

SUPPLEMENTAL FIGURES

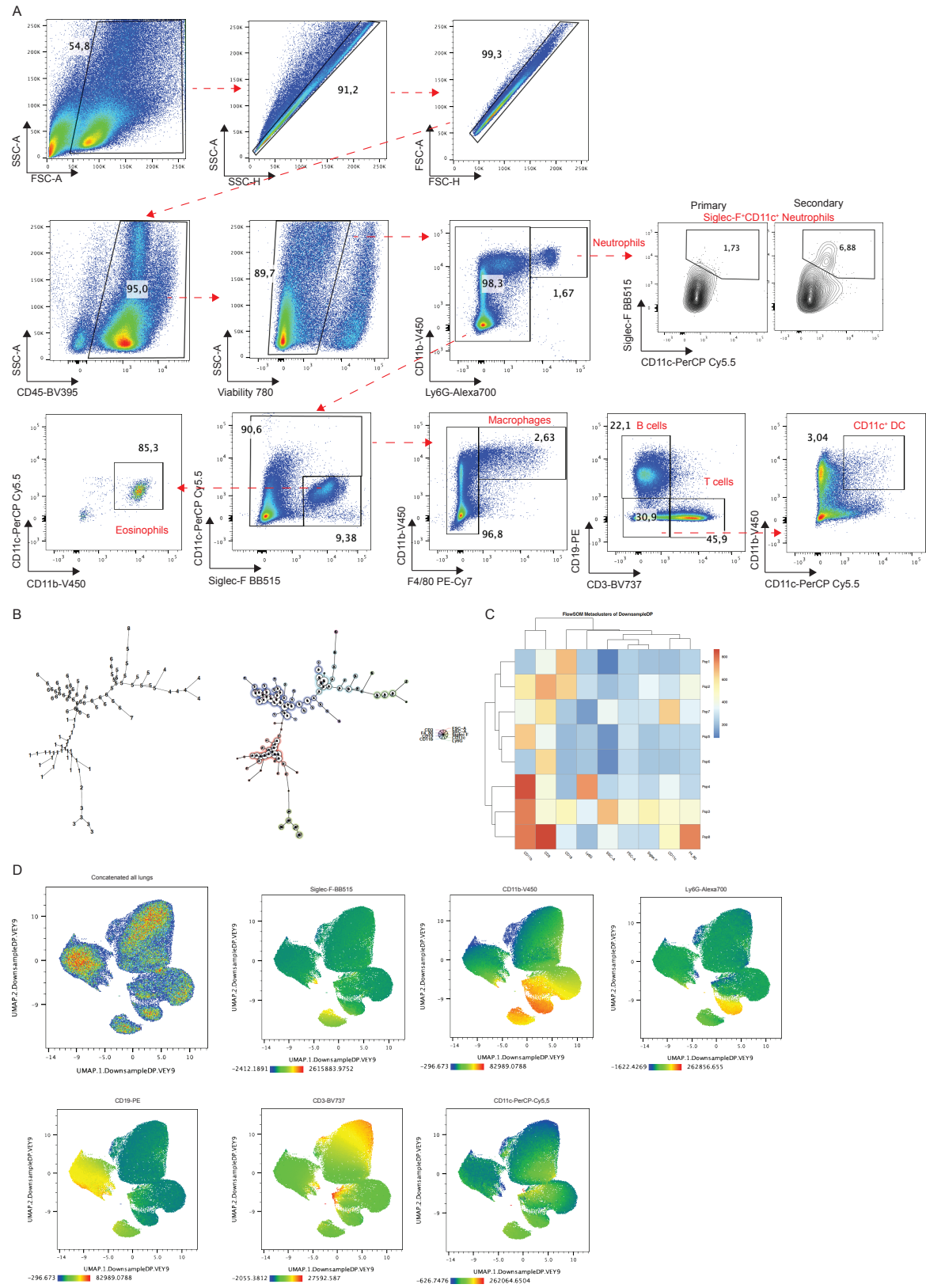


Figure S1. Gating strategy and Uniform Manifold Approximation of live CD45⁺ cells isolated from the lungs of mice challenged with RSV A2.

