



Serum amyloid A is a soluble pattern recognition receptor that drives type 2 immunity

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The molecular basis for the propensity of a small number of environmental proteins to provoke allergic responses is largely unknown. Herein, we report that mite group 13 allergens of the fatty acid-binding protein (FABP) family are sensed by an evolutionarily conserved acute-phase protein, serum amyloid A1 (SAA1), that promotes pulmonary type 2 immunity. Mechanistically, SAA1 interacted directly with allergenic mite FABPs (Der p 13 and Blo t 13). The interaction between mite FABPs and SAA1 activated the SAA1-binding receptor, formyl peptide receptor 2 (FPR2), which drove the epithelial release of the type-2-promoting cytokine interleukin (IL)-33 in a SAA1-dependent manner. Importantly, the SAA1-FPR2-IL-33 axis was upregulated in nasal epithelial cells from patients with chronic rhinosinusitis. These findings identify an unrecognized role for SAA1 as a soluble pattern recognition receptor for conserved FABPs found in common mite allergens that initiate type 2 immunity at mucosal surfaces.

Allergic diseases of the respiratory system such as asthma are initiated by aberrant type 2 immunity to an exclusive group of otherwise innocuous environmental proteins, called allergens. Although extensive efforts have been focused on defining the common motifs underlying allergenicity¹, the immunological mechanisms by which allergens induce type 2 immune responses are still not well understood. Recent evidence suggests that dysregulated allergen sensing through pattern recognition receptors (PRRs) and the aberrant release of innate type 2 mediators, such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) at mucosal surfaces^{2–4}, are central to the initiation or amplification of existing allergic respiratory diseases in genetically susceptible individuals.

While PRRs are classically thought of as membrane-bound Toll-like receptors (TLRs) or cytoplasmic (nucleotide-binding oligomerization domain (NOD)-like receptors/retinoic acid-inducible gene (RIG)-like receptors), several other evolutionarily ancient but secreted molecules constitute a distinct class of soluble PRRs (sPRRs). sPRRs, including pentraxins, are constitutively expressed at low basal concentrations by a variety of cells and can rapidly increase to signal the presence of pathogens, regulate the migration of leukocytes and modulate tissue responses in extracellular compartments such as the alveolar space⁵. As such, they are part of humoral innate defense mechanisms that protect barrier tissues from microorganisms and potential injuries. Although sPRRs have been linked to allergic inflammation in the lung, their role in type 2 immunity at this environmentally exposed site remains poorly understood.

In the respiratory tract, airway epithelial cells (AECs) are a major source of sPRRs that include the acute-phase reactant serum amyloid A (SAA)⁶. The products of the *SAA1/2* genes, the SAA1 and SAA2 proteins, have been classically viewed as highly inducible, liver-derived factors in response to infection or trauma⁷, but baseline expression has been documented in many histologically normal human extrahepatic tissues including the alveolar epithelial lining⁶. As sPRRs, SAA proteins may serve as opsonins that enhance bacterial clearance and block viral entry into cells^{8,9}. In contrast to high-density lipoprotein (HDL)-bound SAA1 in the serum¹⁰, locally produced, lipid-free SAA1 can promote proinflammatory cytokine production, the recruitment of immune cells and epithelial wound repair¹¹ through a number of potential SAA receptors¹². These SAA1 functions, however, are not operative under physiological conditions^{13,14}, probably requiring ligand-induced activation of SAA1.

In vitro, lipid-free SAA1 is prone to self-assemble into hexamers, which may have implications for SAA1 functions and may be modulated by ligand binding and/or the extent of SAA expression^{15,16}. While SAA recognition of bacteria in the gut is essential for immunity associated with the T_H17 subset of helper T cells¹⁷, much less is known about SAA innate immune sensing mechanisms in the lung¹⁸. Although a positive association between elevated sputum and airway SAA1 concentrations and asthma prevalence has been established in several independent studies^{19–22}, a causative role for SAA1 in allergic airway inflammation has remained elusive. Herein, we demonstrate a previously unrecognized role for SAA1 as an innate environmental sensor in type 2 immunity through its

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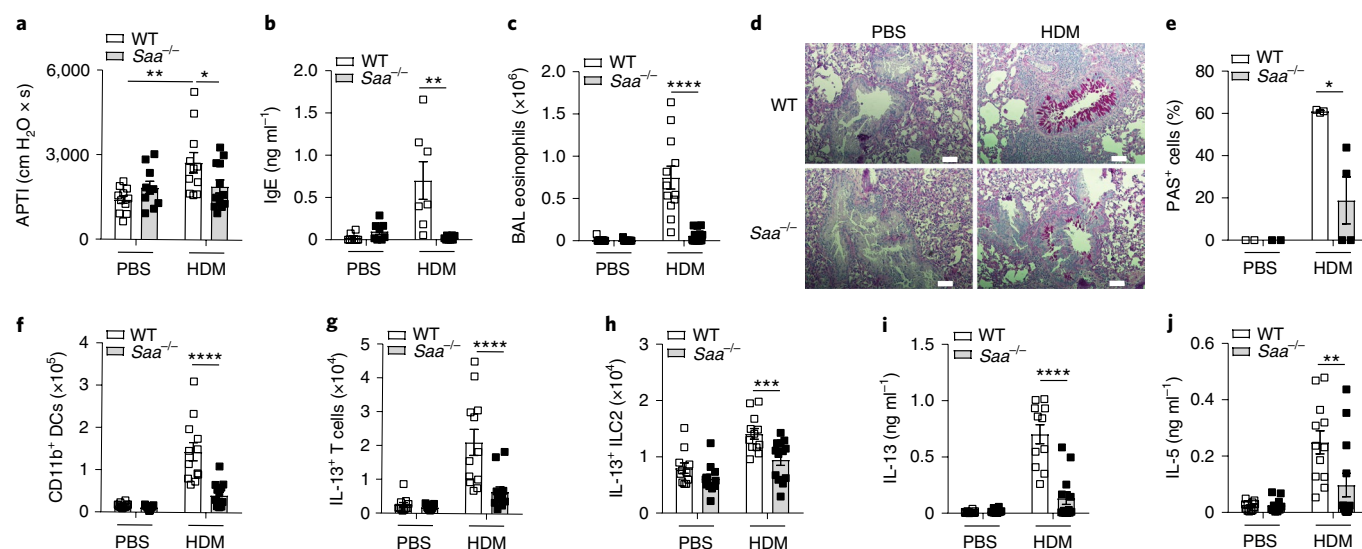


Fig. 1 | SAA drives HDM-induced allergic airway inflammation. The allergic phenotype in WT and *Saa*^{-/-} C57BL/6 mice sensitized and challenged with HDM was analyzed 72 h after the last challenge. Control mice received PBS at both sensitization and challenge. **a–e**, Airway responses to cholinergic stimulation shown as airway pressure over time (APTI, **P* = 0.0378, ***P* = 0.0019; **a**), total serum IgE concentrations (***P* = 0.0019; **b**), BAL eosinophilia (**c**) and histological examination of airway inflammation (**d,e**). Sections were stained for mucus production with periodic acid Schiff (PAS; scale bars, 100 μm) reagent and the bar graphs represent the percentage of mucus-positive cells in the airways (**P* = 0.0241). **f–j**, Numbers of CD11b⁺ DCs (**f**), IL-13⁺CD3⁺CD4⁺ cells (**g**) and IL-13⁺ ILC2 cells (***P* = 0.0003; **h**) in the lungs, and T_H2 cytokine production from HDM-restimulated lung cells (***P* = 0.0018; **i,j**). The data represent the means ± s.e.m. of pooled data from 2 independent experiments containing *n* = 11 WT PBS, *n* = 12 WT HDM, *n* = 11 *Saa*^{-/-} PBS and *n* = 13 *Saa*^{-/-} HDM animals per group (**a,c,f–j**); *n* = 2 WT PBS, *n* = 3 WT HDM, *n* = 2 *Saa*^{-/-} PBS and *n* = 4 *Saa*^{-/-} HDM animals per group (**d,e**); or are representative of 2 experiments with *n* = 8 WT PBS, *n* = 7 WT HDM, *n* = 8 *Saa*^{-/-} PBS and *n* = 8 *Saa*^{-/-} HDM animals per group (**b**). The *P* values were calculated with a two-tailed test using one-way analysis of variance (ANOVA) with Dunnett's post hoc (**b,c,e–j**) or Holm-Sidak's (**a**) analysis that compares WT HDM. *****P* ≤ 0.0001.

interaction with allergenic FABPs resulting in FPR2-dependent initiation of type 2 inflammation.

Results

SAA1 drives type 2 immune responses in vivo. To examine whether SAA1 (the predominant SAA isoform) is critical for allergen-driven aspects of type 2 immunity in the lungs, wild-type (WT) and *Saa*^{-/-} (lacking expression of SAA1.1 and its less abundant isoform SAA2.1) C57BL/6 mice were exposed to an extract of the house dust mite (HDM) species *Dermatophagoides pteronyssinus*, a complex source of allergens associated with type 2 responses in the lung³ (Extended Data Fig. 1a). While HDM-exposed WT mice developed increased airway hyperresponsiveness (AHR) in response to methacholine challenge compared to PBS-exposed mice, *Saa*^{-/-} mice were largely protected from HDM-induced AHR (Fig. 1a). In addition, *Saa*^{-/-} mice had reduced total serum IgE concentrations, bronchoalveolar lavage (BAL) eosinophilia, and goblet cell metaplasia of the airway lumen as compared to control animals (Fig. 1b–e). The suppression of the allergic phenotype was associated with significantly lower numbers of CD11c⁺CD11b^{hi}MHCII^{hi} dendritic cells (DCs), IL-13-secreting CD3⁺CD4⁺ (T_H2) and type 2 innate lymphoid (ILC2) cells and reduced T_H2-cytokine production by HDM-restimulated lung cells (Fig. 1f–h and Extended Data Figs. 2 and 3). The contribution of SAA1 to allergen-driven type 2 immunity was confirmed in an independent approach locally administering SAA1-neutralizing antibodies in the lungs of BALB/c mice (Extended Data Fig. 1b). Consistent with genetic deficiency, SAA1 neutralization throughout the allergen exposure regimen reduced cardinal markers of allergic asthma including AHR and numbers of type 2 effector cells (Extended Data Fig. 4a–g). To determine whether SAA signaling was also necessary for the innate manifestation of type 2 immunity, we analyzed the secretion of epithelial

type 2 cytokines that are central drivers of the allergic response²³. BAL concentrations of IL-33 were significantly increased after a single inhalational HDM exposure in WT mice, while baseline BAL IL-33 amounts in *Saa*^{-/-} mice were unchanged. HDM exposure failed to augment IL-25 concentrations in WT or *Saa*^{-/-} mice, and TSLP was not detected in the BAL (Extended Data Fig. 1c and Fig. 2a–c). Allergen-triggered epithelial IL-33 release²⁴ leads to the expansion of IL-13-secreting lung-resident ILC2 cells^{25,26} that initiate and maintain local allergic responses. WT C57BL/6 mice had elevated numbers of IL-13⁺ ILC2 cells in their lungs compared with *Saa*^{-/-} mice (Fig. 2d). Similarly, inhibiting the local effects of SAA1 through HDL- or antibody-mediated neutralization in BALB/c mice caused significantly reduced numbers of HDM-induced lung IL-13⁺ ILC2 cells (Extended Data Fig. 1d and Fig. 2e). Local overexpression of SAA1, which raised baseline SAA1 concentrations, was not sufficient to induce spontaneous expansion of IL-13-producing ILC2 cells in BALB/c mice. Only in the presence of HDM, overexpression of SAA1 resulted in enhanced numbers of IL-13⁺ ILC2 cells as compared to control mice (Extended Data Fig. 1e and Fig. 2f). This indicates that, even at increased local concentrations, SAA1 requires interaction with HDM allergens to boost its type 2-mediating properties and the expansion of IL-13⁺ ILC2 cells.

SAA1 is a sPRR for FABPs. In the lung, SAA is predominantly synthesized by the airway epithelium⁶ and secreted into the alveolar space⁵, where it has important immune-protective functions^{13,27}. A functional interaction of SAA1 has been described for the bacterial ligand outer membrane protein A (OmpA), facilitating clearance of Gram-negative bacteria⁹. Notably, the β-barrel structure of OmpA is related to those of lipocalins²⁸, a protein family that includes the highly conserved arthropod group 13 allergens of FABPs²⁹. Thus, we hypothesized that, given the structural homology to OmpA,

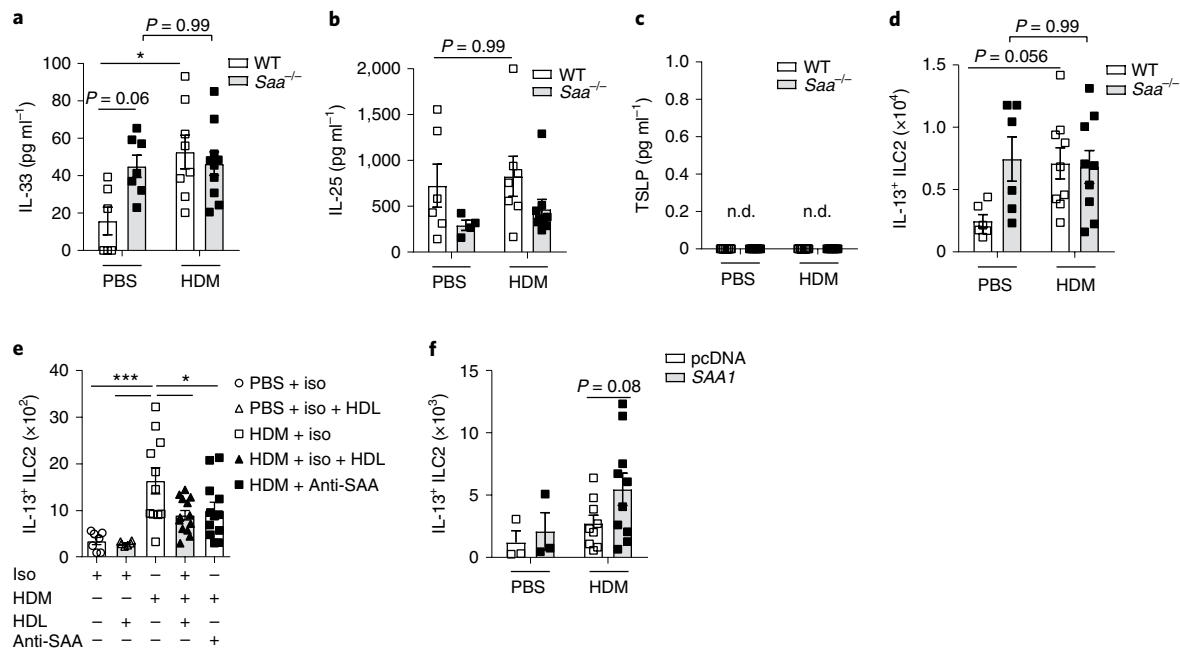


Fig. 2 | SAA signaling is necessary for the innate manifestation of type 2 immunity. **a–c**, Increase of the type 2 cytokines IL-33 (**P* = 0.0138; **a**), IL-25 (**b**) and TSLP (**c**) in the BAL of WT and *Saa*^{-/-} mice 16 h after a single dose of PBS or HDM. n.d., not detected. **d**, Numbers of IL-13⁺ ILC2 cells in the lungs of these mice. **e**, Effects of local SAA1 neutralization in WT BALB/c mice through HDL (**P* = 0.0117) or local SAA antibody blockade (anti-SAA, **P* = 0.0392) of WT BALB/c mice receiving isotype (Iso), HDM + isotype (HDM + iso) or HDM + anti-SAA (HDM + anti-SAA). **f**, Effects of SAA1 overexpression on numbers of Lin⁺CD45⁺CD25⁺ST2⁺IL-13⁺ ILC2 cells in the lungs. For overexpression, WT mice were injected retro-orbitally with a SAA1 overexpression plasmid (SAA1, gray bars) or a non-coding control vector (pcDNA, open bars). The data represent the means ± s.e.m. of pooled data from 2 independent experiments containing *n* = 6 WT PBS, *n* = 8 WT HDM, *n* = 7 *Saa*^{-/-} PBS and *n* = 10 *Saa*^{-/-} HDM animals per group (**a–c**); *n* = 5 WT PBS, *n* = 9 WT HDM, *n* = 6 *Saa*^{-/-} PBS and *n* = 9 *Saa*^{-/-} HDM animals per group (**d**); *n* = 7 PBS + iso, *n* = 6 PBS + iso/HDL, *n* = 11 HDM + iso, *n* = 13 HDM + iso/HDL and *n* = 12 HDM + anti-SAA animals per group (**e**); *n* = 3 pcDNA PBS, *n* = 9 pcDNA HDM, *n* = 3 SAA1 PBS and *n* = 10 SAA1 HDM animals per group (**f**). The *P* values were calculated with a two-tailed test using one-way ANOVA with Dunnett's post hoc analysis (**a–e**) or a two-sided Student's *t*-test (**f**). ****P* ≤ 0.001.

epithelial-derived SAA1 may function as a secreted sensor for mite FABPs that activates epithelial effector functions.

To evaluate a potential interaction between SAA1 and HDM allergens, HDM extract was blotted following SDS–polyacrylamide gel electrophoresis (PAGE) and probed with recombinant human SAA1 (rSAA1) and a SAA-specific monoclonal antibody. This analysis revealed an immunoreactive band of approximately 15 kDa, the reported molecular weight of Der p 13, the HDM FABP (Fig. 3a, HDM). Owing to their high structural similarities and sequence identity (>80%), we used recombinant Blo t 13 (rBlo t 13) from the tropical mite *Blomia tropicalis* as a representative of the mite FABP family in subsequent experiments. SAA was able to interact with rBlo t 13 (14.8 kDa), but not with the other major HDM allergens (rDer p 2 and rDer p 23) that have a similar migratory behavior in SDS–PAGE (15.9 and 14 kDa, respectively; Fig. 3a). To confirm a direct SAA1–mite FABP interaction, we used native PAGE. Binding of other proteins alters the electrophoretic mobility, which depends on both the protein's charge and hydrodynamic size. In the presence of Blo t 13, SAA1 shifted to higher masses and a SAA1 species that overlapped with Blo t 13 was detected by anti-SAA1 in a concentration-dependent manner, suggesting co-migration of SAA1 with Blo t 13 and the formation of SAA1–Blo t 13 complexes (Fig. 3b). Similarly, using a sequence-specific antiserum to the carboxy (C) terminal tail of human SAA1 (amino acids 89–104)³⁰ altered electrophoretic mobility and stronger binding of the antiserum was detected when Blo t 13 was present, despite similar loading (Extended Data Fig. 5a). This result suggests that SAA1 ligand binding might lead to an altered conformation of the protein, making the C-terminal tail more available for antibody binding. Furthermore,

when chemically crosslinked by glutaraldehyde, which offers a direct method of identifying transient and stable protein interactions, the formation of SAA1 dimers and lower-molecular-weight oligomers was reduced by increasing amounts of Blo t 13 (Fig. 3c and Supplementary Table 1). No higher-molecular-weight complexes of SAA1–Blo t 13 could be detected in this approach.

