

1 **Concerted neuron-astrocyte gene expression declines in aging and schizophrenia**
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19
20 Human brains vary across people and over time; such variation is not yet understood in
21 cellular terms. Here we describe a striking relationship between people's cortical neurons
22 and cortical astrocytes. We used single-nucleus RNA-seq to analyze the prefrontal cortex
23 of 191 human donors ages 22-97 years, including healthy individuals and persons with
24 schizophrenia. Latent-factor analysis of these data revealed that in persons whose cortical
25 neurons more strongly expressed genes for synaptic components, cortical astrocytes more
26 strongly expressed distinct genes with synaptic functions and genes for synthesizing
27 cholesterol, an astrocyte-supplied component of synaptic membranes. We call this
28 relationship the Synaptic Neuron-and-Astrocyte Program (SNAP). In schizophrenia and
29 aging – two conditions that involve declines in cognitive flexibility and plasticity^{1,2} – cells
30 had divested from SNAP: astrocytes, glutamatergic (excitatory) neurons, and GABAergic
31 (inhibitory) neurons all reduced SNAP expression to corresponding degrees. The distinct
32 astrocytic and neuronal components of SNAP both involved genes in which genetic risk
33 factors for schizophrenia were strongly concentrated. SNAP, which varies quantitatively
34 even among healthy persons of similar age, may underlie many aspects of normal human
35 interindividual differences and be an important point of convergence for multiple kinds of
36 pathophysiology.

37 **INTRODUCTION**

38

39 In natural, non-laboratory settings – in which individuals have diverse genetic inheritances,
40 environments and life histories, as humans do – almost all aspects of biology exhibit quantitative
41 variation across individuals³. Natural variation makes it possible to observe a biological system
42 across many contexts and potentially learn underlying principles^{4,5}.

43

44 Here we sought to recognize changes that multiple cell types in the human brain
45 characteristically implement together. The need to be able to recognize tissue-level gene-
46 expression programs comes from a simple but important idea in the physiology of the brain and
47 other tissues: cells of different types collaborate to perform essential functions, working together
48 to construct and regulate structures such as synaptic networks.

49

50 We analyzed the prefrontal cortex of 191 human brain donors by single-nucleus RNA-seq
51 (snRNA-seq) and developed a computational approach, based on latent-factor analysis, to
52 recognize commonly recurring multicellular gene-expression patterns in such data. Tissue-level
53 programs whose expression varies across individuals could provide new ways to understand
54 healthy brain function and also brain disorders, since disease processes likely act through
55 endogenous pathways in cells and tissues. A longstanding challenge in genetically complex
56 brain disorders is to identify the aspects of brain biology on which disparate genetic effects
57 converge; here we applied this idea to try to better understand schizophrenia.

58 **RESULTS**

59

60 **Dorsolateral prefrontal cortex snRNA-seq**

61

62 We analyzed the dorsolateral prefrontal cortex (dlPFC, Brodmann area 46), which serves
63 working memory, attention, executive functions, and cognitive flexibility⁶, abilities which decline
64 in schizophrenia and with advancing age^{1,2}. Analyses included frozen post-mortem dlPFC from
65 191 donors (ages 22-97, median 64), including 97 without known psychiatric conditions and 94
66 affected by schizophrenia (**Extended Data Fig. 1 and Supplementary Table 1**). To generate
67 data that were well-controlled across donors and thus amenable to integrative analysis, we
68 processed a series of 20-donor sets of dlPFC tissue each as a single pooled sample (or “village”
69⁷) (**Fig. 1a**), then, during computational analysis, used combinations of many transcribed SNPs
70 to identify the source donor of each nucleus (**Fig. 1a-b** and **Extended Data Fig. 2**).

71

72 Each of the 1,218,284 nuclei was classified into one of seven cell types – glutamatergic neurons
73 (43% of all nuclei), GABAergic neurons (20%), astrocytes (15%), oligodendrocytes (12%),
74 polydendrocytes (oligodendrocyte progenitor cells, 5.5%), microglia (3.6%), and endothelial
75 cells (1.3%) (**Fig. 1c and Extended Data Fig. 3**) – as well as neuronal subtypes defined in
76 earlier taxonomies (**Fig. 1d-e and Extended Data Figs. 4 and 5**). Each donor contributed
77 nuclei of all types and subtypes (**Extended Data Figs. 3, 6, and 7**), though subsequent
78 analyses excluded eleven atypical samples (**Extended Data Fig. 3d**).

79

80

81 **Inference of multicellular gene-programs**

82

83 The data revealed substantial inter-variation in cell-type-specific gene expression levels, with
84 highly expressed genes in each cell type exhibiting a median coefficient of variation (across
85 donors) of about 15%.

86

87 Inter-individual variation in gene expression almost certainly arises from cell-type-specific gene-
88 expression programs, and could in principle also be shaped by concerted changes in multiple
89 cell types. To identify such relationships, we applied latent factor analysis, a form of machine
90 learning which infers underlying factors from the tendency of many measurements to fluctuate
91 together⁸; critically, we analyzed cell-type-resolution data from all cell types at once, using inter-
92 individual variation to enable the recognition of relationships between expression patterns in
93 different cell types (**Fig. 1f**). Each inferred factor was defined by a set of gene-by-cell-type
94 loadings (revealing the distinct genes it involves in each cell type) and a set of expression levels
95 (of the factor) in each donor (**Fig. 1f**).

96

97 Ten latent factors together explained 30% of inter-individual variation in gene expression levels;
98 these factors appeared to be independent of one another in their gene utilization patterns
99 (“loadings”) and their expression levels across the individual donors (**Extended Data Fig. 8a-d**).

100 Inter-individual variation in the factors’ inferred expression levels arose from inter-individual

101 variation within each 20-donor experimental set (**Extended Data Fig. 8e**). Each factor was
102 primarily driven by gene expression in one or a few cell types (**Fig. 1g**).
103

104 Schizophrenia associated with just one of these latent factors (LF4) (**Fig. 1h, Extended Data**
105 **Fig. 9a-e, and Supplementary Table 2**) – a factor that also associated with donor age (**Fig. 1i**).
106 Donors with and without schizophrenia both exhibited the decline in LF4 with age (**Fig. 1i and**
107 **Extended Data Fig. 1c-d**). Joint regression analysis confirmed independent reductions of LF4
108 expression by age and in schizophrenia, and detected no effect of sex (**Supplementary Table**
109 **3**).
110

111 Factors similar to LF4 emerged in all analyses testing LF4's robustness to analysis parameters
112 (**Extended Data Fig. 10**). Individuals' LF4 expression scores also did not correlate with
113 medication use, time of day at death, post-mortem interval, or sequencing depth (**Extended**
114 **Data Fig. 9f-k**). We also found evidence that the LF4 constellation of gene-expression changes
115 manifests at a protein level (**Extended Data Fig. 11**).
116

117
118 **Neuronal and astrocyte genes driving LF4**
119

120 Of the 1,000 gene/cell-type expression traits with the strongest LF4 loadings, 99% involved
121 gene expression in glutamatergic neurons (610), GABAergic neurons (125), or astrocytes (253)
122 (**Fig. 1g**). LF4 involved similar genes and expression effect directions in glutamatergic and
123 GABAergic neurons but a distinct set of genes and effect directions in astrocytes (**Fig. 2a and**
124 **Extended Data Fig. 9m**). To identify biological processes in LF4, we applied gene set
125 enrichment analysis (GSEA,⁹) to the LF4 gene loadings, separately for each cell type.
126

127 In both glutamatergic and GABAergic neurons, LF4 involved increased expression of genes with
128 synaptic functions (**Fig. 2b, Extended Data Fig. 9l and Supplementary Table 4**). The
129 strongest synaptic enrichments for both glutamatergic and GABAergic neurons involved the
130 synaptic vesicle cycle and the presynaptic compartment; the core genes driving these
131 enrichments encoded components of the SNARE complex and their interaction partners
132 (*STX1A*, *SNAP25*, *SYP*), effectors and regulators of synaptic vesicle exocytosis (*SYT11*,
133 *RAB3A*, *RPH3A*), and other synaptic vesicle components (*SV2A*, *SYN1*). In glutamatergic
134 neurons, LF4 also appeared to involve genes encoding postsynaptic components, including
135 signaling proteins (*PAK1*, *GSK3B*, *CAMK4*) and ion channels and receptors (*CACNG8*, *KCNN2*,
136 *CHRN2*, *GRM2*, *GRIA3*).
137

138 Persons with schizophrenia and persons of advanced age exhibited reduced levels of synapse-
139 related gene expression by cortical neurons of all types (**Fig. 2c and Extended Data Fig. 12**).
140 In astrocytes, LF4 involved gene-expression effects distinct from those in neurons (**Fig. 2a and**
141 **Extended Data Fig. 9m**). Gene sets with roles in fatty acid and cholesterol biosynthesis and
142 export, including genes that encode the SREBP1 and SREBP2 transcription factors and their
143 regulators and targets, were positively correlated with LF4 and under-expressed in the cortical
144 astrocytes of donors with schizophrenia (**Fig. 2d and Supplementary Table 4**) or advanced

145 age (**Extended Data Fig. 13a**). These effects appeared to be specific to astrocytes relative to
146 other cell types (**Extended Data Fig. 14**).
147
148

149 **Concerted neuron-astrocyte expression**

150

151 To understand these results in terms of specific biological activities, we focused on gene sets
152 corresponding to neuronal synaptic components and three kinds of astrocyte activities:
153 adhesion to synapses, uptake of neurotransmitters, and cholesterol biosynthesis (**Methods: Selected gene sets**).
154

155

156 The proportion of astrocyte gene expression devoted to each of these three astrocyte activities
157 strongly correlated with the proportion of neuronal gene expression devoted to synaptic
158 components (**Fig. 2e and Extended Data Fig. 15**), even after adjusting for age and case-
159 control status (**Extended Data Fig. 16**). Donors with schizophrenia, as well as donors with
160 advanced age, tended to have reduced expression of these genes (**Fig. 2e and Extended Data**
161 **Fig. 13**).
162

163

164 Because this gene expression program involves concerted effects upon the expression of
165 (distinct) genes for synaptic components in neurons and astrocytes, we call it “SNAP” (Synaptic
166 Neuron-Astrocyte Program), though it also involves genes with unknown functions and more-
167 modest expression effects in additional cell types. We use donors’ LF4 expression scores to
168 measure SNAP expression.
169

170 **Astrocyte gene-programs and SNAP**

171

172 To better appreciate the astrocytic contribution to SNAP, we further analyzed the RNA-
173 expression data from 179,764 individual astrocytes. Analysis readily recognized a known,
174 categorical distinction among three subtypes of adult cortical astrocytes: protoplasmic
175 astrocytes, which populate the gray matter and were the most abundant subtype; fibrous
176 astrocytes; and interlaminar astrocytes (**Fig. 3a and Extended Data Fig. 17a-d**). Neither
177 schizophrenia nor age associated with variation in these subtypes’ relative abundances
178 (**Extended Data Fig. 17e-f**).
179

180

181 We then identified latent factors that collectively explained 25% of quantitative gene-expression
182 variation among individual astrocytes (using cNMF, ¹⁰) (**Extended Data Fig. 18a-b**). The factors
183 appeared to capture diverse biological activities, including translation (cNMF1); zinc and
184 cadmium ion homeostasis (cNMF7); and inflammatory responses (cNMF8) (**Supplementary**
185 **Table 5**). One factor (cNMF2) corresponded to the astrocyte component of SNAP (**Extended**
186 **Data Fig. 18c-e and Supplementary Table 6**); the strong co-expression relationships in SNAP
187 were thus robust to the computational approach used (**Extended Data Figs. 18c-e and 19**).
188

188 Because cNMF2 is informed by variation in the single-astrocyte expression profiles, we consider
189 it a more precise description of the astrocyte-specific gene-expression effects in SNAP, and
190 refer to it here as SNAP-a. Across donors, average astrocyte expression of SNAP-a associated
191 even more strongly with schizophrenia case-control status and with age (**Fig. 3b-e and**
192 **Extended Data Fig. 18f-i**).

193

194 The strongest positive gene-set associations to SNAP-a involved adhesion to synaptic
195 membranes and intrinsic components of synaptic membranes (**Supplementary Table 5**). The
196 20 genes most strongly associated with SNAP-a (**Extended Data Fig. 20**) included eight genes
197 with roles in adhesion of cells to synapses (*NRXN1*, *NTM*, *CTNND2*, *LSAMP*, *GPM6A*,
198 *LRRC4C*, *LRRTM4*, and *EPHB1*) (reviewed in ^{11,12}). SNAP-a also appeared to strongly recruit
199 genes encoding synaptic neurotransmitter reuptake transporters: *SLC1A2* and *SLC1A3*
200 (encoding glutamate transporters EAAT1 and EAAT2), and *SLC6A1* and *SLC6A11* (encoding
201 GABA transporters GAT1 and GAT3) were all among the 1% of genes most strongly associated
202 with SNAP-a.

203

204 We sought to relate SNAP-a to an emerging appreciation of astrocyte heterogeneity and its
205 basis in gene expression ¹³. An earlier analysis of astrocyte molecular and morphological
206 diversity in mice identified gene-expression modules based on their co-expression relationships
207 ¹⁴. SNAP-a exhibited the strongest overlap ($p = 3.5 \times 10^{-4}$, $q = 0.015$ by GSEA) (**Supplementary**
208 **Table 5**) with the module that had correlated most closely with the size of the territory covered
209 by astrocyte processes (the “turquoise” module in ¹⁴, with overlap driven by genes including
210 *EZR* and *NTM*). A potential interpretation is that SNAP-a supports these perisynaptic astrocytic
211 processes (PAPs ¹⁵).

212

213 Earlier work has identified “reactive” astrocyte states induced by strong experimental
214 perturbations and injuries, and described as polarized cell states ¹⁶. We found that more than
215 half of the human orthologs of markers for these states were expressed at levels that correlated
216 negatively and in a continuous, graded manner with SNAP-a expression (**Extended Data Fig.**
217 **21**). At the single-astrocyte level, SNAP-a expression exhibited continuous, quantitative
218 variation rather than discrete state shifts (**Extended Data Fig. 18f-g**), consistent with
219 observations of abundant astrocyte biological variation less extreme than experimentally
220 polarized states ¹⁷.

221

222 We performed an analogous cNMF analysis on the RNA-expression profiles of 75,929
223 glutamatergic neurons, focusing on a single, abundant subtype so that the variation among
224 individual cells would be driven primarily by dynamic cellular programs rather than by subtype
225 identity (**Fig. 3f**). One factor corresponded to the neuronal gene-expression effects of SNAP; we
226 refer to this factor as SNAP-n (**Fig. 3g-j and Supplementary Table 7**). Like SNAP-a, average
227 expression of SNAP-n was associated with age and with schizophrenia (**Fig. 3i-j**). SNAP-n and
228 SNAP-a associated with each other still more strongly, even in a controls-only, age-adjusted
229 analysis, highlighting the close coupling of neuronal and astrocyte gene expression (**Extended**
230 **Data Fig. 22**). Although SNAP-n associated with synaptic gene-sets, the specific genes driving

231 these enrichments were distinct from those driving SNAP-a (**Fig. 3k, Extended Data Fig. 23, and Supplementary Table 8**).

233

234 Expression of SNAP-a and SNAP-n associated with the expression of many transcription factors
235 and their predicted targets, and engaged distinct pathways in astrocytes and neurons (**Fig. 3k and Extended Data Figs. 22c and 24b**): for example, SREBP1 and its well-known
236 transcriptional targets ¹⁸ in astrocytes, and JUNB (AP-1) and its well-known targets ^{19,20} in
237 neurons (**Extended Data Fig. 25**). (The latter may reflect average neuronal activity levels in the
238 PFC, which neuroimaging has found to decline (“hypofrontality”) in schizophrenia ²¹.) SNAP-a
239 expression in astrocytes also associated with a RORB regulon (under-expressed in SNAP-low
240 donors) and a KLF6 regulon (over-expressed) (**Fig. 3k and Extended Data Fig. 24b**); common
241 genetic variation at *RORB* and *KLF6* associates with schizophrenia ²².

242

243

244 **Schizophrenia genetics and SNAP**

245

246 A key question when studying disease through human post-mortem tissue is whether
247 observations involve disease-causing/disease-exacerbating processes, or reactions to disease
248 circumstances such as medications. We found no relationship between SNAP expression and
249 donors' use of antipsychotic medications (**Extended Data Fig. 9j-k**), or between cholesterol-
250 biosynthesis gene expression in astrocytes and donors' statin intake (**Extended Data Fig. 14b**),
251 but this does not exclude the possibility that astrocytes are primarily reacting to disease-
252 associated synaptic hypofunction in neurons, as opposed to contributing to such hypofunction.
253 Human genetic data provide more-powerful evidence, since inherited alleles affect risk or
254 exacerbate disease processes rather than being caused by disease. We thus sought to
255 evaluate the extent to which SNAP-a and SNAP-n involved genes and alleles implicated by
256 genetic studies of schizophrenia.

257

258 Earlier work ²²⁻²⁴ has found that genes expressed most strongly by neurons (relative to other cell
259 types), but not genes expressed most strongly by glia, are enriched for the genes implicated by
260 genetic analyses in schizophrenia ²²⁻²⁴; we replicated these findings in our data (**Fig. 4a and**
261 **Supplementary Note**). However, such analyses treat cell types as fixed levels of gene
262 expression (“cell identities”), rather than as collections of dynamic transcriptional activities;
263 SNAP-a involves a great many genes that are also strongly expressed in other cell types.

264

265 We found that the genes dynamically recruited by SNAP-a in astrocytes were 14 times more
266 likely than other protein-coding genes to reside at genomic loci implicated by common genetic
267 variation in schizophrenia ($p = 5 \times 10^{-25}$, 95% confidence interval (CI): 8.7-24, by logistic
268 regression) and 7 times more likely to have strong evidence from rare variants in schizophrenia
269 (95% CI: 2.3-21, $p = 5 \times 10^{-4}$, by logistic regression) (**Supplementary Note**).

270

271 To evaluate whether common variation in the genes recruited by SNAP-a contributes more
272 broadly to schizophrenia risk, beyond these strongest associations, we used gene-level
273 association statistics from the largest schizophrenia genome-wide association study to date ^{22,25}.

275 As expected, the strongest neuron-identity genes (as defined in the earlier work) exhibited
276 elevated schizophrenia association, while the strongest astrocyte-identity genes did not (**Fig. 4a**
277 and **Supplementary Note**). In the same analysis, however, the genes most strongly associated
278 with SNAP-a and SNAP-n were highly significant as additional predictive factors, particularly the
279 genes associated with SNAP-a (**Fig. 4a**). Analysis by linkage disequilibrium (LD) score
280 regression²⁶ also confirmed enrichment of schizophrenia risk factors among SNAP-a genes
281 (**Extended Data Fig. 26**).

282
283 Polygenic risk involves thousands of common alleles across the genome, whose effects
284 converge upon unknown biological processes. A polygenic risk score (PRS) for schizophrenia
285 associated with reduced expression of SNAP but not with the other latent factors (**Fig. 4b** and
286 **Extended Data Fig. 27**). Higher polygenic risk also associated with deeper decline in SNAP
287 among persons with schizophrenia (**Fig. 4b**).
288

289 To better understand such relationships, we explored the relationship of SNAP-a to genetic risk
290 through two specific genes: Neurexin-1 (*NRXN1*) and complement component 4 (*C4*).
291 Exonic deletions within *NRXN1* greatly increase risk for schizophrenia^{27,28}. Our data indicated
292 that astrocytic, but not neuronal, *NRXN1* expression was reduced in persons with schizophrenia
293 and among persons over 70 years of age (**Fig. 4c** and **Extended Data Fig. 28a-b**). Inter-
294 individual variation in astrocytic *NRXN1* expression strongly associated with SNAP-a (**Fig. 4d**).
295

296 Increased copy number of the complement component 4 (*C4A*) gene more-modestly increases
297 risk for schizophrenia²⁹; far more inter-individual variation in *C4* gene expression (>80%) arises
298 from unknown, dynamic effects on *C4* expression^{29,30}. We found that astrocytes, rather than
299 neurons or microglia, are the main site of *C4* (including *C4A* and *C4B*) RNA expression in
300 human prefrontal cortex (**Fig. 4e** and **Extended Data Fig. 28c**). Donors with lower-than-
301 average expression of SNAP-a tended to have greatly increased *C4* expression: such donors
302 included 43 of the 44 donors with highest *C4* expression levels, and their astrocytes expressed
303 3.2-fold more *C4* than astrocytes in donors with above-average expression of SNAP-a did (**Fig.**
304 **4f**). *C4* expression was also greatly increased among donors over 70 years of age (**Extended**
305 **Data Fig. 28d-e**).

306 **DISCUSSION**

307

308 Here we discovered SNAP (Synaptic Neuron-Astrocyte Program), concerted gene-expression
309 programs implemented by cortical neurons and astrocytes to corresponding degrees in the
310 same individuals. SNAP expression varied even among neurotypical control brain donors and
311 may be a core axis of human neurobiological variation, with potential implications for cognition
312 and plasticity that will be important to understand.

313

314 SNAP appears to involve many genes that contribute to synapses and to astrocyte-synapse
315 interactions (**Figs. 2 and 3k, Supplementary Table 9, and Extended Data Figs. 20 and 23**)
316^{31,32}. The genes associated with SNAP-a suggested a potential role in supporting perisynaptic
317 astrocyte processes, motile astrocyte projections whose morphological plasticity and
318 interactions with synapses can promote synaptic stability¹⁵. Diverse lines of work increasingly
319 reveal a key role for astrocytes in regulating the ability of synaptic networks to acquire and learn
320 new information, for example by lowering thresholds for activity and synaptic plasticity^{33,34}.

321

322 An intriguing aspect of SNAP involved the astrocytic regulation of genes with roles in fatty acid
323 and cholesterol biosynthesis and cholesterol export, which strongly correlated (across donors)
324 with expression of synaptic-component genes by neurons (**Fig. 2d-e**). Earlier research has
325 defined a potential rationale for this neuron-astrocyte coordination: synapses and dendritic
326 spines – synapse-containing morphological structures – require large amounts of cholesterol
327 that astrocytes supply³⁵. Declines in cholesterol biosynthesis have previously been noted in
328 mouse models of brain disorders^{36,37} that (like schizophrenia and aging) involve cognitive
329 losses, cortical thinning, and reduction in neuropil.

330

331 Schizophrenia and aging both brought substantial reductions in SNAP expression (**Fig. 1i-j**).
332 Neuropsychologic, neuroimaging, and neuronal microstructural studies have long noted similar
333 changes in schizophrenia and aging^{1,2,38-47}. Inherited genetic risk for schizophrenia associates
334 with decreased measures of cognition in older individuals^{48,49}, and schizophrenia greatly
335 increases risk of dementia later in life⁵⁰. Our results suggest that these relationships between
336 schizophrenia and aging arise from shared cellular and molecular changes.

337

338 Under-expression of SNAP could in principle underlie longstanding microstructural observations
339⁴¹⁻⁴⁷ of reduced numbers of dendritic spines – synapse-containing morphological structures – on
340 cortical neurons in aged humans and primates and in persons with schizophrenia. These
341 microstructural observations appear to arise from highly plastic thin spines and thus may reflect
342 reduced rates of continuous synapse formation and stabilization (rather than pruning of mature
343 synapses)⁴²⁻⁴⁷. The gene-expression changes we observed in human dlPFC (**Fig. 2c**) suggest
344 that cortical neurons of all types, including glutamatergic and GABAergic neurons, may be
345 affected by such changes.

346

347 It is intriguing to consider whether pharmacotherapies or other interventions could be developed
348 to promote SNAP as a way to address cognitive symptom domains in schizophrenia and aging

349 such as cognitive flexibility, working memory, and executive function deficits, continuous and
350 disabling features which are typically not improved by available treatments ¹.

351

352 An important future direction will be to determine the extent to which SNAP is present in other
353 brain areas, and the relationship of SNAP to molecular and physiological changes in dendrites,
354 synapses, and perisynaptic astrocyte processes. Additional questions involve the molecular
355 mechanisms that accomplish neuron-astrocyte coordination and the extent to which SNAP
356 supports learning and/or cognitive flexibility.

357

358 SNAP was made visible by human inter-individual biological variation. Though controlled
359 laboratory experiments usually try to eliminate genetic and environmental variation, natural
360 variation may be able to reveal cell-cell coordination and regulatory programs in many tissues
361 and biological contexts, offering new ways to identify pathophysiological processes within and
362 beyond the human brain.

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473 **FIGURES**

474

475 **Figure 1. Identification of concerted multi-cellular gene-expression changes common to**
476 **schizophrenia and aging.**

477

478 **a**, Generation of snRNA-seq data, in a series of 20-donor “villages”.

479

480 **b**, Uniform manifold approximation and projection (UMAP, colored by donor) of the RNA-
481 expression profiles of the 1,218,284 nuclei analyzed from 191 donors.

482

483 **c**, Assignments of nuclei to cell types (same projection as in **b**).

484

485 **d-e**, Assignments of nuclei to **(d)** glutamatergic ($n = 524,186$) and **(e)** GABAergic ($n = 238,311$)
486 neuron subtypes.

487

488 **f**, Latent factor analysis. Cell-type-resolution expression data from all donors and cell types
489 were combined into a single analysis. Latent factor analysis identified constellations of gene-
490 expression changes that consistently appeared together.

491

492 **g**, Cell type-specificity of the latent factors inferred from 180 donors, shown as cell-type
493 distributions of the 1,000 most strongly loading gene/cell-type combinations per factor. Factors
494 4-7 and 10 are strongly driven by gene-expression co-variation spanning multiple cell types.

495

496 **h**, Association of schizophrenia with inter-individual variation in the expression levels of the ten
497 latent-factors in Fig. 1g, shown as a quantile-quantile plot comparing the ten factors' observed
498 schizophrenia associations ($-\log_{10}$ p-values) to the distribution of association statistics expected
499 by chance; only LF4 significantly associates with schizophrenia. See also Extended Data Fig.
500 10.

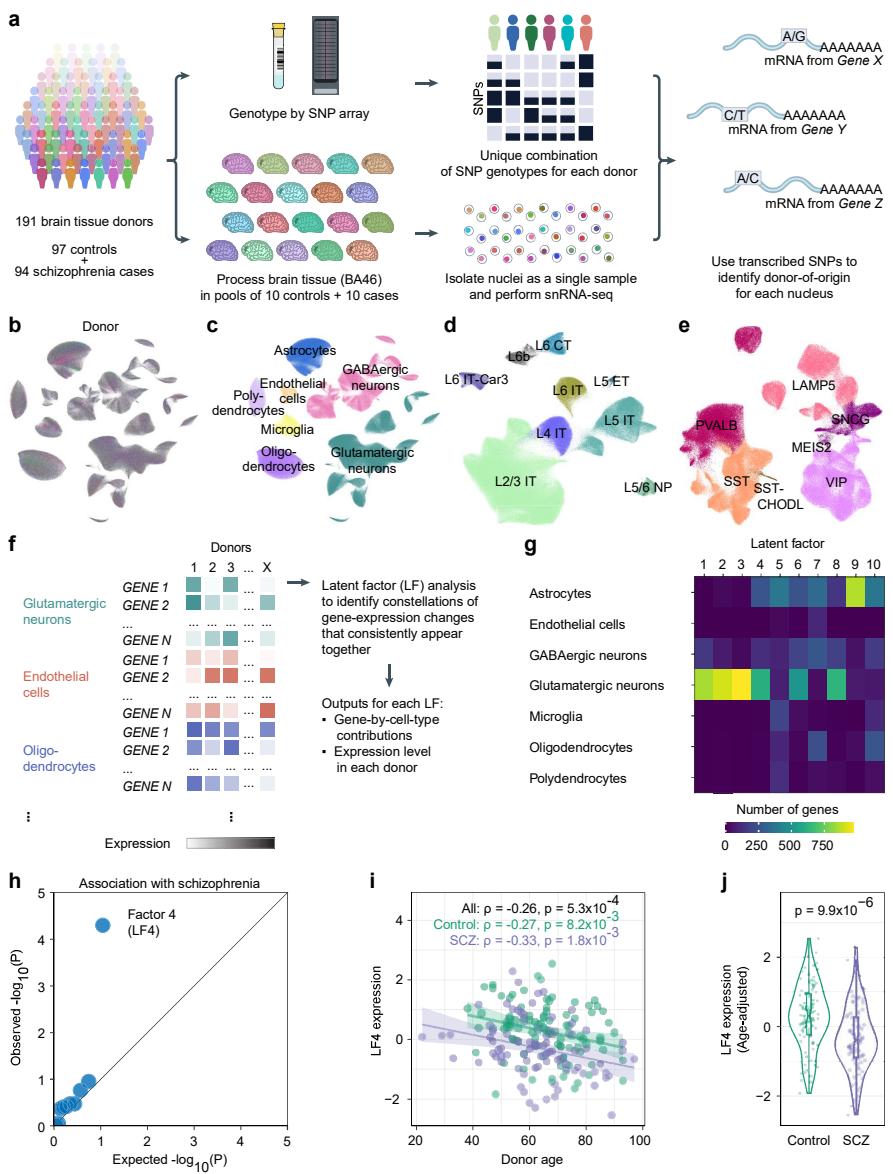
501

502 **i**, Relationship of quantile-normalized Latent Factor 4 (LF4) donor expression levels to age
503 (Spearman's ρ ; $n = 180$ donors). Shaded regions represent 95% confidence intervals.