- (A)** Gating strategy used for the determination of each cell population. (red) name of the final gate. Immune cell populations isolated from the lungs were first gated based on the FSC-A and SSC-A, and single cells were gated based on SSC-A and SSC-H, then by FSC-A and FSC-H. Live (Viability Dye 780^{neg}) were then further gated on CD45 expression. The subpopulations of the indicated immune cells were gated based on specific surface markers.
- (B)** FlowSOM Metacluster tree representation using a maximum of 8 metaclusters of a concatenated file including both primary and secondary infected lungs (FlowSOM v3.0.18; Grassen et al.)
- (C)** Relative expression of each marker by population.
- (D)** Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP v4.0.3; <https://arxiv.org/abs/1802.03426>) visualisation of the live CD45⁺ cells isolated from the lungs.). Representation of the relative expression of each marker on the UMAP plot.

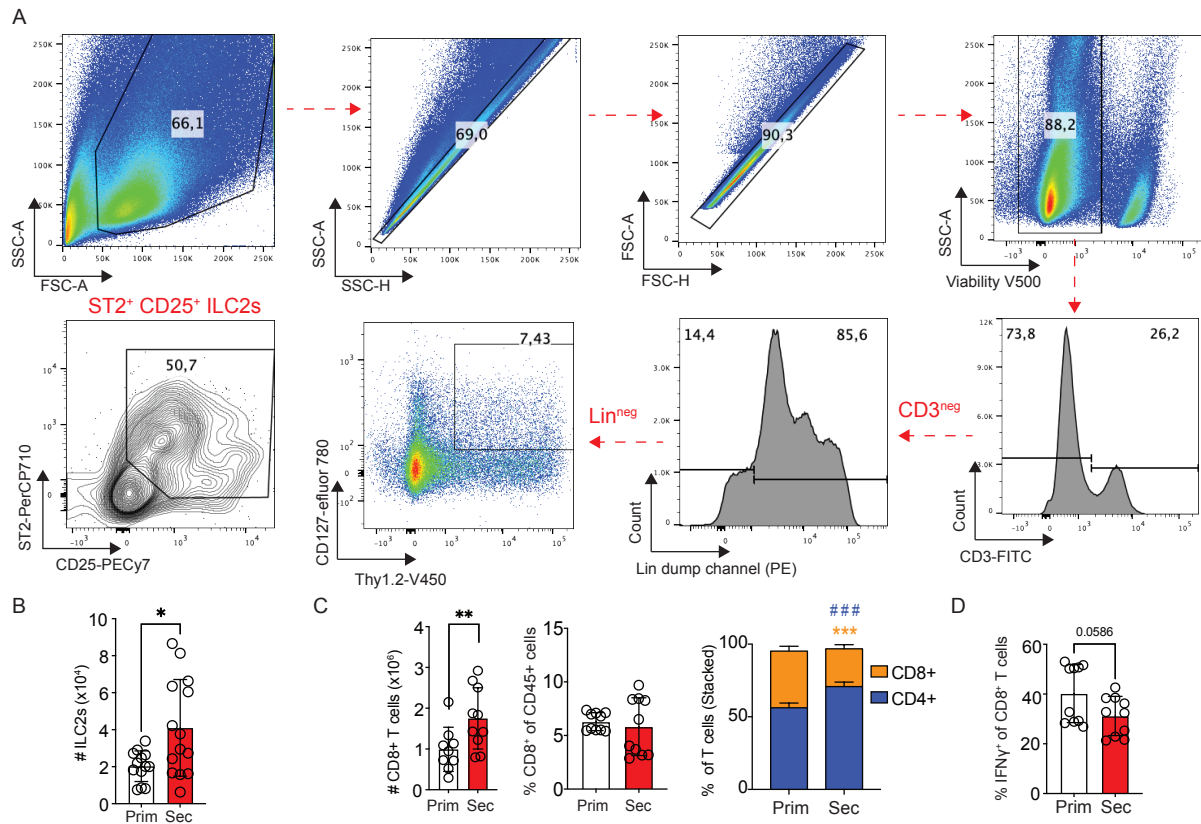


Figure S2. Re-challenge with RSV A2 promotes a rapid expansion of ILC2s and CD8⁺ T cells in the lungs of adult mice.

