Mapping Central Nervous System Immune Cells (II)

- Interdisciplinary Technical Journal Club: special series on Laboratory Animal Science -

Silvia Sorce

4th December 2018

Outline

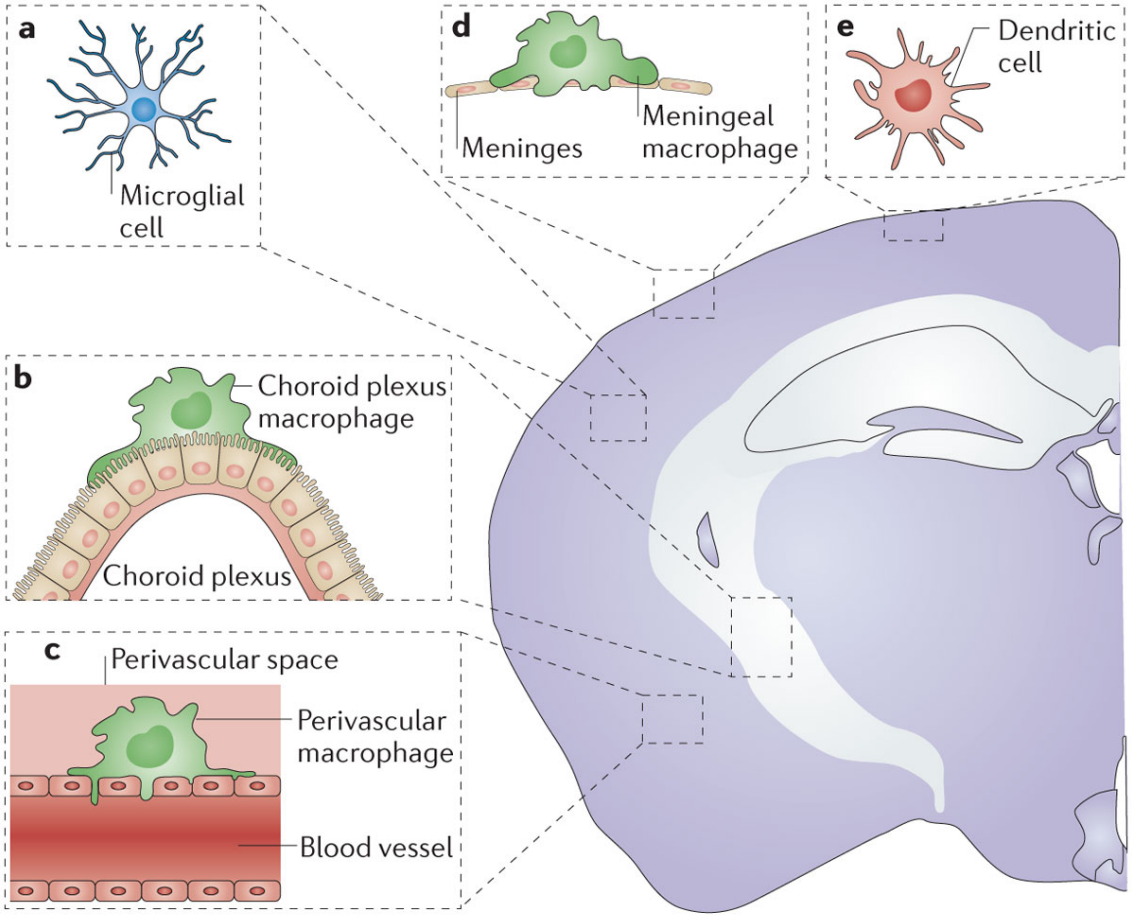
✧ Introduction: microglia and other myeloid cells in the CNS

✧ How to define them→ transcriptomics

- DAM microglia (Keren-Shaul et al., *Cell* 2017)
- Meta-analysis (Friedmann et al., *Cell reports* 2018)
- Development and injury (Hammond et al., *Immunity* 2018)

✧ Useful resource tools

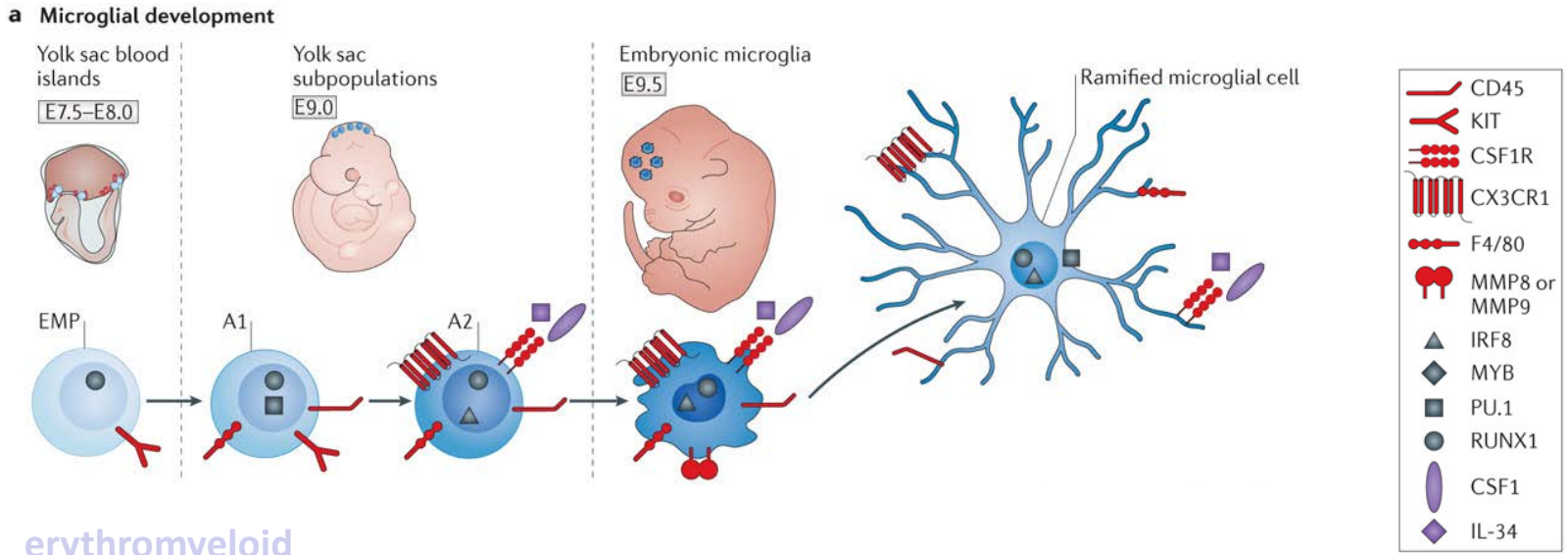
Myeloid cell types in the CNS



Under homeostatic conditions, the brain hosts several heterogeneous populations of myeloid cells that are located at distinct sites, where they execute homeostatic and surveillance tasks.
Within the brain parenchyma, microglia (part **a**) with small delineated processes actively screen the intraneuronal space for incoming threats, whereas macrophages can be found in the outer boundaries of the brain, such as the choroid plexus (part **b**), perivascular space (part **c**) and in the meninges (part **d**).
Blood-derived dendritic cells (part **e**) are present at low numbers in the same locations as macrophages.

Prinz and Priller. *Nature Reviews Neuroscience* **volume 15**, pages 300–312 (2014)

Embryonic and postnatal development of microglia in mice



erythromyeloid progenitors (EMPs)→ **A1 stage → A2 cells → early microglia**

Microglia and brain border macrophages (which reside in the perivascular space, meninges, and choroid plexus) are derived **from the same pool of yolk sac hematopoietic progenitors** and migrate to the brain at the same time in development (Goldmann et al., 2016)

When microglia infiltrate the brain parenchyma and are exposed to brain-derived signals that they achieve their unique identity

Prinz and Priller. *Nature Reviews Neuroscience* **volume 15**, pages 300–312 (2014)

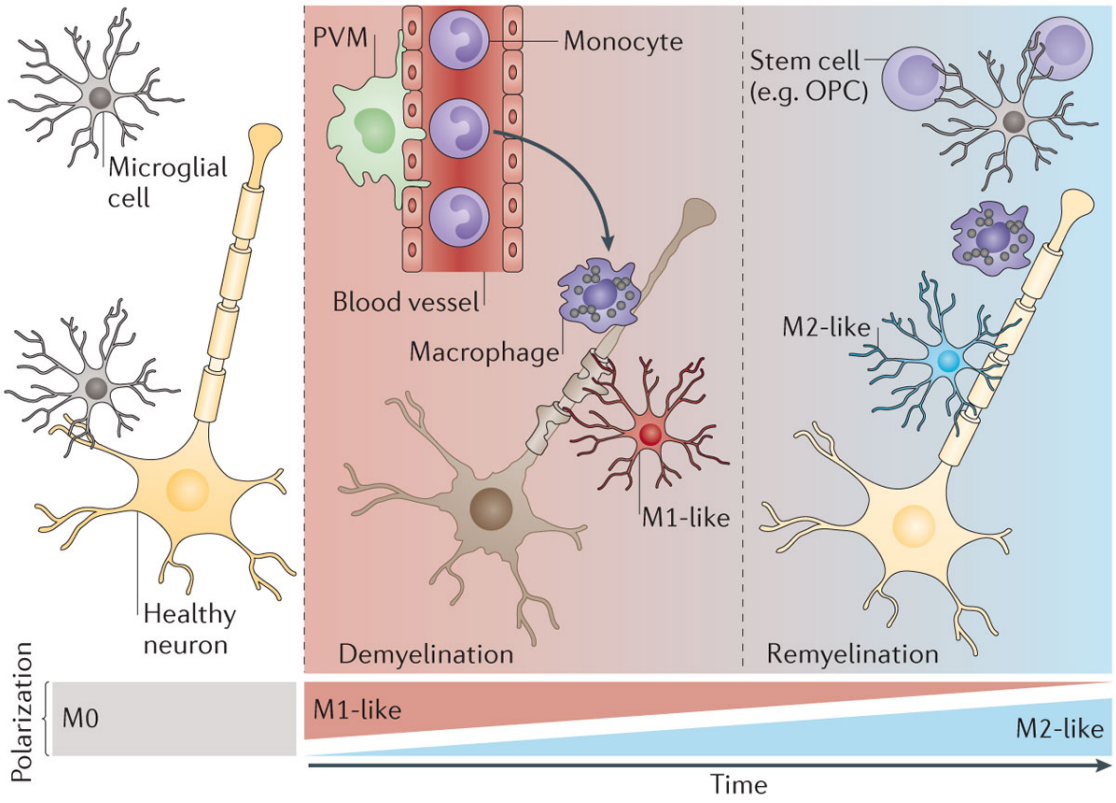
Microglia functions

→ Microglia are essential for **maintaining the health and function** of the brain

→ **During development**:
- pruning synapses
- modulating neurogenesis
- phagocytosing apoptotic cells
- regulating synapse plasticity and myelin formation

→ **In response to injury, pathology, or aging:**
- rapid proliferation
- migration to the site of pathology
- phagocytosis of cells and debris
- production of the cytokines and chemokines necessary to stimulate microglia and other brain and immune cells.

Functional reprogramming of microglia and macrophages in response to brain injury



Under physiological conditions,microglia are continuously surveying their microenvironment. We have named this so-called **'resting' state of microglia M0**.

Neuronal dysfunction or damage can activate microglia to **produce pro-inflammatory cytokines (M1-like polarization).** Depending on the degree of homeostatic disturbances, leukocytes (not shown) may be recruited from the bloodstream. Peripherally derived macrophages (purple) and perivascular macrophages (PVMs) also participate in the inflammatory response.

As a result of the passage of time, the type of brain injury or environmental factors, microglia and/or peripherally derived monocytes and macrophages may acquire **an anti-inflammatory phenotype, which causes them to remove debris and promote regeneration (M2-like polarization)**. This may entail the recruitment and differentiation of local stem and progenitor cells, such as oligodendroglial progenitor cells (OPCs) for remyelination. However, it is important to note that the activation states of microglia and macrophages are not strictly dichotomous but are part of a spectrum of functional states.

Prinz and Priller. *Nature Reviews Neuroscience* **volume 15**, pages 300–312 (2014)

Open question

In neurodegenerative diseases, microglia contribution is:

- ❑ beneficial but insufficient
- ❑ effective at early disease stages but lose their efficacy later on
- ❑ detrimental with disease progression
- ❑ …

REVIEW

Microglia: Scapegoat, Saboteur, or Something Else?

Adriano Aguzzi,[1*] Ben A. Barres,[2] Mariko L. Bennett[2*]

Microglia are resident immune cells in the brain and spinal cord. These cells provide immune surveillance and are mobilized in response to disparate diseases and injuries. Although microglial activation is often considered neurotoxic, microglia are essential defenders against many neurodegenerative diseases. It also seems increasingly likely that microglial dysfunction can underlie certain neurological diseases without an obvious immune component.

Why single-cell RNA-seq?

→ **Heterogeneous cell populations** currently isolated based on a **small set of surface markers** : limited in resolving the heterogeneity, niche specificity, complexity

→ **Conflicting results** regarding their role/profile during **disease onset and progression**

→ **Different profiles** based on the **type of injury or disease**

→ To identify and molecularly describe distinct groups of microglia

Cell

Article

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

Hadas Keren-Shaul,[1,6] Amit Spinrad,[1,2,6] Assaf Weiner,[1,3,6,*] Orit Matcovitch-Natan,[1,2,6] Raz Dvir-Szternfeld,[2] Tyler K. Ulland,[4] Eyal David,[1] Kuti Baruch,[2] David Lara-Astaiso,[1] Beata Toth,[5] Shalev Itzkovitz,[5] Marco Colonna,[4] Michal Schwartz,[2,7,*] and Ido Amit[1,7,8,*]

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http://dx.doi.org/10.1016/j.cell.2017.05.018

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Resource

Diverse Brain Myeloid Expression Profiles Reveal Distinct Microglial Activation States and Aspects of Alzheimer's Disease Not Evident in Mouse Models

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https://doi.org/10.1016/j.celrep.2017.12.066

Resource

Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes

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Cell

Article

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

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http://dx.doi.org/10.1016/j.cell.2017.05.018

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→ **Definition of Disease Associated Microglia (DAM)**

5xFAD: five human familial AD gene mutations

- Contains **2 transgenes**:

- **mutant human amyloid beta** (A4) **precursor protein** (*APP*) cDNA sequence (altered to include the APP K670N/M671L (Swedish) + I716V (Florida) + V717I (London) Familial Alzheimer's Disease (FAD) mutations) inserted into exon 2 of the mouse *Thy1* gene.

- **mutant human presenilin 1** (Alzheimer disease 3) (*PSEN1* or PS1) cDNA sequence (altered to include the PS1 M146L + L286V FAD mutations) inserted into exon 2 of the mouse *Thy1* gene

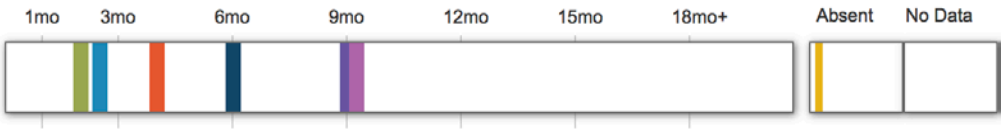
- Both transgenes were added together in equal proportions and **co-injected into the pronuclei of single-cell "C57/B6xSJL" hybrid embryos**.

- Founders from the highest APP expressing line (Tg6799) were **bred with (B6/SJL)F1 for more than 10 generations** with stable germline transmission and expression of both transgenes, demonstrating that these "5XFAD" mice breed as single transgenics.

https://www.jax.org/strain/006554

5xFAD: five human familial AD gene mutations

PHENOTYPE CHARACTERIZATION



Tangles	Absent.
Neuronal Loss	Neuron loss in cortical layer 5 and subiculum.
Plaques	Amyloid deposition begins at 1.5 months and reaches high levels especially in subiculum and deep cortical layers. Aβ42 also accumulates intraneuronally in an aggregated form within the soma and neurites starting at 1.5 months (Oakley et al., 2006).
Gliosis	Gliosis begins at 2 months (Oakley et al., 2006).
Synaptic Loss	Synaptic markers synaptophysin, syntaxin, and PSD-95 decrease with age and are significantly reduced by 9 and 12 months.
Changes in LTP/LTD	LTP is normal in young animals, but becomes impaired around 6 months (Kimura et al., 2009); specifically, in hippocampal slices from < 4-month-old mice, I/O curves of fEPSPs were not different from those of wild-type controls, but the I/O responses at Schaffer collateral-CA1 synapses at 6 months were impaired.
Cognitive Impairment	Impaired spatial memory in Y-maze test at 4-5 months. Impaired stress-related memory, specifically significantly lower levels of contextual freezing at 6 months. Impaired remote memory stabilization at < 4 months.

- On the mixed C57BL/6 and SJL background (see MMRRC stock **34840**, intraneuronal Abeta-42 accumulation is observed starting at 1.5 months of age, just prior to amyloid deposition and gliosis, which begins at two months of age.
- On a congenic C57BL/6J genetic background (see MMRRC stock 34848) it has been the observation of the MMRRC that this phenotype is not as robust as that demonstrated in the mixed C57BL/6 and SJL background

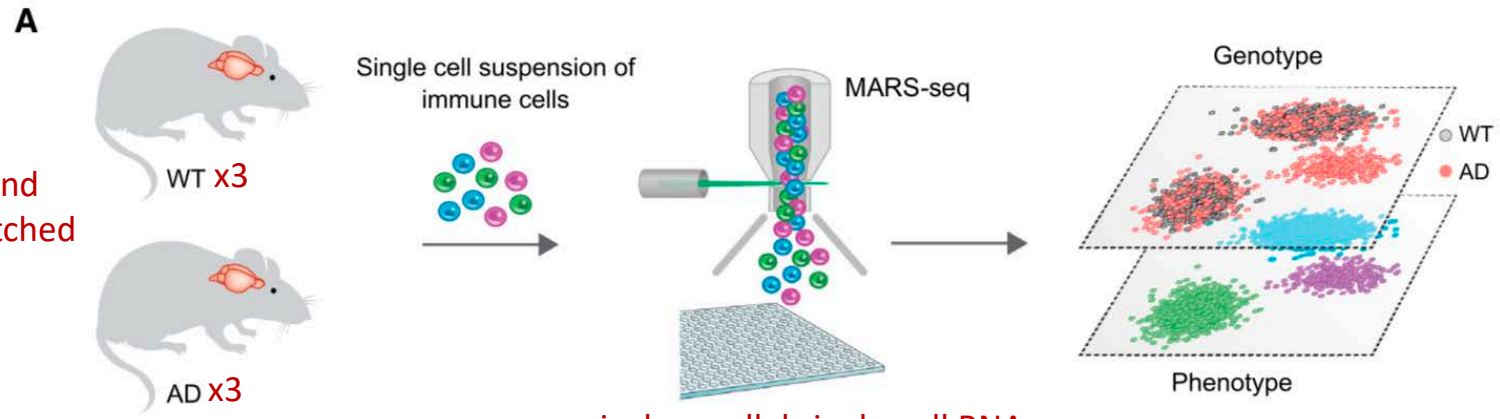
https://www.jax.org/strain/006554

https://www.alzforum.org/research-models/5xfad

Experimental plan

8,016 CD45⁺ single cells from full brain

phenograph



massively parallel single-cell RNA-seq

Experimental Models: Organisms/Strains		
Mouse: 5XFAD Tg6799	The Jackson Laboratory	34840-JAX
Mouse: C57BL/6 WT	Harlan	N/A
Mouse: SOD1-G93A	The Jackson Laboratory	002726
Mouse:Trem2$^{-/-}$	Generated in the Laboratory of Dr. Marco Colonna	N/A

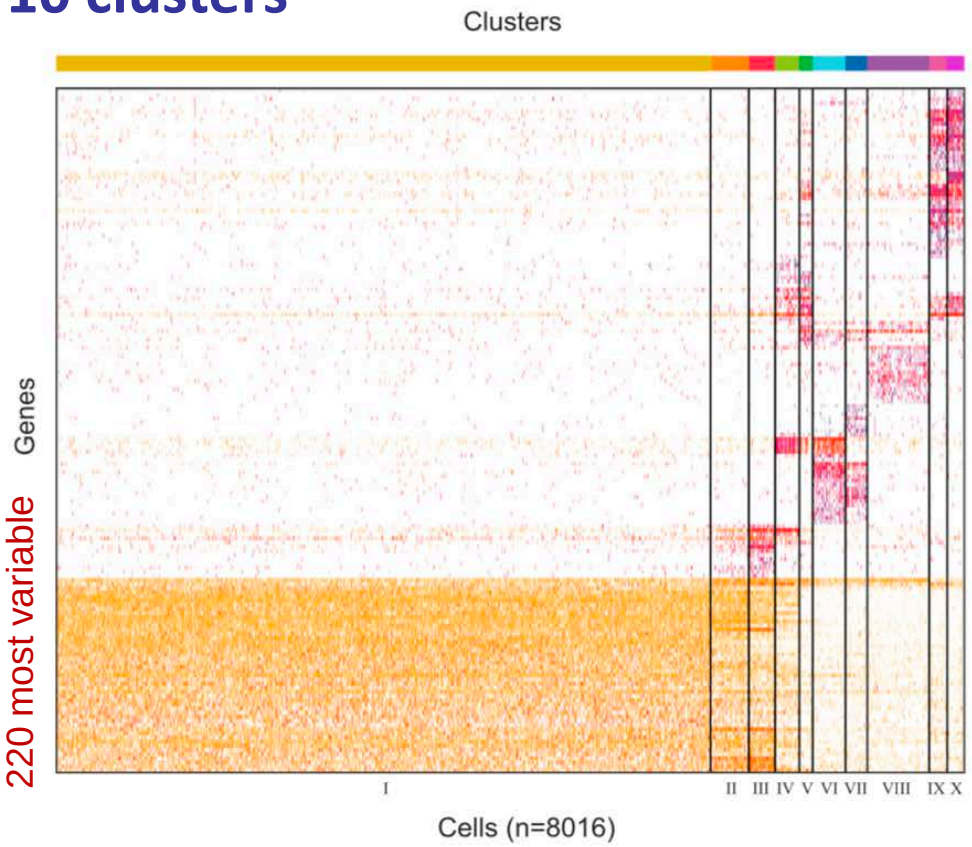
Phenograph → based on t-SNE analysis (t-Distributed Stochastic Neighbor Embedding)

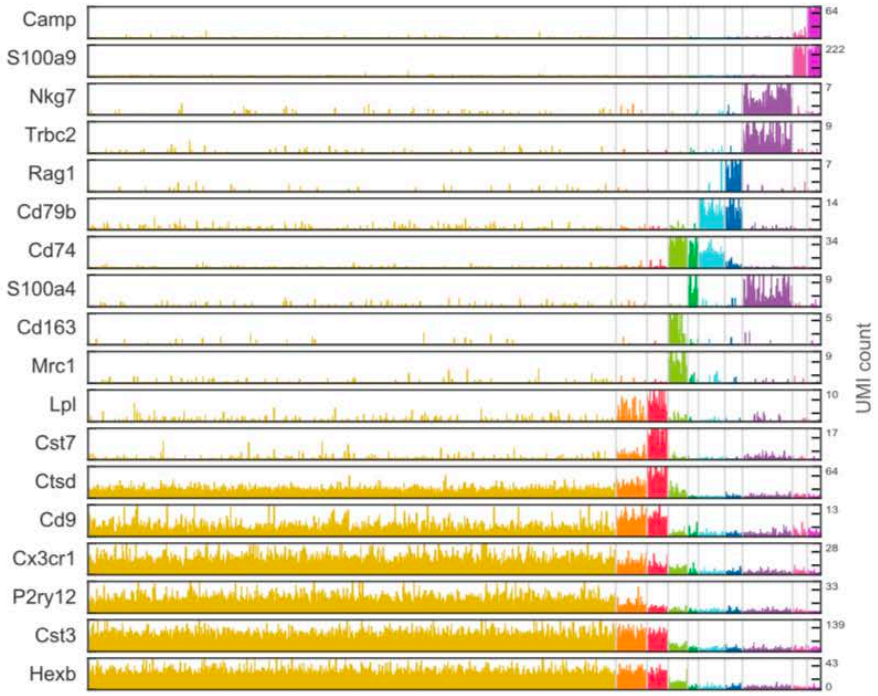
500 most variable genes to define subpopulations

clusters annotation was done manually based on the expression of a large number of hallmark genes, for example, CD3 for T cells, S100A6 for granulocytes and Hexb, Cst3 and Cx3cr1 for microglia

Keren-Shaul et al., *Cell* 2017

10 clusters





The expression level (Unique Molecular Identifier; UMI count) of selected marker genes for each cluster (I–X) is shown on the right.

