Use of spatial transcriptomics and proteomics to understand Alzheimer’s Disease

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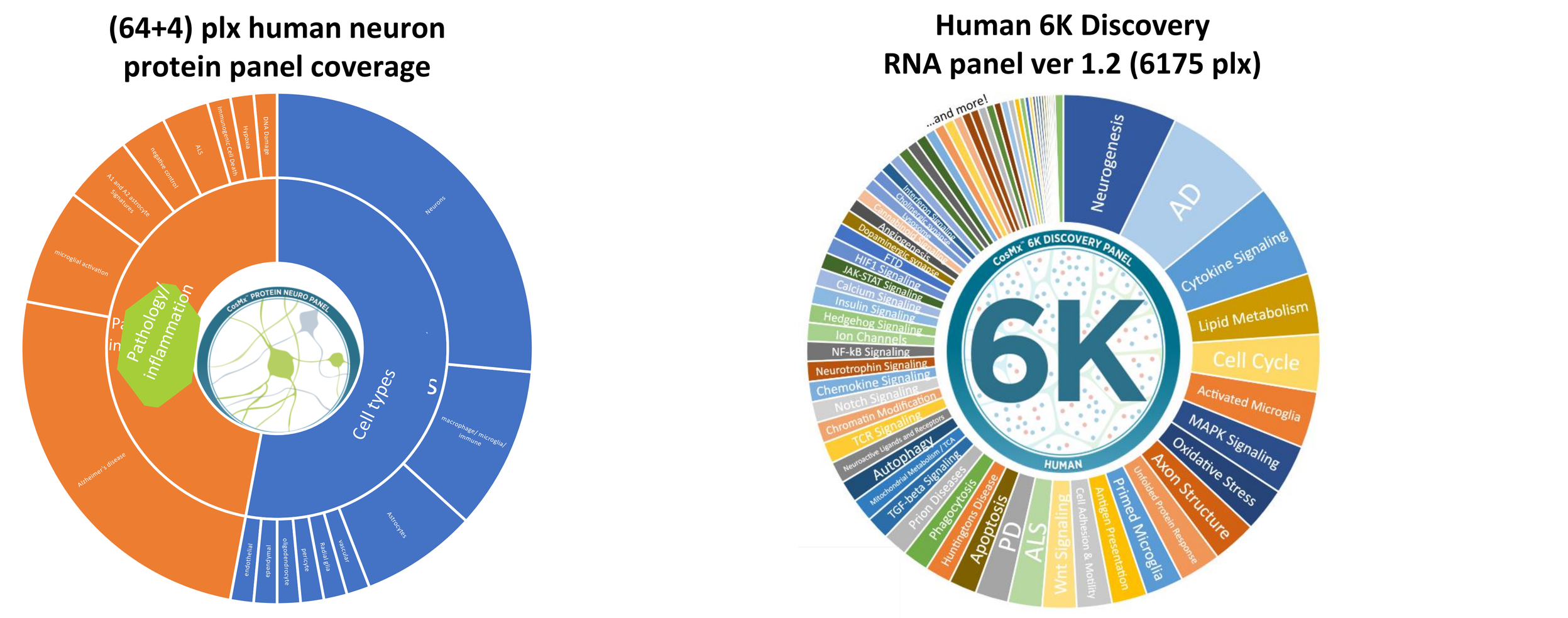
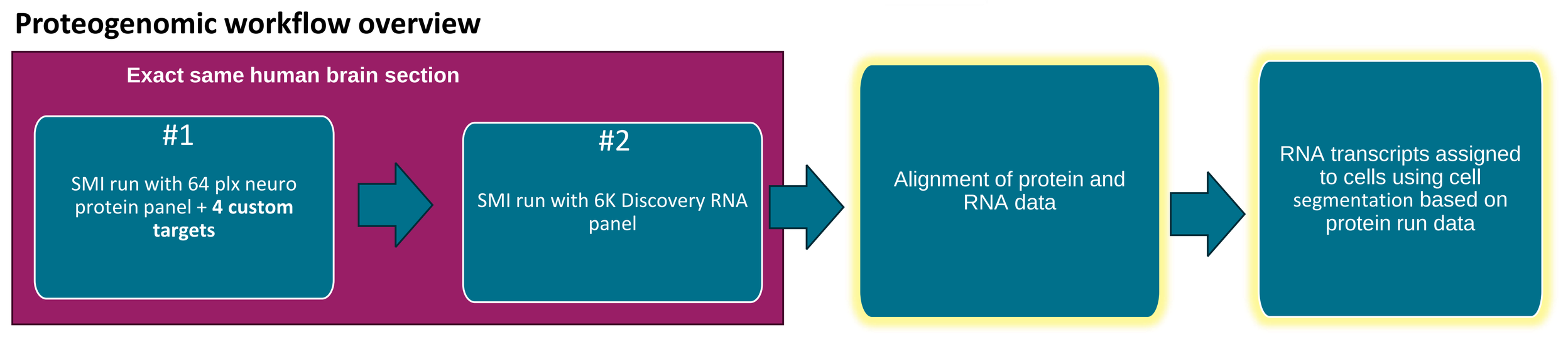
Introduction

