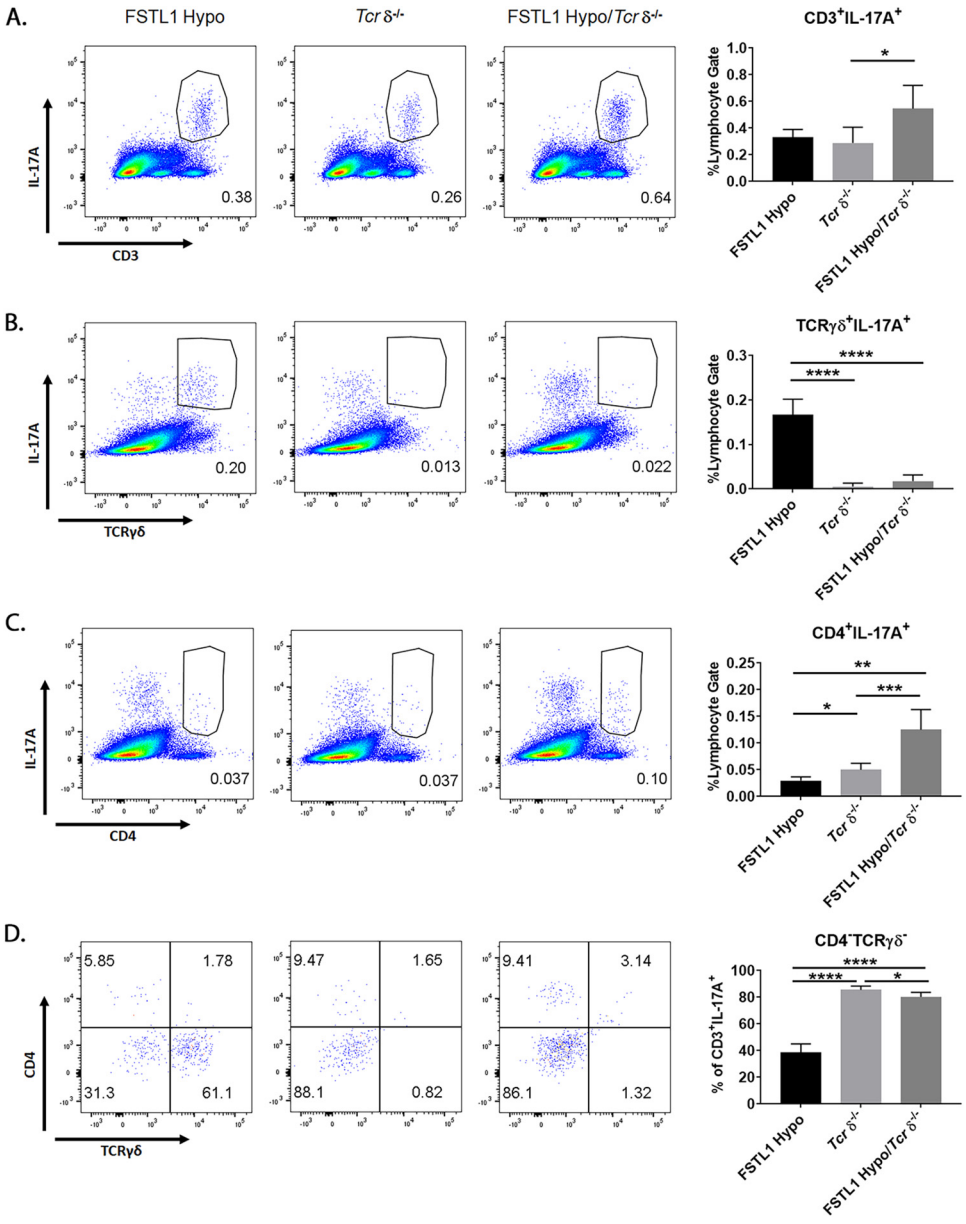

FIG 6 FSTL-1 Hypo-TCR$\delta^{-/-}$ mice have exaggerated, compensatory lung-resident IL-17A$^+$-producing cells. Percentage of CD3$^+$IL-17A$^+$ (A), TCR$\gamma\delta^+$IL-17A$^+$ (B), and CD4$^+$IL-17A$^+$ (C) cells in uninfected FSTL-1 Hypo, Tcr$\delta^{-/-}$, and FSTL-1 Hypo/Tcr$\delta^{-/-}$ lungs by flow cytometric analysis. (D) Percentage of CD4$^+$TCR$\gamma\delta^+$ cells of CD3$^+$IL-17A$^+$ population by flow cytometry in FSTL-1 Hypo, Tcr$\delta^{-/-}$, and FSTL-1 Hypo/Tcr$\delta^{-/-}$ uninfected lungs. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

FSTL-1 regulation of γδ-17 T cells. FSTL-1 Hypo mice expressed elevated proinflammatory cytokines, including IL-17A, following *K. pneumoniae* infection, which was associated with improved lung burden, consistent with the protective role of type-17 immune responses against bacterial lung infection, although the impact of FSTL-1 on IL-17A-producing cells in the lung has not previously been known.

