

Microglial Sensome Landscapes Across Tauopathies

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The role of the microglial sensome in neurodegeneration

Microglia detect and respond to neurodegenerative pathology, a response mediated in part by the *microglial sensome*.

The microglial sensome is a unique cluster of transcripts, within microglia, encoding proteins for sensing endogenous ligands and microbes.

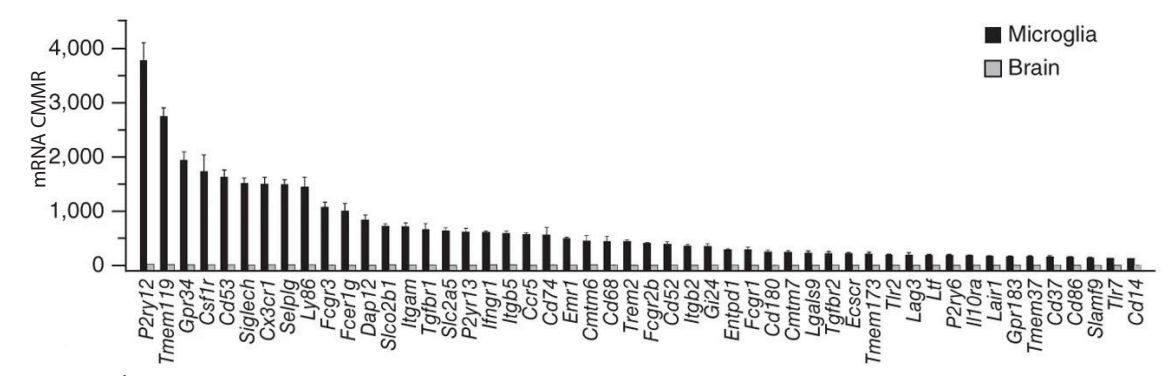


Figure 1. The microglial sensome identified by direct RNA sequencing from Hickman et al. 2013. Bar graph depicts a portion of the top 100 transcripts with the highest enrichment of microglia versus brain. This gene collection was termed the microglial sensome. Expression levels of the genes of the microglial sensome in copies of mRNA per million mapped reads (CMMR) in microglia and brain. Values represent the mean standard deviation of three different experiments carried out with microglia pooled from 22, 10, and 20 mice and three pools of RNA from two brains each.

How does the microglial sensome function across neurodegenerative diseases?

Given that neurodegenerative diseases exhibit distinct pathological and cognitive features, we sought to determine if the microglial sensome encodes proteins differently based on the unique disease pathology and environment.

