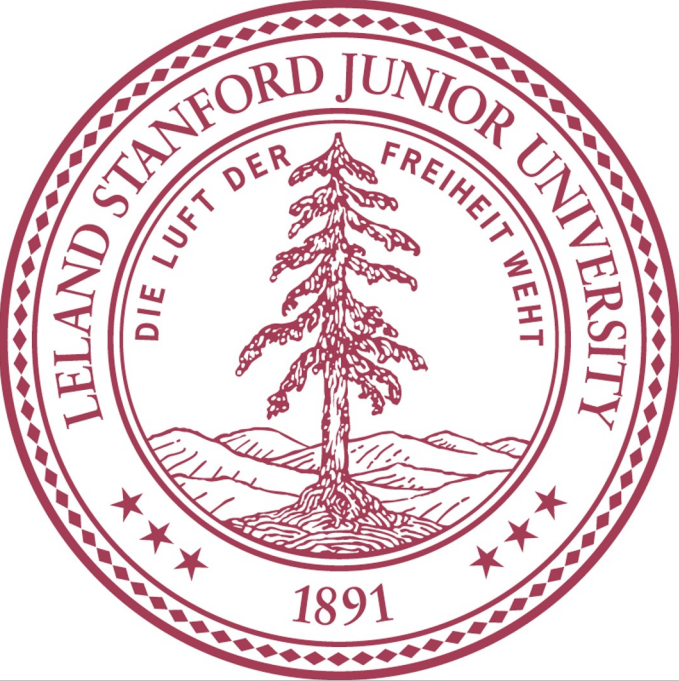




High Throughput Single Pre-synapse Multiplex Proteomics in Alzheimer's Disease

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Abstract

Synaptic molecular characterization at scale remains challenging in the human brain. We developed a mass cytometry–based method, **SynTOF (synaptometry by time of flight)**, to measure several dozen antibody probes across **>20 million single-synapse events** from human brains (healthy, Alzheimer's disease (AD), Lewy body disease (LBD), nonhuman primates (NHPs)), and PS/APP mice. Synaptic molecular integrity was comparable between human samples with short post-mortem interval (PMI) and NHPs with no PMI. As an internal control, human synapses from LBD samples showed reduced caudate dopamine transporter. While Aβ was not detected in human synapses, it was present in PS/APP transgenic mouse single-synapse events. In contrast, human synapses in AD exhibited increased hippocampal pathologic tau, elevated hippocampal CD47, reduced DJ1, and, in AD with dementia, higher ApoE levels. These findings were independently validated using multiplex ion beam imaging of brain sections. Using a recently developed, modified SynTOF probe set designed to target metabolic endpoints across multiple brain regions, our preliminary data showed that elastic net models reliably distinguished controls from AD. In contrast, discrimination between AD and resilient individuals was weaker, driven by opposing regulation of metabolic, synaptic, immune, and protein-aggregation pathways rather than unique markers, highlighting substantial pre-synaptic molecular overlap between AD dementia and resilience to AD. Our work demonstrates that large-scale, high-dimensional, single-synapse profiling can uncover novel molecular insights into human neurodegenerative diseases and may inform future studies of resistant and resilience states.