To determine whether SAA1 and FABP–SAA1 interactions were necessary for HDM-driven epithelial IL-33 release, we analyzed IL-33 secretion in the human AEC line BEAS-2B. Short interfering RNA (siRNA)-targeted SAA1 knockdown in BEAS-2B cells significantly impaired HDM-triggered IL-33 release (Fig. 3d), which was not associated with de novo IL-33 protein synthesis or a loss of cell viability (Extended Data Fig. 5b,c). Other HDM-induced mediators such as IL-6 and IL-8 remained unchanged by loss of SAA1 expression (Extended Data Fig. 5d–f). Further, and consistent with the ability to interact with SAA1, out of several major and minor mite allergens tested, only Blo t 13 induced IL-33 release in BEAS-2B cells, which was dependent on SAA1 (Fig. 3e,f). In addition, the major allergens from cat dander (Fel d 1) and birch pollen (Bet v 1) failed to induce IL-33 release (Fig. 3e). HDM is a complex mixture of many allergenic proteins, but not all might have IL-33-releasing ability. To more directly test the HDM FABP homologue, Der p 13, and its individual contribution to HDM-induced IL-33 release, we utilized two complementary approaches. Monoclonal antibody-mediated neutralization or depletion of Der p 13 in HDM extract decreased IL-33 to near baseline amounts in BEAS 2B cells (Fig. 3g) but did not affect other HDM-induced cytokines such as IL-6 (Extended Data Fig. 5g). Similarly, BALB/c mice challenged with Der p 13-depleted HDM extract showed significantly reduced

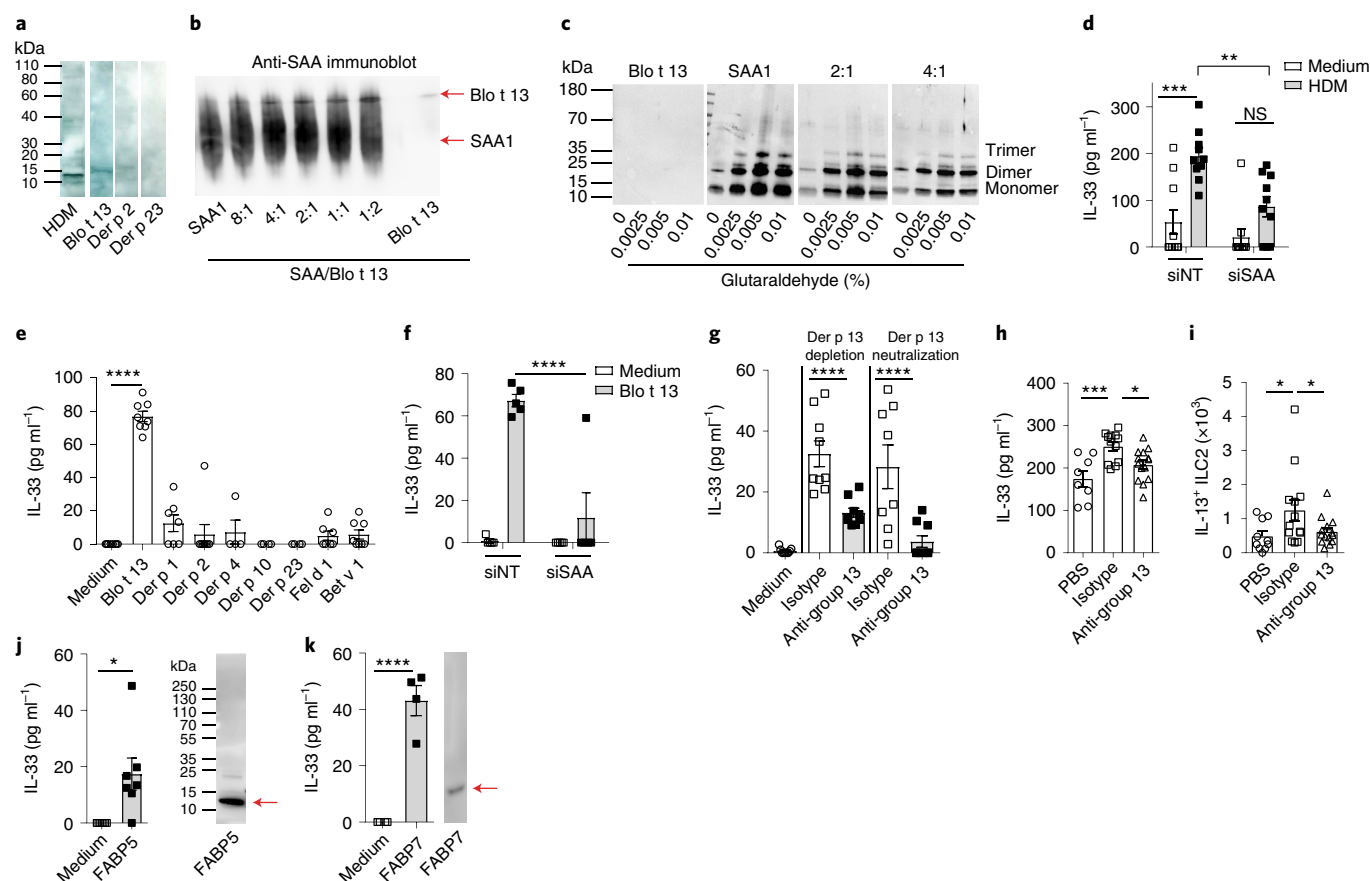


Fig. 3 | SAA1 is a soluble pattern recognition molecule for mite FABPs. **a**, HDM, rBlo t 13, rDer p 2 or rDer p 23 were separated by SDS-PAGE followed by detection with rSAA1 and a mouse monoclonal antibody specific for human SAA1. **b**, Migration of SAA1 (1 mg ml⁻¹) in PBS was analyzed in the presence of increasing amounts of the mite FABP Blo t 13 (4:1 (0.25 mg ml⁻¹), 2:1 (0.5 mg ml⁻¹), 1:1 (1 mg ml⁻¹) and 1:2 (2 mg ml⁻¹) of Blo t 13) by native PAGE followed by immunoblot analysis using mouse monoclonal antibody specific for human SAA1 (R&D Systems; MAB30196). **c**, SAA1 (1 mg ml⁻¹) was chemically crosslinked in the presence of Blo t 13 added at a ratio of 1:2 or 1:4 (as indicated) using 0.0025% and 0.01% glutaraldehyde and analyzed by immunoblot using sequence-specific (amino acids 14–30) rabbit antiserum for human SAA1 (densitometric analysis is shown in Supplementary Table 1). **d**, HDM-induced IL-33 concentrations in BEAS-2B cells after siRNA-mediated silencing of SAA1 (siSAA1) or transfection with non-targeting scrambled siRNA (siNT) (**P = 0.0046; ***P = 0.0002, not significant (NS) = 0.1911). **e**, IL-33 amounts induced by individual mite or unrelated major cat (Fel d 1) and birch pollen (Bet v 1) allergens (10 µg ml⁻¹). **f**, Blo t 13-induced IL-33 in BEAS-2B cells with siRNA-mediated silencing of SAA1 (siSAA1) or transfected with non-targeting scrambled siRNA (siNT). **g**, IL-33 amounts induced by Der p 13-depleted HDM extract (left) and HDM extract where Der p 13 was neutralized (100 µg ml⁻¹; right). In both cases, a monoclonal antibody specific for mite group 13 allergens was used. **h**, BAL IL-33 levels (*P = 0.0209, ***P = 0.006; **h**) and number of Lin⁺CD45⁺ST2⁺IL-13⁺ ILC2 cells (**i**) in the lungs of WT BALB/c mice receiving a single intratracheal (i.t.) challenge with 100 µg of Der p 13-depleted HDM extract (anti-group 13) as compared to isotype-treated control extract (isotype; *P = 0.0423). **j**, IL-33 amounts induced in the BEAS-2B cell line (left panels) and SAA1 binding (right panels) by the human FABPs FABP5 (*P = 0.0207; **j**) and FABP7 (**k**). Cropped images are shown. The data are shown as means ± s.e.m. and are pooled data from 2 (**d**, **e**, **g**) or 3 (**h**, **i**) independent experiments or representative of 2 (**k**) or 3 independent experiments (**f**) each containing at least *n* = 4 biologically independent samples or *n* = 8 PBS, *n* = 12 HDM-isotype and *n* = 14 HDM-anti-group 13 (**h**) or *n* = 10 PBS, *n* = 13 HDM-isotype and *n* = 14 HDM-anti-group 13 (**i**) animals per group. The immunoblots are representative of an experimental *n* = 2 (**a**–**c**, **j**, **k**). The *P* values were calculated with a two-tailed test using one-way ANOVA with Tukey's multiple comparisons test (**d**, **e**, **f**), Dunnett's (**h**) or Holm-Sidak's (**i**) post hoc analysis, or a two-tailed Student's *t*-test with Welch's correction (**g**, **j**, **k**). *****P* ≤ 0.0001.

BAL IL-33 concentrations and lower numbers of IL-13⁺ ILC2 cells in the lungs as compared to mice receiving isotype-treated extract (Fig. 3*h*, *i*). Der p 13 not only contributed to acute airway inflammation but also critically modulated the magnitude of the allergic phenotype in the full asthma protocol (Extended Data Fig. 1*b* and 6). These results show that SAA1 was necessary for HDM-driven epithelial IL-33 release. Blo t 13 and Der p 13 were sufficient to recapitulate the effects of HDM on IL-33 release, which supports our hypothesis that arthropod group 13 allergens are sensed by the SAA1 innate immune recognition pathways to induce a strong type 2 response at mucosal surfaces.

Conserved surface residues are shared among not only mite homologues but also other FABPs (for example, from humans),

which have received attention lately as potential regulators of allergic airway inflammation³¹. Like group 13 allergens, human epidermal and brain FABPs, FABP5 and FABP7, respectively, triggered IL-33 release in BEAS-2B cells and were recognized by SAA1 in immunoblots (Fig. 3*j*, *k*). Cytoplasmic FABPs have been also identified in excretory/secretory products of a variety of parasites including *Schistosoma mansoni*³². The adult stages of *S. mansoni* release FABP-containing exosome-like particles into host tissues to acquire essential fatty acids for their survival³³. We found that *Saa*^{-/-} mice showed impaired lung inflammatory responses following exposure to an extract of adult *S. mansoni* worms containing the *S. mansoni* FABP, Sm14 (ref. 32) (Extended Data Fig. 1*f*), associated with significantly lower numbers of CD11b⁺ DCs in the lungs, reduced effector

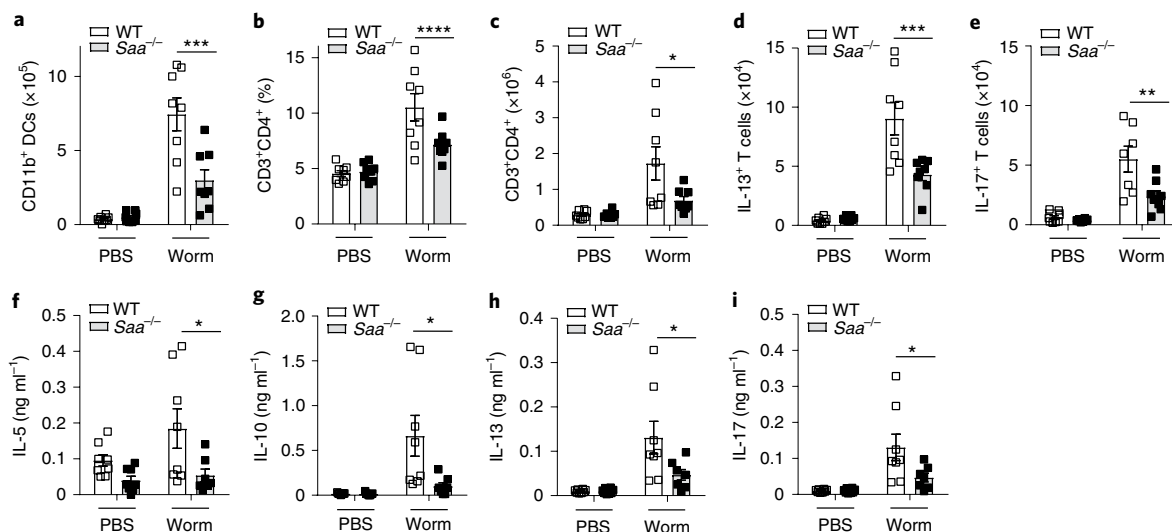


Fig. 4 | T_H2 skewing triggered by *S. mansoni* worm extract is impaired in *Saa*^{-/-} mice. The parasitic worm *S. mansoni* (a Puerto Rican isolate) is routinely maintained in the laboratory by cycling between the intermediate snail host, *Biomphalaria glabrata*, and outbred ICR mice as definitive hosts. **a**, Numbers of CD11b⁺ DCs in the lungs of WT and *Saa*^{-/-} C57BL/6 mice treated with PBS or *S. mansoni* worm extract ($***P=0.0002$). **b,c**, Frequency (**b**) and total numbers (**c**) of CD3⁺CD4⁺ T cells ($*P=0.014$). **d,e**, T_H2 ($***P=0.0003$; **d**) and T_H17 ($**P=0.0014$; **e**) cells in the lungs of these mice. **f-i**, Cytokine production from lung cells restimulated with worm extract (IL-5: $*P=0.019$, IL-10: $*P=0.011$, IL-13: $*P=0.017$, IL-17: $*P=0.017$). The data represent means $\pm$ s.e.m. of pooled data from 2 independent experiments containing a total of $n=8$ animals in each group. The P values were calculated with a two-tailed test using one-way ANOVA with Dunnett's post hoc analysis that compares WT worm to counterparts. $****P\leq 0.0001$.

T cell expansion and impaired T_H cytokine secretion (Fig. 4). Our data indicate specificity of SAA1 for FABPs and provide evidence of a broader context of the SAA1–FABP interaction in regulating IL-33 release that might have implications for other chronic inflammatory diseases associated with increased SAA1 concentrations or helminth infections.

SAA1 dissociation initiates the release of IL-33 from AECs. SAA1 can adopt multiple conformations (monomer, dimer and hexamer) through a number of hydrogen-bonding interactions between the C-terminal tail (amino acids 89–104) and the α -helix bundles¹⁵ (Fig. 5a). These interacting residues contribute to the overall protein stability and generate numerous surface structures to bind antigenic ligands and cell surface receptors. Hence, we hypothesized that the structure of SAA1 might be related to its ability to interact with allergens and drive type 2 responses.

Indeed, we found that HDM-triggered SAA1 functions were not regulated by de novo protein translation and/or secretion (Extended Data Fig. 7a,b). Rather, untreated BEAS-2B cells secreted SAA1 as a lipid-free oligomer of a calculated molecular weight between 60 and 80 kDa into the supernatant (Fig. 5b, medium) that likely corresponds to a SAA1 hexamer as described previously^{15,16}. Within minutes after HDM treatment, hexameric SAA1 dissociated into dimers and monomers (Fig. 5b, HDM), suggesting that IL-33 release is associated with SAA1 dissociation. Similarly, the mite FABP Blo t 13 triggered a concentration-dependent decrease in SAA1 hexamer that was associated with increasing IL-33 concentrations (Extended Data Fig. 7c–e). Furthermore, when using lipid-free rSAA1, increased IL-33 concentration could be detected only in the presence of dissociated forms of SAA1. While SAA1 at $0.1\text{--}1\text{ }\mu\text{g ml}^{-1}$ exclusively separated as a hexamer and induced only very low amounts of IL-33 (Fig. 5c,d), higher SAA1 concentrations allowed the recovery of dimers and monomers along with increased IL-33 concentrations. In this setting, SAA1 hexamer might be stabilized by binding of lipids (for example, retinoic acid) contained in the cell culture medium³⁴. Increasing the SAA1 concentration, however, could limit the availability of stabilizing lipids, resulting in

hexamer dissociation and the exposure of IL-33-triggering epitopes. Complexing rSAA1 to anti-SAA synergistically enhanced the release of IL-33, suggesting that binding might favor SAA1 monomers by preventing formation of lipid-stabilized hexamers. This indicates a structure–function relationship of SAA1 that depends on ligand interaction to activate its type 2-promoting effects. Differences in the quaternary structure might thus also help explain the pleiotropic physiological functions of SAA1 and the presence of multiple SAA receptors¹².

Notably, antisera to amino acids 40–63 and 68–84 of SAA1 (ref. 30) induced a robust IL-33 release from BEAS-2B cells (Fig. 5e). These surface patches are involved in SAA hexamerization and the binding of lipids via the hydrophobic pocket^{15,16,34}, which contribute to the overall stability of hexameric SAA. Perturbing this stability through binding of FABPs might facilitate SAA1 dissociation and the amplification of IL-33 release. Consistent with this assumption, introducing a tryptophan to alanine substitution at position 53 of the amino-acid sequence (W53A) to mutate the hydrophobic core of human SAA1 resulted in significantly higher IL-33 amounts after HDM stimulation in cells overexpressing SAA1(W53A) as compared to WT SAA1 (Fig. 5f). Similarly, ECs that had been grown in medium depleted of lipophilic molecules³⁴ showed higher IL-33 release in response to HDM (Fig. 5g) compared to cells maintained in non-depleted medium. These findings provide a potential structural basis for the type 2 immunity-promoting effects of SAA1.

SAA1 signals via FPR2 to induce type 2 immune responses. We next asked which of the commonly known SAA1 receptors (FPR2, TLR4, CD36 and P2YR) were involved in mediating IL-33 release. Both FPR2 and TLR4 significantly contributed to the HDM-induced IL-33 release (Fig. 6a,b). However, when BEAS-2B cells overexpressed SAA1, blocking of TLR4 with lipopolysaccharide (LPS) from *Rhodobacter sphaeroides* (LPS-RS) could no longer suppress IL-33 release, while HDM-induced IL-33 was still inhibited by WRW4 ($71.5\pm 34.4\%$ inhibition $P<0.0001$), a selective and potent antagonist of FPR2 (Fig. 6c). Moreover, targeting CD36 or P2Y receptors had no inhibitory effect on HDM-triggered IL-33 secretion

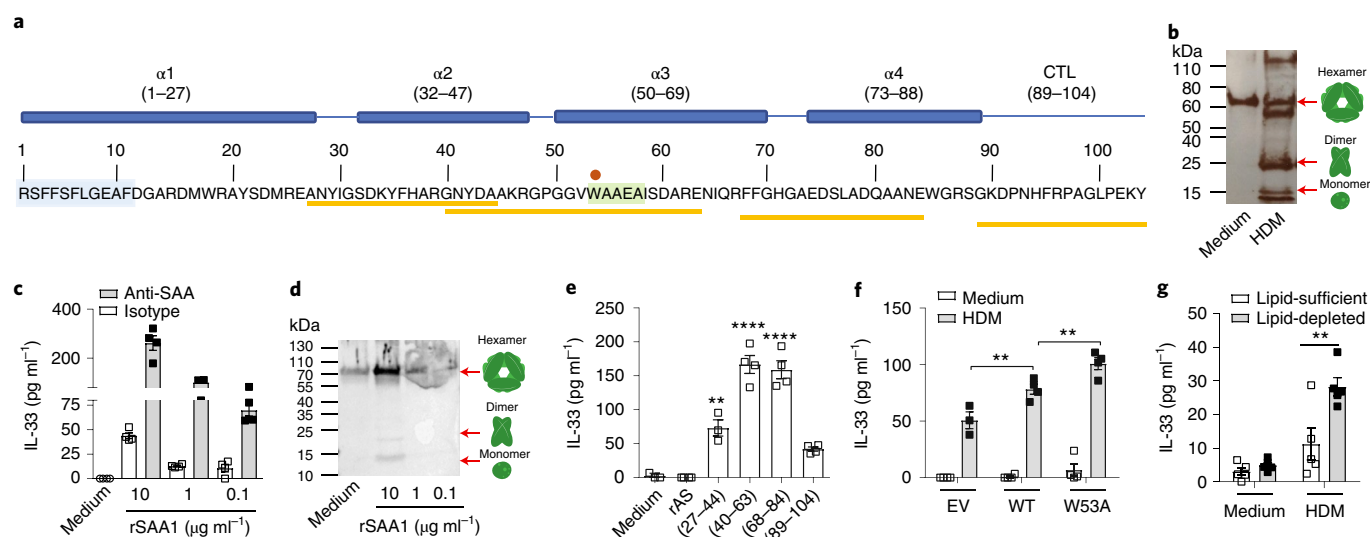


Fig. 5 | HDM-induced IL-33 is dependent on SAA1 dissociation. **a**, The sequence of SAA1. Indicated are: secondary structure α -helices (α 1–4), loops (connecting lines between α -helices), and the C-terminal tail (CTL). Amino acids located within the hydrophobic core of the SAA1 hexamer are shaded in green. The epitopes recognized by affinity-purified IgGs to residues 27–44, 40–63, 68–84 and 89–104 are indicated by yellow lines (used in **e**). The substitution site at the hydrophobic core is indicated by an orange dot (used in **f**). C-terminal deletion of amino acids (Δ 1–11) is shaded in blue. **b**, BEAS-2B supernatants were cleared of lipid-bound SAA1 by pulling down HDL-bound SAA1 using polyclonal goat anti-ApoA1. Cleared supernatants were immunoblotted with monoclonal mouse anti-human SAA1 (Acris). **c**, IL-33 secretion induced by rSAA1 alone (open bars) or in complex with a mouse monoclonal antibody specific for human SAA1 (anti-SAA, filled bars) in the BEAS-2B cell line. **d**, Supernatants of cells either left untreated (medium) or stimulated with rSAA and immunoblotted with a monoclonal antibody specific for human SAA (R&D Systems; MAB30196). **e**, IL-33 release from BEAS-2B cells after incubation with sequence-specific rabbit antiserum specific for human SAA1 (** $P=0.0029$). **f**, HDM-triggered IL-33 release in BEAS-2B cells transfected with empty plasmid control (EV), WT SAA1 overexpression plasmid or a SAA1 plasmid with a W53A substitution to mutate the hydrophobic core of SAA1 (WT to EV ** $P=0.0032$; WT to W53A ** $P=0.0085$). **g**, HDM-induced IL-33 amounts in ECs grown in medium supplemented with charcoal-stripped fetal bovine serum albumin (FBS; ** $P=0.0027$). Cropped immunoblot images are shown. The data are shown as means $\pm$ s.e.m. and are representative of 2 independent experiments (**c,d,f,g**) or 3 (**e**), each containing at least $n=4$ biologically independent samples. The blots are representative of 2 (**d**) and 4 (**b**) independent experiments. The P values were calculated with a two-tailed test using one-way ANOVA with Tukey's multiple comparisons test (**e,g**) or Dunnett's post hoc analysis (**f**). **** $P\leq 0.0001$.

from BEAS-2B cells, while inhibition of TLR2 induced only a marginal reduction in IL-33 (Fig. 6d–f). Consistent with these findings, when we overexpressed an amino-terminal-truncated form of SAA1 (Δ 1–11) that shows reduced avidity for FPR2 (ref. 35), the IL-33-enhancing capability of WT SAA1 was lost (Fig. 6g). These results suggested that FPR2 is the dominant receptor mediating SAA1-induced IL-33 release in the airway epithelium.

Three closely related FPR isoforms have been identified in humans: FPR1–3. While *FPR1* and *FPR2* showed comparable messenger RNA expression in BEAS-2B cells after HDM stimulation, *FPR3* mRNA abundance was very low (Extended Data Fig. 8a). Its predominant intracellular distribution³⁶ also makes FPR3 an unlikely target for HDM-induced IL-33 release. While FPR1 overexpression significantly decreased IL-33 release after HDM exposure, FPR2 overexpression enhanced IL-33 release as expected (Extended Data Fig. 8b,c). This suggests that overexpression of FPR1 could override type 2 immunity-promoting signals delivered through FPR2 (ref. 37). The effects of FPR2 gain or loss of function were highly selective for IL-33, as the concentrations of other HDM-triggered cytokines such as IL-6 or IL-8 were either not or only minimally modulated (Extended Data Fig. 8d–g).

To further demonstrate that the SAA1–FPR2 axis is critical for HDM-induced responses, we blocked FPR2 signaling in vivo in BALB/c mice using WRW4. Similar to SAA1 deficiency, local delivery of WRW4 (Extended Data Fig. 1g,h) improved the pulmonary function in WRW4-treated mice, which was accompanied by reduced HDM-induced IgE synthesis, diminished eosinophilic lung inflammation and reduced mucus production concomitant with decreased HDM-induced *Il5* and *Il13* mRNA expression in

WRW4-treated mice (Fig. 7a–f). WRW4-treated mice also showed significantly reduced IL-33 BAL concentrations, reduced lung IL-13⁺ ILC2 cell numbers and reduced IL-13 released into the BAL (Fig. 7g–i) in an innate model of HDM exposure as compared to mice treated with HDM alone. Importantly, the SAA1–FPR2 axis seemed to be specific for the recognition of allergenic FABPs, as an extract of *Alternaria alternata*, a mold causing respiratory allergy, had no effect on SAA1 hexamer dissociation in EC culture supernatants and the *A. alternata*-triggered IL-33 release occurred independently of the SAA1–FPR2 signaling axis (Extended Data Fig. 9a–e). Supporting this observation, *Saa* deficiency had no effect on *A. alternata*-induced type 2 responses in vivo (Extended Data Figs. 1i and 9f–j). These data are consistent with recent findings that the IL-33-releasing capacity of *A. alternata* is due to its serine proteolytic cleavage of the protease-activated receptor 2 leading to ATP signaling³⁸.

