Single-cell transcriptomic analysis identifies neocortical developmental differences between human and mouse

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Abstract

Background: The specification and differentiation of neocortical projection neurons is a complex process under precise molecular regulation; however, little is known about the similarities and differences in cerebral cortex development between human and mouse at single-cell resolution.

Results: Here, using single-cell RNA-seq (scRNA-seq) data we explore the divergence and conservation of human and mouse cerebral cortex development using 18,446 and 7,610 neocortical cells. Systematic cross-species comparison reveals that the overall transcriptome profile in human cerebral cortex is similar to that in mouse such as cell types and their markers genes. By single-cell trajectories analysis we find human and mouse excitatory neurons have different developmental trajectories of neocortical projection neurons, ligand-receptor interactions and gene expression patterns. Further analysis reveals a refinement of neuron differentiation that occurred in human but not in mouse, suggesting that excitatory neurons in human undergo refined transcriptional states in later development stage. By contrast, for glial cells and inhibitory neurons we detected conserved developmental trajectories in human and mouse.

Conclusions: Taken together, our study integrates scRNA-seq data of cerebral cortex development in human and mouse, and uncovers distinct developing models in neocortical projection neurons. The earlier activation of cognition -related genes in human may explain the differences in behavior, learning or memory abilities between the two species.

Keywords:

Cerebral cortex development, Cross-species clustering, Neocortical projection neurons, Cognition

Background

The mammalian cerebral cortex develops via a complex process of cell proliferation, differentiation, and migration events. The molecular features of the human brain at gestational weeks 7-23 are similar to those of the mouse at postnatal days 14.5 to birth (P0) and reveal gene expression differences between the two species [1, 2]. Interneurons and projection neurons, which are the two major classes of neurons, populate the neocortex. Interneurons show largely GABAergic, connecting locally within the neocortex, and are generated by progenitors in the ventral telencephalic (inhibitory cortical interneuron producing) radial glia, before migrating from sub pallial ganglionic eminences into the cortex [3-6]. By contrast, projection neurons are excitatory, which are generated by progenitors in the dorsal telencephalic (excitatory cortical neuron producing) radial glia [7-9].

Despite immense efforts over the past decades to study the cell types, function, differentiation for the mammalian cerebral cortex, especially human and mouse, large differences and conservation are observed in human versus animal models [10-12]. Elucidating the conserved, divergent and cellular architecture of developing the cerebral cortex, especially projection neurons developments, may highlight to understanding susceptibility to neurological diseases and the occurrence of cognition and memory abilities in the early stages.

Recently, with the advance of next-generation sequencing (NGS) techniques, single-cell RNA sequencing (scRNA-seq) technologies has been paved the way for study cell phenotype and cell behavior at single-cell resolution [13, 14]. More significantly, scRNA-seq can overcome the heterogeneity of cross-species analysis, bypassing the need for pure cell sorting strategies [15, 16]. Moreover, although many studies have been reported the single species cerebral cortex using scRNA-seq, there is no systematic research to

integrate the human and mouse scRNA-seq data, and compare the conservation and divergence of developing neocortex.

Here we comprehensively characterize the conservation and divergence of the cerebral cortex program between human and mouse [9, 17, 18]. We present a cross-species cell atlas and in which no obvious differences in cell type composition was observed. However, we identified different developing trajectories of projection neurons that in human the subventricular zone (SVZ) migration to deeper neocortical layers and more superficial layers are two independent processes. Additionally, the deeper and upper layers are more precisely differentiated in human than those in mouse, and lack of neuron migration signal in human. By comparing excitatory neurons gene expression across species, we also observed significant expression differences of function genes for cell migration, cognition, learning and memory in humans compared with mice. We next evaluated whether further refinement of neuronal subtypes occurs as neurons mature, indicating that human neuronal subtypes resolve from coarse laminar distinctions to refined transcriptional states in the later development, whereas mouse may not show precise subtype differentiation. In summary, we show the importance of cross-evolutionary comparison to better characterize the differences of human and mouse projection neurons development, and the relevance of these to cognition pathway. In conclusion, our study offers essential clues for researching neurodevelopment, especially for the projection neurons development, and the differences of occurrence of cognition ability in human and mouse.

RESULTS

Integration of single-cell transcriptomics of the human and mouse cerebral cortex

In order to compare cellular and molecular processes of the late corticogenesis between human and mouse, we collected published scRNA-seq data sets of human and mouse cerebral cortex at different development stages [9, 17, 18]. We reanalyzed the transcriptome profiles of 18,446 mouse neocortical cells at two key time points for corticogenesis development including 10,836 cells at embryonic day 14.5 (E14.5) from six biological replicates (Fig. 1A), and 7,610 cells at birth (P0) from three biological replicates (Fig. 1A). Because 7- to 23-post-conception-week (pcw) human brain cells are analogous to E14.5 to P0 mouse brain cells (Fig. 1A) [1, 2], we incorporated the transcriptome profiles of 6,655 human brain cells including 100 cells between 5.85 and 7 pcw, and 800 cells between 23-37 pcw (Fig. 1A). To ensure that cells from different species and data sets can be properly integrated, we performed unbiased clustering using Uniform Manifold Approximation and Projection (UMAP) [19]. The major cell lineages in cerebral cortex such as excitatory neurons, inhibitory neurons, neuronal progenitor cells and glial cells were able to be identified using their lineage markers [20, 21] (Fig. 1D, E). Moreover, there was no obvious bias because the majority of the cell types could be identified in all three data sets (Fig. S1C).

Because the number of mouse cells (18,446) was about 3 times higher than that of human cells (6,655) which may cause bias, we randomly extracted 6,655 mouse cells in further analysis. Finally, 13,278 cells (6,655 mouse cells and 6,623 human cells) were used (Fig. 1A) in further analysis which covers all major cell types (Fig. 1D, E).

Identification and characterization of cortical cell types

129 We first compared the cell composition and found that mouse had more interneuron than
130 human (Fig. 2A). We next compared the cell number in each cluster and found that cluster
131 06 Inh_striatal and cluster 09 Layer [19P] had more cells in mouse (20%-30%) than in
132 human (70%-80%). In contrast, cluster 16 Microglia and 17 Mesenchyme had more human
133 cells (80%-90%) (Fig. 2B).
134
135 We next examined the correlation among clusters by cross-species clustering (Fig. 2C).
136 As there were still considerable species differences between humans and mice, we used
137 the integrated matrix. Remarkably, excitatory neuron exhibited a profile highly similar to
138 interneuron, and Glia showed itself a high degree of correlation (Fig. 2C). Interestingly,
139 Cluster 16 Microglia were distinct from excitatory neuron, interneuron and other Glia cells
140 (Fig. 2C). We also calculated the correlation using human and mouse cells respectively
141 and found that human and mouse had similar results with cross-species (Fig. S2A, B).
142
143 We checked the top 10 highly expressed genes of each cluster and found most of them
144 were consistent among human and mouse (Fig. 2D, S2C, D). For example, two SVZ
145 clusters (Clusters 00 SVZ and 01 SVZ) were featured by predominant expression of
146 *EOMES* (*TBR2*) and *ZBTB20* [22-24]. Cells in Cluster 01 SVZ also expressed markers of
147 both excitatory (*NEUROD6*) and inhibitory (*CALB2*) neurons [25-27] which exhibited the
148 expression patterns of rostral migratory steam [18]. Cells in deep-layer populations
149 (Clusters 02 Exc deep and 03 Exc deep) exhibited high expression levels of *CTIP2* and
150 *BCL11B* [28]. Cells in Clusters 04 Exc upper and 05 Exc upper expressed upper layer
151 marker *MEF2C* [8, 29]. We identified NEUROD6 and NEUROD1 kept consistently high
152 expression in excitatory inhibitory, and EOMES expressed in subventricular zone (SVZ),
153 and STMN2 expressed strongly in deep and upper layer and increased the levels of
154 expression with neuron maturation, which conversed the transformation known genes
155 expression trajectories in neurogenesis among human and mouse (from EOMES to

