Supplemental Materials:
Electronic cigarettes disrupt lung lipid homeostasis and innate immunity independent of nicotine

Authors: Matthew C. Madison[1,2], Cameron T. Landers[1,2], Bon-Hee Gu[3], Cheng-Yen Chang[1,2], Hui-Ying Tung[3], Ran You[3], Monica J. Hong[1,3] Nima Baghaei[1], Li-Zhen Song[1], Paul Porter[3], Nagireddy Putluri[4], Ramiro Salas[5], Brian E. Gilbert[6], Ilya Levental[7], Matthew J. Campen[8], David B. Corry[1-3,9,10], Farrah Kheradmand*[1-3,9,10]

Affiliations:

[1] Department of Medicine, Baylor College of Medicine, Houston, TX, USA.

[2] Interdepartmental Program in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX, USA.

[3] Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA.

[4] Department of Molecular and Cell Biology, Baylor College of Medicine, Houston, TX, USA.

[5] Department of Psychiatry, Baylor College of Medicine, Houston, TX, USA.

[6] Department of Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA.

[7] Department of Integrative Biology and Molecular Pharmacology, University of Texas Health Science Center, Houston, TX, USA.

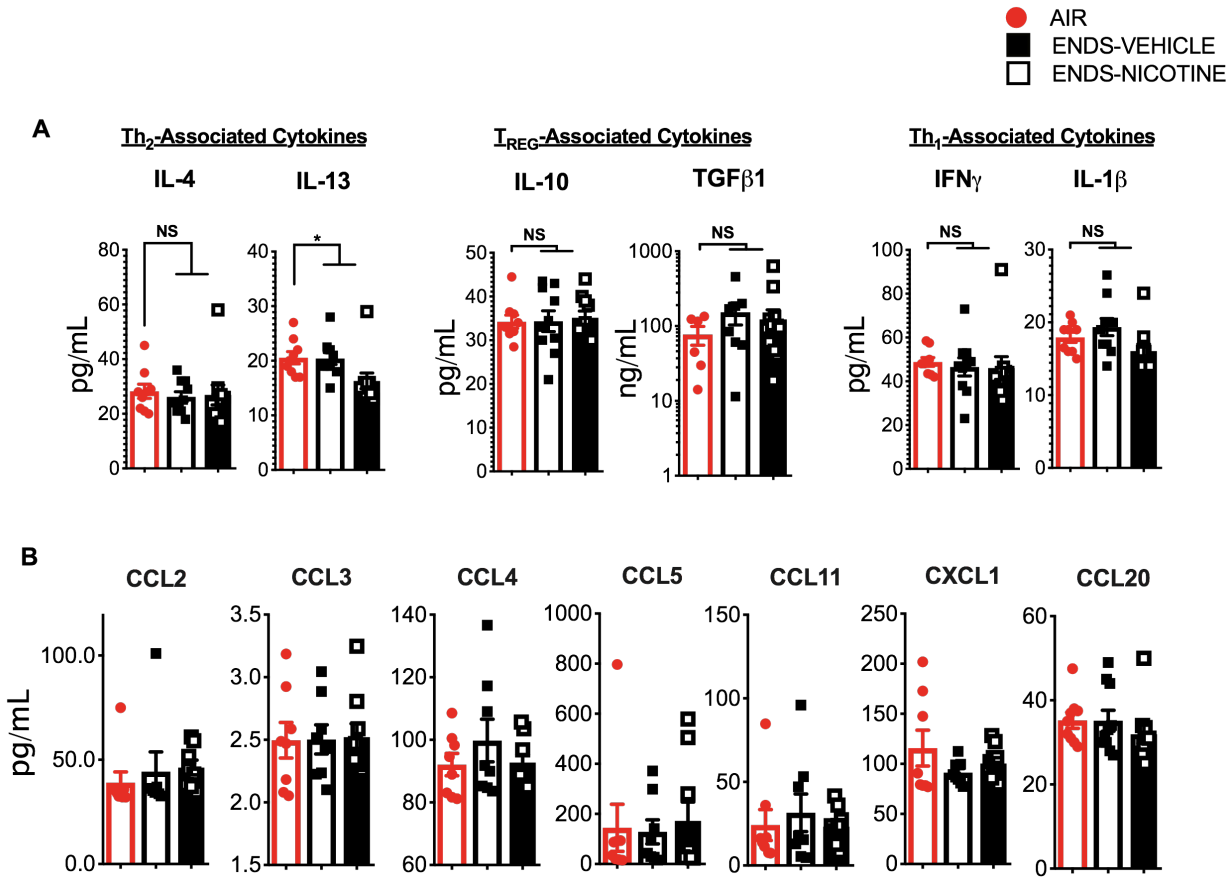
[8] Department of Pharmaceutical Sciences, College of Pharmacy, University of New Mexico, Albuquerque, NM, USA

[9] Biology of Inflammation Center, Baylor College of Medicine, Houston, TX, USA.

[10] Center for Translational Research on Inflammatory Diseases (CTRID), Michael E. DeBakey VA Medical Center, Houston, TX, USA .

