1 **Integrative Network Analysis Reveals Novel Moderators of Aβ-Tau Interaction in**

2 **Alzheimer's Disease**

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Abstract

Background: Although interactions between amyloid-beta and tau proteins have been implicated in Alzheimer's disease (AD), the precise mechanisms by which these interactions contribute to disease progression are not yet fully understood. Moreover, despite the growing application of deep learning in various biomedical fields, its application in integrating networks to analyze disease mechanisms in AD research remains limited. In this study, we employed BIONIC, a deep learning-based network integration method, to integrate proteomics and protein–protein interaction data, with an aim to uncover factors that moderate the effects of the Aβ-tau interaction on mild cognitive impairment (MCI) and early-stage AD.

Methods: Proteomic data from the ROSMAP cohort were integrated with protein–protein interaction (PPI) data using a Deep Learning-based model. Linear regression analysis was applied to histopathological and gene expression data, and mutual information was used to detect moderating factors. Statistical significance was determined using the Benjamini-Hochberg correction ($p < 0.05$).

Results: Our results suggested that astrocytes and GPNMB+ microglia moderate the Aβ-tau interaction. Based on linear regression with histopathological and gene expression data, GFAP and IBA1 levels and *GPNMB* gene expression positively contributed to the interaction of tau with Aβ in non-dementia cases, replicating the

34   results of the network analysis.

35   **Conclusions**: These findings indicate that GPNMB+ microglia moderate the Aβ-tau

36   interaction in early AD and therefore are a novel therapeutic target. To facilitate further

37   research, we have made the integrated network available as a visualization tool for the

38   scientific community (URL: https://igcore.cloud/GerOmics/AlzPPMap).

39

40   **Keywords**

41   Alzheimer's disease, Tau, Amyloid β, microglia, astrocyte, GPNMB, proteomics,

42   network integration, moderator, interaction effects

43

44   **Background**

45   Alzheimer's disease (AD) is the most common form of dementia,

46   characterized by extracellular amyloid-beta (Aβ) plaques and intracellular tau protein

47   tangles, which are believed to be central to its pathology.[1–3] Recent studies have

48   reported an interaction between Aβ and tau proteins in the pathogenesis of AD,

49   contributing to disease progression.[4–9] For instance, Lee et al.[7] revealed that

50   Aβ-tau interactions are associated with the propagation of tau. In practice, lecanemab,

51   an anti-Aβ antibody, which has been shown to slow cognitive decline in patients with

52   early-stage AD, reduces total tau protein and P-Tau181 levels in cerebrospinal

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53 fluid.[10] This suggests that Aβ contributes to the effect of tau in AD. Moreover, several

54 phenotypes are associated with the Aβ-tau interaction, such as astrocyte reactivity,

55 blood pressure, vascular burden, and microglia[11–15]; however, the detailed

56 mechanisms are not fully understood. Various cellular perturbations have been

57 identified through single-nucleus RNA sequencing (snRNA-seq) in AD.[16–22] For

58 example, a recent snRNA-seq study[17] revealed the presence of Trem2-dependent

59 disease-associated microglia (DAM) and a unique Serpina3n+C4b+ reactive

60 oligodendrocyte population in 5XFAD mice. In human AD, distinct glial phenotypes

61 including *IRF8*-driven reactive microglia and oligodendrocytes with impaired

62 myelination and metabolic adaptation to neuronal degeneration have been observed.

63 However, the specific cell types and effects of Aβ-tau interactions are yet to be

64 elucidated.

65   Network analyses based on gene or protein co-expression have revealed

66 gene groups and associated pathways correlated with pathological and clinical factors

67 in AD.[23–25] Additionally, network integration methods based on multi-omics and

68 other data types have been developed.[26–29] By integrating both physical and

69 functional interactions simultaneously, network integration analyses can identify

70 detailed molecular relationships. However, this approach has largely been applied to

71 cancer. AD is a complex disease involving various pathologies, and network

72   integration is a promising approach for determining comprehensive physical and

73   functional interactions involving Aβ and tau in AD.

74   In this study, we investigated moderating factors in the Aβ-tau interaction

75   using a systematic network integration approach. The aim of this study was to

76   elucidate the cell types and moderating factors in the interaction between Aβ and tau

77   proteins in early-stage AD. Figure 1 shows the graphical abstract of the study. First, we

78   integrated the proteomics and protein–protein interaction (PPI) networks by using

79   BIONIC,[30] a network integration algorithm. In the downstream analysis, glial

80   cell-related proteins, including those associated with microglia and astrocytes, were

81   enriched around *APP* and *MAPT* subnetworks, which encode the precursors of Aβ

82   and tau, respectively. Further analyses of microglial subtypes revealed enrichment in

83   GPNMB+ microglia. As a validation, linear regression analysis based on

84   histopathological and gene expression data showed that interactions between Aβ and

85   IBA1, GFAP, and *GPNMB*, were positively related to tau levels. Our findings highlight

86   that deep learning-based network integration can serve as a novel framework for

87   elucidating complex disease mechanisms. The integrated network, made available as

88   a visualization tool for the scientific community, not only enhances our understanding

89   of the Aβ-tau interaction but also provides a basis for generating experimental

90   validation ideas and developing more effective treatments for AD. By leveraging this

91   tool, researchers can gain insights into the intricate molecular interactions at play in

92   AD, fostering the discovery of new therapeutic targets.

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94

95   **Methods**

96   **Proteomics data from the ROSMAP cohort**

97   For the proteomic analysis, ROSMAP tandem mass tag (TMT) proteomics data were

98   obtained. TMT proteomics is a technique for the simultaneous quantitative analysis of

99   protein expression in multiple samples using LC-MS/MS and is commonly employed

100   in AD research.[24,25] The ROSMAP data set included TMT proteomics data for 400

101   cases with differences in cognitive status (Alzheimer's dementia, other dementia, MCI,

102   or no impairment). Data normalization and batch correction were conducted according

103   to previous described procedures.[31] Four outlier samples were excluded from

104   subsequent analyses based on a principal component analysis. In this study,

105   ROSMAP TMT data for MCI and early AD cases were included. Early AD was defined

106   according to previously reported criteria: meeting the NIA-AA core clinical criteria for

107   probable AD dementia and having a global CDR score of 0.5 to 1.0 and a CDR

108   memory box score of 0.5 or greater at screening and baseline.[10] Additionally, the

109   MMSE effectively discriminates between CDR stages 0.5, 1, 2, and 3 but performs

110   poorly in the separation of CDR stages zero and 0.5. The previously established

111   MMSE ranges were 30 for no, 26–29 for questionable, 21–25 for mild, 11–20 for

112   moderate, and 0–10 for severe dementia.[32] We defined early AD as having an

113   MMSE score of ≥21.

114

115   **Proteomics and PPI data integration using a Graph Attention Network-based**

116   **method**

117   To integrate the proteomics and PPI data, total values were calculated for each gene

118   symbol in the proteomics dataset. Pearson correlation coefficients were derived from

119   the proteomics data, retaining only data with Benjamini-Hochberg (BH)-corrected

120   p-values of <0.05. Normalization was performed to set maximum values to 1 and

121   minimum values to 0. For PPI, STRING[33] filtering was used to obtain physical

122   interactions within *Homo sapiens* with confidence scores exceeding 0.7, and

123   normalization was performed to set the maximum values to 1 and minimum values to 0.

