

Comprehensive And Unbiased Plasma Proteomics With Proteograph: Uncovering Novel Protein Candidates For Mild Cognitive Impairment

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Mild cognitive impairment (MCI) is a precursor to Alzheimer’s disease (AD), underscoring the need for reliable biomarkers for early identification. Here, we employed an automated nanoparticle-enrichment sample preparation platform combined with liquid chromatography–tandem mass spectrometry (LC–MS/MS) to facilitate comprehensive and unbiased detection of low-abundance proteins (LAPs). Through profiling of plasma proteomes from 42 individuals with MCI and 13 cognitively normal controls (NC), we identified 35 differentially expressed proteins (DEPs), of which four prioritized candidates were significantly correlated with Mini-Mental State Examination (MMSE) scores. Composite panels of candidate proteins showed potential to discriminate MCI, *APOE4*-carrier MCI, and amnesic MCI subtypes cases from NC, potentially providing insight into pathophysiological mechanisms underlying cognitive impairment. **These findings highlight novel blood-based protein candidates uncovered by overcoming key limitations of conventional plasma proteomics, with potential to support MCI diagnosis.**

INTRODUCTION

METHODS

RESULTS

CONCLUSION

Mild cognitive impairment (MCI), a prodromal stage of Alzheimer’s disease (AD), requires scalable early detection beyond neuropsychological testing and invasive or costly imaging and cerebrospinal fluid assays. Blood-based biomarkers are promising, but conventional plasma proteomics is limited by the extreme dynamic range of blood proteins, which hinders significant signals from low-abundance. Proteograph™, a nanoparticle-enabled automated sample-processing platform coupled with LC–MS/MS, expands plasma proteome coverage to support discovery of protein candidates for MCI detection and subtype stratification.