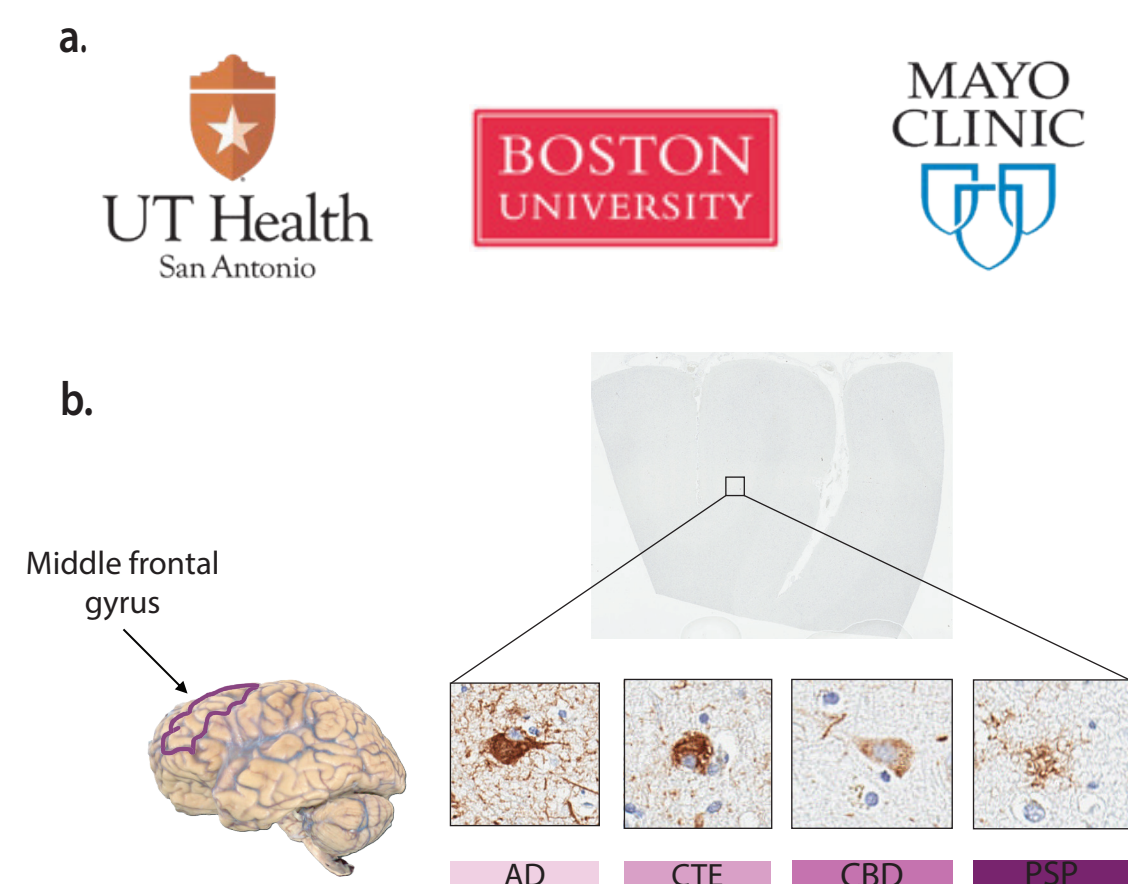


Figure 2. Methods used to explore the role of the microglial sensome in neurodegeneration. a) Decedent donated brains with pathologically confirmed Alzheimer's disease (AD), chronic traumatic encephalopathy (CTE), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) at the following Alzheimer's Disease Research Center brain banks: Glenn Biggs Institute at UT Health San Antonio, Boston University, and Mayo Clinic. b) The middle frontal gyrus of formalin-fixed, paraffin-embedded postmortem brain tissue (n= 47) were cut into serial sections and stained with antibodies against neurodegenerative pathology and sensome markers.