124   Proteomic and PPI data were integrated using a recently reported method,

125   BIONIC,[30] which integrates multiple biological networks while preserving

126   information using graph attention networks.[34] Default values were used for the

127   BIONIC hyperparameters. For both proteomics and PPI data, 4,787 proteins common

128   to both datasets were retained. After integrating the two networks, 512-dimensional

129 features were output for each protein.

130

Clustering analysis and dimension reduction using features derived from

BIONIC

Considering that the data derived from neural networks are nonlinear, clustering was

performed following a method similar to that used in snRNA-Seq analyses.[35] After

feature calculation using BIONIC and subsequent scaling to Z-scores, Euclidean

distances were computed to create a distance matrix. Then, setting k = 25, the

k-nearest neighbor method was used to calculate an adjacency matrix and form a

graph structure. This graph structure was then subjected to clustering using the

Louvain method[36] (resolution = 1) to classify each protein into clusters.

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Cluster centroid analysis using BIONIC-derived features

After implementing BIONIC to evaluate the networks derived from proteomic and PPI

data, a clustering analysis of proteins was performed. To analyze the relationships

between clusters, the average of the feature values for each cluster was calculated as

its centroid. Pearson correlation coefficients and Euclidean distances between the

clusters were calculated. Furthermore, the network of clusters was visualized using

the reciprocal values of the Euclidean distances as the weights of the edges.

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Cell type enrichment analysis based on human brain and microglia snRNA-seq

data

For a cell type enrichment analysis, publicly available human brain[37] and

microglia[21,22] snRNA-seq data were used. Cell annotations for each dataset were

based on publicly available data. The AddModuleScore function was performed using

the Seurat package[38] to calculate the average expression levels in each protein

community. The combined score was calculated by multiplying the expression level by

the proportion of positive cells in each community.

157

Cell type composition analysis based on human brain and microglia

snRNA-seq data

For a cell type composition analysis, publicly available human microglia[21,22]

snRNA-seq data were used. ANOVA was used to compare the frequencies of different

microglial subtypes across various conditions (Control, Aβ+, and Aβ+Tau+). Next, for

the microglial subtypes "Microglia_GPNMB_LPL" and "Microglia_GPNMB_PLAT," a

Tukey HSD test was used to determine specific differences between groups (for cases

where ANOVA showed $p < 0.05$).

166

Gene Ontology functional enrichment analysis

A Gene Ontology (GO) analysis was used to explore the biological functions of our

targeted gene set. The GO biological process (GObp) dataset version 2023.1 was

downloaded from the Molecular Signatures Database (MsigDB)

(https://www.gsea-msigdb.org/gsea/msigdb). For the analysis, the enrichment

function in the clusterProfiler package was used, and values of p < 0.05, after BH

correction, were considered significant. The Rrvgo[39] software was used to

summarize the GO terms.

Modulators of the Aβ-tau interaction

To explore modulators of the Aβ-tau interaction based on proteomics data, a slightly

modified version of the MINDy algorithm[40] was employed. Briefly, (1) Euclidean

distances for APP and MAPT were calculated using BIONIC features, and common

elements within the top 5% with respect to proximity were selected for analyses. (2)

For each candidate modulator, the top and bottom 35% of proteins with respect to

expression levels were segregated, and mutual information for APP and Tau was

calculated for each group. Mutual information, which measures the interdependence

between two random variables X and Y, was defined using equation (1) as follows:

$$MI(X, Y) = \sum x \in X \sum y \in Y p(x, y) \log \big(p(x) p(y) p(x, y) \big) \# (1)$$

185 Here,

186 *p(x,y)* represents the joint probability distribution of *X* and *Y*.

187 *p(x)* and *p(y)* are the marginal probability distributions of *X* and *Y*, respectively.

188 The logarithm base 2 was used.

189 Subsequently, (3) the difference in mutual information between the top and bottom

190 35% groups was calculated using equation (2):

$$\Delta MI = |MI(MAPTMtop35, APPMtop35) - MI(MAPTMbottom35, APPMbottom35)| \#(2)$$

191 Here, M_{top35} and $M_{bottom35}$ indicate the APP and MAPT groups in the top 35% and

192 bottom 35% of protein expression levels, respectively. This differentiation is crucial for

193 understanding the difference in mutual information between the two groups, allowing

194 for a clear analysis of the Aβ-Tau interaction modulators. Bootstrapping with 1,000

195 iterations was then used to compare mutual information, with values of $p < 0.05$

196 deemed significant.

197 Next, factors moderating Aβ-Tau interactions were extracted using data obtained from

198 BIONIC and a network analysis was conducted. Among candidate modulators

199 identified in the MINDy analysis, HSPA5, which is closely associated with Aβ[41] and

200 Tau[42], was included in the analysis. The inner product of the BIONIC-derived

201 features was calculated to determine protein similarity, retaining the top 5% for

202 network construction. From this network, the top 5% of factors based on Euclidean

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203   distances to *APP*, *MAPT*, and *HSPA5* were retained to form a subnetwork. Community

204   detection within this subnetwork was performed using the leading eigenvector function

205   of the iGraph package.[43]

206

207   **Neuropathological and RNA-seq datasets**

208   The neuropathological and RNA-Seq datasets used in this study were obtained from

209   previously published works.[44] Neuropathological data include quantitative

210   immunostaining results for the microglial marker IBA1, astrocyte marker GFAP, Aβ,

211   tau, and phosphorylated tau. The datasets included 377 samples collected from the

212   cortical grey (parietal and temporal) and white matter (parietal) as well as the

213   hippocampus. Additionally, the frequency distributions of age, sex, CERAD scores,

214   and Braak scores among the participants were evaluated, differentiating between the

215   no-dementia and dementia groups. Age was compared between the dementia and

216   no-dementia groups using a t-test, whereas sex, Braak score, and CERAD score were

217   compared between the groups using Fisher's exact test.

218

219   **Linear regression analysis for exploring interaction terms**

220   To explore moderating factors in the Aβ-tau interaction, a linear regression analysis

221   was used to evaluate interaction effects, as described previously.[13] Measurements

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222 were performed on formalin-fixed, paraffin-embedded tissue sections (5 µm). For Aβ,

223 the percentage of area covered by Aβ immunoreactivity was used, identifying both

224 Aβ40 and Aβ42. The analysis focused on the percentage of the area covered by Tau2

225 immunoreactivity, identifying mature neurofibrillary tangles and dystrophic neurites.

226 AT8, an antibody that recognizes phospho-tau (Ser202, Thr205), was also utilized.

227 Interaction effects with Aβ were investigated using immunoreactivities of IBA1 and

228 GFAP and gene expression levels of *GPNMB*. IBA1 was measured as the percentage

229 of area covered by IBA1 immunoreactivity, GFAP as the percentage of area covered

230 by GFAP immunoreactivity (identifying activated astrocytes), and *GPNMB* gene

231 expression levels were quantified as fragments per kilobase of exon per million reads

232 mapped (FPKM). The interaction effects were evaluated using the *t*-test, with values of

233 $p < 0.05$ considered significant.

234

235 **Trajectory analysis of human microglia snRNA-seq data**

236 A trajectory analysis was conducted using human microglial snRNA-seq data and the

237 Slingshot package. The default parameters for the slingshot function were utilized,

238 and the getCurves function was used with the following parameters: approx_points =

239 300, threshold = 0.01, stretch = 0.8, allow.breaks = FALSE, and shrink = 0.99. From

240 the dataset reported by Sun et al.,[21] only microglia were extracted from immune cells

241 for clustering. Clustering was performed using the Seurat package, following the

242 tutorial provided by the Satija Lab

243 (https://satijalab.org/seurat/articles/pbmc3k_tutorial). The settings were

244 FindVariableFeatures with nfeatures = 4000, FindNeighbors, RunUMAP with dims =

245 1:10, and FindClusters with resolution = 0.5. To visualize gene expression over time,

246 ggplot2 was used and regression curves were generated using generalized additive

247 models (GAM).

