1 **A global multiregional proteomic map of the human cerebral cortex**

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22 **Running title: Proteomic map of the human cerebral cortex**

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25

26 **Abstract**

27 The Brodmann area (BA)-based map is one of the most widely used cortical maps
 28 for studies of human brain functions and in clinical practice; however, the molecular
 29 architecture of BAs remain unknown. The present study provided a global
 30 multiregional proteomic map of the human cerebral cortex by analyzing 29 BAs.
 31 These 29 BAs were grouped into 6 clusters based on similarities in proteomic patterns:
 32 the motor and sensory cluster, vision cluster, auditory cluster and Broca's area,
 33 Wernicke's area cluster, cingulate cortex cluster, and heterogeneous function cluster.
 34 We identified 474 cluster-specific and 134 BA-specific signature proteins whose
 35 functions are closely associated with specialized functions and disease vulnerability of
 36 the corresponding cluster or BA. The findings of the present study could provide
 37 explanations for the functional connections between the anterior cingulate cortex and
 38 sensorimotor cortex and for anxiety-related function in the sensorimotor cortex. The
 39 brain transcriptomic and proteomic comparison indicated that they both could reflect
 40 the function of cerebral cortex, but showed different characteristics. These proteomic
 41 data are publicly available at the Human Brain Proteome Atlas
 42 (www.brain-omics.com). Our results may enhance our understanding of the molecular
 43 basis of brain functions and provide an important resource to support human brain
 44 research.

45 **Key words**

46 Brodmann areas; proteomics; cerebral cortex; cingulate cortex; sensorimotor cortex

47

48 Introduction

49 The human cerebral cortex, the outer layer of the cerebrum, exhibits great
50 complexity in its histological structure and cellular organization ¹. In 1909,
51 Korbinian Brodmann distinguished a total of 52 subareas based on the differences in
52 cytoarchitectural organization across the cortex; these areas were later termed
53 Brodmann areas (BAs) ². Subsequently, BA-based map has become one of the most
54 widely used cortical maps for the investigation of the brain functions and in clinical
55 practice. BAs are often correlated with certain cortical functions. For example,
56 Broca's speech and language area is consistently localized in BAs 44 and 45.
57 cytoarchitecture ^{1,2}, imaging ^{1,3}, and functional roles ³ of BAs have been extensively
58 studied, and comprehensive human brain MRI imaging ¹, histology ¹, and connectome
59 maps ³ have been investigated.

60 Molecular annotations, especially at the protein level, of various BAs will provide
61 a deeper understanding of the molecular basis for diverse brain functions.
62 Transcriptomic and proteomic maps of the brain have been generated to study the
63 molecular basis of distinct cytoarchitecture and functions of various BAs ¹. The Allen
64 Institute for Brain Science has generated a transcriptional atlas of the human brain by
65 comprehensive profiling of nearly 900 anatomically precise subdivisions using
66 microarrays ¹. The human neocortex is characterized by a relatively homogeneous
67 transcriptional pattern; the molecular topography of the neocortex is similar to its
68 cytoarchitecture. At the protein level, several studies generated a protein atlas of the
69 mouse brain and identified some brain region-restricted proteins ^{4,5} or cell
70 type-specific proteins ⁴. In 2014, Kim *et al* drafted a proteomic profile of the human
71 frontal cortex from a pool of three individuals and identified 9,060 proteins ⁶. In 2017,
72 Becky *et al.* ⁷ performed a proteomic survey of 7 postnatal human brain regions,
73 including two cerebral cortex regions, the cerebellar cortex, the hippocampus, and
74 other neurological nuclei, ranging from early infancy to adulthood. Individual
75 variations due to age and sex were considerably lower than variations between brain

76 regions. Importantly, the differences in brain cytoarchitecture, development, and
77 function are better represented by changes at the protein level than by changes at the
78 transcriptional level.

79 Previous omics studies have increased our understanding of the brain function.
80 However, some important scientific aspects have not been investigated. Functional
81 annotation of the brain demonstrated that several brain regions are functionally
82 connected but are not spatially adjacent, such as BA41/42 (auditory cortex) and BA
83 44/45 (Broca's area and language production)^{3,8}. However, the data on the brain
84 transcriptome showed a relatively homogeneous expression pattern in the neocortex,
85 and molecular topography in this area is similar to spatial topography. Proteins are the
86 main functional operators in all cells. Proteomic map of the cerebral cortex may
87 provide new insight into this scientific context. Previous studies of the brain proteome
88 investigated only a few BAs, which are not sufficient to illustrate these aspects. Thus,
89 a comprehensive proteomic map of various BAs of the human cerebral cortex is
90 urgently needed.

91 The present study used 29 BA postmortem specimens to generate a global
92 multiregional proteomic map of the human cerebral cortex. Initially, an extensive
93 proteomic library of BA46 was generated. Then, 29 BAs were quantitatively analyzed
94 using the 2DLC/MS/MS approach combined with isobaric tags for relative and
95 absolute quantitation (iTRAQ) labeling. Clustering analysis of 29 BAs was performed
96 based on the similarities in protein expression patterns. Cluster- and BA- signature
97 proteins were identified and analyzed using bioinformatics. The major functional
98 brain areas, including the cingulate cortex, motor cortex, sensory cortex, language
99 area, and visual cortex, were functionally annotated using proteomic data. The
100 transcriptomes of above BAs were also analyzed and the comparison of proteome and
101 transcriptome on BA functions was presented. The results of the present study provide
102 a molecular basis for BA functions. Furthermore, the database provides an important
103 resource to support brain research.

104 **Results**

105 **A broad and comprehensive atlas of brain proteome**

106 A single human brain specimen was obtained from the Human Brain Bank
107 facilitated by the China Human Brain Bank Consortium. The brain specimen was
108 collected from a 54-year-old willed male donor who died of lung cancer and did not
109 have any history of brain metastasis, trauma, or other diseases. The samples of 29
110 BAs of the left cerebral hemisphere were collected with a postmortem delay of less
111 than 6 hours. Hematoxylin-eosin staining (HE staining) of the samples showed that
112 the brain was cancer-free (Supplementary Figure 1). The protocols for brain tissue
113 acquisition, dissection, and sample preparation were under consistent and stringent
114 quality control (see Materials and Methods) based on the standardized operational
115 protocol (SOP) of the Human Brain Bank of China ⁹.

116 BA46 (dorsolateral prefrontal cortex (DFC)) played an important role in many
117 cognitive processes and had been comprehensively characterized in previous
118 proteomic studies ^{6,7}. Therefore, deep proteomic profiling of BA 46 was performed
119 using the 2DLC/MS/MS approach (Figure 1A) to construct a Chinese reference brain
120 protein library. A total of 8,600 proteins (Supplementary Table 1 A-B, data quality in
121 Supplementary Figure 2A) were identified in the present study, corresponding to 73%
122 and 89% of the proteins identified in the studies of Kim *et al* ⁶ and Becky *et al.* ⁷,
123 respectively. Additional 979 (11.4%) proteins were identified by brain proteome
124 analysis for the first time (Figure 1B and Supplementary Table 1 A-B). Thus, we
125 generated a broad and high-coverage proteomic dataset with identification depth
126 similar to that achieved by the two most comprehensive datasets reported in previous
127 studies^{6,7}.

128 Then, to provide a comprehensive protein abundance analysis, the proteome
129 abundance in BA46 was calculated by the intensity-based absolute quantification
130 (iBAQ) method ¹⁰. A total of 7,129 proteins were successfully quantified, and their

131 abundances spanned 7 orders of magnitude (Supplementary Figure 3). Approximately
132 4% of the most abundant proteins (292 proteins) accounted for 75% of the total
133 protein abundance and were considered high-abundance brain proteins in the present
134 study. The remaining 20% and 5% of the total protein abundances corresponded to
135 1,513 mid-abundance proteins and 5,324 low-abundance proteins, respectively
136 (Figure 1C, Supplementary Table 1C). Gene ontology (GO) analysis revealed that
137 nervous system structure and function were enriched in this brain proteome,
138 especially in the case of high-abundance proteins (Figure 1D, Supplementary Table
139 1D), implying the important role of high-abundance proteins in brain functions.