Determining that IL-17A production in the FSTL-1 Hypo lung is dominated by γδT cells suggested a novel function of FSTL-1, namely, controlling innate type-17 cell immunity in the lung. Interestingly, the observation that FSTL-1 Hypo mice have increased γδT-17 cell abundance prior to infection argues that FSTL-1 likely regulates natural, rather than inducible, γδT-17 cell development. Furthermore, the mechanism(s) by which FSTL-1 confers this phenotype (i.e., regulation of γδT cell thymic differentia-

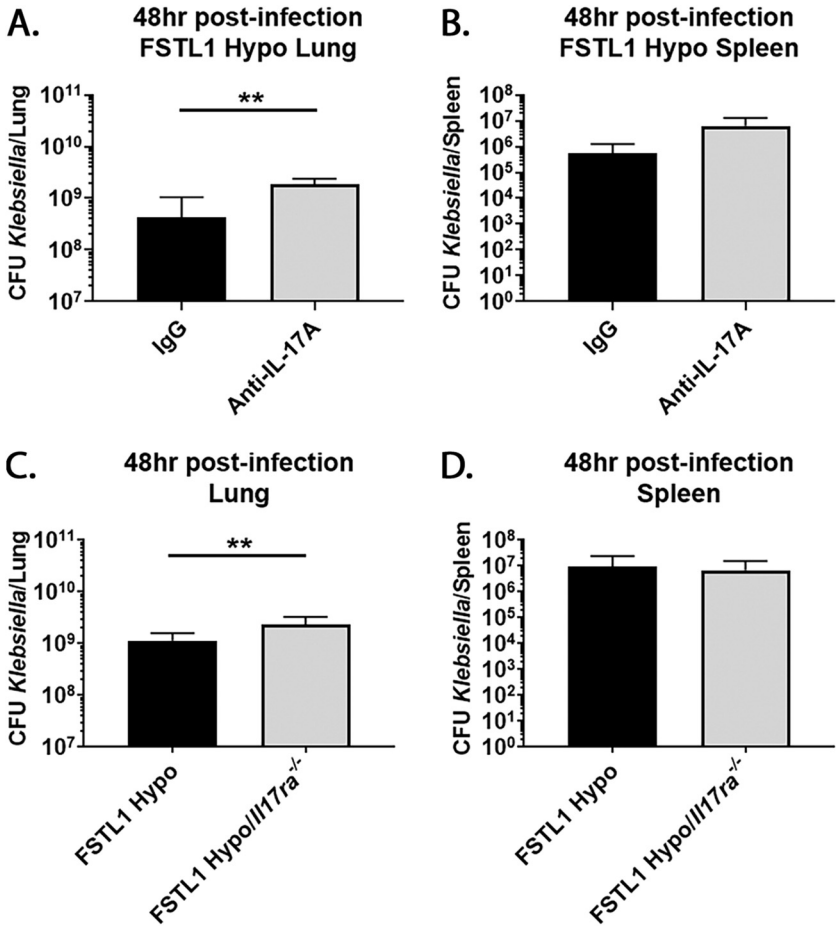

FIG 7 Attenuation of IL-17A signaling in FSTL-1 Hypo mice increased *Klebsiella pneumoniae* lung burden. *Klebsiella pneumoniae* infectious burden at 48 h in the lung (A) and spleen (B) of FSTL-1 Hypo mice treated with anti-IL-17A neutralizing antibody or IgG control. *Klebsiella pneumoniae* infectious burden in the lung (C) and spleen (D) at 48 hours postinfection in FSTL-1 Hypo and FSTL-1 Hypo/Il17ra$^{-/-}$ mice. **, $P < 0.01$. Data for A and B are representative of 3 individual experiments; data from C and D are combined from two individual experiments.

tion, proliferation, apoptosis, or cell extrinsic factors) remain to be defined. However, the findings that FSTL-1 Hypo mice have enhanced control of *K. pneumoniae* infection are the first identification of a potential therapeutic role for FSTL-1 in pulmonary host defense.

