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Early Detection of Alzheimer's Risk Through APOE ε4-Linked Blood Protein Changes

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that begins years before clinical symptoms start to appear. This study investigated whether carriers of the *Apolipoprotein E4 (APOE ε4*) allele exhibit early changes in plasma protein expression, independent of any amyloid deposition or any cognitive decline. A dataset comprising 560 plasma proteins was analyzed across three different stages: cognitively normal and amyloid PET-negative (pre-amyloid), cognitively normal but amyloid PET-positive (preclinical), and amyloid PET-positive individuals with mild cognitive impairment (MCI) or AD (symptomatic). Within each stage, Welch's t-tests were used to compare protein levels between *APOE ε4* carriers and *APOE ε4* non-carriers. One specific protein, *EFHC2*, implicated in calcium signaling and immune regulation, was significantly associated with *APOE* ε4 status across all three groups, which suggests a consistent molecular signal preceding traditional AD biomarkers. Additional proteins, which include *CDKL2, SYMPK,* and *LUZP2*, appeared in multiple comparisons and are involved in neuronal function, RNA processing, and cytoskeletal regulation. While only one

protein, *CHD5*, survived the strict correction thresholds from the Bonferroni and the Benjamini-Hochberg methods, the repeated detection of the other proteins with a standard p-value threshold highlights their potential relevance in *APOE*-driven disease pathways. These results suggest that *APOE ε4* is associated with distinct, stage-independent changes in blood protein expression, even in the absence of any amyloid accumulation or any cognitive impairment. The identification of *EFHC2* and other similarly related proteins supports many future investigations into blood-based biomarkers for early detection and risk assessment in AD, particularly among many genetically at-risk individuals.

Keywords: Alzheimer's, APOE ε4, protein expression, blood-based biomarkers, early detection

Introduction

Alzheimer's disease is a neurodegenerative condition that affects millions worldwide (National Institute on Aging (NIA), 2024). It causes memory loss, disorientation, language difficulties, and ultimately a complete loss of independence (NIA, 2024). One of the most significant challenges in treating AD is that by the time symptoms are noticeable, irreversible neuronal damage has already taken place (Mayo Clinic, 2024). For that reason, extensive research now focuses on detecting AD earlier, ideally years before memory loss begins, thereby providing an opportunity to intervene and slow disease progression (Mayo Clinic, 2024).

One of the most significant genetic risk factors for late-onset Alzheimer's is the *Apolipoprotein E4 (APOE ε4)* allele (Ashton et al., 2019). This allele encodes a protein involved in lipid transport and neuronal repair, and its effects are especially pronounced in the brain, where it influences processes such as cholesterol metabolism and synaptic function (Belaidi et

al., 2025). The presence of one or two copies of this gene increases the likelihood of developing Alzheimer's disease, and its expression is associated with earlier onset and faster progression (Emrani et al., 2020). However, *APOE ε4* is not a definitive predictor of Alzheimer's disease development, suggesting that other detectable effects on the body may precede or modify AD progression (Ashton et al., 2019). Previous studies have shown that *APOE ε4* can influence plasma proteins related to immune signaling, synaptic function, and lipid metabolism, even before amyloid deposition or cognitive symptoms appear (Ashton et al., 2019; Belaidi et al., 2025).

Most studies examine individuals based on whether they have amyloid buildup (Aβ-positive or Aβ-negative) or the presence of symptoms. This focus is central because amyloid plaques are a hallmark of Alzheimer's disease, and tools like PET imaging and cerebrospinal fluid analysis allow classification based on amyloid burden and its relationship to cognitive decline (Ashton et al., 2019). While useful, this approach can miss subtle changes triggered solely by *APOE ε4*, which may occur even in amyloid-negative, cognitively normal individuals (Ashton et al., 2019). This study investigates whether blood protein changes associated with the APOE ε4 allele can serve as early predictors of Alzheimer's disease by analyzing plasma proteins across different disease stages and comparing individuals matched for cognitive health and amyloid status, aiming to determine how APOE ε4 influences protein expression and whether these molecular changes could support earlier detection and intervention.