140 Finally, to investigate specific proteomic profiling in BAs and explore the
141 associations between BA protein expression and function, we used iTRAQ-based
142 quantitative proteomics to generate a region-resolved proteome map of the brain
143 covering 29 BAs with important significance for research and clinical applications
144 (Figure 1A and Table 1). A total of 4,308 proteins were quantified in all BAs. The
145 batch effect of 4 batches was evaluated by qualitative and quantitative analysis. The
146 median CV of four batches was around 6% (Supplementary Figure 2A), and the Venn
147 diagram of protein identification in four batches (Supplementary Figure 2B) showed
148 high overlapping. The log ratio distributions of 29 iTRAQ quantification
149 results (Supplementary Figure 2C) showed similar normal distribution, and the
150 detailed ratio distributions of each BA in four batches (Supplementary Figure 2D)
151 showed similar distributions.

152 A total of 1,241 proteins were characterized by interarea variability (the
153 difference between the maximum and minimum ratios ≥ 2 , detailed in Method
154 section). The Human Brain Proteome Atlas (www.brain-omics.com) was constructed
155 to enhance visualization and use of these data, which are publicly accessible
156 (Supplementary Figure 4).

157 **Brain proteomic atlas reflects functional parcellations**

158 Proteins are the main components and functional operators in the cells; thus, BAs

with similar protein expression patterns may have similar cytoarchitecture or perform similar functions. Based on the similarities of protein expression patterns, K-means clustering¹¹ was used for the classification of BAs. The consensus cumulative distribution function (CDF) plot and silhouette plot (**Supplementary Figure 5**) based on the consensus matrix were used to group 29 BAs into 6 clusters (detailed data in Methods section, Figure 2A, and Table 1).

The cingulate cortex cluster (cluster 4) included BAs in the cingulate cortex, subgenual cingulate cortex, and entorhinal cortex. All these BAs belong to the transition areas between the allocortex (3 layers of neuronal cell bodies) and neocortex (6 layers of neuronal cell bodies). Therefore, the cytoarchitecture of the cingulate cortex cluster was different from that of the neocortex¹². At the proteome level, the cingulate cortex cluster was characterized by protein patterns distinct from those of other clusters, which were mainly located in the neocortex (Figure 2A), implying that the brain proteome can reflect the differences in cytoarchitecture between the cingulate cortex and the neocortex.

The remaining 23 BAs within the neocortex were grouped into 5 clusters, and BAs within a single cluster were functionally similar. For example, the vision cluster (cluster 6) comprised a group of BAs (BAs 17, 18, 19, 37, and 34) engaged in visual processing (Figure 2B, Table 1). BAs 17, 18, and 19 are located in the occipital lobe, and BA37 is adjacent to BA19. All four BAs correspond to classic visual processing regions¹³⁻¹⁵. BA34 is located in the parahippocampal gyrus and is not spatially connected to other four BAs, although BA34 was reported to be related to visual processing¹⁶. Another example is the auditory and Broca's area cluster, which is composed of several spatially separated but functionally related BAs (Figure 2B). BA44/45 of this cluster (Broca's area and motor language area), BA8 (writing language area)¹⁷ and BA7 (engaged in speech¹⁸) are directly involved in language processing, whereas BA41/42 (auditory cortex) and BA26/29/30 (engaged in sound processing)¹⁹ are associated with auditory processing, which is indirectly linked to language processing^{3,8}.

188 Interestingly, two language processing BAs, BA22 (classic Wernicke's area and
189 sensory language area) and adjacent BA21 (also considered a part of Wernicke's area
190 ²⁰), were clustered into the Wernicke's area cluster. This cluster was characterized by
191 proteomic patterns distinct from the auditory and Broca's area cluster. To illustrate the
192 differences in protein expression between these two clusters, we compared differential
193 proteins between the two clusters. A total of 88 and 108 proteins were expressed at
194 higher levels in the Wernicke's area cluster and auditory and Broca's area cluster, with
195 a 1.5-fold change, respectively (Supplementary Table 3). Differentially expressed
196 proteins were enriched in metabolic pathways, suggesting metabolic differences
197 between the two language regions (Supplementary Figure 6A). These proteins are also
198 related to neurotransmitter pathways ²¹, including glutamate, γ -aminobutyric acid
199 (GABA), and dopamine pathways (Supplementary Figure 6B) ²¹. In particular, the
200 GABA receptor was expressed at a higher level in the auditory and Broca's area
201 cluster compared to that in the Wernicke's area cluster in agreement with previous
202 reports on an important role of the GABA receptor in the physiological functions of
203 Broca's area. An inhibitor of the GABA receptor causes a dysfunction in Broca's
204 area and induce stuttering ²², and a GABA agonist improves chronic Broca's aphasia²¹.
205 Therefore, these differentially expressed proteins provide valuable information about
206 the intrinsic mechanism of language processing.

207

208 **Cluster/BA signature proteins linked to specialized function of clusters/BAs**

209 BAs with similar protein expression patterns tend to have similar cytoarchitecture
210 and functions; thus, cluster signature proteins (proteins that were expressed at a high
211 level in a certain cluster) may provide information about specialized functions of a
212 cluster. Iterative signature analysis (ISA, details described in the Methods section)²³
213 was used to identify a total of 474 cluster signature proteins in the present study
214 (Figure 3A and Supplementary Table 4A). Pathway, function, and disease annotations
215 of these proteins are shown in Supplementary Table 4B-E, Figure 3B-3C, and

216 Supplementary Figure 7A-7B.

217 The cingulate cortex cluster had the largest number of signature proteins, 266 out
 218 of all 474 signature proteins. These proteins included 76 (26.3%) and 140 (52.6%)
 219 high- and mid-abundance proteins, respectively (Figure 3D and Supplementary Figure
 220 7C-7E), corroborating the results regarding the differences in cytoarchitecture and
 221 protein expression between the cingulate cortex cluster and the neocortex. Functional
 222 analysis showed that these signature proteins are enriched in metabolic pathways,
 223 including important enzymes for oxidative phosphorylation (NADH dehydrogenase),
 224 glycolysis (ALDOA, PGK1, PGAM1, and ENOA), glycogen turnover (TPIS), and
 225 amino acid synthesis (ASS1 and PDHX) (Figure 3E), indicating high metabolic
 226 activity of the cingulate cortex. These results are in agreement with the metabolic
 227 analysis of the cingulate cortex reported in a previous study²⁴. A total of 21 signature
 228 proteins were involved in cognition, emotion, and memory (Supplementary Table 4F),
 229 consistent with the functional characteristics of the cingulate cortex²⁴⁻²⁶. These results
 230 are in agreement with the functional characteristics of the cingulate cortex. Disease
 231 annotations indicated that proteins related to neurological degenerative diseases
 232 (Huntington's disease, Parkinson's disease, and Alzheimer's disease) were enriched in
 233 the signature proteins of the cingulate cortex cluster. Several key proteins associated
 234 with signaling in Alzheimer's and Parkinson's diseases were expressed at high levels
 235 in the cingulate cortex cluster (Supplementary Figure 7F-7G). These proteins include
 236 tau (MAPT) and alpha-synuclein (SYUA), which are important biomarkers for
 237 Alzheimer's disease^{27,28} and Parkinson's disease²⁹, respectively (Figure 3F). The
 238 pathological accumulation of these two proteins is etiologically correlated with the
 239 development of Alzheimer's²⁸ and Parkinson's diseases²⁹. The data of Western blot
 240 analysis showed that the protein expression patterns of MAPT and SYUA were
 241 consistent with the patterns detected by proteomic analysis (Supplementary Figure
 242 8A-B). The results of IHC-Fr also demonstrated that MAPT and SYUA were
 243 expressed at a high level in BA25 compared to that in BA4 (Supplementary Figure
 244 8C). The results of this study demonstrated the regional distribution of MAPT and

245 SYUA for the first time, adding new information about pathogenesis and vulnerability
246 of these two neurodegenerative diseases in the cingulate cortex. Furthermore, another
247 brain (brain 2) was used to validate the cluster-specific signature proteins by parallel
248 reaction monitoring (PRM). Detailed clinical information on brain 2 is provided in the
249 Methods section. PRM analysis validated that 28 signature proteins were expressed at
250 a high level in cingulate cortex cluster (Supplementary Table 5A-5B).

