

Proteomic Mapping of the Amyloid- β Interactome in Down Syndrome Offers Insights into Alzheimer's Disease Pathways

Mitchell Marta Ariza, PhD¹, Lauren C Tang, PhD¹, Manon Thierry, PhD^{1,2}, Dominique Leitner, PhD¹, Beatrix Ueberheide, PhD¹ and Thomas Wisniewski, MD¹

1 NYU Grossman School of Medicine, New York, NY, USA
2 Institut du Cerveau (Paris Brain Institute), Sorbonne Université/CNRS UMR 7225/Inserm U1127/APHP, Hôpital de la Pitié-Salpêtrière, Paris, France.



Mitchell Martá-Ariza, PhD
Associate Research Scientist
at the Alzheimer's Disease Res...



INTRODUCTION

Down syndrome (DS) provides an important human context for studying Alzheimer's disease (AD), as chromosome 21 triplication and *APP* overexpression drive early age-associated amyloid- β (A β) deposition and AD-related neuropathology. Although A β plaques are one defining feature of AD, their protein environment extends beyond A β and may influence plaque biology and disease progression. Previously, we characterized the A β plaque proteome in DS and AD, identifying a conserved plaque-associated protein signature linked to APP metabolism, lysosomal pathways, and immune responses.

Here, we extend this work by defining the A β interactome in DS using A β immunoprecipitation followed by mass spectrometry, and by comparing candidate A β -associated proteins with proteins detected in amyloid plaques and cerebral amyloid angiopathy (CAA).

METHOD

Frozen frontal cortex tissue from adults with DS and confirmed AD neuropathology was homogenized and incubated with the MOAB-2 antibody for A β immunoprecipitation, with IgG samples used as controls. Immunoprecipitated protein complexes were analyzed by mass spectrometry (MS) to identify proteins associated with A β -containing fractions. Candidate A β -associated proteins were prioritized based on enrichment relative to IgG controls and SAINT probability scoring. Functional characterization was performed using Gene Ontology over-representation analysis and protein-protein interaction network analysis. Proteomic findings were further compared with previously generated A β plaque and cerebral amyloid angiopathy proteomic datasets, and MDK protein was evaluated by immunohistochemistry.