The observations of FSTL-1 Hypo/TCR$\delta^{-/-}$ mice highlight several insights into the cellular requirements and tissue-specific nature of the FSTL-1 Hypo phenotype. Because the FSTL-1 Hypo/TCR$\delta^{-/-}$ and FSTL-1 Hypo mice have similar *K. pneumoniae* burdens in the lung, this suggests that $\gamma\delta$T cells are not required for the lung-specific effects of FSTL-1. Conversely, FSTL-1 Hypo/TCR$\delta^{-/-}$ have a bacterial dissemination similar to that of TCR$\delta^{-/-}$ mice, suggesting that FSTL-1-mediated control of dissemination requires $\gamma\delta$T cells, or a subset thereof. Of note, Moore et al. have shown that TCR$\delta^{-/-}$ mice exhibit compensatory host responses to *K. pneumoniae* pulmonary infection, as we also observed; they also noted an unaltered ability of the TCR$\delta^{-/-}$ mice to recruit neutrophils, which we also observed in the TCR$\delta^{-/-}$ and FSTL-1 Hypo/TCR$\delta^{-/-}$ mice (data not shown), as well as, to produce compensatory IL-17A levels. Nonetheless, the pulmonary immune response of FSTL-1 Hypo suggests a role for FSTL-1 in the development of resident IL-17A-producing cells, including $\gamma\delta$T-17 cells. Furthermore, by showing that IL-17A is critical for FSTL-1 Hypo protection, this work implicated FSTL-1 in regulating the development, activation, and function of IL-17-producing cells, although the mechanism remains unknown. Further examination of the link between FSTL-1 and IL-17A

production in the lung (and other tissues) is needed to determine the cytokines and cells involved in FSTL-1 dependent type-17 immunity.

Within the lung, FSTL-1 has critical developmental, homeostatic, and repair functions (12, 13, 21, 25). Although we recently identified that the FSTL-1 Hypo mouse develops spontaneous pulmonary emphysema, a contribution of type-17 immune responses was not identified in that model. However, FSTL-1 Hypo mice did have reduced *Nr4a1* expression, and *in vitro* exogenous FSTL-1 maintained *Nr4a1* suppression of NF-κB in macrophages. Other work has shown *Nr4a1*/Nur77 inhibit Th17 immune responses, although the role of *Nr4a1* in pulmonary host defense is poorly understood (42, 43). The experimental approaches to study FSTL-1 *in vivo* are complicated by the perinatal lethality of germ line *Fstl1* knockout (12). Further study of the interplay of FSTL-1, Nr4a1, and type-17 immunity in the lung is warranted.

We have shown a novel role for FSTL-1 as an impactful component of host immunity during *Klebsiella pneumoniae* pulmonary infection, by impacting lung-resident immune cell IL-17 production. The present findings reinforce the necessity of innate γδT cell and IL-17 responses in effective pulmonary immunity to *Klebsiella* infection and highlight a potential strategy of immunomodulation for enhancing bacterial clearance in the mammalian lung.

MATERIALS AND METHODS

Animal experiments. Wild-type C57BL/6 (WT; Jackson Laboratory, Bar Harbor, ME), FSTL-1 Hypo, TCRδ$^{-/-}$ (B6-TCRdelta^{-}, B6.129P2-Tcrd^{tm1Mom}/J; Jackson Laboratory, Bar Harbor, ME), IL17ra$^{-/-}$ (Il17ra^{tm1Koll}, Il17ra KO; Jackson Laboratory, Bar Harbor, ME), FSTL-1 Hypo/Tcrδ$^{-/-}$, and FSTL-1 Hypo/IL17ra$^{-/-}$ mice were bred, housed, and subjected to experimental protocols in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh School of Medicine. FSTL-1 Hypo/Tcrδ$^{-/-}$ mice were generated in our institution by crossing FSTL-1 Hypo mice with Tcrδ$^{-/-}$ mice. FSTL-1 Hypo/IL17ra$^{-/-}$ were generated by crossing FSTL-1 Hypo mice with IL17ra$^{-/-}$ mice. Novel double-transgenic strains were verified by PCR using allele-specific primers and resolution with agarose gel electrophoresis as described (10, 44) (Fig. S1A and B). *In vivo* studies were performed with 8- to 15-week-old female and male mice. For *in vivo* neutralization of IL-17A, anti-IL-17A (17F3, BioXcell) or an isotype control (MOPC-21, BioXcell) antibody was administered at 200 μg of intratracheal (i.t.) antibody per mouse both and 400 μg of intraperontaneal (i.p.) antibody on the day prior to infection (day −1), the day of infection (day 0) and on day +1. On day 2, mice were administered only 400 μg i.p. antibody per mouse.

