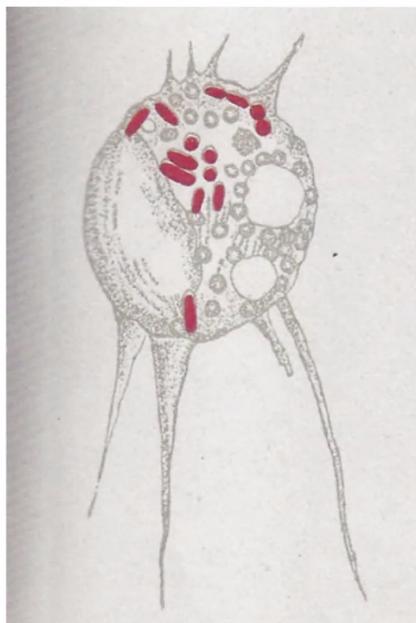


Exploiting dynamic enhancer landscapes to decode macrophage phenotypes in health and disease

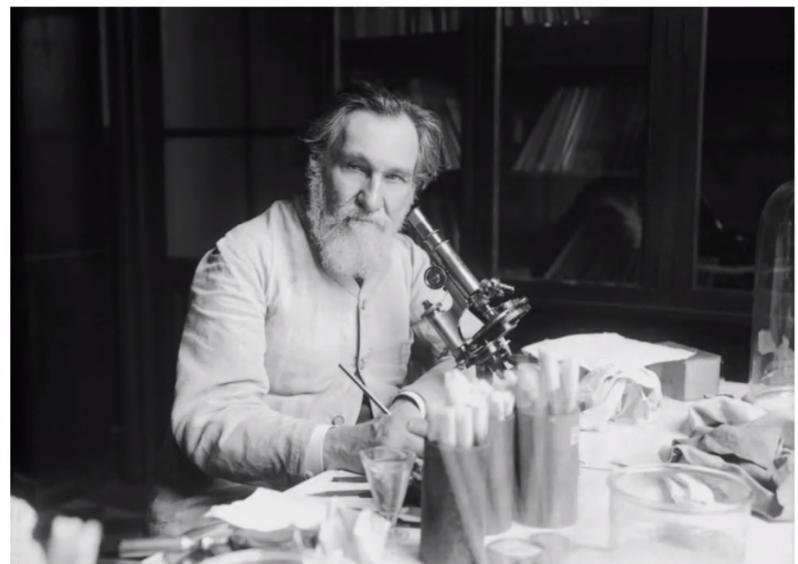
Christopher K. Glass, MD, PhD
Department of Cellular and Molecular Medicine
Department of Medicine
UC San Diego



Macrophages play essential roles in the response to infection and injury



Guinea pig macrophage with *E. coli* bacteria



Metchnikoff in his Pasteur Institute laboratory, 1913.

From Metchnikoff's *Immunity and Infectious Diseases*

Specialized homeostatic functions of resident tissue macrophages

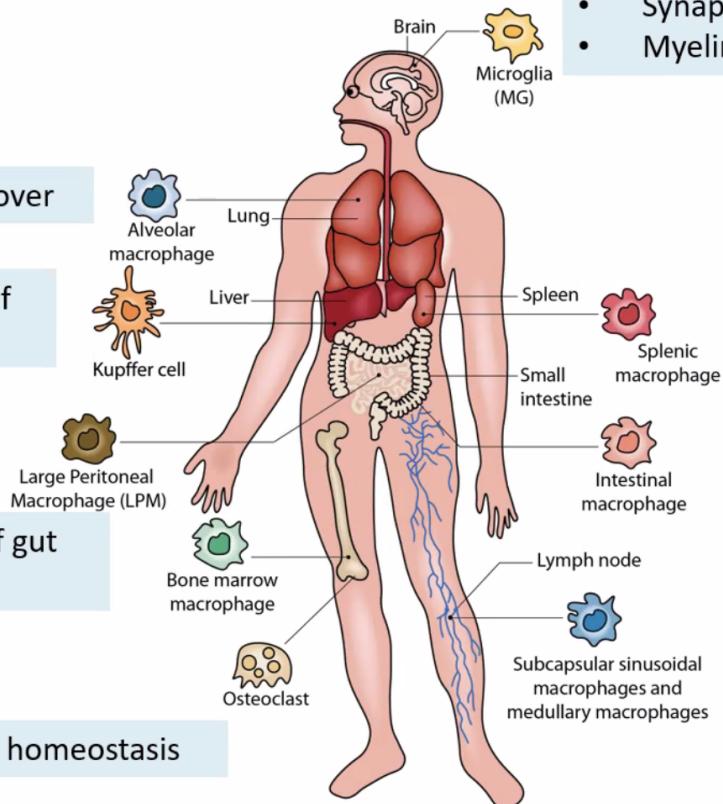
- Surfactant turnover

- Detoxification of portal blood

- Regulation of gut immunity

- Bone homeostasis

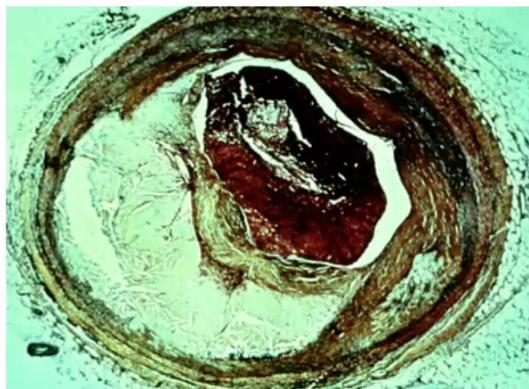
- Synapse refinement
- Myelination



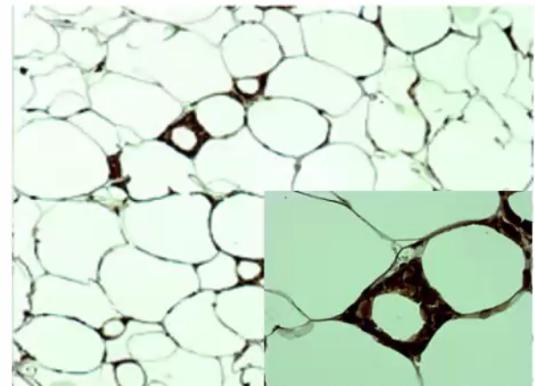
Adapted from PJ Murray, TA Wynn, Nature Reviews Immunology 2011

Roles of macrophages in human disease

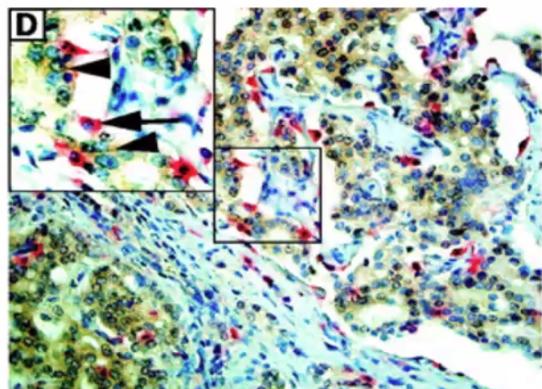
Macrophage foam cells
in atherosclerosis



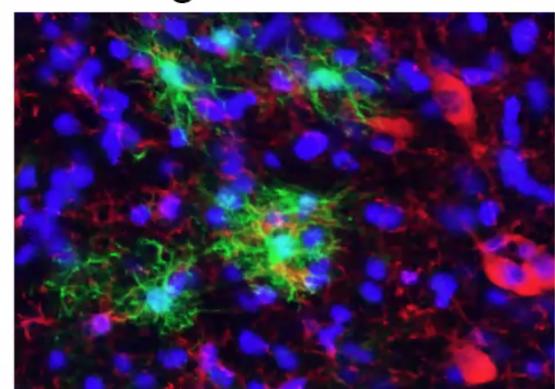
Adipose tissue macrophages
in insulin resistance



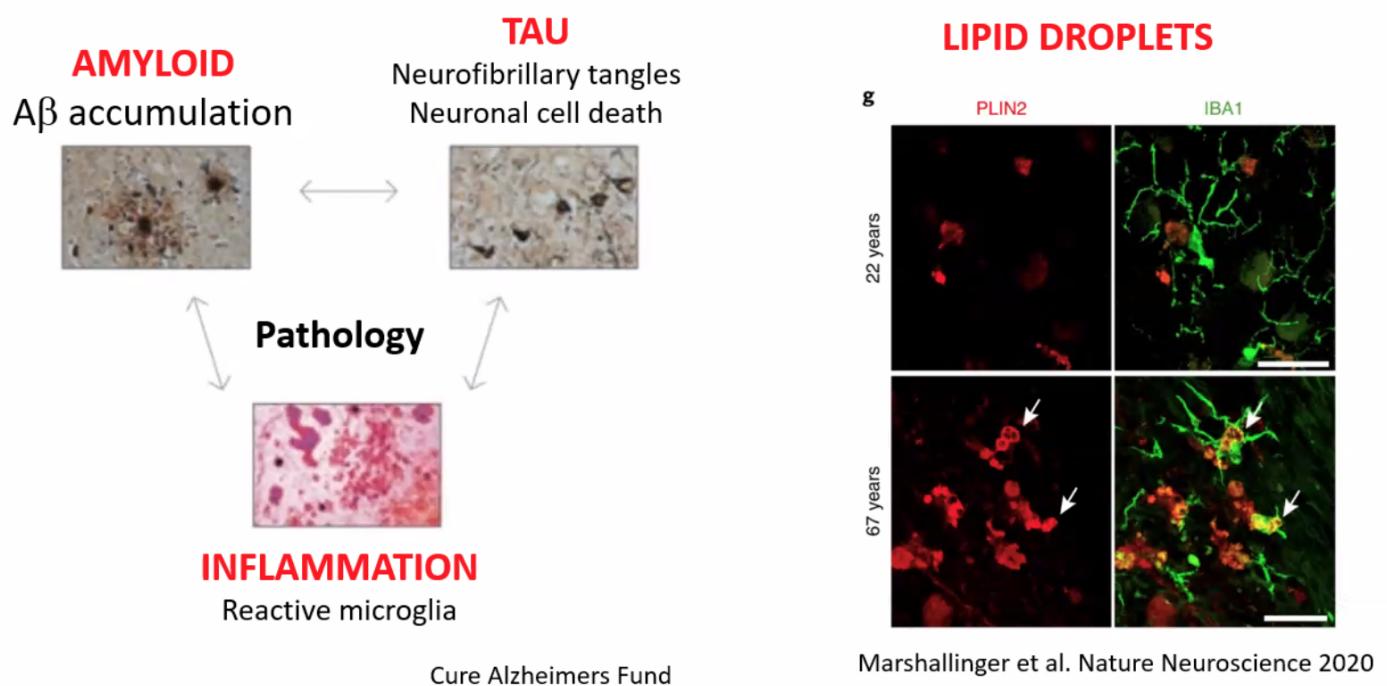
Tumor-associated macrophages
in cancer



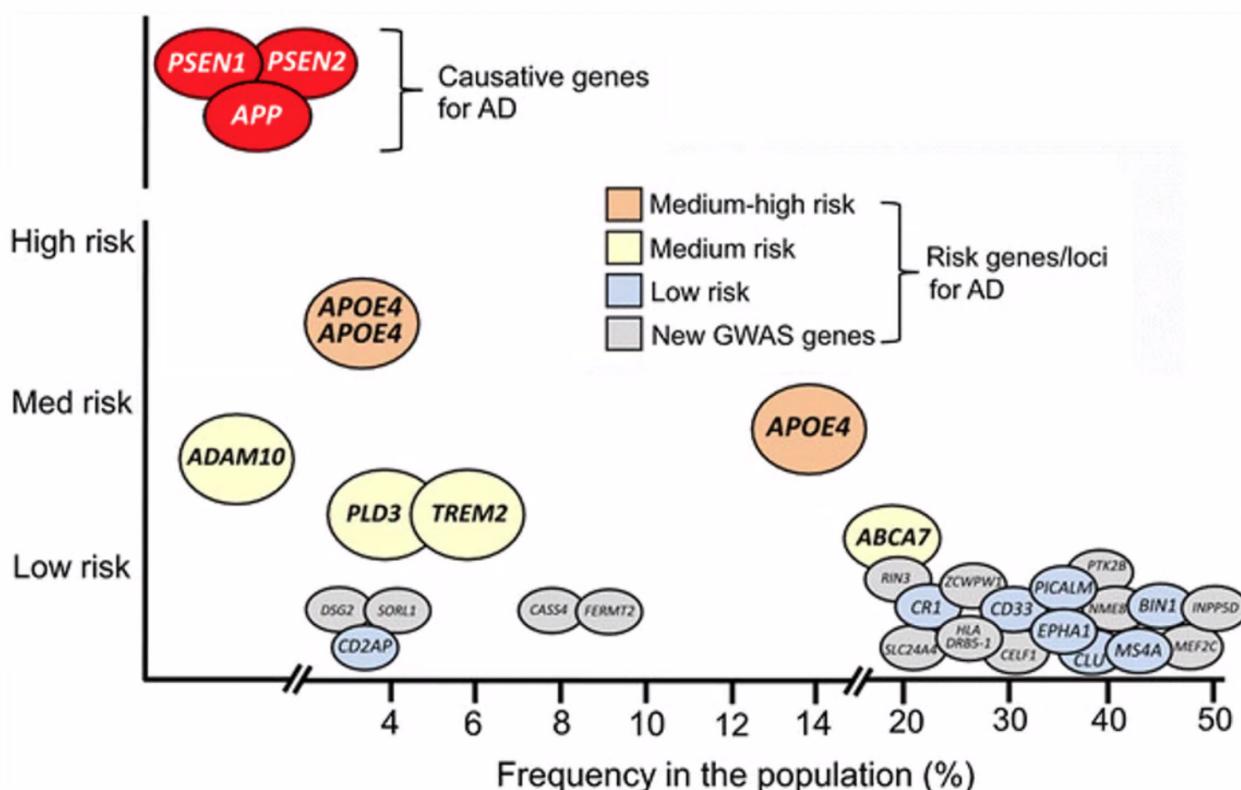
Activated microglia in
neurodegenerative disease