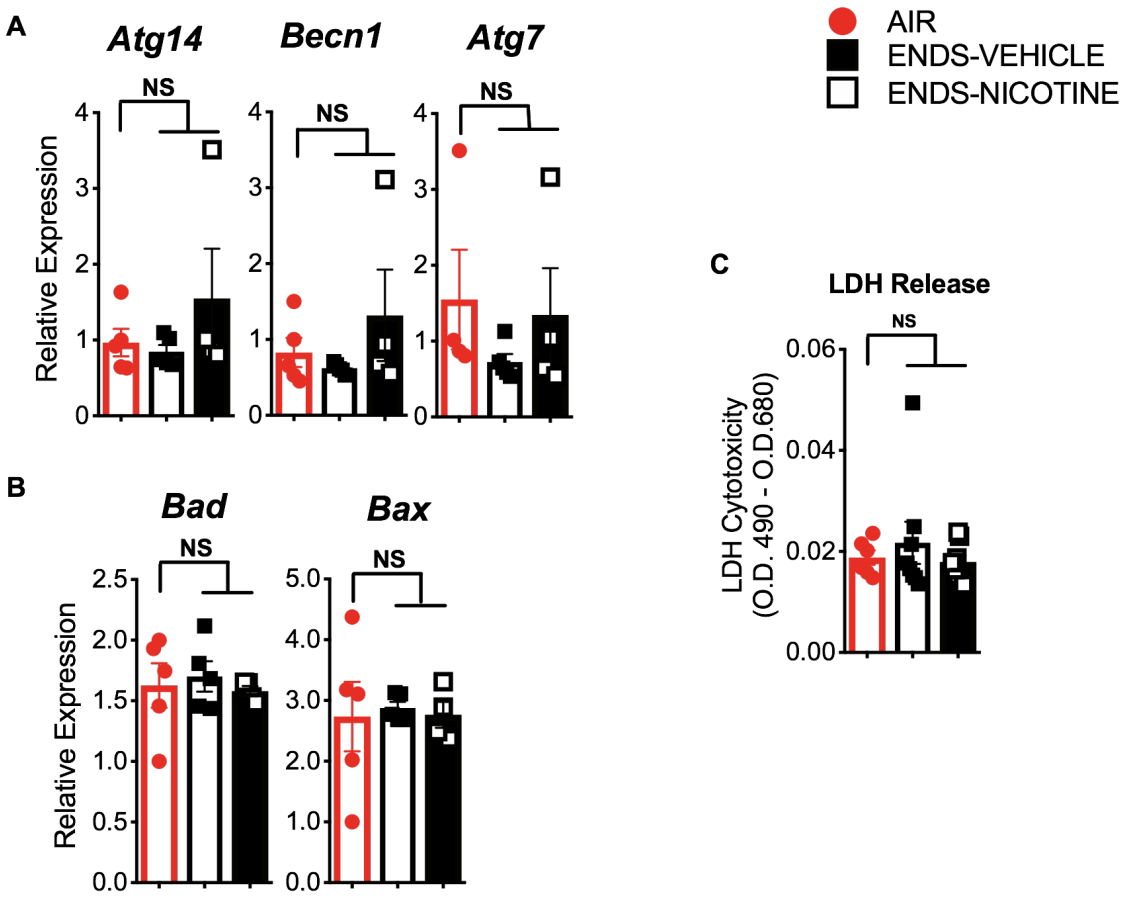
*To whom correspondence should be addressed: Farrah Kheradmand, M.D. (farrahk@bcm.edu)
1 Baylor Plaza Mailstop -M915A
Houston, TX 77030
(713) 798-8622

Supplemental Figure 1.



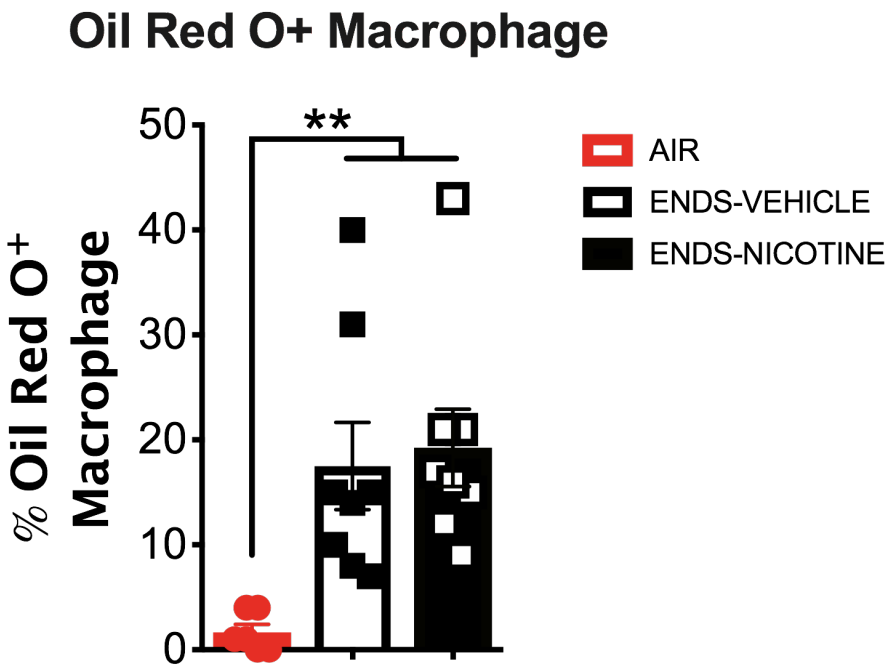
Supplemental Figure 1. ENDS exposure does not encourage Th1, Th2, or Treg-associated cytokine or chemokine profiles in the lung. (A) Representative multiplex cytokine analyses for IL-4, IL-13, IL-10, TGFβ1, IFNγ, and IL-1β from lung homogenate supernatant of mice exposed to AIR, ENDS-VEHICLE, or ENDS-NICOTINE for a 4-month period. The quantified results are expressed as means ± S.E.M. n=7 to 10 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons. (B) Representative multiplex chemokine analyses for CCL2, CCL3, CCL4, CCL5, CCL11, CXCL1, CCL20 from bronchoalveolar lavage supernatants of mice exposed to AIR, ENDS-VEHICLE, or ENDS-NICOTINE for a 4-month period. The quantified results are expressed as means ± S.E.M. n=7 to 10 per group.