504

505 **j**, Quantile-normalized LF4 donor scores ($n = 93$ controls, 87 cases), adjusted for age. P-value
506 is from a two-sided Wilcoxon rank-sum test. In the violin plot, boxes show interquartile ranges;
507 whiskers, 1.5x the interquartile interval; central lines, medians; notches, confidence intervals
508 around medians.

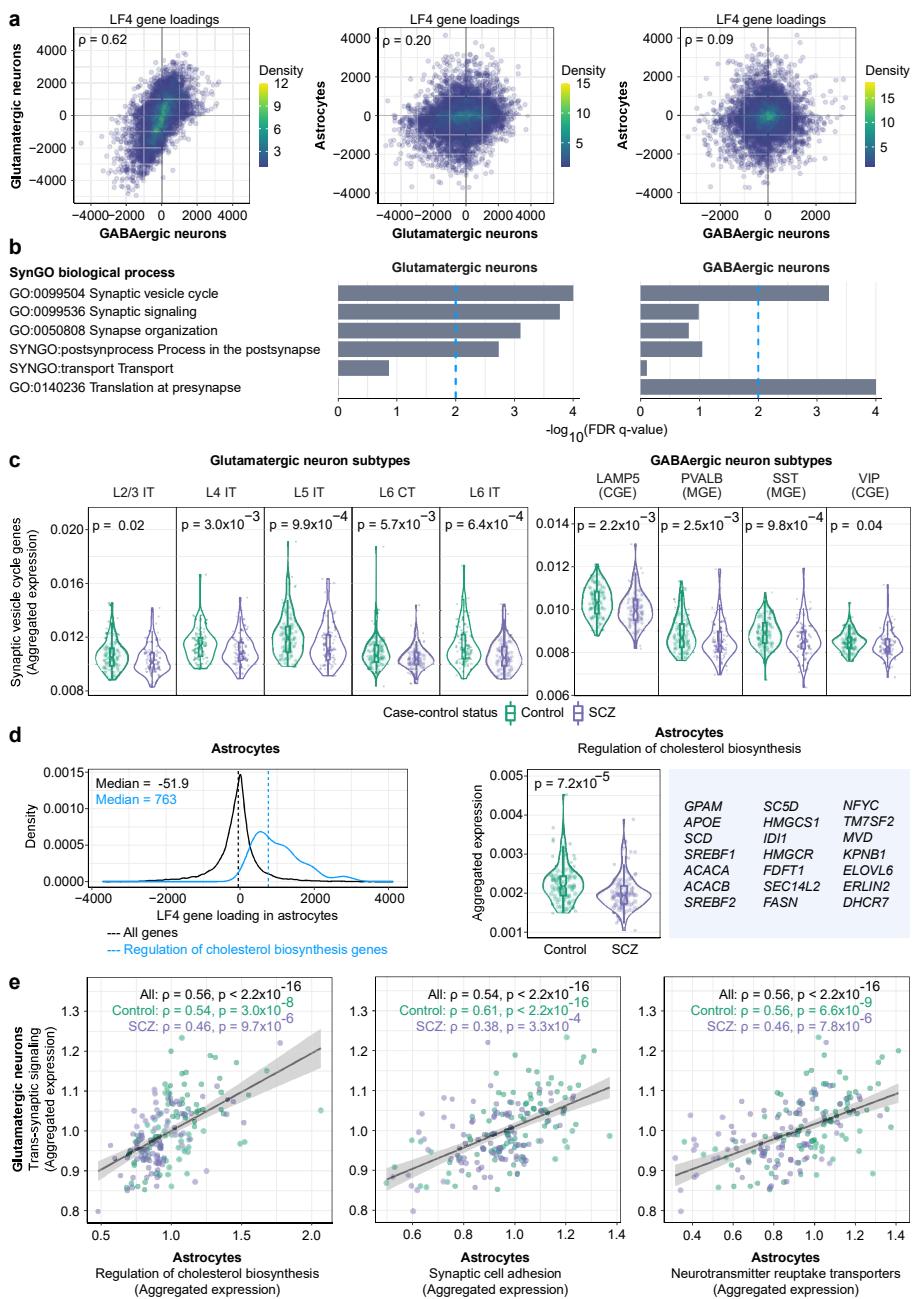
Figure 1



509 **Figure 2. A Synaptic Neuron-Astrocyte Program (SNAP) revealed by Latent Factor 4**
510 **(LF4).**

511
512 **a**, Comparisons of SNAP gene recruitment between cell types. Shown, in each pairwise cell-
513 type comparison, are the latent-factor (LF4) gene loadings of all genes expressed (≥ 1 UMI per
514 10^5) in both cell types in the comparison (Spearman's ρ ; $n = 10,346, 11,232, 11,217$ genes
515 respectively).
516
517 **b**, Concentrations of synaptic gene sets (as annotated by SynGO) in LF4's neuronal
518 components.
519
520 **c**, Fraction of gene expression (UMIs) devoted to synaptic vesicle-cycle genes in subtypes of
521 glutamatergic and GABAergic neurons, across 180 donors. P-values for case-control
522 comparisons are from a two-sided Wilcoxon rank-sum test. Box plots show interquartile ranges;
523 whiskers, 1.5x the interquartile interval; central lines, medians; notches, confidence intervals
524 around medians.
525
526 **d**, Left, distributions of astrocytes' LF4 gene loadings for (black) all expressed genes ($n =$
527 18,347) and (blue) genes annotated for functions in cholesterol biosynthesis ($n = 21$) (hereafter
528 referred to as "cholesterol biosynthesis" genes according to their GO annotation, though
529 subsets contribute to cholesterol export and/or to synthesis of additional fatty acids). Right,
530 proportion of astrocytic gene expression devoted to the annotated cholesterol biosynthesis
531 genes shown, across 180 donors. P-value is from a two-sided Wilcoxon rank-sum test. Box
532 plots show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians;
533 notches, confidence intervals around medians.
534
535 **e**, Concerted gene-expression variation in neurons and astrocytes. Relationships (across 180
536 donors) of astrocytic gene expression related to three biological activities (synapse adhesion,
537 neurotransmitter uptake, cholesterol biosynthesis) to neuronal gene expression related to
538 synapses (Spearman's ρ). Quantities plotted are the fraction of all detected nuclear mRNA
539 transcripts (UMIs) derived from these genes in each donor's astrocytes (x-axis) or neurons (y-
540 axis), relative to the median expression among control donors. Shaded regions represent 95%
541 confidence intervals for the estimated slopes.

Figure 2



542 **Figure 3. Biological states and transcriptional programs of astrocytes and L5 IT**
543 **glutamatergic neurons in schizophrenia.**

544

545 **a-c**, UMAP of RNA-expression patterns from 179,764 astrocyte nuclei from 180 donors. Nuclei
546 are colored by **(a)** astrocyte subtype, **(b)** schizophrenia affected/unaffected status, and **(c)**
547 expression of the astrocyte component of SNAP (referred to as SNAP-a).

548

549 **d**, Relationship of donors' quantile-normalized SNAP-a expression scores to age (Spearman's
550 ρ ; $n = 180$ donors). Shaded regions represent 95% confidence intervals.

551

552 **e**, Distributions of SNAP-a donor scores (age-adjusted and quantile-normalized) for persons
553 with and without schizophrenia ($n = 93$ controls, 87 cases). P-value is from a two-sided
554 Wilcoxon rank-sum test. Box plots show interquartile ranges; whiskers, 1.5x the interquartile
555 interval; central lines, medians; notches, confidence intervals around medians.

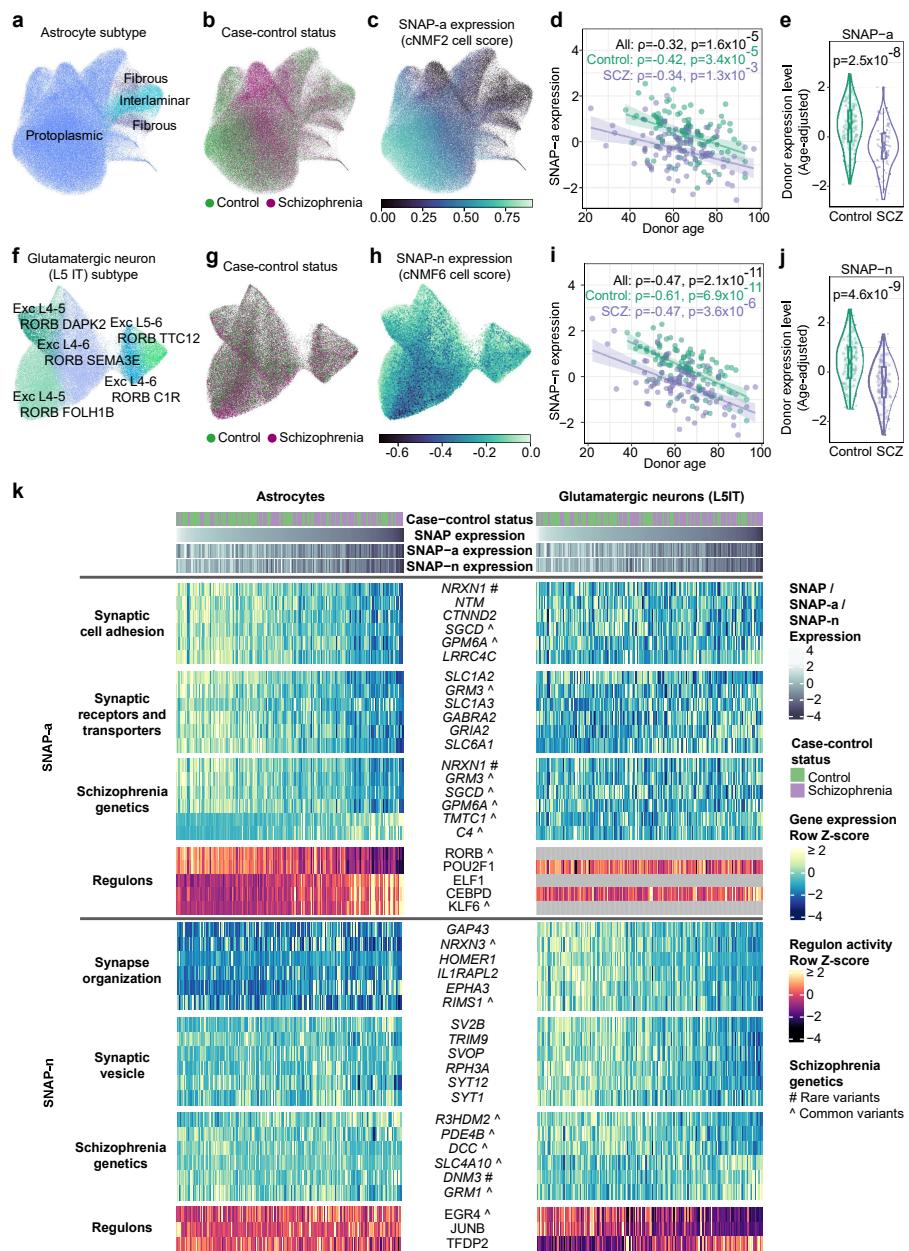
556

557 **f-j**, Similar plots as in **a-e** for the L5 IT glutamatergic neuron contribution to SNAP (referred to as
558 SNAP-n; $n = 75,929$ nuclei).

559

560 **k**, Heatmap showing variation in expression levels of a select set of strongly SNAP-recruited
561 genes across the astrocytes (left) and glutamatergic neurons (right) of 180 brain donors, who
562 are ordered from left to right by SNAP expression levels, in both the left and right panels. One
563 set of genes (SNAP-a, above) exhibits co-regulation in astrocytes; a distinct set of genes
564 (SNAP-n, below) exhibits co-regulation in neurons. Genes annotated by ^ are at genomic loci
565 implicated by common genetic variation in schizophrenia ²². Gray bars indicate that regulon
566 activity was not detected.

Figure 3



567 **Figure 4. Relationship of SNAP to schizophrenia genetics.**

568

569 **a**, Enrichment of schizophrenia genetic association (from common variants, using MAGMA to
570 generate a schizophrenia association Z-score for each gene) in the 2,000 genes most
571 preferentially expressed in glutamatergic neurons and astrocytes, or the 2,000 genes whose
572 expression is most strongly recruited by SNAP-n and SNAP-a. Values plotted are $-\log_{10}$ p-
573 values from a joint regression analysis in which each gene set is an independent and competing
574 predictive factor. See also **Supplementary Note**.

575

576 **b**, Relationship of donors' SNAP expression (quantile-normalized) to donors' schizophrenia
577 polygenic risk scores (Spearman's ρ ; $n = 180$ donors; PGC3 GWAS from ²²). Shaded regions
578 represent 95% confidence intervals.

579

580 **c**, *NRXN1* expression (per 10^5 detected nuclear transcripts) in each cell type in individual
581 donors ($n = 93$ controls, 87 cases). P-values are from a two-sided Wilcoxon rank-sum test. Box
582 plots show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians;
583 notches, confidence intervals around medians.

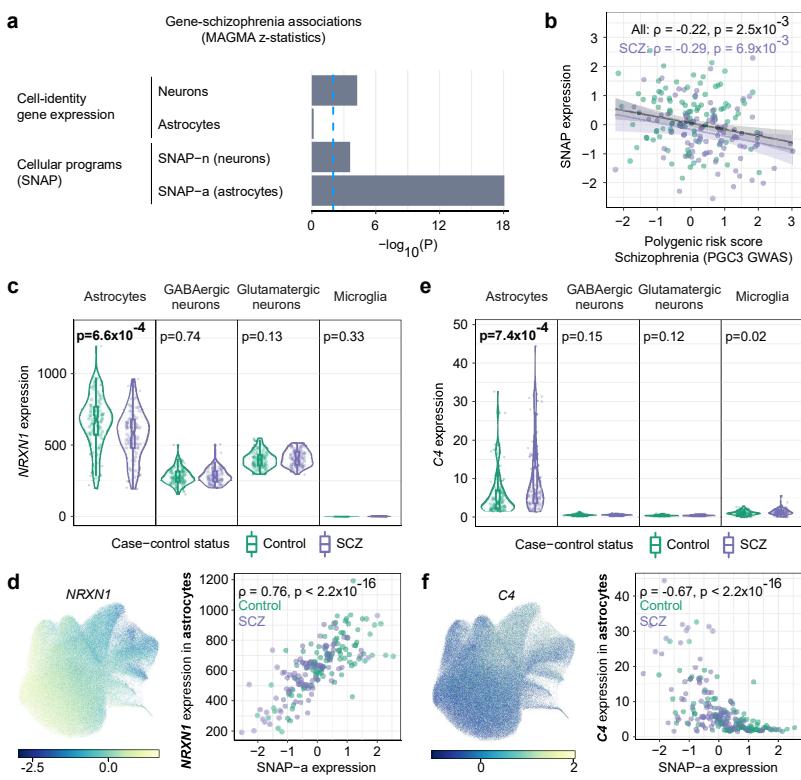
584

585 **d**, Left, *NRXN1* expression in individual astrocytes (using the same projection as in **Fig. 3a-c**).
586 Values represent Pearson residuals from variance stabilizing transformation. Right, relationship
587 of the 180 donors' *NRXN1* expression in astrocytes to SNAP-a expression (Spearman's ρ).

588 **e-f**, Similar plots as in c-d, here for complement component 4 (C4).

589

Figure 4



590 **METHODS**

591

592 **Ethical compliance**

593

594 Brain donors were recruited by the Harvard Brain Tissue Resource Center/NIH NeuroBioBank
595 (HBTRC/NBB), in a community-based manner, across the USA. Human brain tissue was
596 obtained from the HBTRC/NBB. The HBTRC procedures for informed consent by the donor's
597 legal next-of-kin and distribution of de-identified post-mortem tissue samples and demographic
598 and clinical data for research purposes are approved by the Mass General Brigham Institutional
599 Review Board. Post-mortem tissue collection followed the provisions of the United States
600 Uniform Anatomical Gift Act of 2006 described in the California Health and Safety Code section
601 7150 and other applicable state and federal laws and regulations. Federal regulation 45 CFR 46
602 and associated guidance indicates that the generation of data from de-identified post-mortem
603 specimens does not constitute human participant research that requires institutional review
604 board review.

605

606

607 **Donors for single nucleus RNA-seq**

608

609 Donor information with anonymized donor IDs is available in **Supplementary Table 1**.
610 Consensus diagnosis of schizophrenia was carried out by retrospective review of medical
611 records and extensive questionnaires concerning social and medical history provided by family
612 members. Several regions from each brain were examined by a neuropathologist. We excluded
613 subjects with evidence for gross and/or macroscopic brain changes, or with clinical history
614 consistent with cerebrovascular accident or other neurological disorders. Subjects with Braak
615 stages III or higher (modified Bielchowsky stain) were excluded. None of the subjects had
616 significant reported history of substance dependence within 10 or more years from death, as
617 further corroborated by negative toxicology reports. Absence of recent substance abuse is
618 typical for samples from the HBTRC, which receives exclusively community-based tissue
619 donations.

620

621 Exposure to psychotropic and neurotropic medications was assessed on the basis of medical
622 records. Estimated daily milligram doses of antipsychotic drugs were converted to the
623 approximate equivalent of chlorpromazine as a standard comparator ⁵¹. These values are
624 reported as lifetime, as well as last six months' of life, grams per patient. Exposure to other
625 classes of psychotropic drugs was reported as present or absent.

626

627

628 **Single-nucleus library preparation and sequencing**

629

630 We analyzed the dlPFC (Brodmann area 46 (BA46)), which exhibits functional and
631 microstructural abnormalities in schizophrenia ^{52,53} and in aging ⁴⁶. Frozen tissue blocks
632 containing BA46 were obtained from the HBTRC. We used single-nucleus rather than single-cell
633 RNA-seq to avoid effects of cell morphology upon ascertainment, and because nuclear (but not

634 plasma) membranes (but not plasma membranes) remain intact in frozen post-mortem tissue.
635 Nuclear suspensions from frozen tissue were generated following the protocol we have made
636 available at dx.doi.org/10.17504/protocols.io.4r3l22e3xl1y/v1. To ensure that batch compositions
637 were balanced, researchers were not blinded to the batch allocation or processing order of each
638 specimen. To maximize the technical uniformity of the snRNA-seq data, we processed sets of
639 20 brain specimens (each consisting of affected and control donors) at once as a single pooled
640 sample. Specimens were allocated into batches of 20 specimens per batch, ensuring that the
641 same number of cases and age-matched controls (10 per group), and men and women (10 per
642 group) were included in each batch. Some donors were re-sampled across multiple batches to
643 enable quality control analyses (**Extended Data Fig. 2**). Specimens from cases and age-
644 matched controls were also processed in alternating order within each batch. Researchers had
645 access to unique numerical codes assigned to the donor-of-origin of each specimen as well as
646 basic donor metadata (e.g. case-control status, age, sex).

647

648 Some 50 mg of tissue was dissected from the dIPFC of each donor – sampling across the
649 cortical layers and avoiding visible concentrations of white matter – and used to extract nuclei
650 for analysis. GEM generation and library preparation followed the 10X Chromium Single Nuclei
651 3' v3.1 protocol (version #CG000204_ChromiumNextGEMSsingleCell3'v3.1_Rev D). We
652 encapsulated nuclei into droplets using approximately 16,500 nuclei per reaction, understanding
653 that about 95% of all doublets (cases in which two nuclei were encapsulated in the same
654 droplet) would consist of nuclei from distinct donors and thus be recognized by the Dropulation
655 analysis⁷ as containing combinations of SNP alleles from distinct donors. cDNA amplification
656 was performed using 13 PCR cycles.

657

658 Raw sequencing reads were aligned to the hg38 reference genome with the standard Drop-seq
659 (v2.4.1)⁵⁴ workflow, modified so that reads from C4 transcripts would not be discarded as multi-
660 mapping (see Methods below, **C4: MetaGene discovery**). Reads were assigned to annotated
661 genes if they mapped to exons or introns of those genes. Ambient / background RNA were
662 removed from digital gene expression (DGE) matrices with CellBender (v0.1.0)⁵⁵ remove-
663 background.

664

665

666 Genotyping and donor assignment from snRNA-seq data

667

668 We used combinations of hundreds of transcribed SNPs to assign each nucleus to its donor-of-
669 origin, using the Dropulation software (v2.4.1)⁷. Previous Dropulation analyses of stem cell
670 experiments have used whole-genome sequence (WGS) data on the individual donors for such
671 analyses⁷. For the current work, we developed a cost-efficient approach based on SNP array
672 data with imputation. Genomic DNA from the individual brain donors was genotyped by SNP
673 array (Illumina GSA).

674

675 Raw Illumina IDAT files from the GSAMD-24v1-0_20011747 array (2,085 samples) and
676 GSAMD-24v3-0-EA_20034606 array (456 samples) were genotyped using GenCall (v3.0.0)⁵⁶
677 and genotypes were phased using SHAPEIT4 (v4.2.2)⁵⁷ by processing the data through the

678 MoChA workflow (v2022-12-21)^{58,59} (<https://github.com/freeseek/mochawdl>) using default
679 settings and aligning markers against GRCh38. APOE genotypes for marker rs429358 were
680 removed due to unreliable genotypes. To improve phasing, genotypes from the McLean cohort
681 were combined with genotypes from the Genomic Psychiatry Cohort with IDAT files available
682 also from the GSAMD-24v1-0_20011747 array (5,689 samples)⁶⁰. After removing 128 samples
683 recognized as duplicates, phased genotypes were then imputed using IMPUTE5 (v1.1.5)⁶¹ by
684 processing the output data from the MoChA workflow using the MoChA imputation workflow and
685 using the high coverage 1000 Genomes reference panel for GRCh38⁶² including 73,452,470
686 non-singleton variants across all the autosomes and chromosome X. Only SNPs with imputation
687 quality INFO > 0.95 were used for donor assignments. Using this approach, we found that
688 99.6% of nuclei could be assigned confidently to a donor (**Extended Data Fig. 2b**).
689

690 To evaluate the accuracy of this method of donor assignment, we genotyped a pilot cohort of 11
691 donors by both whole-genome sequencing (WGS) and by SNP array. Importantly, the two
692 methods had 100% concordance on the assignment of individual nuclei to donors, validating
693 both our computational donor-assignment method and the sufficiency of the SNPs-plus-
694 imputation approach (**Extended Data Fig. 2a**). SNP data for the individual donors are available
695 in NeMO (accession number nemo:dat-bmx7s1t).
696

697 Following donor assignment, DGE matrices from all libraries in each batch (7 to 8 libraries per
698 batch) were merged for downstream analyses.
699

700 701 **Cell-type assignments**

702 All classification models for cell assignments were trained using scPred (v1.9.2)⁶³. DGE
703 matrices were processed using the following R and python packages: Seurat (v3.2.2)⁶⁴,
704 SeuratDisk (v0.0.0.9010)⁶⁵, anndata (v0.8.0)⁶⁶, numpy (v1.17.5)⁶⁷, pandas (v1.0.5)^{68,69}, and
705 Scanpy (v1.9.1)⁷⁰.
706

707 708 Cell types

710 *Model training*

711 The classification model used for cell-type assignments was trained on the DGE matrix from
712 batch 6 (BA46_2019-10-16), which was annotated as follows. Nuclei with fewer than 400
713 detected genes and 100 detected transcripts were removed from the DGE matrix from this
714 batch. After normalization and variable gene selection, the DGE matrix was processed through
715 an initial clustering analysis using independent component analysis (ICA, using fastICA (v1.2-1)
716⁷¹) as previously described⁷². This analysis produced clustering solutions with 43 clusters of
717 seven major cell types (astrocytes, endothelial cells, GABAergic neurons, glutamatergic
718 neurons, microglia, oligodendrocytes, polydendrocytes) that could be identified based on
719 expression of canonical marker genes (markers in **Extended Data Fig. 3**). (We note that ~9%
720 of cells within clusters annotated as endothelial cells do not express canonical endothelial cell
721

722 markers, but rather those of pericytes; these ~1,400 cells have been grouped together with
723 endothelial cells for downstream analyses.) scPred was trained on this annotated DGE matrix,
724 and the resulting model was subsequently used to make cell-type assignments for the
725 remaining batches' DGE matrices.

726

727 *Filtering*

728

729 Following an initial cell-type classification using the above model, the DGE matrices were
730 filtered further to remove any remaining heterotypic doublets missed by scPred. First, raw DGE
731 matrices from each of the 11 batches were subsetted to form separate DGE matrices for each
732 of the 7 major cell types (77 subsetted DGE matrices total). Each subsetted DGE matrix was
733 normalized using sctransform (v0.3.1) ⁶⁴ with 7,000 variable features, scaling, and centering. For
734 each cell type, normalized DGE matrices from the 11 batches were merged and clustered
735 together in Scanpy (v1.9.1) ⁷⁰ using 50 PCs, batch correction by donor using BBKNN (v1.5.1) ⁷³,
736 and Leiden clustering using a range of resolutions. The most stable clustering resolution for
737 each cell type was selected using clustree (v0.4.4) ⁷⁴. Clusters expressing markers of more than
738 one cell type were determined to be heterotypic doublets; cell barcodes in these clusters were
739 discarded from the above DGE matrices, and these filtered DGE matrices were then carried
740 forward for integrated analyses across batches.

741

742 Neuronal subtypes

743

744 Classification models for neuronal subtypes were trained using DGE matrices from ⁷⁵ that were
745 subsetted to glutamatergic or GABAergic neuron nuclei in middle temporal gyrus (MTG). While
746 a similar dataset exists for human brain nuclei from primary motor cortex (M1) ⁷⁶, we only
747 trained the model on the MTG dataset as M1 lacks a traditional layer 4 (L4), while BA46 does
748 have a L4.

749

750 The neuronal subtypes in this dataset include glutamatergic neuron subtypes of distinct cortical
751 layers and with predicted intratelencephalic (IT), extratelencephalic (ET), corticothalamic (CT),
752 and near-projecting (NP) projection patterns, as well as the four cardinal GABAergic neuron
753 subtypes arising from the caudal (CGE: *LAMP5+*, *VIP+*) and medial (MGE: *PVALB+*, *SST+*)
754 ganglionic eminences.