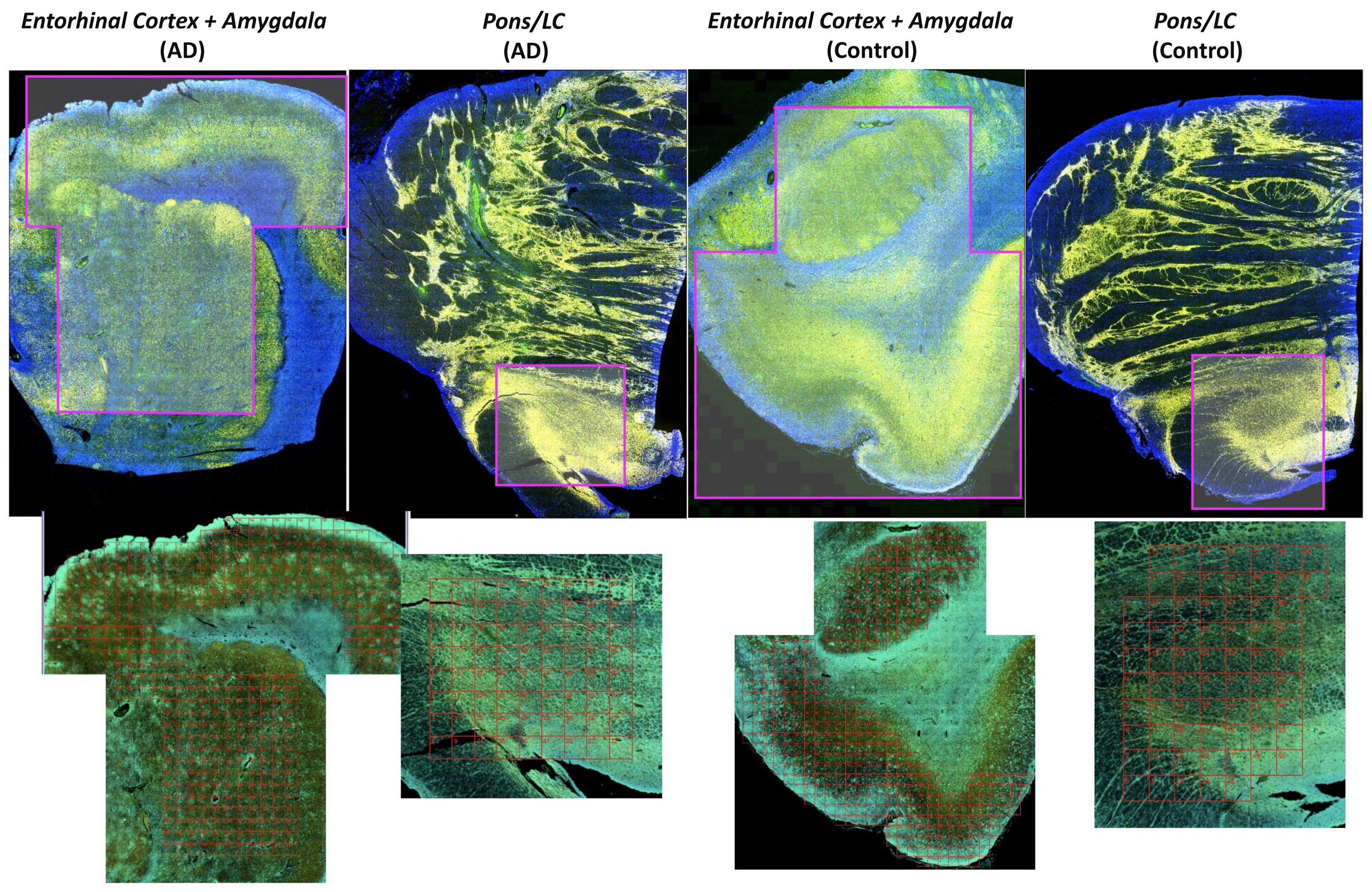
Alzheimer’s Disease (AD) is characterized by the pathological accumulation of amyloid-β (Aβ) plaques and hyperphosphorylated Tau, driving neurodegeneration through selective vulnerability of neurons and non-neuronal cells in key brain regions such as the Entorhinal Cortex (EC), Locus Coeruleus (LC), hippocampus, and prefrontal cortex. These regions form critical connection hubs that degenerate sequentially or concurrently, highlighting the importance of establishing spatial atlases to uncover underlying disease mechanisms. This study employed **the CosMx™ Spatial Molecular Imager (SMI)** platform for high-plex detection of **6,000 RNAs** and **68 proteins** in FFPE brain sections from the EC, LC, and Amygdala, providing detailed spatial context for identifying vulnerable cell populations and molecular signatures linked to AD pathology. In the EC, we observed strong co-expression of RTN1 and MEG3 near amyloid plaques, indicating region-specific vulnerability. In the LC, fewer LC-NE neurons and increased hyperphosphorylated Tau were noted, suggesting early neurodegenerative changes. Aβ42/Aβ40 staining also revealed heightened microglial density in plaque niches, with genes associated with cognitive impairment correlating with Aβ42/Aβ40 ratios. Using spatial transcriptomics and proteomics, we identified 21 distinct RNA/protein clusters via unsupervised Leiden clustering, offering a comprehensive map of cellular neighborhoods across regions. This analysis further uncovered niche-associated genes, many linked to AD pathogenesis. The SMI multi-omics approach allowed for the creation of spatial atlases of neurons and non-neuronal cells, providing insights into the spatial relationships between Aβ plaques, Tau tangles, and their surrounding cellular environments. These findings underscore region-specific vulnerabilities and intercellular communication pathways involved in AD, offering potential biomarkers and therapeutic targets for modulating AD progression and slowing the spread of pathology in its early stages.