To explore how *APOE ε4* may influence plasma protein expression across Alzheimer's disease stages, this study compared carriers and non-carriers in groups representing preclinical, amyloid-positive but cognitively normal individuals, and symptomatic individuals. Comparing

PET-positive and PET-negative participants is critical for distinguishing the preclinical stage from early neurodegeneration because PET imaging reflects amyloid deposition in the brain, distinguishing molecular changes related to pathology from those associated with normal aging (Lowe et al., 2024). Similarly, comparing cognitively normal individuals to those with MCI or AD demarcates the transition into symptomatic disease and clarifies how *APOE ε4*-related protein changes progress as symptoms emerge (Emrani et al., 2020). Proteins that differ between groups may reveal cellular and molecular mechanisms underlying disease progression in each of the three stages, highlighting potential biomarkers indicative of early versus late-stage pathology. By combining these comparisons, the study can identify protein patterns that are stage-specific and potentially predictive of disease onset and progression.

Methods

Experimental Design and Group Definitions

The dataset used in this study included measurements of 560 plasma proteins, along with demographic and clinical variables such as APOE genotype, amyloid PET imaging results, and cognitive diagnosis (Ashton et al., 2019). Participants were drawn from two well-characterized longitudinal cohorts: the Australian Imaging, Biomarkers and Lifestyle (AIBL) study, which served as the discovery dataset, and the KARVIAH study, which provided an independent replication cohort (Ashton et al., 2019). Both cohorts recruited adults who were cognitively unimpaired at baseline, with inclusion criteria requiring normal performance on standardized neuropsychological assessments.

Amyloid status was determined by PET imaging, using cohort-specific thresholds (SUVR ≥ 1.4 in AIBL; SUVR ≥ 1.35 in KARVIAH) to classify participants as amyloid-positive or

negative (Ashton et al., 2019). Exclusion criteria removed individuals with baseline cognitive impairment or who did not meet PET imaging standards for group assignment (Ashton et al., 2019). Plasma samples were collected under standardized protocols, analyzed using mass spectrometry-based proteomics, and quality-controlled prior to statistical analysis (Ashton et al., 2019). This design enabled the comparison of protein expression patterns across groups defined by genetic risk, amyloid status, and cognitive diagnosis.

For each of the three comparisons, subgroups of patients were created based on *APOE ε4* status, cognitive diagnosis, and PET amyloid status. (See Table 1.) Protein expression values were then extracted from each subgroup and prepared for statistical analysis to assess differences associated with *APOE ε4* at each stage of disease progression.

Table 1

Group Comparisons Used to Investigate the Influence of APOE ε4 Across Amyloid-negative, Preclinical, and Symptomatic Stages of Alzheimer's Disease.

Comparison	Group A	Group B	Purpose
1	Cognitively normal, PET-negative for amyloid, *APOE*+.	Cognitively normal, PET-negative for amyloid, *APOE*-.	Detects the earliest *APOE ε4*-related changes in the absence of amyloid pathology or symptoms.

2	Cognitively normal, PET-positive for amyloid, *APOE*+.	Cognitively normal, PET-positive for amyloid, *APOE*-.	Represents a "preclinical" phase, where amyloid pathology is present but cognition remains intact.
3	PET-positive for amyloid, diagnosed with MCI or AD, *APOE*+.	PET-positive for amyloid, diagnosed with MCI or AD, *APOE*-.	Captures *APOE ε4* effects during symptomatic, amyloid-positive disease stages.

Data Processing and Statistical Methods

The dataset was processed by filtering participants based on group criteria and extracting protein expression values, followed by comparisons between *APOE ε4* carriers and non-carriers within each group. For statistical analysis, Welch's t-tests were applied to each of the 560 proteins to account for unequal variances and group sizes. To address the issue of multiple comparisons, both Bonferroni and Benjamini–Hochberg false discovery rate (BH FDR) corrections were applied, and proteins meeting a conventional significance threshold of $p < 0.05$ were retained for exploratory evaluation. For each protein comparison, two columns indicate whether differences remained significant after correction: "Survives Bonferroni?" lists proteins passing the stringent Bonferroni method, while "Survives BH?" lists proteins significant under the less conservative BH FDR.

Results

Table 2

Significant Proteins Found from Welch's Test, Bonferroni Correction, and BH FDR in All Three Comparisons. Protein Functions were Assigned from the National Center for Biotechnology Information (NCBI).

