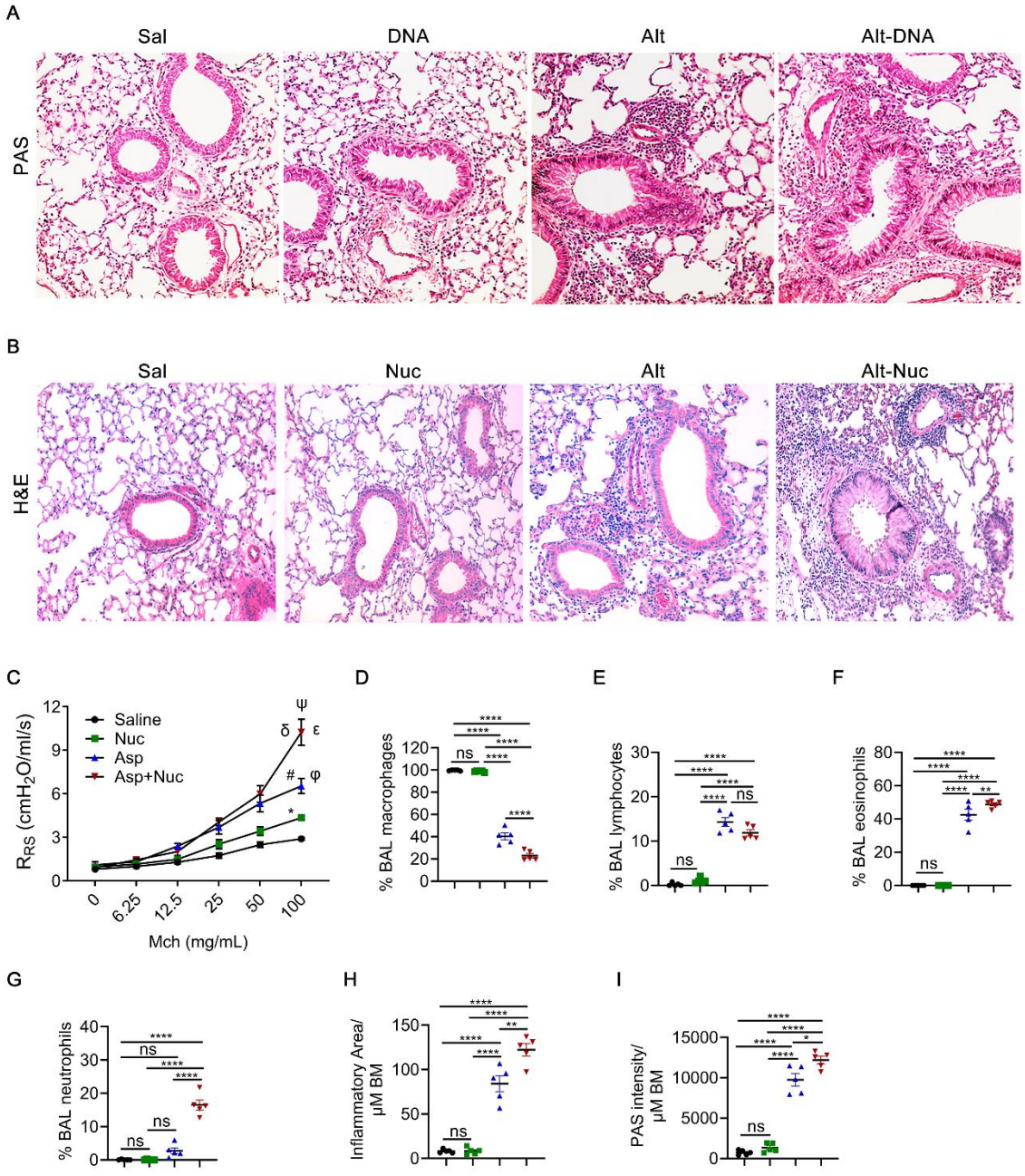
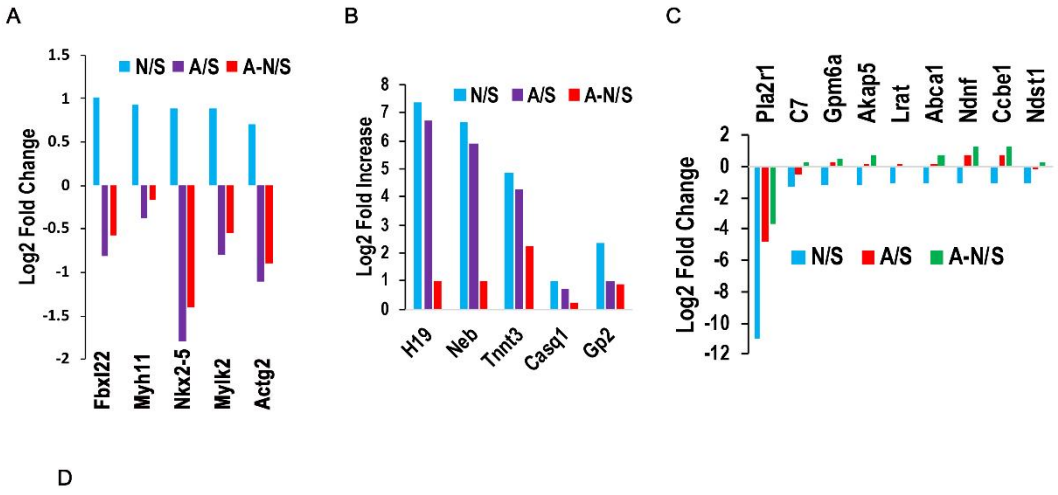


