High-plex spatial proteomics for neuroscience identifies single-cell and spatial niches in neurodegenerative cortical tissues

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1. Overview

Spatial proteomics has revolutionized our understanding of tissue biology and is poised to transform neuroscience. Robust workflows are required to (1) generate spatial proteomic data for brain tissues and (2) analyze them to yield single-cell spatial phenotyping insights, including extracellular structures. We present a quantitative multiplex immunofluorescence approach to study brain tissues using the **CellScape™ Precise Spatial Proteomics (PSP)** platform. With cell profiling and spatial analysis of brain tissues in mind, we developed an antibody panel to label neuronal, glial, vascular, and extracellular structures in mouse and man.

A further requirement for the deployment of spatial proteomics in neuroscience is the availability of computational tools for multiplex imaging data. Precise cell segmentation is required to capture fine cellular arborizations to ensure that multiplexed marker signals are mapped correctly to individual cells. Furthermore, diverse cellular and extracellular aggregates are clinically relevant biomarkers for several neurodegenerative disorders and hence need to be accurately captured. To address these challenges, we used the **Ariadne.ai SPATIAL™** platform, which enable precise segmentation of brain cells, including those with complex shapes like astroglia. The platform also supports the detection and pathological classification of extracellular protein aggregates, enabling comprehensive analyses of spatial biomarkers associated with both disease and normal function.

2. CellScape whole-slide multiplex brain imaging

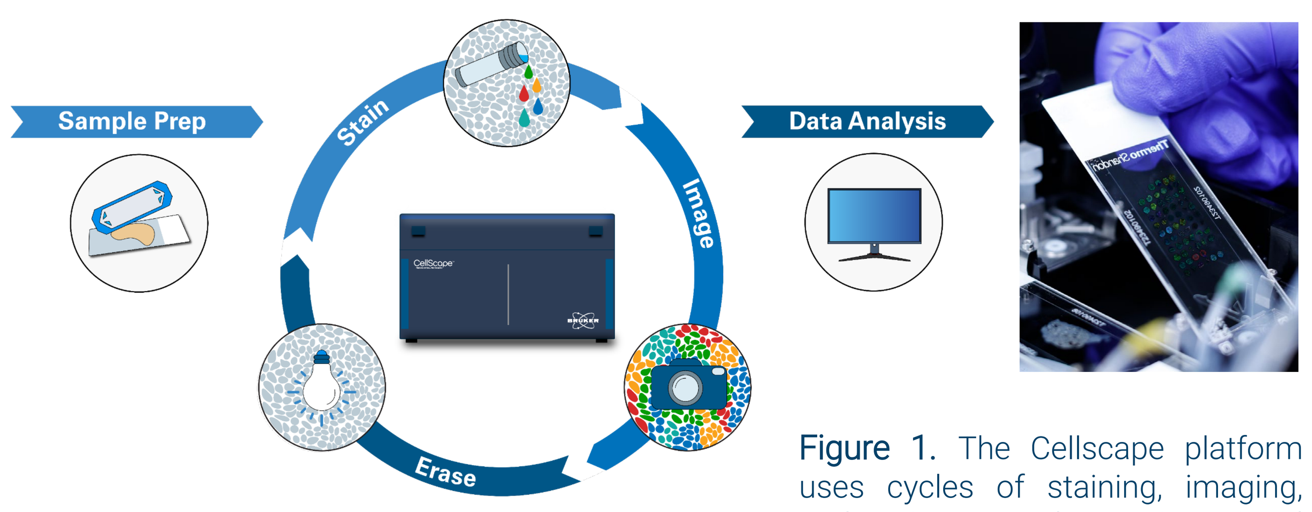


Figure 1. The Cellscape platform uses cycles of staining, imaging, and EpicIF non-destructive signal removal with single and subcellular resolution. Tissues are mounted on standard microscope slides and enclosed in microfluidic chambers for immediate use or long-term storage. Staining and imaging are then conducted automatically, on-instrument, and in real-time.

