Exposure to air pollution triggers wide gene expression changes in the brains of transgenic Alzheimer's mice.

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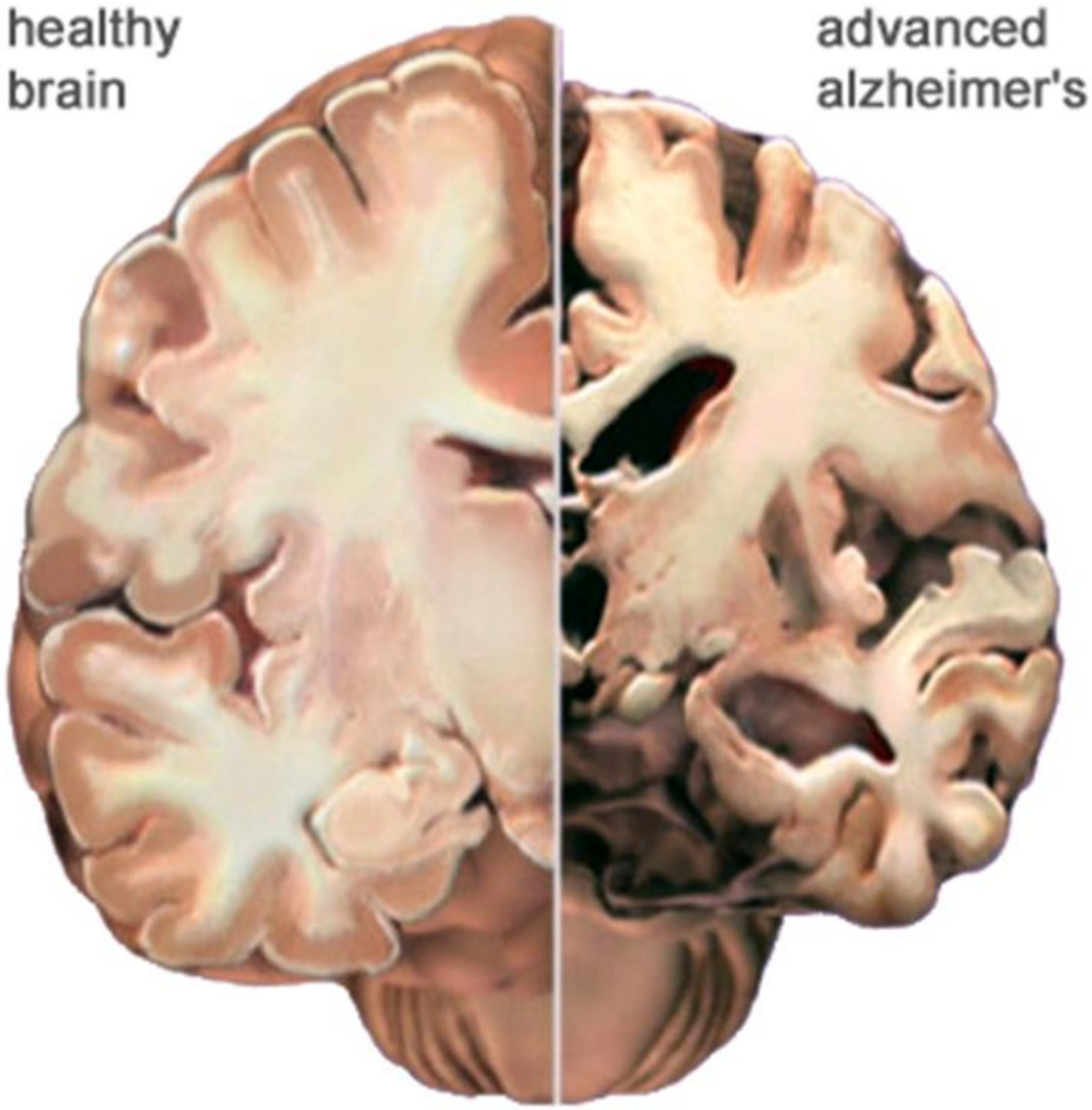
Introduction

- Air pollution is the single most threat to human health.
- Little is known about the effects of air pollution on the brain.
- Including its association with Alzheimer's and Parkinson's diseases.

Alzheimer’s Disease in numbers



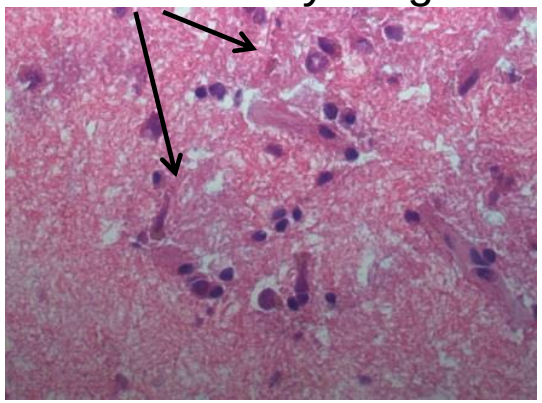
https://www.alz.org/braintour/healthy_vs_alzheimers.asp

- **50 million** people were suffering from dementia in 2016 and **75.6 million** people will have dementia in 2030.
- AD is the leading cause of dementia and is responsible for 60-70% of its diagnoses.
- AD develops slowly, and memory loss occurs late in the disease. Yet, AD is most diagnosed based on cognitive deficits, which ultimately results in late detection and a poor prognosis.

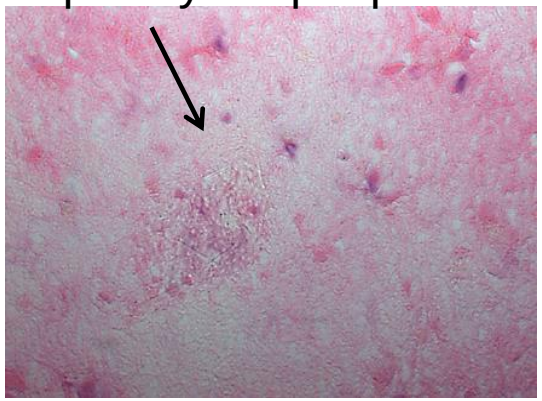
Alzheimer's Disease common pathologies

A. H&E

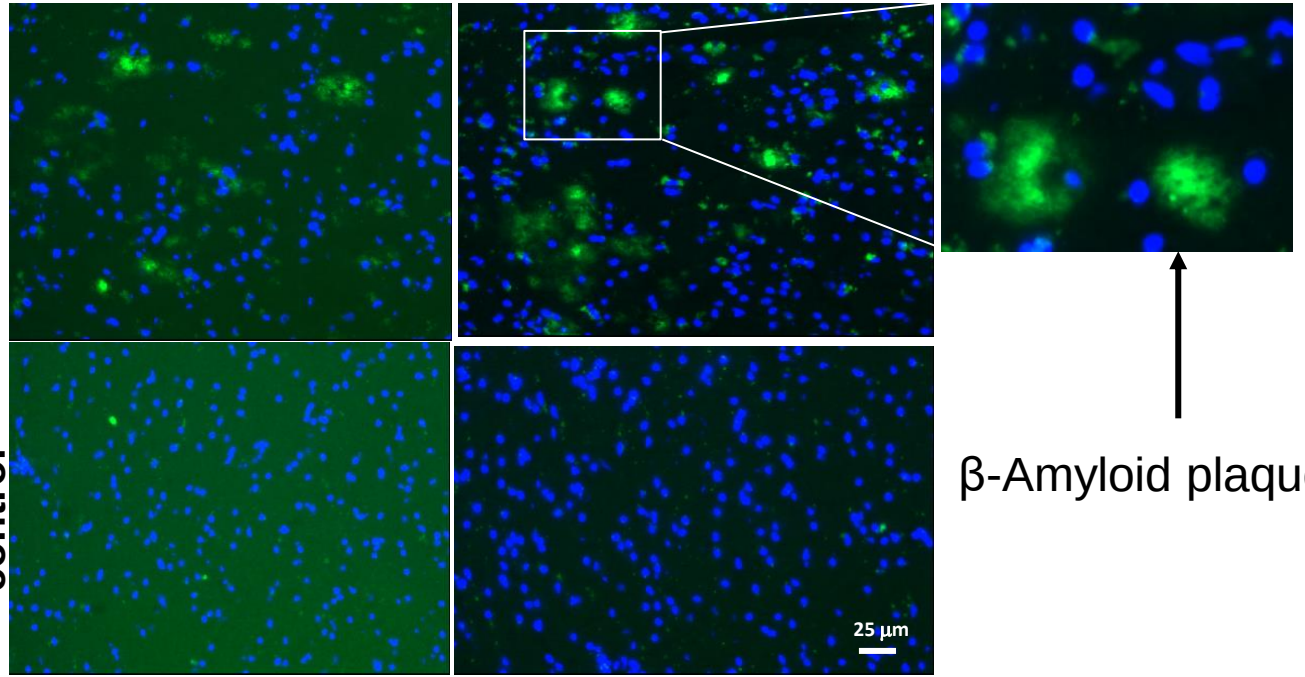
Neurofibrillary tangles



β-Amyloid plaque



B. Immunofluorescence

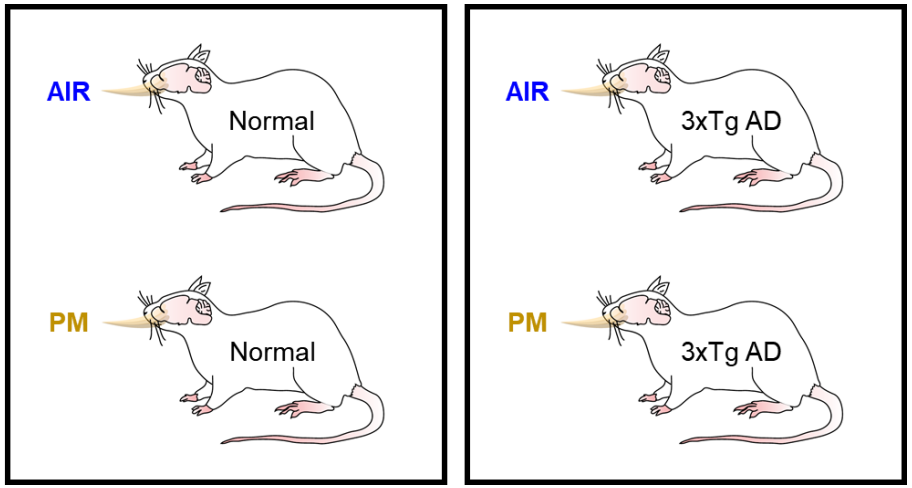


Most common hallmarks of AD

- Aβ1-42 peptide – soluble intracellular as well as extracellular deposits known as senile plaques
- Neurofibrillary tangles (NFTs) resulted from Tau protein hyperphosphorylation and misfolding
- Brain atrophy in humans
- Neuroinflammation
- Oxidative stress
- One of the first regions affected by the disease is the **Hippocampus**

