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Supplementary Materials for

Eosinophils increase airway sensory nerve density in mice and in human asthma

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/10/457/eaar8477/DC1)

Movie S1 (.mp4 format). Quantitative 3D model of airway epithelial sensory nerves from a healthy nonasthmatic subject.
Movie S2 (.mp4 format). Quantitative 3D model of airway epithelial sensory nerves from a subject with moderate persistent asthma.
Movie S3 (.mp4 format). Quantitative 3D model of airway epithelial sensory nerves costained for substance P from a subject with moderate persistent asthma.

SUPPLEMENTARY MATERIALS

Supplemental Figure S1

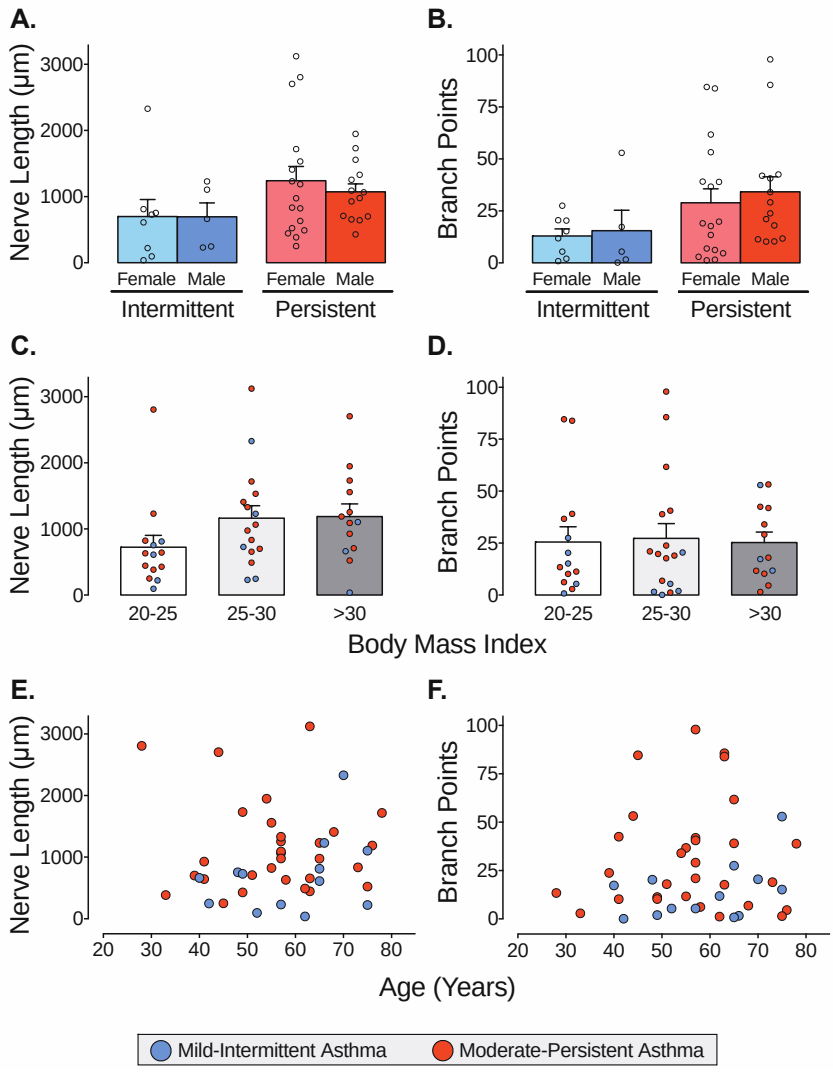

Fig. S1. Airway innervation was not associated with sex, body mass index, or age.

A-B) Nerve length and branch points in men and women with mild-intermittent (Intermittent, blue) or moderate-persistent (Persistent, red) asthma. n=13 and 31 respectively. **C-D)** Nerve length and branch points in patients with asthma stratified by body-mass index. Dot colors correspond with asthma treatment group. Mild-intermittent asthmatics are labeled blue and

moderate-persistent asthmatics are labeled red. n=43. **E-F)** Correlation between nerve length or branch points and patient age. Dot colors correspond with asthma treatment group. Mild-intermittent asthmatics are labeled blue and moderate-persistent asthmatics are labeled red. n=43. A-D data are presented as means ± SEM. Statistical significance was determined by *t* test (unpaired, two tailed; A-B), one-way ANOVA with a Bonferroni *post-hoc* test (C-D) and linear regression analysis (E-F).