Representative images of sensome and neuropathological protein abundance

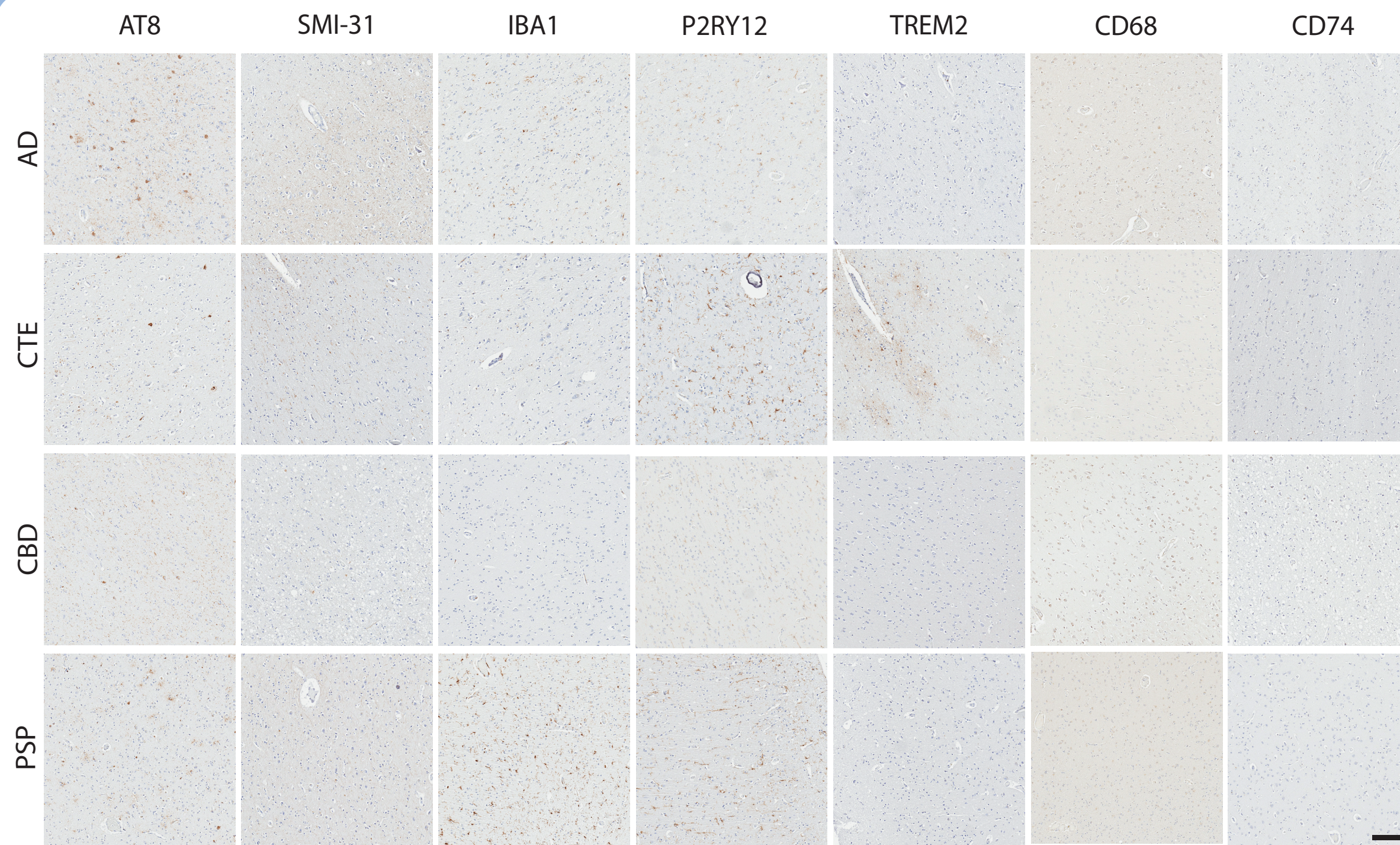


Figure 3. Representative images of sensome and neuropathological protein abundance in AD, CTE, CBD, and PSP. Representative immunohistochemistry images from AD, n= 13, CTE, n= 14, CBD, n= 9, and PSP, n= 11 post-mortem human brains (middle frontal gyrus), utilizing antibodies against AT8 (phosphorylated tau), SMI-31(phosphorylated neurofilament), IBA1(pan-microglia), P2RY12 ("homeostatic" microglia), TREM2 (disease-associated microglia), CD68 (reactive microglia), and CD74 (antigen presenting microglia). Labeled tissue slides were visualized, imaged, and digitized on a Leica Aperio AT2 slide scanner, and analyzed using Aperio ImageScope pathology software. Scale bar: 400 μm.

