HHS Public Access

Author manuscript

Cell Rep. Author manuscript; available in PMC 2023 April 15.

Published in final edited form as:

Cell Rep. 2023 March 28; 42(3): 112208. doi:10.1016/j.celrep.2023.112208.

LMAN1 is a receptor for house dust mite allergens

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SUMMARY

Development of therapies with the potential to change the allergic asthmatic disease course will require the discovery of targets that play a central role during the initiation of an allergic response, such as those involved in the process of allergen recognition. We use a receptor glycocapture technique to screen for house dust mite (HDM) receptors and identify LMAN1 as a candidate. We verify the ability of LMAN1 to directly bind HDM allergens and demonstrate that LMAN1 is expressed on the surface of dendritic cells (DCs) and airway epithelial cells (AECs) *in vivo*. Overexpression of LMAN1 downregulates NF-κB signaling in response to inflammatory cytokines or HDM. HDM promotes binding of LMAN1 to the FcRγ and recruitment of SHP1. Last, peripheral DCs of asthmatic individuals show a significant reduction in the expression of LMAN1 compared with healthy controls. These findings have potential implications for the development of therapeutic interventions for atopic disease.

Graphical Abstract

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AUTHOR CONTRIBUTIONS

J.T.T.-A. was involved in the conceptualization and design of the study. Experimentation was performed by M.H.M., L.G.S., V.S.V., M.L., T.J.J., D.J.H., and J.T.T.-A. Validation was performed by M.H.M. and J.T.T.-A. Formal analysis was provided by X.Z. Resources were provided by Y.Z., B.Z., and J.T.T.-A. M.H.M., T.J.J., X.Z., B.Z., and J.T.T.-A. wrote the original draft of the manuscript. J.T.T.-A. and B.Z. acquired the funding to support the project. J.T.T.-A., L.G.S., and M.H.M. performed the additional experimentation and wrote the changes for the revised, final manuscript.

DECLARATION OF INTERESTS

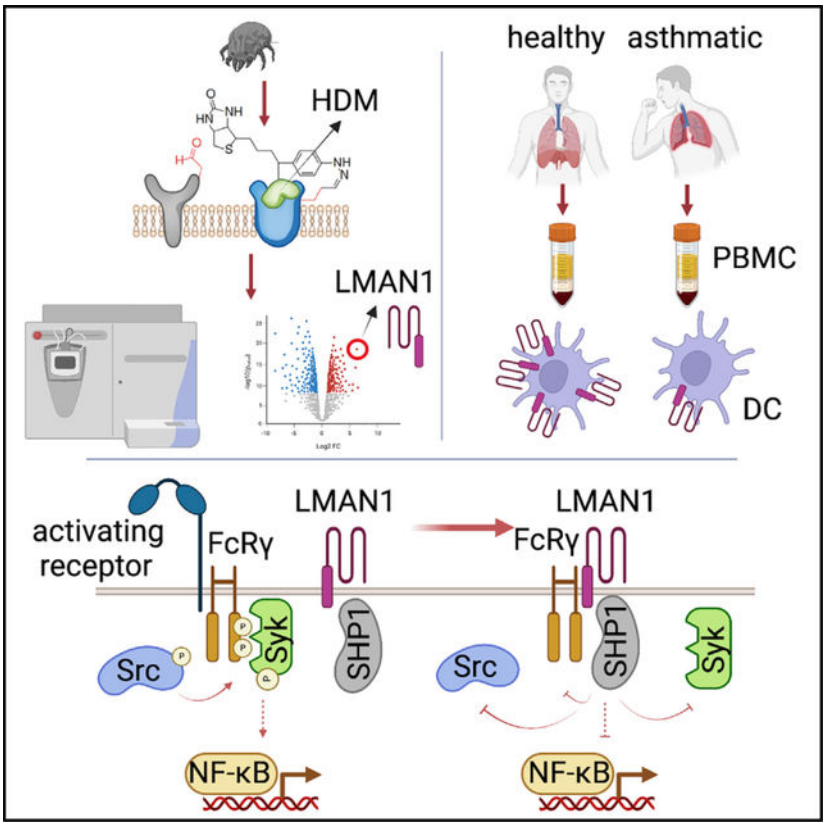
The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2023.112208.



In brief

Miller et al. describe the identification of LMAN1 as a receptor for HDM using an unbiased receptor glycocapture screen. Engagement of LMAN1 by HDM restrains inflammatory responses. Such regulatory mechanisms may be disrupted in asthmatic individuals who show reduced expression of LMAN1 on DCs.

INTRODUCTION

Allergic sensitization is strongly correlated with the development of atopic diseases such as allergic asthma. Current estimates report that >70% of asthmatic individuals are sensitized to at least one allergen, with house dust mite (HDM) being the most common sensitizer. While impressive progress has been made in the development and translation of novel biologics for treatment of asthma (Omalizumab [anti-immunoglobulin (Ig)E],[1–10] Mepolizumab [anti-interleukin(IL)-5],[11–14] Reslizumab [anti-IL-5],[14–20] Benralizumab [anti-IL-5Rα],[14,21–23] and Dupilumab [anti-IL-4Rα][24–26]), use of such agents are limited to individuals with severe asthma whose disease remains uncontrolled with mainstay therapies and only if they meet certain treatment criteria (high IgE levels or high eosinophil levels). These current biologics, as well as many that are still in development, focus on T helper 2 (Th2) high disease, targeting mediators central to sustaining the type 2-driven asthmatic disease process. Thus, despite the efficacy of these therapies for most asthmatic individuals who fall within this endotype, the limitations of use, high cost, and clinical non-responsiveness of a

significant proportion of patients who meet the treatment criteria, underscore the need for identifying new drug targets for this chronic inflammatory disease.

Most therapies under development target mediators produced late in the allergic asthmatic response with the goal of alleviating asthmatic symptoms. However, there is evidence to suggest that interfering with events that occur early during the allergic cascade have a significant impact on modulating the disease course and even have the potential to be curative. In support of this, allergen immunotherapy (AIT), which has been demonstrated to act at the level of the dendritic cell (DC) to promote the development of regulatory T cells (Tregs) or T helper type 1 (Th1) cells instead of Th2, can result in long-term remission (essentially curative).[27] Similarly, anti-TSLP therapy (Tezepelumab by AstraZeneca and CSJ117 by Novartis),[28–31] which also interferes with early events during allergic sensitization, has shown remarkable efficacy in clinical trials, resulting in significant and clinically meaningful decreases in annualized asthma exacerbation rates as well as decreased blood and sputum eosinophil levels, fraction of exhaled nitric oxide (FeNO) and serum IgE.[30] Like AIT, anti-TSLP therapies also work by modulating the phenotype and function of DCs, in turn, affecting the subsequent T cell polarization. These studies suggest that interfering with early allergen sensitization events, such as those occurring at the level of the DC, are likely to be efficacious. One of the earliest steps in this process is the recognition of allergens.

Initial binding of allergens is mediated by airway epithelial cells (AECs) and DCs. Upon exposure to allergens, AECs release the alarmins IL-25, IL-33, and TSLP, which play pivotal roles in activating DCs and imparting a DC phenotype that can prime naive $CD4^+$ T cells toward the Th2 lineage.[32–37] Such alarmins also function to enhance and sustain Th2 function. Both AECs and DCs (specifically, type 2 conventional DCs [cDC2s]) as well as the interaction between these cell types, have been shown to be essential in driving sensitization to HDM.[38–44] Given the key functions of DCs in recognition of allergens, integration of environmental cues (from AECs, basophils, ILC2s, etc.) and serving as a bridge to adaptive immunity, we have chosen to focus on this particular cell type.

While immune sensing of bacteria, viruses, and fungi are almost exclusively mediated by the recognition of pathogen-associated molecular patterns by pattern recognition receptors, the same cannot be said for allergens. Numerous studies have been performed to identify molecular or biochemical features shared by multiple allergens that might point to a common mode of allergen recognition. However, such efforts have been met with limited success. More recent research indicates that allergens such as HDM appear to be sensed through a wider array of mechanisms that include the activation of protease activated receptors (PARs),[45–48] triggering of pathways involved in sensing cellular stress or damage (endoplasmic reticulum [ER] stress response, mechanosensing, release of ATP),[49–51] and the activation of sensory neurons.[52] Thus, the lack of one main ligand or molecular pattern combined with the unpredictability of potential receptors used, has made targeting the process of allergen recognition somewhat arbitrary. In this work, we use an unbiased receptor glycocapture technique that can incorporate a complex ligand mixture such as HDM to identify receptors for this allergen on DCs. By validating our findings using biochemical, *in vitro*, *in vivo*, and *ex vivo* systems, we propose that such unbiased

approaches are not only feasible but may be necessary to identify mechanisms of allergen recognition that, in the future, may be therapeutically targeted in the setting of allergic disease.

RESULTS

Identification of LMAN1 as a candidate receptor for HDM

In order to identify receptors for HDM, we used CaptiRec, an unbiased ligand-receptor glycocapture technique.[53] HDM (experimental ligand) and transferrin (control ligand) were each separately conjugated to the TriCEPs reagent. THP-1 human monocytic cells differentiated into DC cells[54] were mildly oxidized prior to incubation with TriCEPs conjugated ligands to allow for receptor capture. Cells were then washed and pellets sent out for purification of receptor-ligand complexes and mass spectrometry analysis (DualSystems). The volcano plot indicates identified receptors for each ligand with those falling within the white area considered as likely candidate receptors and those falling within the gray area as unlikely (Figure 1). Successful identification of the transferrin receptor (TFR1) using the control ligand transferrin confirms the technical soundness of the assay (Figure 1, left side of volcano plot) while use of the experimental ligand HDM led to the identification of lectin, mannose binding 1 (LMAN1), CD36, and F205C as candidate HDM receptors (Figure 1, right side of volcano plot). Of these, LMAN1 and CD36 were considered as highly likely based on fold enrichment and number of peptides identified (Figure S1). For proteomic analysis, at least two peptides are required for confident identification of proteins and for this reason, F205C was considered an unlikely candidate. Given that CD36 had previously been described as a receptor for phosphorylcholine moieties on HDM,[55] subsequent studies were focused on verifying LMAN1 as a *bona fide* HDM receptor.

