

Integrative Multiomics Profiling of Microglia Across Alzheimer's Disease Progression

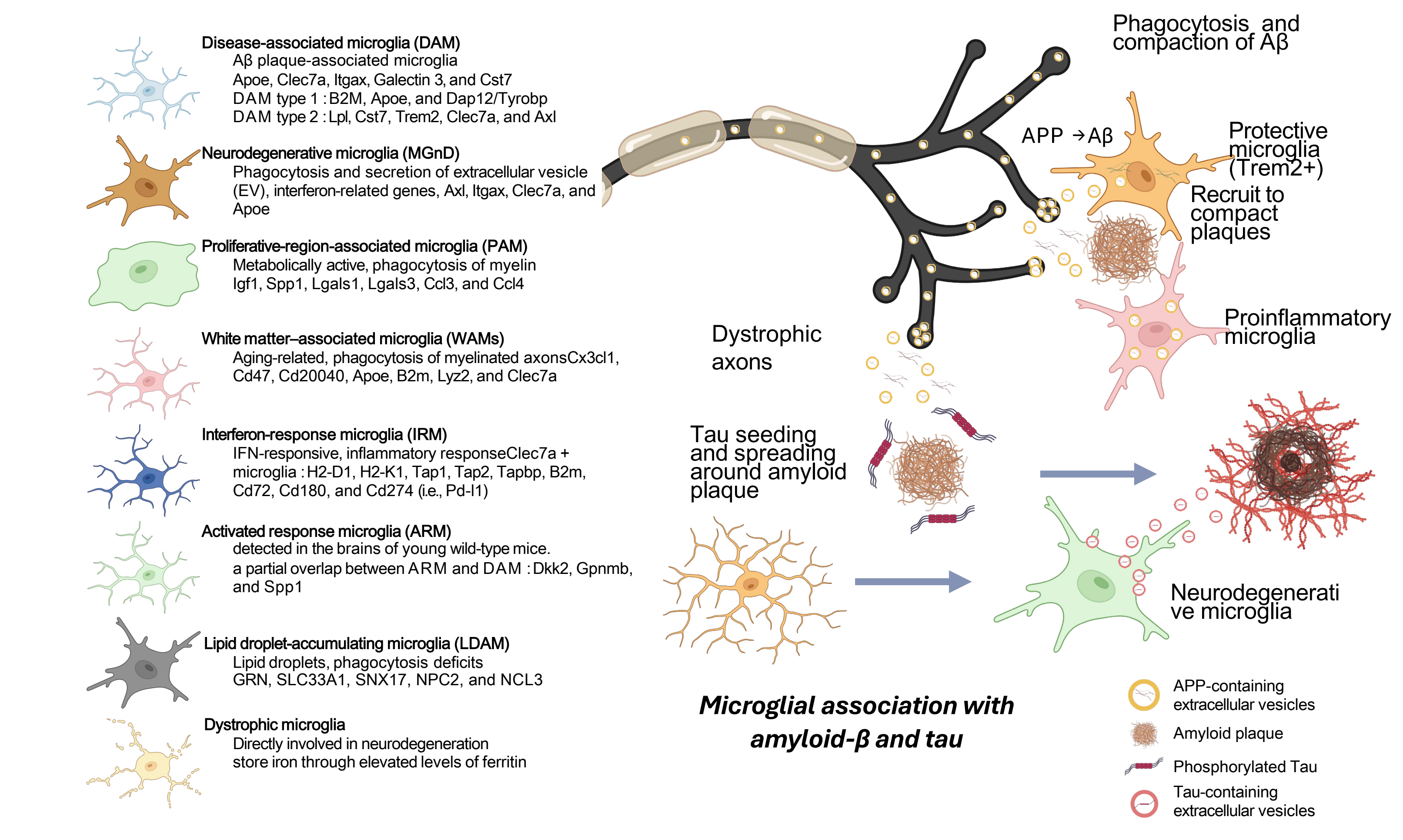
Jaemyung Jang¹, Seungeun Yeo², Soonbong Baek¹, Hyun Jin Jung¹, Youngshik Choe^{1*}

