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Microglia protein profiles in CSF across Alzheimer's disease clinical stages

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Microglia are implicated in the progression of Alzheimer's disease (AD) pathology from its earliest stages, suggesting that cerebrospinal fluid (CSF) microglia profiling across clinical AD stages can aid in treatment development and monitoring. We analyzed two CSF cohorts (n = 834) that span from unimpaired controls to preclinical and dementia AD stages, identifying 109 dysregulated microglia-related proteins. Enrichment analyses revealed innate immune processes and cellular recruitment in preclinical AD, whereas AD dementia revealed adaptive immunity and macrophage responses. Next, we aligned the in vivo microglia protein profiles with ex vivo-derived microglial transcriptomic signatures, such as disease-associated microglia phenotypes. Transcriptomic signatures were not specific to either clinical stage but spanned both. We classified an 18-protein panel highlighting distinct changes between the preclinical and dementia stages. Our findings underscore the potential of microglia-based biomarker research for AD staging, offering insights into microglia dynamics in clinical AD stages and how transcriptomic signatures translate to proteomic profiles.

AD is a multifactorial disease resulting from the complex interaction of various risk factors involving age, genetics and environment[1,2]. The interaction of these factors triggers various pathological processes that advance the disease. Herein, neuroinflammation and microglia are increasingly recognized as important drivers of neurodegeneration due to AD[3–11]. Although microglia account for only about 10% of the total brain cells, their increasingly evidenced phenotypic diversity suggests a vital and complex involvement in the brain's functioning[3,4,6,12]. It is hypothesized that microglia are primed[13] to remove harmful hallmark AD plaques, but, as this pathology is progressively amassed, microglia remain chronically activated and become dysfunctional[8,12,14]. These processes are worsened with aging; aged microglia lose their normal functions, and they may become immunogenic[14]. Capturing microglial states in vivo across the clinical stages of AD is essential for the development of novel therapies aimed at modifying microglia functioning to halt AD, a challenge that has yet to be met.

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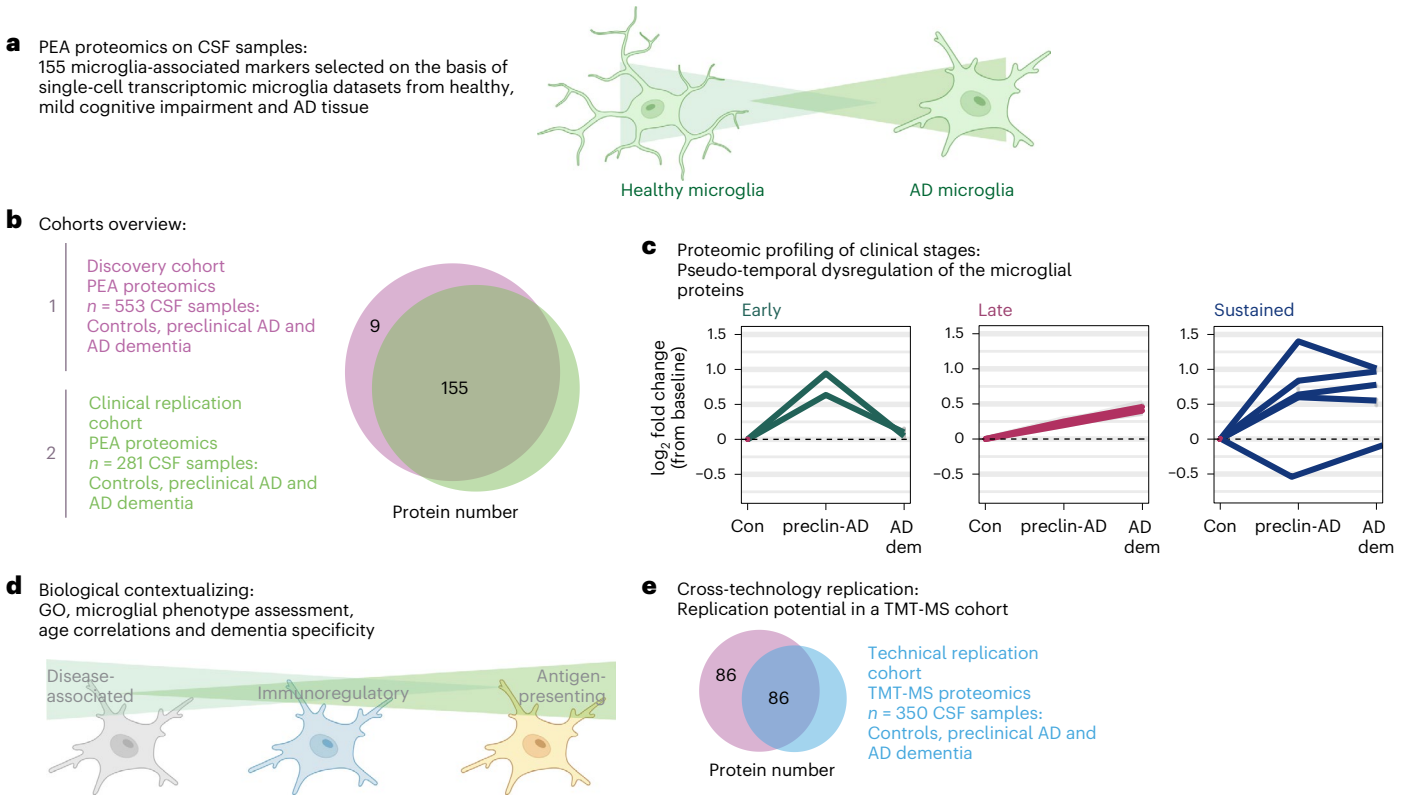

Fig. 1 | Overview of the study. a, From the PEA proteomics in both discovery and replication cohorts, 155 proteins were identified as microglia related based on alignment with single-cell and bulk transcriptomic datasets[15,16,18], as described further in Methods, and were measured in both the discovery and clinical replication cohorts (Supplementary Table 2). **b**, Cohort overview of the discovery and replication cohorts included in the main analysis. **c**, Proteomic profiling was assessed on a pseudo timeline of cross-sectional clinical profiles: cognitively unimpaired controls (Con), preclin-AD and AD dem. **d**, On the previously determined patterns of dysregulated microglial proteins, we assessed Gene Ontology (GO) analysis, matching to a microglial transcriptomic atlas for signatures such as disease-associated, age correlations and dementia specificity. **e**, Cross-technology replication of the patterns in a TMT-MS cohort measuring 350 CSF samples with a total protein number of 86 overlapping with the proteins in the discovery cohort. Icons in **a** and **d** created in BioRender; Hok-a-hin, Y. https://biorender.com/2h4y3uf (2026).

Considerable progress has recently been made in CSF proteomics and single-cell transcriptomics[15–19], the latter establishing the foundations for developing a single-cell microglia atlas[20]. Transcriptomics of ex vivo microglia reveal disease-associated heterogeneous phenotypes[15,16,18], including disease-associated microglia (DAM) phenotypes in AD brain biopsy[18], postmortem tissue[4] and experimental models[3,4,21]. DAM phenotypes have been consistently shown to exhibit temporal heterogeneity[4,21–23], and this may be reflected by abnormal changes in the levels of secreted proteins in the CSF[3,12,21], a valuable in vivo proxy for assessing AD pathology[10]. Additionally, microglial activation in AD is hypothesized to present with two peaks: an early protective one and a later proinflammatory one[24,25]. Human CSF studies show this duality in several microglia proteins, including sTREM2 (refs. 26–30), MIF[27] and sAXL[10], that are elevated in earlier stages of AD, possibly as a protective response to the accumulation of amyloid pathology[10,25,28,31–33]. Employing data from both single-cell transcriptomics and proteomics may reveal the CSF profiles of microglia signatures across stages of the AD continuum[3,18,20,34].

In the present study, we combined available transcriptomic datasets with our high-throughput proximity extension assay (PEA) dataset of CSF from individuals presenting with preclinical AD and AD dementia[35–37]. PEA proteomics is an antibody-based multiplexing proteomic established method for the identification of biomarker candidates that are translatable to single immunoassays and panels[35–40], critical for clinical and trial implementation. We identified and independently validated microglia-related protein profiles over the clinical stages of AD, alongside assessing their translation to transcriptomic microglia phenotypes in CSF. We applied a classification model to delineate a panel of proteins that can distinguish between preclinical asymptomatic AD and late symptomatic AD, with the aim to elucidate CSF-derived microglia-associated differentially expressed proteins (DEPs) between the two stages. For all AD microglia-associated CSF proteins of interest, we examined the effects of age, the specificity for AD compared to other dementia types and their replicability using an orthogonal proteomics technique. Capturing these proteomic profiles can provide insights into microglia activity reflected in the CSF during the transition to preclinical AD and to the AD dementia stage, which is important for the development of targeted biomarkers and treatments.

Results

The study design is summarized in Fig. 1. The discovery cohort consisted of 553 individuals spanning the AD clinical stages: a control group confirmed cognitively unimpaired with a normal CSF AD biomarker profile (controls, $n = 271$); a preclinical AD group with a confirmed AD CSF biomarker profile (preclin-AD, $n = 48$); and an AD dementia group with a confirmed AD CSF biomarker profile (AD-dem, $n = 234$) with CSF PEA proteomics data available (Supplementary Table 1). Validation of these proteins was conducted in the independent clinical cohort with PEA proteomics available with the same grouping (controls, $n = 198$; preclin-AD, $n = 40$; and AD-dem, $n = 43$; Supplementary Table 1). From these CSF PEA proteomic datasets, we selected 155 microglia-associated proteins by comparison to single-cell and bulk transcriptomic datasets of microglia isolated from fresh surgery and frozen autopsy brain tissue[15,16,18] (Supplementary Table 2). We analyzed the normalized protein expression (NPX) levels of these microglial markers to identify early and/or

