Impact of combustion-generated particles on respiratory response to viral infection

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Disclosure to Learners

- Financial relationships with relevant companies within the past 24 months:
  - None

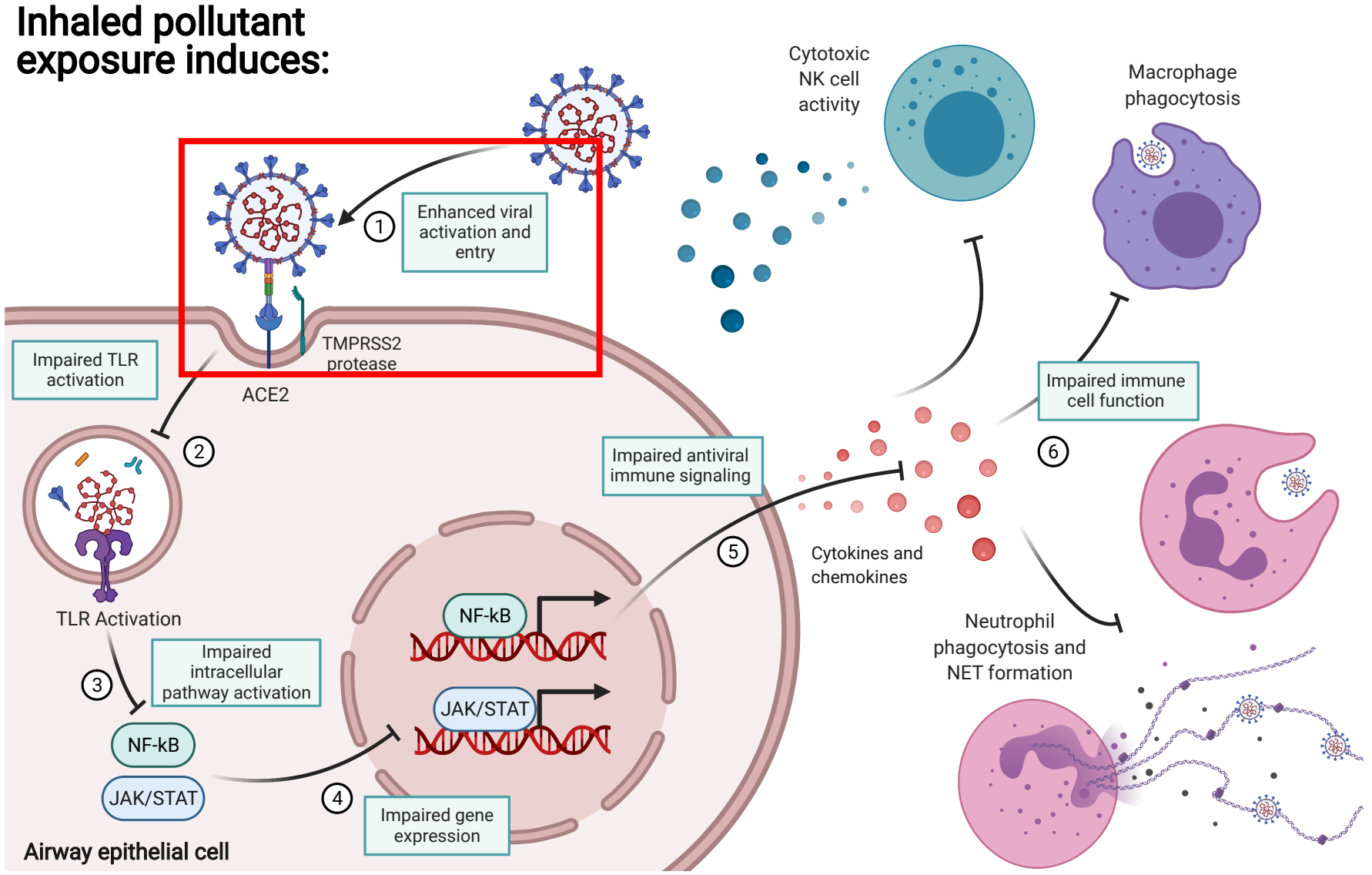
Air Pollution and SARS-CoV-2 – Epidemiological Studies from around the World

- Large portions of air pollution throughout the world are generated via combustion (wildfires, burning of fossil fuels, biomass burning, etc.) (Lightly et al., 2000)
- During the SARS outbreak in 2002, SARS patients coming from areas of high air pollution were more than twice as likely to die from the disease (Cui et al., 2003)
- Long-term exposure to air pollution was associated with increased COVID-19 severity (Conticini et al., 2020)
- Higher historical $PM_{2.5}$ exposures are positively associated with higher county-level COVID-19 mortality (Wu et al., 2020)
- Environmental factors (air quality index) are associated with daily number of COVID-19 cases (Ma et al., 2021)

Why could what we breathe affect viral infections?