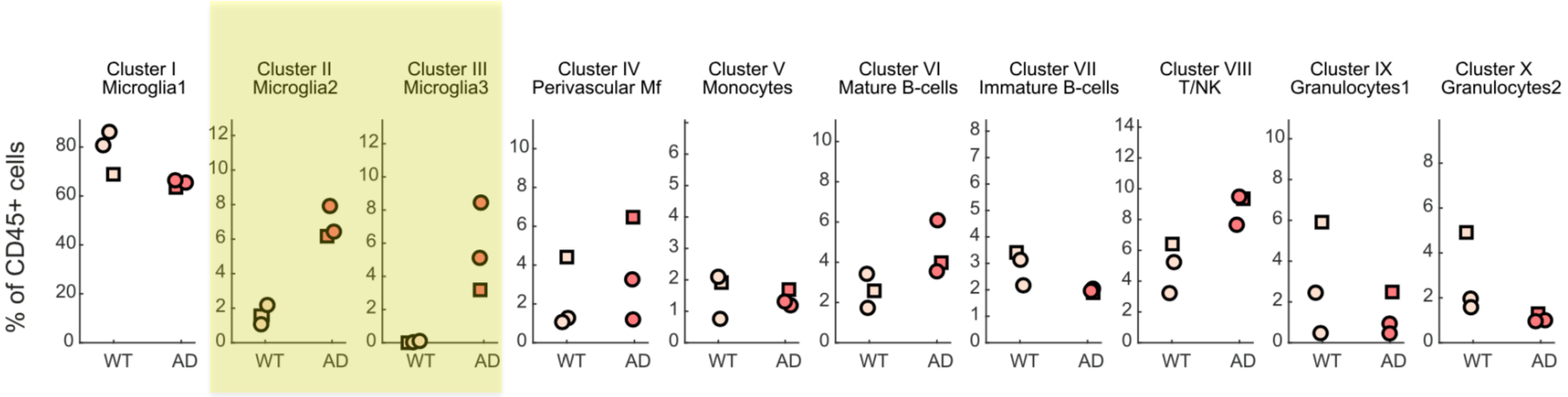
unsupervised graph-based clustering

cluster I: a large group of microglia cells
clusters II (4.2%) and III (2.8%): two small groups of cells, which displayed expression of microglial genes (Cst3 and Hexb) with an additional unique signature of lipid metabolism and phagocytic genes such as Apolipoprotein E (*Apoe*), lipoprotein lipase (*Lpl*), and Cystatin F (*Cst7*)

cluster IV: perivascular macrophage group
cluster V: monocyte state
clusters VI–VIII: several lymphocytes sub groups (B cells, T cells, natural killer [NK] cells;)

Keren-Shaul et al. *Cell* 2017

Contribution of wild-type versus 5XFAD to each group of cells



Dot plot showing the <u>percentage of WT (beige) or AD (red) cells out of the total CD45+ cells</u> in each of the clusters identified in (B).
Each data point, circle (female) or square (male), represents an independent single-cell experiment performed on an individual animal.

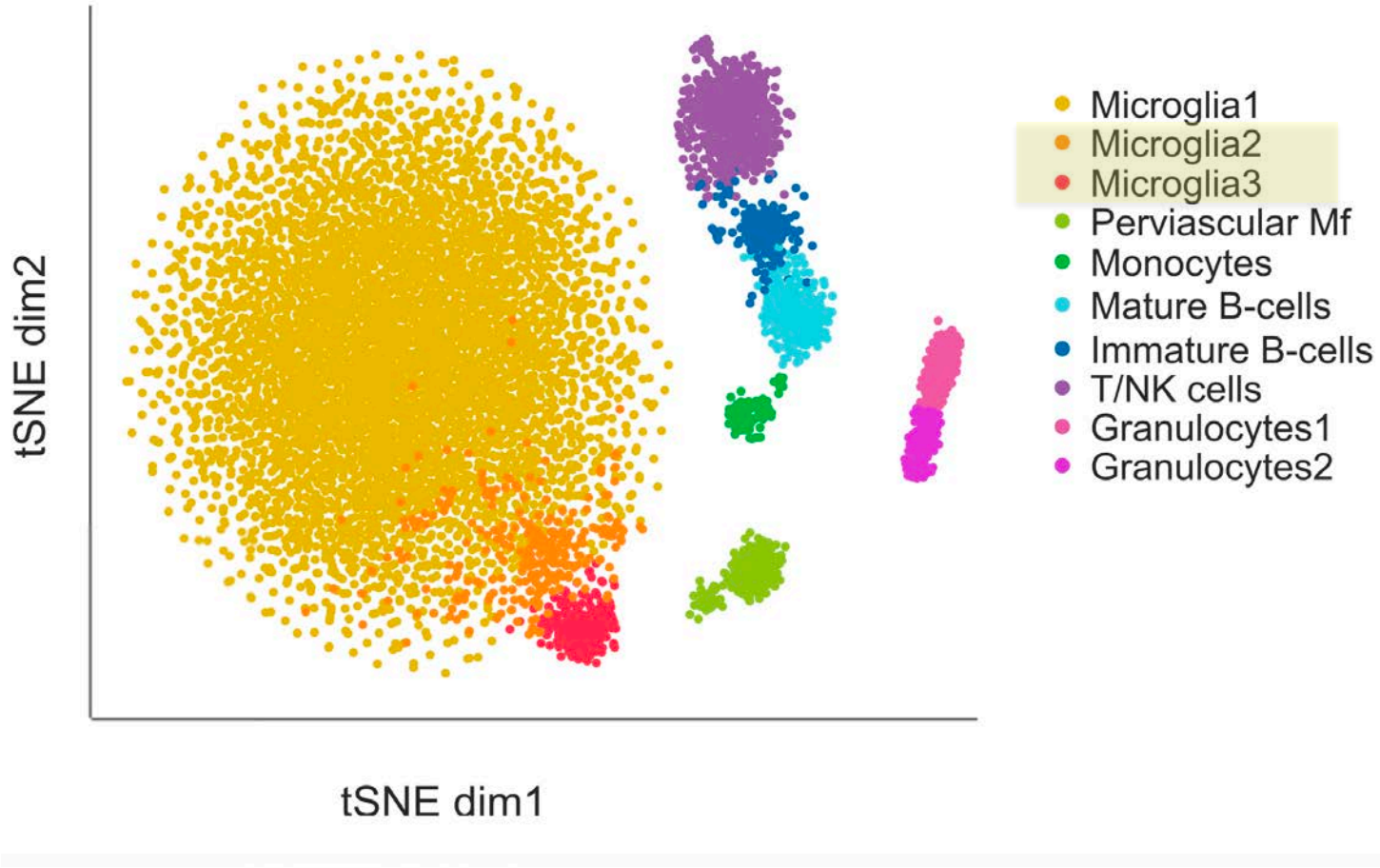
similar percentage of cells in perivascular macrophages, monocytes, group I microglia, granulocytes, lymphocytes

group II and III microglia represent distinctive microglia states observed in AD, but not in the WT background, and we define this state as

disease-associated microglia (DAM)

Keren-Shaul et al., *Cell* 2017

Projection of the cells using t-distributed stochastic neighbor embedding (t-SNE)

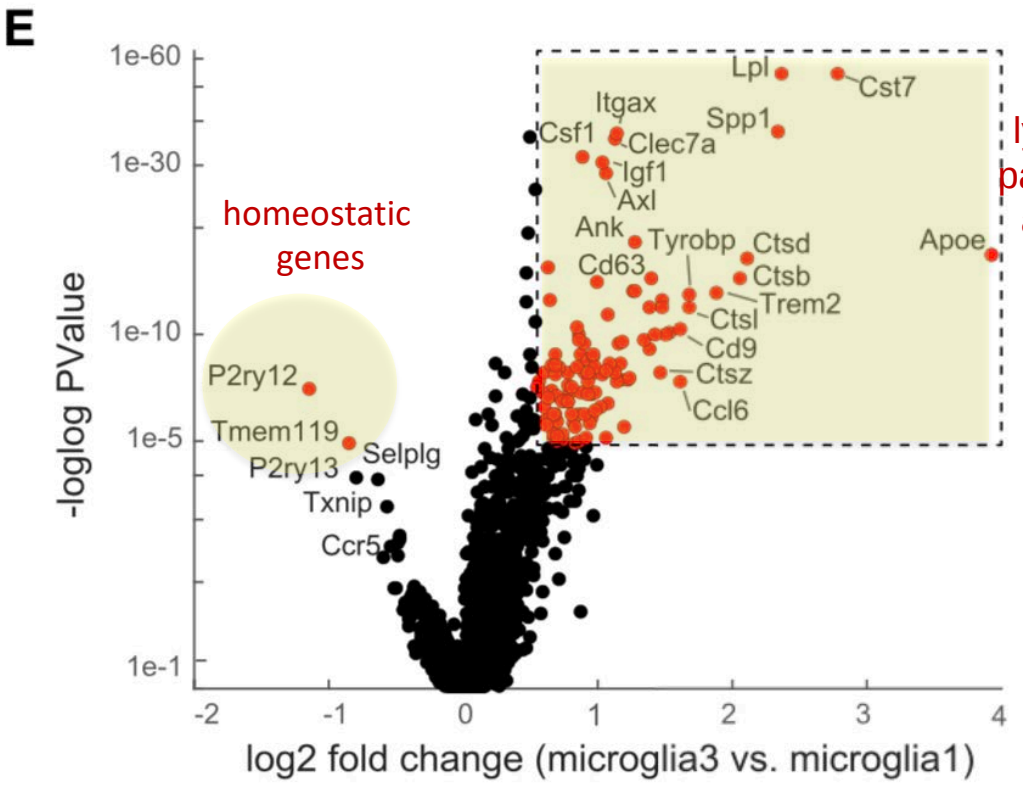


→ the DAM group in proximity to the microglia territory and distinct from the monocytes and perivascular macrophages

→ Group 3 > group 2: group 2 intermediate state

Keren-Shaul et al., *Cell* 2017

DEGs between DAM (microglia3) to homeostatic microglia (microglia1)



→ key marker genes of microglia, e.g. *Hexb* and *Cst3:* group II and III similar to group I

→ **reduction in** the expression levels of several microglia **homeostatic genes**, including the purinergic receptors *P2ry12/P2ry13, Cx3cr1*, and *Tmem119*

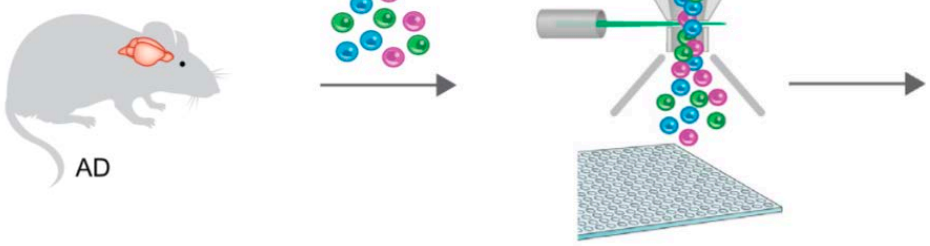
→ **genes are upregulated in DAM including several known AD risk factors, such as *Apoe, Trem2, Ctsd***

→ Table S2: top 500 different genes group 3 vs group 1 (471 UP, 29 DOWN)

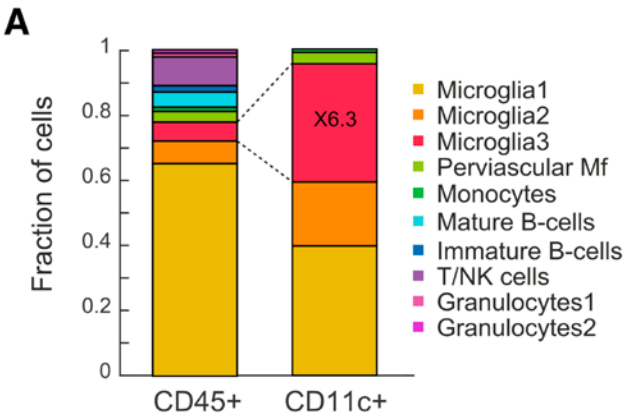
Keren-Shaul et al., *Cell* 2017

Time course of DAM isolated from 5xFAD

1, 3, 6, 8 months
whole brain



1,358 CD11c+ cells

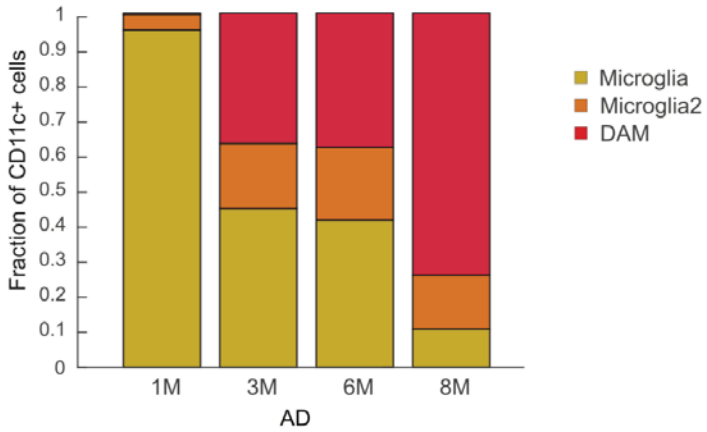


In 6-month-old mice

→ no cells with a DAM signature that are CD11c negative

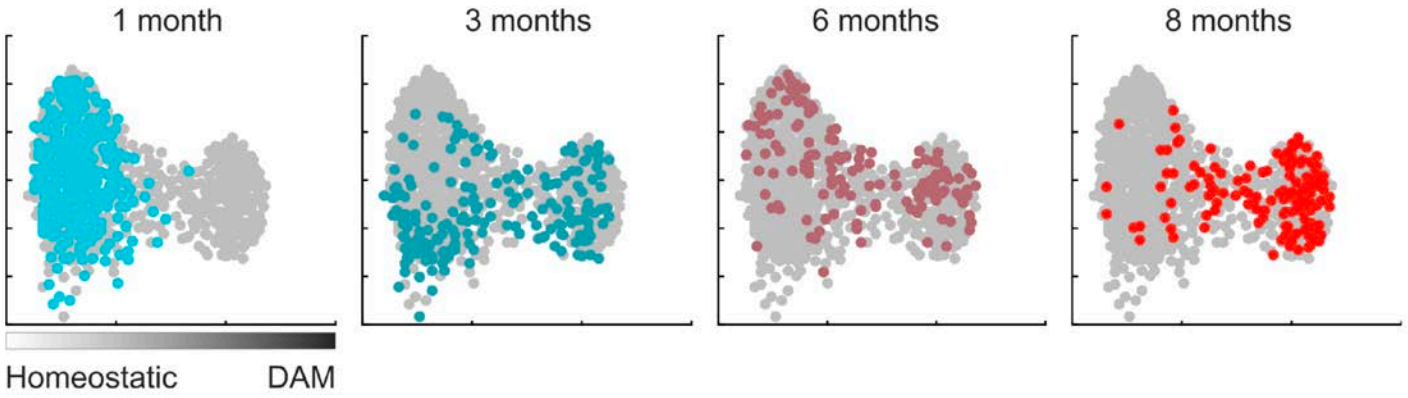
→ CD11c⁺ cells: mixture of various myeloid cells, including microglia, perivascular macrophages, and monocytes

→ *in silico* removed all myeloid contaminants from the time course data and analyzed the remaining <u>893 DAM and microglial cells</u>



Keren-Shaul et al., *Cell* 2017

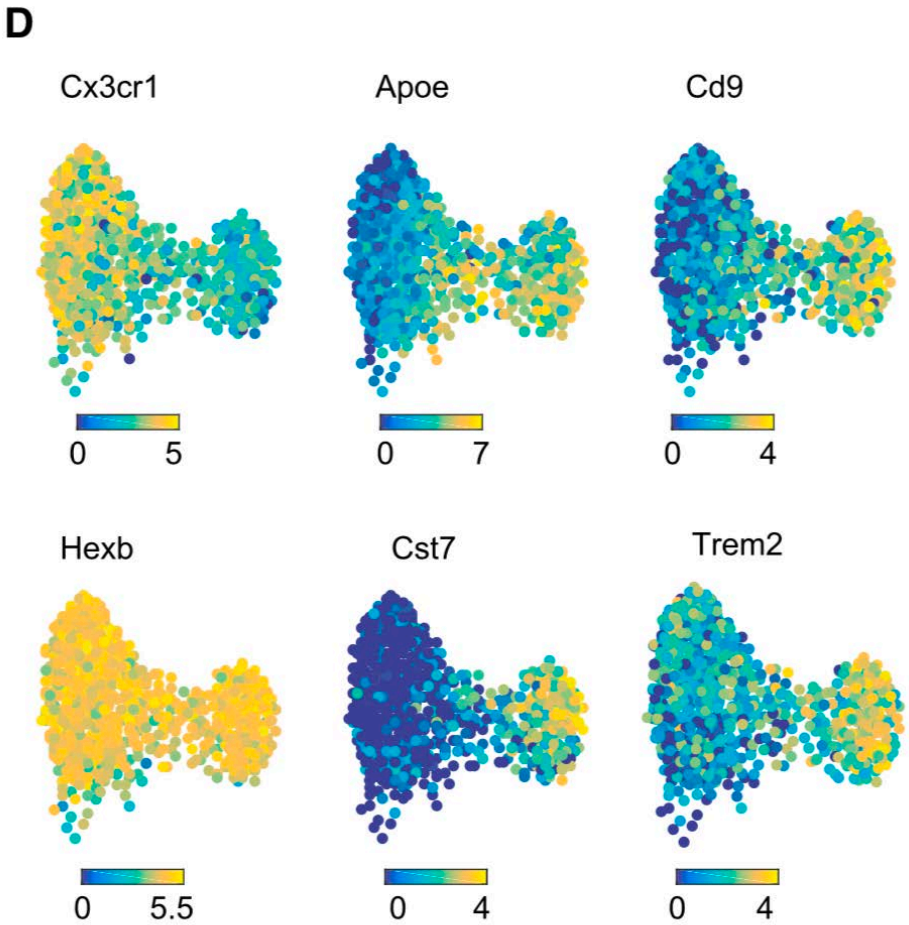
Transition from homeostatic microglia to DAM population as a function of disease progression



projection of the 893 single cells taken from the AD mouse at each time point along disease progression (1, 3, 6, 8 months; color) on the background of all microglia/all time points (gray). x axis refers to the transition axis from homeostatic microglia to DAM

Keren-Shaul et al., *Cell* 2017

Transition from homeostatic microglia to DAM population as a function of disease progression: key markers



some genes **do not change** their expression as a function of microglia transition (***Hexb***)

some genes display a **decrease** in gene expression along this activation axis (***Cx3cr1***)

some show an **increase** in their gene expression (***Apoe, Lpl, CD9, Cst7, Trem2***)

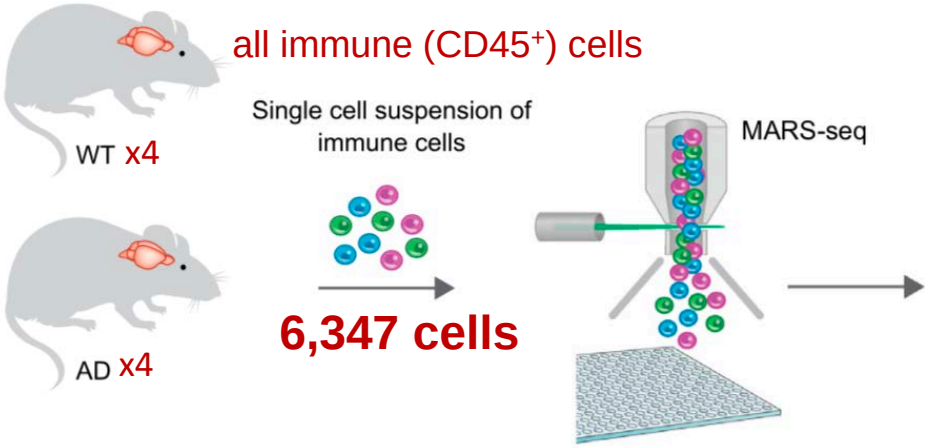
Keren-Shaul et al., *Cell* 2017

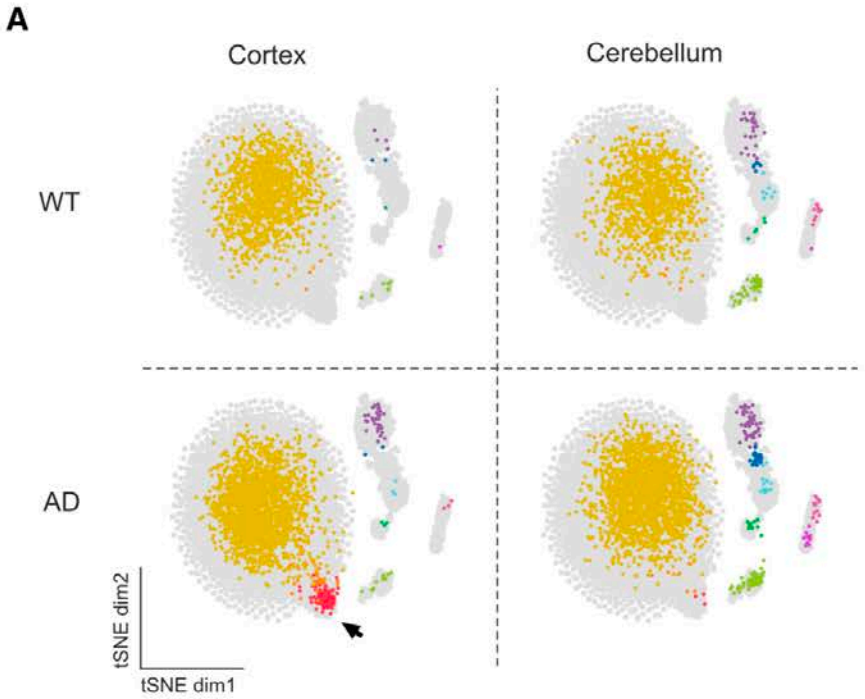
Two brain regions: cortex *vs.* cerebellum

