



Spatial transcriptomic imaging reveals an attenuated amyloid response to amyloid pathology in 5xFAD brains following viral encephalitis

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INTRODUCTION

Alzheimer's disease (AD) patients display severe memory and cognitive impairments due to aberrant protein aggregation, chronic neuroinflammation, and synaptic/neuronal damage leading to degeneration in the brain. An early characteristic of AD pathology is the build-up of amyloid-beta (A β) plaques and neurofibrillary tau tangles, yet causal factors of AD pathogenesis remain undefined and obstruct the establishment of effective treatments. While AD is widely accepted as multifactorial, numerous risk factors have been attributed with increase A β plaque deposition in the brain, impacting the timing and onset of AD pathology. Notably, many genome-wide association studies (GWAS) have implicated genes increasing AD risk with roles involved in immunity and inflammation expressed in myeloid cells, which can reside in the central nervous system (CNS) as microglia.

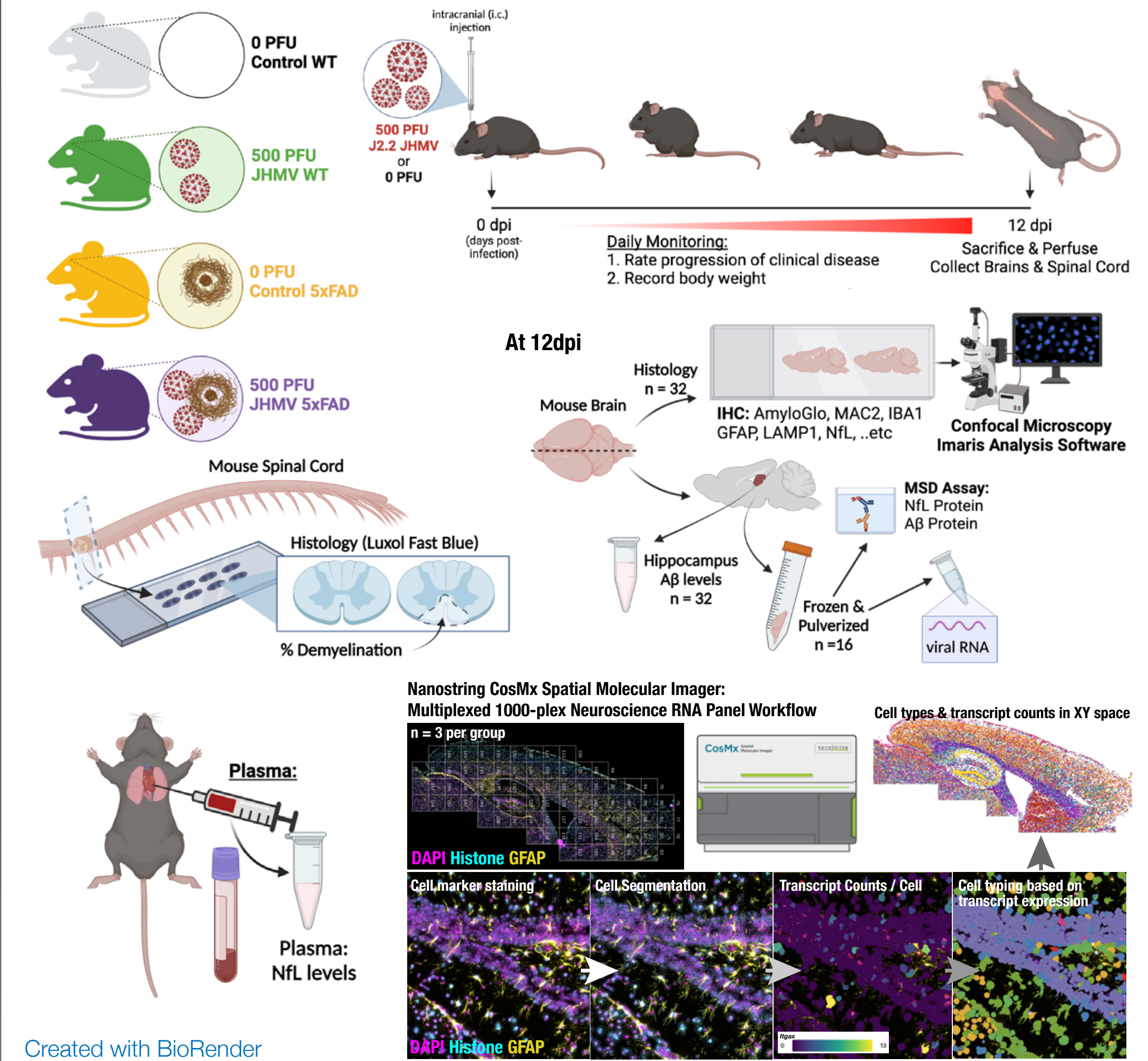
Recently, epidemiological research and post-mortem analysis have also associated viral encephalitis (i.e., viral-induced neuroinflammation) as a major risk factor for AD, highlighting the importance of better understanding how viral-induced inflammation and neuroimmune mechanisms within the brain may impact AD pathologies such as A β plaque deposition. This is further highlighted by the increasing frequency of novel viral pathogens like coronaviruses such as SARS-CoV-2, which the long-term neurological sequelae on AD pathology and neurodegeneration remain unknown. Using the neurotropic JHM strain of murine coronavirus, we have previously reported the robust inflammatory response to control viral replication within the brain. Acute JHMV infection within the brain upregulates cytokine expression, which results in orchestrated infiltration of peripheral immune cells (i.e., monocyte/macrophages and T-cells) to aid in host defense. Specifically, infiltration of viral-specific T-cells into the brain upregulates interferon-gamma (IFN- γ) secretion, which activates infiltrating macrophages to control viral replication. However, viral RNA/antigen will continue to persist within white matter tracts leading to sustained inflammatory macrophages and immunopathogenic responses within the CNS, leading to chronic viral encephalitis and demyelination.

