Figure E1

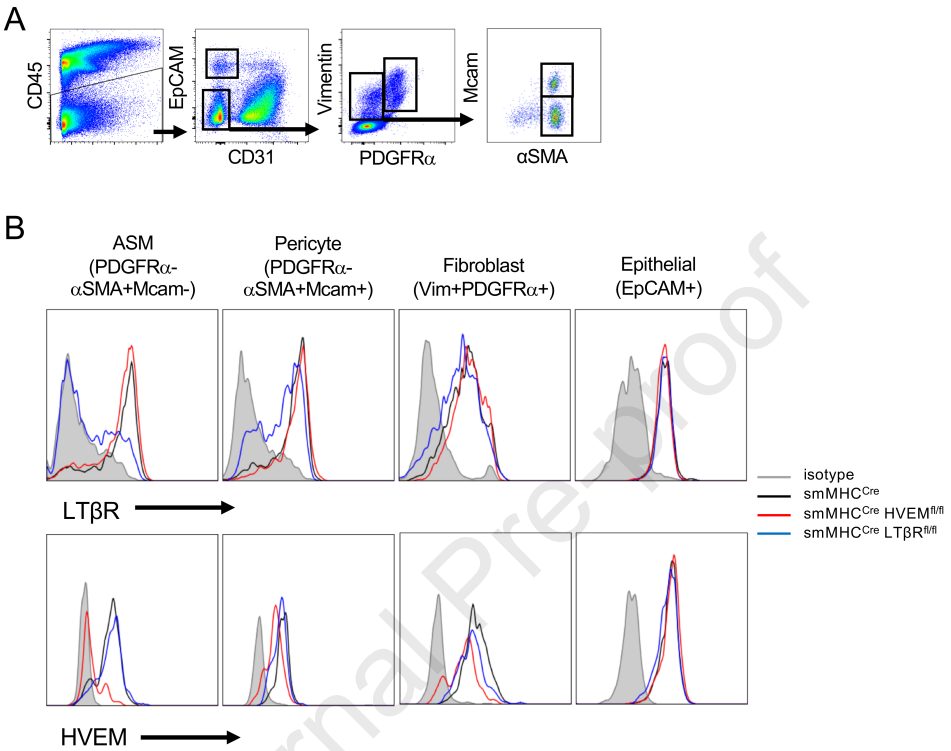

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