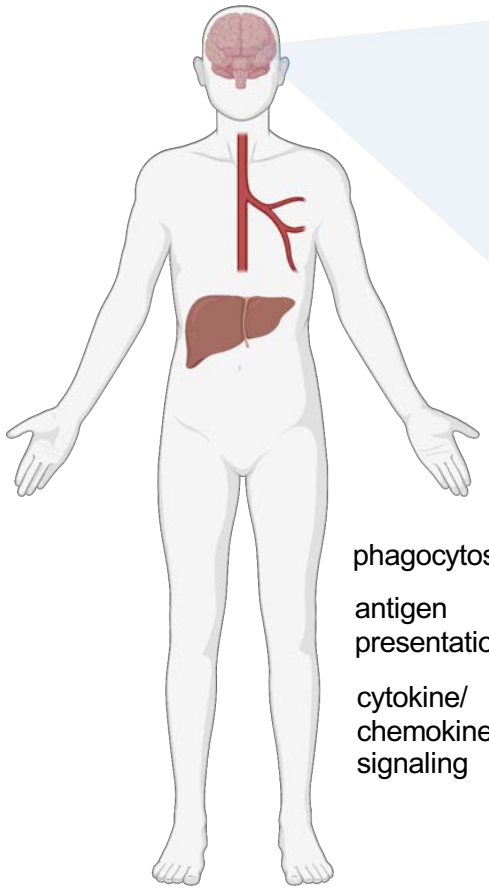
COLUMBIA | COLUMBIA UNIVERSITY IRVING MEDICAL CENTER

CTCN
THE CENTER FOR TRANSLATIONAL & COMPUTATIONAL NEUROIMMUNOLOGY

Characterizing Microglia Gene Expression Programs and Regulatory Networks in Neurodegenerative Disease

Victoria Marshe, PhD
Postdoctoral Research Scientist
Center for Translational and Computational Neuroimmunology
Columbia University Irving Medical Centre
Mentor: Philip De Jager, MD PhD

Microglia are CNS-resident macrophages that are highly responsive to their environment



MYELOID-LINEAGE IMMUNE CELLS

Microglia
CNS-resident

yolk-sac derived

Macrophages
peripheral, tissue-resident

Monocytes
circulating

bone-marrow derived

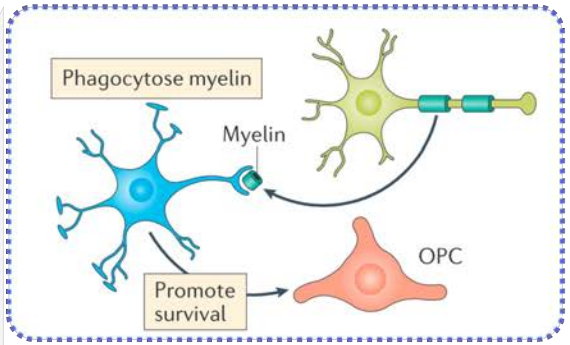
phagocytosis

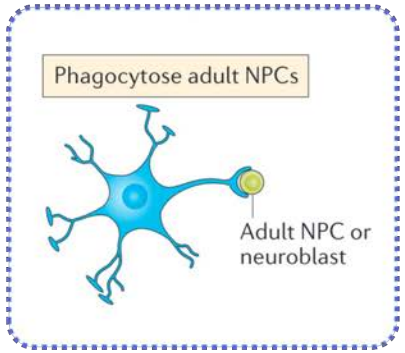
antigen presentation

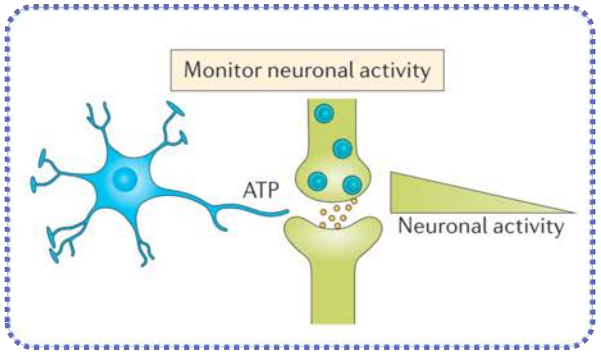
cytokine/ chemokine signaling

*also dendritic cells

HOMEOSTASIS

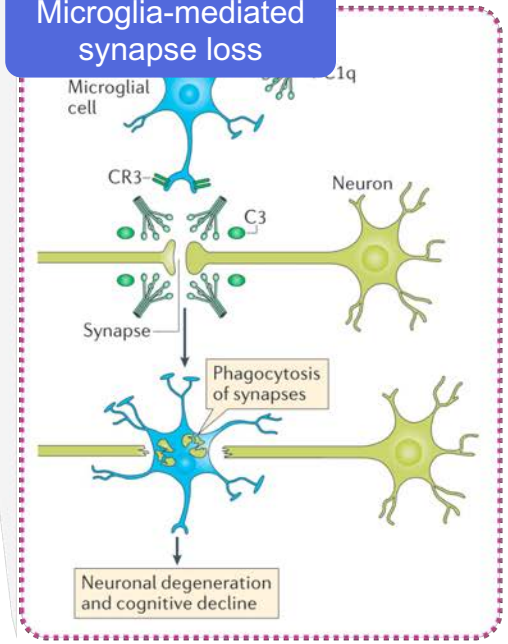




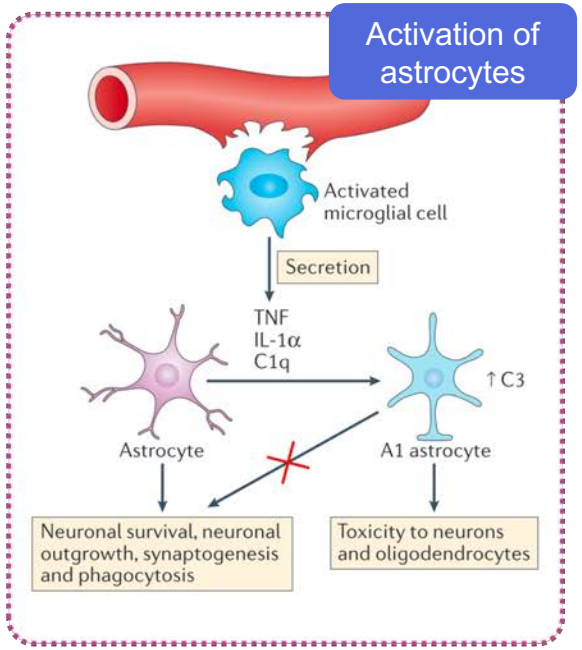


DISEASE & INJURY

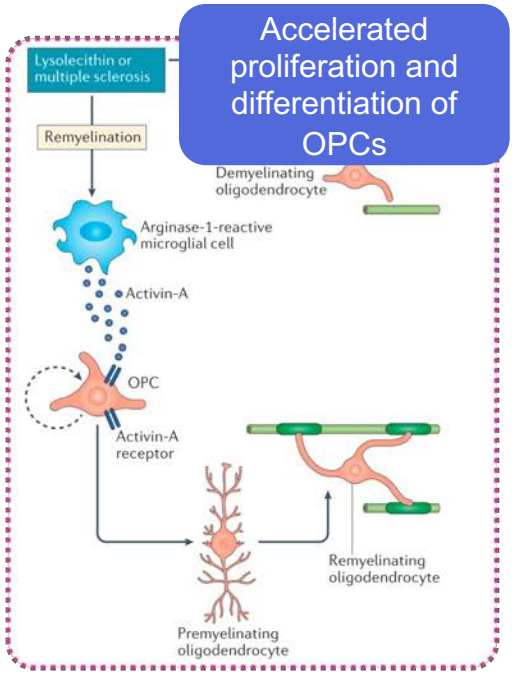
Microglia-mediated synapse loss



Activation of astrocytes

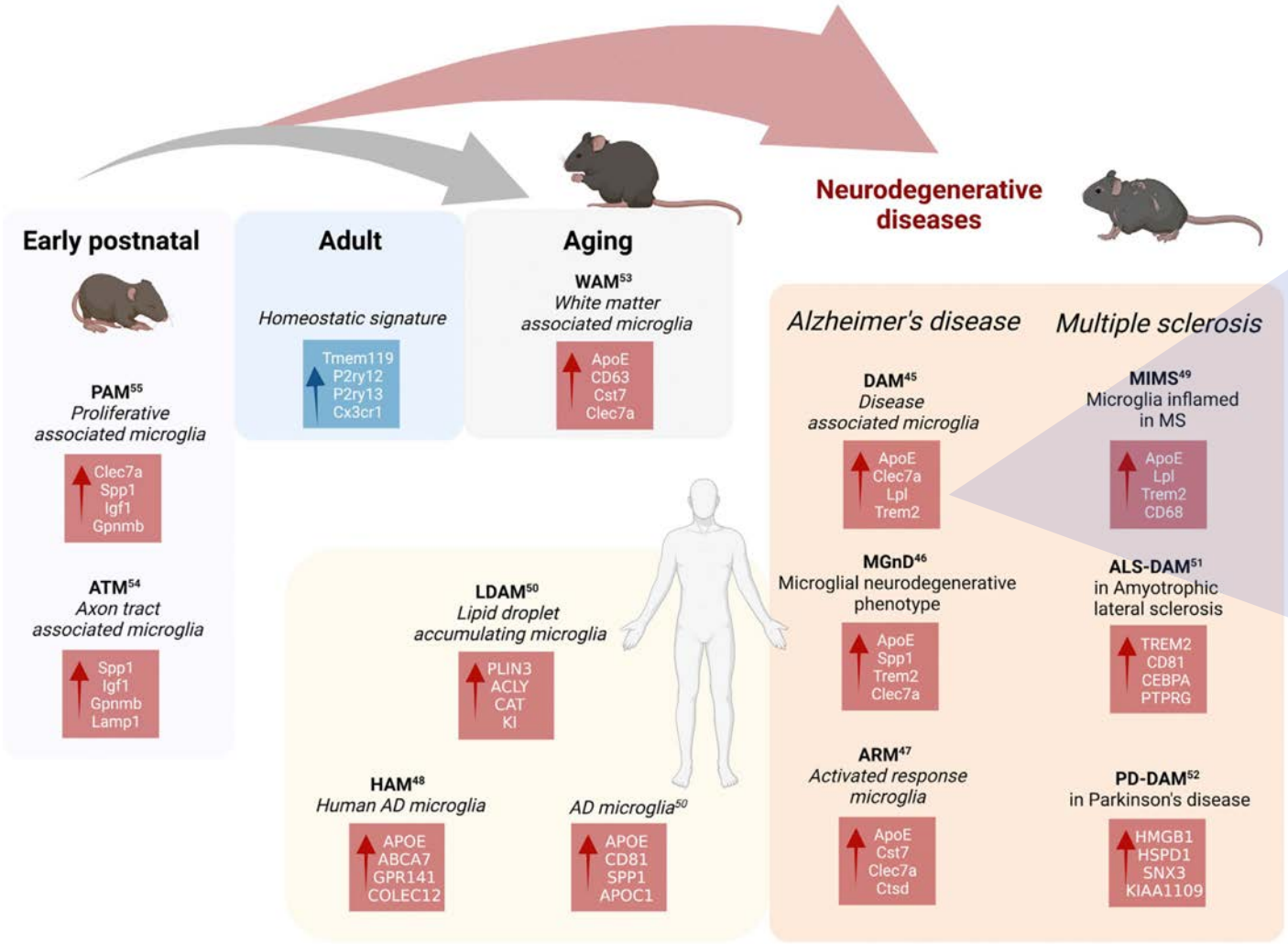


Accelerated proliferation and differentiation of OPCs

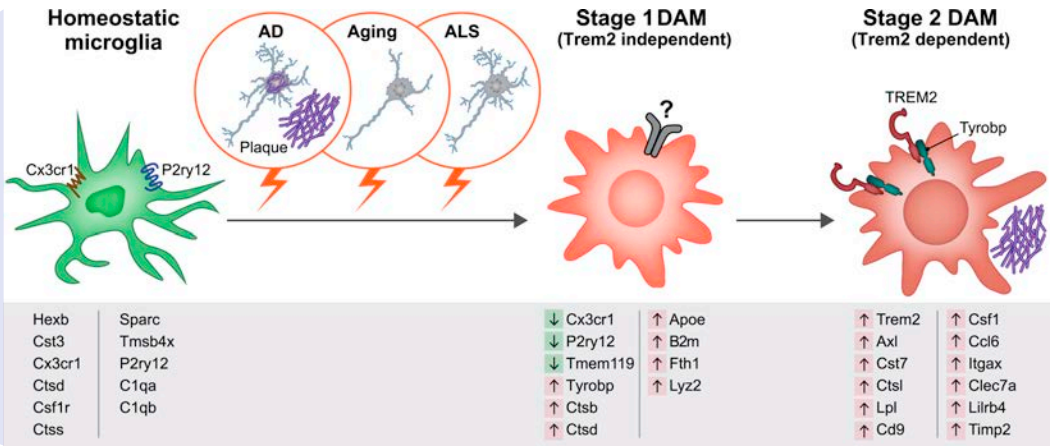


Adapted from Li & Barres. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol* (2018).