Dysregulated SAA–FPR2 axis in type 2 allergic diseases in humans. To test whether the SAA1–FPR2 axis was dysregulated in primary human tissue, we examined expression of SAA1 and FPR2 in primary nasal ECs from patients with chronic rhinosinusitis (CRS), who are commonly sensitized to HDMs³⁹. We found highly elevated SAA1 and FPR2 expression in ECs from individuals with CRS (Fig. 8a,b and Supplementary Table 2) as compared to cells from controls. Following HDM stimulation, CRS donor nasal ECs (Supplementary Table 3) showed significantly increased IL-33 secretion along with a more robust SAA1 hexamer dissociation as compared to control donor ECs (Fig. 8c–e). Together these data suggested that dysregulated IL-33 release in patients with CRS

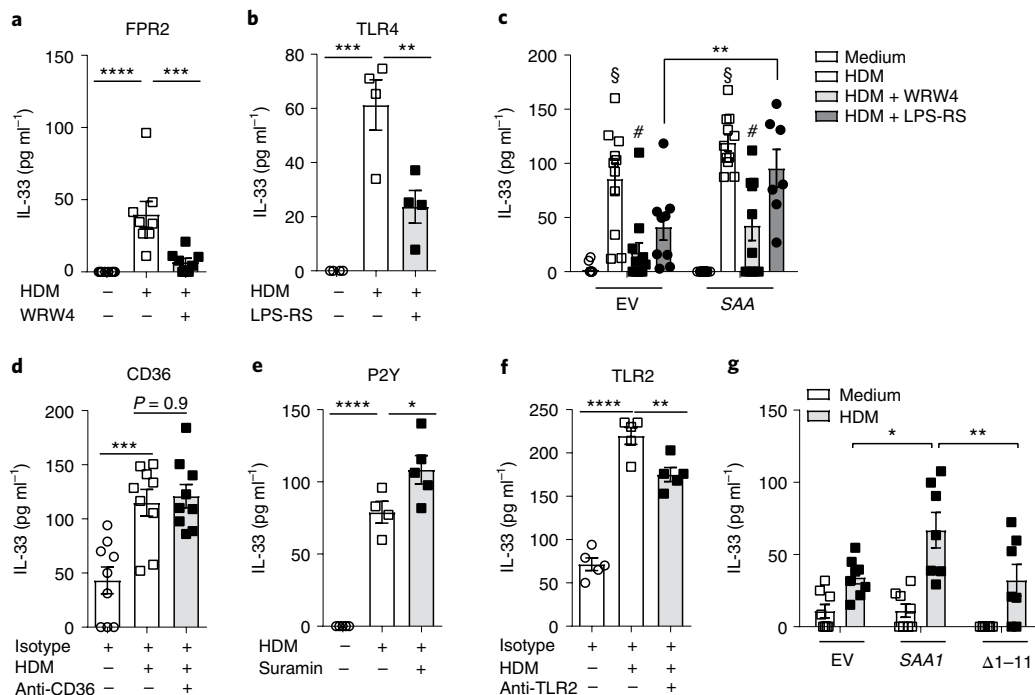


Fig. 6 | SAA1 signals via the FPR2 receptor to induce IL-33. **a,b**, IL-33 concentrations in BEAS-2B cells blocking the SAA-binding receptors FPR2 (WRW4, 12 μ M; *** P =0.0006; **a**) and TLR4 (LPS-RS, 10 μ g ml⁻¹; ** P =0.0046, *** P =0.0002; **b**). **c**, Effects of FPR2 or TLR4 blockade in cells transfected with empty plasmid control (EV) or SAA1 overexpression plasmid. ** P =0.0047, $\$P$ =0.056, # P =0.22 between EV and SAA plasmid. **d-f**, Blockade of CD36 (human CD36-blocking antibody (anti-CD36); 10 μ g ml⁻¹; *** P =0.0005; **d**), the P2 receptor antagonist suramin (100 μ M; * P =0.028; **e**) and TLR2 (anti-human TLR2 IgA, anti-TLR2, 10 μ g ml⁻¹; ** P =0.0055; **f**). **g**, HDM-triggered IL-33 release in BEAS-2B cells transfected with EV, WT SAA1 overexpression plasmid or a SAA1 plasmid with a deletion of the C-terminal amino acids 1–11 (Δ 1–11; * P =0.01, ** P =0.0078). The data are representative of 2–3 independent experiments (**b,d-f**) or pooled data from 2 independent experiments (**a,c,d,g**) each containing at least n =4 biologically independent samples and are depicted as means $\pm$ s.e.m. The P values were calculated with a two-tailed test using one-way ANOVA with Dunnett's (**a,b,d-g**) or Tukey's (**c**) post hoc analysis. **** P ≤0.0001.

might result from aberrant SAA1 dissociation. A similar trend of upregulated *SAA1* and *FPR2* expression was observed in primary bronchial ECs from asthmatics (Extended Data Fig. 10a,b and Supplementary Table 4). Furthermore, concentrations of circulating SAA1 were higher in the serum of patients with a HDM allergy (Fig. 8f and Supplementary Table 5). These results support a role for a dysregulated SAA1–FPR2 axis in allergic diseases.

Discussion

Pattern recognition at mucosal surfaces is essential to maintain tissue homeostasis. However, when dysregulated, innate sensing of otherwise harmless environmental proteins causes an acute innate immune response favoring allergic sensitization and type 2 inflammation in the lungs^{3,4}. Given the different classes of allergens that can elicit allergic responses, multiple unique innate allergen recognition mechanisms have likely evolved for their detection.

Our studies identify a previously unrecognized mechanism of allergen sensing by SAA1 that initiates type 2 immunity through the release of IL-33, which is among the earliest cytokines secreted and has been reproducibly associated with asthma susceptibility in several genome-wide association study. Our results using *Saa*^{-/-} mice or blocking SAA1-triggered signaling pathways indicated that, in the absence of SAA, the early release of IL-33 and the first wave of ILC2-derived IL-13 in response to initial allergen sensitization were decreased compared with WT or control mice. These mice were also resistant to the development of AHR, concomitant with substantial reductions in all of the hallmarks of allergic asthma. Although SAA1 is known to bind several receptors, we demonstrate that its interaction with FPR2 specifically, leads to airway epithelial

IL-33 release, and the development of allergic inflammation in vivo. IL-33 also recruits and activates DCs that skew naive CD4⁺ T cell differentiation towards a T_H2 phenotype^{40,41}. The decreased frequency of CD11b⁺CD11c⁺MHCII^{hi} DCs in the lungs and impaired T_H2 responses in the *Saa*^{-/-} mice likely result from both impaired IL-33 activation of DCs and lack of ILC2-derived IL-13 to support proper induction and maintenance of T_H2 responses^{41,42}. These are likely to be additive effects. In summary, our findings broaden our understanding of how certain aeroallergens such as mite FABPs are sensed by the airway epithelium and reveal a role for local SAA1 in the initiation and maintenance of type 2 immune responses acting upstream of the IL-33–ILC2–IL-13 axis.

SAA1 has pleiotropic effects on the immune system, illustrating the need for tight molecular regulation of this protein. In addition to liver-derived SAA, basal expression of SAA proteins has been detected in several mucosal tissues including the lung⁶. Although the exact mechanisms of SAA1 regulation at these tissue sites are not well understood, the ability of SAA1 to adopt different oligomeric states may have important functional implications for promoting local inflammation^{15,16,34,43,44}. Our results show that SAA1 produced by the airway epithelium is not activated via upregulation of its expression or release, but through dust mite allergen-driven dissociation of 'low-activity' SAA1 oligomers. This was associated with active, non-cytolytic IL-33 release from AECs. In support of this hypothesis and in agreement with other studies^{45,46}, elevated amounts of SAA1 per se were not sufficient to trigger type 2 immune responses (for example, IL-33 release and higher numbers of ILC2 cells), but the presence of HDM causing its dissociation was necessary to fully activate its type 2 immunity-promoting effects.

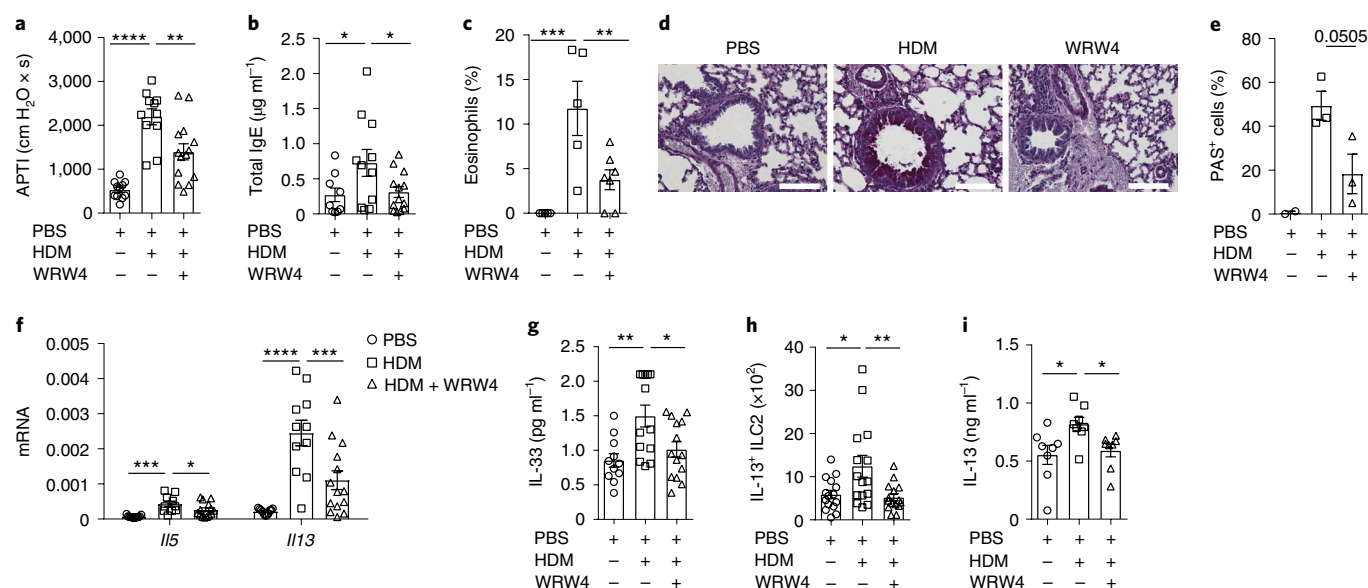


Fig. 7 | The FPR2 axis controls sensitization to HDM by regulating IL-33-mediated ILC2 cell activation and Th2 cytokine production. **a**, Airway responses to cholinergic stimulation shown as APTI measured on day 17 in PBS-, HDM- or HDM + WRW4-treated (4 mg per kg body weight (mg/kg)) WT BALB/c mice ($^{**}P=0.0022$). **b**, Total IgE serum concentrations of treated mice (HDM to PBS $^{*}P=0.0483$; HDM to WRW4 $^{*}P=0.0486$). **c**, Eosinophilic infiltration into the lungs ($^{**}P=0.0094$ and $^{***}P=0.0009$). **d,e**, Histological examination of airway inflammation. Sections were stained for mucus production with PAS (scale bars, 100 μ m). **f**, Lung expression of *Il5* ($^{*}P=0.0421$ and $^{***}P=0.0002$) and *Il13* ($^{***}P=0.0009$) mRNA. **g-i**, BAL IL-33 ($^{*}P=0.0175$ and $^{**}P=0.0029$; **g**), frequency of Lin⁺CD45⁺ST2⁺IL-13⁺ ILC2 cells ($^{*}P=0.0125$ and $^{**}P=0.0058$; **h**) and BAL IL-13 (HDM to PBS $^{*}P=0.0169$; HDM to WRW4 $^{*}P=0.0398$; **i**) in the lung 24 h after a single i.t. challenge with PBS, HDM (100 μ g) or HDM in combination with the FPR2 inhibitory peptide WRW4 (4 mg/kg). The data represent the means $\pm$ s.e.m. of pooled data from 2 experiments containing $n=11$ PBS, $n=11$ HDM and $n=14$ HDM + WRW4 animals per group (**a,b,f**); $n=2$ PBS, $n=3$ HDM and $n=3$ HDM + WRW4 animals per group (**d,e**); $n=11$ PBS, $n=13$ HDM and $n=14$ HDM + WRW4 animals per group (**g**); $n=15$ PBS, $n=15$ HDM and $n=15$ HDM + WRW4 animals per group (**h**); or are representative of 2 (**c**) or 3 (**i**) independent experiments with $n=5$ PBS, $n=5$ HDM and $n=7$ HDM + WRW4 animals per group (**c**) and $n=8$ animals in each group (**i**), respectively. The P values were calculated with a two-tailed test using one-way ANOVA with Dunnett's post hoc (**a-c,e,g-i**) or Holm-Sidak's (**f**) post hoc analysis that compares HDM to PBS and WRW4 counterparts. $^{***}P\leq 0.0001$.

Complexing rSAA1 to anti-SAA1, which likely maintains SAA1 in its dissociated form, greatly enhanced its IL-33-releasing activity. These data support our contention that only SAA1 present in its dissociated form is able to drive type 2 immunity.

Our finding that changes in the oligomeric organization of SAA1 are important to unveil its full bioactivity is consistent with our understanding of the need for tight control of other acute-phase proteins found in high quantities in serum (for example, complement components and pentraxin family members, such as C-reactive protein). The mechanisms whereby SAA1 oligomers, which are relatively unstable in vitro^{16,44}, gain stability in vivo are likely heterogeneous. A common feature seems to be the association with small, nonpolar molecules via the hydrophobic pocket of SAA1 (ref.³⁴). Previously, monomeric SAA1 was identified as a sPRR that can directly target Gram-negative bacteria through the bacterial ligand OmpA (ref.⁹). One could speculate that this 'fatty acid binding sink' evolved to inhibit pathogen growth at mucosal surfaces through sequestration of essential nutrients. Notably, the β -barrel structure of OmpA is related to the structurally conserved family of cytosolic FABPs that are present in a spectrum of species including humans and have also been found as allergens in arthropods (mite FABPs)^{29,47}. Indeed, when group 13 allergens were depleted from HDM extracts, the IL-33/ILC2-inducing activity of HDM was abolished. Consistent with our findings, other HDM allergens do not induce IL-33 protein release from ECs, but might enhance this process by proteolytic processing of IL-33 into highly active, shorter mature forms^{24,48}. Therefore, mite FABPs appear to be the main regulators of IL-33 release and initiation of innate type 2 immunity at mucosal surfaces. The requirement for allergenic components such as mite FABPs likely explains why no

enhancement of type 2 immunity was observed in the study by Ather et al.¹⁸ in which mice sensitized to ovalbumin (a non-FABP protein) in the presence of rSAA showed elevations in IL-17A (ref.⁴⁹). Similarly, in the context of colonization with single filamentous bacteria, SAA1 drives Th17 immune responses. SAA-induced Th17 responses have been linked to TLR2 activation and it is currently unknown whether SAA is dissociated in this scenario.

Our discovery that the lung type 2 immune response can be activated by sensing of allergenic FABPs might have implications beyond the context of asthma in the setting of tissue injury or helminth infection. While a FABP-containing *S. mansoni* extract induced type-2-polarized immune responses in WT mice, *Saa*^{-/-} mice were protected. Helminth FABPs show the typical β -barrel fold previously resolved for other FABP homologues, with the *Schistosoma* FABP being partially similar to that of the mite FABP Blo t 13 (refs.^{50,51}). While SAA1 sensing of parasitic or endogenous FABPs is likely advantageous to ward off attack by fellow parasites and initiate an immediate tissue repair process, we found that the SAA1-FPR2 pathway was chronically upregulated in respiratory ECs from patients with asthma or CRS. HDM stimulation further enhanced this signaling pathway through increased SAA dissociation. This provides evidence that the elevated SAA amounts may be linked to HDM sensitization and the hyperactivated IL-33-ILC2 axis previously reported in these patients^{39,52,53}. Interestingly, proteins encoded by different alleles of SAA1 differ in two amino acids within the ligand-binding hydrophobic pocket³⁴, which may contribute to variations in stability/biological activity and receptor specificity. In fact, SAA1 allelic isoforms have altered PRR selectivity⁵⁴, suggesting that differential allele usage may explain the

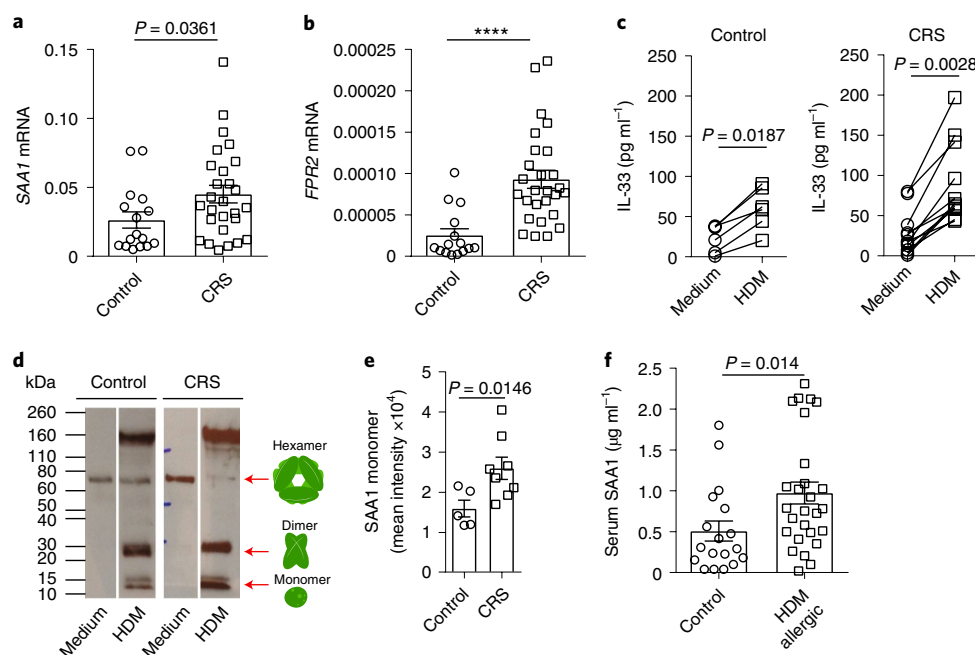


Fig. 8 | Epithelial SAA1 dysregulation in human type 2 immune responses. **a,b**, Basal SAA1 (**a**) and FPR2 (**b**) mRNA expression in nasal ECs of patients with CRS and matched control donors. **c**, HDM (100 µg ml⁻¹)-triggered IL-33 concentration in primary nasal ECs of patients with CRS (right) as compared to controls (left). **d**, Dissociation of hexameric SAA was analyzed by separating lipid-free and lipid-bound SAA by HDL pulldown using a polyclonal goat antibody specific for human ApoA1 and immunoblotting with a monoclonal mouse antibody specific for human SAA1 (Acris). **e**, A bar graph representing quantitative analysis of SAA monomer band intensities (LI-COR Image Studio Software). **f**, SAA1 protein amounts in sera of control donors and individuals with a HDM allergy. The data represent the means ± s.e.m. of *n* = 16 controls and *n* = 27 patients with CRS (**a,b**); *n* = 6 controls and *n* = 12 patients with CRS per group (**c**); *n* = 5 controls and *n* = 8 patients with CRS per group (**e**); and *n* = 18 controls and *n* = 27 patients with a HDM allergy per group (**f**). A representative immunoblot of one control and one patient with CRS (**d**). Cropped images are shown. The *P* values were calculated with a two-tailed test using Student's *t*-test with Welch's correction. *****P* ≤ 0.0001.

enhanced SAA1–IL-33 responses in the presence of HDM in individuals with allergies.

Collectively, we report that, at mucosal surfaces, SAA1 exists in physiologically and functionally different states. An encounter with allergenic FAPBs such as the group 13 mite allergens triggers the dissociation of a 'low-activity' SAA1 hexamer into monomers with pro-type 2 signaling capacity that initiate type 2 immune responses and asthma pathogenesis. This pathway that evolved to recognize either pathogens or endogenous danger signals released following cellular stress or damage may have additional clinical implications. Our results suggest that, in spite of the low specific IgE-binding frequency in individuals with allergies, FAPBs might have sustained and widely distributed lung inflammatory effects in exposed individuals. Initiation of type 2 immune responses is a critical first step in the development of an allergic response upstream of IgE production and allergen–IgE binding. Recognition by PRRs and initiation of innate immune responses are similarly important properties of allergens that should be included as novel molecular biomarkers. This classification should help inform a more precise endotype-driven asthma treatment⁵⁵ and provide the basis for the design of preventive and/or therapeutic anti-inflammatory agents to effectively block allergen recognition and the ensuing inflammatory cascade. The link between SAA1 activation and the IL-33–IL-13 axis may also provide insights into the underlying pathogenesis of a plethora of chronic inflammatory diseases in which SAA1 plays an important pathogenic role including atherosclerosis, rheumatoid arthritis, Alzheimer's or Crohn's disease.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information,

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Methods

Mice. BALB/c mice were originally obtained from Taconic and bred in-house at the Bloomberg School of Public Health animal facility. *Saa^{-/-}* mice (lacking expression of SAA1.1 and its less abundant isoform SAA2.1)⁴⁰ on the C57BL/6 background were a gift from M. C. de Beer (University of Kentucky Medical Center, KY, US). Mice were housed in a specific pathogen-free animal facility in micro-isolator cages. For all experiments, sex- and age-matched mice were used. All in vivo experiments were approved by the Institutional Animal Use and Care Committee at Johns Hopkins Bloomberg School of Public Health and the Medical University of Vienna (BMFWF-66.009/0380-WF/V/3b/2017). All mice used for *S. mansoni* egg isolation were maintained by certified personal (certificate number CZ 02627) in accredited laboratories of the Institute of Immunology and Microbiology of the First Faculty of Medicine, Charles University and the General University Hospital in Prague (accreditation number 8615/2019-MZE-17214). *S. mansoni* (a Puerto Rican isolate) is routinely maintained in the laboratory under approval number MSMT - 7063/2017-2 for *S. mansoni* laboratory life-cycle maintenance by cycling between the intermediate snail host, *B. glabrata*, and outbred ICR mice as definitive hosts according to protocols published previously⁵⁶. Briefly, mice were euthanized six weeks post infection by an intra-peritoneal injection of ketamine (Narkamon 5%; 1.2 ml/kg body weight) and xylazine (Rometar 2%; 0.6 ml/kg body weight) containing heparin (200 U ml⁻¹). Mice were dissected by opening of the abdominal and thoracic cavity. Adult female and male worms were recovered from the mice by perfusion via the hepatic portal vein. Collected worms were repeatedly washed in RPMI medium before homogenization. Extracts were sterile-filtered before use.

