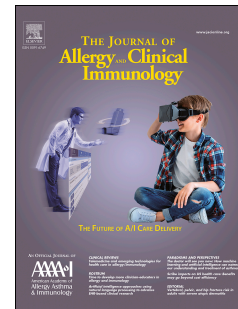


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Dual function of Langerhans cells in skin TSLP-promoted Tfh cell differentiation in mouse atopic dermatitis

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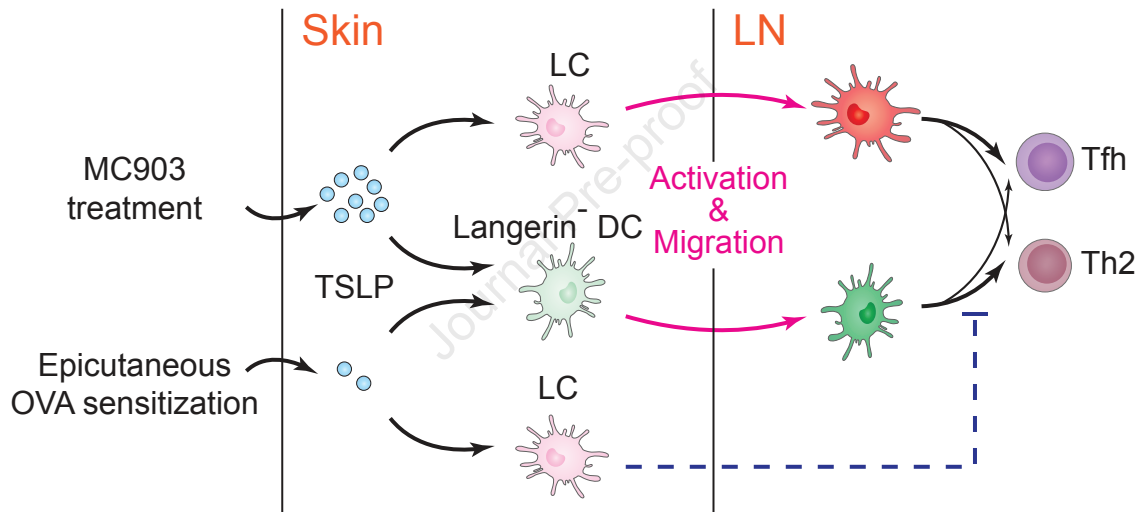
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Dual functions of LCs in skin TSLP-promoted Tfh and Th2 cell differentiation in mouse atopic dermatitis



Title:**Dual function of Langerhans cells in skin TSLP-promoted Tfh cell differentiation in mouse atopic dermatitis**

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Abstract

Background: Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases, usually occurring early in life, and often preceding other atopic diseases like asthma. Th2 cell has been believed to play a crucial role in cellular and humoral response in AD, but accumulating evidences have shown that T follicular helper (Tfh) cell, a critical player in humoral immunity, is associated with disease severity and plays an important role in AD pathogenesis.

Objective: We aimed at investigating how Tfh cells are generated during the pathogenesis of AD, particularly what is the role of keratinocyte-derived cytokine TSLP and Langerhans cells (LCs).

Methods: We employed two experimental AD mouse models, triggered by the overproduction of TSLP through topical application of MC903, or induced by epicutaneous allergen ovalbumin (OVA) sensitization.

Results: We demonstrated that the development of Tfh cells and GC response were crucially dependent on TSLP in MC903 model and OVA sensitization model. Moreover, we found that LCs promoted Tfh cell differentiation and GC response in MC903 model, and the depletion of Langerin⁺ DCs or selective depletion of LCs diminished the Tfh/GC response. By contrast, in the model with OVA sensitization, LCs inhibited Tfh/GC response and suppressed Th2 skin inflammation and the subsequent asthma. Transcriptomic analysis of Langerin⁺ and Langerin⁻ migratory DCs revealed that Langerin⁺ DCs became activated in MC903 model, whereas these cells remained inactivated in OVA sensitization model.

Conclusion: Together, these studies revealed a dual functionality of LCs in TSLP-promoted Tfh and Th2 cell differentiation in AD pathogenesis.

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Capsule Summary

This study demonstrates that keratinocyte-derived cytokine TSLP plays a critical role in promoting not only Th2 but also Tfh/GC response in the pathogenesis of atopic dermatitis, which implicates a dual function of epidermal Langerhans cells.

Key messages:

- TSLP is critically involved in mounting Tfh/GC response in mouse AD driven by MC903 or OVA-sensitization.
- LCs promote Tfh/GC response in MC903-induced AD.
- LCs suppress Tfh/GC response and Th2 skin inflammation in OVA sensitization-induced AD.

Key words:

Atopic dermatitis; TSLP; Dendritic cells; Langerhans cells; Tfh; Th2; allergen sensitization; mouse

Abbreviations:

AD: atopic dermatitis

BAL: Bronchoalveolar lavage

CT: Wild-type Control

DC: Dendritic cell

DEG: Differentially expressed genes

DT: Diphtheria toxin

- 77 DTR: Diphtheria toxin receptor
- 78 EDLN: Ear-draining lymph node
- 79 GC: Germinal center
- 80 H&E: Hematoxylin and eosin
- 81 IHC: Immunohistochemistry
- 82 Ig: Immunoglobulin
- 83 *i.n.: Intranasal*
- 84 *i.p.: Intraperitoneal*
- 85 LC: Langerhans cell
- 86 LMP: laser-assisted skin microporation
- 87 LN: Lymph node
- 88 NT: Non-treated
- 89 PAS: Periodic Acid Schiff
- 90 PCA: Principle component analysis
- 91 Tfh: T follicular helper
- 92 Th2: T helper type 2
- 93 ELISA: Enzyme-linked immunosorbent assay
- 94 TS: Tape stripping
- 95 TSLP: Thymic stromal lymphopoietin
- 96

Introduction

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases which affects up to 20% of children and 3% of adults worldwide, with increasing prevalence in the industrialized countries during the last 30 years ¹. AD is characterized by chronic cutaneous inflammation, T helper type 2 (Th2) response and hyper immunoglobulin IgE. Patients suffering from AD often present genetic risk factors in the form of mutations affecting the skin barrier structure or the immune system ². Onset of AD usually occurs early in life and may lead to allergen sensitization, which can trigger the progression from AD to other atopic diseases such as asthma/allergic rhinitis, in a process called “atopic march” ^{3,4}.

It has been recognized that Th2 cell response is critically implicated in the pathogenesis of AD. Previous studies from us and others using mouse models have established a central role of the cytokine thymic stromal lymphopoietin (TSLP) expressed by epidermal keratinocytes in promoting Th2 cell response and driving the pathogenesis of AD ⁵⁻⁸. In addition to Th2 cell response, humoral immune response is another key feature of AD, with increased serum IgE and IgG1 levels associated with AD, which contribute to AD pathology and the atopic march ^{9,10}. For a long time, Th2 cell has been believed to play a crucial role both in cellular response and humoral response, e.g. helping B cells to produce Igs. However, such knowledge has been challenged with the identification of T follicular helper (Tfh) cell, which emerges to be a critical player in humoral immunity and T cell memory ¹¹.

In lymphoid organs, Tfh cell differentiation process is believed to begin with an initial dendritic cell (DC) priming of naive CD4⁺ T cells, which undergo a cell-fate decision with the acquisition of master transcription factor Bcl6 expression and chemokine receptor CXCR5 expressed on cell surface to become early Tfh cells, of which CXCR5 promotes their

122 migration from T cell zone to the B cell follicles^{12, 13}. The full differentiation and
123 maintenance of Tfh cells implicate the Tfh cell-B cell interaction, leading to GC Tfh cells
124 which are phenotypically defined by their high expression of CXCR5 and PD-1¹⁴. It has been
125 shown that Tfh cells coordinate generation of the GC, initiate help for antigen-specific B cells,
126 and promote selection of high-affinity B cells and differentiation into either memory B cells
127 or long-lived plasma cells¹⁵. Recent studies have identified Tfh cells as an important source
128 of IL-4, a master regulator in type 2 immunity which was previously thought to be produced
129 by Th2 cells, for providing critical B-cell help by its anti-apoptotic and IgE and IgG1 class
130 switch effects¹⁶. In addition, it was reported that Tfh cells produce IL-4 in a GATA3-
131 independent manner¹⁷, suggesting distinct mechanisms employed by Tfh and Th2 cells in the
132 regulation of IL-4.

133 Since their initial identification, the biological functions of Tfh cells and their
134 mechanisms of action in the onset and development of diseases have been studied in
135 autoimmunity, infectious diseases, immunodeficiencies and vaccination¹⁸. Less is known on
136 Tfh cells in the context of AD and other atopic diseases, but more and more evidences have
137 suggested that Tfh cells are associated with disease severity and Tfh cells play an important
138 role in the pathogenesis¹⁹⁻²¹. In human, alteration of circulating Tfh cells is correlated with
139 severity of the disease in children with AD²², or with the comorbid association of allergic
140 rhinitis with asthma²³, and allergen-specific T follicular helper cell counts are correlated with
141 specific IgE levels and efficacy of allergen immunotherapy²⁴. In mice, it has been reported
142 that Tfh cells are important for house dust mite-induced asthma²⁵ or peanut allergy²⁶.

143 Despite of these accumulating evidences showing the importance of Tfh cells in atopic
144 diseases, how Tfh cells and humoral responses are generated and regulated in AD remained to
145 be investigated. In this study, by employing two experimental AD mouse models, one
146 triggered by the overexpression of TSLP in mouse skin through topical application of MC903

147 ^{6, 7, 27}, and the other one induced by epicutaneous allergen ovalbumin (OVA) sensitization, we
148 demonstrated that skin TSLP plays a crucial role in driving/promoting Tfh cell differentiation
149 and GC response, in addition to its recognized role in promoting Th2 cell response. Moreover,
150 we investigated the role of skin DCs in mediating the Tfh cell differentiation. We uncovered a
151 dual functionality of epidermal langerhans cells (LCs) in TSLP-promoted Tfh/Th2 cell
152 differentiation in AD pathogenesis, and further explored the molecular insights by
153 transcriptomic analyses, thus shedding new light onto the long-standing controversy of LCs in
154 skin immunity.

155

Methods

Details on the methods used in this study are described in the Methods section in this article's Online Repository, including Experimental mice; MC903 topical application; Epicutaneous OVA sensitization and airway challenge; Depletion of Langerin⁺ DCs or LCs in mice; Cell preparation for flow cytometry analyses; Surface staining for flow cytometry analyses; LN cell culture and antigen stimulation; RNA sequencing; BAL cell analyses; ELISA; Histopathology; IHC staining; RNA in situ hybridization and Statistics.

Results

Topical MC903 treatment induces TSLP-dependent Tfh cell differentiation and GC response

We have previously reported that topical treatment with MC903, a low calcemic analog of vitamin D3, induces the overproduction of TSLP (TSLP^{over}) and the pathogenesis of AD^{6,7}. To examine the Tfh cell differentiation and GC response in MC903-induced AD model, Balb/c wildtype (WT) mouse ears were topically treated every other day from day (D) 0 to D10 with MC903 and ear-draining lymph nodes (EDLN) were analyzed at D0, D7 and D11 (**Fig 1A**). Results showed that the frequency and number of CXCR5⁺ PD-1⁺ Tfh cells were both increased in MC903-treated WT mice at D7 and further augmented at D11 (**Fig 1B**). We next examined the expression of IL-4, a key signal provided by Tfh cells to sustain B cell maturation, by taking use of *Il4/Il13* dual reporter 4C13R^{Tg/0} mice, in which AmCyan and dsRed are expressed under the control of IL-4 and IL-13 regulatory elements, respectively²⁸. In agreement with a previous report²⁹, CXCR5⁺ PD-1⁺ Tfh cells in EDLNs express IL-4 (AmCyan) but not IL-13 (dsRed) (**Fig E1A**), and the IL-4 expression by Tfh

cells was augmented in MC903-treated 4C13R^{Tg/0} mice at both D7 and D11 (**Fig 1C**). Together, MC903 treatment induces not only Tfh cell differentiation but also the production of IL-4 by Tfh cells.

To examine whether the induction of Tfh cells in MC903 model is triggered by TSLP, mice lacking TSLP (*Tslp*^{-/-})⁶ were subjected to MC903 treatment. Results showed that these mice exhibited highly diminished Tfh cell frequency and number at D7 and D11, compared to WT mice (**Fig 1B**). By breeding *Tslp*^{-/-} with 4C13R^{Tg/0} to generate *Tslp*^{-/-}/4C13R^{Tg/0} mice, we showed that MC903-induced IL-4 expression in Tfh cells was abrogated in the absence of TSLP (**Fig 1C**). In agreement with the recognized role of TSLP in Th2 cell differentiation, we showed that the MC903-induced IL-4- or IL-13-expressing CXCR5⁻ CD4⁺ non-Tfh cells (representing Th2 cells) were also abrogated in *Tslp*^{-/-} mice (**Fig E1B**). These results indicate that the overproduction of TSLP triggers not only Th2 cell differentiation, but also Tfh cell differentiation and IL-4 expression by these cells.

Next, we examined the GC response in MC903-treated Balb/c WT mice. The number of GC B cells, identified as GL-7⁺ CD95⁺ B cells, exhibited an increase in MC903-treated WT mice at D11, but not at D7 (**Fig 1D**). Such increase was abrogated in MC903-treated *Tslp*^{-/-} mice (**Fig 1D**). This was confirmed by immunofluorescence (IF) staining for GCs (**Fig E2A**). In addition, both IgG1⁺ and IgE⁺ B cells exhibited an increase in their numbers in MC903-treated WT mice at D11, which was also abrogated in MC903-treated *Tslp*^{-/-} mice (**Fig 1E**). Of note, we observed that most of the IgG1⁺ B cells were GL-7⁺ CD95⁺ (**Fig E1C**), suggesting that these cells harbor a GC phenotype; however, this was not the case for IgE⁺ B cells (**Fig E1C**).

Taken together, these data indicate that the overproduction of TSLP triggers Tfh cell differentiation and GC response.

Depletion of Langerin⁺ DCs or LCs diminishes the TSLP^{over}-triggered Tfh/GC response

LCs reside in the epidermis as a dense network of immune system sentinels, in close proximity to keratinocytes. We then asked whether LCs mediate the TSLP^{over}-triggered Tfh/GC response. To this aim, we first employed Langerin-DTR knock-in mice (Lang^{DTR}) in which Langerin⁺ cells, including LCs and Langerin⁺ dermal DCs, express the human diphtheria toxin receptor (DTR) and can thus be depleted upon injection of diphtheria toxin (DT)³⁰. Lang^{DTR} mice and their wildtype control littermates were intraperitoneally (*i.p.*) injected with DT at D-2, D0 and every 4 days to maintain the depletion of Langerin⁺ cells (named Lang^{DEP} and CT respectively), and were subjected to topical MC903 treatment (**Fig 2A**). Results showed that the TSLP^{over}-triggered Tfh cell differentiation was largely diminished in Lang^{DEP} mice (**Fig 2B**). The expression of IL-4 (AmCyan) by Tfh cells was also reduced in Lang^{DEP}/4C13R^{Tg/0} mice (**Fig 2C**). Accordingly, GC B cell number was lower and IgG1⁺ (however not in IgE⁺) B cell number was significantly decreased (**Fig 2D**). Therefore, these results indicate that Langerin⁺ DCs play an important role in mediating the TSLP^{over}-induced Tfh/GC response.

As LCs and Langerin⁺ cDC1s were both depleted in Lang^{DEP} mice, we next examined whether LCs mediate Tfh cell differentiation by depleting selectively LCs using two strategies: one took use of the differential recovery time between LCs and Langerin⁺ cDC1s after DT-induced depletion as previously reported³¹ (**Fig E3A-B**), and the other one employed human Langerin-DTR (huLang^{DTR}) mice in which DT injection efficiently depletes LCs but not Langerin⁺ cDC1s³² (**Fig E3C-D**). In both cases, we showed that the selective depletion of LCs led to a decrease in frequency and number of Tfh cells, suggesting an important role for LCs in TSLP^{over}-triggered Tfh cell differentiation.

Epicutaneous OVA sensitization induces a TSLP-dependent Tfh cell differentiation and GC response

We have previously reported that TSLP plays a crucial role for promoting skin sensitization to allergens, using an experimental mouse protocol in which OVA sensitization through tape-stripped (TS) skin leads to an allergic AD inflammation, accompanied by Th2 cell response, and an increased production of OVA-specific IgG1 and IgE in sera ³³. Here, we developed a novel experimental protocol, in which Precise Laser Epidermal System (P.L.E.A.S.E.[®]) ³⁴ was used to disrupt skin barrier and to generate patterned micropores in mouse skin. This protocol allowed us to deliver allergens to micropores at precise depths of the epidermis, thereby achieving a higher efficiency and reproducibility of allergen sensitization through the skin compared with experiments based on TS. We showed that micropores at a depth of 30 μ m (30 μ m-LMP) on Balb/c WT mouse ears reached basal layer of ear epidermis (**Fig 3A**). ELISA analyses indicated that the protein level of TSLP increased at 48 hours after treatment (**Fig 3B**), in agreement with the previous studies showing that barrier disruption induces TSLP production in mouse ³³ and human skin ³⁵. Notably, such level of TSLP was comparable to our previously reported TSLP level in TS skin ³³, although it was much lower compared to that of MC903-treated skin (**Fig 3B; see also Fig E13B**). The administration of OVA did not further induce the TSLP level (**Fig 3B**). *In situ* hybridization showed that TSLP RNA expression was restricted to epidermal keratinocytes in LMP skin (**Fig 3C**).

As expected, OVA treatment on LMP ears (named “LMP/OVA”; **Fig 3D**) induced a Th2-type skin inflammation in TSLP-dependent manner, showing that OVA sensitization-induced infiltration of eosinophils and basophils (**Fig E4A-B**), Th2 cytokines (IL-4 and IL-13) expression by T cells in the skin (**Fig E4C**) and by CXCR5⁺CD4⁺ cells in EDLNs (**Fig**

E4D), were all abolished in mice lacking TSLP. Examination of EDLNs revealed that both frequency and number of Tfh cells were increased in LMP/OVA- compared to LMP/PBS-treated WT mice, and such increase was largely diminished in *Tslp*^{-/-} mice (**Fig 3E**). Note that LMP/PBS was not sufficient to induce Tfh cell differentiation (despite of the induction of TSLP), but LMP plus OVA together promoted Tfh/GC response which was TSLP-dependent (**Fig 3E**). Moreover, IL-4 production by Tfh cells was augmented in LMP/OVA-treated *Tslp*^{+/+}/4C13R^{Tg/0} mice but not *Tslp*^{-/-}/4C13R^{Tg/0} mice (**Fig 3F**). GC B cell number analyzed by flow cytometry (**Fig 3G**) and GC size analyzed by immunofluorescence (**Fig E2B**) both showed an increase in LMP/OVA-treated WT mice, and this increase was abrogated in the absence of TSLP. IgG1⁺ and IgE⁺ B cell numbers were also increased in LMP/OVA-treated WT mice, and they were much lower in LMP/OVA-treated *Tslp*^{-/-} mice (**Fig 3G**). Accordingly, serum levels of OVA-IgG1 and OVA-IgE were decreased in *Tslp*^{-/-} mice compared to WT mice upon LMP/OVA treatment (**Fig 3H**). Together, these results demonstrate that TSLP is crucially required for epicutaneous OVA sensitization-induced Th2 and Tfh/GC responses.

Depletion of Langerin⁺ DCs or LCs augments the Tfh/GC response induced by epicutaneous OVA sensitization

Based on the above data from MC903-induced AD, we had expected that Langerin⁺ DCs would be crucially required for epicutaneous OVA-induced Tfh/GC response. To our surprise, when subjected to 30μm-LMP/OVA sensitization (**Fig 4A**), Lang^{DEP} mice did not exhibit a reduction in frequency and number of CXCR5⁺ PD-1⁺ Tfh cells, instead they tended to be higher compared to CT mice (**Fig 4B**). More strikingly, IL-4 expression by Tfh cells was higher in EDLN from LMP/OVA-treated Lang^{DEP}/4C13R^{Tg/0} mice (**Fig 4C**). Accordingly, the GC B cell, IgG1⁺ and IgE⁺ B cell number were not reduced in

LMP/OVA-treated Lang^{DEP} mice (**Fig 4D**), and serum OVA-specific IgE and OVA-specific IgG1 were higher or tended to be higher (**Fig 4E**). Thus, in contrast to our expectation, Langerin⁺ DCs are not required for the Tfh/GC response in LMP/OVA-induced AD model; instead, they appear to play a counteracting role.

Because LCs are located on the suprabasal layer of the epidermis, we suspected that Langerin⁺ cells would be only required in Tfh cell differentiation when allergens are encountered superficially on the skin. To test this possibility, LMP was performed at the depth of 11 μ m, which disrupted only the cornified layer of the epidermis (**Fig 5A**). We observed that the 11 μ m-LMP induced also the production of TSLP, even though its level was lower compared to 30 μ m-LMP (**Fig 5B**). Treatment of wildtype control (CT) ears with 11 μ m-LMP/OVA induced significant increases (although milder than 30 μ m-LMP/OVA) in Tfh cell frequency as well as GC B cell number, which were all abolished in *Tslp*^{-/-} mice (**Fig 5C**), indicating that, despite of a low induction of TSLP, the Tfh/GC response promoted by 11 μ m-LMP/OVA is still crucially dependent on TSLP. However, when Lang^{DEP} mice were subjected to 11 μ m-LMP/OVA treatment, they exhibited a significant increase in the frequency of Tfh cells, in IL-4 expression by Tfh cells, as well as in GC B cell, IgG1⁺ and IgE⁺ B cell numbers in EDLNs (**Fig 5D-F**), accompanied by augmented serum levels of OVA-IgG1 and OVA-IgE (**Fig 5G**). Similar results were also obtained with huLang^{DEP} mice (**Fig 5H-I**), indicating that LCs significantly counteract the Tfh/GC response induced upon the 11 μ m-LMP/OVA sensitization.

Furthermore, we sought to compare antigen-specific Tfh cells between CT and huLang^{DEP} mice using an activation-induced marker assay³⁶. In this assay, the stimulation of LN suspensions with specific antigen drives upregulation of CD154 (CD40L), CD25 and OX40 on Tfh cells, providing a sensitive method for quantifying antigen-specific Tfh cells in mice³⁶. We showed that *in vitro* stimulation with OVA drove the upregulation of

CD154, OX40 and CD25 in EDLN-derived Tfh cells from LMP/OVA-sensitized CT mice; and such upregulation was significantly higher in Tfh cells from LMP/OVA-sensitized huLang^{DEP} mice (**Fig 5J**), thus indicating a stronger OVA-specific Tfh cell differentiation in huLang^{DEP} mice upon OVA sensitization.

Together, these data indicate that LCs suppress the TSLP-dependent Tfh/GC response in epicutaneous OVA sensitization model.

Langerin⁺ DCs or LCs limit epicutaneous OVA-induced Th2 skin inflammation and the subsequent asthma

Having observed the opposite role of Langerin⁺ DCs or LCs in Tfh/GC response in the two mouse AD models, we further explored their involvement in the induction of Th2 cell response. Upon MC903 treatment, Lang^{DEP}/4C13R^{Tg/0} mice exhibited a slight decrease in IL-4 and a tendency of decrease in IL-13 production by CXCR5⁺CD4⁺ cells in EDLN (**Fig E5A**), or by TCRβ⁺ cells in dermis (**Fig E5B**), which suggests a role, even though minor, for Langerin⁺ DCs in the development of Th2 cell response. In contrast, upon 30μm-LMP/OVA treatment, Lang^{DEP}/4C13R^{Tg/0} mice exhibited a higher Th2 cell response in both skin (**Fig 6A**) and EDLN (**Fig E6**). This was in accordance with the observation that LMP/OVA-sensitized Lang^{DEP} mice exhibited a stronger skin inflammation (**Fig 6B**), accompanied with an increase in eosinophils and basophils (**Fig 6C**). Moreover, when subjected to 11μm-LMP/OVA sensitization, both Lang^{DEP} and huLang^{DEP} mice exhibited an enhanced AD-like skin inflammation compared to CT mice (**Fig E7**). Therefore, contrary to their minor role in promoting Th2 cell response in MC903-AD, LCs suppress the Th2 cell response in OVA-AD.

We further examined whether Langerin⁺ DCs limit the atopic march. Upon *intranasal* (*i.n.*) OVA challenge following epicutaneous allergen sensitization (**Fig 6D**), the Lang^{DEP}

mice developed a much stronger asthmatic inflammation compared with CT mice, exhibiting an increase in the number of eosinophils in bronchoalveolar lavage fluid (BAL) (**Fig 6E**), and in RNA expression of Th2 cytokines IL-4, IL-5 and IL-13, as well as chemokine receptor CCR3 (eosinophils) and MCPT8 (basophils) by BAL cells (**Fig 6F**). In addition, H&E staining of lung sections of OVA-treated Lang^{DEP} mice revealed an increased peribronchial and perivascular infiltration, and PAS staining showed an enhanced goblet cells hyperplasia (**Fig 6G**). Similar results were obtained with huLang^{DEP} mice (**Fig E8 A-F**), indicating that LCs counteract the asthma development following epicutaneous allergen sensitization. To exclude the possibility that the enhanced asthmatic inflammation is due to any depletion of lung DCs during the intranasal challenge, we subjected huLang^{DEP} mice to *i.p.* sensitization with OVA/alum and *i.n.* OVA challenge, and observed that these mice developed similar asthmatic inflammation as wildtype control mice (**Fig E8 G-H**). This suggests that the limitation of asthma inflammation by LCs is indeed due to their role in suppressing the epicutaneous allergen sensitization.

Taken together, these studies reveal opposite roles of LCs in two AD models: in MC903-AD, LCs play an important role in priming Tfh/GC response; they participate but to a lesser extent in promoting Th2 responses. In OVA-AD, LCs are neither required for Tfh/GC nor Th2 responses, instead, they suppress OVA-induced Tfh/GC and Th2 responses as well as the “atopic march”.

Langerin⁺ migratory DCs from MC903-AD but not from OVA-AD mice present profound transcriptomic changes

We next conducted transcriptomic studies to explore molecular insights underlying the opposite roles of Langerin⁺ DCs in Tfh and Th2 cell differentiation in MC903-AD and OVA-AD, by taking use of Lang^{GFP} mouse line in which GFP reports the expression of

Langerin³⁰. Lang^{GFP} mice were treated with MC903 (at D0, D2 and D4) or LMP/OVA (at D0 and D3), and at D5, Langerin⁺ (GFP^{pos}) and Langerin⁻ (GFP^{neg}) migratory DCs (migDCs) were sorted from EDLNs of non-treated (NT), MC903- or LMP/OVA-treated mice, and proceeded to mRNA sequencing (**Fig E9A**). The time point at D5 was selected to compare gene expression patterns of migDCs at the initiation stage of Tfh and Th2 cell differentiation.

Principle component analysis (PCA) for the RNAseq data revealed that the Pos_MC (GFP^{pos} migDCs from MC903-treated Lang^{GFP} mice) was clearly separated from the Pos_NT (GFP^{pos} migDCs from non-treated Lang^{GFP} mice); however, the Pos_OVA (GFP^{pos} migDCs from LMP/OVA-treated Lang^{GFP} mice) was inseparable from the Pos_NT (**Fig 7A**). Correspondingly, analyses of differentially expressed genes (DEGs) in Pos_MC vs Pos_NT identified 756 upregulated and 559 downregulated genes (with a fold change >1.5 and adjusted p<0.05; **Fig 7B**); in a sharp contrast, the comparison of Pos_OVA vs Pos_NT revealed only 39 upregulated and 9 downregulated genes (**Fig 7B**). Therefore, in MC903-AD, Langerin⁺ migDCs undergo profound transcriptomic changes, but in OVA-AD, they present almost no, or very little, transcriptomic changes.

As to Langerin⁻ migDCs, PCA showed that Neg_MC (GFP^{neg} migDCs from MC903-treated Lang^{GFP} mice), Neg_OVA (GFP^{neg} migDCs from LMP/OVA-treated Lang^{GFP} mice) and Neg_NT (GFP^{neg} migDCs from non-treated Lang^{GFP} mice) were all clustered away from each other (**Fig 7A**). Analyses of DEGs identified 710 upregulated and 698 downregulated genes for Neg_MC vs Neg_NT; and 431 upregulated and 427 downregulated genes for Neg_OVA vs Neg_NT (**Fig 7B**), suggesting that Langerin⁻ migDCs present major transcriptomic changes in both MC903-AD and OVA-AD, with considerable numbers of overlapped DEGs (249 upregulated and 215 downregulated).

