Integrated Systems Analysis Deciphers Transcriptome and Glycoproteome Links in Alzheimer's Disease

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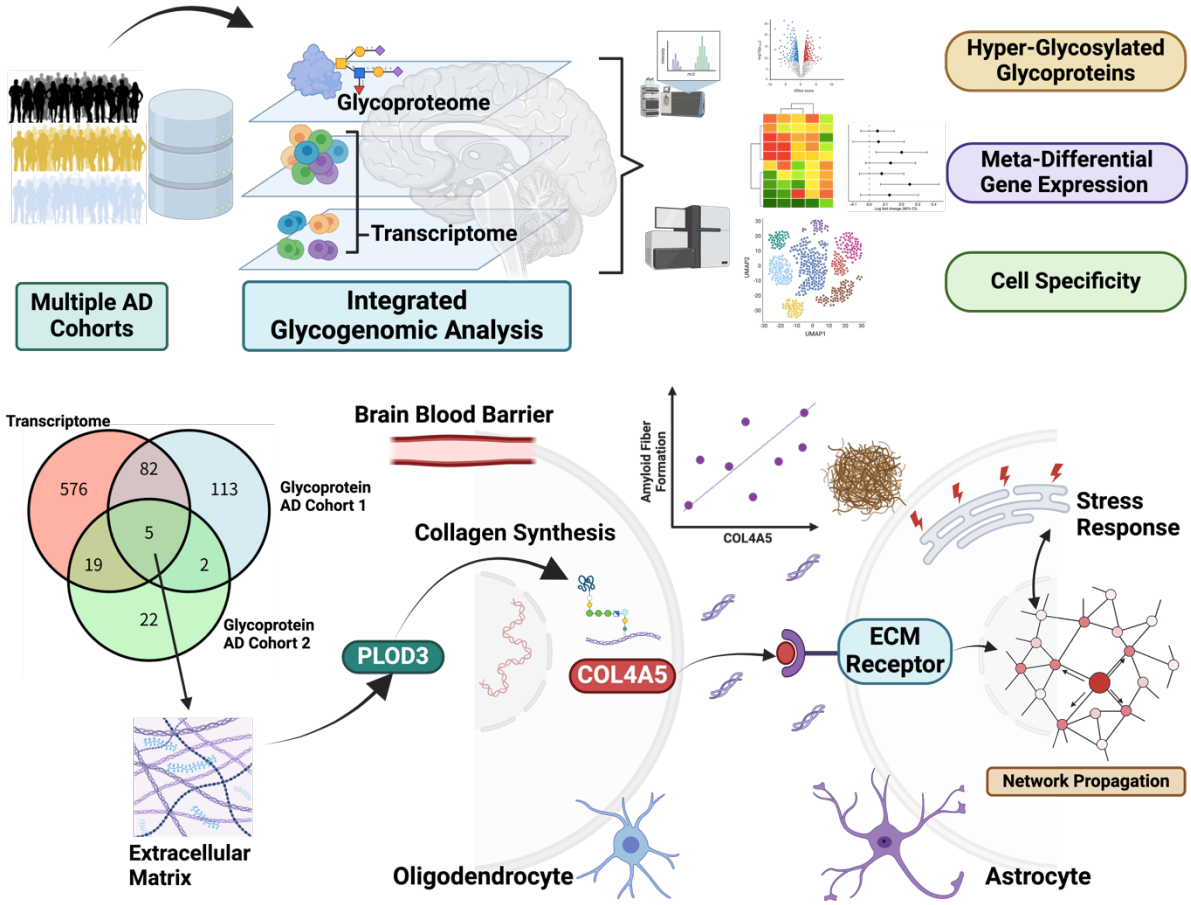
Graphical Abstract



Abstract

Glycosylation is increasingly recognized as a potential therapeutic target in Alzheimer's disease. In recent years, evidence of Alzheimer's disease-specific glycoproteins has been established. However, the mechanisms underlying their dysregulation, including tissue- and cell-type specificity, are not fully understood. We aimed to explore the upstream regulators of aberrant glycosylation by integrating multiple data sources using a glycogenomics approach. We identified dysregulation of the glycosyltransferase PLOD3 in oligodendrocytes as an upstream regulator of cerebral vessels and found that it is involved in COL4A5 synthesis, which is strongly correlated with amyloid fiber formation. Furthermore, COL4A5 has been suggested to interact with astrocytes via extracellular matrix receptors as a ligand. This study suggests directions for new therapeutic strategies for Alzheimer's disease targeting glycosyltransferases.

Keywords:

Glycoproteomics, Transcriptome, Glycosyltransferase, Extracellular Matrix, Alzheimer's disease

42 **Introduction**

43 Alzheimer's disease (AD) is an age-related neurodegenerative disease[1,2]. The primary
44 causes are neurogenic cell loss, accumulation of misfolded proteins, oxidative stress, and
45 inflammatory responses[3]. Genomic, transcriptomic, and epigenetic mechanisms have been
46 intensively examined[4]. However, our knowledge of the post-translational modifications that
47 regulate cellular functions and interactions between cells is still lacking[5]. In particular,
48 glycosylation is the most diverse and abundant post-translational modification among protein
49 modifications[6].

50 Protein glycosylation is a complex multistep process involving approximately 200
51 different glycosyltransferases[6–8]. There are 16 major glycosylation pathways, including lipid
52 glycosylation, N-glycosylation, O-glycosylation, C-mannosylation, lipid glycosylation, and
53 glycosylphosphatidylinositol (GPI)-anchored synthesis. Recently, glycomics / glycoproteomics
54 analysis of the human AD postmortem brain[9,10], serum,[11–17] and cerebrospinal fluid[18–20] for N-
55 glycosylation, the most abundant glycosylation pathway, has revealed dysregulated
56 glycoproteins. In addition, the biological functions of abnormal glycans in AD pathology have
57 been reported in some cases; for example, it is known that inhibition of BACE1 glycosylation
58 reduces the cleavage of the amyloid β precursor protein (APP) [21–23]. However, most biological
59 functions of glycosylation in the pathogenesis of AD are poorly understood.

60 Glycan structures are not independent of the DNA template, and glycosylation
61 depends on a combination of approximately 200 glycosyltransferases and 500 related
62 proteins[6–8]. Thus, their dysregulation may act as an upstream regulatory factor that triggers
63 abnormal glycosylation processes[24]. In addition, it is difficult to elucidate biological
64 glycosylation mechanisms at the single-cell resolution using glycomics / glycoproteomics alone
65 because current technology is limited to probing with glycosylation-specific antibodies and
66 glycan-binding proteins, such as lectin [6]. Therefore, a glycogenomics approach that integrates
67 genomics, or functional genomics, and glycoproteomics is critical for a comprehensive

68 understanding of biological glycosylation pathways[24,25].

69 Here, we present the factors upstream of aberrant glycosylation in AD. We performed
70 an integrated analysis of bulk and single-cell/nuclear transcriptomic and glycoproteomics data
71 from human AD brain tissues. In particular, we showed that the extracellular matrix (ECM)
72 is a common signature in the glycoproteome and transcriptome, and that ECM gene
73 expression signatures are enriched for cerebral vascular-related pathways. We identified
74 Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 3 (PLOD3) as an upstream
75 glycosyltransferase common to ECM pathway. Through an integrated analysis of multiple
76 single-cell expression data, we showed that PLOD3 is involved in the regulation of collagen
77 type IV alpha 5 chain (COL4A5), which is strongly correlated with amyloid fiber formation.
78 Cell–cell interaction and signaling pathway analyses suggested that PLOD3-COL4A5 cascade
79 is involved in the stress response via the ECM receptor in astrocytes.

80

81 **Results**

82 **Hyperglycosylated proteins are primarily enriched in the ECM**

83 To examine the association between the molecular pathogenesis of AD and
84 glycosylation, we accessed glycoproteomic data consisting of 2 cohorts of postmortem brain
85 tissue from AD patients [9,10] (**Figure 1A**). The first dataset consisted of dorsolateral prefrontal
86 cortex tissue from 8 neuropathologically confirmed AD cases and 8 age-matched controls. The
87 second dataset comprised a subset of the ROSMAP cohort[14]. Glycoproteomic analysis was
88 performed on the postmortem brains of 10 patients with asymptomatic AD, 10 patients with
89 symptomatic AD, and 10 healthy brains in which none of the above were present.

90 In each cohort, 92 and 10 AD-specific hyperglycosylated proteins (**Supplementary**
91 **Table S1**) were identified, respectively, and pathway enrichment analysis was performed
92 (**Figure 1A**). Among the pathways significantly enriched in the 2 cohorts, we identified the
93 ECM pathway as the most common pathway among 7 pathways (**Figure 1B, C**). The

94 relationship between AD and ECM has recently been recognized as a new molecular
95 pathogenesis, along with other major pathological hypotheses[26,27]. ECM components contain
96 glycoproteins, including glycosylated proteoglycans and collagen, as major elements[27], and
97 many glycosylations play important roles in ECM formation and maintenance.
98
99 **Meta-analysis of the transcriptome reveals that glycogenes are enriched in the ECM**
100 We explored the upstream factors that regulate ECM hyperglycosylation in AD. We
101 accessed the AD Knowledge Portal (https://adknowledgeportal.synapse.org), which contains
102 postmortem brain transcriptome data from multiple cohorts of patients with AD, and compiled
103 gene expression data. The glycogene set consisting of 214 glycosyltransferases was defined
104 using the gene list in the Glycogene Database (GGDB: https://acgg.asia/ggdb2/)[28] and
105 literature[6,29,30] (**Figure 2A, Supplementary Table 2**). This gene set was also categorized
106 according to its glycosylation pathway and synthesis steps (**Figure 2A**).
107 We derived transcriptional signatures of glycogenes based on a meta-analysis. We
108 identified 46 differentially expressed genes (DEGs) in the glycogenes (**Figure 2B, Table S3**).
109 We mapped glycogenes to glycosylation pathways to determine the pathways enriched for the
110 DEGs (**Figure 2C**). Glycosyltransferases were differentially expressed in all pathways (**Figure**
111 **2C**), indicating that the signals triggering aberrant glycosylation had already been observed
112 at the transcriptional level.
113 Next, we analyzed the biological functions of these glycogene signatures. The 779
114 globally enriched biological pathways were estimated based on the effect size from the
115 differential expression obtained by meta-analysis using all genes (FDR < 5%) (**Figure 2D,**
116 **Supplementary Table S2–S4**). Subsequently, a post-hoc enrichment analysis was performed to
117 infer which glycosylation pathways were associated with these enriched biological pathways
118 (**Figure 2D, Supplementary Table S5**). Significant glycosylation pathways were extracted with
119 a hypergeometric test as the final estimation results (**Figure 2D, Table S4–S5; p-value < 5%**).

120 We found that the ECM is a common biological signature of the transcription and glycosylation
121 layers in AD (**Figure 3A**). The ECM cluster was strongly associated with the hydroxyl
122 galactose glycosylation pathway (**Figure 2D**).
123
124 **PLOD3 is identified as a functional hub glycogene for ECM**
125 Next, we performed an in-depth analysis of the glycogenes that play a central role in
126 the ECM. Of the 779 globally enriched pathways, we constructed a bipartite graph consisting
127 of glycogene–pathway relationships based on 48 pathways, including the differentially
128 expressed glycogenes (**Figure 3B**). We inferred the glycogene importance based on the number
129 of neighboring pathways, that is, the network degree (**Figure 3C**). As a result, PLOD3 was
130 identified as a hub glycogene with the highest degree (**Figure 3C**).
131 PLOD3 is an enzyme that mediates essential glycosylation during the early stages of
132 collagen formation[31]. In general, collagen is broadly modified by the hydroxylation of proline
133 and lysine and glycosylation of specific hydroxylysine residues[32]. Hydroxylation of lysine is
134 catalyzed by PLOD3[33,34]; hydroxylysine undergoes further glycosylation, and COLGALT1
135 transfers galactose, which are critical steps for maintaining collagen integrity[32].
136 To further confirm the results at the gene expression level, we examined whether
137 the changes in PLOD3 expression were consistent among the AD cohorts included in the meta-
138 analysis. We found that PLOD3 was consistently upregulated in individual cohort studies
139 (**Figure 3D**), and the expression signatures of ECM organization and collagen formation
140 showed a consistent overexpression trend (**Figure 3D**). Based on this analysis, we
141 hypothesized that hyperglycosylation of the ECM in AD brain tissue is mediated by PLOD3.
142
143 **PLOD3 is expressed in oligodendrocytes and co-expressed with COL4A5**
144 We sought to determine the cellular origin of the PLOD3 and collagen genes. First,
145 we accessed the scRNA-seq data of normal brain tissue from the Human Protein Atlas