¹Developmental Disorders & Rare Diseases Research Group, Korea Brain Research Institute

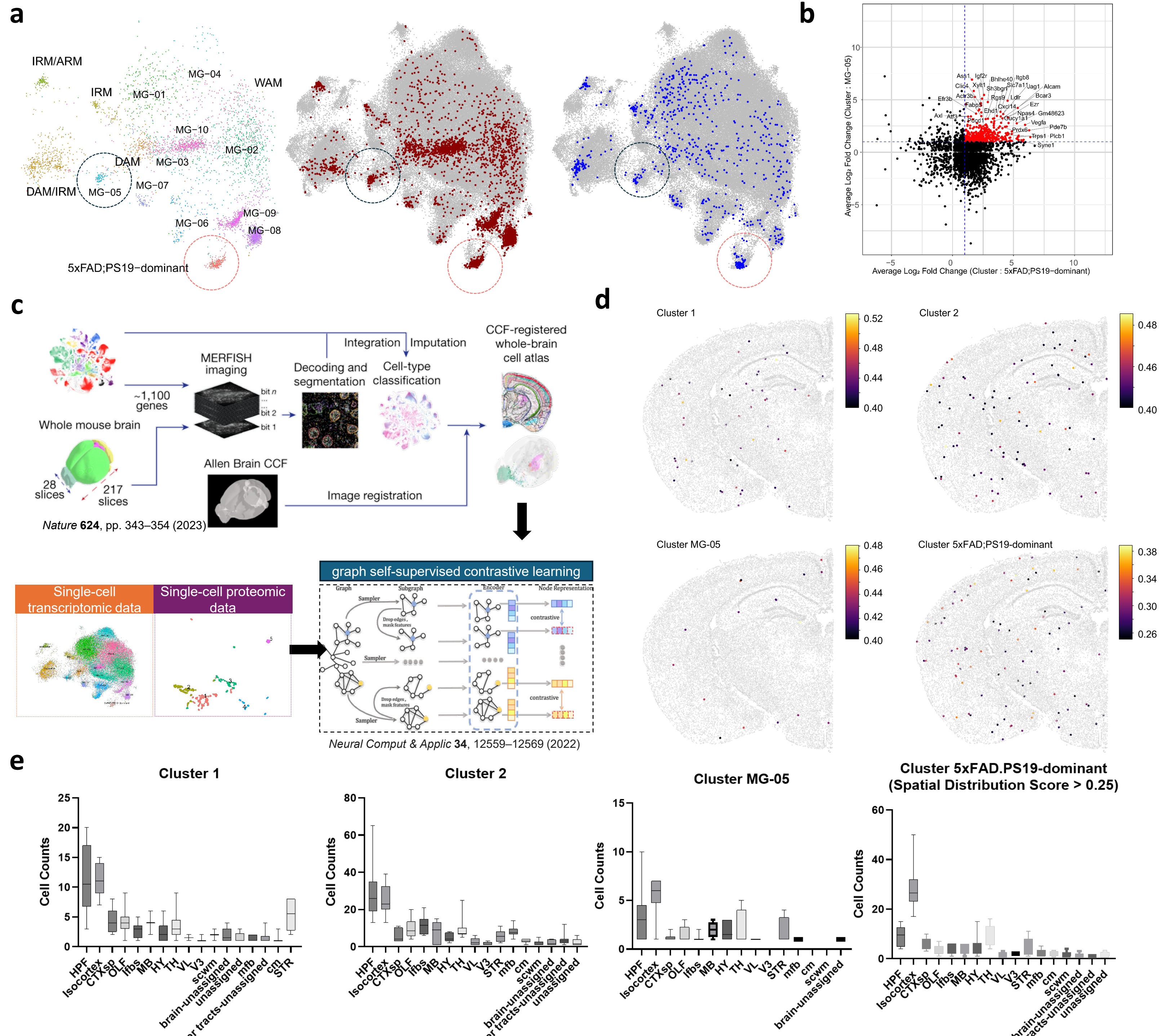
²Gut-Brain System Regulation Korean Medicine Research Center, Dongshin University

