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RNA-binding motif RBM3 suppresses IL5, IL13, and IL17 production in innate lymphoid cells and reduces lung inflammation

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Allyssa Nicholle Strohm

Committee in charge:

Professor Taylor A. Doherty, Chair
Professor Cressida Madigan, Co-chair
Professor Lisa McDonnell

2021

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The Thesis of Allyssa Nicholle Strohm is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

University of California San Diego

2021

DEDICATION

I would like to dedicate this to my Mom, Grandma, and family. Thank you for always supporting me and my dreams, and for your unconditional love.

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Results discussed here are currently under review for publication. The thesis author will be a co-author of this material.

This thesis contains material as it appears in the submitted manuscript, currently under review, titled “RNA-binding protein RBM3 negatively regulates innate lymphoid cells (ILCs) and lung inflammation”. Badrani, Jana; Strohm, Allyssa; Doherty, Taylor. The thesis author is a co-author of the manuscript.

ABSTRACT OF THESIS

RNA-binding motif RBM3 suppresses IL5, IL13, and IL17 production in innate lymphoid cells and reduces lung inflammation

by

Allyssa Nicholle Strohm

Master of Science in Biology

University of California San Diego, 2021

Professor Taylor Doherty, Chair
Professor Cressida Madigan, Co-chair

Group 2 innate lymphoid cells (ILC2s) promote type 2 inflammation in pulmonary diseases through the release of cytokines. ILC2s are a subset ILCs that also includes ILC1s and ILC3s. In response to an allergen assault, the airway epithelium releases alarmin cytokines that

activate ILC2s to robustly produce inflammatory type 2 cytokines. However, while the processes that cause ILC2 activation and potentiation of lung inflammation are well studied, mechanisms surrounding the suppression of ILC function during an allergen assault are poorly understood. To determine the role of the highly expressed RNA binding protein RNA Binding Motif 3 (RBM3) on ILC2s, we challenged *rbm3-/-* and wild type (WT) mice with the fungal allergen *Alternaria alternata* and observed changes in cytokine production using flow cytometry. Our data shows that *rbm3-/-* mice have increased production of the type 2 cytokines IL5, IL13, but also the ILC3 or Th17 cytokine IL17A, compared to WT mice. Flow cytometry revealed that the *rbm3-/-* ILCs had increased cytokine production specific to IL5, IL13, and IL17. These findings confirm that RBM3 directly regulates the function of ILCs upon activation by an allergen. Ultimately, RBM3 may serve a protective role in preventing excessive lung damage and inflammation in allergic airway diseases.

Introduction

Asthma is a chronic pulmonary obstructive disease that causes shortness of breath, chest tightness, and inflammation characterized by the presence of a type of white blood cells known as eosinophils (eosinophilia) (Bousquet et al. 1990). Historically, eosinophilia and mucus production associated with type 2 inflammatory lung diseases are often directly attributed to the function of adaptive immune T helper 2 (Th2) cells (Karta et al. 2016). However, in the last decade, innate lymphoid cells (ILCs) have emerged as key regulators of inflammation and fibrosis in several lung diseases including pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), and asthma (Halim et al. 2012; Hams et al. 2014; Silver et al. 2016). As the innate counterpart to T cells, ILCs are bone-marrow derived cells that lack the antigen receptors for T and B cells (Artis and Spits 2015). Instead of responding to antigen receptor stimulation by a specific foreign substance, ILCs respond to alarmin proteins released during tissue damage (Kotas and Locksley 2018).

Similar to T-cell function, ILCs respond to a stimulus by producing small proteins called cytokines that have different targeted effects on inflammation. ILCs can be separated into 3 categories based on the cytokines they produce (Vivier et al. 2018). ILC1s include natural killer (NK) cells and produce interferon-gamma (IFN-γ); ILC2s produce the type 2 cytokines interleukin 4 (IL-4), IL-5, and IL-13; ILC3s produce Th17 cytokines IL-17 and IL-22 (Vivier et al. 2018). While all subsets of ILCs are tissue resident cells that are found at different mucosal linings throughout the body, ILC2s are the most common ILC population within the lung (Mindt et al. 2018). As tissue resident cells in the lung, ILC2s provide a critical immediate response to irritants and allergens (Gasteiger et al. 2015).

ILC2s play critical roles in the onset of type 2 inflammation in mice upon challenge with allergens such as papain, ovalbumin, house dust mite, and fungal allergen *Alternaria alternata* (Klein Wolterink et al. 2012). Upon allergen assault, the epithelial tissue will release alarmin cytokines IL33, thymic stromal lymphopoietin (TSLP), and/or IL25 which activate ILC2s to produce the type 2 cytokines (Divekar and Kita 2015; Vivier et al. 2018). Once released, IL4 stimulates immunoglobulin (Ig) E and IgG antibody synthesis from B cells, while IL5 and IL13 will induce eosinophilia, mucus production, and airway hyperresponsiveness, resulting in an asthmatic phenotype (Paul and Zhu 2010; Lambrecht et al. 2019). Therefore, by understanding how ILCs are regulated when activated via allergen assault, insight can be gained for future novel therapeutic targets for allergic asthma.

While there are several studies that examine the activation of ILC2s in an airway, there are few studies that address how ILC2 cytokine production is regulated outside of cytokines and mediators. Previous work from our group has illustrated a role for the cysteinyl leukotriene receptor 1 (CysLT1R) in potentiating type 2 cytokine production from ILC2s (Doherty et al. 2013). Moreover, other groups have identified cytokines that suppress ILC2s ability to produce a type 2 response (Duerr et al. 2016; Moro et al. 2016). While these studies and others have identified specific mechanisms for regulating type 2 cytokine production from ILCs through CysLT receptors, lipid mediators, and other regulatory cytokines, our knowledge of novel mechanisms of ILC2 suppression is limited.

One mechanism of suppression of immune cell cytokine production may involve post-transcriptional control by the actions of RNA-binding proteins (RBPs). RBPs directly or indirectly act on mRNA to encourage or halt translation (Stoecklin and Anderson 2006). Furthermore, RBPs are important players in the stability of mRNA and help regulate mRNA

transcription, splicing, and intracellular mRNA trafficking (Gebauer et al. 2020). While the roles of some RBPs in the processing of immune cell mRNA has been examined, the function of many RBPs remains to be elucidated (Turner and DÍaz-Muñoz 2018).

Our lab has discovered via RNA-sequencing that RNA binding motif 3 (RBM3) is a highly expressed RBP in ILCs (Badrani et al, unpublished). RBM3 is tied to maintaining cell viability and increasing cell proliferation during cold shock-induced stress (Danno et al. 1997; Wellmann et al. 2010). No previous reports have identified a role for RBM3 in ILCs. Our lab has found increased RBM3 expression on ILCs that were activated with the fungal allergen *Alternaria*, suggesting RBM3 is a potential novel regulator of ILCs during type 2 inflammatory responses (Badrani et al, unpublished).

Through the use of *rbm3-/-* mice, our lab demonstrated that upon ILC activation by *Alternaria* or alarmin cytokine IL33, RBM3 negatively regulates the ILC2-driven pulmonary type 2 response as well as IL-17 cytokine production (Badrani et al, unpublished). This negative regulation was observed through increased production of the type 2 cytokines IL-5, IL-13, and increased production of Th17 cytokine, IL-17, in *rbm3-/-* mice compared to wild-type (WT) mice. Furthermore, our lab identified an overexpression of CysLT1R on ILCs in *rbm3-/-* mice. The overexpression of the CysLT1R could prove a potential mechanism for the increase in type 2 cytokines, but ongoing work will confirm this conjecture (Badrani et al, unpublished).