- Concurrent exposures:
  - We breathe over 12,ooo Liters of air per day – contains both infectious pathogens and air pollutants
- Target the same tissue
  - Respiratory epithelium is the primary target for both viral pathogens and inhaled pollutants
- Rely on similar host defense functions
  - Innate immune cells are the primary and initial response for pollutants and viral infections

Potential Interactions Between Air Pollutants and SARS-CoV2



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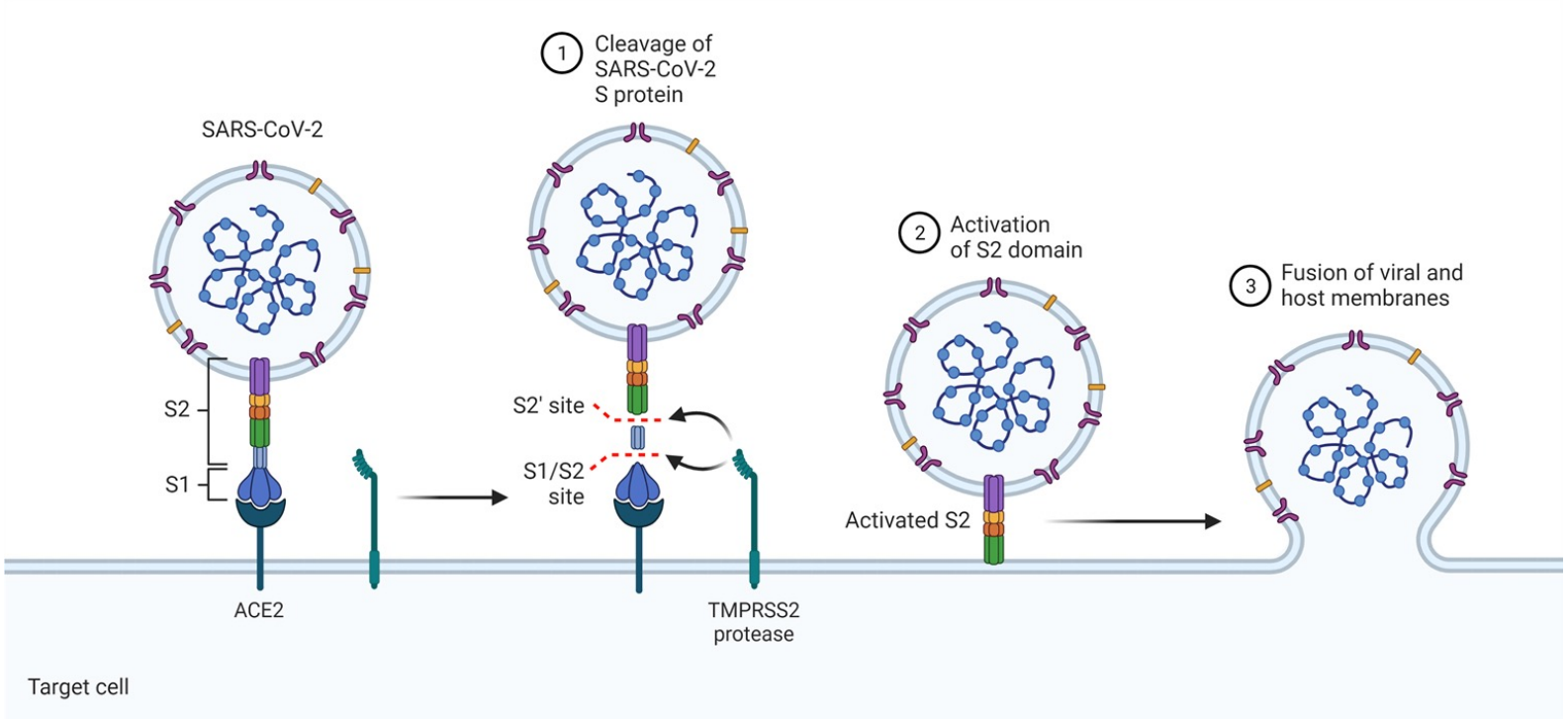
Viral Entry

