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Supplementary information

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Plasma proteomic associations with Alzheimer's disease endophenotypes

In the format provided by the authors and unedited

Supplementary Discussion

Complement

Complement proteins deserve special attention as immune-related proteins in AD. Complement deposition in brain has been observed to be associated with both normal and aberrant synaptic pruning processes[1-4], and complement proteins may also play a role in cerebrovascular dysfunction in AD[5]. In our study, complement proteins were observed to be positively correlated between plasma and brain compartments, with co-expression preserved between brain, CSF, and plasma, and plasma proteins positively associated with cerebral Aβ strongly overlapped with brain and CSF complement modules. We also observed an association between complement in plasma and cerebral microinfarcts, as well as risk for incident cognitive impairment. It therefore seems likely that increased complement levels in plasma could serve as a risk factor for multiple pathways that lead to cognitive decline in AD. Targeting certain proteins in the complement cascade could represent an effective therapeutic approach in AD[6].

Amyloid biomarkers

Proteins measured on the SomaScan v4.1 7k platform were not able to outperform plasma pTau217 as a marker of cerebral β-amyloidosis. However, the Aβ-associated markers identified in this study could be useful additions to current amyloid, tau, and neurodegeneration (ATN) biomarker panels by providing information on other biological pathways that are affected by cerebral Aβ. These pathways may have variation across individuals due either to variation in the biochemical nature of amyloid deposits themselves or to the brain response to amyloid deposition, and therefore biomarkers of these pathways could allow for a more precision-based diagnostic approach than is possible using pTau217 alone. Such proteins could also potentially serve as therapeutic markers of downstream treatment response to Aβ-based therapies.

Lipoproteins

Not all lipoproteins mapped to the lipoprotein module, and a notable lipoprotein independent of this module was APOA4. APOA4 was positively associated with cognitive function and was also positively associated between plasma and brain compartments. APOA4 is a major component of HDL, suggesting direct communication of HDL proteins between the periphery and the CNS. Some studies have shown increased plasma HDL cholesterol (HDL-c) levels are associated with better cognitive function[7], whereas other studies have shown increased HDL-c levels are associated with increased dementia risk[8]. Additional studies to dissect the associations and mechanisms linking different protein components of lipid transport and metabolism with cognitive function are needed.

Biomarkers of Co-pathologies

Multiple studies have investigated protein markers for vascular, Lewy body, and TDP-43 co-pathologies commonly observed in AD. Plasma levels of placental growth factor (PGF) have been observed to be increased in individuals with small vessel cerebrovascular disease as assessed by white matter hyperintensities on MRI[9]. We did not observe an association between PGF and cerebrovascular lesions assessed by neuropathological examination in ROSMAP, which could be due to the different methods of vascular lesion assessment or variation between measurement techniques. As noted above, ECM proteins such as SMOC1 were positively associated with CAA, consistent with their increased levels in vessels with CAA pathology[10-12]. Matrix metalloproteinase (MMP) and tissue inhibitors of matrix metalloproteinase (TIMP) proteins have also been associated with multiple different types of cerebrovascular lesions[13,14]. We measured multiple MMPs in our study. MMP16 was nominally negatively associated with CAA, and other MMPs trended towards an association with either CAA (MMP8 and MMP12 positively associated; MMP13 and MMP16 negatively associated) or microinfarcts (MMP17 positively associated).

Increased CSF levels of MMP12 have recently been associated with white matter hyperintensities, microbleeds and infarcts, consistent with the MMP12 signal in our plasma dataset[15]. We measured TIMPs 1-4 and observed a positive association between TIMP1 levels and both microinfarcts and CAA. CSF dopamine decarboxylase (DDC) levels have previously been associated with Lewy bodies[16], although plasma levels do not seem to display the same association[17]. We also did not observe an association between plasma DDC levels and Lewy bodies. However, we did observe a negative association between peptidyl-glycine alpha-amidating monooxygenase (PAM) levels in plasma and Lewy bodies, consistent with a negative association with Lewy bodies previously observed for this protein in CSF[16]. Finally, hepatoma-derived growth factor-related protein 2 (HDGFL2) in CSF has been identified as a potential biomarker for TDP-43 pathology[18,19]. We observed a negative association of this protein with TDP-43 levels in brain, suggesting that plasma levels of HDGFL2 may also serve as a biomarker for TDP-43.