755

756 We made the following adjustments to the MTG annotations prior to model training. First, as
757 subtype-level annotations (e.g. L5 IT, as used in ⁷⁶ for M1) were not available for the MTG
758 dataset, we inferred these based on M1/MTG cluster correspondences (from Extended Data
759 Fig. 10 in ⁷⁶). Second, we reassigned the following glutamatergic neuron types in MTG from the
760 L4 IT subtype (as inferred by integration with M1 in ⁷⁶) to the L2/3 IT subtype: Exc L3–5 RORB
761 FILIP1L, Exc L3–5 RORB TWIST2, and Exc L3–5 RORB COL22A1. This was on the basis of
762 their properties described in other studies – for example, the Exc L3–5 RORB COL22A1 type
763 has been described as a deep L3 type by Patch-seq ⁷⁷ – and by the expression of their marker
764 genes on a two-dimensional projection of the RNA-expression profiles of glutamatergic neuron
765 nuclei (**Extended Data Fig. 4**).

766 Feature plots for neuronal subtypes (**Extended Data Fig. 4 and Extended Data Fig. 5**) were
767 generated using markers from the repository in <https://bioportal.bioontology.org/ontologies/PCL>
768 (v1.0, 2020-04-26)^{75,76,78}, specifically those for neuronal subtypes from MTG.

769

770 Astrocyte subtypes

771

772 Normalized, filtered DGE matrices from the 11 batches were merged and clustered together in
773 scanpy using 8 PCs, batch correction by donor using bbknn⁷³, and Leiden clustering using a
774 range of resolutions. The most stable resolution that created distinct clusters for putative
775 astrocyte subtypes (resolution 1.3) was selected using clustree⁷⁴. Feature plots for astrocyte
776 subtypes previously described in both MTG and M1^{75,76} (**Extended Data Fig. 17**) were
777 generated using markers from the repository in <https://bioportal.bioontology.org/ontologies/PCL>
778 (v1.0, 2020-04-26)^{75,76,78}. Leiden clusters were assigned to one of three astrocyte subtypes on
779 the basis of expression of these subtype markers.

780

781

782 **Donor exclusion**

783

784 Donors were excluded on the basis of unusual gene-expression profiles and/or cell-type
785 proportions (potentially related to agonal events) as outlined below.

786

787 Expression

788

789 Donors with fewer than 1,000 total UMIs in any cell type were first excluded. Next, for each cell
790 type, gene-by-donor expression matrices comprising the remaining donors were scaled to
791 100,000 UMIs per donor and filtered to the top expressing genes (defined as having at least 10
792 UMIs per 100,000 for at least one donor; these were among the top 12-19% of expressed
793 genes). These filtered expression matrices by cell type were merged into a single expression
794 matrix that was used to calculate each donor's pairwise similarity to the other donors (Pearson
795 correlations of log₁₀-scaled expression values across genes). The median of these pairwise
796 correlation values was determined to be the conformity score for each donor. To identify
797 outliers, these donor conformity scores were converted to modified Z-scores (M_i) for each donor
798 as described in⁷⁹:

799

800
$$M_i = 0.6745 * (x_i - \bar{x}) / MAD$$

801 where

802 x_i : The donor's conformity score

803 \bar{x} : The median of donor conformity scores

804 MAD: The median absolute deviation of donor conformity scores

805

806 Donors whose modified Z-scores had absolute values > 5 were excluded. This approach
807 flagged a total of 5 donors (1 who had low UMI counts and 4 who were outliers on the basis of
808 expression).

809 Cell-type proportions

810

811 Each donor's pairwise similarity to the other donors was determined on the basis of cell-type
812 proportions (i.e., the values plotted in **Extended Data Fig. 3c-d**). Donor conformity scores and
813 modified Z-scores based on these values were calculated for each donor using the same
814 approach described above for expression values. Donors whose modified Z-scores had
815 absolute values > 15 were excluded. This approach flagged a total of 9 donors, 2 of whom were
816 also flagged as expression outliers.

817

818 Between the two approaches, a total of 11 unique donors were flagged as outliers (4 control, 7
819 schizophrenia) and excluded from downstream analyses.

820

821

822 Latent factor analysis

823

824 snRNA-seq data

825

826 Our approach was to (i) create a gene-by-donor matrix of expression measurements for each of
827 seven cell types; (ii) concatenate these matrices into a larger matrix in which each gene is
828 represented multiple times (once per cell type); and (iii) perform latent factor analysis ^{8,80} on this
829 larger matrix. We selected probabilistic estimation of expression residuals (PEER) ⁸¹ over other
830 approaches (e.g. PCA) for inferring latent variables as it is more sensitive and less dependent
831 on the number of factors modeled. A major pitfall to avoid when performing latent factor analysis
832 is obtaining highly correlated factors due to overfitting. The latent factors we have inferred are
833 independent from each other when we compare their gene loadings (**Extended Data Fig. 8c**),
834 enabling us to proceed with downstream analyses based on these factors.

835

836 Raw, filtered DGE matrices from each of the 11 batches were subsetted to form separate DGE
837 matrices for each of the 7 major cell types (77 subsetted DGE matrices total). For each
838 subsetted DGE matrix, cell barcodes from outlier donors were excluded, the DGE matrix was
839 normalized using sctransform (v0.3.1) ⁶⁴ with 3,000 variable features, and the output of Pearson
840 residual expression values (with all input genes returned) was exported to a new DGE matrix.
841 For each cell type, these new expression values in the 11 normalized DGE matrices were
842 summarized across donors (taking the sum of residual expression values) to create a gene-by-
843 donor expression matrix. Each of these expression matrices was filtered to the top 50% of
844 expressed genes (based on feature counts scaled to 100,000 transcripts per donor), yielding
845 expression matrices with approximately 16,000 to 18,000 genes per cell type. Within each
846 expression matrix, each gene name was modified with a suffix to indicate the cell type of origin
847 (e.g. ACAP3 to ACAP3_astrocyte), and the 7 expression matrices were combined to produce a
848 single expression matrix with expression values from all 7 cell types for each donor (see **Fig. 1f**
849 for schematic). This expression matrix was used as the input to latent factor analysis with PEER
850 (v1.0) ⁸¹ using default parameters and a range of requested factors k .

851

852 Though we looked for correlations of these factors with technical variables, these analyses were
853 negative, with one exception: Latent Factor 2 (LF2) appeared to capture quantitative variation in
854 the relative representation of deep and superficial cortical layers in each dissection (**Extended**
855 **Data Fig. 8f**).

856

857 Latent factor donor expression values were adjusted for age by taking the residuals from a
858 regression of the donor expression values against age.

859

860 To improve the visualization of latent factor donor expression values while leaving the results of
861 statistical analyses unchanged, quantile-normalized values were calculated using the formula
862 $qnorm(rank(x) / (length(x)+1))$. Figure legends indicate when these quantile-normalized values
863 are used.

864

865 Proteomics data

866

867 Protein intensities from the *LRRK2* Cohort Consortium (LCC) cohort in ⁸² were downloaded from
868 the ProteomeXchange Consortium (dataset identifier PXD026491) and subset to those peptides
869 that passed the Q-value threshold in at least 25% of all analyzed samples. These were further
870 subset to intensities from control donors without the *LRRK2* G2019S mutation and without
871 erythrocyte contamination ($n = 22$ donors). After normalization of the protein intensities with
872 *sctransform* (v0.3.1) ⁶⁴, the output of Pearson residual expression values (with all input proteins
873 returned) was exported to a new matrix. This matrix of normalized protein intensities was used
874 as the input to latent factor analysis with *PEER* (v1.0) ⁸¹ using default parameters.

875

876 For comparisons of CSF protein loadings to SNAP gene loadings in **Extended Data Fig. 11**,
877 each gene in SNAP was represented by a single composite loading representing gene loadings
878 from all cell types. This composite loading was determined for each gene by first calculating the
879 median expression of each gene (in each cell type), then calculating a new loading onto SNAP
880 weighted across cell types by these median expression values.

881

882

883 **Rhythmicity analysis**

884

885 For **Extended Data Fig. 9f**, rhythmicity analyses were performed as in ⁸³ using scripts from
886 (<https://github.com/KellyCahill/Circadian-Analysis->) and donors' time of death in zeitgeber time
887 (ZT). Analyses also used the following packages: *lme4* (v1.1-31) ⁸⁴, *minpack.lm* (v1.2-4) ⁸⁵.

888

889

890 **Gene set enrichment analysis**

891

892 For gene set enrichment analysis (GSEA) ^{9,86} on latent factors inferred by *PEER*, the C5 Gene
893 Ontology collection (v7.2) ^{87,88} from the Molecular Signatures Database ^{89,90} was merged with
894 SynGO (release 20210225) ⁹¹'s biological process (BP) and cell component (CC) gene lists.
895 Gene sets from this merged database that were enriched in each latent factor were identified

896 with GSEAPreranked in GSEA (v4.0.3)^{9,86} using 10,000 permutations and gene loadings as the
897 ranking metric.

898

899 For astrocyte latent factors inferred by cNMF¹⁰, GSEA was performed as described above with
900 the addition of the following custom gene sets to the database:

901

- 902 PGC3_SCZ_GWAS_GENES_1TO2_AND_SCHEMA1_GENES: A gene set comprising
903 genes implicated in human-genetic studies of schizophrenia, including genes at 1-2 gene
904 loci from GWAS (PGC3,²² and genes with rare coding variants (FDR < 0.05 from²³).
- 905 Gene sets for each of the seven astrocyte subclusters identified in¹⁴.
- 906 Gene sets for each of the 62 “color” module eigengenes identified by WGCNA in¹⁴.
- 907 Gene sets for each of the six astrocyte subcompartments analyzed in⁹², comprising genes
908 encoding the proteins that were unique to or enriched in these subcompartments.

909

910 For L5 IT glutamatergic neuron latent factors inferred by cNMF, GSEA was performed as
911 described above with the addition of the following custom gene sets to the database:

- 912 PGC3_SCZ_GWAS_GENES_1TO2_AND_SCHEMA1_GENES: A gene set comprising
913 genes implicated in human-genetic studies of schizophrenia, including genes at 1-2 gene
914 loci from GWAS (PGC3,²² and genes with rare coding variants (FDR < 0.05 from²³).

915

916

917 Selected gene sets

918

919 Based on the results of the gene set enrichment analyses (GSEA) described above, we
920 selected several of the top-enriched gene sets for further analyses. These are referred to in the
921 figures with labels modified for brevity, but are described in further detail below. Lists of genes in
922 each gene set are in **Supplementary Table 9**.

923

- 924 “Integral component of postsynaptic density membrane” (**Extended Data Fig. 13, Extended**
925 Data Fig. 15, and Extended Data Fig. 16): core genes contributing to the enrichment of
926 GO:0099061 (v7.2, integral component of postsynaptic density membrane) in the
927 glutamatergic neuron component of LF4 (SNAP).
- 928 “Neurotransmitter reuptake transporters” (**Fig. 2e, Extended Data Fig. 13, Extended Data**
929 Fig. 15, and Extended Data Fig. 16): genes from among the 100 genes most strongly
930 recruited by cNMF2 (SNAP-a) with known functions as neurotransmitter reuptake
931 transporters. These include core genes contributing to the enrichment of GO:0140161 (v7.2,
932 monocarboxylate: sodium symporter activity) in SNAP-a.
- 933 “Presynapse” (**Extended Data Fig. 13, Extended Data Fig. 15, and Extended Data Fig.**
934 16): core genes contributing to the enrichment of GO:0098793 (v7.2, presynapse) in the
935 GABAergic neuron component of LF4 (SNAP).
- 936 “Regulation of cholesterol biosynthesis” (**Fig. 2d-e, Extended Data Fig. 13, Extended Data**
937 Fig. 14, Extended Data Fig. 15, Extended Data Fig. 16, and Extended Data Fig. 24d):
938 core genes contributing to the enrichment of GO:0045540 (v7.2, regulation of cholesterol
939 biosynthetic process) in the astrocyte component of LF4 (SNAP). This enrichment is of

940 interest as cholesterol is an astrocyte-supplied component of synaptic membranes ^{35,93,94}.
941 Products of this biosynthetic pathway also include other lipids and cholesterol metabolites
942 with roles at synapses, including 24S-hydroxycholesterol, a positive allosteric modulator of
943 NMDA receptors ⁹⁵. Although we refer to this gene set by this label based on its annotation
944 by GO, we note that subsets of these genes contribute to cholesterol export and/or to
945 synthesis of additional fatty acids.

- 946 • “Schizophrenia genetics” (**Fig. 3k and Extended Data Fig. 24a**): prioritized genes from ²³
947 (FDR < 0.05) or ²².
- 948 • “Synapse organization” (**Fig. 3k**): core genes contributing to the enrichment of GO:0050808
949 (v7.2, synapse organization) in cNMF6 (SNAP-n).
- 950 • “Synaptic cell adhesion” (**Fig. 2e, Fig. 3k, Extended Data Fig. 13, Extended Data Fig. 15,**
951 **Extended Data Fig. 16, and Extended Data Fig. 24a**): genes from among the 20 genes
952 most strongly recruited by cNMF2 (SNAP-a) with known functions in synaptic cell-adhesion.
953 This biological process was selected due to the enrichment of GO:0099560 (v7.2, synaptic
954 membrane adhesion) in SNAP-a.
- 955 • “Synaptic receptors and transporters” (**Fig. 3k, Extended Data Fig. 24a, and Extended**
956 **Data Fig. 24c**): genes from among the 100 genes most strongly recruited by cNMF2 (SNAP-
957 a) with known functions as synaptic receptors and transporters.
- 958 • “Synaptic vesicle” (**Fig. 3k**): core genes contributing to the enrichment of GO:0008024 (v7.2,
959 synaptic vesicle) in cNMF6 (SNAP-n).
- 960 • “Synaptic vesicle cycle” (**Fig. 2c and Extended Data Fig. 12**): core genes contributing to
961 the enrichment of GO:0099504 (v7.2, synaptic vesicle cycle) in the glutamatergic and
962 GABAergic neuron components of LF4 (SNAP).
- 963 • “Trans-synaptic signaling” (**Fig. 2e, Extended Data Fig. 13, and Extended Data Fig. 16**):
964 core genes contributing to the enrichment of GO:0099537 (v7.2, trans-synaptic signaling) in
965 the glutamatergic neuron component of LF4 (SNAP).

966
967 Gene sets displayed in **Fig. 2b** are the SynGO terms most strongly enriched in each top-level
968 category (among biological processes: process in the presynapse, synaptic signaling, synapse
969 organization, process in the postsynapse, transport, and metabolism respectively).
970
971

972 **Analysis of astrocyte and glutamatergic L5 IT neuron gene-expression programs**

973
974 Consensus non-negative matrix factorization
975
976 Consensus non-negative matrix factorization (cNMF) (v1.2) ¹⁰ was performed on both astrocyte
977 and glutamatergic L5 IT neurons. We used cNMF because of its scalability to the astrocyte and
978 glutamatergic L5 IT neuron data sets. The cNMF protocol detailed in their github tutorial for
979 PBMC cells
980 (https://github.com/dylkot/cNMF/blob/master/Tutorials/analyze_pbmc_example_data.ipynb) was
981 followed for the initial data filtering and analysis. For both data sets, data was filtered to remove
982 cells with fewer than 200 genes or 200 UMIs. Genes expressed in fewer than 10 cells were

983 removed. Factorization was run on raw counts data after filtering, with iterations of factorization
984 run for each k (factors requested), with a k ranging from 3 to 30.

985

986 The astrocyte raw counts data contained 179,764 cells and 42,651 genes, of which 0 cells and
987 9,040 genes were excluded. Based on PCA of the gene expression matrix and the cNMF
988 stability report, factorization with $k=11$ was selected for further analysis. The 11 cNMF factors
989 together explained 25% of variation in gene expression levels among single astrocytes.

990

991 The L5 IT raw counts data contained 75,929 cells and 42,651 genes, of which 0 cells and 8,178
992 genes were excluded. Based on the PCA of the gene expression matrix and the cNMF stability
993 report, factorization with $k=13$ was selected for further analysis. The 13 cNMF factors together
994 explained 44% of variation in gene expression levels among single L5 IT glutamatergic neurons.
995 To align the direction of interpretation across all 3 analyses (SNAP, SNAP-a, and SNAP-n), we
996 took the negative of cNMF Factor 6 (SNAP-n) cell scores, gene loadings, and donor scores.

997

998 The latent factor usage matrix (cell by factor) was normalized prior to analysis to scale each
999 cell's total usage across all factors to 1.

1000

1001 Co-varying neighborhood analysis

1002

1003 To further assess the robustness of the astrocyte gene-expression changes represented by
1004 SNAP and SNAP-a, we employed a third computational approach, co-varying neighborhood
1005 analysis (CNA) (v0.1.4) ⁹⁶. The protocol detailed in their github tutorial
1006 (<https://nbviewer.org/github/yakirr/cna/blob/master/demo/demo.ipynb>) was followed for data
1007 preprocessing and analysis.

1008

1009 Pilot association tests to find transcriptional neighborhoods associated with schizophrenia case-
1010 control status were first performed using the default value for Nnull. These pilot analyses
1011 evaluated the effects of batch correction (by batch or donor) and covariate correction (by age,
1012 sex, PMI, number of UMIs, or number of expressed genes). Nearly all analyses yielded highly
1013 similar neighborhoods associated with case-control status with the same global p-value
1014 ($p=1\times 10^{-4}$), with the exception of batch correction by donor which yielded $p=1$. The final
1015 association test described in **Extended Data Fig. 19** was performed with an increased value for
1016 Nnull (Nnull=1000000) and without additional batch or covariate correction.

1017

1018

1019 **Regulatory network inference**

1020

1021 The goal of pySCENIC ^{97,98} is to infer transcription factors and regulatory networks from single
1022 cell gene expression data. The pySCENIC (v0.11.2) protocol detailed in the github tutorial for
1023 PBMC cells (https://github.com/aertslab/SCENICProtocol/blob/master/notebooks/PBMC10k_SCENIC-protocol-CLI.ipynb) was followed for the initial data filtering and analysis.
1024 For both astrocytes and L5 IT glutamatergic neurons, data was filtered to remove cells with
1025

1026 fewer than 200 genes, and genes with fewer than 3 cells. Cells with high MT expression (>15%
1027 of their total transcripts) were removed.

1028

1029 The gene regulatory network discovery adjacency matrix was inferred by running Arboreto on
1030 the gene counts matrix and a list of all transcription factors provided by the authors
1031 (https://resources.aertslab.org/cistarget/tf_lists/allTFs_hg38.txt) to generate an initial set of
1032 regulons. This set was further refined using ctx, which removes targets that are not enriched for
1033 a motif in the transcription factor using a provided set of human specific motifs
1034 (<https://resources.aertslab.org/cistarget/motif2tf/motifs-v9-nr.hgnc-m0.001-o0.0.tbl>) and cis
1035 targets (https://resources.aertslab.org/cistarget/databases/homo_sapiens/hg38/refseq_r80/mc9nr/gene_based). Finally, auCell was run to generate the per-cell enrichment scores for each
1036 discovered transcription factor.

1037

1038

1039 **Super-enhancer analysis**

1040

1041 Preparation of input BAM files: FASTQ files of bulk H3K27ac ChIP-seq data from middle frontal
1042 gyrus ⁹⁹ were downloaded from GEO (accessions GSM4441830 and GSM4441833).
1043 Demultiplexed FASTQ files were trimmed with Trimmomatic (v0.33) ¹⁰⁰ using the parameter
1044 SLIDINGWINDOW:5:30. Trimmed reads were aligned to the hg38 reference genome with
1045 Bowtie2 (v2.2.4) ¹⁰¹ using default parameters. Uniquely mapped reads were extracted with
1046 samtools (v1.3.1) ¹⁰² view using the parameters -h -b -F 3844 -q 10.

1047

1048

1049 Preparation of input constituent enhancers: FitHiChIP interaction files for H3K27ac from middle
1050 frontal gyrus ⁹⁹ were downloaded from GEO (accessions GSM4441830 and GSM4441833).
1051 These were filtered to interacting bins (at interactions with q-value < 0.01) that overlap bulk
1052 H3K27ac peaks in the one-dimensional HiChIP data in both replicates. Next, these bins were
1053 intersected with IDR-filtered scATAC-seq peaks in isocortical and unclassified astrocytes (peaks
1054 from clusters 13, 15, 17, downloaded from GEO accession GSE147672 ⁹⁹). Unique coordinates
1055 of these filtered regions were converted to GFF files.

1056

1057 Super-enhancers (SEs) were called with ROSE (v1.3.1) ^{103,104} using the input files prepared
1058 above and the parameters -s 12500 -t 2500. Coordinates of promoter elements for *H. sapiens*
1059 (Dec 2013 GRCh38/hg38) were downloaded from the Eukaryotic Promoter Database (EPD) ¹⁰⁵
1060 using the “EPDnew selection tool” (https://epd.expasy.org/epd/EPDnew_select.php) ¹⁰⁶. Using
1061 these sets of coordinates, FitHiChIP loops that overlap bulk H3K27ac peaks and scATAC-peaks
1062 in astrocytes were subset to those that contained a promoter in one anchor and a SE in the
1063 other anchor. Binomial smooth plots were generated as in ¹⁰⁷.

1064

1065

1066 **Heritability analyses**

1067

1068 MAGMA

1069

1070 Summary statistics from ²² were uploaded to FUMA (v1.5.6) ¹⁰⁸ web server
1071 (<https://fuma.ctglab.nl>). Gene-level Z-scores were calculated using SNP2GENE with the
1072 “Perform MAGMA” function (MAGMA v1.08) and default parameter settings. The reference
1073 panel population was set to “1000G Phase3 EUR”. The MHC region was excluded due to its
1074 unusual genetic architecture and linkage disequilibrium. MAGMA Z-scores were then used for
1075 downstream analyses as described in the **Supplementary Note**.

1076

1077 Stratified LD score regression

1078

1079 To partition SNP-heritability, we used Stratified LD score regression (S-LDSC) (v1.0.1) ²⁶, which
1080 assesses the contribution of gene expression programs to disease heritability. First, for analysis
1081 of astrocyte-identity genes, we computed (within the BA46 region only), a Wilcoxon rank sum
1082 test on a per-gene basis using presto (v1.0.0) ¹⁰⁹ between astrocytes and all other cell-types; for
1083 analysis of astrocyte-activity genes (SNAP-a), we sorted all genes expressed in astrocytes by
1084 their SNAP-a loadings and took the top 2,000 genes. We then converted each gene set into
1085 annotations for S-LDSC by extending the window size to 100kb (from the transcription start site
1086 and transcription end site), and ordered SNPs in the same order as the .bim file (from phase 3
1087 of the 1000 Genomes Project ¹¹⁰) used to calculate the LD scores. We then computed LD scores
1088 for annotations using a 1 cM window and restricted the analysis to Hapmap3 SNPs. We
1089 excluded the major histocompatibility (MHC) region due to both its high LD and high gene
1090 density. We used LD weights calculated for HapMap3 SNPs for the regression weights. We
1091 then jointly model the annotations corresponding to our gene expression program, as well as all
1092 protein coding genes, and the baseline model (baseline model v1.2). We tested for enrichment
1093 of SNP heritability on the traits listed below. The LDSC script, “munge_sumstats.py” was used
1094 to prepare the summary statistics files. We used the resultant p-values, which reflect a one-
1095 sided test that the coefficient (τ) is greater than zero, as a determinant as to whether our cell
1096 type gene expression programs are enriched for SNP-heritability of a given trait ¹¹¹.

1097 We used summary statistics from the following studies in **Extended Data Fig. 26**: ADHD ¹¹²,
1098 ALS ¹¹³, Alzheimer’s disease ¹¹⁴, age of smoking initiation ¹¹⁵, autism ¹¹⁶, bipolar disorder (all,
1099 type I, and type II) ¹¹⁷, cigarettes per day ¹¹⁵, educational attainment ¹¹⁸, epilepsy (all, focal,
1100 generalized) ¹¹⁹, height ¹²⁰, IQ ¹²¹, insomnia ¹²², neuroticism ¹²³, OCD ¹²⁴, schizophrenia ²², PTSD
1101 ¹²⁵, risk ¹²⁶, subjective well-being ¹²⁷, smoking cessation ¹¹⁵, smoking initiation ¹¹⁵, Tourette’s ¹²⁸,
1102 ulcerative colitis ¹²⁹.

1103

1104

1105 **Polygenic risk scores**

1106

1107 Clumped summary statistics for schizophrenia (from ²²) across 99,194 autosomal markers were
1108 downloaded from the Psychiatric Genomics Consortium portal (file
1109 PGC3_SCZ_wave3_public.clumped.v2.tsv). After liftOver of markers to GRCh38 using custom
1110 tools, 99,135 markers were available for scoring. We processed the output data from the
1111 MoChA imputation workflow ^{58,59} using BCFtools (v1.16) and the MoChA score (v2022-12-21)
1112 ^{58,59} workflow (<https://github.com/freeseek/score>) to compute schizophrenia polygenic scores
1113 across all 2,413 imputed samples from the McLean cohort.

1114

1115 **C4**

1116

1117 MetaGene discovery

1118

1119 Genes that have high sequence homology are typically difficult to capture by standard UMI
1120 counting methods. Reads from these regions map to multiple locations in the genome with low
1121 mapping quality, and are ignored by many gene expression algorithms. MetaGene discovery
1122 leverages that high sequence similarity by looking for UMIs that consistently map to multiple
1123 genes at low mapping quality consistently across many cells.

1124

1125 Each UMI is associated with a single gene if at least one read from the UMI uniquely maps to a
1126 single gene model. If all reads are mapped at low quality to multiple genes, then assignment of
1127 that UMI to a specific gene model is ambiguous, and that UMI is associated with all gene
1128 models. By surveying a large number of cells, a set of gene families are discovered where UMIs
1129 are consistently associated with sets of genes. This discovery process finds expected sets of
1130 gene families with high sequence homology directly from the mapping, such as *C4A/C4B*,
1131 *CSAG2/CSAG3*, and *SERF1A/SERF1B*.

1132

1133 These UMIs are then extracted in the counts matrix as a joint expression of all genes in each
1134 set. We prefer to calculate expression as the joint expression of all genes in the set because the
1135 priors in the data prevent confidently distributing these ambiguous UMIs. For example, *C4A* and
1136 *C4B* have very few UMIs that map uniquely to either gene in the set (8 UMIs, < 0.5% of all UMIs
1137 captured for this set of genes), which is weak prior to proportionally assign ambiguous UMIs to
1138 the correct model.

1139

1140 This approach was validated for *C4* expression by generating a reference genome that
1141 contained only one copy of *C4*. This allowed each UMI to map uniquely to the single remaining
1142 copy of the gene using standard tools. The custom reference approach and joint expression of
1143 *C4A/C4B* via the metagene approach was concordant in 15,664 of 15,669 cells tested
1144 (**Extended Data Fig. 28c**).

1145

1146 Imputation of *C4* structural variation

1147

1148 Phased copy number calls for structural features of the *C4* gene family were obtained by
1149 imputation using Osprey, a new method for imputing structural variation. The total copy number
1150 of *C4* genes, the number of copies of *C4A* and *C4B*, and the copy number of the polymorphic
1151 HERV element that distinguishes long from short forms of *C4*²⁹ were imputed into the McLean
1152 cohort using a reference panel based on 1000 Genomes⁶².

1153

1154 An imputation reference panel was constructed for GRCh38 using 2604 unrelated individuals
1155 (out of 3202 total) from 1000 Genomes. SNPs were included in the reference panel if (a) they
1156 were within the locus chr6:24000000-34000000 but excluding the copy-number variable region

1157 chr6:31980001-32046200 and (b) they were not multi-allelic and (c) they had an allele count
1158 (AC) of at least 3 when subset to the 2604 reference individuals.

1159

1160 The imputation reference panel was merged with genotypes for the McLean cohort obtained
1161 from the GSA genotyping arrays. Markers not appearing in both data sets were dropped and the
1162 merged panel was phased with SHAPEIT4 (v4.2.0)⁵⁷ using default parameters plus "--
1163 sequencing" and the default GRCh38 genetic map supplied with SHAPEIT.

