Characterization and allergic role of IL-33-induced neutrophil polarization

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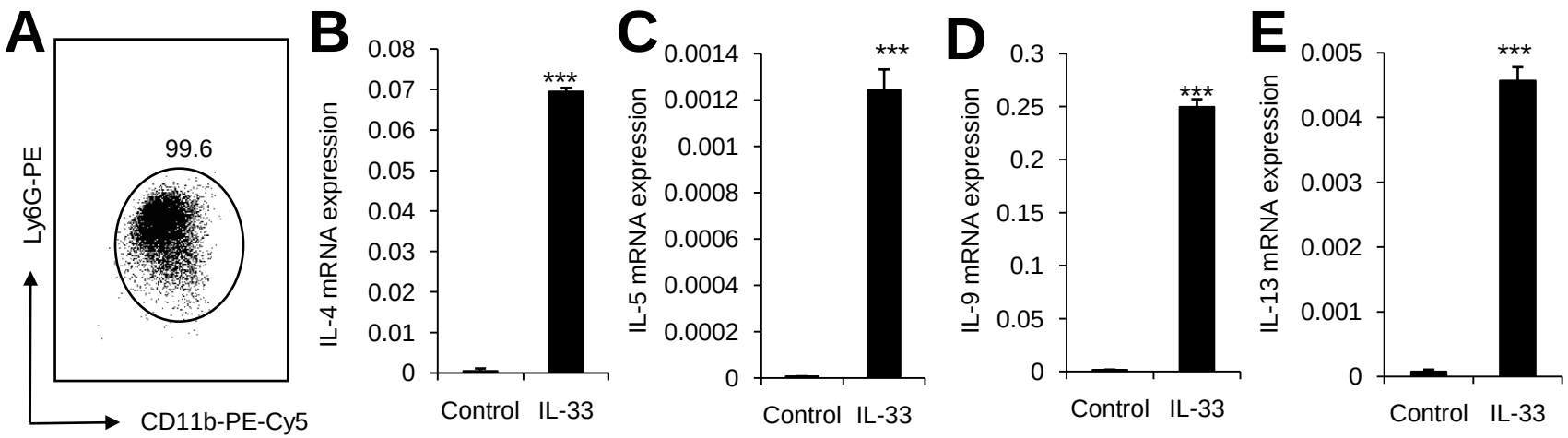
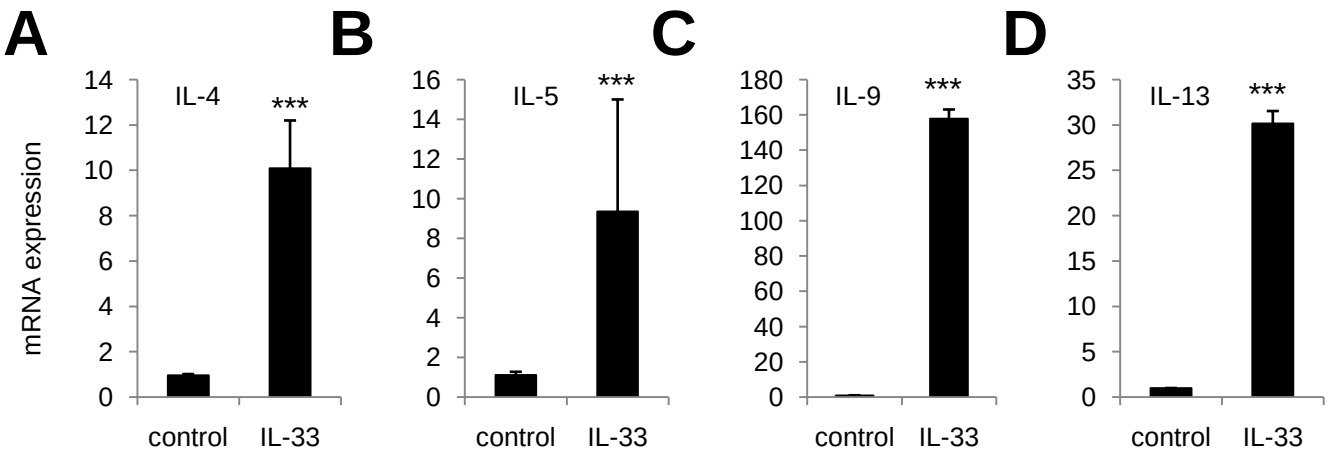