Materials & methods

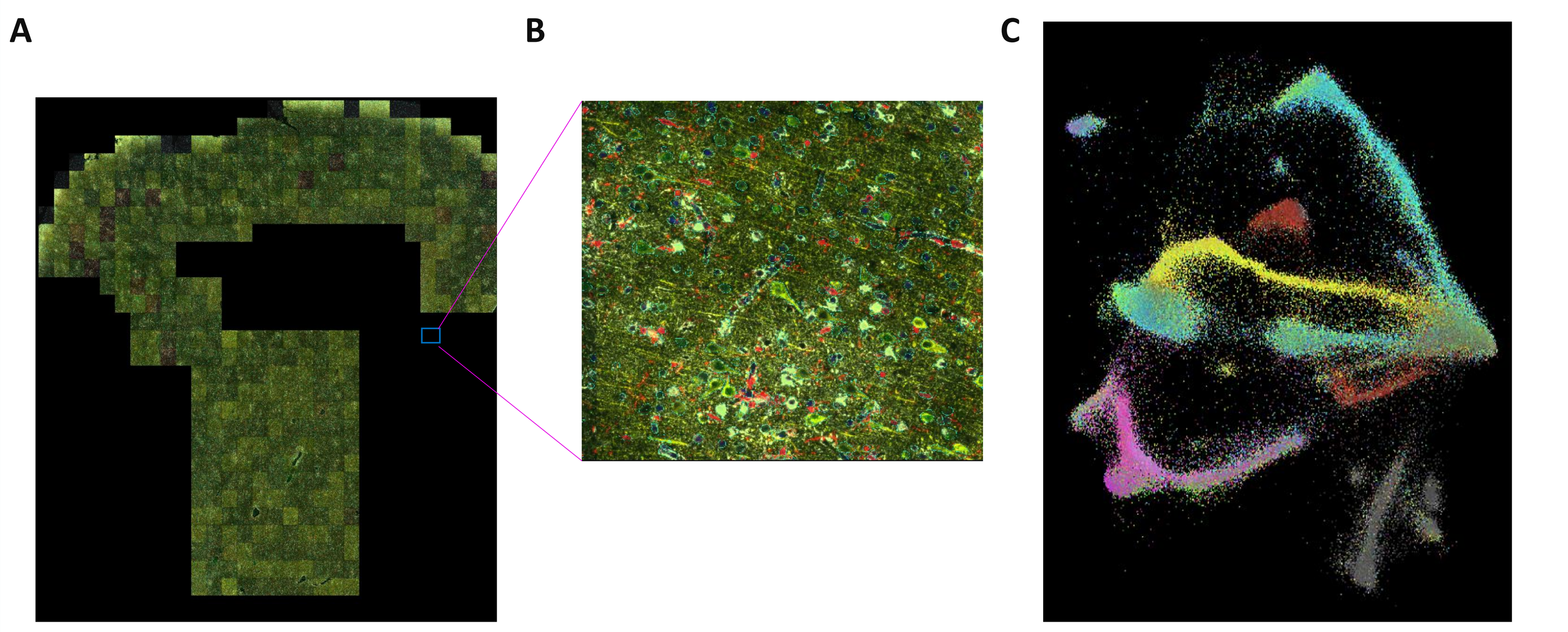




The CosMx™ Spatial Molecular Imager (SMI) platform is used for high plex detection of 6,000 RNAs and 68 proteins in the same FFPE human brain sections in Entorhinal cortex (EC), Amygdala and Locus Coeruleus (LC) in Pons. This proteogenomic assay involves first detecting proteins with oligonucleotide barcode-conjugated antibodies and then exposing sections to protease digestion and detecting RNAs with barcoded RNA probes