1164

1165 Reference copy numbers for the *C4* structural features on GRCh38 were obtained for the 3202
1166 1000 Genomes samples using a custom pipeline based on Genome STRiP (v2.0)¹³⁰. Source
1167 code for this pipeline is available on Terra (<http://app.terra.bio>)¹³¹. Briefly, the pipeline uses
1168 Genome STRiP to estimate total *C4* copy number and HERV copy number from normalized
1169 read depth-of-coverage, then estimates the number of copies of *C4A* and *C4B* using maximum-
1170 likelihood based on reads that overlap the *C4* active site (coordinates chr6:31996082-31996099
1171 and chr6:32028820-32028837). These copy number genotypes were then subset to the 2604
1172 unrelated individuals.

1173

1174 The structural features were imputed into the merged imputation panel using Osprey (v0.1-9)
1175^{132,133} by running ospreyIBS followed by osprey using default parameters plus "-iter 100", the
1176 SHAPEIT4 genetic map for GRCh38 chr6, and a target genome interval of chr6:31980500-
1177 32046500.

1178

1179 The output from Osprey was post-processed using a custom R script (refine_C4_haplotypes.R)
1180 that enforces constraints between the copy-number features and recalibrates the likelihoods
1181 considering only "possible" haplotypes. The enforced constraints are that the *C4A*+*C4B* copies
1182 must equal total *C4* and that the HERV copy number must be less than or equal to *C4* copy
1183 number.

1184

1185

1186 Source data and visualization

1187

1188 In addition to the software cited above, we used Color Oracle (v1.3)^{134,135} as well as the
1189 following packages to prepare the source data and figures in this manuscript.

1190

1191 Python (v3.8.3): matplotlib (v3.5.2)¹³⁶, seaborn (v0.10.1)¹³⁷.

1192

1193 R (v4.1.3): cluster (v2.1.2)¹³⁸, ComplexHeatmap (v2.10.0)^{139,140}, data.table (v1.14.8)¹⁴¹,
1194 DescTools (v0.99.48)¹⁴², dplyr (v1.1.2)¹⁴³, gdata (v2.19.0)¹⁴⁴, ggforce (v0.4.1)¹⁴⁵, ggplot2
1195 (v3.4.2)¹⁴⁶, ggpmisc (v0.5.3)¹⁴⁷, ggpointdensity (v0.1.0)¹⁴⁸, ggpubr (v0.5.0)¹⁴⁹, ggrastr (v1.0.2)
1196¹⁵⁰, ggrepel (v0.9.3)¹⁵¹, grid (v4.1.3)¹⁵², gridExtra (v2.3)¹⁵³, gtable (v0.3.3)¹⁵⁴, matrixStats
1197 (v0.63.0)¹⁵⁵, pheatmap (v1.0.12)¹⁵⁶, plyr (v1.8.8)¹⁵⁷, purrr (v1.0.1)¹⁵⁸, RColorBrewer (v1.1-3)¹⁵⁹,
1198 readxl (v1.4.2)¹⁶⁰, reshape2 (v1.4.4)¹⁶¹, scales (v1.2.1)¹⁶², splitstackshape (v1.4.8)¹⁶³, stats
1199 (v4.1.3)¹⁵², stringi (v1.7.12)¹⁶⁴, stringr (v1.5.0)¹⁶⁵, tidyverse (v1.3.0)¹⁶⁶, viridis (v0.6.2)¹⁶⁷.

1200

1201 **DATA AVAILABILITY**

1202

1203 Sequencing data generated in this study and processed sequencing files are available through
1204 the Neuroscience Multi-omic Data Archive (NeMO) (RRID:SCR_016152) at
1205 <https://assets.nemoarchive.org/dat-bmx7s1t>. The data are available under controlled use
1206 conditions set by human privacy regulations. To access the data, the requester must first create
1207 an account in DUOS (<https://duos.broadinstitute.org>) using their institutional email address. The
1208 Signing Official from the requester's institution must also register in DUOS to issue the
1209 requester a Library Card Agreement. The requester will then need to fill out a Data Access
1210 Request through DUOS, which will be reviewed by the Broad Institute's Data Access
1211 Committee. Once a request is approved, NeMO will be notified to authorize access to the data.
1212 Processed expression data can also be queried using an interactive public web interface that
1213 we created (<https://dlpfc.mccarrolllab.org/app/dlpfc>). Source data with anonymized donor
1214 IDs are provided with this paper.

1215

1216 The following publicly available datasets were also analyzed: ProteomeXchange Dataset
1217 PXD026491⁸² and Gene Expression Omnibus Series GSE147672⁹⁹.

1218

1219

1220 **CODE AVAILABILITY**

1221

1222 Software and core computational analysis to align and process sequencing reads and perform
1223 donor assignment are freely available: <https://github.com/broadinstitute/Drop-seq>. Published or
1224 publicly available software, tools, algorithms, and packages are cited with their version numbers
1225 in the text and Reporting Summary. Other custom code is available upon request from the
1226 corresponding authors.

1227

1228

1229 **ACKNOWLEDGEMENTS**

1230

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1237 Jorstad for advice on glutamatergic neuron subtype classification; Frank Koopmans for SynGO
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1242 tissue donors and their families, without whom this study would not be possible.

1243

1244

1245 **AUTHOR CONTRIBUTIONS**

1246
1247 E.L., S.A.M., and S.B. designed the study. E.L., M.G., N.R., and S.A.M. developed and
1248 evaluated experimental strategies for snRNA-seq from pooled human brain tissue. E.L., M.G.,
1249 N.R., A.L., and C.D.M. prepared and dissected tissue, performed snRNA-seq, and prepared
1250 sequencing libraries. E.L., J.N., M.G., and S.A.M. performed sequencing, alignment, and
1251 quality-control analyses. E.L., J.N., A.W., and S.A.M. developed analysis pipelines. E.L. and
1252 S.A.M. analyzed the data with input from S.A.M., S.B., J.N., and N.K. B.H. performed analyses
1253 of C4. G.G. performed imputation and calculated polygenic risk scores. J.S.V. and S.B. provided
1254 tissue donor metadata. S. Gerges calculated MAGMA Z-scores and performed heritability
1255 enrichment analyses with S-LDSC. S.K. developed the scPred analysis pipeline and the RNA-
1256 expression web resource. S. Ghosh developed the pySCENIC analysis pipeline. J.M.E., K.F.,
1257 and S.B. evaluated and provided tissue for snRNA-seq experiments. D.M. contributed to
1258 analysis pipelines. L.S. contributed to tissue sample management and standardization of the
1259 single-nucleus library preparation and sequencing protocol. A.N., M.H., and K.I. contributed to
1260 project management and sequencing. E.L., S.A.M., and S.B. wrote the paper with input from co-
1261 authors.

1262

1263

1264 **COMPETING INTERESTS**

1265

1266 The authors declare no competing interests.

1267

1268 **ADDITIONAL INFORMATION**

1269

1270 Supplementary Information is available for this paper.

1271

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1273 (smccarro@broadinstitute.org), Sabina Berretta (sberretta@mclean.harvard.edu), or Emi Ling
1274 (eling@broadinstitute.org).

1275

1276

1277 **SUPPLEMENTARY NOTE**

1278 **Synaptic Neuron-Astrocyte Program (SNAP) in the genetics of schizophrenia**

1279 **Cell-identity gene expression and schizophrenia genetics: replication of earlier results**

1280

1281 Many earlier studies ¹⁻³ have found that genes most strongly expressed by neurons relative to
1282 other CNS cell types, but not genes most strongly expressed by astrocytes or other glia, are
1283 enriched for the genes implicated by human-genetic studies in schizophrenia. We first
1284 replicated these findings using the data from the current experiments. Genes that were
1285 preferentially expressed in neurons (as defined by the criteria used in the earlier studies)
1286 exhibited enrichment for schizophrenia-risk genes and alleles by a variety of analysis methods,
1287 but genes that were preferentially expressed in astrocytes did not.

1288

1289 For example, the following is an analysis of common-variant association signals (MAGMA gene-
1290 level Z-scores) versus these sets of “cell-type preferentially expressed genes” (as defined by the
1291 methods of earlier work and applied to the current data), for neurons and astrocytes. For
1292 neurons, for example, this gene-set comprises the 2,000 genes for which neurons exhibit the
1293 highest quantitative expression levels relative to other cell types. As expected, we see strong
1294 significance for neurons but not for astrocytes:

1295

1296

```
1297 > summary( lm( df2$magma.z ~ (cell.type.expr.ranks$neurons < 2000) +  
1298   (cell.type.expr.ranks$astrocytes < 2000) )$coefficients  
1299   Estimate Std. Error t value Pr(>|t|)  
1300   (Intercept) 1.05572978 0.01390269 75.937099 0.000000e+00  
1301   cell.type.expr.ranks$neurons < 2000TRUE 0.16819618 0.03764630 4.467801 7.954875e-06  
1302   cell.type.expr.ranks$astrocytes < 2000TRUE 0.05412946 0.03764630 1.437843 1.504975e-01
```

1303

1304

1305 (Here we used a single, composite “neuronal” set of expression values for the analysis, but we
1306 had very similar results when we used specific types or subtypes of cortical neurons, reflecting
1307 the strong correlation among their gene-expression levels.)

1308

1309 Thus, these well-established and expected relationships are also visible in the current, human
1310 cell-type-specific expression data.

1311

1312 **Cellular programs and schizophrenia genetics**

1313

1314 The above analysis, like other analyses to date, treats cell types as fixed levels of cell-identity
1315 gene expression, rather than as dynamic biological entities that utilize gene expression in ways
1316 whose variation is also meaningful. Cell-identity gene expression actually tells us little about
1317 SNAP-a: of the 500 genes most strongly recruited by SNAP-a, more than 90% are also robustly
1318 expressed in neurons and/or other glia of various types; less than half (203 of 500) are most
1319 strongly expressed in astrocytes, reflecting that biological functions such as synaptic adhesion
1320 and neurotransmitter uptake are also performed by neurons. Rather, it is the close
1321 transcriptional co-regulation of these genes in astrocytes by SNAP-a that appears to strongly
1322 distinguish astrocytes from neurons (**Fig. 3k**).

1323

1324 Cell types would ideally be considered, not only in terms of static cell-identity gene expression,
1325 but by their repertoires of *dynamic* transcriptional responses, such as SNAP-a, the set of
1326 astrocyte gene-expression changes that appear to be implemented in tandem with synaptic
1327 gene-expression changes in neurons (SNAP-n). To do so, we started with the 500 genes
1328 whose expression is most strongly recruited by SNAP-a (as defined by the gene loadings on
1329 this latent factor, and reflecting the fraction of their single-cell expression variance that is
1330 explained by the latent factor or cell state). We first asked whether these 500 genes are
1331 enriched for strong (genome-wide significant) associations to common and rare variants in
1332 schizophrenia. These SNAP-a-defined genes were 14 times more likely (than other protein-
1333 coding genes) to reside at genomic loci implicated by common genetic variation in
1334 schizophrenia ($p = 5 \times 10^{-25}$, 95% confidence interval: 8.7–24, by logistic regression, based on
1335 this SNAP-a-500 gene set containing 26 of the 98 protein-coding genes at 105 loci at which
1336 associated haplotypes involved SNPs in just 1–2 genes). These genes were also 7 times more
1337 likely (than other protein-coding genes) to have strong evidence from rare variants in
1338 schizophrenia (95% CI: 2.3–21, $p = 5 \times 10^{-4}$, by logistic regression, based on the SNAP-a-500
1339 gene set containing 4 of the 32 genes implicated at FDR<0.05 by the SCHEMA Consortium or
1340 by rare, intragenic deletions). Note that SNAP-a was significant even in models in which
1341 “preferential expression in neurons” was a competing predictive factor:

1342

1343

1344 Genes with common variation implicated in schizophrenia
1345 (loci at which associated haplotypes involved SNPs in just 1-2 genes)

1346

```
1347 > summary( glm( df2$in.scz.gwas.12 ~ (cell.type.expr.ranks$astrocytes < 2000) +  
1348 (cell.type.expr.ranks$neurons < 2000) , family=binomial(link='logit') ) )$coefficients  
1349  
1350 (Intercept) Estimate Std. Error z value Pr(>|z|)  
-5.511932 0.1388874 -39.6863400 0.000000e+00  
1351 cell.type.expr.ranks$astrocytes < 2000TRUE 0.134320 0.3412139 0.3936534 6.938370e-01  
1352 cell.type.expr.ranks$neurons < 2000TRUE 1.323195 0.2280745 5.8015912 6.568853e-09
```

1353

1354

```
1355 > summary( glm( df2$in.scz.gwas.12 ~ (cell.type.expr.ranks$astrocytes < 2000) +  
1356 (cell.type.expr.ranks$neurons < 2000) + (df2$SNAPa.rank < 500) , family=binomial(link='logit') ) )  
1357 $coefficients  
1358 (Intercept) Estimate Std. Error z value Pr(>|z|)  
-5.7144114 0.1482871 -38.536128 0.000000e+00  
1359 cell.type.expr.ranks$astrocytes < 2000TRUE -0.5614668 0.3633355 -1.545312 1.222708e-01  
1360 cell.type.expr.ranks$neurons < 2000TRUE 1.2475955 0.2320574 5.376238 7.605827e-08  
1361 df2$SNAPa.rank < 500TRUE 2.6729857 0.2584410 10.342732 4.514813e-25
```

1363

1364

1365

1366 Genes with rare variation implicated in schizophrenia
1367 (SCHEMA FDR<0.05 + NRXN1)

1368

```
1369 >summary( glm( df2$in.schema ~ (cell.type.expr.ranks$astrocytes < 2000) +  
1370 (cell.type.expr.ranks$neurons < 2000) + (df2$SNAPa.rank < 500) , family=binomial(link='logit') ) )  
1371 $coefficients  
1372 (Intercept) Estimate Std. Error z value Pr(>|z|)  
-6.5952346 0.2363882 -27.900012 2.667152e-171  
1373 cell.type.expr.ranks$astrocytes < 2000TRUE -0.8651288 0.7686021 -1.125587 2.603402e-01  
1374 cell.type.expr.ranks$neurons < 2000TRUE 0.6479477 0.4681928 1.383933 1.663788e-01  
1375 df2$SNAPa.rank < 500TRUE 1.9568408 0.5652152 3.462116 5.359452e-04
```

1377

1378

1379

1380 In the above analysis of genes implicated by rare variants, baseline expression in neurons was
1381 not significant. The implication of neuronally-expressed genes in the study by the SCHEMA

1382 Consortium used a different type of analysis, which used a Wilcoxon rank-sum test to evaluate
1383 whether SCHEMA genes had higher levels of neuron-preferential expression than other protein-
1384 coding genes did. In that analysis, in Figure S17 of the SCHEMA paper ², about a third of the
1385 neuronal types tested yielded p-values less than 0.05, whereas no non-neuronal cell types did.
1386 We repeated this analysis with the data from the current study, with several subtypes of neurons
1387 yielding nominally significant results (p<0.05) but not astrocytes (p=0.64), in accordance with
1388 the earlier finding. When we applied an analogous analysis to the gene loadings for SNAP-a, it
1389 was highly significant (p = 8 x 10⁻⁵).

1390

1391

```
1392 > wilcox.test( df2$SNAPa.rank[which(df2$in.schema==1)], df2$SNAPa.rank[which(df2$in.schema==0)] )  
1393  
1394 Wilcoxon rank sum test with continuity correction  
1395  
1396 data: df2$SNAPa.rank[which(df2$in.schema == 1)] and df2$SNAPa.rank[which(df2$in.schema == 0)]  
1397 W = 125676, p-value = 7.696e-05  
1398 alternative hypothesis: true location shift is not equal to 0
```

1399

1400

1401 To evaluate, beyond these top genetic associations, whether common genetic variation in the
1402 genes recruited by SNAP-a contributes more broadly to schizophrenia risk, we further utilized
1403 the gene-level association statistics provided by MAGMA analysis ^{1,4}, which evaluates, for every
1404 gene, the tendency of common patterns of genetic variation (as identified by principal
1405 components analysis) to have elevated levels of association. To integrate across these more-
1406 subtle genomic signals, we also used a larger number of genes prioritized by SNAP-a. The
1407 2,000 genes whose expression is most strongly recruited by SNAP-a had elevated MAGMA z-
1408 scores for association to schizophrenia (p < 2 x 10⁻²⁰), while astrocyte-identity gene expression
1409 did not (p = 0.53).

1410

```
1411 > summary( lm( df2$magma.z ~ (cell.type.expr.ranks$astrocytes < 2000) + (df2$SNAPa.rank <  
1412 2000)))$coefficients  
1413  
1414 (Intercept) Estimate Std. Error t value Pr(>|t|)  
1415 cell.type.expr.ranks$astrocytes < 2000TRUE 1.04298801 0.01349453 77.2896942 0.000000e+00  
1416 df2$SNAPa.rank < 2000TRUE -0.02363672 0.03795977 -0.6226781 5.335046e-01  
1417  
1418
```

1419

1420

1421

1422

1423

1424

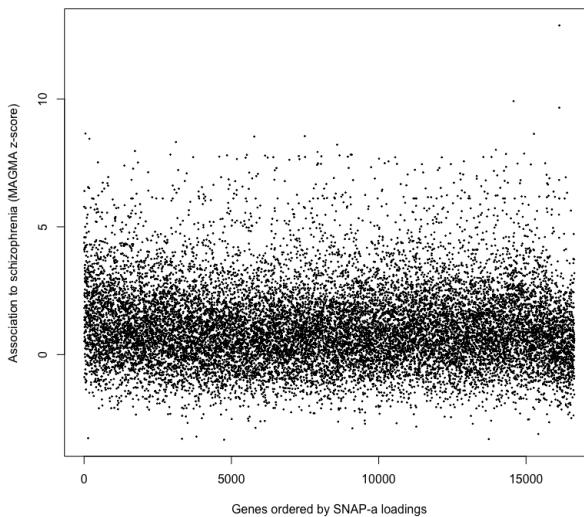
1425

1426

Since the number of genes in the SNAP-a gene set is a somewhat arbitrary parameter of this analysis, we explored the relationship of this enrichment to the gene depth (on the SNAP-a-ranked gene list) used in analysis. The results for eight gene depths are summarized in the table below. Genetic signals were most strongly concentrated at the top of the SNAP-a gene list (as seen by the regression coefficient estimate, first column); however, concentration was still present at greater gene depths, and the statistical significance of the enrichment (as estimated by the test statistic, third column) increased in more-inclusive analyses up to about 2,000 genes, at which point it began to drop.

1427	Gene depth used	Estimate	Std. Error	t value	Pr(> t)
1428					
1429	100	1.02480691	0.15924204	6.4355299	1.264059e-10
1430	200	0.86780224	0.11293191	7.6842959	1.623806e-14
1431	400	0.61147742	0.08089709	7.5587070	4.282410e-14
1432	1000	0.44671707	0.05218176	8.5607889	1.217565e-17
1433	2000	0.35203328	0.03795977	9.2738519	2.010865e-20
1434	3000	0.26788176	0.03200028	8.3712313	6.150143e-17
1435	4000	0.18779209	0.02875229	6.5313782	6.705553e-11
1436	8000	0.04179646	0.02440499	1.7126192	0.08680125

1437
1438 This relationship can also be recognized visually in a plot of MAGMA z-score vs. genes ordered
1439 by their SNAP-a gene loadings, which suggests that enrichment is strongest among the genes
1440 ranked most highly by SNAP-a (far left on plot) gene list but that enrichment continues, albeit
1441 more modestly, over the top 2,000 or so genes.



1442
1443
1444
1445 We also included neuronal-identity gene expression (as defined by the method used in the
1446 earlier studies) and SNAP-n-recruited genes in the regression analysis, as independent and
1447 competing predictive factors. All three were significant in a joint analysis, and the signal for
1448 SNAP-a genes was not attenuated by the inclusion of the two neuronal gene sets:
1449

```
1450 > summary( lm( df2$magma.z ~ (cell.type.expr.ranks$astrocytes < 2000) +  
1451 (cell.type.expr.ranks$neurons < 2000) + (df2$SNAPa.rank < 2000) + (df2$SNAPn.rank < 2000) ))  
1452 $coefficients  
1453 (Intercept) Estimate Std. Error t value Pr(>|t|)  
1454 1.01002575 0.01469833 68.7170433 0.000000e+00  
1455 cell.type.expr.ranks$astrocytes < 2000TRUE -0.01596758 0.03819846 -0.4180164 6.759406e-01  
1456 cell.type.expr.ranks$neurons < 2000TRUE 0.15147290 0.03767309 4.0207191 5.827665e-05  
1457 df2$SNAPa.rank < 2000TRUE 0.33732706 0.03807661 8.8591666 8.861497e-19  
1458 df2$SNAPn.rank < 2000TRUE 0.14417190 0.03947272 3.6524436 2.605539e-04
```

1459
1460 In the above result, both SNAP-n genes and neuronally-preferentially-expressed genes
1461 contributed independently to explaining gene-schizophrenia associations (MAGMA z-statistics),
1462 suggesting that – in neurons as in astrocytes – information about dynamic gene-expression
1463 programs can provide additional information beyond the information provided by cell-identity
1464 gene expression.
1465

1466 Finally, we used LD score regression ⁵ to evaluate per-SNP heritability enrichment across 27
1467 brain phenotypes. Baseline astrocyte-identity gene expression (top 2,000 genes) did not exhibit
1468 heritability enrichment for any of the 27 brain phenotypes tested (**Extended Data Fig. 26a**).
1469 SNAP-a (most strongly recruited 2,000 genes) exhibited per-SNP heritability enrichment ($p = 4 \times$
1470 10^{-5}) for schizophrenia, nominal significance ($p < 0.01$) for smoking cessation and autism, and
1471 was not significant for the other 24 phenotypes tested (**Extended Data Fig. 26b**).
1472

1473 **References**

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1481 5. Finucane, H. K. *et al.* Partitioning heritability by functional annotation using genome-wide
1482 association summary statistics. *Nat. Genet.* **47**, 1228–1235 (2015).
1483

1484 **SUPPLEMENTARY TABLES**

1485

1486 **Supplementary Table 1. Summary of human tissue donor metadata.**

1487 Donor metadata table. Sample details include sex, age, post-mortem interval (PMI, when
1488 available), schizophrenia case-control status, and inclusion in experimental batches.

1489

1490 **Supplementary Table 2. Donor expression levels and gene loadings for latent factors.**

1491 Tables of donor expression levels and genes-by-cell-types loadings for each of the 10 latent
1492 factors inferred by PEER.

1493

1494 **Supplementary Table 3. Regression analysis of LF4 donor expression levels.**

1495 Joint regression analysis of LF4 donor expression levels with age, sex, and schizophrenia case-
1496 control status as independent variables.

1497

1498 **Supplementary Table 4. Gene set enrichment analysis (GSEA) results for LF4 by cell
1499 type.**

1500 Tables of gene sets enriched in each cell type's component of LF4 (at FDR < 0.05) from a
1501 preranked gene set enrichment analysis (GSEA) using LF4 gene loadings.

1502

1503 **Supplementary Table 5. Gene set enrichment analysis (GSEA) results for latent factors
1504 enriched in astrocytes.**

1505 Tables of gene sets enriched in astrocyte latent factors discovered by cNMF (at FDR < 0.15)
1506 from a preranked gene set enrichment analysis (GSEA) using gene loadings for each factor.

1507

1508 **Supplementary Table 6. Donor expression levels and gene loadings for SNAP-a.**

1509 Tables of donor expression levels (mean cell scores by donor) and gene loadings for SNAP-a
1510 (astrocyte latent factor 2 inferred by cNMF).

1511

1512 **Supplementary Table 7. Donor expression levels and gene loadings for SNAP-n.**

1513 Tables of donor expression levels (mean cell scores by donor) and gene loadings for SNAP-n
1514 (L5 IT glutamatergic neuron latent factor 6 inferred by cNMF).

1515

1516 **Supplementary Table 8. Gene set enrichment analysis (GSEA) results for SNAP-n.**

1517 Table of gene sets enriched in SNAP-n (at FDR < 0.15) from a preranked gene set enrichment
1518 analysis (GSEA) using gene loadings for SNAP-n.

1519

1520 **Supplementary Table 9. Genes in selected gene sets.**

1521 Table of genes in selected gene sets used in analyses. Descriptions of selected gene sets are
1522 in Methods.

1523 **EXTENDED DATA FIGURES**

1524

1525 **Extended Data Figure 1. Ages of brain tissue donors.**

1526

1527 **a**, Distribution of the ages of brain donors ($n = 191$ donors).

1528

1529 **b**, Distributions of donors' ages by schizophrenia status, displayed as a quantile-quantile plot
1530 that compares ages of unaffected control donors ($n = 97$ donors) to ages of donors with
1531 schizophrenia ($n = 94$ donors).

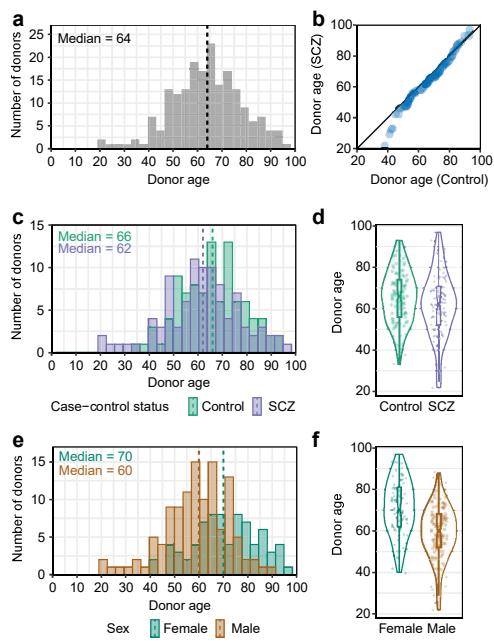
1532

1533 **c-d**, Distributions of donors' ages separated by schizophrenia status ($n = 97$ unaffected and 94
1534 affected), displayed as **(c)** histograms and **(d)** violin plots.

1535

1536 **e-f**, Distributions of donors' ages, separated by sex ($n = 75$ women and 116 men), displayed as
1537 **(e)** histograms and **(f)** violin plots. Note that while female brain donors are on average older
1538 than male donors, expression of SNAP (LF4) did not associate with sex in either a naive or age-
1539 adjusted analysis (**Extended Data Fig. 9d-e**), nor in a simultaneous regression on age, sex,
1540 and schizophrenia affected/unaffected status (**Supplementary Table 3**).

Extended Data Figure 1



1541 **Extended Data Figure 2. Single-donor assignment and sequencing metrics.**

1542

1543 **a**, Validation of the computational assignment of nuclei to individual brain donors whose
1544 genomes have been analyzed (individually) by SNP array-genotyping plus imputation. The
1545 matrix displays the concordance of single-donor assignment between whole-genome
1546 sequencing (WGS) (y-axis) and SNP array + imputation (x-axis) for a pilot set of 11 donors
1547 whose genomes were analyzed by both methods. (Accuracy of donor assignment when WGS
1548 data are available has been previously shown by ⁷.) Each row/column corresponds to one of the
1549 11 donors, and each entry in the table displays the number of nuclei that were assigned to a
1550 given donor (at a false discovery rate of 0.05).

1551

1552 **b**, Density plot showing the fraction of all nuclei that were determined to be “singlets” (containing
1553 alleles from just one donor); $n = 1,262,765$ assignable singlets out of 1,271,830).

1554

1555 **c**, Density plot showing donor-assignment likelihoods (as false discovery rates, on a log scale)
1556 for the 1,271,830 singlet nuclei.

