

Advancing spatial discovery proteomics: Interrogation of Alzheimer's disease and Parkinson's disease neural tissue with GeoMx® DSP and a novel 1200+ plex Discovery Proteome Atlas

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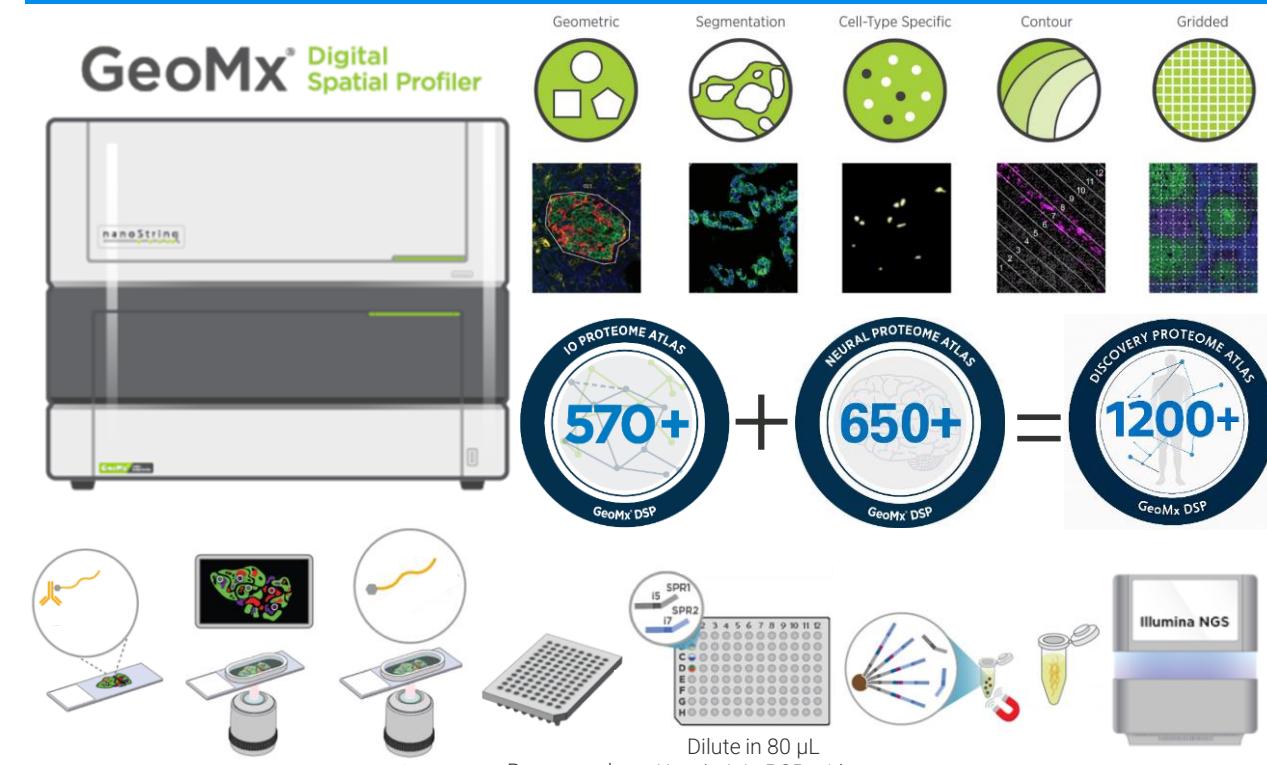
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Abstract

Spatial proteomics is a transformative approach to resolve tissue heterogeneity in complex biological systems including the human brain. Whereas high-plex spatial transcriptomics has transformed tissue analyses, spatial proteomics has been limited by the low number of targets and limited proteome coverage, especially for targets critical to the latest neurobiology advances.

