

# **Health Effects of California Wildfire PM<sub>2.5</sub> Across the Lifespan**

## **Final Report**

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## Abstract

The objective of this study is to determine the long-term health impact of wildfire smoke exposure during neonatal development. To complete this objective, we investigated a cohort of CNPRC female rhesus monkeys that were born within three months prior to the Trinity and Humboldt County wildfires of June/July 2008 and lived outdoors throughout their lives. We conducted a nonterminal and minimally invasive study in thirteen-year-old adult female monkeys born in the spring of 2008 and exposed to wildfire smoke particulate matter  $\leq 2.5$  micrometers in diameter (PM<sub>2.5</sub>) during infancy in the summer of 2008. As an experimental control for the effects of wildfire smoke PM<sub>2.5</sub>, we compared our findings in thirteen-year-old adult female monkeys that were born in the spring of 2009 and not exposed to wildfire smoke PM<sub>2.5</sub> during infancy in the summer of 2009. Our primary assessments for long-term health impacts of neonatal wildfire smoke PM<sub>2.5</sub> exposure included (1) evaluation of the peripheral blood response to bacterial ligands (n=30 per group), (2) conduction of high-resolution computerized tomography (HRCT) imaging of the thoracic wall (n=11-16 per group), and (3) collection of activity monitoring data (n=11 per group). When compared with control adult monkeys, peripheral blood cells from wildfire smoke PM<sub>2.5</sub>-exposed adult monkeys showed enhanced cytokine synthesis when cultured with microbial ligands. Wildfire smoke PM<sub>2.5</sub>-exposed adult monkeys had increased activity levels and reduced sleep duration compared with controls. Wildfire smoke PM<sub>2.5</sub>-exposed adult monkeys also displayed a shift in timing of daily activity, suggesting an altered circadian rhythm. Computational analysis of thoracic HRCT scans showed a trend toward larger airway radius, as well as significantly larger numbers of blood vessels in lower lung lobes in wildfire smoke PM<sub>2.5</sub>-exposed adult monkeys. We conclude that early life exposure to wildfire smoke PM<sub>2.5</sub> can result in immune activation and lung remodeling that persists with maturity along with evidence of sleep dysregulation.

## Executive Summary

### Background

Wildfires are a significant source of air pollution and are predicted to increase in frequency as a result of climate change. Despite becoming a public health concern, the impact of acute wildfire smoke inhalation on development of chronic disease is unknown, particularly in susceptible populations such as young children. Inflammation of the respiratory tract is a consistent observation of acute air pollutant exposure in both children and adults. However, the establishment of persistent lung function decrements is a distinguishing characteristic of air pollutant exposures in children, with growing epidemiologic evidence to suggest health outcomes are retained with maturity and precede the development of chronic obstructive pulmonary disease in adults. Understanding the health effects of wildfire smoke exposure in pediatric populations is currently limited by restrictions on experimental assessment and invasive methodology. To address this knowledge gap in an animal model that is most relevant to the study of pediatric populations, we have previously reported evidence of immune dysregulation and lung function decrements in a cohort of adolescent rhesus macaque monkeys that were exposed as infants to high concentrations of ambient PM<sub>2.5</sub> from Northern California wildfires<sup>1, 2</sup>. For this current study, we proposed to continue assessment of immune and pulmonary changes in adult rhesus macaque monkeys due to early life wildfire smoke exposure into adulthood. Because HRCT scans from our prior research suggested reduced lung function, we also assessed activity levels of wildfire smoke-exposed animals by implementation of collar-based accelerometers for use in half acre field cages.

### Methods

Adult female monkeys were born and reared in an outdoor environment within three months prior to the Trinity and Humboldt County summer wildfires in 2008, which produced significant episodes of PM<sub>2.5</sub> within 2.7 miles of the CNPRC. All study animals lived outdoors throughout their lives. Peripheral blood was collected from monkeys while undergoing routine health evaluations or immediately prior to imaging. High resolution computerized tomography (HRCT) imaging of the thoracic cavity was conducted on a subset of animals evaluated for peripheral blood responses. Peripheral blood mononuclear cells (PBMC) prepared from blood samples were cultured with the bacterial ligand lipopolysaccharide (LPS) and assessed by Luminex and ELISA. LPS is a component of gram-negative bacteria cell walls and can rapidly elicit an innate immune response that mimics a bacterial infection both *in vivo* and *in vitro*. HRCT scans were analyzed using Functional Respiratory Imaging technology to quantify structural changes and estimate functional deficits. Because rhesus macaque monkeys are seasonal breeders, a second cohort born in the spring of 2009 served as a control group; biospecimen collection and imaging were conducted in subsequent years to normalize for age. Quantitative tracking data for activity and sleep were obtained using CamNtech Actiwatch monitors that were recorded every 30 seconds for one week. Activity monitors were worn by 13- and 14-year-old monkeys using 3D-printed collars; all animals remained housed in large social groups in half-acre field cages during data

collection. Serum cortisol levels were measured in historical samples obtained from 2008 wildfire smoke-exposed and 2009 control monkeys during infancy.

### Results

PBMC cultures generated from peripheral blood samples of adult female monkeys exposed to early life wildfire smoke PM<sub>2.5</sub> showed increased expression of proinflammatory cytokines (interleukin-6 (IL-6), interleukin-8(IL-8), interleukin-1 $\beta$ , macrophage inflammatory protein-1 $\beta$ ) and chemokine ligand 18 following LPS treatment relative to controls. Independent of LPS treatment, levels of monocyte chemoattractant protein-1 and macrophage derived chemokine protein in PBMC cultures were reduced in wildfire smoke PM<sub>2.5</sub>-exposed monkeys. Serum C-reactive protein was also reduced in peripheral blood from wildfire smoke PM<sub>2.5</sub>-exposed monkeys. Computational analysis of thoracic wall HRCT scans showed a trend toward larger airway radius, as well as significantly larger numbers of blood vessels in lower lung lobe in animals exposed to wildfire smoke PM<sub>2.5</sub>. Activity monitoring accelerometer data showed increased levels of activity and reduced sleep duration in wildfire smoke PM<sub>2.5</sub>-exposed adult monkeys. Historic measures of serum cortisol from wildfire smoke PM<sub>2.5</sub>-exposed animals during infancy show significant depression of responsiveness following a dexamethasone challenge.

### Conclusions

Our findings demonstrate that early-life ambient wildfire smoke PM<sub>2.5</sub> exposure in rhesus macaque monkeys is associated with dysregulation of immune response that persist across the lifespan. We also detected increased pulmonary blood vessel density and a trend toward larger airway radius in wildfire smoke-exposed animal, which indicates an overall change in lung architecture that is not reversible. Additional findings include evidence of sleep disturbances and altered cortisol responses in wildfire smoke-exposed animals. The collective parameters measured in this study may translate into increased susceptibility to infectious disease, lung pathology, and other chronic health conditions in adult human populations who have experienced comparable exposures, with the caveat that animals evaluated in this study were housed outdoors throughout the 2008 wildfire event.

## Introduction

As climate change continues and housing density near rural regions increase, wildfires are predicted to escalate in severity, frequency, and proximity to populated areas <sup>3, 4</sup>. Wildfire events pose a direct health hazard to human inhabitants of affected areas due to burn injuries and exposure to toxic fumes from housing materials. Wildfires also emit a significant amount of fine particulate matter into the atmosphere that can travel hundreds of miles from the site of origin, but there is currently little known about the public health outcomes of smoke exposures outside the immediate perimeter of burned regions <sup>5, 6</sup>. Exposure to ambient fine particulate matter (aerodynamic diameter < 2.5 µm; PM<sub>2.5</sub>) has been reported to elicit adverse effects on human health, with direct links to cardiovascular and pulmonary emergency room visits. There is also growing evidence that not all PM<sub>2.5</sub> are equivalent with regards to toxicity and health outcomes can differ widely depending upon geographic locations of PM<sub>2.5</sub> <sup>7</sup>. Data on the impact of chemical composition for wildfire derived PM<sub>2.5</sub> on human health are currently limited; however, it has been reported in murine studies that collected wildfire PM<sub>2.5</sub> is more toxic on an equal mass basis than PM<sub>2.5</sub> derived from other combustion-sources <sup>8, 9</sup>.

Experimental studies linking air pollutant exposures with pathologic outcomes in the respiratory tract have been well-documented in multiple laboratory animal models (reviewed in <sup>10, 11</sup>). For human subjects, direct evidence for a causal relationship between air pollution exposure and respiratory disease is controversial due to statistically confounding variables imposed by geographic locations, diet, and other environmental factors. A recent retrospective analysis of individuals born during the 1952 Great Smog in England showed increased frequency of self-reported childhood asthma in association with an atypical 4 day window of high PM<sub>10</sub> concentrations resulting from coal burning and cold weather conditions, a finding notable for showing linkage of disease with a limited period of extreme exposure <sup>12</sup>. Multiple physiologic parameters are believed to enhance susceptibility to air pollution health effects in children, such as increased metabolic rate and larger lung surface area per unit of body weight compared to adults <sup>13</sup>.

Health effects of PM<sub>2.5</sub> and ozone in children have been given special consideration by the Environmental Protection Agency and guidelines have been established based upon epidemiologic studies linking reduced lung function with ambient air pollution exposure in children <sup>14-17</sup>. Exposure to high levels of PM<sub>2.5</sub> during early life has been associated with long-term changes in pulmonary function and immunity <sup>18-20</sup>. An observed increased respiratory tract infection for pediatric populations in association with elevated particulate matter has also been reported, which suggest a link between air pollutant exposures and host pathogen defense deficits in young children <sup>21-23</sup>. Early life exposure to PM<sub>2.5</sub> has been associated with increased prevalence of asthma, allergies, and upper respiratory infections in young children <sup>24, 25</sup>, as well as decreased force expiratory volume in one second (FEV<sub>1</sub>) and forced vital capacity (FCV) later in life <sup>19, 20, 26</sup>. Air pollutant exposures outside of the first year life are not as strongly associated with persistent lung function and immune decrements later in life <sup>1, 20</sup>, emphasizing the importance of infancy as a particularly vulnerable period of childhood development for susceptibility to chronic health issues.

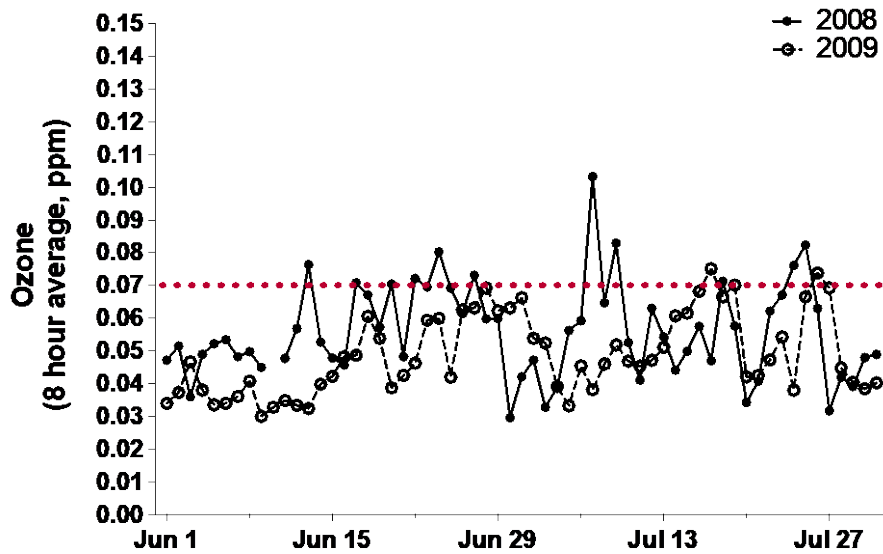
The research proposed in this study is focused on determining whether an early life acute PM<sub>2.5</sub> exposure event can elicit health outcomes that persist into adulthood. We have previously reported that air pollutant exposure to infant rhesus monkeys significantly alters innate immune responses later in life <sup>1, 27</sup>. However, the progression of immune and respiratory changes as a result of early life acute PM<sub>2.5</sub> exposure events and relation to disease is currently unknown. The scope of the work described in this report exclusively utilizes non-invasive measures of health outcomes following early life exposure to wildfire smoke derived PM<sub>2.5</sub>, such that long term longitudinal assessment and potential translation of findings to human subjects is feasible.

In this study, we evaluated age-matched cohorts of CNPRC adult female rhesus macaque monkeys that experienced significantly different levels of wildfire derived PM<sub>2.5</sub> during infancy. Outdoor-housed rhesus monkeys born in the spring of 2008 were exposed to high levels of PM<sub>2.5</sub> derived from the Trinity/Humboldt wildfire episodes in June and July of that year. Outdoor-housed rhesus monkeys born in the spring of 2009 experienced normal levels of urban-based PM<sub>2.5</sub>. Adult animals were non-invasively evaluated for immune and respiratory function by analysis of peripheral blood samples and imaging of the thorax. The impact of exposure on daily activity levels was also measured.

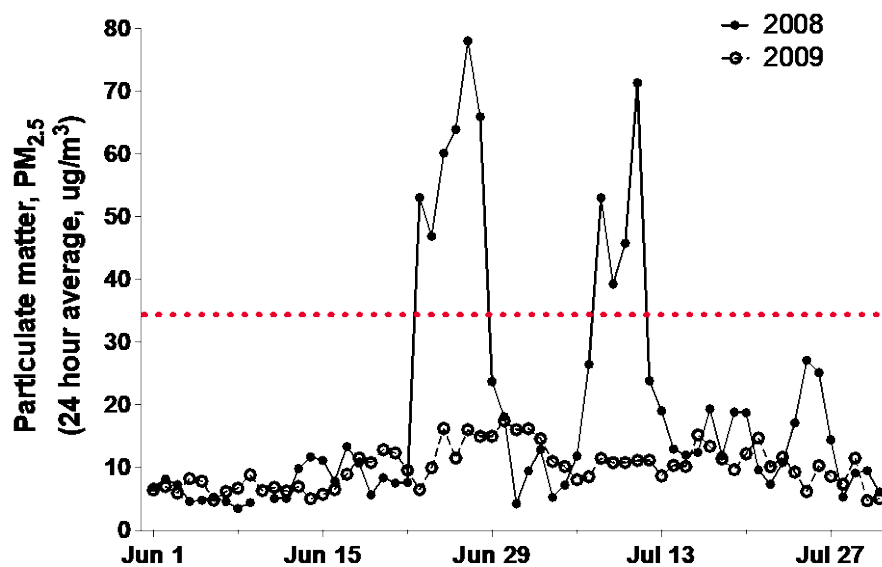
## Materials and Methods

### UC Davis Campus Air Quality Data for the 2008 Humboldt and Trinity County Fires

During the summer of 2008, the Sacramento valley experienced multiple days of elevated concentrations of particulate matter, due to persistent ambient wildfire smoke from Northern California fires. As shown in Figure 1, a comparison of daily 8-hour ozone concentration detected by a California Air Resources Board sampling station in Yolo County located within 2 miles of the CNPRC shows similar patterns between June/July 2008 versus June/July 2009, with a single day in June 2008 and a single day in July 2008 that exceeded the current NAAQS standard of 0.070 ppm/8-hour period. In comparison, there were two episodes in the months of June and July 2008, consisting of 4-6 days each, where PM<sub>2.5</sub> levels exceeded the current NAAQS standard of 35 ug/m<sup>3</sup> per 24-hour period (Figure 2). PM<sub>2.5</sub> levels correlated with a dry low-pressure system on June 20-22, 2008, that produced dry lightning igniting approximately 2000 forest fires across Humboldt County in Northern California, which is located approximately 270 miles from the University of California, Davis campus. In Yolo County, where the CNPRC is located, air quality improved June 26-July 5 2008 due to onshore winds and Delta breeze, but declined July 7-10 2008 when winds calmed. In addition to the data presented within the time frame from Figures 1 and 2, the 8-hour daily average for ozone concentration was 0.099 ppm in 2008 and 0.082 ppm in 2009. Although the 8-hour daily average ozone declined between 2008 and 2009, it should be recognized that these values still exceed the state standard of 0.070 ppm. While these data represent the most accurate measures of air quality within the immediate vicinity of the CNPRC, it should be acknowledged that other sources of inhaled materials may have been present during the period of time in which infant monkeys were housed outdoors. For example, properties adjacent to the CNPRC consist of University of California, Davis agricultural fields; pesticide use is restricted but plant-derived allergens are unavoidable. While the confounding effects of agriculture and allergens cannot be eliminated in the ambient environment of CNPRC outdoor-housed animals, it should be noted that all animals (wildfire smoke exposed and controls) enrolled in the study remained on the CNPRC physical property (~100 acres) for the duration of the 13-year window of assessment, were housed in comparable caging and experienced minimal changes in diet and husbandry methods.