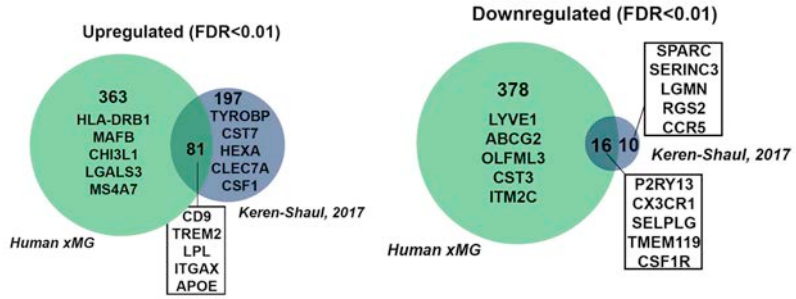
Microglial heterogeneity has been captured across disease-associated signatures



Paolicelli *et al. Neuron* (2022).



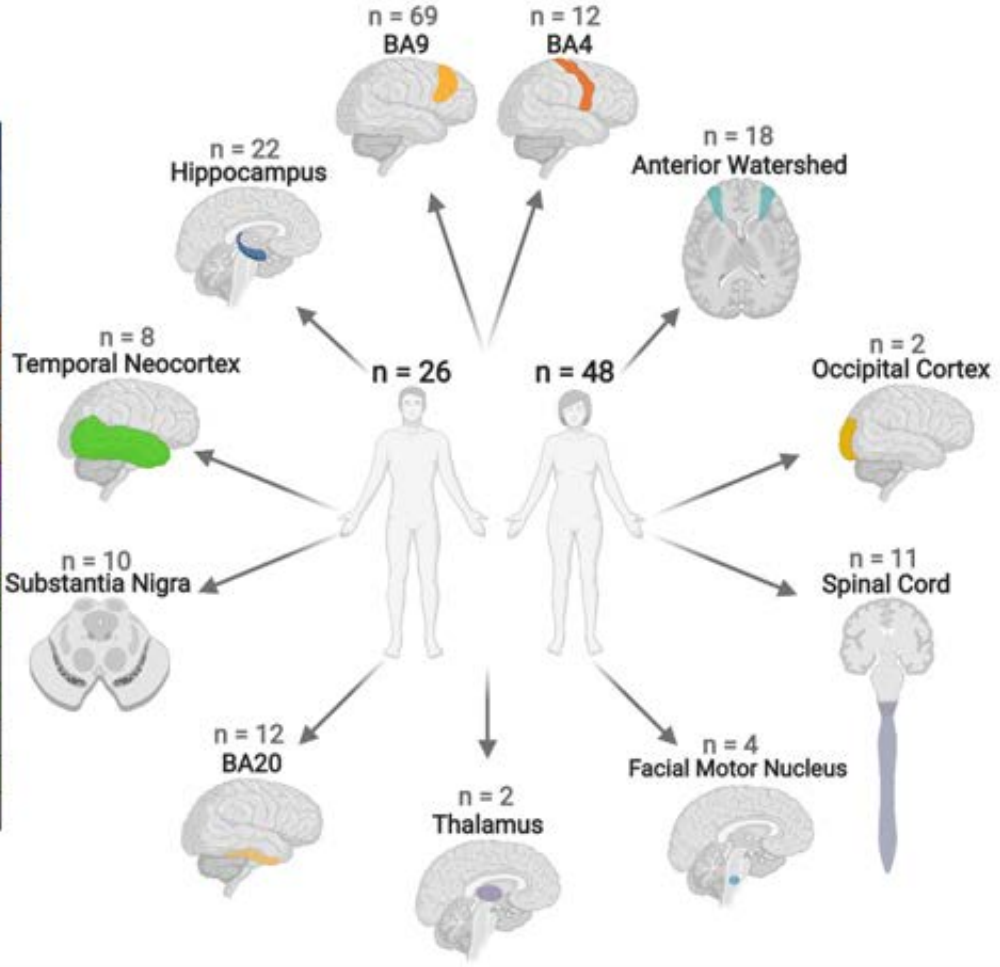
Keren-Shaul *et al. Cell* (2017).



Hasselman *et al. Neuron* (2019).

Defining a cross-disease atlas of microglia signatures

Diagnosis	
Alzheimer's Spectrum	
LOAD	35
EOAD	2
MCI	4
ALS Spectrum	
ALS	9
ALS/FTD	2
FTD	1
Parkinsonism Spectrum	
DLBD-PD	5
PSP	3
Other	
TLE	7
MS	2
HD	1
Stroke Lesion	1
DNET	1
Control	1



Dr. John Tuddenham
CTCN, Columbia University

Banner Sun Health Research Institute (Sun City, AZ)

Brigham and Women's Hospital (Boston, MA)

Massachusetts General Hospital (Boston, MA)

New York Brain Bank (New York, NY)

Rocky Mountain MS Center (Denver, CO)

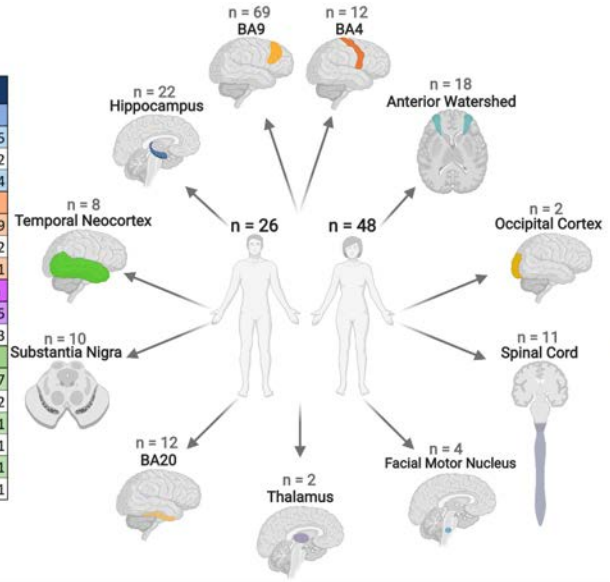
Rush University Medical Center (Chicago, IL)

UW Medical Center (Seattle, WA)

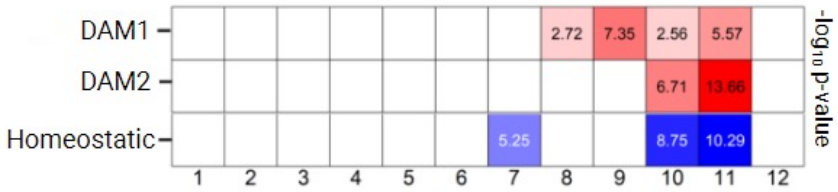
Tuddenham. *et al.* A cross-disease human microglial framework identifies disease-enriched subsets and tool compounds for microglial polarization. biorXiv (2022).

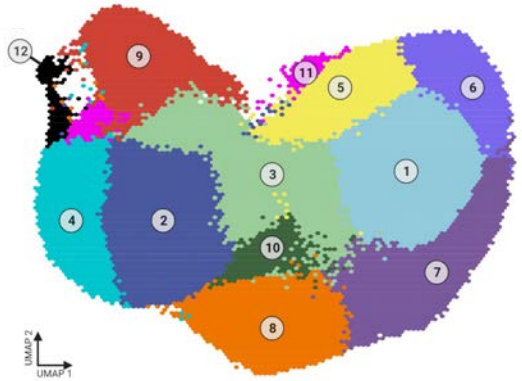
Live microglia show diverse subsets with unique expression signatures

Diagnosis	
Alzheimer's Spectrum	
LOAD	35
EOAD	2
MCI	4
ALS Spectrum	
ALS	9
ALS/FTD	2
FTD	1
Parkinsonism Spectrum	
DLBD-PD	5
PSP	3
Other	
TLE	7
MS	2
HD	1
Stroke Lesion	1
DNET	1
Control	1

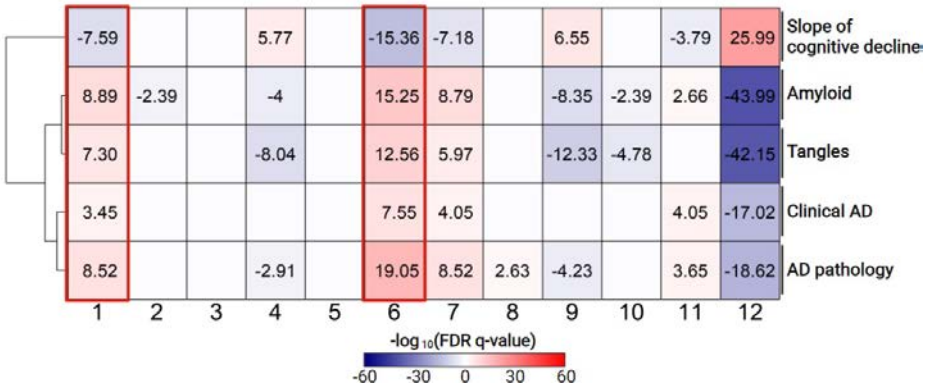


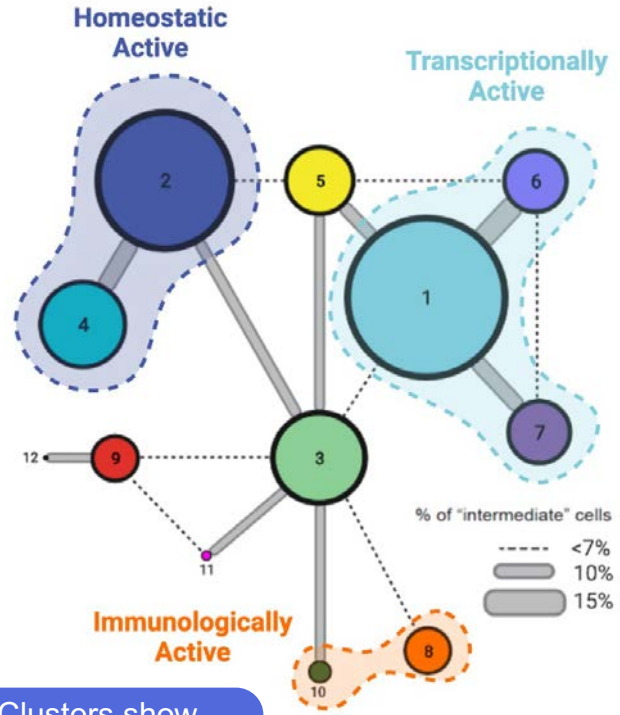
Clusters show variable enrichment for the DAM signature





Some cluster shows signatures associated with disease





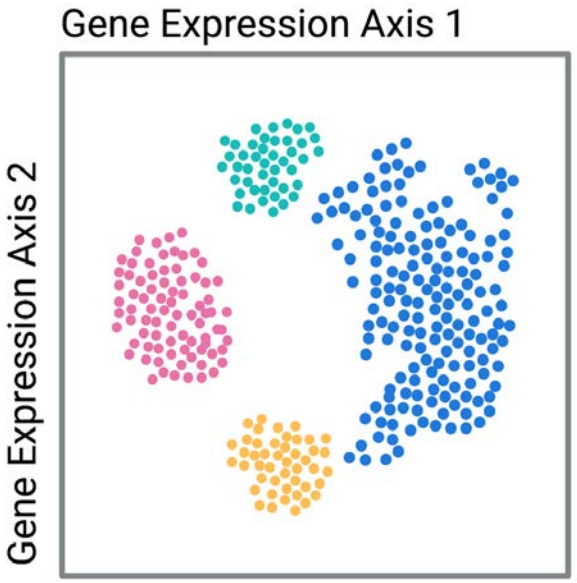
Clusters show similarity and share transcription signatures

Tuddenham. *et al.* A cross-disease human microglial framework identifies disease-enriched subsets and tool compounds for microglial polarization. biorXiv (2022).
Olah et al. Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat Commun*, 2020.

