

1 **ZBTB7A regulates MDD-specific chromatin signatures and astrocyte-mediated stress**
2 **vulnerability in orbitofrontal cortex**

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77 I.M. wrote the manuscript. S.L.F. performed motif analyses on ATAC-seq data, viral cloning and
78 generation, cell culture experiments, viral manipulations, mouse behavior, molecular experiments
79 on ZBTB7A, chemogenetics experiments, RNA-sequencing, mouse ATAC-sequencing, and
80 RNA-sequencing analysis. M.C. performed IHC imaging. A.A, A.E.L, R.M.B., F.C., and A.F.S.
81 assisted with animal viral surgeries and behaviors. S.L.F. and A.A. generated CSDS behavior for
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83 validations, and contributed data interpretation for these experiments. J.F.F. performed FANS-
84 ATAC-sequencing sample and library preparation. J.B. contributed analyses of ATAC-sequencing
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86 deconvolution, and footprinting analyses. P.R. designed and supervised generation of FANS-
87 ATAC sequencing, as well as contributed data analysis and interpretation of the data. M.E.H.
88 prepared a pipeline for preprocessing of ATAC-seq data and contributed to QC of ATAC-seq data.
89 R.D.C. assisted with mouse operant behavior paradigms. I.G performed calcium imaging and P.S.
90 supervised analysis. S.S., W.F.P, and F.K.S contributed flow cytometry assessments of mouse
91 OFC. A.K.F. and R.D.B. performed electrophysiological recordings and data analysis. A.M. and

92 S.J.R. contributed TRAP-seq data. K.G. and C.A.T. performed and supervised human
93 postmortem tissue banking. A.R. and L.S. performed pre-processing of mouse ATAC-sequencing
94 and supervised mouse data analysis.

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119 **Summary**

120 Hyperexcitability in the orbitofrontal cortex (OFC) is a key clinical feature of anhedonic domains
121 of Major Depressive Disorder (MDD). However, the cellular and molecular substrates underlying
122 this dysfunction remain unknown. Here, cell-population-specific chromatin accessibility profiling
123 in human OFC unexpectedly mapped genetic risk for MDD exclusively to non-neuronal cells, and
124 transcriptomic analyses revealed significant glial dysregulation in this region. Characterization of
125 MDD-specific cis-regulatory elements identified ZBTB7A – a transcriptional regulator of astrocyte
126 reactivity – as an important mediator of MDD-specific chromatin accessibility and gene
127 expression. Genetic manipulations in mouse OFC demonstrated that astrocytic *Zbtb7a* is both
128 necessary and sufficient to promote behavioral deficits, cell-type-specific transcriptional and
129 chromatin profiles, and OFC neuronal hyperexcitability induced by chronic stress – a major risk
130 factor for MDD. These data thus highlight a critical role for OFC astrocytes in stress vulnerability
131 and pinpoint ZBTB7A as a key dysregulated factor in MDD that mediates maladaptive astrocytic
132 functions driving OFC hyperexcitability.

133

134 **Introduction**

135 Major Depressive Disorder (MDD) is a leading cause of disability worldwide¹ and involves
136 corticolimbic network disruptions associated with recurrent episodes of negative affect, cognitive
137 impairment, somatic deficits, and anhedonia²⁻⁵. Although relatively understudied in human MDD,
138 the orbitofrontal cortex (OFC) processes affective valence and motivational value in humans,
139 monkeys, and rodents – making it a key prefrontal area involved in the anhedonic symptomatic
140 domains of MDD (i.e., loss of pleasure or motivation)². Functional imaging studies have
141 consistently identified significant OFC changes in MDD patients and demonstrated that OFC
142 hyperactivity correlates with the severity of anhedonic and negative rumination symptoms,
143 suicidality, antidepressant treatment responses, and pathogenic trajectories of the disorder⁶⁻¹⁶. A
144 recent RNA sequencing study that profiled multiple brain regions in human MDD identified the

145 OFC as a region displaying the highest number of differentially expressed genes in female
146 patients, and second highest overall¹⁷, with distinct alterations in gene expression programs
147 identified in comparison to other prefrontal cortical areas. Despite the OFC's clear involvement in
148 MDD, the molecular and cellular substrates underlying these functional alterations remain poorly
149 understood.

150
151 Disease-related cellular phenotypes are determined by spatiotemporally precise gene expression
152 programs induced by transcription factors (TFs) that interact with their corresponding cis-
153 regulatory DNA elements (CREs) in a cell-type-specific manner¹⁸⁻²¹. Chromatin accessibility
154 profiling can be used to identify the full repertoire of active CREs within a given cell-type, and is
155 thus an essential step towards understanding the regulatory drivers of disease pathology. Here,
156 using FANS-coupled ATAC-seq in human postmortem OFC, we found that both genetic risk
157 variants for MDD and MDD-specific CREs were localized to non-neuronal cell populations
158 (primarily glia). We further found that MDD-specific CREs were significantly enriched for binding
159 sites of the chromatin remodeler ZBTB7A, a putative regulator of astrocyte reactivity that was
160 upregulated in MDD OFC and was found to regulate the expression of MDD-specific CRE target
161 genes.

162
163 Extending these studies to mice, we found that Zbtb7a is upregulated following chronic stress
164 exposures (a major risk factor for MDD in humans) in astrocytes specifically, and not in microglia
165 or neurons. We further found that Zbtb7a activity in OFC astrocytes is both necessary and
166 sufficient for behavioral stress responsivity using bidirectional, astrocyte-specific Zbtb7a
167 manipulations in preclinical mouse models of stress. Cell-type-specific ATAC-seq and RNA-seq
168 revealed that Zbtb7a mediates chromatin accessibility in astrocytes to promote aberrant gene
169 expression programs related to astrocyte reactivity, including increased inflammatory signaling
170 and impaired synaptic regulation, which led to cell non-autonomous disruption of glutamate
171 signaling pathways in OFC neurons. Furthermore, using electrophysiological recordings and

172 chemogenetic manipulations, we found that Zbtb7a-mediated astrocyte reactivity promotes OFC
173 neuronal hyperexcitability in response to a mild subthreshold stressor, and that this increased
174 OFC excitability mediates maladaptive social avoidance behaviors following chronic stress. In
175 sum, the results of this cross-species study link stress-induced increases in Zbtb7a expression,
176 similar to that observed in human MDD, with astrocyte reactivity and OFC neuronal
177 hyperexcitability, revealing an important mechanism of stress-induced behavioral deficits related
178 to MDD.

179
180 **Results**
181

182 ***Chromatin accessibility profiling in neuronal vs. non-neuronal cells of OFC identifies glial-
183 specific regulatory signatures of human MDD***

184 To first investigate gene expression alterations that are associated with MDD diagnosis in human
185 OFC, we performed bulk RNA-seq on postmortem OFC tissues from 20 MDD cases vs. 19
186 matched healthy controls (**Figure 1A, Figure S1A**). While neuronal hyperactivity is a well-
187 characterized clinical feature of MDD OFC pathology, both differential expression analysis and
188 weighted gene correlation network analysis (WGCNA)²² implicated robust alterations in glial cell
189 function and inflammatory responses in MDD, suggesting a key role for non-neuronal cell
190 dysregulation in this region (**Figure 1B-C, Figure S1B-D**). To assess distinct patterns of
191 chromatin accessibility in neuronal vs. non-neuronal (primarily glial) nuclei of human MDD OFC,
192 we implemented FANS (Fluorescence-Activated Nuclear Sorting) coupled with ATAC-seq (Assay
193 for Transposase-Accessible Chromatin followed by Sequencing) on nuclear preparations
194 obtained from these 20 MDD cases vs. 19 matched healthy controls (**Table S1**). We performed
195 extensive quality control assessments of the ATAC-seq libraries to yield a total of 70 high quality
196 sample libraries (**Figure S1E-N, and Table S2**). To define the regulatory programs that specify
197 each cell population, we identified active Open Chromatin Regions (OCRs) in neuronal and non-
198 neuronal samples, which accounted for 4.79% and 2.65% of the genome, respectively (**Figure**
199 **1D-E**). Using a curated reference dataset²³, we confirmed that OCR sets in the FAN-sorted

201 populations displayed expected cell-type enrichment patterns (**Figure 1F**). In accordance with
202 previous findings²⁰, neuronal OCRs were found to be more distal to transcription start sites (TSSs)
203 compared to non-neuronal OCRs, reflecting a more complex regulatory scheme and higher levels
204 of functional diversity among neuronal subtypes (**Figure 1F, Figure S1F**). Because the majority
205 of genetic variants that influence human disease are located within non-coding regulatory regions
206 of the genome²⁰, we next investigated the enrichment of common risk variants for MDD in the
207 detected OCR datasets. We calculated the heritability coefficient²⁴ for each set of OCRs, stratified
208 by genomic context (**Figure 1G**), and identified significant enrichment for MDD-associated genetic
209 variants only in non-neuronal-specific promoter OCRs, but not in any of the neuronal OCR sets²⁵
210 (**Figure 1G**). These findings indicate that active regulatory elements within non-neuronal OFC
211 cells are relevant to the genetic risk for affective disorders.

212
213 We next assessed differential accessibility at OCRs in each nuclei population to identify putative
214 CREs that were specific to MDD-diagnosis. Consistent with both our RNA-seq analysis and
215 heritability coefficient calculations, we observed differential chromatin accessibility between MDD
216 vs. controls only in non-neuronal OCRs (203 CREs, **Figure 1H, Figure S1N-P, Table S3**). We
217 also observed significant correlations between MDD-OCRs and bulk RNA-seq signatures from
218 this same patient cohort (**Figure S1Q**). Inflammatory gene targets associated with these MDD-
219 specific CREs were found to display significant expression changes in FAN-sorted non-neuronal
220 nuclei from MDD vs. control subjects, such as lower levels of Nuclear Corepressor 2 (NCOR2),
221 which was recently identified as a negative regulator of astrocyte-specific reactivity pathways²⁶
222 (**Figure S1R**). Finally, to characterize the biological processes regulated by MDD-specific CREs
223 in glia, we performed gene set enrichment analysis (GSEA)²⁷, which revealed significant changes
224 in pathways associated with glial activation, including NF- κ B-induced inflammation, cytokine-
225 mediated cascades, lipid metabolism, and toll-like receptor signaling²⁸⁻³⁰ (**Figure 1I, Table S4**).
226 Together, these data demonstrate that MDD-specific CREs mediate cellular stress responses that

227 are known to be disrupted in MDD³⁰⁻³², and converge with previous reported evidence that glial
228 inflammatory stress pathways play a role in the pathophysiology of MDD, particularly in OFC³³⁻³⁶.

229
230 ***Identification of a key transcription factor regulating MDD-specific OCRs: ZBTB7A***

231 To identify potential transcriptional regulators of these MDD-specific CREs, we implemented TF
232 motif discovery analysis³⁷, and identified a motif that was significantly enriched (57 motif
233 occurrences out of 202 MDD-specific CREs; **Figure 2A**). In order to characterize the functional
234 role of this enriched regulatory motif, we performed gene ontology (GO) analysis³⁸, which
235 revealed significant associations between this enriched motif and gene targets involved in the
236 regulation of inflammatory response (e.g. cytokine pathways and NF- κ B cascades) (**Figure 2B**),
237 confirming that this motif is involved in the same regulatory processes that are enriched in MDD-
238 specific OCRs (**Figure 1I**). Out of the top five candidate TFs with binding motifs that matched the
239 enriched motif sequence, only one of these candidates was expressed at detectable levels in
240 human brain and was also dysregulated between MDD and controls: ZBTB7A (**Figure 2C-2D**),
241 which displayed significant upregulation in MDD OFC at both the mRNA and protein levels
242 (**Figure 2E-F** and **Figure S2B**). These findings that are in accordance with previous profiling
243 studies³⁹. ZBTB7A is a chromatin regulatory factor with pleiotropic effects (both repressive and
244 activating) and has been shown to coordinate alterations in chromatin structure that are necessary
245 for NF- κ B dependent inflammatory gene expression in the context of several types of cancers
246 (notably gliomas) and inflammatory conditions⁴⁰. However, its contributions to psychiatric disease
247 have not yet been explored⁴¹.

248
249 To confirm that ZBTB7A is differentially bound to chromatin in MDD OFC, we carried out
250 footprinting analysis⁴² and calculated ZBTB7A binding predictions within all identified OCR sets.
251 Consistent with motif enrichment analysis, we observed 43.8% more bound ZBTB7A sites that
252 were specific to non-neuronal vs. neuronal cells, with higher occupancy (3.4x) of ZBTB7A sites in
253 non-neuronal MDD cases compared to controls, making it one of the top five most differentially

254 bound TFs genome-wide between MDD and controls (**Figure 2G-H**). One illustrative example
255 demonstrating ZBTB7A binding and increased chromatin accessibility in MDD is *PRR5L* (proline
256 rich 5 like gene), a previously identified MDD biomarker gene involved in stress
257 responsiveness^{43,44}, which displayed increased chromatin accessibility in MDD cases in multiple
258 intronic OCRs. However, the only FDR-significant MDD-specific OCR associated with this gene
259 overlapped with two ZBTB7A binding sites (**Figure 2I**), both of which displayed differential binding
260 in MDD vs. controls.

261 ZBTB7A was recently identified a master transcriptional regulator of astrocyte inflammatory
262 activation in a recent report using CRISPR screens in human iPSC-derived cells, alongside other
263 more well-characterized factors such as STAT3 and RELA⁴⁵. Consistent with its potential role as
264 a regulator of astrocyte reactivity, pathway analyses revealed that both astrocyte-specific genes
265 and ZBTB7A targets were enriched in differentially expressed genes (DEGs) between MDD vs.
266 controls and in gene targets of non-neuronal promoter OCRs (which were enriched for MDD-
267 related genetic variants) (**Figure 2J**, **Figure S2C**). Similarly, a previously published MDD OFC
268 RNA-seq dataset also observed ZBTB7A upregulation in MDD, and DEGs in this study were
269 significantly enriched for ZBTB7A targets, demonstrating that altered ZBTB7A regulation in OFC
270 is observed across heterogeneous human MDD cohorts¹⁷ (**Figure S2D**). In addition, ZBTB7A
271 target genes identified in previously published ChIP-seq datasets showed robust overlap with
272 astrocyte-specific genes (using ARCHS4 human tissue expression reference genes), further
273 suggesting that ZBTB7A is involved in astrocyte function (**Figure S2E**). Consistent with these
274 findings, MDD-specific CREs were found to display significant enrichment for astrocyte/microglia
275 regulatory elements when overlapped with reference panels from human cell-type-specific ATAC-
276 seq data²³ (note that these two cell-types were sorted together in this dataset) (**Figure 1F**).
277

278

279 Given that we observed increases in both ZBTB7A expression and regulatory activity in human
280 MDD OFC, we next focused on determining whether ZBTB7A expression may also be increased
281 in the context of chronic social stress in mice, an etiologically relevant preclinical model for the
282 study of human MDD. Chronic social defeat stress (CSDS) involves 10 days of exposure to a
283 larger, aggressive mouse during daily social defeat sessions that involve 5-10 minute bouts of
284 physical aggression, followed by 24 hours of sensory exposure to produce continuous
285 psychological stress. CSDS induces robust behavioral deficits in mice that are similar to that
286 observed in human MDD, including reward insensitivity and social avoidance⁴⁶ (**Figure 2K**).
287 Importantly, this paradigm also models natural variation in stress vulnerability, as approximately
288 30% of wild-type mice that go through CSDS do not exhibit behavioral deficits related to chronic
289 stress and are termed stress-resilient (vs. stress-susceptible). Using this CSDS procedure, we
290 found that *Zbtb7a* protein was upregulated in bulk OFC tissues from stress-susceptible subjects,
291 but not in control or stress-resilient animals, 48 hours after the final defeat session ($n = 8$ control,
292 $n = 11$ stress-susceptible, $n = 9$ stress-resilient) (**Fig 2L**). We also observed that *Zbtb7a*
293 expression was persistently increased in OFC of a separate cohort of stress-susceptible mice 21
294 days after CSDS ($n = 12$ control, $n = 13$ stress-susceptible), demonstrating that *Zbtb7a*
295 upregulation is maintained for prolonged periods following stress exposures (**Figure S2F-G**). To
296 next determine whether chronic stress leads to *Zbtb7a* upregulation within specific brain cell-
297 types, we utilized Magnetically Activated Cell Sorting (MACs) to isolate astrocyte-, neuron-, and
298 microglia-enriched cell fractions from the OFC of a separate CSDS cohort ($n = 4$ /group, with 3
299 pooled animals/ n) (**Figure S2H-J**). While we observed ~2.7x higher expression of *Zbtb7a* mRNA
300 in neurons vs. astrocytes in unstressed animals (consistent with previously published single-cell
301 seq profiles⁴⁷), IHC immunostaining showed that *Zbtb7a* protein was also expressed at robust
302 levels in mouse OFC astrocytes (**Figure S2K-M**). Importantly, we found that *Zbtb7a* mRNA was
303 increased exclusively in astrocytes following chronic stress exposures, with no significant
304 differences observed in neurons or microglia (**Figure 2M-O**). Finally, we examined *Zbtb7a*

305 expression in an astrocyte-specific Translating Ribosome Affinity Purification coupled to
306 sequencing (TRAP-Seq) dataset ($n = 3$ control, $n = 5$ stress-susceptible, $n = 4$ stress-resilient).
307 Here, we found that *Zbtb7a* mRNA translation was significantly upregulated in frontal cortical (but
308 not hippocampal or striatal) astrocytes of stress-susceptible mice, compared to both control and
309 stress-resilient animals – suggesting that astrocytic *Zbtb7a* levels may correlate with behavioral
310 stress responsivity (**Figure 2P**).

311
312 To next determine if ZBTB7A regulates gene targets associated with MDD-specific CREs, we
313 assessed the impact of overexpressing ZBTB7A (OE) in human primary cortical astrocytes using
314 a lentivirus. In this astrocyte-enriched human cell culture system, ZBTB7A OE was found to
315 significantly alter the expression of numerous genes regulated by MDD-specific CREs. ZBTB7A
316 OE also increased the expression of prominent genes within the NF- κ B pathway, which was
317 found to be altered in our human MDD dataset (**Figure S2N-Q**). To explore if ZBTB7A might
318 increase in astrocytes under inflammatory conditions, we next treated both human and mouse
319 primary astrocyte-enriched cell cultures with lipopolysaccharide (LPS) to stimulate an
320 inflammatory response, which resulted in a significant upregulation of *ZBTB7A/Zbtb7a* expression
321 compared to saline, further linking this chromatin regulator to cellular reactivity pathways in
322 astrocytes (**Figure S2R-S**).

323
324 ***Astrocytic Zbtb7a in rodent OFC is necessary to induce behavioral deficits associated with***
325 ***chronic stress***

326
327 Our human data identified ZBTB7A as an enriched chromatin regulator in glial MDD-specific
328 CREs in OFC, and further validations in mouse models suggested that astrocyte-specific *Zbtb7a*
329 activity may play important roles in behavioral stress responsivity. Therefore, we next set out to
330 determine whether astrocyte-specific knockdown (KD) of *Zbtb7a* might be sufficient to attenuate
331 maladaptive behavioral responses to chronic stress. To do so, we designed a *Zbtb7a*-targeting
332 microRNA (miR) construct with a GFP reporter and cloned this construct – vs. a non-gene

333 targeting scrambled miR-GFP control – into an astrocyte-specific GFAP promoter-driven AAV
334 vector for viral packaging into AAV6³⁸. We confirmed preferential expression of GFP transgene
335 expression in GFAP+ cells using MACs isolated astrocytes, and validated the efficiency of *Zbtb7a*
336 KD in transduced mouse OFC tissues (**Figure S3A-C**). We next transduced OFC of male mice
337 with AAV6-GFAP-Zbt-miR (Zbt-KD) vs. miRNA-negative-GFP (GFP) viruses prior to CSDS, with
338 half of each viral group being assigned to either control or CSDS conditions ($n = 7$ GFP control,
339 $n = 9$ *Zbtb7a* KD control, $n = 18$ GFP chronic stress, $n = 19$ *Zbtb7a* KD chronic stress) (**Figure**
340 **3A**). Post-CSDS, we found that astrocyte-specific *Zbtb7a* KD in OFC was sufficient to fully rescue
341 chronic stress-induced social avoidance observed in GFP-expressing animals, with no significant
342 changes observed in Zbt-KD non-stressed mice (**Figure 3B**). Importantly, *Zbtb7a* KD also
343 rescued anhedonia-like behavior post-CSDS in two different measures of saccharin reward
344 sensitivity: a Pavlovian cue-reward association task, in which mice learn to associate a signal light
345 with reward delivery, as well as an operant reward learning task requiring lever-pressing in
346 response to a cue light to receive rewards (in a separate cohort of $n = 7$ GFP control, $n = 7$ *Zbtb7a*
347 KD control, $n = 8$ GFP chronic stress, $n = 9$ *Zbtb7a* KD chronic stress) (**Figure 3C-F**). Whereas
348 chronically stressed mice learned the reward contingencies of these tasks slower than controls,
349 we observed a significant increase in the number of rewards earned for the *Zbtb7a* KD chronically
350 stressed mice compared to GFP stressed animals. Together, these results indicate that *Zbtb7a*
351 activity in OFC astrocytes is a key contributor to behavioral stress responsivity, including social
352 avoidance and reward insensitivity, following chronic psychosocial stress experiences.