248

249 **Heatmaps of CERAD and Braak scores in the ROSMAP cohort data**

250 To investigate whether GPNMB protein expression moderates the Aβ-tau interaction

251 in patients with MCI and early AD in the ROSMAP cohort, correlations between

252 CERAD and Braak scores were evaluated. The CERAD score in the ROSMAP cohort

253 is a semi-quantitative measure of neuritic plaque density, classified into four scores

254 ranging from 4 to 1, corresponding to no AD, possible AD, probable AD, and definite

255 AD, respectively. The Braak score is a semi-quantitative measure of the severity of

256 neurofibrillary tangle pathology, ranging from 0 to 6, corresponding to Braak stages

257 I–VI. For the top 35% and bottom 35% of patients based on GPNMB protein

258 expression, the frequency of each CERAD and Braak score was visualized using a

259 heatmap.

260

Establishment of AlzPPMap

Using the R Shiny app, we developed a tool named AlzPPMap to analyze and visualize the integrated network. By inputting one or more gene names, the tool allows users to select a percentage of neighboring genes within the integrated network. The selected percentage of data is then used to construct and display a subnetwork.

Results

Study design

The analysis workflow, including an overview of the datasets, methods, and results, is presented in Supplementary Fig. S1. We extracted proteomics data for patients with MCI or early-stage AD, constructed a co-expression network, and integrated it with a PPI network using BIONIC, a deep learning-based method. To characterize the integrated data, we utilized enrichment analysis and whole snRNA-seq data (Fig. 2). Using proteomics data, we searched for factors associated with tau and Aβ (Fig. 3), constructed a subnetwork of tau, Aβ, and moderators, and performed cell type and functional enrichment analyses (Fig. 4). We validated these findings using immunohistochemical data from a separate cohort (Fig. 5). Additionally, for the subnetworks, we obtained microglia-specific snRNA-seq data to perform a microglial cell subtype enrichment analysis (Fig. 6). Finally, we validated these

279   findings using immunohistochemical and gene expression data (Fig. 7).

280

281   **Integration of protein co-expression and physical PPI networks**

282   We constructed an integrated framework as a basis for analyzing the effects of

283   PPIs and their moderators in the AD postmortem brain. To this end, we integrated

284   proteomic data and a physical PPI network to construct a functional-physical PPI

285   model. We employed BIONIC,[30] a deep learning method designed to integrate

286   multiple biological networks while preserving their integrity (Fig. 2a). BIONIC uses a

287   graph attention network[34] to integrate multiple networks, maintain the distinctive

288   characteristics of each, and generate new features. It shows good performance[30]

289   and has been used in analyses of gene interactions in cancer.[45]

290   First, a functional protein interaction network was constructed based on

291   protein abundance data. We compiled postmortem proteome profiles for patients with

292   MCI and early-stage AD from the dorsolateral prefrontal cortex, which were available

293   from the Religious Order Study (ROS) and Rush Memory and Aging Project (MAP)

294   cohort (https://adknowledgeportal.synapse.org/). Next, we generated a PPI network

295   using the STRING database[33] (https://string-db.org/) and constructed subnetworks

296   by prioritizing "high confidence" interactions. Using BIONIC, the integrated framework

297   was embedded into a lower-dimensional vector space, and the embedded coordinates

298   were obtained in a 512-dimensional space. Our downstream analysis used 512

299   integrated network features.

300

301   **Characterizing integrated protein network modules**

302   Next, we characterized the biological context captured by the integrated

303   network by evaluating the cell types and biological functions associated with the

304   protein modules. To investigate these modules, we built k-nearest neighbor graphs of

305   proteins based on the integrated network to obtain clusters (see Methods and Figs. 2a,

306   b). The cell types and biological processes corresponding to each module were

307   inferred using publicly available snRNA-seq data[37] and Gene Ontology terms. The

308   modules in the integrated network contained cell type signatures (Fig. 2c) and were

309   enriched in biological processes related to these cell types (Supplementary Table S1

310   and Fig. 2d). For example, module 1 was characterized by endothelial cells, microglia,

311   and astrocytes, modules 5, 17, 22, and 27 were associated with oligodendrocytes,

312   modules 9 and 13 with astrocytes, and module 11 with neurons (Fig. 2c). Consistent

313   with the cell type results, in module 5, terms related to oligodendrocytes, such as

314   "Ensheathment of neuron" and "Oligodendrocyte differentiation," were identified,

315   whereas module 11 was predominantly enriched in synapse-related terms (Fig. 2d).

316

Identification of *APP* and *MAPT* as adjacent modules in the integrated network

We explored the inter-modular relationships within the integrated network by specifically examining modules that included *APP* and *MAPT*, which serve as markers for Aβ and tau, respectively. The *APP* gene was located in module 11 and *MAPT* was located in module 12. Additionally, *APOE*, which plays a significant role in the accumulation and aggregation of Aβ, was assigned to module 1. We then constructed a network of module–module interactions (Fig. 2e, and Supplementary Fig. S2). Notably, these three modules were in close proximity in the network, with a strong correlation between the *APP* and *MAPT* modules (Pearson correlation coefficient = 0.7667, p < 0.001). Additionally, strong correlations were observed between the *APOE* module and both the *APP* (r = 0.6479, p < 0.001) and *MAPT* modules (r = 0.6289, p < 0.001). Furthermore, within the network, the *MAPT* module was closest to the *APP* module based on Euclidean distances; the *APP* module was second closest to the *MAPT* module. These module-to-module correlations in early-stage AD support the interactions between *APOE*, *APP*, and *MAPT*. [46,47]

Next, we evaluated whether similar findings could be obtained without network integration. Employing the STRING database with settings for high confidence and physical interactions alone, no interactions were detected between the *APP* and *MAPT* modules. Similarly, *APOE* did not interact with the other genes (Supplementary

336   Fig. S3). When examining protein expression data separately, *APP* and *MAPT* did not

337   show significant correlations (Pearson's correlation coefficient = -0.0465, p = 0.61).

338   These results underscore the importance of our integrated network approach in

339   capturing the biological interactions between Aβ and tau, which are crucial for

340   understanding early-stage AD development.

341

342   **Stress response factors moderate Aβ-tau interactions**

343   Next, we aimed to identify factors that could moderate the Aβ-tau interaction

344   based on the integrated network. For this purpose, we prioritized proteins with strong

345   moderating effects on APP-MAPT interactions against proteins close to the *APP* and

346   *MAPT* modules. We utilized the computational framework of Modulator Inference by

347   Network Dynamics (MINDy)[40] (Fig. 3a). Briefly, MINDy calculates mutual information

348   for interaction pairs (such as *APP* and *MAPT*), conditioned by the expression of the

349   modulator, which reflects the modulating effect. In the present analysis, we extracted

350   the neighboring proteins of the *APP* and *MAPT* modules in the integrated network (see

351   Methods, "Modulators of the Aβ-tau interaction"). We then stratified the samples from

352   the proteomics dataset into groups representing the top and bottom 35% of

353   expression for each neighboring protein and evaluated differences in the magnitude of

354   mutual information between *APP* and *MAPT* (Fig. 3a). We analyzed 312 neighboring

19

355 proteins and identified the 11 most significant modulators (Fig. 3b). These candidates

356 included factors involved in the cellular stress response (*HSPD1* and *HSPA5*),[48]

357 ubiquitin-proteasome system (*USP48*, *KBTBD6*, *UBE2D4*, and *STAMBPL1*),[49–51]

358 and apoptosis and cellular responses (*MAP3K5*).[51] These results suggest that

359 proteostasis involving stress response genes is related to the Aβ-tau interaction.