Treatment protocols. Allergic airway inflammation was induced in 6–8-week-old WT C57BL/6 or *Saa^{-/-}* C57BL/6 mice following allergen sensitization on day 0 with HDM, *A. alternata* or worm extract (1 µg in 30 µl of PBS) i.t. Mice, anesthetized using sevoflurane (AbbVie), were subsequently challenged intranasally with 10 µg of HDM, *A. alternata* or worm extract in 30 µl of PBS on days 7–11 and mice were euthanized on day 11. Control mice received PBS in all experiments at the time of sensitization and the challenge phase. In receptor blockade experiments, WT BALB/c mice were treated with HDM (100 µg) or HDM in combination with WRW4 (4 mg/kg mouse) or vehicle control (DMSO) in a total of 40 µl i.t. In early time-point experiments, mice received a single HDM dose or HDM plus WRW4 and were euthanized as indicated. For in vivo SAA1 neutralization, WT BALB/c mice were exposed i.t. to PBS, HDM (100 µg) or HDM in combination with goat anti-mouse SAA1 (R&D Systems, 5 µg per mouse) and were euthanized as indicated. Normal goat IgG was used as an isotype control (BioXcell). For the in vivo overexpression of SAA1, a SAA1 expression plasmid was complexed to polyethylenimine (PEI; In vivo-JetPEI) and administered intravenously to mice. Successful transfection was monitored by quantitative real-time PCR with reverse transcription and secreted SAA1 protein in the BAL. Control mice were transfected with an empty vector control. Mice were exposed i.t. to PBS or HDM 48 h after initial installation of plasmid DNA.

Assessment of allergen-induced AHR. To evaluate airway responses, mice were anesthetized by intraperitoneal administration of ketamine (100 mg/kg)/xylazine (5 mg/kg), and paralyzed with decamethonium bromide (25 mg/kg; Sigma-Aldrich), intubated and respiration at a rate of 120 breaths min⁻¹ with a constant tidal volume (0.2 ml) 72 h after final allergen challenge. Methacholine (30 mg ml⁻¹ for BALB/c) and 200 mg ml⁻¹ for C57BL/6; Sigma-Aldrich) was injected into the inferior vena cava or delivered by nebulization and dynamic airway pressure (cm H₂O × s) was followed for 5 min. Serum and BAL fluid were collected, processed and analyzed as described previously⁵⁷. In brief, blood was collected to measure total serum IgE concentrations. To collect BAL fluid, lungs were lavaged with Hank's balanced salt solution (Invitrogen). Cells were recovered by centrifugation and total cells were counted (trypan blue exclusion). Slides were prepared and stained with Siemens Diff-Quik stain (Siemens Healthcare) for differential cell counts.

Lung cell isolation and in vitro culture. Murine lungs were excised, minced and digested in serum-free RPMI containing Liberase CI (0.5 mg ml⁻¹, Roche) and DNase I (0.5 mg ml⁻¹, Sigma) at 37 °C for 45 min. Cells were washed with RPMI containing 10% (v/v) FBS and viable cells were counted by trypan blue exclusion. For HDM-specific restimulation, whole lung cells were cultured at 250,000 cells per well of a 96-well plate (250 µl final volume) with HDM (30 µg ml⁻¹) and cell culture supernatants were collected for cytokine measurements 72 h later.

FACS analysis and cell sorting. All staining reactions were performed at 4 °C following incubation with FcBlock (TruStain fcX antibody, BioLegend) for 30 min. Dead cells were excluded using the fixable viability dye Zombie Aqua (BioLegend). Monocytes (CD11b^{hi}CD11c^{hi}Ly6G^{hi}Ly6C^{hi}), neutrophils (CD11b^{hi}Ly6G⁺), CD11b⁺ DCs (CD11c⁺CD11b⁺Ly6G⁺MHC^{hi}), alveolar macrophages (CD11c⁺CD11b^{lo}CD317⁺Ly6C⁺SSC^{hi}), plasmacytoid DCs (CD11c⁺CD11b⁺CD317⁺Ly6C⁺) and CD103⁺ DCs (CD11c⁺CD11b⁺CD103⁺) were quantified using anti-CD11c–Alexa Fluor 700 (HL3; BD Pharmingen) or anti-CD11c–PE–Cy7 (HL3; BD Pharmingen), anti-CD11b–PE–Cy7 (M1/70; BD Pharmingen)

or anti-CD11b–APC–Cy7 (M1/70; BioLegend), and anti-Ly6C–Pacific Blue (HK1.4, BioLegend), anti-Ly6G–APC–Cy7 (1A8, BioLegend), anti-MHCII–PE (M5/114.15.2, BioLegend), anti-CD103–PerCP–Cy5.5 (2E7) or anti-CD317–APC (eBio129c). CD3e (145-2C11; BD Pharmingen) and CD4 (RM4-5; eBioscience) staining was used to identify T cells. For measurement of intracellular cytokines, cells were stimulated with phorbol 12-myristate 13-acetate (PMA; 100 ng ml⁻¹) and ionomycin (1 µg ml⁻¹) for 16 h at 37 °C. Monensin and brefeldin A (eBiosciences) were added for the last 4 h of incubation. Cells were fixed, permeabilized and stained with antibodies to IL-13 (eBio13A).

For ILC2 flow, cells were plated at 2 × 10⁶ cells ml⁻¹, and restimulated with PMA/ionomycin overnight as described above before staining with PerCP–Cy5.5 or APC–Cy7-conjugated lineage marker monoclonal antibodies (FcεRI, MAR-1, BioLegend; CD3e, 145-2C11, BD Pharmingen; TCRβ, H57-597, BD Pharmingen; B220, RA3-682, BD Pharmingen; CD49b, DX5, eBioscience; CD11b, M1/70, BD Pharmingen; CD11c, N418; BioLegend; Gr1, RB6-85C, BD Pharmingen; NK1.1, PK136, BioLegend), Alexa Fluor700-conjugated CD45 (30-F11; BD Pharmingen), BV605-conjugated CD90.2 (53-2.1, BD), APC-e780-conjugated CD25 (PC615, eBioscience), PE–Cy7-conjugated ICOS (C398.4A; BioLegend), BV421-conjugated IL-33Rα (DIH9, BioLegend) and FITC-conjugated T1/ST2 (DJ8, MD Bioproducts). After cell surface staining, cells were fixed, permeabilized and stained for intracellular IL-13-PE. Cells were analyzed using an LSRII flow cytometer (BD Biosciences) equipped with three laser lines (405 nm, 488 nm and 633 nm). Compensation of spectral overlap and data analysis were performed using the FACSDiva software (BD Biosciences).

Primary human nasal ECs. Primary human nasal ECs were prepared as described previously⁵⁸. The research protocol was approved through the Johns Hopkins Institutional Review process, and all participants gave signed informed consent. Inclusion criteria included continuous symptoms of rhinosinusitis as defined by the American Academy of Otolaryngology–Head and Neck Surgery Chronic Rhinosinusitis Task Force for greater than 12 weeks, computed tomography of the sinuses revealing isolated or diffuse sinus mucosal thickening or air–fluid levels, and nasal polyps visible on diagnostic endoscopy⁵⁸. Mucosal tissue removed during endoscopic sinus surgery was digested at 4 °C overnight in Ham's F12 medium containing 0.01% protease Sigma type XIV (Sigma) and supplemented with antibiotics (100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, 2.5 µg ml⁻¹ amphotericin B and 50 µg ml⁻¹ gentamicin, Gibco). Digestion in protease was performed, with separation of cells by agitation the following day. Cells were then separated by straining, washed and seeded at a density of ≥ 1.5 × 10⁴ cells cm⁻², onto Vitrogen 100-coated (1:75 in sterile water; Cohesion) P-100 dishes in bronchial epithelium growth medium (BEGM). ECs were then grown at 37 °C for 24 h and washed with Hank's balanced salt solution to remove debris. The medium was then changed every 48 h until the cells reached confluence. Primary sinonasal ECs were grown in BEGM medium. At 80% confluency, cells were trypsinized and seeded into a 96-well culture plate pre-coated with fibronectin–collagen. Cells were starved overnight in medium without bovine pituitary extract at 90% confluency and treated with HDM (200 µg ml⁻¹) for 2 h the following day. The supernatants were collected for IL-33 ELISAs and cells were collected for RNA isolation. The patients' characteristics are listed in Supplementary Tables 2 and 3.

Bronchial EC culture. ECs were isolated from bronchial biopsies of healthy individuals, individuals with mild steroid-naïve asthma and patients with severe asthma (Supplementary Table 4). Individuals were recruited from the Asthma Clinic at Institut Universitaire de Cardiologie et de Pneumologie de Québec (Canada). The ethics committee board approved the study and all participants gave written informed consent. The individuals with asthma were diagnosed according to the American Thoracic Society criteria. Participants with asthma were atopic individuals, and had a positive skin prick test to common aeroallergens, methacholine PC₂₀ (the provocative concentration of methacholine that results in a 20% drop in the forced expiratory volume in 1 s (FEV₁)) of <8 mg ml⁻¹ and FEV₁ > 70% predicted, with only intermittent use of β₂-agonists. Control participants were non-atopic, non-smoking individuals, with no history of asthma or airway and systemic diseases. None of the individuals with asthma had an asthma exacerbation or respiratory tract infection in the preceding four weeks before entry into the study. Bronchial ECs were characterized and cultured as previously described^{59,60}. RNA was extracted from near-confluent cultures.

Sera from patients with a HDM allergy. Approval from the ethics committee of the Medical University of Vienna was obtained for studies with human plasma and informed consent was obtained from the volunteer donors (EK1538/2014). Individuals diagnosed with a HDM allergy were included in this study. The diagnosis of allergy was based on a characteristic history of clinical symptoms (allergic conjunctivitis, rhinitis, urticaria and asthma) and on at least one of the following diagnostic criteria: a positive skin prick test or a positive ImmunoCAP test. The characteristics of the patients are summarized in Supplementary Table 5.

Proteins, expression constructs and cell lines. Extracts of HDM (from *D. pteronyssinus*) and *A. alternata* were from Greer Laboratories. The FPR2-selective antagonist WRW4 was from Calbiochem (Calbiochem, Merck). LPS-RS was

from InVivoGen. rFABP5 and rFABP7 were from Sino Biological, lipoxin A 4 (LXA4) was from Cayman Chemical, and rBlo t 13 and a monoclonal antibody to Blo t 13 were produced as previously described^{47,61}. HDL (isolated from human plasma) was from Sigma (Sigma-Aldrich). Human FPR2 (no. SC322591), human SAA1 (no. RC202738) and murine rFPR1 (no. MR221675) were from Origene. pcDNA3.1 was from Invitrogen, Thermo Scientific. Human SAA1 and On-TARGET plus Non-targeting siRNA (Smart pool: ON-TARGETplus siRNA) were from GE Dharmacon. The BEAS-2B and Caco-2 cell lines were from the American Type Culture Collection (ATCC). BEAS-2B cells were maintained in complete medium composed of BEBM base medium (Lonza) containing bovine pituitary extract (52 µg ml⁻¹), hydrocortisone (0.5 µg ml⁻¹), human endothelial growth factor (0.5 ng ml⁻¹), epinephrine (0.5 µg ml⁻¹), transferrin (10 µg ml⁻¹), insulin (5 µg ml⁻¹), retinoic acid (0.1 ng ml⁻¹), triiodothyronine (6.5 ng ml⁻¹) and gentamycin (50 µg ml⁻¹) (BEGM SingleQuots; Lonza) on collagen–fibronectin (BD Biosciences)-coated flasks in an atmosphere of 5% CO₂ at 37°C. Cells were sub-seeded at the second–third passage and used for subsequent experiments. Cell viability was determined by morphology and trypan blue exclusion. FBS and charcoal-stripped FBS were from Gibco (Gibco). Human rSAA1 was from Pepprotech or produced in-house. Codon-optimized human SAA1 featuring a TEV-cleavable N-terminal hexahistidine tag was expressed in BL21 DE3 star *Escherichia coli* (Invitrogen). The culture was grown to an optical density at 600 nm of 0.6 at 37°C in terrific broth (Carl Roth GmbH + Co. KG) shaking at 180 r.p.m. before setting the incubator to 16°C and allowing the culture to cool and grow for another 45 min. Expression was induced by adding isopropyl-β-D-thiogalactoside at a final concentration of 1 mM and the culture was left overnight. The protein was purified by nickel affinity chromatography from the soluble fraction before incubation with TEV protease (1 mg TEV to 100 mg SAA1) at 4°C overnight to remove the purification tag. A second pass through on an IMAC column was performed to remove uncleaved SAA1, tags and the His-tagged TEV protease, before a final polishing step of size-exclusion chromatography on an S200 Increase 10/300 (GE Healthcare Life Science) into PBS was performed.

EC stimulation and transfections. For stimulation with allergen extracts, 3 × 10⁴ BEAS-2B ECs were seeded per well of a 96-well plate in complete medium and grown until they reached confluence. For plasmid-directed overexpression studies, 1 × 10⁴ BEAS-2B cells were transfected with 0.1 µg plasmid DNA per well coding for human FPR2 and human SAA1, or empty pcDNA3.1 vector using FuGENE HD (Roche) for 48 h. The SAA1(W71A) mutant was generated using the QuikChange II site-directed mutagenesis kit (Agilent Technologies). Oligonucleotides (F 5'-GGG TGT CAG CAG CGA TGG GGC TCG GG-3') and (R 5'-CCC GAG CCC GAT CGC TGC TGA CAC CC-3') were used to delete the WT sequence ⁴¹RSFFSFLGEAF⁴¹, resulting in the deletion of these 11 residues to produce human SAA1mΔ1–11 leading to a SAA mutant with diminished FPR2 avidity. Sanger sequencing (Genetics Resource Core Facility, Johns Hopkins School of Medicine, Baltimore, MD, USA) was used to confirm correct DNA sequences. siRNA-mediated knockdown was performed according to the manufacturer's recommendation. Briefly, BEAS-2B ECs, plated at 1 × 10⁴ per well of a 96-well plate in antibiotic-free medium, were transfected with 25 nM siRNA or non-targeting siRNA in a final volume of 100 µl using DharmaFECT transfection reagent (Thermo Scientific). In lipid-depleted medium experiments, Caco-2 cells were used and maintained in IMDM medium supplemented with charcoal-stripped FBS.

For all experiments, cells were serum-starved overnight before allergen-stimulation (HDM used at 100 µg ml⁻¹; *A. alternata* used at 25 µg ml⁻¹) for 30 min and 24 h. Cell culture supernatants were taken after the indicated time points and analyzed for secreted cytokines by ELISA and cells were lysed in TRIzol (Invitrogen) for RNA isolation. In selected experiments, pharmacological agents and proteins were used to investigate receptor dependency, including the FPR2 antagonist WRW4 (12 µM), the P2-purinergic receptor (P2YR) antagonist suramin (10 µM, Sigma), the TLR4 antagonist LPS (LPS-RS, 10 µg ml⁻¹, InVivoGen), a TLR2-neutralizing antibody (anti-human TLR2 IgA, 10 µg ml⁻¹, InVivoGen), a CD36-blocking antibody (clone JC63.1, 10 µg ml⁻¹, Cayman), LXA4 (100 nM), SAA1 (0.5 µg ml⁻¹) and mouse anti-human SAA1 (0.25–0.5 µg ml⁻¹, DM1004B, Acris). For Der p 13 neutralization and depletion experiments, a monoclonal antibody to its closely related homologue Blo t 13 was used⁶¹. Controls include vehicle and a matched isotype control.

HDL pulldown experiments and SAA1 ligand binding. To determine whether HDM treatment influences the association/dissociation rate of SAA–HDL or its quaternary structure, a straightforward procedure of HDL immunoprecipitation via its major protein component, apolipoprotein A-I (ApoA1), was used. HDM- and medium (control)-treated cell culture supernatants were incubated overnight at 4°C with polyclonal goat anti-human ApoA1 (10 µg ml⁻¹; R&D Systems). HDL-antibody complexes were separated from cell culture supernatants by magnetic separation using protein-G-coated magnetic beads (SureBeads Protein G, Bio-Rad). HDL-depleted supernatants and HDL precipitates were analyzed for their SAA1 content by immunoblotting; immunoreactivity was tested with mouse anti-human SAA1 (DM1004B, Acris; MAB30196, R&D Systems) or sequence-specific antibodies as described previously³⁰. For SAA1 ligand binding experiments, the recombinant FABPs (rFABPs) and extracts were first

resolved by SDS–PAGE and blotted onto nitrocellulose membrane: the minor allergen of *B. tropicalis*, rBlo t 13, and the major HDM allergens rDer p 2 and rDer p 23, as well as whole HDM extract. Membranes were incubated with rSAA1 at 1 µg ml⁻¹ pre-adsorbed to 0.5 µg ml⁻¹ mouse monoclonal anti-SAA1 (Invitrogen) overnight. Detection was by streptavidin–horseradish peroxidase and chemiluminescence using ECL (Bio-Rad) and Biomax film (Kodak) or with a chemiluminescence detection system (ChemiDoc Bio-Rad). For glutaraldehyde crosslinking experiments, rSAA1 at 1 mg ml⁻¹ in PBS was incubated at 20–22°C for 1 h with increasing amounts of Blo t 13 (1 mg ml⁻¹ (1:1); 2 mg ml⁻¹ (1:2); and 4 mg ml⁻¹ (1:4)). Samples were chemically crosslinked with glutaraldehyde (Sigma) concentrations ranging from 0.0025% to 0.01% (wt/vol) for 5 min at 20–22°C. The crosslinking reaction was stopped by adding Laemmli sample buffer and samples were analyzed by SDS–PAGE followed by immunoblotting with anti-human SAA1. In native SDS–PAGE experiments, a fixed concentration of SAA1 of 1 mg ml⁻¹ was incubated with increasing amounts of Blo t 13 (4:1 (0.25 mg ml⁻¹ Blo t 13), 2:1 (0.5 mg ml⁻¹ Blo t 13), 1:1 (1 mg ml⁻¹ Blo t 13) and 1:2 (2 mg ml⁻¹ Blo t 13)) for 2 h at 20–22°C, separated by native PAGE and immunoblotted with polyclonal sequence-specific anti-SAA1 to amino acids 14–30 of human SAA1 (ref.³⁰).

Determination of SAA and cytokine concentrations. Human SAA1 antibody pairs were purchased from Novex (Novex, Thermo Fisher Scientific) or the human SAA1 DuoSet ELISA development kit was used (DY3019-05, R&D Systems). Mouse SAA1 capture antibody was from Thermo Fisher (D9H4L41) and mouse SAA1 detection antibody was from R&D Systems (BAF2948). Human IL-33 ELISA and mouse IL-33 cytokine ELISA kits were from R&D Systems. Mouse IL-25 and TSLP ELISA kits were from Invitrogen. ELISA kits specific for mouse IL-5, IL-10, IL-11, IL-13, IL-22, IL-17A, IFN-γ and GM-CSF were from R&D Systems or analyses were performed using the Luminex system (Luminex 100IS, Biomedica) described previously⁶².

Isolation of RNA and quantitative real-time PCR. Total RNA was isolated from whole mouse lung tissue samples or FACS-sorted cell populations frozen individually in TRIzol reagent (Invitrogen), and total RNA was extracted according to the manufacturer's instructions. Individual sample RNA (1 µg) was reverse-transcribed using Superscript II (Invitrogen) and a mixture of oligo (dT) and random primers. Real-time PCR was performed on a StepOnePlus PCR system (Applied Biosystems) using iTaq DNA polymerase (Bio-Rad). Data were analyzed by the ΔCT method using the SDS 2.1 software (Applied Biosystems). Expression of the target genes was normalized to the mRNA abundance of the housekeeping gene *Rps14*. For human samples, target gene expression was normalized to that of eukaryotic elongation factor (EEF) and ubiquitin c (UBC). Primer sequences used are listed in Supplementary Table 6.

Statistical analysis. All data were analyzed using GraphPad Prism 6 and 7 software. Unless otherwise indicated, all values are represented as mean values ± s.e.m. The figure legends denote the specific statistical tests used for each experiment. *P* values of less than 0.05 were considered significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets supporting the conclusions of this article are available from the corresponding authors upon request. Source data for Figs. 3, 5 and 8 and Extended Data Figs. 5, 7 and 9 are presented with the paper.

