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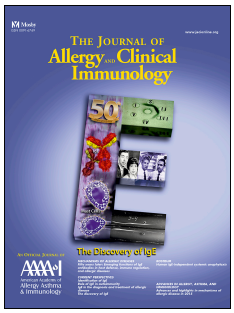


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# Chronic IL-33 expression predisposes to viral-induced exacerbations of asthma by increasing type-2 inflammation and dampening antiviral immunity

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**Abstract**

Background: Rhinovirus infection triggers acute exacerbations of asthma. IL-33 is an instructive cytokine of type-2 inflammation whose expression is associated with viral load during experimental rhinovirus infection of asthmatic subjects.

Objective: To determine whether anti-IL-33 therapy is effective during disease progression, established disease, or viral exacerbation using a preclinical model of chronic asthma and in vivo human primary airway epithelial cells (AECs).

Methods: To model disease onset, progression, and chronicity, mice were exposed to pneumonia virus of mouse and cockroach extract in early-life and later-life, then challenged with rhinovirus. Interventions included anti-IL-33 or dexamethasone at various stages of disease. AECs were obtained from asthmatic and healthy patients, and treated with anti-IL-33 following RV infection.

Results: Anti-IL-33 decreased type-2 inflammation in all phases of disease; however, the ability to prevent airway smooth muscle growth was lost after the progression phase. After the chronic phase, IL-33 levels were persistently high and rhinovirus challenge exacerbated the type-2 inflammatory response. Treatment with anti-IL-33 or dexamethasone diminished exacerbation severity and anti-IL-33, but not dexamethasone, promoted antiviral IFN expression and decreased viral load. RV replication was higher and IFN-lambda lower in asthmatic compared to healthy AECs. Anti-IL-33 lowered RV replication and increased IFN-λ at the gene and protein level.

Conclusion: Anti-IL-33 or dexamethasone suppressed the magnitude of type-2 inflammation during a rhinovirus-induced acute exacerbation, however only anti-IL-33 boosted antiviral immunity and lowered viral replication. The latter phenotype was replicated in RV infected

human AECs, suggesting that anti-IL-33 therapy has the additional benefit of enhancing host defence.

**Key messages:**

- IL-33 blockade abrogates type-2 inflammation during all phases of disease but the ability to modulate airway remodelling is lost in established disease
- Chronic IL-33 expression predisposes to rhinovirus-induced exacerbation of disease in vivo, and suppresses antiviral immunity in ex vivo human AEC cultures
- Anti-IL-33 decreases type-2 inflammation, lowers viral burden, and promotes antiviral immunity during a RV-induced exacerbation

**Capsule summary:**

Using a preclinical model of chronic asthma and primary human AECs we show that anti-IL-33 boosted IFNs and lowered RV replication, suggesting that in addition to attenuating type-2 inflammation, anti-IL-33 therapy may enhance host defence.

**Key words:**

IL-33; rhinovirus; asthma; exacerbation; antiviral

**Abbreviations:**

AEC: Airway epithelial cell

ASM: Airway smooth muscle

BALF: Bronchoalveolar lavage fluid

CRE: Cockroach extract

IFN: Interferon

IL: Interleukin

ILC2: Type-2 innate lymphoid cell

PVM: Pneumonia virus of mice

RSV: Respiratory syncytial virus

## Introduction

Genome-wide association studies have associated variants in or near the *interleukin (IL)-33* and the *IL-33 receptor* (also known as ST2 and IL-1RL1) loci with asthma risk (1-3). IL-33 is constitutively expressed by epithelial and mesenchymal cells in the airway wall, and is primarily located in the nucleus in the steady-state (4, 5). However, in response to cellular stress, injury, or death, it is released into the extracellular space where it acts as a cytokine (6). The expression of IL-33 is elevated in the lungs and airways of patients with asthma, and correlates with disease severity (7-9). IL-33 levels are also elevated in mouse models of asthma, where its blockade is protective (10-12). In the context of eosinophilic asthma, IL-33 is pathogenic because it induces type-2 inflammation through the expansion and activation of type-2 innate lymphoid cells (ILC2s) and CD4+ T helper 2 (Th2) cells. These cells produce type-2 cytokines, namely IL-4, IL-5 and IL-13, which act on structural cells and type-2 effector cells, such as mast cells and eosinophils, to promote the cardinal features of asthma. IL-33 can also directly activate type-2 effector cells, and is therefore a central orchestrator of type-2 immunity. Accordingly, several monoclonal antibodies against IL-33 and its receptor have been developed, and have now entered phase I clinical trials (13).

Consistent with an important role in asthma pathogenesis, known asthma triggers, such as allergens and respiratory viral infections (most notably respiratory syncytial virus [RSV] and rhinovirus [RV]) induce IL-33 release (14). Severe lower respiratory tract viral infections in early life, especially in children who later become sensitised to aeroallergens, are a major risk factor for asthma inception (15-19), and it is noteworthy that the impaired lung function observed in early-life often persists through childhood and into later-life, when disease becomes increasingly chronic (20, 21). To simulate the human epidemiology, we recently developed a preclinical model of asthma by co-exposing mice to cockroach allergen and pneumonia virus of mice (PVM), the mouse homologue of RSV, both in early-life, to model

disease inception, and later-life, to model disease progression (22). In this chronic multi-hit model, antibody-mediated neutralisation of IL-33 in early- and later-life ablated the development of the cardinal features of asthma, including airway hyperreactivity, airway inflammation and airway remodelling. Additionally, we demonstrated that IL-33 acts as a potent suppressor of antiviral immunity, attenuating the induction of type I and III IFNs and increasing viral load, and thereby predisposing to severe viral bronchiolitis in early-life. However, we treated the mice during disease onset in early-life, and hence the effect of IL-33 neutralisation on disease progression or on established disease, as will be the clinical scenario in which anti-IL-33 treatments are to be directed in the near future, remained unknown.

In a separate mouse model of virus-induced asthma, Sendai virus infection was found to induce an early spike in IL-33 expression following by a second, more intense phase that persisted for several weeks after the virus was cleared, implicating a switch from acute to chronic IL-33 release related to long-lived alterations to epithelial progenitor cells (23). In light of our recent findings demonstrating a hitherto unrecognised suppressive effect of IL-33 on antiviral immunity, we sought to identify whether IL-33 expression was long-lived in our virus/allergen model. Although it is recognised that the majority of asthma exacerbations in both children and adults are associated with a respiratory RV infection (24, 25), and that this phenotype is associated with an IL-33$^{high}$IFN$^{low}$ cytokine microenvironment (14, 26), it is not known whether IL-33 predisposes to the impaired antiviral immunity observed during an exacerbation or as shown in virus infected primary AECs from asthmatic patients (14, 27, 28). Hence, we sought to address whether anti-IL-33 therapy would decrease susceptibility to a RV-mediated acute exacerbation of asthma in a mouse model of chronic asthma on two fronts: by decreasing type-2 inflammation and by increasing anti-viral immunity. To translate our findings, we also analysed the effect of anti-IL-33 on the antiviral response of human hAECs from healthy and asthmatic patients infected with RV.

## Experimental Procedures

Detailed methods are available in the online supplement.

*Co-virus and allergen-induced asthma and rhinovirus-induced exacerbation*

Specific pathogen-free BALB/c mice were intranasally inoculated with PVM (1 pfu at 7 days old, 20 pfu at 49 days old; strain J3666) (29) and exposed to 1 μg of cockroach allergen (CRE; Greer Laboratories) as per study design (Fig 1A) (22). Anti-IL-33 or isotype control antibody (200 μg, Pfizer, Inc) or dexamethasone (75 mg, Sigma-Aldrich) were administered as per study designs. For RV-1B exacerbation, mice were intranasally inoculated with RV-1B ($5x10^6$ $TCID_{50}$). All studies were approved by The University of Queensland Animal Ethics Committee.

*Human bronchial epithelial cell culture*

Primary hAECs from healthy and asthmatic patients were obtained from Lonza. Cells were grown in Bronchial Epithelial Growth Medium (BEGM) (Lonza) and expanded to passage 2 prior to experimentation. hAECs were seeded into 24-well plates and grown to 80% confluence prior to infection with RV-16 or RV-1B (MOI 1). RV-16 and RV-1B stocks were generated from infected cultures of HeLa cells as previously described (30). After 2 hours the viral inoculum was removed, replaced with fresh media and treated in the absence or presence of anti-IL-33 (1 μg/mL; cat number RDSAF3625, R&D Systems). At 48 hours post infection, supernatants were removed and snap-frozen and the AECs collected for RNA isolation (detailed below).

*Flow cytometry*

Flow cytometry was performed on bronchoalveolar lavage fluid (BALF) cells or lung tissue digest cells as previously described (31). Single cell suspensions were incubated with

fluorochrome-conjugated antibodies (details in online supplement). Dead cells were excluded when samples were collected with an LSR Fortessa X-20 (BD Biosciences) and the data analysed with FACSDiva v8 (BD Biosciences) and FlowJo v8.8 (Treestar).

*Immunohistochemistry*

Paraffin-embedded lung sections were prepared as previously described (29). Lung sections were stained with anti-IL-33, anti-pro-SPC, anti-HMGB1, anti-α-SM actin and anti-Muc5ac, as detailed in online supplement.

*Measurement of protein expression*

Murine IL-33 (R&D), IL-25 (Biolegend), and IL-5 (BD Biosciences) expression was quantified by ELISA and IL-13 (Enhanced Sensitivity Flex Set, BD Biosciences) by cytokine bead array. Human IFN-λ and IL-33 was quantified by ELISA (R&D).

*Quantitative real time PCR*

Total RNA was isolated with TriReagent solution followed by phenol-chloroform extraction. Reverse transcription was performed using M-MLV reverse transcriptase then qRT-PCR performed using SYBR Green and the primers described in Table S1.

*Statistical analyses*

GraphPad Prism version 7.0 software (La Jolla, California) was used for all statistical analyses. A Student's t-test, one-way ANOVA with a Tukey post-hoc test or two-way ANOVA with a Sidak post-hoc test were applied as appropriate. A $P$ value $<0.05$ was considered statistically significant.

