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IL-17 receptor signaling in the lung epithelium is required for mucosal chemokine gradients and pulmonary host defense against *K. pneumoniae*

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Summary

The cytokine IL17, and signaling via its heterodimeric IL-17RA/IL-17RC receptor, is critical for host defense against extracellular bacterial and fungal pathogens. Polarized lung epithelial cells express IL-17RA and IL-17RC basolaterally. However, their contribution to IL-17-dependent pulmonary defenses in vivo remains to be determined. To address this, we generated mice with conditional deletion of *Il17ra* or *Il17rc* in *Scgb1a1*-expressing club cells, a major component of the murine bronchiolar epithelium. These mice displayed an impaired ability to recruit neutrophils into the airway lumen in response to IL17, a defect in bacterial clearance upon mucosal challenge with the pulmonary pathogen *Klebsiella pneumoniae,* and substantially reduced epithelial expression of the chemokine *Cxcl5*. Neutrophil recruitment and bacterial clearance were restored by intranasal administration of recombinant CXCL5. Our data show that IL-17R signaling in the lung epithelium plays a critical role in establishing chemokine gradients that are essential for mucosal immunity against pulmonary bacterial pathogens.

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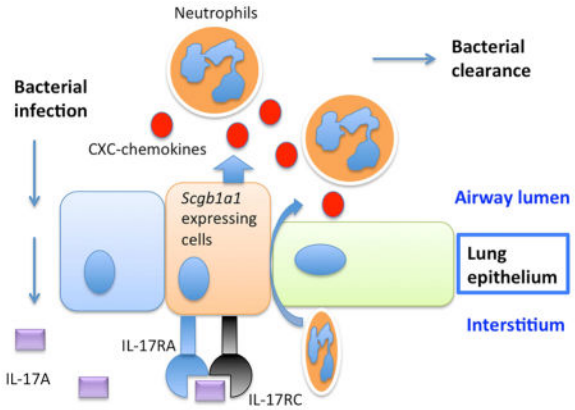
Authors Contributions:
KC and JKK designed all the experiments and wrote the manuscript. KC performed all the in vivo experiments with the assistance of GT, CJE, MB and AVG. TE scored the histology. EEW, WE and DMR participated in the characterization of the flox mice and performed in vitro experiments. TW and WC provided assistance on RNA-seq analysis. SLG and JSL provided the reagents for the experiments involving the *Il17re*$^{-/-}$ mice and helped with manuscript writing.

Graphical abstract



Introduction

The IL-17 family of cytokines includes 6 members, IL-17 (IL-17A), IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F, which are produced by multiple cell types (Pappu et al., 2011). Signaling by IL-17 family cytokines is mediated by members of the IL-17 receptor family, IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE (Gaffen, 2009). While IL-17RA is shared among many IL-17 family members, IL-17RC is the unique receptor for IL-17 and IL-17F. These two members share the highest degree of homology and show similar functions. Numerous studies have demonstrated that IL-17 and IL-17F are critical molecules in mucosal host defense and inflammatory diseases (Chen and Kolls, 2013; Kumar and Subramaniyam, 2015; Way et al., 2013). Infections and inflammatory diseases increase the accumulation of IL-17-producing cells in lung tissue. Polarized airway epithelial cells respond to IL-17 via the basolateral expression of IL-17RA (McAllister et al., 2005) and IL-17RC (Kuestner et al., 2007), and these cells can produce antimicrobial proteins and several neutrophil chemoattractants including several ELR^+ C-X-C chemokines. These pathways are thought to be critical for the function of IL-17 in mucosal immunity. Consistent with this, germ line IL-17RA KO show reduced CXCL2 and G-CSF expression in a model of intrapulmonary *K. pneumoniae* infection (Ye et al., 2001). Using bone marrow chimeric mice, several groups have shown that radioresistant tissue resident cells rather than radiosensitive cells of hematopoietic origin are essential for IL-17R signaling in various tissues (Ge et al., 2014; Linden et al., 2005; Meng et al., 2012; Oh et al., 2011). However, this bone marrow chimeric approach does not address what specific cell type is responsible for IL-17 mediated responses. Furthermore, a subset of alveolar macrophages has also been shown to be radioresistant (Guilliams et al., 2013), which complicates the interpretation of data generated from bone marrow chimeras. To study the function of epithelial IL-17RA signaling, we generated lung epithelial specific conditional IL-17RA KO mice using *Scgb1a1-cre* mice which deletes in club cells, a major component of the bronchiolar epithelium in mice (Simon et al., 2006). Deletion of *Il17ra* or *Il17rc* in lung epithelium resulted in compromised clearance of *K. pneumoniae* in the lung, including the drug resistant isolate expressing New Delhi Metallo-beta-lactamase-1 (2010). This impairment was associated with reduced neutrophil recruitment and a substantial reduction in epithelial

expression of *Cxcl5*, a gene encoding the neutrophil chemoattractant CXCL5 (LIX). The mucosal immune defect in conditional *Il17ra* mice could be restored with mucosal administration of CXCL5. Our data underscore the essential role of epithelial IL-17R expression in mucosal immunity against *K. pneumoniae* by controlling mucosal chemokine gradients that are essential for optimal neutrophil recruitment.