**Figure 1. Daily 8 hour average concentration of ozone from June 1-July 31 on the UC Davis campus.** Daily average readings for ozone were obtained from a California Air Resources Board sampling site located on the UC Davis campus, within two miles of the California National Primate Center. Daily readings for 2008 (dotted line) and 2009 (solid line) are compared over time. The red dotted line designates the NAAQS standard of 0.070 ppm/8 hours. Note there is one day in June with a missed reading.

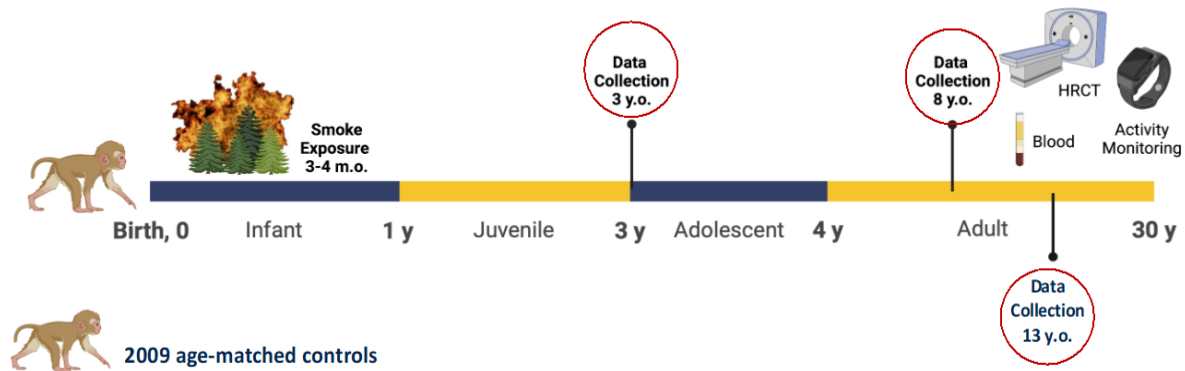


**Figure 2. Daily 24-hour average concentration of PM<sub>2.5</sub> from June 1-July 31 on the UC Davis campus.** Daily average readings for PM<sub>2.5</sub> were obtained from a California Air Resources Board sampling site located on the UC Davis campus, within two miles of the CNPRC. Daily readings for 2008 (dotted line) and 2009 (solid line) are compared over time. The red dotted line designates the NAAQS standard of 0.035 ug/m<sup>3</sup>/ 24 hours ppm. Note there is one day in June with a missed reading.

## Animals

To determine the potential long-term health impacts of acute PM<sub>2.5</sub> exposures, we evaluated parameters of immune and respiratory function in outdoor housed CNPRC rhesus monkeys. Genetically diverse, thirteen-year-old adult female rhesus macaque monkeys (*Macaca mulatta*) were born and raised in CNPRC outdoor housing within three months prior to the Trinity and Humboldt County summer wildfires in 2008. Adult female rhesus macaque monkeys born and raised in CNPRC outdoor housing in 2009 served as exposure controls. Enrolled study animals were exclusively housed outdoors prior to the assessment period. Study animals were selected from more than 15 different CNPRC outdoor field cages that house multiple genetically diverse social groups. CNPRC social groups in outdoor housing may consist of familial lines or newly introduced animals, with an age range spanning from birth through geriatric. The CNPRC electronic medical record system was used to initially identify potential subjects based upon sex and birth date relative to the peak of PM<sub>2.5</sub> in June/July (approximately three months of age); study animal cohorts were established by random selection of females from the pool of identified potential subjects. Control animals were comparably selected (born within identical seasonal time frame) and age-matched to exposed animals. A schematic of the study design is shown in Figure 3.

CNPRC animals housed in outdoor field cages are fed a commercial diet (Lab Diet #5038, Purina Mills International, St. Louis, MO) supplemented with fruits and vegetables. CNPRC produce is seasonal and sourced from a single vendor. The gestational length of captive rhesus macaque monkeys at the CNPRC is on average 166.5 days<sup>28</sup>. Dams nurse infant monkeys until approximately 3 months of age. Developmental stages in the rhesus monkey are as follows: newborn, 24 hours postnatal; neonate, 0–1 month; infant, 1–12 months; juvenile, 12–24 months; adolescent, 2–4 years; and young adult, 4–8 years, 8–18 adult, 19–30+ geriatric. Females will begin reproducing as early as 4 years of age, but most frequently 5–6 years of age. Peripheral blood for serum and PBMC isolation was collected from adult animals and their offspring in outdoor field cages during bi-annual comprehensive health assessments conducted by CNPRC veterinary staff. Thoracic imaging was separately conducted on animals that were transported from outdoor field cages. Because rhesus monkeys are seasonal breeders, a second cohort of study animals born in the spring of 2009 served as the control group; biospecimen collection and imaging were staggered in subsequent years such that age at evaluation was held constant. Offspring of the adult control group served as the age-matched control of offspring from the adult wildfire smoke PM<sub>2.5</sub> group. Care and housing of animals before, during and after evaluations complied with the provisions of the Institute of Laboratory Animal Resources and conforms to practices established by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The University of California Institutional Animal Care and Use Committee approved all animal procedures.



### In Vitro Stimulation of Peripheral Blood Mononuclear Cells with Microbial Ligands

To assess the responsiveness of innate immune function in animals evaluated in this study, we tested cultures of peripheral blood cells by *in vitro* stimulation with microbial ligands. Peripheral blood was evaluated from 20-25 animals per study group based upon our prior findings in this animal exposure group (adult females born in 2008 n=25; adult females born in 2009 n=20)<sup>2</sup>. Peripheral blood mononuclear cells (PBMC) were used in the study as this population consisting of lymphocytes and monocytes is known to express Toll-like receptors that recognize a broad range of microbial ligands. PBMC were prepared from blood samples by Histopaque 1077 gradient centrifugation and cryopreserved prior to culture<sup>29</sup>. For consistency, experiments with thawed peripheral blood mononuclear cells were conducted after all blood samples were collected from a single year. Cryopreserved PBMC were revived and allowed to equilibrate for 1 h at 37°C. Thawed peripheral blood mononuclear cells were cultured in serum free AIM-V medium supplemented with 2 mM L-Glutamine (Invitrogen, Carlsbad, CA) in 96 well tissue culture plates at a concentration of  $2 \times 10^5$  cells/100 ml. LPS (E. Coli 026:B6, Sigma-Aldrich, St. Louis, MO) was diluted in AIM-V media at a concentration of either 5 or 50 ng/ml and added to cultures at the start of incubation. Cultures were maintained for 6 or 24 hours at 37°C in 5% CO<sub>2</sub>. At the end of 6 hours (ELISA) or 24 hours (Luminex), media supernatants were collected by centrifugation of cultures to remove cells. Supernatant was stored at -80°C until analysis by ELISA or Luminex. All assays were conducted with identical media and reagent lots.

### ELISA/Luminex Assays

IL-6 and IL-8 protein concentration in serum and PBMC culture supernatants were measured by ELISA. IL-6 ELISAs were performed with human IL-6 ELISA kit (Invitrogen, San Diego, CA). IL-8 ELISAs were performed with the human IL-8 DuoSet ELISA kit (R&D Systems, Minneapolis, MN). Rhesus C-reactive protein (CRP) ELISA was performed with the Monkey CRP ELISA kit (Life Diagnostics, West Chester, PA). Rhesus anti-Tetanus Toxoid ELISAs were performed with the human Tetanus IgG ELISA kit (IBL, Minneapolis, MN). Luminex was performed using the Monkey Cytokine 29-plex panel (Thermo Fisher Scientific, San Diego, CA). All ELISA/Luminex samples were conducted in duplicate. ELISA was conducted for all study animals. Luminex was conducted on a subset of study adult female monkeys (n= 23 adult female monkeys exposed to early wildfire smoke PM<sub>2.5</sub>; n= 17 adult female monkey controls).

### Activity Monitoring

Quantitative tracking data for activity and sleep were obtained using CamNtech Actiwatch monitors that were recorded every 30 seconds for one week. Activity monitors were worn by 13- and 14-year-old monkeys using 3D-printed collars; all animals remained housed in large social groups in half-acre field cages during data collection. Serum cortisol levels were measured in historical samples from 2008 wildfire exposed and 2009 control infant monkeys.

### High Resolution Computed Tomography Imaging

Thoracic imaging was conducted to obtain non-invasive measures of lung function and remodeling in animals following acute PM<sub>2.5</sub> exposures. Imaging data was obtained from

11-16 animals per study group (Adult females born in 2008 n=16; Adult females born in 2009 n=11). Adult female monkeys used for high resolution computed tomography (HRCT) imaging studies were inspected via ultrasound to ensure less than 30 days of gestation prior to imaging procedures. Animals were sedated and intubated for HRCT imaging. HRCT images were collected on a GE Discovery 610 PET/CT Imaging System. HRCT images were performed at 3 separate external pressures: 20 mm Hg (full), 10 mm Hg (partial), and 0 mm Hg (baseline).

### Functional Respiratory Imaging Analysis

To quantify physiologic and structural changes in the lung of animals undergoing HRCT, scan data was converted into 3D models of airways and lung lobes using Mimics (Materialise, Leuven, Belgium) a commercially available validated software package (Food and Drug Administration, K073468; Conformité Européenne certificate, BE 05/1191.CE.01). Other software used included; TGrid 14.0 (Ansys Inc, Canonsburg, PA) for 3D meshing and Fluent 14.0 (Ansys Inc, Canonsburg, PA) for CFD simulations.

Segmentation of the tracheobronchial tree was done using directional thresholding with automated leakage detection. Automatic airway segmentation was performed up to the point where no distinction could be made between the intra-luminal and alveolar air. Following automated segmentation of the bronchial tree, the airways were manually checked. Missing branches were added to the bronchial tree and incorrect branches were deleted when necessary. The respiratory tract was reconstructed down to the level of airways with a diameter of 1–2 mm, beyond this point, the HRCT resolution is insufficient to distinguish alveolar from intraluminal air. The segmented airway tree was converted into a 3D model that was smoothed using a volume compensation algorithm. The smoothed model was trimmed perpendicular to the airway centerline at the trachea (using the middle point of the superior side of the sternum as a landmark) and at each terminal bronchus. Remaining artifacts due to noise in the HRCTs were then manually removed from the model. Finally, a series of manual quality checks were performed. Total time for the automated steps and manual quality checks varied from 2 to 6 hours per scan.

Functional Respiratory Imaging (FRI) is comprised of a combination of airway segmentation, lung volume segmentation by lobe, and airway resistance calculations based on computational flow simulation using boundary conditions provided by the lobe expansions from FRC to TLC, allowing calculation of lobe volume (iVlobe), airway volume (iVaw), as well as their specific values (corrected for lobe volume). Values for these parameters were calculated on a lobar level. By means of application of CFD on the segmented airway model, the airway resistance (iRaw) was calculated<sup>30</sup>. Lungs were split into lobes by identification of the fissure lines from the CT scan. This allowed determination of total lung volume and of the volume of each lobe individually.

FRI has been validated by comparison with gamma scintigraphy and single-photon emission computerized tomography<sup>30, 31</sup>.

### Statistical Analysis

Statistical analysis was performed using GraphPad Prism 10.0 software (San Diego, CA). Significance between adult groups or offspring groups was determined with one-way ANOVA or Welch's t-test. All data are reported as mean  $\pm$  SD or mean  $\pm$  SEM

as appropriate for each measured parameter. A  $p$  <value of 0.05 or less was considered statistically significant.

## Results

### Task #1

**Select 13-year-old California National Primate Research Center (CNPRC) monkeys housed outdoors during and subsequent to the 2008 Humboldt and Trinity County summer wildfire events.**

#### Summary of Enrolled Study Animals

Adult female monkeys surveyed within this study were identified based upon age relative to the peak PM<sub>2.5</sub> exposure period in 2008 (n=25)(Table 1). Age-matched female monkeys born in 2009 selected in an analogous fashion (n=20)(Table 2). The average weight of 13-year-old animals born in 2008 was 8.62 kg  $\pm$  1.26 kg, while the average weight of 13-year-old animals born in 2009 was 9.15  $\pm$  2.21 kg. There were no statistically significant differences in weight between the two animal cohorts studied, as measured by an unpaired t test. To compare outcomes from our previously reported study<sup>1</sup>, we obtained blood samples from animals during field cage health assessments that take place in the months of June-November or at the time of HRCT scanning. With one exception, blood samples from animals undergoing HRCT scans were obtained just prior to the imaging procedure.

Date of Birth	Age on June 24, 2008 (days)	Weight (kg)
2/24/08	121	11.22
2/25/08	120	7
2/25/08	120	6.6
2/28/08	117	9.7
3/3/08	114	9.23
3/5/08	112	9.63
3/10/08	107	7.48
3/26/08	91	6.68
3/27/08	90	8.22
3/27/08	90	9.86
3/28/08	89	9.07
3/31/08	86	8.2
4/1/08	85	7.49
4/9/08	77	10.76
4/12/08	74	7.43
4/19/08	67	9.12
4/28/08	58	7.6
5/3/08	53	8.01
5/8/08	48	8.56
5/9/08	47	7.46
5/9/08	47	10.12
5/22/08	34	8.3
5/22/08	34	8.56
5/30/08	26	9.9
6/10/08	15	9.34

**Table 1. Age and weight distribution of CNPRC female monkeys born in 2008 evaluated in this study.** The average weight for evaluated female monkeys born in 2008 was 8.62 kg  $\pm$  1.26.