## Results

### *Treatment with anti-IL-33 during the progression and chronic phases reduces many key features of the asthmatic phenotype*

We have previously developed a preclinical model of asthma that simulates the synergistic interactions between lower respiratory tract infections and low-dose allergen exposure (22). Critically, this model (see Fig. 1A for study design) deliberately uses a low dose of virus and low dose of cockroach allergen that when administered alone is not sufficient to induce the hallmark features of asthma. Moreover, we identified that the onset of disease is critically dependent on the timing of virus and allergen co-exposure in early life. Before assessing the effectiveness of anti-IL-33 as a therapeutic, we first wanted to assess whether the timing of the second virus and allergen exposure was critical for disease progression. When the CRE exposure ('C1') was delayed until 7 days (day 49; Supp Fig 1A) after the secondary viral infection, the mice no longer developed the cardinal features of asthma (eosinophilia, neutrophilia, mucous hypersecretion and airway smooth muscle [ASM] growth), as occurs when the CRE was administered 3 days after the virus challenge (day 45; Supp Fig 1B-E). Of note, when the mice were treated with CRE at day 45 and killed 4 days later (i.e. in the absence of the second-fourth CRE challenge; 'C2-C4'), the mice did present with asthma-like pathologies (Supp Fig 1F-J). Collectively, these findings justify the separation of the model into three distinct phases: onset, progression, and chronicity (Fig. 1A), and allowed us to test the effectiveness of anti-IL-33 when administered during disease progression or as a therapeutic agent in established disease.

To assess the effectiveness of IL-33 neutralisation on disease progression, mice were inoculated with PVM and CRE, and then treated intraperitoneally with anti-IL-33 or control isotype-matched antibody, starting in the progression phase and continuing through the

chronic phase (Fig. 1A). To compare with a conventional therapy, an additional group of mice was treated with the corticosteroid dexamethasone (75 µg). Mice treated with the isotype control had elevated numbers of airway eosinophils and neutrophils, coupled with elevated levels of ILC2s, total CD4 T cells, and CD4 Th2 cells (identified by ST-2 expression) (32) as compared to mice exposed to vehicle (i.e. non-PVM, non-CRE) (Fig 1B-F). Consistent with our previous findings (22), IL-5 and IL-13 production was not detectable 72 hr post challenge (data not shown). However, the cellular inflammation was associated with features of airway remodelling including mucus hypersecretion and increased ASM mass (Fig 1G-H). In response to treatment with either anti-IL-33 or dexamethasone, the number of airway eosinophils was significantly decreased compared to mice treated with the isotype control. In contrast, neither treatment affected neutrophils, lymphocytes, regulatory T cells, mononuclear cells or the total cell count in the BAL (Fig 1B-C, Supp Fig 2 and data not shown). Both treatments also significantly reduced lung ILC2s and CD4+ Th2 cells and mucus hypersecretion (Fig 1D-G); however, only anti-IL-33 prevented the increase in ASM mass (Fig 1H).

*IL-33 blockade during the chronic phase affects type-2 immunity but not airway remodelling*

To address the effectiveness of anti-IL-33 or dexamethasone in established disease, mice were treated 24 hr prior to each of the CRE exposures in the chronic phase ('C2-C4'; Fig 2A). Using this regimen, neither treatments had any substantive effect; anti-IL-33 only reduced airway eosinophils and neutrophils (Fig 2B-C and Supp Fig 3), while dexamethasone decreased CD4+ Th2 cells (although this was not significant) (Fig 2D-F). We reasoned that the lack of efficacy may relate to a lack of 'coverage' of IL-33, and assessed the effect of a twice/weekly dosing regimen. In addition to the parameters affected by the once/weekly dosing, ILC2s, CD4+ Th2 cells, and mucus hypersecretion were then also significantly

decreased by anti-IL-33, while twice/weekly dexamethasone only affected ILC2s in the lung (Fig 2B-G). Notably, neither treatment affected ASM growth (Fig 2H).

*Established respiratory disease induces long-term changes to structural cells of the lung*

We next investigated whether the structural alterations would persist in the absence of virus or allergen challenge (Fig 3A). As expected, ASM mass remained significantly elevated when assessed 4 weeks (day 94) after the final CRE exposure (Fig 3B). Although the extent of the mucus hypersecretion and other measures of type-2 inflammation had resolved (data not shown), the concentration of IL-33 in the BALF was significantly greater at 94 dpi than at 69 dpi. IL-33 (red) was expressed primarily in pro-surfactant protein C (SPC)-positive (green) alveolar epithelial cells in both vehicle and PVM/CRE exposed mice; however, the intensity of the IL-33 immunoreactivity was far greater in the mice exposed to PVM/CRE (Fig 3D). We and others have recently identified that high mobility group box 1 (HMGB1), another nuclear alarmin, contributes to the cytokine cascade that amplifies type-2 inflammation (33, 34). The levels of cytoplasmic HMGB1 in the bronchial epithelium were significantly greater in the mice co-exposed to PVM/CRE, and remained elevated at day 94 (Fig. 3E), suggesting that the effects of virus/allergen co-exposure on airway structural cells are long-lived and persist in the absence of overt cellular inflammation.

*Exacerbation with rhinovirus induces type-2 inflammation*

In light of the persistent airway remodelling and IL-33 production, and also our recent findings that IL-33 suppresses antiviral immunity during disease onset (22), we postulated that even without a recent allergen exposure, mice with features of chronic asthma would be more susceptible to an acute exacerbation triggered by RV exposure. To test our hypothesis, healthy (vehicle-exposed) and diseased (PVM/CRE co-exposed) mice were inoculated four weeks after the final CRE challenge with RV-1B ($5x10^6$ $TCID_{50}$) which unlike most RV

strains (e.g. RV-16) can infect and replicate in mice (35) (Fig 4A). Three days after RV inoculation, both airway eosinophils and neutrophils were significantly elevated in the diseased mice as compared to the healthy controls. Similarly ILC2s, CD4+ Th2 cells and mucus hypersecretion were significantly greater in the diseased mice (Fig. 4B-G and Supp Fig 4). This phenotype was associated with a significant increase in IL-13 and IL-5 levels in the BALF (Fig. 4H-I). Importantly, the hyper-inflammatory response to RV inoculation was not evident in mice that had been exposed to CRE alone, PVM alone, or PVM alone in early-life with PVM/CRE co-exposure in later-life (Fig 4B-I). Moreover, RV inoculation of healthy mice did not induce significant IL-33 or IL-25 release (Supp Fig 4A-B). As expected in the short 3 day timeframe, RV-1B inoculation did not increase ASM mass in any of the treatment groups (data not shown). IL-33 was not detectable in the BALF at 1 or 3 days post RV challenge (data not shown).

*Blocking IL-33 prior to RV-exacerbation decreases type-2 inflammation*

This unique preclinical model allowed us to test whether immuno-neutralisation of IL-33 would decrease the magnitude of type-2 inflammation in response to an acute RV-induced exacerbation in the setting of chronic asthma. PVM/CRE co-exposed mice were treated twice/week with dexamethasone, anti-IL-33 or isotype-matched control, in the four weeks between the final CRE challenge and the RV inoculation (Fig 5A). Anti-IL-33 significantly attenuated the number of eosinophils and neutrophils in the airways, and ILC2s and CD4+ Th2 cells in the lungs as compared to mice treated with the isotype control (Fig 5B-F and Supp Fig 5). Treatment with dexamethasone was similarly or more effective at dampening the inflammatory cell response (Fig 5B-F), and of note, both treatments decreased the number of CD4+ T cells in the lung prior to the RV exacerbation (Fig 5E). Furthermore, both treatments significantly decreased the expression of IL-13 and IL-5 in the airway compared with isotype-matched treatment group (Fig 5G-H). Despite the pronounced suppression of

type-2 inflammation, only dexamethasone treatment significantly reduced epithelial Muc5ac expression (Fig 5I). Interestingly, however, treatment with anti-IL-33, but not dexamethasone, significantly decreased the fraction of bronchial epithelial cells with cytoplasmic HMGB1 prior to the RV-induced exacerbation (Fig. 5J). Following RV infection, expression of cytoplasmic HMGB1 was dramatically diminished, regardless of treatment group, suggestive of its extracellular release. When measured in the BALF at 1 dpi, HMGB1 levels were significantly elevated in the isotype- or dexamethasone-treated mice, but not in the anti-IL-33 treated mice (Fig 5K).

*IL-33 blockade prior to exacerbation boosts antiviral immunity*

In light of our previous finding that IL-33 suppresses antiviral immunity during PVM infection in early-life (22), we questioned whether IL-33 affects the host response to RV infection. It was noteworthy that the viral load in the diseased (isotype-treated) mice was significantly greater than that of the healthy (naïve) mice, similar to the delayed clearance observed in RV-inoculated asthmatics (14, 26) (Fig. 6A). Moreover, this phenotype aligned with an attenuated IFN-α response in the lungs (Fig. 6B). Strikingly, when the diseased mice were pre-treated with anti-IL-33, the viral load and expression of IFN-α, IFN-λ and IFN-γ in the lungs was equivalent to that of the healthy mice (Fig. 6B). In stark contrast, dexamethasone treatment did not lower viral load and did not modulate IFN gene expression.

*Anti-IL-33 increases antiviral immunity in healthy and asthmatic AECs*

In light of our findings in mice, we postulated that IL-33 may contribute to the loss of viral control and impaired IFN production observed previously in *in vitro* cultured AECs from asthmatic patients (27, 28, 36-38). Using hAECs obtained from healthy and asthmatic patients, we confirmed that viral replication (both RV-16 or RV-1B; MOI 1) was significantly greater in hAECs from subjects with asthma (Fig. 7A-B). Strikingly, pre-

290 treatment with anti-IL-33 significantly decreased RV-16 replication in both healthy and
291 asthmatic cells (Fig. 7A). A similar trend was observed with RV-1B infected hAECs (Fig.
292 7B). RV-16 or RV-1B induced IFN-λ gene expression was significantly lower in hAECs
293 from asthmatic patients and was significantly up-regulated upon pre-treatment with anti-IL-
294 33 regardless of disease status (Fig. 7C-D). At the protein level, RV-16 stimulated IFN-λ
295 production was significantly increased from the hAECs of asthmatic patients when IL-33 was
296 neutralised (Fig. 7E-F). IFN-β gene expression was not significantly different between
297 healthy and asthmatic patients, however IFN-β transcripts were significantly increased
298 following treatment with anti-IL-33 in the asthmatic patients in response to either virus strain
299 (Fig. 7G-H). Although IL-33 protein was not detectable in the supernatant at 48 hr post
300 infection, (Supp Fig 6A-B); IL-33 mRNA levels were significantly elevated following RV
301 infection (Fig. 7I-J).

## Discussion

In this study, we used a preclinical mouse model of chronic asthma that simulates the synergistic interplay between viral and allergen exposure, and which has four distinct phases: onset, progression, chronic, and exacerbation. We found that anti-IL-33 treatment during the progression and chronic phase, or during the chronic phase alone, or prior to the exacerbation phase alone, attenuated the magnitude of type-2 inflammation and asthma-like pathologies in the airway wall. In general, anti-IL-33 was as effective at ameliorating disease as treatment with the corticosteroid dexamethasone, although there were subtle differences depending on the timing of treatment. However, the most stark difference was observed in the exacerbation phase: whereas dexamethasone had no effect on antiviral host defence, anti-IL-33 promoted antiviral immunity and lowered viral burden. Importantly, the suppressive effect of IL-33 on antiviral immunity was confirmed in RV-16 or RV-1B infected hAECs from healthy and asthmatic patients.