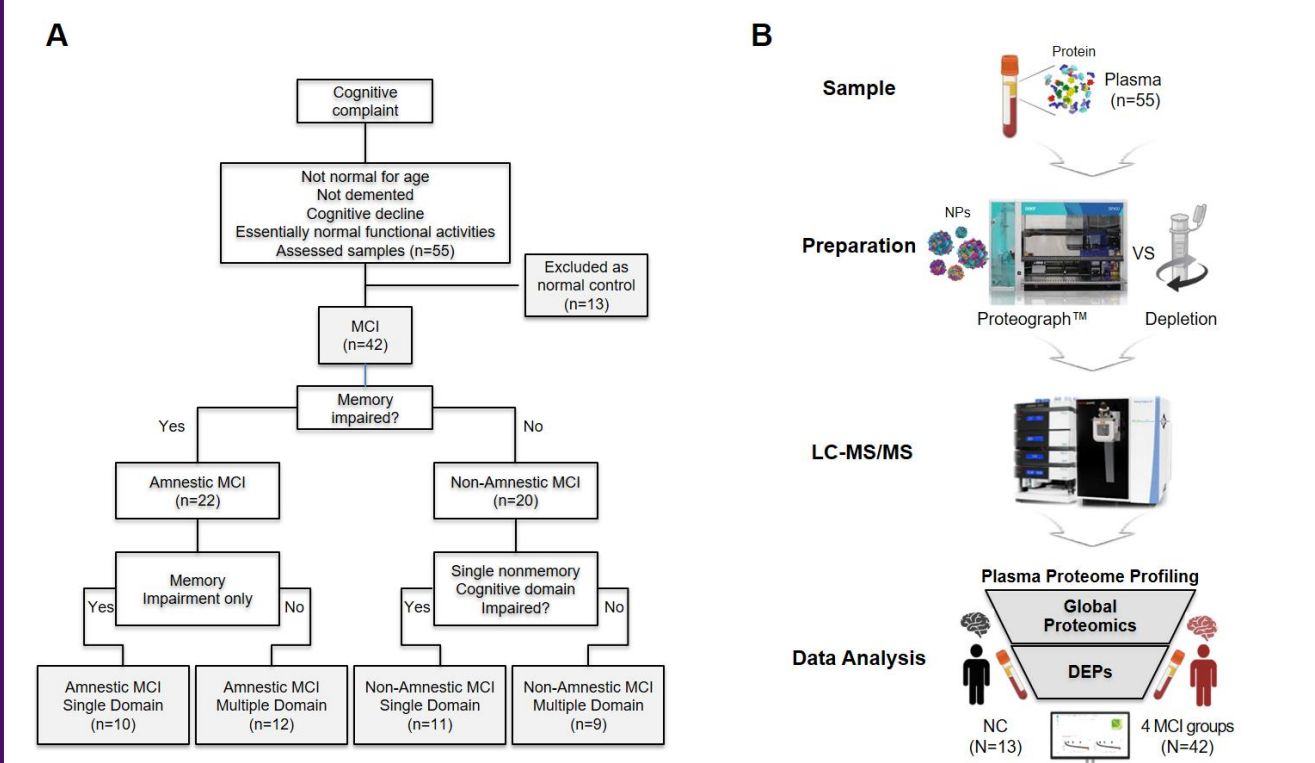


Figure 1. MCI patients classification, study design and proteomics workflow. (A) Flowchart of MCI-subtype decision process for diagnosis of participants. (B) Proteograph-based proteomics workflow for MCI cohort.

Characteristics	Total (n=55)	NC (n=13)	MCI					P Value ¹	P Value ²	P Value ³
			All (n=42)	amMCI (n=22)	asMCI (n=10)	naMCI (n=10)	naMCI (n=10)			
Age (year), mean ± SD	75.3 ± 7.71	74.1 ± 7.16	75.6 ± 7.92	77.0 ± 7.94	74.0 ± 10	76.2 ± 5.07	75.1 ± 8.47	0.515	0.866	0.840
Sex, n (%)										
Male	34 (100%)	7 (21%)	27 (79%)	9 (29%)	3 (30%)	7 (21%)	7 (21%)	0.726	0.240	0.163
Female	21 (100%)	6 (22%)	15 (71%)	3 (14%)	2 (10%)	6 (29%)	4 (19%)			
Education (year), mean ± SD	7.28 ± 4.9	8.1 ± 4.39	6.8 ± 4.91	5.5 ± 6.3	9.2 ± 3.77	4.8 ± 4.12	7.5 ± 4.11	0.204	0.112	0.171
<i>APOE4</i> , n (%)	10 (100%)	2 (20%)	8 (80%)	1 (10%)	3 (30%)	1 (10%)	0.765	0.457	0.331	
MMSE, mean ± SD	22.9 ± 4.27	25.1 ± 3.09	22.2 ± 4.38	20.8 ± 5.51	23.8 ± 3.33	20.7 ± 3.71	23.6 ± 3.85	0.014	0.040	0.177

Table 1. Demographic and clinical characteristics of subjects.

Plasma from 55 participants (42 MCI; 13 controls) were processed using Proteograph™ (five nanoparticles; automated reduction / alkylation / digestion / cleanup) and profiled by high-resolution data-independent acquisition (DIA) LC–MS/MS, with performance compared against top-14 depletion plus filter-aided sample preparation. Proteins were quantified using DIA-NN (library-free; 1% false discovery rate; match-between-runs). Differentially expressed proteins (DEPs) were identified by two-sample t-tests ($P < 0.05$; fold change ≥ 1.5), followed by Gene Ontology (GO) / Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. Candidates were prioritized by Spearman correlation with MMSE and evaluated using receiver operating characteristic (ROC) analyses of single proteins and composite panels for MCI and subtype classification.