156 STMN2) [9, 30] (Fig. 2D). Three interneuron clusters (Clusters 06 Inh_striatal, 07 Inh and
157 08 Inh) were characterized by expression of *GAD2* and *NRXN3*. Specifically, in Cluster 08
158 Inh we found high expression of *LHX6*, *SST* and *MAF* indicating 08 Inh is somatostatin
159 (SST) expressing interneurons but not parvalbumin (PV). *LHX6* is a transcription factor
160 associated with PV and SST interneuron [31, 32], whereas *SST* and *MAF* regulate the
161 potential of interneurons to interneurons to acquire SST+ interneuron identity [33].
162 Interneuron migrate tangentially from the ganglionic eminences, and ganglionic
163 eminences (Cluster 13 GE) expressed high levels of inhibitory marker *DLX1* and
164 proliferation markers *TOP2A* and *MKI67* [34]. RG marker *HES5* was in two RG clusters
165 (Clusters 10 RG and 11 RG), while Cluster 11 RG expressed high levels of *MKI67* and
166 *TOP2A* genes. We also found *GAP43*, a key regulator in early brain development, was
167 constitutively expressed in all these cell types in both human and mouse [35].
168
169 We identified Clusters 06 Inh_striatal and 09 Layer[19P] as inhibitory neurons and they
170 had similar gene expression patterns and cell numbers in human and mouse. Striatal
171 inhibitory neurons (Cluster 06 Inh_striatal) expressed inhibitory markers *GAD2* and lower-
172 layer marker *BCL11B* [36]. Layer 19-P (Cluster 09 layer[19P]) expressed canonical Cajal-
173 Retzius cell markers *RELN* and Purkinje cell marker *PCP4*. In addition, they also
174 expressed non-coding RNAs *SNHG11* and *MEG3* (Fig. 2D, S2C). Microglia (Cluster 16
175 Microglia) had higher cell numbers in human than in mouse and exhibited low expression
176 correlation with other clusters. They expressed microglial markers such as *LAPTM5*,
177 *C1QA*, *C1QB*, and *C1QC* [37], as well as several microglia-specific pathways such as
178 TREM2-TYROBP pathway [37-40] (Fig. 2D). Interestingly, Cluster 20 Unknown exhibited
179 an intermediate state between excitatory neuron and interneuron: it had high expression
180 correlation with both excitatory neuron and interneuron, and expressed several marker
181 genes such as *STMN2* (excitatory neuron) and *NNAT* (interneuron) (Fig. 2D, S2C, D).
182
183 **Different developmental trajectories of excitatory neuron between human and**

184 **mouse**

185

186 To compare the developmental trajectories in excitatory neuron between human and
187 mouse, we split the data into 6,623 human cells and 6,655 mouse cells, and performed
188 unbiased clustering using UMAP again. We identified 23 clusters and also observed
189 lineage separation of the major cell types (Fig. S3A, B, C, D). We used *NEUROD6* as
190 marker gene and identified 6 excitatory neuron clusters in human (Cluster 00 SVZ, 01 Exc
191 deep, 02 Exc deep, 03 Exc upper, 04 Exc upper and 05 Exc upper) and 7 excitatory neuron
192 clusters in mouse (Cluster 00 SVZ, 01 SVZ, 02 Exc deep, 03 Exc deep, 04 Exc deep, 05
193 Exc upper and 06 Exc upper) (Fig. S3A, B). We then reconstructed the corticogenesis
194 developmental trajectories using Monocle analysis. Both human cells and mouse cells
195 were distributed along pseudo-temporally ordered paths from SVZ to neocortical layers,
196 including deeper neocortical layers (layer VI, then layer V) and superficial layers (layer IV,
197 then layer II/III) [28] (Fig. 3A, B). Surprisingly, we found that the developmental trajectory
198 in mouse was a continuous process without any branch, in which SVZ migrated to deeper
199 neocortical layers and then to superficial layers. By contrast, in human this process had
200 two branches in which SVZ migrated to both deeper neocortical layers and superficial
201 layers (Fig. 3A, B, S3E, F). Consistent with this observation, we found that SVZ marker
202 *EOMES* was specifically expressed in the beginning of trajectories, deep-layer marker
203 *BCL11B* was specifically expressed in the upper branch in human, whereas migratory and
204 upper-layer markers *POU3F2* and *SATB2* were specifically expressed in the lower branch,
205 which was different from those in mouse trajectory (Fig. 3A, B, S3I). More important, when
206 we further split the data based on development stages, we still found that the lower-layer
207 neurons were present in 7 to 23 pcw in human and E14.5 and P0 in mouse, with high
208 expression of *BCL11B*. Meanwhile, a large number of Layer II-IV (upper layer) cells at P0
209 in mouse and 20-23 pcw in human was identified, with high expression of *SATB2* (Fig.
210 S3G, H).

211

212 We identified 109 differentially expressed genes in human that may determine cell fate
213 commitment. They were fall into 4 clusters which exhibited successive waves of gene
214 expression in different branches (Fig. 3E). In addition, Gene Ontology (GO) enrichment
215 analysis revealed that the functions of genes predominantly expressed at the SVZ branch
216 were enriched in cerebral cortex development, olfactory bulb development and olfactory
217 lobe development as previously reported [41]. Among these genes, transcription factor
218 *PAX6* is crucial for SVZ neurogenesis and also acts via retinoid signaling to regulate eye
219 development [42] suggesting that eye development may have relative to SVZ
220 neurogenesis (Fig. 3E). Lower-layer from human cortex showed increased expression of
221 genes for modulation of synaptic signaling and chemical synaptic transmission, which
222 have a mechanistic link with learning and memory by plasticity regulation [43]; whereas
223 upper layer CPN from human cortex exhibited increased expression of genes involved in
224 regulation of sodium ion transport and calcium-mediated signaling (Fig. 3E). Genes
225 related to forebrain neuron fate commitment and forebrain neuron differentiation were
226 expressed in both lower-layer and upper layer. More interestingly, the functions of genes
227 that were highly expressed in SVZ, lower layer and upper layer were all enriched in
228 cognition and learning (Fig. 3E).

229

230 We further analyzed the migratory marker genes expression in neocortical. We found
231 these genes had continuously increased expression in SVZ, and decreased expression in
232 deep-layer and upper-layer in human. Specifically, *NRP1* and *PALMD*, which were
233 involved in the regulation of cell morphogenesis and cerebral cortex cell migration,
234 exhibited increased expression in the SVZ lineage and then declined in deep layer and
235 upper layer lineage, suggesting that neuronal migration in projection neurons in the
236 cerebral cortex may occur from the SVZ's maturity stage to the early development of deep
237 and shallow layers, rather than the late deep layer [44] (Fig. S3J, K). By contrast, in mouse

238 cerebral cortex we found migratory genes showed increased expression in SVZ and deep
239 layer, and decreased expression in upper layer suggesting that in mouse the deep layer
240 continuously process cell migration from SVZ to upper layer. Representative genes
241 include *Meis2*, *Nrp1* and *Pou3f2*, whose functions are relative to cell migration and
242 regulation of cell morphogenesis involved in differentiation (Fig. S3K).
243
244 **Divergence in neocortical between human and mouse**
245
246 In order to better understand the distinct layers in neocortical in human and mouse, we
247 performed a comparison of cortical expression models through single-cell analysis,
248 including deeper neocortical cortex (layer VI, then layer V) and late-born neurons migrate
249 through deep layers to gradually fill more shallow layers (layer IV, then layer II/III). Pairwise
250 comparison of human and mouse cortical layer data sets identified a large number of
251 differentially expressed genes (DEGs) (Fig. 4A). We observed a considerably bigger
252 number of differentially expressed genes in human than in mouse, meanwhile we also
253 found some co-expression marker genes, for example, fez family zinc finger 2 *Fezf2* (high
254 expressed in deeper layers), special AT-rich sequence binding protein 2 (*SATB2*) and
255 *POUEF2*, a marker of layer II/III with function of migration and differentiation [28]. More
256 interestingly, we detected the differentially expressed gene: *CLSTN2*, high expressed in
257 human deep layer and mouse upper layer, which significantly associated with pathway of
258 cognitive and memory behavior and reduction in functional inhibitory synapses [45],
259 suggesting that genes, relating to cognition and memory pathways, expressed earlier in
260 human than mouse.
261
262 Pathway analysis of two layers specific to humans revealed enrichment of axon
263 development, such as axon guidance and axonal fasciculation, compared with mouse (Fig.
264 4B). Additionally, enriched pathway contained in many aspects, including visual system,