***Klebsiella pneumoniae* infection.** *Klebsiella pneumoniae* strain 396, a hypervirulent mucoid K1 serotype clinical isolate (37), was grown overnight at 37°C and 250 rpm in tryptic soy broth (TSB) and then the following day was subcultured in TSB at a dilution of 1:1,000 for 2 hours at 37°C and 250 rpm to achieve log-phase growth. Bacteria were pelleted and washed with sterile phosphate-buffered saline (PBS) at a centrifugation speed of 5,000 × *g* for 5 minutes. The cell pellet was diluted in sterile PBS and given at 1×10^4 CFU per mouse via intratracheal (i.t.) administration. At 24 or 48 hours following *K. pneumoniae* administration, mice were sacrificed and lung and spleen were harvested. To assess lung bacterial burden and bacterial dissemination by CFU, the left lung lobe and spleen, respectively, were collected in sterile PBS and subjected to homogenization. The right lung lobe was collected in TRIzol reagent (Invitrogen) for RNA isolation and subsequent gene expression analysis or collected in sterile PBS for collagenase digestion and flow cytometry.

Real-time PCR. RNA was isolated from lung lobe in TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA was normalized and transcribed into cDNA using iScript reverse transcription supermix (Bio-Rad) according to manufacturer's protocol. The following primer sequences/assays were used: *Hprt* (Mm03024075_m1), *Fstl1* (Mm00433371_m1), *Il-17a* (Mm00439618_m1), *Csf3* (Mm00438334_m1), *Cxcl1* (Mm04207460_m1), *Cxcl2* (Mm00436450_m1), *Cxcl5* (Mm00436451_g1), *Il22* (Mm00444241_m1), *Il23a* (Mm00444241_m1), *Il23r* (Mm00444241_m1), *Lcn2* (Mm00444241_m1), *Gapdh* (forward, 5′-AAT GTG TCC GTC GTG GAT CTG A-3′; reverse, 5′-GAT GCC TGC TTC ACC ACC TTC T-3′), and *Trdc* (forward, 5′-TGTTGCTTGTCTGGTGAAAGAT-3′; reverse 5′-GCACTGTACTTCCCGCTGG-3′). Depending on the primer set, real-time PCRs were run on a Bio-Rad CFX96 real-time system using iQ SYBR green supermix (Bio-Rad) or TaqMan universal PCR mastermix (Applied Biosystems).

Enzyme-linked immunosorbent assay. Lung homogenates were assayed for mouse FSTL-1 and the IL-17A protein. Lung homogenates were generated by homogenization of left lung lobe in 1 ml sterile PBS. The abundance of mouse FSTL-1 protein in lung homogenates was measured by an inhouse sandwich enzyme-linked immunosorbent assay (ELISA) protocol. A capture antibody (MAB1738; R&D Systems) was adhered to the bottom of a flat-bottomed 96-well plate overnight at 4°C. The plate was washed five times with wash buffer (PBS containing 0.05% Tween 20). The plate was then blocked with blocking buffer (PBS containing 1% bovine serum albumin and 5% sucrose) for 1 hour at room temperature. Standards and experimental samples were incubated for 1 hour at room temperature and then washed as previously stated. A biotinylated detection antibody (BAF1738; R&D Systems) was added for 1 hour at room temperature and then washed. Streptavidin-

horseradish peroxidase (HRP) (18-4100-51; eBioscience/ThermoFisher) was added, incubated for 20 minutes at room temperature, and then washed. An OptEIA 3,3′,5,5′ tetramethylbenzidine (TMB) substrate kit (BD Biosciences) was used to visualize protein concentration. A total of 1 M sulfuric acid was used to stop the substrate reaction, and the plate was read at a wavelength of 450 nm on a Synergy H1 plate reader (BioTek) using Gen5 v2.01 software. The commercially available Mouse IL-17A (homodimer) ELISA Ready-SET-Go! Kit (Invitrogen) was used according to the manufacture protocol's for assessing IL-17A protein levels.

Flow cytometry. Single-cell suspensions were obtained by digestion of lung lobe in Dulbecco's modified Eagle medium (DMEM) containing 4 mg/ml collagenase (Sigma-Aldrich) and 0.2 mg/ml DNase (Sigma-Aldrich) at 37°C with agitation for 1 hour, and then the digest was strained through a 70-μm filter. ACK lysis buffer (Gibco) was used to lyse red blood cells according to the manufacturer's protocol. Cell labeling was performed using the Foxp3/transcription factor staining buffer set (Invitrogen eBioscience) according to the manufacturer's protocol. Cells were labeled with the following antibodies: anti-IL-17A (eBiol7B7), anti-CD3 (17A2), anti-TCR$\gamma\delta$ (eBioGL3), anti-CD4 (RM4-5), and anti-CD8 (53-6.7). Data acquisition was performed on an LSR Fortessa analyzer (SORP) using FACSDiva software v8.0.1. Data were analyzed using FlowJo software v10.1 (TreeStar).