* Corresponding author : dallarae@kbr.i.re.kr

Neuroimmune in AD



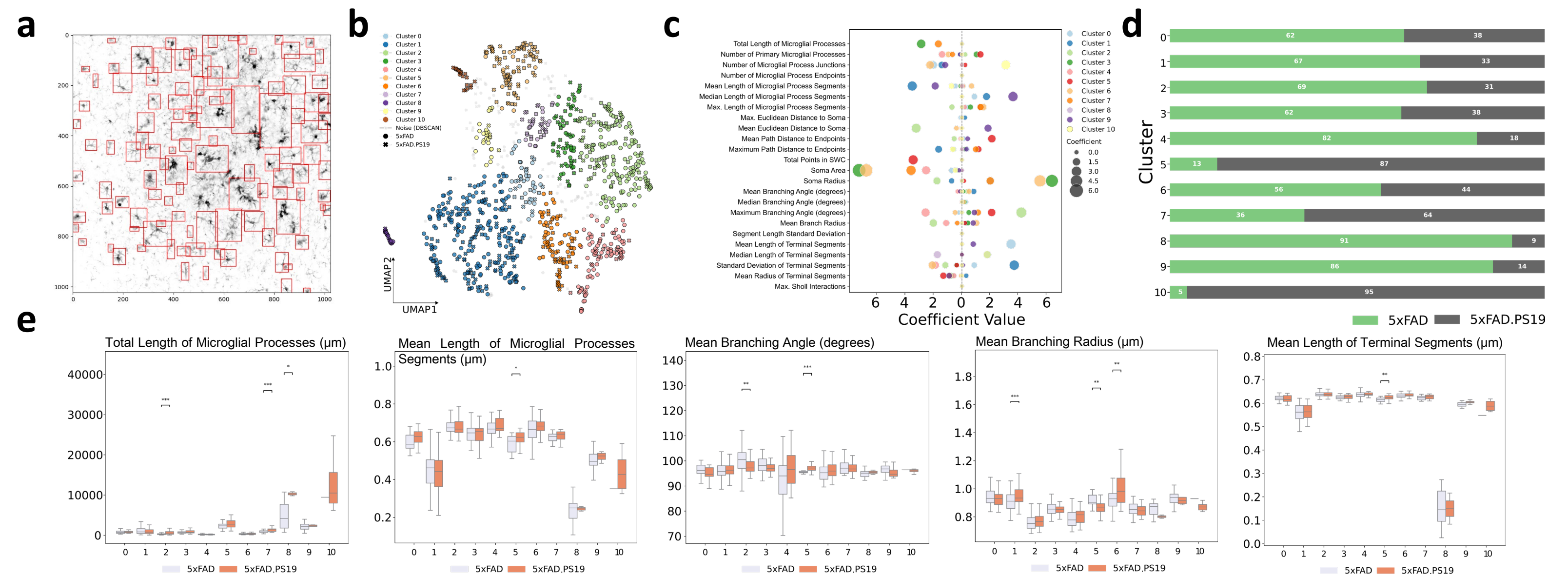
Spatial single-cell proteo-transcriptomic map of brain immune