146 (v22)[35,36]. We found that PLOD3 was co-expressed with COL4A5 in oligodendrocytes (**Figure**
147 **4A**). These two genes showed distinct oligodendrocyte-specific expression signatures (**Figure**
148 **4B**). We also accessed a human AD cohort of single-nucleus RNA-seq data for the entorhinal
149 cortex (GSE138852)[37]. The entorhinal cortex is one of the brain regions that shows
150 neurodegeneration in the early stages of AD[38–40]. The cohort included both non-cognitive
151 impairment (NCI) and AD brain. Six cell types were identified: microglia, astrocytes, neurons,
152 oligodendrocyte progenitor cells, oligodendrocytes, and endothelial cells (**Figure 4C**). PLOD3
153 and COL4A5 were highly expressed in oligodendrocytes (**Figure 4C**). These genes were also
154 predominantly expressed in the AD group (**Figure 4C**).
155
156 **COL4A5 consistently correlated with amyloid fiber formation in multiple cohort studies**
157 COL4A5 is partially correlated with amyloid plaque accumulation[41]. However, this
158 finding has not been validated in large clinical samples. We tested whether COL4A5
159 expression was significantly correlated with APP expression. We analyzed the bulk RNA-seq
160 data used in the meta-analysis and examined their relationship with APP gene expression
161 separately for each brain region. The results showed that COL4A5 was strongly correlated
162 with the APP gene in all datasets (**Figure 4D**). Furthermore, we defined the gene signatures
163 of the amyloid plaque formation pathway and analyzed the correlation between their
164 eigengene expression and COL4A5 in the same way. As expected, a strong correlation was
165 confirmed (**Figure 4D**). PLOD3 was evaluated similarly, showing a weaker correlation than
166 COL4A5, but it was significant in several datasets (**Supplementary Figure 1**).
167
168 **Cerebrovasculature most strongly associated with ECM dysregulation**
169 We explored whether overexpression of the PLOD3–COL4A5 axis is involved in
170 biological processes in the AD brain. First, we analyzed the biological pathways that best
171 explained ECM activity. We used AES-PCA[42–44], a principal component analysis (PCA)-based

172 regression model with ECM activity as the outcome variable and all other biological pathway
173 activities as predictors, for each AD cohort used in the meta-analysis (**Figure 5A,**
174 **Supplementary Table S6**). The estimated p-values were statistically combined using Fisher's
175 method (**Figure 5A**). Four of the top 10 enriched genes were associated with the vascular
176 system (**Figure 5A**) and were overexpressed in the AD group at the expression level (**Figure**
177 **5B**). We hypothesized that the PLOD3–COL4A5 axis is involved in the cerebrovascular
178 microenvironment.
179

180 **PLOD3 and COL4A5 are expressed in oligodendrocytes of the cerebrovasculature**
181 **microenvironment**

182 We analyzed recently reported scRNA-seq data from the vascular microenvironment
183 of the human brain (GSE16357)[45]. These data were used to quantify gene expression by VINE-
184 seq in the cerebral blood vessels in 8 NCI and 9 AD samples (**Figure 5C**). Gene expression was
185 quantified in 143,793 cells from 14 cell types, including vascular endothelial cells (arterial,
186 capillary, and venous), mural smooth muscle cells (SMCs), pericytes, astrocytes, macrophages,
187 T cells, and perivascular and medullary fibroblasts (**Figure 5C**). We examined cell types
188 expressing PLOD3 and COL4A5, which were most strongly expressed in oligodendrocytes
189 (**Figure 5D, E**). In contrast, other type IV collagens were mainly expressed in pericytes and
190 SMCs, which is consistent with the fact that type IV collagen constitutes the vascular
191 basement membrane[46].
192

193 **Oligodendrocytes interact with astrocytes via the COL4A5 ligand**

194 Next, we analyzed the biological functions and pathways mediated by the PLOD3–
195 COL4A5 axis in the cerebrovascular microenvironment. According to the KEGG pathway
196 analysis, COL4A5 may contribute to cell-to-cell communication via ECM ligand receptors
197 (hsa04512). We analyzed how the PLOD3–COL4A5 axis of oligodendrocytes mediates

198 intercommunication between cell types. CellChat[47] allows for the estimation of cell–cell
199 interactions for each signaling pathway. We estimated cell–cell interactions based on collagen
200 signaling pathways in the AD group. Oligodendrocytes interacted with astrocytes via the
201 COL4A5 ligand and CD44 receptor (**Figure 6A**). This was verified using NicheNet[48], another
202 intercellular communication estimation algorithm. Among oligodendrocytes, COL4A5 was
203 identified as one of the most promising candidates (**Figure 6B**). In addition to CD44 identified
204 by CellChat, SDC4, DDR2, ITGB8, and ITGAV were predicted to be astrocyte receptors
205 (**Figure 6B**). These receptors were highly expressed in astrocytes (**Figure 6C**).
206
207 **COL4A5 ligand is involved in the regulatory cascade of the astrocyte stress response**
208 We performed a detailed analysis of signaling pathways to understand the biological
209 functions of COL4A5-mediated interactions between oligodendrocytes and astrocytes. We
210 integrated the predicted COL4A5 ligand–receptor pairs (CD44, SDC4, DDR2, ITGB8, and
211 ITGAV) into the prior knowledge of the signaling network constructed from multiple
212 perturbation experiments and databases using NicheNet. The results indicated that the
213 COL4A5 ligand targeted and activated B-cell/CLL lymphoma 6 (BCL6) and serum and
214 glucocorticoid-regulated kinase 1 (SGK1) via ECM receptors in astrocytes (**Figure 6D**). BCL6
215 is a transcription factor and master regulator of humoral immunity and B-cell
216 lymphomagenesis, while SGK 1 encodes a serine/threonine protein kinase that plays an
217 important role in cellular stress responses[49–51]. Both genes were found to be expressed in
218 astrocytes (**Figure 6E**).
219 Based on these results, we inferred the biological functions of the BCL6 and SGK1
220 gene modules in astrocytes. An astrocyte-specific co-expression network was constructed
221 based on gene expression using the hdWGCNA algorithm[52] (**Figure 6F**). Next, we applied the
222 random walk with restart (RWR) algorithm[53], which is a network propagation algorithm
223 starting from BCL6 and SGK1 on the astrocyte-specific network topology (**Figure 6F**). The

224 RWR allows for the evaluation of the proximity of the network between BCL6, SGK1, and
225 other neighboring genes. Based on these results, we prioritized the top 30 neighbors (**Figure**
226 **6G**). GO analysis of these neighboring gene groups revealed that they were enriched mainly
227 for processes involved in stress response (**Figure 6H**). These enriched pathways were also
228 observed in the GO analysis of BCL6 and SGK1 and were independently identified using the
229 network propagation method (**Supplementary Figure 3**).
230

231 **Discussion**

232 Our knowledge of the involvement of glycosylation, a major post-translational
233 modification, in the pathogenesis of AD is lacking. We systematically explored the
234 pathogenesis and driving factors based on an integrated analysis of the emerging dimensions
235 of glycosylation in combination with transcriptomics.

236 In the brain tissue of patients with AD, hyperglycosylation in the ECM is the main
237 signature shared by the glycome and transcriptome, and the glycosyltransferase PLOD3 is an
238 upstream regulator that acts as a functional hub. PLOD3 is predominantly expressed in
239 oligodendrocytes in AD brain tissue and the cerebrovasculature and is co-expressed with
240 COL4A5. Importantly, COL4A5 significantly correlated with APP levels and the activity of the
241 amyloid fiber formation pathway. Single-cell/nuclear analysis revealed that COL4A5 is a
242 ligand for oligodendrocytes that can mediate cell–cell interactions via ECM receptors on
243 astrocytes. In addition, signaling pathway network analysis identified BCL6 and SGK1 as its
244 target genes, and their neighboring genes in the astrocyte-specific network analysis revealed
245 that these two genes are involved in the regulation of the stress response.

246 The involvement of the ECM in AD has been supported by a large amount of
247 literature[27,54–58]. The physiological roles of the ECM are diverse and include developmental
248 regulation, tissue homeostasis, cell migration, cell proliferation, cell differentiation, neuronal
249 plasticity, and neurite growth[59]. In particular, the ECM is extensively involved in the

250 dysregulation of perineuronal networks in AD[58,60–68], which are involved in the maintenance
251 of spatial structure, neuronal plasticity, scaffolding,[69] and the regulation of aggregation; it is
252 also involved in amyloid protein dynamics[27,70–75] and brain–blood barrier integrity [41,54,76–79].
253 As glycoproteins are the major components of the ECM[55,59,80], glycan synthesis is important
254 for ECM homeostasis in the brain. The enrichment of dysregulated glycoproteins in the ECM
255 is natural in this sense (**Figure 1A, B**).

256 We discovered that PLOD3 was enriched in the ECM and upregulated in the AD
257 meta-analysis (**Figure 2B–D**). PLOD3 is a multifunctional enzyme, and in addition to its role
258 as a lysyl hydroxylase, it has collagen galactosyltransferase and glucosyltransferase
259 activity[34,81–83]. Although no direct evidence of PLOD3 in AD has been reported, it is known to
260 play an essential role in the formation of collagen, a major component of ECM[84]. For instance,
261 defects in PLOD3 (or lysyl hydroxylase 3; LH3) have been implicated in inherited connective
262 tissue disorders and have been shown to cause cerebral small vessel injury[85,86], maintenance
263 of the structural integrity of cerebral blood vessels, and the regulation of inflammatory
264 processes[87]. This enzyme is also a promising biomarker of AD, as its expression has been
265 reported to fluctuate in cell-free RNA expression in blood samples from patients with AD[88].

266 PLOD3 mediates glycosylation during early collagen formation[31]. Type IV collagen is
267 an essential protein in the cerebral vasculature of patients with AD and is responsible for
268 network formation in the basement membrane. Indeed, in our analysis of single-cell
269 expression levels in cerebral vessels, many type IV collagens
270 (COL4A1/COL4A2/COL4A3/COL4A4) were predominantly expressed in pericytes and SMCs
271 (**Figure 5E**). In contrast, COL4A5 behaves differently from other type IV collagens and is
272 predominantly expressed in oligodendrocytes. Oligodendrocytes have been shown to stably
273 bind to cerebral blood vessels by zonation analysis based on single-cell/nuclear sequencing
274 analysis[89,90] and electron microscopy[91]. Interestingly, data from multiple studies support that
275 COL4A5 is strongly correlated with APP and amyloid fiber formation (**Figure 4D**), suggesting

276 a relationship with amyloid plaque accumulation. This may be relevant because the
277 overexpression of type IV collagen generally leads to an increase in cortical basement
278 membrane thickness and has been implicated in the degeneration of cerebral vascular
279 structures[55]. The functional role of type IV collagen in AD cerebrovasculature should be
280 examined in detail in future studies.

281 We also performed an in silico analysis of cell–cell interactions. COL4A5 functioned
282 as a ligand in oligodendrocyte–astrocyte interactions (**Figure 6A**). Analysis of the signaling
283 pathway network suggested that this cell–cell interaction may contribute primarily to the
284 stress response via SGK1 or BCL6 (**Figure 6D–H**). SGK1 is known to be transcriptionally
285 upregulated under cellular stress[49–51]. On the other hand, both factors have also been reported
286 to be involved in inflammatory responses in the central nervous system. Recent studies have
287 shown that inhibition of SGK1 can suppress the NF-κB-mediated inflammatory pathway in
288 glial cells[92]. There is also evidence that BCL6 plays a central role in regulating astrocytes and
289 NF-κB in response to inflammatory stimuli and disorders[93]. Indeed, in our glycoprotein
290 analysis, the immune response pathway was enriched next to the ECM (**Figure 1B, C**), and
291 inflammatory cytokines were also significantly associated with the ECM organization
292 pathway at the transcriptome level (**Figure 5A, Supplementary Figure S2A**). Inflammatory
293 pathways are key signatures in the AD brain; however, their mechanisms of action in the
294 stress response remain unclear. Further examination of the mechanisms underlying BCL6-
295 and SGK1-mediated stress responses is required.

296 This study has several limitations. First, the AD glycomic analysis was limited to N-
297 type glycans. Therefore, evidence of ECM hyperglycosylation should be verified in future
298 studies using comprehensive glycoproteomic data. Second, the AD cohort data used in the
299 meta-analysis were limited to those deposited on the AD knowledge portal. To establish a
300 higher level of evidence, data from other large cohort studies should be included. Third, single-
301 cell sequencing data were collected from several different sources; therefore, there is no

302 guarantee that the results reflect the differential expression results of the bulk sequencing
303 used in the meta-analysis. It is expected that this limitation can be overcome in the future as
304 multilayered omics data are collected. However, validation, including experimental
305 approaches, is required.