Results

Phenotypic characterization of IL-17R$^{fl/fl}$ mice

Targeting of *Il17ra* was achieved by introducing LoxP sites flanking exon 3 of the *Il17ra* gene, and targeting of *Il17rc* was accomplished by adding LoxP sites flanking exons 2 and 3 of the *Il17rc* gene (Kumar et al., 2016). To demonstrate that this would generate a functional null allele, skin fibroblasts from homozygous *Il17ra*$^{fl/fl}$ mice were transduced with adenoviruses encoding the *Cre* recombinase (AdCRE). As shown in (Supplementary Fig. 1A), fibroblasts treated with AdCRE showed substantially reduced expression of IL-17 induced genes such as *Csf3* and *Il6,* which was also associated with diminished *Il17ra* expression. Importantly, the expression of *Il17rc* was not affected. Cre-mediated recombination was also observed in vivo by standard PCR analysis in the spleen of *Il17ra*$^{fl/fl}$ mice receiving intravenous AdCRE (Supplementary Fig. 1B). To further validate the functional deletion of the *Il17ra* gene in vivo, we crossed the *Il17ra*$^{fl/fl}$ mice to *E2a-Cre* mice (Lakso et al., 1996). This line carries a *Cre* transgene under the control of the adenovirus *E2a* promoter that targets expression of Cre to the early mouse embryo, resulting in Cre-mediated recombination in a wide range of tissues. Flow cytometry analysis on peripheral blood confirmed the absence of surface IL-17RA expression in the *Il17ra*$^{fl/fl}$*E2a-cre*$^{+}$ mice (Supplementary Fig. 1C) while the deletion of *Il17rc* in the *Il17rc*$^{fl/fl}$*E2a-cre*$^{+}$ mice did not seem to affect IL-17RA expression on these cells (Supplementary Fig. 2A). However, the percentage of Gr-1^{+} neutrophils in the peripheral blood was reduced in the *Il17ra*$^{fl/fl}$*E2a-cre*$^{+}$ mice (Supplementary Fig. 2B). This is consistent with the phenotype observed in the global *Il17ra*$^{-/-}$ mice (Kelly et al., 2005; Smith et al., 2008). To study the functional loss of *Il17ra* in the lungs of these mice, we used an intranasal delivery model (Herjan et al., 2013) where application of IL-17 in wild type mice induces a massive neutrophil influx into the airways (Fig. 1A&B). Global deletion of *Il17ra* abolished IL-17-induced pulmonary neutrophilia in the *Il17ra*$^{fl/fl}$*E2a-cre*$^{+}$ compared to wild type mice (Fig. 1A&B). Nearly identical defects in IL-17-induced neutrophil influx to the airways were observed in *Il17rc*$^{fl/fl}$*E2a-cre*$^{+}$ mice (Fig. 1A&B). This defect was also accompanied by a significant reduction in IL-17-dependent cytokines in the BAL such as G-CSF and IL-6 (Fig. 1C). The *Il17rc*$^{fl/fl}$*E2a-cre*$^{+}$ mice were still able to respond to intranasal CXCL1, an IL-17 regulated neutrophil chemoattractant, suggesting deletion of IL-17RC did not affect CXCR2 ligand mediated recruitment (Supplementary Fig. 2C). Taken together, these data demonstrate that functional deletion of the *Il17ra* and *Il17rc* alleles is successfully achieved in vivo, and these floxed mice can be used in the generation of tissue specific conditional KO mice.

Defective PMN responses and bacterial clearance in the IL17RA lung conditional KO mice

We have previously reported that polarized lung epithelium only respond to IL-17 applied basolaterally due to the site-specific expression of IL-17RA and IL-17RC (Kuestner et al.,

2007; McAllister et al., 2005). To determine if the lung epithelium is required for IL-17-mediated responses in vivo, we crossed the *Il17ra*$^{fl/fl}$ and *Il17rc*$^{fl/fl}$ mice to *Scgb1a1-cre* transgenic mice with the transgene expression specifically targeting the airway epithelium (Simon et al., 2006). Other groups have also reported that *Scgb1a1-Cre* is expressed in non-ciliated epithelial cells (Bertin et al., 2005; Rawlins et al., 2009). *Scgb1a1-Cre* mediated recombination in the lungs of the mice was confirmed by conventional PCR detecting the recombined allele (Supplementary Fig. 3A). In addition, there was significantly reduced expression of *Il17ra* transcript from bronchial brush samples of these mice (Supplementary Fig. 3B). The *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice displayed significantly reduced neutrophil numbers in the BALs after administration of recombinant IL-17 intranasally compared to littermate *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{-}$ controls (Fig. 2A). To assess the neutrophil recruitment in response to endogenous IL-17, we administered intranasal IL-1β+IL-23, a cytokine combination that triggers potent IL-17 release from lung γδ T-cells (Dubin et al., 2012; Price et al., 2012). The *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice also displayed significantly reduced neutrophil numbers in the BAL fluid (Fig. 2B) after intranasal IL-1β+IL-23 treatment. The defects in neutrophil recruitment in the Cre^{+} mice were not due to a lack of IL-17 production, since type 17 cytokines including IL-17A, IL-17F and IL-22 were equally induced in the Cre^{+} and Cre^{-} mice (Fig. 2C). These data suggested that epithelial IL-17R is critical for IL-17 mediated neutrophil influx in the lungs. Furthermore, the *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice were unable to control bacterial infection in the lungs following mucosal challenge with *K. pneumoniae* (Fig. 2D), demonstrating that epithelial IL-17RA signaling is required for bacterial clearance in in this model. We also performed hematoxylin and eosin (H&E) staining of the lungs to assess histologically the impact of defective IL-17 signaling in the *Scgb1a1* expressing cells but found similar degrees of tissue inflammation in both the Cre^{+} and Cre^{-} mice (Fig. 3A & B), suggesting IL-17R signaling in the epithelium is more important in recruiting bacterial killing neutrophils than in promoting epithelial integrity.

Loss of Cxcl5 expression in the IL17RA lung conditional KO mice

To explore the mechanism that leads to the impaired neutrophil recruitment and consequent mucosal bacterial clearance defect in the IL-17R epithelial conditional KO mice, we first performed RNA-seq analysis on the bronchial epithelium of the *Il17rc*$^{fl/fl}$*Scgb1a1-cre*$^{-}$ and the *Il17rc*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice after intranasal IL-17 challenge. Our analysis identified 694 genes that were differentially expressed in the *Il17rc*$^{fl/fl}$*Scgb1a1-cre*$^{-}$ mice when compared to the *Il17rc*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice, defined as those with a minimum of a 1.2-fold change in expression (p-value <0.05). Genes that have been reported to be regulated by IL-17 in vitro were highly up-regulated in vivo including chemokines (*Ccl20, Cxcl1, Cxcl5*), cytokines (*Csf3, Il6*), and other genes controlled by IL-17 (*Lcn2, Nfkbiz, Pigr*) (Fig. 4A). Gene set enrichment analysis (GSEA) of the data showed enrichment of the Cytokine-cytokine receptor interaction and Chemokine signaling KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (Fig. 4B). Using the same dataset and the Upstream Regulator Analytic from the Ingenuity Pathway Analysis software, we identified a numbers of signaling pathways related to innate immunity (Lipopolysaccharide, TLR4, RELA) and inflammation (IFNG, TNF, IL1B, TGFB1) (Fig. 4C). Real-time RT-PCR analysis on *Cxcl1, Cxcl5, Csf3* and *Il6* transcription in mice received the same treatment validated the RNA-seq results