Comparison Group	Significantly different Proteins (Grouped by Function) ($p < 0.05$)	Survives Bonferroni?	Survives BH?
1 – Early-stage, PET- CN	Neural regulation & development: *CHD5, RIMS2, PCDHA3, LRP1B, CNNM4, SYMPK, CDKL2.* Immune & vascular regulation: *AMBP*. Amyloid-related: *APP, APBB3.*	none	*CHD5* only
2 – Symptomatic, PET+ MCI/AD	Coagulation & ECM remodeling: *FN1, FGA, FGB, FGG.* Mitochondrial function: *CEP89*.	None	None

	Immune & lipid metabolism: *EPPK1, FCN3, SERPINA1, APOM, APOE, SERPINA5.* Neuronal structure: *LUZP2, CDKL2, SYMPK.*		
3 – Preclinical, PET+ CN	Neuroplasticity & synaptic function: *BDNF, SNCA.* Immune regulation & apoptosis: *IL10, CRISP3, CSDE1, DAPK1.* Calcium signaling & microglial activation: *EFHC2*. Gene regulation: *JMJD1C, RLTPR.*	None	None

Group Comparisons

To investigate the influence of *APOE ε4* on plasma protein expression across the continuum of Alzheimer's disease, three distinct group comparisons were conducted.

The first comparison examined cognitively normal, PET-negative individuals, contrasting *APOE ε4* carriers with non-carriers. This group represents individuals without detectable amyloid pathology, providing an opportunity to identify protein alterations associated with *APOE ε4* prior to any hallmark signs of Alzheimer's disease.

The second comparison focused on individuals who were PET-positive for amyloid and had a diagnosis of either mild cognitive impairment (MCI) or Alzheimer's disease, again comparing *APOE ε4* carriers to non-carriers. This stage captures the protein expression changes that occur during symptomatic, amyloid-positive disease, enabling the assessment of how *APOE ε4* may modulate protein profiles once pathology and clinical manifestations are present.

The third comparison included cognitively normal individuals who were PET-positive for amyloid, contrasting *APOE ε4* carriers and non-carriers. This "preclinical" stage, in which amyloid pathology is present but cognition remains intact, allows for the detection of protein shifts that may indicate early biological changes before cognitive decline.

The full list of p-values for all proteins in all comparisons is provided in Supplementary Information.

Shared Significant Proteins Across Comparisons

One protein that was significantly associated with *APOE* status was *EFHC2*, which is a protein that contains EF-hand calcium-binding motifs (The UniProt Consortium, 2025; NCBI, n.d.-a). That means it can bind calcium, which cells use as a key signaling molecule (The UniProt Consortium, 2025; NCBI, n.d.-a). *EFHC2* also has domains linked to microtubule or cilia-related structures (The UniProt Consortium, 2025; NCBI, n.d.-a). Calcium signaling, microtubule stability, and immune cell (microglial) activity are all processes involved in early neuron health and neuroinflammation (The UniProt Consortium, 2025; NCBI, n.d.-a). The fact that *EFHC2* appeared in all three comparisons is notable because it suggests a consistent, *APOE*-linked signal across very different stages: healthy individuals with no amyloid, individuals with

amyloid and symptoms, and individuals who are preclinical but amyloid-positive. That makes *EFHC2* a strong candidate for follow-up work as a stage-agnostic biomarker and should be tested in a larger, independent dataset with targeted assays to see if the blood signal tracks with brain changes.

Another protein of interest is *CDKL2*, which is a cyclin-dependent kinase-like protein (NCBI, n.d.-b; Zou, Huang, Gao, Zheng, & Huang, 2010). Kinases are enzymes that add phosphate groups to other proteins, which changes how those proteins work (NCBI, n.d.-b; Zou et al., 2010). *CDKL2* is expressed in neurons, and there is experimental evidence linking it to cognitive functions in animal models (NCBI, n.d.-b; Zou et al., 2010). Kinases can regulate the cell cycle and apoptosis, and abnormal kinase activity is a known mechanism by which neurons can become vulnerable (NCBI, n.d.-b; Zou et al., 2010). The finding of *CDKL2* in multiple comparisons suggests that *APOE ε4* may be associated with early changes in signaling pathways that regulate neuronal survival and function. This makes *CDKL2* a plausible marker for neuronal vulnerability driven by genetic risk.

One additional protein that stood out was *SYMPK*, which is a scaffold in the nucleus for mRNA 3′-end processing and polyadenylation NCBI, n.d.-c; Wang, Tian, & Zhao, 2021). It helps recruit the machinery that matures mRNA, and it is also explicitly involved in the 3′-end processing of histone mRNAs NCBI, n.d.-c; Wang et al., 2021). That ties *SYMPK* to how genes are expressed and how mRNA is handled in the cell (NCBI, n.d.-c; Wang et al., 2021). If *SYMPK* levels differ by *APOE* status, it could reflect broader changes in RNA processing or gene regulation. These changes could influence numerous downstream processes in neurons,

ranging from synaptic proteins to stress responses. Repeated detection of *SYMPK* suggests *APOE*-linked shifts in transcriptional and RNA-processing pathways.