1557

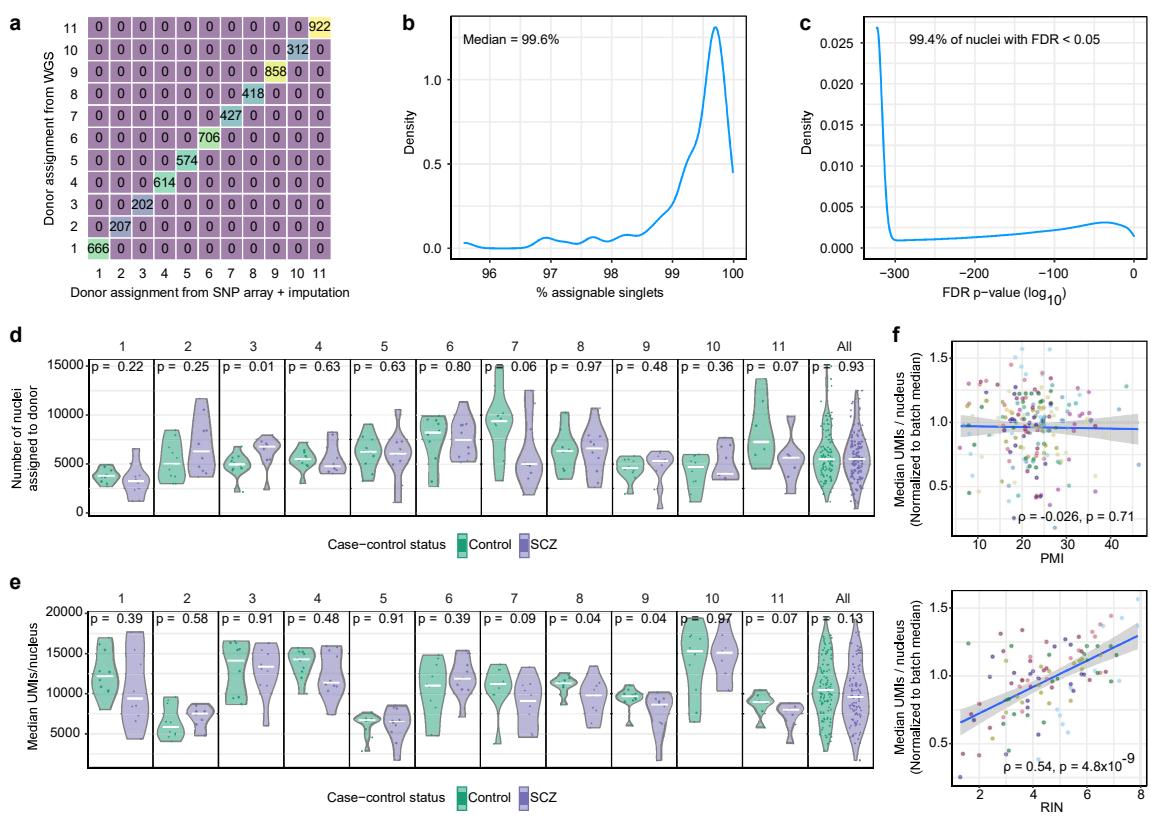
1558 **d**, Number of nuclei assigned to each donor in each of 11 batches or (rightmost panel) across
1559 all batches, separated by schizophrenia case-control status ($n = 10$ controls and 10
1560 schizophrenia cases per batch). P-values from a two-sided Wilcoxon rank-sum test comparing
1561 the affected to the unaffected donors are reported at the top of each panel. Central lines
1562 represent medians.

1563

1564 **e**, Median number of UMIs ascertained per donor in each batch or (rightmost panel) across all
1565 batches, separated by schizophrenia case-control status ($n = 10$ controls and 10 schizophrenia
1566 cases per batch). P-values from a two-sided Wilcoxon rank-sum test comparing the affected to
1567 the unaffected donors are reported at the top of each panel. Central lines represent medians.

1568 **f**, Relationship of median UMIs/nucleus (normalized to the median value of the donors in each
1569 donor’s batch) to (top) post-mortem interval (PMI) and (bottom) RIN score (Spearman’s ρ).
1570 Colors represent different batches. Shaded regions represent 95% confidence intervals.

Extended Data Figure 2



1571 **Extended Data Figure 3. Cell-type classification and composition analysis.**

1572

1573 **a**, Two-dimensional projection of the RNA-expression profiles of the 1,218,284 nuclei analyzed
1574 from 191 donors, reproduced from **Fig. 1c**. Nuclei are colored by their assignments to the major
1575 cell types present in Brodmann area 46 (BA46). The same projection is used in panel **b**.

1576

1577 **b**, Expression levels of canonical marker genes of cell types in BA46. Values represent Pearson
1578 residuals from variance stabilizing transformation (VST).

1579

1580 **c**, Relative representation of each cell type among nuclei ascertained from each donor. Donors
1581 are ordered by their anonymized research IDs at the Harvard Brain Tissue Resource Center.

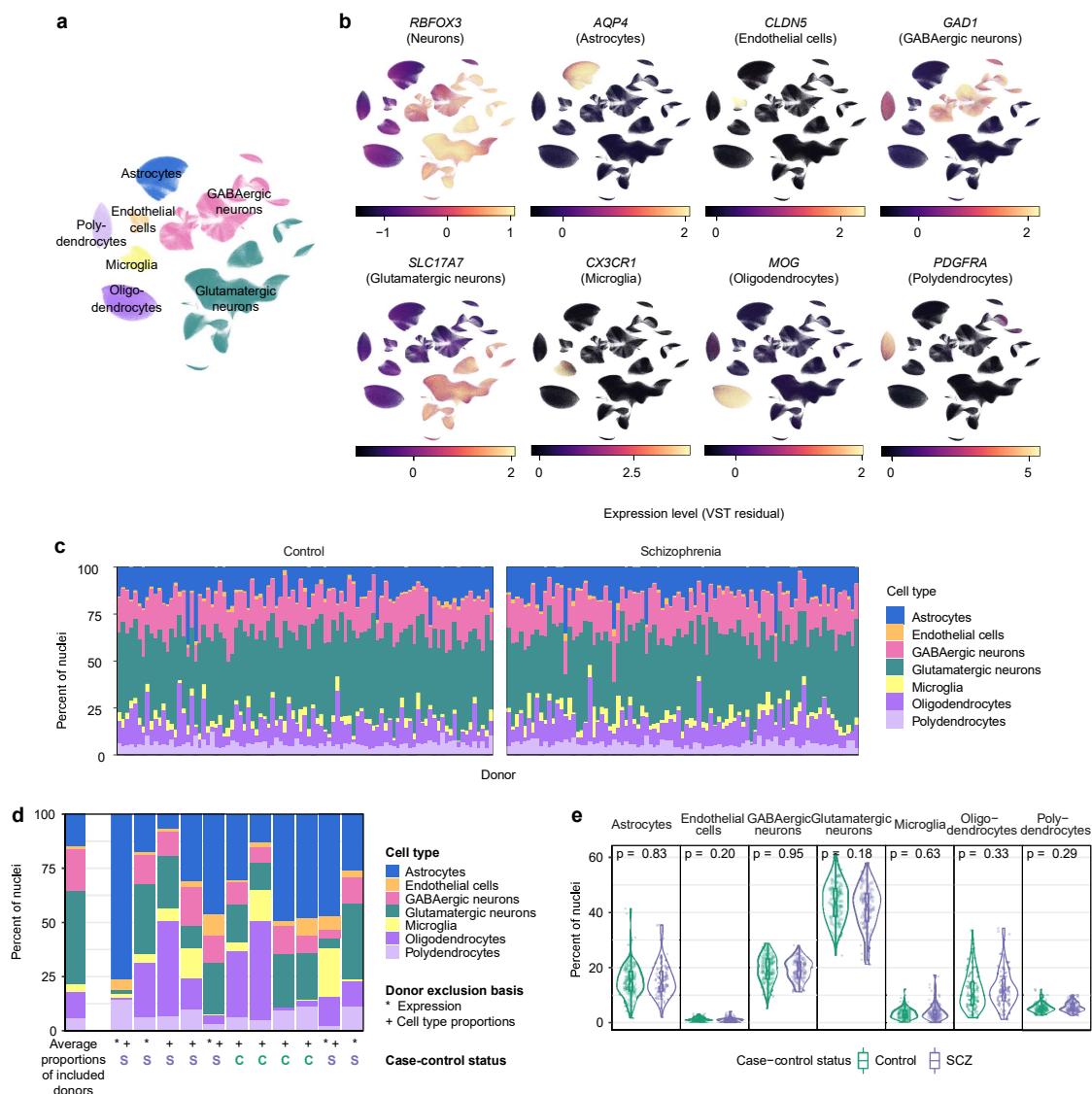
1582

1583 **d**, Cell-type proportions detected in 11 donors whom we excluded from subsequent analyses,
1584 with the basis of exclusion (unusual cell-type proportions and/or expression profiles) indicated
1585 under each donor. For comparison, average cell-type proportions of the 180 donors included in
1586 subsequent analyses are displayed to the left (donors from panel **c**).

1587

1588 **e**, Cell-type proportions ascertained in the BA46 tissue samples; data points are separated by
1589 schizophrenia status ($n = 93$ unaffected and 87 affected). P-values from a two-sided Wilcoxon
1590 rank-sum test comparing the affected to the unaffected donors are reported at the top of each
1591 panel. Box plots show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines,
1592 medians; notches, confidence intervals around medians.

Extended Data Figure 3



1593 **Extended Data Figure 4. Expression of glutamatergic neuron-subtype marker genes.**

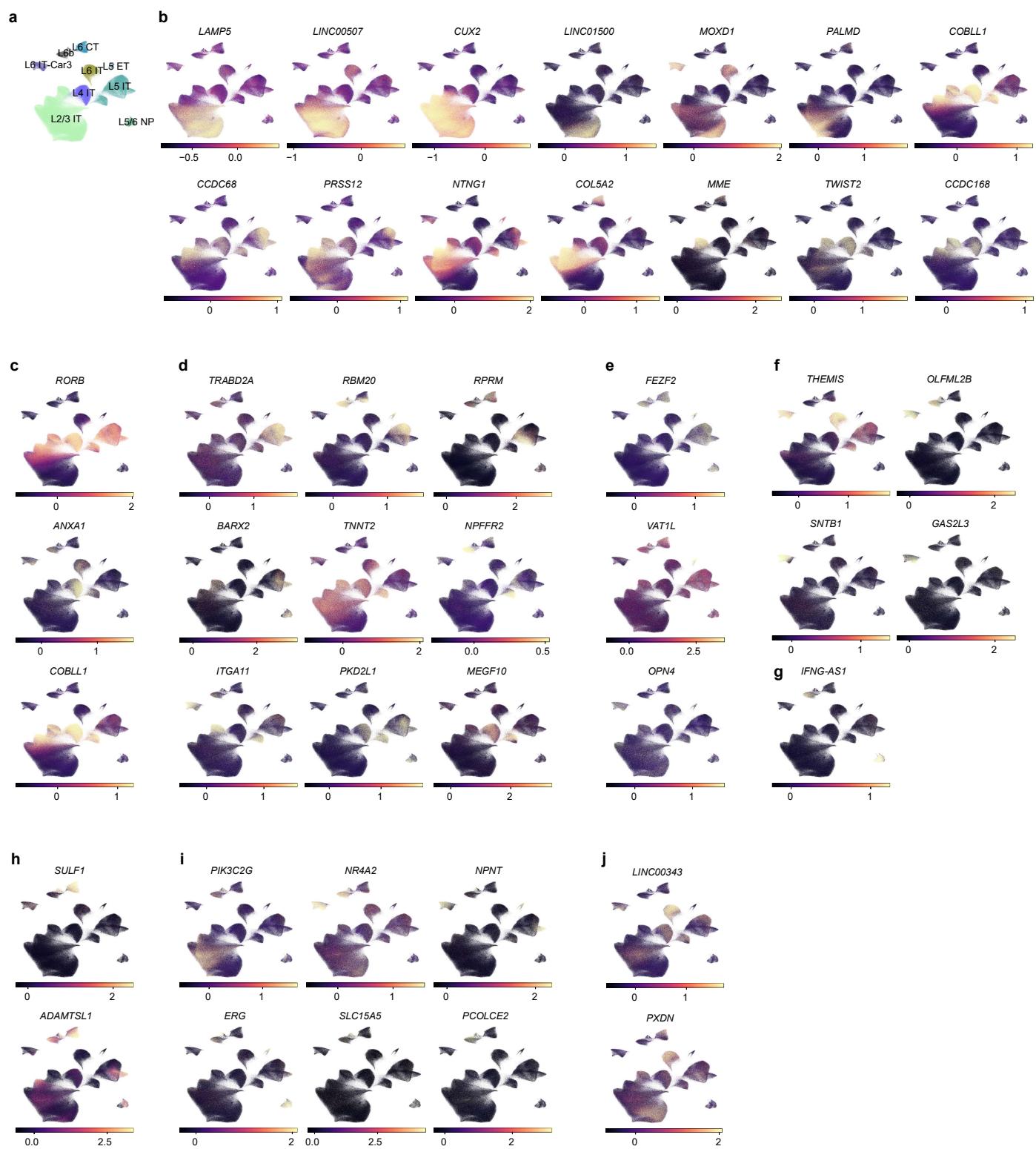
1594

1595 **a**, Two-dimensional projection of the RNA-expression profiles of 524,186 glutamatergic neuron
1596 nuclei, reproduced from **Fig. 1d**. Nuclei are colored by their assignments to subtypes of
1597 glutamatergic neurons using classifications from ⁷⁵ and ⁷⁶. The same projection is used in panels
1598 **b** to **j** below.

1599

1600 **b-j** Expression levels of marker genes for subtypes of **(b)** L2/3 IT, **(c)** L4 IT, **(d)** L5 IT, **(e)** L5 ET,
1601 **(f)** L6 IT-Car3, **(g)** L5/6 NP, **(h)** L6 CT, **(i)** L6b, and **(j)** L6 IT glutamatergic neurons. Markers are
1602 from ⁷⁵ or from transcriptomically similar subtypes in ⁷⁶. Values represent Pearson residuals from
1603 variance stabilizing transformation (VST).

Extended Data Figure 4



1604 **Extended Data Figure 5. Expression of GABAergic neuron-subtype marker genes.**

1605

1606 **a**, Two-dimensional projection of the RNA-expression profiles of 238,311 GABAergic neuron
1607 nuclei, reproduced from **Fig. 1e**. Nuclei are colored by their assignments to subtypes of
1608 GABAergic neurons using classifications from ⁷⁵ and ⁷⁶. The same projection is used in panels B
1609 to H below.

1610

1611 **b-h**, Expression levels of marker genes for subtypes of **(b)** PVALB, **(c)** SST-CHODL, **(d)** MEIS2,
1612 **(e)** SST, **(f)** LAMP5, **(g)** SNCG, and **(h)** VIP GABAergic neurons. Markers are from ⁷⁵ or from
1613 transcriptomically similar subtypes in ⁷⁶. Values represent Pearson residuals from variance
1614 stabilizing transformation (VST).

Extended Data Figure 5



1615 **Extended Data Figure 6. Glutamatergic neuron-subtype composition analysis across**
1616 **donors.**

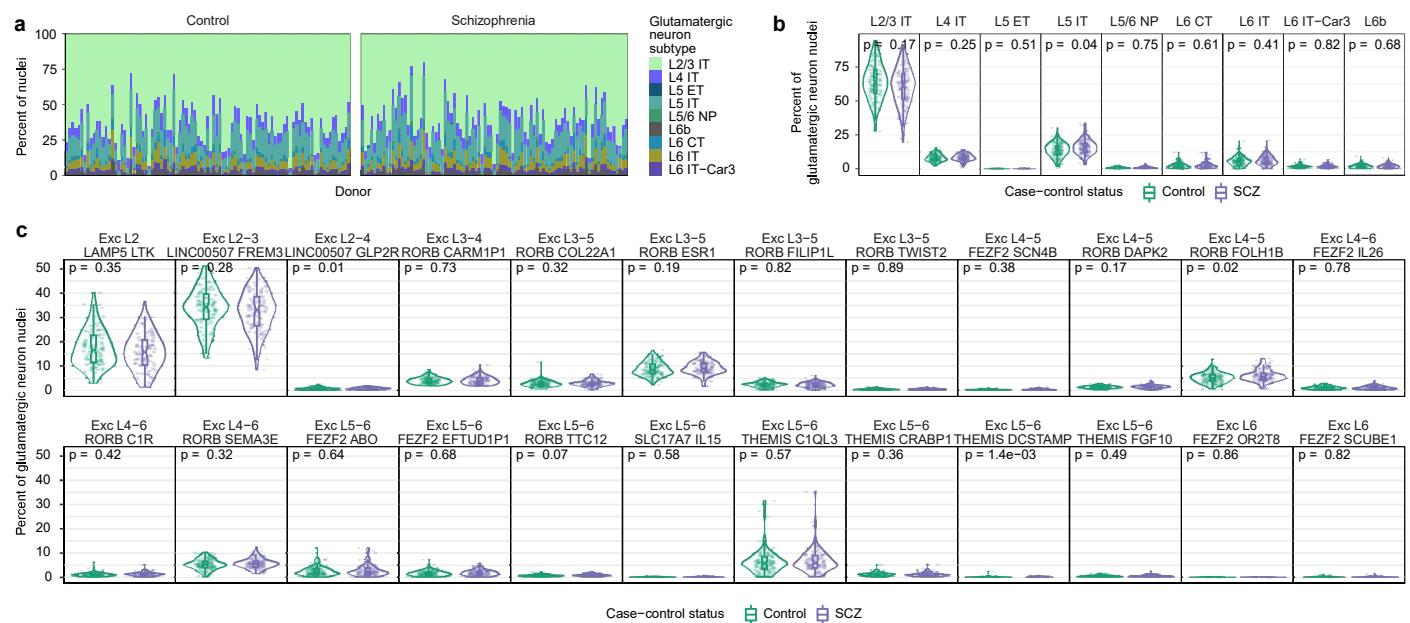
1617

1618 **a**, Relative representation of each glutamatergic neuron subtype among nuclei ascertained from
1619 each donor. Donors are ordered by their anonymized research IDs at the Harvard Brain Tissue
1620 Resource Center.

1621

1622 **b-c**, Proportions of **(b)** glutamatergic neuron subtypes and **(c)** subtypes of these subtypes
1623 (defined in ⁷⁵) by schizophrenia status ($n = 93$ unaffected and 87 affected). P-values from a two-
1624 sided Wilcoxon rank-sum test comparing the affected to the unaffected donors are reported at
1625 the top of each panel. Box plots show interquartile ranges; whiskers, 1.5x the interquartile
1626 interval; central lines, medians; notches, confidence intervals around medians.

Extended Data Figure 6



1627 **Extended Data Figure 7. GABAergic neuron-subtype composition analysis across**
1628 **donors.**

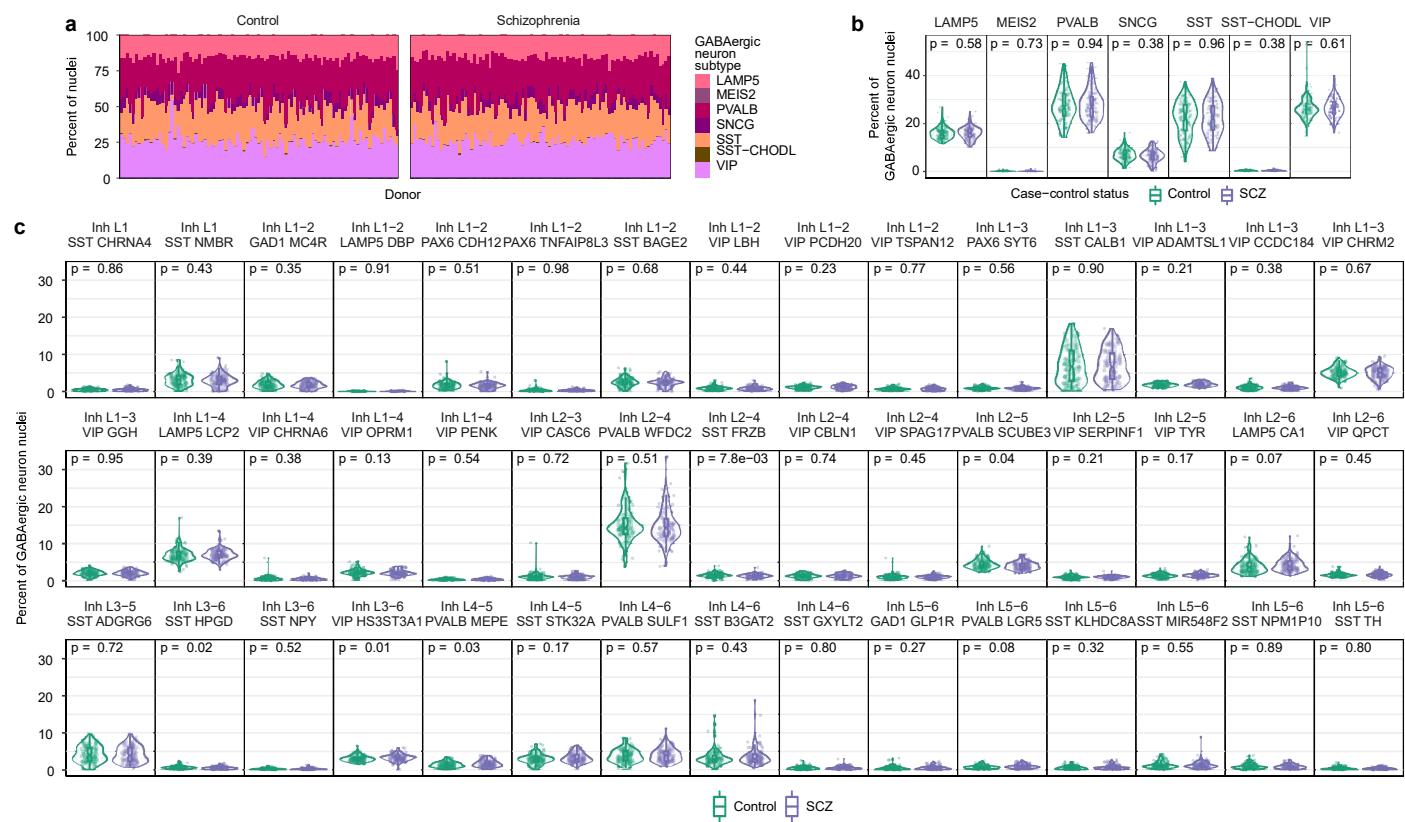
1629

1630 **a**, Relative representation of each GABAergic neuron subtype among nuclei ascertained from
1631 each donor. Donors are ordered by their anonymized research IDs at the Harvard Brain Tissue
1632 Resource Center.

1633

1634 **b-c**, Proportions of **(b)** GABAergic neuron subtypes and **(c)** subtypes of these subtypes (defined
1635 in ⁷⁵) by schizophrenia status ($n = 93$ unaffected and 87 affected). P-values from a two-sided
1636 Wilcoxon rank-sum test comparing the affected to the unaffected donors are reported at the top
1637 of each panel. Box plots show interquartile ranges; whiskers, 1.5x the interquartile interval;
1638 central lines, medians; notches, confidence intervals around medians.

Extended Data Figure 7



1639 **Extended Data Figure 8. Properties of the latent factors inferred from snRNA-seq data.**

1640

1641 **a**, Total % variance in expression explained by latent factors with different numbers of
1642 requested factors k .

1643

1644 **b**, Fraction of variance explained by each latent factor in an analysis with 10 requested factors.

1645 **c-d**, Independence of latent factors, visualized as Pearson correlation heatmaps of factors' **(c)**
1646 gene loadings ($n = 125,437$ gene/cell-type combinations) and **(d)** donor scores ($n = 180$
1647 donors).

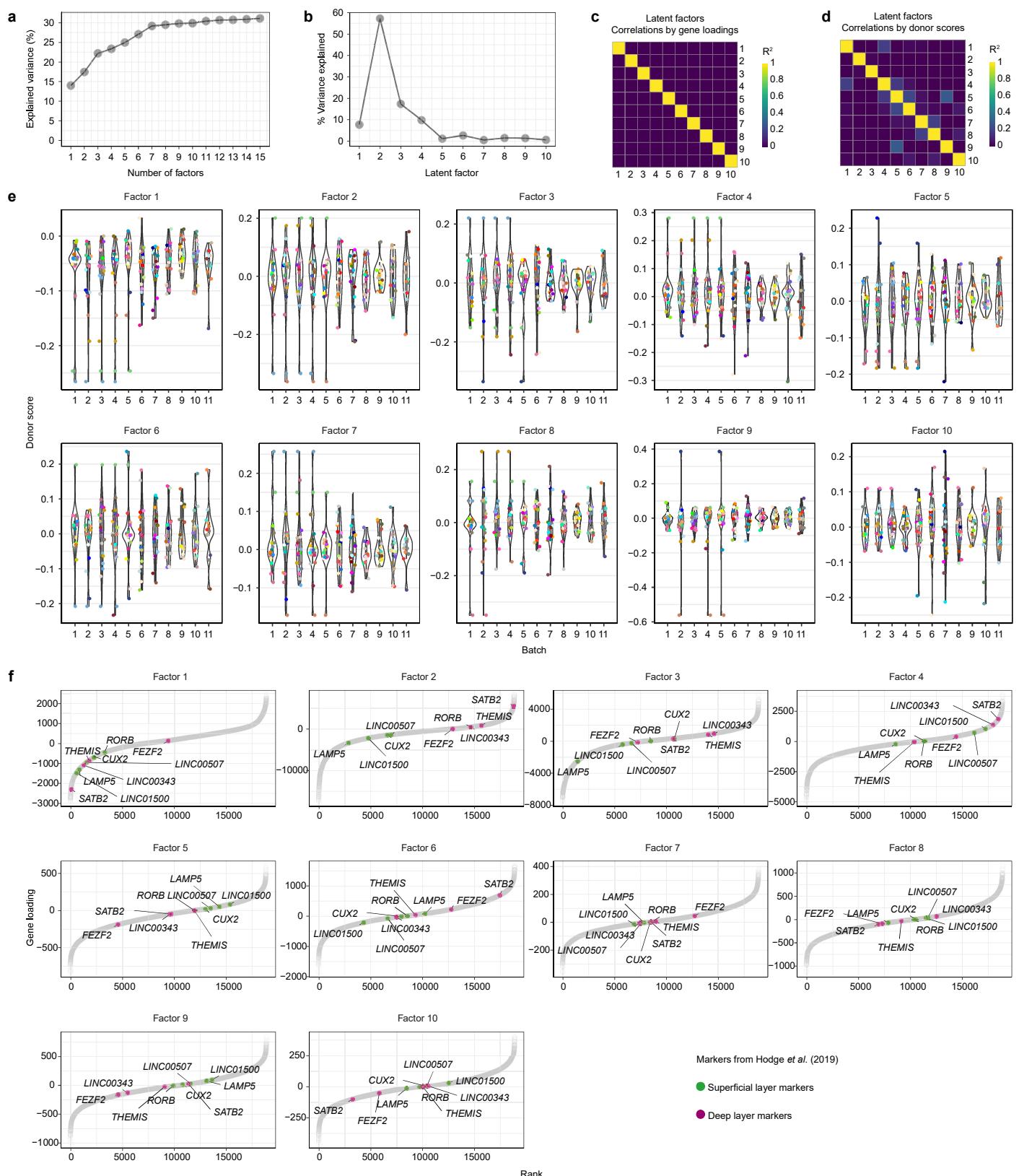
1648

1649 **e**, Expression level of each latent factor (panels) in each donor (points), split by batch ($n = 20$
1650 donors per batch).

1651

1652 **f**, Relationship of latent factors to markers of superficial and deep cortical layers from ⁷⁵.
1653 Markers label dominant classes of glutamatergic neurons (superficial: *LAMP5*, *LINC00507*,
1654 *RORB*; deep: *THEMIS*, *FEZF2*) or spatially restricted subtypes (superficial: Exc L2 LAMP5 LTK,
1655 marked by *CUX2* and *LINC01500*; deep: Exc L5-6 THEMIS C1QL3, marked by *SATB2* and
1656 *LINC00343*). Factor 2 exhibits the most distinct segregation of these superficial and deep layer
1657 markers when genes are ranked by their loadings onto each factor. $n = 18,830$ genes
1658 expressed in glutamatergic neurons; colored dots are plotted over the dots of genes not among
1659 the markers listed above (grey).

