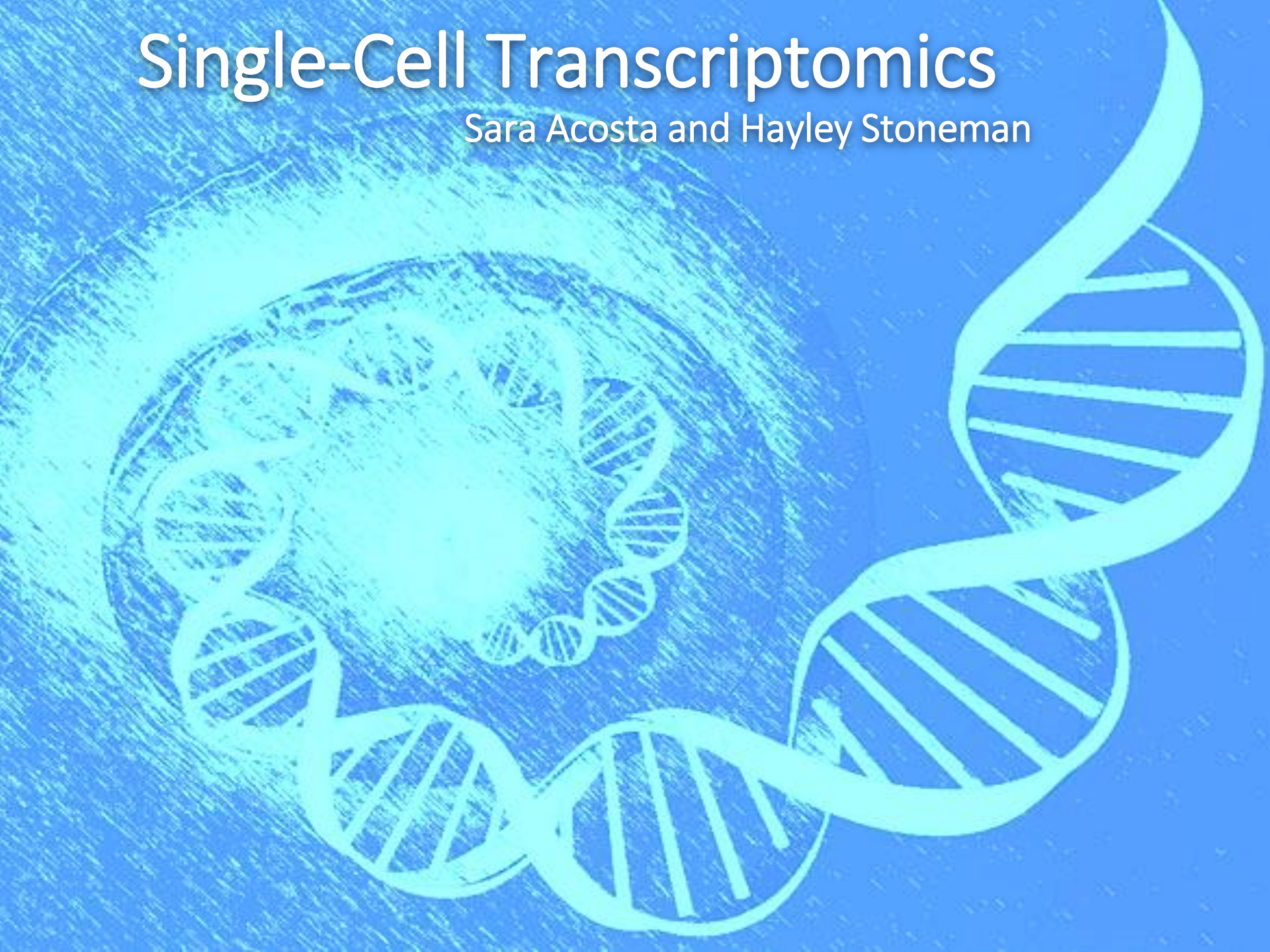
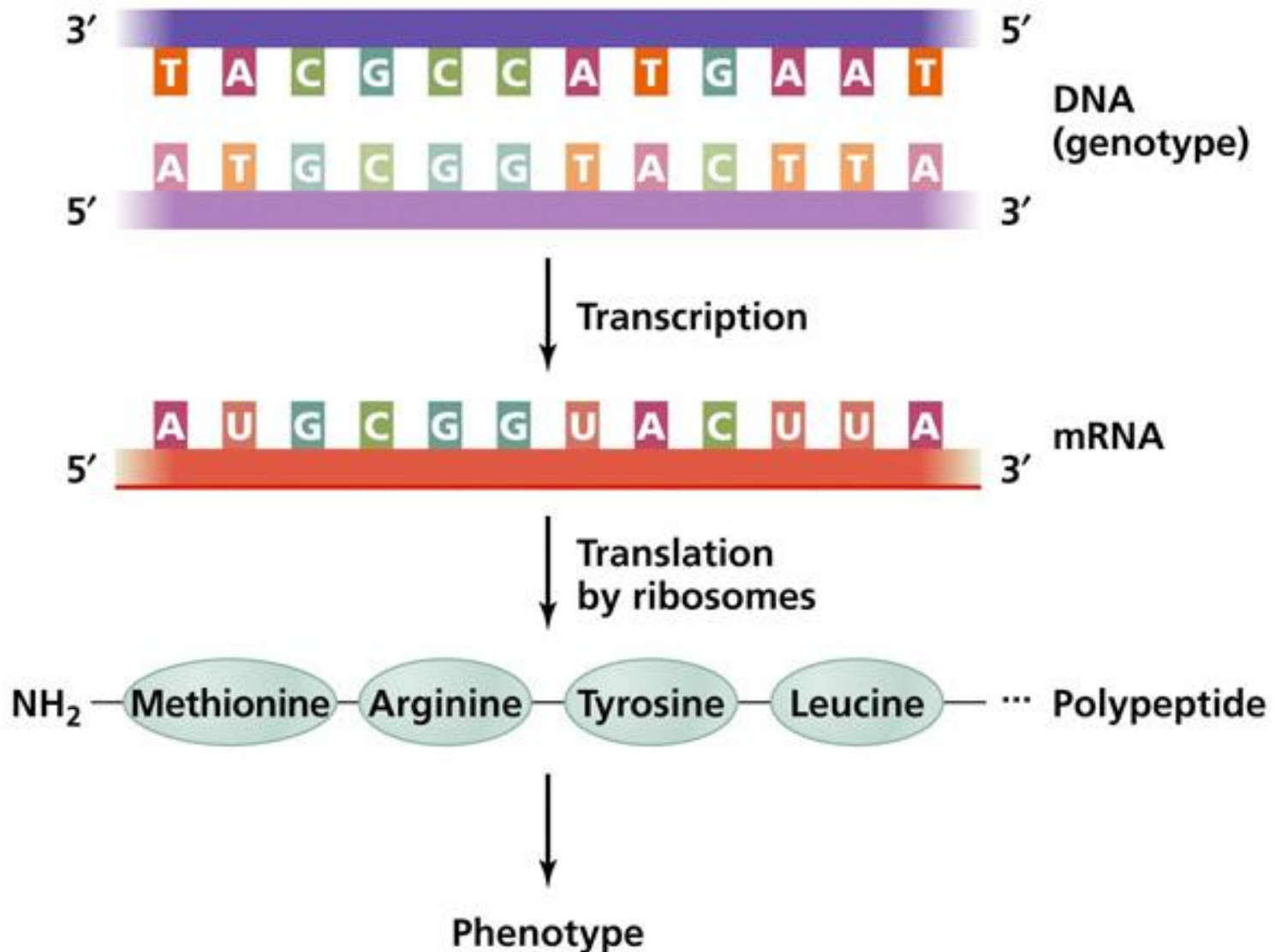


Single-Cell Transcriptomics

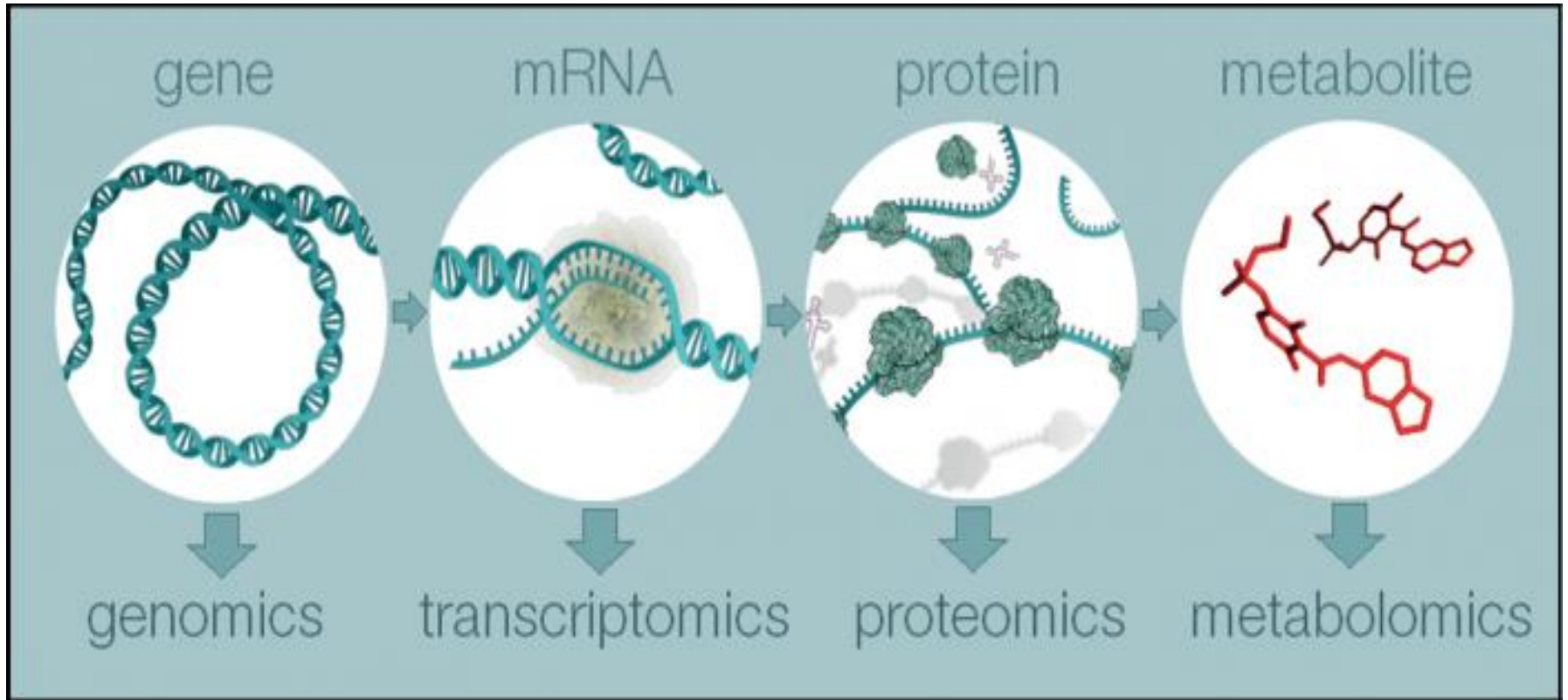
Sara Acosta and Hayley Stoneman



What is transcription?



What is transcriptomics?

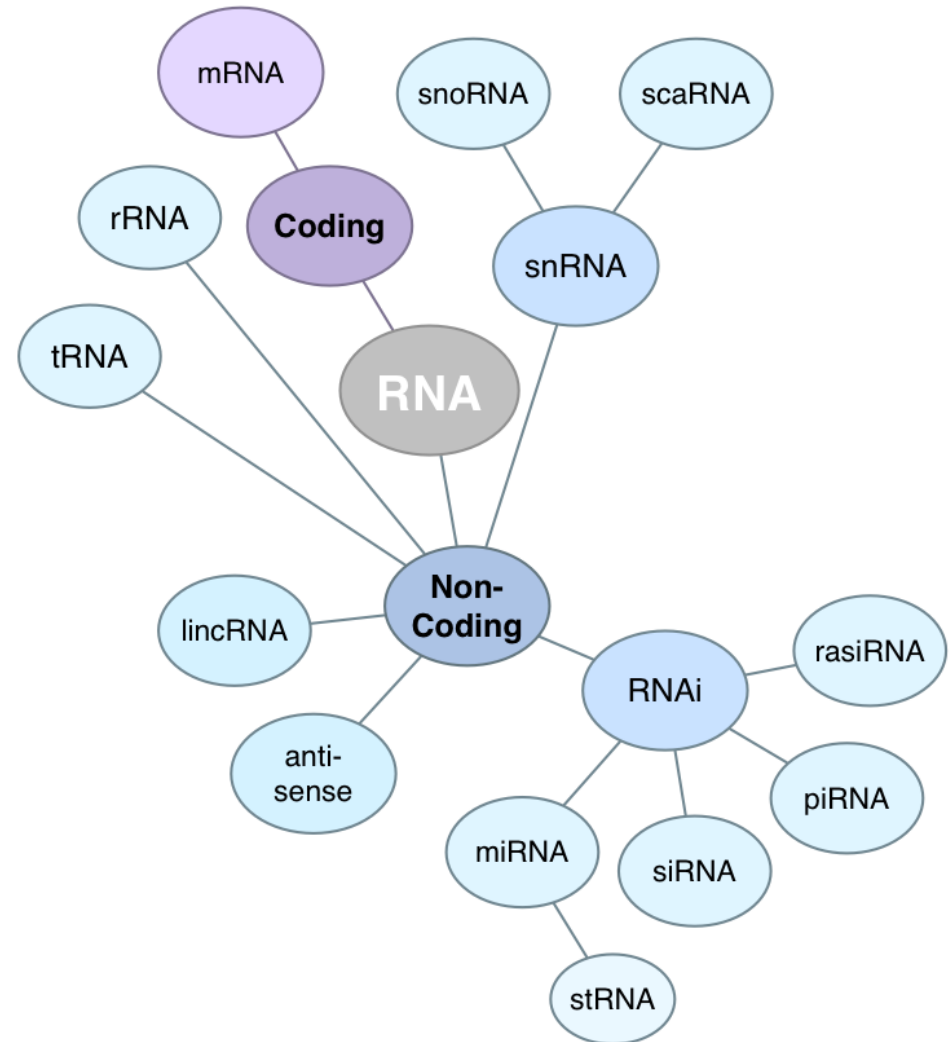


The study of the complete set of RNA transcripts

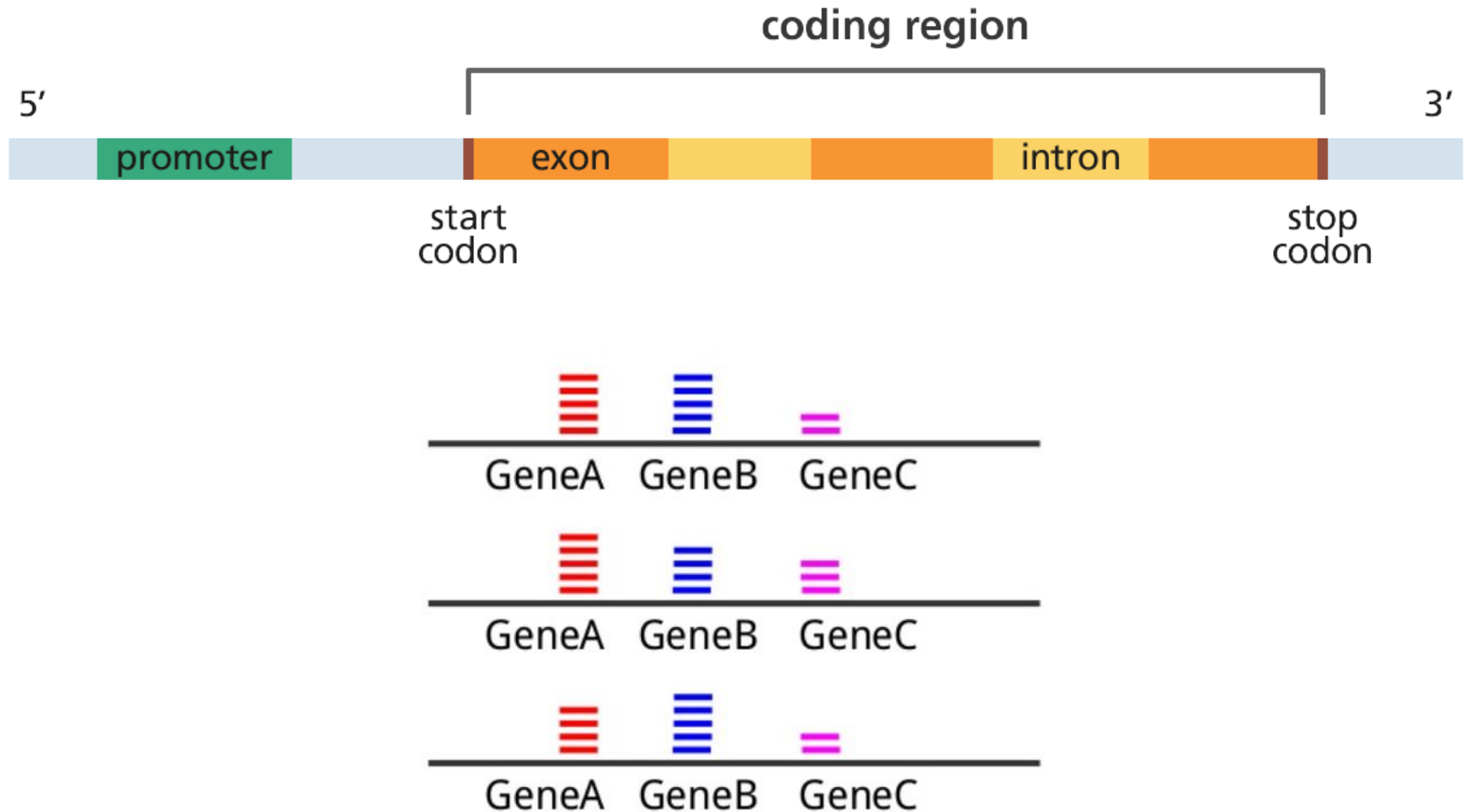
What molecules does transcriptomics study?



