

# EVs - early biomarkers of environmental toxicity?

Findings and approaches from population-based studies

**Andrea A. Baccarelli, MD, PhD**

Director, Laboratory of Precision Environmental Health  
Chair, Department of Environmental Health Sciences  
[andrea.baccarelli@columbia.edu](mailto:andrea.baccarelli@columbia.edu)



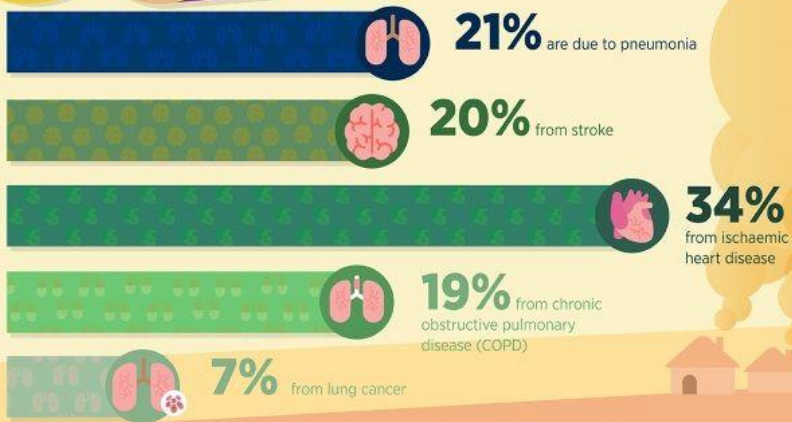
COLUMBIA  
UNIVERSITY

MAILMAN SCHOOL  
of PUBLIC HEALTH

# DEATHS LINKED TO OUTDOOR AND HOUSEHOLD AIR POLLUTION



**7 million** people die prematurely every year from air pollution – both household and outdoor. Among these deaths:



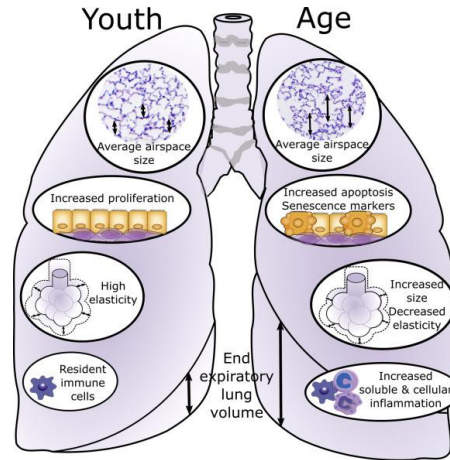
CLEAN AIR FOR HEALTH

#AirPollution

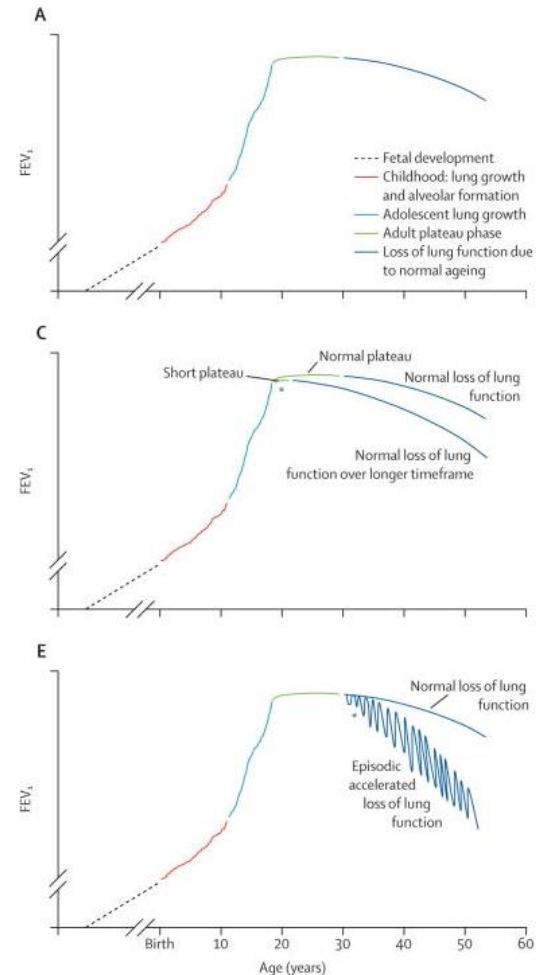


The health effects of ambient and household air pollution

# Respiratory diseases in older people are leading causes of death and disability in the world

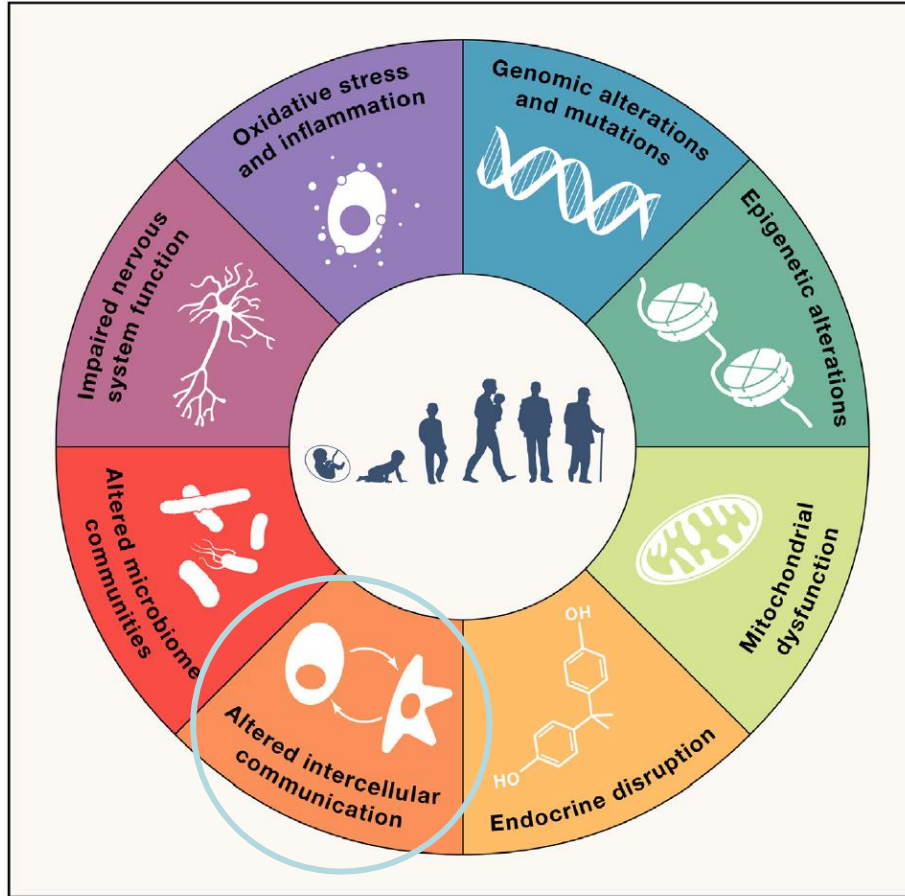


Bowdish ME, et al., Chest 2018  
 Rennard SI, et al. Lancet 2015