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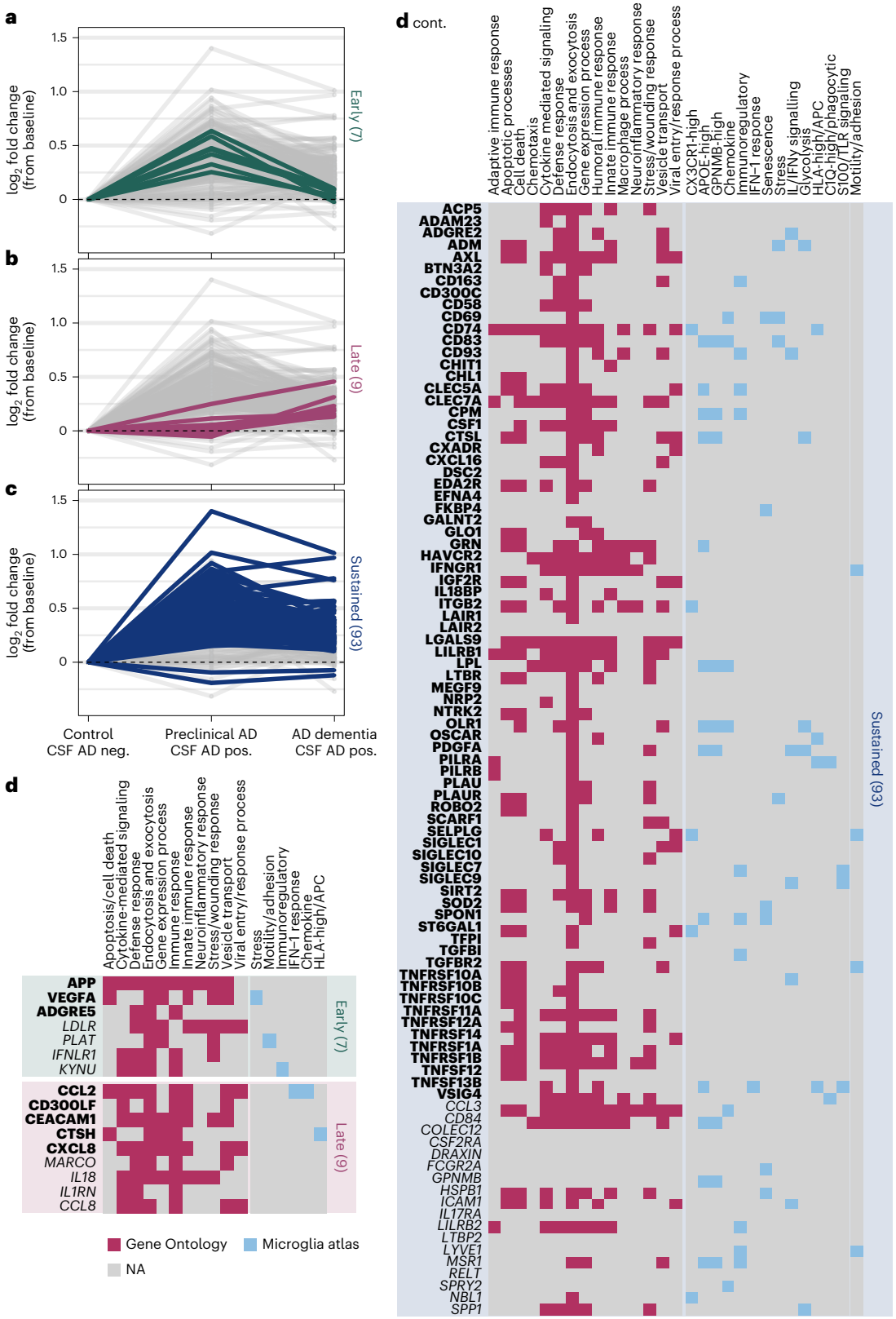

Fig. 2 | Differential protein abundance profiles and their functional context. **a**–**c**, Plotted mean log_2fold changes of each protein from the clinical groups to the control baseline (CSF AD biomarker negative (neg.)). Each line represents a protein significantly dysregulated for the stage assessed: **a**, in dark green, shows the seven proteins significantly upregulated in the early stage (preclinical AD stage, CSF AD biomarker positive (pos.)); **b**, in magenta, shows the nine proteins dysregulated in the late stage (AD dementia, CSF AD biomarker pos.); and **c**, in dark blue, shows the 93 proteins significantly dysregulated early and sustained to the late stage. **d**, Proteins dysregulated in the three profiles divided into functional categories. Bold lettering, proteins that significantly replicated the trend in both the discovery and replication cohorts; italics, proteins that follow the trends insignificantly in the replication cohort. Proteins are categorized into groups with shared functionality based on Gene Ontology enrichment analyses (magenta) and the microglial atlas (light blue). cont, continued; TLR, Toll-like receptor response.

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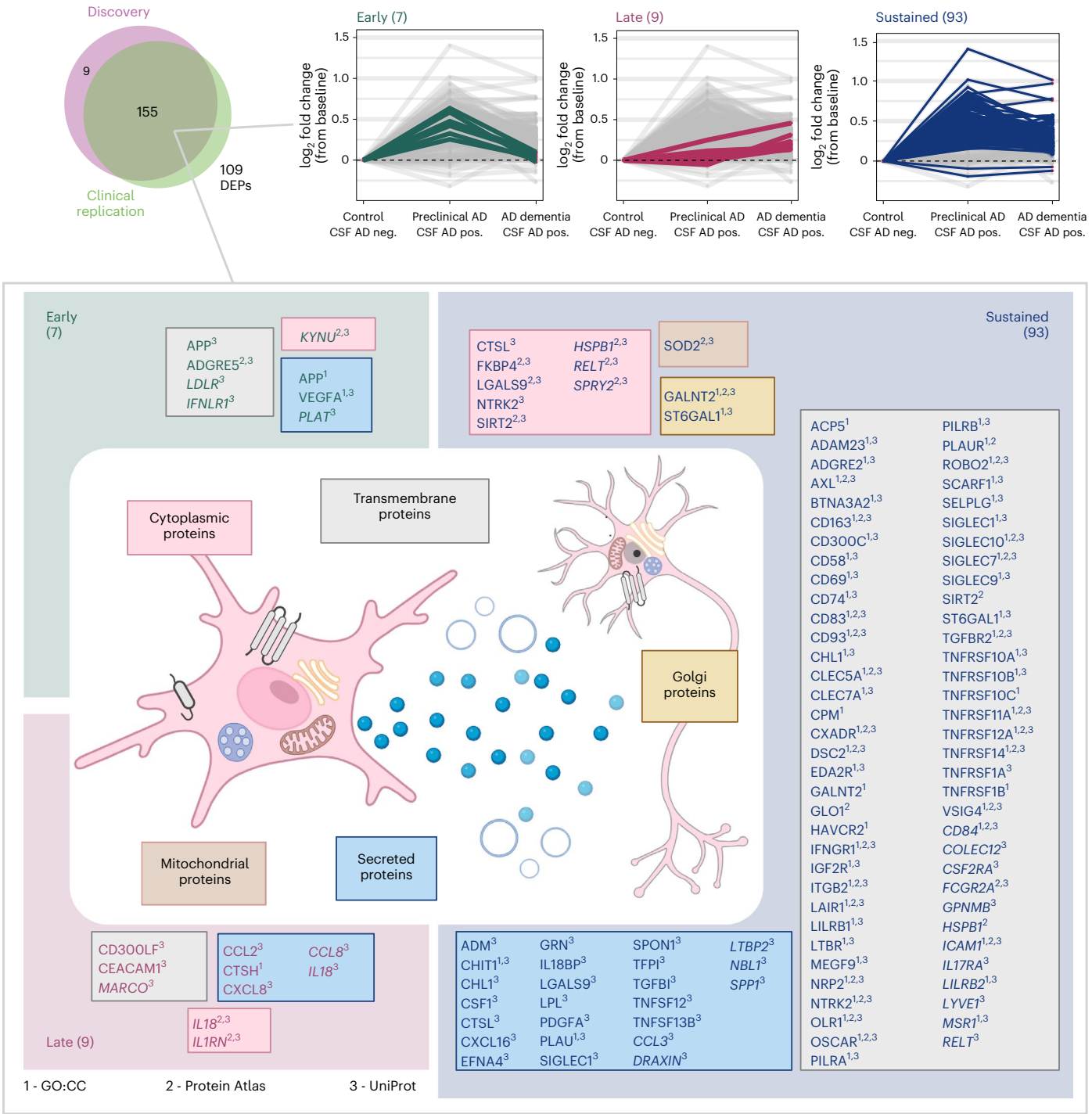

Fig. 3 | Subcellular location of the 109 DEPs from each of the three patterns. Schematic representation of the 109 DEPs from each pattern and their subcellular localizations from three sources (GO:CC, Protein Atlas and UniProt; Supplementary Table 6). Icons created in BioRender; Hok-a-hin, Y. https://biorender.com/2h4y3uf (2026). GO:CC, Gene Ontology Cellular Component.

late dysregulation patterns through an ANCOVA with Tukey's post hoc test and false discovery rate (FDR) correction ($q < 0.05$).

First, to explore the biological significance of microglia-associated CSF proteins dysregulated only in the preclinal AD group (that is, 'Early profile'), dysregulated only in the AD dementia stage (that is, 'Late profile') or with sustained dysregulation in both stages (that is, 'Sustained profile'), we conducted functional enrichment analyses using Gene Ontology. Second, to understand whether the CSF protein profiles can translate into distinct transcriptomic microglial phenotypes, we employed the recently published single-cell transcriptomic microglia atlas[20]. In brief, this atlas is based on transcriptomic data from live microglia cells isolated from a diverse set of patients (detailed further in the Methods). These data were factorized to define biologically relevant transcriptomic signatures capturing the main axes of heterogeneity in human microglia[20]. Our proteins of interest were matched to the top 100 markers for each transcriptomic signature in this transcriptomic atlas, to determine whether specific microglial signatures could be associated with distinct clinical AD groups from CSF.

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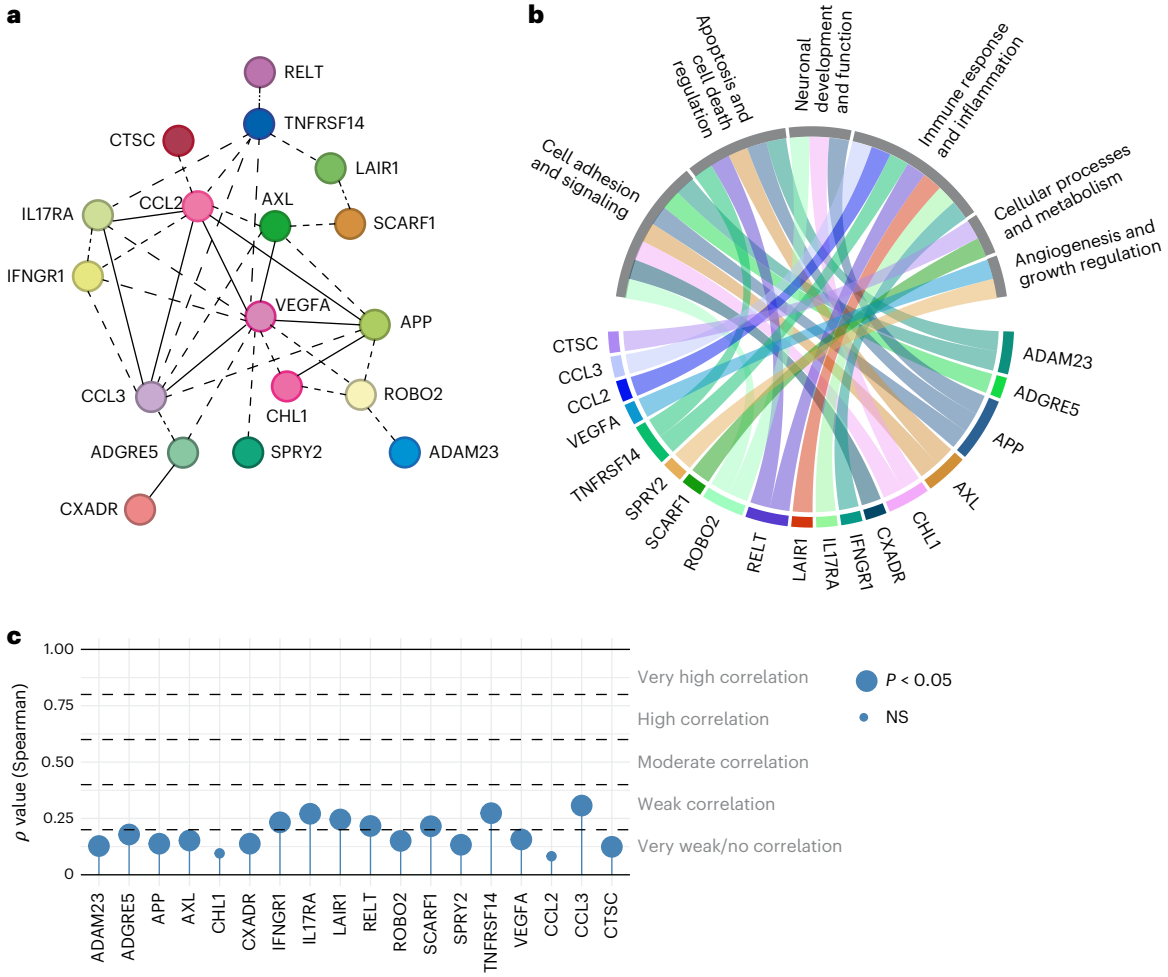