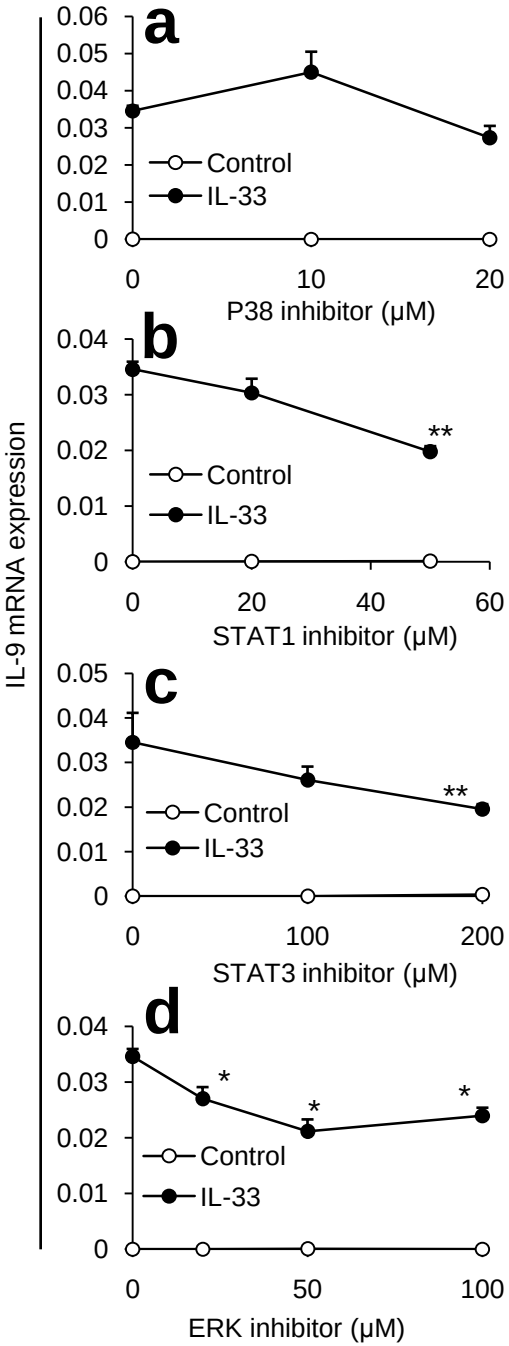
Supplementary Figure 1. Highly purified CD11b$^+$Ly6G$^+$ cells from bone marrow express high levels of Th2/Th9-type cytokines after IL-33 stimulation.

(**A**) The proportion of neutrophils (CD11b$^+$Ly6G$^+$) sorted from bone marrow was detected by flow cytometric analysis. Quantitative PCR analysis of IL-4 (**B**) , IL-5 (**C**), IL-9 (**D**) and IL-13 (**E**) expressions in sorted neutrophils driven by IL-33 (100ng/ml) for 24 hours. Data were shown as mean±SD (n=3). ***p<0.001 compared with IL-33-untreated control cells.