Neurons		Other Markers	
DNA	Tau (P-S199)	IBA1	CD34
NeuN	Tau (P-S214)	GFAP	Vimentin
MAP2	Tau (P-S404)	α-SMA	Amyloid β
NRGN	Tau (P-T396)	APBB1	APOE
Tau	Tau (P-T231)	p62	Ubiquitin

3. Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS) mosaic expression

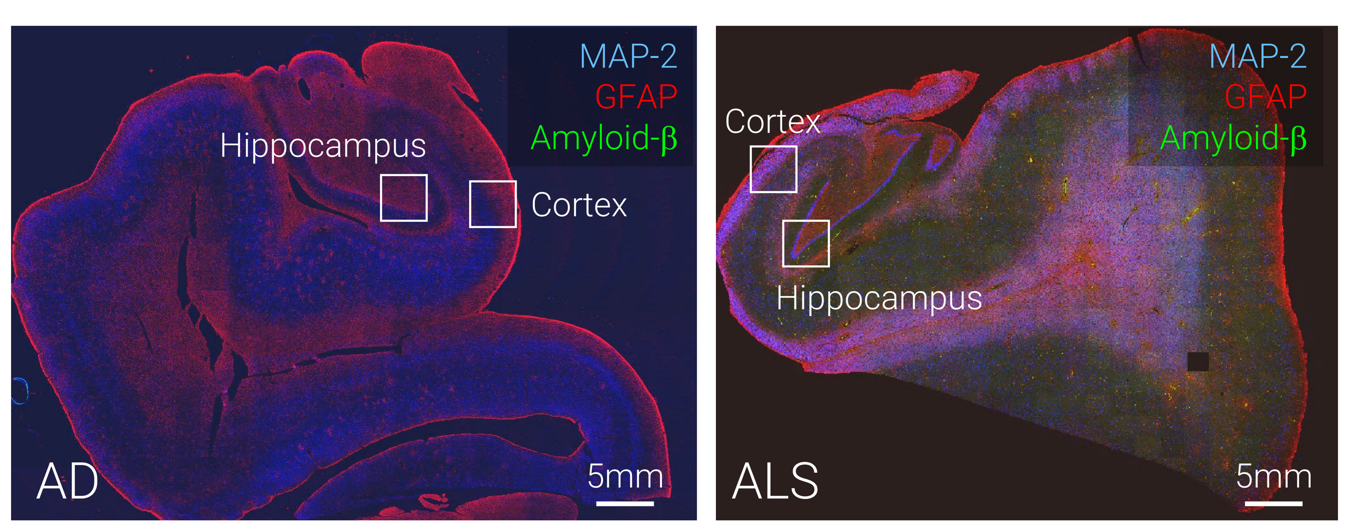


Figure 2. Post-mortem FFPE human brain samples from AD and ALS cases stained with the mIF panel shown above. White boxes indicate regions of interest in the hippocampus and cortex.