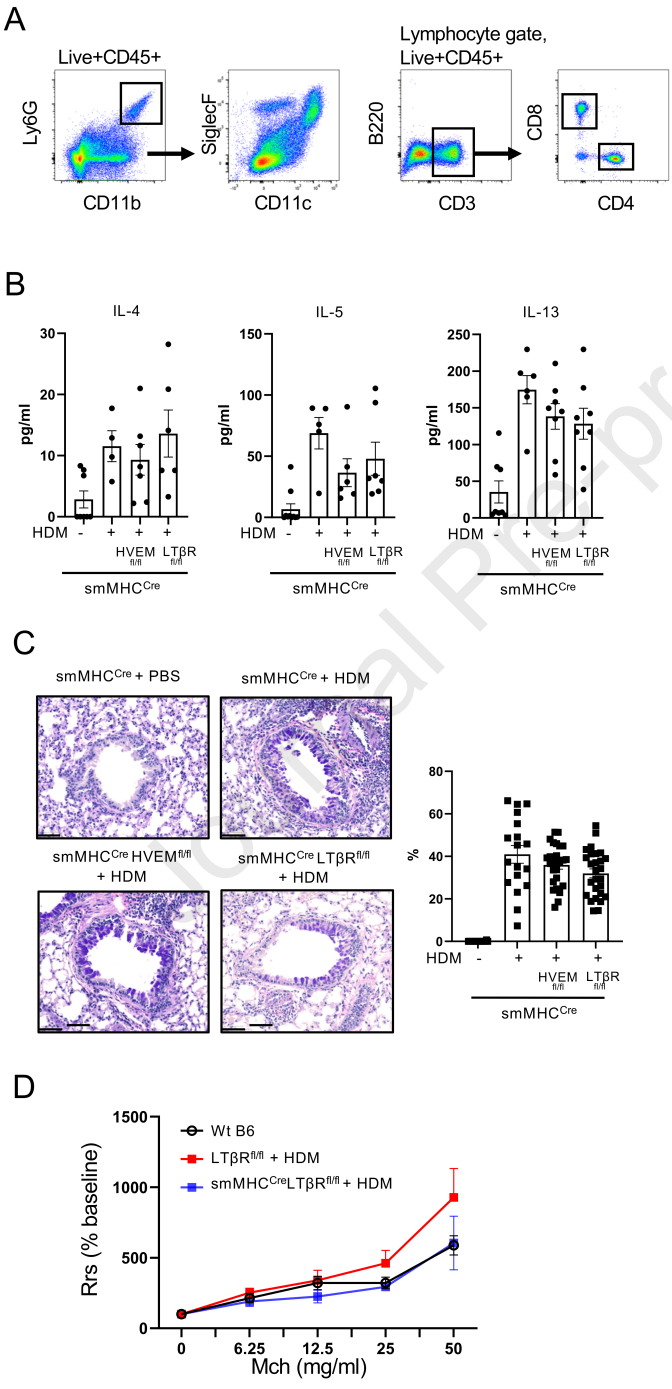
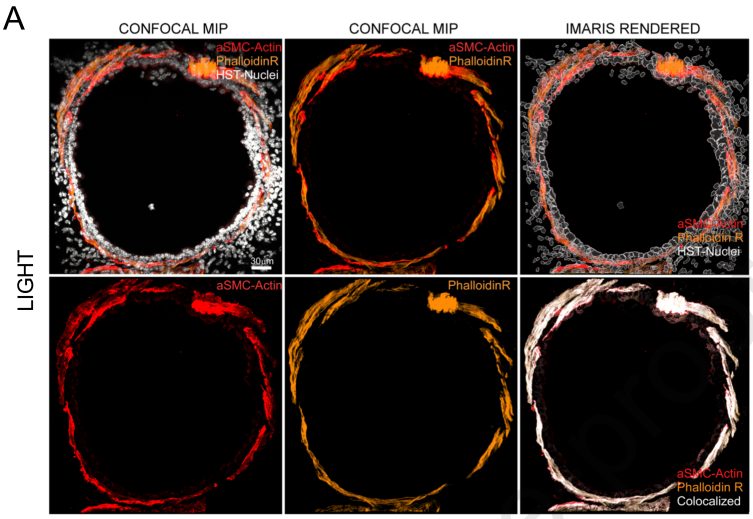
Figure E2