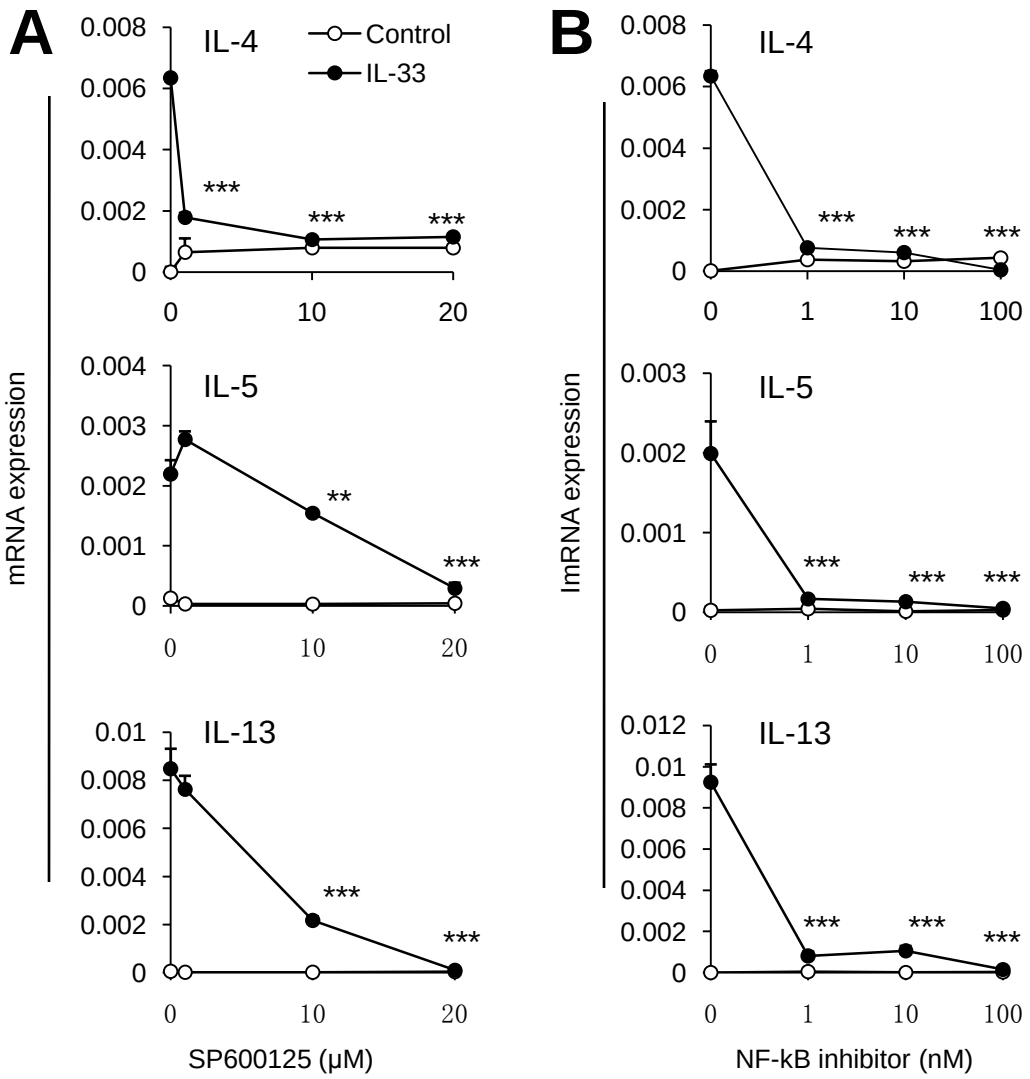
Supplementary Figure 2. Human neutrophils from peripheral blood express high levels of Th2/Th9-type cytokines after IL-33 stimulation.

(**A**) Human neutrophils were isolated by gradient centrifugation from peripheral blood. Quantitative PCR analysis of IL-4 (**B**) , IL-5 (**C**), IL-9 (**D**) and IL-13 (**E**) expressions in neutrophils stimulated by IL-33 (10 ng/ml). Data were shown as mean±SD (n=3). ***p<0.001 compared with IL-33-untreated control cells.