(Supplementary Fig. 4). To confirm the findings on chemokines and cytokines related to neutrophil recruitment, we assayed cytokines and chemokines related to neutrophil recruitment in the BAL fluid 24 hours post IL-17 challenge. The levels of G-CSF and IL-6 production in the BAL were reduced in the conditional knockout mice (Fig. 5A). Additionally, ligands for CXCR2, an essential receptor for neutrophil recruitment, CXCL1 (KC) and CXCL5 (LIX) were also analyzed. CXCL1, encoded by *Cxcl1*, was reduced while CXCL5, encoded by *Cxcl5*, was substantially reduced in the *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice. To determine if these genes are transcriptionally regulated as suggested by our RNA-seq analysis, we performed real-time RT-PCR but found all were equally expressed in RNA samples obtained from whole lung homogenate (Fig. 5B). However, when gene expression was specifically assayed in the lung epithelium using an optimized mouse bronchial brushing technique, *Cxcl5* expression was found to be significantly reduced in *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice compared to littermate *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{-}$ controls (Fig. 5B). These data suggest that epithelial *Cxcl5* expression is critically regulated by epithelial *Il17ra* expression (Fig. 5B). To investigate the functional relevance of CXCL5 we designed a CXCL5 rescue experiment in the conditional knockout mice. To assess early neutrophil recruitment in *K. pneumoniae* infection, we chose an earlier time point, 4h after infection, as the bacterial burden in the conditional KO mice were substantially higher 24h after infection, which could confound interpretations. At 4h post infection, the *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ had significantly less neutrophils in the BAL as compared to the *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{-}$ mice while administration of rCXCL5 intranasally 2h after infection substantially increased the neutrophil counts in the BAL (Fig. 5C). Additionally, at 24h after infection, administration of rCXCL5 at two hours post-infection substantially improved the bacterial clearance of *K. pneumoniae* in the *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice (Fig. 5D and Supplementary Fig. 5).

Defective PMN responses and bacterial clearance in IL17RC lung conditional KO mice

IL-17RA is utilized by several IL-17 family members, thus the defects observed in the *Il17ra*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice could be due defective signaling of several IL-17 family members other than IL-17 or IL-17F. IL-17C, which signals through IL-17RA and IL-17RE, was indeed induced in normal human bronchial epithelial (NHBE) cells in response to IL-17 stimulation (Ray et al., 2015) (Supplementary Fig. 6A). Transcription of *Il17c* was also induced in vivo in both the intranasal IL-17 challenge as well as *K. pneumoniae* infection (Supplementary Fig. 6B). However, IL-17C/IL-17RE signaling did not seem to contribute to the induction of chemokine responses and cytokines in response to intranasal IL-17, since neutrophil recruitment and the expression of *Cxcl1*, *Cxcl2*, *Cxcl5* and *Il6* did not change in *Il17re*$^{-/-}$ mice (Supplementary Fig. 6C&D). IL-17RE is the unique receptor subunit for IL-17C signaling (Swamy and Hayday, 2011). When infected with live *K. pneumoniae*, the *Il17re*$^{-/-}$ mice had a similar bacterial burden in the lungs as the wild type controls (Fig. 6). To assess the IL-17C/IL-17RE signaling in the lungs specifically, we sought to block IL-17C in vivo with recombinant IL-17RE Fc protein. To assess the specificity of IL-17RE Fc, mice were treated with IL-17RE Fc in addition to IL-17C. IL-17C was sufficient to induce neutrophil recruitment into the BAL fluid and this was reduced in mice administered IL-17RE Fc, validating that IL-17RE Fc effectively blocks the IL-17C signaling (Fig 6B). However, blocking IL-17C with IL-17RE Fc showed no significant impact on bacteria

burden or IL-17 related gene expression in the lungs after *K. Pneumoniae* infection (Fig. 6C&D). These data suggest that IL-17C signaling is not required for the clearance of *K. pneumoniae*, emphasizing the important role of IL-17A, the other ligand for IL-17RA, in host defense against *K. pneumonia.*

In contrast to IL-17RA, IL-17RC is used only by IL-17A and IL-17F (Toy et al., 2006). To confirm the requirement of epithelial IL-17RC in host defense against bacterial infection, we challenged the *Il17rc*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice with a drug resistant *K. pneumoniae* isolate expressing New Delhi Metallo-beta-lactamase-1 intranasally. The *Il17rc*$^{fl/fl}$*Scgb1a1-cre*$^{+}$ mice indeed demonstrated a defect in controlling of the bacterial burden in the lungs (Fig. 6E).

Discussion

The potential role of lung epithelium in orchestrating chemokine gradients and subsequent recruitment of neutrophils to the airways has been hypothesized for several years (Dubin and Kolls, 2008). This hypothesis is supported by in vitro data showing that the lung epithelial cells are capable of producing various chemokines including IL-8 and CXCL5 upon IL-17 stimulation (Manni et al., 2014). In situ hybridization staining on the lungs from *K. pneumoniae* infected mice also demonstrated that the mRNA expression of *Cxcl1* and *Cxcl2* are co-localized within the lung epithelium and administration of IL-17 augmented hybridization signals (Aujla et al., 2008). Moreover, recent data using bone marrow chimeras in *Cxcl5*$^{-/-}$ mice also demonstrated that non-myeloid cells are the cellular source of CXCL5 (Balamayooran et al., 2012; Liu et al., 2011). These two studies using two different models, smoke induced lung inflammation and *E. coli* infection respectively, demonstrated that radioresident cells but not hematopoietic cell-driven CXCL5 is important for mediating lung inflammation. Experiments with bone marrow chimeric mice in an *M. tuberculosis* model also demonstrated that the radioresistant lung-resident cells but not the hematopoietic lineage are responsible for CXCL5-dependent PMN recruitment (Nouailles et al., 2014).