Consistent with the findings of others, we observed that IL-33 blockade decreased the cellular inflammation associated with Th2 immunity, including CD4+ Th2 cells, ILC2s, and eosinophils (10-12) (Fig. 1 & 2). Unfortunately, due to the lungs collapsing during forced oscillation technique we could not assess the effect of IL-33 blockade on airway hyperreactivity. However, we recently reported that anti-IL-33 also prevents ASM hyperplasia when administered during onset and progression of asthma (22), and we confirm these findings here by showing that treatment during both the progression and chronic phases is similarly effective. However, we also revealed that commencing treatment after the progression phase has no effect on ASM remodelling (Fig. 2). These data are concordant with clinical reports indicating the ASM becomes more fixed and difficult to reverse in adults with asthma (39, 40), and highlight the need for asthma interventions in early-life when the ASM is more amenable to treatment, perhaps as consequence of greater plasticity.

In the steady-state, IL-33 is primarily expressed by non-haematopoietic cells, including epithelial cells (alveolar and bronchiolar) and endothelial cells (4, 5, 8, 9). We previously reported that PVM/CRE co-exposure increases IL-33 expression in bronchial epithelial cells during disease onset (22), however when we assessed the levels of IL-33 following the progression and chronic phase, we found that IL-33 was primarily expressed by alveolar epithelial cells, even after four weeks without an environmental stimulus (Fig. 3). This phenotype was associated with high levels of IL-33 in the airway lumen, and reminiscent of the findings observed in a mouse model of Sendai virus infection (23), although our model incorporated multiple exposures, more typical of disease aetiology. In contrast to the Sendai virus model, the expression of IL-13, which can feed-forward to amplify IL-33 levels (41), was not elevated. Rather the phenotype in our model was similar to that observed in children with severe asthma, who express greater levels of IL-33 in structural cells in the absence of an elevated IL-13 signal (12). Additionally, we identified that the cytoplasmic expression of HMGB1, another alarmin that is constitutively expressed in the nucleus of barrier cells and contributes to type-2 inflammation (33, 34), remained significantly elevated in bronchial epithelial cells long after the final CRE exposure. Further studies are needed to identify the molecular mechanism that leads to the elevated and persistent expression of IL-33 and HMGB1 as this may identify a novel target(s) for therapeutic intervention.

Acute exacerbations of asthma are a major cause of morbidity and mortality, and present a considerable health care burden. Current therapeutic options to manage exacerbations are suboptimal: corticosteroids reduce exacerbations by 50% (42), anti-IL-5 therapy allows for steroid sparing but again only reduces exacerbations by 50% (43, 44), while type-I IFN therapy fails to improve symptoms during a viral exacerbation (45). To address this unmet need, more representative and predictive preclinical models of viral exacerbation are required (46). Disappointingly, inoculation with RV shortly after the final allergen challenge in acute

models of allergic asthma (using ovalbumin or house dust mite) induces an inflammatory response that is short-lived (lasting <24 hr) and predominantly neutrophilic (47-51), contrary to the clinical phenotype (14, 26). By contrast, RV challenge after the chronic phase in our model induced a mixed granulocytic response that was associated with increased numbers of ILC2 and CD4+ Th2 cells and a marked increase in the production type-2 cytokines (Fig. 4). Importantly, this phenotype was not present in mice that had been exposed to cockroach alone or even the mice that had been subjected to all exposures bar the CRE administration in early-life. Thus, viral bronchiolitis in early-life increased the susceptibility to an RV-triggered exacerbation of asthma in later-life. The molecular processes that underpin this 'switch' in early-life remain elusive; however the long-lived effects may reflect epigenetic alterations in progenitor epithelial cells and/or immune cell populations, and are evidently activated upon RV infection.

In light of the ability of RV to induce IL-33 release from human bronchial epithelial cells (14) and the elevated IL-33 levels in the chronic phase of our model, we hypothesised that the neutralisation of IL-33 prior to the inoculation with RV would diminish the severity of the exacerbation. Similar to corticosteroid treatment, anti-IL-33 significantly decreased the magnitude of the type-2 response (e.g. eosinophils, ILC2s, type-2 cytokines) induced in response to RV challenge (Fig. 5). However, in contrast to the progression and chronic phase, anti-IL-33 did not reduce epithelial Muc5ac expression induced by the RV infection, perhaps due to cytokine redundancy (52, 53). Both treatments also decreased the number of CD4+ T cells in the lungs prior to the exacerbation. Interestingly, pre-treatment with anti-IL-33, but not dexamethasone, decreased the number of bronchial epithelial cells expressing cytoplasmic HMGB1 prior to RV challenge and as a consequence, the concentration of HMGB1 released into the BALF was significantly decreased following the RV-induced exacerbation. This is important as we and others have shown that HMGB1 is a type-2

instructive cytokine (33, 34), like IL-33. Alarmins commonly initiate feed-forward mechanisms to amplify innate immunity (33). Our findings implicate a role for IL-33 in maintaining the dysregulated and proinflammatory epithelium that is now recognised to be a hallmark feature of asthma.

RV-stimulated IFN-λ and IFN-β production has been shown to be impaired in bronchial epithelial cells from asthmatic or allergic subjects (27, 28, 54), although other studies did not replicate this phenotype (55, 56). Intriguingly, in response to experimental RV infection, viral load was significantly higher and correlated with nasal IL-33 expression, in asthmatics compared with healthy subjects (14); however, whether IL-33 directly caused the loss of viral control was not addressed. In our preclinical model, antiviral cytokine expression was markedly impaired in diseased compared to healthy mice following RV inoculation, implicating the existence of a negative-regulatory process in the diseased mice. In light of our recent findings demonstrating that IL-33 suppresses antiviral immunity (22), we postulated that following the chronic phase, persistently elevated IL-33 levels would affect host immunity. Consistent with this theory, anti-IL-33 treatment prior to the RV exacerbation restored the production of antiviral cytokines and diminished the level of RV viral copies to that observed in RV inoculated healthy mice (Fig. 6). In contrast, dexamethasone treatment had no effect on viral load or the dampened IFN production; thus the beneficial effects of dexamethasone on the pro-inflammatory response appear to be outweighed by their detrimental impact on local antiviral immunity, at least in the short-term. Importantly, through immune-neutralisation of human IL-33, we confirmed that RV-induced IL-33 dampens antiviral immunity and increases viral replication in human AECs obtained from both healthy and asthmatic patients (Fig. 7). Although IL-33 protein expression was not detectable in the supernatant, possibly due to its degradation, we observed a significant up-regulation in IL-33 mRNA levels in RV-1B stimulated hAECs from asthmatic patients.

Taken together, our findings suggest that anti-IL-33 protects against virus-induced exacerbation via two mechanisms: decreasing type-2 inflammation and restoring antiviral immunity. Studies are now urgently required in man to confirm the anti-type-2 effects of neutralising IL-33, but perhaps more importantly, it will be critical to assess whether IL-33 blockade restores antiviral immunity and epithelial homeostasis *in vivo*, as there may be the potential to return the airway epithelium to the healthy steady-state.

In conclusion, we present a novel preclinical model of chronic asthma that recapitulates much of the human epidemiology and highlights a critical role for IL-33 in the disease progression, chronicity, and exacerbations. IL-33 blockade attenuates type-2 inflammation in all phases of disease, although its beneficial effects against ASM remodelling are lost once disease is established. Uniquely, we show that anti-IL-33 ameliorates RV-induced exacerbation by suppressing type-2 inflammation, restoring antiviral immunity, increasing viral clearance, and decreasing alarmin expression in the bronchial epithelium. Our findings *in vivo* were replicated *in vitro* using healthy and asthmatic hAECs infected with RV. Anti-IL-33 boosted antiviral immunity in both healthy and asthmatic cells and controlled viral burden. Monoclonal antibodies targeting IL-33 or its receptor have now entered Phase I trials. With phase II trials on the horizon, it will be important to take the opportunity to assess whether IL-33 blockade restores host defence as well as suppressing type-2 inflammation.

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Figure legends

**Figure 1. IL-33 blockade during the progression and chronic phase prevents type-2 inflammation and airway remodelling.** (A) Study design. 7 day old (0 days post infection [dpi]) neonatal mice were infected with pneumonia virus of mice (PVM) and exposed to cockroach allergen (CRE) 3 days later. Mice were then reinfected 6 weeks later and exposed to CRE weekly. Mice were treated with anti-IL-33, relevant isotype control or dexamethasone as indicated. (B) Eosinophils and (C) Neutrophils in the BALF. (D) Type 2 innate lymphoid cells (ILC2), (E) CD4 T cells and (F) Th2 cells in the lung. (G) Mucous production in the lung, quantified as a score of Muc5ac expression. (H) Airway smooth muscle area. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice. Box and whisker plots show quartiles (boxes) and range (whiskers), n=5-8 mice/group, representative of 2 experiments.

**Figure 2. IL-33 blockade during the chronic phase prevents type-2 inflammation but does not alter ASM remodeling.** (A) Study design. Mice were treated once or twice weekly with anti-IL-33, isotype control or dexamethasone prior to second cockroach allergen (CRE) in reinfection. (B) Eosinophils and (C) Neutrophils in the BALF. (D) Type 2 innate lymphoid cells (ILC2), (E) CD4 T cells and (F) Th2 cells in the lung. (G) Muc5ac score. (H) ASM. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice, at that dosing regimen. Box and whisker plots show quartiles (boxes) and range (whiskers) n=6-8 mice/group, representative of 2 experiments.

**Figure 3. IL-33 and HMGB1 are persistently elevated in asthmatic mice.** (A) Study design. Mice were euthanized at 69 or 94 days post primary infection. (B) Airway smooth muscle (ASM) area. (C) IL-33 in the BALF. (D) IL-33 immunofluorescence. Red = IL-33, green = pro-surfactant protein C (pro-SPC), blue = DAPI. Scale bar = 50 µm. (E) Cytoplasmic high mobility group box 1 (HMGB1) expression in airway epithelial cells. * denotes significance compared with vehicle treated mice. # denotes significance between time points. Box and whisker plots show quartiles (boxes) and range (whiskers), n=5-7, representative of 2 experiments.

**Figure 4. Rhinovirus infection induces an exacerbation only in mice with established disease**. (A) Study design. Asthma was established as before then mice were rested for four weeks before inoculation with rhinovirus (RV)-1B ($5x10^6$ TCID50). (B) Eosinophils and (C) Neutrophils in the BALF. (D) Type 2 innate lymphoid cells (ILC2), (E) CD4 T cells and (F) Th2 cells in the lung. (G) Muc5ac score. (H) IL-13 and (I) IL-5 expression in the BALF. * denotes significance compared with vehicle treated mice. # denotes significance compared with PVM/CRE/PVM/CRE/RV mice. Box and whisker plots show quartiles (boxes) and range (whiskers), n=6-9, representative of 2 experiments.