353
354 ***Knockdown of Zbtb7a in rodent OFC astrocytes significantly reverses cell-type specific***
355 ***gene expression signatures associated with chronic stress***

356
357 To explore the molecular changes associated with *Zbtb7a* KD in the context of chronic stress, we
358 next performed bulk RNA-seq profiling on virally transduced OFC tissues from a separate cohort
359 of animals ($n = 4$ GFP control, $n = 4$ *Zbtb7a* KD control, $n = 8$ GFP chronic stress, $n = 8$ *Zbtb7a*
360 KD chronic stress). Threshold-free Rank-Rank Hypergeometric Overlap (RRHO)⁴⁸ analysis

361 revealed transcriptome-wide patterns of reversed gene expression between Zbt-KD stress vs.
362 GFP stress and GFP stress vs. GFP controls, demonstrating that Zbtb7a KD reverses overall
363 gene signatures induced by chronic stress in OFC, maintaining a profile more similar to that of
364 control animals (**Figure 3G, Figure S3E**). Consistent with previous reports that increased Zbtb7a
365 drives NF- κ B activation, transcriptome-wide GSEA analysis demonstrated that Zbtb7a KD
366 reversed the upregulation of inflammatory response gene sets induced by chronic stress, with
367 cytokine production being the most significantly downregulated gene set between Zbt-KD stress
368 and GFP stress (**Figure S3F**). Unsupervised clustering of 1,583 DEGs at FDR <.1 between CSDS
369 and controls showed that both Zbt-KD stress and Zbt-KD controls display an intermediate gene
370 expression phenotype that clusters between controls and chronic stress (**Figure 3H**). Odds ratio
371 analysis revealed significant overlap between DEGs that were up in GFP stress and down in Zbt-
372 KD stress (37.8% reversed, Adj.pval = 4.2e-104), and genes that were down in GFP stress and
373 up in Zbt-KD stress (50.5% reversed, Adj.pval = 1.2e-209) (**Figure 3I**). Interestingly, rescued
374 DEGs were also enriched for genes involved in synaptic organization, neurotransmitter regulation,
375 and calcium/ionic transport, suggesting that astrocytic Zbtb7a KD in the context of chronic stress
376 may alter astrocyte function to have cell non-autonomous effects on neuronal transmission
377 (**Figure 3J**).

378
379 Therefore, to better define the effects of astrocytic Zbtb7a KD specifically on astrocyte gene
380 expression, we next performed RNA-seq on MACs-isolated astrocytes from Zbtb7a KD vs. GFP
381 groups (+/-) CSDS ($n=4$ GFP control, $n=4$ Zbtb7a KD control, $n=4$ GFP chronic stress, $n=5$,
382 Zbtb7a KD chronic stress, with 3 pooled OFC astrocyte fractions per n). Similar to bulk OFC
383 tissues, Zbtb7a KD significantly reversed transcriptome-wide gene expression in astrocytes
384 compared to GFP stress animals (**Figure 3K-L**). Furthermore, approximately 96% (112/117) of
385 the DEGs (FDR<.1) between the Zbtb7a KD stress and GFP stress groups were rescued,
386 including a gene previously identified to be important for chronic stress behavioral responses -

387 *Dusp6*¹⁷, and the glial-specific glutamate transporter *Slc1a2* (**Figure 3M**). Pathway analysis
388 demonstrated that upregulated pathways in chronic stress were associated with astrocyte
389 reactivity (e.g., cell motility and morphological remodeling), while downregulated genes were
390 involved in critical astrocyte functions, such as metabolic homeostasis and regulation of ionic
391 transport and synaptic signaling — pathways that were also enriched for DEGs that were rescued
392 by Zbtb7a KD (**Figure 3N**).

393
394 To examine potential cell non-autonomous effects of astrocytic Zbtb7a KD, we next profiled
395 MACs-isolated OFC neurons from the same cohort of Zbtb7a KD vs. GFP animals (+/- CSDS) (n
396 = 4 GFP control, n = 4 Zbt-KD control, n = 4 GFP chronic stress, n = 5, Zbt-KD chronic stress,
397 with 3 pooled OFC neuronal fractions per n) (**Figure 3O-P**). Comparing these data with astrocyte-
398 specific profiles, we confirmed that both astrocyte and neuronal fractions demonstrated cell-type
399 specific expression patterns for respective population markers⁴⁹ (**Figure S3H**). Interestingly, we
400 found that Zbtb7a KD specifically within astrocytes also led to cell non-autonomous effects on
401 neuronal gene expression in the context of stress, including a reversal of genes associated with
402 glutamate transmission (**Figure 3Q**). These data suggest that during chronic stress, astrocytes
403 lose normal homeostatic processes that may have downstream consequences on OFC neuronal
404 activity, effects that are attenuated by reducing Zbtb7a activity in astrocytes specifically.

405
406 **Zbtb7a regulates chromatin accessibility patterns in astrocytes associated with chronic**
407 **stress**

408 Since ZBTB7A was previously identified as a chromatin remodeling protein involved in multiple
409 cell-signaling pathways, including NF- κ B inflammation⁴⁰, we next sought to confirm whether
410 astrocyte-specific manipulations of Zbtb7a alter chromatin accessibility patterns in the context of
411 chronic stress. To do so, we performed ATAC-seq on MACs-isolated astrocytes from virally-
412 infected OFC tissues from the four groups of animals described above (n = 4 GFP control, n = 4
413 Zbt-KD control, n = 5 GFP stress, n = 5 Zbt-KD stress, with each n composed of 3 pooled OFC

415 samples), and found that promoters displaying chronic stress-induced accessibility were enriched
416 for Zbtb7a targets, as were less accessible promoter regions in Zbt-KD stress vs. GFP stress
417 animals, indicating that these chromatin profiles reflect Zbtb7a regulatory activity in astrocytes
418 (**Figure S3N**). Differential accessibility analyses demonstrated that Zbtb7a KD rescued astrocyte-
419 specific chromatin accessibility patterns induced by chronic stress, with 42.3% (603/1391, Adj.
420 Pval = 2e-89) of up events and 65.5% (2044/3117, Adj.Pval = 0e+00) of down events displaying
421 opposing accessibility compared to GFP stress mice (**Figure S3P**). In addition, Zbt-KD stress
422 ATAC-seq profiles correlated significantly with Zbt-KD stress gene expression changes detected
423 in our astrocyte-specific RNA-seq dataset, and exhibited a reversed pattern of anti-correlation
424 with chronic stress OCRs (**Figure S3Q-R**). Rescued OCRs were found to be enriched for
425 pathways involved in astrocyte reactivity, including ion homeostasis, ECM alterations, and cellular
426 morphogenesis (**Figure S3S**). Importantly, among the genes reversed by Zbtb7a KD for both
427 gene expression and chromatin accessibility were the astrocyte-specific glutamate clearance
428 transporter gene *Slc1a2* (also known as *Eaat2*), which modulates neuronal excitability through
429 maintenance of glutamatergic tone (note that *Slc1a2* has consistently been shown to be
430 downregulated following chronic stress^{50,51}). These findings again highlight that Zbtb7a-mediated
431 astrocyte dysfunction during chronic stress may affect neuronal function through regulation of
432 glutamate clearance and synaptic excitability (**Figure S3T**).

433
434 ***ZBTB7A overexpression in astrocytes is sufficient to induce behavioral deficits following***
435 ***an innocuous mild subthreshold stressor***

436 In order to explore mechanistic roles for astrocytic Zbtb7a upregulation in stress vulnerability and
437 to assess whether increased Zbtb7a is sufficient to elicit a behavioral stress response, we
438 packaged an OE construct for *ZBTB7A* into the same AAV6-GFAP viral vector used for KD
439 experiments (**Figure S4A-E**). Our previous data suggested that increased *ZBTB7A* may be
440 associated with increased vulnerability to stress-related behavioral deficits. Therefore, for OE
441 experiments, we utilized the sub-threshold social defeat paradigm (SSDS) – which involves only

442 a single day of social defeat and does not induce behavioral deficits in wild-type animals⁵² -- to
443 assess whether ZBTB7A OE in OFC astrocytes is sufficient to promote a pro-susceptibility
444 behavioral phenotype after a normally innocuous mild stressor ($n=10$ GFP control, $n=8$ ZBT-OE
445 control, $n=20$ GFP SSDS, $n=18$ ZBT-OE SSDS) (**Figure 4A**).

446
447 We found that astrocyte-specific ZBTB7A OE significantly increased behavioral deficits following
448 acute stress compared to GFP mice, including heightened social avoidance and anhedonic
449 reward insensitivity in the saccharin Pavlovian and operant reward tasks (in a separate cohort of
450 mice, $n = 7$ -10/group) (**Figure 4B-F**). ZBTB7A OE + acute stress also induced deficits in reward-
451 based reversal learning, which is a well characterized OFC-dependent task⁵³, suggesting that
452 ZBT-OE in astrocytes impairs OFC function following a mild stressor (**Figure 4G-H**). Furthermore,
453 although ZBT-OE SSDS animals did not display significant differences in anxiety-like behaviors
454 in the open field test, they did exhibit a significant increase in immobility in the forced swim test
455 (**Figure 4I-J**). In contrast, GFP-SSDS mice displayed distinct proadaptive behaviors in response
456 to acute stress, with no significant differences observed between GFP SSDS mice and GFP
457 controls. Notably, ZBTB7A OE alone did not affect stress-related behaviors in ZBT-OE control
458 mice. This is in agreement with previous reports that ZBTB7A acts mainly to transduce cellular
459 signals through orchestration of chromatin accessibility⁴⁰, and in the absence of NF- κ B activation,
460 ZBTB7A OE does not induce an inflammatory response on its own – though it may prime
461 chromatin states toward heightened stress responses following subsequent adverse
462 experiences⁴⁰.

463
464 **ZBTB7A overexpression in astrocytes induces transcriptome-wide alterations in gene
465 expression and chromatin accessibility related to inflammatory signaling and neuroactive
466 communication following a mild stress**

467
468 To explore the molecular correlates of these behavioral results, we next performed bulk RNA-seq
469 on virally transduced OFC tissues -/+ SSDS ($n = 5$ GFP control, $n = 5$ ZBT-OE control, $n = 7$ GFP
470 SSDS, $n = 6$ ZBT-OE SSDS, 1 OFC per n). Both RRHO analysis and unsupervised clustering of

471 DEGs (1,929 genes, FDR<.1) between the two SSDS groups revealed a robust pattern of
472 transcription induced by ZBTB7A OE, while the ZBT-OE control exhibited a positive correlation
473 with GFP SSDS – indicating that ZBTB7A OE in the absence of a mild stressor does not disrupt
474 overall transcriptomic states, in agreement with our behavioral data (**Figure 4K-M**).

475
476 In addition, although there were only a small number of DEGs between GFP control and GFP
477 SSDS (19 genes, FDR<.1), they included well-characterized stress-related genes, such as an
478 increase in the resilience-related gene *Fkbp5* and a decrease in the inflammatory cytokine *Cxcl12*.
479 Transcriptome-wide, inflammatory gene sets were found to be significantly downregulated in GFP
480 SSDS vs. GFP control mice, demonstrating that the behavioral resilience observed after an
481 exposure to acute stress in GFP SSDS mice likely involves pro-adaptive transcriptional responses
482 (**Figure S4F**). Overlaps between significant DEGs (FDR < 0.1) in ZBT-OE SSDS vs. GFP SSDS
483 and DEGs with a more relaxed cutoff (Pval <.05) in GFP SSDS vs. GFP Control revealed
484 significant reversal of genes in both directions (32.6%, Adj. Pval = 2.53e-47 and 35.6%, Adj. Pval
485 = 8.0e-134), suggesting that overexpression of ZBTB7A in astrocytes reverses proadaptive gene
486 expression responses associated with resilience to a mild stressor (**Figure 4N**). ZBT-OE + mild
487 stress altered molecular pathways related to astrocyte activation, such as regulation of ionic
488 transport, cellular adhesion/chemotaxis, and synaptic organization, all of which were oppositely
489 regulated in the GFP-SSDS vs. GFP control group (**Figure 4O**). Finally, flow cytometry analysis
490 confirmed that astrocytic ZBTB7A OE + acute stress significantly increased functional markers of
491 neuroinflammation in OFC, with higher percentages of microglia expressing activated markers
492 observed (note that the overall number of astrocytes or microglia was not altered between
493 conditions) (**Figure S4G-H**).

494
495 Next, we focused on differences between the two acute stress groups, where ATAC-seq and
496 RNA-seq profiling of MACs-isolated OFC astrocytes from a separate cohort of mice ($n = 4$ GFP
497 SSDS, $n = 4$ ZBT-OE SSDS) revealed robust changes in accessibility (6,094 differentially

498 accessible regions) that correlated significantly with observed differential gene expression
499 patterns (715 DE genes, FDR<.1) (**Figure S4I-K**). Importantly, astrocyte-specific ZBTB7A OE +
500 mild stress induced chromatin accessibility changes that overlapped significantly with those
501 detected following chronic stress (Adj. Pval = 4e-152 for downregulated genes, Adj.Pval = 5e-208
502 for upregulated genes), suggesting that ZBTB7A-mediated chromatin remodeling may be a
503 central regulatory feature controlling astrocytic dysfunction during chronic stress exposures
504 (**Figure S4L-M**). Indeed, ZBTB7A OE + acute stress induced similar alterations in gene
505 expression to those observed following chronic stress and were associated with astrocyte
506 reactivity pathways, including cellular morphology and synaptic regulation (**Figure 4P-R**). In
507 addition, neuronal specific RNA-seq profiles demonstrated an increase in cellular stress
508 response, as well as decreases in neurotransmitter transport and synaptic organization, indicating
509 that astrocyte-specific ZBTB7A OE may induce behavioral stress susceptibility through
510 modulation of astrocytic-induced OFC neuronal hyperactivity (**Figure 4Q-R**).

511
512 ***ZBTB7A overexpression in OFC astrocytes potentiates synaptic transmission***

513 To determine whether ZBTB7A upregulation is associated with changes in astrocyte calcium
514 signaling, we next imaged 2D mouse primary mixed cortical cultures of neurons and glia (including
515 astrocytes) using the genetically encoded calcium indicator GCaMP6f. Primary mixed cultures
516 were transduced with AAV1-hSyn-GCaMP6f or AAV5-gfaABC1D-cyto-GCaMP6f to ensure
517 selective expression solely in neurons or astrocytes, respectively (**Figure S5A-E**). To elicit a
518 subthreshold-like adaptive cellular response, we treated cultures with a low-dose of LPS (LPS_{low}),
519 approximately 1-10% of a typical inflammatory dose⁵⁴⁻⁵⁶, which has previously been used to
520 examine neuroprotective effects of mild LPS treatment in mixed culture models^{55,57-59}. In
521 GCaMP6f astrocytes, ZBT-OE significantly increased calcium transient frequency both at
522 baseline and after LPS_{low} treatment compared to an empty vector control virus, suggesting that
523 ZBTB7A OE disrupts adaptive astrocyte plasticity to subthreshold stressful stimuli (**Figure S5B**).

524 Furthermore, neuronal calcium events, which are a proxy marker for action potentials, were also
525 found to be significantly increased in co-cultures treated with the astrocyte-specific ZBT-OE virus
526 + LPS_{low} compared to empty vector controls (**Figure S5C**). These data indicate that ZBTB7A OE
527 in astrocytes leads to increased astrocyte activity, impairing both astrocytic and neuronal
528 adaptations to a mild stressful stimulus.

529
530 To assess if these astrocytic ZBTB7A-induced increases in neuronal activity occur *in vivo*, we
531 next investigated whether astrocyte specific ZBTB7A OE affects functional measures of synaptic
532 transmission in mouse OFC following SSDS. We utilized our GFAP-driven AAV virus to OE either
533 ZBTB7A or GFP in OFC astrocytes (+/-) SSDS, followed by electrophysiological slice recordings
534 to assess the impact of ZBTB7A OE on synaptic transmission post-exposure to a SSDS mild
535 stressor (**Figure 5A**). We first plotted an input-output (I-O) curve of field excitatory postsynaptic
536 potentials (fEPSPs) in response to presynaptic stimuli. We observed a significant increase in I-O
537 curves in ZBT-OE SSDS vs. GFP SSDS, suggesting that ZBTB7A OE in astrocytes induces
538 potentiation of postsynaptic responses following an acute stress (**Figure 5B-C**). We next applied
539 stimulation protocols to assess the dynamics of presynaptic vesicle mobilization and release.
540 During the stimulation train, we detected a significant difference in fEPSP amplitude between
541 ZBT-OE SSDS vs. GFP SSDS, suggesting a faster depletion of the readily releasable pool of
542 vesicles, which is correlated with higher probability of presynaptic release (**Figure 5D-E**)⁶⁰.
543 Together, these data indicate that astrocytic ZBTB7A OE + acute stress is sufficient to induce
544 increased OFC neuronal excitability, a hallmark feature of human MDD.

545
546 **Astrocytic ZBTB7A-induced OFC neuronal hyperexcitability mediates behavioral vulnerability**
547 **to stress**

548
549 To determine if increased OFC neuronal activity represents a functional link between ZBTB7A-
550 mediated astrocyte reactivity and behavioral vulnerability to stress, we utilized an inhibitory
551 Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetic

552 approach to silence OFC neurons while simultaneously overexpressing ZBTB7A in surrounding
553 astrocytes. We first confirmed that I.P. injection of the DREADD agonist Deschloroclozapine⁶¹
554 (DCZ) on its own did not alter previously observed patterns of behavioral deficits in ZBT OE vs.
555 GFP mice post-stress in the absence of the DREADD, and that neuronal G_i DREADD expression
556 without DCZ agonist activation does not affect previously observed SI phenotypes (**Figure S5F-H**). We then performed the SSDS paradigm on a separate cohort of male mice that were injected
557 intra-OFC with the both the pAAV-hSyn-hM4D(G_i)-mCherry vector to express the inhibitory G_i
558 DREADD in neurons, and either the AAV-GFAP-ZBTB7A OE virus or the rAAV6-GFAP-GFP
559 empty control vector in astrocytes (**Figure 5F**). Note that both groups underwent the SSDS
560 paradigm. Prior to the social interaction test, half of each viral group was injected with either
561 vehicle or DCZ to silence OFC neuronal firing (**Figure 5G**). In the G_i + vehicle-injected mice, ZBT
562 OE + acute stress resulted in reduced social interaction behavior vs. GFP SSDS, as previously
563 observed. However, for DCZ-injected mice, we observed a significant rescue in SI deficits,
564 indicating that silencing of OFC activity prior to the social interaction test led to amelioration of the
565 pro-stress susceptibility effects of astrocyte-specific ZBTB7A OE in the context of a mild stressor
566 (**Figure 5H**). Together, these findings point to an astrocytic ZBTB7A-induced increase in synaptic
567 connectivity driving maladaptive stress susceptibility, which is consistent with clinical reports of
568 astrocyte dysfunction and neural hyperactivity in human MDD OFC¹⁰.

570
571 **Discussion**

572 Overall risk for MDD is determined by complex interactions between genetic and environmental
573 factors that disrupt frontolimbic function. Although MDD has primarily been studied in the context
574 of neuronal plasticity, recent studies suggest that dysregulation of glial cell activity, and astrocytes
575 specifically, may be a key contributor to MDD pathophysiology^{35,36,51,62}. The OFC exhibits distinct
576 functional changes from other frontal cortical regions in chronic stress and MDD¹¹, however the
577 molecular pathways driving these alterations within specific cell-types are not well understood.

578 Here, implementing both RNA-seq and FANs-coupled ATAC-seq in postmortem MDD OFC
579 tissues, we identified significant glial dysfunction in depressed individuals vs. controls, including
580 alterations in inflammatory pathways, cellular metabolism, and ionic homeostasis. Using unbiased
581 cell-population-specific epigenomic profiling, we identified a key chromatin regulator of MDD-
582 specific CREs in human depression, ZBTB7A, which was found to be upregulated in MDD and
583 displayed significantly higher motif occupancy in MDD non-neuronal cells vs. controls. We
584 validated the relevance of this chromatin regulator to astrocyte-mediated chronic stress
585 phenotypes using preclinical mouse models for the study of MDD, and demonstrated that
586 astrocyte-specific ZBTB7A regulatory activity in OFC bidirectionally mediates molecular,
587 electrophysiological, and behavioral alterations induced as a consequence of chronic stress
588 exposures.

589
590 Our human postmortem ATAC-seq data, together with functional validation experiments in
591 preclinical mouse models, demonstrated that astrocytic ZBTB7A may act as a pathogenic driver
592 of astrocyte dysfunction in MDD by inducing inflammatory reactivity and compromising normal
593 astrocyte-mediated regulation of synaptic function⁶³ and glutamatergic signaling^{63,64}. Our mouse
594 data also revealed that ZBTB7A upregulation acts to reverse normal adaptive mechanisms that
595 promote stress resilience. Importantly, our rodent data are consistent with clinical reports of OFC
596 neural hyperactivity in human MDD¹⁰, and raise the intriguing potential of targeting astrocytic
597 ZBTB7A, as well as its downstream substrates mediating OFC dysfunction, therapeutically.
598 Overall, these findings support a critical role for astrocyte plasticity in the pathophysiology of MDD
599 and stress-related disorders and highlight the power of using epigenomic profiling to investigate
600 novel regulatory mechanisms driving aberrant cellular phenotypes in complex disease states.

601
602 **Methods**

603 ***Note that complete statistical information for Extended Data Figures is provided within the***
604 ***Extended Statistics Table S7***

605
606 **Human postmortem samples**
607 Postmortem human orbitofrontal cortex (Brodmann Area 11) tissues from 39 Caucasian subjects
608 (20 cases, 19 controls) were obtained from the Human Brain Collection at the University of Texas
609 Southwestern (UTSW) (IRB approval for tissue banking at UTSW). Tissue preservation was
610 achieved as previously described⁶⁵. Brains were placed on wet ice and transported to the UTSW
611 Brain Bank facilities. Tissues were sliced, flash frozen in 2-methylbutane at -40°C, and stored in
612 sections conserving anatomical landmarks at -80°C. OFC tissues were later sectioned from
613 frozen slices. For each subject, the cause of death was determined by the Coroner Office, and
614 toxicological screens were performed to obtain information on medication and illicit substance use
615 at their time of death. The MDD group consisted of 20 (9 male and 11 female) individuals who
616 met the Structured Clinical Interview for DSM-V (Diagnostic and Statistical Manual of Mental
617 Disorders-V) Axis I Disorders: Clinician Version (SCID-I) criteria for Major Depressive Disorder.
618 The control group comprised 19 subjects (12 male and 7 female Caucasians) with no history of
619 MDD. Groups were matched for age, post-mortem interval and RNA integrity number (RIN). For
620 all subjects, psychological autopsies were performed, giving us access to detailed information on
621 psychiatric and medical histories, as well as other relevant clinical and sociodemographic data
622 (see **Table S1**).