- (A) Representative gating strategy of the flow cytometry of
- (B) Cell number of ILC2s (Lin⁻, Thy1.2⁺, CD127⁺, CD25⁺, ST2⁺) at day 4 p.i.. Student T test. ***p<0.001
- (C) Number and frequency of CD8⁺ T cells in the lungs at day 4 p.i. Student T test. **p<0.01 and stacked frequency of CD4⁺ and CD8⁺ T cells (CD3⁺) in the lungs of mice at day 4 post-infection. Student T test. ### (CD4⁺ T cells) = p<0.001, *** (CD8⁺ T cells) = p<0.001
- (D) Frequency of IFN γ -producing CD8⁺ T cells isolated from the lungs at day 4 p.i. and exposed to PMA + ionomycin + GolgiStopTM for 3 hours. Student T test.

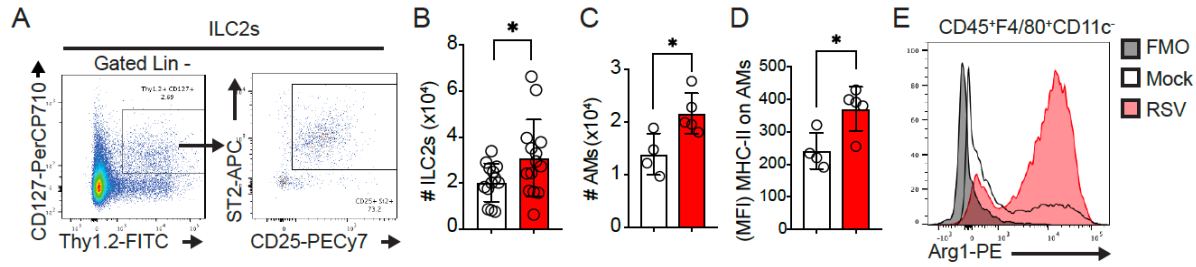


Figure S3. The neonatal innate immune response is skewed towards type 2 innate responses. RSV A2 (10^6 TCID₅₀/g) was instilled intra-nasally in 5–7-day-old BALB/c mice. The lungs were isolated for cell analysis 4 days later. Compiled data of 3 independent experiments.

- (A) Representative flow cytometry of Lin⁻ (CD49b, B220, CD3, F4/80, CD11c, CD11b, Ly6G, TCR $\gamma\delta$), Thy1.2⁺, CD127^{hi}, KLRG1⁺, CD25⁺, ST2⁺ lung resident ILC2s.
- (B) Numbers of ILC2s isolated from the lungs of neonate mice. Student T-test. * $p < 0.05$
- (C) Number of CD45⁺, Ly6G^{int}, CD11c⁺, F4/80⁺ pulmonary alveolar macrophages (AMs) assessed by flow cytometry on 5 mice per group at day 4. Student T-test. * $p < 0.05$
- (D) Mean fluorescence intensity (MFI) of MHC class II (I-A^d) on AMs at day 4. Student T-test. * $p < 0.05$
- (E) Representative histogram of intracellular Arginase 1 (Arg1-PE) expression on CD45⁺ F4/80⁺ CD11c⁺ lung macrophages isolated cells at day 4.