Absolute cell counts. Lung was digested and subjected to red blood cell lysis as prepared for flow cytometry. Total lung cells were enumerated with 0.2% trypan blue solution and an Invitrogen Countess automated cell counter. Percentages of lung CD11b$^+$Ly6G$^+$ neutrophils were obtained by flow cytometry using primary antibodies against mouse CD11b (M1/70) and Ly6G (1A8), LSR Fortessa, and FlowJo software v10.1. The absolute neutrophil number per mouse lung was enumerated using the total lung cell digest count and the flow cytometry percentage of CD11b$^+$Ly6G$^+$ cells. Lung CD3$^+$TCR$\gamma\delta^+$ cells were enumerated for absolute cell count in the same manner using flow cytometry antibodies, anti-CD3 (17A2), and anti-TCR$\gamma\delta$ (eBioGL3).

Statistical analysis. Data preparation and statistical analysis were performed with GraphPad Prism software v8. For analyses comparing two groups of data, an unpaired *t* test with assumed Gaussian distribution was utilized. For a concurrent analysis of three or more data sets, a nonmatching or nonpairing ordinary one-way analysis of variance (ANOVA) with assumed Gaussian distribution, where the mean of each data set was compared to the mean of every other data set, was utilized. Statistical significance was assigned to data analyses yielding probability values less than five percent ($P < 0.05$).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

ACKNOWLEDGMENTS

M.H., J.A.D., J.P., and B.T.C. conceived, designed, and executed studies; analyzed and interpreted results; and drafted and revised the manuscript globally. T.E. analyzed and interpreted results and drafted and revised the manuscript. R.H. conceived, designed FSTL-1 hypomorphic mouse, and interpreted *in vivo* studies. J.K.K. refined the studies, reviewed results, and revised the manuscript. All authors have given final approval for publication and agree to be accountable for the integrity of the information contained in the manuscript.

M.H., J.A.D., J.P., B.T.C., and T.E. do not have a commercial or other association that might pose a conflict of interest. R.H. has a patent null issued. J.K.K. reports grants from Novartis, personal fees from Boehringer Ingelheim, and personal fees from pH Pharma outside the submitted work; in addition, J.K.K. has a patent, PCT/US2020/025518 "Multivalent Vaccines Derived From Klebsiella Out Membrane Proteins," pending.

This work was supported by NIH grants F30AI114146 (T.E.), R01AI073556, T32AR052282 (R.H.), R35HL139930 (J.K.K.), and K08HL128809 (B.T.C.) and in part by the UPMC Children's Hospital of Pittsburgh (B.T.C.).

REFERENCES

1. Magill SS, O'Leary E, Janelle SJ, Thompson DL, Dumyati G, Nadle J, Wilson LE, Kainer MA, Lynfield R, Greissman S, Ray SM, Beldavs Z, Gross C, Bamberg W, Sievers M, Concannon C, Buhr N, Warnke L, Maloney M, Ocampo V, Brooks J, Oyewumi T, Sharmin S, Richards K, Rainbow J, Samper M, Hancock EB, Leaptrot D, Scalise E, Badrun F, Phelps R, Edwards JR, Emerging Infections Program Hospital Prevalence Survey Team. 2018. Changes in prevalence of health care–associated infections in U.S. hospitals. N Engl J Med 379:1732–1744. https://doi.org/10.1056/NEJMoa1801550.
2. CDC. 2019. Antibiotic threats in the United States, 2019. US Department of Health and Human Services, CDC, Washington, DC.
3. Moore TA, Moore BB, Newstead MW, Standiford TJ. 2000. Gamma delta-T cells are critical for survival and early proinflammatory cytokine gene expression during murine Klebsiella pneumonia. J Immunol 165:2643–2650. https://doi.org/10.4049/jimmunol.165.5.2643.
4. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, Shellito JE, Bagby GJ, Nelson S, Charrier K, Peschon JJ, Kolls JK. 2001. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J Exp Med 194:519–527. https://doi.org/10.1084/jem.194.4.519.
5. Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, Odden