306 Our results suggest that glycosylation is involved in the pathogenesis of AD through
307 several unknown mechanisms. Our results also indicate that glycogenomics analysis
308 integrating genetic approaches is a promising method for highlighting the biological functions
309 of glycans and the molecular pathogenesis of diseases at a single-cell resolution. Data on AD
310 glycoproteomics in human subjects are limited. However, as glycoproteomic analysis
311 technology matures, it will be applied to various disease areas, and a vast amount of
312 glycoproteomic data will be accumulated in the next decade. In the near future, the
313 glycogenomics approach will play an important role as a bridge between the established AD
314 genetic pathology and the emerging dimensional omics field of glycoproteomics.

315

316 **Methods**

317 **Glycoproteomics enrichment analysis**

318 The first set of glycoproteomics data[9] was used for enrichment analysis. This dataset
319 was analyzed for glycoproteins overexpressed in the AD group (BRAAK ≥ 5) and the normal
320 group (BRAAK ≤ 2), as defined in the original paper, using the canonical pathway collection
321 of MSigDB (c2.cp.v2022.1.Hs.symbols.gmt). All genes were analyzed as backgrounds using the
322 fedup package in R (https://github.com/rosscm/fedup), and the top 30 significantly enriched
323 pathways were identified. The second set of glycoproteomics data[9] was analyzed in the same
324 manner. Comparisons were made between the symptomatic group (BRAAK ≥ 5 and CERAD 1
325 or 2), the asymptomatic group (BRAAK ≥ 3 and CERAD 1 or 2), and the normal group (BRAAK
326 ≤ 2 and CERAD 4), as defined in the original paper. Glycoproteins specifically identified in the
327 symptomatic group were extracted. Enrichment analysis was performed to identify the top 30

328 significantly enriched pathways.

329

330 **Meta-analysis**

331 Meta-analysis using RNA-seq harmonization of AMP-AD followed the published AD-
332 CONTROL analysis protocol (https://github.com/th1vairam/ampad-DiffExp/tree/df3efa793f,
333 379730bae6d4c9e62910fb2c37e525/gene_level_analysis). First, meta-information was used
334 for data from 3 cohorts (ROSMAP, MSSM, and Mayo), including seven different brain regions,
335 to define patients with definitive late-onset AD from a clinical and neuropathological
336 perspective, that is, neurofibrillary changes, neuritic amyloid plaques, and cognitive
337 dysfunction. The AD control group consisted of patients with AD.

338 AD controls were defined as patients with few plaques and neurofibrillary changes
339 and no cognitive impairment; in ROSMAP, LOAD cases were those with a BRAAK of 4 or more,
340 a CERAD score of 2 or less, and a cognitive diagnosis of probable AD with no other causes
341 (cogdx = 4); LOAD controls were those with a BRAAK of 3 or less, a CERAD score of 3 or more,
342 and a cognitive diagnosis of "no cognitive impairment" (cogdx = 1). For the MSBB, LOAD cases
343 were defined as those with a CDR score of at least 1, a BRAAK score of at least 4, and a
344 CERAD score of at least 2. LOAD cases were similarly defined as those with a CDR score of
345 0.5 or less, a BRAAK of 3 or less, and a CERAD of 1 or less as LOAD controls. In Mayo, cases
346 were defined based on neuropathology, with LOAD cases defined as having a BRAAK score of
347 4 or higher and LOAD controls as having a BRAAK score of 3 or lower.

348 A meta-analysis using a mixed-effects model was performed to determine the
349 differences in the expression levels of each gene in each of the seven brain regions in each
350 cohort. Effect sizes were estimated using the restricted maximum likelihood method based on
351 the standard mean difference using Hedge. The Metacont function from the meta package of
352 R was used for the analysis. The p-values were corrected for multiple testing by "fdr" using
353 the p.adjust function from the stats package.

354

355 **Enrichment map**

356 Gene Set Enrichment Analysis (GSEA) was performed on all genes included in the
357 meta-analysis. The gene set was c2.cp.v2022.1.Hs.symbols from the MsigDB collection, which
358 was loaded using Enrichment Map in Cytoscape and drawn using default settings. After
359 drawing the pathways, we manually classified them into several categories and created
360 several clusters in the network. The list of glycan-related genes manually defined for each
361 glycosylation pathway was then analyzed by post-hoc analysis using the hypergeometric test
362 and the Wilcoxon test, and pathways with FDR ≤ 5% and that were significant by two tests
363 were extracted. Significant pathways in the two tests were extracted.

364

365 **Functional hub glycogene identification**

366 Among the enriched pathways based on the same GSEA results as the enrichment
367 map, only pathways containing glycogenes were extracted, and from these, a two-part graph
368 of the pathway–glycogene was extracted. Based on the two-part graphs obtained, each gene
369 was ranked according to its degree of expression. The glycogene with the highest degree was
370 defined as the functional hub glycogene. The results of querying the extracted PLOD3 to the
371 String database (v11) are shown in **Figure 2D**. Forest plots of PLOD3 are shown with
372 estimated effect sizes and 95% confidence intervals from the meta-analysis. For pathway
373 activity, GSEA was performed using the R fgsea package, with gene ranks for effect sizes for
374 each cohort and c2.cp.v2022.1.Hs.symbols for the gene set. Normalized enrichment scores
375 were used for the forest plots.

376

377 **Cell-type specificity of PLOD3**

378 For the cell-type specificity of healthy tissues, information was obtained from the
379 Human Proteome Atlas (V22) website by entering the gene name. For data on the entorhinal

380 cortex, information was obtained by entering gene names from http://adsn.ddnetbio.com/.

381

382 **Pathway-based PCA regression and GSEA**

383 Pathway-based PCA is a PCA-based method for analyzing pathways and phenotypic
384 associations[43,44,94]. The R Bioconductor PathwayPCA package[42] was used for the analysis.
385 Using region-specific gene expression data from each AD cohort (Mayo, MSSM, and ROSMAP),
386 we specified the mean expression levels of the ECM pathway component genes as the ECM
387 pathway activity for the objective variable and each pathway other than the ECM pathway
388 for the explanatory variables. The gene set used was c2.cp.v2022.1.Hs.symbols from MSigDB.
389 The pathway names containing "ECM," "Extracellular," or "Collagen" were defined as ECM
390 pathways. The genes involved in these pathways were defined as signatures.

391 The p-values of the list of pathways significantly associated with ECM were combined
392 using Fisher's method to calculate the integrated p-value. For the calculation, the log-sum
393 function of the R metapackage[95] was used, and the p-values of the individual datasets were
394 entered for each pathway. In addition, we cross-checked whether significantly related
395 pathways were sufficiently enriched at the expression level. Focusing on the top 10 pathways,
396 we applied GSEA based on the gene set c2.cp.v2022.1.Hs.symbols from MSigDB using the
397 effect sizes of the 3 cohort meta-analysis as the gene rank. To further validate that the top 10
398 pathway activities tended to increase by cohort and region, the means of the effect sizes and
399 confidence intervals were calculated for the signature genes and illustrated as forest plots.

400

401 **Analysis of brain vasculature with scRNA-seq**

402 Count data were preprocessed using the Seurat package in R. Normalization, feature
403 selection with VST, scaling, and dimensional reduction using PCA and UMAP were performed.
404 The cell types were visualized using those previously identified in an original paper[45]. Next,
405 for each cell type, variation analysis between the AD and cognitively normal groups was

406 performed using Seurat's FindMarkes function, and enrichment analysis for the identified
407 groups of DEGs was performed using the R fedup package. The c2.cp.v2022.1.Hs.symbols from
408 MSigDB was used as the gene set to determine which cell types were enriched in ECM-related
409 pathways. We selected gene sets with pathway names containing "ECM," "Extracellular,"
410 "Matrisome," or "Collagen" in the pathway name. The enriched p-values were further
411 transformed as -Log10(FDR) from the multiple-test-corrected FDR, considered as
412 differentially expressed activity signals, and visualized using a heatmap. The expression
413 levels per cell type were obtained by querying https://twc-stanford.shinyapps.io/human_bbb/
414 for PLOD3.
415
416 **Cell–cell interaction and signaling network analysis**
417 Cell–cell interactions were analyzed using the R package CellChat[47]
418 (https://github.com/sqjin/CellChat). Oligodendrocytes and astrocytes identified with CellChat
419 were further analyzed using another algorithm, NicheNet[48] (https://
420 github.com/saeyslab/nichenetr). For a detailed analysis, the ligand–receptor prior information
421 was input by integrating the ligand–receptor pair information used in CellChat with the
422 ligand–receptor pair information used in NicheNet. This was also used for the signal network
423 analyses. A pre-built model was downloaded
424 (https://github.com/saeyslab/nichenetr/blob/master/vignettes/model_construction.md), and
425 both the ligand–receptor information and expression information were identified in the cell–
426 cell interactions.
427
428 **Astrocyte cell-type specific network propagation**
429 For astrocyte-specific network construction using cerebrovascular scRNA-seq, the
430 Toplogical Overlap Measure (TOM) was estimated using hdWGCNA[52], and edges were further
431 defined only if they had a TOM above the 90th percentile as a threshold. The network

432 propagation method was applied using the R package RandomWalkRestartMH[53]. In other
433 words, we performed an RWR starting from SGK1 and BCL6 in the obtained network topology.
434 The 30 most relevant neighbors were narrowed down and plotted using the R package igraph.
435 The R package fedup was used for the enrichment analysis. To estimate the transcriptional
436 activity of BCL6, curated regulon information was first obtained using the R package
437 DoRothEA[96], and transcription factor target genes were estimated using the Viper[97] algorithm.
438 The R package decoupleR[98] was used for the analysis.
439

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Yusuke Matsui: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original draft, Writing - Reviewing and Editing, Visualization, Funding acquisition. **Akira Togayachi**: Resources, Data Curation, Writing - Reviewing and Editing **Kazuma Sakamoto**: Writing - Reviewing and Editing **Kiyohiko Angata**: Writing - Reviewing and Editing. **Kenji Kadomatsu**: Supervision, Writing- Reviewing and Editing, Project administration, Funding acquisition. **Shoko Nishihara**: Conceptualization, Supervision, Writing- Reviewing and Editing, Project administration.

Conflict of interest

The authors declare no competing interests.

Data availability

The glycoproteomics datasets were compiled and used from the supplementary files published in the respective papers[9,10]. For the AMP-AD transcriptome dataset, data were obtained from the RNAseq Harmonization Study (RNAseq Harmonization) Repository (syn21241740). Single-cell transcriptome data in the entorhinal cortex were obtained from GSE138852. Brain vascular data were downloaded from GSE163577.

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713 **Figure 1 - Hyperglycosylated proteins are primarily enriched in the ECM**.

714 A Analysis of glycoprotein data from 2 AD cohorts, using glycoproteins from prefrontal tissues of 2
715 independent AD cohorts. The first cohort (AD cohort 1) consisted of 8 samples each from healthy subjects
716 and those with AD, and the second cohort (AD cohort 2) consisted of 10 samples each from healthy subjects,
717 asymptomatic AD, and symptomatic AD. In each cohort, 92 and 10 AD-specific glycoproteins were identified,
718 respectively.

719 B Pathway enrichment of AD-related glycoproteins. Over-representation analysis of AD-specific
720 glycoproteins was performed.

721 C Significantly enriched pathways that were common in both cohorts are shown. The horizontal axis is the
722 p-value representing the enrichment, which is the logarithm of the nominal p-value multiplied by a negative
723 value.

724 Figures were created with BioRender.com.

725 **Figure 2 - Meta-analysis of the global transcriptome reveals that glycogenes are enriched in the ECM.**
726 A Number of glycogenes constituting the glycosylation pathway used for transcriptome analysis.
727 B Meta-analysis of differential gene expression in multiple AD cohorts. Transcriptome data from 3 AD
728 cohorts: the Mayo cohort (n = 313), the MSSM cohort (n = 315), and the ROSMAP cohort (n = 1168). A meta-
729 analysis of DEGs based on gene-level expression levels (FDR < 5%) was performed; 46 glycogenes were
730 identified as DEGs. In the volcano plot, the horizontal axis represents the effect size summarizing the
731 difference in expression between the non-AD and AD groups across cohorts, and the vertical axis represents
732 the log of the p-value from the meta-analysis (bottom is 10) multiplied by a negative value.
733 C Mapped glycosyltransferase DEGs. In total, 46 glycogene DEGs were mapped. Genes overexpressed in the
734 meta-analysis are shown in red, genes underexpressed are shown in blue, and genes that did not show
735 significant mutations are shown in gray. Genes are classified into 16 major glycosylation pathways,
736 including initiation, core elongation, elongation/branching, capping, and sulfation. Glycosyltransferases
737 with and without pathway specificity are also distinguished.
738 Figure created by BioRender.com.