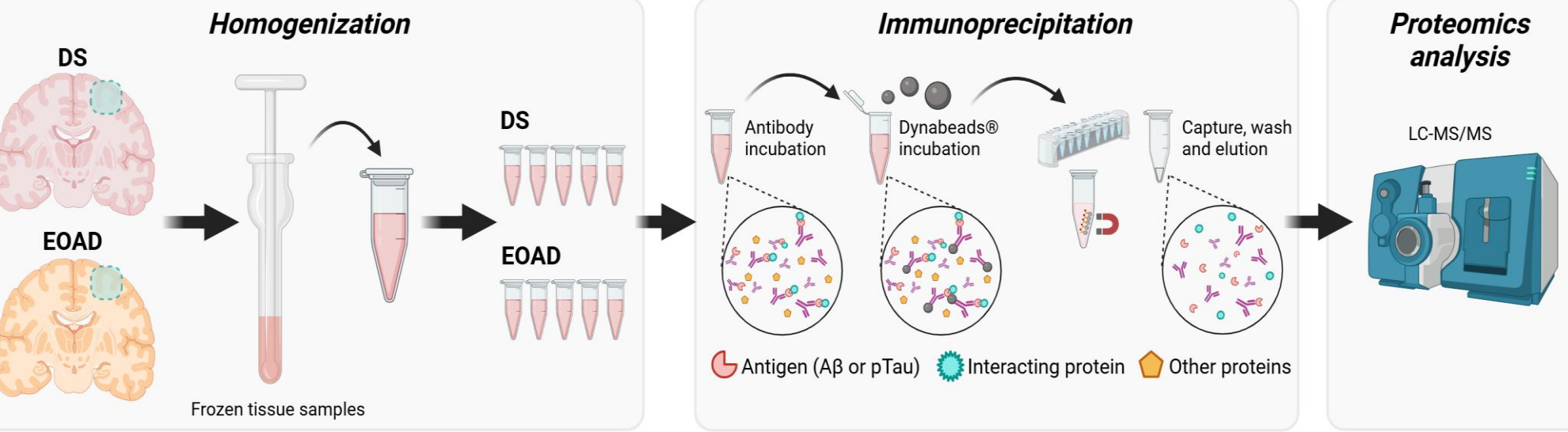


Figure 1. A β immunoprecipitation. Frozen brain samples from individuals with DSAD (n=8) were homogenized and incubated with MOAB-2 antibody against A β peptides, followed by mass spectrometry for protein quantification. Figure created in BioRender.com.

RESULTS

A β immunoprecipitation enriches A β and candidate A β -associated proteins.

