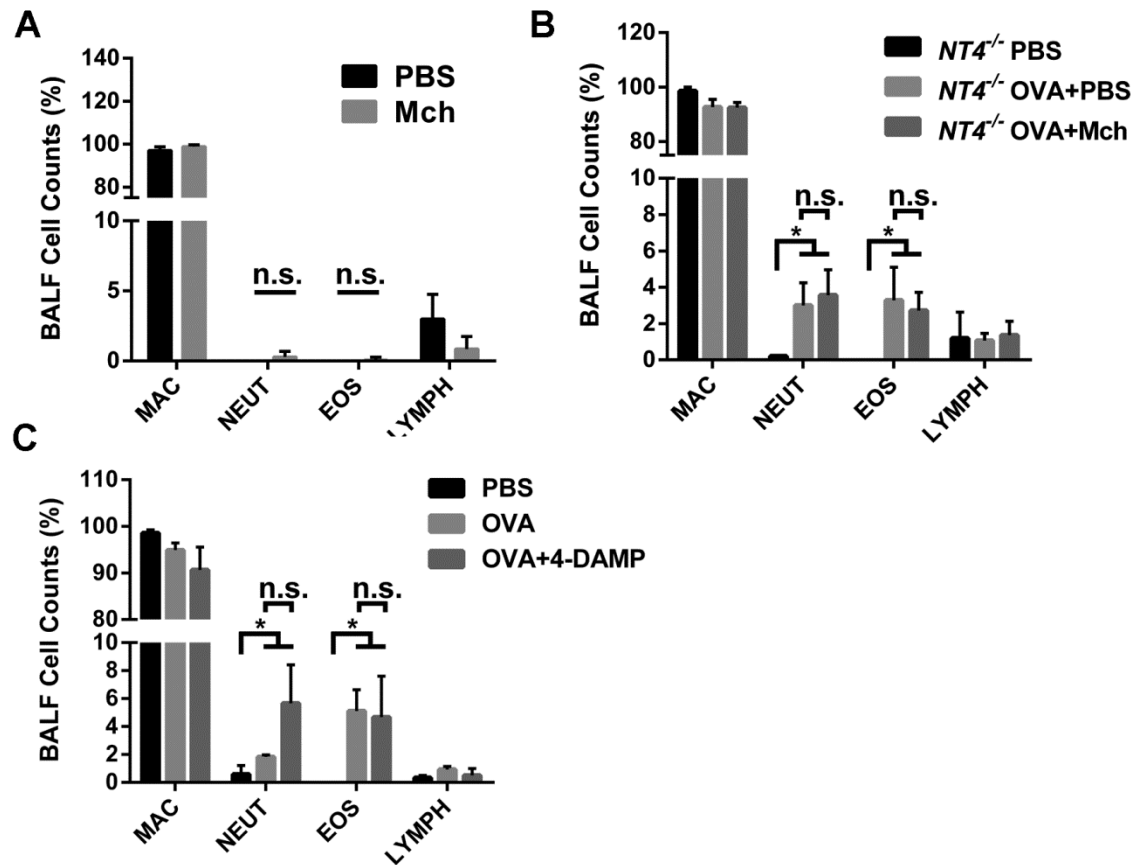
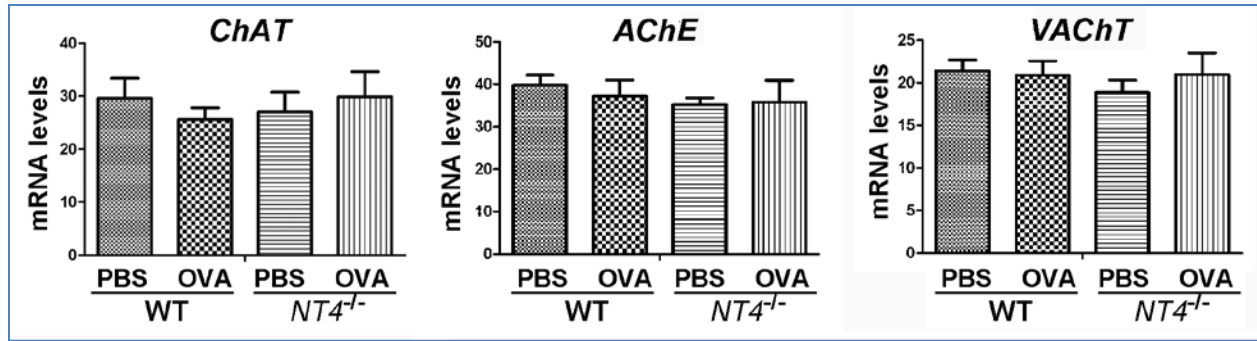