# The hallmarks of environmental aging

Peters, Nawrot, Baccarelli  
Cell 2021

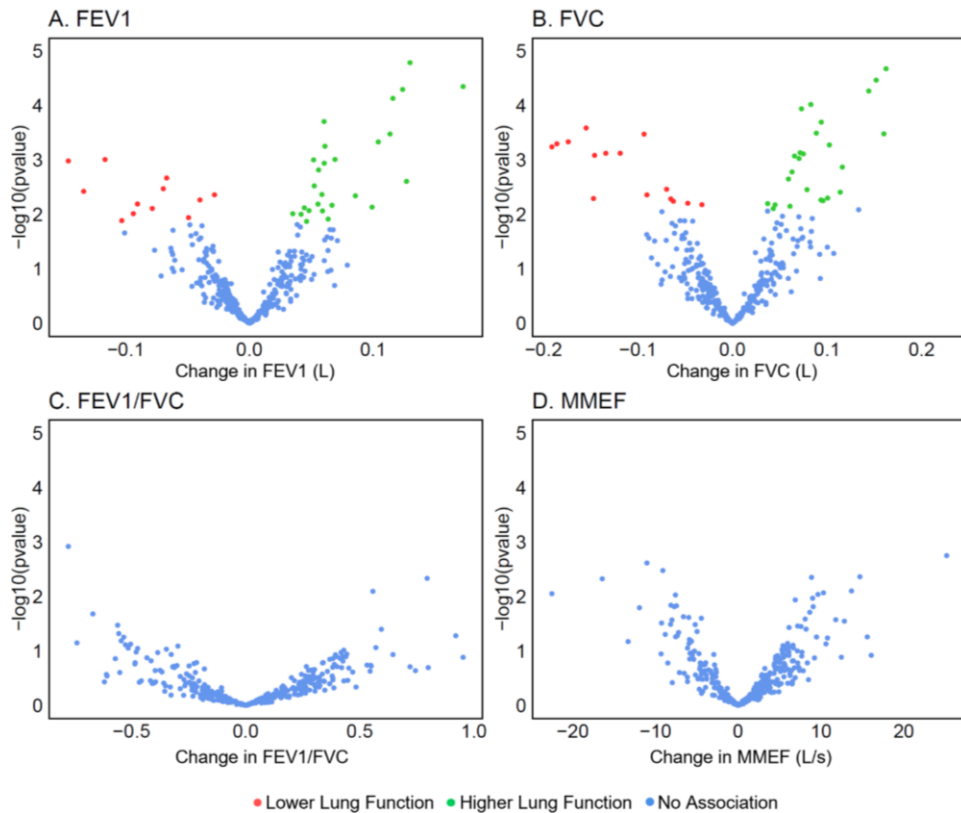


- Prospective cohort
  - Enrolled 2,280 men in New England in 1961-70
  - Aged 21 to 80 free of chronic disease
  - One visit every 3-5 years
  - 656 participants still active between 1996-15
- EV miRNAs
  - From plasma using Norgen Biotek plasma/serum circulating and exosomal RNA kit
  - 381 EV-miRNAs detected in >70% samples
- Lung function
  - FEV1 = Forced Expiratory Volume in 1 second
  - FVC = Forced Vital Capacity
  - MMEF = Maximal Mid-Expiratory Flow

## The VA Normative Aging Study (NAS)

EV miRNA analysis performed in collaboration with Louise Laurent Lab





FEV1 = Forced Expiratory Volume in 1 Second; FVC = Forced Vital Capacity;  
MMEF = Maximal Mid-Expiratory Flow  
Models adjusted for age, BMI, smoking pack-years, and METs

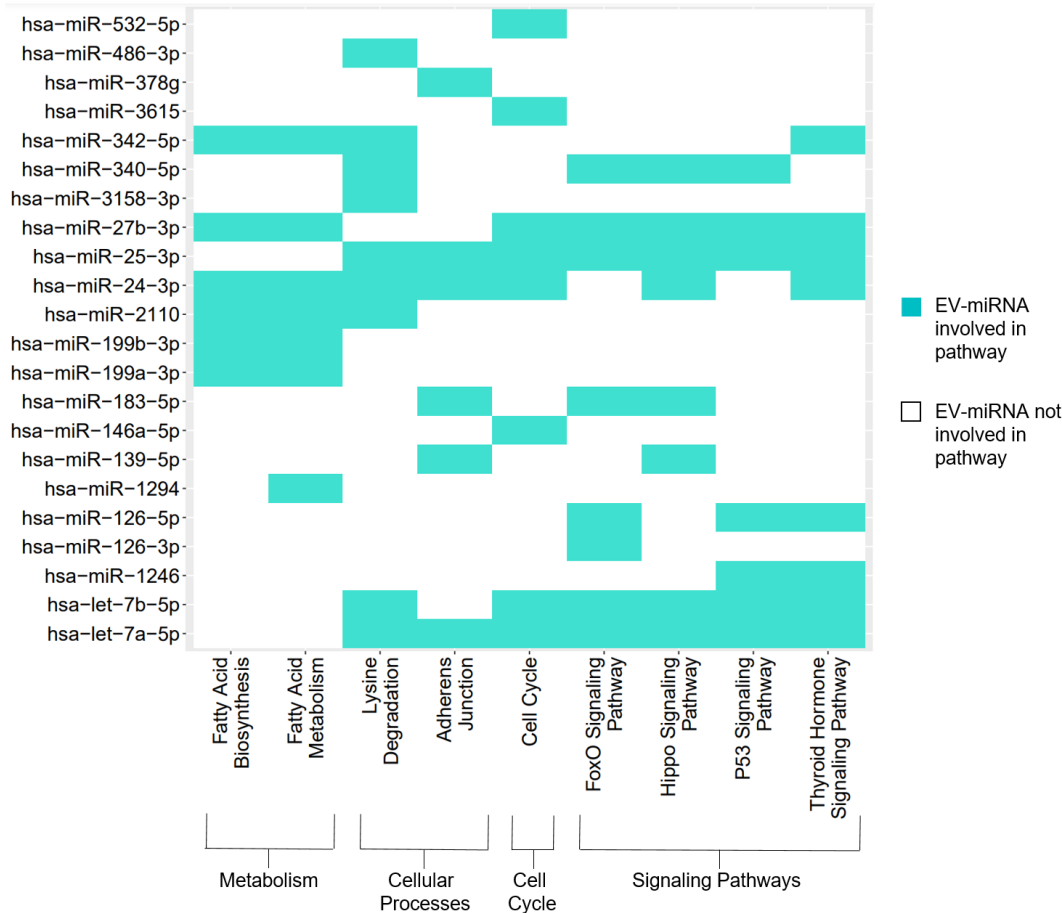
33 EV-miRNAs were associated with baseline FEV1 and FVC