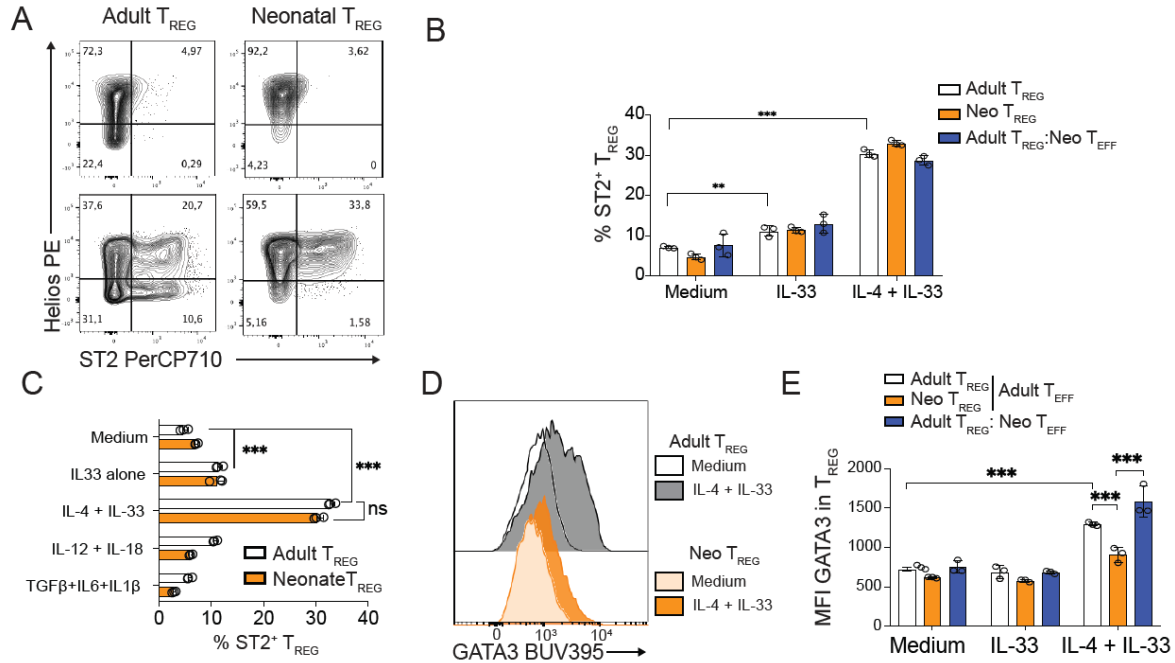


Figure S4. Neonatal T_{REG} cells readily express ST2 *in vitro*.

7 day old (Neonatal) and 8 week old (Adult) $CD4^+CD25^{hi}$ T cells were FACs isolated from the spleen of BALB/c and cultured in the presence of CTV-labelled adult $CD4^+CD25^-$ T cells at a 1:4 ratio in the presence of Mitomycin C-treated adult APCs ($CD4^+$ cells) and soluble $\alpha CD3$ for 72 hours. The cells were exposed to rIL-33 (10ug/ml) or a combination of IL-4 (10ug/ml) + rIL-33 (10ug/ml). Two-way ANOVA. Tukey's test. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

- (A) Representative plot of the expression of Helios and ST2 in CTV- $CD4^+$ Foxp3⁺ T cells at 72 hours.
- (B) Frequency of ST2⁺ among CTV- $CD4^+$ Foxp3⁺ T_{REG} cells at 72 hours.
- (C) Frequency of ST2⁺ T_{REG} between neonatal and adult cells in distinct polarizing conditions.
- (D) Representative histogram of the expression of GATA3 between neonatal and adult $CD4^+$ Foxp3⁺ T_{REG} cells.
- (E) Geometric mean fluorescence intensity of GATA3 in live $CD4^+$ Foxp3⁺ T cells.