623
624 **FANS sorting of neuronal and non-neuronal nuclei**
625 50mg of frozen brain tissue was homogenized in cold lysis buffer (0.32M Sucrose, 5 mM CaCl₂,
626 3 mM Mg(Ac)₂, 0.1 mM, EDTA, 10mM Tris-HCl, pH8, 1 mM DTT, 0.1% Triton X-100) and filtered
627 through a 40µm cell strainer. The flow-through was underlaid with sucrose solution (1.8 M
628 Sucrose, 3 mM Mg(Ac)₂, 1 mM DTT, 10 mM Tris-HCl, pH8) and subjected to ultracentrifugation
629 at 24,000 rpm for 1 hour at 4°C. Pellets were thoroughly resuspended in 500µl DPBS and
630 incubated in BSA (final concentration 0.1%) and anti-NeuN antibody (1:1000, Alexa488
631 conjugated, Millipore) under rotation for 1 hour, at 4 °C, in the dark. Prior to FANS sorting, DAPI

632 (Thermoscientific) was added to a final concentration of 1 μ g/ml. DAPI positive neuronal (NeuN+)
633 and non-neuronal (NeuN-) nuclei were sorted into tubes pre-coated with 5%BSA using a BD-
634 FACSaria flow cytometer (BD Biosciences) equipped with a 70 μ m nozzle (**Figure S6**). 39 tissue
635 dissections from 1 brain region were subjected to FANS, resulting in 78 (39 NeuN- and 39 NeuN+)
636 distinct nuclear populations.

637
638 **RNA-sequencing**

639 For human postmortem OFC, ~25mg of pre-sectioned flash-frozen tissue was utilized for RNA
640 extraction. For mouse studies, animals were euthanized, and brains were removed whole and
641 flash frozen (for bulk sequencing), or processed fresh for cell-type specific isolation with
642 magnetically-activated cell sorting (MACs). Brains were sectioned at 100 μ m on a cryostat (bulk)
643 or brain block (MACs), and GFP was illuminated using a NIGHTSEA BlueStar flashlight to
644 microdissect virally infected tissues with a 2mm punch. For both human and mouse experiments,
645 OFC tissues were homogenized in Trizol (Thermo Fisher), and RNA was isolated on RNeasy
646 Minelute Microcolumns (Qiagen) following manufacturer's instructions. Following elution, samples
647 were enriched for mRNA via polyA tail selection beads, and mRNA libraries were prepared using
648 the Illumina Truseq RNA Library Prep Kit V2 (#RS-122-2001). Libraries were pooled and
649 sequenced on the Illumina Novaseq platform, with an average read count of approximately 20
650 million paired-end reads per sample. RNA-seq data was pre-processed and analyzed as
651 previously described⁶⁶. Briefly, FastQC (Version 0.72) was performed on the concatenated
652 replicate raw sequencing reads from each library to ensure minimal PCR duplication and
653 sequencing quality. Reads were aligned to the hg38 or mouse mm10 genome using HISAT2
654 (Version 2.1.0) and annotated against Ensembl v90. Multiple-aligned reads were removed, and
655 remaining transcript reads were counted using featurecounts (Version 2.0.1). For mouse RNA-
656 sequencing experiments with multiple groups, RUVg⁶⁷ was performed to normalize read counts
657 based on empirically determined control genes that do not vary between control and stress groups

658 (i.e. genes with $p\text{-val} > 0.5$ based on a first-pass differential expression analysis performed prior
659 to RUVg normalization). For human RNA-seq and mouse RNA-seq experiments with two groups,
660 RUVr⁶⁷ was performed to normalized read counts based on the residuals from a first-pass GLM
661 regression of the unnormalized counts on the covariates of interest. The number of factors of
662 variation, or RUV k , for each experiment is listed in **Table S5**). DESEQ2⁶⁸ (Version 2.11.40.6)
663 was used to perform pairwise differential expression analyses between indicated comparisons.
664 Differentially expressed (DE) genes (listed in **Table S5** for each experiment) were defined at
665 FDR<0.1. Threshold free Rank-Rank Hypergeometric Overlap (RRHO) maps were generated to
666 visualize transcriptome-wide gene expression concordance patterns as previously described⁴⁸,
667 using RRHO2 (Version 1.0). For RRHO comparing ATAC-seq vs. RNA-seq, signed log p -value
668 from the RNA-seq DESEQ2 output was ranked for each transcript that was also associated with
669 a differentially accessible peak in the ATAC-seq.

670
671 For the human MDD dataset, we used the WGCNA package (Version 1.71)²² to construct the co-
672 expression network for the top 2000 most variable genes in the set. We chose a suitable soft
673 threshold power of 7 for scale-free network construction with the function pickSoftThreshold. The
674 resulting gene co-expression network was visualized as the heatmap based on dissimilarity of
675 TOM with hierarchical clustering dendrogram, and the number of genes in each module was
676 counted. The correlation between modules and the trait of MDD was assessed by the Pearson
677 correlation coefficients, with students t -test, and a p value of < 0.05 was considered statistically
678 significant. Gene ontology (GO) enrichment analysis was performed for genes in each significant
679 module (and for GO analyses on DE genes from other experiments) with gprofiler(GO), idep
680 (TRANSFAC/JASPAR databases) with total detected genes as background, and enrichR (for cell-
681 type and human disease databases) to test for overrepresented gene categories in our list of DE
682 genes. FDR for representative GO terms from the top 10 terms is calculated based on nominal
683 P -value from the hypergeometric test. Gene Set Enrichment Analysis was performed using the

684 ClusterProfiler package (Version 4.6.0) against GO to calculate gene set enrichment scores, and
685 gene sets were ranked by adj. p-value⁶⁹. Odds Ratio analyses were carried out on DE gene lists
686 using the *GeneOverlap* R package version 1.26.0⁷⁰.

687
688 **Generation of human ATAC-seq libraries**

689 ATAC-seq reactions were performed using an established protocol⁷¹ with minor modifications.
690 Following FANS, 50,000 sorted nuclei were centrifuged at 500 ×g for 10 min, 4°C. Pellets were
691 resuspended in transposase reaction mix (25 µL 2x TD Buffer (Illumina Cat #FC-121-1030) 2.5
692 µL Tn5 Transposase (Illumina Cat #FC-121-1030) and 22.5 µL Nuclease Free H2O) on ice.
693 Samples were incubated at 37°C for 30 min and then purified using the MinElute Reaction
694 Cleanup kit (Qiagen Cat #28204) according to the manufacturer's instructions. Following
695 purification, library fragments were amplified using the Nextera index kit (Illumina Cat #FC-121-
696 1011), under the following cycling conditions: 72°C for 5 minutes, 98°C for 30 seconds, followed
697 by thermocycling at 98°C for 10 seconds, 63°C for 30 seconds, and 72°C for 1 minute for a total
698 of 5 cycles. In order to prevent saturation due to over-amplification, a 5µl aliquot was then
699 removed and subjected to qPCR for 20 cycles to calculate the optimal number of cycles needed
700 for the remaining 45 µL reaction. The additional number of cycles was determined as follows: (1)
701 Plot linear Rn vs. Cycle (2) Calculate the # of cycles that corresponds to 1/4 of maximum
702 fluorescent intensity. In general, we found adding 4-6 cycles to this estimate yielded optimal
703 ATAC-seq libraries, as determined by analysis on Bioanalyzer High Sensitivity DNA Chips
704 (Agilent technologies Cat#5067-4626). Libraries were amplified for a total of 13–19 cycles.
705 Following PCR, ATAC-seq libraries were resolved on 2% agarose gels and fragments ranging in
706 size from 100bp-1Kbp were excised and purified (Qiagen Minelute Gel Extraction Kit – Qiagen
707 Cat#28604). Libraries were quantified by quantitative PCR (KAPA Biosystems Cat#KK4873) prior
708 to sequencing. Libraries were sequenced on Hi-Seq2500 (Illumina) obtaining 2x50 paired-end

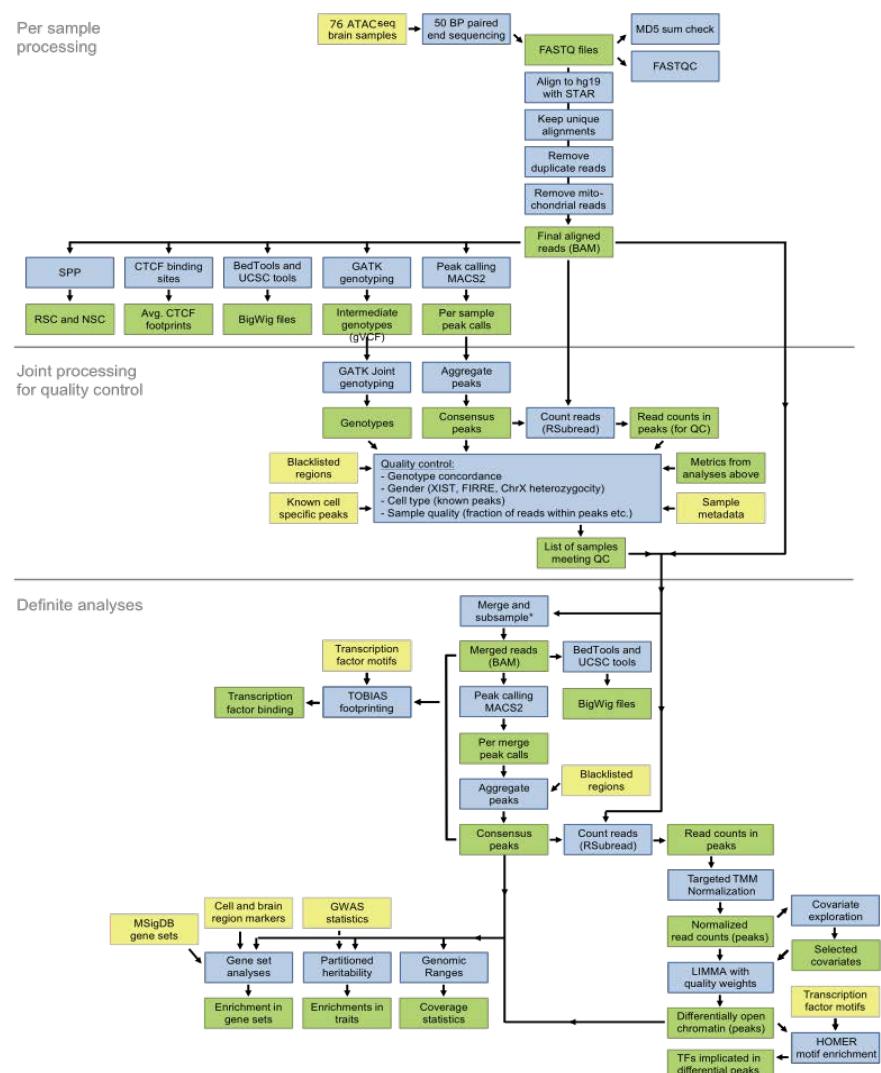
709 reads. After quality controls (see below), 70 ATAC-seq libraries were retained for downstream
 710 analysis.

711
 712 **Data Processing**

713 We provide a summary of
 714 the data processing pipeline

715 to the right. The
 716 preprocessing of ATAC-seq

717 samples involved the
 718 following steps: (1) per
 719 sample processing, (2) joint
 720 processing for quality
 721 control, and (3) analyses of
 722 samples meeting the quality
 723 control criteria. Yellow: input
 724 data. Blue: analyses.
 725 Green: processed data.



726
 727 **Alignment**

728 Raw sequencing reads
 729 were generated by the

730 sequencing center demuxed and with adaptors trimmed. FASTQ files were linked to the sample
 731 clinical and demographics metadata based on pooling ID's and barcodes. Reads were
 732 subsequently aligned to the hg19 reference genome with the pseudoautosomal region masked
 733 on chromosome Y with the STAR aligner (v2.5.0)⁷², using the following parameters: --
 734 alignIntronMax 1, --outFilterMismatchNmax 100, --alignEndsType EndToEnd, --
 735 outFilterScoreMinOverRead 0.3, --outFilterMatchNminOverRead 0.3. Having a coordinate-

736 sorted BAM, we further excluded reads that: (1) were mapped to more than one locus using
737 samtools⁷³; (2) were duplicated using PICARD (v2.2.4; <http://broadinstitute.github.io/picard>); and
738 (3) mapped to the mitochondrial genome.

739
740 **Genotype calling**

741 Genotypes were called by GATK (v3.5.0)⁷⁴. We performed: (1) indel-realignment; (2) base score
742 recalibration; and (3) joint genotype calling across all samples for variants having a phred-scaled
743 confidence threshold ≥ 10 . We excluded clustered variants, variants in ENCODE blacklisted
744 regions⁷⁵, and variants not present in dbSNP v146⁷⁶. Genotype concordance between samples
745 was assessed using both the kinship coefficient calculated by KING v1.9⁷⁷ and the fraction of
746 concordant genotype calls. For these analyses, we kept only variants with minor allele frequencies
747 (MAF) $\geq 25\%$. The two approaches yielded comparable results, with both indicating a clear and
748 unambiguous separation of samples. Using this approach, we were able to confirm that neuronal
749 and non-neuronal libraries supposedly originating from the same subject showed markedly higher
750 genotype concordance score compared to the comparison with unrelated samples (**Figure S1M**).

751
752 **Sex determination of samples**

753 The sex of the samples was assessed using three metrics: (1) the heterozygosity rate of
754 chromosome X genotype calls outside the pseudoautosomal regions. For this, we removed
755 variants with MAF $< 5\%$. A high heterozygosity rate can indicate contamination in male samples.
756 (2) The read counts of OCRs adjacent to *FIRRE* and *XIST* genes that are predominantly
757 expressed in females. (3) Read counts in OCRs on chromosome Y outside the pseudoautosomal
758 region. Using this approach, we detected and excluded two samples that were supposed to
759 originate from a male subject but they were genetically females. After their removal, all remaining
760 samples matched the expected sex characteristics (**Figure S1L**).

761

762 **Quality control of ATAC-seq samples**

763 For each sample, we calculated the following metrics: (1) total number of initial reads; (2) number
764 of uniquely mapped reads; (3) fraction of reads that were uniquely mapped and additional metrics
765 from the STAR aligner; (4) Picard duplication and insert metrics; (5) rate of reads mapped to the
766 mitochondrial genome; (6) PCR bottleneck coefficient (PBC), which is an approximate measure
767 of library complexity estimated as (non-redundant, uniquely mapped reads)/(uniquely mapped
768 reads); (7) normalized strand cross-correlation coefficient (NSC) and relative strand cross-
769 correlation coefficient (RSC), which are metrics that use cross-correlation of stranded read density
770 profiles to measure enrichment independently of peak calling; (8) fraction of reads in peaks
771 (FRiP), which is the fraction of reads that fall in detected peaks (see below for peak calling) and
772 similarly the fraction of reads in only blacklisted peaks and the ratio between these two metrics.

773 **Table S2** describes the main QC metrics. On average, we obtained more than 27 million uniquely
774 mapped paired-end reads per sample. The rate of reads that mapped to the mitochondrial
775 genome was below 2% since we generated ATAC-seq libraries using FANS separated nuclei,
776 instead of whole cells. The bigWig tracks for each sample were manually inspected. A total of six
777 libraries were excluded, having failed QC (including sex check) and/or visual inspection in IGV,
778 leaving 70 libraries that were subjected to further analysis (**Table S2**).

779
780 **Peak calling and read quantification**

781 First, we merged the BAM-files of samples of the same diagnosis and cell type and subsampled
782 to a uniform depth of, at most, 454 million paired-end reads. We subsequently created bigWig
783 files and called peaks using these merged bam files and created a joint set of peaks requiring
784 each peak to be called in at least one of the merged BAM-files. Peaks for OCRs were called by
785 MACS (v2.1)⁷⁸, using the following parameters⁷⁹: `--keep-dup all --shift -100 --extsize 200 --nomodel`. After removing peaks overlapping the blacklisted genomic regions, 371,820 peaks
786 remained. Next, we counted how many reads for each sample overlapped consensus peaks using
787

788 the featureCounts function in RSubread⁸⁰ (v.1.15.0). We counted fragments (defined from paired-
789 end reads), instead of individual reads. This resulted in a sample by peak matrix of read counts,
790 obtained using the following parameters: *allowMultiOverlap* = *F*, *isPairedEnd* = *T*, *strandSpecific*
791 = *0*, *requireBothEndsMapped* = *F*, *minFragLength* = *0*, *maxFragLength* = *2000*, *checkFragLength*
792 = *T*, *countMultiMappingReads* = *F*, *countChimericFragments* = *F*.

793

794 **Differential analysis of chromatin accessibility**

795 We performed a statistical analysis of chromatin accessibility to detect genomic regions with
796 significant differences in chromatin structure among neuronal and non-neuronal cells.

797 First, we used the sample-by-peak read count matrix (70 samples by 371,820 OCRs). We
798 subsequently excluded 1,178 OCRs using a criteria of “ $CPM \geq 1$ in at least 10% of the samples”,
799 resulting in our final sample-by-peak read count matrix (70 samples by 370,642 OCRs). From
800 here, we applied the trimmed mean of M-values (TMM)⁸¹ to normalize the read count followed by
801 quantile normalization to achieve a balanced distribution of reads across samples of the same
802 cell type.

803 **Covariate exploration:** Next, we tested whether we could find biological or technical sample-level
804 covariates that affect the observed read count. For these covariates (e.g. number of peaks called
805 in the sample, FRiP, chrM metrics, RSC and NSC, and Picard insert metrics), we normalized to
806 the median of the cell. All 63 covariates were then tested for inclusion in differential analysis as
807 detailed in the following: As a starting point for building the model to explain chromatin
808 accessibility in the peaks, we selected cell type by diagnosis (2x2=4 levels) and sex (2 levels). To
809 select additional covariates, we sought a good “average model” of chromatin accessibility over all
810 OCRs. For each additional tested covariate, we asked how many OCRs showed an improved
811 Bayesian Information Criterion (BIC) score minus how many showed a worse BIC score when the
812 covariate was added to the “base” linear regression model. Here, we required that at least 5% of
813 the OCRs showed a change of 4 in the BIC score, corresponding to “positive” evidence against

814 the null hypothesis⁸². However, no covariate satisfied the BIC score criteria for inclusion. We were
815 unable to find any covariate even after adjusting the threshold of minimal BIC (tested values = {2,
816 4, 10}) and/or minimal fraction of OCRs exceeding this threshold (tested values = {2%, 5%}).
817 Overall, our final model included 2 variables (cell type by diagnosis [4] and sex [2]³⁹), where the
818 number of levels for factor variables is noted here in square brackets. This model accounted for
819 5 DF.

820 Differential analysis: We used the *voomWithQualityWeights* function from the *limma* package⁴¹ to
821 model the normalized read counts. Then, we performed differential chromatin accessibility
822 analysis by fitting weighted least-squares linear regression models for the effect of each variable
823 on the right-hand side on accessibility of each OCR:

824 *chromatin accessibility ~ cell type:diagnosis + Sex + (1|Person_ID)*

825 Validation of differential OCRs: To validate the relevance of differential OCRs, we applied the
826 following strategies: permutation test and machine-learning test. For the former one, we randomly
827 permuted MDD case/control status ($n = 100$ permuted datasets) and performed differential
828 analysis using the same setting as for primary analysis. We measured (i) whether the sets of
829 differential OCRs on permuted datasets are smaller compared to non-permuted datasets and (ii)
830 whether the *P*-value rankings of differential OCRs on non-permuted datasets are close to normal
831 distribution. For machine learning validation, we trained six machine learning models for
832 prediction of MDD case/control status built on the reported set of (i) differential OCRs and (ii) the
833 same number of randomly selected OCRs. We applied the repeated 5-fold cross-validation (k_{repeat}
834 = 10) and, additionally, we repeated the whole process 10 times with different sets of randomly
835 selected OCRs. Then, we measured an improvement of prediction performance of the classifier
836 based on differential OCRs over classifiers utilizing random OCRs. The following machine
837 learning methods were tested, using the default setting in R-package⁸³: Naive Bayes (nb),
838 Random forest (rf), Nearest neighbor (knn), Logistic regression (multinom), SVM with linear kernel
839 (svmLinear), and SVM with polynomial kernel (svmPoly).

840

841 **Annotation of OCRs and gene set enrichment analysis**

842 We determined the genomic context per each OCR based on its proximity to the closest gene as
843 assigned by ChIPSeeker⁸⁴. For this, we created a transcript database using GenomicFeatures⁸⁵
844 and Ensembl genes. The genomic context was defined as promoter (+/- 3kb of any TSS), 5'-UTR,
845 3'-UTR, exon, intron, and distal intergenic. We used GREAT approach⁸⁶ to assign OCRs to genes
846 and perform enrichment analysis with combined set of Gene Ontology²⁷, biological processes with
847 the curated canonical pathways from REACTOME⁸⁷, KEGG⁸⁸, and PID⁸⁹, all accessed from
848 MSigDB 6.0⁹⁰. We further pruned highly similar gene sets by iteratively removing those with a
849 Jaccard index ≥ 0.5 , preferentially keeping the bigger gene set. This resulted in 4,590 gene sets
850 (biological processes and pathways).

851

852 **Overlap of OCRs with common variants in MDD**

853 To determine whether the sets of neuronal, non-neuronal, and consensual OCRs as well as
854 differential OCRs are enriched for common MDD GWAS variants⁹¹, we calculated partitioned
855 heritability using LD-sc²⁵. This analysis assesses if common genetic variants in the genomic
856 regions of interest explain more of the heritability for a given trait than genetic variants not
857 overlapping the genomic regions of interest, normalized by the number of variants in either
858 category. The algorithm allows for correction of the general genetic context of the annotation using
859 a baseline model of broad genomic annotations (like coding, intronic, and conserved regions). By
860 using this baseline model, the algorithm focuses on enrichments above those expected from the
861 general genetic context of the interrogated regions. We excluded the broad MHC-region (chr6:25-
862 35MB) and, otherwise, used default parameters.

