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1 **Human neural networks with sparse TDP-43 pathology reveal NPTX2 misregulation in**  
2 **ALS/FTLD**

3

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28 **Human cellular models of neurodegeneration require reproducibility and longevity, which is**  
29 **necessary for simulating these age-dependent diseases. Such systems are particularly needed**  
30 **for TDP-43 proteinopathies<sup>1,2</sup>, which involve human-specific mechanisms<sup>3-6</sup> that cannot be**  
31 **directly studied in animal models. To explore the emergence and consequences of TDP-43**  
32 **pathologies, we generated iPSC-derived, colony morphology neural stem cells (iCoMoNSCs) via**  
33 **manual selection of neural precursors<sup>7</sup>. Single-cell transcriptomics (scRNA-seq) and**  
34 **comparison to independent NSCs<sup>8</sup>, showed that iCoMoNSCs are uniquely homogenous and**  
35 **self-renewing. Differentiated iCoMoNSCs formed a self-organized multicellular system**  
36 **consisting of synaptically connected and electrophysiologically active neurons, which matured**  
37 **into long-lived functional networks. Neuronal and glial maturation in iCoMoNSC-derived**  
38 **cultures was similar to that of cortical organoids<sup>9</sup>. Overexpression of wild-type TDP-43 in a**  
39 **minority of iCoMoNSC-derived neurons led to progressive fragmentation and aggregation,**  
40 **resulting in loss of function and neurotoxicity. scRNA-seq revealed a novel set of misregulated**  
41 **RNA targets coinciding in both TDP-43 overexpressing neurons and patient brains exhibiting**  
42 **loss of nuclear TDP-43. The strongest misregulated target encoded for the synaptic protein**  
43 **NPTX2, which was consistently misaccumulated in ALS and FTLD patient neurons with TDP-43**  
44 **pathology. Our work directly links TDP-43 misregulation and NPTX2 accumulation, thereby**  
45 **highlighting a new pathway of neurotoxicity.**

46 TAR DNA-binding protein 43 (TDP-43) accumulates in affected neurons from patients with  
47 neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar  
48 dementia (FTLD)<sup>1,2</sup>. TDP-43 is an essential RNA-binding protein<sup>10-12</sup> that is tightly autoregulated via  
49 binding to its own mRNA<sup>13-16</sup>. At the physiological state, TDP-43 is predominantly nuclear and directly  
50 controls the processing of hundreds of its RNA targets<sup>13,17</sup>. Conversely, TDP-43 forms pathological  
51 aggregates in disease, which are neurotoxic *per se*, featuring a potency that correlates with disease  
52 duration in FTLD patients<sup>18,19</sup>. Moreover, the aggregates trap newly synthesized TDP-43, leading to  
53 nuclear clearance and loss of its normal function<sup>1,20,21</sup>. This effect has detrimental consequences as it  
54 leads to broad splicing misregulation<sup>13,22</sup>, including the inclusion of cryptic exons in specific TDP-43  
55 RNA targets<sup>23</sup>, such as *STMN2*<sup>3,4</sup> and *UNC13A*<sup>5,6</sup>. Both these RNA targets are neuronal and human-  
56 specific, and their levels were recently found to be significantly reduced in brains of patients with TDP-  
57 43 proteinopathies, directly linking the loss of TDP-43 nuclear function to neurodegeneration.

58 The recognition of *STMN2* and *UNC13A* motivated the development of fully human experimental  
59 models for TDP-43 proteinopathies in order to decipher the key targets and downstream pathological  
60 mechanisms of TDP-43 misregulation. Induced pluripotent stem cell (iPSC)-based systems offer this  
61 possibility, and several breakthroughs were made in recent years with this technology, including the  
62 generation and characterization of numerous iPSC lines from ALS and FTLD patients<sup>24</sup>, the recognition  
63 of early neuronal phenotypes in human neurons with ALS-linked mutations<sup>25,26</sup> and disease-linked  
64 transcriptomic signatures<sup>27</sup>. Yet, most studies with iPSC-derived neurons from patients with TDP-43  
65 proteinopathies reported low to no TDP-43 pathologies<sup>28-30</sup>, potentially due to the early maturation state  
66 of human neurons in culture.

67 ***iCoMoNSCs are uniquely homogeneous*** We generated a self-renewing human neural stem cell line  
68 (designated iCoMoNSCs) via manual selection based on their colony morphology<sup>7</sup>, from induced  
69 pluripotent stem cells (iPSCs), which we derived from normal human skin fibroblasts through episomal  
70 reprogramming (Extended Data Fig. 1a). iCoMoNSCs were stable across at least 24 passages retaining  
71 their characteristic radial morphology in cell clusters and karyotype, as well as expression of NSC-  
72 specific markers (Fig. 1a, Extended Data Fig. 1b-f). To determine the homogeneity of our iCoMoNSCs,  
73 we performed single-cell RNA sequencing (scRNA-seq) of two biological replicates at passage 22. Pre-  
74 processing, quality control and filtering yielded >8300 cells per replicate with a median number of ~2000  
75 detected genes and ~4800 unique molecular identifiers (UMIs) (Extended Data Fig. 1g,h), which were  
76 separated in tightly associated clusters (Extended Data Fig. 1i), mostly defined by cell cycle stage  
77 (Extended Data Fig. 1j) and composed of cells from both biological replicates (Extended Data Fig. 1k,l),  
78 demonstrating that our iCoMoNSCs were extremely homogeneous. The classical NSC marker genes  
79 *NES*, *SOX2*, *NR2F1* and *CDH2*, as well as *IRX2* and *SOX1*, were expressed in a subset of cells from  
80 all clusters with similar levels (Extended Data Fig. 1m), suggesting that the majority of cells were true,  
81 self-renewing neural stem cells at different cell cycle stages. We then identified cluster marker genes  
82 (Extended Data Fig. 2a) and interrogated the expression of sets of known cell type marker genes  
83 (Extended Data Fig. 2b). This showed that most of the cells were in G1/S phase (clusters 0, 1 and 5),  
84 followed by ~19% of cells (cluster 2) marked by classical cell cycle-associated genes (G2/M). For a  
85 minority of the cells, markers and specifically expressed genes indicated lower multipotent states with  
86 either gliogenic (~13% of cells, cluster 3) or neurogenic (~8% of cells, cluster 4) nature. Finally, for a  
87 very small percentage of cells (~0.3%, cluster 6) the expression of neuron-specific genes and cluster  
88 markers demonstrated their committed neuroblast nature (Extended Data Fig. 1i,l, Extended Data Fig.  
89 2a-b). Taken together, our data showed that up to 79% of all cells present in our iCoMoNSCs were true,  
90 self-renewing neural stem cells.

91 To corroborate our results, we integrated our data with previously published scRNA-seq datasets from  
92 independent human neural stem cells (NSCs)<sup>8</sup>, which were distributed amongst our iCoMoNSCs in all  
93 clusters (Fig. 1b-d, Extended Data Fig. 2c). This allowed us to refine our cluster annotation (Fig. 1c).  
94 Next, we compared the cluster abundances of all samples individually and found that the iCoMoNSCs  
95 and the iPSC-derived AF22 and Ctrl7 lines had the most similar cell distributions, whereas the SAi2 line  
96 that was derived from human fetal hindbrain primary NSCs showed slightly different cell cycle  
97 distribution and approximately 9-fold less neurogenic cells (Fig. 1c,d). Despite the similarities to the  
98 independent NSC lines, our iCoMoNSCs contained significantly fewer (between 3 and 20 times)  
99 committed neuroblasts, represented in cluster 6 (Fig. 1d), indicating that our iCoMoNSCs consist  
100 primarily of self-renewing neural stem cells and significantly fewer postmitotic, committed neuroblasts  
101 than the independent NSC lines.

102 ***iCoMoNSC neurons form functional networks*** Upon differentiation (Extended Data Fig. 3a), our  
103 iCoMoNSCs consistently generated mixed neuronal and glial multilayer cultures (Fig. 1e,f)<sup>31,32</sup>. After  
104 1.5 months of differentiation, these cultures consisted of approximately 30% of NEUN<sup>+</sup> neurons, a  
105 percentage that stabilised at approx. 35% at later time points (Fig. 1e, Extended Data Fig. 3b,c). In  
106 contrast, the number of Ki67<sup>+</sup> proliferating cells dropped from virtually 100% in iCoMoNSCs (Extended



107 Data Fig. 3d) to ~5% (Extended Data Fig. 3e). To investigate the presence of synaptic markers, we first  
108 immunolabeled 3-month-old cultures for SYP (synaptic vesicles; Fig. 1g) and SNAP-25 (synaptic  
109 vesicle fusion machinery; Fig. 1h) and found a typical punctate pattern. To further assess synapse  
110 formation, we prepared synaptoneurosomes (SNS)<sup>33,34</sup>, a subcellular preparation, enriched in resealed  
111 presynaptic and postsynaptic structures. SNS fractions from 3-month-old human neural cultures were  
112 analyzed by transmission electron microscopy (TEM), which revealed typical synaptic morphology,  
113 consisting of both presynaptic vesicles and postsynaptic densities (Fig. 1i). Immunoblots of total lysates  
114 and SNS fractions, prepared from the human neural cultures, confirmed the enrichment of synaptic  
115 markers (Fig. 1j).

116 To assess the functionality of these synapses, we first performed *in vitro* two-photon calcium imaging  
117 after bolus-loading of the 3-month-old cultures with the membrane-permeable ester form of the calcium  
118 indicator Oregon Green BAPTA-1. Calcium transients of recorded neuronal somata demonstrated that  
119 the cultures indeed displayed sparse spontaneous activity patterns (Extended Data Fig. 3f). To formally  
120 confirm neuronal activity in our cultures, we assessed their electrophysiological properties via whole-  
121 cell patch-clamp measurements. 3.5-month-old patched neurons (Extended Data Fig. 3g) had a  
122 hyperpolarized resting membrane potential ( $-59.7 \pm 4.3$  mV,  $n = 7$ ) and fired single or multiple action  
123 potentials upon depolarizing current injection (10 out of 11 neurons, Extended Data Fig. 3h-i). Voltage-  
124 clamp recordings showed rapidly inactivating inward currents and slowly inactivating outward currents,  
125 typical for Na<sup>+</sup>- and K<sup>+</sup>-currents, respectively (peak Na<sup>+</sup> current density:  $-86.7 \pm 20.5$  pA/pF; peak K<sup>+</sup>  
126 current density:  $149.4 \pm 28.0$  pA/pF;  $n = 10$ ; Extended Data Fig. 3j). Collectively, these analyses  
127 demonstrated that iCoMoNSC-derived neurons contained voltage-dependent channels and were  
128 electrophysiologically active with a hyperpolarized resting membrane potential.

129 To investigate whether iCoMoNSC-derived neurons were interconnected and displayed coordinated  
130 activity, we used high-density microelectrode arrays (Extended Data Fig. 4b; HD-MEAs)<sup>35,36</sup>. Neural  
131 cultures were plated onto the HD-MEAs after approximately 1, 3 and 6 months of differentiation and  
132 were then allowed to reconnect for one month before recording to compare cultures denoted in the rest  
133 of the manuscript as young (1.5-2 months), middle (3-4 months) and old (7-7.5 months) (Extended Data  
134 Fig. 4a). Young cultures exhibited a lower burst activity than middle and old (Fig. 1k). We then analyzed  
135 burst metrics (Fig. 1l, Extended Data Fig. 4c,f,j)<sup>37</sup> and found a significant decrease in burst duration  
136 (BD) between young and middle cultures ( $p < 0.025$ ), as well as a decrease in the inter-burst interval  
137 coefficient of variation (IBI cv) ( $p < 0.025$ ) (Fig. 1l, Extended Data Fig. 4f,h). The increase of IBI cv in  
138 young cultures compared to middle (Fig. 1l, Extended Data Fig. 4h) suggested that early maturation  
139 stages were characterized by irregular bursts. Additionally, for 30% of the young cultures a network  
140 analysis could not be conducted as bursts were undetectable, which is indicative of still-developing  
141 synaptic connections. In contrast, older cultures showed detectable bursts in the majority of MEAs  
142 (>90%). Additionally, the percentage of active electrodes (AE) increased 2.4-fold ( $p < 0.001$ ) from young  
143 to old cultures (Fig. 1l, Extended Data Fig. 4j).

144 We next compared the single-cell and subcellular resolution metrics (Fig. 1m, Extended Data Fig.  
145 4d,e,k-t)<sup>38</sup>. We found a 1.2-fold increase ( $p < 0.025$ ) in inter-spike interval coefficient of variation (ISI cv)<sup>37</sup>  
146 between young and old cultures (Fig. 1m, Extended Data Fig. 4l), indicating more irregular firing rates

147 at later developmental stages. In addition, a 0.7-fold lower mean firing rate (MFR) in young compared  
148 to old cultures demonstrated an increase in spontaneous activity over time (Fig. 1m, Extended Data Fig.  
149 4m). Longer-lasting action potential recovery times were evidenced by the peak-to-trough ratio metric  
150 (PtV), which increased from young to middle ( $p < 0.025$ ) and from young to old cultures ( $p < 0.025$ ) (Fig.  
151 1m, Extended Data Fig. 4p).

152 Next, the subcellular resolution features branch length (BL) and action-potential-propagation velocity  
153 were used to compare the three developmental stages. Significant differences were found in the neuron  
154 BL between young and middle ( $p < 0.025$ ), and between young and old cultures ( $p < 0.001$ ), indicating  
155 different functional maturation stages (Fig. 1m, Extended Data Fig. 4t). Principal component analysis  
156 (PCA) showed a separation of all three maturation stages based on all 15 analyzed parameters  
157 (Extended Data Fig. 4u). Taken together, these functional metrics indicated increased maturation upon  
158 aging of iCoMoNSC-derived neurons in culture.

159 ***iCoMoNSC neurons and glia resemble brain organoids*** To further characterize our iCoMoNSC-  
160 derived cultures, we performed scRNA-seq of young, middle and old (Extended Data Fig. 4a), in  
161 biological duplicates. After processing we retained 3500-8500 cells per sample with 2800-4800  
162 detected genes and 6000-16000 UMIs. Cells were distributed across 19 clusters, which self-organized  
163 with groups of clusters on the top left quadrant representing neurons, whereas the bottom left quadrant  
164 contained clusters of astrocytes and other glial cells (Fig. 2a). Neuronal and astrocytic clusters showed  
165 increasing maturation over time in differentiation, which was evident upon visualization of all  
166 experimental (Fig. 2b) or cell cycle (Fig. 2c) stages. Cluster abundance analysis (Extended Data Fig.  
167 5a-c) and the visualization of individual, cell type-specific markers revealed that our cultures matured  
168 over time: NSC-specific markers, such as *SOX2*, *NQO1*, had high expression in iCoMoNSCs (Extended  
169 Data Fig. 5d). Astrocyte maturation was highlighted by marker *GFAP* (astrocytes), which was expressed  
170 in middle and old neural cultures, *PTPRZ1* (oligodendrocyte progenitor cells, OPCs) was detected in  
171 all samples and *DCN* (pericytes) in young and middle (Extended Data Fig. 5e). Neuronal maturation is  
172 highlighted by markers *SYP* (neuronal) and *SLC32A1* (GABAergic), which were detected in young,  
173 middle and old cultures, whereas *SLC17A6* (glutamatergic) was mostly detected in old cultures  
174 (Extended Data Fig. 5f). Clusters were manually annotated based on analysis of cluster markers  
175 (Extended Data Fig. 5b,g), known markers (Extended Data Fig. 6), CoDex (Cortical Development  
176 Expression) viewer<sup>39</sup>, PanglaoDB database<sup>40</sup> and UCSC Cell Browser. In line with the increasing  
177 maturation over time in differentiation, young neurons were annotated as young inhibitory (cluster 2),  
178 excitatory (cluster 3) and dopaminergic neurons (cluster 13), as well as apoptotic neurons (cluster 15),  
179 while at the middle stage, their annotations converted into maturing (cluster 6) or excitatory  
180 glutamatergic (cluster 7) and in the old cultures, neuronal subtypes were clearly defined as GABAergic  
181 (cluster 5) and glutamatergic (cluster 7). Similarly, gliogenic clusters were annotated as glial precursors  
182 (cluster 18), radial glia/early astrocytes (cluster 10), radial glia (cluster 12) and glia (cluster 11) in young  
183 cultures, young astrocytes (cluster 9) and glia (cluster 11) at the middle stage, and mature astrocytes  
184 (cluster 4), astrocytes (cluster 16), and OPCs (cluster 8) in the old cultures. A small percentage of glial  
185 cells (between 0.16 – 0.7%) was identified as pericytes (cluster 17), regardless of the maturation stage  
186 (Fig. 2a-c, Extended Data Fig. 4a, Extended Data Fig. 5a-f).