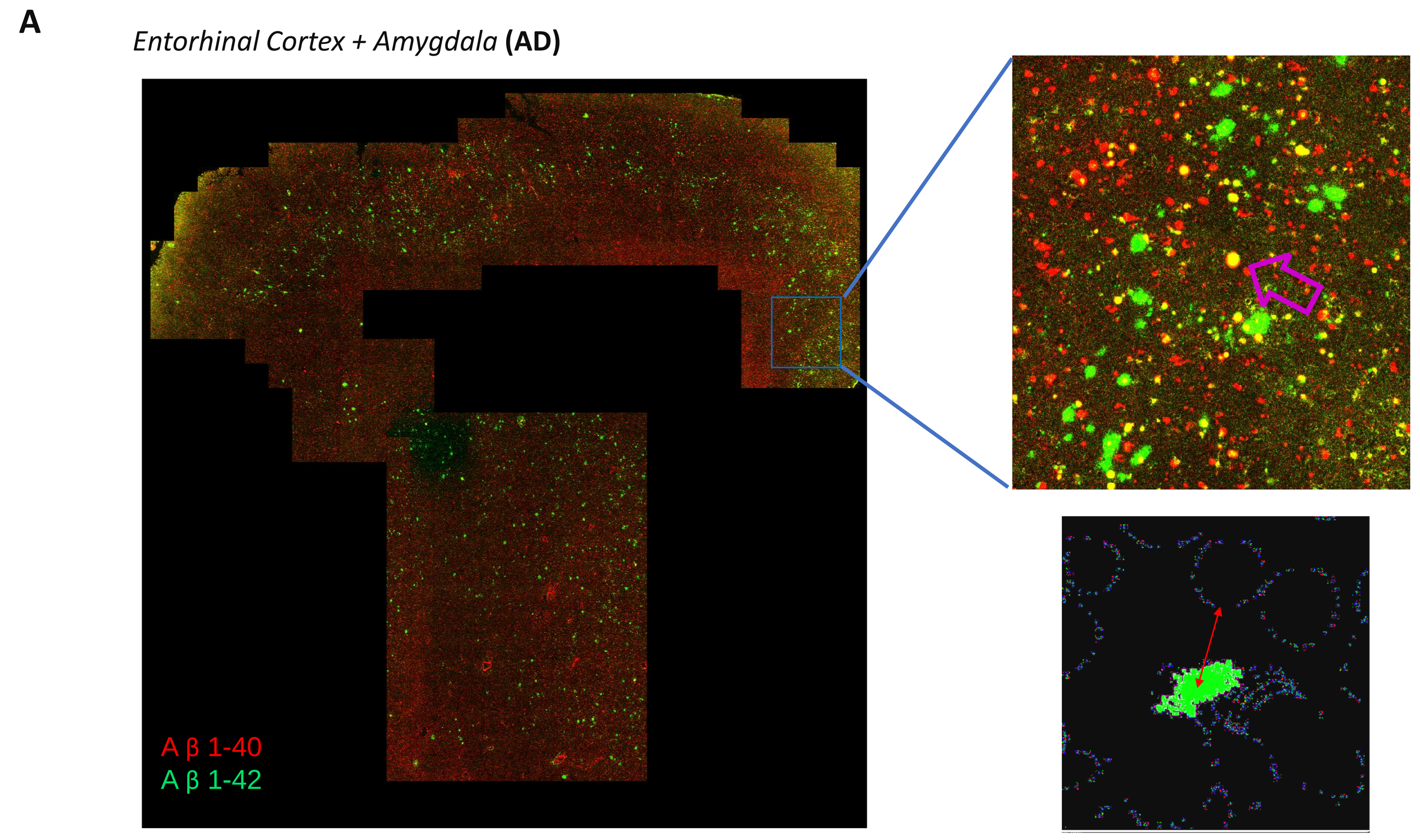


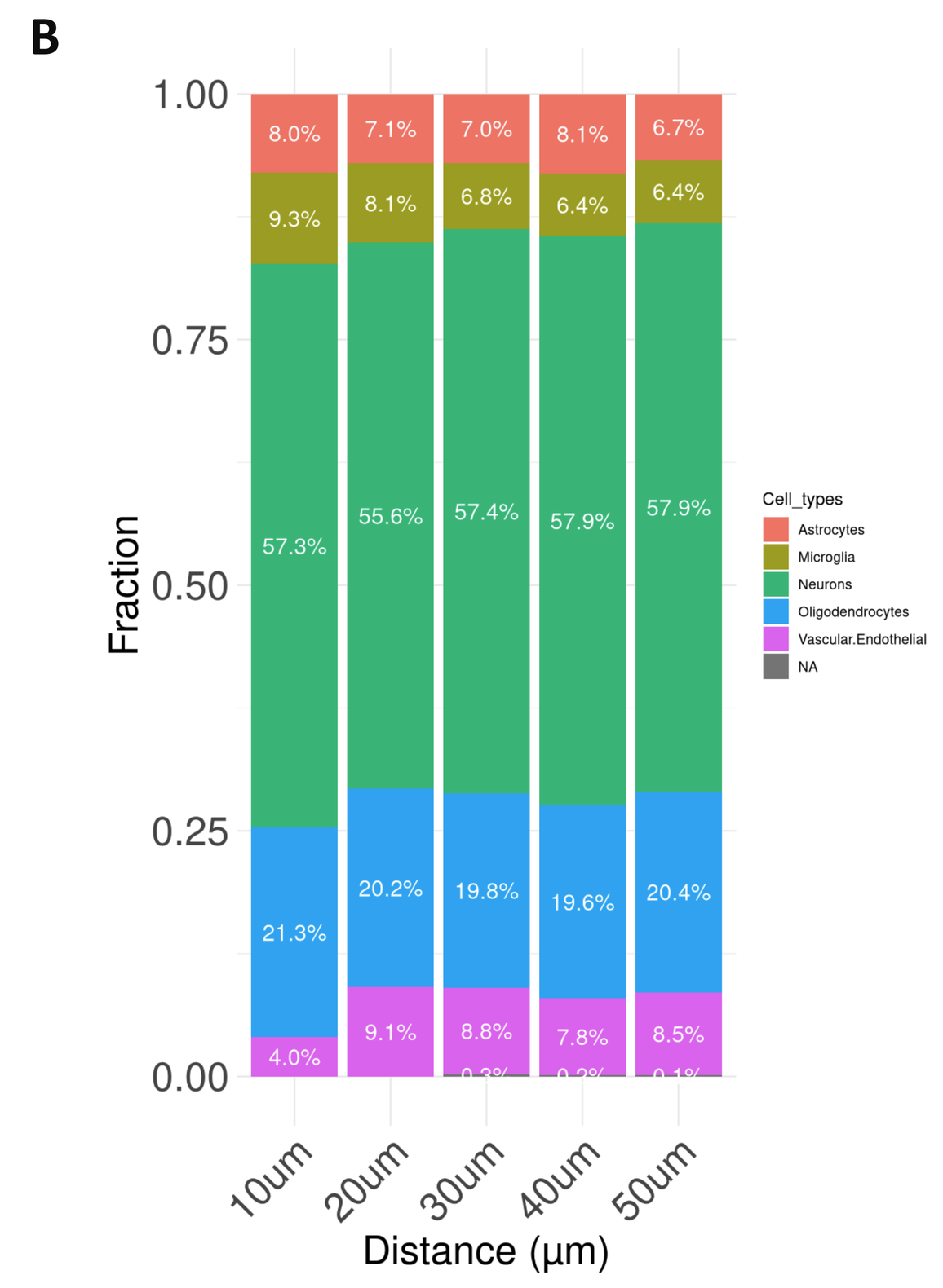
4 FFPE human brain sections across entorhinal cortical (EC + Amygdala)n & Pons (LC) regions of 2 male donors

