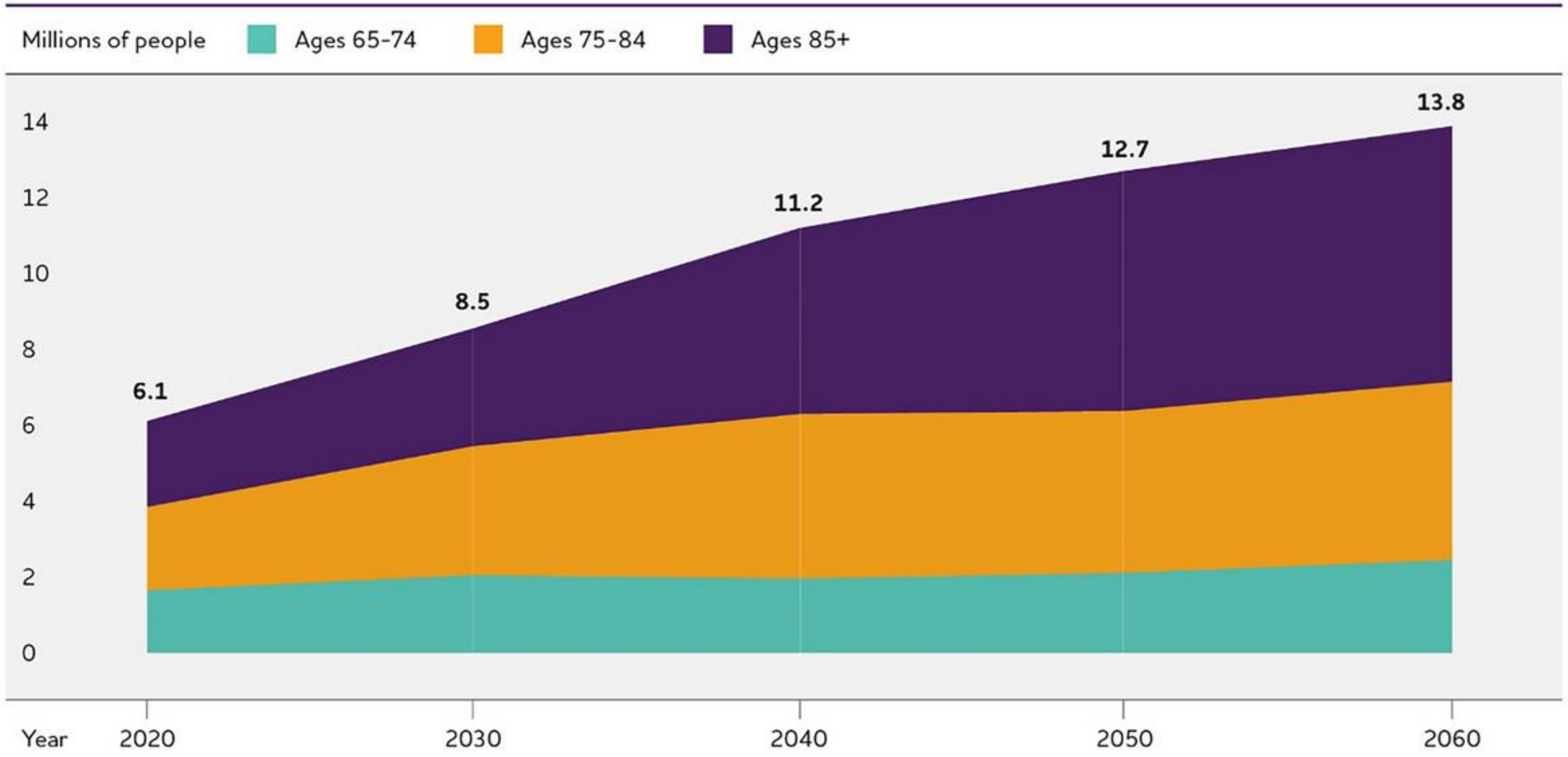
Sex and *APOE* genotype influence microglial gene expression and states in Alzheimer’s disease

Corbin S.C. Johnson[†], Katherine E. Prater[†], Alexandra N. Cochoit, Isa Smith, Michelle Casad, Gala Filippova, Shannon E. Rose, Joel B. Berletch, Christine M. Disteche*, Jessica E. Young*, and Suman Jayadev*

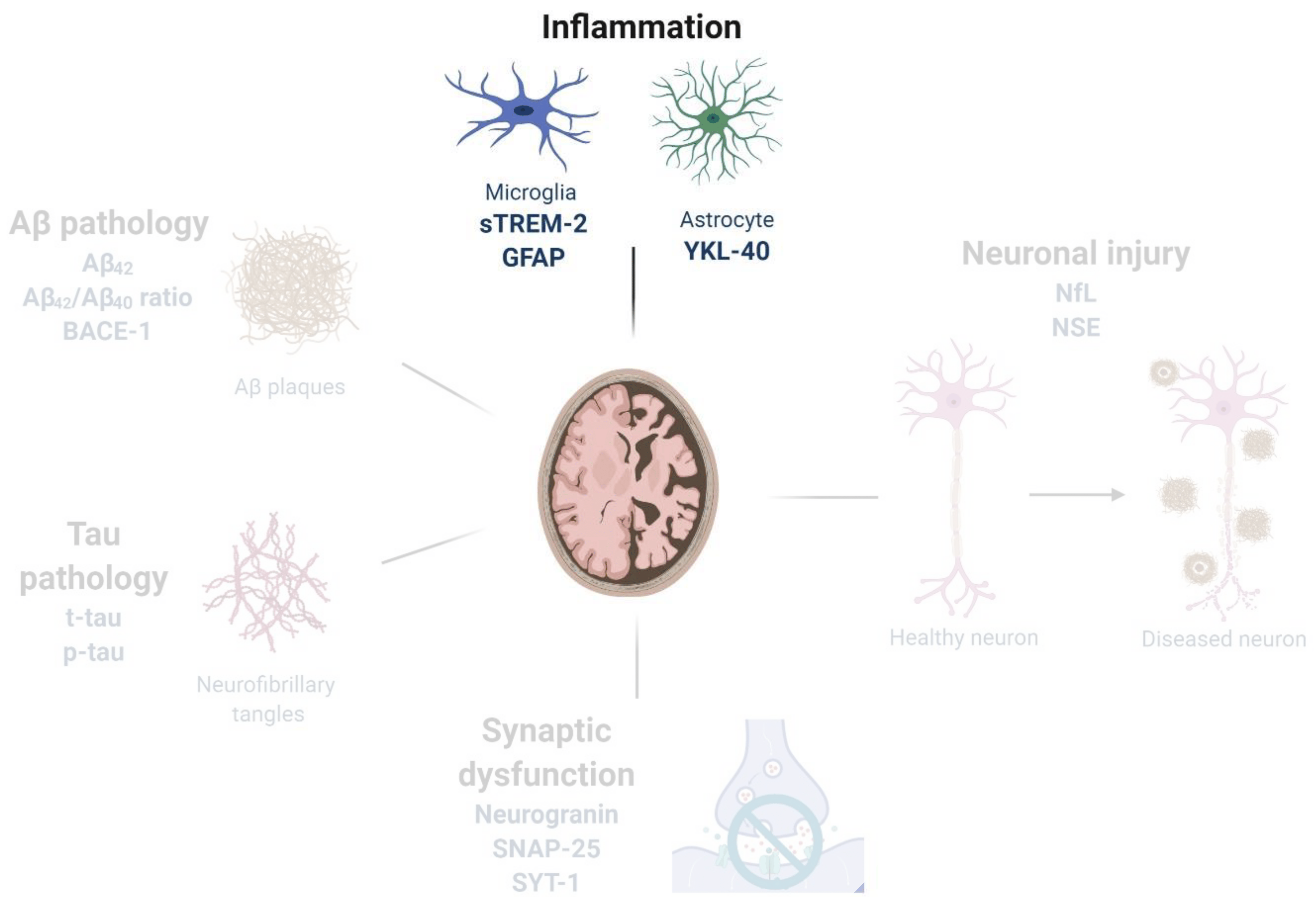
Adult Changes in Thought (ACT) Symposium, 14 May 2025

