Supplementary Figures and Tables

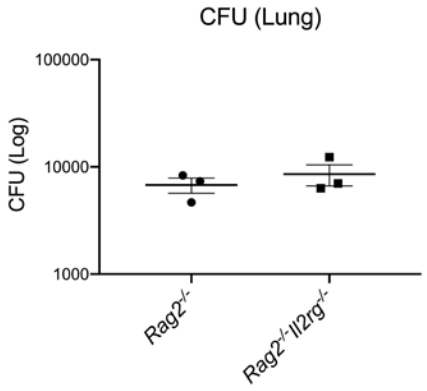

Figure S1. Lung CFU comparing between *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice 12 hours post infection with 10^6 CFU ST258 C4

6-8 week old male *Rag2*$^{-/-}$ mice and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were infected with 10^6 ST258 C4 strain, and 12 hours after challenge lungs were harvested. Lung CFU was not significantly different between groups (A; *n* = 3, representative of two independent experiments). Data are presented as mean ± SEM.

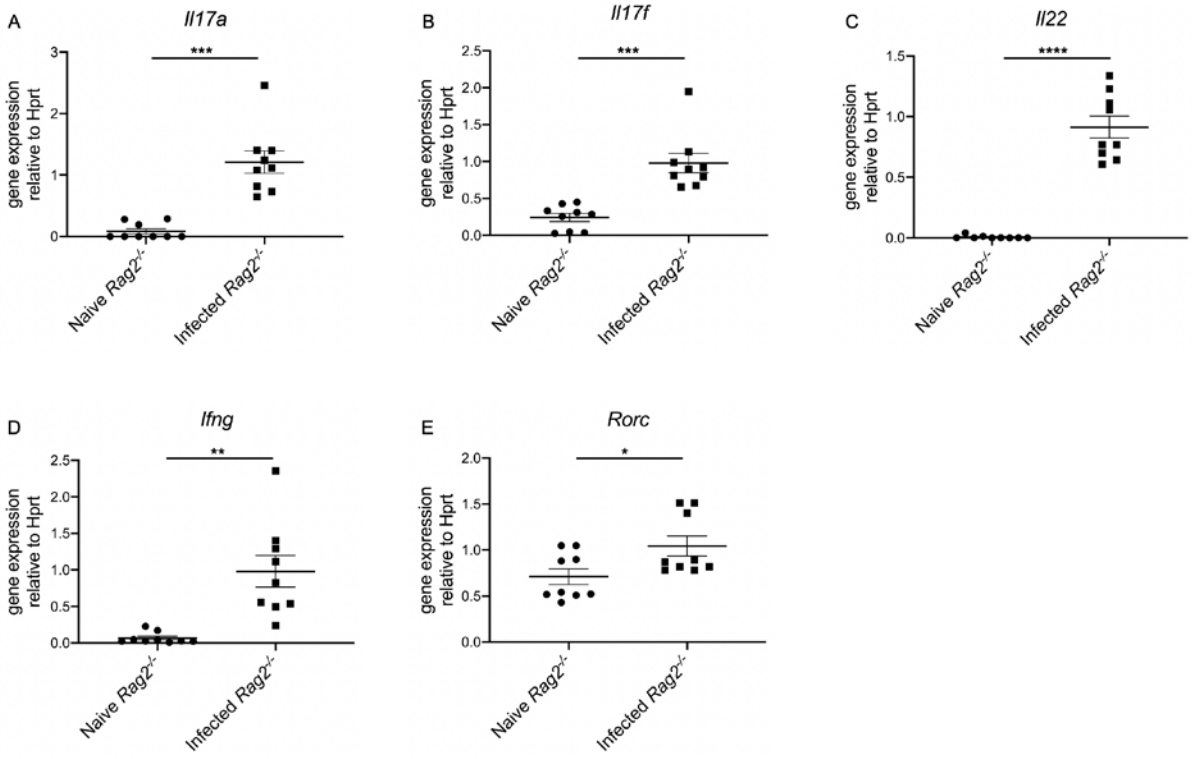

Figure S2. Real time RT-PCR of genes in naive and infected *Rag2*$^{-/-}$ mice

6-8 week old male *Rag2*$^{-/-}$ mice were infected with 10^6 ST258 C4 strain, and 12 hours after challenge lungs were harvested, and gene expression assay was performed by real time RT-PCR (*n* = 9, Data are pooled from two independent experiments). (A) *Il17a*, (B) *Il17f*, (C) *Il22*, (D) *Ifng*, and (E) *Rorc* mRNA expression in the lungs of naïve *Rag2*$^{-/-}$ and infected *Rag2*$^{-/-}$ mice are shown. Data are presented as mean ± SEM. Significant differences are designated by using unpaired t-test. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$