360 To further identify proteins contributing to the Aβ-tau interaction from the

361 moderator candidates, we investigated proteins known to physically interact with Aβ

362 and/or tau sourced from the Amyloidome[41] and Tau Interactome.[42] Consequently,

363 Heat Shock Protein Family A (Hsp70) Member 5 (*HSPA5*) was identified. *HSPA5*, a

364 marker of ER stress associated with AD,[52,53] has been shown to interact in vitro

365 with Aβ and tau, mitigating their toxicity[54–58]. Although in vivo studies have

366 identified it as a candidate therapeutic target[59,60], the detailed mechanisms of

367 action and its relevance in the Aβ-tau interaction are not fully understood[61].

368

369 **Glial cells are closely related to the APP-MAPT interaction in the integrated**

370 **network**

371 We further investigated whether stress responses involving *HSPA5* are

372 involved in Aβ-tau interactions and identified related cell types and biological

373 pathways. To address this, we exploited the integrated network extensively and

20

374   reconstructed *HSPA5* and its neighboring subnetworks as well as *APP* and *MAPT* (Fig.

375   4a). Note that the subnetwork included other significant moderators, *STAMBPL1* and

376   *MAP3K5*, suggesting that these factors, in addition to *HSPA5*, could be associated

377   with the Aβ-tau interaction. This subnetwork was characterized by functions in

378   neuronal death, positive regulation of kinase activity, cellular response to chemical

379   stress, synapse organization, and regulation of autophagy, confirming its relation to

380   stress response pathways (Fig. 4b, see also Supplementary Table S2).

381   We classified the subnetwork proteins into six communities based on topology

382   and then estimated the cell types and biological functions associated with each

383   community (Figs. 4c, d, and Supplementary Table S3). We found that glial cells were

384   the primary cell type related to the stress response subnetwork (Fig. 4c). Additionally,

385   the combined score for each community, calculated by multiplying the expression level

386   by the proportion of cells, showed enrichment for glial cells (Supplementary Fig. S4a).

387   Similar results were obtained using snRNA-seq data from two different cohorts[21,22]

388   and different regions (Supplementary Figs. S4b, c). However, communities 2 and 6

389   were excluded from this analysis owing to the small number of genes included. In the

390   APP-related Community 3, oligodendrocytes were enriched in protein folding,

391   oxidative stress responses, neuronal death, and autophagy. Community 4, which

392   contained *MAPT* and *HSPA5*, was characterized by microglia and excitatory neurons

393   with enrichment in kinase activity. Other communities such as communities 1 and 5

394   were also enriched in glial cells (Figs. 4c, d). Microglia were detected as a feature of

395   three of the four significantly enriched submodules (Fig. 4c).

396

397   **Histopathological data analysis validated the roles of microglia and astrocytes**

398   **in the Aβ-tau interaction in early AD**

399   To further investigate the effect of Aβ-tau interaction, we examined publicly

400   available histopathological (ACT study) data.[44] This dataset contains extensive

401   multimodal histopathological information, including Aβ and tau pathology. The

402   distributions of age, sex, CERAD score, and Braak score in groups with and without

403   dementia are shown in Supplementary Fig. S5. Among these parameters, CERAD

404   and Braak scores showed significant differences between the groups (Fisher's exact

405   test, $p = 0.00237$ and $p = 0.000468$, respectively). In the no-dementia group, the

406   average CERAD score was 1.476 and the average Braak score was 3.421. Braak

407   stages I and II indicate that neurofibrillary tangles are confined mainly to the entorhinal

408   region of the brain, whereas stages III and IV involve the limbic regions, such as the

409   hippocampus.

410   We analyzed the effect of the interaction between tau and Aβ using a linear

411   modeling approach. Tau2, an antibody for a mature neurofibrillary tangles and

412 dystrophic neurites, was used as the dependent variable, whereas Aβ, along with

413 GFAP or IBA1 (activated astrocyte or microglia markers, respectively), was used as

414 the independent variable. We investigated the interaction between GFAP or IBA1 and

415 Aβ, stratifying the analysis by the dementia status and across four brain regions (Fig.

416 5). For GFAP, a significant positive contribution to the interaction was observed in the

417 hippocampus of both the dementia and no-dementia groups (HIP, $\beta = 43.59$, $p = 0.022$

418 and $\beta = 24.852$ and $p = 0.001$, respectively) and in the temporal neocortex of the

419 no-dementia group (TCx, $\beta = 13.873$ and $p < 0.001$). In the case of IBA1, a positive

420 contribution trend was observed in the HIP of the no-dementia group, though the

421 relationship was not significant ($\beta = 4.678$ and $p = 0.338$), whereas a significant

422 positive contribution was observed in the TCx ($\beta = 4.352$ and $p < 0.001$). Conversely,

423 in the dementia group, a significant negative contribution was noted in the TCx ($\beta =$

424 -11.124, $p = 0.04$; Fig. 5b and Supplementary Table S4). Analyses of AT8, an antibody

425 that recognizes phospho-tau (Ser202 and Thr205) showed the same trend in the

426 no-dementia group: GFAP ($\beta = 33.912$ and $p = 0.235$ in HIP; $\beta = 62.732$ and $p < 0.001$

427 in TCx) and IBA1 ($\beta = 14.442$ and $p = 0.36$ in HIP; $\beta = 16.344$ and $p < 0.001$ in TCx)

428 (Supplementary Table S5). Of note, interactions were observed only in the HIP and

429 TCx regions, which are vulnerable in patients with early-stage AD.[62]

430   The average CERAD and Braak scores in the no-dementia group mentioned

431   earlier suggest that, although not exhibiting dementia, this group exhibited mild

432   progression of AD pathology (Supplementary Figs. S5c, d). Collectively, these results

433   support the hypothesis that astrocytes and microglia moderate the Aβ-tau interaction

434   in early-stage AD.

435

436   **GPNMB+ microglia function as moderators of the Aβ-tau interaction**

437   We further examined microglial subtypes that contribute to Aβ-tau interactions.

438   Microglia-overexpressing neuroinflammation-related processes can be assigned to

439   subtypes that exhibit different cellular states under different AD conditions.[63] We

440   evaluated whether a certain subtype of microglia moderates the Aβ-tau interaction in

441   early-stage AD. We accessed recently reported snRNA-seq data[22] from frontal

442   cortex biopsies of 52 patients with hydrocephalus (NPH). This cohort included

443   samples with confirmed amyloid and tau pathologies, in which AD was in an

444   asymptomatic stage. Of the 52 living biopsies, Aβ plaques were identified in 19

445   subjects, Aβ plaques and phosphorylated tau pathology in eight subjects, and no

446   pathology in the remaining 25 subjects; for subjects with Aβ pathology, the subsequent

447   onset of AD was determined in longitudinal studies. In total, 892,828 high-quality

448   single-nucleus profiles were included in analyses. Here, we focused on the microglial

449   cell populations.