251 The motor and sensory cluster had 99 signature proteins. These proteins were
252 enriched in the protein kinase A (PKA) pathway (Figure 3B). The PKA pathway is
253 associated with brain motor function (neuronal plasticity in motor neurons^{30,31}) and
254 sensation (pain sensitization in sensory neurons^{32,33}). Striatal-enriched protein
255 tyrosine phosphatase (STEP), a molecule downstream of PKA, is associated with
256 motor skill learning in the motor cortex³⁴. Other signature proteins with potentially
257 interesting functions include the neuron survival protein EMP2A and mitochondrial
258 28S ribosomal protein S34 (MRPS34) (Figure 3F). The functional loss of EMP2A was
259 reported to cause sensorimotor cortex excitability in Lafora disease³⁵. MRPS34
260 mutation causes delayed psychomotor development and neurodevelopmental
261 deterioration in Leigh syndrome³⁶. Immunological analysis confirmed that EMP2A
262 and MRPS34 were expressed at a high level in the motor and sensory cluster
263 (Supplementary Figure 8). Two signature proteins of motor and sensory cluster,
264 EMP2A and PTN5, were validated by PRM (Supplementary Table 5A-5B).

265 The vision cluster included 49 signature proteins enriched in the
266 methyl-CpG-binding protein 2 (MECP2)-regulated transcription factor pathway.
267 MECP2 and transcriptionally regulated transcription factors are involved in
268 neurological system and neuropsychiatric disorders³⁷ (Figure 3B). MECP2 plays an
269 important role in normal development of the central nervous system and maintenance
270 of neurological functions. Mutation of the MECP2 gene leads to Rett syndrome,
271 which is an autism spectrum-associated disorder with visual impairment³⁸. In a mouse
272 model, knockout of MECP2 causes progressive dysfunction of the visual cortex and
273 rapid regression of visual acuity³⁹. Three signature proteins of vision cluster, MECP2,

274 TAGLN, and CAVN1, were confirmed to be expressed at high level by PRM
275 (Supplementary Fig 10B, Supplementary Table 5A-5B). Additionally, Becky et al ⁷
276 reported a proteomic comparison of BA17 (primary visual cortex) and BA46 (DFC) in
277 11 individuals ranging from early infancy to adulthood. By comparing the 49 vision
278 cluster signature proteins in present study with Becky's study, 16 were expressed at
279 high level in primary visual cortex in both studies (Supplementary Table 5E).

280 Additionally, we focused on BA signature proteins (protein abundance in one or two
281 BAs that is more than twofold higher than the median abundance) to investigate
282 BA-specific functions. A total of 134 BA signature proteins were identified based on
283 the analog criteria suggested by Uhlen *et al* ⁴⁰ (details are provided in the Methods
284 section), (Figure 4A and Supplementary Table 4G). These BA signature proteins
285 included several proteins closely associated with the corresponding brain functions. In
286 the cingulate cortex, superoxide dismutase [Cu-Zn] (SOD1) was identified as a
287 signature protein in the anterior cingulate cortex (BA32 and BA24). SOD1 mutation
288 interferes with GABAergic neurotransmission, leading to cortical neuronal loss in this
289 brain area⁴¹. In the neocortex, FAM107B was identified as a signature protein in
290 BA41/42 (auditory cortex) (Figure 4B). Knockout of FAM107B leads to hearing
291 impairment in mice⁴². Three BA signature proteins, transgelin-2 (BA17 and BA18),
292 RAD23A (BA40), and FAM107B (BA41/42), were validated by Western blot (Figure
293 4B, Supplementary Figure 9). Five BA signature proteins, NEFH (BA4), PMP2
294 (BA4), CALB2 (BA28), TAGLN (BA17 and BA18), and FAM107B (BA41/42), were
295 validated to be expressed at a high level in corresponding BAs in brain 2
296 (Supplementary Table 5C-5D). Two primary visual cortex signature proteins, TAGLN
297 and H1FO were also found to be expressed at high level in primary visual cortex in
298 Becky's study⁷ (Supplementary Table 5E).

299 Overall, these findings demonstrated intrinsic links between specialized functions
300 of the brain clusters and BAs and molecular functions of the corresponding signature
301 proteins, which may help to understand the mechanisms of physiological or
302 pathophysiological functions of the brain.

303 **Molecular annotation of uncanonical functions of BAs**

304 **Functional connection between the motor cortex and anterior cingulate cortex**

305 The anterior cingulate cortex is tightly connected to the motor cortex through
 306 neuronal cell projections according to the results of fluorescence labeling imaging in
 307 rats⁴³. In humans, Caruana *et al.* demonstrated that the anterior cingulate cortex plays
 308 important roles in the control of complex motor behaviors according to the results of
 309 intracortical electrical stimulation⁴⁴. Molecular evidence of the connection between
 310 these two functional brain regions requires additional investigation.

311 Proteomic data obtained in the present study demonstrated that a series of
 312 proteins were expressed at a high level in the primary motor cortex (BA4) and
 313 anterior cingulate cortex (BA33) (Figure 5A, Supplementary Table 6A). Most of these
 314 proteins are associated with motor neuron structure and function (Figure 5B).
 315 Neurofilament proteins (NEFL, NEFM, NEFH, and INA) are the main components of
 316 motor neurons⁴⁵ and are necessary for the maintenance of the number, morphology,
 317 viability, and regenerative capability of motor neurons⁴⁶⁻⁴⁸. Myelin basic protein
 318 promotes myelination of motor neurons⁴⁹. MIF⁵⁰ and PVALB⁵¹ are associated with
 319 amyotrophic lateral sclerosis, a neurodegenerative disease targeting motor neurons⁵².
 320 Thus, these proteins may represent molecular basis of the functional connections
 321 between the primary motor cortex (BA4) and anterior cingulate cortex (BA33) and
 322 may also play a role in the motor functions of the anterior cingulate cortex.

323 **The motor and sensory cluster may be involved in anxiety**

324 Anxiety disorder is a disease characterized by excessive and persistent worry and
 325 fear and is very common worldwide (lifetime prevalence is 5~25% of the population),
 326 representing a substantial economic burden^{53,54}. The sensorimotor cortex has been
 327 reported to be associated with social anxiety disorder⁵⁵ and anxiety-related
 328 personality traits⁵⁶ according to the results of electrophysiological studies. However,
 329 the molecular basis of anxiety in the sensorimotor cortex requires further
 330 investigation.

Proteomic data obtained in the present study indicated that 30 of the 1,241 proteins with inter area variability are involved in anxiety or anxiety-like behaviors according to IPA (Supplementary Table 6B). Clustering analysis indicated that nearly half (14, 46.6%) of these 30 proteins were expressed at a high level in the motor and sensory cluster (Figure 5C). Some of these 14 proteins, including ADCY8, GRIA1, GRIN2A, PLCB4, SLITRK5, NOS1, and GRM2, formed a protein interaction and regulation network and participated in the expression of anxiety and anxiety-like behaviors (Figure 5D). PLCB4⁵⁷ and NOS1⁵⁸ were expressed at a high level in the medial septum and the hippocampus (two anxiety behavior-related regions), respectively, and inhibition of these genes in the regions with high levels of expression increased anxiolytic effects in a mouse model. GRIN2A and GRIA1 are two important glutamate receptors involved in the function of the sensorimotor cortex⁵⁹ and also involved in anxiety. Knockout of GRIN2A⁶⁰ and GRIA1⁶¹ causes a decrease in anxiety in a mouse model. Thus, we hypothesized that these proteins are expressed at a high level in the motor and sensory cluster and may be involved in anxiety-related functions in the sensorimotor cortex, which may explain the molecular basis of anxiety-related processes in the sensorimotor cortex.

Transcriptomic characterization of brain BAs

The transcriptomes of brain BAs were explored to reveal the characteristics of the brain functions. 29 BAs by RNA-seq were analyzed, and 21 samples passed the quality control (RIN $\geq$ 6, 28S/18S $\geq$ 0.7, gene mapping $\geq$ 40%) and were used for further functional analysis. An average of 17,137 transcripts was detected based on the RNA-seq data (Supplementary Table 7A, Supplementary Figure 10). Comparison of the brain proteome library and the transcriptomic database of BA46 identified 8,411 transcriptomic entries (97.8%) for 8,600 proteins detected by LC-MS (Figure 6A). The proteomic data covered 48.8% of the transcriptomic data, covering 61% with expression level >1 read per kilobase per million mapped (RPKM) and 78.4% with expression level >10 RPKM (Figure 6B).

359 A total of 17,091 genes were quantified in 21 BAs. PCA of the transcriptomic data
360 showed that BA4 (primary motor cortex) was different from other BAs in agreement
361 with the finding of the Allen Institute that a series of genes are specifically enriched in
362 the primary motor cortex¹. The cingulate cortex cluster tended to be partially
363 separated from other BAs, which was partially consistent with the proteomic results.
364 However, four functionally distinct clusters distinguished by proteomic analysis could
365 not be found based on the transcriptomic data (Figure 6C).

