Supplementary data for

Targeting IL-17A/glucocorticoid synergy on CSF3 expression in neutrophilic airway diseases

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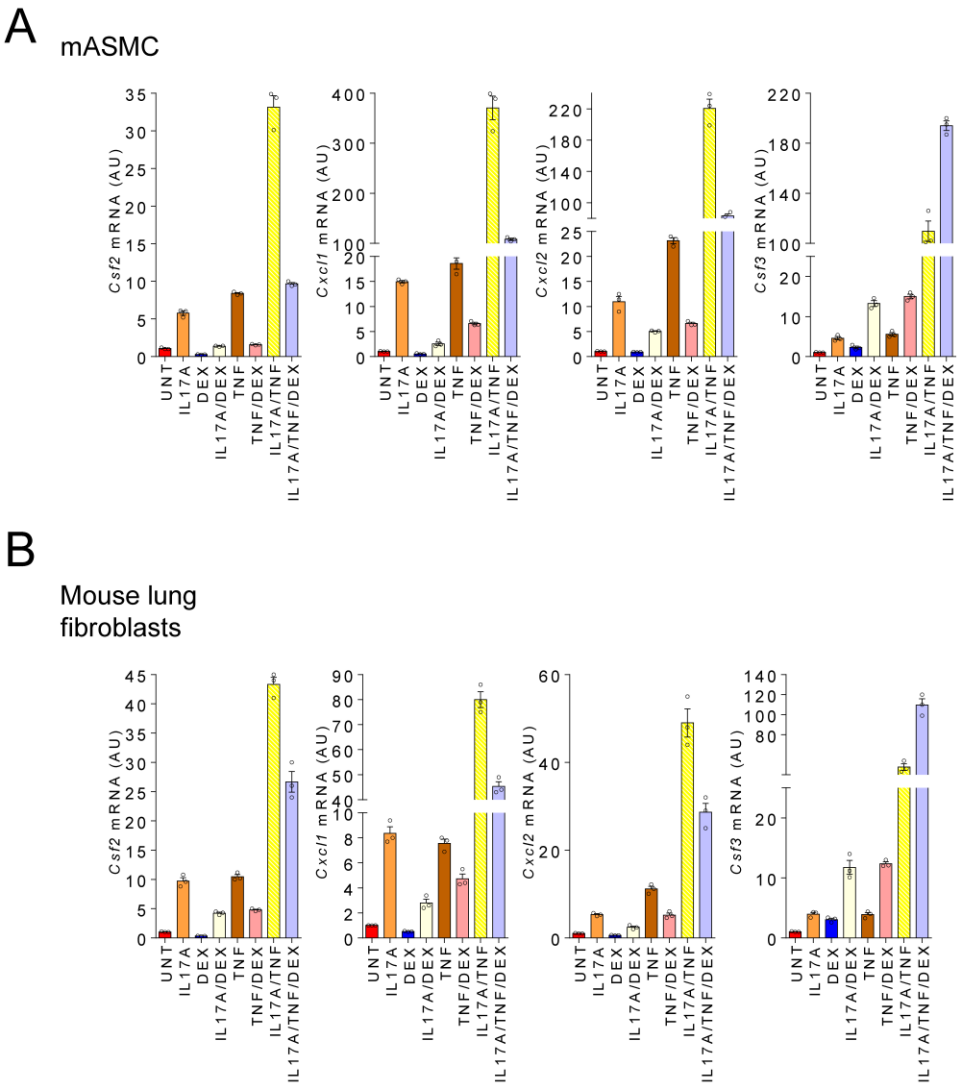
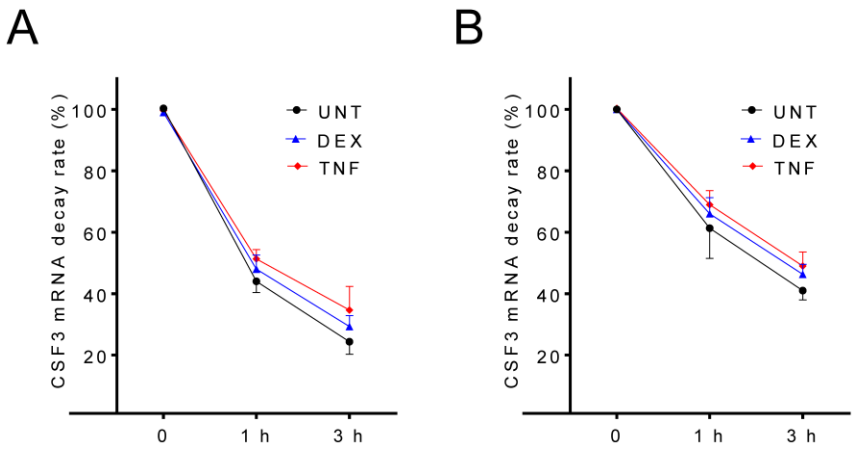
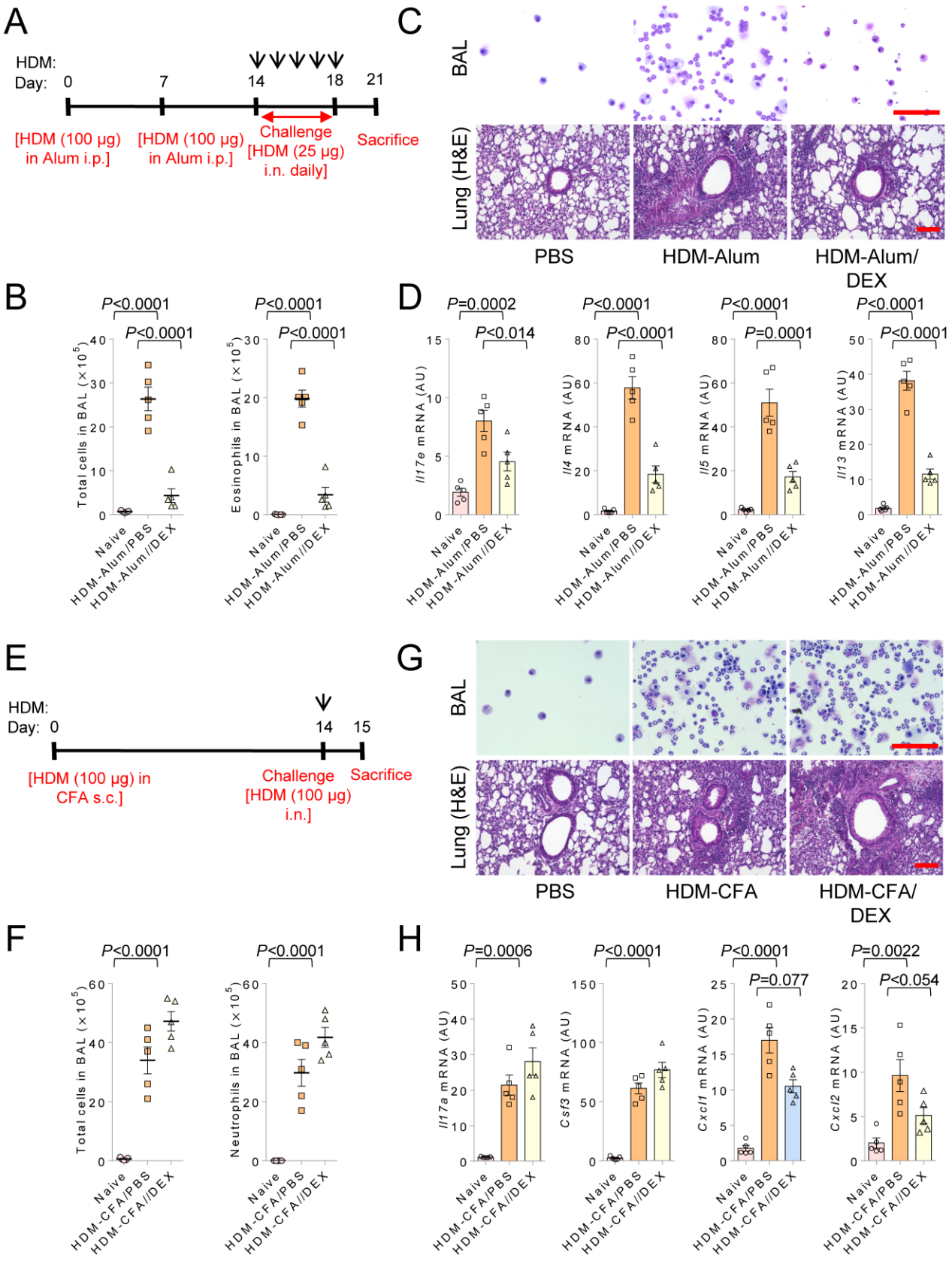