Highly multiplexed screening: 351 phenotypic and 82 metabolic markers

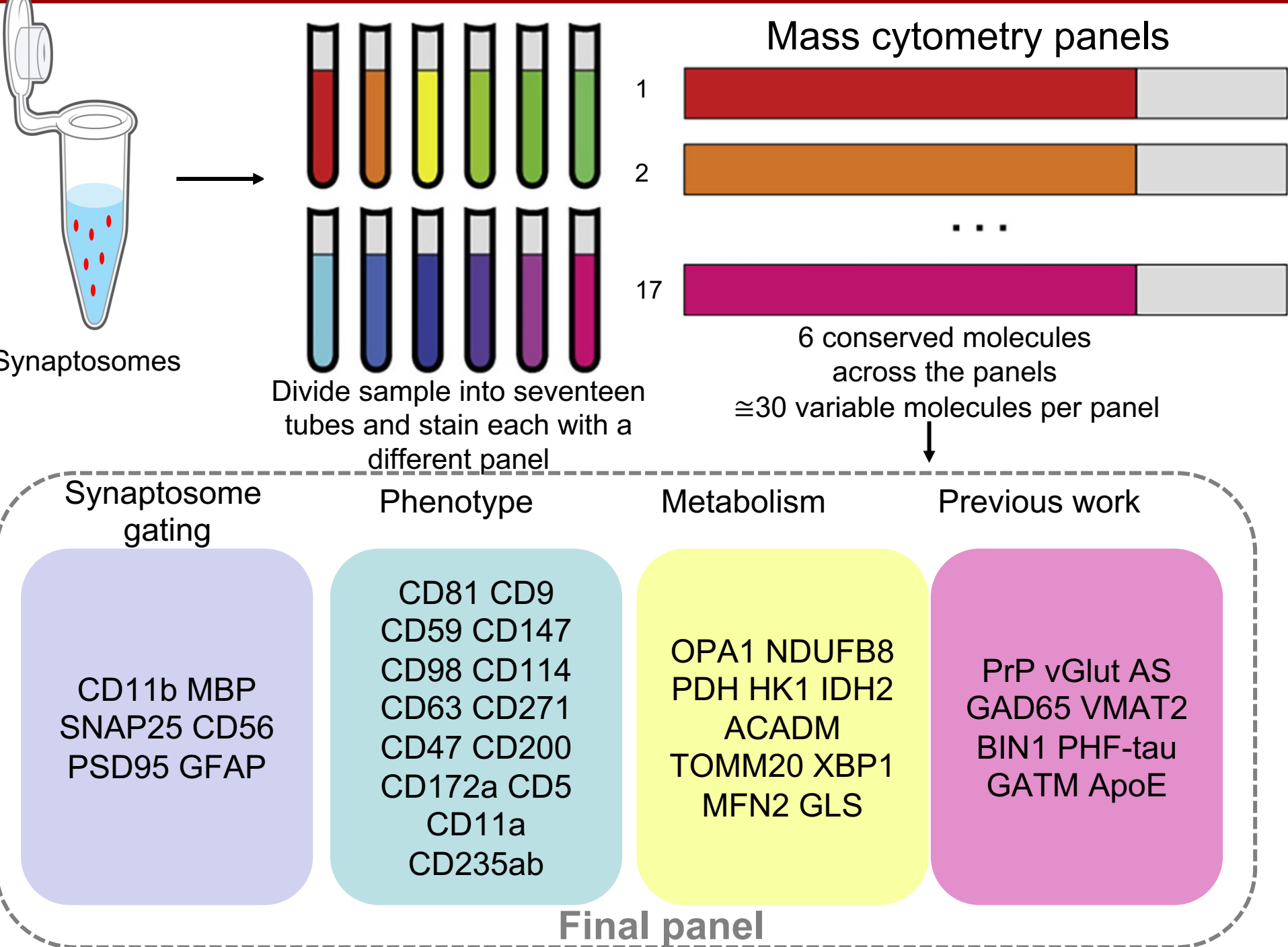


Figure 1. We used pooled human brain synaptosomes across multiple regions and donors. Antigens were classified as present or absent using a stringent threshold. Positive markers were validated against brain-tissue-associated literature.