Pathological features of Alzheimer's Disease

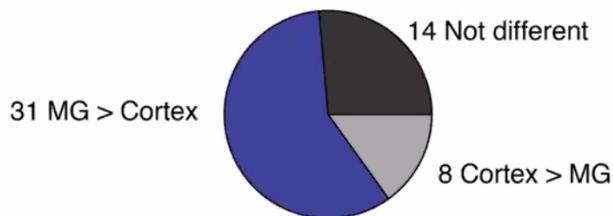
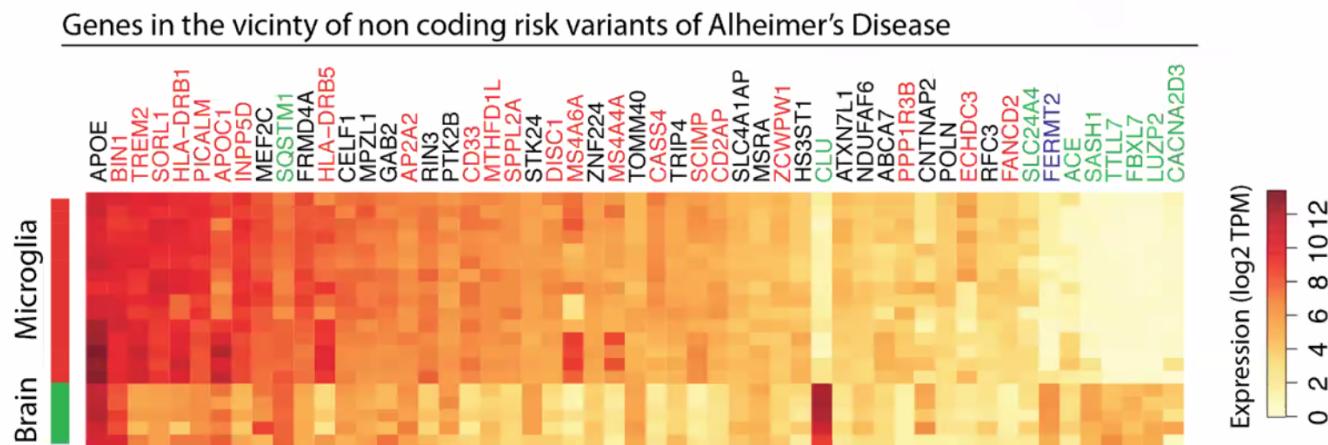


Genetic risk factors for Alzheimer's Disease



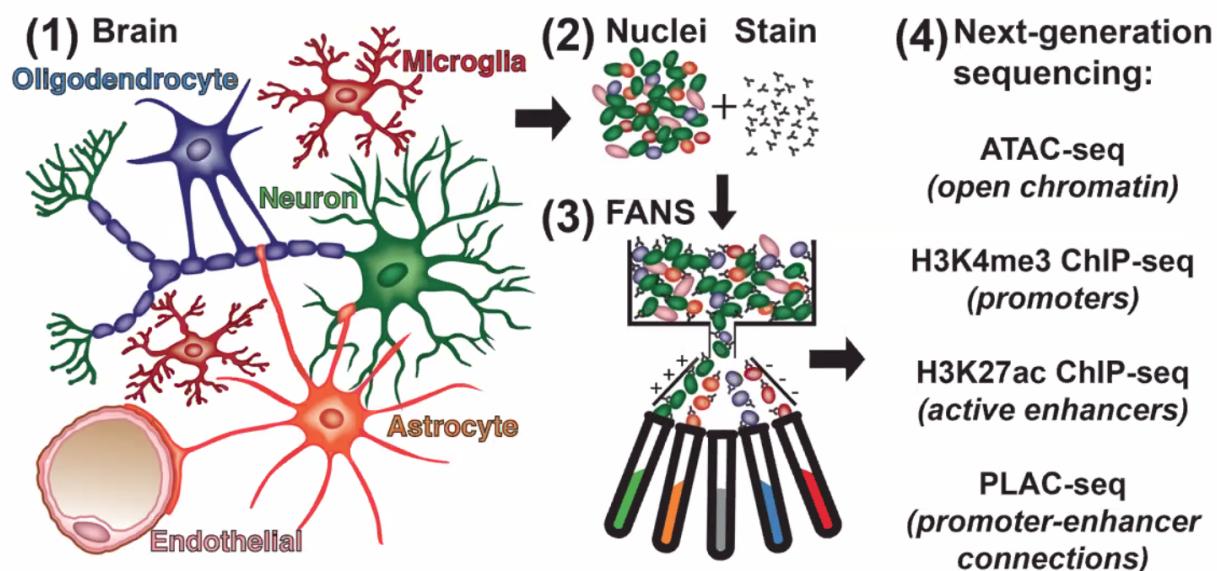
Yamazaki, et al. (2016). CNS drugs

Expression of genes associated with AD risk variants in human MG vs whole brain



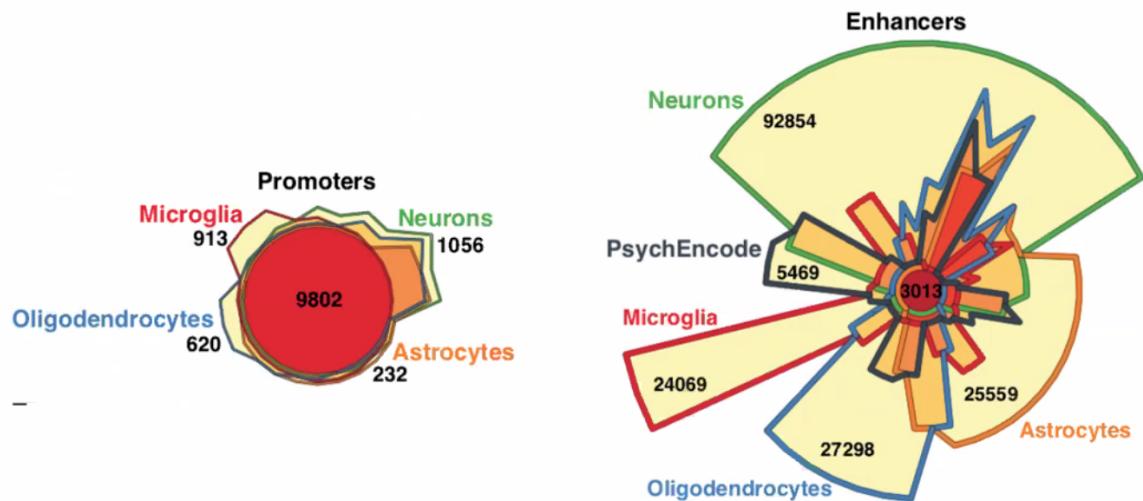
Gosselin, Skola, Coufal, et al., Science 2017

Use of nuclear sorting to define cell-specific enhancer atlases in the human brain



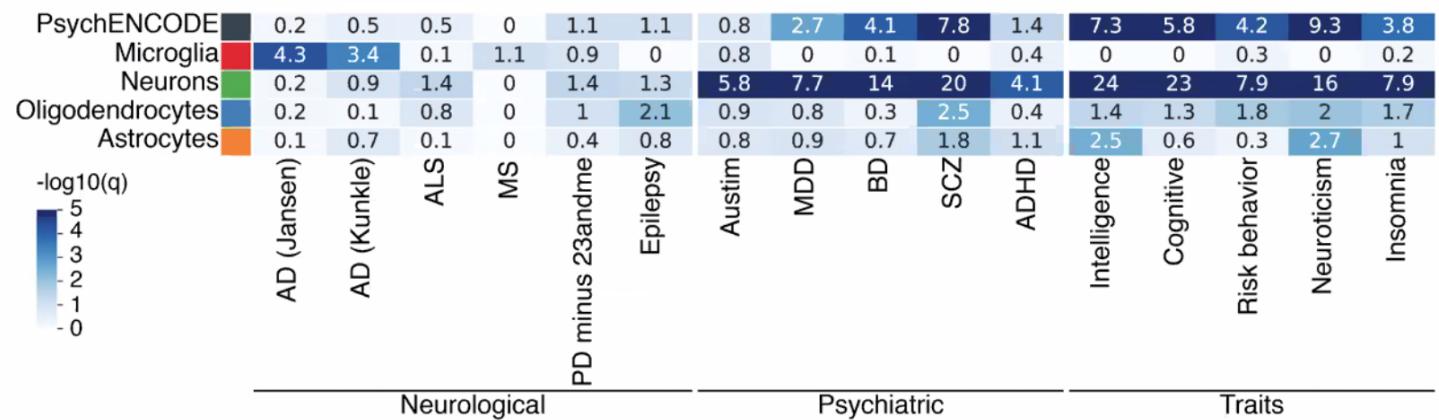
Nott, Holtman, Coufal, et al., Science 2019

Cell-specific enhancers are much more numerous than promoters



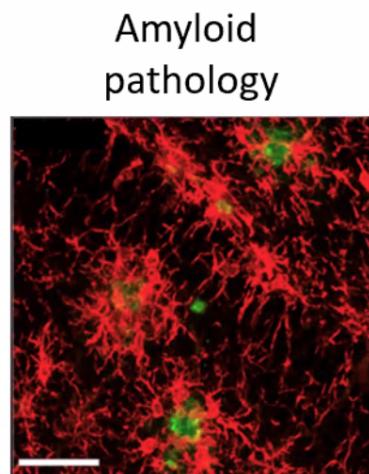
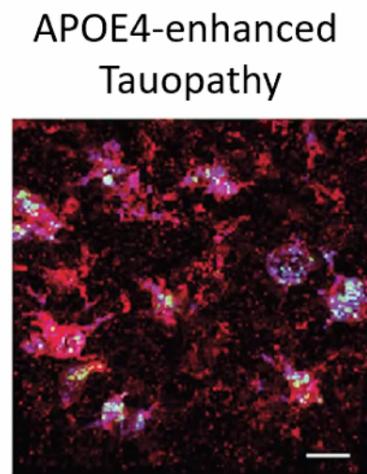
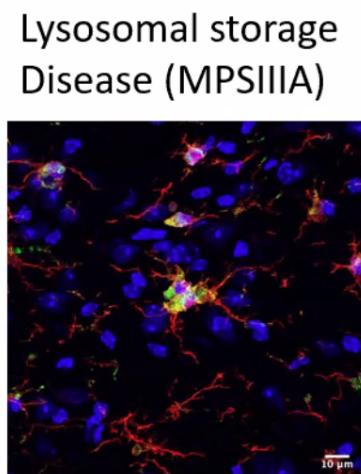
Nott, Holtman, Coufal, et al., Science 2019

Brain regulatory regions are enriched for disease-associated genetic variants



Nott, Holtman, Coufal, et al., Science 2019

Decoding microglia phenotypes in three models of neurodegenerative disease



Trem2-independent Neuropathic?