Date of Birth	Age at June 24, 2009 (days)	Weight (kg)
3/3/09	114	7.9
3/6/09	111	9.88
3/16/09	101	6.91
4/3/09	83	8.31
4/5/09	81	9.73
4/6/09	80	12.02
4/8/09	78	6.98
4/8/09	78	10
4/9/09	77	8.68
4/10/09	76	10.02
4/10/09	76	6.94
4/17/09	69	8.68
4/18/09	68	14.12
4/20/09	66	9.44
5/7/09	49	8.34
5/17/09	39	6.79
5/21/09	35	6.54
5/26/09	30	14.34
5/23/09	33	8.66
6/1/09	25	8.72

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## **Task #2**

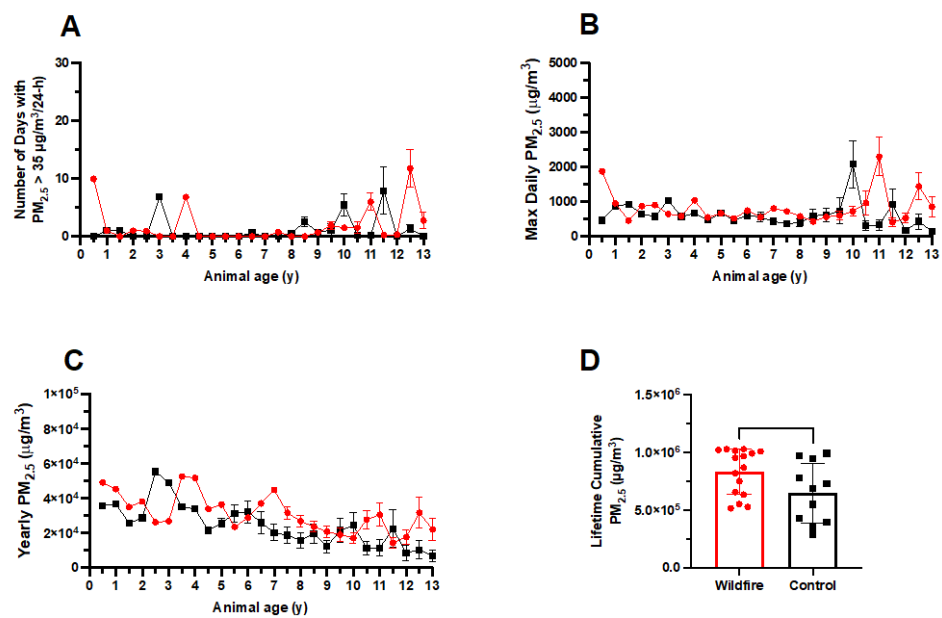
**Measure cumulative exposure to particles <2.5 micrometer in diameter PM<sub>2.5</sub> and O<sub>3</sub> for 13-year-old CNPRC monkeys housed outdoors during and subsequent to the 2008 Humboldt and Trinity County summer wildfire events.**

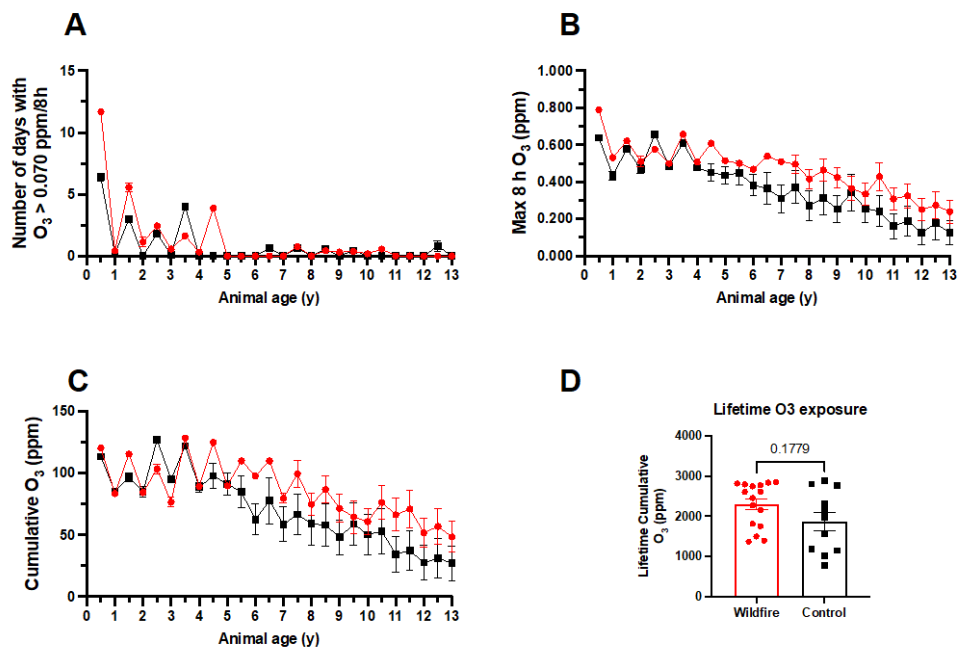
### **Summary of Cumulative Exposure**

The lifetime exposure profiles of the 2008 and 2009 animals surveyed in this study were limited to animal subjects for HRCT scan analysis. Data were sourced from a California Air Resources Board monitoring station, located on the UC Davis campus 2.7 miles from the CNPRC. Values for PM<sub>2.5</sub> and ozone were obtained from the AQMIS2 tool (<https://www.arb.ca.gov/aqmis2/aqdselect.php>) using the “Davis-UCD Campus” recording site. The cumulative amount of PM<sub>2.5</sub> and ozone experienced by each study animal over six-month intervals was quantified, starting from date of birth and terminating at the time of HRCT scan completion, which was approximately 13 years of age. The CNPRC electronic medical record system was used to determine the daily location (outdoor versus indoor) of each study animal during the period of assessment. Air quality data on dates spent indoors in HEPA filtered housing for observation and veterinary intervention purposes were excluded from animals on an individual basis. It is important to note that between the January 1, 2008 and December 31, 2022, 4.3% of days had no PM<sub>2.5</sub> data recorded, 1.9% of days had no ozone data recorded, 2.4% of PM<sub>2.5</sub> data points were negative values and 0.2% of ozone data were negative values. While the uncertainty range for reference instrumentation used to collect these data are not publicly available, it is expected that these values fall within a measure of uncertainty associated with analytical instruments and are within the EPA Federal Method Detection Limits guidelines ([https://www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure\\_rev2\\_12-13-2016.pdf](https://www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf)).

In the sampled cohort of adult female monkeys, animals experienced approximately 13.3 more days with PM<sub>2.5</sub> levels greater than the National Ambient Air Quality Standards (NAAQS) set for PM<sub>2.5</sub> (35 µg/m<sup>3</sup> for a 24-h average, EPA 40 CFAR 50) in 2008 in comparison with 2009 (Figure 4A). Cumulatively, across an thirteen-year lifespan, the adult female monkeys exposed to early life wildfire smoke PM<sub>2.5</sub> experienced approximately 22% more PM<sub>2.5</sub> than control animals (p<0.05; Figure 4D).

Analysis of ambient ozone concentrations revealed few differences in peak, average, or cumulative exposure between wildfire-exposed and control cohorts (Figure 5). However, adult female monkeys exposed to early life wildfire smoke PM<sub>2.5</sub> experienced approximately 11.7 more days with ozone concentrations above the NAAQS set for ozone (0.070 ppm/8-hour average, EPA 40 CFAR 50, Figure 5A) relative to control animals. Although the 8-hour daily average ozone declined between 2008 and 2009, it should be recognized that these values still exceed the state standard of 0.070 ppm. Although elevated ozone in 2008 did not correspond with elevations of PM<sub>2.5</sub> it is possible that ozone is increased due to elevated wildfire smoke<sup>32</sup>.



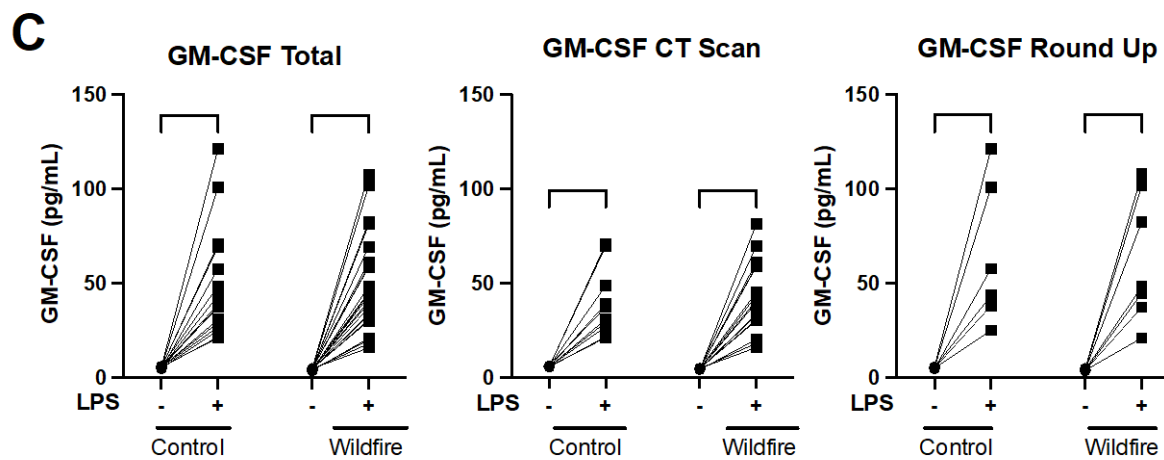
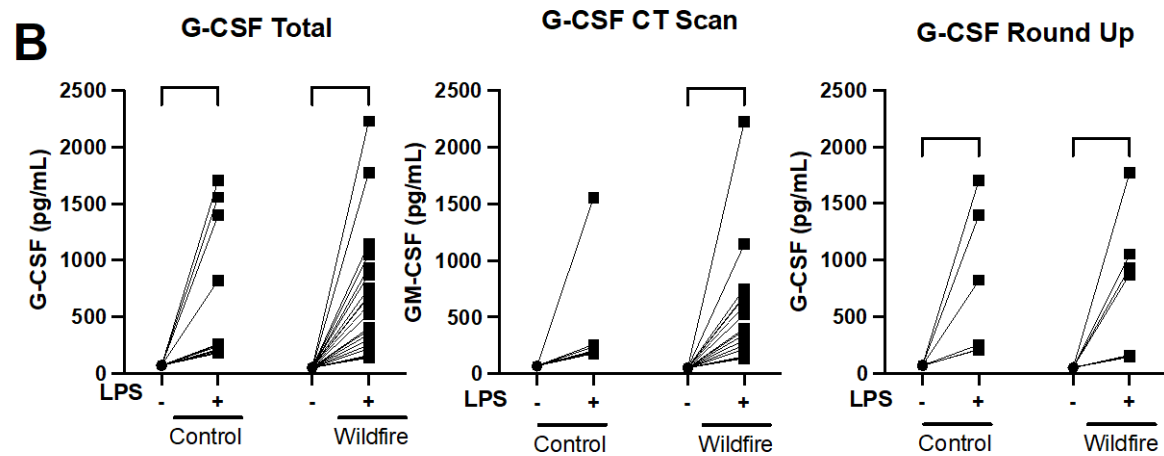
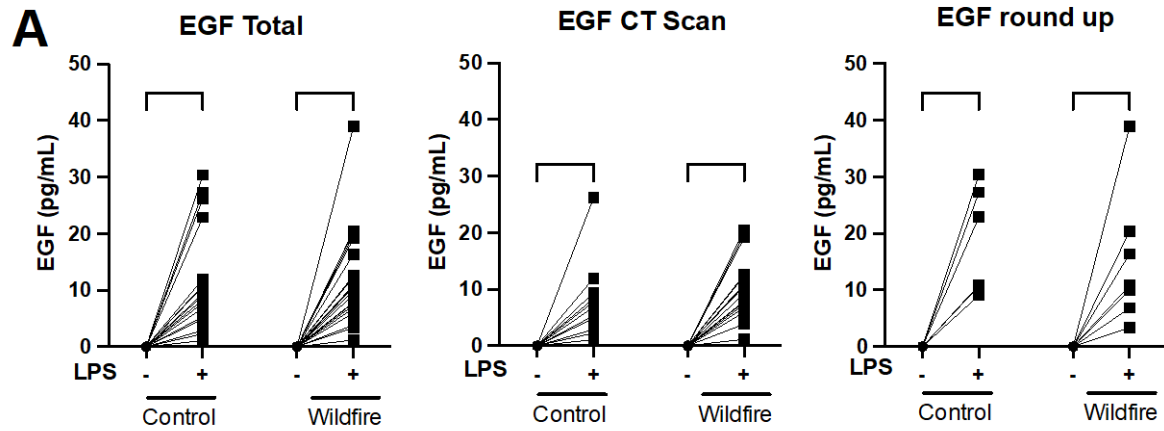


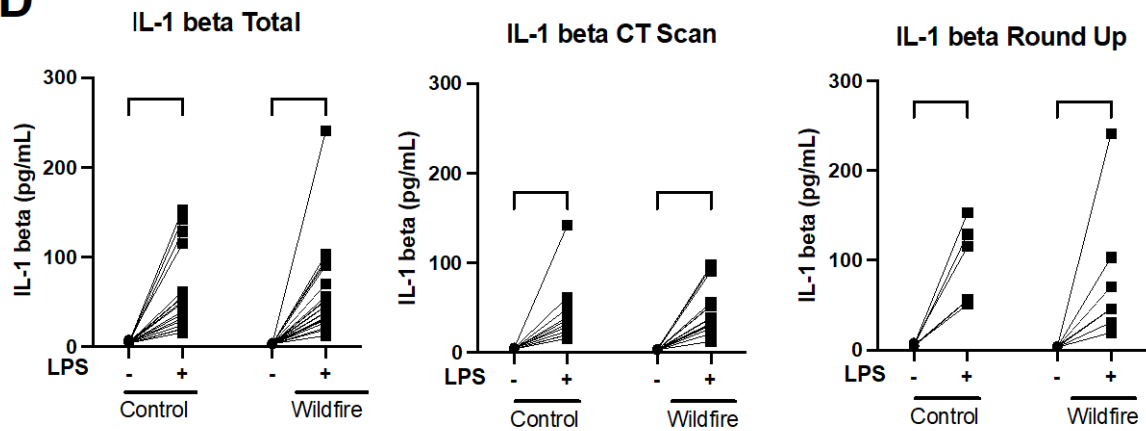
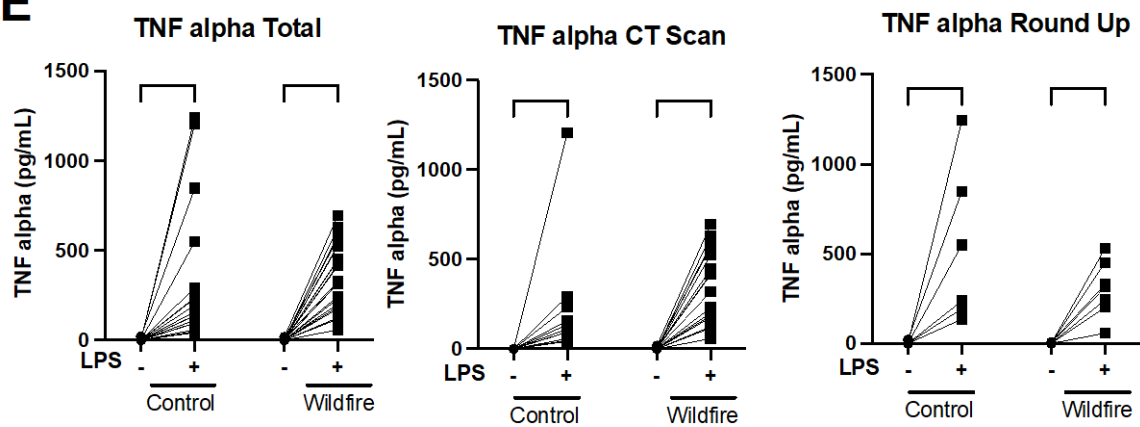
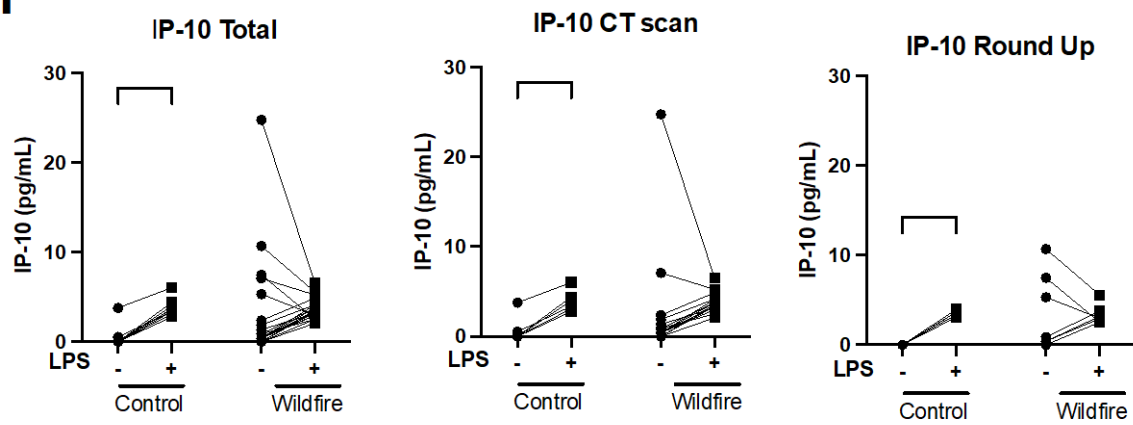
### **Task #3**