**Figure 5. IL-33 blockade prevents type-2 inflammation during an RV-exacerbation**. (A) Study design. Mice were treated twice weekly with anti-IL-33, isotype or dexamethasone in the four weeks prior to rhinovirus (RV)-1B infection. (B) Eosinophils and (C) Neutrophils in the BALF. (D) Type 2 innate lymphoid cells (ILC2), (E) CD4 T cells and (F) Th2 cells in the lung. (G) IL-13 and (H) IL-5 expression in the BALF. (I) Muc5ac score. (J) Cytoplasmic high mobility group box 1 (HMGB1) expression in airway epithelial cells. (K) HMGB1 expression in the BALF. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice. Data are mean±SEM, n=7-9 mice/group, representative of 2 experiments.

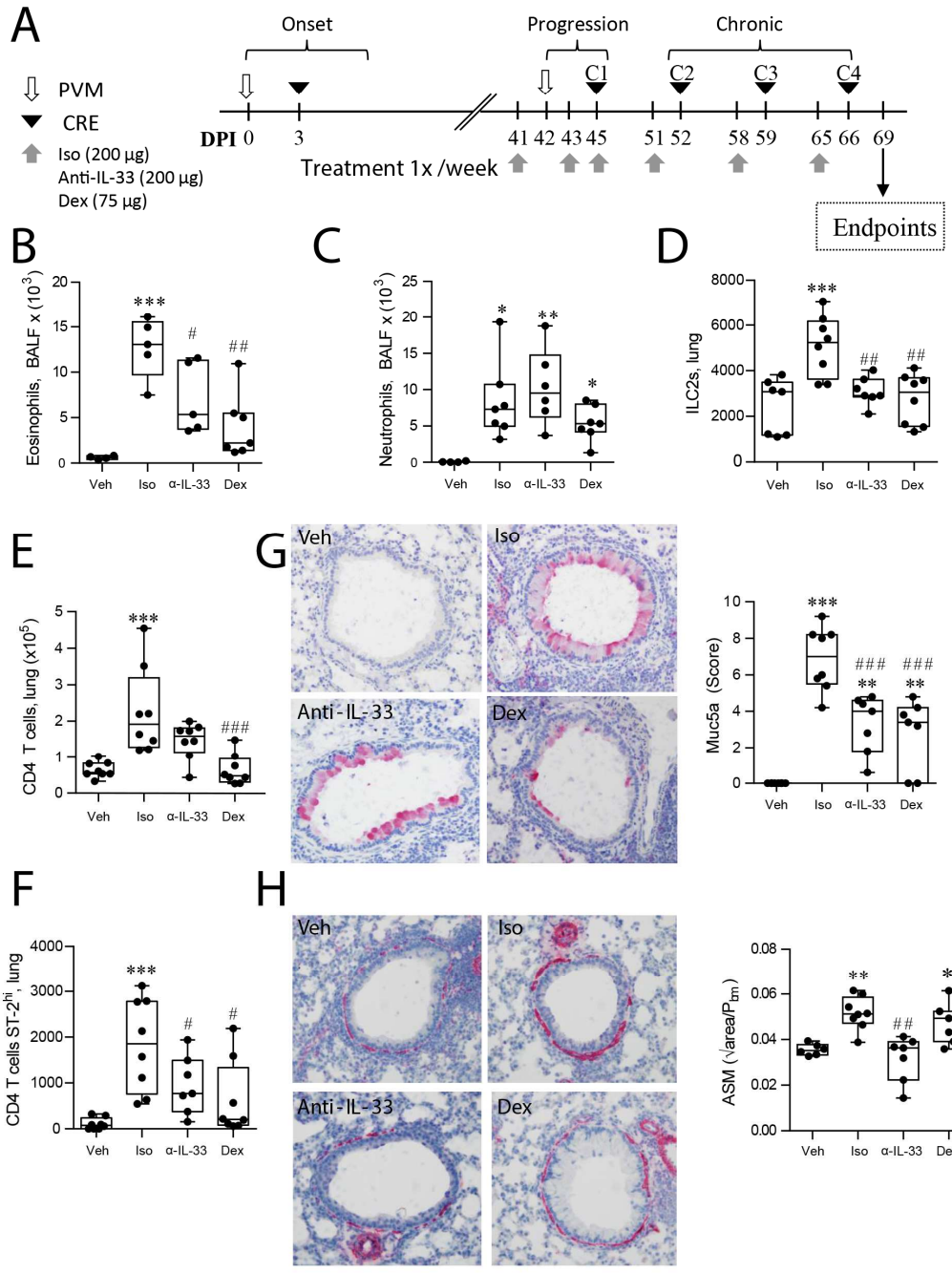
**Figure 6. Treatment with anti-IL-33 promotes antiviral immunity during RV-exacerbation**. Mice were euthanized 1 day post rhinovirus (RV)-1B infection. (A) RV-1B viral copies were measured by qPCR. (B) Gene expression of interferon (IFN)-α, IFN-λ and IFN-γ. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice. Box and whisker plots show quartiles (boxes) and range (whiskers), n=5-7 mice/group, representative of 2 experiments.

**Figure 7. Anti-IL-33 blockade boosts antiviral immunity of RV infected hBECs.** Healthy (open circles) and asthmatic (closed) primary hAECs were infected with RV-16 or RV-1B (MOI 1). **(A)** RV-16 and **(B)** RV-1B viral copies were measured by qPCR. **(C)** Gene expression of IFN-λ following RV-16 or **(D)** RV-1B infection. **(E)** IFN-λ expression in supernatant following RV-16 or **(F)** RV-1B infection. **(G)** Gene expression of IFN-β following RV-16 or **(H)** RV-1B infection. **(I)** IL-33 expression in uninfected (UI) samples and RV-16 or **(J)** RV-1B infected hAECs. * denotes significance within groups. # denotes significance between healthy and asthmatic cells. n=5 patients/group, in duplicate.

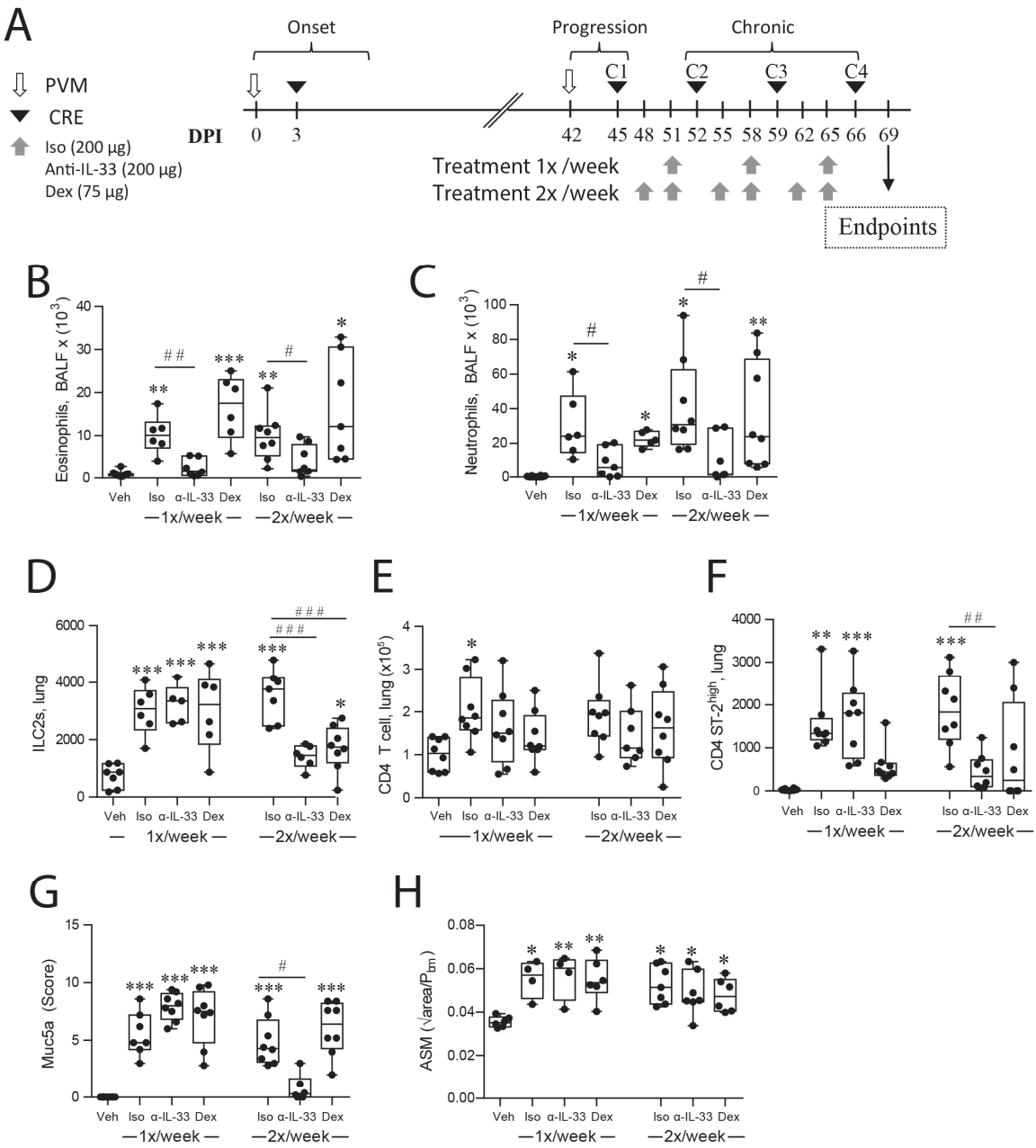
# Table S1

| *Name* | *Oligonucleotide Primer* |
|---|---|
| RV-1B | Forward: 5'- CAATGGGTGTTGTACTCTGTTATTC -3' |
| | Reverse: 5'- CCTGGAGAGTTGCCTACTATTG -3' |
| RV-16 | Forward: 5' – CCCTGAATGTGGCTAACCTT -3' |
| | Reverse: 5' – GAAACACGGACACCCAAAGTA -3' |
| mHprt | Forward: 5'- AGGCCAGACTTTGTTGGATTTGAA -3' |
| | Reverse: 5'- CAACTTGCGCTCATCTTAGGCTTT -3' |
| mIFN-α4 | Forward: 5'- CAGCATCTACAAGACCCACAA -3' |
| | Reverse: 5'- GCAGGTCACATCCTAGAGAAC -3' |
| mIFN-λ2 | Forward: 5'- GATTGCCACATTGCTCAGTTC -3' |
| | Reverse: 5'- CTTCTCAAGCAGCCTCTTCTC -3' |
| mIFN-γ | Forward: 5'- TCTTGAAAGACAATCAGGCCATCA -3' |
| | Reverse: 5'- GAATCAGCAGCGACTCCTTTTCC -3' |
| hACTB | Forward: 5'- TACGCCAACACAGTGCTGTCT -3' |
| | Reverse: 5' – TCTGCATCCTGTCGGCAAT -3' |
| hIFN-λ2 | Forward: 5' – CTCTGTCACCTTCAACCTCTTC - 3' |
| | Reverse: 5' – ATCTCAGGTTGCATGACTGG - 3' |
| hIFN-β | Forward: 5' – CTCTGGCACAACAGGTAGTAG - 3' |
| | Reverse: 5' – GGAAAGAGCTGTAGTGGAGAAG - 3' |
| hIL-33 | Forward: 5' – CCACTGAGGAAAGAGCCATAG – 3' |
| | Reverse: 5' – TGAGCCTATCGTTTGGAACTG – 3' |