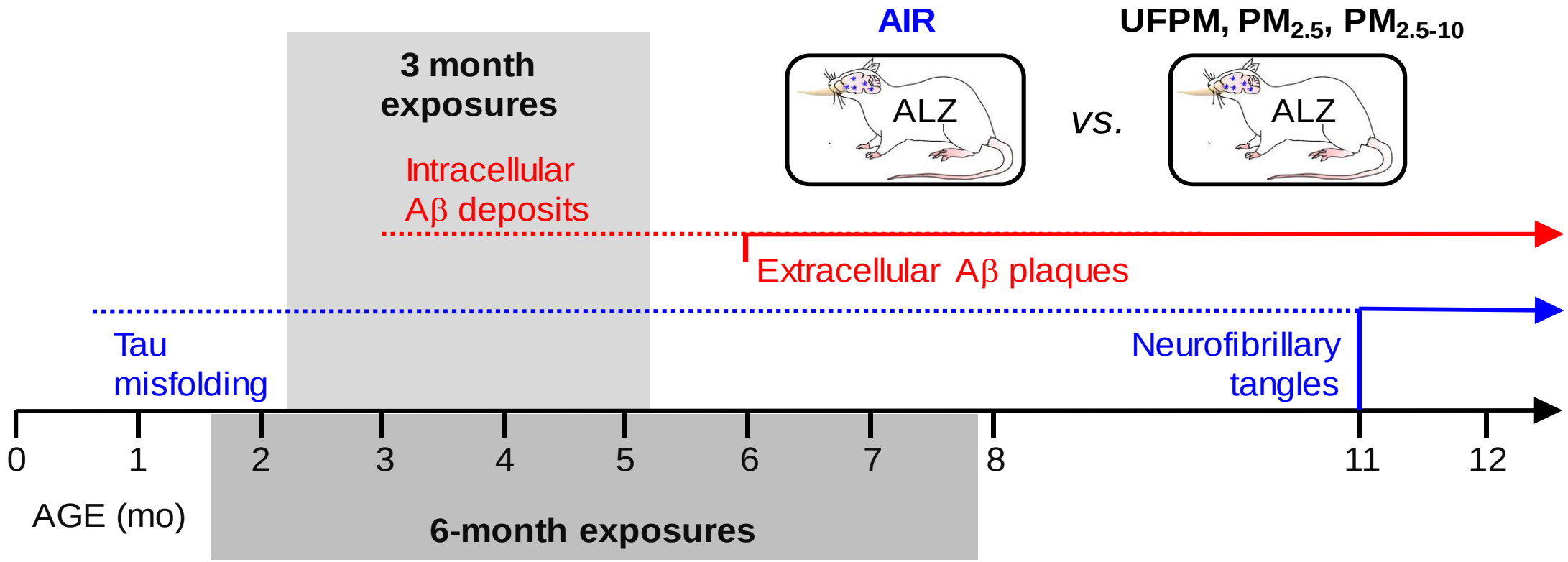
Patil R. et al. *Macromolecular Bioscience*, 2015

Can polluted air affect humans with genetic pre-disposition for Alzheimer's disease?



Study design:

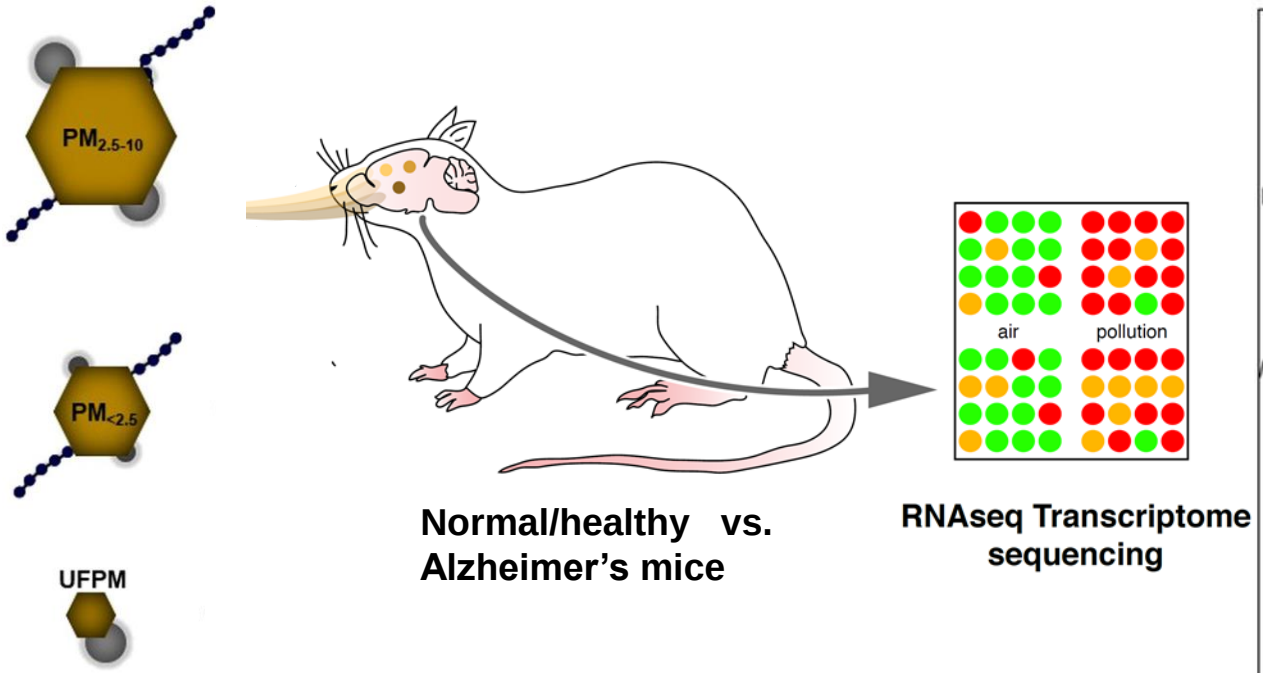
- 3xTg AD mice were divided into 4 groups for each of a **3- and 6-month** pollution exposure.
- The same experiment was also performed with healthy Balb/C mice.
- 3xTg AD mice contains 3 genetic modifications: Psen1 (presanilin1) mutation, APPSwe and tauP301L transgenes (Tg(APPSwe,tauP301L)1Lfa)



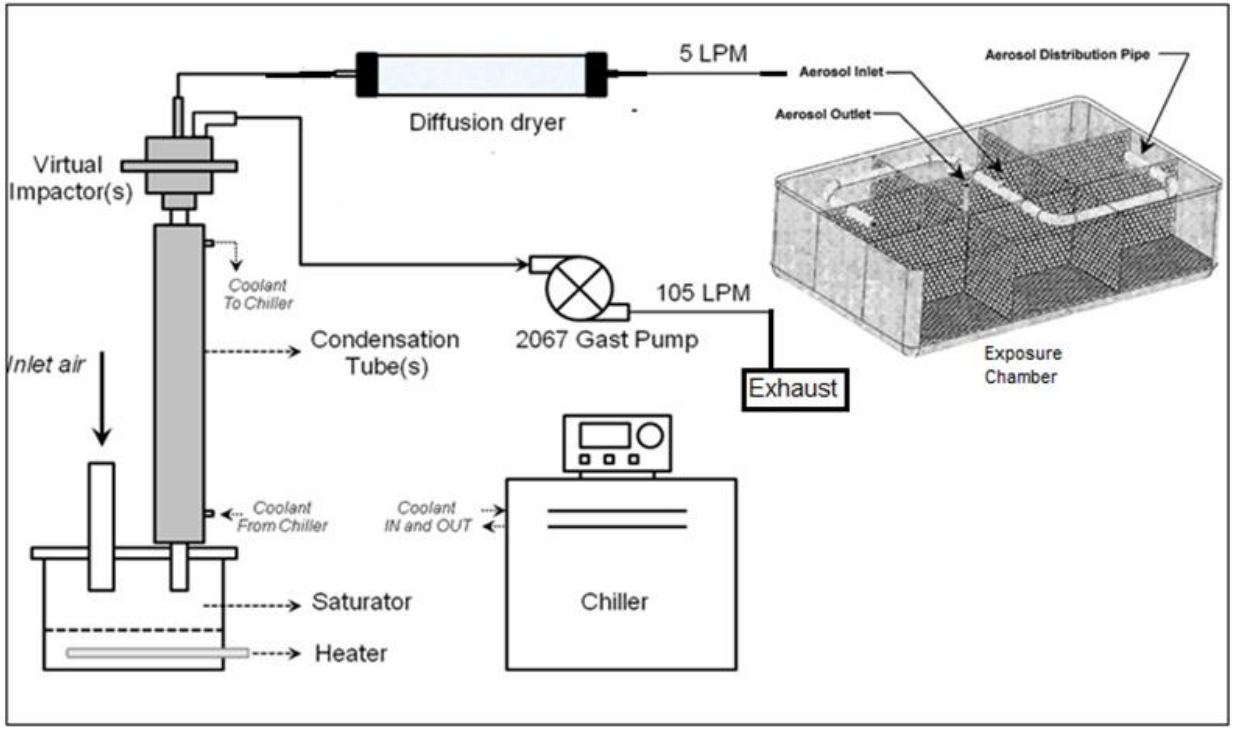
Can polluted air affect humans with genetic pre-disposition for Alzheimer's disease?

Study design:

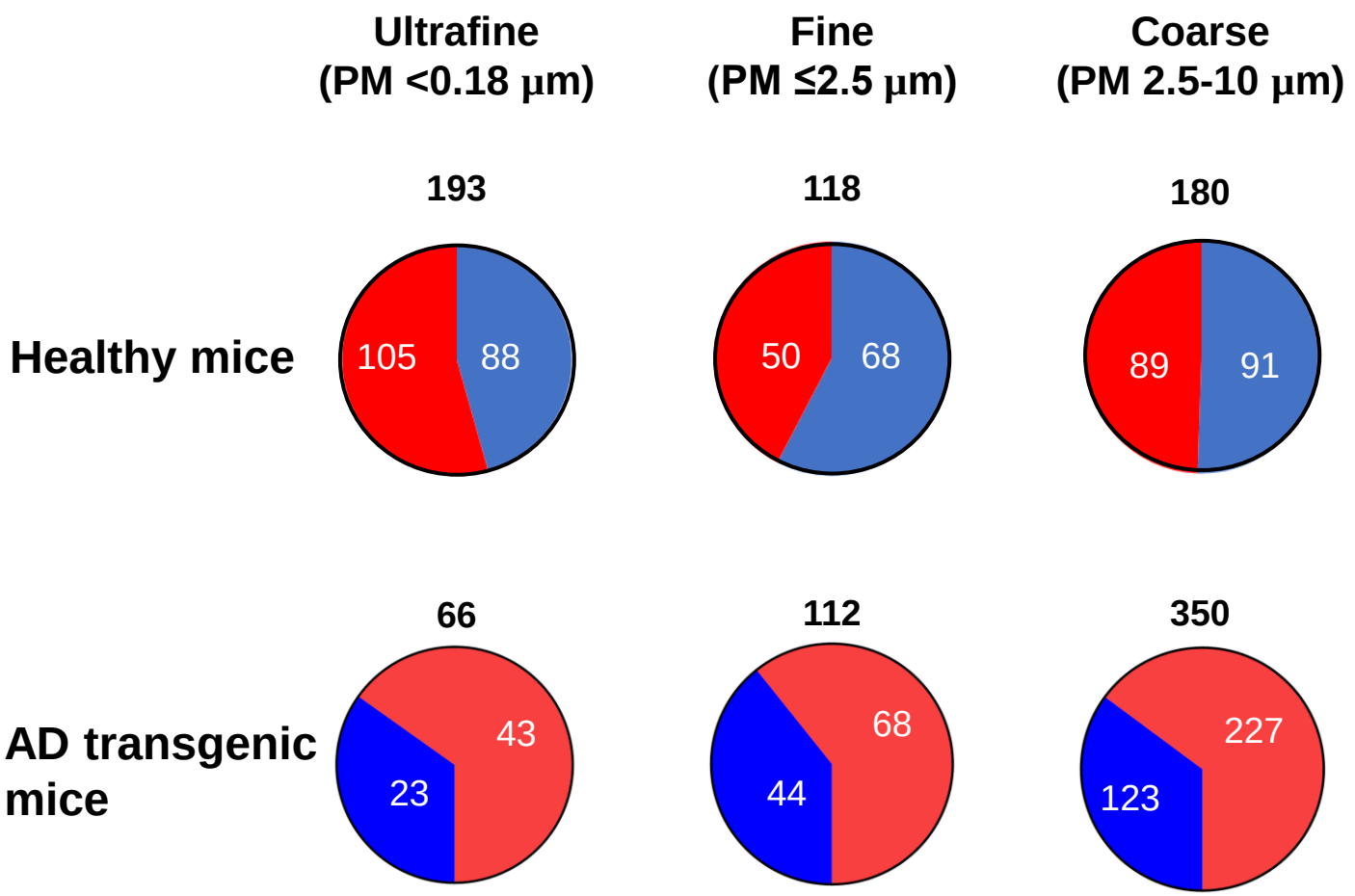
- Mice were exposed separately to particulate matter (PM) defined by size as **ultrafine** (**PM <0.18 μm**), **fine (PM ≤2.5 μm**) or **coarse (PM 2.5-10 μm**) using a state-of-the-art air pollution exposure system.
- Mice were exposed to PM for 5 hours per day for 5 days each week.
- The VACES, shown schematically in the right figure, was used to conduct PM exposures.
- RNA sequencing and analysis (of the mice brains) were performed, as well as immunohistochemistry.



The Versatile Aerosol Concentration Enrichment System (VACES)



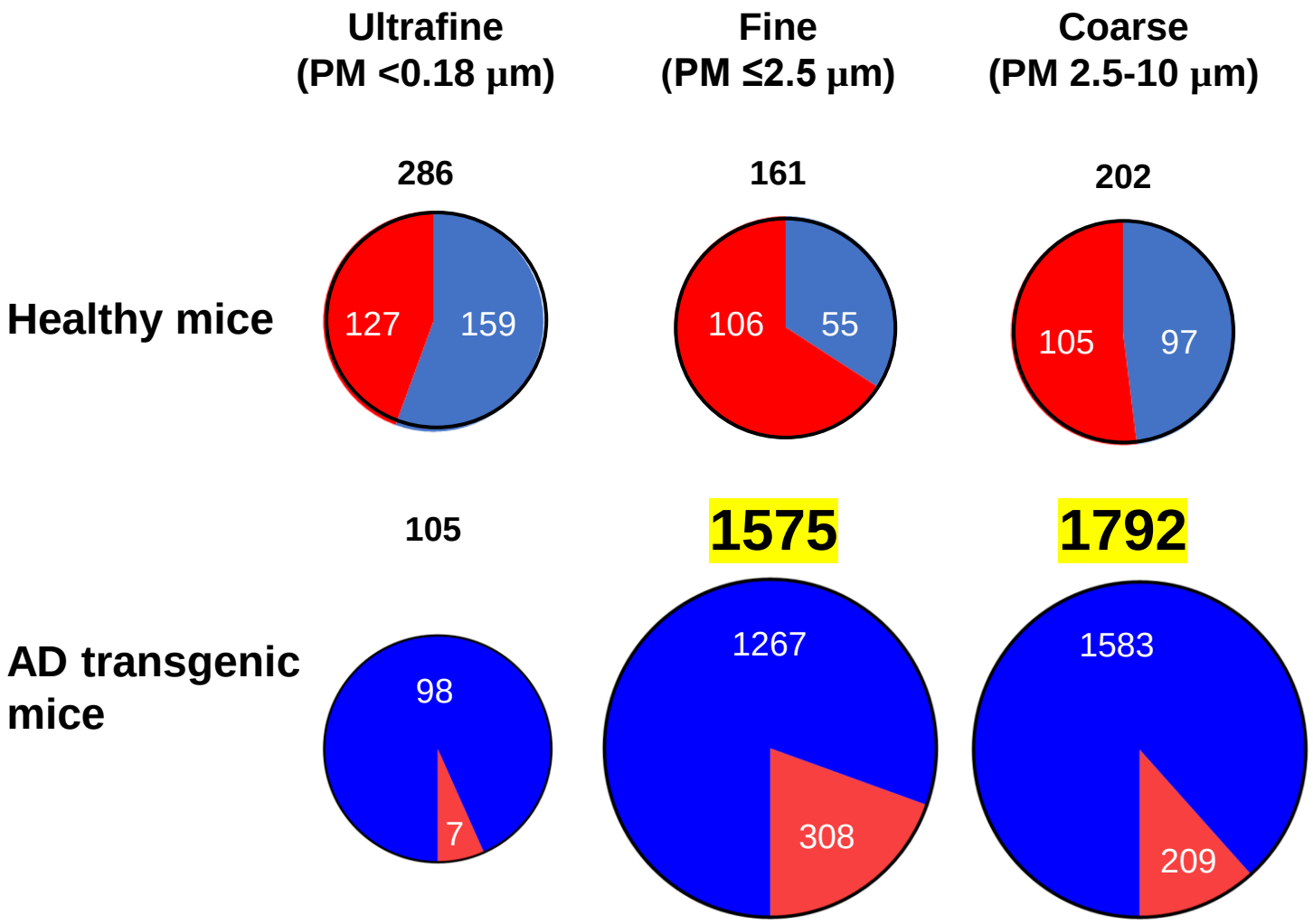
Gene dysregulation: AD Vs. Normal at **3-month** exposure to PM



Blue: downregulated genes
Red: upregulated genes

- Differentially expressed genes (DEG) were calculated by comparing gene expression in each PM exposure group separately with expression levels in the clean air exposure control group.
- DEGs were deemed significant at ≥1.5-fold change and $p \leq 0.05$ significance.
- Different mice strains (AD vs. healthy) gene dysregulation is different and connected directly to PM type.
- For **AD mice**, most genes are **upregulated** following **3-month** exposure in all PM groups.
- While healthy mice the highest gene dysregulation is seen in UFPM, the AD mice are affected mostly by coarse PM.

Gene dysregulation in exposed mice: AD Vs. Normal at **6-month** exposure

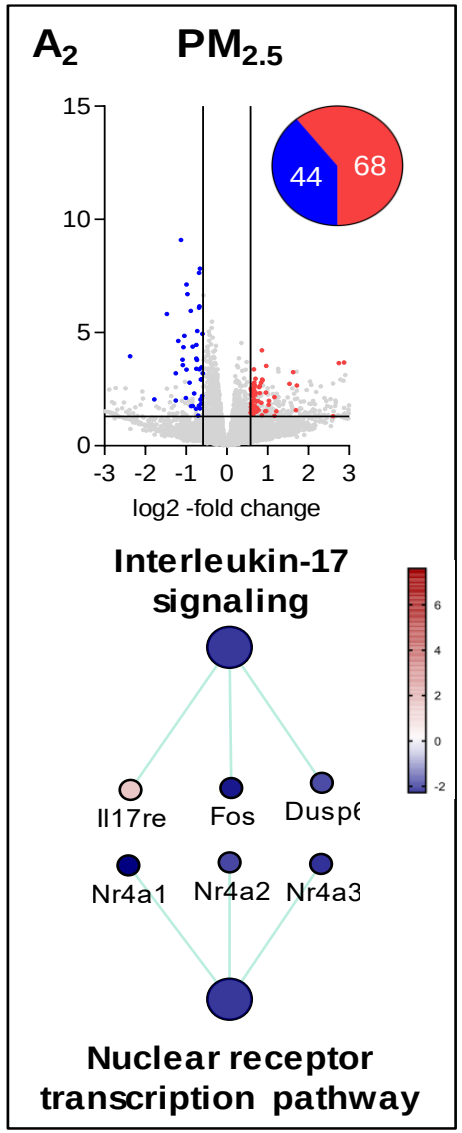


Blue: downregulated genes
Red: upregulated genes

- While the dysregulation in genes of healthy mice at 6 month remains moderate, for AD mice a massive dysregulation is evident for coarse and fine PM exposure groups.
- At 6-month exposure, all **AD 3xTg** exposure groups reveals more **downregulated** genes than upregulated ones.
- For healthy mice, the highest dysregulation is again detected at the UFPM group.