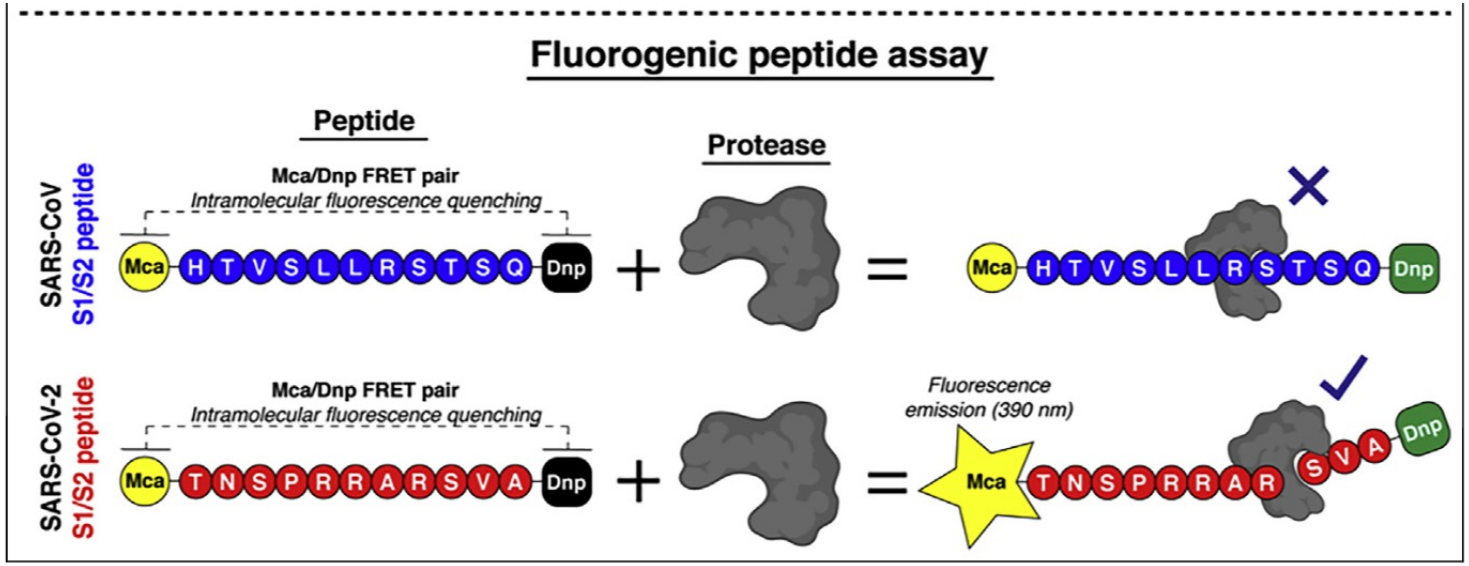


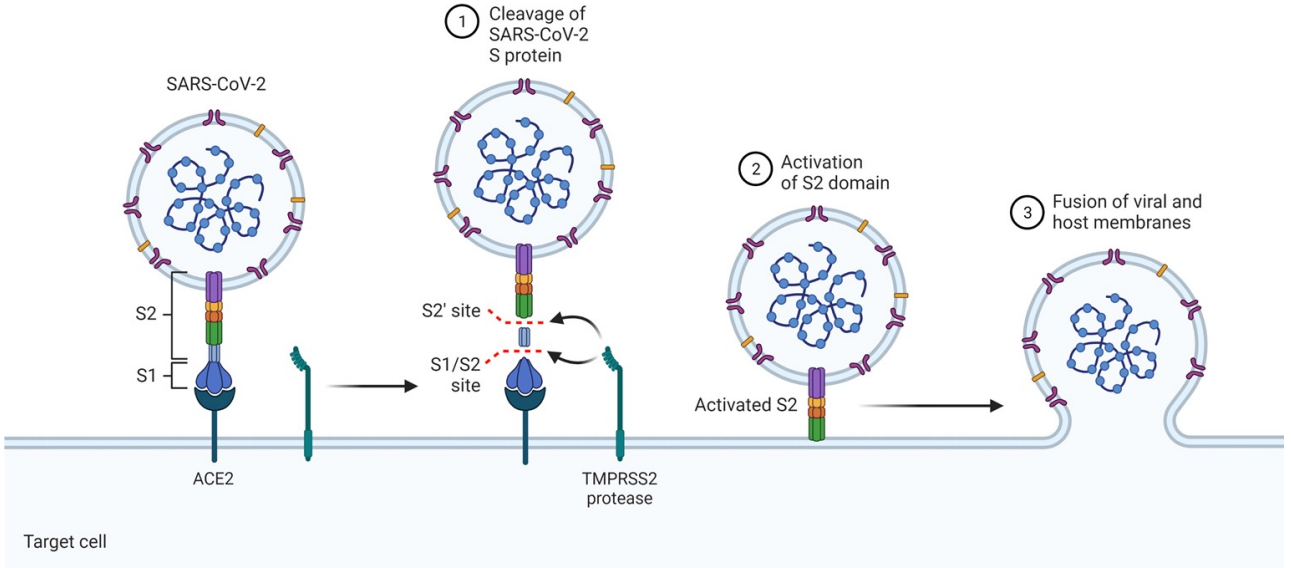
Viral activation for infection requires cleavage of the viral fusion protein

Pollutant-induced Modification of Viral Receptors/Proteolytic Activation

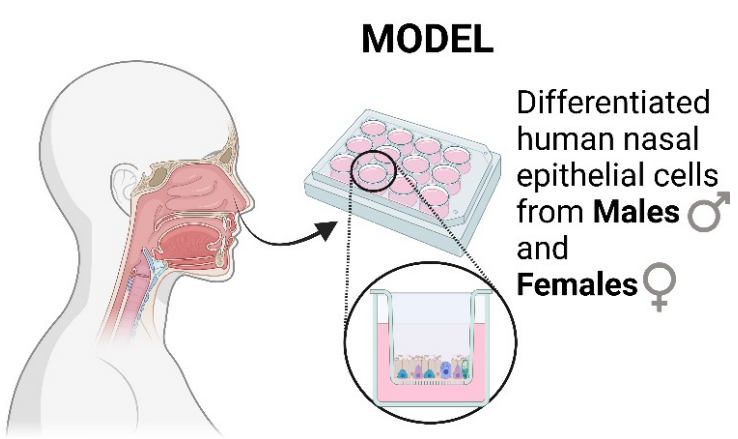
- Proteolytic Cleavage/Activation of SARS-CoV2 requires proteases, such as TMPRSS2, furin, cathepsins, etc.
- Exposure to diesel exhaust increased expression of ACE2 and TMPRSS2 in human pluripotent stem cell-derived alveolar epithelial cells and alveolar organoids (Kim et al., 2020)
- Expression of ACE2 and TMPRSS2 might be regulated by several consensus motifs for binding of the aryl hydrocarbon receptor (AhR), a common pathway activated by ambient air pollutants (Watzky et al., 2020;Lawal 20217)
- Exposure to ozone increases secreted levels of TMPRSS2 and decreases levels of SLPI (antiprotease), which was linked to increased viral entry of influenza virus (Kesic et al., 2012)

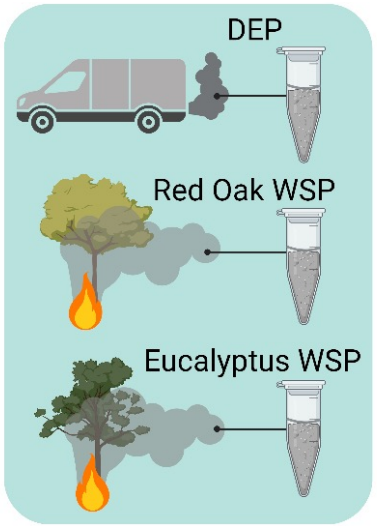
Internally Quenched Fluorescent Peptides to Assess Cleavage Activity



