Breathing in Trouble - When Particles Strike

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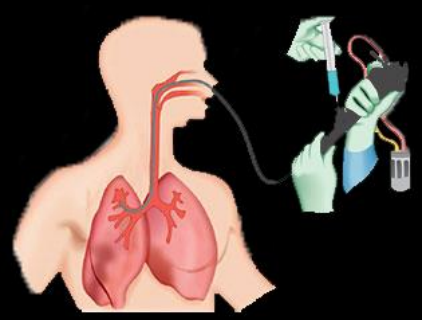
Background - IPF

- Pulmonary fibrosis affects millions of people worldwide[1,2]
- Average life expectancy of 3-5 years after diagnosis for IPF[3]
- Only 20% of patients survive 5 years after diagnosis[3]
- An estimated 40,000 lives are lost each year in the U.S. from IPF[1,4]
- Lung macrophages have a decisive role in fibrotic remodeling of the injured lung
  - Monocyte-derived macrophages contribute to the pathogenesis of pulmonary fibrosis[5-8]

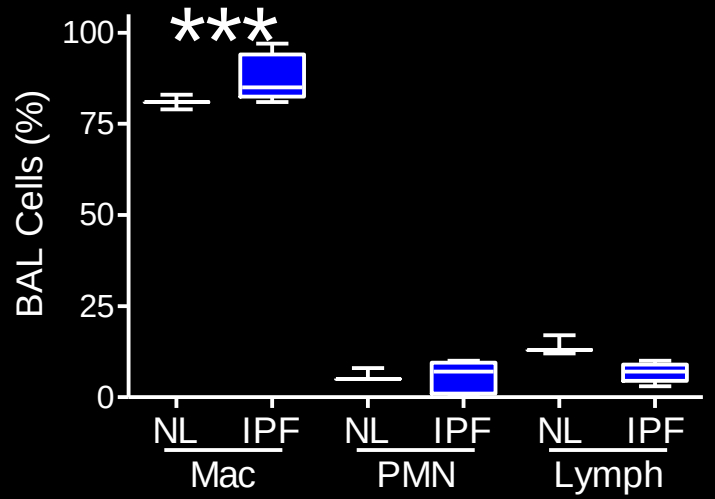
[1]Raghu *et al.* (2006) *Am. J. Respir. Crit. Care Med.*
[2]Hutchinson *et al*. (2014) *Ann Am Thorac Soc.*
[3]Raghu *et al.* (2011) *Am. J. Respir. Crit. Care Med.*
[4]Hutchinson *et al.* (2015) *Eur. Respir. J.*
[5]Misharin *et al.* (2017) *J. Exp. Med*.
[6]McCubbrey *et al.* (2018) *Am. J. Respir. Cell Mol. Biol.*
[7]He *et al.* (2019) *JCI Insight*.
[8]Larson-Casey *et al.* (2019) *J. Clin. Invest*.

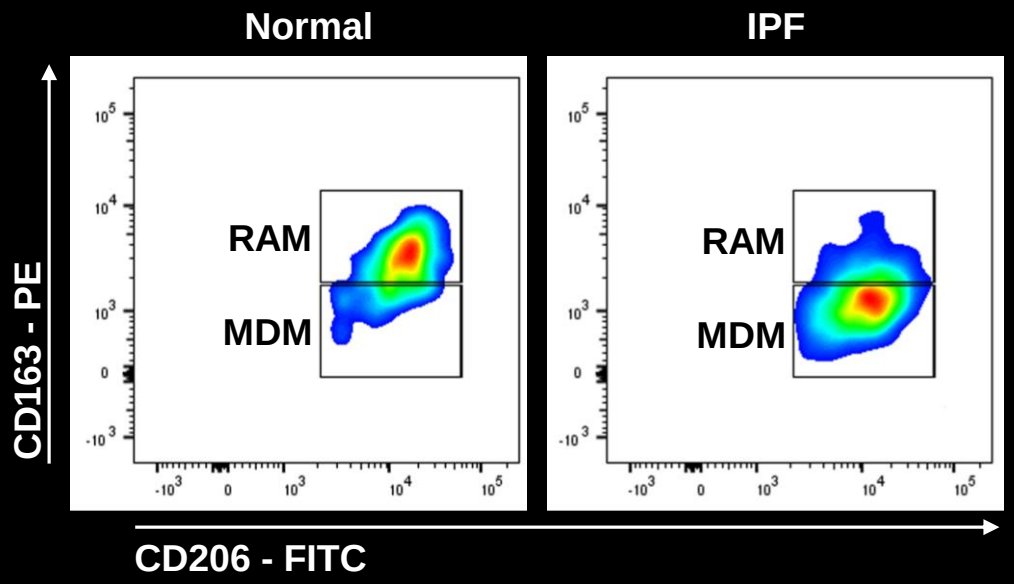
Recruited MDMs are increased in IPF lung



Isolate
Lung macrophages

Flow cytometry





MDM = monocyte-derived macrophages: $CD11b^{+}HLA\text{-}DR^{++}CD206^{++}CD169^{+}CD163^{+}$
RAM = residential alveolar macrophages: $CD11b^{+}HLA\text{-}DR^{++}CD206^{++}CD169^{+}CD163^{++}$

Larson-Casey *et al.* (2020) *PLOS ONE.*

Background - Acute Exacerbations of IPF

- Sudden acceleration of the disease that leads to a significant decline in lung function
- Up to 85% mortality rate during or immediately after AE-IPF[1]
- Median survival after AE-IPF is ~3-4 months[2]
- AE-IPF may be triggered by:
  - Viral infection
  - Lung procedures/operations
  - Aspiration
  - Pulmonary toxicity from medication
  - Environment: air pollution

?

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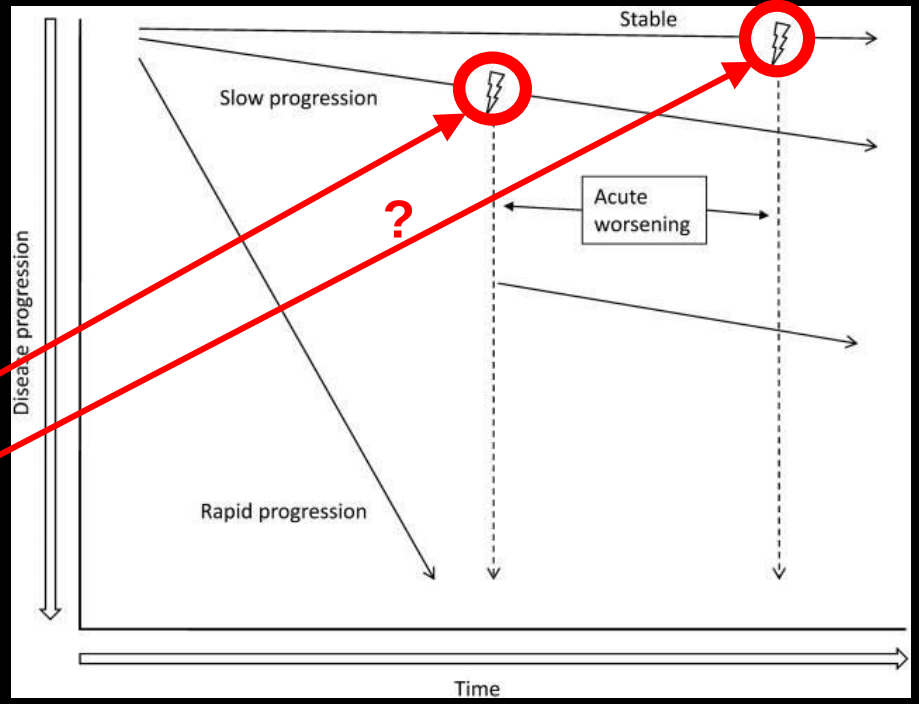
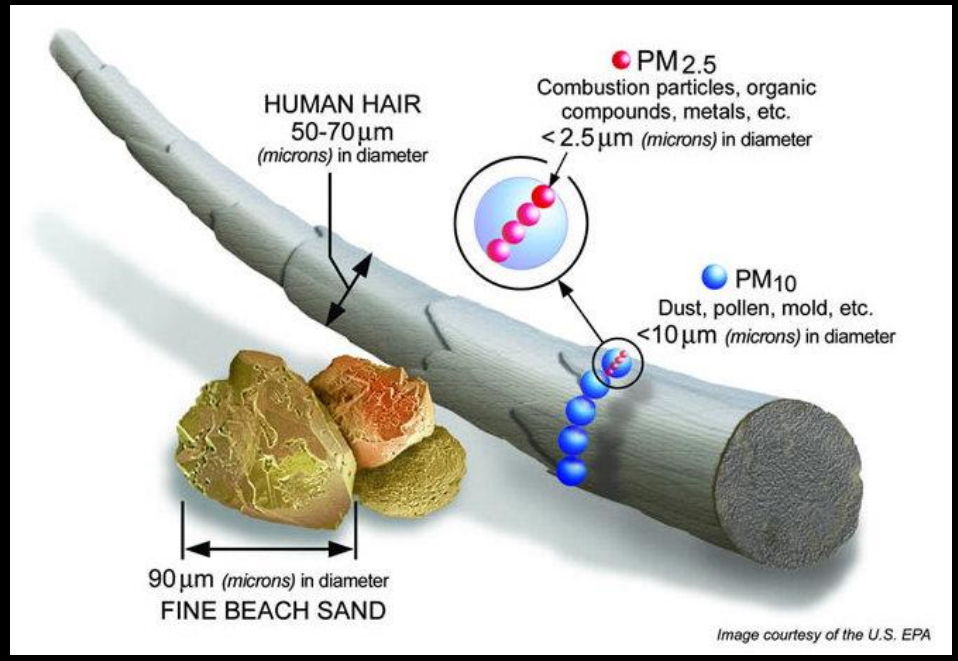