265 facial nerve, trigeminal nerve and motor neuron in human — more specifically, retinal
266 ganglion cell axon guidance, facial nerve development, trigeminal nerve development,
267 motor neuron axon guidance and regulation of synaptic transmission, glutamatergic (Fig.
268 4C). In contrast, some pathways were enriched in mouse, mainly in cell fate commitment,
269 neuron fate commitment and neuron migration (Fig. 4C).
270
271 To continuously process research in neocortical in human and mouse, we expanded our
272 analysis included SVZ. Pairwise comparison of human and mouse SVZ and upper cortical
273 layers scRNA-seq transcriptomics data identified a large number of DEGs (Fig. S4A). The
274 number of DEGs detected in mouse was 6 times higher than that derived from human.
275 SVZ marker genes, *EOMES* and *NHLH1*, related to cell-type determination within
276 developing neurons, upper layer markers, *SATB2* and *MEF2C*, had co-expression in
277 human and mouse. We found more than 200 significant gene ontology (GO) terms in
278 human and mouse respectively. Specifically, for human, pathways focused on neuron
279 migration and axon development. In addition, human-specific genes also revealed
280 enrichment of “eye development”, “visual system development”, and “sensory system
281 development”. For mouse, we detected mouse-specific pathways “learning or memory”,
282 and “synapse organization” (Fig. S4B). we also identified the DEGs between SVZ and
283 deeper layer among human and mouse (Fig. S4C). The number of DEGs are equal in
284 human and mouse (Fig. S4D).
285
286 We next examined the correlation between each cluster in neocortical between human
287 and mouse (Fig. 4D). We identified mouse SVZ and mouse upper layer showed
288 remarkably low degree correlation (Fig. 4D). In contrast, the correlation of human SVZ
289 with human deep layer were nearly similar to those of human SVZ with human upper layer
290 (Fig. 4D). Taken together, we conclude that the differences of deeper layer and upper layer
291 has larger in human than mouse, while the differences of SVZ and upper layer has larger

292 in mouse than human (Fig. 4D).

293

294 Having defined the populations of SVZ, deeper layer and upper layer cells, we further used
295 CellPhoneDB to perform an unbiased ligand receptor interaction analysis between these
296 populations [46]. To detect how neocortical projection neurons are generated in human,
297 we focused functional analyses on interactions with SVZ, deeper layer and upper layer
298 (Fig. 4E). SVZ expressed *PTN* and *MDK*, signaling to receptors *ALK* on upper layer, that
299 are known to regular nervous system development, function, and repair, especially,
300 regulating the sympathetic neuron growth during development and aberrant signaling to
301 neuroblastoma predisposition [47-49] (Fig. 4E). SVZ expressed *PTN* and *MDK*, signaling
302 to the receptor tyrosine phosphatase family *PTPRZ1* on deeper and upper layers, which
303 shows the significant function in activate cell growth, migration and cellular activities (Fig.
304 4E). More importantly, *PTPRZ1* interaction with *MDK* promotes neuron migration [50]. SVZ
305 expressed high levels of *NOTCH3* interacting with receptor *DLL3* on deeper layer cells
306 and upper layer cells, which could modulate cellular differentiation, fates for terminal
307 neuronal differentiation and links to progression toward a neuronal fate in the developing
308 cortex [51, 52](Fig. 4E). In addition, ephrin family showed high level of expression in
309 human cortical. Firstly, *EPHA8_EFNA5* could promotes neurite outgrowth and axon
310 guidance [53], which showed significance in process SVZ to deep layer (Fig. 4E).
311 *EFNA1_EPHA4* involves in dendritic spine morphogenesis in deep layer to upper layer
312 [54] (Fig. 4E). Deep layer expressed *WNT* genes interacting with receptors *FZD4* and
313 *ROR1* on upper-layer cells. *WNT5A_ROR1* promotes the formation of presynaptic [55]
314 (Fig. 4E). Upper layer expressed Atypical Chemokine Receptor 2 ligands (*ACKR2*)
315 receptors that are known to be required for immature/mature dendritic cells [56] (Fig. 4E).
316 *ESAM_ESAM* helps cell-cell adhesion in deep layer to upper layer (Fig. 4E).

317

318 In summary, our trajectory, cell-type and unbiased ligand-receptor interactions analysis

319 between mouse and human indicates that human and mouse have differences in
320 development neocortical models, the correlation between deep layer and upper layer are
321 lower in human than mouse, and the ligand-receptor analysis indicate that deep layer and
322 upper layer may not have various neuron migration interactions in human. These
323 phenomena may therefore show SVZ migration to appropriate layers are relatively
324 independent in human neocortical projection neurons.
325
326 **Characterization of the excitatory neuron gene expression pattern across species**
327
328 To better understand the divergence and conservation of excitatory neuron gene
329 expression across evolution, we used developmental time course reconstructed by
330 monocle analysis to process the further analysis of gene expression pattern. Although a
331 large part of genes keep conservation across evolution, some genes showed converse
332 expression trends in human and mouse. We defined two gene expression clusters, with
333 cluster a representing up-regulated genes during the frontal development excitatory
334 neuron in human, mainly including SVZ cells (Fig. 5A), and in mouse, the expression
335 patterns are converse (Fig. 5B). Cluster a contained genes *VCAN*, *BCL11B*, *SOX5*, *PTN*
336 and *CNR1*, whereas Cluster b contained genes *ST18*, *KLHL13* and *NHLH2* (Fig. 5A, B).
337 We focused some genes have key functions in neuron development, for example, we
338 detected *CNR1* have various difference in expression pattern, in human, it expressed in
339 SVZ cells, whereas it expressed in deeper and upper layer cells in mouse, in other words,
340 CNR1 expressed earlier in human than mouse in cortex (Fig. 5A, B, C). CNR1, known as
341 cannabinoid receptor 1, is expressed in the peripheral nervous system and central
342 nervous system and combine cannabinoid to regulate the release either glutamate or
343 GABA, and the endocannabinoids (*eCBs*) and their receptors (*CNR1*) express in cortex
344 that can act as the significant function as cell proliferation and migration and axon
345 guidance during the critical stages of brain development [57-62]. In addition, *CNR1* also