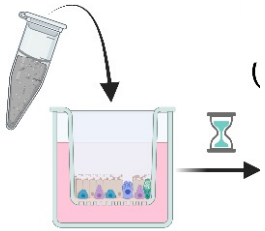


WSP Enhance Cleavage of SARS-CoV2 Peptide

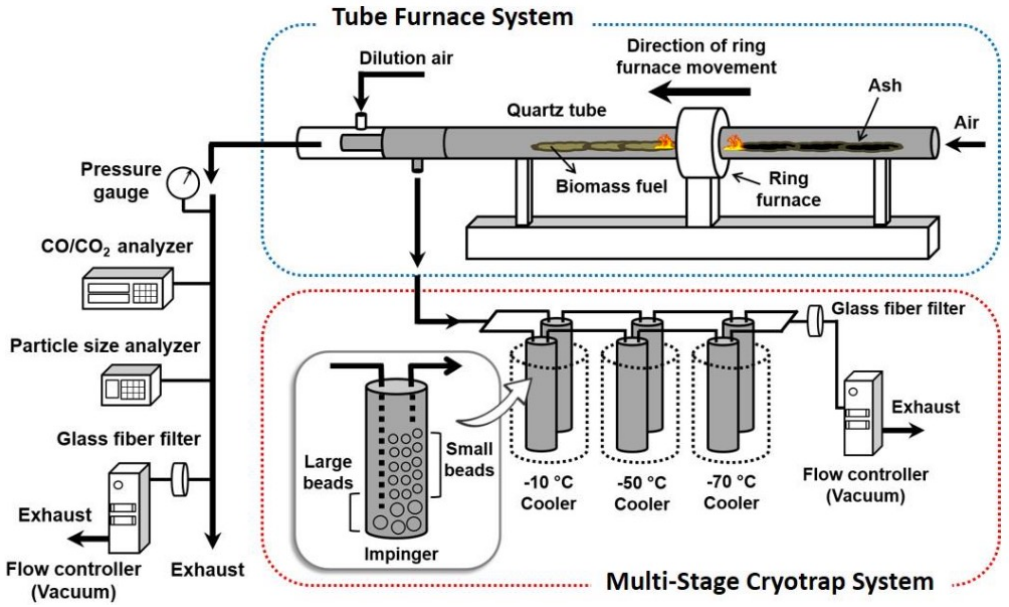


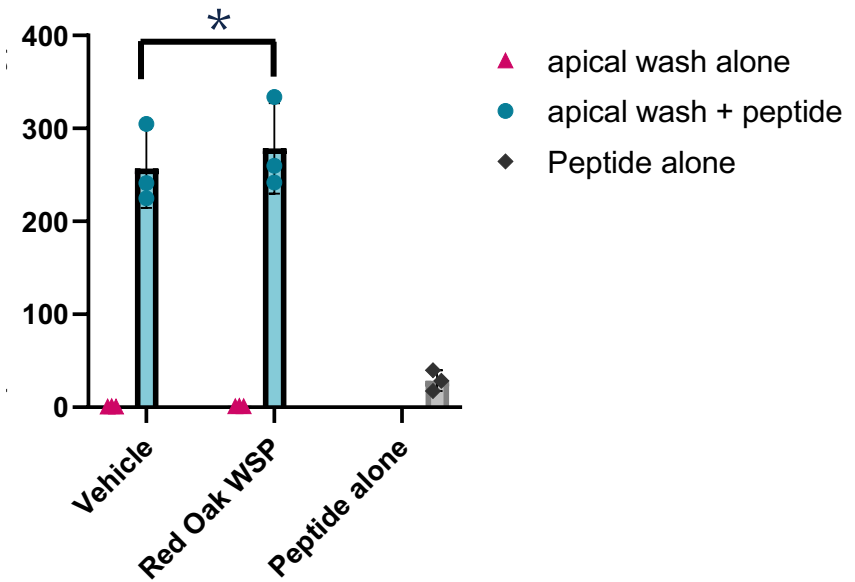


22 µg/cm² particulate suspension (or vehicle)