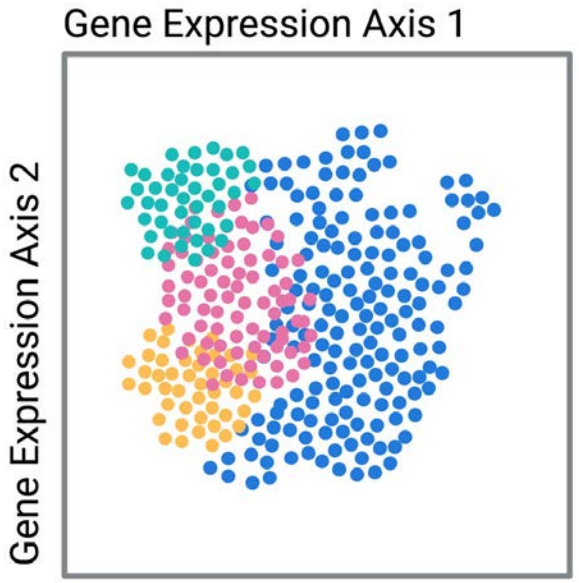
Dr. John Tuddenham
CTCN, Columbia University

Shifting Towards Understanding The Continuous Nature Of Cellular Expression Programs

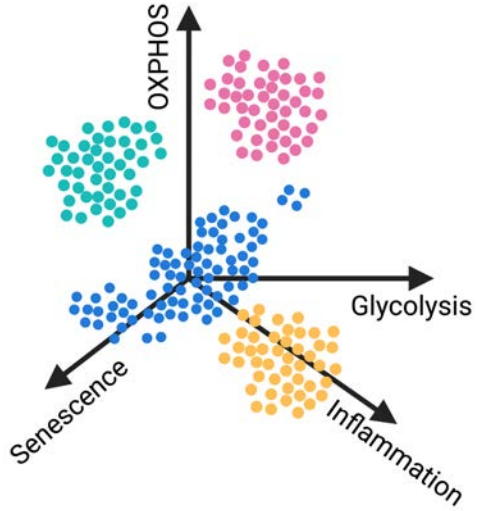
Scenario 1

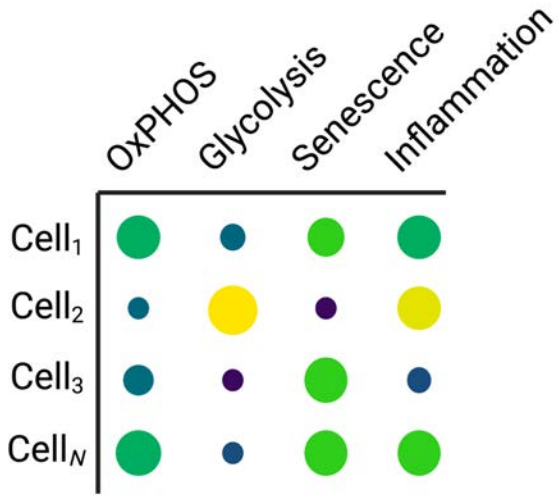


Scenario 2



Scenario 3

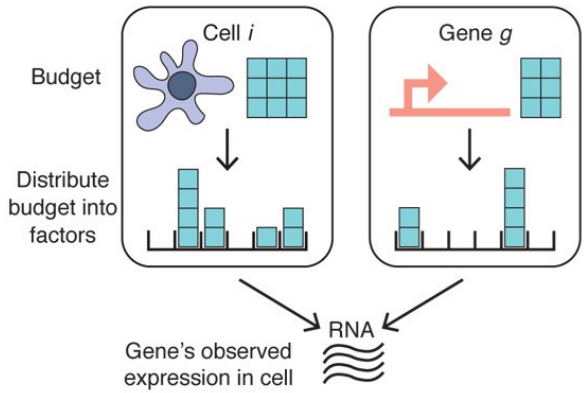


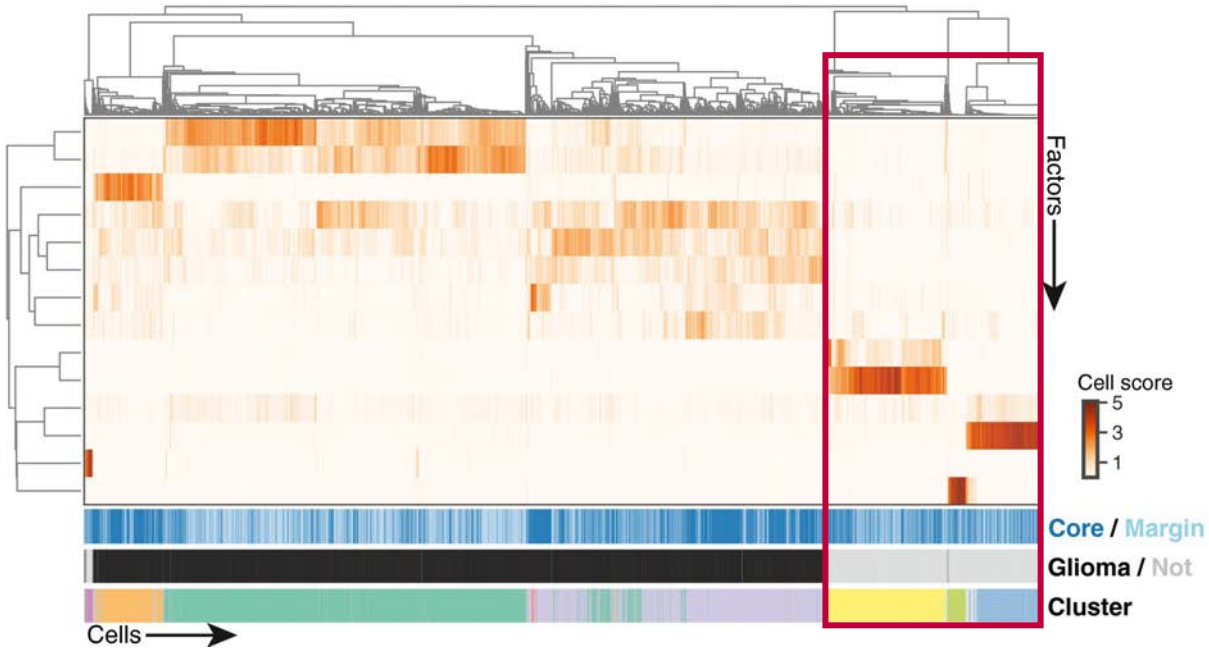


See Cembrowski & Menon. Continuous Variation within Cell Types of the Nervous System. *Trends Neurosci* 2018;**41**:337–48 for conceptual description.

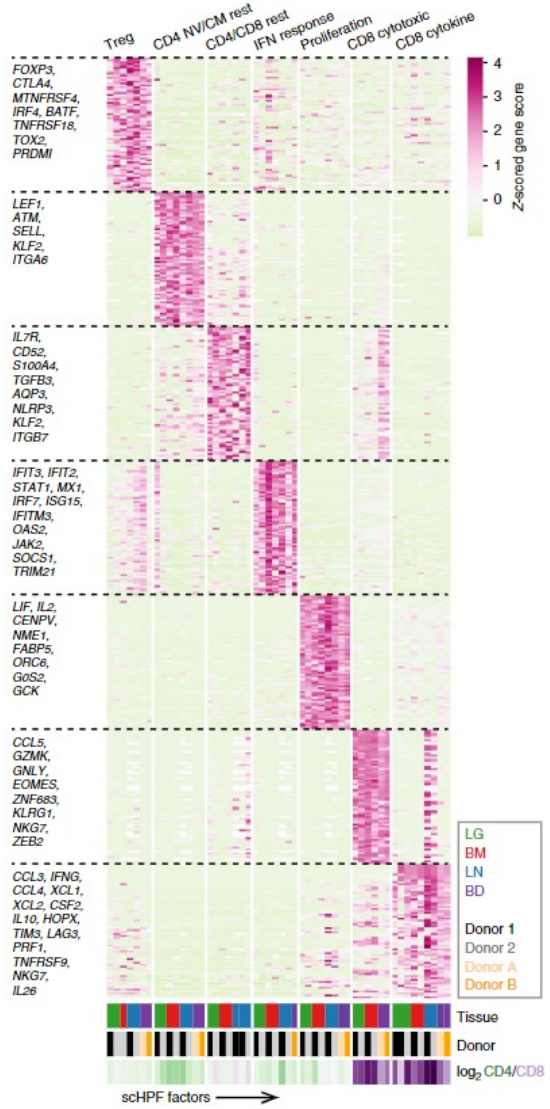
A High Dimensional Reference Map Of Human T Cell Activation In Highlights The Potential Of Understand Expression Programs

scHPF: single-cell Hierarchical Poisson Factorization





Levitin et al. *Mol Syst Biol* 2019;15:e8557.



Szabo et al. *Nat Commun* 2019;10:4706.

Dr. Peter Sims
Systems Biology
Columbia

20

ACT Study

Updated live microglia dataset

DIAGNOSES

AD spectrum (97)
(LOAD, EOAD, MCI)
89.5 [47-107] years
248,341 cells

ALS/FTD spectrum (14)
(ALS, FTD, ALS-FTD, ALS-PSP)
62 [33-101] years
52,232 cells

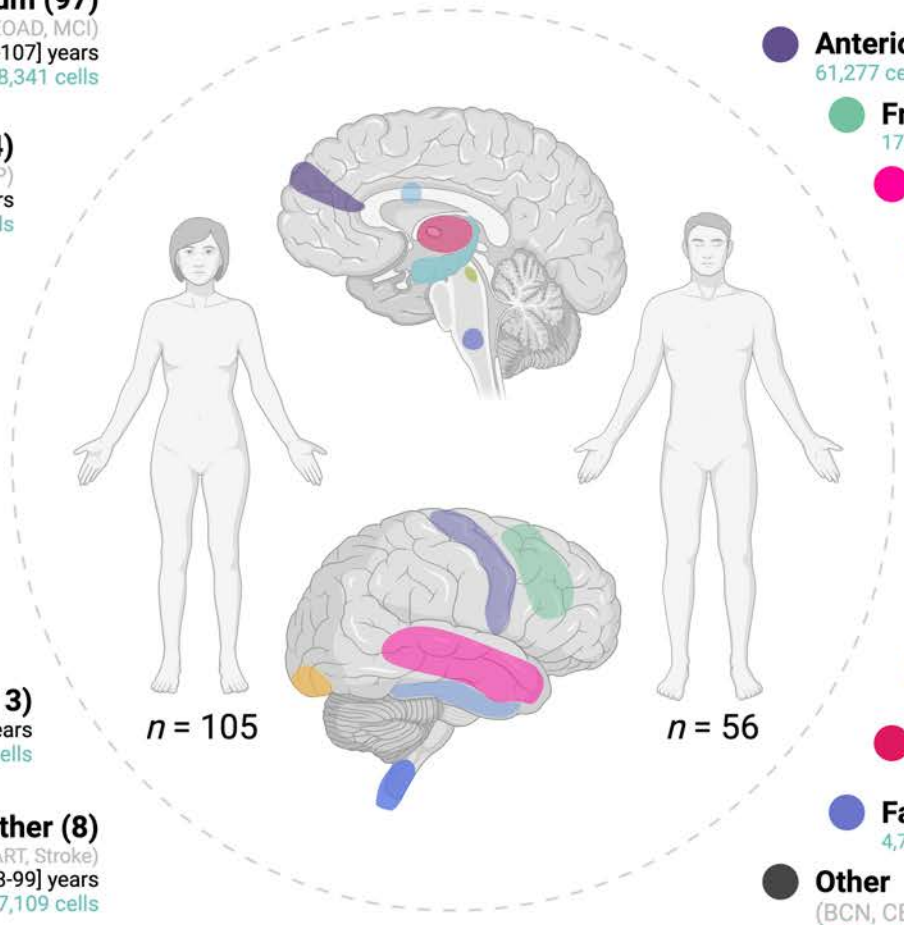
Huntington's disease (4)
62 [33-101] years
11,087 cells

Multiple sclerosis (12)
(PPMS, SPMS)
68 [47-89] years
43,982 cells

PD spectrum (13)
(DLBD, DLBD-PD, MSA, PD, PSP)
80 [70-89] years
30,775 cells

Temporal lobe epilepsy (13)
35 [2-60] years
37,562 cells

Other (8)
(Control, DNET, ET, PART, Stroke)
62 [43-99] years
17,109 cells



TISSUES

Anterior watershed (AWS)
61,277 cells

Frontal cortex (BA9/46)
174,744 cells

Temporal neocortex
37,888 cells

Temporal cortex (BA20/21)
20,610 cells

Motor cortex (BA4)
18,393 cells

Visual cortex (BA17)
1,251 cells

Hippocampus
63,036 cells

Substantia nigra
17,181 cells

Spinal cord
13,103 cells

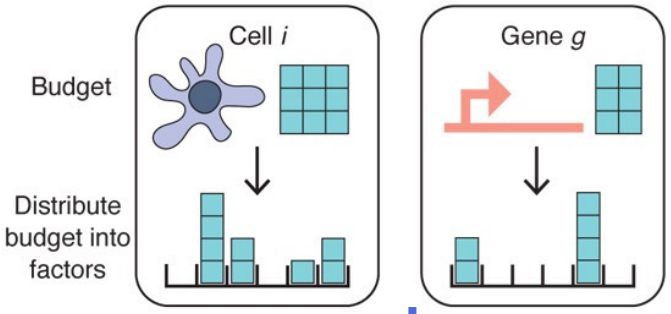
MS lesion/perilesion
10,625 cells

Thalamus/pulvinar
10,587 cells

Facial nucleus
4,771 cells

Other
(BCN, CB, DN, DNET, OC, STN, BA9 SBWM)
7,622 cells

scHPF: single-cell Hierarchical Poisson Factorization



MODEL

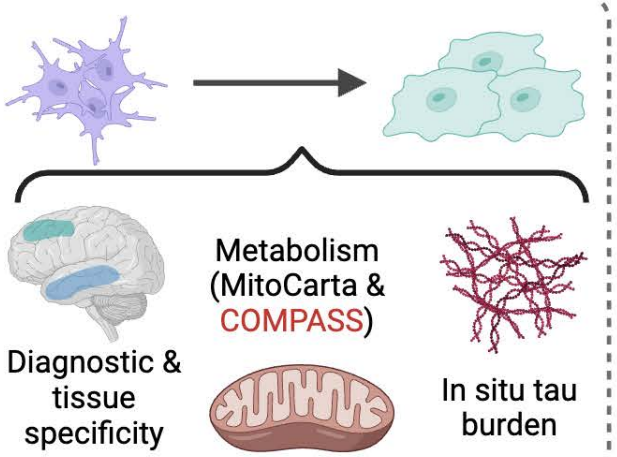
	F1	F2	F3
$Cell_1$	1.2	5.6	2.2
$Cell_2$	0.3	4.3	3.5
$Cell_3$	0.8	2.1	7.8
$Cell_N$	4	0.3	5.5