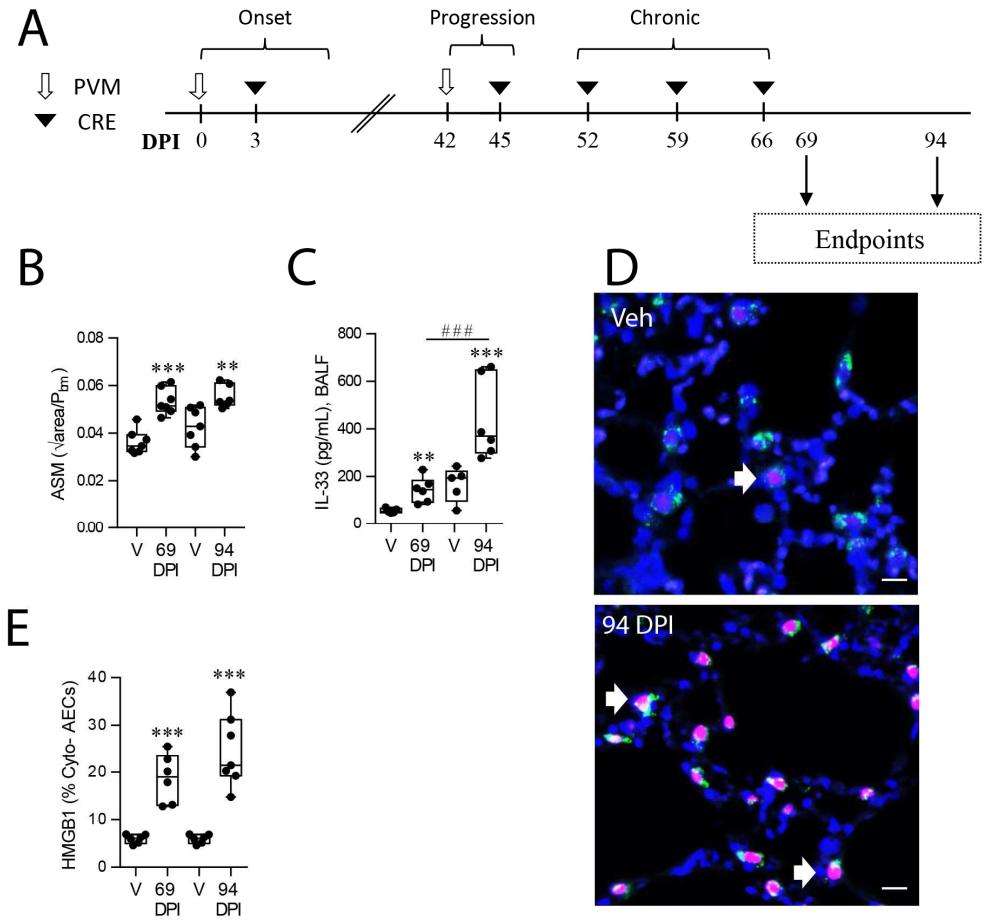
# Figure 1


A
Onset
Progression
Chronic
PVM
CRE
Iso (200 µg)
Anti-IL-33 (200 µg)
Dex (75 µg)
DPI 0 3 41 42 43 45 51 52 58 59 65 66 69
C1 C2 C3 C4
Treatment 1x /week
Endpoints
B
Eosinophils, BALF x (10^3)
C
Neutrophils, BALF x (10^3)
D
ILC2s, lung
E
CD4 T cells, lung (x10^5)
F
CD4 T cells ST-2^hi, lung
G
Veh
Iso
Anti-IL-33
Dex
Muc5a (Score)
H
Veh
Iso
Anti-IL-33
Dex
ASM (√area/P_bm)
Veh Iso α-IL-33 Dex

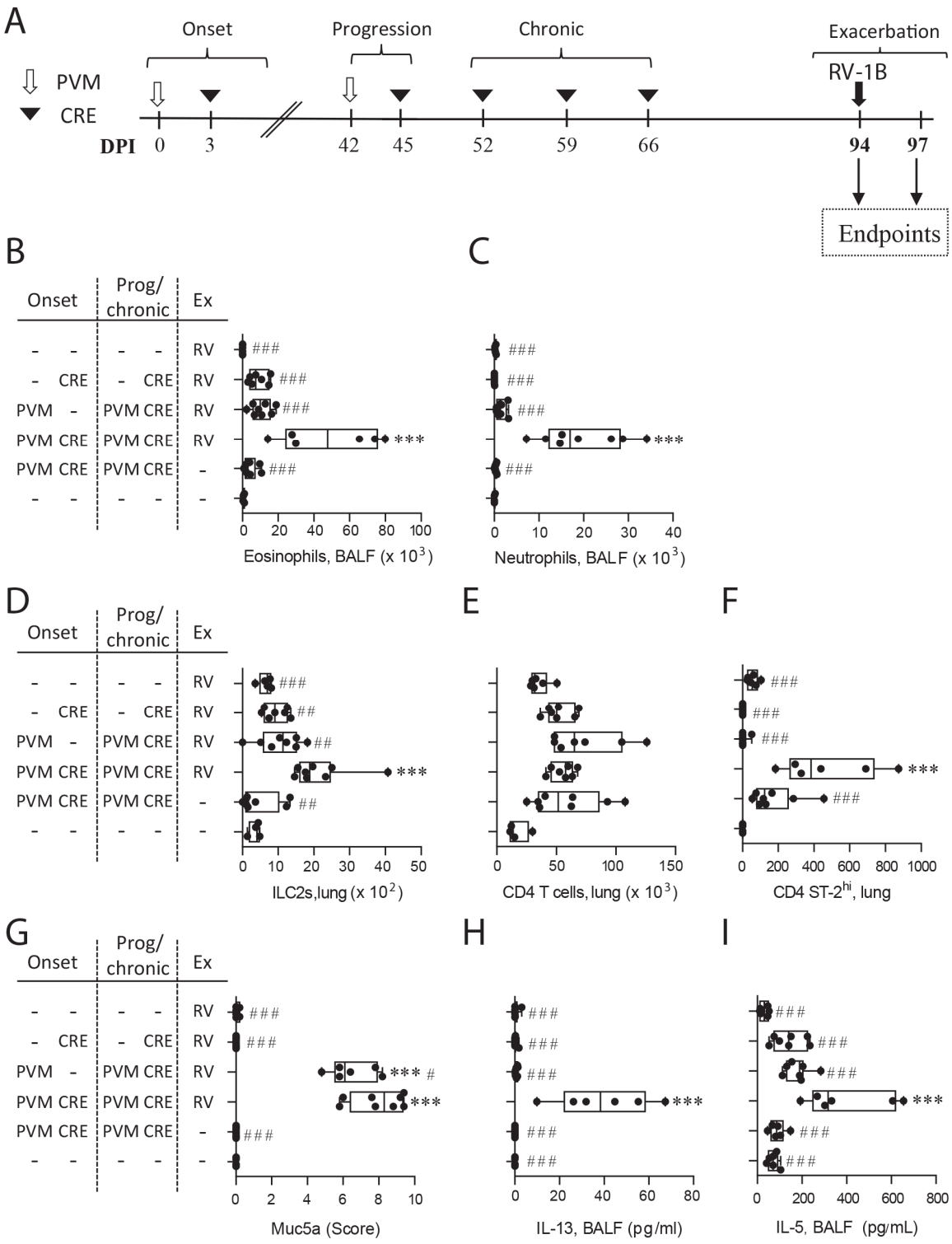
# Figure 2


A
PVM
CRE
Iso (200 µg)
Anti-IL-33 (200 µg)
Dex (75 µg)
DPI
Onset
Progression
Chronic
C1
C2
C3
C4
0 3 42 45 48 51 52 55 58 59 62 65 66 69
Treatment 1x /week
Treatment 2x /week
Endpoints
B
Eosinophils, BALF x (10^3)
C
Neutrophils, BALF x (10^3)
D
ILC2s, lung
E
CD4 T cell, lung (x10^5)
F
CD4 ST-2^high, lung
G
Muc5a (Score)
H
ASM (√area/P_tm)
Veh Iso α-IL-33 Dex Iso α-IL-33 Dex
—1x/week— —2x/week—