Figure S3. Gating analysis on cytokine positive cells in the scRNAseq dataset

Single cell RNA sequencing was performed 12 hours post infection with 10^6 CFU ST258 C4 on 6-8 week old male *Rag2*$^{-/-}$ lung cells using CD45 selection after depletion of Ly6G$^+$ cells. (*n* = 2/group). Then we gated on cytokine positive cells. (A) *Icos*$^+$*Il17a*$^+$, (B) *Il22*$^+$, (C) *Ccr6*$^+$, (D) *Rora*$^+$, (E) *Rorc*$^+$, (F) *Ncr1*$^+$, and (G) *Il5*$^+$ are shown as red dots in the dot plots.

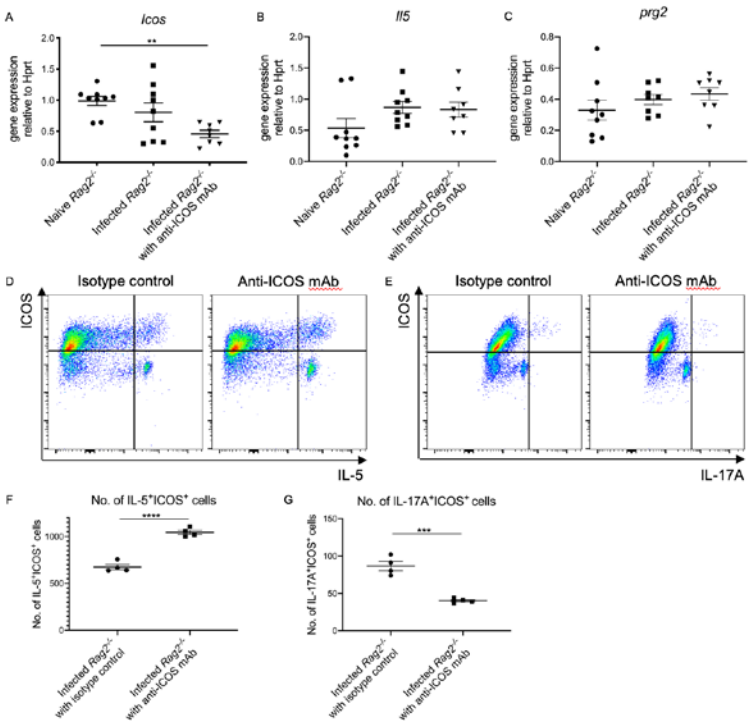

Figure S4. Effect of anti-ICOS mAb on type 2 genes

6-8 week old male *Rag2*$^{-/-}$ mice were infected with 10^6 CFU ST258 C4 strain via intratracheal, and some mice were administered with 500µg anti-ICOS mAb via intraperitoneally once 2 hours before infection. Twelve hours after inoculation lungs were harvested and real time RT-PCR was performed. (mean ± SEM, *n* = 9, Data are pooled from two independent experiments). (A) *Icos,* (B) *Il5* and (C) *prg2* mRNA expression in the naïve *Rag2*$^{-/-}$, infected *Rag2*$^{-/-}$ and infected *Rag2*$^{-/-}$ pretreated with anti-ICOS mAb are shown. Lung digested cells were enriched for innate lymphoid cells to analyze via flow cytometry. Flow gating strategies were conducted as CD45$^+$CD127$^+$Lin$^-$ (*n* = 4, two independent experiments). Representative FACS plots and the quantification of them showed the IL-5$^+$ICOS$^+$ producing cells (D, F) and IL-17A$^+$ICOS$^+$ producing cells (E, G) in infected *Rag2*$^{-/-}$ mice pretreated with isotype control or anti-ICOS mAb. Significant differences for (A), (B), and (C) are designated by using one-way ANOVA test followed by Tukey's multiple comparisons test, and for (F) and (G) by unpaired t test. **, P < 0.01, ***, P < 0.001, ****, P < 0.0001.

