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1  **$\gamma\delta$  T cells amplify *Blomia tropicalis*-induced allergic airway disease**

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**Journal of Allergy and Clinical Immunology**

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**Section designation:** Letter to the Editor

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27

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38

### 39 **Authorship Contributions:**

40 M.L.M conceived and designed the experiments.

41 A.B., C.D., F.M., and J.R.V. performed the research.

42 A.B., C.D., J.R.V., and M.L.M. analysed the data.

43 M.L.M. wrote the manuscript

44

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- 51 • IL-4-producing V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cells promote allergic airway inflammation in a *Blomia*  
52 *tropicalis* model of asthma
- 53 • Human peripheral blood  $\gamma\delta$  T cells are activated in atopic *B. tropicalis* subjects  
54 compared to non-atopic donors.

55

### 56 **Key Words**

57 *Blomia tropicalis*;  $\gamma\delta$  T cells, atopy; asthma; airway hyperreactivity; lung, IL-4, patients

58

59 **Short title:**  $\gamma\delta$  T cells and *Blomia tropicalis* allergic immune responses

60

### 61 **ABBREVIATIONS**

62

63 AHR: airway hyperreactivity

64 BALF: bronchoalveolar lavage fluid

65 WT: wild type

66 *To the Editor:*

67

68 Allergic asthma is a chronic and heterogeneous immunological disease characterized by  
69 airway inflammation, hyper-IgE production, mucus hypersecretion and airway hyperreactivity  
70 (AHR). The most studied house dust mites (HDM) respiratory allergens belong to  
71 *Dermatophagoides pteronyssus* and *Dermatophagoides farinae* mite species. However, the  
72 storage mite *Blomia tropicalis* represents an important allergenic source and sensitization to  
73 its allergens are commonly associated with typical clinical manifestations of allergy,  
74 including asthma (1-3). Although these mites may coexist in tropical and subtropical regions,  
75 *B. tropicalis* is the major allergen in some countries (4, 5). A limited number of reports have  
76 focused on the allergic processes involved in the immune responses triggered by *B. tropicalis*  
77 (3, 6, 7). Here, we used allergic asthma murine models and *ex vivo* analysis of *B. tropicalis*  
78 allergic subjects to better explore the role of immune cells in allergic responses obtained in  
79 the context of *B. tropicalis* exposure.

80 Mice received *B. tropicalis* allergens exclusively administered intranasally (without  
81 adjuvant) for both sensitization and challenge phases (Appendix S1). This model reproduced  
82 the major allergic asthma hallmarks, namely enhanced BALF cell counts accompanied by  
83 airway eosinophilia and airway hyperresponsiveness (AHR) (Figure 1A and B). We  
84 addressed the involvement of IL-4, as a major Th2 cytokine by comparing lung inflammation  
85 in IL-4-deficient (IL-4<sup>-/-</sup>) and wild-type (WT) mice. The lack of IL-4 resulted in the absence  
86 of the typical airway eosinophilia and AHR observed in WT mice following *B. tropicalis*  
87 sensitization and challenge (Figure S1A and B). These results show that IL-4 is required for  
88 *B. tropicalis* allergen-induced asthma in our model.

89 Distinct immune cells, such as iNKT cells and  $\gamma\delta$  T cells, may be capable to secrete  
90 IL-4 and favour asthmatic inflammatory Th2-cytokine-dependent responses (8, 9). As  
91 previously described, iNKT cells can be divided into three major subsets, iNKT1 (IFN- $\gamma$  and

92 IL-4 producers), iNKT2 (IL-4 producers) and iNKT17 (IL-17 producers) (10). These subsets  
93 were present in the lung of *B. tropicalis* treated mice (Figure S2A). No major differences  
94 were observed concerning their ability to secrete IL-4, IL-13 or IFN $\gamma$  but the frequency of IL-  
95 17-producing iNKT cells was enhanced when compared to controls (Figure S2A). However,  
96 this enhanced frequency was not critical for airway inflammation since iNKT cell-deficient  
97 mice presented similar airway eosinophilia than WT mice (Figure S2B).

98         Concerning  $\gamma\delta$  T cells, including the V $\gamma$ 1.1<sup>+</sup> (hereafter mentioned as V $\gamma$ 1<sup>+</sup>)  $\gamma\delta$  T cell  
99 subset, we found that their number were higher in the lung of *B. tropicalis*-treated than in  
100 NaCl-treated mice (Figure S3). It is noteworthy that airway eosinophilia and AHR were  
101 significantly reduced in *B. tropicalis*-sensitized and -challenged TCR $\delta$ <sup>-/-</sup> mice when compared  
102 to WT controls (Figure 1C, 1D). Further, IgE levels in the serum, Mucin-5 mRNA expression  
103 and mucus deposition in the lung were also impaired in TCR $\delta$ <sup>-/-</sup> compared to WT mice  
104 (Figure S4). Previous reports have shown that the influence of  $\gamma\delta$  T cells on allergic responses  
105 may be complex (9). Here, we focused our analysis on V $\gamma$ 1<sup>+</sup> and V $\gamma$ 1<sup>-</sup>  $\gamma\delta$  T cell subsets  
106 (Figure S5A). Among  $\gamma\delta$  T cells, IL-4 and IL-13 were mainly produced by the V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cell  
107 subset in the lungs of the asthmatic mice (Figure S5B and C). Of note, the frequency of IL-4<sup>+</sup>  
108 and IL-13<sup>+</sup> among gated V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cells from *B. tropicalis*-treated mice was higher than in  
109 controls (Figure S5B). These cells also secreted higher levels of IFN $\gamma$  and IL-17 in asthmatic  
110 mice (Figure S5B). In contrast, the percentage of IL-4<sup>+</sup>, IL-13<sup>+</sup>, IL-17<sup>+</sup> and IFN $\gamma$ <sup>+</sup> among  
111 gated V $\gamma$ 1<sup>-</sup> subset was similar in mice treated with *B. tropicalis* and controls (Figure S5B and  
112 C). These findings indicating that cytokine-producing V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cells may be critical for this  
113 inflammatory allergic response.