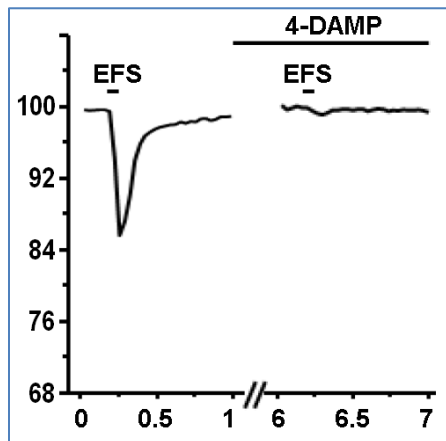
SUPPLEMENTARY FIGURES



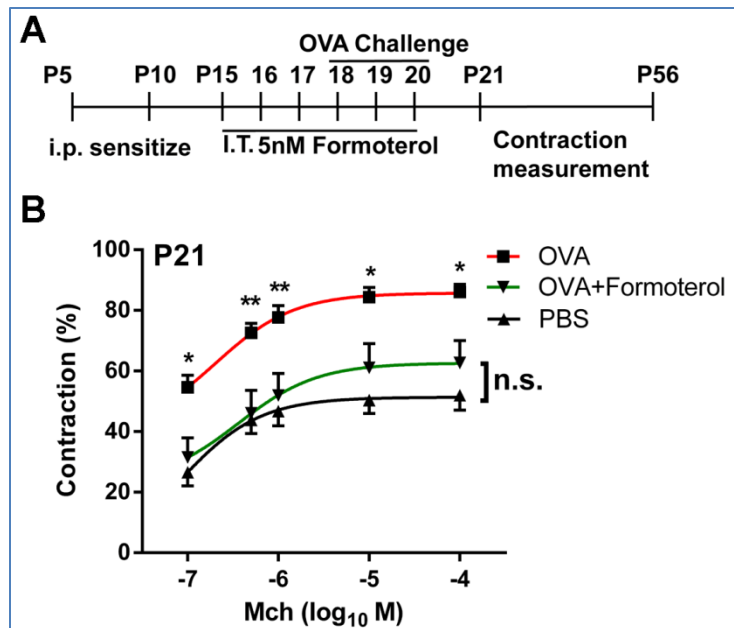
Supplementary Figure 1. Cholinergic signaling had no effect on allergic inflammation. Inflammation was assessed by differential immune cell counts in bronchoalveolar lavage. Percentages of macrophages (Mac), eosinophils (Eos), lymphocytes (Lymph), and neutrophils (Neut) are shown. (A) Wild type mice were nebulized with PBS or Mch between P15-P20. Mice were analyzed at P21. (B) PBS- and OVA-exposed *NT4*^{-/-} mice were nebulized with saline or Mch between P15-P20. Mice were analyzed at P21. (C) PBS- and OVA-exposed wild type mice were treated with and without 4-DAMP between P15-P20. Mice were analyzed at P21. Bar graphs represent mean±SEM of data from at least 4 mice for each group. *P<0.05.