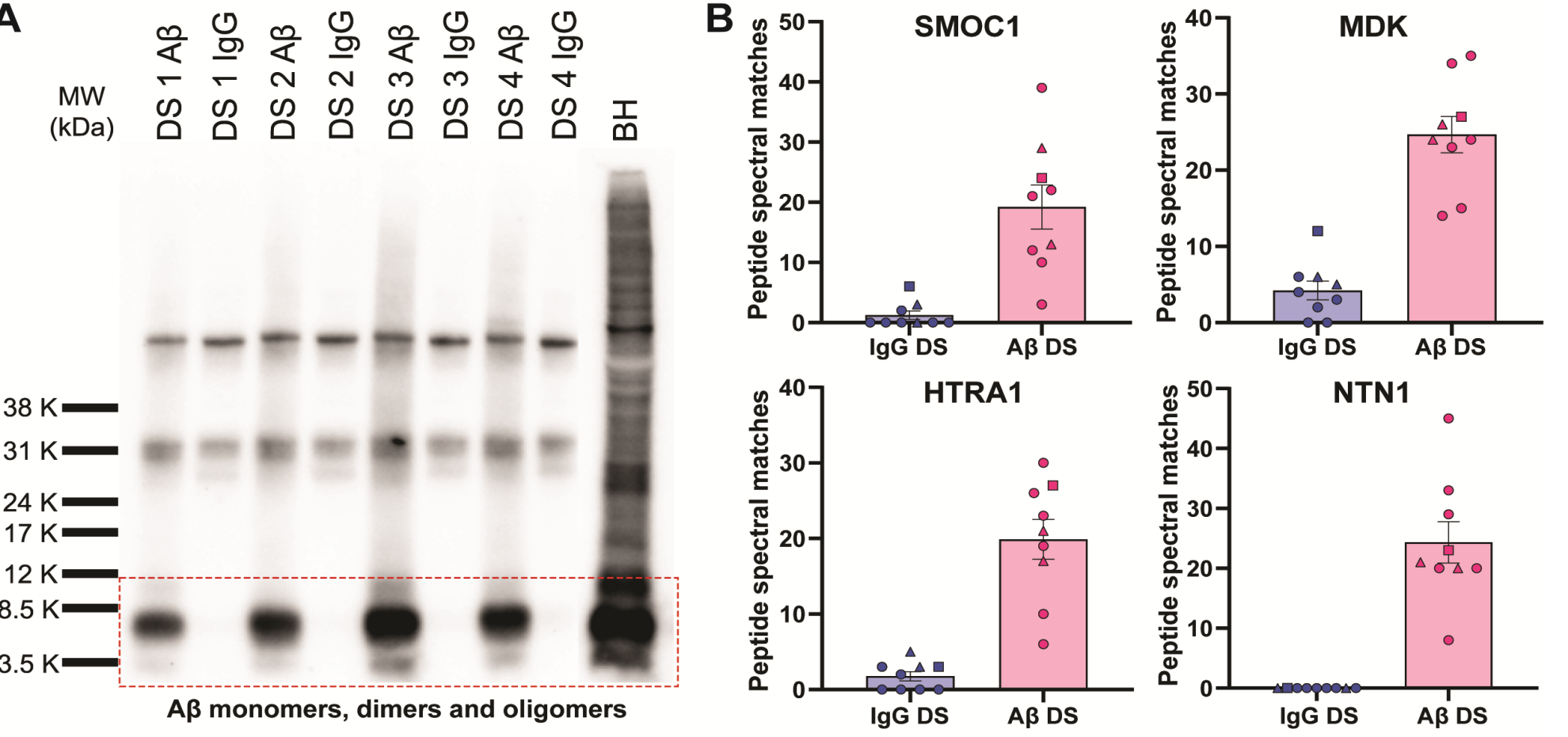


Figure 2. A. Representative western blot of A β IP and IgG control samples from four DS cases, with one brain homogenate lane included as a non-IP reference. Red dotted box indicates the approximate molecular weight corresponding to A β species. **B.** Peptide spectral matches for selected proteins of interest identified by MS in IgG control and A β IP samples, supporting their enrichment in A β IP fractions relative to IgG controls.

RESULTS

A β interactome profiling identifies candidate A β -associated proteins linked to neuronal signaling, synaptic maintenance, ATP synthesis and complement activation.