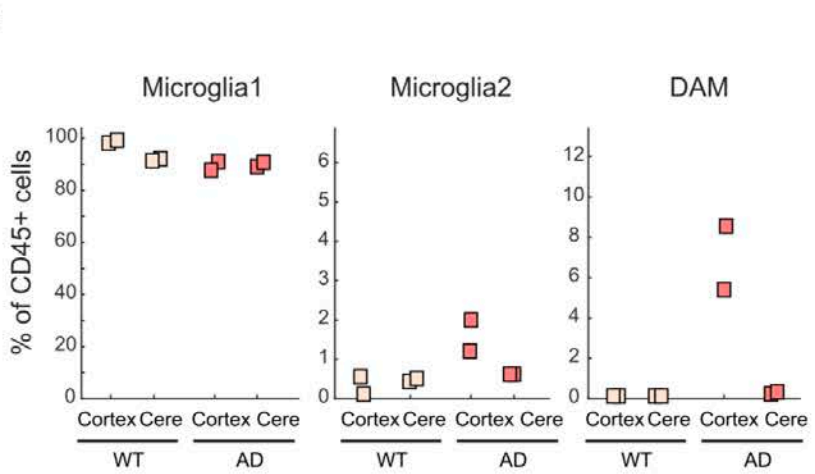
6-month-old

Cortex (affected)

Cerebellum (not affected)



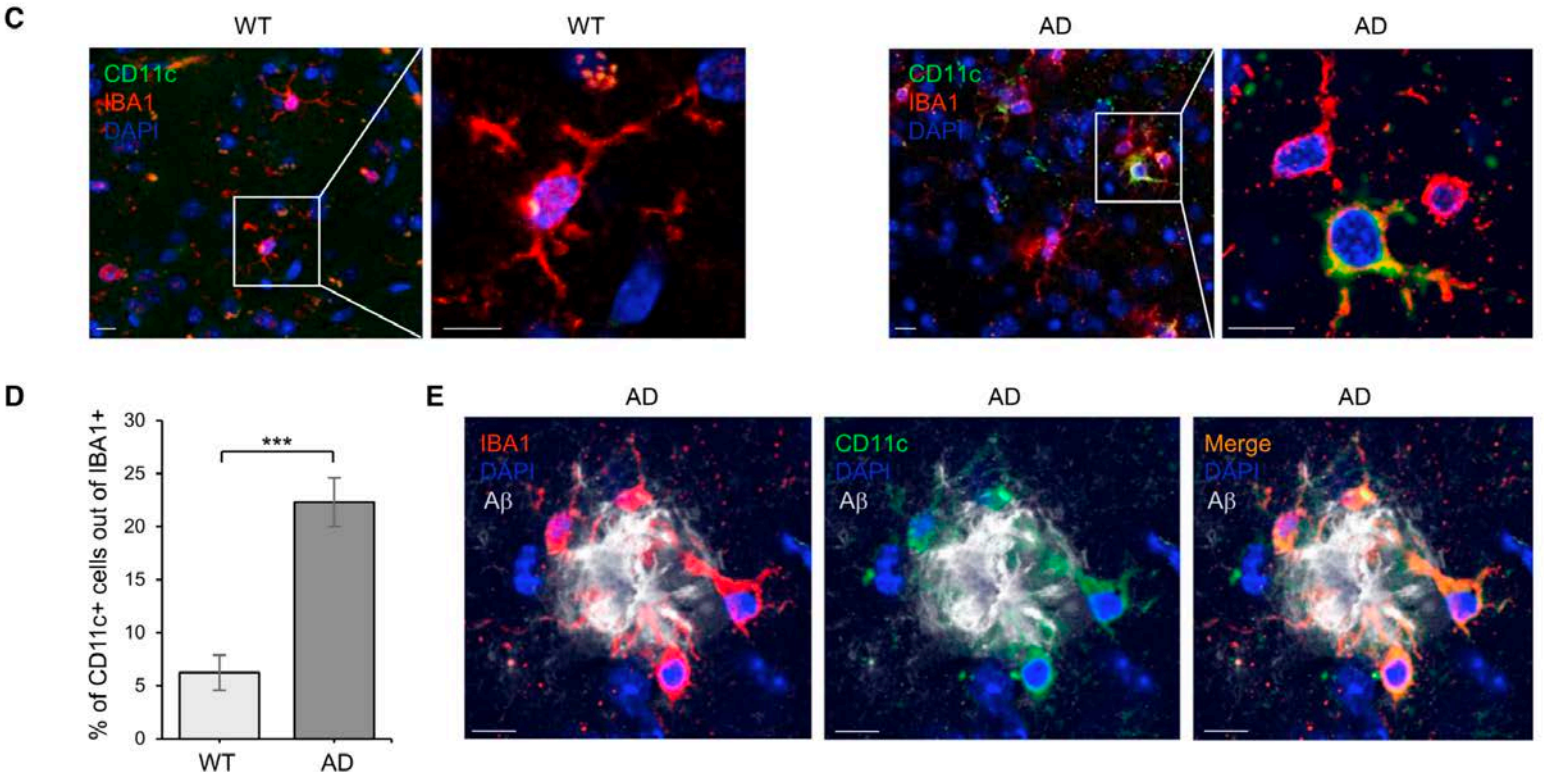




Keren-Shaul et al., *Cell* 2017

Localization of DAM

→ Staining for Cd11c/Iba1



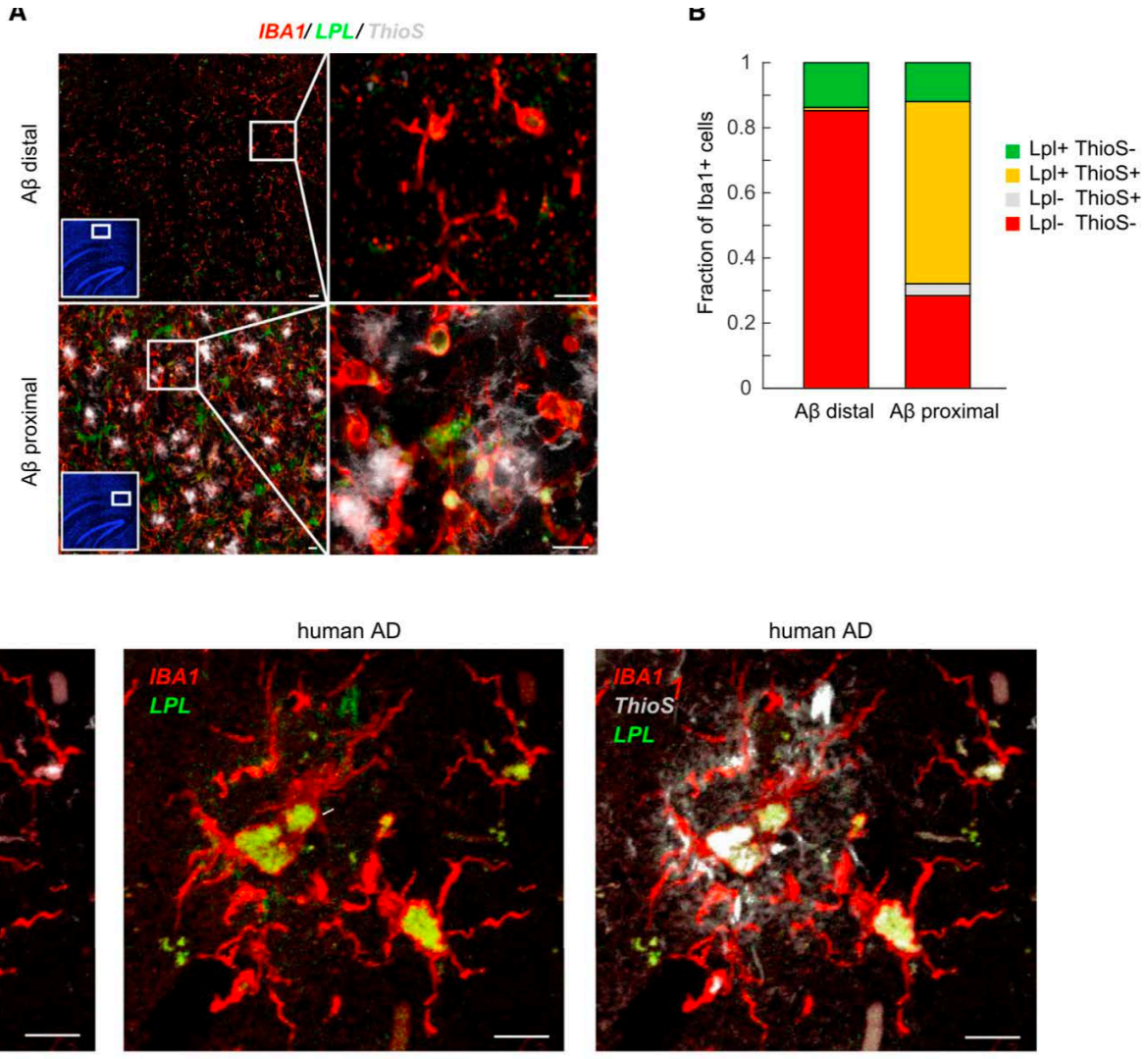
22.3% overlap in AD *vs.* 6% in WT mice

DAM population cells localized in the vicinity of the Aβ plaques

Keren-Shaul et al., *Cell* 2017

DAM are phagocytic : *Lpl staining*

***Lpl*: DAM-specific gene previously identified as an AD risk factor**

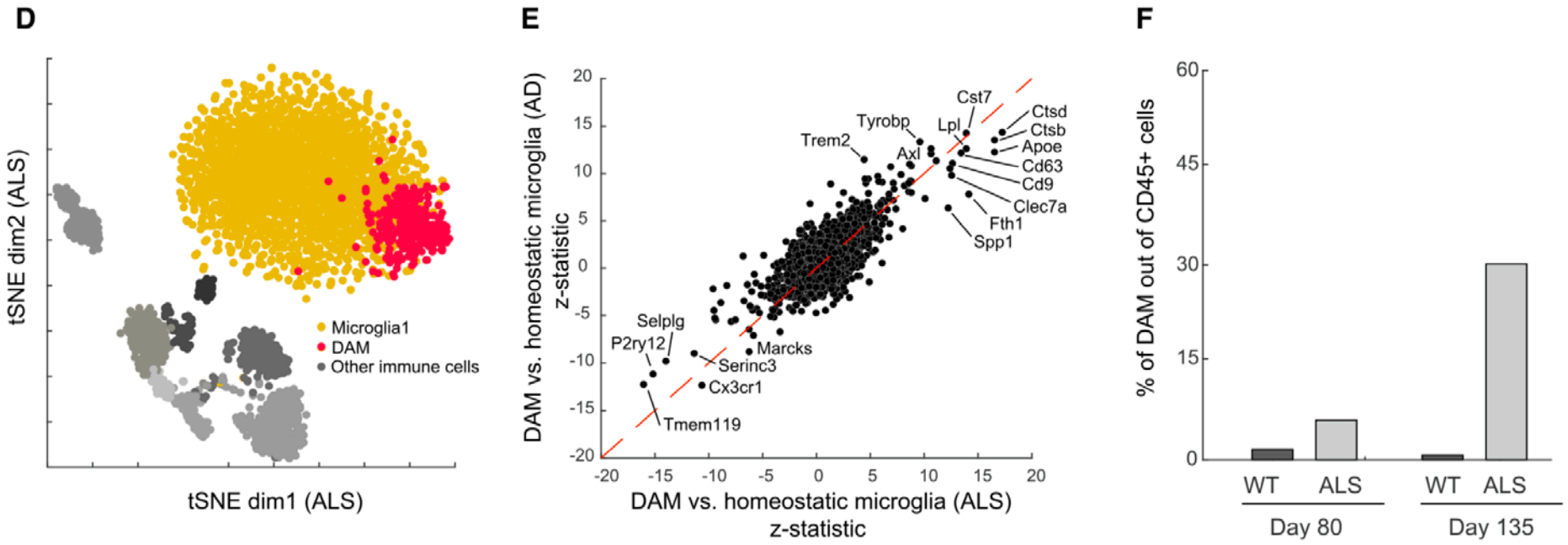


microglia containing Thioflavin-S-labeled particles are mostly clustered in close vicinity of Aβ plaques

Keren-Shaul et al., *Cell* 2017

DAM are also present in ALS mouse model

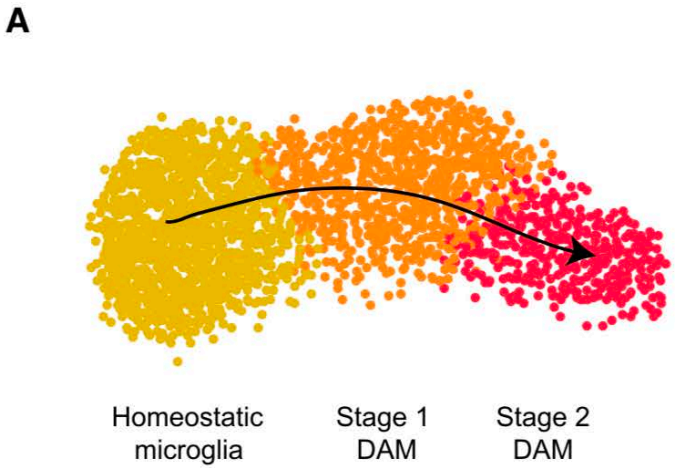
3,194 CD45^{+} cells from the spinal cords of mSOD1 (G93A) mice at early (day 80) and late (day 135) disease progression stages



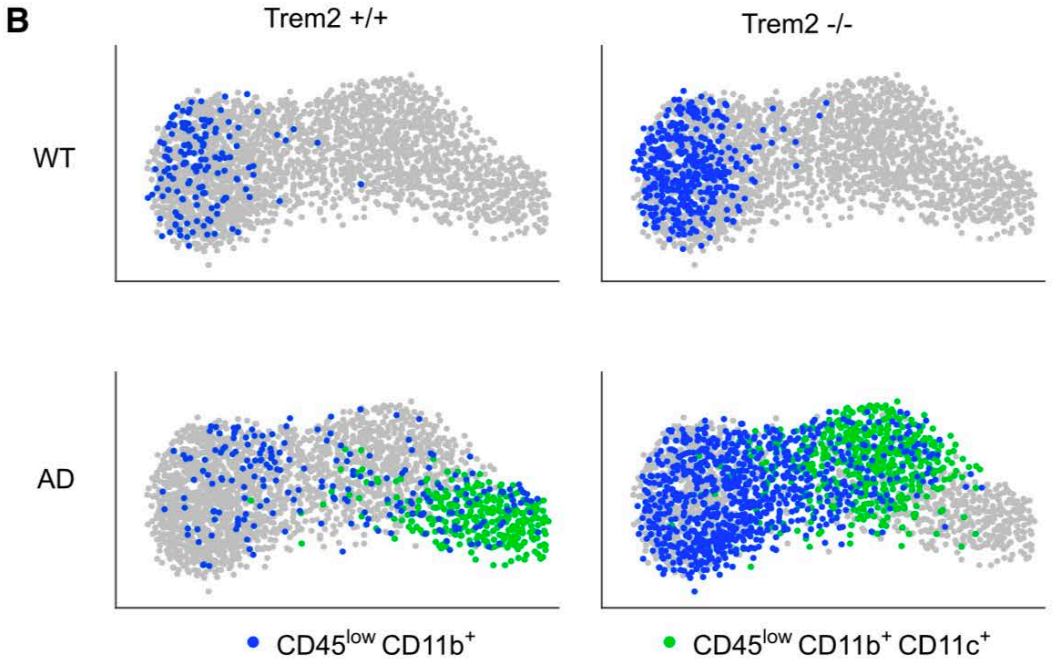
Keren-Shaul et al., *Cell* 2017

DAM and *Trem2*

Single-cell RNA-seq of DAM, using CD11c and CD11b enrichments, from whole brains of *Trem2*$^{+/+}$ 5XFAD and *Trem2*$^{-/-}$ 5XFAD mice together with matched WT and *Trem2*$^{-/-}$ controls, altogether 3,864 cells



spectrum of transcriptional states from homeostatic microglia toward the DAM state



the intermediate state, which expressed only a partial set of the DAM program, Tyrobp, Apoe, B2m, and Ctsd, but not the majority of the lipid metabolism and phagocytic pathway genes (e.g., Lpl), was much more abundant in the Trem2 knockout experiment.

Keren-Shaul et al., *Cell* 2017

Conclusion

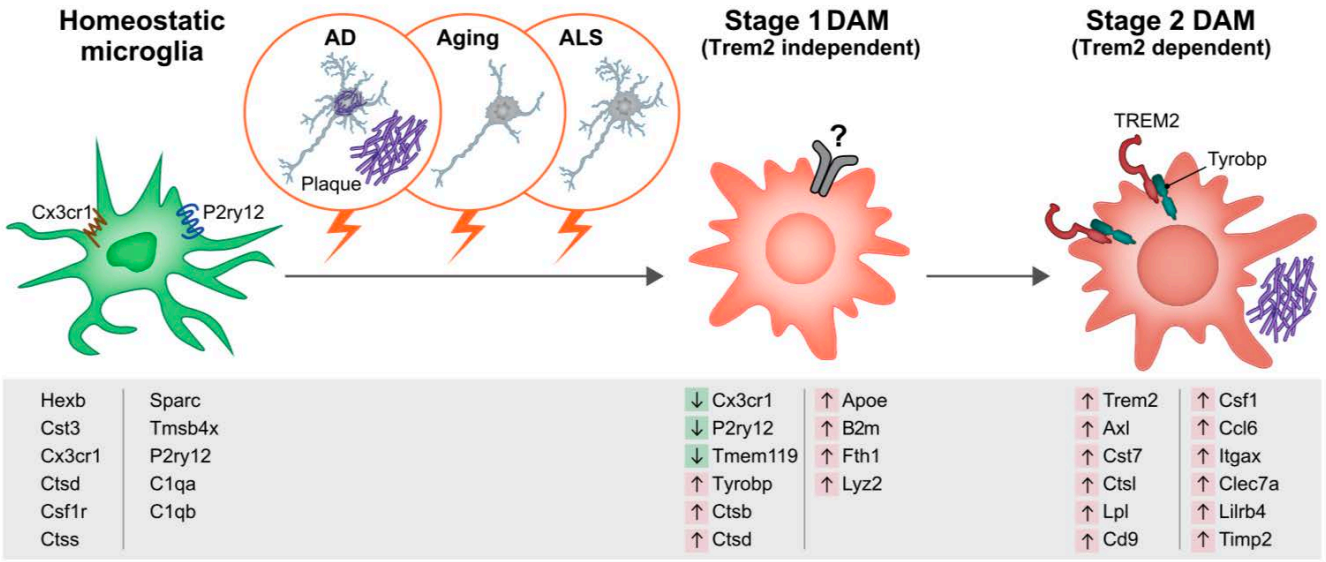


Figure 6. DAM Are Regulated through a Two-Step Activation Mechanism

Schematic illustration showing microglia switching from homeostatic to stage 1 DAM (Trem2-independent) and stage 2 DAM (Trem2-dependent) following signals such as those associated with AD pathology, aging, and ALS pathology. Key genes involved in each stage are shown below each condition. Arrows indicate up (red) or down (green) regulation of the gene in the specific stage.

step 1: initial activation through an unknown mechanism leads to an intermediate state in a Trem2-independent mechanism

step 2: secondary activation signal that is Trem2-dependent and involves upregulation of phagocytic and lipid metabolism genes such as *Cst7* and *Lpl*

→ Production of new research (therapeutic) tools based on specific markers

Keren-Shaul et al., *Cell* 2017

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Diverse Brain Myeloid Expression Profiles Reveal Distinct Microglial Activation States and Aspects of Alzheimer's Disease Not Evident in Mouse Models

Brad A. Friedman,[1,9,*] Karpagam Srinivasan,[2] Gai Ayalon,[2] William J. Meilandt,[2] Han Lin,[2] Melanie A. Huntley,[1] Yi Cao,[1] Seung-Hye Lee,[2] Patrick C.G. Haddick,[3,6] Hai Ngu,[4] Zora Modrusan,[5] Jessica L. Larson,[1,7] Joshua S. Kaminker,[1,2] Marcel P. van der Brug,[3,8] and David V. Hansen[2,*]

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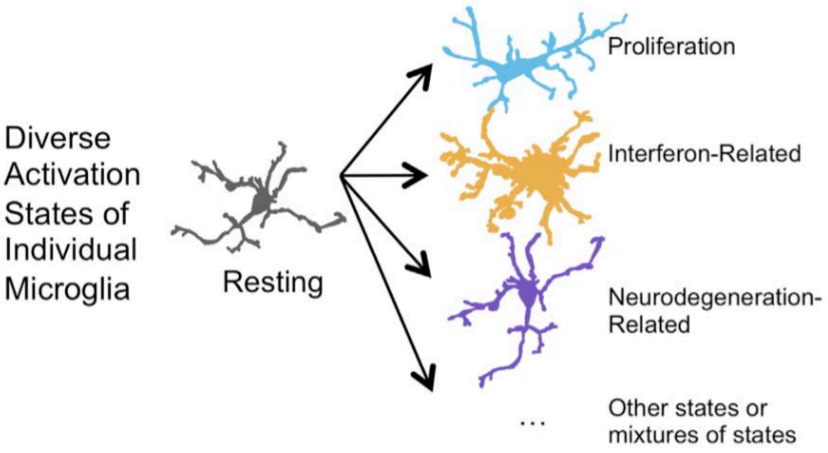
*Correspondence: friedman.brad@gene.com (B.A.F.), hansen.david@gene.com (D.V.H.)
https://doi.org/10.1016/j.celrep.2017.12.066



Experimental plan: meta-analysis

→ gene expression studies of acutely isolated microglia/myeloid cells from adult mouse brains (or spinal cords)

→ most common strategies were selection of $CD11b^{+}$, $CD11b^{+}$;$CD45^{int}$, $Cx3cr1::GFP^{+}$ cells by fluorescence-activated cell sorting (FACS)