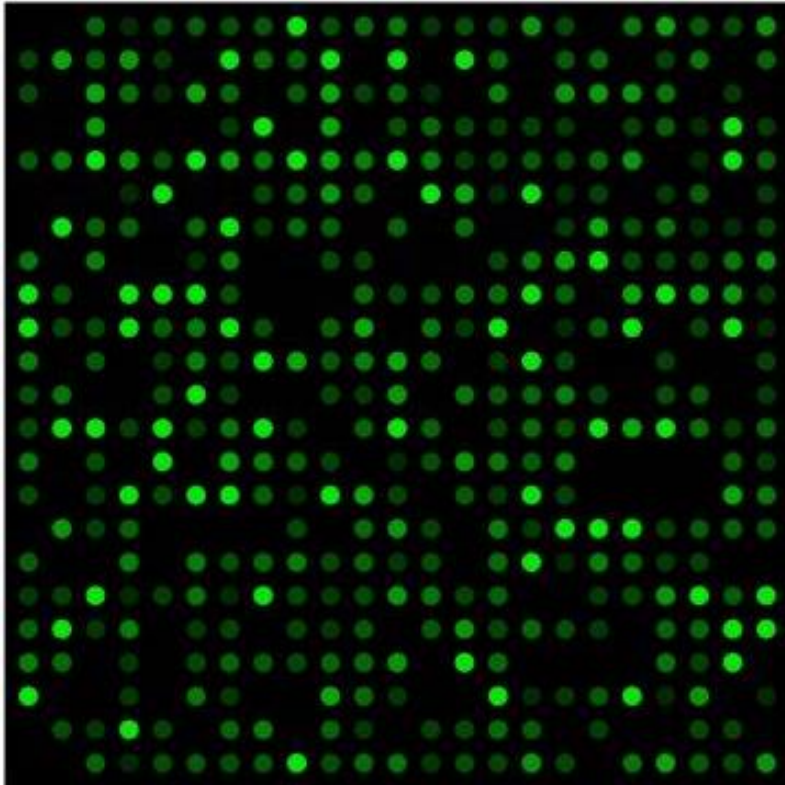
Messenger RNA (mRNA)



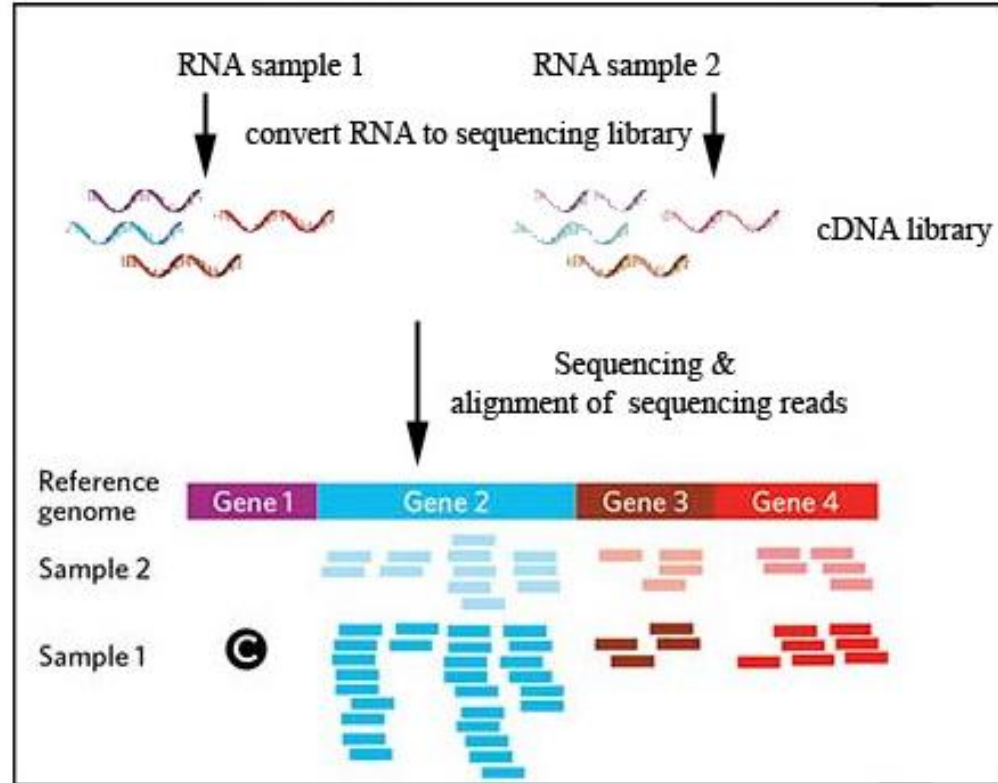
What can we discover through transcriptomics?



What methods are used to quantify the transcriptome?

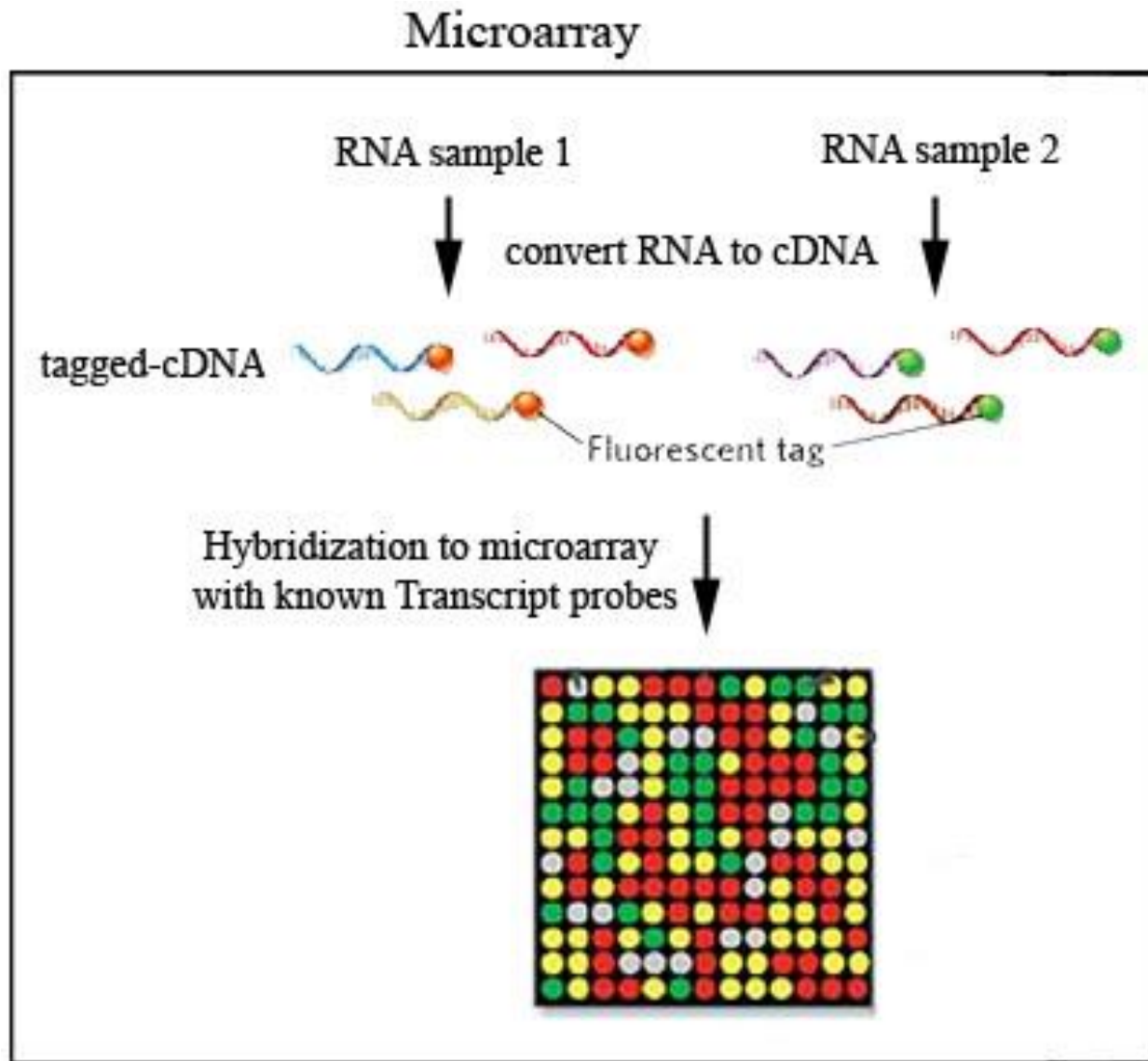


Microarray

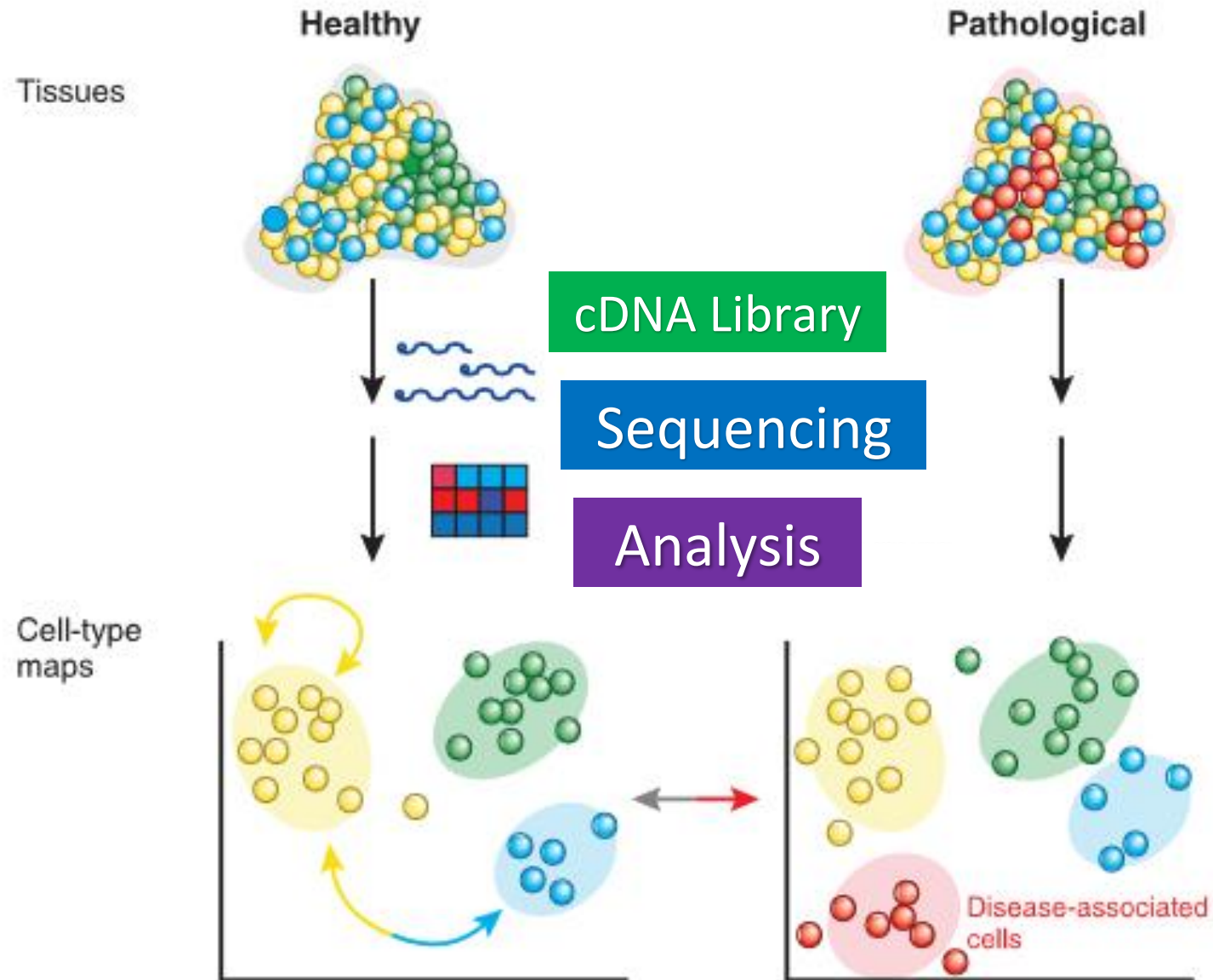


RNA Sequencing

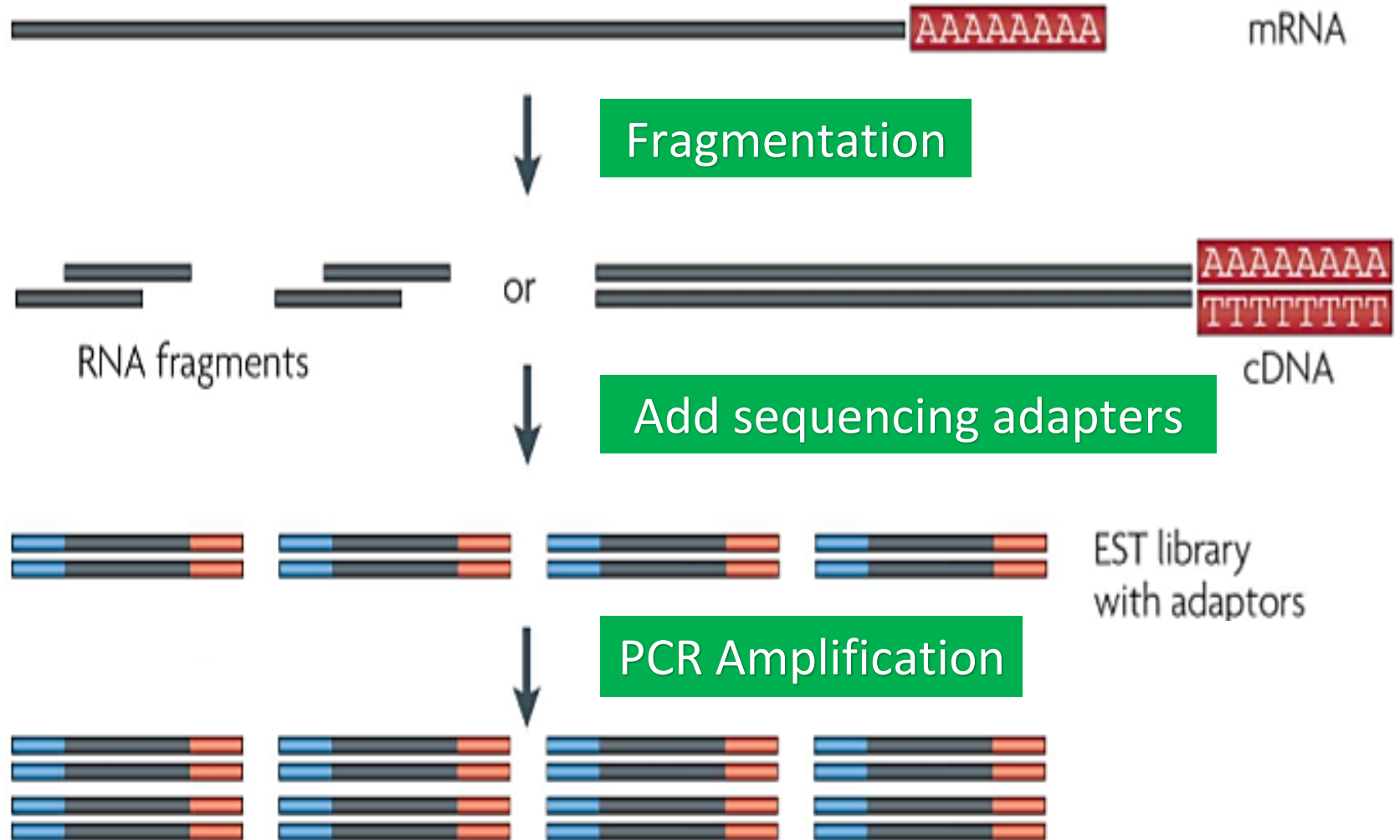
How do microarrays work?



How does RNA-Sequencing work?



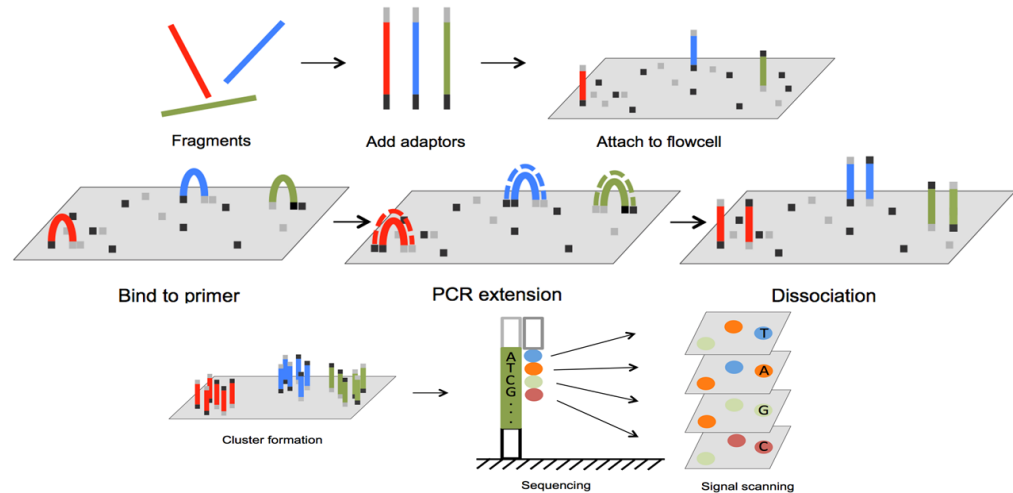
How is the cDNA library created?



How is the cDNA library sequenced?

High Throughput Sequencing

Illumina



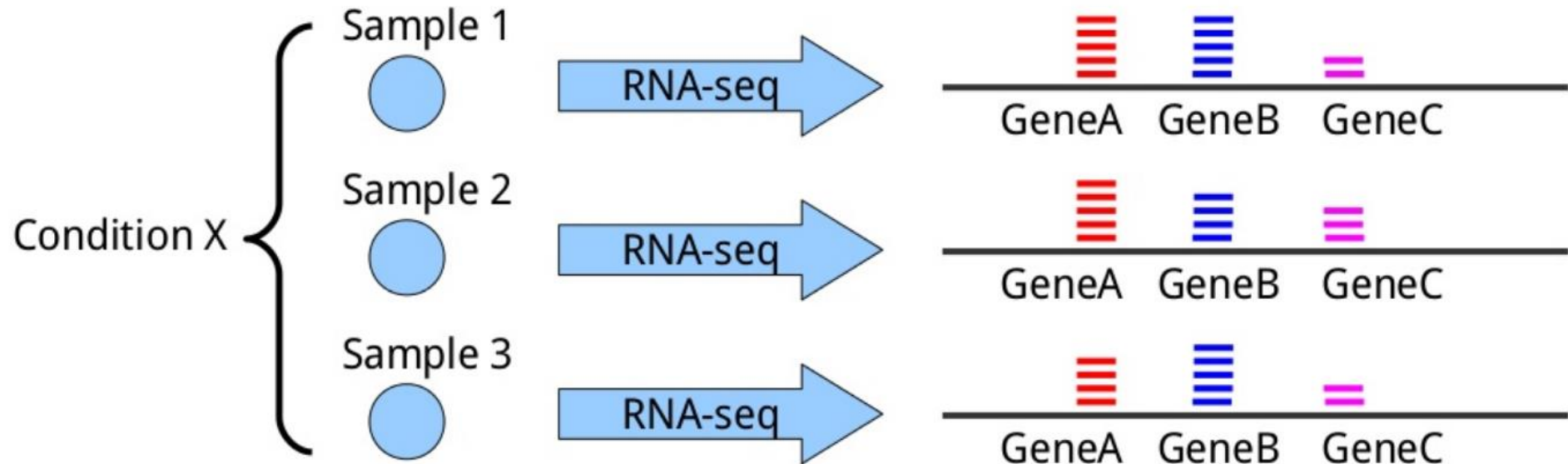
Sequence processing

Alignment

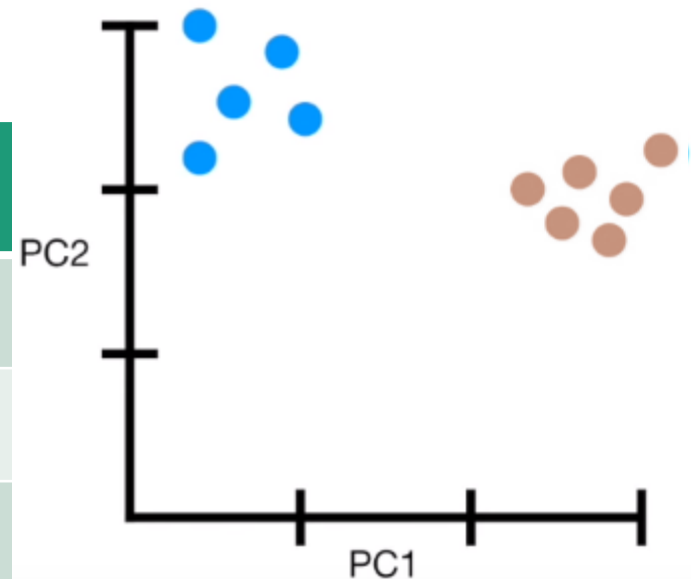
GATAGGTGTGACTACGCCCCATGAAGCGGCACTGACTATGAGACGCATGCTAACCCCGCGCGATATATATACGCGACGATGACTATATAGCTCGACTGCCATGACAAAAGTGAAGCCGCATATCTGCTGGGTA