Here we introduce the Human Discovery Proteome Atlas (DPA), a 1,200+ plex antibody-based proteomic assay for the GeoMx® Digital Spatial Profiler (DSP) platform. The GeoMx DPA assay is the highest spatial proteomics plex on the market with wide coverage of proteins that target critical neurobiology, immunology, and oncology processes. We profiled 32 ROIs (regions of interest) across cortical layers of the parietal and occipital lobes in brain sections from an Alzheimer's disease patient and compared them to similar regions in normal control tissue. We also profiled the spatial proteomic signatures in the postcentral gyrus, frontal lobe, temporal lobe, and cerebellum of Alzheimer's disease and normal tissues. We found differential expression of numerous protein targets between the disease and normal tissue that are spatially organized within brain regions. Additionally, we profiled thalamus tissue from a Parkinson's disease patient and characterized the proteomic signature from multiple regions. We further characterized the specificity, sensitivity, and reproducibility of DPA in tumor tissue microarrays with 50+ tumor cases from various organs including the brain (e.g. atypical meningioma, malignant ependymoma of the right occipital lobe, anaplastic oligodendrogloma, glioblastoma). By capturing spatial information on over 1,200 antibody targets, DPA provides comprehensive insights into heterogeneous tissues with complex disease mechanisms, offering a discovery portal for novel therapeutic or biomarker identification.

GeoMx Digital Spatial Profiler and NGS Readout



GeoMx Discovery Proteome Atlas (DPA) is the Highest Plex Assay for Spatial Proteomics

