Impacts of Early-Life Wildfire Smoke Exposure on WNT Pathway Signaling Molecules and the Developing Distal Airways of Rhesus Macaques

By

MEGHNA SIVAPRASAD

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Integrative Pathobiology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

_____

Lisa Miller, Chair

_____

Nicholas Kenyon

_____

Hong Ji

Committee in Charge

2024

CONTENTS

1. ABSTRACT

With the advent of anthropogenically driven climate change, there is growing concern about the increasing severity of wildfires worldwide. Exposure to wildfire smoke has emerged as a critical public health issue, particularly for infants, whose developing lungs are highly vulnerable to pulmonary injury. While the overall effects of wildfire air pollutants on adult populations have been extensively studied, revealing significant impacts on respiratory and cardiovascular health, the implications of wildfire smoke on the particularly vulnerable infant population, and its consequences for lung development and function, remain largely unknown.

Previous studies conducted by our research group using a rhesus macaque model have shown dysregulations in the expression of molecules involved in WNT pathway signal transduction in response to ozone exposure, a common secondary pollutant from wildfire smoke. This pathway plays a major role in lung development, suggesting that wildfire air pollutants such as $PM_{2.5}$ may modulate WNT signaling in the lungs, potentially affecting distal airway development.

In this study, we hypothesize that early-life wildfire smoke exposure is associated with altered alveolar growth and dysregulation of WNT pathway signaling molecules in the lungs of infant rhesus macaques. Lung tissue from a cohort of infants housed outdoors during the 2018 Camp Fire in Northern California (n=4, 5–6 months old) and indoor housed age-matched archived control animals (n=4, 6 months old), were used for this study. Biospecimens were analyzed for WNT11 and WNT3a expression using immunohistochemical techniques, western blots, and RT-qPCR, and overall alveolar growth was assessed using morphometric techniques.

Our findings indicate that wildfire smoke exposure was associated with alterations in the expression of molecules in the WNT pathway at the protein, gene, and tissue levels, with increased expression of non-canonical WNT signaling molecules (WNT11) and decreased expression in canonical signaling molecules (WNT3a) compared to indoor housed controls. However, analysis of the distal airways showed no significant changes in morphometric parameters of alveolar growth, suggesting that the short-term exposure to wildfire smoke, as assessed within the parameters of this study, was not associated with alterations in distal lung development.

2. ACKNOWLEDGMENTS

The completion of this thesis is due to the contributions of many people to whom I am extremely grateful, and I extend the most heartfelt thanks to them all. First and foremost, I would like to express my deepest gratitude to Dr. Miller, for her steadfast support, guidance, and encouragement throughout my master's journey. Her teachings and mentorship have allowed me to grow so much in these last two years and I am extremely grateful for the countless hours she has dedicated to helping me refine my research skills and develop my scientific thinking.

To my colleagues at the Lisa Miller lab, Krista, Taylor, Diwash, Alexa, Noah, Chris, Vuvi, Alexander and Gabrielle, thank you for creating a wholesome, collaborative, and motivating environment. I can truly say this thesis would not have been possible without the many discussions, brainstorming sessions, guidance and support you have shared, and I am truly grateful for it all.

I am also deeply thankful to Dr. Nicholas Kenyon, and Dr. Hong Ji, for agreeing to serve on my committee and for their insightful feedback and contributions. Their expertise and advice have positively shaped the direction of my research, and I am honored to have had their guidance in the completion of this thesis.

I would like to specially thank Dr. Jisoo Han and the Department of Molecular and Cellular Biology, who provided me with the opportunity to serve as a Teaching Assistant, offering not only financial support but also a chance to discover my passion for mentorship and teaching and the ability to grow as an educator and mentor. I also thank Dr. Devin Horton and Dr. Erin Kent for always being there to provide a listening ear, support, advice, and encouragement throughout all my endeavors in graduate school.

I would also like to give my heartfelt appreciation to my friends in GGIP who have been there for me through thick and thin. Your endless encouragement, support, and laughter have been essential in keeping me grounded and bringing positivity and joy throughout this journey.

Last but certainly not the least, I would like to thank my whole family back in India, for being my pillars of unwavering support, love, and encouragement. I have achieved more than I thought I was capable of and none of it would be possible without you. I thank you with all my heart.

3. CHAPTER 1: INTRODUCTION

1.1 Climate change and wildfires : A growing global threat

Climate change refers to the growing long-term alterations in temperature, weather patterns, and other aspects of the Earth's climate system, due to natural or human activities. Since the 1800s, these changes can primarily be attributed to human activities, which have caused an increase in greenhouse gases in the atmosphere, trapping heat and causing global warming (1). The impacts of climate change and global warming are widespread, affecting the natural environment and ecosystems, human societies, and economies. Rising sea levels, more frequent extreme weather events, and shifts in biodiversity are some of the notable manifestations of these changes. However, one of the more concerning consequences of the broadening impacts of climate change is wildfires (2).

Wildfires are uncontrolled, unplanned, and unwanted fires that burn natural wildland vegetation as well as man-made structures. Over the past two decades, wildfires have become more severe as a result of natural and anthropogenic mechanisms related to climate change. Rising global temperatures from human activities, like burning of fossil fuels, have caused prolonged heatwaves, led to hotter and drier conditions, creating an environment that is more conducive to wildfires (3). Deforestation and changes in land use reduce vegetation's ability to absorb CO_2, further accelerating global warming. These higher temperatures cause vegetation to dry out more rapidly, increasing its potential for ignition. Climate change also impacts precipitation patterns, leading to prolonged periods of drought, which reduce soil moisture and increase the chance of fire outbreaks (4).

Climate change is also linked to changes in wind patterns and the frequency of lightning strikes, both of which contribute to the increasing severity of wildfires. Strong winds cause fires to spread more rapidly over larger areas, while lightning strikes, combined with dry conditions, can serve as an ignition source for new fires (2). This increasing severity of wildfires also contributes to a vicious natural cycle: as wildfires burn, they release a significant amount of greenhouse gases, which further exacerbate global warming, increasing the likelihood of even more severe fires in the future. The impacts of these wildfires are not confined to the regions where they occur and have global consequences such as compounding global warming, loss of biodiversity, and severe public health issues (5).

In North America, particularly in the western United States and Canada, there have been devastating wildfire seasons in the past few decades, with some of the worst fires on record occurring in California (2003, 2008, 2018, 2020), Hawaii (2023), Texas (2011), and Canada (2017, 2018, 2023) (6-9). The smoke from these fires caused severe air quality issues that affected millions of people and lead to declarations of public health emergencies. The 2019-2020 bushfire season, known as "Black Summer," saw unprecedented fires across Australia, fueled by record-breaking temperatures and prolonged drought. These fires destroyed vast areas of land, killed, or displaced nearly 3 billion animals, and caused billions of dollars in damages (8). Southern Europe, particularly countries like Greece, Spain, and Portugal, have experienced increasingly severe wildfires. In 2021, Greece faced one of its worst fire seasons in decades, with extreme heatwaves and dry conditions contributing to the rapid spread of fires. In 2017 and 2019, Portugal experienced particularly devastating wildfires in the central and northern regions of the country (9).

There has been a surge in wildfire events in regions of the Amazon rainforest and the Sahel Congo Basin in Africa. In Asia, prominent blazes have been reported in China, in the Greater Khingan Mountains (2003) and the Hengduan Mountains (2017), and in India, with fires burning in the forests of the Western Ghats (2016), Uttarakhand (2019), and the northeastern states (2021). These fires have led to extensive destruction, loss of biodiversity, and pose serious threats to residents in these regions (10,11). The occurrence of catastrophic wildfire events across various continents highlight the urgent need for global cooperation in comprehensive and coordinated efforts to address climate change.

1.2 Wildfire air pollution : Chemical composition, variability, and impact on human health

Wildfires are a major source of air pollution globally. Air pollution refers to the presence of excessive quantities of harmful substances or chemicals in the Earth's atmosphere. These pollutants can be gases, particulate matter, chemicals, or biological molecules that pose risks to human health, ecosystems, and the environment (12). The composition of air pollutants, and consequently the impacts from exposure to these pollutants, vary greatly based on the source of pollution, such as natural sources, traffic-related, or industrial emissions. Wildfires contribute to worsening air pollution through the combustion of vegetation, other forms of biomass, or urban structures. The smoke generated from these blazes is a complex, heterogeneous mixture of gases, fine particles, organic chemicals, heavy metals, and more (13). This smoke can travel large distances, impacting air quality and public health in regions far removed from the fire's origin. The composition and chemical makeup of wildfire smoke is highly variable and depend on the type of

biomass that is burned, weather conditions during the fire, the temperature of the fire, and the fire's location (14).

Wildfires produce particulate matter, specifically PM_{10} and $PM_{2.5}$ (particulate matter of 2.5 or 10 micrometers or less), whose fine size allows deep penetration into the lungs and absorption into the bloodstream. $PM_{2.5}$ can disperse over a greater area and settle more slowly than larger particles, resulting in prolonged exposure times (15). While the differential toxicity of wildfire $PM_{2.5}$ versus other sources has not been fully characterized, recent animal toxicological studies have shown that wildfire $PM_{2.5}$ can have a higher toxic effect on the lungs than the same mass of $PM_{2.5}$ from other sources (16-18). High levels of carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NOx), and sulfur dioxide (SO_2) are released with the combustion of vegetation and biomass. Exposure to high concentrations of these chemicals can affect the cardiovascular system, have neurological effects, and impact the respiratory system. Wildfire smoke also contains volatile organic compounds (VOCs), which are a group of organic chemicals that can easily evaporate into the air, like benzene and formaldehyde, which affect the respiratory system and can be carcinogenic (19). The incomplete burning of organic matter that occurs with wildfires can cause the formation of polycyclic aromatic hydrocarbons (PAHs), which can be carcinogenic and have also been linked to reproductive toxicity (20). While ground-level ozone is not directly produced by wildfires, it is a significant component of wildfire smoke as it is produced when VOCs and nitrogen oxides react in the presence of sunlight. Ozone is a powerful respiratory toxicant that has been linked with several respiratory conditions and impaired lung development (21). The combustion of man-made structures also impact the composition of wildfire smoke, with increases in the concentration of dioxins and heavy metals in the ambient atmosphere. The combined and

cumulative effects of these pollutants may contribute to increased risks of respiratory and cardiovascular diseases, and long-term impacts to overall health (22,23).

Depending on the conditions that lead to wildfires, what is burning, and where the fire occurs, the composition of wildfire smoke varies. For example, in the Amazon rainforest, with wet and dense vegetation, fires produce smoke that is rich in organic carbon and results in a greater release of particulate matter, whereas fires in drier forests like those seen in North America (Canada, western United States) tend to release more black carbon, which further traps heat and contributes to climate warming (24). Sulfur-rich soils, as seen in Hawaii, and fires in agricultural regions (where nitrogen-rich fertilizers are used), as seen in northern India, will produce higher amounts of SO_2 and NOx (25). Smoldering fires, where the main blaze is quenched and smoldering flames are left to die out on their own, as seen in the peatlands of Indonesia and in the boreal forest regions, produce larger quantities of incomplete combustion products, including PAHs, VOCs, CO, and methane (26,27).

Exposure to wildfire smoke air pollution can impact major organ systems in the body, particularly the cardiovascular and respiratory systems. The severity of these effects depends on the concentration of pollutants, duration of exposure, and individual susceptibility. Inhalation of these pollutants can lead to acute respiratory symptoms like coughing, inflammation, exacerbation of asthma and COPD, and increased risk of stroke and heart failure in people with preexisting cardiovascular conditions.

1.3 Effects of air pollution exposure on the respiratory system.

Air pollution continues to be a global public health concern, with numerous studies demonstrating its harmful effects on human health (28). The respiratory system, particularly vulnerable to pollutants, serves as both the first point of contact and the initial line of defense against airborne particles and gases. Direct exposure to these pollutants leads to a range of adverse health outcomes in the respiratory system (29). The impact of air pollution on the respiratory system directly correlates with increased healthcare utilization, including hospital admissions, emergency room visits, and outpatient consultations related to air pollution exposure.

Experimental studies have characterized acute lung injuries (ALI) triggered by airway pollutants. Inflammation is central to the biological mechanisms underlying air pollution toxicity (30). This pro-inflammatory response is generally attributed to increased immune reactions and oxidative stress. For example, increased inflammatory responses following $PM_{2.5}$ exposure have been linked to higher concentrations of reactive oxygen species (ROS), cytotoxicity in lung macrophages, aberrant activation of innate immunity (including neutrophilia and eosinophilia), and the production of inflammatory cytokines in the alveolar epithelium (31-33). Studies conducted on airway bronchial epithelial cells have also shown that exposure to air pollutant like $PM_{2.5}$ and ozone can cause oxidative stress, ROS mediated inflammation, mitochondrial dysfunction and increase in the expression of proinflammatory cytokines. (34,35) (Fig.1)

Air pollutants adversely affect respiratory epithelial barrier integrity, contributing to the development of obstructive lung diseases (36). Exposure to air pollutants decreases trans-epithelial electrical resistance (TEER), impairs mucociliary clearance (MCC), and initiates epithelial-mesenchymal transition and tissue remodeling (37-39). These exposures may also increase susceptibility to pathogens, raising the risk of morbidity and mortality from airway infections

(40,41). Wildfires, which produce bioaerosols containing fungal and bacterial cells, may transmit inhaled microbes to humans (42). Air pollutants may also impair cellular pathogen clearance and reduce the expression and function of host defense peptides (HDPs) like human cathelicidin and secretory leukocyte protease inhibitor (SLPI), in response to lung pathogens like *Pseudomonas aeruginosa*, *Aspergillus*, *Streptococcus pneumoniae*, and respiratory syncytial virus (RSV) (43,44). Both short-term and long-term exposure to particulate matter has been linked to epigenetic modifications, including DNA methylation and post-translational modifications (PTMs) (H3 histone modifications) (46). Most epigenetic investigations in association with air pollutants focus on global DNA methylation and gene specific CpG methylations (45-47). The lung microbiome, which is essential for respiratory health, is also influenced by environmental factors such as air pollution, with evidence of increased microbial diversity (48).

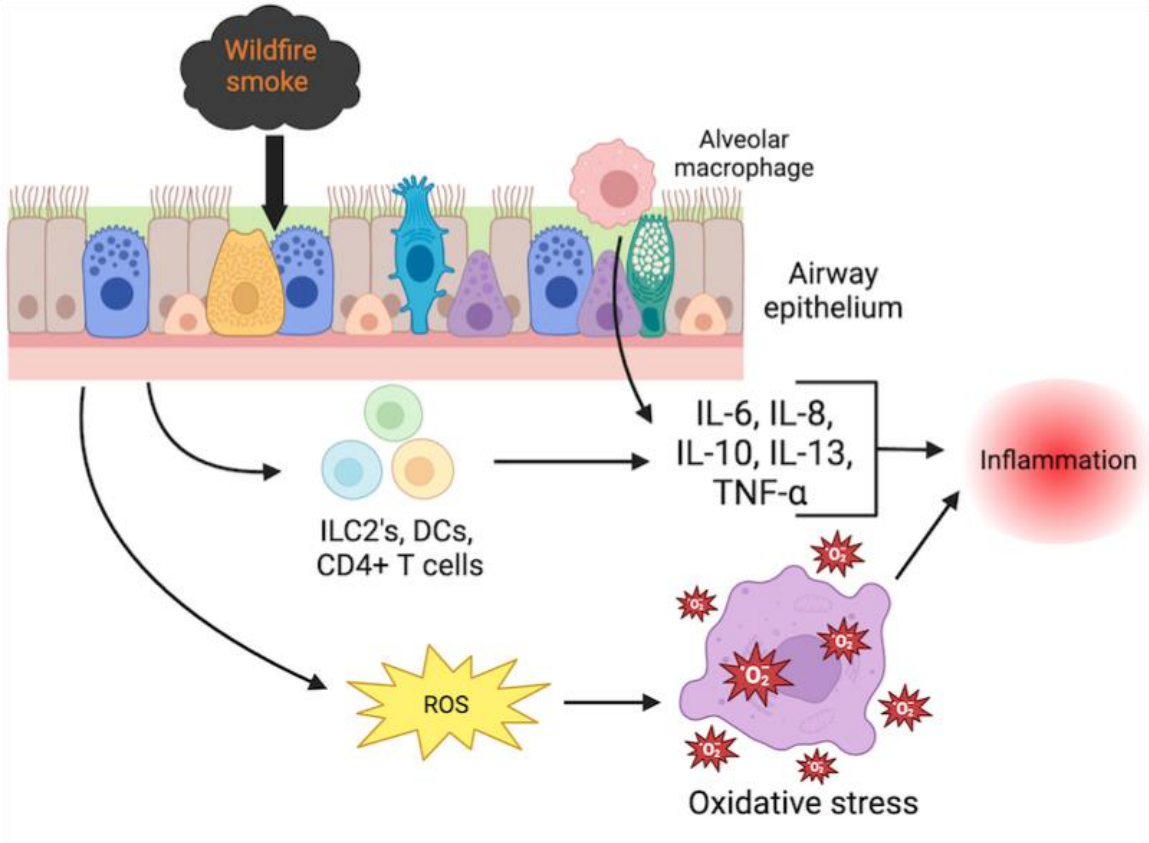

Figure 1: Mechanisms of wildfire smoke induced respiratory inflammation

Several observational studies have documented the acute and chronic effects of air pollution on respiratory health (49-51). Acute air pollution exposure refers to the immediate or short-term health impacts that manifest within hours or days of exposure to high pollutant concentrations. These effects, which are often reversible once the exposure ceases, include airway irritation, coughing, shortness of breath, and throat irritation (52). Acute exposure can trigger inflammatory responses, impair normal respiratory function, and exacerbate pre-existing conditions such as asthma or chronic obstructive pulmonary disease (COPD) (52). Studies have shown that pollutants like ozone and wildfire PM can worsen asthma symptoms, leading to increased healthcare utilization (53-55). Acute exposure to wildfire air pollution has also been linked to increased hospital admissions for respiratory infections, such as bronchitis and pneumonia, particularly in vulnerable populations (56). Even healthy individuals can experience temporary decreases in lung function following short-term exposure to pollutants (57).

Chronic air pollution exposure elicits long-term health impacts that develop over months or years. The effects are often irreversible and can lead to lasting damage to the respiratory system (58). Epidemiological studies have linked chronic exposure to air pollution to long-term decreases in lung function, and other cardiopulmonary conditions (59). While the mechanisms are not well-defined, long-term exposure to pollutants like PM and ozone may cause chronic inflammation in the airways, leading to obstructive pathologies such as COPD (60,61). Experimental studies using murine models have described persistent inflammation, oxidative stress responses, structural changes like fibrosis and damage to airway epithelium in response to chronic air pollution exposure (62,63). Studies conducted in non-human primates have also shown long term health effects, with altered immune profiles, alveolar growth, excess prenatal loss, and increased risk of developing respiratory conditions (64,65).

Longitudinal studies have shown that individuals living in areas with high levels of air pollution face a greater risk of developing COPD (66). Epidemiological studies have associated chronic exposure to $PM_{2.5}$ with an increased risk of lung cancer. The International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) classify PM as a Group 1 carcinogen (67). Furthermore, chronic exposure to air pollution has been linked to a gradual decline in lung function over time, as measured by decreases in lung capacity and airflow rates (68-70). Studies have shown that individuals in highly polluted areas experience a more rapid decline in lung function with age compared to those in less polluted regions (71). For example, studies conducted in Montana revealed decreased long-term lung function in elderly populations exposed to the 2017 wildfire season, with lung function decrements persisting for over three years after the initial exposure (72). Experimental evidence of lung function decrements was also observed in a cohort of adolescent rhesus macaque monkeys exposed to the 2008 Californian wildfires in early life (73). (Fig. 2)

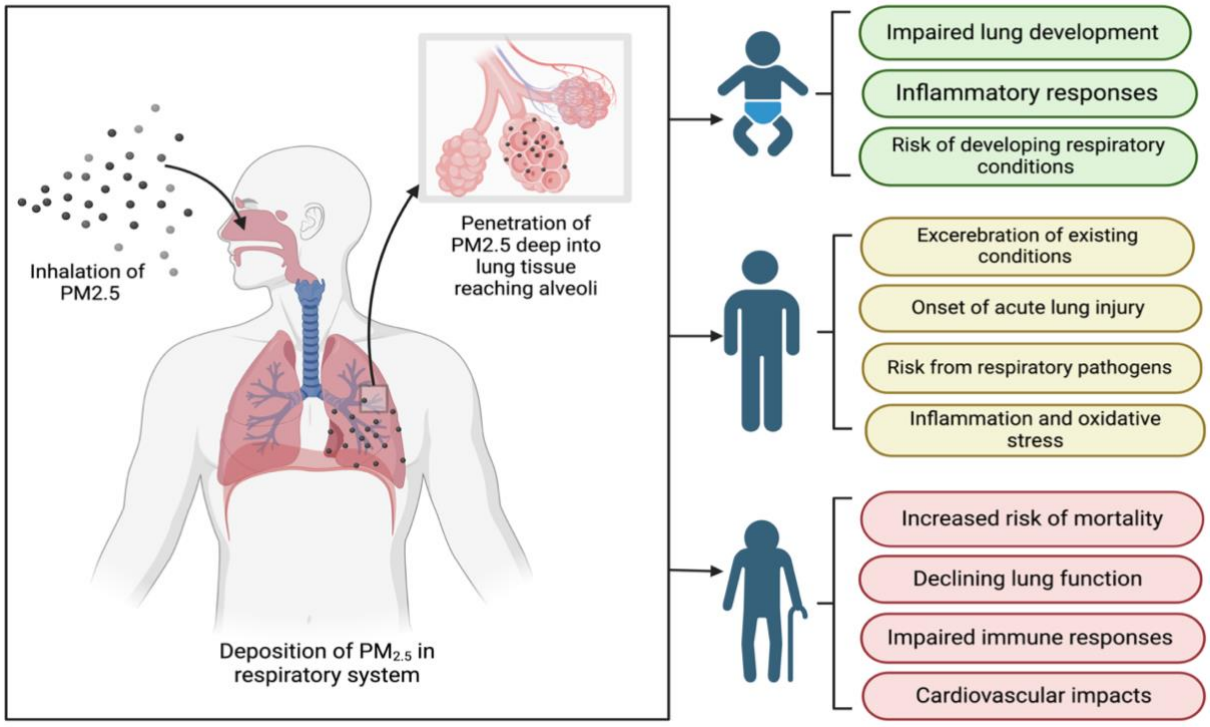

Figure 2: Impacts of $PM_{2.5}$ exposure on respiratory health across all ages

1.4 Impacts of wildfire smoke exposure on vulnerable populations

As discussed in the previous section, smoke released during wildfire events poses a serious threat to public health. However, there are vulnerable populations that face a greater risk to adverse health outcomes following exposure to harmful air pollution compared to those in the general populations (74,75). Vulnerable populations are groups of people who are more susceptible to the adverse effects from environmental hazards like wildfire smoke (76,77). There are several factors that contribute to the heightened risk faced by those that are classified as vulnerable populations. Their physiological sensitivity or less robust physiological system make them vulnerable to harmful effects from these hazards, their pre-existing health conditions that make them more likely to face exacerbated symptoms and the existence of socioeconomic disparities that lead to protective resources and healthcare being inaccessible to these groups (78). Infants and children, whose respiratory systems are still developing; elderly populations, who face age-related declines in health immune function; people with preexisting respiratory, cardiovascular, or inflammatory conditions; pregnant individuals; and people from low-income communities are all considered vulnerable populations (79).

The acute effects from these exposures can manifest more rapidly and have a more severe and significant impact on their overall health. Vulnerable populations can experience heightened respiratory symptoms such as coughing, wheezing, shortness of breath and throat irritations in response to smoke inhalation (80). Studies conducted during the 2007 San Diego wildfires observed a significant increase in emergency department visits for respiratory symptoms particularly in children and the elderly (81). Individuals who have preexisting respiratory conditions such as asthma, COPD could experience exacerbations to their conditions that require medical attention or hospitalizations.

Many studies have linked significant increases in healthcare utilization from asthma related hospitalizations, emergency department (ED) visits with exposure to wildfire air pollutants (82-89). Studies into the 2008 Northern Californian wildfires showed a linear increase in asthma hospitalizations and asthma related ED visits for every 5 ug/m^3 increase in $PM_{2.5}$ concentrations along with increase in asthma relief medication and corticosteroid prescriptions (82). Several studies have also described positive associations of air pollution exposure and exacerbations in COPD related hospitalizations, ED visits and healthcare usage (90-92). A study during the 2002 wildfires in Colorado, found that wildfire PM worsened COPD symptoms with elevations persisting more than 24 hrs after initial exposure (90). Increasing COPD hospitalizations were also observed with 10ug/m^3 increases in $PM_{2.5}$ concentration (92). A study into the 2008 northern Californian wildfires showed positive associations for COPD related ED visits but no associations for COPD related hospitalizations (82).