Spatial distribution of axonal Aβ-responsive microglia

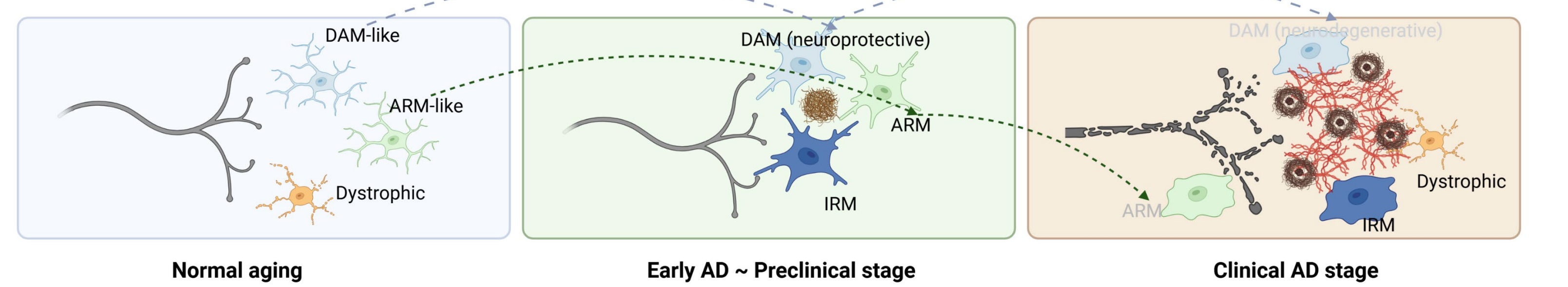
(a) UMAP plot displaying the disease-associated microglial subtypes. Colors represent transcriptional clusters identified through Louvain algorithm with multilevel refinement clustering (b) Differential marker expression analysis. Four-way plot depicting cluster markers (fold change > 2, adjusted P < 0.01) observed in 5xFAD;PS19-dominant versus MG-05 (c) Data integration workflow using a graph self-supervised contrastive learning method (d) Spatial mapping of axonal Aβ-responsive microglial cells. The 5xFAD;PS19-dominant and MG-05 clusters from single-cell transcriptomics and clusters 1 and 2 from single-cell proteomics are mapped within the mouse brain using the MERFISH spatial transcriptomics dataset (Zhuang-ABCA-1; 2.8 million cells) (e) Regional microglial distribution (Spatial Distribution Score > 0.4). Bar plot quantifying cluster-specific cell counts across 10 consecutive coronal sections. HPF : Hippocampal formation, CTSp : Cortical subplate, OLF : Olfactory areas, MB : Midbrain, TH : Thalamus, HY : Hypothalamus, VL : lateral ventricle, STR : Striatum, V3 : third ventricle

Morphological characterization of axonal Aβ-responsive microglia



(a) Representative confocal image of 1,077 axonal Aβ-responsive microglia from 5xFAD or 5xFAD;PS19 mouse brain (b) UMAP visualization of microglia morphology and cluster identification (c) Feature importance value for cluster discrimination (Logistic Regression Classifier) (d) Proportion of experimental groups within each cluster (e) Comparison of key morphological features between 5xFAD and 5xFAD;PS19 groups across clusters (* p < 0.05, ** p < 0.01, *** p < 0.001)

Conclusion



Specific microglial subsets associated with neuropathological states such as DAM, ARMs exist within the normal brain. Employing single-cell transcriptomic and proteomic analyses of microglia revealed axonal Aβ-responsive microglial cell types. Spatial mapping of microglial subtypes healthy brain visualizes vulnerable neural areas in AD.