Supplementary Fig. 1. (**A and B**) Representative PAS and H&E staining of the lung tissue from Sal, Alt, Nuc and Alt+Nuc treated B6 mice as per **Fig. 2A**. (**C**) AHR following Saline, Nuc, Aspergillus (Asp) or Asp+Nuc exposure in B6 mice as measured by flexiVent. *(Sal vs Nuc) P=0.01; $^{\varphi}$(Sal vs Asp) P<0.0001; $^{\varepsilon}$(Sal vs Asp+Nuc) P<0.0001; $^{\#}$(Nuc vs Asp) P<0.0001; $^{\delta}$(Nuc vs Asp+Nuc) P<0.0001; $^{\Psi}$(Asp vs Asp+Nuc) P=0.002. . (n=5; 2-way ANOVA, Tukey's multiple comparisons test).

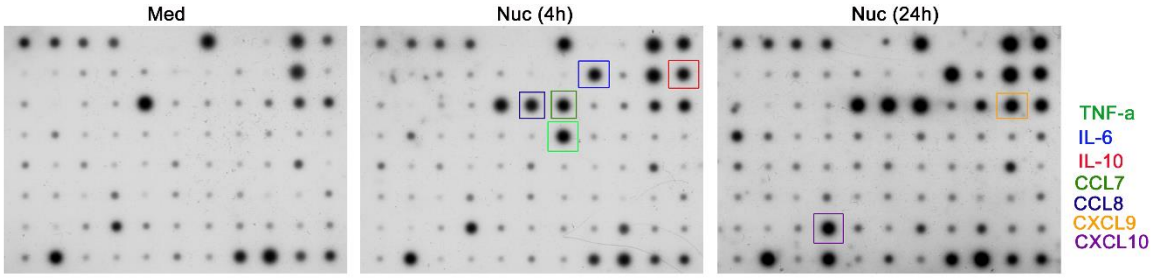
(**D-G**) Differential counts of BAL macrophages, lymphocytes, eosinophils and neutrophils. (**H and I**) Morphometric quantification of lung inflammation and mucus production following H&E and PAS staining of the lung tissue (groups were color-coded as in C). (n=5; 2-way ANOVA, Tukey's multiple comparisons test). Data are presented as mean+/-SEM, **P*<0.05, ***P*<0.01, ****P*<0.001 *****P*<0.0001, ns=not significant.