While recent work by Badrani et al has shown that RBM3 negatively regulates type 2 and IL-17 cytokines, these findings raise an important question. The question is whether ILC2s are also producing IL-17 (which has previously been described as "ILC2-17s") or whether separate populations of ILC3s in the airway are responsible for IL-17 production that is increased in *rbm3-/-* mice. Considering that these cytokine increases were originally observed as independent

cytokine increases in broad ILC heterogenous populations, we aimed to examine whether IL-5, IL-13, and IL-17 cytokines are being produced by the same ILCs "ILC2-17s" or by independent ILC3s (Badrani et al, unpublished). This is important as ILC2-17s have been found in some settings to be more pathogenic and also termed "inflammatory ILC2s".

To address this question, my recent work examined the production of both IL5 and IL17 as well as IL13 and IL17 production from ILCs via dual cytokine staining and flow cytometry. Our preliminary data shown below suggests that ILC2s in *rbm3*-/- mice directly are also producing IL-17 upon allergen challenge supporting that these are ILC2-17s or inflammatory ILC2s.

Results

a)

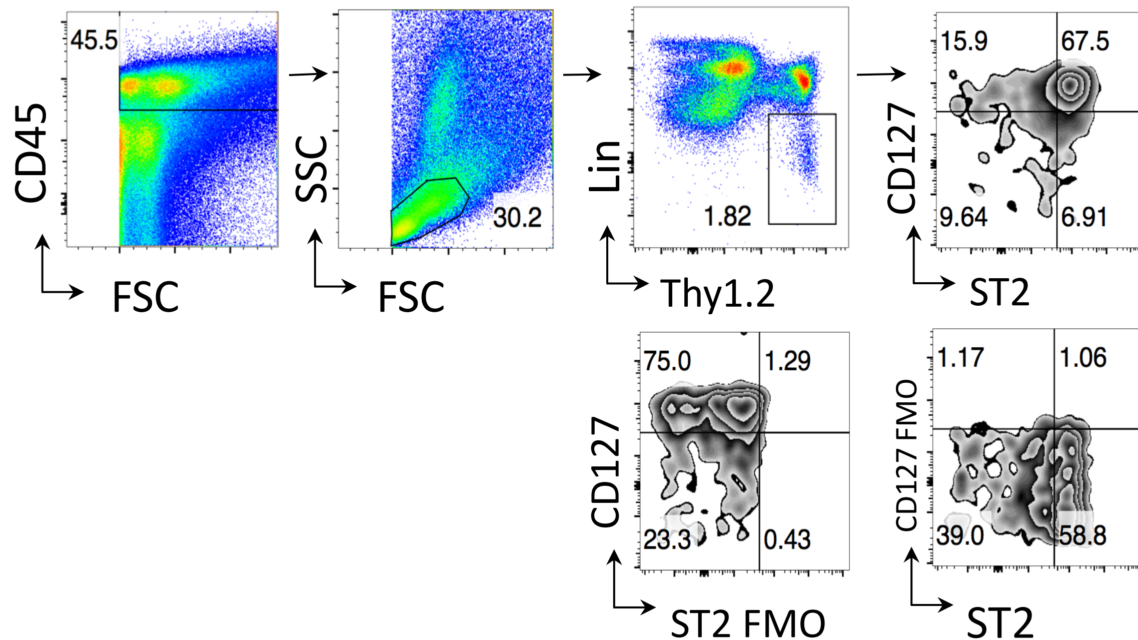


Figure 1 RBM3 is induced by *Alternaria* and is highly expressed in mouse ILCs.
Wild-type mice were challenged with 50 μg of *Alternaria* four times over 10 days to activate and expand the ILC population. (a) ILCS were gated as CD45+ Lineage- Thy1.2+ Lymphocytes with ILC subsets identified based on ST2 and CD127 expression. Four subsets of ILCs (ST2+ CD127+, ST2+CD127-, ST2-CD127+, ST2-CD127-) were sorted and collected for bulk RNA-sequencing and RBM3 transcriptome analysis. Gating was based on full minus one controls (FMOs) and representative FACS plots are shown. (b) Heatmap showing the relative expression of 207 RBPs in the sorted ILC subsets. Centroid linkage or Manhattan clustering used. (c) Heatmap showing the relative expression of the top 25 highly expressed RBPs in the four ILC subtypes. RBPs involved in splicing or homeostatic cell cycle were excluded. Centroid linkage or Manhattan clustering used. (d) RBM3 expression in lung ILCs from naïve, PBS, or *Alternaria* challenged mice. Grey= isotype, Black= Naïve, Blue= PBS, Red= *Alternaria* (after 24hrs).

Figure 1 continued

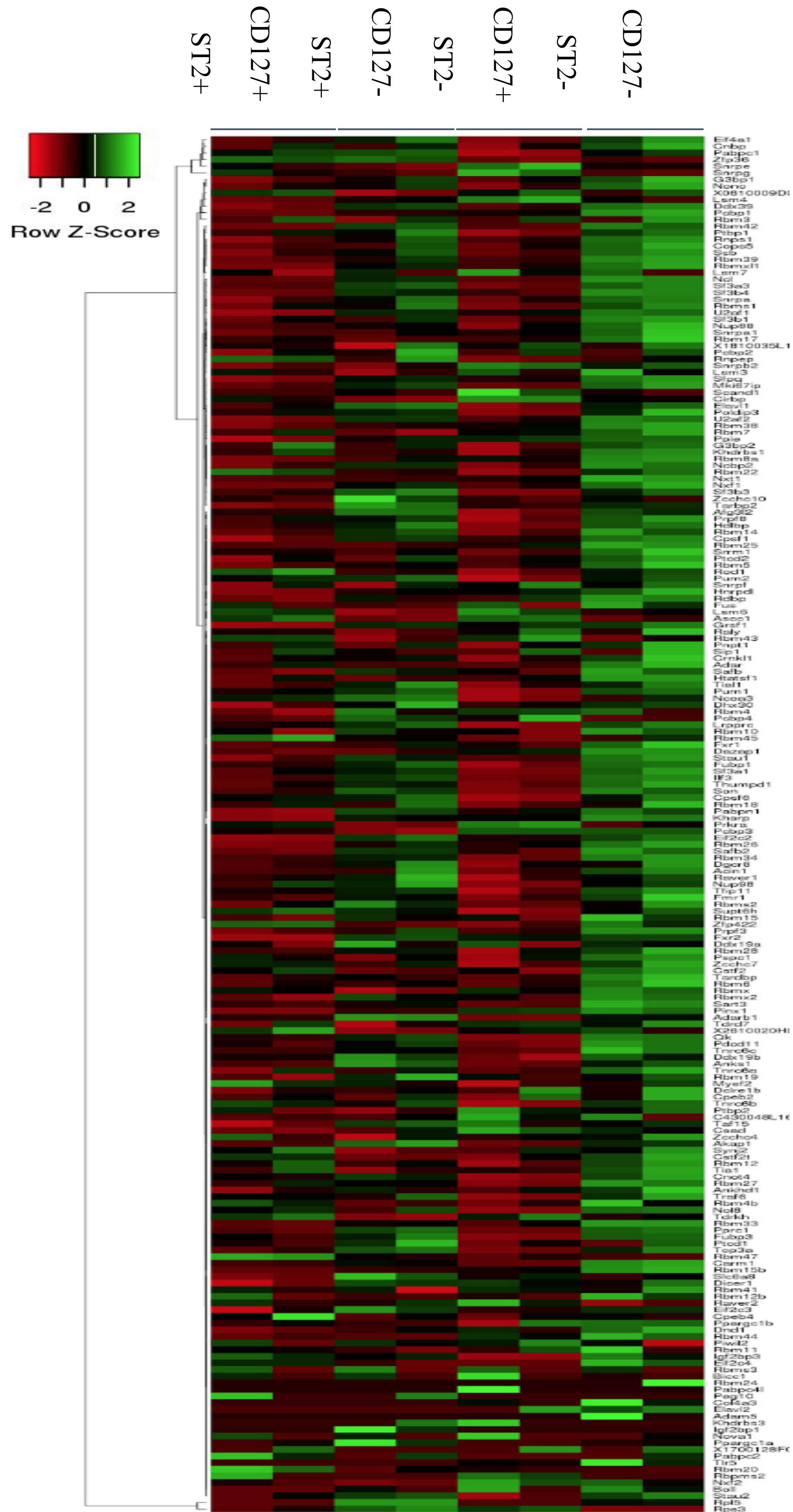
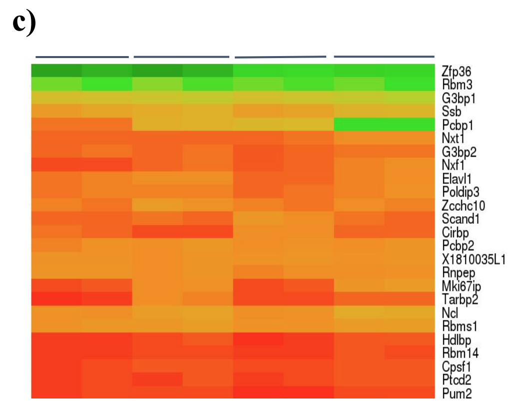