863

864 **Motif Matching**

865 In order to identify candidates for DNA-binding proteins with recognition motifs enriched in our
866 MDD-specific OCR set, we utilized the RSAT suite *peak-motifs*, a computational pipeline that

867 discovers motifs in input sequences, and compares them with position-specific scoring matrix
868 (PSSM) transcription factor databases^{24,92}. Input sequences are scanned to predict binding sites,
869 and the background model is a Markov chain of order 2 trained on the input sequences. Using
870 *peak-motifs*, word-based analysis was first performed on the MDD-specific OCR set (n=183
871 sequences) with hexanucleotides ($k = 6$) and heptanucleotides ($k = 7$). The tool combines four
872 pattern-discovery algorithms that utilize overrepresentation and positional bias as two criteria to
873 detect significant oligonucleotide, which are then used as seeds to build probabilistic description
874 of motifs (PSSMs), indicating residue variability at each position of the motif. Discovered motifs
875 were compared with the JASPAR nonredundant core database of known transcription factor
876 binding motifs to predict associated transcription factors (using *compare-matrices*). Several
877 metrics are computed to measure the similarity between each matrix pair (including Pearson
878 correlation, width normalized correlation). These metrics are converted to ranks, and a mean rank
879 is computed to enable comparison between candidate factors. The *peak-motifs* pipeline
880 discovered a motif (**Figure 2A**) that was significantly enriched in MDD-specific OCR sequences.
881 The distribution of this motif within OCR sequences is shown in **Figure 2A**, indicating a relatively
882 higher number of sites near sequence centers. For the top 5 candidate transcription factors
883 identified as matches to this motif, the bar graph in **Figure S2A** displays the consensus score
884 from the Human Protein Atlas⁹³ for expression in human brain for each factor. The mRNA
885 expression data is derived from deep sequencing of RNA (RNA-seq) from 37 different normal
886 tissue types.

887
888 In order to characterize the functional role for the discovered motif from the *peak-motifs* pipeline,
889 we utilized GOMo (v5.3.3), from the MEME-suite of tools⁹⁴ (**Figure 2B**). This approach calculates
890 associations between a user-specified DNA regulatory motif [expressed as a position weight
891 matrix (PWM)] and Gene Ontology (GO) terms, by computing an association score between the
892 (putative) targets of the input TF motif and each GO term in the GO map. An empirically generated

893 p-value for the enrichment of the GO term is also computed for the association score for each GO
894 term with respect to the motif, based on the rank sum test null model.

895
896 **Footprinting analysis**
897 To determine the bound/unbound status of transcription factors in neuronal and non-neuronal
898 cells as well as in MDD cases and controls, we performed footprinting analysis using TOBIAS (v.
899 0.12.4)³⁸. Following the settings from our previous study⁴², we searched for the presence of 431
900 motifs representing 798 transcription factors (some motifs are shared due to their high similarity)
901 in consensus OCRs of four merged BAM files representing both cell types & MDD diagnosis
902 status. First, we ran the TOBIAS module ATACorrect to correct for Tn5 insertion bias in input
903 BAM files, followed by TOBIAS ScoreBigwig to calculate footprinting scores across OCRs. Then,
904 TOBIAS BINDetect combined footprinting scores with the information of transcription factor
905 binding motifs to evaluate the individual binding positions of each transcription factor and
906 determine whether a given position was bound by a given transcription factor or not for each
907 condition, i.e. cell type and brain region. Finally, TOBIAS PlotAggregate was used to visually
908 compare the aggregated footprints for select motifs.

909
910 **QPCR**
911 In order to measure mRNA gene expression for gene targets of interest, FAN-sorted nuclei
912 (**Figure S1R**) from human postmortem OFC tissues were prepared as described above, with
913 addition of RNase inhibitor in the sorting collection buffer, and pelleted for RNA extraction.
914 Cultured human primary astrocytes (HPA) were washed with sterile PBS, scraped and pelleted
915 for RNA extraction. For both nuclei samples and HPA cell samples, pellets were resuspended in
916 RLT lysis buffer with 10% B-mercaptoethanol (B-ME), homogenized with a 22g needle and
917 syringe, combined with equal volume 70% ethanol, and applied to Qiagen micro minelute column.
918 RNA was washed, treated with DNAase, and eluted in 13ul of RNase-free water, according to
919 manufacturer's instructions.

920
921 To measure *ZBTB7A* in bulk brain tissues, postmortem human OFC tissues were sectioned into
922 50 mg sections. Frozen mouse brains were sliced into 1mm coronal slices in a brain matrix, and
923 2mm OFC punches were removed. For both human and mouse tissues, sections were
924 homogenized in Trizol (Thermo Fisher #15596026) with a motorized pestle, followed by
925 chloroform extraction and precipitation with 70% ethanol. Samples were applied to a Qiagen micro
926 minelute column, and RNA was washed, treated with DNase, and eluted according to
927 manufacturer's instructions into 13ul RNase-free water. For all RNA samples (derived from nuclei,
928 cells, or brain tissues), 500ng of total RNA was utilized to synthesize cDNA using the Bio-Rad
929 script cDNA synthesis kit (#1708891). From this reaction, 4 ng of cDNA was used to perform
930 qPCR with PowerUp™ SYBR™ Green Master Mix (#A25742), according to the manufacturer's
931 instructions. Target gene CT values were averaged over 3 replicates, normalized to the reference
932 gene (human brain – *HPRT1*, mouse brain – *Gapdh*), and the $\Delta\Delta CT$ was calculated. Graphs show
933 experimental group fold change relative to controls, mean +/- SEM. Full list and sequences of
934 primers used can be found in **Table S8**.

935
936 **Western Blot**
937 In order to measure protein expression, postmortem human OFC tissues were sectioned into 50
938 mg sections. Frozen mouse brains were sliced into 1mm coronal slices, and 2mm OFC punches
939 were removed. For both human and mouse tissues, sections were homogenized in 200ml RIPA
940 cell lysis buffer, 1X protease inhibitor cocktail and 1X phospho-stop inhibitor using a 1ml dounce
941 homogenizer. Following homogenization, lysates were briefly sonicated with a probe sonicator for
942 five 1s pulses. Protein concentrations were measured using the DC protein assay kit (BioRad),
943 and 20 ug of protein was loaded onto 4-12% NuPage BisTris gels (Invitrogen) for electrophoresis.
944 Proteins were then fast-transferred to nitrocellulose membranes and blocked for 1 hr in 5% milk
945 in PBS + 0.1% Tween 20 (PBST), followed by incubation with primary antibodies overnight at 4°

946 C with rotation. The following antibodies were used: monoclonal rabbit anti-zbtb7a (Abcam #
947 ab175918) (1:1000) for human blots, rabbit anti-Zbtb7a (Abcam #ab106592) (1:1000) for mouse
948 blots, as well as rabbit anti-Gapdh (Abcam #ab9485) (1:10,000), and rabbit anti-H3.3 (Abcam
949 #ab1791). After overnight primary antibody incubation, membranes were washed 3x in PBST (10
950 min) and incubated for 1 hr with horseradish peroxidase conjugated anti-rabbit (BioRad 170-6515,
951 lot #: 64033820) secondary antibodies (1:10000; 1:50000 for anti-Gapdh antibody, BioRad) in 5%
952 milk/PBST at RT. After three final washes with PBST, bands were detected using enhanced
953 chemiluminescence (ECL; Millipore). Densitometry was used to quantify protein bands using
954 ImageJ Software (NIH). Target protein measurements were normalized to Gapdh bands, and
955 experimental group fold change was calculated relative to controls. Raw blots and indication of
956 representative images utilized can be found in **Figure S6**.

957
958 **Animals**

959 C57BL/6J mice were purchased from The Jackson Laboratory (Stock #024694). All procedures
960 were done in accordance with NIH guidelines and the Institutional Animal Care and Use
961 Committees of the Icahn School of Medicine at Mount Sinai.

962
963 **Male Chronic Social Defeat Stress Paradigm**

964 In order to investigate the expression of Zbtb7a in the context of a mouse model of stress, the
965 Chronic Social Defeat Stress (CSDS) in males was performed as described previously⁹⁵. Briefly,
966 a cohort of 20 8-week old male C57BL/6J mice were randomly assigned to either the control or
967 stress condition. Animals in the stress group underwent 10 consecutive days of a single 7-minute
968 defeat session with an unfamiliar CD1 retired breeder male that had been previously screened
969 for aggression towards C57BL/6J mice. Following the defeat session, the C57 mice spent 24
970 hours in the same cage as the CD1, separated by a perforated divider to allow for sensory contact.
971 Control animals spent 24 hours in the same cage as a different male C57BL/6J for each day of
972 the 10 day paradigm, separated by a perforated divider. The Social Interaction (SI) test was

973 performed as described previously⁴⁶. Briefly, in the first trial, the subject mouse was allowed to
974 freely explore an arena with an empty mesh cage inside an interaction zone. In the second trial,
975 a CD1 target was put in the mesh cage, and the mouse was again allowed to explore the arena.
976 Trials are recorded and scored by Ethovision software: SI ratio score was calculated as (time
977 spent in interaction with target)/(time spent in interaction zone without target). Control mice
978 typically have scores ≥ 1.0 , indicating increased time spent investigating the unfamiliar mouse. In
979 the stress mice, scores < 1.0 are defined as “avoidant” and mice are described as stress
980 susceptible, while scores > 1.0 are defined as “non avoidant” and the mice are described as stress
981 resilient. In a typical CSDS experiment, approximately 30% of WT mice will segregate into the
982 stress resilient group⁹⁶.

983
984 **Viral Constructs**

985 Viral vector constructs were generated as previously described⁵². Briefly, ZBTB7A overexpression
986 plasmids (Origene Cat. #RC222759) were cloned into either a Lentiviral CMV-driven construct for
987 use in cell culture experiments (shown in **Figure S2**) or a GFAP-GFP Adeno-associated virus
988 (AAV) construct (Addgene plasmid #50473) for use in animal experiments (utilized in **Figure 3**
989 **and Figure 4**). Lentiviral vectors contained either a ZBTB7A-HA tagged overexpression construct
990 or an empty vector expressing RFP. AAV vectors contained either an ZBTB7A OE construct or
991 GFP. For AAV vectors utilized in **Figure 3**, a miRNA targeting endogenous Zbtb7a was generated
992 using the BLOCK-iT™ Pol II miR RNAi Expression Vector Kit with EmGFP (Thermo # K493600),
993 in addition to a scramble negative control (Thermo # K493600) (miR-neg) which forms a hairpin
994 structure just as a regular pre-miRNA, but does not target any known vertebrate gene. Constructs
995 were packaged into GFAP driven AAV expression vectors to generate AAV-GFAP-Zbtb7a-miR-
996 GFP and AAV-GFAP-mir-neg-GFP. Purified plasmids were sent to GENEWIZ for sequence
997 validation. Plasmids were sent to Cyagen Biosciences for packaging into Lentivirus or AAV6
998 serotype viruses at high titer ($>10^{12}$ units).

999
1000 Negative control sequence without 5' overhangs:
1001 GAAATGTACTGCGCGTGGAGACGTTTGGCCACTGACTGACGTCTCCACGCAGTACATT
1002
1003 Oligos used for Zbtb7a KD:
1004 NM_010731.3_1062_top:
1005 TGCTGTAGAAGTCCAAGCCATTGCAGGTTTGGCCACTGACTGACCTGCAATGTTGGACTTCTA
1006 NM_010731.3_1062_bottom:
1007 CCTGTAGAAGTCCAACATTGCAGGTCAGTCAGTGGCAAAACCTGCAATGGCTTGGACTTCTAC
1008
1009 **Primary Human Astrocyte Cell Culture and LPS treatment**
1010 Primary Human Astrocytes isolated from human cerebral cortex and frozen at first passage were
1011 purchased from ScienCell (#1800) and cultured in Astrocyte medium (ScienCell #1801) on 50ug/ml
1012 coated Matrigel-coated plates (BD #354230). Cells were treated with lentivirus particles at MOI =
1013 ~2 to overexpress ZBTB7A or RFP. Approximately 72 hours after lentivirus transduction, PHAs
1014 were treated with 2ug puromycin to positively select for cells expressing each construct. After 6
1015 days of selection, cells were collected for molecular analyses (**Figure S2O-Q**). For testing
1016 ZBTB7A mRNA expression in inflammatory conditions, the cells were treated with either saline or
1017 LPS (Sigma Cat. # L2630) at 1 ug/ml for 8 hours, and collected for molecular analyses (**Figure**
1018 **S2R**).
1019
1020 **Primary Mouse Astrocytes**
1021 Primary astrocytes were cultured from frontal cortical dissections of mouse pups at P1, as
1022 previously described ⁹⁷. Briefly, cortices were dissociated, and diluted in 10% Fetal Bovine Serum
1023 (OmegaSci, FB-11)/1% penicillin-streptomycin in DMEM (Gibco, 11995-065) and plated at a
1024 density of one brain per uncoated T75 flask. On DIV 1, plates were tapped to dislodge neurons
1025 and the media was changed to remove floating cells. Remaining astrocytes were maintained and
1026 grown to confluence and seeded at a density of approximately 3x10⁶ cells/plate for subsequent
1027 experiments. Once confluent, the cells were treated with either saline or LPS (Sigma Cat. #
1028 L2630), and collected for molecular analyses (**Figure S2S**).

1029
1030

TRAP-sequencing Data

1031 Polyribosome immunoprecipitation was performed as described⁹⁸. Briefly, mice were put through
1032 the CSDS paradigm, as described above, and sacrificed by rapid decapitation. Brain regions were
1033 dissected. Brain tissue was homogenized and homogenates were centrifuged to remove cell
1034 debris, and NP-40 (EMD Biosciences) and DHPG (Avanti Polar Lipids) were added, followed by
1035 another centrifugation step. The supernatant, which contains the ribosomes, was subjected to
1036 immunoprecipitation using anti-EGFP antibodies conjugated to Protein G magnetic Dynabeads
1037 (Invitrogen). The beads were washed, and RNA was extracted using Trizol reagent following the
1038 manufacturer's protocol. RNA was further purified on RNeasy columns (Qiagen). RNA was
1039 amplified using the Ovation RNA-seq System V2 (NuGEN). Library preparation and amplification
1040 was performed by the Rockefeller University Genomic Facility, and libraries were sequenced on
1041 the Illumina HiSeq platform.

1042
1043

Immunohistochemistry

1044 Mice were anesthetized with intraperitoneal (i.p.) injection of ketamine/xylazine (10/1 mg/kg), and
1045 then perfused transcardially with ice cold phosphate buffered saline (PBS) followed by ice cold
1046 4% paraformaldehyde (PFA) in PBS. Next, brains were post-fixed in 4% PFA overnight at 4° C
1047 and then transferred into 30% sucrose in PBS for two days. Brains were then cut into serial 40
1048 µm coronal slices in a cryostat at -20C. Free floating slices containing OFC were washed 3x in
1049 tris buffered saline (TBS), incubated for 30 min in 0.2% Triton-X in TBS to permeabilize tissue,
1050 and then incubated for 1 hr at RT in blocking buffer (0.3% Triton-X, 3% donkey serum in TBS).
1051 Brain slices were then incubated overnight on an orbital rotator at 4 degrees C with primary
1052 antibodies. 24 hours later, brain slices were washed 3x in TBS and then incubated for 2 hrs at
1053 room temperature (RT) with a fluorescent-tagged AlexaFluor 680 secondary antibody. Brain
1054 sections were then washed 3x in TBS, incubated with DAPI (1:10000, lot #: RK2297251, Thermo
1055 Scientific 62248) for 5 min at RT, mounted on Superfrost Plus slides (Fischer Scientific) and then

1056 coverslipped with Prolong Gold (Invitrogen). Immunofluorescence was visualized using a confocal
1057 microscope (Zeiss LSM 780). For quantification of Zbtb7a overlap with Gfap, images were split
1058 into respective color channels, and we calculated the Mander's Correlation Coefficient⁹⁹ using the
1059 *coloc2* package (version 2.0.2) on FIJI, which performs pixel intensity correlation and statistical
1060 testing.

1061
1062 Antibodies used:

1063 *Primary*

1064 Chicken-anti-GFAP (astrocyte marker): Thermo Scientific # PA1-10004 (1:1000)
1065 Mouse-anti-NeuN (neuronal marker): Millipore # MAB377 (1:1000)
1066 Note viruses utilized express eGFP (Zbt-KD/GFP and ZBT-OE/GFP) and mCherry (Gi DREADD)

1067
1068 *Secondary*

1069 Goat-anti-chicken Alexaflour 680: Thermo Scientific # A32934 (1:500)
1070 Donkey-Anti-mouse Alexaflour 680: Thermo Scientific # A32788(1:500)
1071 Donkey-anti-Rabbit Alexaflour 568: Abcam #ab175470 (1:500)
1072 Goat-Anti-Armenian hamster: Jackson Immunoresearch #127-545-099 (1:500)

1073
1074 Animal Surgeries

1075 Male C57BL/6J mice were anesthetized with a ketamine/xylazine solution (10/1 mg/kg) i.p.,
1076 positioned in a stereotaxic frame (Kopf instruments) and 1 μ l of viral construct was infused
1077 bilaterally into the OFC using the following coordinates; AP, 2.6 mm; ML, \pm 1.2 mm; V, 2.8 mm,
1078 angle 10°). Following surgery, mice received meloxicam (1 mg/kg) s.c. and topical antibiotic
1079 treatments for 3 days. All behavioral testing or electrophysiological recordings commenced 21
1080 days after surgery to allow for maximal expression of the viral constructs.

1081
1082 Magnetic Cell sorting

1083 For magnetic-activated cell sorting, we collected virally-infected fresh OFC tissues, pooling 3 mice
1084 per n , and performed the MACs protocol, following manufacturer's instructions. Briefly, OFC
1085 tissues were removed and washed in cold D-PBS, and tissue was dissociated using the Adult

1086 Brain Dissociation Kit, mouse and rat (Miltenyi # 130-107-677) enzyme kit in combination with the
1087 gentleMACs Octo Dissociator with Heaters (Miltenyi # 130-096-427). Samples were strained with
1088 MACs SmartStrainers (70uM, Miltenyi# 130-098-462), and spun at 300g for 10 minutes at 4C.
1089 Myelin debris was removed using myelin removal beads (Miltenyi # 130-096-733) in combination
1090 with the autoMACs Pro Separator. Samples were magnetically labeled with Anti-ACSA-2
1091 Microbeads (Miltenyi #130-097-678) to isolate astrocytes with the autoMACs Pro Separator with
1092 the positive selection program. The negative fraction was subsequently incubated with Adult Non-
1093 neuronal Cell biotin-antibody cocktail (Miltenyi #130-126-603), followed by anti-biotin microbeads
1094 (Miltenyi #130-126-603) and then processed on the autoMACs Pro Separator to isolate neuronal
1095 cells via negative selection. For validation experiments in **Figure S2H-J**, negative fractions
1096 following astrocyte isolation were further processed with anti-Cd11b microbeads (Miltenyi #130-
1097 093-634) to isolate microglia, followed by incubation with anti-Pdgfra microbeads (Miltenyi # 130-
1098 094-543) to isolate immature oligodendrocytes, using the autoMACS Pro Separator prior to
1099 isolation of neuronal fraction, as described above. Isolated astrocyte and neuronal cells were then
1100 counted, with 50K cells separated for ATAC-seq, and the remainder of cells used for RNA
1101 extraction via trizol, followed by cleanup using the Qiagen Minelute kit. For the mouse ATAC-seq,
1102 MACs-isolated cells were processed according to the OMNI-ATAC protocol¹⁰⁰, which has been
1103 optimized for fresh cells.

1104
1105 **Mouse ATAC-seq Differential Accessibility Analysis:**

1106 Raw sequencing reads were aligned to the mouse genome (mm10) using default settings of
1107 HISAT2¹⁰¹. Only uniquely mapped reads were retained. Alignments were filtered using
1108 SAMtoolsv1.19¹⁰² to remove duplicate reads. Peak calling was performed using MACSv2.1.124
1109 with settings --nomodel --shift -100 --extsize 200. Peaks were filtered for FDR < 0.05. Differential
1110 analyses were performed using diffReps20 with a window size of 1 kb. A default p-value cutoff of
1111 0.0001 was used. Peaks and differential sites were further annotated to nearby genes or

1112 intergenic regions using the region analysis tool from the diffReps package. DiffReps outputs can
1113 be found in **Table S6**.

1114
1115 **Reward Sensitivity Tasks and Operant Saccharin Behavior:**

1116 Animals were single housed and given restricted access to water (4h/day for 4d) before the start
1117 of the behavioral training. During the course of the experiment, mice were given access to water
1118 for 2h each day (post-session). Reward-learning training was performed as previously
1119 described¹⁰³, with minor modifications.

1120
1121 The first stage of the experiment was four days of Pavlovian cue-reward association training for
1122 reinforcement with 0.2% saccharin-solution. Modular standard mouse operant chambers
1123 enclosed in light and sound blocking cubicles were used, equipped with white house lights and
1124 ventilation fans - interior dimensions: 55.69 × 38.1 × 40.64 cm; exterior dimensions:
1125 63.5 × 43.18 × 44.45 cm; walls: 1.9 cm) (MedAssociates, Fairfax, VT). Each chamber contained
1126 two retractable levers and one central reward magazine containing a dipper calibrated to provide
1127 ~50ul of liquid saccharin reward per each reinforcement. Each daily session was 40-min (with
1128 operant levers retracted), in which mice learned to introduce their noses into the central reward
1129 magazine to get saccharin rewards, which were delivered every 60 s. A cue light above the
1130 magazine signaled reward delivery. Correct and incorrect saccharin retrieval was detected via
1131 infrared beam breaks upon head entry in the magazine and automatically recorded by MedPC
1132 software.

1133
1134 Next, mice were put through 5-7 days of 1 h sessions of operant learning training, in which mice
1135 were conditioned to lever press on a fixed-ratio 1 (FR1) schedule for *ad libitum* saccharin
1136 reinforcement. The basic settings were: session onset was indicated by illumination of the house
1137 light , and extension of both active and inactive levers; one active lever response (FR1) initiated

1138 magazine-cue light illumination and subsequent reward delivery, and following retrieval a 2.5 s
1139 inter-trial interval (ITI) was initiated; the session terminated after 1 hr.

1140
1141 In OE studies, after lever-press training as described above, mice were further trained on a
1142 reversal learning paradigm, using two levers positioned left and right of the central liquid reward
1143 magazine. For the baseline phase, mice went through 1 session/day for 8 days of training: On
1144 FR1, a response at the correct lever initiated magazine light and reward delivery,; the session
1145 terminated after 30 min. At the reversal phase, the previously incorrect lever was now correct
1146 and *vice versa*, so that non-reward-shift behavior was required; reversal testing lasted for an
1147 additional 8 days.