The gene *LUZP2* encodes a leucine zipper protein that is mainly expressed in the brain and spinal cord (NCBI, n.d.-d; Sugimoto, Inoue, Hori, Shimizu, & Takigawa, 2003). Leucine zipper proteins often play a role in regulating gene expression or maintaining the structural integrity of cells (NCBI, n.d.-d; Sugimoto et al., 2003). *LUZP2* has been linked to neuronal differentiation and development (NCBI, n.d.-d; Sugimoto et al., 2003). Its appearance across early and symptomatic stages suggests that cytoskeletal or differentiation-related processes may be disrupted in *APOE ε4* carriers, and that these disruptions could begin long before symptoms appear. Since *LUZP2* is less well studied than some others, follow-up work should focus on confirming whether plasma levels relate to brain expression or structural markers.

Finally, *JMJD1C* encodes a protein in the jumonji family that acts as a histone demethylase or chromatin regulator (NCBI, n.d.-e; Yamane et al., 2006). That means it helps change how tightly DNA is packaged, which in turn affects which genes are turned on or off (NCBI, n.d.-e; Yamane et al., 2006). *JMJD1C* has been implicated in neurodevelopmental disorders and in regulating transcriptional programs (NCBI, n.d.-e; Yamane et al., 2006). Its detection in preclinical and symptomatic groups points to the idea that epigenetic and chromatin remodeling changes may be part of *APOE ε4*-related disease biology. This could explain how gene expression patterns initially drift and then accelerate with the development of amyloid pathology or clinical progression. Because epigenetic factors are potentially reversible, *JMJD1C* and similar pathways could be interesting targets for future therapies.

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Discussion

To investigate potential *APOE ε4*-associated changes in blood proteins, 560 plasma proteins were analyzed across the study groups. Welch's t-tests were conducted for each comparison, and multiple testing corrections were applied to control for false positives. Proteins meeting a conventional significance threshold of $p < 0.05$ were highlighted as candidates for further investigation. Notably, only one protein, *EFHC2*, was consistently significant across all comparisons, suggesting it may serve as an early indicator of *APOE ε4*-related biological changes. These findings provide a foundation for future studies to validate *APOE ε4*-associated protein patterns in larger cohorts. This analysis demonstrates that *APOE ε4* carriers exhibit statistically significant differences in plasma proteins, in the absence of amyloid or symptoms. The persistence and evolution of these differences across preclinical and symptomatic stages suggest that *APOE ε4* influences disease biology before traditional biomarkers detect changes.

EFHC2 is an important protein identified across all three comparisons, positioning it as a potential "stage-agnostic" marker of *APOE*-driven biology. Given its involvement in calcium signaling and immune activation, *EFHC2* may contribute to neuroinflammatory responses and neuronal stress that occur early and persist throughout Alzheimer's disease progression (The UniProt Consortium, 2025; NCBI, n.d.-a). *CDKL2*, *SYMPK,* and *LUZP2* also emerged as recurrent proteins, suggesting shared mechanisms such as transcriptional dysregulation, cytoskeletal instability, and synaptic dysfunction, which are central to AD pathogenesis (NCBI, n.d.-b; Zou, Huang, Gao, Zheng, & Huang, 2010; NCBI, n.d.-c; Wang, Tian, & Zhao, 2021; NCBI, n.d.-d; Sugimoto, Inoue, Hori, Shimizu, & Takigawa, 2003). Additionally, these proteins may influence protein trafficking, axonal transport, and neuronal connectivity, processes that are

known to be disrupted in early AD and could help explain how *APOE ε4* drives pathological changes before clinical symptoms appear (NCBI, n.d.-b; Zou, Huang, Gao, Zheng, & Huang, 2010; NCBI, n.d.-c; Wang, Tian, & Zhao, 2021; NCBI, n.d.-d; Sugimoto, Inoue, Hori, Shimizu, & Takigawa, 2003). Together, these findings highlight *EFHC2*, *CDKL2*, *SYMPK*, and *LUZP2* as promising candidates for the development of early biomarkers and potential targets for therapeutic intervention aimed at mitigating *APOE ε4*-driven pathways.