Gene dysregulation in AD mice: **pathways initiated** following **3-month** of **fine PM** exposure

Global DEG & reactome gene enrichment in **Alzheimer's** mice at **3** months



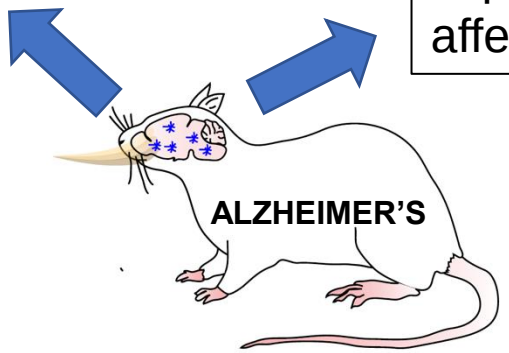
Blue: downregulated genes
Red: upregulated genes

Main pathways dysregulated:

- G alpha (i) signalling events
- Antimicrobial peptides
- Neutrophil degranulation
- Alternative complement activation and activation of C3 and C5 (complement)
- PD-1 signaling - MHC class II antigen presentation
- Nuclear Receptor transcription pathway

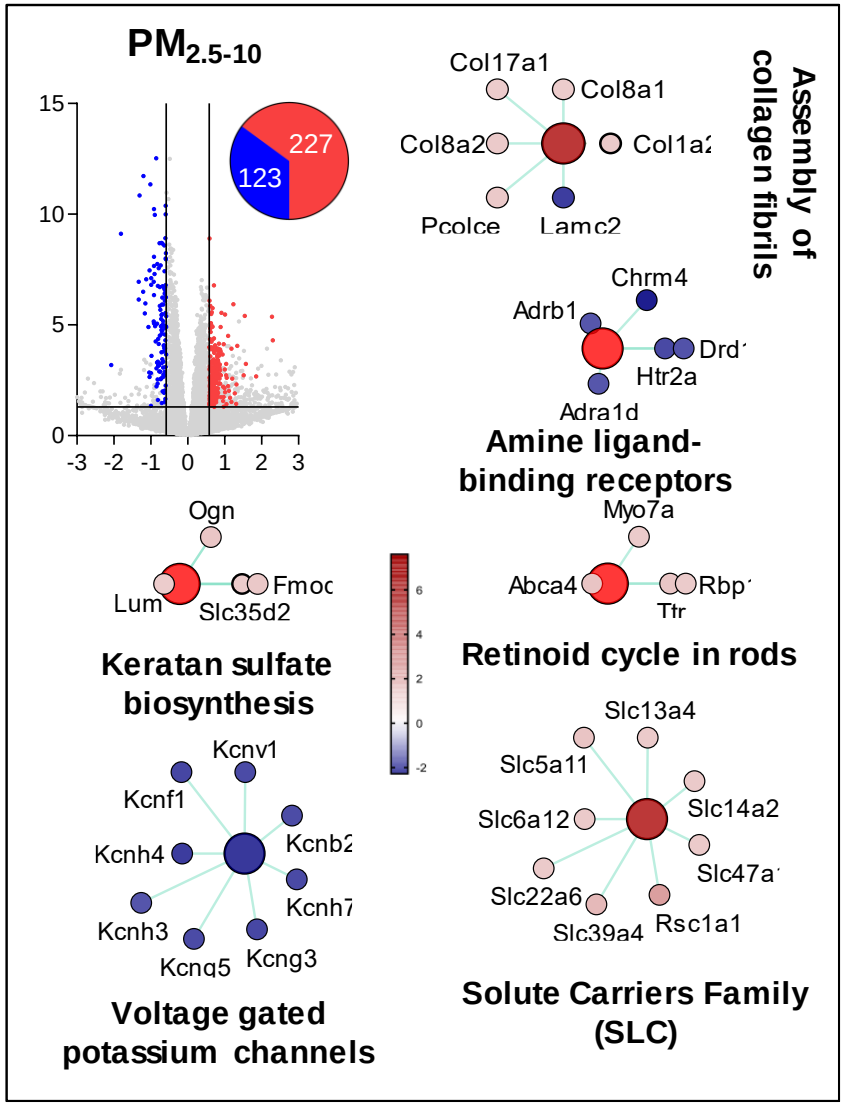
The immune system activated

Gene expression is affected



Gene dysregulation in AD mice: **pathways initiated** following **3-month** of **coarse PM** exposure

Global DEG & reactome gene enrichment in **Alzheimer's** mice at **3** months



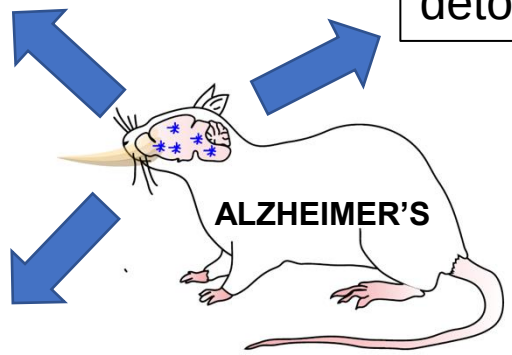
Blue: downregulated genes
Red: upregulated genes

Main pathways dysregulated:

- Voltage gated Potassium channels
- Keratan sulfate biosynthesis
- G alpha (q) signalling events
- Common Pathway of Fibrin **Clot Formation** and activation of C3 and C5 (**complement immune system**).
- **Collagen** biosynthesis and modifying enzymes, chain trimerization and assembly of collagen fibrils and multimeric structures.
- Transport of bile salts and organic acids, metal ions and amine compounds (solute carrier family). The **SLC family** function includes active efflux transport in the blood-brain barrier (BBB) which work as a **detoxification system** in the brain by facilitating **removal of xenobiotic** compounds.

The immune system activated

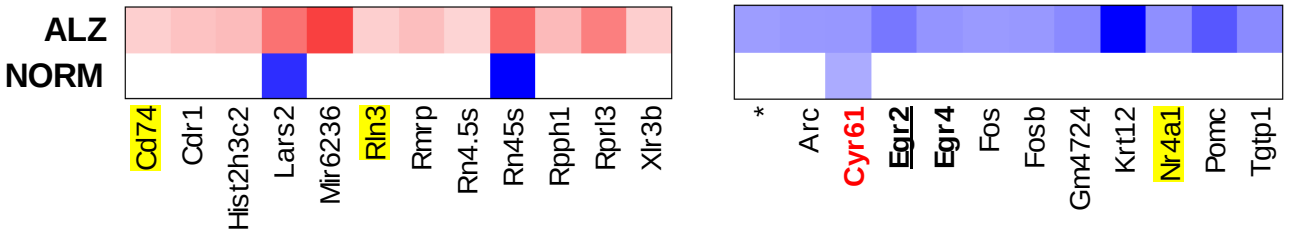
Carriers for brain detoxification increased



Coagulation process initiated

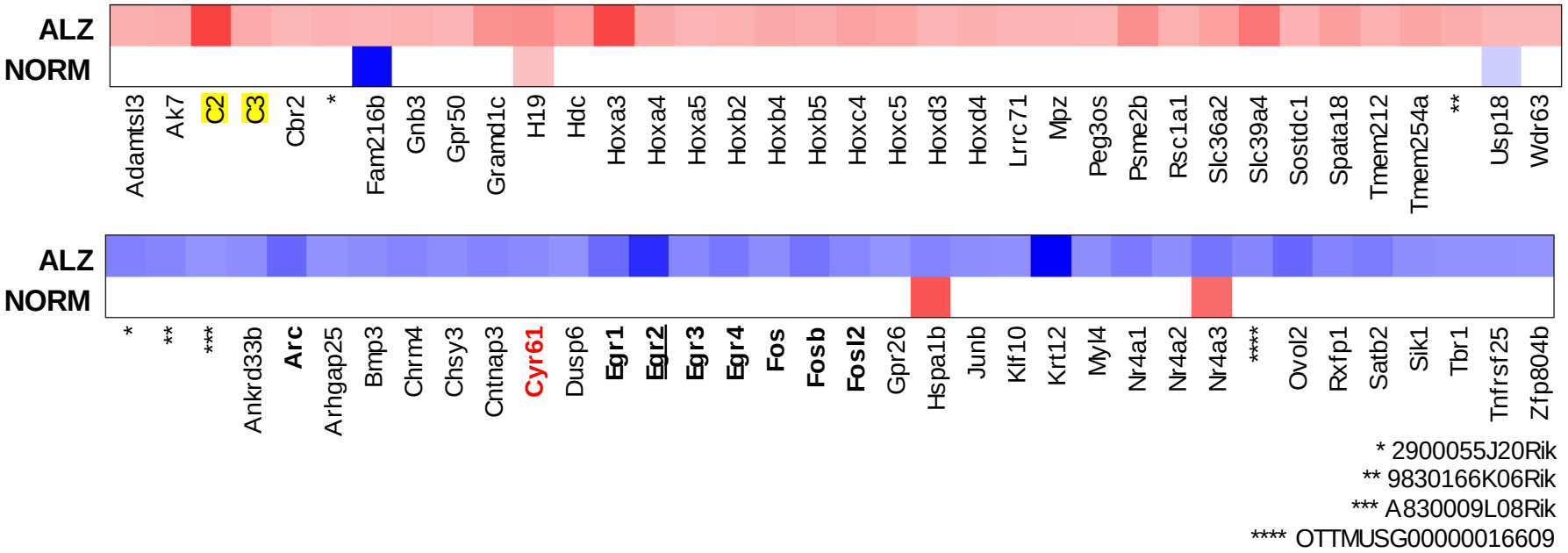
Top 10% Differentially expressed genes in coarse PM exposure: AD vs normal following **3-month** exposure

A) Top 10% DEG in $PM_{2.5}$



* 8430408G22Rik

B) Top 10% DEG in $PM_{2.5-10}$



* E230008N13Rik
** Tnfsf12Tnfsf13

* 2900055J20Rik
** 9830166K06Rik
*** A830009L08Rik
**** OTTMUSG00000016609

Blue: downregulated genes
Red: upregulated genes

(A) In healthy mice following 3-month <u>fine PM</u> exposure:

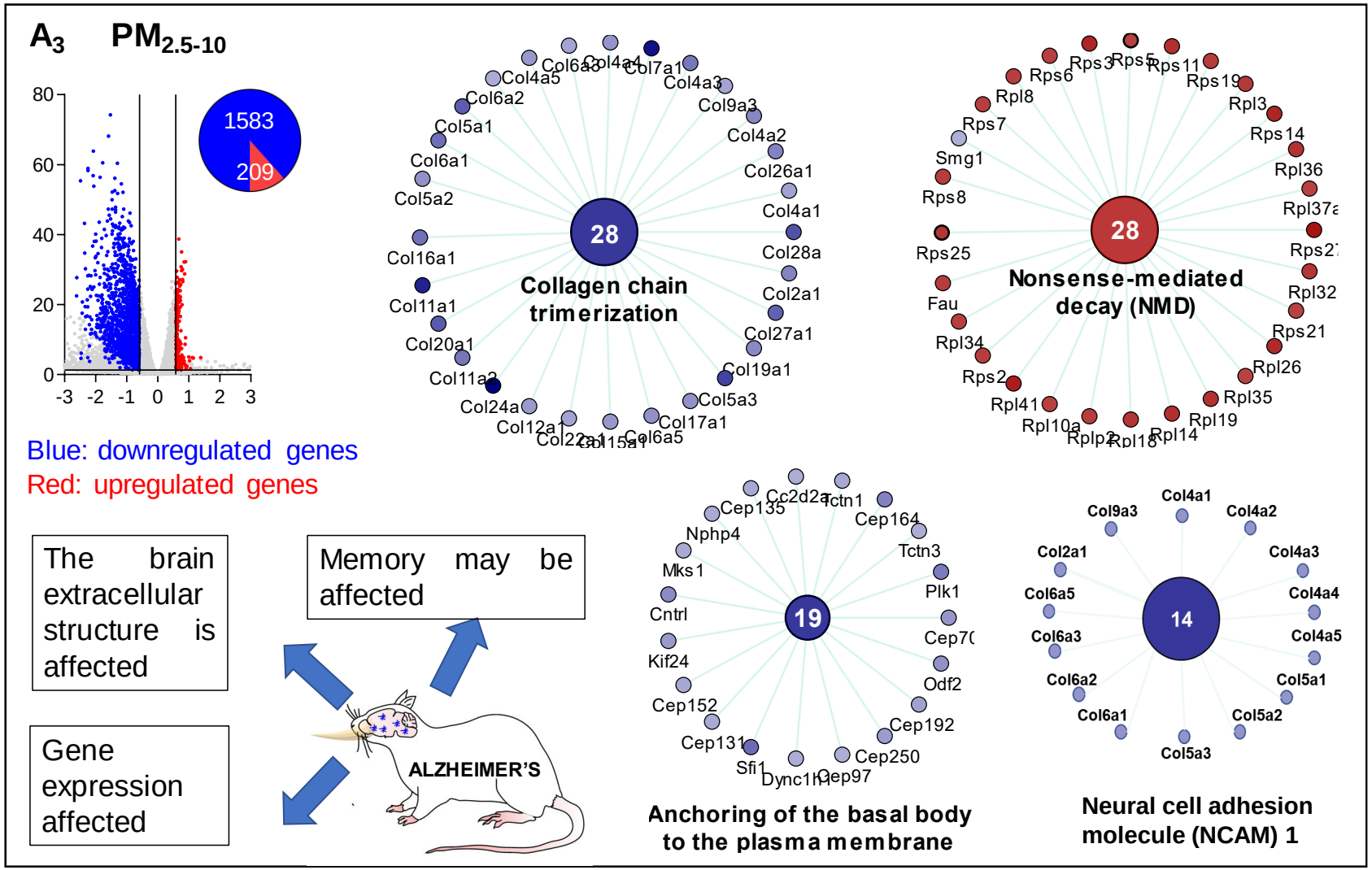
- G alpha **(i)** signalling events affected and evidence of neutrophil degranulation is seen
- **No** evidence of PD-1 signaling pathway dysregulation, complement activation, MHC class II antigen presentation or Nuclear Receptor transcription pathway dysregulation.