Extended Data Figure 8



1660 **Extended Data Figure 9. Properties of Latent Factor 4 (LF4).**

1661

1662 **a**, Expression of each latent factor by case-control status ($n = 93$ controls and 87 cases). P-
1663 values are from a two-sided Wilcoxon rank-sum test. Box plots show interquartile ranges;
1664 whiskers, 1.5x the interquartile interval; central lines, medians; notches, confidence intervals
1665 around medians.

1666

1667 **b**, Expression of LF4 by case-control status, split by sex (female: $n = 31$ controls and 39 cases;
1668 male: $n = 62$ controls and 48 cases). P-values are from a two-sided Wilcoxon rank-sum test.
1669 Box plots show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines,
1670 medians; notches, confidence intervals around medians. Note that the more-modest p-value for
1671 the females-only analysis relative to the males-only analysis appears to represent the smaller
1672 sample (70 females vs. 110 males) rather than a weaker relationship to schizophrenia status;
1673 please see also **Extended Data Fig. 18h**.

1674

1675 **c**, Similar plots as in **b**, here displaying LF4 expression values adjusted for donor age.

1676

1677 **d**, Expression of LF4 by sex, split by case-control status (controls: $n = 31$ females and 62
1678 males; cases: $n = 39$ females and 48 males). P-values are from a two-sided Wilcoxon rank-sum
1679 test. Box plots show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines,
1680 medians; notches, confidence intervals around medians.

1681

1682 **e**, Similar plots as in **d**, here displaying LF4 expression values adjusted for donor age.

1683

1684 **f-k**, Relationship of LF4 expression measurements to other available donor and tissue
1685 characteristics: **(f)** time of death in zeitgeber time (ZT), with rhythmicity analyses performed as
1686 in ⁸³; **(g)** post-mortem interval; **(h)** number of nuclei sampled; **(i)** number of UMIs sampled; **(j)**
1687 use of psychiatric medications (left column) across each donor's lifespan or (right column) in the
1688 last 6 months prior to death; and **(k)** use of clozapine. Correlation coefficients in **g-j** are
1689 Spearman's ρ . P-values in **k** are from a two-sided Wilcoxon rank-sum test. Box plots show
1690 interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians; notches,
1691 confidence intervals around medians.

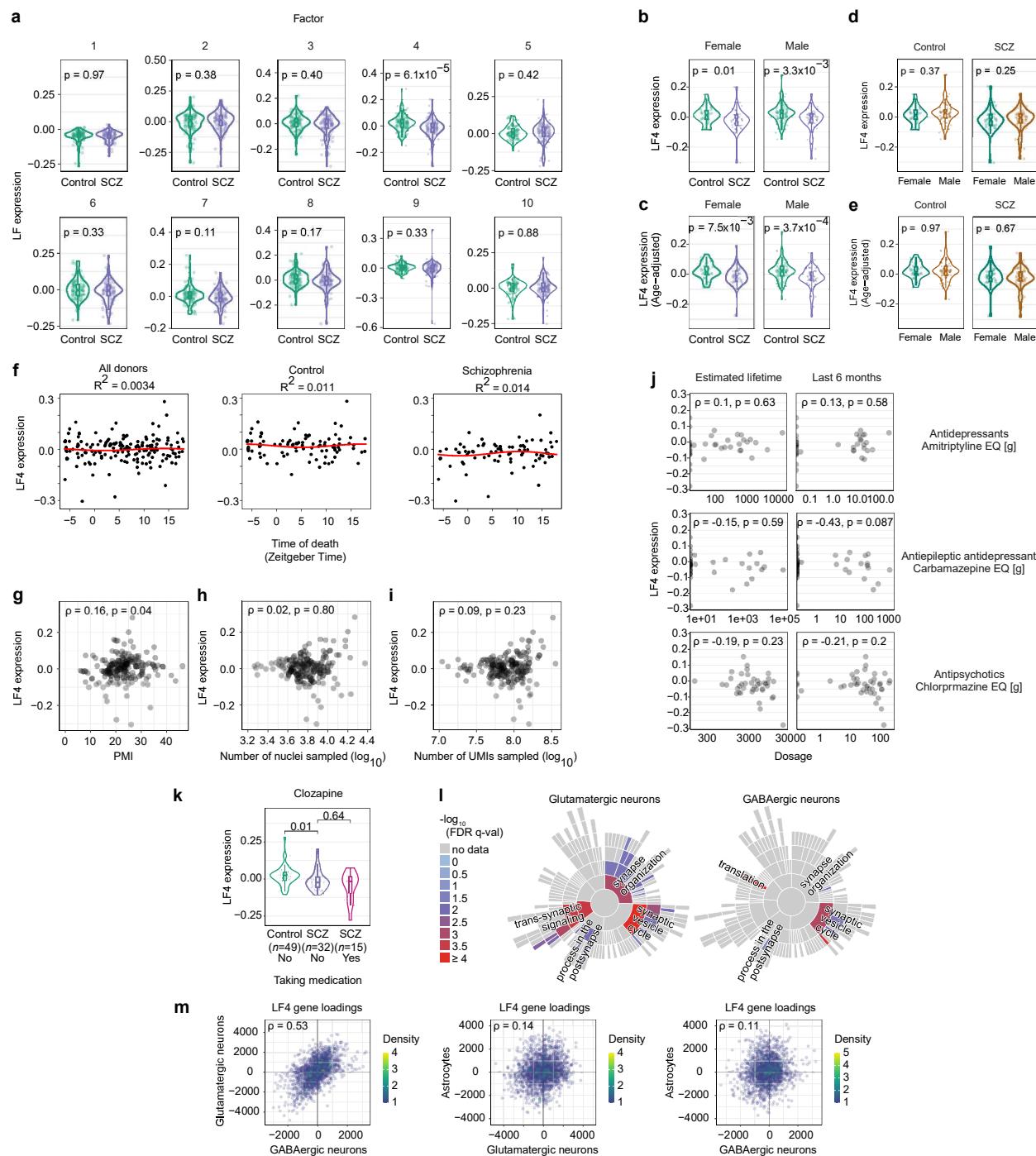
1692

1693 **l**, Concentrations of the strongest enriched neuronal gene-expression changes in LF4 among
1694 synaptic functions as annotated by SynGO ⁹¹. Plots show categories of SynGO biological
1695 processes.

1696

1697 **m**, See also **Fig. 2a**. LF4 involves broadly similar gene-expression effects in glutamatergic and
1698 GABAergic neurons, and a distinct set of gene-expression effects in astrocytes. Genes plotted
1699 are the protein-coding genes that are expressed (at levels of at least 10 UMIs per 10^5) in both
1700 cell types (Spearman's ρ ; $n = 1,538$, 1,067, and 1,131 genes respectively).

Extended Data Figure 9



1701 **Extended Data Figure 10. Robustness of Latent Factor 4 (LF4) to analysis parameters.**

1702

1703 LFs similar to LF4 were identified in **(a)** analyses with different numbers of factors ($n = 180$ donors), **(b)** a controls-only analysis ($n = 93$ donors), and **(c)** a cases-only analysis ($n = 87$ donors).

1706

1707 **a**, Column 1: Association of latent-factor expression levels with schizophrenia case-control status, shown as a quantile-quantile plot that compares observed $-\log_{10}$ p-values to the distribution of $-\log_{10}$ p-values expected under a null hypothesis ($n = 15, 20$, and 30 factors). The observed p-values were calculated for each latent factor by a two-sided Wilcoxon rank-sum test of latent factor expression levels (by donor) between cases and controls. In all analyses, LF4 is the factor that deviates the most from the line of unity and displays the strongest association with schizophrenia case-control status. Column 2: Expression of LF4 by case-control status ($n = 93$ controls and 87 cases). P-values are from a two-sided Wilcoxon rank-sum test. Box plots show interquartile ranges; whiskers, 1.5 times the interquartile interval; central lines, medians; notches, confidence intervals around medians. Shaded regions represent 95% confidence intervals. Column 3: Comparison of gene loadings ($n = 125,437$ gene/cell-type combinations) that demonstrates the relationship of LF4 inferred from an analysis requesting 10 factors to LF4 inferred from an analysis requesting $15, 20$, or 30 factors (Spearman's ρ). Shaded regions around regression lines represent 95% confidence intervals. Column 4: Comparison of donor expression levels ($n = 180$ donors) that demonstrates the relationship of LF4 inferred from an analysis requesting 10 factors to LF4 inferred from an analysis requesting $15, 20$, or 30 factors (Spearman's ρ). Shaded regions around regression lines represent 95% confidence intervals.

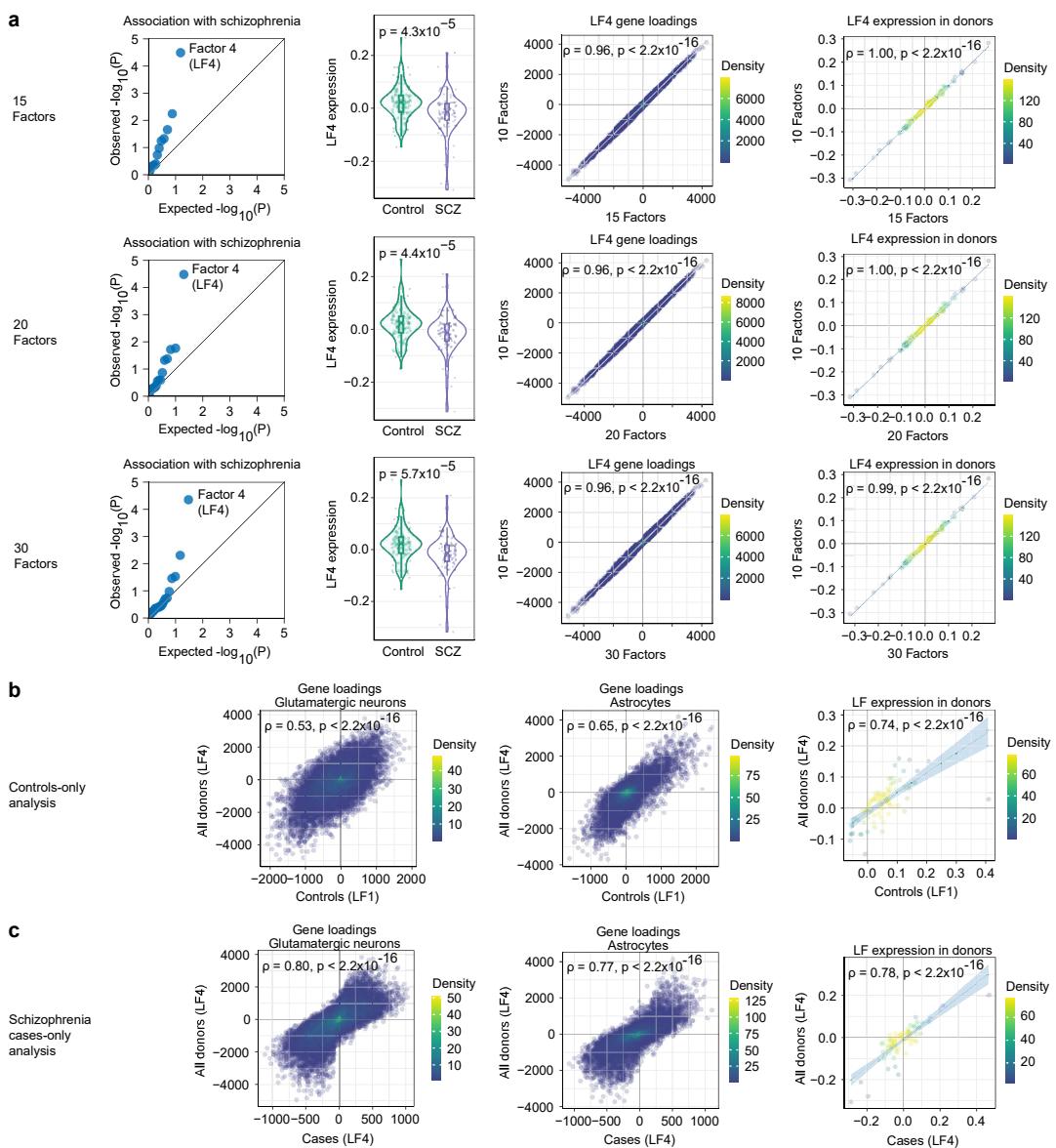
1724

1725 **b**, Column 1: Comparison of gene loadings from glutamatergic neurons ($n = 18,829$ genes) that demonstrates the relationship of LF4 inferred from an analysis of all donors to LF1 inferred from an analysis of only control donors (Spearman's ρ). Shaded regions around regression lines represent 95% confidence intervals. Column 2: Similar plot as in Column 1, here plotting gene loadings from astrocytes ($n = 18,346$ genes). Column 3: Comparison of donor expression levels ($n = 180$ donors) that demonstrates the relationship of LF4 inferred from an analysis of all donors to LF1 inferred from an analysis of only control donors (Spearman's ρ). Shaded regions around regression lines represent 95% confidence intervals.

1733

1734 **c**, Similar plots as in **b**, here for the relationship of LF4 inferred from an analysis of all donors to LF4 inferred from an analysis of only donors with schizophrenia.

Extended Data Figure 10



1736 **Extended Data Figure 11. Latent factor analysis of cerebrospinal fluid (CSF) proteomics**
1737 **data from different individuals identifies a factor resembling SNAP.**

1738

1739 To assess the biological significance of SNAP, we also sought evidence that SNAP manifests in
1740 the proteins that can be sampled from cerebrospinal fluid (CSF). We analyzed available data
1741 from a mass-spectrometry proteomics analysis of cerebrospinal fluid (CSF) from 22 healthy
1742 human donors ⁸², performing a latent factor analysis that is conceptually analogous to our
1743 analysis (in **Fig. 1f**) of cell-type-specific RNA-expression measurements in the brain donors (but
1744 of an independent data set, derived from a distinct set of donors). The top latent factor in
1745 analysis of the CSF proteomics data (explaining >15% of inter-individual variation in CSF
1746 protein measurements) bore a strong resemblance to SNAP.

1747

1748 **a**, Relationship of SNAP gene loadings to the top latent factor in an analysis of inter-individual
1749 variation in CSF protein levels (CSF LF1) using quantitative protein abundance measurements
1750 from ⁸² (Spearman's ρ ; $n = 1,341$ genes/proteins shared between both analyses). For SNAP,
1751 each gene is represented by a single composite loading representing gene loadings from all cell
1752 types (weighted by its median expression in each cell type). Shaded region represents 95%
1753 confidence interval.

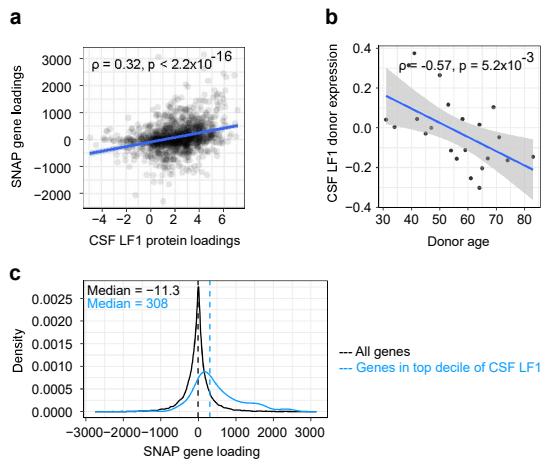
1754

1755 **b**, Relationship of CSF LF1 donor scores to age (Spearman's ρ ; $n = 22$ donors). Shaded region
1756 represents 95% confidence interval.

1757

1758 **c**, Density plot showing distribution of SNAP gene loadings for (black) all genes and genes
1759 encoding proteins that are strongly recruited (top decile) by (blue) CSF LF1. Distributions were
1760 found to be different by Wilcox test ($p = 2.1 \times 10^{-28}$, two-sided Wilcoxon rank-sum test).

Extended Data Figure 11

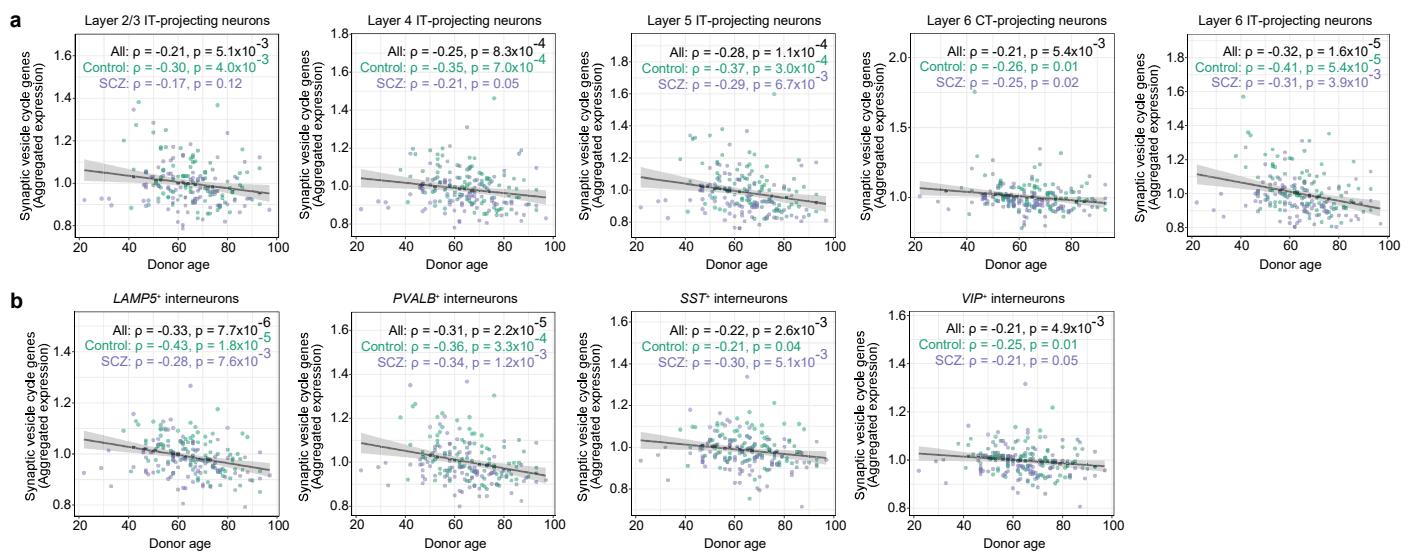


1761 **Extended Data Figure 12. Relationship of synaptic vesicle cycle gene expression in**
1762 **neuronal subtypes to advancing age.**

1763

1764 **a-b**, See also **Fig. 2c**. Neuronal expression of synaptic vesicle cycle genes in the most
1765 abundant subtypes of **(a)** glutamatergic and **(b)** GABAergic neurons (across 180 donors),
1766 plotted against donor age (Spearman's ρ). Expression values are the fraction of all UMIs in each
1767 donor (from the indicated subtype) that are derived from these genes, normalized to the median
1768 expression among control donors. Shaded regions represent 95% confidence intervals. The
1769 observed decline in schizophrenia and aging was consistent with earlier observations that
1770 expression of genes for synaptic components is reduced in schizophrenia¹⁶⁸ and with advancing
1771 age¹⁶⁹.

Extended Data Figure 12



1772 **Extended Data Figure 13. Relationship of gene-set expression in astrocytes and neurons**
1773 **to advancing age and schizophrenia.**

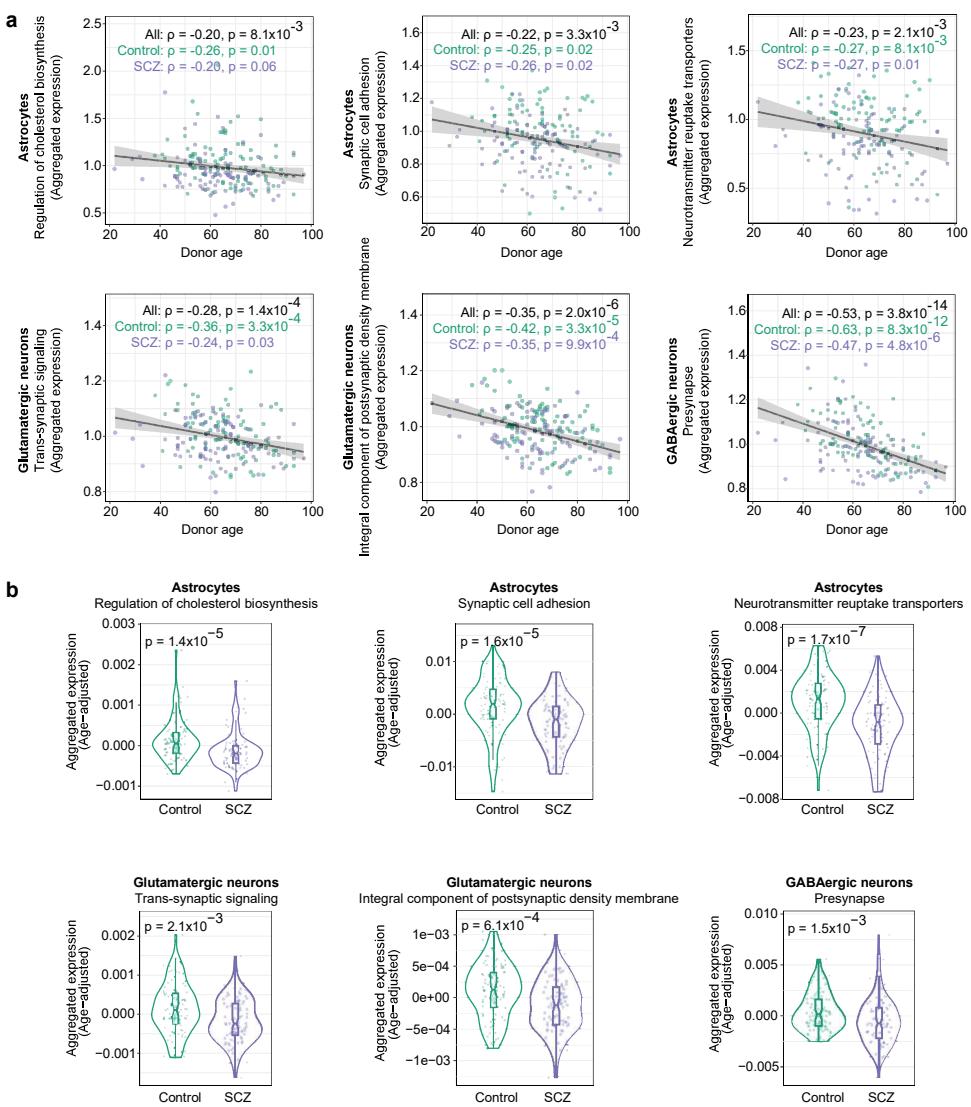
1774

1775 **a**, Expression of gene sets enriched in the astrocyte and neuronal components of LF4 (across
1776 180 donors), plotted against donor age (Spearman's ρ). Expression values are the fraction of all
1777 UMIs in each donor (from the indicated cell type) that are derived from these genes, normalized
1778 to the median expression among control donors. Shaded regions represent 95% confidence
1779 intervals.

1780

1781 **b**, Expression (by donor, separated by schizophrenia case-control status; $n = 180$ donors) of
1782 gene sets enriched in the astrocyte and neuronal components of LF4. Expression values are the
1783 fraction of all UMIs in each donor (from the indicated cell type) that are derived from these
1784 genes, adjusted for donor age. P-values from a two-sided Wilcoxon rank-sum test comparing
1785 the affected to the unaffected donors are reported at the top of each panel. Box plots show
1786 interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians; notches,
1787 confidence intervals around medians.

Extended Data Figure 13



1788 **Extended Data Figure 14. Expression of cholesterol-biosynthesis genes in cortical cell**
1789 **types.**

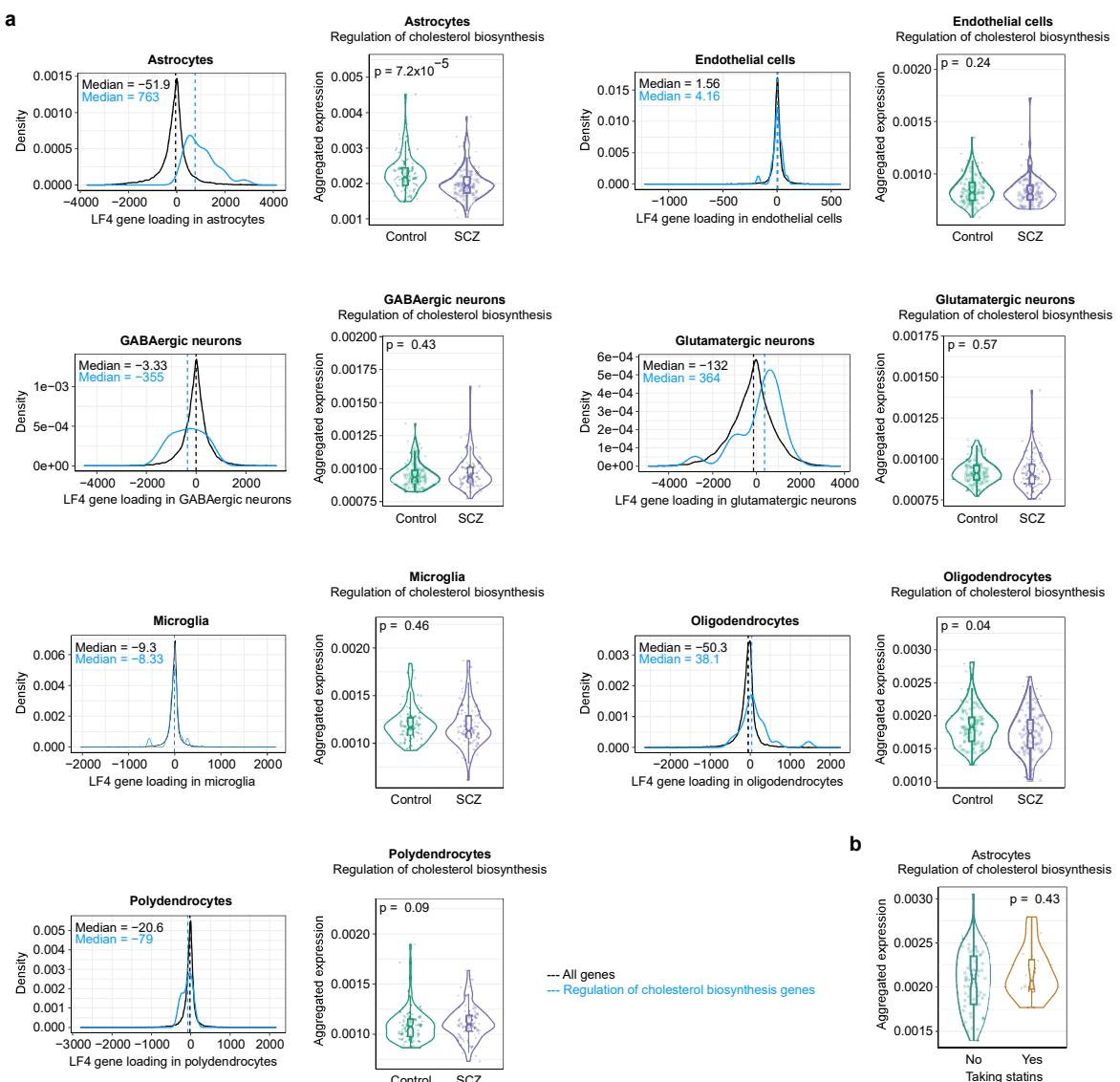
1790

1791 **a**, See also **Fig. 2d**. For each cortical cell type: (Left) Distributions of LF4 gene loadings for
1792 (black) all expressed genes and (blue) specifically for genes annotated by GO as having roles in
1793 cholesterol biosynthesis (core genes contributing to the enrichment of GO:0045540 (“cholesterol
1794 biosynthesis genes”) in that cell type’s component of LF4. (Right) Each cell type’s expression of
1795 cholesterol biosynthesis genes (by donor, split by schizophrenia case-control status; $n = 180$
1796 donors). Expression values are the fraction of all UMIs in each donor (from the indicated cell
1797 type) that are derived from these genes. P-values are from a two-sided Wilcoxon rank-sum test
1798 comparing the affected to the unaffected donors. Box plots show interquartile ranges; whiskers,
1799 1.5x the interquartile interval; central lines, medians; notches, confidence intervals around
1800 medians.