To recapitulate AD-like neuropathologies, we utilized the 5xFAD mouse strain, which is a well-characterized preclinical model that reproduces robust A β plaque accumulation and chronic neuroinflammation through reactive glial cells surrounding plaques. To investigate microbial interactions with AD pathology, we infected 5xFAD mice with JHMV to determine how acute viral encephalitis induced by a neurotropic murine coronavirus (impacts established A β plaque deposition during late-stage disease).

METHODS & APPROACH

Animals: All rodent experiments were performed in accordance with animal protocols approved by the Institutional Animal Care and Use Committee at the University of California, Irvine (UCI), 6-month and 10-month-old (male and female) 5xFAD hemizygous mice and age-matched C57BL/6 wildtype mice were used in this study.

JHMV Infection: Mice were infected intracranially (i.c.) with 500 PFU of JHM virus in 30 μ L of sterile Hanks balanced sterile solution (HBSS); control mice received 30 μ L of HBSS via i.c. injection. The mice were observed daily for clinical disease progression and body weight loss. At 12 days post-infection (dpi), the mice were sacrificed and spinal cords were isolated to evaluate demyelination. Brains were also isolated to assess viral titers, amyloid-beta (A β) burden, and immunohistochemical staining. Plasma samples were also collected to assess plasma neurofilament light-chain (NFL) levels using Mesoscale Discovery (MSD) R-Plex assays. Fixed-frozen brains were sectioned for spatial transcriptomic imaging using the Bruker Nanostring CosMx Spatial Molecular Imaging platform.



1. JHMV infection induces similar weight loss, clinical disease progression, and control of viral replication in 5xFAD mice at 12 days p.i.