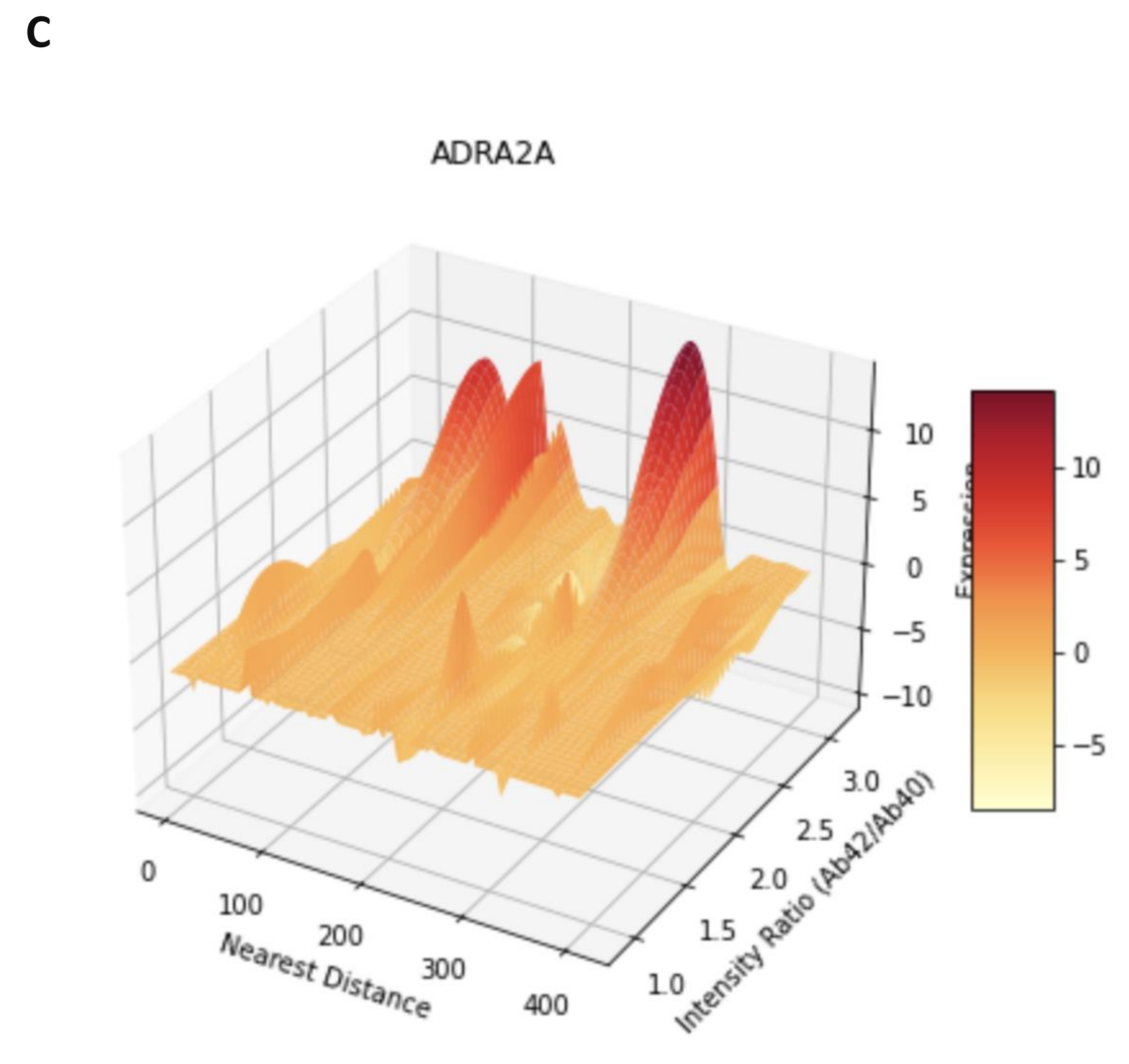


A, CosMx SMI allows simultaneous profiling of RNA and protein targets and is compatible with formalin-fixed, paraffin-embedded (FFPE) tissues. B, New Protein-based cell segmentation using advanced neuro-specific model & workflow: successful captures cells of diverse shapes & sizes along w/ their protrusions & axons. C, Cell populations and their annotations are projected onto a reference-defined UMAP for visualization.