Figure 3 - PLOD3 is identified as a hub glycogene for the ECM.

A Comparison of AD trascriptome and glycoprotein signatures. Common pathways are shown.

B Relationship between glycogenes and globally enriched pathways. Orange nodes represent globally enriched pathways, and green nodes represent glycogen enriched in each pathway.

C Functional hub glycogenes in globally enriched pathways. To identify functional hub glycogenes involved in multiple pathways, we constructed a pathway-gene bipartite graph, calculated the degree of each glycogene (number of genes directly connected to the pathway), and ranked the importance of each glycogene. The vertical axis of the bar graph represents the order of each glycogene.

D Activity changes in PLOD3, ECM, and collagen formation in AD brains in each transcriptome cohort. Forest plots of Log2 fold changes in PLOD3, ECM organization, and collagen formation activity between non-AD and AD are plotted by cohort and brain region.

DLPFC: dorsolateral prefrontal cortex; STG: superior temporal gyrus; PHG: parahippocampal gyrus; IFG: inferior frontal gyrus; FP: frontal pole; TCX: temporal cortex; CBE: cerebellum. Dots indicate estimated mean effect sizes, bar widths are 95% confidence intervals of the estimates, and vertical lines with red dots indicate zero (no change).

754 **Figure 4 - PLOD3 is expressed in oligodendrocytes and is co-expressed with COL4A5**.

755 A Cell-type specificity of PLOD3 in healthy brain tissues. Cell clusters obtained from gene expression in
756 healthy brain tissue by Human Protein Atlas (v22) scRNA-seq, and the Transcripts Per Million (TPM) in
757 each cluster. PLOD3 and COL4A5 are highly expressed in oligodendrocytes and belong to the same cluster.
758 B Expression levels of PLOD3 and COL4A5 per cell type.
759 C Cellular specificity of PLOD3 and collagen in the enthorhinal cortex. Scatter plots show the cluster
760 structure of cell populations projected by UMAP to 2D coordinates based on gene expression; the first panel
761 shows cell types, the second non-AD and AD, the third and fourth panels show cell type-specific expression
762 of PLOD3 and COL4A5 in oligodendrocytes, respectively.
763 D Correlation of COL4A5 with the expression of APP (upper panel) and the activity of amyloid fiber
764 formation (lower panel) for each cohort and each region.
765 DLPFC: dorsolateral prefrontal cortex; STG: superior temporal gyrus; PHG: parahippocampal gyrus; IFG:
766 inferior frontal gyrus; FP: frontal pole; TCX: temporal cortex; CBE: cerebellum.

767 **Figure 5 - Cerebrovasculature most strongly associated with ECM dysregulation.**

768 A Pathways significantly associated with the activity of the ECM organization were estimated for each
769 cohort tissue using the AES-PCA model. The p-values estimated for each cohort and for each brain tissue
770 were estimated as integrated p-values, and the top 10 pathways are shown in the figure. Figures were
771 generated by BioRender.com.

772 B Enrichment of pathways involving the cerebrovasculature in AD with gene set enrichment analysis
773 (GSEA) (FDR < 5%). Forest plots shown below each enrichment plot indicate Log2 fold change for each
774 pathway in each cohort and each region.

775 C Analysis using cerebrovascular scRNA-seq data (8 NCI, 9 AD).

776 D Expression of PLOD3 per cell type.

777 E Expression of type IV collagen per cell type.

778 DLPFC: dorsolateral prefrontal cortex; STG: superior temporal gyrus; PHG: parahippocampal gyrus; IFG:
779 inferior frontal gyrus; FP: frontal pole; TCX: temporal cortex; CBE: cerebellum.

Figure 6 - COL4A5 ligand is involved in the regulatory cascade of the astrocyte stress response.

A Estimated cell-to-cell communication based on ECM ligand–receptor expression.

B Receptor candidates for oligodendrocyte-derived COL4A5 ligands predicted to bind in astrocytes.

C Cell type-specific expression levels of receptors for COL4A5.

D COL4A5-mediated signaling pathways and target genes in astrocytes.

E Expression levels of target genes BCL6 and SGK1 per cell type.

F Analysis flow of the exploration of neighboring genes and functional estimation using network propagation in astrocyte-specific co-expression networks.

G Top 30 neighboring genes estimated by network propagation based on BCL6 and SGK1.

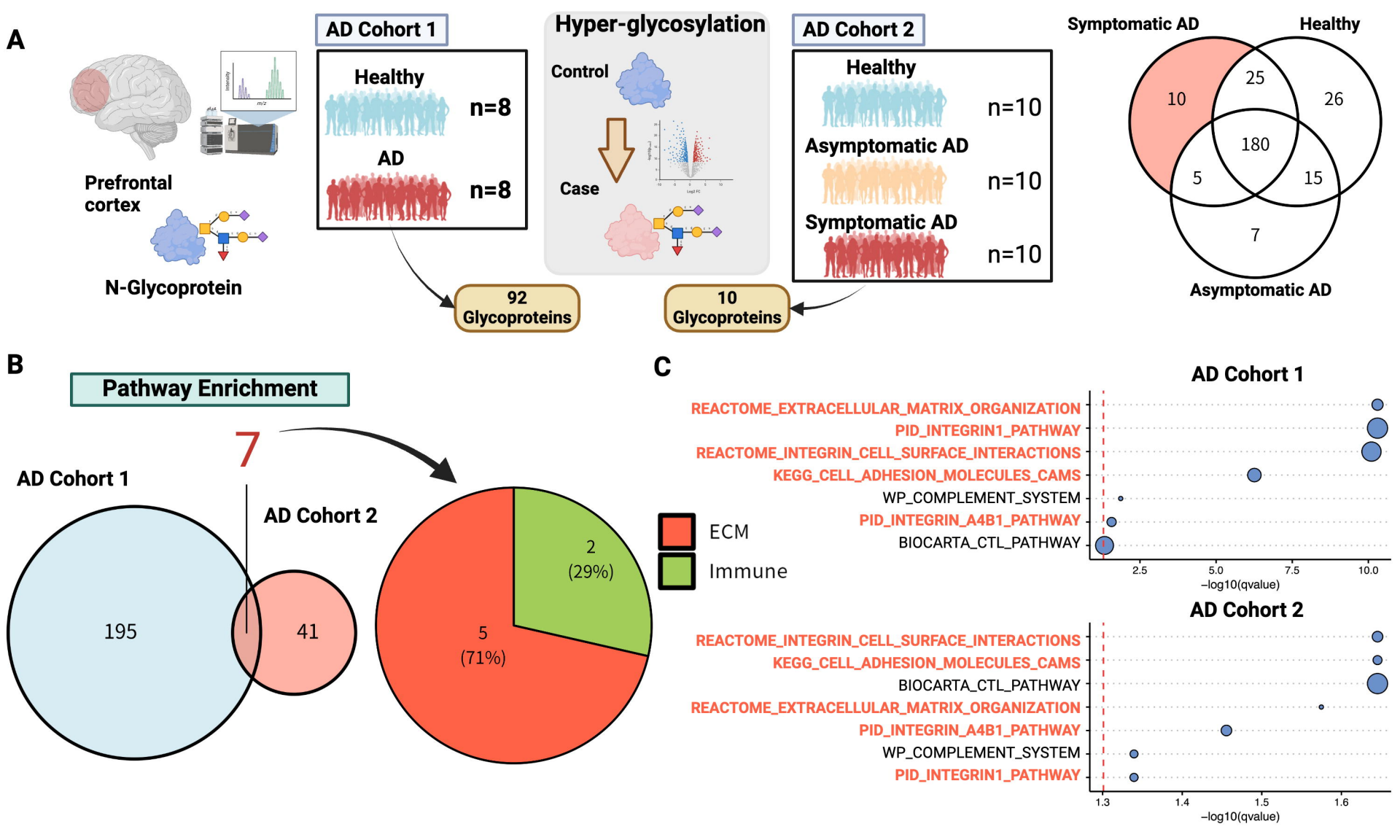
H Gene set analysis of BCL6 and SGK1 neighbor genes.

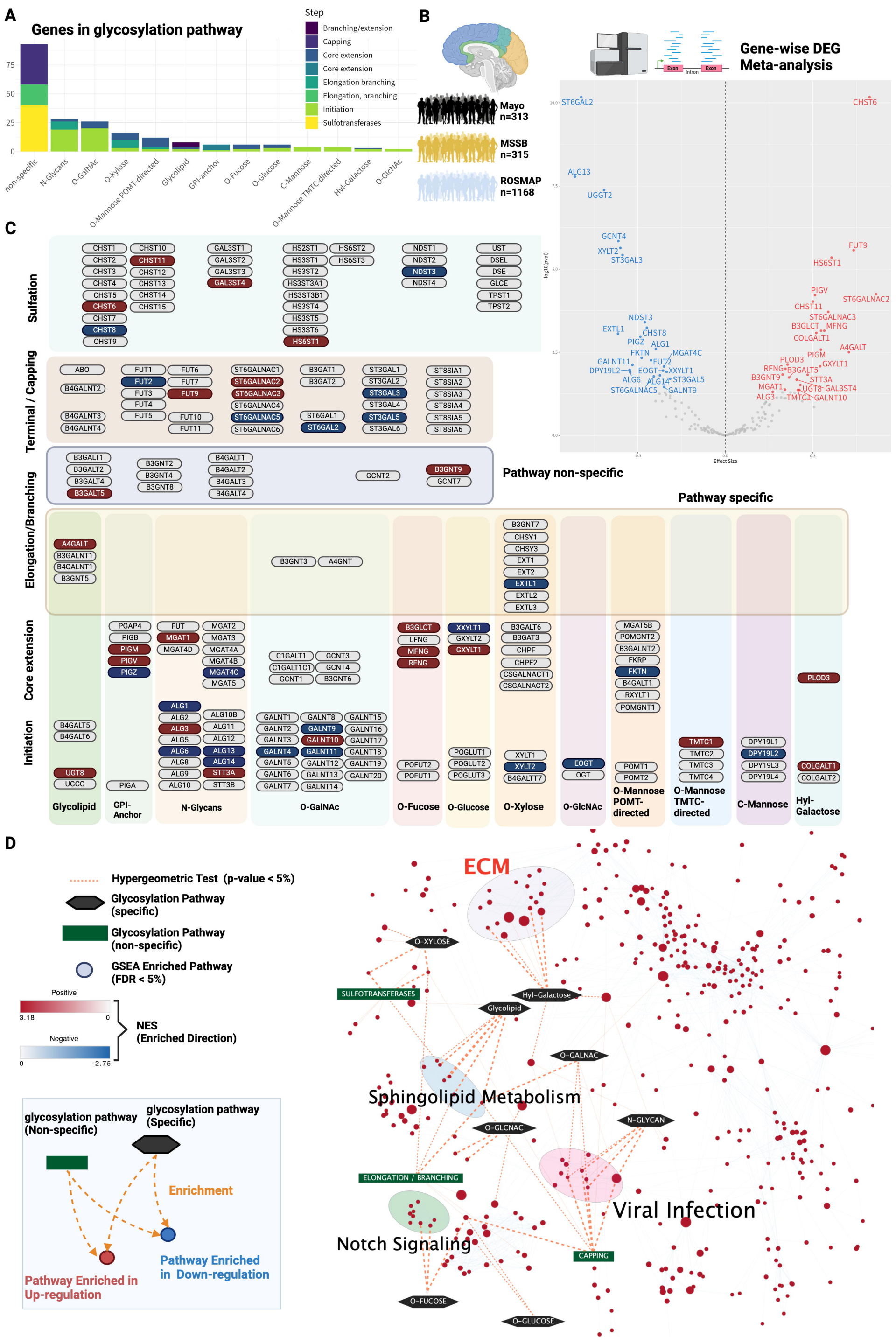
790 **Supplementary Figure 1 - Correlation of PLOD3 with APP activity (upper panel) and amyloid fiber formation**
791 **(lower panel) for each cohort and each region.**
792 DLPFC: dorsolateral prefrontal cortex; STG: superior temporal gyrus; PHG: parahippocampal gyrus; IFG:
793 inferior frontal gyrus; FP: frontal pole; TCX: temporal cortex; CBE: cerebellum.