366 The BA signature genes at the transcriptomic level were used to explore BA-specific
367 functions. A total of 1,319 BA signature genes were identified (Figure 6D and
368 Supplementary Table 7B). Functional analysis indicated that BA signature genes were
369 closely involved in the functions of corresponding BA. In cingulate cortex, a total of
370 13 signature genes of BA 28 were involved in memory function, in agreement with
371 the functional characteristics of the ventral entorhinal cortex (BA28)
372 ²⁶(Supplementary Table 7C). In neocortex, motor neuron related proteins (NEFH,
373 NEFM)^{46,47} were identified as signature genes of primary motor cortex (BA4), and
374 were also expressed at high level in BA4 in our proteomic data. In Auditory
375 cortex (BA41/42), SMPX was identified as a signature gene. Previous study reported
376 that mutation of SMPX was associated with X-linked deafness type 4⁶².

377 A total of 4,250 genes were quantified based on the data of transcriptomic and
378 proteomic results in 21 BAs (Supplementary Table 7D). Gene products for all region
379 pairs were classified based on fold-change similarity between mRNA and protein
380 (details described in the Methods section). An average of 39.5% of the genes were
381 classified into “no change” (≤ 2 -fold for both protein and RNA) category, and 17.4%
382 of the genes were classified into “agree” category (same direction of changes (>2 -fold)
383 and same magnitude of fold changes) or “partially agree” category (same direction of
384 changes (> 2 -fold) and different magnitude of fold changes) category. In addition,
385 20.6% or 15.7% of the genes were considered “protein only” (≥ 2 -fold change in
386 protein level only) or “RNA only” (≥ 2 -fold change in mRNA level only), respectively.

387 In contrast, 6.7% of the genes corresponded to opposite differences in the mRNA and
388 protein levels (> 2-fold) (“disagree” group) (Figure 6E).

389 To show the differences between transcriptome and proteome on brain functions,
390 BA4 (Primary motor cortex) and BA46 (DFC) were compared and the scatter plots of
391 the mRNA and protein fold change between them were shown in Figure 6F
392 (Supplementary Table 7E, other BA pair comparison data in Supplementary Figure
393 11). According to IPA functional annotation of the differential gene products between
394 BA4 and BA46, Progressive motor neuropathy and Parkinsonism related genes were
395 enriched in “protein only” and “partially agree” categories, which could reflect the
396 characteristics of the Primary motor cortex. In contrast, the cognitive impairment and
397 the depressive disorder related genes showed a bias toward “mRNA only” categories,
398 reflecting the characteristics of the DFC. Additionally, the neurodevelopment related
399 genes were enriched in the “agree” category, consistent with the Becky’s findings ⁷
400 (Figure 6G, Supplementary Table 7F).

401

402 Discussion

403 In this study, we constructed a Chinese cerebral cortex proteomic atlas of 29 BAs
404 from a donor without neurological diseases. Similarities in the protein expression
405 patterns indicated that these 29 BAs can be grouped into six clusters. The cluster- and
406 BA signature proteins can provide molecular evidence for annotation of
407 cytoarchitecture, function, and disease vulnerability of these clusters or the
408 corresponding BAs. All data are publicly available in the Human Brain Proteome
409 Atlas (HBPA) (www.brain-omics.com).

410 In this brain proteome atlas, BAs with similar protein expression patterns have
411 similar functions, even though they are not spatially adjacent, which reflects
412 functional parcellations. BAs within the cingulate cortex cluster and vision cluster are
413 characterized by both spatial proximity and functional relevance. However, BAs
414 within the auditory and Broca's area cluster are not directly adjacent and are
415 functionally connected (speech and auditory). These results indicated that the
416 proteomic map mainly reflects the functional parcellations of the human cerebral
417 cortex. Furthermore, proteomic similarity of BAs may provide molecular annotations
418 for functional connections between BAs. For example, BA38 (temporal pole) is an
419 enigmatic region with largely unknown function⁶³. The brain connectome indicated a
420 functional connection between BA3/1/2-BA38 and BA21/22-BA38³. The results of
421 fMRI experiments indicated that BA38 is involved in speech comprehension and
422 considered a part of "extended Wernicke's area"^{64,65}. A study on Braille reading in
423 blind individuals using brain imaging methods indicated that BA38 can
424 integratesensory-motor and language comprehension.⁶³ In the present study, BA38
425 was characterized by protein expression patterns similar to both the sensorimotor
426 cortex (BA3/1/2, BA4, and BA6) in the motor and sensory cluster and Wernicke's
427 area (BA21 and BA22). We suggest that BA38 may play integratory roles in both
428 sensory-motor functions and language comprehension.

429 Additionally, the present study identified 474 cluster- and 134 BA signature
 430 proteins. These proteins reveal intrinsic links between their molecular functions and
 431 specialized functions of the brain regions. The cingulate cortex contains an extremely
 432 large number of signature proteins compared to that of other neocortex regions. These
 433 signature proteins were enriched in metabolic pathways, mainly glycolysis, glycogen
 434 turnover, and amino acid synthesis. Previous studies demonstrated that astrocytes had
 435 unique metabolic pathways of brain glucose metabolism compared to those in neurons
 436 ⁶⁶. First, glycolysis is predominant in astrocyte glucose metabolism. Second, the
 437 storage and turnover of glycogen are mainly concentrated in astrocytes. Third, TCA
 438 cycle-derived amino acids, such as aspartate (Asp), glutamate (Glu), glutamine (Gln),
 439 arginine (Arg), and γ -aminobutyric acid (GABA), are synthesized in astrocytes using
 440 astrocyte-specific enzymes ⁶⁶. The combination of previous reports and proteomic
 441 data obtained in the present study indicates the activation of astrocyte metabolism in
 442 the cingulate cortex, which may correspond to metabolic characteristics of the
 443 cingulate cortex. Additionally, the results of the present study suggest that some
 444 signature proteins in the cingulate cortex cluster participate in the pathogenesis of
 445 some neurological diseases, such as MAPT in Alzheimer's disease and SYUA in
 446 Parkinson's disease. Previous studies have shown that the accumulation of these two
 447 proteins in the cingulate cortex is a pathological feature of the corresponding diseases
 448 ^{28,29}. Abnormalities in the cingulate cortex play a central role in the development of
 449 Alzheimer's ²⁴ and Parkinson's diseases ^{67,68}. These signature proteins may be
 450 investigated to determine molecular mechanisms of neurological diseases, especially
 451 in some specific brain regions. Thus, information about these proteins may
 452 substantially improve our understanding of the molecular basis of brain functions and
 453 pathogenesis.

454 Furthermore, the data of the present study provide molecular annotations of the
 455 noncanonical functions of brain regions. Molecular function analysis of these data
 456 provides molecular basis for functional connections between the anterior cingulate
 457 cortex and motor cortex and may explain the molecular basis of anxiety in the

458 sensorimotor cortex. These findings promote our understanding of the molecular
459 mechanisms of the noncanonical functions of brain regions. Therefore, proteomic data
460 obtained in the present study may be used to investigate new functions of BAs or new
461 functional connections between BAs.

462 In present study, the comparison of BA transcriptome and proteome indicated
463 that the proteomic data could separate the cerebral cortex into 6 clusters, which were
464 associated with BA's function. However, transcriptomic data did not show similar
465 classification, which was consistent with Allen results¹. Above results indicated that
466 brain proteomic data might preferably reflect BA function. Functional analysis of the
467 transcriptome suggested that it could also reflect the function characteristics of BAs.
468 17.4% gene showed similar differential expression trend as the protein (such as
469 neurodevelopment), and some BA signature genes (NEFH, NEFL in primary motor
470 cortex) also showed consistent expression patterns as the protein. Moreover, 15.7%
471 gene only differentially expressed in mRNA level, and some of which were associated
472 with BA function (cognition impairment, depressive disorder in DFC). Above data
473 demonstrated that transcriptome and proteome showed different characteristics of
474 brain functions. According to previous studies⁷, in neuron mRNA and proteins might
475 locate in different brain regions. Synapse proteins were transmitted from the nucleus
476 into another BA through axon, so they could be detected in both BAs. However,
477 mRNA was limited to the source BA containing cell body, so they were only
478 identified in source BA. Thus, such genes would be differentially expressed only on
479 mRNA level. Above study could partially account for the differences between
480 transcriptome and proteome.