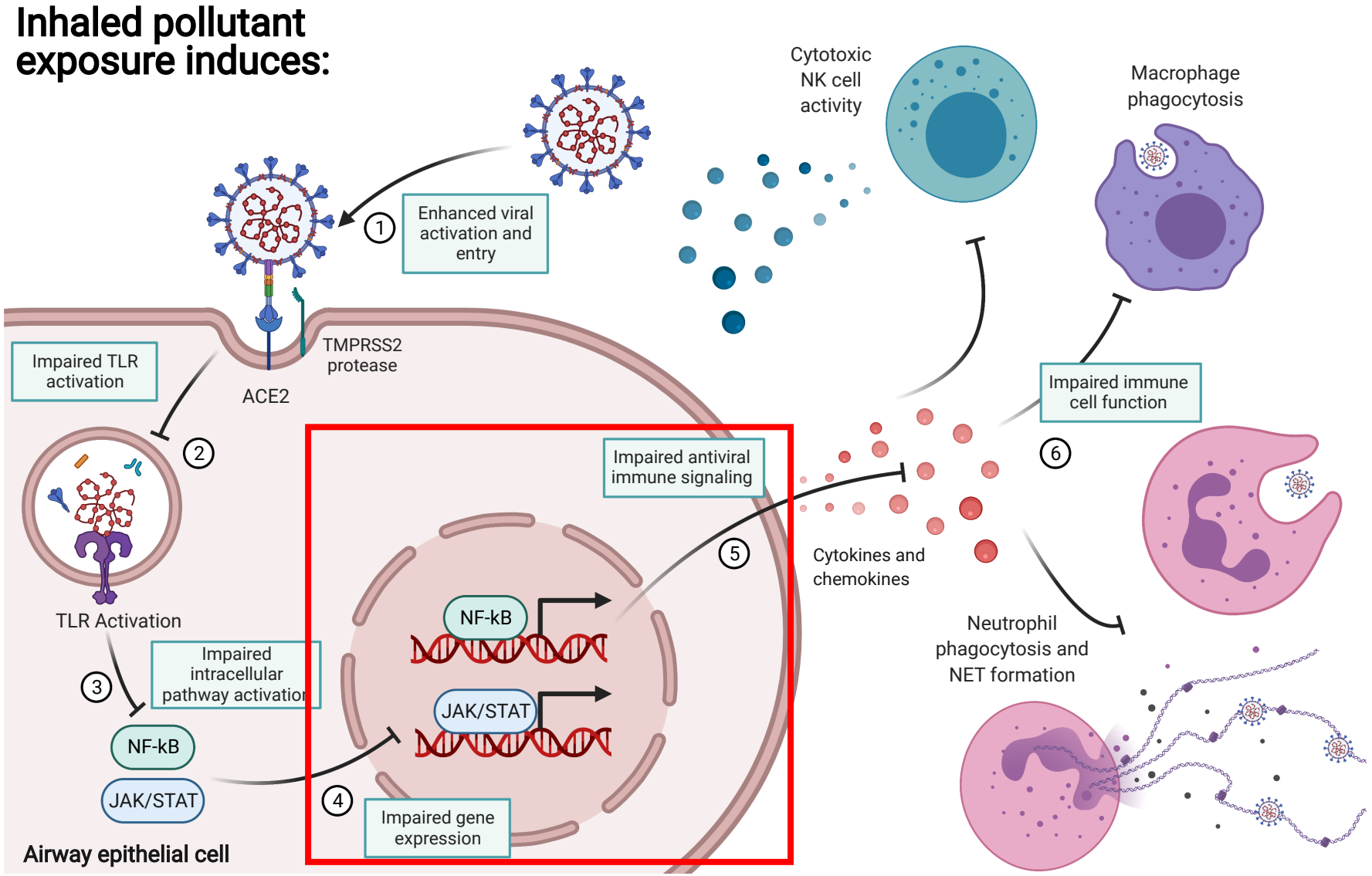


Apical washes from particle exposed and unexposed hNECs for cleavage assay





Potential Interactions Between Air Pollutants and SARS-CoV2



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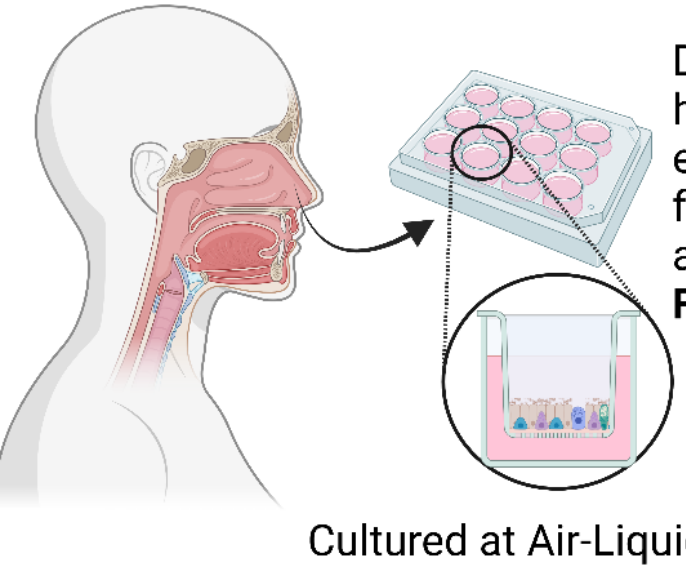
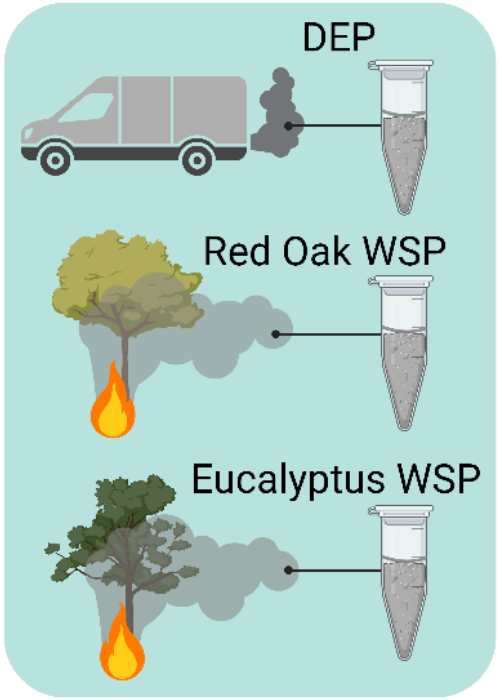
Experimental Outline

MODEL



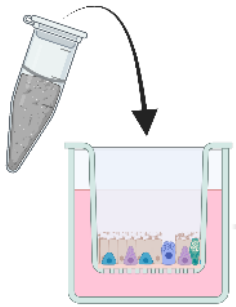
Differentiated human nasal epithelial cells from **Males** ♂ and **Females** ♀