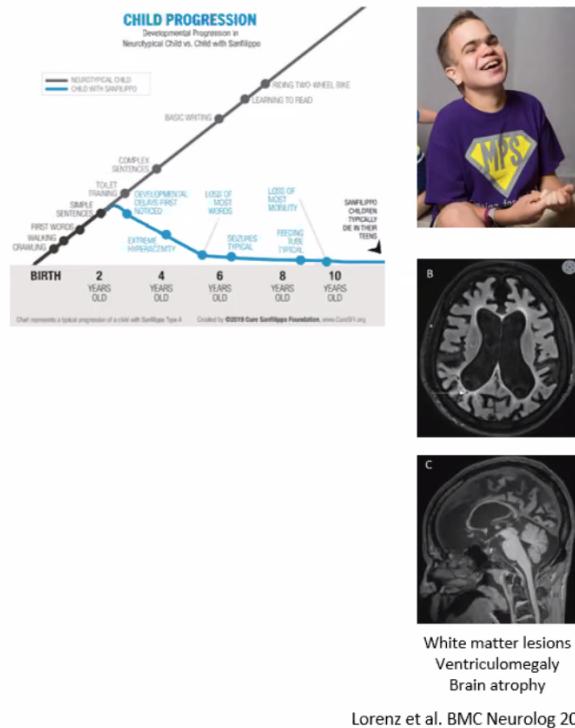
Trem2-independent Neuropathic

Trem2-dependent Neuroprotective

Mucopolysaccharidosis Type IIIA (MPS-IIIa)

- **Most common & severe neurodegenerative LSD¹**

- Childhood onset --> rapid disease progression¹
- Significant neurological deterioration by 4-6 years of age¹

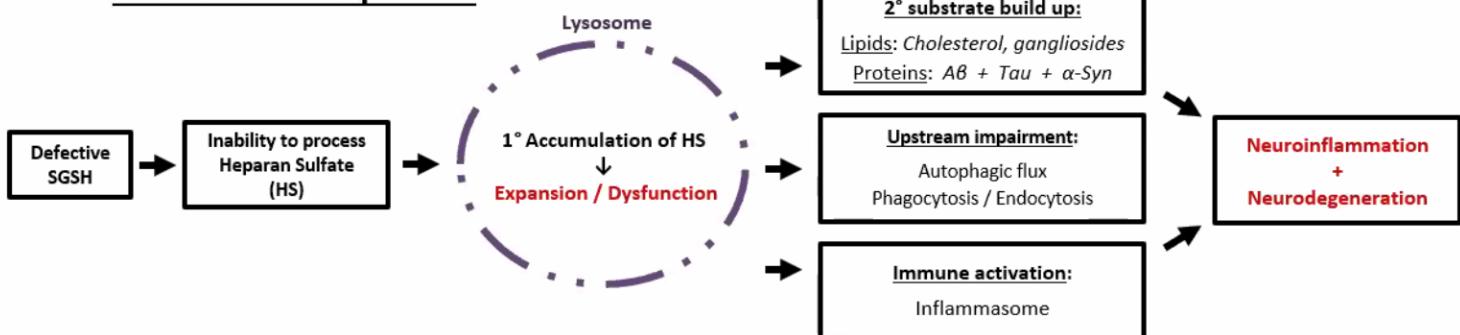


Lorenz et al. BMC Neurolog 2022

- **Cause:** Lysosomal enzyme defect

- *Homozygous LoF mutations in lysosomal Sulfatase (SGSH)*

- **General disease process:**

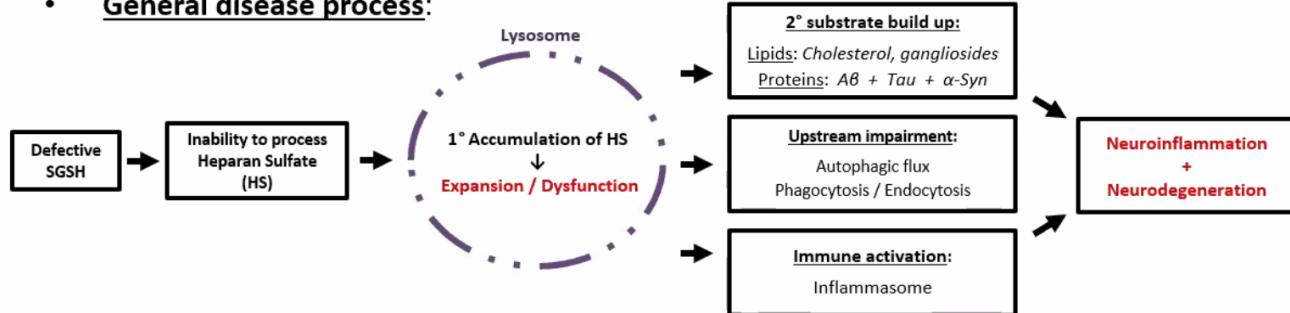


¹Valstar et al. J Inherit Metab Dis 2008

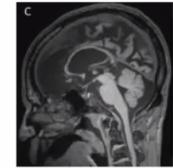
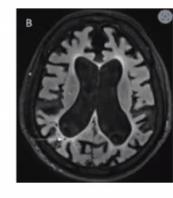
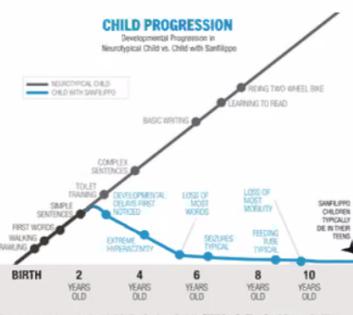
Lorenz e

Mucopolysaccharidosis Type IIIA (MPS-IIIA)

- **Most common & severe neurodegenerative LSD¹**
 - Childhood onset --> rapid disease progression¹
 - Significant neurological deterioration by 4-6 years of age¹
- **Cause:** Lysosomal enzyme defect
 - *Homozygous LoF mutations in lysosomal Sulfatase (SGSH)*
- **General disease process:**



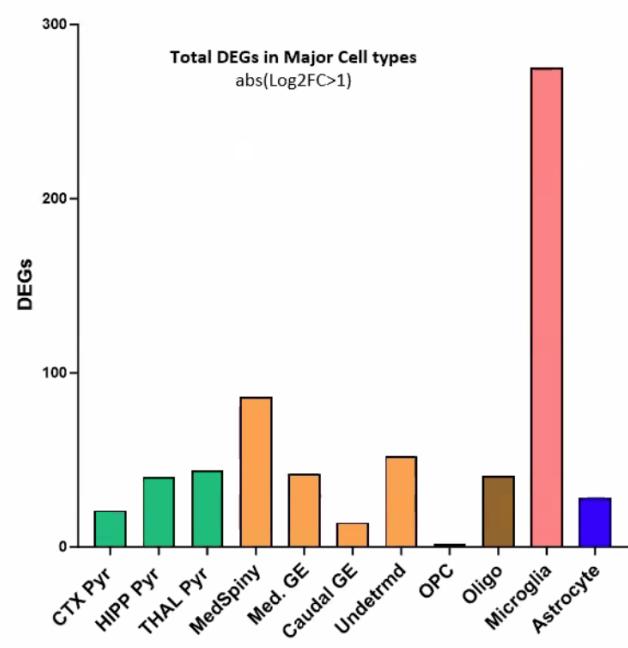
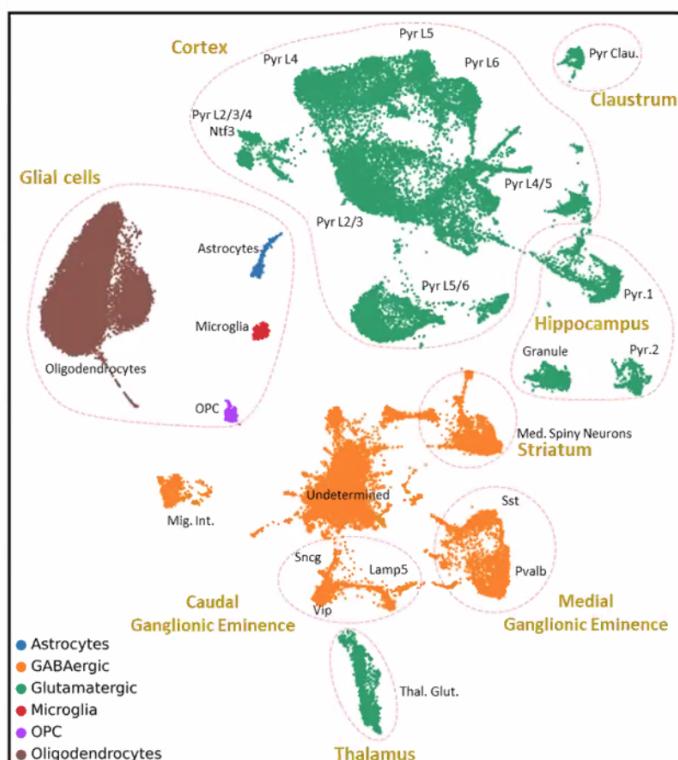
/alstar et al. J Inherit Metab Dis 2008



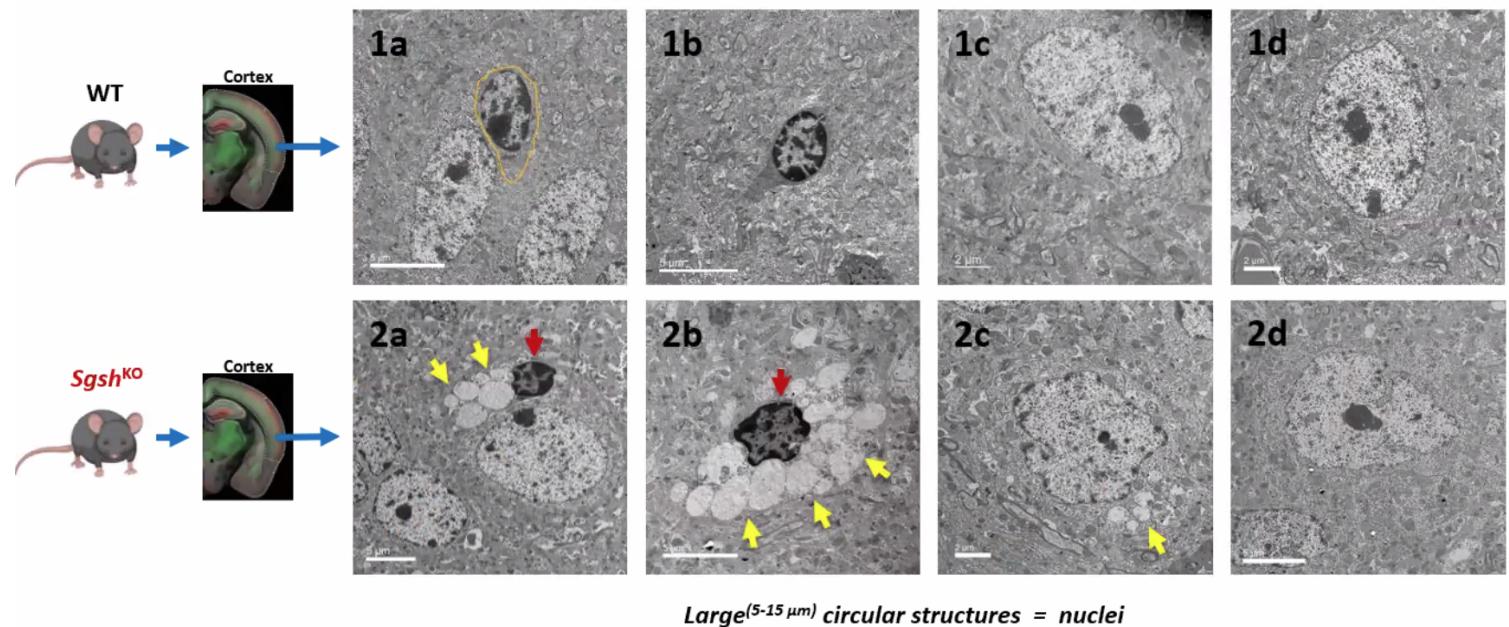
White matter lesions
 Ventriculomegaly
 Brain atrophy