However, there is a lack of data confirming this idea using targeted genetic approaches that can identify specific cell types responsible for CXCL5 production. In this study, *Il17ra*$^{fl/fl}$ mice were crossed to lung epithelial specific *Scgb1a1-cre* mice to establish mice with epithelial conditional deletion of *Il17ra*. These mice displayed reduced neutrophil influx into the airways upon intranasal IL-17 challenge. As a consequence, the lung conditional KO mice were also unable to control mucosal *K. pneumoniae* infection. Analyses of gene expression in the bronchial epithelium as well as the whole long homogenate revealed that there was a major defect in *Cxcl5* expression, which was responsible for the inability of the host to control the bacterial burden. To confirm that this phenotype was IL-17A/IL-17F specific, we crossed the *Il17rc*$^{fl/fl}$ mice to the *Scgb1a1-cre* mice and found that these mice phenocopied the *Il17ra*$^{fl/fl}$ mice.

We also found that epithelial IL-17R expression is also required for the controlling the growth of the NDM-1+ strain of *K. pneumoniae* suggesting that compromised epithelial IL-17R signaling may increase susceptibility to infection caused by extracellular pathogens

including the antibiotic resistant strains. Indeed, humans with deficiencies in IL-17RA, IL-17RC and IL-17F have been shown to have increased susceptibility to Candida species and develop Chronic mucocutaneous candidiasis (Ling et al., 2015; Puel et al., 2011). Alternatively perhaps IL-17RA or IL-17RA signaling could be augmented to enhance treatment of multi-drug resistant *K. pneumoniae* infections. The accompanying paper by Conti et al. also demonstrated a critical role for epithelial IL-17R signaling in oral mucosal immunity against *C. albicans*. In this study, a new CRE-expressing transgenic mouse line specific for the oral and esophageal epithelium, i.e., the $K13^{CRE}$ line, was crossed to $Il17ra^{fl/fl}$ mice to generate mice lacking IL-17RA exclusively in the oral/esophageal epithelium. When challenged with *Candida albicans*, these conditional IL-17R deficient mice phenocopied global $Il17ra^{-/-}$ mice in many aspects including increased mucosal fungal burden, disease symptoms, and gene expression patterns (Conti, 2016). However, the defective effector molecule in these mice was β-defensin 3, which differs somewhat from CXCL5 observed in the $Il17ra^{fl/fl}Scgb1a1\text{-}cre^{+}$ mice. This may reflect different functions of different types of epithelial cells as K13 expression is restricted to the internal stratified squamous epithelial in the oral cavity (Winter et al., 1990) while *Scgb1a1* is mostly expressed in cuboidal, non-ciliated, secretory club cells of the bronchiolar epithelium (Simon et al., 2006).

Several groups have observed that blood neutrophil counts are reduced in IL-17R-deficient mice (Kelly et al., 2005; Smith et al., 2008) and this is consistent with our observations in our $Il17ra^{fl/fl}E2a\text{-}cre^{+}$ mice. This developmental defect could lead to the reduced neutrophil influx in the lung upon intranasal IL-17 administration. These observations led us to generate the epithelial specific KO mice by crossing the IL-17R flox mice to the *Scgb1a1-cre* line. *Scgb1a1-cre* did efficiently mediate recombination of *Il17ra* in the lungs (Supplementary Fig. 3). However, the lung epithelium is heterogeneous and deletion of IL-17R in only one cell type might be expected to exhibit only a partially impaired response. This may explain why only one principal CXCR2 ligand, *Cxcl5*, was reduced in the $Il17ra^{fl/fl}$ $Scgb1a1\text{-}cre^{+}$ mice following IL-17 challenge (Fig. 5A&B). The gene expression from lung homogenate is comparable between two groups since most cells in the lungs from the epithelial IL-17R KO mice still express the IL-17 receptors. For example airway smooth muscle cells and fibroblasts have been shown to produce chemokines in response to IL-17 (Ivanov and Linden, 2007; Kwofie et al., 2015). This study establishes a critical role of epithelial IL-17RA/RC signaling to achieve the necessary chemokine gradients for lumenal egress of neutrophils in the lung. Further studies crossing the $Il17ra^{fl/fl}$ mice with additional epithelial specific CRE lines are needed to determine the exact roles of epithelial IL-17R in pulmonary host defense as well as chronic lung inflammation in other cell lineages. For example, a recently established mouse model incorporated a tamoxifen-inducible Cre recombinase (Cre-ERT2) under the control of the human surfactant protein C (SPC) promoter which deletes in distal lung epithelium (Gui et al., 2012). Crossing this line with the IL-17R flox mice would permit ablation of IL-17R in the distal lung in a temporally controlled manner.

Another study using an *E. coli* pneumonia model demonstrated that a bacterial clearance defect when ablating Onconstatin-M during pneumonia was also dependent on the epithelial expression of *Cxcl5* (Traber et al., 2015). These studies together with the present study

emphasize the important role of epithelial derived CXCL5-secreting cells in orchestrating PMN recruitment to bacterial infection in the lung. Indeed, the essential role of CXCL5 in multiple models demands a more comprehensive analysis of this chemokine, which shares receptors with many others such as CXCL1 and CXCL2.

Taken together, our study demonstrated that epithelial IL-17R signaling is essential in regulating chemokine pathways in vivo, particularly *Cxcl5* expression and subsequent recruitment of neutrophils for the clearance of bacteria in the lungs. Loss of epithelial IL-17R signaling may compromise host defense against extracellular bacteria and targeting this molecular pathway may provide clinical benefits for patients with pneumonia.

Experimental Procedures

Animal Studies

8–10 week old mice were used for in vivo studies. The *Il17ra*$^{fl/fl}$ and *Il17rc*$^{fl/fl}$ mice were generated at Ozgene and have been recently described (Kumar et al., 2016). *Il17re*$^{-/-}$ mice were acquired from Genentech (Conti et al., 2015). Recombinant cytokines or 10^4 *K. pneumoniae* were given to isoflurane anesthetized mice in cold sterile PBS (50μL) by intranasal injection. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Experimental K. pneumoniae Infection

Klebsiella pneumoniae ATCC strain 43816, serotype 2 or BAA-2146 (NDM1+) (ATCC, Rockville, MD) isolates were grown in 100 mL of tryptic soy broth (Difco, Detroit, MI) for 18 h at 37°C. The culture was then diluted at 1:100 and grown for 2 additional hours to reach early logarithmic phase. The concentration of *K. pneumoniae* was determined by measuring the absorbance at 600 nm. Bacteria were pelleted by centrifugation at 5,000 rpm for 15 min, washed twice in cold PBS, and resuspended at the desired concentration. 24h after infection, mice were sacrificed and left lungs were harvested in TriZol for RNA extraction, right lungs were harvested in sterile PBS for bacterial burden (CFU) and Luminex (Millipore) on the Bioplex reader (Bio-Rad).