Cohort differences

The cohorts analyzed in this study were different in several ways that could have influenced the results. BioHermes reflected a real-world population with racial and ethnic diversity roughly matching U.S. population demographics, and enrollment was performed at clinical trial sites using procedures similar to those used in AD therapeutic studies. ROSMAP was older (average age 84 compared to approximately age 70 in the other cohorts), mostly female (72%), and nearly all white (97%), whereas the Emory cohorts reflected a population enrolled from a clinical site and had more racial diversity (20-30% African American). Because BioHermes was approximately twice the size as Emory GADRC or ROSMAP, any unique characteristics of the enrolled participants or the amyloid assessment method (PET) in this cohort would have been weighted more heavily in the meta-analysis results. One such difference in BioHermes was the relatively large proportion of participants who had cognitive impairment and negative amyloid PET scans,

indicating the likely presence of other neuropathologies leading to cognitive impairment. Despite these differences, the proteins most strongly associated with cerebral Aβ were found to be similar across cohorts except for ROSMAP, where the associations were overall weaker compared to the other cohorts and had less overlap. It is possible the associations in ROSMAP were significantly influenced by the method of amyloid assessment (neuropathology), but this seems less likely and because we adjusted for age, sex, and race as needed in the analyses, it seems more likely that other factors such as pre-analytical variation may have influenced the associations with cerebral Aβ in this cohort. BioHermes and Emory GADRC were also most similar in associations with cognitive function, whereas ROSMAP and Emory Other had less overlap. Despite these cohort differences, a strength of the analysis was integration of information across cohorts and endophenotype assessment methods in meta-analyses to identify proteins robust to different sources of variation.

Limitations

We could not adjust for kidney function, which when severely compromised can affect plasma levels of pTau217. However, a very small fraction (0.6%) of the Emory GADRC cohort had kidney disease reported in the National Alzheimer's Coordinating Center Uniform Data Set, and the Bio-Hermes cohort is a community-based population that has clinical characteristics similar to those enrolled in clinical trials of disease-modifying treatments which typically exclude individuals with severe kidney disease. Although it is unlikely that our results were significantly influenced by kidney disease, future studies will ideally adjust for this potential confound. Plasma is an inherently complex matrix that is significantly influenced by pre-analytical factors. Even though samples were collected under different protocols, the scale and multi-cohort nature of our study increases confidence that the protein associations reported are not confounded by technical variance. Development of additional analytical methods beyond the ones we describe in this study

that adjust for pre-analytical variance will help to increase statistical power to observe protein-trait associations. Another caveat is the time delay between plasma sampling and autopsy in the ROSMAP cohort. Although we adjusted for this potential confound in our models and it did not appear to have a large effect for most proteins, future studies that analyze plasma more proximate to autopsy in other cohorts will be needed to validate our findings on associations between blood and brain proteins. Multiple proteomic platform measurements, with blood and brain proteomes potentially measured on the same platform, and brain proteomes obtained from multiple brain regions would also be helpful to assess and validate blood-to-brain associations. In addition, future proteomic studies that explicitly incorporate post-translationally modified (PTM) forms of proteins may also yield additional protein isoforms strongly associated with AD endophenotypes, with pTau217 as a prime example.

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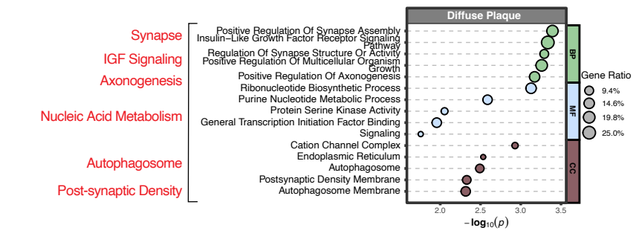
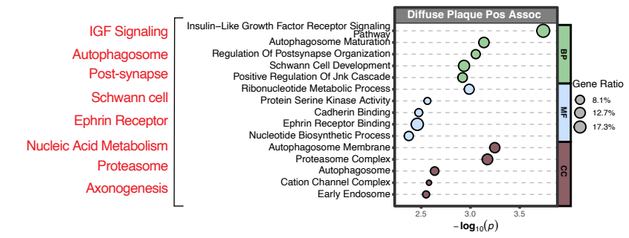
All Associations