481 This study established a Chinese proteome atlas of the brain; however, the
482 following issues need to be resolved in the future studies. First, we drafted a global
483 multiregional proteomic map of the left hemisphere of a single brain. Previous
484 proteomic and transcriptomic studies suggested that individual variations in age and
485 gender were substantially lower than variations across brain regions^{1,7}. Proteomic

486 atlas generated in the present study can represent common characteristics of the
487 normal human cerebral cortex to some extent; however, future studies should consider
488 inclusion of multiple individuals balanced with regard to gender, age, and brain
489 hemisphere sampling and should use more sensitive and accurate proteomic
490 technologies. Second, this pilot atlas includes only 29 BAs with important brain
491 functions, and future studies should include additional important regions, such as the
492 cerebellar cortex, hippocampus, and other important nerve nuclei, to generate a more
493 comprehensive atlas.

494

495 **Materials and methods**

496 **Tissue procurement.**

497 Samples from frozen postmortem human brain specimens were obtained
 498 following the SOP of the Human Brain Bank of China facilitated by the China
 499 Human Brain Bank Consortium (see Qiu *et al.*⁹ for details on sample collection,
 500 handling, and preservation). Basic demographic information, medical information,
 501 and medical records of the donor were obtained. Brain 1 specimens were collected
 502 from a 54-year-old willed male donor who died of lung cancer and did not have any
 503 history of brain metastasis, trauma, or other diseases. Brain 2 was collected from a
 504 93-year-old willed female donor who died of heart failure and did not have
 505 neurological diseases. The scalp was incised along the coronal plane from the ear to
 506 top of the skull and turned over to expose the skull. The skull was cut apart at the
 507 sagittal suture starting 1 cm above the eyebrows and continuing to the occipital
 508 protuberance, and the dura was removed. The cerebrum, brain stem, cerebellum, and
 509 part of the cervical cord were completely removed and temporarily preserved in cold
 510 saline. Twenty-nine samples of the cortex from various BAs were dissected from the
 511 left cerebral hemisphere and stored at -80°C for protein extraction. Routine
 512 hematoxylin/eosin, silver, and immunohistochemical staining procedures for
 513 amyloid-beta, p-tau, and alpha-synuclein were performed in the visual cortex, inferior
 514 parietal lobule, superior and middle temporal gyri, medial frontal cortex, amygdala,
 515 anterior cingulate gyrus, hippocampus, basal ganglia, cerebellum, middle brain,
 516 cerebral pons, and medulla in the right cerebral hemisphere of the brain sample; the
 517 data were used for pathological diagnosis to exclude neurodegenerative
 518 diseases. According to the Standardized Operational Protocol⁹, we performed HE
 519 staining of the brain areas, including BA40, 24, 17, 41/42/22, to confirm that brain is
 520 cancer-free.

521 **Ethical statements**

522 Brain tissue was collected after obtaining consent from the donor and the next of

kin using a signed informed consent form and the project was approved by the Institutional Review Board of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (approval number: 009-2014).

Sample preparation

A total of 29 brain tissue samples were selected for proteomic analysis (80 mg each). The samples were rinsed with PBS until washing fluid was clear, and each sample was lysed with lysis buffer (containing 8 M urea, 2.5 M thiourea, 4% CHAPS, 65 mM DTT, and 50 mM Tris-HCl) in a homogenizer on ice. The lysate was centrifuged at $14,000 \times \text{rpm}$ at 4°C for 20 min, and the supernatant was collected. The Bradford method was used to measure the protein concentration. Proteins were digested by the FASP method, as previously described⁶⁹. The peptides were extracted by an Oasis C18 extraction column and quantified by the BCA method.

iTRAQ labeling and 1D off-line separation

Digested peptides were labeled with 8-plex iTRAQ reagent according to the manufacturer's protocol (Sciex, Framingham, MA). The 28 Brodmann areas were randomly divided into 4 groups (Supplementary Table 2) and labeled by iTRAQ 8-plex agent. In each group, BA46 was used as common internal reference sample. The labeled samples within each experiment were mixed together. To reduce sample complexity, the peptide samples were fractionated through a high-pH RPLC system. The samples were reconstituted in 100 μL of buffer A (1 mM ammonia, pH = 10) and loaded onto a column (4.6 mm $\times$ 250 mm, Xbridge C18, 3 μm , Waters, Milford, MA). The elution gradient was composed of 5–25% buffer B (90% ACN, 1 mM ammonia, pH = 10) at a flow rate of 0.8 mL/min for 60 min. 60 fractions were collected, dried, and resuspended in 0.1% formic acid; the fractions were pooled into 20 samples using a stepwise concatenation strategy to combine every 20th fraction (1, 21, 41; 2, 22, 42; ...). A total of 100 fractions from a group of unlabeled samples and four groups of iTRAQ-labeled samples were further analyzed by LC-MS/MS.

550 LC-MS/MS analysis

551 The pooled mixtures of **iTRAQ**-labeled samples were analyzed using a
 552 self-packed RP C18 capillary column (75 μm ×100 mm, 1.9 μm). The elution gradient
 553 was composed of 5–30% buffer B (0.1% formic acid and 99.9% ACN) at a flow rate
 554 of 0.3 $\mu\text{L}/\text{min}$ for 45 min. An LTQ Orbitrap Fusion Lumos instrument (Thermo Fisher
 555 Scientific, Waltham, MA) was used to acquire raw data. The following parameters
 556 were used for MS data acquisition: top speed data-dependent mode (3 s) was used for
 557 full scan; full scans were acquired in an Orbitrap at a resolution of 60,000; the
 558 MS/MS scans were acquired at a resolution of 15,000 with charge state screening
 559 (excluding precursors with unknown charge state or +1 charge state); 38% normalized
 560 collision energy in HCD mode; 30s dynamic exclusion (exclusion size list 500); 1.6
 561 Da isolation window. Each fraction from the **iTRAQ**-labeled sample was run twice.
 562 For label-free analysis, 32% normalized collision energy in HCD was used, other
 563 parameters were identical to the **iTRAQ**. Each fraction from the unlabeled sample was
 564 run three times.

565 Database search

566 The MS/MS spectra were searched by Proteome Discoverer software (v3.1,
 567 Thermo Fisher Scientific) using the SwissProt human database downloaded from the
 568 UniProt website (www.UniProt.org). Trypsin was selected as the cleavage specificity
 569 parameter, and allowed a maximum of two missed cleavage sites. **For iTRAQ**
 570 **quantification**, carbamidomethylation of cysteine and **iTRAQ** 8-plex labels were set
 571 as fixed modifications, and the oxidation of methionine, deamidation of asparagine
 572 and glutamine, and carbamylation of lysine and peptide N-terminus were used as
 573 dynamic modifications. The searches were performed using a peptide tolerance of 10
 574 ppm and a protein tolerance of 0.02 Da. A 1% false-positive rate at the protein
 575 level was set as a filter, and each protein had to contain at least 1 unique peptide. **For**
 576 **label-free quantification**, carbamidomethylation of cysteine was used as a fixed
 577 modification, and other search parameters were identical to the parameters used for

iTRAQ quantification. The abundance of each protein was estimated by Progenesis software (Nonlinear Dynamics, version 4.0), and the iBAQ value of each protein was calculated based on the abundance of a protein divided by the theoretical polypeptide number of the corresponding protein.

Bioinformatics analysis of the iTRAQ data of 29 BAs

Each iTRAQ experiment contained seven BA samples and a common internal reference sample (BA46). In each experiment, the abundance ratios of the seven samples in BA46 were calculated, and the average protein abundance ratios of two technical replicates were used for further analysis. To ensure reliability of the data, the missing values in any two technical replicates of any group were excluded from subsequent analysis. The abundance ratios of proteins identified in all four experiments were merged into a single table in which each row represented a protein and each column represented a BA. In the column for BA46, all protein ratios were filled with 1. Then, the ratios were log2 transformed and normalized to a column median of 0 and a row median of 0. Thus, the positive value was higher than median, and the negative value were lower than median. Through row-wise normalization, the expression pattern of each row/protein in all the samples could be better exhibited, and it would be helpful for following cluster analysis and signature proteins filtering.

For better cluster analysis and signature protein selection the proteins without inter-area variability were filtered. The fold changes of all proteins between all BA pairs were calculated. The distribution of log2 fold change showed that the and Q3 + 1.5 interquartile range was 1.57. A strict filter (fold change > 2) was used as the threshold of outliers, which would be helpful to signature proteins. To identify the differential proteins between Wernicke's area cluster and auditory and Broca's area cluster, a relative relax threshold (1.5 fold change) was used to find more differential proteins, which would be helpful for pathway or function enrichment analysis.