Raghu *et al.* (2011) *Am J Respir Crit Care Med.*

[1]Song *et al.* (2011) *Eur. Respir. J.*
[2]Kishaba *et al.* (2014) *Lung.*

Air Pollution & AE-IPF

- Over 120 million Americans live in communities where air pollution exceeds air quality standards[1]
- Adverse effects of air pollution exposure are well-established in patients with COPD and asthma, however; the mechanism(s) that air pollution induces AE-IPF is not known[2,3].
- Air pollution exposure is associated with increased rate of decline in lung function, number of AE, and mortality in IPF[4,5,6,7].
- Increased $PM_{2.5}$ and PM_{10} exposures are risk factors for mortality in patients with IPF[4,8].
- $PM_{2.5}$,PM_{10} and NO_2 exposure are associated with accelerated lung function decline in IPF patients[5]



[1]American Lung Association. (2023) State of the Air 2023.
[2]Landrigan *et al.* (2018) *Lancet*.
[3]Fann *et al.* (2012) *Risk Anal.*
[4]Sesé *et al.* (2018) *Thorax*.
[5]Winterbottom *et al.* (2018) *Chest.*
[6]Tahara *et al.* (2021) *Respir*. Res.
[7]Tomos *et al.* (2021) *Environ*. Health.
[8]Johannson *et al.* (2014) *Eur. Respir. J.*

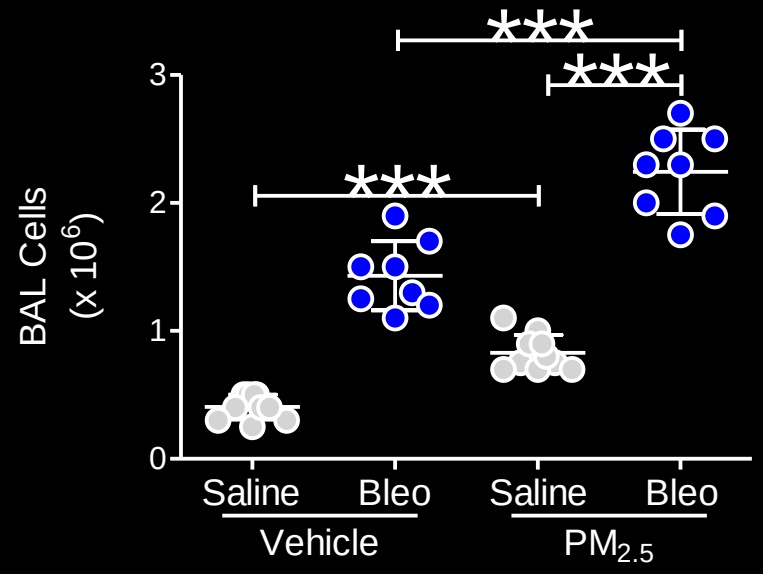
$PM_{2.5}$ increases number of cells in BAL

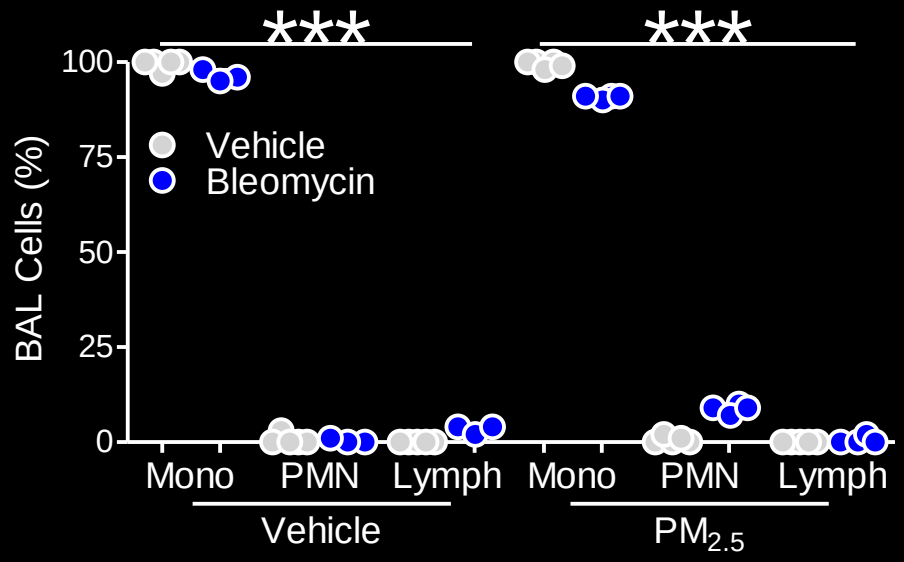
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections
at day 21

BAL at Day 28

Cell count/differential



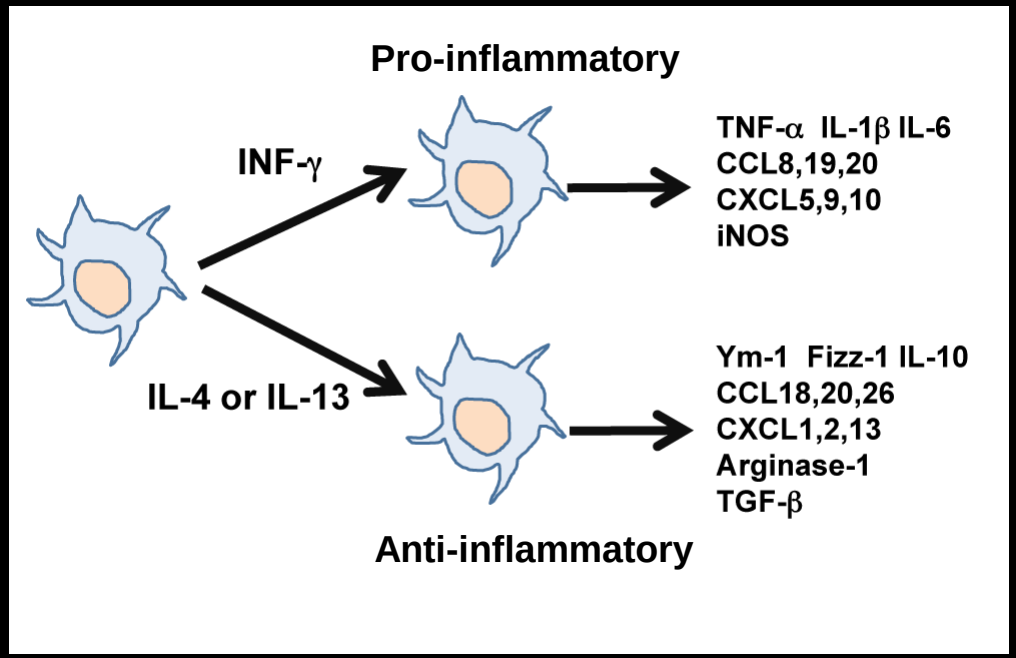


Larson-Casey *et al.* under review.

Monocyte/macrophage phenotype

- Monocyte/macrophage function varies depending on location and environment
- Monocyte/macrophages develop mixed phenotypes in complex pathological conditions
- In the IPF lung, macrophages polarize to an anti-inflammatory phenotype

- Monocyte/macrophage phenotype in $PM_{2.5}$-mediated AE-IPF is not known



He and Carter (2015) *J Clin Cell Immunol.*

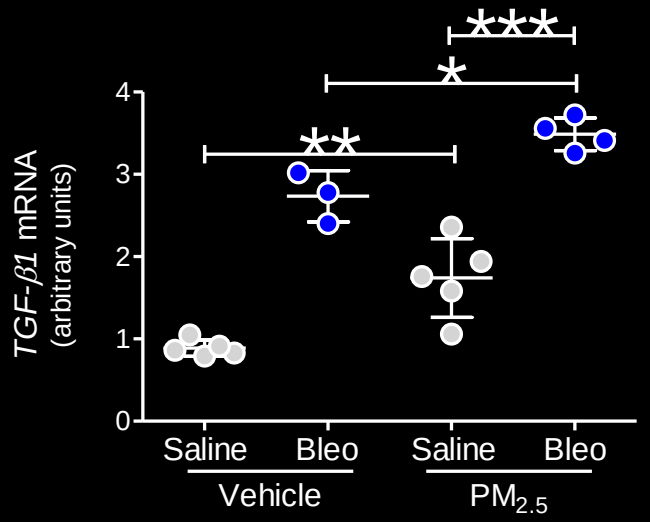
$PM_{2.5}$ mediates anti-inflammatory & pro-inflammatory phenotype

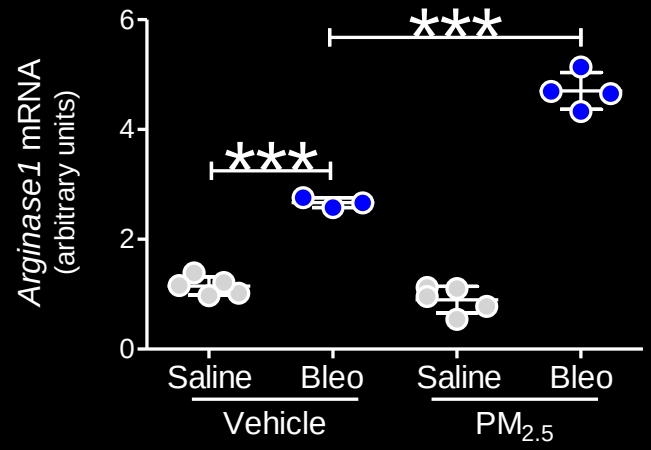
Saline or Bleomycin
1.75 U/kg

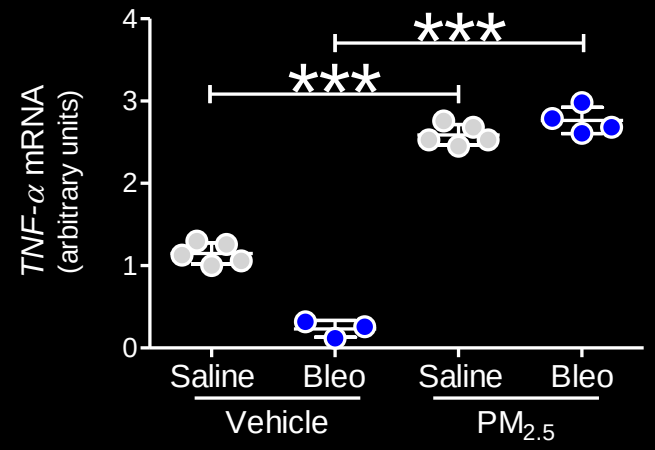
Daily $PM_{2.5}$ (i.t) injections
at day 21