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Author contributions

U.S., S.L. and M.W.-K. designed the study. U.S. and S.L. performed the experiments and analyzed the data with help from N.G., X.X., J.P., N.Y. and W.F.P. C.K., B.K. and P.A.T. performed experiments and provided technical assistance with the allergic phenotype involving *Saa*^{-/-} mice. G.H. produced and characterized rSAA1.1. J.D. provided

S. mansoni worm extracts. L.C. and L.P. made *B. tropicalis* rFABP and *B. tropicalis* FABP-specific monoclonal antibodies. E.M. provided peptide-specific SAA antibodies and performed SAA1 antibody blots. S.R. provided serum samples from control individuals and patients with a HDM allergy. A.P.L. provided samples from control individuals and patients with CRS. J.C. isolated and prepared AECs from controls and individuals with asthma. W.F.P. and E.M. thoroughly revised the manuscript. U.S. and M.W.-K. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

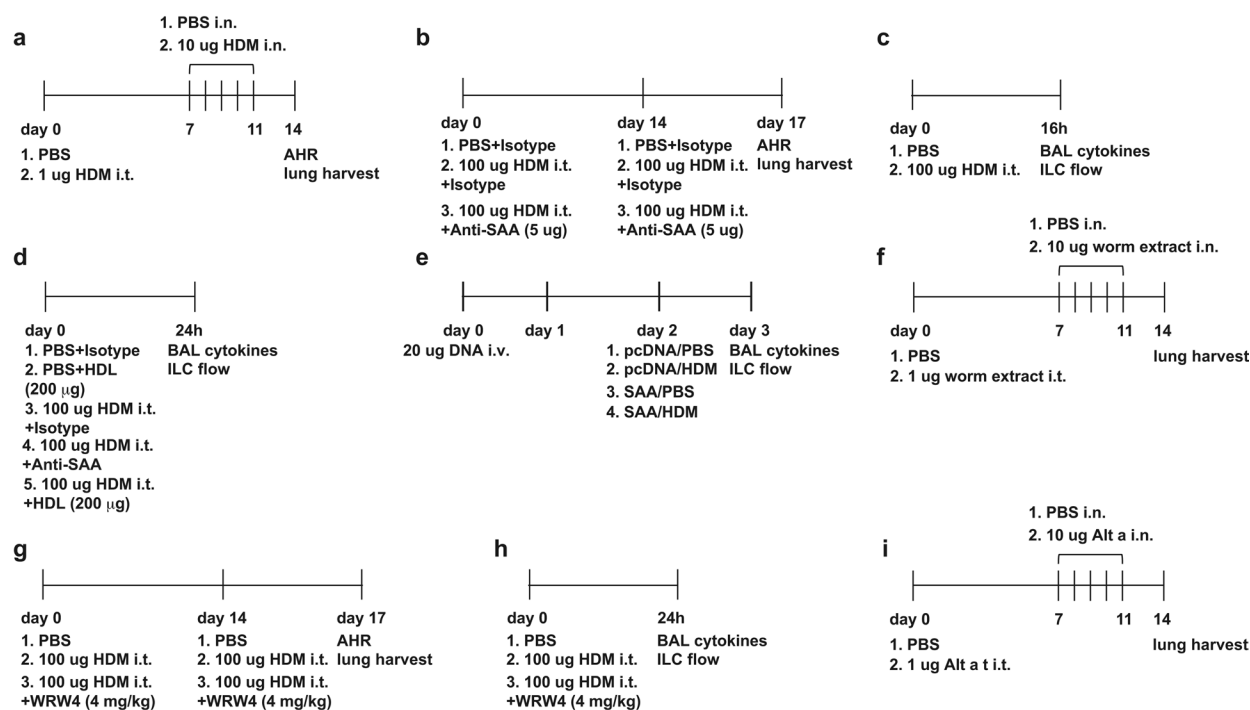
Extended data is available for this paper at <https://doi.org/10.1038/s41590-020-0698-1>.

Supplementary information is available for this paper at <https://doi.org/10.1038/s41590-020-0698-1>.

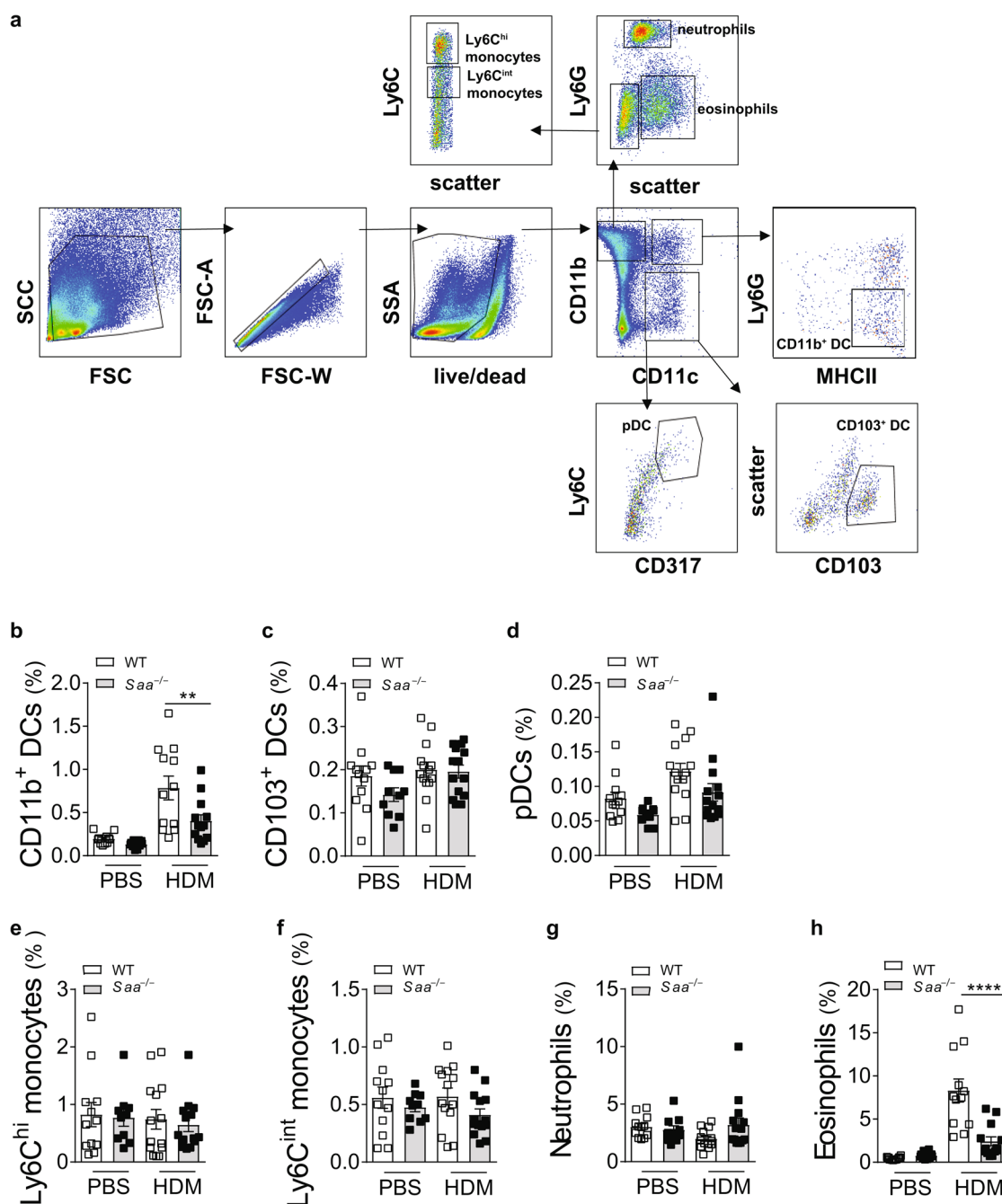
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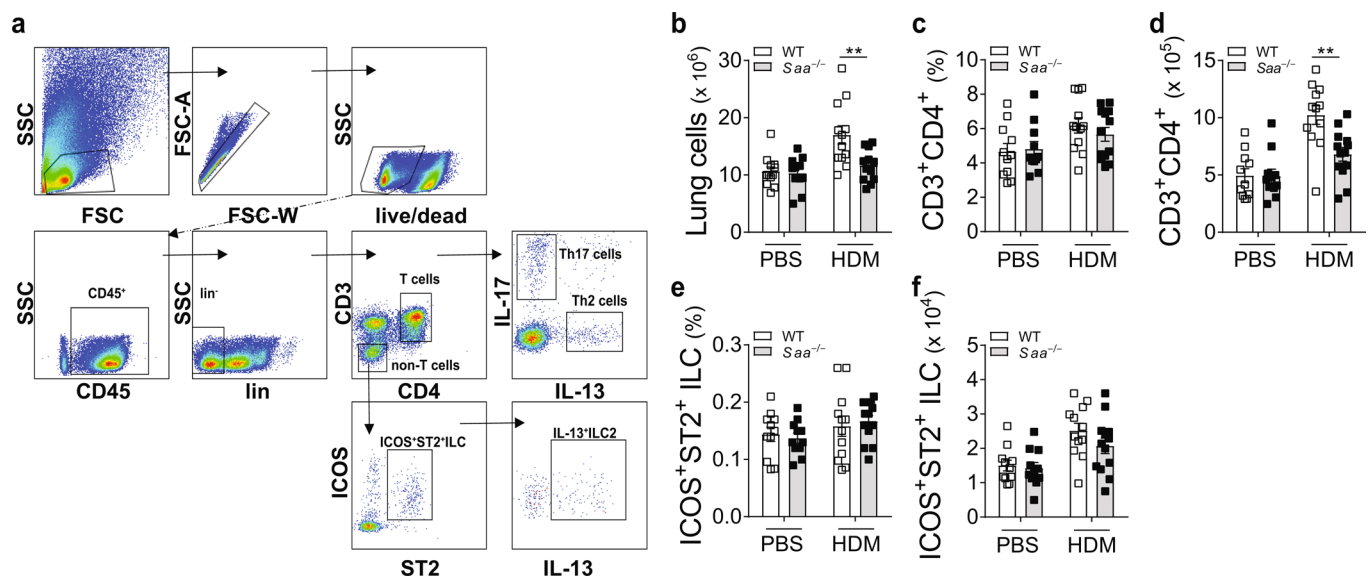
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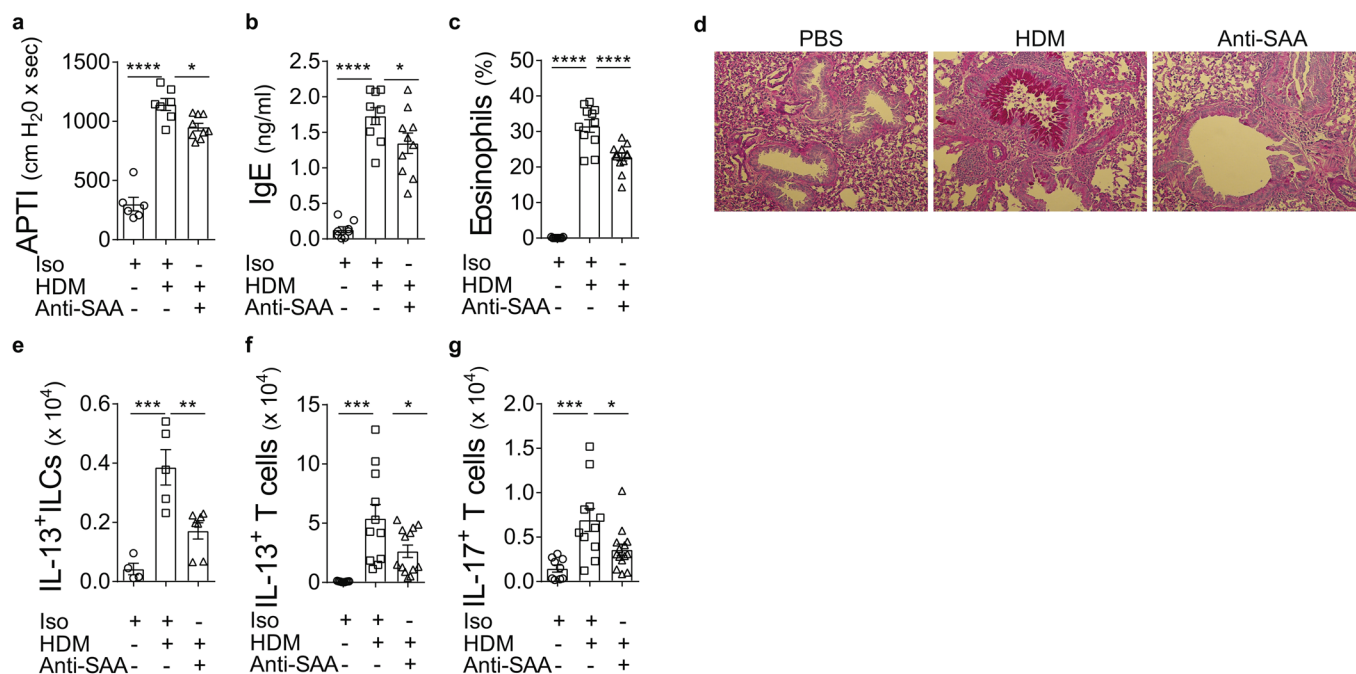
Extended Data Fig. 1 | Experimental design. **a**, Model of allergen-induced airway hyperresponsiveness (AHR) for wild-type (WT) and *Saa*^{-/-} mice (both C57BL/6 background). Mice were sensitized i.t. on day 0 (1 μ g) and i.n. on days 7–11 with 10 μ g of HDM extract. Airway measurements were performed 72 h after the last allergen challenge (used in Fig. 1a–j and Extended Data Figs. 2 and 3). **b**, For SAA1 antibody blockade, we used an established mouse model of allergen-induced AHR sensitizing WT BALB/cJ mice i.t. on day 0 and 14 with 100 μ g of HDM extract + isotype control, or HDM + anti-SAA. Airway measurements and tissue harvests were performed 72 h after the last allergen challenge (used in Extended Data Fig. 4). **c**, In short-term exposure protocols WT and *Saa*^{-/-} mice (both C57BL/6 background) received a single HDM challenge (100 μ g) were sacrificed 16 h later. **d**, In short-term exposure protocols BALB/cJ mice received a single HDM challenge (100 μ g) + isotype control, HDM challenge + HDL (200 μ g) and isotype control, or HDM + anti-SAA and were sacrificed 24 h later (used in Fig. 2e). **e**, For overexpression of SAA1 *in vivo*, mice were injected 20 μ g of DNA complexed to polyethylenimine at day 0, exposed to PBS or HDM 48 h later and ILC2s as well as BAL cytokines measurements were performed on day 3 (used in Fig. 2f). **f**, WT and *Saa*^{-/-} mice were sensitized i.t. on day 0 (1 μ g) and i.n. on days 7–11 with 10 μ g with extracts from the parasitic worm *Schistosoma mansoni* (a Puerto Rican isolate). Tissues were harvested 72 h after the last allergen challenge (used in Fig. 4). **g**, For FPR2 blockade (WRW4, 2 mg/kg), WT BALB/cJ mice were sensitized and challenged i.t. on day 0 and 14 with 100 μ g of HDM extract. Airway measurements were performed 72 h after the last allergen challenge (used in Fig. 7a–f). **h**, In short term exposure experiments, BALB/cJ mice received a single HDM challenge (100 μ g) or HDM + WRW4 and were sacrificed 24 h later (used in Fig. 7g, h). **i**, Model of *A. alternata* (Alt a)-induced airway inflammation for WT and *Saa*^{-/-} mice. Mice were sensitized i.t. on day 0 (1 μ g) and i.n. on days 7–11 with 10 μ g of Alt a extract. Tissues were harvested 72 h after the last allergen challenge (used in Extended Data Fig. 9f–j).



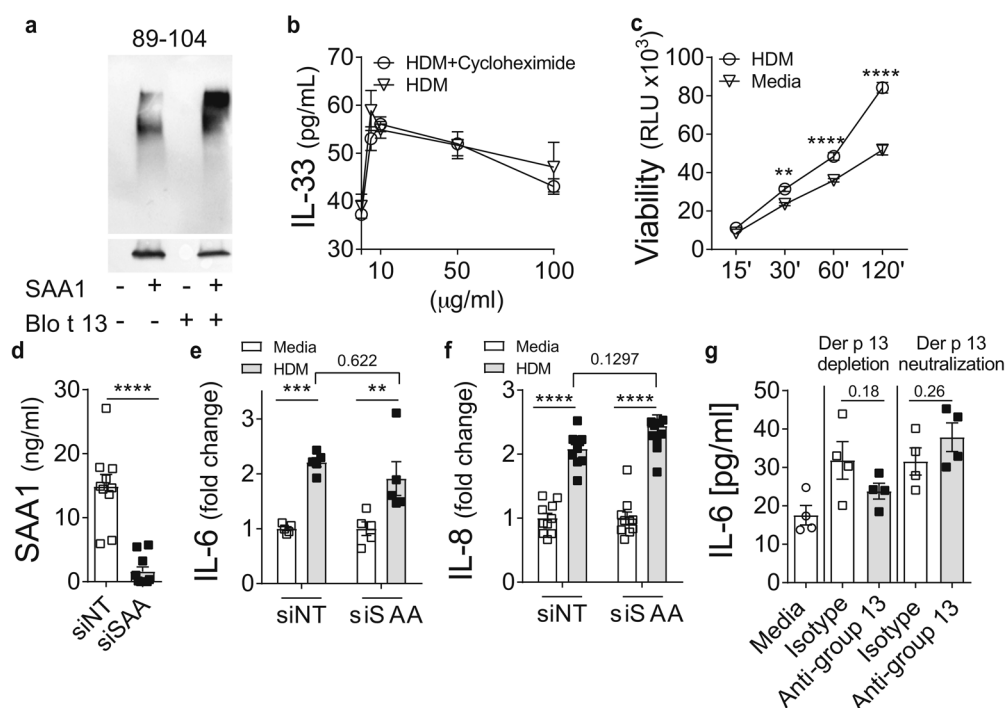
Extended Data Fig. 2 | Reduced recruitment of CD11b⁺ DCs in *Saa*^{-/-} mice after allergen exposure. Allergic phenotype in WT and *Saa*^{-/-} C57BL/6 mice sensitized and challenged with PBS or HDM was analyzed seventy-two hours after the last challenge. **a**, Lung cell gating strategy used in Fig. 1, Fig. 4 and Extended Data Figs. 6 and 9. Frequency of dendritic cell (DC) populations (**b-d**), monocytes (**e-f**) and granulocytes (**g-h**) in the lungs of WT and *Saa*^{-/-} mice used in Fig. 1. Data represent means $\pm$ s.e.m. of pooled data from 2 independent experiments containing n=11 WT PBS, n=12 WT HDM, n=11 *Saa*^{-/-} PBS and n=13 *Saa*^{-/-} HDM animals per group. *P* values were calculated with a two-tailed test using one-way analysis of variance (ANOVA) with Dunnett's post hoc analysis that compares WT HDM to counterparts. ***P* = 0.0039, *****P* $\leq$ 0.0001.



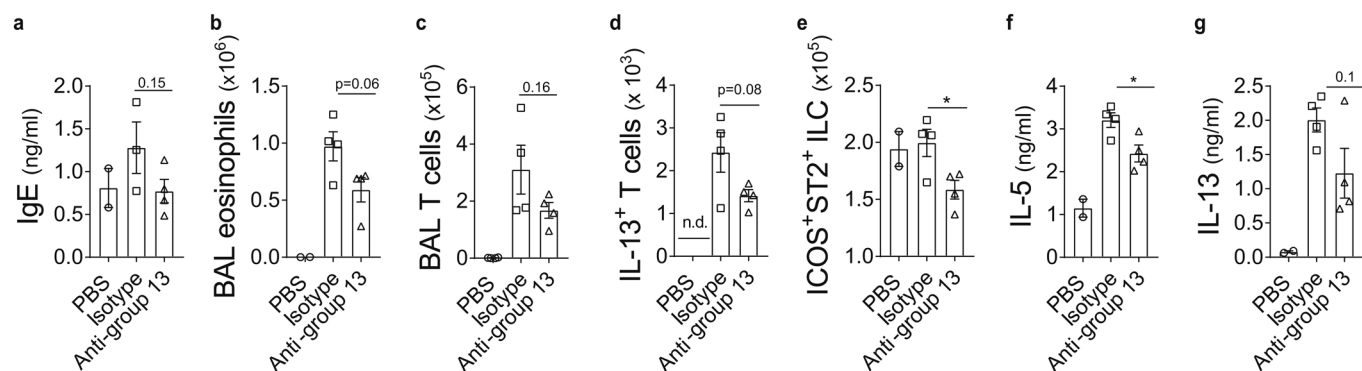
Extended Data Fig. 3 | Reduced migration of CD3⁺CD4⁺ T cells to the lungs of *Saa*^{-/-} mice after allergen exposure. Allergic phenotype in WT and *Saa*^{-/-} C57BL/6 mice sensitized and challenged with PBS or HDM was analyzed seventy-two hours after the last challenge. **a**, ILC and T cell gating strategy (used in Fig. 1g, h, Fig. 4b–e and Extended Data Fig. 9h–j). **b**, Total lung cell counts (***P* = 0.0073), **(c)** frequency and **(d)** numbers of CD3⁺CD4⁺ T cells (***P* = 0.0014) and **(e)** frequency and numbers of **(f)** ICOS⁺ST2⁺ ILCs in the lungs of WT and *Saa*^{-/-} mice. Data represent pooled data presented as means $\pm$ s.e.m. from 2 independent experiments containing *n*=11 WT PBS, *n*=12 WT HDM, *n*=11 *Saa*^{-/-} PBS and *n*=13 *Saa*^{-/-} HDM animals per group. *P* values were calculated with a two-tailed test using one-way analysis of variance (ANOVA) with Dunnett's post hoc analysis that compares WT HDM to counterparts.