Table S1. Raw data of airway nerve modeling and eosinophilia in mice.

Proximal Trachea

Nerve Length						**Mean**	**SEM**
WT	1102	892	629	1644	1145	**1082.4**	**167.6**
Il5tg	1366	1690	2148	3282		**2121.6***	**418.6**
PHIL	633	1123	1118	933		**952.0**	**115.1**
Il5tg/PHIL	1727	2179	1108	1203		**1554.5**	**248.9**

Branch Points						**Mean**	**SEM**
WT	42	43	19	62	32.8	**39.6**	**7.0**
Il5tg	65	72	86	112		**83.8***	**10.4**
PHIL	14	30	37	24		**26.3**	**4.8**
Il5tg/PHIL	59	70	34	32		**48.7**	**9.6**

Carina

Nerve Length									**Mean**	**SEM**
WT	270	458	361	481	674	388	395	306	**416.6**	**44.4**
Il5tg	566	1243	640	1181	583				**842.6***	**151.6**
PHIL	265	696	433	427	320	300	405	569	**426.9**	**51.2**
Il5tg/PHIL	463	319	407	389					**394.7**	**29.8**

Branch Points									**Mean**	**SEM**
WT	6	11	7	8	11.7	9	8	5	**8.2**	**0.8**
Il5tg	10	24	15	16	12				**15.4***	**2.4**
PHIL	5	12	9	7	5	4	8	13	**7.9**	**1.2**
Il5tg/PHIL	4	7	8	5					**6.0**	**0.9**

BAL Eosinophils						**Mean**	**SEM**
WT	0	0	0	0	0	**0.0**	**0.0**
Il5tg	6	7	8	6	30	**11.4***	**4.8**
PHIL	0	0	0	0	0	**0.0**	**0.0**
Il5tg/PHIL	0	0	0	0	0	**0.0**	**0.0**

* $p < 0.05$ compared to all other groups

Supplementary Movies:

Movie S1. Quantitative 3D model of airway epithelial sensory nerves from a healthy nonasthmatic subject. Representative video of three-dimensional nerve modeling in an intact bronchoscopic airway biopsy from a non-asthmatic healthy subject. Airway epithelial nerve models are generated from raw images immunolabeled with antibody to the pan-neuronal protein PGP9.5 (green) and DAPI (blue). Branch points are represented as red spheres in the model. Nerve models allow precise quantification of nerve length and branching. Videos were created in Imaris.

Movie S2. Quantitative 3D model of airway epithelial sensory nerves from a subject with moderate persistent asthma. Representative video of three-dimensional nerve modeling in an intact bronchoscopic airway biopsy from a subject with moderate-persistent asthma. Airway epithelial nerve models generated from raw images immunolabeled with antibody to the pan-neuronal protein PGP9.5 (green), eosinophil peroxidase (pink) and DAPI (blue) allow quantification of nerve morphology and eosinophils in airway parenchyma. Branch points are represented as red spheres in the model. Subjects with moderate-persistent asthma have significantly increased airway innervation manifesting as longer nerve length and more branching compared to mild-intermittent asthma and non-asthmatic healthy subjects.

Movie S3. Quantitative 3D model of airway epithelial sensory nerves costained for substance P from a subject with moderate persistent asthma. Representative video of three-dimensional nerve modeling co-stained for substance P in an intact bronchoscopic airway biopsy

from a subject with moderate-persistent asthma. Airway epithelial nerves were immunolabeled with antibody to the pan-neuronal protein PGP9.5 (green), substance P (pink) and DAPI (blue). Branch points are represented as red spheres in the model. Neuronal substance P expression was significantly increased in moderate-persistent asthma compared to mild-intermittent asthma and non-asthmatic healthy subjects.