Figure S1. $CD4^+$ and $CD8^+$ T cells are increased in allografts. Transplanted lungs from B6→B6 and B10→B6 transplants were harvested at different time-points and analyzed by flow cytometry. (A) Frequency of $CD4^+$ and (B) frequency of $CD8^+$ T cells in the lymphocyte gate in the transplanted left lung at days 14 (n = 2 for B6→B6; n = 4 for B10→B6), 21 (n = 5 for B6→B6; n = 12 for B10→B6) and 28 (n = 3 for B6→B6; n = 5 for B10→B6) post-transplant, compared to B6 → B6, analyzed by unpaired *t* test, $^{**}P < 0.01$.

Figure S2. Characterization of IL-17A producing cells in lung allografts. Left lungs from B10 mice were transplanted into untreated, anti-CD4 antibody or isotype treated B6 recipients and were analyzed at Day 21 post-transplant by flow cytometry. (A) Representative dot plots of isotype antibody treated control lung allograft showing percentage of different IL-17A producing cellular subsets on gated IL-$17A^+$ lymphocytes. (B) Representative dot plots of an untreated allograft depicting proportion of iNKT cells ($TCR\beta^+$ PBS57/CD1d $tetramer^+$) on gated IL-$17A^+$ lymphocytes; n=3. (C) The stacked bar graph indicating average proportion of different cell types on gated IL-$17A^+$ lymphoyctes in anti-CD4 antibody and control isotype antibody treated lung allografts; n=6 (control), n=7 (anti-CD4) except for $\gamma\delta^+$ T cells of IL-$17A^+$ lymphoyctes where n=7 (control), n=8 (anti-CD4).

Figure S3. Mediastinal lymph nodes of $STAT3^{CD4-/-}$ mice are deficient in Th17 cells and IL-$17A^+$ lymphocytes are mainly γδ T cells. Left lungs from B10 mice were transplanted into $STAT3^{fl/fl}$CD4-Cre negative (WT) or $STAT3^{CD4-/-}$ littermates and mediastinal lymph nodes were analyzed at Day 21 post-transplant by flow cytometry. (A) Representative dot plots showing IL-17A producing $CD4^+$ T cells when gated on total lymphocytes in mediastinal lymph nodes from the transplanted WT and $STAT3^{CD4-/-}$ animals. Number in quadrants represents percentage of cells. (B) Frequency of IL-17A producing $CD4^+$ T cells in mediastinal lymph nodes when gated

on total lymphocytes in transplanted mice. $^{**}P<0.005$ (Mann-Whitney *U* test). (C) Representative dot plots showing percentage of IL-17A expressing cells on gated lymphocytes in mediastial lymph nodes. (D) The frequency of IL-17A producing cells in mediastinal lymph nodes as assessed by flow cytometry when gated on lymphocytes. (E) Dot plots showing analysis of γδ T cells on gated IL-17A cells in lymph node at day 21 of transplant. (F) Percentage of γδ T cells of IL-17A^{+} lymphocytes. $^{***}P<0.0001$ (unpaired *t*-test). (A, C, E) The number in dot plots indicates percentage. (B, D, F) n=6 for WT and STAT3$^{CD4-/-}$.

Figure S4. STAT3 deficient CD4^{+} T cells are skewed towards Th1 phenotype. Left lungs from B10 mice were transplanted into STAT3$^{fl/fl}$.CD4-Cre negative (WT) or STAT3$^{CD4-/-}$ littermates and analyzed at day 21 post-transplant. (A) Representative dot plots showing percentage of IFNγ^{+} lymphocytes on gated CD4^{+} T cells in native and transplanted lung from WT and STAT3$^{CD4-/-}$ mice. Number in dot plots indicates percentage. (B) Percentage and absolute number of IL-17A^{+}CD4^{+} T (Th1) cells, absolute number normalized to the weight of the lung, one-way ANOVA with *post hoc* Newman-Keuls multiple comparison test was used for analysis, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, n=6 for WT, n=7 for STAT3$^{CD4-/-}$. (C) Relative mRNA expression of indicated genes in transplanted lungs as quantified using Real Time PCR. Data are plotted relative to WT and normalized to β-actin. Analyzed using Mann-Whitney *U* test, n=4 for WT, n=8 for STAT3$^{CD4-/-}$.