Christina Eckhardt

Eckhardt et al., under review



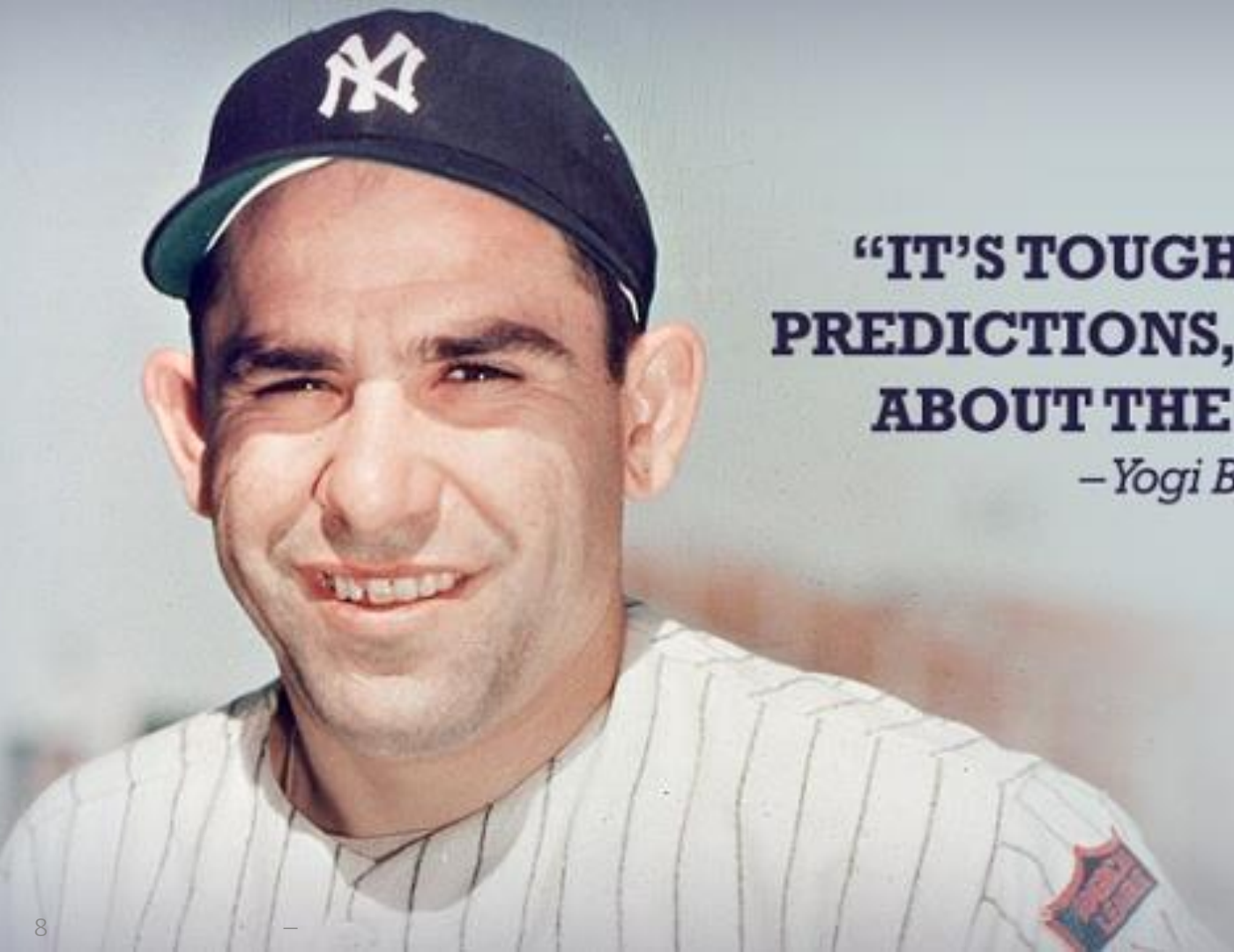


Nine KEGG biological processes related to metabolism, cellular processes, and signaling pathways were enriched



Christina Eckhardt

Eckhardt et al., under review



**“IT’S TOUGH TO MAKE  
PREDICTIONS, ESPECIALLY  
ABOUT THE FUTURE.”**

*– Yogi Berra*

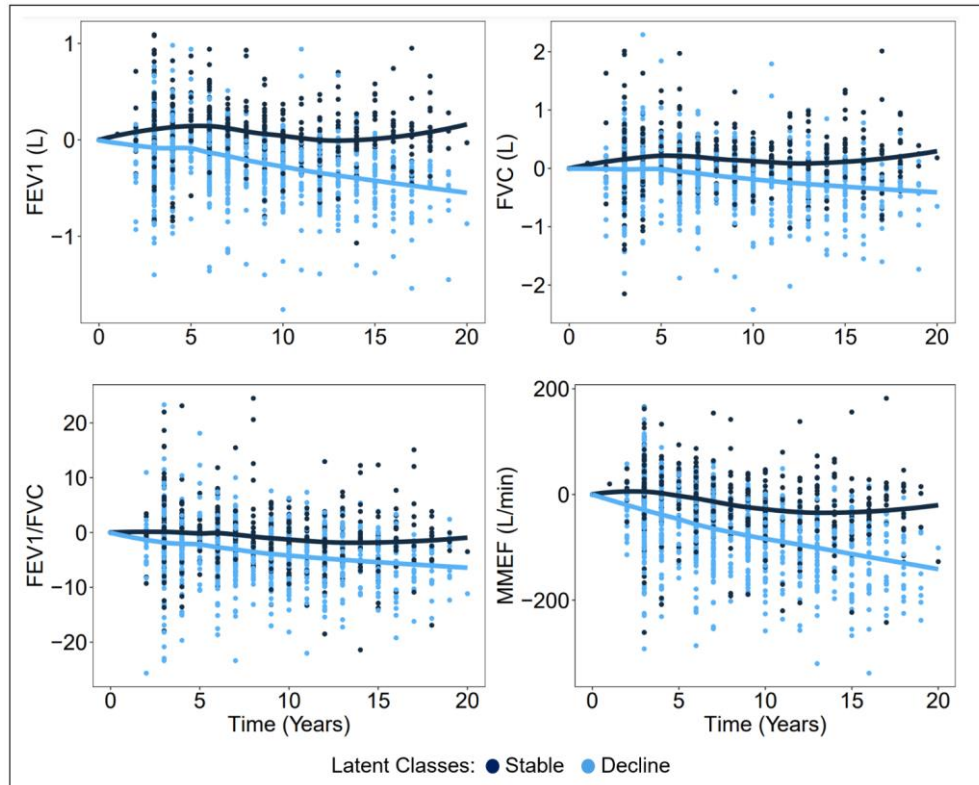


# Multivariate latent class growth modeling identified two lung function trajectories



Christina Eckhardt

Eckhardt et al., under review



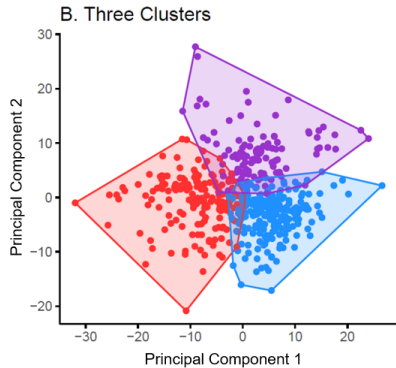
FEV1 = Forced Expiratory Volume in 1 Second; FVC = Forced Vital Capacity;  
MMEF = Maximal Mid-Expiratory Flow  
Models adjusted for age