Exposure to air pollution has been shown to increase the risk of cardiovascular (CV) health issues especially in individuals with pre-existing conditions (93-98). Some studies have corroborated the relationship between $PM_{2.5}$ and healthcare utilization for cardiac arrests, myocardial infarction, congestive heart failure, and ischemic heart disease (96,98,101). Several large cohort studies into CV mortality from $PM_{2.5}$ exposures show significant positive associations with one study targeting wildfire specific $PM_{2.5}$ (94,99,100). Research into California wildfires has linked worsening cardiovascular health to wildfire smoke, with increased risks of myocardial infarctions, ischemic heart disease, heart failure, dysrhythmia, pulmonary embolisms, and ischemic strokes following exposure (95).

Chronic effects from these exposures even at lower levels can have long term consequences, especially from vulnerable populations (105). Prolonged exposure to particulate matter can lead to the development or worsening of respiratory conditions like asthma, bronchitis, and COPD in children and the elderly (106). These associations have been described in several epidemiological and clinical studies (107-110). Long-term exposure to air pollution is associated with an increased risk of cardiovascular disease, including hypertension, coronary artery disease, and heart failure (111-114). Pregnant people exposed to air pollution are also at a risk of preterm births, low birthrates, and developmental issues in their children (115-117). Positive associations have been made between wildfire smoke exposure and risk of preterm births and low birth weight from epidemiological and population studies conducted in China, USA, and Brazil (118-120). Along with these impacts to long term cardio-pulmonary health, air pollution and wildfire smoke exposure also have impacts on lung function in children and adults. Children who had lagged exposure to the wildfires in the Brazilian Amazon had reduced PEF (peak expiratory flow) with increasing concentrations of PM_{10} and $PM_{2.5}$ (120). Studies conducted after the 1997 Indonesian brushfires showed that adult males had reduced lung function that persisted up to 10 years from initial exposure, while children exposed to the fires seemed to have recovered lung function within the same period (121). Lung function decrements were also observed in a cohort of adolescent rhesus macaque monkeys who were exposed to the 2008 Northern California wildfires in early life (during infancy). This suggests that detrimental effects from wildfire smoke persist as the lung develops (122). (Fig. 3)

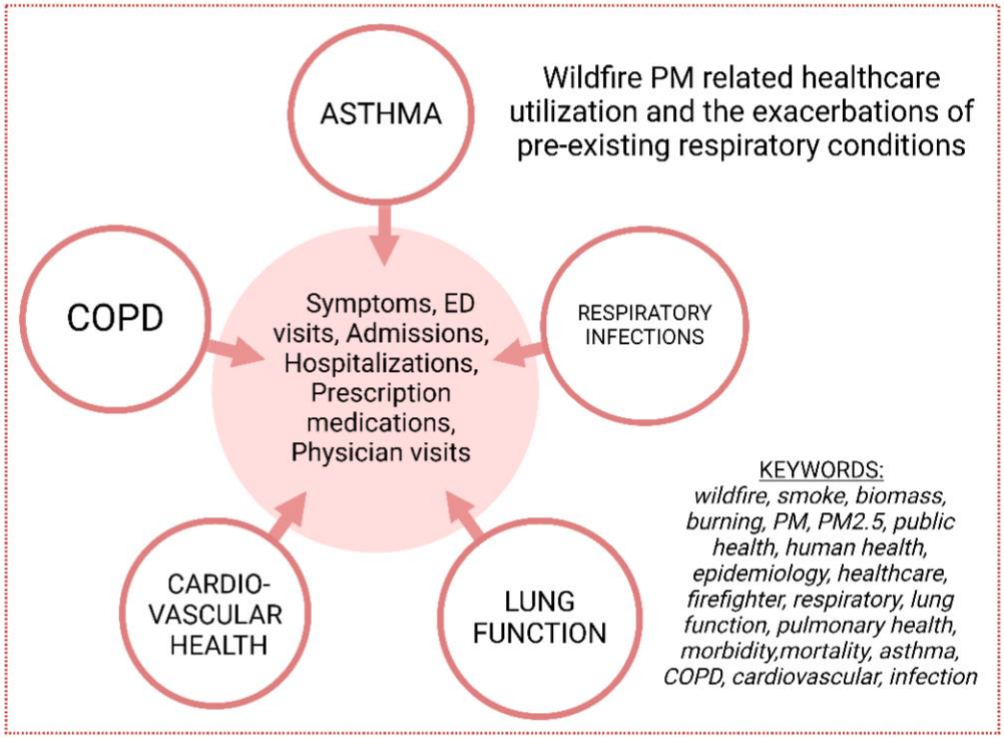

Figure 3: Associations between wildfire and cardio-respiratory conditions using healthcare utilization data

1.5 Infant lung development and the significance of cell signaling pathways

While there are 5 distinct stages that are involved in the fetal morphogenesis of the lungs, at the time of birth the lungs have not fully matured (123,124). Postnatal lung development is a critical phase in human respiratory physiology, involving complex morphological and functional changes that continue well beyond birth and extend into early adulthood (125). This period is crucial for establishing healthy respiratory function and influencing long-term respiratory health. The lungs undergo significant growth and differentiation during postnatal development, which contributes to an increase in lung volume and size along with the maturation and growth of the airways (125). Between infancy and young adulthood, we see linear increase in lung volume in response to changes in the body weight and dimensions (126). These developments ensure the functionality of the lungs and its ability to meet the respiratory needs and metabolic needs of the body as we transition from infancy to adulthood.

Most of the developmental milestones seen in the postnatal lung involve the maturation and expansion of the distal lung and airways. It consists of an alveolarization phase that occurs from birth to 1-2 years of age and late alveolarization stage that occurs from early childhood to adulthood (126). Alveolarization is characterized by the sudden massive appearance of alveoli in the lung in the first 6 months of life called 'bulk alveolarization'. This phase of the differentiation of alveolar airspaces continues till about 24 months from birth. Late alveolarization involves the addition of new alveoli till 8 years of age, followed by a period of slow repetitive expansion of existing alveoli and corresponding increase in airways size till late adolescence or early adulthood (125). At birth the distal airways contain 0-50 million alveoli which through postnatal development expands to encompass the 300 million alveoli that are seen at adulthood (125). Postnatal lung development also involves significant growth and expansion of the lung tissue. During this period, the lung's conducting airways, including the bronchi and bronchioles, also undergo maturation. The branching pattern of the airways becomes more complex, and the airway smooth muscle and connective tissue develop, which contributes to the regulation of airflow and lung compliance (125,126).

The various signaling pathways in the lung are crucial for regulating key processes during different stages of lung development. These pathways interact within a complex network, ensuring precise regulation of lung development and integrating various developmental cues to ensure coordinated progression of lung development (127). The WNT/β-catenin signaling pathway is highly involved in branching morphogenesis, lung progenitor cell proliferation, differentiation, and maintenance, as well as distal airway development (128). The SHH (Sonic Hedgehog) pathway defines structural aspects of proximo-distal airway modeling and is crucial in epithelial-mesenchymal crosstalk and mesenchymal cell differentiation (129). The FGF (fibroblast growth

factor) pathway is essential for mesenchymal patterning, airway smooth muscle cell generation, pulmonary vascular development, and distal lung formation (130). Notch signaling regulates cell-cell communication during development, determining proximal and distal epithelial cell fates, and plays a role in alveolar differentiation and capillary formation (131). TGF-β signaling is involved in lung progenitor cell development, homeostasis, extracellular matrix formation, and repair (132). The BMP pathway contributes to alveolar epithelial cell differentiation, distal lung structure formation, and maintenance of cell proliferation and apoptosis (133).

The dynamic interplay between these developmental pathways ensure the normal functional maturation of the lungs. Any disruptions, by external environmental factors (air pollution) to the signaling or the expression of molecules involved in these pathways could lead to detrimental consequences to the processes of lung development. (Fig.4)

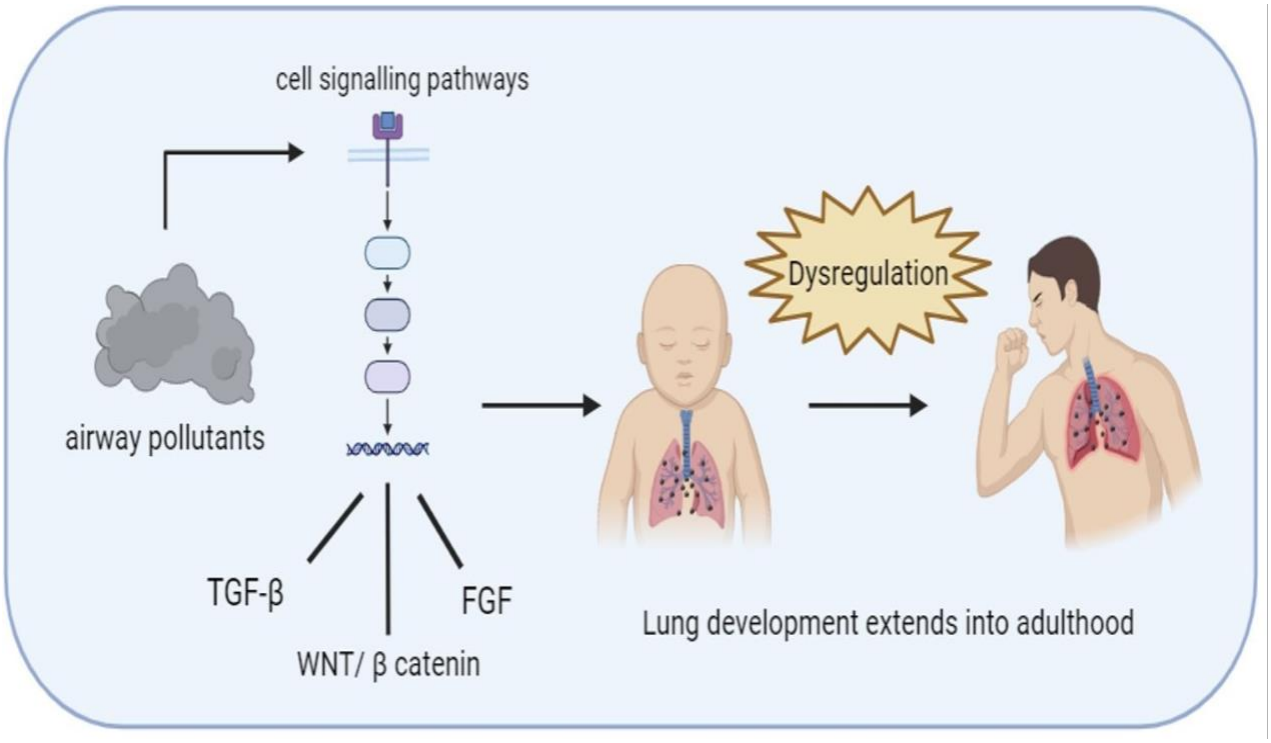

Figure 4: Effects of air pollution exposure on cell signaling pathways could alter post-natal lung development.

1.6 Air pollution and impaired lung development.

Postnatal lung development is highly susceptible to impairments in lung growth and respiratory system function as a result of exposure to air pollution (134,135). Exposure to pollutants during these susceptible periods has been associated with altered growth trajectory and lung function (136). Several studies have described the adverse effects of air pollution exposure, including outdoor ambient air pollution, traffic-related pollution, and oxidant air pollution, on lung development (137). Lung function decrements have been observed in children from 0-10 years old. Short and long-term exposure during childhood has shown reduced growth FEV_1 (forced expiratory capacity for 1 second), FVC (forced vital capacity), PEF (peak expiratory flow), with larger deficits observed with longer and higher levels of exposure. (138-143)

Exposure to air pollution during the first year of life was associated with worse lung function decrements at 8 years of age compared to exposure outside of this critical window (142). Cohort studies conducted in Oslo and China have also reported lung function deficits in children exposed to high concentrations of ambient air pollution (143). Animal models of lung development have also suggested that air pollution can have detrimental effects on the developing lung. Specifically, studies conducted in mice showed that chronic exposure to PM caused reduced lung growth and lung function decrements (144). Similar impairments to lung growth were seen in neonatal rats in response to airway toxicants. Nonhuman primates (rhesus macaques) exposed to oxidant air pollutants like ozone had impaired distal airway development with structural changes, alterations to normal alveolarization and alveolar development and reduced size of distal airways (145,146). Lung function decrements with reductions to vital capacity, total lung capacity, airway resistance, and reduced lung volumes were also observed in non-human primates' response to wildfire air pollution (147).

Previous work by our research group has also shown the effects of air pollution exposure on pathways of lung development in rhesus macaques. The WNT pathway, specifically signaling molecules involved in the non-canonical arm of that pathway have shown dysregulation in the lungs post cyclic ozone exposure in rhesus macaques. (Fig. 5) Infants and young adults were exposed to episodic ozone at 0.5 ppm for 5 months, starting at either one or thirty-one months of age, and assessed for WNT pathway signaling molecule expression (148). Given that ozone is one of the major secondary pollutants in wildfire smoke, we can speculate that wildfire could have a similar dysregulating effect upon this pathway, the consequences of which would be seen in alterations to lung development.

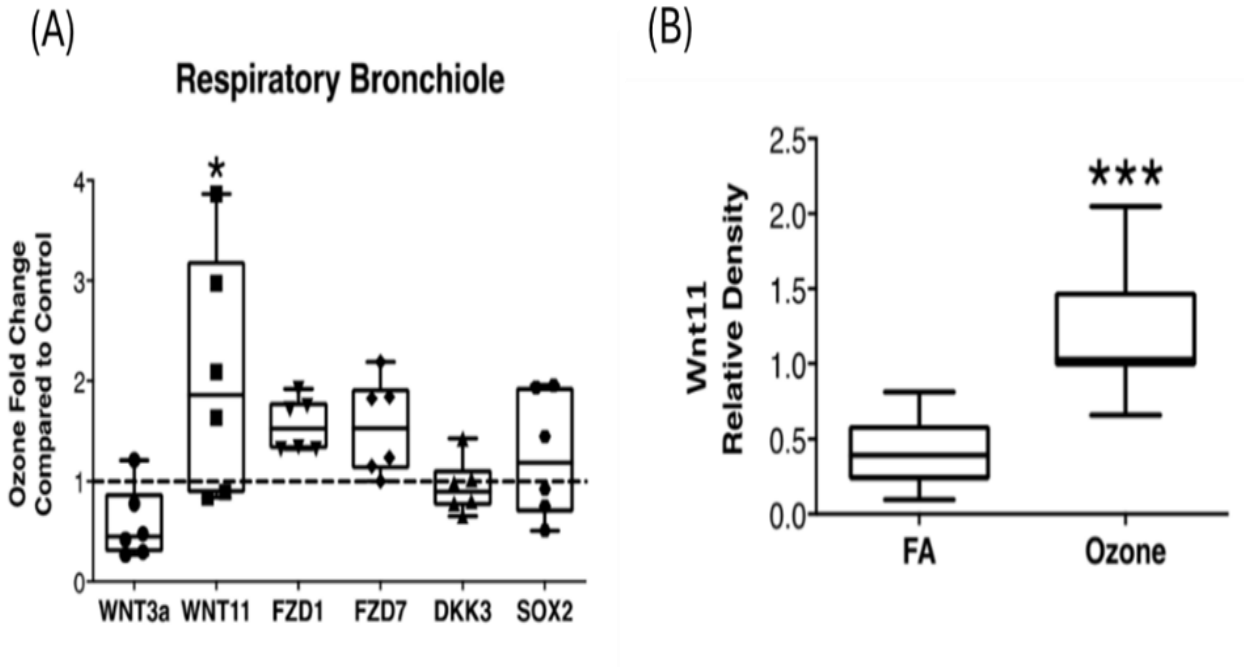

Figure 5 : Non-Canonical WNT11 dysregulation post cyclic ozone exposure. (A) Relative gene expression of targets involved in WNT signaling in the respiratory bronchioles. (B) Relative density of WNT11 protein in lungs of filtered air controls compared to ozone exposed animals.

1.7 Need for expanded research objectives: Gaps in current state of wildfire health effects research

The investigation of health outcomes associated with wildfire smoke exposure are often retrospective and observational. These methodologies involve the analysis of existing data to draw conclusions about prior exposures. Researchers use historical air quality data, wildfire events, and administrative healthcare data such as morbidity and mortality, hospitalizations, and medications. Human epidemiologic studies can be challenging as it is difficult to obtain adequate statistical power due to the sporadic nature, smaller sizes of affected populations, range of affected areas and the inability to account for all confounding factors and variables involved in environmental events like wildfires. It is also difficult to compare findings from different studies due to the variability in wildfire smoke chemistry and lack a universally accepted standard to approximate exposure data.

Most studies on the health effects from wildfires exposures have focused on adult populations, primarily with an emphasis on subjects with pre-existing cardiac, respiratory, or inflammatory conditions. These effects have been described in several epidemiological studies and healthcare data analyses, as indicated in the above sections. The attention given to these populations is warranted since adults form a larger fraction of the general population and are more readily available to participate or have their health data collected as part of comprehensive research efforts. While the epidemiological evidence provided from these populations have been extremely valuable in the efforts to understand the effects of wildfire on human health, there is a growing need to expand our studies to encompass broader demographics of vulnerable populations such as infants and children.

In the case of infants and children, there is ample evidence to suggest that exposure to ambient air pollution can affect respiratory health and lung function (135-137). Infants and children make up a particularly vulnerable population, who do not have adequate representation in wildfire health impact studies. This proves to be a major concern given their unique physiological characteristics, such as immature respiratory and immune systems and higher respiration rates (resulting in higher intake of air and thus pollutants compared to adults). They also spend a disproportionate amount of time outdoors and have higher levels of activity. This makes them more susceptible to adverse effects from environmental hazards such as wildfires. Understanding the effects of wildfire smoke on infant respiratory health is important since it has long-term implications on overall lung health. Any disruptions to the respiratory system's critical growth period, which extends into early adulthood, could have prolonged, persistent consequences on lung function, increased risk of developing chronic respiratory diseases like asthma, and heightened sensitivity to respiratory irritants later in life.

It is imperative to expand the scope of these studies to include addressing the unique phenomena occurring when infants and children are exposed to wildfire smoke. The differences in lung growth patterns and immune responses in younger populations compared to adults may lead to long-term health consequences that are currently critically under-researched. By addressing these gaps, we can develop specialized interventions that not only mitigate immediate health risks but also ensure the wellbeing of all vulnerable populations. This approach will contribute to building more comprehensive and effective public health strategies, ultimately enhancing public health outcomes on a broader scale.

In summary, more extensive research is needed to explore how early-life disruptions affect lung development and pulmonary health. Previous studies have shown associations between airway pollutants, their effects on lung function, and changes in the expression of molecules involved in lung development following air pollution exposure. Based on our prior research with experimental ozone exposure, we propose that exposure to wildfire pollutants may also have detrimental effects on lung development. Exposure to wildfire smoke may disrupt the normal growth and maturation of alveoli, potentially leading to impaired respiratory function. Additionally, the dysregulation of key signaling pathways such as WNT could hinder critical processes involved in lung development, exacerbating the negative impact on respiratory health. Thus, in this thesis we hypothesize that:

a. **Early life exposure to wildfire smoke is associated with altered alveolar growth and development in the distal lung of infant rhesus macaques.**
b. **Early life exposure to wildfire smoke is associated with dysregulation of WNT pathway signaling molecules in the distal lung of infant rhesus macaques.**

4. CHAPTER 2: IMPACTS OF WILDFIRE SMOKE ON AIR QUALITY

2.1 Background

The Northern California Camp Fire, which ignited on November 8, 2018, represents the deadliest and most destructive wildfire in California's history. This catastrophic event resulted in 85 fatalities and the burning of 153,336 acres of land. Additionally, over 18,000 structures were destroyed, with damages estimated at a minimum of $16.5 billion (149, 150). The wildfire was exacerbated by extreme weather conditions, including high winds and low humidity. These factors, combined with over 200 days without rainfall—a consequence of a statewide drought linked to climate change—contributed to the rapid spread of the fire through highly desiccated vegetation (151,152). Originating in the Sierra Nevada Mountain range in Butte County, California, smoke plumes from the Camp Fire traveled across the Sacramento Valley and the San Francisco Bay Area, due to strong winds and dry conditions (153). The extensive smoke coverage from the Camp Fire severely degraded air quality in affected regions within Northern California. Smoke plumes from the Camp Fire were observed hundreds of miles away, impacting air quality as far as San Francisco and Sacramento for approximately two weeks (154). Although pollutant concentrations in these areas were lower than those near the fire, regional air quality exceeded safe levels ('Hazardous' as designated by the EPA AQI score of 0-100), prompting widespread health warnings and advisories. The EPA sets National Ambient Air Quality Standards (NAAQS) for air pollutants which can be harmful to public health and the environment: $PM_{2.5}$ - 35 $\mu g/m^3$, Ozone – 0.070ppm and NO_2 – 0.100ppm. The Camp Fire burned for a total of 17 days, creating severe health risks and environmental damage before being fully contained on November 25, 2018 (153).

Unlike wildfires that primarily burn vegetation, the Camp Fire burned a substantial number of structures, resulting in wildfire smoke with unique characteristics (154). Chemical analysis of $PM_{2.5}$ (particulate matter of 2.5 μm or less) collected during the Camp Fire showed elevated levels of metals such as zinc, lead, calcium, iron, and manganese in the wildfire smoke, with these pollutants observed in the air as far as San Jose and Modesto (155). Following the initial impacts, the highest levels of pollutants were observed between November 13 and 16, with concentrations returning to safe levels (EPA NAASQ standards) by November 22 (156).

During the Camp Fire, $PM_{2.5}$ concentrations exceeded national averages observed from 2010 to 2017. Specifically, maximum $PM_{2.5}$ concentrations recorded between November 8 and 22 were more than three times higher than levels observed in previous years, representing a 300% increase from historical averages (157). During the Camp Fire, $PM_{2.5}$ concentrations within the Bay Area remained above 50 $\mu g/m^3$ for approximately two weeks after the fire was extinguished, with localized spikes exceeding 400 $\mu g/m^3$ during this period (158). Over the entirety of the Camp Fire event, the average $PM_{2.5}$ concentration was 64 $\mu g/m^3$, almost double the 24-hour standard. Furthermore, the Sacramento Valley experienced high levels of $PM_{2.5}$, with maximum concentrations exceeding 400 $\mu g/m^3$. These concentrations, 10 times higher than the 24-hour $PM_{2.5}$ standard, are classified as "Hazardous" according to the EPA's Air Quality Index (AQI) scale, indicating severely poor and unhealthy conditions (158).

To understand the impact of Camp Fire smoke on the Sacramento Valley region and characterize the pollutants present in the atmosphere, we collected and analyzed air quality data throughout the entire duration of the wildfire. This data will provide contextual insights into the potential effects of this exposure on the lung development of non-human primates housed at the CNPRC.

2.2 Materials and Methods

Air quality data acquisition and analysis

Air quality data was acquired from monitoring sites managed by the California Air Resources Board (CARB). CARB is an agency that oversees all air pollution efforts and monitors and regulates air quality across the state of California. CARB operates an extensive network of air quality monitoring sites located throughout California to assess pollution levels and ensure compliance with state and federal air quality standards. These sites continuously monitor and collect real-time data on a wide range of air pollutants. The impact of the wildfire on air quality in Northern California was analyzed and the concentrations of key wildfire smoke pollutants during November 2018, specifically during the period of the Camp Fire (11/08/2018 - 11/25/2018) and from November 2019 as air quality reference during a non-wildfire year, was examined. Data was retrieved from CARB's openly accessible air quality databases, focusing on 3 specific pollutants in wildfire smoke – particulate matter ($PM_{2.5}$), ozone, nitrogen dioxide (NO_2) and temperature. This process involved extracting daily measurements of these pollutants, and ambient temperature, from the AQMIS (Ambient Quality Management Information System) database (159). The analysis centered on monitoring sites within the Sacramento Valley air basin, a region that was severely impacted by the Camp Fire. These sites included Sacramento County (site #34295), Yolo County (site #57557-Davis and site #57582-Woodland), Placer County (site #31811), Sutter County (site #51898), Butte County (site #04625), and Shasta County (site #45567). The collected data was compiled and organized to facilitate the analysis of pollutant levels at these key locations throughout the month.