114         The enhanced frequency of IL-4-producing V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cells in the lung of *B. tropicalis*-  
115 treated mice (Figure S5B) led us to the question whether airway eosinophilia and AHR could

116 be restored by transferring fully competent  $V\gamma 1^+ \gamma\delta$  T cells to *B. tropicalis*-sensitized and -  
117 challenged  $TCR\delta^{-/-}$  mice. This was the case since the adoptive transfer of 25 000  $V\gamma 1^+ \gamma\delta$  T  
118 cells from WT mice one hour before the first challenge restored airway inflammation,  
119 particularly for airway eosinophilia, and AHR in  $TCR\delta^{-/-}$  mice (Figure 1E and F). In contrast,  
120 adoptive transfer of  $V\gamma 1^+ \gamma\delta$  T cells from  $IL-4^{-/-}$  mice had no effect (Figure 1E and F). The  
121 failure to develop an effective AHR response in  $TCR\delta^{-/-}$  mice and the inability of  $V\gamma 1^+ \gamma\delta$  T  
122 cells from  $IL-4^{-/-}$  mice to restore AHR can be ascribed to the lack of IL-4 production by  $V\gamma 1^+$   
123  $\gamma\delta$  T cells, which compromises their contribution to asthmatic symptoms. Thus, IL-4-  
124 producing  $V\gamma 1^+ \gamma\delta$  T cells are required for airway inflammation and AHR in our *B. tropicalis*  
125 asthma model.

126 We further addressed the relevance of  $\gamma\delta$  T cells to atopic immune process observed in  
127 *B. tropicalis* allergic subjects. We obtained blood samples from subjects clinically classified  
128 as atopic or non-atopic (no allergic responses to any tested allergen) to *B. tropicalis* allergens  
129 (Table S1). The frequency of peripheral blood  $\gamma\delta$  T cells was significantly lower in *B.*  
130 *tropicalis* atopic than non-atopic individuals (Figure 2A and S6). However, these cells were  
131 more activated in atopic subjects, as they spontaneously produced higher levels of  $IFN\gamma$  and  
132 IL-4 than those of non-atopic donors (Figure 2B, 2C and S6). The frequency of IL-17-  
133 producing  $\gamma\delta$  T cells was similar in atopic and non-atopic subjects (Figure 2D and S6).  
134 Overall, these observations suggest that  $\gamma\delta$  T cells may be involved in immune allergic  
135 processes in *B. tropicalis* atopic patients. Further studies are required to determine the major  
136 blood  $\gamma\delta$  T cell subsets activated in these patients.

137 This study has limitations since the precise nature of the stimuli (*B. tropicalis* antigens  
138 or cytokines) required to induce IL-4 production by  $\gamma\delta$  T cells and the analysis of these  
139 lymphocytes in BALF from subjects susceptible to *B. tropicalis* remain to be determined.

140 However, our findings provided many insights into how  $\gamma\delta$  T cells may contribute to immune  
141 allergic responses triggered by *B. tropicalis*. First,  $\gamma\delta$ -deficient mice provided unequivocal  
142 evidence that the absence of  $\gamma\delta$  T cells profoundly impairs the allergic asthma symptoms  
143 induced by *B. tropicalis* sensitization and challenge under defined experimental conditions.  
144 Moreover, airway eosinophilia and AHR in response to *B. tropicalis* were achieved only  
145 when V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cells from WT, but not from IL-4<sup>-/-</sup>, mice were adoptively transferred to  $\gamma\delta$ -  
146 deficient mice. Consistent with this result,  $\gamma\delta$  T cells were activated in *B. tropicalis* atopic  
147 patients, as they produced higher levels of IFN $\gamma$  and IL-4 than non-atopic donors. In  
148 conclusion, our results suggest an unanticipated role for  $\gamma\delta$  T cells in allergic immune  
149 responses induced by *B. tropicalis*.

150

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200  
201 **LEGENDS**

202  
203 **Figure 1:**  $\gamma\delta$  T cells were required for asthma symptoms induced by *B. tropicalis*. (A, C, E)  
 204 The number of total cells (Total), monocyte (Mono), eosinophil (Eosino), neutrophil (Neutro)  
 205 and lymphocyte (Lympho) were determined in BALF of wild-type (WT) or  $\text{TCR}\delta^{-/}$  asthmatic  
 206 (*B. tropicalis*) or non-asthmatic (NaCl) mice (n=7 to 20). (B, D, F) Lung resistance (Rtr) was  
 207 measured 24h after the last *B. tropicalis* challenge or controls (NaCl) (n=7 to 15). (E, F) BALF  
 208 cell counts (E) and lung resistance (Rtr) were measured 24h after the last *B. tropicalis* challenge  
 209 of WT,  $\text{TCR}\delta^{-/}$ ,  $\text{TCR}\delta^{-/}$  mice adoptively transferred with sorted  $\text{V}\gamma 1^{+}$   $\gamma\delta$  T cells from WT or  
 210  $\text{IL-4}^{-/}$  mice (n=5 to 10). \* p<0.05; \*\*p<0.01; \*\*\*\*p<0.0001.

211  
212 **Figure 2.**  $\gamma\delta$  T cells were activated in *B. tropicalis* atopic patients. (A) Percentages of  $\gamma\delta$  T cells  
 213 in PBMC from non-atopic versus *B. tropicalis* atopic subjects. (B, C, D) Frequency of  
 214 spontaneous  $\text{IFN}\gamma^{+}$  (B),  $\text{IL-4}^{+}$  (C) and  $\text{IL-17A}^{+}$  (D) cells among gated  $\gamma\delta$  T cells from non-atopic  
 215 versus *B. tropicalis* atopic subjects.