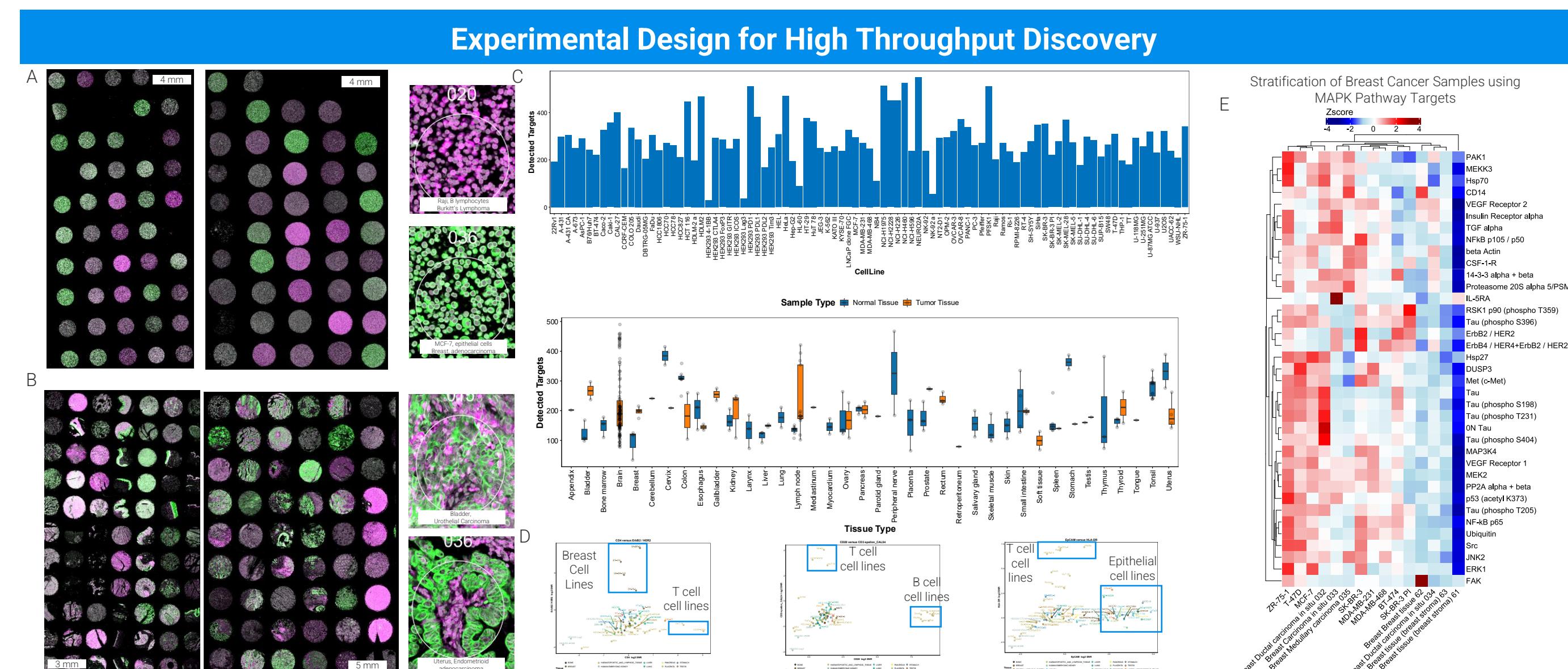
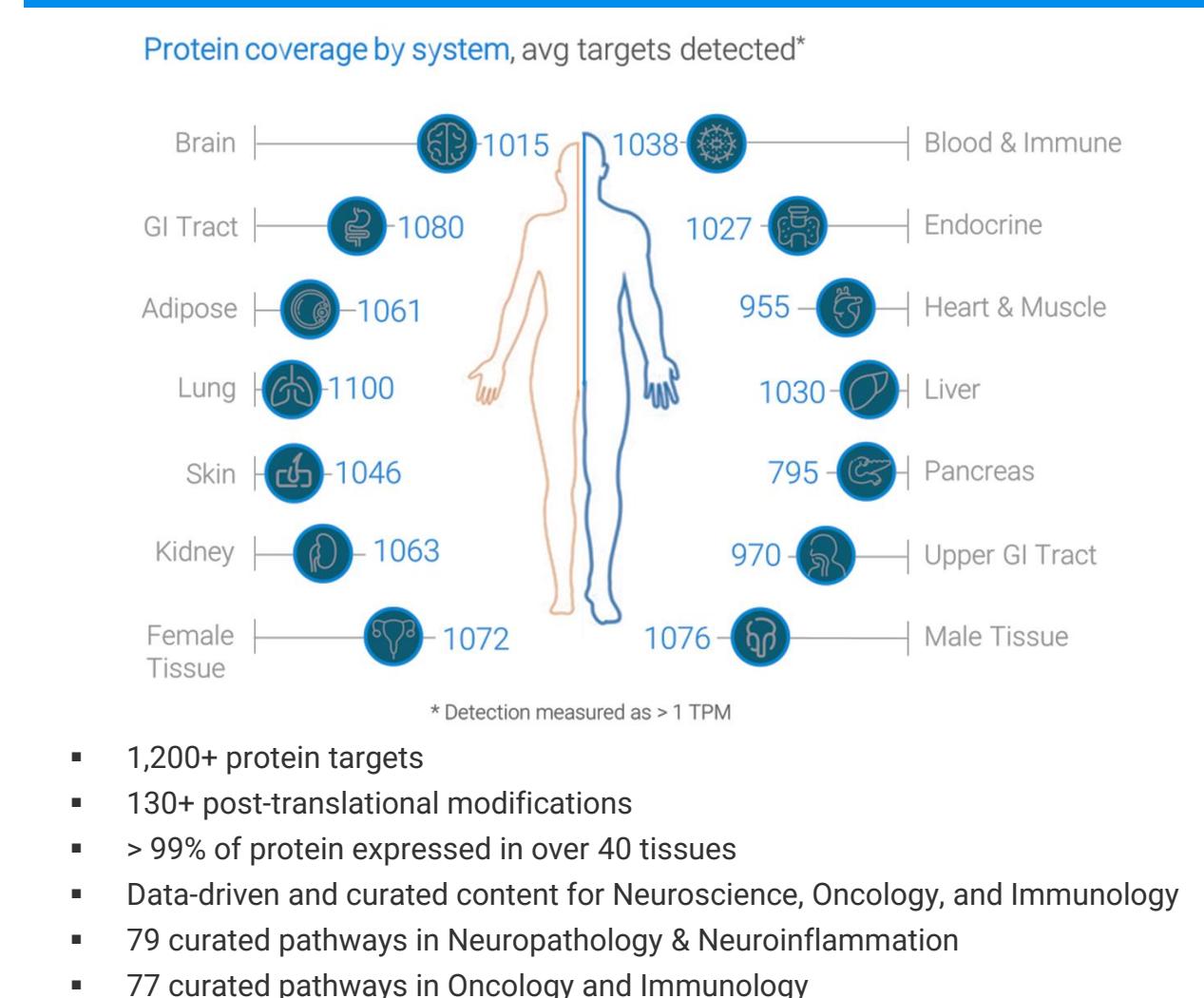