Alzheimer’s disease (AD) prevalence is increasing

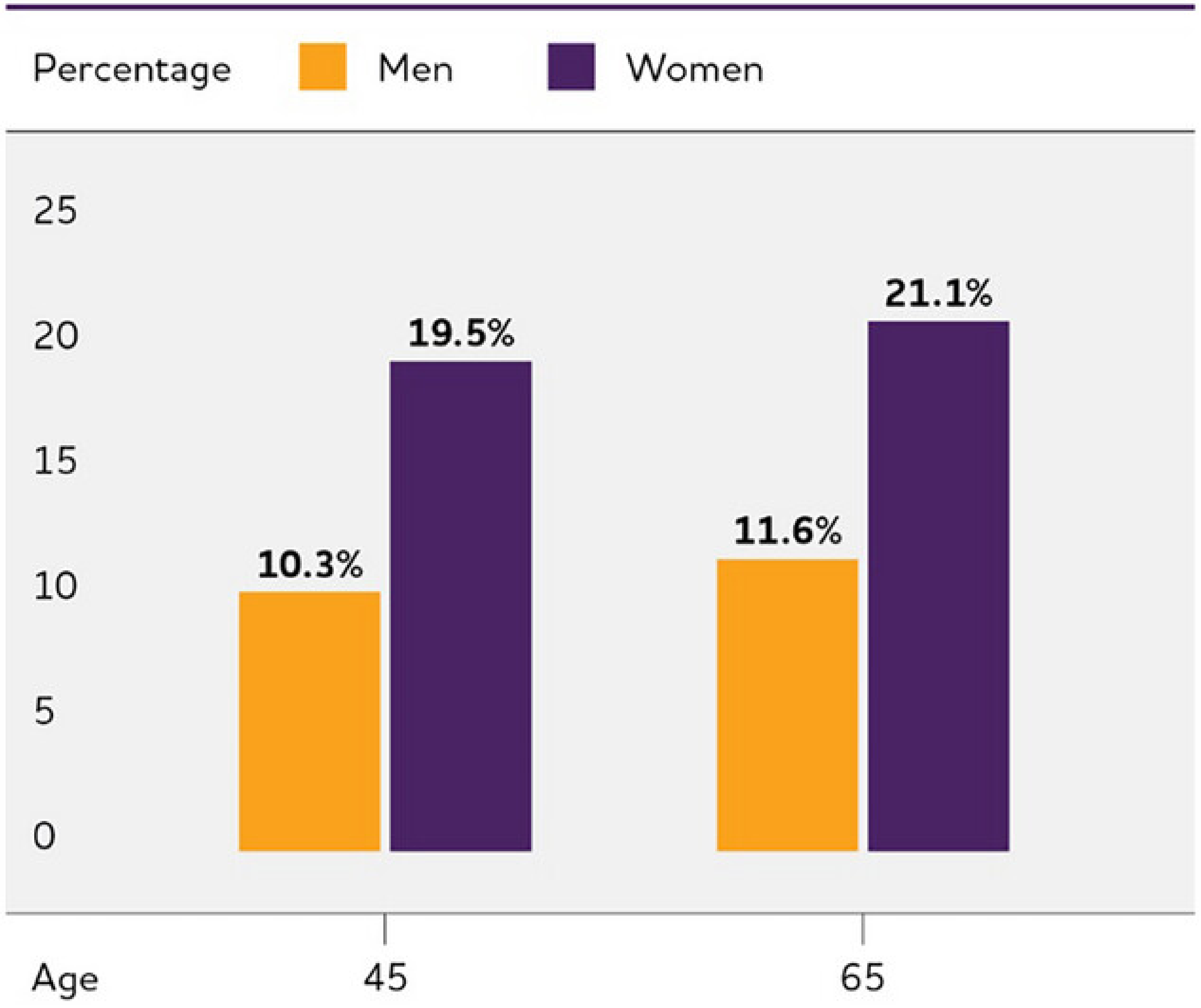


Adapted from Rajan et al. (2021) *Alzheimers Dement.*

Alzheimer's disease pathological hallmarks



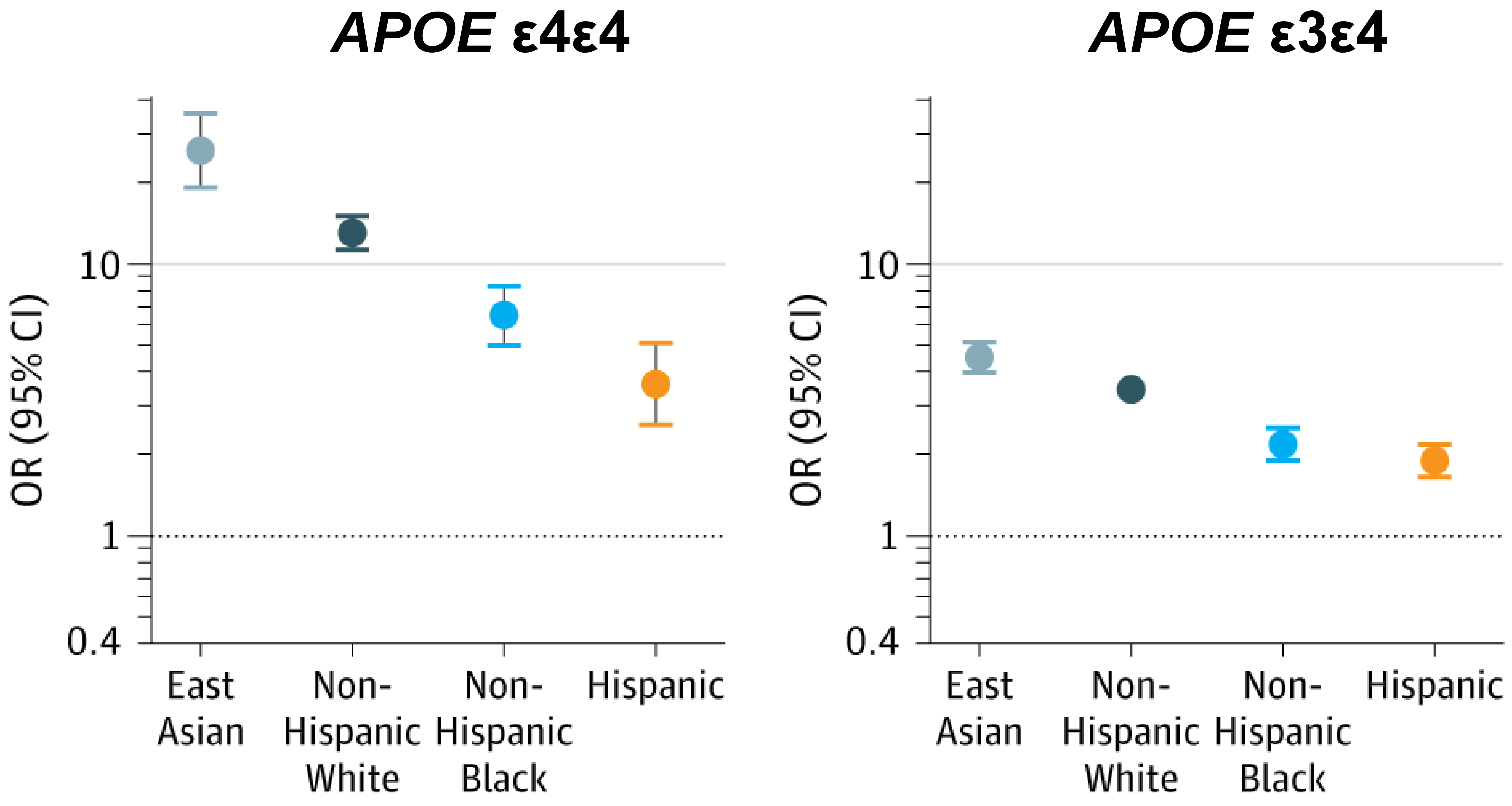
AD risk for women is double



Adapted from Chêne et al. (2015) *Alzheimers Dement.*

APOE genotype alters AD risk

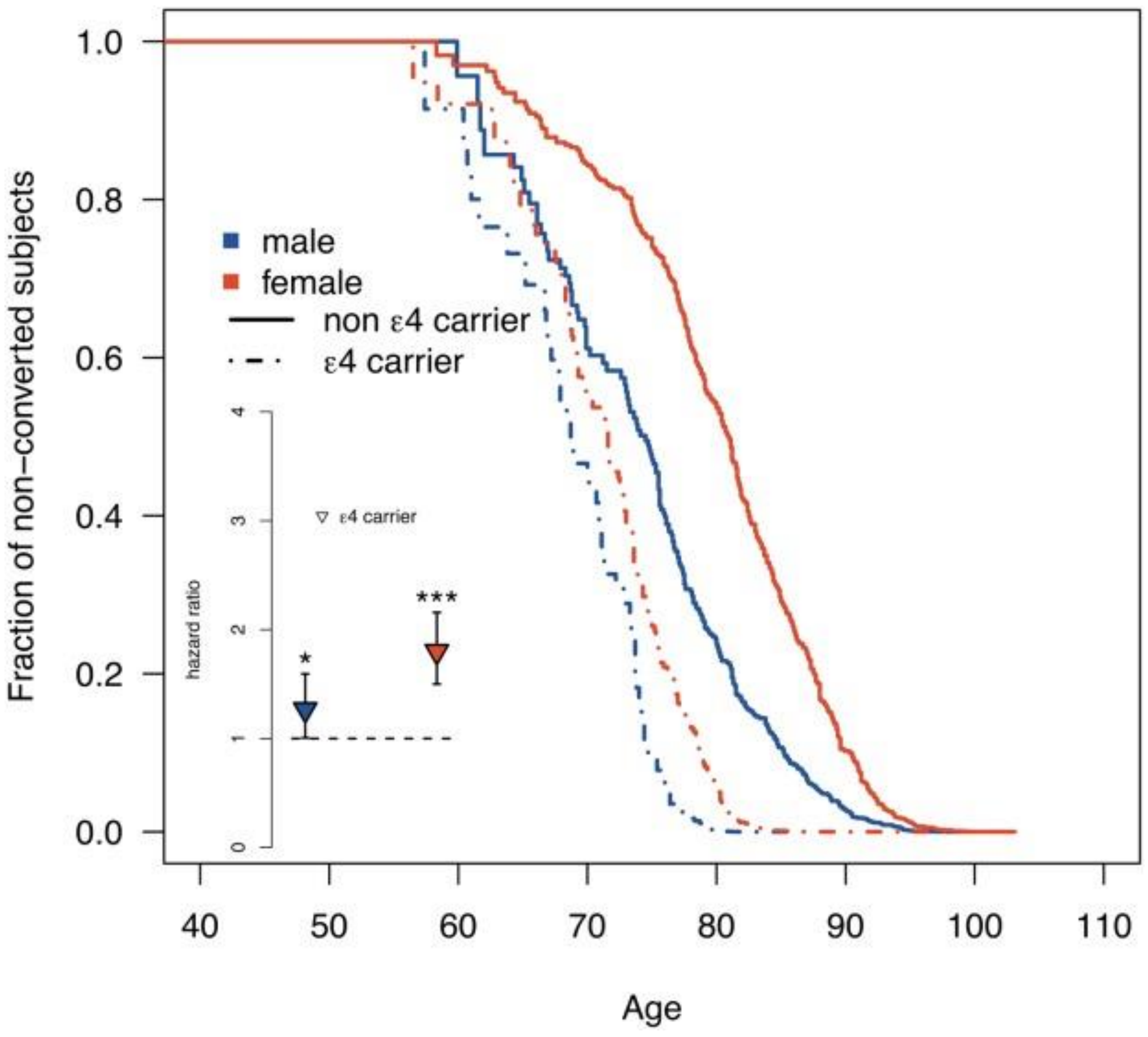
APOE ε4 allele increases AD risk



Belloy et al., (2023) *JAMA Neurol.*

Apolipoprotein E (*APOE*) and Alzheimer's disease

Including interaction between sex and *APOE*



Altmann et al., (2014) *Ann. Neurol.*

Research questions

- How do biological sex and *APOE* genotype alter the function of microglia in individuals with AD?
- How do biological sex and *APOE* genotype alter the morphology (shape) of microglia in individuals with AD?

Research questions

- How do biological sex and *APOE* genotype alter the function of microglia in individuals with AD?