Fig. 4 | Biological contextualization of the identified dysregulated proteins between the symptomatic AD stages. a, STRING-generated protein–protein interaction network. Query proteins (colored nodes) with a combined score threshold of 150 are shown. Edges represent functional associations: solid lines indicate high confidence scores (>400); dashed lines denote lower confidence scores (<400). **b**, Functional enrichment analysis circos plot. Broadly grouped significantly enriched GO:BP terms ($P_{FDR} < 0.05$, two-tailed) are displayed on the upper arc, with associated proteins on the lower arc. **c**, Lollipop plot representing the two-tailed Spearman's ρ correlation values of age in the cognitively unimpaired group with negative CSF AD biomarker profiles of the 18 proteins of interest. Larger sized dot represents $P < 0.05$; smaller dot represents non-significance. GO:BP, Gene Ontology Biological Processs; NS, not significant.

CSF proteins uniquely upregulated in the preclinical AD stage ('Early profile')

The proteomic profiling of our discovery and clinical replication cohorts identified a first profile with seven proteins elevated exclusively in the preclinical AD group compared to both the control and dementia stages (Fig. 2a and Supplementary Table 3). Of these, three proteins significantly replicated in both cohorts, and four more proteins that were found in our discovery cohort followed the same trend in the clinical replication cohort, although insignificantly; so all seven were included in the biological enrichment analyses. The preclinical AD profile had significant associations with varied inflammatory and immune processes, defense and wounding responses, chemotaxis and signaling (Fig. 2d). From the seven proteins, APP, ADGRE5 (CD97), LDLR and IFNLR1 are transmembrane receptors; VEGFA and PLAT are secreted proteins; and KYNU is a cytoplasmic protein (Fig. 3 and Supplementary Table 6). All are related to microglial functioning and may activate microglia[41–43]. Moreover, processes linked to endocytosis and exocytosis were enriched, potentially highlighting the glial function and surveillance. Three proteins—VEGFA, KYNU and PLAT—overlapped with microglial signatures in the transcriptomic atlas[18,20,44] (Fig. 2d and Supplementary Table 2), specifically linked to stress, immunoregulatory and motility/adhesion, respectively. Although functionally of interest, no defined signature could be attributed exclusively to the 'Early profile'. Collectively, the Gene Ontology analysis and the overlap with microglial signatures suggest that proteins elevated exclusively during the preclinical AD phase may overall reflect increased cellular recruitment, activation of broad immune processes and enhanced motility for surveillance.

CSF proteins uniquely upregulated in the AD dementia stage ('Late profile')

The second proteomic profile that arose in our discovery and clinical replication cohorts revealed nine proteins elevated exclusively in the AD dementia group compared to the control and the preclinical AD stage (Fig. 2b and Supplementary Table 4). Out of the nine proteins, five significantly replicated the 'Late profile', and four more proteins that were found in our discovery cohort followed the same trend in the clinical replication cohort, although insignificantly; so all nine proteins were included in the biological enrichment analyses. Functional enrichment analysis revealed similar processes as in our 'Early profile', although with more proteins enriched in innate and humoral immunity and cytokine signaling; from these nine proteins, CCL2 (MCP-1), CTSH, CXCL8 (MCP-2), IL-18 and CCL8 (MIF-1α) are secreted chemokines and cytokines, whereas CD300LF (CLM-1), CEACAM1, MARCO and IL-1RN are found

either as transmembrane proteins or in the cytoplasm (Figs. 2d and 3 and Supplementary Table 6). When mapping to the transcriptomic microglia atlas, only two proteins—CCL2 and CTSH—overlapped with microglial signatures (Fig. 2d and Supplementary Table 2): CCL2 with interferon 1 (IFN-1) response and chemokine signatures, whereas CTSH mapped to the antigen-presenting cell (APC) signatures (HLA^{high}). This means that no defined microglial signature could be assigned to only the 'Late profile'. Taken together, the findings suggest an increase in immune response in the AD dementia stage compared to the early stage of the disease, alongside increased proinflammatory activity and cellular death proteins.

CSF proteins dysregulated in the preclinical AD stage and sustained into the AD dementia stage ('Sustained profile')

In the third profile of our discovery and clinical replication cohorts, 75 proteins were significantly increased in the preclinical AD stage compared to controls, with this change remaining significant into the AD dementia stage (Fig. 2c and Supplementary Table 5). In addition to these 75 proteins, 18 other proteins in this pattern in our discovery cohort were also significantly dysregulated (16 increased and two decreased) in at least one of the stages in the clinical replication cohort, although insignificantly. Most of these proteins (67) are transmembrane proteins, 24 are secreted, eight are cytoplasmic, two are Golgi and one is mitochondrial (Fig. 3 and Supplementary Table 6). Functional enrichment analysis pointed to an association with several immune and transmembrane proteins particularly enriched in apoptotic processes and adaptive, humoral and innate immune system processes, along with cytokine signaling (Fig. 2d), which fits with a combination of the above-described profiles unique for the disease stages. Notably, 44 of the 93 pattern-replicating proteins (both significantly and insignificantly) overlapped with assigned signatures in the microglia atlas, highlighting the involvement of 14 proteins from the total of 20 proteins (70%) within the disease-associated signature ($GPNMB^{high}$ and $APOE^{high}$), five of the eight (62.5%) with a homeostatic signature ($CX3CR1^{high}$), 17 of the 26 (65%) with the inflammation signature (immunoregulatory, chemokine and IFN-1 response), six of the nine (67%) with the antigen presentation signature (HLA^{high}/APC and $C1Q^{high}$/phagocytic), nine of the 16 (56%) with the innate immunity signature (IL/IFNγ and S100/TLR signaling) and 10 of the 14 (71%) with the stress and senescence signature, among others (Fig. 2d and Supplementary Table 2). Together, the notion that the far majority of altered proteins fall in this profile suggests a profound upregulation of microglia activity throughout the two stages and that this is not limited to a specific microglia subtype reflected in the CSF.

Classification of CSF proteins dysregulated specifically between the preclinical AD and AD dementia stages

Following the above identification of the three protein profiles over the AD disease stages, we narrowed the focus onto refining a list of proteins that could be suitable for capturing the changes occurring within the progression from preclinical AD to AD dementia. Classification analysis of the microglia proteins of interest identified key proteins whose abundance patterns best distinguish between the early and late clinical AD stages and may best reflect the changing biology. We focused on CSF proteins in our discovery cohort that were most significantly differentially expressed (standardized difference > ±0.2, $q < 0.05$) between the preclinical AD and AD dementia stages. The 18 identified proteins are represented in Supplementary Table 7. Of these, 15 decreased across the early and late stages—ADAM23, ADGRE5, APP, AXL, CHL1, CXADR, IFNGR1, IL17RA, LAIR1, RELT, ROBO2, SCARF1, SPRY2, TNFRSF14 and VEGFA—whereas three increased across the early and late stages: CCL2, CCL3 and CTSC. These proteins originate from the three profiles: 'Early profile' (ADGRE5, APP and VEGFA) and 'Late profile' (CCL2) and the remainder in the 'Sustained profile' (ADAM23, AXL, CHL1, CXADR, IFNGR1, IL17RA, LAIR1, RELT, ROBO2, SCARF1, SPRY2, TNFRSF14, CCL3 and CTSC).

To begin understanding if these proteins work in an orchestrated manner to affect microglia processes, we performed STRING analysis for protein–protein interactions[45]. This highlighted links between the proteins with CCL2, CCL3 and VEGFA as hubs of connectivity between the classified proteins (Fig. 4a). Functional analyses of these proteins showed broad functions in neuronal, immune response, cell signaling and apoptotic processes (Fig. 4b). The transcriptomic microglial signatures in the microglia atlas overlapped with only four proteins: CCL2, CCL3 and SPRY2 with the chemokine signature and IFNGR1 with the motility/adhesion signature (Fig. 2d and Supplementary Table 7). The later stage increase includes two proinflammatory chemokines and a protease involved in immune regulation, suggesting a shift toward chronic immune activation and potential protein dysfunction. By contrast, the 15 proteins that decrease are primarily immune receptors or receptor signaling pathways, which may indicate less cellular communication in later stages.

To expand the possible utility of this panel, we explored the discriminative power of these proteins between the two stages of symptomatic AD. We performed an internal cross-validation with all 18 proteins together yielding an area under the curve (AUC) of 0.94 (95% confidence interval: 0.83–1.00) and with the proteins' individually tested AUCs ranging between 0.68 and 0.81 (Supplementary Table 7), providing an initial indication of their potential for staging. Although the strength of differential expression was supported in our replication cohort (standardized mean difference > ±0.2), only four proteins in this cohort replicated the significance (APP, CHL1, CXADR and IL17RA; $P < 0.05$). In line with this, the cross-validation in the clinical replication cohort on the 18 proteins together yielded a high overall AUC of 0.88 (0.80–0.96) and with the individually tested proteins resulting in low to medium AUCs ranging between 0.47 and 0.69 (Supplementary Table 7).

Age effects

As a major risk factor for AD, age may trigger pathogenic processes at different stages of AD progression and, as such, may modulate the protein abundances in CSF. To assess the age effects, we calculated the correlation with age on the levels of our CSF proteins of interest in our control (cognitively unimpaired) group. For the 109 replicated proteins in the previous early, late and sustained profiles, EDA2R and HSPB1 are the only proteins with a moderate correlation with age ($\rho > 0.4$, $P < 0.05$), whereas most proteins showed a weak correlation (ρ: 0.2–0.4; 61/109), a very weak correlation (ρ: 0–0.2; 35/109) or no correlation ($P > 0.05$; 11/109) with age (Extended Data Fig. 1). From our panel of 18 proteins discriminating the preclinical versus dementia stages (Fig. 4c), the levels of CCL2 and CHL1 did not correlate with age, whereas the remainder showed a weak or very weak correlation. We performed our receiver operating characteristic (ROC) analysis of this panel correcting for age, which led to minimal AUC changes of 0–0.05 (Supplementary Table 9). These subtle or inexistent effects of age support that dysregulations in our profiles may reflect disease effects.