Figure 1. GeoMx Digital Spatial Profiler (DSP) experimental design for high-throughput discovery applications in Spatial Proteomics. GeoMx exported images of scanned slide and region-of-interest (ROIs) showing 5 μm thick section of FFPE tissue or cell panel microarrays used in this study. **(A)** CPA46 and CPA49 representing 89 cell lines from 20+ human tissue types. **(B)** TMA800 (tumor tissues) and TMA999 (normal tissues) representing 30+ profiled human tissue types. Slides stained with fluorescent morphology antibody markers to guide ROI selection. PanCk+ for epithelial cells (green), CD45+ for immune cells (magenta), and DNA stain (grey). At least 1 ROI of 200 μm diameter circles was selected per cell line or core. **(C)** Box plots of number of detected targets in each 200 μm ROI in the cell lines and tumor arrays samples. Each ROI is represented by a bar or transparent circle on the box plot. Protein detection defined by signal-to-noise ratio (SNR) of 5 where noise is geometric mean of IgG internal negative controls. **(D)** Scatterplots of different protein expression patterns across a range of tissue types and cell lines. **(E)** Stratification of breast cancer tissues and breast cell lines with protein targets in the MAPK pathway. Protein targets with a signal-to-noise ratio (SNR) of ≥ 5 and observed at least once were used to generate the clustered heatmap. Color is based on z-score transformed log2 SNR transformed counts.