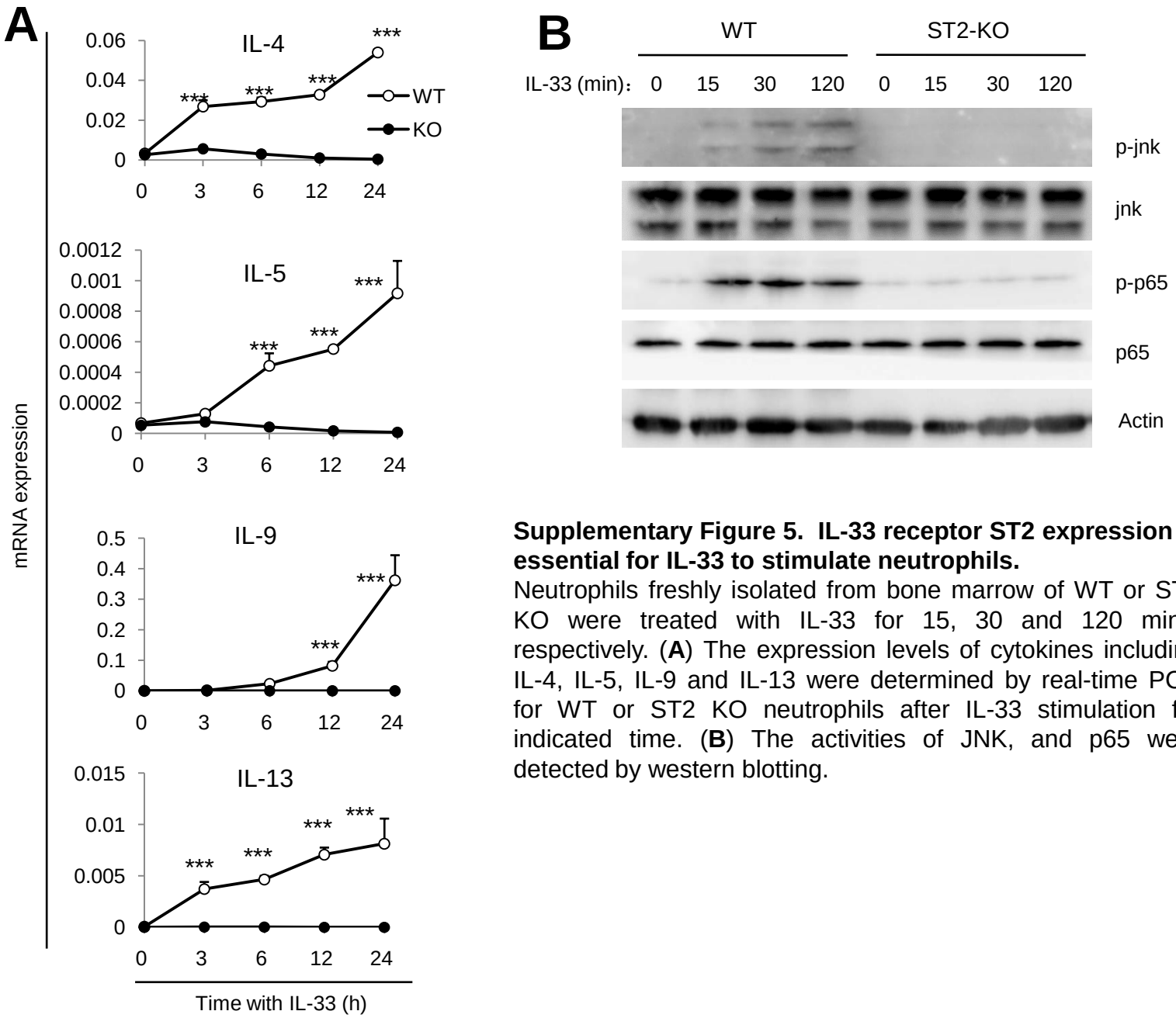
Supplementary Figure 3. Inhibition of p38 MAPK, STAT1, STAT3 and ERK activation slightly decrease the IL-9 mRNA expression in N9 cells.

Neutrophils were pretreated with the indicated concentrations of p38 MAPK, STAT1, STAT3 and ERK inhibitors for 30 mins and then stimulated with IL-33 for 24 hours. IL-9 mRNA expression were detected by real-time PCR. Data were shown as mean±SD (n=3). **P*<0.05 and ***P*<0.01 compared with inhibitor-untreated cells.