Figure 1 continued

c)



d)

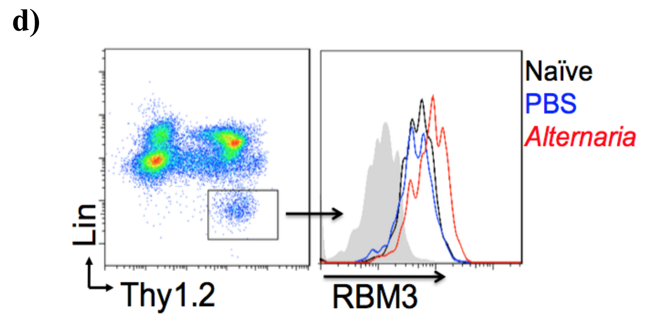


Figure 2 RBM3 suppresses lung inflammation in *Alternaria*-challenged mice.
WT and *rbm3-/-* mice were challenged with 10μg *Alternaria* three times over 7 days. Data is representative of 3 independent experiments (n=4 per experiment). (a) Total BAL and Lung eosinophil cell numbers and representative FACS plots. (b) Total BAL and Lung neutrophil cell numbers and representative FACS plots. WT and *rbm3-/-* mice were challenged with 20μg and 10μg *Alternaria* four times over 10 days. Data is representative of 4 independent experiments (n=4 per experiment). (c) Total BAL and lung eosinophils. (d) Total BAL and lung neutrophils. (e) Type 2 cytokine levels detected in BAL fluid via ELISA. (f) H&E and PAS lung sections at 20X; scale bar is 100μM. Images are representative of airways from 4 mice per group. *$p<0.05$, **$p<0.01$, ***$p<0.001$, Mann-Whitney Test.

Figure 2 continued

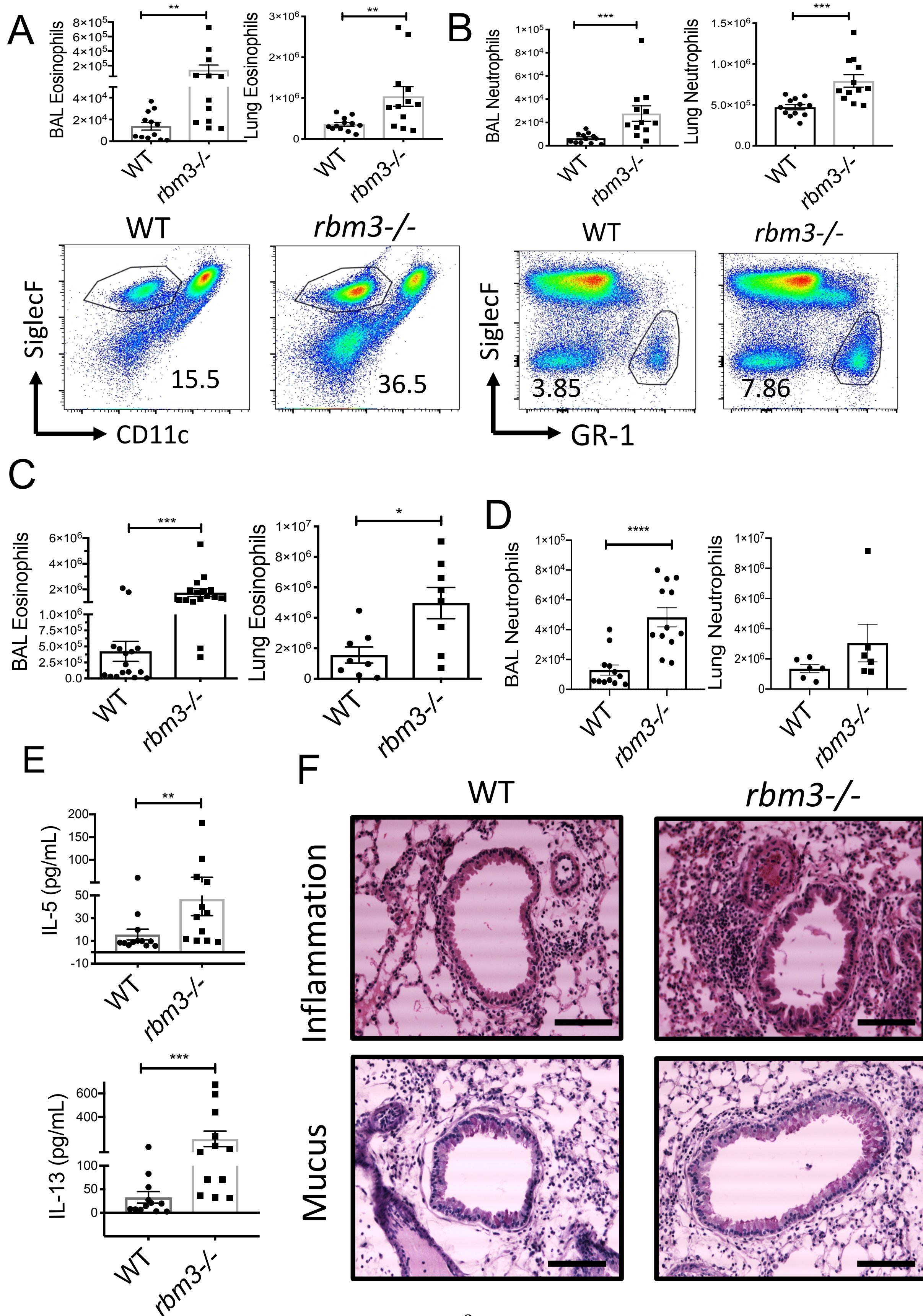


Figure 3 Increased pulmonary ILC type 2 and 17 cytokine secretion in *rbm3-/-* mice. WT and *rbm3-/-* mice were challenged with 20μg and 10μg *Alternaria* four times over 10 days. Data are representative of 4 independent experiments (n=4 per group). (a) Total lung lineage-Thy1.2+ ILC numbers and representative FACS plots with ILC percentage. (b) Total number of Ki67 expressing ILCs and representative FACS plots with Ki67-producing cell percentage. Gating based on isotype control. (c) Total IL5 and IL13 producing ILCs in the lung and representative FACS plots with IL5 and IL13-producing cell percentages. Gating is based on isotype control. Mice were challenged with 25μg *Alternaria* three times over 7 days. (d) Total IL17A production and representative FACS plots of IL17A percentages. Data are representative of 4 mice per group. *Rag2-/-* and *rbm3-/-rag2-/-* mice were challenged with 20μg *Alternaria* four times over 10 days. Data are representative of 4 mice per group. (e-g) Cell totals for ILCs, Ki67-producing ILCs, IL5-producing ILCs, and IL13-producing ILCs. (h) Total BAL and lung eosinophils. Unpaired T test, *p<0.05, **p<0.01, ***p<0.001.