450   We performed an enrichment analysis of each microglial subtype for the

451   response-related subnetwork communities (communities 1–6) based on the

452   integrated network. GPNMB+ microglia were significantly enriched in community 1

453   (Fig. 6). The expression level and proportion of cells identified as the GPNMB+

454   microglial subtype was high for the community 1 signature score (Fig. 6a). In addition,

455   we compared the compositions of cell subtypes across different statuses in the same

456   cohort. The frequency of GPNMB+ microglia was significantly higher in Aβ+ and

457   Aβ+Tau+ samples than in the control group (Supplementary Fig. S6 and

458   Supplementary Table S6). The combined score for Community 1, calculated by

459   multiplying the expression level and the proportion of cells with positive expression,

460   further confirmed that the GPNMB+ microglial subtype was the highest ranked among

461   the examined subtypes (Fig. 6b). Importantly, the GPNMB+ microglial subtype is

462   expressed in the early stages of AD and expands as the disease progresses.[22]

463   To further validate the involvement of GPNMB+ microglia in the Aβ-tau

464   interaction in early-stage AD, we used another large-scale snRNA-seq data set for

465   microglia subtypes in AD,[21] including transcriptome data for 194,000 single-nuclear

466   microglia and multiple AD pathological phenotypes in 443 human subjects. The cell

467   subtypes were annotated based on a previous study.[22] We found the same trend

468   towards enrichment for GPNMB+ in Module 1 (Figs. 6c, d). These results suggest

469   that the stress response module moderating Aβ-tau interactions in early AD

470   corresponds to GPNMB+ microglia.

471

472   **Aβ-tau stress response mediators are activated prior to GPMNB+ microglial**

473   **activation**

474   We further examined the relationship between stress response-related

475   mediators of the Aβ-tau interaction and GPNMB+ microglia. Previous studies have

476   reported that ER stress induces GPNMB expression in neurons but not in

477   microglia.[64] We hypothesized that stress response mediators are involved in the

478   transition of microglia from a homeostatic state to an activated GPNMB+ state in the

479   human brain. To test this hypothesis, trajectory analysis of the two snRNA-seq

480   datasets used in the previous analyses was performed using Slingshot.[65] Briefly, in

481   this analysis, cells are embedded in the manifold space based on expression

482   similarity between cells. This trajectory reflects the cellular state and can be treated

483   as a "pseudo-time" axis. Multiple branched trajectories were identified. For each

484   trajectory, we identified the GPNMB+ microglial coordinates on the pseudo-time

485   trajectories (Supplementary Figs. S7a, c).

486   Next, we analyzed the points at which the candidate Aβ-tau-moderating

487   factors related to the stress response predicted using MINDy were expressed in the

488   pseudo-time analysis. In both datasets, the moderator proteins were highly

489   expressed prior to GPNMB+ microglia in all pseudo-time trajectories (Supplementary

490   Figs. S7b, d). Similar results were obtained for *HSPA5*, a moderating factor based on

491   the amyloidome (i.e., the collection of amyloid peptides and related proteins, peptides,

492   and other molecules)[41] and tau interactome.[42] These results suggest that stress

493   factors moderating the Aβ-tau interaction may act through the activation of GPNMB+

494   microglia.

495

496   **GPMNB protein expression is correlated with the clinical severity of AD**

497   We evaluated the hypothesis that GPMNB+ microglia moderate Aβ-tau

498   interactions in early AD. In particular, we examined whether *GPMNB* could serve as a

499   biomarker for the clinical severity of AD.

500   For this purpose, we assessed brain tissue protein expression and clinical

501   parameters from the ROSMAP cohort data using CERAD and Braak scores as

502   indicators of the clinical severity of AD. We stratified the patients into two groups

503   based on *GPNMB* protein expression: the top 35% and bottom 35%. The *GPNMB*

504   high- and low-expression groups corresponded to the GPNMB+ microglia-activated

505   and non-activated groups, respectively. Within the ROSMAP cohort, the CERAD

506   score is a semi-quantitative measure of neuritic plaques, where 1 indicates definite

507 AD, 2 is probable AD, 3 is possible AD, and 4 represents no AD. The top 35% showed

508 greater disease progression based on both CERAD and Braak scores than that of the

509 bottom 35% (Figs. 7a, b).

510

511 **GPNMB shows significant Aβ-tau interaction effects in the preclinical stage**

512 We further examined whether *GPNMB* moderates the effect of the Aβ-tau

513 interaction in early-stage AD. To this end, we assessed the histopathological and AD

514 diagnostic information from the ACT study as well as gene expression data. We

515 tested interaction effects of *GPNMB* gene expression on Aβ and tau by linear

516 regression analyses (Fig. 7c, Supplementary Tables S7 and S8).

517 There were significant positive interaction effects of *GPNMB* gene expression

518 on Aβ-tau in the non-dementia group in the HIP and TCx brain regions (β = 0.089, p =

519 0.02 and β = 0.026, p < 0.001 in HIP and TCx, respectively). Notably, no significant

520 interaction effects were observed at the same sites in the dementia group.

521 Collectively, these findings suggest that GPNMB+ microglia and astrocyte moderate

522 the Aβ-tau interaction in the early stages of AD (Fig. 7d).

523

524 **Establishment of an integrated network analysis tool, AlzPPMap**

525 Finally, we developed a tool named AlzPPMap (Alzheimer's disease

28

526   Proteomics PPI Integrated Network Map) for the analysis and visualization of

527   integrated networks. This tool enables users to analyze the integrated functional

528   network derived from proteomics data alongside physical networks derived from PPIs.

529   Users can select one or more genes of interest and, as demonstrated in this study,

530   choose a specific percentage of closely related genes within the network based on

531   their proximity to the selected gene(s). The selected genes are then used to construct,

532   visualize, and detect communities within a subnetwork. AlzPPMap facilitates the

533   identification of molecules and molecular pathways that are both functionally and

534   physically associated with the target genes. The tool can be accessed at:

535   https://igcore.cloud/GerOmics/AlzPPMap.

536

Discussion

538   The number of individuals with AD is expected to continue increasing,[66]

539   making the development of effective treatments a pressing issue. As mentioned in the

540   Introduction, whereas Aβ and tau proteins are known to contribute to AD pathology,

541   the details of their involvement remain unclear.[1,2] The specific interactions between

542   them have been reported,[4–7] but not the underlying mechanisms. In this study, we

543   employed a deep learning-based network integration analysis to investigate the

544   mechanisms of the Aβ-tau interaction in patients with early-stage AD. The study was

545   conducted in four steps: (1) network integration, (2) identification of moderators and

546   subnetwork construction, (3) validation analysis using histopathological data, and (4)

547   development of a user-friendly web application.

548   In the first phase, we constructed an integrated functional-physical interaction

549   network where two different data types—protein co-expression and physical

550   PPIs—were combined into a single network using BIONIC.[30] Interestingly,

551   enrichment analysis and snRNA-seq data showed that the integrated network

552   captured relevant cell types and pathways. We also confirmed that Aβ and tau were

553   present in neighboring modules in the integrated network. Notably, neither

554   co-expression information nor physical interaction data alone could capture the

555   Aβ-tau interaction, emphasizing the need to evaluate functional and physical

556   interactions simultaneously. Co-expression network analyses are widely used in AD

557   research,[23–25] but it is difficult to represent physical interactions, such as Aβ-tau

558   interactions or the amyloidome. Therefore, an approach that integrates networks

559   based on different types of biological information may be effective in elucidating the

560   complex molecular pathogenesis of AD.