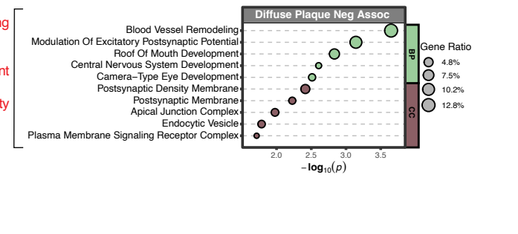
Positive Associations

Negative Associations

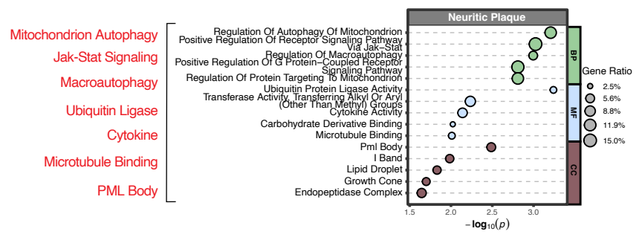
Diffuse Plaque

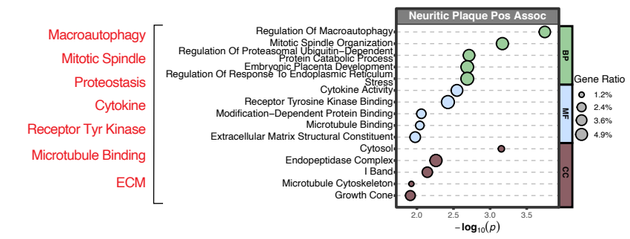


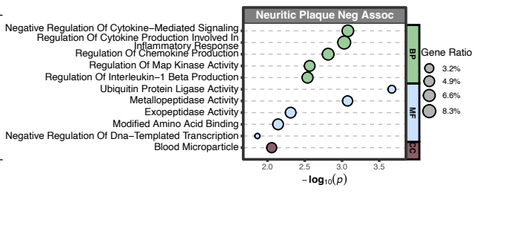




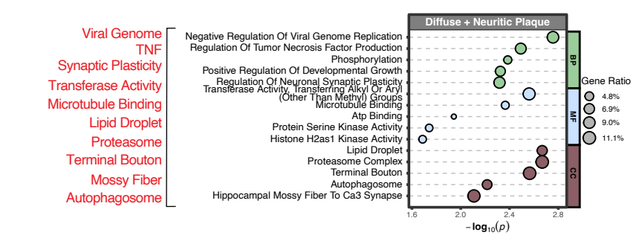
Neuritic Plaque

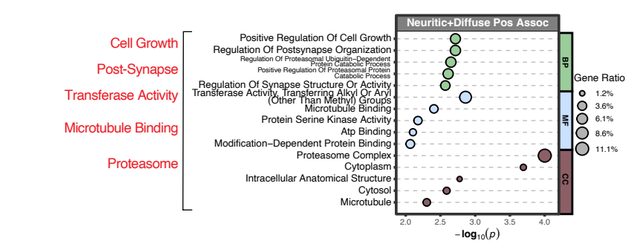


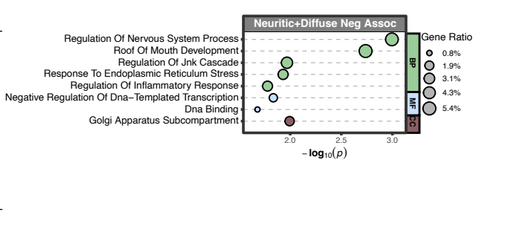