# miRNA clusters and lung function trajectory

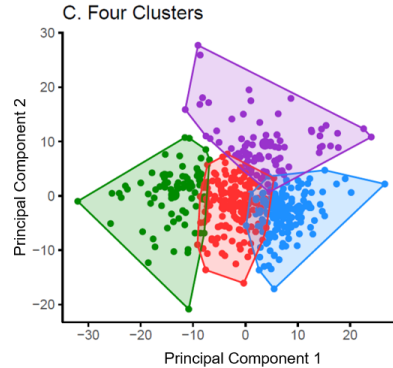


Christina Eckhardt

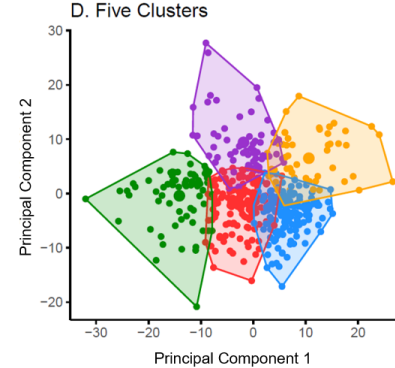
Clusters of miRNAs defined by k-means clustering  
 Cluster 1 (purple) was consistently associated with higher risk of belonging to the declining lung function trajectory class



Cluster: ■ C1 ■ C2 ■ C3



Cluster : ■ C1 ■ C2 ■ C3 ■ C4



Cluster : ■ C1 ■ C2 ■ C3 ■ C4 ■ C5

	N	RR	95% CI	p
Cluster 2 (C2)	159	Ref	Ref	Ref
Cluster 1 (C1)	99	1.19	1.05, 1.35	0.008
Cluster 3 (C3)	215	0.95	0.83, 1.09	0.50

	N	RR	95% CI	p
Cluster 3 (C3)	146	Ref	Ref	Ref
Cluster 1 (C1)	81	1.16	1.00, 1.33	0.047
Cluster 2 (C2)	79	1.03	0.87, 1.22	0.75
Cluster 4 (C4)	157	0.95	0.82, 1.10	0.51

	N	RR	95% CI	p
Cluster 3 (C3)	144	Ref	Ref	Ref
Cluster 1 (C1)	60	1.24	1.06, 1.44	0.006
Cluster 2 (C2)	50	1.15	0.96, 1.39	0.13
Cluster 4 (C4)	142	1.01	0.86, 1.18	0.89
Cluster 5 (C5)	77	1.08	0.90, 1.29	0.41

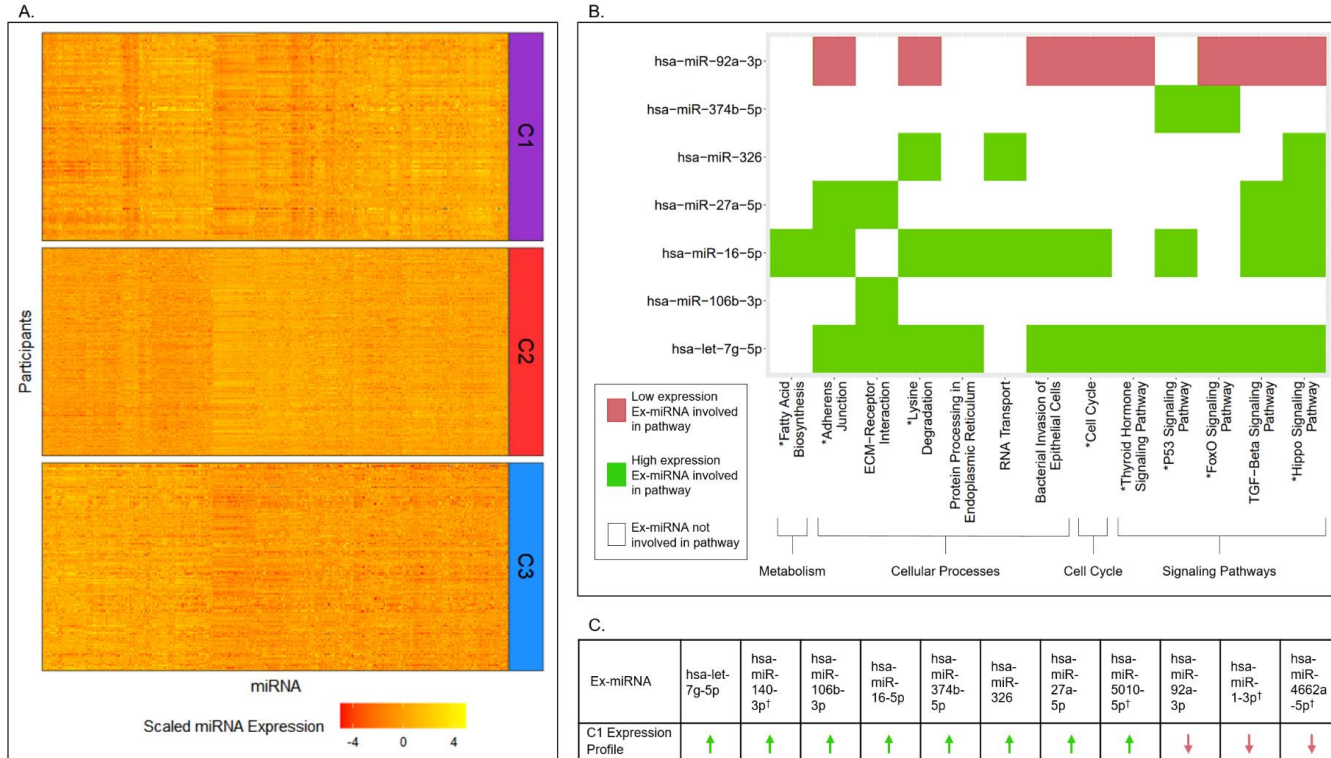
CI = Confidence interval. N = Number of participants. P = p-value. RR = Relative Risk  
 Models adjusted for age, BMI, smoking pack-years, and METs

Eckhardt et al.,  
 under review

# LASSO regression identified 11 EV-miRNAs that differentiated the C1 cluster from other groups



Christina Eckhardt



Eckhardt et al.,  
under review

## nature aging

Xu Gao, Brent Coull, Xihong Lin,  
Pantel Vokonas, Avron Spiro 3rd,  
Lifang Hou, Joel Schwartz &  
Andrea A. Baccarelli