LMAN1 binds to HDM allergen extract and purified major HDM allergens on the cell surface

To validate LMAN1 as a true receptor for HDM, FLAG-tagged LMAN1 was overexpressed in HEK293 cells and affinity purified prior to use in a binding assay with increasing amounts of *Dermatophagoides pteronyssinus* extract (D. pter) or with the major HDM allergen Der p 1. This resulted in a significant amount of interaction for both D. pter and Der p 1, which occurred in a dose-dependent fashion (Figure 2A). LMAN1 is more widely recognized as a cargo receptor, shuttling a select group of proteins between the ER, ERGIC and Golgi.[56] To confirm whether LMAN1 also transits to the cell surface for interaction with HDM, we performed confocal microscopy on unpermeabilized dendritic cells and observed colocalization between LMAN1 and the HDM allergen Der p 1 at discrete regions on the cell surface (Figure 2B). Additionally, it has been reported that LMAN1 exists endogenously as homohexamers.[57] Thus, we also conducted a series of cellular binding assays to confirm interactions between oligomeric LMAN1 and HDM. Murine DC2.4 dendritic cells or human THP-1 monocytic cells were virally transduced with appropriate vectors to under or overexpress LMAN1. Total and cell surface LMAN1 expression for each cell line was confirmed (Figure S2). A flow cytometry-based cellular binding assay between each of the cell lines and biotinylated HDM extract or biotinylated HDM allergens was then performed. In each case, for both the murine DC2.4 cell line and

the human THP-1 cell line, significantly less HDM was bound when cells underexpressed LMAN1 and significantly more HDM was bound when cells overexpressed LMAN1 (Figure 2C). This was true whether total HDM allergens (D. pter) or purified allergens (Der p 1) were used (Figure 2C). The same trend was also observed when a different dust mite species was used (*D. farinae*, [Figure S3]). Altogether, these data suggest that LMAN1 can directly bind to HDM allergens on the cell surface.

LMAN1 binds to HDM in a CRD-dependent manner

LMAN1 serves as a cargo receptor for various mannosylated ligands[58–61] and certain HDM allergens have also been demonstrated to be mannosylated.[62] LMAN1 is composed of an N-terminal signal sequence (blue), a carbohydrate recognition domain (CRD, purple), a helical or stalk domain (Helix, green), a transmembrane region (orange), and a short cytoplasmic region (pink) (Figure 3A). In order to determine whether the CRD domain of LMAN1 is responsible for binding to HDM, we transiently transfected HEK293 cells with either wild-type (WT) LMAN1, LMAN1 lacking the CRD domain (ΔCRD), LMAN1 lacking the Helix domain (ΔHelix), or LMAN1 in which the N156 residue within the CRD domain was mutated to alanine (N156A) (Figure 3A). The N156 LMAN1 residue has been shown to be important for binding calcium and binding of LMAN1 to various mannosylated cargo has been demonstrated to be calcium dependent.[63] Thus, disruption of LMAN1 calcium binding indirectly impairs carbohydrate binding. Expression of each of these constructs was verified by SDS-PAGE and western blot and cell surface expression of LMAN1 was also assessed by FACS (Figure 3A). Such cells were then subjected to a flow cytometry-based binding assay similar to Figure 2. We observed that when transfected LMAN1 lacked the CRD or was mutated at the N156 residue, binding to D pteronyssinus extract was significantly reduced compared with when WT LMAN1 was transfected (Figure 3B). Such reduction in binding was not observed when LMAN1 lacked the Helix region (Figure 3B). A similar and even more significant trend was observed when binding to Der p 1 was assessed (Figure 3B). This would be in line with previous studies which report that Der p 1 (as well as other *D. pteronyssinus allergens*) are highly mannosylated.[62] Altogether, these data suggest that LMAN1 binds mannosylated HDM allergens via its CRD.

Resident lung DCs and AECs express LMAN1

In order to determine whether expression of LMAN1 was relevant to binding and/or uptake of HDM *in vivo*, we administered fluorescent HDM intratracheally (i.t.) into naive mice. We observed two populations of cells: an HDM^{lo} population and an HDM^{hi} population (Figure 4A). When we additionally stained for LMAN1, we discovered that LMAN1 was expressed largely by the HDM^{hi} population, suggesting a positive correlation between LMAN1 expression and efficient HDM binding and/or uptake (Figure 4A). To determine the identity of these $HDM^{hi}LMAN1^{+}$ cells, we performed flow cytometric analysis of different lung cell populations. We found that LMAN1 was expressed at a low level on alveolar macrophages and highly expressed on various lung DC populations (cDC2s, cDC1s, and pDCs, ~95%) (Figures 4B and 4C). We additionally found LMAN1 expression on $EpCAM^{+}$ AECs (~80%) (Figure 4D). In order to determine whether LMAN1 was also present on cells recruited to the lung, we performed an HDM model of asthma and subjected d14 BAL cells to FACs (Figure S4). We observed that LMAN1 was present on a small (~15%) but

distinct population of T cells and a larger fraction of B cells (~37%) and neutrophils (~42%) (Figure S4). These data demonstrate that LMAN1 is expressed basally on multiple cell types relevant to allergen sensing in the lung, in particular, on lung DCs and AECs and can also be found expressed on subpopulations of recruited T cells, B cells, and neutrophils.

LMAN1 downregulates NF-κB activation in response to inflammatory stimuli or HDM

In order to identify a potential function for LMAN1, we stimulated each of the previously generated LMAN1 under or overexpressing cell lines with or without HDM. We observed that when LMAN1 was underexpressed, there was a trend for an increase in the production of various inflammatory chemokines in response to HDM compared with a control DC cell line (Figure S5). The inverse seemed to be true when LMAN1 was overexpressed (Figure S5). Given that expression of many of these inflammatory chemokines is under the control of nuclear factor (NF)-κB, we tested whether LMAN1 could downregulate NF-κB activation using a dual-luciferase assay. We stimulated murine 3T3 cells or human HEK293 cells with the inflammatory cytokine tumor necrosis factor (TNF)-α to induce NF-κB activation and then transfected in increasing amounts of LMAN1. For either condition (mouse or human), we observed a significant dose-dependent reduction in NF-κB activation with increasing amounts of LMAN1 transfected (Figure 5A). To confirm such effects also occurred when a relevant cell type and stimulus was used, we stimulated LMAN1 overexpressing or control dendritic cells with HDM for different timepoints and assessed activation of NF-κB via SDS-PAGE and western blot. In the setting of LMAN1 overexpression, there was an appreciable reduction in the phosphorylation of IκBα when compared with the control cell line (Figure 5B). When we stimulated LMAN1 knockdown cells with HDM, the converse was observed (Figure 5B). In order to determine whether the same might hold true for primary cells, we stimulated WT BMDCs with HDM in the presence of LMAN1 blocking antibodies and observed enhancement of phosphor-IkBα and phospho-Syk (Figure 5C). Likewise, knockdown of LMAN1 in WT BMDCs using electroporated small interfering RNA (siRNA) also resulted in increased activation of NF-κB (Figure 5D). Endotoxin is a known constituent of dust mite extracts. In order to determine whether the presence of endotoxin was confounding the results, we also treated LMAN1 overexpressing or control dendritic cells with LPS for different timepoints and assessed activation of NF-κB via SDS-PAGE and western blot (Figure S6B). Not only were the kinetics of activation of the different NF-κB components different when compared with activation with HDM (Figure S6A), we also failed to see a reduction in NF-κB activation with increased levels of LMAN1, suggesting that the regulatory effects of LMAN1 were specific to HDM. Thus, increased LMAN1 can downregulate NF-κB activation in response to TNF-α stimulation in epithelial cells or in response to HDM exposure in DCs, while knockdown or blocking of LMAN1 promotes NF-κB activation in response to HDM exposure in DCs.

HDM promotes binding of LMAN1 to the FcRγ and recruitment of SHP1

The intracellular region of LMAN1 is extremely short (12 amino acids). Aside from residues involved in ER exit and retention, there are no obvious signaling motifs present. In order to determine whether LMAN1 uses signaling adaptors such as the FcRγ (FCER1G) to mediate negative regulation of NF-κB, we transiently transfected HEK 293 cells with LMAN1 and FcRγ and performed reciprocal immunoprecipitation experiments. As seen

in Figure 6A, immunoprecipitation of LMAN1 via its HA tag was able to pull down the FcRγ. Likewise, immunoprecipitation of FcRγ via its FLAG tag was able to pull down LMAN1. In order to determine whether this interaction also occurs endogenously and whether it could give us a clue into mechanisms used by LMAN1, we stimulated control (empty vector) and LMAN1 overexpressing (LMAN1 oex) DC2.4 cell lines with HDM and immunoprecipitated the FcRγ. We found that HDM stimulation promoted binding of LMAN1 and FcRγ at around 60 mins in control DC2.4 cells while LMAN1 and FcRγ were already found to be associated when LMAN1 was overexpressed (Figure 6B). In addition, the kinetics of LMAN1 binding to FcRγ also coincided with the recruitment of SHP1, a well-known negative regulator of many FcRγ and CLR-mediated pathways (Figure 6B). To determine whether association with FcRγ and SHP1 is a common mechanism used by LMAN1 to downregulate other signaling pathways, we also tested whether we could see interaction of these components using tumor necrosis factor (TNF)-α stimulation of DC2.4 cells. Indeed, we observed a transient interaction of all components, albeit with a different kinetics compared with HDM (Figure S6C). Last, in order to see whether this interaction was also occurring in primary cells, we treated BMDCs with HDM and immunoprecipitated the FcRγ (Figure S6D). Interestingly, while we did observe this FcRγ-LMAN1-SHP1 interaction in primary BMDCs, the complex appeared to be together basally and dissociated upon HDM-induced NF-κB activation. As we hypothesized the complex likely to associate again at later timepoints to help terminate the response, we performed an extended stimulation (Figure S6D). Indeed, enhancement of the FcRγ-LMAN1-SHP1 complex was observed after overnight stimulation (Figure S6D). Altogether, these data suggest that LMAN1 and FcRγ interact endogenously in a stimulus-dependent manner and recruit SHP1 to downregulate activated pathways.

LMAN1 cell surface expression is downregulated in the peripheral DCs of asthmatic individuals compared with healthy controls

Given our biochemical and cellular experiments indicating LMAN1 binding to HDM, our flow cytometry experiments indicating expression of LMAN1 on airway DCs and AECs *in vivo* and the fact that a large proportion of asthmatic individuals are sensitized to HDM, we sought to determine whether we could detect changes in the cell surface expression of LMAN1 in peripheral DCs of individuals with asthma. FACs analysis was performed on peripheral blood mononuclear cells (PBMCs) from healthy and asthmatic individuals (Table S1) to determine the expression of LMAN1 on the following populations: Basophils, $CD123^+$ DCs, $CD123^-CD11c^+$ DCs, Total $CD11c^+$ DCs, $CD14^+$ $CD16^-$ Monocytes, $CD14^+CD16^+$ Monocytes and $CD14^-$ $CD16^+$ Monocytes. We observed a significant decrease in the cell surface expression of LMAN1 on all DC populations ($CD123^+$ DCs, $CD123^-CD11c^+$ DCs, Total $CD11c^+$ DCs) of asthmatic individuals compared with healthy controls (Figure 7). Importantly, this decrease in LMAN1 expression in asthmatic individuals was not observed for basophils or any monocyte population assessed (Figure 7). To assess whether similar trends existed for lung resident cells, we additionally probed a publicly available single-cell RNA-sequencing dataset[64,65] (https://www.lungcellatlas.org/) [66] for LMAN1 expression. When comparing expression in cells from the lower airway, LMAN1 expression was reduced in eight out of the 10 different epithelial subtypes examined in asthmatic individuals compared with healthy controls (ionocytes, submucosal,

mucous ciliated, goblet, ciliated, basal activated, basal cycling and basal 2)(Figure S7). In addition, when non-epithelial cells were interrogated, LMAN1 expression was also reduced in lung resident DCs (total or activated) in asthmatic individuals compared with healthy controls (Figure S7). Taken together, these data suggest that reduction in the expression of LMAN1 (either at the mRNA level or at the cell surface) is associated with the asthmatic disease state.