187 To determine the level of maturation of the emerging cell types within our iCoMoNSC-derived cultures,  
188 we integrated our data with two brain organoid scRNA-seq datasets<sup>9</sup> (Fig. 2e, Extended Data Fig. 5h)  
189 and identified 22 clusters (Fig. 2d). Cells from the two datasets were mixed in most clusters, but overall  
190 the datasets occupied different parts of the two-dimensional space indicating subtle transcriptomic  
191 differences within cell types, potentially depicting differences in their developmental stages. With the  
192 exception of four clusters consisting primarily of cells from our neural samples or organoids, all other  
193 clusters contained cells originating from both systems. The clusters specific to the iCoMoNSC-derived  
194 cultures were composed of iCoMoNSCs (1 and 3), OPCs (14) and maturing neurons (18). The organoid-  
195 specific clusters 2 and 20 contained iPSCs, or neuroepithelial-like/retina progenitors (6), as well as  
196 cycling ventral and dorsal progenitors (11) (Fig. 2d,f). Overlapping neuronal populations included  
197 midbrain/hindbrain cells (cluster 7), intermediate progenitors and early cortical neurons/ventral  
198 progenitors (cluster 16), ventral progenitors and cortical neurons (clusters 0, 4, 8, 10 and 13).  
199 Overlapping glial subtypes were labeled as radial glia (clusters 5, 9, 12 and 15) and early radial  
200 glia/neuroepithelial-/neuroectodermal-like cells (cluster 17), whereas cluster 21 cells were annotated  
201 as choroid-plexus/mesenchymal-like cells. Altogether, our neural model intersected with human cortical  
202 brain organoids within clusters representing intermediate and ventral progenitors, early and late cortical  
203 neurons and radial glial cells. Differences were driven by the source cells, i.e., NSCs in our model and  
204 iPSCs in the brain organoid models. These results demonstrate that our neural model contained  
205 neuronal and glial cells of a transcriptional maturation level similar to that in brain organoids.  
206 Notwithstanding the differences in the source cells, these data indicate that, even in the absence of a  
207 3D organ-like cell organization, neuronal and glial maturation in our iCoMoSCS-derived neural model  
208 is comparable to that in cortical organoids.

209 ***Induced TDP-43 pathology in a minority of neurons*** TDP-43 pathology characterizes affected brain  
210 regions of patients with TDP-43 proteinopathies, yet it was recently reported that only <2% of cortical  
211 cells show pathological changes in TDP-43 in FTL<sup>41</sup>. To simulate this, we transduced young  
212 iCoMoNSC-derived neural networks with human TDP-43-HA-expressing lentiviral vector<sup>42</sup> (Extended  
213 Data Fig. 7), with a titer designed to reach ~2% of transgenic cells in the network at 1 week of induction  
214 (Fig. 3a-b). Yet the percentage of transgenic neurons in the network gradually decreased (Fig. 3b),  
215 indicating that TDP-43-HA overexpression was toxic to human neurons. We then analyzed TDP-43-HA  
216 biochemically, using SarkoSpin, a method for the specific enrichment of pathological TDP-43 species,  
217 recently developed in our lab<sup>18</sup>. Interestingly, we detected progressive accumulation of TDP-43-HA in  
218 the SarkoSpin pellet, accompanied by fragmentation and appearance of high molecular weight bands  
219 and smear indicative of aggregation (Fig. 3c, Extended Data Fig. 8a), reminiscent of postmortem TDP-  
220 43 proteinopathy patient brains<sup>18</sup>. Specifically, in total cell lysates, we detected progressive  
221 fragmentation of full-length TDP-43-HA, to its 35 kDa and 25 kDa C-terminal fragments over time  
222 (Extended Data Fig. 8b). Both TDP-43 fragments accumulated in the SarkoSpin pellet, while full-length  
223 TDP-43-HA was present in the soluble fraction (SarkoSpin supernatant) at 2 weeks post induction, but  
224 was progressively redistributed to the SarkoSpin pellet in later time points (Fig. 3c). Collectively, our  
225 data demonstrate that overexpression of wild-type TDP-43-HA in human neurons resulted in  
226 progressive aggregation and fragmentation and the loss of TDP-43-HA-overexpressing cells.

227 Surprisingly, while we found no evidence that TDP-43-HA overexpressing neurons develop TDP-  
228 43<sup>p403/404</sup>-positive inclusions (Fig. 3a), the latter emerged and amplified over time within non-transgenic  
229 neurons present in the same neural network (Extended Data Fig. 8c,d), showing that pathological TDP-  
230 43 changes extend beyond the initially affected cells. At early time points, TDP-43<sup>p403/404</sup> signal  
231 appeared in the form of small, dot-like pre-inclusions that were largely confined within the soma  
232 (Extended Data Fig. 8e), whereas at later time points, these inclusions were larger and additionally  
233 present within neuronal processes (Extended Data Fig. 8f). This indicates progressive maturation of  
234 TDP-43 inclusions into aggregates in aged human neural networks.

235 ***Distinct transcriptional profile in neurons with TDP-43 pathology*** To understand the effect of  
236 sparse TDP-43-HA overexpression and related pathology in human neural networks, we induced its  
237 expression for 2 or 4 weeks, before harvesting the cells for scRNA-seq. Samples were analyzed at our  
238 middle stage (~3 months), consisting of both inhibitory and excitatory neurons (Fig. 2a,b) interconnected  
239 into functional networks (Fig. 1k). Between 6000-10000 cells per sample were retained after  
240 preprocessing and filtering with a median number of detected genes of 4300-5100 and median number  
241 of UMIs between 15000 and 20000. We identified 17 clusters (Fig. 3d) with a very similar cell type  
242 distribution (apoptotic, glial/astrocytic, maturing inhibitory and excitatory neurons) to our non-  
243 transduced middle-aged samples (Fig. 2a,b). We quantified the expression of the TDP-43-HA transcript  
244 in all samples using the scRNA-seq analysis pipeline Alevin<sup>43</sup> and identified marker genes for each  
245 cluster. This analysis revealed a single cluster (number 12) that was almost exclusively composed of  
246 cells overexpressing TDP-43-HA (1.3% of cells in cluster 12 are from the OFF sample, 43.3% from ON  
247 2W, 39.3% from ON 4W r1 and 16.1% from ON 4W r2) (Fig. 3e, Extended Data Fig. 8g). Cells in cluster  
248 12 had an increase in total TDP-43 expression (log<sub>2</sub>FC 2.96), compared to all other neuronal clusters,  
249 and we were able to detect the transduced construct in 84.4% of cluster 12 cells, but only 2.6% of all  
250 other neuronal cells. Overexpression of TDP-43-HA over a period of 2 or 4 weeks altered the expression  
251 of several genes that were identified as cluster 12 markers and were either upregulated (Fig. 3f) or  
252 downregulated (Fig. 3g) when compared to all other neuronal clusters. Among the top 10  
253 downregulated genes was *STMN2*<sup>3,4</sup> a previously known human-specific RNA target of TDP-43.  
254 *UNC13A*<sup>5,6</sup>, another recently discovered TDP-43 target was not among the top 10 markers, but was  
255 also significantly downregulated in TDP-43-overexpressing cells (Fig. 3g), validating the relevance of  
256 our model for human disease. Excitingly, several marker genes that have not yet been associated with  
257 or directly connected to ALS or FTLD neuropathology, but are linked to neuronal survival and/or death,  
258 have been found upregulated in cluster 12. Among those, Neuronal Pentraxin 2 (*NPTX2*), a neuron-  
259 specific gene, also known as neuronal activity-regulated protein (*NARP*), is secreted and involved in  
260 long-term neuronal plasticity. To understand if any of these RNAs were directly bound by TDP-43, we  
261 analyzed previously published iCLIP datasets from control and FTLD patient brain samples<sup>17</sup>.  
262 Interestingly, with the exception of *C4orf48* and *GADD45A*, all other cluster 12-misregulated RNAs are  
263 indeed TDP-43 binding targets and the binding is altered in FTLD compared to control patient brains  
264 (Fig. 4a).

265 ***NPTX2 is misaccumulated in FTLD and ALS patients*** We then investigated if any of our newly  
266 identified cluster 12 marker genes were altered in ALS and FTLD patient brain samples. To that end,

267 we re-analysed the recently reported RNA-seq dataset comparing the transcriptomic profiles of single  
268 nuclei from individual FTLD-ALS neurons with (TDP-43-negative) or without (TDP-43-positive) nuclear  
269 clearance<sup>44</sup>, which is a consequence of pathological TDP-43 accumulation and sequestration of  
270 functional protein. Out of the nine cluster 12-upregulated RNAs, six were significantly altered, and four  
271 were significantly upregulated in TDP-43-negative nuclei. The strongest upregulation was in *NPTX2*  
272 mRNA, which was increased two-fold in TDP-43-negative FTLD-ALS patient neurons compared to  
273 controls (Fig. 4b). Moreover, iCLIP data<sup>17</sup> analysis showed that TDP-43 directly bound *NPTX2* mRNA  
274 (Fig. 4a), primarily at its 3'UTR within a highly GU-rich region (Fig. 4c), the sequence specifically  
275 identified by the RNA recognition motifs of TDP-43<sup>13,45,46</sup>. Importantly, this TDP-43/*NPTX2* interaction  
276 was reduced in FTLD patient brains, as shown by the loss of iCLIP crosslinks, marking positions of  
277 direct protein-RNA interactions. Indeed, while in control brains, five TDP-43 crosslinks were identified  
278 on *NPTX2* 3'UTR, none were detected in FTLD brains (Fig. 4c). Collectively these data suggest that  
279 TDP-43 directly regulates *NPTX2* mRNA levels via binding on its 3'UTR, an event that is disturbed in  
280 human neurons with TDP-43 pathology.

281 To understand if *NPTX2* protein is specifically altered in neurons with TDP-43 pathology, we then used  
282 immunofluorescence in our human neural networks and found that this normally secreted and  
283 synaptically-localized protein<sup>47,48</sup> is aberrantly accumulated within neuronal somata and processes of  
284 TDP-43-HA-expressing neurons (Fig. 4d, Extended Data Fig. 9a). Validating our cellular model and  
285 analysis, affected neurons also depicted increase in *MEF2A* and decrease in *STMN2* levels (Extended  
286 Data Fig. 9b-c), as we predicted (Fig. 3f-g). We then wondered if *NPTX2* accumulation also occurred  
287 in patient brains (Supplementary Table 1). To this end, we used immunofluorescence for *NPTX2*, TDP-  
288 43<sup>p403/404</sup> and *MAP2* (neuronal marker) and found that while *NPTX2* levels were very low in adult human  
289 neurons of patients, it accumulated into dense inclusions in both the somata and processes of neurons  
290 with TDP-43 pathology in the granule cell layer of the dentate gyrus of the hippocampus of both FTLD-  
291 TDP Type A and Type C<sup>49,50</sup> patients as well as in the frontal cortex of FTLD-A patients and in the  
292 primary motor cortex of an ALS patient (Fig. 4e,f, Extended Data Fig. 10). Interestingly, *NPTX2* did not  
293 appear to co-aggregate with TDP-43, suggesting that its loss of function, rather than a gain, is  
294 responsible for this novel *NPTX2* pathology. Overall, our work identified aberrant *NPTX2* accumulation  
295 as a direct consequence of TDP-43 misregulation in disease, suggesting a novel neurotoxic pathway  
296 for TDP-43 proteinopathies.

297 **Discussion** Long-term self-renewing NSC lines derived from ES or iPSCs provide an excellent source  
298 of expandable cells<sup>7,8,51</sup> for creating models of neurogenesis and CNS-related diseases. We generated  
299 iCoMoNSCs and showed that they represent a uniquely homogenous population of self-renewing NSCs  
300 with the potential to give rise to mature and diverse neuronal and glial subtypes. Notably, only a very  
301 small percentage (~0.3%) of iCoMoNSCs was identified as committed neuroblasts. Upon terminal  
302 differentiation, iCoMoNSCs consistently generated mixed neuronal and glial cultures with  
303 electrophysiologically active neurons that formed interconnected networks with spontaneous  
304 coordinated activity. Importantly, scRNA-seq confirmed the progressive maturation of neuronal and glial  
305 subtypes comparable to that in human brain organoids<sup>9</sup>.



306 We then explored how overexpression of TDP-43 in a minority of neurons would affect these neural  
307 networks over time, aiming to simulate the situation in the diseased brain that reportedly contains only  
308 ~2% of cells with TDP-43 pathology<sup>44</sup>. After 4 weeks of TDP-43-HA overexpression, we observed a  
309 drop in soluble TDP-43-HA levels paralleled by progressive insoluble TDP-43 aggregation and C-  
310 terminal fragmentation<sup>52</sup>, as well as progressive loss of TDP-43-HA-positive cells. scRNA-seq revealed  
311 that cells with TDP-43 overexpression and pathology were characterized by a distinct transcriptional  
312 profile, with downregulation of *STMN2*<sup>3,4</sup> and *UNC13A*<sup>5,6</sup>, indicating loss of TDP-43 function.  
313 Importantly, this approach uncovered a novel set of up- and downregulated genes. Remarkably, with  
314 the exception of two, all were direct RNA targets of TDP-43 and their binding was previously found to  
315 be altered in the human FTLD brain<sup>17</sup>.

316 Contrasting our novel RNA targets with RNA-seq data from TDP-43-negative cortical neurons of  
317 ALS/FTLD patients<sup>44</sup> highlighted *NPTX2* as the most significantly upregulated RNA in human patients.  
318 TDP-43 binds *NPTX2* mRNA at its 3'UTR, an event that is disturbed in FTLD<sup>17</sup>, suggesting a repressive  
319 role of this interaction. Importantly, the binding mode and increased *NPTX2* RNA levels indicate a  
320 regulation mechanism distinct from the previously described cryptic exon suppression<sup>23</sup> recently shown  
321 for *STMN2*<sup>3,4</sup> and *UNC13A*<sup>5,6</sup>. Notably, this misregulation led to aberrant accumulation of NPTX2  
322 protein, which we consistently observed not only in our cellular model, but also in the brains of ALS and  
323 FTLD patients. Indeed, NPTX2 accumulation in patients reliably marked cells with TDP-43 pathology,  
324 without co-aggregation of the two proteins. Interestingly, NPTX2 was recently shown to be decreased  
325 in the CSF of symptomatic genetic FTLD patients, in a manner correlating with clinical severity<sup>53</sup>.  
326 Collectively, these data indicate that while global NPTX2 levels decrease with age<sup>54</sup> and in dementia<sup>53-</sup>  
327 <sup>56</sup>, neurons with TDP-43 pathology have increased levels of NPTX2, likely due to loss of TDP-43 binding  
328 on *NPTX2* mRNA.

329 What could the consequences of this aberrant increase of NPTX2 levels in adult neurons be? NPTX2  
330 is a neuronal pentraxin and an immediate early gene regulated by neuronal activity<sup>48,57,58</sup>. It is a Ca<sup>2+</sup>-  
331 dependent lectin<sup>48</sup> that is secreted from presynaptic terminals<sup>47,48</sup>, where it regulates synaptogenesis  
332 and glutamate signaling via AMPA receptor (AMPA) clustering<sup>57,59,60</sup>. NPTX2 binding to neuronal  
333 pentraxin receptor (NPTXR)<sup>59</sup> mediates synaptic maintenance, plasticity and postsynaptic  
334 specialization<sup>57,61</sup> in both excitatory and inhibitory synapses<sup>61</sup>. NPTX2 enhances glutamate receptor 1  
335 function, and its upregulation causes synaptic remodeling through recruiting Ca<sup>2+</sup>-permeable AMPARs  
336 on the neuronal membrane<sup>62</sup>. Glutamate excitotoxicity has long been proposed to be one of the major  
337 pathological pathways selectively killing vulnerable neurons in ALS<sup>63</sup> and other neurodegenerative  
338 diseases<sup>64</sup>. Since excitotoxicity is primarily propagated via Ca<sup>2+</sup> influx through Ca<sup>2+</sup>-permeable  
339 receptors<sup>65</sup>, it is attractive to speculate that NPTX2 malfunction in neurons with TDP-43 pathology may  
340 increase their vulnerability to glutamate excitotoxicity in ALS and FTLD. We propose that NPTX2 may  
341 be a key neuronal target of TDP-43 and that its aberrant increase in affected neurons may trigger a  
342 pathological cascade leading to neuronal loss in ALS/FTLD. In this context, NPTX2 may represent an  
343 important therapeutic target for TDP-43 proteinopathies, along with the recently discovered *STMN2* and  
344 *UNC13A*.