1148
1149 **Subthreshold Social Defeat Paradigm**
1150 In order to investigate the role of Zbtb7a in stress vulnerability, we performed the Subthreshold
1151 variant of the Social Defeat Paradigm (SSDS) on a cohort of 8 week old C57BL/6J male mice that
1152 were injected with either the rAAV6-GFAP-Zbtb7a OE construct or the rAAV6-GFAP-GFP empty
1153 control vector into the OFC 3 weeks previously. Half of each virus group was randomly assigned
1154 to the stress group or control group. The stress group underwent the SSDS paradigm as
1155 described previously¹⁰⁴. Briefly, the stress mice were subjected to three 5-min defeat sessions
1156 with an aggressive CD1 male mouse consecutively on a single day, separated by a 15-minute
1157 rest period. The experimental mouse then spent 24 hours in the aggressor home cage, separated
1158 by a perforated divider to allow sensory exposure to the aggressor, and was then tested for social
1159 interaction as described above. Note that WT mice do not show behavioral deficits after the SSDS
1160 paradigm.

1161
1162 **Forced Swim**

1163 The forced swim test (FST) was similarly conducted as previously described⁵². Mice were placed
1164 in a 4 liter glass beaker with 2L of room-temperature water for 7 minutes. Each session was
1165 recorded and scored by a blinded observer to record the number of seconds each mouse was
1166 immobile during the last 4 minutes of the test.

1167
1168 **Singe-cell suspension preparation and Flow Cytometry**

1169 Single-cell suspensions from the brain tissue were prepared as described previously¹⁰⁵. Briefly,
1170 virally-transduced OFC tissue was dissected, minced and digested with 450 U/ml collagenase I,
1171 125 U/ml collagenase XI, 60 U/ml DNase I and 60 U/ml hyaluronidase (Sigma) in PBS for 40 min
1172 at 37 °C. Samples were passed through a 70-µm cell strainer and mixed with 30% percoll layered
1173 on top of 70% percoll. The percoll gradient was centrifuged at 500 g for 30 min with the brake off.
1174 The cell fraction was collected and washed with PBS before downstream applications. Total viable
1175 cell numbers were quantified using counting beads (Thermo Fisher Scientific). Cell suspensions
1176 were stained with the antibody cocktail in PBS supplemented with 2% FBS and 0.5% BSA. The
1177 following monoclonal antibodies were used for flow cytometry analyses at a dilution of 1/700: anti-
1178 CD45 (BioLegend, clone 30-F11, 103147), anti-CD11b (BioLegend, clone M1/70, 101226), anti-
1179 CD11c (Biolegend, clone N418, 117333), anti-TREM2 (R&D Systems, clone 237920,
1180 FAB17291P), anti-P2RY12 (Biolegend, clone S16007D, 848003), anti-ASCA2 (Miltenyi Biotec,
1181 clone REA969, 130-116-245), anti-MHCII (BioLegend, clone M5/114.152, 107602) and anti-
1182 CCR2 (R&D systems, clone 475301, MAB55381). Viable cells were identified through negative
1183 staining for Zombie NIR (BioLegend). Data were acquired on a Cytek Aurora and analyzed with
1184 FlowJo (Tree Star). Flow cytometry gating strategy shown in **Figure S6** included all cells, singlets,
1185 live cells and cell populations were identified as astrocytes (ACSA2⁺CD45⁻) or microglia
1186 (CD45^{mid}P2RY12⁺CD11b⁺).

1187
1188 **Electrophysiology**

1189 Male C57BL/6J mice (age approximately 60 days) were deeply anesthetized with isoflurane and
1190 then decapitated, followed by rapid removal and chilling of the brain. Coronal slices (300 μ m thick)
1191 were prepared using a Comprestome vibrating microtome (Precisionary, Natick, MA), in ice-cold
1192 sucrose cutting solution (in mM: 215 sucrose, 2.5 KCl, 1.6 Na₂HPO₄, 26 NaHCO₃, 4 MgSO₄, 1
1193 CaCl₂ and 20 glucose). The slices then were transferred to artificial cerebrospinal fluid (ACSF; in
1194 mM: 120 NaCl, 3.3 KCl, 1.2 NaHPO₄, 26 NaHCO₃, 1 MgSO₄, 2 CaCl₂ and 11 glucose; pH 7.2, 300
1195 mOsM; bubbled with 95% O₂/5% CO₂) at 32°C for 30 minutes, after which they were transferred
1196 to room temperature ACSF and allowed to recover for at least one hour. Recordings were
1197 obtained in a submersion recording chamber superfused with ACSF (1 mL/min) at room
1198 temperature. A concentric bipolar stimulating electrode was placed in layer 1 of the orbital frontal
1199 cortex to evoke synaptic responses using a 100 μ s stimulus delivered by an IsoFlex stimulus
1200 isolator (AMPI, Jerusalem, Israel). A glass Ag/AgCl electrode filled with ACSF recorded field
1201 excitatory synaptic potentials (fEPSPs) from layer 5. Recordings were acquired using Axoclamp
1202 2A and Axopatch 1D amplifiers, Digidata 1440A analog-digital convertor, and pClamp software
1203 10 (all from Molecular Devices, San Jose, CA). Signals were low-pass filtered at 2 kHz and
1204 digitized at 10 kHz. An input-output (I-O) curve was constructed by recording fEPSPs in response
1205 to stimuli ranging from 100-800 μ A (average of three fEPSPs per stimulus strength, recorded at
1206 intervals of 20 seconds between stimuli, starting with the lowest intensity). Given the proximity of
1207 the recording electrode to the stimulating electrode within respective layers of the OFC, we plotted
1208 peak amplitude (instead of peak slope), to avoid effects of recording artifacts. Following
1209 construction of an I-O curve, the stimulus intensity that evoked a fEPSP of ~50% of maximum
1210 amplitude was used in rundown experiments. Separate slices from the same animals were used
1211 for the rundown experiments. For rundown experiments, a single 30-s train was delivered at 10
1212 Hz after establishment of a stable baseline. The percentage change in fEPSP amplitude from
1213 baseline was calculated. All data were graphed as means \pm SEM.
1214

1215 **Calcium Imaging**

1216 Calcium imaging was performed in 2D primary mixed cultures of mouse cortical neurons and glia
1217 (including astrocytes) to assess neuronal and astrocytic activity, using the genetically encoded
1218 calcium indicator GCaMP6f. Primary mixed cultures were transduced with AAV1-hSyn-GCaMP6f
1219 (Addgene # 100837-AAV1) or AAV5-gfaABC1D-cyto-GCaMP6f (Addgene # 52925-AAV5) to
1220 ensure selective expression solely in neurons or in astrocytes, respectively, at least 5 days prior
1221 to the imaging sessions. Both GCaMP6f-astrocyte and GCaMP6f-neuronal cultures were treated
1222 with the AAV-GFAP-ZBTB7A OE vector for ZBT-OE conditions. In GCaMP6f-neuron cultures, to
1223 control for the astrocyte AAV treatment, “control virus” conditions were additionally treated with
1224 an AAV5-RFP empty vector. Neurons and astrocytes activity were imaged independently after
1225 14-23 DIV, in mixed cultures plated on poly-d-lysine matrix (0.1 mg/mL, gibco #A38904-01)
1226 coated 10 mm glass coverslips. A Nikon Eclipse TE2000-U microscope with a 10X objective was
1227 used to image the coverslips with the mixed primary cultures mounted on a diamond-shaped
1228 chamber. To excite and detect GCaMP6f fluorescence, a 480 nm LED (Mic-LED-480A,
1229 Prizmatix), a HQ480/40x excitation filter, a Q505LP dichroic mirror, and a HQ535/50m emission
1230 filter (Semrock) were used. Emitted fluorescence was projected onto a sCMOS Zyla chamber
1231 camera (VSC-01910, Andor) and sampled at 8.87 fps for GCaMP6f-expressing neurons (284x240
1232 pixels, 3x3 binning) and 4.7 fps for GCaMP6f-expressing astrocytes (160x135 pixels, 4x4
1233 binning). Nikon Elements software (NIS-Elements AR 5.20.01) was used to control light source
1234 and sCMOS camera.

1235
1236 To record spontaneous neuronal or astrocytic calcium spikes, mixed cultures were continuously
1237 perfused during fluorescence recording with artificial cerebrospinal fluid buffer (ACSF), with the
1238 following composition (in mM): NaCl 125, KCl 5, D-Glucose 10, HEPES-Na 10, CaCl₂ 3.1, MgCl₂
1239 1.3. (pH adjusted to 7.4 with HCl and osmolarity corrected with sucrose to 290-300 mOsm).

1240 Perfusion was gravity fed (flow rate of 0.065 ml/s) and controlled with a ValveBank8 II (AutoMate
1241 Scientific Inc.).

1242
1243 GCaMP6f-expressing neurons or astrocytes ROIs were segmented, and raw fluorescence data
1244 were background corrected and extracted using Nikon Elements software. $\Delta F/F$ was calculated
1245 as $(F_t - F_{\min})/F_{\min}$, being F_t = raw fluorescence at time t, and F_{\min} = minimum fluorescence for the
1246 entire trace. A low-pass Butterworth filter was used to denoise the $\Delta F/F$ trace, and an adaptive
1247 iteratively reweighted Penalized Least Squares (AirPLS) based algorithm¹⁰⁶ was applied to
1248 baseline correct the $\Delta F/F$ trace for drift, using R-Studio (R version 4.0.3). Spike detection was
1249 performed using a custom script in R that applied specific criteria for neuronal and astrocytic
1250 calcium events. For neurons, action potential-derived Ca^{2+} spikes had the following criteria
1251 (framerate acquisition of 8.87 fps): duration < 45 frames, rise phase \geq 3 frames, fall phase \geq 3
1252 frames, rise phase \leq fall phase, peak height $> 4 \times \text{SD}$ (for ROIs with $\text{SD} < 20$), and peak height $>$
1253 $3 \times \text{max background signal}$. For astrocytes, Ca^{2+} events detected fell in the following criteria
1254 (framerate acquisition of 4.7 fps): duration < 100 frames, rise phase \geq 10 frames, fall phase \geq
1255 10 frames, peak height $> 4 \times \text{SD}$ (for ROIs with $\text{SD} < 20$), and peak height $> 4 \times \text{max background}$
1256 signal. Final n of cells per condition was as follows: astrocyte-GCaMP6f ($n=623$ cells control virus
1257 saline, $n= 559$ cells ZBT OE saline, $n=747$ cells control virus LPS, and $n=517$ cells ZBT OE LPS)
1258 and neuron gCaMP6f ($n=135$ cells control virus saline, $n= 1277$ cells ZBT OE saline, $n=238$ cells
1259 control virus LPS, and $n=1324$ cells ZBT OE LPS). Statistical analysis was performed in
1260 GraphPad Prism 8.4.3.

1261
1262 **Chemogenetic Manipulation**

1263 In order to determine if neuronal hyperexcitability contributes to the observed behavioral effects
1264 of ZBTB7A OE, we performed the Subthreshold variant of the Social Defeat Paradigm (SSDS) on
1265 a cohort of 8 week old C57BL/6J male mice that were injected with the pAAV-hSyn-hM4D(Gi)-
1266 mCherry to express the inhibitory Gi DREADD (Addgene #50475-AAV2), in combination with

1267 either the rAAV6-GFAP-Zbtb7a OE construct or the rAAV6-GFAP-GFP empty control vector into
1268 the OFC 3 weeks previously. Half of each virus group was randomly assigned to the ZBT OE or
1269 GFP viral group. Both viral groups underwent the SSDS paradigm as described previously¹⁰⁷. The
1270 experimental mouse then spent 24 hours in the aggressor home cage, separated by a perforated
1271 divider to allow sensory exposure to the aggressor. The mice were then single housed for 24
1272 hours, and then half of each viral group was injected with either the DREADD agonist
1273 Deschloroclozapine⁵² (Tocris # 7193) at 1ug/kg in 1% DMSO or vehicle. Fifteen to twenty minutes
1274 post-injection, the mice were tested for social interaction as described above.

1275
1276 **Acknowledgements**

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1281 R01MH109677 (P.R.), U01MH116442 (P.R.) and R01MH110921 (P.R.), F31 MH116588 and F99
1282 NS125774 (S.L.F.).

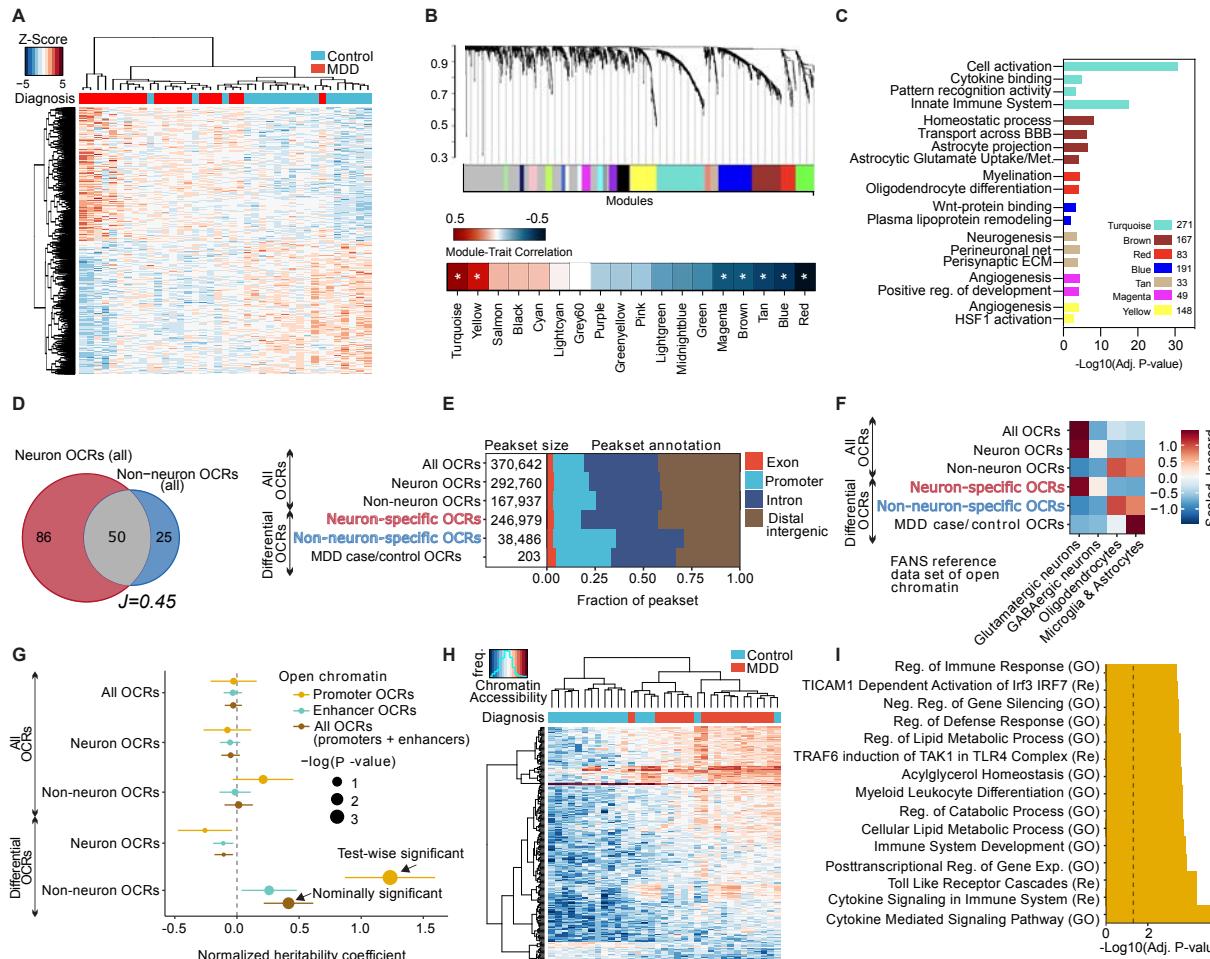
1283
1284 **Data availability**

1285 Raw (FASTQ files) and processed ATAC-seq data (OCRs, and raw / normalized count matrices)
1286 have been deposited in Gene Expression Omnibus and are accessible through GEO Series
1287 accession number GSE149871. Browsable UCSC genome browser tracks of processed data are
1288 available at: https://labs.icahn.mssm.edu/roussos-lab/mdd_atacseq. Raw and processed RNA-
1289 seq data and mouse astrocyte-specific ATAC-seq data is accessible through GEO Series
1290 accession number GSE214922.
1291 External validation sets: RNA-seq of MDD case/control postmortem human brains (GSE102556),
1292 TRAP-seq of astrocyte specific CSDS (GSE139684).

1293

1294 **Main Figures and Figure Legends**

1295 **Note that full statistics information is provided in the Supplemental Statistics Table S7.**



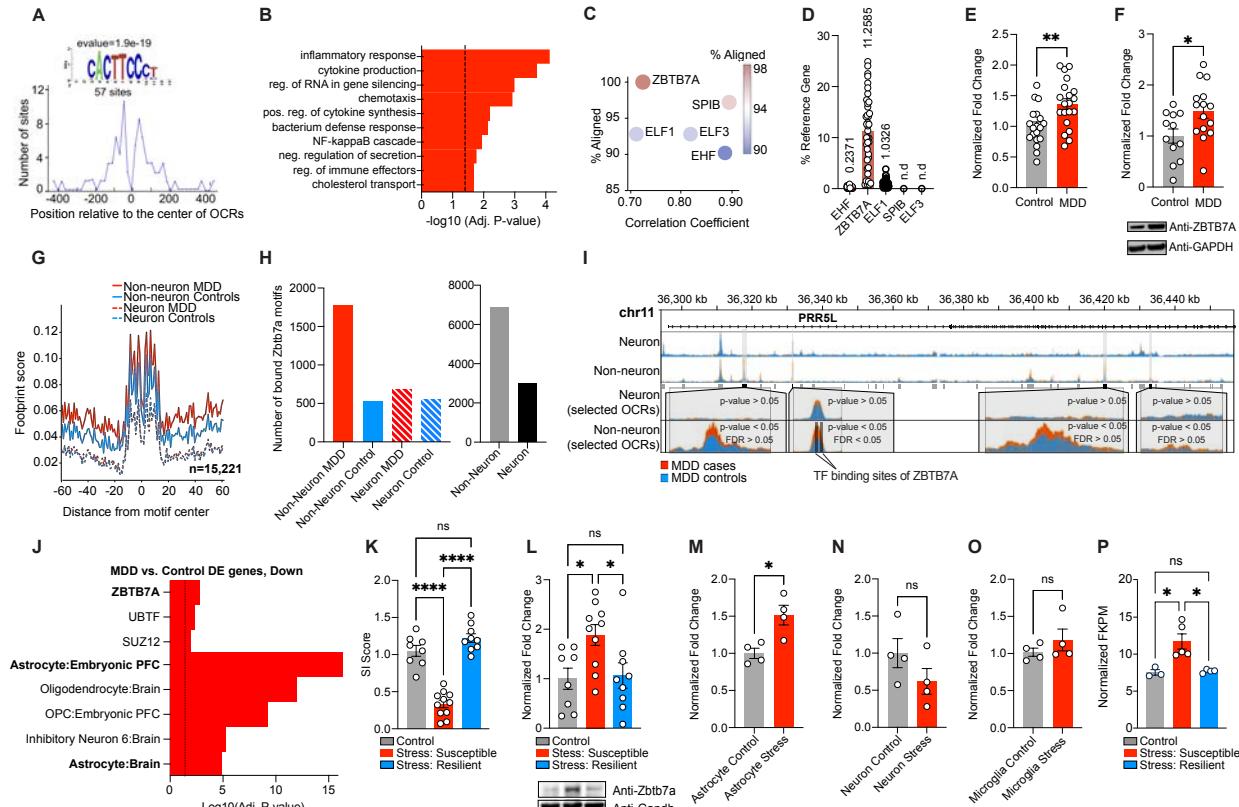
1296

1297 **Fig. 1. Chromatin accessibility profiling in neuronal vs. non-neuronal cells identifies glial**
1298 **regulatory signatures of human MDD in OFC.**

1299

1300 **(A)** Clustering of MDD case and control samples at 1412 differentially expressed (DE) genes
1301 (rows, FDR < 0.1). **(B)** The co-expression modules identified by weighted gene correlation
1302 network analysis (WGCNA) [top] and heatmap of co-expression module correlation with MDD
1303 trait. * indicates Adj. P < .05 significance of correlation. **(C)** Gene Ontology (GO) analysis for
1304 genes in significant co-expression modules. **(D)** Venn diagram of shared and distinct open
1305 chromatin between neuronal and non-neuronal samples. Numbers indicate megabases of OCRs,
1306 "J" indicates the Jaccard index. **(E)** Proportions of all and differential OCRs stratified by genomic
1307 context. **(F)** Overlap of all and differential OCRs with a reference study of lineage-specific brain
1308 open chromatin atlas⁶¹. **(G)** Enrichment of common genetic variants in MDD²³ with all and
1309 differential OCRs when assayed by LD-score regression. Sets of OCRs were further stratified by

1310 genomic context to “Promoter OCRs” overlapping the 3kb window around TSS and “Enhancer
1311 OCRs”. **(H)** Clustering of MDD case and control non-neuronal samples at 203 differentially
1312 accessible OCRs (rows). **(I)** Overlap between gene sets representing biological processes and
1313 pathways with the set of 203 differentially accessible OCRs between MDD cases and control. Top
1314 15 enriched pathways are shown (BH-adjusted p-value < 0.05). Dashed line indicates nominal
1315 significance. “GO”: gene ontology, “Re”: REACTOME.