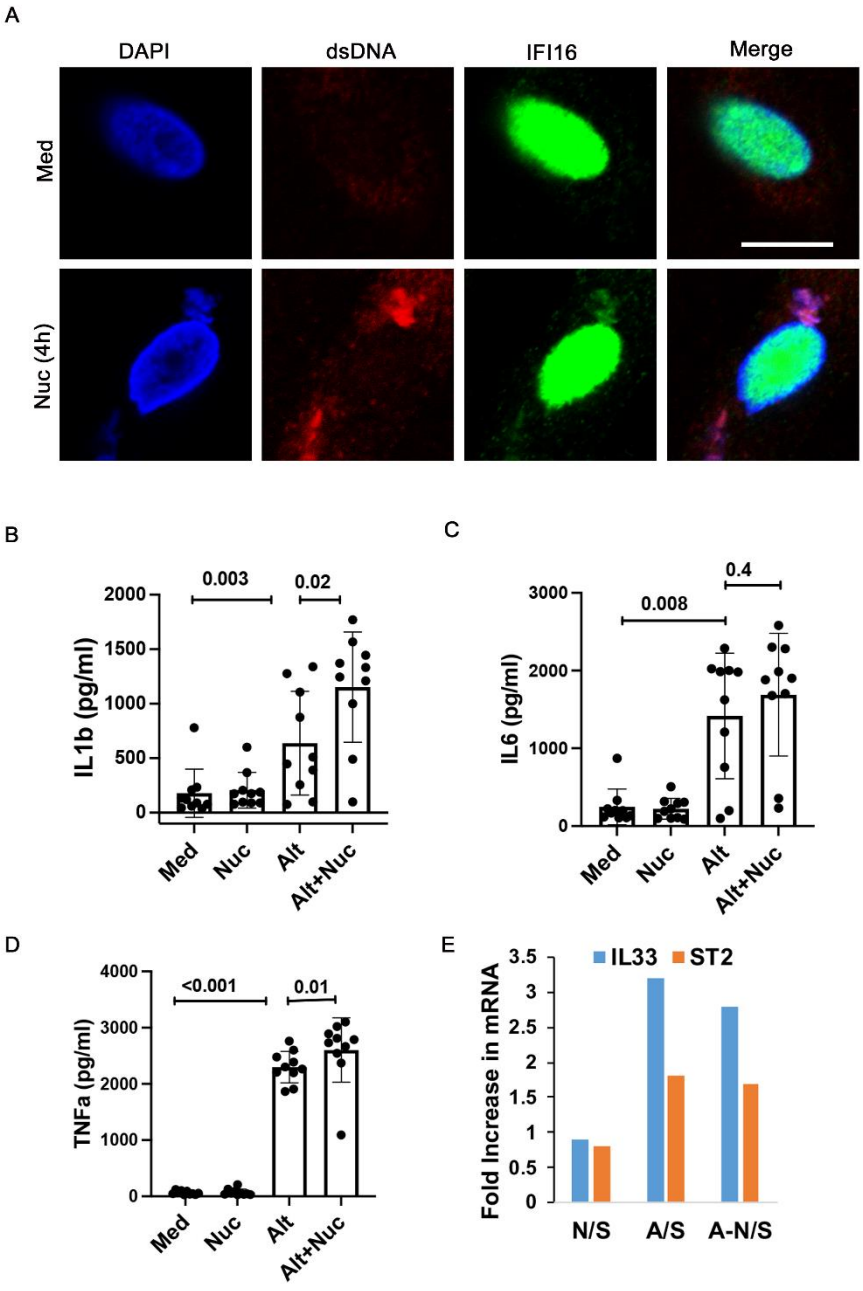
D

PANTHER Overrepresentation Test, GO Ontology	
GO Biological Process	FDR (AN vs A)
Regulation of modification of postsynaptic actin cytoskeleton (GO:1905274)	3.46E-02
Activation of innate immune response (GO:0002218)	9.11E-04
Positive regulation of NIK/NF-kappaB signaling (GO:1901224)	1.18E-04
Cellular response to interferon-beta (GO:0035458)	9.44E-03
Body fluid secretion (GO:0007589)	1.02E-02

Supplementary Fig. 2. Top Nuc up- and down-regulated genes in the lung from Nuc-treated mice and comparison with Sal, Alt and Alt-Nuc treated mice. (**A**) Genes that were selectively upregulated by Nuc but inhibited by Alt and Alt-Nuc. (**B**) Genes upregulated by all three treatment protocols--Nuc, Alt and Alt-Nuc. (**C**) Genes down-regulated by Nuc. (**D**) Top GO biological processes in Alt-Nuc vs Alt treatment groups.

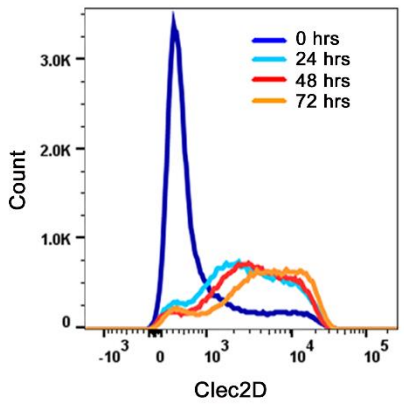


Supplementary Fig. 3. Nuc-induced cytokines/chemokines. Human BAL macrophages were cultured with medium (Med), and Nuc for 4 and 24 hr and the culture supernatant was assayed for 80 cytokines/chemokines using the human cytokine array C5 from RayBiotec. The cytokine/chemokine that was upregulated by Nuc is shown in color-coded square symbol.