CHARACTERIZATION

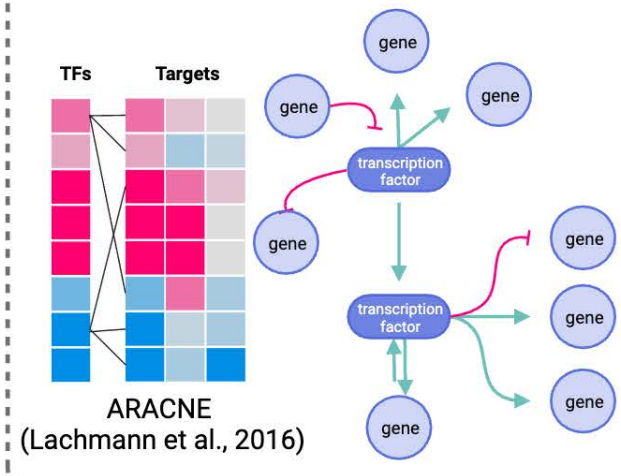
Biological enrichment (GO)
Disease-outcome associations
External validation

Analysis Overview

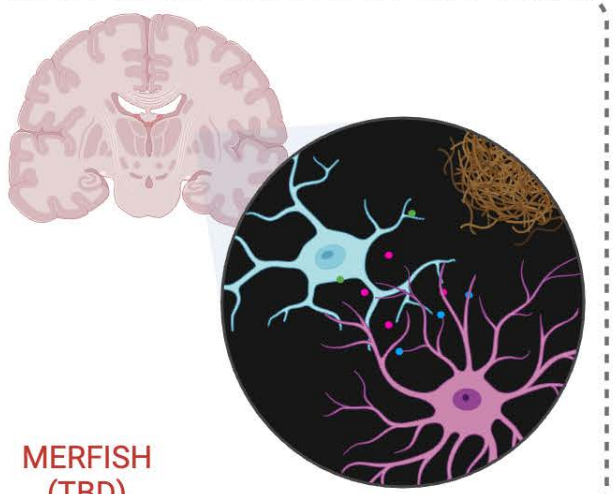
BIOLOGICAL CHARACTERIZATION



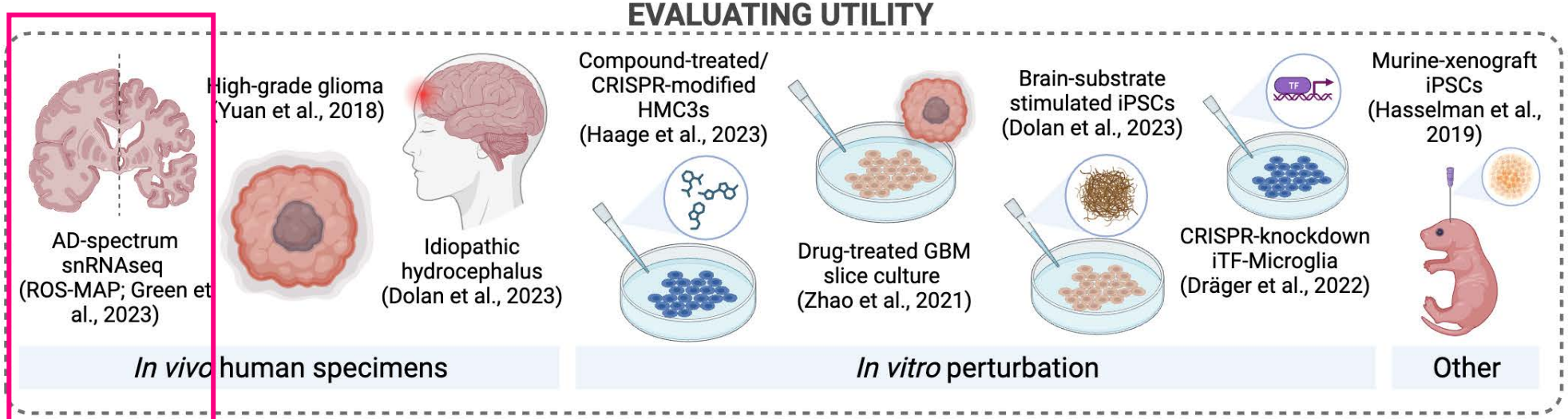
REGULATORY NETWORK



IN SITU VALIDATION



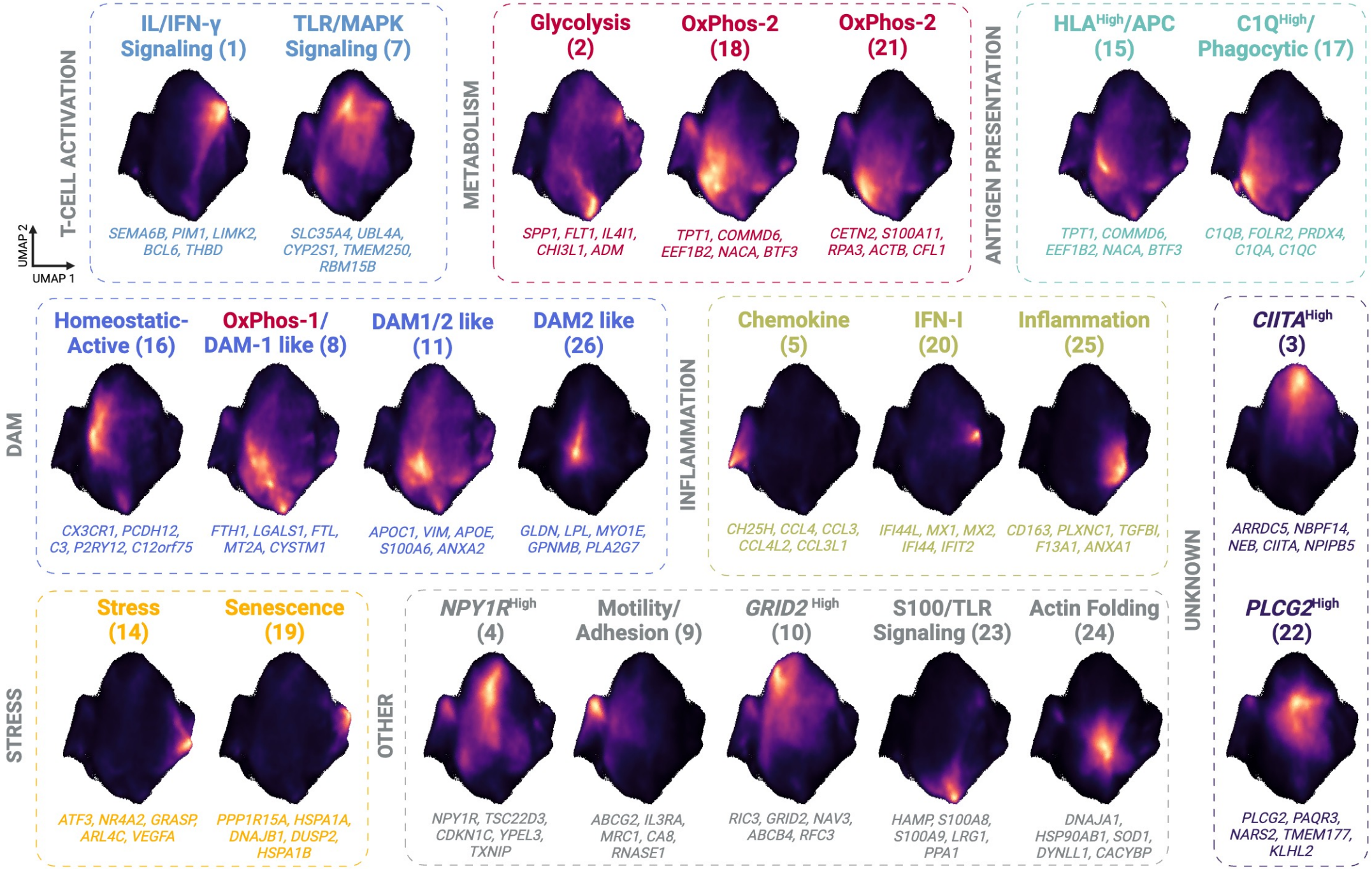
EVALUATING UTILITY



TAKE AWAYs

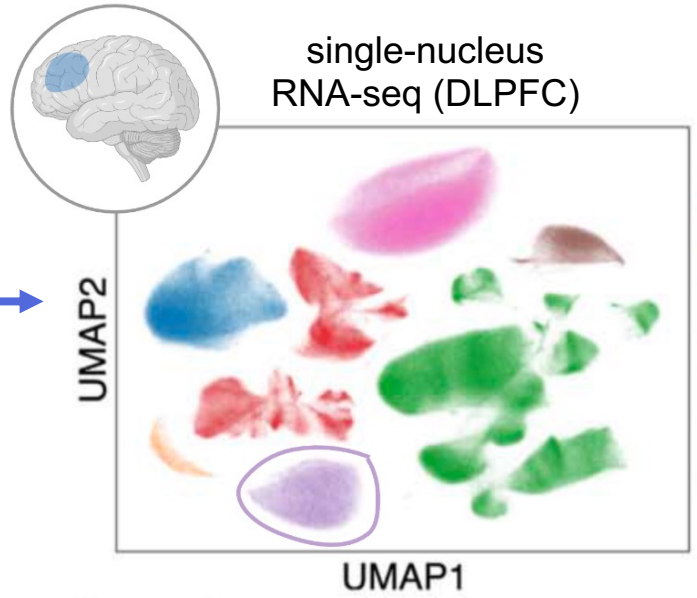
- Expression programs are biologically relevant
- Programs show diagnostic specificity, with some distinction between white and grey matter
- Program have shared and unique TF regulation
- The paradigm is broadly applicable across cellular compartments, as well as model systems with and without chemical, genetic, and biological perturbation

Expression programs in microglia across disease and brain regions

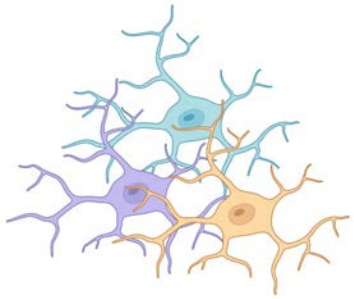


Projecting single-nucleus RNAseq data onto the 23-factor single-cell model

Religious Orders Study (ROS)
Memory and Aging Project (MAP)
(*n* = 424)

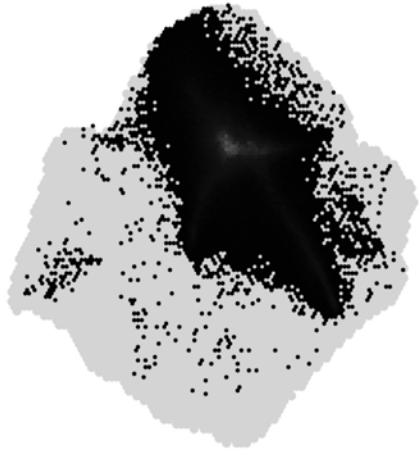


Extracting
microglial only



87,319 cells

Projection



Gilad Green,
Hebrew University

Masashi Fujita,
Columbia University

Green et al. bioRxiv (2023)

Expression programs show substantial overlap with DEGs for clustering-based microglia subtypes

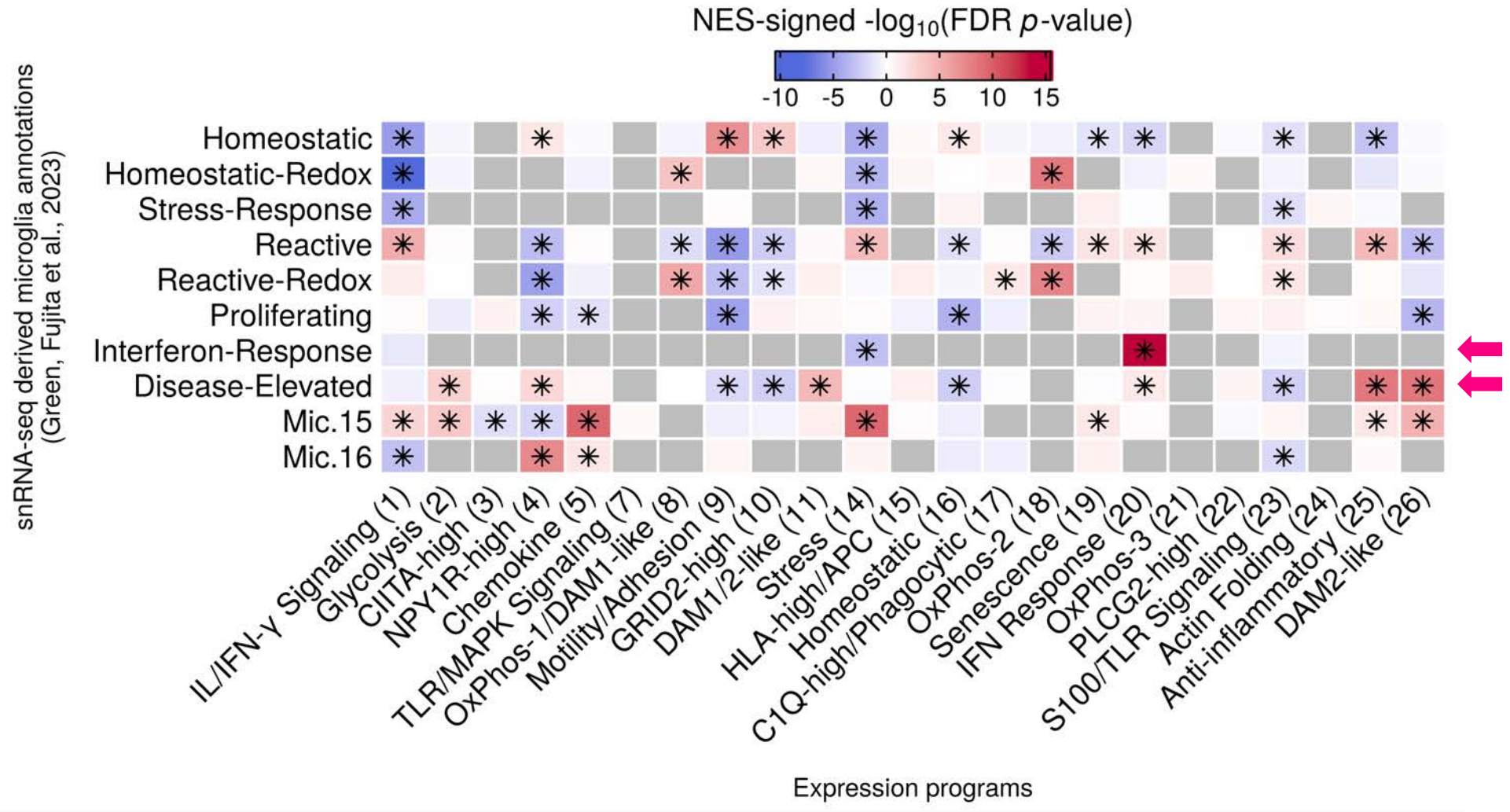
Clustering-based microglia subtypes (snRNA-seq)