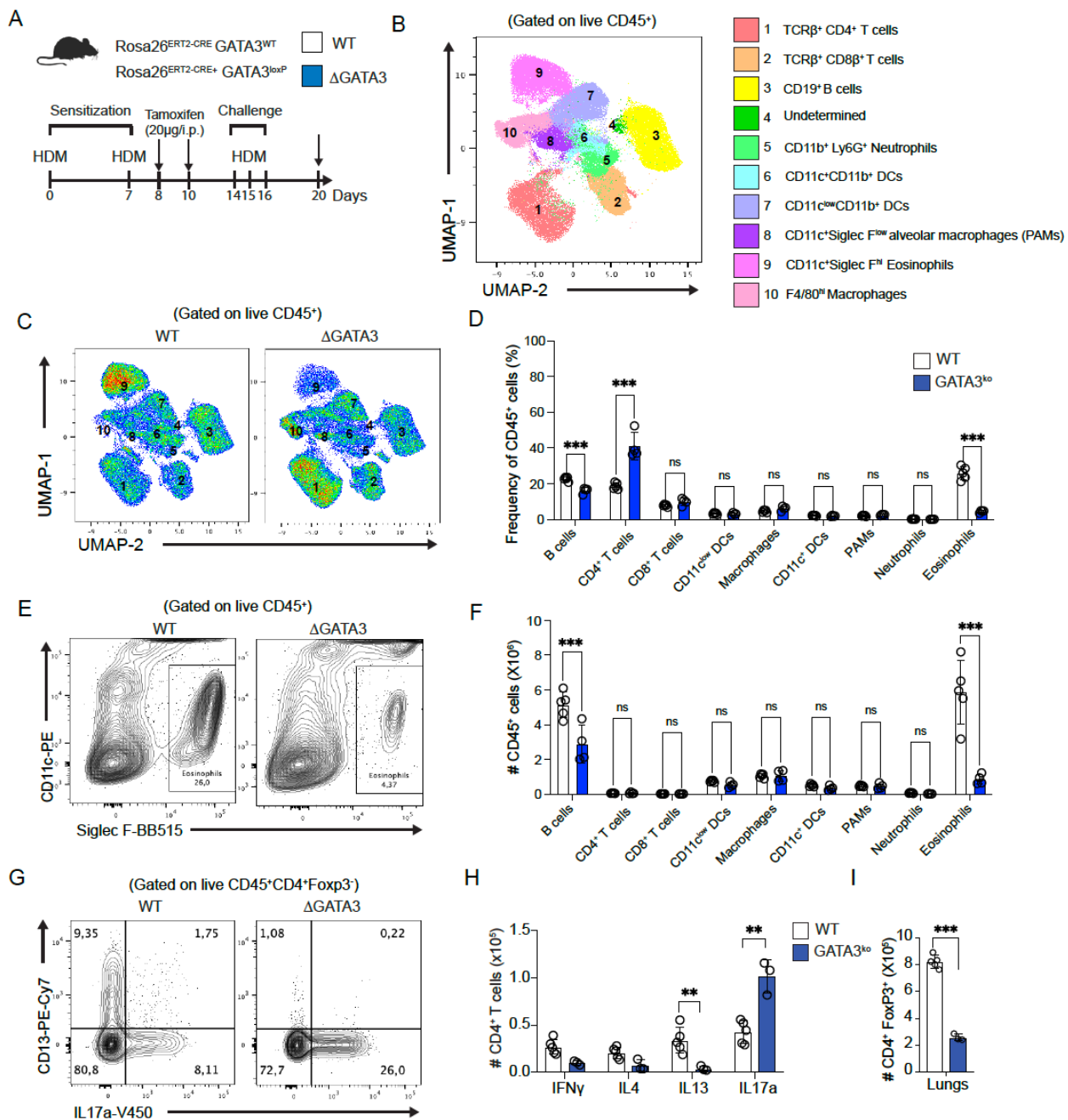


Figure S5. The sustained expression of the transcription factor GATA3 is required to promote exacerbated eosinophilia and T_H2 responses in the lungs.

- (A) 8-10 week old female mice from ERT2-CRE GATA3^{wt} (WT) or ERT2-CRE GATA3^{loxP} (GATA3^{ko}) were sensitized with 10µg/mouse of HDM (*Dermatophagoides pteronyssinus* extract) at day 0 and 6. In between the sensitisation and the challenge, the mice received 20ug of tamoxifen (days 8 and 10), before 3 consecutive days of HDM challenge and necropsy at day 20. Representative of two independent experiments (n=4-5/group; N=2).
- (B) Uniform Manifold Approximation and Projection for Dimension Reduction (UMAP v4.0.3; <https://arxiv.org/abs/1802.03426>) visualisation of the live CD45⁺ cells isolated from the lungs using an unbiased FlowSOM (v3.0.18; Grassen et al. 2015) clustering algorithm to visualise each cluster by Cluster Explorer (v1.7.6; Joseph Trotter, FlowJO Exchange).
- (C) UMAP representation of the distinct cells isolated from the lungs of mice at day 20 of one representative mouse of each group.
- (D) Frequency of each described population among CD45⁺ cells isolated from the lungs at day 20. Two-way ANOVA. Tukey's test. ***p<0.001
- (E) Representative flow cytometry plot of Siglec-F and CD11b expression among live CD45⁺ cells isolated from the lungs at day 20. Box shows the gating for eosinophils.
- (F) Number of each described population among CD45⁺ cells isolated from the lungs at day 20. Two-way ANOVA. Tukey's test. ***p<0.001
- (G) Representative flow cytometry plot of IL-13 and IL17A expression among live CD45⁺ CD4⁺ Foxp3⁻ cells isolated from the lungs at day 20 and treated for 3 hours with PMA + Ionomycin + GolgiStopTM.
- (H) Number of IFN γ , IL-4, IL13 and IL17A expressing T cells in the lungs at day 20. Two-way ANOVA. Tukey's test. **p<0.01
- (I) Number of FoxP3⁺ CD4⁺ T cells isolated from the lungs at day 20. Student T test. ***p<0.001