216





**Table S1:** Characteristics of the adults included in the study

	<b>Non-atopic</b>	<b>Bt-Atopic</b>	<b>p</b>
Number	15	13	
Age, years (mean $\pm$ SE)	30.8 $\pm$ 2.1	29.6 $\pm$ 2.8	0.41
Sex (male/female)	7/8	7/6	0.83
<b>SPT results (n/%)</b>			
<i>Blomia tropicalis</i>	0/0	13/100	
<i>Dermatophagoides pteronyssinus</i>	0/0	9/69	
<i>Dermatophagoides farinae</i>	0/0	8/61	
<i>Aspergillus fumigatus</i>	0/0	5/38	
<i>Canis familiaris</i>	0/0	5/38	
<i>Alternaria alternata</i>	0/0	4/30	
<i>Penicillium notatum</i>	0/0	4/30	
<i>Felis domesticus</i>	0/0	2/15	
<i>Cladosporium herbarum</i>	0/0	1/8	
<i>Periplaneta americana</i>	0/0	0/0	
<b>Others</b>			
Clinical manifestation (past 6 mo)	0/0	13/100	
Regular use of antihistamines	0/0	13/100	

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SPT - Skin prick test; mo - months

# $\gamma\delta$ T cells amplify *Blomia tropicalis*-induced allergic airway disease

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## On-line repository

### METHODS

#### Mice and reagents

Eight to ten-week-old specific pathogen-free C57BL/6J, IL-4<sup>-/-</sup> and TCR $\delta$ <sup>-/-</sup> mice were bred in our facility. All animal experiments were carried out according to the guidelines for care and use of animals approved by the French Institutional Committee (APAFIS#4105-201511171831592).

#### Airway allergen sensitization and challenge model.

To induce allergic airway inflammation, we adapted a previously described protocol (1). In brief, mice were sensitized and challenged by intranasal administrations of *B. tropicalis* extracts (Greer laboratories, USA) at day 0 (D0) with 100 $\mu$ g/mouse then at D7, D9, D11, D14 and D16 with 50 $\mu$ g/mouse.

Twenty-four hours after the last challenge, mice were anesthetized with a mixture of ketamine (150 mg/kg) and xylazine (400  $\mu$ g/kg) and their tracheas were cannulated (tracheostomy with ligation). FlexiVent apparatus (SCIREQ) was used to access airway-specific resistance (Rn, tidal volume of 10 ml/kg at a respiratory rate of 150 breath/min in response to increasing doses of aerosolized acetyl- $\beta$ -methylcholine chloride (methacholine; Sigma-Aldrich). Assessments were performed at least three times and the maximum R value obtained after each dose of methacholine was used for the measure.

Airway inflammation was assessed in cytospin preparations of cells in bronchoalveolar lavage fluid (BALF, 3 x 0.5 mL washes with PBS) that were stained with May-Grünwald/Giemsa (Merck). For some experiments, BALF cells were also analysed by flow cytometry.

#### Adoptive transfer

In some experiments, 25 x 10<sup>3</sup> electronically sorted V $\gamma$ 1<sup>+</sup> or V $\gamma$ 1<sup>-</sup>  $\gamma\delta$  T cells were adoptively transferred intravenously (i.v.) to *B. tropicalis* immunized mice 1h before the first challenge (D7). Mice were then further challenged at at D7, D9, D11, D14 and D15 with 50 $\mu$ g/mouse, as described above.

#### Leucocytes from lung tissues

Lung tissues were cut into pieces using a GentleMACS Dissociator (Miltenyi Biotec) and treated with collagenase type 4 (Thermo Fischer Scientific) plus DNase I (Roche). The lymphocyte-enriched fraction was collected at the 35-70% interface of Percoll gradients (GE Healthcare). Cells were immediately stained or stimulated for 4h with 10<sup>-8</sup> M PMA and 1  $\mu$ g ml<sup>-1</sup> ionomycin, in the presence of 10  $\mu$ g ml<sup>-1</sup> brefeldin A (all from Sigma-Aldrich).

#### Immunofluorescence.

Murine cells were incubated with CD1d-PBS57-APC tetramers, anti-TCR $\beta$ -APCeFluor780, anti-V $\gamma$ 1.1-PE and anti-TCR $\gamma\delta$ -BV605 (Biolegend). Fixable viability dye was used to exclude dead cells (ThermoFischer Scientific). For intracellular staining, cells were further fixed with 4% PFA, washed, and permeabilized with 0.5% saponin (Sigma-Aldrich), and then incubated

51 with anti-IL-17-PerCPCy5.5, anti-IL-4-PE-Cy7, anti-IFN- $\gamma$ -V450, anti-anti-IL-13-PE-eF610  
52 or isotype control (eBiosciences). The cells were washed and fluorescence was detected using  
53 a LSRFortessa (Becton Dickinson) and further analysed using the FlowJo 10.4.1 software  
54 (Tree Star).

55

#### 56 **$\gamma\delta$ T cell sorting.**

57 Splenocytes from WT or IL-4<sup>-/-</sup> mice, isolated as previously described (2, 3), were labelled  
58 with anti-V $\gamma$ 1-PE and anti-TCR $\gamma\delta$ -BV605, as described above, and then sorted using a  
59 FACS Aria cell sorter (Becton Dickinson).