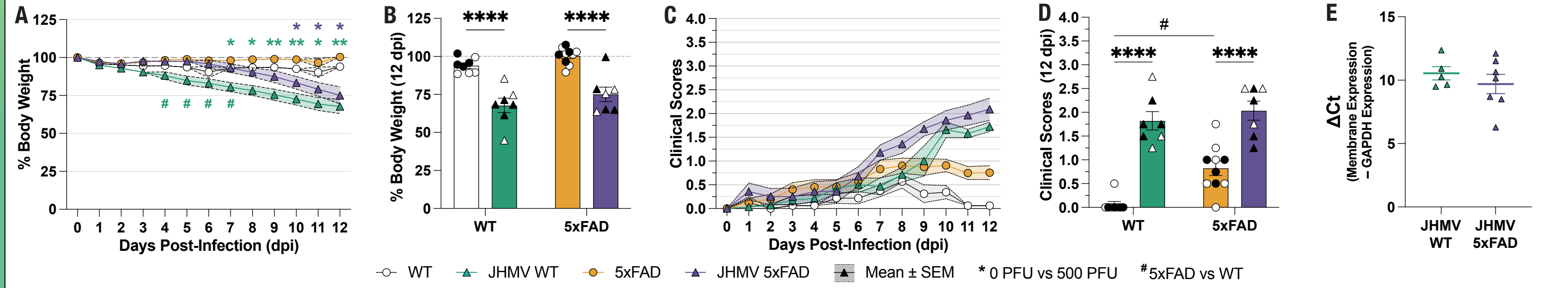


Figure 1. JHMV infection leads to reduced body weights and increased clinical disease severity, NFL plasma levels, and demyelination in WT and 5xFAD mice. Line chart depicting percent changes in initial body weights (A) and scores of clinical disease progression (B) from uninfected WT (white), JHMV-infected WT (yellow), JHMV-infected 5xFAD mice (orange) across each day post-infection. Comparing percent body weight (C) and daily clinical disease scores (D) at 12dpi in JHMV-infected mice compared to vehicle controls in both WT and 5xFAD groups. (E) qPCR demonstrate similar transcript levels of viral membrane protein RNA within homogenized brains of JHMV-infected WT and 5xFAD mice at 12dpi. n = 7-10. Data is presented as mean $\pm$ SEM. Two-way ANOVA followed up Holm-Sidak post-hoc tests to examine biologically relevant interactions. *p<0.05, **p<0.01, ***p<0.0001, ****p<0.0001

2. Dense-core amyloid-beta (A β) plaque volumes are reduced in subiculum brain regions of JHMV-infected 5xFAD mouse brains at 12dpi

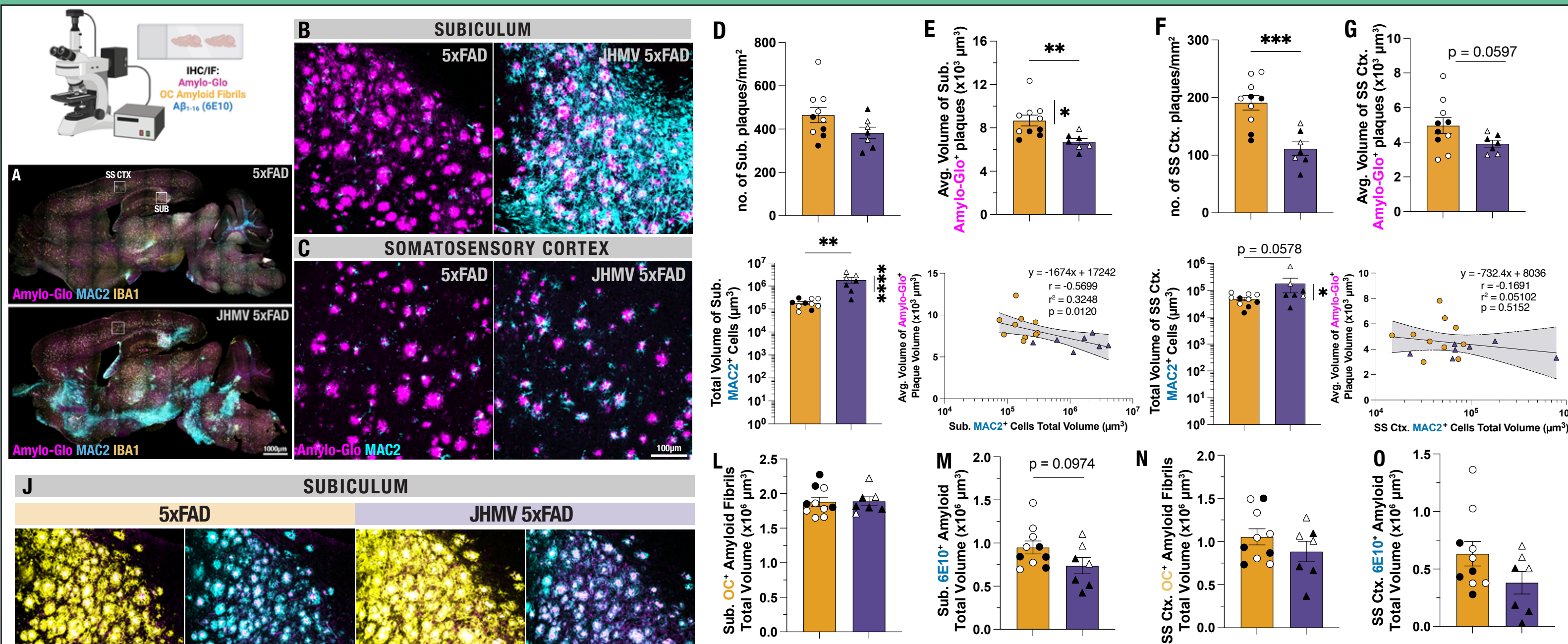
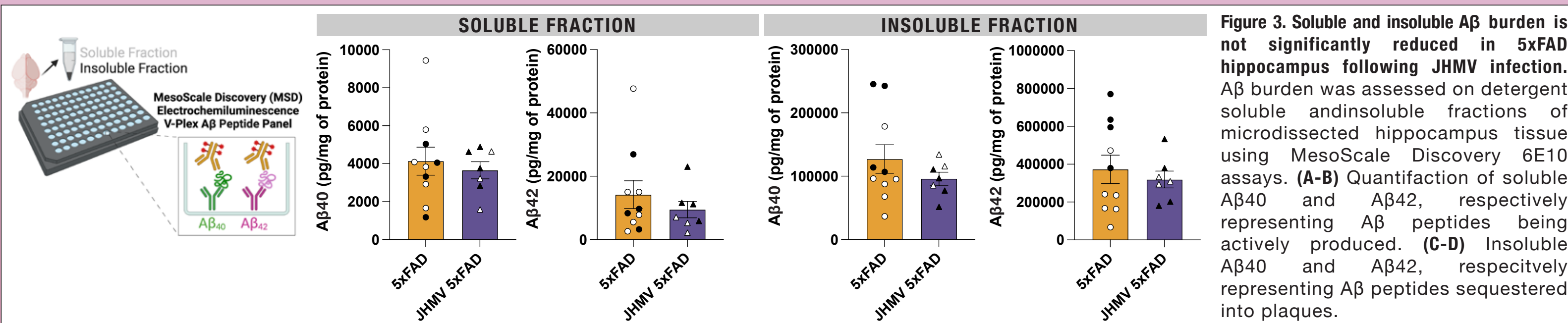