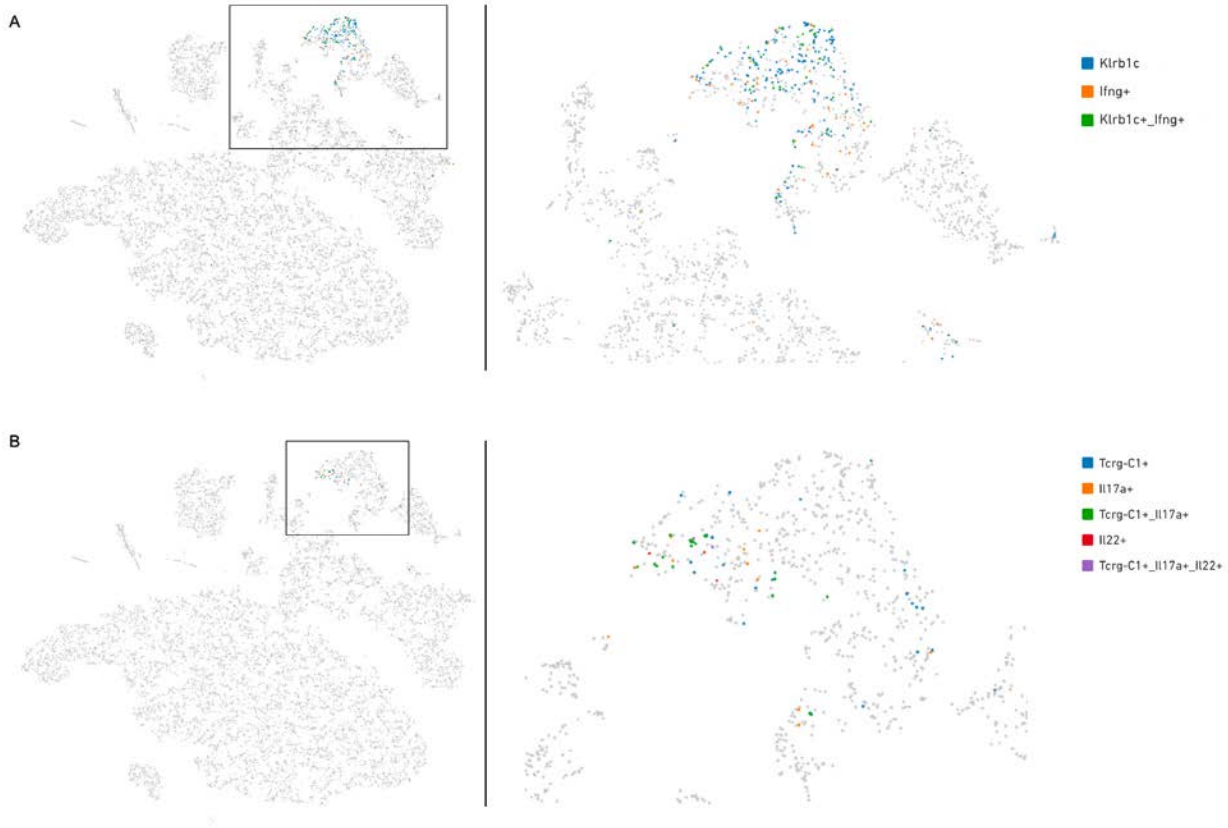

Figure S5. Single cell RNA sequencing on FK506 treated WT mice

TSNE from the scRNAseq data 12 hours post infection of ST258 C4 in C57BL/6 mice with FK506 treatment on the expression of *Ifng*, *Il17a* and *Il22* are shown. *Ifng* expression was mostly observed in NK cells (A), whereas *Il17a* and *Il22* expressing cells were clustered in γδ T-cells (B) (*n* = 2 mice/group).

