

Single Cell Transcriptomics & Role of microglia in restricting Alzheimer's



Diane Xue and Franklin Yeo

Preview

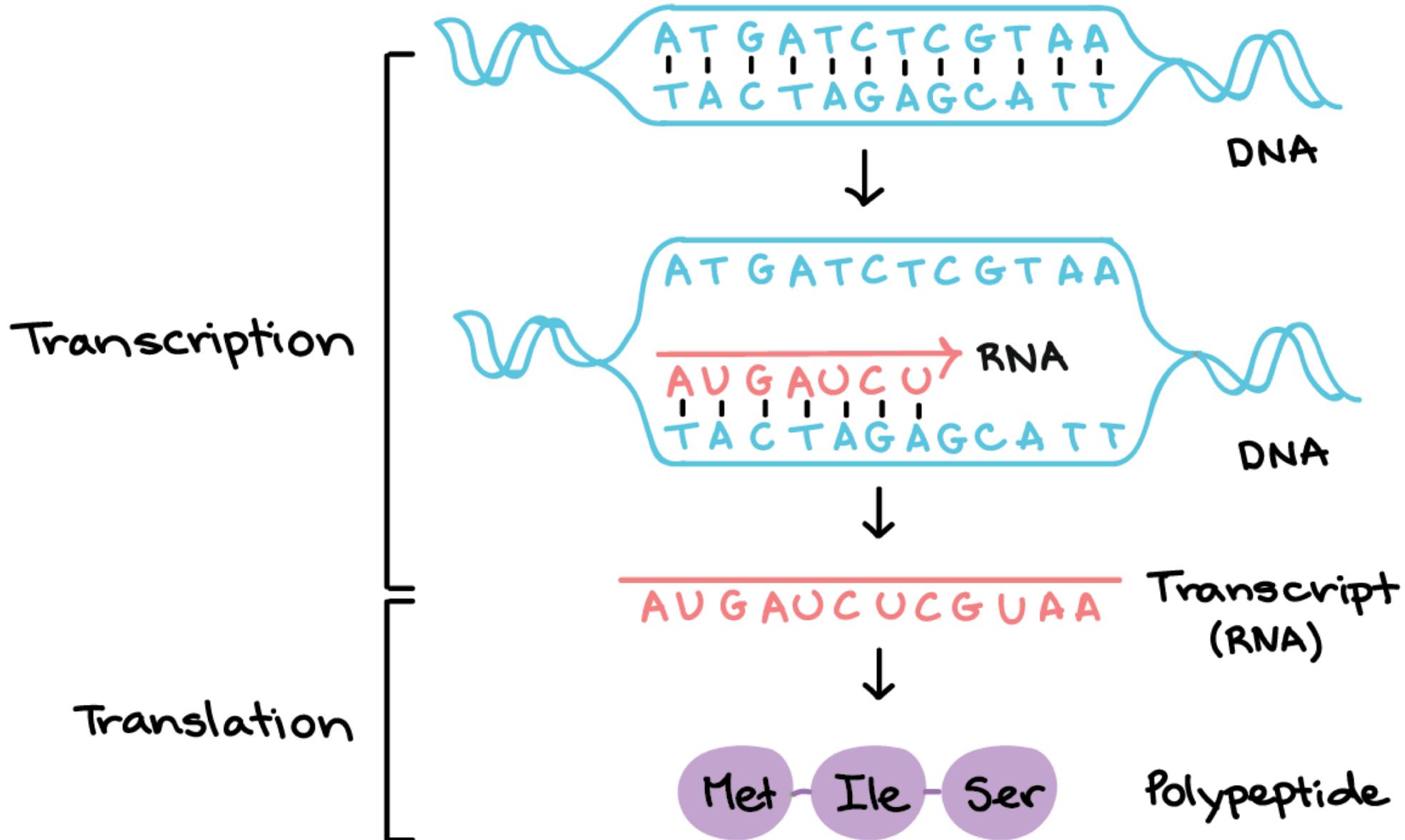
RNA-sequencing

Single Cell Transcriptomics

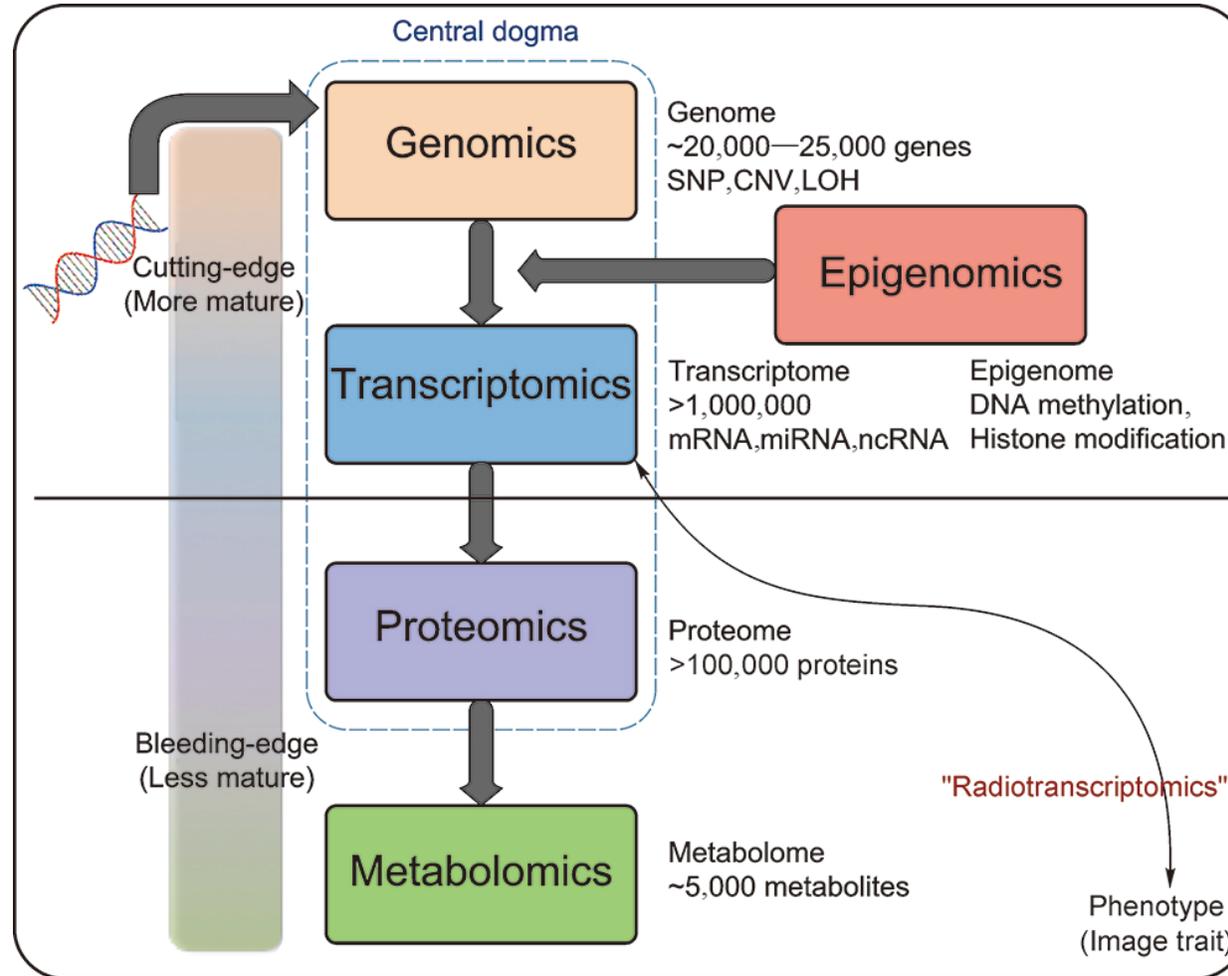
Association of Microglia with Alzheimer's Disease

Future implications for neurodegenerative disease treatment

What is transcription?

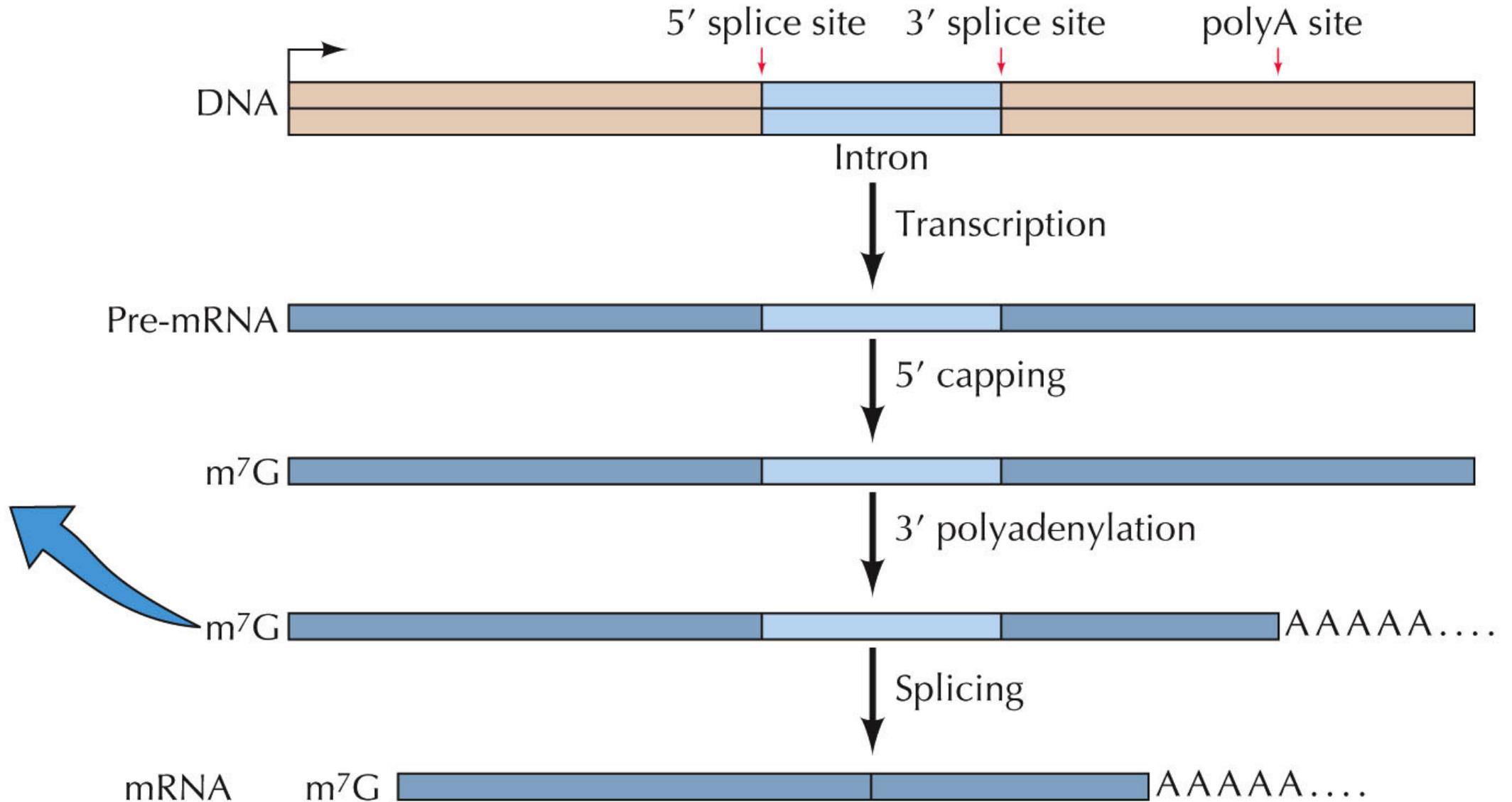


What is transcriptomics, and why is it important?

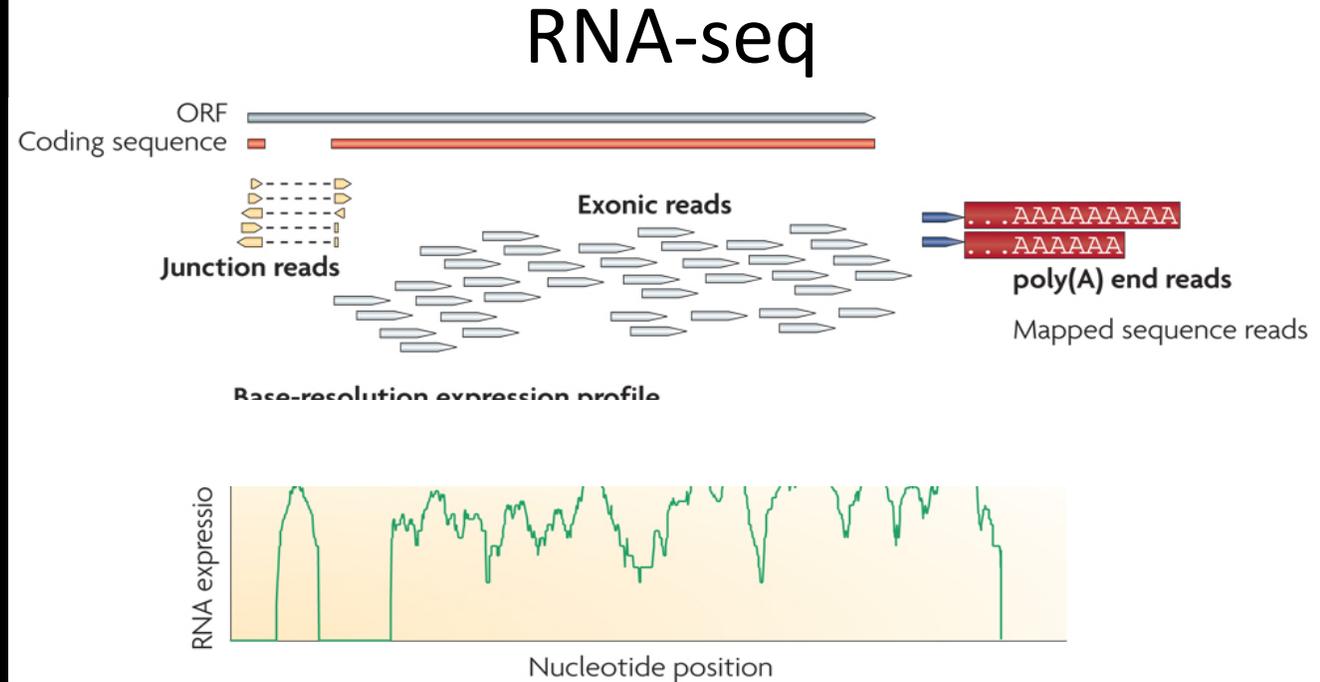
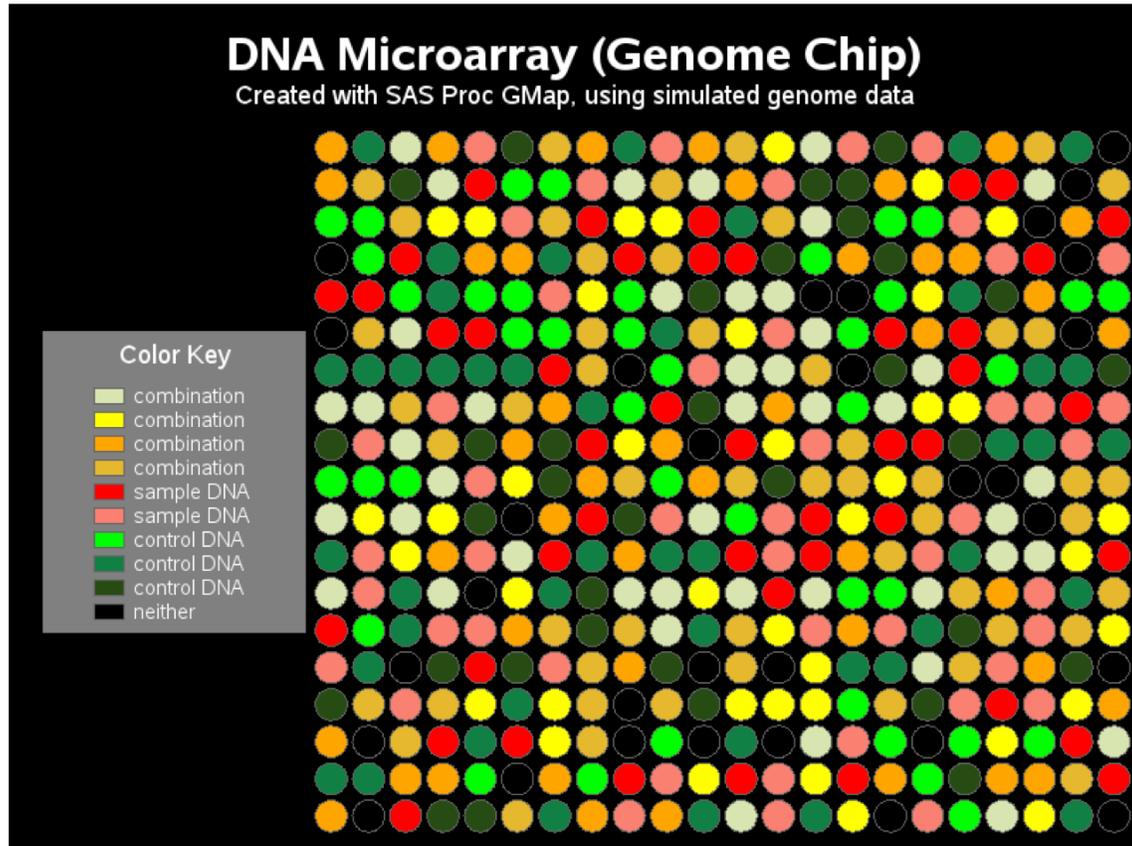