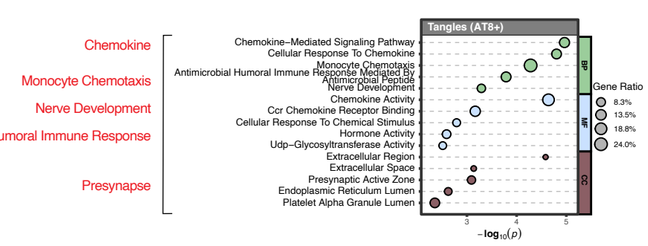
Diffuse + Neuritic Plaque

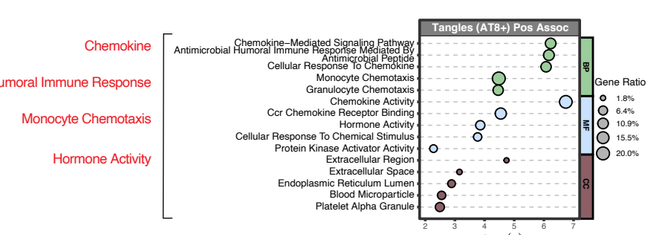


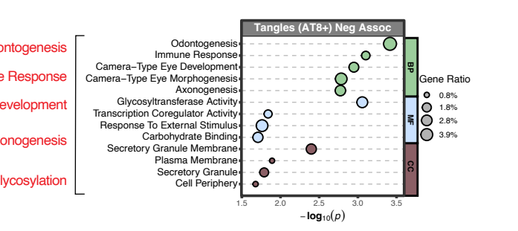




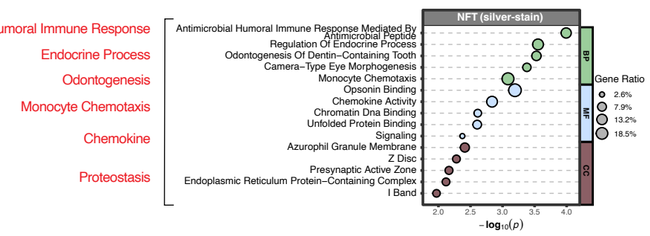
Tangles (AT8+)

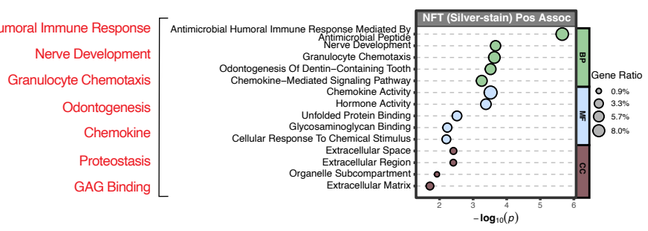


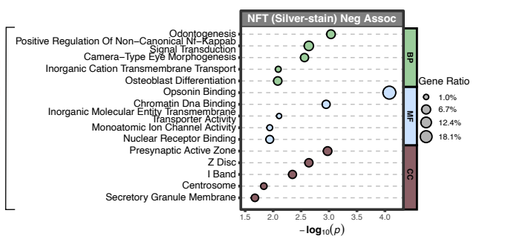




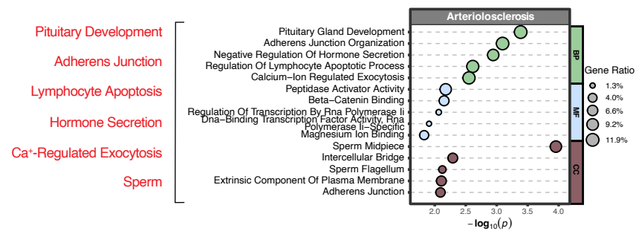
NFT (Silver-stain)