(B) In healthy mice following 3-month of <u>coarse PM</u> exposure:

- G alpha **(i)** signalling events affected
- **No** evidence for complement activation or collagens dysregulation.

Gene dysregulation in AD mice: **pathways** following **6-month** of **coarse PM** exposure

Transcriptome analysis and gene enrichment for 6-month exposures

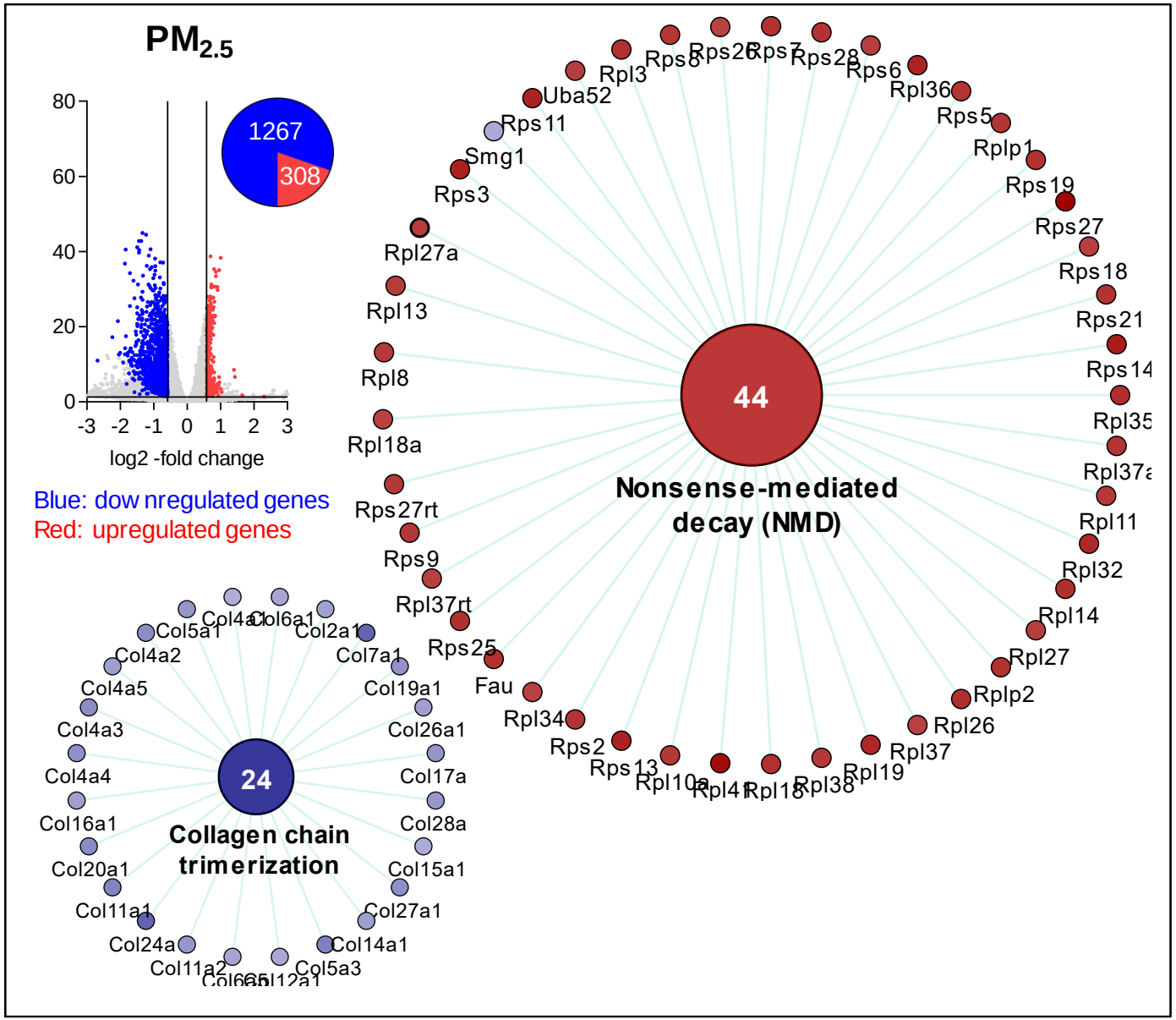


Main pathways dysregulated:

- **Collagen** chain trimerization, biosynthesis and modifying enzymes, extracellular matrix organization, collagen degradation, **ECM** proteoglycans, Laminin interactions, Crosslinking of collagen fibrils, Non-integrin membrane-ECM interactions, Integrin cell surface interactions.
- **Nonsense Mediated Decay (NMD)** independent of the Exon Junction Complex (EJC) and NMD enhanced by the Exon Junction Complex (EJC). Nonsense-mediated mRNA decay is a surveillance pathway that exists in all eukaryotes.
- **NCAM1** interactions - Neural cell adhesion molecule (NCAM) has been implicated as having a role in cell–cell adhesion, neurite outgrowth, synaptic plasticity, and **learning and memory**.

Gene dysregulation in AD mice: **pathways** following **6-month** of **fine PM** exposure

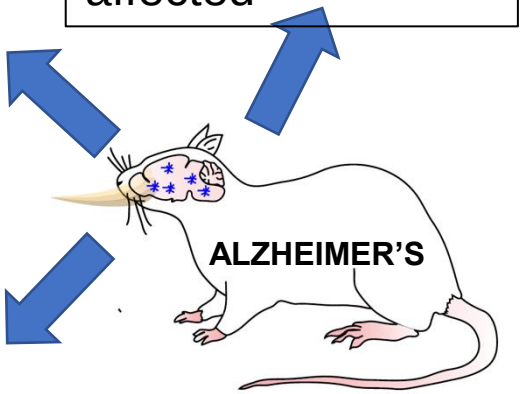
Transcriptome analysis and gene enrichment for 6-month exposures



The brain extracellular structure is affected

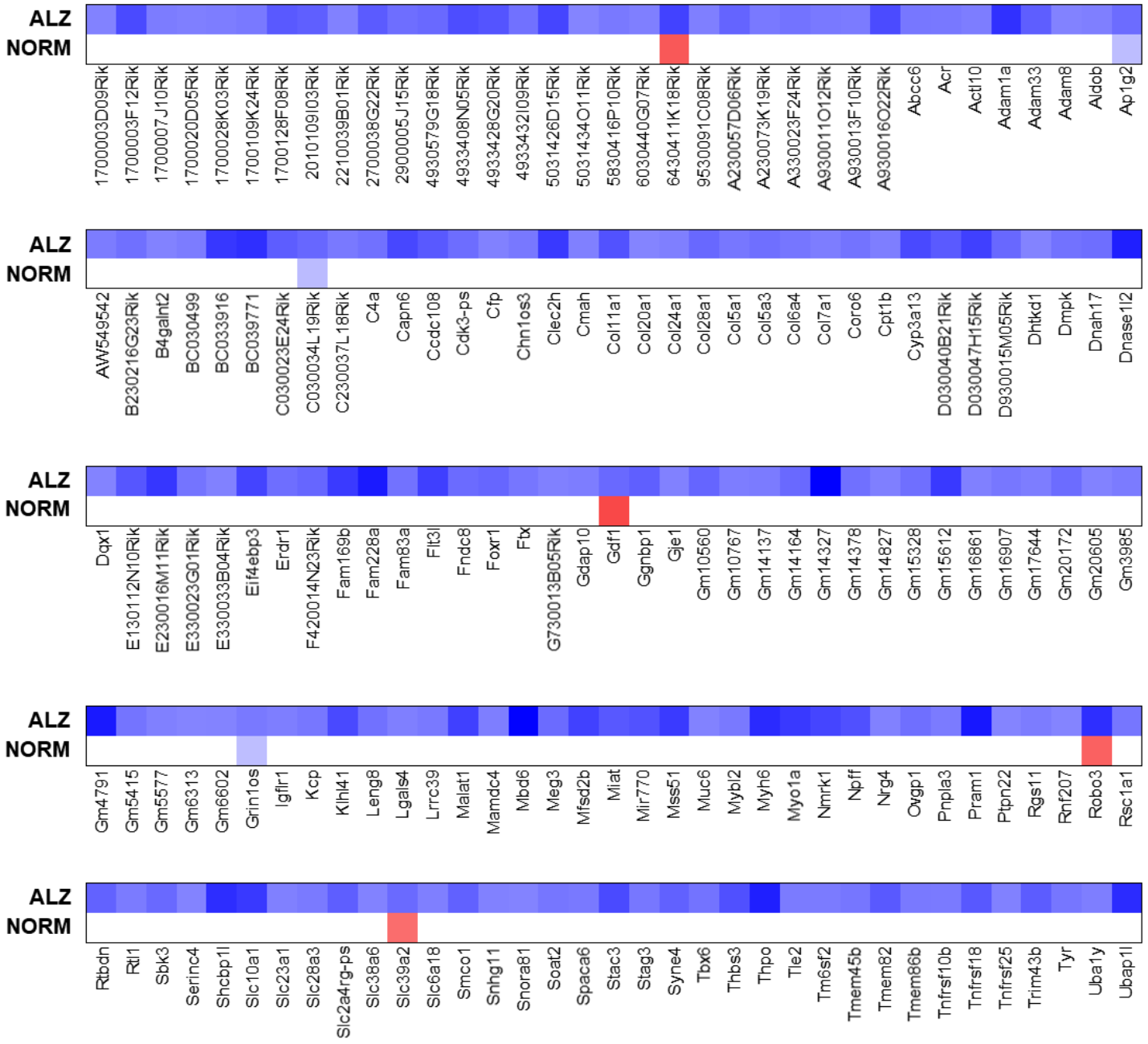
Gene expression affected

Memory may be affected



Main pathways dysregulated for fine PM 6-month exposure in AD 3xTg mice is as the coarse PM exposure dysregulation.