Figure 3 continued

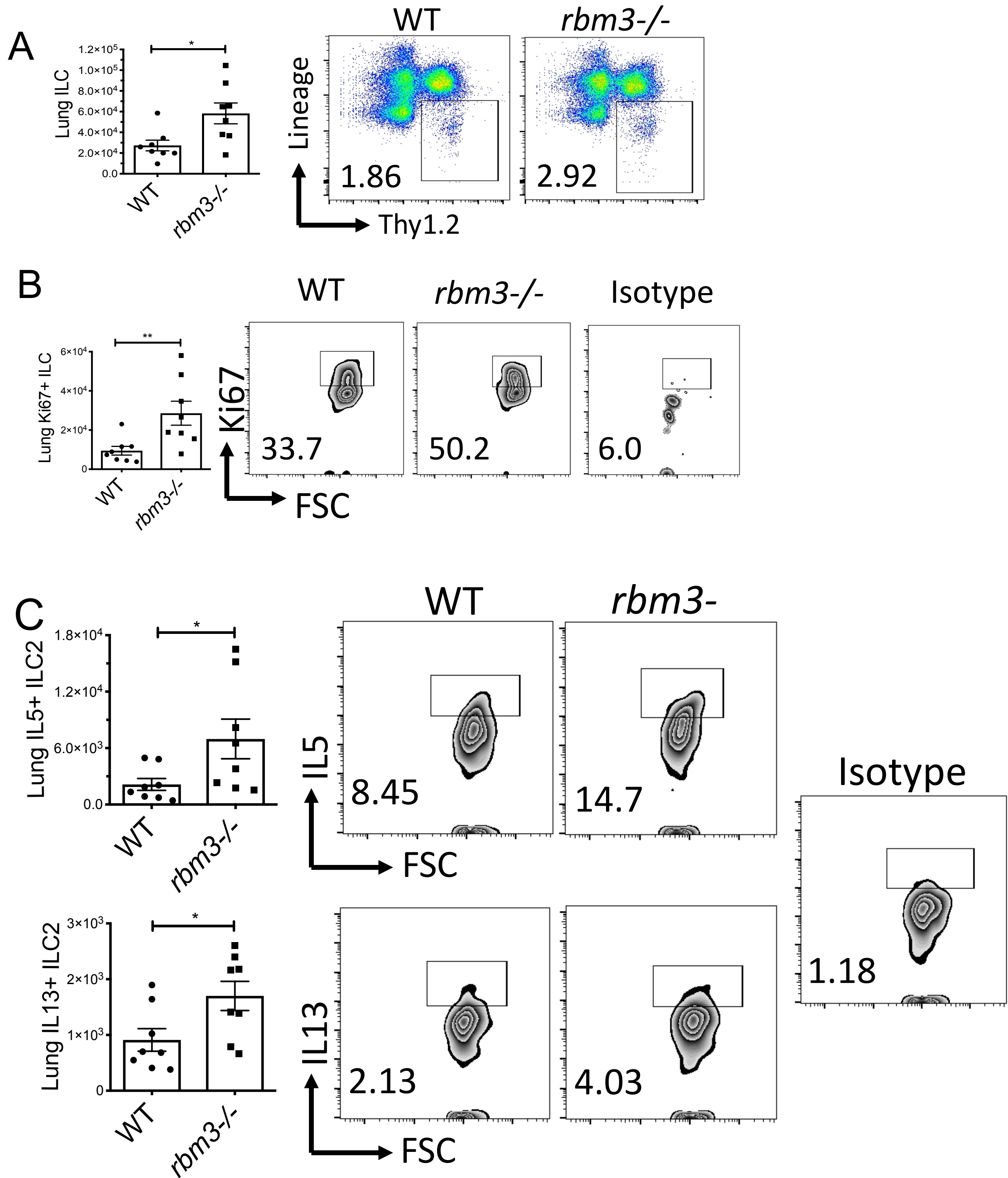
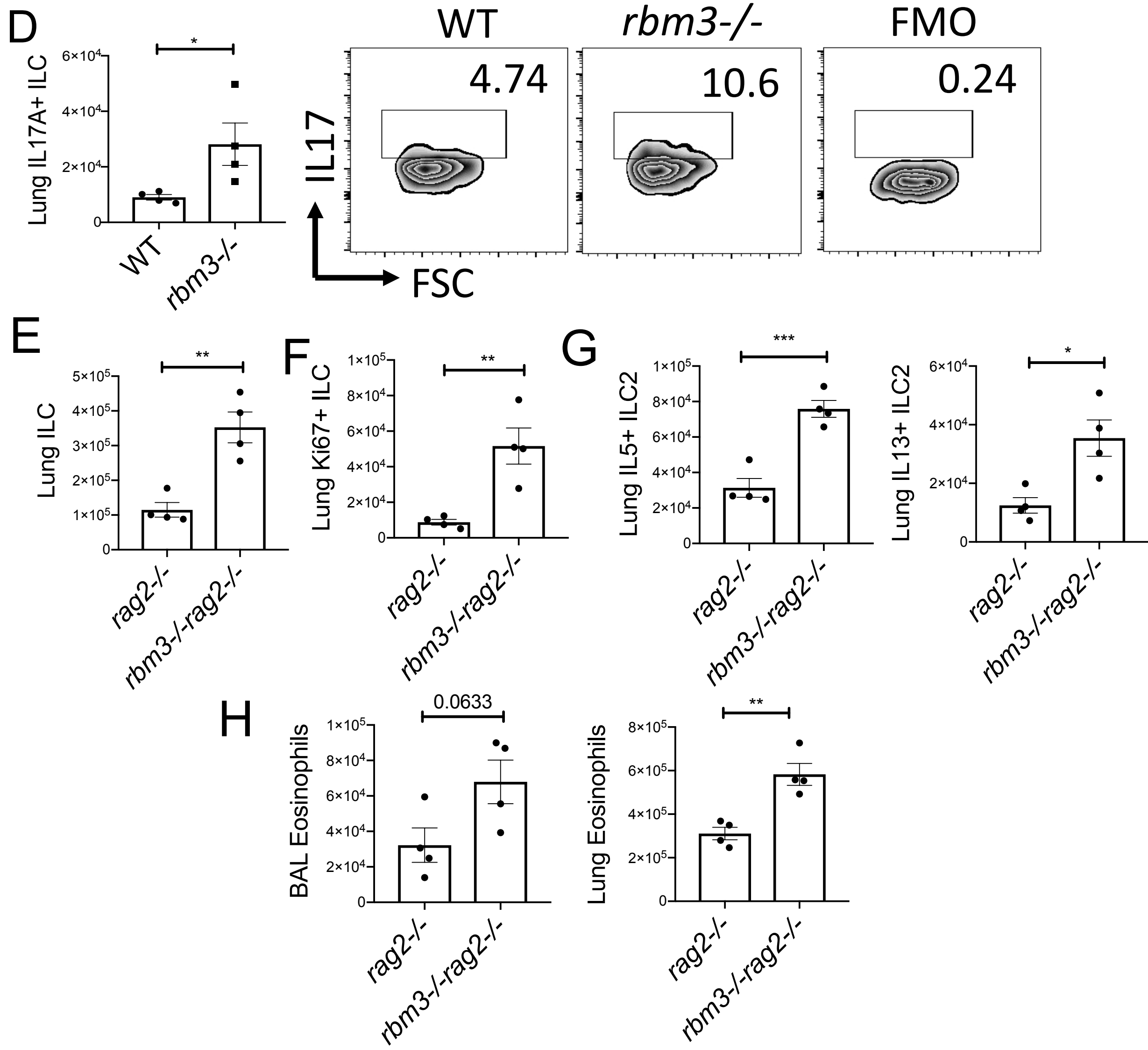