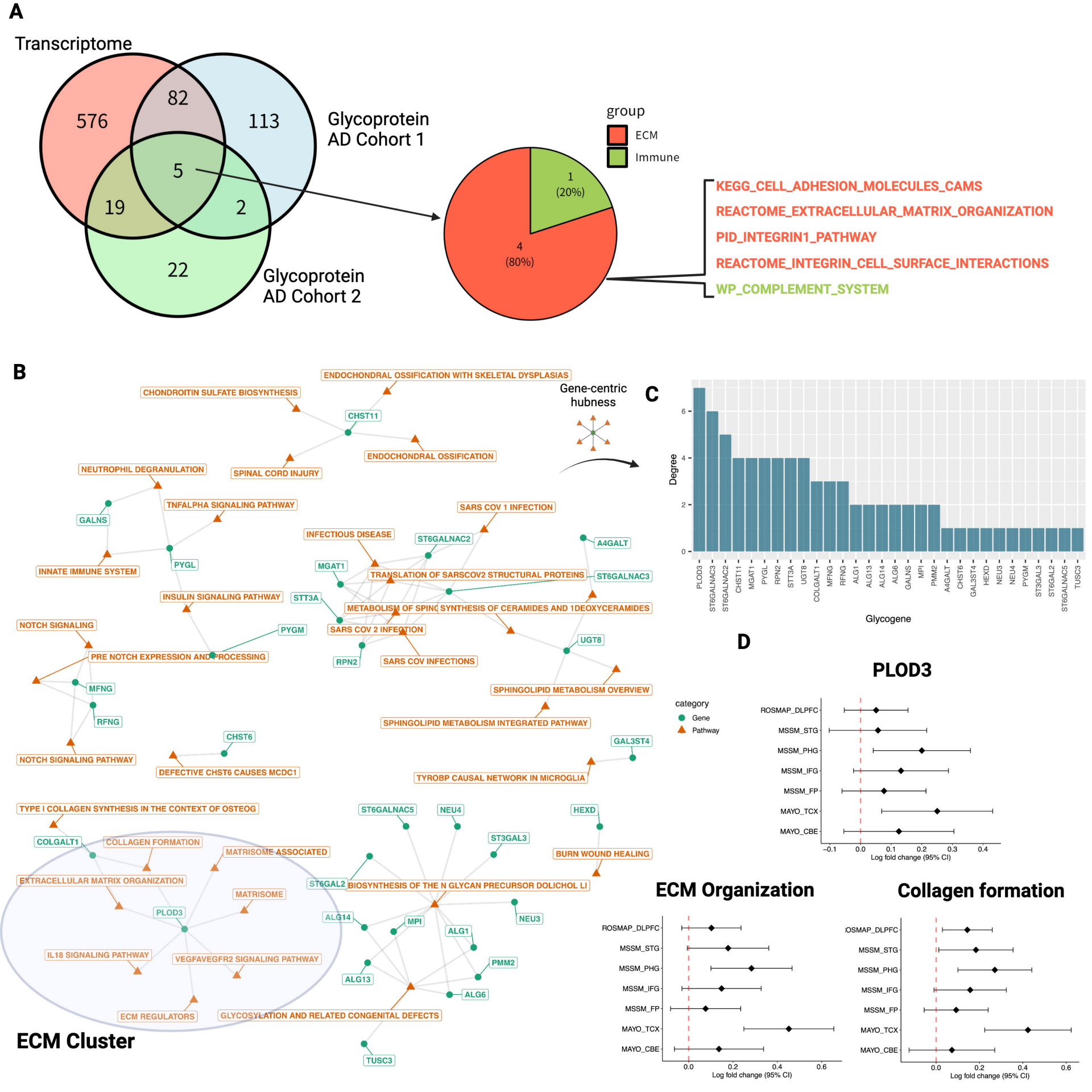
794 **Supplementary Figure 2 - ECM activity is strongly associated with inflammatory cytokines.**

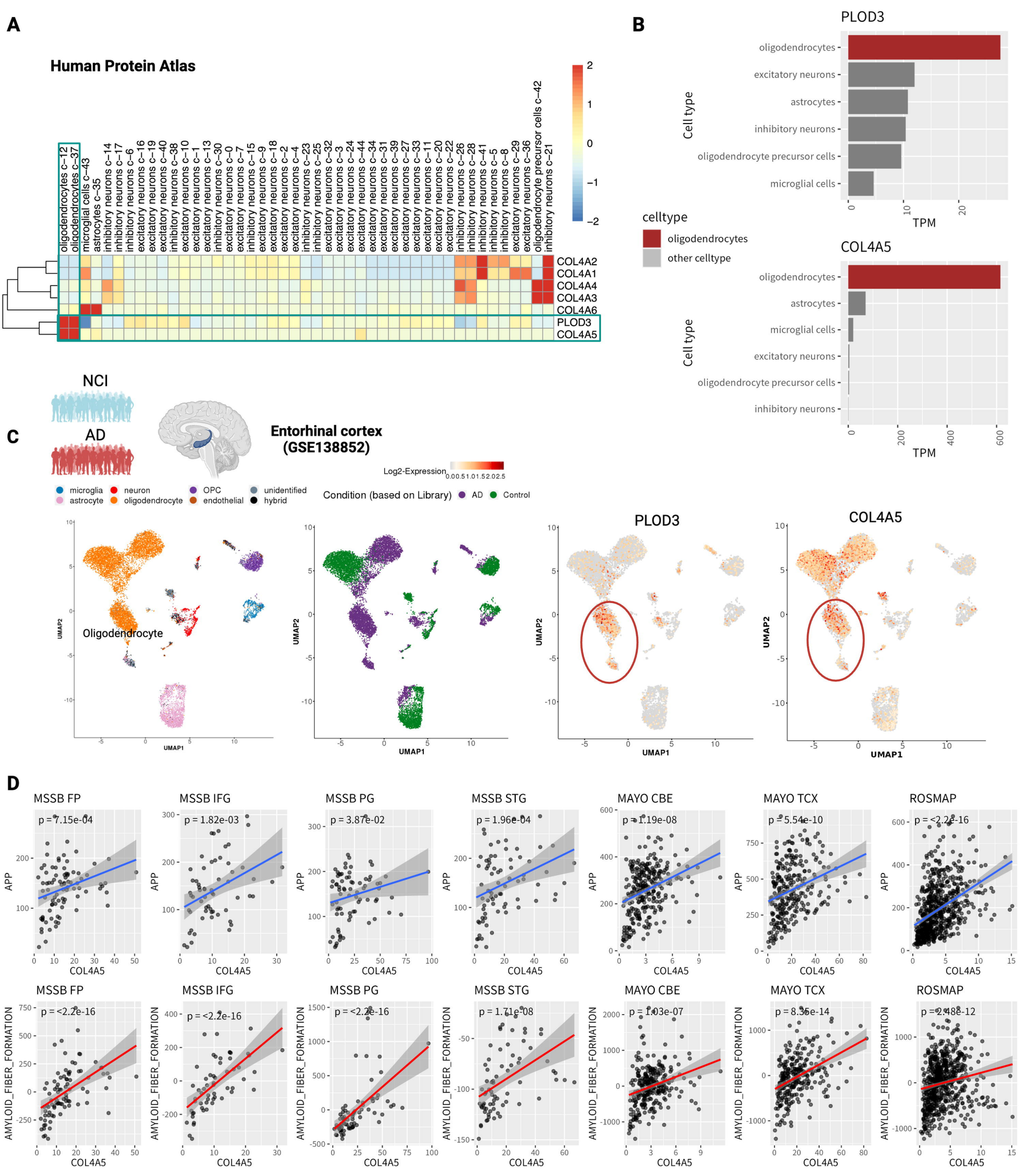
795 A Enrichment of pathways involving the immune system in AD with GSEA (FDR < 5%). Forest plots shown
796 below each enrichment plot indicate Log2 fold change for each pathway in each cohort and each tissue.
797 B Pathways significantly associated with ECM activity obtained by applying AES-PCA for each cohort and
798 region.
799 DLPFC: dorsolateral prefrontal cortex; STG: superior temporal gyrus; PHG: parahippocampal gyrus; IFG:
800 inferior frontal gyrus; FP: frontal pole; TCX: temporal cortex; CBE: cerebellum.

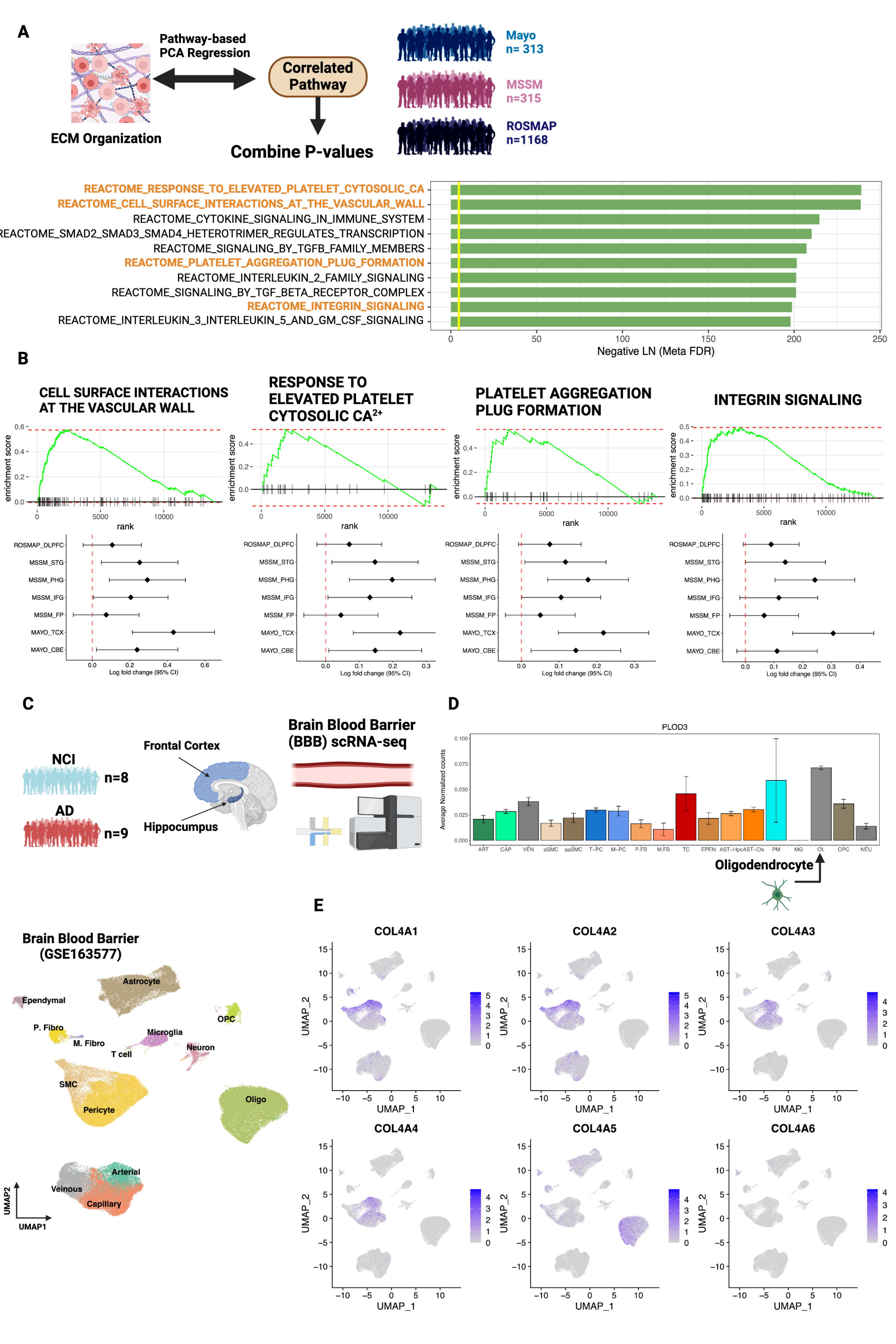
801 **Supplementary Figure 3 - COL4A5 ligand is involved in the regulatory cascade of the astrocyte stress**
802 **response.**
803 A Top 30 neighboring genes estimated by network propagation based on BCL6.
804 B Gene set analysis of BCL6 neighbor genes.
805 C Top 30 neighboring genes estimated by network propagation based on SGK1.
806 D Gene set analysis of SGK1 neighbor genes.