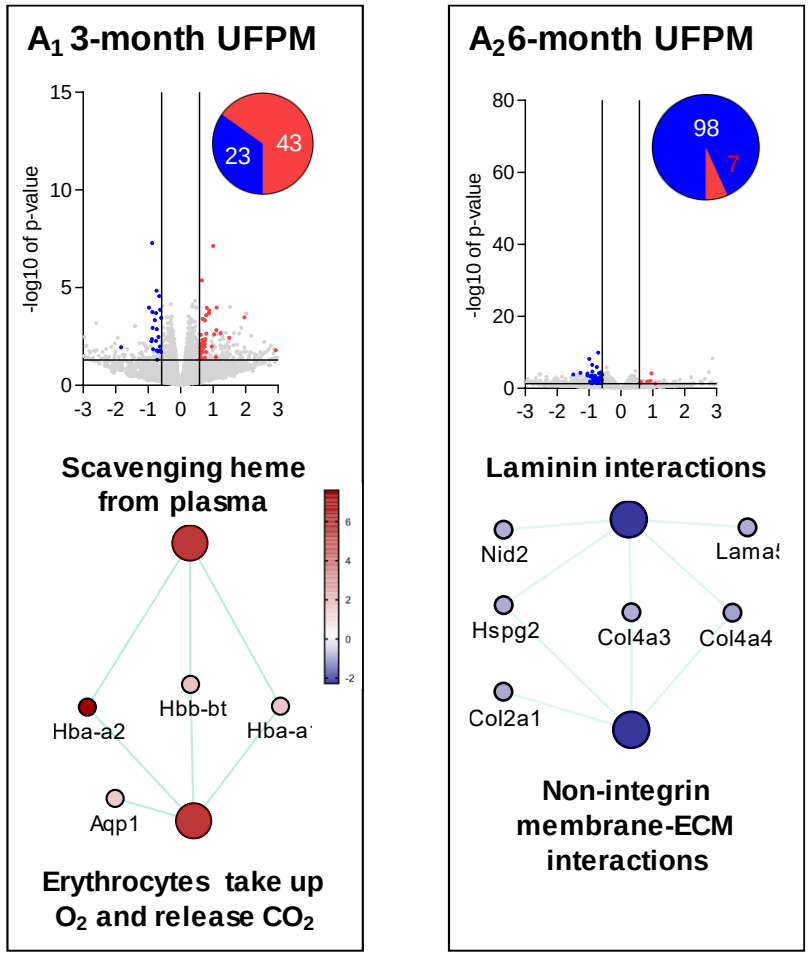
Top 10% Differentially expressed genes in coarse PM exposure: AD vs normal following coarse PM 6-month exposure



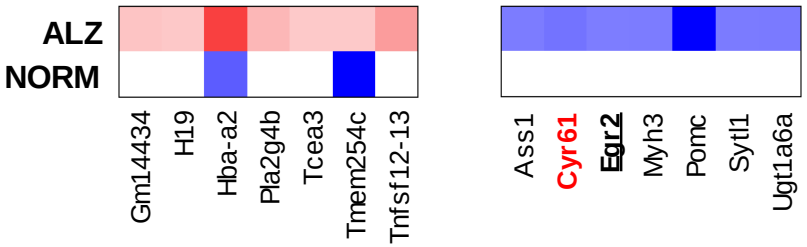
- The top 10% of DEG as well as the pathways mentioned before following 6-month exposure are unique to the AD mice.
- More genes are downregulated than upregulated in AD coarse 6-month exposure.
- Some of the genes are upregulated but are with low expression.

Gene dysregulation in AD mice: pathways seen following 3- and 6-month of UFPM

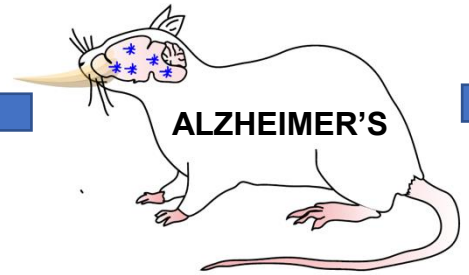
(**A**) Global DEG & reactome gene enrichment in **Alzheimer's** mice



(**B**) Top 10% DEG in UFPM



The brain extracellular structure is affected



Memory may be affected

A_1) Main pathways dysregulated at 3-month exposure:

- G alpha (i) signalling events
- Peptide ligand-binding receptors
- Neutrophil degranulation
- changes in cellular processes related to oxygenation of blood and hemoglobin

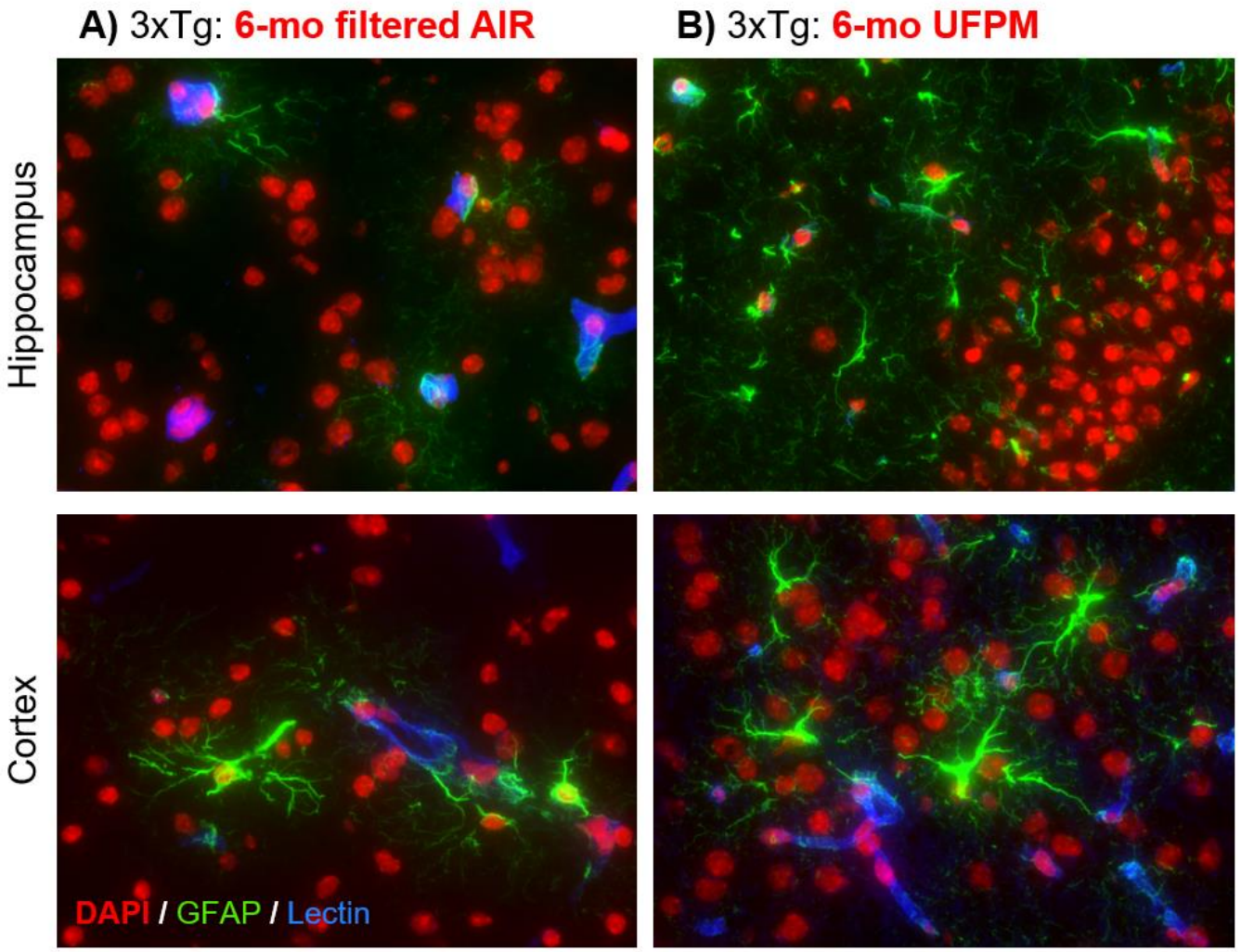
A_2) Main pathways dysregulated at 6-month exposure:

- Laminin interactions, Non-integrin membrane-ECM interactions, **Extracellular matrix** organization, Integrin cell surface interactions, ECM proteoglycans, Collagen chain trimerization, assembly of collagen fibrils and other multimeric structures, crosslinking of collagen fibrils, collagen biosynthesis and modifying enzymes, collagen degradation
- **NCAM1** interactions
- Platelet degranulation

B) Top 10% DEG following 3-month exposure to UFPM:

Suggests different genes and pathways affected compared to healthy mice.

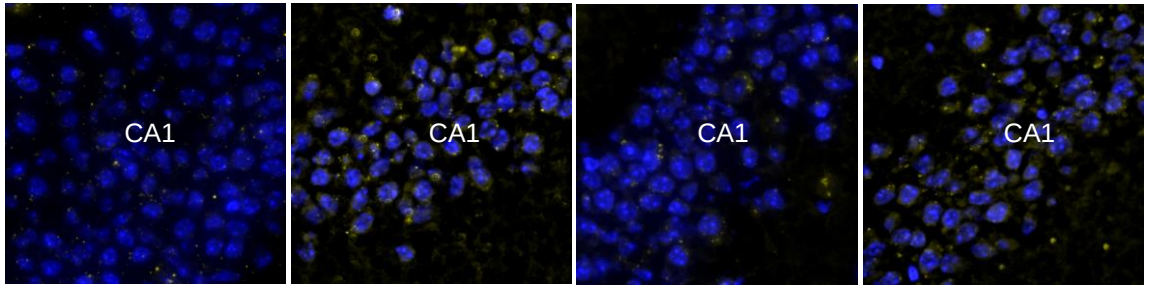
AD inflammation: even in UFPM, immune cells are activated



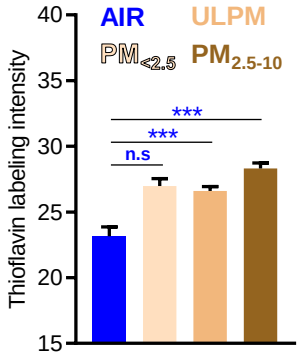
- Hippocampal and cortical brain regions of 3x Tg mice exposed for 6 months to filtered air (A) and UFPM (B).
- The numbers / density of astroglia in the UFPM exposure group appears to be elevated in the hippocampus (compare left and right images in top panel).