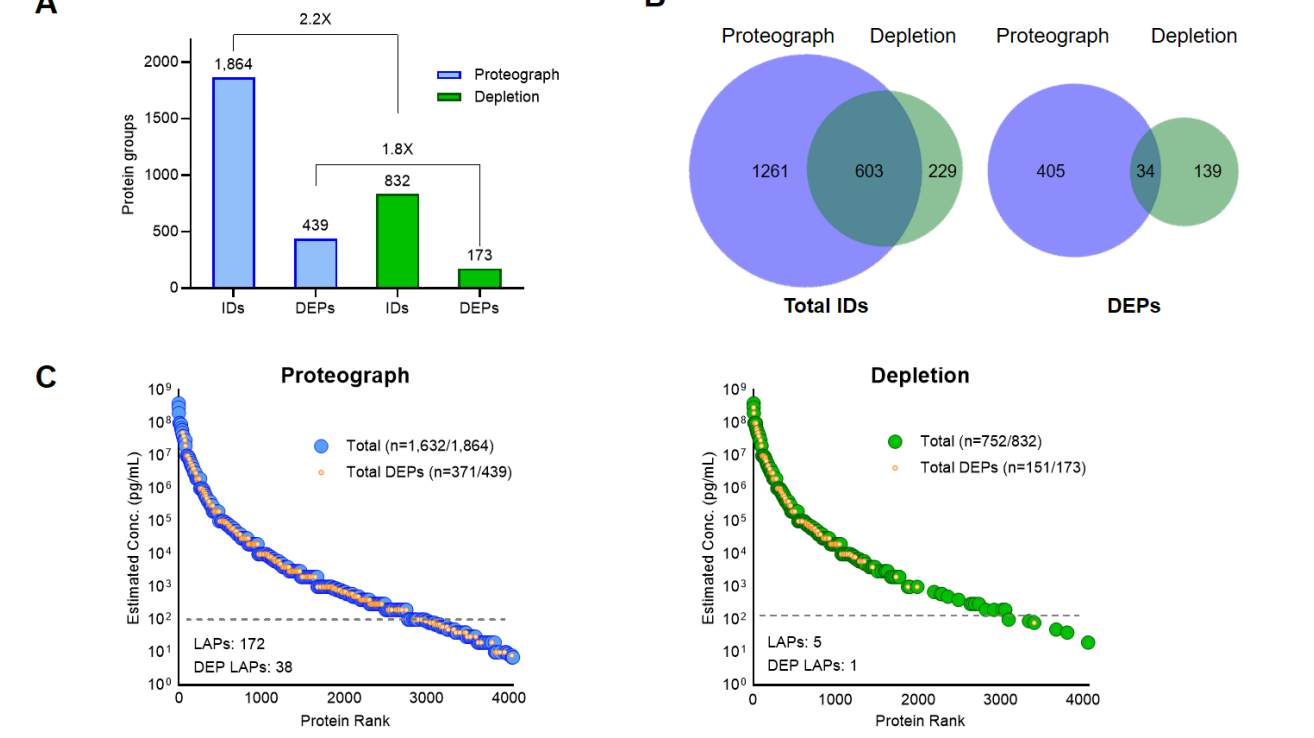


Figure 2. Performance of the Proteograph™ vs conventional depletion workflow. (A and B) Numbers shown in each panel indicate the number of proteins mapped to the Human Plasma Proteome Project (HPPP) database (numerator) out of the total number of detected proteins (denominator) for each workflow (Proteograph™ and depletion). (C) The Proteograph enhanced dynamic range coverage of the plasma proteome compared to depletion method, as demonstrated by the waterfall plot.