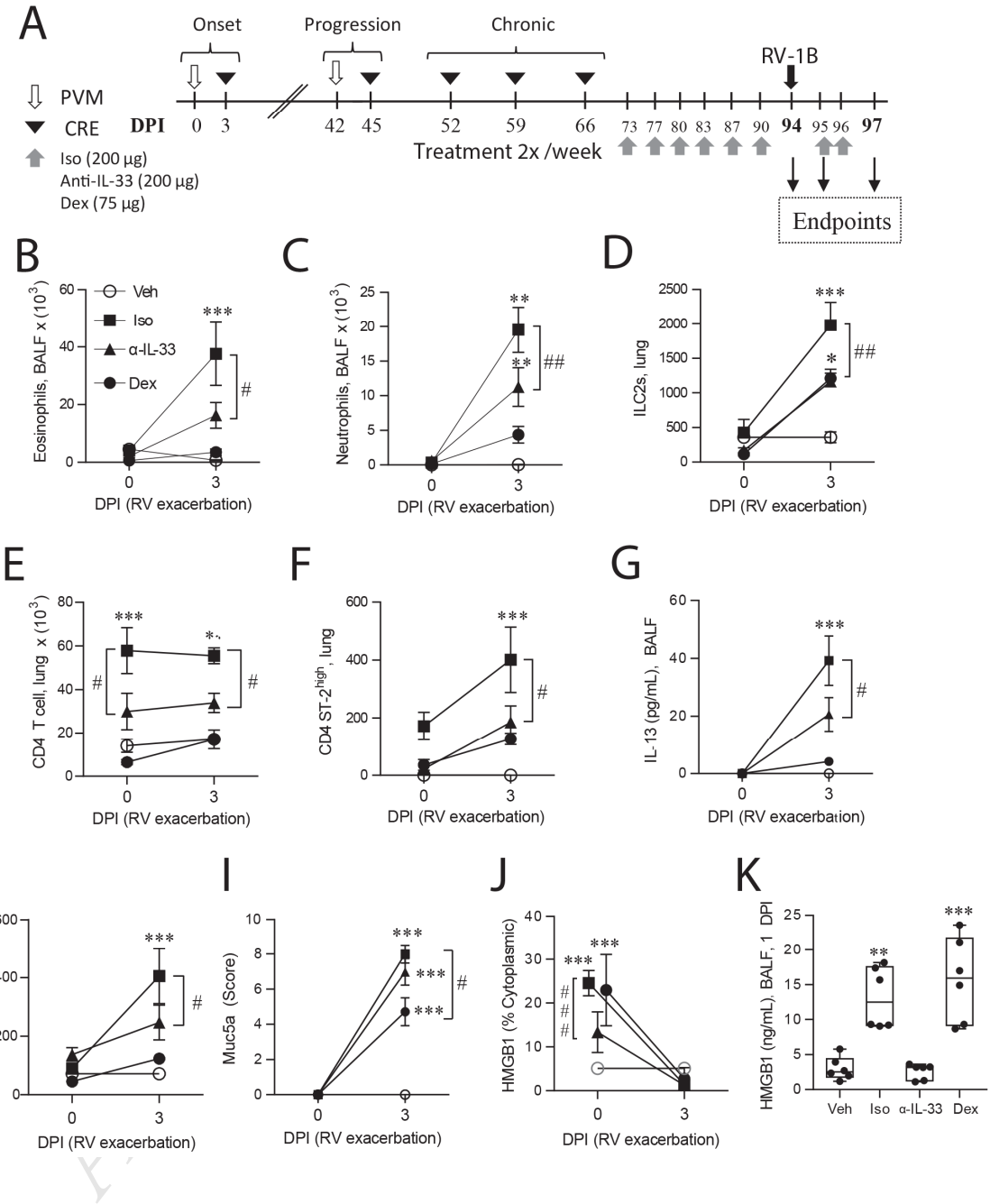
# Figure 3


A
Onset
Progression
Chronic
PVM
CRE
DPI 0 3 42 45 52 59 66 69 94
Endpoints
B
ASM (√area/Pm)
0.08
0.06
0.04
0.02
0.00
***
**
V 69 DPI V 94 DPI
C
IL-33 (pg/mL), BALF
800
600
400
200
0
###
**
***
V 69 DPI V 94 DPI
D
Veh
94 DPI
E
HMGB1 (% Cyto- AECs)
40
30
20
10
0
***
***
V 69 DPI V 94 DPI

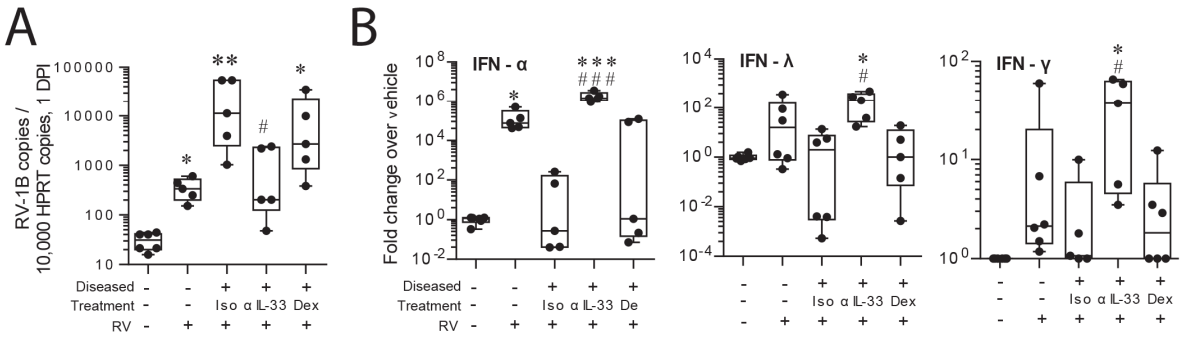
# Figure 4


A
Onset
Progression
Chronic
Exacerbation
PVM
CRE
RV-1B
DPI 0 3 42 45 52 59 66 94 97
Endpoints
B
C
Onset
Prog/ chronic
Ex
Eosinophils, BALF (x 10^3)
Neutrophils, BALF (x 10^3)
D
E
F
ILC2s,lung (x 10^2)
CD4 T cells, lung (x 10^3)
CD4 ST-2^hi, lung
G
H
I
Muc5a (Score)
IL-13, BALF (pg/ml)
IL-5, BALF (pg/mL)

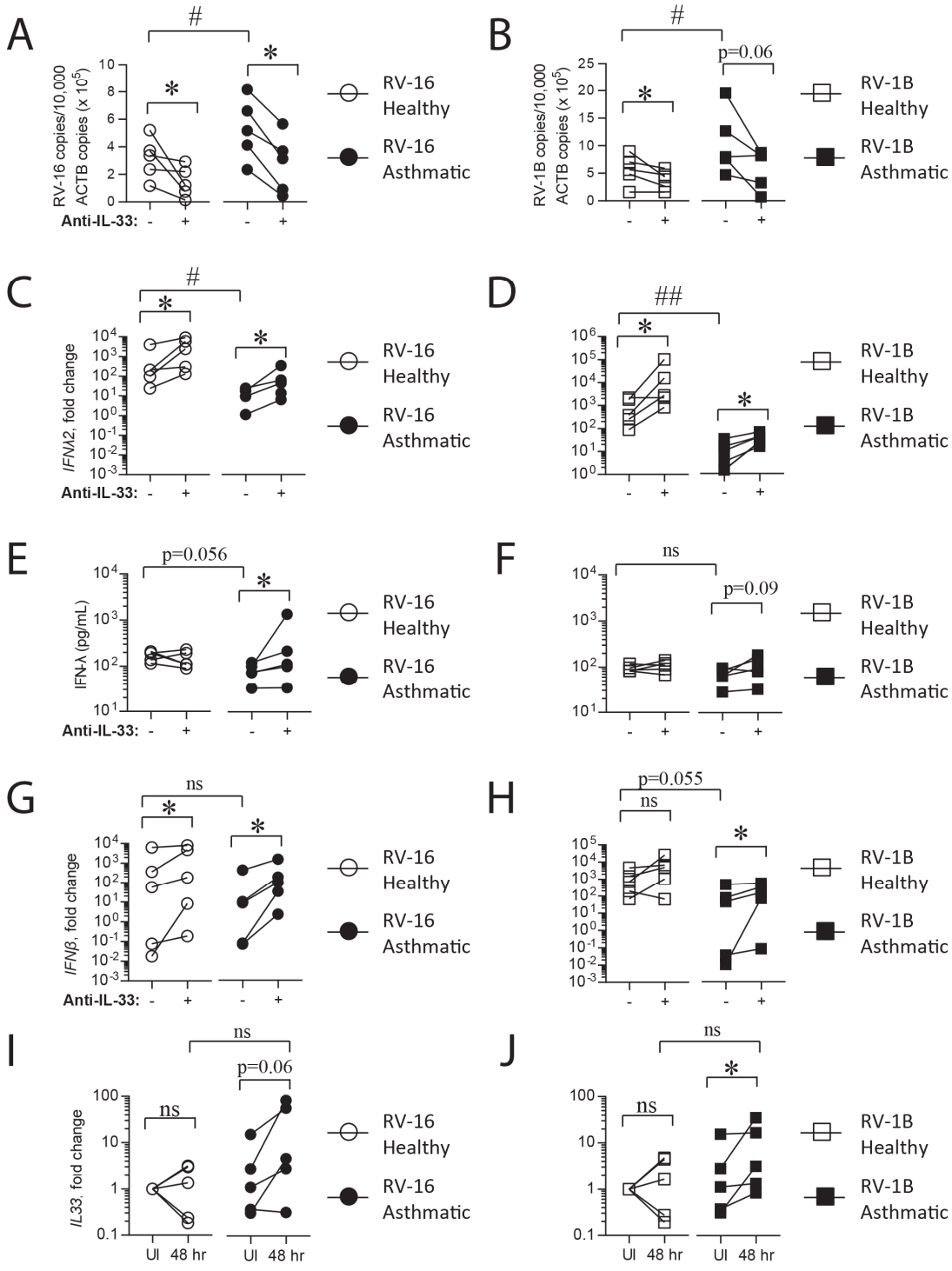
# Figure 5


A
Onset
Progression
Chronic
RV-1B
PVM
CRE
DPI
0 3 42 45 52 59 66 73 77 80 83 87 90 94 95 96 97
Treatment 2x /week
Iso (200 µg)
Anti-IL-33 (200 µg)
Dex (75 µg)
Endpoints
B
Eosinophils, BALF x (10^3)
Veh
Iso
α-IL-33
Dex
DPI (RV exacerbation)
C
Neutrophils, BALF x (10^3)
DPI (RV exacerbation)
D
ILC2s, lung
DPI (RV exacerbation)
E
CD4 T cell, lung x (10^3)
DPI (RV exacerbation)
F
CD4 ST-2^high, lung
DPI (RV exacerbation)
G
IL-13 (pg/mL), BALF
DPI (RV exacerbation)
H
IL-5 (pg/mL), BALF
DPI (RV exacerbation)
I
Muc5a (Score)
DPI (RV exacerbation)
J
HMGB1 (% Cytoplasmic)
DPI (RV exacerbation)
K
HMGB1 (ng/mL), BALF, 1 DPI
Veh
Iso
α-IL-33
Dex

# Figure 6



# Figure 7


A
RV-16 copies/10,000 ACTB copies (x 10^5)
Anti-IL-33: - + - +
RV-16 Healthy
RV-16 Asthmatic
B
RV-1B copies/10,000 ACTB copies (x 10^5)
p=0.06
RV-1B Healthy
RV-1B Asthmatic
C
IFNλ2, fold change
D
E
IFN-λ (pg/mL)
p=0.056
F
p=0.09
G
IFNβ, fold change
H
p=0.055
I
IL33, fold change
p=0.06
UI 48 hr
J

# Chronic IL-33 expression predisposes to viral-induced exacerbations of asthma by increasing type-2 inflammation and dampening antiviral immunity

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**Online Supplement**

*Co-virus and allergen-induced asthma and rhinovirus-induced exacerbation*

Specific pathogen-free BALB/c mice were intranasally inoculated at 7 days of age with 1 PFU of PVM (strain J3666) (1) or vehicle (DMEM + 10% FCS) under light isofluorane-induced anaesthesia. Mice were re-inoculated 6 weeks later (49 days old) with 20 PFU of PVM or vehicle diluent. Mice were intranasally exposed to 1 µg of cockroach allergen (Greer Laboratories) (CRE) or vehicle, at 10 and 52, 59, 66, and 73 days of age, as indicated and euthanised at 76 days of age (2). Anti-IL-33 or isotype control antibody (200 µg, Pfizer, Inc) or dexamethasone (75 mg, Sigma-Aldrich) were administered as per study designs. For RV exacerbation, mice were rested for four weeks following final CRE, then inoculated with RV-1B ($5x10^6$ TCID50) (i.n.). All studies were approved by The University of Queensland Animal Ethics Committee.