Specificity for AD

As an indication of specificity of the findings for AD compared to two non-AD dementias, we expanded the discovery analysis within the entire discovery cohort that included CSF from patients with a diagnosis of dementia with Lewy bodies[36] (DLB; $n = 110$) or frontotemporal dementia[37] (FTD; $n = 143$) (full demographics in Supplementary Table 8a). Six out of seven proteins showed an increase in only the preclinical AD stage, aside from KYNU, which showed an increase in DLB (Extended Data Fig. 2). From the 'Late profile', three out of nine proteins were specific for AD. In the 'Sustained profile', 59 out of 93 proteins showed specificity for AD (57 increased in both AD stages and two decreased in both AD stages). This supports that most of the dysregulation is specific to AD, whereas the reminder may be related to more generic aspects of neurodegenerative diseases.

Technique specificity

Lastly, we performed an orthogonal technique replication—namely, tandem mass tag mass spectrometry (TMT-MS). We compared the results in our discovery cohort, PEA proteomics, to a group of patients measured with TMT-MS (n = 137; demographics in Supplementary Table 1). This analysis, with 86 proteins measured in both techniques, showed that 81% of the proteins exhibited similar upregulation or downregulation patterns across all profiles, although many lacked statistical significance (Supplementary Tables 3–5 and Extended Data Fig. 3). Profile-specific replication rates were 64% for the 'Early profile', 71% for the 'Late profile' and 84% for the 'Sustained profile'. Eleven proteins demonstrated significantly consistent patterns in both the technical replication cohort and the discovery cohort. Of those, APP replicated in the 'Early profile', and 11 proteins (DSC2, GLO1, GRN, HAVCR2, IL18BP, ITGB2, SOD2, SPON1, TNFRSF12A, TNFRSF14 and VSIG4) replicated in the 'Sustained profile'. These findings underscore the robustness of our proteomic results across different measurement techniques.

Sensitivity analyses

To evaluate the potential influence of tau pathology on these markers, we stratified groups according to AT (amyloid–tau) status. Although the A+T− group was relatively small (n = 25; Supplementary Table 8b), resulting in reduced statistical power, our findings suggest that tau may be the principal driver of differential protein expression, with 91% of proteins reaching significance only after T+ status (Supplementary Table 9). We also examined the impact of *APOE ε4* carriership by repeating our analyses stratified by *APOE* genotype. Here, the preclinical AD groups were limited in size (*APOE ε4* n = 27; *APOE ε2/ε3* n = 19; Supplementary Table 8c), which may have affected statistical power. Nevertheless, our results show that 53% of the 109 proteins remain unchanged, whereas 20% are specifically associated with *ε4* carriership, particularly in the late and sustained groups (Supplementary Table 9).

Discussion

In the present study, we aligned single-cell transcriptomic microglia states with CSF proteomics to describe the presence and dynamics of microglia-associated CSF proteins and signatures over the AD clinical stages. Proteomic analysis highlighted specific proteins upregulated in the preclinical AD stage (including APP, VEGFA and ADGRE5) and in the AD dementia stage (including CCL2, CTSH and CXCL8) as well as proteins that are dysregulated across both stages (including ITGB2, VSIG4 and DSC2). Functional enrichment analysis and alignment to microglial transcriptomic signatures suggested that preclinical proteins reflect increased cellular recruitment and an initial immune response, possibly representing a microglial-associated innate response to AD pathology. In the late stage, there were adaptive and humoral immune processes and apoptosis, and, throughout both stages, there was a complex pattern of immune activation and cellular death. Surprisingly, when we aligned the in vivo protein profiles with ex vivo single-cell microglial transcriptomic signatures, such as the previously described DAM, these were not specific to either clinical stage but spanned both. Our findings offer insights into microglia-associated signatures in CSF, underscoring the potential of microglial biomarker research for staging of AD and how single-cell transcriptomic signatures translate to CSF proteomic profiles. This provides a conceptual framework and foundation for tracking the microglia-associated contribution over the AD disease course for treatment development and has a potential application for predicting clinical onset of AD. Previous studies explored AD staging through CSF proteomics[40,46–49] with some even identifying glial proteins that emerge during asymptomatic phases—for example, SMOC1 and ITGAM associated with early changes and amyloid pathology[40]. However, these studies did not specifically examine microglia-associated proteins across AD stages, potentially overlooking more subtle alterations arising from this minority cell population relative to neurons.

Using the single-cell transcriptomic signatures of phenotypes from the microglia atlas[18,20,44], we aimed to determine whether these phenotypes can be reflected in secreted proteomics, in relation to disease stages. Overall, we recognized a few microglia signature markers indicative of inflammation, stress and motility in the early AD stage and, in the late stage, few additional markers of inflammation and adaptive immunity with antigen presentation. As may be expected, the sustained profile containing 75 proteins significantly replicating in both cohorts (Fig. 2c,d) allowed for better mapping of transcriptomic signatures to the CSF proteome, containing markers of the following transcriptomic signature groups in order of percentage of proteins that could be mapped: homeostatic (83%), stress and senescence (80%), inflammation (76%), antigen presentation (71%), innate immunity (64%) and DAM (63%). The homeostatic signature in the transcriptomic atlas[20], CX3CR1^{high}, consists of the chemokine receptor CX3CR1, regulating and stabilizing microglia[50]. The DAM signature consisted of two subdivided groups—GPNMB^{high} and APOE^{high}–representing temporal disease-associated phenotypes from experimental and pathology studies[3,4]. APOE^{high} and GPNMB^{high} signatures both include high loadings for TREM2 (refs. 20,51) with the GPNMB^{high} signature being more akin DAM2 signature in murine models[4,20]. Interestingly, in the sustained profile, the multitude of signatures that we could assign proteins to are possibly reflecting that different microglia phenotypes can be detected in CSF at the same time. It may reflect a fundamental difference between tissue and bodily fluids (that is, tissue has specific spatial and complex cellular organizations while CSF is a cell-sparse medium), as a DAM signal arises in CSF without distinct phenotypic switching. Crucially, a recent spatial proteomics study[52] explored the possibility of a microglial state continuum in postmortem brain tissue, highlighting a continuous gradient of microglial marker activation throughout healthy and AD tissue rather than following discrete stages. Alternatively, the lack of stage specificity could be due to individual heterogeneity of the disease progression in AD[53] or a mismatch between clinical and biological progression[54]. These findings have implications for clinical trials, where it may, thus, be possible to detect target engagement toward each of these microglia phenotypes.

In the preclinical stage of AD—that is, asymptomatic individuals with positive AD CSF biomarker profiles—three proteins show unique upregulations: APP, VEGFA and ADGRE5. Microglia have been shown to respond early to APP, the amyloid precursor protein cleaved into amyloid-β making up AD plaques[4,55,56]. Although APP is a neuronal marker, APP transcripts were detected in the available transcriptomic datasets of healthy and diseased microglia, leading to the inclusion in our data. The presence of APP here may reflect proximity to APP-expressing neighboring cells or context-dependent microglial expression under pathology[25], which may decrease in latter stages due to cell loss. Vascular endothelial growth factor A (VEGFA), typically protective for cognitive function and vascular integrity, when increased in AD, it increases oxidative stress and accelerates disease progression[57]. ADGRE5 (CD97 antigen subunit α) is part of the complement activation and leukocyte migration pathways, often associated with early stages of AD[58,59]. Early changes in protein levels may be associated with tau pathology, as demonstrated by the AT sensitivity analysis in which most proteins lost significance at the A+T− stage. This observation aligns with previous studies indicating that immune activation is linked to increasing tau levels[60]. As leukocyte infiltration occurs, proinflammatory macrophages are attracted to the site of injury by chemokines such as CCL2 (also known as monocyte chemoattractant protein 1 (MCP-1)), CCL3 (MIP-1α) and CXCL8 (MCP-2)[61], which increase in the dementia stage. Amyloid plaques may further lead to elevated CCL2 expression in monocytes[62], possibly explaining the observed increase in CCL2 during late-stage AD. Our sensitivity analysis suggests that *APOE ε4* carriership may slightly influence progression into the dementia stage, with a subset of proteins showing upregulation mainly driven by *ε4* into the late stage[63]. With the advancement into the

dementia stage, regulators of neuroinflammation decrease in the CSF, as captured by our panel of 18 proteins in our preclinical AD versus AD dementia profile. This potential decline in immune regulation, coupled with ongoing immune cell infiltration at the site of injury, suggests that initial neuroinflammation increases may confer protection, but, as this inflammation continues to be exacerbated over time, it becomes neurotoxic[59,64,65]. Interestingly, these effects were not correlated with chronological age in our study and may, therefore, be independent of processes triggered by aging. Nearly one-third of the proteins identified in our study (31/109) have been functionally characterized in genetic, animal and cell models, particularly in relation to inflammation and AD (Supplementary Table 6). This overlap demonstrates that many of the proteins identified from the CSF profiles have been established within experimental inflammatory processes. Several other proteins within the early and sustained expression patterns remain unexplored and warrant further investigation, as they may provide insights into aging and neurodegeneration and offer potential targets for future therapeutic development.

Our study has notable limitations and several strengths to consider. A key limitation lies in the challenge of the translation from ex vivo transcriptomics to clinically applicable measurements, due to biological and technical complexity[66]. Although CSF offers a direct read-out of ongoing biological processes in the brain, its proteomic coverage remains limited compared to transcriptomics, and transcript levels may not accurately reflect secreted protein dynamics. There are also differences in detected transcripts per cell type per study. Here, we used single-cell transcriptomic datasets for our protein selection and signature alignment instead of the single-nucleus transcriptomics datasets, due to the lower amounts of transcripts in the nucleus as compared to the whole cell[67]. Due to the scarcity of cell-type-specific proteins[38,68], contributions from astrocytes, neurons or other cell types cannot be excluded in our analysis. Additionally, with increasing age and disease, disruption of the blood–brain barrier[69] and impairment of the glymphatic system[70] can increase the influx of peripheral proteins into the CSF, further limiting the precise determination of the source of the proteins. To address the translational gap between proteomics and transcriptomics, we integrated the largest single-cell microglia transcriptomics datasets with CSF proteomics. Future strategies focusing on bridging the brain-to-CSF translation gap could include single-cell brain transcriptomics, postmortem tissue and paired proteomics, refining methods to unambiguously assign cell type origin in the CSF proteome. Second, our study is on cross-sectional groups within the cohorts, which required us to estimate temporal patterns. Long-term longitudinal studies with repeated CSF samples are required to test dynamics of CSF proteins more directly. Third, our final 18 candidates of interest were identified using targeted antibody-based proteomics. Although this approach may limit the breadth of candidate proteins, it enhances translatability to immunoassays, as previously demonstrated[71,72]. Technical limitations of our study include (1) the absence of TREM2 in our discovery cohort and (2) the small sample sizes for our sensitivity analyses. Additionally, the cohorts were predominantly white, and, therefore, it can be valuable to study the generalizability in other populations. Finally, proteomic studies are inherently variable due to the multiple technologies employed for biomarker discovery; therefore, another strength of our study is that we solidified our candidate proteins through external validations. We integrated a large cohort dataset of transcriptomic-based human microglial signatures with data from two independent human CSF clinical cohorts and from two different proteomic technologies. Improving the biofluid biomarker staging addresses an important need in the AD field[54]. Although the prioritized proteins in our study were not significantly replicated in the validation cohort, they demonstrated high classification performance in both cohorts (AUC > 0.88 and AUC > 0.96), suggesting strong combined discriminative power. The lack of replication may be attributable to differences in dementia group sample sizes and age distributions between cohorts. Providing the framework to bridge transcriptomic and proteomic data represents a crucial step toward clinical translation of the substantial evidence for microglia involvement in AD.