Genome sequence

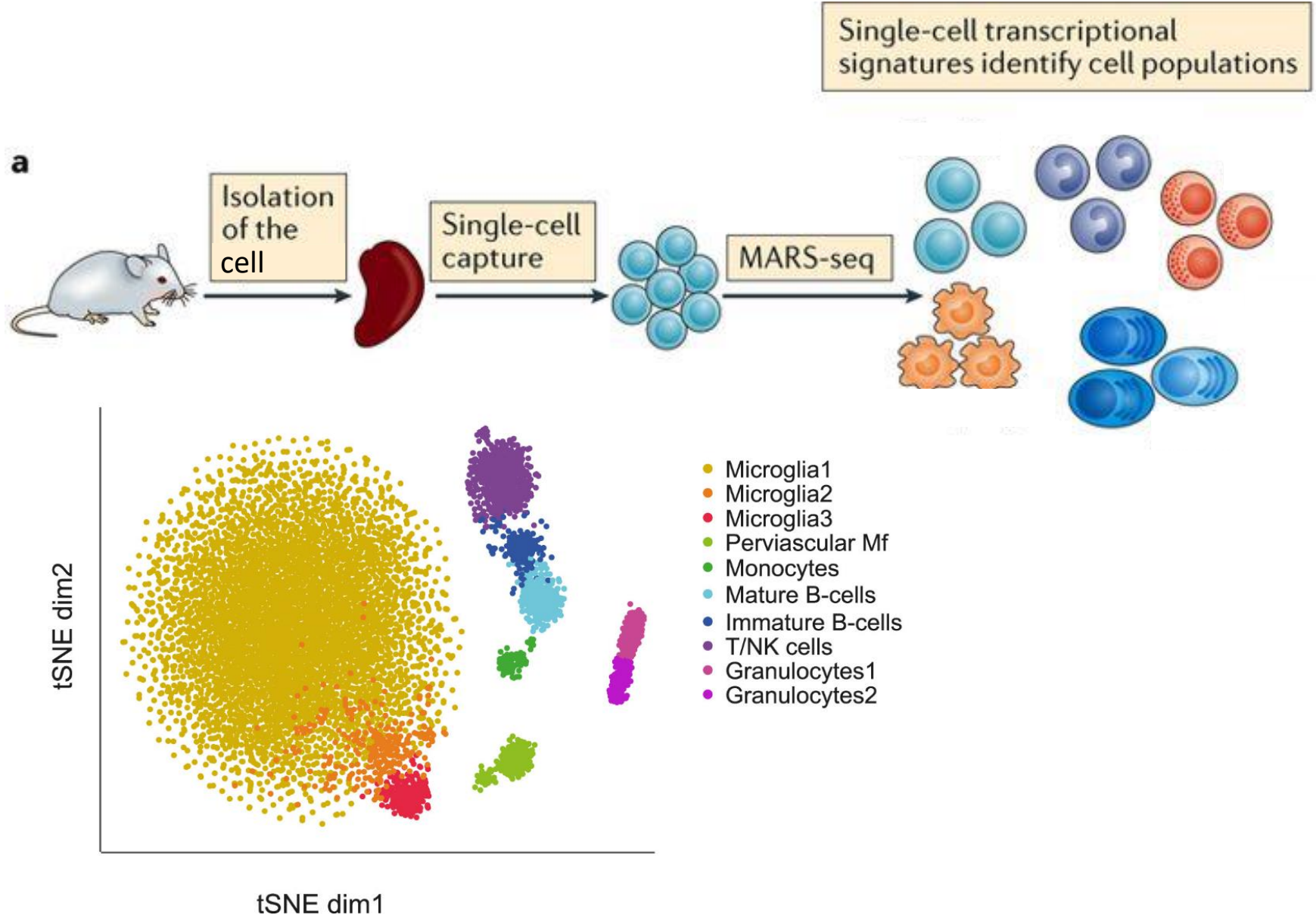
How is the data analyzed?



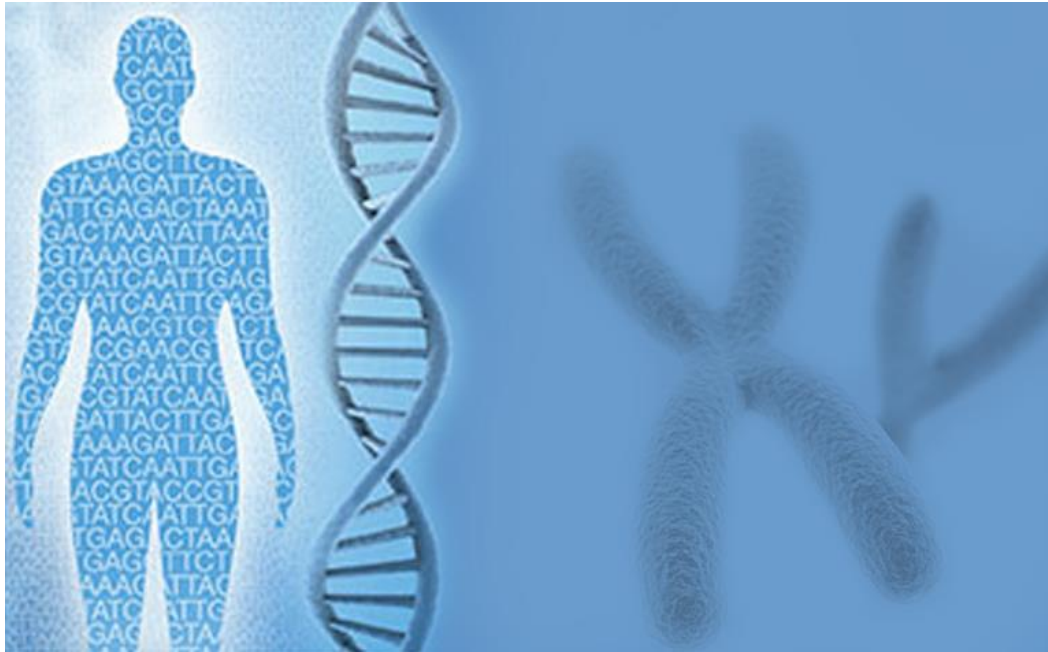
	Normal Cell #1	Normal Cell #2	Mutated Cell #3	...
<i>Gene1</i>	30	23	5	...
<i>Gene2</i>	24	18	2	...
<i>Gene3</i>	5	9	22	...



What is single-cell transcriptomics?



What are the advantages of RNA-seq?

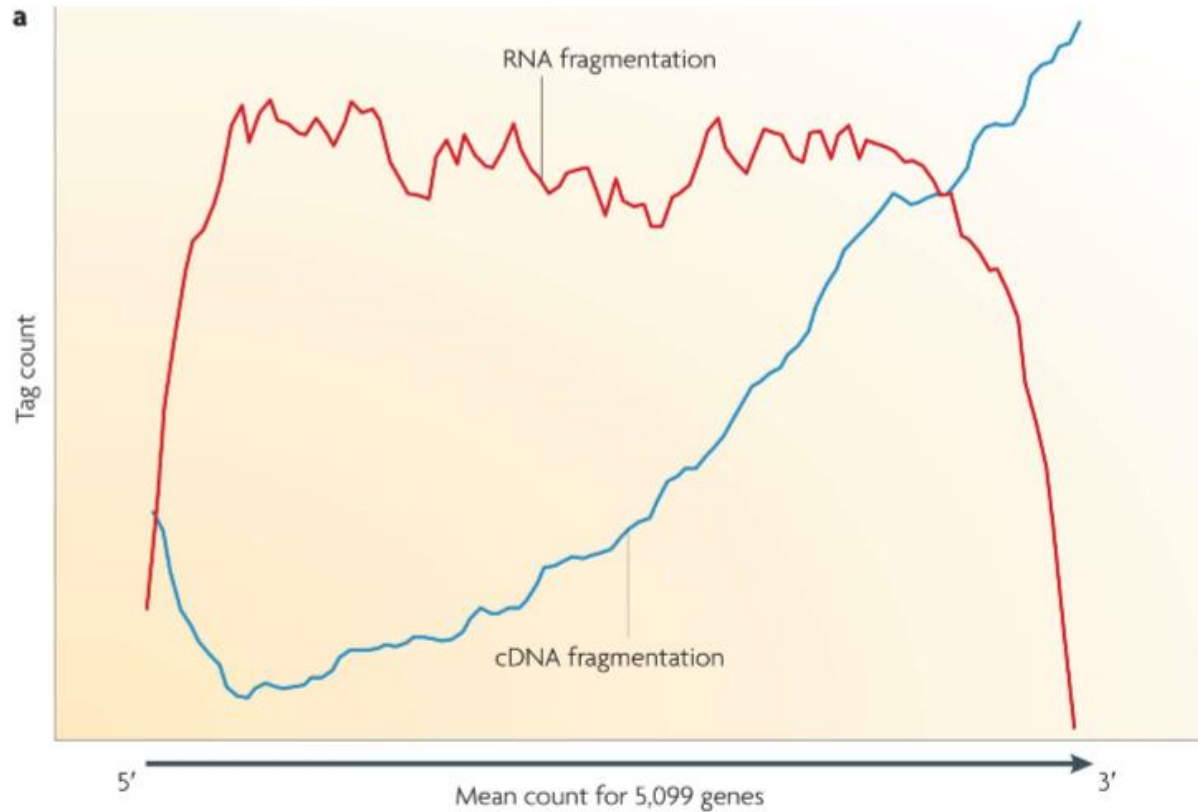


**High sensitivity for
different expression levels**

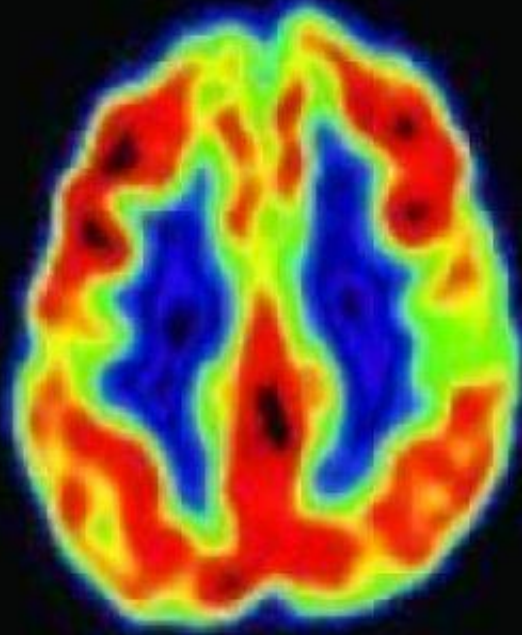
Low RNA input (~1 ng)

**No reference transcripts
required**

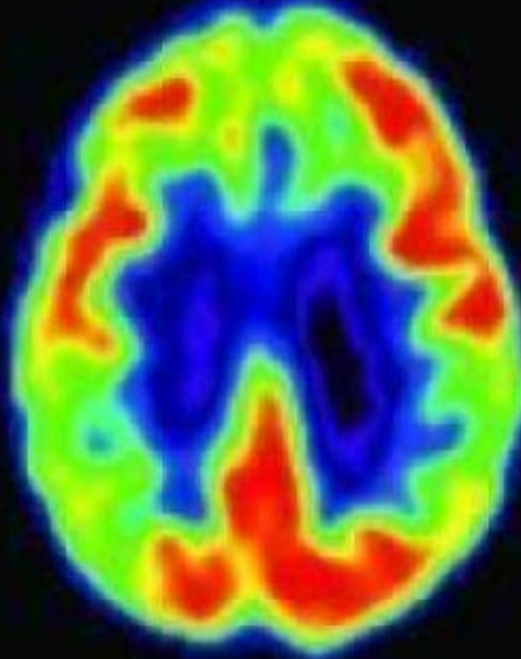
Challenges?



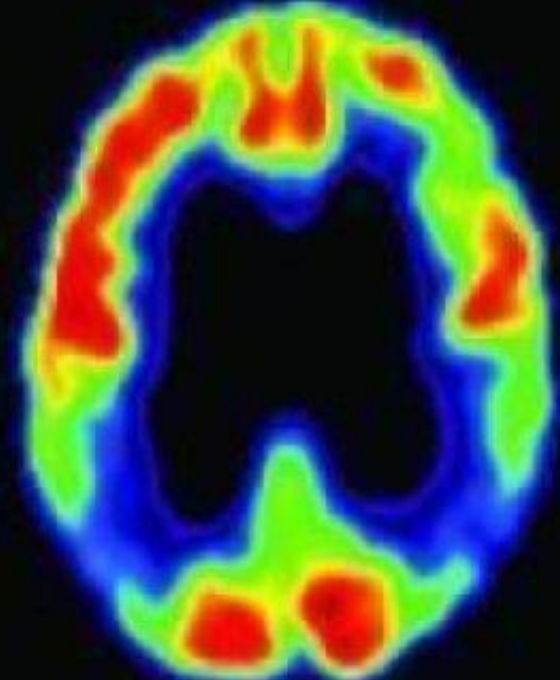
What is Alzheimer's Disease (AD)?



Normal



Mild cognitive
impairment



Alzheimer's
disease

Progressive disease developing memory loss and other cognitive abilities

ALZHEIMER'S

TOP 10 EARLY SIGNS



MEMORY LOSS



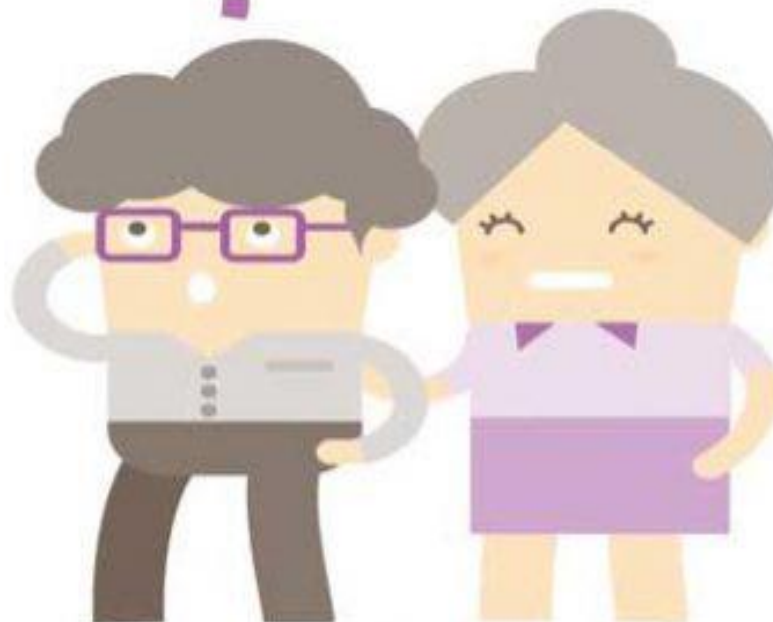
**CHANGES
IN MOOD**



**MISPLACING
BELONGINGS**



**HARD TO COMPLETE
FAMILIAR TASK**



**CONFUSION OF
TIME AND PLACE**



**SOCIAL
WITHDRAWAL**



**POOR
JUDGEMENT**

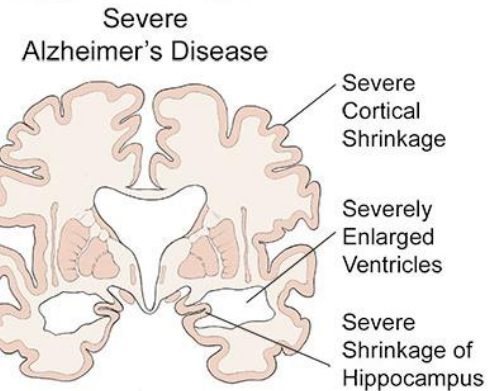
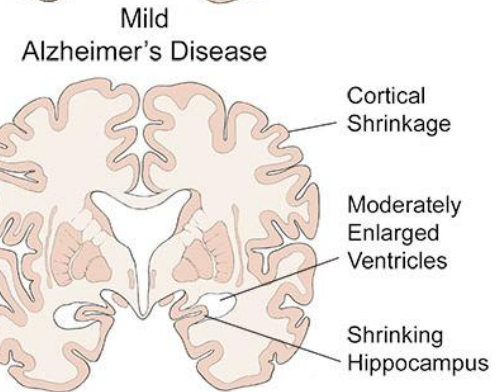
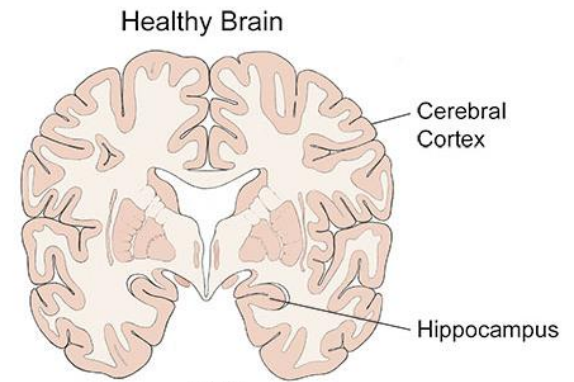
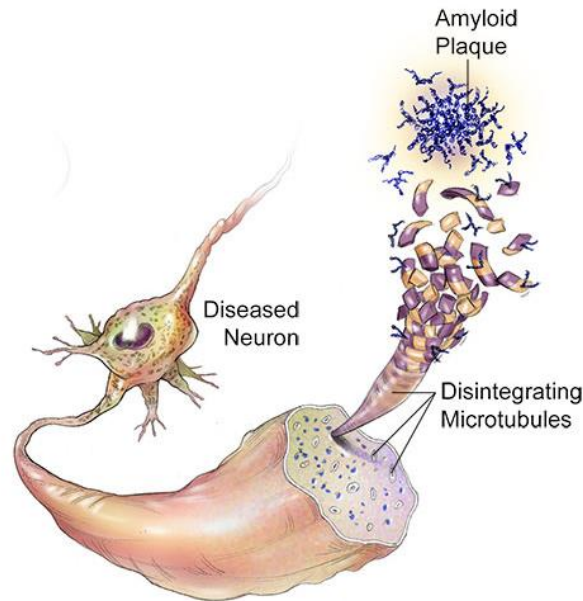
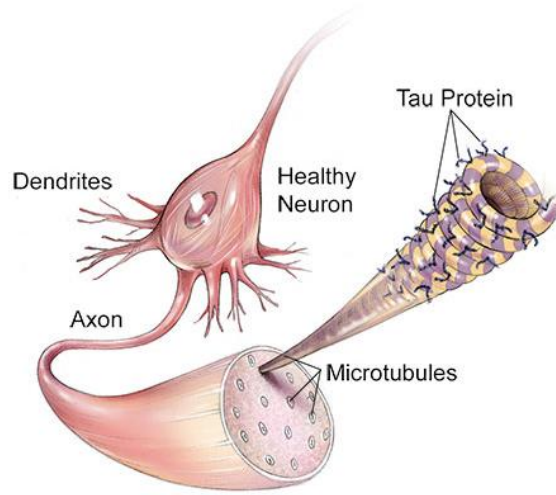