60

#### 61 **mRNA expression.**

62 RNAs were extracted using the RNeasy Plus Minikit (Qiagen) including a DNase treatment.  
63 Then RNA was reverse transcribed using the High Capacity RNA-to-cDNA Kit  
64 (ThermoFisher Scientific), according to the manufacturer's instructions. Primers and probes  
65 for real-time PCR were provided by ThermoFisher Scientific under references: Mucin 5b  
66 (Muc5b): Mm00466391\_m1 and HPRT: Mm01545399\_m1. All reactions were performed in  
67 triplicate with TaqMan® Fast Advanced Master Mix according to the supplier's instructions  
68 for a Step One Plus apparatus (ThermoFisher Scientific). All data were normalized to the  
69 internal standard, namely HPRT expression in each sample, and expressed as relative  
70 expression using the  $\Delta\Delta$ Ct method versus the reference sample.

71

#### 72 **Histology of the airways.**

73 Lungs were fixed with 10% formalin via the trachea, removed and stored in 10% formalin.  
74 Lung tissues were embedded into paraffin and 3 $\mu$ m sections were stained with periodic acid  
75 Schiff (PAS).

76

#### 77 **Patient samples**

78 Blood samples were collected from subjects who were previously clinically classified as  
79 atopic or non-atopic individuals and voluntarily subjected to a skin prick test (SPT) to  
80 confirm the atopic state. These individuals were classified into two groups: as Bt- atopic  
81 individuals (clinically allergic and reactive to *Blomia tropicalis* extract, with co-reaction to at  
82 least one allergen), and non-atopic individuals (without any clinical allergy symptoms and not  
83 reactive to any tested allergen). Additional information about these individuals is shown in  
84 table 1.

85 Each sample of peripheral blood mononuclear cells (PBMCs) was provided from a  
86 different donor. The ethics committees at the HCor and the School of Medicine at the  
87 University of São Paulo approved this study (CAAE: 15507613.4.0000.0060).

88

#### 89 **Skin prick test (SPT) and blood sample collection**

90 The SPT were performed in accordance with European standards (4) with an adapted panel of  
91 allergens that included the profile of Brazilian allergens (i.e., *Blomia tropicalis*, *Canis*  
92 *familiaris*, *Periplaneta americana*, *Aspergillus fumigatus*, *Penicillium notatum*, *Alternaria*  
93 *alternata*, *Cladosporium herbarum*, *Dermatophagoides pteronyssinus*, *Dermatophagoides*  
94 *farinae*, and *Felis domesticus* - IPI ASAC), as previously described (5).

95 Briefly, one drop of each allergen extract or controls was applied to the volar forearm  
96 and a superficial skin puncture was made using a hypodermic needle (Alko, Brazil). After 15  
97 minutes, the results were considered positive when wheals measure reach a diameter of 3 mm  
98 greater than that of the negative control. We excluded patients who used antihistamines,  
99 glucocorticosteroids or certain other systemic drugs that can influence the SPT results within  
100 15 days before the test or with severe eczema or dermatographism.

101

## Human flow cytometry

Flow cytometry using human PBMCs to evaluate phenotype and spontaneous intracellular cytokine production were performed as previously described (6, 7). Briefly, PBMC separations were performed using Ficoll-Paque Plus (GE Healthcare) after centrifugation and suspensions of PBMCs were phenotypic evaluated *ex vivo* or cultured to intracellular cytokine production evaluation. PBMCs were stained with mouse anti-human  $\gamma\delta$ TCR-FITC, CD161-PECy5 or isotype control antibodies (BD Pharmingen).

To evaluate intracellular cytokine production, PBMCs were cultured in RPMI 1640 medium containing 10% FBS and 1  $\mu$ g/mL of Brefeldin A (Sigma-Aldrich) for 24 hours without additional stimulus. Cultured PBMCs were then washed, fixed with formaldehyde and stained with mouse anti-human  $\gamma\delta$ TCR-FITC, IL-17A-Alexa700, IL-4-PECy7, IFN- $\gamma$ -HorizonV450 or isotype control antibodies (BD Pharmingen) in 100  $\mu$ L of PBS containing 0.05% saponin. Thirty minutes later, PBMCs were washed, fixed, acquired using a LSRFortessa cytometer (BD Biosciences, USA), and analysis was performed using FlowJo software 10.1 (Tree Star).

## Statistics

Data are expressed as means  $\pm$  SEM. The AHR values were analysed with repeated- measures 2-way ANOVA followed by Bonferroni correction as a post-hoc test. All other values were analysed with Mann-Whitney U test. Results were considered significant at a *P* value of 0.05 or less (\**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001). Data were analyzed using GraphPad Prism version 6 (GraphPad Software).

## LEGENDS

**Figure S1:** IL-4-producing cells were required for asthma symptoms induced by *B. tropicalis*. (A) The number of total cells (Total), monocyte (Mono), eosinophil (Eosino), neutrophil (Neutro) and lymphocyte (Lympho) were determined in BALF of wild-type (WT) or IL-4<sup>-/-</sup> asthmatic (*B. tropicalis*) mice. (B) Lung resistance (Rtr) was measured 24h after the last *B. tropicalis* challenge or controls (NaCl) (n=7 to 15).

**Figure S2:** iNKT cells were not required for asthma symptoms induced by *B. tropicalis*. (A) A representative Facs profile showing the gating strategy used to identify iNKT (CD1d tetramer<sup>+</sup>TCR $\beta$ <sup>+</sup>) cells and the percentage of IFN $\gamma$ , IL-4, IL-13 and IL-17A positive cells, and their respective isotype controls, among gated iNKT cells in the lungs of WT asthmatic (*B. tropicalis*) or non-asthmatic (NaCl) mice. (B) The number of total cells (Total), monocyte (Mono), eosinophil (Eosino), neutrophil (Neutro) and lymphocyte (Lympho) were determined in BALF of wild-type (WT) or iNKT (J $\alpha$ 18<sup>-/-</sup>)-deficient asthmatic (*B. tropicalis*) mice. (n=5 to 7).