Cultured at Air-Liquid Interface

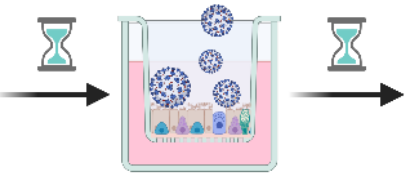


METHOD

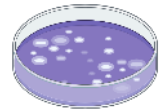
22 µg/cm² particulate suspension (or vehicle)



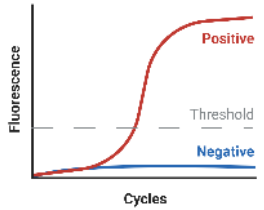
SARS-CoV-2 infection (or vehicle)



0, 24, or 72 h p.i. sample collection

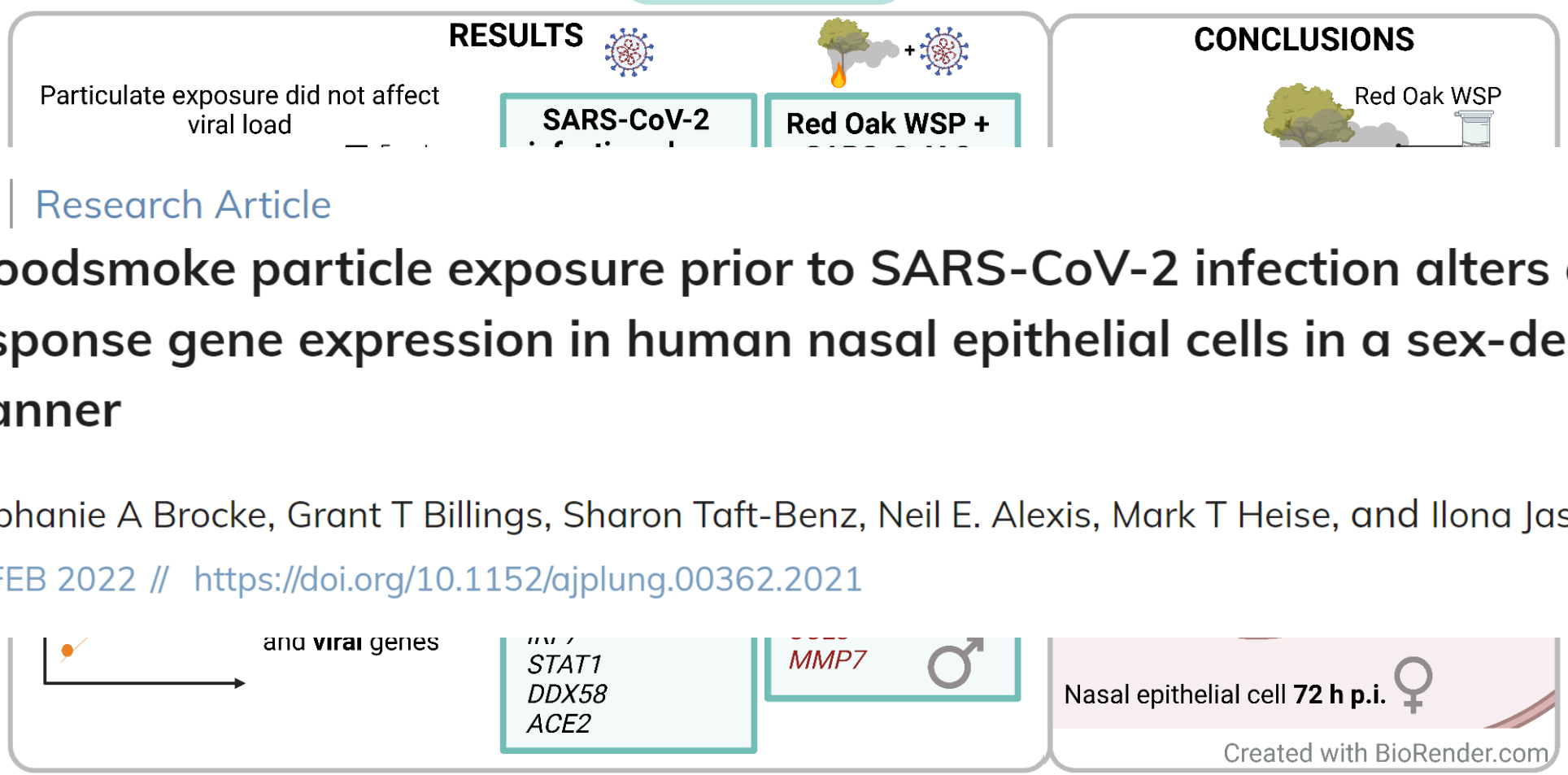


viral titer



gene expression

WSP Modify SARS-CoV2 Antiviral Gene Expression in a Sex-dependent Manner



Research Article

Woodsmoke particle exposure prior to SARS-CoV-2 infection alters antiviral response gene expression in human nasal epithelial cells in a sex-dependent manner

Stephanie A Brocke, Grant T Billings, Sharon Taft-Benz, Neil E. Alexis, Mark T Heise, and Ilona Jaspers*