Supplemental Figure 2.



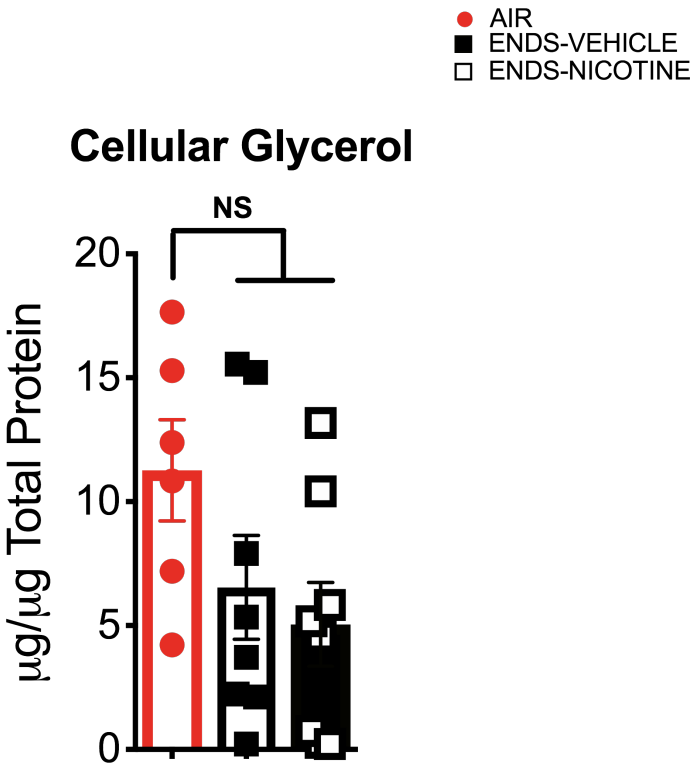
Supplemental Figure 2. BAL cells from ENDS-exposed mice fail to show increases in markers of apoptosis and autophagy. (A) Representative gene expression analysis for autophagy mediators: *Atg7*, *Atg14*, and *Beclin1*. cDNA was generated from RNA derived from cells pelleted from BAL fluid. The quantified results are expressed as means ± S.E.M. n≥4 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons. (B) Representative gene expression analysis for pro-apoptotic mediators: *Bad* and *Bax*. cDNA was generated from RNA derived from cells pelleted from BAL fluid. The quantified results are expressed as means ± S.E.M. n=4 to 6 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons. (C) Representative cytotoxicity analysis using a colorimetric, lactate dehydrogenase release assay with BAL fluid supernatant. The quantified results are expressed as means ± S.E.M. n=6 to 7 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons.

Supplemental Figure 3.