Consensus clustering was performed using the Consensus Cluster Plus package of R¹¹ to assess the similarities in the proteome profiles of BAs. K-means clustering with

606 Euclidean distance was performed 1,000 times on the subsampled data. The optimal
607 number of clusters was selected using the consensus matrix, consensus cumulative
608 distribution function (CDF) plot, and silhouette plot. The consensus matrix showed
609 the best clustering results at $k = 3$. However, the CDF of consensus increased at
610 greater k values. At $k = 6$, a relative increase in CDF reached a very small value. The
611 silhouette plot at $k = 6$ showed a mean silhouette score of 0.72, and all scores were
612 positive, indicating that clusters were well separated. Thus, BAs were clustered into
613 six clusters.

614 Cluster signature proteins were identified by a biclustering algorithm known as
615 iterative signature algorithm (ISA). ISA was implemented using the isa2 package for
616 R²³. Biclustering was restricted to upregulated modules in both rows and columns
617 with a score threshold of 0.7. For all six clusters, modules that included at least 80%
618 BAs inside a cluster and no BAs outside of the cluster were selected. Proteins in the
619 selected modules with scores that passed the threshold of 0.7 were considered
620 signature proteins for this cluster.

621 An adequate cutoff ratio was determined to identify proteins overexpressed in
622 specific BAs. The differences in the log2 protein abundance ratios between all BA
623 pairs for all proteins were calculated, and the data were normally distributed with a
624 mean of 0.00 and a standard deviation (sd) of 0.35. The log2-fold change cutoff was
625 then determined to be 1, which approximated the mean + 3 * sd. Thus, an area
626 signature protein was defined as a protein with abundance level in one or two
627 particular BAs of at least twofold of the median abundance level in all 29 BAs.

628 **Downstream Bioinformatics analysis**

629 All proteins of interest were analyzed using the David database
630 (<http://david.ncifcrf.gov/>) and compared with the whole human genome for GO
631 enrichment analysis. For IPA analysis, the signature or differential proteins were
632 evaluated using IPA software (Ingenuity Systems, Mountain View, CA), and the
633 enrichment of the disease, function, and canonical pathway categories were analyzed

634 according to the P-value rankings. For reactome analysis, cluster signature proteins
635 were mapped to the reactome database in the pathway category according to the
636 P-value rankings. For KEGG analysis, cluster signature proteins were mapped to the
637 KEGG database in the pathway category according to the P-value rankings.

638 **IHC and Western blot**

639 Western blot analysis of brain samples was performed to validate the proteomic
640 quantitation of several selected candidate proteins (tau, alpha-synuclein, EPM2A,
641 MRPS34, TAGLN, FAM107B, and RAD23A). Proteins extracted from the brain
642 tissues were separated by SDS-PAGE and electro-transferred to a PVDF membrane
643 (Millipore, Bedford, MA). The membrane was blocked with 2% (v/v) BSA for 2 h at
644 room temperature and incubated with primary antibodies and a peroxidase-conjugated
645 secondary antibody. The membranes were visualized using Immobilon Western
646 chemiluminescent horseradish peroxidase substrate (Millipore), and the bands were
647 analyzed by ImageJ software.

648 Formalin-fixed, paraffin-embedded brain tissue samples were used for IHC-Fr
649 analysis. The tissue sections were deparaffinized and rehydrated in xylene and a
650 graded ethanol series, and antigen retrieval was performed in a pressure cooker by
651 boiling in sodium citrate buffer (pH 6.0) for 2 min. Then, endogenous peroxidases
652 were blocked with 0.3% H₂O₂ for 15 min, and the samples were incubated in the
653 presence of 2% fetal calf serum for 20 min. Primary antibodies were incubated at 4°C
654 overnight, and peroxidase-labeled polymer conjugated to anti-mouse, anti-rabbit, or
655 anti-goat immunoglobulins was incubated for 1 h. The sections were counterstained
656 with Mayer's hematoxylin and dehydrated, and the images were acquired under a
657 microscope.

658 Primary antibodies for Western blot and IHC-Fr against MAPT (rabbit
659 monoclonal, ab76128), SYUA (rabbit monoclonal, ab138501), EPM2A (rabbit
660 monoclonal, ab129110), MRPS34 (mouse monoclonal, HPA042112, Sigma), TAGLN
661 (rabbit polyclonal, ab14106), and RAD23A (mouse monoclonal, ab55725) were

662 purchased from Abcam (Cambridge, UK). Antibodies against FAM107B (rabbit
663 polyclonal, ab122566) were purchased from Sigma-Aldrich (St Louis, MO).

664 **PRM analysis**

665 Selected cluster and BA signature proteins were validated in 27 BAs of brain 2 by
666 PRM. To estimate the quality of the data, we used the mixed sample as quality control
667 (QC) during the analysis, before and after all samples, and every 7-8 samples. Two
668 technical repeats of each sample were assayed. The average Pearson correlation
669 coefficient of QC samples was 0.97, indicating the reproducibility of the QC.

670 The samples were analyzed on a C18 RP self-packed capillary LC column (75
671 $\mu\text{m} \times 100 \text{ mm}$). The elution gradient was composed of 5-30% buffer B (0.1% formic
672 acid, 99.9% ACN) with a flow rate of 0.5 $\mu\text{L}/\text{min}$ for 45 min. An LTQ Orbitrap Fusion
673 Lumos instrument was used to acquire raw data. Following parameters was used for
674 MS data acquisition: PRM mode; full scans and MS/MS scans were acquired in
675 Orbitrap at a resolution of 60,000 and 15,000, respectively; 32% normalized collision
676 energy in HCD mode; 30s dynamic exclusion; The isolation window was 4, and the
677 schedule mode window was 7 min.

678 PRM data processing was performed by Skyline 19.1 software as previous
679 described ⁶⁹. The peptide abundance was normalized to TIC in each sample, which
680 was extracted by Progenesis QIP software (Waters).

681 **RNA seq and data processing**

682 RNA-seq was performed by BGI cooperation (Shenzhen, China). For
683 transcriptome data analysis, read counts were normalized using the median of ratios
684 normalization method in DESeq2 to eliminate the variations in sequencing depth and
685 RNA composition in the samples ⁷⁰. Normalized counts were logarithmically
686 transformed with base 2. A total of 17,091 transcripts with read counts > 1 in at least
687 50% of the samples were retained for downstream analysis. Transcriptomic and
688 proteomic data were matched by gene symbols.

689 **Comparison between the proteome and transcriptome**

690 The genes commonly quantified at the mRNA and protein levels were quantitatively
 691 compared. Both transcriptomic and proteomic datasets were log2 transformed by
 692 zero-mean normalization. The gene products for all possible region pairs were
 693 classified based on their fold-change similarity between mRNA and protein. Genes
 694 with the same direction of the changes at the RNA and protein levels and with the
 695 same magnitude of fold change (the variability in protein level between BAs was <
 696 2-fold of that at the mRNA level, assigned to the “agree” category); the genes with the
 697 same direction of change but with different magnitude of fold change (larger than
 698 2-fold) at the RNA and protein levels were assigned to the “partially agree” category.
 699 The genes that had no significant inter regional changes in either RNA or protein
 700 levels (within 2-fold) between BAs were assigned to the "no change" category. The
 701 genes that had variable levels of corresponding proteins between various BAs (larger
 702 than 2-fold) but did not vary at the mRNA level (within 2-fold) were assigned to the
 703 "protein only" category. The genes that had variable mRNA levels (larger than 2-fold)
 704 but did not vary at the protein level (within 2-fold) were defined as "mRNA" only.
 705 The genes that showed opposite changes in mRNA and protein levels were assigned
 706 to the “disagree” category.

707

708 **CRedit author statement**

709 **Zhengguang Guo:** formal analysis, funding acquisition, validation, writing - original
 710 draft. Chen Shao: methodology, formal analysis, software visualization, funding
 711 acquisition, and writing - review & editing. Yang Zhang: conceptualization, writing -
 712 review & editing. Wenying Qiu: resources, investigation. Wenting Li: resources.
 713 Weimin Zhu: project administration. Qian Yang: validation. Yin Huang: software. Lili
 714 Pan: visualization. Yuepan Dong: visualization. Handan Sun: investigation. Xiaoping
 715 Xiao: investigation. Wei Sun: investigation, project administration, funding
 716 acquisition, writing - review & editing. Chao Ma: project administration, funding
 717 acquisition. Liwei Zhang: conceptualization, project administration, funding
 718 acquisition, supervision. All authors read and approved the final manuscript.