**Study of the Transcriptome:
the collection of transcripts of genes**

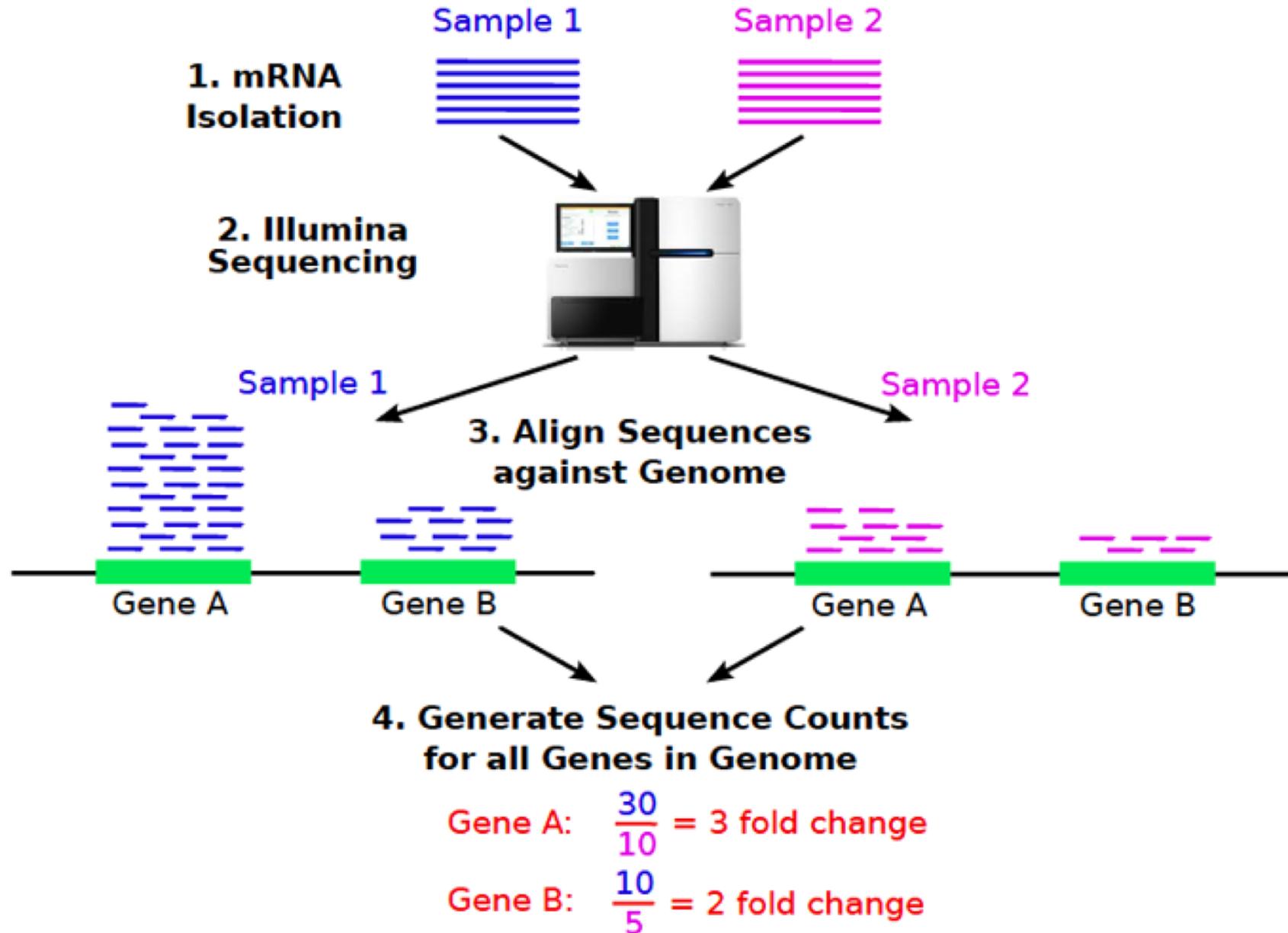
What does transcriptomics allow us to do?



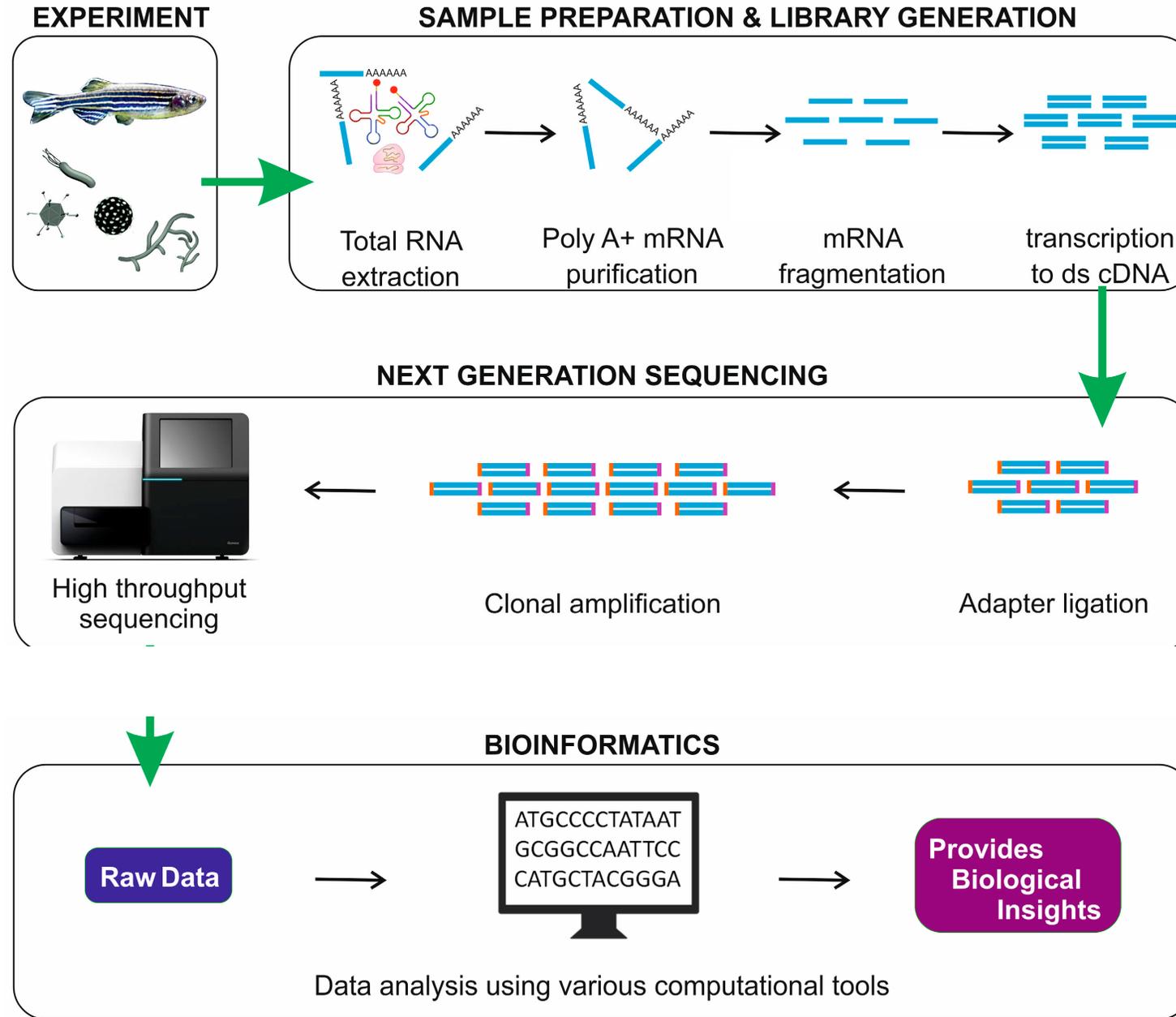
What techniques are utilized in transcriptomics?



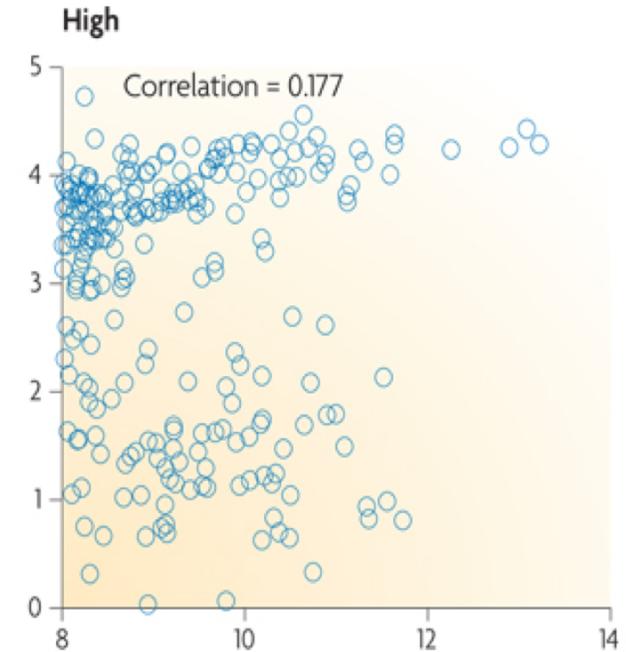
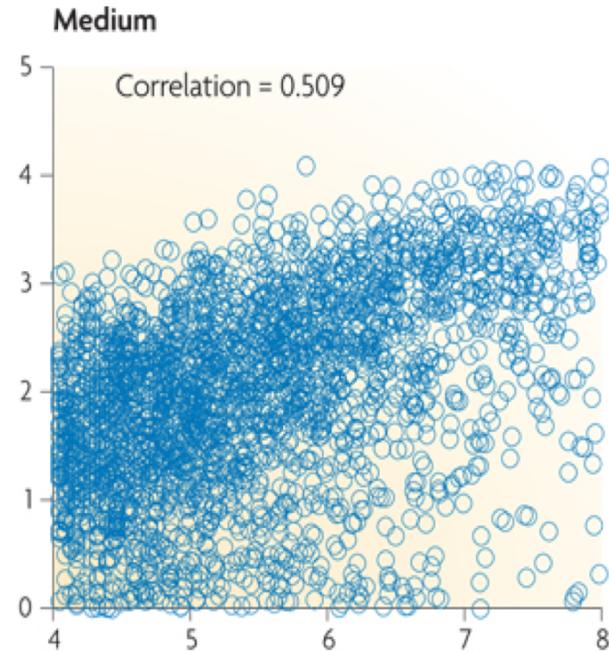
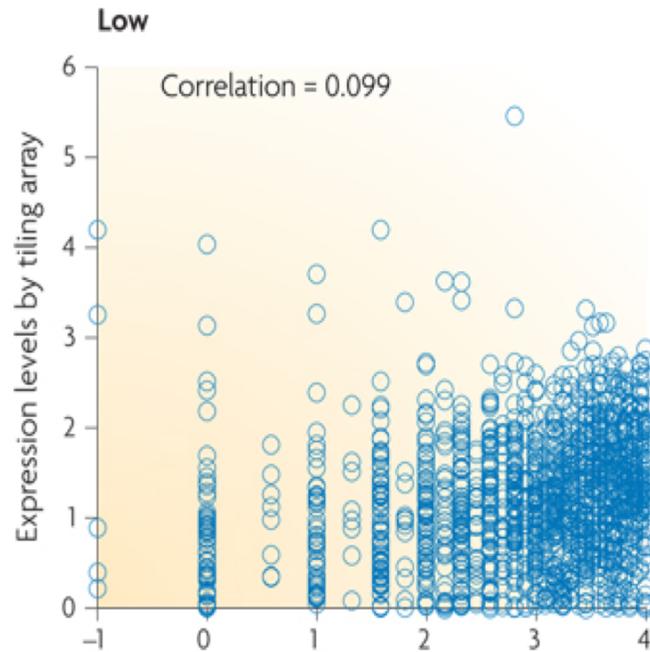
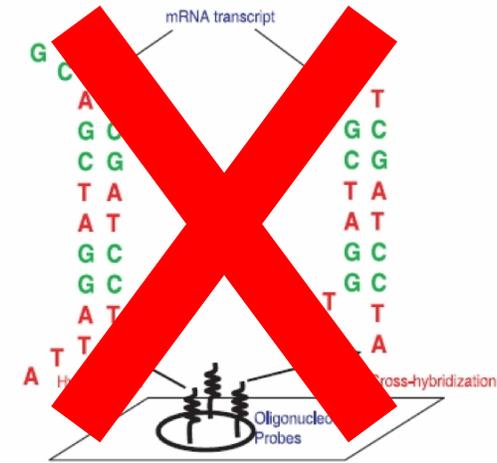
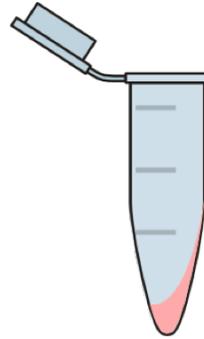
What is RNA-seq?



What is the work flow of a transcriptomic assay?

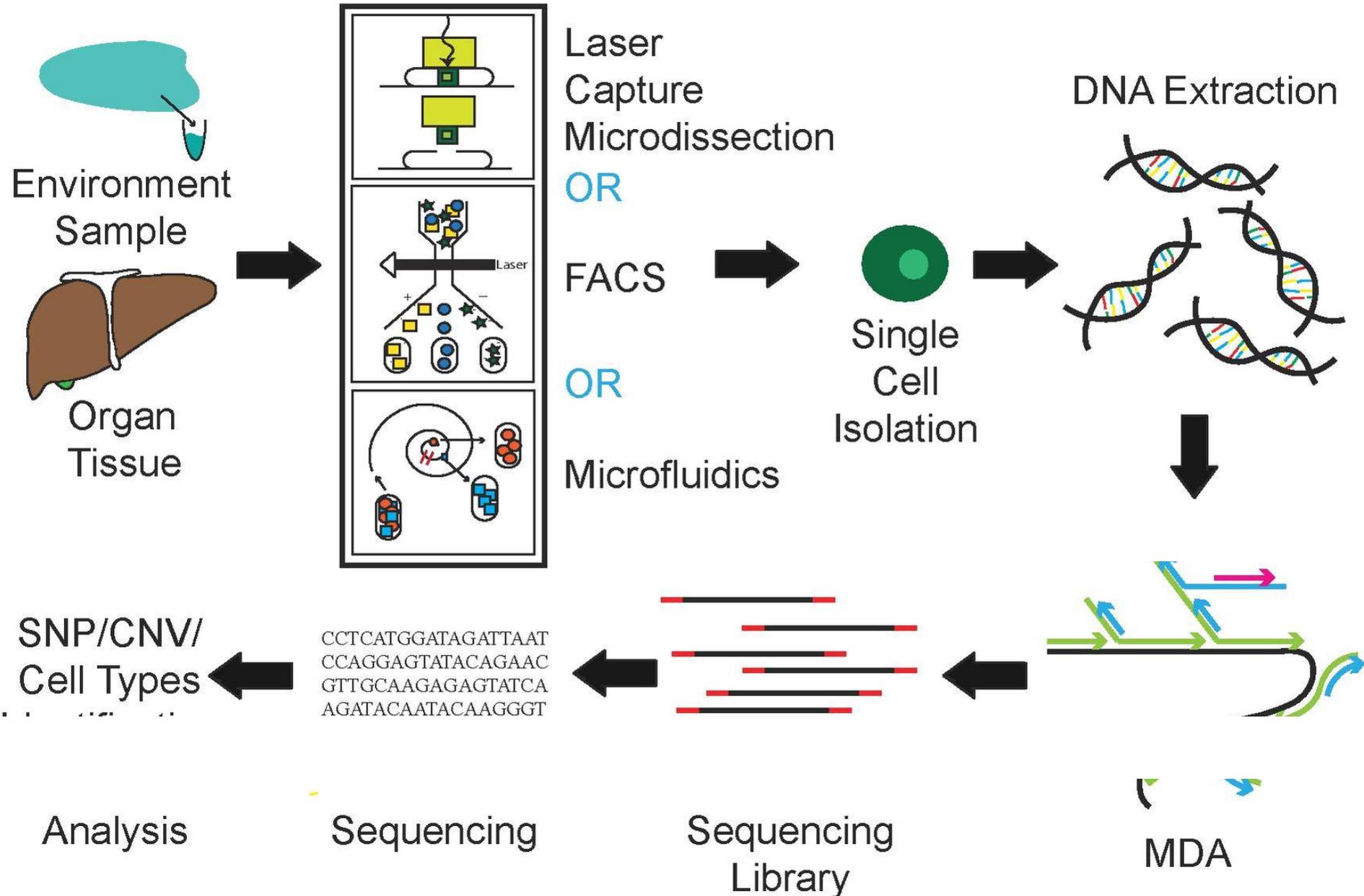


What are the advantages of RNA-Seq?