Lorenz et al. BMC Neurolog 2022

Microglia are the most strongly affected brain cell type in Sgsh KO mice

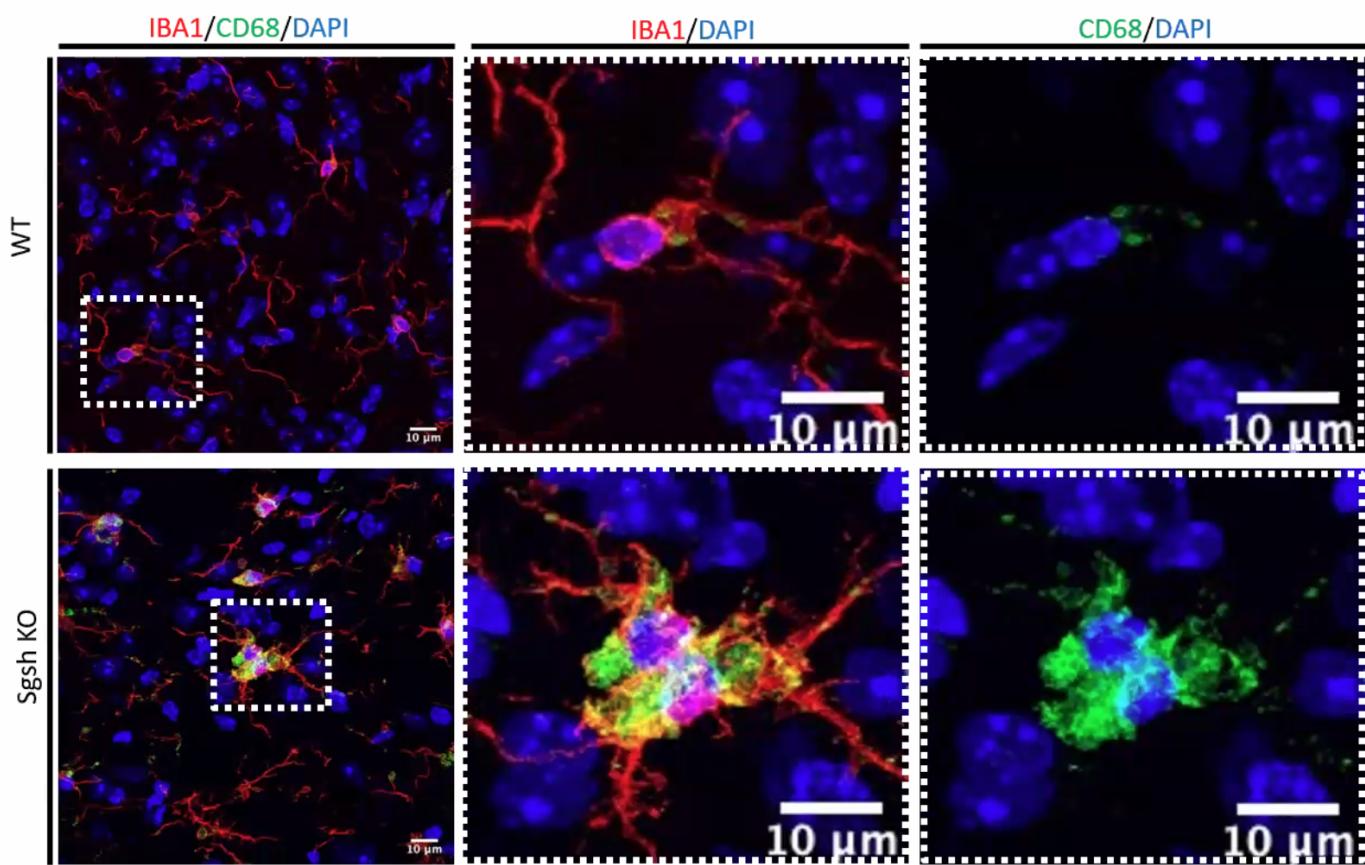


Microglia are the most strongly affected brain cell type in Sgsh KO mice



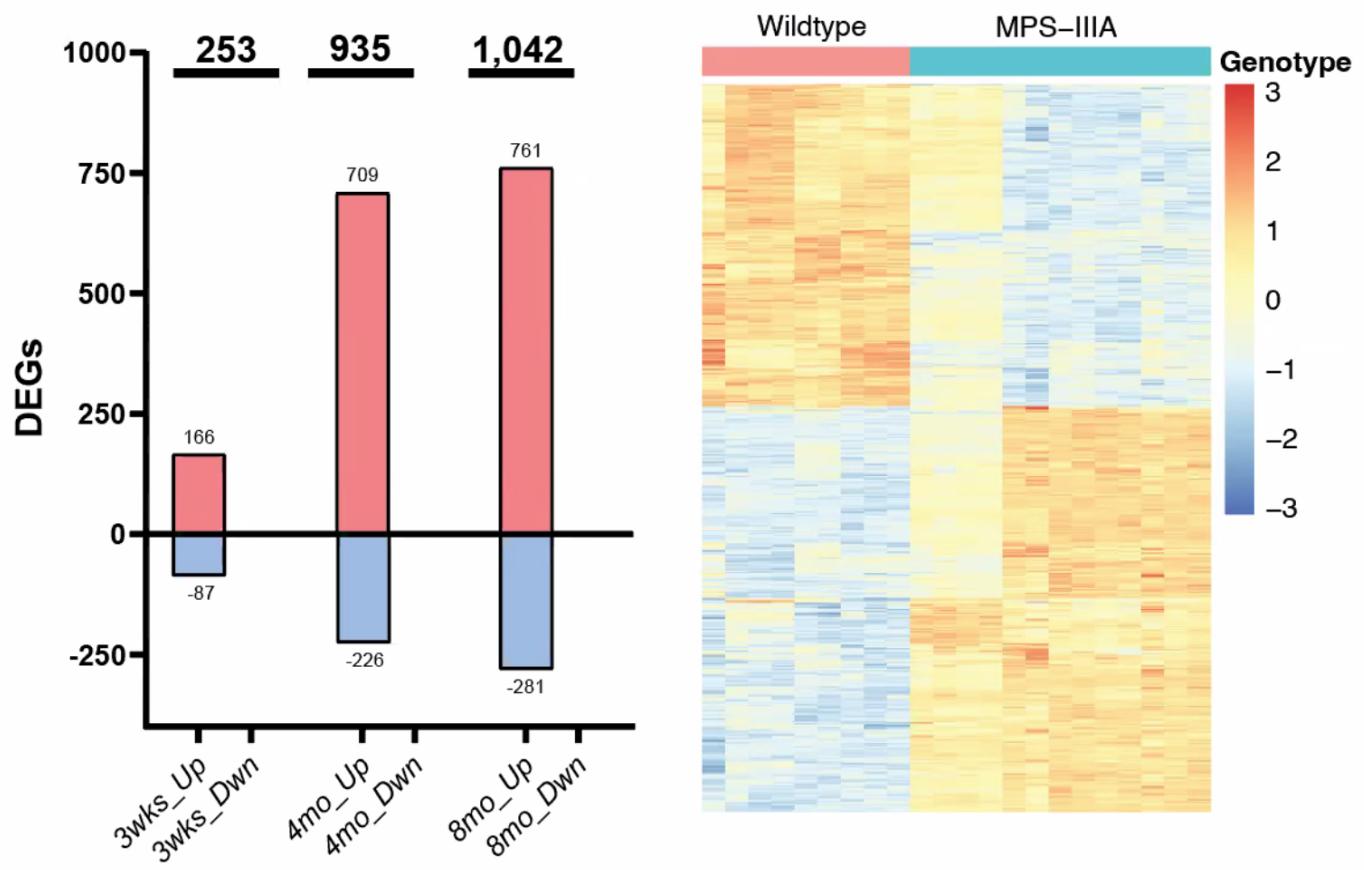
Large($^{5-15} \mu\text{m}$) circular structures = nuclei

Microglia are the most strongly affected brain cell type in Sgsh KO mice



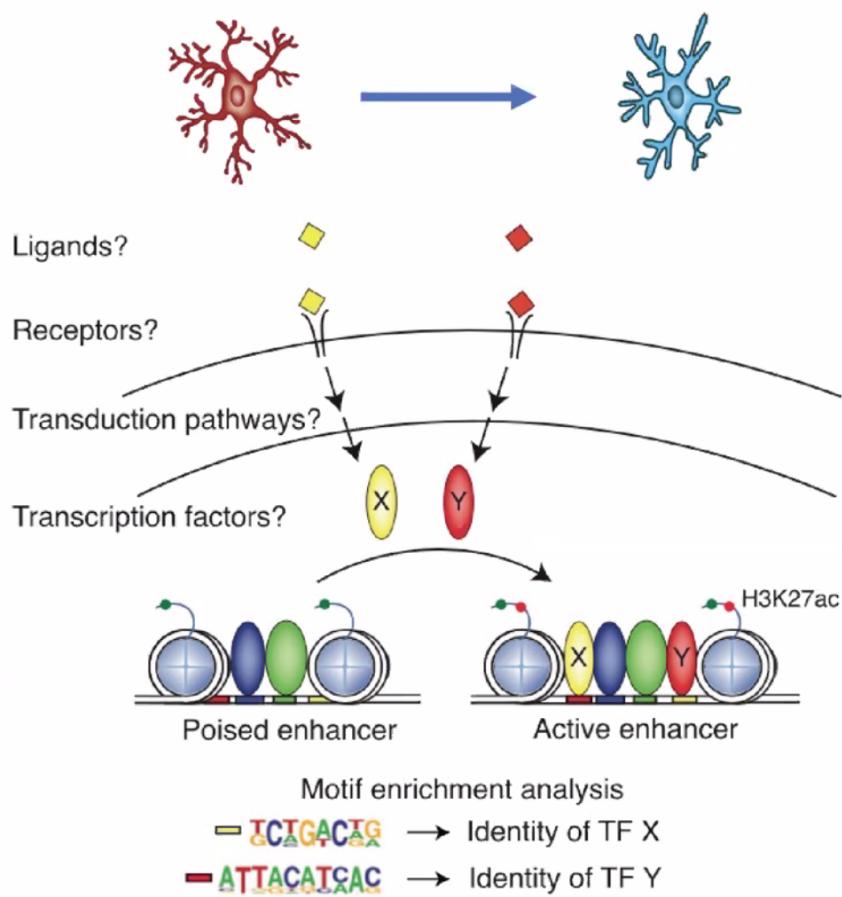
Joy Zhou, Ben Li

Sgsh KO leads to a progressive lysosomal stress response in microglia



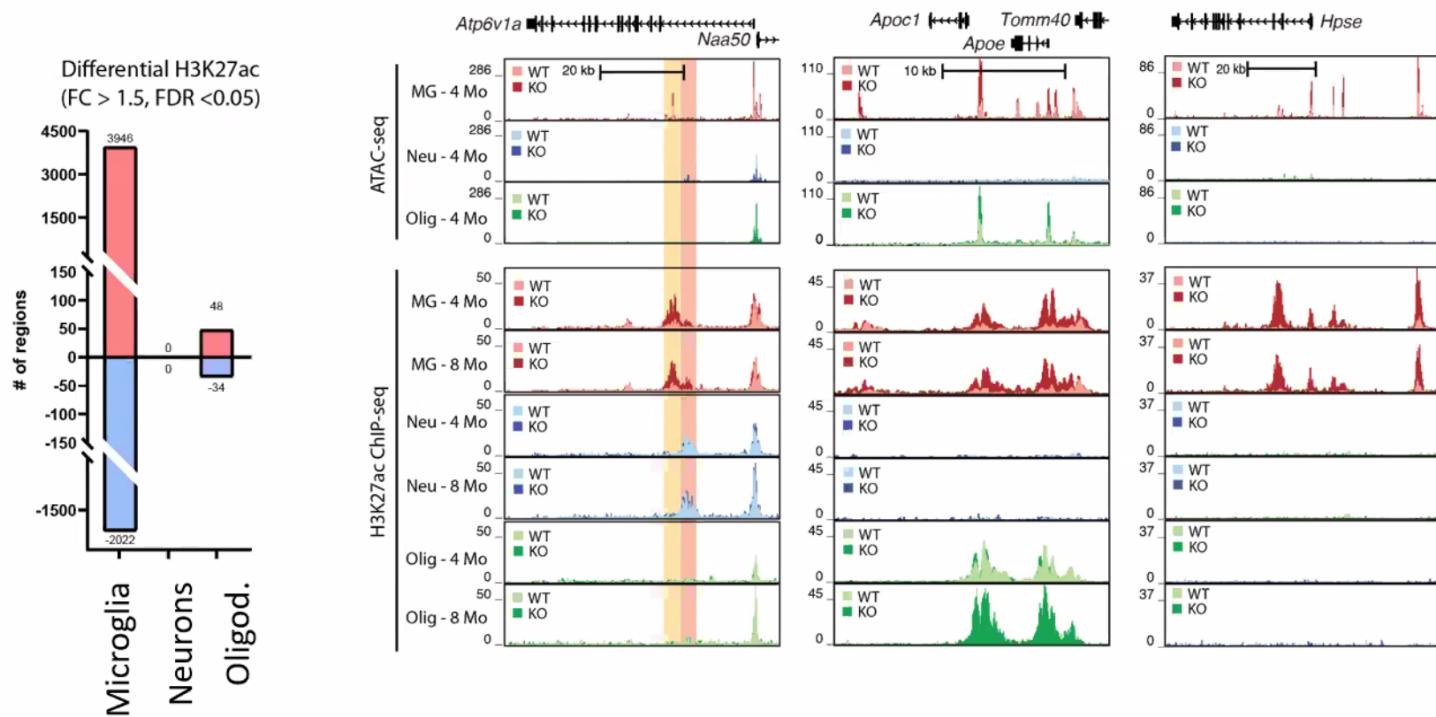
Chris Balak, Johannes Schlachetzki

Exploiting dynamic enhancer landscapes to decode macrophage phenotypes in health and disease