Animals

All animals used for this study were obtained from the breeding colony at the California National Primate Research Center (CNPRC) at the University of California, Davis. Care and housing of animals complied with the provisions of the Institute of Laboratory Animal Resources and conformed to practices established by the American Association for Accreditation of Laboratory Animal Care. Animal protocols were reviewed and approved by the University of California, Davis, Institutional Animal Care and Use Committee (IACUC). Exposed groups consisted of wildfire smoke-exposed infant rhesus macaque monkeys (n=4) housed in outdoor enclosures at the CNPRC during the 2018 Northern California Camp Fire. The average concentrations of various air pollutants during this period are detailed in figure 7, as reported by the California Air Resources Board (CARB) air quality monitoring site (#57577), located 2.2 miles from the CNPRC. Biospecimens from animals in exposed groups were obtained from the CNPRC Pathology Unit; animals were euthanized due to health conditions unrelated to exposure or the respiratory tract. Age-matched control animals (n=4) were housed indoors in facilities equipped with a MERV13 filtration system (75% filtration capacity for 3.0 to 0.3 μm size particles). Biospecimens from controls were obtained from archived specimens from a previous study. 1-2 months following the initial exposure to wildfire smoke, necropsy was performed on the animals in the exposed group for biospecimens collection.

Study design and biospecimens

Lungs collected from wildfire exposed and indoor housed infants were used in this study for experiments conducted in Chapters 3 and 4 (Fig. 6A). Different lung lobes were separately processed for downstream experimental analyses. The left caudal lobes were paraffin-embedded and used for histology and morphometry. The right caudal lobes were snap-frozen in liquid N_2 for protein extractions and Western blots, and the right cranial lobes were inflated with RNAlater for RT-qPCR (Fig. 6B).

Group	Wildfire smoke event	Sex	Approximate Age during wildfire exposure	Age at Necropsy	Sample Size
Wildfire smoke-exposed	2018 Camp Fire	50% male 50% female	5-6 months	6-7 months	4
Indoor housed	N/A	50% male 50% female	N/A	6 months	4

Table 1 : Descriptive demographics for groups used in study

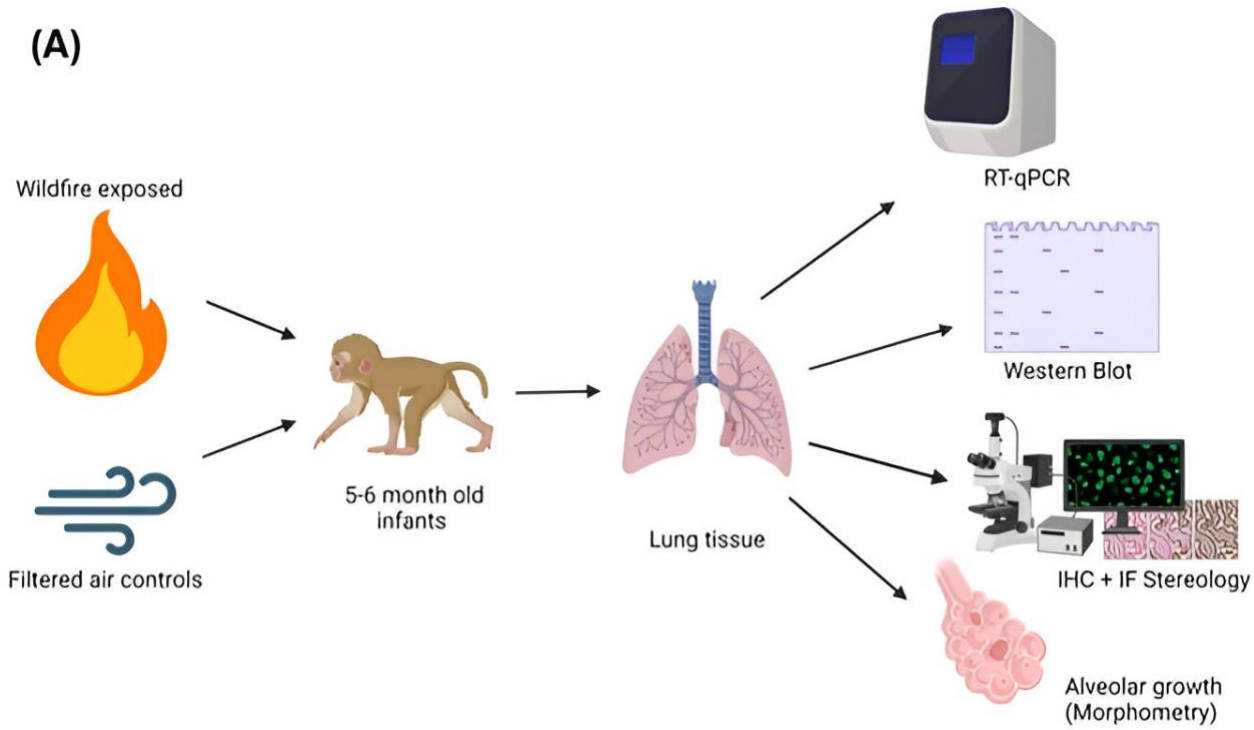

Figure 6: (A) Study design and experimental set up

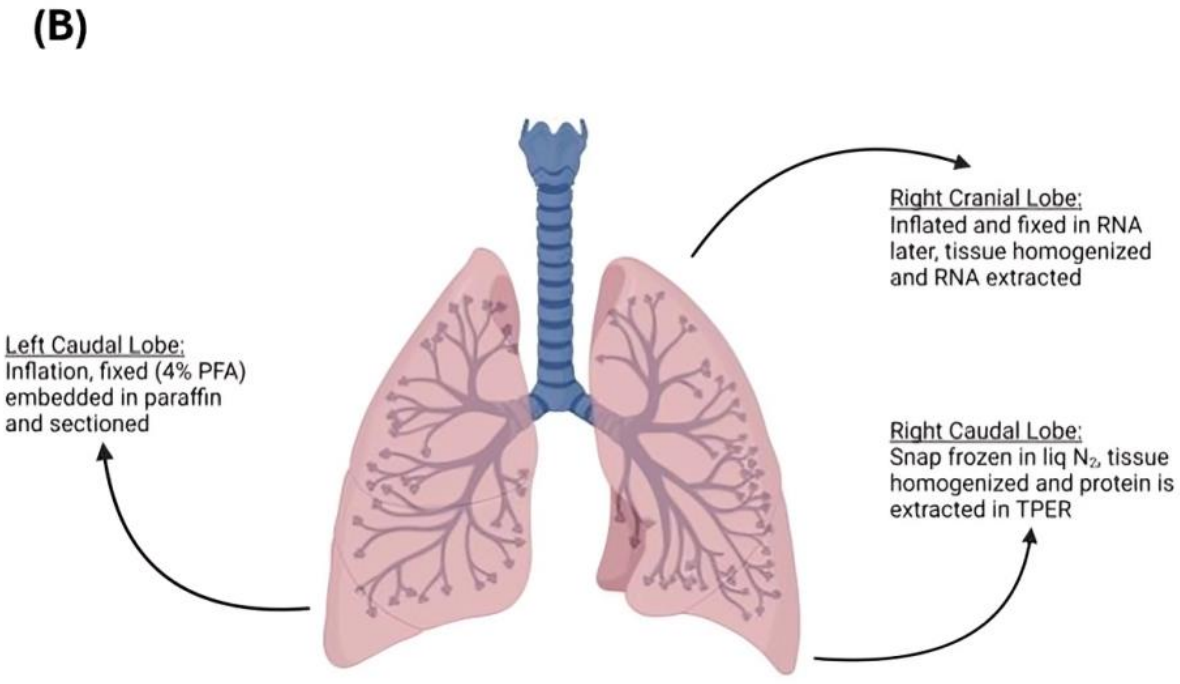

Figure 6: (B) Biospecimens and lung utilization

2.3 Results

The objective of this analysis was to quantify various air pollutants generated during the period of time when the Camp Fire affected air quality in regions that immediately affect the California National Primate Research Center. The comparative analysis of air quality in Davis, specifically at UC Davis, is particularly relevant because it reflects the pollutant concentrations to which the nonhuman primates at the California National Primate Research Center (CNPRC) were exposed while housed outdoors. These animals experienced fluctuations in pollutant levels during the Camp Fire, providing crucial context for interpreting the biological and physiological responses observed in subsequent studies. The end of the Camp Fire period was determined when the blaze reached 100% containment on November 25th, 2018, and air quality returned to safe levels as designated by the EPA (0-100 AQI score).

$PM_{2.5}$ concentrations in this region remained elevated for a period of 12 days during the Camp Fire weeks, with the highest concentration of 184.7 µg/m³ observed on November 15 (≈ 150 µg/m³ increase from federal standard of 35 µg/m³) (Fig. 7A). Ozone levels were elevated (above concentrations during other Camp Fire smoke days) for a period of 4 days during the Camp Fire weeks, with concentrations reaching 0.61 ppm during peak smoke days but did not reach levels higher than national AQ standards (Fig. 7B). NO_2 concentrations were elevated (compared to concentrations seen in the same period during a non-wildfire year) for a period of 10 days during the Camp Fire, but still remained well below federal standards. Concentrations fluctuated between 0.019 and the highest concentrations of 0.39 ppm toward the end of the fire period (Fig. 7C). While the overall temperature did decrease throughout November, during peak smoke days between November 10 and 15, temperatures were steadily elevated by almost 6°C (Fig. 7D). During the same period in 2019 (non-wildfire year), we observe fluctuations in the concentrations of

pollutants, but it does not reach peak hazardous levels. A comparison of the concentrations of the pollutants of interest in a non-wildfire year (2019) (Fig. 8) shows the stark difference in air quality during wildfire seasons and ambient air quality levels in Yolo County (Davis region).

The impact of the Camp Fire's smoke plumes extended to regions as far as the San Francisco Bay Area and the Sacramento Valley. For this analysis, we focused on five major counties within the Sacramento Valley air basin—Yolo, Placer, Butte, Sacramento, and Sutter—affected by the wildfire smoke (Fig. 9). $PM_{2.5}$ concentrations in these regions were all substantially elevated, with the highest concentrations reaching over 400 $\mu g/m^3$ and remaining elevated for almost 11 days (compared to federal standard of 35 $\mu g/m^3$). The highest concentrations of $PM_{2.5}$ were seen in Butte County (the epicenter of the blaze) and Sutter County (Fig. 9A). Ozone levels were elevated (compared to concentrations during non-smoke days) for 5 days across all counties, with the highest concentration being 0.062 ppm. The highest concentrations of ambient ozone were detected in Sacramento and Placer Counties (Fig. 9B). Varying but consistently elevated levels of ambient NO_2 were observed in all the regions of interest during the entire Camp Fire period (compared to concentrations on non-smoke days), with Placer, Butte, and Sutter counties being most heavily impacted by raised NO_2 concentrations. However, these concentrations were also well below federal standards and all the regions showed similar sharp declines in NO_2 concentrations back to normal levels toward the end of the Camp Fire period (Fig. 9C). Unlike air pollutants, temperature was minimally affected by the wildfire smoke and ambient air pollution, as most areas in the Sacramento Valley steadily decreased or did not fluctuate much at all (Sutter County), with a few that showed increased temperatures of 3-4°C for a period of 5 days in the middle of the Camp Fire period, indicating that temperature was less affected by wildfires compared to air pollutants (Fig. 9D).

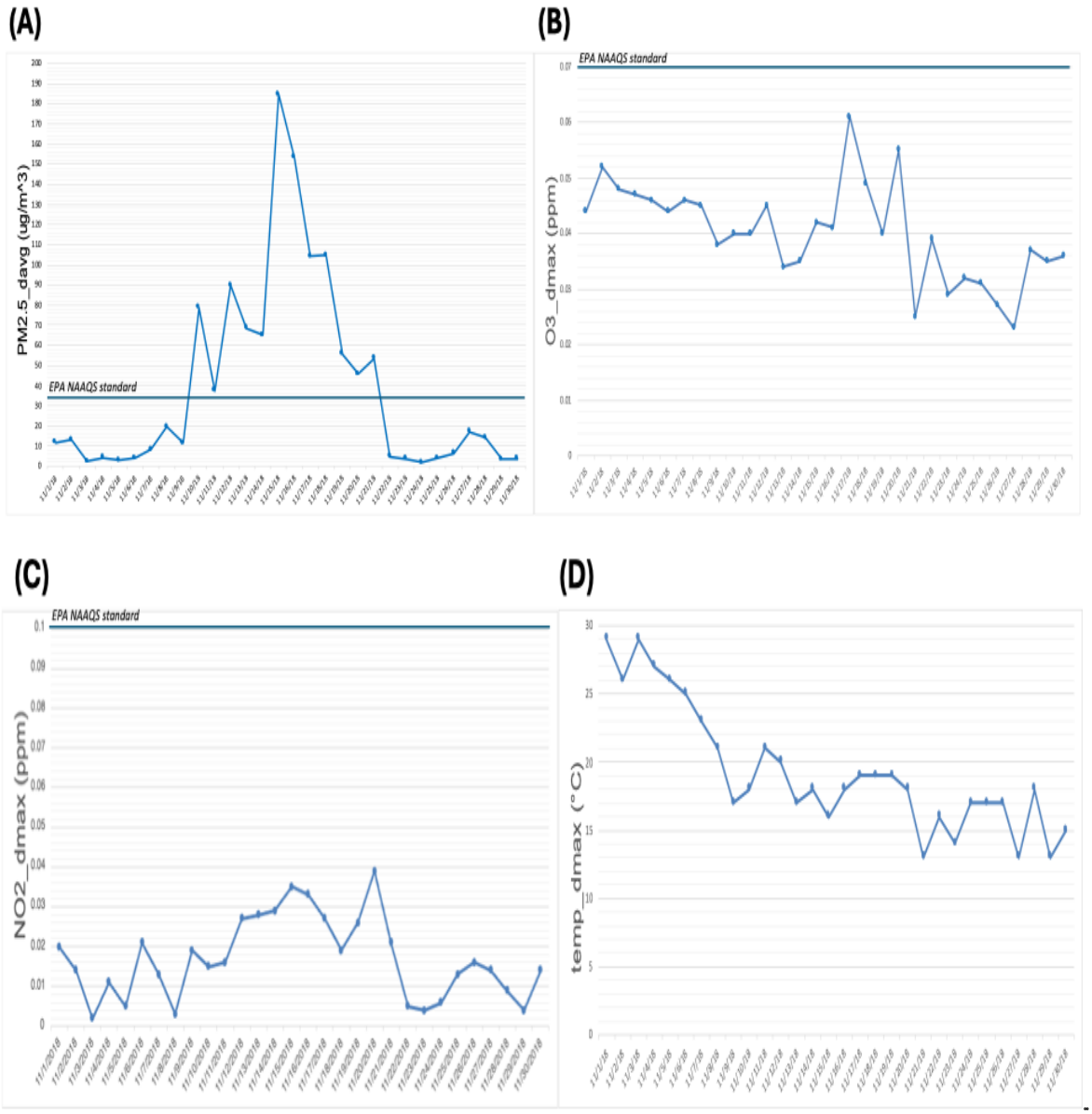

Figure 7: Air quality analysis of (A) $PM_{2.5}$, (B) Ozone, (C)NO_2 and (D) Temperature during November 2018 (Camp Fire: 11/08/2018–- 11/25/2018) in the Davis-UC Davis region in Yolo County.

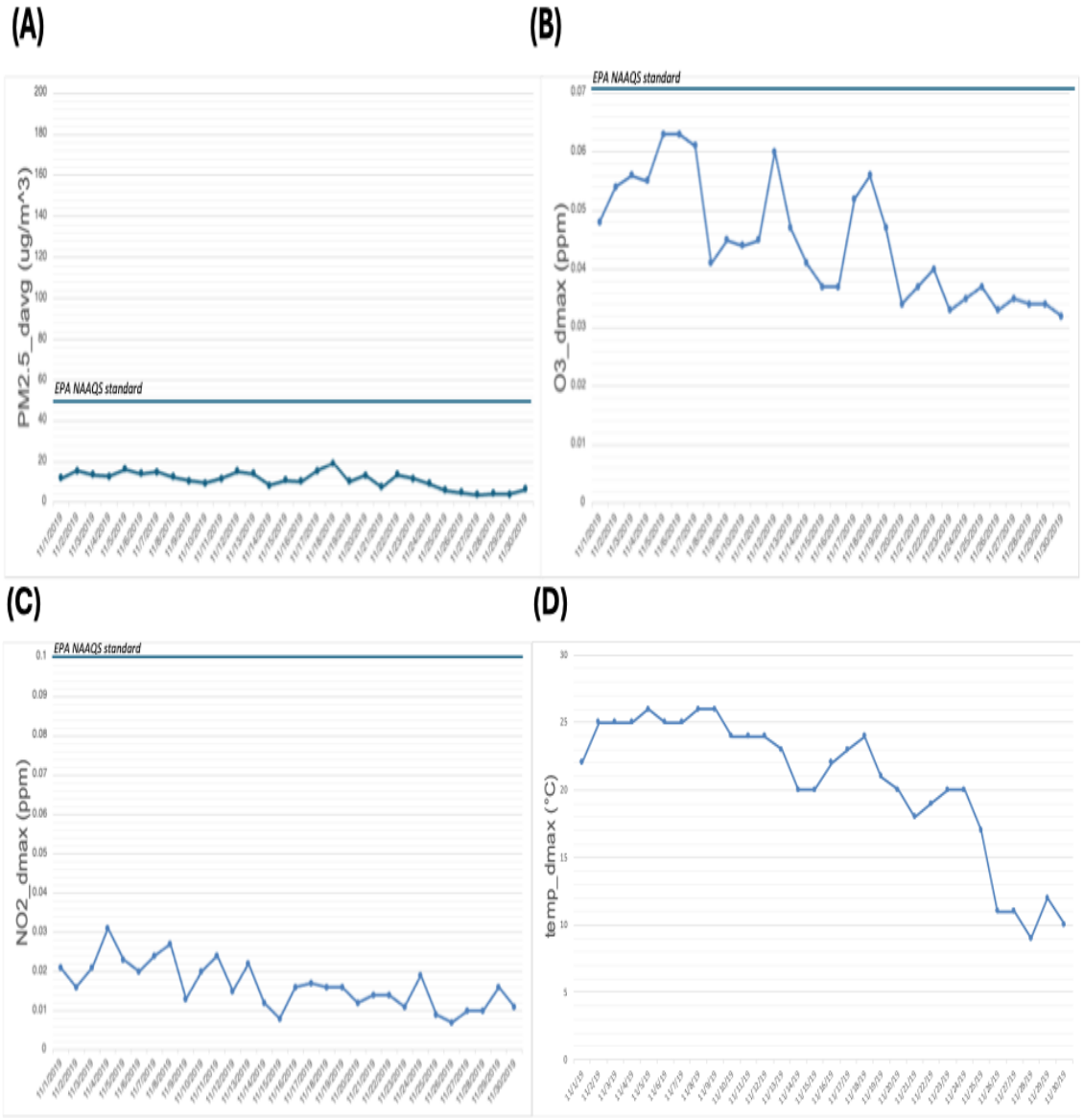

Figure 8: Reference air quality analysis of (A) PM2.5, (B) Ozone, (C) NO_2 and (D) Temperature during November 2019 (non- wildfire period) in the Davis-UC Davis region in Yolo County.

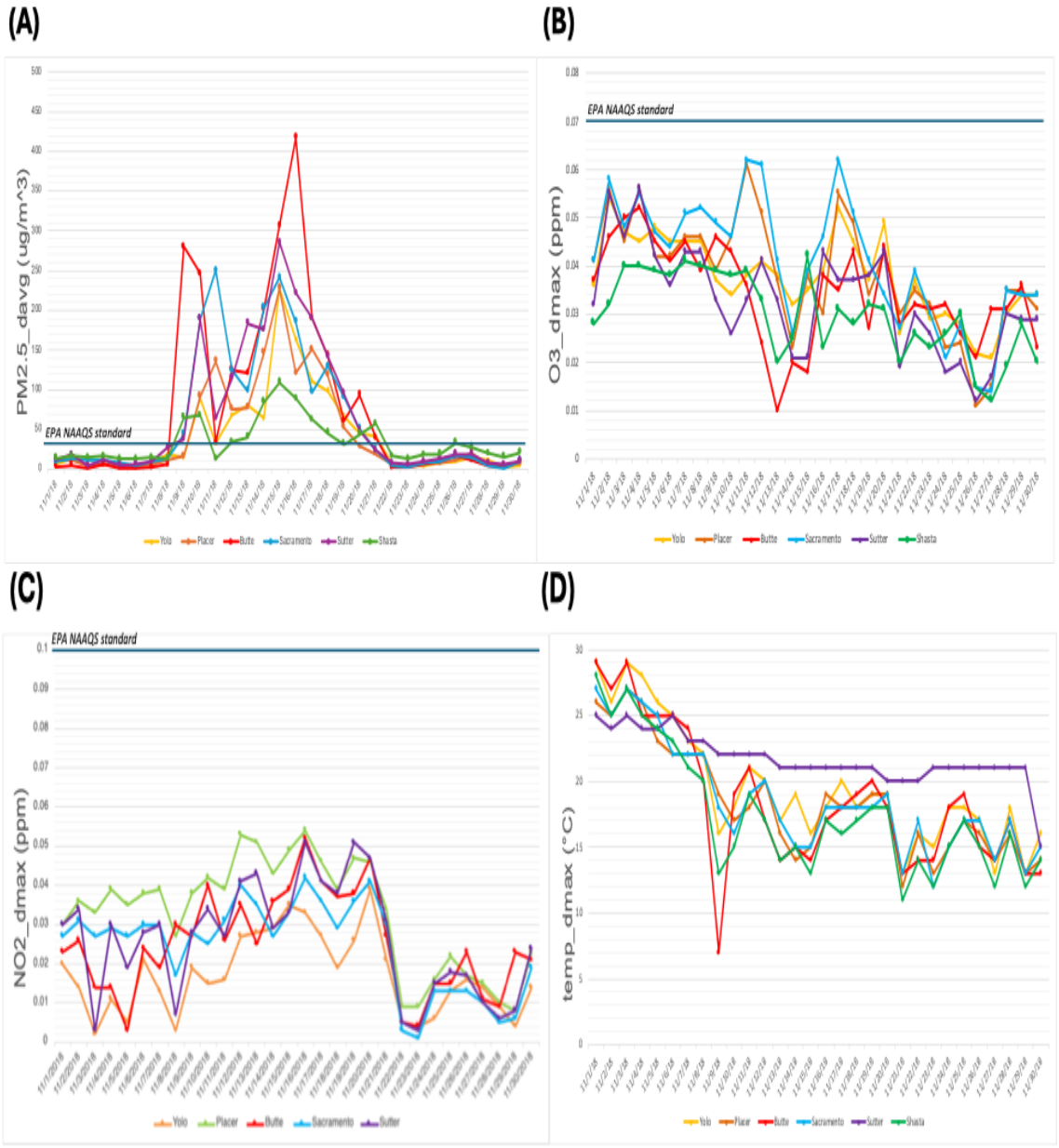

Figure 9: Air quality analysis of (A) $PM_{2.5}$, (B) Ozone, (C) NO_2 and (D) Temperature during November 2018 (Camp Fire: 11/08/2018–- 11/25/2018) in affected counties in the Sacramento valley air basin including Yolo (yellow), Placer (orange), Butte (red), Sacramento (blue), Sutter (purple) and Shasta (green) counties.

2.4 Discussion

The data from this analysis illustrates how the Camp Fire impacted air quality across Davis and the broader Sacramento Valley region. Elevated levels of key pollutants, including $PM_{2.5}$, ozone (O_3), and nitrogen dioxide (NO_2), were observed over an extended period in all major areas analyzed. These findings are particularly relevant when considering the potential long-term health risks posed by wildfire smoke pollutants to populations across California, especially vulnerable groups at higher risk for adverse effects from direct and prolonged exposure to harmful pollutants. (160) One of the key findings from the data analyses was the sustained elevation of $PM_{2.5}$ levels, which remained high for nearly two weeks.

This prolonged exposure to $PM_{2.5}$, which can penetrate deep into the lungs, poses serious respiratory health risks. The peaks observed in ozone and NO_2 levels may further compound these risks, as these pollutants can also exacerbate conditions such as asthma and other respiratory diseases. The data highlights the Camp Fire's widespread impact, showing increases in pollutant levels across multiple counties in the Sacramento Valley air basin. This trend likely reflects a broader pattern of declining air quality across Northern California (161).