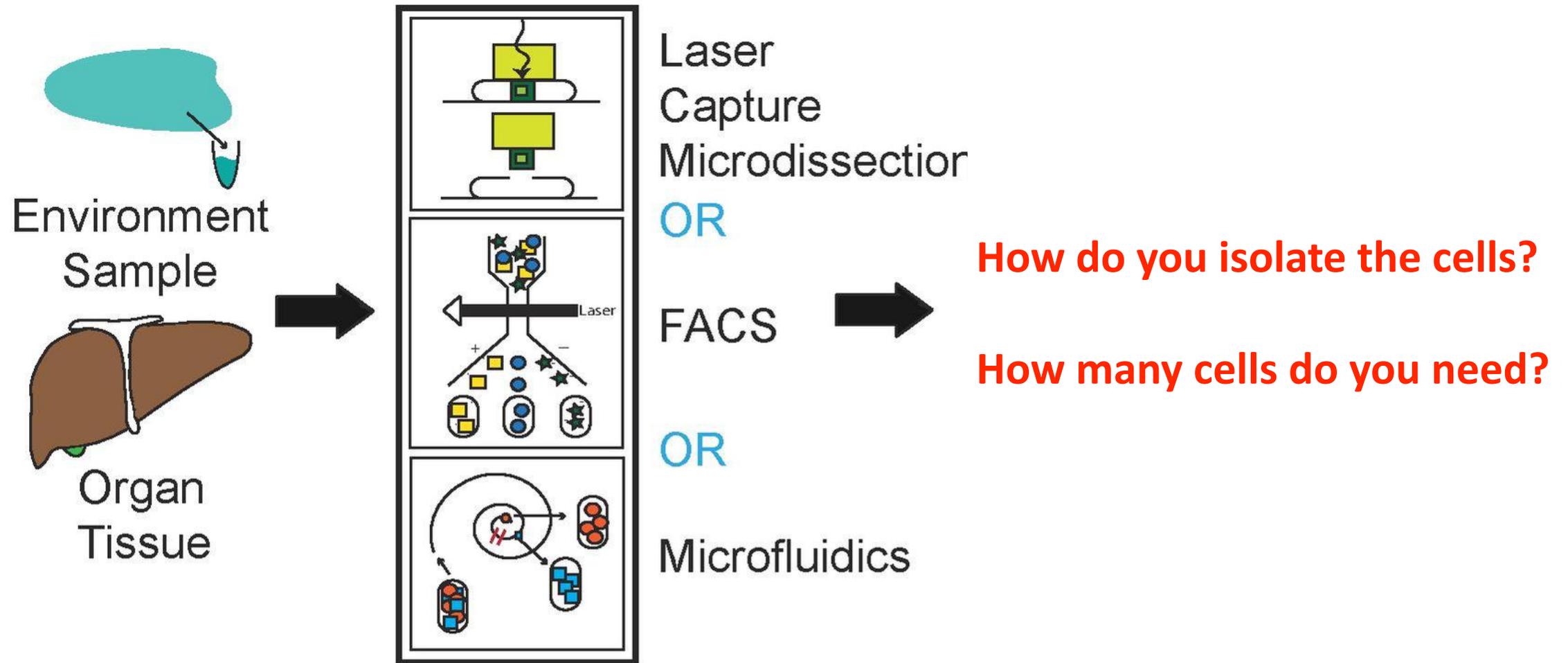


Large Dynamic Range

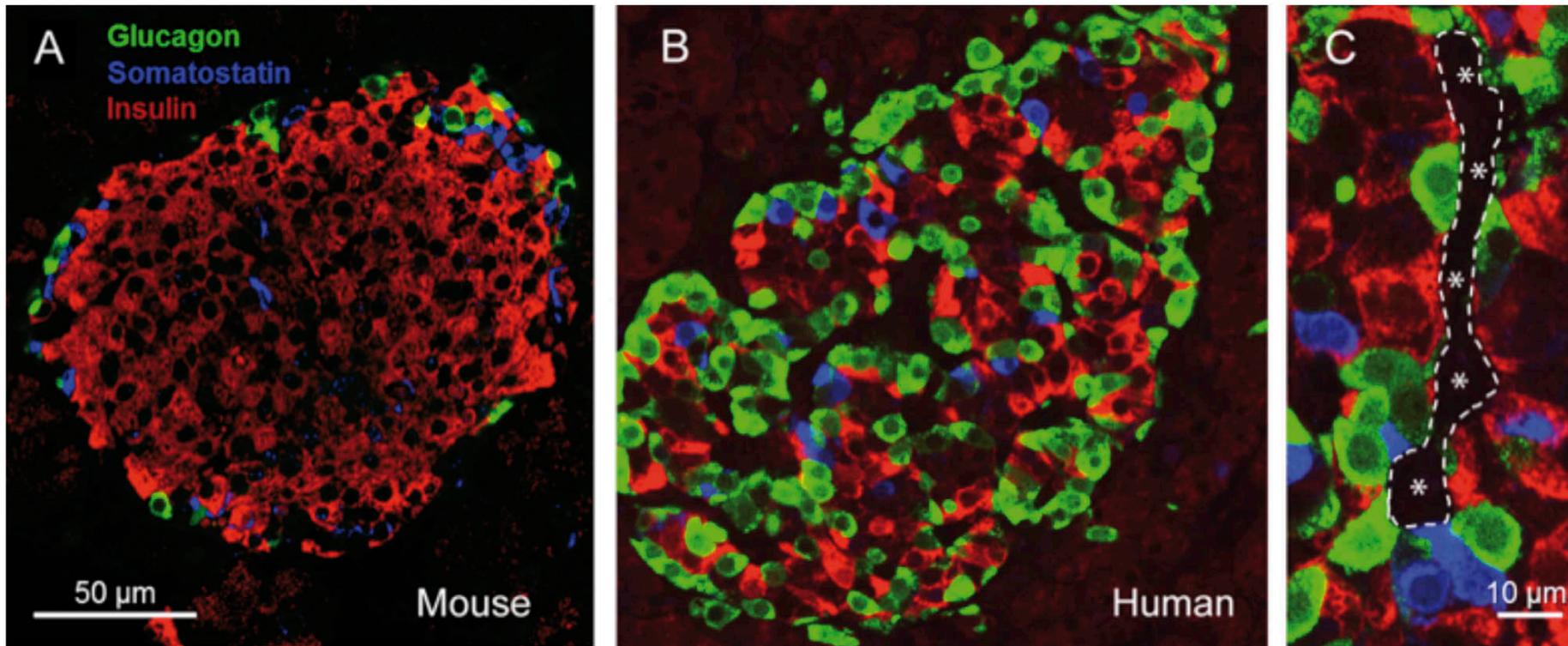
What is Single Cell Transcriptomics?



What needs to be considered before doing single cell RNA-seq?

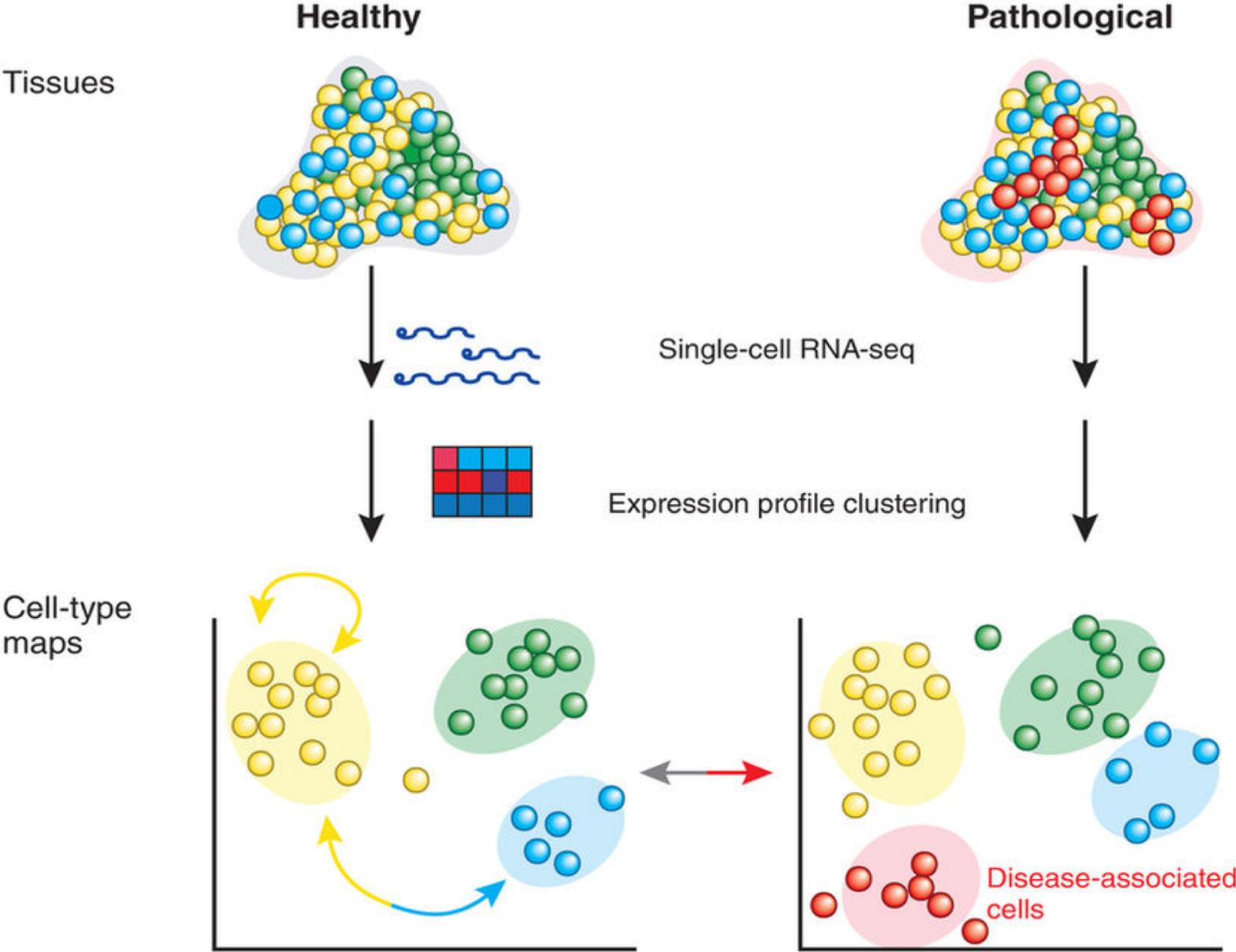


What needs to be considered before doing single cell RNA-seq?

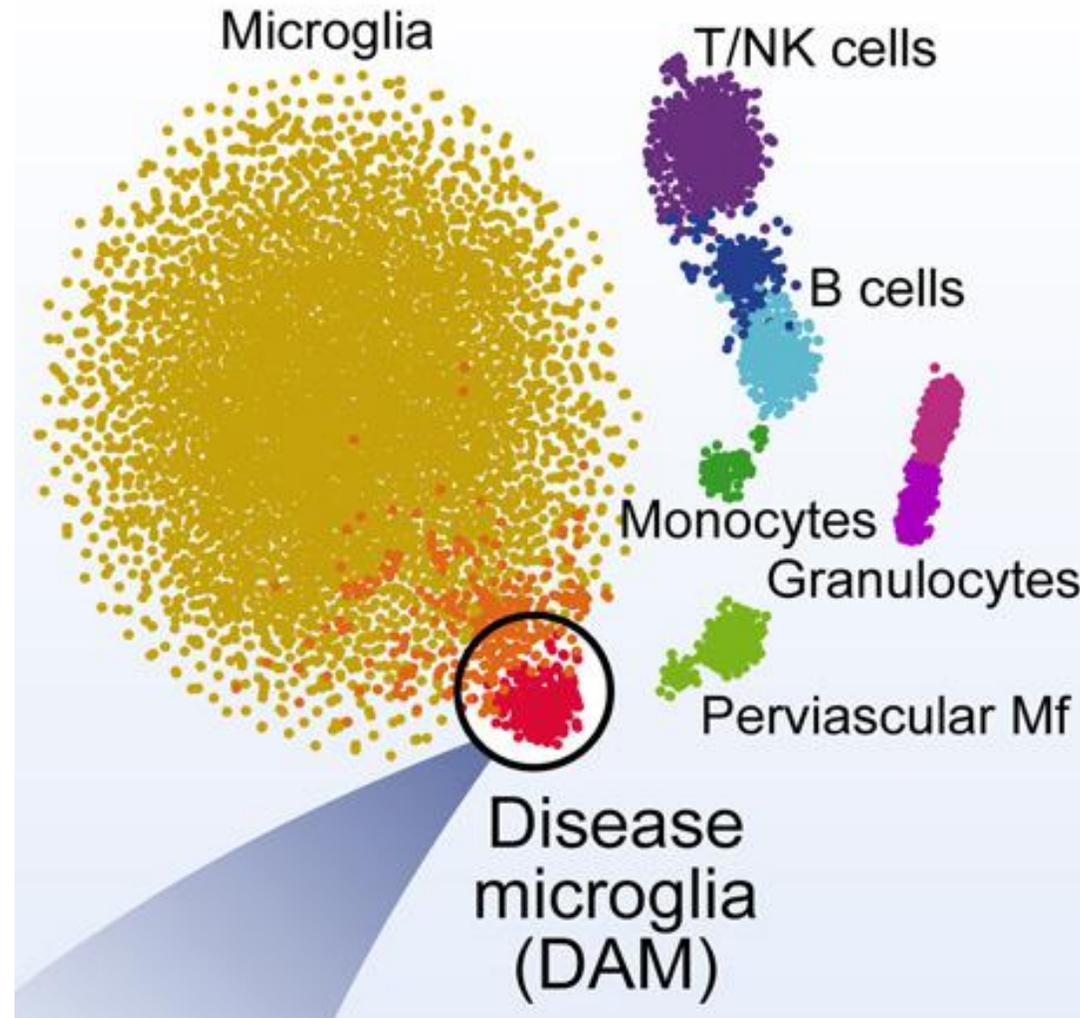


Spatial single cell transcriptomics would allow better understanding of intercellular communication

How is Single-cell RNA-seq utilized in research?