↓

Subtype differentially expressed genes (DEGs)

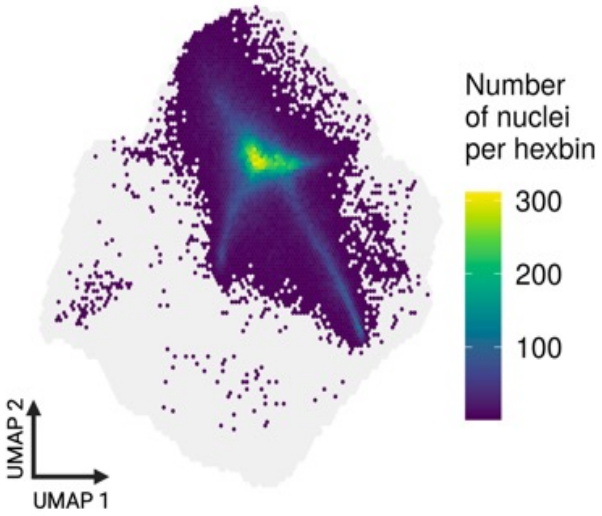
↓

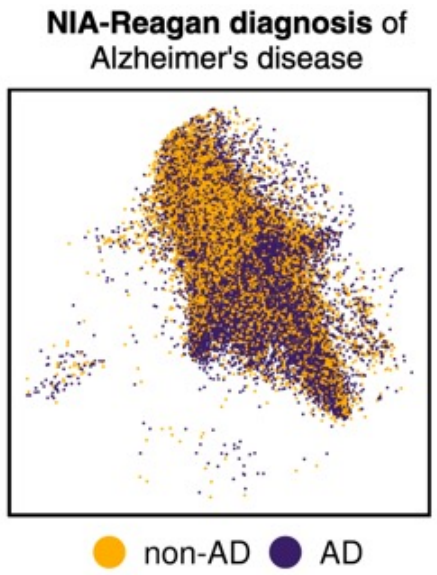
Fast gene set enrichment analysis (FGSEA) w/ top 100 factor markers

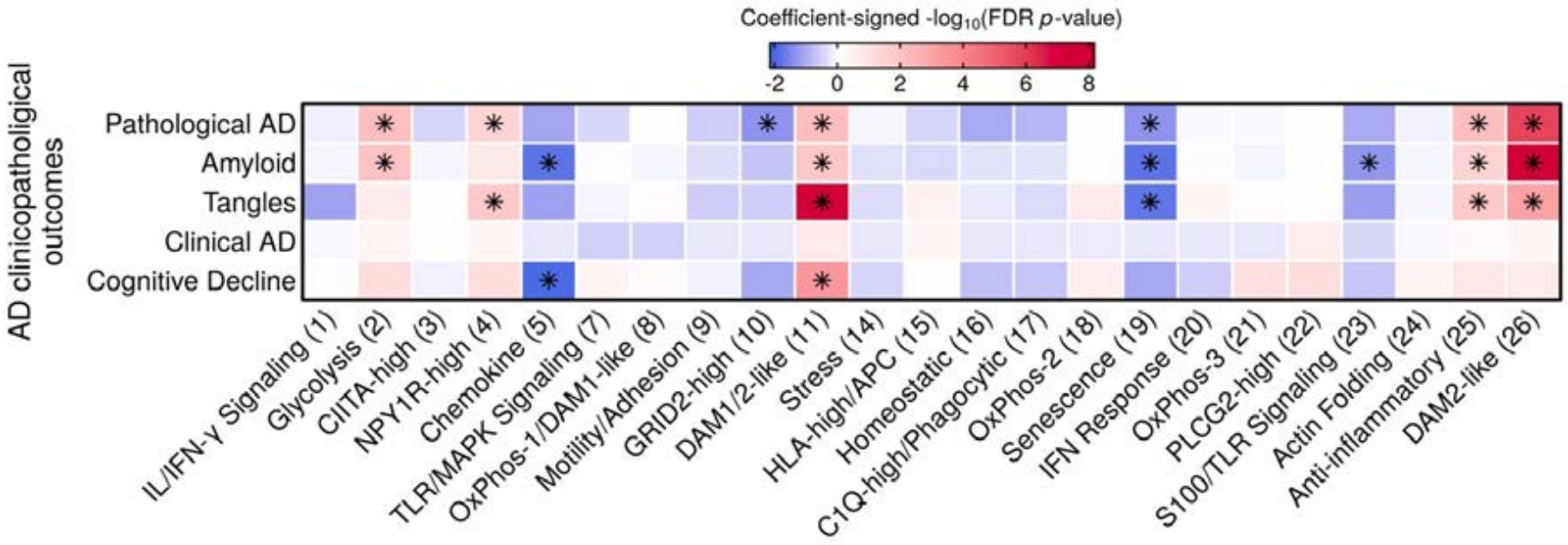


Significance levels: *FDR-corrected *p*-value < 0.05.

Expression programs are broadly applicable and capture microglial perturbations associated with disease







Linear regressions for donor-level expression program scores (mean-aggregated across cells) adjusted for study, age at death, sex, and postmortem interval. For cognitive decline, the model was also adjusted for years of education.
*FDR p-value < 0.05

miloR: Detecting perturbed cell states as differentially abundant graph neighborhoods

nature biotechnology

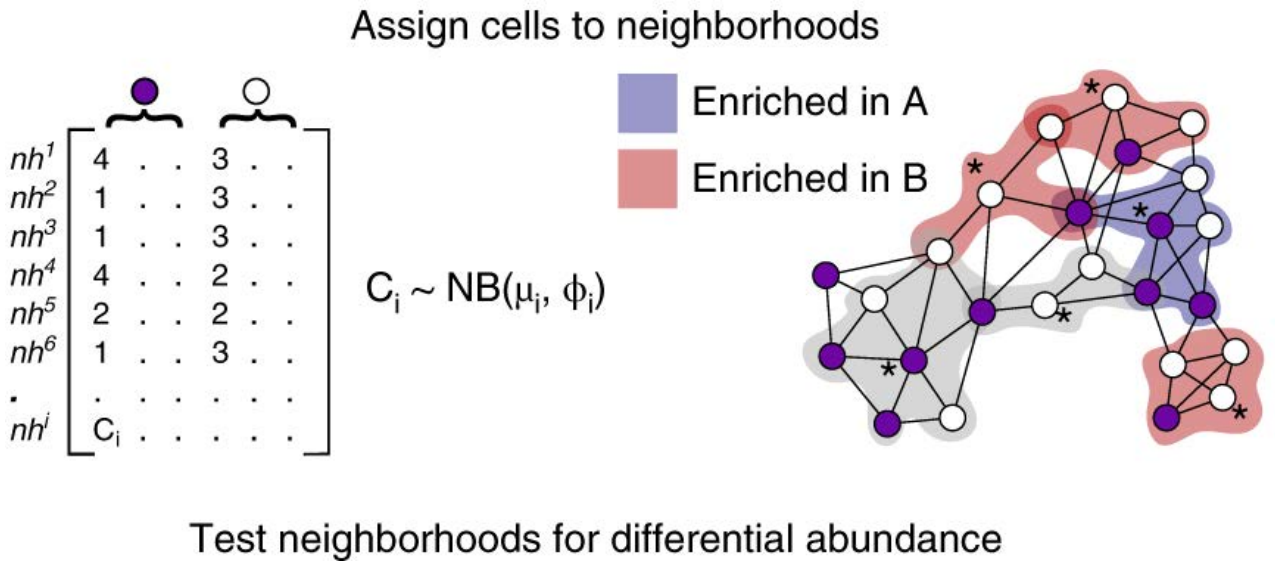
ARTICLES

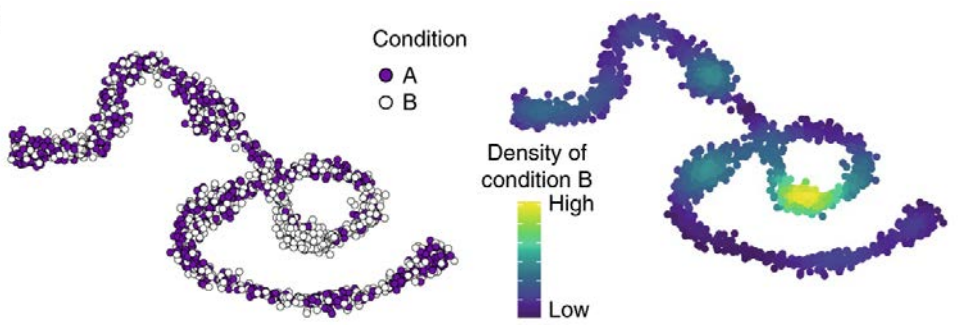
https://doi.org/10.1038/s41587-021-01033-z

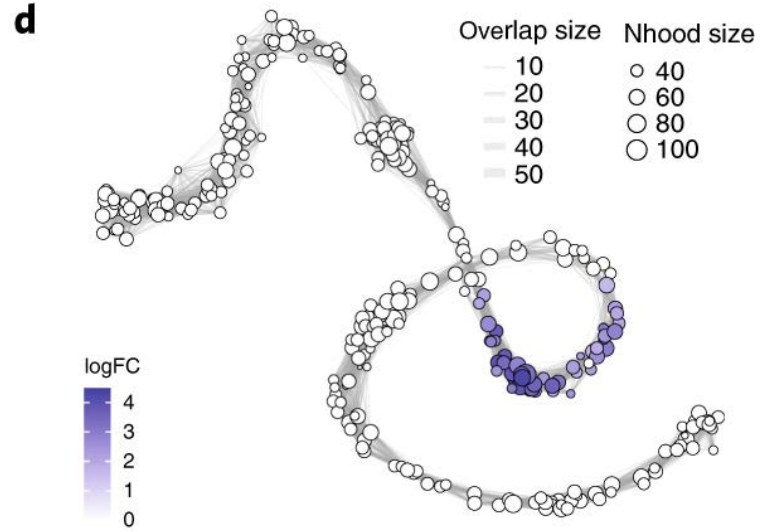
Check for updates

Differential abundance testing on single-cell data using *k*-nearest neighbor graphs

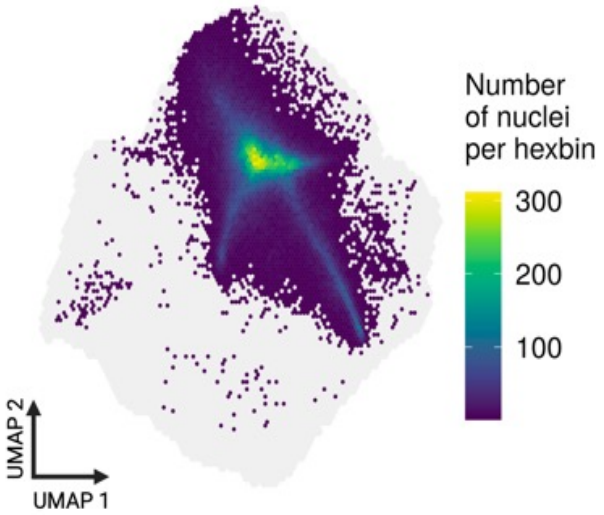
Emma Dann[1], Neil C. Henderson[2,3], Sarah A. Teichmann[1,4], Michael D. Morgan[5,6] ✉ and John C. Marioni[1,5,6] ✉