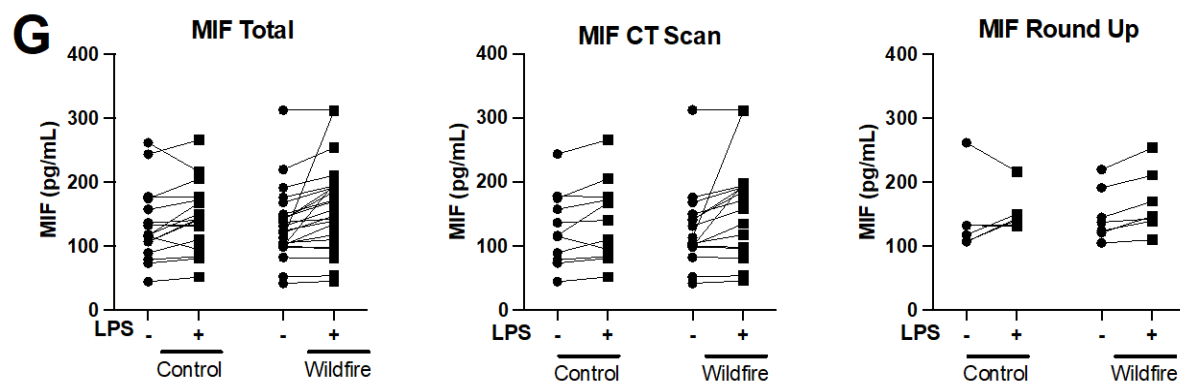
**Determine if wildfire smoke exposure can result in persistent dysregulation of immune function in 13-year-old CNPRC monkeys housed outdoors during and subsequent to the 2008 Humboldt and Trinity County summer wildfire events.**

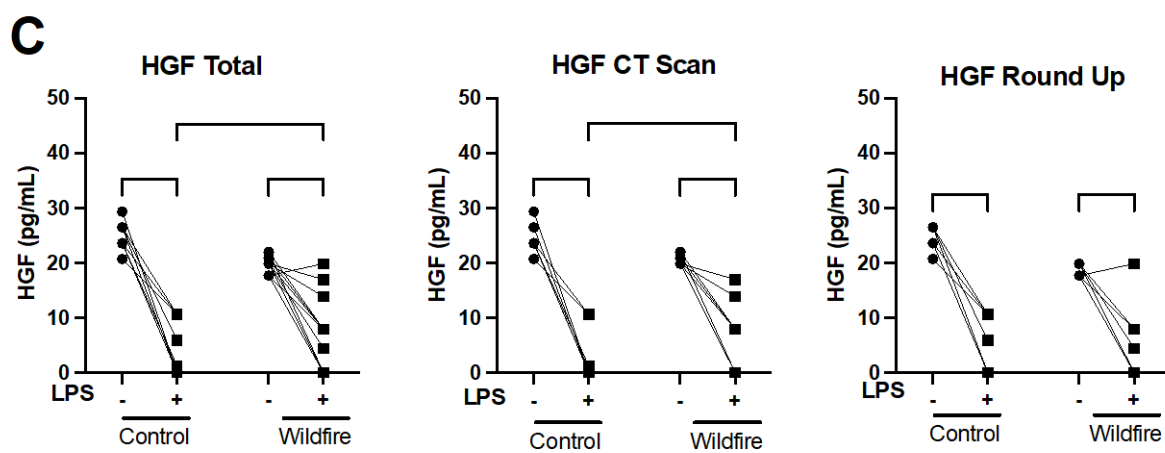
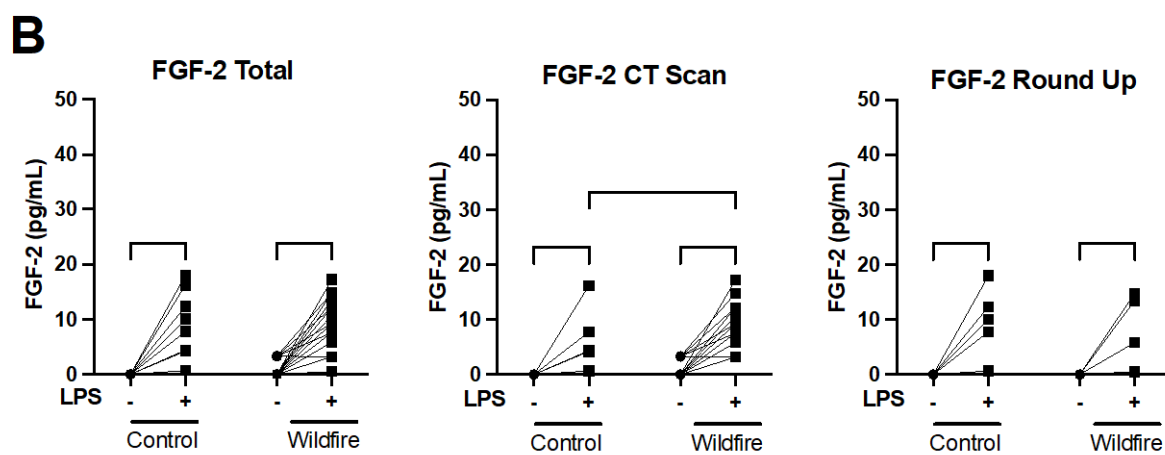
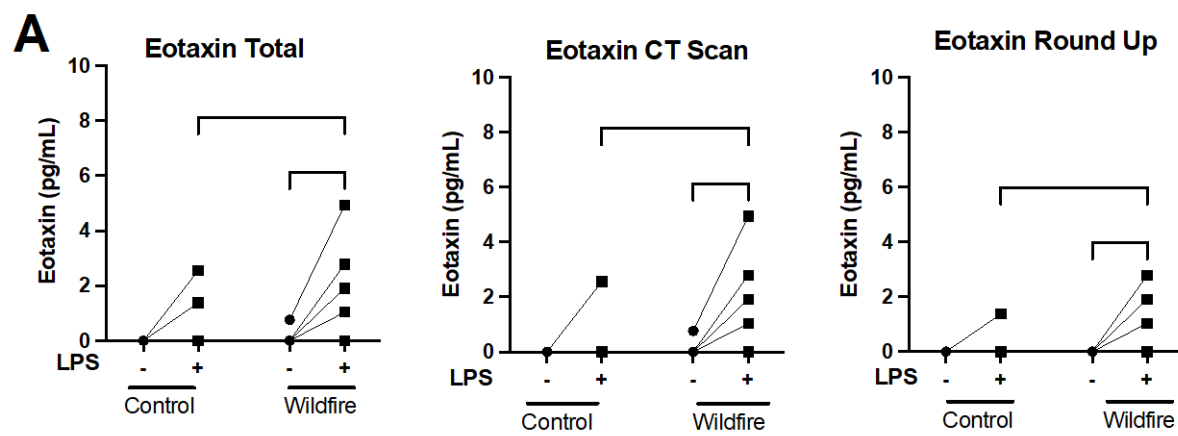
#### **Analysis of Immune Profiles**

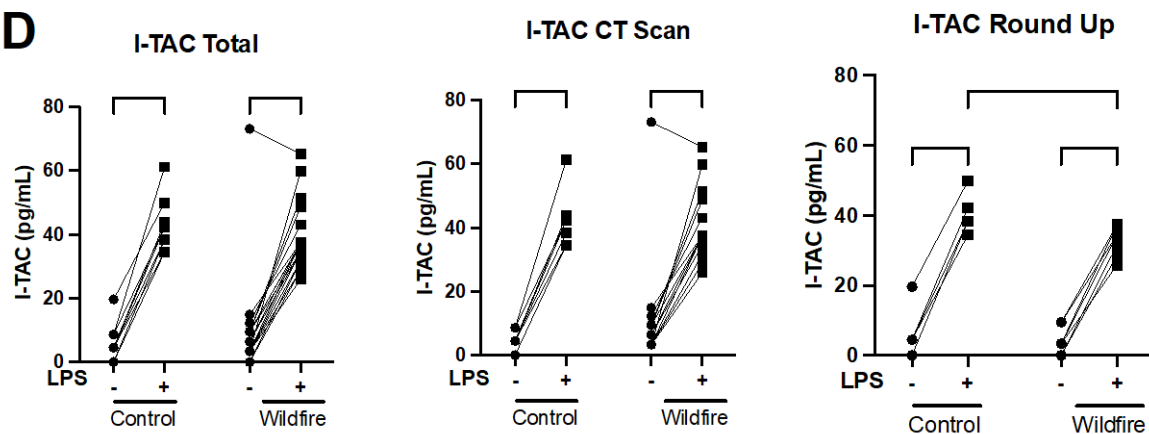
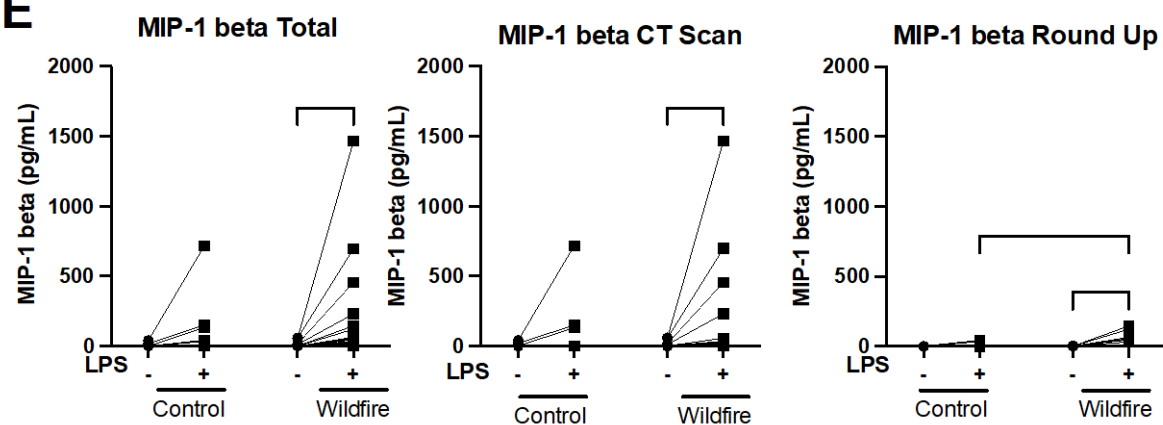
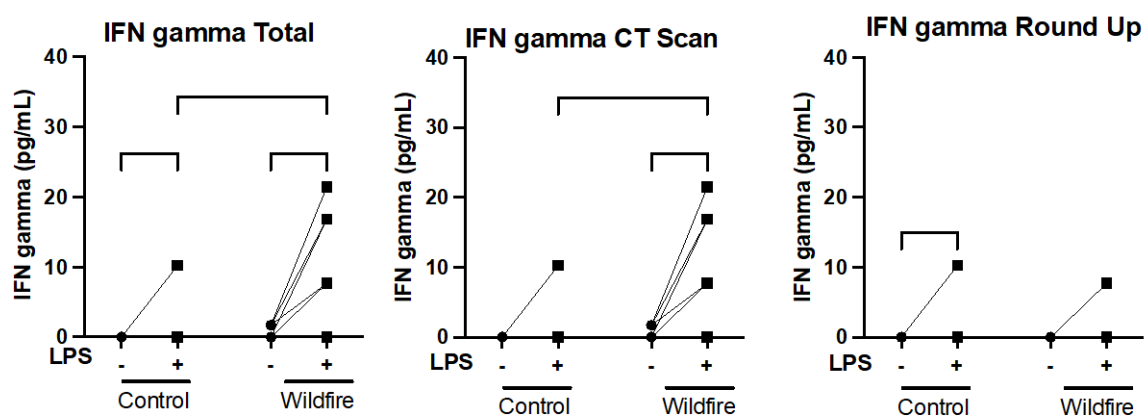
To determine whether the immune response to an infectious challenge might be compromised in animals exposed to wildfire smoke during early life, an *in vitro* PBMC culture assay established from peripheral blood samples obtained from study animals was used to assess for the ability of circulating immune cells to respond to a microbial ligand challenge as previously described <sup>1</sup>. Cytokine responses following 24-hour treatment of PBMC cultures with the Toll-like receptor 4 agonist, LPS, were measured with a non-human primate 29-plex Luminex panel. LPS is a component of gram-negative bacteria cell walls and can rapidly elicit an innate immune response that mimics a bacterial infection both *in vivo* and *in vitro*. All 29 cytokines tested by the panel were detectable by cultured PBMC following LPS treatment. Of the 29 cytokines, 22 were determined to be significantly different between exposed (born in 2008) and control (born in 2009) adult animals at thirteen years of age (Figure 6). In addition to undergoing a Luminex panel, we also evaluated the dose-dependent secretion of IL-8 and IL-6 in response to LPS treatment. At 6 hours (Figure 8) and 24 hours (Figure 9) we detected differential cytokine secretion in association with wildfire smoke exposure. As with our Luminex panel, with some cytokines, there were differences in whether animals were undergoing CT scans versus outdoor round up blood collection. We do not yet understand the basis for the differences, although it is likely due to animal stress.