Figure S1

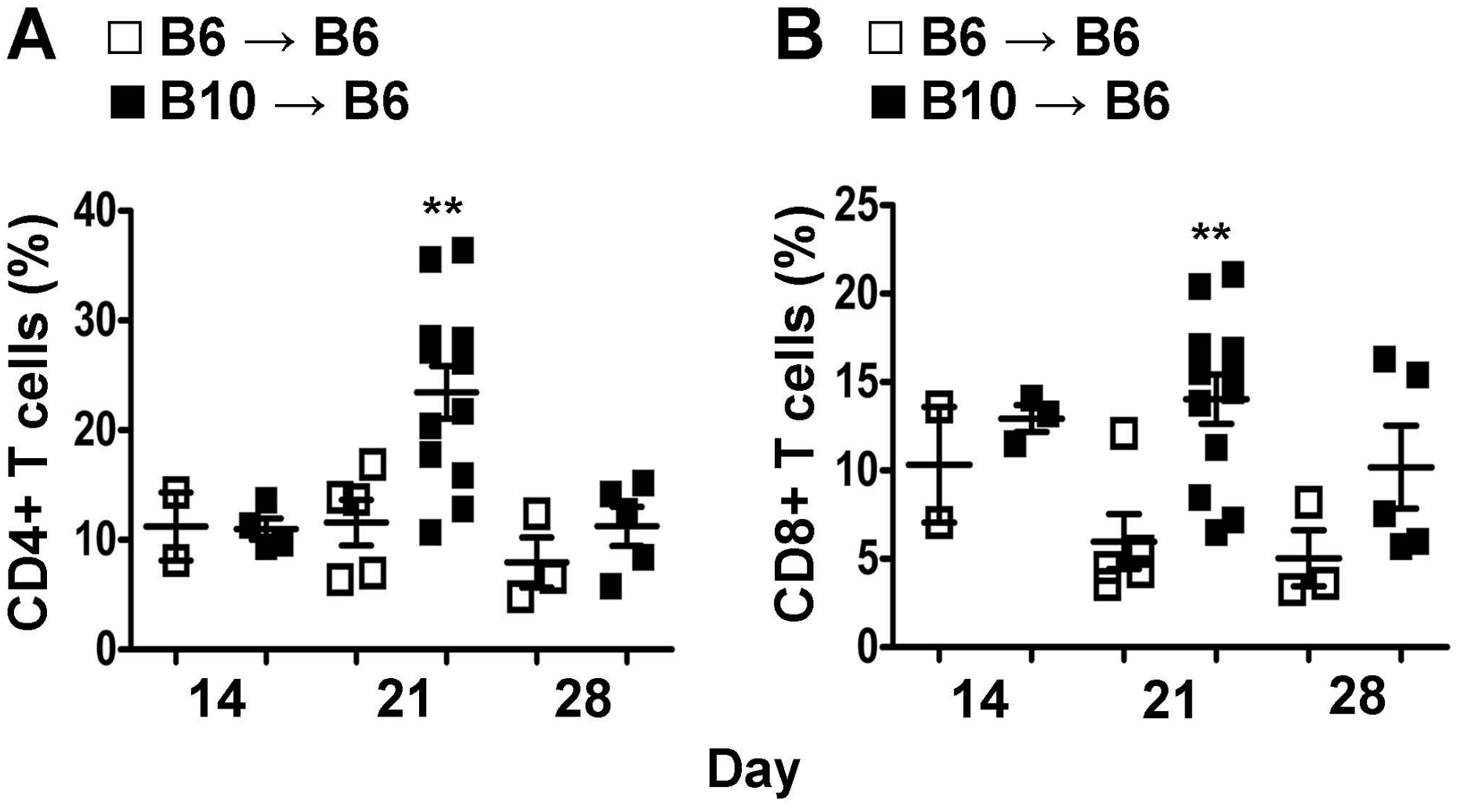

A
□ B6 → B6
■ B10 → B6
CD4+ T cells (%)
40
30
20
10
0
**
14
21
28
B
□ B6 → B6
■ B10 → B6
CD8+ T cells (%)
25
20
15
10
5
0
**
14
21
28
Day