Figure 2. JHMV infection is associated with reduced dense-core A β plaque volumes in 5xFAD mice. A β plaque burden was assessed using Amylo-Glo staining for dense-core plaques, E610 for A β ₁₋₁₆ and OC for amyloid fibrils in 30 μ m sagittal brain sections. Lgals3 expression in myeloid cells was assessed using MAC2 antibody immunostaining. (A) Representative whole-brain images of 12dpi JHMV-infected and control 5xFAD mice. (B) Representative 20X confocal images of subiculum (B) and somatosensory cortex (C) depicting Amylo-Glo plaques and MAC2-myeloid cells. Quantification of plaque densities (D, F) and average plaque volumes (E, G), and total volume of MAC2+ cells per FOV of JHMV-infected and control 5xFAD brain sections. Scatterplot and linear regression depicting the relationship of total volume of MAC2+ cells and average plaque volumes in subiculum and somatosensory cortex in 5xFAD mice. (H, J) Representative 20X confocal images in subiculum and somatosensory cortex, respectively. Total volume quantification of OC+ and E610+ plaques in subiculum (K, L) and somatosensory cortex (M, N). n = 7-10. Data presented as mean $\pm$ SEM. Two-tailed, unpaired t-tests were used to compare mean differences between groups. *p<0.01, **p<0.0001, ***p<0.0001

3. JHMV infection does not significantly reduce A β 40 nor A β 42 levels in detergent soluble and insoluble fractions of 5xFAD hippocampus tissue



4. Spatial transcriptomic imaging enables sequencing and gene expression analysis with single-cell resolution and spatial information