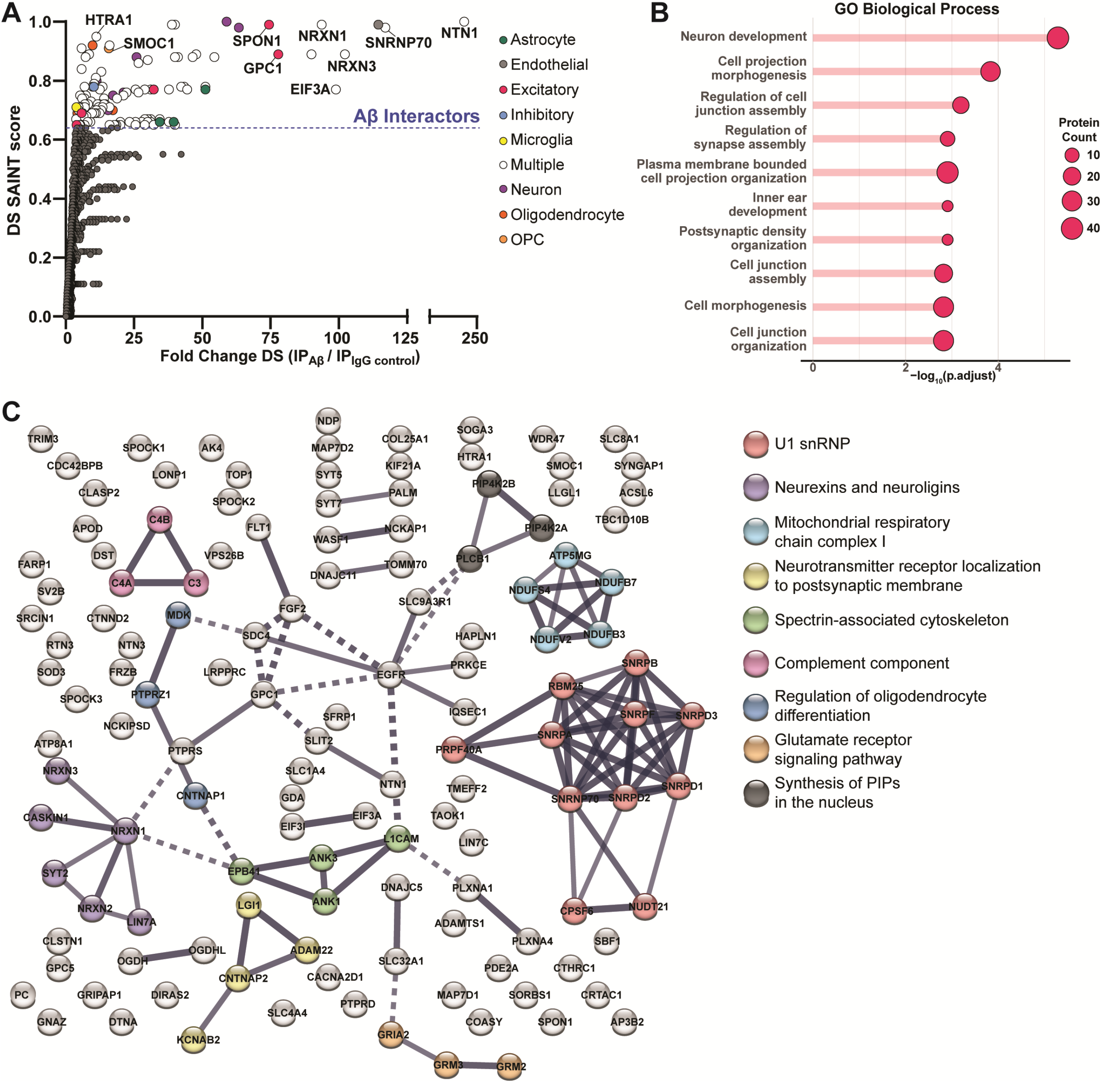


Figure 3. Functional characterization of probable A β interactors. **A.** A β IP-MS identified probable A β interactors based on fold change relative to IgG controls and SAINT score ≥ 0.65 . **B.** GO biological process analysis of probable A β interactors revealed top enriched pathways related to neuronal development, cell projection morphogenesis, cell junction assembly, and synapse assembly * $p < 0.05$. **C.** STRING v12.0 protein-protein interaction network analysis was performed using high-confidence interactions (score ≥ 0.700) and MCL clustering (inflation = 2.5), identifying clusters related to small nuclear ribonucleoprotein complexes, neuroligin/neuroligin-mediated synaptic maintenance, ATP synthesis, neurotransmission, cell clearance, and complement activation.

MDK localizes to amyloid plaques and CAA in DS brain tissue.