Figure 3 continued



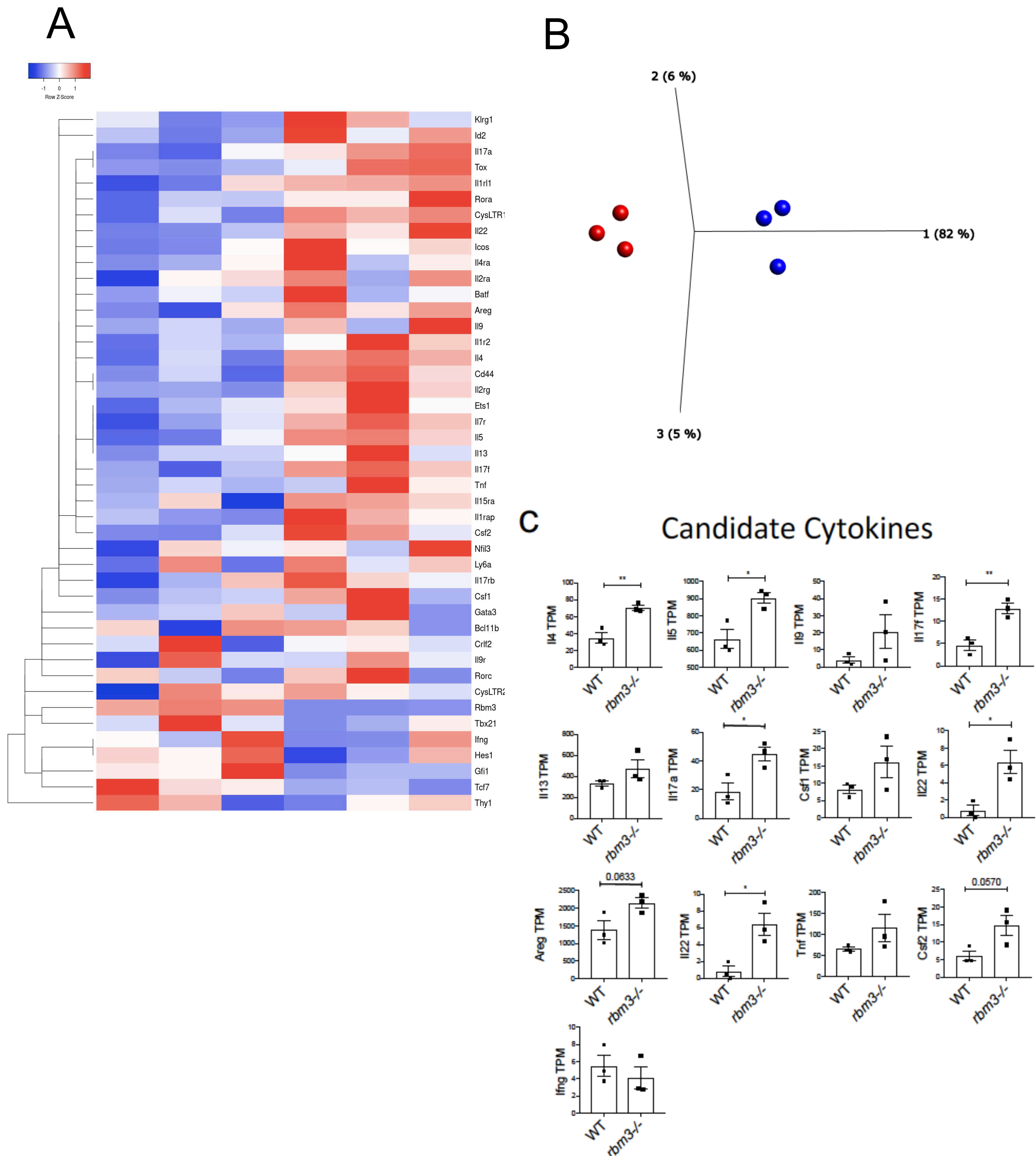
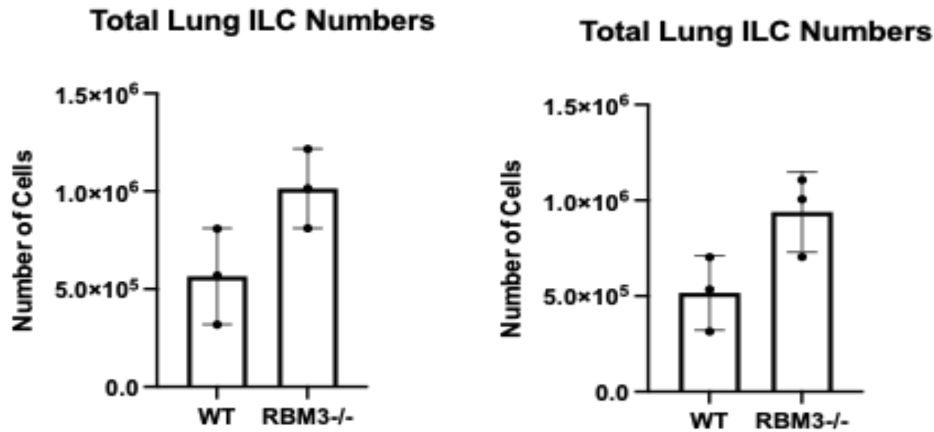


Figure 4 Transcriptome analysis reveals activated ILC programs. WT and *rbm3-/-* mice were intranasally challenged with 25μg *Alternaria* three times over 7 days. Lineage-Thy1.2+ ILCs were FACS sorted and purified. Bulk RNA-sequencing was performed. (a) Relative expression levels of select ILC transcripts. One minus Kendall's correlation and single linkage used. (b) Principal component analysis (PCA) of WT (blue) and *rbm3-/-* (red) samples. (c) Transcripts per million of select cytokine transcripts. *p<0.05, **p<0.01, unpaired T test

a)



b)