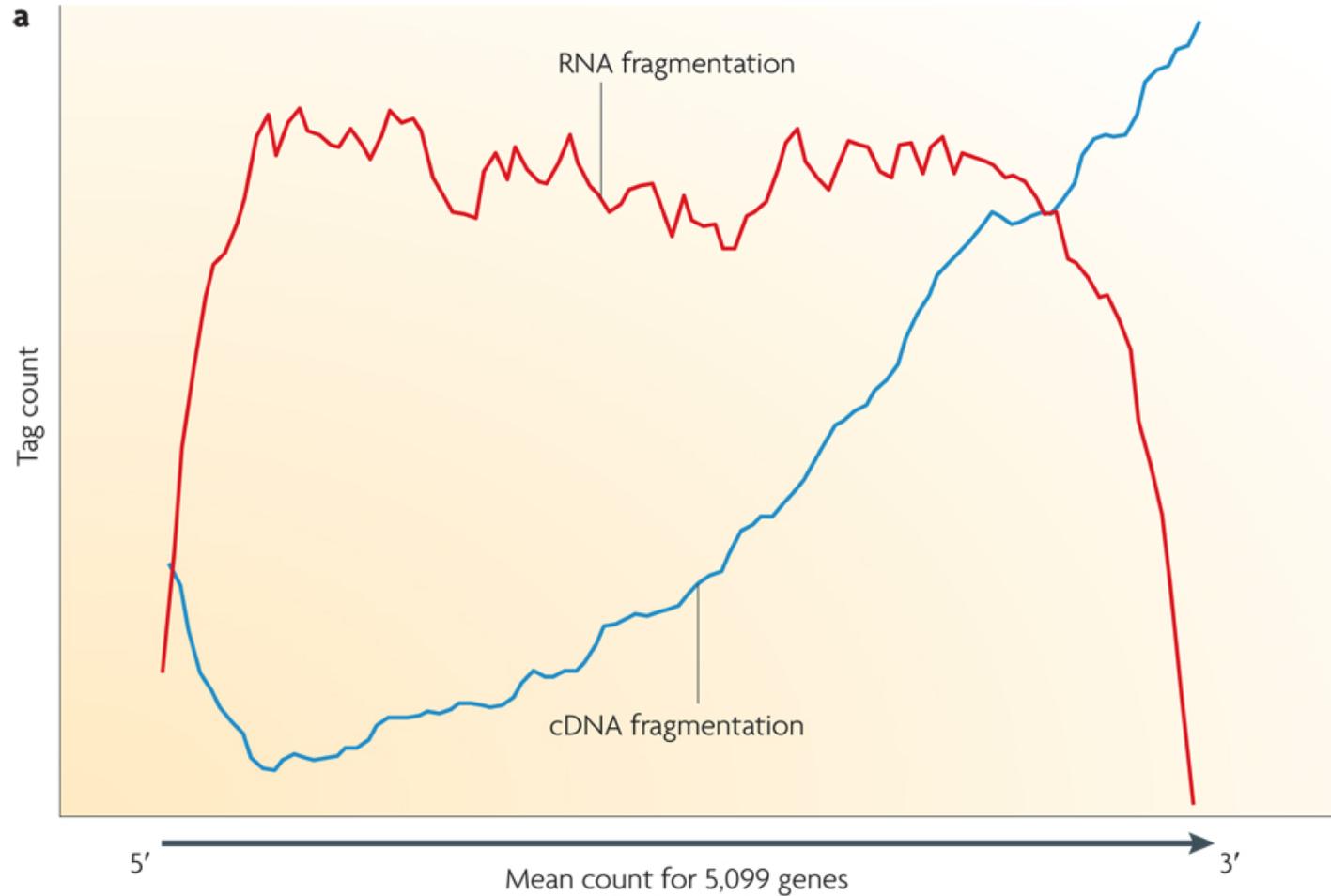


What has Single Cell RNA-seq allowed us to do?



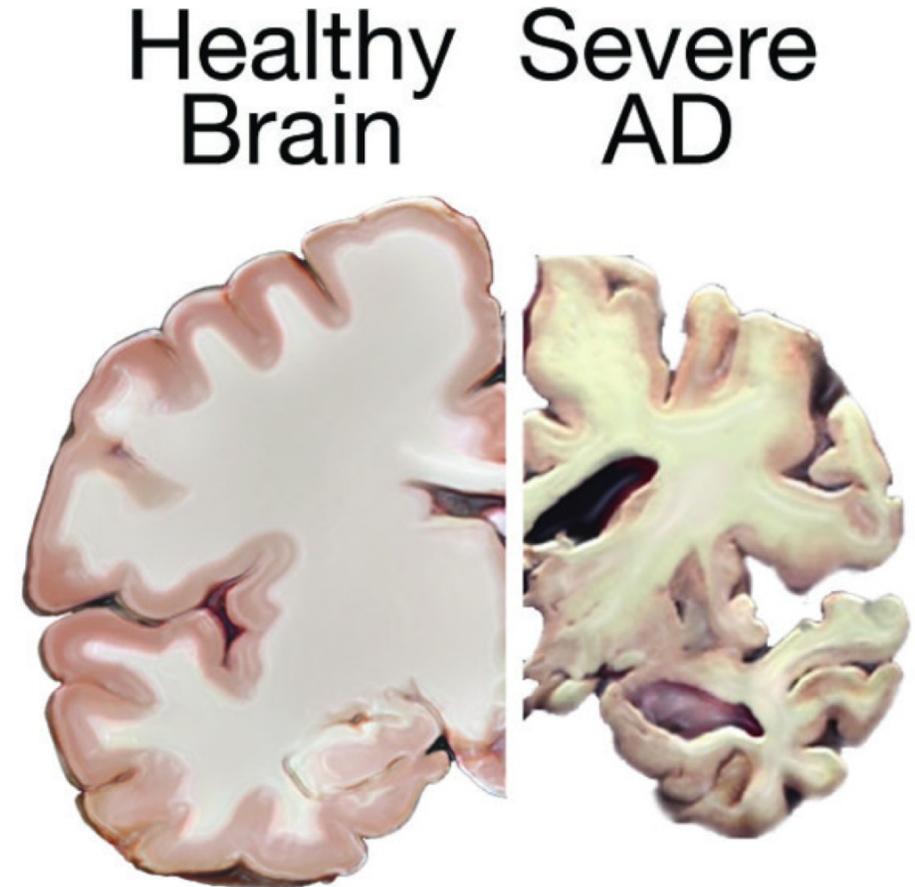
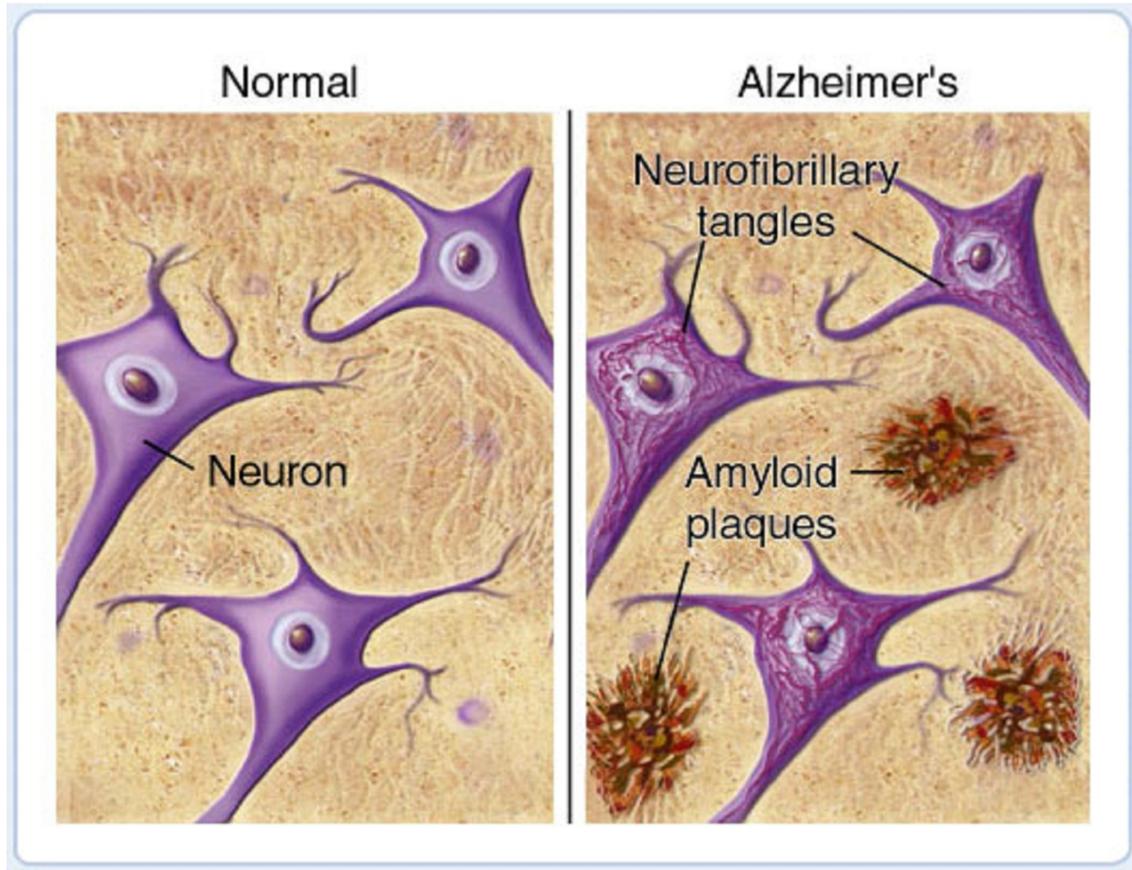
What are some challenges of RNA-Seq?

Library Construction



Inaccurate expression level detection due to bias

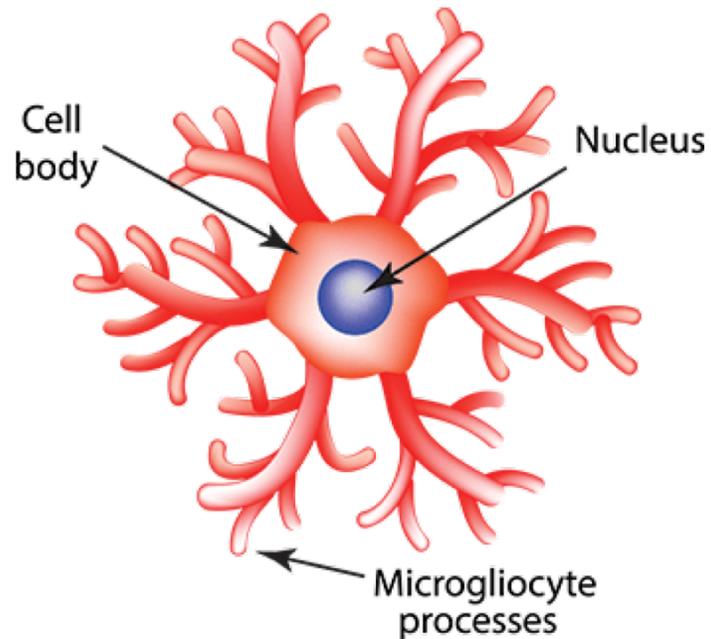
What is Alzheimer's Disease (AD)?



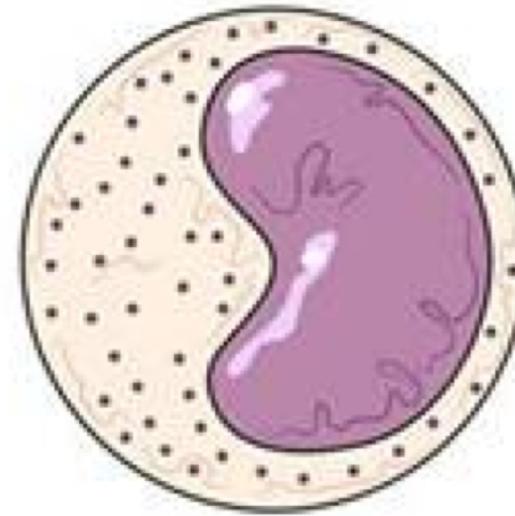
Neurodegenerative disease in the brain that causes dementia

What is known about Immune Cells in Alzheimer's?

MICROGLIA

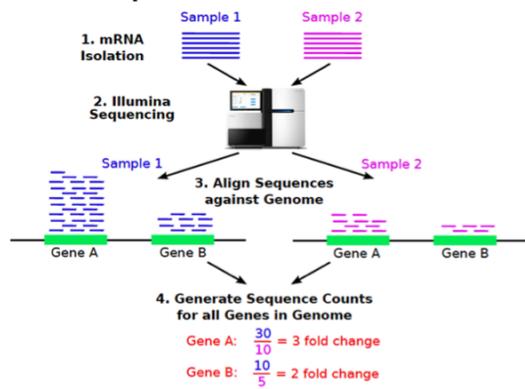


Monocyte



Conflicting results regarding systemic immunity, recruited **monocytes**, and tissue-resident **microglia**

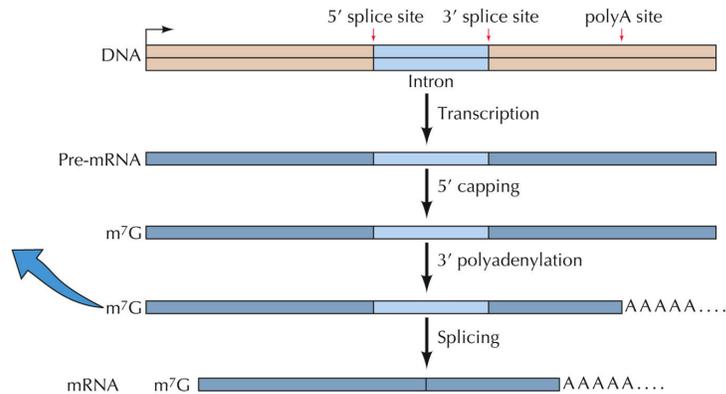
What is RNA-seq?



Summary

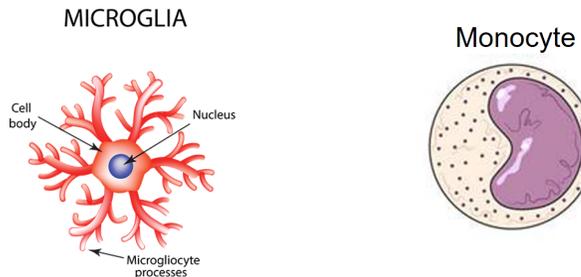
RNA-seq can help determine transcriptional structures

What does transcriptomics allow us to do?



Transcriptomics is the study of the RNA products of a gene

What is known about Immune Cells in Alzheimer's?



Single Cell RNA-seq allows characterization of disease associated cells

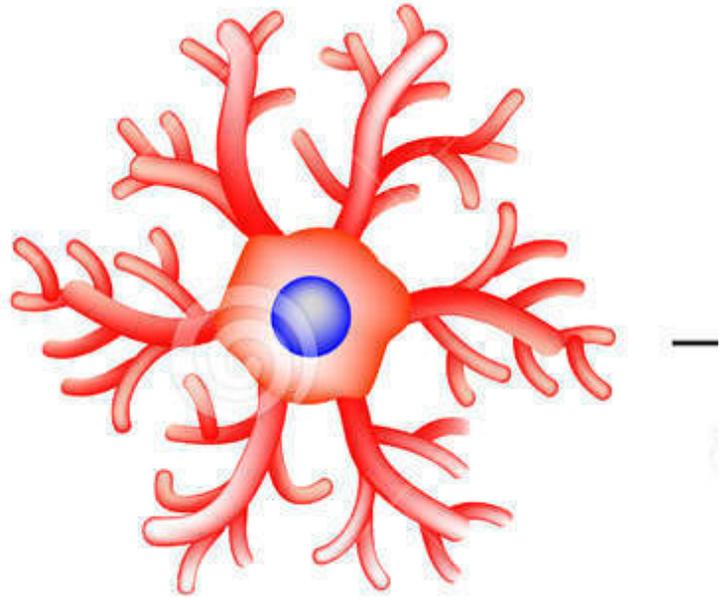
Conflicting results regarding systemic immunity, recruited **monocytes**, and tissue-resident **microglia**

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

Keren-Shaul, et al. 2017. Cell, Volume 169, Issue 7



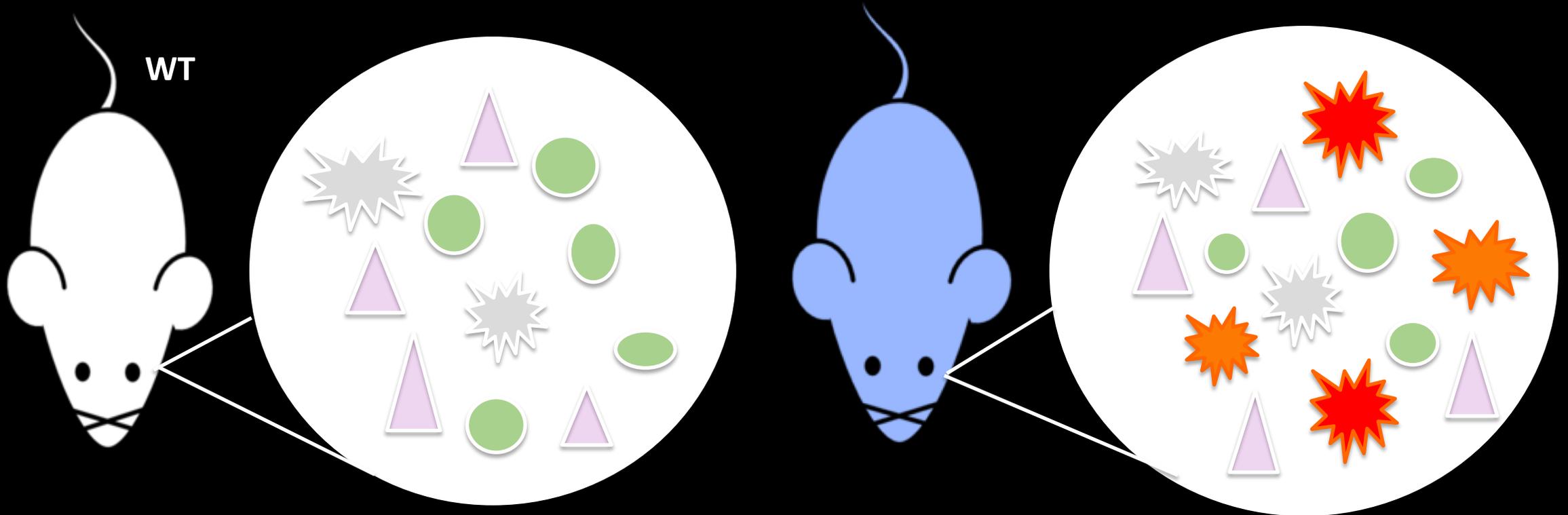
What are Microglia?