In conclusion, we show potential for the assessment of microglial phenotypes throughout the AD continuum from human CSF, showing that secreted proteins align to specific microglial phenotypes as the DAM signature. We highlight several promising protein biomarkers that can aid in inflammatory staging of patients as well as increase understanding of the contribution of microglia spanning the clinical AD stages. Furthermore, our widespread upregulation of microglial proteins throughout the AD continuum adds evidence to the increasing body of evidence of sustained microgliosis during AD progression, with relatively subtle changes over the disease course.

Methods

Ethics statement

Ethical approval was given by the institutional ethical review boards of each center, and all participants gave written informed consent. For the discovery cohort and the technical replication cohort: VU University Medical Center (VUmc): AD CSF biobank METC number 00–211; discovery cohort: additional samples from the University of Pennsylvania: language and cognitive impairment in Parkinson's disease and Parkinson's disease with dementia or DLB IRB069801; clinical replication cohort: DELCODE is an observational longitudinal memory clinic-based multicenter study whose protocol was approved by the ethics committees of the medical faculties of all participating sites: the ethics committees of Berlin (Charité, University Medicine), Bonn, Cologne, Göttingen, Magdeburg, Munich (Ludwig-Maximilians-University), Rostock and Tübingen. The process was led and coordinated by the ethics committee of the medical faculty of the University of Bonn. The registration number of the trial at the ethics committee in Bonn is 117/13.

Cohort description

The study was performed as a retrospective investigation in CSF samples from a cohort of individuals from the Amsterdam Dementia Cohort (ADC)[73] and the Center for Neurodegenerative Disease Research at the University of Pennsylvania. All participants underwent standard neurological and cognitive assessments, and diagnosis was assigned according to international consensus criteria for mild cognitive impairment[74] and AD[75]. CSF Aβ42 and total-Tau (tTau), referred to as AD CSF biomarkers, were analyzed locally using commercially available kits (ADC: ELISA INNOTEST $A\beta_{1-42}$, hTAUAg, Fujirebio; University of Pennsylvania: Luminex xMAP INNO-BIA AlzBio3, Luminex). A positive CSF AD biomarker profile was defined locally as increased $tTau/A\beta_{42}$ in the cohorts from the ADC (>0.46 (ref. 76)) and the University of Pennsylvania (>0.30 (ref. 77)). We excluded one patient who progressed to FTD, two to progressive supranuclear palsy and two to vascular dementia. To capture the AD continuum, we selected a total of 553 individuals with CSF measurements for $A\beta_{42}$, tTau and proteomics data available, comprising three groups: cognitively unimpaired control group with negative AD CSF biomarker profiles and in whom objective cognitive investigations were normal (that is, criteria for mild cognitive impairment, dementia or any other neurological or psychiatric disorder not fulfilled[73]) (controls; $n = 277$, mean age 58 years, 36.5% females); preclinical AD group with positive AD CSF biomarker profiles with no objective cognitive symptoms (preclin-AD; $n = 48$, mean age 68 years, 58.3% females); and AD dementia group with positive AD CSF biomarker profiles and fulfilled the dementia criteria[73] ($n = 234$, mean age 66 years, 40.6% females) for the main analysis (Supplementary Table 1). The discovery cohort was split based on AT status for sensitivity analyses, defining A+ as an $A\beta_{1-42}$ concentration of <1,092 pg ml^{-1} and T+ as a pTau181 concentration of >24 pg ml^{-1} or a tTau concentration of >235 pg ml^{-1} for a small subset of patients who did not have pTau181 measurements[78] (demographics in Supplementary Table 8b).

Protein-level measurements and selection

For our discovery cohort, samples were randomly distributed across plates within each batch, including bridging samples, for proteomics measurement at the Olink facility, which was blinded to the clinical details of the samples. A total of 1,196 CSF protein levels were quantified using 11 Olink Target 96 multiplex antibody-based protein panels based on PEA technology (Cardiometabolic, Cardiovascular II and III, Cell Regulation, Development, Immune Response, Inflammation, Metabolism, Neurology, Oncology II and Organ Damage; Olink Proteomics) as in our previous studies[27,35,36,79]. After quality control and normalization, the data were provided in the relative protein quantification unit, NPX, which is in a $\log_2$ scale. All characteristics and validation data for each assay are available at the manufacturer's website (https://olink.com/).

Based on overlap with human microglia transcriptomic datasets in both healthy and AD samples from the MGEnrichment tool[80], our protein list overlapped with two RNA sequencing datasets for homeostatic proteins[15,16] along with a single-cell RNA sequencing dataset[18] for AD states of microglia, resulting in a total of 172 unique proteins, which were refined based on the independent clinical cohort availably (described below) to 155 total proteins included for the statistical analysis (transcriptomic sources for these proteins are presented in Supplementary Table 2). In addition, we obtained an indication of the cell type specificity with the EWCE (expression weighted cell type enrichment) R package[81] in a whole brain cell single-nucleus dataset from ref. 82. In this set, 133 of our protein transcripts were detected, of which 98 in microglia, with 44 of these 98 transcripts showing to be enriched for microglia compared to the other cell types (Supplementary Table 2).

Validation cohorts and disease specificity analysis

For the clinical replication, we focused on replication in an independent cohort, using the extensive DZNE-Longitudinal Cognitive Impairment and Dementia Study (DELCODE) cohort, an observational longitudinal memory clinic-based multicenter study in Germany[83]. The CSF AD profile was measured on a V-PLEX Aβ Peptide Panel 1 (6E10) Kit (K15200E) and a V-PLEX Human Total Tau Kit (K151LAE) (Meso Scale Diagnostics). Cutoff values for abnormal concentrations of $A\beta_{42}$ (<496 pg ml^{-1})[84] and cutoff values established in Bonn based on clinical non-impaired control samples for tau (>470 pg ml^{-1}) were used to split the groups. This independent clinical cohort was measured using the Olink 3072 Explore panel, which includes 3,072 assays, of which 156 (90.7%) of the microglial proteins of interest overlapped. The cohort was split into the same groups as the discovery cohort: cognitively unimpaired (n = 198, mean age 69 years, 52% females), preclinical AD (n = 40, mean age 73 years, 27.5% females) and AD dementia (n = 43, mean age 75 years, 67.4% females) (Supplementary Table 1). Statistical analyses were performed individually in this cohort and compared to the results from the discovery cohort without the need for batch or platform corrections.

For the technical replication, we focused on mass spectrometry cohort replication. A subset of the ADC cohort was measured using TMT-MS[85]; CSF biomarkers and cutoffs are the same as in the discovery cohort. The overlap with the discovery protein amounted to 109 total microglial proteins (Fig. 1a), of which 88 (51.2%) were measured in at least 50% of samples. The technical replication cohort was labeled according to the cognitive state and biomarker profiles using the same definitions as our discovery cohort: cognitively unimpaired controls (n = 126, mean age 59 years, 34.1% females), preclinical AD (n = 38, mean age 65 years, 50% females) and AD dementia (n = 186, mean age 65 years, 49.5% females) (Supplementary Table 1).

We additionally assessed, broadly, the specificity for the AD continuum by an ANCOVA comparison, the same as in our previous analysis, within our complete discovery cohort[35] that includes CSF from patients with diagnoses of DLB[36,86] (n = 110, mean age 69 years, 16.4% females) and FTD[37,87] (n = 143, mean age 62 years, 46.9% females) (additional demographics in Supplementary Table 8a).

Statistics and reproducibility

Data processing was performed in RStudio (version 2023-10-31) using R (version 4.3.2). No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications[35,36,40]. Data distribution was assumed to be normal, but this was not formally tested. Differential protein expression across the groups in the discovery cohort was performed using a one-way ANCOVA with age and sex as covariates, followed by Tukey's post hoc test, and for multiple testing using FDR correction for the number of proteins. Differential protein expression across the groups in the clinical replication cohort was performed using a single-stratum one-way ANCOVA with age and sex as covariates comparing each group to the control group and corrected using FDR. We performed classification modeling on the selected proteins of interest that showed a standardized difference between the preclinical AD versus the AD dementia levels of more than ±0.2 and that had a significant FDR-correct P value, q value, of less than 0.05 in the profiling. Cross-validation in both the discovery and replication cohorts was performed, and the predictive performance of all models was assessed by comparing ROC curves and their AUCs. TMT-MS results are on a z-score scale, and, therefore, to ease comparison, the NPX values of the discovery and clinical replication cohorts were z-scored, and the same analyses were applied.

Differential protein expression across the groups in the technical replication cohort was performed using one-way ANCOVA with age, sex and multiple comparison testing correction and FDR, as in the discovery cohort. The replication between the two proteomic techniques was calculated by comparing the patterns of dysregulation for each profile (that is, proteins dysregulated in the same direction in both divided by the total proteins measured in both, as a percentage). The age correlation analyses were performed using the cor.test function from 'stats' (version 3.6.2) in R with the method set on 'spearman' for non-parametric tests on all proteins of interest. All plots were plotted using ggplot2 (version 3.5.1) and circlize for circos plot (version 0.4.16) in R. The figures were compiled in Adobe Illustrator, and icons in Figs. 1 and 3 were extracted from BioRender.

Biological pathway analyses and phenotypic assessment (transcriptomic atlas)

To understand the biological context of the proteins, we analyzed the dysregulated proteins in the three profiles together using clusterProfiler[88] (version 4.10.1) for Gene Ontology enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment using, as background, the entire list of proteins measured in the PEA panels and analyzing the significantly dysregulated proteins per group and the proteins that followed the same trend of dysregulation although insignificantly. Significant Gene Ontology functional terms (P < 0.05) were grouped into general categories based on their main function to broadly understand their functions.

Second, the proteins were assessed based on their overlap with transcriptomic-based microglial phenotypes[18,89,90]. The assessment was done by overlapping results with the microglial transcriptomic atlas derived from single-cell hierarchical Poisson factorization[89,90] clustering on single-cell RNA sequencing of microglial cells of various diseased samples[18,20,44]. The top 100 markers associated with each transcriptomic signature were assigned to 26 groups and subgroups (innate immunity: IL/IFNγ signaling, TLR/MAPK signaling and S100/TLR signaling; disease-associated: APOE^{high} and GPNMB^{high}; antigen presentation: HLA^{high}/APC and C1Q^{high}/phagocytic; metabolic: glycolysis, OxPhos-1, OxPhos-2 and OxPhos-3; inflammation: chemokine, IFN-1 response and immunoregulatory; cellular stress: stress and senescence; homeostatic: CX3CR1^{high}; other: NPY1R^{high}, motility/adhesion, GRID2^{high} and actin folding; and unknown: CIITA^{high} and PLCG2^{high}). In Supplementary Tables 2 and 7, we highlight (in gray) the markers that had any detectable association over score 1 for each signature in the whole dataset (not only the top 100).