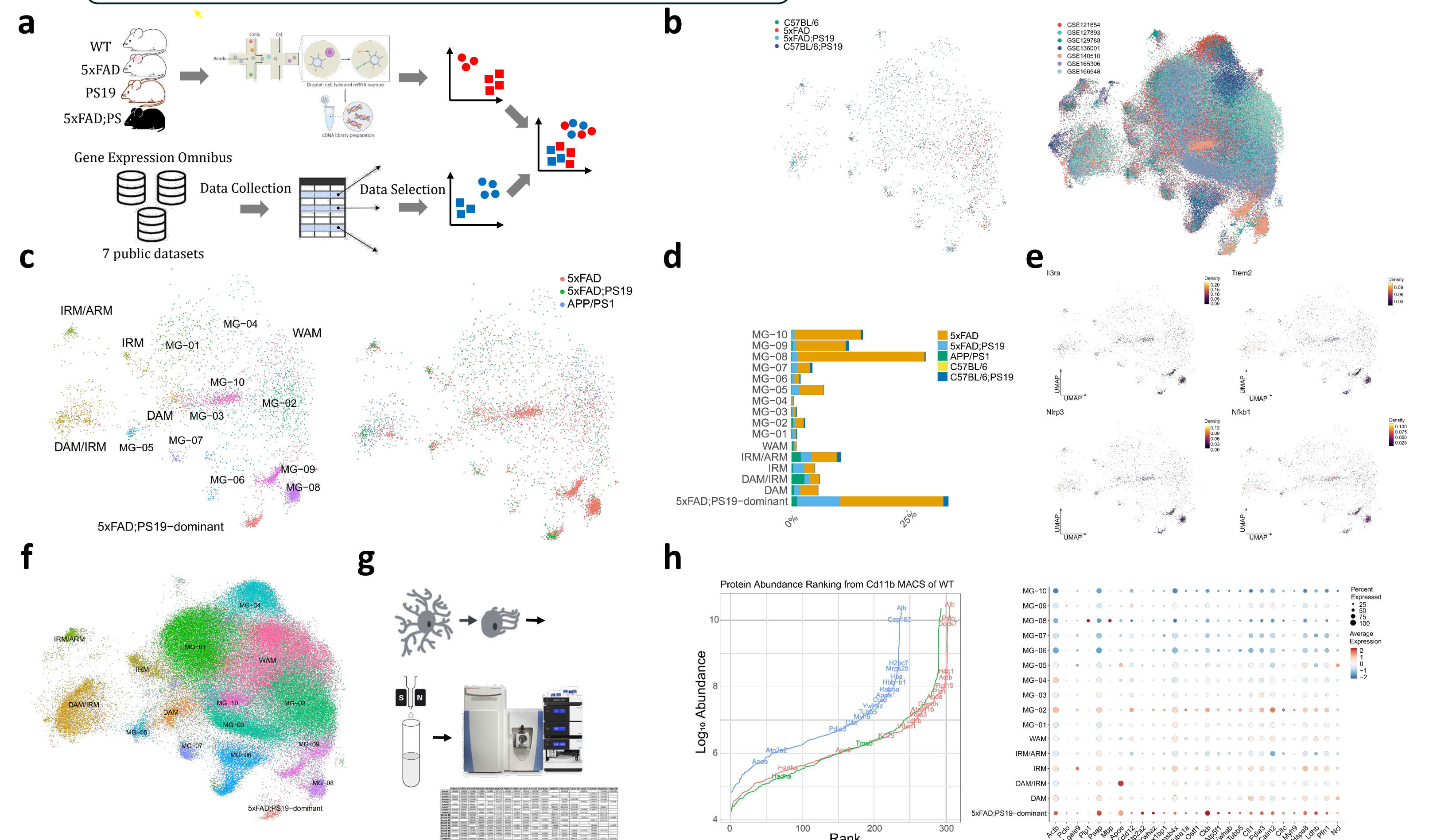
References

- Chen, L., Huang, J., Li, J. et al. Self-supervised graph representation learning using multi-scale subgraph views contrast. *Neural Comput & Applic* 34, 12559–12569 (2022).
- Gerrits E, et al. Distinct amyloid-beta and tau-associated microglia profiles in Alzheimer's disease. *Acta Neuropathol* 141, 681–696 (2021).
- Grubman A, et al. Transcriptional signature in microglia associated with Abeta plaque phagocytosis. *Nat Commun* 12, 3015 (2021).
- Hammond TR, et al. Single-Cell RNA Sequencing of Microglia throughout the Mouse Lifespan and in the Injured Brain Reveals Complex Cell-State Changes. *Immunity* 50, 253–271 e256 (2019).
- Hao Y, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. *Nat Biotechnol* 42, 293–304 (2024).
- Long Y, et al. Spatially informed clustering, integration, and deconvolution of spatial transcriptomics with GraphST. *Nat Commun* 14, 1155 (2023).
- Ochocka N, et al. Single-cell RNA sequencing reveals functional heterogeneity of glioma-associated brain macrophages. *Nat Commun* 12, 1151 (2021).
- Phifer S KV, Ferrero E. granulator: Rapid benchmarking of methods for "in silico" deconvolution of bulk RNA-seq data. *J. 1.12.0 edn. R package* (2024).
- Safaiyan S, et al. White matter aging drives microglial diversity. *Neuron* 109, 1100–1117 e1110 (2021).