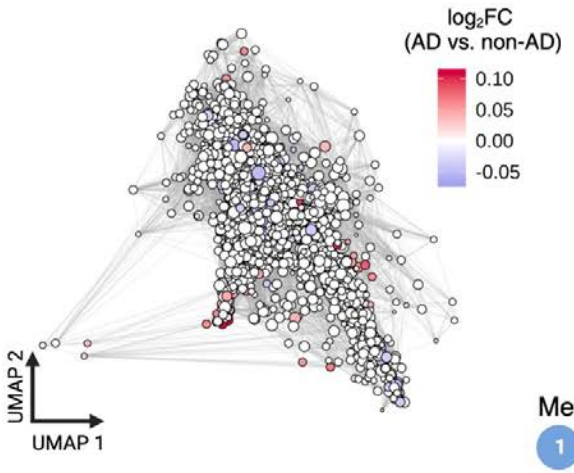


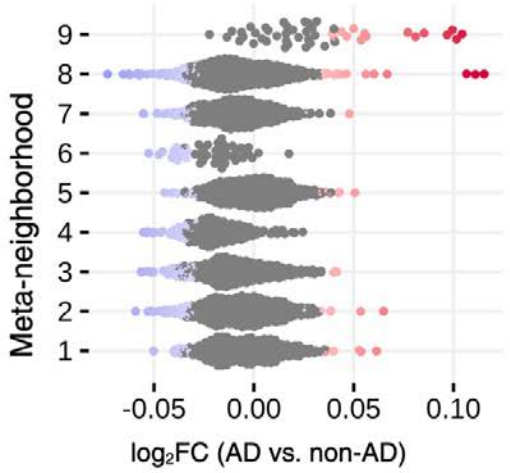


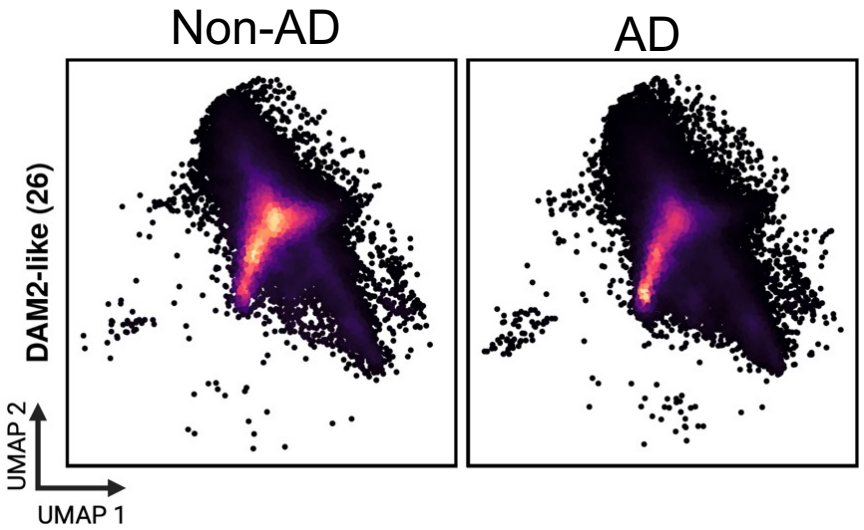


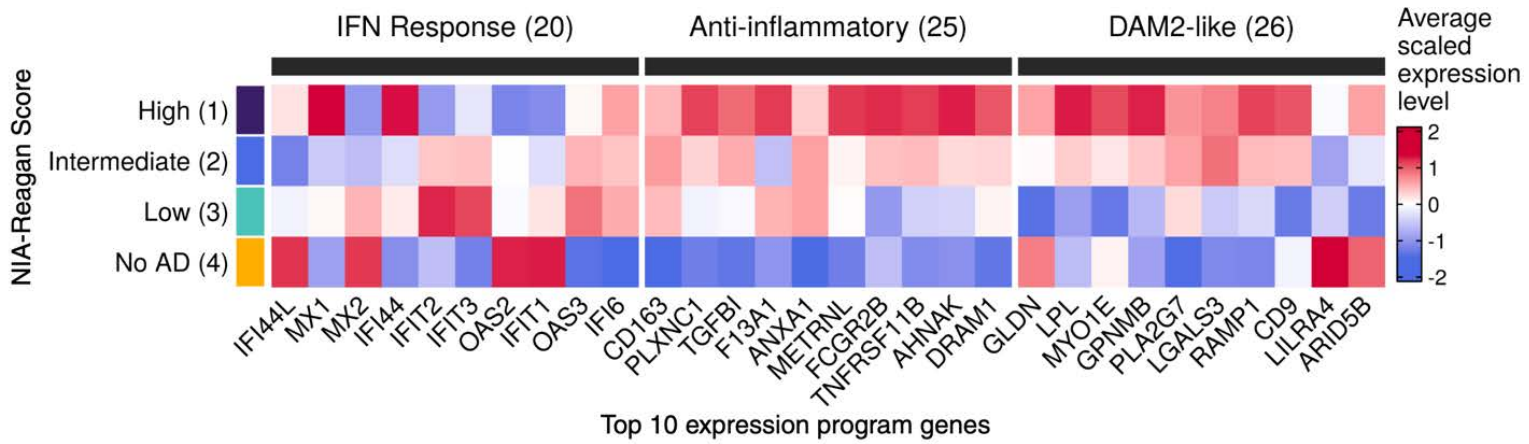
AD microglia show a differentially abundant subset of microglia characterized by high DAM2-like (26) expression





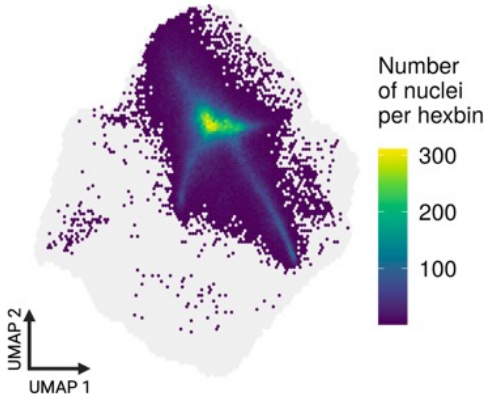


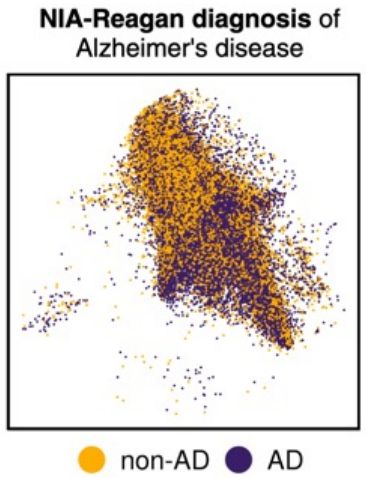




Expression programs capture stable patterns across independently-generated datasets

ROS-MAP snRNA-seq
(Green, Fujita et al.)





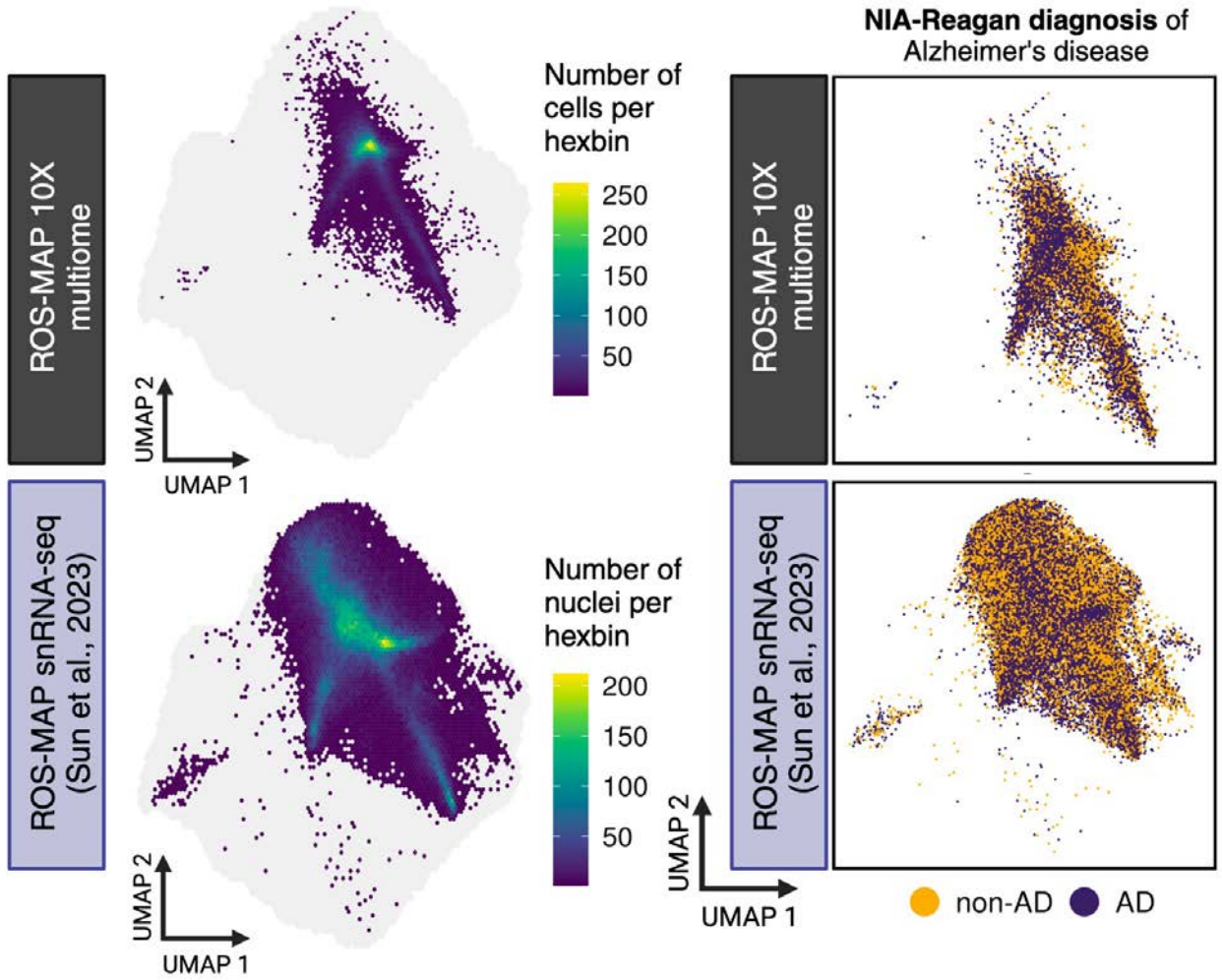
ROS-MAP 10X multiome
(scRNA-seq + ATAC-seq)

27,399 transcriptomes
232 donors (no overlap)

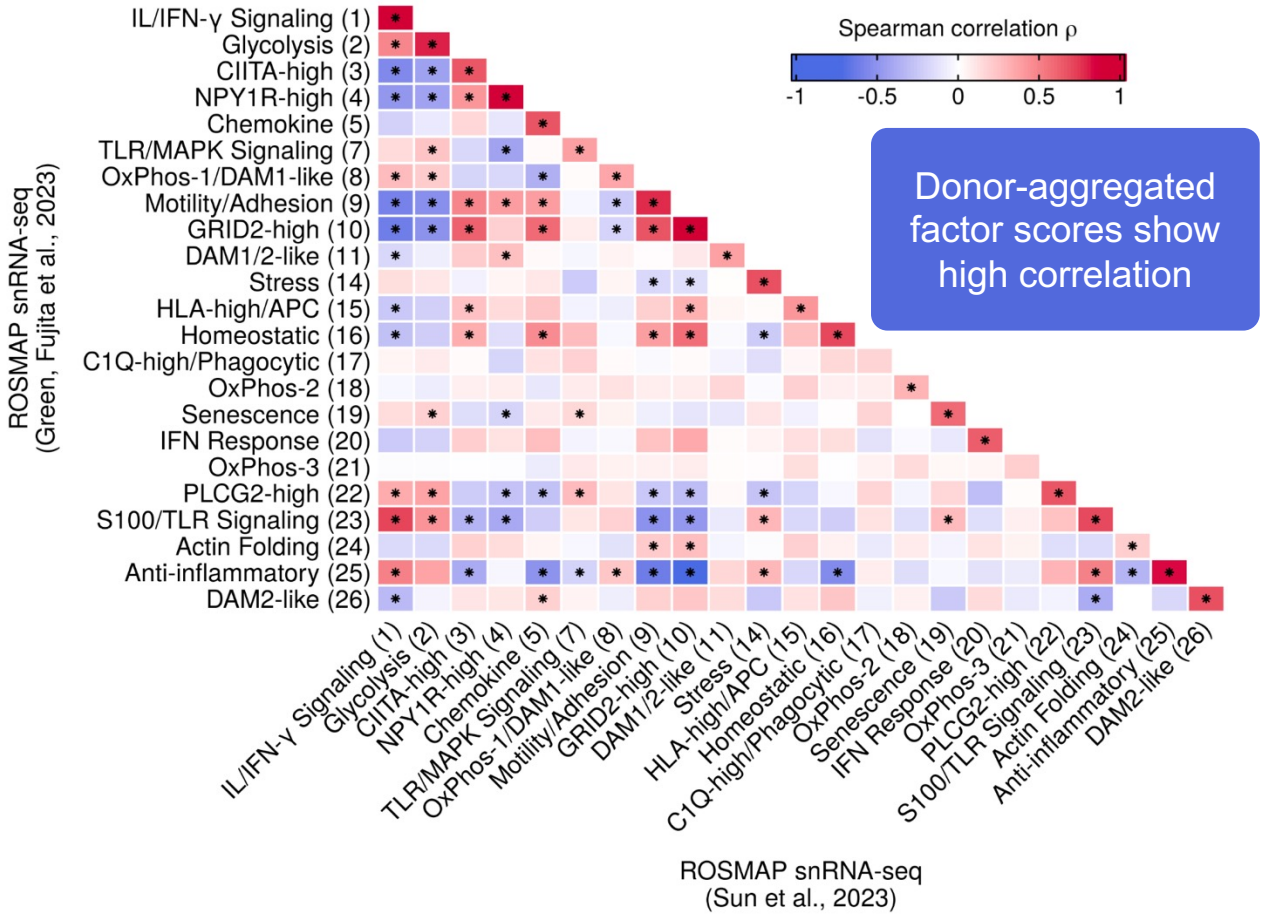
ROS-MAP snRNA-seq
(Kellis lab)