AR, Shellito JE, Bagby GJ, Nelson S, Kolls JK. 2005. Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. J Exp Med 202:761–769. https://doi.org/10.1084/jem.20050193.
6. Cai S, Batra S, Lira SA, Kolls JK, Jeyaseelan S. 2010. CXCL1 regulates pulmonary host defense to Klebsiella infection via CXCL2, CXCL5, NF-kappaB, and MAPKs. J Immunol 185:6214–6225. https://doi.org/10.4049/jimmunol.0903843.
7. Chan YR, Liu JS, Pociask DA, Zheng M, Mietzner TA, Berger T, Mak TW, Clifton MC, Strong RK, Ray P, Kolls JK. 2009. Lipocalin 2 is required for pulmonary host defense against Klebsiella infection. J Immunol 182:4947–4956. https://doi.org/10.4049/jimmunol.0803282.
8. Chen K, Eddens T, Trevejo-Nunez G, Way EE, Elsegeiny W, Ricks DM, Garg AV, Erb CJ, Bo M, Wang T, Chen W, Lee JS, Gaffen SL, Kolls JK. 2016. IL-17 receptor signaling in the lung epithelium is required for mucosal chemokine gradients and pulmonary host defense against K. pneumoniae. Cell Host Microbe 20:596–605. https://doi.org/10.1016/j.chom.2016.10.003.
9. Murakami T, Hatano S, Yamada H, Iwakura Y, Yoshikai Y. 2016. Two types of interleukin 17A-producing gammadelta T Cells in protection against pulmonary infection with Klebsiella pneumoniae. J Infect Dis 214:1752–1761. https://doi.org/10.1093/infdis/jiw443.
10. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, Schwarzenberger P, Shellito JE, Kolls JK. 2001. Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. Am J Respir Cell Mol Biol 25:335–340. https://doi.org/10.1165/ajrcmb.25.3.4424.
11. Yang Y, Liu J, Mao H, Hu YA, Yan Y, Zhao C. 2009. The expression pattern of Follistatin-like 1 in mouse central nervous system development. Gene Expr Patterns 9:532–540. https://doi.org/10.1016/j.gep.2009.07.001.
12. Geng Y, Dong Y, Yu M, Zhang L, Yan X, Sun J, Qiao L, Geng H, Nakajima M, Furuichi T, Ikegawa S, Gao X, Chen YG, Jiang D, Ning W. 2011. Follistatin-like 1 (Fstl1) is a bone morphogenetic protein (BMP) 4 signaling antagonist in controlling mouse lung development. Proc Natl Acad Sci U S A 108:7058–7063. https://doi.org/10.1073/pnas.1007293108.
13. Sylva M, Li VS, Buffing AA, van Es JH, van den Born M, van der Velden S, Gunst Q, Koolstra JH, Moorman AF, Clevers H, van den Hoff MJ. 2011. The BMP antagonist follistatin-like 1 is required for skeletal and lung organogenesis. PLoS One 6:e22616. https://doi.org/10.1371/journal.pone.0022616.
14. Görgens SW, Raschke S, Holven KB, Jensen J, Eckardt K, Eckel J. 2013. Regulation of follistatin-like protein 1 expression and secretion in primary human skeletal muscle cells. Arch Physiol Biochem 119:75–80. https://doi.org/10.3109/13813455.2013.768270.
15. Miyabe M, Ohashi K, Shibata R, Uemura Y, Ogura Y, Yuasa D, Kambara T, Kataoka Y, Yamamoto T, Matsuo K, Joki Y, Enomoto T, Hayakawa S, Hiramatsu-Ito M, Ito M, Van Den Hoff MJ, Walsh K, Murohara T, Ouchi N. 2014. Muscle-derived follistatin-like 1 functions to reduce neointimal formation after vascular injury. Cardiovasc Res 103:111–120. https://doi.org/10.1093/cvr/cvu105.
16. Wei K, Serpooshan V, Hurtado C, Diez-Cunado M, Zhao M, Maruyama S, Zhu W, Fajardo G, Noseda M, Nakamura K, Tian X, Liu Q, Wang A, Matsuura Y, Bushway P, Cai W, Savchenko A, Mahmoudi M, Schneider MD, van den Hoff MJ, Butte MJ, Yang PC, Walsh K, Zhou B, Bernstein D, Mercola M, Ruiz-Lozano P. 2015. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature 525:479–485. https://doi.org/10.1038/nature15372.
17. Chen F, Hu Q, Huang H, Chen B, Xia Y, Liu W. 2017. Follistatin-like protein 1 increases transepithelial resistance in kidney epithelial cells through Akt signaling. Mol Med Rep 16:4341–4347. https://doi.org/10.3892/mmr.2017.7113.
18. Liu X, Liu Y, Li X, Zhao J, Geng Y, Ning W. 2017. Follistatin like-1 (Fstl1) is required for the normal formation of lung airway and vascular smooth muscle at birth. PLoS One 12:e0177899. https://doi.org/10.1371/journal.pone.0177899.
19. Jiang H, Zhang L, Liu X, Sun W, Kato K, Chen C, Li X, Li T, Sun Z, Han W, Zhang F, Xiao Q, Yang Z, Hu J, Qin Z, Adams RH, Gao X, He Y. 2020. Angiocrine FSTL1 (follistatin-like protein 1) insufficiency leads to atrial and venous wall fibrosis via SMAD3 activation. Arterioscler Thromb Vasc Biol 40:958–972. https://doi.org/10.1161/ATVBAHA.119.313901.
20. Ma R, Wang M, Gao S, Zhu L, Yu L, Hu D, Zhu L, Huang W, Zhang W, Deng J, Pan J, He H, Gao Z, Xu J, Han X. 2020. miR-29a promotes the neurite outgrowth of rat neural stem cells by targeting extracellular matrix to repair brain injury. Stem Cells Dev 29:599–614. https://doi.org/10.1089/scd.2019.0174.