4. Segmentation of neurons and glia on Ariadne.ai SPATIAL

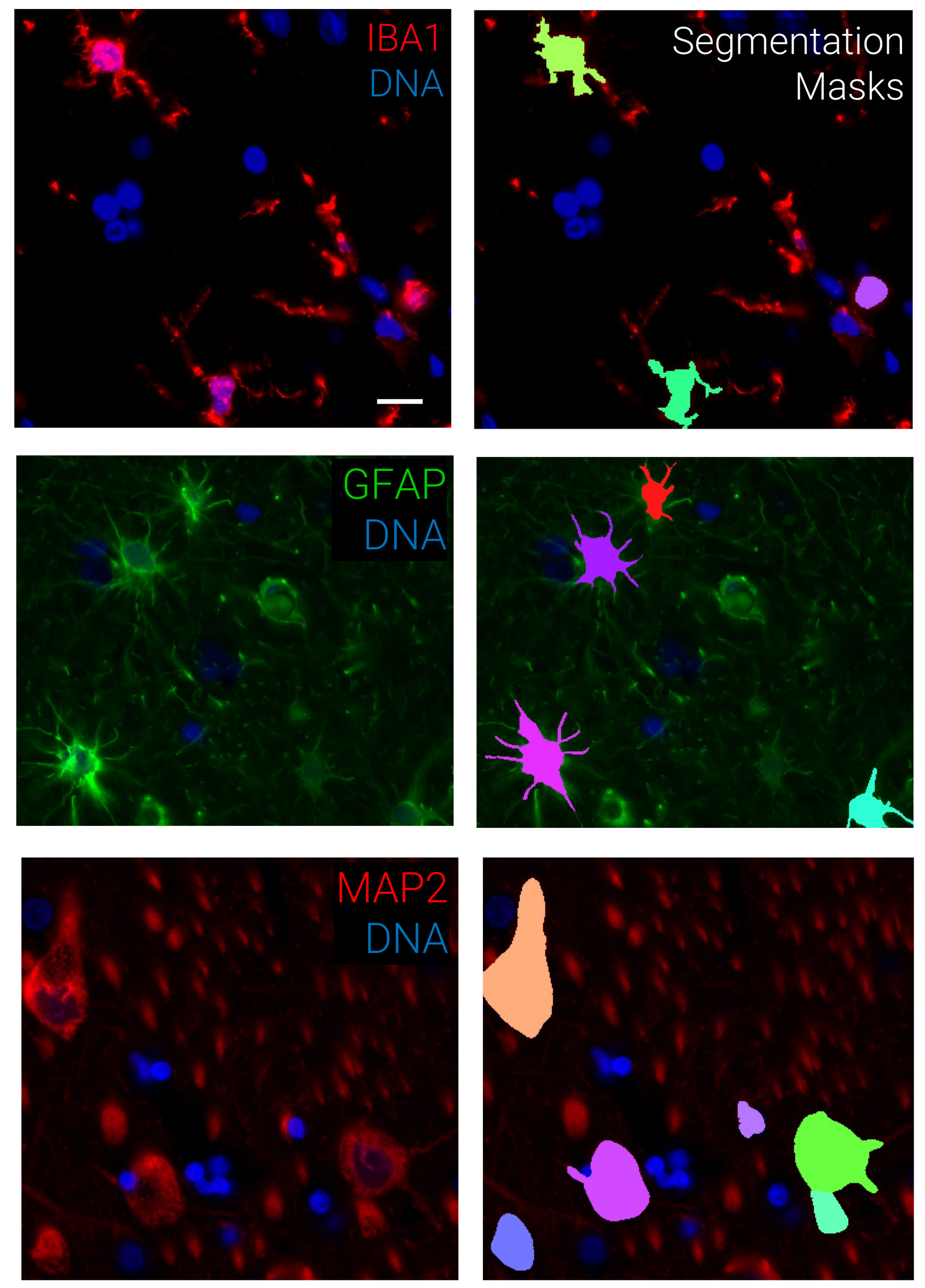


Figure 3. Segmentation of neurons and glia. Instance segmentation masks and corresponding channels used to generate them are shown for neuronal nuclei (NeuN), microglia (IBA1), astrocytes (GFAP), and MAP2-positive neuronal soma. Scale bar: 10 μm (applies to all panels).