- Sala Frigerio C, et al. The Major Risk Factors for Alzheimer's Disease: Age, Sex, and Genes Modulate the Microglia Response to Abeta Plaques. *Cell Rep* 27, 1293–1306 e1296 (2019).
- Sun N, et al. Human microglial state dynamics in Alzheimer's disease progression. *Cell* 186, 4386–4403 e4329 (2023).
- Wu T, et al. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *The innovation* 2, (2021).
- Ximerakis M, et al. Single-cell transcriptomic profiling of the aging mouse brain. *Nat Neurosci* 22, 1696–1708 (2019).
- Zhang M, et al. Molecularly defined and spatially resolved cell atlas of the whole mouse brain. *Nature* 624, 343–354 (2023).
- Zhang X, Smits AH, van Tilburg GB, Ova H, Huber V, Vermeulen M. Proteome-wide identification of ubiquitin interactions using UbiA-MS. *Nat Protoc* 13, 530–550 (2018).
- Zhou Y, et al. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med* 26, 981 (2020).
- Zhu B, et al. Trem2 deletion enhances tau dispersion and pathology through microglia exosomes. *Mol Neurodegener* 17, 58 (2022).

Acknowledgement

This research was by KBRI (25-BR-02-01, 25-BR-05-01), the Ministry of Trade, Industry and Energy (No. 2410006472), and by the Ministry of Science and ICT (No. 2022R1A5A202954611).

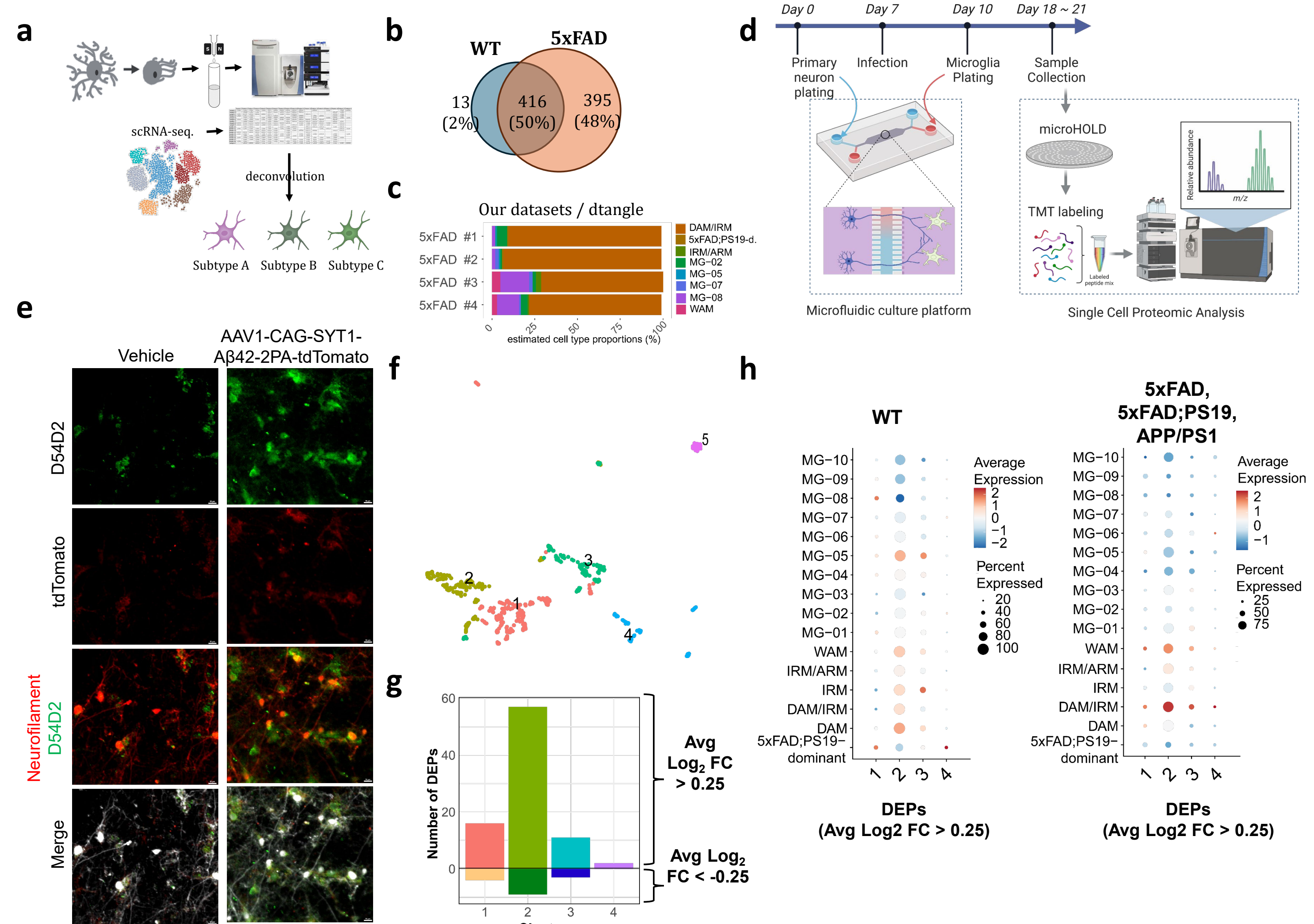
M-Galactic – multi-omics atlas of brain immune