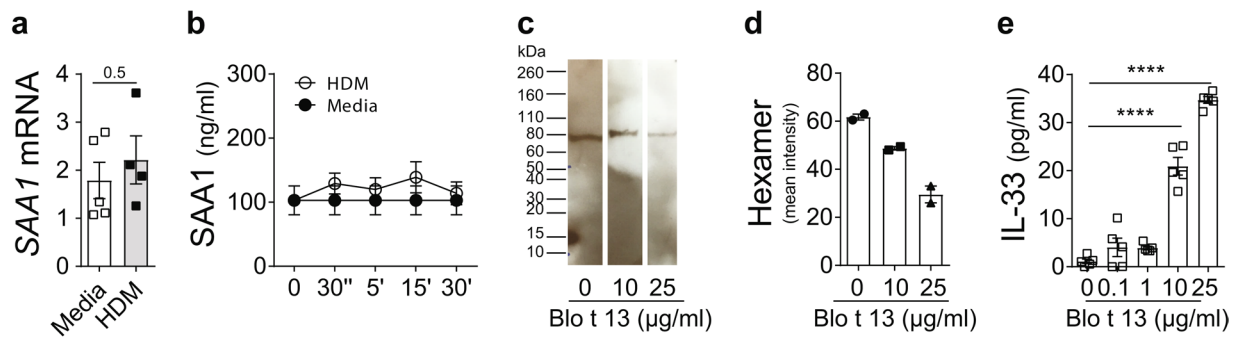
Extended Data Fig. 4 | Airway SAA1-neutralization ameliorates the allergic phenotype. **a**, AHR (* $P = 0.0105$), **(b)** total serum IgE concentrations (* $P = 0.0265$), **(c)** eosinophil infiltration into the lungs, and **(d)** PAS stained lung sections of isotype (iso), HDM+isotype (HDM), or HDM+SAAab-treated (anti-SAA) WT BALB/c mice. Antibodies were administered at 5 μ g/i.t. Frequency of **(e)** Lin⁻CD45⁺ST2⁺IL-13⁺ ILC2s (** $P = 0.0027$, *** $P = 0.0002$), **(f)** T_H2 and (* $P = 0.0260$, *** $P = 0.0001$) **(g)** T_H17 cells (* $P = 0.0149$, *** $P = 0.0005$) in the lungs of these mice. Data represents means $\pm$ s.e.m. of pooled data from 2 independent experiments containing (a) $n=6$ PBS+iso, $n=7$ HDM+iso, $n=9$ HDM+anti-SAA animals per group; **(b, c, f, g)** $n=9$ PBS+iso, $n=11$ HDM+iso, $n=13$ HDM+anti-SAA animals per group or are representative of 2 independent experiments with **(e)** $n=4$ PBS+iso, $n=5$ HDM+iso, $n=7$ HDM+anti-SAA animals per group. P values were calculated with a two-tailed test using one-way analysis of variance (ANOVA) with Dunnett's post hoc analysis that compares HDM to iso and anti-SAA counterparts. **** $P \leq 0.0001$.



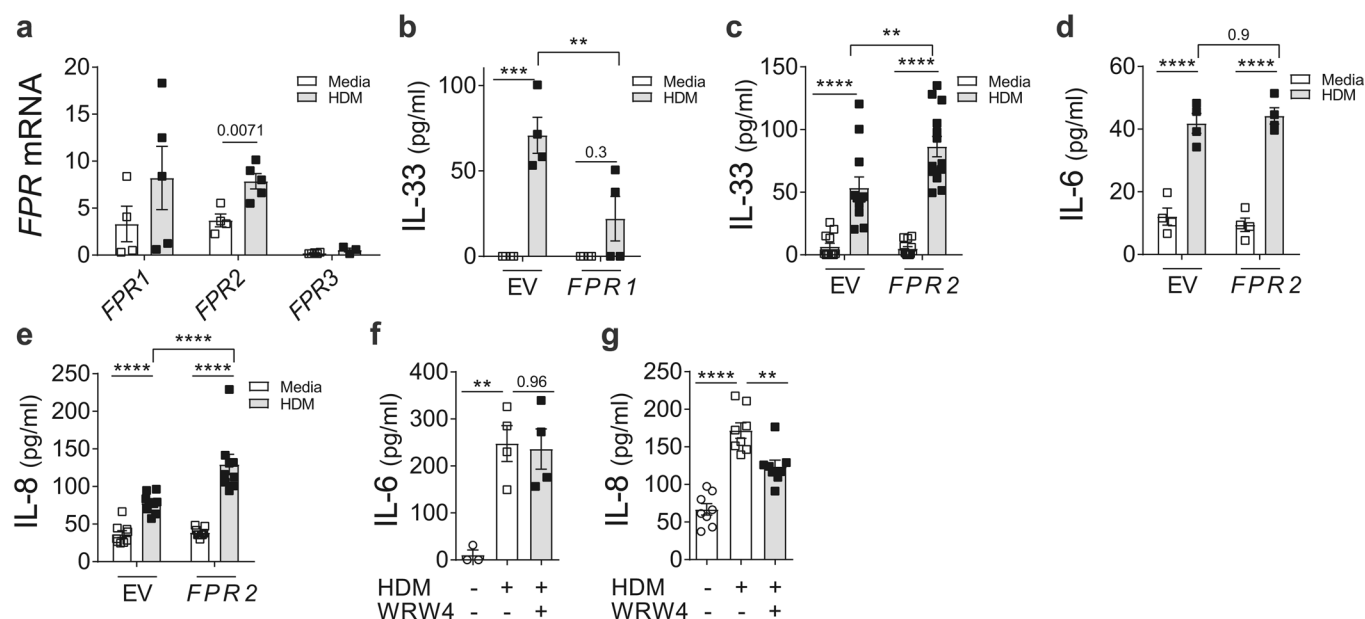
Extended Data Fig. 5 | SAA1 is a pattern recognition molecule for mite-derived and human cytosolic FABPs. **a**, Migration of SAA1 (1 mg/ml) in IMDM media was analyzed in the presence of the mite FABP rBlo t 13 (1 mg/ml) by native PAGE followed by immunoblot analysis using a sequence-specific antiserum (amino acid 89-104) raised against human SAA1. **b**, Effects of the protein synthesis inhibitor cycloheximide on IL-33 concentrations in BEAS-2B cells treated for 30 min with HDM (100 $\mu\text{g/mL}$). **c**, Cell viability of BEAS-2B cells after HDM exposure over time as measured by continuous reduction of a cell viability substrate by viable cells ($**P = 0.0041$). SAA1 concentrations in cells after (**d**) siRNA-mediated silencing of SAA1 (siSAA1) or non-targeting scrambled siRNA (siNT). Effects of SAA1 on HDM-induced IL-6 ($**P = 0.0086$, $***P = 0.0007$) and IL-8 release in cells with siRNA mediated knockdown of SAA1 (siSAA1) (**e** and **f**). **g**, HDM-induced IL-6 amounts after Der p 13-depletion and/or neutralization using a monoclonal antibody specific for mite group 13 allergens (anti-group 13). Data are shown as means $\pm$ s.e.m. and are representative of 2 (**b**, **e**) or 3 (**c**) independent experiments or pooled data from 2 independent experiments (**d**, **f**) each containing at least $n=4$ biologically independent samples. Representative analysis of SAA1 migration patterns in the presence of Blo t 13 using sequence-specific rabbit antiserum raised against human SAA1 (aa 89-104) (**a**). IL-6 amounts were analysed for one representative experiments with $n=5$ biologically independent samples (**g**). Cropped images are shown. P values were calculated with a two-tailed test using one-way analysis of variance (ANOVA) followed by Dunnett's post (**b**) or Tukey's hoc analysis (**e**, **f**), two-way ANOVA followed by Dunnett correction (**c**) or Student's t -test (**d**, **g**). $***P \leq 0.001$; $****P \leq 0.0001$.



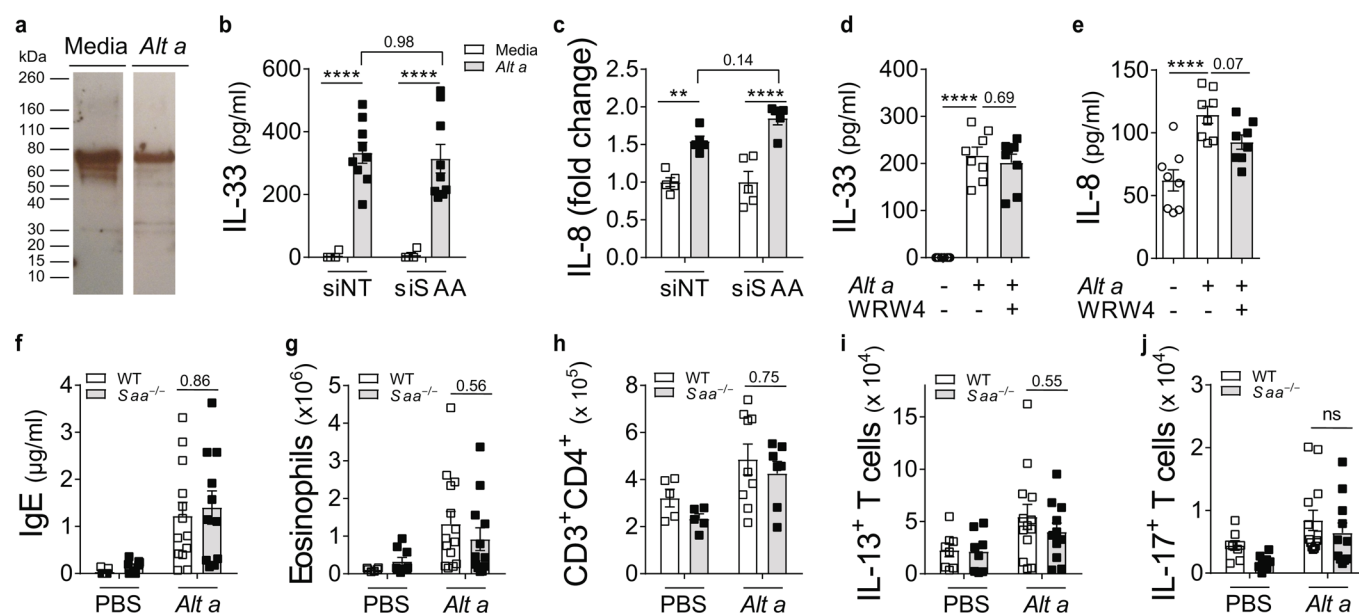
Extended Data Fig. 6 | Der p 13-depleted HDM has decreased T_H2 skewing capacity. **a**, Total IgE serum concentrations and numbers of BAL (**b**) eosinophils and (**c**) T cells in WT BALB/c mice undergoing a full allergen-exposure protocol using HDM that was depleted using either an isotype control antibody or a monoclonal antibody specific for mite group-13 allergens. **d**, Numbers of mediastinal lymph node IL-13⁺ CD4⁺ T cells (n.d., not detected) and (**e**) lung ILC2 ($*P = 0.029$). **f–g**, Cytokine production from HDM-restimulated lung cells ($*P = 0.024$). Data represent mean $\pm$ s.e.m. of $n=2$ PBS, $n=4$ HDM-isotype depleted, $n=4$ HDM- anti-group 13-depleted comparing HDM-isotype to α -group 13-depleted extract group using two-tailed unpaired t-test.



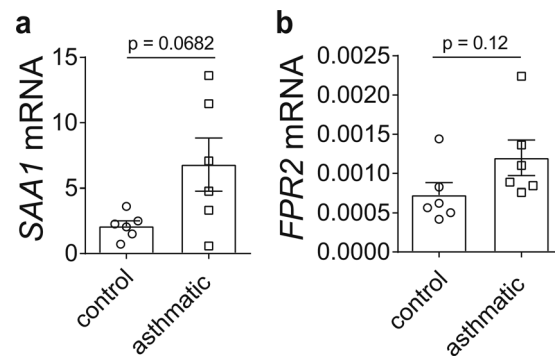
Extended Data Fig. 7 | HDM-induced IL-33 is dependent on SAA1 dissociation. **a**, mRNA and **(b)** protein amounts of SAA in response to HDM (100 μg/ml). **c**, SAA1 hexamer after rBlo t 13 stimulation was analyzed as described in Fig. 5b. **d**, Bar graph represents quantitative analysis of SAA hexamer using LI-COR Image Studio Software. **e**, Concentration-dependent IL-33 release from BEAS-2B cells induced by rBlo t 13. Data are shown as means ± s.e.m. and are pooled data from 2 independent experiments (**b**, **e**) each containing n=4-5 replicate wells or representative of 2-3 independent experiments (**d**). SAA1 mRNA expression, normalized to the average of housekeeping genes, is presented as mean value ± s.e.m. (n = 5). Immunoblot is representative of an experimental n=2. Cropped images are shown. P values were calculated with a two-tailed test using Student's t-test (**a**), two-way analysis of variance (ANOVA) followed by Dunnett's correction (**b**) or one-way ANOVA with Dunnett's post hoc analysis (**d**, **e**). ****P ≤ 0.0001.



Extended Data Fig. 8 | The SAA1-FPR2 axis regulates IL-33. **a**, mRNA expression of the FPR family members FPR1, FPR2 and FPR3 at baseline (filled bars) or after 2 h of HDM stimulation (white bars). **b**, HDM-triggered IL-33 amounts in BEAS-2B cells overexpressing human *FPR1* (** $P = 0.0068$, *** $P = 0.0003$). **c**, IL-33 secretion in BEAS-2B cells overexpressing human *FPR2* or cells transfected with an empty vector (EV; pcDNA3.1) (** $P = 0.0028$). HDM-induced IL-6 and IL-8 amounts in BEAS-2B cells overexpressing *FPR2* (**d** and **e**) or blocking the *FPR2* receptor **f** (** $P = 0.0043$) and **g** (** $P = 0.0021$)) using WRW4. Data presented as means $\pm$ s.e.m. and is representative of 2 independent experiments each containing at least $n = 4$ biologically independent samples (**b**, **d**, **f**) or pooled data from 2 independent experiments (**c**, **e**, **g**). mRNA expression, normalized to the average of housekeeping genes, is presented as mean values $\pm$ s.e.m. ($n = 5$ biologically independent samples) performed in duplicates (**a**). P values were calculated with a two-tailed test using Student's t -test (**a**) or one-way analysis of variance (ANOVA) with Tukeys multiple comparison test (**b-e**) or Dunnett's post hoc analysis (**f**, **g**). **** $P \leq 0.0001$.



Extended Data Fig. 9 | SAA1-IL-33 axis is specific to HDM and not induced by *A. alternata*. **a**, Immunoblot of SAA1 after *A. alternata* (*Alt a*) stimulation of BEAS-2B cells performed as described in Fig. 5b. *Alt a*-induced IL-33 and IL-8 (** $P = 0.0044$) secretion in BEAS-2B cells after siRNA-mediated silencing of SAA1 (siSAA1) (**b** and **c**) or WRW4-mediated FPR2 blockade (**d** and **e**). **f**, Total serum IgE concentrations, (**g**) eosinophil numbers and frequency of (**h**) CD3⁺CD4⁺, (**i**) T_H2 and (**j**) T_H17 cells in the lungs of PBS or *Alt a*-treated WT and *Saa*^{-/-} C57BL/6 mice. Immunoblots are representative of an experimental $n=2$ (**a**). Data are presented as means $\pm$ s.e.m. and represent pooled data from 2 independent experiments (**b**, **d**, **e**, **f**, **g**, **i**, **j**) or show one representative experiment (**c**) each containing at least $n=4$ biologically independent samples or $n=8$ WT PBS, $n=13$ WT *Alt a*, $n=8$ *Saa*^{-/-} PBS and $n=11$ *Saa*^{-/-} *Alt a* animals per group (**f**, **g**, **i**, **j**) or $n=5$ WT PBS, $n=9$ WT *Alt a*, $n=5$ *Saa*^{-/-} PBS and $n=7$ *Saa*^{-/-} *Alt a* animals per group (**h**). Cropped images are shown. P values were calculated with a two-tailed test using one-way analysis of variance (ANOVA) with Tukeys multiple comparison test (**a**, **b**) or Dunnett's post hoc analysis (**d**-**j**). **** $P \leq 0.0001$ siNT = non-targeting siRNA; siSAA1 = SAA1-targeting siRNA. ns=not significant.



Extended Data Fig. 10 | Dysregulated SAA and FPR2 expression in patients with asthma. Basal (a) SAA1 and (b) FPR2 expression in bronchial epithelial cells from individuals with asthma and matched controls. Data represents means $\pm$ s.e.m. of (a and b) $n=6$ control and $n=6$ individuals with asthma per group. *P* values were calculated with a two-tailed test using Student's *t*-test with Welch's correction.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Flow data were collected using the FACSDiva software 6.2. Immunoblot signals were detected by streptavidin-horseradish peroxidase and chemiluminescence using ECL (Biorad) and Biomax film (Kodak) or detected with a chemiluminescence detection system (ChemiDoc Bio-Rad, Berkeley, CA, US). Real-time PCR was performed on a StepOnePlus PCR system (Applied Biosystems, Foster City, CA, US). Cytokine data was collected using a Multiskan GO apparatus equipped with the SkanIt software 4.1.0.43 and 6.0.1.6 (Thermo Fisher) or a BioTek microplate reader (Fisher Scientific). For multiplexed cytokine measurements the the Luminex system (Luminex 100IS, Biomedica) was used. Airway hyperresponsiveness was followed using a RC fine pointe site (Buxco FinePointe Resistance and Compliance, DSI, Harvard Bioscience, Inc) or using a FlexiVent (SciReq, Saint-Urbain, Quebec, Canada).

Data analysis

Flow cytometry data was collected and analyzed using FACSDiva software 6.2 and FlowJo v10.5.3. Western blot images were analyzed using the LI-COR Image StudioLite Ver 5.3 or ImageJ 1.48v Software. Real-time PCR data were analyzed by the deltaCT method and using the SDS 2.1 software (Applied Biosystems). For cytokine data the SkanIt software (Thermo Fisher), Gen5™ Reader Control and Data Analysis Software 2.0 or xPONENT Software Solutions 3.1.871.0 were used. Airway hyperresponsiveness was analyzed using the FinePoint Software Research Suite v2.4.6 or Flexiware Software. Prism 7 was used for generation of graphs and statistical analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets supporting the conclusions of this article are included within the article and its supplementary information files.

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size for in vivo studies was based on previous data published using similar mouse models, stimuli and endpoint measurements (e.g. AHR and IgE). In general, we routinely used 4-8 mice per genotype and condition. For in vitro experiments, reasonable sample sizes (4-5 replicate wells) were chosen to ensure adequate reproducibility of results and was based on our previous studies.
Data exclusions	In in vivo experiments, animals were excluded in which HDM sensitization was not properly established due to technical issues or due to technical problems during APTI measurements and which would have rendered the outcome measures meaningless. Rout and Grubb's outlier test were run with default parameters in Prism to detect and exclude significant outliers.
Replication	Biological and technical replicates have been performed and the information on replicates is included in each figure legend. Most experiments have been reproduced 2-3 times with comparable results. Whenever representative data is shown, the experimental data were reproduced with similar results.
Randomization	For in vivo experiments, sex and age-matched mice derived from different breeding pairs were assigned randomly for each experiment according to their genotypes. Healthy donors were randomly selected. In vitro experiments were not randomized.
Blinding	The investigators were not blinded to experimental conditions, as all in vivo experiments were conducted and analyzed by one person. Human samples were labeled as numeric numbers and analyzed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used for flow cytometry:
 CD11c-Alexa Fluor 700, HL3, #560583, BD Pharmingen 1/400
 CD11c-PE-Cy7, N418, #117317, BioLegend 1/500
 CD11b-PE-Cy7 or APC-Cy7 M1/70, #557657 BD Pharmingen ; M1/70, #101216 BioLegend 1/500
 Ly6G-Pacific Blue, HK1.4, #128032, BioLegend 1/400
 Ly6G-BV605, 1A8, #127639 BioLegend 1/300
 MHCII-PE, M5/114.15.2, # 07608, BioLegend 1/2000

CD103-PerCP-Cy5.5, 2E7, #121416, BioLegend 1/200
 CD317-APC, eBio129c, #17-3171-82, eBioscience 1/200
 CD3e-PerCP-Cy5.5, 145-2C11, #551163, BD Pharmingen) 1/400
 CD4-FITC, RM4-5, #11-0042-82, eBioscience 1/400
 IL-13-APC, eBio13A, #47-7133-82, eBioscience 1/150
 FceRI-PerCP-Cy5.5 or APC-Cy7, MAR-1, #134325 #134319, BioLegend 1/400
 TCR β -PerCP-Cy5.5 or APC-Cy7, H57-597, #560657 #560656, BD Pharmingen 1/200
 B220-PerCP-Cy5.5 or APC-Cy7, RA3-6B2, #552094 #561101, BD Pharmingen 1/400
 CD49b-PerCP-Cy5.5 or APC-Cy7, DX5, #A15420 #46-5971-82, eBioscience, 1/200
 CD11b-PerCP-Cy5.5 or APC-Cy7, M1/70, #561114 #557657, BD Pharmingen, 1/500
 CD11c-PerCP-Cy5.5 or APC-Cy7, N418, #117327 #117323, BioLegend, 1/500
 Gr1-PerCP-Cy5.5 or APC-Cy7, RB6-8C5, #552093 #557661 BD Pharmingen 1/400
 NK1.1-APC-Cy7, PK136, #108727 #108723, BioLegend 1/250
 CD45-Alexa Fluor700, 30-F11, #560510, BD Pharmingen 1/400
 CD90.2-BV605, 53-2.1, #563008, BD Pharmingen 1/250
 CD25-APC-e780, PC61.5, #47-0251-82, eBioscience 1/250
 ICOS-PE-Cy7, C398.4A, #313519, BioLegend 1/250
 T1/ST2-FITC, DJ8, #101001F, MD Bioproducts 1/150
 IL-33 α -BV421, DIH9, # 145309, Biolegend 1/250

The following antibodies were used for cytokine ELISA:

Anti-human SAA1 antibody pairs were purchased from Novex (#KHA0011 Novex, Thermo Fisher Scientific) or human SAA1 DuoSet ELISA (#DY3019-05, R&D Systems). Human IL-33 (AF3625 capture antibody and BAF3625 detection antibody), IL-6 (#DY206) and IL-8 (#DY208) ELISA were from R&D Systems. Mouse SAA1 capture antibody (D9H4L41) from Thermo Fisher (#700830) and mouse SAA1 detection antibody from R&D Systems (#BAF2948). Mouse IL-25 (#88-7002-88) and TSLP (#88-7490-88) ELISA were from Invitrogen. Mouse IL-33 ELISA was from R&D Systems (#DY3626). Mouse cytokine ELISA kits specific for IL-5 (#DY405), IL-13 (#DY413), IL-17A (#DY421), and IFN (γ) (DY421) were from R&D Systems or eBioscience IL-5 (TRFK5 #14-7052-81/TRFK4 #13-7051-81), IL-13 (eBio13A #12-7133-82/eBio1316H #13-7135-81), IL-17A (eBio17B7 #16-7135-81/eBio17CK15A5 #13-7135-81), and IFN (γ) (AN-18 #14-7313-81/R4-6A2 #13-7312-81) were.