345

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487

## 488 **Figure Legends**

489

490 **Figure 1. iCoMoNSC neurons form functional networks.** **a**, Immunofluorescence of iCoMoNSCs for  
491 NSC markers. **b**, UMAP of iCoMoNSCs integrated with 768 cells from three independent NSC lines  
492 and **(c)** corresponding annotated clusters. **d**, Percentage of cell distribution per sample across clusters.  
493 **e**, Immunofluorescence of human neural cultures stained with neuronal or **(f)** astrocytic markers.  
494 Synaptic marker immunofluorescence at 4 months for **(g)** SYP and **(h)** SNAP-25. **i**, Electron microscopy  
495 of SNS showing pre- and postsynaptic compartments. **j**, Western blots of SNS fractions. **k**, Population  
496 spike time histograms (top) and raster plots (bottom) of neuronal networks. **l**, Heatmaps of percentage  
497 changes in network or **(m)** single-cell and subcellular metrics features. **n**, Spike-sorted axonal branches.  
498 Scale bars, **(a,e,f)** 50  $\mu\text{m}$ , **(g,h)** 10  $\mu\text{m}$ , **(i)** 250 nm.

499

500 **Figure 2. iCoMoNSC neurons and glia partially resemble brain organoids.** **a**, UMAP of young,  
501 middle and old iCoMoNSC-derived neural cultures. Colors highlight manually annotated clusters, **(b)**  
502 experimental or **(c)** cell cycle stage. **d**, All samples from **(a)** integrated with organoid datasets.  
503 Annotation of clusters consisting of both iCoMoNSC and organoid cells are indicated. **e**, Same UMAP  
504 as **(d)** highlighting the experimental stage origin. **f**, Cell distribution across all clusters. Stars indicate  
505 clusters with cell composition of at least 10% per origin.

506 **Figure 3. Distinct transcriptional profile in neurons with induced TDP-43 pathology.** **a**,  
507 Immunofluorescence of TDP-43-HA in iCoMoNSC neurons. Scale bar, 25  $\mu$ m. **b**, Quantification of TDP-  
508 43-HA-positive neurons over time. Each dot represents a biological replicate. **c**, Western blots of  
509 SarkoSpin supernatants (top) and pellets (bottom). Line separates independent experiments. **d**, UMAP  
510 of single-cell RNA-seq TDP-43 overexpression experiment. Colors indicate clusters, dashed lines  
511 highlight different cell types. Red dashed line depicts cluster 12, containing cells expressing TDP-43-  
512 HA. **e**, UMAP highlighting TDP-43-HA expression confined in cluster 12. **f**, Dot plot with the scaled  
513 average expression of the top cluster 12-upregulated and **(g)** -downregulated, including the significantly  
514 downregulated *UNC13A*, marker genes when compared to all other neuronal clusters.

515

516 **Figure 4. NPTX2 is misaccumulated in FTLD and ALS patients.** **a**, Plot shows the number of iCLIP  
517 crosslinks<sup>17</sup> in both FTLD patient and control brain samples in mRNA of the cluster 12 up- and -  
518 downregulated genes. **b**, Log<sub>2</sub> fold change in RNA expression of the top 10 cluster 12-upregulated  
519 markers between TDP-43-negative and TDP-43-positive neuronal nuclei from FTLD patients<sup>44</sup>. **c**,  
520 Location of iCLIP crosslink sites in *NPTX2* in both control (green) and FTLD patient (red) human brain<sup>17</sup>.  
521 Zoom-in: iCLIP crosslinks (green) and UG- or GU-repeats (orange). **d**, Only TDP-43-HA-  
522 overexpressing neurons showed detectable *NPTX2* levels in immunofluorescence. **e**,  
523 Immunofluorescence of human brain sections: FTLD-A hippocampus and **(f)** ALS primary motor cortex  
524 demonstrating inclusion-like *NPTX2* signal only in MAP2-positive neurons containing TDP-43<sup>p403/404</sup>-  
525 positive aggregates in the cytoplasm. Scale bars, **(d)** 20  $\mu$ m, **(e)** 10  $\mu$ m, **(f)** 30  $\mu$ m.

526

## 527 **Methods**

528

### 529 **iPSC-derived, colony morphology neural stem cells (iCoMoNSCs)**

530 Similarly to our previous study on human ESCs<sup>7</sup>, iPSC colonies were manually picked and partially  
531 dissociated into smaller clumps and transferred into non-adhesive culture dishes and induced to form  
532 embryoid bodies (EBs) in the presence of EB medium (iPS media without bFGF). After 5-7 days, EBs  
533 were transferred onto Poly-L-Ornithine (Sigma P4957-50ML; 20  $\mu$ g/ml in sterile water Gibco 10977035,  
534 1 hour at 37°C followed by 3 washes with PBS Gibco 10010015) / Laminin (Gibco 23017-015; 5  $\mu$ g/ml  
535 in PBS, 1 hour at 37°C) coated dishes (P/L) and left to adhere in NSC media (DMEM/F12 Gibco  
536 21331046; 0.5X B27- supplement Gibco 12587-010, 0.5X N2 supplement Gibco 17502-048; 1X  
537 GlutaMAX Gibco 35050-061; 25 ng/ml bFGF Gibco PHG0261). Formation of neural rosettes was  
538 observed within 4-10 days. Neural rosettes were manually dissected and picked under EVOS™ XL  
539 Core Imaging System (LifeTech AMEX1000), and after dissociation re-plated onto fresh P/L-coated  
540 dishes in NSC media. After 2-5 days, new and smaller rosettes appeared (R1 rosettes) with the  
541 presence of heterogeneous contaminating cells. The R1 rosettes were then manually dissected, picked  
542 and dissociated into smaller clumps and re-plated onto fresh P/L dishes. After further 2-5 days, new  
543 rosettes (R2) with minimal contaminating cells appeared. R2 rosettes were then routinely monitored to  
544 identify small groups of radially organized cells that were present outside of the neural rosettes and  
545 represented an independent “clone-like population”, the iCoMoNSCs. These small patches of

546 iCoMoNSCs were then manually picked and transferred onto freshly P/L-coated 24 well plates. Clones  
547 that showed clear radial and consistent morphology, good attachment, survival and proliferation upon  
548 transfer were enzymatically detached using 0.05% Trypsin (Gibco 15400-054 diluted in PBS) and 1X  
549 Defined Trypsin Inhibitor (Gibco R-007-100) and expanded for numerous passages and banked in NSC  
550 freezing media (NSC media + 10% DMSO). Upon further expansion to P9-13, iCoMoNSCs lines were  
551 prepared for karyotype check at Cell Guidance Systems (according to CellGS fixed sample protocol).  
552 iCoMoNSCs clone 10/80 was used in the study. iCoMoNSCs are available upon request.

553

#### 554 **Differentiation of iCoMoNSCs into human neural networks**

555 iCoMoNSCs were plated in NSC media at 75 000 cells/cm<sup>2</sup> onto Matrigel-coated (Corning #354234;  
556 ~0.15 mg/ml diluted in cold DMEM/F12 Gibco #11330032 and incubated at least 1 hour at 37°C) 6 well  
557 plates and left to recover and proliferate to reach ~95% confluency. At this point, NSC media was  
558 switched to “D3” differentiation media (DMEM/F12 Gibco #11330032; 0.5X B27+ supplement Gibco  
559 #17504-044, 1X N2 supplement Gibco #17502-048; 1X GlutaMAX Gibco #35050-061; 1X  
560 Penicillin/Streptomycin Sigma #P4333-100ML) supplemented with 5 µM Forskolin (Cayman #AG-CN2-  
561 0089-M050), 1 µM synthetic retinoid Ec23 (Amsbio #AMS.SRP002-2), 500 nM Smoothed agonist  
562 SAG (Millipore #5666600) for the first 5 days. On the days 6-10, Ec23 was increased to 2 µM. On days  
563 11-25, Ec23 was decreased to 10ng/ml, SAG to 50nM and BDNF (PeproTech # 450-02), GDNF  
564 (PeproTech # 450-10) and CNTF (Alomone labs #C-240) were added at 20 ng/ml. At day 26 and  
565 onwards, media was switched to maturation media (1:1 DMEM/F12:Neurobasal (Gibco #21103049)  
566 mix; 1X B27+ supplement, 1X N2 supplement; 1X GlutaMAX, 5 µM Forskolin, BDNF, GDNF, CNTF,  
567 NT-3 (PeproTech #450-03) and IGF-1 (Stem Cell #78022) all at 20 ng/ml and 10 µM cAMP. Media was  
568 changed daily and almost completely for the first 10 days, whereas from this point on only 2/3 of media  
569 was changed 3 times a week.

570

#### 571 **Cloning of the AutoTetON cassette, lentiviral vector preparation and transduction**

572 Using NEBuilder HiFi DNA Assembly Cloning Kit (NEB #E5520), human wild-type, full length TDP-43  
573 with a C-terminal HA tag from a published pcDNA5 plasmid containing the human TDP-43 cDNA  
574 sequence<sup>66</sup> was inserted into our autoregulatory, all-in-one TetON cassette (AutoTetON; build based  
575 on Markusic et. al 2005<sup>42</sup>), which was previously inserted into a pLVX lentiviral transfer vector (Clontech  
576 # 632164), while deleting CMV-PGK-Puro, generating Auto-TDP-43-HA lentiviral transfer vector.  
577 AutoTetON cassette was build using both NEBuilder and Q5 site directed mutagenesis (NEB #E0554S)  
578 kits and consists of the Tet-responsive promoter  $P_{\text{tight}}$ , consisting of seven tet operator sequences  
579 followed by the minimal CMV promoter (sequence source pCW57.1; Addgene #41393), driving the  
580 inducible expression of the downstream TDP-43-HA, followed by downstream IRES2 sequence  
581 (sequence source Addgene #60857), which is immediately followed by T7 tag fused to SV40 NLS,  
582 which was fused to rtTA-Advanced (sequence source Addgene #41393), which made the rtTA  
583 predominantly nuclear, making it readily available for the system, while the T7-tag made rtTA a useful,  
584 independent marker of the transgenic cells. See Extended Data Fig. 7 for more details.



585 Auto-TDP-43-HA was then packaged into lentivirus (LV) via co-transfection with CMV-Gag-Pol (Harvard  
586 #dR8.91) and pVSV-G (Clontech, part of #631530) plasmids into production HEK293T cells adapted to  
587 grow in serum-free conditions (OHN media; based on Opti-MEM (ThermoFisher #11058-021),  
588 supplemented with 0.5% B27- (ThermoFisher #12587-010), 0.5% N2 (ThermoFisher #175020-01), 1%  
589 GlutaMAX (ThermoFisher #35050038) and bFGF (25ng/ml; ThermoFisher #PHG0261)), which reduces  
590 the expression of the GOI from the transfer vector (i.e. it eliminates traces of tetracyclines in the FBS)  
591 as well as eliminates serum-carry over into the LV supernatant. Medium was changed the following  
592 morning and supernatants were then collected 48 hours post transfection (36 hours post media  
593 change), centrifuged at (500g, 10 min, 4°C), filtered through Whatman 0.45µm CA filter (GE  
594 #10462100) and concentrated using Lenti-X™ Concentrator (Takara #631232) according to the  
595 producer instructions (overnight incubation). The resulting lentiviral pellets were then resuspended in  
596 complete neuronal maturation media to achieve 10x concentrated LV preparations, which were titrated  
597 using Lenti-X™ GoStix™ Plus (Takara #631280). 10x concentrate of Auto-TDP-43-HA LV was then  
598 used at 1600ng (of lentiviral p24 protein as per GoStix Value (GV)) per well of a 6 well plate of  
599 differentiated human neural cultures (~2 months old) along with 3µg/ml of polybrene (Sigma-Aldrich  
600 #TR-1003-G), pipetting the LV concentrate directly onto the culture (drop-wise). Complete neuronal  
601 maturation media was then added to reach 750µl total. Medium was exchanged completely the  
602 following day. TDP-43-HA expression was induced by 1µg/ml of Doxycycline (DOX; Clontech #631311)  
603 when needed.

604

#### 605 **Patient post-mortem brain immunofluorescence**

606 Formalin-fixed, paraffin-embedded hippocampal, frontal or primary motor cortex patient (ALS, FTL-  
607 TDP Type A, FTL- TDP Type C) sections (see **Supplementary table 1**) were used. All FTL- tissue  
608 samples were donated to Queen Square Brain Bank for Neurological Disorders at UCL Queen Square  
609 Institute of Neurology with full, informed consent. Anonymized autopsy ALS sample was collected by  
610 the Institute of Neuropathology at UZH. According to Swiss law, anonymized autopsy tissues do not fall  
611 within the scope of the Human Research Act and may be used in research. Sections were  
612 deparaffinized in three Xylene rounds (5 minutes each) and rehydrated in decreasing EtOH washes (2x  
613 100% for 10 minutes; 2x in 95% for 5 minutes; 80, 70 and 50% for 5 minutes each) and finally  
614 submerged in MilliQ water for 10 minutes. Antigen retrieval was then performed by microwave heat  
615 treatment in sodium citrate buffer (0.01M, pH 6.0). Sections were then cooled down on ice for 10  
616 minutes and once quickly washed in PBS, followed by 3x PBS washes for 5 minutes at RT before  
617 blocking with blocking buffer (BB; 5% normal donkey serum Sigma-Aldrich #S30-M, 3% BSA (Sigma  
618 #A4503) and 0.25% Triton-X100 (Sigma #T9284) in PBS for 30 minutes at RT. Primary antibodies  
619 (Supplementary Table 2) were then diluted in BB and 300 µl of the antibody mix was evenly put per  
620 slide/section and covered by parafilm. Slides were put into a wet and dark incubation chamber and  
621 incubated overnight at RT. Slides were rinsed once in PBS and then washed 3x 5 minutes in PBS on a  
622 shaker. Secondary antibodies (Supplementary Table 2) diluted in BB, centrifuged for 30 minutes at  
623 15000 x g at RT and 500 µl of the antibody mix was evenly put per slide/section and put into the  
624 incubation chamber to incubate for 2.5 hours at RT. Slides were then rinsed once in PBS, washed 1x

625 5 minutes in PBS on a shaker and to stain the nuclei (DNA), 500  $\mu$ l of DAPI solution (Thermo Scientific  
626 #62248; diluted to 1 $\mu$ g/ml in PBS) was evenly added onto the slide/section and incubated for 10 minutes  
627 at RT in the incubation chamber. Slides were rinsed once in PBS, washed 2x 5 minutes in PBS on a  
628 shaker, followed by Sudan Black (0.2% in 70% EtOH; Sigma #199664) autofluorescence quench for 10  
629 minutes at RT on shaker. Slides were rinsed 6 times in PBS and left washing in PBS until mounted and  
630 coverslipped in ProLong Diamond Antifade Mountant (ThermoFisher #P36961) and then left to dry in  
631 the chemical cabinet overnight at RT in the dark. Mounted sections were stored at 4°C in the dark.  
632 Stained patient brain sections were imaged using Leica SP8 Falcon inverted confocal for high power,  
633 high resolution microscopy (63X oil objective; 1.7 or 3x zoom; 2096x2096 pixels at 0.059 $\mu$ m/pixel or  
634 1848x1848 pixels at 0.033 $\mu$ m/pixel, respectively, approx. 20 z-steps per stack at 0.3 $\mu$ m). White laser  
635 and HyD detector settings were kept the same for each staining combination and all imaged conditions.  
636 Huygens professional (Scientific Volume Imaging, Hilversum, Netherlands) was used to deconvolute  
637 the stacks and the deconvoluted images were further post-processed in Fiji<sup>67</sup> to produce a 3D projection  
638 for data visualization (1st image of the 3D projection is shown in figures). Post-processing settings were  
639 kept the same for all images of all sections from all patients (except for DAPI and MAP2 channels for  
640 certain sections when higher brightness settings were used to reach intensity that allowed proper  
641 visualization).

642

### 643 **High-Density Microelectrode Arrays**

644 CMOS-based HD-MEAs<sup>35</sup> were used to record the extracellular action potentials of iCoMoNSC-derived  
645 human neural networks. The HD-MEA featured 26,400 electrodes, organized in a 120  $\times$  220 grid within  
646 a total sensing area of 3.85  $\times$  2.10 mm<sup>2</sup>. The electrode area was 9.3  $\times$  5.45  $\mu$ m<sup>2</sup>, and the center-to-  
647 center electrode distance (pitch) was 17.5  $\mu$ m, which allows for recording of cell electrical activity at  
648 subcellular resolution. Up to 1,024 electrodes could be simultaneously recorded from in user-selected  
649 configurations. The HD-MEA featured noise values of 2.4  $\mu$ V<sub>rms</sub> in the action-potential band of 0.3 - 10  
650 kHz and had a programmable gain of up to 78 dB. The sampling frequency was 20 kHz.

#### 651 *HD-MEA Recordings*

652 The recording setup was placed inside a 5% CO<sub>2</sub> cell-culture incubator at 37°C. Recordings were  
653 performed using the “Activity Scan Assay” and “Network Assay” modules, featured in the MaxLab Live  
654 software (MaxWell Biosystems AG, Zurich, Switzerland), as previously described<sup>37</sup>. The spontaneous  
655 neuronal activity across the whole HD-MEA was recorded using 6,600 electrodes at a pitch of 35  $\mu$ m in  
656 7 electrode configurations for 120 seconds. The most “active” 1,024 electrodes were then used to record  
657 network electrical activity for 300 seconds. Active electrodes were identified based on their firing rate,  
658 and, among those, the 1,024 electrodes featuring the highest firing rates were selected.

#### 659 *HD-MEA Metrics*

660 We used metrics similar to those described in Ronchi et al., 2021<sup>37</sup> to characterize and compare the  
661 neuronal cultures; we used network, single-cell and subcellular-resolution metrics. As network metrics  
662 (Extended Data Fig.6a) we used the burst duration (BD), inter-burst interval (IBI), inter-burst interval  
663 coefficient of variation (IBIcv)<sup>37</sup>. As single-cell metrics (Extended Data Fig.6b) we used the mean firing  
664 rate (MFR), mean spike amplitude (MSA), and the inter-spike interval coefficient of variation (ISICv)<sup>37</sup>.



665 Additionally, we included the following extracellular waveform metrics (Extended Data Fig.6c), extracted  
666 from SpikeInterface<sup>38</sup>, an open-source Python-based framework to enclose all the spike sorting steps:

- 667 1. Half width half maximum (HWHM), half width of trough of AP wave at half amplitude.
- 668 2. Peak-to-trough ratio (Ptr), ratio of peak amplitude with respect to amplitude of trough.
- 669 3. Peak to valley (PtV), time interval between peak and valley.
- 670 4. Repolarization slope (RepS), slope between trough and return to baseline.
- 671 5. Recovery slope (RecS), slope after peak towards recovery to baseline.

672 As subcellular-resolution metrics, we extracted the action potential propagation velocity (Vel)<sup>37</sup> and the  
673 axon branch length (BL).

674 The percentage of active electrodes (AE) was also computed to measure the overall number of  
675 electrodes that could detect action potentials<sup>37</sup>.

#### 676 *HD-MEA Data Analysis*

677 Data analysis was performed using custom-written codes in MATLAB R2021a and Python 3.6.10 and  
678 are available upon request.

679 Spike sorting was performed to identify single units in the extracellular recordings. We used the  
680 Kilosort<sup>268</sup> software within the SpikeInterface<sup>38</sup> framework and the corresponding default parameters.  
681 We automatically curated the spike sorting output using the following parameters ([link](#)):

- 682 1. Inter-spike interval violation threshold (ISIt) = 0.5

683 The ISIt takes into account the refractory period, which follows every action potential (AP). The  
684 assumption is that if two APs occur within a too short time interval, they most likely come from two  
685 different neurons.

- 686 2. Firing rate threshold (FRt) = 0.05

687 The FRt sets the minimum firing rate of a neuron to be considered as “good” unit.

- 688 3. Signal-to-noise ratio threshold (SNRt) = 5

689 The SNRt takes into account the ratio between the maximum amplitude of the mean AP waveform and  
690 the noise characteristics of the specific channel.

- 691 4. Amplitude cutoff (ACt) = 0.1

692 The ACt takes into account the false-negative rate, thus the fraction of spikes per unit with an amplitude  
693 below the detection threshold.

- 694 5. Nearest neighbors hit rate (NNt) = 0.9

695 After computing the principal component for a unit, the NNt is used to check on the fraction of the  
696 nearest neighbors that fall into the same cluster.

#### 697 *HD-MEA Statistical Analysis*

698 Statistical comparisons to compare samples from more than two populations were performed using the  
699 Kruskal-Wallis H test. In case the null hypothesis was rejected, we performed a post-hoc Dunn test with  
700 Sidák correction for multiple comparisons (Dunn-Sidák multiple-comparison test).

701 Statistical analysis was performed in MATLAB R2021a.

702

703

704

## 705 **scRNA sequencing of human neural networks**

706 Duplicates (except for TDP-43-HA OFF and TDP-43-HA 2 weeks samples) of human neural networks  
707 (young (1.5 months), middle (3 months) and old (7.5 months)) and TDP-43-HA experiment samples at  
708 middle stage, were dissociated into single-cells suspension using Papain Dissociation System  
709 (Worthington #LK003150), passed through 70 $\mu$ m and 40 $\mu$ m cell strainers (Falcon #07-201-431 and  
710 #07-201-430), and resuspended in HIB++ media (Hibernate™-E Medium medium (Gibco #A1247601)  
711 supplemented with EDTA (1mM final; Invitrogen #AM9260G), HEPES (10mM final; Gibco #15630080),  
712 with 1X B27+ supplement (Gibco #17504-044), 1X N2 supplement (Gibco #17502-048); 1X GlutaMAX  
713 (Gibco #35050-061), BDNF (PeproTech #450-02), GDNF (Alomone labs #G-240), CNTF (Alomone  
714 labs #C-240), NT-3 (PeproTech #450-03) and IGF-1 (Stem Cell #78022) all at 20 ng/ml)) to 1000 cells  
715 per  $\mu$ l using CASY Cell Counter (Innovatis AG).

716

## 717 **Single-cell RNA sequencing (scRNA-seq) using 10X Genomics platform**

718 The quality and concentration of the single-cell preparations were evaluated using an haemocytometer  
719 in a Leica DM IL LED microscope and adjusted to 1,000 cells/ $\mu$ l. 10,000 cells per sample were loaded  
720 in to the 10X Chromium controller and library preparation was performed according to the  
721 manufacturer's indications (Chromium Next GEM Single Cell 3' Reagent Kits v3.1 protocol). The  
722 resulting libraries were sequenced in an Illumina NovaSeq sequencer according to 10X Genomics  
723 recommendations (paired-end reads, R1=28, i7=8, R2=91) to a depth of around 50,000 reads per cell.  
724 The sequencing was performed at Functional Genomics Center Zurich (FGCZ).

725

## 726 **Single-cell data analysis**

727 Data was processed with CellRanger for demultiplexing, read alignment to the human reference  
728 genome (GRCh38) and filtering to generate a feature-barcode matrix per sample. Cell doublets were  
729 removed with scDbtFinder<sup>69</sup> and outlier cells were detected and filtered with the "scater" R package<sup>70</sup>.  
730 In short, cells with more than 3 median-absolute-deviations away from the median number of UMIs, the  
731 number of features and the percentage of mitochondrial genes were removed. For the 7.5 months old  
732 samples, we additionally filtered cells with less than 2000 UMIs and less than 1500 detected features.  
733 For the TDP-43 overexpression experiment, we additionally filtered cells with less than 5000 UMIs and  
734 less than 2500 detected features.

735 Seurat v3<sup>71</sup> was used for log-normalization and to identify the top 2000 highly variable genes per  
736 sample. Louvain clustering was always performed with resolution 0.4 based on a shared nearest  
737 neighbor graph constructed from the top 20 principal components (PC). The Uniform Manifold  
738 Approximation and Projection (UMAP)<sup>72</sup> was always computed from the top 20 PCs. Cell cycle scores  
739 were computed with Seurat using a list of G2/M and S phase markers from Kowalczyk et al. 2015<sup>73</sup>.

740 Marker genes that are upregulated in one cluster compared to any other cluster with a log-fold change  
741 of at least 2 were identified with the findMarkers function from the scran R package<sup>74</sup>, which runs  
742 pairwise *t*-tests and combines the results into a ranked list of markers for each cluster. Heatmaps with  
743 marker gene expression show scaled mean log-counts of all cells in each cluster.

744 The two iCoMoNSC samples were integrated with Seurat using canonical-correlation analysis (CCA)<sup>75</sup>,  
745 the data was scaled and number of UMIs and the percentage of mitochondrial UMIs was regressed out  
746 before clustering.

747 The scRNA-seq data from three different NSC lines<sup>8</sup> was downloaded from ArrayExpress (accession  
748 number E-MTAB-8379) and integrated with our iCoMoNSCs using Seurat, the data was scaled and the  
749 number of UMIs was integrated out before clustering.

750 The human organoid (404b2 and H9) scRNA-seq data<sup>9</sup> was downloaded from ArrayExpress (accession  
751 number E-MTAB-7552). Our iCoMoNSC and human neural cultures (1.5, 3 and 7.5 months old) were  
752 integrated with cells from human brain organoids using Seurat, the data was scaled and the number of  
753 UMIs and the percentage of mitochondrial UMIs were regressed out before clustering.

754 In the four samples of the TDP-43 overexpression experiment, we additionally quantified the expression  
755 of the TDP-43-HA transcript, the total TDP-43 transcripts (endogenous TDP-43 transcript and TDP-43  
756 sequence of the lentiviral construct and the lentiviral construct (transcribed part without the TDP-43  
757 sequence) with Alevin<sup>43</sup> using the list of identified barcodes from CellRanger as whitelist. The counts  
758 were added to the filtered feature-barcode matrix from CellRanger. The total TDP-43 log2FC between  
759 cluster 12 and all other neuronal clusters was computed using the subset of cells with total TDP-43 UMI  
760 count > 0 (91.1% of cells in cluster 12 and 29.5% of all other neuronal cells).

761

762 Additional Methods are detailed in the Supplementary Information.

763

#### 764 **Data availability**

765 scRNA-seq data will be made publicly available upon publication.

766

#### 767 **Code availability**

768 All code for the scRNA-seq data analysis is deposited on github  
769 [https://github.com/khembach/neural\\_scRNAseq](https://github.com/khembach/neural_scRNAseq) as a workflow project<sup>76</sup>. All plots and results can be  
770 accessed as well: [https://khembach.github.io/neural\\_scRNAseq](https://khembach.github.io/neural_scRNAseq).

771

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798

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817

### 818 **Author contributions**

819 Conceptualization of the study was carried by M.H.P., K.M.B., S.R. and M.Po. M.H.P. generated iPSCs,  
820 iCoMoNSCs and differentiated them into neural networks, carried out cloning and lentiviral vector  
821 preparation, biochemistry and cell and human brain immunofluorescence as well as imaging and data  
822 analysis and prepared samples for calcium imaging, patch clamp, MEA and single-cell RNA sequencing  
823 experiments. K.M.B. analyzed the single-cell RNA sequencing data and re-analysed iCLIP and single

824 nuclei RNA sequencing datasets. S.R. cultured neural cultures on the MEAs, performed MEA  
825 recordings, developed MEA metrics and analysed all recorded MEA data. V.I.W. performed human  
826 brain immunofluorescence and imaging and prepared samples for single-cell RNA sequencing. Z.M.,  
827 E.M.H., and M.Pa. performed cell immunofluorescence and imaging, F.L. contributed to biochemistry  
828 experiments and S.S. performed synaptoneurosome isolation and western blots. V.H., and I.D.  
829 performed and analysed whole cell patch-clamp recordings. A.V.D.B. performed and analysed two  
830 photon calcium imaging. K.F., A.A. and T.L. provided human brain sections, neuropathological  
831 consultation and critical input on the study. D.B. provided critical feedback on the iCoMoNSC generation  
832 and characterization. M.D.R. provided feedback on sequencing data analyses. M.D.R., T.K., M.M.,  
833 A.H., M.H.P. and M.Po. provided supervision. M.H.P., K.M.B., S.R., V.I.W. and M.Po. wrote and edited  
834 the manuscript and prepared figures. M.Po. directed the entire study. All authors read, edited, and  
835 approved the final manuscript.

836

### 837 **Competing interest declaration**

838 The authors declare no competing interests.

839

### 840 **Additional information**

841 Supplementary Information is available for this paper.

842

843 Correspondence and requests for materials should be addressed to [magdalini.polymeridou@uzh.ch](mailto:magdalini.polymeridou@uzh.ch)

844

### 845 **Extended Data Figures legends**

846 **Extended Data Figure 1. iCoMoNSCs characterization.** **a**, Phase contrast images from different  
847 stages of iCoMoNSC generation. Human fibroblasts (left) were reprogrammed into iPSC colonies (2nd  
848 left), which formed embryoid bodies (middle) and then generated neural rosettes (2nd right). Patches  
849 of morphologically distinct colonies migrated out of rosettes and were isolated as clones (right). **b**,  
850 Phase contrast image showing the overall homogeneous morphology and pinwheel growth organisation  
851 of the iCoMoNSCs. NSC-marker immunofluorescence of iCoMoNSCs at early passage 6 (**c, d**) and late  
852 passage 17 (**e, f**) with NES, SOX2 and PLZF (**c, d**) or ZOI (**e, f**). Violin plots showing the number of  
853 genes (**g**) or UMIs (**h**) detected in all iCoMoNSCs by scRNA-seq in two biological replicates. **i**, UMAP  
854 of two integrated replicate samples of iCoMoNSCs clustering into 7 clusters, (**j**) same UMAP  
855 representing cell cycle stages or (**k**) showing the biological replicates whose distribution across the  
856 clusters is shown in (**l**). **m**, UMAP with normalized expression of selected genes across all iCoMoNSCs.  
857 Scale bars, (**a**) from left to right: 100  $\mu$ m, 500  $\mu$ m, 500  $\mu$ m, 500  $\mu$ m, 50  $\mu$ m, (**b**) 150  $\mu$ m, (**c-f**) 50  $\mu$ m.

858

859 **Extended Data Figure 2. Cluster markers and known marker genes of iCoMoNSCs.** **a**, Heatmap  
860 showing top cluster markers of iCoMoNSCs clusters. **b**, Heatmap showing gene expression of a set of  
861 known markers amongst the iCoMoNSCs clusters. **c**, UMAP of iCoMoNSCs integrated with 768 cells  
862 from three human neural stem cell lines (NES) showing individual samples in different colors.

863

864 **Extended Data Figure 3. Differentiation of iCoMoNSCs.** **a**, Schematics of iCoMoNSCs differentiation  
865 protocol. **b**, Immunofluorescence of neuronal markers MAP2/NFM, NEUN and DCX at 4 weeks of  
866 differentiation. **c**, Quantification of NEUN-positive cells over time in differentiation reaching approx. 40%  
867 at 12 weeks. Each data point represents an independent field of view normalized to DAPI<sup>+</sup> nuclei. **d**,  
868 KI67 immunofluorescence of iCoMoNSCs at passage 6. **e**, Quantification of KI67-positive cells over  
869 time in differentiation showing a drop to 5% at 12 weeks. Each data point represents an independent  
870 field of view normalized to DAPI<sup>+</sup> nuclei. **f**, 2-photon calcium imaging of neural networks bolus loaded  
871 with the AM ester form of Oregon Green BAPTA-1 showed typical firing neurons (neurons 1. and 3.).  
872 **g**, Infrared DIC image of whole-cell patch clamped human neuron at 4 months. **h**, Example voltage  
873 responses of a neuron elicited by tonic current injection. **i**, Average action potential and input resistance.  
874 **j**, Example current evoked by voltage steps from -60 mV to +40 mV (duration, 100 ms) from a holding  
875 voltage of -80 mV. Data recorded from the neuron in (**g**, **h**). **k**, Peak Na<sup>+</sup> and K<sup>+</sup> current densities  
876 plotted vs. voltage. Scale bars, (**b,d**) 50  $\mu$ m, (**f**) 100  $\mu$ m, (**g**) 20  $\mu$ m.

877

878 **Extended Data Figure 4. Schematics of network and single-cell properties and individual plots**  
879 **from data shown in figure 1l-n.** **a**, Timeline of the experiment: starting from iCoMoNSCs (0 months),  
880 neural cultures were differentiated, sequenced and plated on MEAs at young (1.5 months), middle (3  
881 months) and old (7 months) stages. **b**, HD-MEA chip mounted on a printed circuit board featuring a cell  
882 culture chamber (ring) and the microelectrode array in the center (green). Scale bar, 4 mm. **c**,  
883 Representative spike time histogram recorded from 1,020 electrodes used to illustrate how network  
884 metrics were extracted. **d**, Representative spontaneous APs recorded from one electrode, used to  
885 illustrate how single-cell metrics were extracted. **e**, Representative action potential (AP) used to  
886 illustrate how features were extracted from the AP shape. **f-t**, Box plots representing all results  
887 illustrated in **Fig. 1l, m**.

888

889 **Extended Data Figure 5. Neuronal and glial maturation of iCoMoNSC-differentiated human**  
890 **neural cultures.** **a**, Heatmap of cell distribution from all experimental stages (in biological duplicates)  
891 amongst all clusters. **b**, Top cluster marker genes for each cluster. **c**, UMAP of iCoMoNSCs and young,  
892 middle and old human neural cultures with two replicates each showing cells from individual samples  
893 in different colors. **d**, UMAP with normalized expression of selected NSC, (**e**) glial and (**f**) neuronal  
894 marker genes across all samples. **g**, Heatmap with the expression of the top cluster markers from (**b**)  
895 from our aging experiment. **h**, UMAP of young, middle and old iCoMoNSC-derived neural cultures  
896 integrated with organoid datasets highlighting the cell origin.

897

898 **Extended Data Figure 6. Heatmap with the gene expression of known marker genes amongst all**  
899 **clusters from our aging experiment.**

900

901 **Extended Data Figure 7. Autoregulatory, all-in-one TetON cassette and its function.** **a**,  
902 Schematics of our improved, autoregulatory, all-in-one TetON cassette, which consists of a single tight  
903 TRE (*P<sub>tight</sub>*) promoter driving inducible expression of TDP-43-HA, which is linked via IRES2 to T7-



904 tagged rtTA with SV40 NLS fused to its N-terminus to increase its nuclear localisation, making it a  
905 nuclear marker of transgenic cells. **b**, Schematics showing DOX OFF stage (left) in which no transgenic  
906 mRNA or protein could be detected. Upon addition of doxycycline (DOX ON stage; right), the nuclear,  
907 T7-tagged rtTA binds the Tet operators in the TRE promoter, which induces overexpression of TDP-  
908 43-HA as well as it leads to steady expression of rtTA needed for the whole cassette to function. The  
909 system is reversible.

910

911 **Extended Data Figure 8. TDP-43-HA-induced pathology in human neural networks. a**, SOD1  
912 Western blot (WB) of SarkoSpin fractions of human neural networks overexpressing TDP-43-HA for 2,  
913 4 or 9 weeks. **b**, WB on total homogenates (SarkoSpin input). SOD1 was used as a loading control. **c**,  
914 Immunofluorescence with phospho-specific (S403/404) anti-TDP-43 antibody revealed inclusion-like  
915 structures in the soma of TDP-43-HA-negative neurons. **d**, Quantification of TDP-43<sup>p403/404</sup>-positive  
916 cells over 4 weeks of TDP-43-HA overexpression. TDP-43<sup>p403/404</sup>-positive inclusions were later found  
917 localized to neurites at 5 weeks (**e**) and occasionally grew into-aggregate like structures at 9 weeks of  
918 TDP-43-HA overexpression (**f**). **g**, UMAP highlighting the four different samples of the single-cell RNA-  
919 seq TDP-43-HA experiment. Scale bars, 25  $\mu$ m.

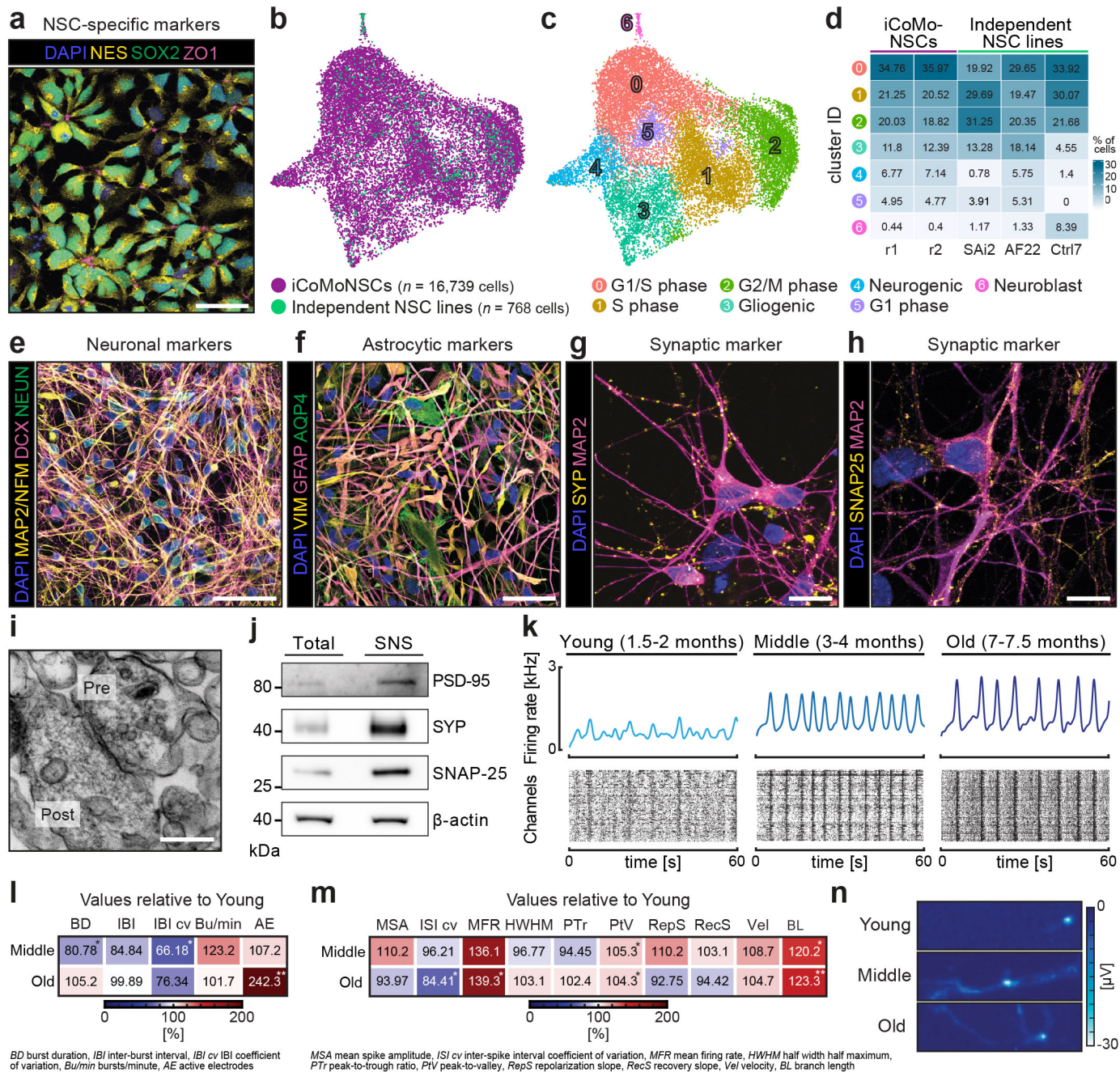
920

921 **Extended Data Figure 9. Immunofluorescence of cluster 12 misregulated genes in human**  
922 **neurons overexpressing TDP-43-HA. a**, Immunofluorescence of human neural networks transduced  
923 to overexpress TDP-43-HA for 4 weeks demonstrating that only the TDP-43-HA-positive neurons  
924 expressed elevated levels of (**a**) NPTX2 in the soma and neurites, (**b**) MEF2A in the nucleus and (**c**)  
925 lowered levels of STMN2 in the soma and processes as demonstrated with 2 different antibodies. Scale  
926 bars, 20  $\mu$ m.

927

928 **Extended Data Figure 10. NPTX2 immunofluorescence in FTLD-A, FTLD-C and ALS patient brain**  
929 **sections. Additional immunofluorescence of multiple FTLD and ALS cases demonstrating NPTX2**  
930 **accumulation and inclusions in neurons with TDP-43<sup>p403/404</sup>-positive aggregates in the hippocampus in**  
931 **(a) different FTLD-A cases, (b) different FTLD-C cases and in the primary motor cortex of an ALS case**  
932 **(c). d**, Note that while the NPTX2 pathology could be observed in the cortex of both FTLD and ALS  
933 patients, it is only present when the TDP-43<sup>p403/404</sup>-positive aggregate-containing neurons could still be  
934 identified by the MAP2 staining (middle and right) as opposed to the disintegrated, “ghost neurons” that  
935 left behind only the TDP-43<sup>p403/404</sup>-positive aggregates (left). Scale bars, (**a,b,d**) 10  $\mu$ m, (**c**) 20  $\mu$ m.

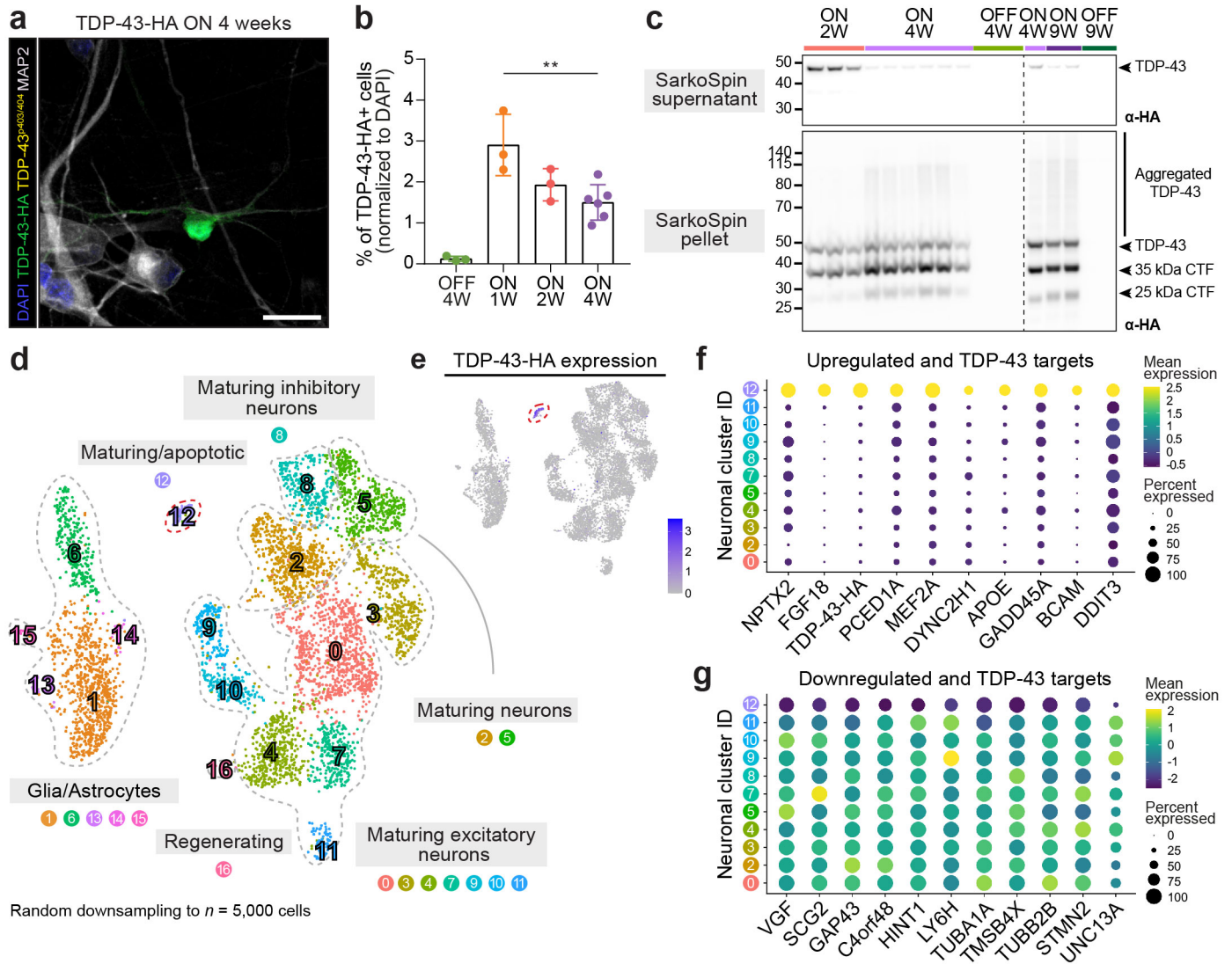




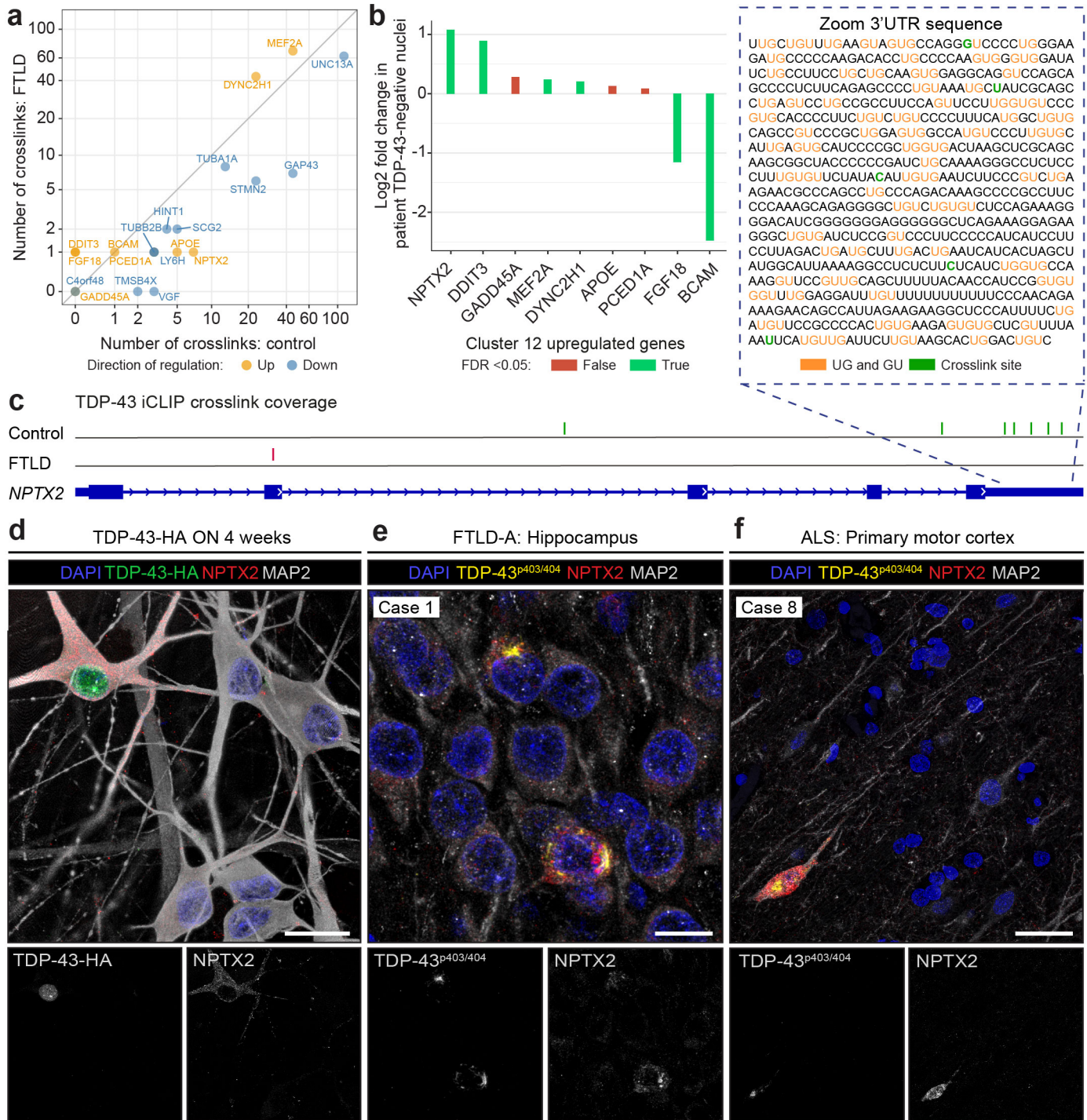
**Figure 1. iCoMoNSC neurons form functional networks.** **a**, Immunofluorescence of iCoMoNSCs for NSC markers. **b**, UMAP of iCoMoNSCs integrated with 768 cells from three independent NSC lines and **(c)** corresponding annotated clusters. **d**, Percentage of cell distribution per sample across clusters. **e**, Immunofluorescence of human neural cultures stained with neuronal or **(f)** astrocytic markers. Synaptic marker immunofluorescence at 4 months for **(g)** SYP and **(h)** SNAP-25. **i**, Electron microscopy of SNS showing pre- and postsynaptic compartments. **j**, Western blots of SNS fractions. **k**, Population spike time histograms (top) and raster plots (bottom) of neuronal networks. **l**, Heatmaps of percentage changes in network or **(m)** single-cell and subcellular metrics features. **n**, Spike-sorted axonal branches. Scale bars, **(a,e,f)** 50  $\mu$ m, **(g,h)** 10  $\mu$ m, **(i)** 250 nm.





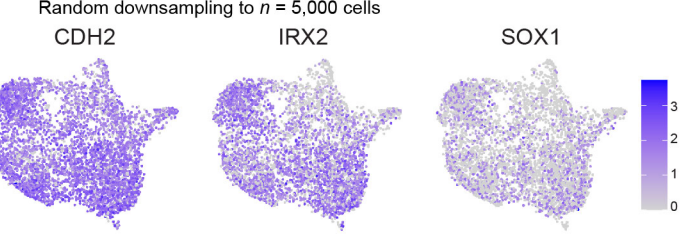
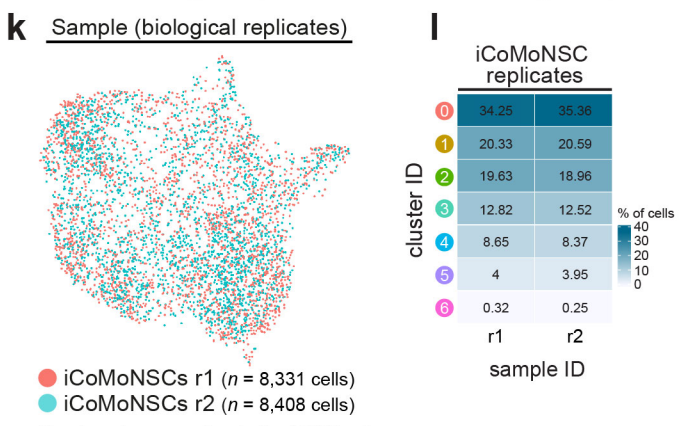
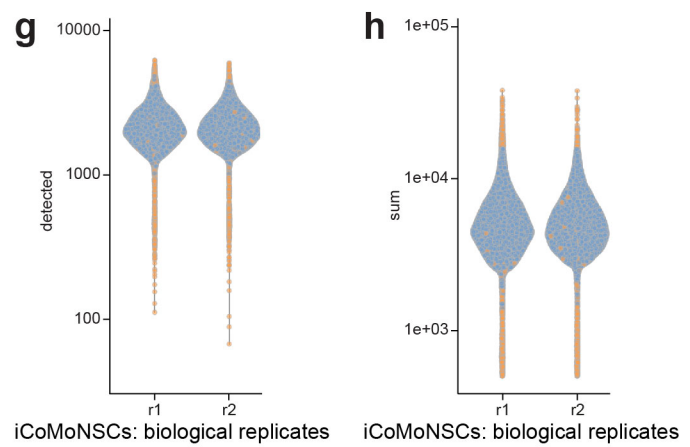
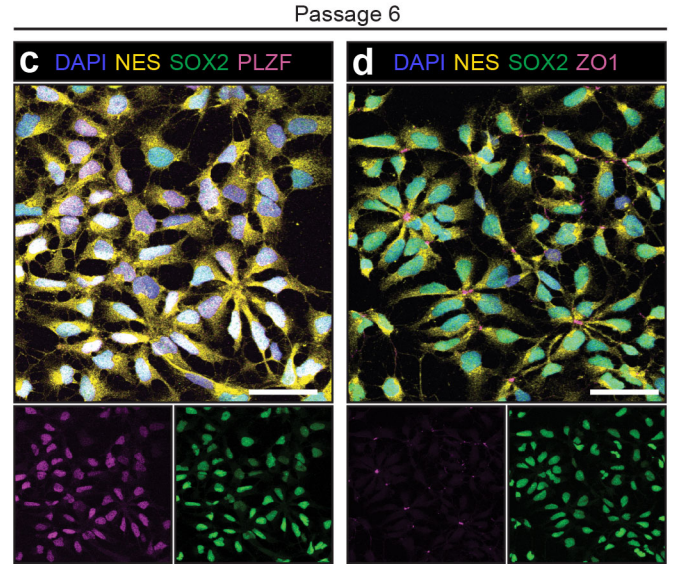
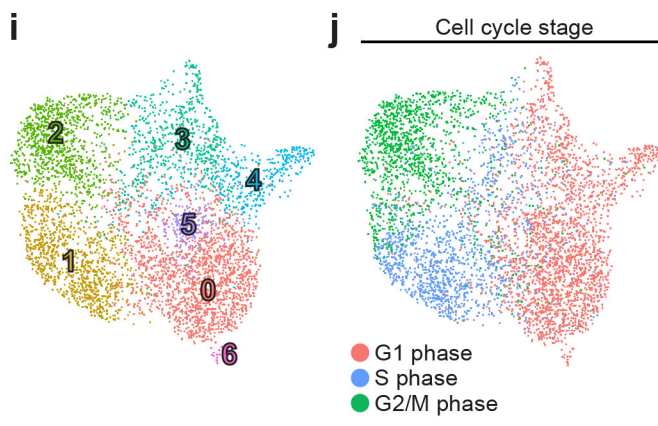
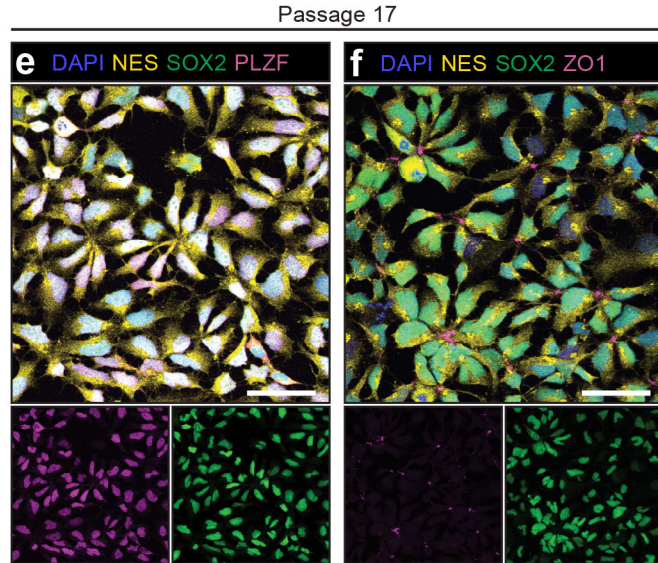
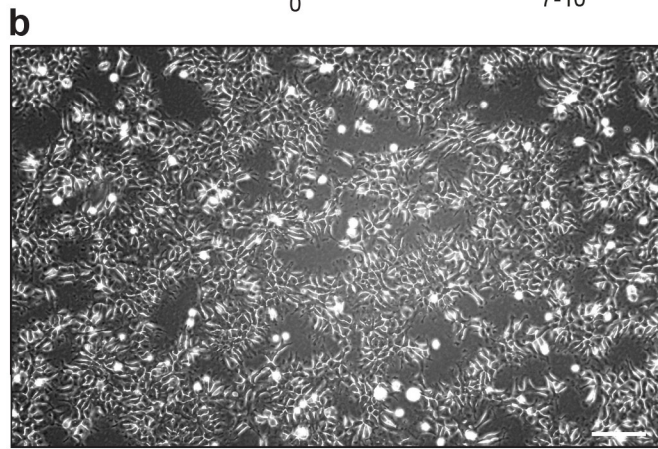
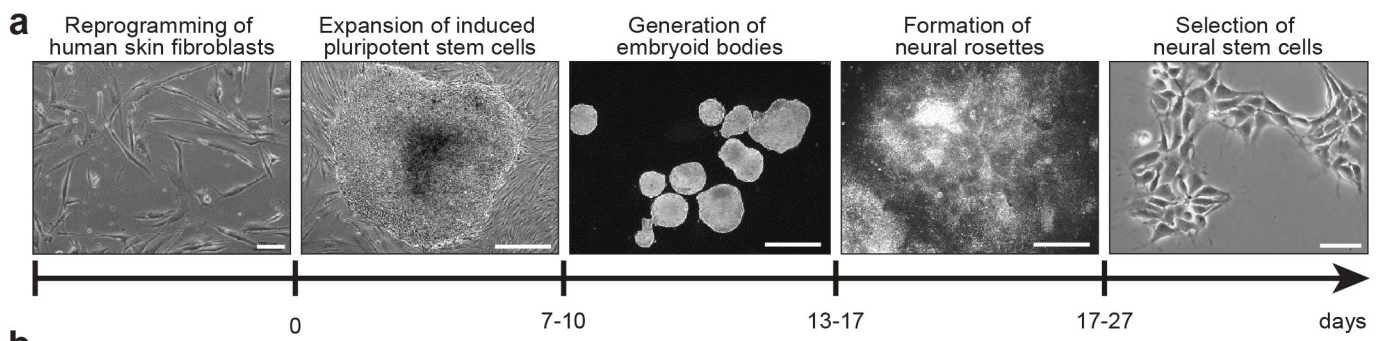


**Figure 3. Distinct transcriptional profile in neurons with induced TDP-43 pathology.** **a**, Immunofluorescence of TDP-43-HA in iCoMoNSC neurons. Scale bar, 25  $\mu$ m. **b**, Quantification of TDP-43-HA-positive neurons over time. Each dot represents a biological replicate. **c**, Western blots of SarkoSpin supernatants (top) and pellets (bottom). Line separates independent experiments. **d**, UMAP of single-cell RNA-seq TDP-43 overexpression experiment. Colors indicate clusters, dashed lines highlight different cell types. Red dashed line depicts cluster 12, containing cells expressing TDP-43-HA. **e**, UMAP highlighting TDP-43-HA expression confined in cluster 12. **f**, Dot plot with the scaled average expression of the top cluster 12-upregulated and **(g)** -downregulated, including the significantly downregulated UNC13A, marker genes when compared to all other neuronal clusters.

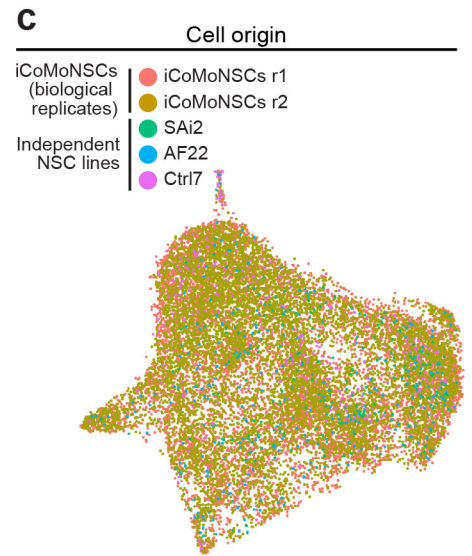
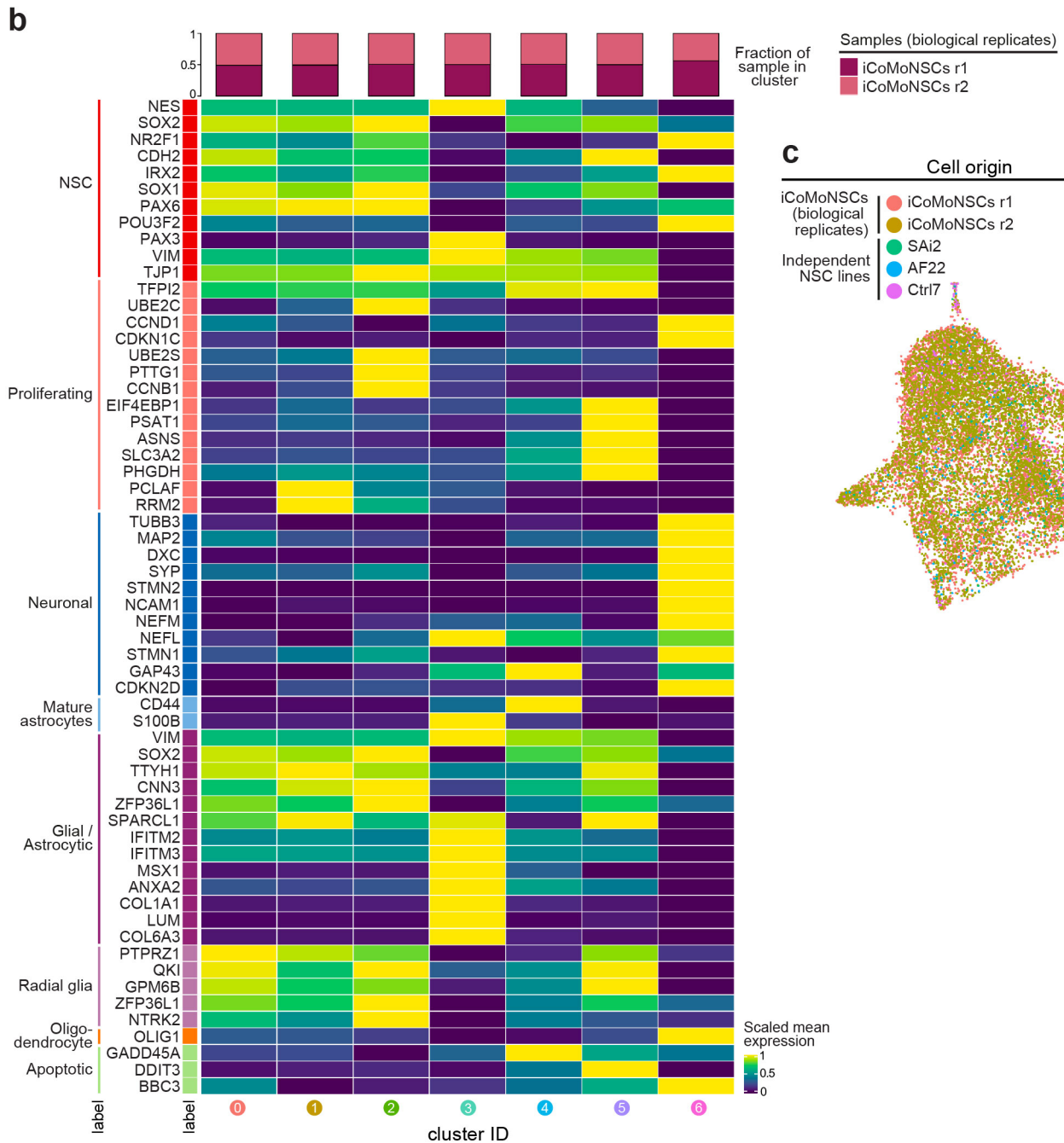
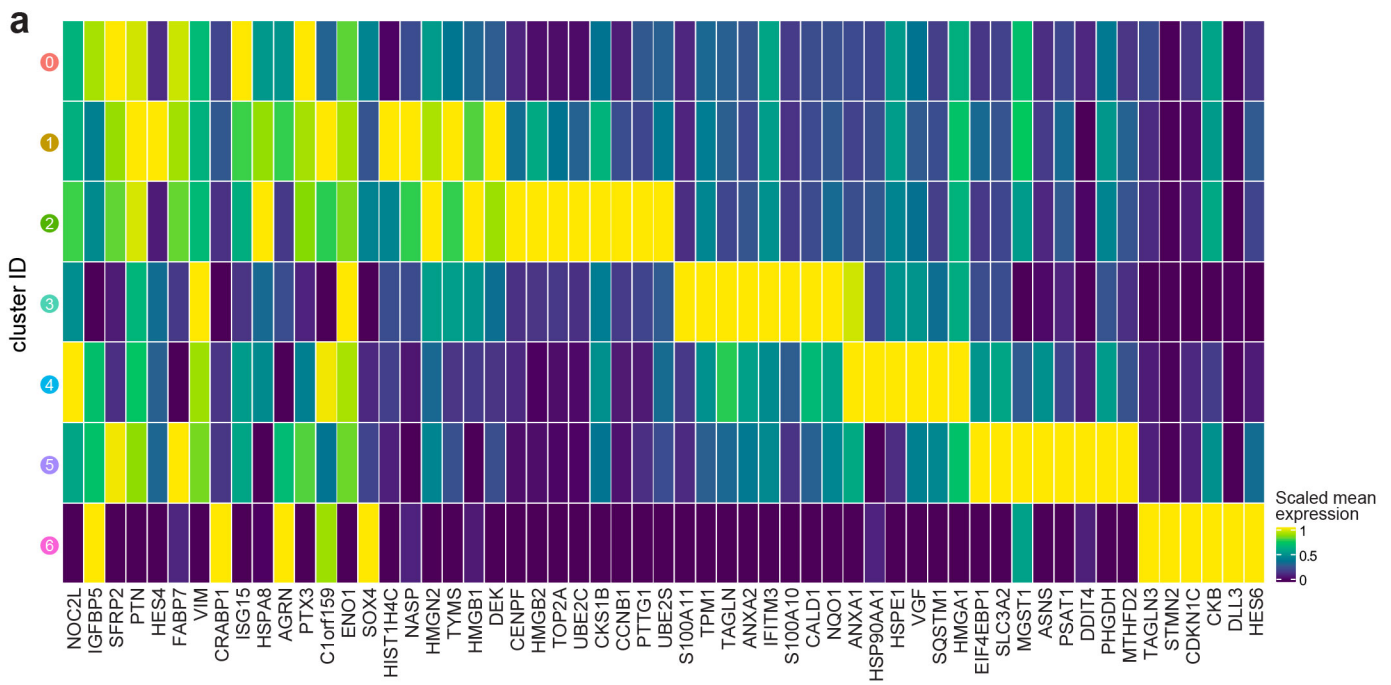


**Figure 4. NPTX2 is misaccumulated in FTLD and ALS patients.** **a**, Plot shows the number of iCLIP crosslinks<sup>17</sup> in both FTLD patient and control brain samples in mRNA of the cluster 12 up- and -downregulated genes. **b**, Log<sub>2</sub> fold change in RNA expression of the top 10 cluster 12-upregulated markers between TDP-43-negative and TDP-43-positive neuronal nuclei from FTLD patients 47. **c**, Location of iCLIP crosslink sites in *NPTX2* in both control (green) and FTLD patient (red) human nuclei brain<sup>17</sup>. Zoom-in: iCLIP crosslinks (green) and UG- or GU-repeats (orange). **d**, Only TDP-43-HA-overexpressing neurons showed detectable NPTX2 levels in immunofluorescence. **e**, Immunofluorescence of human brain sections: FTLD-A hippocampus and **(f)** ALS primary motor cortex demonstrating inclusion-like NPTX2 signal only in MAP2-positive neurons containing TDP-43<sup>p403/404</sup>-positive aggregates in the cytoplasm. Scale bars, **(d)** 20  $\mu$ m, **(e)** 10  $\mu$ m, **(f)** 30  $\mu$ m.



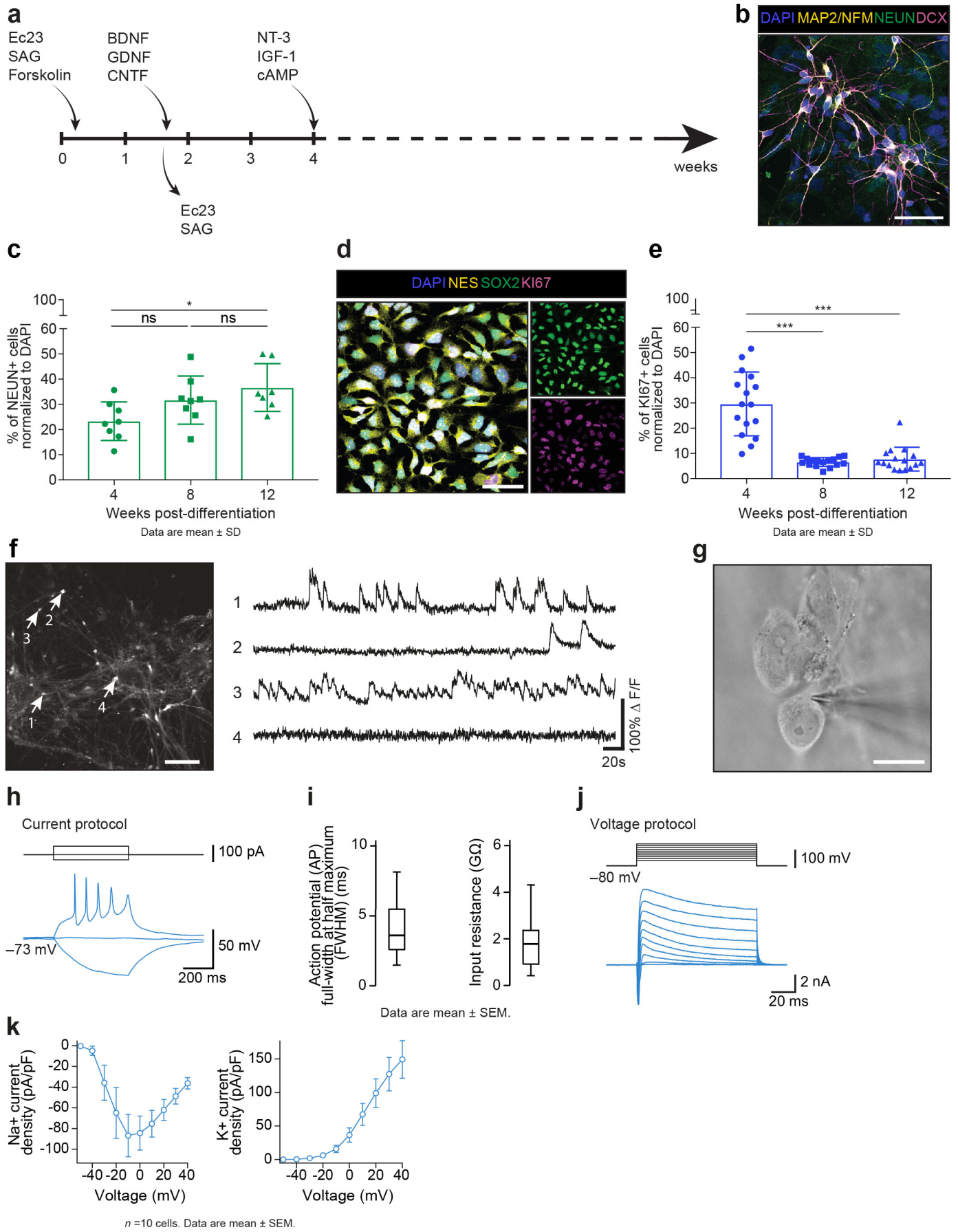


Extended Data Fig. 1

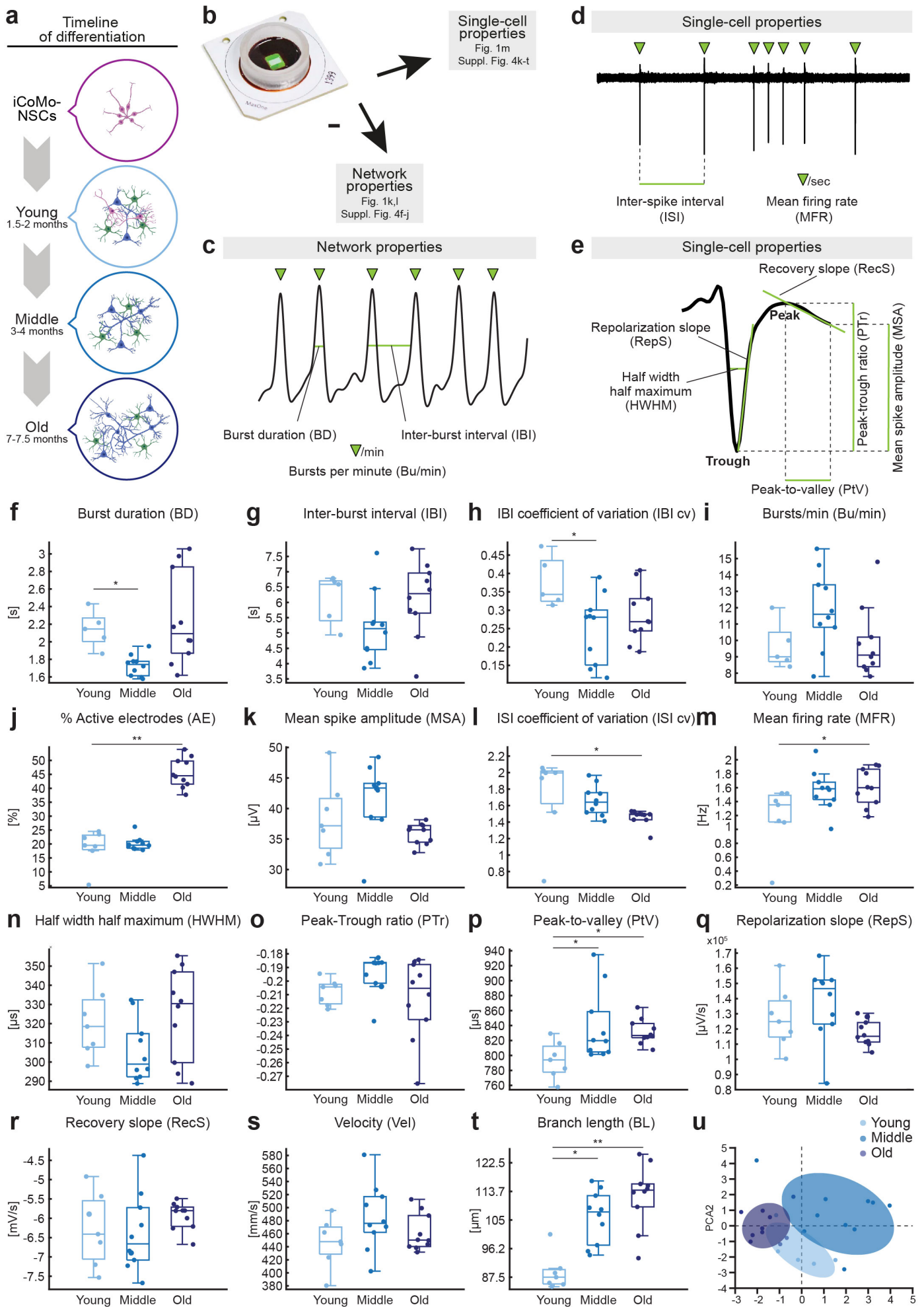


Extended Data Fig. 2

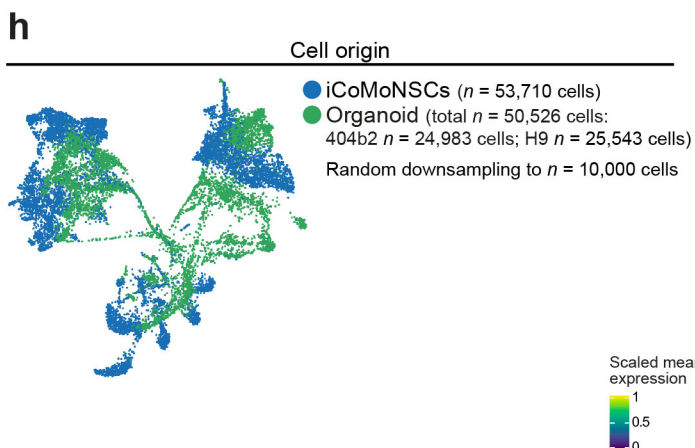
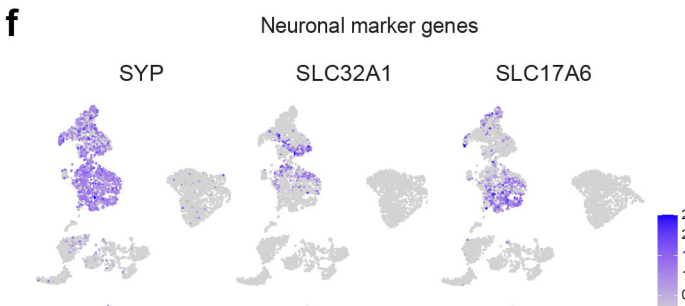
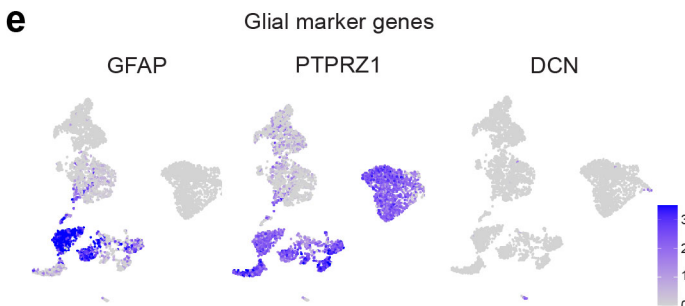
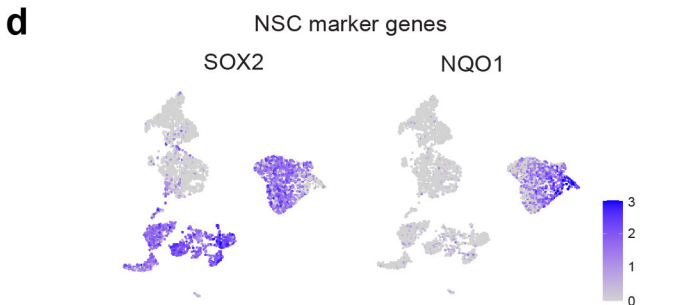
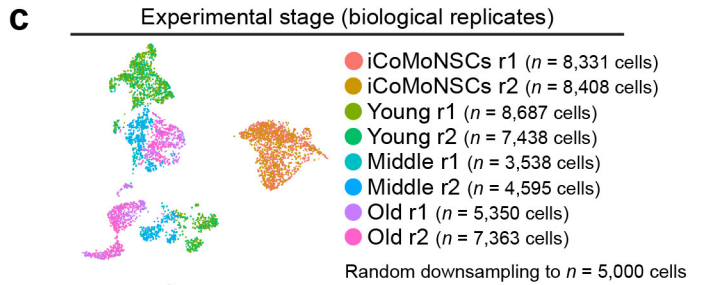
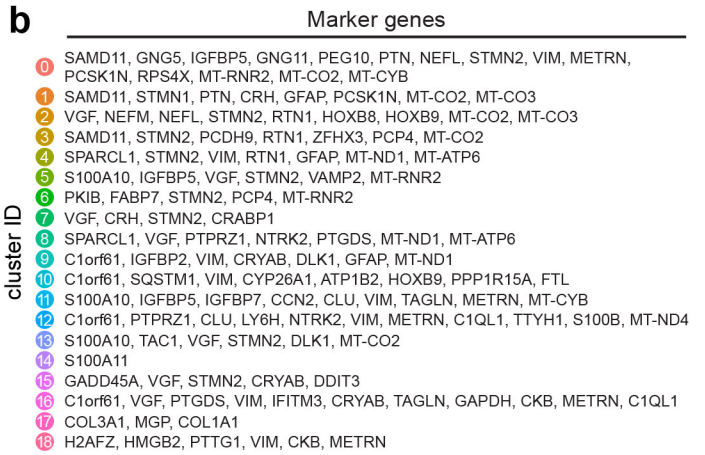
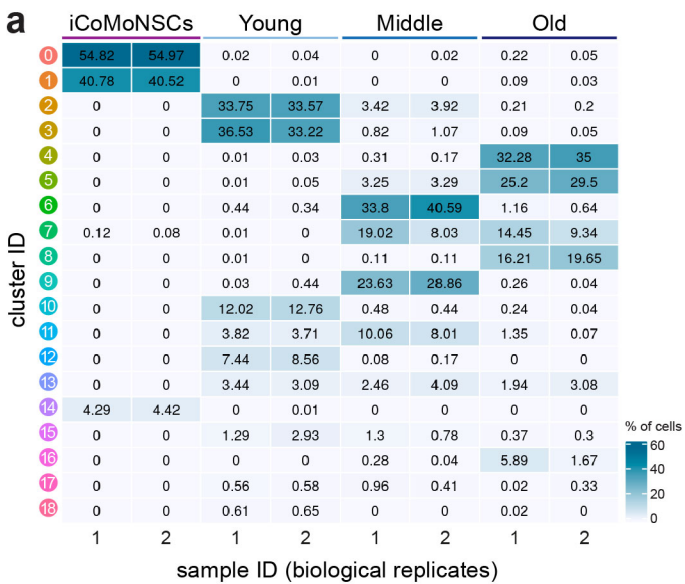