DISCUSSION

The present study identifies LMAN1 as a receptor for HDM allergens. LMAN1, also known as ERGIC-53, is a type I membrane receptor that mainly functions as a cargo receptor for a select set of glycoproteins, mediating their transport between the ER and the Golgi compartments. While other C-type lectins have been implicated in the recognition of HDM (Dectin-1,[67] Dectin-2,[68] DC-SIGN,[69,70] Mannose Receptor[71,72]), no L-type lectin has been shown to fulfill this role. As there are only four mammalian L-type lectins described and all are involved in protein sorting in luminal compartments, identification of LMAN1 as an allergen receptor would have been unlikely without using an unbiased strategy such as receptor glycocapture. With regard to disease relevance, LMAN1 has not previously been identified in GWAS studies as a susceptibility gene for atopy or allergic asthma. However, LMAN1 was previously reported as being significantly downregulated in the setting of asthma using microarray analysis.[73] Likewise, single-cell transcriptomic analysis of various lung cell populations in healthy versus asthmatic individuals indicated decreased expression of this protein in lung DCs and AECs in the setting of disease[64] (Figure S7).[65,66] One other study exploring the association of SNPs with lung function decline among cotton textile workers, found that an SNP within the intron of LMAN1 (rs10515978) was suggestive for accelerated decreases in forced expiratory volume (FEV_1), a measure of lung function.[74] Thus, approaches such as the one used in this work could complement existing techniques to identify additional receptors of interest that might be missed due to the limitations or bias of current methods (i.e., focus on common variants with large effect sizes for GWAS). It also demonstrates that complex ligands such as allergen mixtures can successfully lead to identification of candidate receptors despite low abundance of individual allergen-conjugates. Indeed, co-identification of CD36 using this screen serves as additional verification in the success of this effort, CD36 having also been identified as an HDM receptor by an independent group.[55]

While this discovery is exciting, it does raise a number of interesting questions. First, why would a protein involved in luminal protein sorting function in the recognition of allergens at the cell surface? Although the majority of LMAN1 appears to cycle between the ER, ERGIC, and Golgi, a subset of LMAN1 likely escapes COP1 retention and traffics to the cell surface where it is then available for allergen capture and endocytosis. Such cell surface localization of LMAN1 has been either observed or suggested by multiple groups.[75–77] Proteomic studies have reported endogenous LMAN1 within early endosomes.[76] Other investigators have described the ability of certain proteins to promote re-localization of LMAN1 to the cell periphery.[77] LMAN1 was also found to associate with Rab3GAPs, which are involved in vesicular membrane transport and fusion.[77] Our own flow cytometry data, binding assays, and (non-permeabilized) confocal images are in support of this model.

The confocal images also indicate the presence of cell surface LMAN1 in discrete regions, similar to the clusters observed for DC-SIGN.[78,79] In this regard, LMAN1 could act in a similar manner and aid in endocytosis of bound allergens for subsequent antigen processing and presentation to T cells.[80] However, this has yet to be empirically proven.

Second, our finding that LMAN1 expression levels inversely correlate with NF-κB activation and cytokine secretion in response to stimulation with HDM, the observation that LMAN1 promotes recruitment of the well-known signaling regulator SHP1, and the lower expression of LMAN1 on the peripheral DCs of asthmatic individuals, suggests that LMAN1 may function as a negative regulator of allergic responses. Given that many C-type lectin receptors can act either as an activating receptor or an inhibitory receptor depending on the context in which they are stimulated, it would be important to demonstrate this function conclusively using *in vivo* models of asthma and knockout animals. Furthermore, the finding that LMAN1 is primarily present on the cell surface of DCs and AECs, two cell types intimately involved in the proximal events during allergic sensitization, would argue that further examination of the importance of this receptor in the recognition and response to HDM within each cell type is warranted. The data obtained from such studies would give credence to the development of approaches aimed at manipulating this receptor to modulate the allergic response. This is an area of great interest for our laboratory and one that we are currently pursuing.

Third, it would be important to determine the consequences of differential engagement of LMAN1 (high versus low avidity ligands), co-engagement of heterologous receptors alongside LMAN1, and potential oligomerization or cooperation of LMAN1 with other CLR members, on the signaling outcomes mediated through this receptor. Ligand avidity has been demonstrated to influence whether ITAM-coupled receptors elicit activating or inhibitory signals.[81–84] Likewise, while co-engagement of TLRs can result in synergistic signaling for some lectins,[85–88] inhibition of signaling through heterologous receptors has also been described.[89–94] Specifically, CLR-mediated modulation of TLR-induced NF-κB activation appears as a recurring theme.[89,92,95] LMAN1 may similarly serve as an immunomodulatory receptor. Furthermore, formation of heteromeric complexes has been documented for various lectins such as MCL and Mincle,[96] Dectin-2 and Dectin-3,[97] and MR and DC-SIGN,[98] leaving open the possibility that this may also occur for LMAN1. Thus, ascertaining whether LMAN1-mediated signaling outcomes can likewise be “tuned” a particular way, will require further work carefully examining each of these factors.

Last, recognition of HDM mediated by LMAN1 appears to be dependent on glycosylation (likely mannosylation). This implies that LMAN1 may serve as a general sensor of other mannosylated allergens or other mannosylated antigens. Mannosylation is a common modification present on the proteins or lipids of many allergens (Ara h 1 from peanut, Bla g 2 from cockroach, Can f 1 from dog dander), bacteria (ManLAM of Mycobacterium tuberculosis, CPS of Streptococcus pneumoniae), fungi (cell walls of Saccharomyces cerevisiae and Candida albicans), and viruses (gp120 of HIV, spike of SARS-CoV-2). While beyond the scope of this work, should this hold true, it would expand the implications of this study beyond just allergen sensing.

Overall, we have demonstrated the utility of using an unbiased receptor glycocapture approach to identify LMAN1 as a receptor for HDM. The finding that LMAN1 is expressed on the surface of airway resident cells important to the allergic response, coupled with a role in downregulating inflammatory signaling in DCs, and the observed differential expression of this receptor in asthmatic individuals, suggests that modulation of LMAN1 levels may hold promise as a potential therapeutic strategy. Further work will need to be undertaken to determine whether LMAN1-targeted therapies (at the DC or AEC-level) will be efficacious for the treatment of atopic disorders such as allergic asthma.

Limitations of the study

While this study has demonstrated that cell surface LMAN1 on DCs appears to act as a regulatory receptor for HDM, this research is not without its limitations. A large part of the work was performed using DC cell lines that may respond differently from primary cells. Thus, while the overall conclusions obtained are likely universal (ability of LMAN1 to bind HDM, ability of LMAN1 to regulate inflammatory signaling, ability to associate with FcRγ and recruit SHP1), various factors that differ between cell lines and primary cells (basal activation states, sensitivity to stimulus, level of maturation), could all influence exact outcomes, such as the kinetics of the response being evaluated.

Furthermore, whether the observed regulatory function of LMAN1 also holds true for the various primary DC subsets and whether the regulatory mechanisms employed differ from cell type to cell type, remains to be tested. In addition, details of the molecular basis underlying the FcRγ-LMAN1-SHP1 association still need elucidating. For lectin receptors that use FcRγ as an adaptor, association is mediated through the cytoplasmic or transmembrane regions of the lectin where there is the presence of a charged arginine. LMAN1 does have such a residue at the beginning of the cytosolic domain (R499 human, R506 mouse) and it remains to be seen whether the observed complex is mediated through this association under endogenous conditions. Furthermore, the upstream mechanisms by which the FcRγ-LMAN1-SHP1 complex is able to inhibit NF-κB activation still need to beexplored. Last, aswe point out a role for this receptor at the cell surface, it is yet to be determined whether LMAN1 cell surface expression can also be influenced by inflammatory or maturation stimuli in a manner similar to other immune lectins.

STAR*METHODS

Detailed methods are provided in the online version of this paper and include the following:

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Justine T. Tigno-Aranjuez (justine.tigno-aranjuez@ucf.edu).

Materials availability—Plasmids generated in this study will be provided to interested parties upon request under an MTA with UCF. DC2.4 cell lines stably under or overexpressing LMAN1 will be provided to interested parties upon request under an MTA

with Dr. Kenneth L. Rock for use of derivatives of his original materials. THP-1 cell lines stably under or overexpressing LMAN1 will be provided to interested parties upon request under an MTA with UCF. Items generated in this manuscript are also listed within the key resources table.

Data and code availability—All data reported in this manuscript will be shared by the corresponding author upon request.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animals—Eight to twelve week old C57BL/6J mice (Jackson Laboratories; Bar Harbor, ME) (Cat# 000664) of both sexes were used for the studies. Mice were housed in the specific pathogen free, AALAC-accredited, animal facility at the UCF Health Science Campus at Lake Nona. All procedures were conducted in accordance with animal protocols approved by the University of Central Florida Institutional Animal Care and Use Committee (IACUC).

Human subjects—Human subjects included in the study ranged from 21–49 years in age and included both sexes. Additional (deidentified) information for each individual can be found in Table S1 [T1]. Human studies were conducted in accordance with a protocol reviewed and approved by the University of Central Florida Institutional Review Board (SBE-16-12711) and informed consent was obtained from all subjects involved.

Cell lines—DC 2.4 cells (kindly provided by Dr. Kenneth L. Rock, UMass)[99] were cultured in RPMI (Corning; Corning, NY) (Cat# 10040CM) containing 10% FBS (MilliporeSigma; Burlington, MA) (Cat# F0926), 1x antibiotic-antibiotic (Gibco; Waltham, MA) (Cat# 15240062) and 2mM L-glutamine (Gibco; Waltham, MA) (Cat# 25030081). THP-1 cells (ATCC; Manassas, VA) (Cat# TIB-202) were cultured using RPMI supplemented with 10% FBS, 1x antibiotic-antibiotic and 2mM L-glutamine. HEK 293T and NIH/3T3 cells (ATCC; Manassas, VA) (Cat# CRL-3216 and CRL-1658 respectively) were cultured using DMEM (Corning; Corning, NY) (Cat# 10013CV) supplemented with 10% FBS and 1x antibiotic-antimycotic. Cell lines stably transduced to underexpress or overexpress LMAN1 were additionally grown in the presence of Puromycin at 1:1500 (Invivogen; San Diego, CA) (Cat# ant-pr-1).