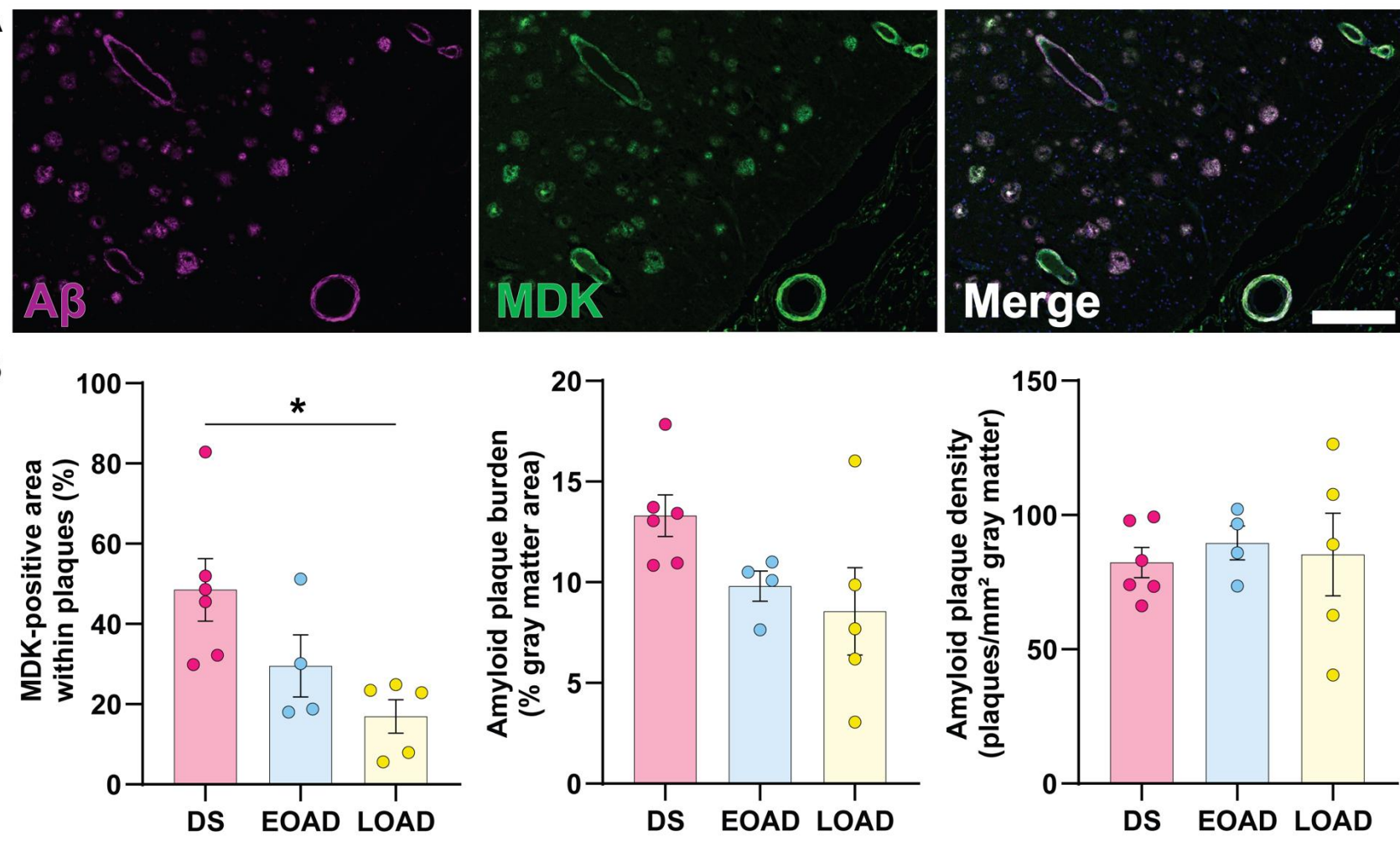


Figure 4. Preliminary MDK immunohistochemical validation in DS. **A.** Representative DS brain tissue images showing A β , MDK, and merged staining. Scale bar 200 μ m. **B.** Quantification of MDK-positive area within plaques, amyloid plaque burden, and amyloid plaque density across DS, EOAD, and LOAD cases. MDK-positive plaque area was significantly higher in DS compared with LOAD, while amyloid plaque burden and plaque density did not differ between cohorts. * $p < 0.05$.

RESULTS

A β interactome proteins overlap with amyloid plaque and CAA proteomic signatures.