**Figure S3:**  $\gamma\delta$  T cells and the V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cell subset were enhanced in the lung of *B. tropicalis* asthmatic mice. (A) A representative Facs profile showing the gating strategy used to identify TCR $\gamma\delta$ <sup>+</sup> and V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cells. (B) The number of total  $\gamma\delta$  T cells and of the V $\gamma$ 1<sup>+</sup>  $\gamma\delta$  T cell subset were determined in the lungs of wild-type asthmatic (*B. tropicalis*) or non-asthmatic (NaCl) mice (n=5 to 10).

**Figure S4:** Impaired seric IgE and mucus production in the lung of TCR $\delta$ <sup>-/-</sup> *B. tropicalis* asthmatic mice. (A) Total IgE levels in the serum of WT and TCR $\delta$ <sup>-/-</sup> mice following *B. tropicalis* or NaCl administration (B) Mucin-5 mRNA expression assessed by quantitative RT-PCR in the lung of WT and TCR $\delta$ <sup>-/-</sup> mice following *B. tropicalis* or NaCl administration.

153 Data are representative of two experiments. (C) Representative PAS-stained lung histology  
154 sections of controls (NaCl) and *B. tropicalis*-sensitized and -challenged WT and TCR $\delta^{-/-}$  mice.  
155 Following *B. tropicalis* administration, the airways of WT mice contained more PAS-stained  
156 mucus-producing cells than TCR $\delta^{-/-}$  mice.  
157

158 **Figure S5.** Distinct cytokine production by lung V $\gamma 1^{+}$  and V $\gamma 1^{-}$   $\gamma\delta$  T cell subsets. (A) Flow  
159 cytometric gating strategy used to identify TCR $\delta^{+}$ V $\gamma 1^{+}$  and TCR $\delta^{+}$ V $\gamma 1^{-}$   $\gamma\delta$  T cell subsets and  
160 the frequency of these cells in the lung of *B. tropicalis*- versus NaCl-treated mice. (B, C) A  
161 representative Facs profile showing the percentage of IFN $\gamma$ , IL-4, IL-13 and IL-17A positive  
162 cells among gated TCR $\delta^{+}$ V $\gamma 1^{+}$  (B) and TCR $\delta^{+}$ V $\gamma 1^{-}$  (C)  $\gamma\delta$  T cell subsets and their frequency  
163 in the lungs of WT asthmatic (*B. tropicalis*) or non-asthmatic (NaCl) mice. \*  $p < 0.05$ . (D)  
164 Facs profile represents the respective IL-13 versus IL-4 and IL-17A versus IFN- $\gamma$  isotype  
165 controls.  
166

167 **Figure S6.**  $\gamma\delta$  T cells were activated in *B. tropicalis* atopic patients. (A) Gate strategy to  
168 obtain viable lymphocytes. (B) A representative Facs profile showing the gating strategy used  
169 to identify TCR $\gamma\delta^{+}$  cells and the percentage of IFN $\gamma$ , IL-4, IL-13 and IL-17A positive cells  
170 among gated TCR $\gamma\delta^{+}$  cells.  
171

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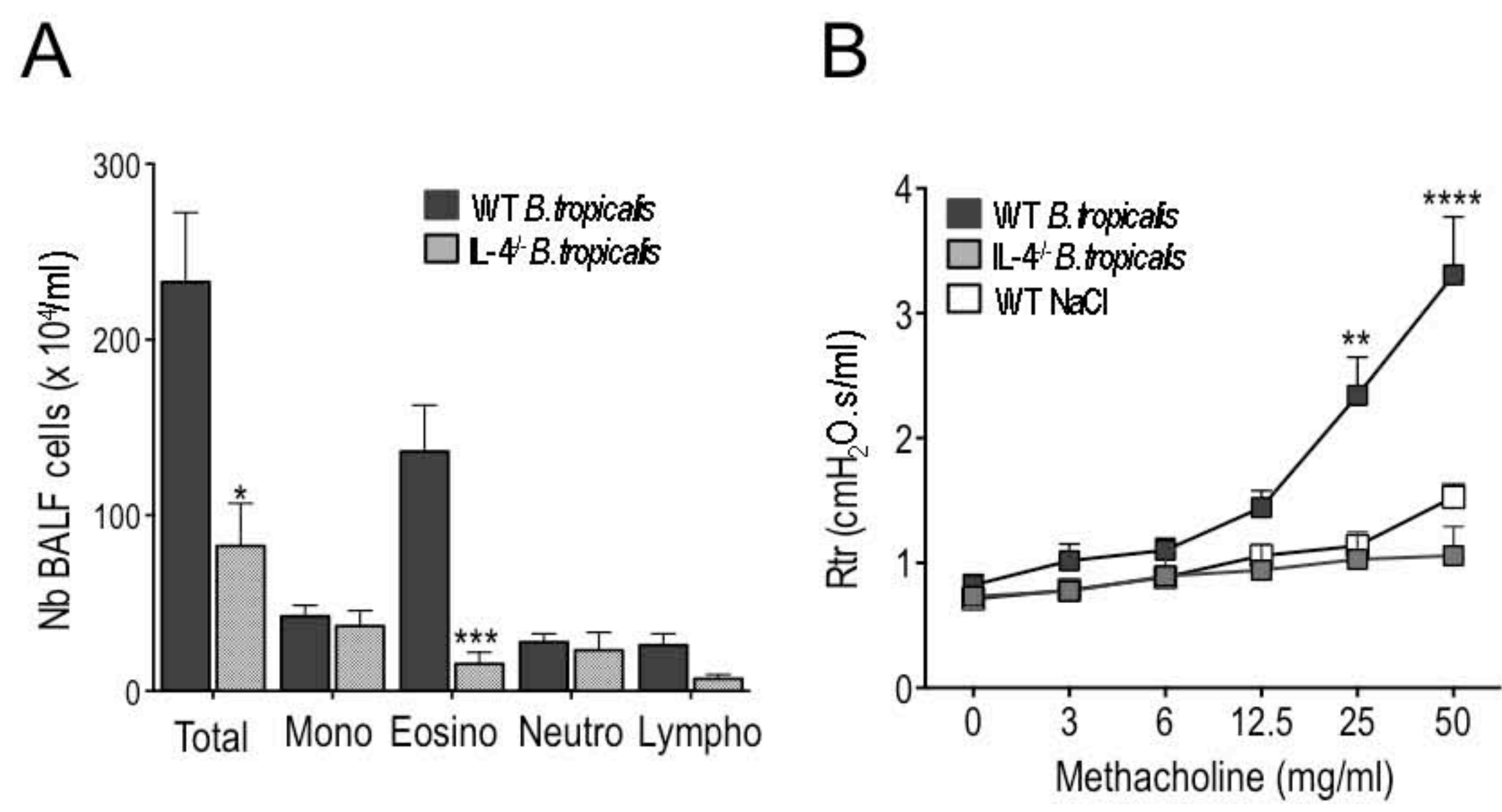