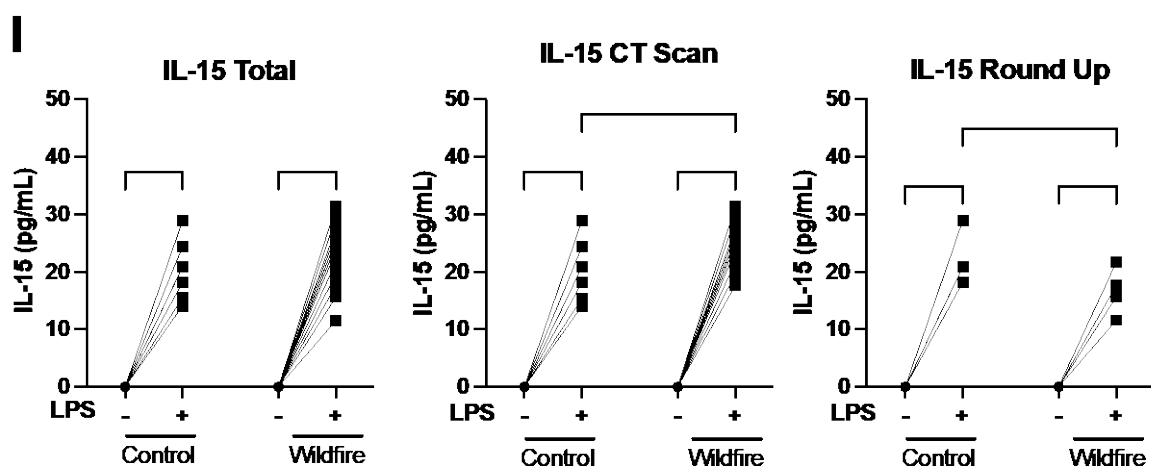
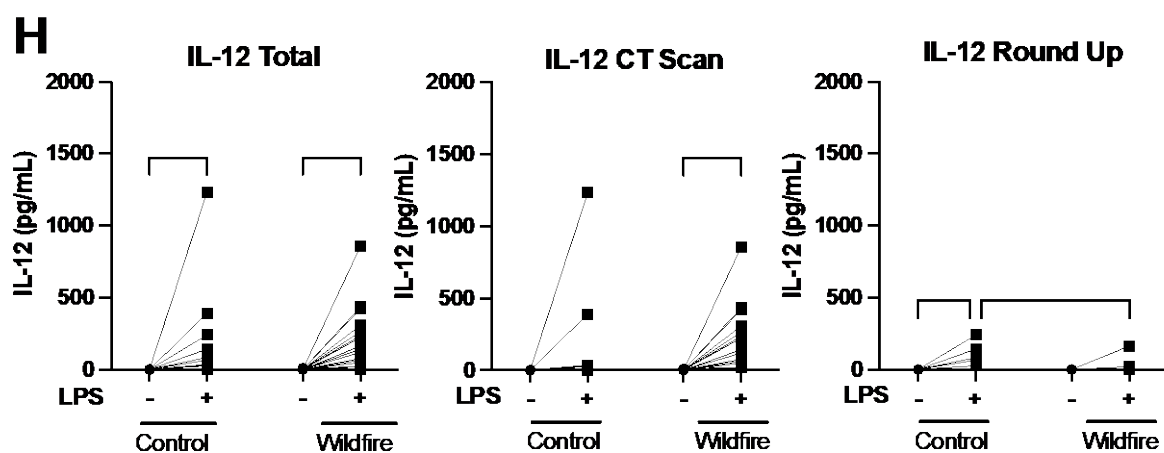
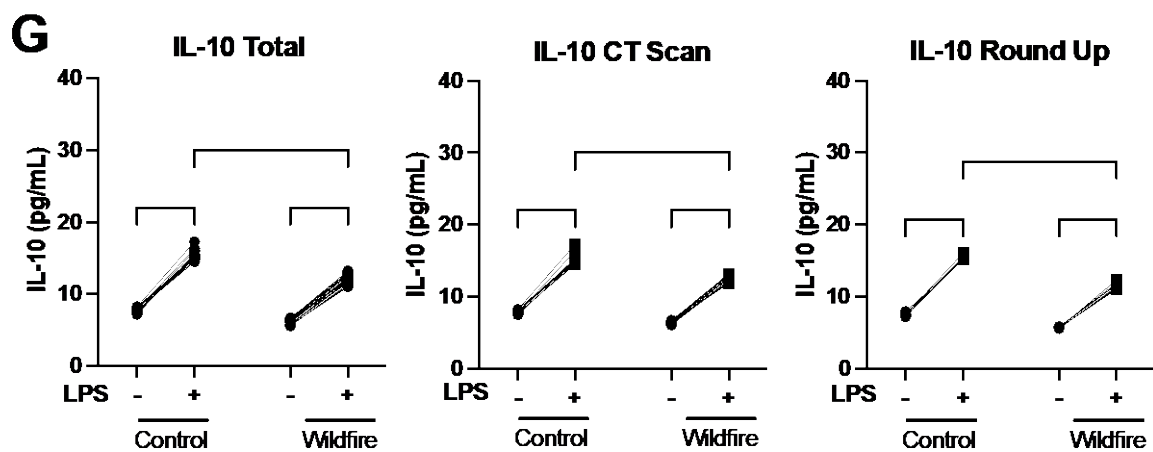


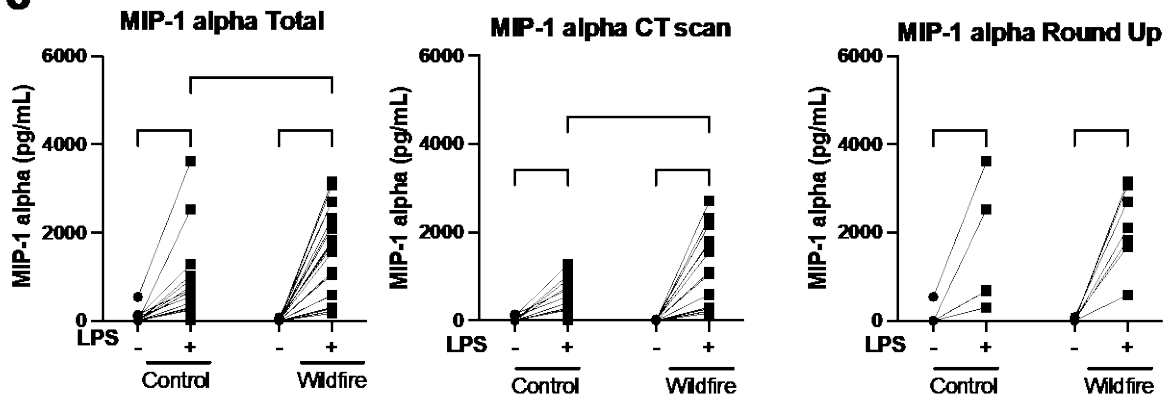
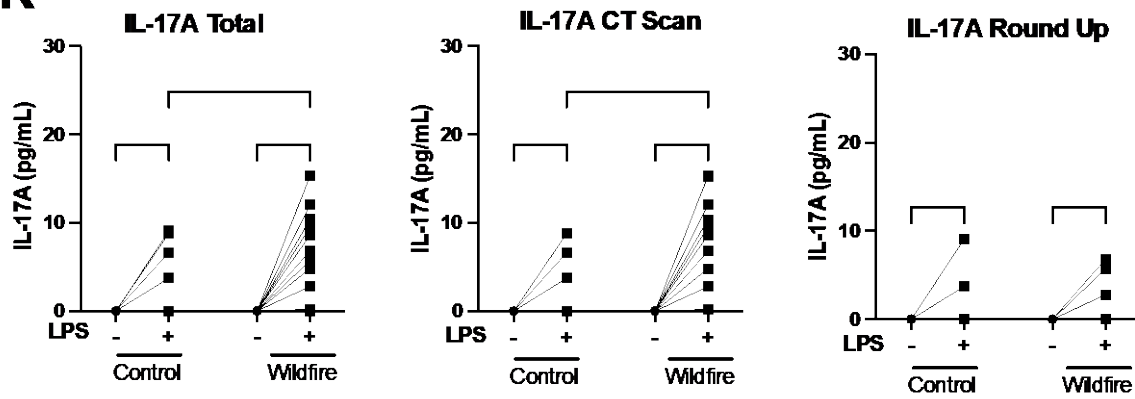
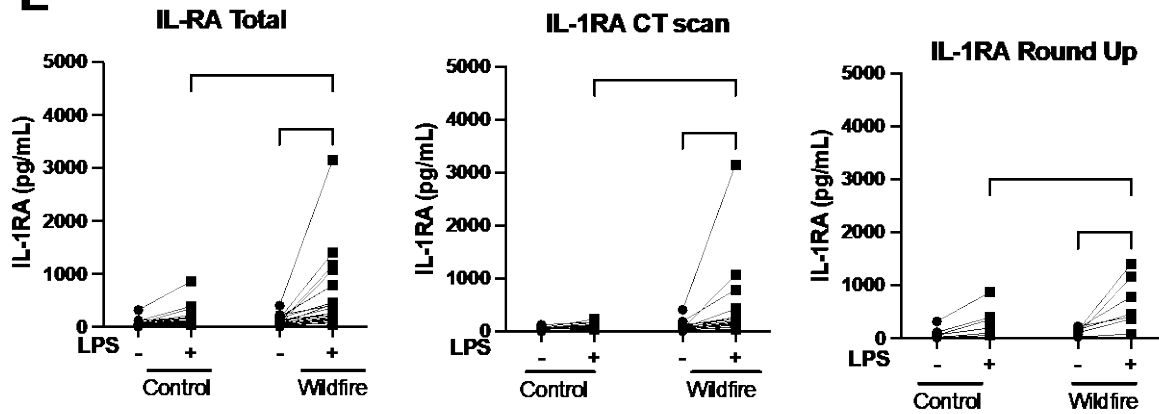
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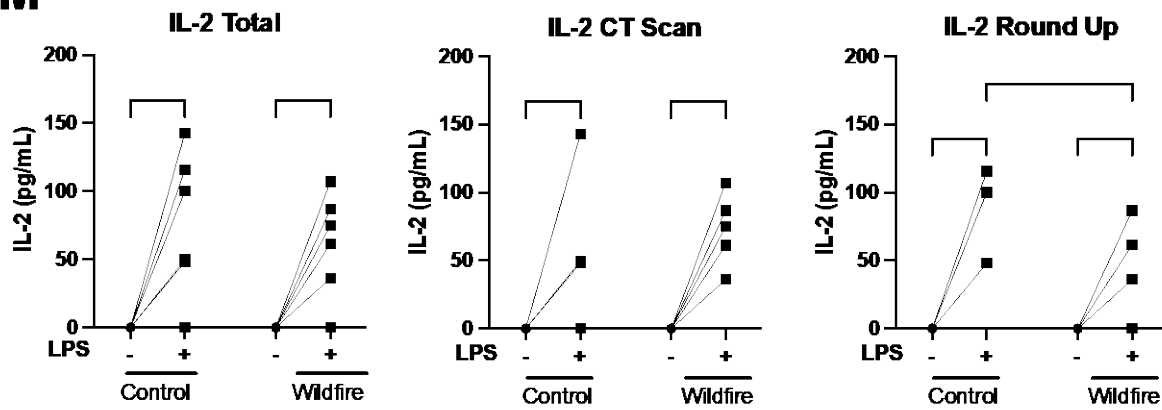
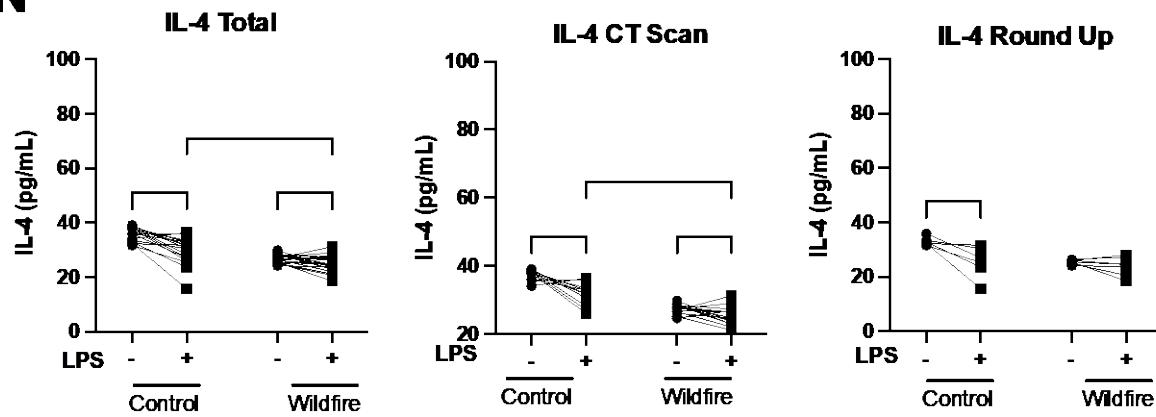
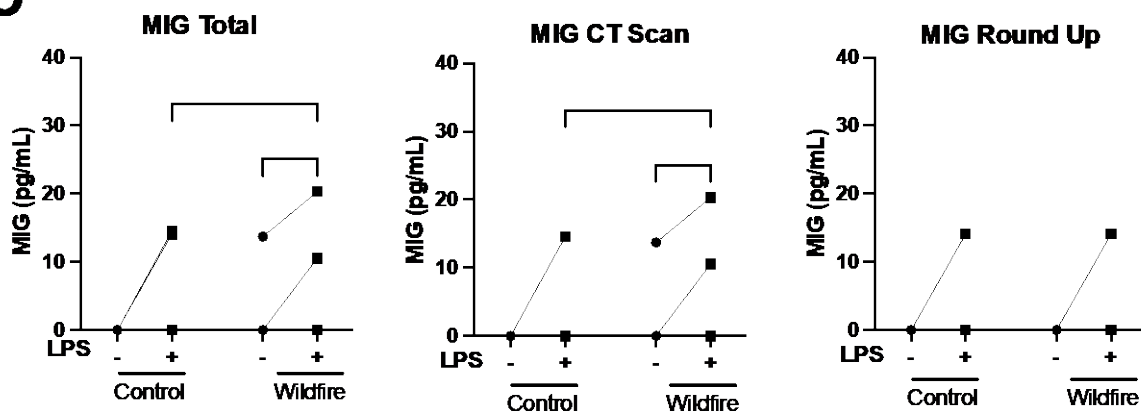


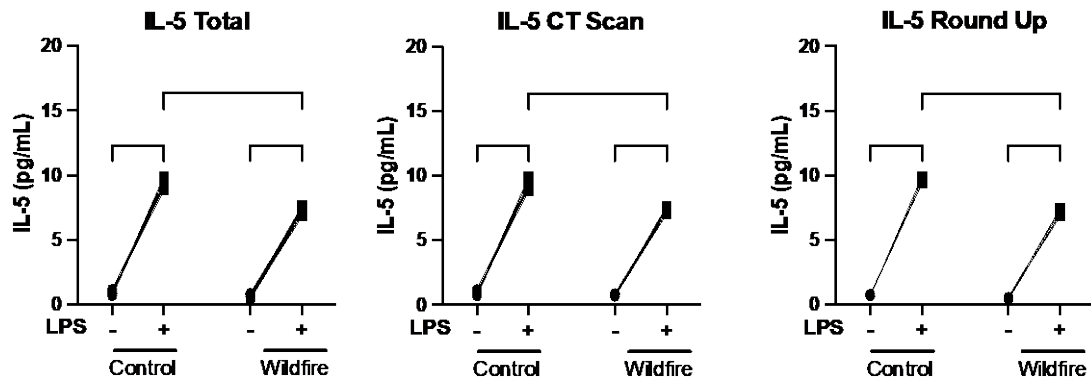
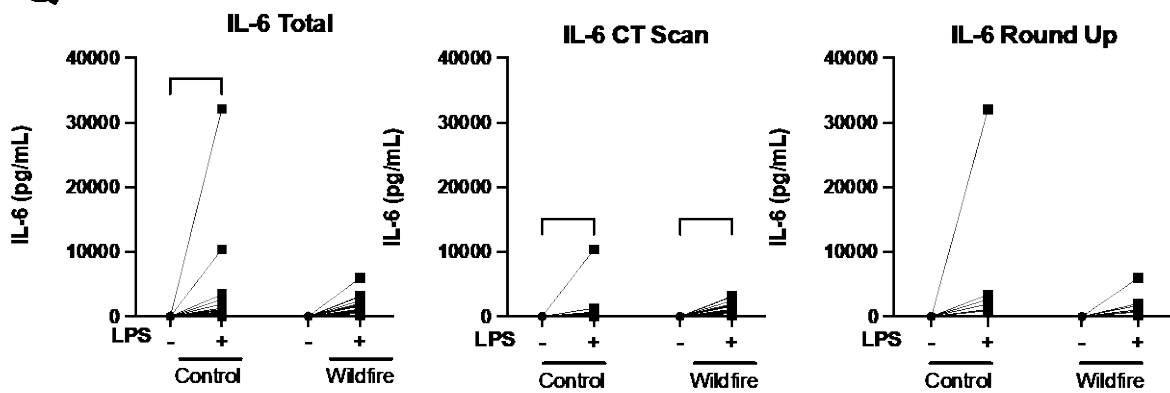
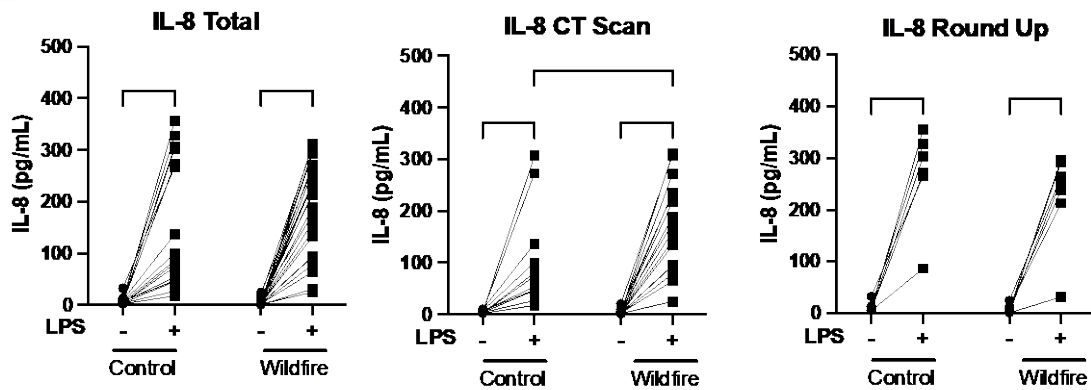