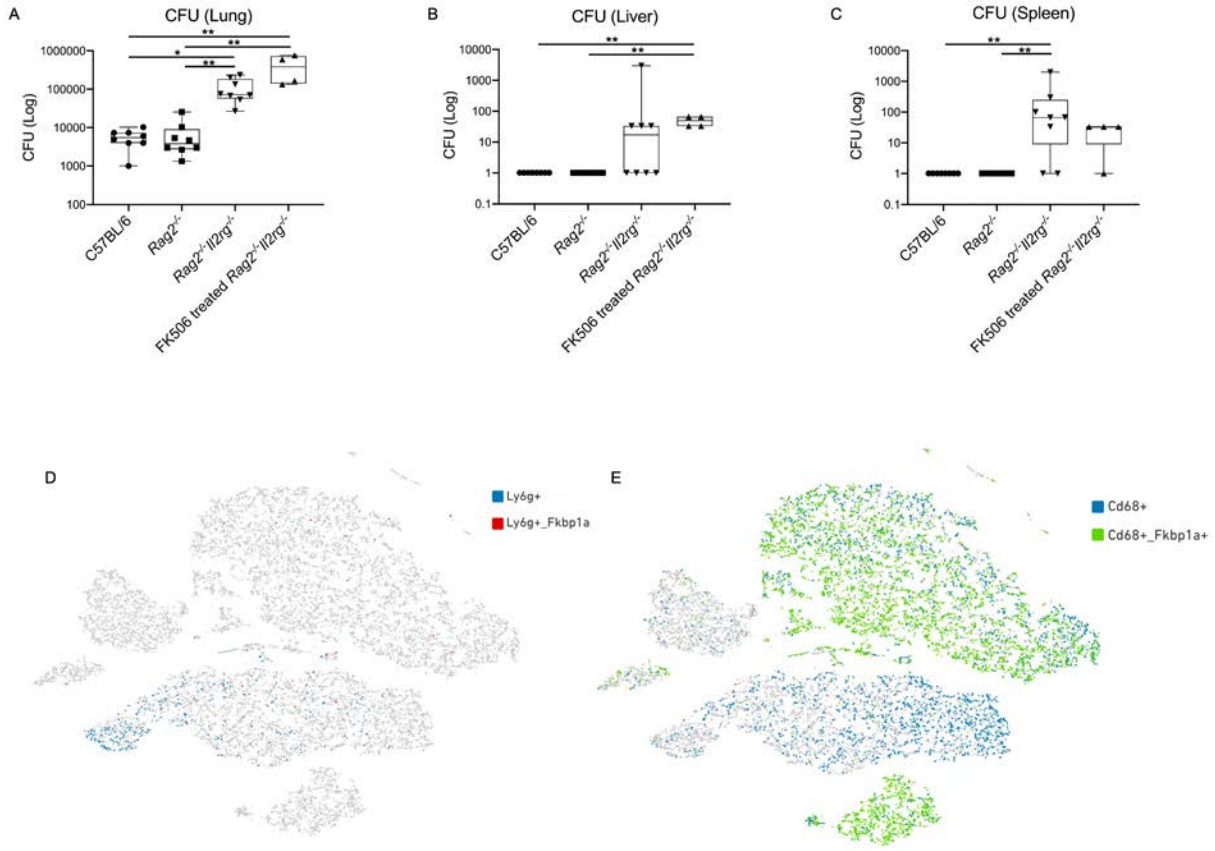

Figure S6. Effect of FK506 on myeloid cells in ST258 C4 infection

Forty-eight hours post intratracheal challenge with 10^6 CFU ST258 C4, 6-8 week old male C57BL/6 mice, *Rag2*$^{-/-}$ mice, *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice, and FK506 treated *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were euthanized (*n* = 4 mice/group). FK506 treatment on *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice showed the slight trend to increase the lung CFU (A), but not significant, and did not affect the dissemination to the liver (B) and spleen (C). Data are presented as box and whisker plots showing median, first and third quartiles, and maximum and minimum values. Significant differences are designated by using Kruskal-Wallis test followed by Dunn's multiple comparisons test. *, $P < 0.05$, **, $P < 0.01$. TSNE plots of scRNAseq data in ST258 C4 infected *Rag2*$^{-/-}$ mice attests that (D) 8.1% in Ly6g$^+$ cells (red) and (E) 60.7% in CD68$^+$ cells (green) are also *Fkbp1a*$^+$.

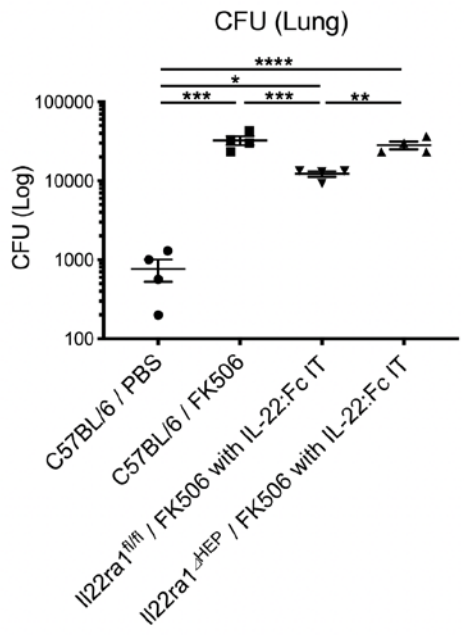

Figure S7. Effect of intratracheal IL-22:Fc and endogenous *Il22ra1* on infection