Resting Microglia

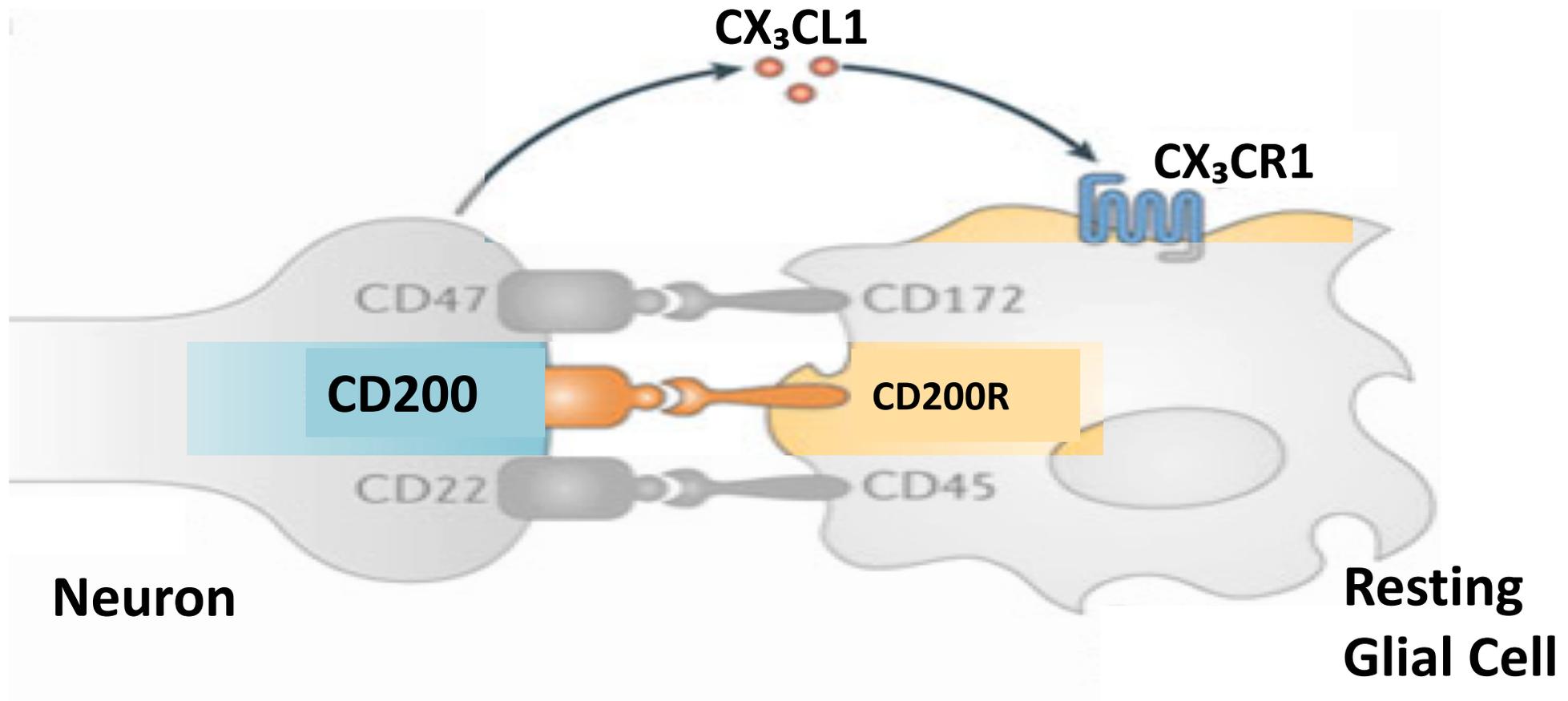
The primary immune cells in the central nervous system

What are **Disease-Associated Microglia**?



Microglia that are observed in Diseased mice, but not WT mice

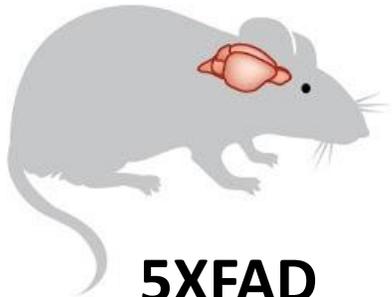
How is Microglial Immune Activity Regulated?



Checkpoint Mechanisms (inhibitory pathways)

How did the authors characterize the immune cells involved in AD?

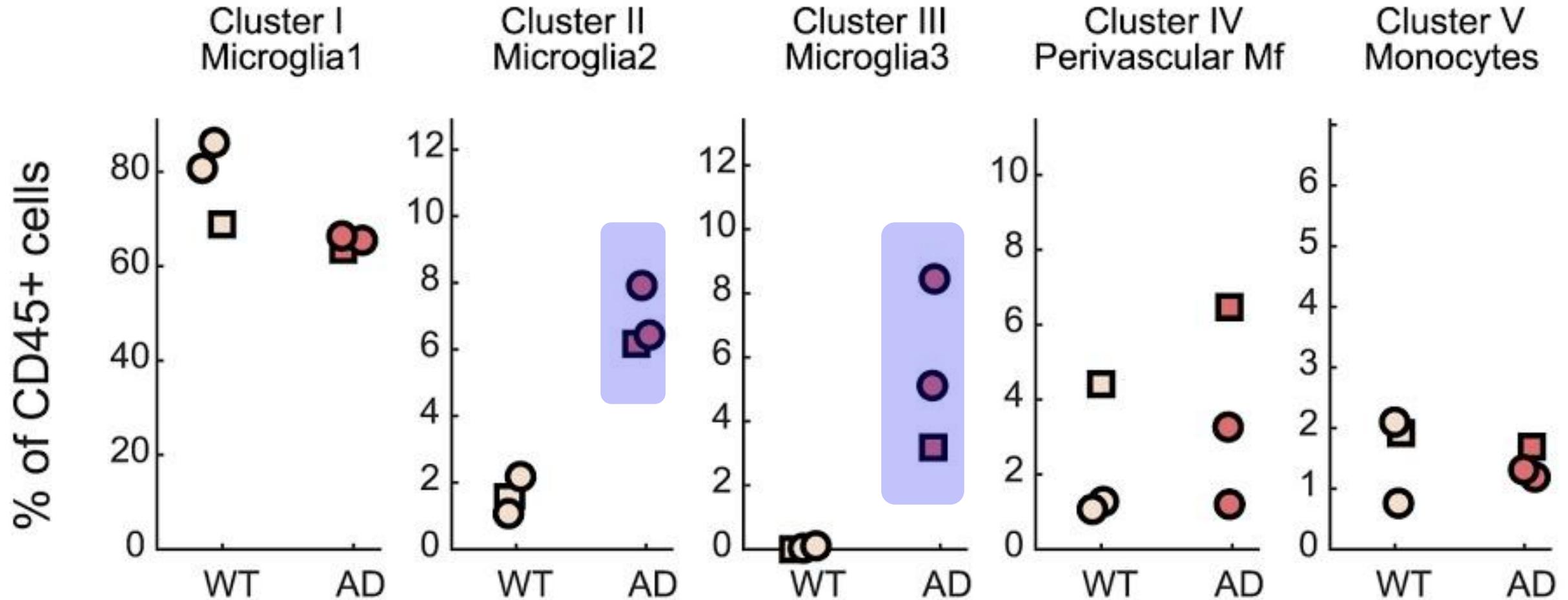
Wild-Type



5XFAD

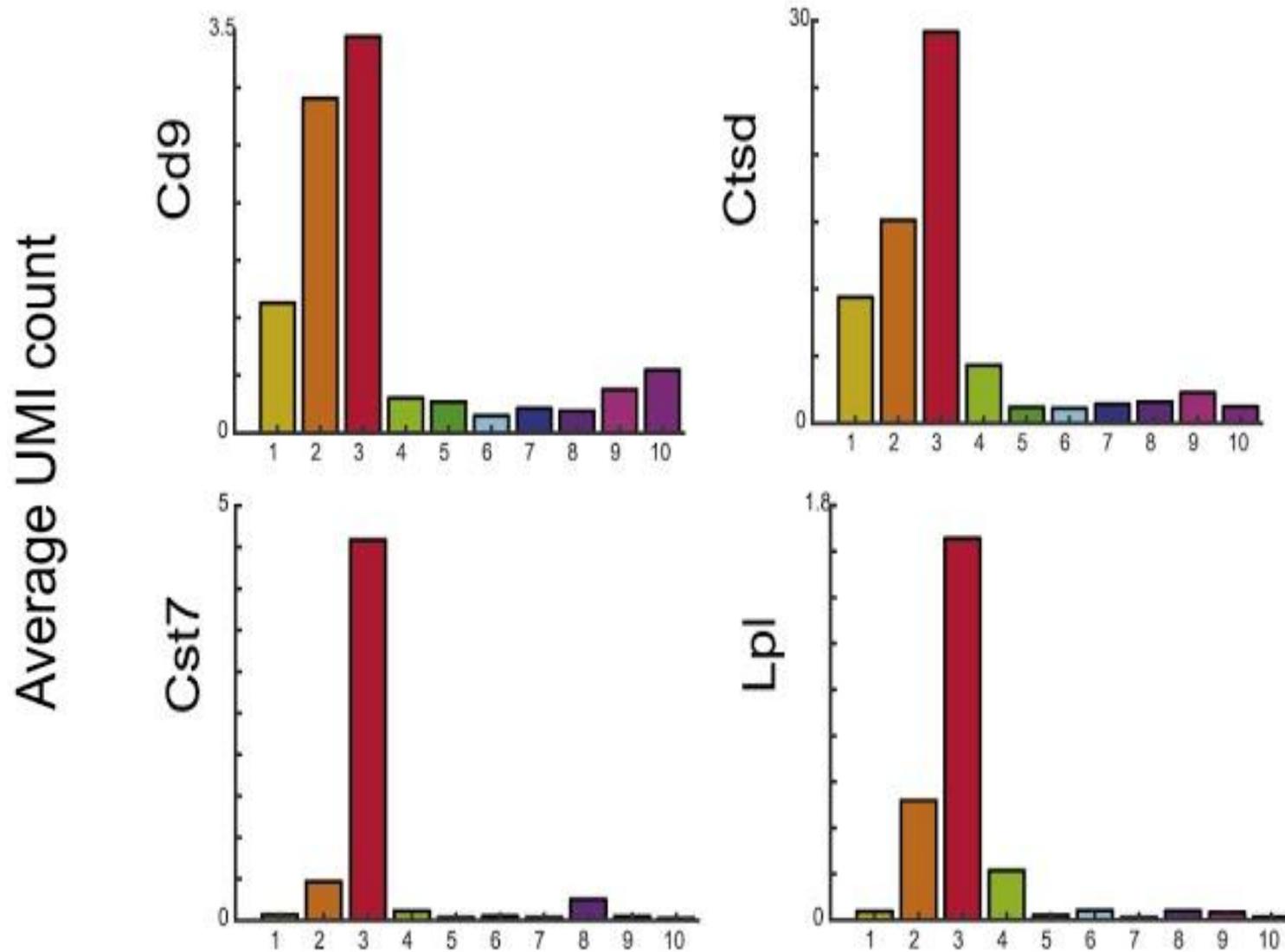
Massively Parallel RNA-seq to characterize immune cells

Which immune cells are involved in AD?



DAM = Microglia 2 and 3 observed in AD but not WT mice

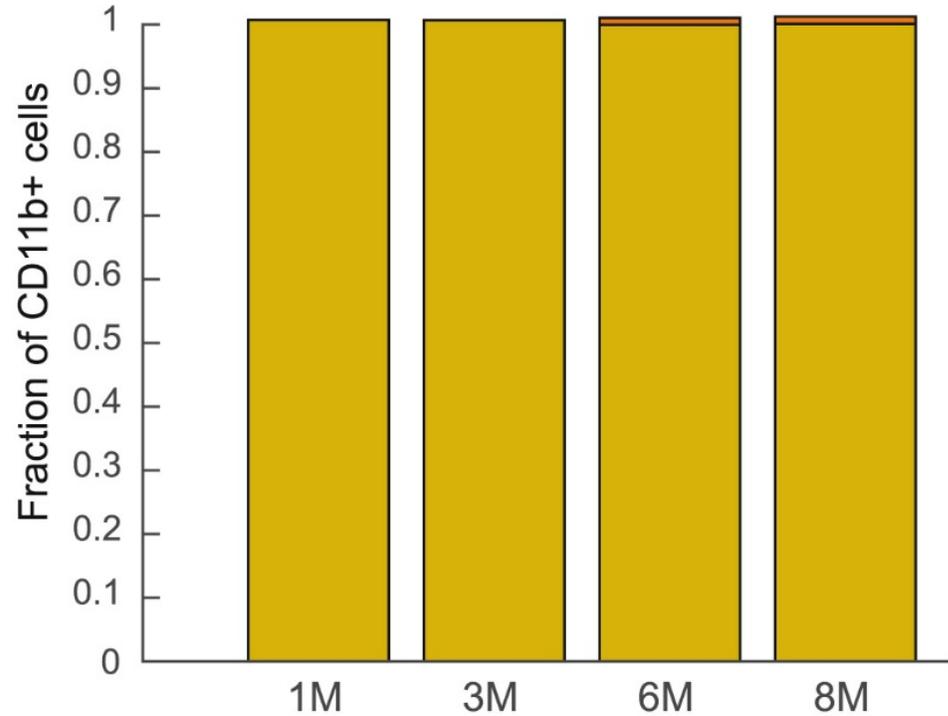
What are the unique molecular identifiers of Disease-Associated Microglia?



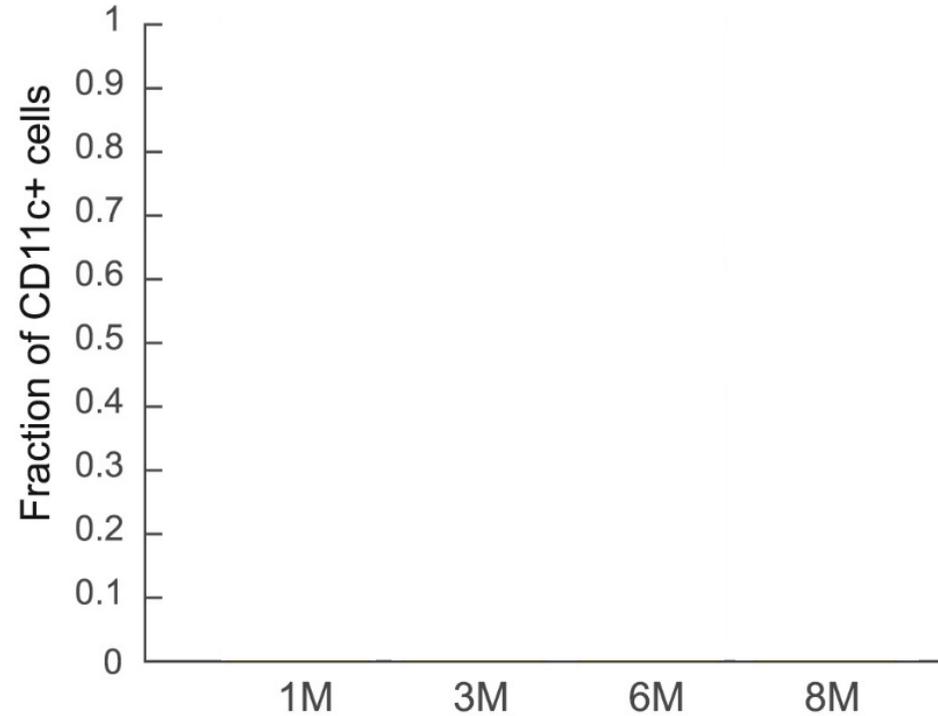
Genes expressed at a higher rate in Clusters **2** and **3**

Why is Cluster 2 gene expression lower than Cluster 3?