Resolving the plaque niche in Entorhinal Cortex/Amygdala using spatial omics

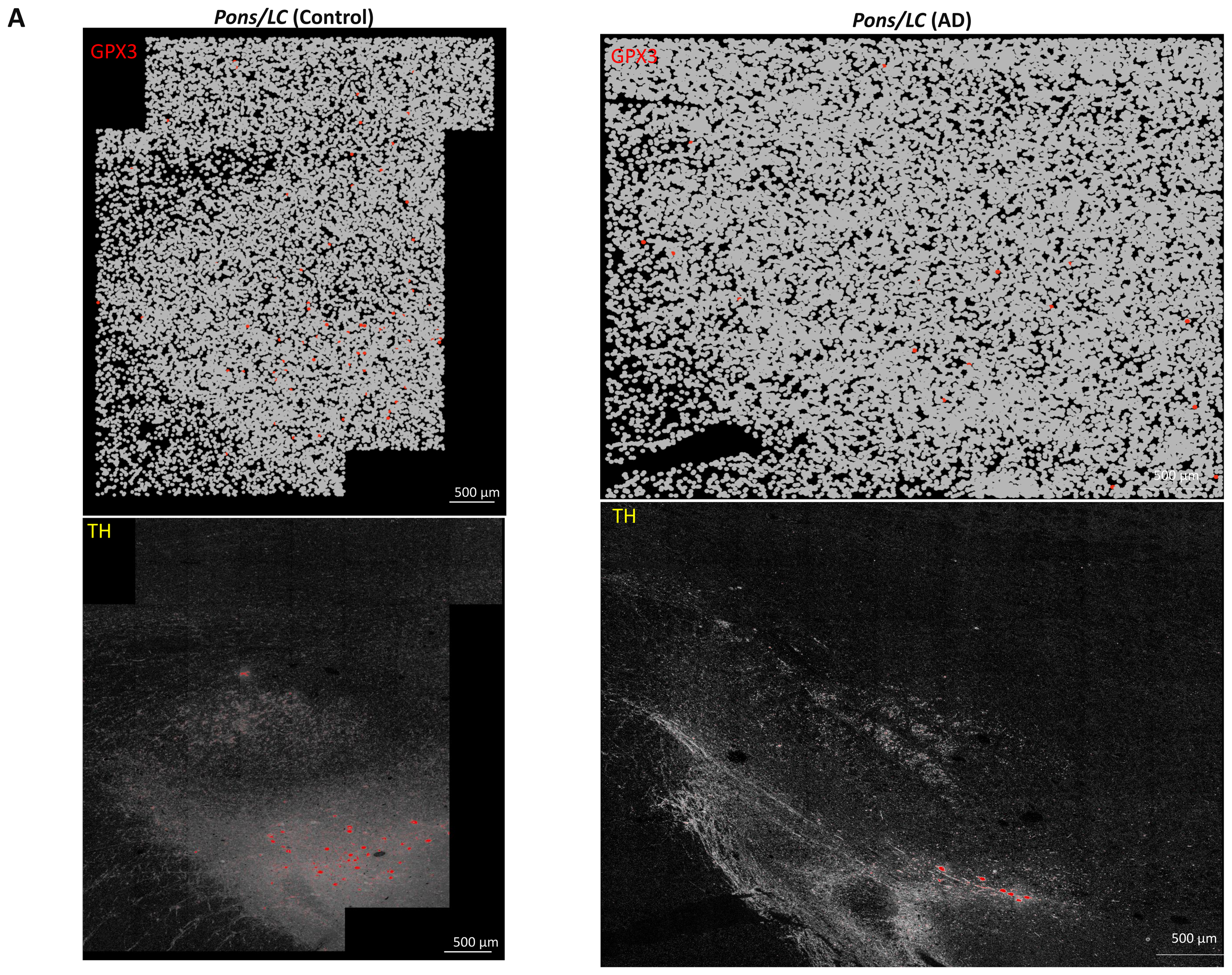


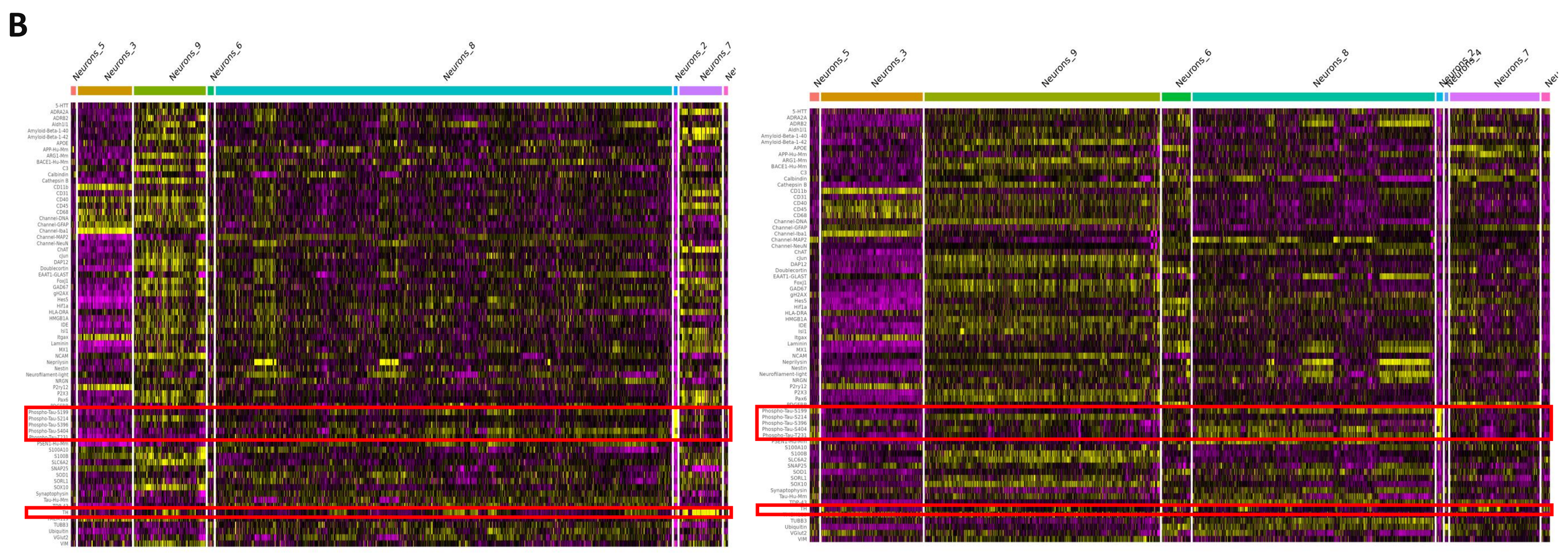




A, These sections were directly stained for Aβ42 and Aβ40 using monoclonal Aβ antibodies. Amyloid plaques were segmented, and both cellular and transcriptomic responses to the plaques were evaluated according to their distance to the plaque. B, The density of microglia increased within plaque niches. C, Some genes implicated in cognitive impairment in Alzheimer’s disease were found to correlate with the Aβ42/Aβ40 ratio and proximity to the plaques.

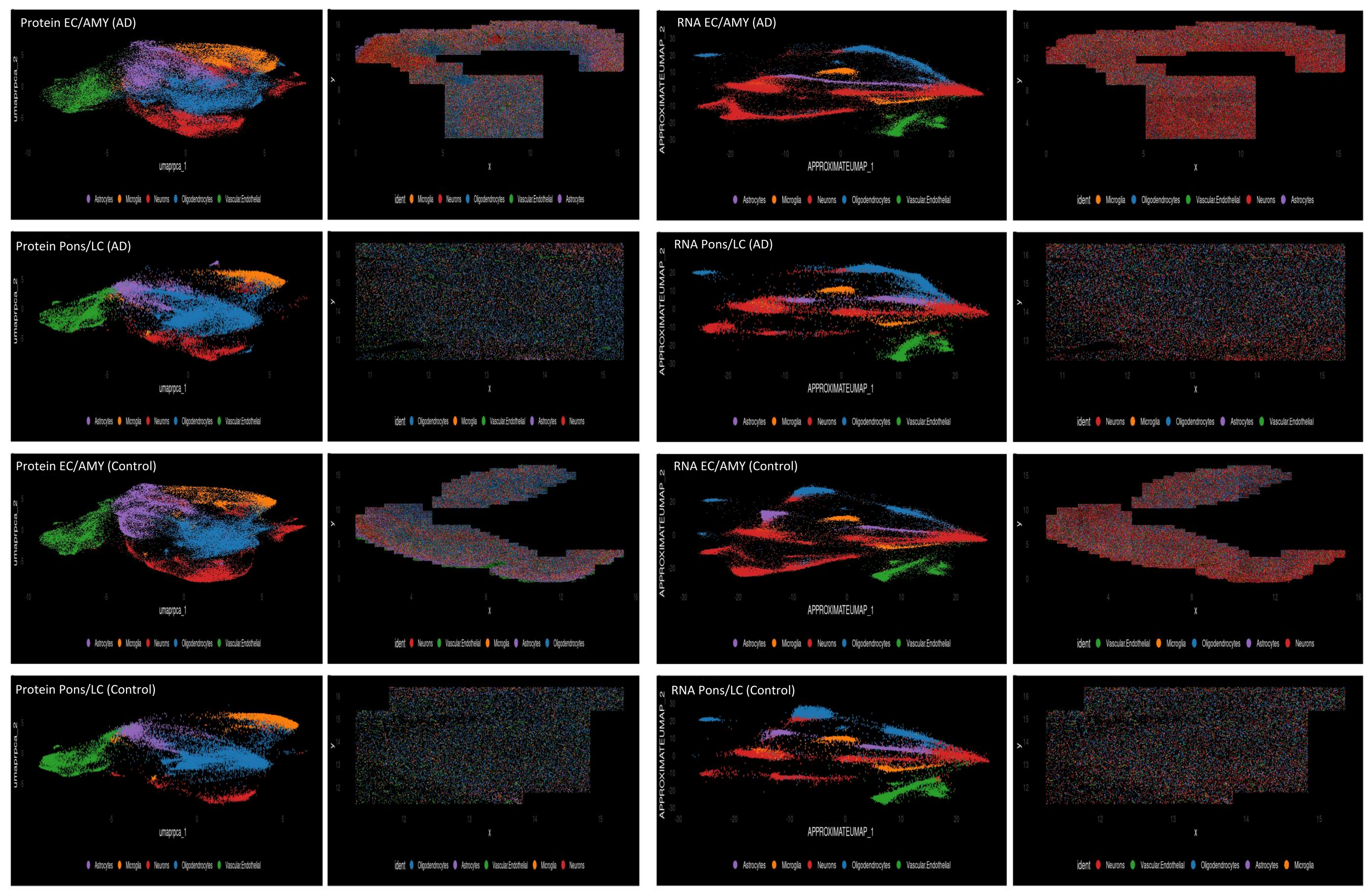
Spatial gene expression, (NE) neurons and hyperphosphorylated-tau in human locus coeruleus (LC)