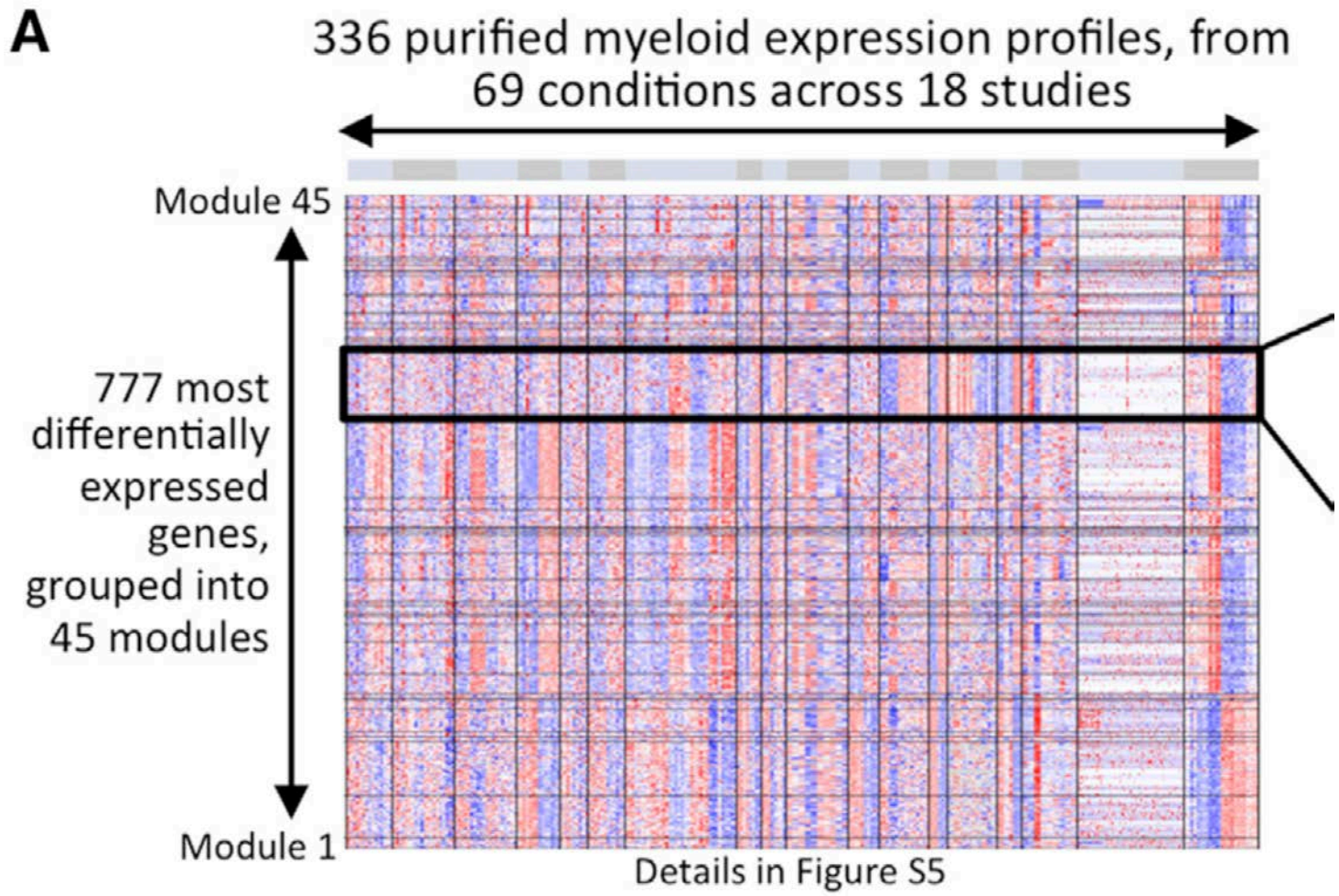
→ at least 3 replicates per treatment group

→ database included **18 datasets** spanning **69 different conditions** and **336 individual expression profiles** across a range of

- neurodegenerative, neoplastic, inflammatory, infectious disease models
- different developmental stages,
- different brain regions,
- myeloid cell subtypes

Friedman et al., *Cell Reports 22*, 832–847, January 16, 2018

45 modules of co-regulated genes

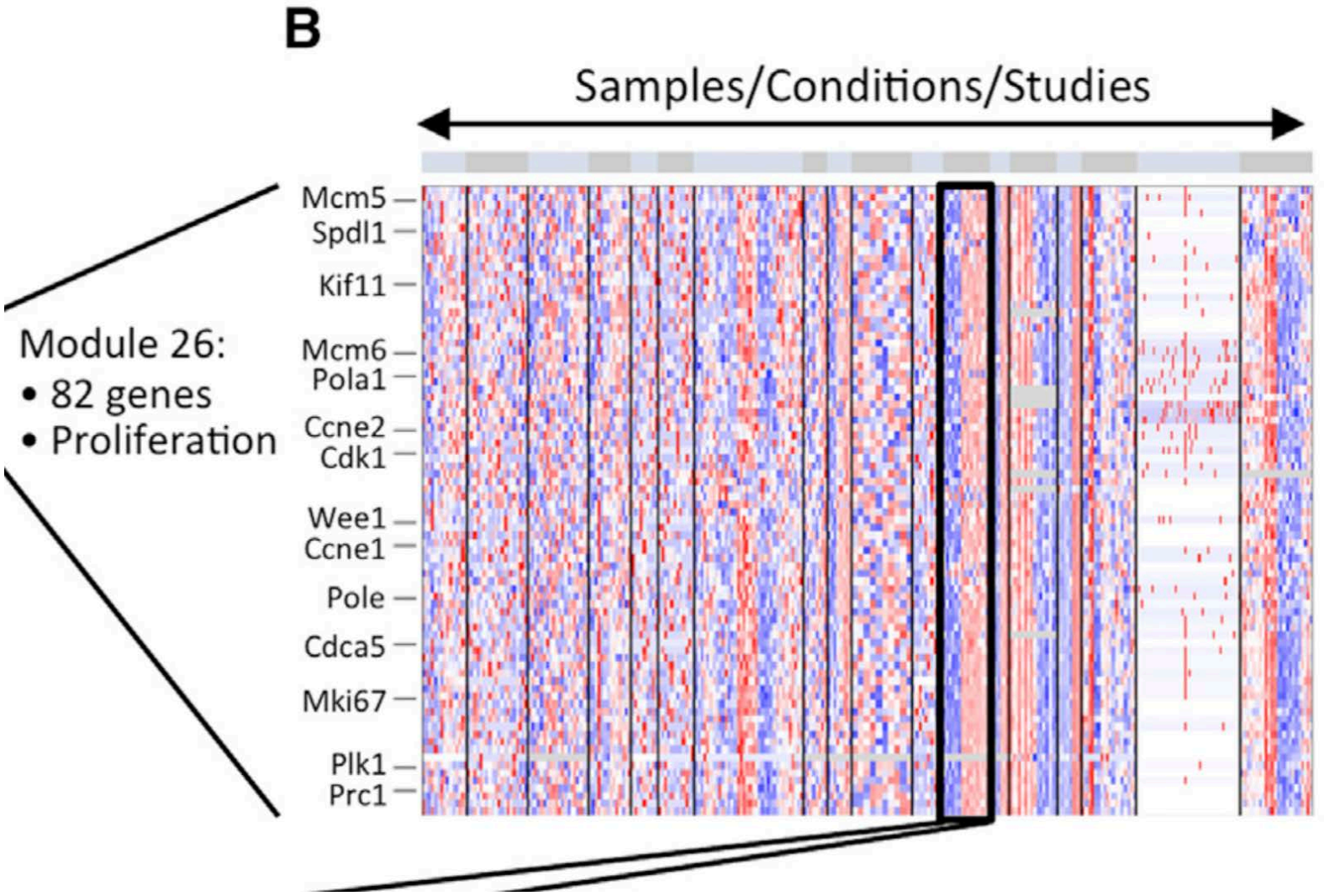


Heatmap of within-study-normalized gene expression (*Z* score) for the 777 genes (rows) **differentially expressed in at least 7 comparisons** in 18 different studies (columns).
Hierarchical clustering identified **45 modules of co-regulated genes**

→ Data S1: excel file with list of all genes/all studies

Friedman et al., *Cell Reports*, 2018

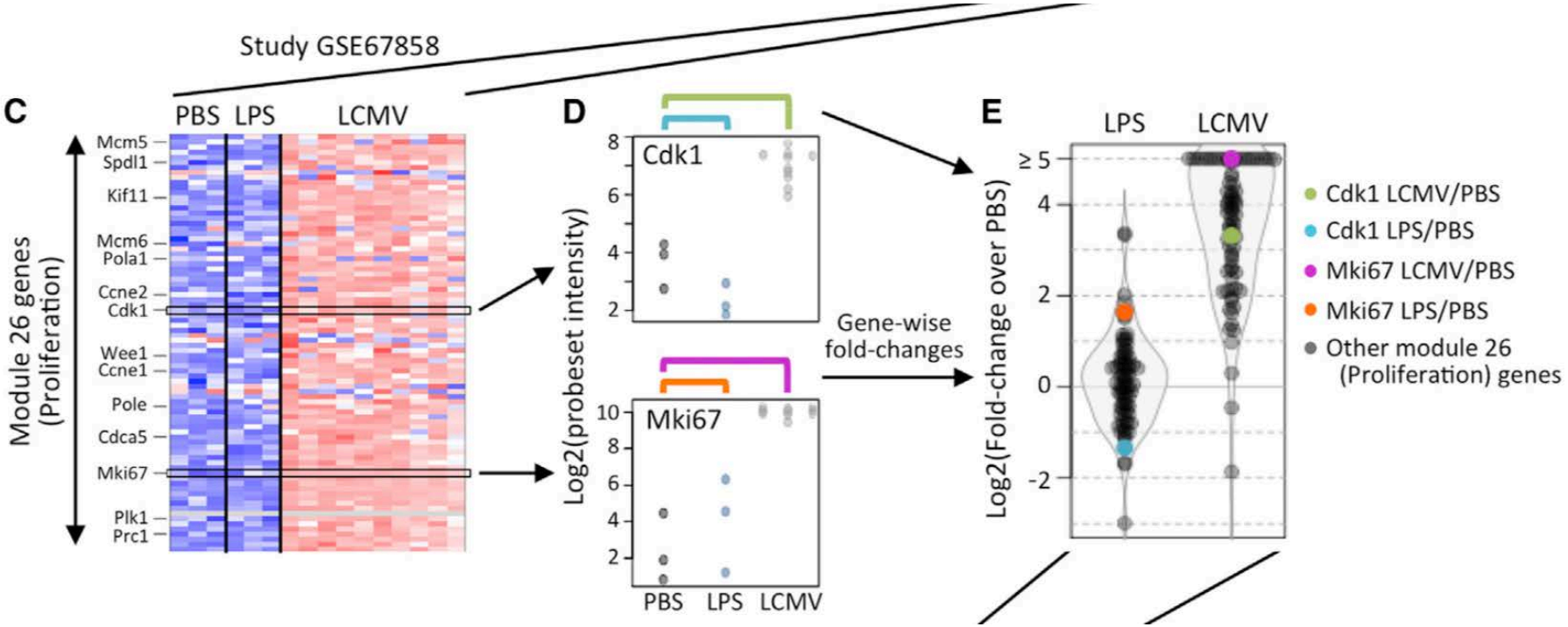
Module 26: proliferation-related



82 genes of module 26, which are enriched for proliferation-associated genes → all studies

Friedman et al., *Cell Reports*, 2018

Module 26: proliferation-related



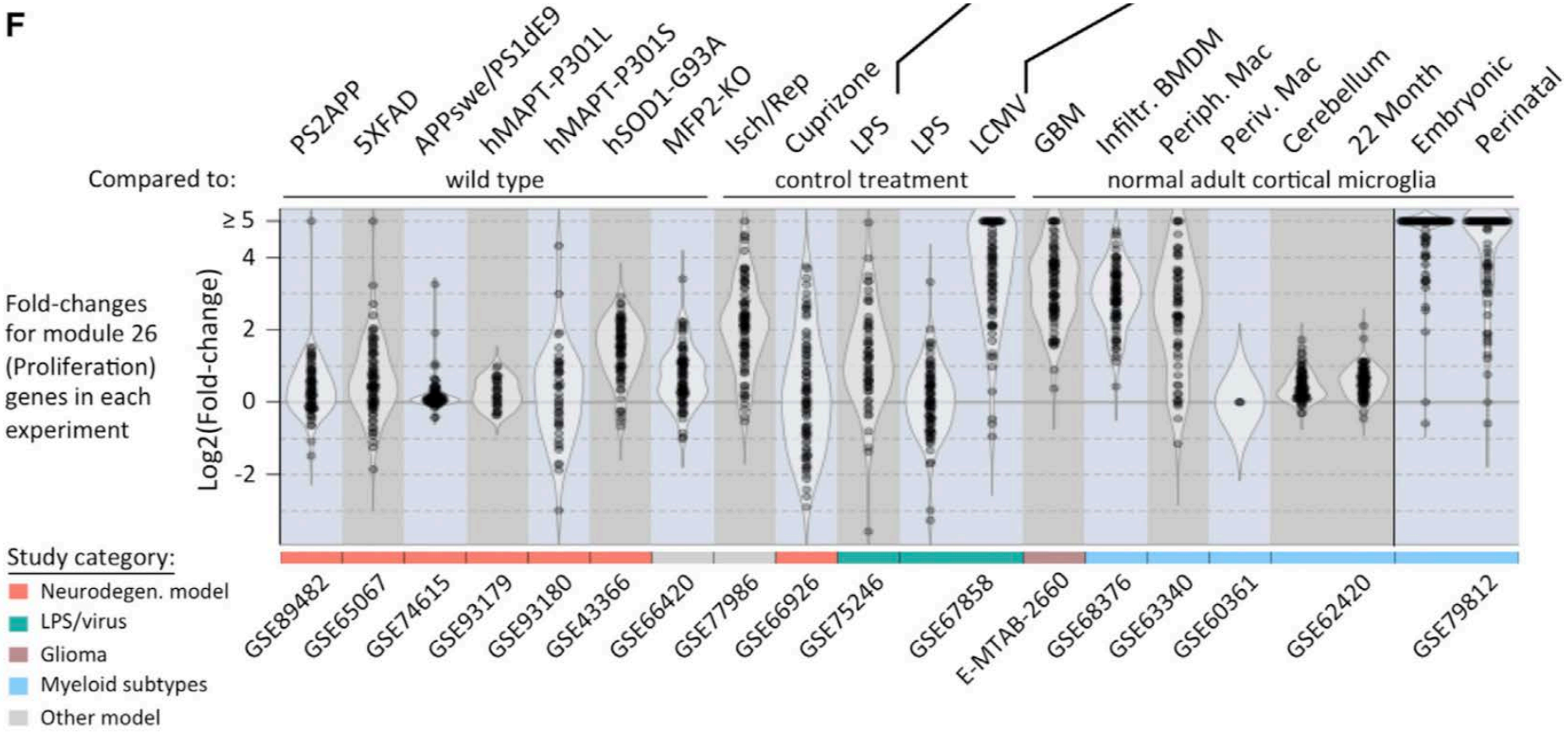
C) 82 genes of module 26, which are enriched for proliferation-associated genes → only study GSE67858 from mice injected with PBS, lipopolysaccharide (LPS), or **lymphocytic choriomeningitis virus (LCMV)**. Proliferation genes were induced by LCMV but not by LPS

D) Expression levels of two genes from the module for individual samples in the three experimental groups

E) Differential expression of each gene in the module, in LPS- or LCMV-treated animals relative to PBS

Friedman et al., *Cell Reports*, 2018

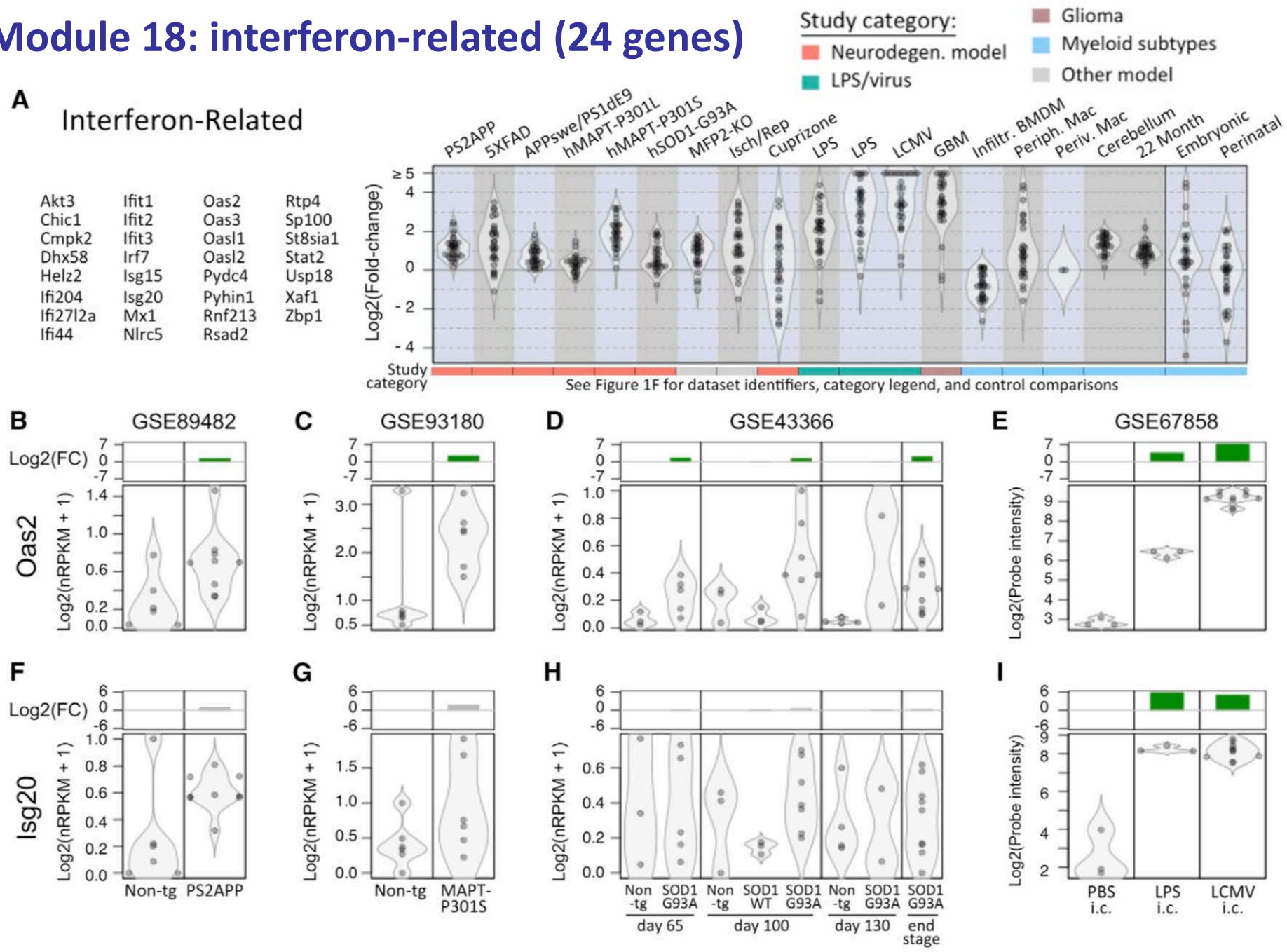
Module 26: proliferation-related



Differential expression of each gene in module 26, in many more conditions in the database, as well as **embryonic and perinatal** compared to adult brain myeloid cells.
Each point represents the differential expression of 82 genes in the module for one comparison.

Friedman et al., *Cell Reports*, 2018

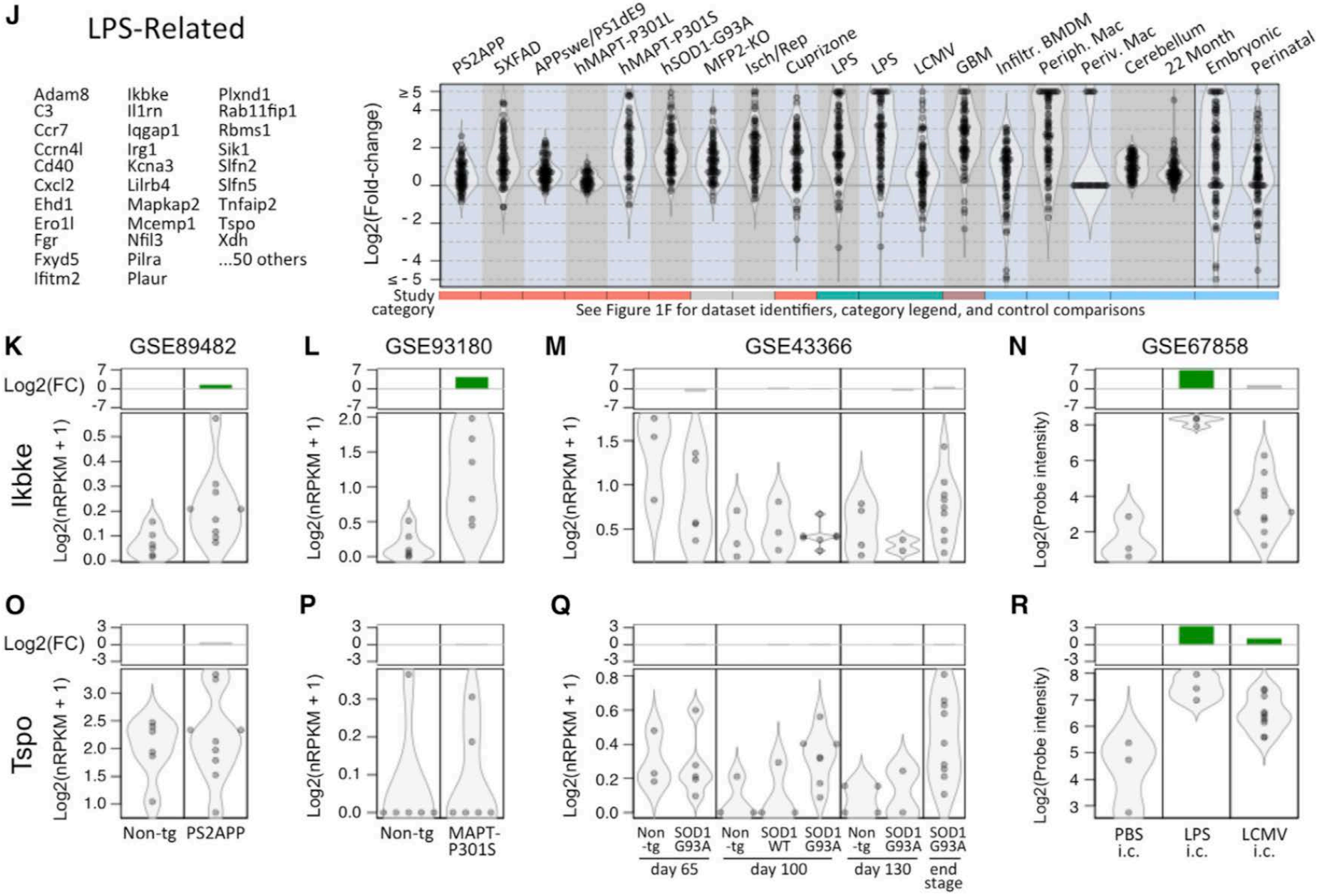
Module 18: interferon-related (24 genes)