ElasticNet and UMAP

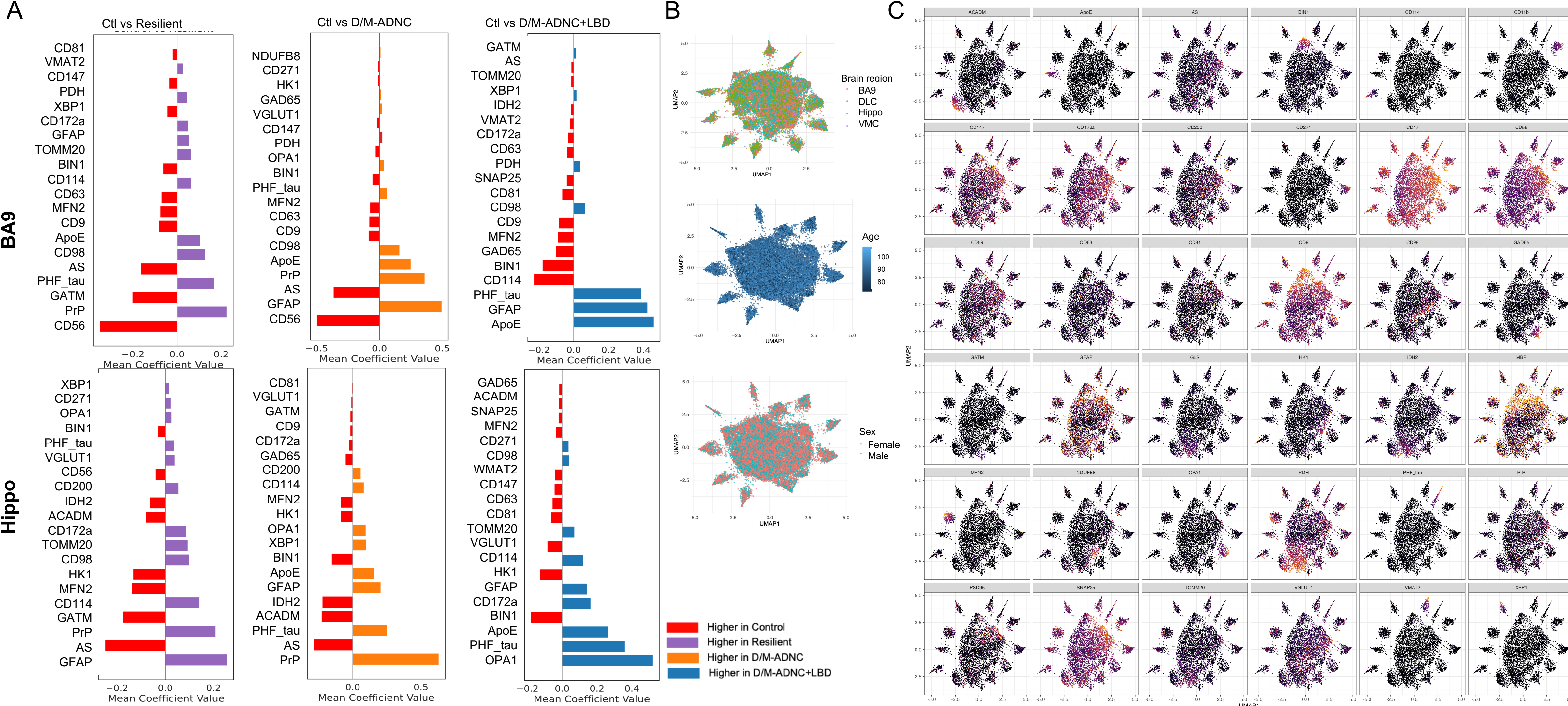
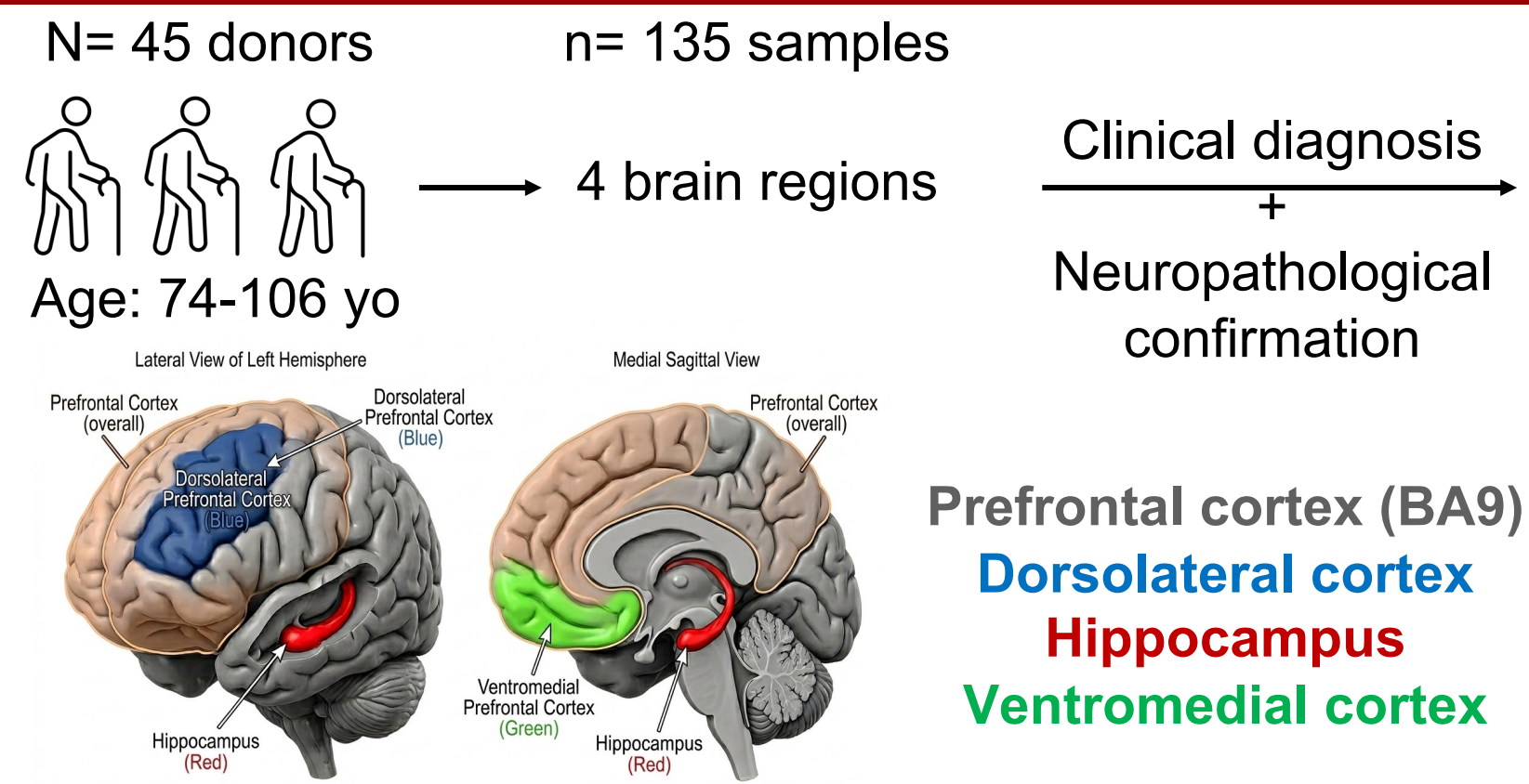