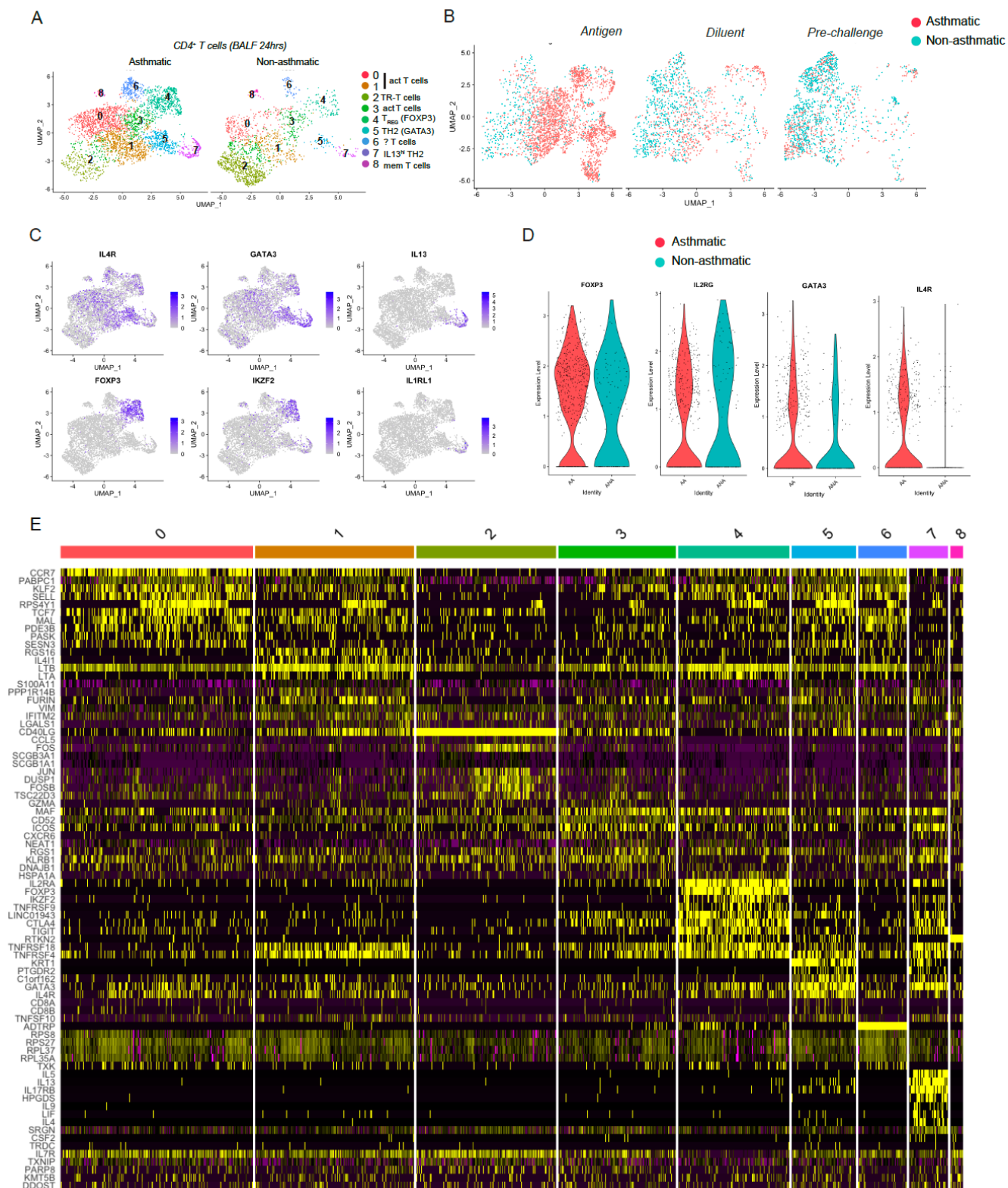
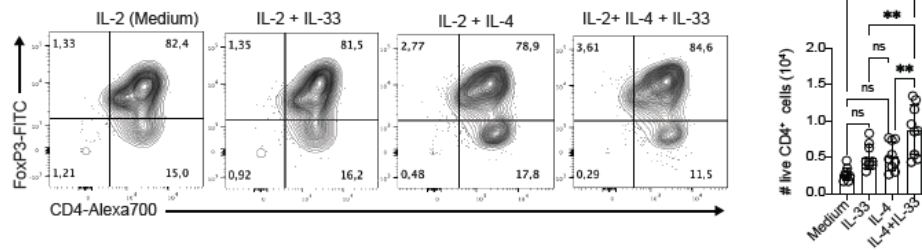