5. Segmentation and classification of pathological protein aggregates (Aβ and Tau)

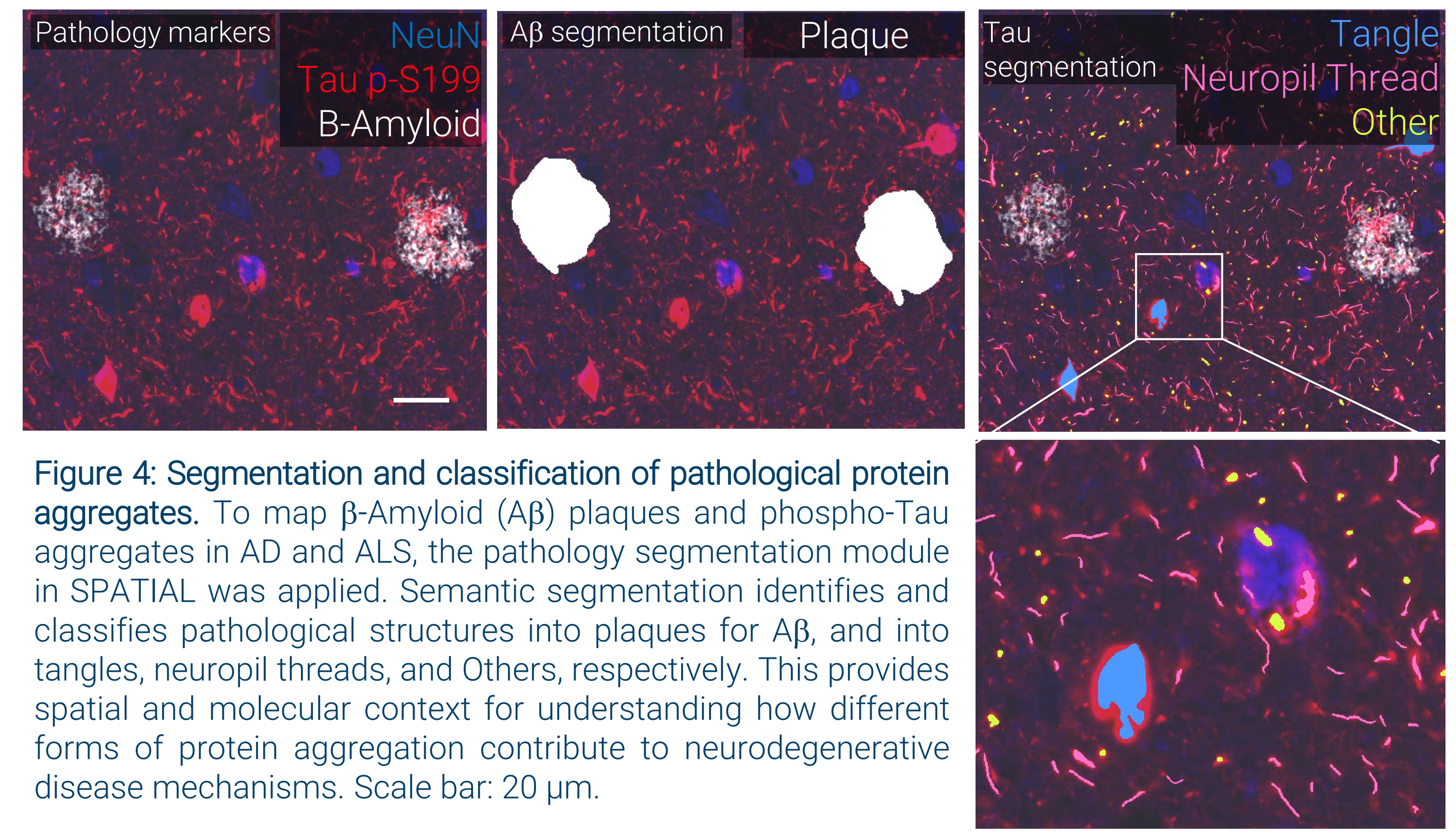
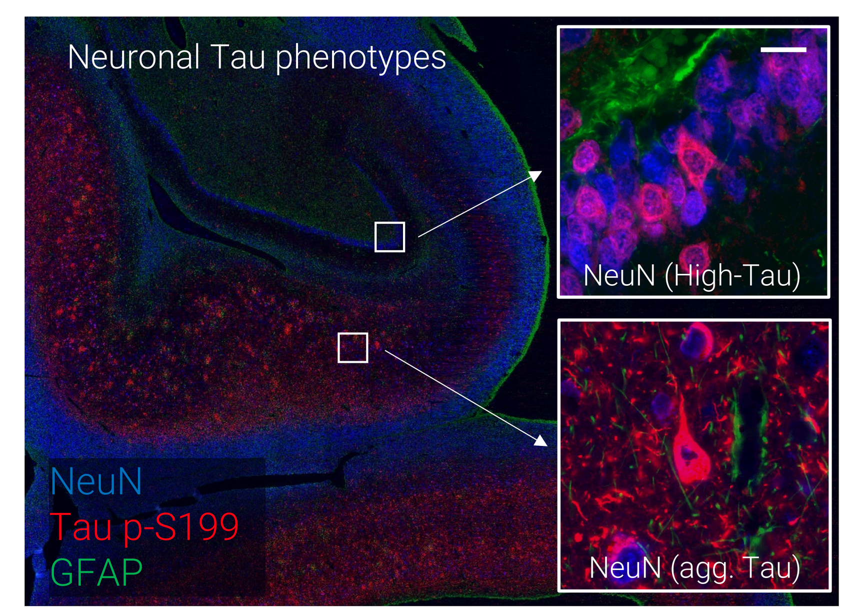