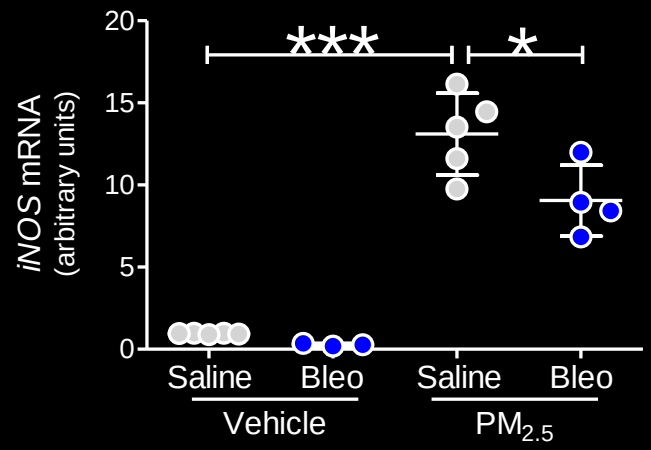
BAL at Day 28

mRNA analysis









Larson-Casey *et al.* under review.

Hypothesis

Exposure to $PM_{2.5}$ regulates recruitment of monocytes that display a mixed phenotype to promote fibrotic progression.

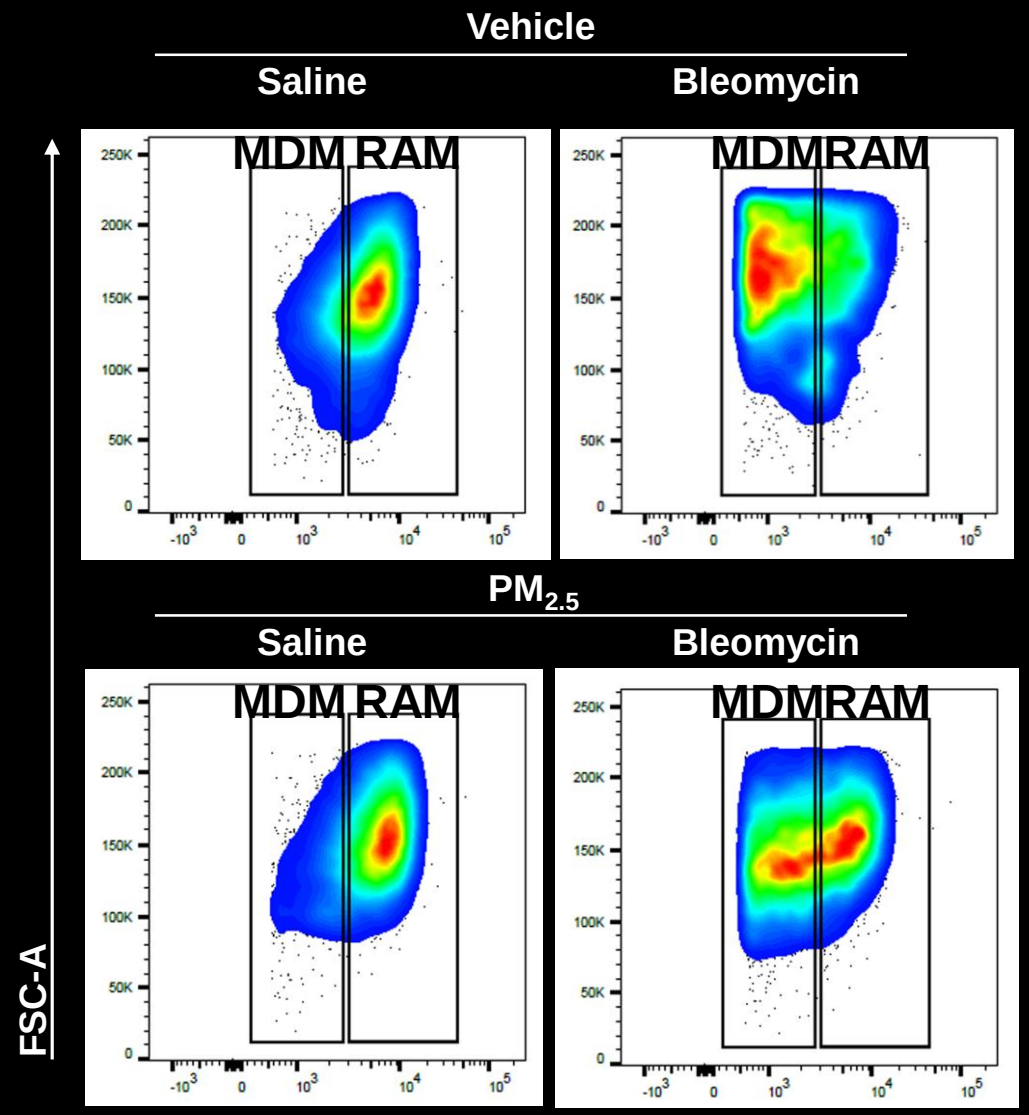
$PM_{2.5}$ does not increase MDM recruitment

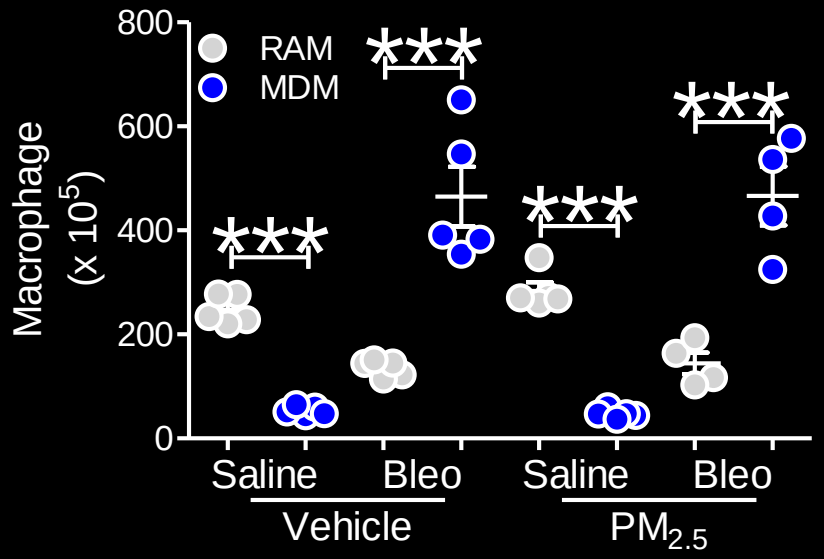
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections
at day 21

BAL at Day 28

Flow cytometry





MDM = monocyte-derived macrophages: $CD45^{+}CD11b^{+/-}Ly6G^{-}CD64^{+}Ly6C^{-}Siglec\ F^{lo}$

RAM = residential alveolar macrophages: $CD45^{+}CD11b^{+/-}Ly6G^{-}CD64^{+}Ly6C^{-}Siglec\ F^{hi}$

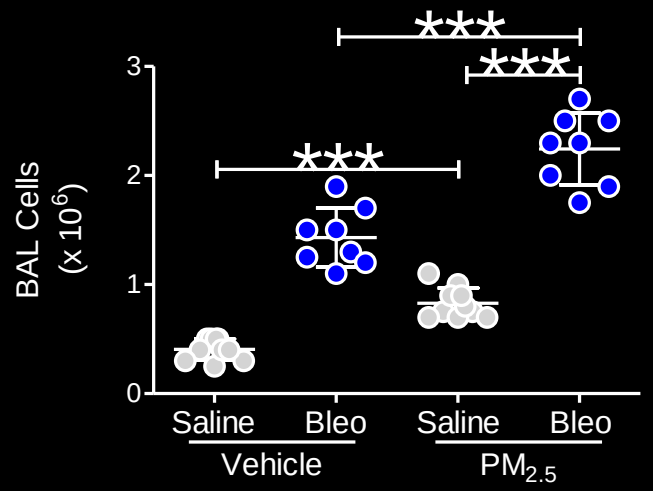
$PM_{2.5}$ increases CCL2 in BAL fluid

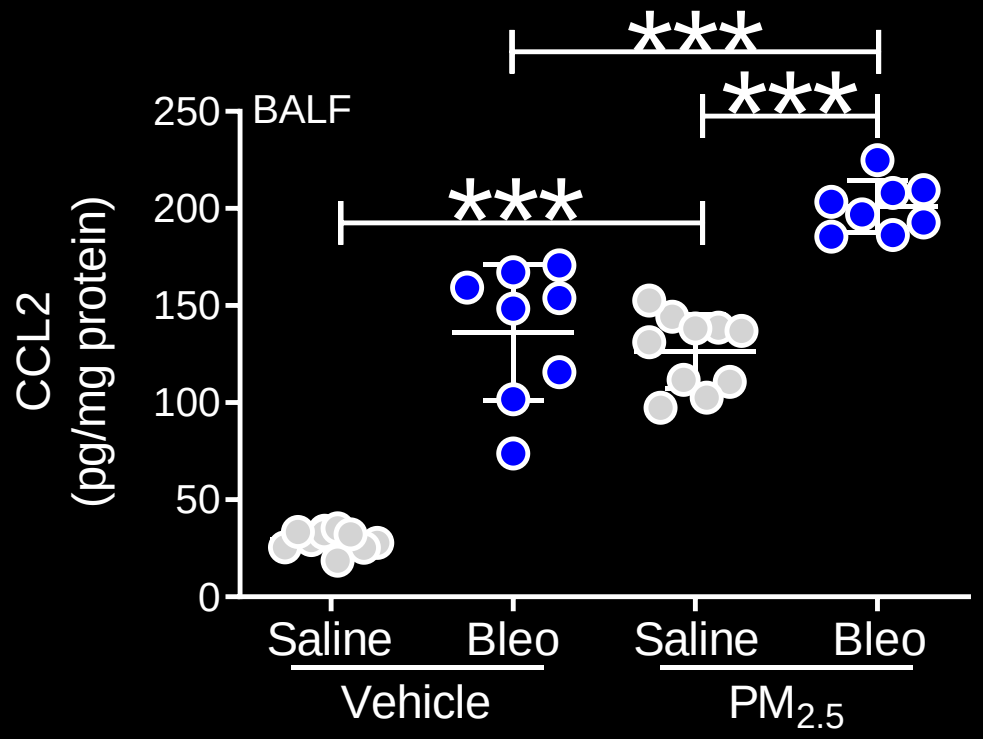
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections
at day 21