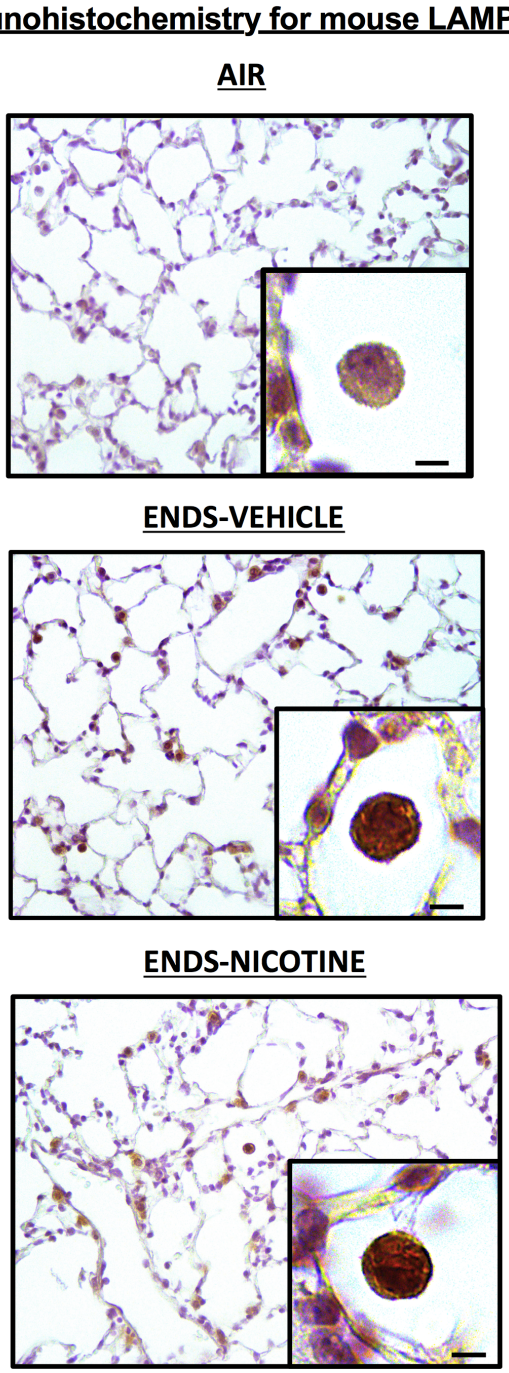
Supplemental Figure 3. BAL cells from ENDS-exposed mice exhibit significant Oil Red O positivity following 4-month exposure. Representative Oil Red O quantification of macrophages from cytospin preparations of bronchoalveolar lavage fluid from AIR, ENDS-VEHICLE, and ENDS-NICOTINE groups exposed for a 4-month period. The quantified results are expressed in terms of mean % oil red o positive macrophages ± S.E.M. n=6 to 7 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons.

Supplemental Figure 4.



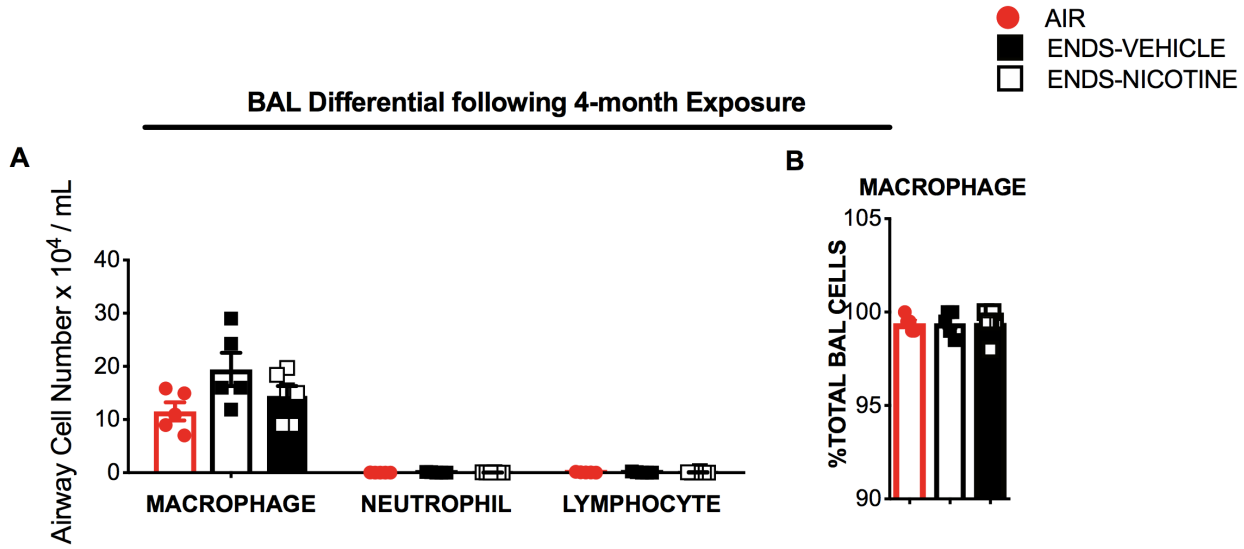
Supplemental Figure 4. BAL cells from ENDS-exposed mice do not accumulate glycerol following 4-months of exposure. Representative values acquired using a colorimetric detection assay for total glycerol. The quantified results are expressed as means ± S.E.M. n=6 or n=8 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons.

Supplementary Figure 5.