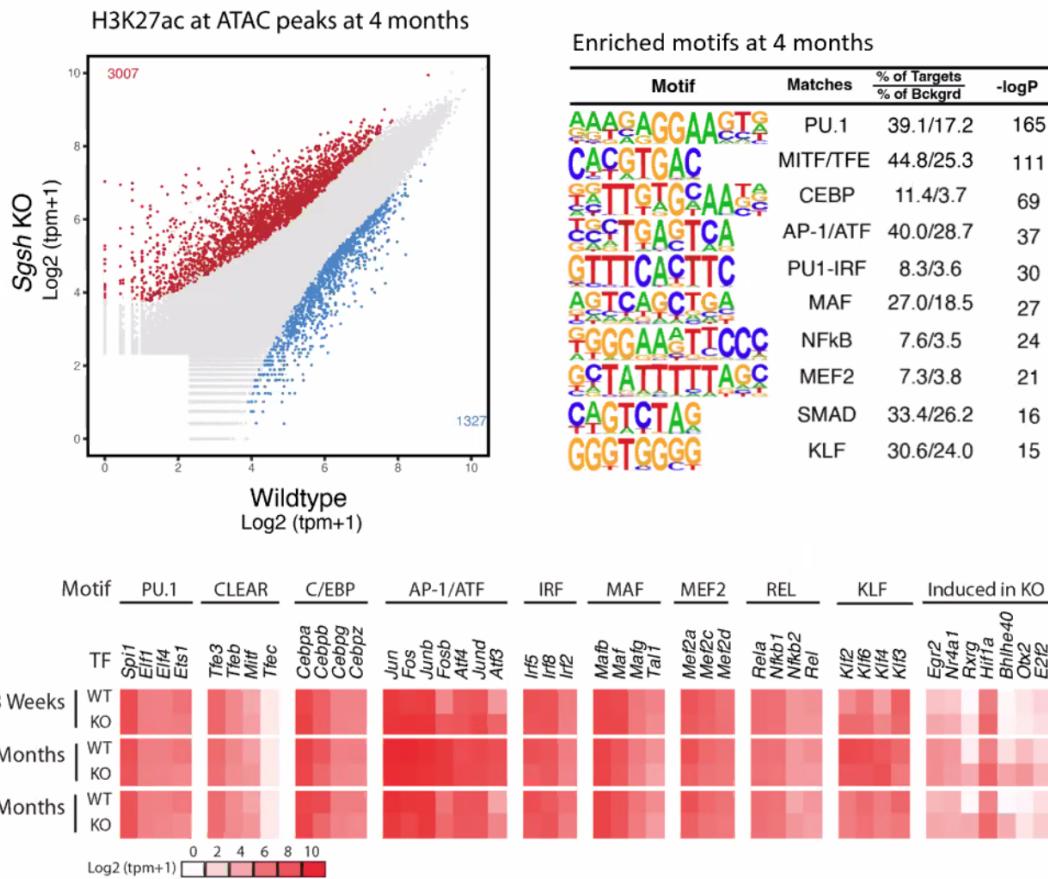
Microglia-selective alterations in the epigenetic landscape in *Sgsh* KO mice

ATAC-seq and H3K27ac ChIP-seq

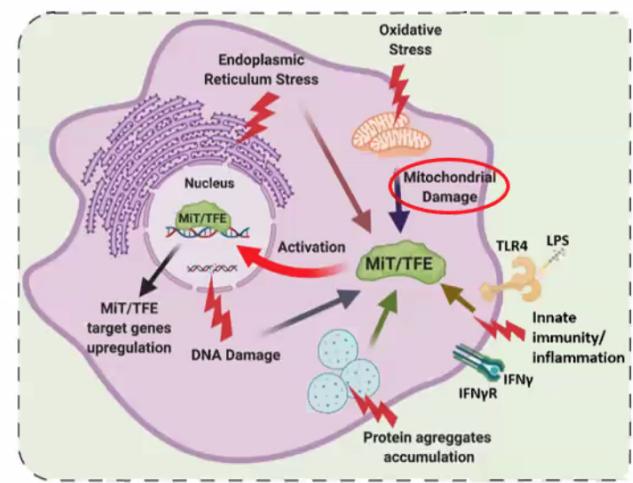
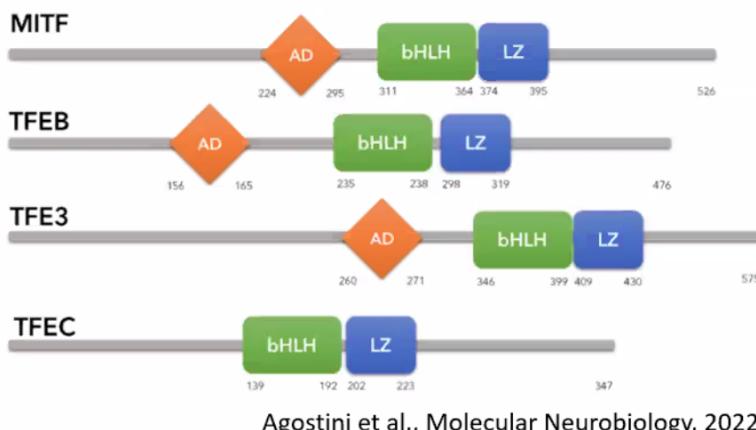


Chris Balak

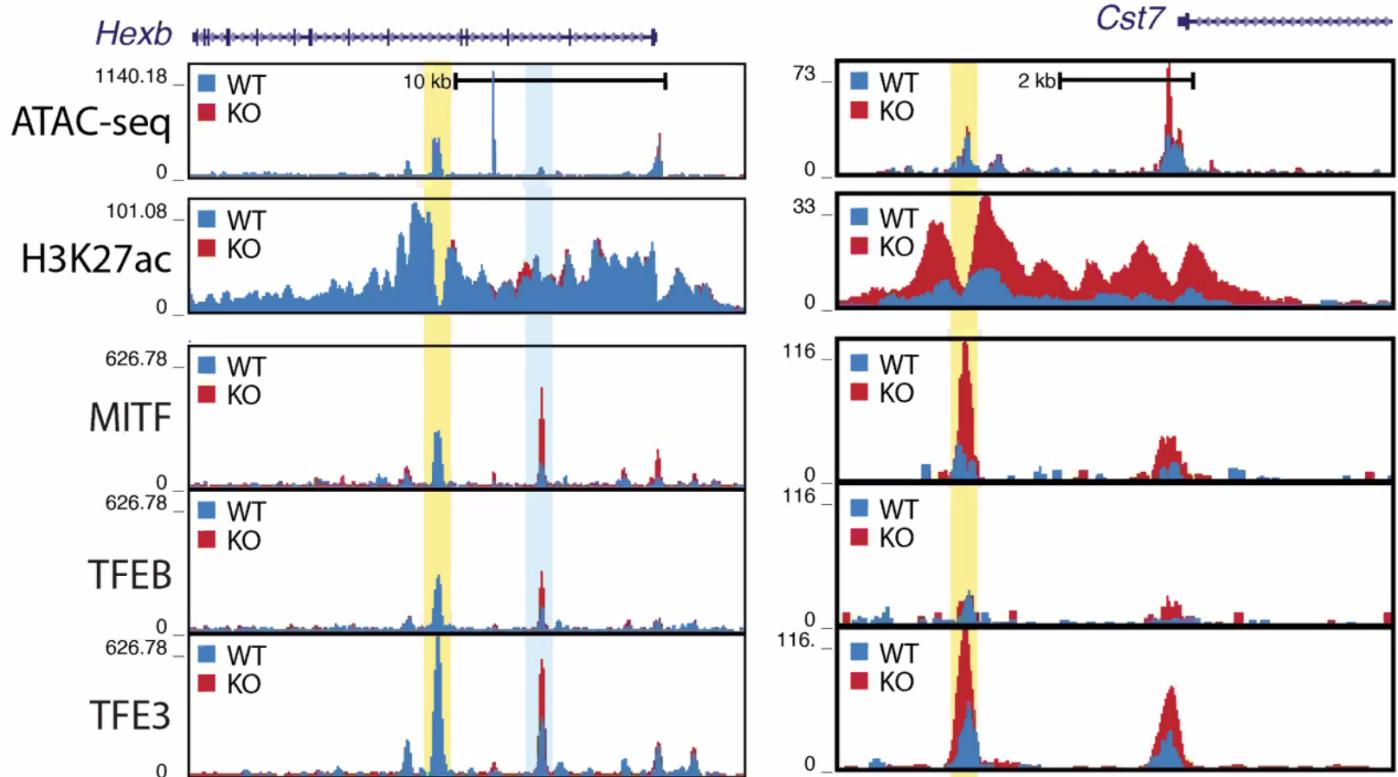
Inference of transcriptional drivers of the lysosomal stress response in *Sgsh* KO microglia



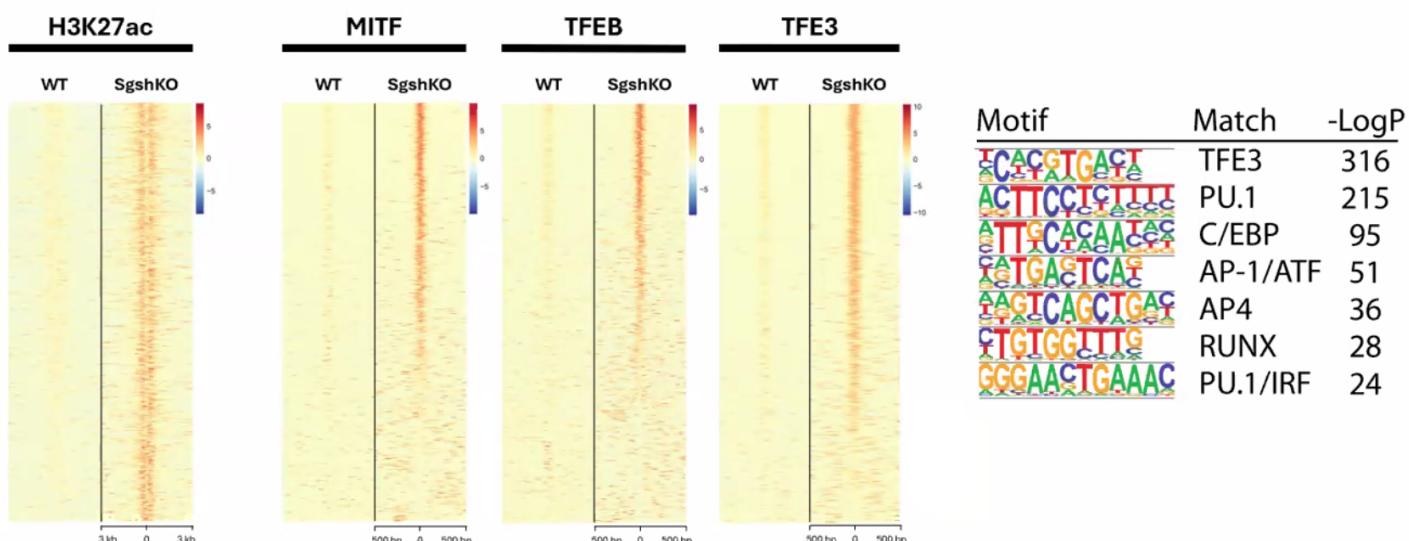
The MITF / TFE family of transcription factors



Constitutive and induced binding of MITF, TFE3 and TFE3 in *Sgsh* KO microglia



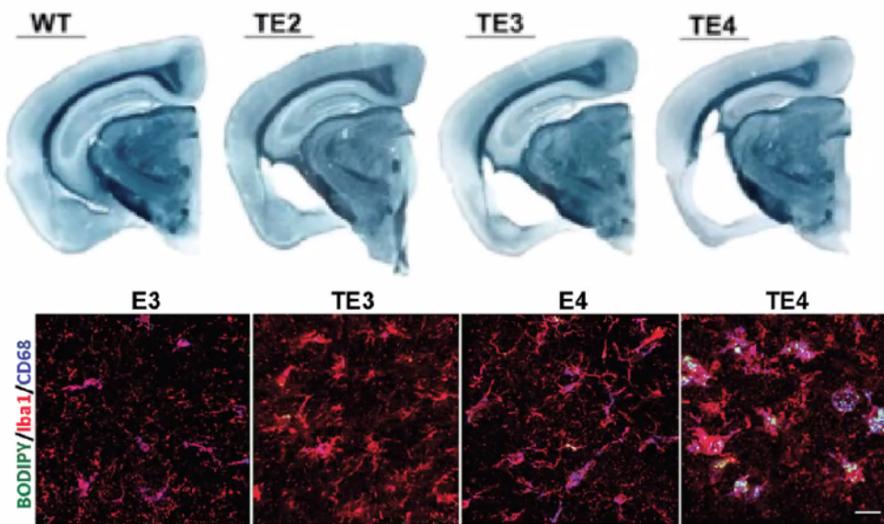
Increased binding of MITF, TFEB and TFE3 in *Sgsh* KO microglia is strongly associated with increased H3K27ac and gene activation