Figure S1

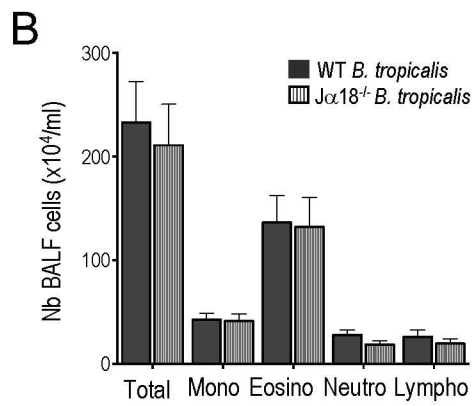
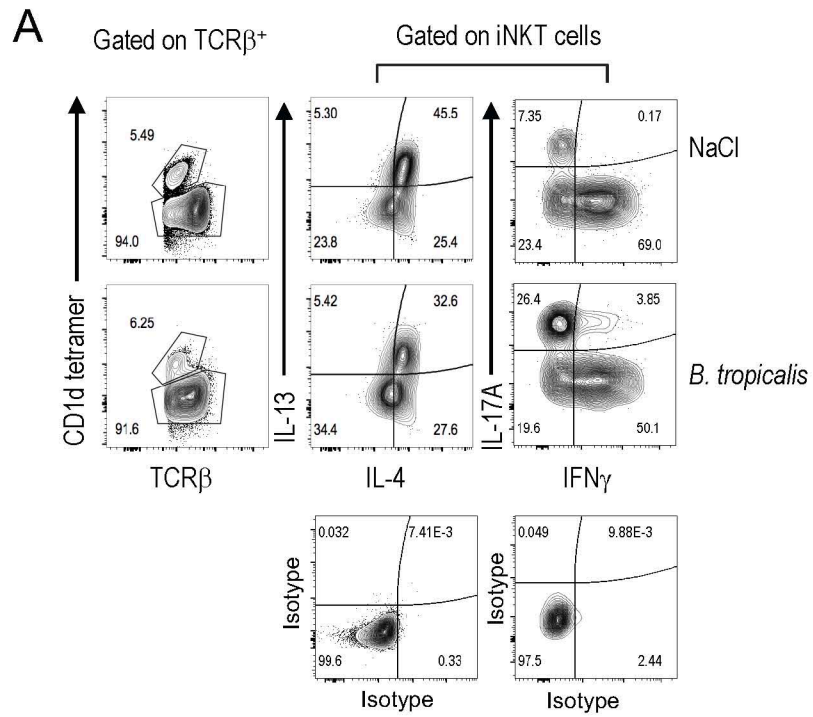