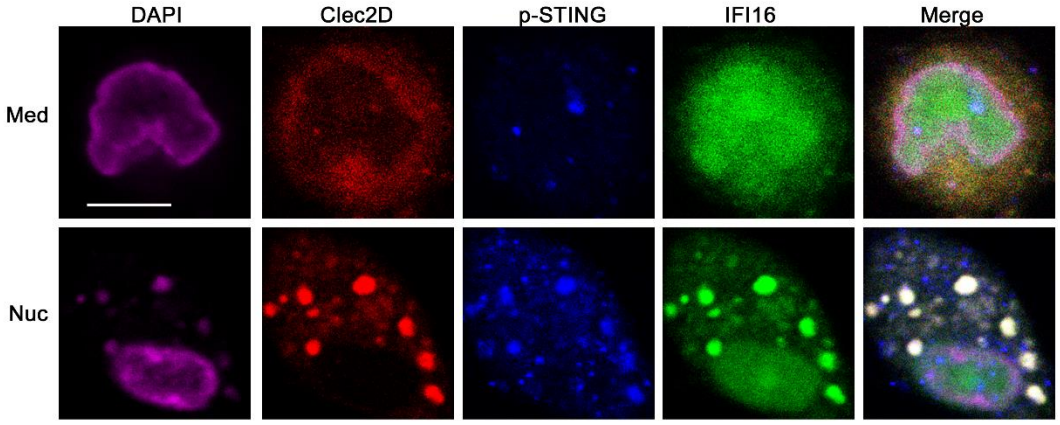


Supplementary Fig. 4. Nuc uptake and induction of cytokines. (**A**) Nuc uptake by human BAL macrophages. Macrophages were incubated with Nuc or medium for 4 hr, stained for cytosolic dsDNA (red) and IFI16 (green) and counterstained with DAPI. The anti-dsDNA antibody specifically detects cytosolic but not nuclear dsDNA. One representative image is shown. Scale bar, 5 μm. (**B-D**) Cytokine production by monocytes. Blood monocytes from asthma patients were cultured with. Medium, Nuc (10 μg/ml), Alt (10 μg/ml) and Alt + Nuc (10 μg/ml each) for 24 hr and