Katherine Prater, PhD

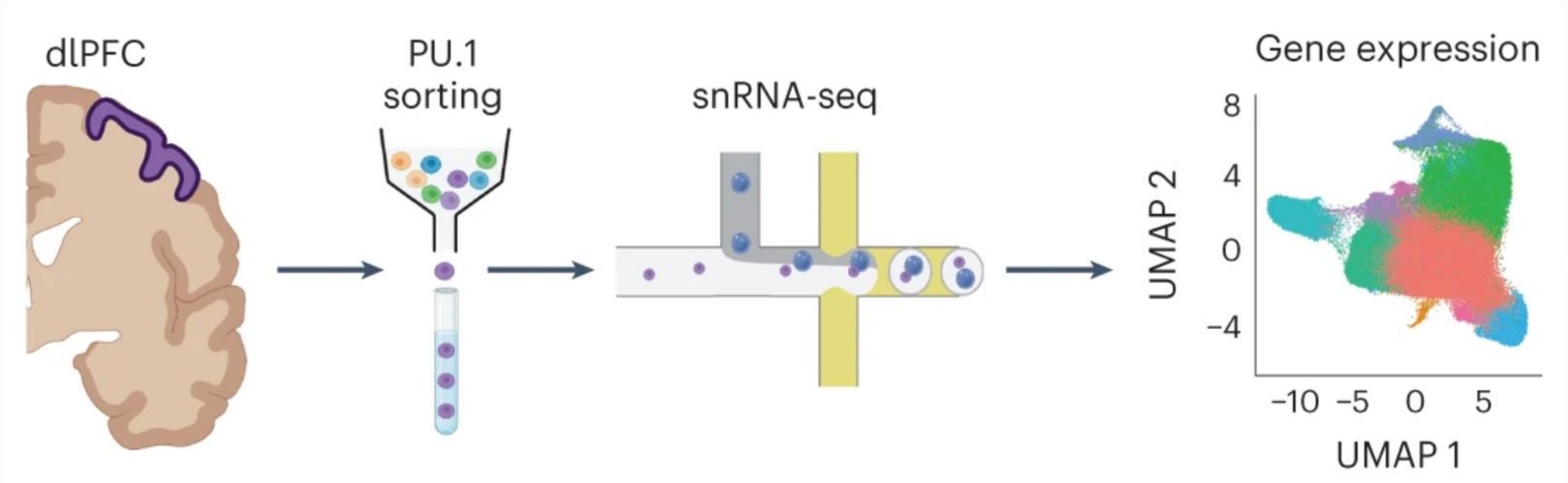
Lexi Cochoit

Isa Smith

Donor demographics

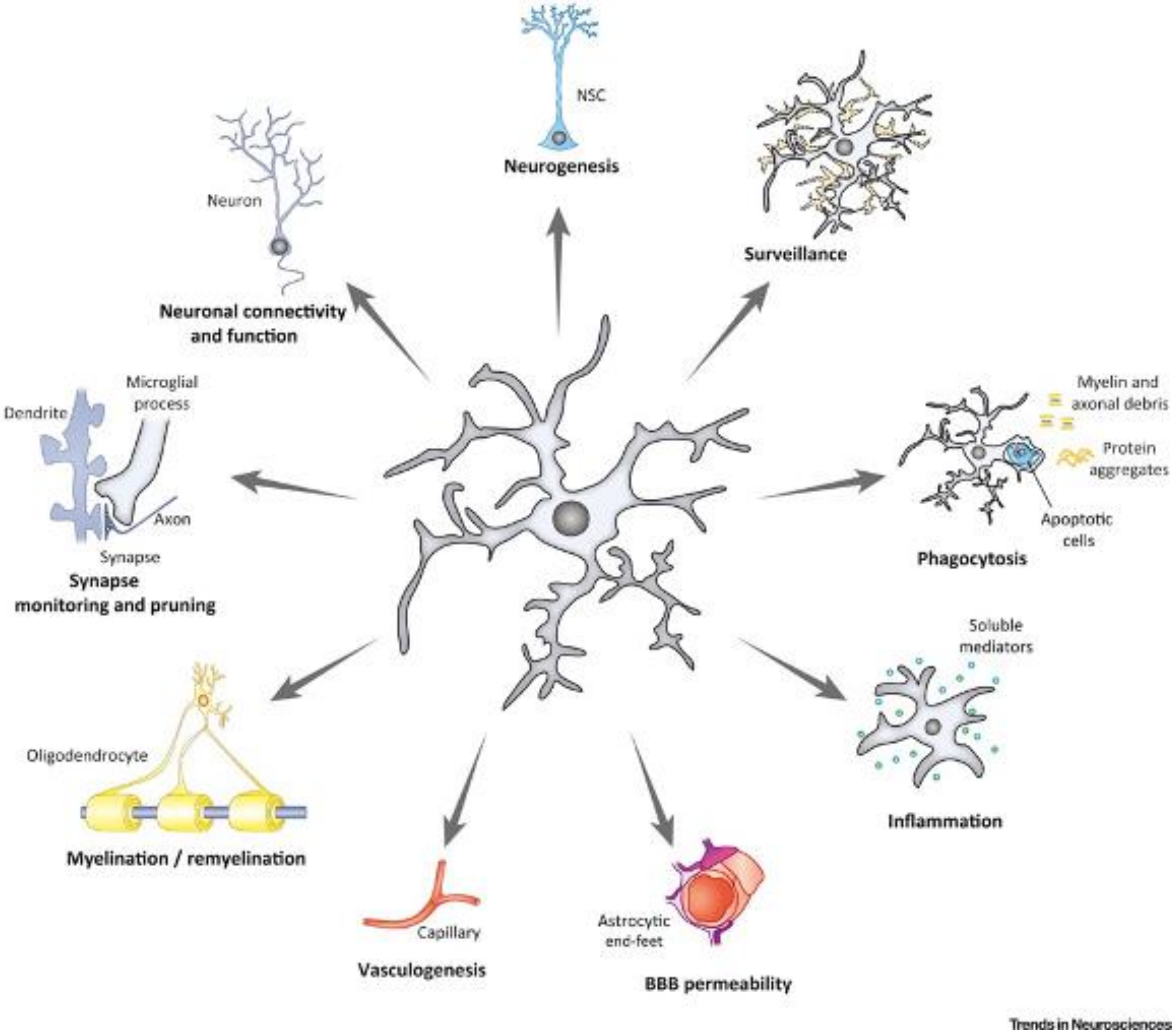
Sex	*APOE* Genotype	n	In ACT Study	ADNC (s.d.)	Age at Death (s.d.)	Post-mortem Interval (s.d.)
Female	ε3ε3	23	20	2.4 (0.5)	93.1 (6.2)	6.0 (1.8)
	ε3ε4	18	7	2.8 (0.4)	85.9 (9.4)	6.3 (2.7)
	ε4ε4	5	1	3 (0)	76.4 (9.2)	6.0 (3.4)
Male	ε3ε3	15	12	2.6 (0.5)	91.6 (5.9)	6.0 (2.8)
	ε3ε4	17	9	2.5 (0.5)	81.0 (10.5)	5.5 (2.0)
	ε4ε4	3	0	2.7 (0.6)	74.7 (0.6)	5.9 (2.6)
Total		81	49	2.6 (0.5)	87.0 (9.9)	6.0 (2.3)