Studying non-human primates (NHPs) and their exposure to wildfire events in a single location, with access to air quality data from a nearby monitoring site, offers a unique and controlled approach to understanding the effects of pollution on these animals. By housing the NHPs in a consistent environment with minimal variation in surrounding conditions, researchers can more accurately attribute observed health effects to specific pollutants. This controlled setting also facilitates longitudinal studies, enabling the examination of chronic exposures and the developmental impacts of air pollution as the NHPs age. These findings can then be used for comparative studies, providing predictive insights into potential human health outcomes.

However, translating these findings to human studies presents some challenges due to the complexity and variability of human behaviors and environments. Human studies are limited by the difficulty in conducting controlled experiments and often require the reliance on observational studies, which are more prone to confounding factors. Additionally, humans are exposed to a wide range of environments, making it challenging to measure consistent and accurate long-term exposure. Furthermore, human health is influenced by a complex interplay of factors, including genetics, diet, lifestyle, socioeconomic status, and pre-existing health conditions, which complicates the ability to isolate the specific effects of pollutants (162).

The analysis relied on data from fixed monitoring stations that are generally located in more densely populated areas, which may not fully capture the variability in air quality across fires in different locations. Future research should aim to provide a more comprehensive picture by including a wider range of pollutants, like carbon monoxide (CO), elemental and organic carbons and polycyclic aromatic hydrocarbons (PAHs), more comprehensive pollutant metrics like VOC profiles and employing air quality monitoring techniques that focus on long-term trends and cumulative effects over time.

In summary, the analysis of air quality during the Camp Fire reveals the far-reaching impact of wildfire smoke on ambient air conditions. These findings also emphasize the need for robust public health strategies during wildfire events and the necessity for emergency response plans that include public advisories, access to protective measures (air filtration and masks), health support services, and community engagement.

5. CHAPTER 3: IMPACTS OF WILDFIRE SMOKE ON ALVEOLAR GROWTH

3.1 Background

Postnatal alveolar growth is a fundamental process in lung development, ensuring that the lungs are adequately prepared for the critical task of gas exchange following birth and is characterized by the expansion and growth of the distal lungs and addition of more alveoli (162). In humans, initial alveolar development starts just before birth and continues for the first 6 months of life and terminates around 2 years of age and continued growth and expansion of alveoli occurs from 8 years old and extends into early adulthood (163-165). At birth, the lungs possess a limited number of primitive alveolar structures, known as saccules, which are the precursor to mature alveoli found in the adult lung. The maturation of these saccules into a fully functional alveolar network occurs through alveolarization, which is most active during the early years of life (166). Alveolarization involves the formation of new septa, which divide the existing saccules into smaller, more numerous alveoli, thereby increasing the surface area available for gas exchange. This is crucial to enable the lungs to meet the increased oxygen demands of the growing body (167). Initially, the septa that form within the saccules are immature, characterized by a double-layered capillary network separated by connective tissue. As development progresses, a process called microvascular maturation takes place, where these double-layered networks fuse into a single-layered capillary network within the septa (168). This transition is essential for the structural maturation of the alveoli, ensuring that they are capable of supporting efficient gas exchange (169). However, alveolarization does not stop once the initial phase of septation and capillary maturation is complete. The lungs continue to undergo a secondary phase of alveolarization, known as continued alveolarization, even as they grow. During this phase, new septa continue to form from

pre-existing mature septa that already possess a single-layered capillary network. However, the rate of alveolar formation during continued alveolarization is slower than classical alveolarization that occurs earlier in life (168,169).

In later stages, newly formed septa may sometimes lack a complete capillary network on the alveolar surface. Angiogenesis ensures the alveoli remain well-vascularized and functional. The development of alveolar septa begins with the accumulation of components that create the structural framework needed for septa to rise and divide airspaces, establishing the essential alveolar architecture (170). As the septa mature and capillary networks integrate, the lungs enhance their ability to support the body's oxygen needs. Alveolarization and microvascular maturation are closely linked processes that continue as long as the lungs are growing (171). This ongoing development ensures that the lungs are able to adapt to the body's changing needs, providing sufficient surface area for gas exchange even as the body grows. Any disruptions to this process of alveolarization during these critical periods in early life could have detrimental effects on long term lung function trajectories and overall health (172).

Exposure to air pollution can disrupt this delicate process. Air pollutants, such as particulate matter (PM), ozone (O_3), and nitrogen dioxide (NO_2), can interfere with the signaling pathways that regulate these processes of postnatal lung development. These pollutants induce oxidative stress and inflammation, which can inhibit the normal process of alveolarization (173). As a result, septa may form incompletely or abnormally, reducing the overall number of alveoli and diminishing the surface area available for gas exchange and impair the lungs' ability to efficiently oxygenate blood. In the long term, these disruptions can lead to chronic respiratory issues, such as asthma and reduced lung function, highlighting the importance of protecting the developing lungs from harmful environmental exposures (175).

Overall, postnatal development and alveolar growth are dynamic and continuous processes, crucial for the maturation and functionality of the lungs. From the formation of septa and double-layered capillary network to alveolarization, each step has a vital role to ensure the lungs can meet the demands of the body. The efficiency and effectiveness of these processes are essential for proper lung function and ensuring the body receives the oxygen it needs to thrive. Any disruption in alveolarization or microvascular maturation could have profound implications for respiratory function later in life. Therefore, ensuring the proper development of alveolar structures is crucial for respiratory function and maintenance of long-term lung health.

The hypothesis of this study is that early life exposure to wildfire smoke is associated with altered alveolar growth and development in the distal lung of infant rhesus macaques. This hypothesis is based on the known vulnerability of infants to environmental hazards and previous studies that have shown that exposure to air pollutants (ozone) can cause structural alterations to the distal airways in the developing lungs of rhesus macaques (145-147). Thus, we propose that wildfire air pollutants could similarly affect alveolar growth and lung development. To test this hypothesis, we used a morphometric approach to measure alveoli in lung tissue sections obtained from monkeys that had been exposed to wildfire smoke at infancy. Morphometry focuses on measuring and analyzing the shapes, sizes, and spatial relationships of structures in the tissue in two dimensions. Sampling from various fields across the tissue sections allows for the collection of representative data from the whole lobe, enabling quantification of the proportion of lung volume occupied by airspaces versus tissue in a non-biased fashion. Morphometric analysis of the alveoli also identifies structural variations, which may reflect developmental alterations due to pollutant exposure. This approach can help inform comprehensive frameworks for assessing the effects of wildfire smoke on alveolar development.

3.2 Materials and Methods

Morphometry

To analyze alveolar structures, one paraffin-embedded lung block was selected for each wildfire smoke exposed animal (n=4) or indoor housed animal (n=4) from distal locations in the lung lobe primarily containing respiratory bronchioles, terminal bronchioles, and alveoli. These animals were exposed to variable concentrations of wildfire air pollutants as described in chapter 2 which included $PM_{2.5}$, ozone, and nitrogen dioxide. Duplicate sections were prepared, and the slides were stained with H&E to visualize the alveoli. Brightfield images are captured using Leica Microsystems DMS 7000 T fluorescent microscope at 200x magnification. Sampling is conducted from randomly selected fields to ensure an accurate representation of alveolar structures (n=20 per section) (180-183).

Lung morphometric analysis was performed using AlveolEye, a recently developed AI-assisted software (178). This tool integrates artificial intelligence, computer vision, and classical image processing to improve the accuracy and reliability of lung morphometry from histological images. It calculates metrics like mean linear intercept (MLI) and airspace volume density (ASVD). MLI estimates alveolar size through systematic serial measurements of the alveolar epithelium using a testing grid overlay. ASVD quantifies the proportion of lung volume occupied by airspaces versus tissue structures, using a grid point counting technique that overlays an evenly spaced lattice of random points on images containing both airspaces and tissue (176,177). Images were processed using AlveolEye, which automatically determines the optimal threshold by Otsu's method (optimizes the separation of tissue structures from the background by minimizing the intra-class variance of pixel intensities) (181). Images undergo post-processing, and the chord length framework is applied, (275 chord lines–highest accuracy with AUC_{ROC} score >0.8) and MLI and

ASVD are calculated (180). These values are averaged across the sampling pool for each section. To ensure accuracy, analysis was performed in duplicate with consistent assessment settings across images. Statistical analysis was performed using JMP software.

3.3 Results

H&E-stained lung slides from wildfire exposed and indoor housed animals were imaged to identify alveolar structures in the lung epithelium. Fields of view from the stained alveoli that were sampled and imaged across the section (n=20/animal) were compiled and imported to the AI assisted morphometric analysis software, AlveolEye. Morphometric parameters of alveolar size and air space volume was determined by the software. MLI and ASVD were calculated by AlveolEye using sampled images from distal lung sections stained with H&E and were used to identify if the exposure to wildfire caused structural changes in the alveoli. The morphometric analysis revealed no significant structural alterations in the alveoli of infants exposed to wildfire smoke compared to those housed indoors. The mean linear intercept (MLI) calculated from the alveoli of wildfire smoke-exposed animals were not significantly different from those in the indoor housed group (Fig. 10A). While there was a slight increase in the average size of alveoli in the wildfire-exposed group, this difference can be attributed to the presence of a single outlier (highest value in wildfire-exposed group) rather than a consistent effect of wildfire exposure. The analysis of air space volume density (ASVD) also did not show any significant differences between wildfire smoke-exposed and indoor housed animals (Fig.10B). The proportion of airspace volumes in the alveoli was consistent across both groups, with no statistically significant changes observed.

.

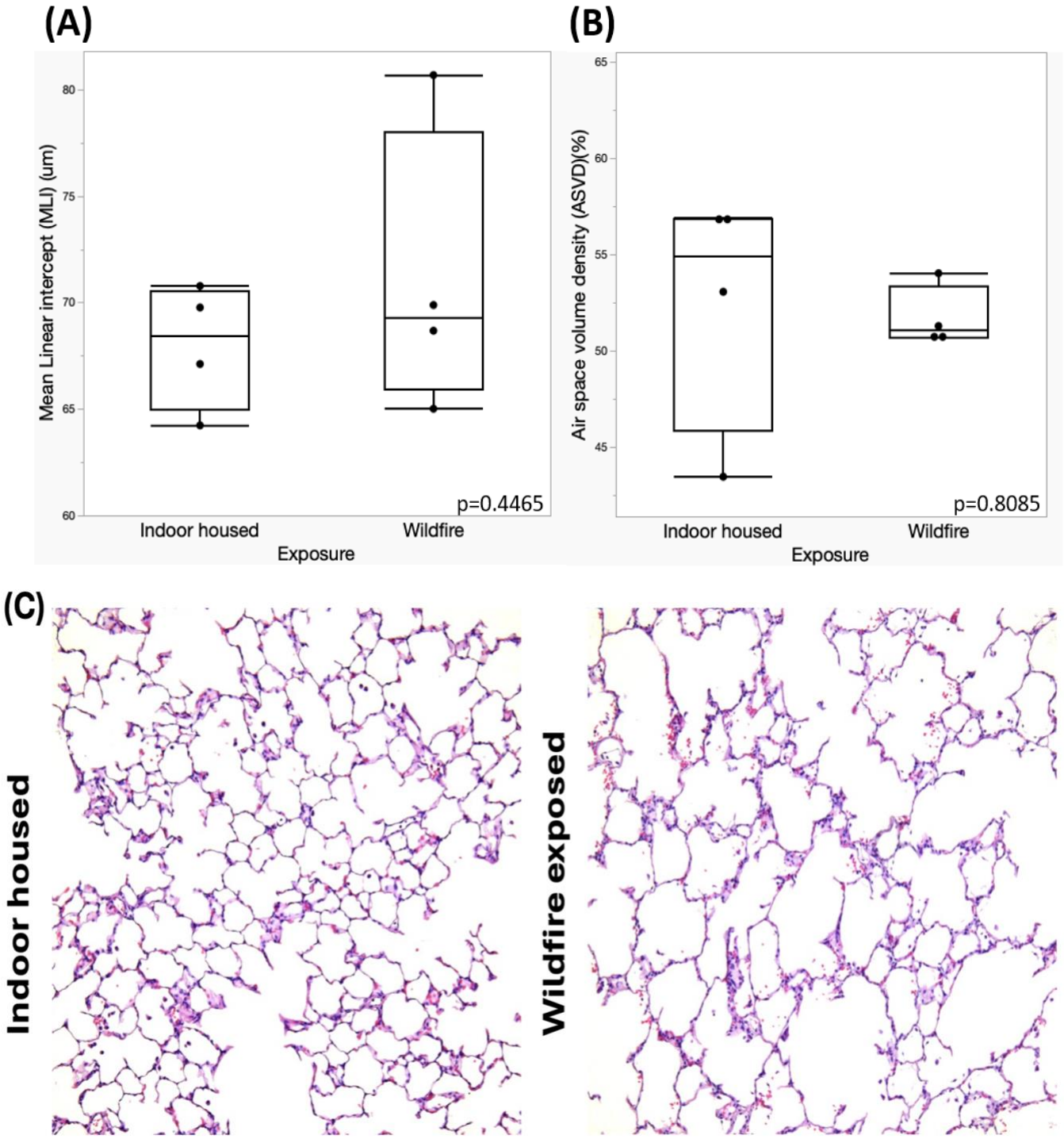

Figure 10: Lung morphometric analysis of (A) mean linear intercept (um) of alveoli and (B) air space volume density (%) of alveolar epithelium of the distal left caudal lobes of infant rhesus macaques post short-term wildfire exposure. (C) Representative images of H&E-stained alveolar epithelium in indoor housed and wildfire-exposed lungs (100x)

3.4 Discussion

The study aimed to assess the impact of short-term wildfire smoke exposure on alveolar growth in the infant rhesus macaque lung. Using AI-assisted morphometric analysis software, AlveolEye, this study used key histological parameters like mean linear intercept (MLI) and airspace volume density (ASVD) to determine if wildfire smoke exposure caused any structural alterations in the alveoli.

The findings revealed no significant differences between wildfire smoke-exposed and indoor housed groups in terms of alveolar structure. Both MLI and ASVD measurements did not show statistically significant changes (MLI $p= 0.4465$, ASVD $p= 0.8085$). This suggests that short-term exposure to wildfire smoke does not result in measurable morphological alterations in alveolar structures, as modeled in this study. This is an important finding, as it indicates that the structural integrity of the alveolar epithelium remains intact despite exposure to wildfire smoke. However, the study's results do not rule out the possibility of other non-structural effects or the potential for structural damage under conditions of prolonged or more intense exposure.

Researchers have employed various methods to quantify alveoli and assess the effects of pollutants on lung development and function. Traditional techniques include stereological sampling, morphometric analysis, and microscopy. Studies on murine models, have used methods such as grid line intersection counting, Cavalieri's principle, point counting, and stereological sampling to show septal thickening, decreased total alveolar air space volume, and a reduction in alveolar numbers after exposure to pollutants like ozone and $PM_{2.5}$ (183-186). In non-human primates, methods like stratified sampling, the disector and fractionator techniques, and point intersect counting have identified structural alterations, including larger-sized alveoli and fewer non-alveolarized airway generations, in the lungs of these animals, suggesting impaired distal

airway development (187-190). Similarly, morphometric analysis of human lung tissue has revealed significant structural damage following air pollution exposure. These findings highlight the critical role of these quantification methods in understanding the impacts of environmental pollutants on lung development and function (191,192).

In contrast, our approach using AlveolEye software offers a novel method for alveolar quantification. While traditional stereological techniques are reliable, they require manual sampling and counting, which can be time-consuming and prone to human error. AlveolEye utilizes AI-driven algorithms to analyze lung tissue samples, enhancing the efficiency, accuracy, and consistency of morphometric measurements. The software integrates automated grid placement with the open-source multidimensional image viewer plugin, napari, enabling advanced visualization and analysis. By automating these processes, AlveolEye reduces human error, increases consistency, and allows for the analysis of larger datasets. However, these advantages do not necessarily mean that this method is superior to traditional approaches. Extensive further studies are needed to compare and validate these findings. Therefore, this outcome should be considered a preliminary finding rather than a definitive conclusion, highlighting the need for ongoing research and validation.

This study allows us to understand the impacts of wildfire smoke exposure on distal lung development, however there are a few limitations that need to be addressed. Firstly, our model does not account for the inherent differences in animal and lung size, and the overall variability in developmental rates typically observed in an outbred population. Additionally, we focused solely on the effects of very short-term exposure during a narrow developmental window. This may have resulted in less pronounced effects compared to the effects of prolonged exposures over a more extended developmental period. Such a limited timeframe may not fully capture the potential long-

term consequences of air pollution on lung development. We have also only used one representative distal lung section for this analysis and have not sampled throughout the entire lung. Thus, it might not represent the heterogeneity of structures and pathology present in all the regions of the lung. Our AI-assisted software, AlveolEye, was trained primarily on mouse lungs, which may limit its accuracy in estimating morphometric features in non-human primate lungs.

Alveolar development is crucial for effective lung function, and any disruptions during this critical period could have lasting impacts on respiratory health (173). While this study did not find significant structural changes following short-term wildfire smoke exposure using novel sampling approach, future research should focus on exploring the effects of prolonged wildfire smoke exposure across different stages of lung development to better understand the potential long-term impacts on alveolarization and overall lung function. Understanding these risks is essential for developing strategies to protect vulnerable populations such as infants and reduce the long-term burden of respiratory diseases.

7. CHAPTER 4: IMPACTS OF WILDFIRE SMOKE ON WNT PATHWAY SIGNALING MOLECULES

4.1 Background

Lung development, particularly the formation and maturation of alveoli, involves a complex interplay of multiple signaling pathways that coordinate the growth, differentiation, and interaction of various cell types within the alveolar microenvironment. This dynamic network of signaling pathways include SHH (Sonic Hedgehog) pathway that influences the structural functions of mesenchymal cells, Notch signaling that coordinates differentiation of alveolar epithelial cells, TGF- β signaling that modulates extracellular matrix (ECM) production of mesenchymal cells like fibroblasts and the FGF pathway plays a role in endothelial cell proliferation and migration (129-133). The WNT signaling pathway plays a role in epithelial proliferation and differentiation, epithelial-mesenchymal crosstalk and septation of alveoli (128). Together the interactions of these pathways ensures the proper development of the alveoli and support their capacity to effectively respond to injury from environmental pollutants.

The WNT pathway is essential for various developmental processes in the lung, including cardiopulmonary specification, branching morphogenesis, lung axis formation, and regulation of cell fate determination. It promotes distal lung fates while suppressing proximal ones. It also plays a critical role in epithelial-mesenchymal crosstalk, coordinating growth and differentiation during lung development and is involved in generating spatially, temporally, and regionally distinct cell populations and maintaining stem and progenitor cell populations (193, 194). These cells serve as a reservoir, capable of differentiating into various lung cell types during development and repair. The most prominent role of the WNT pathway during lung development is in the distal lung, particularly in distal lung morphogenesis and bud formation (196). WNT ligands are crucial during

the alveolarization stage, where they contribute to alveologenesis and the regulation, maintenance, and proliferation of alveolar epithelial cells. WNT signaling is vital for maintaining and expanding alveolar progenitor cells, promoting AT2 cell proliferation, and supporting their differentiation into AT1 cells (197).

WNT Signaling Pathway Overview

The WNT signaling pathways play crucial roles in regulating cell fate, migration, proliferation, polarity, organogenesis, and tissue homeostasis. The pathways involve 19 WNT glycoproteins and 10 Frizzled (Fzd) receptors and are divided into two categories: the canonical β-catenin-dependent pathway and the non-canonical β-catenin-independent pathway (184). The canonical pathway, activated by WNT1 class ligands (WNT2, WNT3, WNT3a, WNT8a), induces cell proliferation, differentiation, and maturation by transporting β-catenin to the nucleus. The non-canonical pathway, activated by WNT5a-type ligands (WNT4, WNT5a, WNT5b, WNT7a, WNT11), regulates cellular polarization and migration and modulates canonical signaling. Non-canonical signaling includes the planar cell polarity (PCP) and calcium (Ca^{2+}) pathways (193).

Canonical - β-catenin Dependent Pathway

Canonical signaling is activated when WNT ligands bind to Fzd and LRP5/6 receptors on the cell membrane. This binding recruits a Disheveled (Dvl) protein, which phosphorylates the LRP protein, causing its binding to Axin protein. With Axin bound to LRP, it becomes unavailable to form, a multiprotein destruction complex comprising Axin, serine/threonine kinase glycogen synthase kinase 3 (GSK-3), casein kinase 1 (CK1), the E3-ubiquitin ligase β-TrCP, and protein phosphatase 2A (PP2A). The absence of this destruction complex allows β-catenin to accumulate in the cytoplasm, which then translocates to the nucleus to transcribe WNT target genes. Without WNT ligand binding, Axin cannot bind to LRP and thus becomes available to form the β-catenin

destruction complex, which ubiquitinates β-catenin and leads to its cytoplasmic degradation and transcriptional repression of downstream WNT target genes (194,195). (Fig. 11)

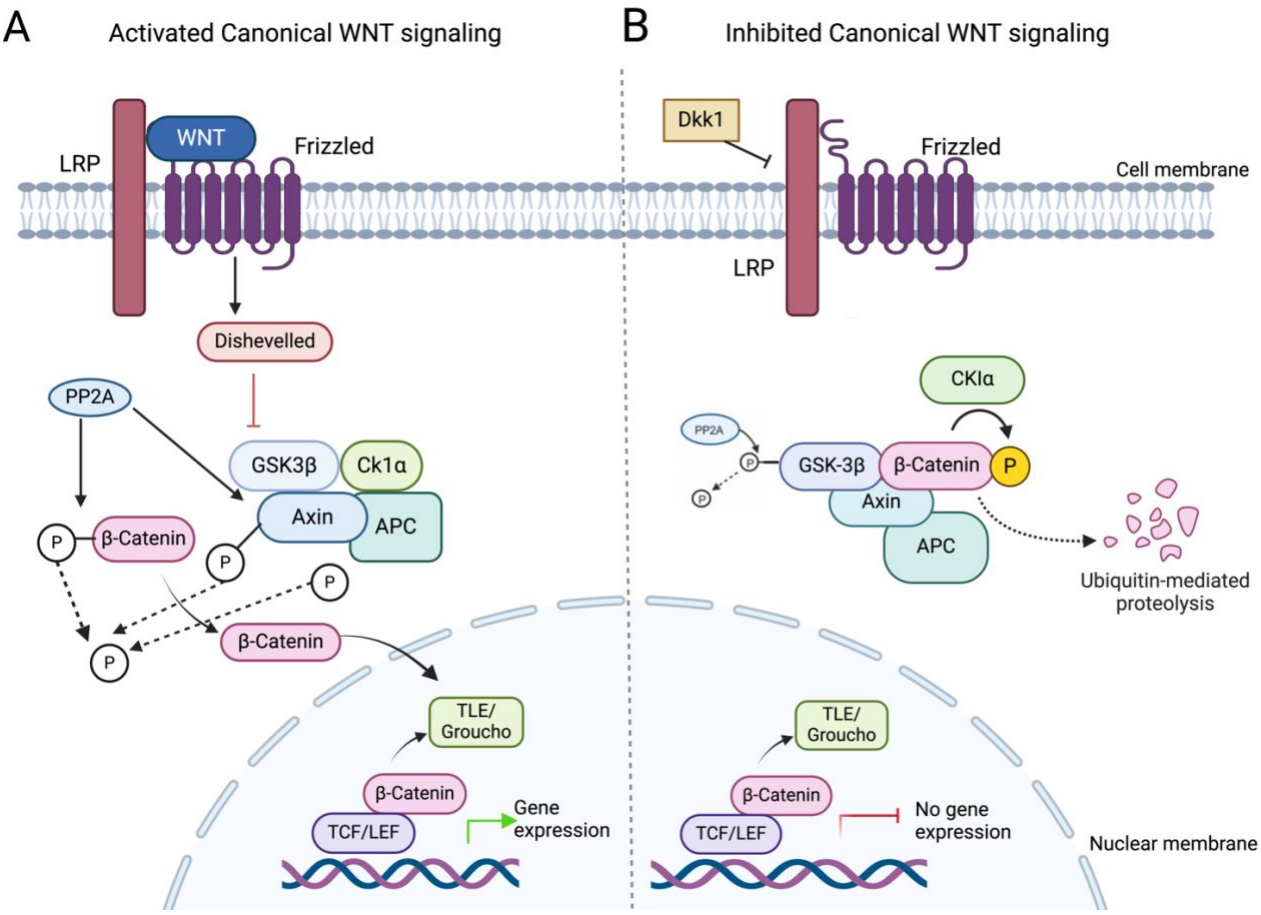

Figure 11: (A) Activation and (B) inhibition of the canonical WNT signaling cascade (*Created with BioRender template)*

Non-canonical – β-catenin independent pathways: PCP and Ca^{2+} pathways.