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735

736 **Competing interests**

737 The authors declare that they have no competing interests.

738

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933 Table 1: Location, function and proteomic clustering of 29 human brain Brodmann
934 areas.

Cluster	Brodmann area	Lobe	Location	Function
Motor and sensory	3/1/2	Parietal Lobe	Postcentralgyrus	Somatosensory system ¹
	4	Frontal Lobe	Precentralgyrus	Primary motor cortex ²
	6	Frontal Lobe	Frontal Lobe	Premotor cortex and supplementary motor cortex ²
	20	Temporal Lobe	Inferior temporal gyrus	Visual processing and recognition ³
	38	Temporal Lobe	Temporal pole	Visceral emotional responses ⁴
Visual	17	Occipital Lobe	Occipital Lobe	Visual processing ⁵
	18	Occipital Lobe	Occipital Lobe	Visual processing ⁵
	19	Occipital Lobe	Occipital Lobe	Visual processing ⁵
	37	Temporal Lobe	Fusiform gyrus	Visual recognition ⁶⁻⁸
	34	Temporal lobe	Parahippocampalgyrus	Neuron information processing; visual processing ⁹
Auditory and Broca's area	8	Frontal Lobe	Prefrontal cortex	Eye movements; <i>writing language</i> ¹⁰
	7	Parietal Lobe	Parietal Lobe	Visuo-motor coordination; <i>speech and language</i> ¹¹
	26/29/30	Cingulate cortex	Retrosplenial cortex	Cognition;memory ¹² ;auditory ¹³
Heterogeneous functions	41/42	Temporal Lobe	Anterior transverse temporal gyrus	Auditory cortex ¹⁴
	44/45	Frontal Lobe	Inferior frontal gyrus	Broca'sarea, language production ¹⁵
	9	Frontal Lobe	Dorsolateral prefrontal cortex	Executive functions, cognition, behaviour ¹⁶

	10	Frontal Lobe	Anterior prefrontal cortex	Executive functions, cognition, memory ¹⁷⁻¹⁹
	39	Parietal Lobe	Angular gyrus	Complex language function, mathematics processing, and spatial cognition ²⁰⁻²⁵
	40	Parietal Lobe	Supramarginalgyrus	Language perception and processing, visual word recognition ^{26,27}
	46	Frontal Lobe	Dorsolateral prefrontal cortex	Executive functions, cognition, behaviour ¹⁶
Wernicke's area	21	Temporal Lobe	Middle temporal gyrus	Wernicke's area, comprehension of written and spoken language ²⁸
	22	Temporal Lobe	Superior temporal gyrus	Wernicke's area, comprehension of written and spoken language ²⁸
	31	Cingulate cortex	Posterior cingulate cortex	Emotion and memory; intrinsic control networks ²⁹
	32	Cingulate cortex	Anterior cingulate cortex	Emotional and cognitive processing ^{30,31}
	33	Cingulate cortex	Anterior cingulate cortex	Emotional and cognitive processing ^{30,31}
Cingulate cortex	23	Cingulate cortex	Ventral Posterior cingulate cortex	Emotion and memory; intrinsic control networks ²⁹
	24	Cingulate cortex	Ventral anterior cingulate cortex	Emotional and cognitive processing ^{30,31}
	25	Cingulate cortex	Subgenual cingulate	Emotion and memory ³²
	28	Temporal Lobe	Ventral entorhinal cortex	Neuron information processing, memory ³³

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1017 **Figure legends**

1018 **Figure 1: A broad and comprehensive atlas of brain proteome covering 29 BAs. A:**

1019 Graphical illustration of the workflow for brain proteome database in Brodmann Area
1020 (BA46) and brain proteomic map in the 29 major BAs. First, an extensive proteomic
1021 library was generated of BA46 by 2D-LC-MS/MS and label-free quantification in
1022 BA46. Then, 29 BAs were quantitatively analyzed by 2D-LC-MS/MS and iTRAQ
1023 quantification. Further, a website, Human Brain Proteome Atlas,
1024 HBPA (www.brain-omics.com) was built. B: Venn diagram of protein number of
1025 BA46 in present and previous studies. C: Accumulation of protein mass from the
1026 highest abundance to the lowest abundance protein in BA46. The high abundance
1027 proteins, (grey, top for 75% of total protein abundance), mid abundance proteins,
1028 (orange, top 75%-95% of total protein abundance), and low abundance proteins (blue,
1029 bottom 5% total protein amount) shown by distinct colors. D: GO enrichment analysis
1030 of the brain proteome, and the high, middle, low abundance brain proteins, and
1031 compared with the whole proteome. The percentages of the proteins in each category
1032 were in bar plot. *: $p < 0.05$.

1033 **Figure 2: Brain proteomic atlas reflects the functional parcellation. A:**

1034 brain areas were divided into 6 clusters, including motor and sensory cluster, vision
1035 cluster, auditory and Broca's area cluster, Wernicke's area cluster, cingulate cortex
1036 cluster, and heterogeneous function cluster. The pearson correlation heatmap of 29
1037 BAs in the left. Red: positive correlation; Blue: negative correlation. The locations of
1038 29 BAs were shown in the right. B: Brief annotations of 29 BAs in the 6 clusters. The
1039 lobe location of each BA was annotated in the inner circle, and the function of each
1040 BA was annotated in the outer circle. Bold: Canonical function; Italic: uncanonical
1041 function.

1042 **Figure 3: Cluster signature proteins linked to specialized function of the cluster. A:**

1043 Heat map of the cluster signature proteins. The normalized protein expression value
1044 z-score represents the protein abundance. Red: high abundance. Blue: low abundance.

1045 B,C: Pathway (B) and disease (C) enrichment analysis of cluster signature proteins in
1046 six clusters. The -Log (p-value) by two-sided hypergeometric test were in
1047 heatmaps.Red:significantly enriched. D: Distribution of the high-, mid-, low-
1048 abundance proteins in the cluster signature proteins. The number of high-, mid-, low-
1049 abundance proteins in each cluster signature proteins were annotated in the bar plot. E:
1050 Heatmap of cingulate cortex cluster signature proteins involved in
1051 glycolysis/gluconeogenesis, oxidative phosphorylation, and biosynthesis of amino
1052 acids. The normalized protein expression value z-score represents the protein
1053 abundance. Red: high abundance. Blue: low abundance. F: The relative expression
1054 value of two motor and sensory cluster signature proteins (MRPS34 and EMP2A) and
1055 two cingulate cortex cluster specific proteins (SYUA and MAPT) in all the 29 BAs.
1056 The motor and sensory cluster and the cingulate cortex cluster were highlighted by
1057 yellow in the left and right panels, respectively.

1058 **Figure 4: BA signature proteins linked to BAs' function.** A: Heat map of the BA
1059 signature proteins in 29 BAs. The normalized protein expression value z-score
1060 represents the protein abundance. Red: high abundance. Blue: low abundance. B: The
1061 relative expression value of three BA signature proteins, TAGLN, RAD23A, and
1062 FAM107B in all the 29 BAs. The BA41/42, BA40, BA17 and BA18 were highlighted
1063 by yellow in the upper, middle, and lower panels, respectively.

1064 **Figure 5: Molecular annotation of the un-canonical functions of BAs.** A: Scatter
1065 plot of protein log2 fold expression value in BA 33 compared with the value in BA4.
1066 Proteins expressed at high level in both BA4 and BA33 highlighted in orange. B: The
1067 co-highly-expressed proteins in BA4 and BA33 were associated with motor neuron
1068 structure and functions. C: Clustering analysis of anxiety-related proteins. The
1069 proteins highly expressed in motor and sensory cluster highlighted in red. D: Protein
1070 network of anxiety-related proteins. Orange: The proteins expressed at high level in
1071 motor and sensory cluster. Green:The proteins expressed at low level in motor and
1072 sensory cluster.