Single nucleus RNA sequencing

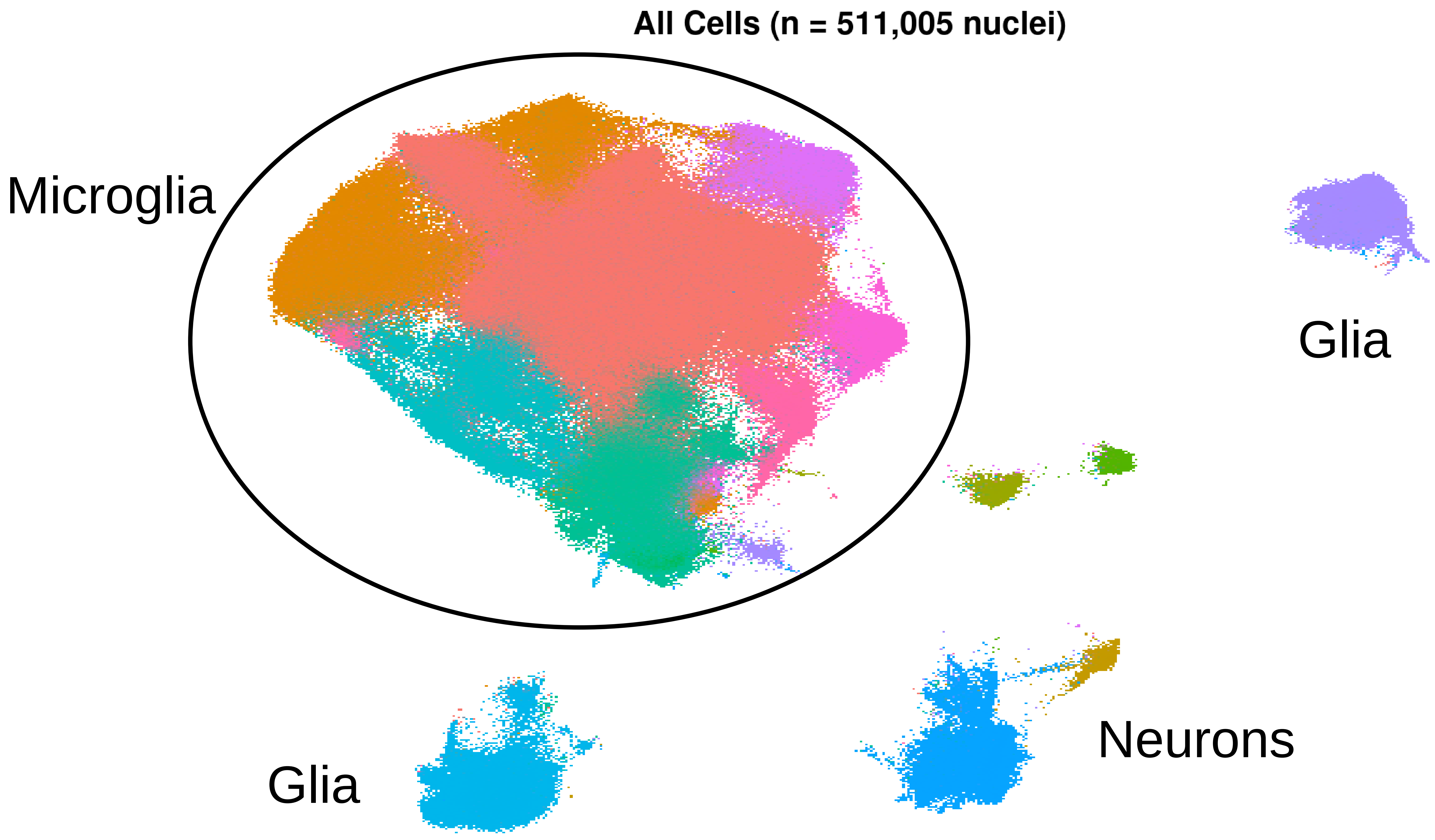


Prater, et al., (2023) *Nature Aging*

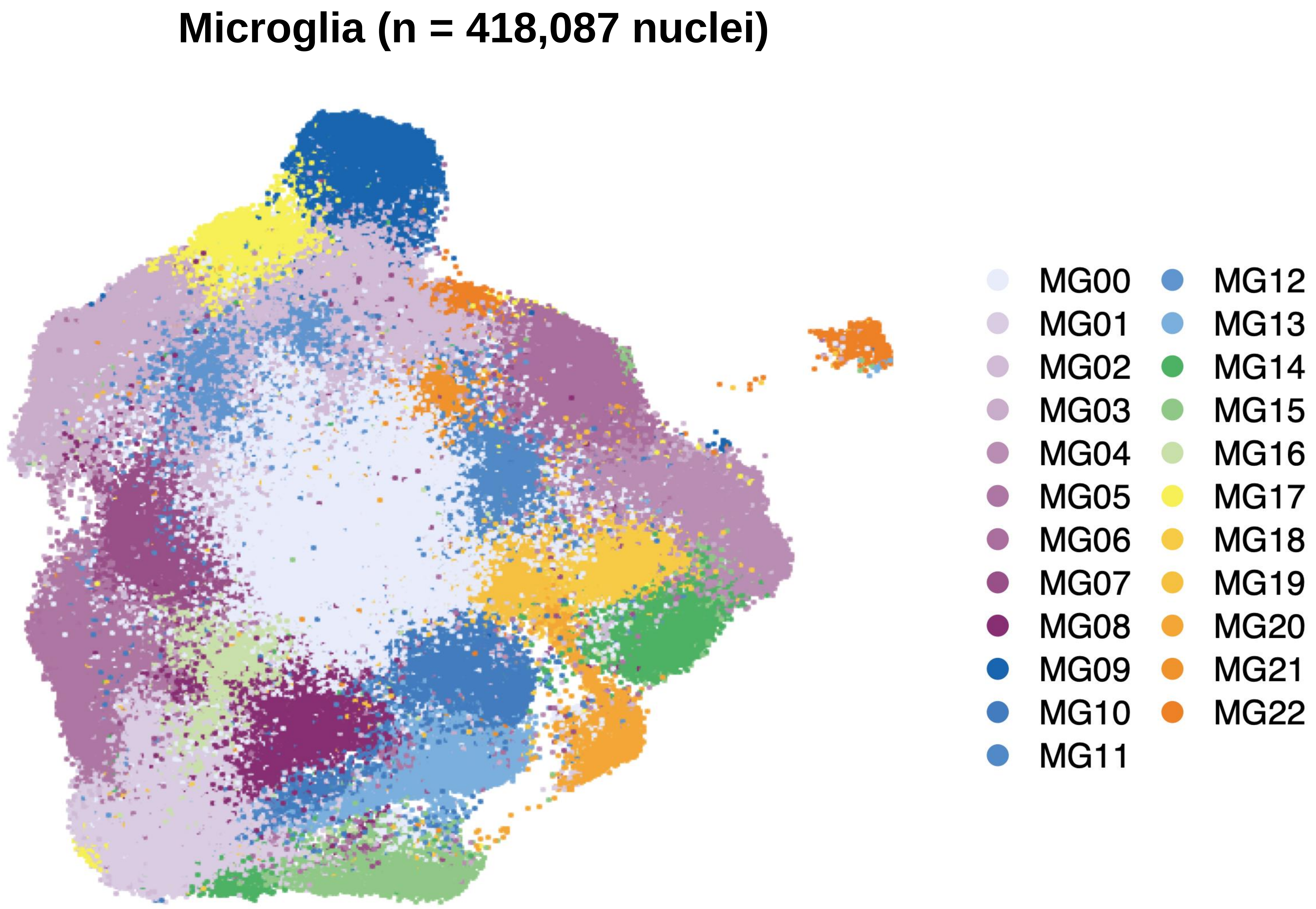
Diversity of microglial functions



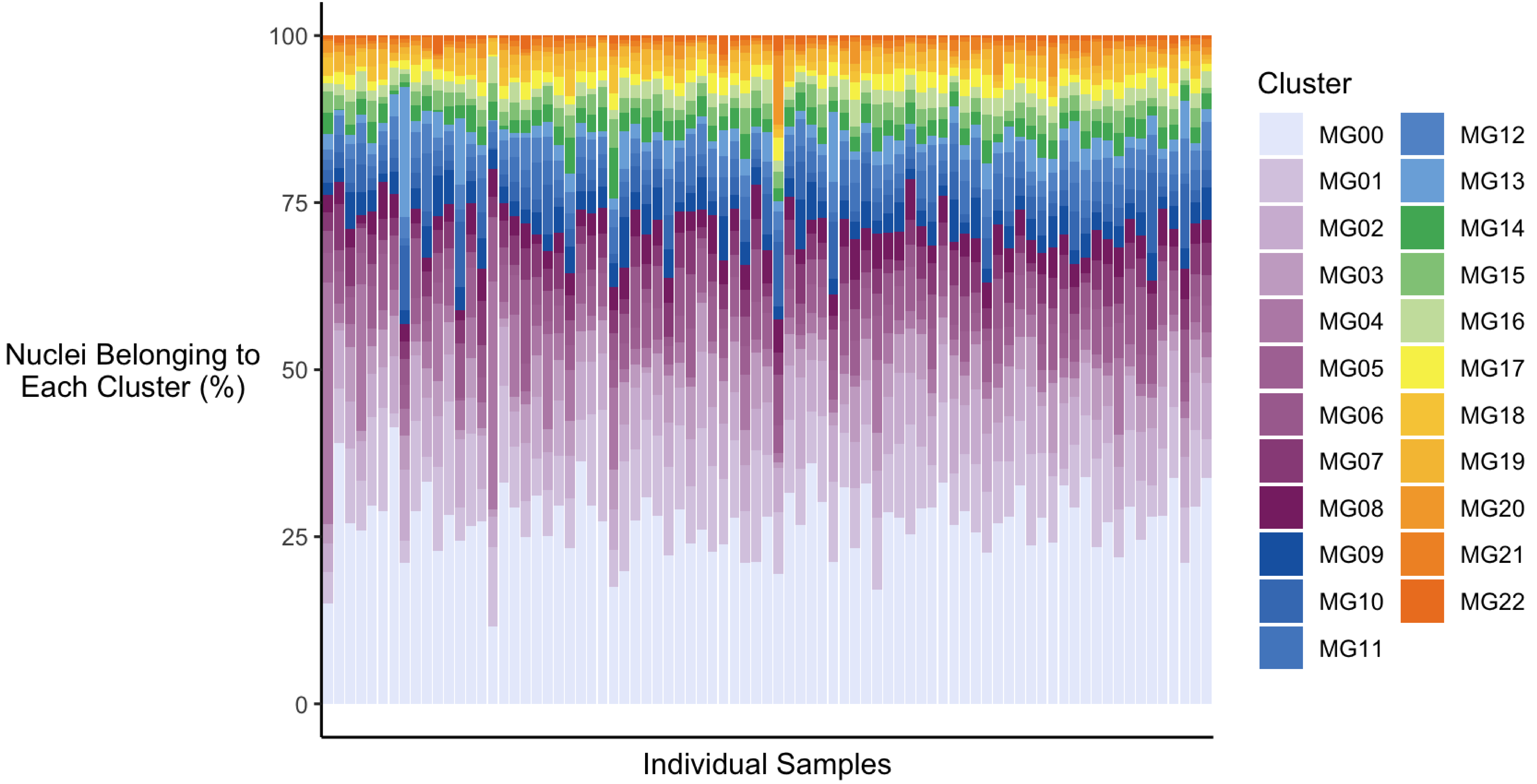
Clustering nuclei



Sub-clustering microglia

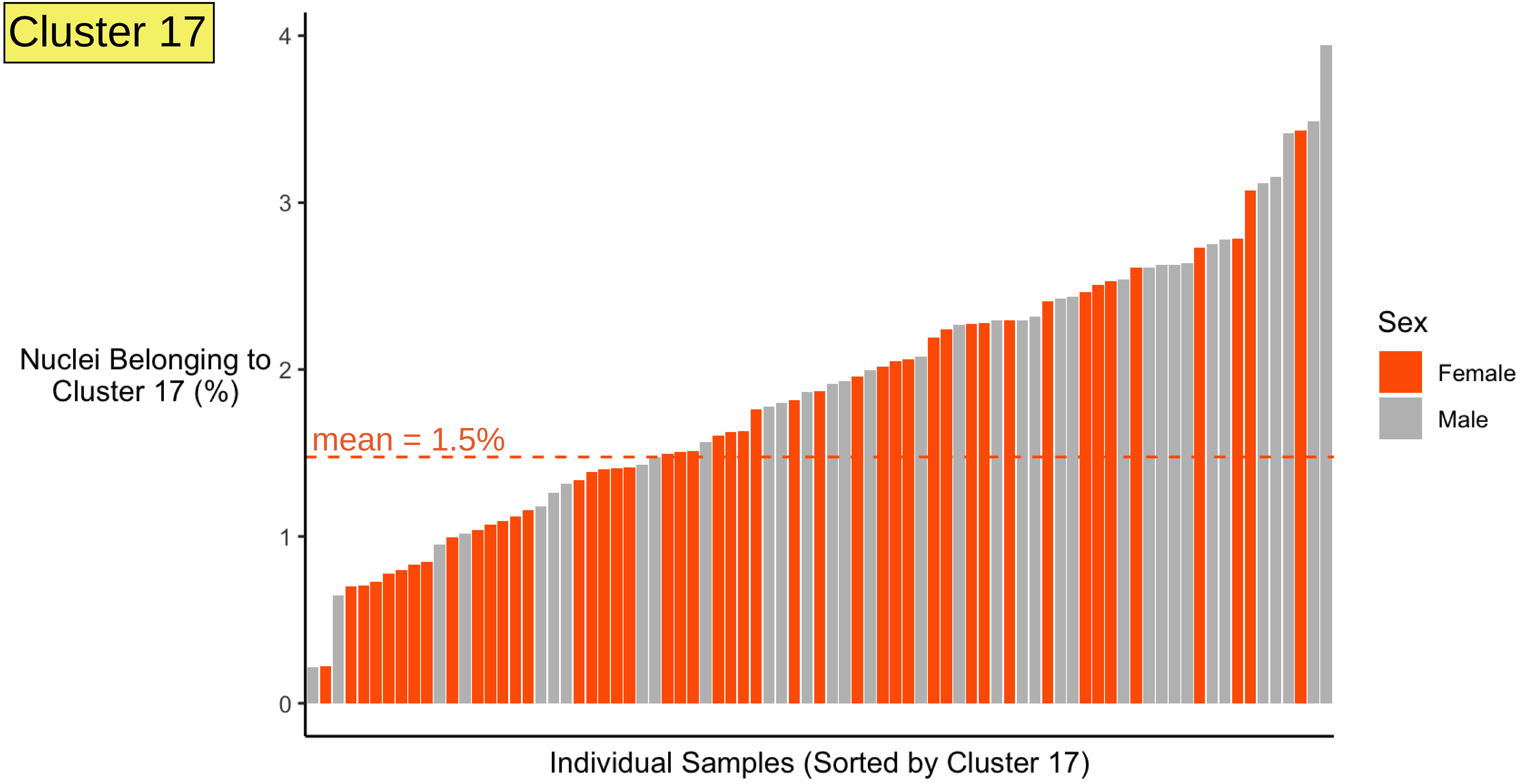


Micgroglial composition by individual



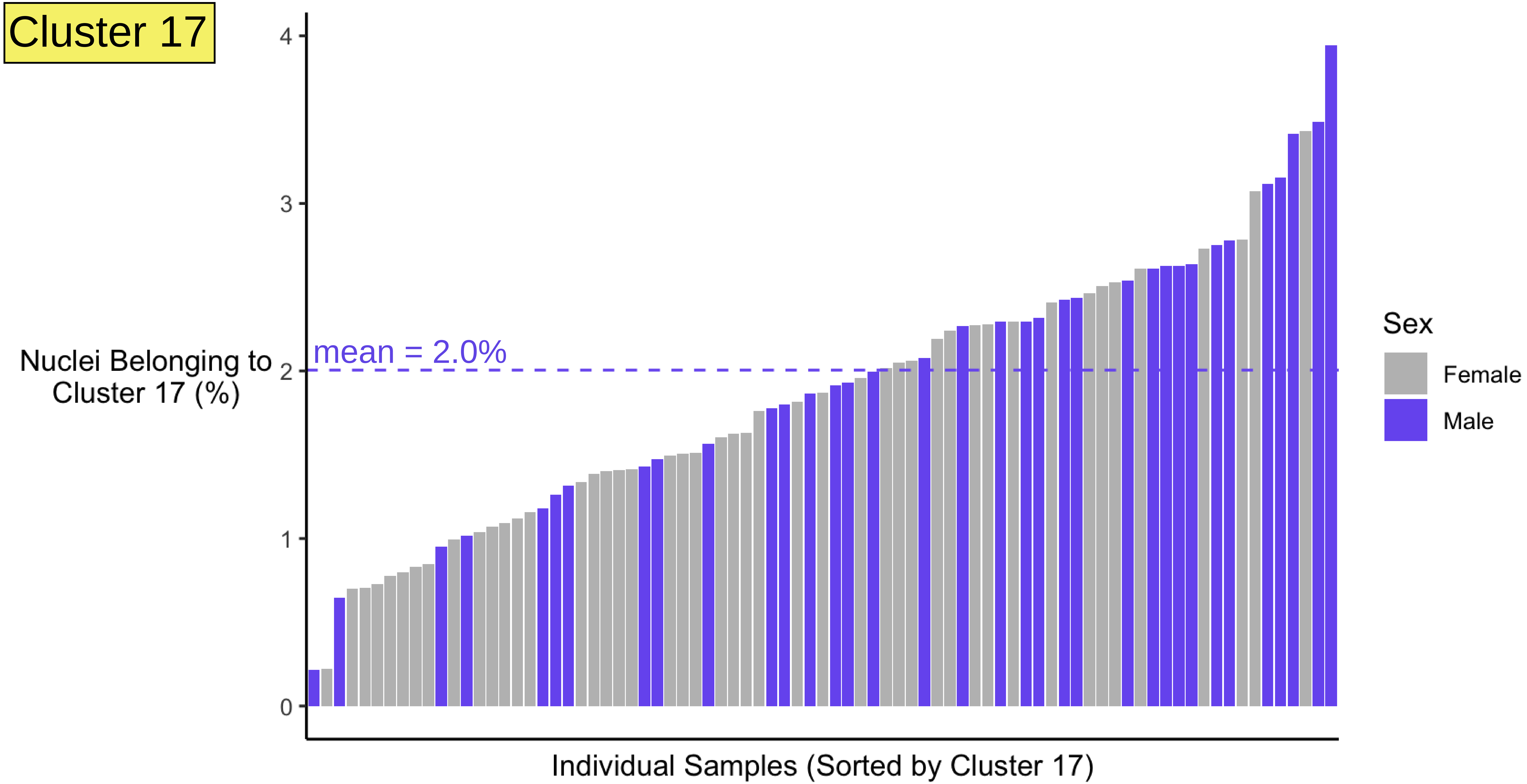
Differential composition of microglial states

Cluster 17

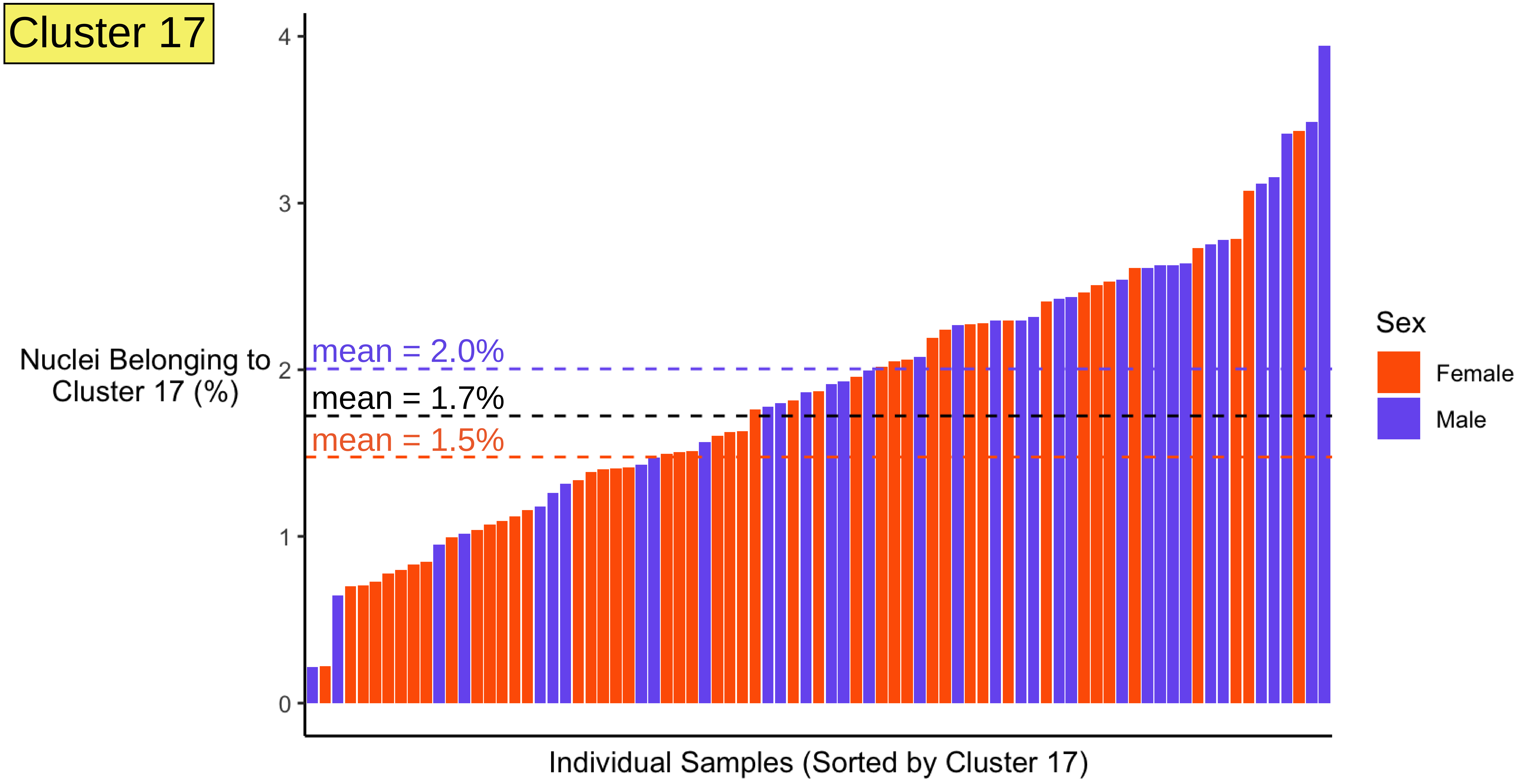


Differential composition of microglial states

Cluster 17



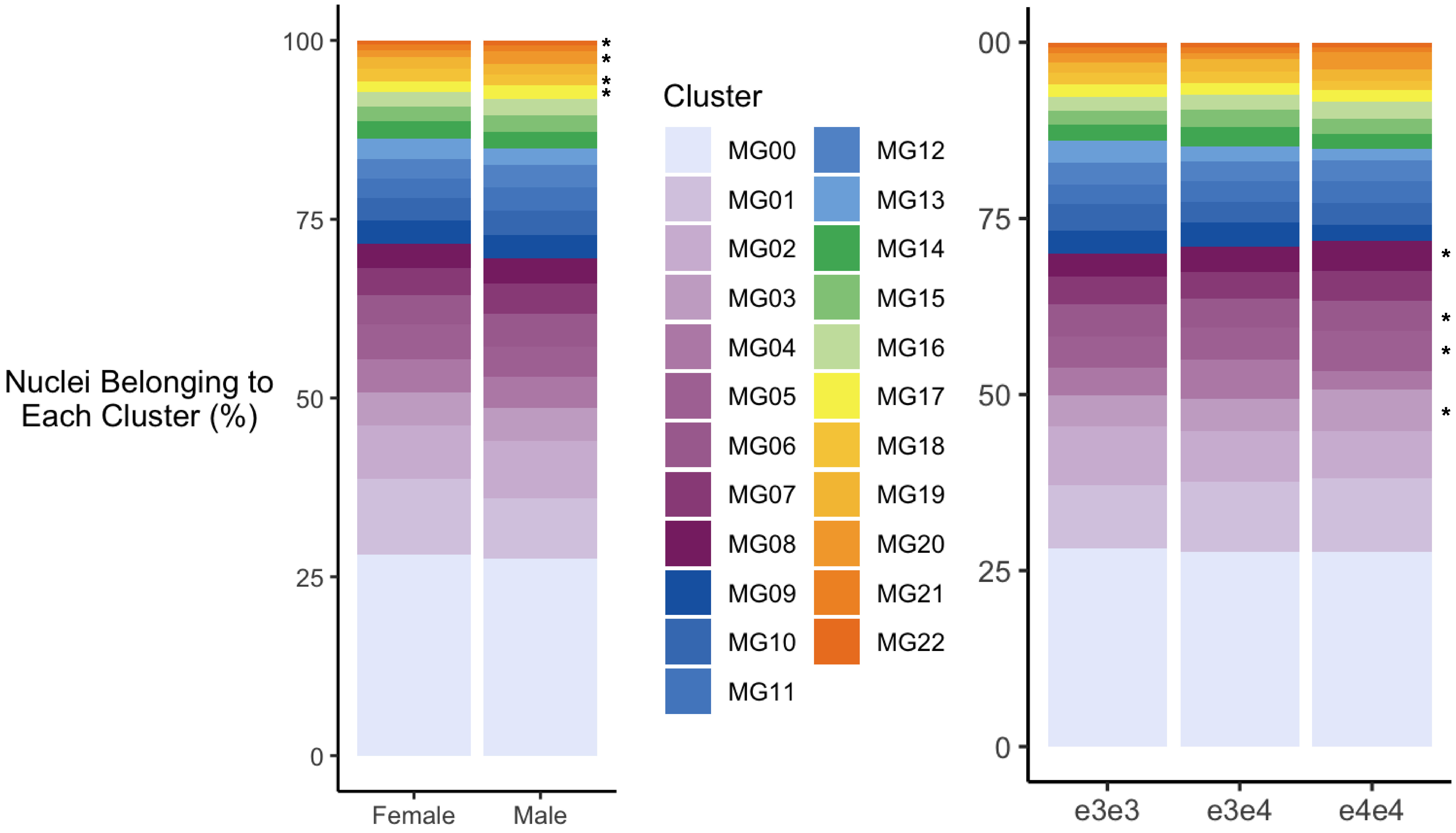
Differential composition of microglial states



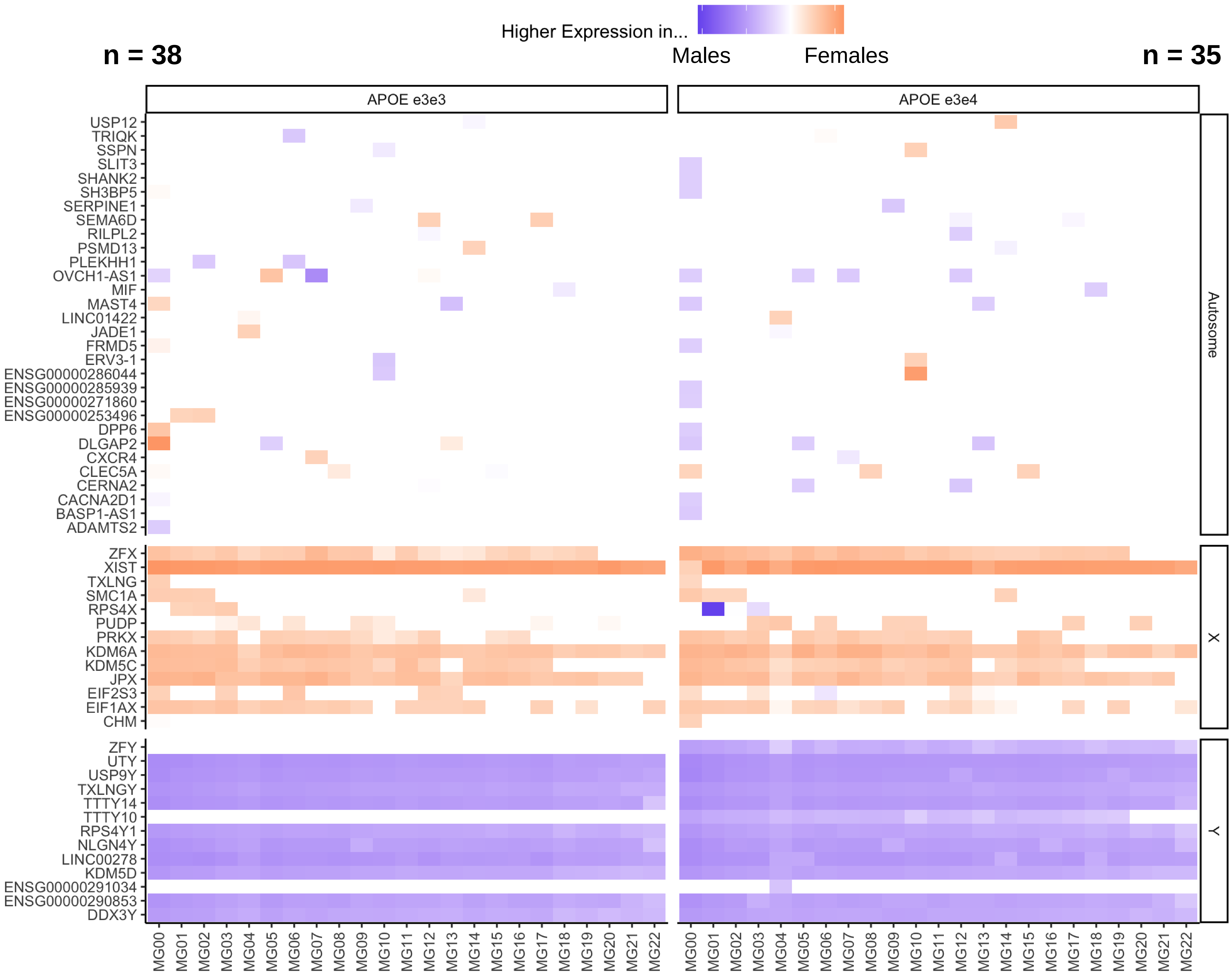
Differential composition of microglial states

percent of sample belonging to cluster ~ *sex* + APOE *genotype* + *sex:*APOE *genotype*