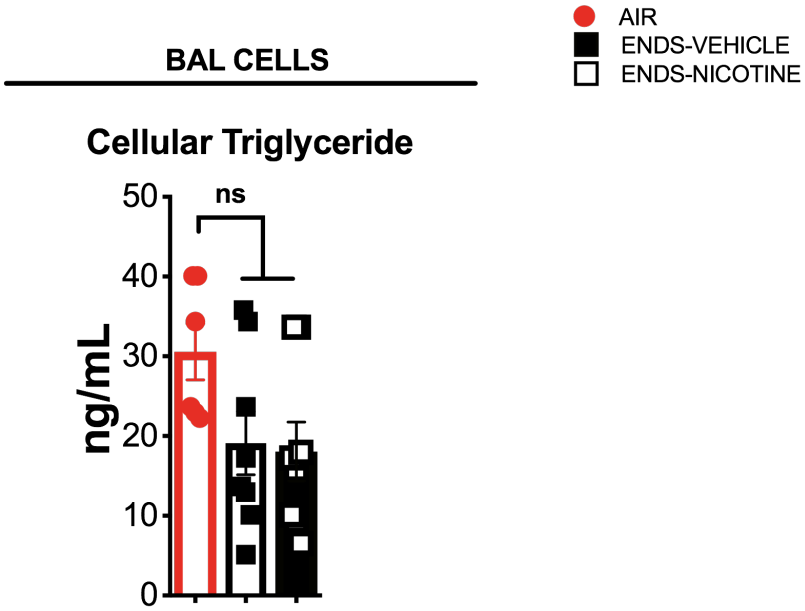
Supplemental Figure 5. ENDS-exposed mice demonstrate stronger staining for lysosomal associated protein-1 (LAMP-1). Representative micrographs of lung tissue from AIR, ENDS-Vehicle, and ENDS-Nicotine exposed mice. Five-µm slices of tissue were immunohistochemically stained for LAMP-1 and subsequently counterstained with hematoxylin. Scale Bar: 20 µm

Supplemental Figure 6.



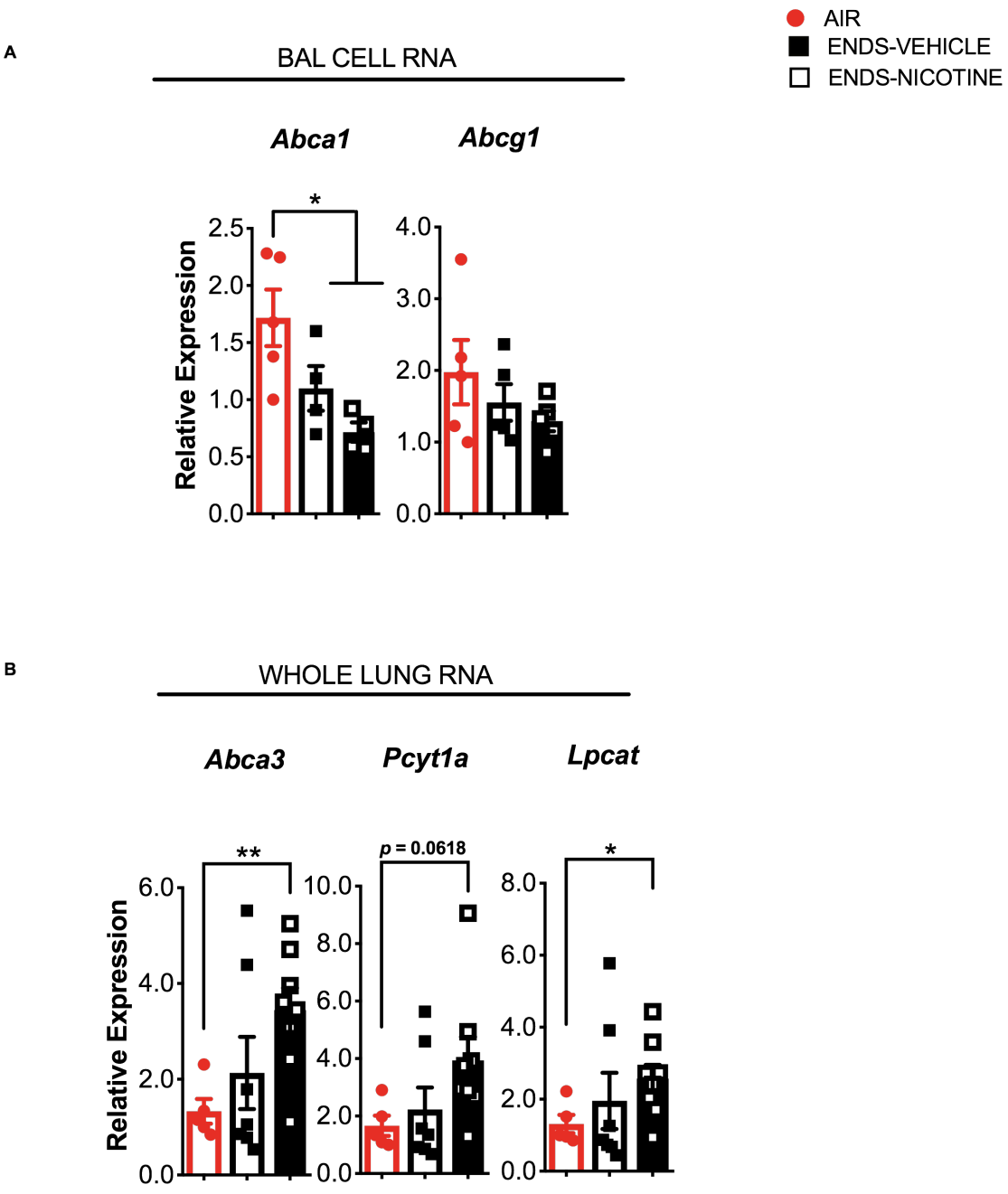
Supplemental Figure 6. BAL Cells from ENDS and Air-exposed are almost exclusively alveolar macrophages. (**A**) Differential bronchoalveolar lavage cell numbers for macrophages, neutrophils, and lymphocytes in the airway. n=5 per group. (**B**) Percentage of macrophage within the total BAL cell population. n=5 per group.