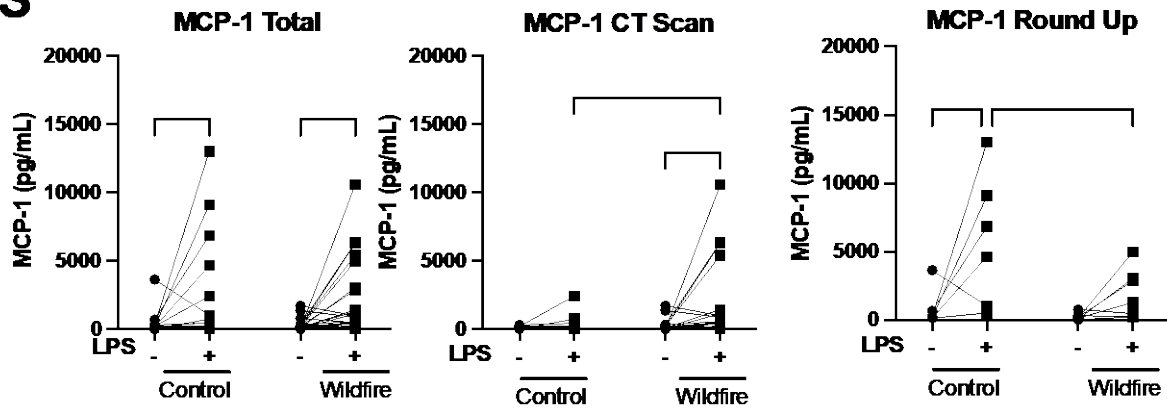
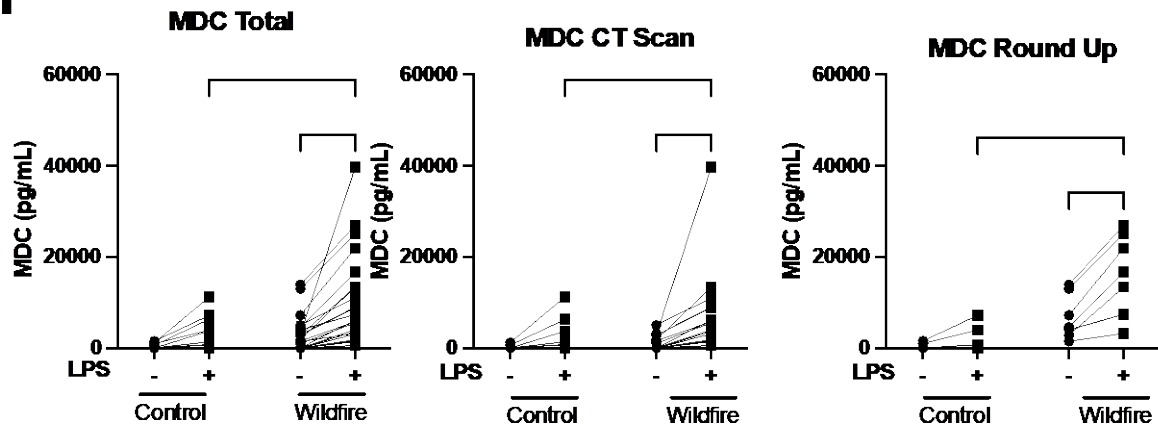
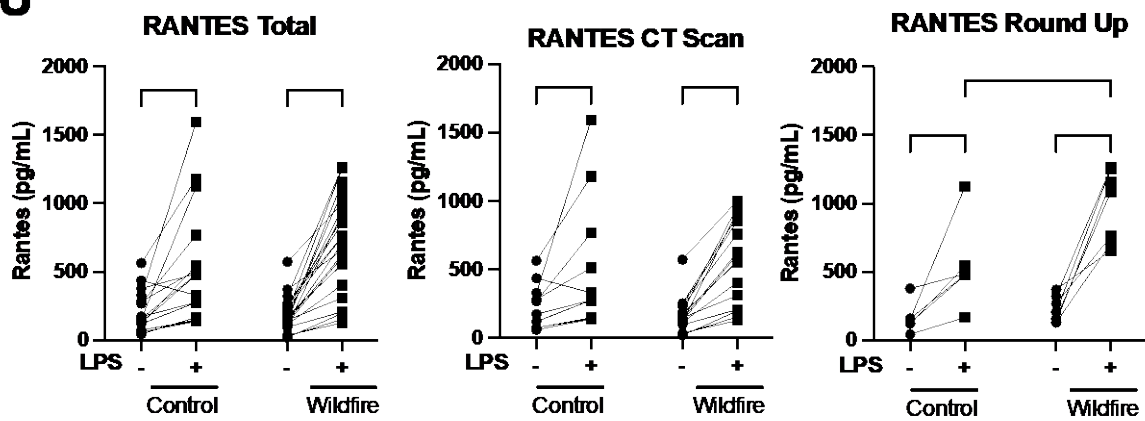
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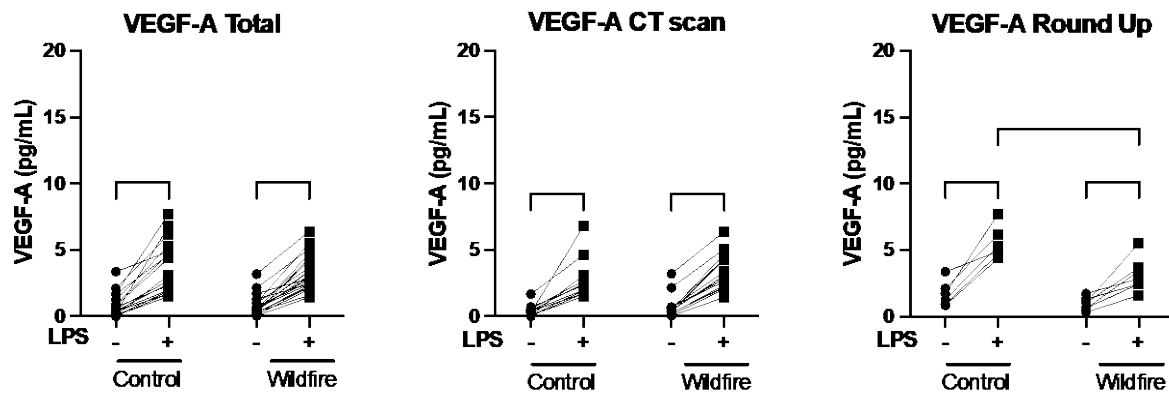
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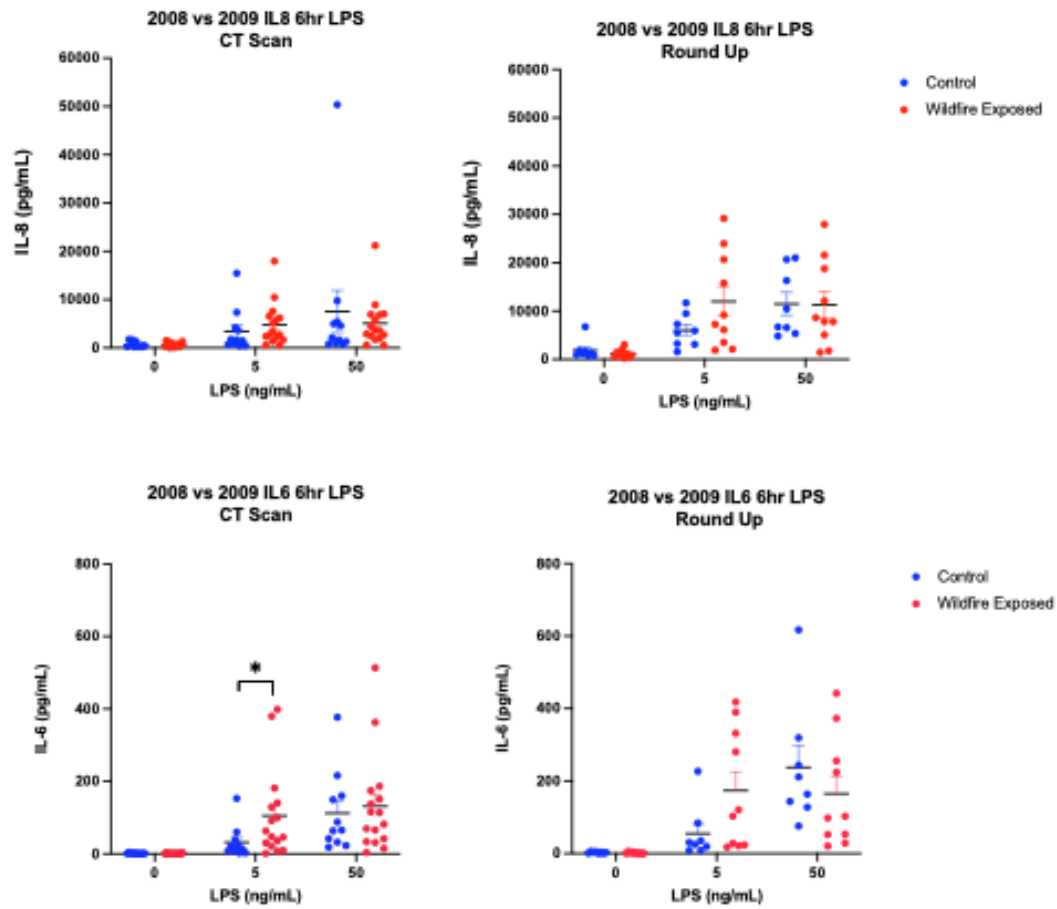
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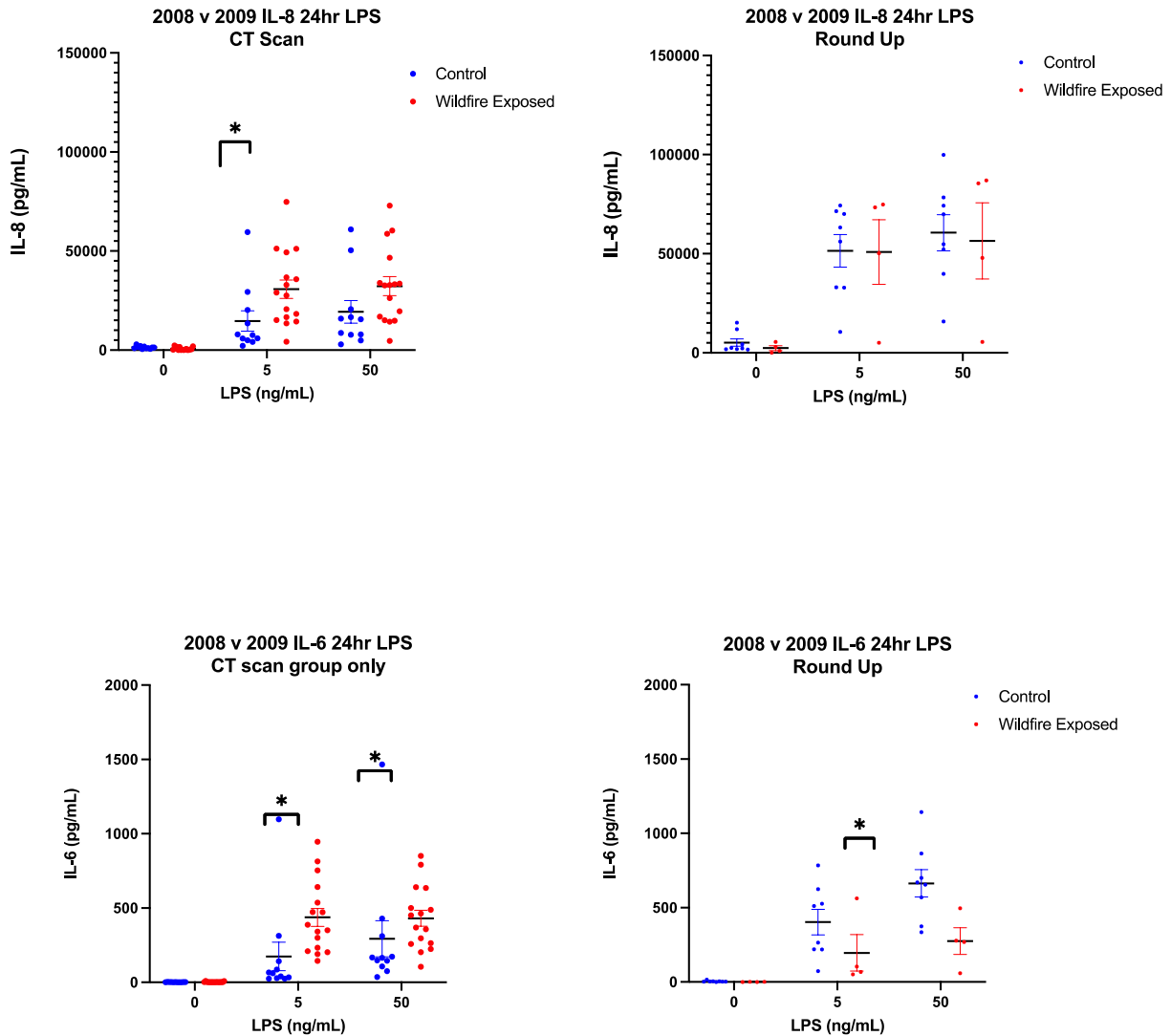
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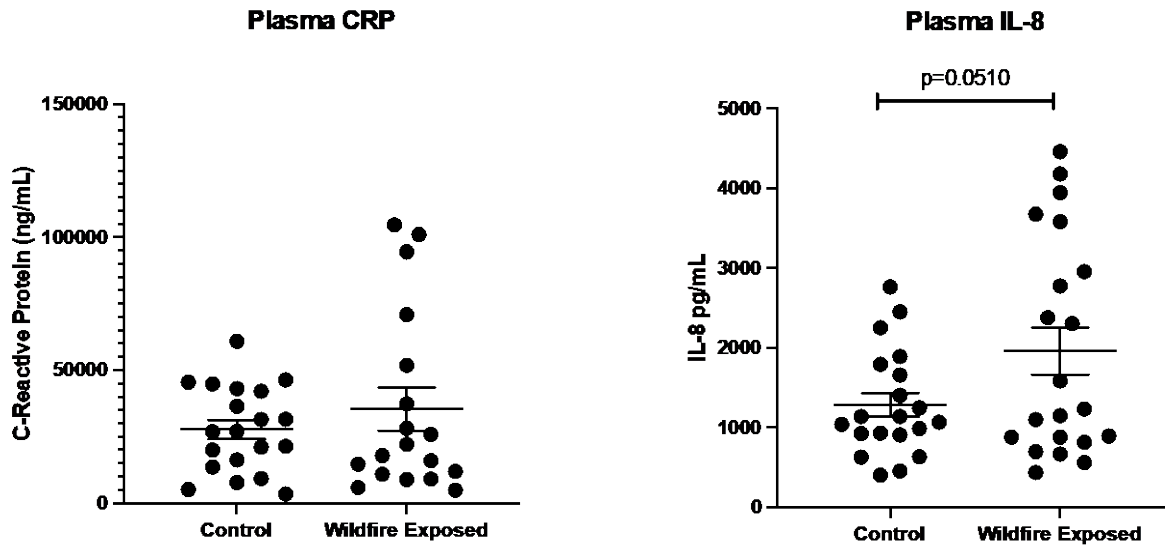
**Figure 8. Association of dysregulated innate immune responses with early life wildfire smoke PM<sub>2.5</sub> exposure in adult female monkeys following 6 hours of LPS treatment.** PBMC from exposed (n=20) or control (n=19) adult female monkeys were stimulated with LPS for 6 hours. Supernatant from cell cultures was collected and measured for IL-8 and IL-6 by ELISA. Animals evaluated at CT scan or outdoor round up were separately graphed for comparison. \* p-value < 0.05.