The panel of 18 proteins were connected using STRING version 11.5 using the STRINGdb[91] (version 2.14.3) in R, using the score threshold 200 on all interaction sources.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The discovery PEA proteomics data that support the findings of this study are available at https://www.synapse.org/PRIDE_AD. The clinical replication PEA proteomics data that support the findings of this study are available from the DELCODE authors, but restrictions apply to the availability of these data, and so they are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from the cohorts' steering committees (contact for and information on data access: https://www.dzne.de/en/research/studies/clinical-studies/delcode/). The technical replication mass spectrometry proteomics data supporting the findings of this study are available through the ADDI workbench (https://doi.org/10.58085/HR6S-2991).

Code availability

No custom code was developed specifically for this study.

References

1. Andrews, S. J. et al. The complex genetic architecture of Alzheimer's disease: novel insights and future directions. *EBioMedicine* **90**, 104511 (2023).
2. Migliore, L. & Coppede, F. Gene–environment interactions in Alzheimer disease: the emerging role of epigenetics. *Nat. Rev. Neurol.* **18**, 643–660 (2022).
3. Deczkowska, A. et al. Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell* **173**, 1073–1081 (2018).
4. Keren-Shaul, H. et al. A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* **169**, 1276–1290 (2017).
5. Smith, A. M. et al. Diverse human astrocyte and microglial transcriptional responses to Alzheimer's pathology. *Acta Neuropathol.* **143**, 75–91 (2022).
6. Takata, K., Ginhoux, F. & Shimohama, S. Roles of microglia in Alzheimer's disease and impact of new findings on microglial heterogeneity as a target for therapeutic intervention. *Biochem. Pharmacol.* **192**, 114754 (2021).
7. Heneka, M. T. TREM1 is the new kid on the block for the aging-associated innate immune response and Alzheimer's disease. *Nat. Immunol.* **25**, 938–940 (2024).
8. Heneka, M. T. et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* **14**, 388–405 (2015).
9. Heneka, M. T., Golenbock, D. T. & Latz, E. Innate immunity in Alzheimer's disease. *Nat. Immunol.* **16**, 229–236 (2015).
10. Pereira, J. B. et al. Microglial activation protects against accumulation of tau aggregates in nondemented individuals with underlying Alzheimer's disease pathology. *Nat. Aging* **2**, 1138–1144 (2022).
11. Heneka, M. T. et al. Neuroinflammation in Alzheimer disease. *Nat. Rev. Immunol.* **25**, 321–352 (2024).
12. Srinivasan, K. et al. Alzheimer's patient microglia exhibit enhanced aging and unique transcriptional activation. *Cell Rep.* **31**, 107843 (2020).
13. Perry, V. H. & Holmes, C. Microglial priming in neurodegenerative disease. *Nat. Rev. Neurol.* **10**, 217–224 (2014).
14. Rim, C., You, M. J., Nahm, M. & Kwon, M. S. Emerging role of senescent microglia in brain aging-related neurodegenerative diseases. *Transl. Neurodegener.* **13**, 10 (2024).
15. Galatro, T. F. et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat. Neurosci.* **20**, 1162–1171 (2017).
16. Gosselin, D. et al. An environment-dependent transcriptional network specifies human microglia identity. *Science* **356**, eaal3222 (2017).
17. Green, G. S. et al. Cellular communities reveal trajectories of brain ageing and Alzheimer's disease. *Nature* **633**, 634–645 (2024).
18. Olah, M. et al. Single cell RNA sequencing of human microglia uncovers a subset associated with Alzheimer's disease. *Nat. Commun.* **11**, 6129 (2020).
19. Paolicelli, R. C. et al. Microglia states and nomenclature: a field at its crossroads. *Neuron* **110**, 3458–3483 (2022).
20. Marshe, V. S. et al. A factor-based analysis of individual human microglia uncovers regulators of an Alzheimer-related transcriptional signature. Preprint at *bioRxiv* https://doi.org/10.1101/2025.03.27.641500 (2025).
21. Mathys, H. et al. Temporal tracking of microglia activation in neurodegeneration at single-cell resolution. *Cell Rep.* **21**, 366–380 (2017).
22. Gao, C., Jiang, J., Tan, Y. & Chen, S. Microglia in neurodegenerative diseases: mechanism and potential therapeutic targets. *Signal Transduct. Target. Ther.* **8**, 359 (2023).
23. Gerrits, E. et al. Distinct amyloid-β and tau-associated microglia profiles in Alzheimer's disease. *Acta Neuropathol.* **141**, 681–696 (2021).
24. Fan, Z., Brooks, D. J., Okello, A. & Edison, P. An early and late peak in microglial activation in Alzheimer's disease trajectory. *Brain* **140**, 792–803 (2017).
25. Baligacs, N. et al. Homeostatic microglia initially seed and activated microglia later reshape amyloid plaques in Alzheimer's disease. *Nat. Commun.* **15**, 10634 (2024).
26. Guerreiro, R. et al. TREM2 variants in Alzheimer's disease. *N. Engl. J. Med.* **368**, 117–127 (2013).
27. Hok, A. H. Y. S. et al. Neuroinflammatory CSF biomarkers MIF, sTREM1, and sTREM2 show dynamic expression profiles in Alzheimer's disease. *J. Neuroinflammation* **20**, 107 (2023).
28. Huang, W., Huang, J., Huang, N. & Luo, Y. The role of TREM2 in Alzheimer's disease: from the perspective of Tau. *Front. Cell. Dev. Biol.* **11**, 1280257 (2023).
29. Suarez-Calvet, M. et al. Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. *Sci. Transl. Med.* **8**, 369ra178(2016).
30. Suarez-Calvet, M. et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol. Med.* **8**, 466–476 (2016).
31. Morenas-Rodriguez, E. et al. Soluble TREM2 in CSF and its association with other biomarkers and cognition in autosomal-dominant Alzheimer's disease: a longitudinal observational study. *Lancet Neurol.* **21**, 329–341 (2022).
32. Brosseron, F. et al. Multicenter Alzheimer's and Parkinson's disease immune biomarker verification study. *Alzheimers Dement.* **16**, 292–304 (2020).
33. Brosseron, F. et al. Characterization and clinical use of inflammatory cerebrospinal fluid protein markers in Alzheimer's disease. *Alzheimers Res. Ther.* **10**, 25 (2018).
34. Mulenge, F. et al. Transcriptomic analysis unveils bona fide molecular signatures of microglia under conditions of homeostasis and viral encephalitis. *J. Neuroinflammation* **21**, 203 (2024).
35. Del Campo, M. et al. CSF proteome profiling across the Alzheimer's disease spectrum reflects the multifactorial nature of the disease and identifies specific biomarker panels. *Nat. Aging* **2**, 1040–1053 (2022).

36. Del Campo, M. et al. CSF proteome profiling reveals biomarkers to discriminate dementia with Lewy bodies from Alzheimer s disease. *Nat. Commun.* **14**, 5635 (2023).
37. Hok-A-Hin, Y. S. et al. Large-scale CSF proteome profiling identifies biomarkers for accurate diagnosis of frontotemporal dementia. Mol Neurodegener 20, 93 (2025). https://doi.org/10.1186/s13024-025-00882-5
38. van der Ende, E. L. et al. CSF proteomics in autosomal dominant Alzheimer's disease highlights parallels with sporadic disease. *Brain* **146**, 4495–4507 (2023).
39. Martino Adami, P. V. et al. Matrix metalloproteinase 10 is linked to the risk of progression to dementia of the Alzheimer's type. *Brain* **145**, 2507–2517 (2022).
40. Pichet Binette, A. et al. Proteomic changes in Alzheimer's disease associated with progressive Aβ plaque and tau tangle pathologies. *Nat. Neurosci.* **27**, 1880–1891 (2024).
41. Manocha, G. D. et al. APP regulates microglial phenotype in a mouse model of Alzheimer's disease. *J. Neurosci.* **36**, 8471–8486 (2016).
42. de Gea, P. et al. VEGF controls microglial phagocytic response to amyloid-β. *Front. Cell. Neurosci.* **17**, 1264402 (2023).
43. Tjong, W. Y. & Lin, H. H. The RGD motif is involved in CD97/ADGRE5-promoted cell adhesion and viability of HT1080 cells. *Sci. Rep.* **9**, 1517 (2019).
44. Tuddenham, J. F. et al. A cross-disease resource of living human microglia identifies disease-enriched subsets and tool compounds recapitulating microglial states. Nat Neurosci 27, 2521–2537 (2024). https://doi.org/10.1038/s41593-024-01764-7
45. Szklarczyk, D. et al. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* **47**, D607–D613 (2019).
46. Guo, Y. et al. Multiplex cerebrospinal fluid proteomics identifies biomarkers for diagnosis and prediction of Alzheimer's disease. *Nat. Hum. Behav.* **8**, 2047–2066 (2024).
47. Higginbotham, L. et al. Integrated proteomics reveals brain-based cerebrospinal fluid biomarkers in asymptomatic and symptomatic Alzheimer's disease. *Sci. Adv.* **6**, eaaz9360 (2020).
48. Whelan, C. D. et al. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. *Acta Neuropathol. Commun.* **7**, 169 (2019).
49. Ali, M. et al. Multi-cohort cerebrospinal fluid proteomics identifies robust molecular signatures across the Alzheimer disease continuum. *Neuron* **113**, 1363–1379 (2025).
50. Zhao, J. et al. The effect of CX3CL1/ CX3CR1 signal axis on microglia in central nervous system diseases. *J. Neurorestoratol.* **11**, 100042 (2023).
51. Lee, S. et al. *APOE* modulates microglial immunometabolism in response to age, amyloid pathology, and inflammatory challenge. *Cell Rep.* **42**, 112196 (2023).
52. Mrdjen, D. et al. Spatial proteomics of Alzheimer's disease-specific human microglial states. *Nat. Immunol.* **26**, 1397–1410 (2025).
53. Gelir, F. et al. Characterizing heterogeneity in Alzheimer's disease progression: a semiparametric model. *Sci. Rep.* **15**, 7660 (2025).
54. Jack, C. R. Jr. et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. *Alzheimers Dement.* **20**, 5143–5169 (2024).
55. Krasemann, S. et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* **47**, 566–581 (2017).
56. Mancuso, R. et al. Xenografted human microglia display diverse transcriptomic states in response to Alzheimer's disease-related amyloid-beta pathology. *Nat. Neurosci.* **27**, 886–900 (2024).
57. Ali, M. & Bracko, O. VEGF paradoxically reduces cerebral blood flow in Alzheimer's disease mice. *Neurosci. Insights* **17**, 26331055221109254 (2022).
58. Aiyaz, M., Lupton, M. K., Proitsi, P., Powell, J. F. & Lovestone, S. Complement activation as a biomarker for Alzheimer's disease. *Immunobiology* **217**, 204–215 (2012).
59. Zhang, W., Xiao, D., Mao, Q. & Xia, H. Role of neuroinflammation in neurodegeneration development. *Signal Transduct. Target. Ther.* **8**, 267 (2023).
60. Morgan, D. G. & Mielke, M. M. Knowledge gaps in Alzheimer's disease immune biomarker research. *Alzheimers Dement.* **17**, 2030–2042 (2021).
61. DiStasi, M. R. & Ley, K. Opening the flood-gates: how neutrophil-endothelial interactions regulate permeability. *Trends Immunol.* **30**, 547–556 (2009).
62. Zhang, Q. et al. Neuroinflammation in Alzheimer's disease: insights from peripheral immune cells. *Immun. Ageing* **21**, 38 (2024).
63. Raulin, A. C. et al. ApoE in Alzheimer's disease: pathophysiology and therapeutic strategies. *Mol. Neurodegener.* **17**, 72 (2022).
64. Arfaei, R. et al. Decoding the role of the CCL2/CCR2 axis in Alzheimer's disease and innovating therapeutic approaches: keeping all options open. *Int. Immunopharmacol.* **135**, 112328 (2024).
65. Wojcieszak, J., Kuczynska, K. & Zawilska, J. B. Role of chemokines in the development and progression of Alzheimer's disease. *J. Mol. Neurosci.* **72**, 1929–1951 (2022).
66. Buccitelli, C. & Selbach, M. mRNAs, proteins and the emerging principles of gene expression control. *Nat. Rev. Genet.* **21**, 630–644 (2020).
67. Slyper, M. et al. A single-cell and single-nucleus RNA-Seq toolbox for fresh and frozen human tumors. *Nat. Med.* **26**, 792–802 (2020).
68. Liu, Y., Beyer, A. & Aebersold, R. On the dependency of cellular protein levels on mRNA abundance. *Cell* **165**, 535–550 (2016).
69. Farrall, A. J. & Wardlaw, J. M. Blood–brain barrier: ageing and microvascular disease – systematic review and meta-analysis. *Neurobiol. Aging* **30**, 337–352 (2009).
70. Rasmussen, M. K., Mestre, H. & Nedergaard, M. The glymphatic pathway in neurological disorders. *Lancet Neurol.* **17**, 1016–1024 (2018).
71. Frangogiannis, N. G. Biomarkers: hopes and challenges in the path from discovery to clinical practice. *Transl. Res.* **159**, 197–204 (2012).
72. Rifai, N., Gillette, M. A. & Carr, S. A. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat. Biotechnol.* **24**, 971–983 (2006).
73. van der Flier, W. M. & Scheltens, P. Amsterdam Dementia Cohort: performing research to optimize care. *J. Alzheimers Dis.* **62**, 1091–1111 (2018).
74. Albert, M. S. et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* **7**, 270–279 (2011).
75. McKhann, G. M. et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* **7**, 263–269 (2011).
76. Duits, F. H. et al. Four subgroups based on tau levels in Alzheimer's disease observed in two independent cohorts. *Alzheimers Res. Ther.* **13**, 2 (2021).
77. Irwin, D. J. et al. Comparison of cerebrospinal fluid levels of tau and Aβ 1-42 in Alzheimer disease and frontotemporal degeneration using 2 analytical platforms. *Arch. Neurol.* **69**, 1018–1025 (2012).