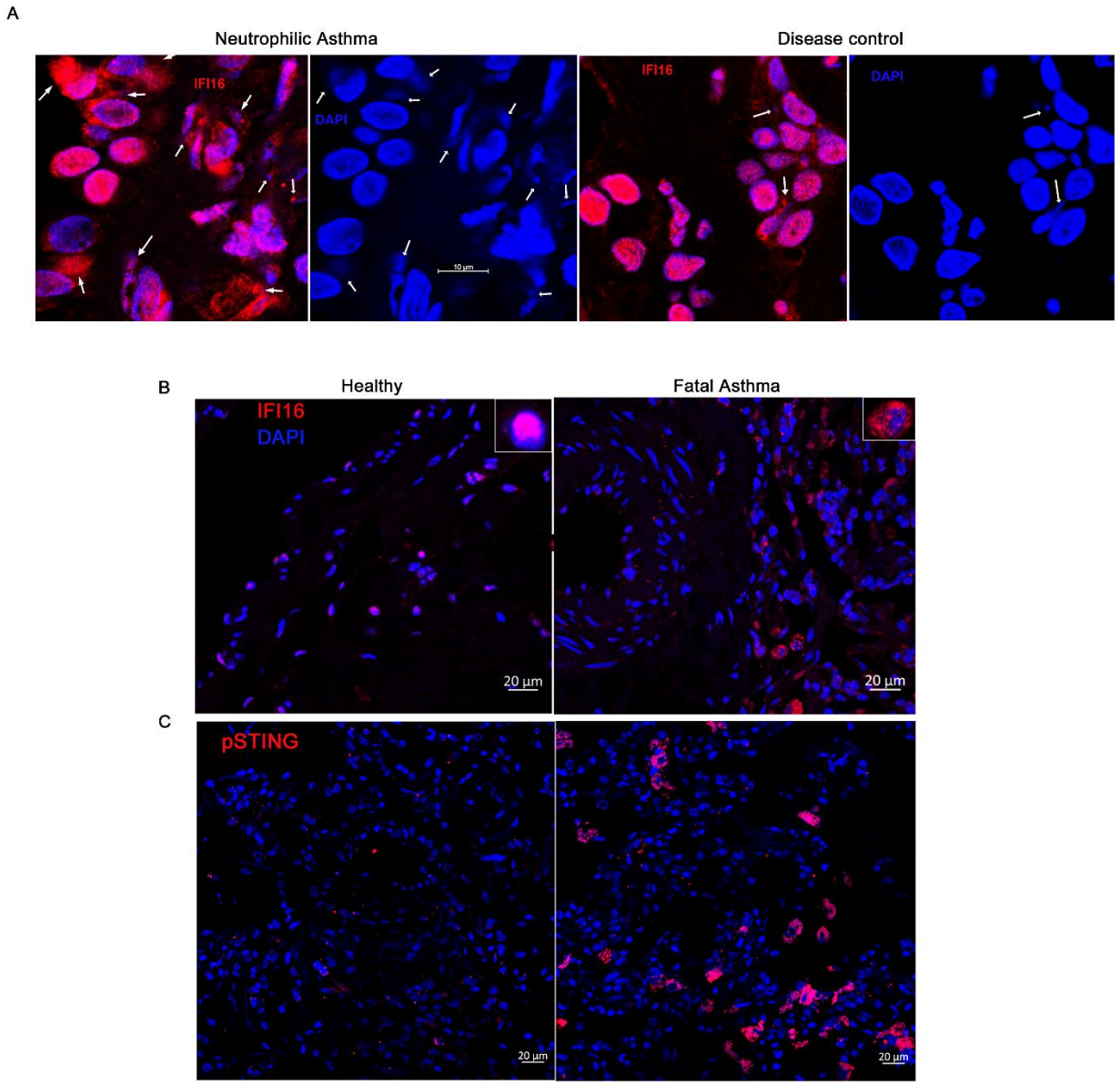
assayed for the indicated cytokines. Statistical analyses by Mann-Whitney U test. (**E**) The increase in mRNA for *il33* and *il1rl1* (ST2) in the lung from the study groups as compared to the Saline (S) group.



Supplementary Fig. 5. Flow cytometric measurement of CLEC2D following stimulation with Alt for 0, 24, 48, and 72 hrs (n=4).

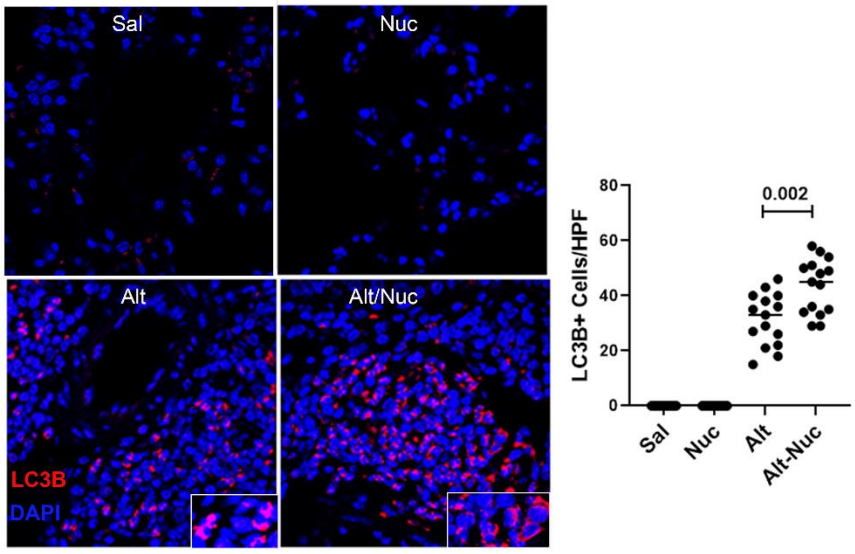


Supplementary Fig. 6. CLEC2D immunostaining. Triple immunostaining of human BAL macrophages for CLEC2D (red), p-STING (blue) and IFI16 (green) and counterstaining with DAPI (pink) following incubation with Nuc for 4 hr. Co-localization (white) of internalized CLEC2D with p-STING and cytosolic IFI16 is shown in the merged image (n=3). Scale bar, 5 μm.