1801

1802 **b**, Expression in astrocytes of cholesterol biosynthesis genes by donor, separated by statin
1803 intake among donors with available medication data ($n = 63$ donors not taking statins and 16
1804 donors taking statins). Expression values are the fraction of all UMIs in each donor’s astrocytes
1805 that are derived from these genes. P-value is from a two-sided Wilcoxon rank-sum test. Box
1806 plots show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians;
1807 notches, confidence intervals around medians.

Extended Data Figure 14



1808 **Extended Data Figure 15. Concerted synaptic investments by neurons and astrocytes.**

1809 See also **Fig. 2e**.

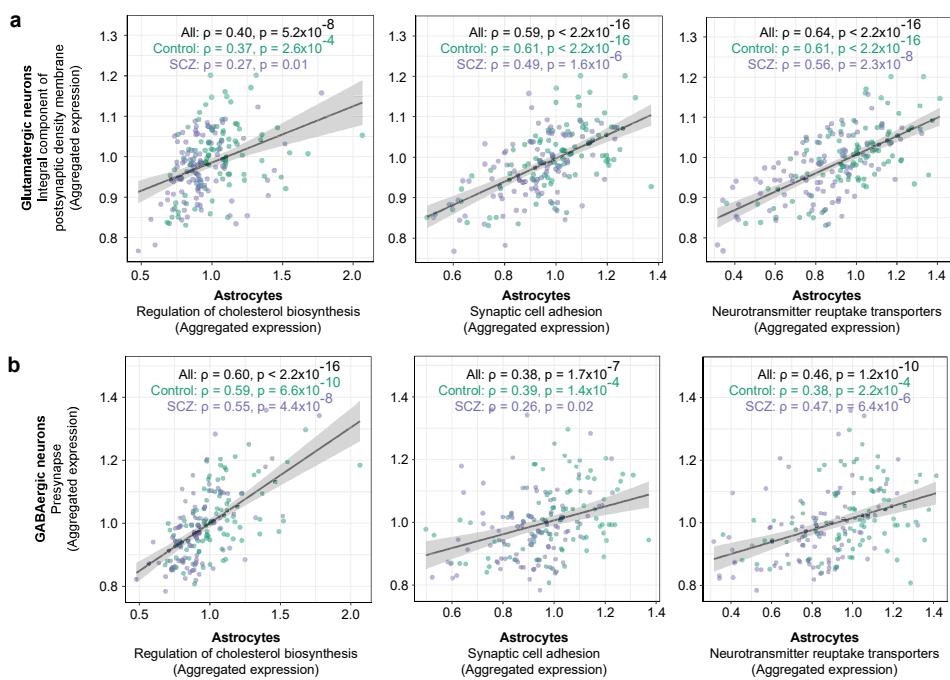
1810

1811 **a**, Relationship of donors' glutamatergic-neuron expression of genes that are integral
1812 components of the postsynaptic density membrane (core genes contributing to the enrichment
1813 of GO:0099061) to astrocyte expression of (top) cholesterol biosynthesis, (middle) synaptic
1814 adhesion, and (bottom) neurotransmitter reuptake transporters (Spearman's ρ). Expression
1815 values are the fraction of all UMIs in each donor (from the indicated cell type) that are derived
1816 from these genes, normalized to the median expression among control donors. Shaded regions
1817 represent 95% confidence intervals.

1818

1819 **b**, Similar plots as in **a**, here for donors' GABAergic-neuron expression of presynapse genes
1820 (core genes contributing to the enrichment of GO:0098793) on the x-axis.

Extended Data Figure 15

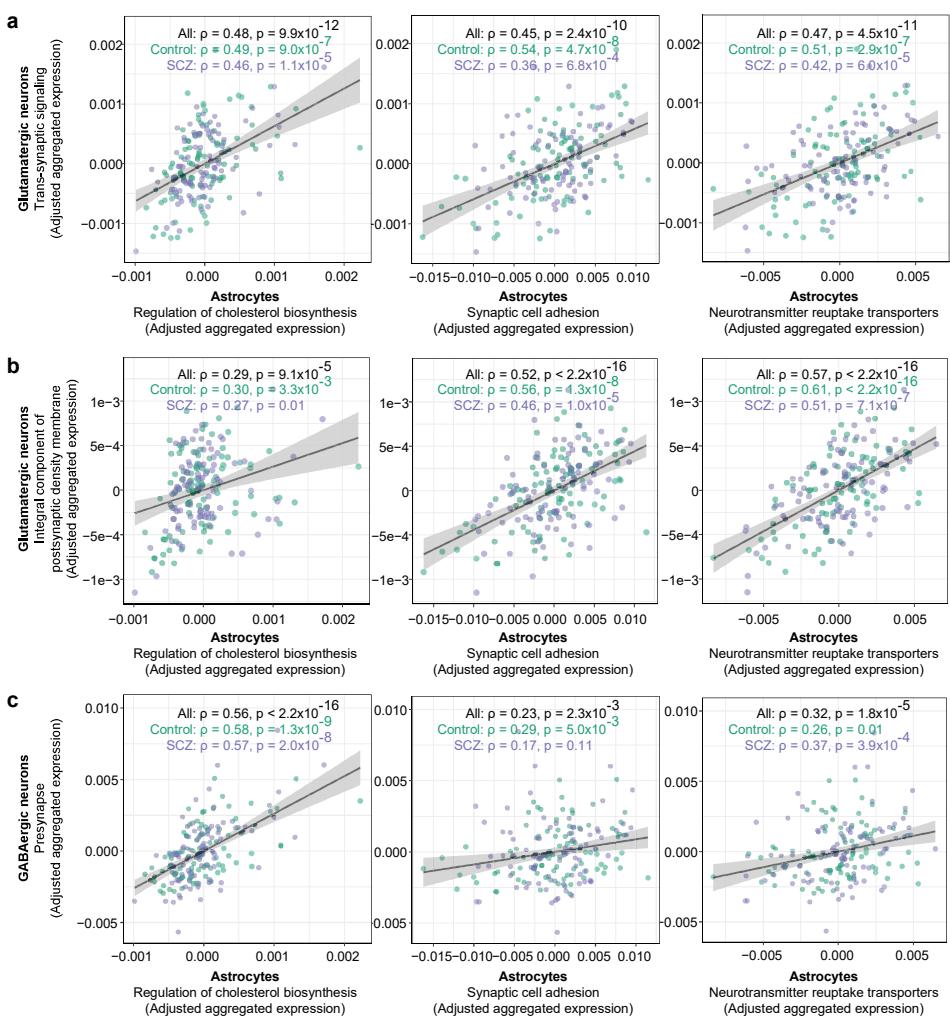


1821 **Extended Data Figure 16. Concerted synaptic investments by neurons and astrocytes,**
1822 **adjusted for age and schizophrenia case-control status.**

1823

1824 **a-c** See also **Fig. 2e**. Relationship of donors' neuronal gene expression to astrocyte gene
1825 expression (Spearman's ρ), adjusted for age and case-control status. Astrocyte gene sets
1826 plotted on the x-axes represent (left) cholesterol biosynthesis, (middle) synaptic adhesion, and
1827 (right) neurotransmitter reuptake transporters. Neuronal gene sets plotted on the y-axes
1828 represent **(a)** trans-synaptic signaling in glutamatergic neurons, **(b)** integral component of
1829 postsynaptic density, and **(c)** presynapse genes. Expression values are the fraction of all UMIs
1830 in each donor (from the indicated cell type) that are derived from these genes, adjusted for
1831 donor age and schizophrenia case-control status. Shaded regions represent 95% confidence
1832 intervals.

Extended Data Figure 16



1833 **Extended Data Figure 17. Astrocyte subtype classification and proportions across**
1834 **donors.**

1835

1836 **a**, Two-dimensional projection of the RNA-expression profiles of 179,764 astrocyte nuclei from
1837 180 donors, reproduced from **Fig. 3a**. Nuclei are colored by their assignments to subtypes of
1838 astrocytes using classifications from ⁷⁵ and ⁷⁶. The same projection is used in panels B to D
1839 below.

1840

1841 **b-d**, Expression levels of marker genes for subtypes of **(b)** protoplasmic astrocytes (*SLC1A3+*)
1842 and non-protoplasmic astrocytes (*SLC1A3-* and *GFAP+*) comprising the **(c)** fibrous (*AQP1+*)
1843 and **(d)** interlaminar (*AQP1-* and *ID3+*, *SERPINI2+*, and *WDR49+*) subtypes. Markers are from
1844 ⁷⁵ or from transcriptomically similar subtypes in ⁷⁶. Values represent Pearson residuals from
1845 variance stabilizing transformation (VST).

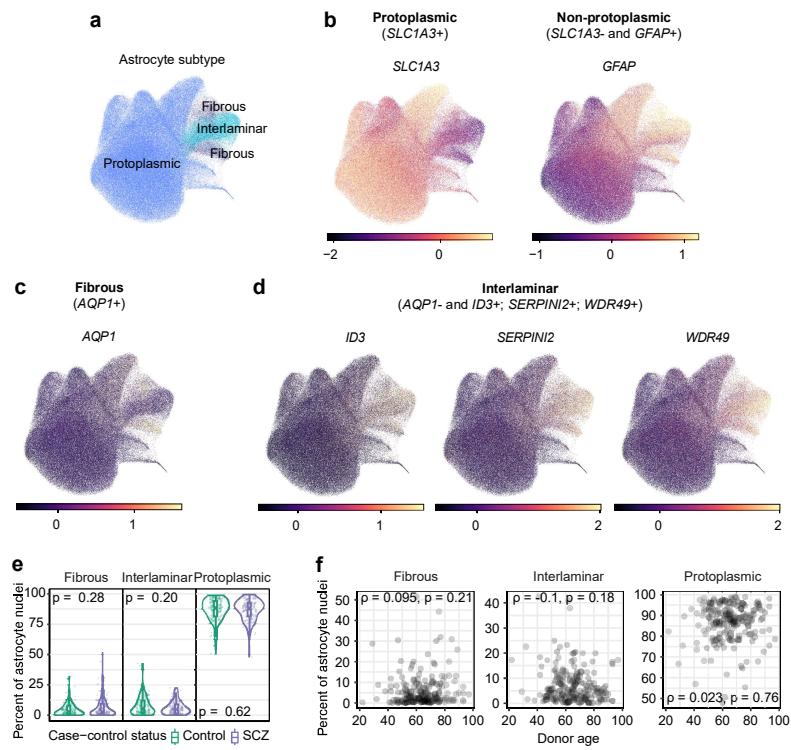
1846

1847 **e**, Proportions of astrocyte subtypes in BA46 by schizophrenia status ($n = 93$ unaffected and 87
1848 affected). P-values from a two-sided Wilcoxon rank-sum test comparing the affected to the
1849 unaffected donors are reported at the top of each panel. Box plots show interquartile ranges;
1850 whiskers, 1.5x the interquartile interval; central lines, medians; notches, confidence intervals
1851 around medians.

1852

1853 **f**, Relationship of sampled astrocyte subtype proportions to donor age (Spearman's ρ).

Extended Data Figure 17



1854 **Extended Data Figure 18. Astrocyte gene-expression programs inferred by cNMF (SNAP-
1855 a) and their relationship to SNAP.**

1856

1857 **a**, Visualization of the trade-off between error and stability of cNMF factors as a function of the
1858 number of factors k . 11 factors were requested based on these results.

1859

1860 **b**, Clustergram of consensus matrix factorization estimates. Each color on the x- and y-axes
1861 represents one of 11 cNMF factors.

1862

1863 **c-d**, Relationship of SNAP-a to SNAP by **(c)** gene loadings ($n = 33,611$ genes) and **(d)** donors'
1864 expression levels of each factor ($n = 180$ donors) (Spearman's ρ). Shaded regions represent
1865 95% confidence intervals.

1866

1867 **e**, UMAP of RNA-expression patterns from 179,764 astrocyte nuclei from 180 donors, using the
1868 same projection from **Fig. 3a-c**. Nuclei are colored by (left) each donor's expression of SNAP or
1869 (right) each cell's expression of the astrocyte component of SNAP (cNMF2, also referred to as
1870 SNAP-a). SNAP-a is reproduced from **Fig. 3c** for comparison with SNAP.

1871

1872 **f**, Distributions of SNAP-a expression levels among astrocytes in each donor, split by
1873 experimental batch. Box plots show interquartile ranges; whiskers, 1.5x the interquartile interval;
1874 central lines, medians.

1875

1876 **g**, Density plots showing distributions of SNAP-a expression levels among astrocytes in each
1877 donor for one representative batch (batch 4) out of 11 batches. Labels in top-right corners
1878 indicate anonymized research IDs at the Harvard Brain Tissue Resource Center. Colors
1879 represent case-control status (green: controls; purple: schizophrenia cases). At the single-
1880 astrocyte level, SNAP-a expression exhibited continuous, quantitative variation rather than
1881 discrete state shifts by a subpopulation of astrocytes, supporting the idea that astrocyte
1882 biological variation extends beyond polarized states^{17,170,171}.

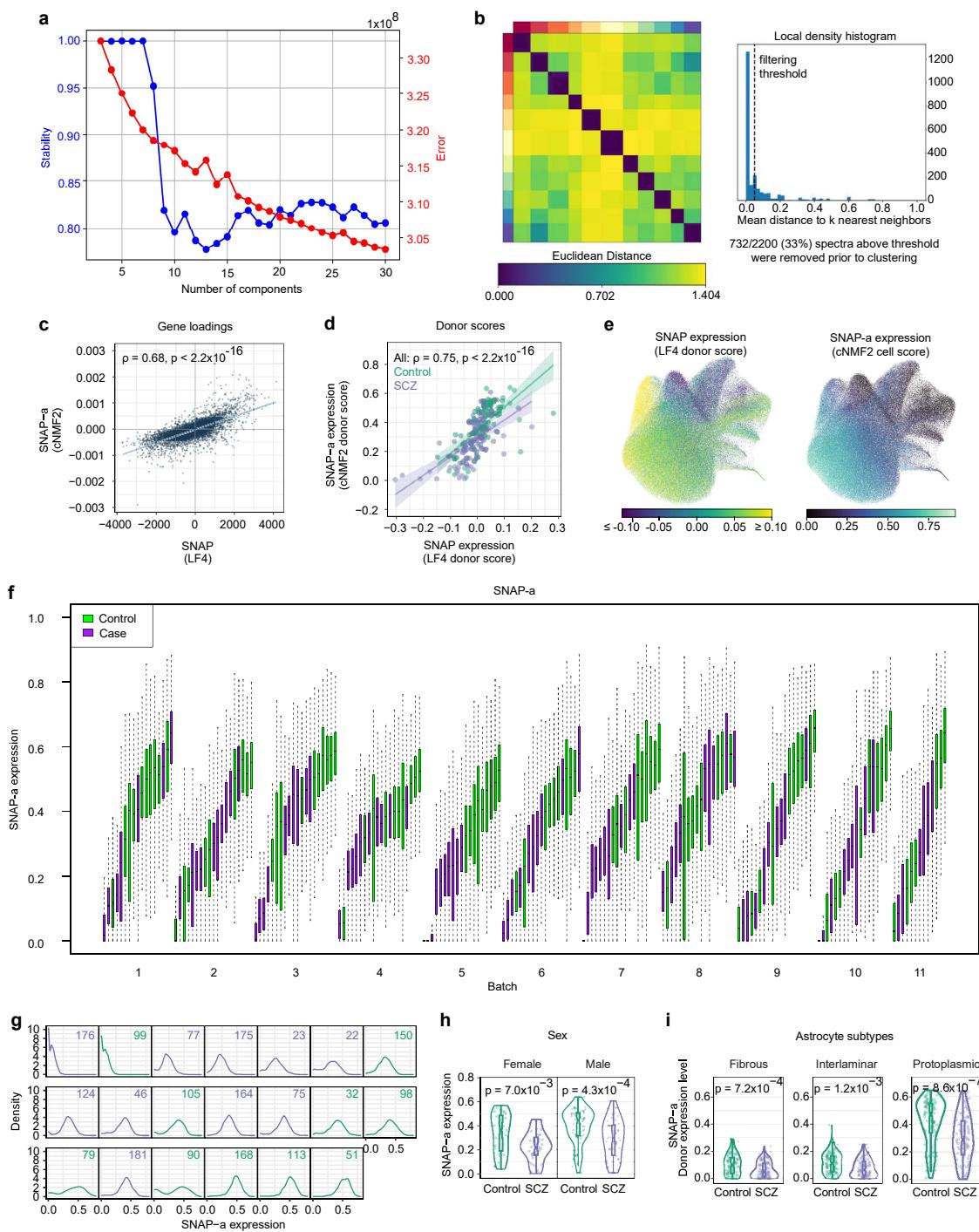
1883

1884 **h**, Distributions of SNAP-a expression levels by case-control status, split by sex. P-values from
1885 a two-sided Wilcoxon rank-sum test comparing the affected to the unaffected donors are
1886 reported at the top of each panel. Box plots show interquartile ranges; whiskers, 1.5x the
1887 interquartile interval; central lines, medians; notches, confidence intervals around medians.

1888

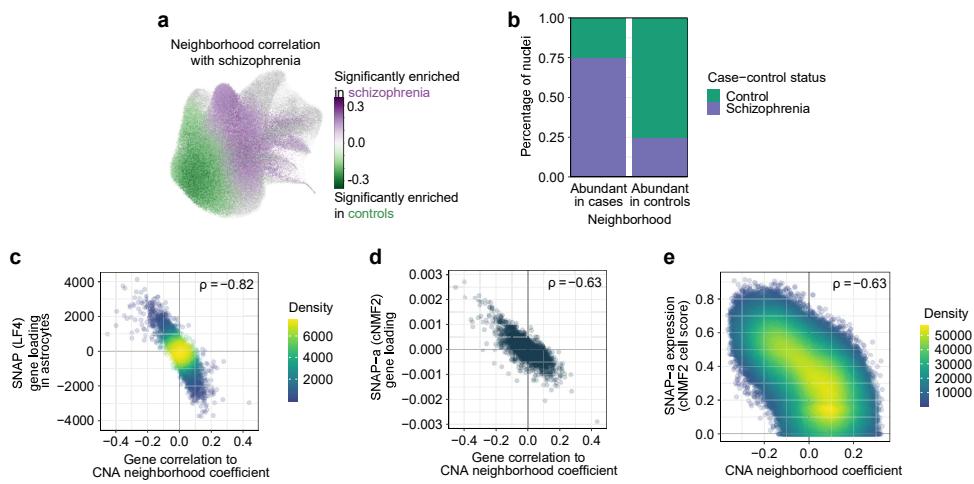
1889 **i**, Distributions of SNAP-a expression levels by case-control status, split by astrocyte subtype.
1890 P-values from a two-sided Wilcoxon rank-sum test comparing the affected to the unaffected
1891 donors are reported at the top of each panel. Box plots show interquartile ranges; whiskers, 1.5x
1892 the interquartile interval; central lines, medians; notches, confidence intervals around medians.

Extended Data Figure 18



1893 **Extended Data Figure 19. Identification of astrocyte transcriptional neighborhoods**
1894 **associated with schizophrenia case-control status by co-varying neighborhood analysis.**
1895 To further assess the robustness of the astrocyte gene-expression changes represented by
1896 SNAP and SNAP-a, we employed a third computational approach, co-varying neighborhood
1897 analysis (CNA)⁹⁶.
1898
1899 **a**, Same projection as in **Fig. 3a-c**, but with points colored according to their transcriptional
1900 neighborhood's correlation to schizophrenia case-control status ($n = 179,764$ astrocyte nuclei
1901 from 180 donors). Among cells whose neighborhood coefficients passed an $FDR < 0.05$
1902 threshold for association, purple indicates high correlation to case status and green indicates
1903 high correlation to control status. All other cells with $FDR > 0.05$ for association are colored in
1904 gray.
1905
1906 **b**, Proportion of nuclei in each of the indicated astrocyte transcriptional neighborhoods that are
1907 assigned to schizophrenia cases and controls ($n = 34,271$ nuclei abundant in cases and 38,327
1908 nuclei abundant in controls).
1909
1910 **c-d**, Relationship of genes' correlation to schizophrenia-associated transcriptional
1911 neighborhoods to **(c)** the astrocyte component of SNAP ($n = 8,997$ shared genes) and **(d)**
1912 SNAP-a by their gene loadings ($n = 9,015$ shared genes) (Spearman's ρ). Genes plotted are the
1913 subsets of protein-coding genes (with expression levels of at least 1 UMI per 10^5) that are
1914 shared between the indicated pairs of analyses.
1915
1916 **e**, Relationship of cell-level neighborhood coefficients for schizophrenia-associated
1917 transcriptional neighborhoods to SNAP-a cell scores (Spearman's ρ ; $n = 179,764$ astrocytes).

Extended Data Figure 19

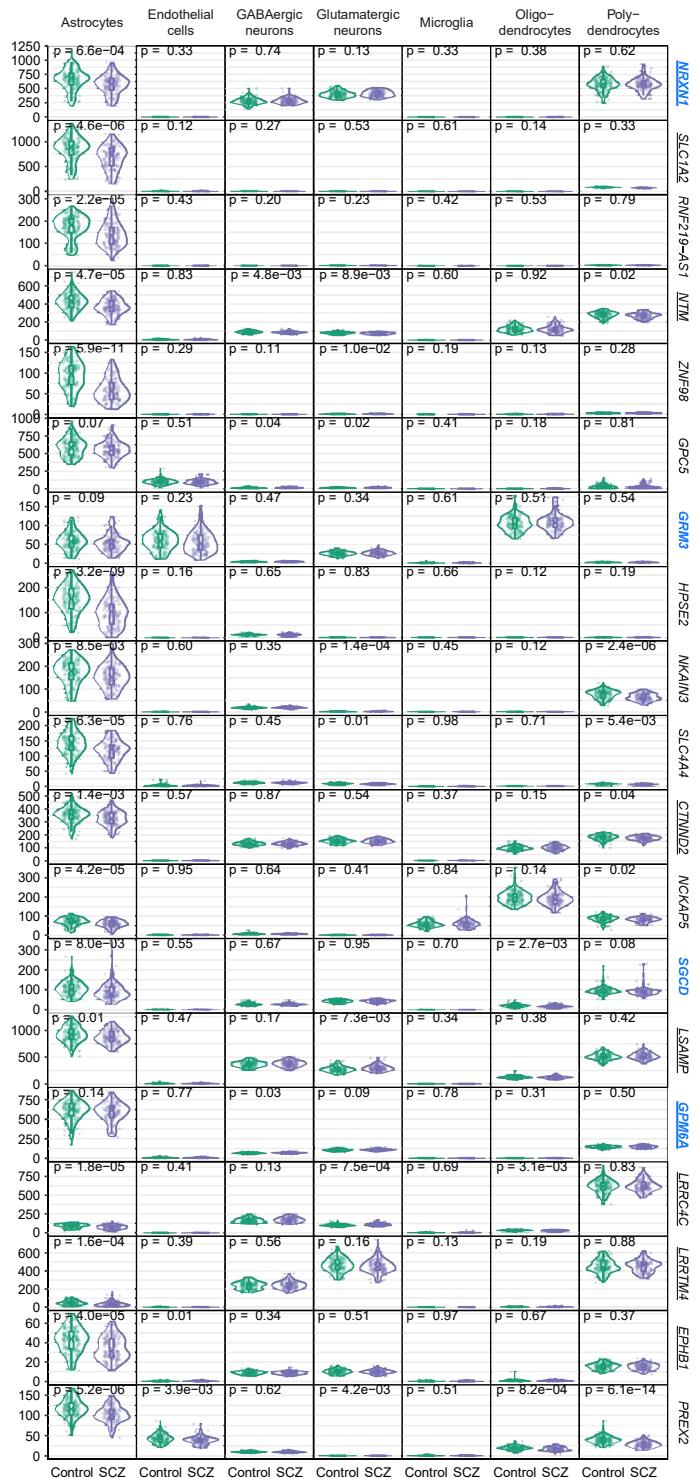


1918 **Extended Data Figure 20. Expression across cell types of genes most strongly recruited
1919 by SNAP-a.**

1920

1921 Expression in each cell type (by donor, separated by schizophrenia status), of the 20 genes that
1922 are most strongly recruited by SNAP-a ($n = 93$ unaffected (green) and 87 affected (purple)
1923 donors). These included eight genes with roles in adhesion of cells to synapses (*NRXN1*, *NTM*,
1924 *CTNND2*, *LSAMP*, *GPM6A*, *LRRC4C*, *LRRTM4*, and *EPHB1*) (as established by earlier work
1925 including ^{172–181} and reviewed in ^{11,12}). P-values from a two-sided Wilcoxon rank-sum test
1926 comparing the affected to the unaffected donors are reported at the top of each panel. Box plots
1927 show interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians;
1928 notches, confidence intervals around medians. Genes that have been strongly implicated in
1929 human genetic studies of schizophrenia are highlighted in blue. Genes with known functions in
1930 synaptic adhesion (listed above) or neurotransmitter uptake (*SLC1A2*) are underlined.

Extended Data Figure 20

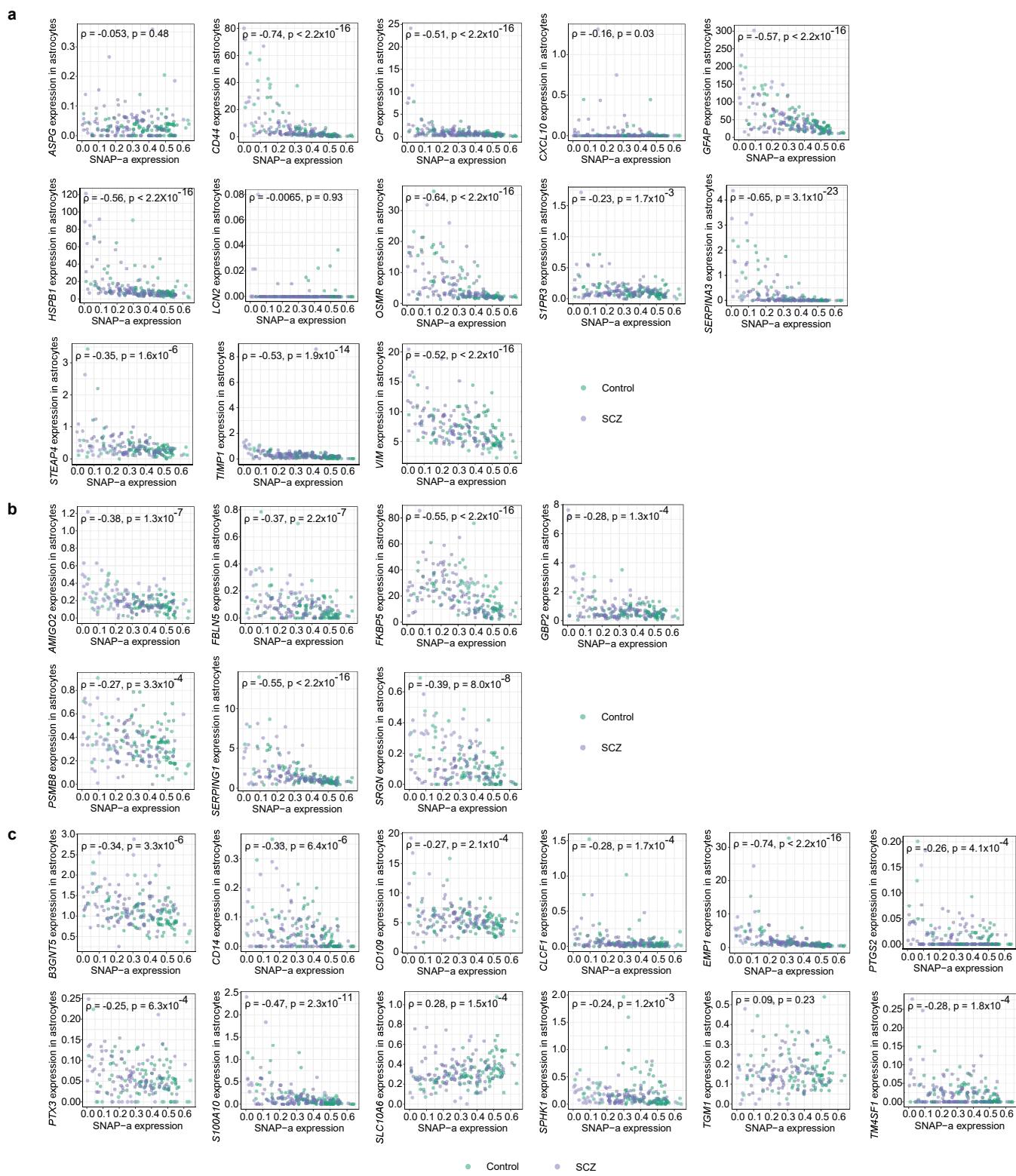


1931 **Extended Data Figure 21. Relationship of reactive astrocyte marker expression to SNAP-**
1932 **a expression.**

1933

1934 Relationship of donors' expression levels of reactive astrocyte marker genes to SNAP-a
1935 expression (Spearman's ρ). Markers are from ¹⁶ and represent **(a)** pan-reactive (PAN), **(b)** A1,
1936 and **(c)** A2 reactive astrocytes.