The following antibodies were used for immunoblotting and depletions:

SAA1 content by Western-blotting was tested with mouse anti-human SAA1 antibody (clone 607 #DM1004B, Acris, Origene; clone 865504 #MAB30196, R&D Systems) or sequence-specific antibodies as described (Malle, E. et al. Mapping of antigenic determinants of purified, lipid-free human serum amyloid A proteins. Scand. J. Immunol. 48, 557-561 (1998). Goat anti-human ApoA1 antibody (R&D systems, Minneapolis, MN, US) was used for HDL pull downs. For Der p 13 neutralization and depletion experiments, a monoclonal antibody directed against its closely related homologue Blo t 13 was used (Labrada, M. et al. Monoclonal antibodies against Blo t 13, a recombinant allergen from *Blomia tropicalis*. Int. Arch. Allergy Immunol. 129, 212-218 (2002).

Validation

All antibodies used for flow cytometry and cytokine ELISA are from commercial sources and have been validated by vendors, and have been titrated and used by our own lab in previous experiments. Validation data for species and application are available on the manufacturer's website (checkable via the clone or catalogue number). Antibodies used for immunoblotting were either purchased from best available vendors and are also validated by the vendors. Sequence-specific antibodies raised against SAA1 peptides have been validated previously in Malle, E. et al. Mapping of antigenic determinants of purified, lipid-free human serum amyloid A proteins. Scand. J. Immunol. 48, 557-561 (1998). The monoclonal antibody directed against its closely related homologue Blo t 13 was validated in Labrada, M. et al. Monoclonal antibodies against Blo t 13, a recombinant allergen from *Blomia tropicalis*. Int. Arch. Allergy Immunol. 129, 212-218 (2002). Controls included matched isotype control as well as positive and negative control samples.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	BEAS-2B and Caco-2 cell lines were from American Type Culture Collection (ATCC, Manassas, VA, US).
Authentication	None of the cell lines have been authenticated.
Mycoplasma contamination	These cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Unless otherwise specified, experiments were performed with mice between 6-8 weeks of age. BALB/cJ mice were originally obtained from Taconic (Rensselaer, NY, US) and bred in-house at the Bloomberg School of Public Health animal facility. For BALB/cJ experiments, age-matched female mice were used. Saa knockout mice on the C57BL/6 background were a kind gift from Maria C. deBeer (University of Kentucky Medical Center, KY, US) and bred in-house at the Medical University of Vienna. WT C57BL/6J mice were originally obtained from Charles River (Sulzfeld, Germany) and bred in-house at the Medical University of Vienna. For C57BL/6J experiments age-matched male and female mice were used in individual experiments. All in vivo
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experiments were approved by the Institutional Animal Use and Care Committee at Johns Hopkins Bloomberg School of Public Health and the Medical University of Vienna .

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field-collected samples.

Ethics oversight

All in vivo experiments were approved by the Institutional Animal Use and Care Committee at Johns Hopkins Bloomberg School of Public Health and the Medical University of Vienna .

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Inclusion criteria for patients with CRS included continuous symptoms of rhinosinusitis as defined by the AAO-HNS Chronic Rhinosinusitis Task Force for greater than 12 weeks, computed tomography of the sinuses revealing isolated or diffuse sinus mucosal thickening or air-fluid levels, and nasal polyps visible on diagnostic endoscopy. The asthmatic subjects were diagnosed according to the American Thoracic Society criteria. Asthmatic subjects were atopic, and had a positive skin prick test to common aeroallergens, methacholine PC20 of <8 mg/ml, and FEV1 >70% predicted, with only intermittent use of β_2 -agonists. Control subjects were non-atopics, non-smokers, with no history of asthma or airway and systemic diseases. Inclusion of allergic individuals suffering from HDM allergy in this study was based on a characteristic history of clinical symptoms (allergic conjunctivitis, rhinitis, urticaria, and asthma) and on at least one of the following diagnostic criteria: a positive skin prick test or a positive ImmunoCAP.

Recruitment

Subjects for the collection of primary nasal epithelial cells were recruited from the Johns Hopkins Outpatient Rhinology Clinic. All subjects gave signed informed consent. Inclusion criteria included continuous symptoms of rhinosinusitis as defined by the AAO-HNS Chronic Rhinosinusitis Task Force for greater than 12 weeks, computed tomography of the sinuses revealing isolated or diffuse sinus mucosal thickening or air-fluid levels, and nasal polyps visible on diagnostic endoscopy. Subjects for the collection of bronchial epithelial cells were recruited from the Asthma Clinic at Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec, QC, Canada). The asthmatic subjects were diagnosed according to the American Thoracic Society criteria. Control subjects were non-atopics, non-smokers, with no history of asthma or airway and systemic diseases. HDM allergic subjects and matched healthy controls for the collection of plasma were recruited at the Medical University of Vienna. Blood samples were collected between 2014–2017 after signing a patient information and an informed consent. Diagnosis was based on a clinical history of typical symptoms like (allergic conjunctivitis, rhinitis and asthma) and a positive ImmunoCAP.

Ethics oversight

In case of primary nasal epithelial cells, the research protocol was approved through the Johns Hopkins Institutional Review process, and all subjects gave signed informed consent. The study with bronchial epithelial cells was approved by the IUCPQ (Quebec, Canada) ethics committee, and all subjects gave written informed consent. For studies with human plasma, approval was obtained from the ethics committee of the Medical University of Vienna and informed consent was obtained from the volunteer donors (EK1538/2014).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Murine lungs were excised, minced, and digested in serum-free RPMI containing Liberase CI (0.5 mg/ml, Roche) and DNase I (0.5 mg/ml, Sigma) at 37°C for 45 min. Cells were washed with RPMI containing 10% (v/v) FBS and viable cells were counted by trypan blue exclusion. All staining reactions were performed at 4°C following incubation with FcBlock (TruStain fcX antibody, Biolegend, San Diego, CA, US) for 30 minutes. Dead cells were excluded using the fixable viability dye Zombie Aqua (Biolegend). For measurement of intracellular cytokines, cells were stimulated with phorbol 12-myristate 13-acetate (PMA; 100 ng/ml) and ionomycin (1 μ g/ml) for 16 h at 37°C. Monensin and brefeldin A (eBiosciences) were added for the last 4 h of incubation. Cells were fixed, permeabilized (Fixation Buffer Cat. No. 420801, Biolegend; Permeabilization Wash Buffer Cat. No. 421002, Biolegend) and stained with antibodies.

Instrument

Flow cytometry data was collected using a LSRII and BD LSRFortessa flow cytometer (BD Biosciences, San Jose, CA).

Software

Flow cytometry data was analyzed using FACS Diva software and FlowJo v10.5.3.

Cell population abundance

No cell sorting was performed in this study.

Gating strategy

Among live (dead cell via viability dye Zombie Aqua (Biolegend)), single cells:

CD4 T cells were gated as: CD3+CD4+ and stained for intracellular IL-13.

For ILC2 flow, cells were stained with PerCP-Cy5.5 or APC-Cy7-conjugated lineage marker mAbs (FcεRI, MAR-1, Biolegend; CD3ε, 145-2C11, BD Pharmingen; TCRβ, H57-597, BD Pharmingen; B220, RA3-682, BD Pharmingen; CD49b, DX5, eBioscience; CD11b, M1/70, BD Pharmingen; CD11c, N418; Biolegend; Gr1, RB6-85C, BD Pharmingen; NK1.1, PK136, Biolegend), Alexa Fluor700-conjugated CD45 (30-F11; BD Pharmingen), BV605-conjugated CD90.2 (53-2.1, BD), APC-e780-conjugated CD25 (PC615, eBioscience), PE-Cy7-conjugated ICOS (C398.4A; Biolegend), BV421-conjugated IL-33Rα (DIH9, Biolegend) and FITC-conjugated T1/ST2 (DJ8, MD Bioproducts). After cell surface staining, cells were fixed, permeabilized and stained for intracellular IL-13-PE.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

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Serum amyloid A is a soluble pattern recognition receptor that drives type 2 immunity

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Bernhard Kratzer² , **Peter A. Tauber**² , **Xiao Xiao**¹, **Nu Yao**¹, **Jan Dvorak**^{4,5,6}, **Luis Caraballo**⁷,
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SAA1 species	Blot 13:SAA 1 relation	
	2:1	4:1
monomer	49.57% $\pm$ 7.4%	54.17% $\pm$ 6.1%
dimer	56.5% $\pm$ 5.2%	50.5% $\pm$ 9.5%
trimer	68.6% $\pm$ 3.7%	80.2% $\pm$ 5.4%

Supplementary table 1. Chemical cross-linking of SAA1 and Blot 13 (used in Fig. 3c). Blot 13-induced decrease in optical density compared to SAA1 alone is shown as mean percent of all glutaraldehyde concentrations for the monomer, dimer and trimer band. Densitometric analysis was performed using Image Studio Lite software. Data represent mean $\pm$ SEM and are pooled from 2 independent experiments with n=8 samples per monomer, dimer and trimer condition.

disease status	age	sex	nasal polyps
control	59	f	no
control	46	f	no
control	66	m	no
control	69	m	no
control	56	m	no
control	20	m	no
control	63	f	no
control	24	m	no
control	43	f	no
control	50	m	no
control	61	f	no
control	44	f	no
control	28	m	no
control	68	f	no
control	58	m	no
control	25	f	no
CRS	32	f	no
CRS	69	m	no
CRS	31	m	no
CRS	67	f	no
CRS	63	f	no
CRS	51	m	no
CRS	64	m	no
CRS	66	m	no
CRS	47	f	no
CRS	80	m	no
CRS	56	f	yes
CRS	n/a	n/a	yes
CRS	68	f	yes
CRS	63	m	yes
CRS	57	m	yes
CRS	31	m	yes
CRS	53	m	yes
CRS	47	m	yes
CRS	52	n/a	yes
CRS	n/a	n/a	n/a
CRS	59	m	no
CRS	73	f	no
CRS	49	m	yes

CRS	30	m	yes
CRS	45	f	no
CRS	79	f	no
CRS	42	m	yes

Supplementary table 2. Patient information for chronic rhinosinusitis patients (used in Fig. 8a and b).

disease status	age	sex	other manifestations and medication	SAA in blot
control	n/a	f	Graves disease	yes
control	60	f	nasal tumor, steroids	yes
control	61	f	n/a	yes
control	50	m	Graves disease, steroids	yes
control	48	m	nasal tumor steroids	no
control	60	f	nasal tumor	yes
control	18	m	n/a	yes
CRS	64	m	mild asthma, steroids	yes
CRS	66	m	steroids	yes
CRS	80	m	localized sinus infection	yes
CRS	n/a	n/a	n/a	no
CRS	58	m	steroids	no
CRS	61	f	polyps, allergies, asthma	yes
CRS	64	f	allergy, asthma	yes
CRS	40	m	polyps, allergies	yes
CRS	77	m	allergies, desensitization	no
CRS	35	f	allergy, asthma	yes
CRS	n/a	n/a	sinus blockage, allergies	yes
CRS	56	m	n/a	no

Supplementary table 3. Patient information for donors used in cell culture experiments

(used in Fig. 8c-e). n/a= not available

disease status	age	sex	PC20	FEV1	IgE reactivity	medication
control	21	f	36.92	3,51 L (106%)	no	no
control	50	m	>256	4 L (80%)	no	no
control	22	f	>128	3,52 L (108%)	no	no
control	38	m	>128	4,4 L (112%)	no	no
control	24	m	41,67	3,28 L (80%)	no	no
control	30	f	40,4	3,36 L (109%)	no	no
mild asthmatic	31	f	3.11	3,78 (127%)	cat hair, cat pelt, dog hair, horse hair, grasses, ragweed, house dust mites	Ventolin as needed
mild asthmatic	26	f	1.1	3,52 (101%)	cat hair, cat pelt, trees, ragweed, house dust mites	Ventolin as needed
mild asthmatic	57	f	n/a	1,26 L (58%)	trees, animals	n/a
mild asthmatic	49	f	n/a	1,78 (67%)	animals, pollens	n/a
severe asthmatic	62	f	n/a	1,53L (66%)	ragweed, house dust mites	1500mcg eq. fluticasone/day
severe asthmatic	64	f	n/a	1,71L (72%)	trees, house dust mites	1125mcg eq. fluticasone/day

Supplementary table 4. Patient information for asthmatics (used in Extended Data. 10a and b). n/a= not available

disease status	HDM specific IgE [kIU/L]	other allergies	total IgE [kIU/L]	SAA1 µg/ml
control	<0.35		24.2	0.04
control	<0.35		223	0.4752
control	<0.35		2	0.9266
control	<0.35		96.2	0.5612
control	<0.35		41.2	0.7836
control	<0.35		2	0.04
control	<0.35		11.8	0.04
control	<0.35		27	1.561
control	<0.35		55.5	0.291
control	<0.35		14.8	0.41652
control	<0.35		9.29	0.22548
control	<0.35		10.2	0.30712
control	<0.35		16.7	1.801
control	<0.35		2	0.1
control	<0.35		97.8	0.23688
control	<0.35		17.9	0.18064
control	<0.35		37.2	0.1536
control	<0.35		146	1.0048
HDM allergic	12.4	x	147	0.924
HDM allergic	65.1	x	411	0.7248
HDM allergic	1.88	x	320	2.0956
HDM allergic	0.96		80.7	2.0858
HDM allergic	1.10		104	1.0198
HDM allergic	1.72	x	111	0.6628
HDM allergic	14.7		167	1.3328
HDM allergic	17.3	x	698	0.499
HDM allergic	1.89		19.8	0.40726
HDM allergic*	<0.35		8.14	2.3106
HDM allergic	6.46	x	158	0.5806
HDM allergic*	<0.35		4.42	0.7532
HDM allergic	1.30	x	506	1.0494
HDM allergic	0.49	x	583	1.957
HDM allergic	4.35		23.5	0.02
HDM allergic	0.91	x	5000	1.0884
HDM allergic	19.9	x	194	0.7874
HDM allergic	0.44	x	81.5	2.123
HDM allergic	1.46	x	127	0.2606
HDM allergic	0.67		15.5	2.1324
HDM allergic	11.80	x	362	0.4896
HDM allergic	4.12		17.2	0.35172
HDM allergic	0.38	x	99	0.20642
HDM allergic	3.77		14.2	0.7814
HDM allergic*	<0.35		19.1	1.026
HDM allergic	15.10		146	0.511
HDM allergic	1.06		8.43	0.1

Supplementary table 5. Patient information for serum samples of HDM allergic patients

(used in Fig. 8f). * indicates patients with positive anamneses but negative CAP results. x = presence of other allergies.

mouse RT primer		
Gene	forward	reverse
<i>Il4</i>	5'-TGAACGAGGTCACAGGAGAA-3'	5'-CGAGCTCACTCTCTGTGGTG-3'
<i>Il5</i>	5'-GCAATGAGACGATGAGGCTT-3'	5'-CCCACGGACAGTTTGATTCT-3'
<i>Il13</i>	5'-TGTGTCTCTCCCTCTGACCC-3'	5'-CACACTCCATACCATGCTGC-3'
<i>Il17</i>	5'-TCCAGAAGGCCCTCAGACTA-3'	5'-TGAGCTTCCCAGATCACAGA-3'
<i>Il33</i>	5'-TCCCAACAGAAGACCAAAG-3	5'-GATACTGCCAAGCAAGGAT-3'
<i>Il25</i>	5'-ATTTCCCCGGCAAAGTTCAG-3	5'-AACGGAGTCTCATGCAGTAGA-3'
<i>Tslp</i>	5'-ACGGATGGGGCTAACTTACAA-3	5'-AGTCCTCGATTTGCTCGAACT-3
<i>Saa1</i>	5'-GTCTTCTGCTCCCTGCTCC-3'	5'-TCATGTCAGTGTAGGCTCGC-3'
<i>Fpr2</i>	5'-ACACCACAGGAACCGAAGAG-3'	5'-CCACCACTTCTGATCCATTCA-3'
<i>St2</i>	5'-CGTGTCCAACAATTGACCT-3'	5'-CAAGTAGGACCTGTGTGCCC-3'
<i>S14</i>	5'-TGGTGTCTGCCACATCTTTGCATC-3'	5'-AGTCACTCGGCAGATGGTTTTCCTT-3'
human RT primer		
Gene	forward	reverse
<i>SAA1</i>	5'-AGCCGAAGCTTCTTTTCGTT-3'	5'-GCCGATGTAATTGGCTTCT-3'
<i>FPR1</i>	5'-CCAGGAGACCCAGACCTAGA-3'	5'-GAGATGTTCGTGGGGAGAGA-3'
<i>FPR2</i>	5'-GCACACAGGAAAAGGAGCTT-3'	5'-GCAGAACAGTGTAGCCAGCA-3'
<i>FPR3</i>	5'-TCCACAGGAATCCAGGAAC-3'	5'-AGGGAGCACCTCCTCAGTTT-3'
<i>TLR2</i>	5'-AACCGGAGAGACTTTGCTCA-3'	5'-CCACTGACAAGTTTCAGGCA-3'
<i>TLR4</i>	5'-GGACTCTGATCCCAGCCAT-3'	5'-TGCCCCATCTTCAATTGTCT-3'
<i>EEF</i>	5'-ATTACAGGGACATCTCAGGCTGAC-3'	5'-CATTCTTGGAGATACCAGCTTCAA-3'

Supplementary table 6. Primer sequence

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☒ | ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

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Software and code

Policy information about [availability of computer code](#)

Data collection

Flow data were collected using the FACSDiva software 6.2. Immunoblot signals were detected by streptavidin-horseradish peroxidase and chemiluminescence using ECL (Biorad) and Biomax film (Kodak) or detected with a chemiluminescence detection system (ChemiDoc Bio-Rad, Berkeley, CA, US). Real-time PCR was performed on a StepOnePlus PCR system (Applied Biosystems, Foster City, CA, US). Cytokine data was collected using a Multiskan GO apparatus equipped with the SkanIt software 4.1.0.43 and 6.0.1.6 (Thermo Fisher) or a BioTek microplate reader (Fisher Scientific). For multiplexed cytokine measurements the the Luminex system (Luminex 100IS, Biomedica) was used. Airway hyperresponsiveness was followed using a RC fine pointe site (Buxco FinePointe Resistance and Compliance, DSI, Harvard Bioscience, Inc) or using a FlexiVent (SciReq, Saint-Urbain, Quebec, Canada).

Data analysis

Flow cytometry data was collected and analyzed using FACSDiva software 6.2 and FlowJo v10.5.3. Western blot images were analyzed using the LI-COR Image StudioLite Ver 5.3 or ImageJ 1.48v Software. Real-time PCR data were analyzed by the deltaCT method and using the SDS 2.1 software (Applied Biosystems). For cytokine data the SkanIt software (Thermo Fisher), Gen5™ Reader Control and Data Analysis Software 2.0 or xPONENT Software Solutions 3.1.871.0 were used. Airway hyperresponsiveness was analyzed using the FinePoint Software Research Suite v2.4.6 or Flexiware Software. Prism 7 was used for generation of graphs and statistical analysis.

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Data

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All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The datasets supporting the conclusions of this article are included within the article and its supplementary information files.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size for in vivo studies was based on previous data published using similar mouse models, stimuli and endpoint measurements (e.g. AHR and IgE). In general, we routinely used 4-8 mice per genotype and condition. For in vitro experiments, reasonable sample sizes (4-5 replicate wells) were chosen to ensure adequate reproducibility of results and was based on our previous studies.
Data exclusions	In in vivo experiments, animals were excluded in which HDM sensitization was not properly established due to technical issues or due to technical problems during APTI measurements and which would have rendered the outcome measures meaningless. Rout and Grubb's outlier test were run with default parameters in Prism to detect and exclude significant outliers.
Replication	Biological and technical replicates have been performed and the information on replicates is included in each figure legend. Most experiments have been reproduced 2-3 times with comparable results. Whenever representative data is shown, the experimental data were reproduced with similar results.
Randomization	For in vivo experiments, sex and age-matched mice derived from different breeding pairs were assigned randomly for each experiment according to their genotypes. Healthy donors were randomly selected. In vitro experiments were not randomized.
Blinding	The investigators were not blinded to experimental conditions, as all in vivo experiments were conducted and analyzed by one person. Human samples were labeled as numeric numbers and analyzed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used for flow cytometry:
 CD11c-Alexa Fluor 700, HL3, #560583, BD Pharmingen 1/400
 CD11c-PE-Cy7, N418, #117317, BioLegend 1/500
 CD11b-PE-Cy7 or APC-Cy7 M1/70, #557657 BD Pharmingen ; M1/70, #101216 BioLegend 1/500
 Ly6G-Pacific Blue, HK1.4, #128032, BioLegend 1/400
 Ly6G-BV605, 1A8, #127639 BioLegend 1/300
 MHCII-PE, M5/114.15.2, # 07608, BioLegend 1/2000

CD103-PerCP-Cy5.5, 2E7, #121416, BioLegend 1/200
 CD317-APC, eBio129c, #17-3171-82, eBioscience 1/200
 CD3e-PerCP-Cy5.5, 145-2C11, #551163, BD Pharmingen) 1/400
 CD4-FITC, RM4-5, #11-0042-82, eBioscience 1/400
 IL-13-APC, eBio13A, #47-7133-82, eBioscience 1/150
 FceRI-PerCP-Cy5.5 or APC-Cy7, MAR-1, #134325 #134319, BioLegend 1/400
 TCR β -PerCP-Cy5.5 or APC-Cy7, H57-597, #560657 #560656, BD Pharmingen 1/200
 B220-PerCP-Cy5.5 or APC-Cy7, RA3-6B2, #552094 #561101, BD Pharmingen 1/400
 CD49b-PerCP-Cy5.5 or APC-Cy7, DX5, #A15420 #46-5971-82, eBioscience, 1/200
 CD11b-PerCP-Cy5.5 or APC-Cy7, M1/70, #561114 #557657, BD Pharmingen, 1/500
 CD11c-PerCP-Cy5.5 or APC-Cy7, N418, #117327 #117323, BioLegend, 1/500
 Gr1-PerCP-Cy5.5 or APC-Cy7, RB6-8C5, #552093 #557661 BD Pharmingen 1/400
 NK1.1-APC-Cy7, PK136, #108727 #108723, BioLegend 1/250
 CD45-Alexa Fluor700, 30-F11, #560510, BD Pharmingen 1/400
 CD90.2-BV605, 53-2.1, #563008, BD Pharmingen 1/250
 CD25-APC-e780, PC61.5, #47-0251-82, eBioscience 1/250
 ICOS-PE-Cy7, C398.4A, #313519, BioLegend 1/250
 T1/ST2-FITC, DJ8, #101001F, MD Bioproducts 1/150
 IL-33 α -BV421, DIH9, # 145309, BioLegend 1/250

The following antibodies were used for cytokine ELISA:

Anti-human SAA1 antibody pairs were purchased from Novex (#KHA0011 Novex, Thermo Fisher Scientific) or human SAA1 DuoSet ELISA (#DY3019-05, R&D Systems). Human IL-33 (AF3625 capture antibody and BAF3625 detection antibody), IL-6 (#DY206) and IL-8 (#DY208) ELISA were from R&D Systems. Mouse SAA1 capture antibody (D9H4L41) from Thermo Fisher (#700830) and mouse SAA1 detection antibody from R&D Systems (#BAF2948). Mouse IL-25 (#88-7002-88) and TSLP (#88-7490-88) ELISA were from Invitrogen. Mouse IL-33 ELISA was from R&D Systems (#DY3626). Mouse cytokine ELISA kits specific for IL-5 (#DY405), IL-13 (#DY413), IL-17A (#DY421), and IFN (γ) (DY421) were from R&D Systems or eBioscience IL-5 (TRFK5 #14-7052-81/TRFK4 #13-7051-81), IL-13 (eBio13A #12-7133-82/eBio1316H #13-7135-81), IL-17A (eBio17B7 #16-7135-81/eBio17CK15A5 #13-7135-81), and IFN (γ) (AN-18 #14-7313-81/R4-6A2 #13-7312-81) were.