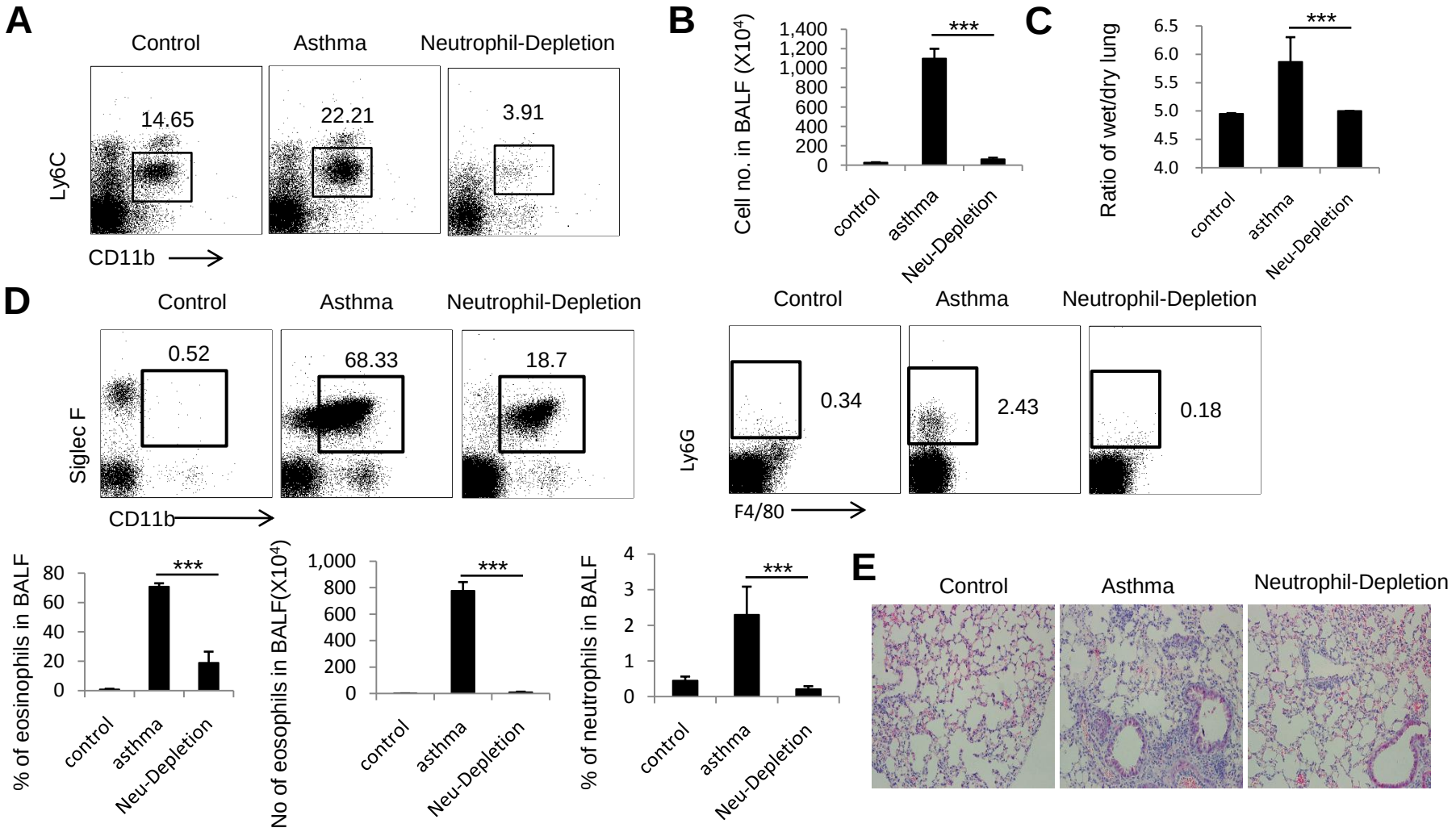


Supplementary Figure 4. Inhibition of JNK and NF-kB activation significantly decreased the IL-4, IL-5 and IL-13 mRNA expressions in IL-33-treated neutrophils.

Neutrophils were pretreated with the indicated concentrations of JNK (**A**) and NF-kB (**B**) inhibitors for 30 mins and then stimulated with IL-33 for 24 hours. IL-4, IL-5 and IL-13 mRNA expressions were detected by real-time PCR. Data were shown as mean±SD (n=3). **P*<0.05, ***p*<0.01 and ****p*<0.001 compared with the inhibitor-untreated control.



Supplementary Figure 5. IL-33 receptor ST2 expression is essential for IL-33 to stimulate neutrophils.
Neutrophils freshly isolated from bone marrow of WT or ST2 KO were treated with IL-33 for 15, 30 and 120 mins, respectively. (**A**) The expression levels of cytokines including IL-4, IL-5, IL-9 and IL-13 were determined by real-time PCR for WT or ST2 KO neutrophils after IL-33 stimulation for indicated time. (**B**) The activities of JNK, and p65 were detected by western blotting.



Supplementary Figure 6. Neutrophil depletion attenuated OVA-induced allergic airway inflammation.
OVA-presensitized WT mice were challenged with aerosolized OVA daily for 4 days as described in materials and methods. Neutrophils were depleted by 0.5 mg anti-Gr-1 depleting antibody on day -1 and day 2 of first aerosolized OVA challenge (A) Neutrophil depletion measured by flow cytometry of $Ly6C^{low}$ $CD11b^{+}$ cells in circulation. (B) Cell numbers in BAL fluid of WT and neutrophil depleted mice after OVA challenge. (C) Ratio of wet/dry lung weights of WT and IL-33-TG mice after OVA challenge was measured as described in methods. (D) Flow cytometric analysis of infiltrated cells like eosinophils in BAL fluid of WT and neutrophil depleted mice after OVA challenge. (E) H&E staining of lung tissues from control and OVA-induced asthma for WT and neutrophil depleted mice were presented. Data are expressed as mean±SD (n=3-5) and are one representative of 3 independent experiments with similar results. *P<0.05, **p<0.01 and ***p<0.001 compared with control mice.