1316

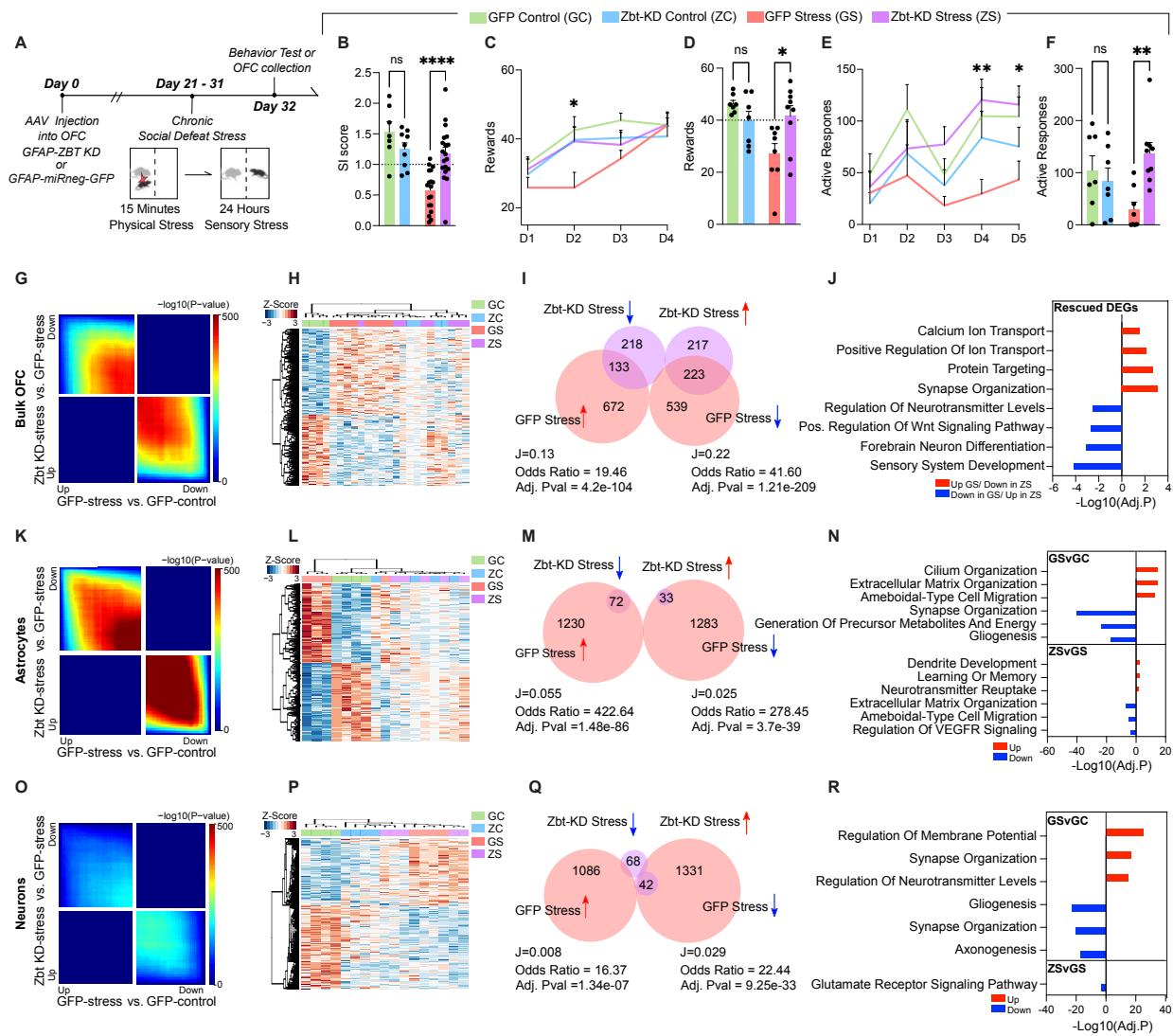
1317 **Fig. 2. Identification of ZBTB7A as a key transcription factor regulating MDD-specific**
1318 **OCRs.**

1319

1320 (A) Distribution of the discovered motif that is significantly enriched ($e\text{-value} = 1.9\text{e-}19$) in MDD-specific OCRs. (B) GO BP terms from MEME-GoMo, based on gene targets of regulatory regions containing the discovered motif. Top 10 most significant terms are shown (BH-adjusted $p\text{-value} < 0.05$). Dashed line indicates $p = 0.05$ significance. (C) Correlation coefficients for TF candidate recognition motifs against discovered motif (x-axis), and percent alignment between TF candidate recognition motifs with discovered motif (y-axis and color key) (D) Percent expression of TF candidate genes (CT value) over reference gene (*HPRT1*). “n.d.” indicates not detected (E) Normalized fold change of *ZBTB7A* transcripts in bulk OFC postmortem human tissues from MDD ($n = 20$) vs. control ($n = 19$) samples. Student’s two-tailed t-test [$t_{37} = 3.215$, $**p = 0.0027$] (F) Normalized fold change of *ZBTB7A* protein in bulk OFC postmortem human tissues from MDD ($n = 15$) vs. control ($n = 12$) samples. Student’s two-tailed t-test [$t_{25} = 2.441$, $*p = 0.0221$] (G) Aggregated footprint scores across *ZBTB7A* transcription factor binding sites that are bound in either MDD or control samples of neuronal or non-neuronal cells. Note that the effect of Tn5 transposase bias is not fully corrected, resulting into unsmoothed signal. (H) Bar graphs for number of bound *ZBTB7A* TFBS detected exclusively in MDD case or control samples from

1335 neuronal and non-neuronal cells (left) and exclusively in non-neuronal and neuronal populations
1336 (mixed MDD/control (right). **(I)** Representative pile-up traces of cell specific ATAC-seq signal
1337 overlapping PRR5L gene. Four OCRs, all being dysregulated between MDD cases and controls
1338 (p-value < 0.05) in non-neuronal cells, are highlighted. The most significantly dysregulated OCR
1339 (FDR<0.05) overlaps two transcription factor binding sites of ZBTB7A. **(J)** GO analysis with
1340 CellMarker Augmented Database²⁵ and CHEA ENCODE Consensus database¹⁰⁸ for genes in the
1341 set of downregulated DE genes from human MDD RNA-seq. **(K)** Social interaction ratio score for
1342 control (n = 8) vs. chronic stress: susceptible (n = 11) vs. chronic stress: resilient mouse (n = 9)
1343 groups. 1-way ANOVA [$F_{2,25} = 66.99$], followed by Tukey's MC test: control vs. stress susceptible
1344 ***p=<.0001, stress susceptible vs. stress resilient ***p=<.0001, control vs. stress resilient ns, p
1345 = .151. **(L)** Normalized fold change protein expression of *Zbtb7a* in mouse OFC bulk tissues
1346 collected from control vs. chronic stress: susceptible vs. chronic stress: resilient mouse groups.
1347 1-way ANOVA [$F_{2,24} = 4.883$], followed by Tukey's MC test: control vs. stress susceptible *p =
1348 0.03, stress susceptible vs. stress resilient *p = 0.039, control vs. stress resilient ns, p = 0.979.
1349 **(M)** Normalized fold change *Zbtb7a* mRNA expression in MACs-isolated astrocytes from
1350 chronically stressed OFC mouse tissues vs. control (n = 4/group). Two-tailed Student's t-test [t_6
1351 = 3.458]. *p = 0.013. **(N)** Normalized fold change *Zbtb7a* mRNA expression in MACs-isolated
1352 neurons from chronically stressed OFC mouse tissues vs. control (n = 4/group). Two-tailed
1353 Student's t-test [t_6 = 1.454]. ns, p = 0.196. **(O)** Normalized fold change *Zbtb7a* mRNA expression
1354 in negative cell fraction post MACs-isolation of astrocytes and neurons, which is enriched for
1355 microglia, from chronically stressed OFC mouse tissues vs. control (n = 4/group). Two-tailed
1356 Student's t-test [t_6 = 1.053]. ns, p = 0.332. **(P)** FKPM values for *Zbtb7a* in astrocyte specific CSDS
1357 TRAP-seq data set [GSE139684], with n = 3 control, n = 5 stress: susceptible, n = 4 stress-
1358 resilient. 1-way ANOVA [$F_{2,9} = 10.01$], followed by Tukey's MC test: control vs. stress susceptible
1359 *p = 0.012, stress susceptible vs. stress resilient *p = 0.01, control vs. stress resilient ns, p =
1360 0.989. All data graphed as means \pm SEM.

1361



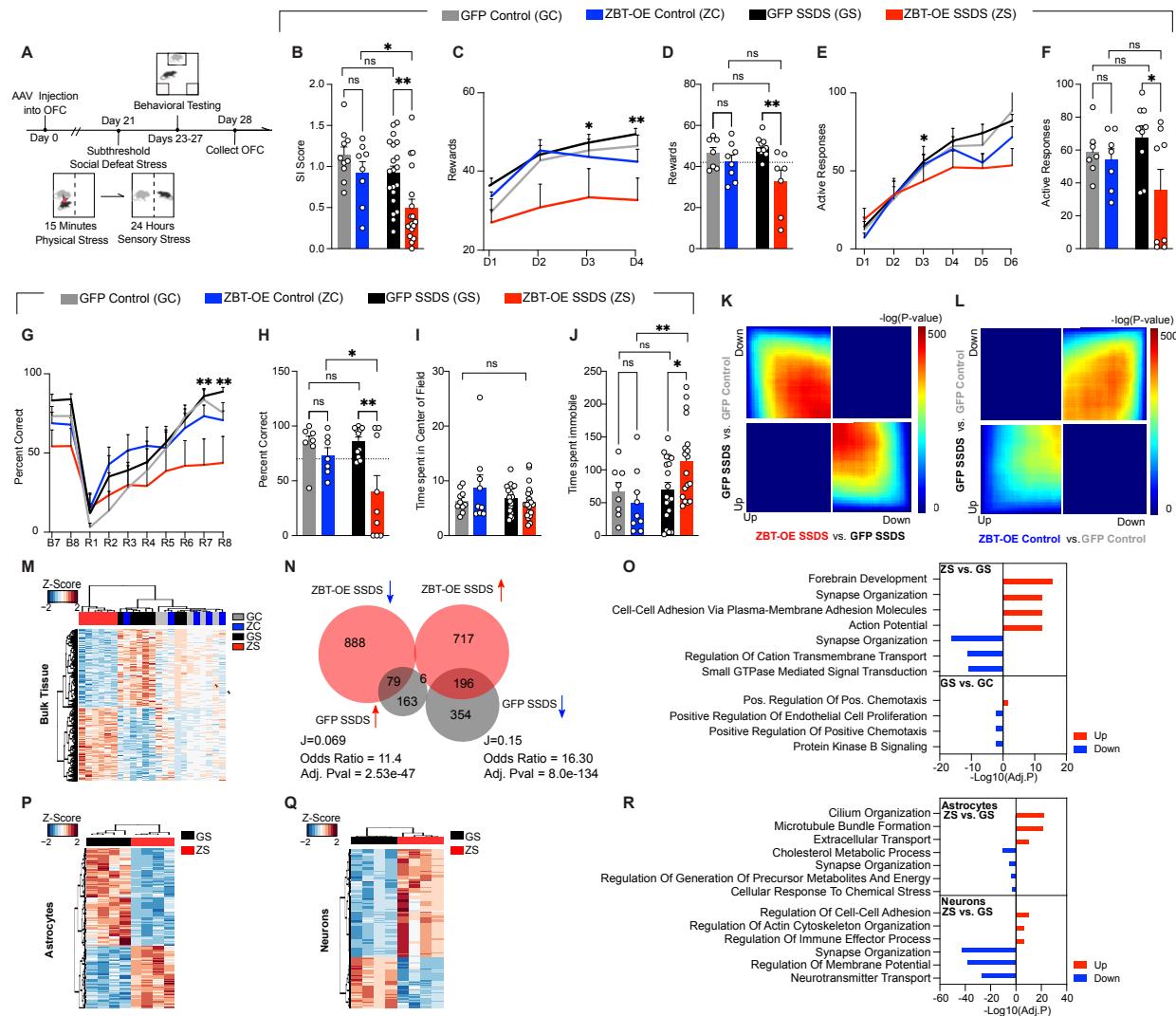
1362

1363 **Fig. 3. Zbtb7a in rodent OFC astrocytes is necessary to promote chronic stress-induced**
1364 **alterations in behavior and gene expression.**

1365

1366 **(A)** Schematic of experimental timeline with CSDS paradigm performed after rAAV6 injection into
1367 OFC, followed by behavioral test and tissue collection for molecular analyses. **(B)** Social
1368 interaction scores. 2-way ANOVA main effect of interaction $F_{1,49} = 13.97$, ***p = 0.0005. Sidak's
1369 MC test, GFP control vs. GFP stress ****p < 0.0001. GFP Stress vs. Zbt-KD stress ****p < 0.0001.
1370 Zbt-KD control vs. Zbt-KD stress ns, p = .8663. GFP control vs. Zbt-KD control ns, p = 0.2958. **(C)**
1371 Pavlovian cue-reward association task. "D" = Day of task. Mixed Effects analysis, main effect of
1372 Test Day x Stress $F_{3,83} = 3.460$ *p = 0.0200. **(D)** Individual values for Day two of task shown in
1373 (C). 2-way ANOVA main effect of Interaction $F_{1,27} = 8.500$ p = 0.0071. Sidak's MC test GFP
1374 control vs. GFP stress **p = 0.0019. GFP stress vs. Zbt-KD stress *p = 0.0119. Zbt-KD control

1375 vs. Zbt-KD stress ns, p=.9208. GFP control vs. Zbt-KD control, ns, p = 0.4086. **(E)** Effort-based
1376 operant reward learning task on FR1 schedule, “D” = Day of task. Mixed Effects analysis main
1377 effect of Virus x Stress [$F_{1,27} = 5.835$] *p = 0.0228. **(F)** Individual values for Day four of task shown
1378 in (E). 2-way ANOVA main effect of Interaction [$F_{1,27} = 8.531$] *p = 0.0070. Sidak’s MC test GFP
1379 control vs. GFP stress, *p = 0.0490. GFP stress vs. Zbt-KD stress **p = 0.0023. Zbt-KD control
1380 vs. Zbt-KD stress ns, p=.1759. GFP control vs. Zbt-KD control ns, p = 0.7740. **(G)** RRHO
1381 comparing gene expression for the indicated comparisons in bulk OFC tissue. Each pixel
1382 represents the overlap between differential transcriptomes, with the significance of overlap of a
1383 hypergeometric test color-coded. **(H)** Clustering of groups at 1,583 DE genes (FDR < 0.1)
1384 between GFP stress and GFP control in bulk OFC. **(I)** Scaled Venn-diagram and odds ratio test
1385 of the overlap between differentially expressed (DE) genes in bulk OFC tissues comparing Zbt-
1386 KD stress vs. GFP stress, with GFP stress vs. GFP control. “J” indicates the Jaccard index. **(J)**
1387 GO analysis for rescued genes in Zbt-KD stress vs. GFP-stress. **(K)** RRHO comparing gene
1388 expression for the indicated comparisons in MACS-isolated astrocytes. Each pixel represents the
1389 overlap between differential transcriptomes, with the significance of overlap of a hypergeometric
1390 test color-coded. **(L)** Clustering of groups at 2,673 DE genes (FDR < 0.1) between GFP stress
1391 and GFP control in MACS-isolated astrocytes. **(M)** Scaled Venn-diagram and odds ratio test of
1392 the overlap between DE genes in MACS-isolated astrocytes comparing Zbt-KD stress vs. GFP
1393 stress, with GFP stress vs. GFP control. “J” indicates the Jaccard index. **(N)** GO analysis for gene
1394 DEGs in GFP-stress vs. GFP control and Zbt-KD stress vs. GFP-stress, separated by up/down
1395 regulation. **(O)** RRHO comparing gene expression for the indicated comparisons in MACS-
1396 isolated neurons. Each pixel represents the overlap between differential transcriptomes, with the
1397 significance of overlap of a hypergeometric test color-coded. **(P)** Clustering of groups at 2,540 DE
1398 genes (FDR < 0.1) between GFP stress and GFP control in MACS-isolated neurons. **(Q)** Scaled
1399 Venn-diagram and odds ratio test of the overlap between DE genes in MACS-isolated neurons
1400 comparing Zbt-KD stress vs. GFP stress, with GFP stress vs. GFP control. “J” indicates the
1401 Jaccard index. **(R)** GO analysis for gene DEGs in GFP-stress vs. GFP control and Zbt-KD stress
1402 vs. GFP-stress, separated by up/down regulation. All data graphed as means \pm SEM.

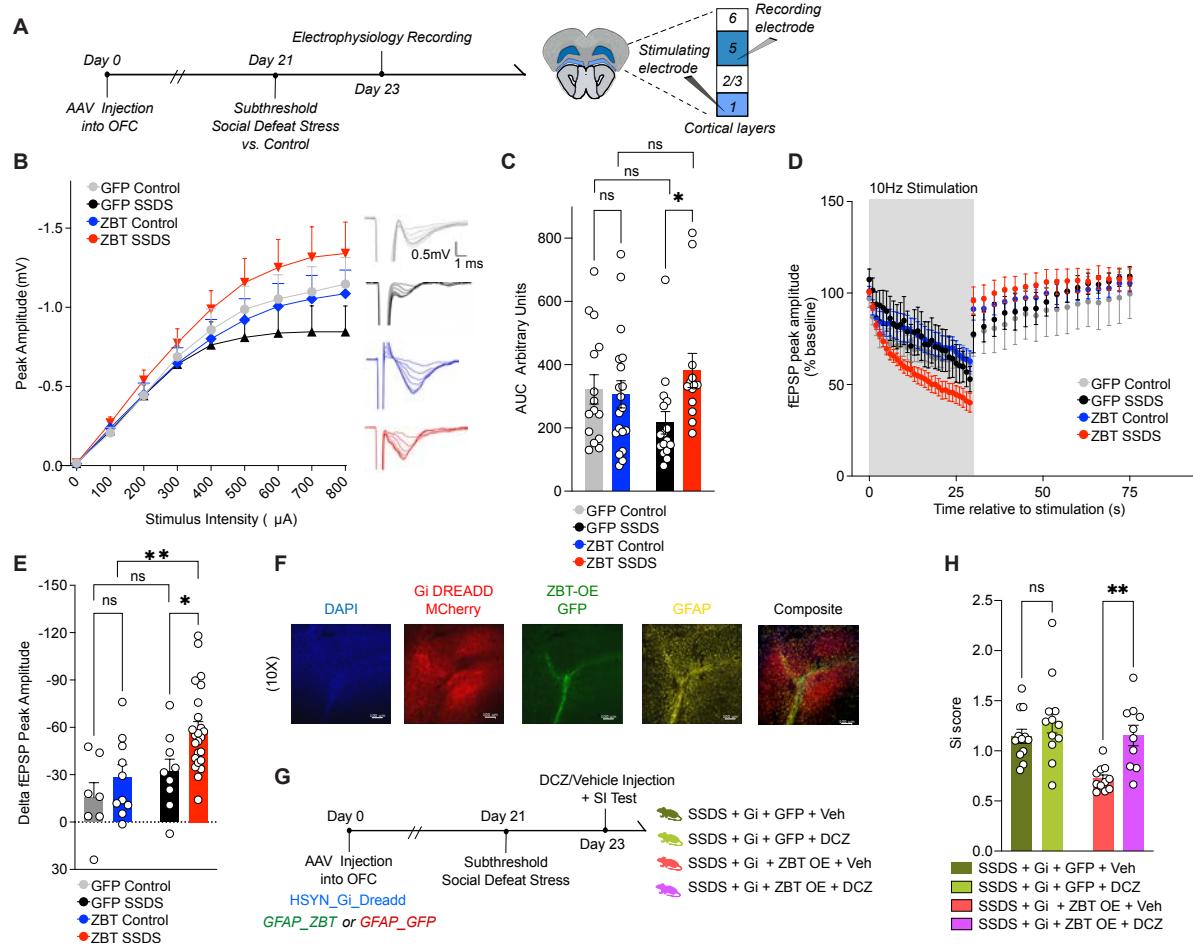


1403

1404 **Fig. 4. ZBTB7A in mouse OFC astrocytes is sufficient to induce chronic stress-mediated**
 1405 **alterations in chromatin accessibility, gene expression, and behavior.**

1406
 1407 (A) Schematic of experimental timeline with subthreshold SSDS mild stress paradigm performed
 1408 after rAAV6 injection into OFC, followed by behavioral tests and tissue collection for RNA-seq.
 1409 (B) Social interaction. 2-way ANOVA main effect of stress [$F_{1,52} = 8.144$], ** $p = 0.0062$, main
 1410 effect of virus [$F_{1,52} = 7.730$], ** $p = 0.0075$. Sidak's MC test, GFP control vs. GFP SSDS ns, $p =$
 1411 0.2788. GFP SSDS vs. ZBT-OE SSDS ** $p = .0041$. ZBT-OE control vs. ZBT-OE SSDS * $p = .0286$.
 1412 GFP control vs. ZBT-OE control n.s. $p = .4480$. (C) Pavlovian cue-reward association task. "D"
 1413 indicates Day of test. 3-way ANOVA, main effect of Virus x Stress [$F_{1,29} = 5.291$] * $p = 0.0288$. (D)
 1414 Individual values for day 2 of task shown in (C). 2-way ANOVA, main effect of virus [$F_{1,28} = 9.759$],
 1415 $p = **0.0041$. Sidak's MC test, GFP control vs. GFP SSDS ns, $p=0.651$. GFP SSDS vs. ZBT-OE
 1416 SSDS ** $p = .0021$. ZBT-OE control vs. ZBT-OE SSDS ns, $p = 0.146$. (E) Operant reward task,

1417 FR1. "D" indicates Day of test. 3-way ANOVA, main effect of Test Day x Virus [$F_{5,149} = 2.823$] *p
1418 = 0.0182. (F) Individual values for day 3 of task shown in (E). 2-way ANOVA, main effect of virus
1419 [$F_{1,27} = 4.408$], *p = 0.0453. Sidak's MC test, GFP control vs. GFP SSDS ns, p=0.709. GFP SSDS
1420 vs. ZBT-OE SSDS *p = .0218. ZBT-OE control vs. ZBT-OE SSDS ns, p = 0.282. (G) Percent
1421 correct trials in reversal learning paradigm. "B" indicates Baseline day, "R" indicates Reversal
1422 phase day. 3-way ANOVA, main effect of Test day x Virus [$F_{9,261} = 4.529$] p < 0.0001. (H)
1423 Individual values for day 7 as shown in (G). 2-way ANOVA, main effect of virus [$F_{1,30} = 9.017$],
1424 **p = 0.0054. Sidak's MC test, GFP control vs. GFP SSDS ns, p=0.9797. GFP SSDS vs. ZBT-OE
1425 SSDS **p = .0013. ZBT-OE control vs. ZBT-OE SSDS *p = 0.0389. GFP control vs. ZBT-OE
1426 control n.s., p = 0.7280. (I) Time spent (s) in the center of the field during open field test. 2-way
1427 ANOVA ns, (J) Forced Swim tests. 2-way ANOVA main effect of interaction [$F_{1,50} = 4.129$], *p =
1428 0.0475, main effect of stress [$F_{1,50} = 4.993$], *p = 0.0475. Sidak's MC test, GFP control vs. GFP
1429 SSDS ns, p=0.9876. GFP SSDS vs. ZBT-OE SSDS **p = 0.0070. (K-L) RRHO comparing gene
1430 expression between indicated comparisons, in the context of mild stress. (M) Clustering at 1,929
1431 DE genes between ZBT-OE SSDS and GFP SSDS. (N) Scaled Venn-diagram and odds ratio test
1432 of the overlap between differentially expressed (DE) genes in bulk OFC tissues comparing ZBT-
1433 OE stress vs. GFP SSDS, with GFP SSDS vs. GFP control. "J" indicates the Jaccard index. Note
1434 for GFP SSDS vs. GFP control, DEGs were defined at pval < 0.05 (O) GO analysis for gene DEGs
1435 in ZBT-OE SSDS vs. GFPSSDS and GFP SSDS vs. GFP control, separated by up/down
1436 regulation. All data graphed as means \pm SEM. (P) Clustering at 715 DE genes between ZBT-OE
1437 SSDS and GFP SSDS astrocytes (n = 4/group). (Q) Clustering at 1,191 DE genes between ZBT-
1438 OE SSDS and GFP SSDS neurons (n = 4/group). (R) GO analysis for DE genes (FDR < 0.1)
1439 between ZBT-OE SSDS and GFP SSDS groups in MACs-isolated astrocytes and neurons,
1440 separated by up/down regulation.