Gene ontology analyses of DEGs in Langerin⁺ migDCs from MC903-treated mice

Next, using the upregulated or downregulated DEGs identified in Pos_MC (vs Pos_NT) as input, we performed cluster analyses of all the groups and generated heat map to visualize trends of expression for genes across the different groups. Results are presented in **Fig E9B** and **Fig E10A**. Further, we performed gene ontology (GO) analyses of the upregulated genes in Pos_MC (**Fig 7C**), and examined whether these genes were also significantly upregulated in Neg_MC (vs Neg_NT), and Neg_OVA (vs Neg_NT). We paid particular attention to the upregulated genes shared in all the three groups (Pos_MC, Neg_MC and Neg_OVA), standing here for “commonly upregulated” genes (highlighted in red in **Fig 7C**), as they could be implicated in TSLP-promoted Tfh and/or Th2, a common feature shared by MC903-AD and OVA-AD. Among them, we found genes related to: 1) “regulation of cell migration”, many of which were reported to facilitate DC migration (*Mmp14*³⁷; *Stat5*³⁸; *Nrp2*³⁹; *Sema7a*⁴⁰); 2) “T cell costimulation”: *Cd80* and *Cd86*⁴¹, *Il2ra*⁴², *Pdcd1lg2* (PD-L2)⁴³, *Cd274* (PD-L1), *Gpr183* (EBI2)^{44,45}; 3) “cytokine signal”: *Il2ra*, *Tnfrsf11b* and *Ccl22*; and 4) “transcription factors” such as *Ikzf4*, *Irf4*, *Stat4* and *Stat5a*.

We examined TSLP signaling pathway among the upregulated genes in Pos_MC. Using the reported TSLP-regulated gene set⁴⁶, we identified *Cd84*, *Cd82*, *Ccl17*, *Ccl22* and *Tnfrsf11b* (in the cluster with higher expression in Pos_MC than Neg_MC), as well as *Cish*, *Cd86*, *Cd80*, *Cd274*, *Il2ra*, *Il6*, *CCR2*, *Tgfb1* (in the cluster with higher expression in Neg_MC than Pos_MC) (**Fig 7D**). In addition, we identified *Irf4*, which has been recently shown to be downstream of TSLP signaling in human migratory LCs⁴⁷. The upregulation of these TSLP-targeting genes by Langerin⁺ migDCs suggests that these cells could be a direct responder to TSLP signaling, although it remains to be demonstrated that TSLP signals through its receptor

on LCs drive their migration/activation. Besides these known TSLP downstream genes, more TSLP pathway genes identified from those “commonly upregulated” genes can be envisaged.

Interestingly, we did not find *Tnfrsf4* (encoding OX40L) among the DEGs in Pos_MC, despite that OX40L was reported to be TSLP-responsive gene and mediate TSLP-promoted Th2⁴⁸ and Tfh⁴⁹ cell differentiation. Actually, OX40L expression by GFP^{pos} cells was barely detected in Pos_NT, Pos_MC or Pos_OVA (**Fig 7E**). On the other hand, OX40L was expressed in Neg_NT, and its expression was further upregulated in Neg_MC and Neg_OVA. Therefore, it is unlikely that OX40L would be responsible for the Tfh-promoting function of Langerin⁺ DCs, while its precise function as a potential TSLP downstream factor in Langerin⁻ DCs remains to be defined (**Fig 7E**).

Among the above-mentioned TSLP-regulated gene, IL-6 has been shown to be a critical cytokine for Tfh cell differentiation^{50, 51}. We thus tested whether IL-6 neutralization decreases Tfh / GC response in MC903-AD. Results showed that IL-6 was not required for the initiation of Tfh cell differentiation and the overall GC reaction (**Fig E11**), although it is possible that its function in Tfh response is redundant with other signals as suggested by Eto et al⁵². Besides IL-6, several other Tfh-promoting factors derived from DCs have been recently reported, including IRF-4⁵³, IL-2Ra^{42, 54} and EBI2 (*Gpr183*)^{44, 45}, whose expression was all “commonly upregulated” in Pos_MC, Neg_MC and Neg_OVA (**Fig 7D**). The role of these potential candidates in TSLP-promoting Tfh cell differentiation remains to be examined.

Finally, among the downregulated genes (**Fig E10B**), less knowledge was available, but we could see *Il12b* (IL-23/IL-12p40), whose expression in DCs was previously reported to be suppressed by TSLP⁵⁵. Other commonly downregulated ones included genes related “T cell costimulation” *Havcr2* (TIM3), *Lgals8* (Galectin 8); “Regulation of cell migration” *Adam15* and *Ptk2* (negative regulators for cell migration) and “regulation of transcription” *Foxc2*, *Thrb*, *Tcf7l2*, *Ehf* and *Lmo2*.

Journal Pre-proof

Discussion

In this study, we analyzed how Tfh cells were generated in two experimental AD mouse models, triggered by the overproduction of TSLP by topical application of MC903, or induced by epicutaneous OVA sensitization. We demonstrated a crucial role for TSLP in promoting Tfh cells and GC response in MC903-AD as well as OVA-AD. Intriguingly, we revealed a dual function of LCs in TSLP-promoted Tfh/Th2 cell differentiation: while they promoted Tfh cell differentiation in MC903-AD, they inhibited Tfh/GC response and suppressed Th2 skin inflammation and the atopic march in OVA-AD.

1) TSLP: critical player for Th2 and Tfh cell response in AD

It has been recognized that TSLP is overproduced in AD lesional skin⁵⁶, however, its expression varies from high to low, which could be related with the cause (e.g. genetic mutation of *Spink5* which induces a high level of TSLP⁵⁷ vs skin barrier impairment which induces a low level of TSLP³⁵), age (e.g. TSLP serum level in AD children is high at early stage and decreases with age⁵⁸), or the nature of disease (e.g. intrinsic or extrinsic AD). Our study demonstrates that no matter in AD models associated with either high or low TSLP expression, TSLP is crucial for promoting Tfh/GC response in AD. Recently, the link between TSLP and Tfh cell differentiation was suggested by the study with human blood DC-T cell coculture system⁴⁹. Thus, the Tfh-promoting function of TSLP appears to be conserved between mouse and human, which suggests that it is relevant and valuable to employ AD mouse models to elucidate mechanisms underlying the TSLP (skin)-Tfh (draining LN) axis, particularly the access of tissue and lymphoid organs is rather limited in human study.

Our data add new evidence that neutralization of TSLP or blocking TSLP downstream pathway will be helpful for reducing Th2 and Tfh cell responses in AD. Notably, TSLP is

crucial for driving the downstream IL-4/IL-13 expression by Th2 cells, as well as IL-4 expression by Tfh cells. Indeed, blocking antibody against IL-4/-13R (Dupilumab), which may actually target both Th2 and Tfh cell responses, has been shown to achieve significant therapeutic effect on AD⁵⁹. Intriguingly, neutralization TSLP antibody Tezepelumab has been demonstrated to significantly reduce annual asthma exacerbation rate in patients with uncontrolled asthma⁶⁰. A recent study with Tezepelumab showed numeric improvements in patients with moderate to severe AD, despite that there were certain limitations in that study including patient selection, use of topical corticosteroids, duration of treatment and uncertain inhibition of TSLP with the dose used⁶¹. Given the preclinical evidence for the role of TSLP in AD, more clinical studies are required to evaluate TSLP as therapeutic target in AD.

It should be also noted that recent studies have recognized the importance of Tfh cells in AD¹⁹⁻²¹, but the *in vivo* function of Tfh remains to be further delineated using AD mouse models. This is challenged by the lack of appropriate tools to deplete Tfh cells. We are under the way to generate mouse line in which DTR can be selectively expressed in Tfh cells, thus allowing the DT-induced depletion of Tfh cells.

2) LCs: function as migratory DCs to promote Tfh cell differentiation

LCs represent one of the most studied but controversial DC subtypes. Our study shows that LCs are importantly engaged in the initiation Tfh cell differentiation and GC response triggered by TSLP^{over} in MC903-AD. This provides new evidence on the Tfh-promoting function of LCs in AD, in addition to several studies reporting the requirement of LCs for humoral responses in other contexts^{62,63,64}. In MC903-AD, we observed that LCs play a dominant role in Tfh cell differentiation, although dermal langerin⁻ DCs may also contribute. On the other hand, langerin⁻ DCs (cDC2) appear to be the major player for the TSLP^{over}-induced Th2, while LCs have somewhat but minor contribution. Nevertheless, to provide

direct evidence for the contribution of cDC2 in TSLP-driven Tfh and Th2 responses in AD, further studies could be performed using DC-specific KO of IRF4 or Dock8 mice, which have impaired development and migration of CD11b⁺ cDC2⁶⁵, or CD301b-DTR mice in which CD301b⁺ cDC2 can be transiently depleted⁶⁶.