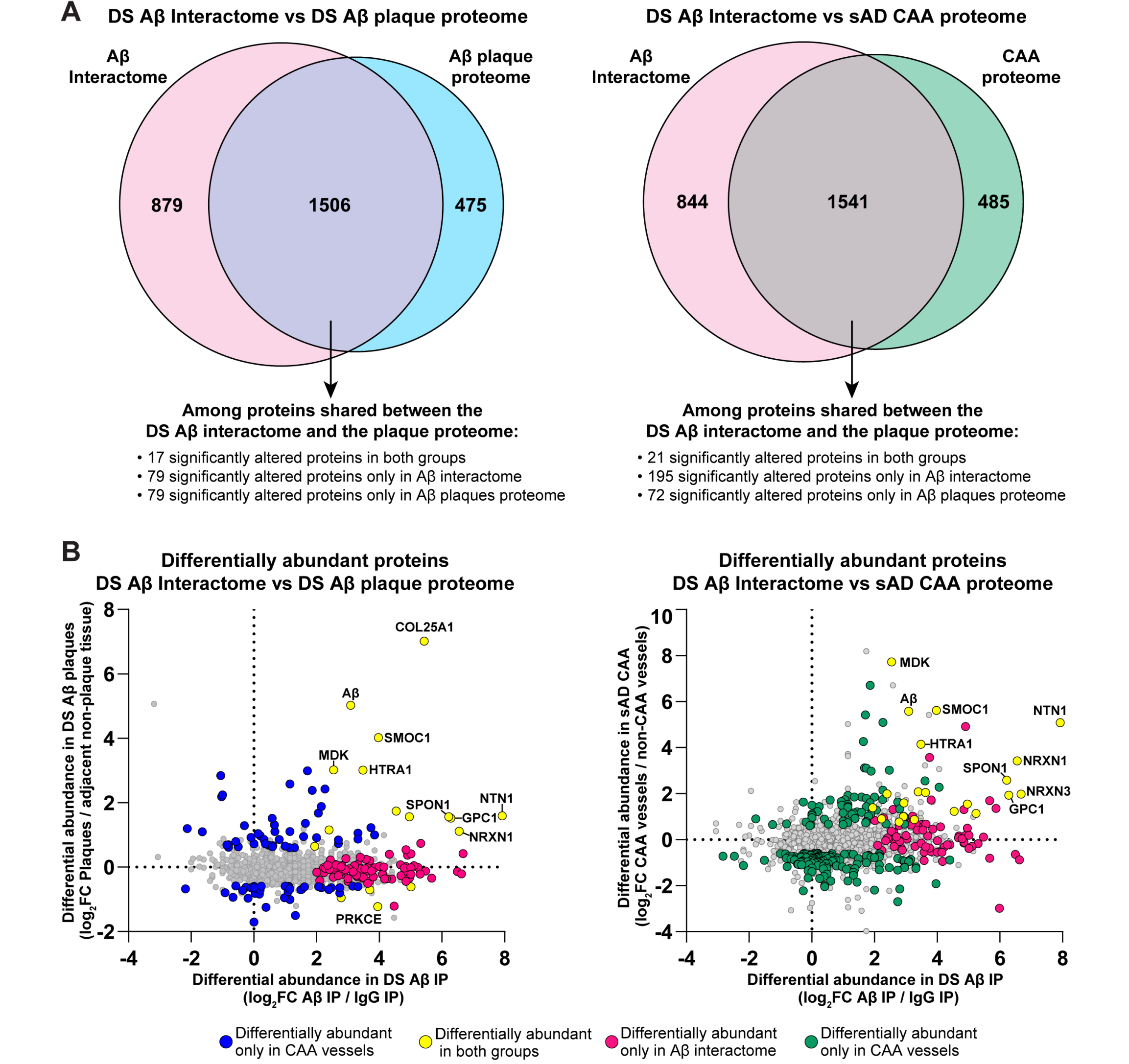


Figure 5. Comparison of A β interactome, amyloid plaque, and CAA proteomes. **A.** Venn/Euler diagrams showing overlap between proteins identified in the DS A β interactome and proteins previously identified in DS amyloid plaques or sporadic AD CAA. Shared proteins were used for the comparisons in panel B. **B.** Scatter plots comparing differential abundance of significantly altered proteins identified in the DS A β interactome (SAINT score ≥ 0.65) with differential abundance of proteins identified in amyloid plaques or CAA proteomics studies (FDR $< 5\%$, FC > 1.5). Proteins differentially abundant in both datasets are highlighted in yellow, while proteins significant only in the A β interactome or only in the lesion proteome are shown separately. Several candidate A β -associated proteins, including MDK, HTRA1, SMOC1, SPON1, GPC1, NTN1, and NRXN1, showed overlap with plaque and/or CAA proteomic signatures, suggesting partial convergence between A β -associated biochemical complexes and amyloid lesion-associated protein changes.

CONCLUSIONS

The A β interactome in DS provides a complementary biochemical layer to localized amyloid plaque proteomics. While plaque proteomics defines the proteins enriched within the amyloid lesion microenvironment, A β immunoprecipitation identifies proteins that co-purify with A β -containing complexes. Integrating these approaches helps distinguish proteins that accumulate in plaques from those more closely associated with A β biochemically, prioritizing candidates that may contribute to amyloid-associated pathways in DS-AD.

With the DS A β interactome, we identified A β -associated proteins linked to neuronal signaling, synaptic organization, RNA-protein complexes, mitochondrial function, and complement-related pathways.

Overlap with amyloid plaque and CAA proteomic datasets suggests partial convergence between A β -associated biochemical complexes and amyloid lesion-associated protein signatures.

MDK, HTRA1, SMOC1, SPON1, GPC1, NTN1, and NRXN1 were prioritized as candidate A β -associated proteins based on A β IP enrichment and overlap with amyloid lesion proteomes.