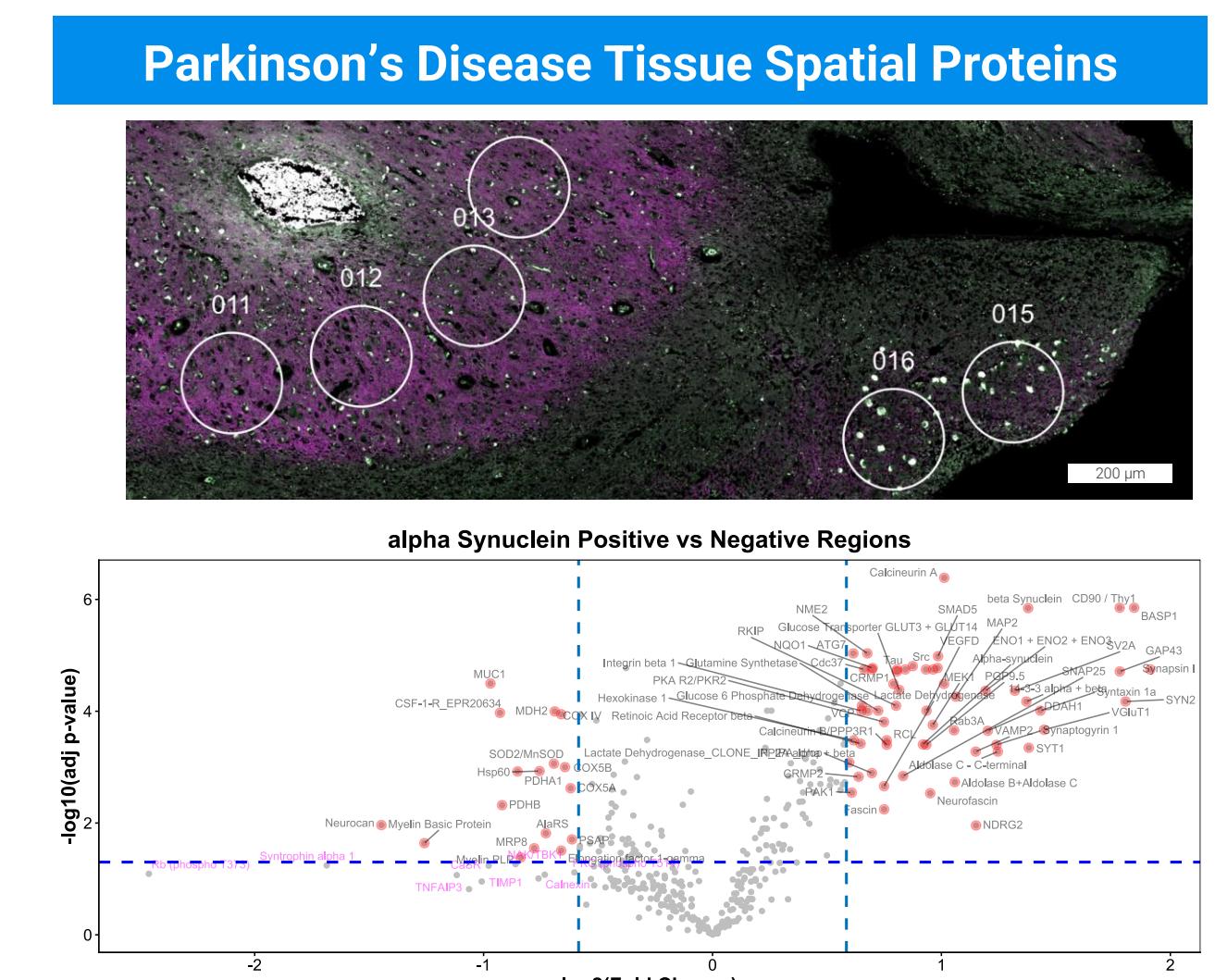
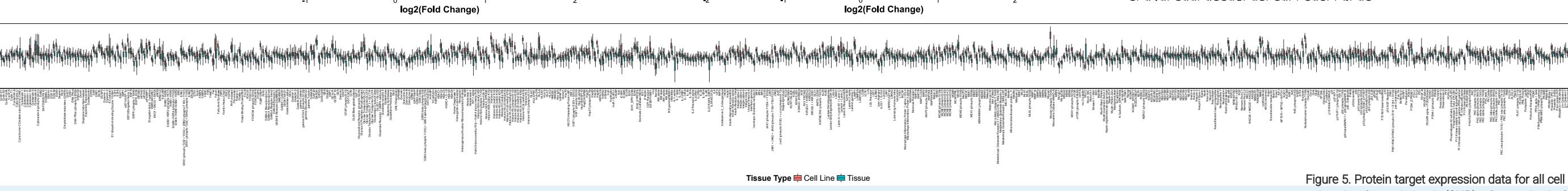