Decoding microglia phenotypes in the MPSIIIA mouse

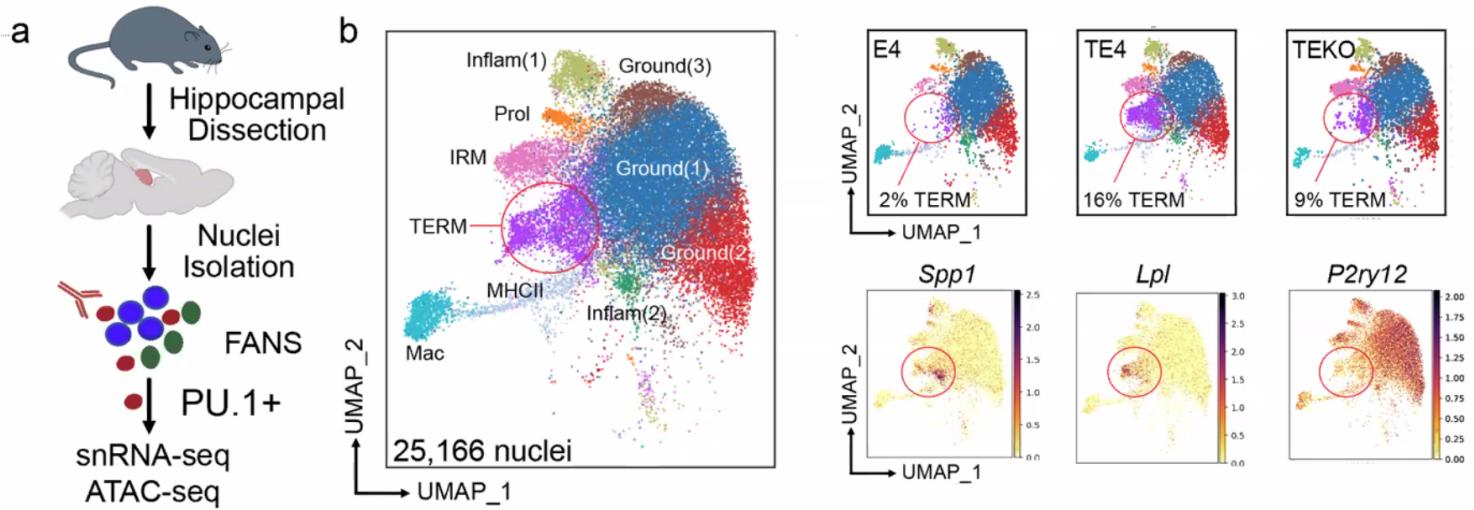
- Loss of function of *Sgsh* primarily affects microglia
- Quantitative analyses of open chromatin and H3K27ac define an epigenetic signature of a lysosomal stress response in microglia
- Motif enrichment analysis enables inference of transcriptional drivers consisting of lineage-determining and signal-dependent transcription factors
- Findings suggest that members of the MITF/TFE family of transcription factors collaborate with C/EBP, PU.1 and AP-1 factors to drive the lysosomal stress response.

Decoding microglia phenotypes in the TE4 mouse model of neurodegeneration



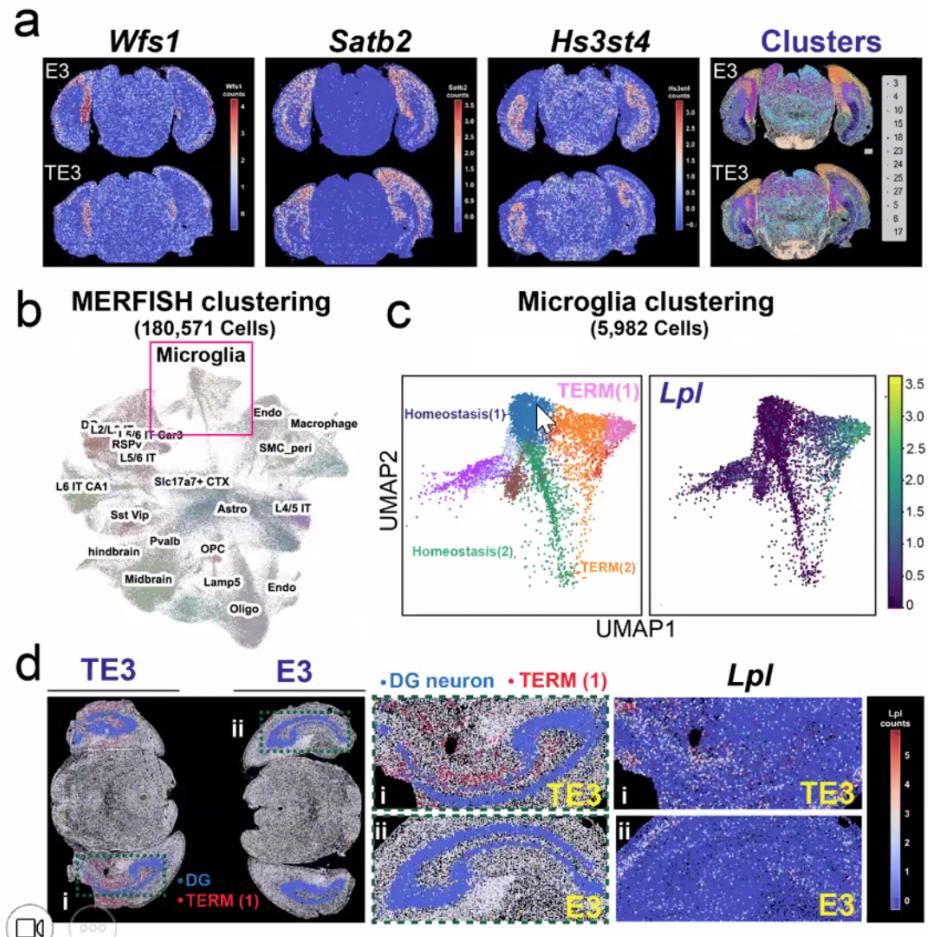
- PS19 Tau and the combination of human APOE3 or APOE4 drive severe neurodegeneration (APOE4 > APOE3)
- Neurodegeneration is strongly dependent on microglia
- Microglia in TE4 mice exhibit evidence of lysosomal dysfunction, including accumulation of lipid droplets.

Identification of Tau/apoE-Responsive Microglia (TERMs) in TE4 mice

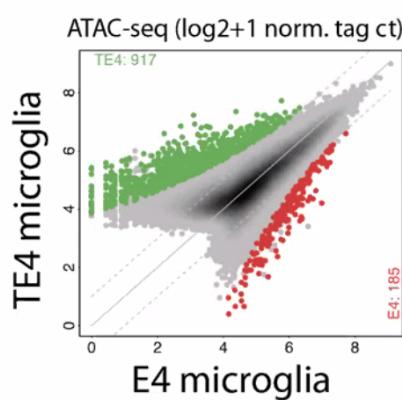
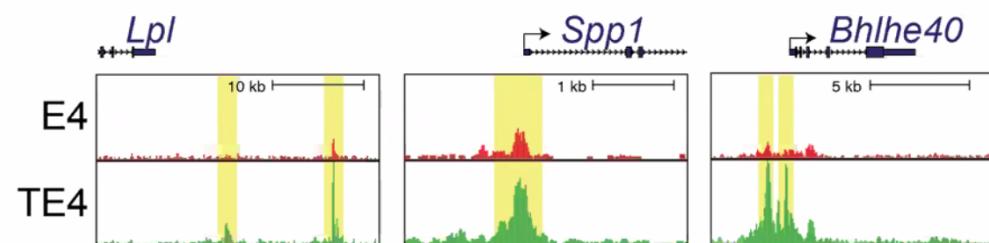


Johannes Schlachetzki

TERMs specifically localize to areas of neurodegeneration in the hippocampus



Quantitative analysis of open chromatin in TE4 microglia enables inference of the drivers of the TERM phenotype

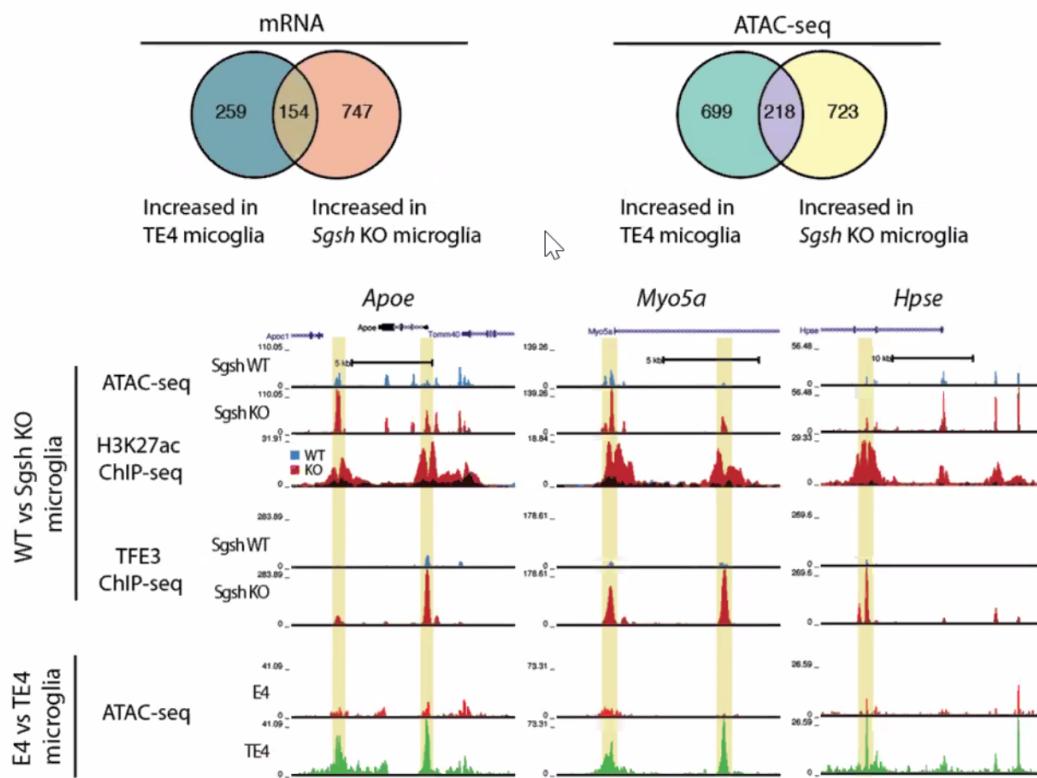


Motif analysis - TE4 peaks >2x TE peaks

Matches	-logP	Matches
403		C/EBP
246		PU.1
140		AP-1/ATF3
139		MITF/BHLH
113		EGR2

Johannes Schlachetzki

The transcriptomic and epigenetic signature of TE4 microglia overlaps with that of Sgsh KO microglia



Chris Balak, Johannes Schlachetzki

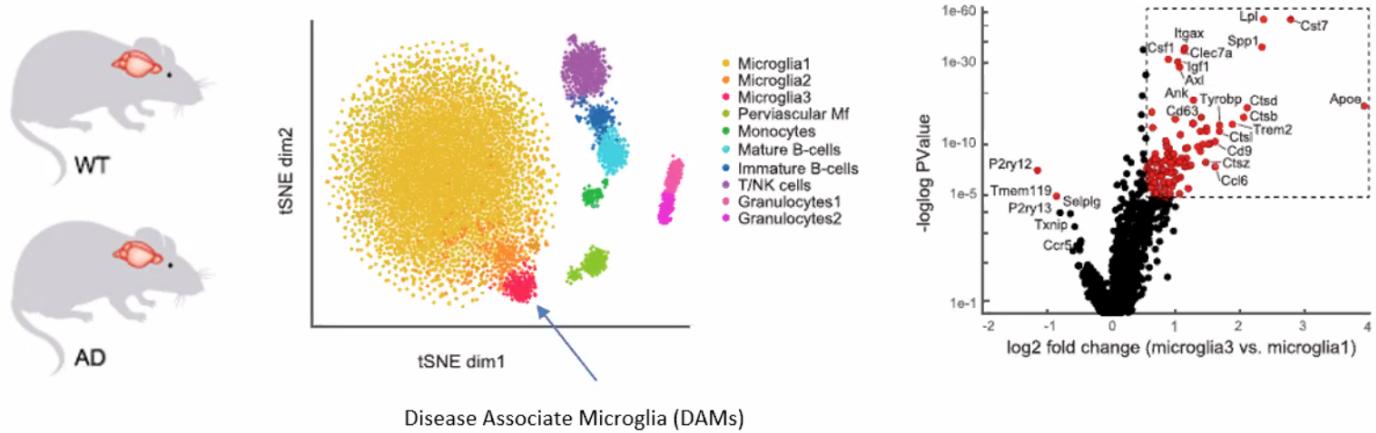
Decoding microglia phenotypes in the PS19 Tau/humanized APOE mouse

- The combination of PS19Tau and human APOE3 or APOE4 induces the development of a Tau/apoE-Responsive Microglia (TERM) phenotype that is associated with neurodegeneration
- Spatial transcriptomic analyses localize TERMS to focal regions of neurodegeneration in the brain
- The altered open chromatin landscape of microglia from TE4 mice suggests that the TERM phenotype is driven by C/EBP, PU.1, AP-1/ATF, MITF/TFE and EGR transcription factors
- The altered chromatin landscapes of TE4 microglia and Sgsh KO microglia exhibit significant overlap, suggesting overlapping mechanisms driving disease phenotypes