Why is Cluster 2 gene expression lower than Cluster 3?



Wild-Type

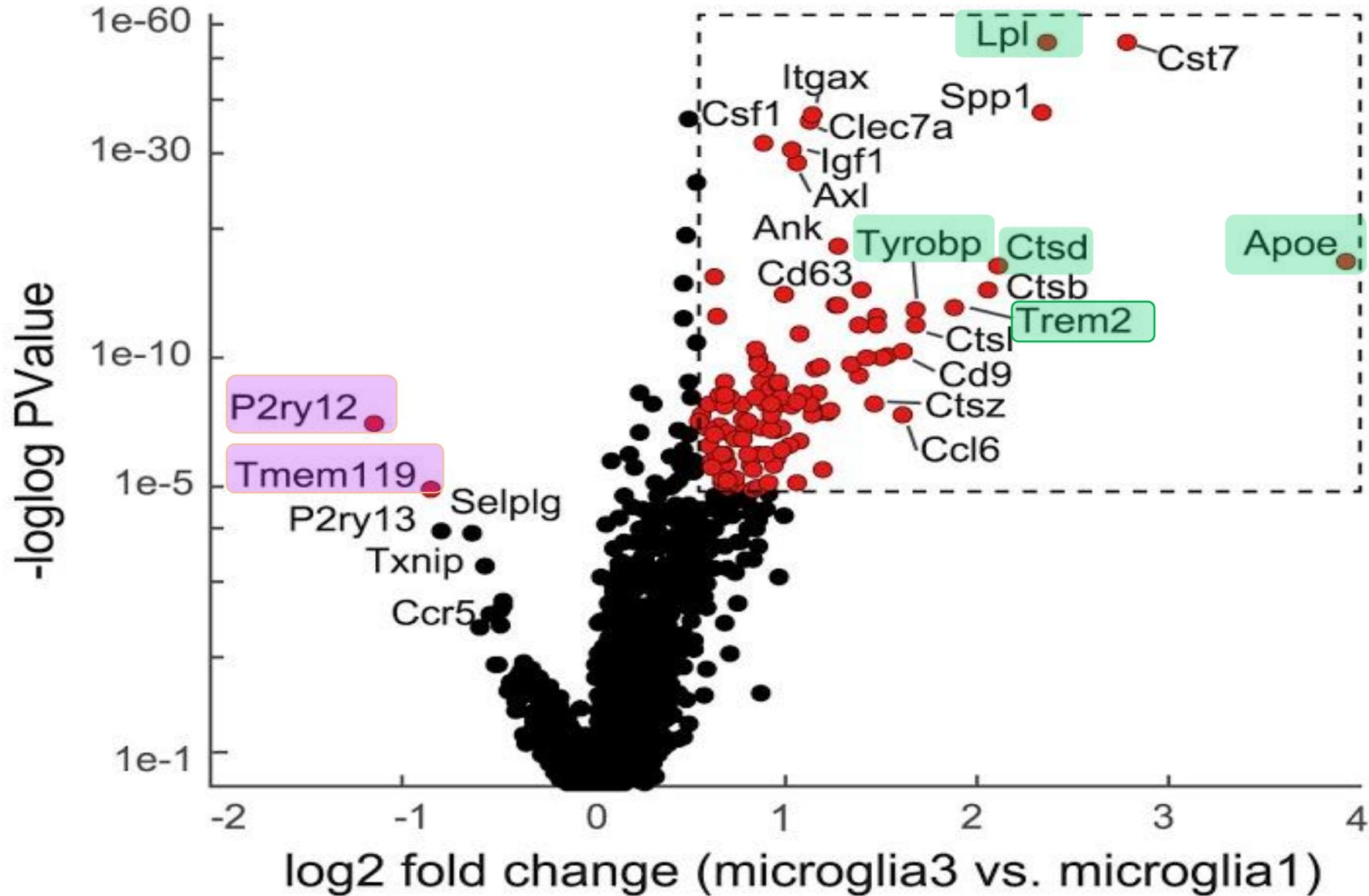


5XFAD

Cluster 1
Cluster 2
Cluster 3

Cluster 2 is associated with an intermediate stage of Alzheimer's Disease

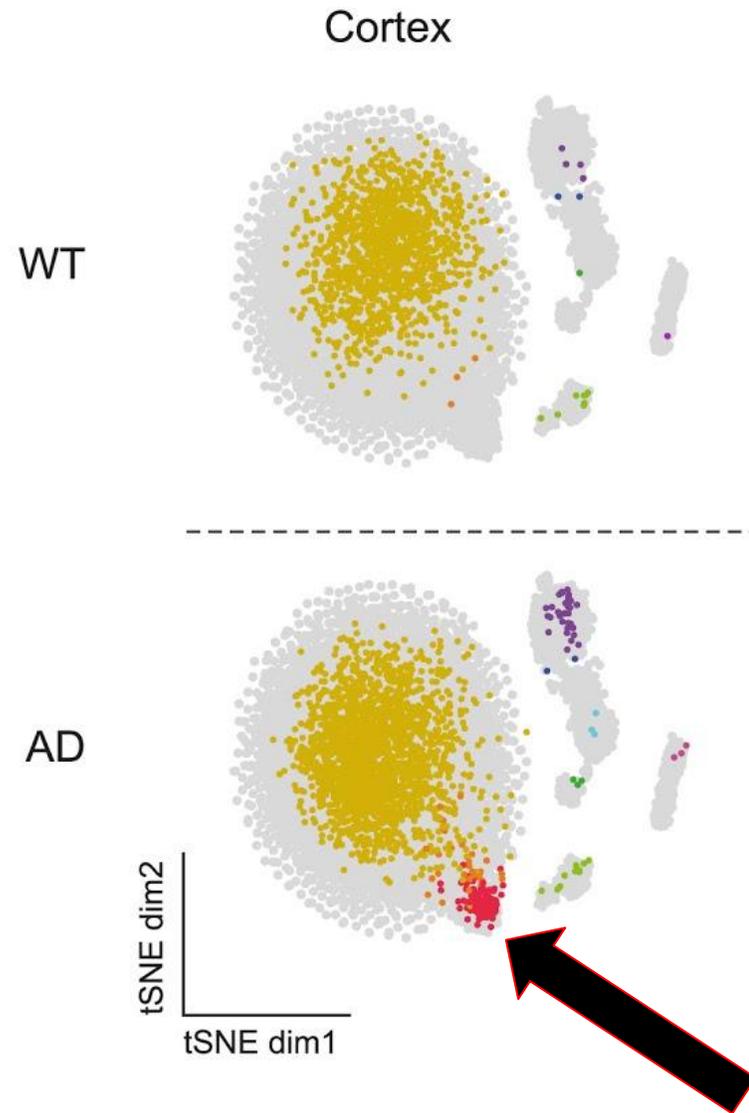
How is DAM gene expression different from homeostatic microglia?



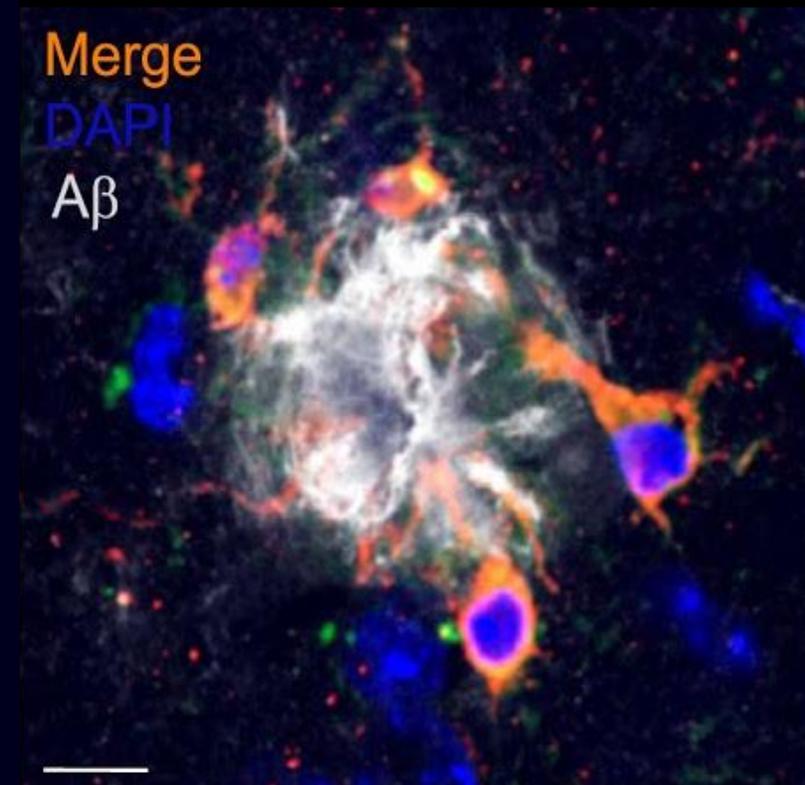
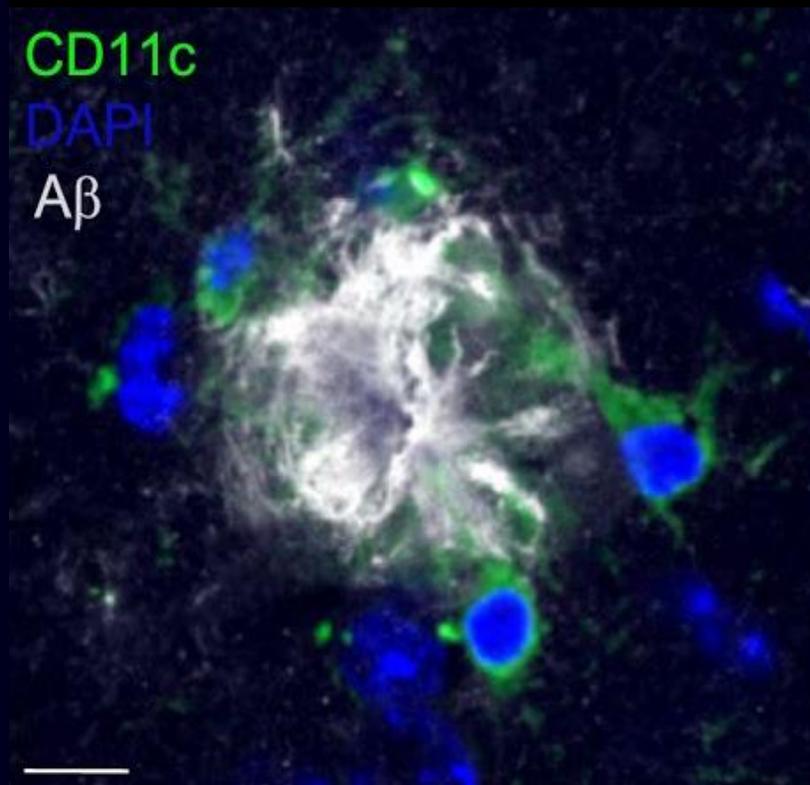
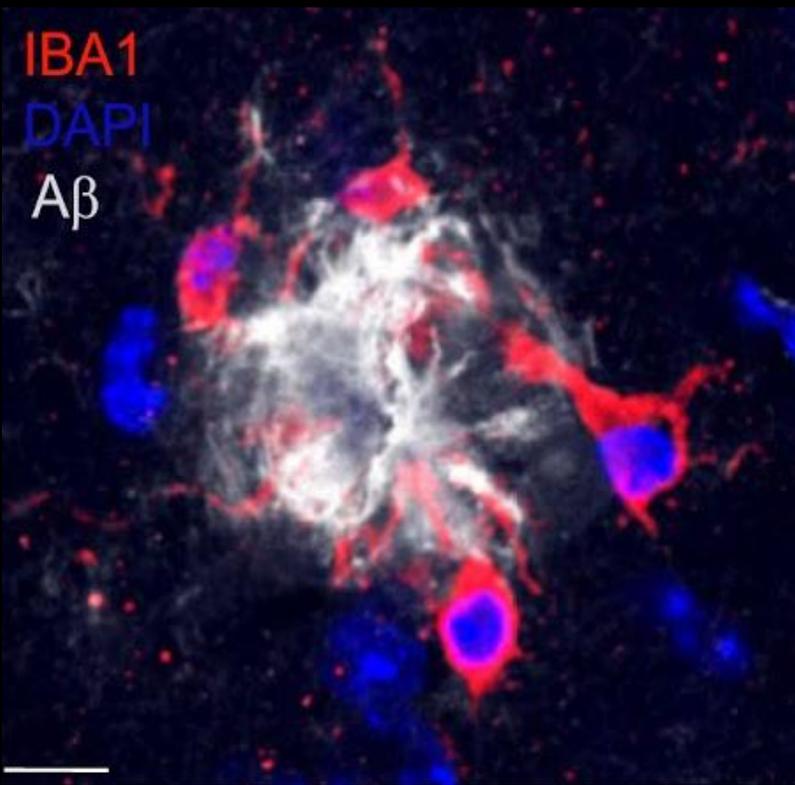
AD Risk Factors

Homeostatic Maintenance

Where are **DAM** localized within the brain?

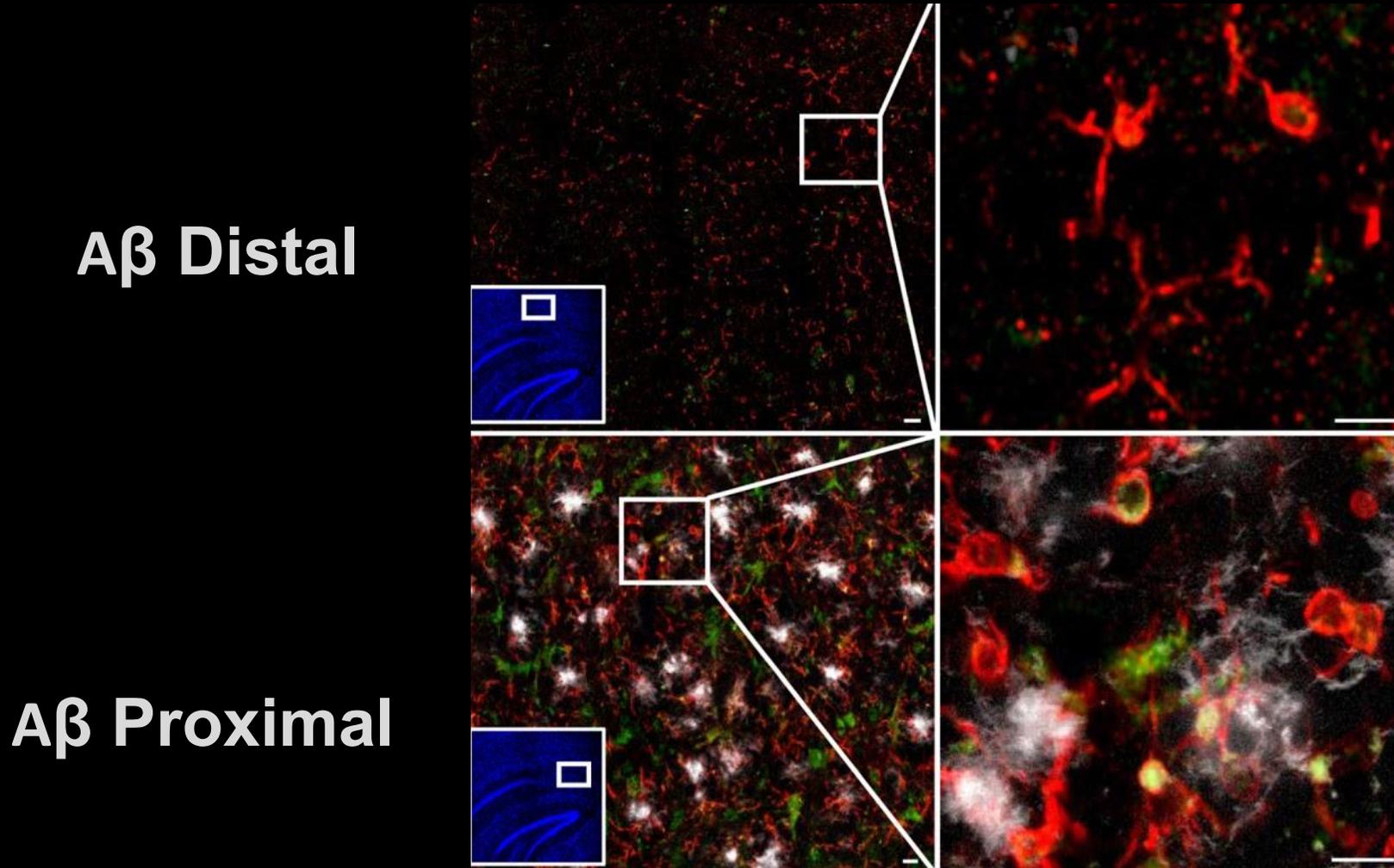


Where in the cortex are the **DAM** located?