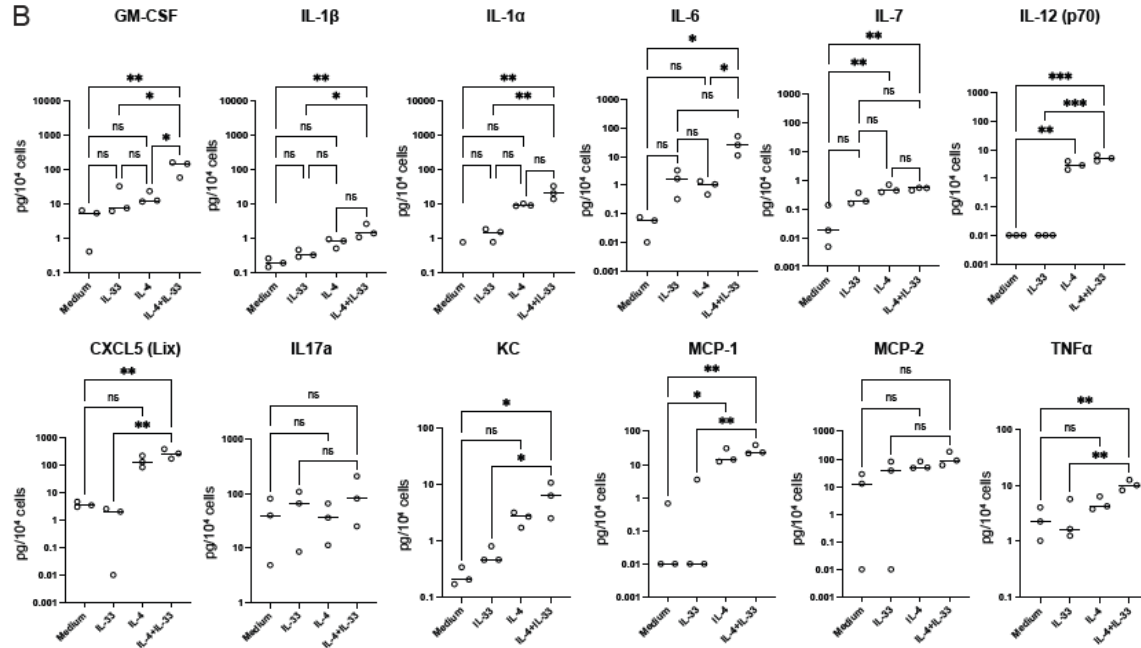
Figure S6. A human house-dust-mite challenge generates abundant GATA3⁺ IL-4R α ⁺ T_{REG} cells in a bronchial alveolar lavage collected from allergic patients