Integrative microglial atlas of transcriptome and proteome

(a) Workflow for the integration of microglial multiomics data to establish M-Galactic. (b) Transcriptomic Data Integration : UMAP plot demonstrating our dataset (n = 2,315) and seven public datasets (n = 156,660) (c) UMAP plot depicting the clustering of microglia across three Alzheimer's disease (AD) mouse models (5xFAD, 5xFAD;PS19, APP/PS1), resulting in 16 distinct clusters (n = 4,856). (d) Bar graph showing changes in the proportion of each cell type across different models (e) Density plot of the expression levels of *Ii3ra*, *Trem2*, *Nlrp3*, and *Nfkb1* genes. (f) UMAP plot illustrating microglial clustering in wild-type (WT) mice comprising 16 clusters (n = 153,791). (g) Schematic of proteomic analysis for enriched microglia from WT mouse brains using CD11b-magnetic-activated cell sorting (MACS) isolation. (h) Rank order distribution of WT microglial proteins based on abundance logarithmic scale. (i) Dot plot highlighting WT microglia proteins identified using CD11b-MACS

Integrative analysis of transcriptome and proteome MG profiles



Single cell microHOLD proteomic analysis reveals distinct Aβ-responsive microglia profiles

(a) Schematic of the in-silico deconvolution of proteomic data (b) Venn diagram illustrating unique proteins identified in proteomic analyses using CD11b MACS. (c) Cell type proportions in four proteomic samples (d) Single-cell proteomic analysis workflow for axonal Aβ-responsive microglia profiles (e) Representative images of neurons and microglia in the microfluidic culture platform (f) UMAP plot shows proteomic profiles of single cells from primary microglia (n = 550). (g) Bar graph of number of differentially expressed proteins (DEP, average Log2FC > 0.25 or < -0.25, P-value < 0.1) between Aβ-expressing and vehicle group (h) Dot plot of gene scores based on DEPs (average Log2FC > 0.25, P-value < 0.1) across 4 clusters of a prior single-cell proteome analysis