*Sample extraction and processing*

Following euthanasia by pentobarbitone overdose, a bronchoalveolar lavage (BAL) was performed with 600 µL of PBS. The BAL fluid was centrifuged at 5,000 rpm, 4°C for 5 min and the supernatant stored at -80$^{o}$C until analysis by cytokine bead array (CBA) or ELISA.

BALF cells were analysed immediately by flow cytometry. Lung lobes were excised and processed as previously described (3). Briefly, the left lung lobe was processed immediately for flow cytometry and the superior right lobe fixed in 10% formalin neutral buffer overnight before storage in 70% ethanol. The post-caval and inferior lobes were pooled and snap frozen before mechanical digestion and clarification, followed by analysis by ELISA. The inferior right lobe was snap frozen before RNA extraction. All snap frozen lungs were stored at -80 $^{0}$C.

*Flow cytometry*

Flow cytometry was performed on BALF cells or lung tissue digest cells as previously described (3). Briefly, single cell suspensions were incubated with anti-FcγRIII/II (Fc block) for 15 min at 4°C then incubated with the following fluorochrome-conjugated antibodies at 4°C for 30 minutes: anti-mouse CD2-FITC (RM2-5), Gr-1–FITC (RB6-8C5), CD11c–FITC (HL3), CD11b–FITC (M1/70), B220-FITC (RA36B2), CD3–FITC (145-2C11) (all Miltenyi Biotec), CD8-PerCP (53-6.7), FoxP3-PE (MF23), CD11b-PerCP-Cy5.5 (clone M1/70), Siglec F-AF647 (clone E50-2440), Ly6G-FITC (clone 1A8), CD4-V500 (RM4-5) (all BD Biosciences), CD45-BV421 (30-F11), ST-2-APC (DIH9), Ly6C-BV570 (clone HK1.4), CD11c-BV785 (clone N418), (all Biolegend), B220-PE (clone RA3-6B2), CD3ε-PE (clone145-2C11), MHCII-APC-eFluor 780 (clone M5/114.15.2) (all eBioscience). 7-AAD (eBioscience) was used to exclude dead cells and cells were gated as demonstrated in Supplementary Figure 7. Samples were collected with an LSR Fortessa X-20 (BD Biosciences) and the data analysed with FACSDiva v8 (BD Biosciences) and FlowJo v8.8 (Treestar).

*Immunohistochemistry*

Paraffin-embedded lung sections were prepared as previously described (1). For immunohistochemistry, lung sections were pretreated with 10% normal goat serum for 30 min. Sections were probed with anti-IL-33 (AF3626, R&D), anti-HMGB1 (Ab 18256, Abcam), anti-pro-SPC (Millipore), anti-α-SM actin (Sigma-Aldrich) and anti-Muc5ac (Thermofisher) overnight at 4°C. Following incubation with appropriate secondary antibodies, immunoreactivity was developed with Fast Red (Sigma-Aldrich) and counterstained with Mayer's hematoxylin (bright field) or with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) (fluorescence). Muc5ac was scored from 1-5 as a percentage of Muc5ac+ AECs and 1-5 for Muc5ac plugging (maximum score = 10) (Scanscope XT, Aperio). Cytoplasmic HMGB1 was quantified as percentage of airway epithelial cells positive for HMGB1 in the cytoplasm. Airway smooth muscle mass around the small airways (defined as a circumference <800 µm) was measured using Scanscope XT software and expressed as area per µm of basement membrane. Bright field photomicrographs were taken at 400x and 1000x magnification using an Olympus BX-51 microscope with an Olympus DP-72 camera at room temperature and acquired using Olympus Image Analysis Software. Fluorescent images were taken using a Leica DMi8 Inverted Confocal microscope and processed using Imaris Image Analysis software.

*Measurement of protein expression*

The limit of detection for each assay is indicated in brackets. IL-33 (R&D Systems) (15 pg/mL), IL-25 (Biolegend) (1 pg/mL), HMGB1 (Chondrex) (0.5 ng/mL) and IL-5 (BD Biosciences) (9 pg/mL) expression was quantified by ELISA. IL-13 (Enhanced Sensitivity Flex Set, BD Biosciences) (9 fg/mL) expression was quantified by CBA.

*Quantitative real time PCR*

Total RNA was isolated from the inferior right lung lobe with TriReagent solution (Ambion) followed by phenol-chloroform extraction. DNAse digestion was performed with Turbo DNAse (Ambion), according to the manufacturer's instructions. Reverse transcription was performed using M-MLV reverse transcriptase and random primers (Invitrogen). qRT-PCR was performed with SYBR Green (Life Technologies) with the primers described in Table S1. RV-1B or RV-16 viral copy number was determined by generating a standard curve of RV-1B or RV-16 viral copies. Expression values were normalized to *Hprt* or *ACTB* and expressed as fold change over vehicle-treated mice or cells, as described (1, 2).

*Statistical analyses*

GraphPad Prism version 5.0 software (La Jolla, California) was used for all statistical analyses. A Student's t-test, one-way ANOVA with a Tukey post-hoc test or two-way ANOVA with a Sidak post-hoc test were applied as appropriate. A $P$ value $<0.05$ was considered statistically significant.

**Supplemental Figure 1. Timing of cockroach allergen (CRE) exposure in reinfection underpins asthma progression**. (A) Study design. CRE-1 was administered 3 (45 DPI) or 7 (49 DPI) days post reinfection with PVM. (B) Eosinophils and (C) Neutrophils in BALF. (~~B~~D) Muc5ac score. (~~C~~E) Airway smooth muscle (ASM) area. (~~D~~F) Study design. Mice were euthanised 4 days post C1 (49 DPI). (G) Eosinophils and (H) Neutrophils in BALF. (~~E~~I) Muc5a score. (~~F~~J) ASM area. * denotes significance compared with vehicle treated mice. # denotes significance compared with 45 DPI CRE mice. Box and whisker plots show quartiles (boxes) and range (whiskers), n=4-8 mice/group, representative of 2 experiments.

**Supplemental Figure 2. IL-33 blockade during the progression and chronic phase.** Mice were treated as per study design in Fig 1A. (A) Total BALF cells. (B) Lymphocytes and (C) Mononuclear cells in the BALF. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice. Box and whisker plots show quartiles (boxes) and range (whiskers), n=5-8 mice/group, representative of 2 experiments.

**Supplemental Figure 3. IL-33 blockade during the chronic phase alone.** Mice were treated as per study design in Fig 2A. (A) Total BALF cells. (B) Lymphocytes and (C) Mononuclear cells in the BALF. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice Box and whisker plots show quartiles (boxes) and range (whiskers), n=6-8 mice/group, representative of 2 experiments.

**Supplemental Figure 4. Rhinovirus infection exacerbation model.** Mice were treated as per study design in Fig 4A. (A) IL-33 and (B) IL-25 in BALF of vehicle mice infected with rhinovirus (RV)-1B alone. (C) Total BALF cells. (D) Lymphocytes and (E) Mononuclear cells in the BALF. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice. Box and whisker plots show quartiles (boxes) and range (whiskers), n=6-9, representative of 2 experiments.

**Supplemental Figure 5. IL-33 blockade during an RV-exacerbation.** Mice were treated as per study design in Fig 5A. (A) Total BALF cells. (B) Lymphocytes and (C) Mononuclear cells in the BALF. * denotes significance compared with vehicle treated mice. # denotes significance compared with isotype treated mice. Data are mean±SEM, n=7-9 mice/group, representative of 2 experiments.

**Supplemental Figure 6. Rhinovirus infection of human airway epithelial cells.** Healthy (open circles) and asthmatic (closed) primary hAECs were infected with RV-16 or RV-1B (MOI 1). (A) IL-33 in the supernatant following RV-16 and (B) RV-1B infection. * denotes significance within groups. # denotes significance between healthy and asthmatic cells. n=5 patients/group, in duplicate.

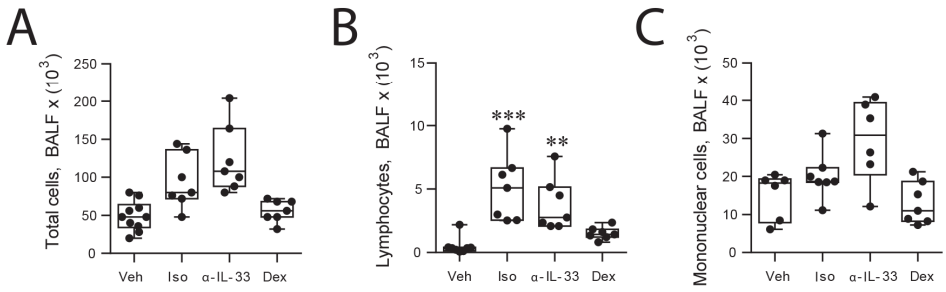
**Supplemental Figure 7. Gating strategy for FACS of inflammatory cell populations.** (A) BALF cells. **(**B) CD4+ T cell populations. (C) ILC2s.