79,188 transcriptomes
235 overlapping donors

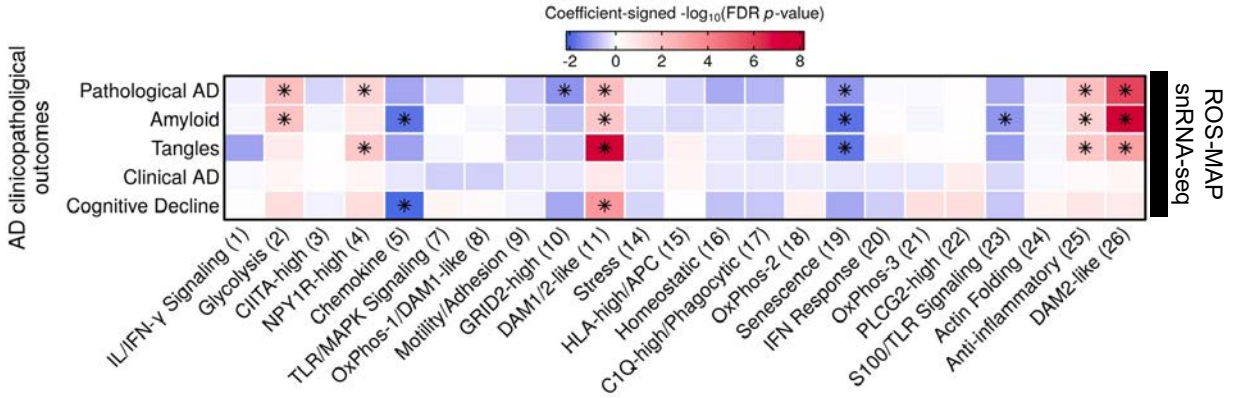
Sun et al. Human microglial state dynamics in Alzheimer's disease progression. *Cell.* 2023;186:4386-4403.e29.

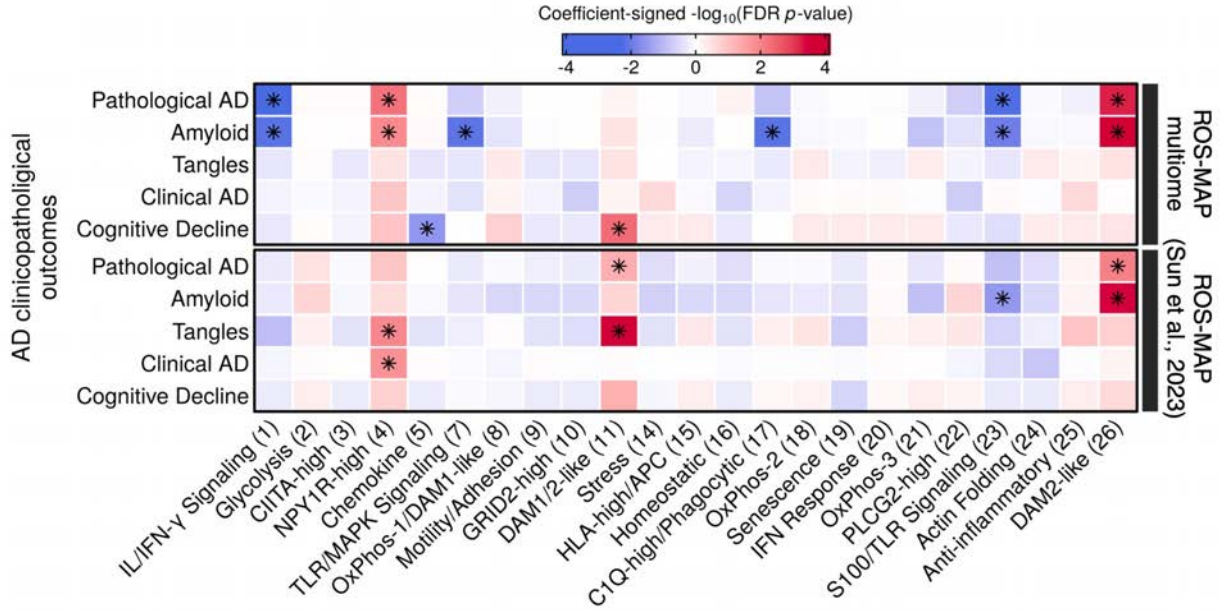


Expression programs capture stable patterns across independently-generated datasets (2)

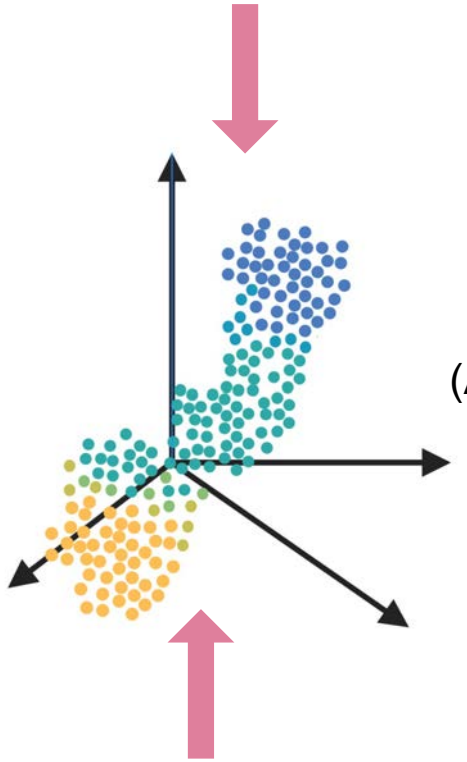


Donor-aggregated factor scores show high correlation





Identifying a putative factor regulatory network using ARACNe

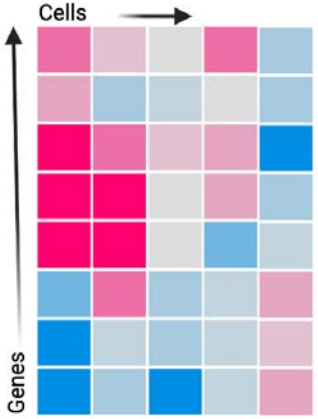


ARACNe
(Algorithm for the Reconstruction of Accurate Cellular Networks)

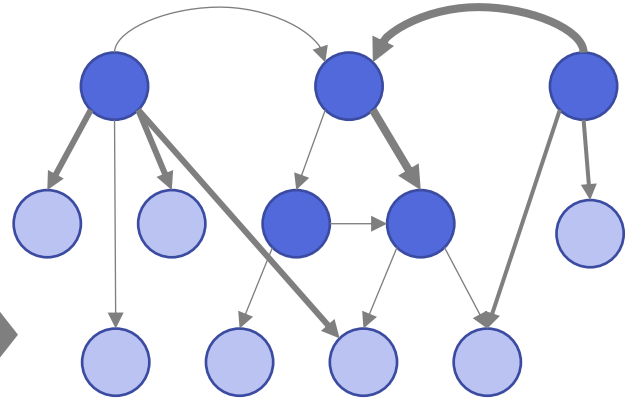
Transcription factors[1]

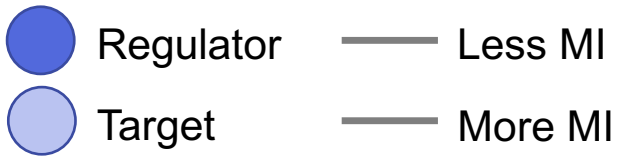
ARID5B
BAZ2B
BCL6
DBP
FOXO3
JAZF1
SPI1
ZBTB16
...

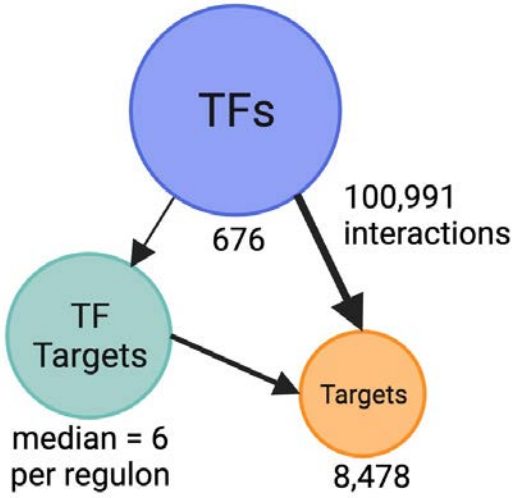
Expression Matrix



Regulons

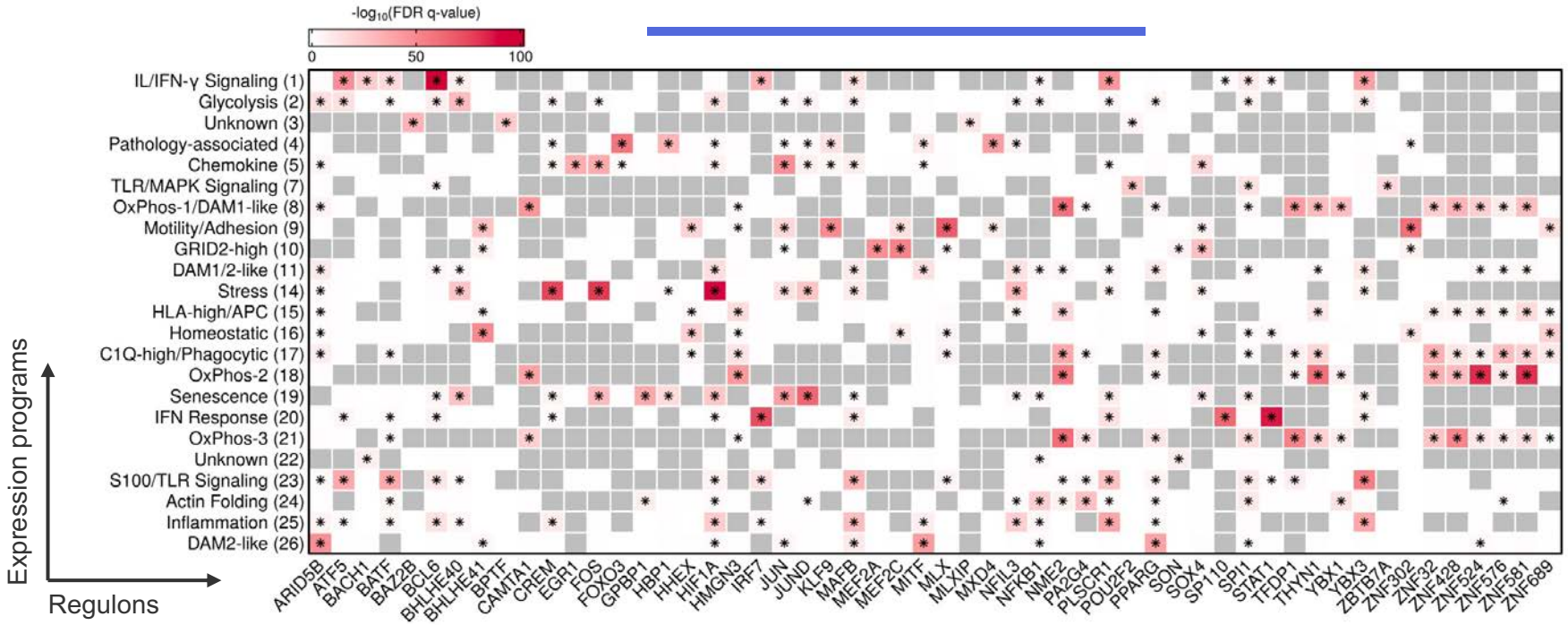




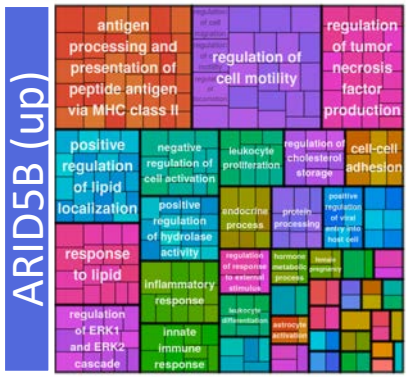


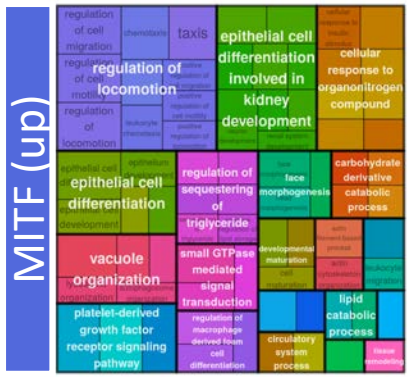
Lambert et al. The Human Transcription Factors. Cell 172, 650–665 (2018).
Margolin *et al.* ARACNE: An Algorithm for the Reconstruction of Gene Regulatory Networks in a Mammalian Cellular Context. *BMC Bioinformatics* **7**, S7 (2006).