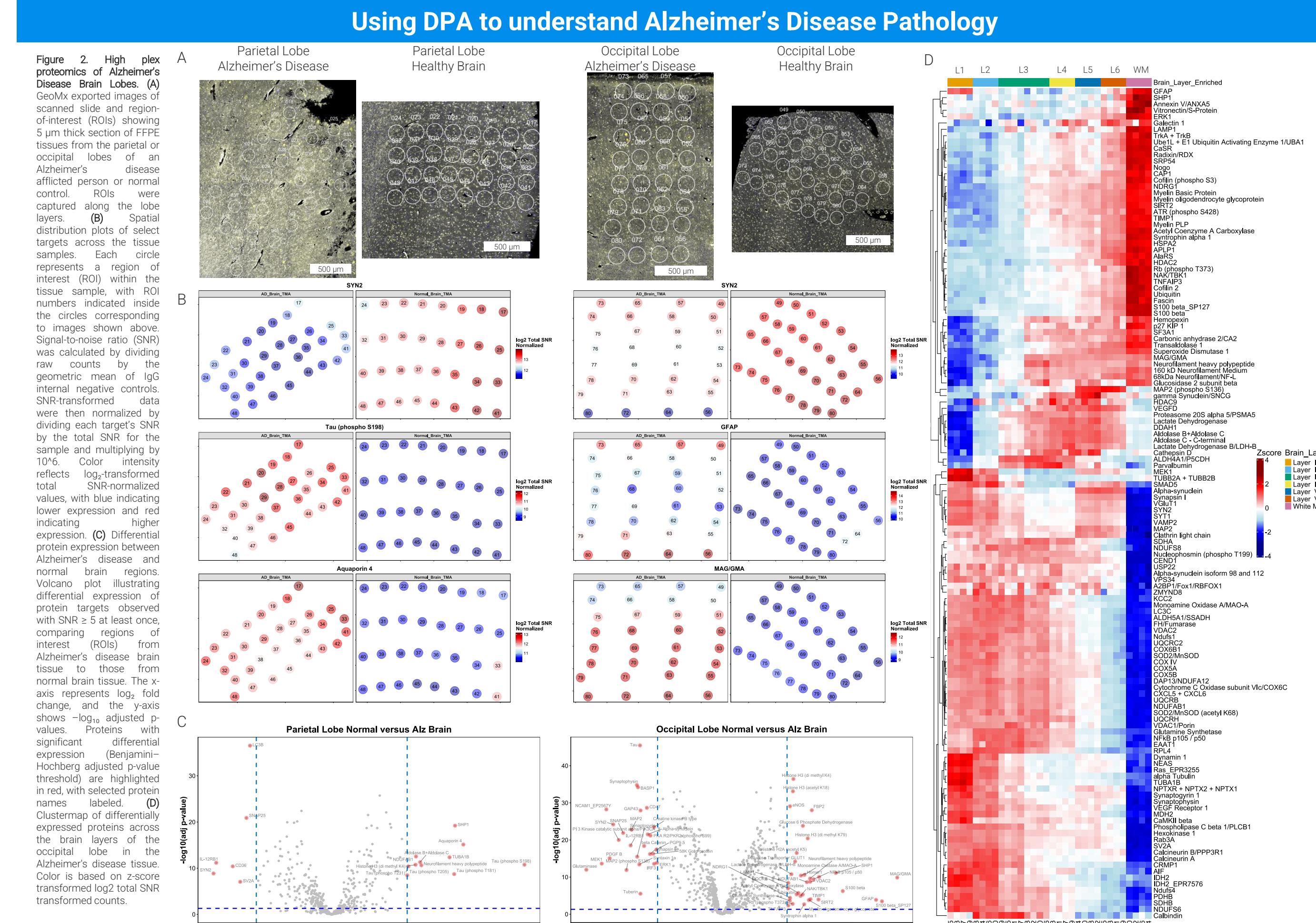