**Figure 9. Association of dysregulated innate immune responses with early life wildfire smoke PM<sub>2.5</sub> exposure in adult female monkeys following 24 hours of LPS treatment.** PBMC from exposed (n=20) or control (n=19) adult female monkeys were stimulated with LPS for 24 hours. Supernatant from cell cultures was collected and measured for IL-8 and IL-6 by ELISA. Animals evaluated at CT scan or outdoor round up were separately graphed for comparison. \* p-value < 0.05.

#### Plasma CRP and IL-8 Protein Analysis

To understand how wildfire smoke exposure might alter systemic immunity, levels of IL-8 protein and CRP were measured in the serum of both adult female monkeys and using standard ELISA methods. We did not detect statistically significant differences in plasma CRP between control and wildfire smoke exposed animals. There was a trend toward increased plasma IL-8 with wildfire smoke exposure which did not reach statistical significance ( $p=0.0510$ ); additional analysis separating outdoor housed versus HRCT scanned animals may distinguish exposure effects.



**Figure 10. Plasma CRP and IL-8 protein concentration following early life wildfire smoke PM<sub>2.5</sub> exposure.** Plasma CRP ELISA was conducted comparing control (n=20) with exposed (n=18) animals. Plasma IL-8 ELISA was conducted comparing control (n=20) with exposed (n=21) animals.

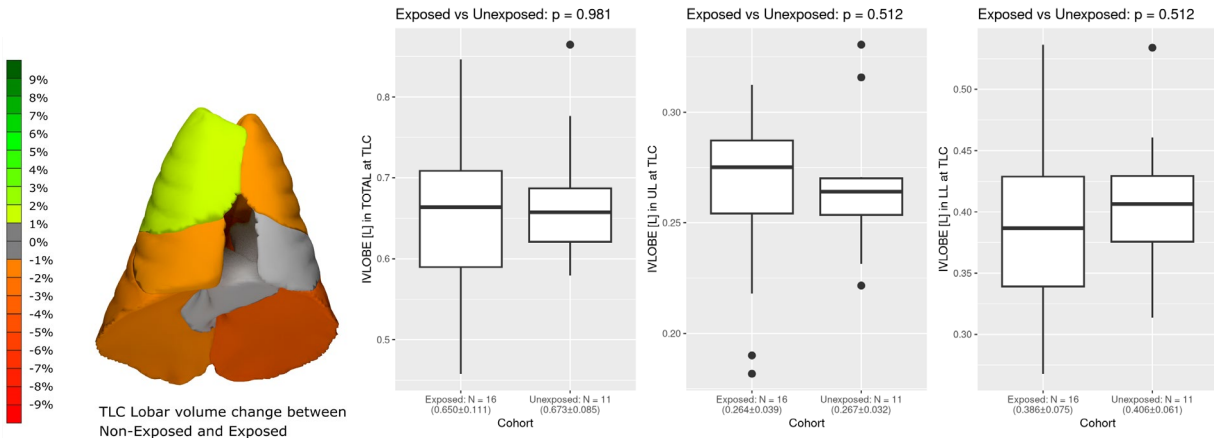
#### **Task #4**

**Determine if parameters of respiratory health including lung volume and remodeling have been persistently compromised with wildfire smoke exposure in 13-year-old CNPRC monkeys housed outdoors during and subsequent to the 2008 Humboldt and Trinity County summer wildfire events.**

#### **Analysis of Lung Structure and Function**

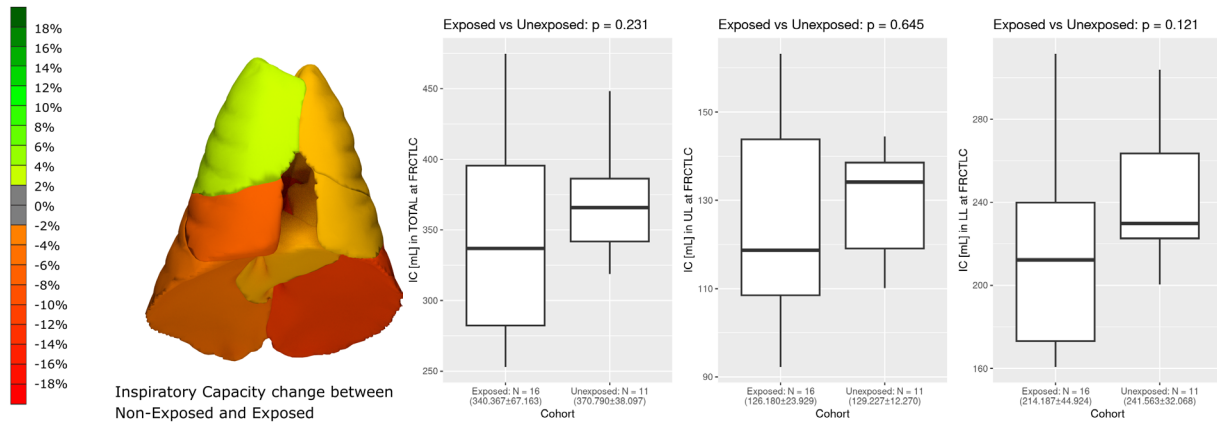
A subset of thirteen-year-old adult female monkeys exposed to early life wildfire smoke PM<sub>2.5</sub> and control animals were randomly selected for evaluation by HRCT. HRCT imaging can be a more clinically relevant methodology to evaluate anatomical and physiological changes present as a result of antecedent wildfire exposure due to the more frequent use of this modality to assess human patients for chronic lung diseases such as pulmonary fibrosis and chronic obstructive pulmonary disease. FRI technology recently developed by an industry partner was used to quantitatively determine if any differences in pulmonary physiology existed between the two cohorts.

Results from HRCT analysis indicate no significant differences in total lung volume and inspiratory capacities as thirteen-year-old adults (Figures 11-12). Ventilation perfusion ratios were also not significantly different. There was a trend ( $p=0.08$ ) toward increased airway radius in the upper lung lobes, as well as a trend ( $p=0.089$ ) toward increased airway radius in lower lung lobes (Figure 13). Analysis of the blood vessel density revealed a significant increase in blood vessel volume for wildfire exposed animals relative to controls, which was limited to the lower lung lobes (Figure 14).



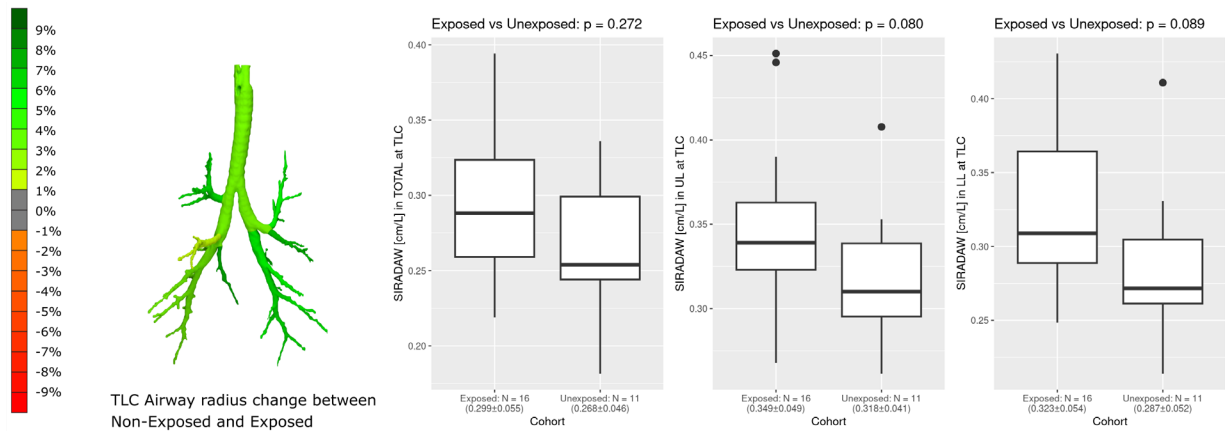
**Figure 11. High resolution computed tomography thoracic wall imaging of adult female monkeys: total lung capacity.**

HRCT images at total lung capacity (TLC) were analyzed to determine total lung volume. Box plots show the average values for lung volume for the total lung (TOTAL), upper lobes only (UL), and lower lobes (LL) +/- standard deviation of n=11-16 monkeys per group. Changes to lobe specific sections are represented as the average as percent deviation by wildfire exposed monkeys compared to controls for lobar volume. Red indicates a decrease, whereas green indicates an increase in the average values for wildfire exposed animals compared to controls.

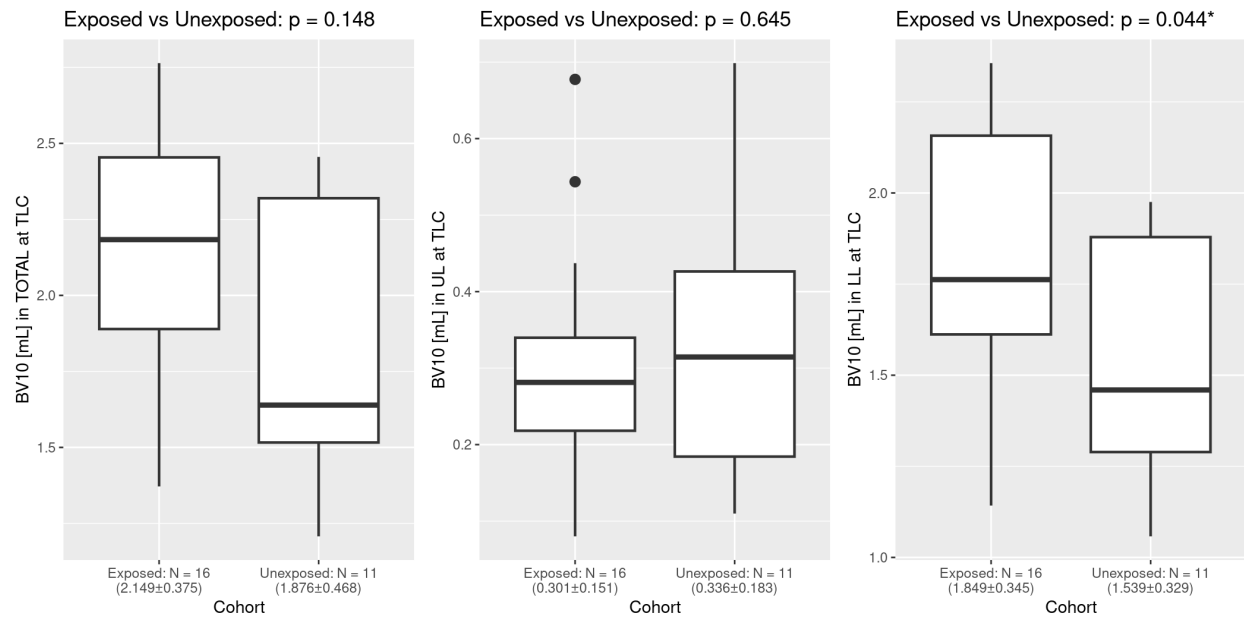


**Figure 12. High resolution computed tomography thoracic wall imaging of adult female monkeys: inspiratory capacity.**

HRCT images at total lung capacity were used to measure inspiratory capacity (IC). Box plots show the average values for inspiratory capacity for the total lung (TOTAL), upper lobes only (UL), and lower lobes (LL)  $\pm$  standard deviation of n=11-16 monkeys per group. Changes to lobe specific sections are represented as the average as percent deviation by wildfire exposed monkeys compared to controls for inspiratory capacity. Red indicates a decrease, whereas green indicates an increase in the average values for wildfire exposed animals compared to controls.



**Figure 13. High resolution computed tomography thoracic wall imaging of adult female monkeys: airway radius.** HRCT images at total lung capacity were analyzed to determine specific airway radius. Box plots show the average values for specific airway radius for the total lung (TOTAL), upper lobes only (UL), and lower lobes (LL) +/- standard deviation of n=11-16 monkeys per group. Changes to lobe specific sections are represented as the average as percent deviation by wildfire exposed monkeys compared to controls for specific airway radius. Red indicated a decrease, whereas green indicated an increase in the average values for wildfire exposed animals compared to controls.



**Figure 14. High resolution computed tomography thoracic wall imaging of adult female monkeys: vascular density.** HRCT images at total lung capacity were analyzed to determine vascular density. Box plots show the average values for vascular density for the total lung (TOTAL), upper lobes only (UL), and lower lobes (LL) +/- standard deviation of n=11-16 monkeys per group. \* $p < 0.044$

## **Task #5**

**Assess whether parameters of respiratory function correlate with activity levels in 13-year-old CNPRC monkeys housed outdoors during and subsequent the 2008 Humboldt and Trinity County summer wildfire events.**

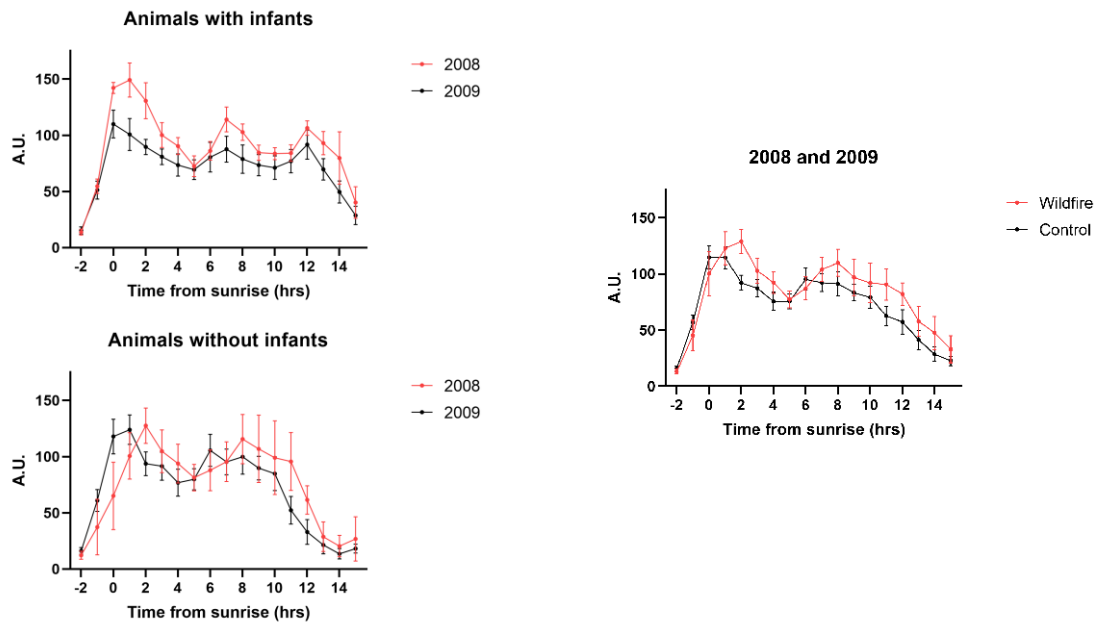
### **Analysis of Activity Monitoring**

Quantitative tracking data for activity and sleep were obtained using CamNtech Actiwatch monitors that were recorded every 30 seconds for one week. Activity monitors were worn by 13- and 14-year-old monkeys using 3D-printed collars; all animals remained housed in large social groups in half-acre field cages during data collection. A larger cohort of animals (n= 11 adult female monkeys exposed to early wildfire smoke PM<sub>2.5</sub>; n= 19 adult female monkey controls) were initially evaluated over a period of 3 months. Because many of the evaluated animals had given birth, there was variation in whether animals carried infants; a subset of nursing animals were selected out of our initial cohort for consistency (n=5 for 2008 and n=7 for 2009). Serum cortisol levels were measured in historical samples from 2008 wildfire exposed and 2009 control infant monkeys.