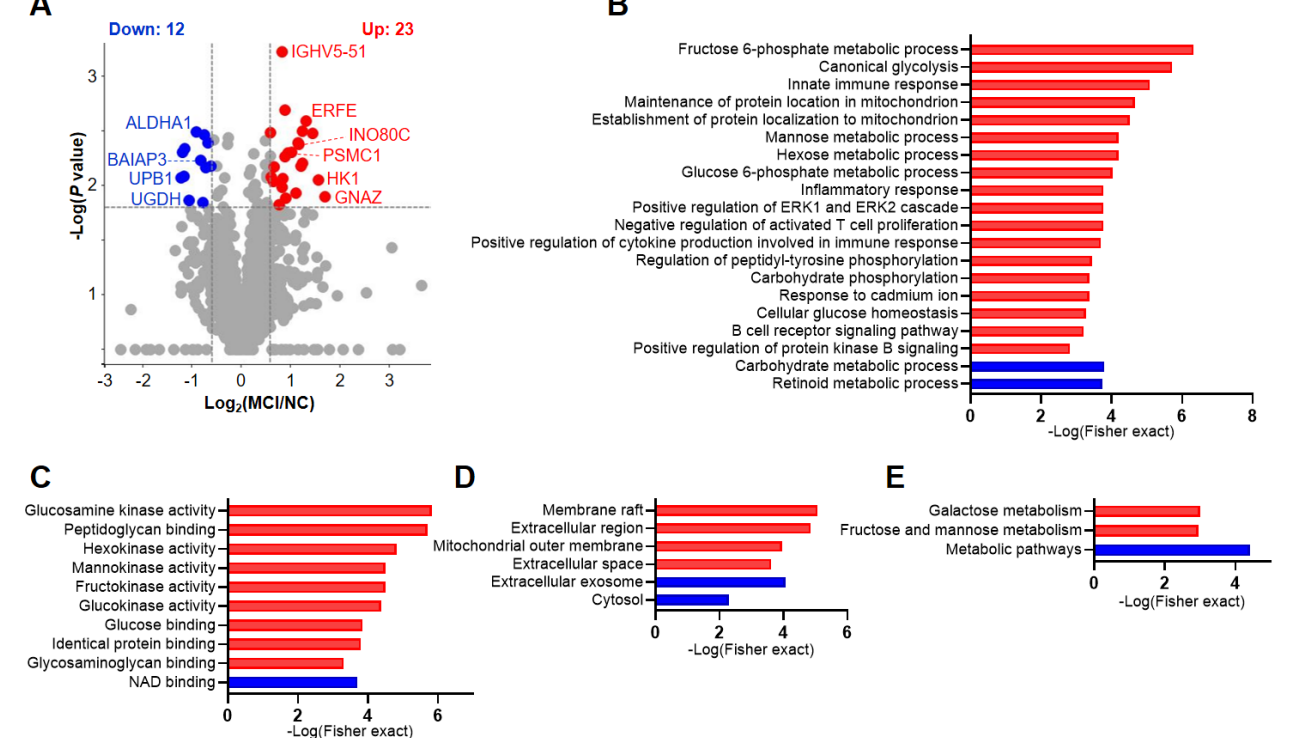


Figure 3. Identified DEPs and functional annotation in the MCI cohort. (A) A volcano plot analysis was conducted to identify DEPs based on a fold change threshold of 1.5 and an unpaired Student’s t-test with a $P < 0.05$. A total of 23 up-regulated DEPs (red dots) and 12 down-regulated DEPs (blue dots) were found to be statistically significant. (B-E) GO and KEGG enrichment analyses were performed for each DEP.