Figure 3. Differential protein expression of α-synuclein positive neurons in different regions of the thalamus from a Parkinson's disease affected patient. The volcano plot displays the differentially expressed proteins between ROIs 11, 12, and 13, highlighting a strong α-synuclein signal (magenta), compared to ROIs 15 and 16, which show weaker signal. All ROIs are 200 μm diameter circles. GeoMx exported image from the sample tissue with MAP-2 in green and DNA in grey.

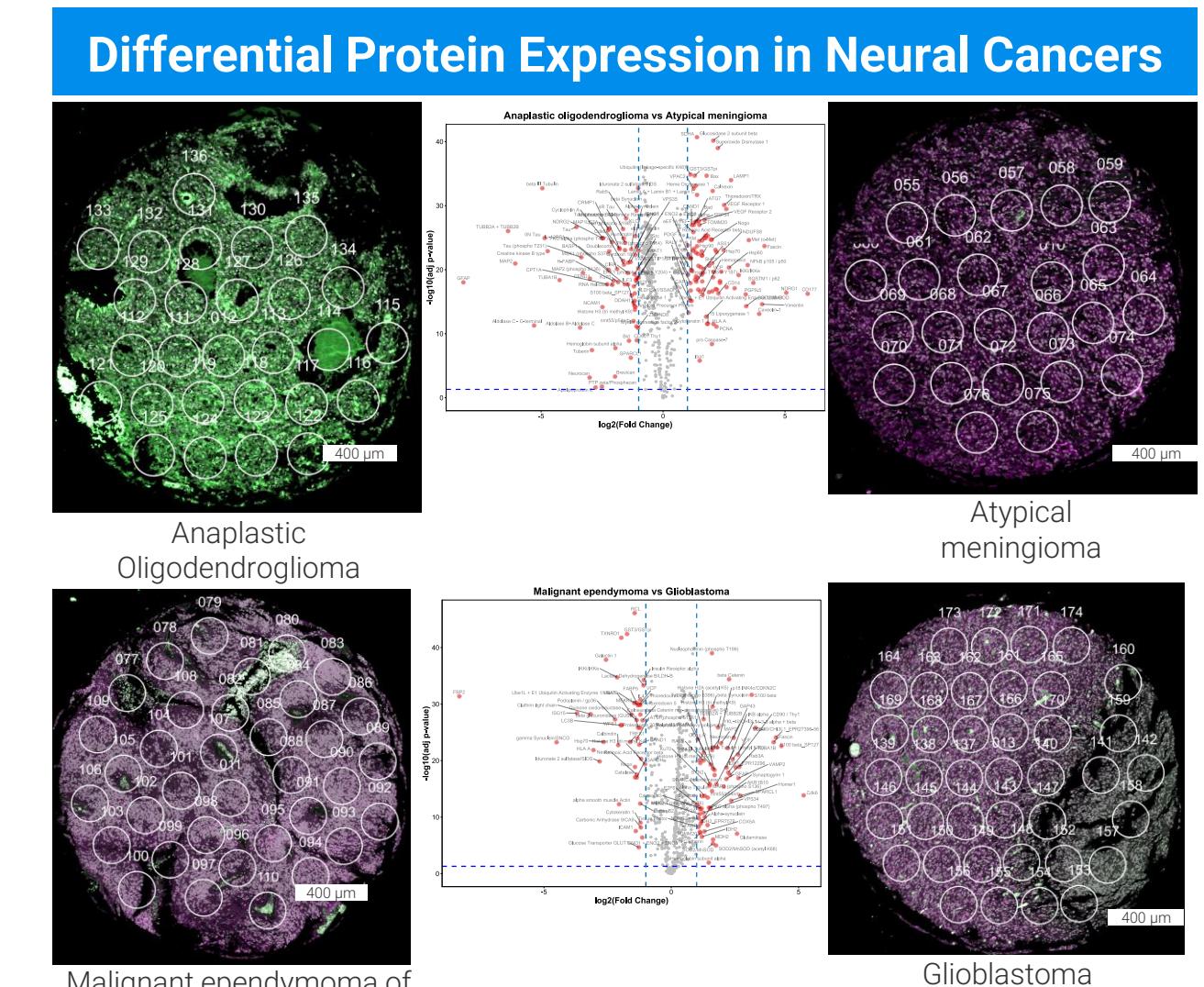


Figure 4. Expansive target list of the Discovery Proteome Atlas enables new biomarker discovery for different neuroscience applications. Comparison of 4 different types of neural cancers. Volcano plots showing the differentially expressed proteins between anaplastic oligodendrogloma and atypical meningioma tissue cores and between malignant ependymoma of the right occipital lobe and glioblastoma tissue cores. GeoMx exported images show the sample patterns of the different cancer cores.

1. Introducing GeoMx Discovery Proteome Atlas (DPA), with broad coverage over the human body to measure 1,200+ proteins and PTMs in space.
2. DPA includes the Neural Proteome Atlas, which targets 650+ proteins targets of importance for neuroscience.
3. High-throughput experimental design using GeoMx Protein Atlases is well suited for discovery applications for novel insights in Neuroscience.