Differential composition of microglial states

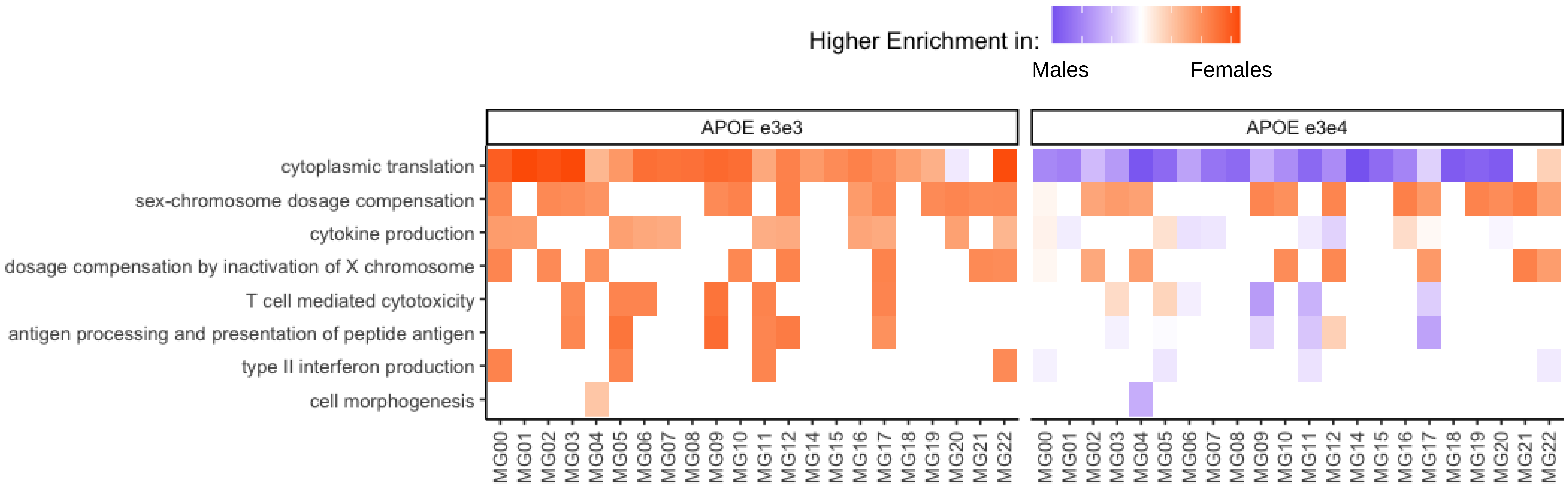


Sex influences expression of 56 genes across clusters



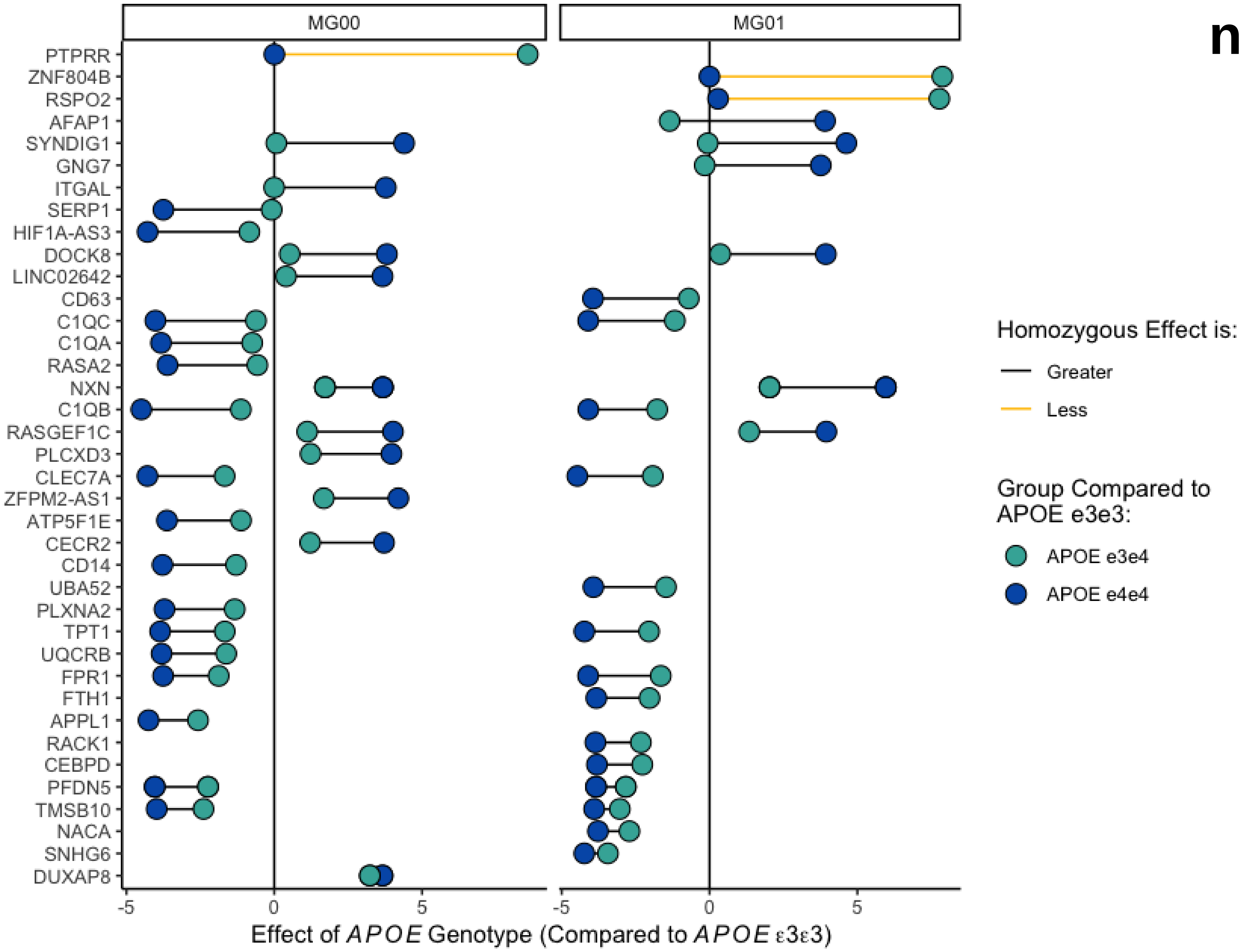
Immune function impacted by sex

Possible evidence of *APOE* genotype interaction



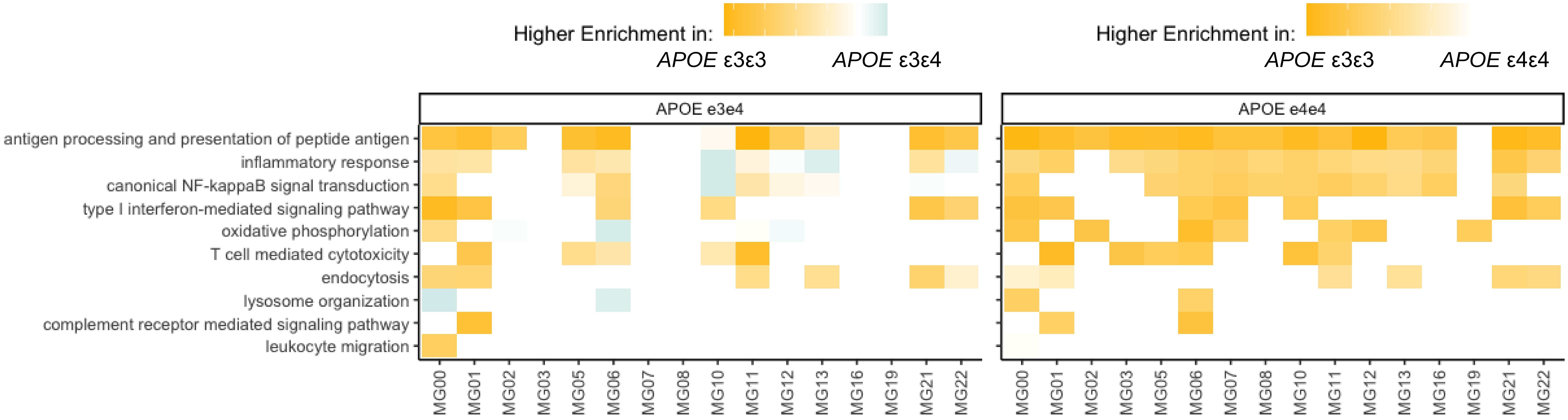
APOE ε4ε4 more extreme in most genes

n = 46 Females



Immune function damped in *APOE* ε4 carriers

Effect pronounced in *APOE* ε4ε4 homozygotes



Summary from genomic analyses

- How do biological sex and *APOE* genotype alter the function of microglia in individuals with AD?
  - Sex and *APOE* genotype both impact composition of microglial states
  - Expression of genes involved in key immune functions broadly impacted, but some microglial states may be more impacted than others
  - Demonstrated interaction between *APOE* genotype and sex, exacerbated in *APOE* ε4ε4 homozygotes

Future directions

Bioinformatic approaches

- Characterize microglial states
- Explore gene regulatory networks that may be driving gene expression differences in microglia
- Analyze gene expression in neurons and other glial cell populations
- Analyze sex differences in the healthy aging individuals in our database

Research questions

- How do biological sex and *APOE* genotype alter the function of microglia in individuals with AD?
- How do biological sex and *APOE* genotype alter the morphology (shape) of microglia in individuals with AD?


Vanessa Souders


Nick Karagas, MD, PhD


Isa Smith


Mason Pirner


Rachel Blaine

Undergraduate Research Symposium

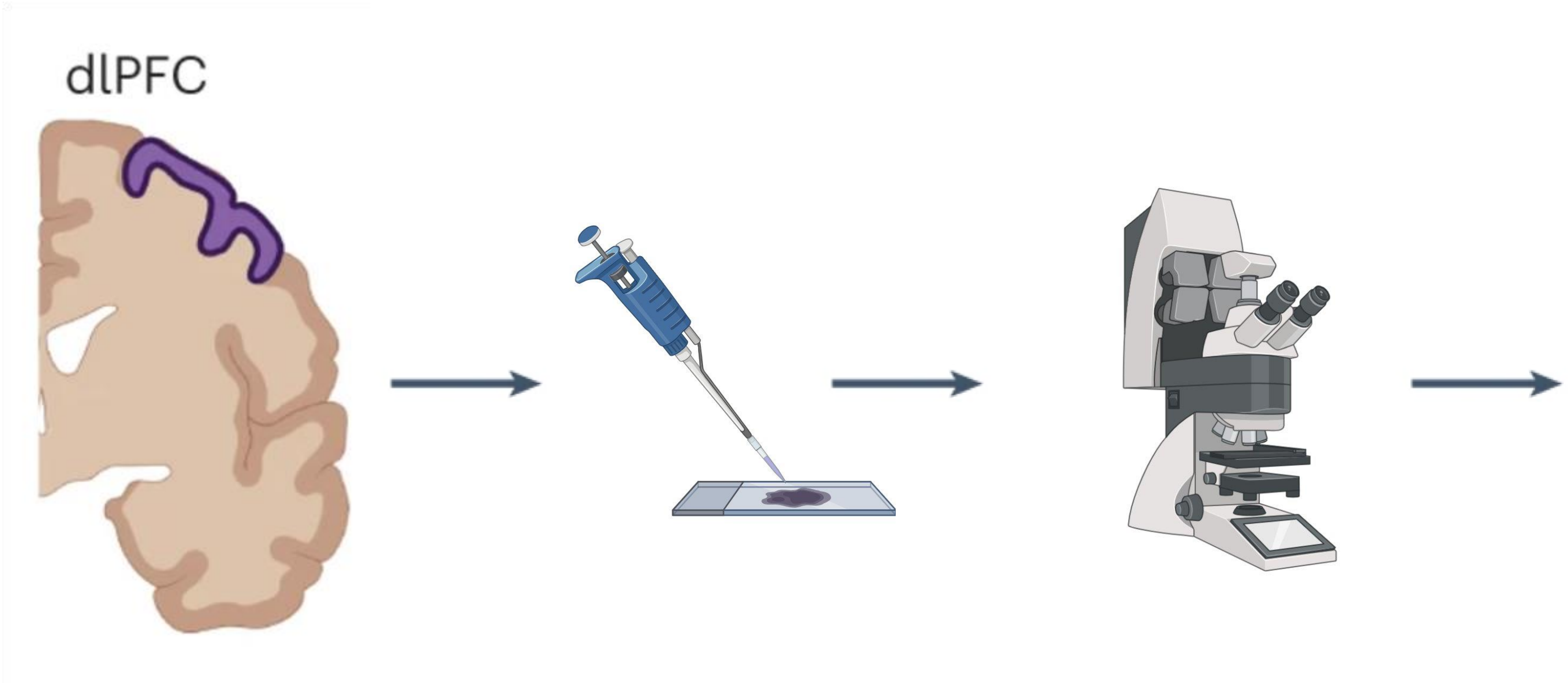
Friday, May 16, 12:30-1:30 PM, Mary Gates Hall