PS2APP **Tau-P301S** **SOD1-G93A**

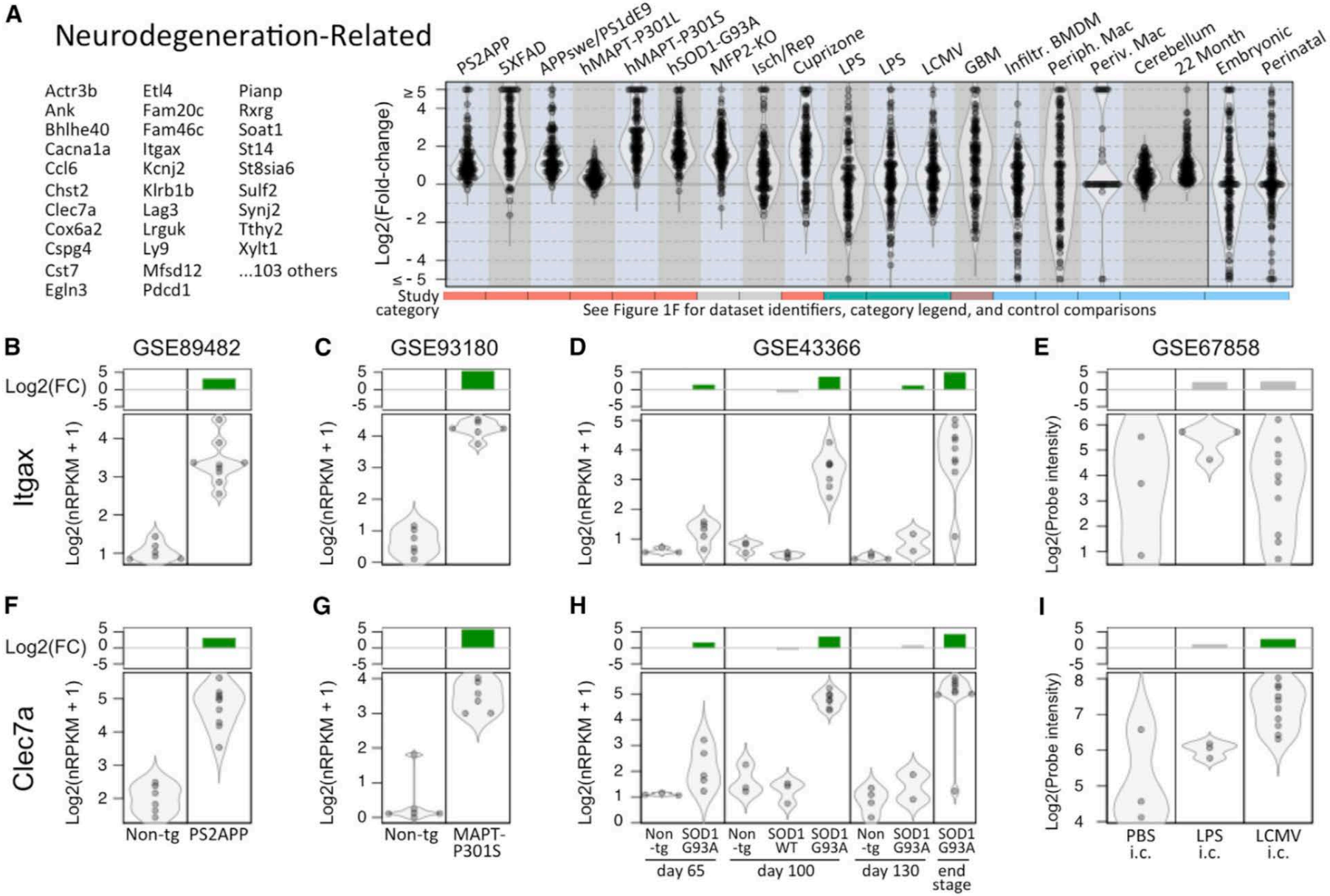
Friedman et al., *Cell Reports*, 2018

Module 10, 12, 13, 17: LPS-related



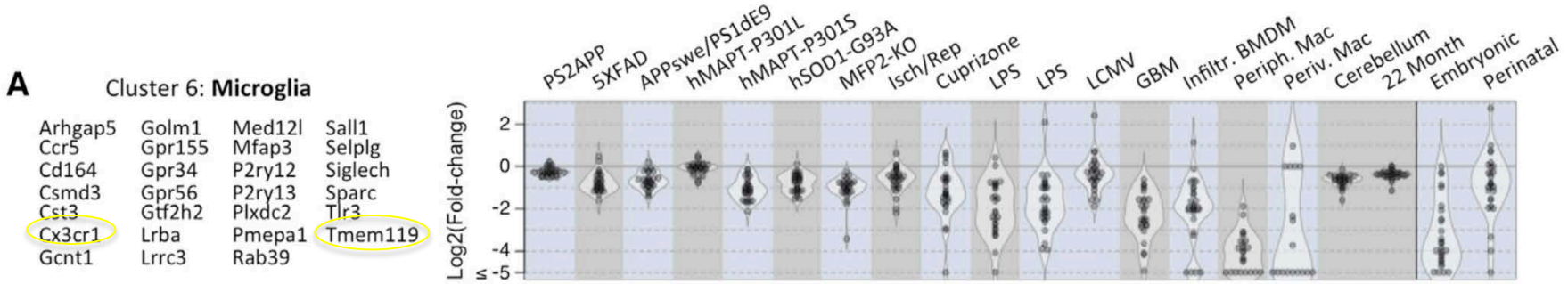
Friedman et al., *Cell Reports*, 2018

Module 24, 25, 36, 37: neurodegeneration-related 134 genes



→ neurodegeneration-related modules represent a special activation state of brain myeloid cells largely distinct from that induced by microbial challenge and characterized by **altered environmental engagement**

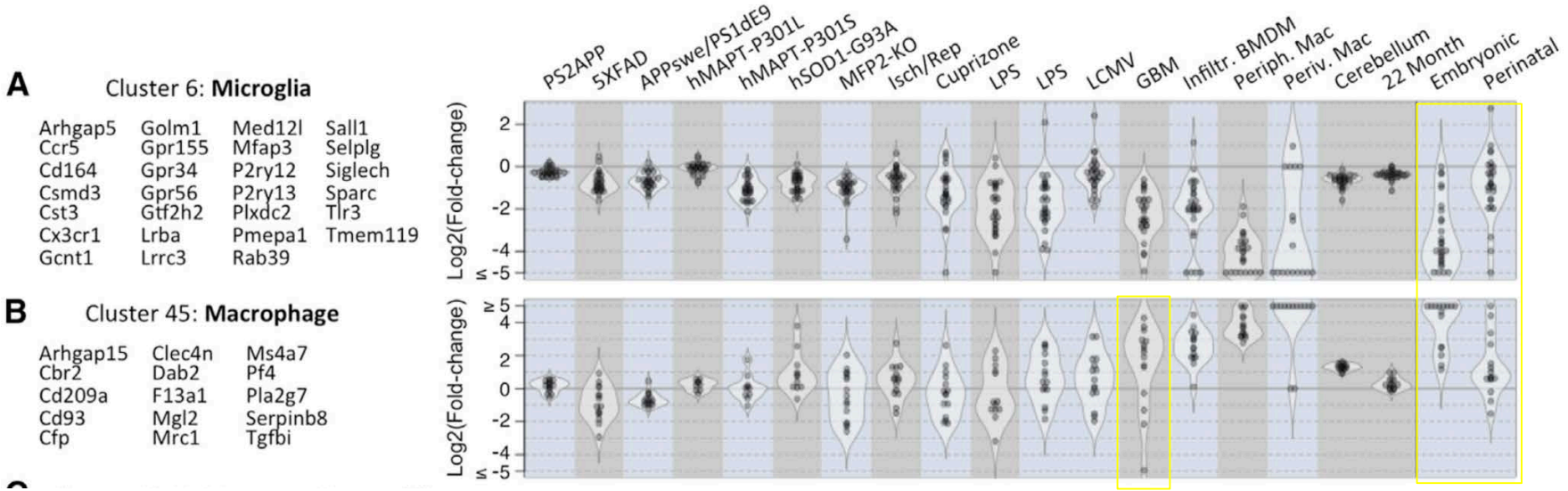
Microglia *vs.* peripheral/infiltrating : microglia module (6)



microglia module (module 6) were unique in their specific elevation in parenchymal microglia relative to perivascular macrophages

!!! Virtually all perturbations **reduced the expression of the microglia module** (and the brain myeloid modules generally), with modest decreases in neurodegenerative models and pronounced reductions with LPS treatment
→ either due to a change in gene expression or to partial replacement of the sorted myeloid compartment with non-microglial cells?

Microglia *vs.* peripheral/infiltrating : macrophage module (45)

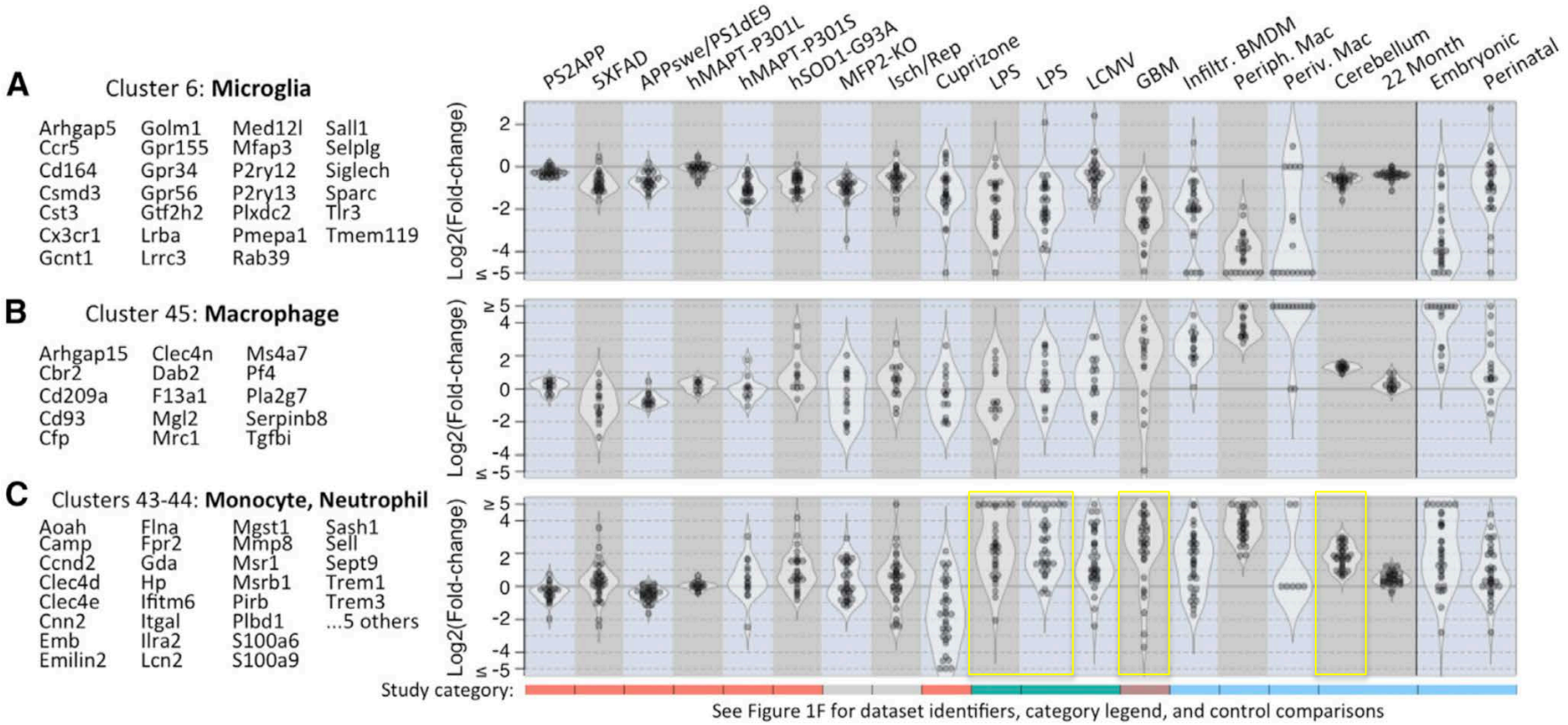


macrophage module (module 45):

- only **glioma** showed pronounced elevation of these genes

- expression of the microglia and macrophage modules was inversely coordinated during brain myeloid cell **development**, with macrophage expression gradually reduced and microglia expression gradually increased from embryonic through perinatal to adult brains

Microglia *vs.* peripheral/infiltrating : monocyte module (43-44)



Monocyte, neutrophil module (module 43-44):

- mostly unchanged in neurodegeneration models
- robustly elevated in **LPS and glioma models**, as well as in **cerebellum**

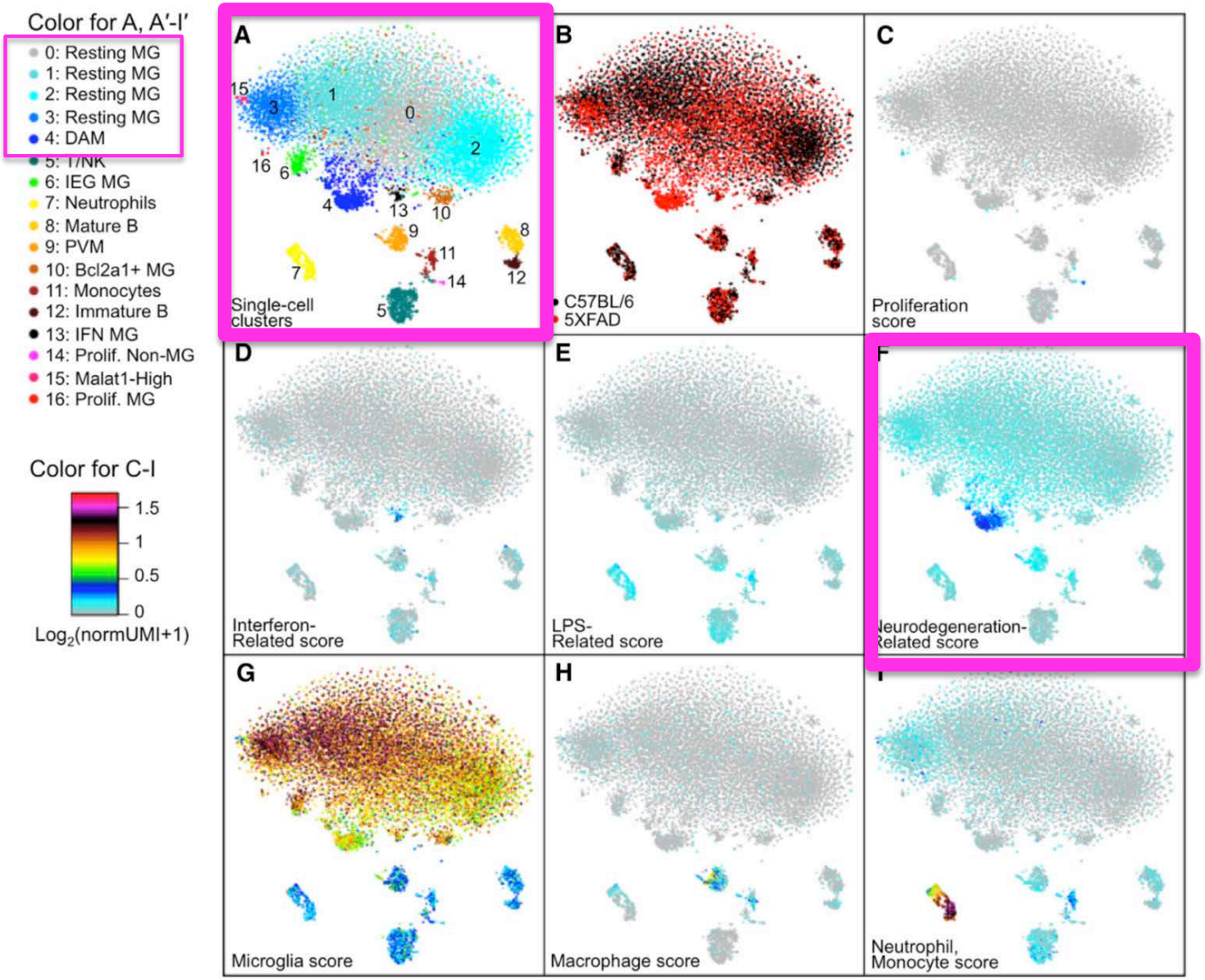
New Microglial Subpopulations Identified by Gene Modules

Open question:

these modules could be induced **concurrently within individual cells**
or
whether they represented **discrete (mutually exclusive) activation states**

we examined their expression in a recently published single-cell RNA-seq survey of $CD45^{+}$ immune cells from the 5XFAD mouse model → Keren-Shaul et al., 2017

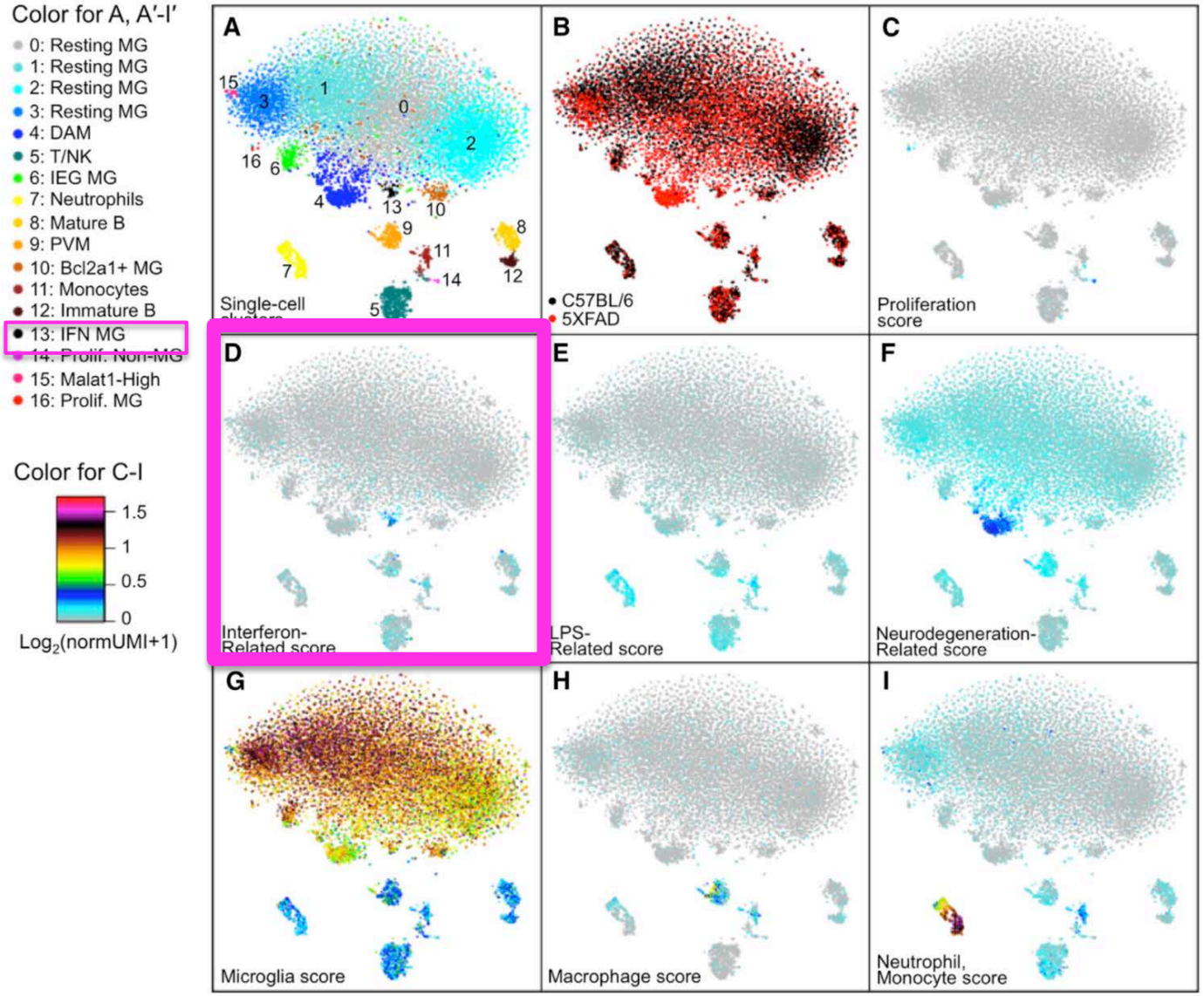
Myeloid Gene Modules Represent Distinct Cell Types and Activation States



→ identified other interesting clusters of microglial cells: discrete, possibly exclusive, microglial states

interferon-related module (cell cluster 13, Figure 6D),
the proliferation module (cell cluster 16, Figure 6C),