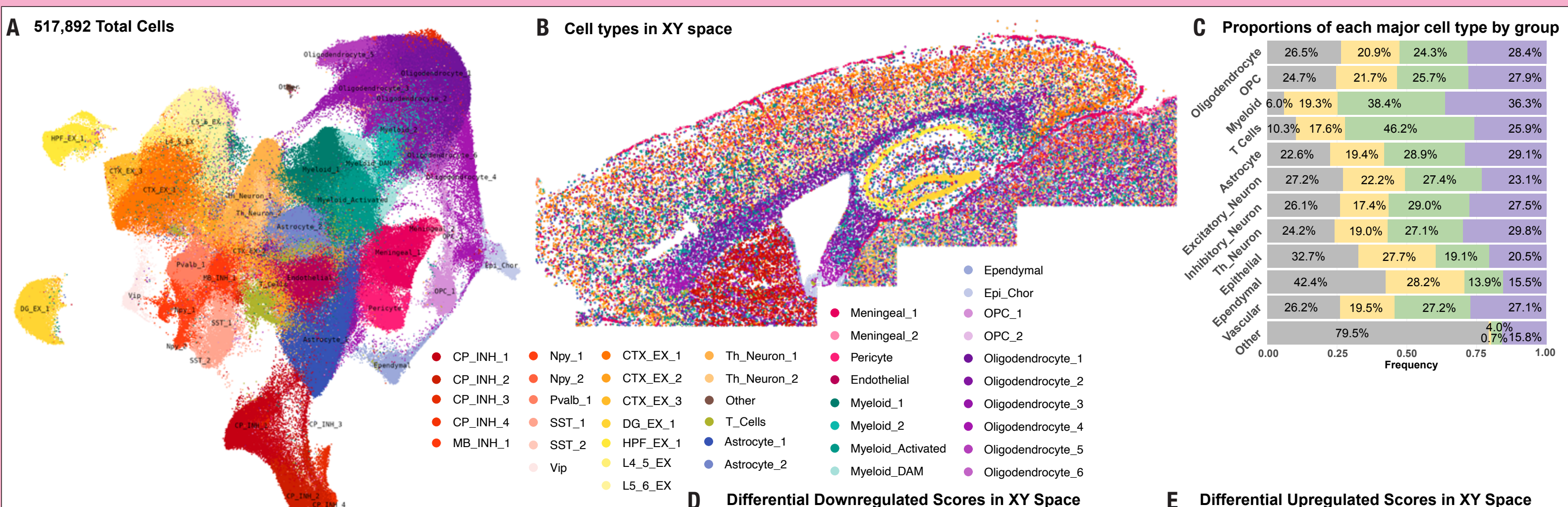


Figure 4. Spatial transcriptomics reveal enhanced microglial response to A β plaques during acute JHMV infection. (A) UMAP of 517,892 cells across 12 hemi-brains (n=3/group). Clustering at 1.0 resolution yields 42 clusters, which were annotated manually based on gene expression and anatomical location in XY space. (B) Clusters plotted in XY space. (C) Proportion of each major cell type, grouped by condition. Percentages normalized for the total number of cells in each group. (D-E) Differential downregulation (D) or upregulation (E) scores in JHMV 5xFAD vs. Control 5xFAD in each cluster plotted in space.

5. Acute viral encephalitis induces widespread transcriptional changes in myeloid cells, diminishing DAM-associated responses to A β plaques