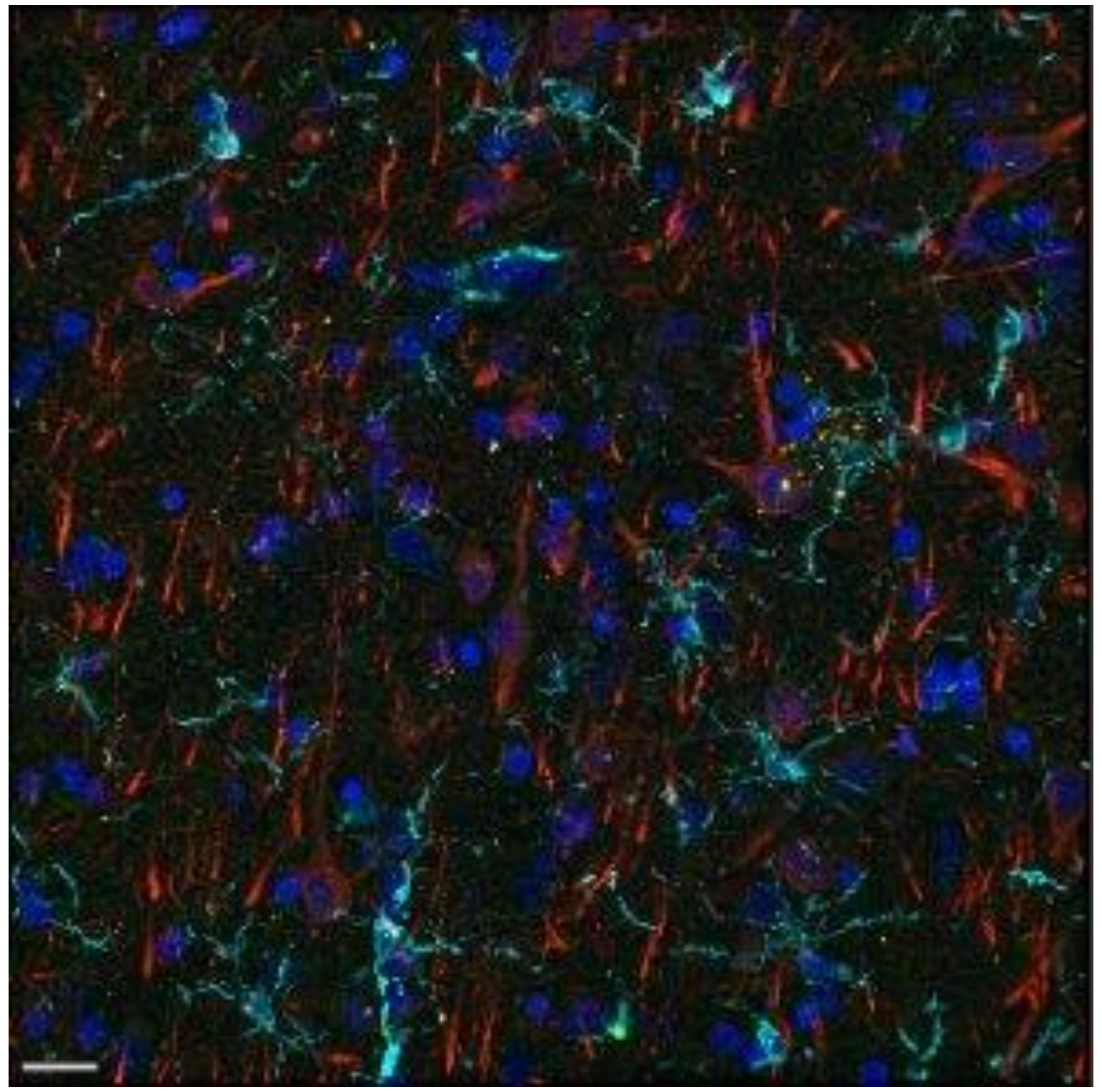
Vanessa Souders

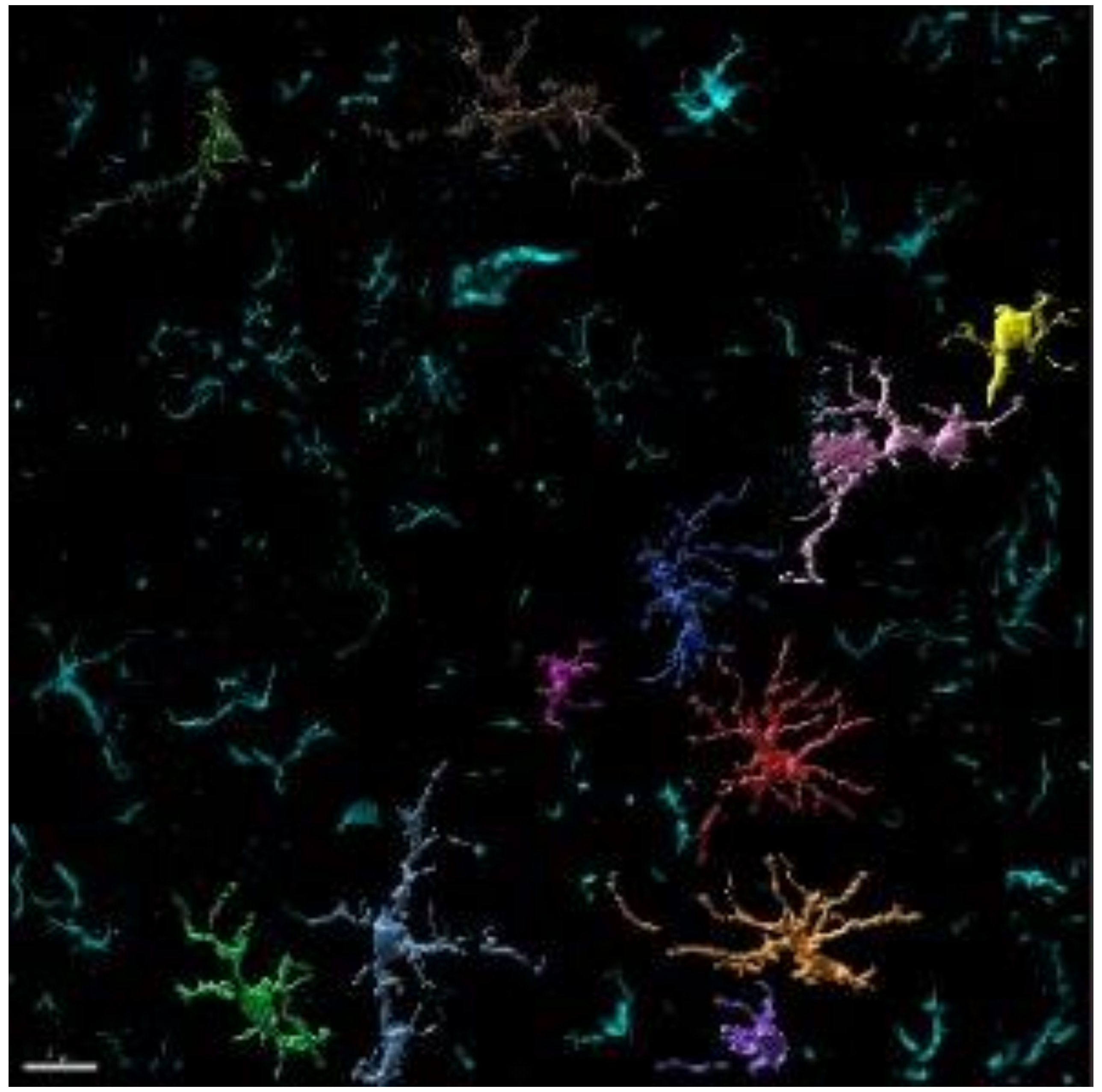
MARY GATES ENDOWMENT FOR STUDENTS

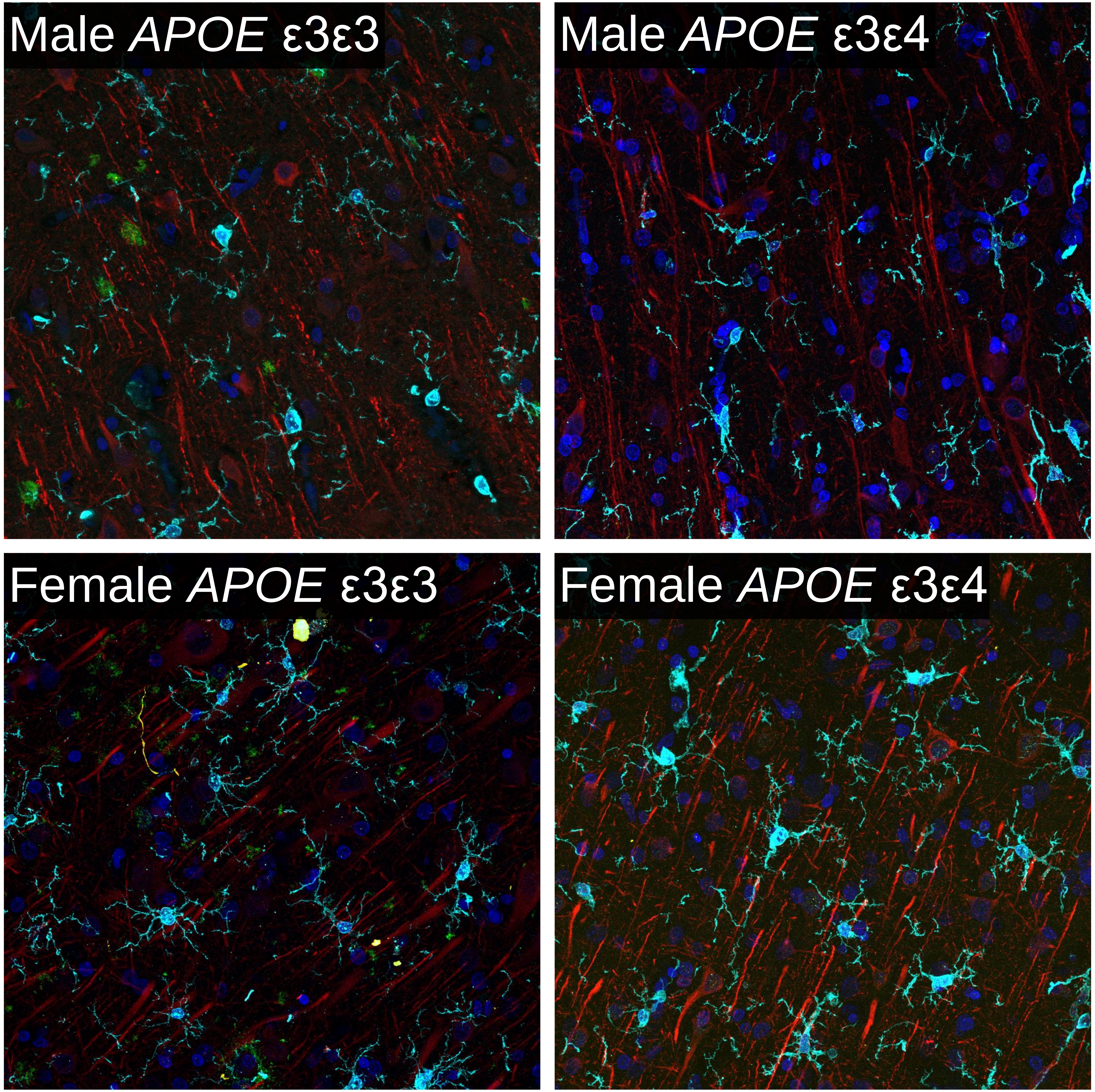
UNIVERSITY *of* WASHINGTON
Undergraduate Academic Affairs

Immunohistochemistry









IHC Staining

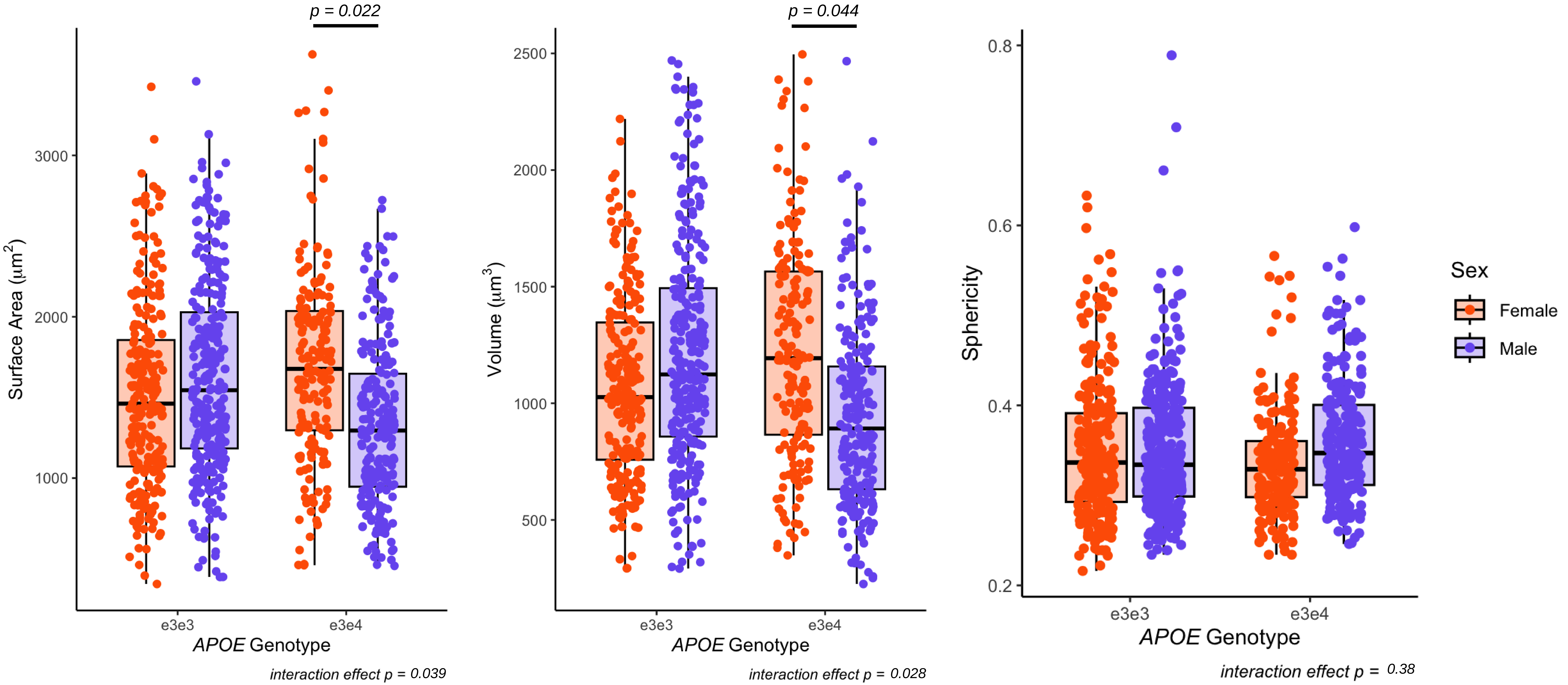
DAPI (nuclei)
MAP2 (neurons)
IBA1 (microglia)
AT8 (pTau)
Abeta (pan-amyloid β)

n = 22 individuals
6 images/person

930 total microglia

Interactive effects of sex and *APOE* on morphology

morphology ~ sex * APOE genotype + cortical layer + (1| identity)