Myeloid Gene Modules Represent Distinct Cell Types and Activation States



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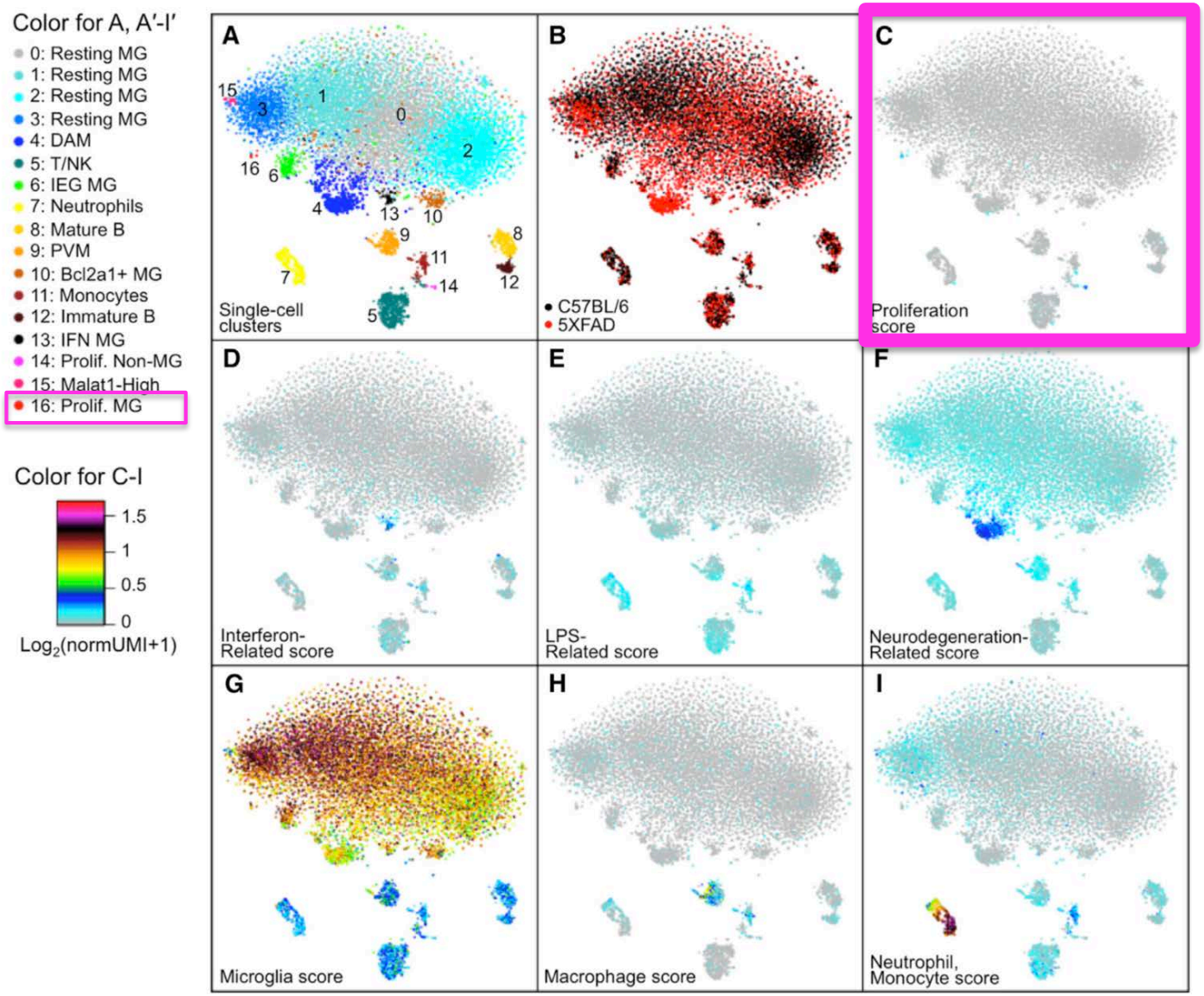
Myeloid Gene Modules Represent Distinct Cell Types and Activation States



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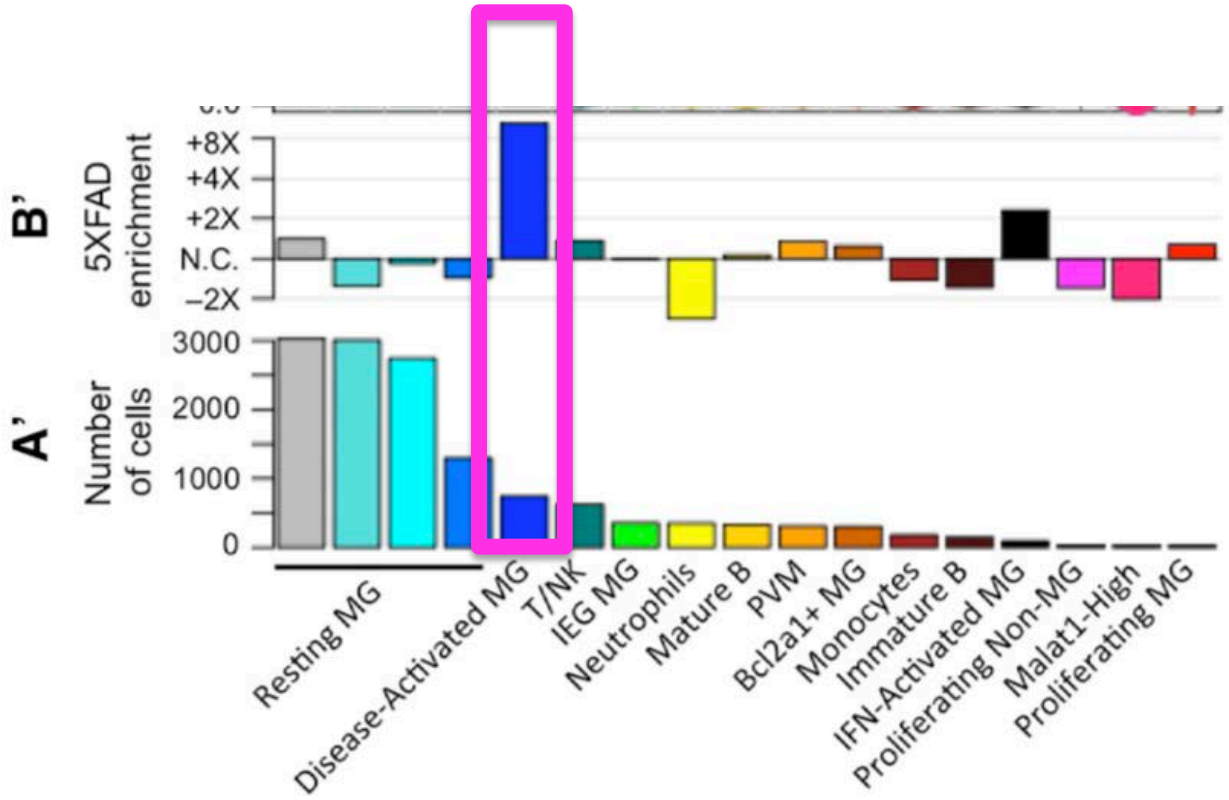
Myeloid Gene Modules Represent Distinct Cell Types and Activation States



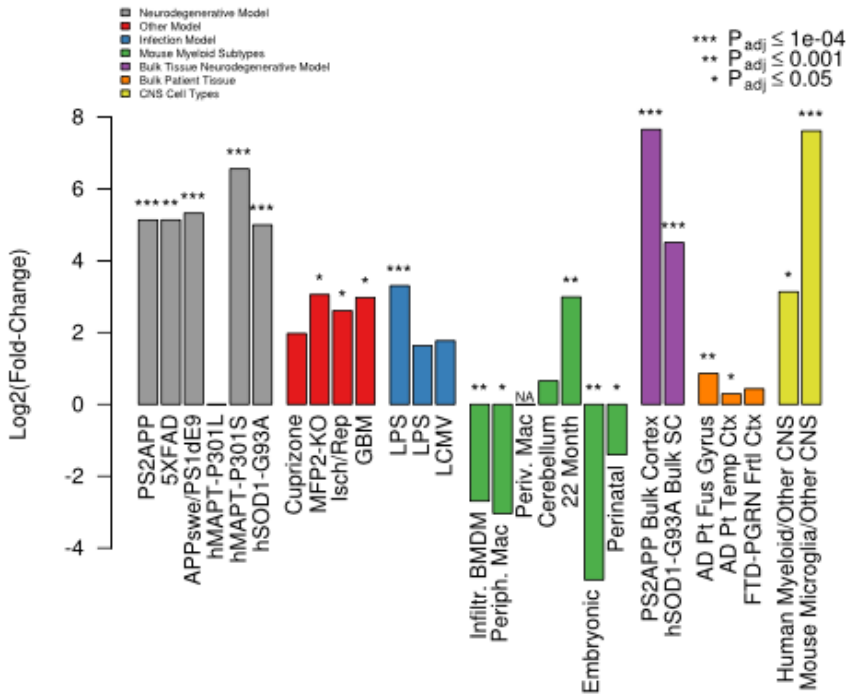
→ identified other interesting clusters of microglial cells: discrete, possibly exclusive, microglial states

interferon-related module (cell cluster 13, Figure 6D),
the proliferation module (cell cluster 16, Figure 6C),

Cst7 data from http://research-pub.gene.com/BrainMyeloidLandscape/

Gene Information

Mouse Entrez Gene ID	13011
Mouse GRCm38 Coordinates	chr2:150570415-150578944:+
Mouse Biotype	protein_coding
Mouse Gene Symbol	Cst7
Mouse Description	cystatin F (leukocystatin)
Mouse Aliases	Cmap
Human Entrez Gene ID	8530
Human Gene Symbol	CST7
AD/PD GWAS Hit	No
Previous MG Gene Sets	NA
Barres Human Cell Types	NA
Barres Mouse Cell Types	microglia
ABA Mouse Cell Types	NA
Myeloid Activation Cluster	Neurodegeneration-Related
Immune-Specific	NA



+ expression plots for each study

Immunity
Resource

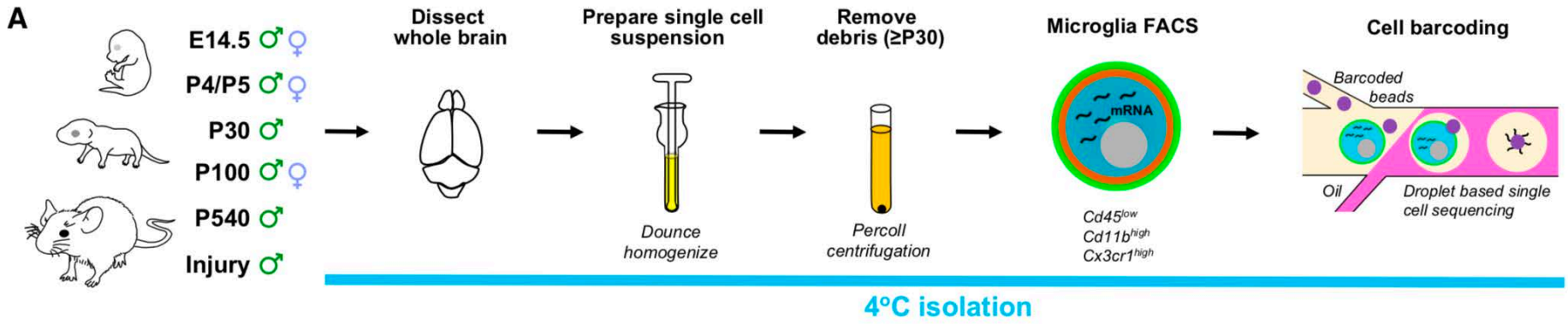
Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes

Timothy R. Hammond,[1,2,3] Connor Dufort,[1] Lasse Dissing-Olesen,[1,2,3] Stefanie Giera,[1,2,7] Adam Young,[6] Alec Wysoker,[3] Alec J. Walker,[1,2,3] Frederick Gergits,[1] Michael Segel,[6] James Nemesh,[3] Samuel E. Marsh,[1,2,3] Arpiar Saunders,[3,5] Evan Macosko,[3] Florent Ginhoux,[8] Jinmiao Chen,[8] Robin J.M. Franklin,[6] Xianhua Piao,[1,2,7] Steven A. McCarroll,[3,5,*] and Beth Stevens[1,2,3,4,9,*]

[1]Boston Children's Hospital, F.M. Kirby Neurobiology Center, Boston, MA, USA
[2]Harvard Medical School, Boston, MA, USA
[3]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA, USA
[4]Howard Hughes Medical Institute, Boston Children's Hospital, Boston, MA, USA
[5]Department of Genetics, Harvard Medical School, Boston, MA, USA
[6]Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK
[7]Boston Children's Hospital, Division of Newborn Medicine, Department of Medicine, Boston, MA, USA
[8]Singapore Immunology Network (SIgN), A*STAR, Biopolis, Singapore
[9]Lead Contact
*Correspondence: mccarroll@genetics.med.harvard.edu (S.A.M.), beth.stevens@childrens.harvard.edu (B.S.)
https://doi.org/10.1016/j.immuni.2018.11.004

Experimental plan

perfusion



Experimental Models: Organisms/Strains		
Mouse: C57BL/6J	Jackson Labs	IMSR_JAX:000664

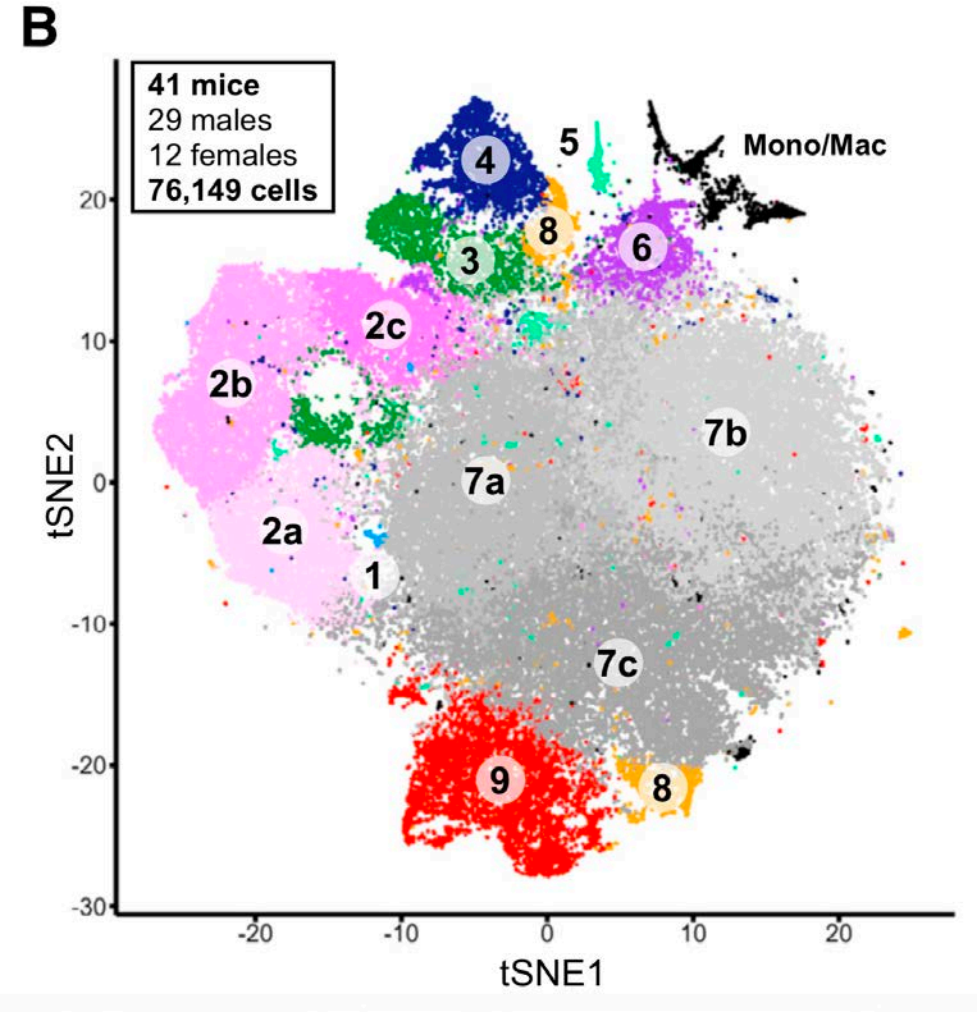
Single-cell RNA-seq of total **76,149 mouse microglia**, 3-4 mice/age, 41 mice in total

comparable sequencing depths (40,000–60,000 reads/cell) and had a similar median unique molecular identifier (UMI) count and median gene number in all ages and conditions

identify, curate, and remove from analysis contaminating cells (including neurons, endothelial cells, and other cell types)

independent components that captured batch or replicate effects were removed before clustering analysis

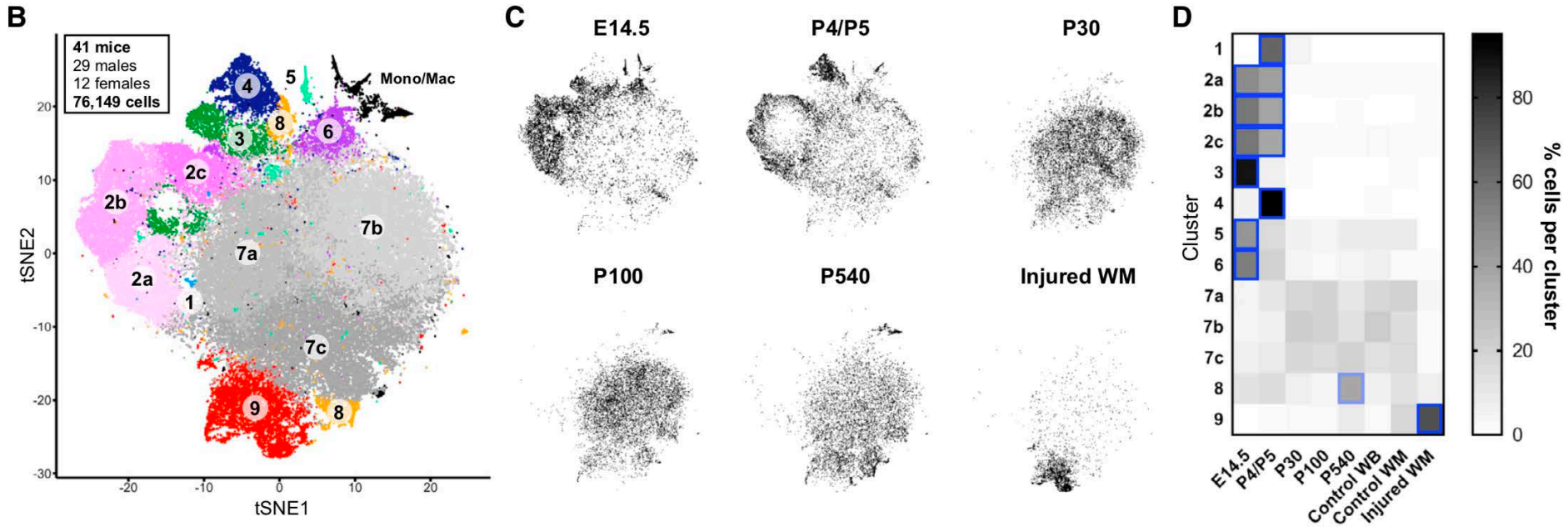
tSNE projection : 9 microglia clusters



tSNE plot of 76,149 cells

In total, **9 microglia clusters** and **1 monocyte/macrophage** (Mono/Mac)-containing cluster were identified across all ages and conditions, including injury

Distinct Subpopulations of Microglia Peak in Number during Early Development



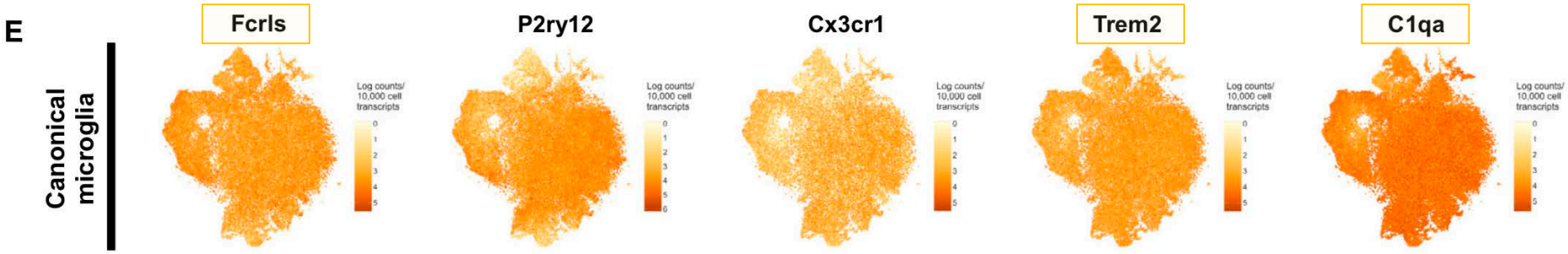
youngest ages (E14.5 and P4/P5) → clusters 1-6
juvenile – adult → clusters 7a to 7c
aged → cluster 8
injured → cluster 9

greatest microglial diversity at the youngest ages

considerably less diversity in juveniles (P30) and adults (P100)

both **aging and injury caused a redistribution of microglial states**

Canonical microglial genes

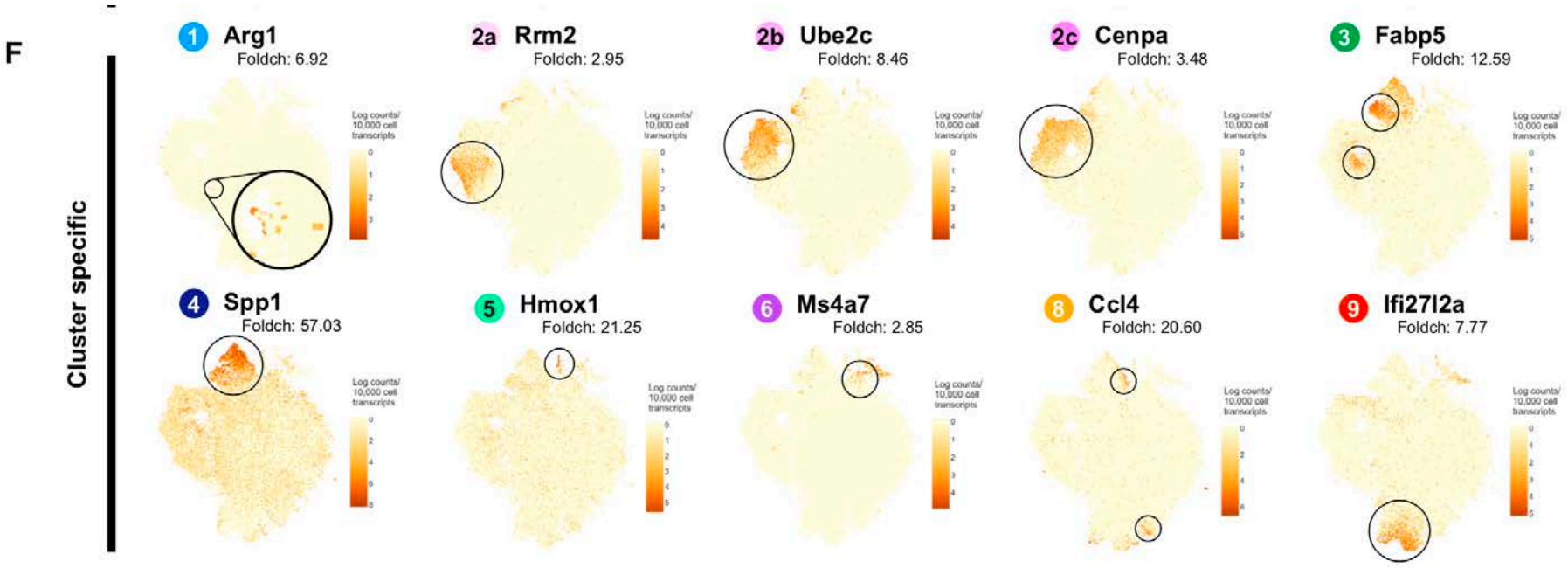


canonical microglial genes (*Fcrls*, *P2ry12*, *Cx3cr1*, *Trem2*, and *C1qa*) were highly expressed by most of the analyzed cells,

but **only three (*C1qa*, *Fcrls*, *Trem2*) were uniformly expressed** in all clusters

P2ry12, *Cx3cr1*, and *Tmem119* (not shown) transcripts were expressed at much lower levels or not at all in certain clusters of microglia from the developing brain