In proximity to A β -plaques

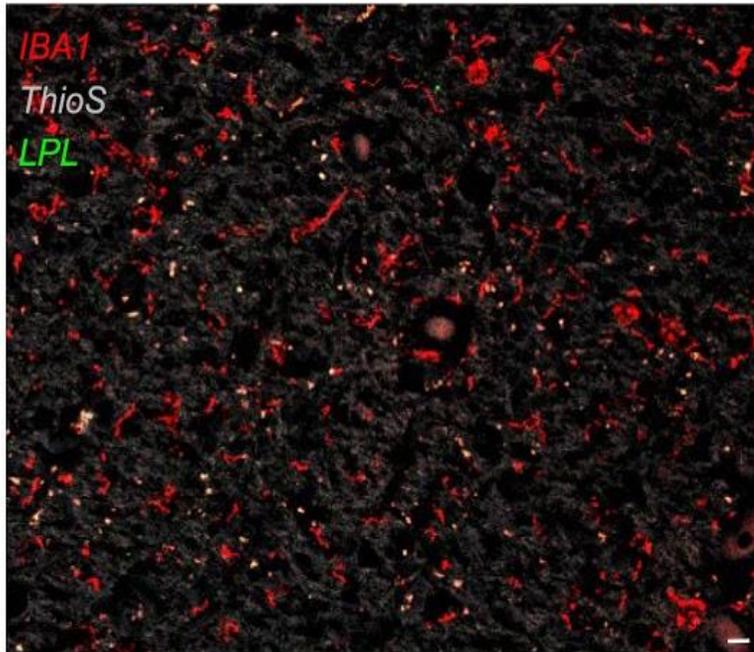
Do **DAM** contain phagocytic particles of A β ?



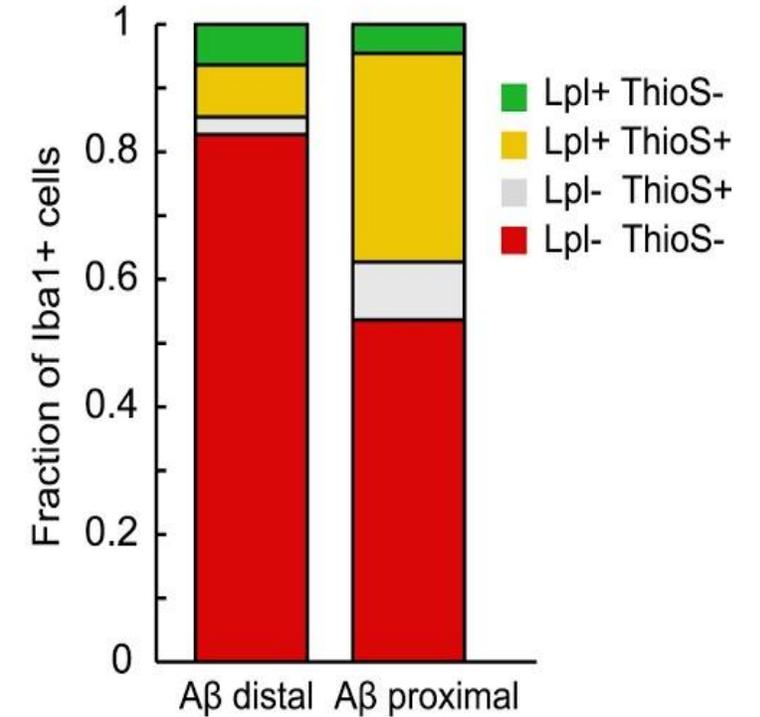
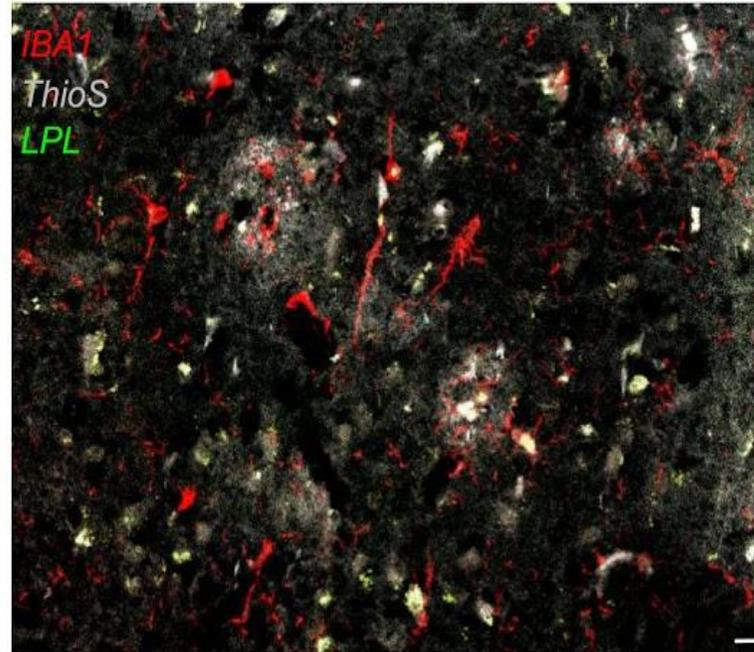
DAM near A β plaques contain high levels of A β particles

Are DAM cells conserved in humans?

human non-AD

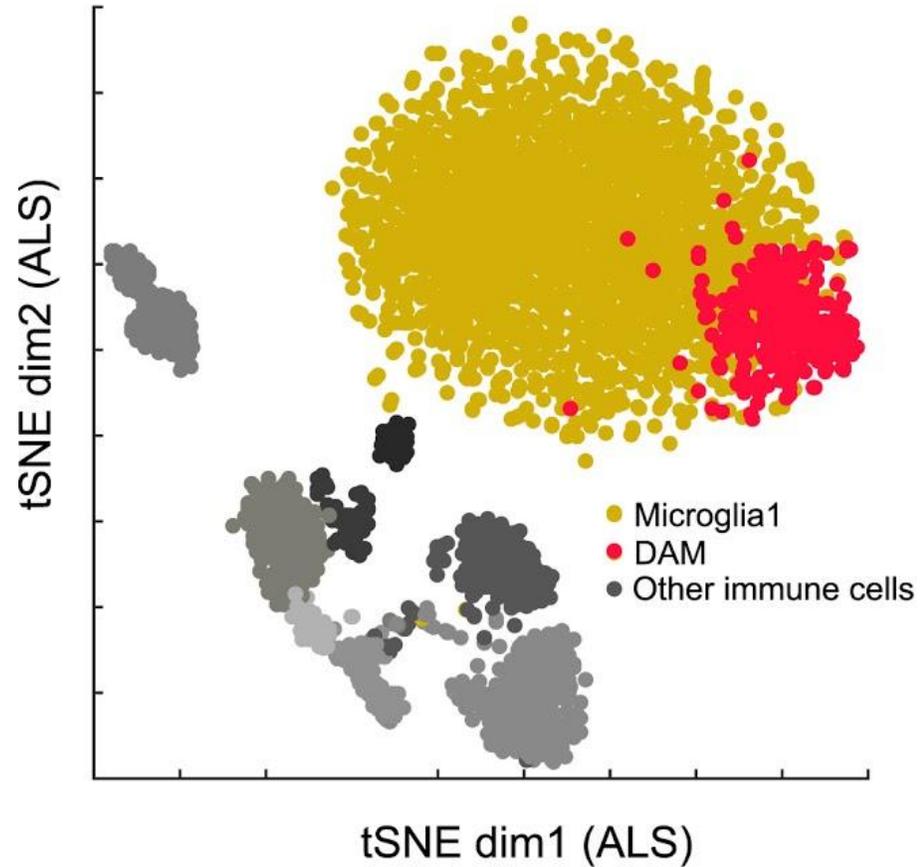


human AD



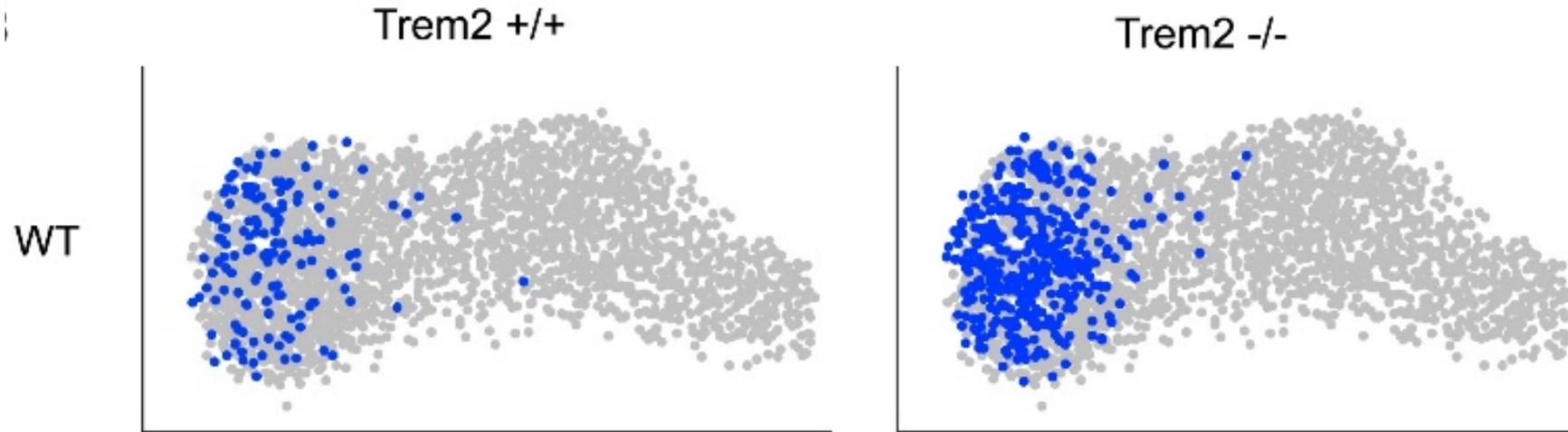
Yes, high Lpl gene expression near Aβ plaques

Are **DAM** cells present in other neurodegenerative diseases?



Yes, distinct group of cells with **DAM** characteristics in ALS

What triggers **DAM** activation?

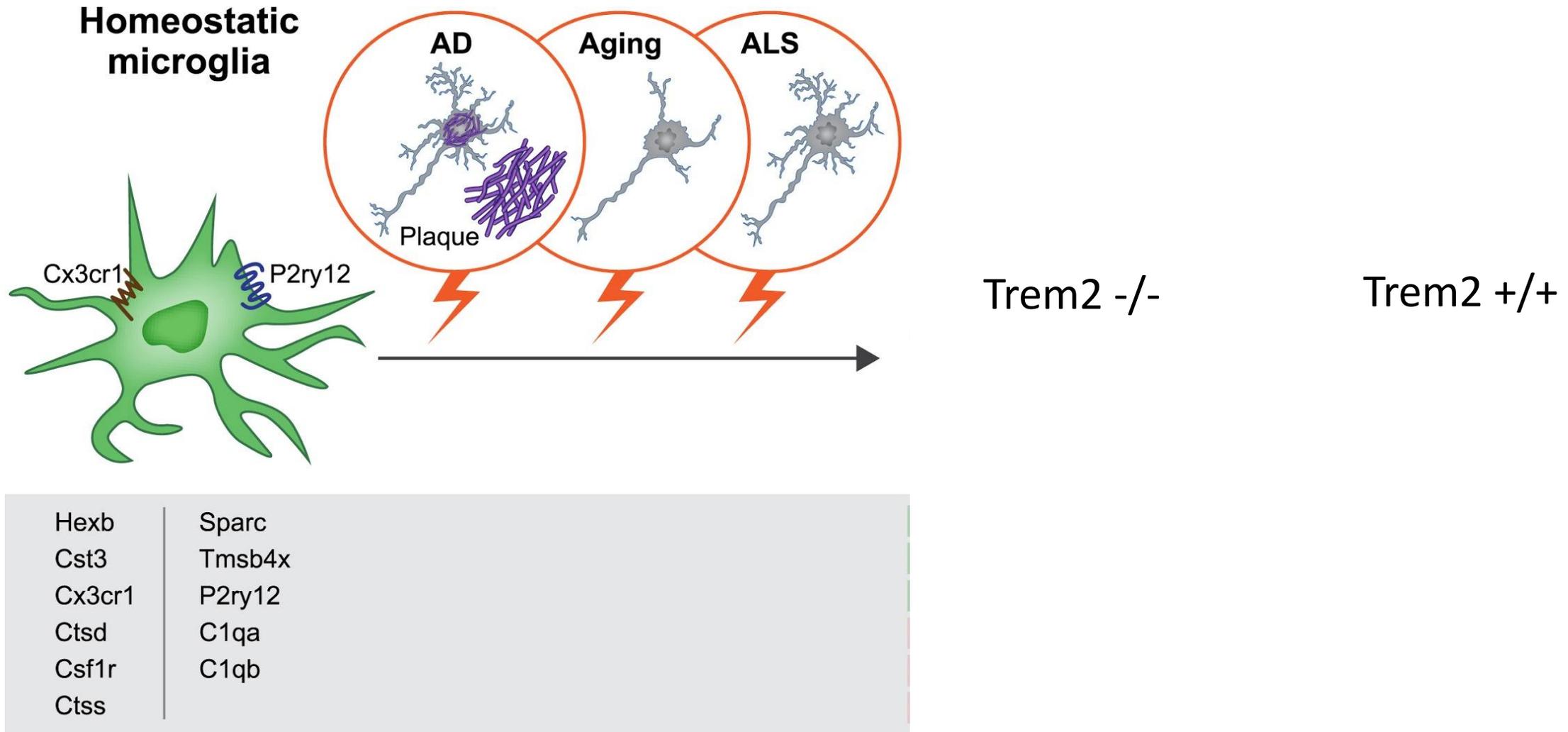


● CD45^{low} CD11b⁺

● CD45^{low} CD11b⁺ CD11c⁺

1. Trem2-independent pathway
2. Trem2-dependent pathway

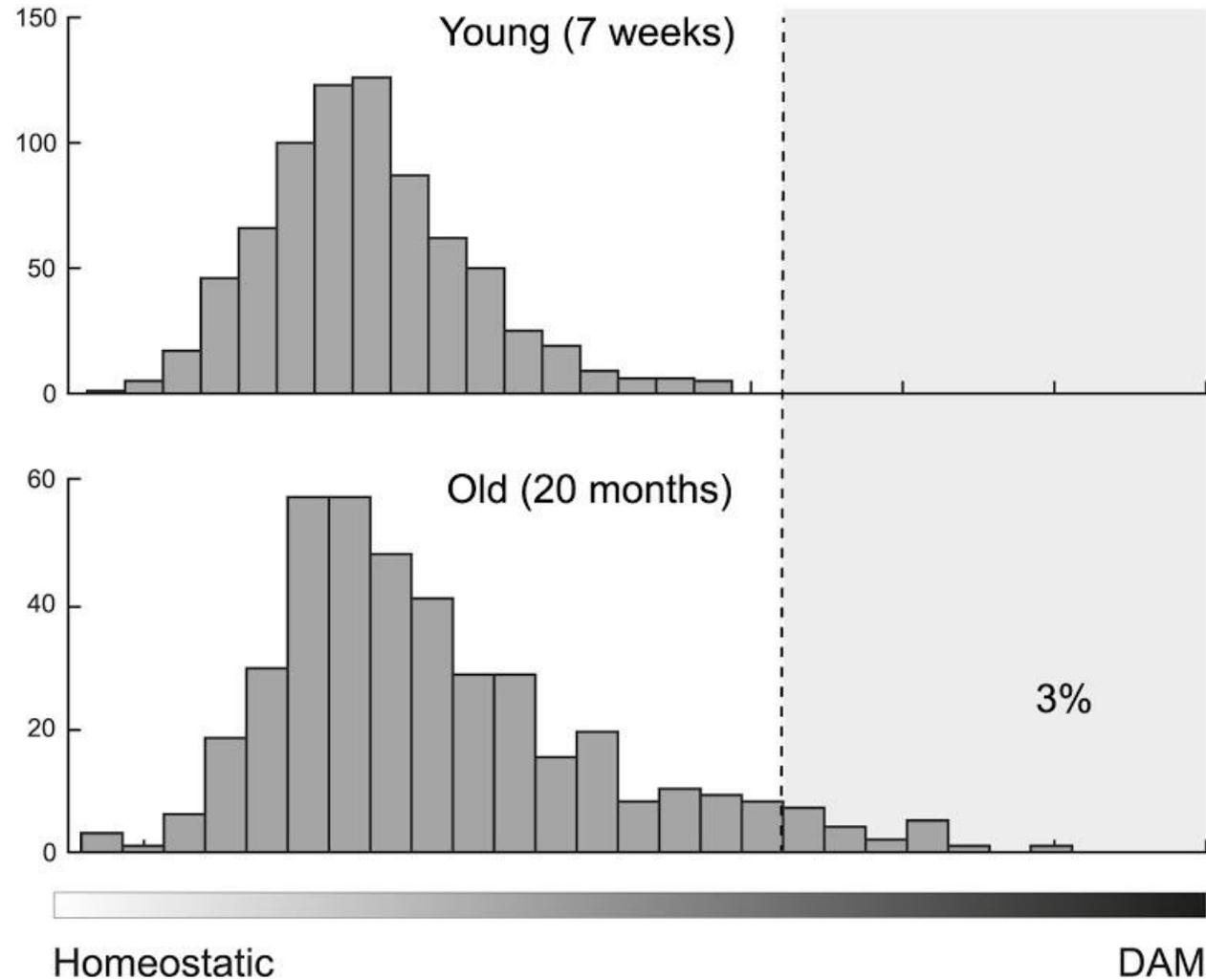
How does gene expression change in each stage?