BAL at Day 28

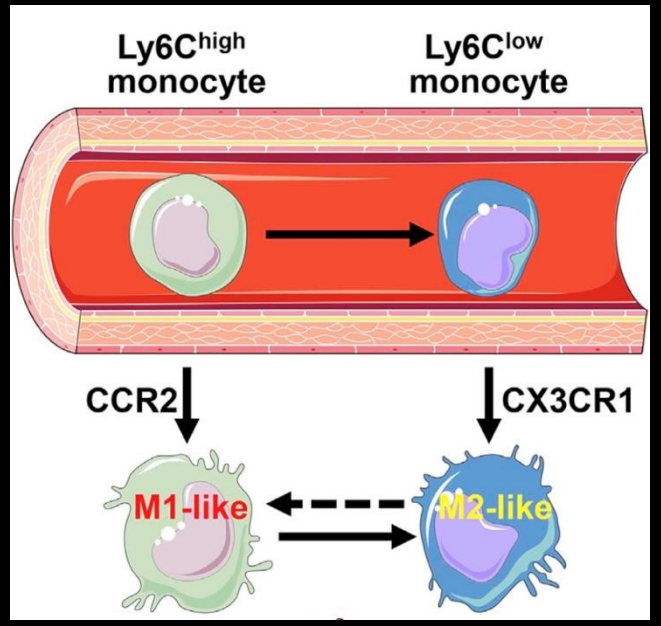
ELISA analysis
on BAL fluid





Larson-Casey *et al.* under review.

Monocyte recruitment during tissue injury in mice



Ma *et al*. (2018) *Transl Res.*

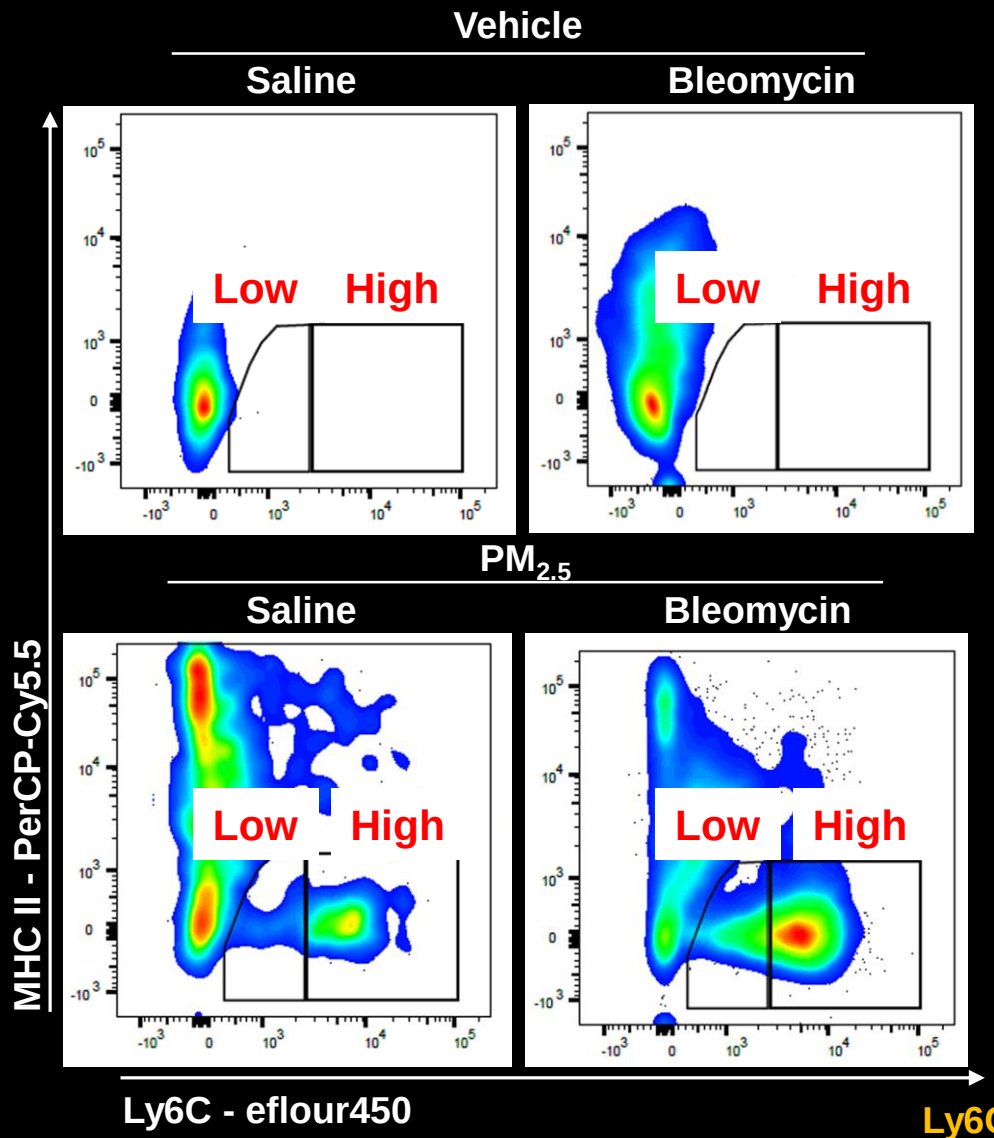
$PM_{2.5}$ recruits Ly6C expressing monocytes

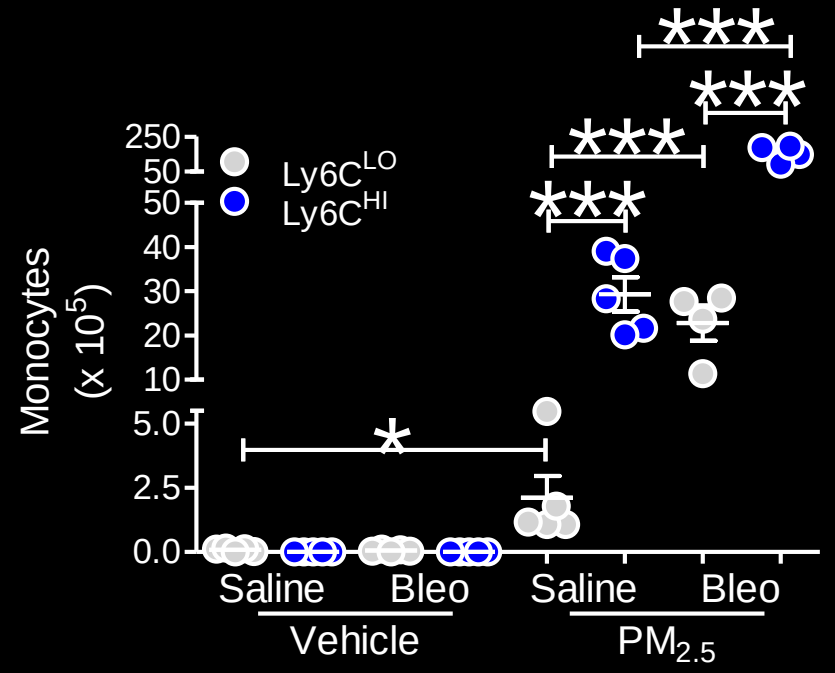
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections
at day 21

BAL at Day 28

Flow cytometry





$Ly6C^{high}$: $CD45^+CD11b^+Ly6G^-CD64^{+/-}$MHC II^-Siglec F^-Ly6C^{hi}
$Ly6C^{low}$: $CD45^+CD11b^+Ly6G^-CD64^{+/-}$MHC II^-Siglec F^-Ly6C^{lo}

$PM_{2.5}$ exposure recruits anti-inflammatory $Ly6C^{LO}$ monocytes & pro-inflammatory $Ly6C^{HI}$ monocytes