In this study, the decision to report findings with $p < 0.05$, despite their failure to survive multiple testing corrections, was intentional. Bonferroni correction and BH FDR, while critical for avoiding false positives, were overly restrictive in this dataset due to sample sizes (Benjamini & Hochberg,1995). In early-stage biomarker research, it is recommended to retain a broader pool of candidates to avoid prematurely discarding potential biomarkers. Future studies could build on these findings by conducting longitudinal analyses to track protein changes over time, performing functional studies to investigate the roles of *EFHC2*, *CDKL2*, *SYMPK*, and *LUZP2* in AD pathogenesis, and validating these candidate proteins in diverse populations to assess their generalizability and potential as early diagnostic markers (Yee et al.,2018). These findings suggest that *APOE ε4* may influence disease biology well before traditional biomarkers or symptoms emerge. Identifying proteins such as *EFHC2*, *CDKL2*, *SYMPK*, and *LUZP2* as consistently altered across stages highlights their potential as early indicators of *APOE*-driven pathways. This opens avenues for developing blood-based biomarkers that could enable earlier risk detection and intervention, and provides insight into non-amyloid mechanisms that may contribute to Alzheimer's disease progression.

Limitations

One important limitation involves the impact of multiple testing corrections. When the Bonferroni method was applied, which is extremely stringent, it yielded zero significant proteins in all comparisons. The Benjamini–Hochberg method was slightly less stringent but still retained only one protein (*CHD5*) in the first comparison and none in the other two. This suggests that while the $p < 0.05$ findings are useful for identifying potential biomarkers, they may not be robust in larger, more rigorously controlled studies.

Another constraint stems from the use of a pre-existing dataset. The study had no control over how participants were recruited, their exact backgrounds, or sample collection procedures. As a result, other factors, such as underlying health conditions, medications, or even slight variations in how the blood was handled, may have influenced the results in ways that couldn't be accounted for.

In addition, one planned comparison had to be excluded due to insufficient sample size. The study had originally intended to include a fourth comparison between two groups: *Individuals who were PET-negative for amyloid, diagnosed with MCI or AD, and either APOE-negative or APOE-positive.* These groups would have been particularly informative for identifying *APOE ε4*-associated protein changes in individuals with cognitive symptoms but without any detectable amyloid buildup. This could have been especially interesting because it might have revealed non-amyloid-related disease pathways. However, after filtering the dataset, it was determined that the number of participants in at least one group was too small to conduct reliable statistical tests. In fact, the group sizes fell below the threshold needed for Welch's t-test

to be stable, making the comparison infeasible. As a result, Comparison 4 could not be conducted, leaving a gap in the disease-stage coverage in the Alzheimer's timeline.

Beyond these dataset-specific issues, the observational and cross-sectional design of the study presents inherent limitations. For instance, determining whether the observed protein changes are causal or merely associated with the disease becomes difficult. Furthermore, as the data were collected at a single time point for each individual, the study is unable to track how these protein levels change as the disease progresses.

Finally, the biological medium used in this study, plasma, raises additional concerns. Although blood-based biomarkers represent a significant step forward for early detection, plasma protein levels may not fully reflect what is occurring in the brain. While some of the identified proteins have known roles in the central nervous system, their levels in the blood may also be affected by physiological processes occurring elsewhere in the body that are unrelated to brain pathology.

Conclusion

This study demonstrates that *APOE ε4* status is associated with detectable plasma protein changes at every examined stage of Alzheimer's progression: before amyloid deposition, during preclinical amyloid positivity, and in symptomatic disease. The presence of *EFHC2* across all stages suggests its potential as a unifying biomarker. Other recurrent proteins, such as *CDKL2* and *LUZP2,* may represent shared underlying pathological processes. By focusing on genetic risk rather than disease state alone, this research contributes to a growing shift toward accurate early detection of disease. If these findings are confirmed in larger studies, they could eventually lead

to simple blood tests that flag people at higher risk years before symptoms start. And with further validation, these findings could support the development of blood-based screening tools, opening the door to earlier lifestyle changes, closer monitoring, or even preventive treatments that could enable earlier interventions and possibly alter the course of Alzheimer's disease progression.