The WNT PCP pathway is activated by the binding of WNT ligands to the Fzd receptor, which phosphorylates Dvl, leading to the recruitment of Inversin, Par6 (polarity protein), and Smad ubiquitination regulatory factor (Smurf). These proteins ubiquitinate Prickle, a protein that normally inhibits WNT/PCP signaling. Activation of Rac1, RhoA, and Profilin can occur without the presence of Prickle, triggering the activation of c-Jun via JNK. The translocation of c-Jun to the nucleus initiates gene transcription. Activated Profilin and RhoA contribute to actin polymerization, which is vital for cell polarity and migration (197,198). (Fig. 12)

The WNT/Ca^{2+} pathway is primarily activated by WNT5a ligand binding to the Fzd2 receptor. This interaction stimulates the release of Ca^{2+} from the endoplasmic reticulum into the cytoplasm. The subsequent increase in cytoplasmic Ca^{2+} levels activates nuclear factor of activated T cells (NFAT), leading to the transcription of WNT-targeted genes (196).

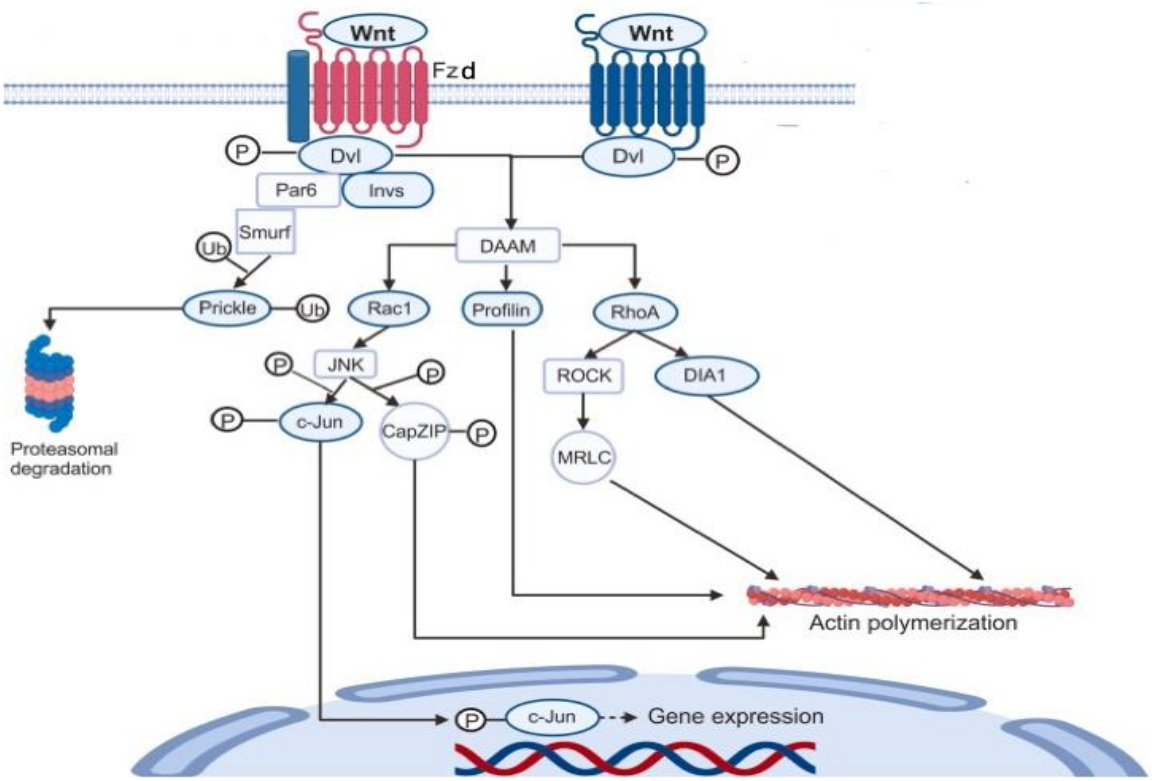

Figure 12: Activation of non-canonical WNT signaling cascade: PCP Cascade (*Created with BioRender templates)*

In this study, we focused on WNT3a and WNT11, to investigate if wildfire smoke exposure altered their expression in the developing lung. The rationale for selecting these target ligands stems from previous research indicating that these signaling molecules are sensitive to dysregulation in response to air pollutant exposure. Previous transcriptomic analyses and studies conducted by our research group have shown that WNT3a (canonical molecule) and WNT11 (non-canonical molecule) were dysregulated in response to cyclic ozone exposure during early life (148). By examining these same ligands, we aimed to determine whether similar disruptions occur following wildfire smoke exposure.

4.2 Materials and Methods

Immunohistochemistry (IHC)

Left caudal lung lobes (distal lung) from 5–6-month-old wildfire smoke-exposed (n=4) or indoor housed (n=4) animals were embedded in paraffin and sectioned into 0.5-micron slides. Slides underwent deparaffinization and rehydration prior to immunostaining. The ImmunoCruz rabbit ABC staining system (sc-2018, Santa Cruz Biotechnology) was used following the manufacturer's instructions. Staining was optimized for minimal background by treating with 1% peroxidase solution, blocking with 3% blocking serum, and applying 30 seconds of DAB chromogen staining. Tissue sections were incubated with the primary antibody. A biotinylated secondary antibody was then added, followed by ABC reagent. The target protein expression was visualized by incubation in peroxidase substrate. Both polyclonal rabbit WNT3a and WNT11 primary antibody (GeneTex) were used at a 1:100 dilution. Slides were visualized under brightfield using the Leica Microsystems DMS 7000 T microscope.

Immunofluorescence (IF)

To perform immunofluorescence staining, 5μm paraffin slides from the left caudal lung lobes (distal lung) of wildfire smoke-exposed (n=4) and indoor housed (n=4) animals were used. Slides were deparaffinized, rehydrated, and underwent heat-induced target retrieval (DAKO antigen retrieval solution, Agilent). Slides were blocked with 5% goat serum in 1x PBS with 0.2% Triton. Following blocking, the slides were incubated with primary antibodies for WNT11 (1:100 GeneTex) and WNT3a (1:100 GeneTex). These antibodies were detected by fluorescently tagged goat anti-rabbit secondary antibodies used at a 1:1000 dilution (Invitrogen AF647, AF488). Sections were also treated with DAPI (1μg/ml) as a nuclear stain. Expression of targets was visualized with the Leica Microsystems DMS 7000 T fluorescent microscope.

Stereological Sampling

The relative expression of these targets is semi-quantified using stereological sampling and image analysis. Stereological sampling uses 2D measurements to provide information about 3D structures by consistent sampling, allowing for conclusions from small numbers of instances. This approach allows for histopathological evaluations of test effects at the tissue level (202).

Fields of view (FOV) from immunofluorescent stained distal lung sections of left caudal lung lobes from animals exposed to either wildfire (n=4) or housed indoors (n=4), targeting WNT11 and WNT3a (refer to the IF section), were used for sampling. Systematic uniform random sampling (SURS) was performed to ensure that every structure/instance of tissue architecture in a single section or FOV had an equal chance of being sampled (203-205). Sampling parameters are defined as follows:

- **Starting Field 'm' (m=5):** Random starting field that is 'm' fields away from the landing FOV (first FOV in the sampling frame). 'm' should be between 1 and 'p'.
- **Sampling Period 'p' or Periodicity (p=6):** Number of fixed frames in a defined region consistently repeated 'p' times across the section, with every pth FOV being sampled.
- **Fraction of Sampling Frame 'f' (f=1/6):** Fraction of the sampling period, where f=1/p.
- **Sampling Size 's' (s=10):** Number of FOVs captured as part of the sampled set (205).

SURS begins by reaching the landing field, then moving the sampling frame 'm' times to reach the starting field. From the mth FOV, sampling starts by moving the sampling frame 'p' times and capturing every pth FOV until the sample size 's' is reached (206). However, this method has limitations, including the absence of SURS for section slicing or selection, potential inconsistencies in the sampling frame due to manual sampling without an automated fractionator, and the assumption of uniform distribution of observed changes across the specimen (206).

Image analysis was performed using ImageJ's threshold analysis to semi-quantify the expression of the targets of interest in the sampled images. ImageJ applies global thresholding to measure the fluorescent intensity of stained ligands, representing target expression. The thresholding process segments the image, distinguishing the features of interest (fluorescent intensity) as the foreground and the rest as background (207). A cutoff value or threshold is set to classify pixels below it as background and those above it as foreground. The thresholds for WNT11 (200 pixels in the foreground) and WNT3a (165 pixels in the foreground) were set using positive and negative controls for each target. Each foreground pixel is assigned a binary value and measured using relevant analysis parameters (208). Fluorescent intensity is normalized to the nuclear stain DAPI to account for cell density variations, ensuring comparability across different samples. Statistical significance was determined using GraphPad Prism software.

Western Blotting

Proteins extracted from right caudal lung lobes were analyzed for WNT11 and WNT3a expression in comparison to age-matched controls. Protein samples were prepared by adding 4x Laemmli sample buffer (Bio-Rad) and 1M DTT (dithiothreitol) as a reducing agent. Protein samples were heated at 95°C for 10 minutes before being resolved on 10% MiniPROTEAN TGX Precast Protein Gels (Bio-Rad) at 45V for 20 minutes, followed by 70-90V for 1-1.5 hours. The transfer was done using the Bio-Rad Trans-Blot Turbo transfer system. The gel was placed between ion reservoir stacks provided with the Trans-Blot Turbo Mini PVDF Transfer Packs. A pre-programmed transfer protocol was run on the system for 3 minutes. The stack was disassembled, and the protein blot was washed with Tris buffer solution containing 0.1% Tween 20 (TBST).

The blots were blocked with EveryBlot blocking buffer (Bio-Rad) for 5 minutes while rocking at room temperature. Blots were incubated overnight at 4°C with rocking in either polyclonal rabbit WNT11 primary antibody (1:1000, GeneTex) or polyclonal rabbit WNT3a antibody (1:1000, GeneTex). The membrane was washed and then incubated for 1 hour while rocking at room temperature with anti-rabbit IgG secondary antibody (1:1000). The blot was washed and incubated with HRP-luminol working solution for enhanced chemiluminescence. Bands were visualized using the Amersham ImageQuant 500 imaging system (Cytiva). ImageJ software was used to perform densitometry and quantify the relative density (mean gray value) of protein bands on the Western blot. Statistical significance was determined using a t-test with JMP software.

RT-qPCR

Lung tissue (right cranial lobes) from wildfire-exposed or indoor housed animals that were stored in RNAlater was used to perform RT-qPCR. Tissues were homogenized in Trizol, and RNA was extracted from the homogenized tissues using the DirectZol RNA Miniprep Plus Kit (Zymo Research). cDNA was then prepared using a high-capacity cDNA reverse transcription kit (Applied Biosystems). Each sample was plated in duplicate to perform qPCR on a 96-well plate. Twenty microliter reactions were made using 2µL of undiluted cDNA and 18µL of master mix containing 1µL of primer probe. All primers were TaqMan probes from Thermo Fisher Scientific (WNT11 - Assay ID: Rh01045904_m1; WNT3a - Assay ID: Rh02851012_m1). The plate was run for 45 cycles. Relative gene expression was calculated using the average cycle (Ct) value of the duplicates and was normalized to GAPDH control. Results were reported as fold change 2^(-ΔCT). Statistical analysis was performed using GraphPad Prism software.

4.3 Results

Early life wildfire smoke exposure is associated with dysregulated expression of WNT signaling proteins in the lung

Western blots were performed using whole lung homogenate to determine if the expression of WNT target proteins was altered in wildfire exposed animals compared to the indoor housed control animals. Our results showed that the expression of non-canonical WNT11 (39kDa) was significantly increased in wildfire-exposed animals compared to those housed indoors. While not statistically significant, there was a decreased expression of canonical WNT3a (38kDa) in wildfire smoke-exposed animals relative to the indoor housed controls. These findings follow a similar trend in target expression observed in animals exposed to ozone. (149). (Fig. 13)

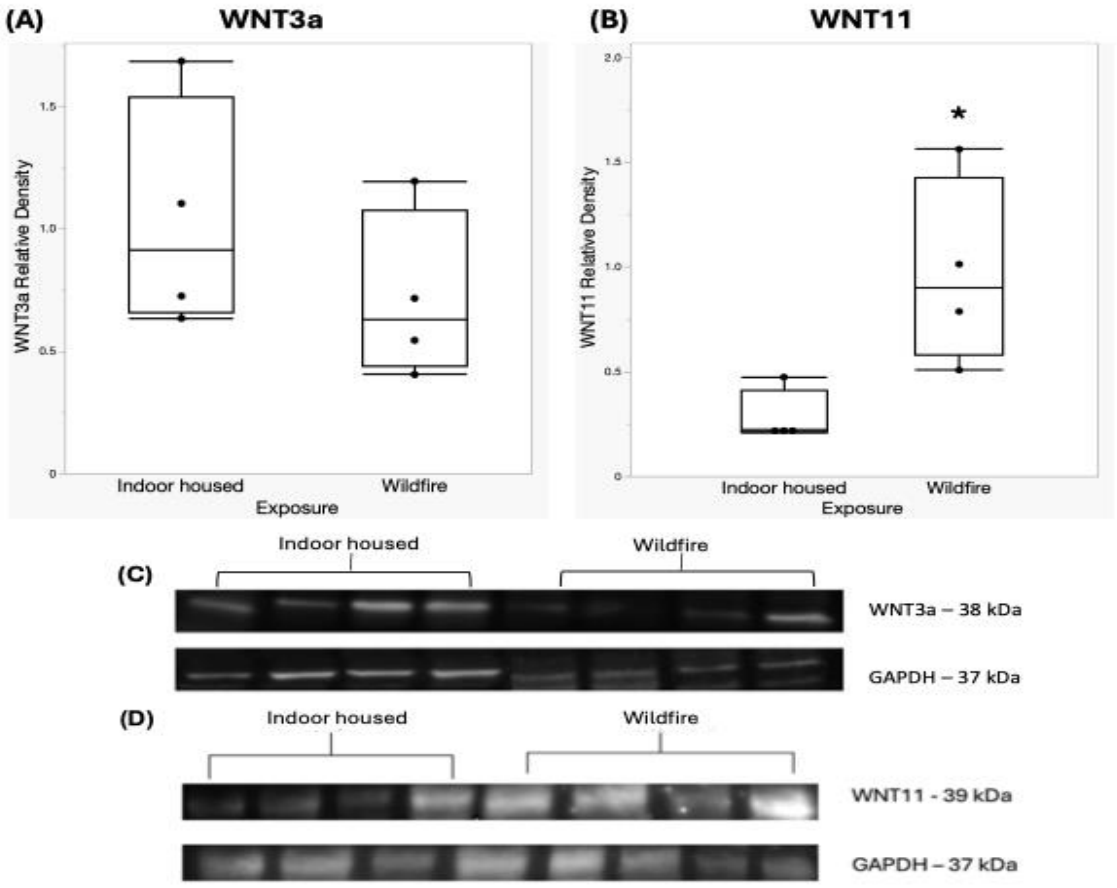

*Figure 13: Relative density of (A) WNT3a and (B)WNT11 in indoor housed controls (n=4) and wildfire exposed animals (n=4), * $p < 0.05$. (C),(D) Representative western blot images for WNT3a and WNT11 resp.*

Early life wildfire smoke exposure is associated with the expression of WNT ligands in the distal lung

Immunohistochemical staining was performed to visually compare the expression and observe the differences in the relative distribution of these targets in the distal lung epithelium of wildfire-exposed and indoor housed animals. Our results showed similar expression as seen in overall protein. Wildfire-exposed animals showed visibly less immunostaining of canonical WNT3a, indicated by the decreased intensity of the brown staining across the tissue compared to the broader staining patterns seen in the alveoli of indoor housed animals. The intensity of immunostaining for non-canonical WNT11, showed in the lung tissue post-wildfire exposure, indicated by the increased intensity of brown staining seen in wildfire-exposed lung tissue compared to the minimal staining intensity seen in indoor housed lung tissue. (Fig. 14)

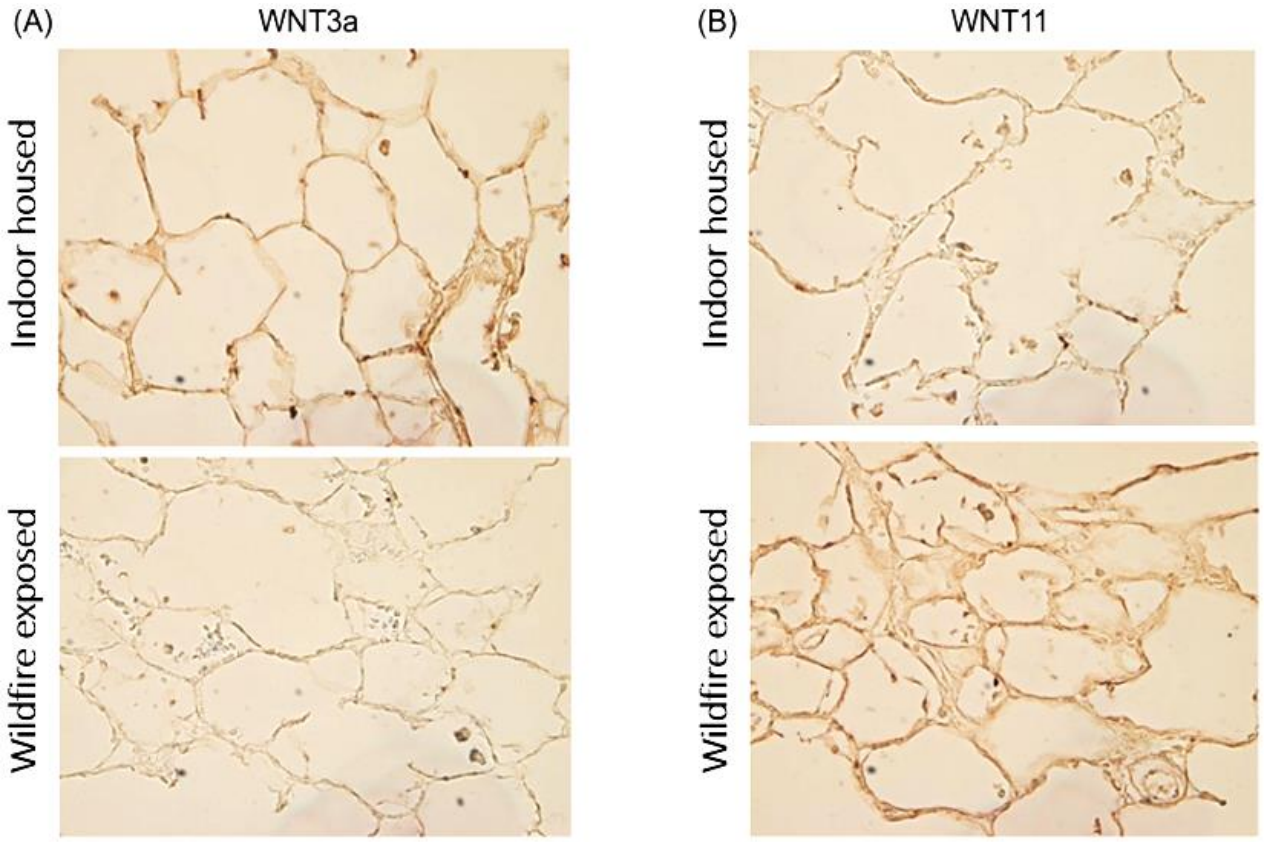

Figure 14:Distribution of (A) WNT3a and (B) WNT11 in the alveolar epithelium of the left caudal lung lobes of indoor housed animals and wildfire exposed animals (Brightfield – 400x)

To investigate the expression of these WNT targets in the lung tissues, immunofluorescence staining was performed on distal lung sections targeting WNT11 and WNT3a. The expression of these targets was measured using the relative fluorescent intensity of these proteins in the sections after being exposed to wildfire smoke or housed indoors. Our results indicate that wildfire exposure caused decreases in overall canonical WNT3a expression across the tissue, indicated by the reduced green fluorescence in wildfire-exposed lungs compared to the fluorescent intensity in indoor housed controls. Wildfire-exposed animals, conversely, showed increased expression of non-canonical WNT11, indicated by the increased red, fluorescent intensity compared to the reduced intensity seen in indoor housed controls. These results are in line with the expression seen in overall protein and immunohistochemical staining. (Fig. 15)

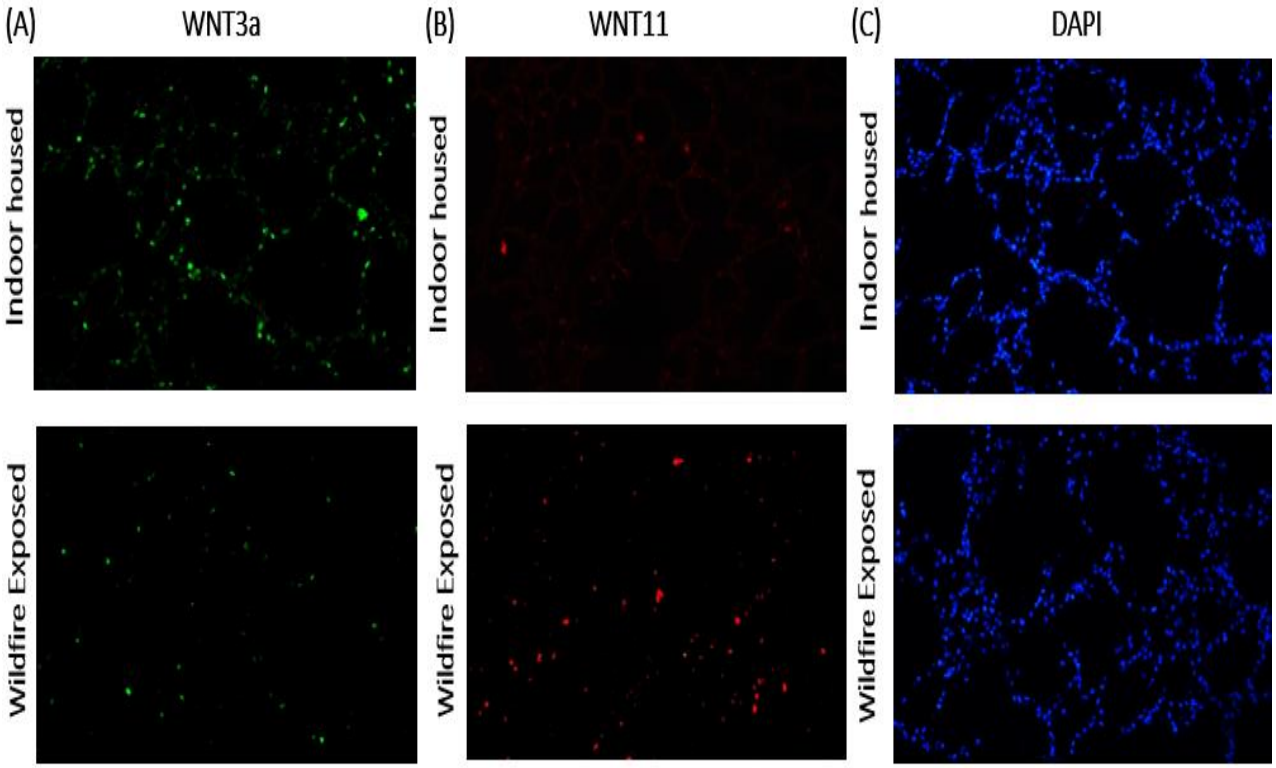

Figure 15: Fluorescent intensity of (A) WNT3a (green signal) and (B) WNT11 (red signal) ligands and (C) DAPI (blue signal) in the alveolar epithelium of the left caudal lung lobes of indoor housed and wildfire exposed animals (Fluorescence – 200x)

The relative expression of these targets in the distal lung, in response to wildfire smoke or indoor ambient air, was determined by performing SURS of fields of fluorescently stained sections of the lung tissue and analyzing images to semi quantify the expression of these ligands in the alveolar epithelium. Our findings revealed that the relative expression of these WNT ligands was altered in the distal lung. Specifically, wildfire smoke-exposed animals exhibited a significantly increased expression of WNT11 compared to the indoor housed controls. Although the change in WNT3a expression was not statistically significant, it displayed a similar downward trend in wildfire-exposed lungs, as seen in overall protein expression and distribution in the alveolar epithelium. (Fig. 16)