Because of elevated activity levels in the wildfire smoke-exposed adult monkeys, we investigated whether measures of stress (cortisol) differed between animal groups. As a part of the CNPRC biobehavioral assessment program, we compiled measures of cortisol levels in infant monkeys exposed to wildfire smoke in 2008. Dexamethasone suppression test measures cortisol regulation and is performed routinely as a behavioral measure for CNPRC infant monkeys. For the measure, blood samples are initially drawn approximately two hours after the infants had been separated from their mothers and relocated to the testing environment; these values are “stressed” values. Blood samples are subsequently drawn at the conclusion of behavioral testing and is consistent with prolonged stress from separation and relocation.



**Figure 15. 3D-printed nylon collar with Actiwatch mini (quarter for scale).**



**Figure 16. Five-day average activity levels for animal cohorts normalized to sunrise.**

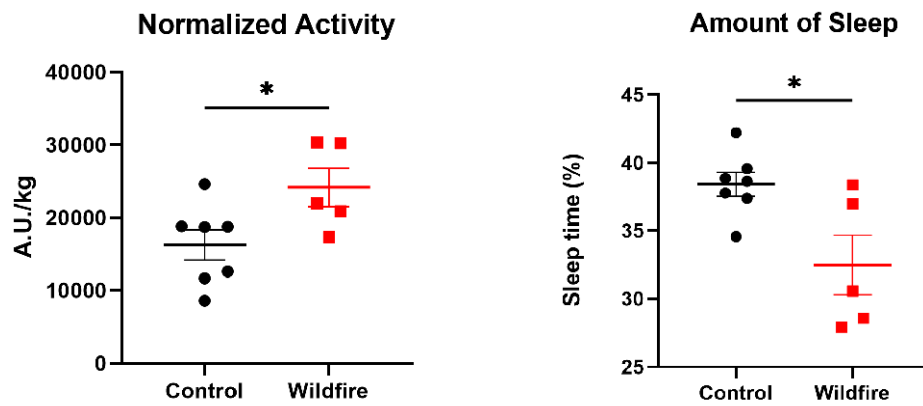
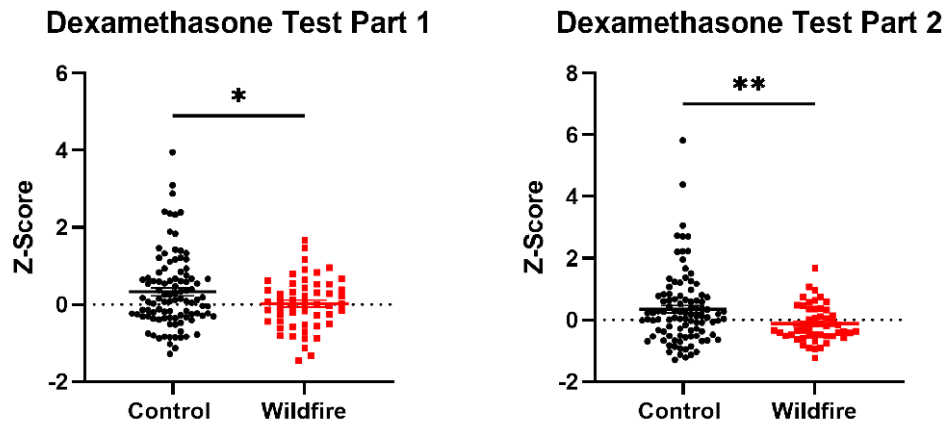


Figure 17. Weight-normalized activity levels and percentage of day spent sleeping for nursing animals. \* $p < 0.05$  compared to control



**Figure 18. Dexamethasone testing Z-scores for 2008 and 2009 cohorts at the California National Primate Research Center.** Dexamethasone suppression test measures cortisol regulation and is performed routinely as a behavioral measure for California National Primate Research Center infant monkeys. Samples for the test were collected within 3 months following the 2008 wildfire event. \* $p < 0.05$  compared to control

- Sample 1 was drawn approximately two hours after the infants had been separated from their mothers and relocated to the testing environment. Consequently, these values are “stressed” values.
- Sample 2 was drawn at the conclusion of behavioral testing and is consistent with prolonged stress from separation and relocation.

## Discussion

The US Environmental Protection Agency recognizes wildfire smoke as a significant source of particulate matter under 2.5 microns diameter (PM<sub>2.5</sub>) emissions, which are expected to increase because of climate change<sup>33, 34</sup>. The National Oceanic and Atmospheric Administration estimates that 200 million people in the United States live in counties affected by wildfire smoke conditions<sup>35</sup>. Exacerbation of pre-existing respiratory conditions, including asthma and COPD, is associated with wildfire events. However, there is a paucity of data on whether wildfire smoke exposure can promote the development of chronic disease<sup>36-43</sup>. Understanding the human health risks of wildfires is complicated by highly variable emissions from combined combustion of biomass and anthropogenic materials at the wildland urban interface. Further, growing epidemiological evidence suggests that particulate matter source and composition can be associated with more severe health outcomes. There is a compelling need to understand the toxicology of wildfire smoke exposures for public health, particularly in vulnerable populations such as young children.

Long term respiratory health effects of early life pollution exposures have been documented in human epidemiology; however, there are limited data on extreme air quality events associated with chronic disease. Bharadwaj et. al. 2016 reported that early life exposure to the Great Smog of London in 1952 significantly enhanced the likelihood of childhood and adult asthma, suggesting that an extreme air quality event can result in health effects that persist with maturity<sup>12</sup>. Our investigation using non-invasive physiological parameters in a nonhuman primate cohort offers a unique opportunity to investigate the long-term health impacts of an acute ambient air pollution event (wildfire smoke PM<sub>2.5</sub>) in a laboratory animal housed in a well-characterized environment at the CNPRC. For this study, we investigated a cohort of CNPRC outdoor housed female adult rhesus monkeys that were born within three months prior to the Trinity and Humboldt County wildfires of June/July 2008. We hypothesized that wildfire smoke PM<sub>2.5</sub> exposure during early life would result in an altered innate immune response to infection, lung function decrements and altered activity levels in adult animals. Our hypothesis was based upon previously published findings in wildfire smoke PM<sub>2.5</sub> exposed adolescent animals<sup>1, 2</sup>. To test this hypothesis, we (1) evaluated the immune response to microbial ligands in blood samples, (2) obtained lung structural data using *in vivo* imaging methods and (3) used accelerometers to measured activity levels in outdoor housed animals.

### *Integration of Results.*

Immune function. Our initial studies in wildfire smoke-exposed juvenile animals, we found that peripheral blood responses to LPS challenge in culture resulted in reduced secretion of IL-6 and IL-8.<sup>1</sup> Comparatively, our current findings in thirteen-year-old adult animals are very similar to our findings in eight year old adult animals, where we observed the IL-6 and IL-8 response to LPS was increased in peripheral blood cultures<sup>2</sup>. We had speculated that inflammaging (systemic shifts in cytokine production are inherent with aging) could explain the shifts in responsiveness relative to younger animals. Inflammaging is defined as the tendency to increase cytokine production in response to

similar stimuli with age; presently however, the mechanism driving this observation is still unclear<sup>44, 45</sup>. Our data from thirteen-year-old animals is consistent with the notion of enhanced inflammatory responses in association with maturation. The enhanced maturation of systemic inflammation with age is further supported by multiplex cytokine analysis. In eight-year-old animals, we had previously detected changes in six cytokines classically associated with monocytes and macrophages. Our most recent findings in thirteen-year-old animals using a similar multiplex panel showed an exposure effect for 22 different cytokines out of the initial panel of 29 cytokines, suggesting a more expansive detrimental effect on the immune system. These results suggest that wildfire smoke exposure elicits a persistently altered immune profile despite the transition from young to older adulthood.

Lung function. Our initial HRCT findings in eight-year-old animals showed significant differences in lung volume and inspiratory capacity between wildfire smoke exposed animals and controls, which was consistent with earlier findings in three-year-old juvenile animals. Interestingly, in our current study we did not detect significant differences in lung volume and inspiratory capacity for thirteen-year-old animals, regardless of exposure. While it is possible that a lack of difference could be due to the animal cohort, it is most likely due to the wildfire smoke-exposed animals initially showing delayed growth parameters. Indeed, human studies of traffic-related air pollution exposure support the concept that early exposures result in delayed lung function trajectories (reviewed in<sup>20</sup>).

Our previous thoracic imaging analysis in eight-year-old animals showed significantly increased specific airway radius and blood vessel density were detected. We speculated that increased pressure generated by the diaphragm during inhalation could have been redistributed to the conducting airways due to increased fibrosis at the alveoli. We postulated that increased blood vessel density might have occurred as a physiological compensatory mechanism to reduced inspiratory capacity. Indeed, previous studies have reported increased angiogenesis in IPF patients with increased IL-8<sup>46</sup>. In our current thirteen-year-old cohort, there was a trend in increased specific airway radius and significantly increased blood vessel density. It should be noted that our sample size for the control animals was reduced due to timing of the data collection; many animals in this age group were pregnant and could not be included for imaging. Although we did not evaluate differences in birth rates between the 2008 and 2009 animal cohorts in this study which may have influenced our ability to collect imaging data, our prior report did find that offspring from the 2008 females showed evidence of immune dysregulation.<sup>2</sup>

An important caveat to our finding that animals exposed to wildfire smoke showed comparable lung volumes and inspiratory capacity to controls at thirteen years of age is that this outcome does not imply a lack of pathology. The imaging approach utilized for this study relies on estimates of lung mechanics, while physiological approaches (e.g. spirometry) are a more direct measure of pulmonary function. The major advantage of the imaging approach is the ability to detect progressive changes in lung architecture, such as enlarged airways and increased blood vessel density. Notably, the persistent lung remodeling detected in thirteen-year-old animals exposed to early-life wildfire smoke is consistent with our prior findings from 2019, despite minimal overlap of

subjects in the animal cohort. These collective findings provide essential evidence of the long-term health impacts of exposure to wildfire smoke in pediatric populations.

Activity monitoring. In blood samples collected within 3 months following the 2008 wildfires or comparatively from 2009 controls, wildfire smoke-exposed animals showed significantly dysregulated serum cortisol relative to control animals. Parental psychosocial-stress, which is often associated with low socioeconomic status, increases the risk of developing childhood asthma with exposure to traffic-related air pollution, however it has not been clear whether air pollution directly contributes to mental health<sup>47</sup>. In support of a causal association, epidemiologic surveys have linked ozone and PM<sub>2.5</sub> with increased prevalence of depression, anxiety, and other psychiatric disorders<sup>48, 49</sup>. Inflammation in conjunction with oxidative stress has been speculated as a key cellular mechanism for air pollution exposure, however recent studies have suggested that PM<sub>2.5</sub> can directly alter circadian rhythm through epigenetic modifications<sup>50, 51</sup>.

Because our prior investigation of wildfire smoke health effects suggested altered lung function, we conducted activity monitoring of animals using accelerometers. Because accelerometers Quantitative tracking data for activity and sleep were obtained using CamNtech Actiwatch monitors that recorded every 30 seconds for one week. Adults that were exposed to wildfire smoke as infants in 2008 showed significantly reduced sleep duration compared to controls and a shift in peak activity relative to controls. We also detected significantly increased activity levels normalized to body weight in wildfire smoke-exposed adults relative to controls.

#### *Relevance.*

Our findings of significantly altered immunity, lung structure and sleep duration is directly relevant to the study of human health outcomes of air pollution because the nonhuman primate animal cohort was directly exposed to ambient wildfire smoke within their outdoor habitat. While the human population living within the Yolo County region may have the capacity to remain indoors during an acute air quality event, variations in building ventilation could influence exposure levels. Our finding of sleep disturbances is of particular interest given recent reports of similar dysregulation in preschoolers in association with early life exposure to PM<sub>2.5</sub><sup>52</sup>.

#### *Recommendations.*

Because the CNPRC rhesus macaques in this study are continuously housed outdoors unless undergoing clinical evaluation, the overall duration of exposure to ambient wildfire smoke PM<sub>2.5</sub> will likely be prolonged relative to the surrounding human population. As such, extrapolating our findings to humans during significant wildfire smoke PM<sub>2.5</sub> episodes should consider the amount of exposure. Moreover, it is possible that the chemistry of wildfire smoke will change throughout the course of a wildfire event, which could lead to variances in the chemical constituents of the smoke. Lastly, the accelerated maturation of the rhesus macaque monkey may also have influenced the outcomes of exposures. Despite these caveats, our collective findings of alterations in immunity, lung structure, and sleep patterns associated with wildfire smoke PM<sub>2.5</sub> provide further evidence linking acute air quality events with chronic health conditions. These results support additional evidence-based research to improve responsiveness to

wildfire events so that the most vulnerable populations are protected from long-term health effects. If funding can be obtained, future studies would examine animals that have been similarly exposed to the 2018 Camp Fire and 2020 August Complex Fire at infancy to assess whether comparable immune, respiratory and sleep dysregulation phenotypes can be observed. Based upon the known levels of PM<sub>2.5</sub> in both the 2018 Camp Fire and 2020 August Complex Fire, it would be expected that we would detect comparable, if not more robust differences in animals exposed to ambient smoke from these wildfire events.

## Summary and Conclusions

The objective of this study was to determine the impact of early life PM<sub>2.5</sub> exposure from wildfire smoke on parameters of immunity, lung function, and activity levels that influence overall health as well as responses to infectious disease. To complete this objective, we investigated a cohort of CNPRC outdoor housed female adult rhesus monkeys that were born within three months prior to the Trinity and Humboldt County wildfires of June/July 2008. We hypothesized that wildfire smoke PM<sub>2.5</sub> exposure during a window of early life would result in dysregulation of innate immune response to infection, lung function decrements and altered activity levels in adult animals. Our hypothesis was based upon previously published findings in wildfire smoke PM<sub>2.5</sub> exposed adolescent animals. To test this hypothesis, we (1) evaluated the immune response to microbial ligands in blood samples, (2) obtained lung structural data using *in vivo* imaging methods and (3) measured activity levels in outdoor housed animals. We found that monkeys that were exposed to ambient wildfire smoke PM<sub>2.5</sub> exposure during infancy showed significantly enhanced systemic inflammation, both at baseline as well as following microbial ligand challenge. We also detected significantly increased pulmonary blood vessel density and a trend toward larger airway radius in monkeys that were exposed to early life wildfire smoke. Lastly, we observed that activity levels of monkeys exposed to early life wildfire smoke was significantly increased, which corresponded to reduced sleep duration and altered cortisol responses to stress. Our observation of a significantly compromised immune response, lung remodeling and sleep disturbances in adult female rhesus monkeys exposed to early life wildfire smoke PM<sub>2.5</sub> suggests that young human subjects who were similarly exposed to wildfire smoke PM<sub>2.5</sub> in 2008 could exhibit a similar health profile, with the caveat that the animals evaluated in this study were housed outdoors throughout the 2008 wildfire event. Because we evaluated the effect of an ambient pollutant exposure on a population of monkeys living outdoors, the amount of PM<sub>2.5</sub> inhaled by individual animals may vary and potentially confound our findings. Further research is needed to determine whether humans with similar exposures to wildfire smoke PM<sub>2.5</sub> can exhibit a similar health profile.

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## Abbreviations

CARB: California Air Resources Board  
CNPRC: California National Primate Research Center  
HRCT: high-resolution computerized tomography  
PM<sub>2.5</sub>: particulate matter  $\leq$  2.5 micrometers in diameter  
PBMC: peripheral blood mononuclear cells  
ELISA: enzyme-linked immunosorbent assay  
LPS: lipopolysaccharide  
IL-6: interleukin-6  
IL-8: interleukin-8  
CRP: C-reactive protein  
FRI: Functional Respiratory Imaging  
iVlobe: lobe volume  
iVaw: airway volume  
iRaw: airway resistance  
HEPA: high-efficiency particulate air