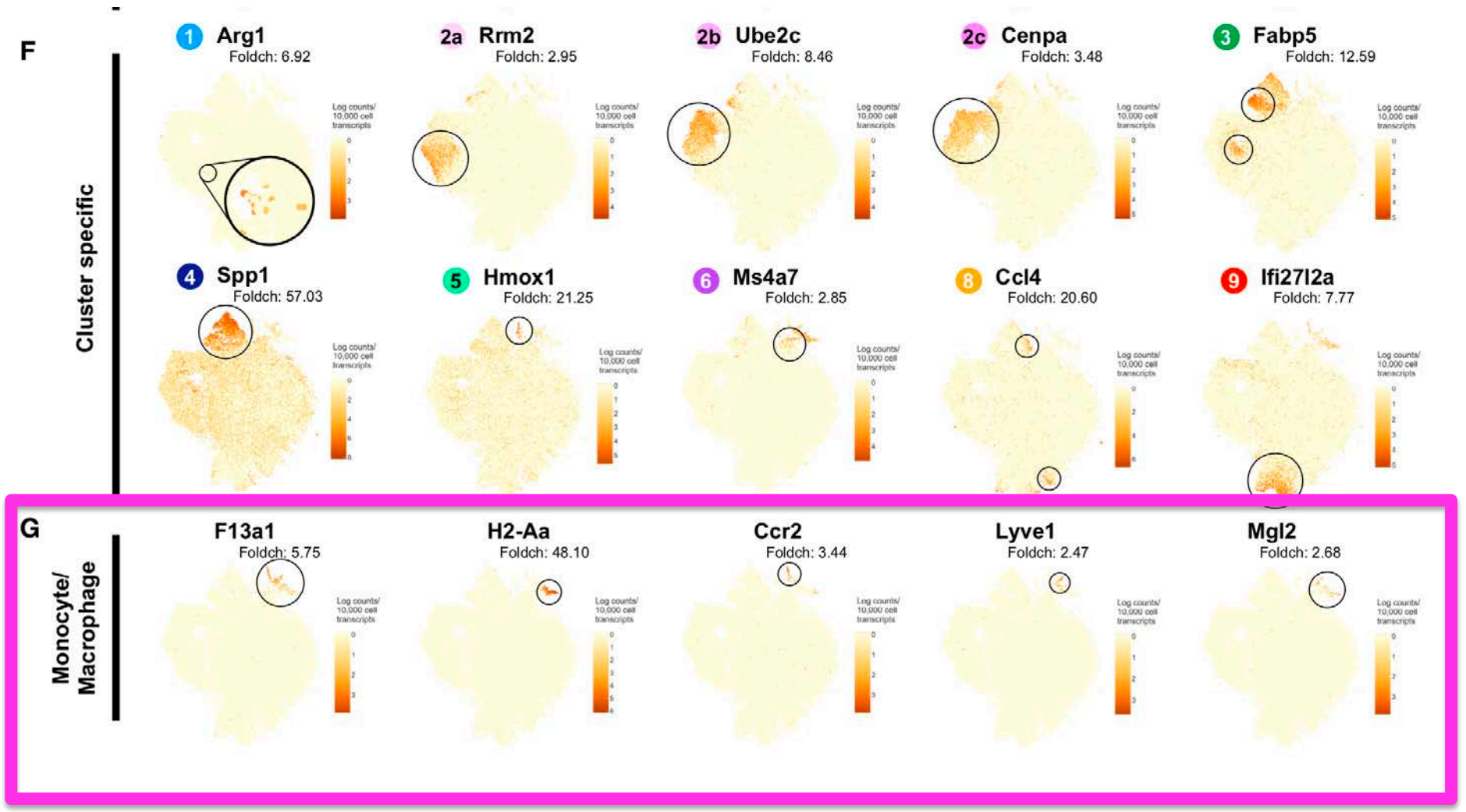
Genes uniques to specific microglial states



youngest ages (E14.5 and P4/P5) → clusters 1-6
Juvenile – adult → 7a to 7c no specific genes
Aged → 8
Injured → 9 interferon, alpha-inducible protein 27 like protein 2A

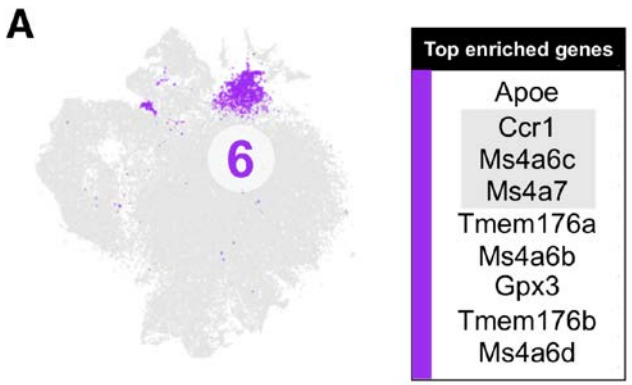
→ multiple specific and definable states that change over the course of development, aging, and injury

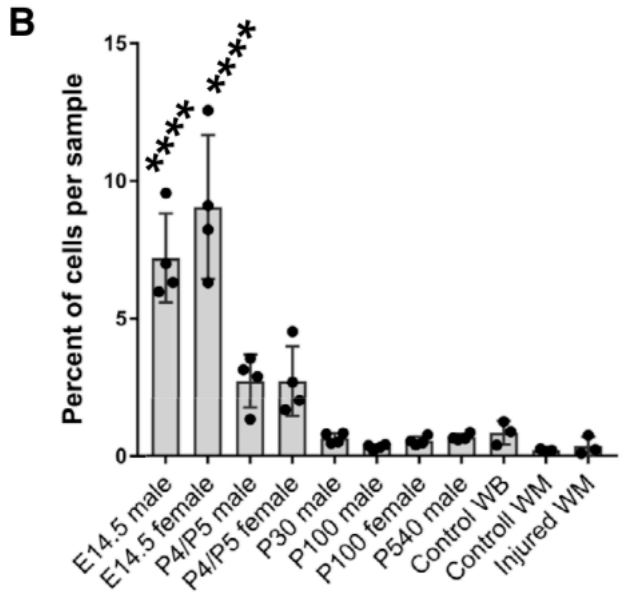
Genes uniques to monocyte/macrophage

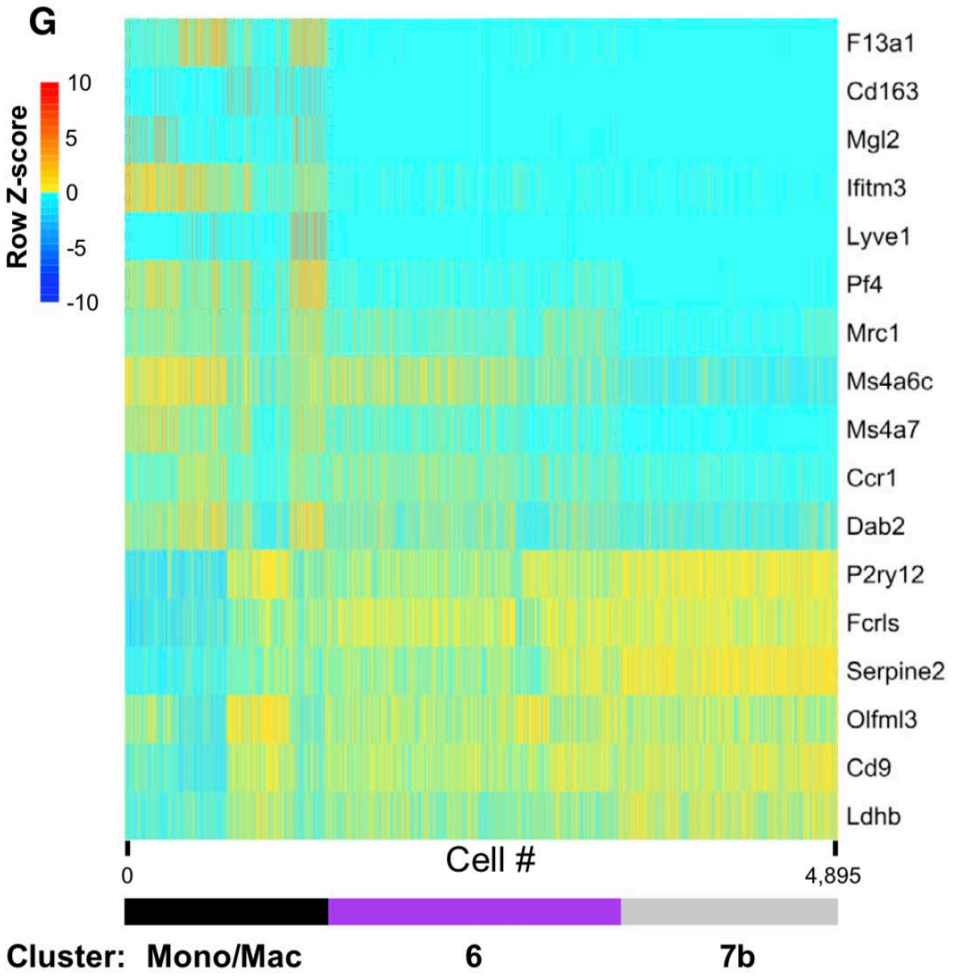


Non-microglial macrophages and monocytes uniquely expressed certain genes

Identification of *Ms4a7*-Expressing Microglia in the Embryonic Brain that Resemble Brain Border Macrophages → E14.5



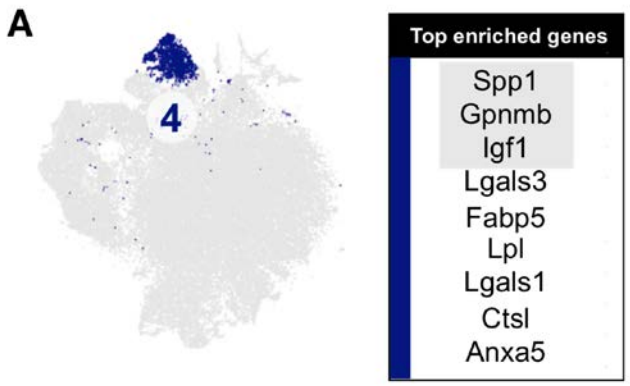




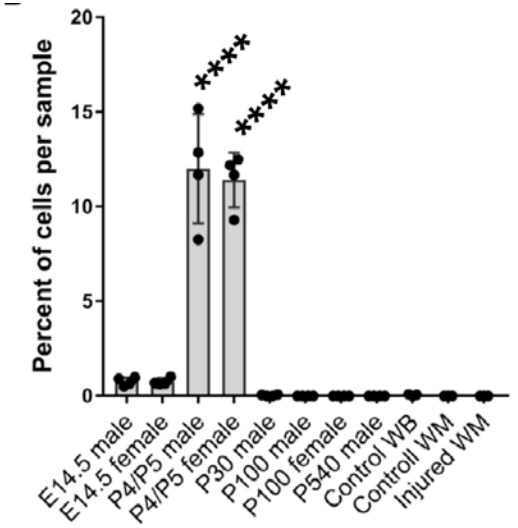
membrane-spanning 4-domains subfamily A (MS4A)

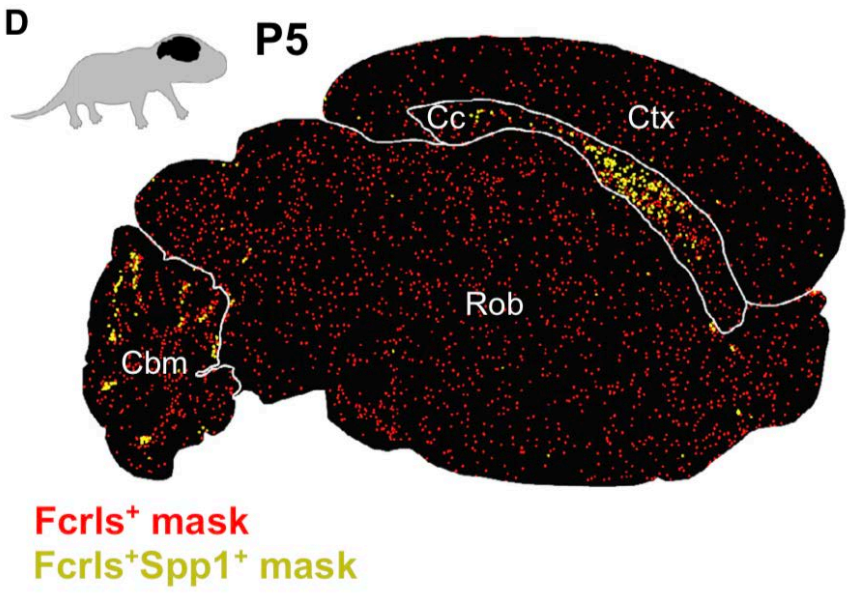
macrophage markers (Mrc1, Ccr1, Dab2) and those found in mature microglia (P2ry12, Fcrls, Serpine2 [cluster 7b]), suggesting an intermediate state

Specialized Axon Tract-Associated Microglia (ATM) Appear during a Restricted Developmental Window → P4/5



amoeboid morphology AND also upregulate the lysosomal markers lysosomal-associated membrane protein 1 (*Lamp1*) and *Cd68*



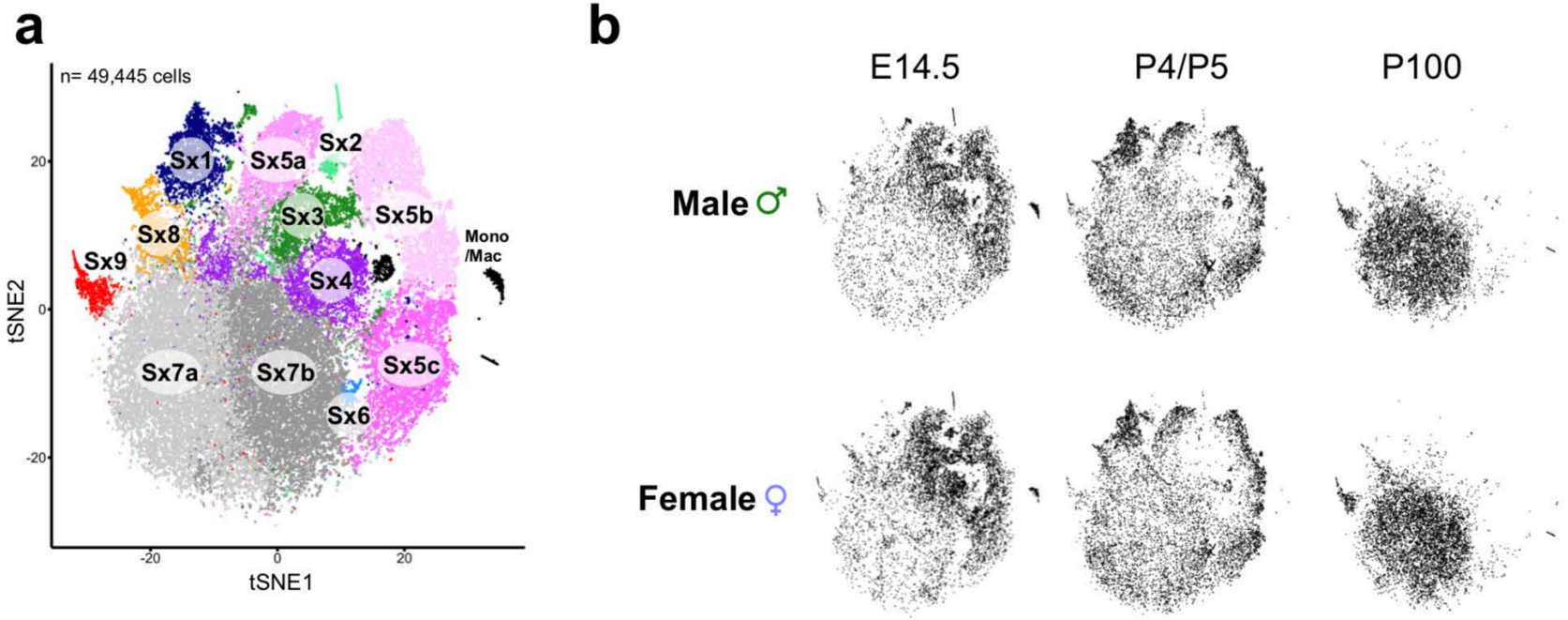


smFISH for *Spp1:* subcortical axon tracts of the corpus callosum in the forebrain, as well as in distinct clusters in the axon tracts of the cerebellum

The axon tracts where ATM were concentrated will eventually become heavily myelinated, but ATM are largely gone before myelination occurs.

Sex Has No Impact on Microglial Diversity or the Number of Cells in Each Subpopulation

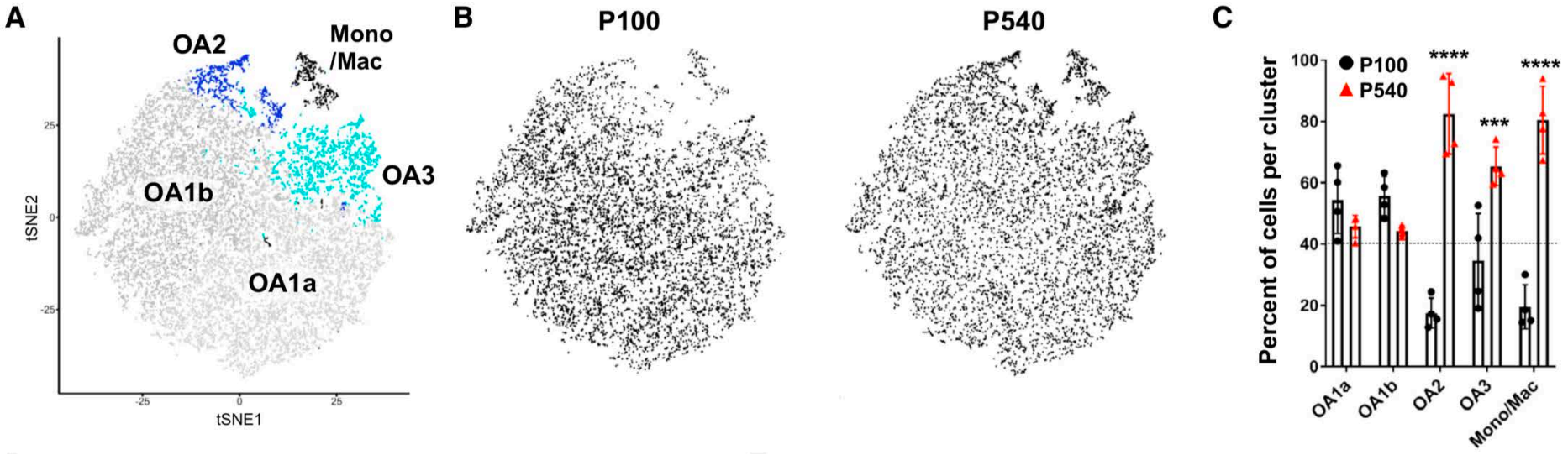
microglia from male and female mice at three major developmental ages: E14.5, P4/P5, and P100



→ showed that microglial diversity was largely unaffected by sex during normal development (only small difference in cluster Sx6)

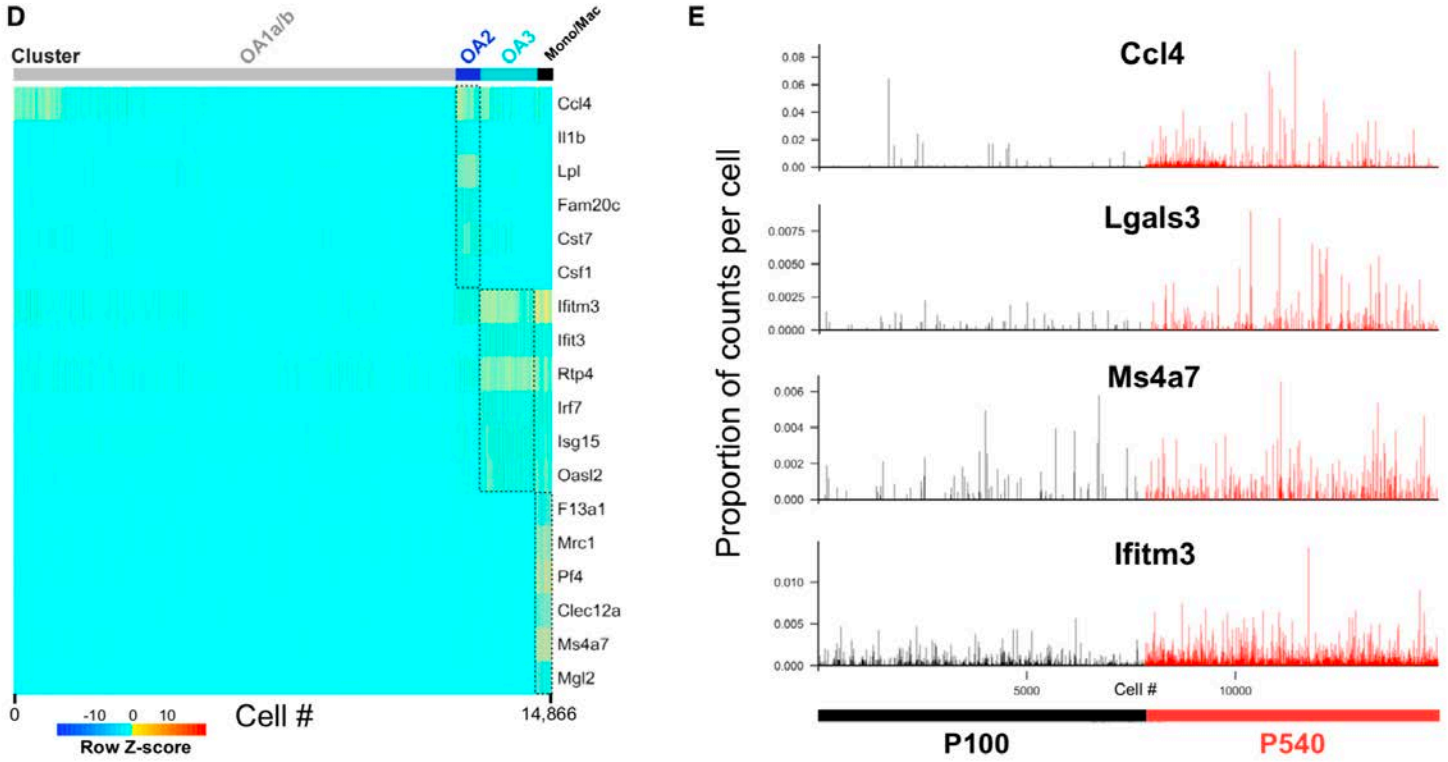
Small Populations of Inflammatory and Interferon-Responsive Microglia Emerge in the Aged Brain: OA2 and OA3 clusters

Direct comparison of P100 and P540 microglia



Two microglia clusters enriched in aging mice (aging clusters **OA2 and OA3**), along with one monocyte and macrophage cluster (Mono/Mac)