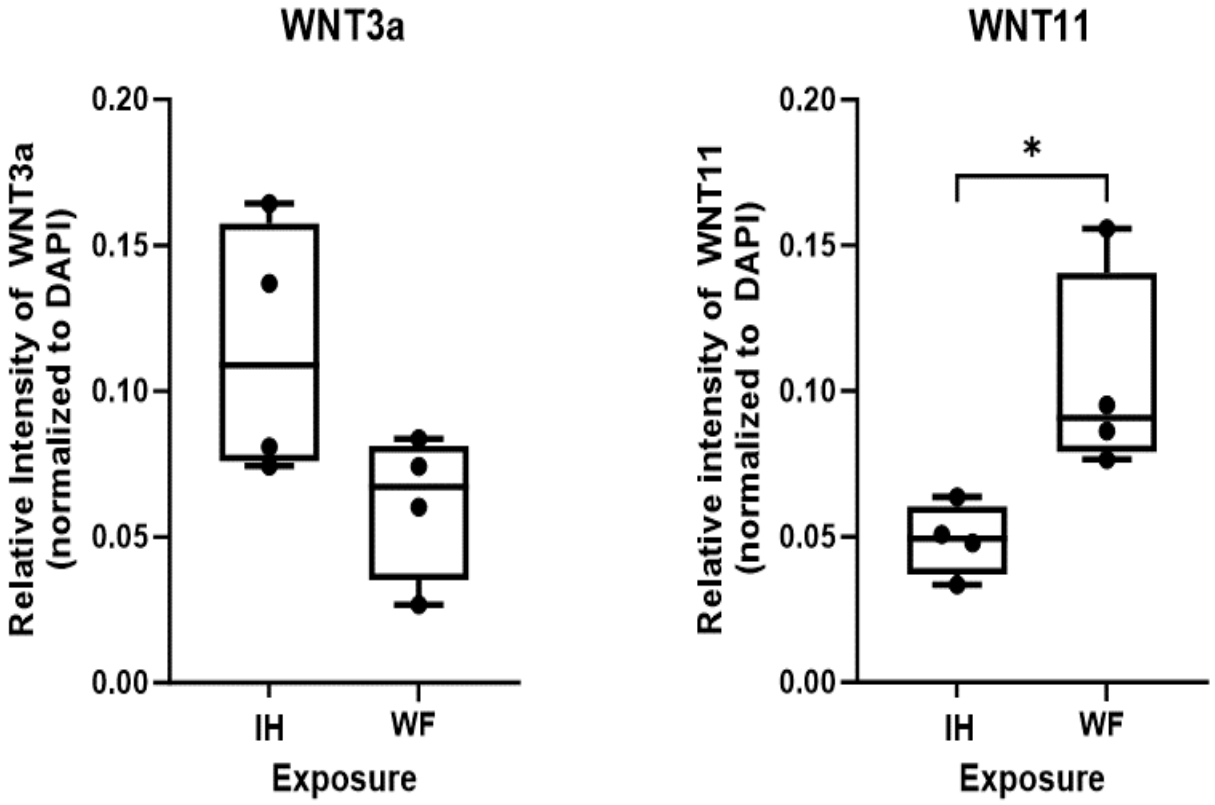

*Figure 16: Relative expression (measured as fluorescent intensity) of (A) WNT3a and (B) WNT11 ligands in the distal lungs of wildfire exposed (n=4) and indoor housed (n=4) animals. *p<0.05*

From our sampled set, we analyzed the percentage of tissue area with positive expression (area fraction), which reflects the relative area of distal lung tissue showing positive WNT target fluorescent signals compared to the overall tissue area in wildfire smoke-exposed and indoor housed lungs. Although no statistically significant differences were found in the area of expression of these ligands in the distal lung, WNT3a ligands exhibited a downward trend, showing a reduced overall area of positive expression in wildfire smoke-exposed animals compared to those housed indoors. In contrast, no differences were observed in the area of expression of WNT11 ligands between the wildfire smoke-exposed group and the indoor housed control group. (Fig. 17)

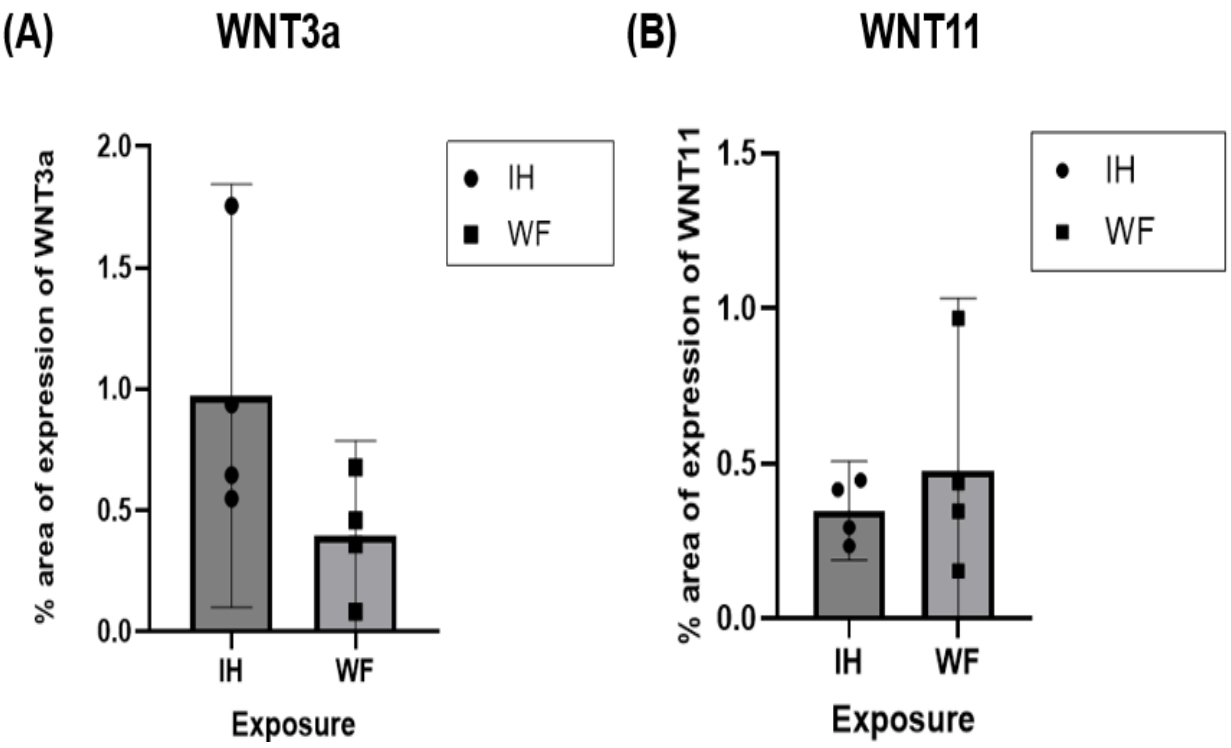

Figure 17: Area of positive expression (measured as area fraction or % of pixels above signal threshold) of (A) WNT3a and (B) WNT11 in wildfire exposed (n=4) and indoor housed animals (n=4)

Early life wildfire smoke exposure is associated with dysregulated gene expression of WNT ligands in the lung

To investigate the impact of wildfire exposure on the expression of genes associated with the canonical (WNT3a) and non-canonical (WNT11) WNT signaling pathways in the lungs, we performed reverse transcription quantitative PCR using the right cranial lung lobes. TaqMan gene expression assays were used to compare the gene expression profiles between wildfire-exposed lungs and those housed indoors. In wildfire-exposed lungs, we observed a significant decrease in the expression of the canonical WNT3a gene compared to its expression in lungs that were exposed to indoor ambient air. Although the expression of the non-canonical WNT11 gene did not show statistically significant differences, there was an upward trend in its expression in wildfire-exposed lungs relative to the indoor housed controls, which is consistent with the trends seen in protein expression. (Fig. 18)

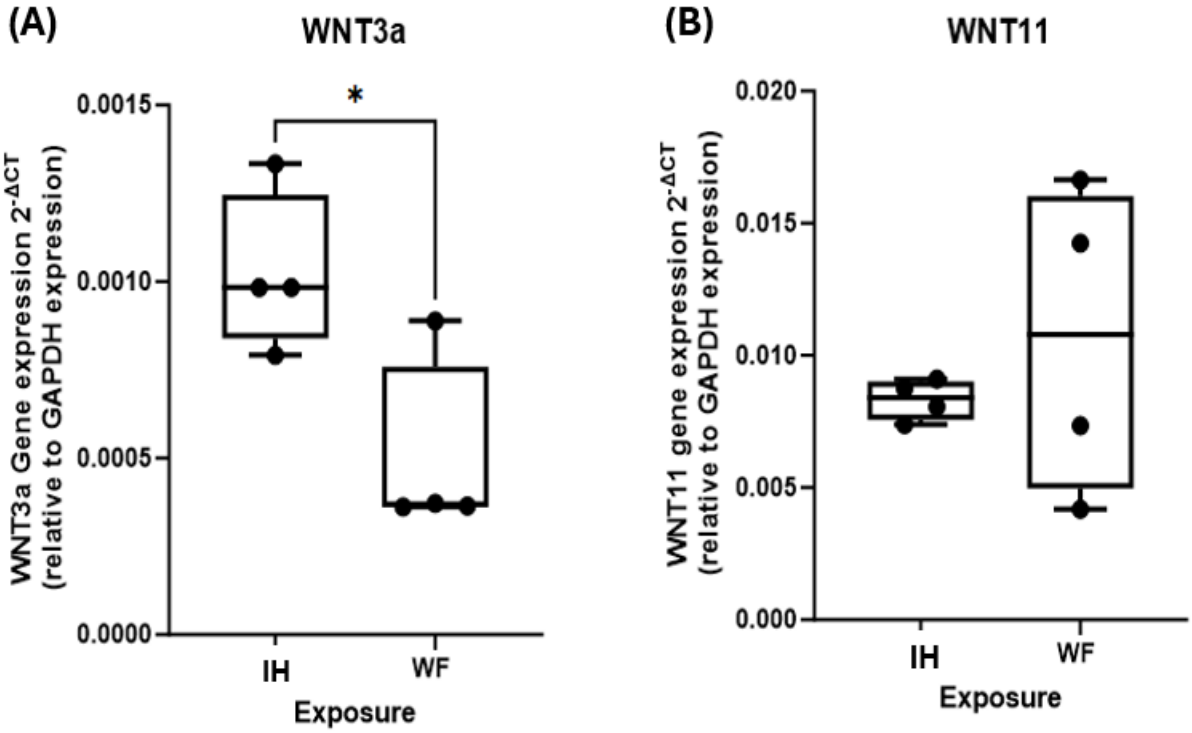

*Figure 18: Gene expression of (A) WNT3a and (B) WNT11 ligands in the right cranial lung lobes of indoor housed (n=4) and wildfire exposed animals (n=4). * $p<0.05$*

4.4 Discussion

Our study aimed to investigate the effects of wildfire smoke exposure on the expression of WNT pathway signaling molecules, specifically the canonical ligand WNT3a and the non-canonical ligand WNT11, in the lungs of infant rhesus macaques. Our findings indicate that early life wildfire smoke exposure is associated with the alteration of the expression of WNT ligands in the lungs, with an increase in non-canonical WNT11 expression and a trend toward reduced canonical WNT3a expression in wildfire smoke-exposed animals. A significant increase in non-canonical WNT11 protein levels was noted post-exposure, while canonical WNT3a protein levels decreased, though not statistically significant, in wildfire-exposed animals compared to those that were housed indoors. Histological analyses of these target ligands in the distal lungs supported the trends seen in protein levels, with wildfire-exposed lungs showing decreased WNT3a immunohistochemical and immunofluorescent staining intensity and a significant increase in WNT11 ligand expression (fluorescent signal intensity) in the distal lung. Gene expression analyses showed a significant decrease in WNT3a mRNA levels in wildfire-exposed lungs, with a corresponding increase in WNT11 mRNA levels, though not statistically significant, compared to those housed indoors. These results align with the observed trends in distal lung alveolar epithelium and protein expression. Collectively, these findings indicate that wildfire smoke exposure can be associated with dysregulated WNT signaling in the distal lung, enhancing non-canonical WNT11 expression while suppressing canonical WNT3a expression. These observed trends in WNT ligand expression are consistent with previous studies on the effects of ozone exposure on pulmonary WNT signaling molecules in rhesus macaques, reinforcing the notion that environmental pollutants, such as wildfire smoke, similarly can impact pulmonary WNT signaling.

Overall, our study offers insights into the effects of wildfire smoke on the cellular and developmental pathways involved in the infant lungs. Combined with previous work done in our laboratory, examining the effects of ozone exposure on lung development, these findings further suggest that exposure to air pollutants during critical phases of lung development triggers distinct molecular responses in the lungs, highlighting that environmental pollutants can have similar impacts on lung development and WNT signaling.. While wildfire PM shares some similarities with other sources of PM such as urban and traffic related PM, wildfire PM can be more complex, due to the combustion of a wide range of organic materials, which may result in a diverse array of toxicants (209). This may result in a more profound impact on lung development compared than other exposures to singular air pollutants like ozone. Nonetheless, this overall impact may ultimately depend on the presence of air pollution, regardless of its source, given the similar disruptions to WNT signaling molecules observed with wildfire smoke and ozone. Thus, the inherent severity of the effects from exposure to either wildfire PM or other pollutants may depend on specific context of the exposure, such as concentration and duration, and not solely on the type of pollutant.

Research has shown that air pollution can impact the function of WNT cell signaling in the lungs (210). Air pollutants impact the functioning of these molecules through disruption of epithelial mesenchymal cross talk, airway remodeling, activation of oxidative stress, and inflammatory responses (210-214). These responses can alter WNT signaling by dysregulating the expression or function of these ligands in the lung epithelium. WNT3a which is involved in cell proliferation and differentiation, when dysregulated could experience disrupted tissue regeneration and repair mechanisms. WNT11, which has roles in cell migration during development, could have

compromised function potentially affecting developmental processes and increasing the susceptibility to diseases.

Given the important roles of both canonical and non-canonical WNT signaling in the lungs, dysregulations in the balance between these signaling pathways post-wildfire exposure could have important implications for long-term lung health and development. The upregulation of non-canonical signaling molecules like WN11 after exposure to air pollutants may suggest reparative efforts by the lung tissue, as this ligand and non-canonical signaling is involved in regeneration, remodeling, and cellular migration. In contrast, the decrease in canonical signaling molecules, like WNT3a which are critical for proliferation, differentiation, and homeostasis, may suggest a suppression of these processes, potentially impairing the lung's ability to recover from the exposure. However, further extensive studies are necessary to fully understand the significance of these dysregulations to the WNT pathways on overall lung function.

While our findings offer a starting point for understanding the effects of wildfire smoke on cellular pathways in the developing lung, several limitations should be considered. Although we observed significant differences in the expression of WNT ligands in the infant lungs, the relatively small sample size limits the robustness of these findings. Further studies with larger sample sizes are needed to accurately characterize the extent and variations in the expression of these ligands. Secondly, our study treats wildfire smoke exposure as a single entity, without distinguishing between the various air pollutants that comprise it. Our study assumes a synergistic effect of the smoke as a whole, potentially overlooking the individual contributions of specific pollutants. Canonical ligands like WNT3a have been mapped to several cell types in the lungs including the alveolar epithelial cells, stromal cells, and niche endothelial cells and WNT11 has been mapped to alveolar epithelial cells and mesenchymal cells in the lungs. Another limitation of our study's

findings is that we did not double stain for a cell phenotype to identify which specific cells were expressing these ligands. Future studies should aim to characterize the expression of these ligands various cell types in response to air pollution. This approach would provide a more comprehensive understanding of the mechanistic disruptions occurring across all cell types in the lung (200,201).

Future research should study the individual effects of the components of wildfire smoke exposure along with understanding how dose and concentration of air pollutants affect WNT signaling in the lungs. Additionally, it is important to recognize that while we have observed associations between WNT ligand expression and wildfire smoke exposure, these cannot be correlated to direct functional or structural changes in lung development, given that the animals only had short-term exposures to a single wildfire event and was limited to a single developmental time point. Further longitudinal studies, encompassing multiple time points and varying exposure levels, would be required to determine if the observed dysregulations in WNT signaling persist, worsen, or resolve over time, and how they might translate into tangible effects on lung developments and health.

In summary, our study demonstrates that early life wildfire smoke exposure is associated with altered expression of WNT signaling molecules in the distal lung, with increased WNT11 expression and decreased WNT3a expression. These findings suggest that WNT signaling pathways could play a role in the response to wildfire smoke in the developing lung, potentially contributing to adverse pulmonary outcomes. Further research is needed to explore the long-term implications of these changes for both lung functioning and development.

4. CHAPTER 5: DISCUSSION AND CONCLUSION

With the broadening impact of anthropogenically driven climate change on the natural environment, it is becoming important to understand how new forms of environmental pollutants such as wildfires can influence human health, both acutely and chronically. The severity of wildfires has increased over the past two decades and is expected to worsen with continued global warming (5-7). The air pollution associated with wildfire events includes $PM_{2.5}$, ozone, SO_2, NO_x, and more, which elicits adverse effects that are particularly problematic for vulnerable subjects such as pediatric and geriatric populations (28,29). Infants and children are particularly vulnerable to environmental exposure as their respiratory and immune systems are still developing and maturing. Disruptions to critical developmental processes, such as alveolarization and airway epithelial cell differentiation, could have long-lasting consequences for lung function, development, and overall health (53). This study aimed to investigate the effects of wildfire smoke exposure on the lung development of infant rhesus macaques exposed to the wildfire smoke from the 2018 Camp Fire in Northern California. It specifically focused on alveolar structure and WNT signaling pathways, which are known to play a crucial role in lung growth and repair.

The 2018 Camp Fire look place in November, which is a window of postnatal development for rhesus macaques that are born in the California National Primate Research Center (CNPRC) in the spring. The analysis of Camp Fire air quality data obtained from a California Air Resources Board monitor within three miles of the CNPRC revealed sustained elevations in $PM_{2.5}$ levels at all sites across the Sacramento Valley, which is concerning given the well-established link between fine particulate matter and respiratory morbidity (83-86). Increases in the concentration of other analyzed pollutants were also observed, though not to the extent seen with $PM_{2.5}$. These elevations were compared to ambient levels of air pollutants during the same period in a non-wildfire year

(2019). Importantly, the infant rhesus macaque monkeys evaluated in this study were housed outdoors in open field cages throughout the Camp Fire event, which resulted in a daily continuous environmental exposure to wildfire smoke during the period of the Camp Fire.

When evaluating the impact of this exposure on the alveolar structure of the distal lung in infant rhesus macaques, our investigation did not find significant changes in key morphometric parameters of lung growth such as mean linear intercept (MLI) and air space volume density (ASVD). These findings would suggest that short-term exposure to wildfire smoke, at least within the parameters assessed in this study, did not induce detectable morphological changes in the alveolar growth when evaluated 1-2 months following the exposure window. Since alveolarization is known to continue beyond infancy and into young adulthood in both rhesus macaques and humans, these results may not reflect the long-term impacts of wildfire smoke exposure on lung development because this prolonged period of lung development may be required to detect significant differences in alveolar growth (163,165,168).

In contrast, our investigation into WNT signaling pathways revealed more pronounced effects. The observed increase in WNT11 expression, a marker of non-canonical signaling, coupled with the decrease in WNT3a expression, a canonical signaling molecule, at the gene, protein, and tissue levels, suggests that wildfire smoke may be associated with dysregulation in the expression and functioning of these critical signaling molecules in the WNT developmental pathways. This dysregulation could have consequences on processes such as cell proliferation, differentiation, and tissue repair, potentially altering downstream processes of lung development.

The findings from this study have important implications for understanding the impacts of wildfire smoke exposure on lung development in vulnerable infant and child populations. While there was a lack of detectable morphometric changes in the alveoli following short-term wildfire

exposure, the disruption in WNT signaling pathways could predispose individuals to respiratory and developmental issues later in life. This has implications for public health strategies aimed at protecting vulnerable populations, as it suggests that interventions should focus on preventing acute respiratory symptoms and potential long-term developmental impacts.

While this study provides a starting point for understanding the effects of wildfire smoke on lung development, it is important to acknowledge its limitations. The relatively short duration of exposure and the focus on a single developmental time point may have limited our ability to detect long-term or cumulative effects. Additionally, the small sample size reduces the statistical power to identify subtle changes in alveolar structure or signaling pathways. Moreover, our study treated wildfire smoke as a single entity without distinguishing between the various pollutants that comprise it. Future research should aim to address these limitations by experimental exposures using defined materials for combustion, multiple developmental time points, and larger sample sizes to fully characterize the impact of wildfire smoke on lung development. Studies should also address the individual effects of specific components of wildfire smoke to better understand their distinct roles in disrupting lung development. Additionally, investigating how these pollutants interact with each other and with genetic factors could provide further insights into the mechanisms underlying the observed dysregulation of WNT signaling.

In conclusion, this thesis dissertation provides evidence of an association between early life wildfire smoke exposure and lung development. It expands our understanding of the interactions between environmental pollutants and mechanisms of lung development, providing a foundation for future research and informing public health strategies aimed at protecting vulnerable populations from the continuing threat of wildfire smoke.

8. BIBLIOGRAPHY

1. Balmes JR. The Changing Nature of Wildfires. Clin Chest Med. 2020;41(4):771-776. doi:10.1016/j.ccm.2020.08.006
2. McKenzie, D., Gedalof, Z. E., Peterson, D. L., & Mote, P. (2004). Climatic change, wildfire, and conservation. Conservation biology, 18(4), 890-902.
3. Wehner, M. F., Arnold, J. R., Knutson, T., Kunkel, K. E., & LeGrande, A. N. (2017b). Ch. 8: Droughts, Floods, and Wildfires.
4. Marlon, J. R., Bartlein, P. J., Walsh, M. K., Harrison, S. P., Brown, K. J., Edwards, M. E., ... & Whitlock, C. (2009). Wildfire responses to abrupt climate change in North America. Proceedings of the National Academy of Sciences, 106(8), 2519-2524.
5. Balmes JR. Where There's Wildfire, There's Smoke. N Engl J Med. 2018;378(10):881-883. Zhuang, Y., Fu, R., Santer, B. D., Dickinson, R. E., & Hall, A. (2021). Quantifying contributions of natural variability and anthropogenic forcings on increased fire weather risk over the western United States. Proceedings of the National Academy of Sciences, 118(45). https://doi.org/10.1073/pnas.2111875118
6. Burke M, Driscoll A, Heft-Neal S, et al. The changing risk and burden of wildfire in the United States. Proc Natl Acad Sci U S A. 2021;118(2). doi:10.1073/pnas.2011048118
7. Hessburg, P. F., Prichard, S. J., Hagmann, R. K., Povak, N. A., & Lake, F. K. (2021). Wildfire and climate change adaptation of Western North American Forests: A case for intentional management. Ecological Applications, 31(8). https://doi.org/10.1002/eap.2432
8. Clarke, H., Cirulis, B., Penman, T. et al. The 2019–2020 Australian forest fires are a harbinger of decreased prescribed burning effectiveness under rising extreme conditions. Sci Rep 12, 11871 (2022). https://doi.org/10.1038/s41598-022-15262-y
9. Anne Ganteaume;Renaud Barbero;Marielle Jappiot;Eric Maillé;. (2021). Understanding future changes to fires in southern Europe and their impacts on the wildland-urban interface . Journal of Safety Science and Resilience, (), –. doi:10.1016/j.jnlssr.2021.01.001
10. Gill, A. Malcolm; Stephens, Scott L.; Cary, Geoffrey J. . (2013). *The worldwide "wildfire" problem. Ecological Applications, 23(2), 438–454.* doi:10.1890/10-2213.1
11. Duc HN, Bang HQ, Quang NX. Modelling and prediction of air pollutant transport during the 2014 biomass burning and forest fires in peninsular Southeast Asia. Environ Monit Assess. 2016;188(2):106. doi:10.1007/s10661-016-5106-9
12. D'Evelyn, S. M., Jung, J., Alvarado, E., Baumgartner, J., Caligiuri, P., Hagmann, R. K., Henderson, S. B., Hessburg, P. F., Hopkins, S., Kasner, E. J., Krawchuk, M. A., Krenz, J. E., Lydersen, J. M., Marlier, M. E., Masuda, Y. J., Metlen, K., Mittelstaedt, G., Prichard, S. J., Schollaert, C. L., Smith, E. B., … Spector, J. T. (2022). Wildfire, Smoke Exposure, Human Health, and Environmental Justice Need to be Integrated into Forest Restoration and Management. Current environmental health reports, 9(3), 366–385. https://doi.org/10.1007/s40572-022-00355-7
13. Black, C., Tesfaigzi, Y., Bassein, J. A., & Miller, L. A. (2017). Wildfire smoke exposure and human health: Significant gaps in research for a growing public health issue. *Environmental toxicology and pharmacology*, *55*, 186–195. https://doi.org/10.1016/j.etap.2017.08.022
14. Liu, J.C., Peng, R.D. The impact of wildfire smoke on compositions of fine particulate matter by ecoregion in the Western US. *J Expo Sci Environ Epidemiol* 29, 765–776 (2019). https://doi.org/10.1038/s41370-018-0064-7
15. Liu, J. C., Mickley, L. J., Sulprizio, M. P., Dominici, F., Yue, X., Ebisu, K., Anderson, G. B., Khan, R. F. A., Bravo, M. A., & Bell, M. L. (2016). Particulate Air Pollution from Wildfires in the Western US under Climate Change. Climatic change, 138(3), 655–666. https://doi.org/10.1007/s10584-016-1762-6
16. Wegesser TC, Pinkerton KE, Last JA. California wildfires of 2008: coarse and fine particulate matter toxicity. Environ Health Perspect. 2009;117(6):893-897. doi:10.1289/ehp.0800166
17. Kim YH, Warren SH, Krantz QT, et al. Mutagenicity and Lung Toxicity of Smoldering vs. Flaming Emissions from Various Biomass Fuels: Implications for Health Effects from Wildland Fires. Environ Health Perspect. 2018;126(1):017011. doi:10.1289/EHP2200 [8-10]
18. Aguilera R, Corringham T, Gershunov A, Benmarhnia T. Wildfire smoke impacts respiratory health more than fine particles from other sources: Observational evidence from Southern California. Nat Commun. 2021;12(1). doi:10.1038/s41467-021-21708-0
19. Korsiak, J., Pinault, L., Christidis, T., Burnett, R. T., Abrahamowicz, M., & Weichenthal, S. (2022). Long-term exposure to wildfires and cancer incidence in Canada: a population-based observational cohort study. *The Lancet Planetary Health*, *6*(5), e400-e409.
20. Zhang, Y., Obrist, D., Zielinska, B., & Gertler, A. (2013). Particulate emissions from different types of biomass burning. *Atmospheric Environment*, *72*, 27-35.