Supplementary Figure 1. Glucocorticoid differentially regulates IL-17A-induced neutrophil-promoting cytokines in mASMCs and lung fibroblasts. Real-time PCR analysis of mRNA expression of indicated cytokines in mASMCs (**A**) and mouse lung fibroblasts (**B**). Cells were untreated (UNT) or treated for 4 h as indicated: PBS (UNT), IL-17A (100 ng/ml), DEX (1 μM), TNF (10 ng/ml). Data represent mean ± SD (n=3 technical replicates). All data are representative of three independent experiments.

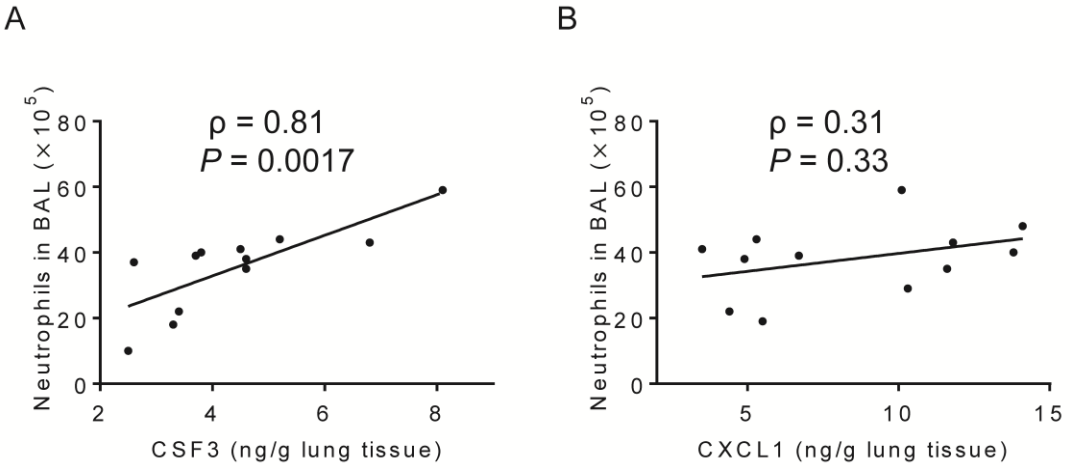


Supplementary Figure 2. DEX and TNF do not stabilize CSF3 mRNA. mRNA degradation assay. hASMCs were pretreated with medium (**A**) or IL-17A (**B**) for 4 h, followed by treatment with Actinomycin D (5 µg/ml) in the absence and presence of DEX or TNF (10) ng/ml). mRNA decay rate was measured as a ratio (percentage) of remaining mRNA at indicated time point to the amount of mRNA at time point 0. Data represent mean ± SEM (n=3 biological replicates). All data are representative of two independent experiments.