6-8 week old male WT C57BL/6 mice, *AlbCre*$^{-/-}$ x *Il22ra1*$^{fl/fl}$ *(Il22ra1*$^{fl/fl}$*) mice,* and *AlbCre*$^{-/+}$ x *Il22ra1*$^{fl/fl}$ *(Il22ra1*$^{\triangle HEP}$*)* mice were treated with vehicle control or 10mg/kg FK506 via intraperitoneally 24 hours prior to intratracheal challenge with 10^6 ST258 C4 strain. FK506 was administered every 24 hours until 24 hours before euthanasia. Some mice were treated with 1µg IL-22:Fc via intratracheally (IT) 30 minutes before infection. These mice were euthanized 48 hours post infection, and lung CFU were assessed (*n* = 4, two independent experiments). Significant differences are designated by using one-way ANOVA test followed by Tukey's multiple comparisons test. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$.

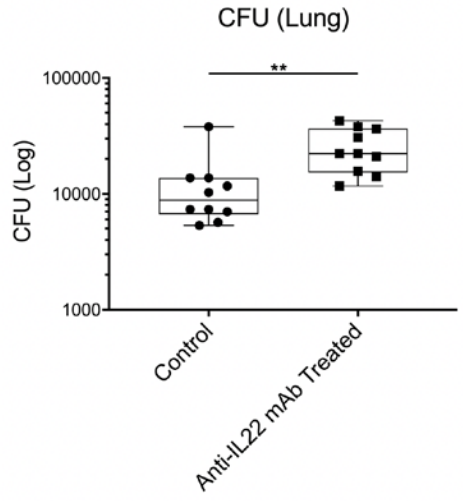

Figure S8. Effect of intratracheal anti-IL-22 mAb on infected WT C57BL/6 mice

6-8 week old male WT C57BL/6 mice were treated with 50μg anti-IL-22 mAb via intratracheal 30 minutes before 10^6 ST258 C4 pulmonary infection. Mice were euthanized and lung bacterial burdens were assayed 24 hours post infection (*n* = 10, Data are pooled from two independent experiments). Data are presented as box and whisker plots showing median, first and third quartiles, and maximum and minimum values. This data was analyzed by Mann-Whitney U test. **, $P < 0.01$.

A

Pathway	p-value
Cytokine-cytokine receptor interaction	1.924-6
Th17 cell differentiation	3.188e-4
Primary immunodeficiency	3.732e-4
Antigen processing and presentation	4.124e-4
Natural killer cell mediated cytotoxicity	0.001
Graft-versus-host disease	0.001
Necroptosis	0.004
Type I diabetes mellitus	0.006
Inflammatory bowel disease	0.014
Allograft rejection	0.031
Autoimmune thyroid disease	0.032

B

Pathway	p-value
Pancreatic secretion	0.001
Gastric acid secretion	0.004
Thyroid hormone synthesis	0.020
Glutamatergic synapse	0.026
Sphingolipid signaling pathway	0.031
Complement and coagulation cascades	0.046
Retrograde endocannabinoid signaling	0.046

Table S1. RNA sequencing comparing between *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice 12 hours post infection with 10^6 CFU ST258 C4

6-8 week old male *Rag2*$^{-/-}$ mice and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were infected with 10^6 ST258 C4 strain, and 12 hours after challenge lungs were harvested. Significantly (A) downregulated or (B) upregulated KEGG pathways were generated by pathway analysis in infected *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice compared to *Rag2*$^{-/-}$ mice are shown.

Pathway	p-value
Antigen processing and presentation	1.292e-4
Herpes simplex infection	0.001
Staphylococcus aureus infection	0.006
Cell adhesion molecules	0.009
Allograft rejection	0.009
Autoimmune thyroid disease	0.009
Intestinal immune network for IgA production	0.010
HTLV-1 infection	0.013
Th17 cell differentiation	0.016
Toxoplasmosis	0.022
Asthma	0.035
Type I diabetes mellitus	0.045
Graft-versus-host disease	0.045
Influenza A	0.046
Leishmaniasis	0.046
Th1 and Th2 cell differentiation	0.047

Table S2. Pathway analysis in FK506 treated WT mice

Significantly downregulated KEGG pathways from pathway analysis using scRNAseq data 12 hours post infection of 10^6 ST258 C4 in C57BL/6 mice with FK506 treatment are shown (*n* = 2 mice/group).