Figure S2



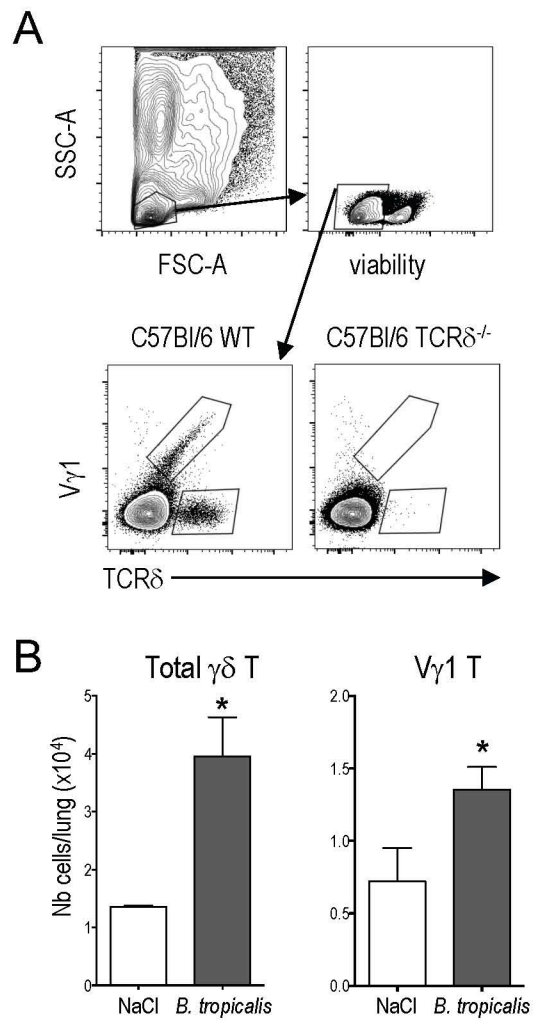


Figure S3

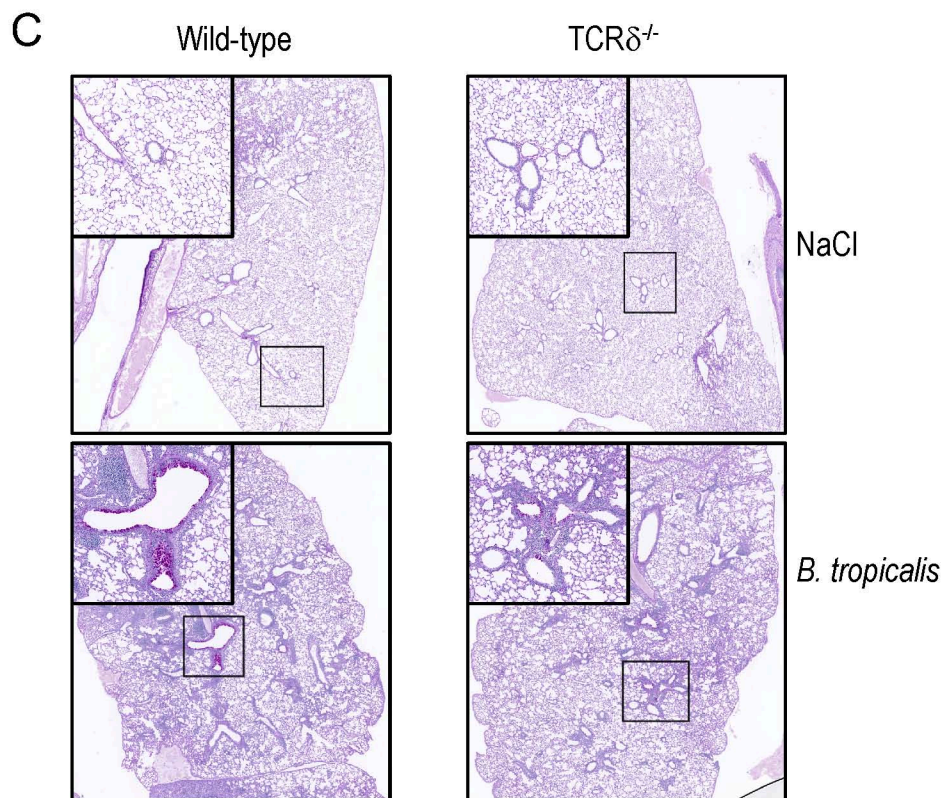
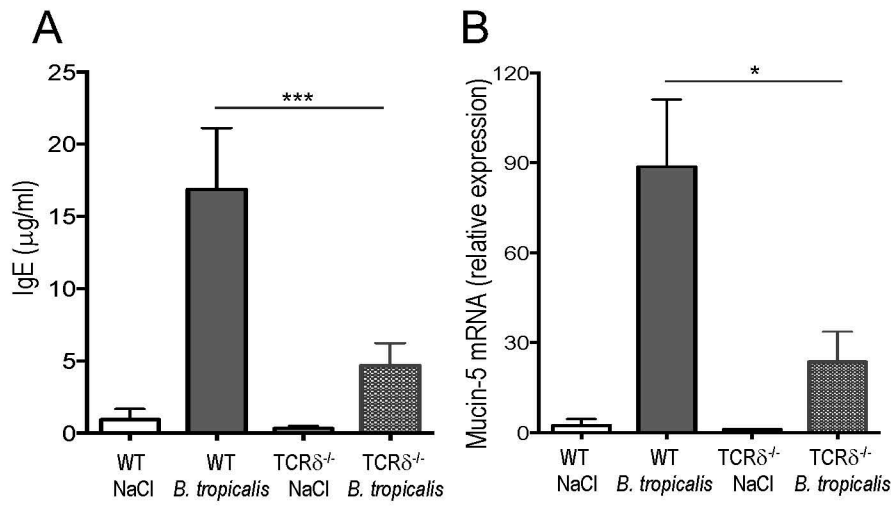