**STRUGGLING TO
COMMUNICATE**

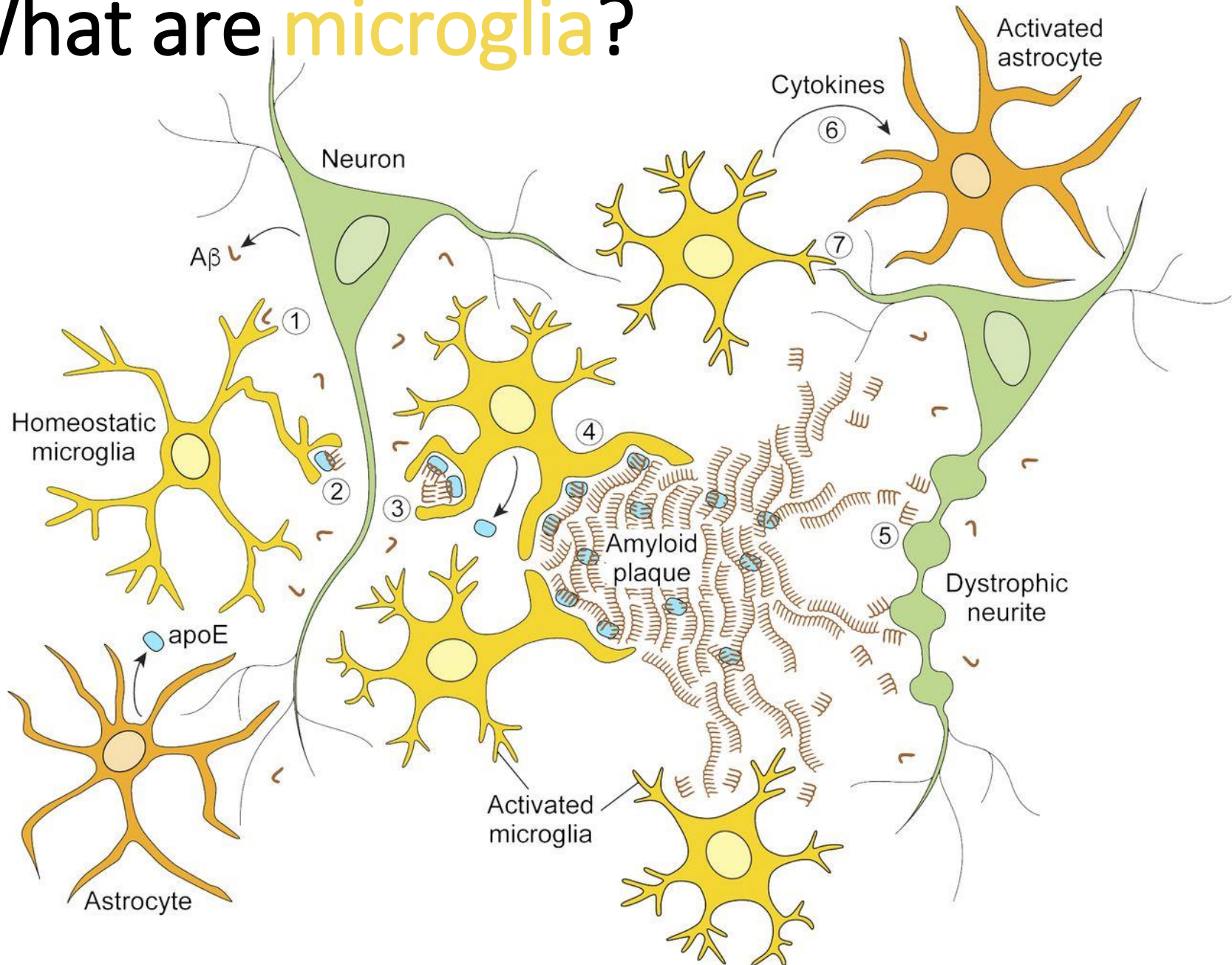


**CHANGES IN
VISION**

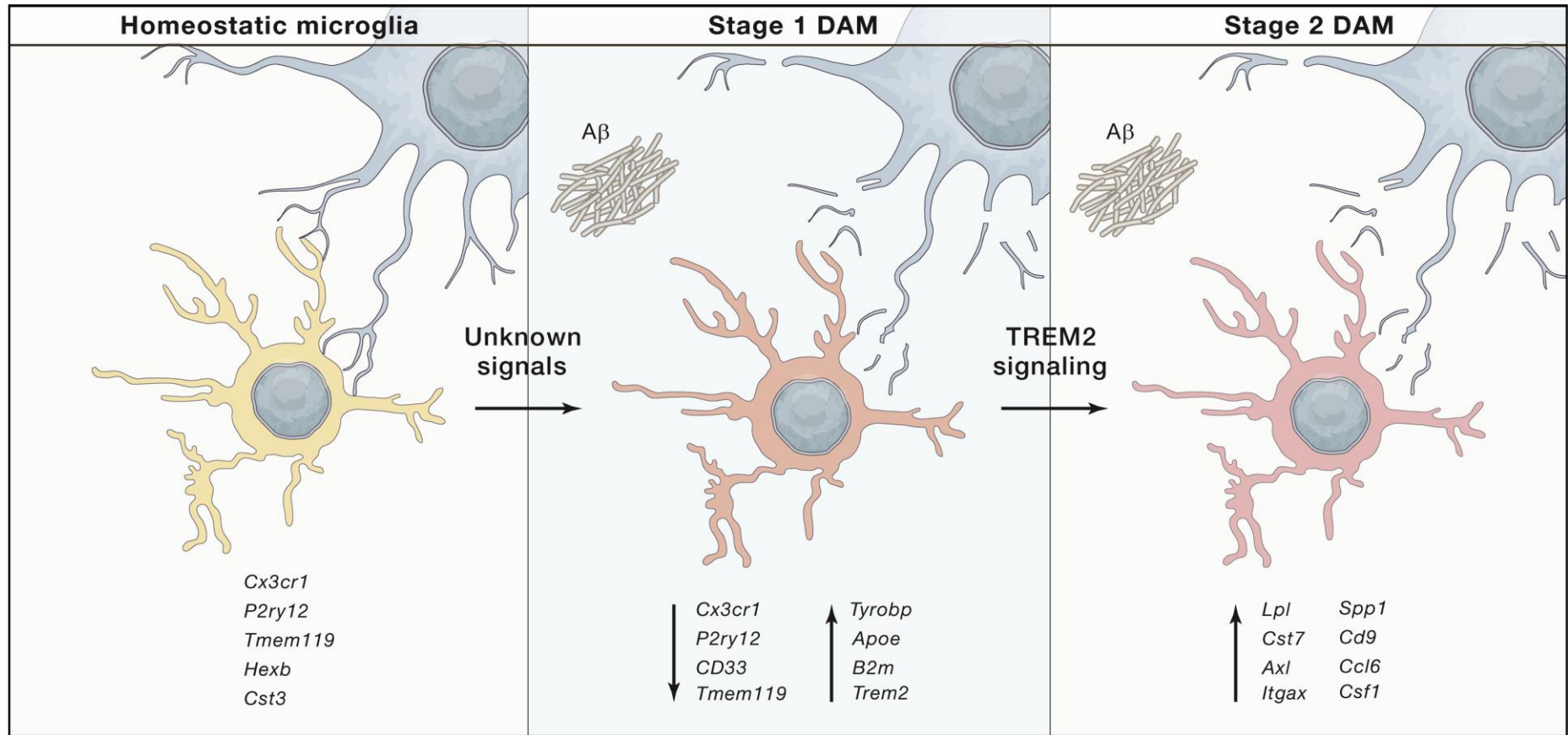
How does Alzheimer's progress?



What are microglia?

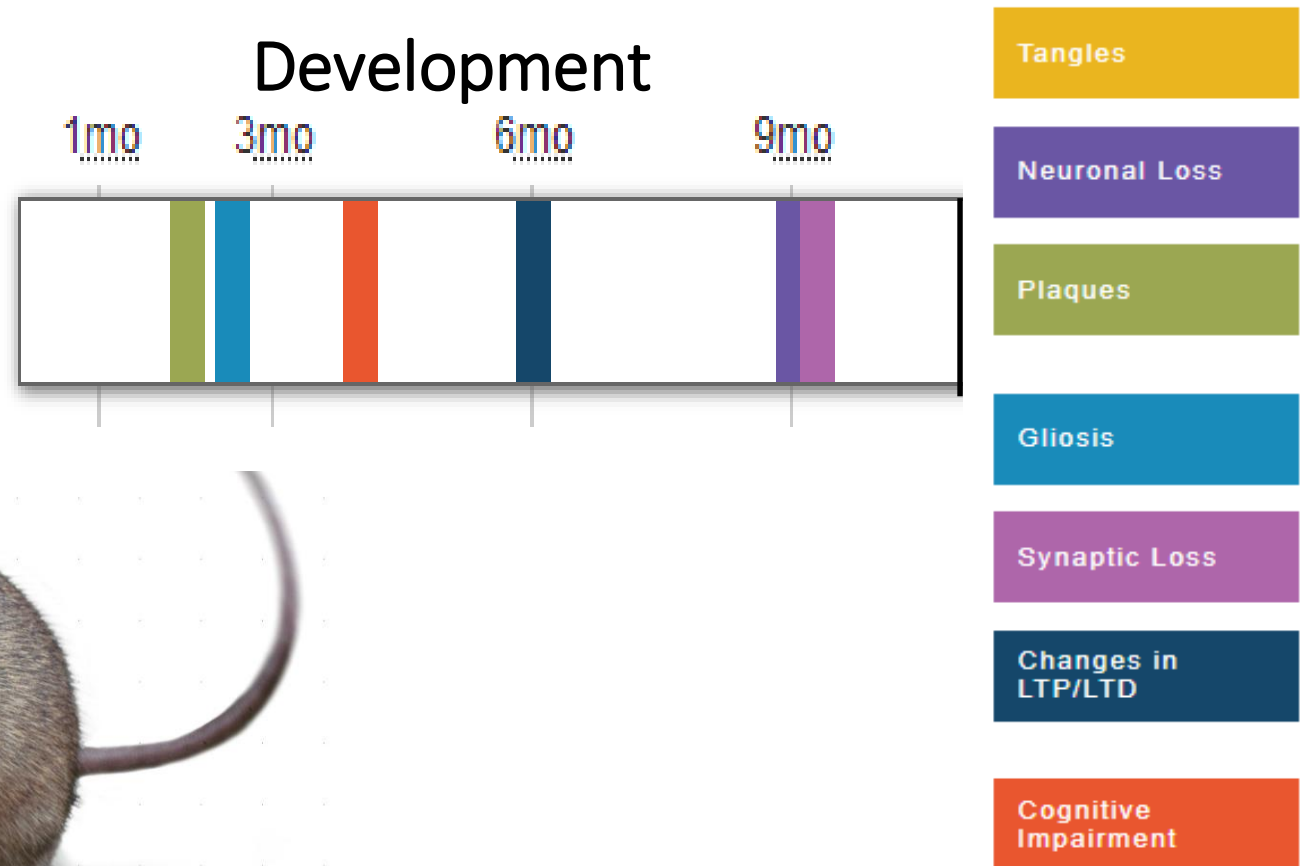


What are Disease-Associated Microglia (**DAM**)?

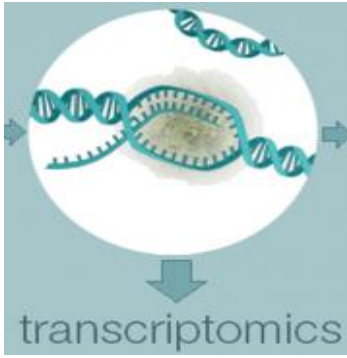


Why use mice as a model organism?

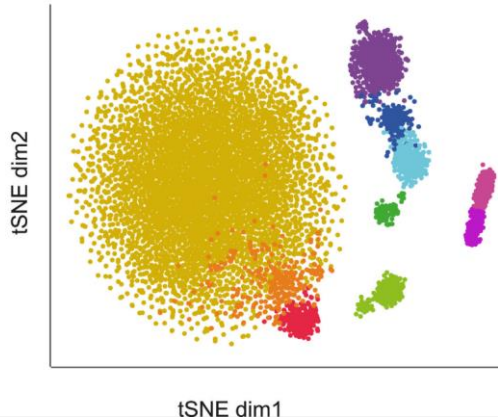
5xFAD mouse model with 5 AD mutations



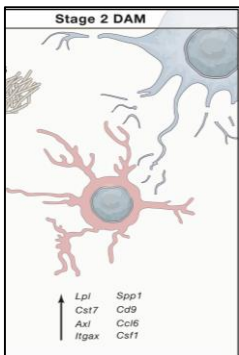
Summary



Transcriptomics is the study of the transcripts of genes.



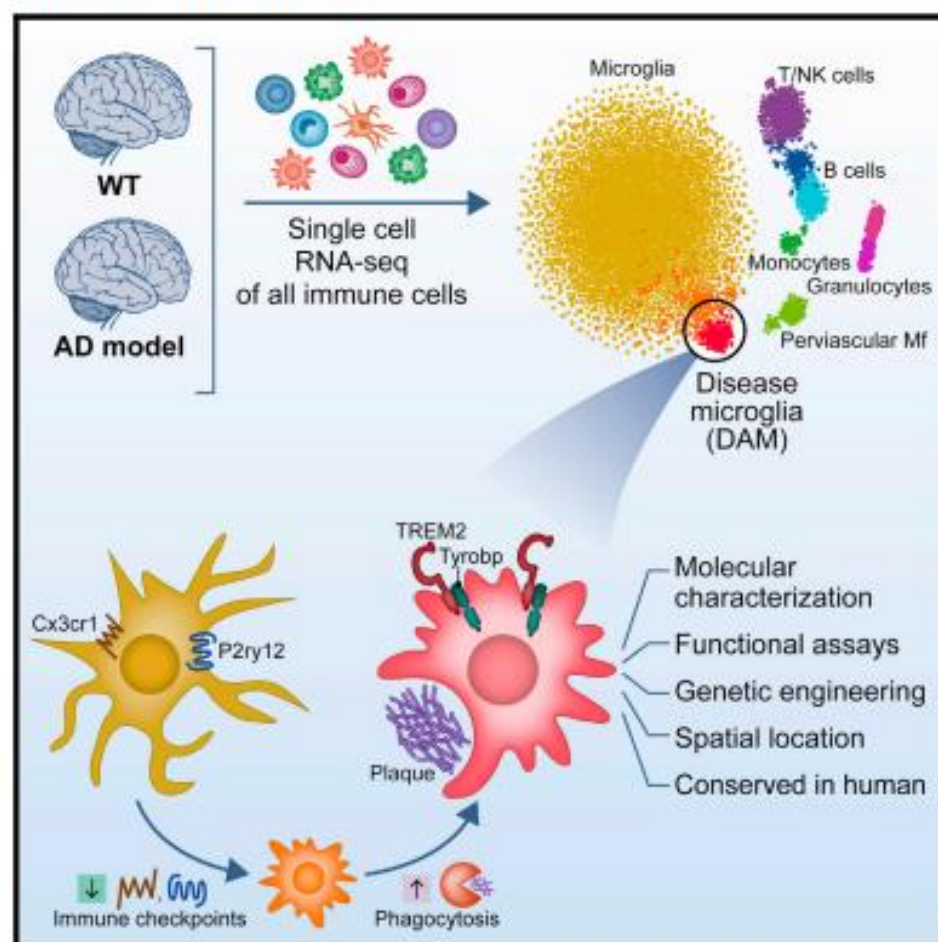
Single-cell RNA sequence can be used to identify expression in distinct cell populations.



Alzheimer's disease is associated to the presence of a type of microglia: **DAM**.

A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease

Graphical Abstract



Authors

Hadas Keren-Shaul, Amit Spinrad,
Assaf Weiner, ..., Marco Colonna,
Michal Schwartz, Ido Amit

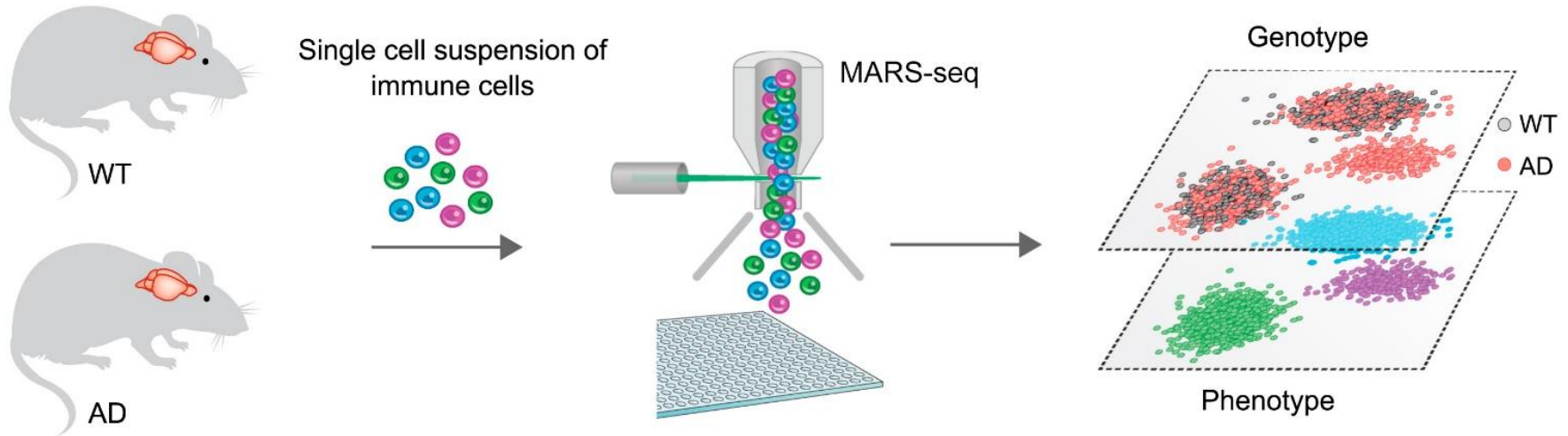
Correspondence

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ido.amit@weizmann.ac.il (I.A.)

In Brief

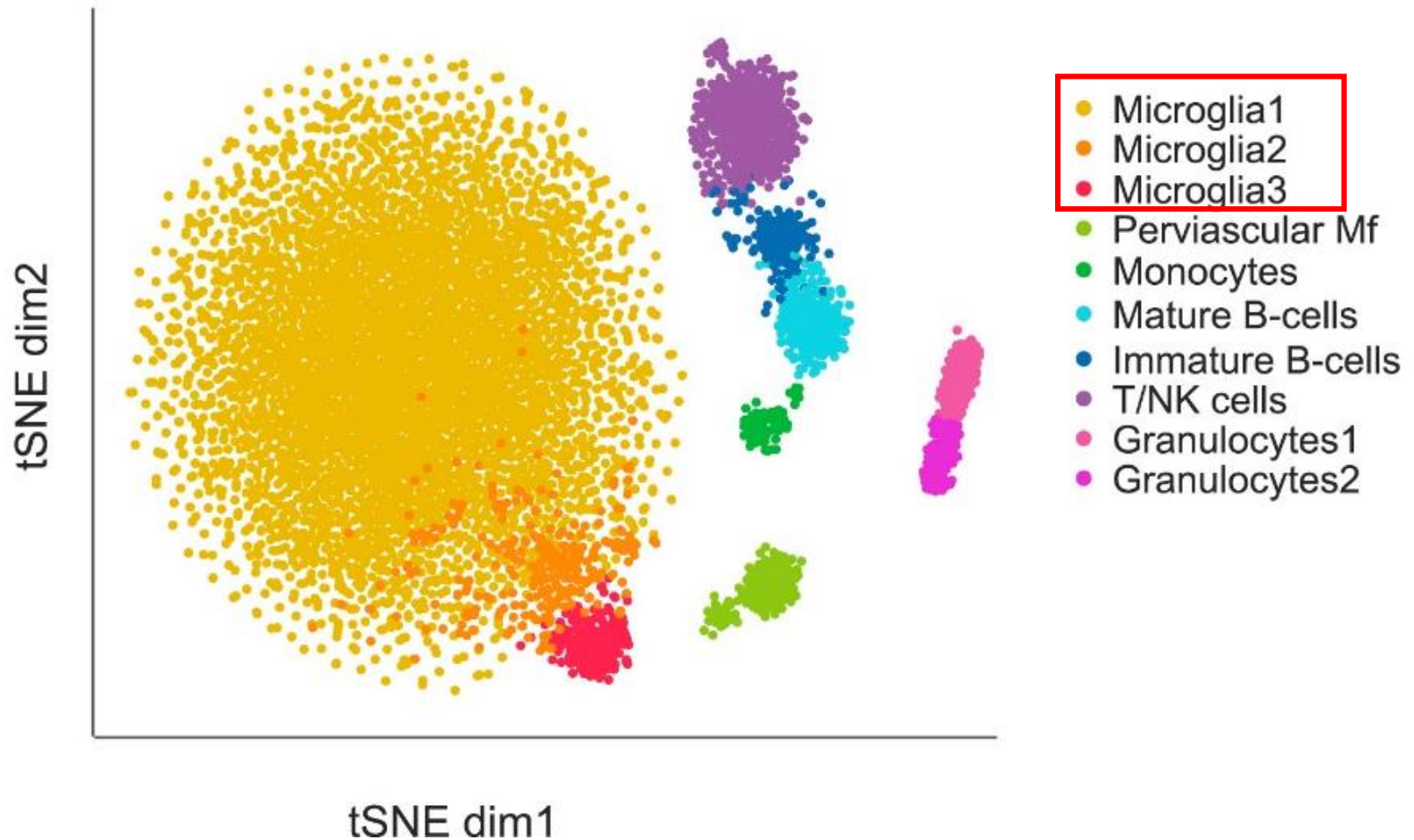
A new type of microglia associated with restricting neurodegeneration may have important implications for treatment of Alzheimer's and related diseases.