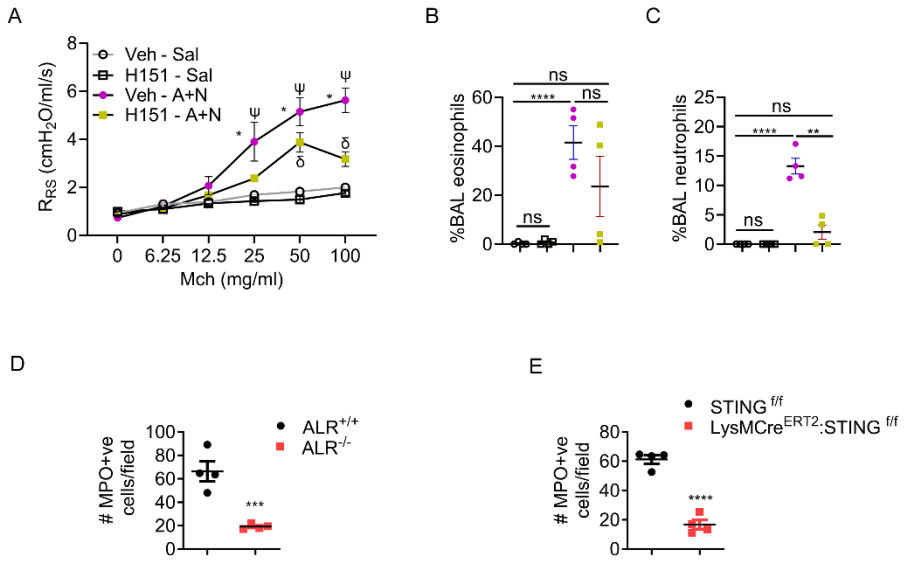


Supplementary Fig. 7. IFI16 and p-STING in the airway tissue from asthma patients. (**A**) Representative immunostaining of endobronchial biopsy specimens from a neutrophilic asthma patient and a disease control subjects for IFI16 (red). The tissue was counterstained with DAPI for DNA. Cytosolic IFI16 co-localizing with DAPI-staining extranuclear DNA is shown by white arrows (n=17 as per **Fig. 5A**). Scale bar, 10 µm. (**B and C**) Representative immunostaining of cadaveric lung sections from a healthy subject and an age- and gender-matched fatal asthma patient

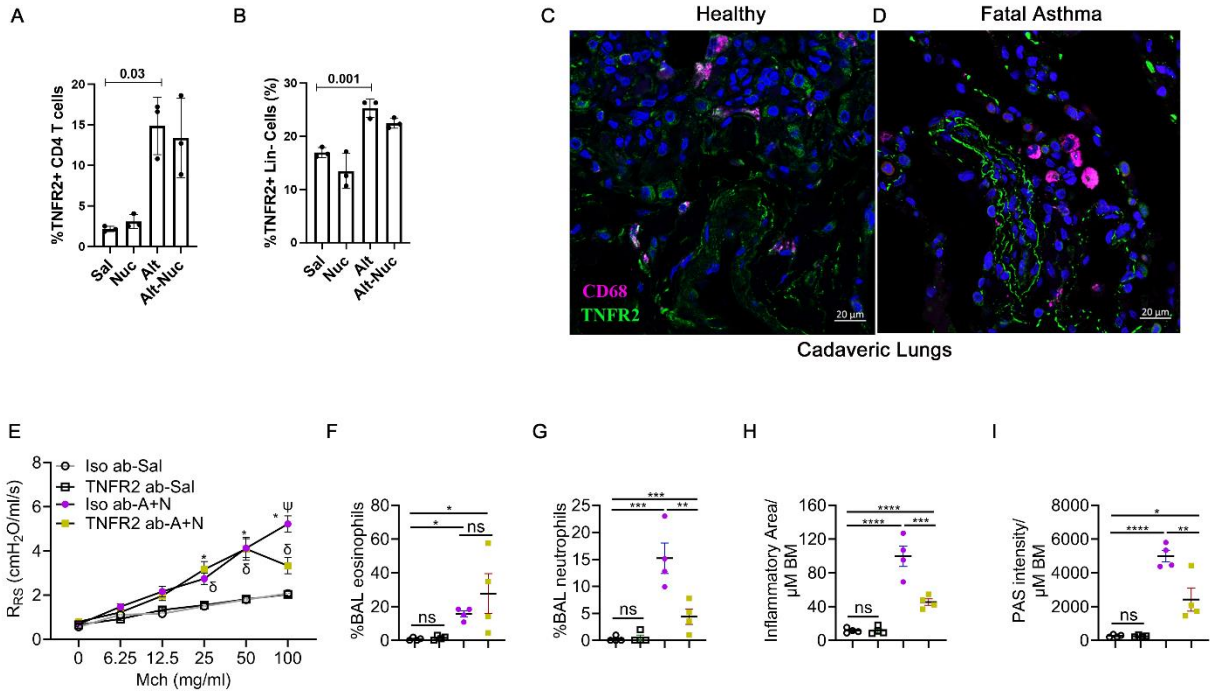
for IFI16 and p-STING (red). Scale bar, 20 μm.