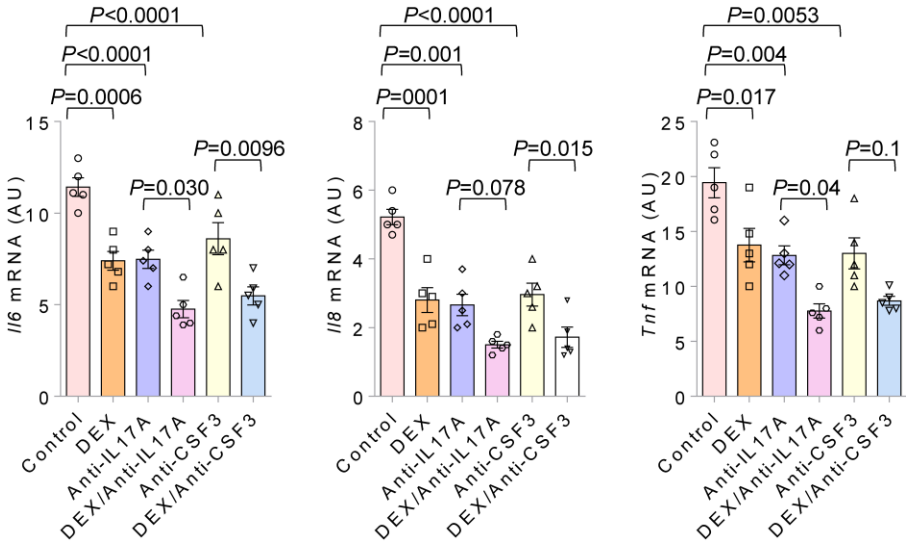


Supplementary Figure 3. Low-dose DEX inhibits type-2 eosinophilic airway inflammation but promotes type-17 neutrophilic airway inflammation in HDM-induced allergic asthma models. (A) Eight-week WT C57BL/6 female mice were subjected to HDM-Alum type-2 asthma model (n = 5 per group). PBS (control) or

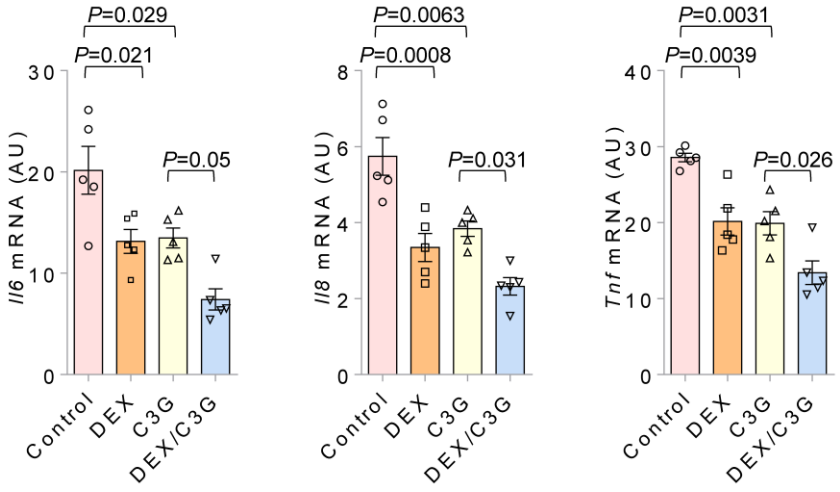
DEX (0.3 mg/kg BW) was administrated into the mice (as described in Materials and Methods). Twenty-four hours after challenging, total and eosinophil counts in the BAL were quantified (**B**). Representative BAL cells were prepared by cytospin and lung tissues were subjected to H&E staining (**C**). (**D**) mRNA expression of lung tissues was quantified by real-time PCR. (**E**) Eight-week WT C57BL/6 female mice were subjected to HDM-CFA type-17 acute asthma model (n = 5 per group). PBS (Control) or DEX (0.3 mg/kg BW) was administrated into the mice (as described in Materials and Methods). Twenty-four hours after challenging, total and neutrophil counts in the BAL were quantified (**F**). Representative BAL cells were prepared by cytospin and lung tissues were subjected to H&E staining (**G**). (**H**) mRNA expression of lung tissues was quantified by real-time PCR. Data represent mean ± SEM. One-way ANOVA was performed, followed by Tukey's multiple comparison test. The multiplicity adjusted P values were calculated for the indicated comparisons. The above data are representative of three independent experiments.