Figure 4: Segmentation and classification of pathological protein aggregates. To map β-Amyloid (Aβ) plaques and phospho-Tau aggregates in AD and ALS, the pathology segmentation module in SPATIAL was applied. Semantic segmentation identifies and classifies pathological structures into plaques for Aβ, and into tangles, neuropil threads, and Others, respectively. This provides spatial and molecular context for understanding how different forms of protein aggregation contribute to neurodegenerative disease mechanisms. Scale bar: 20 μm.



NeuN (High-Tau) - Early Tau accumulation: Neurons with >90th percentile of Tau p-S214

NeuN (agg. pTau) - Advanced aggregation and neurofibrillary pathology: Neurons overlapping >33% with tangle segmentation

Figure 5: Distinct neuronal Tau phenotypes. Phenotypes characterize progressive stages of neuronal tau pathology in neurodegenerative diseases. Scale bar: 15 μm.

7. TAU pathology is shaped by proximity to Aβ plaques

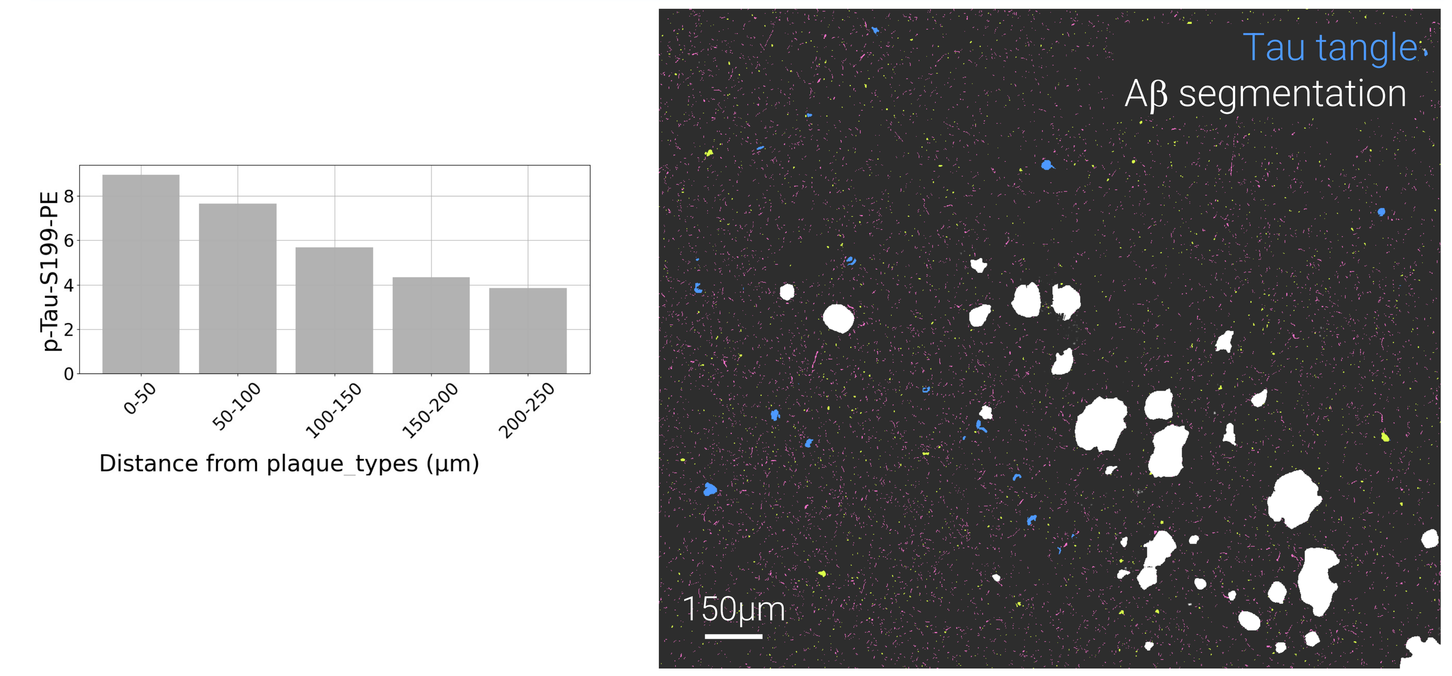


Figure 8. Association of p-Tau S199 and Amyloid plaque in AD cortex. Left: Mean Tau p-S199 signal decreases with increasing distance from Aβ plaques, indicating a spatial association between Tau phosphorylation and plaque proximity. Right: Spatial map showing Aβ plaque segmentation (white) and Tau tangles (blue) within cortical tissue, illustrating enrichment of p-Tau signal near plaques.

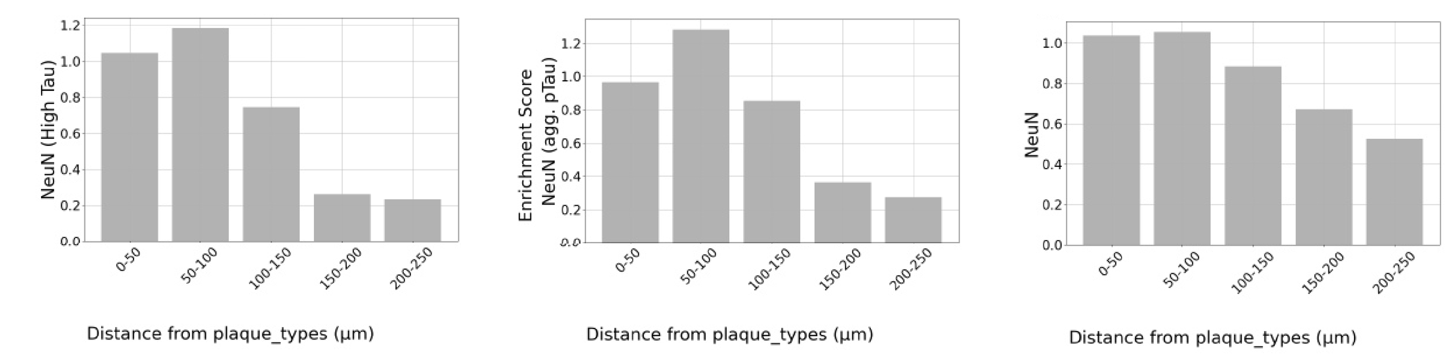


Figure 9. Enrichment of neuronal phenotypes relative to amyloid plaque proximity in AD cortex. Bar plots show the density or enrichment of High-Tau neurons (left), Agg. pTau neurons (center), and total NeuN+ neurons (right) as a function of distance from Aβ plaques. Both Tau-affected phenotypes are enriched near plaques, particularly within 50-100 μm, suggesting a spatial relationship between amyloid deposition and neuronal tau pathology.