Supplementary Fig. 8. Autophagy in the asthma models. Representative immunostaining of lung tissue from Sal, Nuc, Alt and Alt-Nuc treated mice for the autophagy marker LC3B. The quantification of the LC3B+ cells from the 4 study groups is shown in the right panel. Scale bar, 20 μm.

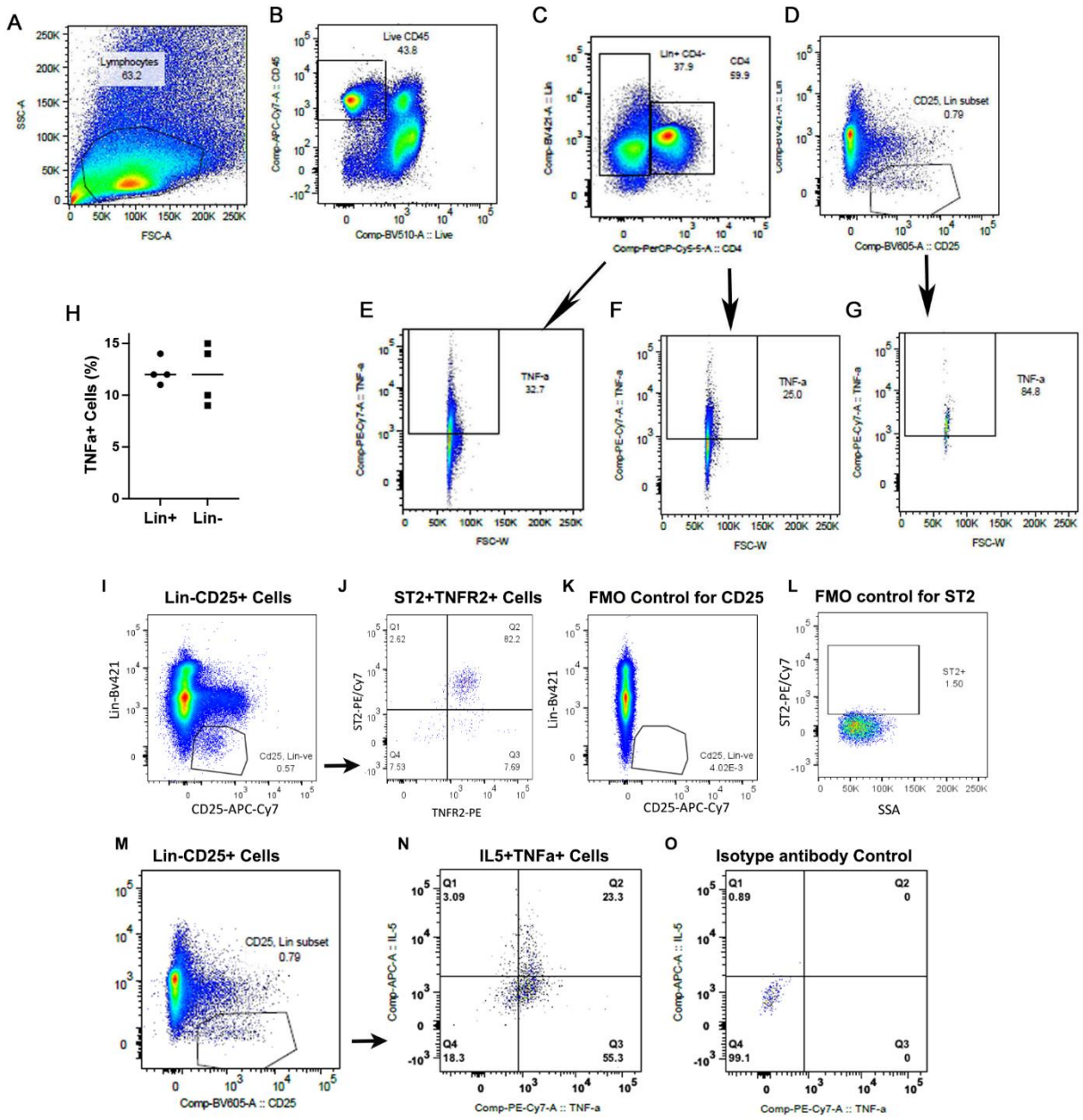


Supplementary Fig. 9. (**A-C**) B6 mice were pretreated i.p. with the STING inhibitor H151 (750 nmol H-151 per mouse in 200 µl 10% Tween-80 in PBS) or vehicle (10% Tween-80 in PBS) 1 hr before i.n. exposure to Alt-Nuc or Sal as per **Fig. 2A**. (A) AHR (lung resistance) in Veh-Sal, H151-Sal, Veh-A+N and H151-A+N groups as measured by flexiVent. *(Veh-Sal vs Veh-A+N) P<0.0001; $^{\delta}$(Veh-Sal vs H151-A+N) P<0.0001; $^{\Psi}$(Veh-A+N vs H151-A+N) P=0.006. (n=4; 2-way ANOVA, Tukey's multiple comparisons test). (**B and C**) Differential counts of BAL eosinophils and neutrophils. (**D and E**) The lung tissue from *ALR*$^{+/+}$, *ALR*$^{-/-}$, *Sting*$^{f/f}$ and *LysM*Cre:*Sting*$^{f/f}$ mice (n=4 per group) that were exposed to Alt-Nuc (as per **Fig. 2A**) was immunostained for neutrophil myeloperoxidase (MPO) and the MPO+ cells were quantified. Comparison made by Student's 2 tailed t-test. Data are presented as mean+/-SEM, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, ns=not significant.



Supplementary Fig. 10. TNFR2 and asthma. (**A and B**) TNFR2 induction by Alt. Isolated lung CD4+ and Lin- cells from the 4 mouse groups (Sal, Nuc, Alt, and Alt-Nuc) were analyzed by FCM (n=3 per group) (**C and D**) Immunostaining for TNFR2 expressing blood vessels