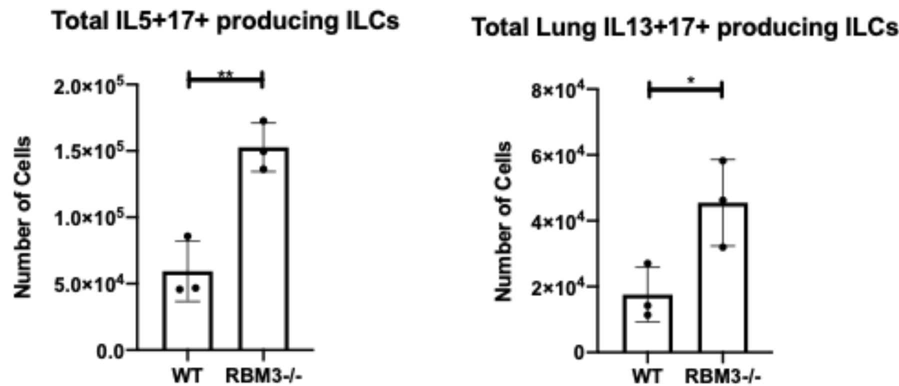
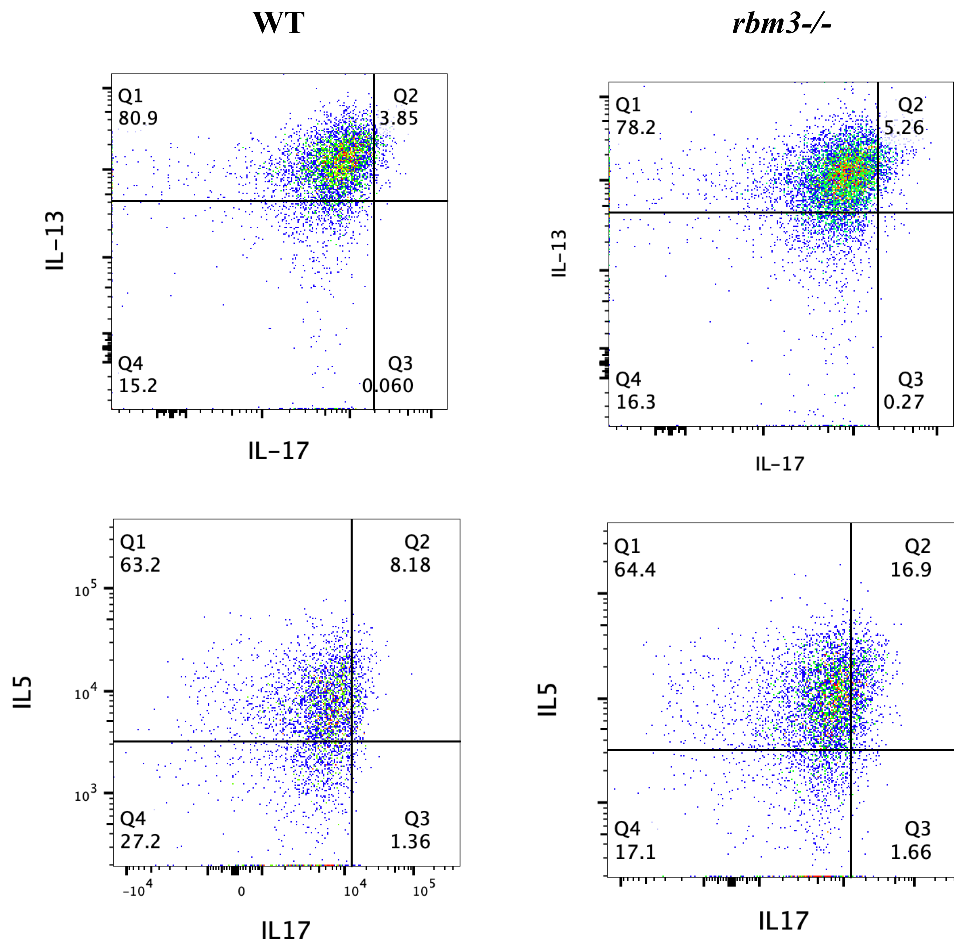


Figure 5 RBM3 suppresses concurrent production of IL5, IL13, and IL17 from ILCs. Three WT and three *rbm3-/-* mice were challenged intranasally with 25μg of *Alternaria* 3 times over 7 days to activate and expand the ILC population *in-vivo*. Lungs were collected and single cell suspensions were stained with fluorescently tagged antibodies to quantify the total number of ILCs (a) and ILCs producing IL5 and IL17 (b) or ILCs producing IL13 and IL17 (b). a) Total number of identified lung ILCs from individual samples stained with IL5 and IL17 (5/17) or IL13 and IL17 (13/17) in WT and *rbm3-/-* mice. Identifies total number of ILCs present between different staining groups. b) Total number of lung ILCs co-producing IL5 and IL17 (IL5+IL17+) or IL13 and IL17 (IL13+IL17+) in WT and *rbm3-/-* mice. Identifies total number of ILCs producing IL5/17 and IL13/17. c) Representative FACS Plots of dual staining of IL5/17 or IL13/17 dual expression. Data are representative of one experiment with 3 mice per group. Each bar represents the average of the 3 data points per group. Error bars represent one standard deviation of each data set. *p<0.05, **p<0.01, unpaired T test.

Figure 5 continued

c)



Results

To assess the which genes are activated upon ILC activation, WT mice were intranasally challenged 4 times over 10 days with 50μg *Alternaria.* Lungs were collected and surface stained as described in the methods, and ILCs were gated as CD45+ Lineage- Thy1.2+ Lymphocytes with ILC subsets identified based on ST2 and CD127 expression. The Lin-Thy1.2+ population was then isolated and FACS purified based on cell surface expression of CD127 and ST2 (Figure 1a). These cells were then prepared for RNA-seq library preparation and sequencing (see methods). RNA-seq revealed that there are 207 highly expressed RBPs in the activated WT Lin-Thy1.2+ subpopulations (Figure 1b). Of these, we previously examined the top 25 highly expressed RBPs amongst the four subsets and found that *zfp36* was the most highly expressed (Figure 1c). However, extensive work has identified a role for *zfp36* in regulating TNF alpha synthesis and secretion (Taylor et al. 1996). Further, *zfp36-/-* mice were prone to develop spontaneous inflammation and autoimmune diseases (Taylor et al. 1996). Since we were interested in studying novel RBP regulation using *in-vivo* asthma models, we explored other potential RBPs that had more viable mouse knock-out models. RBM3 was the second most highly expressed RBP in lung ILCs upon allergen challenge (Figure 1c). To confirm the expression of RBM3 at a protein level in ILCs, WT mice were intranasally administered *Alternaria* and the lung ILCs were examined for RBM3 expression via flow cytometry. Lineage-Thy1.2+ ILCs from *Alternaria*-challenged mice had an increase in RBM3 expression compared to the naïve, PBS, and isotype controls. This data suggests that RBM3 is a highly expressed RBP in activated lung ILCs and may regulate ILC function.