METHOD DETAILS

TriCEPs assay for Ligand-Receptor Capture—THP-1 DCs were generated as previously described.[54] Cell concentrations were adjusted to 5×10^5 cells/mL in RPMI medium and incubated with 100 ng/mL human GM-CSF and 100 ng/mL human IL-4 on days 1 and 3 of the culture (PeproTech; Cranbury, NJ) (Cat# HDC). On day 5, cells were adjusted to 5×10^5 cells/mL in low serum (0.1% FBS) RPMI and 100 ng/mL human GM-CSF, 200 ng/mL human IL-4, 20 ng/mL human TNF-α (PeproTech; Cranbury, NJ) (Cat#

300-01A) and 200 ng/mL ionomycin (MilliporeSigma; Burlington, MA) (Cat# I3909-1ML) were added. On day 8, cells were placed in low serum RPMI without additional cytokines and used on day 9. Differentiation and maturation was confirmed by flow cytometry. Each stimulation condition required 4×10^7 THP-1 DCs. Conjugation of allergen or control ligand was performed by incubating 300 μg of house dust mite extract (Citeq; Groningen, Netherlands) (Cat# 02.01.85) or 300 μg of transferrin (MilliporeSigma; Burlington, MA) (Cat# T0665) with 3.75 μg of TriCEPs reagent according to manufacturer's instructions (DualSystems Biotech; Schlieren, Switzerland). Cells were oxidized by adding LRC buffer and 75 mM $NaIO_4$ followed by incubation for 15 min with shaking at 4C. A ratio of 4×10^7 THP-1 DCs to 300 μg of TriCEPs conjugated ligand was used for the experiments and binding was performed for 90 mins at 4C. Cells were washed with 1x PBS and cell pellets were sent frozen to DualSystems for lysis, purification and mass spectrometry.

Mass spectrometry—The LRC-TriCEPS samples were analyzed on a Thermo LTQ Orbitrap XL spectrometer fitted with an electrospray ion source. The samples were measured in data dependent acquisition mode in a 90 min gradient using a 10cm C18 packed column. The six remaining individual samples in the CaptiRec dataset were analyzed with a statistical ANOVA model. This model assumes that the measurement error follows Gaussian distribution and views individual features as replicates of a protein's abundance and explicitly accounts for this redundancy. It tests each protein for differential abundance in all pairwise comparisons of ligand and control samples and reports the p-values. The p - values are then adjusted for multiple comparisons to control the experiment-wide false discovery rate (FDR). The adjusted p-value obtained for every protein is plotted against the magnitude of the fold enrichment between the two experimental conditions. The area in the volcano plot that is limited by an enrichment factor of 2 fold or greater and an FDR-adjusted p-value less than or equal to 0.01 is defined as the receptor candidate space.

Generation of stable cell lines and cell culture—For generation of DC2.4 or THP-1 cells with knockdown of LMAN1, shRNA against mouse or human LMAN1 in the pLKO.1 lentiviral system was used (Horizon Discovery; Boyertown, PA) (Cat# RMM3981-201783827 (mouse) and Cat# RHS3979-201775306 (human)). For generation of DC2.4 and THP-1 cells with overexpression of LMAN1, pCMV3 SP-N-HA-mouse LMAN1(Sino Biologicals; Wayne, PA) (Cat# MG5A0204-NY) or pCMV3 human-SP-N-HA-human LMAN1 (Sino Biologicals; Wayne, PA) (Cat# HG161166-NY) were subcloned into the pBABE puro vector (Addgene; Watertown, MA) (Cat#1764). For retroviral transduction, pBABE plasmids were used with pUMVC (Addgene; Watertown, MA) (Cat# 8449) and VSV-G (Addgene; Watertown, MA) (Cat# 8454) to generate viral particles. For lentiviral transduction, pLKO plasmids were used with psPAX2 (Addgene; Watertown, MA) (Cat# 12260) and pMD2.G (Addgene; Watertown, MA) (Cat# 12259) to generate viral particles. Viral transduction was carried out for 48hrs and cell selection was carried out using Puromycin.

Transient transfection, SDS/PAGE and western blot—Human WT pED-FLAG-LMAN1, pED-FLAG-LMAN1 DCRD (R44-E296), pED-FLAG-LMAN1 DHelix (G271-N457), and pED-FLAG-LMAN1 N156A were reported previously.[63] Transient transfection

of HEK293T cells or NIH/3T3 was performed using calcium phosphate transfection. Cell lysates for western blot were obtained using cell lysis buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 1 mM β-glycerophosphate, 1 mM PMSF, 1 mM $NaVO_4$, 10 mM calyculin A, protease inhibitor cocktail]. Cell lysates were ran on 10% percent bis/acrylamide gels using a Mini-Protean Tetra cell apparatus and transferred to nitrocellulose membranes using a Criterion blotter (all from Bio-Rad; Hercules, California) (Cat# 1658000, Cat# 1620112, Cat# 1704071). LMAN1 was detected using an α-LMAN1 antibody (Cell Signaling; Danvers, MA) (Cat# 13947) or using an α-FLAG antibody (MilliporeSigma; Burlington, MA) (Cat# F1804). Total protein was detected using stain free gels (Bio-Rad; Hercules, California) (Cat# 1610182).

Purification of LMAN1 and in vitro binding assay—HEK293T cells were transfected with WT pED-FLAG-LMAN1 using calcium phosphate. The following day, cells were washed with 1× PBS and lysed using cell lysis buffer. After centrifugation to pellet the insoluble fraction, lysate supernatants were incubated at 4C overnight with anti-FLAG resin (ThermoFisher; Waltham, MA) (Cat # A36803). Purification was carried out by transferring lysates into a spin column and performing a series of 1× PBS washes. LMAN1 was purified using acid elution. The purity and identity of LMAN1 was verified using SDS/PAGE using stain-free total protein gels or by Western Blot using antibodies against FLAG or LMAN1. A Bradford assay was used to determine the protein concentration of purified LMAN1 (Bio-Rad; Hercules, California) (Cat# 5000006).

For *in vitro* binding assays, Nunc Maxisorp plates were coated overnight with 10μg/ml of purified LMAN1. The following day, plates were washed with 1 × PBS with 1mM Ca^{2+}. Blocking was performed using 1 × PBS containing 1mM Ca^{2+} and 1% BSA. Biotinylated D. pteronyssinus extracts or Der p 1 (Citeq; Groningen, Netherlands) (Cat# 02.01.88 and Cat# 02.01.73) were diluted in 1 × PBS containing 1mM Ca^{2+} and 1% BSA and allowed to bind overnight. After washing, plates were incubated with Streptavidin-HRP (Biolegend; San Diego, CA) (Cat# 405210) and developed using TMB substrate (Invitrogen; Waltham, MA) (Cat# 00420156). For testing blocking ability of antibodies, plate-bound LMAN1 was first incubated with anti-LMAN1 antibody for 4hrs at 4C prior to incubation with biotinylated *D. pteronyssinus* extracts or Der p 1.

Cellular chemokine analysis—DC 2.4 cells were seeded at 0.5×10^6 cells/well in 24-well plates and treated with 10 μg/mL D. *pteronyssinus* extracts (Citeq; Groningen, Netherlands) (Cat# 02.01.85) for 16 hours. Cells were lysed using 120 μL T-PER buffer (ThermoFisher; Waltham, MA) (Cat # 78510). containing protease inhibitors. Samples were analyzed for protein concentration via Bradford Assay and adjusted to 0.5 mg/mL in Legendplex assay buffer. A 13-plex bead-based immunoassay for detection of mouse pro-inflammatory chemokines (Biolegend; San Diego, CA) (Cat# 740451) was performed following the manufacturer's instructions. Samples were acquired using a 6-color Novocyte Cytometer (Agilent; Santa Clara, CA) (Cat# 2010049) and analyzed using the provided Legendplex software (Biolegend; San Diego, CA).

Assessing *in vivo* LMAN1 expression—*D. pteronyssinus* extract (Citeq; Groningen, Netherlands) (Cat# 02.01.85) was labelled with Alexa647 using a commercial kit (Abcam;

Waltham, MA) (Cat# ab269823) and 60 μg was delivered intratracheally. One day later, mice were euthanized and BAL cells obtained. One million BAL cells were stained with an α-LMAN1 or isotype control antibody to determine expression of LMAN1 on cells which had bound or taken up HDM. For flow cytometry of APC subsets, lung tissue was digested and enriched for APCs via Nycodenz gradient centrifugation.[100] For detecting LMAN1 on $CD103^+$ and $CD11b^+$ lung DC populations, the following staining panel was used: PE anti-mouse LMAN1 (OTI1A8), PerCP/Cy5.5 anti-mouse CD103(2E7), PE/Cy7 anti-mouse $I\text{-}A^b$ (AF6-120.1), Alexa647 anti-mouse CD11b (M1/70), APC/Cy7 anti-mouse CD11c (N418). For detecting LMAN1 on lung pDC populations, the following staining panel was used: PE anti-mouse LMAN1 (OTI1A8), Alexa647 anti-mouse CD317(2E7), PerCP anti-mouse CD11b (M1/70), APC/Cy7 anti-mouse CD11c (N418). For AEC staining, lungs were enzymatically digested and homogenized using a lung dissociation kit and GentleMacs (Miltenyi Biotec; Auburn, CA) (Cat # 130-095-927) and stained with PE anti-mouse LMAN1 (OTI1A8) and APC anti-mouse CD326 (G8.8). All cells were blocked prior to staining using anti-mouse CD16/32 (2.4G2).

Cellular binding assays—Detection of HDM or Der p 1 binding was conducted by incubating 1 million cells with 5 μg/mL biotinylated *D. pter* extract (02.01.88), *D. farinae* extract (02.02.88) or Der p 1 (02.01.73), (Citeq Biologics, The Netherlands) for 45 min at 37 C. This was followed by incubation with Streptavidin Alexafluor 647 (Biolegend; San Diego, CA) (Cat# 405237). Live cells were gated and histograms for the relevant channels were generated. All samples were acquired using a 6-color Novocyte cytometer (Agilent; Santa Clara, CA) (Cat# 2010049) and analyzed using NovoExpress software.

NF-κB dual-luciferase assay—Mouse or human LMAN1 constructs (Sino Biologicals; Wayne, PA) (Cat# MG5A0204-NY and Cat# HG161166-NY) were transfected into NIH/3T3 cells or HEK293 cells, respectively, using calcium phosphate transfection. Transfections were performed using increasing concentrations of LMAN1 (0.3–4.5 μg). To measure NF-κB activity, 150 ng of NF-κB-firefly luciferase and 100 ng of CMV renilla luciferase were co-transfected with an empty vector or with LMAN1. After 16 hr incubation, cells were treated with 50 ng/uL mouse or human TNF-α and lysates were harvested 5 hrs after treatment. The dual luciferase assay was performed according to manufacturer instructions (Promega; Madison, WI) (Cat# E1910). Luminescence was read using a Cytation 5 plate reader (Agilent; Santa Clara, CA) (Cat# BTCYT5MPV).

Generation of bone-marrow derived dendritic cells (BMDCs) and siRNA knockdown—Bone marrow from mouse tibias and femurs were counted and grown at 2×10^5 cells/ml in petri dishes with 10mL complete RPMI media containing 20ng/mL GM-CSF (Peprotech; Cranbury, NJ). On day 3, an additional 10mL media with 20ng/mL GM-CSF was added. On day 6, half the media was replenished with 10mL complete RPMI media containing 20ng/mL GM-CSF. At day 7 or 8, cells were used or matured further using 30ng/mL TNF-α for another 48hours. Confirmation of BMDC cell surface phenotype was assessed by FACs analysis.

siRNA knockdown of mLMAN1 was performed via electroporation using a Gene Pulser Xcell system (Biorad; Hercules, CA). The conditions were: 400V, 150μF, 100Ω using 2

× 10^6 cells/cuvette in a 200 μL volume containing 6 μg of control or mLMAN1-specific siRNA (Qiagen; Germantown, MD).

Human samples and flow cytometry staining panels—Human peripheral blood mononuclear cells were isolated via Lymphoprep density gradient centrifugation from whole blood (StemCell; Cambridge, MA) (Cat# 07801). For identification of peripheral $CD123^+$DCs and basophils, the following staining panel was used: FITC anti-human Lin [CD3(UCHT1), CD14(HCD14), CD16(3G8), CD19(HIB19), CD20(2H7), CD56(HCD56)], PE anti-human LMAN1 (OTI1A8), PerCP anti-human HLA-DR (L243), PE/Cy7 anti-mouse CD11c (S-HCL-3), APC anti-human CD123 (6H6). For identification of monocyte populations, the following staining panel was used: FITC anti-human Lin [CD3(SK7), CD19(4G7), CD20(2H7), CD56(HCD56)], PE anti-human LMAN1 (OTI1A8), PerCP anti-human HLA-DR (L243), PE/Cy7 anti-mouse CD11c (S-HCL-3), APC anti-human CD14 (M5E2), APC/Cy7 anti-human CD16(3G8). FMO controls excluded PE anti-human LMAN1 antibody. All cells were blocked prior to staining using Human TruStain FcX. All samples were acquired using a 6-color Novocyte cytometer (Agilent; Santa Clara, CA) (Cat# 2010049) and analyzed using NovoExpress software.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed by. Dr. Xiang Zhu. The specific analyses are described separately for each experiment and indicated within the figure legends. Raw data (MFI, absorbance, luminescence, pixel intensities) were analyzed using GraphPad Prism 9. When ANOVA was performed, a post-hoc Tukey test was conducted for pairwise comparisons to control for Type I error. All statistics tests were two-tailed with $\alpha < 0.05$ considered to be significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This project was supported by an American Lung Association Innovation Award IA-695632 and NIH R01HL162897 to J.T.T.-A. and an NIH R01HL094505 grant to B.Z.

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Highlights

- Receptor glycocapture screen identifies LMAN1 as an HDM receptor
- Binding of LMAN1 to HDM allergens requires LMAN1 CRD
- LMAN1 downregulates NF-κB signaling through binding FcRγ and recruitment of SHP1
- Asthmatic individuals show reduced cell surface expression of LMAN1 on peripheral DCs

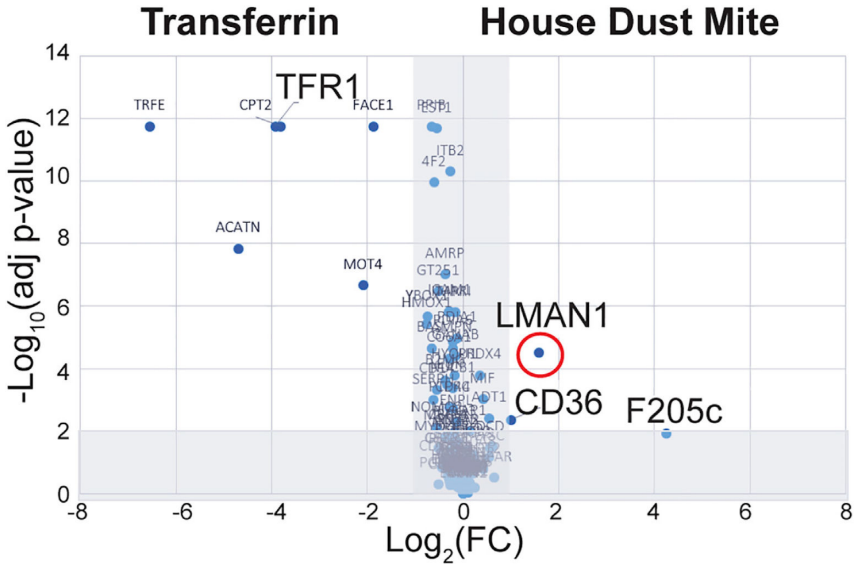

Figure 1. Ligand-receptor capture (LRC)-Tri-CEPs platform identifies LMAN1 as a candidate receptor for HDM

Control (transferrin) or experimental (HDM) ligand was conjugated to TriCEPs reagent and allowed to bind to mildly oxidized THP-1 DCs for receptor capture. Cells were washed and pellets sent to DualSystems for purification of complexes, mass spectrometry, and downstream analysis. Volcano plot showing the receptors identified for transferrin (left) and HDM (right). LMAN1 and CD36 were identified as candidate receptors for HDM. THP-1 DCs: THP-1 cells differentiated into a DC lineage.

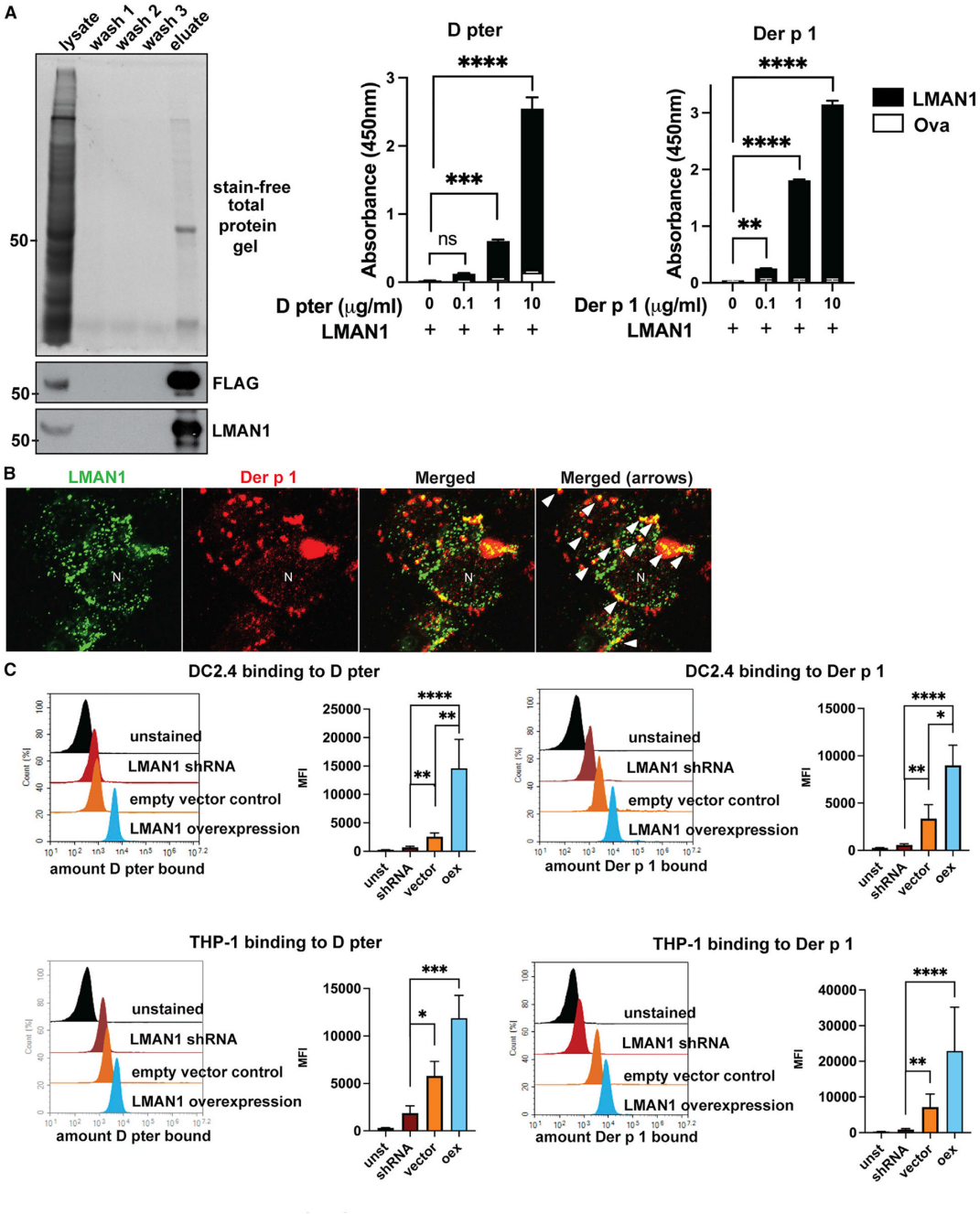

Figure 2. LMAN1 binds to house dust mite (HDM) allergen extract and to purified major HDM allergens on the cell surface

(A) FLAG-tagged human LMAN1 was purified using an affinity resin. SDS-PAGE and western blot were performed to confirm the purity and identity of LMAN1. A direct binding assay between LMAN1 and D pter or Der p 1 was performed by coating plates with purified human LMAN1 (black bars) or ovalbumin (white bars) and allowing biotinylated allergen to bind followed by incubation with Streptavidin-HRP, development and measurement of the resulting absorbance. A dose-dependent binding of allergens to purified human LMAN1 was observed.

(B) DC 2.4 cells stably expressing HA-tagged mouse LMAN1 were allowed to bind biotin-Der p 1 for 45 min at 37°C. Cells were then washed and stained with Streptavidin-A647 (Der p 1) and anti-HA A488 (LMAN1) in the absence of permeabilization. Cells were washed, fixed, and mounted overnight prior to confocal analysis. Images were acquired at 363. Yellow areas (the most prominent are shown by arrowheads) indicate areas of colocalization. N indicates the nucleus. Confocal analysis indicates colocalization of LMAN1 with Der p 1 at the cell surface.

(C) The murine DC2.4 dendritic cell line or human THP-1 monocytic cell line were transduced with the indicated lentiviral vectors to knockdown (shRNA) or overexpress (oex) mouse or human LMAN1, respectively. The resulting cells were selected using Puromycin and LMAN1 cell surface and total expression were verified by western blot and flow cytometry (S2). Biotinylated D pter or purified Der p 1 allergen were allowed to bind to the generated cell lines prior to secondary staining with Streptavidin-Alexa647 and analysis by flow cytometry. Representative histograms for the binding are shown to the left, while quantification of MFI is shown to the right. Data are represented as mean ± SEM. A one-way ANOVA was used to assess significance $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. sh: shRNA knockdown, oex: overexpression, D pter: *Dermatophagoides pteronyssinus*, Der p 1: *Dermatophagoides pteronyssinus* major mite allergen 1. Independent experiments: (A): D pter (n = 4); (A): Der p 1 (n = 3); (C): DC2.4 D pter (n = 7) and Der p 1 (n = 7); (C): THP-1 D pter (n = 5) and Der p 1 (n = 5).

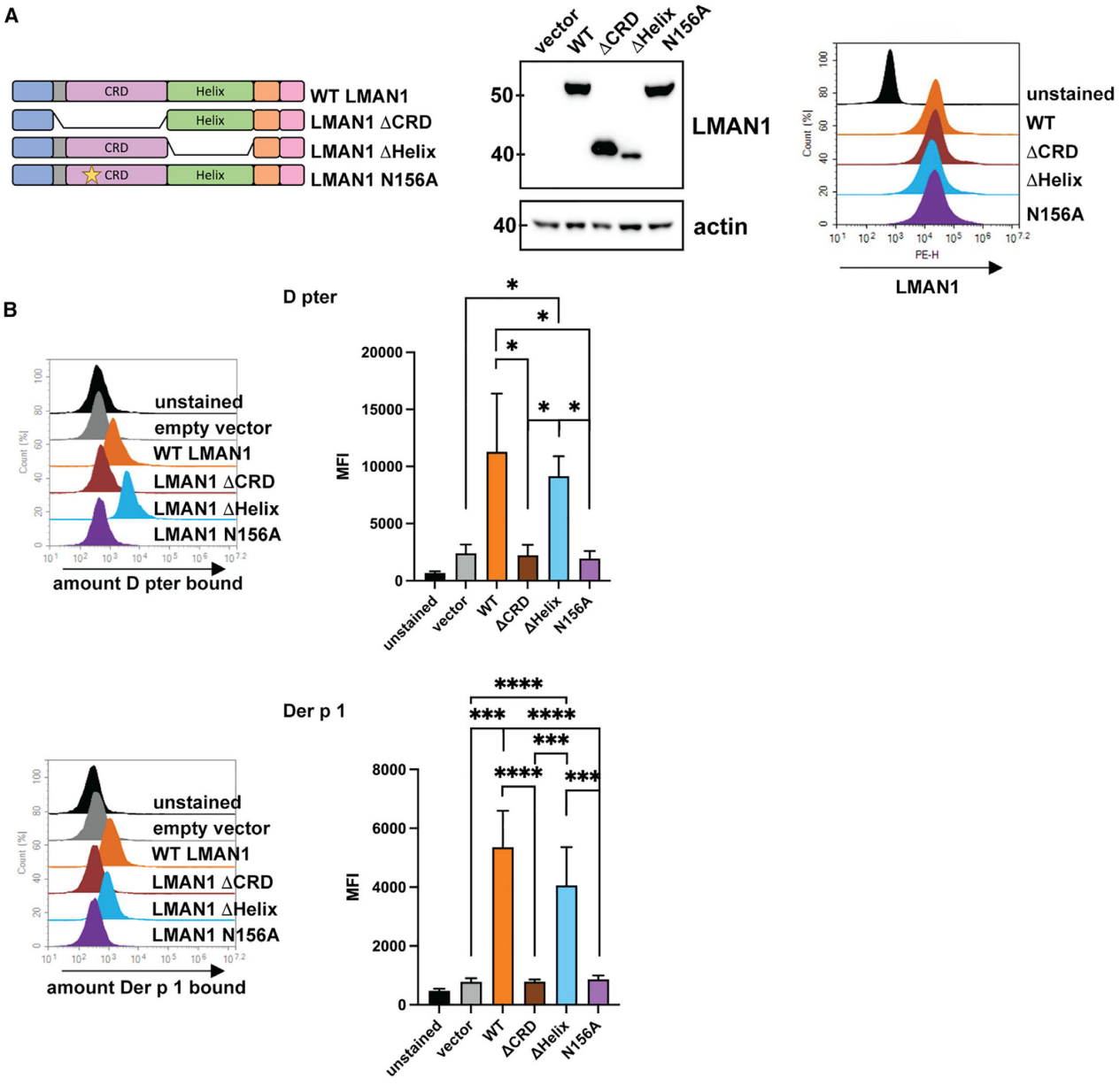

Figure 3. LMAN1 binds to HDM in a carbohydrate recognition domain (CRD)-dependent manner

HEK293 cells were transiently transfected with either WT or mutant human LMAN1 constructs.

(A) Schematic of the different constructs used in the binding experiment and confirmation of total protein expression by SDS-PAGE and western blot and cell surface expression by FACS.

(B) Following transfection, biotinylated D pter or Der p 1 was allowed to bind to the cells prior to secondary staining with Streptavidin-Alexa647 and analysis by flow cytometry. Representative histograms for the binding are shown to the left, while quantification of MFI is shown to the right. Data are represented as mean ± SEM. A one-way ANOVA was used to assess significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. D pter,

Dermatophagoides pteronyssinus; Der p 1, *Dermatophagoides pteronyssinus* major mite allergen 1. Independent experiments: (B): D pter (n = 6), Der p 1 (n = 5).

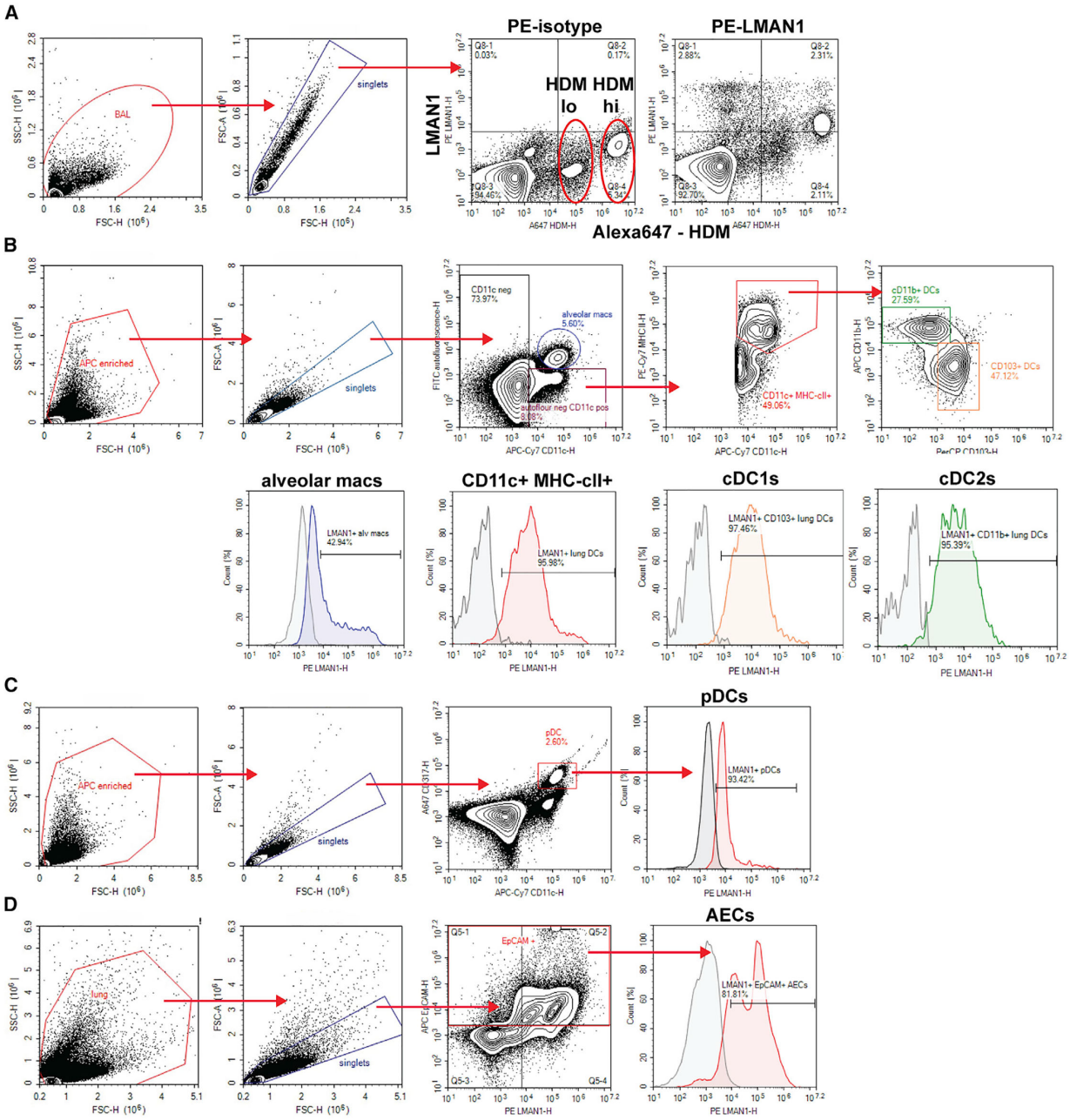

Figure 4. LMAN1 is expressed on the surface of relevant cell types in the murine lung

(A) Alexa 647 labeled HDM (D pter) was intratracheally administered into naive mice. One day later, BAL was harvested and stained with either isotype controls or anti-LMAN1. HDM^{hi} cells expressed LMAN1.

(B–D) (B) Lung tissue was digested and APCs were enriched using Nycodenz gradients. FACS plots for identification of different APC populations and histograms for expression of LMAN1 are shown. LMAN1 is expressed highly on lung cDC1 and cDC2 lung DCs and to a lower extent on alveolar macrophages. LMAN1 is also expressed by (C) pDCs and (D) airway epithelial cells. For airway epithelial cell staining, lung tissues were enzymatically digested but no APC enrichment was performed. Gray histograms represent fluorescence

minus one (FMO) controls. (A)–(D): representative plots are shown for n = 3 experiments performed.

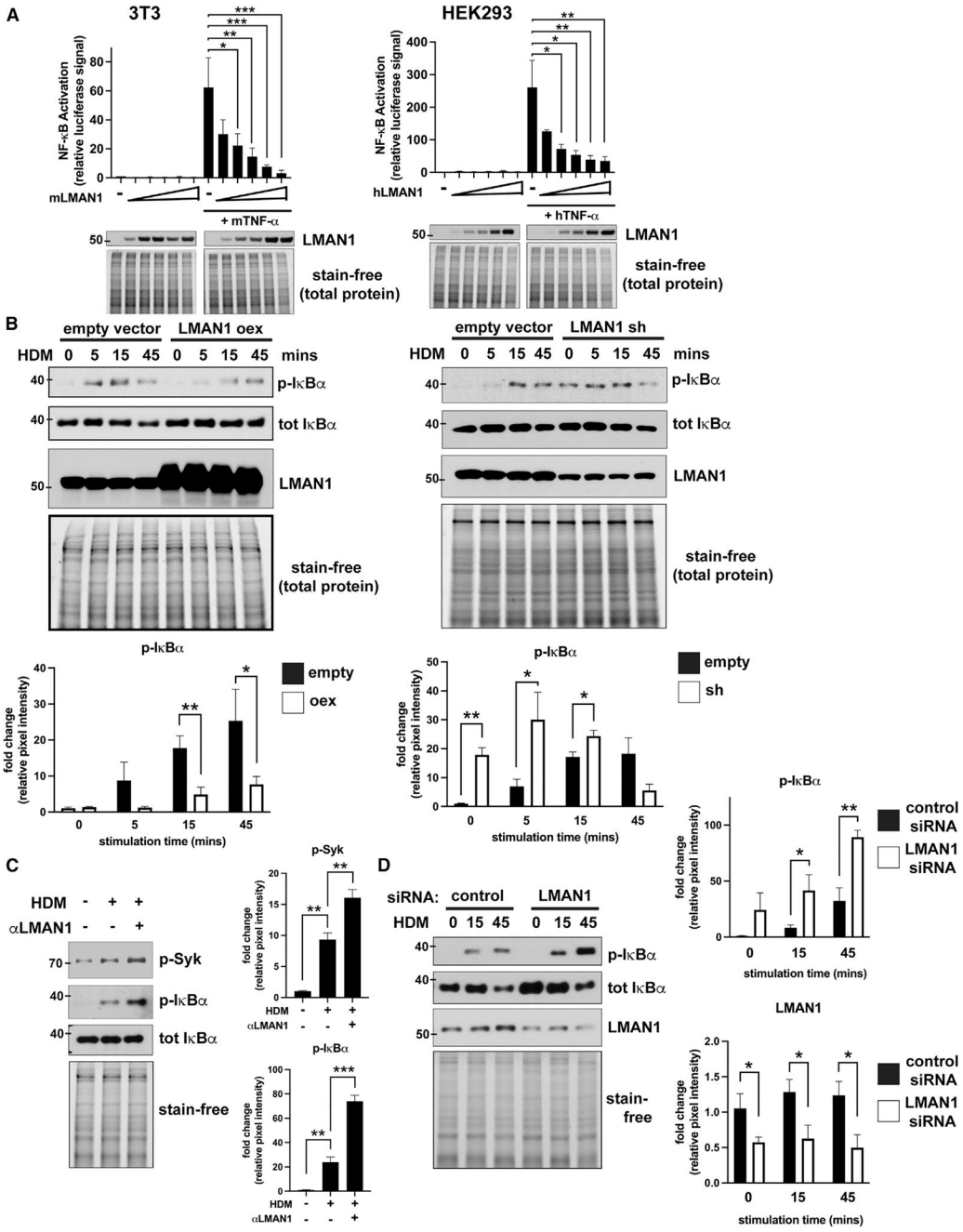

Figure 5. LMAN1 downregulates NF-κB activation in response to inflammatory stimuli or HDM

(A) 3T3 or HEK293 cells were transfected with NF-κB reporters and increasing amounts of mouse or human LMAN1 in the absence or presence of TNF-α stimulation. NF-κB activity in response to TNF-α was decreased by LMAN1 in a dose-dependent manner.

(B) DC2.4 empty vector, LMAN1 overexpressing (oex) or LMAN1 knockdown (sh) cells were stimulated with HDM for the timepoints indicated and lysates were subjected to SDS-PAGE and western blot to assess modification of signaling pathway components. Overexpression of mouse LMAN1 inhibits activation of NF-κB in response to HDM while knockdown of mouse LMAN1 enhances activation of NF-κB in response to HDM.

(C) WT bone marrow-derived dendritic cells (BMDCs) were stimulated with HDM for 45 min in the absence or presence of an LMAN1 blocking antibody and lysates were subjected to SDS-PAGE and western blot to assess modification of signaling pathway components. Blocking LMAN1 enhances activation of NF-κB in response to HDM.
(D) WT BMDCs were electroporated with a control or mouse LMAN1-specific siRNA prior to stimulation with HDM. Lysates were collected at the indicated timepoints and subjected to SDS-PAGE and western blot to assess modification of signaling pathway components. siRNA knockdown of mouse LMAN1 enhances activation of NF-κB in response to HDM. Data are represented as mean ± SEM. A one-way (A and C) or two-way (B and D) ANOVA was used to assess significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Independent experiments: (A): 3T3 (n = 3), (A): HEK293 (n = 3), (B): LMAN1 overexpression (n = 3), (B): LMAN1 shRNA (n = 3), (C): blocking antibody (n = 3), (D): siRNA knockdown (n = 4). Fold change represents the pixel intensity relative to the empty vector cell lines or control siRNA-treated cells at the zero time point, which is set to 1.

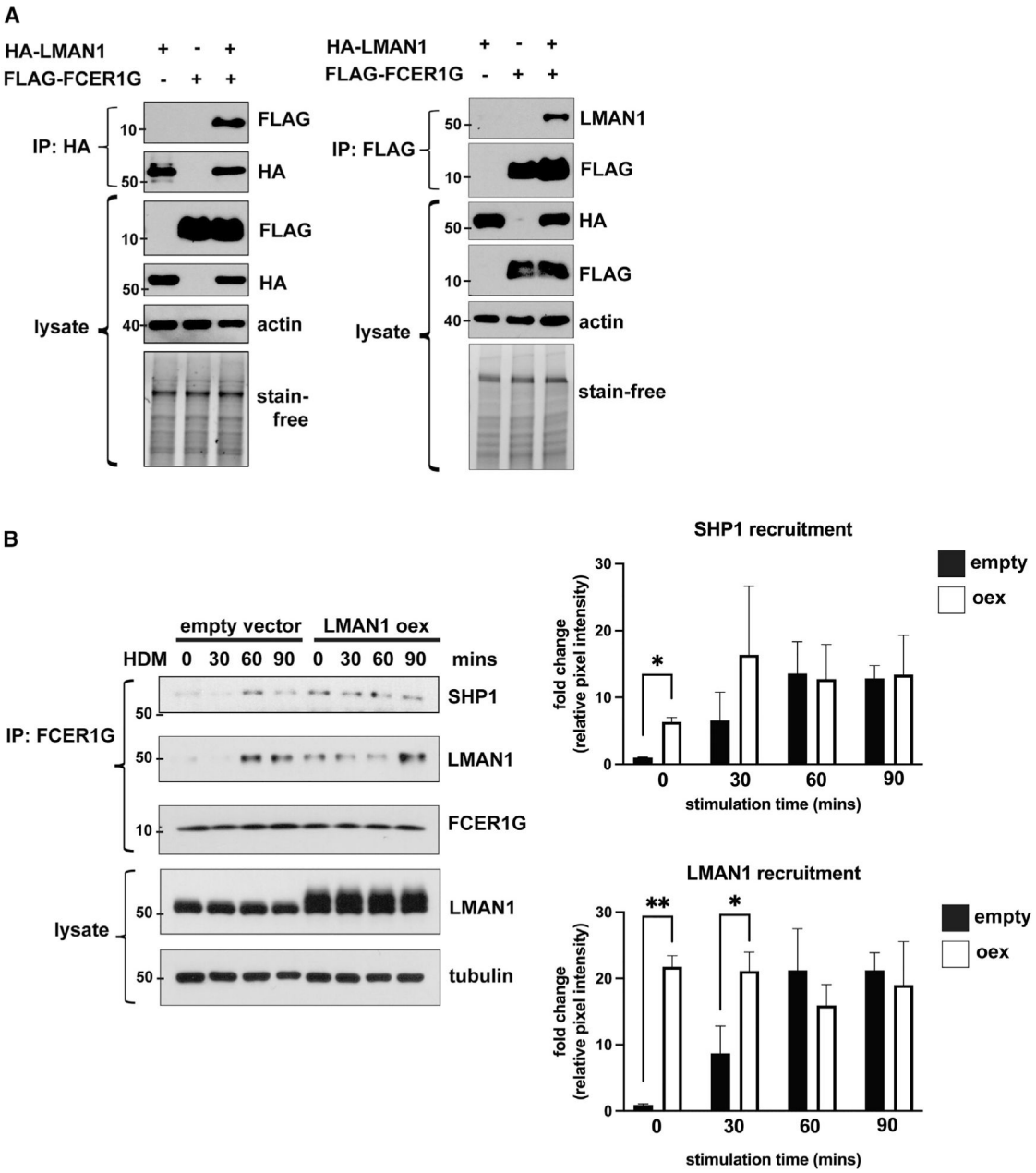

Figure 6. HDM promotes binding of LMAN1 to the FcRγ and recruitment of SHP1

(A) FcRγ and LMAN1 interact in transient overexpression experiments. HEK293 cells were transfected with indicated constructs for mouse LMAN1 and mouse FcRγ. LMAN1 was immunoprecipitated via its HA tag and FcRγ was immunoprecipitated via the FLAG tag. Reciprocal co-IP experiments show binding between FcRγ and LMAN1 when either LMAN1 (left) or FcRγ (right) is pulled down.

(B) FcRγ and LMAN1 interact endogenously in response to HDM. Control (empty vector) or LMAN1 overexpressing (LMAN1 oex) DC2.4 cells were stimulated with HDM for the indicated timepoints and FcRγ was immunoprecipitated. Control cells show HDM-inducible binding of LMAN1 to the FcRγ coincident with recruitment of SHP1 at 60 min while

all three proteins are already basally interacting in LMAN1 overexpressing cells. Data are represented as mean ± SEM. A two-way ANOVA was used to assess significance. *$p < 0.05$, **$p < 0.01$. Independent experiments: (A) (n = 3), (B) (n = 3).