Figure S2

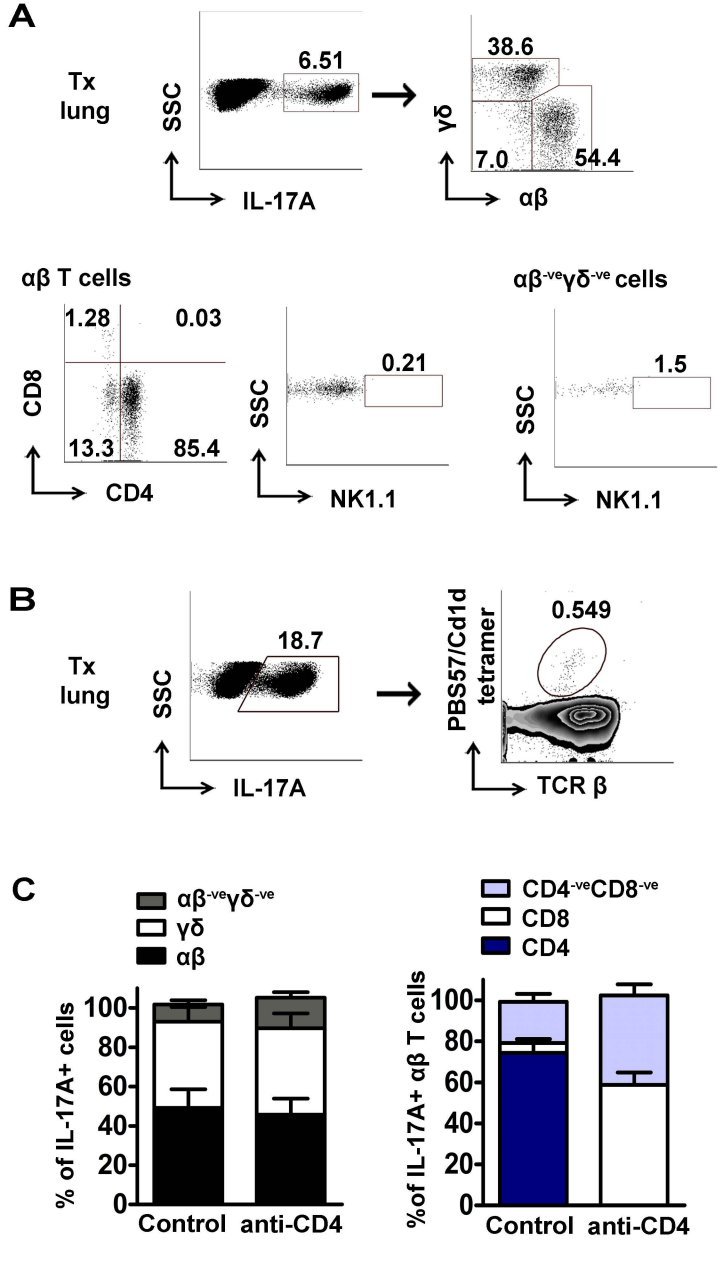

A
Tx lung
SSC
IL-17A
6.51
γδ
αβ
38.6
7.0
54.4
αβ T cells
CD8
CD4
1.28
0.03
13.3
85.4
SSC
NK1.1
0.21
αβ-veγδ-ve cells
1.5
B
Tx lung
18.7
PBS57/Cd1d tetramer
TCR β
0.549
C
αβ-veγδ-ve
γδ
αβ
% of IL-17A+ cells
Control
anti-CD4
CD4-veCD8-ve
CD8
CD4
%of IL-17A+ αβ T cells