Decoding microglia phenotypes in mouse models of amyloid pathology

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

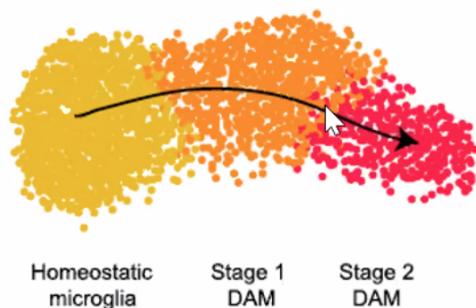
Hadas Keren-Shaul,^{1,6} Amit Spinrad,^{1,2,6} Assaf Weiner,^{1,3,6,*} Orit Matcovitch-Natan,^{1,2,6} Raz Dvir-Szternfeld,² Tyler K. Ulland,⁴ Eyal David,¹ Kuti Baruch,² David Lara-Astaiso,¹ Beata Toth,⁵ Shalev Itzkovitz,⁵ Marco Colonna,⁴ Michal Schwartz,^{2,7,*} and Ido Amit^{1,7,8,*}



Keren-Shaul et al., Cell 2017

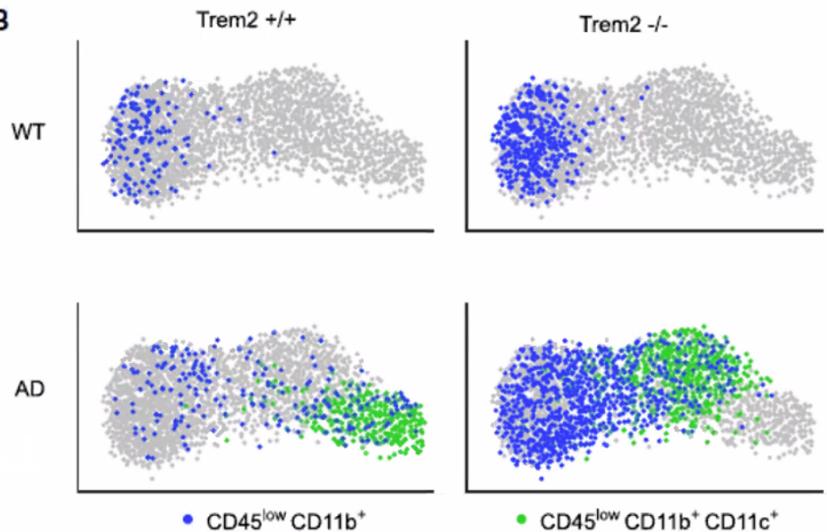
TREM2-independent and TREM2-dependent transitions are required for the DAM phenotype

A



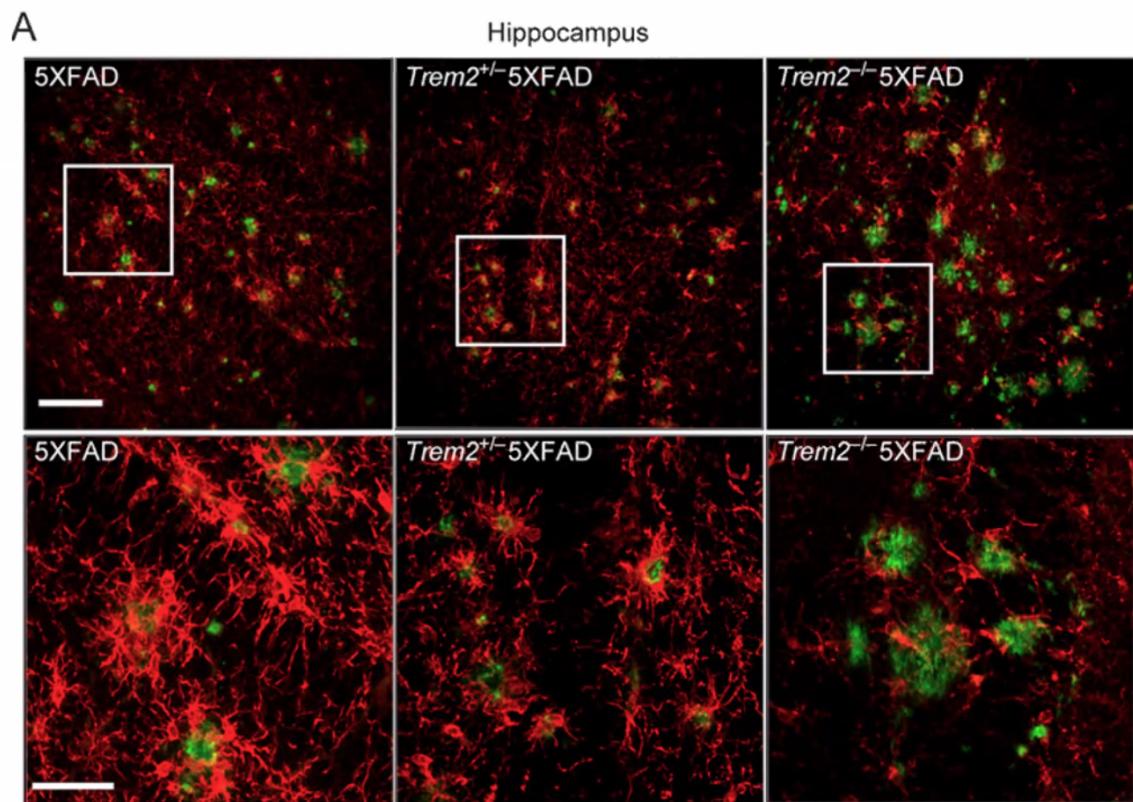
Single cell RNA-seq analysis of microglia in a
mouse model of A-beta amyloid accumulation

B



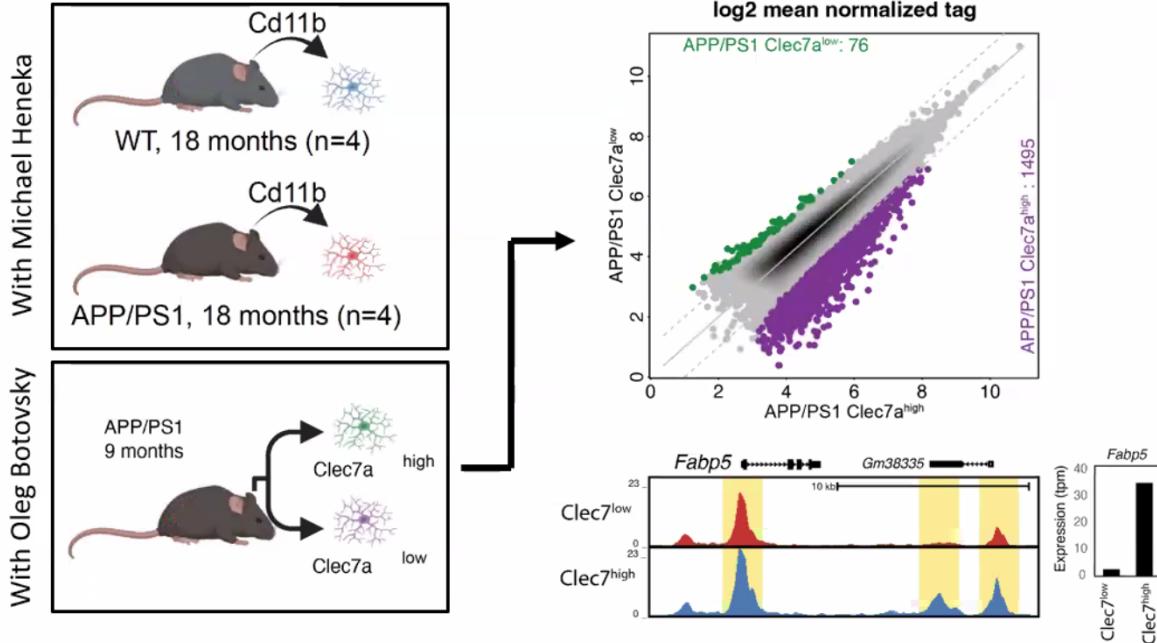
Keren-Shaul et al., Cell 2017

Evidence for Trem2-dependent protective functions of DAMs in Alzheimer's disease

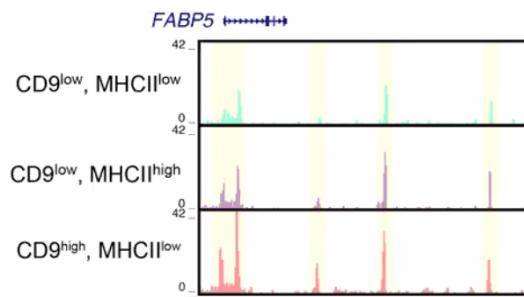
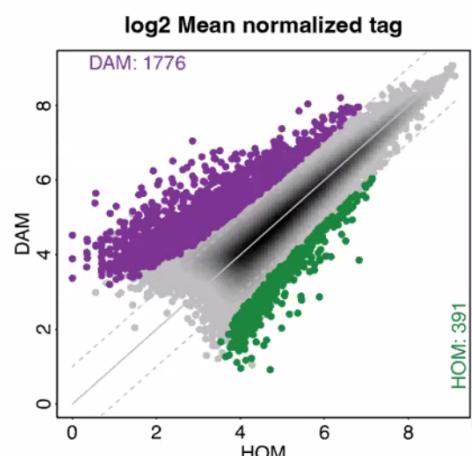
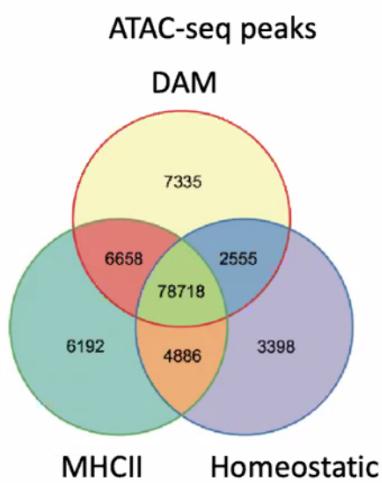
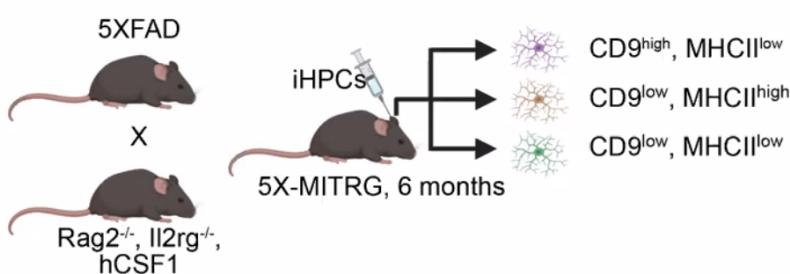


Wang et al., 2015, Cell 160, 1061–1071

Identification of amyloid responsive genomic regions in microglia isolated from APP/PS1 mice



Identification of amyloid responsive genomic regions in human microglia isolated from 5X-MITRG mice



Jonny Hasselman, Matt Blurton-Jones

A similar and extensive set of TF motifs is enriched in amyloid responsive genomic regions in mouse and human microglia

Mouse microglia

Rank	Motif	Best Match
1	SATTCACAACT	C/EBP
2	ACTTCCCTCTT	PU.1
3	GTGAAGTCATA	ATF
4	GTCACGTGACCA	TFE
5	CTAAAAATAG	MEF2
6	CCGCCACACGC	EGR
7	ATGACCTCAT	JUN
8	CTAACCAACAGCC	RUNX
9	TTACTGTTTC	ISRE
10	GTCAGCTGGT	TCF
11	CACGCTTCCAC	NFAT

Human microglia

Rank	Motif	Best Match
1	ACTTCCCTCATTC	PU.1
2	GC GTGGGTGG	EGR
3	TTGCCCAAAATC	CEBP
4	GTCATGTGATC	TFE
5	GTGACTCATCA	ATF
6	TGATGAA	CEBP
7	TCAGCTGACT	TCF
8	TGATGACACAAT	JUN
9	TAACCACA	RUNX
10	GCTAACTC	MAF
11	CTATTTATAGAA	MEF2

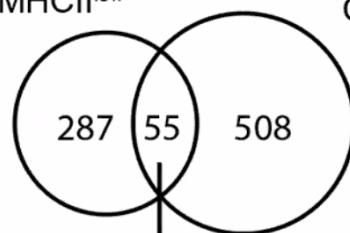


Johannes Schlachetzki

Differentially expressed genes in mouse and human amyloid responsive microglia exhibit much less similarity