1441

1442 **Fig. 5. ZBTB7A in mouse OFC astrocytes induces cell non-autonomous neuronal**
 1443 **hyperexcitability to mediate stress susceptibility.**

1444

1445 (A) Schematic of experimental timeline with subthreshold stress paradigm performed after AAV6
 1446 injection into OFC, followed by slice electrophysiology recordings. (B) Input-output (I-O) curve
 1447 constructed by recording fEPSPs in response to stimuli ranging from 100-800 μ A. 3-way ANOVA,
 1448 main effect of Stimulus Intensity x Virus x Stress [$F_{8,480} = 2.626$] **p = 0.0080. (C) Individual
 1449 values for (I-O) curve, area under curve (A.U.C.). 2-way ANOVA main effect of Interaction [$F_{1,59} =$
 1450 4.062], *p = 0.0484. Sidak's MC test, GFP control vs. GFP stress ns, p=0.1923. GFP control vs.
 1451 ZBT-OE stress *p = 0.0295. ZBT-OE control vs. ZBT-OE stress n.s., p = 0.4230. GFP control vs.
 1452 ZBT-OE control n.s., p= 0.9597. (D) Rundown stimulation from a single 30-s train delivered at 10
 1453 Hz. The percentage change in fEPSP amplitude from baseline was calculated during and post-
 1454 10Hz stimulation. 3-way ANOVA, main effect of Stimulus x Virus [$F_{29,1334} = 3.376$] ***p < 0.0001,
 1455 main effect of stress x virus [$F_{1,46} = 4.356$] *p = 0.0425. (E) Individual values for delta fEPSP
 1456 amplitude (% baseline) between end of 10Hz stimulation and 1s after end of stimulation train. 2-

1457 way ANOVA main effect of virus [$F_{1,46} = 6.115$], $p = 0.0172$, main effect of stress [$F_{1,46} = 8.454$],
1458 ** $p = 0.0056$. Sidak's MC test, GFP control vs. GFP stress ns, $p=0.5172$. GFP Stress vs. ZBT-
1459 OE stress * $p = 0.0207$. ZBT-OE control vs. ZBT-OE stress ** $p = 0.0059$. GFP control vs. ZBT-OE
1460 control n.s., $p= 0.5172$. (F) IHC validation of hsyn-hM4D(Gi)-mCherry (in red) and GFAP-ZBT OE
1461 (in green) localized in astrocytes (GFAP, in yellow) and DAPI (in blue). Images taken at 10x
1462 magnification. (G) Experimental scheme of chemogenetics experiment, in which SSDS is
1463 performed on a cohort of mice expressing hM4D(Gi)-mCherry (+/-) ZBT OE, (+/-) DCZ. (H) Social
1464 interaction. 2-way ANOVA main effect of Virus [$F_{1,41} = 10.11$], ** $p = 0.0028$, main effect of agonist
1465 [$F_{1,41} = 10.65$], ** $p = 0.0022$. Sidak's MC test, Gi + GFP stress + vehicle vs. Gi + GFP stress +
1466 DCZ ns, $p=0.3880$. Gi + ZBT-OE stress + vehicle vs. Gi + ZBT-OE stress + DCZ ** $p = 0.0040$. All
1467 data graphed as means \pm SEM.
1468

1469 **SUPPLEMENTARY TABLES**

1470

1471 **Table S1_demo:** Demographics of the postmortem brain cohort.

1472

1473 **Table S2_qc:** Quality control metrics of ATAC-seq dataset of postmortem brain cohort.

1474

1475 **Table S3_dac:** Differential analysis between neuronal and non-neuronal samples as well as
1476 among MDD cases and controls in ATAC-seq dataset of postmortem brain cohort.

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1478 **Table S4_gsea:** Gene set enrichment analysis for cell type and disease-specific sets of OCRS.

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1480 **Table S5_deseq2:** DESEQ2 outputs for all RNA-seq experiments.

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1482 **Table S6_diffreps:** Diffreps outputs for mouse ATAC-seq experiments.

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1484 **Table S7_stats:** Full statistical information

1485 **Table S8_primers:** Full list and sequences of human primers used in qPCR experiments

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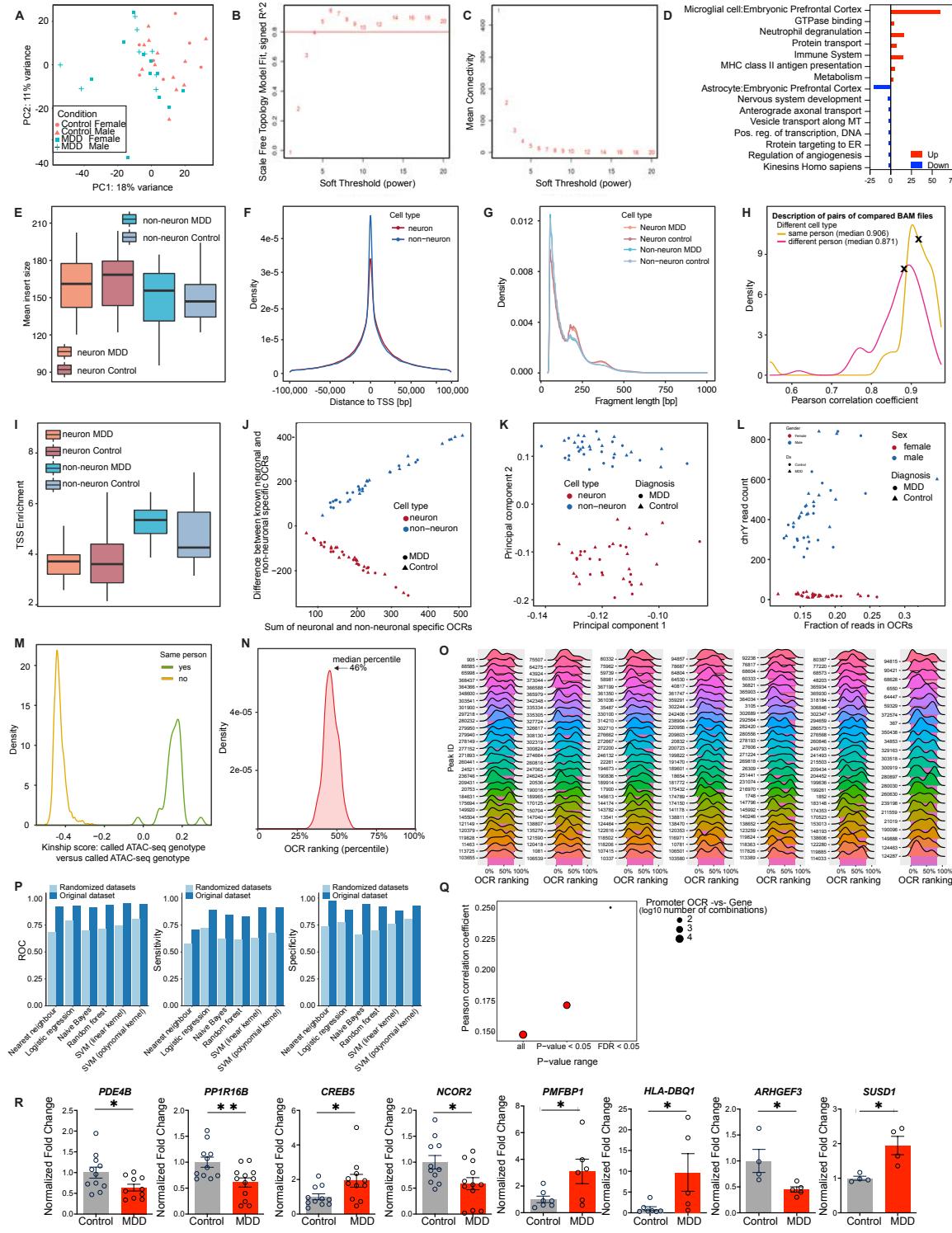
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1773 Supplemental Figures and Figure Legends

1774 Note that full statistics information is provided in the Supplemental Statistics Table S7.

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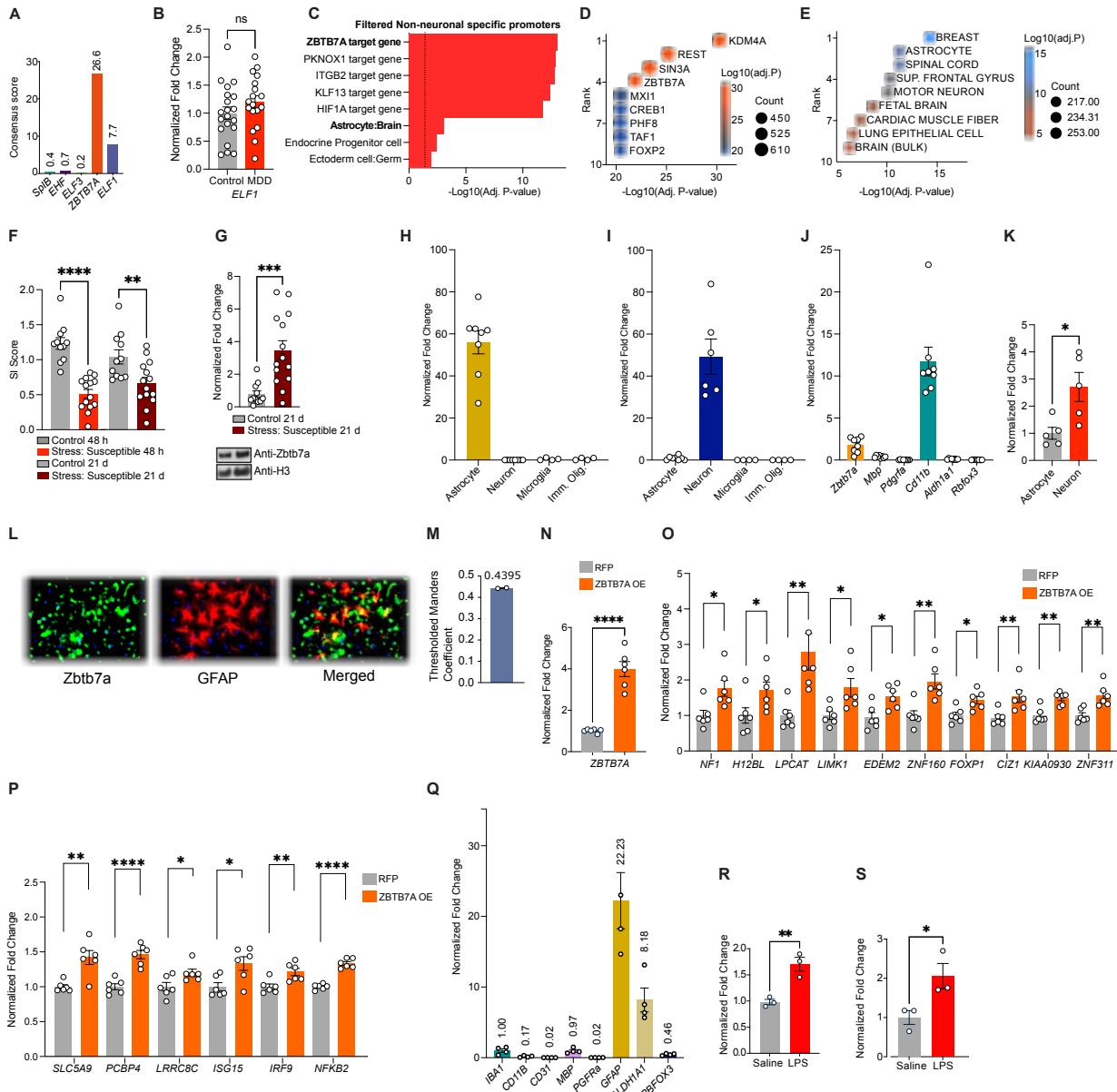
1778 **Supplemental Figure 1. Quality control metrics for human postmortem MDD molecular**
1779 **profiling.**

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1781 **(A)** Principal component analysis of sample gene expression levels. **(B)** Analysis of scale-free fit
1782 index for possible soft-thresholding powers (β). **(C)** Analysis of mean connectivity for possible
1783 soft-thresholding powers. **(D)** GO analysis for 1,450 DE genes between MDD and control groups,
1784 separated by up/down regulation. **(E)** Fraction of uniquely mapped, non-duplicated, non-chrM
1785 paired-end reads compared to all reads in raw sequencing files. **(F)** Number of uniquely mapped,
1786 non-duplicated, non-chrM paired-end reads. **(G)** Fraction of duplicated to uniquely mapped
1787 paired-end reads. **(H)** Fraction of mitochondrial DNA reads to uniquely mapped, non-duplicated
1788 paired-end reads. **(I)** Number of OCRs (called per sample). **(J)** Fraction of reads in OCRs (FRIP).
1789 **(K)** GC-content in consensus set of OCRs; **(L)** Median insert size. For all whisker plots in this
1790 figure: The center line indicates the median, the box shows the interquartile range, whiskers
1791 indicate the highest/lowest values within 1.5x the interquartile range. **(M)** Genotype check based
1792 on pair-wise comparison of genotypes called from ATAC-seq samples. Pairs of neuronal and non-
1793 neuronal samples supposedly originating from the same person have distinctly higher scores
1794 (green line) than pairs of samples from different individuals (yellow line). **(N)** Summary and **(O)**
1795 per-OCR distribution of *P*-value ranking for the reported set of 203 differentially accessible OCRs
1796 within differentially analyses results generated on the datasets of non-neuronal samples with
1797 randomly permuted MDD and Control status ($n=100$ permuted datasets). This analysis proves
1798 that the reported set of 203 differentially accessible OCRs (median percentile of *P*-value is 1%)
1799 are not affected by technical artifacts since their median percentile of *P*-value in the datasets with
1800 permuted MDD and Control status is 46% (further details in Methods: Differential analysis of
1801 chromatin accessibility). **(P)** Performance of machine learning classifiers built on the reported set
1802 of 203 differential OCRs and 203 random OCRs. To enable the robust performance evaluation,
1803 the repeated 5-fold cross-validation was applied ($k_{repeat} = 10$); additionally, the whole process was
1804 repeated 10 times with different sets of 203 randomly selected OCRs. For all whisker plots in this
1805 figure: The center line indicates the median, the box shows the interquartile range, whiskers
1806 indicate the highest/lowest values within 1.5x the interquartile range. Student's two-tailed t-tests
1807 were performed for statistical comparisons, $^*=p<.05$, $^{**}=p<.01$. Data displayed as mean (+/-
1808 SEM).

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1813 **Supplemental Figure 2. Identification and characterization of ZBTB7A in human MDD and**
 1814 **mouse chronic stress OFC.**

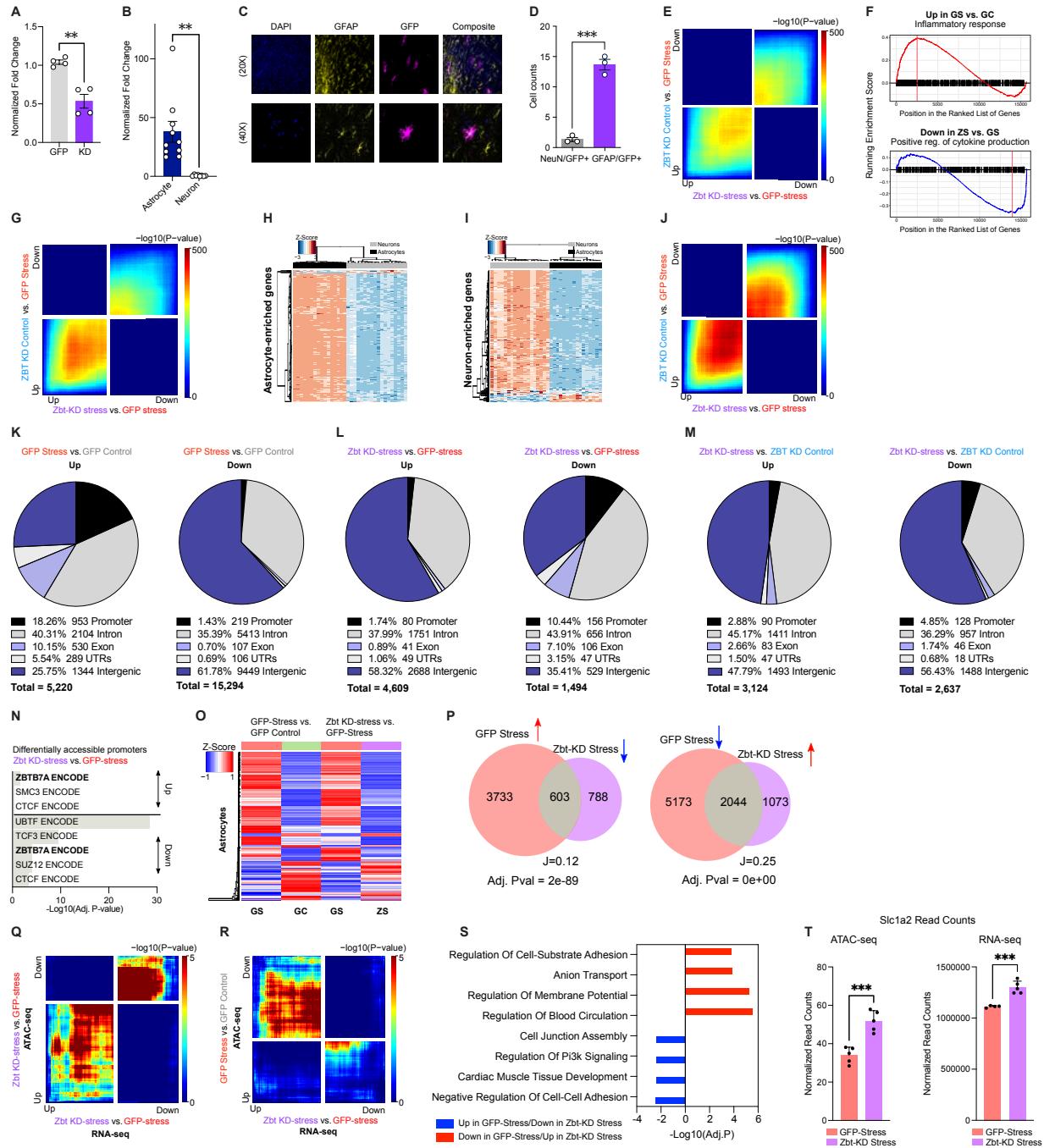
1815

1816 **(A)** Consensus score from the Human Protein Atlas¹ for expression in human brain for each factor.

1817 The mRNA expression data is derived from deep sequencing of RNA (RNA-seq) from 37 different
 1818 normal tissue types. **(B)** Normalized fold change for mRNA expression for ELF1 in bulk human
 1819 OFC tissues, control vs. MDD. **(C)** GO analysis with CellMarker Augmented Database² and CHEA
 1820 ENCODE Consensus database³ for genes in detected non-neuronal specific promoters, filtered
 1821 by logFC > 1, (+/-) 3000bp from TSS **(D)** Overlap between DE genes from MDD vs. control OFC
 1822 tissues⁴ and ENCODE consensus target gene sets via EnrichR, plotted by rank (y-axis) and -

1823 \log_{10} (adjusted *p*-value) on the x-axis and by fill color. Bubble size displays the number of
1824 overlapping genes for each term. **(E)** Overlap between ZBTB7A target genes (from TRANSFAC)
1825 and ARCHS4 human tissue expression reference gene sets via EnrichR. plotted by rank (y-axis)
1826 and $-\log_{10}$ (Adjusted P-value) on the x-axis and by fill color. Bubble size displays the number of
1827 overlapping genes for each term. **(F)** Social interaction ratio for control vs. chronically stressed
1828 CSDS mouse groups at 48 h post-stress and 21 d post-stress. **(G)** Normalized fold change protein
1829 expression of *Zbtb7a* in mouse OFC bulk tissues collected from control vs. chronically stressed
1830 mouse groups at 21 d post-stress. **(H)** qPCR expression data for astrocyte-specific gene *Aldh1a1*
1831 in MACs-isolated cell fractions **(I)** qPCR expression data for neuron-specific *Rbfox3* (*NeuN* in
1832 MACs-isolated cell fractions). **(J)** qPCR expression data for cell type-specific genes in negative
1833 fraction from MACs-isolated astrocyte and neuron cell fractions, showing the negative fraction is
1834 enriched for microglia marker *Cd11b*. **(K)** qPCR expression data for *Zbtb7a* in MACs-isolated
1835 astrocyte vs. neuron cell fractions. **(L)** 20x IHC images showing *Zbtb7a* protein is expressed in
1836 mouse OFC astrocytes, depicts overlap of *Zbtb7a* with astrocyte-specific marker *Gfap*. **(M)**
1837 Thresholded Mander's coefficient describes overlap of color channels of interest. **(N)** Expression
1838 of *ZBTB7A* mRNA in human primary cultured astrocytes treated with *ZBTB7A* OE lentivirus vs.
1839 RFP empty vector control virus. **(O-P)** Bar graph showing normalized fold change of mRNA
1840 expression in ZBT-OE vs. RFP human primary cultured astrocytes for the listed gene targets. **(Q)**
1841 Normalized fold change of cell-type specific marker genes in human primary astrocyte-enriched
1842 cultures. **(R)** Normalized fold change of *ZBTB7A* mRNA expression in cultured human astrocytes
1843 treated with saline vs. LPS. **(S)** Normalized fold change of *Zbtb7a* mRNA expression in cultured
1844 mouse astrocytes treated with saline vs. LPS. Student's two-tailed t-tests or 1-way ANOVA with
1845 MC tests were performed for statistical comparisons. Data presented as mean (+/- SEM). *=*p*<.05,
1846 **=*p*<.01, ***=*p*<.001, ****=*p*<.0001.