561   In the second phase, we used the MINDy[40] algorithm to search for factors

562   that moderate the Aβ-tau interaction using proteomics expression data. We identified

563   ER stress markers such as *HSPA5*, along with other stress response-related factors,

564   including *HSPD1*, *USP48*, *KBTBD6*, *UBE2D4*, *STAMBPL1*, and *MAP3K5*, as

565   potential moderators. Although ER stress responses have been frequently reported to

566   be associated with AD,[52–60] their detailed mechanisms remain unclear. In this

567   study, our findings suggested that stress response pathways were associated with

568   the Aβ-tau interaction, indicating that elucidating how these pathways influence the

569   progression of AD could be a focus for future research. In particular, understanding

570   how ER stress responses are involved in the Aβ-tau interaction and transmitted

571   through glial cells could contribute to the development of novel therapeutic strategies.

572   We next identified molecules, cell types, and pathways involved in the Aβ-tau

573   interaction by constructing subnetworks from the factors identified in the second

574   phase, extracting genes closely related to the two proteins within the integrated

575   network. The results suggested that glial cells, particularly GPNMB+ microglia, are

576   involved in the Aβ-tau interaction.

577   In the third phase, we validated the findings using an independent dataset

578   with histopathological data from the ACT study.[44] Through linear regression

579   analysis, we examined the interaction between GFAP, IBA1, and GPNMB with Aβ in

580   the hippocampus and temporal neocortex in the non-dementia group. Significant

581   positive interactions were detected, further suggesting that astrocytes and GPNMB+

582   microglia are moderators of the Aβ-tau interaction in early-stage AD. GPNMB+

583   microglia, a recently identified subtype, are elevated in the frontal white matter and

584   cortex of patients with AD.[67] Additionally, *GPNMB* is expressed in activated

585   microglia[68] and is associated with AD pathology.[22] Furthermore, the expression of

586   *GPNMB* in microglia is correlated with recently reported subtypes, such as

587   disease-associated microglia (DAM)[63] and microglial neurodegenerative phenotype

588   (MGnD),[69] which are closely associated with plaques and neurodegeneration.

589   However, their specific functions related to Aβ and tau have not been fully elucidated.

590   Our results provide new insights into the functions of GPNMB+ microglia in the

591   pathogenesis of AD.

592   In summary, network integration analysis has been extensively reported in

593   cancer research,[26–30] but its use in AD research remains limited. Through this

594   study, we were able to identify potential moderators of the Aβ-tau interaction by

595   integrating proteomics and PPI data using BIONIC.[30] Future analyses using a

596   framework like the one presented in this study, integrating other omics data, could

597   lead to new insights into the mechanisms of AD and other disease areas. Additionally,

598   in the last phase, we developed a tool named AlzPPMap to evaluate the integrated

599   network constructed in this study. This tool enables the analysis and visualization of

600   relationships between other genes, potentially providing new insights and ideas for

601   experimental validation.

602   This study had several limitations. First, except for the GPNMB+ microglial

603   data[22], all data were derived from postmortem brains. This introduces potential

604   confounding factors between postmortem effects and AD pathology. Second, in the

605   analyses summarized in Fig. 4, protein expression was aggregated by genes, not

606   accounting for multiple isoforms or post-translational modifications, especially in *APP*

607   and *MAPT*. For example, Aβ40 and Aβ42 are produced from *APP* through different

608   processes, and Tau in *MAPT* undergoes various phosphorylation modifications,[70]

609   impacting the disease differently. Histopathological evaluations were used to validate

610   the integrated protein network analysis results. Third, the *GPNMB* gene expression

611   analysis was based on bulk RNA-seq data, which may not specifically reflect

612   GPNMB+ microglia. A detailed analysis of the role of GPNMB+ microglia in AD

613   requires further experimental studies. Fourth, although we applied a significance

614   threshold of $P < 0.05$ with the Benjamini-Hochberg correction, there is ongoing

615   discussion in the field regarding the potential benefits of using a more stringent

616   threshold. Future studies with larger sample sizes could help to further support our

617   findings. Nonetheless, our findings offer valuable new insights into the Aβ-tau

618   interaction and highlight potential directions for the development of AD therapeutics.

619

620

List of abbreviations

Alzheimer's disease (AD), amyloid-beta (Aβ), disease-associated microglia (DAM), mild cognitive impairment (MCI), protein–protein interaction (PPI), Religious Order Study and Rush Memory and Aging Project (ROSMAP), single-nucleus RNA sequencing (snRNA-seq), tandem mass tag (TMT)

Declarations

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643 were obtained from www.radc.rush.edu. Human AD brain single-nucleus RNA

644 sequencing (snRNA-seq) data were downloaded from the NCBI for Biotechnology

645 Information Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/).

646 Microglial snRNA-seq data were accessed at https://braincelldata.org/resource and

647 https://doi.org/10.7303/syn2580853. Additionally, the human neuropathological and

648 RNA sequencing datasets used for the linear regression analysis are available at

649 NIAGADS (https://www.niagads.org/). Figures were created using Bio-Render.com.

650 **Funding**

653

654 **Author contributions**

655 Conceptualization and Methodology: M.Y., K.A., Formal Analysis, Investigation, Data

656 Curation, Writing – Original Draft, Visualization: K.A., Resource, Writing – Review &

657 Editing, Supervision, Project Administration and Funding Acquisition: M.Y.

658

Human Ethics and Consent to Participate declarations

This study utilizes publicly available datasets obtained through appropriate

agreements or public data portals, ensuring compliance with ethical guidelines. The

proteomic data used in this study were accessed from the AD Knowledge Portal

(https://adknowledgeportal.org) under the terms of the data use agreement. These

datasets were collected with the informed consent of participants and ethical approval

from the original study institutions, including ROSMAP cohort studies. Additionally,

neuropathological and single-nucleus RNA sequencing (snRNA-seq) datasets used

in this study were obtained from the NCBI Gene Expression Omnibus (GEO) and

NIAGADS (https://www.niagads.org/), where all samples were collected following

approved institutional protocols, with participants providing informed consent for

research and data sharing. For data requiring restricted access, the necessary

approvals and permissions were obtained from the respective data providers. This

study adhered to all relevant ethical regulations for the analysis of secondary human

data.

Competing interests

The authors declare that there are no competing interests.

Availability of data and materials

All data used in this study are publicly accessible. The proteomic data can be

accessed via the AD Knowledge Portal (https://doi.org/10.7303/syn2580853). Human

AD brain snRNA-seq and microglia snRNA-seq datasets are available at the NCBI

Gene Expression Omnibus (GEO) under accession number GSE174367 and via the

following links: https://braincelldata.org/resource and

https://doi.org/10.7303/syn2580853. The human neuropathological and RNA

sequencing datasets employed for the linear regression analysis can be found at

https://www.niagads.org/datasets/ng00059. Additional information required to

reanalyze the data reported in this study is available from the lead author upon

request.

Code availability

The code used can be found on GitHub

(https://github.com/matsui-lab/AlzPPMap/tree/main).

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951 **Figure legends**

952 **Fig. 1 A graphical abstract of the study**

953 This study consists of the following four steps: 1. Integration of proteomics and

954 protein–protein interaction (PPI) data, 2. Downstream analysis: Identification of

955 moderators involving APP and MAPT, and construction of subnetworks, 3. Validation

956 using pathological histological evaluation data, 4. Development of a user-friendly web

957 application. In addition to step 2 conducted in this study, users can also construct and

958 visualize subnetworks of genes of interest (including interactions between multiple

959 genes).

960 **Fig. 2 Characterization of integrated protein network modules**

961 (a) Schematic representation of the workflow for integrating proteomics and

962 protein–protein interaction (PPI) data and evaluation of integrated features.

963 (b) Stochastic neighbor embedding (tSNE) plot displaying protein modules.