346 likely plays an important role in the development of memory and cognition, and is relative
347 to many emotional, neurological diseases, such as novelty [63-65]. *ST18*, expressing
348 earlier in mouse than human, shows key factor in breast cancer [66] (Fig. 5A, B, D).
349
350 In order to detect the function of cluster a genes and cluster b genes, we found more
351 genes with similar expression patterns to process GO terms, function protein network and
352 transcriptional regulatory relationships analysis. We detected some neuron differentiation
353 and synaptic pathways, including chemical synaptic transmission, cell morphogenesis
354 involved in neuron differentiation, active in human SVZ (Fig. S5E, F). More interesting,
355 axon development, learning or memory, neuron recognition, cognition, regulation of
356 behavior and regulation of synaptic transmission, glutamatergic were more active in the
357 human SVZ than mouse SVZ (Fig. 5E). The key genes, enriching in cognition, learning or
358 memory, neuron differentiation and neuron regeneration pathways, including *PTPRZ1*,
359 *PTN*, *CNR1*, *ATP1A2*, *SOX5* and *JUN*, showed the different expression pattern in human
360 and mouse excitatory neuron, suggesting human probably develop these function,
361 especially for cognition, learning or memory, earlier than mouse (Fig. 5F, S5A, B, C, D).
362 According to protein and transcription factor analysis, we observed key protein or
363 transcription factor: *JUN* connected with *FOS*, as a classic marker for neuronal activation,
364 which has important regulator of axonal regeneration and neuronal activation. [67] (Fig.
365 5A, 5B, S5E, F). Moreover, *JUN-PTN* was detected TF-target regulatory relationship,
366 which directly indicated activation of neuronal migration during later brain development
367 [68-70], and ligand-receptor interaction: activating *PTN_PTPRZ1* in human SVZ to deeper
368 layer and SVZ to upper layer before (Fig. 4E), which activates cell growth, migration and
369 cellular activities [70, 71], and we found these genes have different expression pattern in
370 human and mouse, for human, they showed highly expression in SVZ, whereas *Ptprz1*
371 and *Ptn* performed low expression in mouse SVZ, and increase expression in deeper layer
372 and upper layer cells (Fig. 5A, B, S5A, B), indicating that neuron cell migration performs

373 in human SVZ to deeper layer and upper layer or mouse deeper layer to upper layer
374 process. We also found genes expressing in mouse SVZ, like *ST18* and *UACA*, relative
375 to reactive oxygen species biosynthetic process (Fig. 5D, E).
376
377 **Single-cell transcriptomic analysis identifies human excitatory neuron subsets**
378
379 In order to examine whether the excitatory neuron of human and mouse display similar
380 cell types or contain several subtypes, we further observed the clustering of human and
381 mouse (Fig. S3A, B). Surprisingly, distinct clusters of maturing neurons (04 Exc upper and
382 05 Exc upper) reproducibly emerge according to the distinct expression profiles, including
383 reciprocal expression of PFC (The prefrontal cortex) marker (*ABCA1*) and V1 (Visual
384 cortex) marker (*LPL*) [9] (Fig. 6A). By contrast, the gene with reciprocal expression in
385 human 04 Exc upper and 05 Exc upper don't have similar expression patterns in mouse
386 (Fig. 6B).
387
388 In order to further make sure the cell types, we used known labels to tag the clustering
389 and processed statistics (Fig. S6A, B, C, D), we found that the proportion of PFC and V1
390 cells have remarkable difference in 05 Exc upper (Fig. S6C), suggesting that the coarse
391 laminar distinctions among newborn neurons are resolved into refined transcriptional state
392 in human later development, whereas the phenomenon disappeared in cross-species
393 clustering that the number of PFC and V1 cells in each cluster are similar, especially in
394 excitatory neuron, indicating that the newborn neurons probably don't have refined
395 expression states in mouse among the E14.5 and P0 stages (Fig. S6D).
396
397 In order to detect the biological processing of PFC and V1 in human, We therefore selected
398 expression differentially genes for pathway analysis by gene ontology resource and
399 functional protein association networks (STRING) [72](Fig. 6C, S6D, E, F). Axon guidance,

400 axonogenesis, axon development were active in PFC and V1 (Fig. 6C). Pathway relative
401 to synapse signaling, including synapse organization, modulation of chemical synaptic
402 transmission, regulation of trans-synaptic signaling and synapse structure or activity,
403 synapse maturation, axonal transport of mitochondrion, were more active in V1 (Fig. 6C).
404 In PFC area, we enriched sensory system development and cognition, rather in V1, and
405 in PFC and V1, we all discovered visual system development and eye development (Fig.
406 6C).
407
408 To further examine the notion of typological distinction between PFC and V1 inner human
409 and mouse, we compared distinct co-expression profiles of genes involved in the
410 specification of projection patterns during development, for example, *SATB2* and *BCL11B*
411 co-expressed in human maturing PFC deep layer and upper layer at later stages of
412 development (Fig. 6D). In contrast, neurons in V1 had distinguish expression pattern of
413 *BCL11B* and *SATB2* (Fig. 6D). More interestingly, *Bcl11b* and *Satb2* also had co-
414 expression phenomenon in mouse, but it appeared in the immature neurons, indicating
415 that the early fate specification [18] (Fig. 6D). Then, we found that it is common that
416 maturing PFC neurons co-expressed genes in deeper and upper layer cells, for example,
417 *ABCA1* and *VCAN*, which couldn't express in deep layer in mouse (Fig. 6E, F). Additionally,
418 we focused on the function of these genes, which may play role in intercellular signaling
419 and extracellular structure organization.
420
421 **Conservation of radial glia developmental trajectories**
422
423 The comparison of excitatory neurons is crucial for our understanding of many differences
424 between human and mouse developing cortex. However, they are many cell types that
425 haven't been compared. We further analyzed the developmental trajectories in radial glia.
426 We observed there are two RG clusters in cross-species clustering (cluster 10 RG and 11

427 RG) (Fig. 1D), which had similar human and mouse cells proportions (Fig. 2A, B). During
428 early development, dorsal telencephalic radial glia produces excitatory cortical neurons,
429 while ventral telencephalic produces inhibitory cortical interneuron during 7 to 23 pcw in
430 human that have been reported [9]. We applied principal component analysis (PCA) for
431 cross-species clustering that observed 11 RG migrate to 13 GE (Interneuron migrate
432 tangentially from the ganglionic eminences) and 12 G2/M, and 10 RG migrate to 00 SVZ
433 (Fig. S7A, B), and then, we chose these clusters to reconstruct the trajectories analysis
434 (Fig. 1D), that we found two lineages: from 11 RG to 13 GE and 12 G2/M or 10 RG to 00
435 SVZ (Fig. 7A). Group of cells expressing *MKI67*, markers of proliferating and glia, were
436 detected at early timepoint at the pseudo-time analysis by Monocle, while *EOMES* and
437 *NEUROD6* expressed in SVZ branch, and *GAD2* expressed in GE branch (Fig. 7B). We
438 detected that these developing processes have been emerged at stage 2 (E14.5 in mouse
439 and 7- to 11.5 pcw in human). We use species-specific clusters to analysis with four
440 markers to depict the expression profile (Fig. S7B, C, D, E).
441
442 In order to detect the gene expression relative to cell fate commitment, we identified 80
443 genes highly dispersed in human and mouse. DE genes that fall into 5 clusters and
444 depicted successive waves of gene expression in different branches (Fig. S7F). Genes
445 predominantly expressing at the 11 RG branch enriched in pathways, including nuclear
446 division, mitotic nuclear division and sister chromatid segregation, suggesting that these
447 cells stayed in G2/M stages (Fig. S7F). Genes co-expressed in 10 RG and 11 RG enriched
448 in epithelial cell proliferation, gliogenesis, astrocyte differentiation, as expected in radial
449 glia (Fig. S7F). GE cells expressed genes, relating to cerebral cortex GABAergic
450 interneuron differentiation (Fig. S7F).
451
452 To examined two RG expression profile and enrichment pathways in human and mouse,
453 we detect expression differentially genes in these two clusters (Fig. 7C). 11 RG expressed

454 some proliferating marker genes, including *MKI67*, *TOP2A* and *UBE2C*, and regulate cell
455 cycle genes, like *CDK1* and *TPX2*, which co-expressed in human and mouse ventral
456 telencephalic radial glia (Fig. 7C); while dorsal telencephalic radial glia expressed three
457 kinds genes, *HES5* (RG marker), *EOMES* (SVZ markers) and *DACH1* (enriched in
458 ventricular radial glia cells) [73] (Fig. 7C), that related to olfactory bulb development and
459 olfactory lobe development, which also enriched in SVZ (Fig. 7D).
460
461 As many proliferating and cell cycle genes high expressed in 11 RG, rather than 10 RG,
462 we further analysis the cell cycle of cell types. Excitatory neuron exhibited a profile highly
463 similar to interneuron that stay at S states, and 10 RG kept at G1/S states. In contrast, 11
464 RG, 12 G2/M and 13 GE continued cell division (Fig. 7E, F).
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481 **DISCUSSION**

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483 We have systematically analyzed scRNA-seq data to integrate and compare developing
484 human and mouse cerebral cortex, including identified cell type diversities, developing
485 trajectories, gene expression patterns, functional protein association networks, signal
486 transduction pathways and ligand receptor interaction analysis.