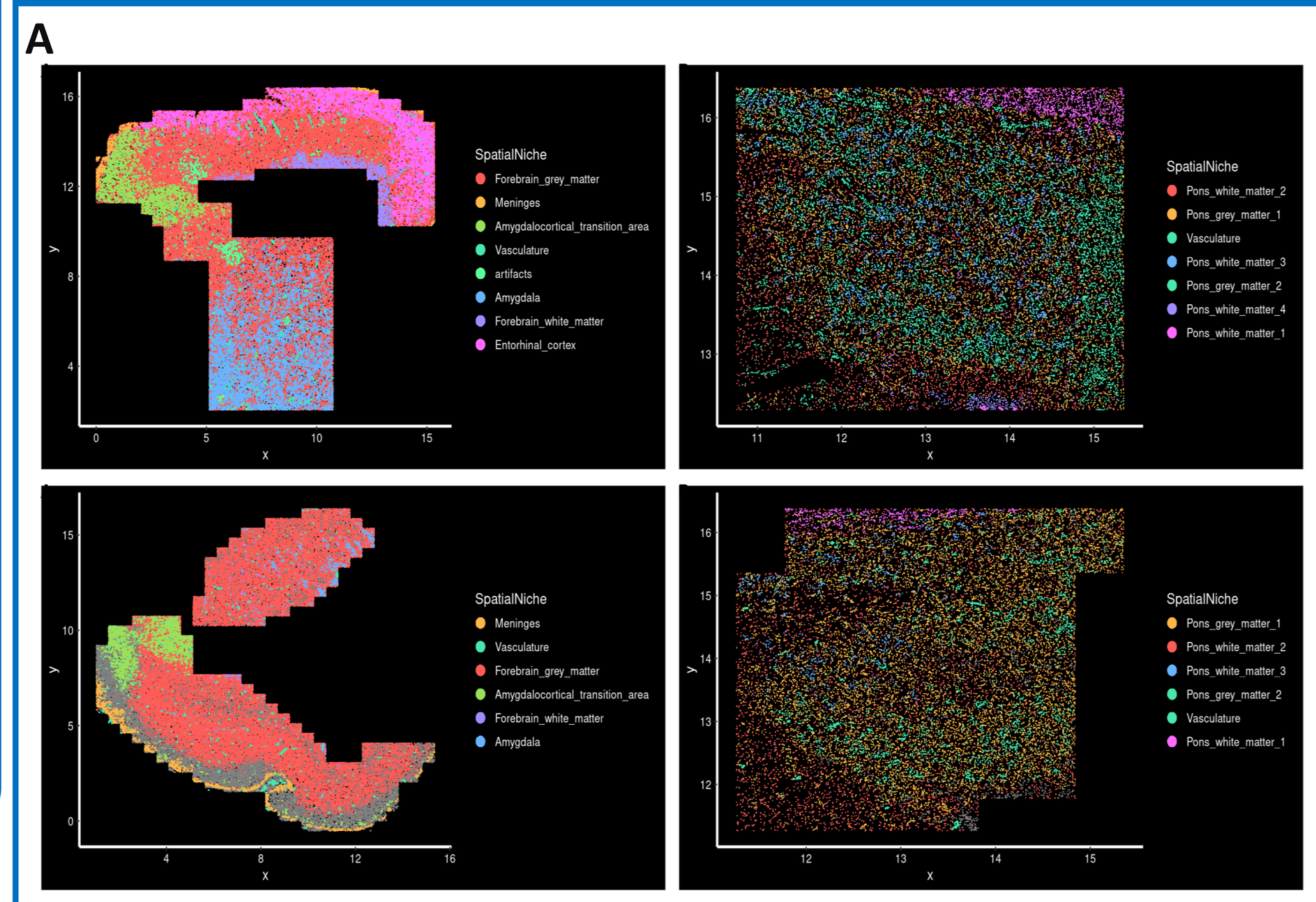
A, LC-associated genes were used to annotate regions from the pons identified as containing LC-NE neurons.
B, In Alzheimer’s disease (AD), the number of these neurons was reduced, and a higher accumulation of hyper-phosphorylated Tau was detected in specific neuron types compared to controls.

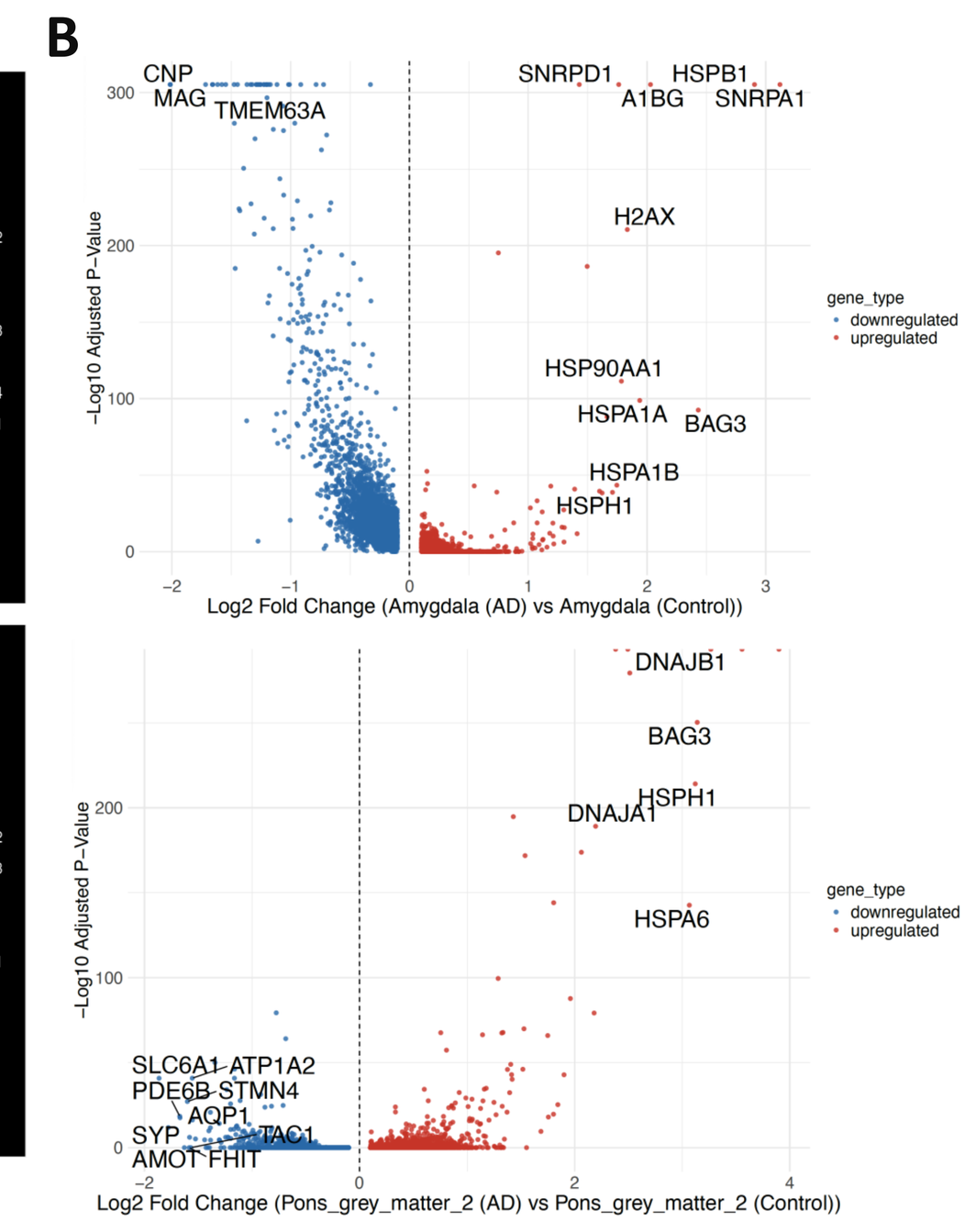
Unsupervised Protein or RNA-based cell typing identifies major cell types as well as their spatial patterns