Extended Data Figure 21



1937 **Extended Data Figure 22. Biological states and transcriptional programs of L5 IT**
1938 **glutamatergic neurons in schizophrenia.**

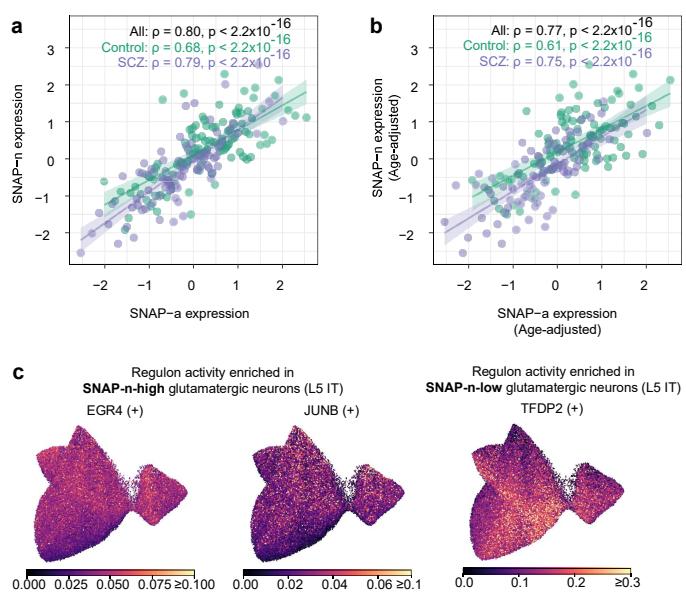
1939

1940 **a-b**, Relationship of SNAP-a to SNAP-n (Spearman's ρ). Values plotted are **(a)** quantile-
1941 normalized and **(b)** donor age-adjusted, quantile-normalized donor scores for each factor.
1942 Shaded regions represent 95% confidence intervals.

1943

1944 **c**, UMAP of regulon activity scores (as inferred by pySCENIC ⁹⁸) from L5 IT glutamatergic
1945 neuron nuclei from 180 donors, using the same projection from **Fig. 3f-h**. Regulons plotted are
1946 the most strongly enriched in L5 IT glutamatergic neurons with high versus low SNAP-n
1947 expression. (+) indicates that the targets of the indicated regulon were found to be upregulated
1948 in expression.

Extended Data Figure 22

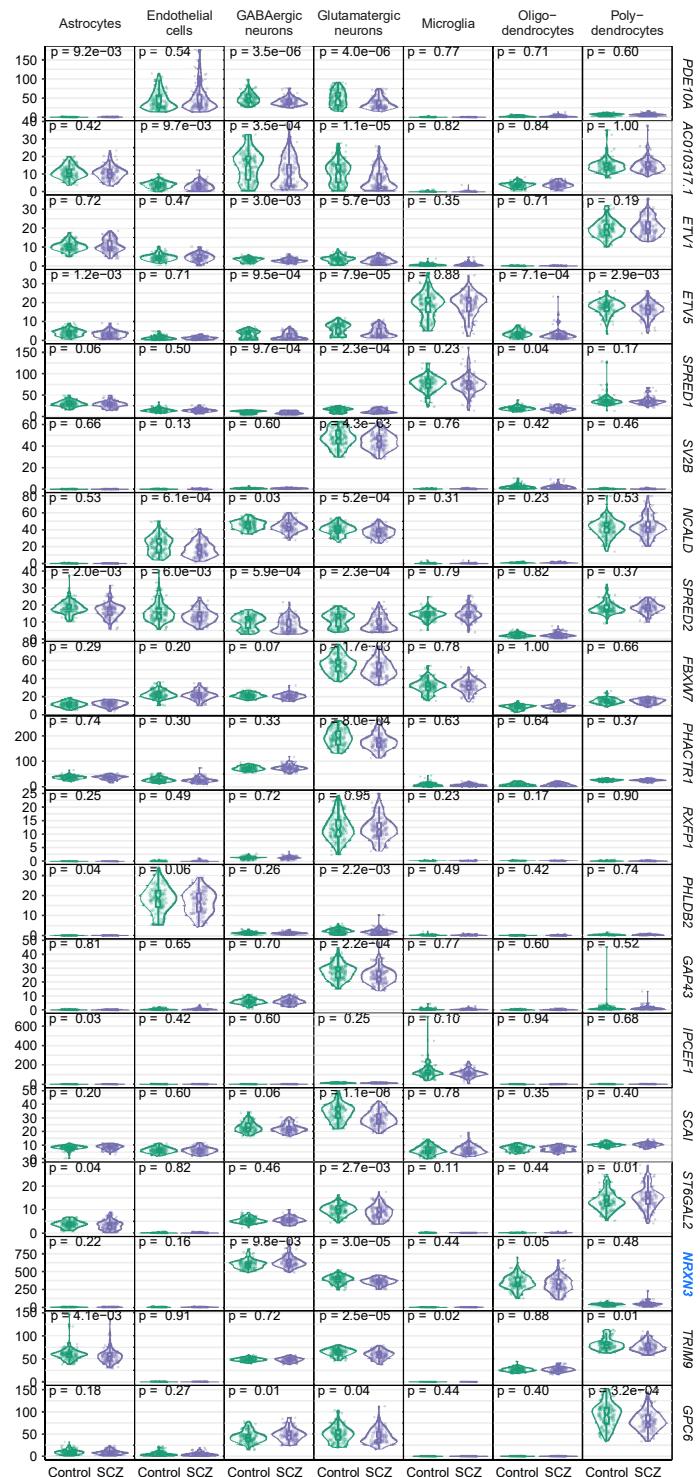


1949 **Extended Data Figure 23. Expression across cell types of genes most strongly recruited
1950 by SNAP-n.**

1951

1952 Expression in each cell type (by donor, separated by schizophrenia case-control status) of the
1953 20 genes that are most strongly recruited by SNAP-n ($n = 93$ controls and 87 cases). P-values
1954 from a two-sided Wilcoxon rank-sum test comparing the affected to the unaffected donors are
1955 reported at the top of each panel. Box plots show interquartile ranges; whiskers, 1.5x the
1956 interquartile interval; central lines, medians; notches, confidence intervals around medians.
1957 Genes that have been strongly implicated in human genetic studies of schizophrenia are
1958 highlighted in blue.

Extended Data Figure 23



1959 **Extended Data Figure 24. Astrocyte gene-expression programs underlying SNAP-a.**

1960

1961 **a**, See also **Fig. 3k**. Concerted expression in (left) astrocytes and (right) GABAergic neurons of
1962 genes strongly recruited by SNAP-a. These were enriched in genes encoding synaptic-adhesion
1963 proteins, intrinsic components of synaptic membranes such as transporters and receptors, as
1964 well as genes strongly implicated in human genetic studies of schizophrenia. Genes in the
1965 “Schizophrenia genetics” heatmap are from among the prioritized genes from ²³ (FDR < 0.05) or
1966 ²². Genes annotated by [^] are from among all genes at loci implicated by common variants in ²²,
1967 regardless of prioritization status.

1968

1969 **b**, UMAP of regulon activity scores (as inferred by pySCENIC ⁹⁸) from 179,764 astrocyte nuclei
1970 from 180 donors, using the same projection from **Fig. 3a-c**. Regulons plotted are the most
1971 strongly enriched in astrocytes with high versus low SNAP-a expression. (+) indicates that the
1972 targets of the indicated regulon are predicted to be upregulated in expression.

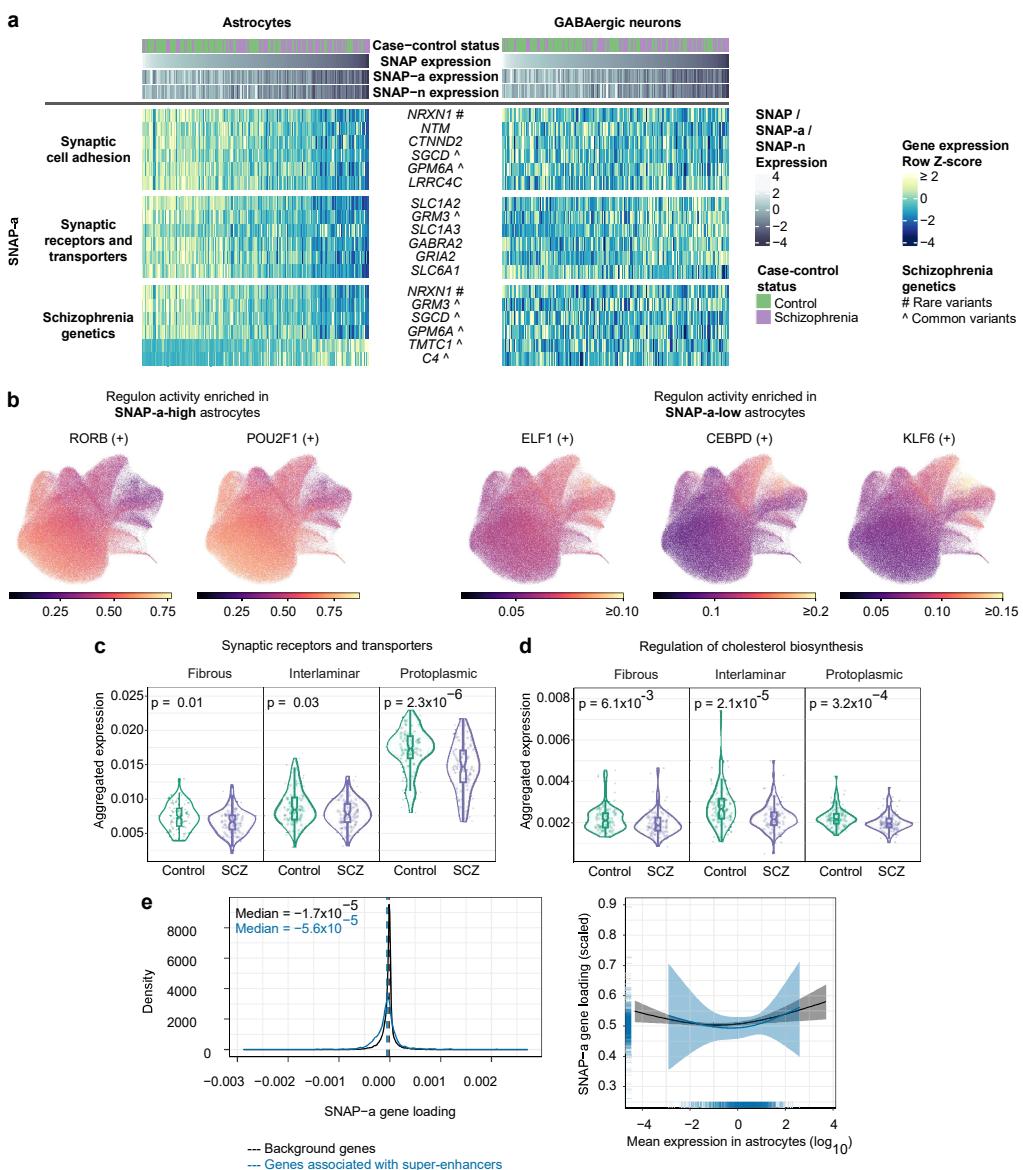
1973

1974 **c-d**, Transcriptional investments (by donor, separated by schizophrenia case-control status) in
1975 **(c)** genes encoding synaptic receptors and transporters and **(d)** cholesterol biosynthesis genes,
1976 in subtypes of astrocytes. Quantities plotted are the fraction of all UMs in each subtype that are
1977 derived from these genes. P-values from a two-sided Wilcoxon rank-sum test comparing the
1978 affected to the unaffected donors are reported at the top of each panel. Box plots show
1979 interquartile ranges; whiskers, 1.5x the interquartile interval; central lines, medians; notches,
1980 confidence intervals around medians.

1981

1982 **e**, Relationship of SNAP-a expression to association with super-enhancers. Genes expressed in
1983 astrocytes were grouped based on whether their promoters were predicted to contact super-
1984 enhancers in astrocytes (using bulk H3K27ac HiChIP and scATAC-seq data from ⁹⁹), and
1985 SNAP-a loadings were compared between the two groups. (Left) Distributions of SNAP-a gene
1986 loadings for (blue) 1,286 genes whose promoters are predicted to contact super-enhancers in
1987 astrocytes and (black) the set of 32,325 remaining expressed background genes. (Right)
1988 Binomial smooth results of scaled SNAP-a gene loadings versus log₁₀-scaled mean expression
1989 values in astrocytes, shown separately for the two groups. Shaded regions represent 95%
1990 confidence intervals.

Extended Data Figure 24



1991 **Extended Data Figure 25. Expression of well-characterized transcriptional programs in**
1992 **SNAP-a and SNAP-n.**

1993

1994 **a**, Concerted expression in (left) astrocytes and (right) L5 IT glutamatergic neurons of target
1995 genes of known transcriptional programs specifically active in SNAP-a or SNAP-n. Genes are
1996 listed in decreasing order by their importance for each regulon as scored by pySCENIC.

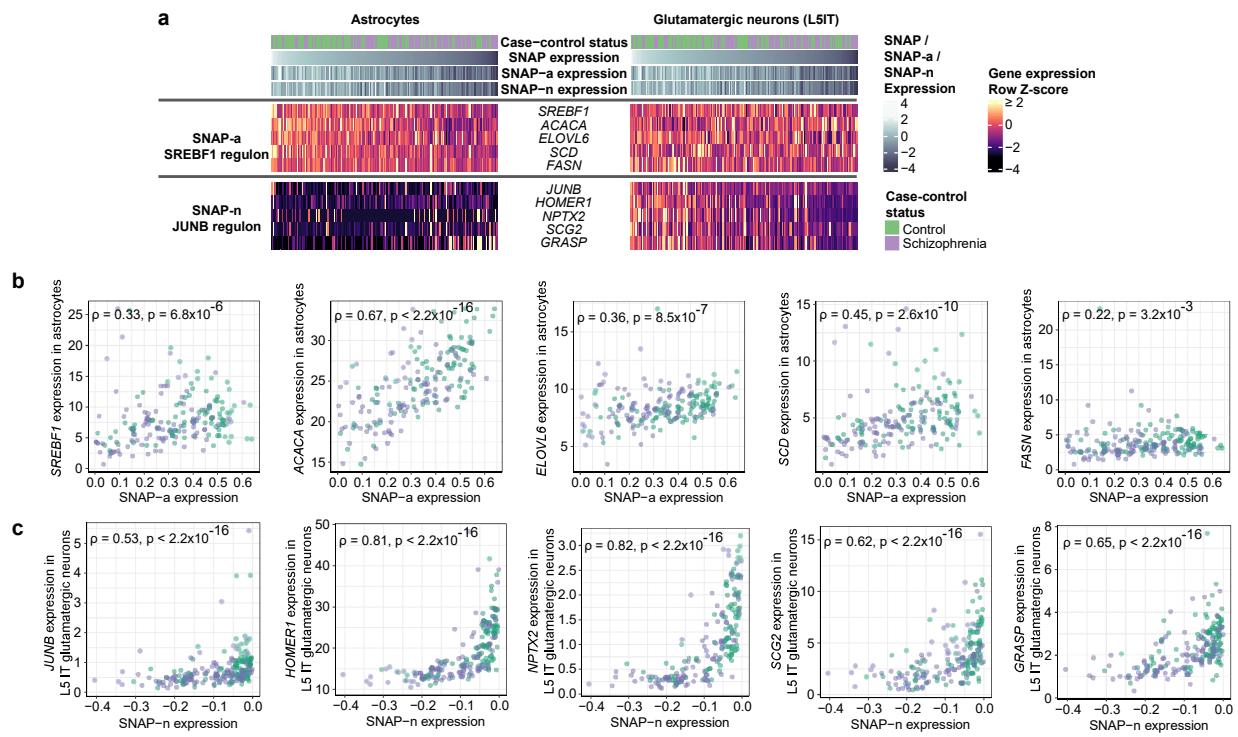
1997

1998 **b**, Relationship of donors' expression levels of known SREBP1 target genes (involved in fatty
1999 acid biosynthesis)^{18,182,183} to SNAP-a expression (Spearman's ρ). Target-gene expression levels
2000 in astrocytes are shown.

2001

2002 **c**, Relationship of donors' expression levels of known JUNB target genes (that are late-
2003 response genes)^{19,20,184} to SNAP-n expression (Spearman's ρ). Target-gene expression levels in
2004 L5 IT glutamatergic neurons are shown.

Extended Data Figure 25

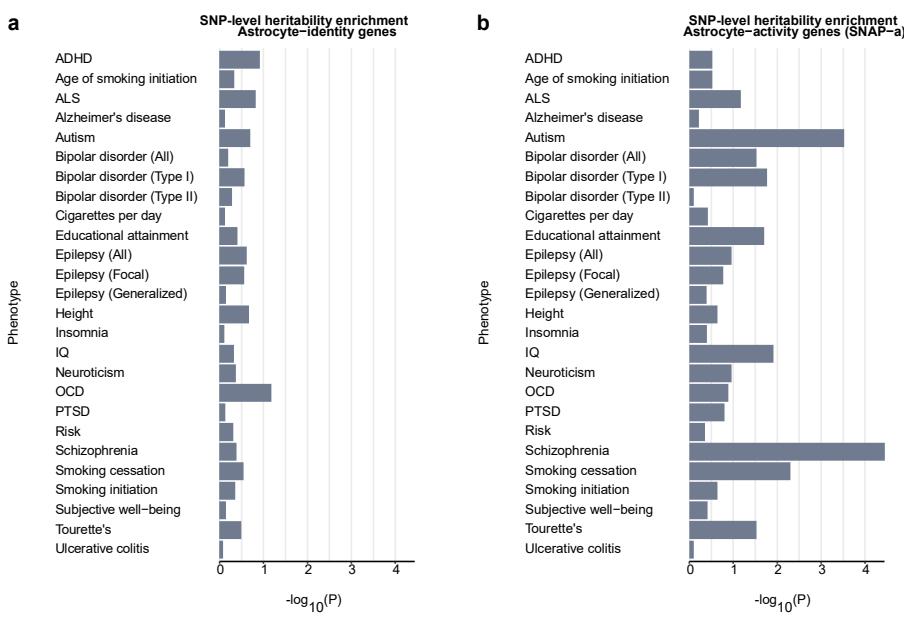


2005 **Extended Data Figure 26. Heritability enrichment for 26 traits among the top 2,000**
2006 **astrocyte-identity or astrocyte-activity (SNAP-a) genes.**

2007

2008 Heritability enrichment analysis for the indicated phenotypes in regions surrounding **(a)** the
2009 2,000 genes most preferentially expressed in astrocytes compared to other cell types or **(b)** the
2010 2,000 genes most strongly recruited by SNAP-a in astrocytes. Summary statistics are from the
2011 following studies: ADHD ¹¹², age of smoking initiation ¹¹⁵, ALS ¹¹³, Alzheimer's disease ¹¹⁴, autism
2012 ¹¹⁶, bipolar disorder (all, type I, and type II) ¹¹⁷, cigarettes per day ¹¹⁵, educational attainment ¹¹⁸,
2013 epilepsy (all, focal, generalized) ¹¹⁹, height ¹²⁰, insomnia ¹²², IQ ¹²¹, neuroticism ¹²³, OCD ¹²⁴,
2014 PTSD ¹²⁵, risk ¹²⁶, schizophrenia ²², smoking cessation ¹¹⁵, smoking initiation ¹¹⁵, subjective well-
2015 being ¹²⁷, Tourette's ¹²⁸, ulcerative colitis ¹²⁹.

Extended Data Figure 26



2016 **Extended Data Figure 27. Calculation of polygenic risk scores for schizophrenia.**

2017

2018 **a**, Association of polygenic risk scores (PRS) for schizophrenia (from PGC3 GWAS, ²²) with
2019 schizophrenia case-control status, displayed as a quantile-quantile plot that compares PRS of
2020 control donors to the PRS of donors with schizophrenia ($n = 191$ donors).

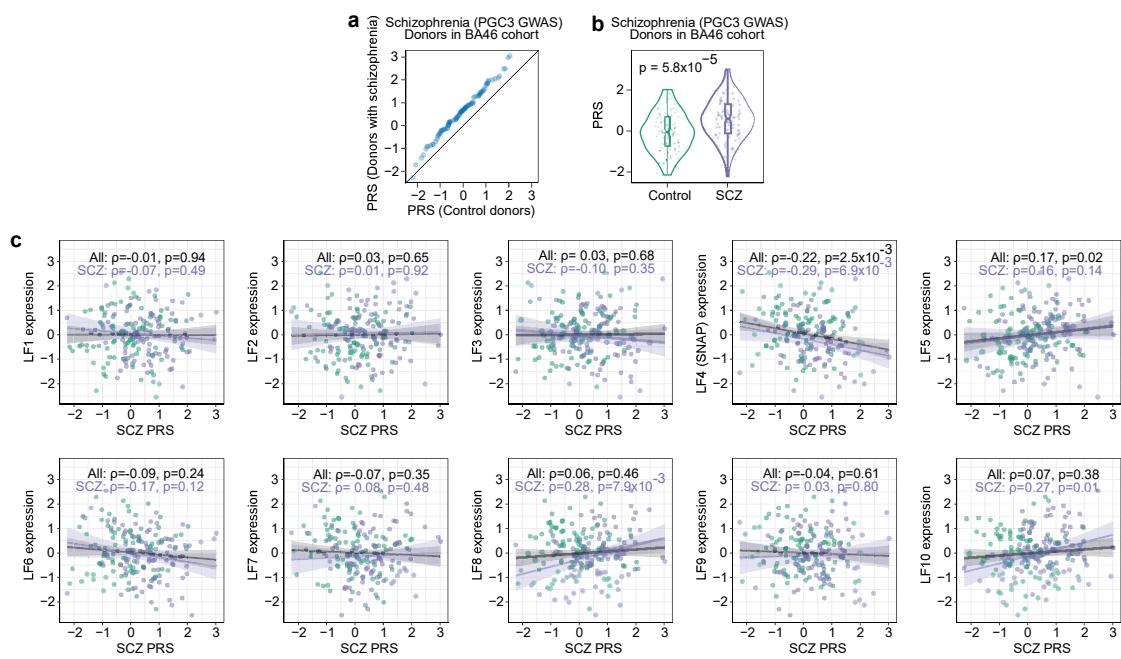
2021

2022 **b**, Distributions of schizophrenia PRS for 94 schizophrenia cases and 97 controls. P-value is
2023 from a two-sided Wilcoxon rank-sum test. Box plots show interquartile ranges; whiskers, 1.5x
2024 the interquartile interval; central lines, medians; notches, confidence intervals around medians.

2025

2026 **c**, See also **Fig. 4b**. Relationship of inter-individual variation in expression of each of the 10
2027 latent factors inferred by PEER (donor scores, quantile-normalized) to donors' polygenic risk
2028 scores (PRSSs) for schizophrenia (Spearman's ρ ; PGC3 GWAS from ²²). Shaded regions
2029 represent 95% confidence intervals. The observed relationship of schizophrenia PRS to
2030 expression of LF4 – which associates with schizophrenia and aging – is consistent with previous
2031 observations that a PRS for schizophrenia also associates with decreased measures of
2032 cognition in older individuals ⁴⁸ and with psychosis in Alzheimer's Disease ¹⁸⁵.

Extended Data Figure 27



2033 **Extended Data Figure 28. Relationship of astrocytic *NRXN1* and *C4* expression to**
2034 **advancing age.**

2035

2036 **a**, Relationship of *NRXN1* expression to age in astrocytes (Spearman's ρ). Shaded region
2037 represents 95% confidence interval.

2038

2039 **b**, Expression of *NRXN1* in astrocytes in control donors, split by donor age ($n = 56$ donors
2040 younger than 70 years old and 37 donors 70 years old or older). P-value is from a two-sided
2041 Wilcoxon rank-sum test. Box plots show interquartile ranges; whiskers, 1.5x the interquartile
2042 interval; central lines, medians; notches, confidence intervals around medians.

2043

2044 **c**, Validation of a metagene computational approach for identifying RNA transcripts (UMIs) from
2045 the *C4* genes. Standard analysis approaches tend to discard sequence reads from *C4A* or *C4B*
2046 because these genes are almost identical in sequence, differing only at a few key positions (far
2047 from the 3' end), such that most reads are discarded due to low mapping quality. To measure
2048 expression of these genes, UMIs were either aligned to a custom reference genome that
2049 contained only one *C4* gene (x-axis) or were processed through a custom pipeline that identified
2050 UMIs associated with sets of gene families with high sequence homology, including *C4A/C4B*
2051 (y-axis). The two approaches (custom reference approach and joint expression of *C4A/C4B* via
2052 the metagene approach) arrived at concordant *C4* UMI counts in 15,664 of 15,669 cells tested.
2053 Note that these measurements do not distinguish between *C4A* and *C4B*.

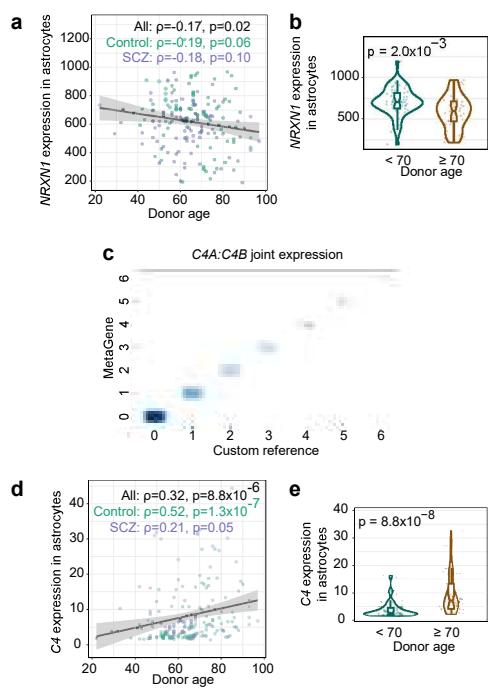
2054

2055 **d**, Relationship of *C4* expression to age in astrocytes (Spearman's ρ). Shaded region
2056 represents 95% confidence interval.

2057

2058 **e**, Expression of *C4* in astrocytes in control donors, split by donor age ($n = 56$ donors younger
2059 than 70 years old and 37 donors 70 years old or older). P-value is from a two-sided Wilcoxon
2060 rank-sum test. Box plots show interquartile ranges; whiskers, 1.5x the interquartile interval;
2061 central lines, medians; notches, confidence intervals around medians.

Extended Data Figure 28



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