487

488 According to previous reports, neocortical projection neurons are generated in an "inside-
489 out" fashion by diverse progenitor types in the VZ and SVZ, for example, cut-like
490 homeobox 2-positive (*Cux2*+) progenitors produce the most superficial-layer (upper) [74,
491 75], which is resided callosal projection neurons (CPN) and high expression marker gene
492 (SATB2), whereas corticofugal-layer (deep) neurons derive from Cux2-negative (*Cux2*–)
493 progenitors, which include a large number of corticothalamic projection neurons (CThPN)
494 that reside in layer VI, and subcerebral projection neurons (SCPN) that reside in layer V
495 and express BCL11B and FEZF2 [28]. As the result of the basis of current evidence, a
496 number of different models are produced, In line with a previous report, we detected the
497 'sequential competence states' model is undesirable in mouse and human [28], because
498 the mouse early developing deep layer (02 EXC deep) expressed an upper layer marker
499 (*SATB2*), a deep layer marker (*BCL11B*), a migratory marker (*TIMA2*) and a transcription
500 factor that is expressed in Layer II–III neurons during their migration and differentiation
501 (*POU3F2*) (Fig. S8B), and human developing trajectories (Fig. 3A), suggesting that there
502 is unreasonable that individual progenitors (*Cux2*–) produce a single neuronal subtype:
503 CThPN at a time [28] (Fig. S8F). More significantly, according to our study, we indicated
504 human and mouse exist two developing models. For human, we observed the
505 reconstructed excitatory neuron trajectory, indicating that the deep and upper layer migrate
506 from SVZ independently (Fig. 3A), whereas the deep layer and upper layer ordering are
507 chaos in mouse developing neocortex trajectory (Fig. 3B), and then, we detected the

508 ligand and receptors have differentially expression in human and mouse (Fig. 4E, S5A, B).

509

510 Additionally, *CUX2* expressed differentially in human and mouse, for example, *CUX2* only
511 expressed in upper layer in human (Fig. S8C), while *Cux2* have expression in maturing
512 mouse deep layer neuron (Fig. S8D), indicating that *Cux2*- positive lineage after
513 generating deep-layer neuronal sub-types change their competence state to express *Cux2*
514 and produce CPN to generate upper-layer. The results may suggest that human is more
515 likely to similar the model "independent lineages" and the model "nested multipotential
516 lineages" is more likely to exist in mouse [28] (Fig. 8A).

517

518 We further summary the divergence and conservation of ligand receptor interaction
519 analysis between human and mouse neocortical projection neurons [46] (Fig. 8B). We
520 predicted the neuron migration and differentiation between SVZ and layers that could
521 potentially be triggered by protein-interactions: *PTN_PTPRZ1* and *MDK_PTPRZ1* [50]
522 (Fig. 8B), meanwhile these expression patterns show high expressed in human SVZ to
523 deep layers and upper layers, and mouse SVZ to deep layers and upper layers, and deep
524 layers to upper layers, which are consistent with our predicted two models: human for
525 "independent lineages" and mouse for "nested multipotential lineages" (Fig. 8B, S8E, F).
526 Then, we observed *NOTCH, EFNA*, *EPHA*, *WNT*, *ACKR* and *CCL* signaling in human
527 deep and upper layer with significant function in the development of synaptic and dendritic
528 cells [53] (Fig. 8B).

529

530 Our data also suggest that cognition-linked genes might form robust groupings according
531 species-specific and cell-type expression profiles. We detected many cognition-relative
532 genes expressed earlier in human than mouse, including *PTPRZ1*, *CNR1*, *PTN*, and
533 *CLSTN2* (Fig. 4A, 5A, B, C, S5A, B), that enriched in cognition, learning and memory and
534 behavior pathways [45, 63, 76] (Fig. 5E, S5F), therefore understanding human cognition

535 development is probably earlier than those of mouse. In conclusion, our single-cell
536 analysis provides an essential resource for future studies directed at understanding the
537 difference developing neocortical trajectories, and excitatory neuron sub-types expression
538 profiles, and cognition-relative genes expression profiles during the early human and
539 mouse brain development.
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562 **METHODS**

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564 **Data processing of scRNA-seq from Chromium system**

565 In the early data processing, we applied the processed gene-cell data matrix without poor-
566 quality cells from published study. We used Seurat package (v3.1.2) to process secondary
567 filtration [77]. Only cells that expressed more than 500 genes were considered, only genes
568 expressed in at least 5 single cells were included for further analysis. Additionally, we
569 selected the homologous genes between human and mouse to process further analysis.
570 In total, 12350 genes across 18446 mouse and 6623 human single cells remained for
571 subsequent analysis. The data were normalized to a total of 1×10^4 molecules per cell for
572 the sequencing depth using the Seurat package. The batch effect was mitigated by using
573 the ScaleData function of Seurat (v3.1.2).

574

575 **Data integration of scRNA-seq and identification of cell types**

576 The Seurat package (v3.1.2) was used to integrate human and mouse scRNA-seq data.
577 We applied the new normalization method 'SCTransform' to process cross-species
578 clustering in order to better operation downstream integration [77]. Then, the Seurat
579 package was used to perform linear dimensional reduction. we used
580 SelectIntegrationFeatures or FindVariableFeatures function from Seurat and further
581 analysis used 'FindIntegrationAnchors' to select 2000 highly variable genes as input for
582 PCA. Then we identified significant PCs based on the JackStrawPlot function. Strong PC1-
583 PC30 were used for UMAP to cluster the cells by FindClusters function with resolution 0.8.
584 Clusters were identified by the expression of known cell-type markers. The markers
585 EOMES, NEUROD6, GAD2 and MKI67 were used to identify the basic cell-type, including
586 progenitor cells, excitatory neurons, inhibitory neurons and astrocytes, respectively.

587

588 **Identification of DEGs between clusters**

589 The DEGs of each cluster were identified using FindAllMarkers function (min.pct = 0,
590 logfc.threshold = 0, test.use = “wilcox”) with the Seurat R package [77]. We used the
591 Wilcoxon rank-sum test (default), and top 10 DE genes ($p < 0.05$) by fold change were
592 identified for each cluster.
593
594 **Correlation Analysis**
595 For correlation analysis of merged human single cell and mouse single cell data, we used
596 integrated data from Seurat package and the Pearson-correlation coefficient calculated in
597 Hmisc package (version 4.3-0), and the relative visualization used package pheatmap
598 (version 1.0.12).
599
600 **Constructing single cell trajectories in cerebral cortex**
601 The Monocle 2 R package (version 2.10.1) were applied to construct single cell pseudo-
602 time trajectories to discover developmental transitions [78]. We used data matrix from
603 Seurat with 2000 highly variable genes into pseudo-time order. we then called
604 ‘differentialGeneTest’ to process DEGs analysis in different cell-types. ‘DDRTree’ were
605 applied to reduce dimensional space and “orderCells” were used to sort cells. The
606 minimum spanning tree on cells and marker genes expression profiles were plotted using
607 the visualization functions “plot_cell_trajectory” for Monocle 2
608
609 **Identifying genes relative to cell fate choices**
610 We analyzed branches in single cell trajectories by BEAM and calILRs function, and we
611 selected genes with q-value < 0.01 and a mean expression value ≥ 0.18, Genes
612 dynamically expressed along the different branches were clustered using the
613 plot_multiple_branches_heatmap function and plot_genes_branched_heatmap function,
614 and Enriched GO terms of cluster-grouped genes were identified using clusterProfiler
615 package (version 3.10.1) [79]. Then, genes were clustered into different clusters by using