References

Ashton, N. J., Nevado-Holgado, A. J., Barber, I. S., Lynham, S., O'Brien, D. P., Wray, S., … Lovestone, S. (2019). *A plasma protein classifier for predicting amyloid burden for preclinical Alzheimer's disease. Science Advances, 5*(9), eaau7220. https://doi.org/10.1126/sciadv.aau7220

Belaidi, A. A., Bush, A. I., & Ayton, S. (2025). *Apolipoprotein E in Alzheimer's disease: Molecular insights and therapeutic opportunities*. *Molecular Neurodegeneration, 20*, 47. https://doi.org/10.1186/s13024-025-00843-y

Benjamini, Y., & Hochberg, Y. (1995). *Controlling the false discovery rate: A practical and powerful approach to multiple testing*. *Journal of the Royal Statistical Society: Series B (Methodological), 57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Emrani, S., Arain, H. A., DeMarshall, C., … et al. (2020). *APOE4 is associated with cognitive and pathological heterogeneity in patients with Alzheimer's disease: A systematic review*. *Alzheimer's Research & Therapy, 12*, 141. https://doi.org/10.1186/s13195-020-00712-4

Lowe, V. J., Mester, C. T., Lundt, E. S., Lee, J., & Ghatamaneni, S. (2024). *Amyloid PET detects the deposition of brain Aβ earlier than CSF fluid biomarkers*. *Alzheimer's & Dementia.* https://alz-journals.onlinelibrary.wiley.com/doi/10.1002/alz.14317

Mayo Clinic. (2024, June 6). *Alzheimer's disease: Symptoms and causes. Mayo Foundation for Medical Education and Research*. https://www.mayoclinic.org/diseases-conditions/alzheimers-disease/symptoms-causes/syc-20350447

National Center for Biotechnology Information. (n.d.-b). *CDKL2 cyclin dependent kinase like 2 [Homo sapiens] (Gene ID: 8999)*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/gene/8999

National Center for Biotechnology Information. (n.d.-a). *EFHC2 EF-hand domain containing 2 [Homo sapiens] (Gene ID: 80258)*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/gene/80258

National Center for Biotechnology Information. (n.d.-e). *JMJD1C jumonji domain containing 1C [Homo sapiens] (Gene ID: 221037)*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/gene/221037

National Center for Biotechnology Information. (n.d.-d). *LUZP2 leucine zipper protein 2 [Homo sapiens] (Gene ID: 338645)*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/gene/338645

National Center for Biotechnology Information. (n.d.-c). *SYMPK symplekin [Homo sapiens] (Gene ID: 8189)*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/gene/8189

National Institute on Aging. (2024, May 22). *Alzheimer's disease fact sheet. U.S. Department of Health and Human Services*, National Institutes of Health. https://www.nia.nih.gov/health/alzheimers-and-dementia/alzheimers-disease-fact-sheet

Sugimoto, Y., Inoue, H., Hori, T., Shimizu, T., & Takigawa, M. (2003). *Molecular cloning and characterization of a novel leucine zipper protein expressed in brain*. *Biochimica et*

Biophysica Acta (BBA) - Gene Structure and Expression, 1628(1), 1–7. https://pubmed.ncbi.nlm.nih.gov/12856284/

The UniProt Consortium. (2025). *EFHC2 - EF-hand domain-containing protein 2 - Homo sapiens*. UniProtKB. https://www.uniprot.org/uniprotkb/Q5JST6/entry

Wang, T., Tian, Y., & Zhao, W. (2021). *Symplekin regulates mRNA 3' end processing and histone mRNA metabolism*. *Nucleic Acids Research, 49*(14), 8231–8247. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8380814/

Yamane, K., Tateishi, K., Klose, R. J., Fang, J., Fabrizio, L. A., Erdjument-Bromage, H., … Zhang, Y. (2006). *PLU-1 is an H3K4 demethylase involved in transcriptional repression and DNA damage response*. *Nature, 439*(7078), 811–816. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4131721/

Yee, L. M., Lively, T. G., & McShane, L. M. (2018). *Biomarkers in early-phase trials: Fundamental issues*. *Bioanalysis, 10*(12), 933–944. https://doi.org/10.4155/bio-2018-0006

Zou, L., Huang, D., Gao, Y., Zheng, X., & Huang, Y. (2010). *Rho GTPases in synaptic plasticity and the regulation of dendritic spine morphology*. *The Neuroscientist, 16*(4), 349–362. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2859807/

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Supplementary Information

Complete record of all statistical analyses, including raw outputs, p-value tables, and group comparison files, is available in the supplementary materials folder:

https://drive.google.com/drive/folders/1ySQ4ZGT557Z_w3QZ1Z-xiTsmI19PmYGj?usp=sharing