03 May 2021

Air pollution

### Air pollution spikes may impair older men's thinking, study finds

Even short, temporary increases in airborne particles can damage brain health, research suggests

### Even short, temporary increases in airborne particles can damage brain health, research suggests



▲ There is growing evidence that exposure to fine particulate matter in the air, largely from road vehicles and industry, is harmful to the brain. Photograph: Dominic Lipinski/PA



## Numerous EV-miRNAs associated with cognitive trajectory over time

- Same cohort (NAS)
- Outcome: Mini-mental state exam (MMSE)
- Modeled MMSE change over time (i.e., time\*miRNA)

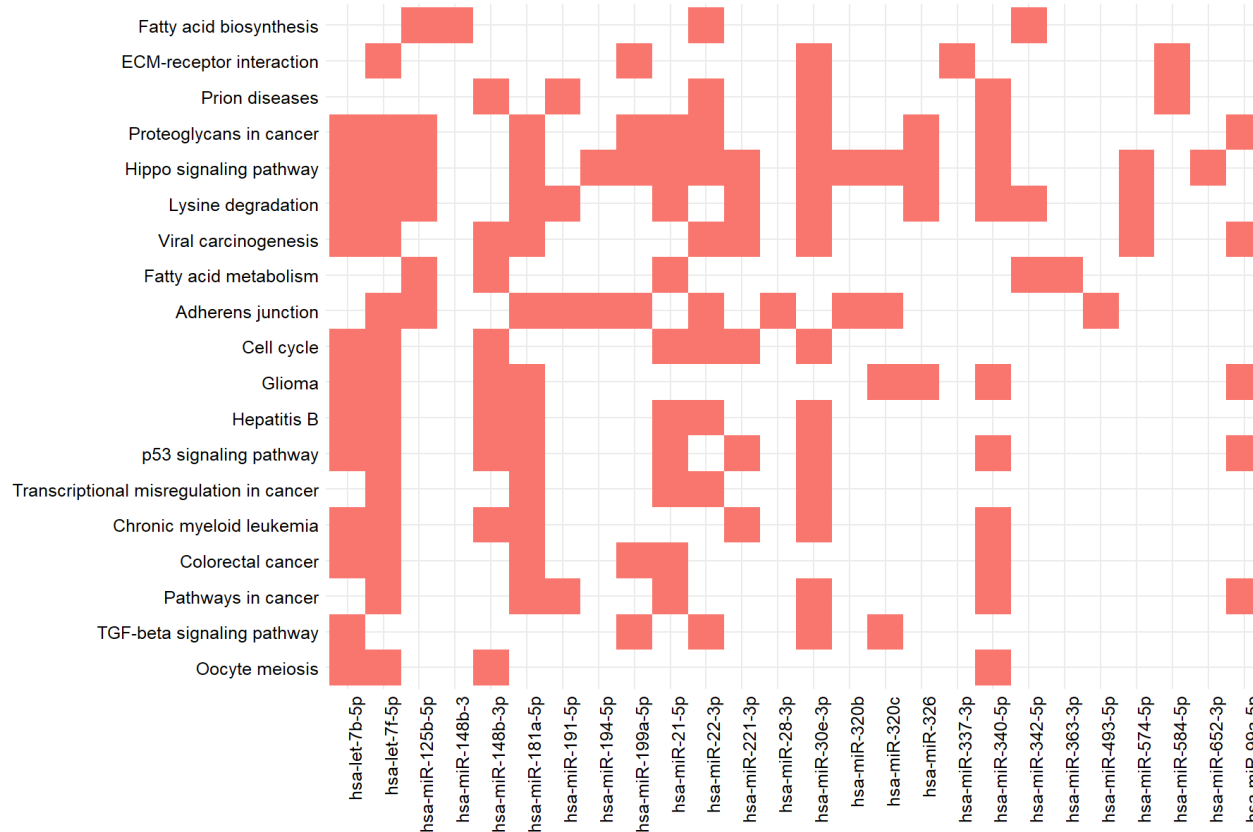


Nicole Comfort

Models adjusted for age, education, alcohol, and smoking status



# KEGG pathways enrichment

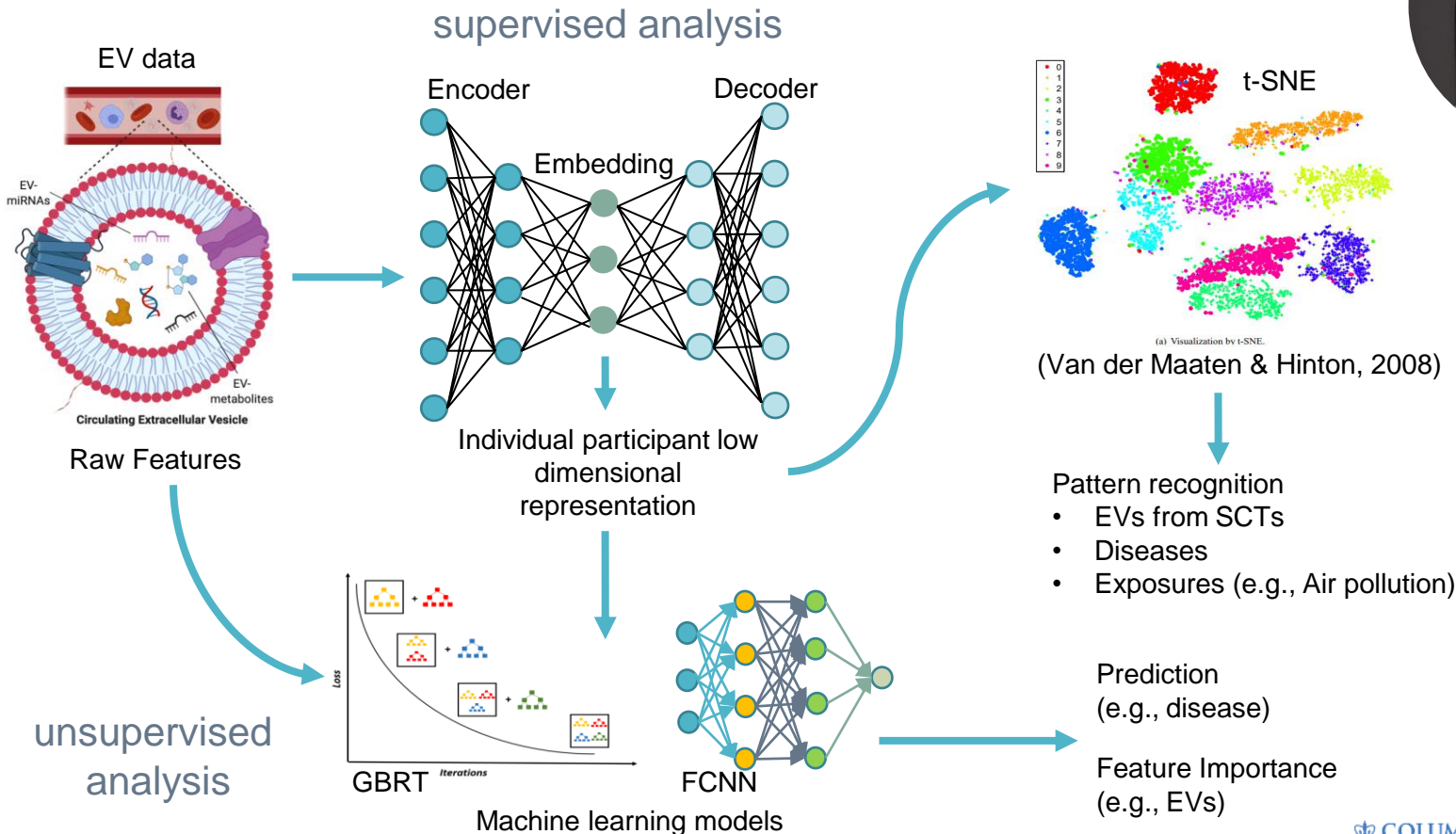


Nicole Comfort