6. Disease and regionally distinct distributions of cell phenotypes and pathological protein aggregates

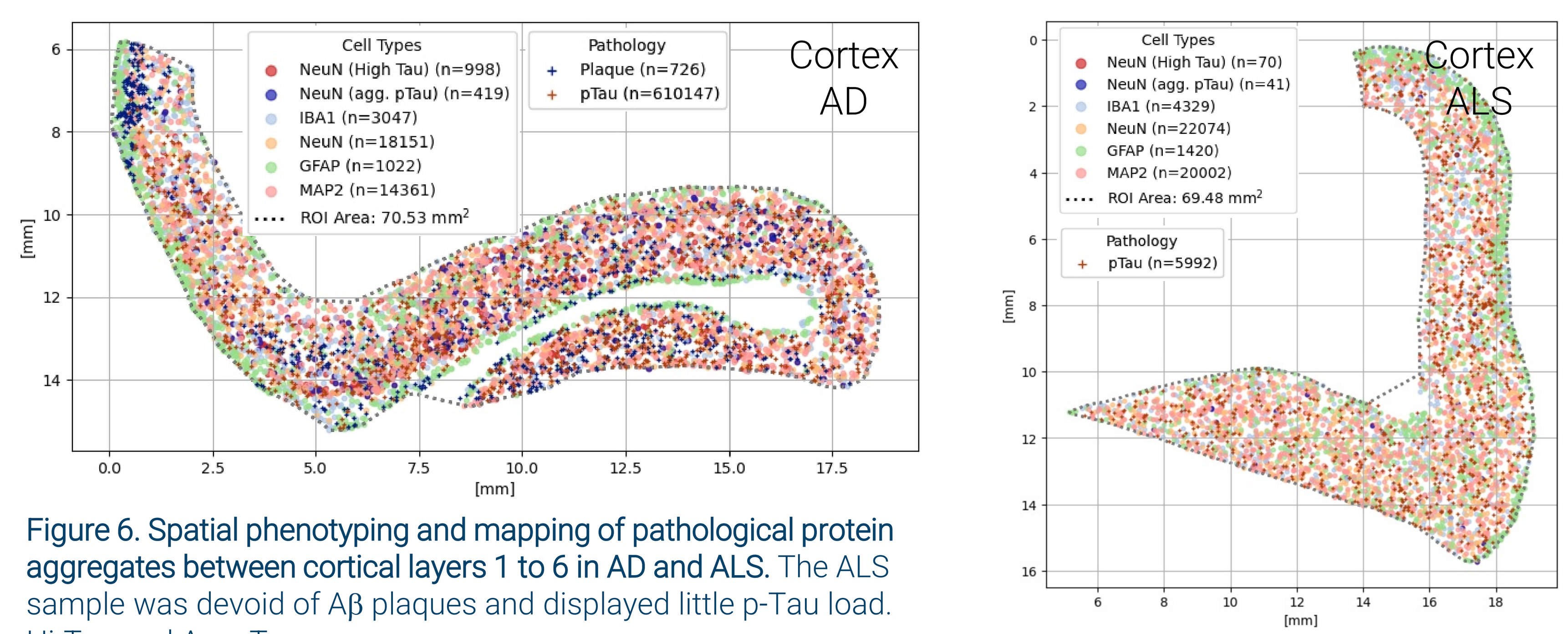
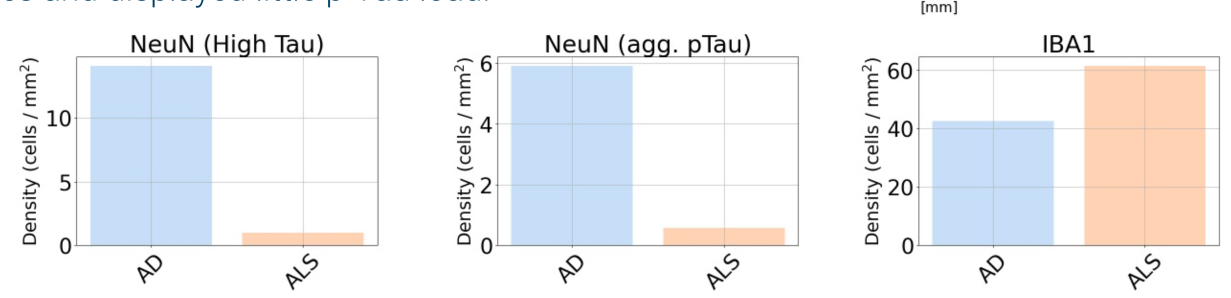


Figure 6. Spatial phenotyping and mapping of pathological protein aggregates between cortical layers 1 to 6 in AD and ALS. The ALS sample was devoid of Aβ plaques and displayed little p-Tau load. Hi-Tau and Agg. Tau neurons were abundant in AD but nearly absent in ALS; interestingly ALS samples harbored more microglia than AD samples despite fewer obvious pathological hallmarks.