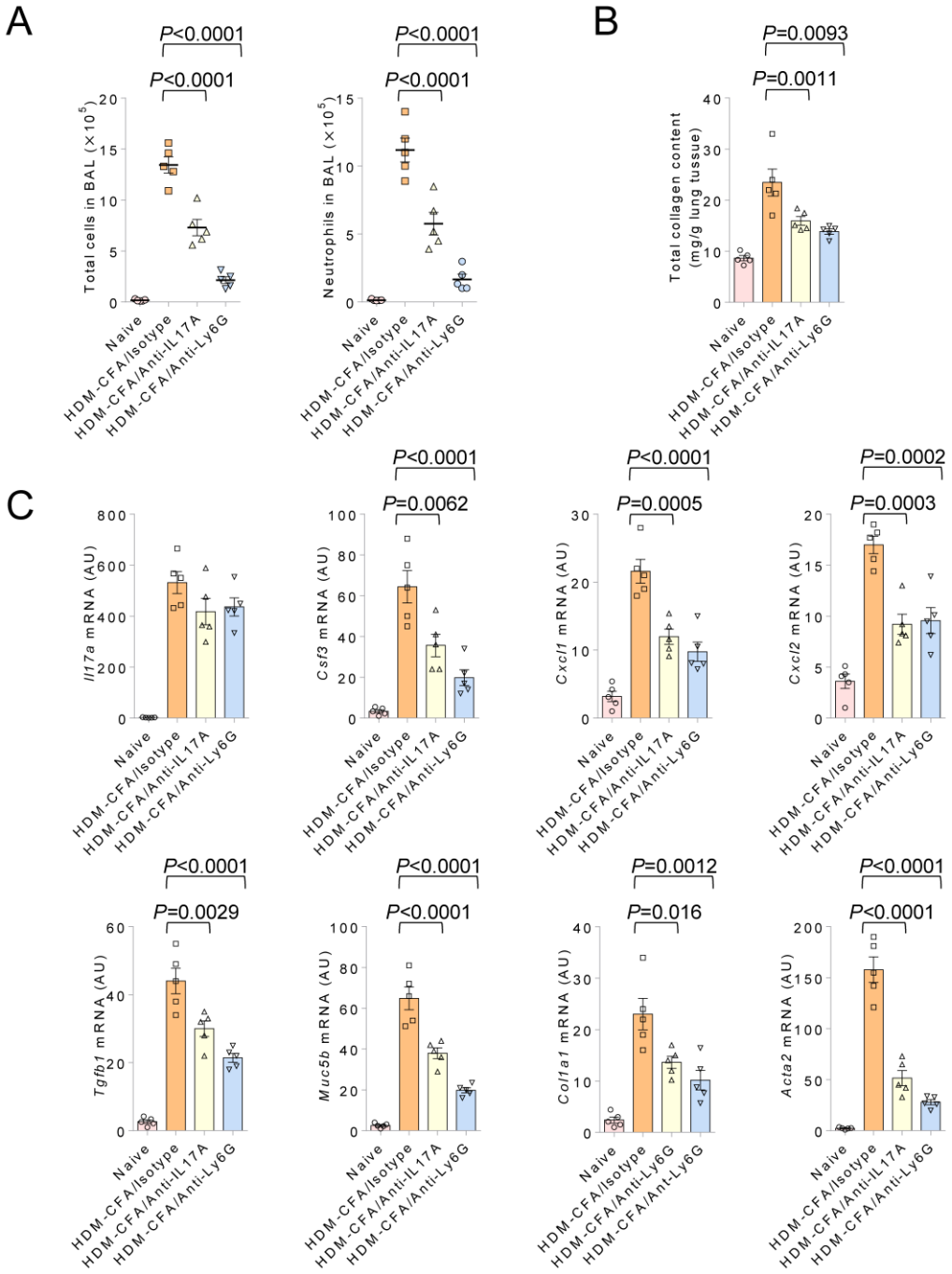
Supplementary Figure 4. CSF3 levels in lung tissues correlate with BAL neutrophil counts in DEX-treated mice on HDM-CFA type-17 acute asthma model. Eight-week WT C57BL/6 female mice were subjected to HDM-CFA type-17 acute asthma model (n = 12). DEX (0.3 mg/kg BW) was administrated into the mice (as described in Materials and Methods). (**A**) Correlation between the levels of CSF3 in lung tissues and neutrophil counts in BAL. (**B**) Correlation between the levels of CXCL1 in lung tissues and neutrophil counts in BAL. ρ = Spearman's rank correlation coefficient.