Unsupervised Leiden clustering identified 21 clusters in RNA and proteins were projected to spatial location

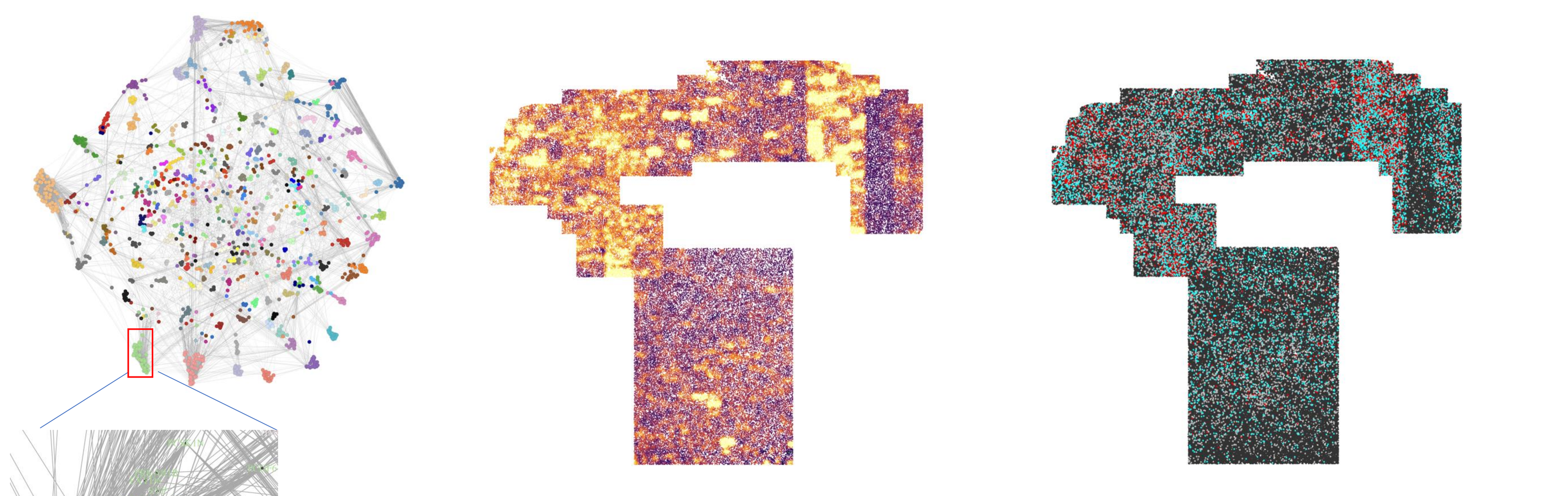
Unsupervised neighborhood analysis identifies tissue architecture and allows studies of cell behavior in healthy vs. AD

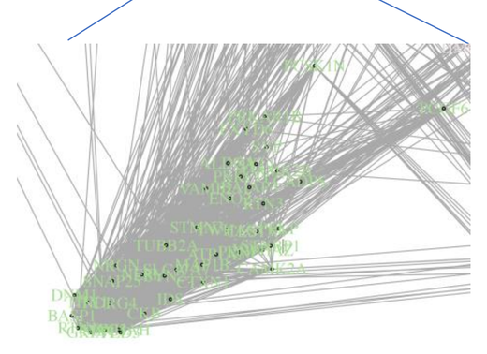




A, To study how cells and their transcriptomes change in response to spatial context, a computationally efficient algorithm was used to partition tissues into distinct ‘niches’ or ‘spatial clusters.’ B, Cell-type-specific, niche-associated genes were then identified, many of which are implicated in Alzheimer’s disease pathogenesis.

InSituCor identifies spatially colocalized gene modules that show differential neighborhood expression in Alzheimer’s entorhinal cortex





RTN1-MEG3

In the entorhinal cortex, RTN1 and MEG3 show strong spatial co-expression (red dots), particularly near amyloid plaques, suggesting a role in Alzheimer’s disease (AD) neurodegeneration. While MEG3 is highly expressed in the amygdala, it lacks significant co-expression with RTN1 (cyan dots). This region-specific pattern, identified using the *InSituCor* method, highlights the entorhinal cortex’s vulnerability to AD and the potential involvement of RTN1 and MEG3 in disease progression.

Summary & Conclusions

This study applied the **CosMx™ Spatial Molecular Imager (SMI)** platform for high-plex detection of RNA and protein targets in **FFPE brain sections** from the **Entorhinal Cortex (EC)**, **Amygdala**, and **Locus Coeruleus (LC)**. The main findings are:

- **Amyloid Plaques**:
  - Aβ42/Aβ40 staining revealed increased microglial density in plaque niches.
  - Genes linked to cognitive impairment correlated with Aβ42/Aβ40 ratio.
- **Locus Coeruleus (LC)**:
  - Fewer LC-NE neurons in AD, with more hyperphosphorylated Tau in specific neurons.
- **Leiden Clustering**:
  - 21 RNA/protein clusters identified, mapped to spatial locations.
- **Spatial Niches**:
  - Niche-associated genes, many tied to AD pathogenesis, were identified.
- **RTN1 and MEG3 Co-expression:**
  - Strong co-expression near amyloid plaques in the entorhinal cortex, highlighting region-specific vulnerability in AD.

Conclusion

CosMx SMI provided key insights into Alzheimer’s, revealing changes in **Aβ plaques**, **microglia**, **LC neurons**, and identifying **niche-specific genes** for potential biomarkers and therapies.