21. Manisalidis, I., Stavropoulou, E., Stavropoulos, A., & Bezirtzoglou, E. (2020). Environmental and Health Impacts of Air Pollution: A Review. *Frontiers in public health*, *8*, 14. https://doi.org/10.3389/fpubh.2020.00014
22. OEHHA, 2019, Wildfire Smoke: A Guide For Public Health Officials Revised 2019, 2019. https://oehha.ca.gov/public-information/general-info/wildfires
23. Sparks, T. L., & Wagner, J. (2021). Composition of particulate matter during a wildfire smoke episode in an urban area. *Aerosol Science and Technology*, *55*(6), 734–747. https://doi.org/10.1080/02786826.2021.1895429
24. Cobelo, I., Castelhano, F. J., Borge, R., Roig, H. L., Adams, M., Amini, H., Koutrakis, P., & Réquia, W. J. (2023). The impact of wildfires on air pollution and health across land use categories in Brazil over a 16-year period. *Environmental Research*, *224*, 115522. https://doi.org/10.1016/j.envres.2023.115522
25. Deshpande, M. V., Kumar, N., Pillai, D., Krishna, V. V., & Jain, M. (2023). Greenhouse gas emissions from agricultural residue burning have increased by 75 % since 2011 across India. *Science of The Total Environment*, *904*, 166944. https://doi.org/10.1016/j.scitotenv.2023.166944
26. Rein, G., & Huang, X. (2021). Smouldering wildfires in peatlands, forests and the arctic: Challenges and perspectives. *Current Opinion in Environmental Science & Health*, *24*, 100296. https://doi.org/10.1016/j.coesh.2021.100296
27. Luo, J., Han, Y., Zhao, Y., Huang, Y., Liu, X., Tao, S., Liu, J., Huang, T., Wang, L., Chen, K., & Ma, J. (2020). Effect of northern boreal forest fires on pah fluctuations across the Arctic. *Environmental Pollution*, *261*, 114186. https://doi.org/10.1016/j.envpol.2020.114186
28. Kelly, F. J., & Fussell, J. C. (2015). Air pollution and public health: emerging hazards and improved understanding of risk. *Environmental geochemistry and health*, *37*, 631-649.
29. Ko, F. W., & Hui, D. S. (2010). Effects of air pollution on lung health. *Clinical Pulmonary Medicine*, *17*(6), 300-304.
30. Pardo M, Li C, He Q, et al. Mechanisms of lung toxicity induced by biomass burning aerosols. Part Fibre Toxicol. 2020;17(1):4. doi:10.1186/s12989-020-0337-x
31. Li N, Hao M, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin Immunol. 2003;109(3):250-265. doi:10.1016/j.clim.2003.08.006
32. Franzi LM, Bratt JM, Williams KM, Last JA. Why is particulate matter produced by wildfires toxic to lung macrophages? Toxicol Appl Pharmacol. 2011;257(2):182-188. doi:10.1016/j.taap.2011.09.003
33. Main LC, Wolkow AP, Tait JL, et al. Firefighter's Acute Inflammatory Response to Wildfire Suppression. J Occup Environ Med. 2020;62(2):145-148. doi:10.1097/JOM.0000000000001775
34. Albano, G. D., Montalbano, A. M., Gagliardo, R., Anzalone, G., & Profita, M. (2022). Impact of Air Pollution in Airway Diseases: Role of the Epithelial Cells (Cell Models and Biomarkers). International journal of molecular sciences, 23(5), 2799. https://doi.org/10.3390/ijms23052799
35. Leclercq, B., Kluza, J., Antherieu, S., Sotty, J., Alleman, L. Y., Perdrix, E., Loyens, A., Coddeville, P., Lo Guidice, J. M., Marchetti, P., & Garçon, G. (2018). Air pollution-derived PM2.5 impairs mitochondrial function in healthy and chronic obstructive pulmonary diseased human bronchial epithelial cells. Environmental pollution (Barking, Essex : 1987), 243(Pt B), 1434–1449. https://doi.org/10.1016/j.envpol.2018.09.062
36. Zaręba Ł, Piszczatowska K, Dżaman K, et al. The Relationship between Fine Particle Matter (PM2.5) Exposure and Upper Respiratory Tract Diseases. J Pers Med. 2024;14(1):98. doi:10.3390/jpm14010098
37. Aghapour M, Ubags ND, Bruder D, et al. Role of air pollutants in airway epithelial barrier dysfunction in asthma and COPD. Eur Respir Rev. 2022;31(163):210112. doi:10.1183/16000617.0112-2021
38. Yasuda M, Inui TA, Hirano S, et al. Intracellular Cl- Regulation of Ciliary Beating in Ciliated Human Nasal Epithelial Cells: Frequency and Distance of Ciliary Beating Observed by High-Speed Video Microscopy. Int J Mol Sci. 2020;21(11):4052. doi:10.3390/ijms21114052
39. Xu Z, Ding W, Deng X. PM2.5, Fine Particulate Matter: A Novel Player in the Epithelial-Mesenchymal Transition? Front Physiol. 2019;10:1404. doi:10.3389/fphys.2019.01404
40. Zhou X, Josey K, Kamareddine L, et al. Excess of COVID-19 cases and deaths due to fine particulate matter exposure during the 2020 wildfires in the United States. Sci Adv. 2021;7(33) . doi:10.1126/sciadv.abi8789
41. Chen X, Liu J, Zhou J, et al. Urban particulate matter (PM) suppresses airway antibacterial defence. Respir Res. 2018;19(1):5. doi:10.1186/s12931-017-0700-0
42. Kobziar LN, Thompson GR 3rd. Wildfire smoke, a potential infectious agent. Science. 2020;370(6523):1408-1410. doi:10.1126/science.abe8116
43. Persson LJP, Aanerud M, Hardie JA, et al. Antimicrobial peptide levels are linked to airway inflammation, bacterial colonisation and exacerbations in chronic obstructive pulmonary disease. Eur Respir 2017; 49:1601328
44. Mushtaq N, Ezzati M, Hall L, et al. Adhesion of Streptococcus pneumoniae to human airway epithelial cells exposed to urban particulate matter. J Allergy Clin Immunol. 2011;127(5):1236-42.e2. doi:10.1016/j.jaci.2010.11.039

45. Gavito-Covarrubias D, Ramírez-Díaz I, Guzmán-Linares J, et al. Epigenetic mechanisms of particulate matter exposure: air pollution and hazards on human health. Front Genet. 2024;14:1306600. doi:10.3389/fgene.2023.1306600
46. Zheng, Y., Sanchez-Guerra, M., Zhang, Z., Joyce, B. T., Zhong, J., Kresovich, J. K., ... & Hou, L. (2017). Traffic-derived particulate matter exposure and histone H3 modification: a repeated measures study. *Environmental research*, *153*, 112-119.
47. Sofer T, Baccarelli A, Cantone L, et al. Exposure to airborne particulate matter is associated with methylation pattern in the asthma pathway. Epigenomics. 2013;5(2):147-154. doi:10.2217/epi.13.16
48. Li N, He F, Liao B, et al. Exposure to ambient particulate matter alters the microbial composition and induces immune changes in rat lung. Respir Res. 2017;18(1):143. doi:10.1186/s12931-017-0626-6
49. Seaton, A., Godden, D., MacNee, W., & Donaldson, K. (1995). Particulate air pollution and acute health effects. *The lancet*, *345*(8943), 176-178.
50. Atkinson, R. W., Ross Anderson, H., Sunyer, J., Ayres, J. O. N., Baccini, M., Vonk, J. M., ... & Katsouyanni, K. (2001). Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. *American journal of respiratory and critical care medicine*, *164*(10), 1860-1866.
51. Dockery, D. W., & Pope, C. A. (1994). Acute respiratory effects of particulate air pollution.
52. Black C, Tesfaigzi Y, Bassein JA, Miller LA. Wildfire smoke exposure and human health: Significant gaps in research for a growing public health issue. Environ Toxicol Pharmacol. 2017;55:186-195. doi:10.1016/j.etap.2017.08.022
53. Reid CE, Brauer M, Johnston FH, et al. Critical review of health impacts of wildfire smoke exposure. Environ Health Perspect. 2016;124(9):1334-1343. doi:10.1289/ehp.1409277
54. Beyene T, Harvey ES, Van Buskirk J, et al. 'Breathing Fire': Impact of prolonged bushfire smoke exposure in people with severe asthma. Int J Environ Res Public Health. 2022;19(12):7419. doi:10.3390/ijerph19127419
55. Mahsin MD, Cabaj J, Saini V. Respiratory and cardiovascular condition-related physician visits associated with wildfire smoke exposure in Calgary, Canada, in 2015: a population-based study. *Int J Epidemiol*. 2022;51(1):166-178. doi:10.1093/ije/dyab206
56. Orr A, Migliaccio CAL, Buford M, Ballou S, Migliaccio CT. Sustained effects on lung function in community members following exposure to hazardous PM2.5 levels from wildfire smoke. Toxics. 2020;8(3):1-14. doi:10.3390/toxics8030053
57. Goldberg, M. (2008). A systematic review of the relation between long-term exposure to ambient air pollution and chronic diseases. *Reviews on environmental health*, *23*(4), 243-298.
58. Rush, B., McDermid, R. C., Celi, L. A., Walley, K. R., Russell, J. A., & Boyd, J. H. (2017). Association between chronic exposure to air pollution and mortality in the acute respiratory distress syndrome. *Environmental Pollution*, *224*, 352-356.
59. Sutherland ER, Make BJ, Vedal S, et al. Wildfire smoke and respiratory symptoms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2005;115(2):420-422. doi:10.1016/j.jaci.2004.11.030
60. Wilgus ML, Merchant M. Clearing the air: Understanding the impact of wildfire smoke on asthma and COPD. Healthcare (Basel). 2024;12(3):307. doi:10.3390/healthcare12030307
61. Barbier, E., Carpentier, J., Simonin, O., Gosset, P., Platel, A., Happillon, M., ... & Garçon, G. (2023). Oxidative stress and inflammation induced by air pollution-derived PM2. 5 persist in the lungs of mice after cessation of their sub-chronic exposure. Environment International, 181, 108248.
62. Michaudel, C., Fauconnier, L., Julé, Y., & Ryffel, B. (2018). Functional and morphological differences of the lung upon acute and chronic ozone exposure in mice. Scientific reports, 8(1), 10611.
63. Júnior, G. R., Costa, N. D. S. X., Belotti, L., dos Santos Alemany, A. A., Amato-Lourenco, L. F., da Cunha, P. G., ... & Mauad, T. (2019). Diesel exhaust exposure intensifies inflammatory and structural changes associated with lung aging in mice. Ecotoxicology and Environmental Safety, 170, 314-323.
64. Miller, L. A. (2019). Are adverse health effects from air pollution exposure passed on from mother to child. University of California, Davis. California Air Resources Board Contract,(15-303).
65. Berns, K., & Haertel, A. J. (2024). Excess prenatal loss and respiratory illnesses of infant macaques living outdoors and exposed to wildfire smoke. American Journal of Primatology, 86(5), e23605..
66. Stowell JD, Geng G, Saikawa E, et al. Associations of wildfire smoke PM2.5 exposure with cardiorespiratory events in Colorado 2011-2014. Environ Int. 2019;133(Pt A):105151. doi:10.1016/j.envint.2019.105151
67. Loomis, D., Huang, W., & Chen, G. (2014). The International Agency for Research on Cancer (IARC) evaluation of the carcinogenicity of outdoor air pollution: focus on China. *Chinese journal of cancer*, *33*(4), 189–196. https://doi.org/10.5732/cjc.014.10028
68. Kurt, O. K., Zhang, J., & Pinkerton, K. E. (2016). Pulmonary health effects of air pollution. Current opinion in pulmonary medicine, 22(2), 138–143. https://doi.org/10.1097/MCP.0000000000000248
69. Götschi, T., Heinrich, J., Sunyer, J., & Künzli, N. (2008). Long-term effects of ambient air pollution on lung function: a review. *Epidemiology*, *19*(5), 690-701.

70. Andersen, Z. J., Hvidberg, M., Jensen, S. S., Ketzel, M., Loft, S., Sørensen, M., Tjønneland, A., Overvad, K., & Raaschou-Nielsen, O. (2011). Chronic obstructive pulmonary disease and long-term exposure to traffic-related air pollution: a cohort study. *American journal of respiratory and critical care medicine*, *183*(4), 455–461. https://doi.org/10.1164/rccm.201006-0937OC
71. Schikowski, T., Sugiri, D., Ranft, U. *et al.* Long-term air pollution exposure and living close to busy roads are associated with COPD in women. *Respir Res* 6, 152 (2005). https://doi.org/10.1186/1465-9921-6-152
72. Orr A, Migliaccio CAL, Buford M, Ballou S, Migliaccio CT. Sustained effects on lung function in community members following exposure to hazardous PM2.5 levels from wildfire smoke. Toxics. 2020;8(3):1-14. doi:10.3390/toxics8030053
73. Black C, Gerriets JE, Fontaine JH, Harper RW, Kenyon NJ, Tablin F, Schelegle ES, Miller LA. Early life wildfire smoke exposure is associated with immune dysregulation and lung function decrements in adolescence. Am J Respir Cell Mol Biol. 2017;56(5):657-666. doi:10.1165/rcmb.2016-0380OC
74. Waisel, D. B. (2013). Vulnerable populations in healthcare. *Current Opinion in Anesthesiology*, *26*(2), 186-192.
75. Flaskerud, J. H., & Winslow, B. J. (1998). Conceptualizing vulnerable populations health-related research. *Nursing research*, *47*(2), 69-78.
76. Madrid, E., Mennie, G. W., & Newton, P. L. (2006). Challenges in vulnerable populations. *JAAPA*, *19*(2), 41-46.
77. Ashkin, E. A. (2018). Vulnerable populations. *Chronic Illness Care: Principles and Practice*, 331-341.
78. Bayram, H., Rice, M. B., Abdalati, W., Akpinar Elci, M., Mirsaeidi, M., Annesi-Maesano, I., ... & Balmes, J. R. (2023). Impact of global climate change on pulmonary health: susceptible and vulnerable populations. *Annals of the American Thoracic Society*, *20*(8), 1088-1095.
79. Makri, A., & Stilianakis, N. I. (2008). Vulnerability to air pollution health effects. *International journal of hygiene and environmental health*, *211*(3-4), 326-336.
80. Black C, Tesfaigzi Y, Bassein JA, Miller LA. Wildfire smoke exposure and human health: Significant gaps in research for a growing public health issue. Environ Toxicol Pharmacol. 2017;55:186-195. doi:10.1016/j.etap.2017.08.022
81. Hutchinson, J. A., Vargo, J., Milet, M., French, N. H. F., Billmire, M., Johnson, J., & Hoshiko, S. (2018). The San Diego 2007 wildfires and Medi-Cal emergency department presentations, inpatient hospitalizations, and outpatient visits: An observational study of smoke exposure periods and a bidirectional case-crossover analysis. *PLoS medicine*, *15*(7), e1002601. https://doi.org/10.1371/journal.pmed.1002601
82. Reid CE, Jerrett M, Tager IB, et al. Differential respiratory health effects from the 2008 northern California wildfires: A spatiotemporal approach. Environ Res. 2016;150:227-235. doi:10.1016/j.envres.2016.06.012
83. Heaney A, Stowell JD, Liu JC, et al. Impacts of fine particulate matter from wildfire smoke on respiratory and cardiovascular health in California. GeoHealth. 2022;6(6) . doi:10.1029/2021GH000578
84. Stowell JD, Geng G, Saikawa E, et al. Associations of wildfire smoke PM2.5 exposure with cardiorespiratory events in Colorado 2011-2014. Environ Int. 2019;133(Pt A):105151. doi:10.1016/j.envint.2019.105151
85. Gan RW, Ford B, Lassman W, et al. Comparison of wildfire smoke estimation methods and associations with cardiopulmonary-related hospital admissions. GeoHealth. 2017;1(3):122-136. doi:10.1002/2017GH000073
86. Reid CE, Brauer M, Johnston FH, et al. Critical review of health impacts of wildfire smoke exposure. Environ Health Perspect. 2016;124(9):1334-1343. doi:10.1289/ehp.1409277
87. Haikerwal A, Akram M, Sim MR, et al. Fine particulate matter (PM2.5) exposure during a prolonged wildfire period and emergency department visits for asthma. Respirology. 2016;21(1):88-94. doi:10.1111/resp.12613
88. Jiao A, Headon K, Han T, Umer W, Wu J. Associations between short-term exposure to wildfire particulate matter and respiratory outcomes: A systematic review. Sci Total Environ. 2024;907:168134. doi:10.1016/j.scitotenv.2023.168134
89. Reid CE, Maestas MM. Wildfire smoke exposure under climate change: Impact on respiratory health of affected communities. Curr Opin Pulm Med. 2019;25(2):179-187. doi:10.1097/MCP.0000000000000552
90. Sutherland ER, Make BJ, Vedal S, et al. Wildfire smoke and respiratory symptoms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2005;115(2):420-422. doi:10.1016/j.jaci.2004.11.030
91. Magzamen S, Gan RW, Liu J, et al. Differential cardiopulmonary health impacts of local and long-range transport of wildfire smoke. GeoHealth. 2021;5(3) . doi:10.1029/2020GH000330
92. Wilgus ML, Merchant M. Clearing the air: Understanding the impact of wildfire smoke on asthma and COPD. Healthcare (Basel). 2024;12(3):307. doi:10.3390/healthcare12030307
93. Chen H, Samet JM, Bromberg PA, Tong H. Cardiovascular health impacts of wildfire smoke exposure. Part Fibre Toxicol. 2021;18(1):1-22. doi:10.1186/s12989-020-00394-8
94. Wettstein ZS, Hoshiko S, Fahimi J, Harrison RJ, Cascio WE, Rappold AG. Cardiovascular and cerebrovascular emergency department visits associated with wildfire smoke exposure in California in 2015. J Am Heart Assoc. 2018;7(8) . doi:10.1161/JAHA.117.007492
95. Ye T, Guo Y, Chen G, et al. Risk and burden of hospital admissions associated with wildfire-related PM2.5 in Brazil, 2000-15: a nationwide time-series study. Lancet Planet Health. 2021;5(11) . doi:10.1016/S2542-5196(21)00173-X

96. Sorensen C, House JA, O'Dell K, et al. Associations between wildfire-related PM2.5 and intensive care unit admissions in the United States, 2006-2015. GeoHealth. 2021;5(8) . doi:10.1029/2021GH000385
97. Vuorio A, Budowle B, Raal F, Kovanen PT. Wildfire smoke exposure and cardiovascular disease-should statins be recommended to prevent cardiovascular events?. *Front Cardiovasc Med*. 2023;10:1259162. Published 2023 Sep 14. doi:10.3389/fcvm.2023.1259162
98. Dennekamp M, Straney LD, Erbas B, Abramson MJ, Keywood M, Smith K, et al. Forest fire smoke exposures and out-of-hospital cardiac arrests in Melbourne, Australia: a case-crossover study. Environ Health Perspect. 2015;123(10):959-964. doi:10.1289/ehp.1408436
99. Haikerwal A, Akram M, Del Monaco A, Smith K, Sim MR, Meyer M, et al. Impact of fine particulate matter (PM2.5) exposure during wildfires on cardiovascular health outcomes. J Am Heart Assoc. 2015;4(7). doi:10.1161/JAHA.114.001653
100. Mott JA, Mannino DM, Alverson CJ, Kiyu A, Hashim J, Lee T, et al. Cardiorespiratory hospitalizations associated with smoke exposure during the 1997 Southeast Asian Forest Fires. Int J Hyg Environ Health. 2005;208(1-2):75-85. doi:10.1016/j.ijheh.2005.01.018
101. Rajagopalan S, Al-Kindi SG, Brook RD. Air pollution and cardiovascular disease: JACC state-of-the-art review. J Am Coll Cardiol. 2018;72(17):2054-2070. doi:10.1016/j.jacc.2018.07.099
102. Rajagopalan S, Landrigan PJ. Pollution and the heart. N Engl J Med. 2021;385(20):1881-1892. doi:10.1056/nejmra2030281
103. Liu C, Chen R, Sera F, Vicedo-Cabrera AM, Guo Y, Tong S, Coelho MS, Saldiva PH, Lavigne E, Matus P. Ambient particulate air pollution and daily mortality in 652 cities. N Engl J Med. 2019;381(8):705-715. doi:10.1056/NEJMoa1817364
104. Tran, H. M., Tsai, F. J., Lee, Y. L., Chang, J. H., Chang, L. T., Chang, T. Y., ... & Chuang, H. C. (2023). The impact of air pollution on respiratory diseases in an era of climate change: A review of the current evidence. Science of the Total Environment, 166340.
105. Kelly, F. J. (2014). Influence of air pollution on respiratory disease. EMJ Respir, 2, 96-103.
106. Schwartz, J. (1993). Particulate air pollution and chronic respiratory disease. Environmental research, 62(1), 7-13.
107. Hooper, L. G., & Kaufman, J. D. (2018). Ambient air pollution and clinical implications for susceptible populations. Annals of the American Thoracic Society, 15(Supplement 2), S64-S68.
108. Bentayeb, M., Simoni, M., Baiz, N., Norback, D., Baldacci, S., Maio, S., ... & Geriatric Study in Europe on Health Effects of Air Quality in Nursing Homes (GERIE) Group. (2012). Adverse respiratory effects of outdoor air pollution in the elderly. The international journal of tuberculosis and lung disease, 16(9), 1149-1161.
109. Marchetti, P., Miotti, J., Locatelli, F., Antonicelli, L., Baldacci, S., Battaglia, S., ... & Marcon, A. (2023). Long-term residential exposure to air pollution and risk of chronic respiratory diseases in Italy: The BIGEPI study. Science of the Total Environment, 884, 163802.
110. Tibuakuu, M., Michos, E. D., Navas-Acien, A., & Jones, M. R. (2018). Air pollution and cardiovascular disease: a focus on vulnerable populations worldwide. Current epidemiology reports, 5, 370-378.
111. Lee, B. J., Kim, B., & Lee, K. (2014). Air pollution exposure and cardiovascular disease. Toxicological research, 30(2), 71-75.
112. Miller, K. A., Siscovick, D. S., Sheppard, L., Shepherd, K., Sullivan, J. H., Anderson, G. L., & Kaufman, J. D. (2007). Long-term exposure to air pollution and incidence of cardiovascular events in women. New England Journal of Medicine, 356(5), 447-458.
113. Grande, G., Ljungman, P. L., Eneroth, K., Bellander, T., & Rizzuto, D. (2020). Association between cardiovascular disease and long-term exposure to air pollution with the risk of dementia. JAMA neurology, 77(7), 801-809.
114. Llop, S., Ballester, F., Estarlich, M., Esplugues, A., Rebagliato, M., & Iniguez, C. (2010). Preterm birth and exposure to air pollutants during pregnancy. Environmental research, 110(8), 778-785.
115. Stieb, D. M., Chen, L., Eshoul, M., & Judek, S. (2012). Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environmental research, 117, 100-111.
116. Heft-Neal, S., Driscoll, A., Yang, W., Shaw, G., & Burke, M. (2022). Associations between wildfire smoke exposure during pregnancy and risk of preterm birth in California. Environmental Research, 203, 111872.
117. Ming, X., Yang, Y., Li, Y., He, Z., Tian, X., Cheng, J., & Zhou, W. (2024). Association between risk of preterm birth and long-term and short-term exposure to ambient carbon monoxide during pregnancy in chongqing, China: a study from 2016-2020. BMC public health, 24(1), 1411. https://doi.org/10.1186/s12889-024-18913-z
118. Jiang, P., Li, Y., Tong, M. K., Ha, S., Gaw, E., Nie, J., ... & Wang, M. (2024). Wildfire particulate exposure and risks of preterm birth and low birth weight in the Southwestern United States. Public Health, 230, 81-88.
119. Requia, W. J., Papatheodorou, S., Koutrakis, P., Mukherjee, R., & Roig, H. L. (2022). Increased preterm birth following maternal wildfire smoke exposure in Brazil. International Journal of Hygiene and Environmental Health, 240, 113901.