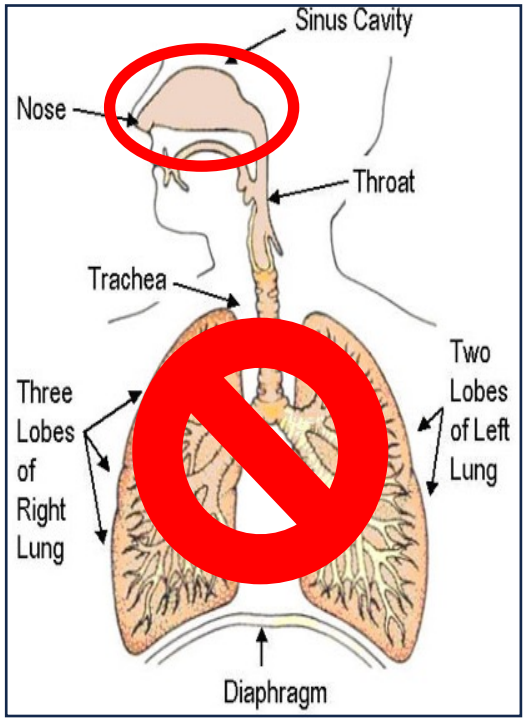
02 FEB 2022 // https://doi.org/10.1152/ajplung.00362.2021

Clinical Studies To Understand Cause-and-Effect Relationships

Human *in vivo* studies of Influenza Infections

fluMist Influenza Virus Vaccine Live, Intranasal

- FluMist™ is a cold-adapted Live Attenuated Influenza Virus (LAIV) vaccine
- "cold-adapted", thus replication limited to nasal cavity (32°C)
- It generates a replicative but self limited viral infection with innate and immune host defense responses
- Provides a safe tool to study influenza virus infections *in vivo*



Model Pollutant Exposures and LAIV - Woodsmoke

- PM derived from Woodsmoke is of increasing public health concern in the US and globally
- Healthy study participants were exposed to either 500 μg/m^3 of wood smoke particulate or air for two hours
- LAIV-induced CXCL10 (a critical IFN-inducible chemokine) levels were suppressed in the nasal mucosa of all participants
- An exposure by sex interaction was observed, with males showing greater inflammation-related gene expression, while in females' host-defense related gene expression was mildly decreased

Wood Smoke Exposure Alters Human Inflammatory Responses to Viral Infection in a Sex-Specific Manner

A Randomized, Placebo-controlled Study

Meghan E. Rebuli[1], Adam M. Speen[1], Elizabeth M. Martin[1,2], Kezia A. Addo[1], Erica A. Pawlak[3], Ellen Glista-Baker[3], Carole Robinette[3], Haibo Zhou[4], Terry L. Noah[1,3,5], and Ilona Jaspers[1,3,5]

[1]Curriculum in Toxicology & Environmental Medicine, [2]Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, [3]Center for Environmental Medicine, Asthma, and Lung Biology, [4]Department of Biostatistics, and [5]Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

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Summary

- Both acute and chronic exposure to air pollutants (particulate and gas phase) affect respiratory host defense
- Epidemiological studies suggest a link between pollutant exposure and COVID-19, but more studies are needed to determine whether other co-factors are modifying this effects
- Organotypic *in vitro* models have uncovered plausible mechanisms by which inhaled air pollutants could affect the susceptibility to SARS-CoV2
- Model virus infections combined with controlled acute pollutant exposures have identified pollutant-induced modification of respiratory virus infection; sex is a biological variable that needs to be considered

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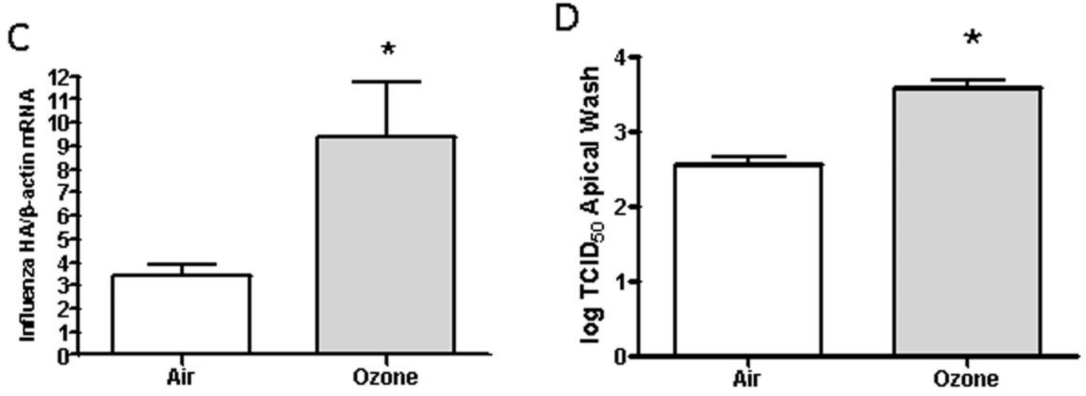
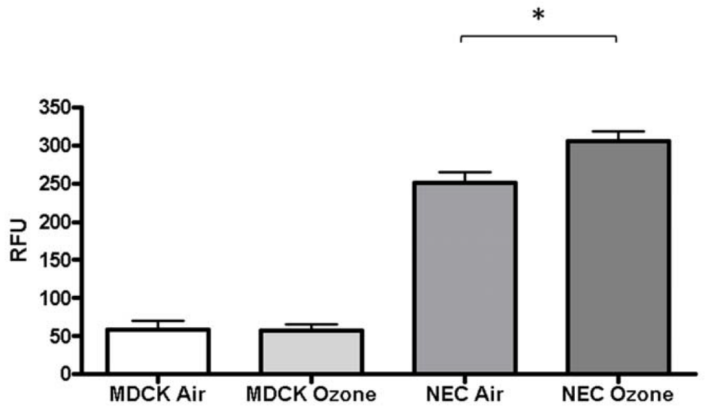
Biorender.com