Figure S4

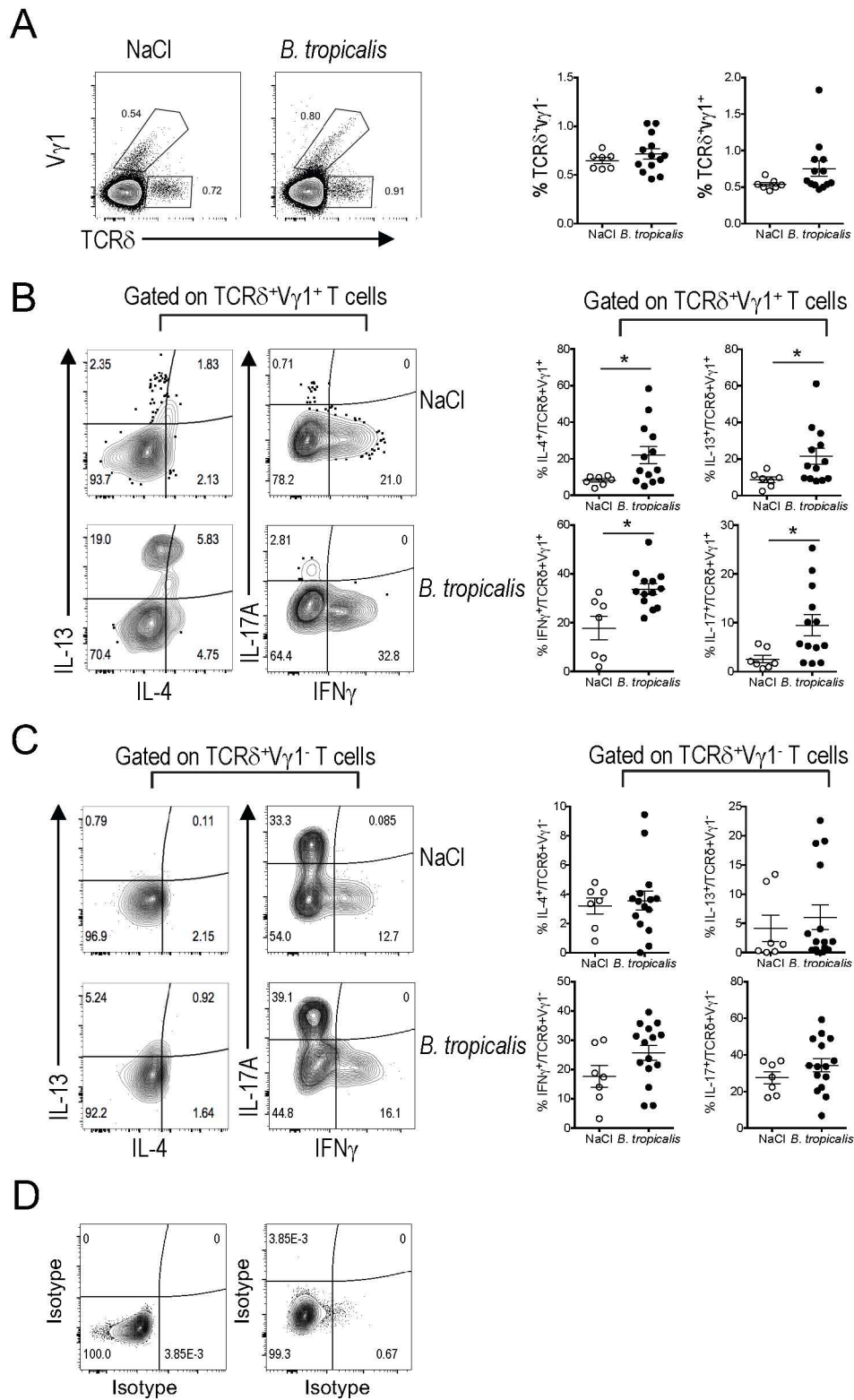


Figure S5

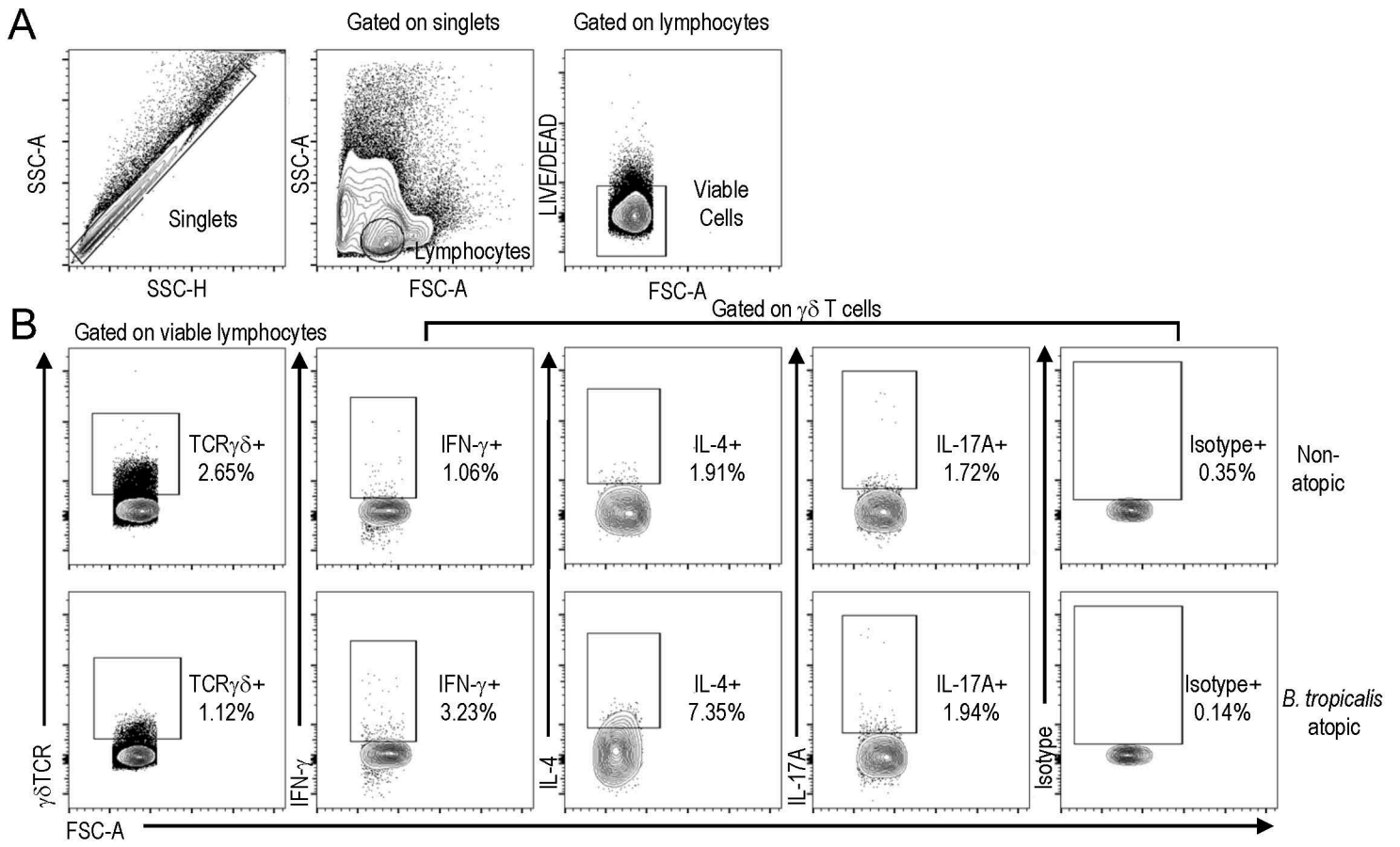


Figure S6