78. Willemse, E. A. J. et al. Diagnostic performance of Elecsys immunoassays for cerebrospinal fluid Alzheimer's disease biomarkers in a nonacademic, multicenter memory clinic cohort: the ABIDE project. *Alzheimers Dement. (Amst.)* **10**, 563–572 (2018).
79. Hok, A. H. Y. S. et al. Thimet oligopeptidase as a potential CSF biomarker for Alzheimer's disease: a cross-platform validation study. *Alzheimers Dement. (Amst.)* **15**, e12456 (2023).
80. Jao, J. & Ciernia, A. V. MGEnrichment: a web application for microglia gene list enrichment analysis. *PLoS Comput. Biol.* **17**, e1009160 (2021).
81. Skene, N. G. & Grant, S. G. Identification of vulnerable cell types in major brain disorders using single cell transcriptomes and expression weighted cell type enrichment. *Front. Neurosci.* **10**, 16 (2016).
82. Habib, N. et al. Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat. Methods* **14**, 955–958 (2017).
83. Jessen, F. et al. Design and first baseline data of the DZNE multicenter observational study on predementia Alzheimer's disease (DELCODE). *Alzheimers Res. Ther.* **10**, 15 (2018).
84. Janelidze, S. et al. CSF Aβ42/Aβ40 and Aβ42/Abβ38 ratios: better diagnostic markers of Alzheimer disease. *Ann. Clin. Transl. Neurol.* **3**, 154–165 (2016).
85. Tijms, B. M. et al. Pathophysiological subtypes of Alzheimer's disease based on cerebrospinal fluid proteomics. *Brain* **143**, 3776–3792 (2020).
86. McKeith, I. G. et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. *Neurology* **89**, 88–100 (2017).
87. Cairns, N. J. et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol.* **114**, 5–22 (2007).
88. Wu, T. et al. clusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb.)* **2**, 100141 (2021).
89. Levitin, H. M. et al. De novo gene signature identification from single-cell RNA-seq with hierarchical Poisson factorization. *Mol. Syst. Biol.* **15**, e8557 (2019).
90. Levitin, H. M., Zhao, W., Bruce, J. N., Canoll, P. & Sims, P. A. Consensus scHPF identifies cell type-specific drug responses in glioma by integrating large-scale scRNA-seq. Preprint at *bioRxiv* https://doi.org/10.1101/2023.12.05.570193 (2023).
91. Szklarczyk, D. et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* **51**, D638–D646 (2023).

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Author contributions

Conceptualization: E.-R.B., C.E.T. and L.V. Methodology: E.-R.B., C.E.T. and L.V. Proteomic analysis: E.-R.B., P.V.M.-A., E.M.V., B.M.T. and L.V. Transcriptomic data analysis: V.S.M. and P.L.D.J. Project administration and funding acquisition for the clinical cohorts and projects (for example, CSF collection, PEA/TMT-MS runs and clinical data): Y.S.H., W.A.B., D.J.I., A.S.C.-P., A.W.L., Y.P., W.M.v.d.F., O.P., J.H.-R., J.P., A.S., J.W., F.J., E.D., K.B., R.P., S.T., C.L., F.B., DELCODE, M.d.C., P.J-V., B.M.T. and A.R. Writing—original draft: E.-R.B., P.V.M.-A., A.R., C.E.T. and L.V. Writing—reviewing and editing: E.-R.B., P.v.B., P.V.M.-A., V.S.M., E.M.V., Y.S.H., W.A.B., D.J.I., A.S.C.-P., A.W.L., Y.P., W.M.v.d.F., O.P., J.H.-R., J.P., A.S., J.W., F.J., E.D., K.B., R.P., S.T., C.L., F.B., M.d.C., R.W., P.J.-V., B.M.T., P.L.D.J., A.R., C.E.T. and L.V. Visualization: E.R.B. Supervision: C.E.T. and L.V.

Competing interests

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Additional information

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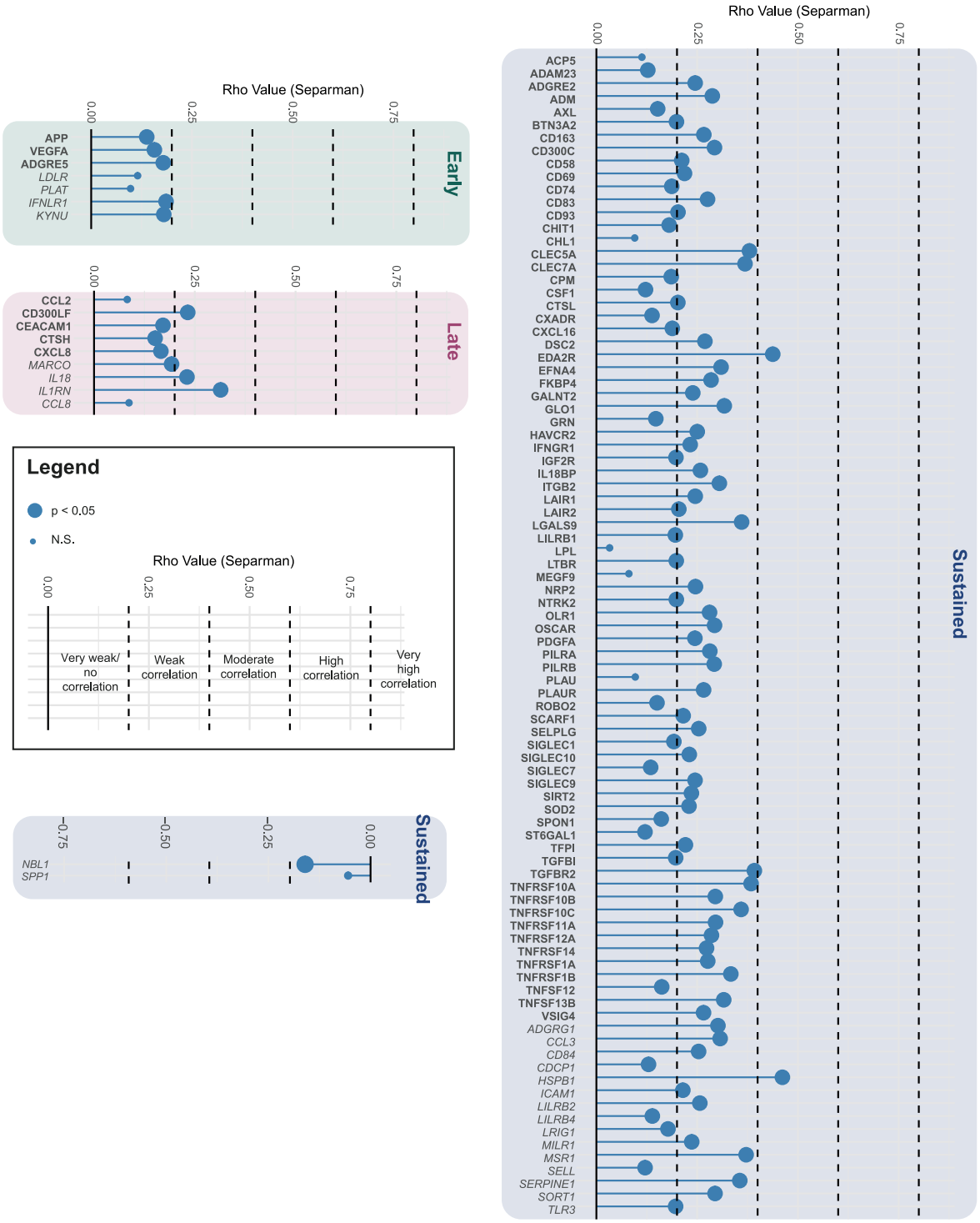

Extended Data Fig. 1 | Age correlations. lollipop plot representing the Spearman rho correlation values of age in the cognitively normal group with negative CSF AD biomarkers for all proteins in the three profiles (7 in the early, 9 in the late, and 93 in the sustained, bold lettering for significant replications and italics for insignificant); extension of Fig. 4c in the main text. Dashed lines delineate the strengths of age-protein R: very weak or none < 0.2, 0.2 > weak < 0.4, 0.4 > moderate < 0.6, 0.6 > strong < 0.8, and 0.8 > very strong < 1. Larger dot represents $p_{FDR} < 0.05$, two-tailed, that is significant, smaller represents insignificant.