and cells, and CD68+ macrophages in cadaveric lungs from a healthy subject and a fatal asthma patient. Scale bar, 20 µm. (**E-I**) Effect of an anti-TNFR2 antibody and an isotype control antibody. B6 mice were pretreated with 100µg of anti-TNFR2 antibody (clone TR75-54.7) or an isotype control antibody on day -1, +2 and +4 and then treated with Alt-Nuc or Sal as per **Fig. 2A**. (**E**) AHR (lung resistance) in Iso ab-Sal, TNFR2 ab-Sal, Iso ab-A+N and TNFR2 ab-A+N groups as measured by flexiVent. *(Iso ab-Sal vs Iso ab-A+N) P<0.0001; $^{\delta}$(Iso ab-Sal vs TNFR2 ab-A+N) P<0.0001; $^{\Psi}$(Iso ab-A+N vs TNFR2 ab-A+N) P<0.0001. (n=4; 2-way ANOVA, Tukey's multiple comparisons test). (**F-I**) Quantification of eosinophils and neutrophils in BAL, morphometric quantification of lung inflammation and mucus production in mice pretreated with Iso ab or TNFR2 ab and treated with Sal or A+N (Groups were color coded as in **E**). (n=4; 2-way ANOVA, Tukey's multiple

comparisons test). Data are presented as mean+/-SEM, *P<0.05, **P<0.01, ***P<0.001 ****P<0.0001, ns=not significant.



Supplementary Fig. 11. Flow cytometric analysis of TNFα+ lymphoid cells. **(A-G)** The flow cytometry gating strategy for detection of TNFα+ cells in Lin+CD4-, Lin+CD4+ and Lin-CD45+ cell populations in the lung cell digest. **(H)** Comparison of TNFα+ cells between Lin+ and Lin- CD45+ lung cells from Alt-treated mice. (**I-L**) Frequency of ST2+TNFR2+ ILC2s in Lin-CD25+ lung cell population and their FMO controls. (**M-O**) Frequency of IL5+TNFα+ cells

in lung ILCs (Lin-CD25+) and their isotype controls.