Luminex

Kits with custom luminex panel including G-CSF, IL-6, CXCL1, CXCL5 and CCL2 were purchased from EMD Millipore Corporation (Billerica, MA) and the assays were performed per manufacturer instructions.

RNA isolation from mouse bronchial brush

The bronchial brushing was performed using a 5–7cm long PE50 tubing (Becton Dickinson), sanding to create roughness (P240 sand paper), and treating with RNaseZap (Ambion). Two sanded tubes were inserted into the right main and left main bronchus separately with gentle brushing (twisting) and immediately placed in the same eppendorff tube containing 1 mL TriZol (life technology). These tubes were vortexed thoroughly before adding chloroform for RNA extraction and RNA carrier Glycoblue (life technology) was added during RNA precipitation. RNA samples were then quantified by spectrophotometer

and subjected to cDNA synthesis and gene expression analysis. RNA sequencing data was analyzed using the *CLC Genomics* Workbench software (QIAGEN) and the raw data have been deposited into the Sequencing Read Archive under SRA accession number SRP087890.

Real Time PCR

RNA was isolated using TriZol reagent (Life Technology) and cDNA was prepared using iScript reverse transcriptase master mix (Bio-Rad, Hercules, CA). Real time PCR was carried out with Bio-rad CFX96 system using TaqMan PCR Master Mix (Life Technologies) and premixed primers/probe sets from Life Technologies.

Flow cytometry

anti-mouse Gr-1, Ly6G, CD11b and F4/80 monoclonal antibodies were purchased from eBioscience. Data were acquired using FACS LSR II (BD Bioscience) and analyzed by FlowJo software (Treestar, CA).

Ear skin fibroblasts isolation and stimulation

Mouse ear skin pieces (app. 3×3mm) were washed with 70% EtOH and transferred into DMEM. Skin pieces were then cut into small pieces using scalpel before incubation for 1hr at 37 °C (water bath) with 0.25% trypsin. Cells were then pelleted and re-suspended in DMEM/10%FCS and fed with fresh medium the next day. IL-17 stimulation was performed once the fibroblasts reached 70% confluence.

Pathology scores

Hematoxylin and eosin–stained slides were coded and scored from 0 (absent) to 4 (severe) for the following parameters: interstitial inflammation, endothelialitis, bronchitis, edema, thrombi, pleuritis and percentage of the lung surface demonstrating confluent (diffuse) inflammatory infiltrate by a pathologist blinded for groups. The total “lung inflammation score” was expressed as the sum of the scores for each parameter.

Statistical Analyses

Unpaired, two-tailed, Student’s t tests, $\alpha = 0.05$, were used to assess whether the means of two normally distributed groups differed significantly. One-way ANOVA analysis was used to compare multiple means. Significance is indicated as $P < 0.05$. All error bars represent the SEM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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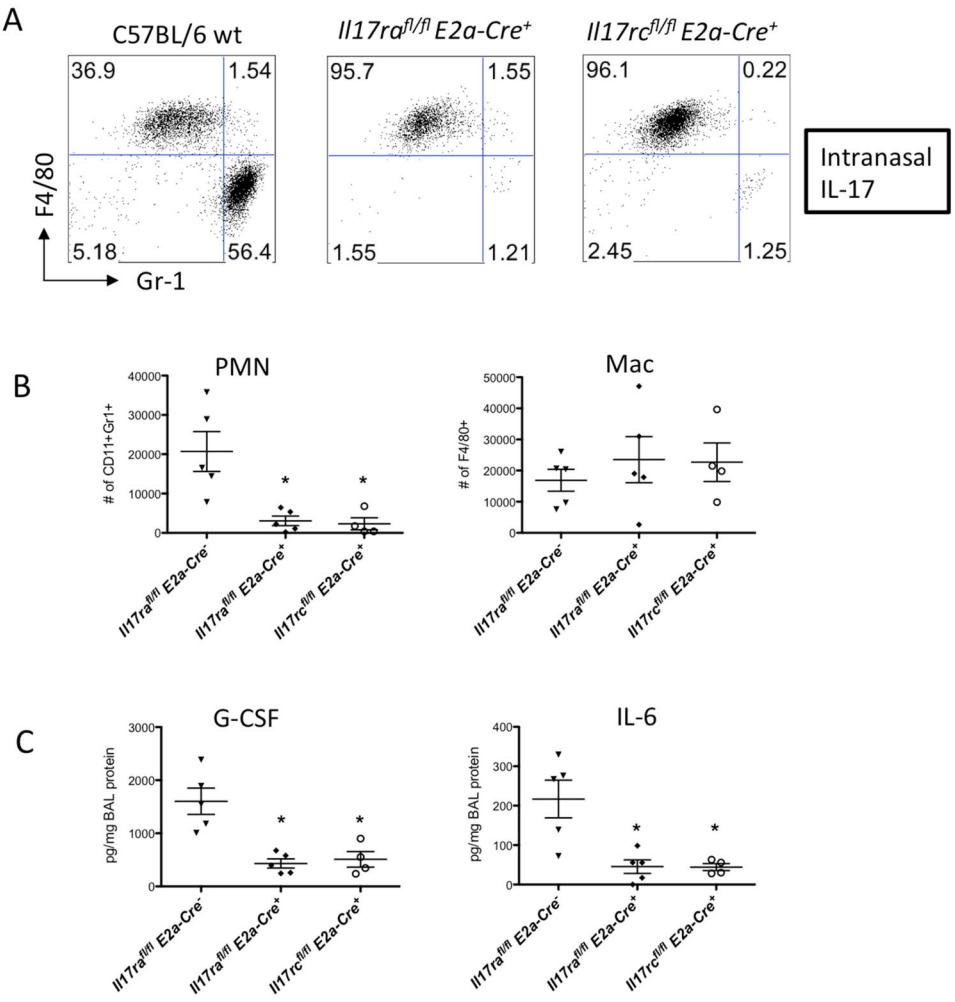

Fig. 1. Characterization of the IL17R$^{fl/fl}$ mice following IL-17 intranasal administration

(A) C57BL/6 mice with indicated genotypes were challenged with recombinant IL-17 intranasally (300ng/mouse) for 24h and BAL cells were harvested. Representative FACS plots of neutrophil (PMN) and Macrophage (Mac) determined by Gr-1 and F4/80 staining were shown. (B) *Il17ra*$^{fl/fl}$ and *Il17rc*$^{fl/fl}$ mice with indicated genotypes were challenged with IL-17 and total numbers of BAL neutrophils and macrophages were enumerated. G-CSF and IL-6 in the BAL fluid were measured by Luminex (C). Data are represented as mean +/− SEM. See also supplementary Fig. 1–2 for further characterization of these mice.