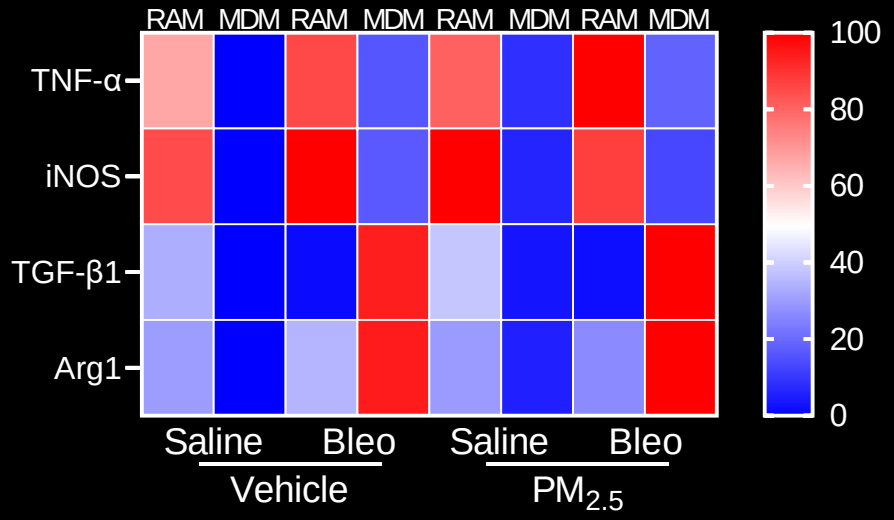
Saline or Bleomycin
1.75 U/kg

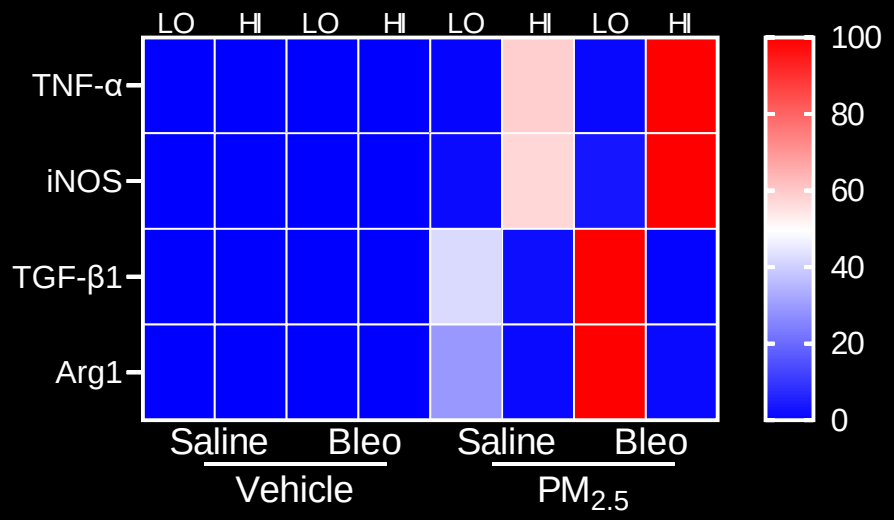
Daily $PM_{2.5}$ (i.t) injections at day 21

BAL at Day 28

Flow cytometry

mRNA analysis





Larson-Casey *et al.* under review.

$PM_{2.5}$ promotes epithelial and endothelial injury

Saline or Bleomycin
1.75 U/kg

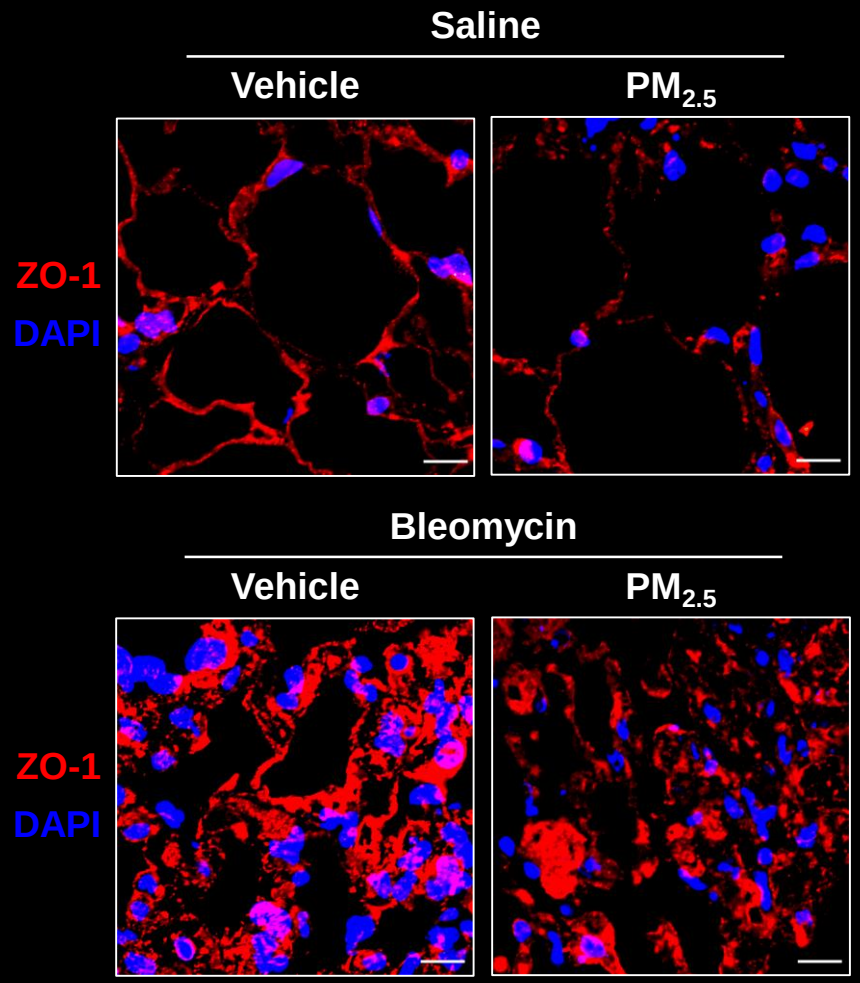
Daily $PM_{2.5}$ (i.t) injections
at day 21

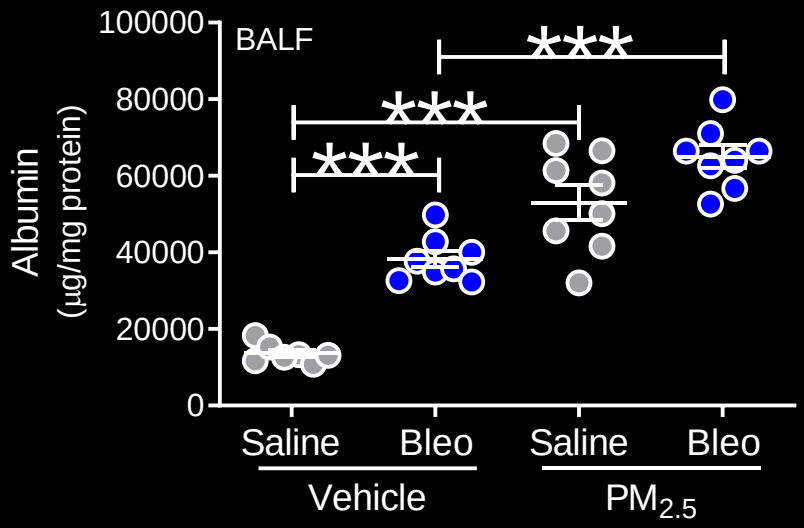
BAL at Day 28

Isolate
lung tissue

ELISA analysis
on BAL fluid

Confocal
analysis





Larson-Casey *et al.* under review.