1. Down-regulation of homeostatic genes

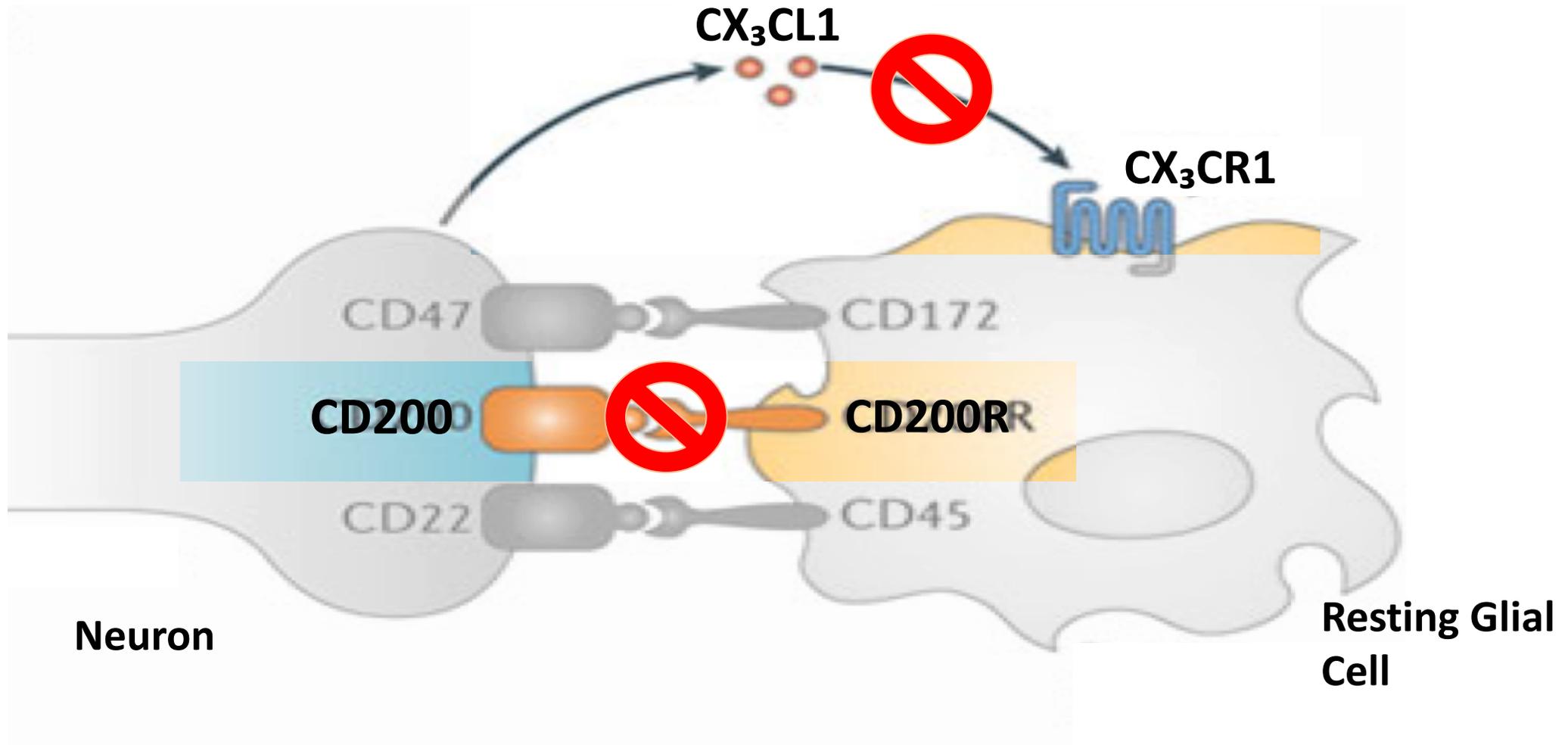
2. Upregulation of phagocytic genes

What are the effects of DAM on Alzheimer's Disease?



DAM mitigates disease through phagocytic activity

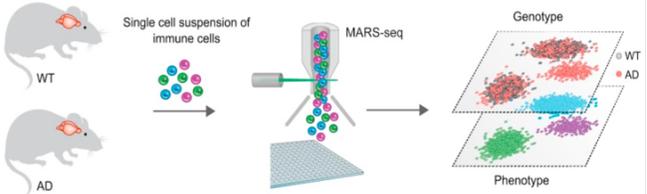
Implications for future neurodegenerative disease treatment?



Targeted blocking of microglia-specific checkpoints

What methods would you use in your own project?

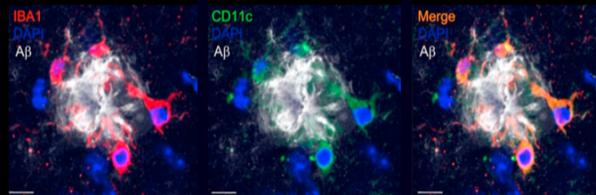
How did the authors characterize the immune cells involved in AD?



Massively Parallel RNA-seq to characterize immune cells

Individual immune cell activity was identified via **RNA-seq**

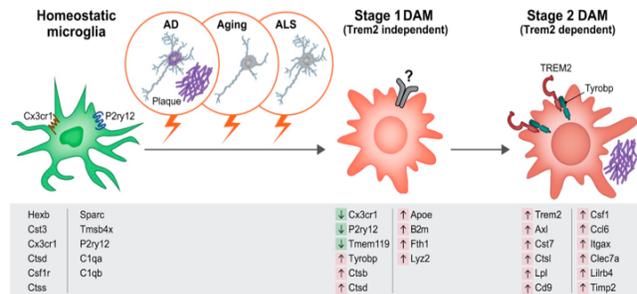
Where in the cortex are the **DAM** located?



Immunostaining validated where the cells are in the brain.

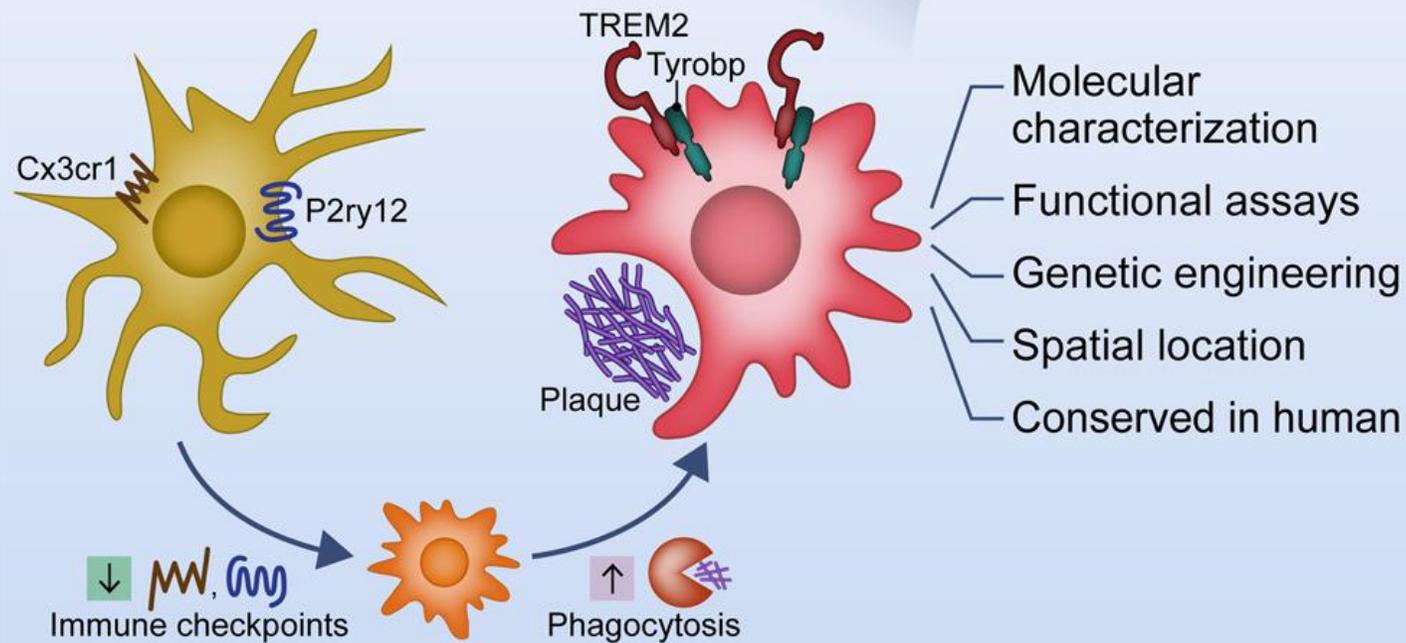
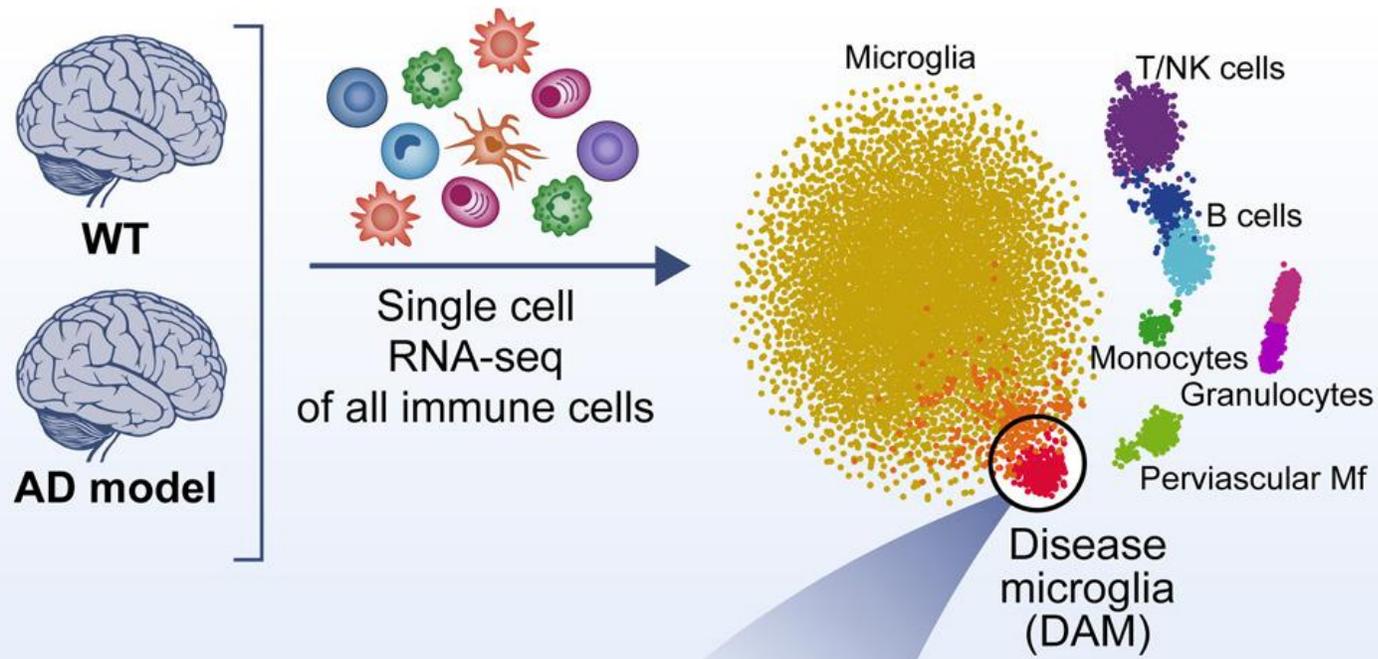
In proximity to Aβ-plaques

What triggers DAM activation?



Comparing **knockout-treatments vs disease-treatments** can determine the pathway of the diseased cell

1. Trem2-independent pathway 2. Trem2-dependent pathway



Will you use Single cell-RNA seq in your own projects?

References

1. <http://jonlieffmd.com/wp-content/uploads/2014/04/bigstock-DNA-cell-chromosome-Illustration-showing-the-forma-32048591.jpg>
2. <https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393>
3. <https://www.intechopen.com/source/html/49419/media/image2.png>
4. <http://www.discoveryandinnovation.com/BIOL202/notes/images/cDNA.jpg>
5. <http://img2-1.timeinc.net/toh/i/g/12/Home-Solutions/05-coins-uses/00-coin-uses.jpg>
6. <http://www.clker.com/cliparts/L/L/F/f/Y/N/eppendorf-open-large-pink-pellet-hi.png>
7. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2949280/bin/nihms229948f2.jpg>
8. <http://bitesizebio.s3.amazonaws.com/content/uploads/2011/07/cDNA-microarray-experiment.jpg>
9. <https://www.intechopen.com/source/html/39517/media/image3.jpeg>
10. <https://info.gbiosciences.com/hs-fs/hub/127518/file-30478736-jpg/images/dna-rna-purification.jpg>
11. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2949280/>
12. <https://production.wordpress.uconn.edu/bioinformatics/wp-content/uploads/sites/15/2013/06/bioinfo3.png>

References (cont.)

13. <http://news.berkeley.edu/2015/07/14/early-human-heart-development-stem-cells-model/>
14. https://www.wur.nl/upload_mm/9/1/e/15dc62dd-461a-4ee4-a304-8d5c1271bcde_shutterstock_52453933_green_genetic_dna_LR_490x330.jpg
15. <http://www.doctortipster.com/wp-content/uploads/2014/11/New-Discovery-of-the-Source-of-Neuron-Death-in-Alzheimers-Disease.jpeg>
16. <https://ghr.nlm.nih.gov/art/large/alzheimer-disease-brain.jpeg>
17. http://www.uwyo.edu/virtual_edge/lab13/images/ascomycota01.jpg
18. https://inbotanicalmood.files.wordpress.com/2014/05/arabidopsis_thaliana_24703_116770.jpg
19. <https://www.sciencenewsforstudents.org/sites/default/files/scald-image/350-inline-1-microglia-iStock-683557250.gif>
20. <http://physiologyplus.com/wp-content/uploads/2017/01/monocytes.png>
21. <https://www.sciencedirect.com/science/article/pii/S0092867417305780?via%3Dihub>
22. <https://www.dreamstime.com/stock-illustration-activation-microglia-neuron-nerve-cell-infographics-vector-illustration-background-image90174529>
23. <http://atvb.ahajournals.org/content/35/4/755>
24. <https://www.ncbi.nlm.nih.gov/pubmed/17504139>
25. <https://i.pinimg.com/originals/28/c8/06/28c8063a0720be1b8e265f8b6b69295d.jpg>
<https://cbd-international.net/effects-cannabis-oil-alzheimers-disease/>
26. <https://www.nature.com/subjects/transcriptomics>
27. <http://ib.bioninja.com.au/standard-level/topic-2-molecular-biology/26-structure-of-dna-and-rna/types-of-rna.html>