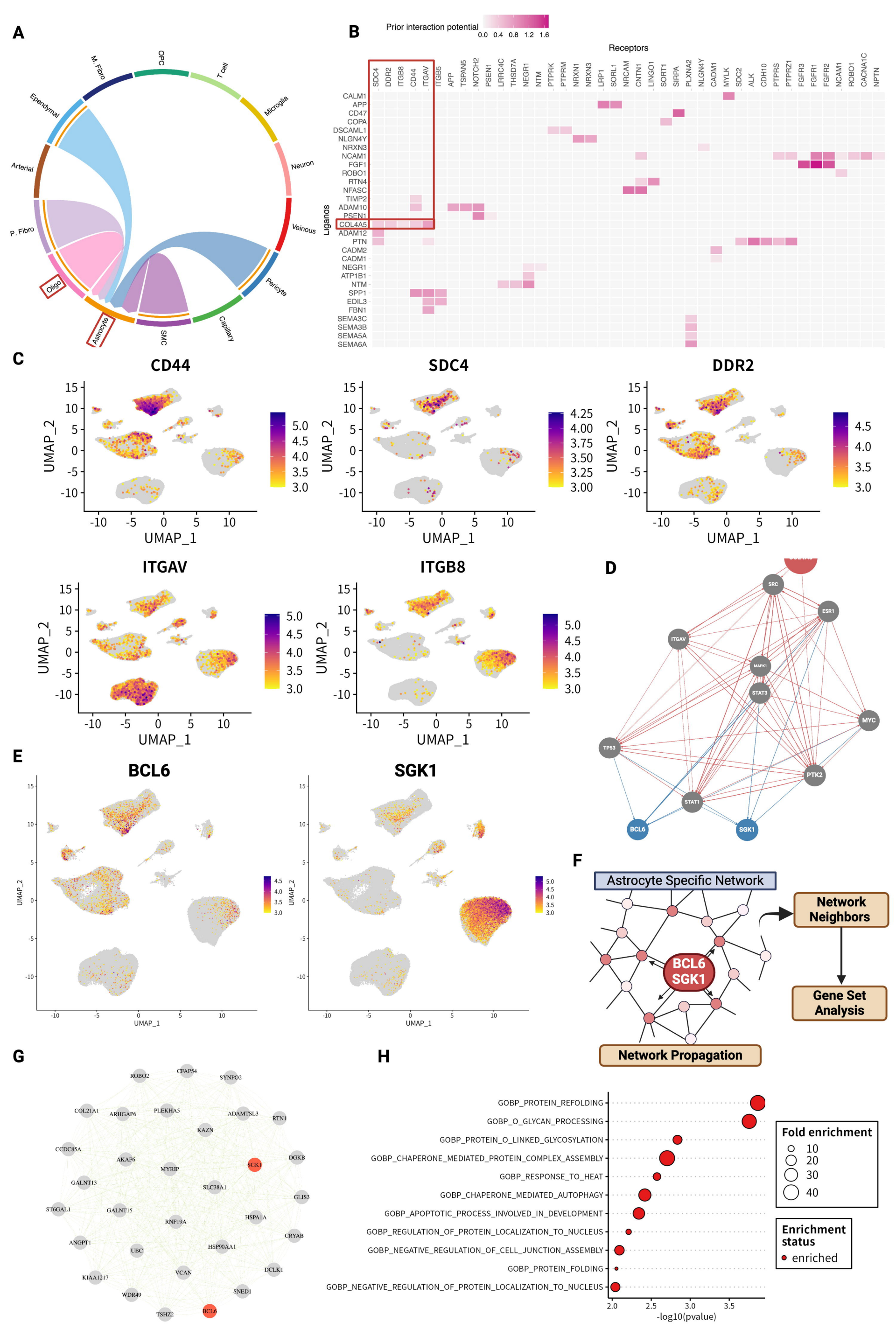

A
Prefrontal cortex
N-Glycoprotein
AD Cohort 1
Healthy
n=8
AD
n=8
Hyper-glycosylation
Control
Case
AD Cohort 2
Healthy
n=10
Asymptomatic AD
n=10
Symptomatic AD
n=10
92 Glycoproteins
10 Glycoproteins
Symptomatic AD
Healthy
Asymptomatic AD
10
25
26
180
5
15
7
B
Pathway Enrichment
AD Cohort 1
AD Cohort 2
7
195
41
5 (71%)
2 (29%)
ECM
Immune
C
AD Cohort 1
REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
PID_INTEGRIN1_PATHWAY
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
KEGG_CELL_ADHESION_MOLECULES_CAMS
WP_COMPLEMENT_SYSTEM
PID_INTEGRIN_A4B1_PATHWAY
BIOCARTA_CTL_PATHWAY
2.5
5.0
7.5
10.0
−log10(qvalue)
AD Cohort 2
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
KEGG_CELL_ADHESION_MOLECULES_CAMS
BIOCARTA_CTL_PATHWAY
REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
PID_INTEGRIN_A4B1_PATHWAY
WP_COMPLEMENT_SYSTEM
PID_INTEGRIN1_PATHWAY
1.3
1.4
1.5
1.6
−log10(qvalue)

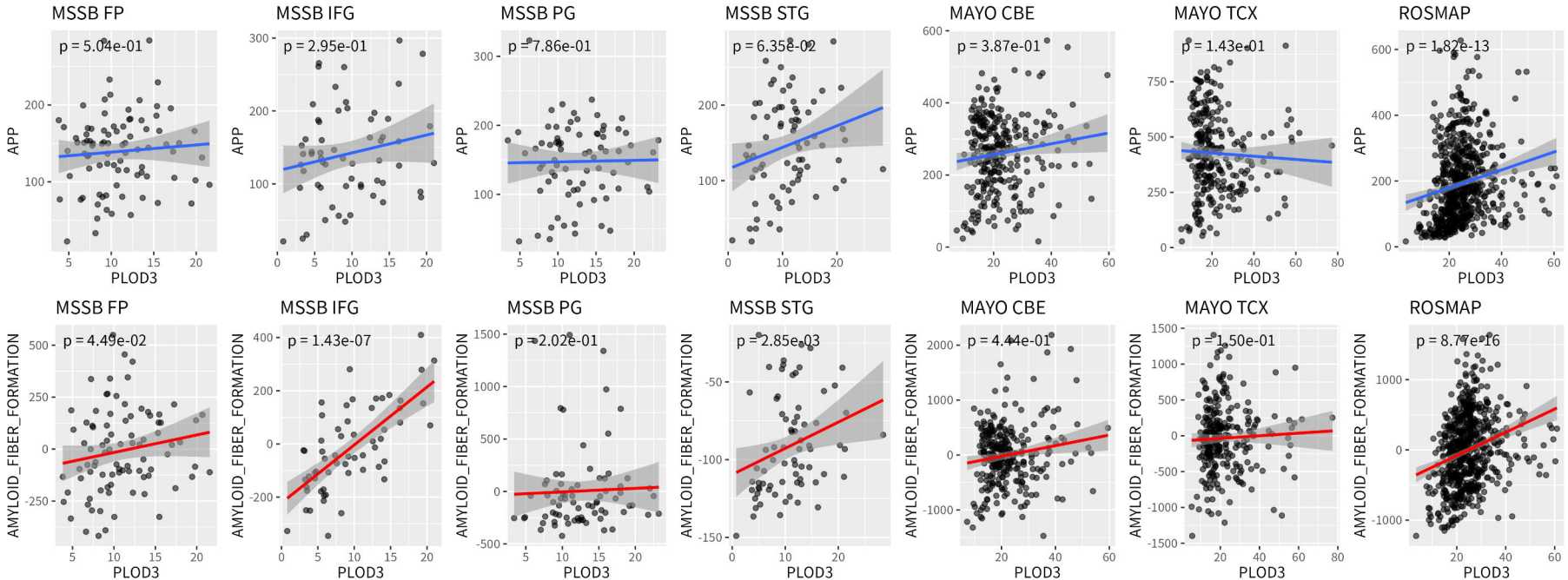

A
Genes in glycosylation pathway
Step
Branching/extension
Capping
Core extension
Core extension
Elongation branching
Elongation, branching
Initiation
Sulfotransferases
non-specific
N-Glycans
O-GalNAc
O-Xylose
O-Mannose POMT-directed
Glycolipid
GPI-anchor
O-Fucose
O-Glucose
C-Mannose
O-Mannose TMTC-directed
Hyl-Galactose
O-GlcNAc
B
Mayo n=313
MSSB n=315
ROSMAP n=1168
Gene-wise DEG Meta-analysis
Exon
Intron
Exon
-log10(pval)
Effect Size
C
Sulfation
Terminal / Capping
Elongation/Branching
Pathway non-specific
Pathway specific
Core extension
Initiation
Glycolipid
GPI-Anchor
N-Glycans
O-GalNAc
O-Fucose
O-Glucose
O-Xylose
O-GlcNAc
O-Mannose POMT-directed
O-Mannose TMTC-directed
C-Mannose
Hyl-Galactose
D
Hypergeometric Test (p-value < 5%)
Glycosylation Pathway (specific)
Glycosylation Pathway (non-specific)
GSEA Enriched Pathway (FDR < 5%)
Positive
3.18
0
Negative
0
-2.75
NES (Enriched Direction)
glycosylation pathway (Non-specific)
glycosylation pathway (Specific)
Enrichment
Pathway Enriched in Down-regulation
Pathway Enriched in Up-regulation
ECM
O-XYLOSE
SULFOTRANSFERASES
Hyl-Galactose
Glycolipid
O-GALNAC
Sphingolipid Metabolism
O-GLCNAC
N-GLYCAN
ELONGATION / BRANCHING
Viral Infection
Notch Signaling
CAPPING
O-FUCOSE
O-GLUCOSE


A
Transcriptome
576
82
113
5
19
2
22
Glycoprotein AD Cohort 1
Glycoprotein AD Cohort 2
group
ECM
Immune
1 (20%)
4 (80%)
KEGG_CELL_ADHESION_MOLECULES_CAMS
REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION
PID_INTEGRIN1_PATHWAY
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS
WP_COMPLEMENT_SYSTEM
B
Gene-centric hubness
ENDOCHONDRAL OSSIFICATION WITH SKELETAL DYSPLASIAS
CHONDROITIN SULFATE BIOSYNTHESIS
CHST11
ENDOCHONDRAL OSSIFICATION
SPINAL CORD INJURY
NEUTROPHIL DEGRANULATION
TNFALPHA SIGNALING PATHWAY
GALNS
INNATE IMMUNE SYSTEM
PYGL
SARS COV 1 INFECTION
INFECTIOUS DISEASE
ST6GALNAC2
A4GALT
MGAT1
TRANSLATION OF SARSCOV2 STRUCTURAL PROTEINS
ST6GALNAC3
INSULIN SIGNALING PATHWAY
STT3A
METABOLISM OF SPING SYNTHESIS OF CERAMIDES AND 1DEOXYCERAMIDES
NOTCH SIGNALING
PYGM
SARS COV 2 INFECTION
UGT8
PRE NOTCH EXPRESSION AND PROCESSING
RPN2
SARS COV INFECTIONS
MFNG
SPHINGOLIPID METABOLISM OVERVIEW
RFNG
SPHINGOLIPID METABOLISM INTEGRATED PATHWAY
CHST6
GAL3ST4
NOTCH SIGNALING PATHWAY
DEFECTIVE CHST6 CAUSES MCDC1
TYROBP CAUSAL NETWORK IN MICROGLIA
TYPE I COLLAGEN SYNTHESIS IN THE CONTEXT OF OSTEOG
ST6GALNAC5
NEU4
HEXD
COLGALT1
COLLAGEN FORMATION
ST3GAL3
MATRISOME ASSOCIATED
BURN WOUND HEALING
EXTRACELLULAR MATRIX ORGANIZATION
ST6GAL2
BIOSYNTHESIS OF THE N GLYCAN PRECURSOR DOLICHOL LI
MATRISOME
PLOD3
ALG14
MPI
NEU3
ALG1
IL18 SIGNALING PATHWAY
VEGFAVEGFR2 SIGNALING PATHWAY
PMM2
ALG13
ALG6
ECM REGULATORS
GLYCOSYLATION AND RELATED CONGENITAL DEFECTS
TUSC3
ECM Cluster
category
Gene
Pathway
C
Degree
Glycogene
PLOD3
ST6GALNAC3
ST6GALNAC2
CHST11
MGAT1
PYGL
RPN2
STT3A
UGT8
COLGALT1
MFNG
RFNG
ALG1
ALG13
ALG14
ALG6
GALNS
MPI
PMM2
A4GALT
CHST6
GAL3ST4
HEXD
NEU3
NEU4
PYGM
ST3GAL3
ST6GAL2
ST6GALNAC5
TUSC3
D
PLOD3
ECM Organization
Collagen formation
ROSMAP_DLPFC
MSSM_STG
MSSM_PHG
MSSM_IFG
MSSM_FP
MAYO_TCX
MAYO_CBE
Log fold change (95% CI)