Final Proof

Figure E3



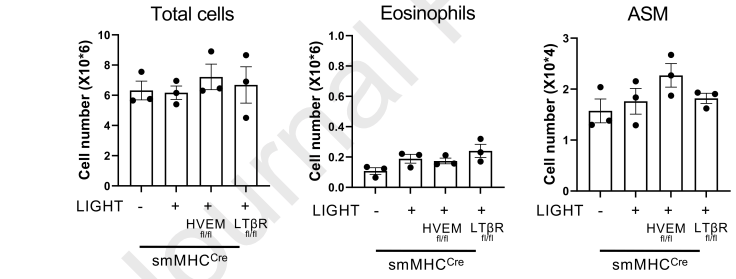


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Figure E4

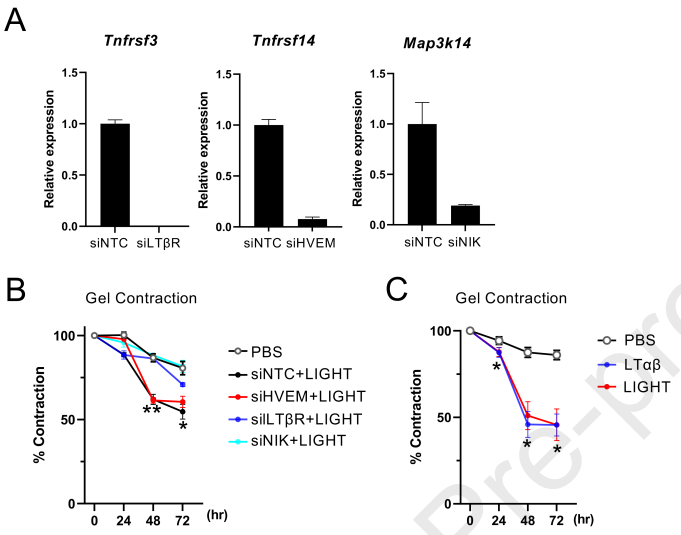

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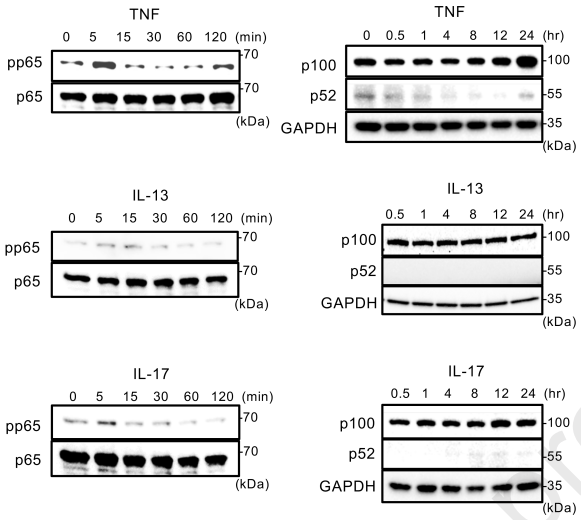
Figure E5



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Supplementary Figure Legends

Figure E1. Flow cytometry analysis of lung structural cells. (A) Flow gating of epithelial cells ($CD45^-EpCAM^+CD31^-$), fibroblasts ($CD45^-EpCAM^-CD31^-Vimentin^{hi}PDGFR\alpha^+$), pericytes ($CD45^-EpCAM^-CD31^-Vimentin^{med}PDGFR\alpha^-\alpha SMA^+Mcam^+$), and ASM ($CD45^-EpCAM^-CD31^-Vimentin^{med}PDGFR\alpha^-\alpha SMA^+Mcam^-$) in mouse lungs. (B) Expression of HVEM and LTβR on gated lung structural cells from $smMHC^{Cre}$, $smMHC^{Cre}HVEM^{fl/fl}$, or $smMHC^{Cre}LT\beta R^{fl/fl}$ mice.

Figure E2. Analysis of inflammatory features in allergen-induced lungs, and AHR in acute lung inflammation. (A) Flow gating of CD45+ lung immune cells; Neutrophils ($CD11b^+Ly6G^+$), Eosinophils ($CD11b^-Ly6G^-CD11c^{lo}SiglecF^+$), Alveolar macrophages ($CD11b^-Ly6G^-CD11c^+SiglecF^+$), $CD4^+$ T cells ($CD3^+CD4^+CD8^-$) and $CD8^+$ T cells ($CD3^+CD4^-CD8^+$). (B) IL-4, IL-5 and IL-13 production in BAL fluid of $smMHC^{Cre}$, $smMHC^{Cre}HVEM^{fl/fl}$, or $smMHC^{Cre}LT\beta R^{fl/fl}$ mice challenged chronically with HDM over 6 weeks (n = 6-8/group). (C) Representative PAS stained lung sections (scale 100 μm) and percentages of PAS-positive bronchial epithelial cells. n =4-5 mice/group, 5 bronchi per mouse. Data representative of 3 experiments. (D) Airway resistance (AHR) after methacholine challenge, measured by Flexivent (n = 4-5 mice/group), in mice acutely challenged with HDM over 14 days. Data means ± SEM.

Figure E3. Bronchial smooth muscle and other cellular changes in mice injected with LIGHT. (A) Confocal images of lung bronchi from mice treated with LIGHT and stained with Phalloidin (orange) and α-smooth muscle actin (red), scale 30 μm. (B) $smMHC^{Cre}$, $smMHC^{Cre}HVEM^{fl/fl}$, or $smMHC^{Cre}LT\beta R^{fl/fl}$ mice were treated intratracheally with LIGHT or PBS, and numbers of total cells, eosinophils, or ASM in lung tissues were analyzed by flow cytometry.

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Figure E4. Involvement of LTβR and NIK in ASM contractility. (A) mRNA expression of Tnfrsf3 (LTβR), Tnfrsf14 (HVEM) and Map3k14 (NIK) in ASM after siRNA targeting. (B) Gel contraction of ASM with siRNA knockdown after stimulation with LIGHT. (C) Gel contraction of ASM stimulated with LIGHT or LTαβ. Means ± SEM from triplicate cultures. Data means ± SEM and representative of 2 experiments. *P < 0.05, **P < 0.01.

Figure E5. TNF, IL-13, and IL-17 activation of canonical or non-canonical NF-κB in ASM. Activation of canonical NF-κB (pp65, left) and non-canonical NF-κB (processing of p100 to p52, right) assessed in human ASM stimulated for various times with TNF, IL-13 or IL-17.

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