Figure S3

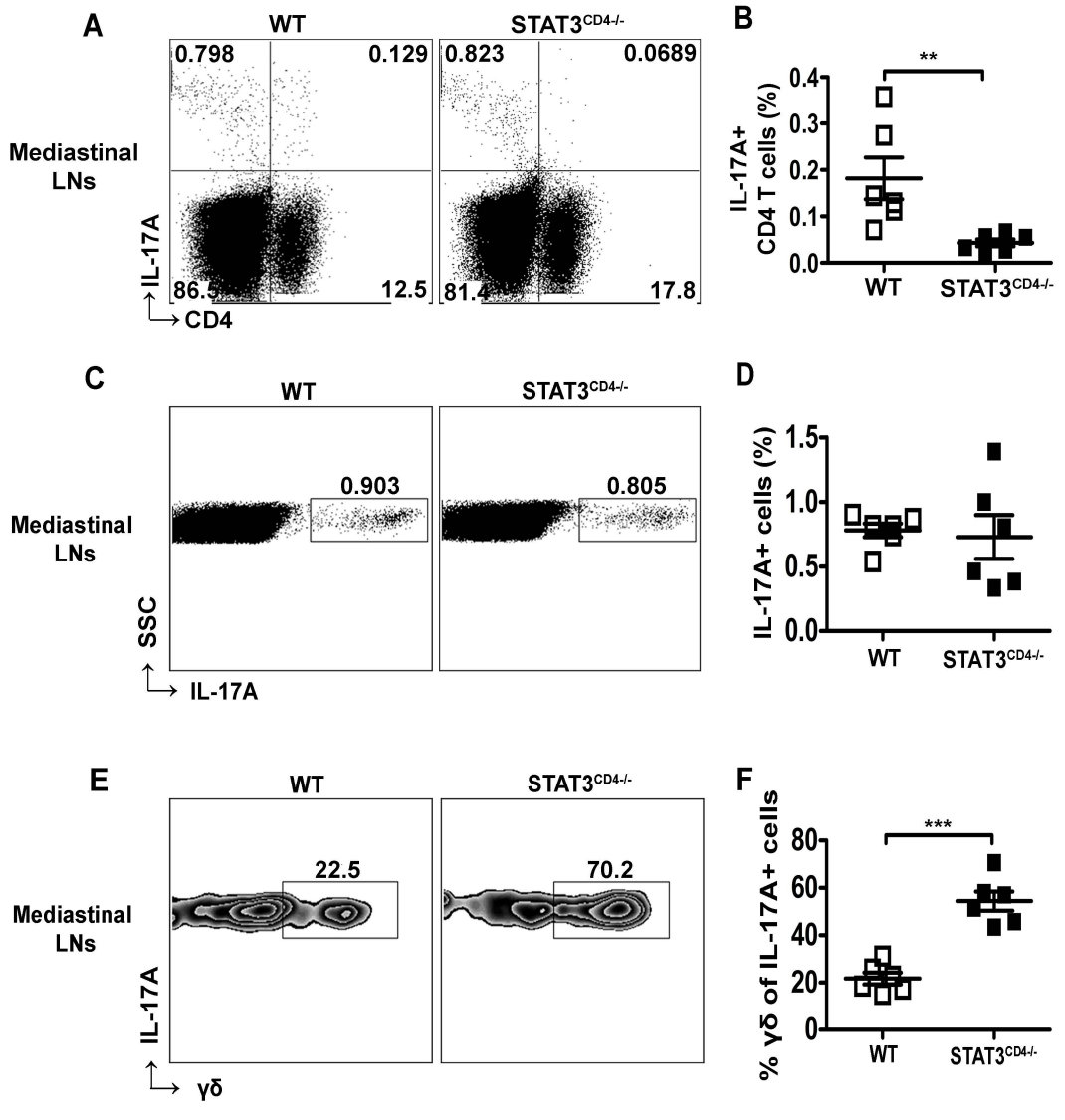