1847



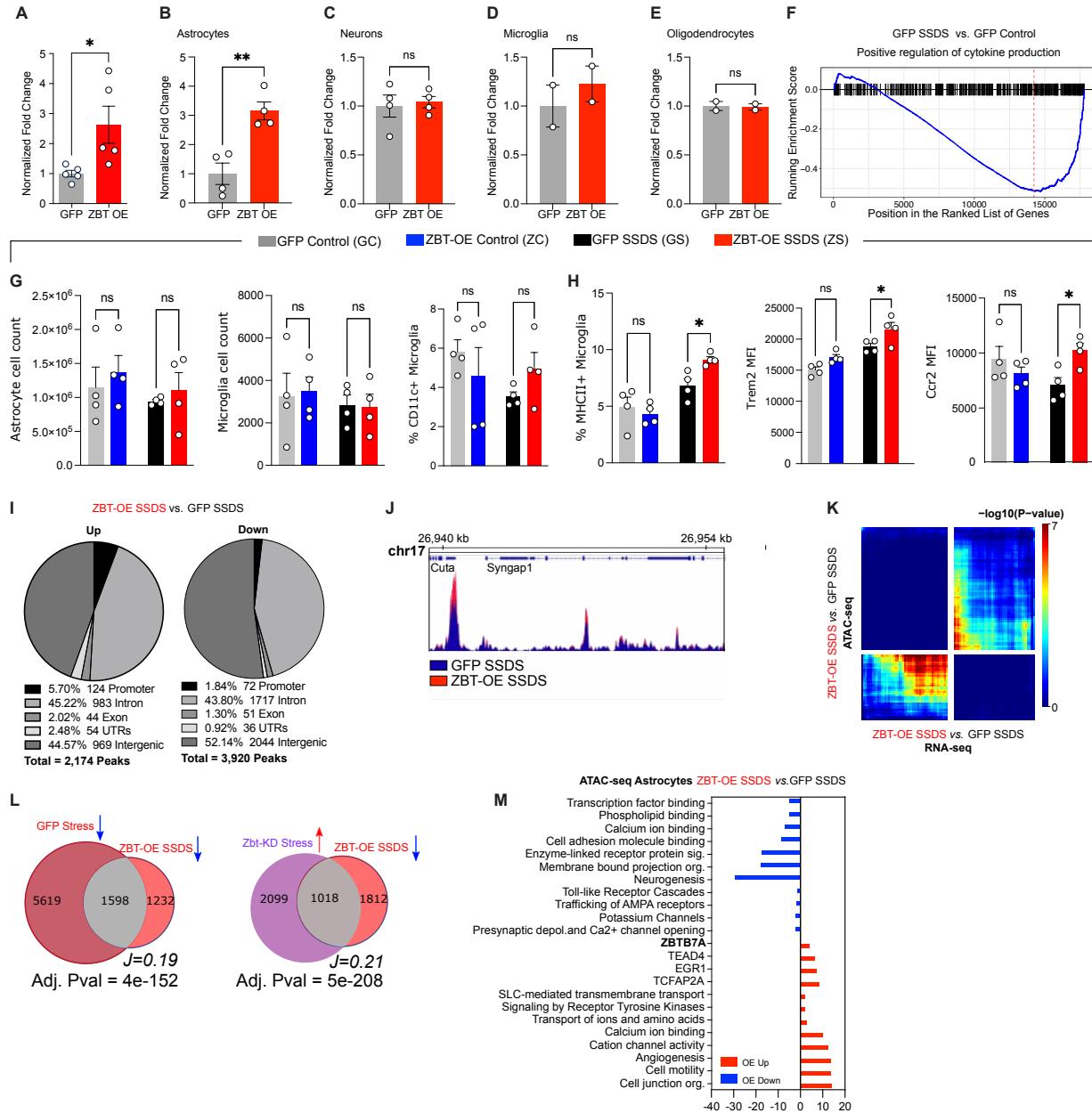
1848

1849 **Supplemental Figure 3. Zbtb7a KD alters cell-type specific chromatin accessibility and**
 1850 **gene expression.**

1851

1852 **(A)** Normalized fold change of qPCR *Zbtb7a* gene expression from OFC tissues transduced with
 1853 Zbt-KD virus vs. miR-neg-GFP (GFP), with n = 4/group. **(B)** qPCR expression levels of the GFP
 1854 transgene in MACs-isolated neurons vs. astrocytes from AAV6-GFAP-miR-neg-GFP virally-
 1855 transduced OFC mouse tissues. **(C)** Representative IHC images of OFC tissues transduced with
 1856 an rAAV6 virus expressing ZBTB7A-GFP (in magenta) overlaid with a nuclear co-stain (DAPI in

1857 blue) and GFAP (in yellow) to show astrocyte-specific expression. **(D)** Cell counts in OFC tissues
1858 transduced with AAV6-ZBTB7A-GFP of cells co-expressing Gfap/Zbtb7a or NeuN/Zbtb7a. **(E)**
1859 RRHO comparing gene expression for the indicated comparisons, in bulk OFC tissue. **(F)** GSEA
1860 enrichment plot for most significantly enriched gene set in GFP Stress vs. GFP Control and ZBT
1861 stress vs. GFP Stress in bulk OFC tissue. The enrichment plot shows a line representing the
1862 running ES for a given GO as the analysis goes down the ranked list. The value at the peak is the
1863 final ES. **(G)** RRHO comparing gene expression for the indicated comparisons, in MACS-isolated
1864 astrocytes. **(H-I)** Heatmaps depict unsupervised clustering of normalized read count values in
1865 MACs-isolated astrocytes and neurons for (H) 239 astrocyte-enriched genes and (I) 279 neuron
1866 enriched genes identified in previous report⁵. **(J)** RRHO comparing gene expression for the
1867 indicated comparisons, in MACS-isolated neurons. **(K-M)** ATAC-seq diffReps analysis of
1868 differential accessibility between indicated conditions. Pie charts indicate distribution of differential
1869 accessibility events, stratified by genomic context for the indicated conditions and separated for
1870 up/down events. **(N)** Gene ontology (GO) pathway analysis of differentially accessible promoters
1871 from Zbt-KD stress vs. GFP stress [less accessible promoters, top] and GFP stress vs. GFP
1872 control [more accessible promoters, bottom]. **(O)** Clustering of groups at 1,138 overlapping
1873 genomic regions between GFP Stress vs. GFP control and Zbt-KD stress vs. GFP stress,
1874 depicting Z-score of log2FC accessibility. **(P)** Scaled Venn diagram and odds ratio analyses of
1875 the number of shared and distinct OCR gene targets between indicated conditions. Numbers
1876 indicate differentially accessible peaks, “J” indicates the Jaccard index. **(Q-R)** RRHO comparing
1877 gene expression and chromatin accessibility for the indicated comparisons. **(S)** GO pathway
1878 analysis of rescued OCR gene targets between Zbt-KD Stress and GFP Stress MACS-isolated
1879 astrocytes ATAC-seq. **(T)** Normalized read counts for accessibility (left) and gene expression
1880 (right) at *Slc1a2* gene in MACS-isolated astrocytes. Data were analyzed with Student's two-tailed
1881 t-tests. * $=p<.05$, ** $=p<.01$, *** $=p<.001$, **** $=p<.0001$. All data graphed as means \pm SEM.



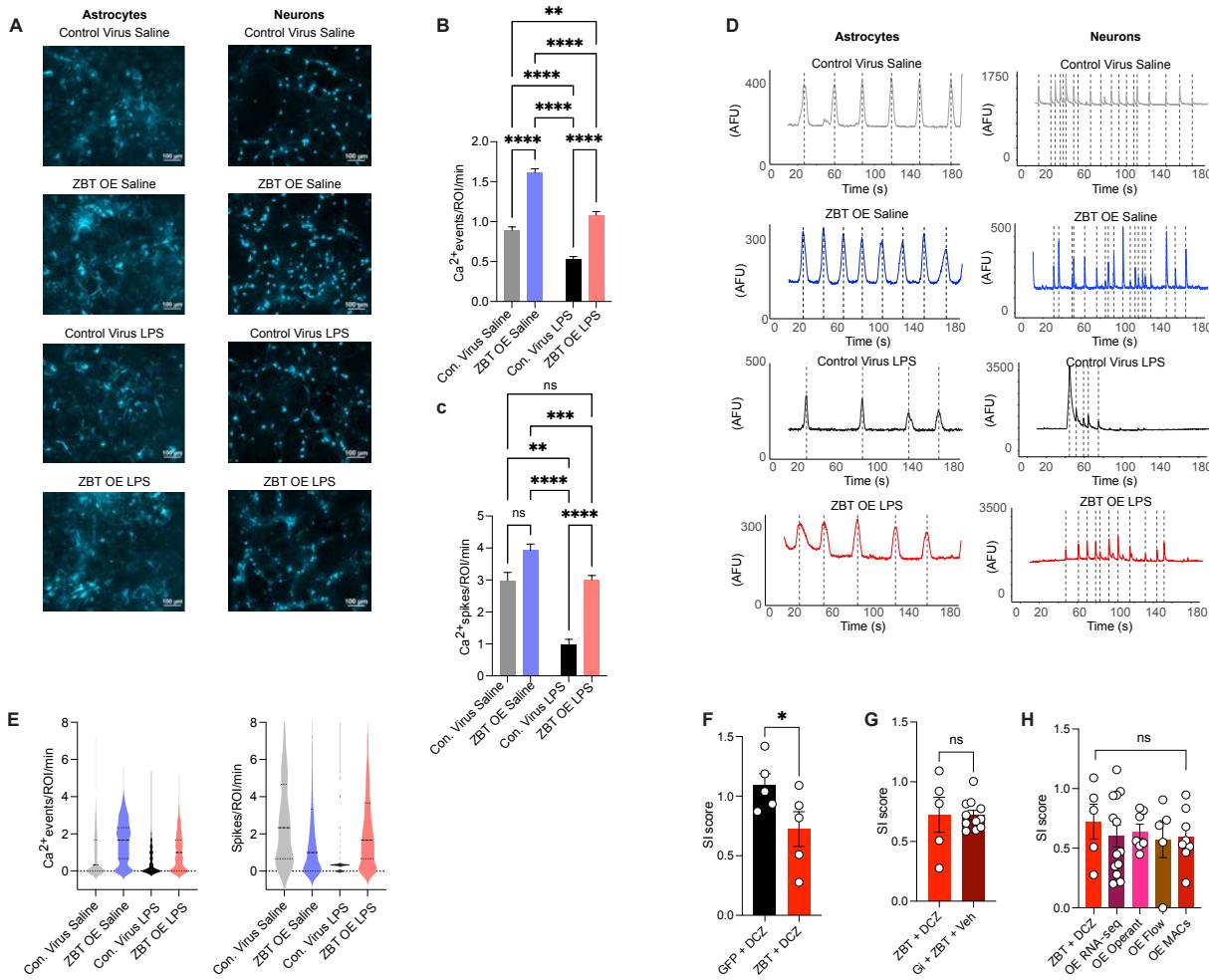
1882

1883 **Supplementary Figure 4. ZBTB7A OE in OFC astrocytes promotes significant alterations**
 1884 **in behavior, chromatin accessibility, and gene expression.**

1885

1886 (A) Normalized fold change of qPCR *Zbtb7a* gene expression from OFC tissues transduced with
 1887 ZBTB7A OE virus vs. GFP, n = 5/group. (B-E) qPCR expression levels of *Zbtb7a* in MACs-
 1888 isolated (B) astrocytes and (C) neurons (D) microglia and (E) oligodendrocytes from AAV6-GFAP-
 1889 ZBT OE transduced virally-transduced OFC mouse tissues, n = 2-4/group. (F) GSEA enrichment
 1890 plot for most significantly enriched gene set in GFP Stress vs. GFP Control in bulk OFC tissue.
 1891 (G) Number of astrocytes [left], and microglia⁶ per organ. (H) Percent CD11c+ microglia [far left],

1892 percent MHCII+ microglia [left], Trem2 MFI⁶ and Ccr2 [far right] MFI⁶ in virally transduced ZBT-
1893 OE vs. GFP mice (+/- SSDS) OFC via flow cytometry, n = 4/group. Gating strategy shown in
1894 **Supplementary Fig. 6. (I)** ATAC-seq diffReps analysis of differential accessibility comparing
1895 ZBT-OE SSDS vs. GFP SSDS. Pie charts indicate distribution of differential accessibility events,
1896 stratified by genomic context. **(J)** Representative pile-up traces of cell specific ATAC-seq signal
1897 overlapping Syngap1 gene. **(K)** RRHO comparing gene expression profile of MACs-isolated
1898 astrocytes with MACS-isolated astrocyte chromatin accessibility for indicated conditions. **(L)** Venn
1899 diagram and odds ratio analysis of the shared and distinct OCRs from ATAC-seq diffreps analysis
1900 between indicated conditions. **(M)** GO pathway analysis of gene targets associated with
1901 differentially expressed [red is more accessible, blue is less accessible] chromatin regions
1902 between ZBT-OE SSDS and GFP OE SSDS. Data were analyzed with Student's two-tailed t-tests
1903 or with 2-way ANOVA, or 3-way ANOVA, followed by 2-Way ANOVAs for MC comparisons,
1904 *= $p<.05$, **= $p<.01$. All data graphed as means \pm SEM.
1905

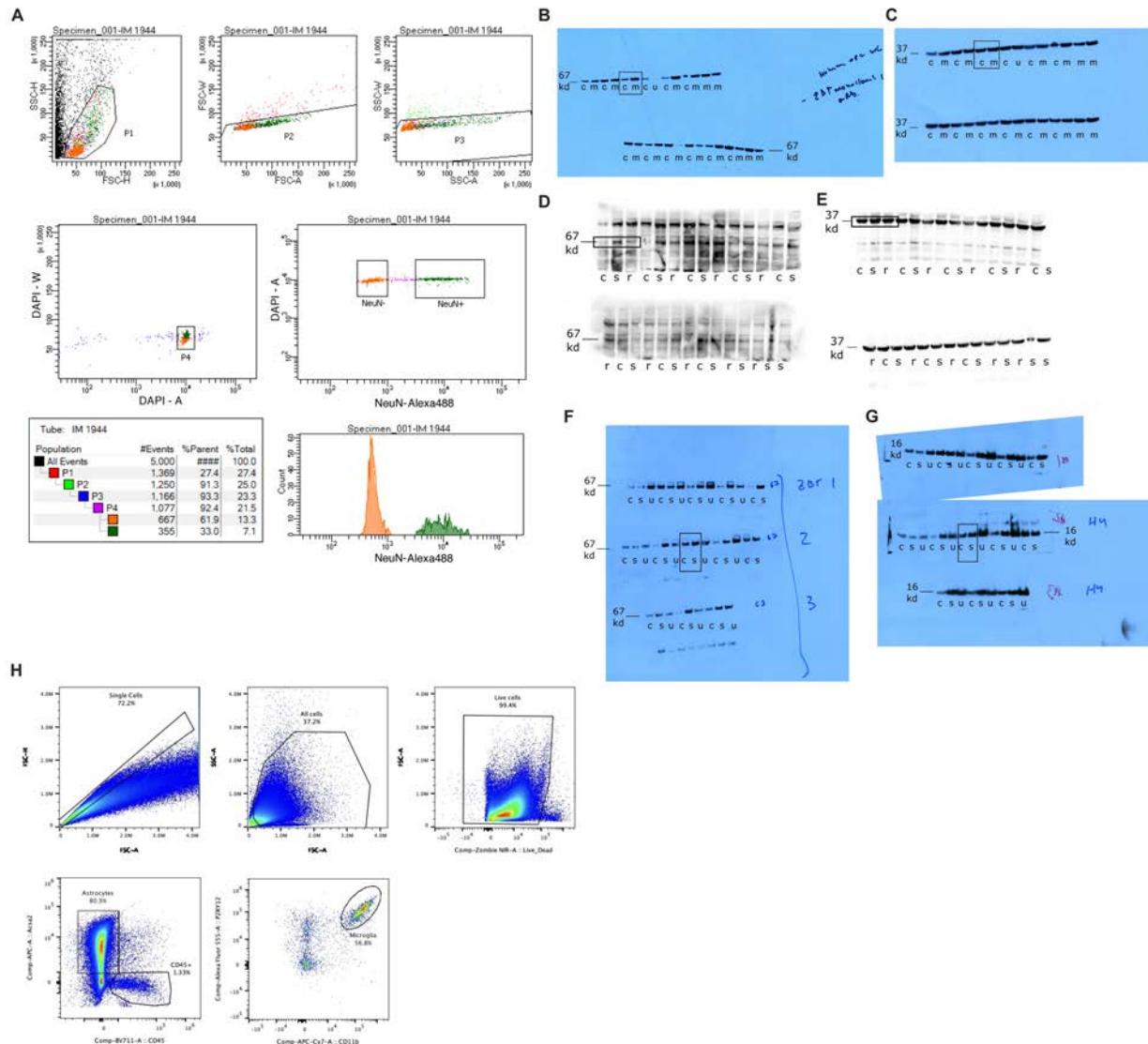


1906

1907 **Supplemental Figure 5. Calcium imaging and chemogenetic manipulations in the context**
 1908 **of astrocyte-specific ZBT7A OE.**

1910 **(A)** Representative images show gCAMP6f-expressing cells in either astrocyte-treated or neuron-
 1911 treated primary co-cultures. **(B)** Mean frequency of Ca^{2+} events detected in astrocytes
 1912 expressing gCAMP6f. “Con.” Indicates Control. Representative traces show **(C)** Mean frequency
 1913 of Ca^{2+} event detected in neurons expressing gCAMP6f. “Con.” Indicates Control. **(D)** Representative traces for calcium event frequencies in astrocytes [left] and neurons⁶. **(E)** Violin
 1915 plots depicting individual values for (right) astrocyte [$n=623$ cells control virus saline, $n= 559$ cells
 1916 ZBT-OE saline, $n=747$ cells control virus LPS, and $n=517$ cells ZBT-OE LPS] and (left) neuronal
 1917 [$n=135$ cells control virus saline, $n= 1277$ cells ZBT-OE saline, $n=238$ cells control virus LPS, and
 1918 $n=1324$ cells ZBT OE LPS] calcium events. **(F)** Social interaction score for ZBT OE SSDS vs.
 1919 GFP SSDS mice injected with DCZ. **(G)** Social interaction score for ZBT-OE SSDS vs. ZBT-OE
 1920 + Gi Dreadd + vehicle. **(H)** Comparison of SI score across multiple cohorts of ZBT-OE SSDS

1921 animals. Data were analyzed with Student's two-tailed t-tests or with 1-way ANOVA plus Tukey's
1922 MC test, * $=p<.05$, ** $=p<.01$, *** $=p<.001$, **** $=p<.0001$. All data graphed as means \pm SEM.
1923



1924

1925 **Supplemental Figure 6. Flow cytometry gating and raw blots**

1926 **(A)** For FANS-coupled ATAC-seq on human postmortem tissues, nuclear populations were
 1927 initially gated by side and forward scatter to differentiate nuclei from cellular debris. Populations
 1928 were then gated based on DAPI staining to identify singlets and to further disregard debris. DAPI
 1929 positive nuclei were subsequently gated based on NeuN staining to differentiate neurons (NeuN⁺)
 1930 from non-neurons (NeuN⁻). Final nuclei population abundance for non-Neurons (NeuN⁻): 70.5%
 1931 (in orange) and for neurons (NeuN⁺): 29.5% (in green). **(B)** Western blot film scan for ZBTB7A in
 1932 bulk human OFC tissue, MDD (labeled "m") vs. controls (labeled "c"). ZBTB7A band at expected
 1933 molecular weight of 67kDa. Note samples labeled "u" are not included in this manuscript due to
 1934 lack of signal (suspected improper nuclear lysis). **(C)** Western blot film scan for housekeeping

1935 gene GAPDH in human OFC, MDD vs. controls. Run on the same membrane as ZBTB7A in (B).
1936 (D) Raw image from chemidoc for western blot film for Zbtb7a in male mouse OFC, 48 hours after
1937 final defeat. CSDS susceptible (labeled "s") vs. CSDS resilient (labeled "r") vs. controls (labeled
1938 "c"). (E) Raw image from chemidoc western blot film for Gapdh loading control in male mouse
1939 OFC, CSDS susceptible vs. resilient vs. controls. Run on the same membrane as Zbtb7a in (D).
1940 (F) Western blot film scan for Zbtb7a in male mouse OFC, 21 days after final defeat. CSDS
1941 susceptible (labeled "s") vs. controls (labeled "c"). Note samples labeled "u" are from an unrelated
1942 study, and not included in this manuscript. (G) Western blot film scan for H3.3 loading control in
1943 male mouse OFC, CSDS susceptible vs. controls (note H3.3 was used for these blots due to use
1944 of nuclear lysates, Gapdh could not be used). Run on the same membrane as Zbtb7a in (F). (H)
1945 Gating strategy used to identify cell populations in the OFC of mouse OE experiments (Fig. S4).
1946
1947

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