616 ‘plot_multiple_branches_pseudotime’ and ‘plot_genes_branched_pseudotime’ to
617 visualize the expression profiles in different cell-types.
618
619 **GO enrichment analysis**
620 Gene ontology (GO) biological process enrichment analysis were performed on differently
621 expressed genes (DEGs) (p-value < 0.05, fold change (FC) > 1) using clusterProfiler
622 package (version 3.10.1) and Metascape [79, 80].
623
624 **Analysing inter-lineage interactions within the neocortex**
625 For comprehensive systematic analysis of inter-lineage interactions within the neocortex,
626 we use CellPhoneDB [46]. We used gene-cell matrix data from Seurat packages after
627 integration as the input data, and we considered only ligands and receptors expressed in
628 greater than 10% of the cells in any given subpopulation. Then, we seted the P-Value 0.05
629 to ensure the prominence. We called ‘cellphonedb plot dot_plot’ to visualize the ligand-
630 receptor interactions within the neocortical projection neuron.
631
632 **Comparing gene expression pattern divergence**
633 The pseudo-time of each cell was assigned by Monocle2, and used median function in R
634 to calculate the mean of pseudo-time in mouse and human trajectories, dividing cells into
635 two categories, including early stage of the cells and the latter stage of cells. and we
636 reconstructed the clustering included 132500 homologous genes within human and
637 mouse by using Seurat package [77, 78]. FindMarkers function helps us to calculate the
638 expression profiles between these two groups, and genes with average expression
639 difference > 0.585 natural log with $P < 0.05$ were selected as DEGs between human and
640 mouse. a part of genes visualized by ‘plot_pseudotime_heatmap’ function.
641
642 **Functional protein association networks analysis**

Functional protein association networks were calculated on DEGs using STRING, the results visualized using Cytoscape [72, 81].

Connecting transcription factors to target genes

To find the potential transcription factors, TRRUST was used for search transcriptional regulatory interactions [82], and the results visulized using Cytoscape [72, 81].

Cell-cycle analysis

In the cell-cycle analysis, we applied scran packge in R (version 1.10.2) to score G1, S and G2/M states of each cells [83]. we selected a cell-cycle related gene set with 43 genes expressed during S and 54 genes expressed during G2/M from Seurat package [77]. These gene sets should be anti-correlated in their expression levels, and cells expressing neither are likely to be in the G1 phase (not cycling).

Statistical analysis

Comparisons between two groups were made using wilcox. Sample size and P values are given in the Figure legends.

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894

Figure1

A

Species	Age	Cells	Tissue	Gene number	Reference
Mouse	E14.5 and P0	18,446/18446	Cerebral cortex	13,250	
Human	5.85 to 37 pcw	6,655/6623	Cerebral cortex	13,250	

Species	Age	Cells	Tissue	Gene number	Reference
Mouse	E14.5 and P0	6,655/6655	Cerebral cortex	13,250	
Human	5.85 to 37 pcw	6,655/6623	Cerebral cortex	13,250	