There have been long debates on the migration, antigen uptake, and T cell differentiation of LCs in different contexts; but transcriptomic study on migratory LCs in skin-draining LNs under inflammatory pathological contexts was lacking. Our transcriptomic data are therefore of value; however, one drawback is that migratory LCs and cDC1 were not separated in Langerin⁺ (GFP^{pos}) migDCs, thus the gene expression data still need to be cautiously interpreted concerning LCs. Nevertheless, we have shown that Langerin⁺ migDCs in EDLN of MC903-induced TSLP^{over} mice presented substantial transcriptional changes, suggesting that the activation and migration of Langerin⁺ DC to the draining LNs underlie its function to prime Tfh cell differentiation in MC903-AD. Indeed, when comparing numbers of GFP^{pos} and GFP^{neg} migratory DCs in EDLN of MC903-treated Lang^{GFP} mice at D5, we observed that both were increased (**Fig E12**), supporting that both Langerin⁺ DCs and Langerin⁻ DCs migrate to draining LNs in MC903-AD.

3) LCs: function as non-migratory cells in the skin to suppress Tfh/Th2 response?

Our study revealed a suppressive role of LCs for epicutaneous OVA-induced Tfh and Th2 cell differentiation. This is in contrast with two previous studies which reported a role of LCs in provoking AD inflammation by using a tape stripping (TS) OVA sensitization model^{67, 68}. To examine whether the discrepancy is due to the different effects of LMP compared to TS, we performed TS/OVA sensitization on mouse ears. Results showed that, similar to LMP/OVA, TS/OVA-sensitized Lang^{DEP} mouse EDLNs exhibited increased frequency and number of Tfh cells, increased IL-4 expression by Tfh cells, higher numbers of GC B cells, IgG1⁺ and

IgE⁺ B cells, with elevated OVA-IgG1 and OVA-IgE in sera (**Fig E13 A-G**). Moreover, when *i.n.* challenged with OVA, Lang^{DEP} mice developed a stronger asthmatic inflammation (**Fig E13 H-I**). Therefore, the discrepancy with the previous reports ^{67, 68} is not explained by the difference of LMP vs TS technique in epicutaneous OVA sensitization; rather, it could be due to other factors remained yet to be determined, such as the allergen application method: topical OVA vs long exposure (2-day) of OVA placed on patch-test tape; the difference of mouse background: Balb/c vs C57Bl/6; or the site of allergen application: ear vs back.

Why are LCs not implicated in the promotion of Tfh/Th2 cell differentiation in EDLN in this context? Transcriptomic analyses showed that in sharp contrast to MC903-AD, Langerin⁺ migDCs in OVA-AD presented almost the same transcriptomic program as in untreated mice, suggesting an absence of migration/activation of these cells. Indeed, Langerin⁺ migDC numbers in EDLNs from LMP/OVA-treated or TS/OVA-treated mice at D5 were nearly unchanged, whereas Langerin⁻ migDC number was increased (**Fig E12**). This is in agreement with previous studies showing that when skin was treated with fluorescence-conjugated OVA ⁶⁶, HDM ⁶⁹, or Dextran ⁷⁰, antigen uptake and transport to draining LNs were mainly exerted by Langerin⁻ DCs. Of note, it was recently shown that LCs can transfer antigen to cDC2 in the context of Langerin mAb-mediated targeting ⁷¹. It will be interesting to see whether this occurs in AD models, and whether efficiency of LC antigen transfer could be altered in the two models, as another possible explication of different implication of LCs in Tfh cell differentiation.

Then how do LCs exert their anti-Tfh/Th2 role in OVA-AD? A recent study showed that LCs played an immunosuppressive role when OVA was applied on the intact skin, in accompany with the induction of IL-10 in LCs in skin-draining LNs ⁷². However, this does not seem to be our case, because Langerin⁺ migDCs in EDLN did not exhibit any transcriptional change of Treg-inducing signals including IL-10 and TGFβ, or RALDH2.

More likely, the anti-Tfh/Th2 effect of LCs is related to their immune suppression function *in situ* in the skin, in keeping with LC ontogeny not only as DCs but also as non-migratory macrophages^{73,74}. It should be further studied how LCs exert such functionality, for example, by limiting the antigen-uptake by cDC2 in the skin, or by promoting local Tregs in OVA-sensitized skin^{75,76}. Transcriptomic analysis of LCs isolated from the OVA-treated skin site may provide further molecular insights.

4) What signals switch the function of LCs?

One intriguing question is what microenvironment cues and molecular signals switch the function of LC between anti-Tfh/Th2 to pro-Tfh/Th2 in AD contexts. Notably, MC903-AD and OVA-AD exhibit similar AD phenotype which is TSLP-dependent, but the quantity of TSLP and the nature of antigen are different in these two models. In MC903-AD, MC903 induced a high production of TSLP⁷ which was sufficient to induce Tfh and Th2 cell differentiation. As there was no administration of experimental allergen, the nature of antigen implicated in T cell differentiation in MC903 model may involve endogenous antigens or microbiota co-existing in the skin. On the other hand, in OVA-AD, the disruption of skin barrier with LMP induced TSLP expression however to a much lower extent. It is possible that LCs sense the quantity of TSLP. Indeed, as a danger signal, TSLP may convert the function of LCs when its level is above certain threshold. *In vitro* studies have shown that TSLP triggers DC migration⁷⁷, or promotes the survival, maturation and migration of human LCs, and allogenic naïve CD4⁺ T cells cocultured with TSLP-conditioned LCs produced cytokines IL-4 and IL-13⁷⁸, but quantitative study on TSLP signaling has never been performed. It will be interesting to explore whether and how quantitative TSLP signaling determines the role of LCs, by conducting *in vivo* or *ex vivo* dose-dependent experiments. In addition, the nature and quantity of antigens can be also involved in the functional switch of

LCs. To unravel such complexity, the emerging mathematic modeling^{79, 80} may eventually help to integrate multiple parameters for a better understanding of functional switch of LCs.

It will be interesting to further explore in AD patients whether and how TSLP levels are correlated with the states and function of LCs. A better understanding of what molecular switch determines the function of LCs either as "pro-Tfh/Th2" or as "anti-Tfh/Th2", and of how LCs exert such functions, will allow us to shape LCs to act in suppressing the skin inflammation, limiting the allergen sensitization through AD skin, thus preventing the progression from AD to asthma. On the other hand, the potential of LCs to induce Tfh cell differentiation and GC response and the subsequent induction of antigen-specific antibodies has been of interest for transcutaneous vaccination^{63, 81}. Therefore, the knowledge we obtain from this study should be also insightful for LC-based skin vaccination, including the use of TSLP at an appropriate level as an effective adjuvant for promoting Tfh cell differentiation and humoral response.

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Figure Legends

FIG 1. Overproduction of TSLP in the skin triggers Tfh differentiation and GC reponse in MC903-induced AD mice. **A**, Experimental protocol. Mouse ears were topically treated with MC903 or ethanol (EtOH; as vehicle control) every other day from day (D) 0 to D10 and EDLNs were analyzed at D0, D7 and 11. **B**, Frequency and number of CXCR5⁺ PD-1⁺ Tfh cells in EDLN from MC903-treated Balb/c wildtype (WT) and *Tslp*^{-/-} mice. **C**, Frequency of IL-4 (AmCyan)⁺ in Tfh cells and cell number of IL-4⁺ Tfh cells in EDLNs. **D-E**, Number of CD95⁺ GL-7⁺ GC B cells, IgG1⁺ B cells and IgE⁺ B cells in EDLNs. Values shown are means $\pm$ SEMs. **B-D**, one-way ANOVA with Tukey's multiple comparison post-hoc test; **E**, unpaired t-test with Welch's correction. Data are representative of 3 independent experiments with similar results.

FIG 2. Depletion of Langerin⁺ cells diminishes the MC903-induced Tfh/GC response. **A**, Experimental protocol. Lang^{DTR} mice and wildtype littermate controls (CT) were *i.p.* injected with DT at D-2 and D0 and then every 4 days. Mouse ears were topically treated with MC903 or EtOH every other day from D0 to D10 and EDLNs were analyzed at D11. **B**, Frequency and number of Tfh cells. **C**, IL-4 (AmCyan) expression by Tfh cells. **D**, Total number of GC B cells, IgG1⁺ and IgE⁺ B cells. Values shown are means $\pm$ SEMs; one-way ANOVA with Tukey's multiple comparison post-hoc test. Data are representative of 3 independent experiments with similar results.

FIG 3. OVA sensitization through laser-microporated (LMP) skin induces TSLP-dependent Tfh/GC response. **A**, H&E staining of untreated or 30 μ m-LMP ears of Balb/c WT mice. The red arrow points to a micropore with the disruption of the epidermis. Scale bar, 100 μ m. **B**,

TSLP protein levels in ears of WT mice at 48h after the indicated treatment. **C**, RNAscope in situ hybridization for TSLP in untreated or 30 μ m-LMP-ears at 48h after the microporation. The black arrow points to one of the positive signals. Scale bar, 50 μ m. **D**, Experimental protocol for OVA epicutaneous sensitization through LMP ears. OVA or PBS (vehicle) were topically applied on LMP ears at D0, D4, D7 and D11 and EDLNs were analyzed at D13. **E-F**, Frequency and cell number of Tfh cells (**E**) and IL-4 (AmCyan) producing Tfh cells (**F**) in EDLNs. **G**, GC B cell, IgG1⁺ and IgE⁺ B cell numbers in EDLNs. **H**, Serum levels of OVA-IgG1 and OVA-IgE. Values shown are mean $\pm$ SEM; one-way ANOVA with Tukey's multiple comparison post-hoc test. Data are representative of 3 independent experiments with similar results.