21. Dong Y, Geng Y, Li L, Li X, Yan X, Fang Y, Li X, Dong S, Liu X, Li X, Yang X, Zheng X, Xie T, Liang J, Dai H, Liu X, Yin Z, Noble PW, Jiang D, Ning W. 2015. Blocking follistatin-like 1 attenuates bleomycin-induced pulmonary fibrosis in mice. J Exp Med 212:235–252. https://doi.org/10.1084/jem.20121878.
22. Gu C, Wang X, Long T, Wang X, Zhong Y, Ma Y, Hu Z, Li Z. 2018. FSTL1 interacts with VIM and promotes colorectal cancer metastasis via activating the focal adhesion signalling pathway. Cell Death Dis 9:654. https://doi.org/10.1038/s41419-018-0695-6.
23. Lau MC, Ng KY, Wong TL, Tong M, Lee TK, Ming XY, Law S, Lee NP, Cheung AL, Qin YR, Chan KW, Ning W, Guan XY, Ma S. 2017. FSTL1 promotes metastasis and chemoresistance in esophageal squamous cell carcinoma through NFkappaB-BMP signaling cross-talk. Cancer Res 77:5886–5899. https://doi.org/10.1158/0008-5472.CAN-17-1411.
24. Campfield BT, Nolder CL, Marinov A, Bushnell D, Davis A, Spychala C, Hirsch R, Nowalk AJ. 2014. Follistatin-like protein 1 is a critical mediator of experimental Lyme arthritis and the humoral response to Borrelia burgdorferi infection. Microb Pathog 73:70–79. https://doi.org/10.1016/j.micpath.2014.04.005.
25. Henkel M, Partyka J, Gregory AD, Forno E, Cho MH, Eddens T, Tout AR, Salamacha N, Horne W, Rao KS, Wu Y, Alcorn JF, Kostka D, Hirsch R, Celedon JC, Shapiro SD, Kolls JK, Campfield BT. 2020. FSTL-1 attenuation causes spontaneous smoke-resistant pulmonary emphysema. Am J Respir Crit Care Med 201:934–945. https://doi.org/10.1164/rccm.201905-0973OC.
26. Miller M, Beppu A, Rosenthal P, Pham A, Das S, Karta M, Song DJ, Vuong C, Doherty T, Croft M, Zuraw B, Zhang X, Gao X, Aceves S, Chouiali F, Hamid Q, Broide DH. 2015. Fstl1 promotes asthmatic airway remodeling by inducing oncostatin M. J Immunol 195:3546–3556. https://doi.org/10.4049/jimmunol.1501105.
27. Campfield BT, Eddens T, Henkel M, Majewski M, Horne W, Chaly Y, Gaffen SL, Hirsch R, Kolls JK. 2017. Follistatin-like protein 1 modulates IL-17 signaling via IL-17RC regulation in stromal cells. Immunol Cell Biol 95:656–665. https://doi.org/10.1038/icb.2017.26.
28. Kudo-Saito C, Fuwa T, Murakami K, Kawakami Y. 2013. Targeting FSTL1 prevents tumor bone metastasis and consequent immune dysfunction. Cancer Res 73:6185–6193. https://doi.org/10.1158/0008-5472.CAN-13-1364.
29. Kudo-Saito C, Ishida A, Shouya Y, Teramoto K, Igarashi T, Kon R, Saito K, Awada C, Ogiwara Y, Toyoura M. 2018. Blocking the FSTL1-DIP2A axis improves anti-tumor immunity. Cell Rep 24:1790–1801. https://doi.org/10.1016/j.celrep.2018.07.043.
30. Chaly Y, Fu Y, Marinov A, Hostager B, Yan W, Campfield B, Kellum JA, Bushnell D, Wang Y, Vockley J, Hirsch R. 2014. Follistatin-like protein 1 enhances NLRP3 inflammasome-mediated IL-1beta secretion from monocytes and macrophages. Eur J Immunol 44:1467–1479. https://doi.org/10.1002/eji.201344063.
31. Miyamae T, Marinov AD, Sowders D, Wilson DC, Devlin J, Boudreau R, Robbins P, Hirsch R. 2006. Follistatin-like protein-1 is a novel proinflammatory molecule. J Immunol 177:4758–4762. https://doi.org/10.4049/jimmunol.177.7.4758.
32. Clutter SD, Wilson DC, Marinov AD, Hirsch R. 2009. Follistatin-like protein 1 promotes arthritis by up-regulating IFN-gamma. J Immunol 182:234–239. https://doi.org/10.4049/jimmunol.182.1.234.
33. Wilson DC, Marinov AD, Blair HC, Bushnell DS, Thompson SD, Chaly Y, Hirsch R. 2010. Follistatin-like protein 1 is a mesenchyme-derived inflammatory protein and may represent a biomarker for systemic-onset juvenile rheumatoid arthritis. Arthritis Rheum 62:2510–2516. https://doi.org/10.1002/art.27485.
34. Chaly Y, Marinov AD, Oxburgh L, Bushnell DS, Hirsch R. 2012. FSTL1 promotes arthritis in mice by enhancing inflammatory cytokine/chemokine expression. Arthritis Rheum 64:1082–1088. https://doi.org/10.1002/art.33422.
35. Ni S, Li C, Xu N, Liu X, Wang W, Chen W, Wang Y, van Wijnen AJ. 2018. Follistatin-like protein 1 induction of matrix metalloproteinase 1, 3 and 13 gene expression in rheumatoid arthritis synoviocytes requires MAPK, JAK/STAT3 and NF-kappaB pathways. J Cell Physiol 234:454–463. https://doi.org/10.1002/jcp.26580.
36. Eddens T, Kolls JK. 2012. Host defenses against bacterial lower respiratory tract infection. Curr Opin Immunol 24:424–430. https://doi.org/10.1016/j.coi.2012.07.005.
37. Chen K, McAleer JP, Lin Y, Paterson DL, Zheng M, Alcorn JF, Weaver CT, Kolls JK. 2011. Th17 cells mediate clade-specific, serotype-independent mucosal immunity. Immunity 35:997–1009. https://doi.org/10.1016/j.immuni.2011.10.018.
38. Lu Y-J, Gross J, Bogaert D, Finn A, Bagrade L, Zhang Q, Kolls JK, Srivastava