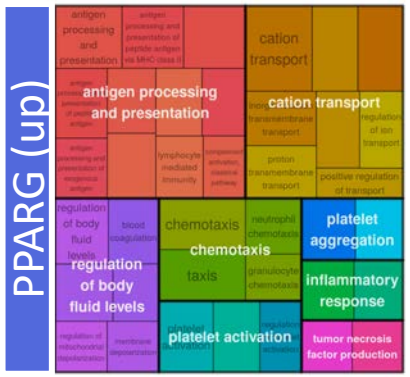
Microglial expression programs show unique and shared regulons which point toward key processes associated with disease

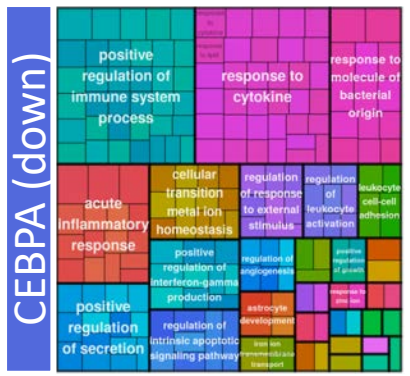


DAM2-like (26)

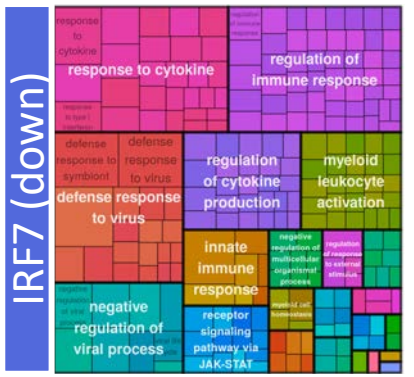








IFN response (20)

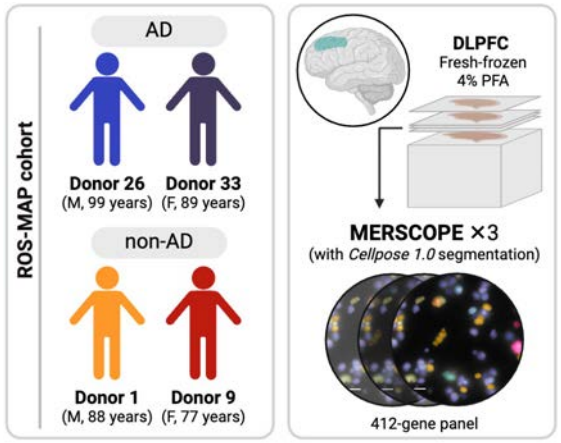


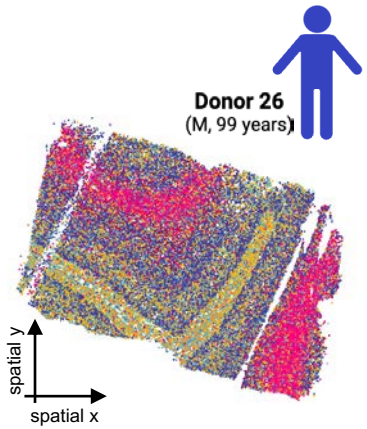
Spatially-resolved MERFISH shows *in situ* association between *ARID5B* and genes comprising DAM2-like factor 26

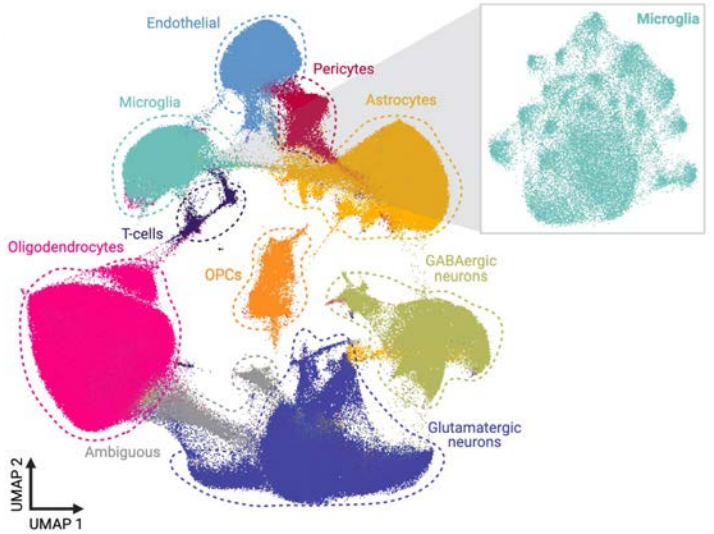
TFs validation

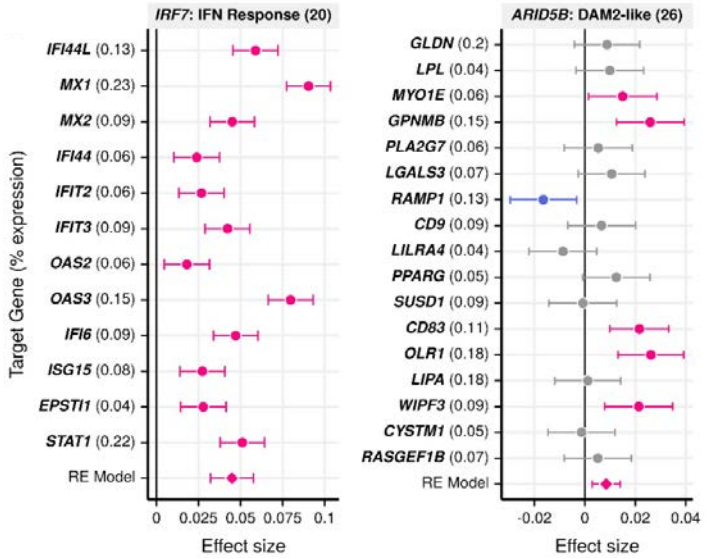
DAM2-like (26)
ARID5B
MITF
PPARG
CEBPA

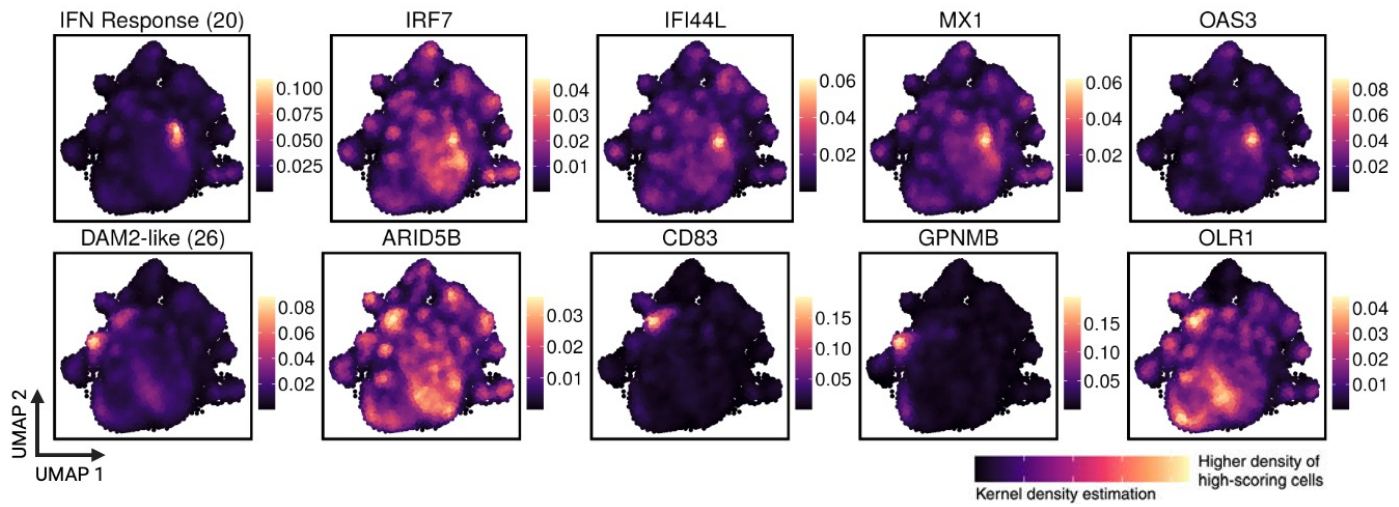
IFN response (20)
IRF7





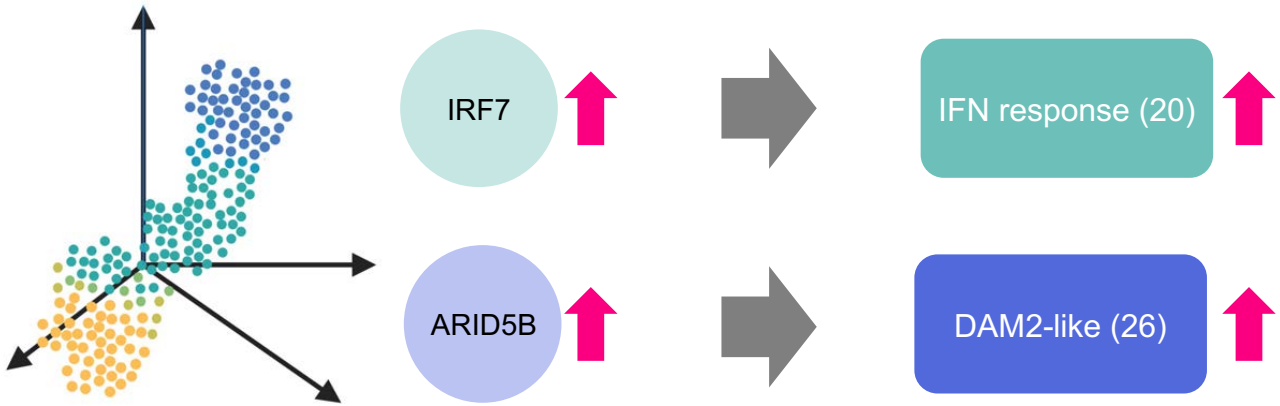






Continuous expression programs and their regulators in microglia: take-aways

- Identifying discrete subtypes of microglia is challenging
- scHPF factorization is allowing us to define continuous expression programs which show biological and disease-associated relevance
- scHPF recapitulates signals across single-cell and single-nucleus data, human-derived and model systems
- There is a complex network of regulation for expression programs, pointing toward factor-specific and shared regulators
- Spatially-resolved single-cell data provides support for *ARID5B* association with greater expression of DAM2-like factor (26)



IRF7
- Enriched TF motif in antiviral subcluster (Sun et al., Cell, 2023)

ARID5B
- Enriched motif peaks in ‘activated’ states (Sun et al., Cell, 2023)

MITF
- DAM enriched TF-regulon and ↑ phagocytic activity in iMGLs (Dolan et al., 2024)
- AD-associated subtype upregulates MITF regulon (Lee et al., medRxiv, 2023)

Sun, N. *et al.* Human microglial state dynamics in Alzheimer’s disease progression. *Cell* (2023).
Dolan *et al.* Exposure of iPSC-derived human microglia to brain substrates enables the generation and manipulation of diverse transcriptional states in vitro. *Nat Immunol* (2023).
Lee *et al. Plasticity of Human Microglia and Brain Perivascular Macrophages in Aging and Alzheimer’s Disease. medRxiv* (2023).

Acknowledgements

Center for Translational and Computational Neuroimmunology (CTCN)

Dr. Philip De Jager
Dr. Natacha Comandante-Lou
Dr. Sinah Engel
Dr. Verena Haage
Dr. Masashi Fujita
Dr. Hans-Ulrich Klein
Dr. Vilas Menon
Dr. Marta Olah
Dr. Mariko Taga
Dr. John Tuddenham
Dr. Ya Zhang
Dr. Lu Zeng

Columbia University
Dr. Peter Sims
Dr. Annie Lee

CUIMC MS Center
Dr. Claire Riley, MD

New York Brain Bank
Dr. Andrew Teich, MD PhD

Hebrew University
Dr. Gilad Green
Dr. Naomi Habib

Banner Sun Health Research Institute (Sun City, AZ)
Dr. Thomas Beach, MD PhD

Massachusetts General Hospital (Boston, MA)
Dr. Bradley Hyman, MD PhD
Dr. Matthew Frosch, MD PhD

NIH NINDS (Bethesda, MD)
Dr. Daniel Reich, MD PhD

Brigham and Women's Hospital (Boston, MA)
Dr. Rani Sarkis, MD, MSc
Dr. Garth Cosgrove, MD
Dr. Jeffrey Helgager, MD
Dr. Golden
Dr. Page Pennell, MD

RUMC/RADC (Chicago, IL)
Dr. David Bennett, MD
Dr. Julie Schneider, MD MS

Rocky Mountain MS Center (Denver, CO)
Dr. John Corboy, MD

University of Washington (Seattle, WA)
Dr. Dirk Keene

Yale University (New Haven, CT)
Dr. David Pitt, MD

FUNDING

CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

NEURODEGENERATION CHALLENGE NETWORK

Biogen

NIH National Institutes of Health