$PM_{2.5}$ mediates progression of pulmonary fibrosis

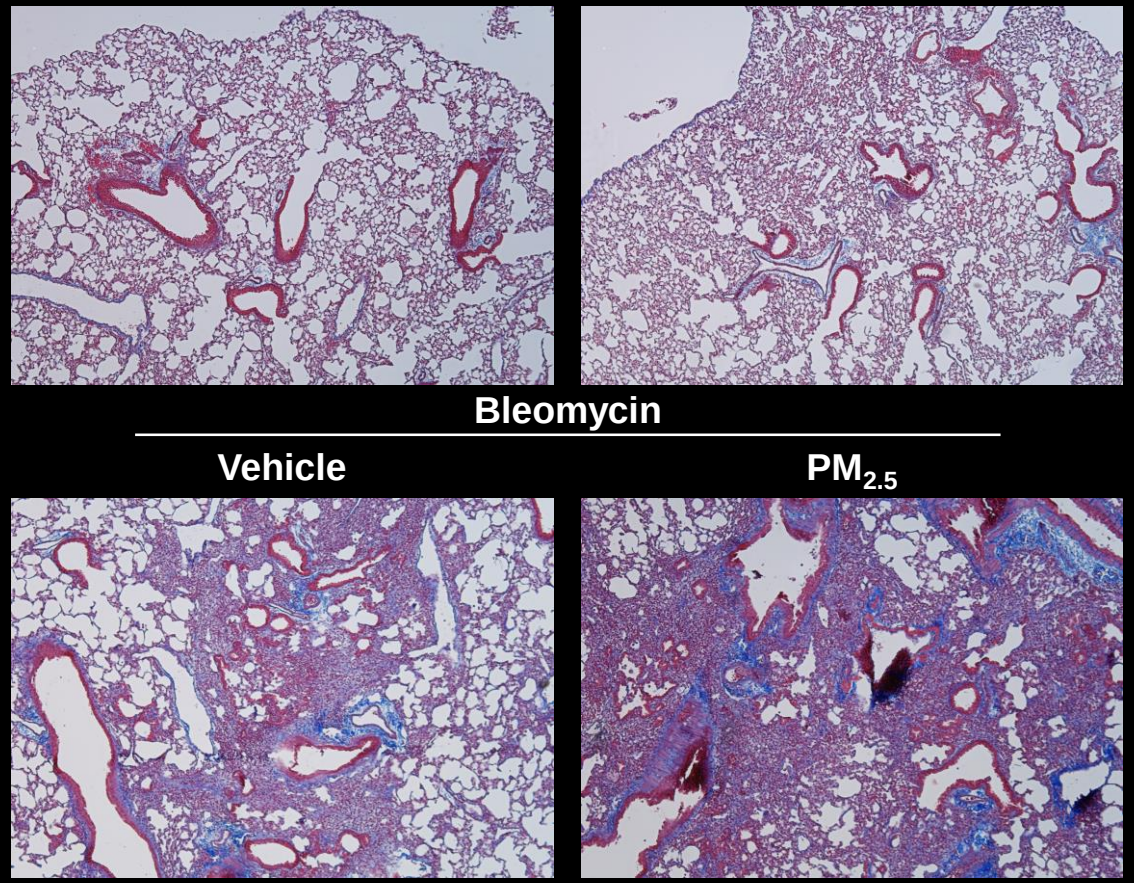
Saline or Bleomycin
1.75 U/kg

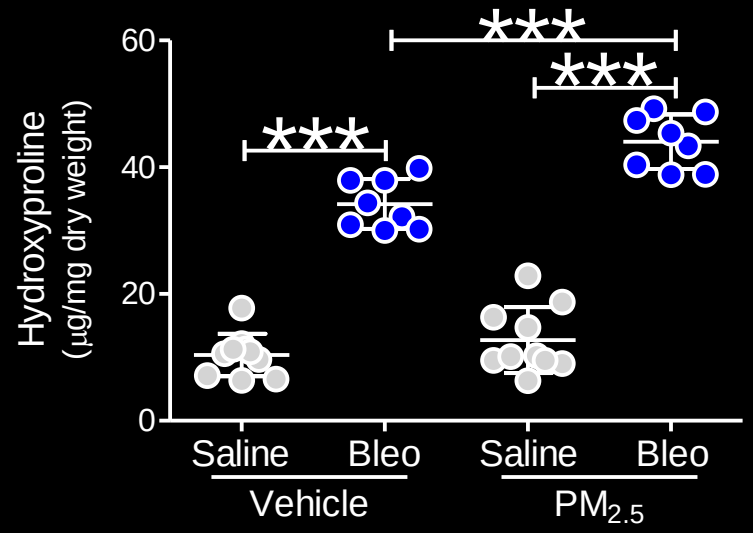
Daily $PM_{2.5}$ (i.t) injections
at day 21

BAL at Day 28

Isolate lung tissue

Masson's trichrome
staining





Larson-Casey *et al.* under revision.

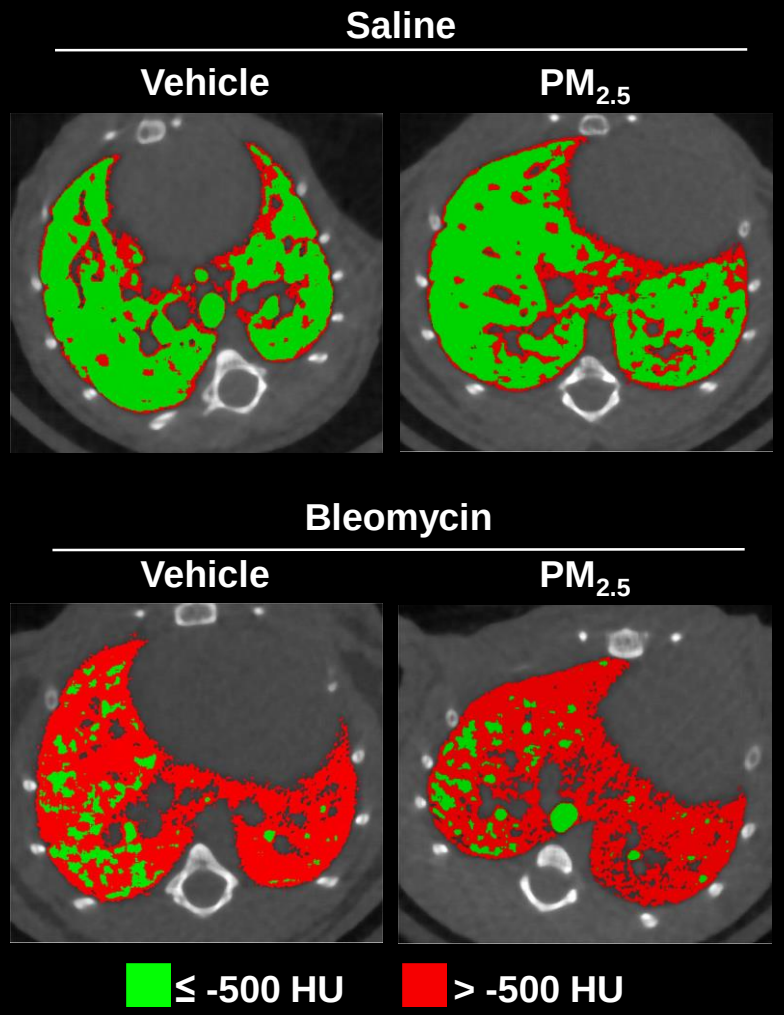
$PM_{2.5}$ mediates progression of pulmonary fibrosis

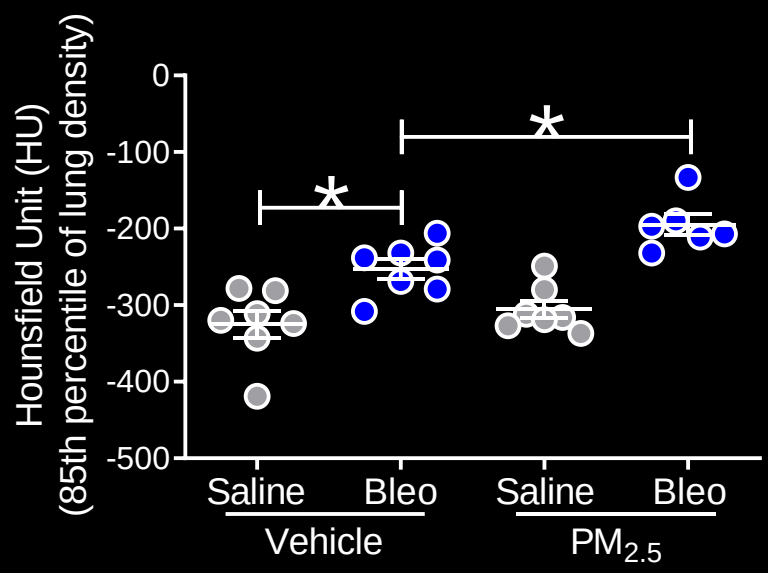
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections
at day 21

BAL at Day 28

Micro-CT imaging





Larson-Casey *et al.* under review.

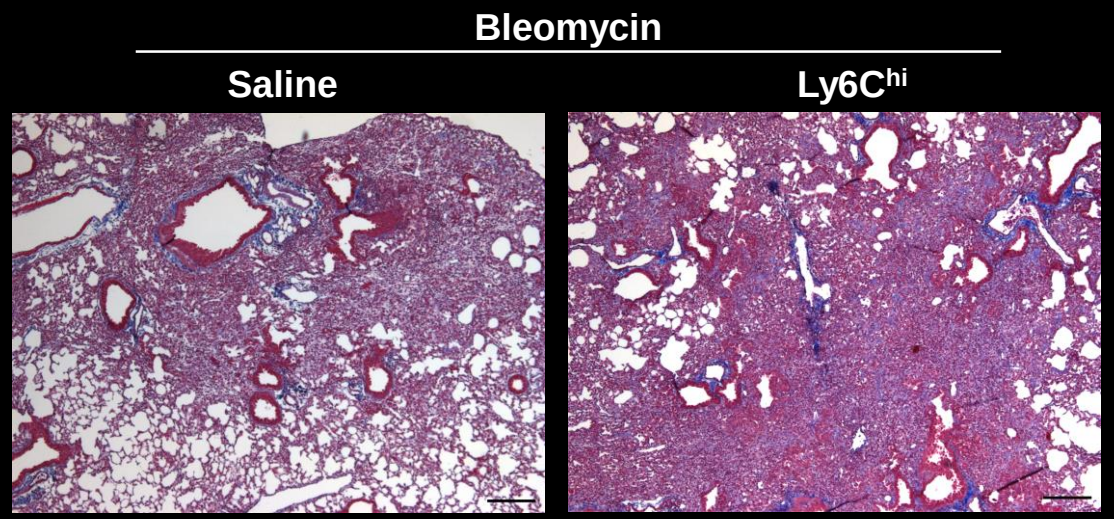
Ly6C^{hi} monocytes drive fibrotic progression

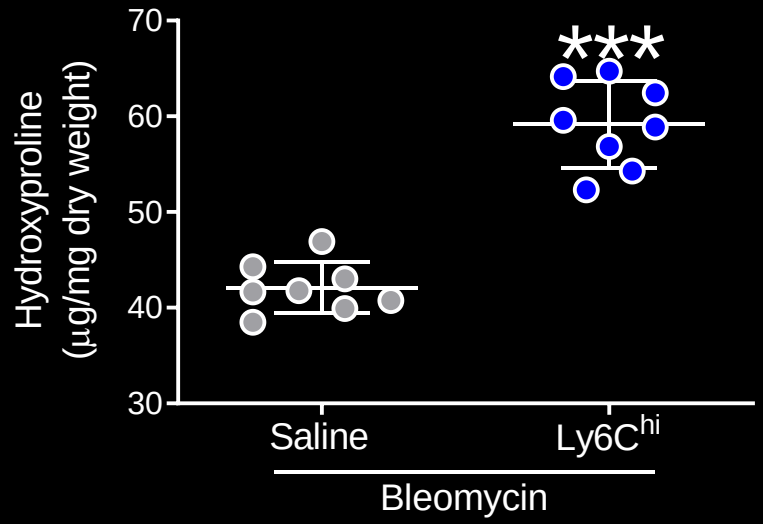
Bleomycin 1.75 U/kg

Ly6C^{hi} monocyte adoptive transfer (i.t) at day 21

BAL at Day 28

Masson's trichrome staining





Larson-Casey *et al.* under review.