What was the experimental setup?



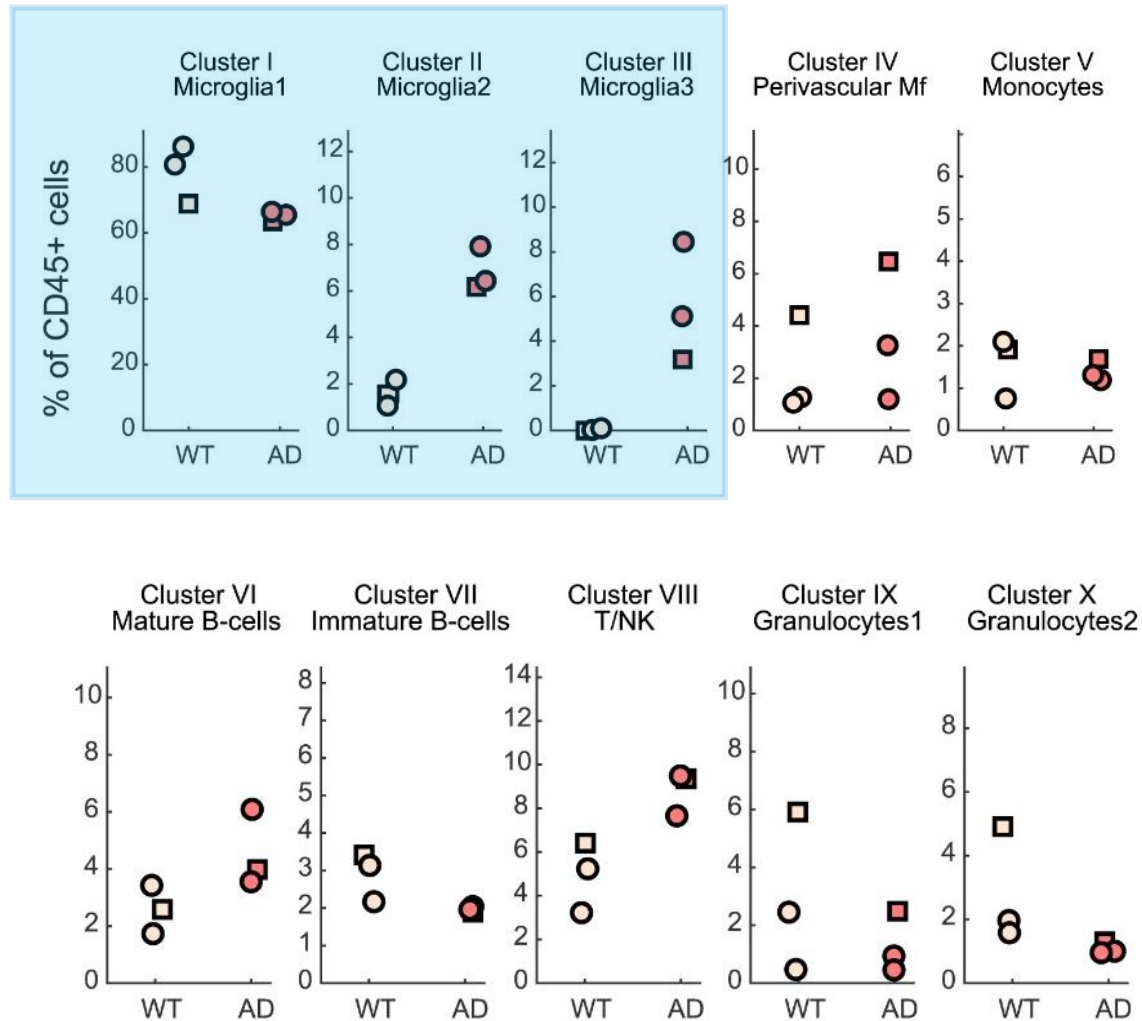
Performed MARS-seq on mice cells

What cell type clusters did they discover?

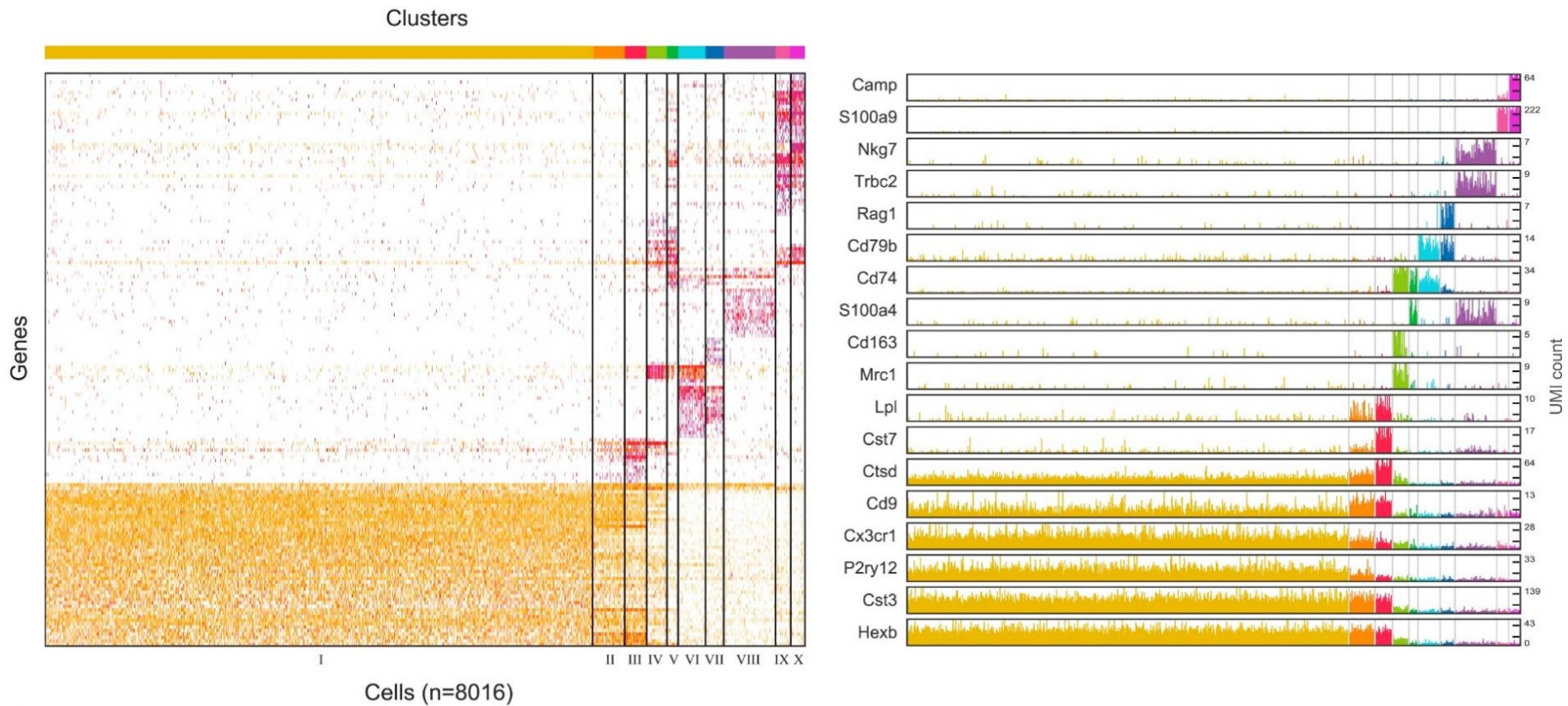


Identified disease associated microglia (**DAM**)

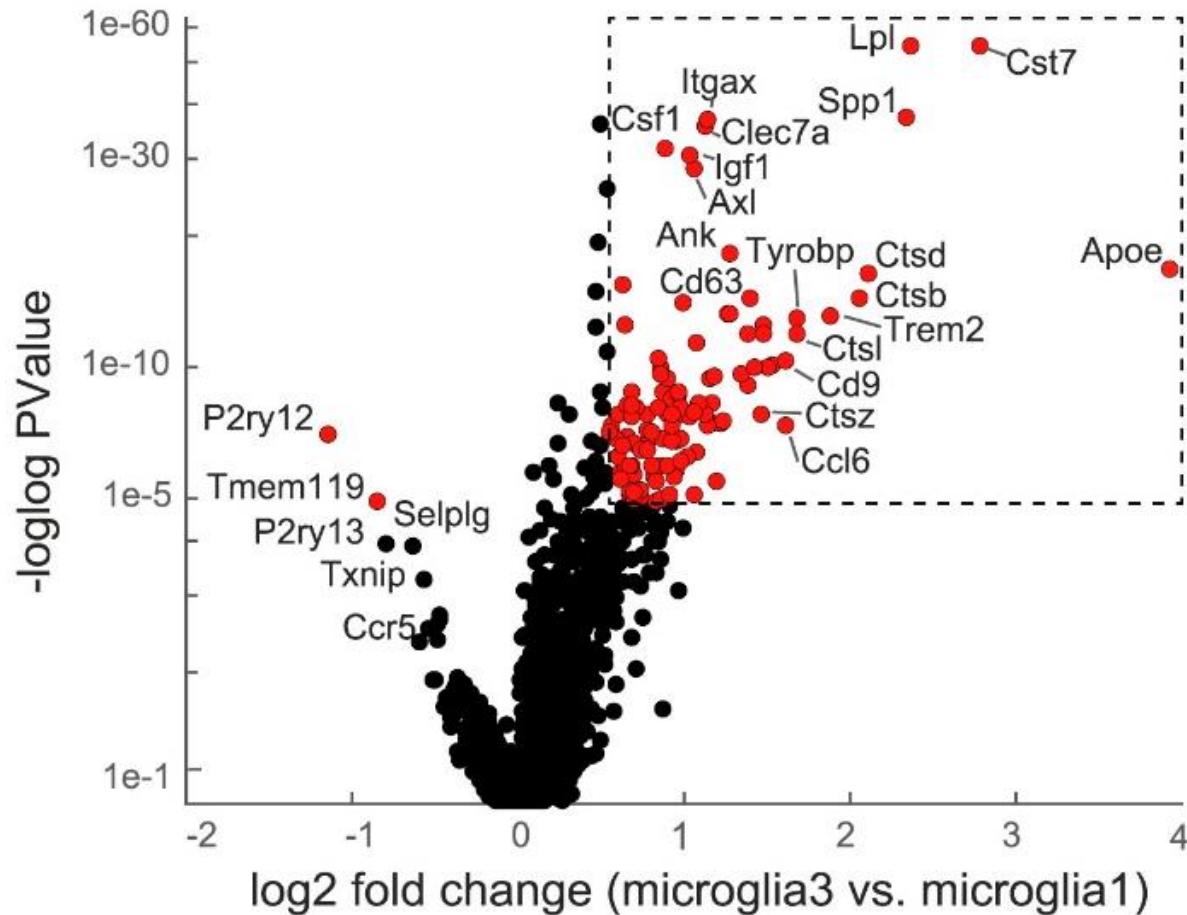
What are the frequency differences?



What are the gene expression profiles?

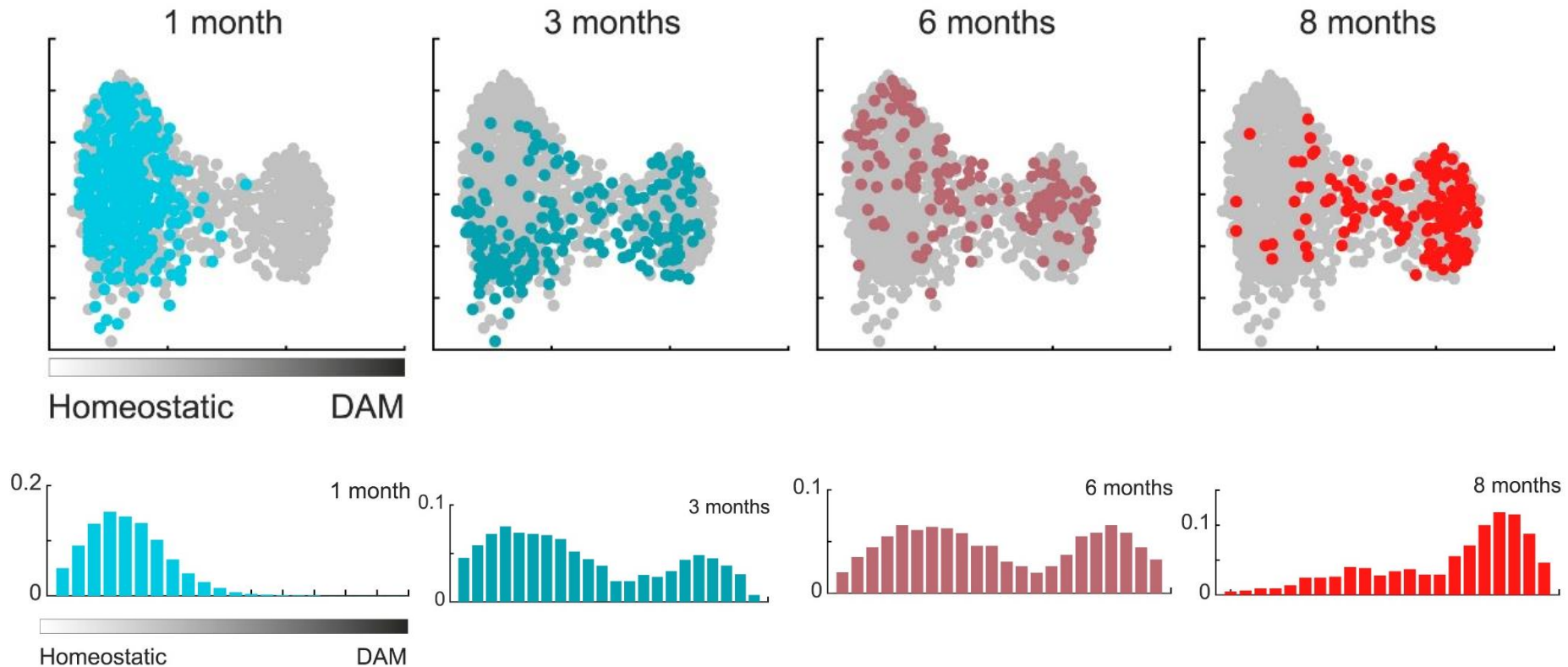


What marker genes exist for DAM?



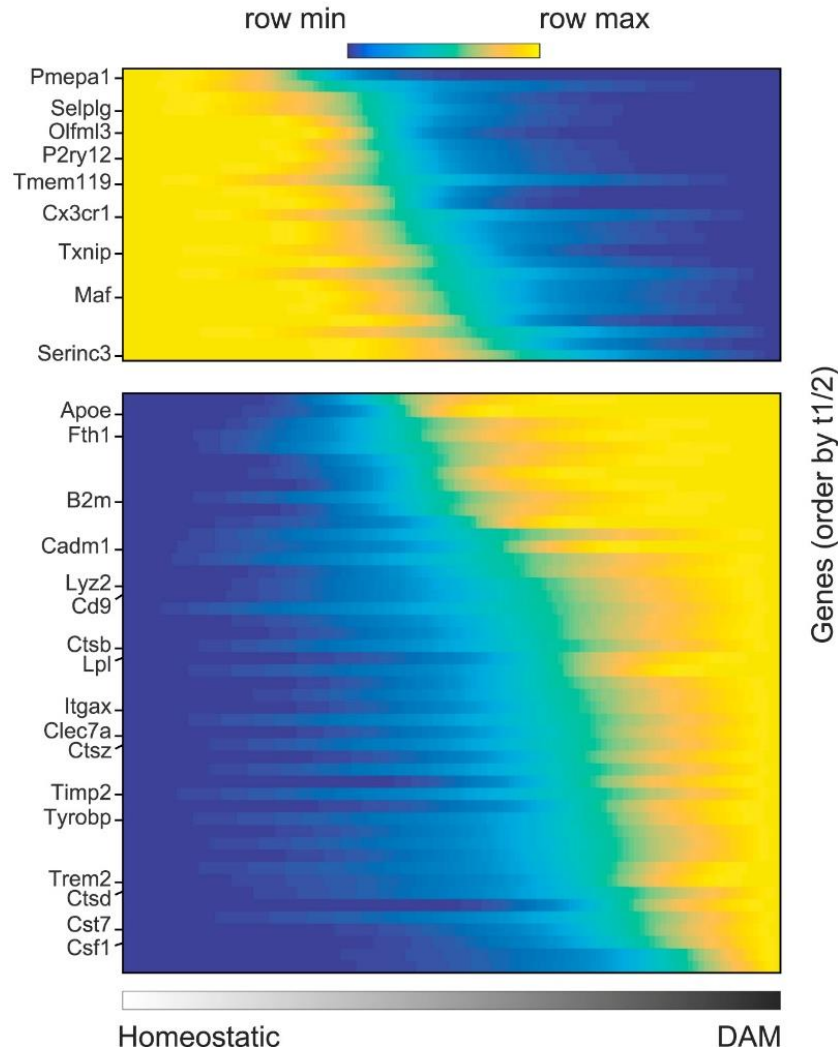
Identified genes differentially expressed in DAM

How common are DAM in AD progression?



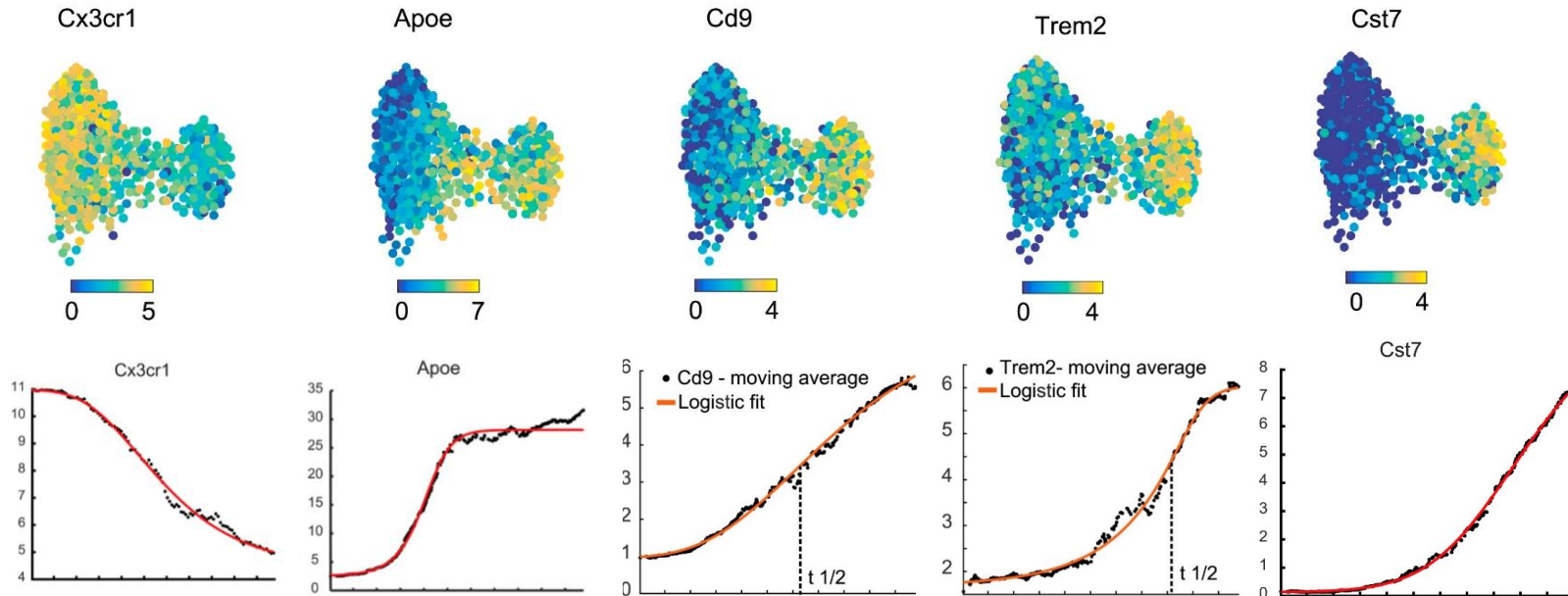
Microglia progress to DAM as AD progresses

How does gene expression change in DAM?