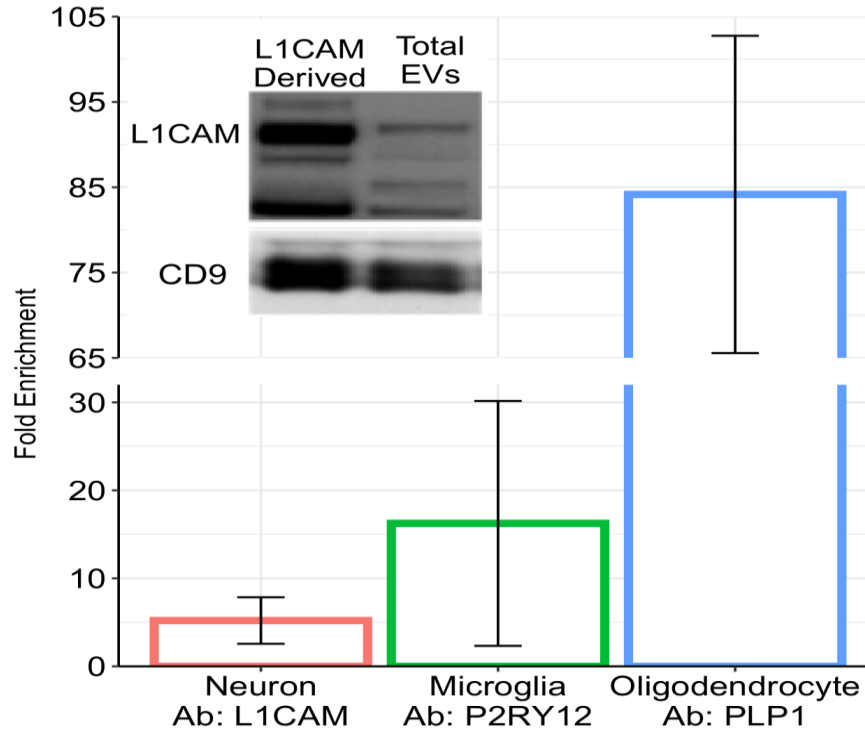
# Machine learning approaches to EV data



Yike Shen



Ongoing work:  
Isolation from blood  
samples of EVs from  
neurons, microglia,  
and oligodendrocytes



Howie Wu

## Brain

- neuronal EVs
- oligodendrocyte EVs
- astrocyte EVs
- microglial EVs

## Lung disease

- lung epithelial cell EVs
- alveolar cell EVs

## Heart disease

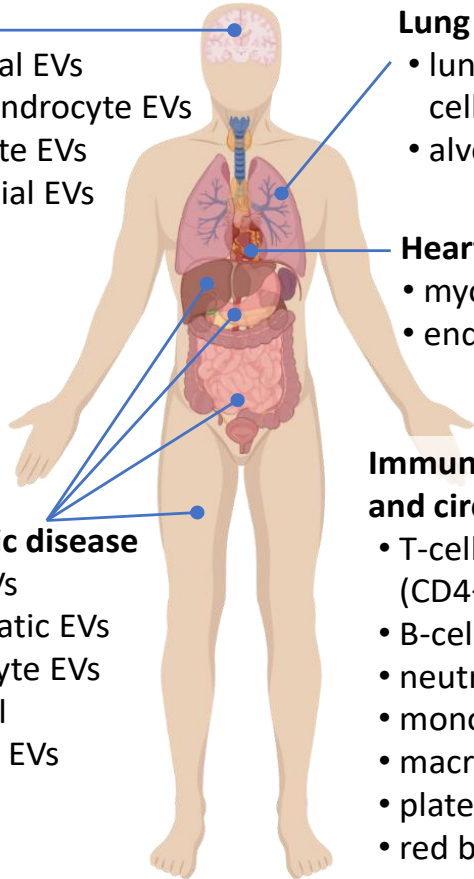
- myocardial EVs
- endothelial EVs

## Immune system and circulating cells

- T-cell EVs (CD4+, CD8+, NK)
- B-cell EVs
- neutrophil EVs
- monocyte EVs
- macrophage EVs
- platelet EVs
- red blood cell EVs

## Metabolic disease

- liver EVs
- pancreatic EVs
- adipocyte EVs
- skeletal muscle EVs



Our 10-year plan for isolating source cell-type specific EVs in plasma/serum

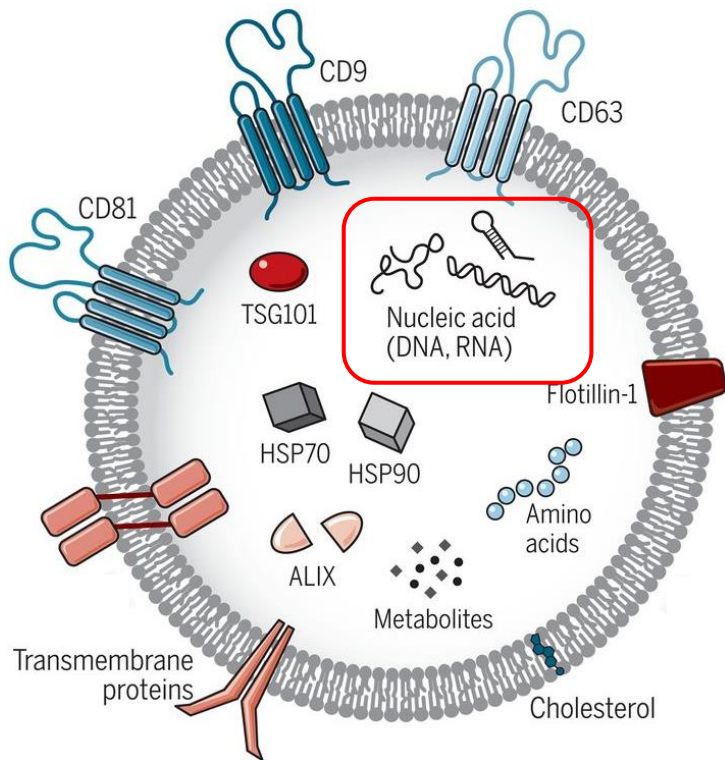
Possible downstream applications:

- RNA sequencing
- metabolomics
- protein/proteomics
- environmental chemicals
- others

# Ongoing work – Development of Seq protocols optimized for human studies and constraints



Howie Wu



Low-input protocols for small RNA seq (<500pg)

Allow us to investigate the encapsulated miRNAs as intercellular communication system



## Next-Generation Sequencing



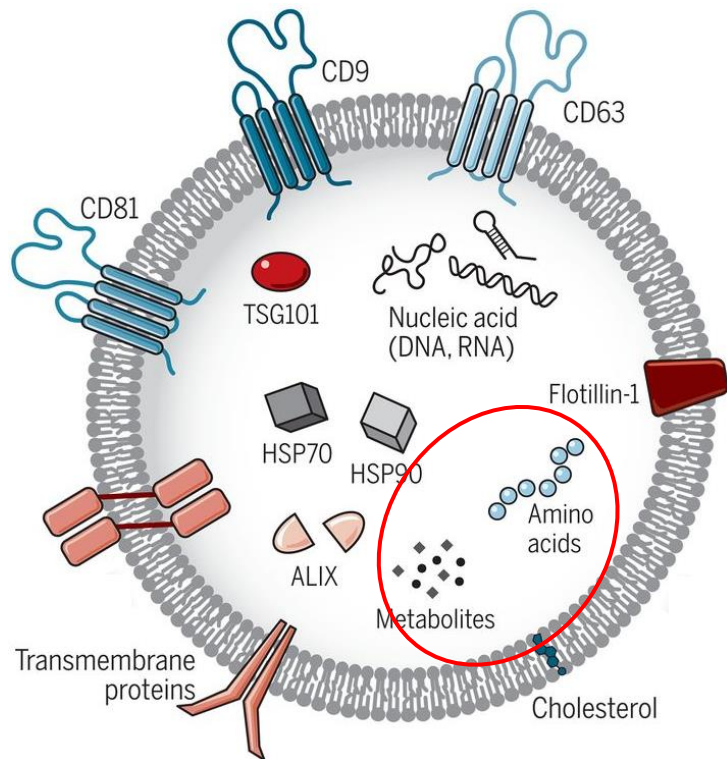
# Ongoing work – Synergy with high-resolution untargeted mass spectrometry



Vrinda Kalia

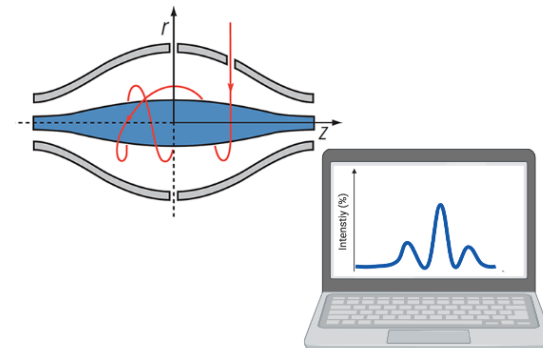
Currently testing and optimizing protocols

- Metabolomics  
Comprehensive capture of EV small molecule cargo
- Exposomics  
Accurate capture of multiple target specific exposures



## Accurate mass detection

Accurate mass and abundance of metabolites



# Next steps

- Data analysis on EVs and air pollution ongoing
- EV-SCTs in large cohort studies
  - miRNAs, metabolomics, chemicals
- Parallel mechanistic studies



# Laboratory of precision environmental health

Thanks to my lab team and all collaborators!

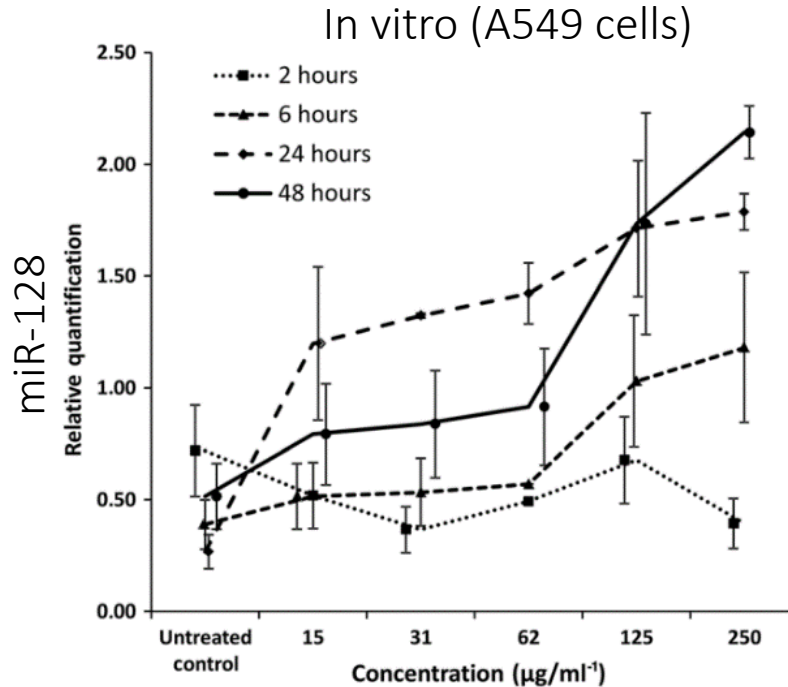
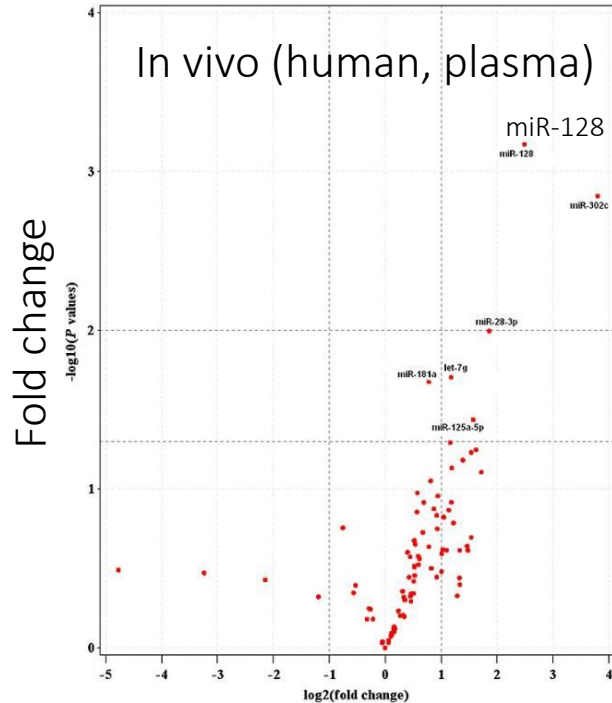
Our research is currently supported by multiple NIEHS/NIH awards:

- R35ES031688, PI: Baccarelli
- R01ES027845, PIs: Baccarelli, Takser
- R01ES032242, PIs: Navas-Acien, Baccarelli
- R01AG069120, PIs: Baccarelli, Hou, Yaffe
- R01ES032242, PIs: Colicino, Baccarelli
- P30ES009089, PI: Baccarelli

# Air pollution and EV-encapsulated miRNA



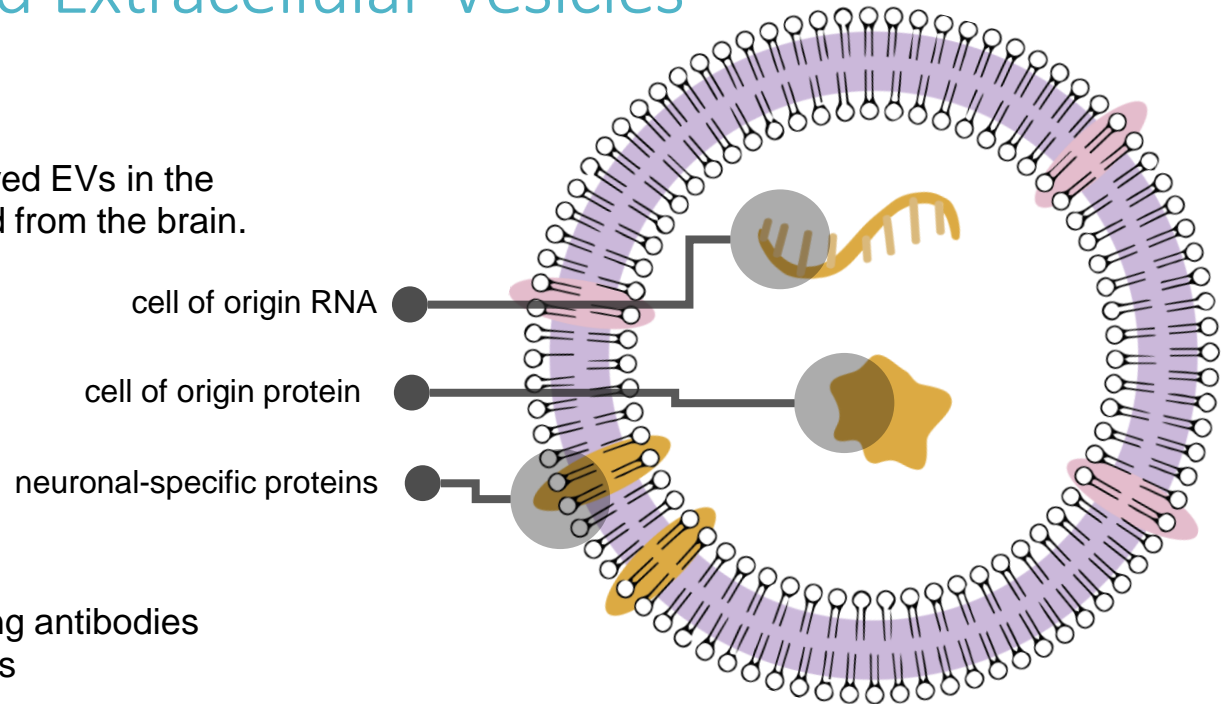
Valentina  
Bollati



Bollati et al. J Appl Tox 2014

# Neuron-derived Extracellular Vesicles

We can isolate neuron-derived EVs in the plasma as they are released from the brain.



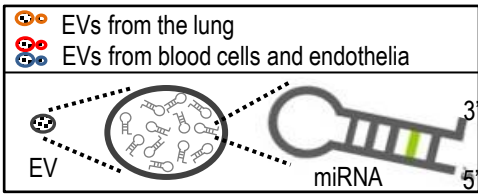
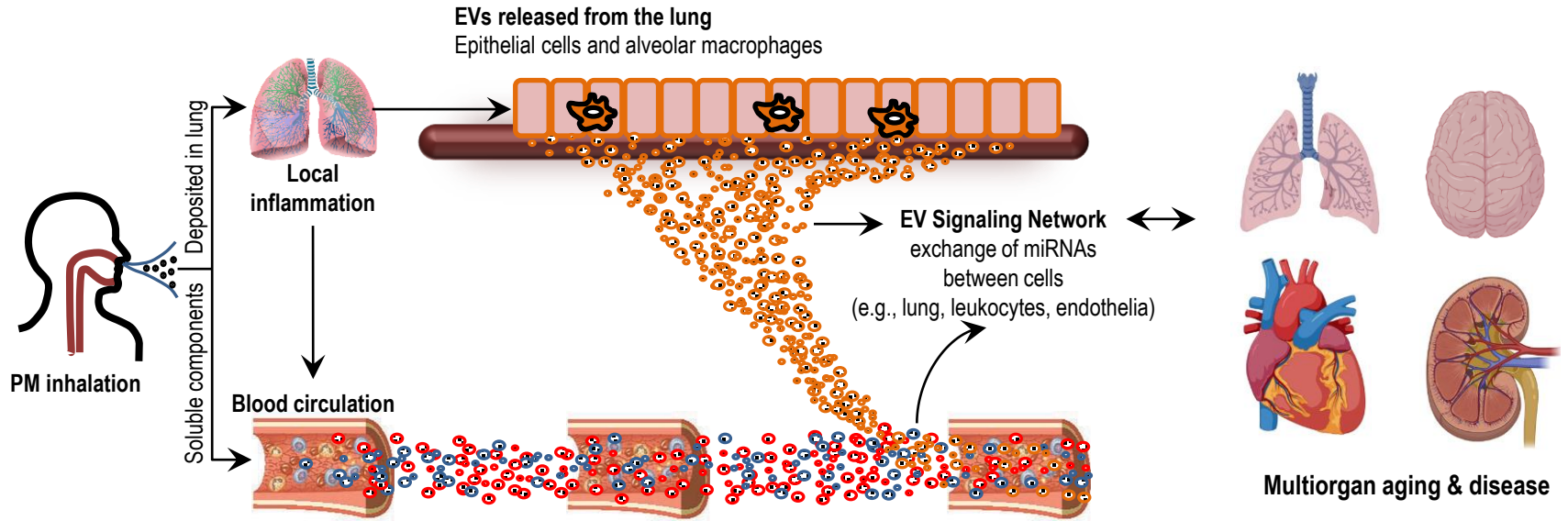
Immunoaffinity isolation using antibodies for neuronal-specific proteins

Courtesy of Neurodex





# EVs, air pollution, and aging – a conceptual model



## REFERENCES

Alharbi et al., Biomed Res Int 2021  
 Rota et al. Sci Rep 2020  
 Alkoussa et al. Crit Rev Toxicol 2020  
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