Supplemental Figure 7.



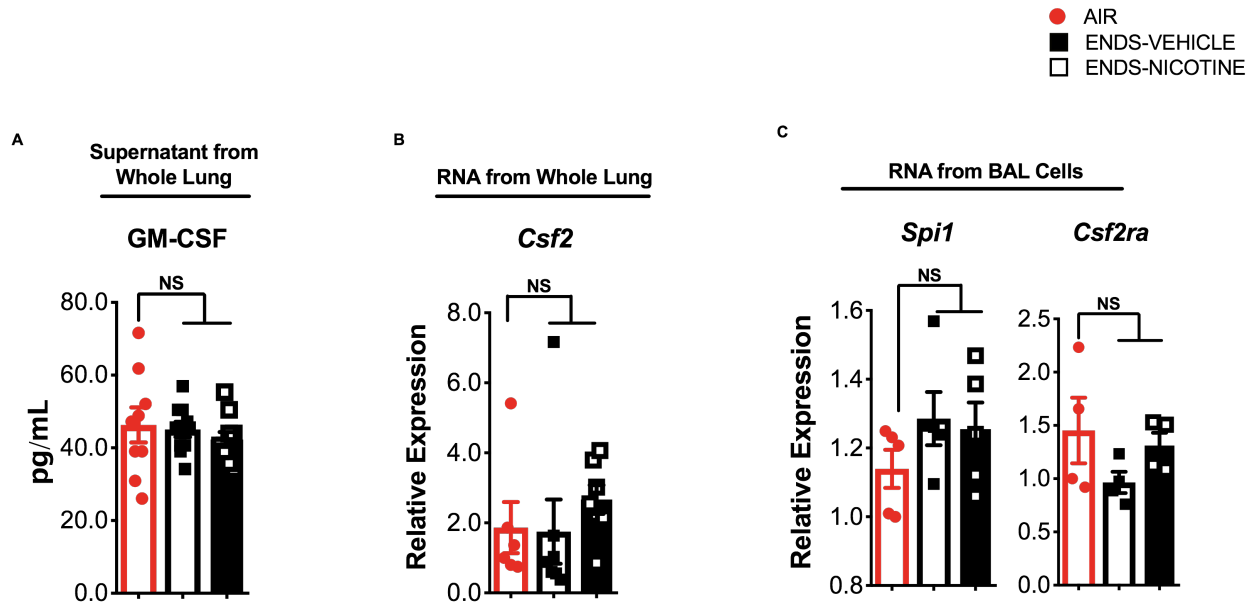
Supplemental Figure 7. Triglycerides are not significantly altered by ENDS exposure. (**A**) Total Triglyceride levels within BAL cells from mice exposed to AIR, ENDS-Vehicle, and ENDS-Nicotine for 4 months. n=5 to 7 per group.

Supplemental Figure 8.



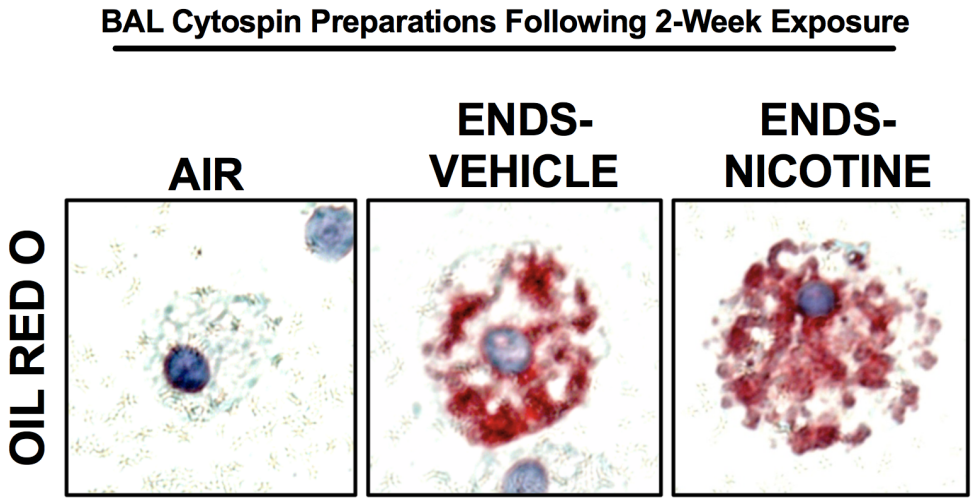
Supplemental Figure 8. ENDS exposure alters the synthesis and handling of lipids in epithelial and airway immune populations. (A) Relative gene expression of lamellar body organizing protein, ABCA3, and phosphatidylcholine-modifying enzymes, PCYT1A (CCTα) and LPCAT. The quantified results are expressed as means ± S.E.M. n=6 or 7 per group. Significance was determined by Student's *t* test. (B) Relative gene expression lipid efflux protein, ABCA1. The quantified results are expressed as means ± S.E.M. n=6 or 7 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons. $^{**}P < 0.01$, $^{*}P < 0.05$.