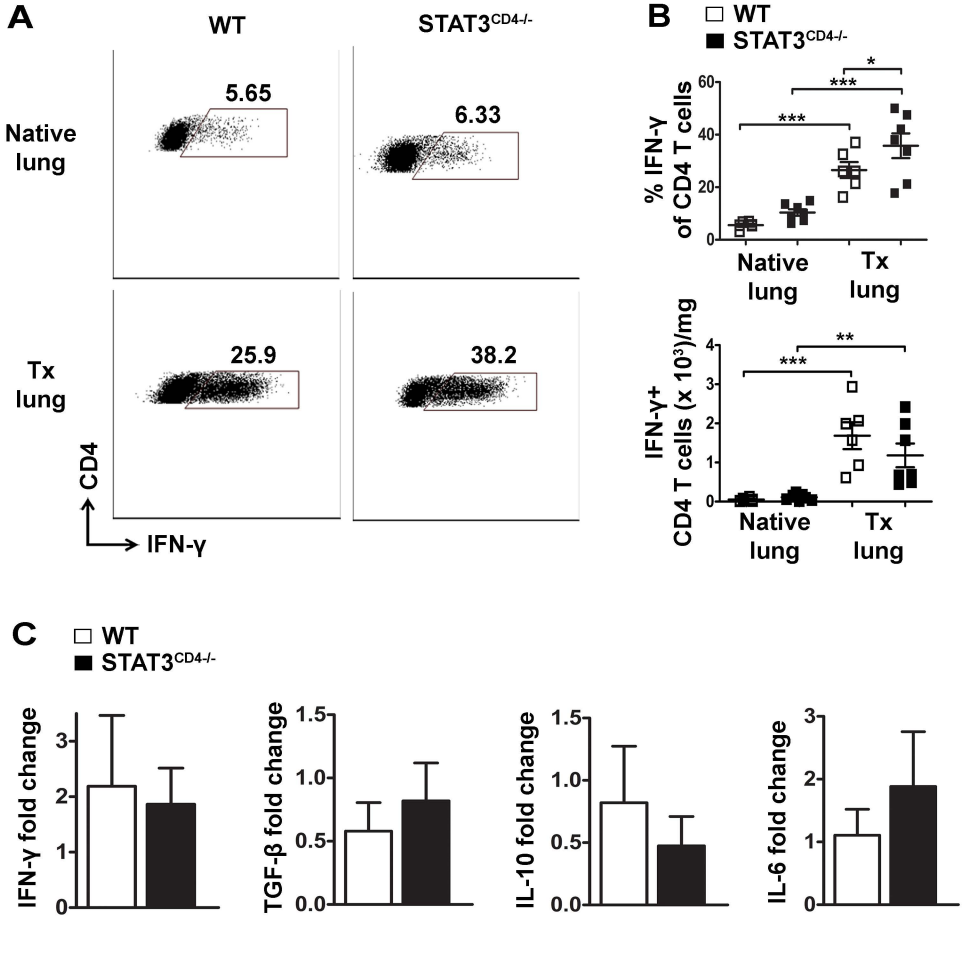