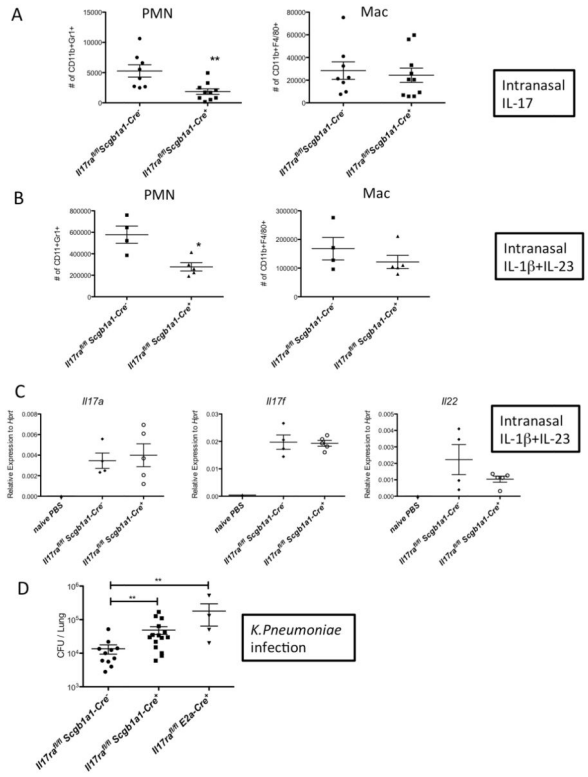

Fig. 2. Defective PMN responses and bacterial clearance in the IL-17RA epithelial conditional KO mice

Il17ra$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$ and *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ mice were challenged with recombinant IL-17 intranasally (300ng/mouse) for 24h and BAL neutrophils and macrophage numbers were determined by FACS (A). *Scgb1a1-Cre* mediated recombination was shown in supplementary Fig. 3. *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$ and *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ mice were challenged with recombinant IL-1β (10ng/mouse) plus IL-23 (500ng/mouse) intranasally for 24h and BAL neutrophils and macrophage numbers were determined by FACS (B). Gene expression of Th17 canonical cytokines from the lungs of IL-1β+IL-23 treated mice were determined by real-time RT-PCR (C). *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$, *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$, and *Il17ra*$^{fl/fl}$ *E2a-Cre*$^{+}$ mice were infected with 10^4 KP-43816 intranasally and sacrificed 24h post infection. Bacterial burden in the lungs were determined by standard CFU assay (D). Data are represented as mean +/− SEM.

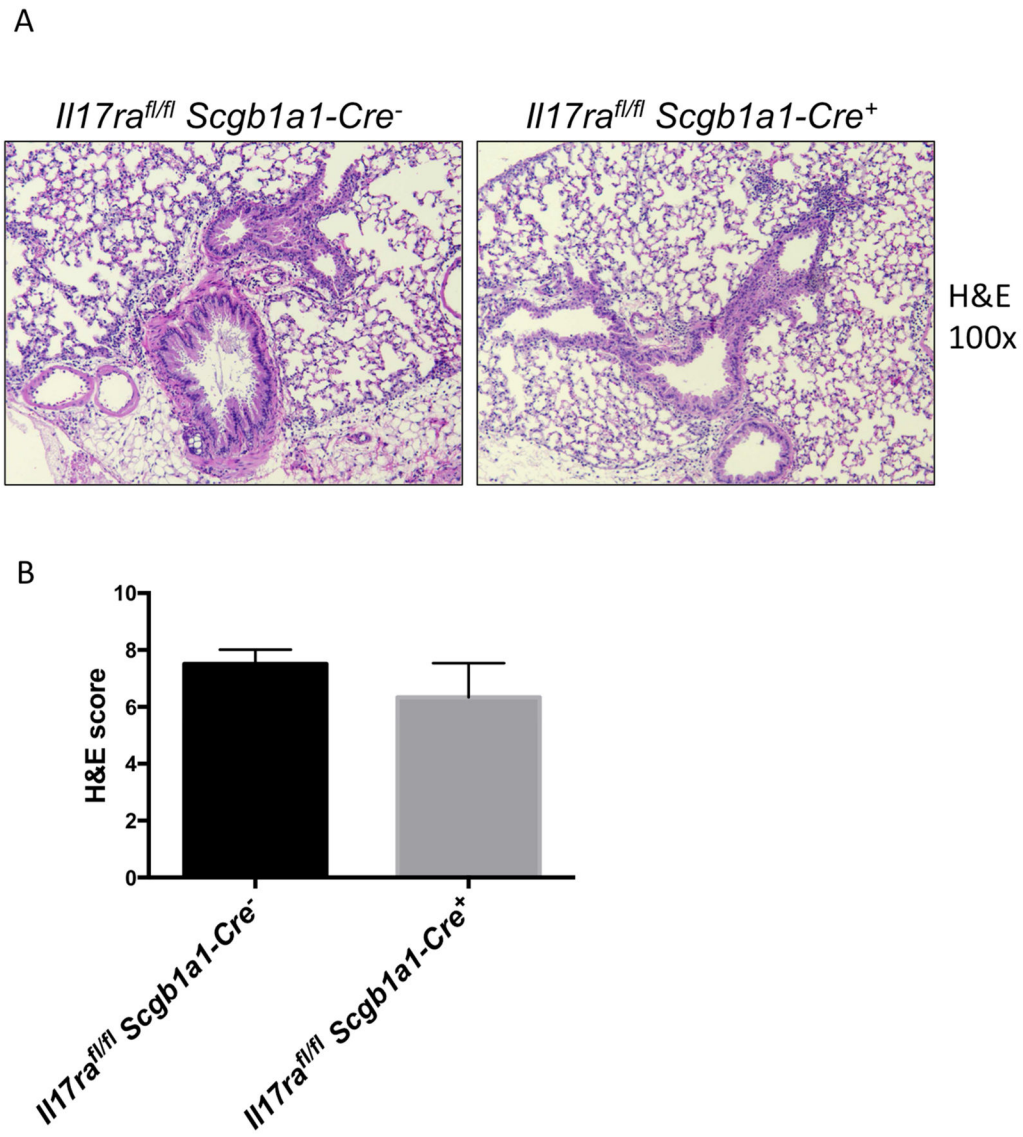

Fig. 3. Histopathology of the IL-17RA epithelial conditional KO mice after *K. pneumoniae* infection

Il17ra$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$ and *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre* mice were infected with 10^4 KP-43816 intranasally and sacrificed 24h post infection. Representative H&E stainings were shown (A) and pathology scores were graphed (B). Data are represented as mean +/− SEM.