Supplemental Figure 9.



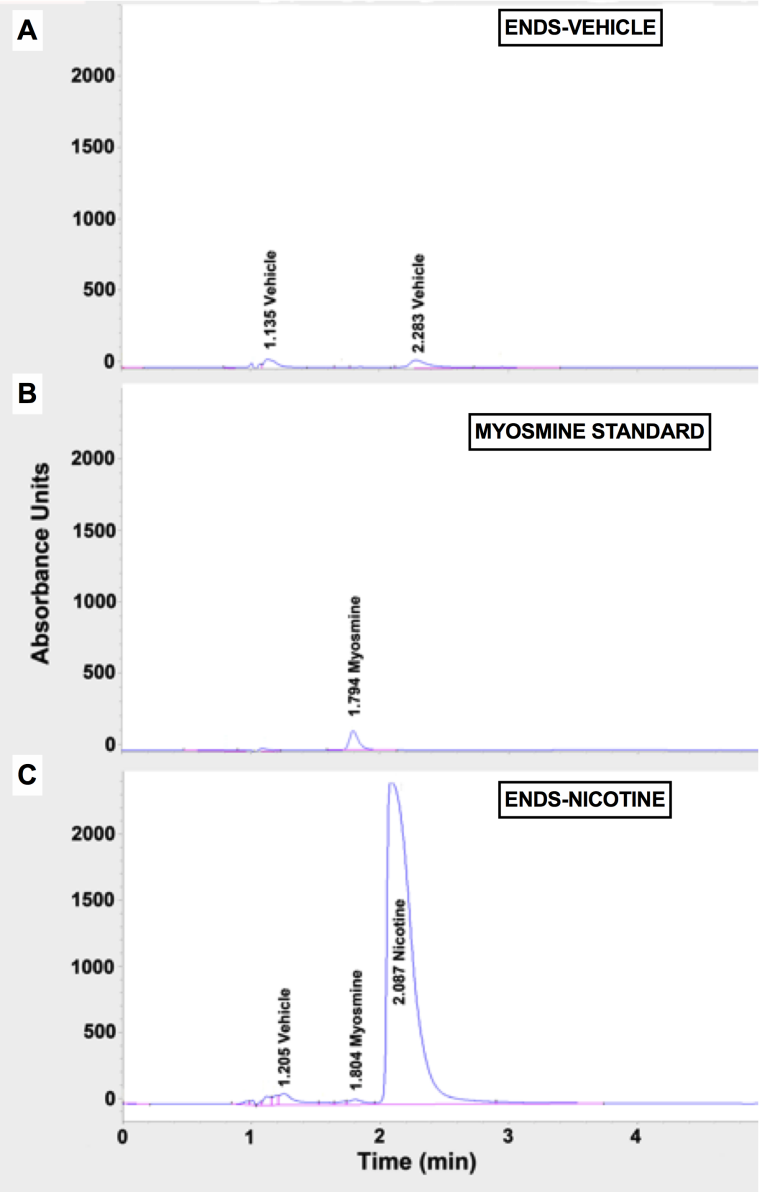
Supplemental Figure 9. GM-CSF-related pathways are unaltered by ENDS exposure. (A) Protein concentrations of GM-CSF from whole lung homogenate supernate. (B) Relative gene expression of GM-CSF's gene, *Csf2,* whole lung tissue. (C) Relative gene expression of GM-CSF receptor, *Csfra2*, and its downstream transcription factor, *Spi1* (PU.1). The quantified results are expressed as means ± S.E.M. n=4 per group. Significance was determined by One-way ANOVA with a Bonferroni correction for multiple comparisons.

Supplemental Figure 10.