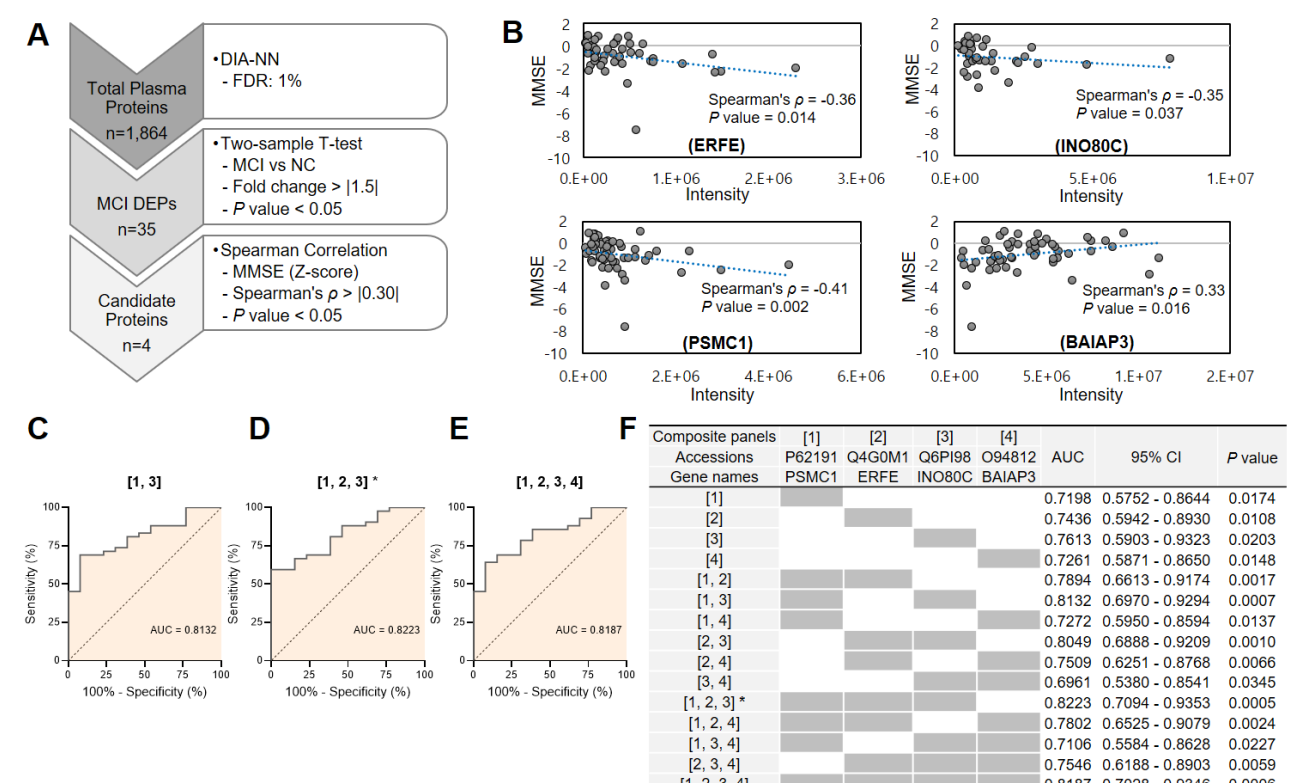


Figure 4. Prioritized candidate proteins associated with clinical diagnostic criteria to discriminate MCI. (A and B) The candidate prioritization workflow and candidate proteins based on clinical-proteome correlation between DEPs and MMSE scores (C-F) ROC curves are presented for selected candidates, showcasing high AUC values that effectively discriminate between MCI groups.

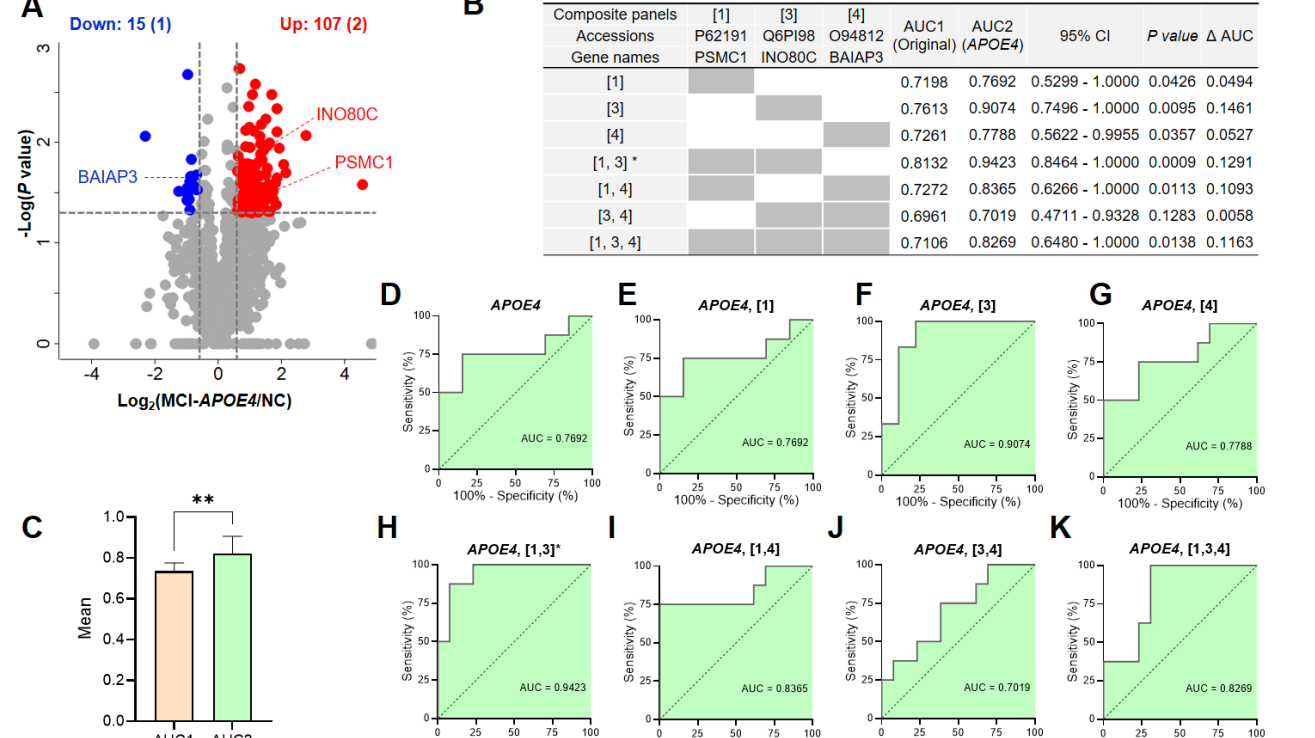


Figure 5. *APOE4*-dependent effects of prioritized candidates on MCI classification. (A) Differential expression analysis between normal controls (NC; $n = 13$) and *APOE4*-carrier MCI ($n = 8$) identified 122 DEPs (107 upregulated and 15 downregulated) (B-K) ROC analyses of composite panels. Comparative analysis between *APOE4*-carrier MCI versus NC (AUC2) and overall MCI versus NC (AUC1) were showed.