964 (c) Upper panels: Uniform Manifold Approximation and Projection (UMAP)

965 visualizations of single-nucleus RNA sequencing (snRNA-seq) data for patients with

966 Alzheimer's disease (AD) (GSE174367)[37] clustered according to cell types (left)

967 and conditions (right) Lower panels: expression levels of proteins within the identified

968 modules.

969 (d) Representative Gene Ontology (GO) terms enriched in each protein module.

970   (e) Network depicting the closeness among modules. Closeness was defined by

971   averaging BIONIC-derived features within modules, calculating the Euclidean

972   distances among modules, and taking reciprocals.

973   **Fig. 3 Inference of APP-MAPT modulators using a mutual information-based**

974   **method**

975   (a) Graphical representation of the Modulator Inference by Network Dynamics

976   (MINDy) analysis.

977   (b) Scatter plot illustrating the relationships between –log10(p-values) and Scores.

978   Points representing p-values less than 0.05 are highlighted in red and are labeled

979   with their corresponding gene symbols.

980   **Fig. 4 Glial cells moderate APP and MAPT interactions**

981   (a) Workflow for network reconstruction from BIONIC analysis results, subnetwork

982   construction using proteins highly associated with intermediary factors, APP and

983   MAPT, and community detection within the network, followed by cell type

984   identification and enrichment analyses for each community.

985   (b) GO enrichment analysis of the subnetwork, categorized by parent terms using the

986   rrvgo[39] software.

987   (c) Representative GO terms enriched in each community.

988   (d) Dotplot displaying expression levels of proteins within each community.

989

Fig. 5 Histopathological analysis validated the roles of microglia/astrocytes as

Aβ-tau moderators in early Alzheimer's disease

(a) Schematic of the analytical workflow for linear regression analyses. Samples were

sourced from four brain regions: frontal white matter (FWM), hippocampus (HIP),

parietal neocortex (PCx), and temporal neocortex (TCx). RNA sequencing and

immunohistochemistry data were used.[44] A linear regression was performed,

repeated for GFAP and IBA1, and Aβ as predictors of tau interactions.

(b) Significant results of linear regression analyses for each of the four distinct brain

regions, with subjects categorized into no-dementia and dementia groups.

Fig. 6 snRNA-seq Analysis Identifying Enrichment for GPNMB+ Microglia in

Community 1

(a, c) Dot plot showing the expression levels of genes aggregated by community in

each microglial subtype.[21,22]

(b, d) Min-max normalized combined score for community 1, calculated by multiplying

the expression level by the proportion of expressing cells.

Fig. 7 GPNMB+ microglia as a possible moderator of the Aβ-tau interaction

(a, b) Heatmaps displaying the correlations between CERAD and Braak scores in

samples categorized by the top (a) and bottom (b) 35% of GPNMB expression.

1008   (c) Significant results of linear regression analyses for each of the four distinct brain

1009   regions, with subjects categorized into no-dementia and dementia groups.

1010   (d) Schematic representation summarizing the findings supporting the roles of both

1011   astrocytes and GPNMB+ microglia in the Aβ-tau interaction.

1012

1013

1014

a

APP

Modulator

MAPT

Top 35% Bottom 35%

$$\Delta MI = $$
$$| MI(\text{MAPT}_{\text{Mtop35}}, \text{APP}_{\text{Mtop35}}) - $$
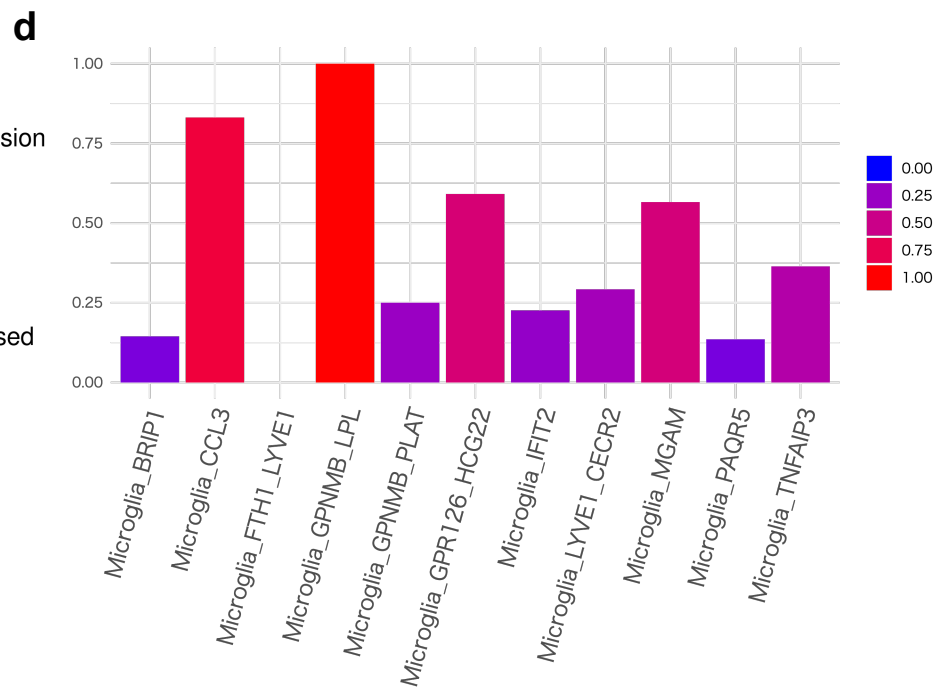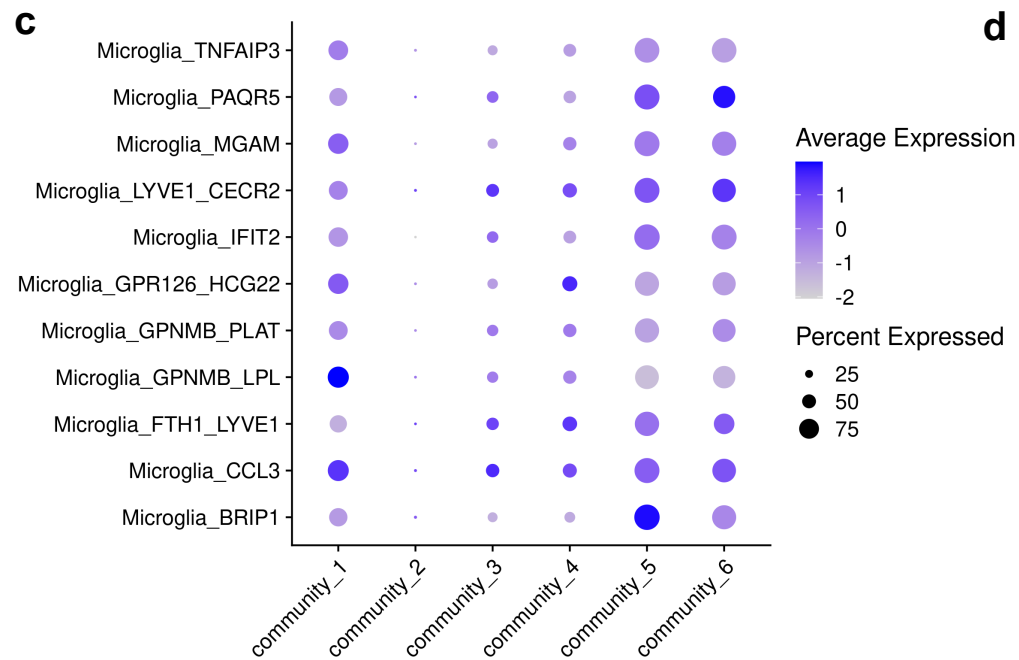$$MI(\text{MAPT}_{\text{Mbottom35}}, \text{APP}_{\text{Mbottom35}}) |$$

$$MI(X,Y) = \sum x \in X \sum y \in Y\, p(x,y)\log(p(x)p(y)p(x,y))$$

Modulator low

MAPT   APP

Modulator high

MAPT   APP

b

Score vs -log10(p)