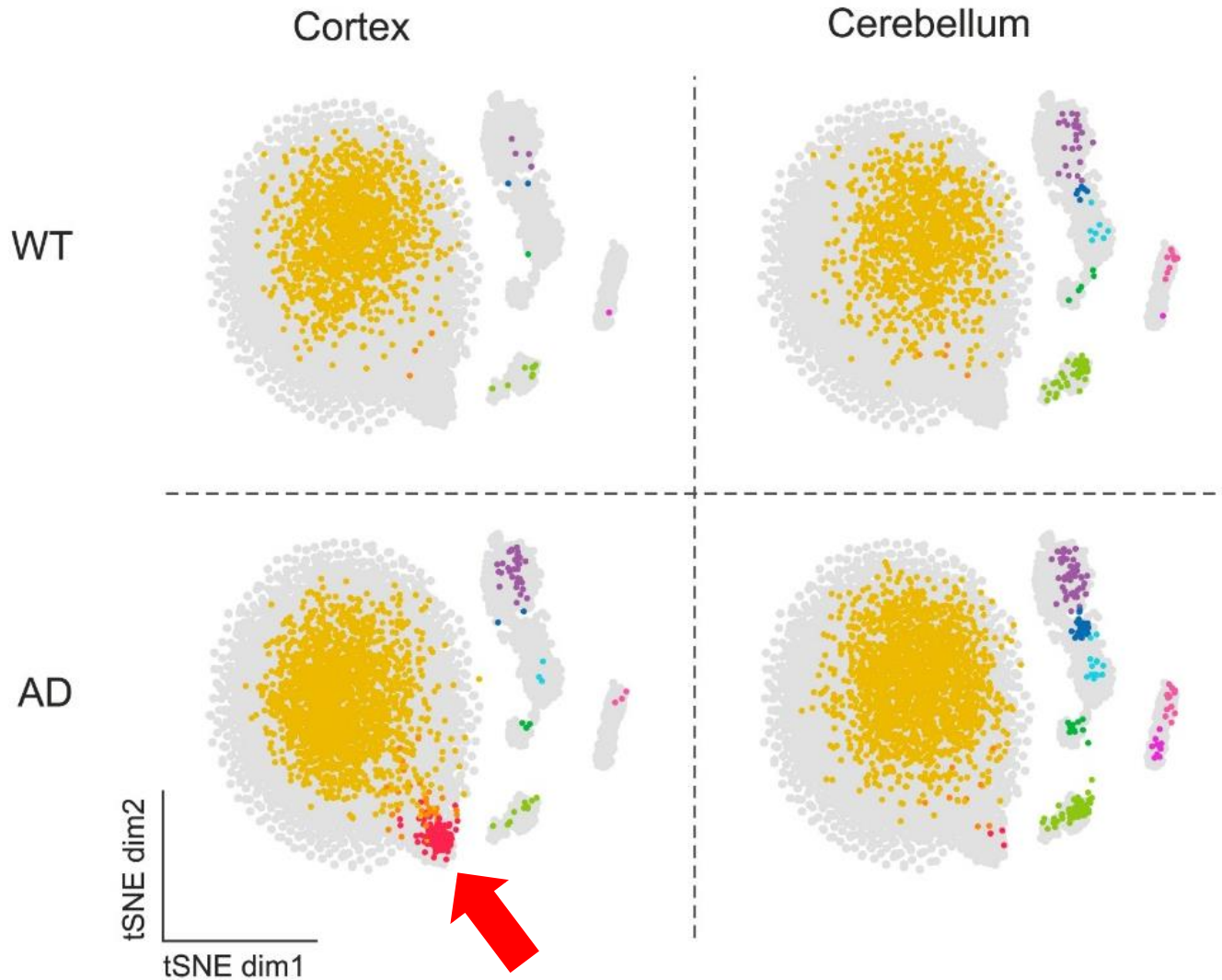
Some genes upregulated some downregulated in DAM

Can gene expression changes in **DAM** be quantified?



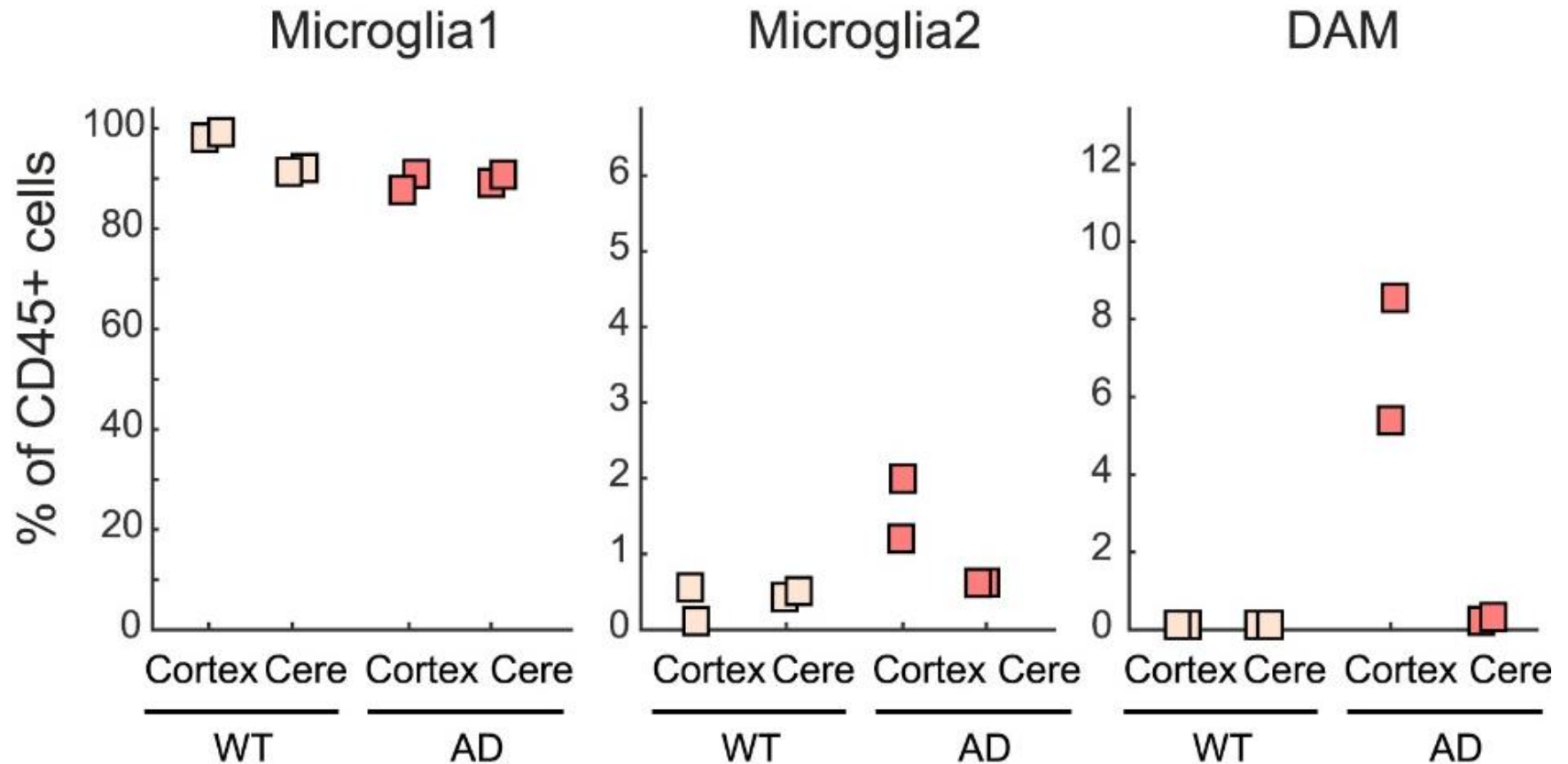
Gene expression can be quantified during progression

Where are **DAM** localized?



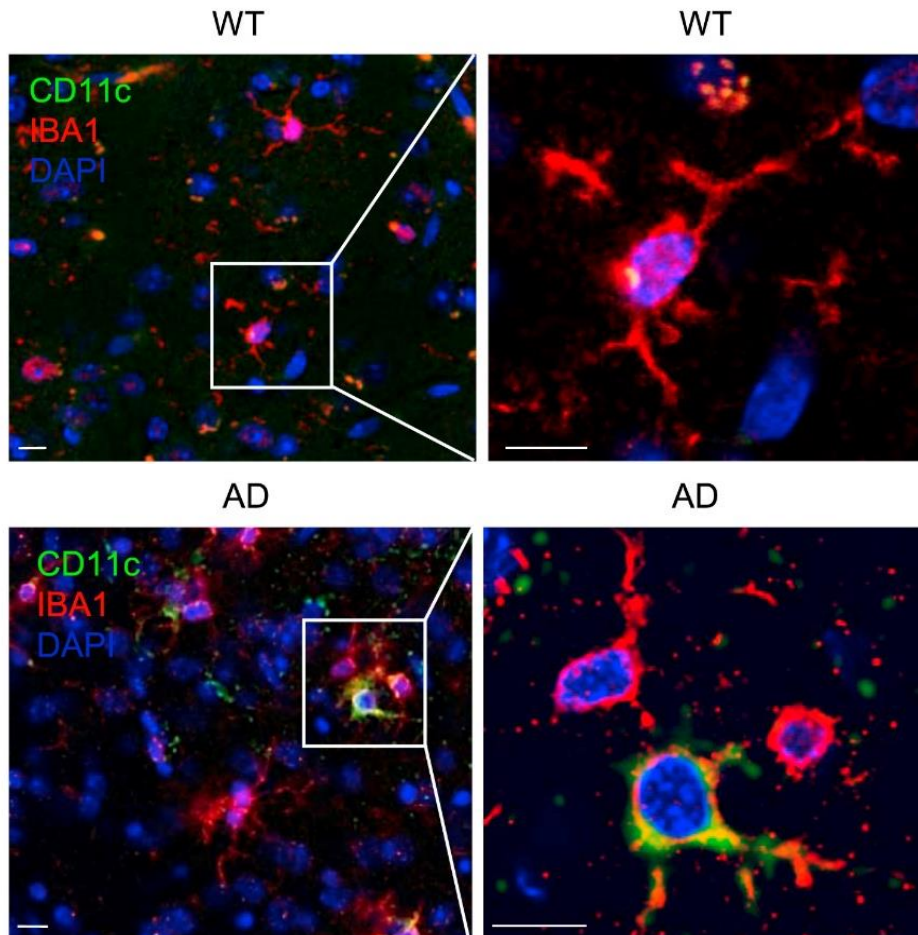
DAM spatially located in cortex only of AD mice

How common are **DAM** in AD brains?



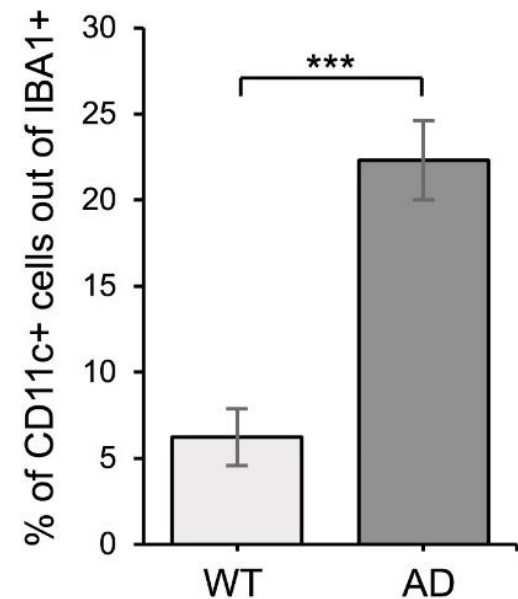
Significantly more **DAM** in AD than WT mouse brains

Are **DAM** and **microglia** markers co-expressed in AD?



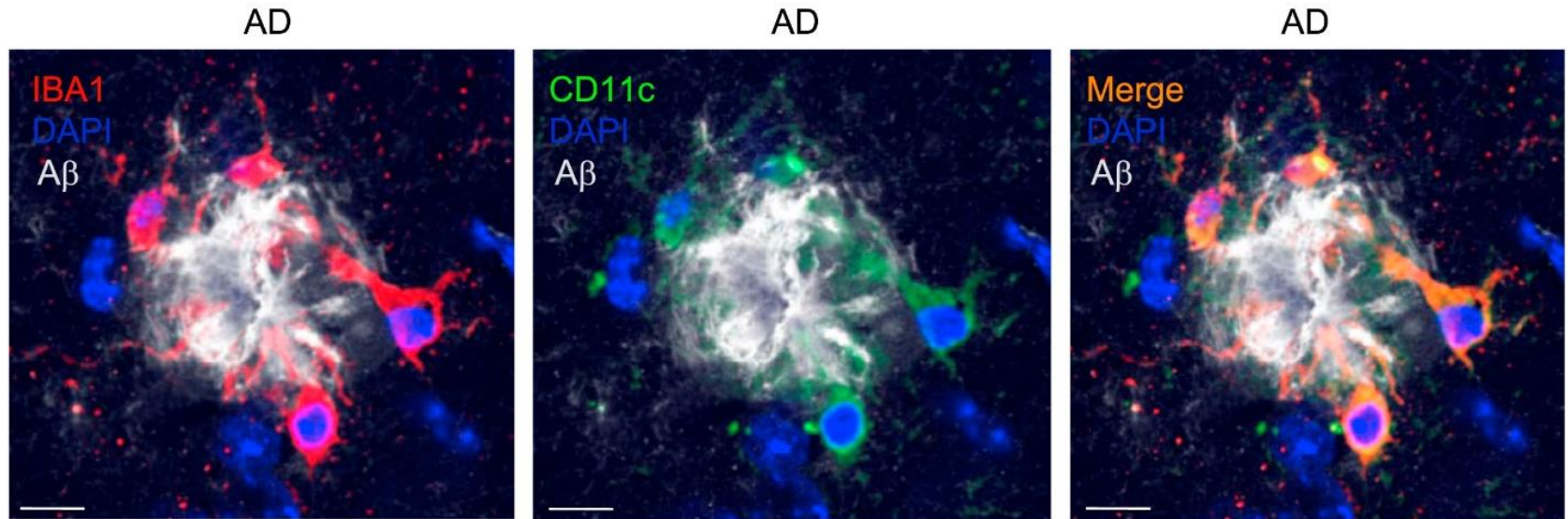
CD11c = DAM

IBA1 = homeostatic microglia



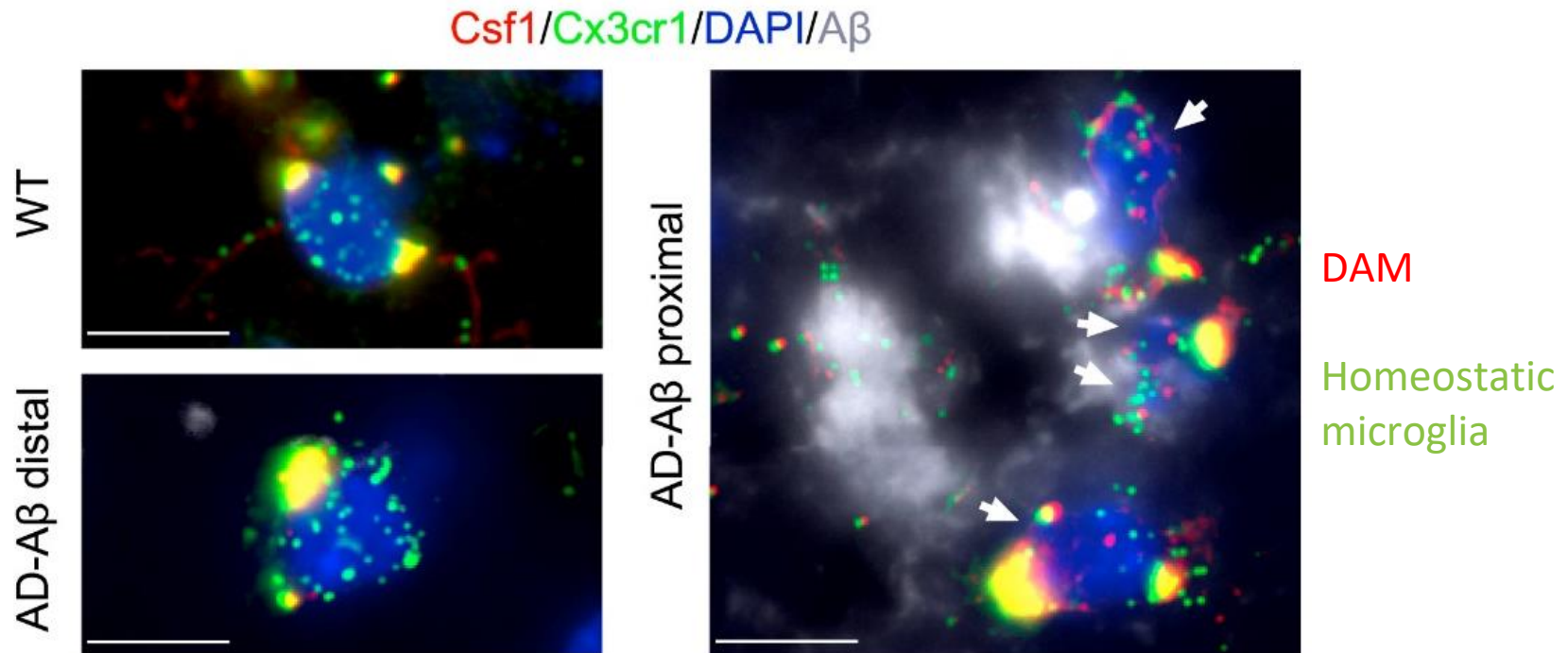
AD single cells have co-expressed **IBA1** and **CD11c**

Where are **DAM** localized in AD?