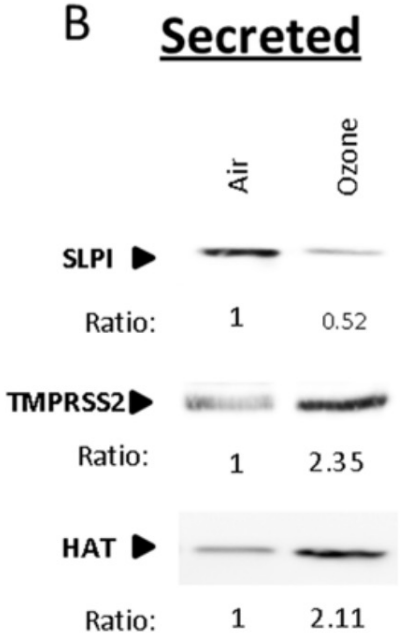
Ozone Exposure enhances influenza infection by increasing protease expression



Ozone exposure increased Influenza A infection of HNECs



Ozone exposure enhanced influenza virus-like particle entry

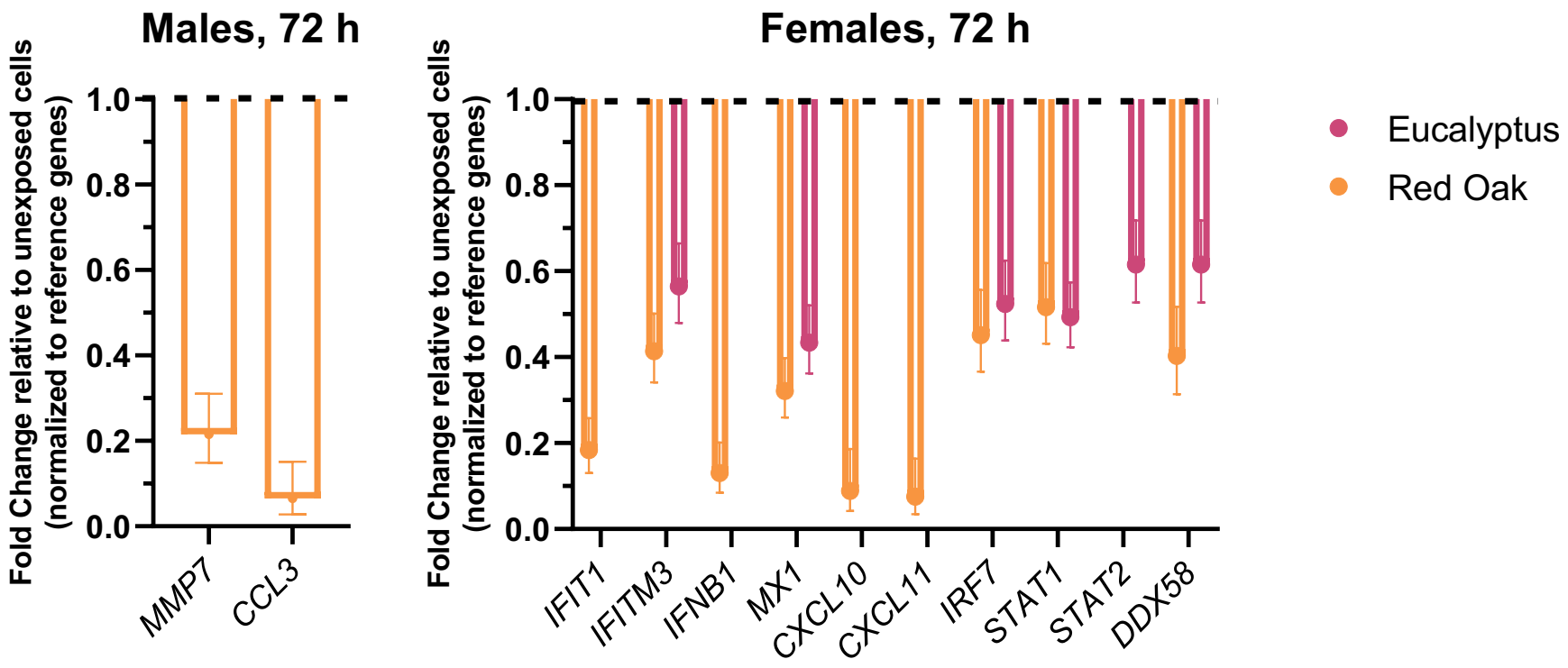


Ozone exposure increased expression of TMPRSS2 and HAT (proteases which activate influenza A) and decreased expression of their antiprotease SLPI

Kesic MJ, et al. 2012. *PLoS One*

Woodsmoke particle exposure suppresses antiviral gene expression, 72 h p.i.

Fold change of statistically significant changes in gene expression,
Sex disaggregated



N=3 biological replicates per bar. Student's t-test. * indicates statistically significant difference from unexposed controls. # indicates statistically significant difference between sexes

Model Pollutant Exposures and LAIV – Diesel Exhaust

Diesel Exhaust Exposure and Nasal Response to Attenuated Influenza in Normal and Allergic Volunteers

Terry L. Noah[1,2], Haibo Zhou[2,3], Hongtao Zhang[3], Katie Horvath[4], Carole Robinette[1,2], Matthew Kesic[2], Megan Meyer[5], David Diaz-Sanchez[6], and Ilona Jaspers[1,2]

[1]Department of Pediatrics, [2]Center for Environmental Medicine, Asthma and Lung Biology, [3]Department of Biostatistics, [4]Curriculum in Toxicology, and [5]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; and [6]United States Environmental Protection Agency, Chapel Hill, North Carolina

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