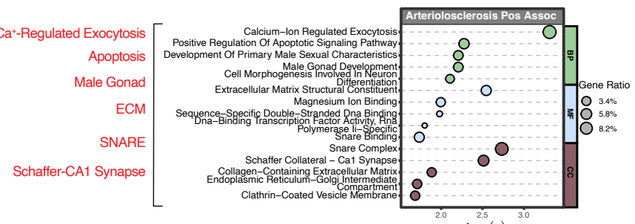


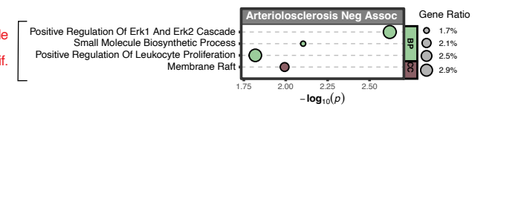




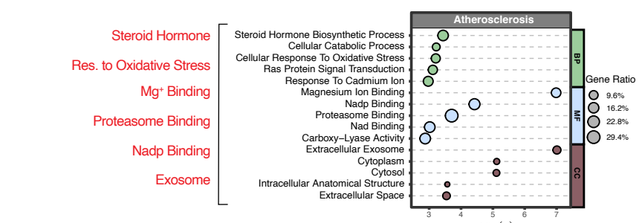
Arteriolosclerosis

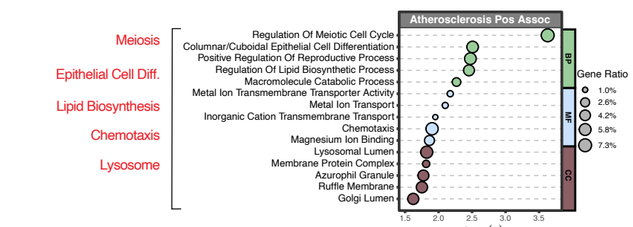


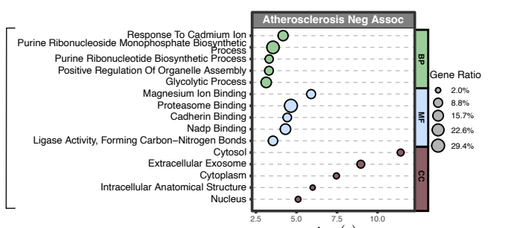




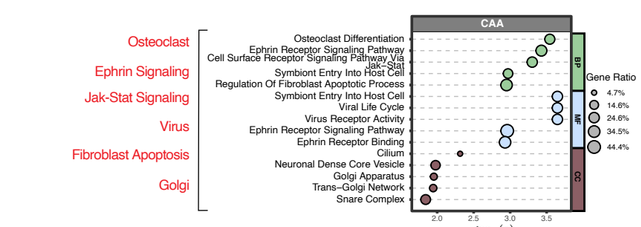
Atherosclerosis

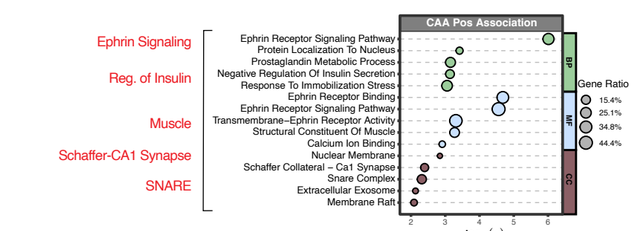


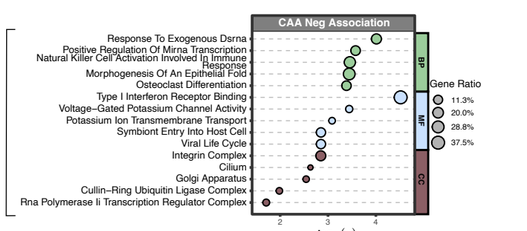




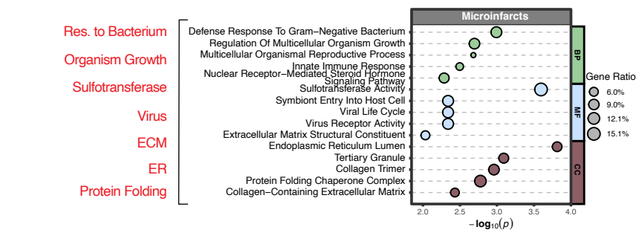
CAA

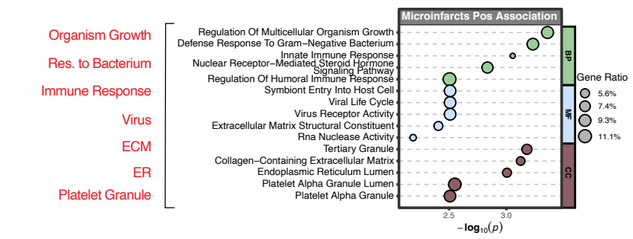


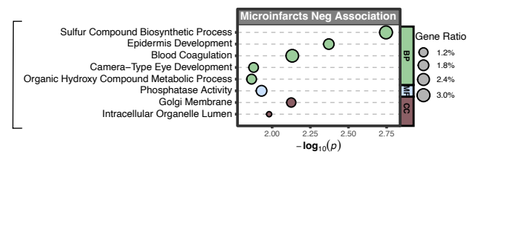




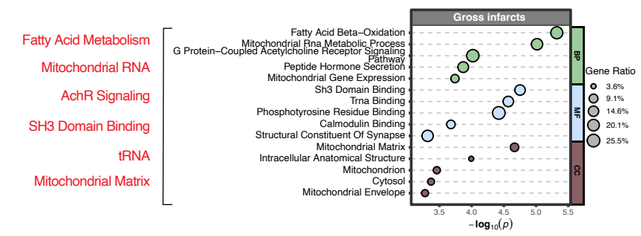
Microinfarcts

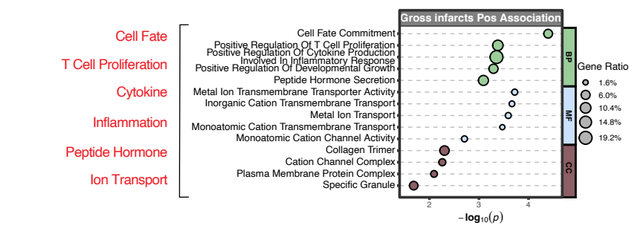


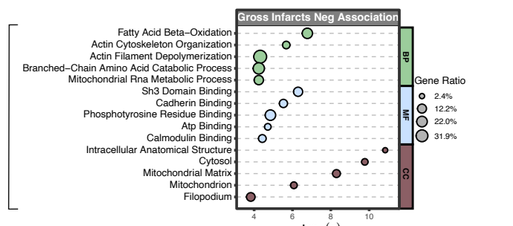




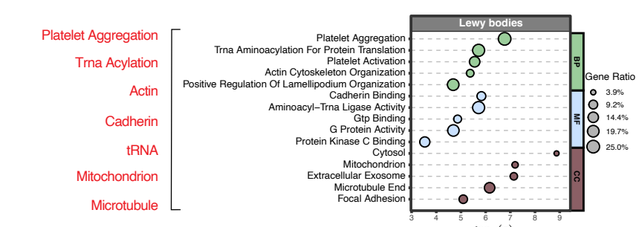
Gross Infarcts