120. Jacobson Lda S, Hacon Sde S, de Castro HA, Ignotti E, Artaxo P, Saldiva PH, et al. Acute effects of particulate matter and black carbon from seasonal fires on peak expiratory flow of schoolchildren in the Brazilian Amazon. PLoS One. 2014;9(9). doi:10.1371/journal.pone.0104177
121. Kim Y, Knowles S, Manley J, Radoias V. Long-run health consequences of air pollution: Evidence from Indonesia's forest fires of 1997. Econ Hum Biol. 2017;26:186-198. doi:10.1016/j.ehb.2017.05.002
122. Black C, Gerriets JE, Fontaine JH, Harper RW, Kenyon NJ, Tablin F, Schelegle ES, Miller LA. Early life wildfire smoke exposure is associated with immune dysregulation and lung function decrements in adolescence. Am J Respir Cell Mol Biol. 2017;56(5):657-666. doi:10.1165/rcmb.2016-0380OC
123. Bush, Andrew. (2016). Lung Development and Aging. Annals of the American Thoracic Society, 13(Supplement_5), S438–S446. doi:10.1513/AnnalsATS.201602-112AW
124. Rao L, Tiller C, Coates C, et al. Lung growth in infants and toddlers assessed by multi-slice computed tomography. Acad Radiol. 2010;17(9):1128-1135. doi:10.1016/j.acra.2010.04.012
125. Burri, Peter H.. (2006). Structural Aspects of Postnatal Lung Development – Alveolar Formation and Growth. Biology of the Neonate, 89(4), 313–322. doi:10.1159/000092868
126. Gauderman W, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med. 2004;351:1057-1067.
127. Caldeira I, Fernandes-Silva H, Machado-Costa D, Correia-Pinto J, Moura RS. Developmental pathways underlying lung development and congenital lung disorders. Cells. 2021;10(11):2987. doi:10.3390/cells10112987.
128. Aros CJ, Pantoja CJ, Gomperts BN. Wnt signaling in lung development, regeneration, and disease progression. Commun Biol. 2021;4(1). doi:10.1038/s42003-021-02759-3.
129. Kugler MC, Joyner AL, Loomis CA, Munger JS. Sonic hedgehog signaling in the lung: From development to disease. Am J Respir Cell Mol Biol. 2015;52(1):1-13. doi:10.1165/rcmb.2014-0132TR.
130. Yang L, Zhou F, Zheng D, Wang D, Li X, Zhao C, Huang X. FGF/FGFR signaling: From lung development to respiratory diseases. Cytokine Growth Factor Rev. 2021;62:94-104. doi:10.1016/j.cytogfr.2021.09.002.
131. Xu, K., Moghal, N., & Egan, S. E. (2012). Notch signaling in lung development and disease. Notch signaling in embryology and Cancer, 89-98.
132. Bartram U, Speer CP. The role of transforming growth factor β in lung development and disease. Chest. 2004;125(2):754-765. doi:10.1378/chest.125.2.754.
133. Frum T, Hsu PP, Hein RF, Conchola AS, Zhang CJ, Utter OR, Anand A, Zhang Y, Clark SG, Glass I, Sexton JZ, Spence JR. Opposing roles for TGFB- and BMP-signaling during nascent alveolar differentiation in the developing human lung. NPJ Regener Med. 2023;8(1). doi:10.1038/s41536-023-00325-z.
134. Gauderman WJ, Urman R, Avol E, Berhane K, McConnell R, Rappaport E, Chang R, Lurmann F, Gilliland FD. Association of improved air quality with lung development in children. N Engl J Med. 2015;372(10):905-913. doi:10.1056/NEJMoa1414123
135. Urman R, McConnell R, Islam T, Avol EL, Lurmann FW, Vora H, Linn WS, Rappaport EB, Gilliland FD, Gauderman WJ. Associations of children's lung function with ambient air pollution: joint effects of regional and near-roadway pollutants. Thorax. 2014;69(6):540-547. doi:10.1136/thoraxjnl-2012-203159
136. Gehring U, Gruzieva O, Agius RM, Beelen R, Custovic A, Cyrys J, Eeftens M, Flexeder C, Fuertes E, Heinrich J, Hoffmann B, de Jongste JC, Kerkhof M, Klümper C, Korek M, Mölter A, Schultz ES, Simpson A, Sugiri D, Svartengren M, Brunekreef B. Air pollution exposure and lung function in children: the ESCAPE project. Environ Health Perspect. 2013;121(11-12):1357-1364. doi:10.1289/ehp.1306770
137. Gauderman W, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med. 2004;351:1057-1067.
138. Li S, Williams G, Jalaludin B, et al. Panel studies of air pollution on children's lung function and respiratory symptoms: a literature review. J Asthma. 2012;49:895-910. doi:10.3109/02770903.2012.719038
139. Peters JM, Avol E, Gauderman WJ, et al. A study of twelve Southern California communities with differing levels and types of air pollution. II. Effects on pulmonary function. Am J Respir Crit Care Med. 1999;159(3):768-775. doi:10.1164/ajrccm.159.3.9804144
140. Barraza-Villarreal A, Sunyer J, Hernandez-Cadena L, et al. Air pollution, airway inflammation, and lung function in a cohort study of Mexico City schoolchildren. Environ Health Perspect. 2008;116:832-838. doi:10.1289/ehp.10527
141. Rojas-Martinez R, Perez-Padilla R, Olaiz-Fernandez G, et al. Lung function growth in children with long-term exposure to air pollutants in Mexico City. Am J Respir Crit Care Med. 2007;176:377-384. doi:10.1164/rccm.200703-394OC
142. Schultz ES, Gruzieva O, Bellander T, Bottai M, Hallberg J, Kull I, Svartengren M, Melén E, Pershagen G. Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study. Am J Respir Crit Care Med. 2012;186:1286-1291. doi:10.1164/rccm.201203-0528OC

143. Wang T, Wang H, Chen J, et al. Association between air pollution and lung development in schoolchildren in China. J Epidemiol Community Health. 2020;74(10):792-798. doi:10.1136/jech-2020-214283
144. Mauad T, Rivero DH, de Oliveira RC, et al. Chronic exposure to ambient levels of urban particles affects mouse lung development. Am J Respir Crit Care Med. 2008;178(7):721-728. doi:10.1164/rccm.200803-436OC
145. Fanucchi MV, Plopper CG, Evans MJ, Hyde DM, van Winkle LS, Gershwin LJ, Schelegle ES. Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. Am J Physiol Lung Cell Mol Physiol. 2006;291(4) –L650. doi:10.1152/ajplung.00027.2006.
146. Avdalovic MV, Tyler NK, Putney L, et al. Ozone exposure during the early postnatal period alters the timing and pattern of alveolar growth and development in nonhuman primates. Anat Rec (Hoboken). 2012;295(10):1707–1716. doi:10.1002/ar.22545.
147. Black C, Gerriets JE, Fontaine JH, Harper RW, Kenyon NJ, Tablin F, Schelegle ES, Miller LA. Early life wildfire smoke exposure is associated with immune dysregulation and lung function decrements in adolescence. Am J Respir Cell Mol Biol. 2017;56(5):657-666. doi:10.1165/rcmb.2016-0380OC
148. Burgess, T. (2019). Effects of early life ozone exposure on Wnt11 expression in the respiratory tract of nonhuman primates. (Order No. 22622027, University of California, Davis). ProQuest Dissertations and Theses, , 27. Retrieved fromhttps://www.proquest.com/dissertations-theses/effects-early-life-ozone-exposure-on wnt11/docview/2309521875/se-2
149. Wang D, Guan D, Zhu S, et al. Economic Footprint of California wildfires in 2018. Nat Sustain. 2020;4(3):252-260. doi:10.1038/s41893-020-00646-7
150. Knorr, W., Dentener, F., Lamarque, J. F., Jiang, L., & Arneth, A. (2017). Wildfire air pollution hazard during the 21st century. Atmospheric Chemistry and Physics, 17(14), 9223-9236.
151. Brewer, Matthew J., and Craig B. Clements. 2020. "The 2018 Camp Fire: Meteorological Analysis Using In Situ Observations and Numerical Simulations" Atmosphere 11, no. 1: 47. https://doi.org/10.3390/atmos11010047
152. Li S, Banerjee T. Spatial and temporal pattern of wildfires in California from 2000 to 2019. Sci Rep. 2021;11(1):8779. doi:10.1038/s41598-021-88131-9
153. US EPA, Camp Fire Response, response.epa.gov/camp-fire-response, 2022
154. California Air Resources Board. Camp Fire Air Quality Data Analysis. 2021
155. Rooney B, Wang Y, Jiang JH, et al. Air Quality Impact of the northern california camp fire of November 2018. Atmos Chem Phys. 2020;20(23):14597-14616. doi:10.5194/acp-20-14597-2020
156. Vu, B. N., Bi, J., Wang, W., Huff, A., Kondragunta, S., & Liu, Y. (2022). Application of geostationary satellite and high-resolution meteorology data in estimating hourly PM2. 5 levels during the Camp Fire episode in California. Remote sensing of environment, 271, 112890.
157. US Forest service, Brown H (2020) The Camp Fire Tragedy of 2018 in California. Fire Management Today 78, 11–2
158. Cascio WE. Wildland fire smoke and human health. Sci Total Environ. 2018;624:586-595.doi:10.1016/j.scitotenv.2017.12.086
159. California Air Resources Board. Air Quality and Meteorological Information (AQMIS) Database. 2018
160. Li S, Banerjee T. Spatial and temporal pattern of wildfires in California from 2000 to 2019. Sci Rep. 2021;11(1):8779. doi:10.1038/s41598-021-88131-9
161. Pope III, C. A. (1998). Epidemiology investigations of the health effects of particulate air pollution: Strengths and limitations. Applied occupational and environmental hygiene, 13(6), 356-363.
162. Bush, Andrew. (2016). Lung Development and Aging. Annals of the American Thoracic Society, 13(Supplement_5), S438–S446. doi:10.1513/AnnalsATS.201602-112AW
163. Zeltner, T. B., & Burri, P. H. (1987). The postnatal development and growth of the human lung. II. Morphology. Respiration physiology, 67(3), 269-282.
164. Butler, J. P., Loring, S. H., Patz, S., Tsuda, A., Yablonskiy, D. A., & Mentzer, S. J. (2012). Evidence for adult lung growth in humans. New England Journal of Medicine, 367(3), 244-247.
165. Rao L, Tiller C, Coates C, et al. Lung growth in infants and toddlers assessed by multi-slice computed tomography. Acad Radiol. 2010;17(9):1128-1135. doi:10.1016/j.acra.2010.04.012
166. Schittny J. C. (2017). Development of the lung. Cell and tissue research, 367(3), 427–444. https://doi.org/10.1007/s00441-016-2545-0
167. Hyde, D. M., Blozis, S. A., Avdalovic, M. V., Putney, L. F., Dettorre, R., Quesenberry, N. J., ... & Tyler, N. K. (2007). Alveoli increase in number but not size from birth to adulthood in rhesus monkeys. American Journal of Physiology-Lung Cellular and Molecular Physiology, 293(3), L570-L579.
168. Burri, P. H. (2006). Structural aspects of postnatal lung development–alveolar formation and growth. Neonatology, 89(4), 313-322.
169. Bourbon, J., Boucherat, O., Chailley-Heu, B., & Delacourt, C. (2005). Control mechanisms of lung alveolar development and their disorders in bronchopulmonary dysplasia. Pediatric research, 57(7), 38-46.

170. Hislop, A. A., Wigglesworth, J. S., & Desai, R. (1986). Alveolar development in the human fetus and infant. Early human development, 13(1), 1-11.
171. Thurlbeck, W. M. (1982). Postnatal human lung growth. Thorax, 37(8), 564-571.
172. Voynow, J. A., & Auten, R. (2015). Environmental pollution and the developing lung. Clinical pulmonary medicine, 22(4), 177-184.
173. Dockery, D. W., Skerrett, P. J., Walters, D., & Gilliland, F. (2005). Development of lung function. Of air pollution on children's health and development, 108.
174. Joshi, S., & Kotecha, S. (2007). Lung growth and development. Early human development, 83(12), 789-794.
175. Davies, G., & Reid, L. (1970). Growth of the alveoli and pulmonary arteries in childhood. Thorax, 25(6), 669-681.
176. Mitzner W. (2008). Use of mean airspace chord length to assess emphysema. Journal of applied physiology (Bethesda, Md. : 1985), 105(6), 1980–1981. https://doi.org/10.1152/japplphysiol.90968.2008
177. Ochs, M., Schipke, J. A short primer on lung stereology. Respir Res 22, 305 (2021). https://doi.org/10.1186/s12931-021-01899-2
178. Alveoleye. Sucre Lab. (n.d.). https://www.sucrelab.org/alveoleye
179. de Santana, J.L., de Lima Vitorasso, R., de Oliveira, M.A., Moriya, H.T. (2019). The Mean Linear Intercept (Lm) in the Lung: An Analysis of Line Segment Lengths. In: Costa-Felix, R., Machado, J., Alvarenga, A. (eds) XXVI Brazilian Congress on Biomedical Engineering. IFMBE Proceedings, vol 70/2. Springer, Singapore. https://doi.org/10.1007/978-981-13-2517-5_73
180. Crowley G, Kwon S, Caraher EJ, et al. Quantitative lung morphology: semi- automated measurement of mean linear intercept. BMC Pulm Med. 2019;19(1):206. Published 2019 Nov 9. doi:10.1186/s12890-019-0915-6
181. Kumar, S., Pant, M., & Ray, A. K. (2012). Segmentation of CT Lung Images Based on 2D Otsu Optimized by Differential Evolution. Advances in Intelligent and Soft Computing, 131 AISC (VOL. 2), 891–902. https://doi.org/10.1007/978-81-322-0491-6_82
182. Knust, J., Ochs, M., Gundersen, H. J. G., & Nyengaard, J. R. (2009). Stereological Estimates of Alveolar Number and Size and Capillary Length and Surface Area in Mice Lungs. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology, 292(1), 113–122. https://doi.org/10.1002/AR.20747
183. Mauad, T., Rivero, D. H., de Oliveira, R. C., Lichtenfels, A. J., Guimarães, E. T., de Andre, P. A., Kasahara, D. I., Bueno, H. M., & Saldiva, P. H. (2008). Chronic exposure to ambient levels of urban particles affects mouse lung development. *American journal of respiratory and critical care medicine*, *178*(7), 721–728. https://doi.org/10.1164/rccm.200803-436OC
184. Bouthillier, L., Vincent, R., Goegan, P., Adamson, I. Y. R., Bjarnason, S., Stewart, M., Guénette, J., Potvin, M., & Kumarathasan, P. (1998). Acute effects of inhaled urban particles and Ozone. The American Journal of Pathology, 153(6), 1873–1884. https://doi.org/10.1016/s0002-9440(10)65701-x
185. de Barros Mendes Lopes, Thais; Groth, Espen E.; Veras, Mariana; Furuya, Tatiane K.; de Souza Xavier Costa, Natalia; Ribeiro Júnior, Gabriel; Lopes, Fernanda Degobbi; de Almeida, Francine M.; Cardoso, Wellington V.; Saldiva, Paulo Hilario Nascimento; Chammas, Roger; Mauad, Thais . (2018). Pre- and postnatal exposure of mice to concentrated urban PM 2.5 decreases the number of alveoli and leads to altered lung function at an early stage of life. Environmental Pollution, 241(), 511–520.doi:10.1016/j.envpol.2018.05.055
186. de Souza Xavier Costa, N., Ribeiro Júnior, G., dos Santos Alemany, A.A. *et al.* Air pollution impairs recovery and tissue remodeling in a murine model of acute lung injury. *Sci Rep* **10**, 15314 (2020). https://doi.org/10.1038/s41598-020-72130-3
187. Edward S Schelegle; Lisa A Miller; Laurel J Gershwin; Michelle V Fanucchi; Laura S Van Winkle; Joan E Gerriets; William F Walby; Valerie Mitchell; Brian K Tarkington; Viviana J Wong; Gregory L Baker; Lorraine M Pantle; Jesse P Joad; Kent E Pinkerton; Reen Wu; Michael J Evans; Dallas M Hyde; Charles G Plopper. (2003). Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. , 191(1), 74–85. doi:10.1016/s0041-008x(03)00218-7
188. Mark V. Avdalovic; Nancy K. Tyler; Lei Putney; Susie J. Nishio; Sherri Quesenberry; Parmjit J Singh; Lisa A. Miller; Edward S. Schelegle; Charles G. Plopper; Thiennu Vu; Dallas M. Hyde. (2012). *Ozone Exposure During thE Early Postnatal Period Alters the Timing and Pattern of Alveolar Growth and Development in Nonhuman Primates. , 295(10), –.* doi:10.1002/ar.22545
189. Fanucchi, M. V.. (2006). *Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. AJP: Lung Cellular and Molecular Physiology, 291(4), L644–L650.* doi:10.1152/ajplung.00027.200
190. Herring, M.J.; Putney, L.F.; St. George, J.A.; Avdalovic, M.V.; Schelegle, E.S.; Miller, L.A.; Hyde, D.M. . (2015). *Early life exposure to allergen and ozone results in altered development in adolescent rhesus macaque lungs. Toxicology and Applied Pharmacology, 283(1), 35–41.* doi:10.1016/j.taap.2014.12.006
191. B. Martonen, T., D. Schroeter, J., Hwang, D., S. Fleming, J., & Conway, J. H. (2000). Human lung morphology models for particle deposition studies. *Inhalation toxicology*, *12*(sup4), 109-121.
192. Respiratory Changes due to Long-term Exposure to Urban Levels of Air Pollution Souza, Marcelo B. et al. CHEST, Volume 113, Issue 5, 1312 – 1318

193. Qin K, Yu M, Fan J, Wang H, Zhao P, Zhao G, Zeng W, Chen C, Wang Y, Wang A, Schwartz Z, Hong J, Song L, Wagstaff W, Haydon RC, Luu HH, Ho SH, Strelzow J, Reid RR, He TC, Shi LL. Canonical and noncanonical Wnt signaling: Multilayered mediators, signaling mechanisms and major signaling crosstalk. Genes Dis. 2023;11(1):103-134. doi:10.1016/j.gendis.2023.01.030
194. Liu, J., Xiao, Q., Xiao, J., Niu, C., Li, Y., Zhang, X., Zhou, Z., Shu, G., & Yin, G. (2022). Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. In Signal Transduction and Targeted Therapy (Vol. 7, Issue 1). Springer Nature. https://doi.org/10.1038/s41392-021-00762-6
195. Lingappan K, Savani RC. The Wnt signaling pathway and the development of bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2020;201(10):1174-1176. doi:10.1164/rccm.202002-0277ED.
196. Li, C., Bellusci, S., Borok, Z., & Minoo, P. (2015). Non-canonical WNT signalling in the lung. In Journal of Biochemistry (Vol. 158, Issue 5, pp. 355–365). Oxford University Press. https://doi.org/10.1093/jb/mvv081
197. Raslan AA, Yoon JK. WNT signaling in lung repair and regeneration. Molecules Cells. 2020;43(9):774-783. doi:10.14348/molcells.2020.0059.
198. Pongracz, J. E., & Stockley, R. A. (2006). Wnt signalling in lung development and diseases. Respiratory research, 7, 1-10.
199. Aros CJ, Pantoja CJ, Gomperts BN. Wnt signaling in lung development, regeneration, and disease progression. Commun Biol. 2021;4(1):1-12. doi:10.1038/s42003-021-02118-w.
200. Raslan, A. A., & Yoon, J. K. (2020). WNT signaling in lung repair and regeneration. Molecules and cells, 43(9), 774-783.
201. De Langhe, S. P., & Reynolds, S. D. (2008). Wnt signaling in lung organogenesis. Organogenesis, 4(2), 100-108.
202. Nyengaard, J. R., & Gundersen, H. J. G. (2006). Sampling for stereology in lungs. In European Respiratory Review (Vol. 15, Issue 101, pp. 107–114). https://doi.org/10.1183/09059180.00010101
203. Ochs, M., & Schipke, J. (2021). A short primer on lung stereology. In Respiratory Research (Vol. 22, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s12931-021-01899-2
204. Knudsen, L., Brandenberger, C., & Ochs, M. (2021). Stereology as the 3D tool to quantitate lung architecture. In Histochemistry and Cell Biology (Vol. 155, Issue 2, pp. 163–181). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00418-020-01927-0
205. Gundersen, H. J. G., Mirabile, R., Brown, D., & Boyce, R. W. (2013). Stereological Principles and Sampling Procedures for Toxicologic Pathologists. In Haschek and Rousseaux's Handbook of Toxicologic Pathology, Third Edition: Volume 1-3 (Vol. 1, pp. 215–286). Elsevier. https://doi.org/10.1016/B978-0-12-415759-0.00008-X
206. Gundersen, H. J. G. (2002). The smooth fractionator. Journal of Microscopy, 207(Pt 3), 191–210. https://doi.org/10.1046/J.1365-2818.2002.01054.X
207. Schroeder, A. B., Dobson, E. T., Rueden, C. T., Tomancak, P., Jug, F., & Eliceiri, K. W. (2021). The ImageJ ecosystem: Open-source software for image visualization, processing, and analysis. Protein Science, 30(1), 234-249.
208. Amber, K. T., & Zikry, J. (2017). Validating the use of ImageJ for the quantitative analysis of immunofluorescence. J Clin Cosmet Dermatol, 1.
209. Aguilera, R., Corringham, T., Gershunov, A. *et al.* Wildfire smoke impacts respiratory health more than fine particles from other sources: observational evidence from Southern California. *Nat Commun* **12**, 1493 (2021). https://doi.org/10.1038/s41467-021-21708-0
210. Lee, K. Y., Cao, J. J., Lee, C. H., Hsiao, T. C., Yeh, C. T., Huynh, T. T., ... & Chuang, H. C. (2015). Inhibition of the WNT/β-catenin pathway by fine particulate matter in haze: Roles of metals and polycyclic aromatic hydrocarbons. Atmospheric Environment, 109, 118-129.
211. Wu, X., Ciminieri, C., Bos, I. S. T., Woest, M. E., D'Ambrosi, A., Wardenaar, R., ... & Gosens, R. (2022). Diesel exhaust particles distort lung epithelial progenitors and their fibroblast niche. Environmental Pollution, 305, 119292.
212. Zou, W., Wang, X., Sun, R., Hu, J., Ye, D., Bai, G., ... & Ran, P. (2021). PM2. 5 induces airway remodeling in chronic obstructive pulmonary diseases via the Wnt5a/β-catenin pathway. International Journal of Chronic Obstructive Pulmonary Disease, 3285-3295.
213. Luo, L., Hong, X., Diao, B., Chen, S., & Hei, M. (2018). Sulfur dioxide attenuates hypoxia-induced pulmonary arteriolar remodeling via Dkk1/Wnt signaling pathway. Biomedicine & Pharmacotherapy, 106, 692-698.
214. Zou, W., Wang, X., Hong, W., He, F., Hu, J., Sheng, Q., ... & Ran, P. (2020). PM2. 5 induces the expression of inflammatory cytokines via the Wnt5a/Ror2 pathway in human bronchial epithelial cells. International journal of chronic obstructive pulmonary disease, 2653-2662.