Summary from microscopic analyses

- How do biological sex and *APOE* genotype alter the morphology (shape) of microglia in individuals with AD?
  - Significant interactive effects of sex and *APOE* genotype on the surface area and volume of microglia
  - Within *APOE* ε3ε4 individuals, females have significantly larger microglia (by surface area and volume)

Future directions

Microscopy approaches

- Expand data set to full cohort
- Characterize microglia on further morphological features
- Employ machine learning approaches to quantify multidimensional aspects of microglial morphology

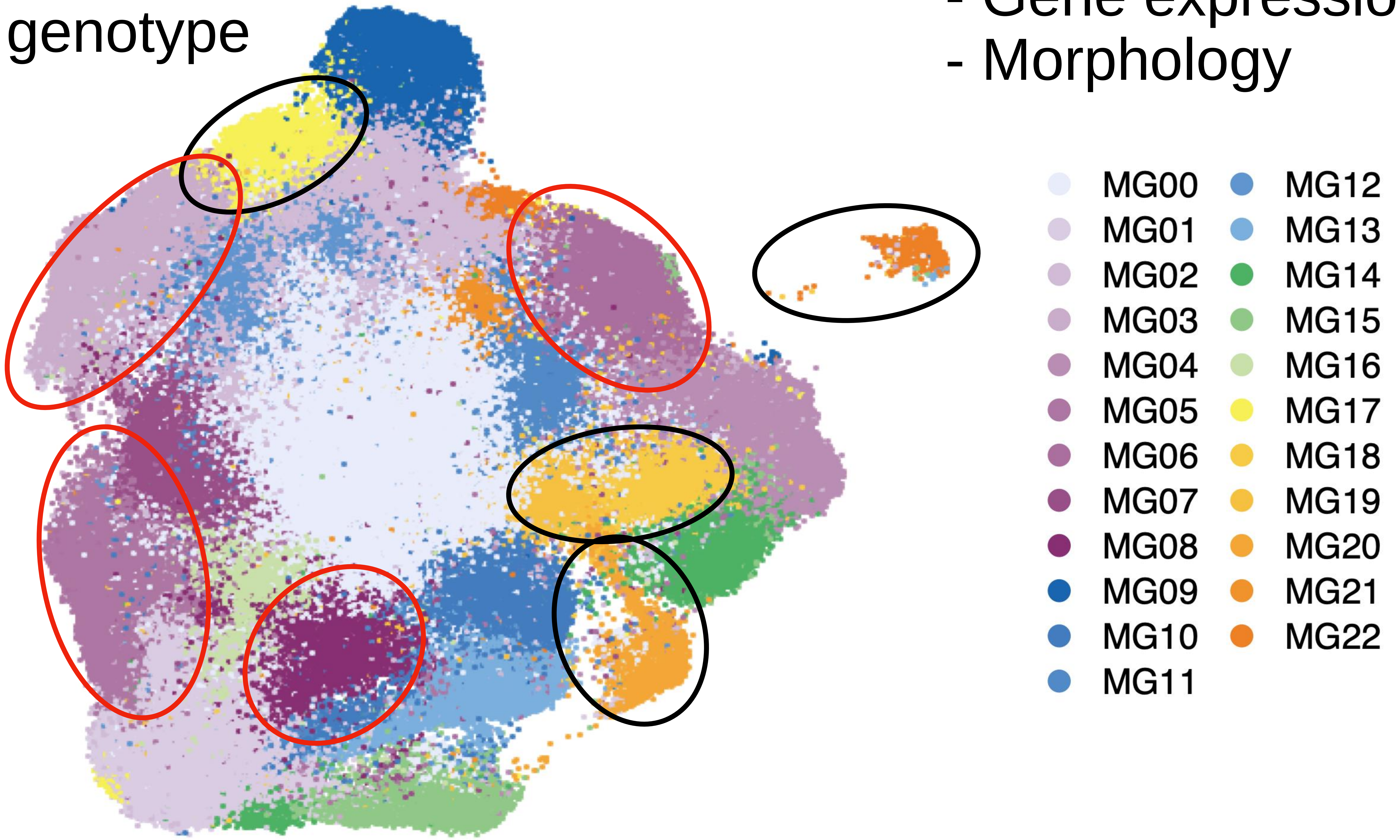
Summary

Proportional differences by sex

Proportional differences by *APOE* genotype

Interactive effects on:

- Gene expression
- Morphology



Acknowledgements

Jayadev Lab
Sumie Jayadev
Katie Prater
Nick Karagas
Arti Parihar
Lexi Cochoit
Isa Smith
Aquene Reid
Vanessa Souders
Mason Pirner
Rachel Blaine
Nikhil Saha
Fevet Ibrahim
Carole Smith
Kevin Gr

Collaborators
Jessica Young
Christine Disteche
Joel Berletch
Kevin Lin
Michelle Casad
Shannon Rose
Gala Filippova
Katharine Hui

Neuropathology Core
C. Dirk Keene
Caitlin Latimer
Amber Nolan
Aimee Schantz
John Campos
Erica Melief

Donors
I have tremendous gratitude for the patients and families of patients who have donated to this study.

Resources
Northwest Genomics Sequencing Core
Lab Medicine and Pathology Flow Core
Hyak supercomputer at UW
Keck Microscopy Center

Funding
NIH RF1-AG063540
NIH RF1-AG051437
U19-AG032438
Ellison Research Fund
Weill Nuerohub
Alzheimer’s Disease Training Program (5T32-AG052354-07)

UW Medicine
DEPARTMENT OF NEUROLOGY

ACT
ADULT CHANGES IN THOUGHT STUDY

Kaiser Permanente Washington
Health Research Institute

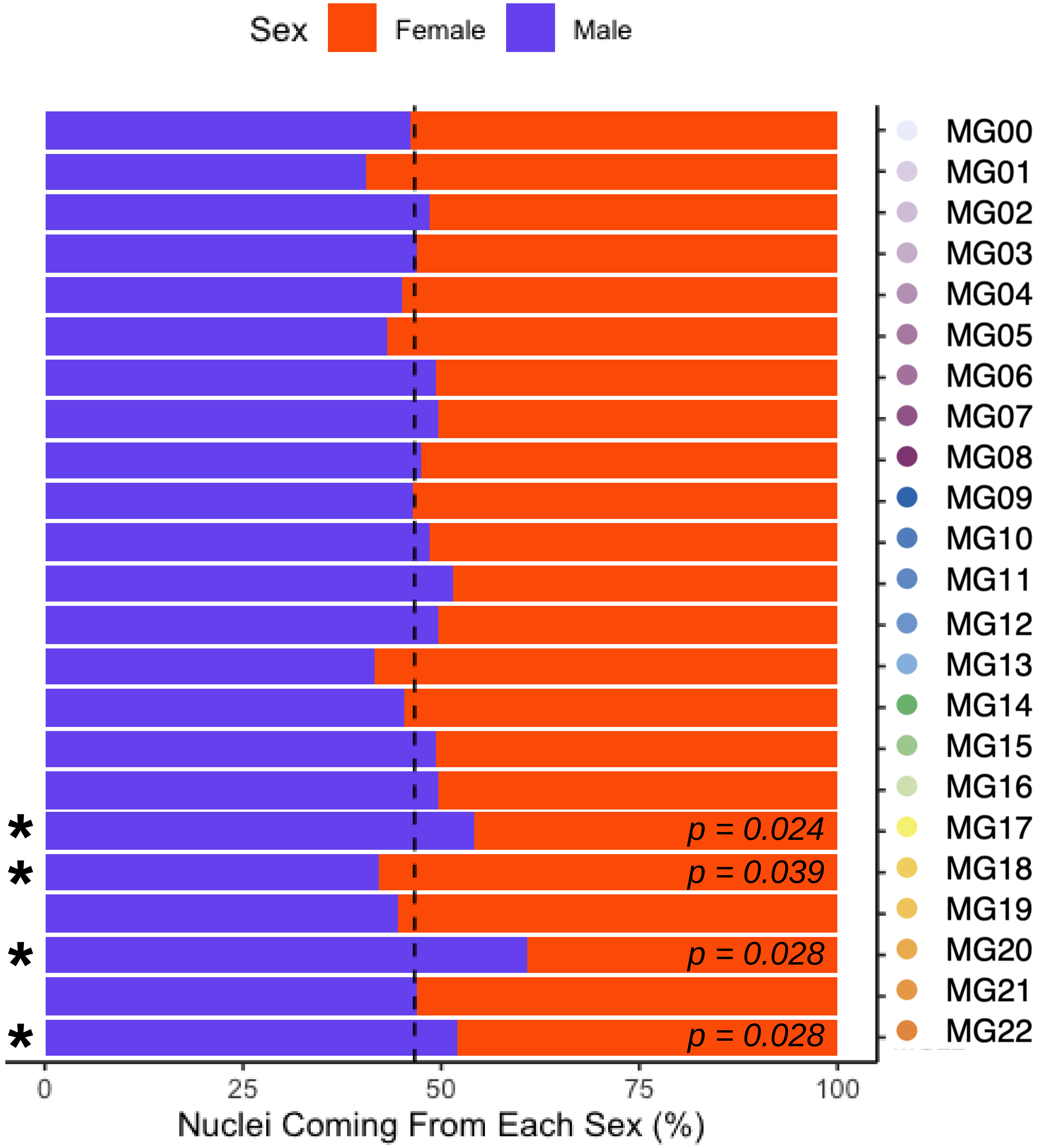
UNIVERSITY of WASHINGTON
ADRC
Alzheimer’s Disease Research Center

Interactive effects of sex and *APOE* on morphology

morphology ~ sex ** APOE ***genotype + cortical layer + (1| identity)

Model	Sex Effect (Female)		*APOE* Effect (ε3ε3)		Interaction Effect		Intercept		Cortical Layer (2/3)	
	β (Std. Err.)	*p*	β (Std. Err.)	*p*	β (Std. Err.)	*p*	β (Std. Err.)	*p*	β (Std. Err.)	*p*
Surface Area (μm^2)	82.28 (129.6)	0.54	156.1 (154.3)	0.33	**-474.4 (211.6)**	***0.039***	1565 (99.07)	6.2×10^{-12}	-15.12 (36.28)	0.677
Volume (μm^3)	134.4 (112.8)	0.25	159.1 (134.4)	0.25	**-445.4 (184.6)**	***0.028***	1084 (85.97)	3.4×10^{-10}	28.57 (27.11)	0.29
Sphericity	4.507×10^{-3} -0.01409	0.75	-8.7159×10^{-3} (0.01673)	0.61	0.02057 (0.02291)	0.38	0.3442 (0.01080)	4.9×10^{-18}	7.832×10^{-3} (4.559×10^{-3})	0.086

Differential composition of microglial states



Differential composition of microglial states