FIG 4. Depletion of Langerin⁺ cells does not reduce but rather tends to augment 30 μ m-LMP/OVA-induced Tfh/GC response. **A**, Experimental protocol. Lang^{DTR} mice and wildtype littermate controls (CT) were *i.p.* injected with DT at D-2, D0 and then every 4 days. Mouse ears were treated by 30 μ m-LMP/OVA or 30 μ m-LMP/PBS at D0, D4, D7 and D11 and EDLNs were analyzed at D13. **B**, Frequency and number of Tfh cells. **C**, IL-4 (AmCyan) expression by Tfh cells. **D**, Number of GC B cells, IgG1⁺ and IgE⁺ B cells. **E**, Serum levels of OVA-specific IgG1 and OVA-specific IgE in 30 μ m-LMP/OVA-sensitized Lang^{DEP} or CT mice. Data are means $\pm$ SEM; **B-D**, one-way ANOVA with Tukey's multiple comparison post-hoc test. **E**, unpaired t-test with Welch's correction. Data are representative of 3 independent experiments with similar results.

FIG 5. Depletion of Langerin⁺ cells or LCs enhances 11 μ m-LMP/OVA-induced TSLP-dependent Tfh/GC response. **A**, H&E staining of untreated or 11 μ m-LMP ears of Balb/c WT mice. The red arrow points to a micropore with the impairment of cornified layer. Scale bar,

100 μ m. **B**, TSLP protein levels in ears of WT mice. **C**, Comparison of Tfh cells and GC B cells in EDLNs from WT or *Tslp*^{-/-} mice. **D-F**, Comparison of Tfh cells (**D**), IL-4 (AmCyan) expression by Tfh cells (**E**) and number of GC B cells, IgG1⁺ B cells and IgE⁺ B cells (**F**) in EDLNs from CT or Lang^{DEP} mice. **G**, Serum OVA-IgG1 and OVA-IgE levels. **H**, Experimental protocol. **I**, Comparison of Tfh cells, GC B cells, IgG1⁺ and IgE⁺ B cells in CT and huLang^{DEP} mice. **J**, Comparison of antigen-specific Tfh cells between LMP/OVA-sensitized CT and huLang^{DEP} mice. EDLNs were *in vitro* stimulated with OVA or PBS (vehicle control), and activation markers CD154, CD25 and OX40 expressed by EDLN-derived Tfh cells were examined. Values shown are mean $\pm$ SEM; one-way ANOVA with Tukey's multiple comparison post-hoc test. Data are representative of 2 independent experiments with similar results.

FIG 6. Langerin⁺ cells counteract LMP/OVA sensitization-induced skin Th2 inflammation and the subsequent asthmatic phenotype. **A**, IL-4 (AmCyan) and IL-13 (DsRed) expression in TCR β ⁺ dermal cells. **B**, H&E staining of mouse ears. **C**, IHC staining of mouse ears with anti-MBP (for eosinophils) or anti-MCPT8 (for basophils). Arrows point to positive signals. **D**, Experimental protocol for OVA epicutaneous sensitization and airway challenge. Mice were *i.p.* injected with DT at D-2, D0 and then every 4 days. Mice were either treated with OVA on LMP ears at D0, D4, D7 and D11 or non-treated (NT). All mice were subjected to *i.n.* instillation with OVA from D9 to D12, and analyzed at D13. **E**, Differential cell counting for eosinophils (Eos), neutrophils (Neutro), lymphocytes (Lympho) and macrophages (Macro) in BAL. **F**, RNA levels of indicated genes in BAL cells by RT-qPCR. **G**, Lung sections were stained with H&E for histology or PAS for goblet cell hyperplasia analyses. B: bronchiole, V: blood vessel. Scale bar, 100 μ m. Values shown are means $\pm$ SEM; one-way ANOVA with

Tukey's multiple comparison post-hoc test. Data are representative of 2 independent experiments with similar results.

FIG 7. Transcriptomic analyses of migratory DCs in EDLNs of Lang^{GFP} mice upon MC903 treatment or epicutaneous OVA sensitization. Lang^{GFP} mice were treated with MC903 at D0, D2 and D4 or 30μm-LMP/OVA on D0 and D3; EDLNs were collected at D5 for cell sorting and RNAseq analyses. **A**, Left, percentage of variability explained by each Principal Component. Right, principal component analyses showing PC1, PC2 and PC3. **B**, Venn diagram showing the number of upregulated and downregulated genes (fold change > 1.5; p < 0.05; raw read > 200 in at least one sample of all groups), and the number of commonly upregulated or downregulated genes between the comparisons, as indicated. Pos_NT, Pos_MC, Pos_OVA: GFP^{Pos} migDCs from non-treated, MC903-treated or LMP/OVA-treated Lang^{GFP} mice; Neg_NT, Neg_MC, Neg_OVA: GFP^{neg} migDCs from non-treated, MC903-treated or LMP/OVA-treated Lang^{GFP} mice. **C**, Selected genes corresponding to gene ontology terms. *, p<0.05; NS, non significant. **D**, Heatmaps of the reported TSLP pathway genes, which are significantly upregulated in Pos_MC vs Pos_NT. **E**, Heatmap of Tnfsf4 (encoding OX40L) from RNAseq data, and RT-qPCR analyses.