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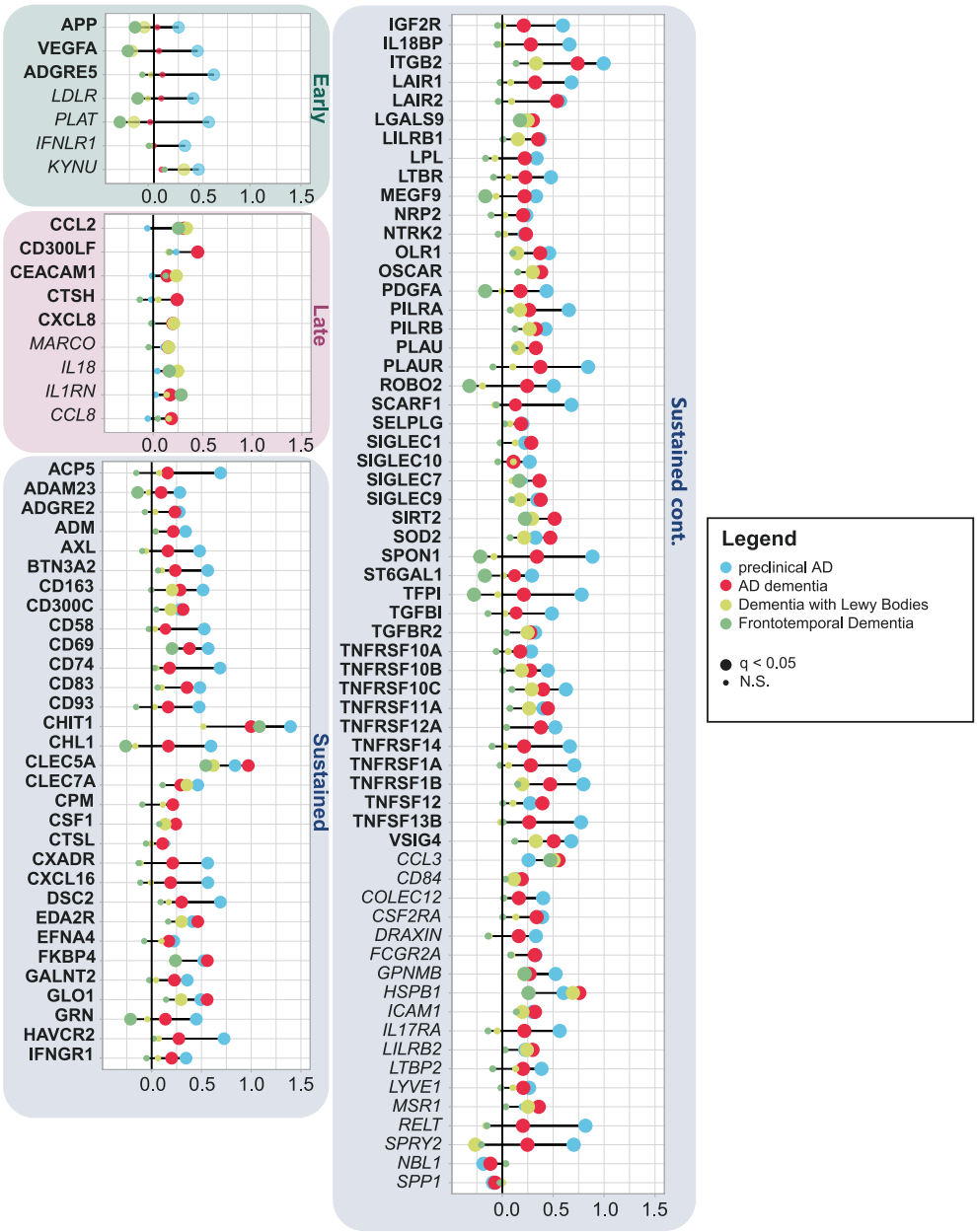

Extended Data Fig. 2 | Assessment across non-AD dementias. lollipop plots showing the $\log_2$(fold change) from the CN baseline of the replicated proteins (7 in the early, 9 in the late, 93 in the sustained, in bold the significantly replicating ones, in italics the non-significant) all within the discovery cohort that includes measures for DLB and FTD patients.

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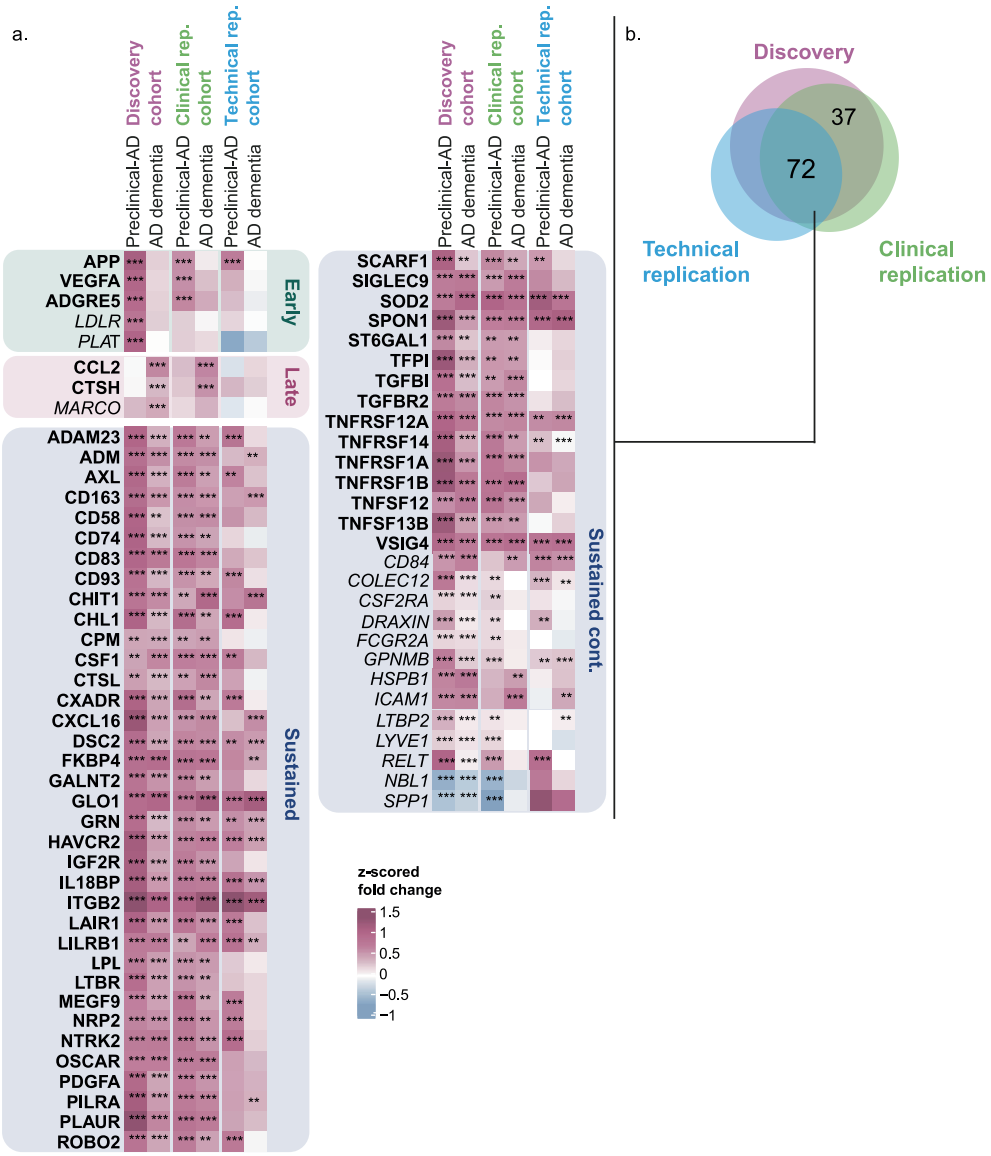

Extended Data Fig. 3 | Replication heatmaps across the three cohorts. **a**. Replication heatmap of the 72 proteins (only from the 109 DEPs, not the total proteins in the cohorts) that are measured in all 3 cohorts over the two proteomic techniques in the analysis (5 out of 7 in the early profile, 3 out of 9 in the late profile, and 64 out of 93 each cohort plotted with the mean z-scored fold change of each protein from the clinical groups stated to the CN baseline. The proteins in bold are the proteins that replicate the patterns. Significant associations are depicted by asterisks. **q < 0.05, ***q < 0.01, ‘ ‘ = N.S. **b**. Venn diagrams of the protein numbers measured in each technique over the three cohorts.

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Software and code

Policy information about availability of computer code

Data collection	No software was used.
Data analysis	R (version 4.3.2) with packages: ggplot2 (3.5.1), stats (3.6.2), ClusterProfiler (4.10.1) and STRINGdb (2.14.3)

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The Discovery PEA proteomics data that support the findings of this study are available at https://www.synapse.org/PRIDE_AD. The clinical replication PEA proteomics data that support this study are not publicly available, but may be provided upon reasonable request to the DELCODE consortium of the DZNE (DZNE). The technical replication mass spectrometry proteomics data support the findings of this study are available through the ADDI workbench (https://fair.addi.ad-datainitiative.org/#/data/datasets/five_csf_proteomic_subtypes_in_ad; https://doi.org/10.58085/HR6S-2991).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex was self-reported. It was included as covariate in analyses specified
Reporting on race, ethnicity, or other socially relevant groupings	Race/ethnicity were not included as covariates in this manuscript. The ADC and DELCODE are relatively homogeneous with the vast majority of White individuals from the Netherlands and Germany
Population characteristics	Information provided in Supplementary table 1
Recruitment	Participants were recruited form the participating memory clinics and provided approved consent as stated in the manuscript.
Ethics oversight	Discovery and technical replication cohorts: Amsterdam UMC, VUmc, AD CSF (biobank METC number 00-211); Clinical replication cohort: University of Bonn (Lfd, Nr. 227/19).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Discovery: n = 553; Clinical replication: n = 281; Technical replication: n = 350
Data exclusions	Proteins with more than 75% of participants having measurements below the limit of detection
Replication	We used three independent cohorts to validate: two with Olink and one with TMT-MS. Analyses were performed once and independently per cohort
Randomization	Randomization was performed during the proteomic measurement of the samples over the various plates per each cohort
Blinding	Proteomic analyses were performed by Olink with blinding to any clinical data.

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Study description	
Research sample	
Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

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Sampling strategy	
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Timing and spatial scale	
Data exclusions	
Reproducibility	
Randomization	
Blinding	

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☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Details about antibody-based proteomic technology is provided by Olink (www.olink.com). ADC: enzyme-linked immunosorbent assay INNOTEST Aβ(1-42), hTAUAg; Fujirebio; Penn: Luminex xMAP INNO-BIA AlzBio3; Luminex
Validation	Extensive validation has been conducted by Olink (www.olink.com), CSF Aβ(1-42), total Tau have been exclusively validated and are routinely used for research and clinical purposes.

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☐	☐	Demonstrate how to render a vaccine ineffective
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☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
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Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Instrument

Software

Cell population abundance

Gating strategy

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Design specifications

Behavioral performance measures

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See Eklund et al. 2016)

Correction

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis