

1 ***JUN* upregulation drives aberrant transposable element mobilization, associated innate immune
2 response, and impaired neurogenesis in Alzheimer's disease**

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16

17 **Abstract**

18

19 Adult neurogenic decline, inflammation, and neurodegeneration are phenotypic hallmarks of
20 Alzheimer's disease (AD). Mobilization of transposable elements (TEs) in heterochromatic
21 regions was recently reported in AD, but the underlying mechanisms are still
22 underappreciated. Combining functional genomics with differentiation of familial and
23 sporadic AD patient derived-iPSCs into hippocampal progenitors, CA3 neurons, and cerebral
24 organoids, we found that upregulation of the AP-1 subunit c-JUN triggers decondensation of
25 genomic regions containing TEs. This leads to cytoplasmic accumulation of TE-derived RNA-
26 DNA hybrids, activation of the cGAS-STING cascade, and increased cleaved caspase-3 levels,
27 suggesting initiation of programmed cell death in progenitor cells and neurons. Notably,
28 inhibiting c-JUN effectively blocks all the downstream molecular processes and rescues
29 neuronal death and impaired neurogenesis in the AD progenitors. Our findings open new
30 avenues for identifying therapeutic strategies and biomarkers to counteract disease
31 progression and diagnose AD in the early, pre-symptomatic stages.

32

33 **Keywords:** Alzheimer's disease, chromatin relaxation, JUN, retrotransposons, RNA-DNA
34 hybrids, cGAS-STING

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39 **Introduction**

40 Alzheimer's disease (AD), the most frequent cause of dementia¹⁻³, is an age-related
41 neurodegenerative disorder characterized by progressive memory loss and decline of
42 cognitive functions. AD's histopathological hallmarks include extracellular Amyloid beta (A β)
43 plaques and intracellular neurofibrillary TAU tangles (NFTs)⁴. One of the first brain regions to
44 show these pathological features is the hippocampus⁵. The subgranular zone of the dentate
45 gyrus within the hippocampus is a human neurogenic niche⁶⁻⁸ which harbors neural stem cells
46 and controls cell fate determination⁹. Interestingly, defects in adult hippocampal
47 neurogenesis are associated with various neurodegenerative disorders, including AD¹⁰⁻¹³.

48 Several studies report alterations in hippocampal neurogenesis in transgenic animal
49 models of AD¹² and an exacerbated decline of adult neurogenesis in AD patients^{14,15}. Notably,
50 these alterations occur in the early stages of the disease¹², suggesting they play a role in
51 triggering the onset of the disease's clinical phenotypes. Impaired neurogenesis likely
52 accelerates and facilitates neurodegenerative progression¹⁶. Moreover, these studies
53 highlight the link between the key molecules of AD (i.e. TAU and A β) and neurogenesis. TAU
54 is involved in the microtubule dynamics required for axonal outgrowth and plays an essential
55 role in hippocampal neurogenesis¹⁷. Recent studies indicate that TAU hyperphosphorylation
56 impairs hippocampal neurogenesis^{18,19}.

57 Increasing evidence suggests that intracellular accumulation of A β also negatively
58 impacts neural precursors cell (NPC) proliferation and neuronal differentiation during
59 hippocampal neurogenesis²⁰⁻²². Additionally, neural stem cell (NSC) fate determination, and
60 therefore neurogenesis, is regulated by MAP kinases²³⁻²⁶. Interestingly, several studies
61 suggest a compelling link between MAPK signaling and AD pathogenesis, revealing that the c-
62 JUN-amino-terminal kinase (JNK) pathway is involved in A β -induced neurodegeneration²⁷⁻³⁰
63 and in the hyperphosphorylation of TAU contributing to the formation of the NFTs³¹⁻³³.
64 Importantly, c-JUN is the downstream effector of the JNK pathway.

65 Phospho-c-JUN is a fundamental member of the AP-1 family of transcription factors,
66 functioning as either homodimers (c-JUN/c-JUN) or as heterodimers (c-JUN/c-FOS, c-
67 JUN/ATF2, c-JUN/MAF)³⁴. Among various other functions, AP-1 modulates cell death
68 signals^{31,32} and promotes the transcription of a series of pro-apoptotic factors, such as TNF- α ,
69 FAS-L, c-MYC and ATF3, which induce cell death by apoptosis³¹⁻³³. Recent lines of evidence
70 suggests that AP-1 can act as a pioneer factor by binding condensed nucleosomes and

71 recruiting the BAF complex to elicit chromatin accessibility³⁵⁻³⁷. Notably, upregulation of *JUN*
72 (which encodes for c-JUN) has been detected in many neurodegenerative diseases, including
73 AD^{32,38}. Nonetheless, the link between aberrant c-JUN activity and the associated
74 neurodegenerative outcomes have not been explored in depth.

75 Finally, there is mounting evidence indicating a role for transposable elements (TEs)
76 in the molecular pathogenesis of AD. More specifically, the disease is characterized by
77 aberrant de-repression and mobilization of TEs found in regions of repressed chromatin,
78 particularly retrotransposons belonging to the long interspersed nuclear elements (LINEs) and
79 long terminal repeat containing transposons (LTRs)³⁹⁻⁴⁶. Yet, the mechanisms leading to TE
80 de-repression and the functional consequences of this phenomenon in AD pathogenesis are
81 understudied, especially in humans.

82 In this study, we leveraged familial and sporadic AD patient-derived induced
83 pluripotent stem cells (iPSCs) and differentiated them into hippocampal progenitors, CA3
84 neurons and cerebral organoids. We demonstrated that c-JUN is the upstream regulator of
85 the transcriptional network altered in AD hippocampal progenitors, and that the aberrant
86 upregulation of *JUN* leads to the de-repression and mobilization of hundreds of TEs.
87 Moreover, we found that aberrant TE mobilization induces a cytoplasmic accumulation of
88 RNA-DNA hybrids, which elicits the activation of the cGAS-STING pathway and, ultimately,
89 activation of caspase-3, suggesting the initiation of programmed cell death. Inhibiting c-JUN
90 phosphorylation blocks this cell-death axis in AD progenitors by maintaining TE repression,
91 ultimately preventing the activation of the downstream pathogenic cascade.

92

93 **Results**

94

95 **iPSC-derived model of human neurogenesis in familial Alzheimer's disease**

96 To investigate the role of c-JUN in the onset of Alzheimer's disease (AD), we started
97 from familial AD and then replicated our findings in sporadic AD. Specifically, we derived
98 hippocampal precursor neurons from AD patients and control human iPSCs. First, we
99 confirmed the pluripotent state and the expression of *JUN* (which encodes the c-JUN protein)
100 in two control (CTRL1 and CTRL2) and two familial AD lines (FAD1 and FAD2). CTRL and FAD
101 lines included both sexes and were derived from individuals of comparable age. The FAD1 cell

102 line contains an *APP* gene duplication and the FAD2 line contains a heterozygous missense
103 mutation in *PSEN2* (*PSEN2*: p.Asn141Ile).

104 The immunofluorescence analysis for the pluripotency markers, NANOG and OCT4,
105 and the immunofluorescence for c-JUN showed that the fraction of cells expressing these
106 markers was not significantly different between CTRL and AD lines, indicating that the AD
107 lines are pluripotent and they have the same percentage of JUN expressing cells than the
108 control lines (**Extended Data Fig. 1a, b**). However, FAD iPSCs showed higher expression levels
109 of c-JUN (**Extended Data Fig. 1b**).

110 We then differentiated the four iPSC lines (CTRL1, CTRL2, FAD1, and FAD2) to
111 hippocampal neural precursor cells (hpNPCs) using a previously published protocol (**Fig. 1a**)⁴⁷.
112 To assess the composition of the obtained hpNPC population, we quantified the expression
113 of the established markers for different hippocampal neural precursor stages via qPCR (Fig.
114 1b). NESTIN defines early precursors, TBR2 and FOXG1 define intermediate progenitors,
115 PROX1 defines late progenitors, and DCX defines neuroblasts⁴⁸ (**Fig. 1b**). We observed that
116 the FAD hpNPCs have impaired neurogenesis, indicated by a robust reduction of early neural
117 stem cells (NSCs; NESTIN-positive cells) and a substantial increase in TBR2- and FOXG1-
118 positive intermediate progenitors (**Fig. 1b**). This result was also confirmed by
119 immunofluorescence (**Fig. 1c, d**). Moreover, the FAD hpNPCs failed to properly differentiate
120 into neuroblasts (**Fig. 1b, d**), demonstrated by the significantly reduced expression of the
121 neuroblast marker, DCX, as well as the reduced percentage of the DCX positive cells (**Fig. 1c**).
122

123 ***JUN* regulates the transcriptional network dysregulated in familial Alzheimer's disease 124 hippocampal neural progenitors**

125 To further investigate the differences between healthy and FAD hippocampal neural
126 progenitors, we characterized their transcriptomes using RNA-sequencing (RNA-seq). After
127 20 days in proliferation medium, we collected the cells to perform RNA-seq. This analysis
128 identified 1,976 differentially expressed genes, 751 of which (38.1%) were downregulated,
129 and 1,225 (61.9%) were upregulated in the FAD progenitors (FDR <5%; log₂(FC) \pm 1.5; **Fig. 2a**).
130 In agreement with RT-qPCR and immunofluorescence data (**Fig. 1**), the early progenitor
131 markers, *NESTIN* and *PAX6*, were downregulated, confirming the impaired
132 neurogenesis in FAD progenitors.

133 Several studies have demonstrated a critical role for WNT signaling in the
134 pathogenesis of AD^{49–55} and in the regulation of adult hippocampal neurogenesis^{56–63}.
135 Accordingly, the expression of several genes involved in both the canonical (*DKK3*, *WNT7A*,
136 *SFRP4*, *WNT2*) and non-canonical (*WNT5A*, *RORA*, *RAC2*) WNT signaling pathways were
137 upregulated in FAD hpNPCs (**Fig. 2a**). Moreover, *DKK1* was expressed more in the FAD lines
138 relative to the CTRLs (**Extended Data Table 1**). *DKK1* is an antagonist of the
139 canonical WNT signaling pathway⁶⁴, which leads to the activation of the WNT/JNK pathway,
140 which ultimately results in increased phosphorylation of c-JUN (encoded by *JUN*)⁶⁵.
141 Furthermore, the expression of the *JUN* gene itself was significantly upregulated in the FAD
142 lines ($\log_2(\text{FC}) = -0.6611642$; $p\text{-value} = 0.00039024$; **Fig. 2a**). Consistent with this, the
143 activation of the WNT/JNK pathway was suggested to play a role in A β oligomer
144 neurotoxicity^{51,66}.

145 We employed the WEB-based GEne SeT AnaLysis Toolkit (WebGestalt)⁶⁷ to identify
146 pathways associated with the 1,976 differentially expressed genes. This analysis revealed that
147 these genes are associated with inflammation, neurogenesis, and neural differentiation, as
148 well as cytoskeleton organization, apoptotic process, and the MAPK cascade (**Fig. 2b**).
149 Notably, Ingenuity Pathway Analysis (Qiagen) identified c-JUN as one of the top enriched
150 transcriptional regulators upstream to all of the differentially expressed genes, suggesting
151 that most of the differentially expressed genes are c-JUN targets (**Fig. 2c**). Moreover, when
152 analyzing the top enriched pathways that included the greatest number of differentially
153 expressed genes, *JUN* was one of only 8 genes shared across all these pathways (Fig. 2d). To
154 functionally test this computational prediction, we performed an immunoblot confirming
155 upregulation of c-JUN and phosphorylated c-JUN in FAD hpNPCs (**Fig. 2e**)

156 Overall, these results indicate that the FAD progenitors are characterized by aberrant
157 activation of the WNT/JNK pathway, an upregulation of *JUN* expression (which encodes for c-
158 JUN), and dysregulation of its target genes.

159

160 **The WNT/JNK pathway is dysregulated in FAD CA3 hippocampal neurons**

161 Recent studies have demonstrated that canonical WNT signaling is inhibited by several
162 pathogenic mechanisms in the brain of AD patients which leads to neural death and synaptic
163 plasticity regulation impairment^{53,54}. To investigate whether aberrant activation of the
164 WNT/JNK pathway was also occurring in AD neurons, we differentiated CTRL and FAD

165 progenitors into CA3 hippocampal neurons using an established protocol (**Extended Data Fig.**
166 **2a**)⁶⁸. Both CTRL and FAD differentiated neurons expressed the specific CA3 markers, such as
167 Glutamate Ionotropic Receptor Kainate Type Subunit 4 (GRIK4) and Secretagogin (SCGN;
168 **Extended Data Fig. 2b**). However, the differentiation of the FAD lines resulted in a reduced
169 number of mature CA3 neurons relative to the CTRL (51.4% in FAD; 87.2% in CTRL, **Extended**
170 **Data Fig. 2b**).

171 We performed RNA-seq on the CA3 neurons and identified 1,105 differentially
172 expressed genes between CTRL and FAD (FDR < 5%; **Extended Data Fig. 2c**). 82 of these genes,
173 including *MAPK10* (*JNK3*), *ROR2*, *WNT7A* and *WNT7B*, are involved in both the WNT/JNK
174 pathway and the MAPK cascade. Notably, using WebGestalt we identified the MAPK cascade
175 as one of the top 10 differentially expressed pathways using WebGestalt (**Extended Data Fig**
176 **2d**).

177 Finally, when analyzing the top enriched pathways that included the greatest number
178 of differentially expressed genes, we found that only *MAPT* is shared across these pathways
179 (**Extended Data Fig. 2e**). *MAPT* encodes for TAU, one of the key proteins in AD
180 pathogenesis^{69,70}, and recent studies have reported a correlation between aberrant c-JUN
181 activity and the formation and maturation of NFTs³².

182 In summary, we observed that the WNT/JNK pathway is dysregulated, not only in FAD
183 hippocampal progenitors but also in the CA3 hippocampal neurons CA3 neurons, suggesting
184 a critical role for c-JUN in both these cell types.

185

186 **Dysregulated chromatin accessibility in FAD hippocampal progenitors**

187 To investigate if the transcriptomic aberrations identified in the FAD lines are
188 associated with significant differences in chromatin accessibility, we performed an ATAC-seq
189 on the CTRL- and FAD-derived progenitors, generating 150 bp long Paired-End reads.

190 We identified 3,100 differentially accessible (DA) regions between CTRL and FAD
191 hpNPCs (FDR < 5%; $\log_2(\text{FC}) \pm 1.5$). Of these regions, 68.35% were accessible in FAD compared
192 to CTRL (**Fig. 3a**: FAD Up). By examining the closest gene to each of the 3,100 DA regions, we
193 found that approximately 14% (430) of these DA regions were located nearest to a
194 differentially expressed gene (i.e. their nearest gene was differentially expressed; **Fig. 3b**),
195 suggesting that there are at least 430 enhancer-gene pairs (or promoter-gene pairs)
196 dysregulated in the FAD progenitors. Of the 430 DA regions, 94.1% were putative enhancers

197 (distance from closest transcription start site or TSS > 1kb; **Fig. 3c**) whereas 5.9% were
198 putative promoters (TSS distance < 1kb).

199 We then performed DNA-based motif analysis (MEME-ChIP) to identify any potential
200 transcription factors underlying the changes in chromatin accessibility in the 3,100 DA
201 regions. Remarkably, the most significantly enriched binding motif was the sequence
202 recognized by JUN (e-value=10⁻⁵¹; **Fig. 3d**).

203

204 **Transposable elements are aberrantly active in FAD hippocampal progenitors**

205 Aberrant de-repression of transposable elements is a hallmark of AD^{42,46}. In
206 agreement, we observed that 1,336 DA regions overlapped with a TE. The top 50 TE copies
207 identified as significantly more accessible (i.e. active) in FAD relative to CTRL progenitors were
208 predominantly (82%) retrotransposons (RTEs). Of these re-activated RTEs, 53.6% were LTRs,
209 while the remaining were more or less equally distributed between LINEs (long interspersed
210 nuclear elements) and SINEs (short interspersed nuclear elements; **Fig. 3e**). This aberrant LTR
211 mobilization in the DA regions of the FAD lines was also investigated at the TE family level
212 (**Fig. 3f**). In detail, 93.4% of the differentially accessible LTRs were endogenous retroviruses
213 (ERVs; **Fig. 3h**). In line with these findings thus far, a motif analysis conducted on just the
214 differentially accessible LTRs also revealed enrichment for the binding motif of JUN (e-value
215 =10⁻⁶¹, **Fig. 3h**).

216 Together, these data indicate that the FAD progenitors are characterized by
217 dysregulated chromatin accessibility at thousands of genomic sites, including hundreds of
218 transposable elements. Moreover, our data suggests that most of these genomic regions are
219 c-JUN target sites, and that aberrant c-JUN activity may underlie this observed chromatin
220 dysregulation.

221 Several recent studies have demonstrated that AP-1 can act as a pioneer transcription
222 factor by recruiting the BAF chromatin remodeling complex at condensed genomic regions to
223 elicit chromatin accessibility^{35,71–73}. Thus, we suggest that aberrantly active AP-1 in FAD
224 progenitors may recruit BAF to its target sites (i.e. at the regions harboring the AP-1 motif),
225 activating the TEs within these genomic regions.

226

227

228 **Aberrant TE mobilization elicits cGAS-STING activation**

229 Several studies have demonstrated that aberrant TE de-repression and mobilization is
230 observed across a variety of neurodegenerative disorders, including AD^{39-41,43-46}. A recent
231 study in blind mole rats (an ageing model for longevity studies) has shown that TE de-
232 repression leads to the cytosolic accumulation of TE-derived RNA-DNA hybrids, which
233 activates the cGAS-STING innate immune pathway leading to cell death⁷⁴. The blind mole rats
234 leverage this mechanism to suppress pre-cancerous cells⁷⁴. We thus set out to investigate if
235 the aberrant TE de-repression observed in the AD brains may also lead to cytosolic RNA-DNA
236 hybrid accumulation, innate immune response and apoptosis, all of which are hallmarks of the
237 disease.

238 Intriguingly, immunostaining conducted on FAD and CTRL progenitors with the S9.6
239 antibody, specific for the detection of RNA-DNA hybrids⁷⁴, displayed significant hybrid
240 accumulation in the cytoplasm of both FAD lines relative to the CTRLs (**Fig. 4a, b**). The RNA-
241 DNA hybrid accumulation could be facilitated by the reduced expression levels of Ribonuclease
242 H (RNase H) observed in the FAD progenitors. Namely, RNase H is specialized to selectively
243 degrade RNA-DNA hybrids present in cells (**Fig.4c**)⁷⁵. Additionally, we detected a robust
244 increase in STING immunofluorescence signal quantified in the FAD progenitors (**Fig. 4a, b**).
245 The upregulation of STING and cGAS in the FAD progenitors was further validated by western
246 blot (**Fig. 4d**). Because activation of the cGAS-STING pathway in cells leads to their death via
247 apoptosis⁷⁶, we assessed whether FAD progenitors were executing this cell death program.
248 Using western blot analysis, we observed an increase in cleaved caspase 3 (CC3) in the FAD
249 lines compared to controls (**Fig. 4d**).

250 These experiments provide a solid mechanistic link between aberrant TE mobilization,
251 cytoplasmic accumulation of RNA-DNA hybrids, and cGAS-STING activation potentially
252 resulting in cell death characterized by caspase-3 activation in AD progenitors.

253

254 **c-JUN inhibition rescues the neurogenic defects observed in the FAD hippocampal 255 progenitors**

256 Our genomic data revealed a distinct role for c-JUN. In fact, many of the differentially
257 expressed genes are known c-JUN-regulated genes, and many of the DA regions harbor the
258 JUN binding motif. Our data also shows that *JUN* is upregulated in the FAD progenitors and
259 the activation of the WNT/JNK pathway may trigger phosphorylation of the upregulated c-
260 JUN, leading to increased aberrant c-JUN activation. We hypothesize that these processes

261 may lead to aberrant AP-1 activity, resulting in the opening of thousands of genomic regions
262 harboring the JUN binding motif, allowing for the de-repression of hundreds of TEs that are
263 typically repressed in neural precursors.

264 To functionally validate our genomic data and test this hypothesis, we treated CTRL
265 and FAD hpNPCs with a synthetic peptide competitor for the binding of JNKs, called c-JUN
266 peptide (see methods). Treatment with this peptide for five days disrupts the interaction
267 between JNK and c-JUN, ultimately inhibiting c-JUN phosphorylation and therefore pathway
268 activation (**Fig. 5a**). Notably, treatment of the progenitors with the c-JUN inhibitor rescues the
269 neurogenic defects previously observed (**Fig. 1**) in the FAD progenitors. Namely, in the c-JUN
270 inhibitor-treated FAD progenitors (hereafter FAD+c-JUN peptide), the expression of TBR2 and
271 FOXG1 becomes comparable to the CTRLs (**Fig. 5b**), suggesting a rescue of the intermediate
272 progenitor pool.

273 Next, we performed RNA-seq on FAD+cJUN peptide and untreated FAD progenitors,
274 using both FAD lines. This experiment led to the identification of 1,034 differentially
275 expressed genes (FDR <5%, log₂(FC) = \pm 1.5; **Fig. 5c**). Notably, nearly a third of these genes
276 (292/1034) were previously identified as differentially expressed when comparing CTRL and
277 FAD progenitors (**Fig. 5d**), suggesting that inhibiting c-JUN phosphorylation rescues a
278 significant fraction of the transcriptomic aberrations observed in the FAD hippocampal neural
279 precursors.

280 WebGestalt analysis on these 292 genes revealed enrichment for neuronal
281 differentiation processes and inflammation (**Fig. 5e**). For 83 of the 292 genes, including the
282 early progenitor marker *PAX6*, the expression is entirely rescued by c-JUN inhibition
283 (**Extended data table 2**). The 83 genes with complete rescue are enriched for pathways
284 associated with neuron differentiation (p-value = 0.00021), neuron development (p-value =
285 0.000324), and neuron generation (p-value = 0.000625).

286 These results demonstrate that c-JUN plays a crucial role in regulating neurogenesis
287 and neuron differentiation in FAD hippocampal progenitors, and that aberrant c-JUN activity
288 underlies a significant fraction of the transcriptomic abnormalities observed in FAD
289 progenitors.

290

291 **c-JUN inhibition rescues TE de-repression, RNA-DNA hybrid formation, cGAS-STING**
292 **activation, and caspase-3 activation**

293 c-JUN inhibition also impacts the observed aberrant opening of chromatin at TE loci.
294 By performing an RT-qPCR on a group of RTEs, selected among those previously identified as
295 aberrantly active in FAD, we demonstrated that c-JUN inhibition results in a significant
296 reduction of TE transcription at both LTR and LINE loci (**Fig. 6a**).

297 Next, we tested if the reduction of aberrant TE mobilization, resulting from c-JUN
298 inhibition (**Extended Data Fig. 3a**) affected RNA-DNA dimer accumulation and consequent
299 cGAS-STING activation. Notably, FAD+cJUN peptide progenitors show a significant reduction
300 of both RNA-DNA hybrid accumulation and cGAS-STING cascade activation relative to
301 untreated cells (**Fig. 6b, c; Extended Data Fig. 3b**). Finally, an immunoblot for cleaved caspase
302 3 indicated that inhibition of the JNK/c-JUN interaction also prevented activation of CC3 in
303 FAD progenitors (**Fig. 6c**).

304 With these results, we demonstrate that by inhibiting c-JUN phosphorylation, the
305 chromatin remains in a repressed state. Therefore, aberrant TE mobilization does not occur,
306 preventing the activation of the cGAS-STING-induced caspase-3 activation.

307

308 **Aberrant c-JUN activity underlies cGAS-STING activation and caspase-3 activation in**
309 **sporadic AD hippocampal progenitors**

310 To test whether the mechanism that we characterized in FAD progenitors is shared
311 between the two AD types (familial and sporadic) we differentiated two sporadic Alzheimer's
312 iPSC lines (SAD1 and SAD2) into hpNPCs, following the same protocol previously described
313 (**Extended Data Fig. 4a**). Notably, an RT-qPCR for the same markers characterized for FAD
314 (NESTIN, TBR2, FOXG1, PROX1, and DCX) also revealed impaired neurogenesis in SAD, with an
315 accelerated differentiation signature consistent with previous studies in sporadic AD
316 (**Extended Data Fig 4b**)⁷⁷. Interestingly, RNA-seq revealed that *JUN* is also upregulated in the
317 SAD progenitors (**Extended Data Fig 4c**) and that the 183 differentially expressed genes in
318 SAD hpNPCs are involved in neurogenesis and neuronal differentiation pathways (**Extended**
319 **Data Fig 5d**). Moreover, as in FAD progenitors, SAD hpNPCs show abnormal chromatin
320 accessibility at TE loci (**Extended Data Fig 4e**).

321 Treatment of SAD progenitors with the inhibitor of c-JUN phosphorylation led to a
322 decrease in cytoplasmic RNA-DNA dimers (**Fig. 7a**), as well as a significant a reduction of
323 cGAS/STING and cleaved caspase 3 activation (**Fig. 7b**). Importantly, these experiments

324 revealed that both AD types (familial and sporadic) share the same aberrantly activated cell-
325 death axis.

326

327 **RTE-derived RNA-DNA hybrids inducing the cGAS-STING cell-death axis are present in**
328 **familial and sporadic AD cerebral organoids**

329 Finally, we tested whether this pathway was also active in cerebral organoids, which harbor
330 both progenitor cells and differentiated neurons in a three-dimentional architecture,
331 resembling the human brain. The FAD, SAD, and CTRL iPSC lines were differentiated into
332 cerebral organoids through an embryoid body intermediate. After 62 days,
333 immunofluorescence was performed on cerebral organoids exhibiting the proper neuronal
334 differentiation (**Extended Data Fig. 5a**). We observed a more significant overall accumulation
335 of RNA-DNA hybrids and an upregulation of STING in FAD and SAD organoids compared to
336 CTRL organoids (**Fig. 7c, Extended Data Fig. 5b**). The activation of the cGAS-STING cell-death
337 axis and the increase in CC3 were also confirmed through western blot (**Fig. 7d**). As expected,
338 the cytoplasmic accumulation of RNA-DNA hybrids was seen in TBR2-positive neural
339 progenitors (**Extended Data Fig 5c**) that are enriched in the FAD organoids (**Extended Data**
340 **Fig 5c**). Interestingly, mature neurons (MAP2-positive) in AD cerebral orgnaoids also have a
341 cytoplasmatic accumulation of RNA-DNA hybrids and an upregulation of STING (**Extended**
342 **Data Fig 5d**). Finally, activating the cGAS-STING cell-death axis in neurons leads to caspase-3
343 activation (**Extended Data Fig 5e**). These organoid-based data further validated our proposed
344 pathogenic mechanism and cascade for both familial and sporadic AD. This suggests that the
345 molecular impairment driven by TE re-activation observed in progenitors, is also maintained
346 in mature neurons in a physiological model of neural differentiation.

347 Overall, this collection of experiments unveils a novel mechanism linking AP-1 to TE
348 mobilization, innate immunity and cell death in AD.

349

350 **Discussion**

351 Alzheimer's disease (AD) is the most common neurodegenerative disorder. Fibrillar
352 deposits of highly phosphorylated TAU protein are a key pathological feature of AD as well as
353 other AD related dementias⁷⁸. Importantly, studies in *Drosophila* have highlighted that TAU
354 hyperphosphorylation and upregulation correlate with global nuclear chromatin relaxation

355 and abnormal transcriptional activation of heterochromatic genomic regions^{40,43}. One of the
356 consequences of this phenomenon is the aberrant mobilization of TEs, which are typically
357 repressed in the genome. Progressive TE de-repression and mobilization in the brain typically
358 correlates with ageing^{39,44}. However this phenomenon is significantly exacerbated in many
359 neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), Multiple
360 Sclerosis, and Alzheimer's^{39,41-46}.

361 Recent studies showed that overexpression of TAU alone, in aging *Drosophila* brains
362 is sufficient to increase the expression of retrotransposons, mostly belonging to the LINE and
363 ERV groups^{40,43}. However, the mechanism linking tauopathies to chromatin relaxation and TE
364 mobilization remains unexplored.

365 Here, we unveiled a cascade of biological processes which links the upregulation of
366 the AP-1 member, c-JUN, to aberrant TE mobilization. We detected an upregulation of c-JUN
367 in hippocampal progenitors derived from both familial and sporadic AD iPSC lines. Further,
368 kinases involved in the regulation and phosphorylation of c-JUN (MAPK/JNK signaling) were
369 also dysregulated in fully differentiated FAD iPSC-derived CA3 hippocampal neurons. We
370 demonstrated that c-JUN upregulation has two main consequences: 1) the dysregulation of
371 hundreds of genes involved in neuronal differentiation and neuron generation 2) the
372 activation of hundreds of RTEs, that harbor the AP-1 binding motif. AP-1 activating repressed
373 chromatin was not an unexpected result; several recent studies have demonstrated that AP-
374 1 can act as a pioneer transcription factor by recruiting the BAF chromatin remodeling
375 complex to its targets to elicit accessibility and activation^{35,36,79}.

376 The second goal of our study was to investigate the consequences of aberrant TE de-
377 repression and mobilization. With our experiments, we demonstrated that abnormal
378 expression of RTEs leads to the accumulation of RTE-derived RNA-DNA hybrids in the
379 cytoplasm of the AD hpNPCs, as well as in AD cerebral organoids. We showed that this triggers
380 the activation of the innate immune response, particularly of the cGAS-STING pathway, which
381 ultimately elicits the accumulation of cleaved caspase-3, a molecular signature of cells
382 undergoing apoptosis⁸⁰. Importantly, we observed this phenomenon in both familial and
383 sporadic AD lines. We were able to explicitly demonstrate that c-JUN facilitates this
384 mechanism and that treating the AD hpNPCs with a c-JUN inhibitor sufficiently decreases this
385 cascade leading to a reduction of cell death.

386 Neuroinflammation plays an important role in the pathogenesis of Alzheimer's
387 disease, and in this context, the cGAS-STING signalling pathway has recently emerged as a
388 key mediator of inflammation in the settings of infection, cellular stress and tissue damage.⁸¹
389 In fact, neuroinflammation is primarily driven by type-I interferons (INFs), and the role of
390 STING in the control of the type-I IFN-mediated response is becoming increasingly
391 appreciated.⁸² Given this premise, the finding of our studies may open new potential
392 therapeutic avenues. For instance, nanobody-based targeting⁸³ of cytoplasmatic RNA-DNA
393 hybrids might provide a new therapeutic approach to counteract the activation of innate
394 immune response and to reduce neuroinflammation, bypassing the side effects of the anti-
395 inflammatory drugs currently involved in the clinical trials. Additionally, experiments on pre-
396 clinical models may be employed to test compounds which could act downstream in the cell
397 death axis demonstrated here. Finally, the cytoplasmic accumulation of RNA-DNA hybrids
398 could be used as an early biomarker for AD in imaging tools for diagnosis.

399 In summary, these lines of evidence point toward a pathological mechanism
400 underlying AD. Future studies on possible therapeutic intervention that would target this
401 mechanism are essential with the goal of identifying therapeutic strategies and early
402 diagnosis for AD and other neurodegenerative disorders.

403

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412 Foundation (D.T., C.S.).

413

414 **Data availability**

415 The original genome-wide data generated in this study have been deposited in the GEO
416 database under accession code GSE213610.

417

418 **Author contributions**

419 M.T., D.T and C.S. designed the experiments. C.S. performed most of the experiments. S.M.B,
420 performed some of the experiments. M.E.C and M.S. contributed to some experiments and
421 analyses. C.S., M.T., S.M.B. and D.T. analyzed the data. C.S. wrote the manuscript with the
422 contribution of all the authors.

423 **Competing interests**

424 The authors declare no competing interests.

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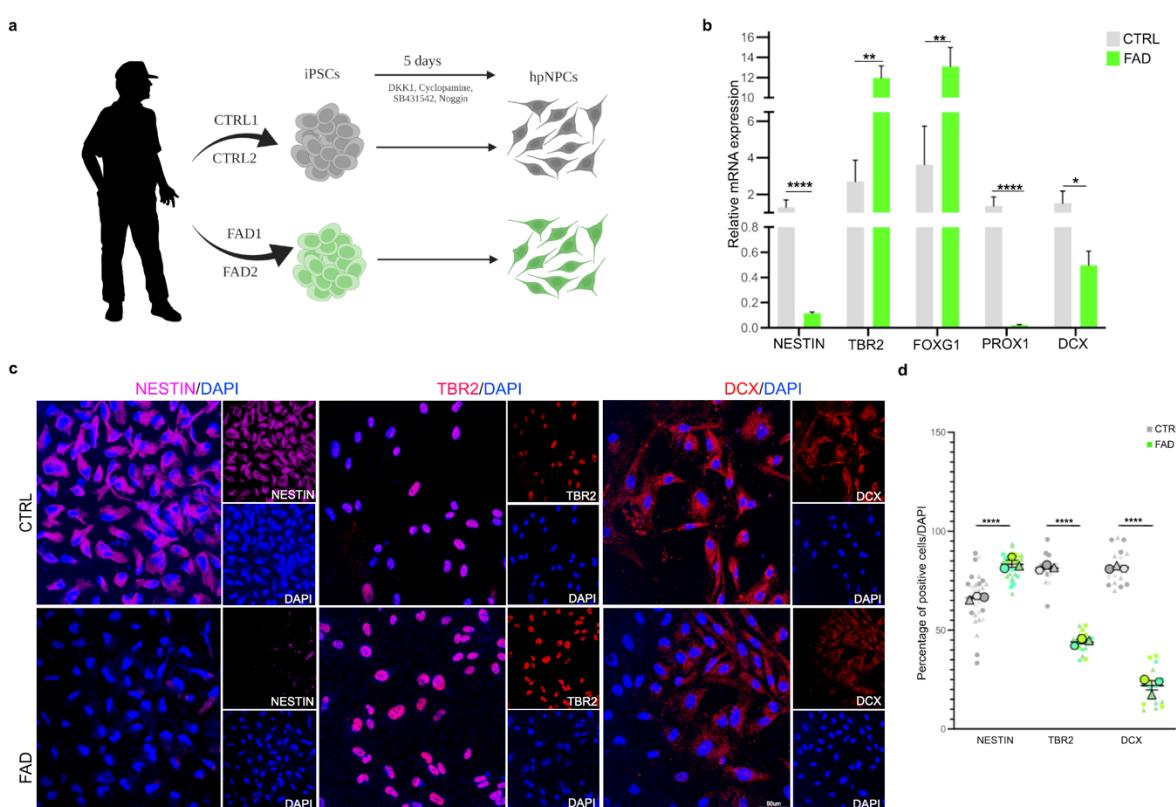
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461 **Figures**

462

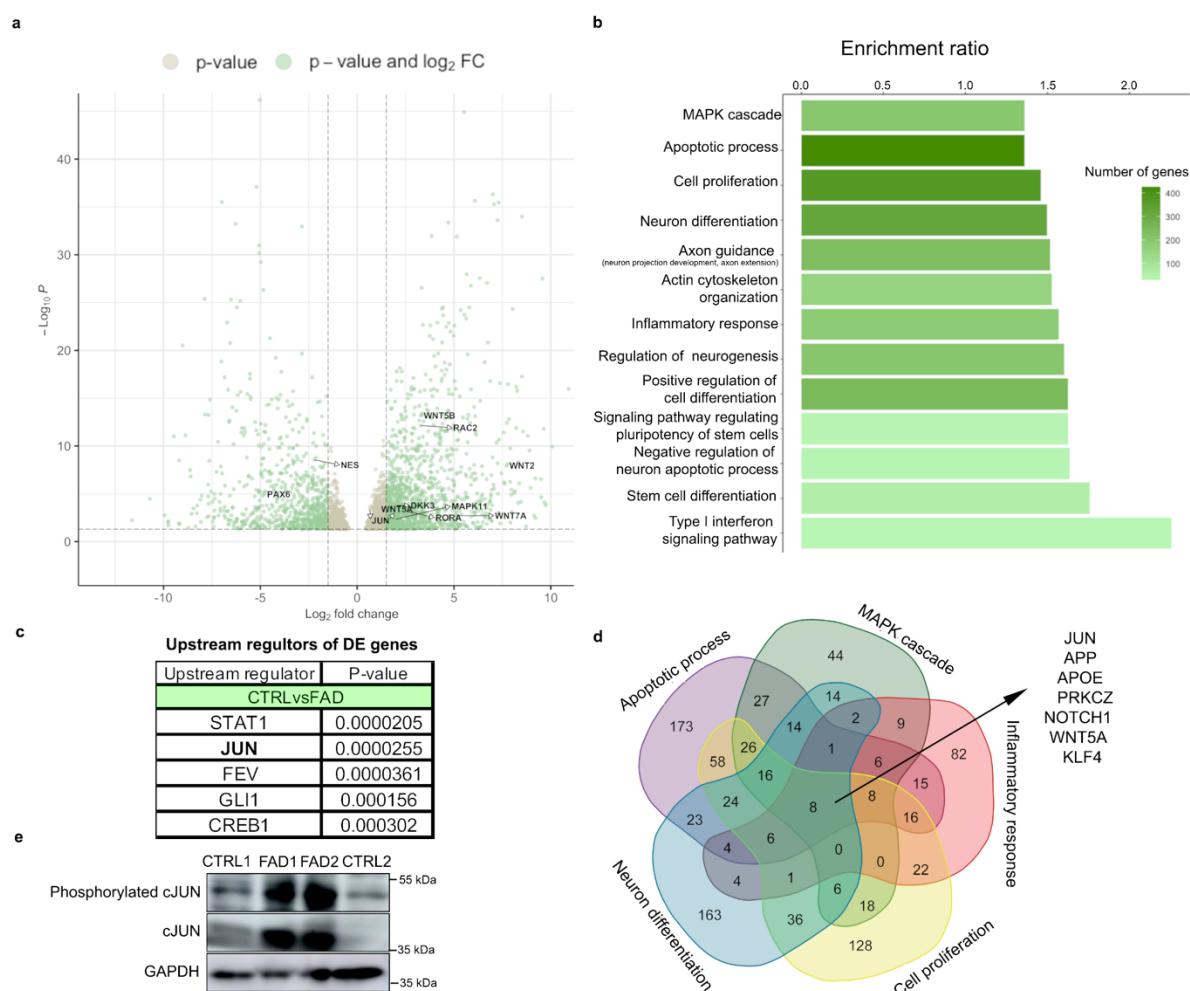


463

464 **Figure 1. FAD iPSC-derived hippocampal neural progenitors display impaired neurogenesis.**

465 **a.** Scheme of the protocol for hippocampal neural progenitor cell (hpNPC) differentiation
466 (made with BioRender.com). iPSCs were derived from the skin fibroblasts of two patients with
467 familial Alzheimer's disease (FAD1 and FAD2) and two healthy controls (CTRL1 and CTRL2),
468 and differentiated into hpNPCs after 5 days in induction media. The hpNPCs were maintained
469 in proliferation media post-induction. **b, c, & d.** qPCR and immunofluorescence for markers
470 of different stages of hpNPC populations. The qPCR shows a neurogenic defect in FAD
471 progenitors, with enrichment for TBR2-positive intermediate progenitors as also confirmed
472 in the immunofluorescence and relative quantification for hpNPC population markers. NESTIN
473 – early precursors; TBR2/FOGX1 – intermediate progenitors; PROX1 – late progenitors; DCX –
474 neuroblasts. Scale bar 50um, 40X magnification. DAPI staining on nuclei in blue.

475

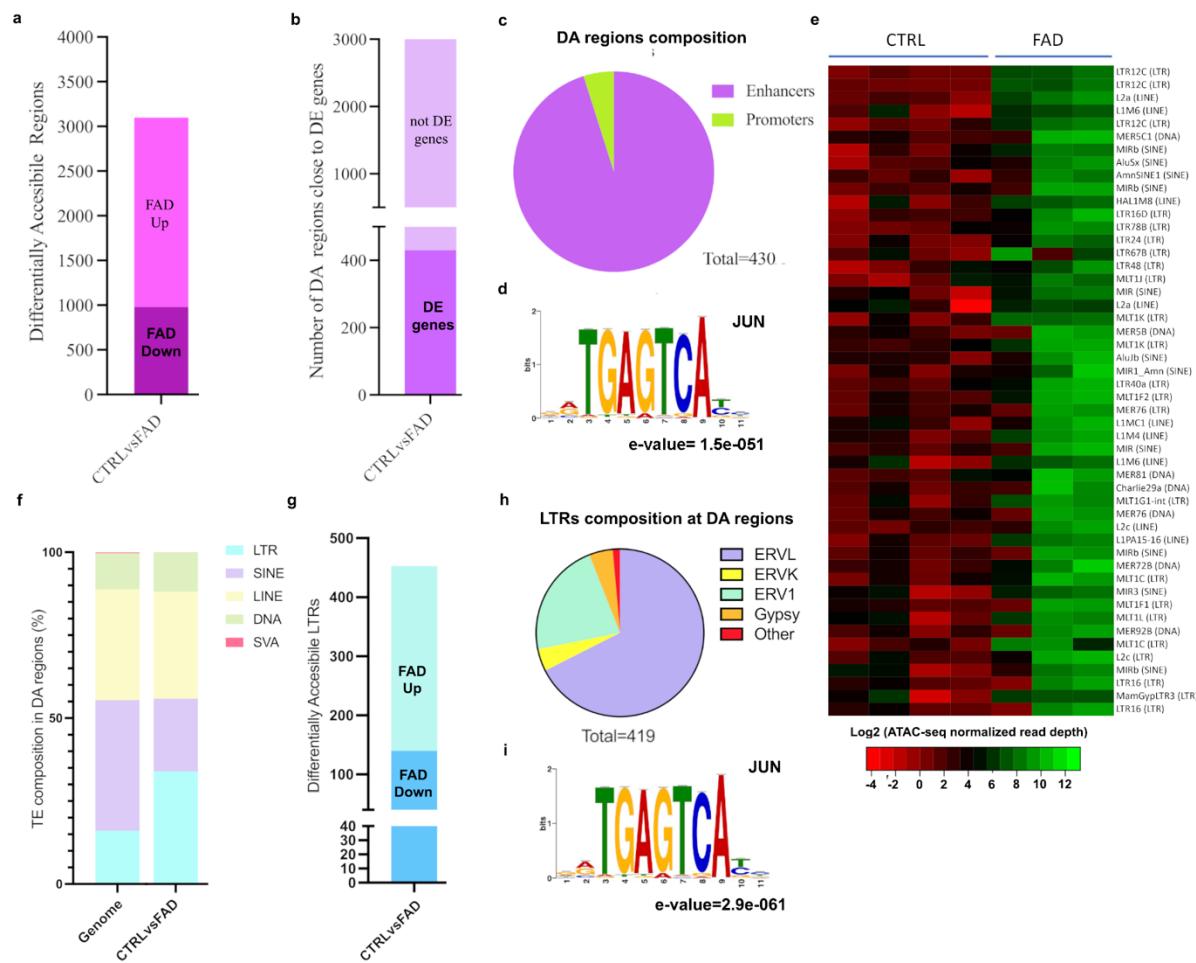


476

477 **Figure 2. *JUN* upregulation causes dysregulated transcriptional networks in FAD hpNPCs.**

478 **a.** Volcano plot showing genes differentially expressed in FAD hpNPCs relative to CTRL
479 hpNPCs. Labeled differentially expressed genes are involved in neurogenesis (*PAX6*, *NES*) and
480 *WNT/JNK* signaling (*WNT2*, *WNT7A*, *WNT5B*, *RAC2*, *RORA*, *MAPK11*, *DKK3*, *WNT5A*). Green =
481 differentially expressed genes passing significance thresholds $p\text{-value} < 0.05$ and $\log_2(\text{fold-}$
482 $\text{change}) > \pm 1.5$; Gray = differentially expressed genes passing significance threshold of $p\text{-}$
483 $\text{value} < 0.05$. **b.** Enriched pathways associated with the 1,976 differentially expressed genes
484 in FAD hpNPCs predicted by WebGestalt. **c.** Top upstream regulators/transcription factors of
485 the 1,976 differentially expressed genes in FAD hpNPCs, as predicted by Ingenuity Pathway
486 Analysis (Qiagen). **d.** Venn diagram showing the genes shared across all of the top five
487 enriched pathways with the most differentially expressed genes (venn diagram made with
488 <https://bioinformatics.psb.ugent.be>). **e.** Immunoblot displaying the upregulation of both c-
489 *JUN* and phosphorylated c-JUN across FAD and CTRL hpNPCs.

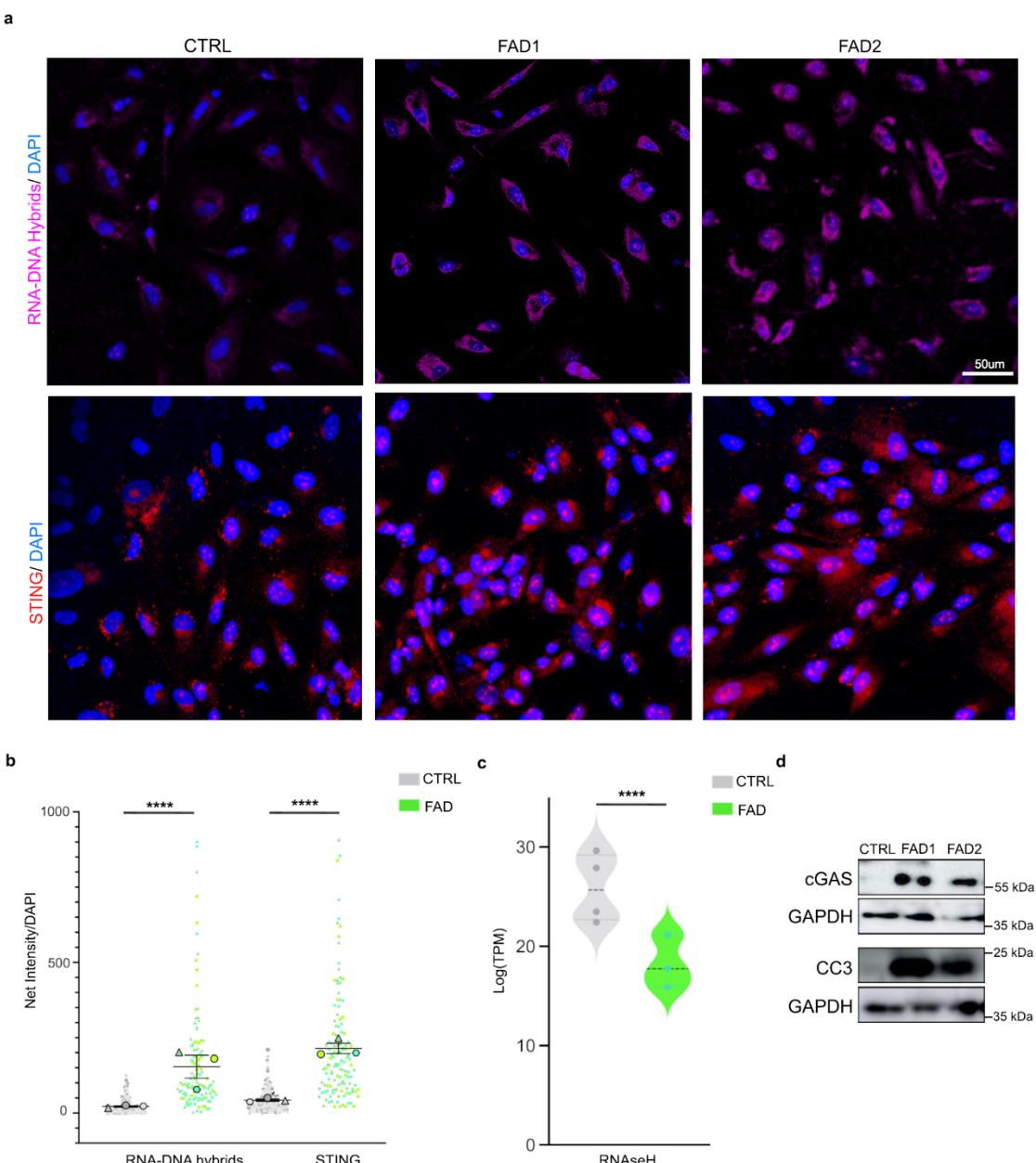
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492 **Figure 3. Differentially accessible transposable elements in FAD hpNPCs.** **a.** Differentially
493 accessible (DA) regions in the FAD hpNPCs compared to CTRL hpNPCs. FAD Up = significantly
494 more accessible in FAD relative to CTRLs; FAD Down = significantly less accessible in FAD
495 relative to CTRLs. **b.** DA regions located near a differentially expressed gene. **c.** DA regions
496 near differentially expressed genes are predominantly enhancers (>1 kb from the
497 transcription start site or TSS). **d.** MEME-ChIP analysis of 3,100 DA regions uncovered an
498 enriched binding motif for JUN. **e.** Heatmap showing the top 50 TE copies identified as
499 significantly more accessible in FAD relative to CTRL hpNPCs. **f, g, h.** Family distribution of the
500 aberrantly active TEs in FAD progenitors. **i.** The aberrantly active LTRs are enriched for the
501 JUN motif.

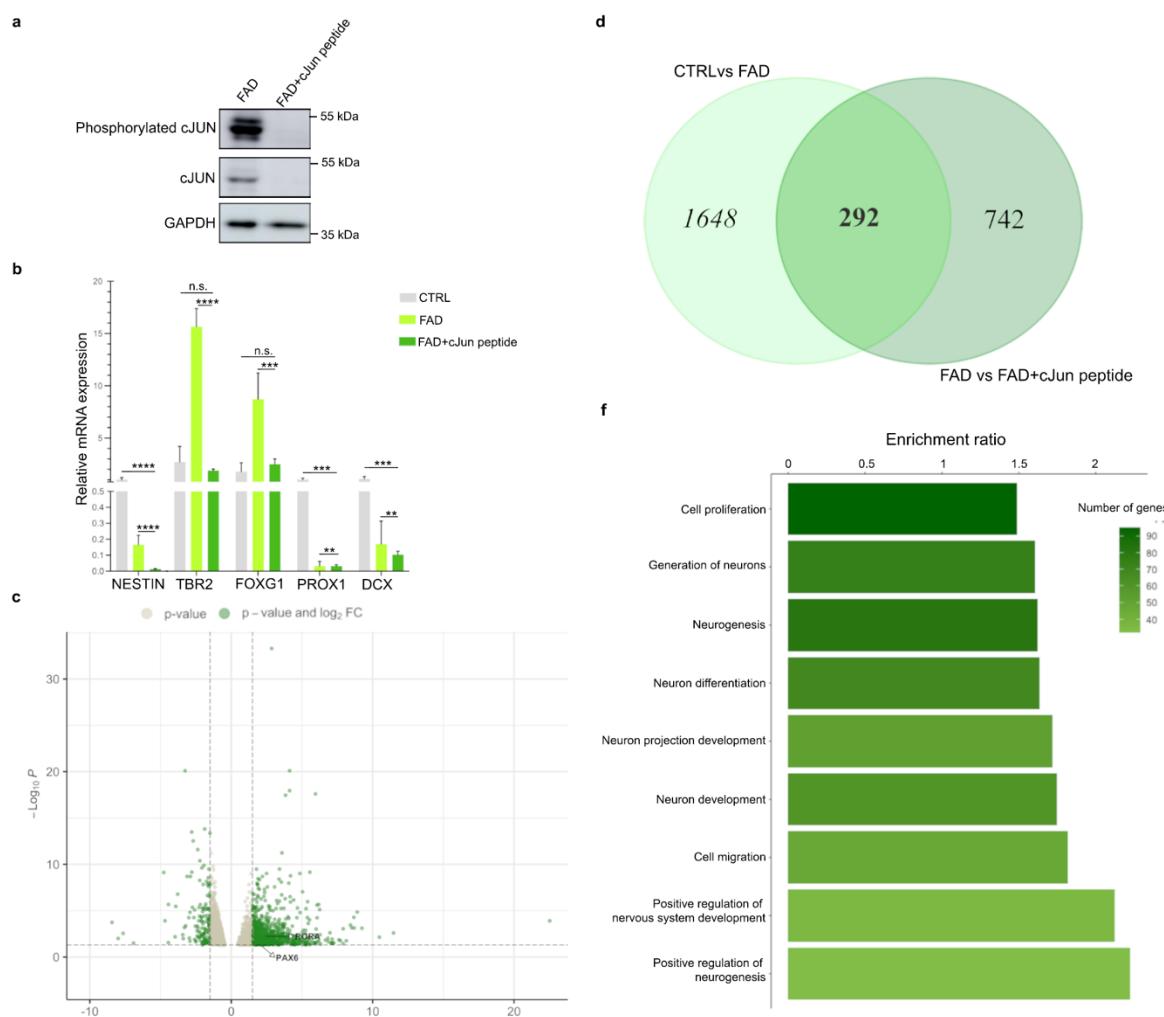
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503

504 **Figure 4. Accumulation of RNA-DNA hybrids triggers the cGAS-STING cascade and CC3**
505 **activation in FAD hpNPCs. a.** Immunofluorescence for RNA-DNA hybrids (S9.6 antibody, pink
506 signal) and STING (red signal) displays the accumulation of RNA-DNA hybrids in the cytoplasm
507 of FAD hpNPCs, and an upregulation of STING. Scale bar 50um, 40X magnification. DAPI
508 staining on nuclei in blue. **c.** Violin plot of $\log_2(\text{TPM})$ for RNaseH in CTRL and FAD hpNPCs.
509 **d.** Immunoblots for cGAS, STING, and cleaved caspase 3 (CC3) in FAD hpNPCs relative to
510 CTRLs.

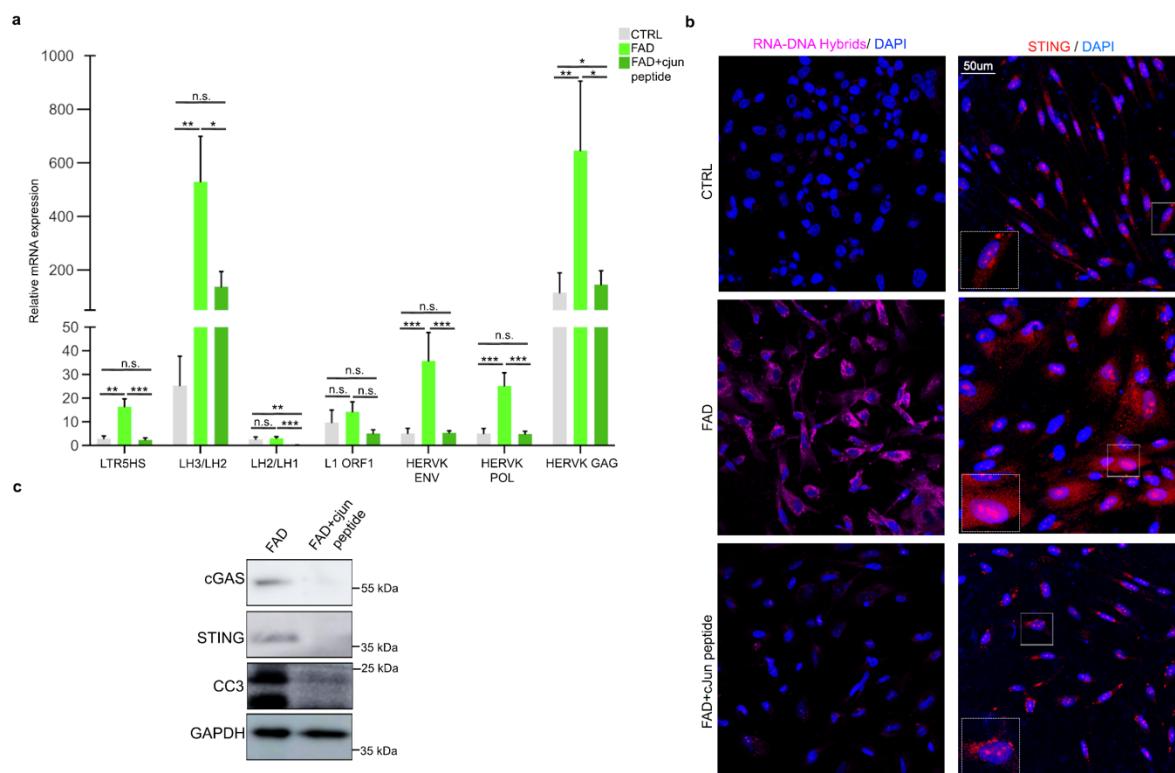
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513 **Figure 5. Inhibiting c-JUN phosphorylation partially rescues the impaired neurogenesis and**
 514 **the gene expression differences in FAD hpNPCs. a. Immunoblot of c-JUN and phosphorylated**
 515 **c-JUN untreated and treated with c-JUN peptide (FAD+c-JUN peptide) in FAD hpNPCs. b. qPCR**
 516 **analysis for hpNPCs markers in CTRL hpNPCs, untreated FAD hpNPCs, and c-JUN peptide-**
 517 **treated FAD hpNPCs c. Volcano plot of differentially expressed genes in FAD+c-JUN peptide**
 518 **relative to untreated FAD. Labeled genes are involved in neurogenesis (PAX6) and WNT/JNK**
 519 **signaling (RORA). Green = differentially expressed genes passing significance thresholds p-**
 520 **value < 0.05 and log₂(fold-change) +/- 1.5; Gray = differentially expressed genes passing**
 521 **significance threshold of p-value < 0.05. d. Venn diagram displaying the genes that were**
 522 **differentially expressed both in the “FAD+c-JUN peptide vs untreated FAD” comparison and**
 523 **in the “FAD vs CTRL” comparison. 292 differentially expressed genes overlap in the two**
 524 **comparisons. (venn diagram was made with <https://bioinformatics.psb.ugent.be>) e.**
 525 **Pathways enriched in the 292 overlapping genes predicted by WebGestalt.**

526

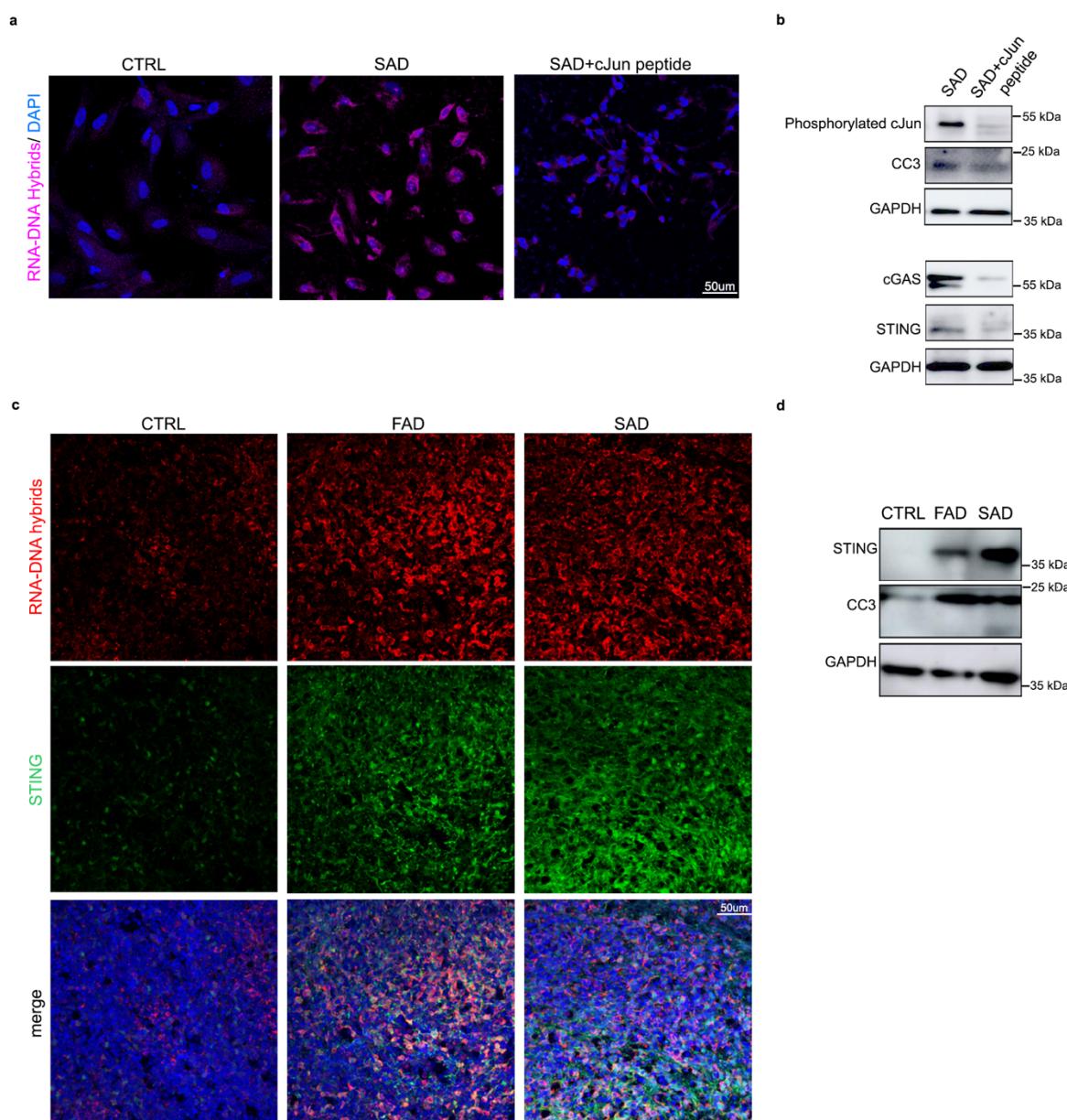


527

528 **Figure 6. Inhibiting c-JUN phosphorylation rescues aberrant TE derepression and the**
529 **activation of the cGAS-STING cascade in FAD progenitors. a.** qPCR analysis for a group of TEs
530 selected among those previously identified as aberrantly active in FAD hpNPC. Primers for
531 HERVK/LTR5HS target individual ORFs from the LTR; Primers for L1-ORF1 target a conserved
532 region in ORF1 of 6x; the other L1 primers target the L1PA2 family; the LH1/LH2 primers target
533 the end of the 3' UTR; The LH1/LH2 primers target the 5' of the other amplicon. **b.**
534 Immunofluorescence for RNA-DNA hybrids (S9.6 antibody, pink signal) and STING (red signal)
535 shows that c-JUN inhibition significantly decreases the accumulation of RNA-DNA hybrids and
536 STING levels in the cytoplasm of FAD hpNPCs. Scale bar 50um, 40X magnification. White dot
537 line boxes represent 2X magnification of the corresponding squared box. DAPI staining on
538 nuclei in blue. **c.** Immunoblots for cGAS, STING, and cleaved caspase 3 (CC3) on FAD+c-JUN
539 peptide hpNPCs relative to FAD progenitors.

540

541



542

543 **Figure 7. TE-derived RNA-DNA hybrids and cGAS-STING activated in SAD progenitors and AD**
544 **cerebral organoids** **a.** Immunofluorescence for RNA-DNA hybrids (S9.6 antibody, pink signal)
545 and STING (red signal) show that c-JUN inhibition significantly decreases the accumulation of
546 RNA-DNA hybrids and STING levels in the cytoplasm of SAD hpNPCs. Scale bar 50μm, 40X
547 magnification. DAPI staining on nuclei in blue. **b.** Immunoblots for phosphorylated c-JUN,
548 cGAS, STING, and cleaved caspase 3 (CC3) on SAD+c-JUN peptide hpNPCs relative to SAD
549 progenitors. **c.** Immunofluorescence for RNA-DNA hybrids (S9.6 antibody, red signal) and
550 STING (green signal) on cerebral organoids (CTRL, FAD, SAD). Scale bar 50μm, 40X

551 magnification. DAPI staining on nuclei in blue. **d**. Immunoblots for STING and cleaved caspase
552 3 (CC3) on AD organoids relative to CTRLs.

553

554 **Materials and Methods**

555

556 **Human iPSC culture**

557 Control and Alzheimer's disease iPSC lines were obtained from the Coriell Institute for Medical
558 Research (Camden, NJ). In particular, we received two control lines (Control line-
559 1:IPSM8Sev3, male, 65 years old and Control line-2: iPSM15Sev4, female, 62 years old) CTRL1
560 and CTRL2 respectively; two familial Alzheimer's disease lines (Familial Alzheimer's line-1:
561 AG25370, female, 80 years old and Familiar Alzheimer's line-2: GM24675, male, 60 years old)
562 FAD1 and FAD2 respectively; and two sporadic Alzheimer's disease lines (Sporadic Alzheimer's
563 line-1: AG27607, female, 69 years old and Sporadic Alzheimer's line-2: GM24666, male, 83
564 years old) SAD1 and SAD2 respectively. All the AD lines excepted for AG25370 were validated
565 in previous studies^{77,83,85,86}.

566 The iPSC lines were expanded in feeder-free, serum-free mTeSR™1 medium (85850,
567 STEMCELL Technologies). Cells were passaged ~1:10 at 80% confluency using EDTA 0.5mM
568 (15575020, Invitrogen) and small cell clusters (50–200 cells) were subsequently plated on
569 tissue culture dishes coated overnight with Geltrex™ LDEV-Free hESC-qualified Reduced
570 Growth Factor Basement Membrane Matrix (A1413302, Fisher-Scientific).

571

572 **hpNPC Differentiation**

573 The iPSC lines were differentiated into hpNPCs as previously described⁴⁷. Briefly, iPSCs were
574 treated with hpNPC induction medium for five days: DMEM/F-12 medium (Invitrogen)
575 supplemented with B-27 (A3582801, Gibco), N-2 (17502048, Gibco), DKK1 (778606,
576 Biolegend), Cyclopamine (C-8700, LC Laboratories), Noggin (597004, Biolegend), and
577 SB431542 (S1067, Selleck Chemicals LLC). At day 6, the hpNPCs were plated in a new geltrex-
578 coated well and cultured in proliferation medium, consisting of DMEM/F-12 medium
579 (Invitrogen) supplemented with B-27 (A3582801, Gibco), N-2 (17502048, Gibco) and 20 ng/ml
580 bFGF (713304, Biolegend).

581

582 **CA3 Neuron Differentiation**

583 The iPSC lines were differentiated into CA3 Neurons as previously described⁶⁸. Briefly, iPSCs
584 were treated with hpNPC induction medium for 15 days. At day 16, the hpNPCs were plated
585 in a new PLO-Laminin double-coated well in Neuron induction medium, consisting
586 in DMEM/F12 medium (11320082, Gibco) supplemented with B-27 (A3582801, Gibco), N-2
587 (17502048, Gibco), BDNF (450-02, Prepotech), Dibutyryl-cAMP (11-415-0, Tocris), laminin
588 (23017015, ThermoFisher Scientific), AA (A4544-25G, Sigma), WNT3a (5036-WN, R&D
589 System). After 3 weeks, the neurons were switched to neuron medium, consisting
590 of DMEM/F-12 medium (Invitrogen) supplemented with B-27 (A3582801, Gibco), N-2
591 (17502048, Gibco), BDNF (450-02, Prepotech), Dibutyryl-cAMP (11-415-0, Tocris), laminin
592 (23017015, ThermoFisher Scientific) and AA (A4544-25G, Sigma) for one week. Mature CA3
593 neurons were then collected for RNA-seq and fixed for immunofluorescence.

594

595 **Cerebral organoid Differentiation**

596 CTRL and AD (FAD and SAD) iPSCs were differentiated into cerebral organoids following a
597 previously published protocol⁸⁶. Briefly, embryoid bodies were formed from CTRL, FAD, and
598 SAD iPSCs and maintained in Essential 8 media (E8 media, A1517001, ThermoScientific)
599 supplemented with ROCK inhibitor (SCM075, Millipore) for 4 days. Neuronal induction was
600 obtained by replacing the E8 media with Neural induction media: DMEM/F12 (11330-032,
601 Invitrogen) supplemented with N-2 (17502048, Gibco), 1% Glutamax (35050-038, Invitrogen),
602 1% MEM-NEAA (M7145, Sigma), and Heparin at a final concentration of 1 µg/ml (H3149,
603 Sigma). After 4-5 days, when neuroepithelium formation was achieved, spheroids were
604 embedded in Matrigel (356234, BD Biosciences) and cultured in Cerebral organoid
605 differentiation media: DMEM/F12 (11330-032, Invitrogen) and Neurobasal (21103049,
606 Invitrogen) (1:1) supplemented with B27 without VitA (12587010, Invitrogen), N2 (17502048,
607 Gibco), Insulin (I9278-5ML, Sigma), 2-Mercaptoethanol (1:100 dilution, 8057400005, Merk),
608 1% MEM-NEAA (M7145, Sigma), and Glutamax (35050-038, Invitrogen). After 4 days in static
609 culture, spheroids were transferred to a shaker and maintained. Half media changes were
610 performed every 3-4 days.

611

612 **hpNPC c-JUN peptide treatment**

613 CTRL, FAD, and SAD hpNPCs were treated with 100µM of c-JUN peptide (19-891, Fisher
614 Scientific) for 5 days in a proliferative condition. This peptide comprises residues 33–57 of the

615 JNK binding (δ) domain of human c-JUN and it is a competitive inhibitor of JNK/c-JUN
616 interaction preventing c-JUN phosphorylation and activation.

617

618 **Processing of organoids**

619 At day 62, the whole organoids were fixed in 4% PFA overnight at 4 °C. After cryoprotection
620 in 30% sucrose (s7903, Sigma), organoids were cryo-sectioned at 20 μ m thickness and slices
621 were analyzed by immunohistochemistry.

622

623 **Immunofluorescence**

624 Immunohistochemistry of iPSCs, hpNPCs, and CA3 neurons was performed in μ -Slide 4 Well
625 Glass Bottom (80426, IBIDI), while organoid IF was performed on 20- μ m serial sections. Upon
626 fixation (4% PFA for 10 minutes), cells were permeabilized in blocking solution (0.1% Triton X-
627 100, 1X PBS, 5% normal donkey serum) and then incubated with the antibody of interest. The
628 total number of cells in each field was determined by counterstaining cell nuclei with 4,6-
629 diamidino-2-phenylindole dihydrochloride (DAPI; Sigma-Aldrich; 50 mg/ml in PBS for 15 min
630 at RT). To improve the efficiency of Tbr2 detection, the cells and the organoid slides, prior to
631 permeabilization and blocking step, were treated with 10 mM sodium citrate (pH = 6) for 10
632 minutes at 95 °C.

633 For RNA-DNA hybrid staining (S9.6 antibody), upon fixation (4% PFA for 10 minutes),
634 cells and organoid slides were permeabilized in PBS 1X 0.5% Triton X-100 for 15 minutes. They
635 were then incubated overnight at -20°C in 100% methanol. The samples were then blocked
636 in 1X PBS 5% NDS for 4 hours at 37°C and followed by overnight incubation with the S9.6
637 antibody.

638 Immunostained cells and organoid slices were analyzed via confocal microscopy using
639 a Nikon A1R+. Images were captured with x40 for hpNPCs and x20 and x60 objectives for
640 organoids and a pinhole of 1.0 Airy unit. Analyses were performed in sequential scanning
641 mode to rule out cross-bleeding between channels. Fluorescence intensity quantification was
642 performed with Fiji and the NIS-Elements AR software. All antibodies are listed in the
643 Antibodies table (Supplementary Table S3).

644

645

646 **Western Blot**

647 For total lysate, cells were harvested and washed three times in 1X PBS and lysed in RIPA
648 buffer (50mM Tris-HCl pH7.5, 150mM NaCl, 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS,
649 500uM DTT) with protease and phosphatase inhibitors. Twenty µg of whole cell lysate were
650 loaded in Novex WedgeWell 4-12% Tris-Glycine Gel (Invitrogen) and separated through gel
651 electrophoresis (SDS-PAGE) in Tris-Glycine-SDS running buffer (Invitrogen). The proteins were
652 then transferred to ImmunBlot PVDF membranes (ThermoFisher) for antibody probing.
653 Membranes were incubated with 10% BSA in 1X TBST for 1 hour at room temperature (RT),
654 then incubated for variable times and concentrations with the suitable antibodies
655 (Supplementary table S3) diluted in 5% BSA in 1X TBST. Membranes were then washed with
656 1X TBST and incubated in the HRP-linked species-specific secondary antibody (1:10000
657 dilution) for one hour at RT. The membrane was visualized using the Pierce ECL Plus Western
658 Blotting Substrate (32132; ThermoFisher) and imaged with an Amersham Imager 680. All
659 antibodies are listed in the Antibodies table (Supplementary Table S3).

660

661 **Real-time quantitative polymerase chain reaction (RT-qPCR)**

662 Cells were lysed in Tri-reagent (R2050-1-50, Zymo Research) and RNA was extracted using the
663 Direct-zol RNA Miniprep kit (Zymo Research). 600ng of template RNA was retrotranscribed
664 into cDNA using RevertAid first strand cDNA synthesis kit (Thermo Scientific) according to the
665 manufacturer's directions. 15ng of cDNA was used for each real-time quantitative PCR
666 reaction with 0.1 µM of each forward and reverse primer, 10 µL of PowerUp™ SYBR™ Green
667 Master Mix (Applied Biosystems) in a final volume of 20 µl, using a QuantStudio 3 Real-Time
668 PCR System (Applied Biosystems). Thermal cycling parameters were set as follows: 3 minutes
669 at 95°C, followed by 40 cycles of 10 seconds at 95°C and 20 seconds at 63°C followed by
670 30 seconds at 72°C. Each sample was run in triplicate. 18S rRNA was used for normalization.
671 Primer sequences are reported in Supplementary Table S4.

672

673 **RNA-Seq**

674 Cells were lysed in Tri-reagent (R2050-1-50, Zymo Research) and total RNA was extracted
675 using Quick-RNA Miniprep kit (R1055, Zymo Research) according to the manufacturer's
676 instructions. RNA was quantified using a DeNovix DS-11 Spectrophotometer while the RNA
677 integrity number (RIN) was checked on an Agilent 2200 TapeStation. Only samples with RIN

678 values above 8.0 were used for transcriptome analysis. RNA libraries were prepared using
679 NEBNext® Poly(A) mRNA Magnetic Isolation Module (E7490S, New England Biolabs),
680 NEBNext® UltraTM II Directional RNA Library Prep Kit for Illumina® (E7760S, New England
681 Biolabs) and NEBNext® UltraTM II DNA Library Prep Kit for Illumina® (E7645S, New England
682 Biolabs) according to the manufacturer's instructions. The libraries were sequenced using an
683 Illumina NextSeq2000, generating 150 bp Paired-End reads.

684

685 **RNA-Seq Analyses**

686 Reads were aligned to hg19 using STAR v2.5⁸⁷ in 2-pass mode with the following parameters:
687 --quantMode TranscriptomeSAM --outFilterMultimapNmax 10 - -outFilterMismatchNmax 10
688 --outFilterMismatchNoverLmax 0.3 --alignIntronMin 21 --alignIntronMax 0 --
689 alignMatesGapMax 0 --alignSjoverhangMin 5 --runThreadN 12 --twopassMode Basic --
690 twopass1readsN 60000000 --sjdbOverhang 100. We filtered bam files based on alignment
691 quality (q = 10) using Samtools v0.1.19 (Li H, et al., 2009). We used the latest annotations
692 obtained from Ensembl to build reference indexes for the STAR alignment. Kallisto (Bray et
693 al., 2016) was used to count reads mapping to each gene. RSEM⁸⁸ was used to obtain FPKM
694 (Fragments Per Kilobase of exon per Million fragments mapped). Differential gene expression
695 levels were analyzed using DESeq2⁸⁹, with the following model: design = ~condition, where
696 condition indicates either CTRL or Alzheimer's disease (FAD or SAD) lines.

697

698 **ATAC-Seq**

699 For ATAC-Seq experiments, 50,000 cells per condition were processed as described in the
700 original ATAC-seq protocol paper⁹⁰. Briefly, 50,000 cells were collected, washed, and lysed.
701 The chromatin was subjected to transposition/library preparation via a Tn5 transposase using
702 the Tegment DNA Enzyme and Buffer Kit (20034197, Illumina) and incubated at 37°C for 30
703 min with slight rotation (300 RPM). Transposed DNA was purified using a MinElute PCR
704 Purification Kit (28004; Qiagen). Transposed DNA fragments were then amplified using a
705 universal and barcoded primer⁹⁰. Thermal cycling parameters were set as follows: 1 cycle of
706 72°C for 5 minutes, 98°C for 30 seconds, followed by 5 cycles of 98°C for 10 seconds, 63°C for
707 30 seconds, and 72°C for 1 min. The amplification was paused and 5ul of the partially
708 amplified, transposed DNA was used for a qPCR side reaction including the universal and
709 sample-specific barcoded primers⁹⁰, PowerUp™ SYBR™ Green Master Mix (Applied

710 Biosystems), NEBNext High-Fidelity 2x PCR Master Mix, and nuclease-free water. The qPCR
711 side reaction parameters were set as follows: 1 cycle of 72°C for 5 minutes, 98°C for 30
712 seconds, followed by 40 cycles of 98°C for 10 seconds, 63°C for 30 seconds, and 72°C for 1
713 min. The Rn vs cycle plot was used to determine the remaining number of PCR cycles needed
714 where 1/3 of the maximum fluorescent intensity corresponds to the cycle number. The
715 remaining partially amplified transposed DNA was fully amplified using the previous
716 parameters with the additional cycle number determined from the qPCR side reaction. The
717 amplified, transposed DNA was purified using AMPure XP beads (A63881, Beckman Coulter)
718 and sequenced using an Illumina NextSeq2000, generating 150 bp Paired-End reads.

719

720 **ATAC-Seq Analyses**

721 After removing the adapters, the sequences were aligned to the reference hg19, using
722 Burrows-Wheeler Alignment tool (BWA), with the MEM algorithm⁹¹. Aligned reads were
723 filtered based on mapping quality (MAPQ > 10) to restrict our analysis to higher quality and
724 likely uniquely mapped reads, and PCR duplicates were removed. All mapped reads were
725 offset by +4 bp for the forward strand and -5 bp for the reverse strand. We called peaks using
726 MACS2⁹², at 5% FDR, with default parameters. We analyzed differential genome accessibility
727 using DESeq2⁸⁹, with the following model: design = ~condition, where condition indicates
728 either CTRL or Alzheimer's disease (FAD or SAD) lines. R v3.3.1. and BEDtools v2.27.1⁹³ were
729 used for all comparative TEs analyses.

730

731 **Statistical and genomic analyses**

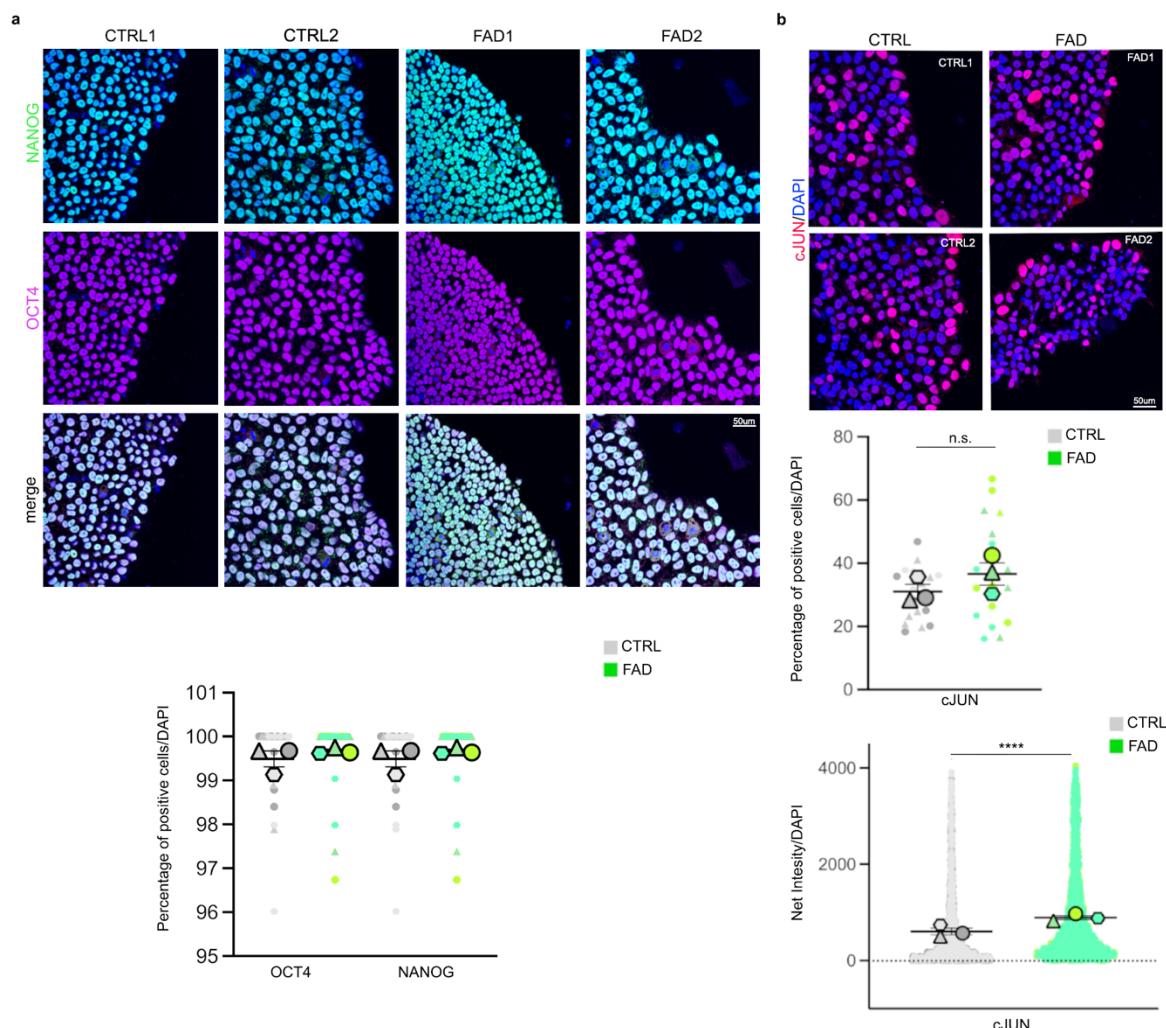
732 All statistical analyses were performed using R v3.3.1. BEDtools v2.27.1⁹³ was used for
733 genomic studies. Pathway analysis was performed with WEB-based GEne SeT AnaLysis Toolkit
734 (<http://www.webgestalt.org>). Motif analyses were performed using the MEME-Suite⁹⁵,
735 specifically with the MEME-ChIP application. Fasta files of the regions of interest were
736 produced using BEDTools v2.27.1. Shuffled input sequences were used as background. E-
737 values < 0.001 were used the threshold for significance. All described results (qPCR analyses
738 and Immunofluorescences) are representative of at least three independent experiments
739 unless specifically stated otherwise. Data were presented as average ± SEM. Statistical
740 analysis was performed using Excel (Microsoft) or GraphPad Prism 8 software (GraphPad).
741 Student's t-test was used for the comparison between two groups. A value of P < 0.05 was

742 considered significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; n.s. , not
743 significant.

744

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746 **Extended Data**



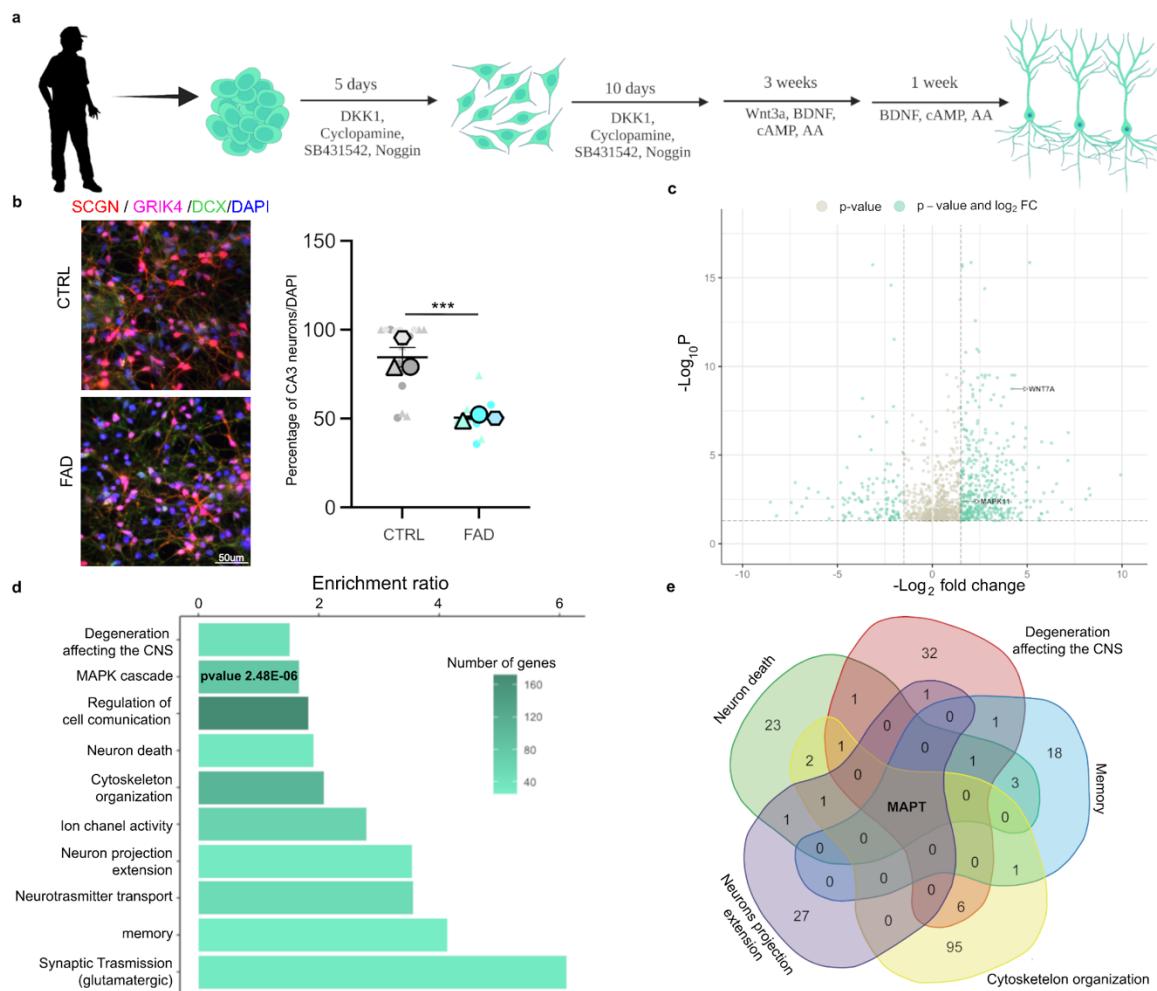
747

748 **Extended Data Figure 1. Pluripotency and JUN expression profiles of CTRL and FAD-derived**
749 **iPSCs. a. and b. Immunofluorescence quantifying the expression of a. pluripotency markers**
750 **OCT4 (purple) and NANOG (green) and b. endogenous c-JUN in iPSCs derived from CTRLs and**
751 **FAD. The quantification of immunofluorescence in panel b is reported as the percentage of**
752 **expressing cells (upper superplot) and expression of Net Intensity (bottom superplot).**

753

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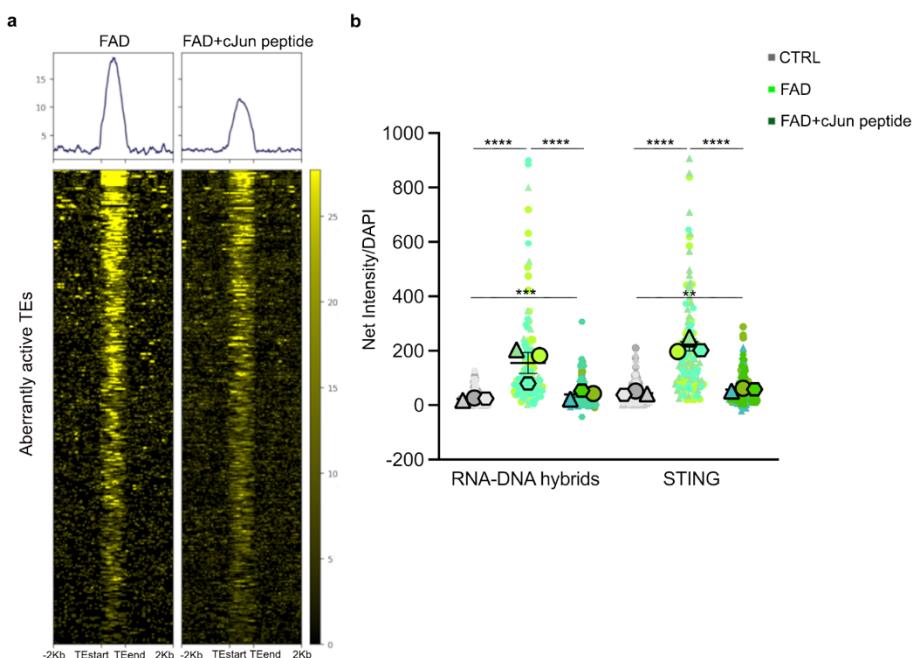
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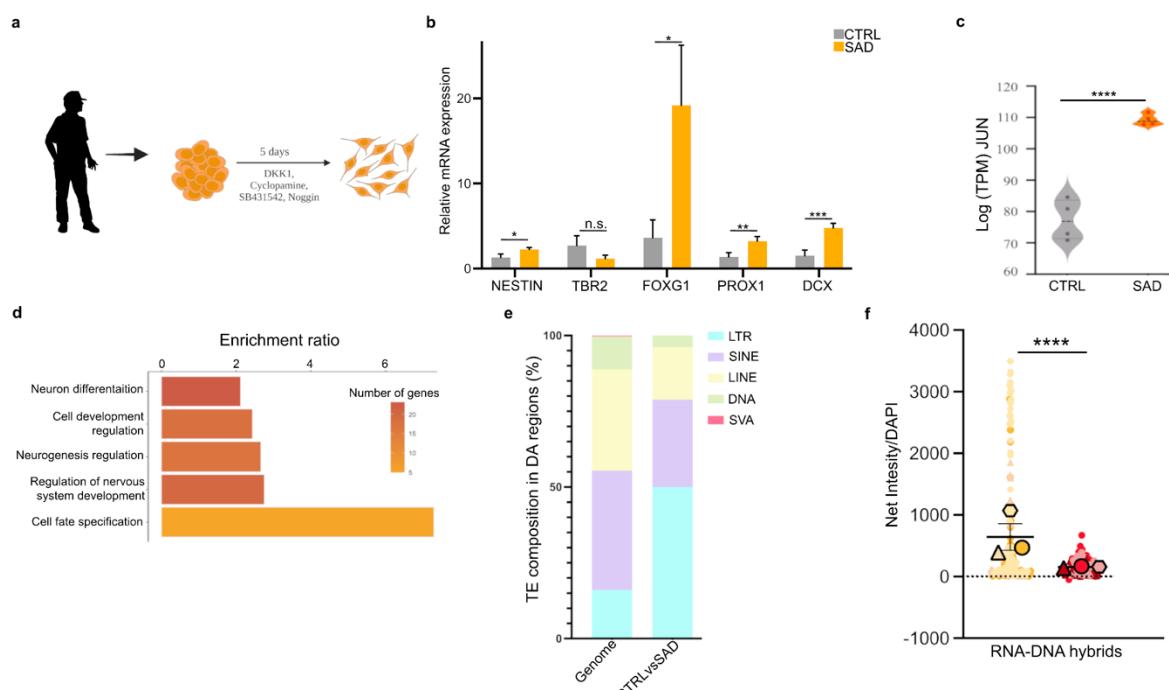
757 **Extended Data Figure 2. Dysregulated transcriptional networks and pathways in FAD CA3**
758 **hippocampal neurons.** **a.** A scheme of the protocol for CA3 hippocampal neuron
759 differentiation (made with Biorender.com). **b.** Immunofluorescence for CA3 neuron markers.
760 GRIK4 (pink) and SCGN (red) were expressed in CTRL and FAD neurons demonstrating their
761 proper differentiation. The relative quantification shows that FAD hpNPCs failed to
762 differentiate into CA3 neurons as demonstrated by the reduced number of SCGN- and GRIK4-
763 positive cells in the FAD neuron culture. Scale bar 50um, 40X magnification. DAPI staining on
764 nuclei in blue. **c.** Volcano plot displaying the 1,105 differentially expressed genes in FAD CA3
765 neurons relative to CTRL CA3 neurons. Teal = differentially expressed genes passing
766 significance thresholds $p\text{-value} < 0.05$ and $\log_2(\text{fold-change}) < 1.5$; Gray = differentially
767 expressed genes passing significance threshold of $p\text{-value} < 0.05$. **d.** Enriched pathways
768 associated with the 1,105 differentially expressed genes in FAD CA3 neurons predicted by
769 WebGestalt. **e.** Venn diagram showing the genes shared across five enriched pathways in AD.
770 (venn diagram was made using <https://bioinformatics.psb.ugent.be>).

771
772



773
774 Extended Data Figure 3. **TE activation induces RNA-DNA hybrid accumulation triggering the**
775 **cGAS-STING cascade and apoptosis in FAD hpNPCs.** **a.** Heatmap showing a reduction in the
776 number of open regions of chromatin in FAD+c-JUN peptide at aberrantly active TEs. **b.**
777 Quantification of the immunofluorescences in Figure 4a.

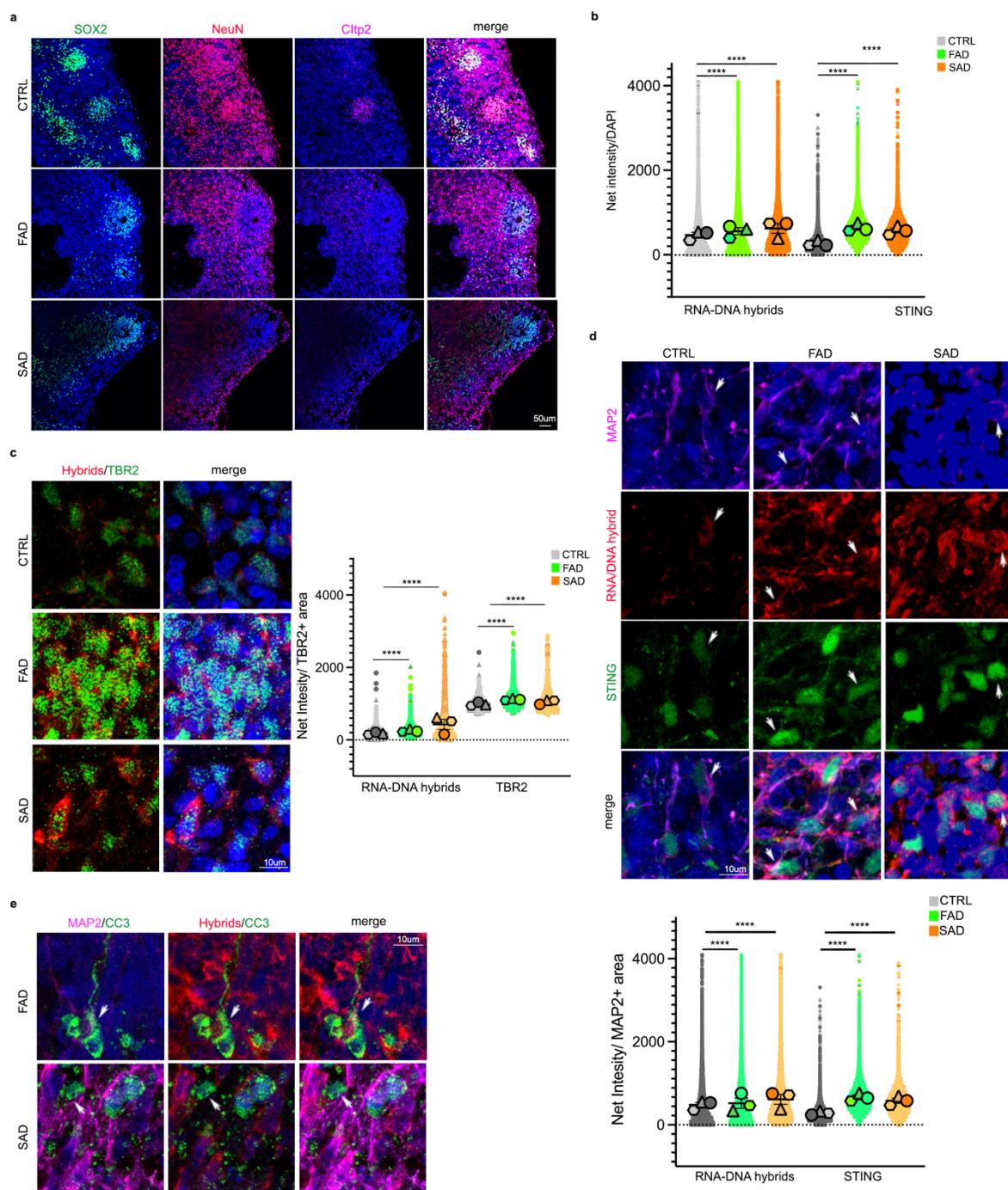
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780 Extended Data Figure 4. **SAD iPSC-derived hippocampal neural progenitors display impaired**
781 **neurogenesis, gene expression dysregulation, and aberrant activation of TEs.** **a.** The scheme
782 of the protocol for hpNPC differentiation (made with Biorender.com). **b.** qPCR analysis for
783 hpNPC population markers. NESTIN – early precursors; TBR2/FOXG1 – intermediate
784 progenitors; PROX1 – late progenitors; DCX – neuroblasts. **c.** Violin plot of $\log_2(\text{TMP})$ for *JUN*
785 in SAD hpNPCs compared to CTRLs. **d.** Pathways enriched in the 183 differentially expressed
786 genes (SAD vs CTRL hpNPCs) predicted by WebGestalt. **e.** TE family distribution of the TEs
787 aberrantly de-repressed in SAD progenitors shows an enrichment for LTRs. **f.** Quantification
788 of the immunofluorescences in Figure 7a.

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791 Extended Data Figure 5. **Characterization of TE-derived RNA-DNA hybrids inducing the cGAS-
792 STING cell-death axis in AD cerebral organoids.** **a.** Immunofluorescence for progenitors
793 (SOX2, green signal), immature neurons (NeuN, red signal), and mature neurons (Citp2, pink
794 signal) in CTRL and AD (FAD and SAD) organoids. Scale bar 50μm, 20X magnification. DAPI
795 staining on nuclei in blue. **b.** Quantification of the immunofluorescences in Figure 7b. **c.**
796 Immunofluorescence for intermediate progenitors (TBR2, green signal) and RNA-DNA hybrids
797 (red signal) in CTRL and AD (FAD and SAD) organoids. Scale bar 10μm, 60X magnification 4X

798 digital zoom. DAPI staining on nuclei in blue. **d.** Immunofluorescence for neurons (MAP2, pink
799 signal) and RNA-DNA hybrids (red signal) and STING (green signal) in CTRL and AD (FAD and
800 SAD) organoids. Scale bar 10um, 60X magnification 4X digital zoom. DAPI staining on nuclei in
801 blue. **e.** Immunofluorescence for neurons (MAP2, pink signal), RNA-DNA hybrids (red signal)
802 and cleaved caspase 3 (CC3; green signal) in FAD and SAD organoids. White arrows indicate
803 MAP2/RNA-DNA hybrid/CC3-positive neurons. Scale bar 10um, 60X magnification 4X digital
804 zoom. DAPI staining on nuclei in blue.

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842 **Extended Data Table1.** TMPs values of genes involved in the WNT pathway that are
 843 differentially expressed in FAD hpNPCs.

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	CTRL	FAD
DKK1	3.692171	9.72627767
DKK3	57.7179857	223.367699
JUN	77.3016	109.701667
RAC2	6.636084	51.6482917
RORA	2.65834244	7.06845833
SFRP4	0.8792335	14.3325863
WNT2	0.01490205	3.81743227
WNT5A	59.2553505	204.736115
WNT7A	0.0380094	0.7898473

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847 **Extended Data Table2.** List of 83 completely rescued genes after the inhibition of c-JUN
 848 phosphorylation.

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Rescued gene after inhibition			
Down-regulated		Up-regulated	
gene	p-value	gene	p-value
SLC38A5	1.13E-08	AC055839.2	0.00503467
PIK3AP1	0.024016038	ACHE	0.00089729
SECTM1	5.33E-09	ACSM3	0.02013152
MFAP3L	0.007974303	ADAMTS18	0.02742898
S100A6	0.031978317	ADD2	0.00303124
TUBA4A	0.026239837	ALKAL2	0.00127631
PCDH10	0.004463934	ANGPT2	0.02758469
NPTX1	7.95E-21	ANKRD7	0.02085884
TREX1	0.00041767	ARHGAP28	0.00111001
		ATCAY	0.02859681
		B4GALNT3	0.00414689
		BMF	0.04384791
		CA14	0.03877118
		CAMK2A	0.03274196
		CCNL	0.04487228
		CD200	0.00474689
		CFAP65	0.0413402
		CLDN10	0.00136135
		CLDN6	0.03073783
		CNMD	8.65E-05
		CNTNAP2	0.0117396
		CRABP2	0.03501695
		DACT1	0.00822037
		DGKB	0.0267694
		DMRTA1	0.04101869
		DOCK8	0.00186351
		DTX4	0.00070423
		EDNRB	0.02369424
		FAM131B	0.01979536
		FBN3	0.01625092
		FGF13	5.58E-05
		FILIP1	0.03914467
		FREM2	0.03102541
		GABRB3	0.02235285
		GLDC	0.00800157
		GNG2	0.00874216
		GPSM3	0.04532086
		HEPH	7.50E-06
		HFM1	0.00807794

Rescued gene after inhibition			
Down-regulated		Up-regulated	
gene	p-value	gene	p-value
		KANK3	0.0178539
		KCNF1	0.0158949
		LAMA1	0.0346366
		LRRN1	8.23E-09
		MAST4	0.00865933
		MMP2	0.01588143
		MMP9	2.13E-06
		MUC3A	0.01962786
		MYCL	0.00662627
		NETO1	0.03666395
		NHSL2	0.0309825
		NOVA1	0.00992984
		PAX6	0.04899031
		PCDHGA6	0.0258056
		PECAM1	0.0008668
		PLCXD3	0.0268678
		PLD6	0.0338688
		PMEL	0.0049156
		PNMA8C	0.0001973
		PTPRN	6.99E-10
		RCAN2	0.0368716
		RIMS2	0.0010725
		SCUBE1	0.01658889
		SDR42E1	0.00675173
		SHC2	0.02540905
		SLC25A34	0.0308753
		SLC2A12	0.00041463
		THBS3	0.02950207
		TMEM130	0.02207853
		TMEM178A	3.43E-09
		USHBP1	0.0483738
		YPEL1	7.85E-09
		ZNF521	0.0098816
		ZNF662	0.04882
		ZSCAN10	0.0348837

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885 **Extended data Table 3.** Table of antibodies used.

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Antibody	Specificity	Host specie	Dilution	Supplier	Reference
OCT4 (MOUSE) Clone 3A2A20 unconjugated	OCT4	mouse	1:200	StemCell Technologies	60093
Human Nanog Antibody	NANOG	goat	1:20	R&D Systems	AF1997
c-Jun Monoclonal Antibody (4H9)	cJUN	mouse	1:1000 WB	Fisher	MA5-15889
Anti-h nestin AF488 25 ug	NESTIN	mouse	1:200	Invitrogen	5016830
Tbr2 Abcam antibody	TBR2	rabbit	1:200	Abcam	ab216870
Phospho-c-Jun (Ser73) Polyclonal Antibody	Phospho cJUN	rabbit	1:1000	Fisher	#44-292G
S9.6	RNA-DNA hybrids	mouse	1:500	Kerafast	ENH001
STING Polyclonal Antibody	STING	rabbit	1:100 IF 1:1000 WB	ThermoFisher	PA5-23381
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit antibody	Cleaved caspase 3	rabbit	1:200 IF 1:1000 WB	CellSignaling	9664T
SCGN Polyclonal Antibody	SCGN	rabbit	1:250	Fisher	PA5-30393
GRIK4 Monoclonal Antibody (8H5G5)	GRIK4	mouse	1:100	Fisher	MA5-31745
Goat Polyclonal Doublecortin antibody	DCX	goat	1:200	Rockland Immunochemicals	600-101-MH8
AB5603 Anti-Sox2 (rabbit polyclonal)	SOX2	rabbit	1:1000	EMD Millipore	AB5603-100UG
RBFOX3/NeuN Antibody (1B7)	NeuN	mouse	1:1000	Novus Biologicals	NBP1-92693
Mab1045 Anti-CTIP2/BCL11B Antibody, clone 25B6, Anti-CTIP2/BCL11B Antibody, clone 25B6	Cipt2	rat	1:1000	EMD Millipore	MABE1045
Anti-cGAS Antibody	cGAS	rabbit	1:1000	Millipore Sigma	ABF124
MAP2 antibody	MAP2	chicken	1:1000	Novus Biologicals	NB300-213
GAPDH antibody (rabbit) D16H11	GADPH	rabbit	1:1000	Cell Signaling Technologies	5174S
c-Jun (60A8) Rabbit mAb	cJUN	rabbit	1:200 IF	Cell Signaling Technologies	9165
Horse Anti-mouse IgG, HRP-linked	mouse IgG	horse	1:10000	Cell Signaling Technologies	7076S
Anti-rabbit IgG, HRP-linked	rabbit IgG	goat	1:10000	Cell Signaling Technologies	7074S
Cy™3 AffiniPure Donkey Anti-Rabbit IgG (H+L)	rabbit IgG	Donkey	1:250	Jackson ImmunoResearch	711-165-152
Alexa Fluor® 594 AffiniPure Donkey Anti-Mouse IgG (H+L)	mouse IgG	Donkey	1:500	Jackson ImmunoResearch	715-585-150

Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG	rabbit IgG	Donkey	1:500	Jackson ImmunoResearch	711-545-152
Alexa Fluor® 488 AffiniPure Donkey Anti-Goat IgG (H+L)	Goat IgG	Donkey	1:500	Jackson ImmunoResearch	705-545-003
Alexa Fluor® 647 AffiniPure Donkey Anti-Mouse IgG (H+L)	mouse IgG	Donkey	1:500	Jackson ImmunoResearch	715-605-150
Goat anti-chicken IgY (H+T) Alexa Flour Plus 647	Chicken IgG	Goat	1:1000	Invitrogen	A32933
Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H+L)	Rat IgG	Donkey	1:500	Jackson ImmunoResearch	712-605-150

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888 **Extended Data Table 4.** List of primers used for qRT-PCR analysis.

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Primer name	sequence
HERVK env fwd	GCTGCCCTGCCAACCTGAG
HERVK env rev	CCTGAGTGACATCCGCTTACC
HERVK gag fwd	AAATAAGACCCAACCGCCAGTAGC
HERVK gag rev	GAATTGCCATGCCTCAGTATCTCC
HERVK pro fwd	GCCGATGAAAAAGCCCGTAAGG
HERVK pro rev	TTGACACTCAGGATTGGCGTTTC
LTR5HS fwd	GGGCAGCAATACTGCTTGT
LTR5HS rev	CAATAGTGGGGAGAGGGTCA
L1 ORF1 fwd	CTCGCAGAAACCTACAAG
L1 ORF1 rev	CCATGTTAGCGCTTCCTTC
LH1 fwd	AAAGACACATGCACWCRTATGTT
LH2 rev	TTTCTCAYTYATAGGTGGGA
LH2 fwd	CATGGAATAYTATGCAGCCATAAA
LH3 rev	TCCCACCTATRARTGAGAA
NES fwd	CTTCAGGACCCAAGCTGGA
NES rev	CAGGTGTCTCAAGGGTAGCAG
TBR2 fwd	ACCTTCTCCAGCGTGTGAG
TBR2 rev	TCCTCGTACCTCTTGCTCCT
FOXG1 fwd	AGAAGAACGGCAAGTACGAGA
FOXG1 rev	TGTTGAGGGACAGATTGCGC
PROX1 fwd	GACTTGAGGTTCCAGAGAGA
PROX1 rev	TGTAGGCAGTTGGGATTG
DCX fwd	TCAGGGAGTGCCTACATTAC
DCX rev	GTTGGGATTGACATTCTGGTG

18S fwd	ATACATGCCGACGGGCGCTG
18S rev	AGGGGCTGACCGGGTTGGTT

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