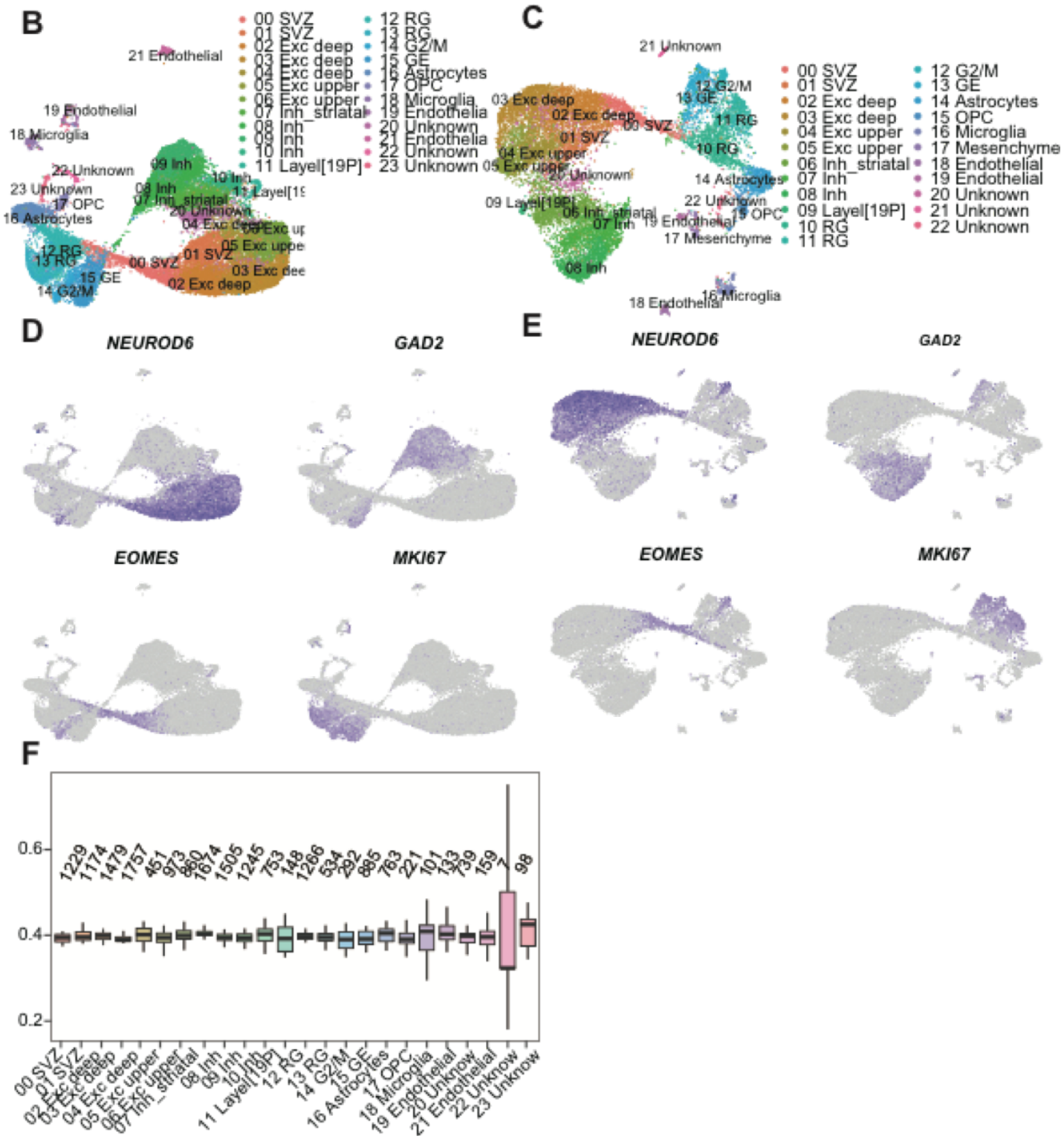


Figure 2

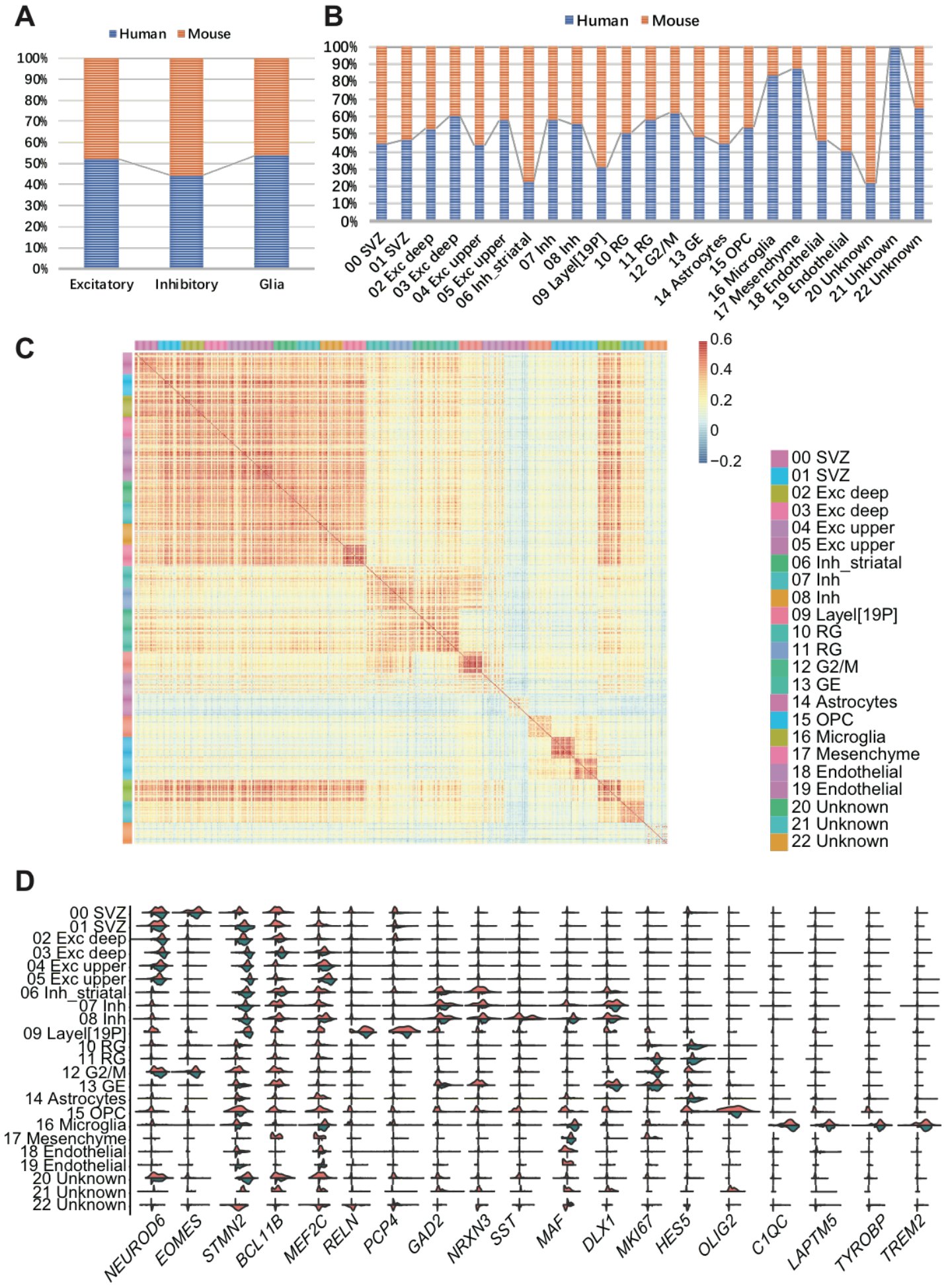

A
Human
Mouse
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Excitatory
Inhibitory
Glia
B
Human
Mouse
00 SVZ
01 SVZ
02 Exc deep
03 Exc deep
04 Exc upper
05 Exc upper
06 Inh_striatal
07 Inh
08 Inh
09 Layel[19P]
10 RG
11 RG
12 G2/M
13 GE
14 Astrocytes
15 OPC
16 Microglia
17 Mesenchyme
18 Endothelial
19 Endothelial
20 Unknown
21 Unknown
22 Unknown
C
0.6
0.4
0.2
0
−0.2
00 SVZ
01 SVZ
02 Exc deep
03 Exc deep
04 Exc upper
05 Exc upper
06 Inh_striatal
07 Inh
08 Inh
09 Layel[19P]
10 RG
11 RG
12 G2/M
13 GE
14 Astrocytes
15 OPC
16 Microglia
17 Mesenchyme
18 Endothelial
19 Endothelial
20 Unknown
21 Unknown
22 Unknown
D
NEUROD6
EOMES
STMN2
BCL11B
MEF2C
RELN
PCP4
GAD2
NRXN3
SST
MAF
DLX1
MKI67
HES5
OLIG2
C1QC
LAPTM5
TYROBP
TREM2