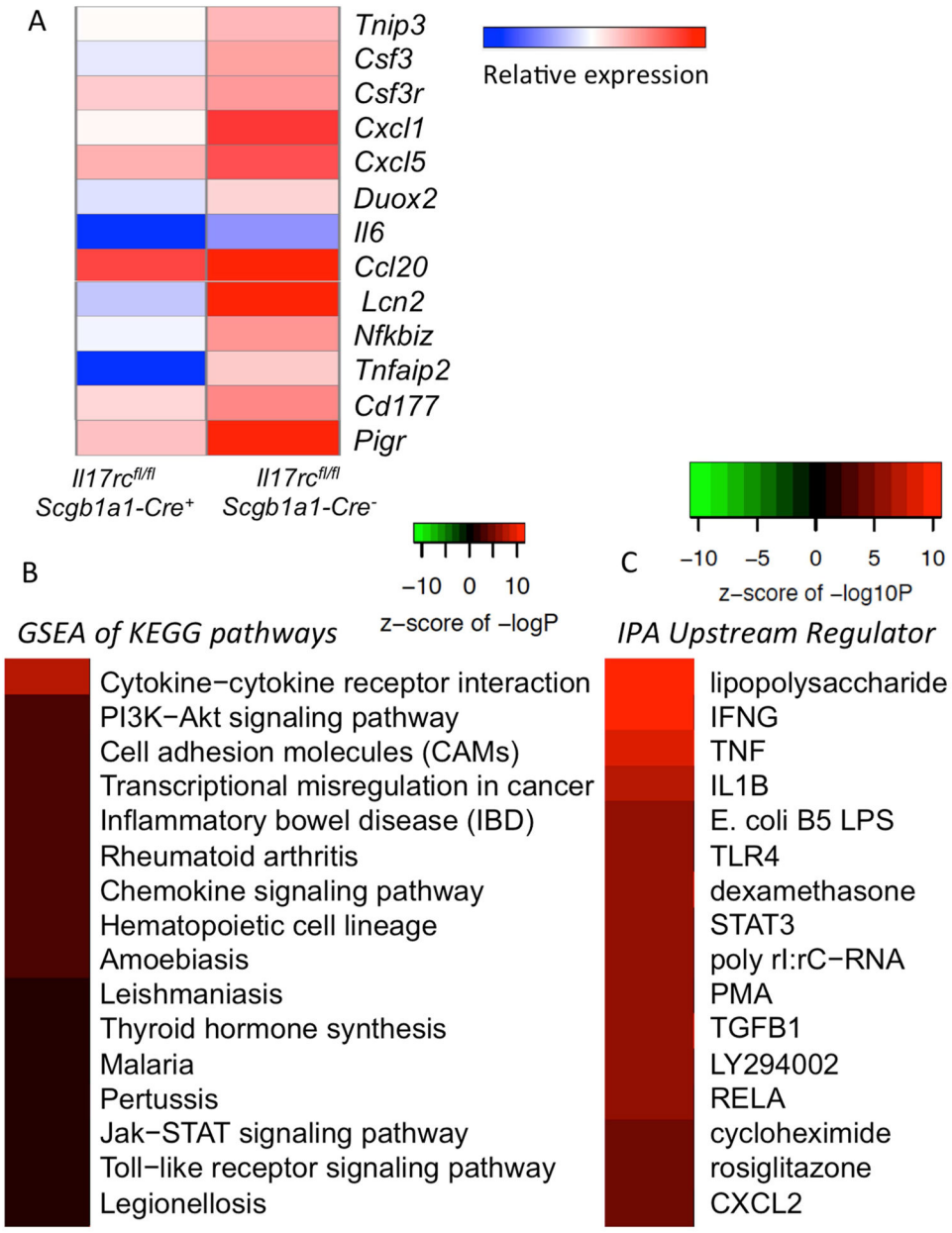

Fig. 4. RNA-seq analysis on mouse bronchial brushes from the IL-17RC epithelial conditional KO mice

Il17rc$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$ and *Il17rc*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ mice were challenged with recombinant IL-17 intranasally (300ng/mouse) for 6h and bronchial brushings were harvested for RNA extraction and RNA-seq analysis. (A). Heat map of selected known IL-17R-dependent genes. (B). Enriched KEGG pathways from the gene set enrichment analysis (GSEA). (C). Enriched pathways using the Upstream Regulator Analytic from the Ingenuity Pathway Analysis software. Real-time RT-PCR validation of these RNA-seq findings was shown in supplementary Fig. 4.