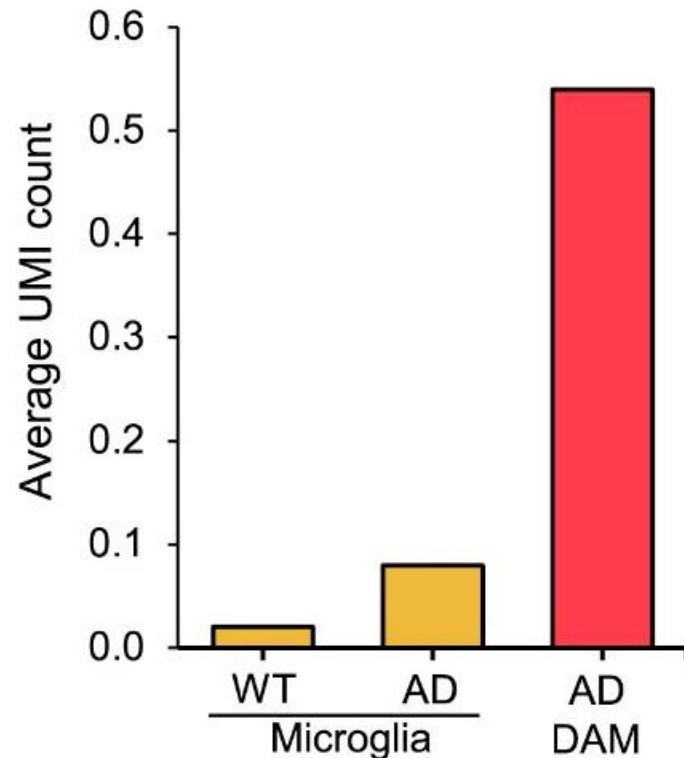
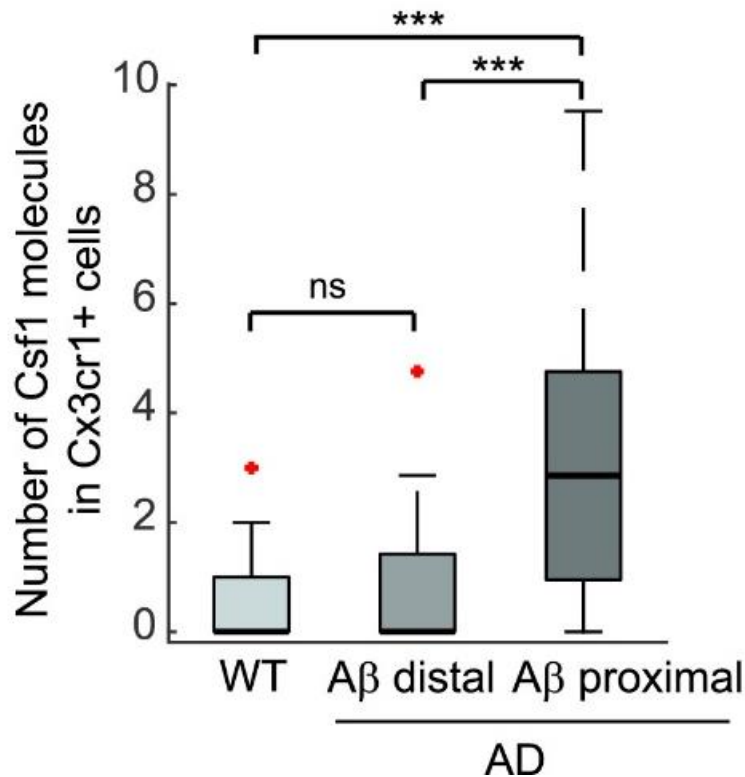
DAM localized in close proximity to plaque foci

Are **DAM** genes localized near plaques?



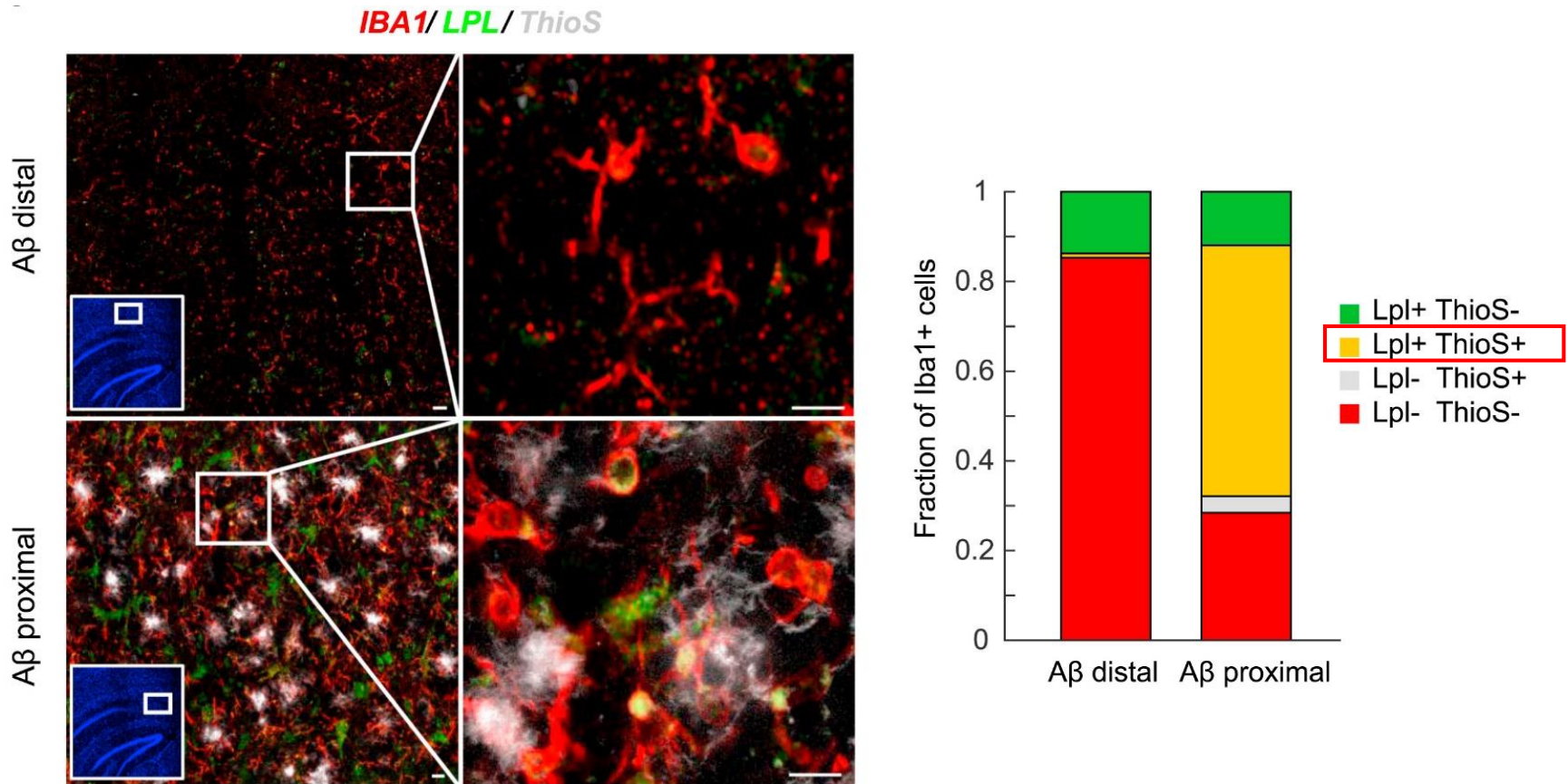
DAM specific genes expressed in microglia near plaques

Can **DAM** localization be quantified?



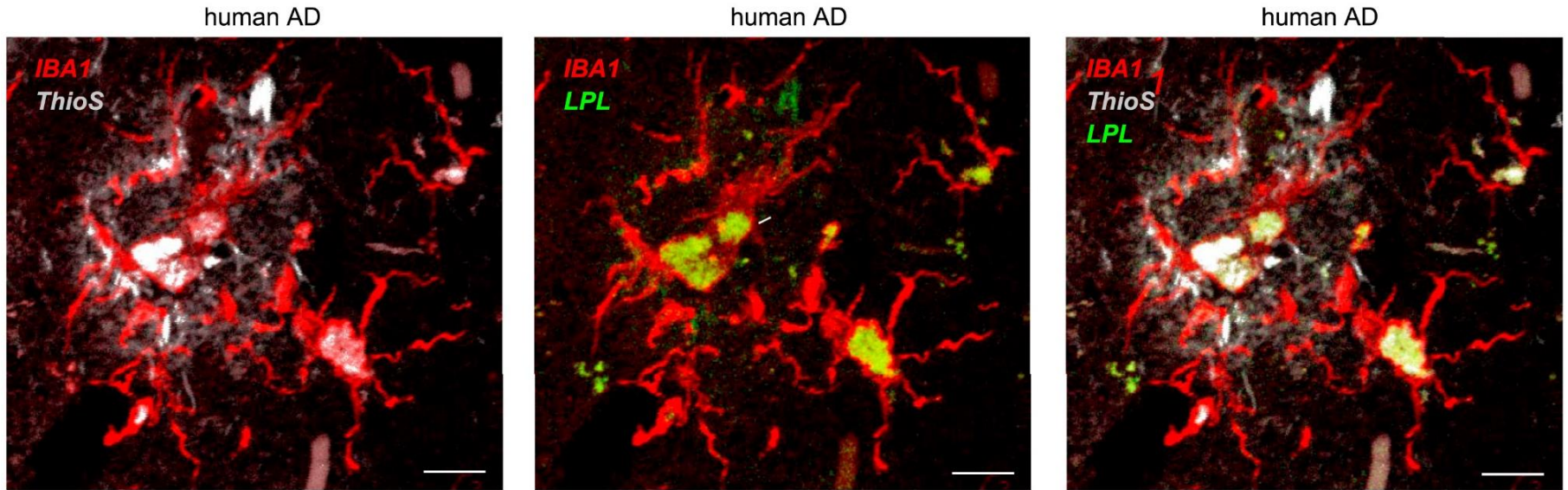
DAM (Csf1) are present in AD near Aβ plaques

Is the same localization pattern true for LPL?



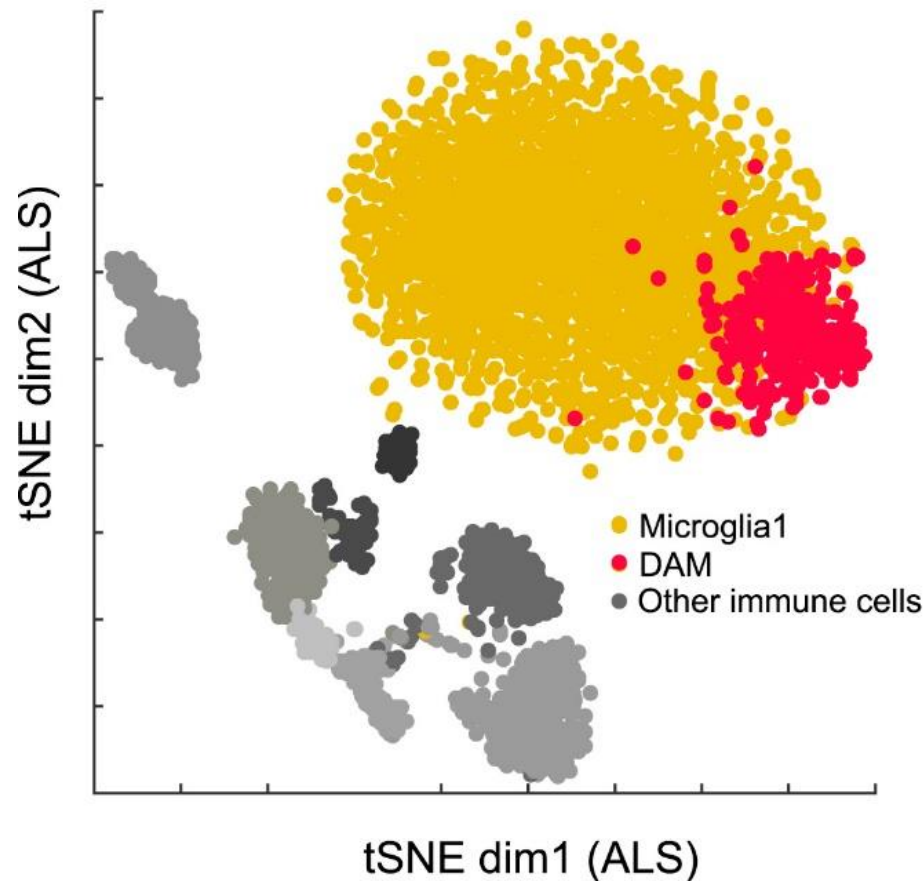
DAM (LPL) are present in AD near Aβ plaques

Are DAM conserved in humans?



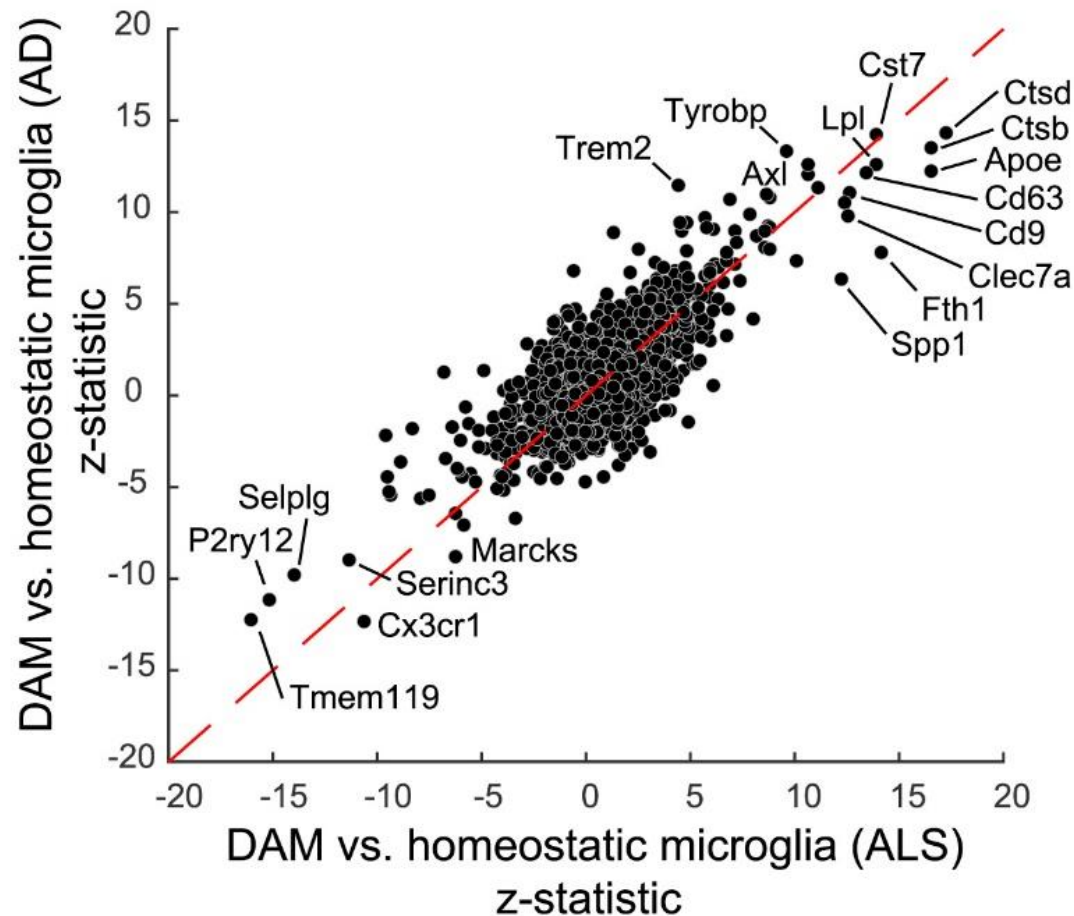
DAM are conserved in humans

Are **DAM** conserved in other neurodegenerative diseases?



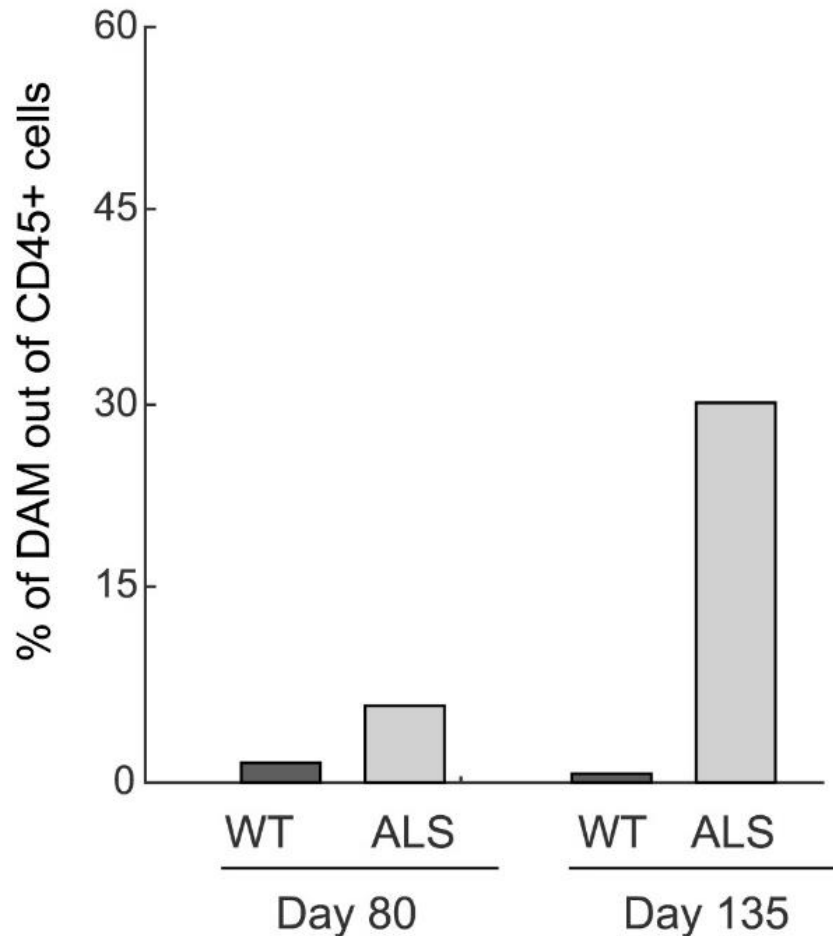
DAM are conserved in ALS mice

What does ALS **DAM** gene expression look like?



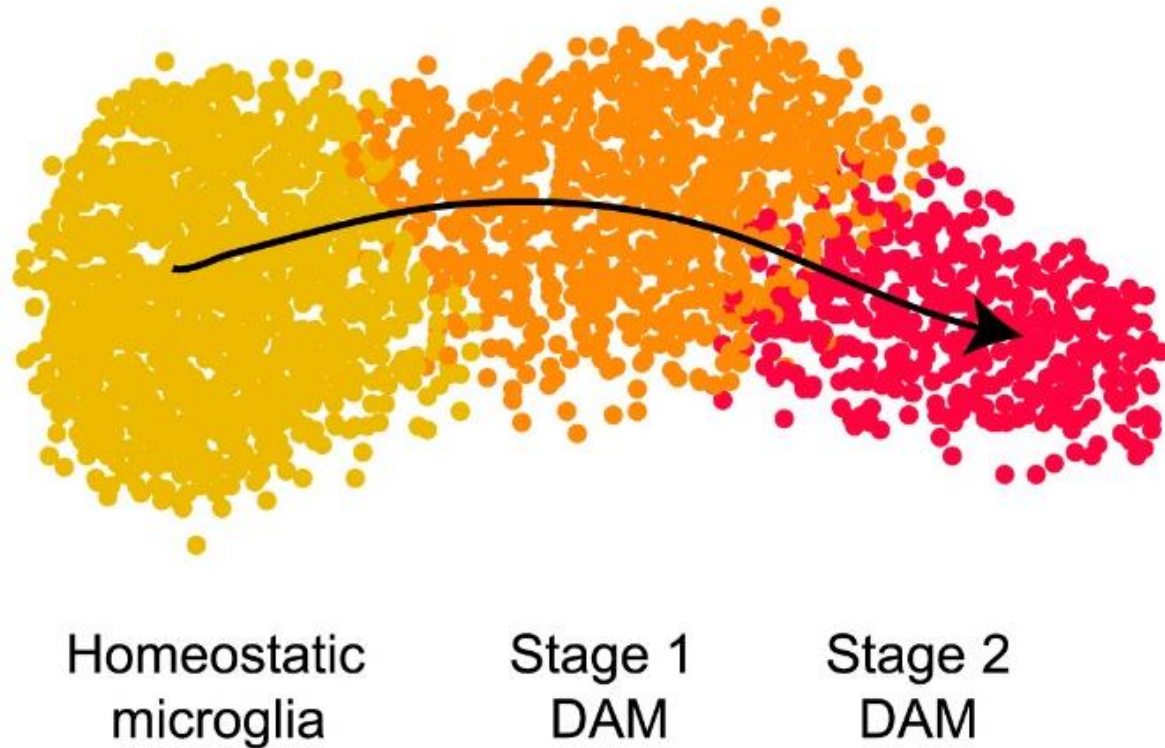
ALS **DAM** have similar gene expression to AD

Do **DAM** increase with ALS progression?