A
Human Protein Atlas
oligodendrocytes c−12
oligodendrocytes c−37
microglial cells c−43
astrocytes c−35
inhibitory neurons c−14
inhibitory neurons c−17
inhibitory neurons c−6
excitatory neurons c−16
excitatory neurons c−19
excitatory neurons c−40
inhibitory neurons c−38
excitatory neurons c−10
excitatory neurons c−1
excitatory neurons c−13
inhibitory neurons c−30
excitatory neurons c−0
excitatory neurons c−7
inhibitory neurons c−15
excitatory neurons c−9
excitatory neurons c−18
excitatory neurons c−2
excitatory neurons c−4
inhibitory neurons c−23
inhibitory neurons c−25
excitatory neurons c−32
excitatory neurons c−3
excitatory neurons c−24
excitatory neurons c−44
excitatory neurons c−34
excitatory neurons c−31
excitatory neurons c−39
excitatory neurons c−27
excitatory neurons c−33
excitatory neurons c−11
excitatory neurons c−20
excitatory neurons c−22
inhibitory neurons c−26
inhibitory neurons c−28
inhibitory neurons c−41
inhibitory neurons c−5
inhibitory neurons c−8
excitatory neurons c−29
excitatory neurons c−36
oligodendrocyte precursor cells c−42
inhibitory neurons c−21
COL4A2
COL4A1
COL4A4
COL4A3
COL4A6
PLOD3
COL4A5
2
1
0
−1
−2
B
PLOD3
Cell type
oligodendrocytes
excitatory neurons
astrocytes
inhibitory neurons
oligodendrocyte precursor cells
microglial cells
0
10
20
TPM
celltype
oligodendrocytes
other celltype
COL4A5
Cell type
oligodendrocytes
astrocytes
microglial cells
excitatory neurons
oligodendrocyte precursor cells
inhibitory neurons
0
200
400
600
TPM
C
NCI
AD
Entorhinal cortex
(GSE138852)
Log2-Expression
0.0 0.5 1.0 1.5 2.0 2.5
microglia
astrocyte
neuron
oligodendrocyte
OPC
endothelial
unidentified
hybrid
Condition (based on Library)
AD
Control
Oligodendrocyte
UMAP1
UMAP2
PLOD3
COL4A5
D
MSSB FP
p = 7.15e-04
MSSB IFG
p = 1.82e-03
MSSB PG
p = 3.87e-02
MSSB STG
p = 1.96e-04
MAYO CBE
p = 1.19e-08
MAYO TCX
p = 5.54e-10
ROSMAP
p = <2.2e-16
APP
COL4A5
MSSB FP
p = <2.2e-16
MSSB IFG
p = <2.2e-16
MSSB PG
p = <2.2e-16
MSSB STG
p = 1.71e-08
MAYO CBE
p = 1.03e-07
MAYO TCX
p = 8.36e-14
ROSMAP
p = 2.48e-12
AMYLOID_FIBER_FORMATION
COL4A5


A
Pathway-based PCA Regression
ECM Organization
Correlated Pathway
Combine P-values
Mayo n= 313
MSSM n=315
ROSMAP n=1168
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA
REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM
REACTOME_SMAD2_SMAD3_SMAD4_HETEROTRIMER_REGULATES_TRANSCRIPTION
REACTOME_SIGNALING_BY_TGFB_FAMILY_MEMBERS
REACTOME_PLATELET_AGGREGATION_PLUG_FORMATION
REACTOME_INTERLEUKIN_2_FAMILY_SIGNALING
REACTOME_SIGNALING_BY_TGF_BETA_RECEPTOR_COMPLEX
REACTOME_INTEGRIN_SIGNALING
REACTOME_INTERLEUKIN_3_INTERLEUKIN_5_AND_GM_CSF_SIGNALING
Negative LN (Meta FDR)
B
CELL SURFACE INTERACTIONS AT THE VASCULAR WALL
RESPONSE TO ELEVATED PLATELET CYTOSOLIC CA2+
PLATELET AGGREGATION PLUG FORMATION
INTEGRIN SIGNALING
enrichment score
rank
ROSMAP_DLPFC
MSSM_STG
MSSM_PHG
MSSM_IFG
MSSM_FP
MAYO_TCX
MAYO_CBE
Log fold change (95% CI)
C
NCI n=8
AD n=9
Frontal Cortex
Hippocampus
Brain Blood Barrier (BBB) scRNA-seq
D
PLOD3
Average Normalized counts
ART CAP VEN aSMC aaSMC T-PC M-PC P.FB M.FB TC EPEN AST-Hpc AST-Ctx PM MG OL OPC NEU
Oligodendrocyte
E
Brain Blood Barrier (GSE163577)
Astrocyte
Ependymal
P. Fibro
M. Fibro
Microglia
T cell
Neuron
OPC
SMC
Pericyte
Oligo
Arterial
Veinous
Capillary
UMAP2
UMAP1
COL4A1
COL4A2
COL4A3
COL4A4
COL4A5
COL4A6
UMAP_1
UMAP_2


A
M. Fibro
OPC
T cell
Microglia
Neuron
Veinous
Pericyte
Capillary
SMC
Astrocyte
Oligo
P. Fibro
Arterial
Ependymal
B
Prior interaction potential
0.0 0.4 0.8 1.2 1.6
Receptors
Ligands
SDC4 DDR2 ITGB8 CD44 ITGAV ITGB5 APP TSPAN5 NOTCH2 PSEN1 LRRC4C THSD7A NEGR1 NTM PTPRK PTPRM NRXN1 NRXN3 LRP1 SORL1 NRCAM CNTN1 LINGO1 SORT1 SIRPA PLXNA2 NLGN4Y CADM1 MYLK SDC2 ALK CDH10 PTPRS PTPRZ1 FGFR3 FGFR1 FGFR2 NCAM1 ROBO1 CACNA1C NPTN
CALM1
APP
CD47
COPA
DSCAML1
NLGN4Y
NRXN3
NCAM1
FGF1
ROBO1
RTN4
NFASC
TIMP2
ADAM10
PSEN1
COL4A5
ADAM12
PTN
CADM2
CADM1
NEGR1
ATP1B1
NTM
SPP1
EDIL3
FBN1
SEMA3C
SEMA3B
SEMA5A
SEMA6A
C
CD44
SDC4
DDR2
ITGAV
ITGB8
UMAP_1
UMAP_2
D
SRC
ESR1
ITGAV
MAPK1
STAT3
MYC
TP53
PTK2
STAT1
BCL6
SGK1
E
BCL6
SGK1
F
Astrocyte Specific Network
BCL6
SGK1
Network Neighbors
Gene Set Analysis
Network Propagation
G
ROBO2
CFAP54
SYNPO2
COL21A1
ARHGAP6
PLEKHA5
ADAMTSL3
RTN1
KAZN
CCDC85A
AKAP6
MYRIP
SGK1
DGKB
GALNT13
SLC38A1
GLIS3
ST6GAL1
GALNT15
RNF19A
HSPA1A
CRYAB
ANGPT1
UBC
HSP90AA1
DCLK1
KIAA1217
VCAN
SNED1
WDR49
TSHZ2
BCL6
H
GOBP_PROTEIN_REFOLDING
GOBP_O_GLYCAN_PROCESSING
GOBP_PROTEIN_O_LINKED_GLYCOSYLATION
GOBP_CHAPERONE_MEDIATED_PROTEIN_COMPLEX_ASSEMBLY
GOBP_RESPONSE_TO_HEAT
GOBP_CHAPERONE_MEDIATED_AUTOPHAGY
GOBP_APOPTOTIC_PROCESS_INVOLVED_IN_DEVELOPMENT
GOBP_REGULATION_OF_PROTEIN_LOCALIZATION_TO_NUCLEUS
GOBP_NEGATIVE_REGULATION_OF_CELL_JUNCTION_ASSEMBLY
GOBP_PROTEIN_FOLDING
GOBP_NEGATIVE_REGULATION_OF_PROTEIN_LOCALIZATION_TO_NUCLEUS
-log10(pvalue)
Fold enrichment
10
20
30
40
Enrichment status
enriched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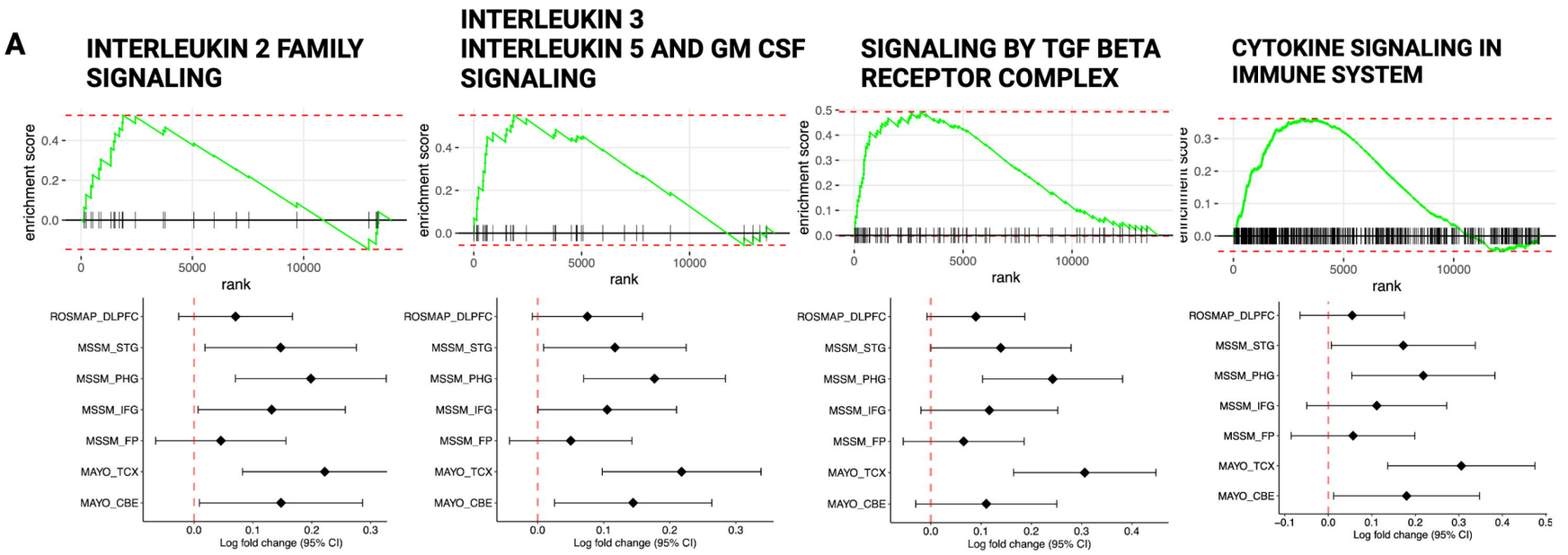
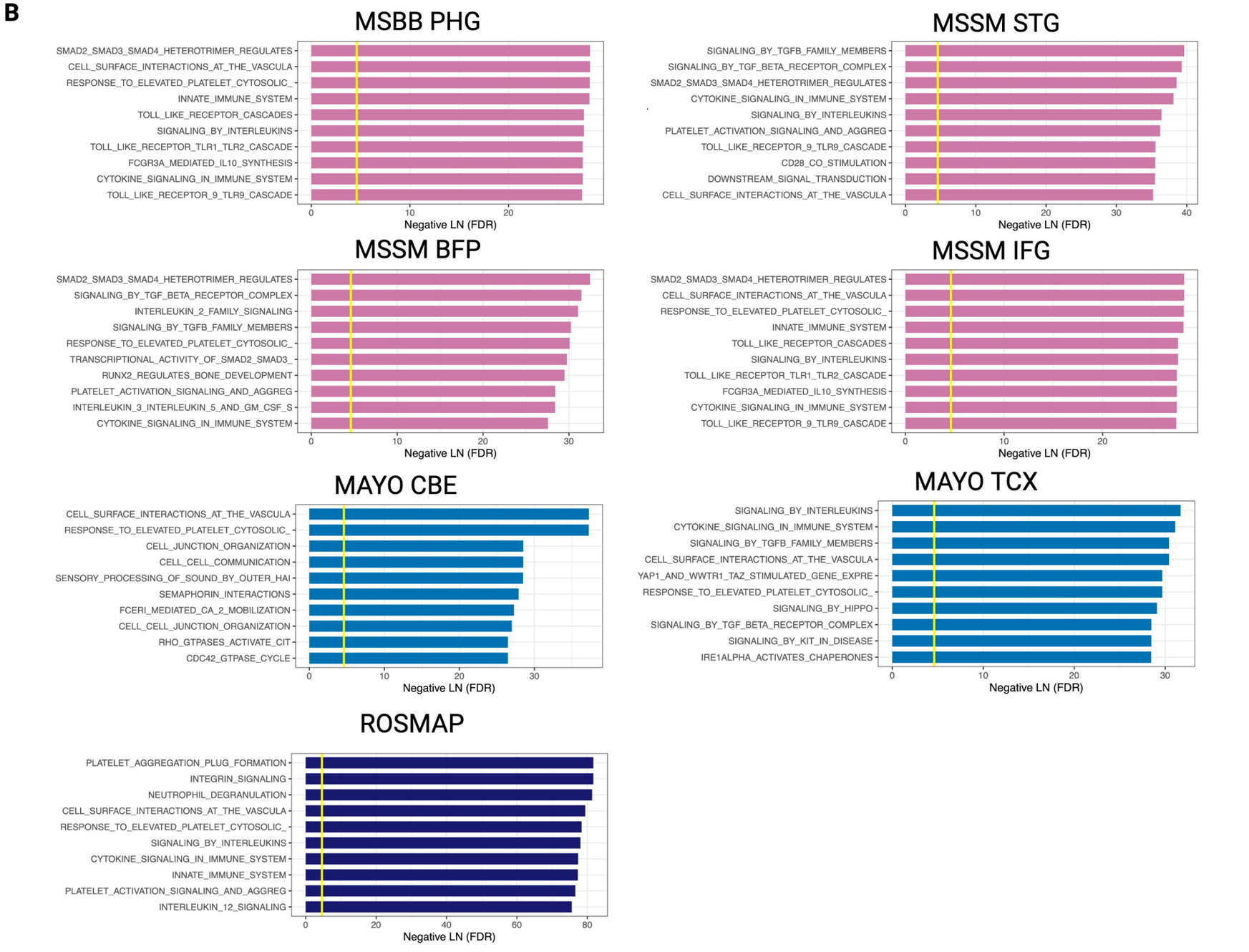
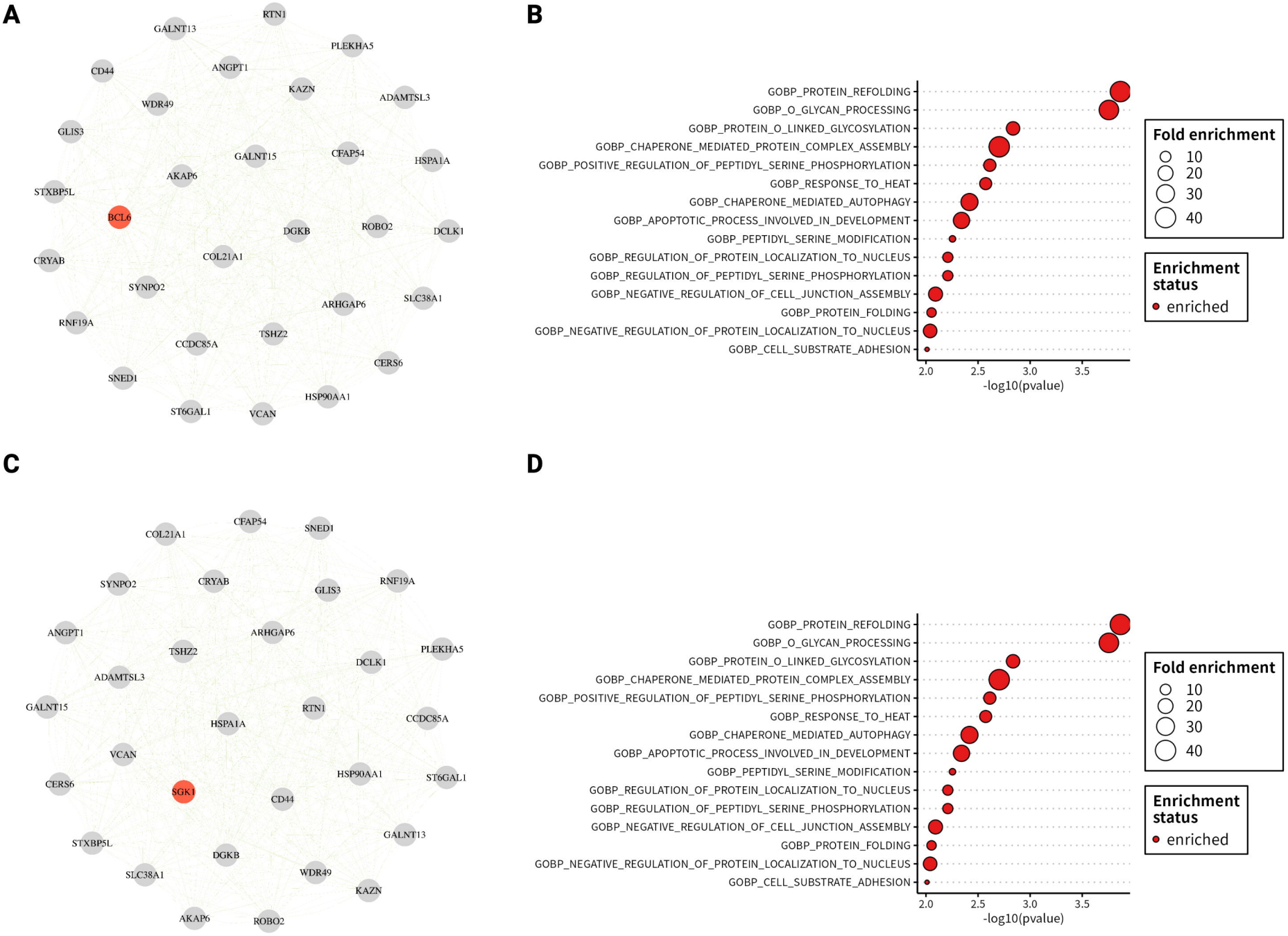
p = 5.04e-01
MSSB IFG
p = 2.95e-01
MSSB PG
p = 7.86e-01
MSSB STG
p = 6.35e-02
MAYO CBE
p = 3.87e-01
MAYO TCX
p = 1.43e-01
ROSMAP
p = 1.82e-13
APP
PLOD3
MSSB FP
p = 4.49e-02
MSSB IFG
p = 1.43e-07
MSSB PG
p = 2.02e-01
MSSB STG
p = 2.85e-03
MAYO CBE
p = 4.44e-01
MAYO TCX
p = 1.50e-01
ROSMAP
p = 8.77e-16
AMYLOID_FIBER_FORMATION
PLOD3