To determine the role of RBM3 *in-vivo*, WT and *rbm3-/-* mice were intranasally challenged with 10μg *Alternaria* three times over 7 days. Lungs and BAL fluid was collected

and analyzed for eosinophils and neutrophils as an endpoint for airway inflammation. *Rbm3-/-* mice had significantly increased BAL and lung eosinophils and neutrophils compared to the WT mice (Figure 2a, b). To confirm this using another protocol, we found the same results when WT and *rbm3-/-* mice were challenged with 20μg *Alternaria* four times over 10 days with *rbm3-/-* mice having increased eosinophilia and neutrophilia in the lung and BAL (Figure 2c, d). Upon examination for the type 2 cytokines IL5 and IL13, challenged *rbm3-/-* mice had significant increases in cytokine production in the BAL compared to WT mice (Figure 2e). H&E and PAS staining revealed increased inflammation and mucus production in the challenged *rbm3-/-* mice (Figure 2f). Overall, compared to WT mice, *rbm3-/-* mice have an increase in airway and lung inflammation when challenged with *Alternaria.*

After observing an increase in type 2 inflammation in *rbm3-/-* mice, we sought to elucidate whether ILCs were being activated and proliferated in *rbm3-/-* mice upon *Alternaria* challenge. Lineage-Thy1.2+ ILCs numbers were increased upon allergen assault compared to the challenged WT mice (Figure 3a). Further, there was a significant increase in the proliferation marker Ki67 for *rbm3-/-* mice compared to the WT mice (Figure 3b). IL5 and IL13 production from ILCs was also significantly increased in *rbm3-/-* mice (Figure 3c). Interestingly, the type 17 cytokine IL17A was significantly increased in ILCs from *rbm3-/-* mice compared to WT mice (Figure 3d). We then used mice that lack the adaptive immune T and B cells (*rag2-/-)* mice to examine the role of RBM3 in the absence of T and B cell interactions. The *rbm3-/-rag2-/-* mice also displayed increased ILC cell numbers and ILC proliferation marker expression compared to single *rag2-/-* mice (Figure 3e, f). Similarly, IL5 and IL13 production was significantly increased in the double knockout mouse compared to the *rag2-/-* mouse (Figure 3g). Lung and BAL eosinophils were also highly expressed in the *rbm3-/-rag2-/-* mice (Figure 3h). Together, this

data indicates a suppressive role for RBM3 upon exposure to *Alternaria* through methods that are independent of adaptive immunity.

Next, to assess RBM3 inhibition of ILC functions, we performed RNA-seq on lung WT and *rbm3-/-* ILCs from *Alternaria*-challenged mice. Gene expression transcripts most commonly related to ILC2 and ILC3 activation were more highly expressed in *rbm3-/-* mice compared to WT mice (Figure 4a). Principal component analysis of the most variable genes resulted in distinct global transcriptional differences between the transcriptomes of WT and *rbm3-/-* mice (Figure 4b). Expression levels for ILC2 and ILC3 cytokine transcripts *il4, il5, il17f, il17a,* and *il22* were significantly increased in *rbm3-/-* mice compared to the WT controls (Figure 4c). Interestingly, *ifng,* an ILC1 cytokine was not significantly different between WT and *rbm3-/-* mice, suggesting that in *Alternaria* challenged mice, there is a specificity of RBM3 to inhibit the type 2 and 17 immune responses.

To determine the direct role of RBM3 inhibition in the concurrent production of the cytokines IL5, IL13, and IL17 from ILCs, WT and *rbm3-/-* mice were intranasally administered *Alternaria.* ILCs were identified as CD45+, Lineage-Thy1.2+ lymphocytes that were dual-stained with fluorescently tagged antibodies for IL-5 and IL-17 or IL-13 and IL-17 production. In this preliminary experiment with low numbers, there were no significant differences (unpaired T test, $p<0.05$) in the total number of lung ILCs between challenged *rbm3-/-* and WT mice. However, there was a significant (unpaired T test, *$p<0.05$, **$p<0.01$) increase in cytokine production of double-positive IL-5 and IL-17 as well as IL-13 and IL-17 in *rbm3-/-* mice compared to WT mice (Figure 5b, c). Overall, there was more IL-5 and IL-17 production from both WT and *rbm3-/-* mice compared to IL-13 and IL-17 production (no significance test

performed) (Figure 5b, c). This data suggests that ILC2-17s are increased in rbm3-/- mice and that RBM3 suppresses the production of IL-5, IL-13, and IL-17 from this population.

Discussion

Our results demonstrate that when activated and expanded with the fungal allergen *Alternaria*, ILCs highly express the RNA binding protein, RBM3. In *rbm3-/-* mice, increased inflammation and induction of type 2 and 17 cytokines in a manner independent of adaptive immunity compared to WT mice suggests that RBM3 has a direct role in the regulation of ILC function. Furthermore, we identified that activated ILCs co-produce the cytokines IL5 and IL17 or IL13 and IL17 and are thus are likely ILC2-17s. *Alternaria* challenge in *rbm3-/-rag2-/-* mice showed a significant increase in cytokine production from ILCs compared to WT mice, indicating that RBM3 is directly interacting and regulating the function of ILCs. This is significant as it suggests that ILCs are responsible for the production of IL5, IL13, and IL17 and not another immune cell such as T cells, which are also known to secrete these cytokines (Lambrecht et al. 2019). Other experiments (not shown) we have performed show that *rbm3*-/- ILCs directly over produce cytokines in vitro and in vivo to the alarmin IL-33 which also supports a direct effect of RBM3 in ILCs.

RBM3 is classically expressed during hypothermic conditions and acts to stabilize mRNA to enhance transcription (Danno et al. 1997). However, more recent research has identified novel protective roles for RBM3 that extend beyond its characterization as a cold shock induced protein. One study found that an overexpression of RBM3 prevents detrimental behaviors initiated by traumatic brain injury (Liu et al. 2020). Another study showed that the overexpression of RBM3 prevented total muscle cell death and apoptosis in mice (Ferry et al. 2011). While previous work from our group identified that RBM3 suppresses the production of the type 2 cytokines IL5 and IL13 as well as IL17, the results found within this study demonstrate that RBM3 is specifically regulating cytokine production from ILCs (Badrani et al, unpublished). The suppression of hyper-inflammatory cytokine activation during allergen assault

potentially demonstrates another novel protective role of RBM3 in preventing lung injury that parallels other identified protective roles of RBM3.

While this study implies that RBM3 suppresses type 2 and 17 cytokine production from ILCs, it is possible and likely that these cytokines are being produced by the ILC subset ILC2s and ILC3s. However, while it is known that ILC2s produce IL5 and IL13 upon activation and ILC3s produce IL17, it was recently shown that a group of ILC2s known as inflammatory ILC2s (iILC2) or ILC2-17s can also produce IL17 (Cai et al. 2019). This iILC2 finding in conjunction with the data here supports that a single ILC population is concurrently producing IL5, IL13, and IL17. While this study supports our novel findings that RBM3 suppresses type 2 and 17 cytokine production from ILCs, further staining for cytokine production from all ILC subsets is needed to identify the sub-populations that RBM3 regulates. Our lab has hypothesized that one potential mechanism of control occurs through the CysLT1R, and planned experiments will deduce such avenues of molecular regulation for RBM3 (Badrani et al, unpublished). These experiments include staining for transcription factor expression to examine whether RBM3 regulates ILC cytokine production at the transcription factor level. Overall, this work is significant as we identified that RBM3 suppresses cytokine production from ILCs and may specifically regulate a novel population of ILC2-17s and may protect lung tissues from inflammatory damage during allergen assault.

This thesis contains material as it appears in the submitted manuscript, currently under review, titled “RNA-binding protein RBM3 negatively regulates innate lymphoid cells (ILCs) and lung inflammation”. Badrani, Jana; Strohm, Allyssa; Doherty, Taylor. The thesis author is a co-author of the manuscript.

Materials and Methods

Mice

Female and male C57BL/6J mice aged 6-12 weeks old were obtained from Jackson Laboratories (Bar Harbor, ME). WT mice were sex and age matched to the *rbm3-/-* mice received from Dr. Peter Vanderklish at Scripps Research and bred in-house. The *rbm3-/-rag2-/-* mice were created through multi-generational crosses of *rbm3-/-* and *rag2-/-* mice and bred in-house. All studies are approved through the University of California, San Diego Institutional Animal Care and Use Committee.