Anti-CCR2 monoclonal antibody inhibits Ly6C^{HI} monocyte recruitment

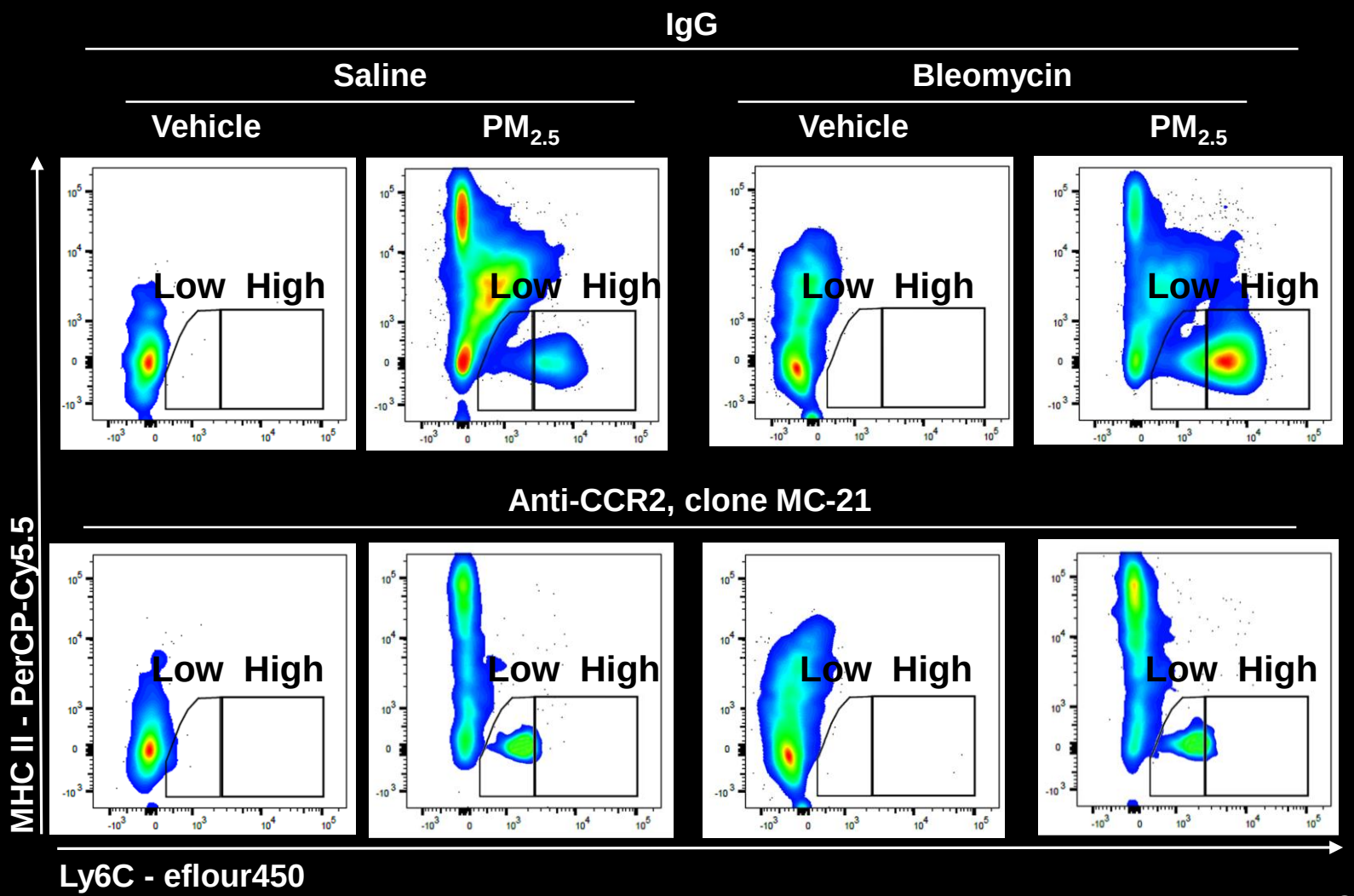
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections at day 21

Daily anti-CCR2 (i.p) injections at day 23

BAL at Day 28

Flow cytometry



Larson-Casey *et al.* under review.

Anti-CCR2 treatment inhibits the pro-inflammatory phenotype

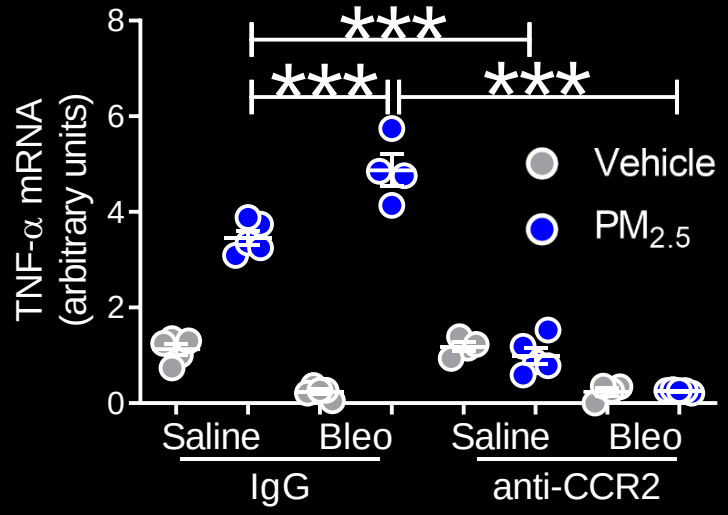
Saline or Bleomycin
1.75 U/kg

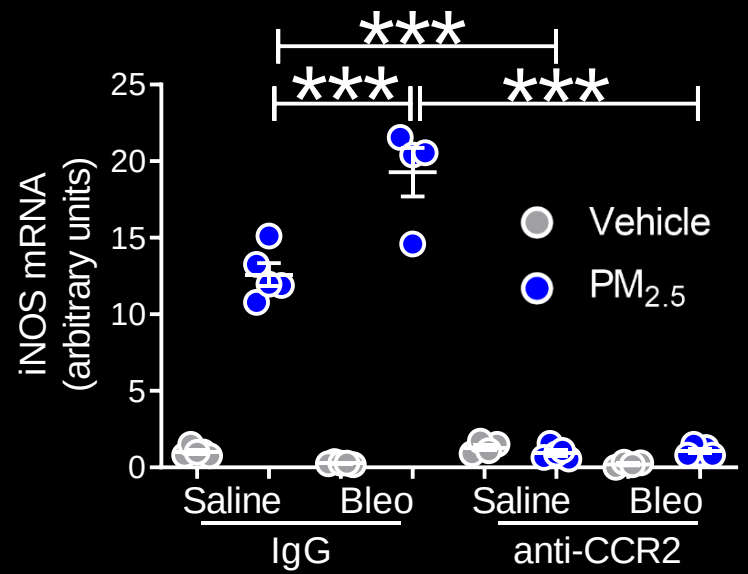
Daily $PM_{2.5}$ (i.t) injections at day 21