Figure 3. Differential synaptosome protein expression profiles and high-dimensional clustering across brain regions and neurodegenerative disease states. **(A)** Tornado plots displaying mean coefficient values from an ElasticNet predictive model identifying proteins that best distinguish neurodegenerative disease states from healthy controls in Brodmann Area 9 (BA9) and the Hippocampus (Hippo). **(B)** UMAP embedding displaying the multidimensional clustering of single synaptosomes across color-coded to evaluate brain region, age, or sex cohorts. **(C)** Faceted UMAP plots showing the single-synaptosome expression profiles and structural localization of individual surface, synaptic, and metabolic markers.

Post-Mortem Alzheimer's Disease cohort



Brain region	Control	D/M-ADNC	D/M-ADNC+LBD	Resilient-ADNC	D/M-LBD	Resilient-LBD
BA9	6	15	11	6	2	1
DLC	2	15	10	6	2	1
Hippo	6	15	11	6	2	1
VMC	4		1			

Figure 2. All procedures were performed in accordance with IRB protocols and informed consent was obtained for all donors. Mean Post-Mortem Interval (PMI) was 4.82± 1.37 hours.

Conclusions

- The synaptosome proteome responds in a region-specific manner—the cortex (BA9) and the hippocampus experience distinct pathological strains and survival responses to the exact same disease classifications.
- Discrimination between AD and resilient individuals was weaker, driven by opposing regulation of metabolic, synaptic, immune, and protein-aggregation pathways rather than unique markers, highlighting substantial pre-synaptic molecular overlap between AD dementia and resilience to AD.
- OPA1 (mitochondrial fusion) is heavily upregulated in the Severe Mixed Pathology (D/M-ADNC+LBD), indicating profound mitochondrial, structural, and inflammatory shifts when Lewy body dementia is co-present with Alzheimer's.