- (A) 10X genomics Single cell analysis data obtained from Alladina et al. 2023 (GSE193816) on cells isolated from the BALF of asthmatic patients and healthy controls challenged with their cognate HDM or cat allergen for 24 hours. The single cell files were obtained raw and underwent quality control. UMAP Clustering CD4⁺ T cells from the BALF of antigen-challenged asthmatic (AS) and healthy controls (HC) at 24hrs.
- (B) UMAP Clustering of CD4⁺ T cells split by sample collection time (Pre-challenge or post-challenge with diluent or antigen).
- (C) Feature Plot of “GATA3”, “IL-13”, “IL1RL1”, “FOXP3”, “IKRZF2” and “IL4R” observed in CD4⁺ T cells obtained from asthmatic and HC at 24 hours.
- (D) VinPlot representation of FOXP3, IKZF2, GATA3, IL4R identified through differential gene expression analysis of cluster 4 (T_{REG}) between asthmatic and non-asthmatic patients.
- (E) Top 10 genes identified in each cluster of CD4⁺ T cells isolated from the BALF of patients prior and after antigen challenge

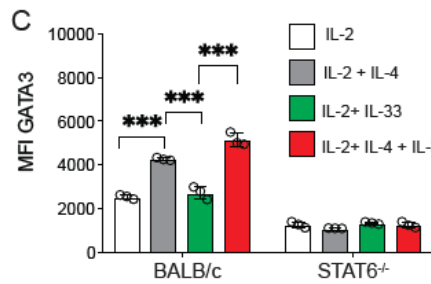
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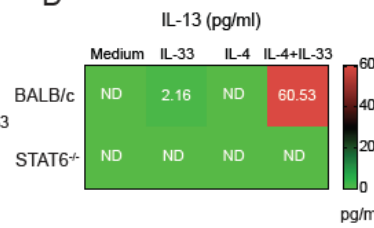
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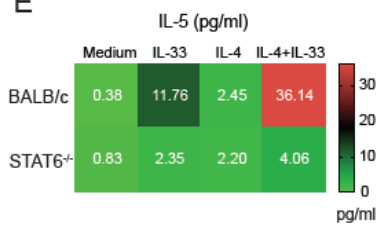
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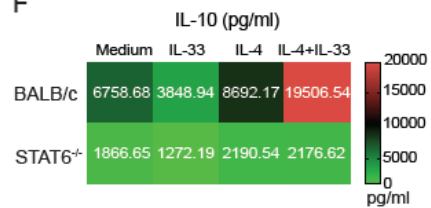
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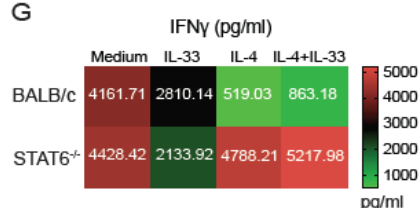


Figure S7. The T_H2-polarization of T_{REG} cells by IL-4 and IL-33 is STAT6-dependent

- (A)** Representative flow cytometry of the total live cells at 72 hours and compiled cells counts of the cultures used for **(B)** Multiplex ELISA (18-plex Eve technologies®) of the supernatants obtained from three independent T_{REG} cell cultures. Splenic GFP⁺ CD4⁺ T cells (B6.Foxp3^{GFPki}) were purified by FACs Aria (99.8% purity) and activated by plated α CD3 (5 μ g/ml) + α CD28 (2 μ g/ml) for 72 hours in the presence of IL-2 (200U/ml), IL-33 (10ng/ml) and/or IL-4 (10ng/ml).
- (C-G)** CD4⁺CD25^{hi} T cells from BALB/C or STAT6^{-/-} mice were sorted and activated by plated α CD3 (5 μ g/ml) + α CD28 (2 μ g/ml) for 72 hours in the presence of IL-2 (200U/ml), IL-33 (10ng/ml) and/or IL-4 (10ng/ml). Medium = IL-2 alone **(C)** Mean fluorescence intensity (MFI) of GATA3 expression in Foxp3⁺ T_{REG} at 72 hours. IL-2 alone (White), IL-2 + IL-4 (Grey), IL-2 + IL-33 (Green) or a combination of IL-2 + IL-4 + IL-33 (Red) **(D-G)** The supernatants were collected and sent for Multiplex ELISA (Eve technologies®). Left scale pg/ml. **(D)** IL-13 (pg/ml), **(E)** IL-5 (pg/ml), **(F)** IL-10 (pg/ml) and **(G)** IFN γ (pg/ml) levels in the supernatants calculated per 10 000 cells. One-way ANOVA. Tukey correction. ***p<0.001; **p<0.01; *p<0.05