Microglial sensome protein levels across tauopathies

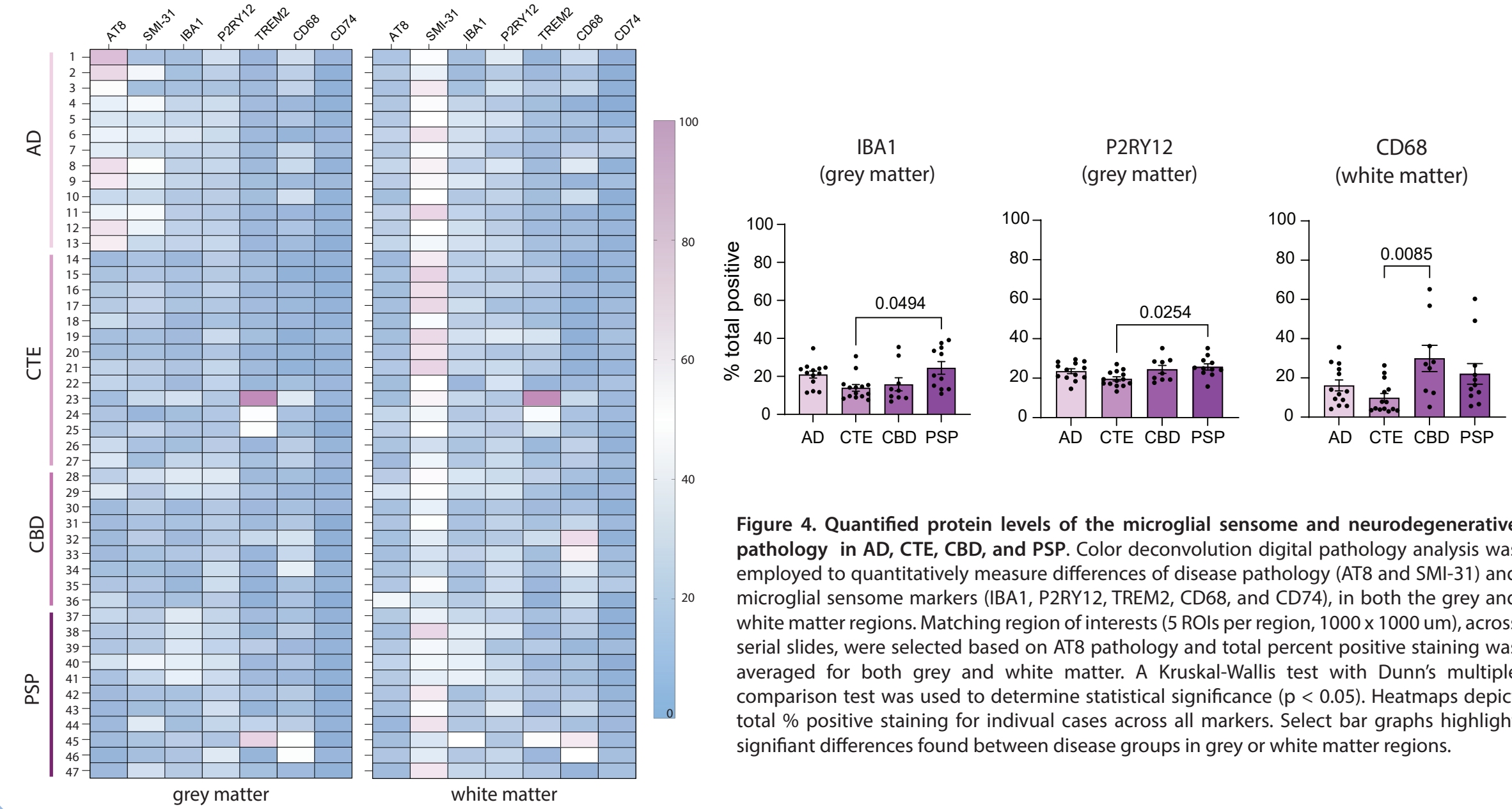


Figure 4. Quantified protein levels of the microglial sensome and neurodegenerative pathology in AD, CTE, CBD, and PSP. Color deconvolution digital pathology analysis was employed to quantitatively measure differences of disease pathology (AT8 and SMI-31) and microglial sensome markers (IBA1, P2RY12, TREM2, CD68, and CD74), in both the grey and white matter regions. Matching region of interests (5 ROIs per region, 1000 x 1000 um), across serial slides, were selected based on AT8 pathology and total percent positive staining was averaged for both grey and white matter. A Kruskal-Wallis test with Dunn's multiple comparison test was used to determine statistical significance (p < 0.05). Heatmaps depict total % positive staining for individual cases across all markers. Select bar graphs highlight significant differences found between disease groups in grey or white matter regions.