Human microglia amyloid-responsive genes

CD9^{high}, MHCII^{low}
HLA-DRB1, CIITA,
MS4A6, MAFB
CD36, MITF, PPARG

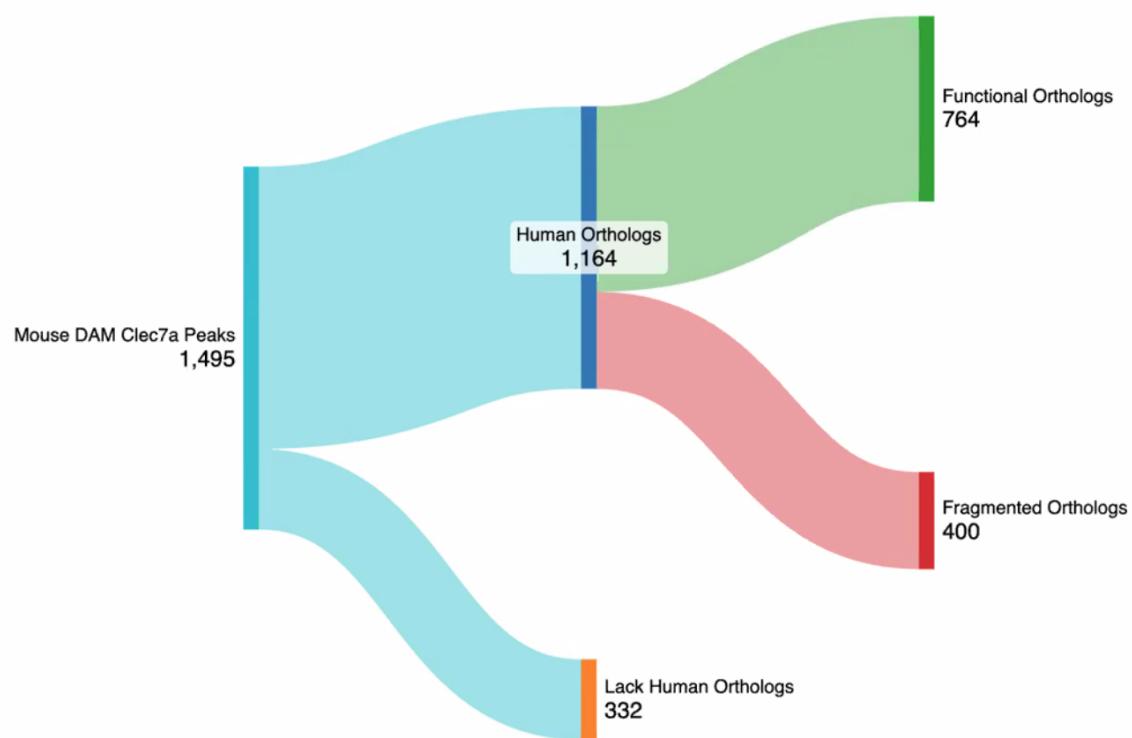


CD9, LPL, ATF3, GPNMB
EGR2, SPP1, MYO1E, FABP3
FABP5, APOC2, LGALS3

Mouse microglia amyloid-responsive genes

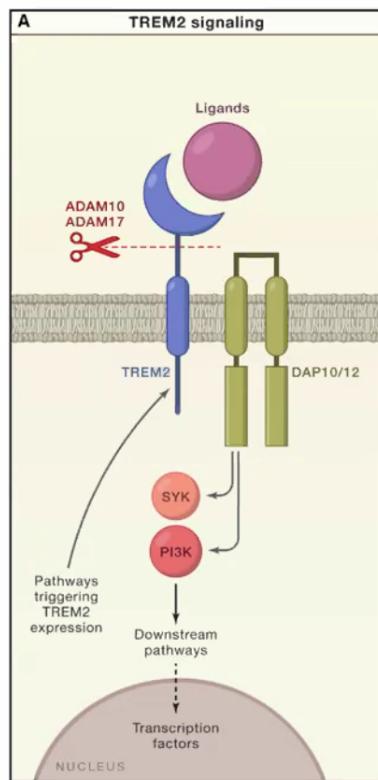
Clec7a^{high}
Clec7a, Hexa, Cd68,
Csf1, Axl, Ch25h,
Itgax, Soat1, Hif1a

Differences in mouse and human cis-regulatory architecture likely explain different responses to amyloid at the RNA level



Fatir Qureshi, Olivia Corradin

Significant knowledge gaps upstream and downstream of TREM2

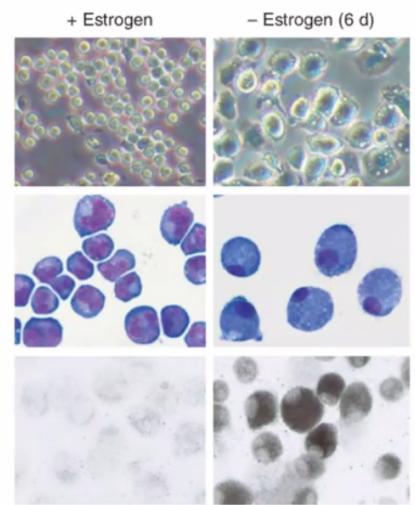
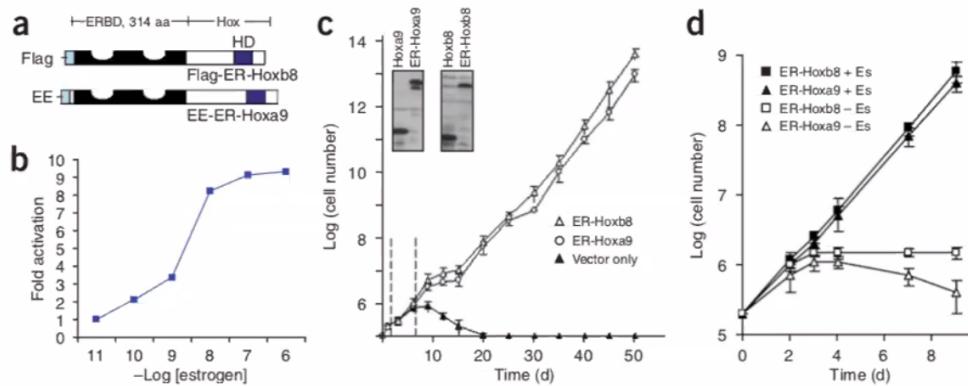


Deczkowska, Weiner and Amit, Cell 181, 1207 2020

ER-HoxB8 conditionally immortalized monocyte progenitors can be differentiated into macrophages

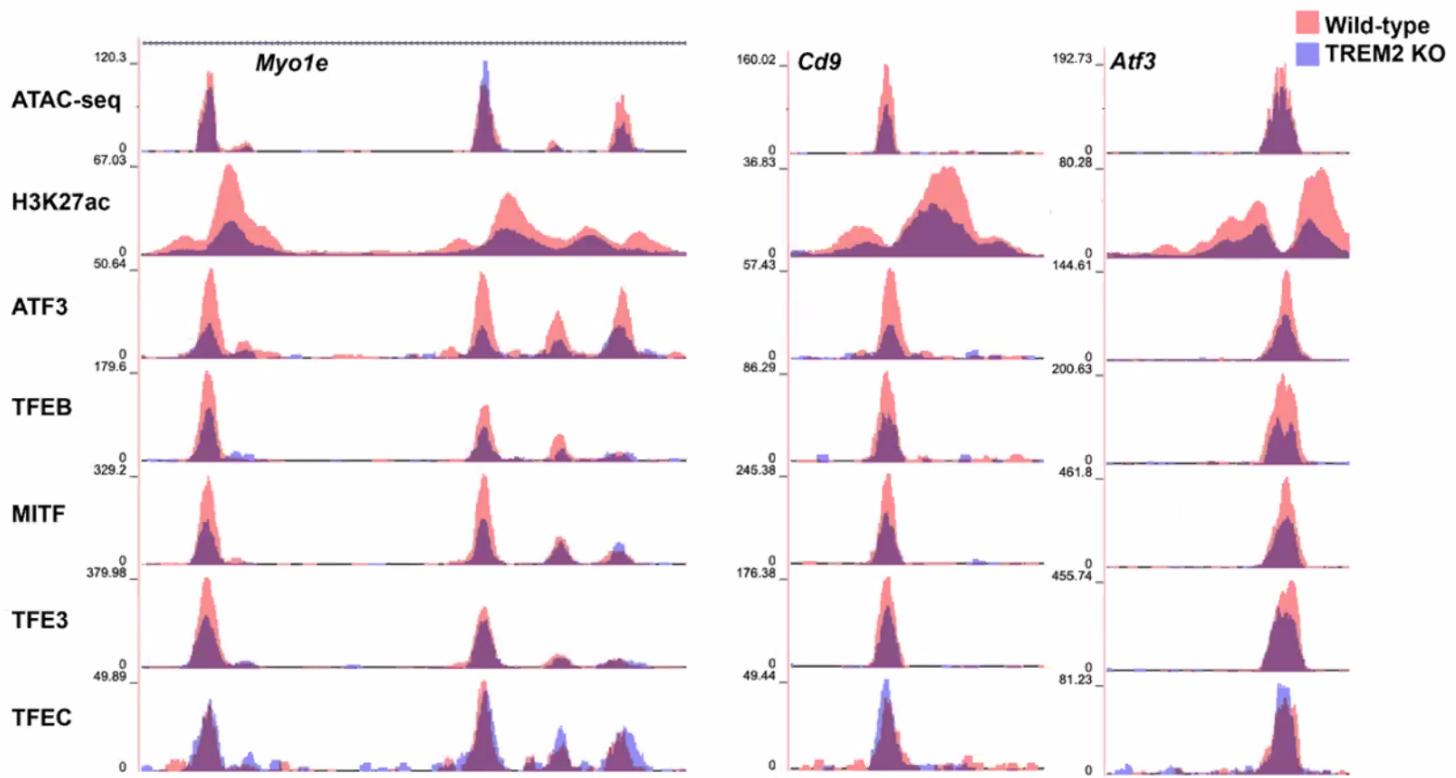
Quantitative production of macrophages or neutrophils *ex vivo* using conditional Hoxb8

Gang G Wang^{1,2}, Katherine R Calvo^{1,3}, Martina P Pasillas¹, David B Sykes^{1,4}, Hans Häcker⁵ & Mark P Kamps¹



Nature Methods 2006

Validation of inferred transcription factor binding at Trem2-dependent genes



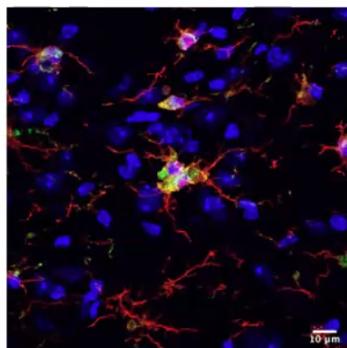
Thomas Prohaska

Decoding amyloid-responsive microglia phenotypes

- Many transcription factors appear to contribute to the microglia response to amyloid pathology and are similar in mouse and human microglia
- There is substantial overlap in putative transcriptional drivers with those implicated in driving *Sgsh* KO microglia and TERM phenotypes
- The transcriptional response to amyloid pathology is substantially different in mouse and human microglia, probably due to differences in cis regulatory architecture
- Trem2 signaling regulates the genome wide binding and activity of ATF3, MITF, TFEB and TFE3

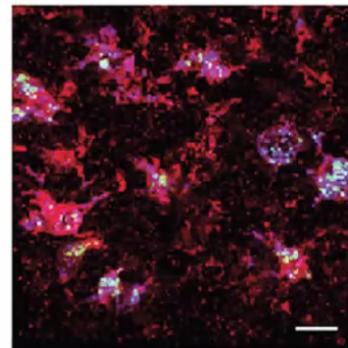
Decoding microglia phenotypes in neurodegenerative diseases

Lysosomal storage disease
(MPSIIIA)



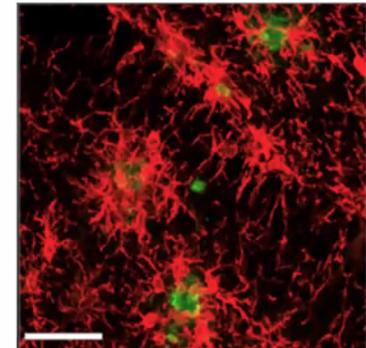
Sgsh KO

APOE4-enhanced
Tauopathy



PS19 Tau X APOE4 (TE4)

Amyloid pathology



5XFAD



PU.1
MITF/TFE
C/EBP
AP-1/ATF
PU.1/IRF



C/EBP
PU.1
AP-1/ATF
MITF/TFE
EGR2



PU.1
EGR
C/EBP
MITF/TFE
AP-1/ATF

Thanks!

Chris Balak
Johannes Schlachetzki
Nathan Spann
Joy Zhou
Thomas Prohaska
Isidoro Cobo
Dan Zhang
Martina Pasillas
Jana Collier
Benjamin Li

UC Irvine
Matt Blurton-Jones
Jonny Hasselmann
Amanda McQuade

Harvard Mecical School
Oleg Butovsky

Luxembourg Centre for Systems Biomedicine
Michael Heneka