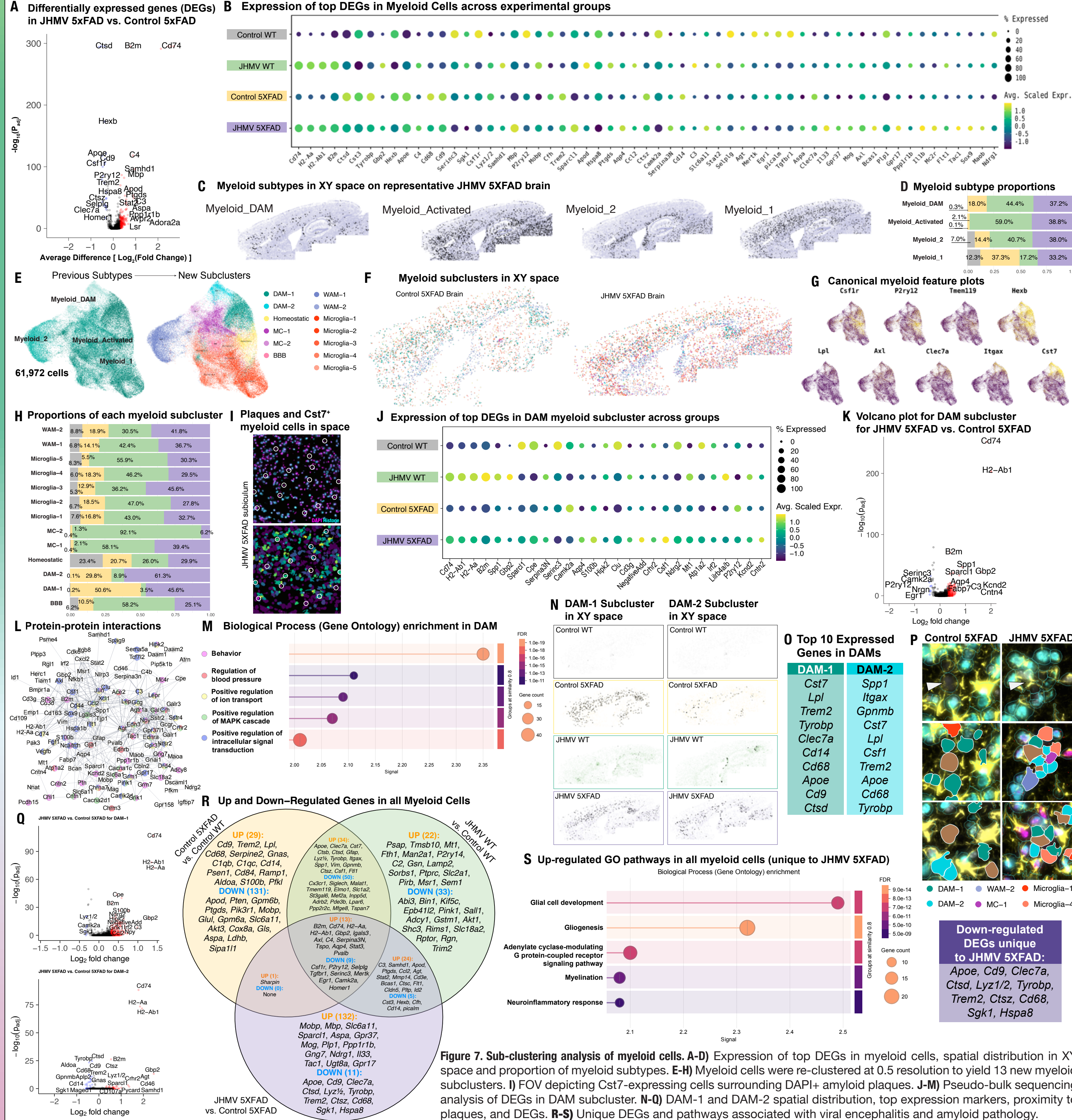


Figure 5. Acute viral encephalitis induces widespread transcriptional changes in myeloid cells, diminishing DAM-associated responses to A β plaques. (A) Differentially expressed genes (DEGs) in JHMV 5xFAD vs. Control 5xFAD. (B) Expression of top DEGs in myeloid cells across experimental groups. (C) Myeloid subtypes in XY space on representative JHMV 5xFAD brain. (D) Myeloid subtype proportions. (E) Myeloid subclusters in XY space. (F) Myeloid subclusters in XY space. (G) Canonical myeloid feature plots. (H) Proportions of each myeloid subcluster. (I) Plaques and Cst7+ myeloid cells in space. (J) Expression of top DEGs in DAM myeloid subcluster across groups. (K) Volcano plot for DAM subcluster for JHMV 5xFAD vs. Control 5xFAD. (L) Protein-protein interactions. (M) Biological process (Gene Ontology) enrichment in DAM. (N) DAM-1 subcluster in XY space. (O) Top 10 expressed genes in DAMs. (P) Down-regulated genes unique to JHMV 5xFAD. (Q) Up-regulated GO pathways in all myeloid cells (unique to JHMV 5xFAD). (R) Unique DEGs and pathways associated with viral encephalitis and amyloid pathology.

CONCLUSIONS & FUTURE DIRECTIONS

Our data demonstrates that coronavirus-induced encephalitis in 5xFAD mice results in acute and robust neuroinflammation within the CNS, leading to:

1. Reduced volumes of Amylo-Glo⁺ dense-core A β plaques within brain regions with high expression of macrophage-like myeloid MAC2/Lgals3 expression and infiltrating CD4⁺ T cells, and CD8⁺ T cells.
2. Upregulated and downregulated DEGs involved in both neuronal function and immunological pathways
3. Spatial transcriptomic imaging reveals dysregulated gene expression in myeloid cell populations, while DAM genes associated with responses to amyloid pathology are uniquely down-regulated.
4. Within DAM subclusters, viral encephalitis increases DAM-2 cells, which associate tightly with plaques.

Future experiments will perturb upstream mechanisms regulating DAM expression in myeloid cells to determine their respective role in reducing A β plaque burden during acute viral encephalitis. We will also utilize spatial proteomic imaging to assess the protein expression changes that occur within cell types proximal to A β plaques during viral encephalitis. Establishing how viral-mediated neuroinflammation impacts the onset/severity of AD pathogenesis and accelerated neuronal dysfunction is highly relevant and can lead the field toward therapeutic targets to control progressive neurodegeneration in patients previously infected with emerging viral pathogens like coronaviruses. It is critical that we define the acute effects and long-term consequences of coronavirus-induced encephalitis on AD to anticipate its repercussions on pathological progression and identify therapeutic interventions to counteract advancing neurodegeneration.

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