A
WT
STAT3CD4-/-
0.798
0.129
0.823
0.0689
Mediastinal LNs
IL-17A
CD4
86.5
12.5
81.4
17.8
B
**
IL-17A+ CD4 T cells (%)
0.4
0.3
0.2
0.1
0.0
WT
STAT3CD4-/-
C
WT
STAT3CD4-/-
0.903
0.805
Mediastinal LNs
SSC
IL-17A
D
IL-17A+ cells (%)
1.5
1.0
0.5
0.0
WT
STAT3CD4-/-
E
WT
STAT3CD4-/-
22.5
70.2
Mediastinal LNs
IL-17A
γδ
F
***
% γδ of IL-17A+ cells
80
60
40
20
0
WT
STAT3CD4-/-

Figure S4


A
WT
STAT3CD4-/-
Native lung
Tx lung
5.65
6.33
25.9
38.2
CD4
IFN-γ
B
WT
STAT3CD4-/-
% IFN-γ of CD4 T cells
Native lung
Tx lung
IFN-γ+ CD4 T cells (x 10³)/mg
C
WT
STAT3CD4-/-
IFN-γ fold change
TGF-β fold change
IL-10 fold change
IL-6 fold change

Supplemental Materials and Methods

Animals

Adult male C57BL/6N (H-2^b) and C57BL/10 (H-2^b) mice were purchased from Harlan Laboratories (Indianapolis, IN). *Stat3*$^{fl/fl}$ mice with a CD4-Cre transgene (designated as STAT3$^{CD4-/-}$) (H-2^b) mice were previously described on a C57Bl/6N background (15, 16). *Stat3*$^{fl/fl}$ littermates negative for the CD4-Cre transgene were used as wild-type (WT) controls in experiments with STAT3$^{CD4-/-}$ mice. γδ T cell deficient mice (TCRδ$^{-/-}$) on C57Bl/6J background were obtained from Jackson Laboratories (Bar Harbor, ME) and TCR δ$^{-/+}$ were generated by breeding with C57Bl/6N from Harlan labs. Mice were housed under specific pathogen-free conditions in the animal care facility at Indiana University or University of Illinois at Chicago. Male animals, 8–12 weeks of age weighing 24-30g, were used as both donors and recipients. All experimental mouse protocols were reviewed and approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee and the University of Illinois at Chicago Animal Care Committee.

Orthotopic lung transplant

A mouse model of orthotopic minor histocompatibility antigen mismatch left lung transplant was used and has been previously described in detail (10). The entire operation was performed using a Prescott's operating microscope (Zeiss 6SFC, Monument, USA) with 20-40× magnification. All surgical procedures were performed utilizing sterile technique. Mice were not treated with antibiotics or immunosuppressive agents. Both donor and recipient were anesthetized with Isoflurane.

Histology

Lungs were inflated via the trachea with 10% neutral buffered formalin solution (Sigma-Aldrich, Missouri, USA), followed by embedding in paraffin. Tissue sections were prepared and stained with H&E or Masson's trichrome stain. Standard clinical criteria for scoring vascular lung rejection were used according to ISHLT guidelines and scoring was done blinded (17). For the severity of fibrosis an arbitrary scale was used with F1= normal; F2= mild fibrosis, F3= moderate fibrosis, F4=severe fibrosis (18). Presence of OB lesions was also determined. For transplanted lungs, 2/3 of the lung was used for histology and the remaining 1/3 was used for flow cytometry. As a control, the upper and middle lobes from the native right lungs were used for histology or flow cytometry.

Flow cytometry

For cell surface staining, cells were stained with antibodies for CD4, CD8, TCRβ, TCR γδ, and NK1.1 in FACS buffer (0.01% NaN_3 + 2% FBA in 1X PBS) for 30 min at 4°C. Cells were stained with PBS57-loaded CD1d tetramer (NIH Tetramer facility, Emory University) for identification of invariant NKT (iNKT) cells. For staining of intracellular cytokines, cells were stained with surface markers, fixed with 2% formaldehyde and permeabilized with 0.5% saponin buffer. After permeabilization, cells were stimulated with PMA (100 ng/ml; Sigma), ionomycin (1 μg/ml; Sigma) for 4 hours and Brefeldin A (10 μg/ml; Sigma) was added for last 3 hours. Cells were then stained with antibodies for IL-17A, IFN- γ and respective isotype controls. The stained cells were acquired on a LSRII (BD Biosciences) and data analysis was performed with Flowjo software (Tree Star, Ashland, OR). The absolute number of cells has been adjusted for the weight of the lung tissue, as only a portion of lung is used for flow cytometry. All antibodies were obtained from BioLegend.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). For comparison of two groups with normal distribution, two-tailed unpaired *t* test was used. One-way ANOVA with *post hoc* Newman-Keuls test was applied for comparisons between multiple groups with normal distribution. For skewed data, Mann-Whitney *U* test was applied. The prevalence of OB between different groups in Table 1 was compared using two-tailed Fisher's exact test. Differences were considered significant at $P<0.05$. All data are expressed as mean ± (SEM).