Supplementary Figure 5. Inflammatory gene expression in lung tissues of mice treated by DEX, anti-IL-17A, and anti-CSF3, alone or in combination in HDM-CFA type-17 acute asthma model. Eight-week WT C57BL/6 female mice (n = 5 per group) were subjected to HDM-CFA type-17 acute severe asthma model and treated as described in Figure 2. AU, fold induction relative to unchallenged control mice. Data represent mean ± SEM. One-way ANOVA was performed, followed by Tukey's multiple comparison test. The multiplicity adjusted P values were calculated for the indicated comparisons. The above data are representative of three independent experiments.

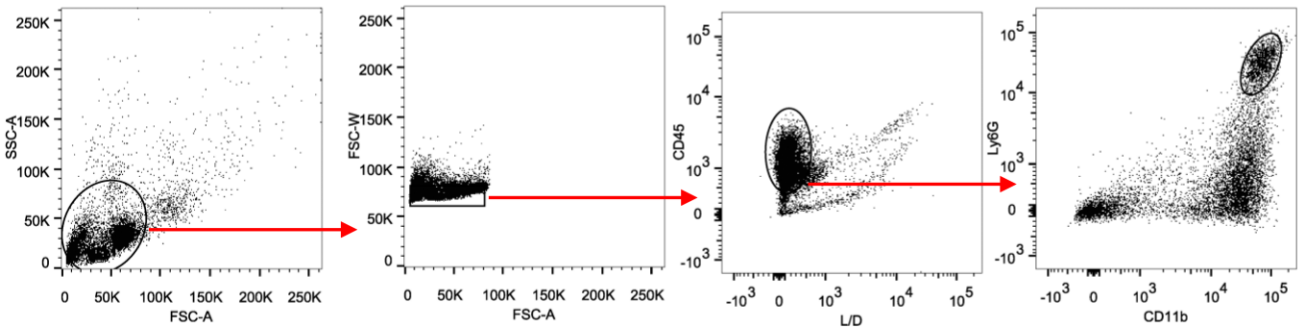


Supplementary Figure 6. Inflammatory gene expression in lung tissues of mice treated by DEX, C3G, alone or in combination in HDM-CFA type-17 acute asthma model. Eight-week WT C57BL/6 female mice (n = 5 per group) were subjected to HDM-CFA type-17 acute severe asthma model and treated as described in Figure 3. AU, fold induction relative to unchallenged control mice. Data represent mean ± SEM. One-way ANOVA was performed, followed by Tukey's multiple comparison test. The multiplicity adjusted P values were calculated for the indicated comparisons. The above data are representative of three independent experiments.



Supplementary Figure 7. Depletion IL-17A and neutrophils alleviates airway inflammation and remodeling in HDM-CFA type-17 chronic asthma Eight-week WT C57BL/6 female mice were subjected to HDM-CFA type-17 chronic asthma model (n = 5 per group). Isotype control, anti-IL17A or anti-Ly6G antibodies were administrated into the mice (as described in Materials and Methods). Twenty-four hours after last challenging, total and neutrophil counts in the BAL (**A**).and total collagen content in lung tissue (**B**) were quantified. (**C**) mRNA expression of lung tissues was quantified by RT-PCR. Data represent mean ± SEM. One-way ANOVA was performed, followed by Tukey's multiple comparison test. The multiplicity adjusted P values

were calculated for the indicated comparisons. The above data are representative of three independent experiments.



Supplementary Figure 8. Gating strategy for lung neutrophils. Lung cells were stained with anti-CD45, anti-CD11b and anti-Ly6G antibodies, followed by tunnel assay using biotinylated dNTP and streptavidin-FITC as shown in Figure. 6C. The neutrophils were gated as $CD45^{+}CD11b^{+}Ly6G^{+}$ cells. L/D, live/dead dye (Zombie NIR™).