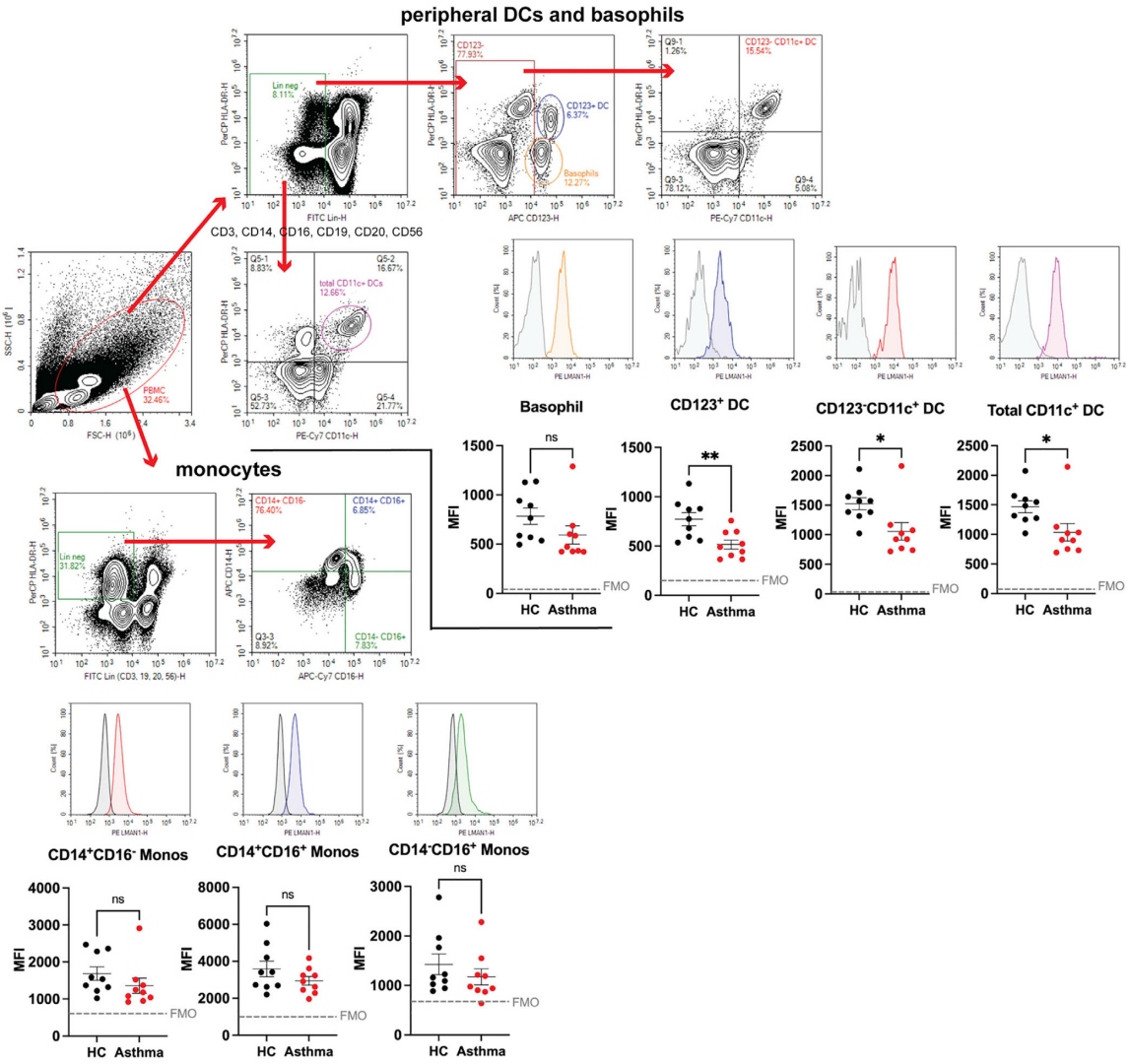

Figure 7. LMAN1 cell surface expression is downregulated in the peripheral DCs of asthmatic individuals compared with healthy controls

PBMCs isolated from healthy controls or asthmatic individuals were stained with appropriate antibody panels to identify LMAN1 expression on peripheral antigen-presenting cells via flow cytometry. Shown are representative FACS plots and corresponding histograms for the indicated subsets. Gray histograms or dashed lines represent FMO controls for each staining panel used. For scatterplots, longer horizontal lines represent the mean and shorter lines represent the SEM. A two-tailed t test was performed to assess significance. *$p < 0.05$, **$p < 0.01$ ($n = 9$/group).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
α-mouse CD16/CD32 (rat) – blocking non-specific staining by flow cytometry	BD Biosciences	Cat# 553142; RRID:AB_394657
α-FLAG, clone M2 (mouse)	Sigma-Aldrich	Cat# F1804; RRID:AB_262044
α-HA-11, clone 16B12 (mouse)	Biolegend	Cat# 901501; RRID:AB_2565006
α-LMAN1, clone OTI1A8 (mouse) – used with secondary reagents for quantitation of LMAN1 cell surface expression by flow cytometry, used for blocking experiments	Novus	Cat# NBP2-03381; RRID:AB_2934291
α-LMAN1, clone E2B6H (rabbit) – Western Blot	Cell Signaling	Cat# 13974; RRID:AB_2798356
PE a-mouse/human LMAN1, clone OTI1A8 (mouse) – for measuring LMAN1 expression on various cell types by flow cytometry	Novus	Cat# NBP2-71152PE; RRID:AB_2934292
PE Mouse IgG1, κ Isotype Control – control for LMAN1 expression on HDM positive cells	BD Biosciences	Cat# 555749; RRID:AB_396091
PerCP/Cy5.5 α-mouse CD103 clone 2E7 (hamster)	Biolegend	Cat# 121416; RRID:AB_2128621
PE/Cy7 α-mouse I-A^b clone AF6-120.1 (mouse)	Biolegend	Cat# 116420; RRID:AB_10575296
Alexa647 α-mouse CD11b clone M1/70 (rat)	Biolegend	Cat# 101218; RRID:AB_389327
APC/Cy7 α-mouse CD11c clone N418 (hamster)	Biolegend	Cat# 117324; RRID:AB_830649
Alexa647 α-mouse CD317 clone 2E7 (rat)	Biolegend	Cat# 127014; RRID:AB_1953289
PerCP α-mouse CD11b clone M1/70 (rat)	Biolegend	Cat# 101230; RRID:AB_2129374
APC α-mouse CD326 clone G8.8 (rat)	Biolegend	Cat# 118213; RRID:AB_1134105
Human TruStain FcX - blocking non-specific staining by flow cytometry	Biolegend	Cat# 422302; RRID:AB_2818986
FITC α-human lineage cocktail [CD3, CD14, CD16, CD19, CD20, CD56] (mouse)	Biolegend	Cat# 348801; RRID:AB_10612570
FITC α-human CD3 clone SK7 (mouse)	Biolegend	Cat# 344804; RRID:AB_2043993
FITC α-human CD19 clone 4G7(mouse)	Biolegend	Cat# 392508; RRID:AB_2750099
FITC α-human CD20 clone 2H7 (mouse)	Biolegend	Cat# 302304; RRID:AB_314252
FITC α-human CD56 clone HCD56 (mouse)	Biolegend	Cat# 318304; RRID:AB_604100
PerCP α-human HLA-DR clone L243 (mouse)	Biolegend	Cat# 307627; RRID:AB_893574
PE/Cy7 α-human CD11c clone S-HCL-3 (mouse)	Biolegend	Cat# 371507; RRID:AB_2650779
APC α-human CD123 clone 6H6 (mouse)	Biolegend	Cat# 306011; RRID:AB_439778
APC α-human CD14 clone M5E2 (mouse)	Biolegend	Cat# 301808; RRID:AB_314190
APC/Cy7 α-human CD16 clone 3G8 (mouse)	Biolegend	Cat# 302018; RRID:AB_314218
α-phospho p105, clone 18E6 (rabbit)	Cell Signaling	Cat# 4806S; RRID:AB_2282911
α-phospho IκBα, clone 5A5 (mouse)	Cell Signaling	Cat# 9246S; RRID:AB_2267145
α-IκBα, clone L35A5 (mouse)	Cell Signaling	Cat# 4814S; RRID:AB_390781
α-phospho p65, clone 93H1 (rabbit)	Cell Signaling	Cat# 3033S; RRID:AB_331284
α-FCER1G, polyclonal (rabbit)	Millipore Sigma	Cat# 06-727; RRID:AB_310227
α-SHP-1, clone E1U6R (rabbit)	Cell Signaling	Cat# 26516S; RRID:AB_2934293
α-beta actin, clone C4 (mouse)	Santa Cruz	Cat# sc-47778; RRID:AB_626632
α-tubulin, clone TU-02 (mouse)	Santa Cruz	Cat# sc-8035; RRID:AB_628408
Biological samples		

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human healthy and asthmatic PBMCs [see S10]	This paper	N/A
Chemicals, peptides, and recombinant proteins		
Dermatophagoides pteronyssinus extract	Citeq	02.01.85
Dermatophagoides pteronyssinus extract, biotinylated	Citeq	02.01.88
Der p 1, biotinylated	Citeq	02.01.73
Dermatophagoides farinae extract, biotinylated	Citeq	02.02.88
Critical commercial assays		
LRC-TriCEPS	DualSystems	N/A
Experimental models: Cell lines		
HEK293T	ATCC	Cat# CRL-3216
THP-1	ATCC	Cat# TIB-202
NIH/3T3	ATCC	Cat# CRL-1658
DC2.4	Dr. Kenneth L. Rock (Shen et al.[99])	N/A
DC2.4 LMAN1 shRNA	This paper	N/A
DC2.4 empty vector	This paper	N/A
DC2.4LMAN1 overexpression	This paper	N/A
THP-1 LMAN1 shRNA	This paper	N/A
THP-1 empty vector	This paper	N/A
THP-1 LMAN1 overexpression	This paper	N/A
Experimental models: Organisms/strains		
C57BL/6J	Jackson Laboratory	Cat# 000664
Recombinant DNA		
pLKO.1-puro-u6-shRNA hLMAN1	Horizon Discovery	Cat# RHS3979-201775306
pLKO.1-puro-u6-shRNA mLMAN1	Horizon Discovery	Cat# RMM3981-201783827
pCMV3 SP-N-HA-hLMAN 1	Sino Biologicals	Cat# HG161166-NY
pCMV3 SP-N-HA-mLMAN1	Sino Biologicals	Cat# MG5A0204-NY
pBABE-puro-HA.11-hLMAN1	This paper	N/A
pBABE-puro-HA.11-mLMAN1	This paper	N/A
pED-FLAG-hLMAN1	Zheng et al.[63]	N/A
pED-FLAG-hLMAN1 ΔCRD	Zheng et al.[63]	N/A
pED-FLAG-hLMAN1 ΔHelix	Zheng et al.[63]	N/A
pED-FLAG-hLMAN1 N156A	Zheng et al.[63]	N/A
pBABE-puro	Addgene	Cat# 1764
PUMVC	Addgene	Cat# 8449
VSV-G	Addgene	Cat# 8454

REAGENT or RESOURCE	SOURCE	IDENTIFIER
psPAX2	Addgene	Cat# 12260
pMD2.G	Addgene	Cat# 12259
Other		
Streptavidin-Alexafluor647	Biolegend	Cat# 405237
Streptavidin-HRP	Biolegend	Cat# 405210
Mouse LMAN1 siRNA	Qiagen	Cat# 1027416
AllStars Negative Control siRNA	Qiagen	Cat# 1027417
A647 conjugation kit	Abcam	Cat# ab269823
α-FLAG affinity resin	Thermo Scientific	Cat# A36803
Zombie green viability dye	Biolegend	Cat# 423112