Aging effect: OA2 cluster → inflammation

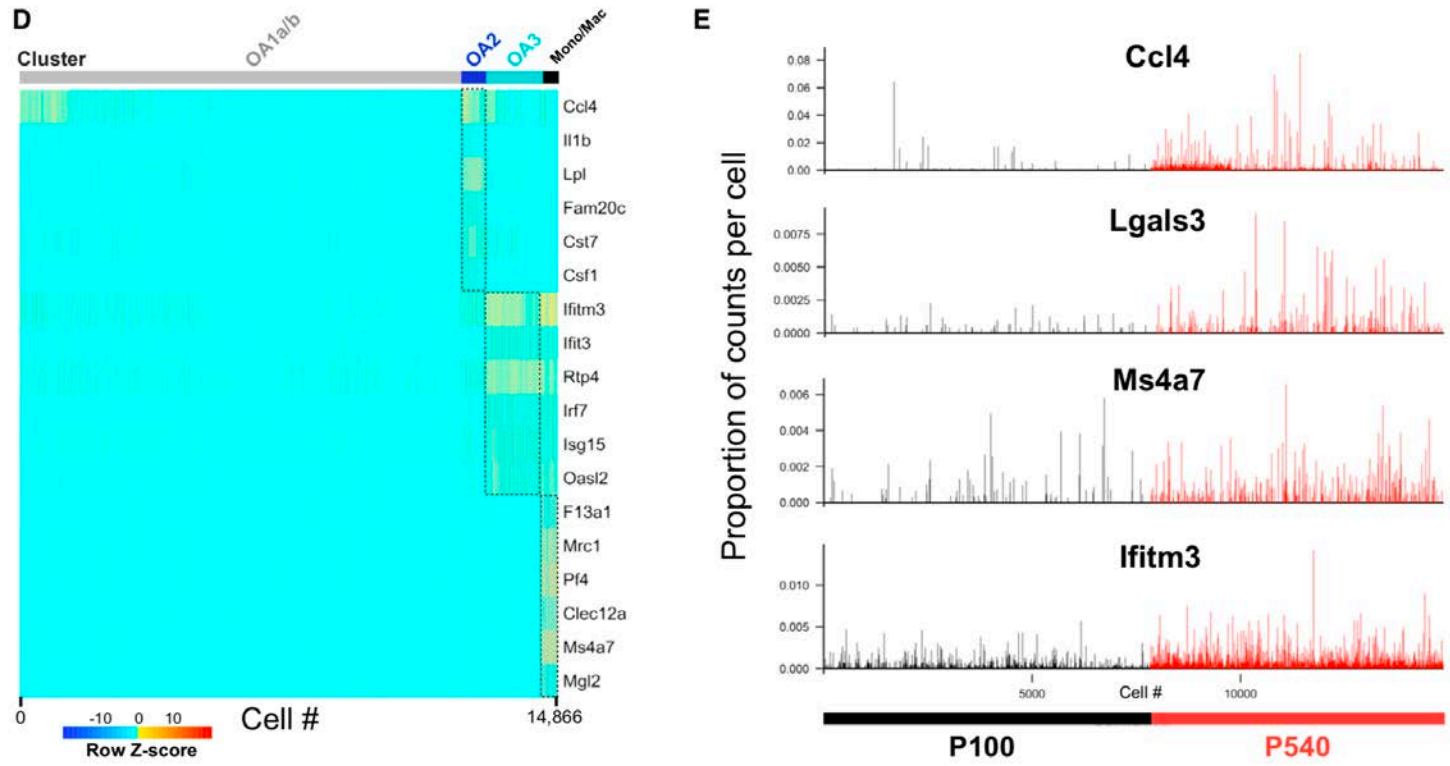


OA2 microglia expressed a number of **inflammatory signals** that were not normally expressed by other populations of microglia *in vivo:*
cystatin F (Cst7), chemokines Ccl4 and Ccl3, as well as the inflammatory cytokine interleukin 1 beta (Il1b)

OA2 microglia are **distributed throughout the adult and aged brain**

→ This increase, coupled with the overall increase in the inflammatory environment in the aged brain (Franceschi et al., 2007), suggests that this small subpopulation of microglia contributes to age-related brain inflammation

Aging effect: OA3 cluster → interferon-response



Cluster OA3 upregulated **interferon-response genes** including interferon induced transmembrane protein 3 (Ifitm3), receptor transporter protein 4 (Rtp4), and 20-50 oligoadenylate synthe- tase-like 2 (Oasl2)

OA3 profile **restricted to a small subset of microglia**

→ the number of microglia that occupy these states form **only a small fraction of microglia**, suggesting that the vast majority of microglia are unaltered or only slightly altered by aging and that local cues like blood brain barrier compromise (Montagne et al., 2015) or microinfarcts (Smith et al., 2012) could drive state changes rather than a brain-wide shift.

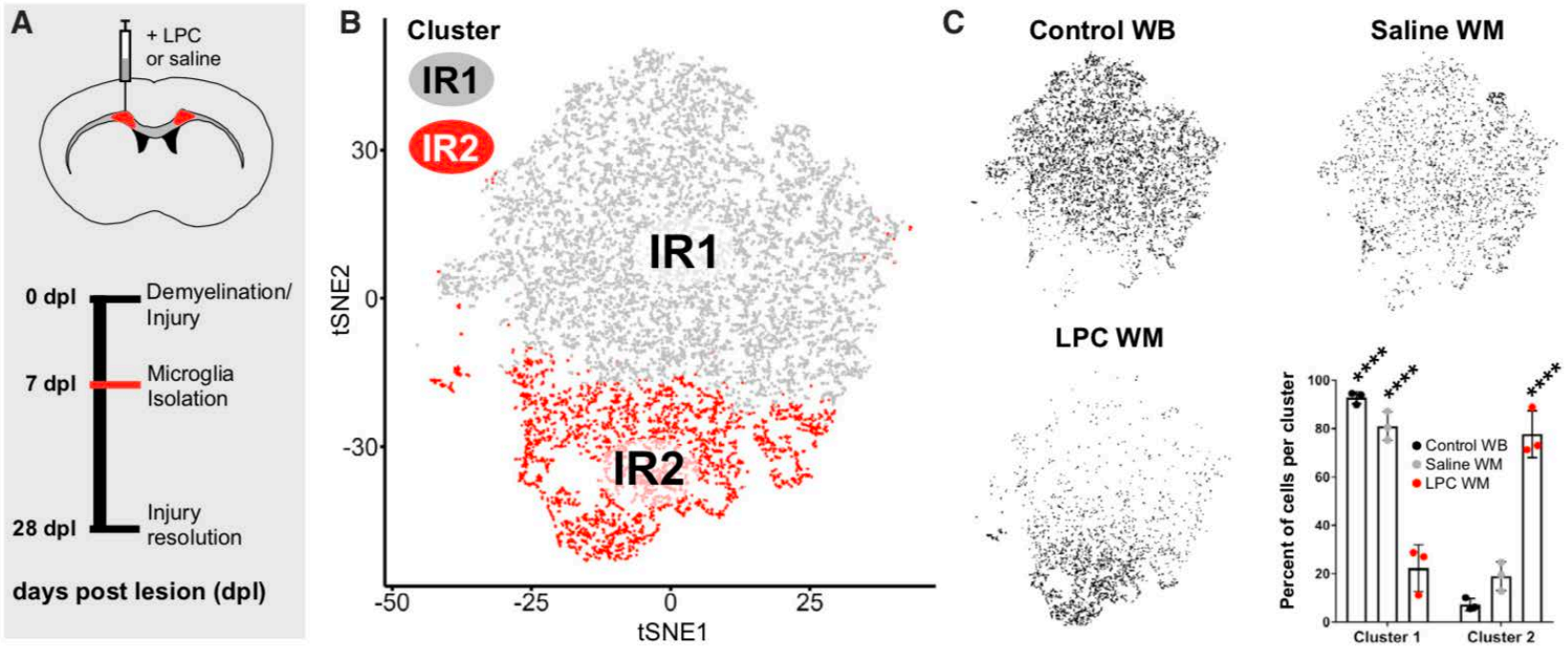
Injury-Responsive Microglia (IRM) in Demyelinated Lesions Exhibit Multiple Activation States

→ focal demyelination of the subcortical white matter in mice is triggered by injection of lysolecithin (LPC)

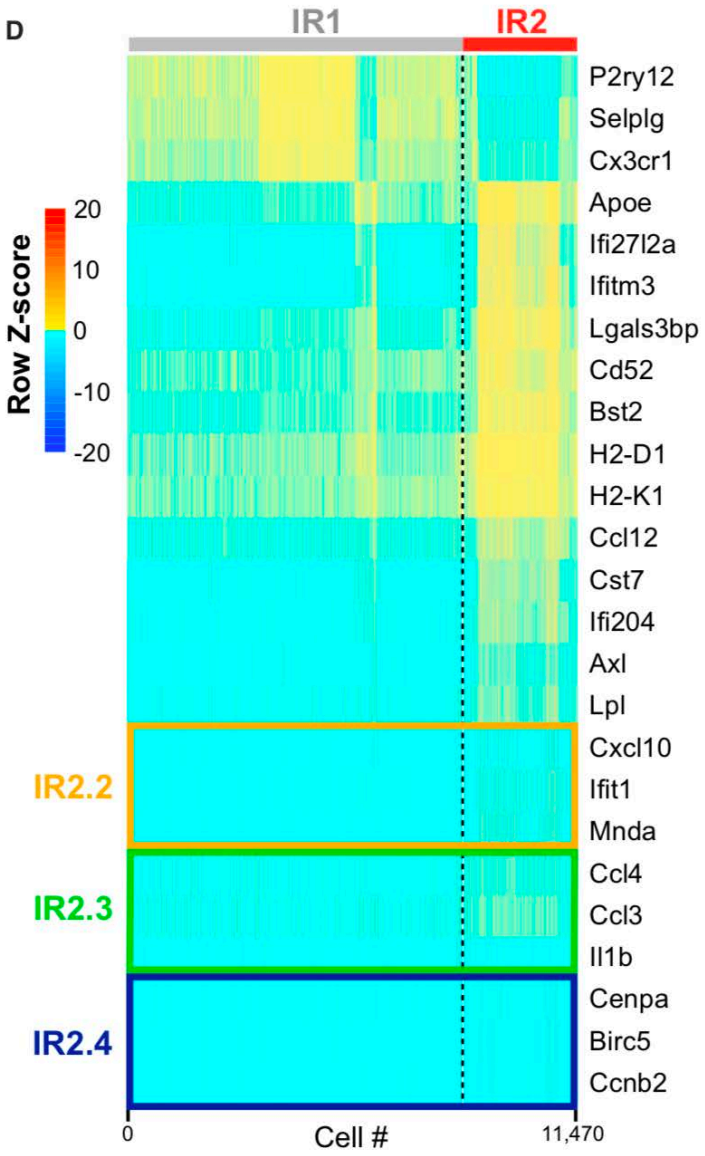
→ white matter from LPC- and saline-injected adult (P100) mice + uninjected P100 whole-brain control samples were collected and processed in parallel

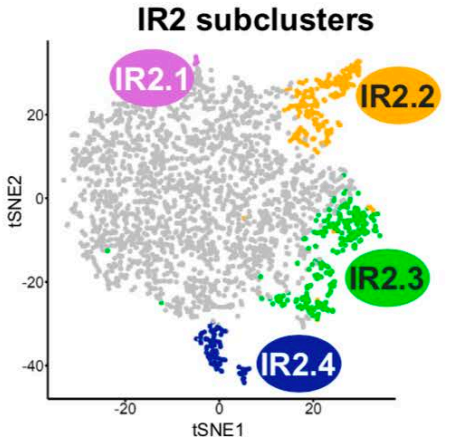
→ 2 major clusters:

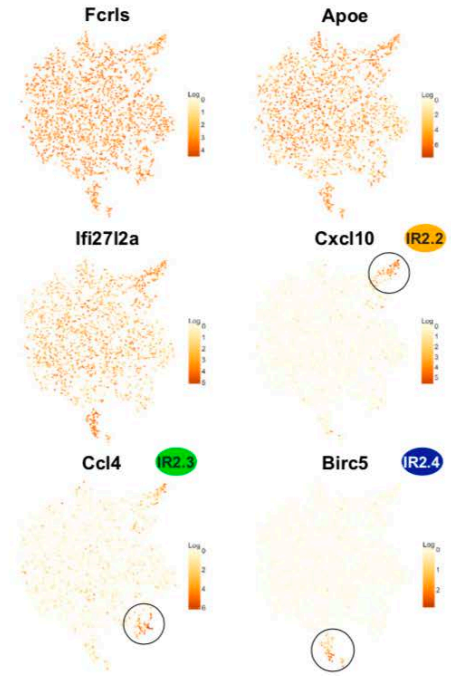
- Injury-responsive cluster 1 (IR1): control microglia
- IR2: microglia from LPC-injected demyelinated lesions



Injury-Responsive Microglia in Demyelinated Lesions Exhibit Multiple Activation States



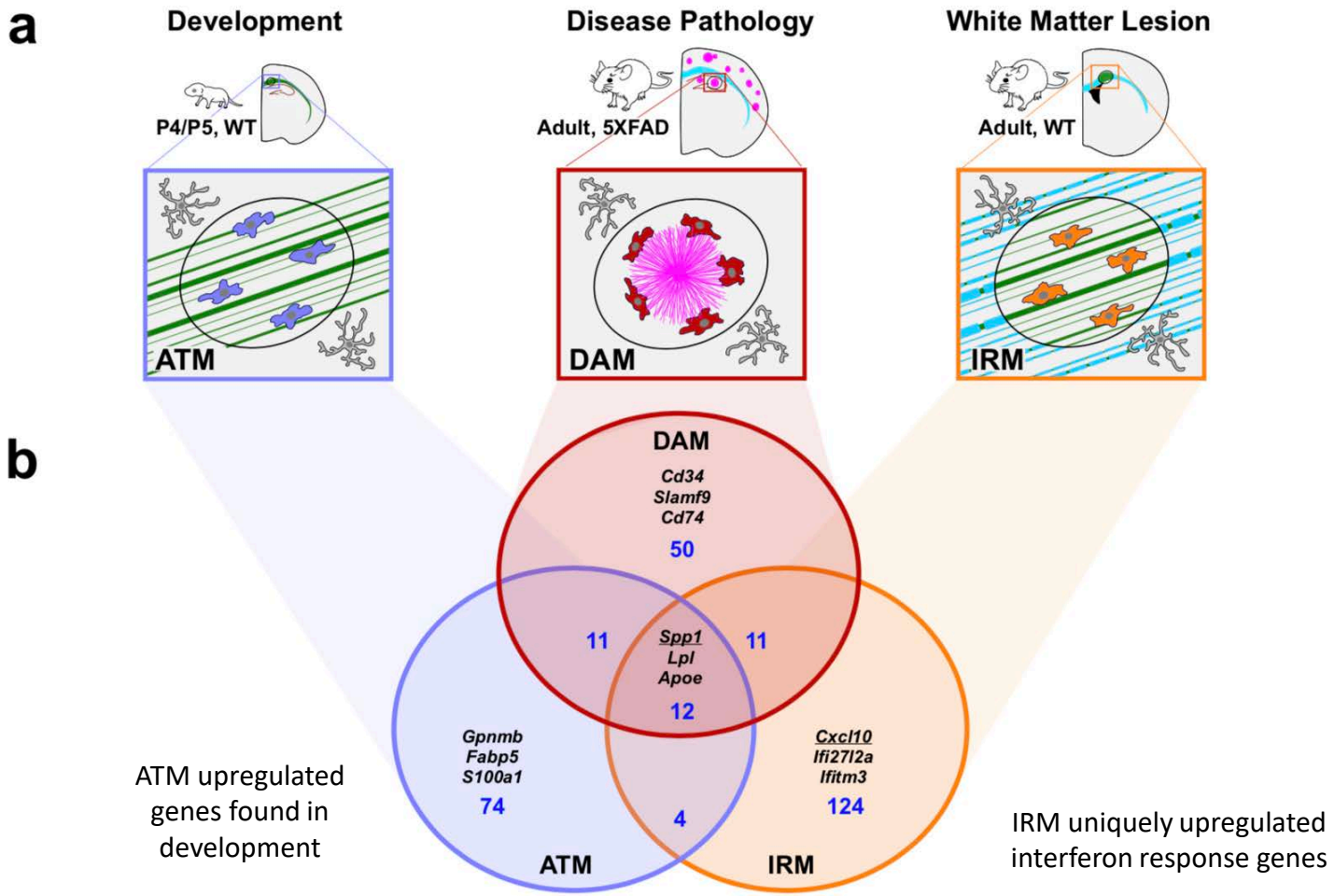




- downregulated expression of the canonical microglial markers *P2ry12* and *Cx3cr1*
- subpopulations within the IR2 cluster*: e.g.* **IR2.4** expressed cell proliferation markers, including Birc5 **IR2.2** upregulated the interferon response gene Cxcl10

Comparison of ATM - DAM - IRM

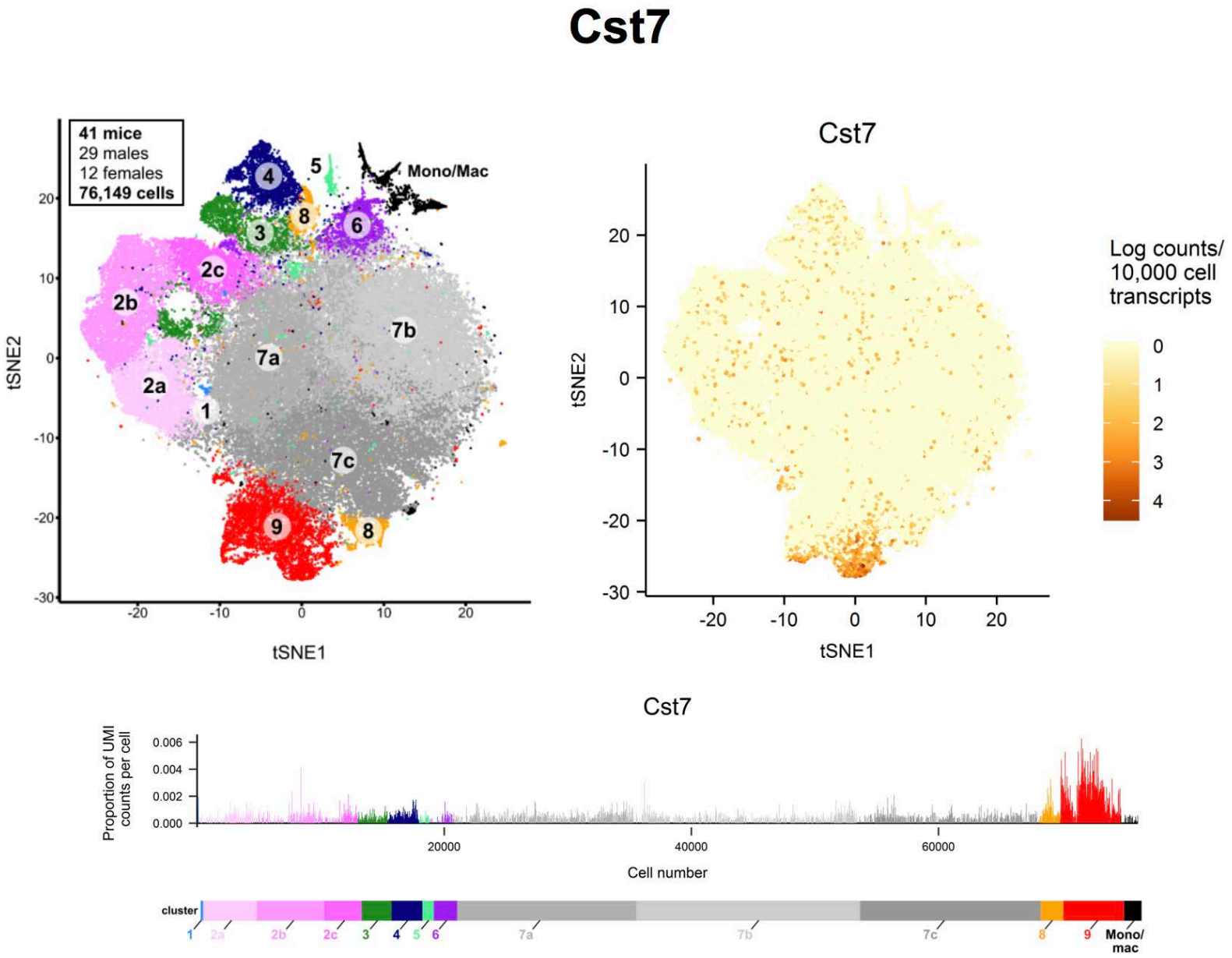
overlap in genes that were upregulated 1.5-fold or higher with a p value of less than 1E-10 → ***Table S2***



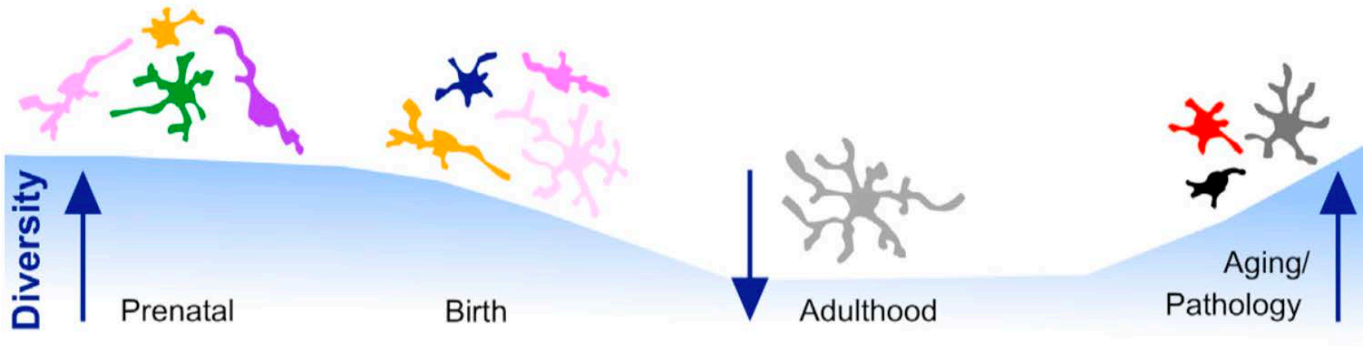
→ all three groups shared a common transcriptional signature of **12 core genes** including *Spp1*, *Lpl*, and *Apoe*

→ each group also expressed a number of unique genes.

Cst7 data from www.microgliasinglecell.com



Conclusion



→ microglia assume **many distinctive states** that change over time, states that can be defined by unique markers and localized within the brain.

→ information required for the **development of new tools**—including new Cre driver lines

→ specific roles of each microglial state will need to be tested directly, using genetic manipulation and other tools as they become available

→ a deeper mechanistic insight into microglia signaling mechanisms

Summary useful tools for further data mining/visualization

- DAM microglia (Keren-Shaul et al., *Cell* 2017):

Table S2: top 500 different genes in DAM (471 UP, 29 DOWN)

- Meta-analysis (Friedmann et al., *Cell reports* 2018)

Data S2 (or S3): excel file with list of all genes/all studies (43MB)

Website: http://research-pub.gene.com/BrainMyeloidLandscape/

- Development and disease (Hammond et al., *Immunity* 2018)

www.microgliasinglecell.com

Thank you!