Daily anti-CCR2 (i.p) injections at day 23

BAL at Day 28

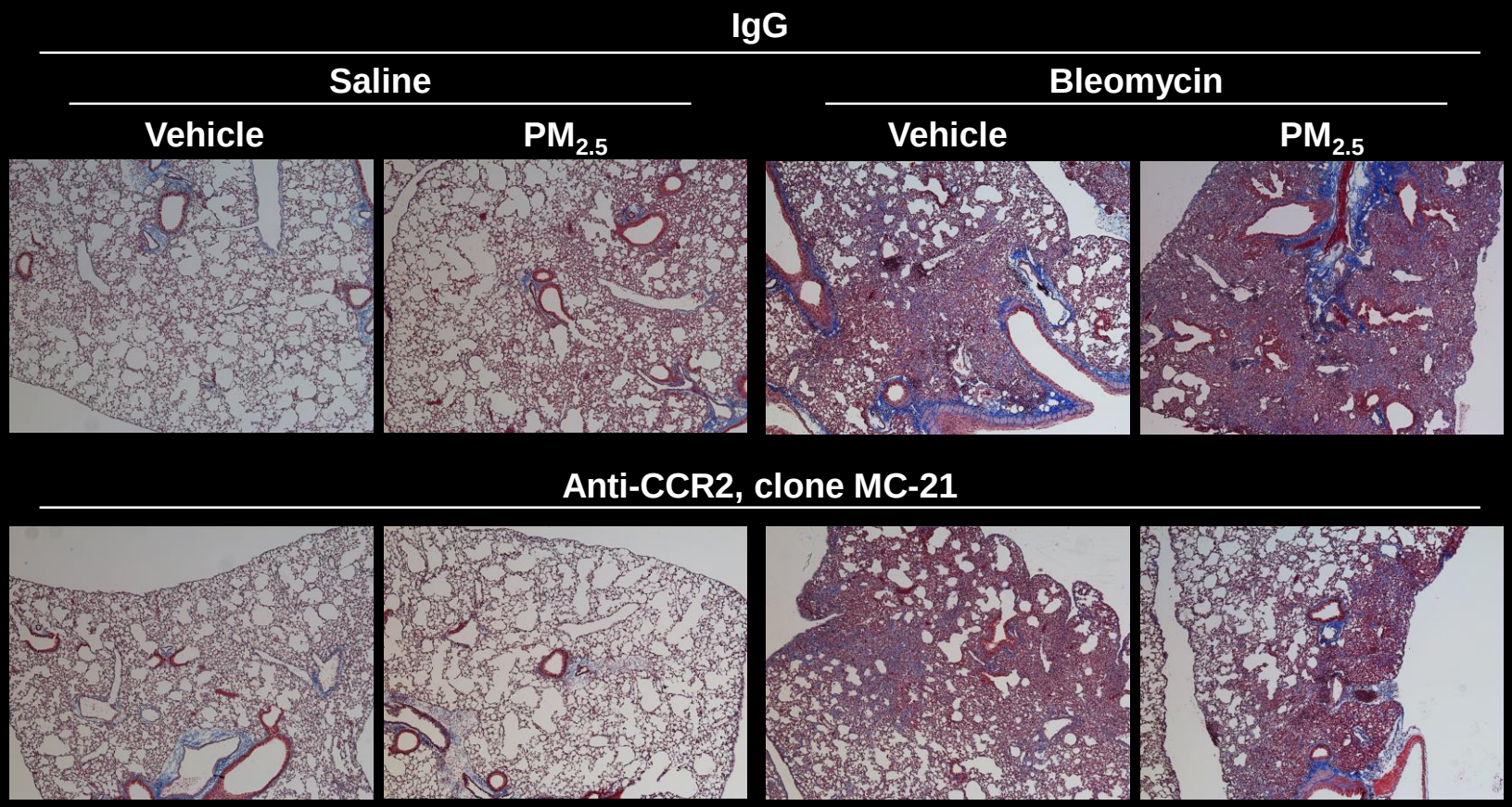
mRNA analysis

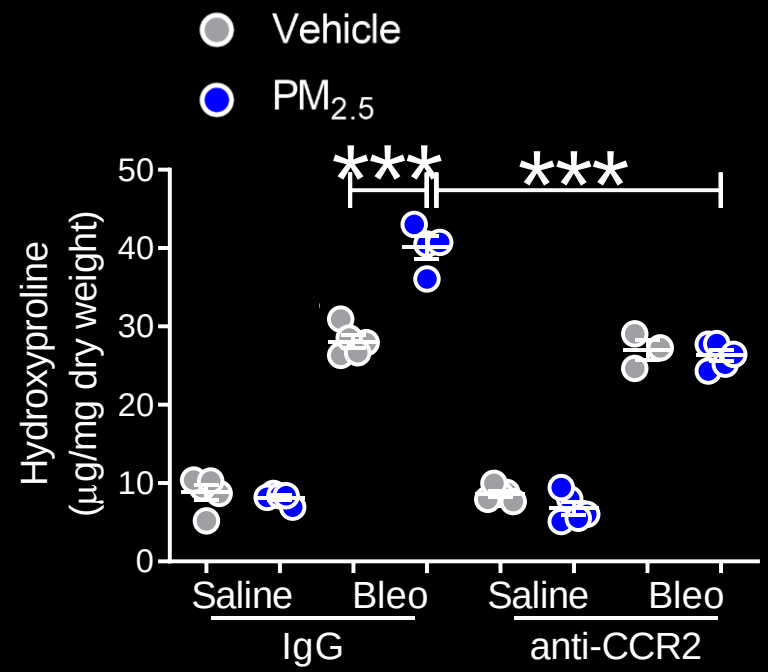




Larson-Casey *et al.* under review.

Anti-CCR2 treatment inhibits $PM_{2.5}$ mediated fibrotic progression





Larson-Casey *et al.* under review.

Anti-CCR2 treatment improves lung function

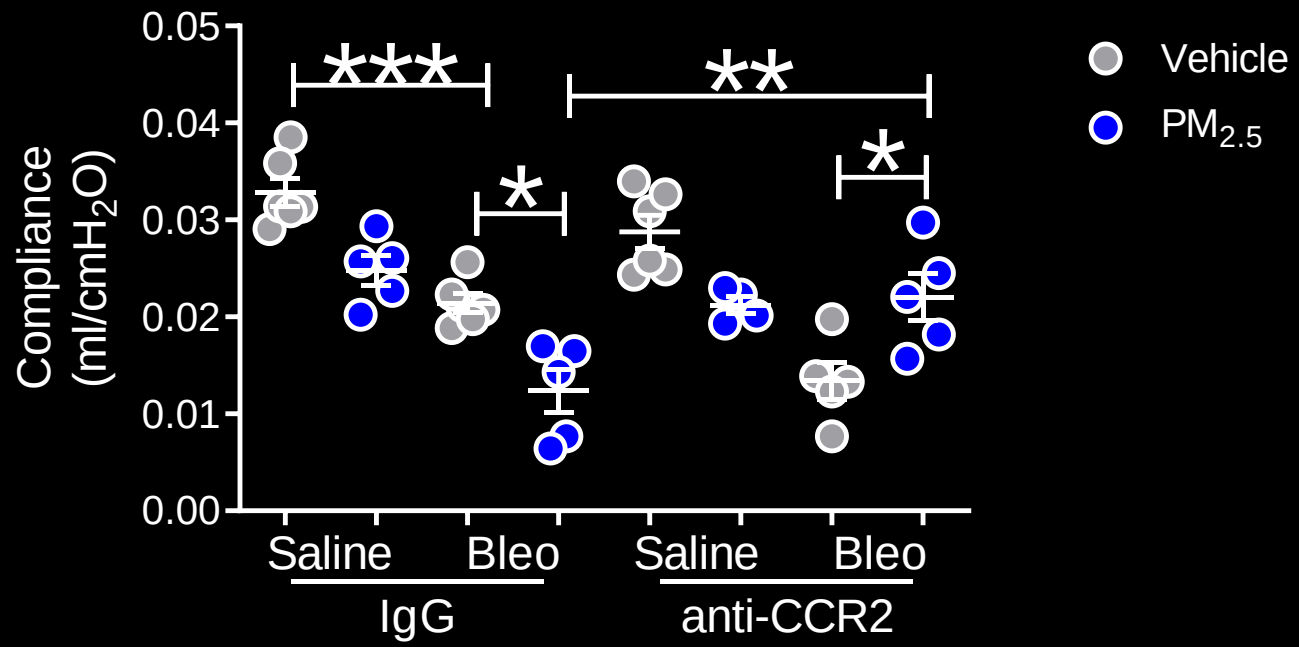
Saline or Bleomycin
1.75 U/kg

Daily $PM_{2.5}$ (i.t) injections
at day 21

Daily anti-CCR2 (i.p)
injections at day 23

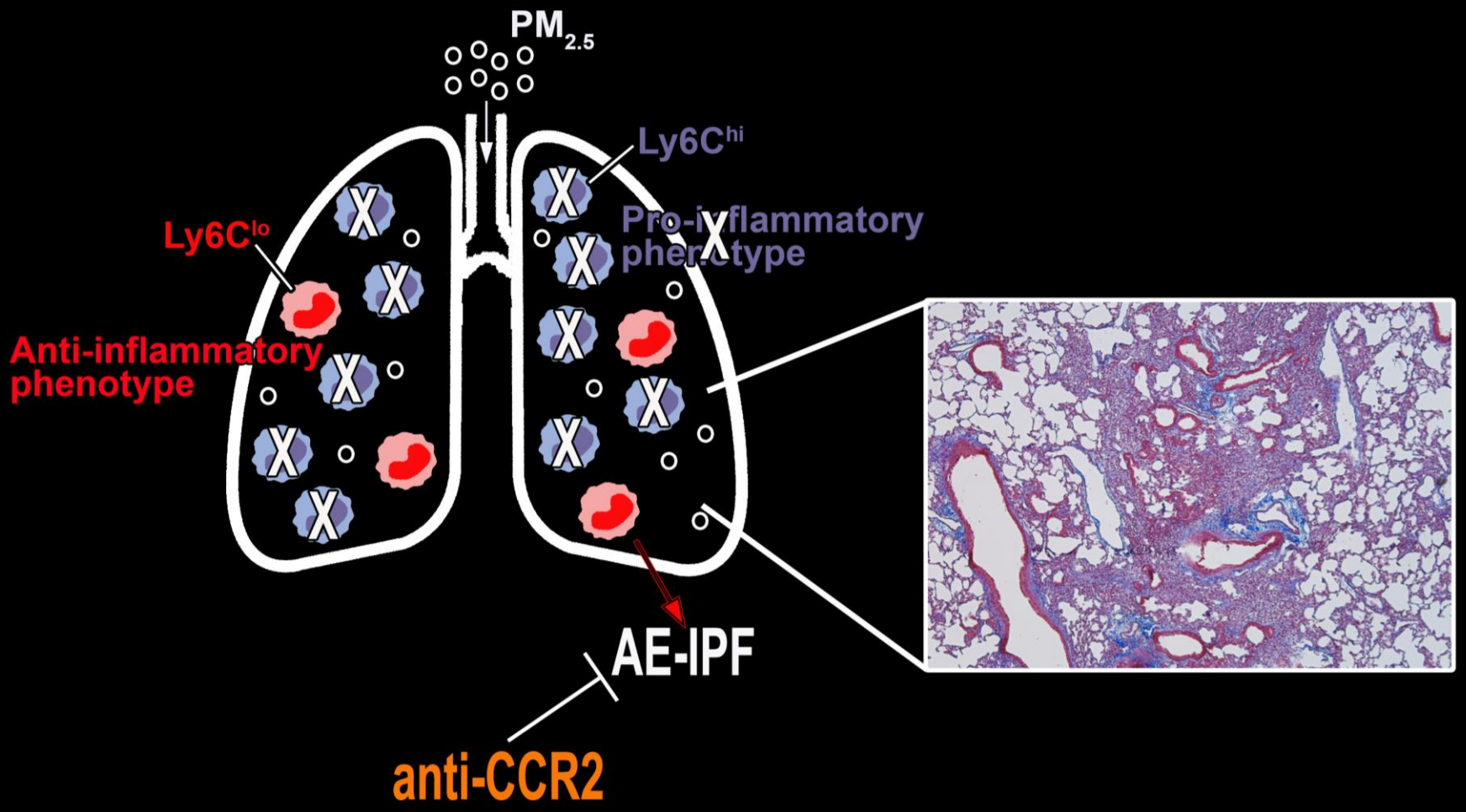
BAL at Day 28

mRNA analysis



Larson-Casey *et al.* under review.

Conclusion


PM2.5
Ly6Chi
Ly6Clo
Pro-inflammatory phenotype
Anti-inflammatory phenotype
AE-IPF
anti-CCR2

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Jyotsana Pandey
Linlin Gu
Ranu Surolia
Steven Duncan
Veena Antony

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PULMONARY, ALLERGY & CRITICAL CARE

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PBF PARKER B. FRANCIS FELLOWSHIP PROGRAM

American Lung Association.

UAB Superfund Research Center
Impact of Airborne Heavy Metals on Lung Disease and the Environment

NIH National Institutes of Health
Turning Discovery Into Health