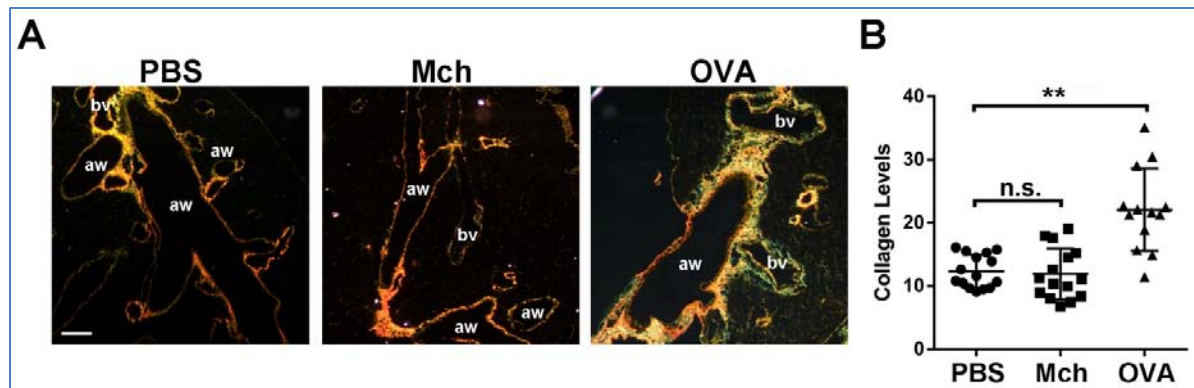
Supplementary Figure 2. Early life OVA exposure and NT4 had no effect on airway epithelium expression of ChAT, AChE, and VACHT. Airway epithelium cells were isolated from wild type and *NT4*^{-/-} mouse lungs at P21 by flow cytometry followed by gene expression analysis. ChAT is the biosynthetic enzyme for acetylcholine. AChE is the major enzyme that degrades acetylcholine. VACHT is the transporter for packaging acetylcholine into secretory vesicles prior to secretion.



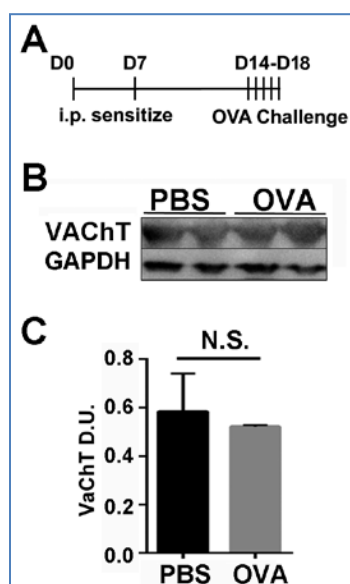
Supplementary Figure 3. EFS-induced airway contraction using lung slices is mediated through ChRM3 activation in response to cholinergic nerve release of acetylcholine. Representative trace showing contractile responses to 5-sec electric field stimulation of lung slices from wild type mice at P21 with and without the presence of a ChRM3 blocker, 4-DAMP (100μM).



Supplementary Fig. 4. Formoterol administration effectively relaxed airway in mice following early life allergen exposure. (A) Scheme of formoterol treatment during OVA sensitization and challenge. Formoterol (50 μ l, 5nM) was given intra-tracheally between P15 and P20. Mice were analyzed at P21 for the efficacy of Formoterol in bronchodilation. (B) Airway reactivity assays using lung slices prepared from saline controls and OVA-exposed mice with and without Formoterol treatment. Each point represents mean $\pm$ SEM of 12-28 airways from 3-5 mice. * $P < 0.05$, ** $P < 0.01$. n.s., not significant.



Supplementary Figure 5. OVA stimulation but not MCh treatment in early life increased collagen deposition around intrapulmonary airways. (A) Representative images under polarized light and (B) quantification of Sirius Red stained collagen in airways from lung sections of PBS, OVA and MCh exposed mice at P21. Sections were stained for 1 hour with Sirius Red dye solution (0.1% of Sirius Red in saturated aqueous picric acid) before mounted for observation under polarized light microscopy. The collagen was quantified by normalizing the birefringent area to the circumference of airway wall. Data was collected from 12-15 sections from 3 mice in each group. Each mark represents the quantification of airways in each section. Scale bar, 150 μ m. AW, airway. BV, blood vessel. n.s., not significant. ** $P < 0.01$.



Supplementary Figure 6. OVA exposure in adulthood had no effect on cholinergic innervation. (A) Scheme of OVA sensitization and challenge in adult mice. Mice were analyzed at day 19. (B) Western blot analysis of VACHT in lungs of control and OVA-exposed adult mice. GAPDH was loading control. (C) Quantification of VACHT levels by densitometry and normalization to GAPDH. Bar graphs represent mean $\pm$ SEM of 6 mice in 3 independent experiments. N.S., not significant.