Lung Inflammation Models

Mice were intranasally challenged with *Alternaria alternata* extract (Greer, Lenoir, NC) diluted in PBS. For the isolation of the ILC subsets via FACS for RNA-seq, mice were challenged with 50μg of *Alternaria* intranasally four times over 10 days. For the isolation of Lineage-Thy1.2+ ILCs via FACS for RNA-seq, mice were challenged intranasally with 25μg of *Alternaria* three times over 7 days. Multiple *Alternaria* challenge models were used on *rbm3-/-* mice. Mice were intranasally challenged three times over 7 days with 10μg or 25μg of *Alternaria* or challenged once with 20μg of *Alternaria* followed by three challenges of 10μg of *Alternaria* over 10 days. *Rag2-/-* mice and *rbm3-/-rag2-/-* mice were challenged with 20μg *Alternaria* four times over 10 days.

BAL and Lung Processing

Bronchoalveolar lavage (BAL) fluid was collected using 2% bovine serum albumin (BSA) (Sigma, St Louis, MO). Supernatant from the first BAL was collected and stored at -20℃

for future ELISA analysis. The whole lung was collected into RPMI and promptly dissociated into a single-cell suspension using the Miltenyi Lung Digest Kit and Dissociator per the company's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were counted using the Novocyte flow cytometer based on size and granularity (ACEA, San Diego, CA).

Histology

In some experiments, the left half of the lung was used for histology. Lungs were perfused and fixed with 4% paraformaldehyde. Hematoxylin and Eosin (H&E) and Periodic-acid Schiff (PAS) staining was performed at the Histology Core in UCSD's Moore's Cancer Center.

ELISA

Samples stored at -20℃ were analyzed using the IL5 and IL13 ELISA kits per R&D protocols (R&D Systems, Minneapolis, MN). Plates were read using a microplate reader model 680 (Bio-Rad Laboratories, Hercules, CA). Microsoft Excel and PRISM by GraphPad (San Diego, CA) were used to analyze ELISA results.

ILC Purification and RNA-Sequencing

FACS isolation and cell preparation for RNA-sequencing was completed as previously described (GEO accession #136156) (Cavagnero et al. 2019). WT and *rbm3-/-* Lin-Thy1.2+ ILCs were sorted with the BD FACSAria II or the BD FACSAria Fusion directly into TrizolLS at the UCSD Human Embryonic Stem Cell Core Facility. RNA-sequencing was performed at the La Jolla Institute.

Total RNA was purified using miRNAeasy kit (Qiagen), quantified and quality of RNA assessed by Fragment Analyzer (Advance Analytical). All samples had an RNA integrity number >9.0 and passed the quality and quantity control steps. Purified total RNA was amplified following the Smart-seq2 protocol and yielded approximately 5ng (Rosales et al. 2018; Picelli et al. 2014). mRNA was captured using poly-dT oligos and directly reverse-transcribed into full-length cDNA using the described template-switching oligo (Picelli et al. 2014; Rosales et al. 2018). cDNA was PCR amplified for 15 cycles and purified using the AMPure XP magnetic bead (0.9:1 (vol:vol) ratio, Beckman Coulter). 1ng of cDNA was used to prepare a NextEra XT sequencing library per each sample (NextEra XT DNA library prep kit and index kits; Illumina). An automated platform generated barcoded Illumina sequencing libraries (Biomek FXP, Beckman Coulter). To reduce assay variability, amplification and sequencing preparations were completed in a 96 well format and quality control steps were included to optimize RNA quality and quantity, the number of PCR preamplification cycles, and fragment library size. The reference genome was mm10 (mouse genome). All samples passed the quality control steps and were pooled at equimolar concentration, loaded and sequenced on the Illumina sequencing platform, Novaseq 6000 (Illumina). Libraries were sequenced to obtain more than 20 million 100 x 100 bp paired end reads (S4 P200 flow cell and sequencing kit; Illumina) mapping uniquely to mouse mm10 mRNA reference.

Sequencing data for this study can be found in the Gene Expression Omnibus under accession #136156. The paired end reads that passed Illumina filters were filtered for reads aligning to tRNA, rRNA, adapter sequences, and spike-in controls. The reads were then aligned to the mm10 reference genome using STAR (v 2.6.1c). DUST scores were calculated with PRINSEQ Lite (v 0.20.3) and low complexity reads (DUST>4) were removed from the BAM

files. The alignment results were parsed via the SAMtools to generate SAM files. The featureCounts program (v1.6.5) was used to find the read counts to each genomic feature. After removing absent features, which had zero counts in all samples, R/Bioconductor package DESeq2 to identify differentially expressed genes among the raw counts from our samples. Differential expression p-values were calculated using the Wald test for differences between the base means of two conditions. These p-values were then adjusted for multiple test correction using Benjamini Hochberg algorithm to control the false discovery rate.

Flow Cytometry

For surface stains, one-million lung and BAL cells were stained. For intracellular stains, two million lung cells were stained. Fc receptors were blocked for 5 minutes using CD16/32 (Biolegend, San Diego, CA). Eosinophils were gated as CD45.2+CD11c-Siglec F+, neutrophils were gated as CD45.2+Siglec F-GR1+ with CD45.2 (PerCP), Siglec-F (PE), GR-1 (APC), and CD11c (FITC). ILCs were gated as Lineage-Thy1.2+ lymphocytes or Lineage-T1ST2+ lymphocytes and were stained using CD45.2 (PerCP), Thy1.2 (APC), T1ST2 (PE), and a lineage cocktail (FITC). The lineage cocktail included markers from Biolegend (CD3e, Ly-6G/Ly-6C, CD11b, CD45R/B220, and TER-119), as well as CD11c, NK1.1, CD5, FceR1, TCRβ, and TCR$\gamma\delta$. The ILC subsets were surface stained for ST2 (APC) and CD127 (PE-Cy7) expression. For nuclear intracellular staining, cells were first surface stained as described above and then permeabilized using the FoxP3 kit (ThermoFisher, Waltham, MA). Cells were then stained for Ki-67 (PE or APC), and RBM3 expression.

For cytokine intracellular staining with the 10-day challenge model, cells were cultured overnight with Golgi Plug (Fisher Scientific, Hampton, NH) at 500,000 cells per well. After

surface staining for ILCs, cells were fixed and permeabilized using the BD kit (BD Biosciences, La Jolla, CA) and stained for IL5 (PE) or IL13 (PE). For cytokine intracellular staining following the *Alternaria* challenges, lung cells were cultured for 3 hours with cell stimulation cocktail (ThermoFisher, Waltham, MA) at 1 million cells per well. The cells were collected and were surface stained for ILCs as aforementioned. After surface staining, cells were fixed and permeabilized with the BD kit and stained for IL5 (PE), IL13 (PE), and/or IL17A (AmCyan). The polyclonal RBM3 antibody used in this study were raised in rabbits and obtained by Dr. Peter Vanderklish at Scripps research and identifies *in-vivo* RBM3 expression as previously described (Pilotte et al. 2009). All flow cytometry was performed using the Novocyte and data was analyzed using FlowJo software (Tree Star, Ashland, OR). All antibodies except RBM3 were purchased from Biolegend, ThermoFisher, or BD Biosciences.

Statistical Analysis

Statistical analysis was performed with PRISM Software (GraphPad Software, La Jolla, CA). P-values were obtained using the Mann-Whitney test or the unpaired t-test and a P-value of less than 0.05 was considered statistically significant, *p<0.05, **p<0.01, ***p<0.001.

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