-log10(p)

3

2

1

0

0.0   0.1   0.2   0.3

Score

FLRT3

HSPD1

USP48

BRD4   RAB30

UBE2D4

RCAN1   KBTBD6

HSPA5

STAMBPL1

MAP3K5

● p.value < 0.05

a

Brain samples
(FWM, HIP, PCx, TCx)

RNA-seq

Immunohistochemistry

Gene Expression Profiles

Samples

IHC scores

$$\text{Tau} = \beta_0 + \beta_1 A\beta + \beta_2 M + \beta_3 (A\beta \times M) + \varepsilon$$

linear regression for the identification of intermediary factors

b

Hippocampus
(No Dementia)

Astrocyte

Amyloid beta

Interaction effect
β=24.9, p=0.001

Tau

Temporal cortex
(No Dementia)

Astrocyte

Amyloid beta

Interaction effect
β=13.9, p<0.001

Tau

Microglia

Amyloid beta
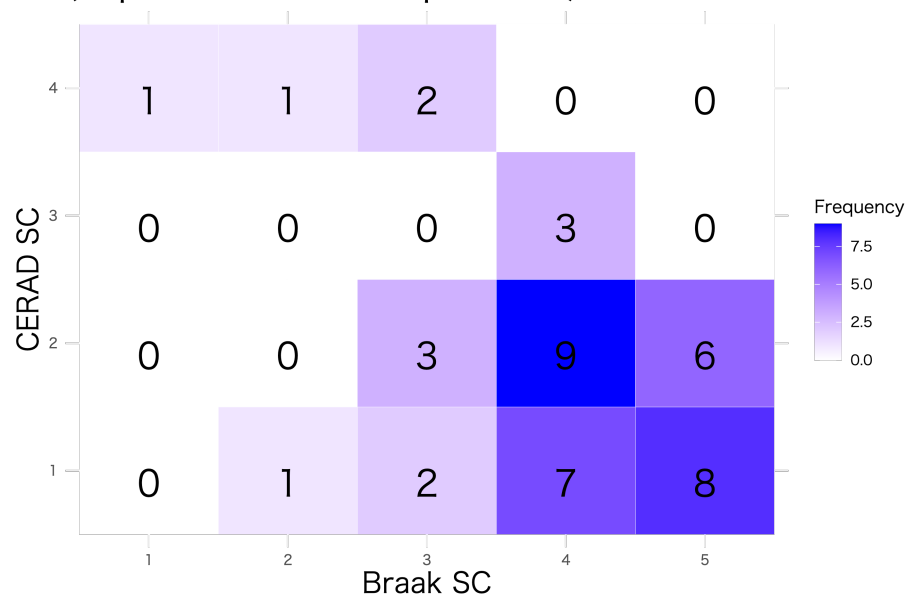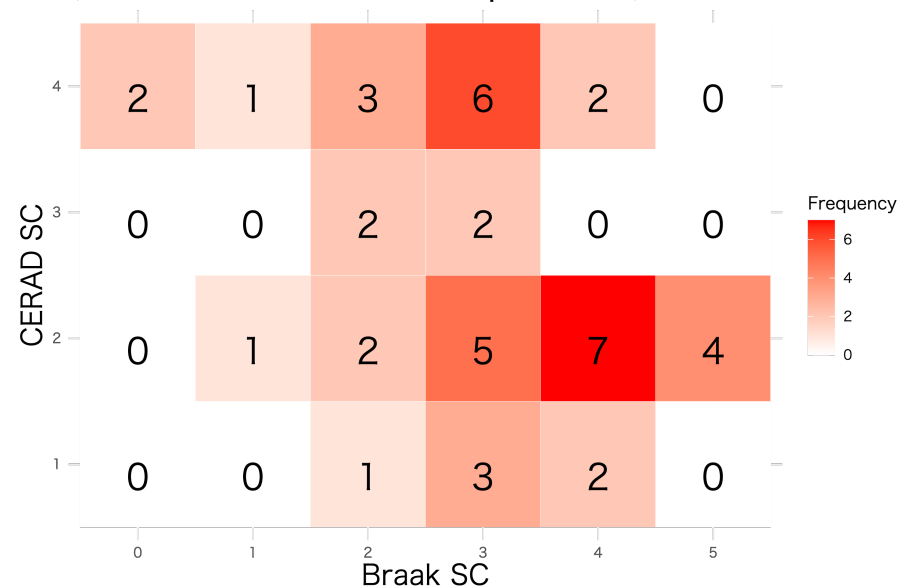
Interaction effect
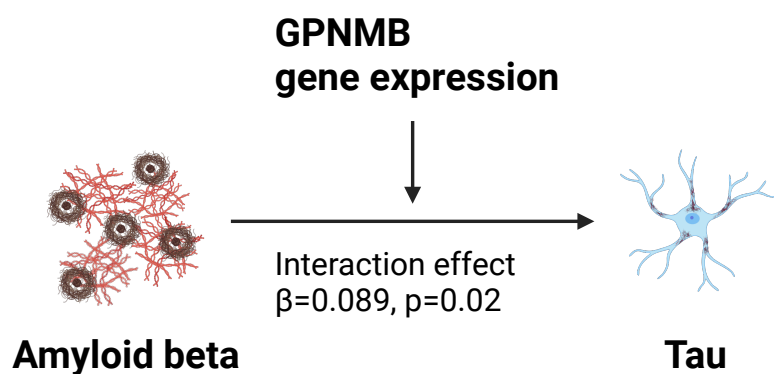β=4.35, p<0.001

Tau

a Heatmap of braaksc and ceradsc (Top 35% GPNMB Expression)

b Heatmap of braaksc and ceradsc (Bottom 35% GPNMB Expression)

c

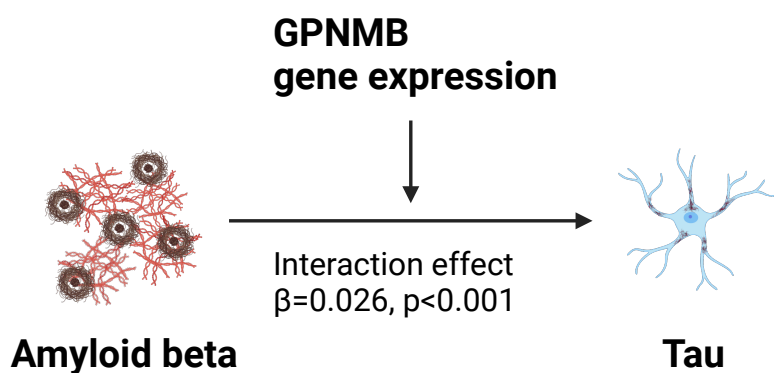Hippocampus (No Dementia)

GPNMB gene expression

Amyloid beta → Tau

Interaction effect β=0.089, p=0.02

Temporal cortex (No Dementia)

GPNMB gene expression

Amyloid beta → Tau

Interaction effect β=0.026, p<0.001

d MCI / early stage Alzheimer's disease

Amyloid beta

GPNMB+ Microglia

Astrocyte

Tau