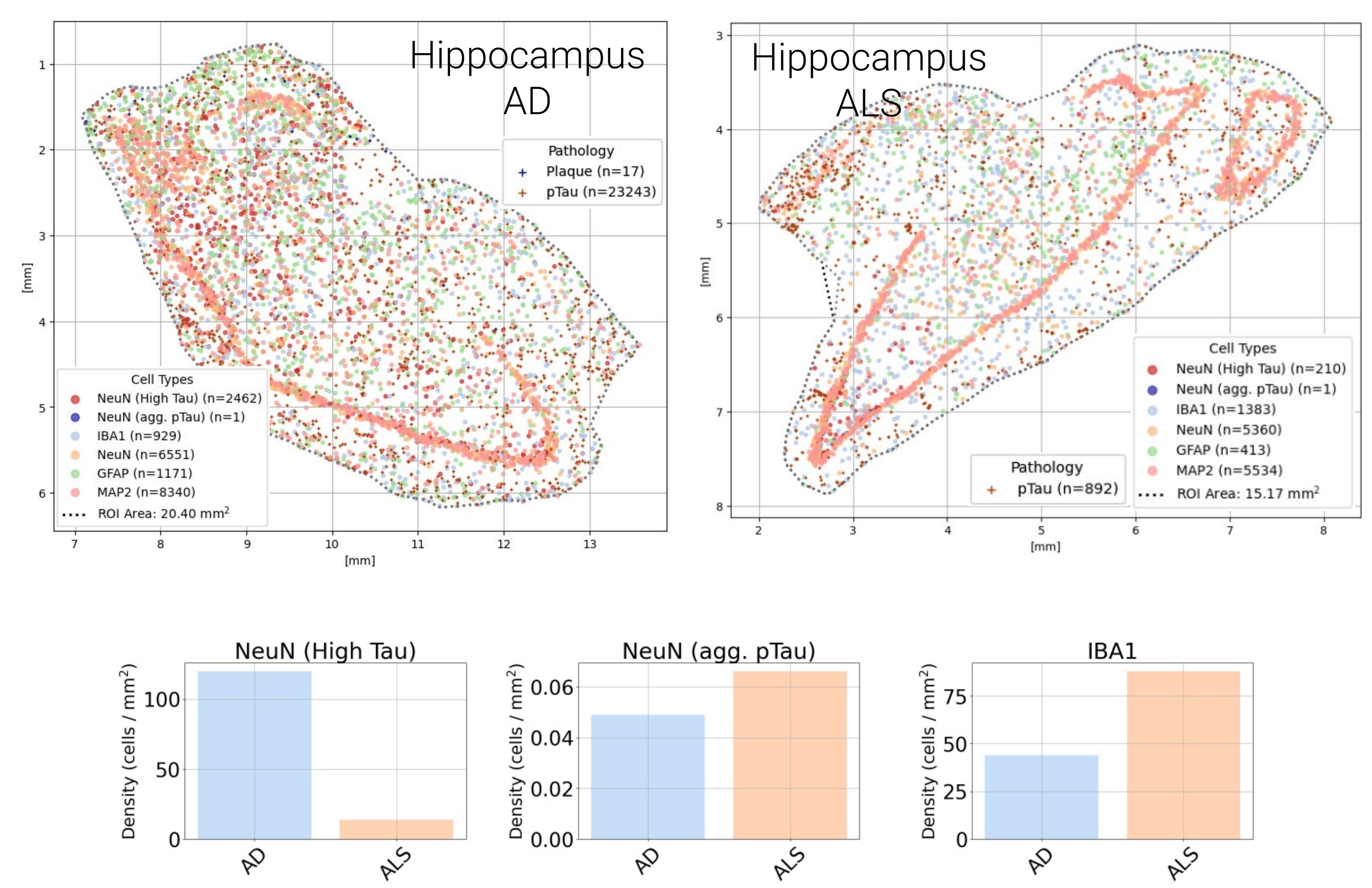


Figure 7. Spatial phenotyping and mapping of pathological protein aggregates in the hippocampus of AD and ALS. Consistent with cortical findings, the ALS hippocampus was devoid of Aβ plaques and contained fewer neurons classified as High-Tau. Interestingly, unlike in the cortex, more neurons exhibited the Agg. pTau phenotype despite the absence of plaques. Microglia were again more abundant in ALS than in AD.

Contact information

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