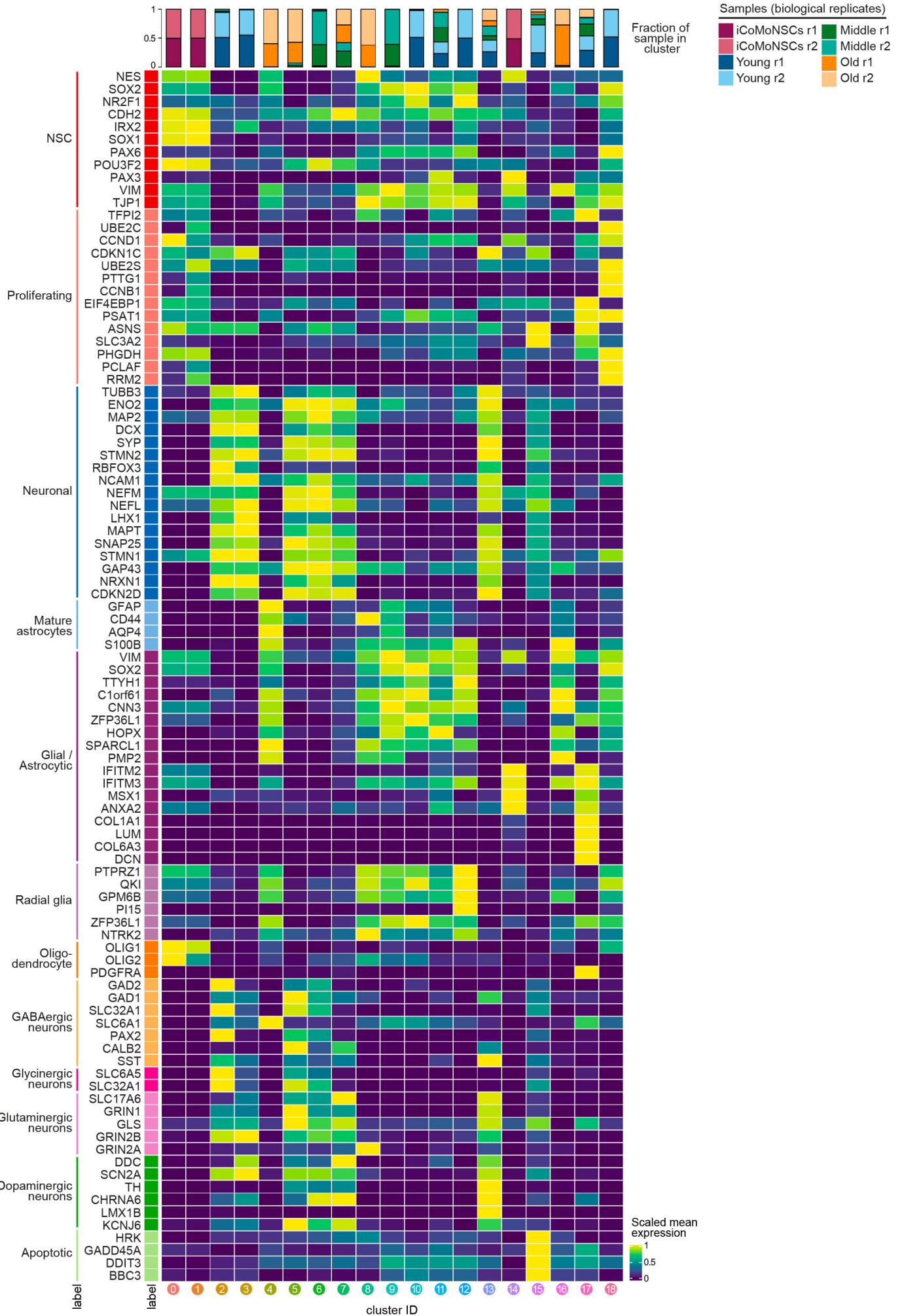




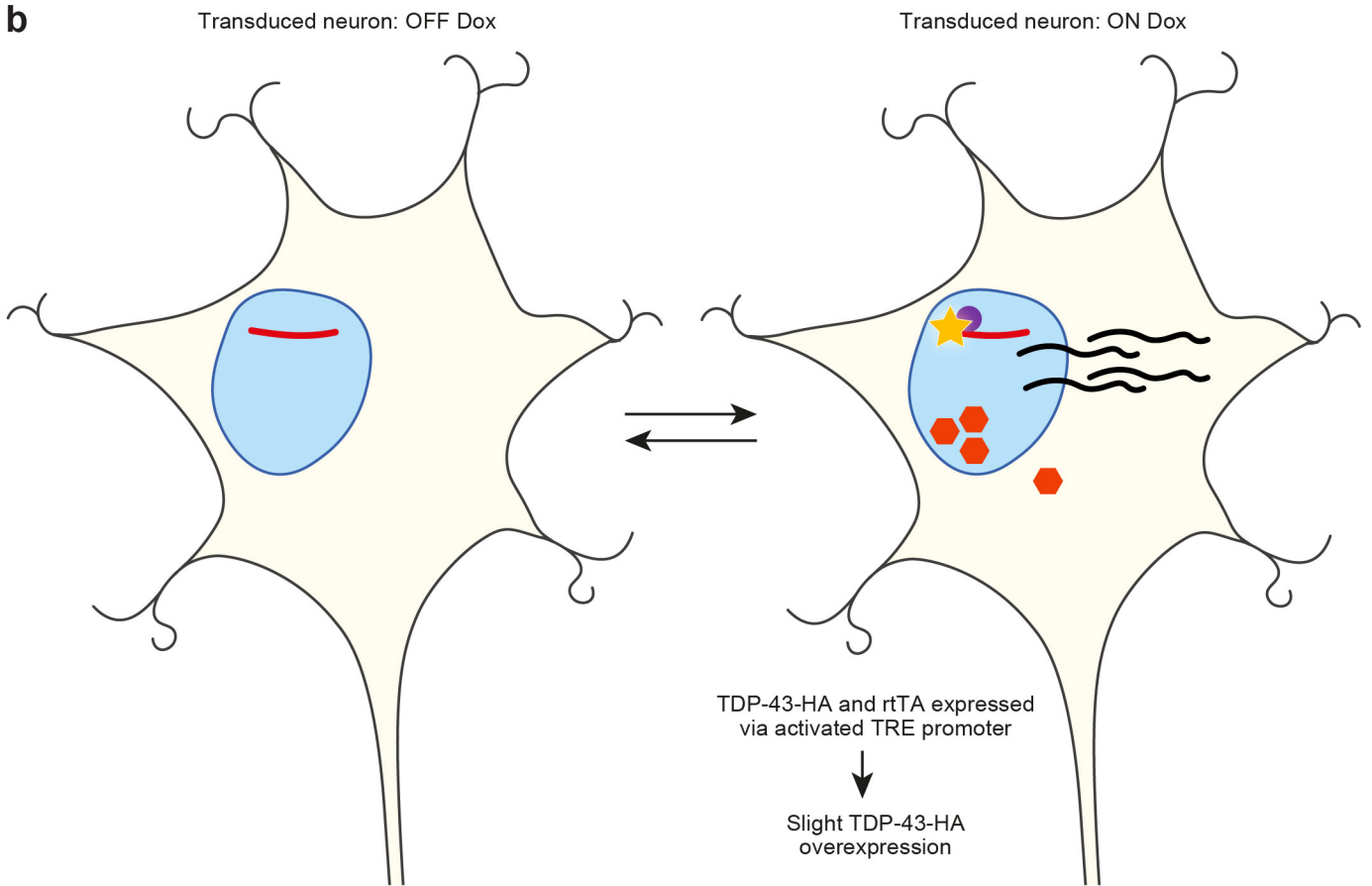
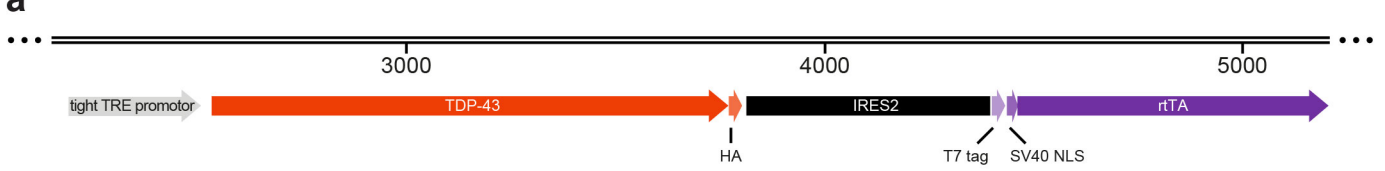


f-t: Each dot represents the respective metric value obtained from one HD-MEA culture. Box plots indicate distribution median value and 25th and 75th percentiles. Asterisks indicate  $p$  values with respect to young cultures: \*  $p < 0.025$ , \*\*  $p < 0.001$ .





Extended Data Fig. 6



Legend

— Transgenic DNA

⬡ TDP-43-HA protein

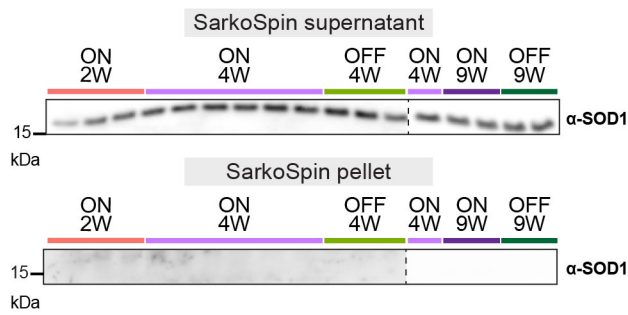
★ Doxycycline (Dox)

~ Transgenic mRNA

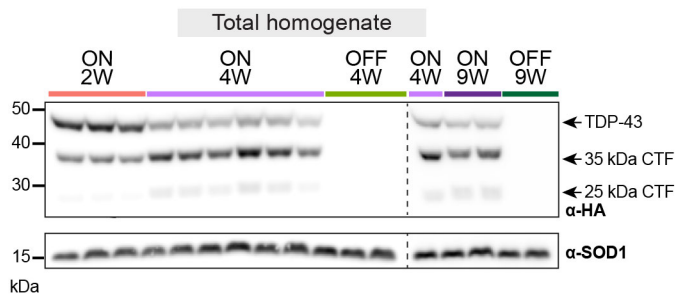
● T7-SV40NLS-rtTA protein



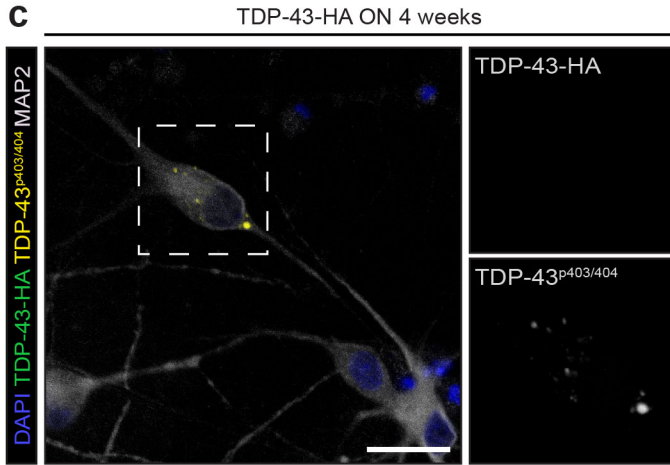
a related to Fig. 3c



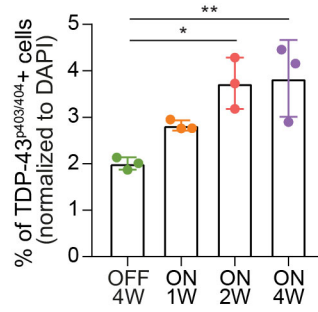
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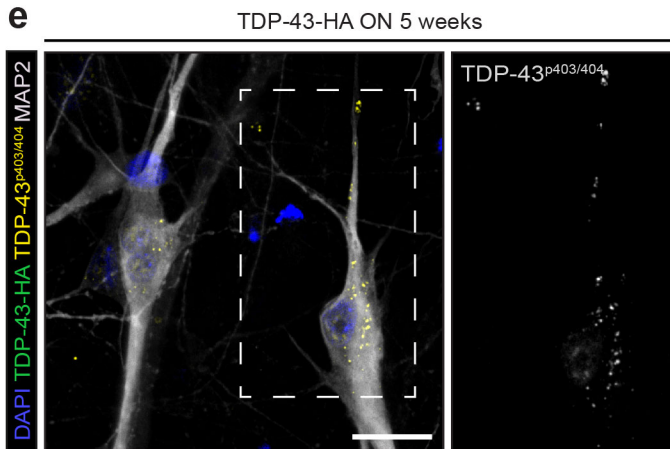
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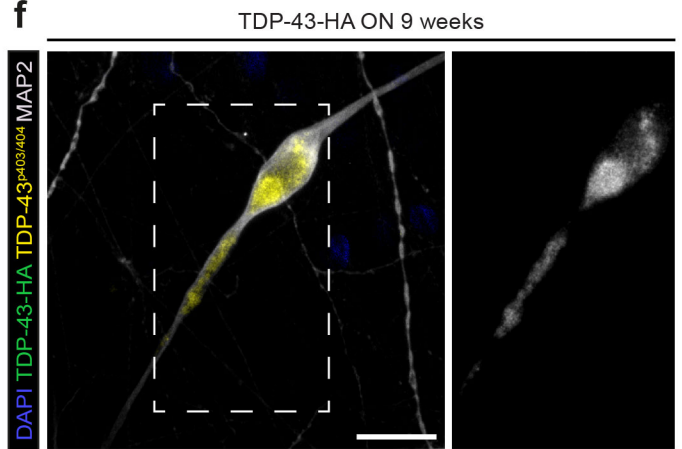
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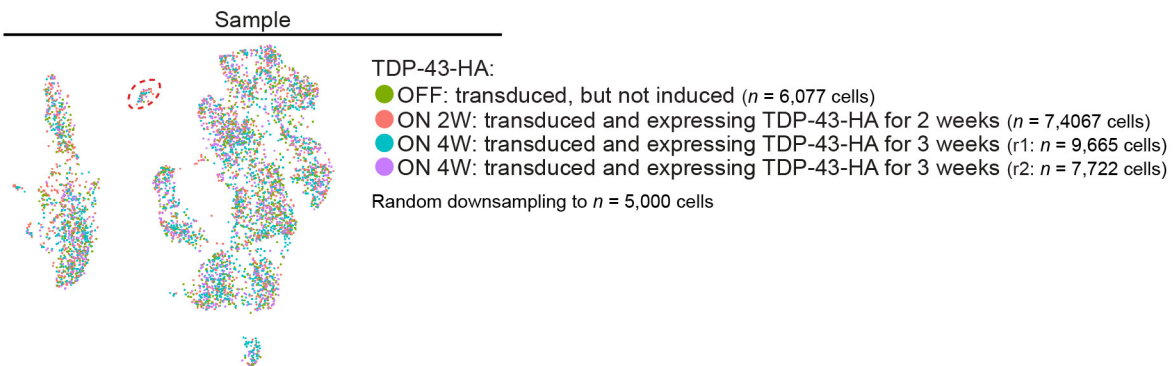
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f

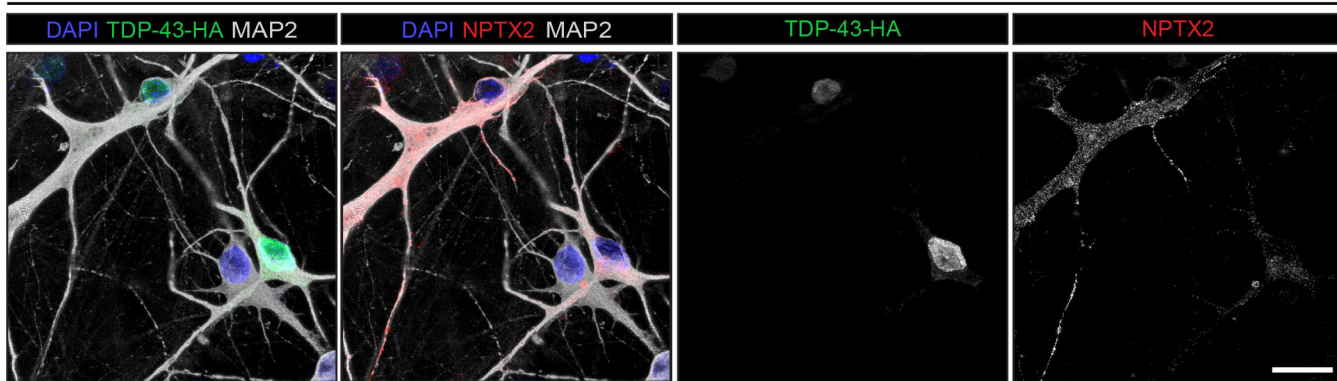


g

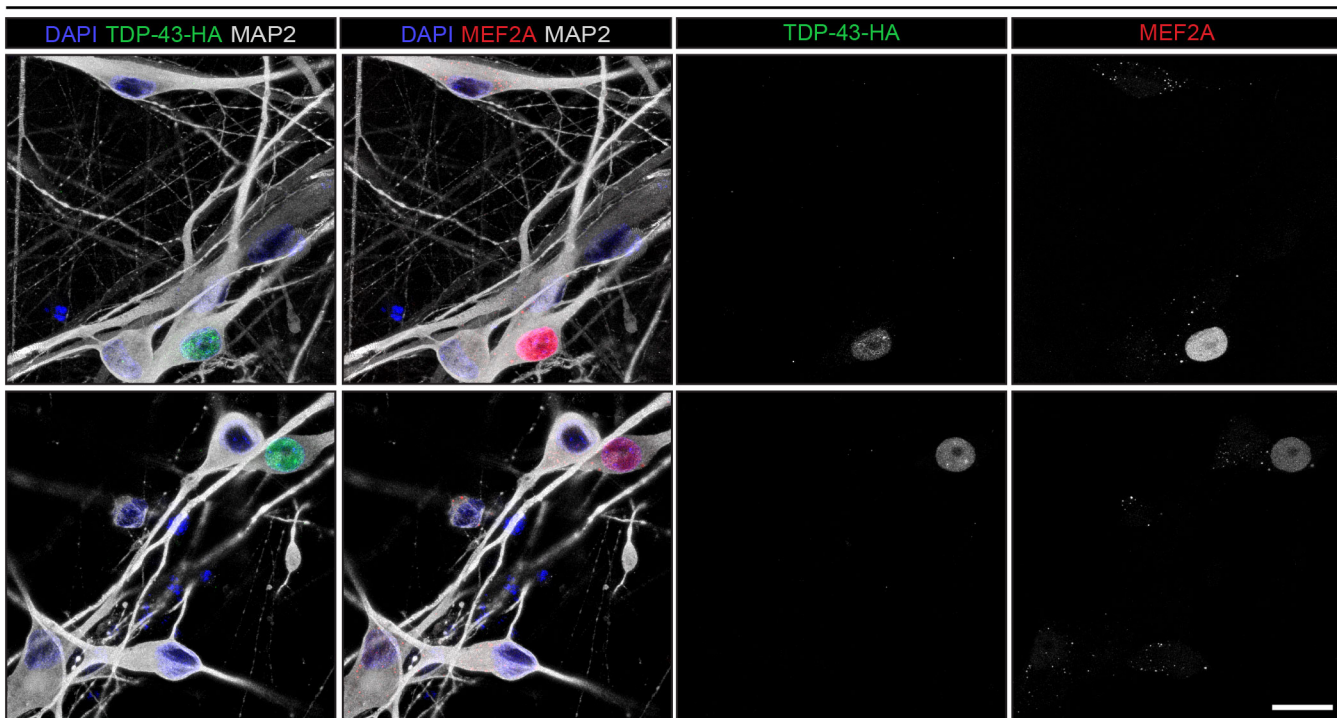




TDP-43-HA ON 4 weeks



TDP-43-HA ON 4 weeks



TDP-43-HA ON 4 weeks