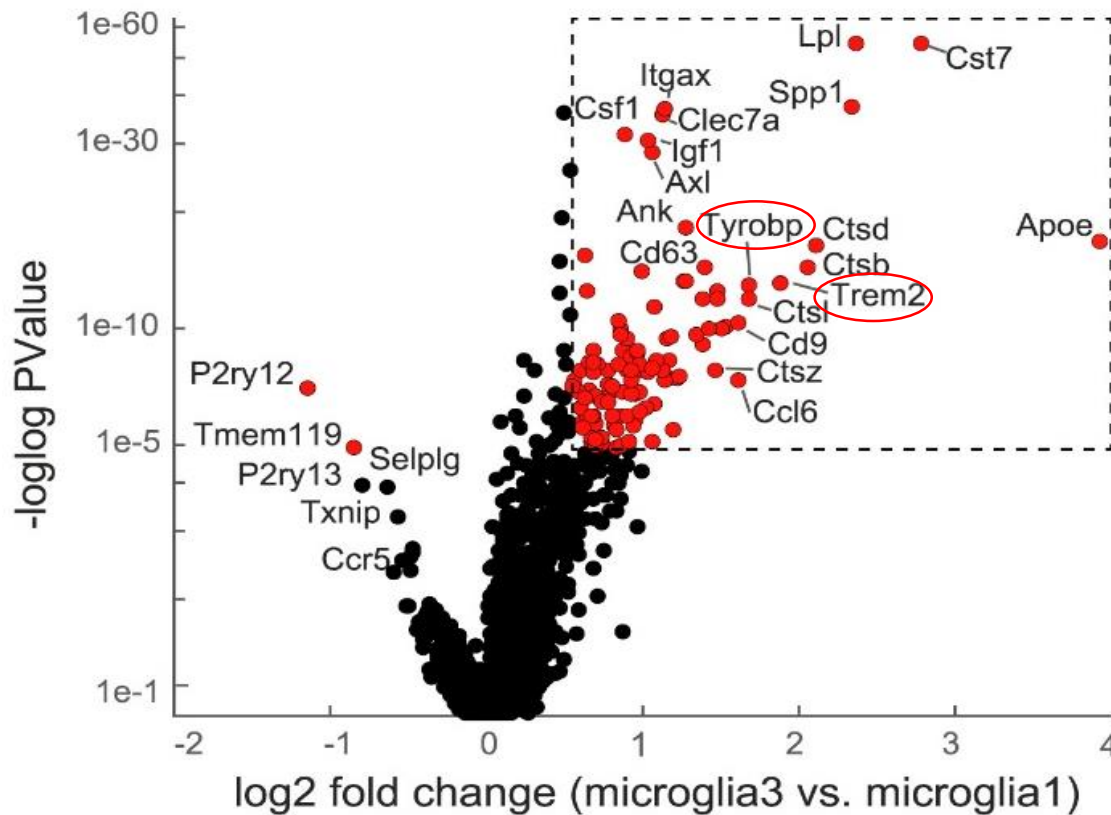
DAM prevalence increases with ALS progression

How do microglia progress to DAM?



Microglia progress through an intermediate to DAM

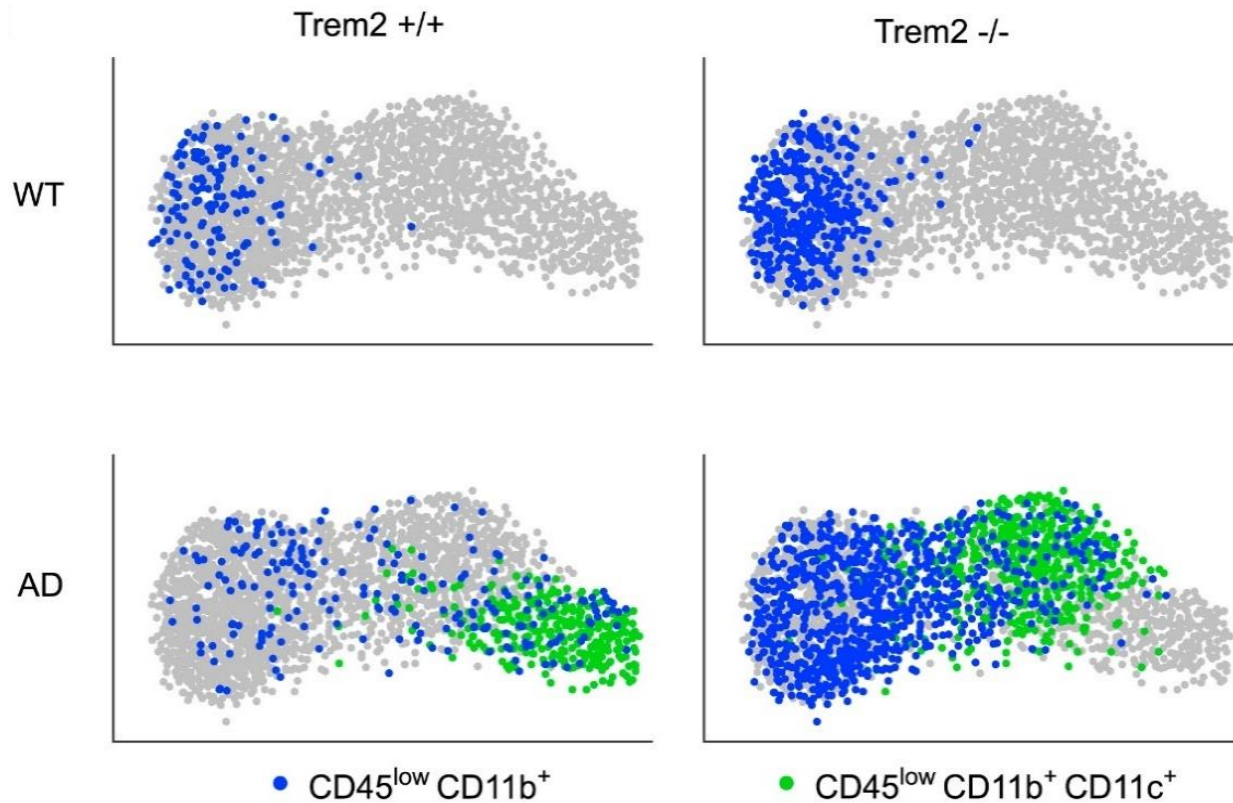
What genes should be used to track activation?



Two form signaling complex

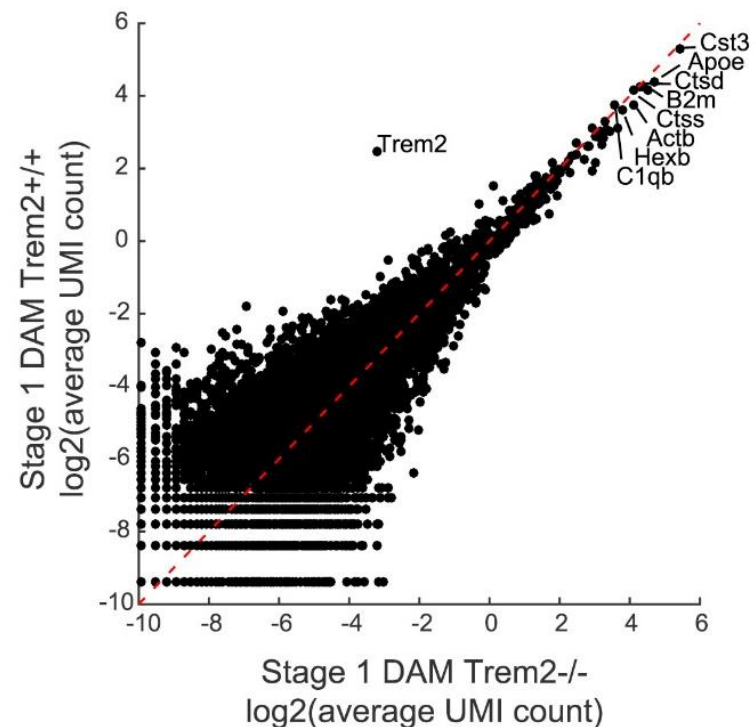
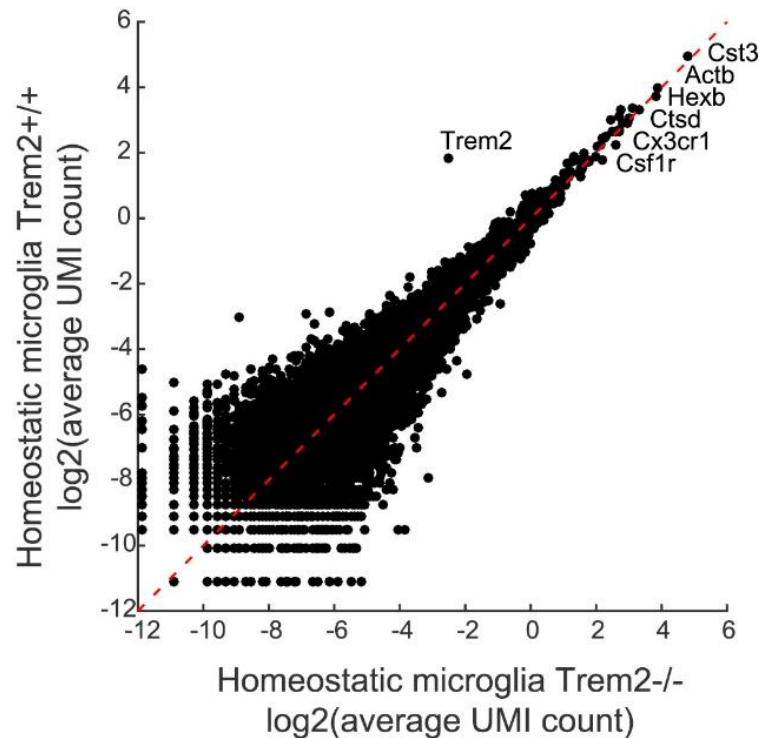
Trem2 associated with AD risk factors

How does Trem2 affect **DAM** progression in AD?



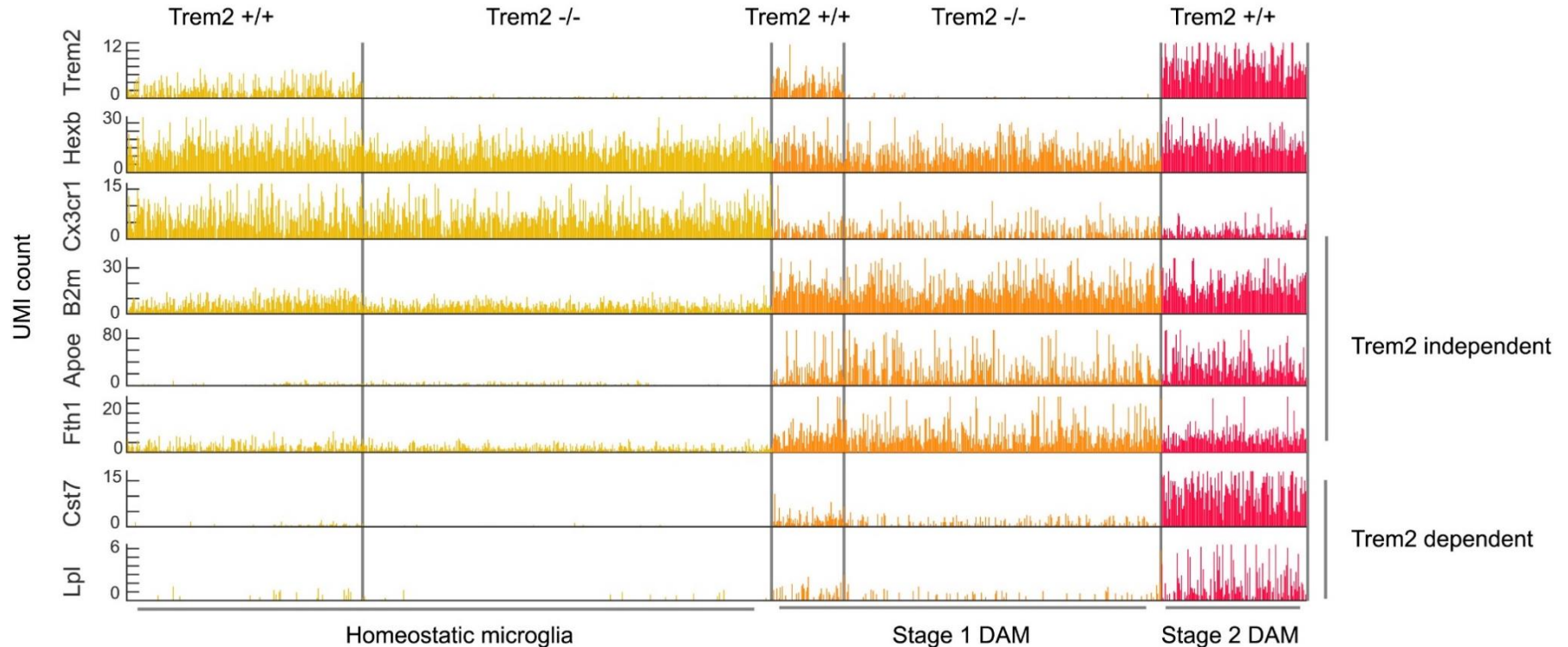
Trem2 $-/-$ in AD have mostly DAM stage 1 (intermediate)

How does Trem2 affect microglia gene expression?



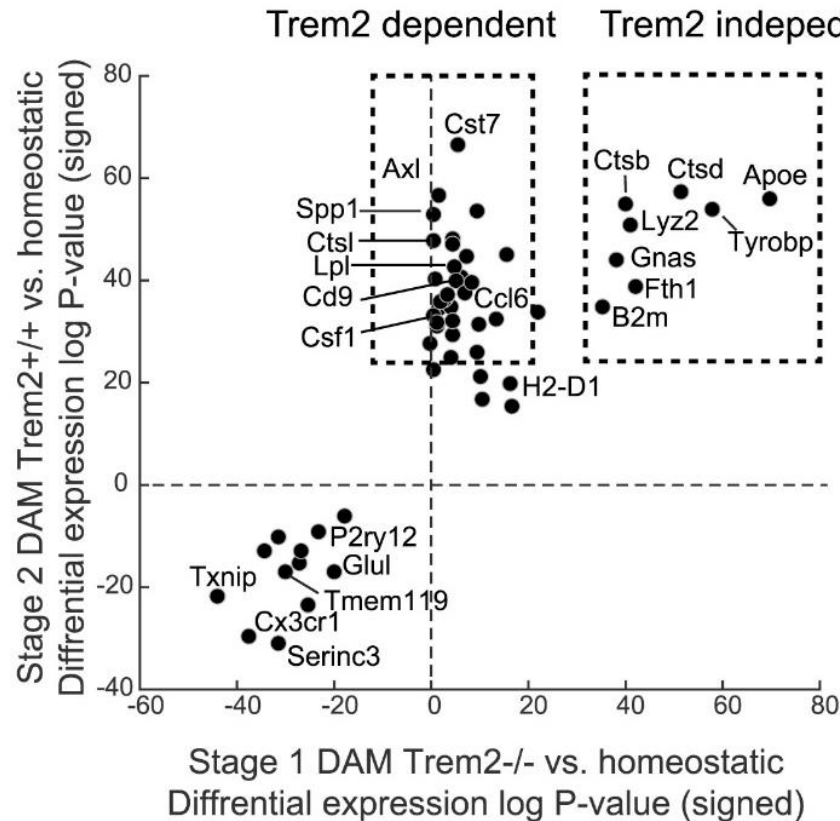
The **DAM** program is similar to previously identified AD program

How does Trem2 affect **DAM** gene expression?



DAM are generated through two-step mechanism

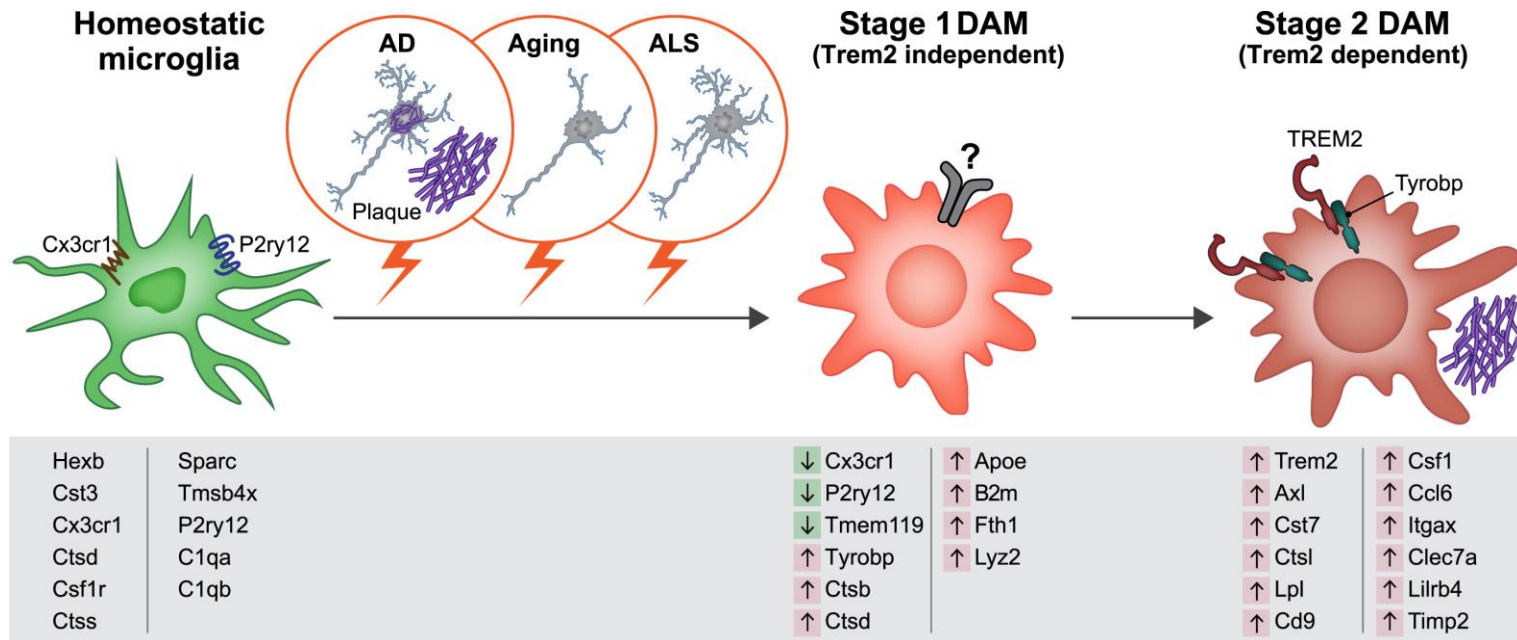
Does gene expression differ between two stages?



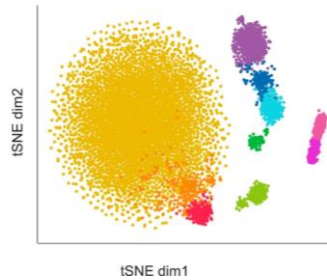
Activated first by Trem2-independent pathway

Activated second by Trem2-dependent pathway

What does the two step activation mechanism involve?



Summary

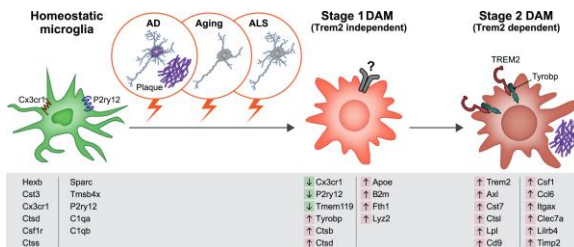
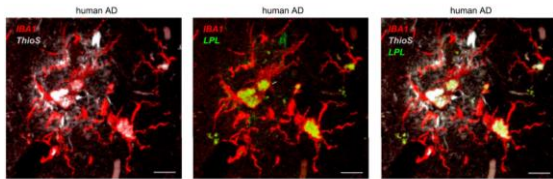


Identified novel microglia type (DAM) in AD

DAM localized near A β plaques

DAM phagocytic and conserved in humans and other neurodegenerative diseases

DAM regulated through 2 step mechanism



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