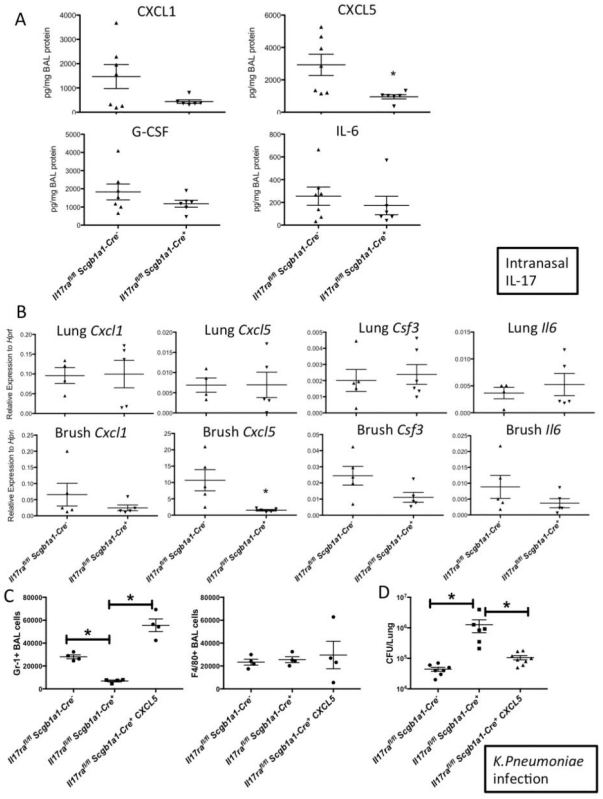

Fig. 5. Loss of *Cxcl5* expression in the IL-17RA epithelial conditional KO mice

Il17ra$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$ and *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ mice were challenged with recombinant IL-17 intranasally (300ng/mouse) for 24h and IL-17 downstream chemokines and cytokines in the BAL fluids were measured by Luminex (A). Gene expression in whole lung homogenates and bronchial brushings were determined by real-time RT-PCR (B). *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{-}$ and *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ mice were infected with 10^4 KP43816 intranasally. 2h post infection, a subgroup of *Il17ra*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ the mice received 1ug recombinant CXCL5 (LIX). Mice were sacrificed at 4h after infection for BAL cell enumeration by FACS (C). A separate cohort of mice were sacrificed at 24h after infection and bacterial burden in the lungs were determined by CFU (D). See also the pathology of these mice in supplementary Fig. 5. Data are represented as mean +/− SEM.

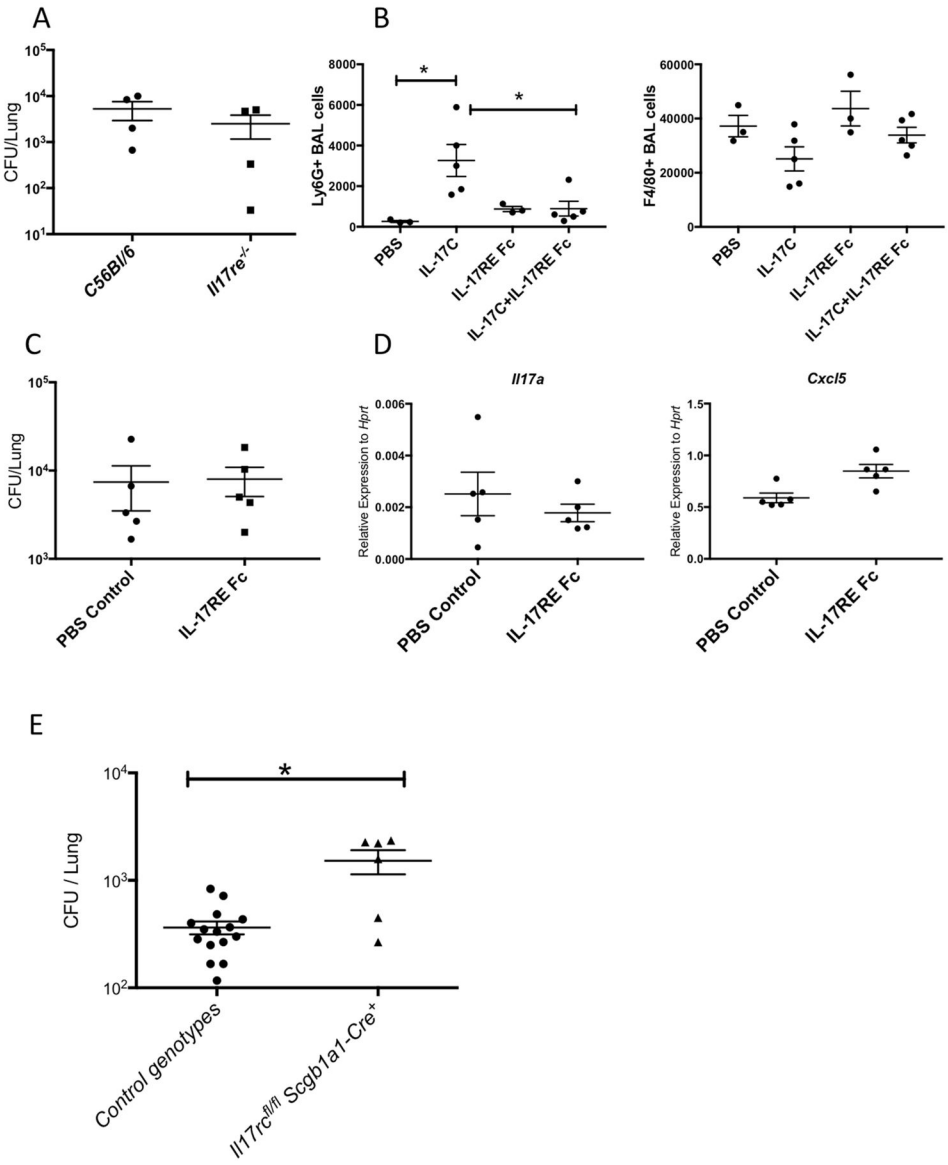

Fig. 6. Defective PMN responses and bacterial clearance in the IL-17RC epithelial conditional KO mice

WT or *Il17re+/−* as well as littermates *Il17re−/−* mice were infected with 10^4 KP-43816 intranasally and sacrificed 24h post infection. Bacterial burden in the lungs were determined by standard CFU assay (A). See also supplementary Fig. 6. C57BL/6 mice were challenged with recombinant IL-17C (500ng/mouse) with or without IL-17RE Fc (500ng/mouse) intranasally and sacrificed at 24h. BAL neutrophils and macrophage numbers were determined by FACS (B). C57BL/6 mice were infected with 10^4 KP43816 intranasally with or without IL-17RE Fc (500ng/mouse) intranasally and sacrificed at 24h. Bacterial burden in the lungs were determined by CFU (C) and gene expression in the lungs were analyzed by real-time RT-PCR (D). Littermate controls as well as *Il17rc*$^{fl/fl}$ and *Il17rc*$^{fl/fl}$ *Scgb1a1-Cre*$^{+}$ mice were infected with 10^5 KP-2146 intranasally and sacrificed 24h post infection.

Bacterial burden in the lungs were determined by CFU (E). Data are represented as mean +/− SEM.