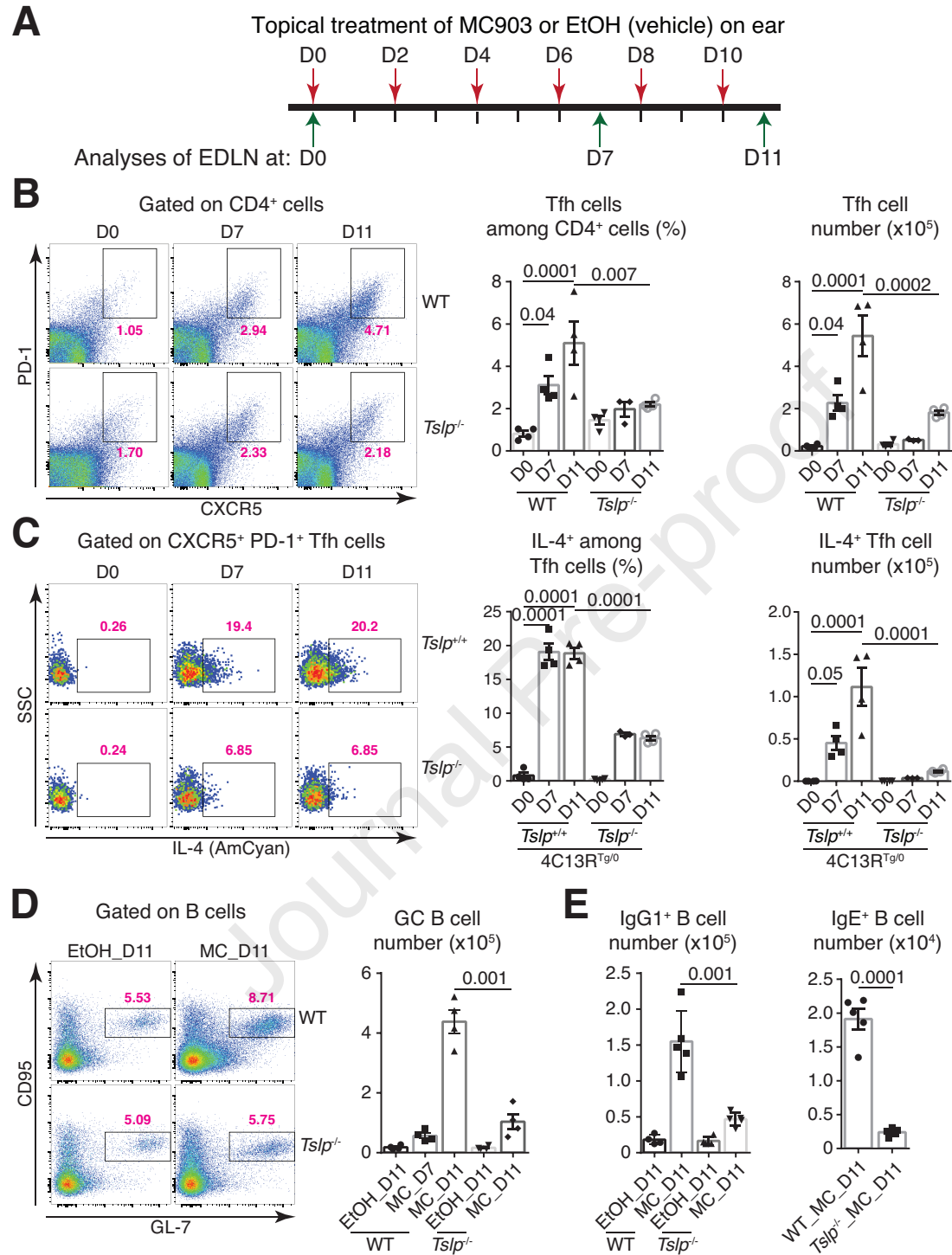


FIG 1

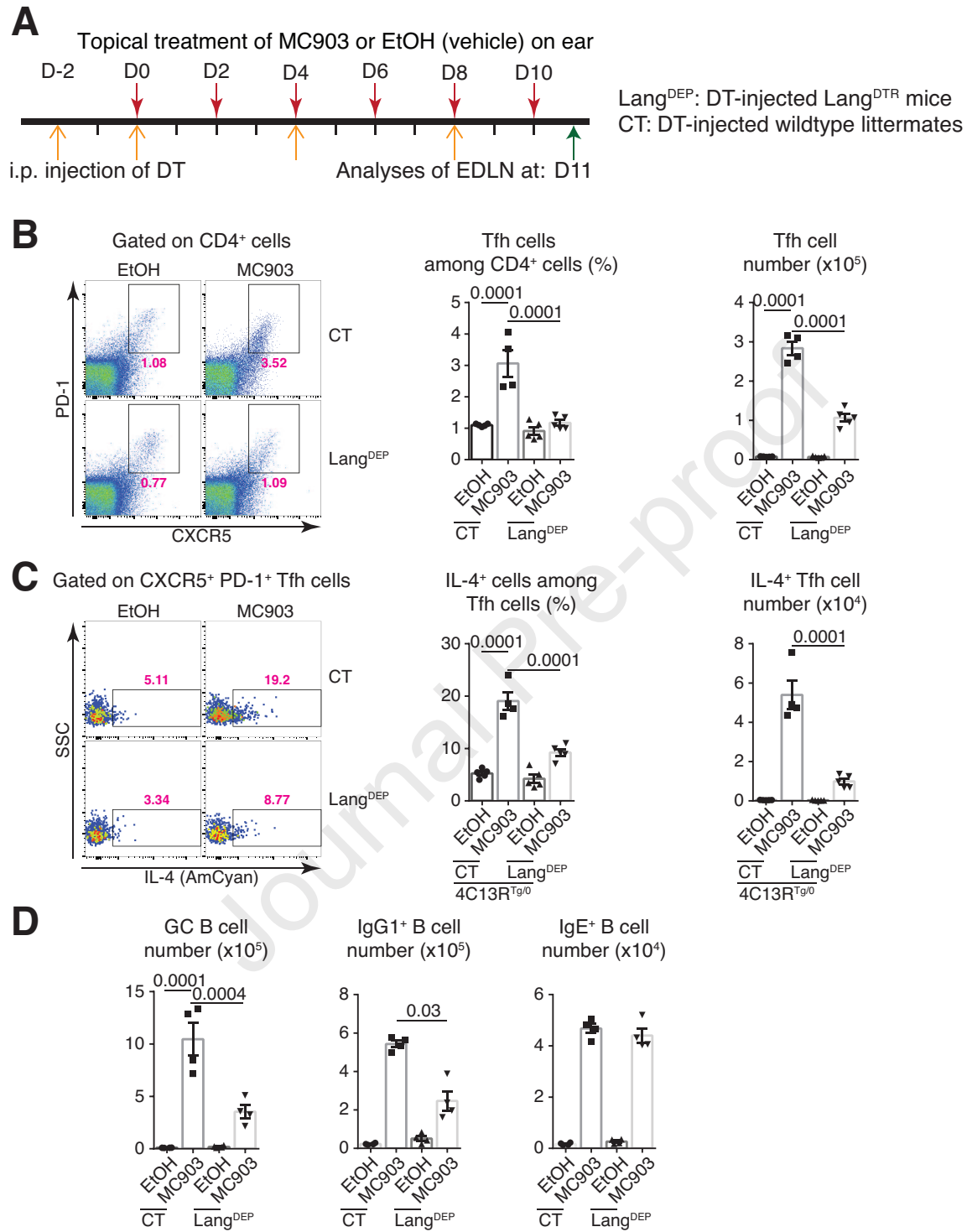


FIG 2

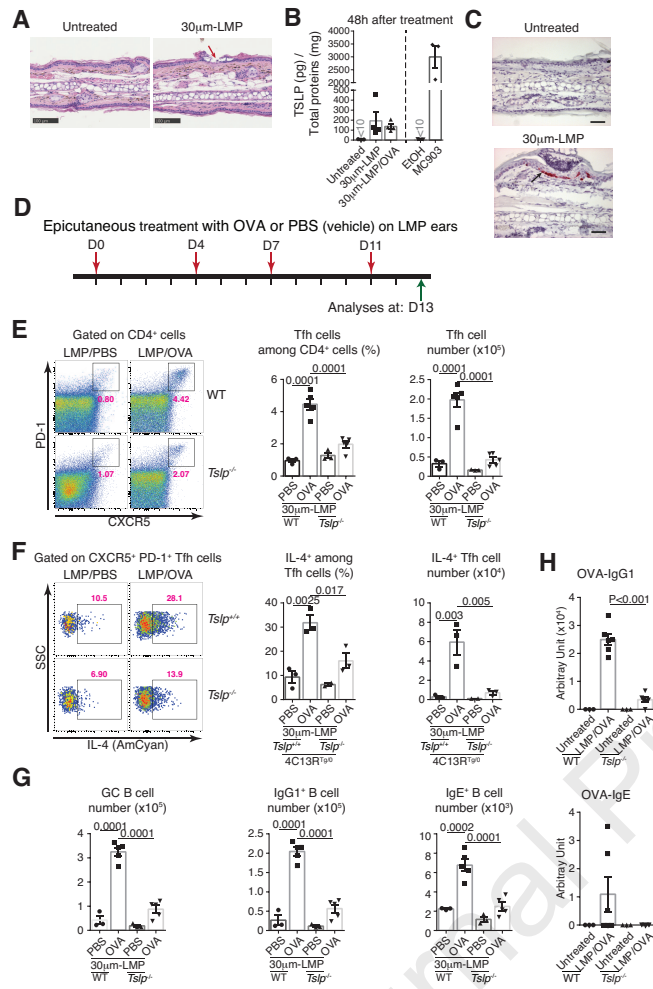


FIG 3

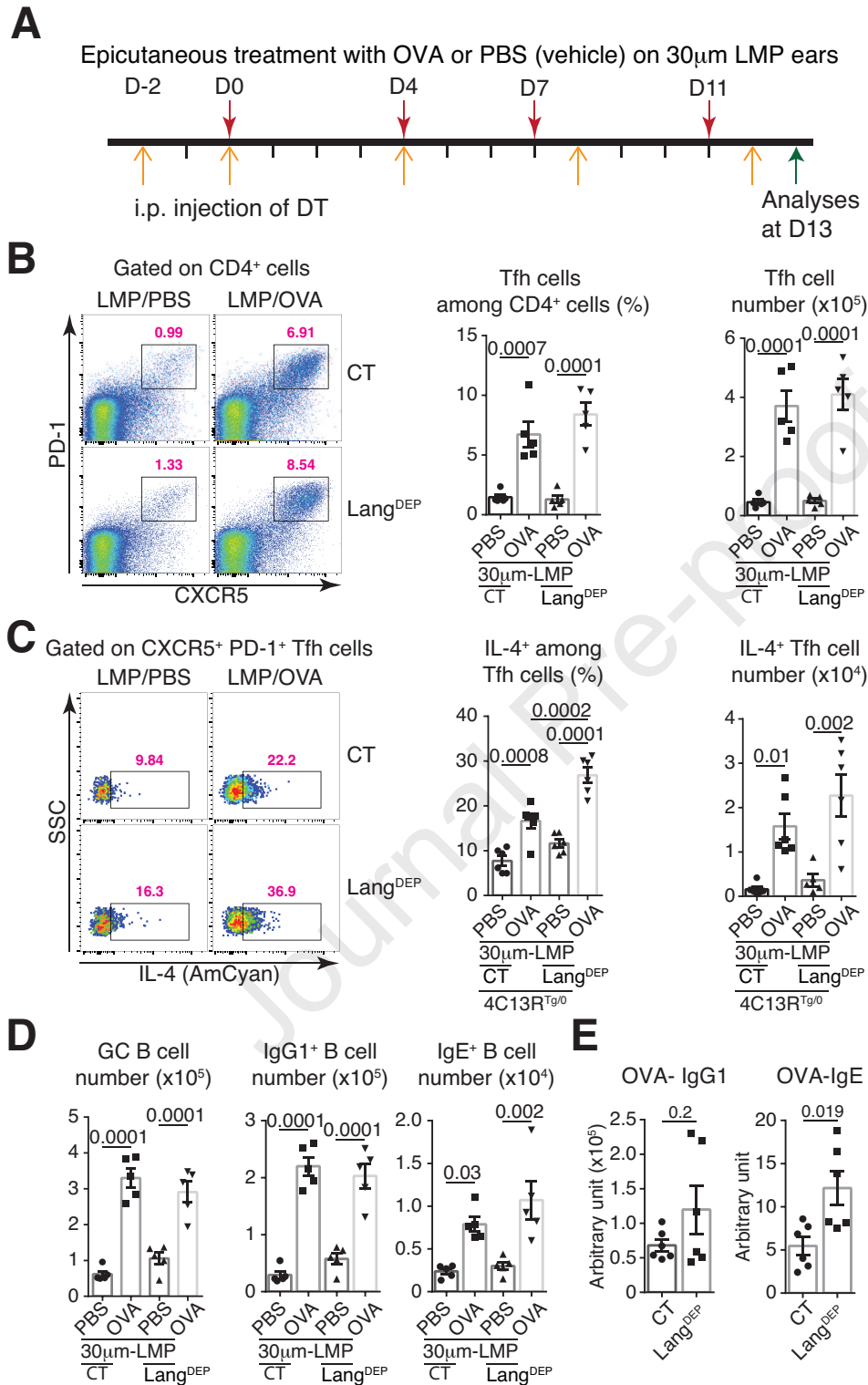