Altered intracellular Aβ pathology in hippocampal neurons after **6-month** PM exposures

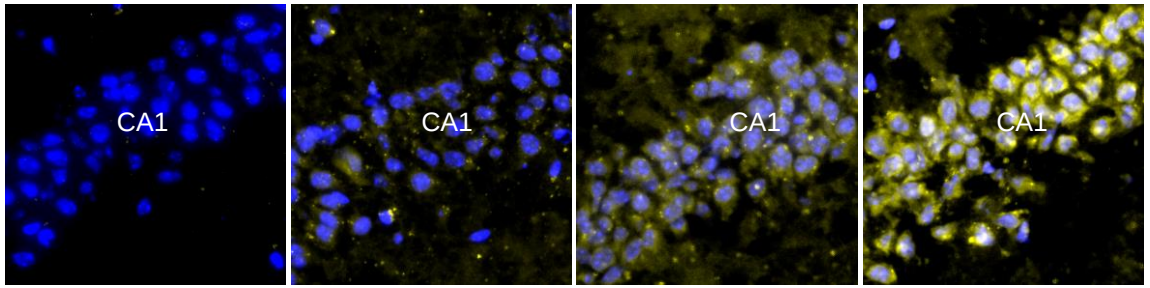
A) Hippocampal CA1 Thioflavin Staining: **3-month** exposure group



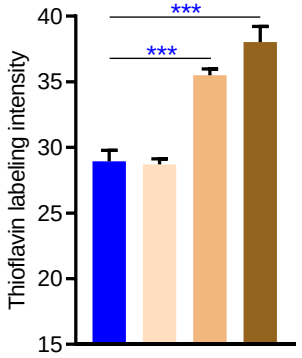
AIR $PM_{<2.5}$ $PM_{2.5}$ $PM_{2.5-10}$



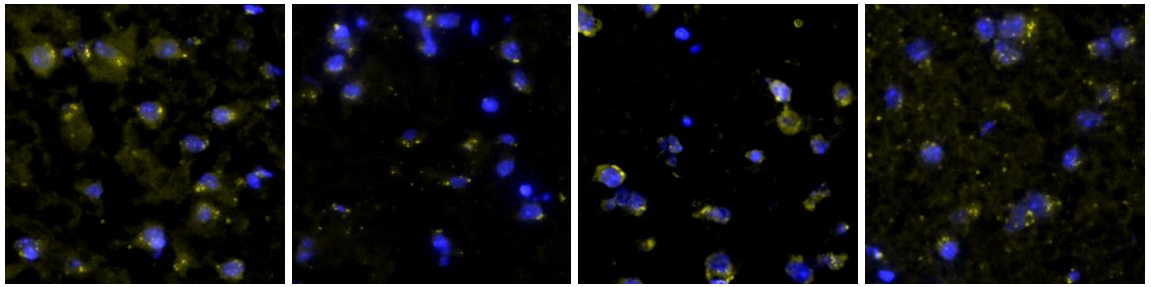
B) Hippocampal CA1 Thioflavin Staining: **6-month** exposure group

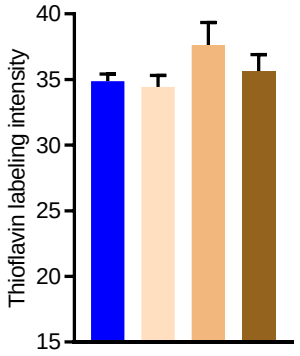


AIR $PM_{<2.5}$ $PM_{2.5}$ $PM_{2.5-10}$



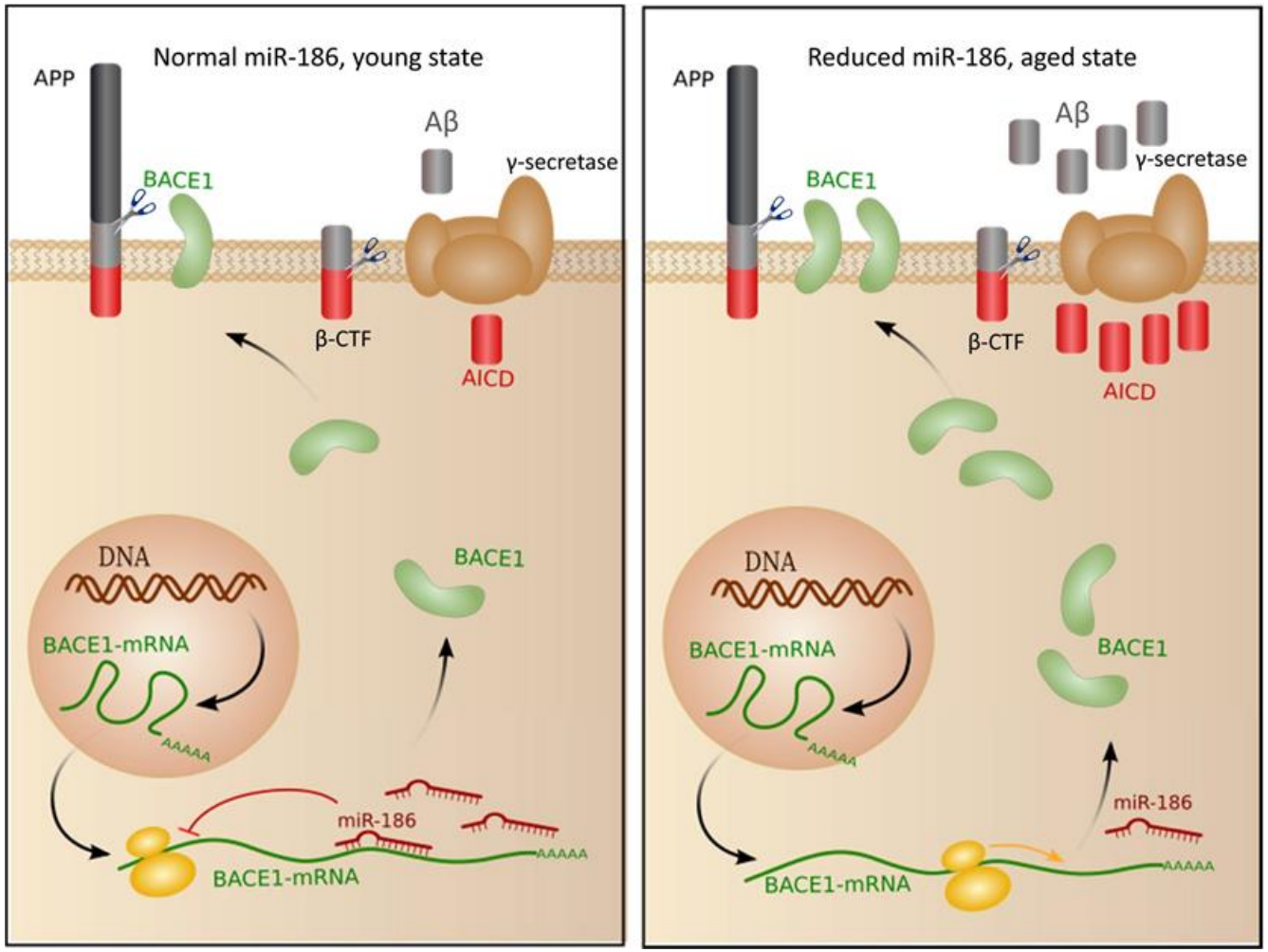
C) Prefrontal **Cortex** Thioflavin Staining: **6-month** exposure group





- Thioflavin-t, was used to stain soluble $A\beta_{1-42}$ and plaques.
- We find a mild increase in thioflavin-T staining in the brains of mice that were exposed to three months of particulate matter, mostly in the **hippocampi area**.
- After six-month long exposures, we observed a **more pronounced increase** in the staining. Notably, staining intensity also increased in mice that were exposed to clean air, which is indicative of the normal progression of AD.
- This increase is clearly visible in side by side comparisons of imaging data from Air, **PM≤2.5** and **PM2.5-10** tissues
- No senile plaques were detected following 6-month exposure.

Amyloid-beta burden: The classic APP/BACE-1 mechanism

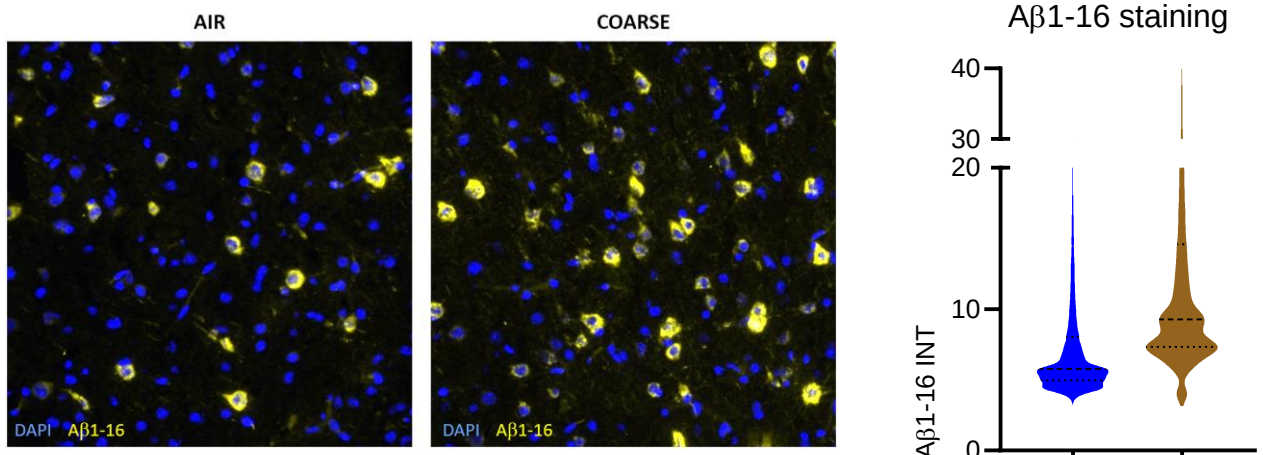


- $A\beta_{1-40}$ and $A\beta_{1-42}$ are cleavage products of a larger trans membranal protein called Amyloid Precursor Protein or APP.
- It is the common consensus that in AD, a burden of $A\beta_{1-42}$ is caused by an increase in an enzyme call beta secretase or BACE-1.
- This enzyme cleaves the APP protein in a location that results in $A\beta_{1-42}$ and not the non-toxic $A\beta_{1-40}$.
- Therefore, a lot of treatment efforts were aimed at inhibiting or blocking BACE-1.
- All BACE-1 targeting treatment to date did not pass clinical trials.