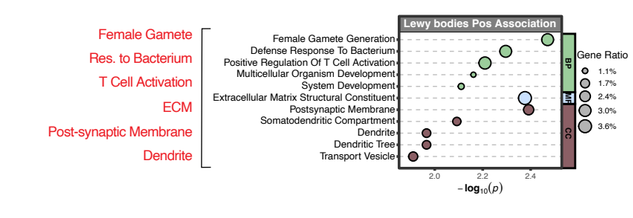


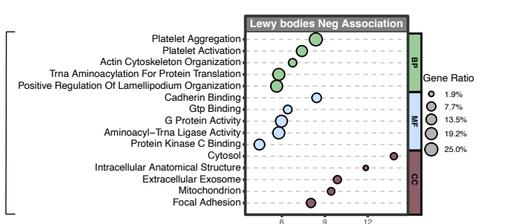




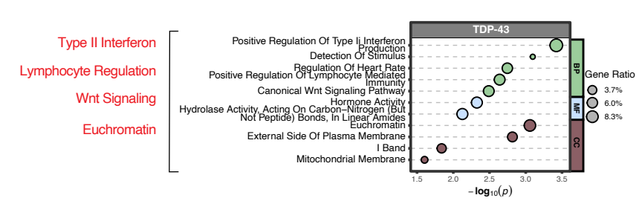
Lewy bodies

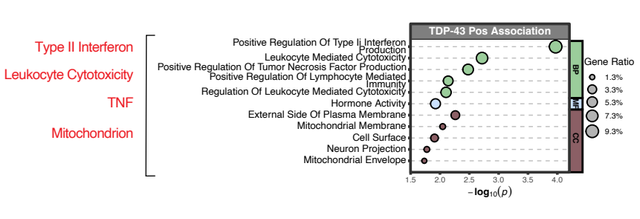


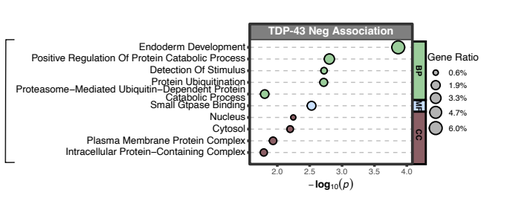




TDP-43







Supplementary Figure 1. Biological Pathways Associated with Neuropathologies in ROSMAP. Gene ontology analysis of proteins associated with different neuropathologies in the ROSMAP cohort in both positive and negative directions (all associations, left) or either positively (middle) or negatively (right), highlighting biological pathway (BP), molecular function (MF), and cellular compartment (CC) terms. A summary of the terms is provided to the left in red.