A, Lundgren A, Forte S, Thompson CM, Harney KF, Anderson PW, Lipsitch M, Malley R. 2008. Interleukin-17A mediates acquired immunity to pneumococcal colonization. PLoS Pathog 4:e1000159. https://doi.org/10.1371/journal.ppat.1000159.
39. Michel M-L, Keller AC, Paget C, Fujio M, Trottein F, Savage PB, Wong C-H, Schneider E, Dy M, Leite-de-Moraes MC. 2007. Identification of an IL-17-producing NK1.1(neg) iNKT cell population involved in airway neutrophilia. J Exp Med 204:995–1001. https://doi.org/10.1084/jem.20061551.
40. Moore TA, Perry ML, Getsoian AG, Newstead MW, Standiford TJ. 2002. Divergent role of gamma interferon in a murine model of pulmonary versus systemic Klebsiella pneumoniae infection. Infect Immun 70:6310–6318. https://doi.org/10.1128/iai.70.11.6310-6318.2002.
41. Roark CL, Simonian PL, Fontenot AP, Born WK, O'Brien RL. 2008. gammadelta T cells: an important source of IL-17. Curr Opin Immunol 20:353–357. https://doi.org/10.1016/j.coi.2008.03.006.
42. Liu X, Wang Y, Lu H, Li J, Yan X, Xiao M, Hao J, Alekseev A, Khong H, Chen T, Huang R, Wu J, Zhao Q, Wu Q, Xu S, Wang X, Jin W, Yu S, Wang Y, Wei L, Wang A, Zhong B, Ni L, Liu X, Nurieva R, Ye L, Tian Q, Bian XW, Dong C. 2019. Genome-wide analysis identifies NR4A1 as a key mediator of T cell dysfunction. Nature 567:525–529. https://doi.org/10.1038/s41586-019-0979-8.
43. Liebmann M, Hucke S, Koch K, Eschborn M, Ghelman J, Chasan AI, Glander S, Schadlich M, Kuhlencord M, Daber NM, Eveslage M, Beyer M, Dietrich M, Albrecht P, Stoll M, Busch KB, Wiendl H, Roth J, Kuhlmann T, Klotz L. 2018. Nur77 serves as a molecular brake of the metabolic switch during T cell activation to restrict autoimmunity. Proc Natl Acad Sci U S A 115:E8017–E8026. https://doi.org/10.1073/pnas.1721049115.
44. Itohara S, Mombaerts P, Lafaille J, Iacomini J, Nelson A, Clarke AR, Hooper ML, Farr A, Tonegawa S. 1993. T cell receptor delta gene mutant mice: independent generation of alpha beta T cells and programmed rearrangements of gamma delta TCR genes. Cell 72:337–348. https://doi.org/10.1016/0092-8674(93)90112-4.