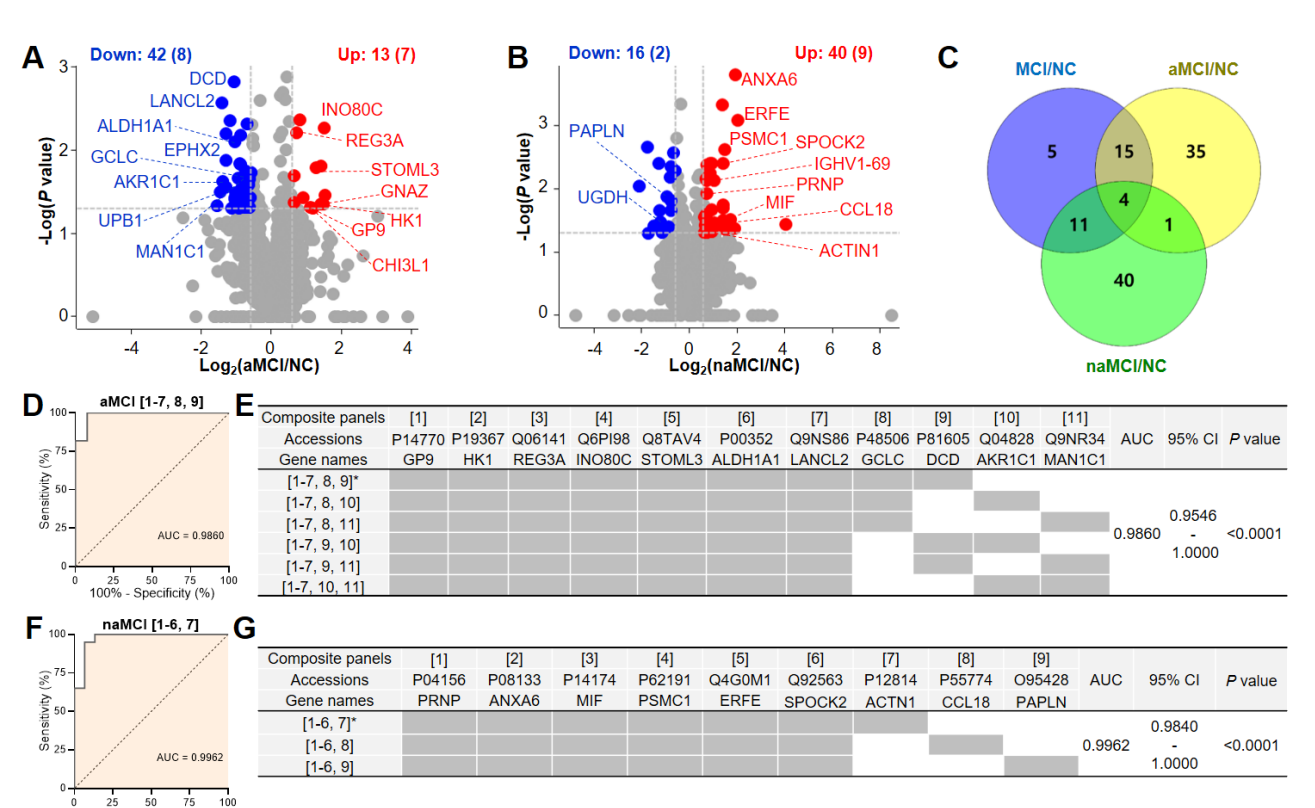


Figure 6. Candidate proteins to discriminate amnesic and non-amnesic MCI. (A and B) DEPs for amnesic MCI (aMCI) and nonamnesic MCI (naMCI) compared to NC are depicted using volcano plots (C-G) The candidate proteins and their composite panels capable of discriminating aMCI from naMCI were analyzed.

Nanoparticle-enabled Proteograph™ plasma proteomics enables comprehensive, unbiased detection of low-abundance proteins and improves discovery yield. The prioritized candidate proteins and composite panels support blood-based identification and subtype stratification of MCI, offering a scalable framework to aid early diagnosis and to elucidate systemic pathophysiology relevant to AD progression.

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