The following antibodies were used for immunoblotting and depletions:

SAA1 content by Western-blotting was tested with mouse anti-human SAA1 antibody (clone 607 #DM1004B, Acris, Origene; clone 865504 #MAB30196, R&D Systems) or sequence-specific antibodies as described (Malle, E. et al. Mapping of antigenic determinants of purified, lipid-free human serum amyloid A proteins. Scand. J. Immunol. 48, 557-561 (1998). Goat anti-human ApoA1 antibody (R&D systems, Minneapolis, MN, US) was used for HDL pull downs. For Der p 13 neutralization and depletion experiments, a monoclonal antibody directed against its closely related homologue Blo t 13 was used (Labrada, M. et al. Monoclonal antibodies against Blo t 13, a recombinant allergen from Blomia tropicalis. Int. Arch. Allergy Immunol. 129, 212-218 (2002).

Validation

All antibodies used for flow cytometry and cytokine ELISA are from commercial sources and have been validated by vendors, and have been titrated and used by our own lab in previous experiments. Validation data for species and application are available on the manufacturer's website (checkable via the clone or catalogue number). Antibodies used for immunoblotting were either purchased from best available vendors and are also validated by the vendors. Sequence-specific antibodies raised against SAA1 peptides have been validated previously in Malle, E. et al. Mapping of antigenic determinants of purified, lipid-free human serum amyloid A proteins. Scand. J. Immunol. 48, 557-561 (1998). The monoclonal antibody directed against its closely related homologue Blo t 13 was validated in Labrada, M. et al. Monoclonal antibodies against Blo t 13, a recombinant allergen from Blomia tropicalis. Int. Arch. Allergy Immunol. 129, 212-218 (2002). Controls included matched isotype control as well as positive and negative control samples.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	BEAS-2B and Caco-2 cell lines were from American Type Culture Collection (ATCC, Manassas, VA, US).
Authentication	None of the cell lines have been authenticated.
Mycoplasma contamination	These cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Unless otherwise specified, experiments were performed with mice between 6-8 weeks of age. BALB/cJ mice were originally obtained from Taconic (Rensselaer, NY, US) and bred in-house at the Bloomberg School of Public Health animal facility. For BALB/cJ experiments, age-matched female mice were used. Saa knockout mice on the C57BL/6 background were a kind gift from Maria C. deBeer (University of Kentucky Medical Center, KY, US) and bred in-house at the Medical University of Vienna. WT C57BL/6J mice were originally obtained from Charles River (Sulzfeld, Germany) and bred in-house at the Medical University of Vienna. For C57BL/6J experiments age-matched male and female mice were used in individual experiments. All in vivo
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experiments were approved by the Institutional Animal Use and Care Committee at Johns Hopkins Bloomberg School of Public Health and the Medical University of Vienna .

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field-collected samples.

Ethics oversight

All in vivo experiments were approved by the Institutional Animal Use and Care Committee at Johns Hopkins Bloomberg School of Public Health and the Medical University of Vienna .

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Inclusion criteria for patients with CRS included continuous symptoms of rhinosinusitis as defined by the AAO-HNS Chronic Rhinosinusitis Task Force for greater than 12 weeks, computed tomography of the sinuses revealing isolated or diffuse sinus mucosal thickening or air-fluid levels, and nasal polyps visible on diagnostic endoscopy. The asthmatic subjects were diagnosed according to the American Thoracic Society criteria. Asthmatic subjects were atopic, and had a positive skin prick test to common aeroallergens, methacholine PC20 of <8 mg/ml, and FEV1 >70% predicted, with only intermittent use of β_2 -agonists. Control subjects were non-atopics, non-smokers, with no history of asthma or airway and systemic diseases. Inclusion of allergic individuals suffering from HDM allergy in this study was based on a characteristic history of clinical symptoms (allergic conjunctivitis, rhinitis, urticaria, and asthma) and on at least one of the following diagnostic criteria: a positive skin prick test or a positive ImmunoCAP.

Recruitment

Subjects for the collection of primary nasal epithelial cells were recruited from the Johns Hopkins Outpatient Rhinology Clinic. All subjects gave signed informed consent. Inclusion criteria included continuous symptoms of rhinosinusitis as defined by the AAO-HNS Chronic Rhinosinusitis Task Force for greater than 12 weeks, computed tomography of the sinuses revealing isolated or diffuse sinus mucosal thickening or air-fluid levels, and nasal polyps visible on diagnostic endoscopy. Subjects for the collection of bronchial epithelial cells were recruited from the Asthma Clinic at Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec, QC, Canada). The asthmatic subjects were diagnosed according to the American Thoracic Society criteria. Control subjects were non-atopics, non-smokers, with no history of asthma or airway and systemic diseases. HDM allergic subjects and matched healthy controls for the collection of plasma were recruited at the Medical University of Vienna. Blood samples were collected between 2014–2017 after signing a patient information and an informed consent. Diagnosis was based on a clinical history of typical symptoms like (allergic conjunctivitis, rhinitis and asthma) and a positive ImmunoCAP.

Ethics oversight

In case of primary nasal epithelial cells, the research protocol was approved through the Johns Hopkins Institutional Review process, and all subjects gave signed informed consent. The study with bronchial epithelial cells was approved by the IUCPQ (Quebec, Canada) ethics committee, and all subjects gave written informed consent. For studies with human plasma, approval was obtained from the ethics committee of the Medical University of Vienna and informed consent was obtained from the volunteer donors (EK1538/2014).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Murine lungs were excised, minced, and digested in serum-free RPMI containing Liberase CI (0.5 mg/ml, Roche) and DNase I (0.5 mg/ml, Sigma) at 37°C for 45 min. Cells were washed with RPMI containing 10% (v/v) FBS and viable cells were counted by trypan blue exclusion. All staining reactions were performed at 4°C following incubation with FcBlock (TruStain fcX antibody, Biolegend, San Diego, CA, US) for 30 minutes. Dead cells were excluded using the fixable viability dye Zombie Aqua (Biolegend). For measurement of intracellular cytokines, cells were stimulated with phorbol 12-myristate 13-acetate (PMA; 100 ng/ml) and ionomycin (1 μ g/ml) for 16 h at 37°C. Monensin and brefeldin A (eBiosciences) were added for the last 4 h of incubation. Cells were fixed, permeabilized (Fixation Buffer Cat. No. 420801, Biolegend; Permeabilization Wash Buffer Cat. No. 421002, Biolegend) and stained with antibodies.

Instrument

Flow cytometry data was collected using a LSRII and BD LSRFortessa flow cytometer (BD Biosciences, San Jose, CA).

Software

Flow cytometry data was analyzed using FACS Diva software and FlowJo v10.5.3.

Cell population abundance

No cell sorting was performed in this study.

Gating strategy

Among live (dead cell via viability dye Zombie Aqua (Biolegend)), single cells:

CD4 T cells were gated as: CD3+CD4+ and stained for intracellular IL-13.

For ILC2 flow, cells were stained with PerCP-Cy5.5 or APC-Cy7-conjugated lineage marker mAbs (FcεRI, MAR-1, Biolegend; CD3ε, 145-2C11, BD Pharmingen; TCRβ, H57-597, BD Pharmingen; B220, RA3-682, BD Pharmingen; CD49b, DX5, eBioscience; CD11b, M1/70, BD Pharmingen; CD11c, N418; Biolegend; Gr1, RB6-85C, BD Pharmingen; NK1.1, PK136, Biolegend), Alexa Fluor700-conjugated CD45 (30-F11; BD Pharmingen), BV605-conjugated CD90.2 (53-2.1, BD), APC-e780-conjugated CD25 (PC615, eBioscience), PE-Cy7-conjugated ICOS (C398.4A; Biolegend), BV421-conjugated IL-33Rα (DIH9, Biolegend) and FITC-conjugated T1/ST2 (DJ8, MD Bioproducts). After cell surface staining, cells were fixed, permeabilized and stained for intracellular IL-13-PE.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Figure 3a

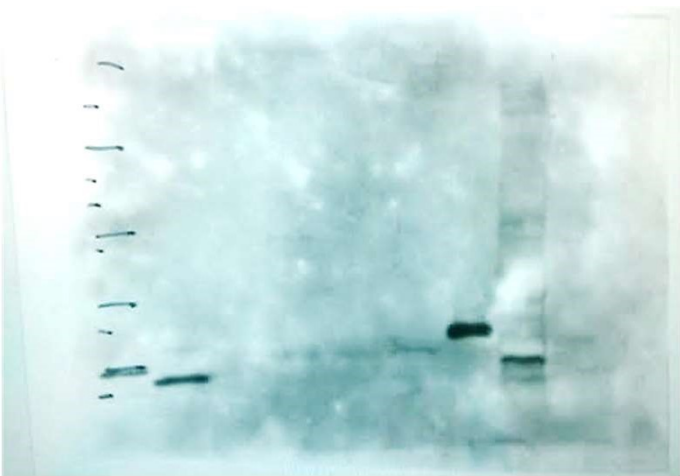


Figure 3b



Figure 3c

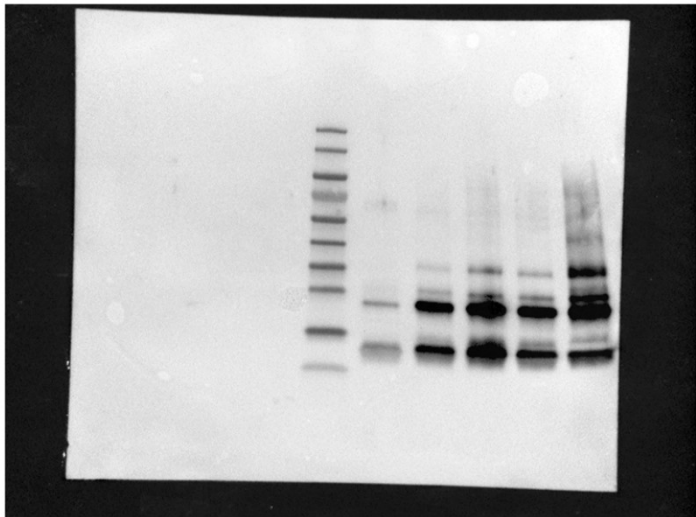


Figure 3c

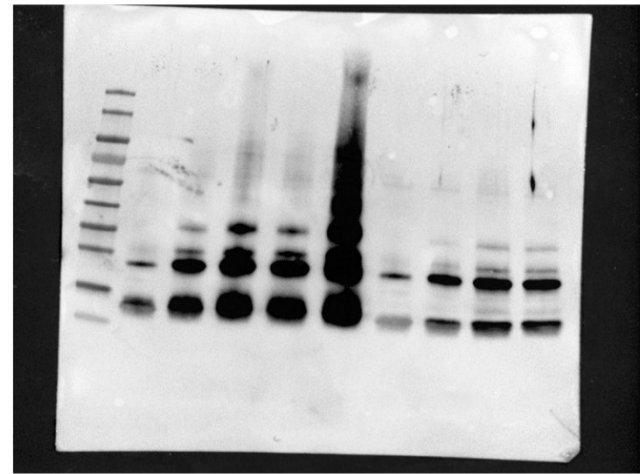


Figure 3j

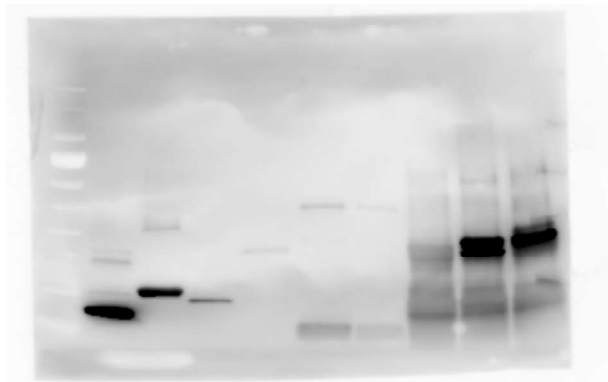


Figure 3k

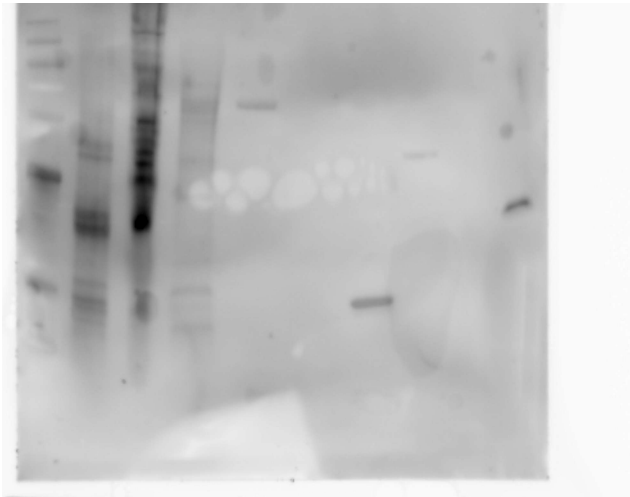




Figure 5b

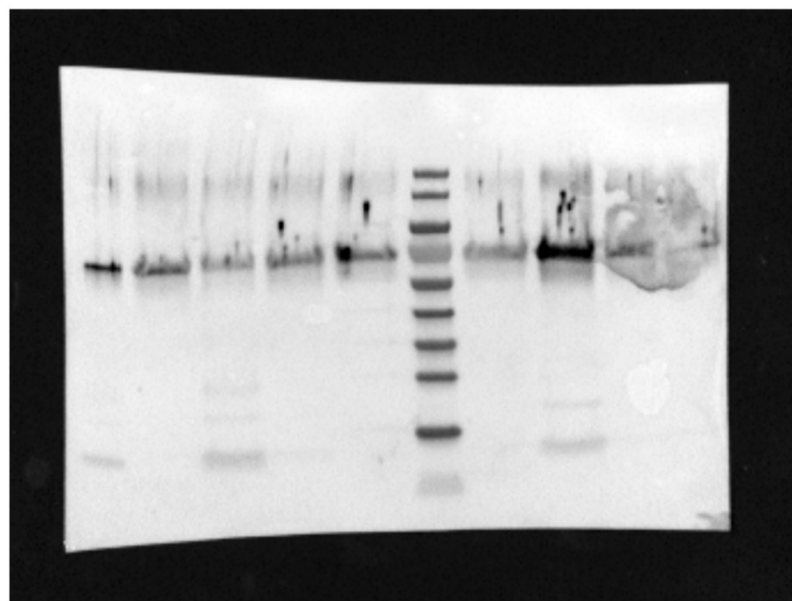


Figure 5d

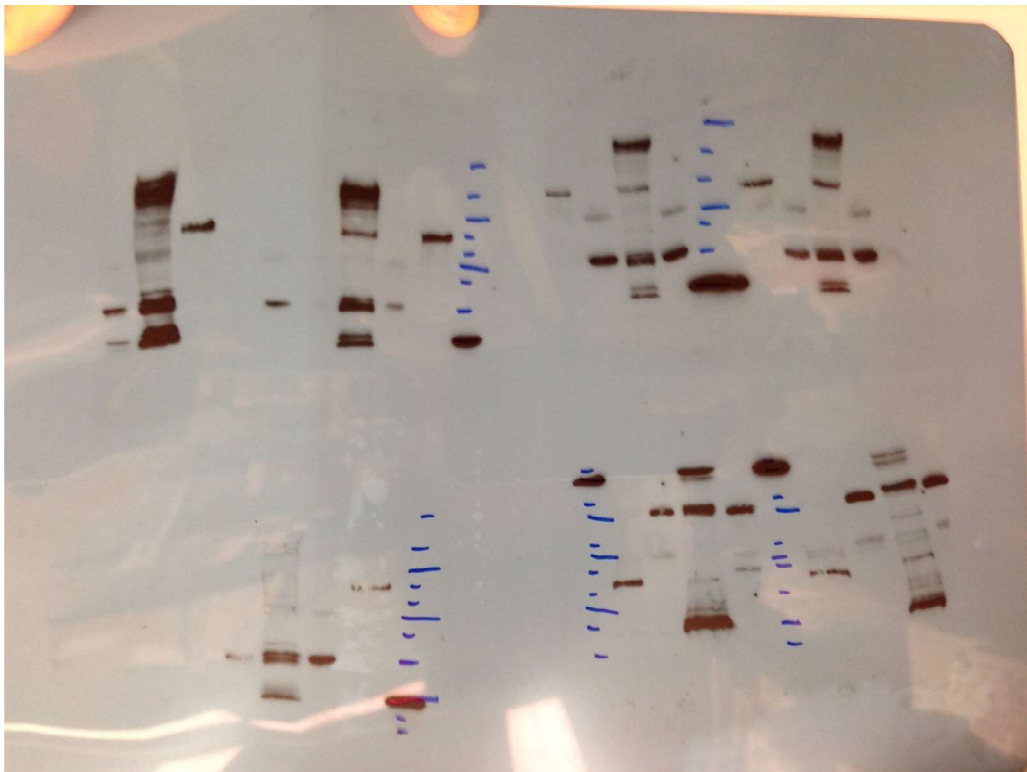
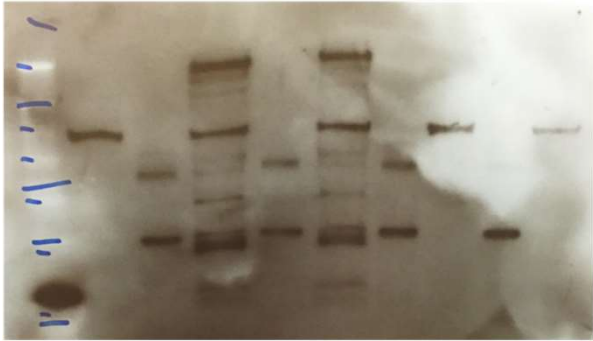
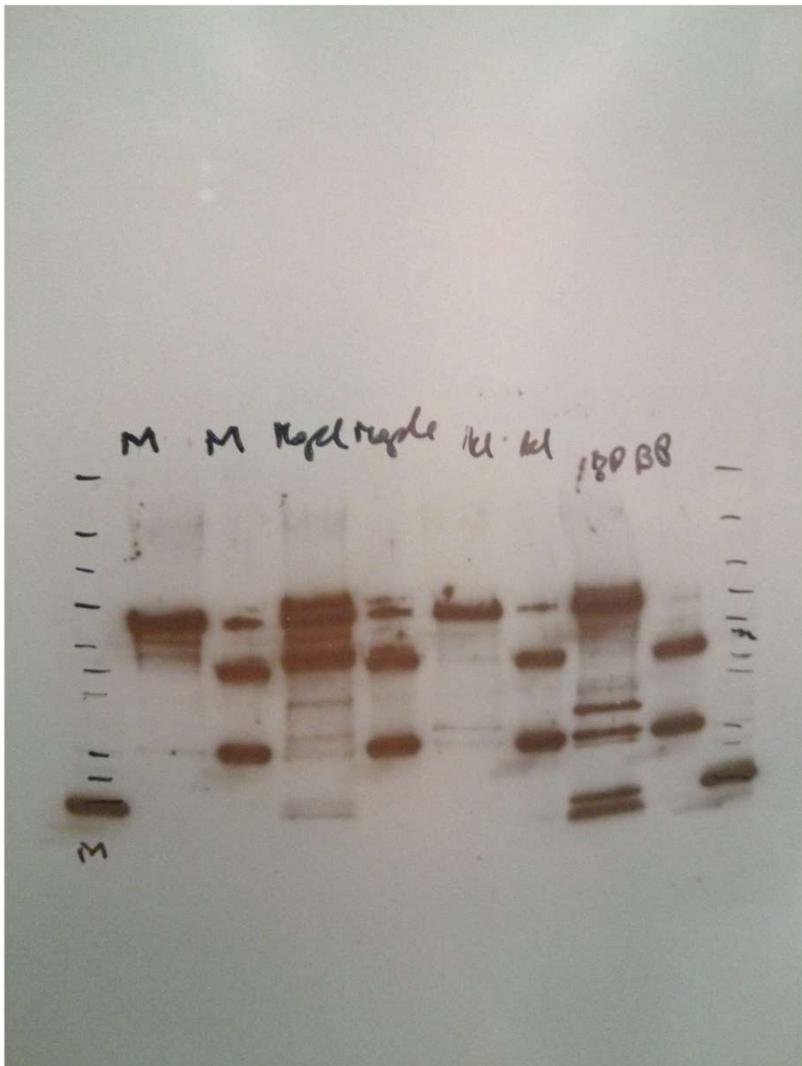


Figure 8d



Extended Data Figure 7c



Extended Data Figure 9a