Figure 6: Comparison of brain proteome and transcriptome. A: Venn diagram of protein and mRNA number in BA46. B: Histogram of the distribution of the gene expression in mRNA and protein level. Orange: Gene identified in protein level. Grey: Genes only identified only in mRNA level. The MS/MS coding genes covered 61% genes expressed at >1 read per kilobase per million mapped (RPKM) and 78.4% of genes expressed at >10 RPKM. C: Principal component analysis of mRNA shows that the BA4 and cluster cingulate cortex were partially separated with the other BAs. D: Heat map of the BA signature genes in 27 BAs. The normalized protein expression value z-score represents the protein abundance. Red: high abundance. Blue: low abundance. E: The Distribution of the relative dominance of no change (grey), agree (green), partially agree (purple), protein only (Blue), mRNA only (orange), and disagree (red) genes. Genes are defined based on the consistency or inconsistency between the mRNA and protein measurements. Agree genes are those with same direction of change in both RNA and protein level, and with the same magnitude of fold change (the variability in protein level between BAs is < 2 fold of that in mRNA level). Partially agree genes are genes with same direction of change, but with different magnitude of fold change (larger than 2 fold) in RNA and protein level. No change genes, both protein and RNA were not changed (within 2 fold) between the BAs. Protein only genes vary between different BAs by protein (larger than 2 fold), but not by mRNA (within 2 fold). mRNA only genes vary by mRNA (larger than 2 fold), but not by protein (within 2 fold). Disagree genes performed opposite direction of change between protein and mRNA. F: Scatter plots of Log2 formed mRNA and protein abundance fold change between BA4 and BA46. grey (no change), Green (agree), purple (partially agree), Blue (protein only), orange (mRNA only), and Red (disagree) genes were defined in E. The inset pie charts illustrated the percentage of the green, purple, blue, orange, and red genes. G: Scatter plots of mRNA and protein fold change for BA4/ BA46 comparison, showing the position of gene products contained in ontological terms of interest. The inset small table showed the percentage of the genes of responding ontological terms in the mRNA only, protein only, agree,

1102 partially agree and disagree categories. The colored lines indicated the threshold of
1103 mRNA gene (orange), protein only gene (blue), and the agree gene (green).

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1105 **Supplementary material**

1106 **Supplementary Figure 1: The HE staining data of two brains. A: Brain 1; B:**
1107 **Brain 2.**

1108 **Supplementary Figure 2: Reliability of iTRAQ analysis pipeline.** A: Technical CV
1109 distributions of label-free and iTRAQ quantification. B: The venn diagram of the
1110 proteins quantified in 4 batches. C: Log ratio distribution of iTRAQ quantification
1111 results. D: The ratio distributions of each run in 4 batches.

1112 **Supplementary Figure 3: Protein abundance distribution of BA46 from the**
1113 **highest abundance to the lowest abundance protein.**

1114 **Supplementary Figure 4:Data visualization in Human Brain Proteome Atlas**
1115 **website.** A: Protein quantification distribution of MAPT in 29 Brodmann areas. B:
1116 Protein expression data in Brodmann area 25(Subgenual area). C: Comparison of the
1117 differential proteins between BA3/1/2, and BA4.

1118 **Supplementary Figure 5: The protein clustering analysis.** A: The clustering is
1119 derived by consensus clustering based on 1,000 resampled data sets, exploring the
1120 range of from k=2 to k=8. B: Based on both consensus cumulative distribution
1121 function (CDF) area curve and the delta plot assessing change in CDF area, the CDF
1122 of consensus increased at greater k values. At k = 6, a relative increase in CDF
1123 reached a very small value. . C: Silhouette plots were performed to evaluate the
1124 Stability of the clustering. The silhouette plot at k = 6 showed a mean silhouette score
1125 of 0.72, and all scores were positive.

1126 **Supplementary Figure 6: Analysis of the differential proteins between**
1127 **Wernicke's area cluster and auditory and Broca's area cluster.** A: IPA-based
1128 Canonical pathway analysis of differential proteins. The -Log (p-value) of the
1129 Canonical pathway term were shown. B: Heatmap of differential proteins involved in
1130 language eloquent regions related neurotransmitter pathways. The normalized protein
1131 expression value z-score represents the protein abundance. Red: high abundance. Blue:
1132 low abundance.

1133 **Supplementary Figure 7: Functional analysis of cluster signature proteins,**
1134 **related to Figure 3.** A: The IPA-based biofunction (A) and KEGG database-based
1135 pathway (B) enrichment analysis of cluster signature proteins.The -Log (p-value) by
1136 two-sided hypergeometric test in heatmap. Red: significantly enriched. C-E: Heat map
1137 of the high-abundance proteins (C), mid-abundance proteins (D) and low abundance
1138 proteins(E) in the 29 BAs. The normalized protein expression value z-score represents

the protein abundance. Red: high abundance. Blue: low abundance. F: Protein expression analysis of cingulate cortex cluster in Parkinson's signaling. G: Protein expression analysis of cingulate cortex cluster in Alzheimer's disease signaling. (Red: highly-expressed in cingulate cortex cluster; Green: lowly-expressed in cingulate cortex cluster)

Supplementary Figure 8: The Western Blot and IHC-Fr results of cluster signature proteins. A: Western Blot results of two motor and sensory cluster signature proteins (MRPS34 and EPM2A) and two cingulate cortex cluster signature proteins (Tau and a-syn). B: Comparison between immunoblotting results and iTRAQ results of above proteins. C: IHC-Fr validation results of two cingulate cortex cluster signature proteins (MAPT and SYUA). Scale bars represent as 50 μ m.

Supplementary Figure 9: The Western Blot and IHC-Fr results of Brodmann area signature proteins. A: Western Blot results of three Brodmann area signature proteins (Transgelin, FAM107B, and RAD23A). B: Comparison between the immunoblotting results and iTRAQ results of above proteins.

Supplementary Figure 10: Quality control of RNA-seq. A: Number of gene detected in RNA-seq analysis of the 21 BAs. B: Box plot of gene expression level of 21 BAs.

Supplementary Figure 11: Scatter plots of mRNA and protein fold change of all regions compared with BA 46. These scatter plots are identically defined as those in Figure 6E.

1160

1161 **Supplementary Table 1: Brain proteome database in BA46.**A:Qualitative data of
1162 BA46 technical repeat 1, 2 and 3. The proteins identified in Kim's study and Becky's
1163 study were annotated. B: Protein identification summary of BA 46. C: Quantitative
1164 data of BA 46. D: The GO analysis results of brain proteome and high-, mid-, and
1165 low-abundance proteins compared with the whole human proteome.

1166 **Supplementary Table 2: iTRAQ quantification data for 29 BAs.** A: Batch 1; B:
1167 Batch 2; C: Batch 3; D: Batch 4. E: Protein identification summary of the human
1168 brain proteome of 29 BAs. F: Normalized protein quantification data of the 4308
1169 proteins in all of 29 BAs. The 1241 proteins with inter-area variability (the difference
1170 between the maximum and minimum ratios ≥ 2) were annotated.

1171 **Supplementary Table 3: Differentially proteins between the Wernicke's area**
1172 **cluster and Auditory and Broca's area cluster.**

1173 **Supplementary Table 4: Cluster/BA signature proteins.** A:List of cluster signature
1174 proteins. B: Biofunction analysis of cluster signature proteins. C: Disease analysis of
1175 cluster signature proteins. D: Canonical pathway analysis of cluster signature proteins.
1176 E: KEGG pathway analysis of cluster signature proteins. F: Cluster cingulate cortex
1177 signature proteins were involved in cognition, emotion, and memory. G: BA signature
1178 proteins.

1179 **Supplementary Table 5: Validation of the Cluster/BA signature proteins. A: PRM**
1180 **results of Cluster signature proteins (peptide level).** B: PRM results of Cluster
1181 **signature proteins (protein level).** C: PRM results of BA signature proteins (peptide
1182 **level).** D: PRM results of BA signature proteins (protein level). E: Comparison of
1183 **Vision cluster/vision cortex signature proteins results between this study and Becky's**
1184 **study.**

1185 **Supplementary Table 6: Molecular annotation of the un-canonical functions of**
1186 **BAs.** A: Proteins highly expressed in both Primary motor cortex (BA4) and Anterior
1187 **cingulate cortex (BA33).** B:Thirty proteins with inter-area variability were involved in
1188 **anxiety or anxiety-like behaviors.**

1189 **Supplementary Table 7. Comparison of brain proteome and**
 1190 **transcriptome.**A:RNA-seq results for the 21 BAs. B: BA signature genes. C:Thirteen
 1191 signature genes involved in memoryin BA28. D. The common genes in transcriptomic
 1192 and proteomic results. E: The differential transcriptomic and proteomic results for
 1193 BA4 and BA46 comparison. F. The disease and biofunction analysis of differential
 1194 genes of BA4 and BA46. The categories mentioned in the manuscript were
 1195 highlighted in yellow.

A

B

C

D

A

Lateral Surface

Medial Surface

B

A

B

C

D

A

B

C

D

E

G

F