Microglial morphometric changes across tauopathies

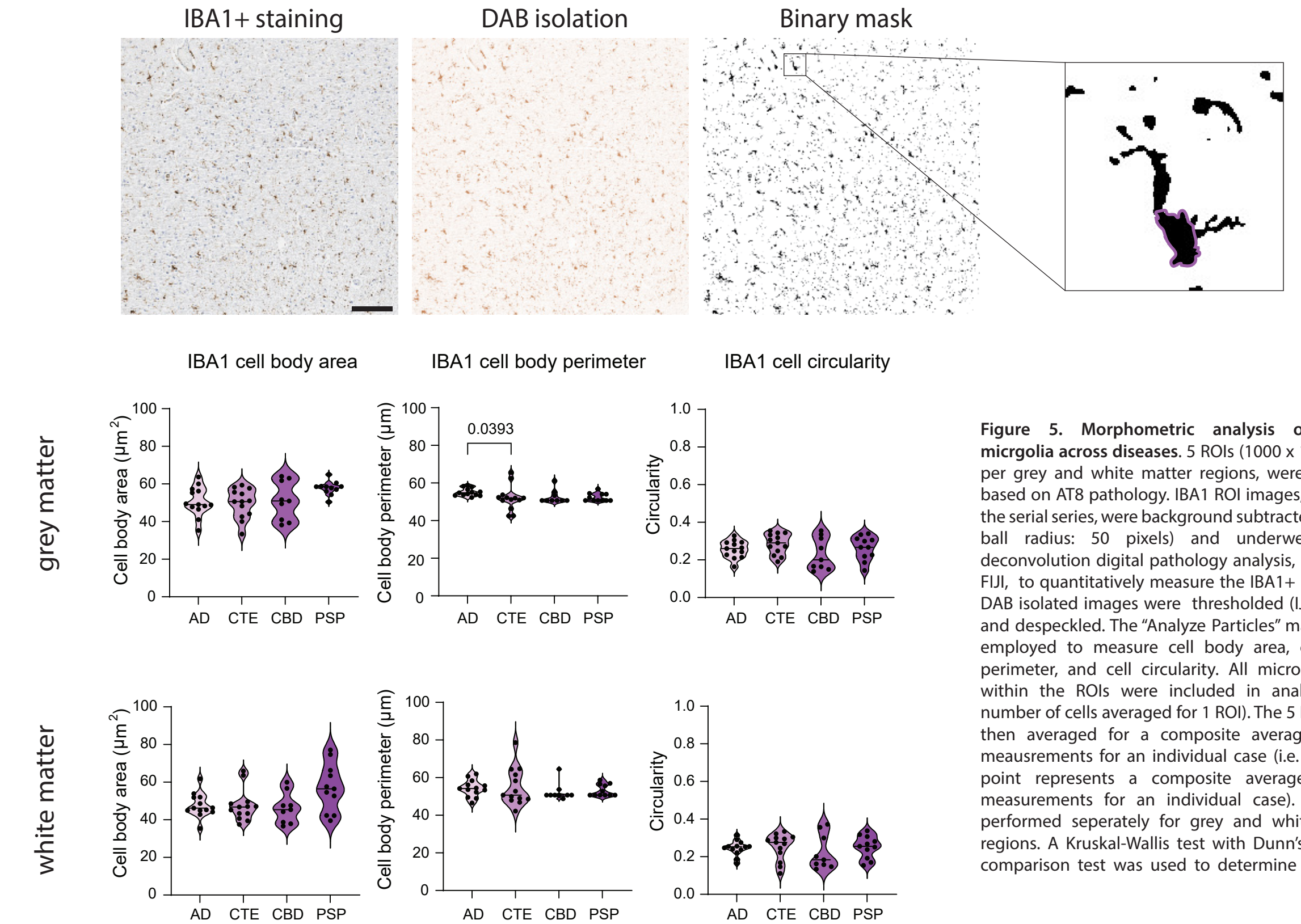


Figure 5. Morphometric analysis of IBA1+ microglia across diseases. 5 ROIs (1000 x 1000 um), per grey and white matter regions, were selected based on AT8 pathology. IBA1 ROI images, from the serial series, were background subtracted (rolling ball radius: 50 pixels) and underwent color deconvolution digital pathology analysis, in ImageJ Fiji, to quantitatively measure the IBA1+ microglia. DAB isolated images were thresholded (J_isodata) and despeckled. The "Analyze Particles" macros was employed to measure cell body area, cell body perimeter, and cell circularity. All microglial cells within the ROIs were included in analysis ("x" number of cells averaged for 1 ROI). The 5 ROIs were then averaged for a composite average of cell measurements for an individual case (i.e. one data point represents a composite average of cell measurements for an individual case). This was performed separately for grey and white matter regions. A Kruskal-Wallis test with Dunn's multiple comparison test was used to determine statistical

Microglial sensome markers in relation to neurodegenerative pathology and each other

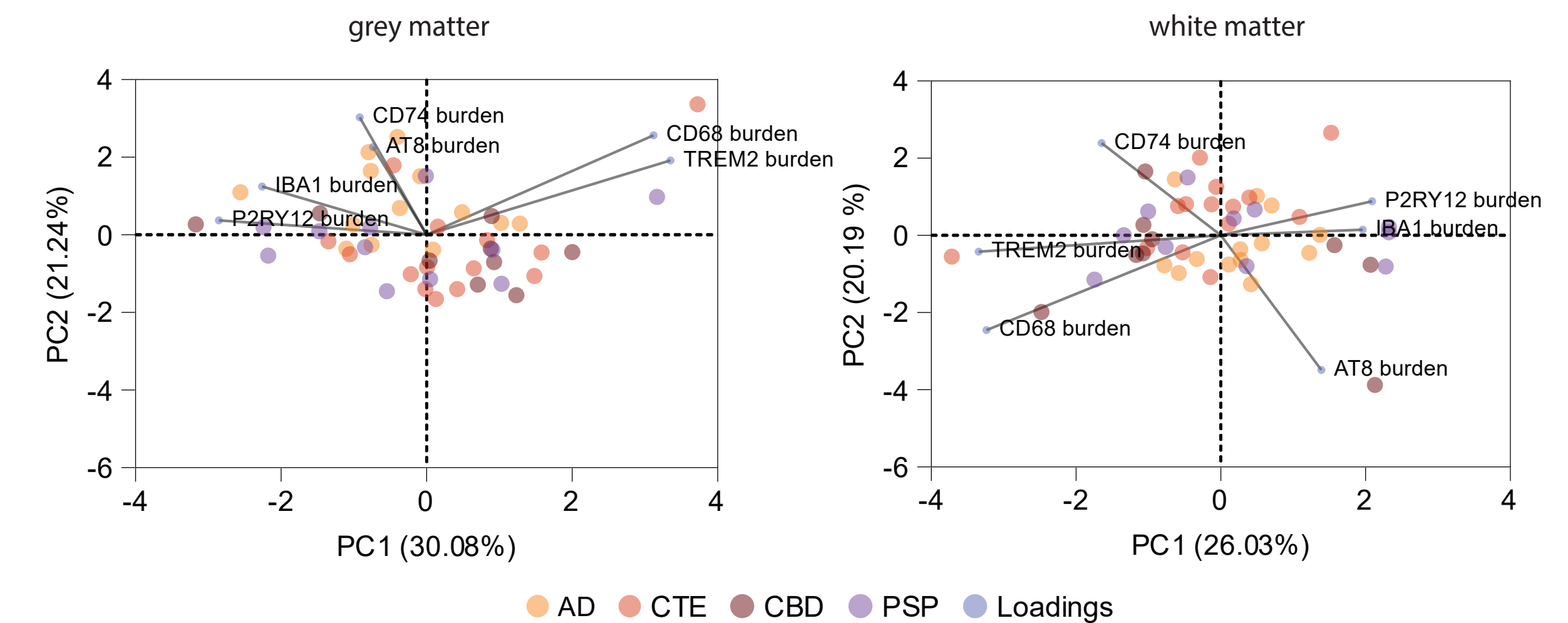


Figure 6. Principle component analysis of the microglial sensome in AD, CTE, CBD, and PSP. Color deconvolution digital pathology analysis was employed to quantitatively measure differences of disease pathology (AT8) and microglial sensome markers (IBA1, P2RY12, TREM2, CD68, and CD74), in both the grey and white matter regions. Matching region of interests (5 ROIs per region, 1000 x 1000 um), across serial slides, were selected based on AT8 pathology and optical density x total percent positive staining was averaged for both grey and white matter. A principle component analysis (PCA) was used to determine variance and distribution of the tauopathies, where PC1 is driven by microglial functional states and PC2 is driven by tau pathology.

Spatial distribution and clustering of the microglial sensome

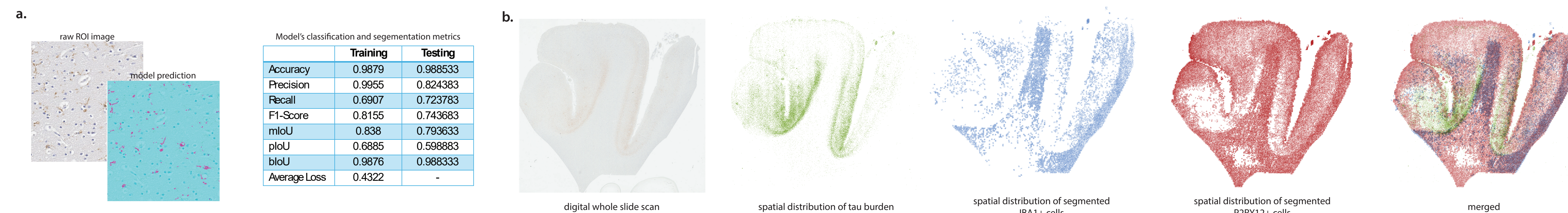


Figure 7. Spatial mapping of the microglial sensome. a) A deep learning pipeline was developed and used to perform microglia cell segmentation on whole slide images. b) Coordinate points from the model segmentation were imported into Genentech's SpatialQFPs R package to visualize the segmented cells and perform clustering analysis.

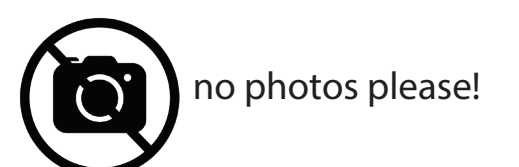
Conclusions

These findings suggest neurodegenerative pathology associated with each disease may elicit distinct microglial adaptations contributing to divergent neurodegenerative pathways.

Future work should explore sensome activity in selectively vulnerable regions of each disease.



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