FIG 4

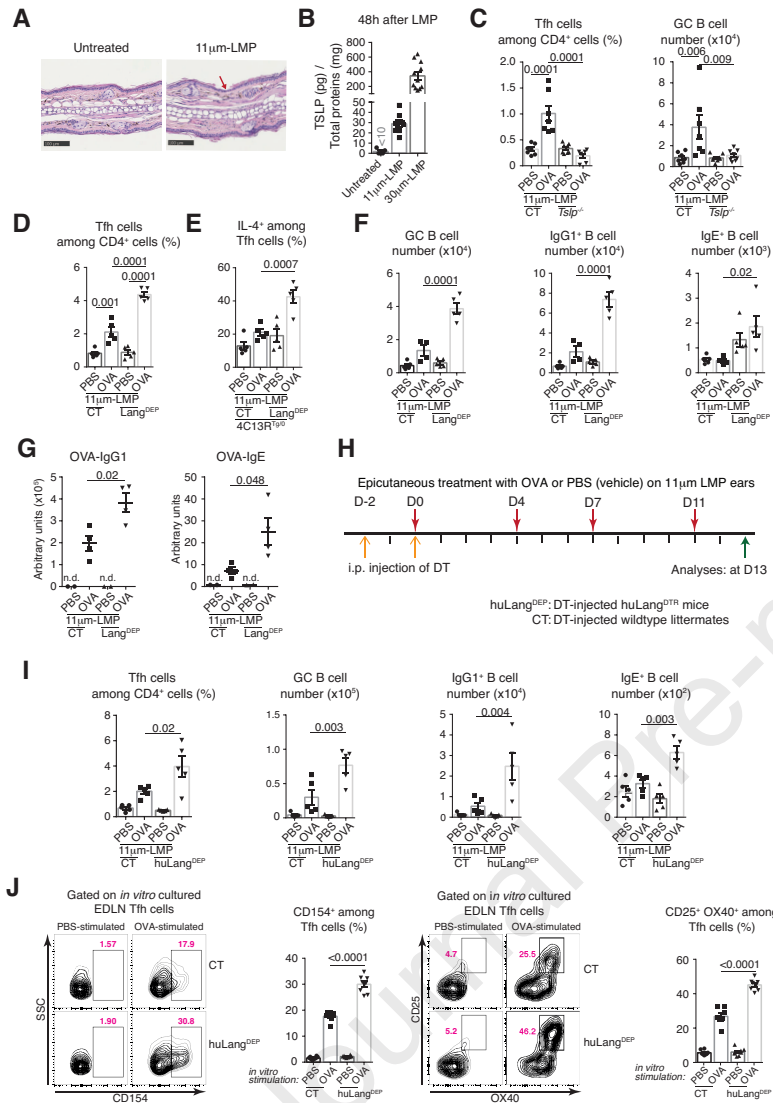


FIG 5

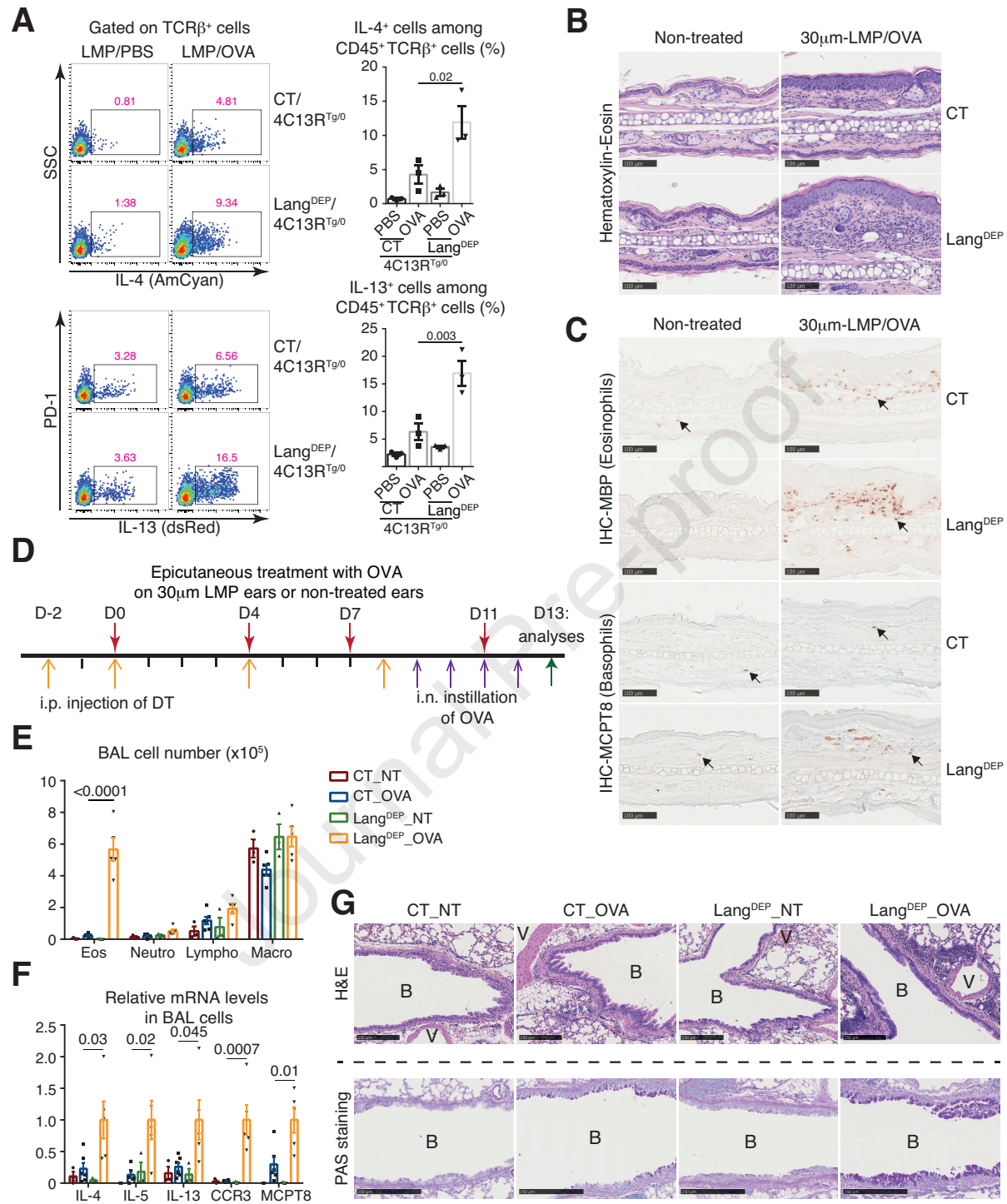


FIG 6

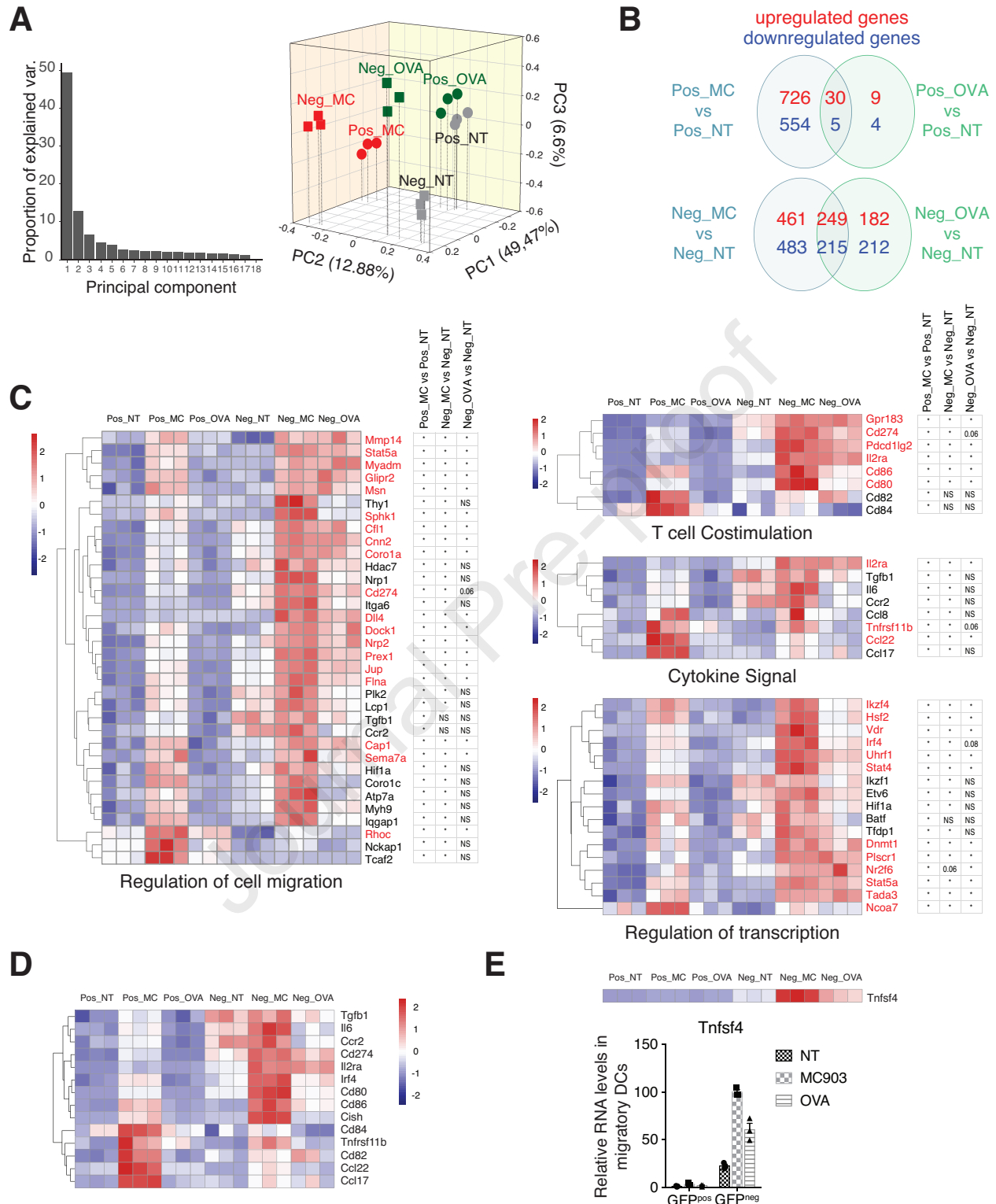


FIG 7