A
INTERLEUKIN 2 FAMILY SIGNALING
INTERLEUKIN 3 INTERLEUKIN 5 AND GM CSF SIGNALING
SIGNALING BY TGF BETA RECEPTOR COMPLEX
CYTOKINE SIGNALING IN IMMUNE SYSTEM
enrichment score
rank
ROSMAP_DLPFC
MSSM_STG
MSSM_PHG
MSSM_IFG
MSSM_FP
MAYO_TCX
MAYO_CBE
Log fold change (95% CI)


B
MSBB PHG
SMAD2_SMAD3_SMAD4_HETEROTRIMER_REGULATES
CELL_SURFACE_INTERACTIONS_AT_THE_VASCULA
RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_
INNATE_IMMUNE_SYSTEM
TOLL_LIKE_RECEPTOR_CASCADES
SIGNALING_BY_INTERLEUKINS
TOLL_LIKE_RECEPTOR_TLR1_TLR2_CASCADE
FCGR3A_MEDIATED_IL10_SYNTHESIS
CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM
TOLL_LIKE_RECEPTOR_9_TLR9_CASCADE
MSSM STG
SIGNALING_BY_TGFB_FAMILY_MEMBERS
SIGNALING_BY_TGF_BETA_RECEPTOR_COMPLEX
PLATELET_ACTIVATION_SIGNALING_AND_AGGREG
CD28_CO_STIMULATION
DOWNSTREAM_SIGNAL_TRANSDUCTION
MSSM BFP
INTERLEUKIN_2_FAMILY_SIGNALING
TRANSCRIPTIONAL_ACTIVITY_OF_SMAD2_SMAD3_
RUNX2_REGULATES_BONE_DEVELOPMENT
INTERLEUKIN_3_INTERLEUKIN_5_AND_GM_CSF_S
MSSM IFG
MAYO CBE
CELL_JUNCTION_ORGANIZATION
CELL_CELL_COMMUNICATION
SENSORY_PROCESSING_OF_SOUND_BY_OUTER_HAI
SEMAPHORIN_INTERACTIONS
FCERI_MEDIATED_CA_2_MOBILIZATION
CELL_CELL_JUNCTION_ORGANIZATION
RHO_GTPASES_ACTIVATE_CIT
CDC42_GTPASE_CYCLE
MAYO TCX
YAP1_AND_WWTR1_TAZ_STIMULATED_GENE_EXPRE
SIGNALING_BY_HIPPO
SIGNALING_BY_KIT_IN_DISEASE
IRE1ALPHA_ACTIVATES_CHAPERONES
ROSMAP
PLATELET_AGGREGATION_PLUG_FORMATION
INTEGRIN_SIGNALING
NEUTROPHIL_DEGRANULATION
Negative LN (FDR)


A
RTN1
GALNT13
PLEKHA5
CD44
ANGPT1
KAZN
ADAMTSL3
WDR49
GLIS3
GALNT15
CFAP54
HSPA1A
AKAP6
STXBP5L
BCL6
DGKB
ROBO2
DCLK1
CRYAB
COL21A1
SYNPO2
SLC38A1
ARHGAP6
RNF19A
TSHZ2
CCDC85A
CERS6
SNED1
HSP90AA1
ST6GAL1
VCAN
B
GOBP_PROTEIN_REFOLDING
GOBP_O_GLYCAN_PROCESSING
GOBP_PROTEIN_O_LINKED_GLYCOSYLATION
GOBP_CHAPERONE_MEDIATED_PROTEIN_COMPLEX_ASSEMBLY
GOBP_POSITIVE_REGULATION_OF_PEPTIDYL_SERINE_PHOSPHORYLATION
GOBP_RESPONSE_TO_HEAT
GOBP_CHAPERONE_MEDIATED_AUTOPHAGY
GOBP_APOPTOTIC_PROCESS_INVOLVED_IN_DEVELOPMENT
GOBP_PEPTIDYL_SERINE_MODIFICATION
GOBP_REGULATION_OF_PROTEIN_LOCALIZATION_TO_NUCLEUS
GOBP_REGULATION_OF_PEPTIDYL_SERINE_PHOSPHORYLATION
GOBP_NEGATIVE_REGULATION_OF_CELL_JUNCTION_ASSEMBLY
GOBP_PROTEIN_FOLDING
GOBP_NEGATIVE_REGULATION_OF_PROTEIN_LOCALIZATION_TO_NUCLEUS
GOBP_CELL_SUBSTRATE_ADHESION
2.0
2.5
3.0
3.5
-log10(pvalue)
Fold enrichment
10
20
30
40
Enrichment status
enriched
C
CFAP54
COL21A1
SNED1
SYNPO2
CRYAB
GLIS3
RNF19A
ANGPT1
ARHGAP6
TSHZ2
PLEKHA5
DCLK1
ADAMTSL3
GALNT15
RTN1
HSPA1A
CCDC85A
VCAN
HSP90AA1
ST6GAL1
CERS6
SGK1
CD44
GALNT13
STXBP5L
DGKB
SLC38A1
WDR49
KAZN
AKAP6
ROBO2
D
GOBP_PROTEIN_REFOLDING
GOBP_O_GLYCAN_PROCESSING
GOBP_PROTEIN_O_LINKED_GLYCOSYLATION
GOBP_CHAPERONE_MEDIATED_PROTEIN_COMPLEX_ASSEMBLY
GOBP_POSITIVE_REGULATION_OF_PEPTIDYL_SERINE_PHOSPHORYLATION
GOBP_RESPONSE_TO_HEAT
GOBP_CHAPERONE_MEDIATED_AUTOPHAGY
GOBP_APOPTOTIC_PROCESS_INVOLVED_IN_DEVELOPMENT
GOBP_PEPTIDYL_SERINE_MODIFICATION
GOBP_REGULATION_OF_PROTEIN_LOCALIZATION_TO_NUCLEUS
GOBP_REGULATION_OF_PEPTIDYL_SERINE_PHOSPHORYLATION
GOBP_NEGATIVE_REGULATION_OF_CELL_JUNCTION_ASSEMBLY
GOBP_PROTEIN_FOLDING
GOBP_NEGATIVE_REGULATION_OF_PROTEIN_LOCALIZATION_TO_NUCLEUS
GOBP_CELL_SUBSTRATE_ADHESION
2.0
2.5
3.0
3.5
-log10(pvalue)
Fold enrichment
10
20
30
40
Enrichment status
enriched