

Figure S1. OVA-sensitized mice show increases in the levels of IgE, airway innervation and NT4 at P15. (a) Serum levels of OVA-specific IgE in PBS- and OVA-exposed pups at P15 were measured by ELISA. Each marker represents a sample. Black horizontal line indicates mean of each group. (b) Western blot analysis of neurofilament (NF(L)) (1:200, Cell Signaling, Cat. 28355) and NT4 in lung homogenates collected at P15 from controls and OVA-exposed mice. GAPDH was loading control. Each lane represents 1 mouse sample. (c, d) Densitometry analysis of NF(L) and NT4 levels based on the results of Western blot assays. Data were normalized to GAPDH. \*\* P<0.01.



**Figure S2. NT4 deficiency has no effect on mast cell number at baseline and following OVA exposure. (a)** Toluidine blue staining and quantification for mast cells in control and OVA-exposed lungs in  $NT4^{-/-}$  mice at P21. Arrows point to stained mast cells. Scale bar, 10 µm. Insert provides an enlarged view of spewed granules from a mast cell after OVA exposure. (b) Quantification of mast cell number in  $NT4^{-/-}$  mice at P21. Data represent the average and SEM from 10 non-overlapping, 100X images (0.015 mm<sup>2</sup>) in each mouse lung and 5 mice for each condition.  $NT4^{-/-}$  mice have similar numbers of mast cells in lungs as wild type mice at baseline and following allergen exposure at P21 (shown in **Figure 3c,d**). \*\*\*P<0.001.



**Figure S3. Early-life exposure to HDMA increases the levels of cholinergic innervation and NT4 at P21.** (a) Experimental scheme of HDMA exposure in neonatal mice. (b) Western blot analysis of NF(L) and VAChT in lung homogenates collected at P21 from controls and HDMA-exposed mice. GAPDH was loading control. Each lane represents 1 mouse. (c, d). Densitometry analysis of VAChT and NF(L) signal in the Western blot assay shown in (b). Data were normalized to GAPDH. (e) Quantitation of the NT4 levels in control and HDMA-exposed mice at P21 by Western blot assay. Data were normalized to GAPDH. \*P<0.05.



Figure S4. Neonatal mice have more mast cells in proportion to the size of the lung than adult mice. (a) Representative images of toluidine blue staining of lung sections in control and OVA-exposed mice at P21 and in adulthood. Arrows point to stained mast cells. Scale bars, 100  $\mu$ m. (b) Quantification of mast cell number in adult lungs. Data represent the average and SEM from 10 non-overlapping, 100X images (0.015 mm<sup>2</sup>) in each adult mouse lung and 3 mice for each condition. \*\*P<0.01. The relative abundance of mast cells in adult mouse lungs is approximately 20-30-fold lower than that in mouse lungs at P21 (shown in Figure 3a-d).