**Supplemental Table 1.** Oligonucleotide sequences used in this study are shown.

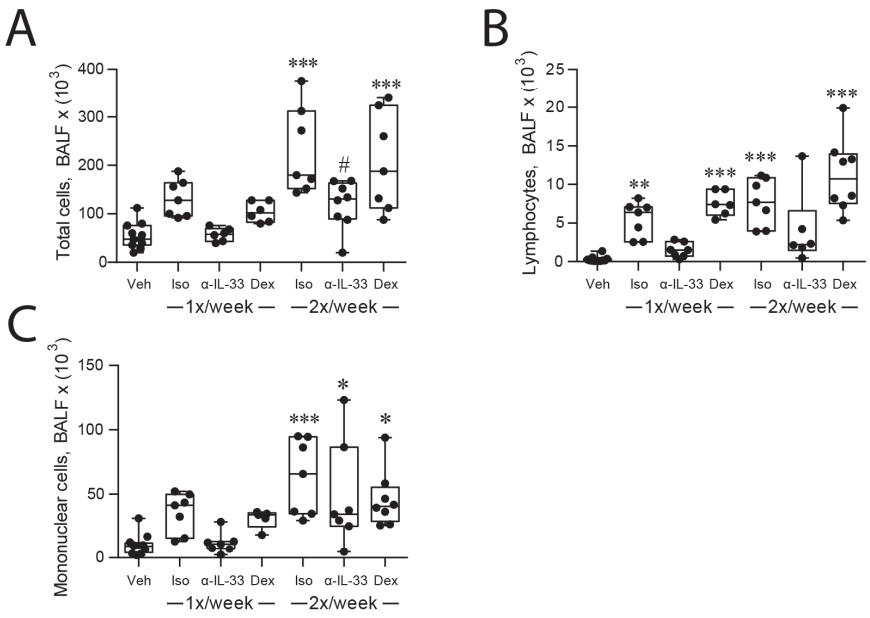
Supplemental References

1. Davidson S, Kaiko G, Loh Z, Lalwani A, Zhang V, Spann K, et al. Plasmacytoid dendritic cells promote host defense against acute pneumovirus infection via the TLR7-MyD88-dependent signaling pathway. J Immunol. 2011;186(10):5938-48.
2. Lynch JP, Werder RB, Simpson J, Loh Z, Zhang V, Haque A, et al. Aeroallergen-induced IL-33 predisposes to respiratory virus-induced asthma by dampening antiviral immunity. J Allergy Clin Immunol. 2016;138(5):1326-37.
3. Kaiko GE, Loh Z, Spann K, Lynch JP, Lalwani A, Davidson S, et al. TLR7 gene deficiency and early-life Pneumovirus infection interact to predispose toward the development of asthma-like pathology in mice. J Allergy Clin Immunol. 2013;131(5):1331-39.
4. Lee JU, Chang HS, Lee HJ, Jung CA, Bae DJ, Song HJ, et al. Upregulation of interleukin-33 and thymic stromal lymphopoietin levels in the lungs of idiopathic pulmonary fibrosis. BMC pulmonary medicine. 2017;17(1):39.
5. Busch DR, Hobbs DBD, Zhou DJ, Castaldi DPJ, McGeachie DMJ, Hardin DME, et al. Genetic Association and Risk Scores in a COPD Meta-Analysis of 16,707 Subjects. American Journal of Respiratory Cell and Molecular Biology.0(ja):null.

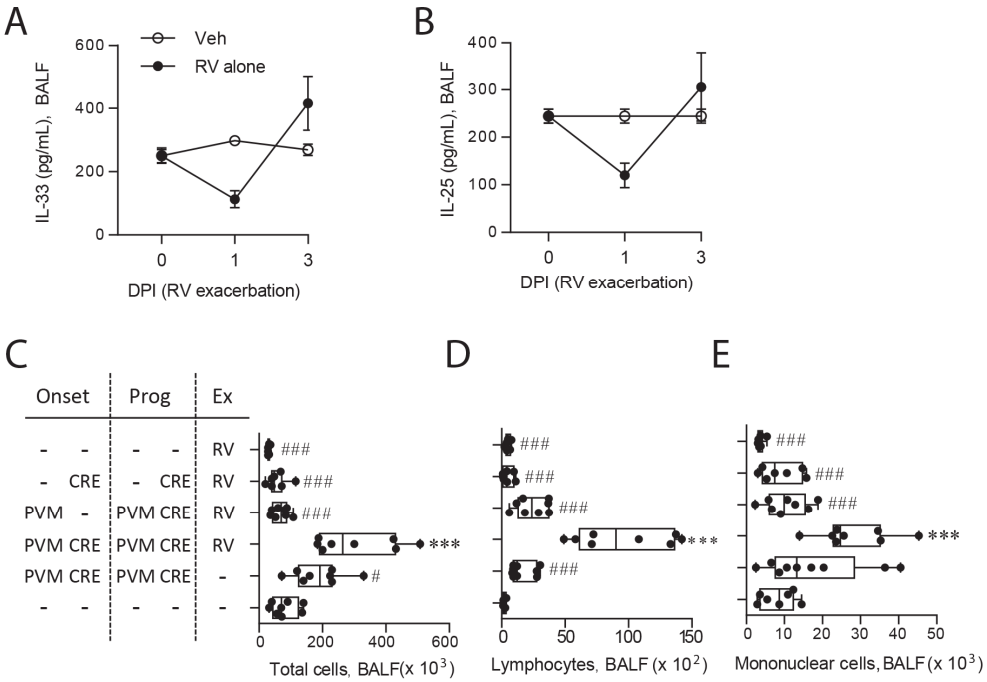
# Supplemental Figure 2



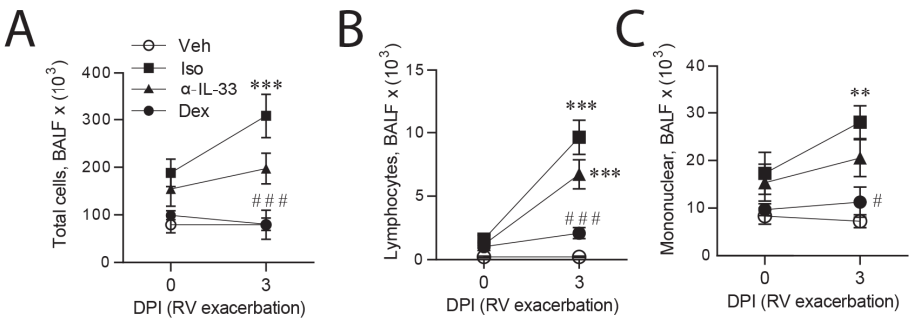
# Supplemental Figure 3



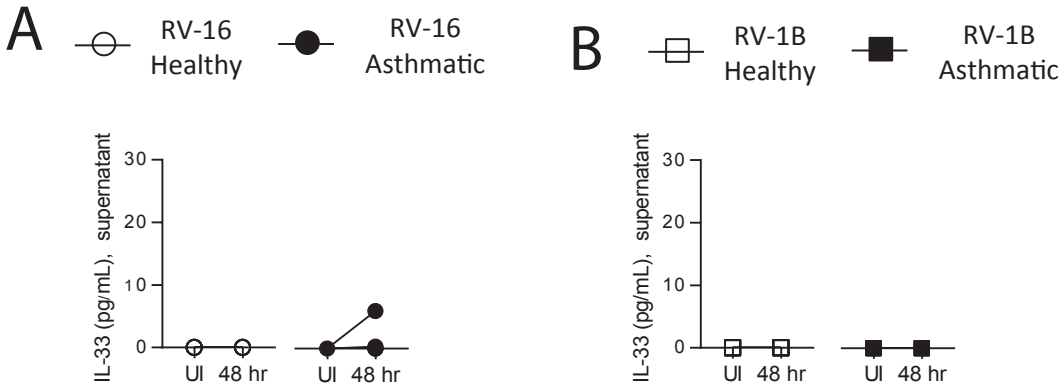
# Supplementary Figure 4



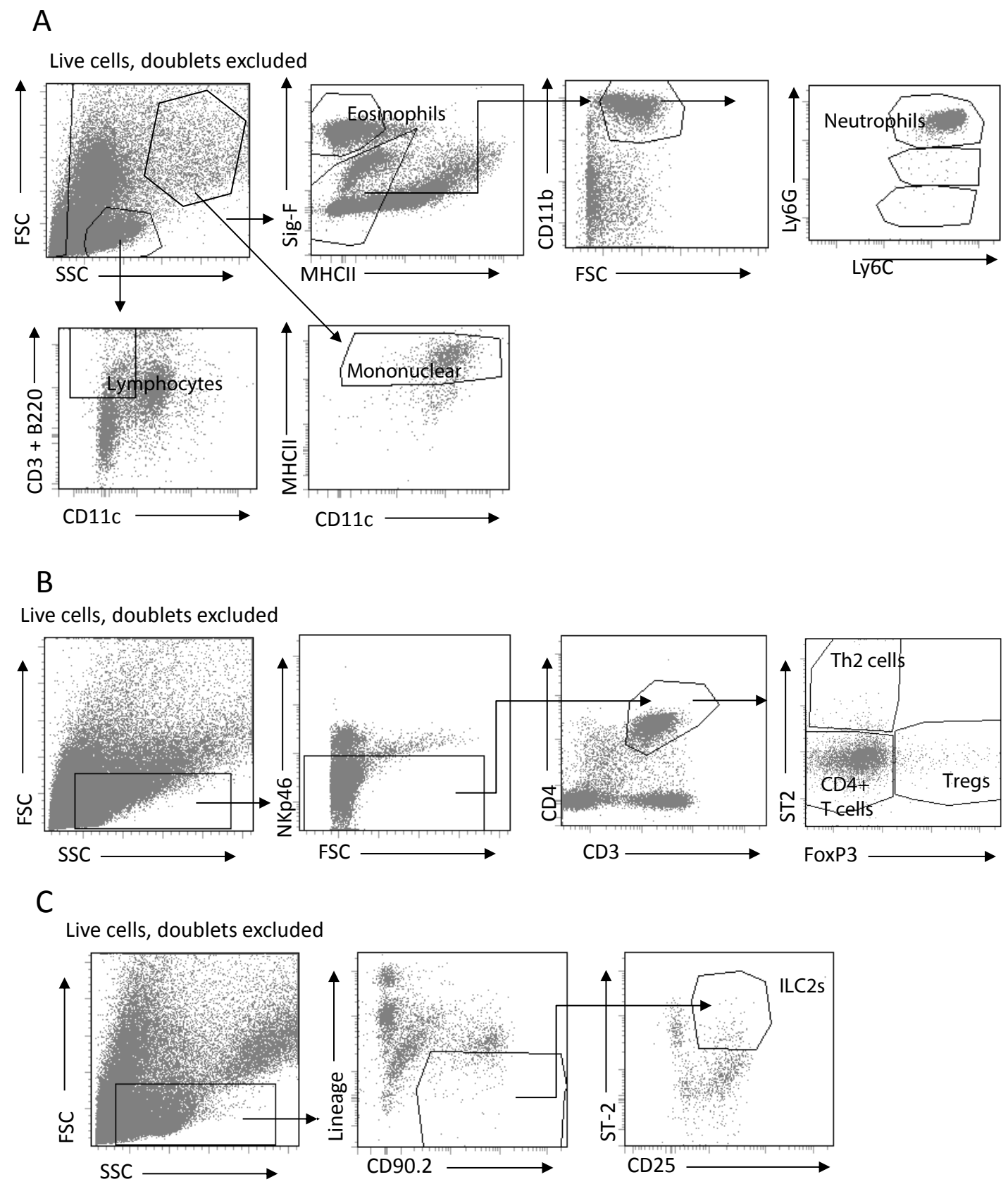
# Supplementary Figure 5



# Supplementary Figure 6



# Supplementary Figure 7



# Supplemental Figure 1