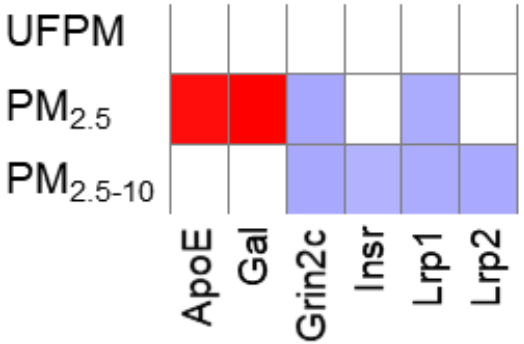
Ben Halima, S.; Siegel, G.; Rajendran, L., miR-186 in Alzheimer's disease: a big hope for a small RNA? *Journal of Neurochemistry* **2016,** *137* (3), 308-311.

Gene dysregulation in AD mice following **6-month** exposure: Aβ and classic mechanisms

(**A**) Hippocampal CA1 $A\beta_{1-16}$: 6-month exposure group (coarse PM exposure)

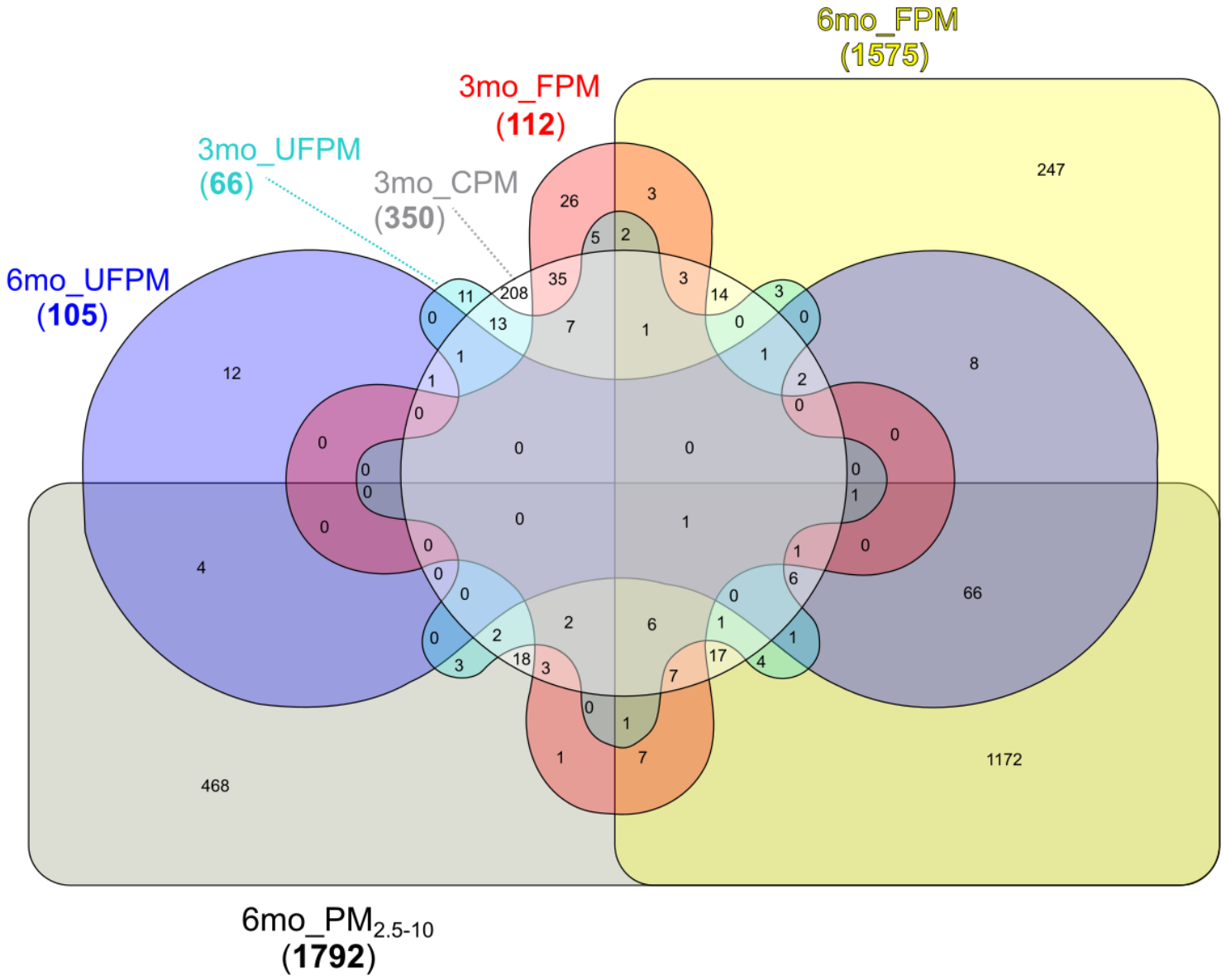


(**B**) Taqman Mouse Alzheimer's Panel (TaqMan #4418723)
Alzheimer's (6months)



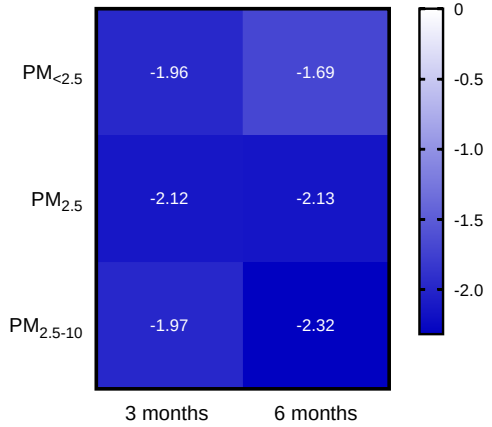
- **(A)** Staining for $A\beta_{1-16}$ confirms high levels of Aβ alloforms in AD mice following 6-month exposure to coarse PM.
- Interestingly, genes such as APP (amyloid precursor protein) and BACE-1 (beta secretase-1) which are often implicated in the Aβ burden in AD are not dysregulated in the RNAseq data for coarse PM 6-month exposed AD mice.
- **(B)** Low density lipoprotein receptor-related protein (LRP) 1 and 2 which have a role in the transport of Aβ alloforms across the BBB, both in and out of the brain, are downregulated.
- This observation supports the possible existence of an additional or alternative mechanism to the classic BACE-1/APP theory, for the amyloid increase and burden seen in AD.

Discovery of 'Unconventional' disease pathways through air pollution study: **CYR61**



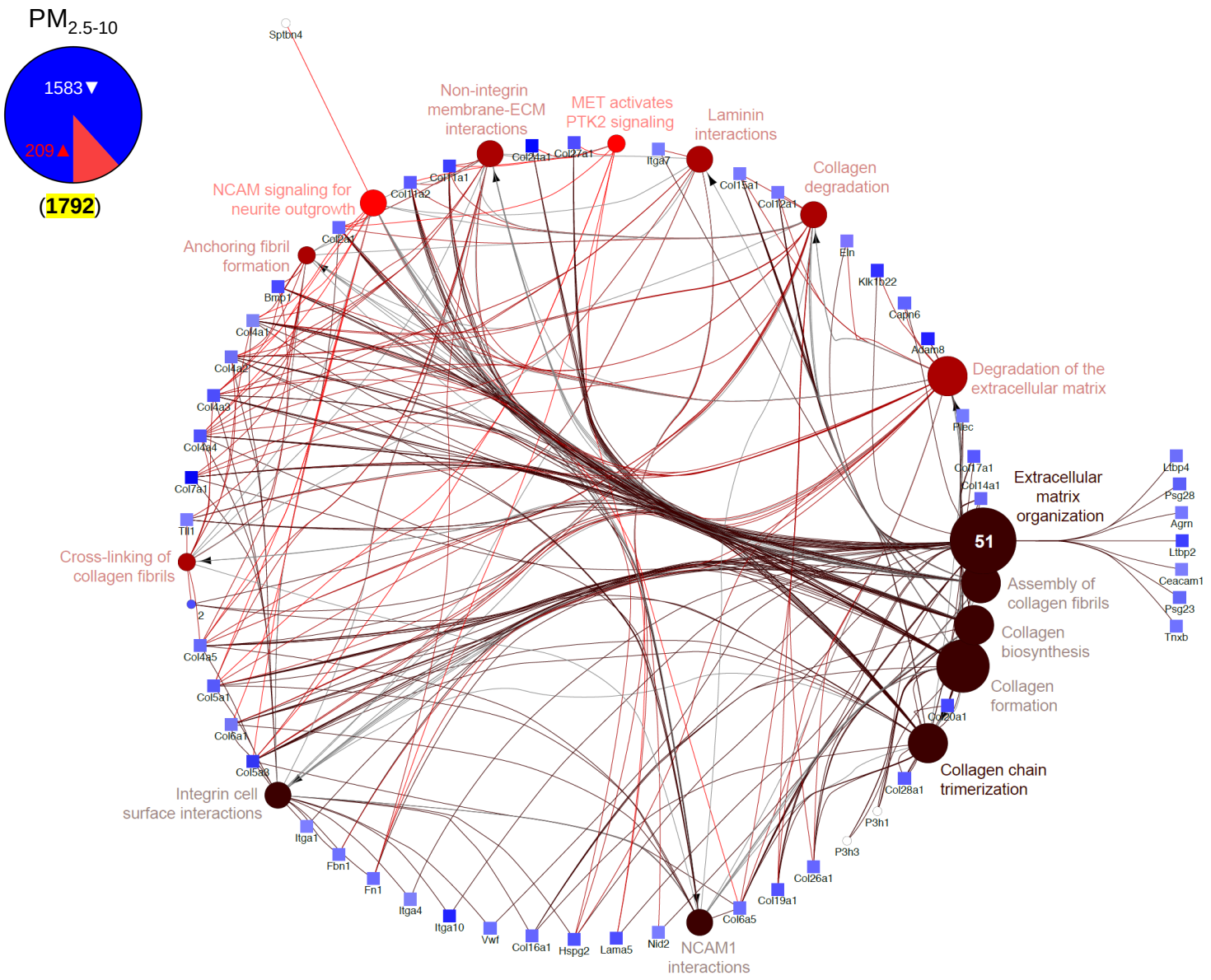
Overlap of DEG in Alzheimer's mice across time and PM conditions

- When all tested conditions following exposure of AD mice to PM were analyzed for overlapping genes, only one gene is consistently downregulated in both 3- and 6-month exposure, in all PM conditions: CYR61.
- In adulthood CYR61 plays important roles in inflammation and tissue repair and is associated with diseases related to chronic inflammation**.**
- Potential target for treatment.



CYR61 heatmap for all exposure conditions

Conclusions and future prospective



- Mice with **pre-disposition** to Alzheimer's are **more sensitive to coarse and fine** PM pollutants than healthy mice.
- Disease **progression in AD** mice exposed to coarse and fine PM was **accelerated**.
- Per the RNAseq analysis, **increased inflammatory response** is evident.
- **Remodeling of Extracellular matrix (ECM)** in Alzheimer's mice exposed to PM2.5-10
- A longer 12-month exposure is planned, as it may reveal additional pathway.
- >> **New biomarkers and targets** that are specifically linked to airborne pollution are being discovered. These biomarkers and targets can bring researches close to a solution and help design new potential **therapeutics**.

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