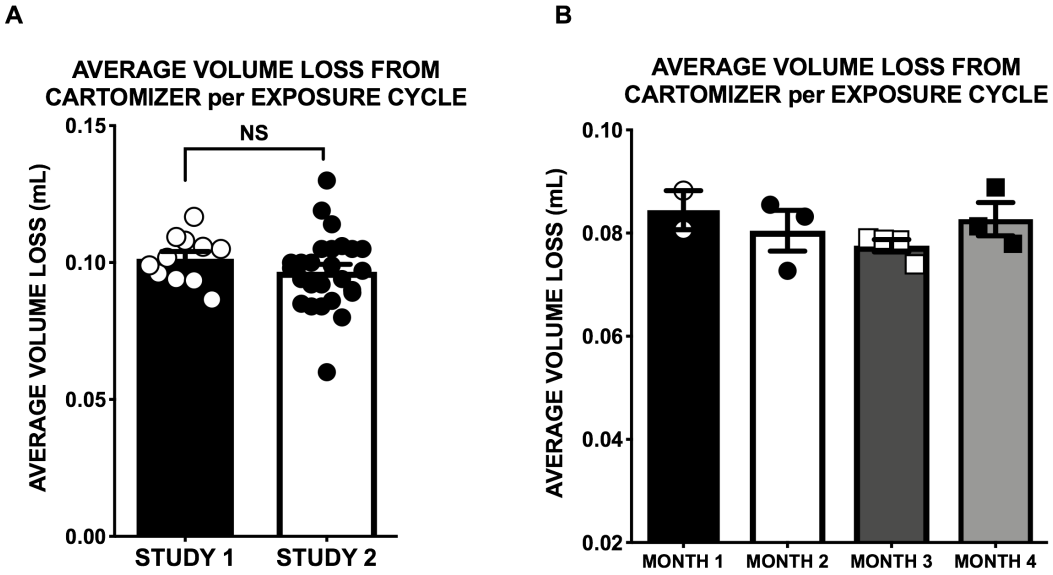
Supplemental Figure 10. Lipid deposition in ENDS-exposed alveolar macrophages at 2-weeks of exposure. Representative Oil Red O staining of cytospin preparations of airway immune cells acquired by bronchoalveolar lavage from mice exposed to AIR, ENDS-VEHICLE, or ENDS-NICOTINE for 2 weeks.

Supplemental Figure 11.



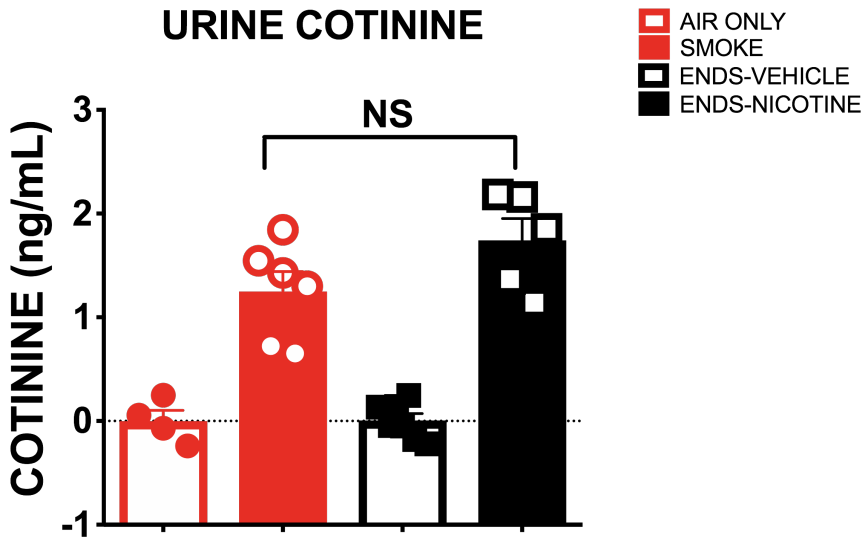
Supplemental Figure 11. ENDS solutions did not contain anabasine or cotinine contaminants. Concentration and purity of the ENDS liquids were confirmed by high performance liquid chromatography (HPLC). Focused was placed on three major known contaminants, anabasine, myosmine and cotinine. Standards for nicotine tartrate (Sigma-Aldrich), myosmine (Alfa Aesar), anabasine (Tocris Biosciences), and cotinine (Alfa Aesar) were obtained from commercial vendors. (**A**) Only traces of the vehicle solvents (PG/VG) were detected in the ENDS-Vehicle E-Juice used in our study. Cotinine, mysomine, nicotine, or anabasine were not detected. (**B**) Mysomine standard used in the analyses. (**C**) Traces of the vehicle solvents and mysomine (24 and 73 µg/ml, respectively) were detected along with nicotine in the ENDS-Nicotine E-Juice used in our study. Cotinine and anabasine were not detected. Analyses were performed on 2 E-Juice samples that were purchased at different time points from the same E-Juice vendor.

Supplemental Figure 12.



Supplemental Figure 12. E-Cigarette Analyses. (A) Average volume loss of solvent (i.e. vapor produced) from the ENDS device determined weekly for two independent studies demonstrates the consistency of eCigarette from study to study. (B) Average volume loss of solvent (i.e., vapor produced) from the ENDS device determined weekly across a 4-month study demonstrates consistent vapor exposure throughout an independent study. The quantified results are expressed as means ± S.E.M. n=2 to 3 per group. Significance was determined by the Student's *t* test.

Supplemental Figure 13.



Supplemental Figure 13. ENDS-Nicotine and Cigarette Smoke-exposed Mice receive equivalent nicotine exposure. Cotinine, a downstream metabolite and surrogate marker for nicotine, concentration from the urine of mice exposed to AIR, SMOKE, ENDs-Vehicle, and ENDS-Nicotine, respectively, 18 hours following exposure. The quantified results are expressed as means ± S.E.M. n=4 to 7 per group. Significance was determined by the Student's *t* test.