Figure 3

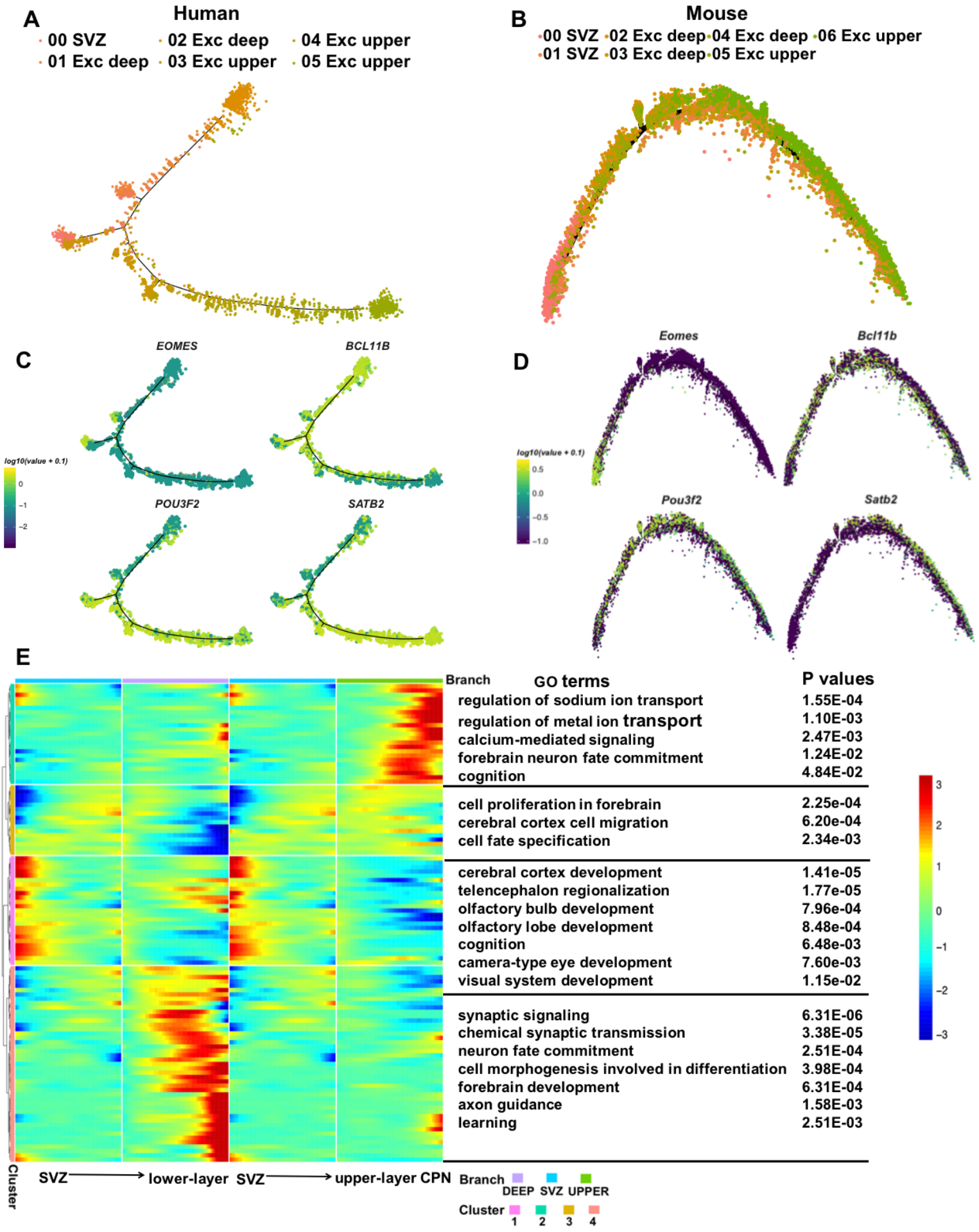

A
Human
00 SVZ
01 Exc deep
02 Exc deep
03 Exc upper
04 Exc upper
05 Exc upper
B
Mouse
00 SVZ
01 SVZ
02 Exc deep
03 Exc deep
04 Exc deep
05 Exc upper
06 Exc upper
C
EOMES
BCL11B
POU3F2
SATB2
log10(value + 0.1)
D
Eomes
Bcl11b
Pou3f2
Satb2
log10(value + 0.1)
E
Branch
GO terms
P values
regulation of sodium ion transport 1.55E-04
regulation of metal ion transport 1.10E-03
calcium-mediated signaling 2.47E-03
forebrain neuron fate commitment 1.24E-02
cognition 4.84E-02
cell proliferation in forebrain 2.25e-04
cerebral cortex cell migration 6.20e-04
cell fate specification 2.34e-03
cerebral cortex development 1.41e-05
telencephalon regionalization 1.77e-05
olfactory bulb development 7.96e-04
olfactory lobe development 8.48e-04
cognition 6.48e-03
camera-type eye development 7.60e-03
visual system development 1.15e-02
synaptic signaling 6.31E-06
chemical synaptic transmission 3.38E-05
neuron fate commitment 2.51E-04
cell morphogenesis involved in differentiation 3.98E-04
forebrain development 6.31E-04
axon guidance 1.58E-03
learning 2.51E-03
Cluster
SVZ → lower-layer SVZ → upper-layer CPN
Branch
DEEP SVZ UPPER
Cluster
1 2 3 4

Figure 4

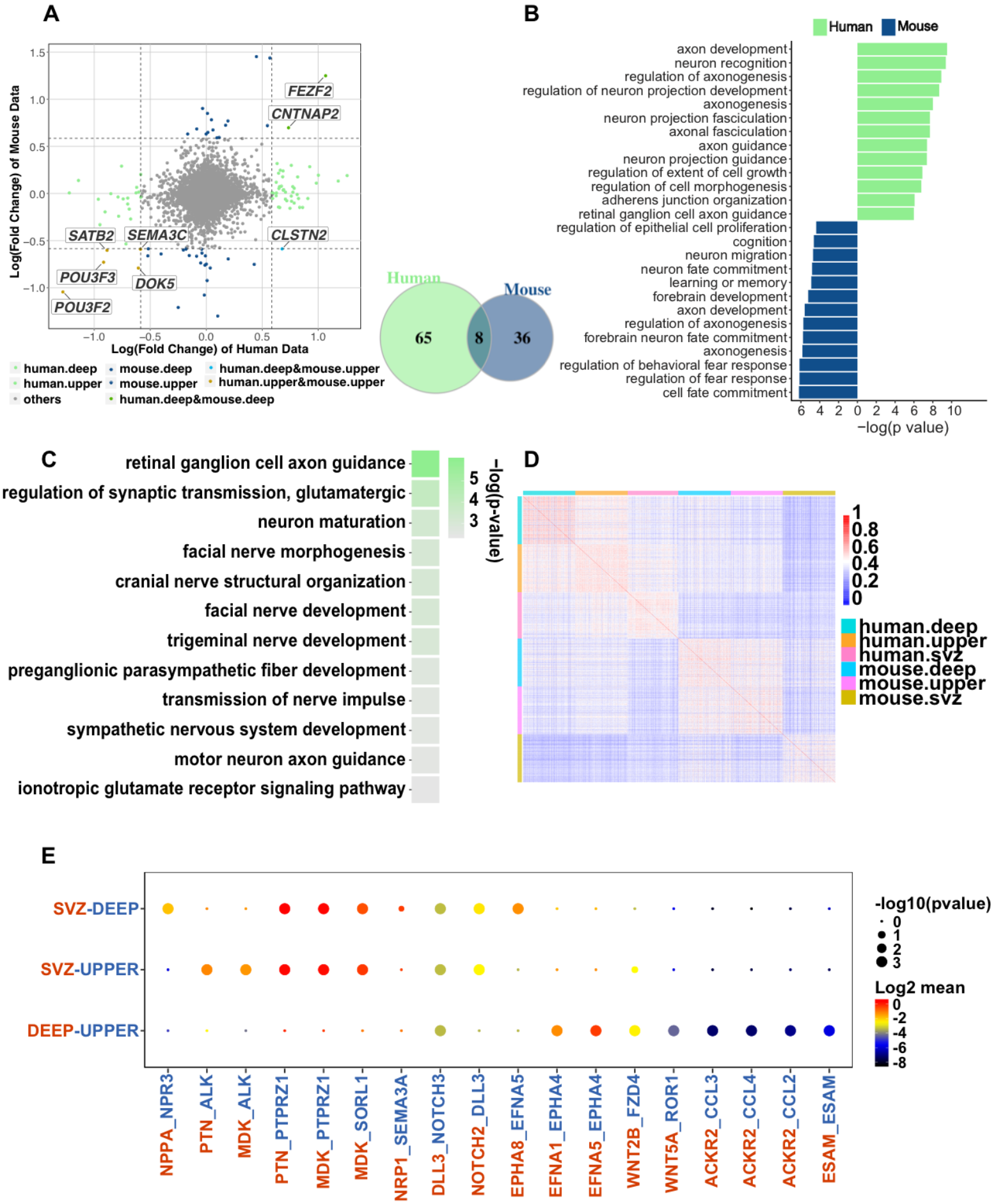

A
Log(Fold Change) of Mouse Data
Log(Fold Change) of Human Data
FEZF2
CNTNAP2
SATB2
SEMA3C
CLSTN2
POU3F3
DOK5
POU3F2
human.deep
human.upper
others
mouse.deep
mouse.upper
human.deep&mouse.deep
human.deep&mouse.upper
human.upper&mouse.upper
Human
Mouse
65
8
36
B
Human
Mouse
axon development
neuron recognition
regulation of axonogenesis
regulation of neuron projection development
axonogenesis
neuron projection fasciculation
axonal fasciculation
axon guidance
neuron projection guidance
regulation of extent of cell growth
regulation of cell morphogenesis
adherens junction organization
retinal ganglion cell axon guidance
regulation of epithelial cell proliferation
cognition
neuron migration
neuron fate commitment
learning or memory
forebrain development
axon development
regulation of axonogenesis
forebrain neuron fate commitment
axonogenesis
regulation of behavioral fear response
regulation of fear response
cell fate commitment
−log(p value)
C
retinal ganglion cell axon guidance
regulation of synaptic transmission, glutamatergic
neuron maturation
facial nerve morphogenesis
cranial nerve structural organization
facial nerve development
trigeminal nerve development
preganglionic parasympathetic fiber development
transmission of nerve impulse
sympathetic nervous system development
motor neuron axon guidance
ionotropic glutamate receptor signaling pathway
−log(p-value)
D
human.deep
human.upper
human.svz
mouse.deep
mouse.upper
mouse.svz
E
SVZ-DEEP
SVZ-UPPER
DEEP-UPPER
NPPA_NPR3
PTN_ALK
MDK_ALK
PTN_PTPRZ1
MDK_PTPRZ1
MDK_SORL1
NRP1_SEMA3A
DLL3_NOTCH3
NOTCH2_DLL3
EPHA8_EFNA5
EFNA1_EPHA4
EFNA5_EPHA4
WNT2B_FZD4
WNT5A_ROR1
ACKR2_CCL3
ACKR2_CCL4
ACKR2_CCL2
ESAM_ESAM
-log10(pvalue)
Log2 mean

Figure 5

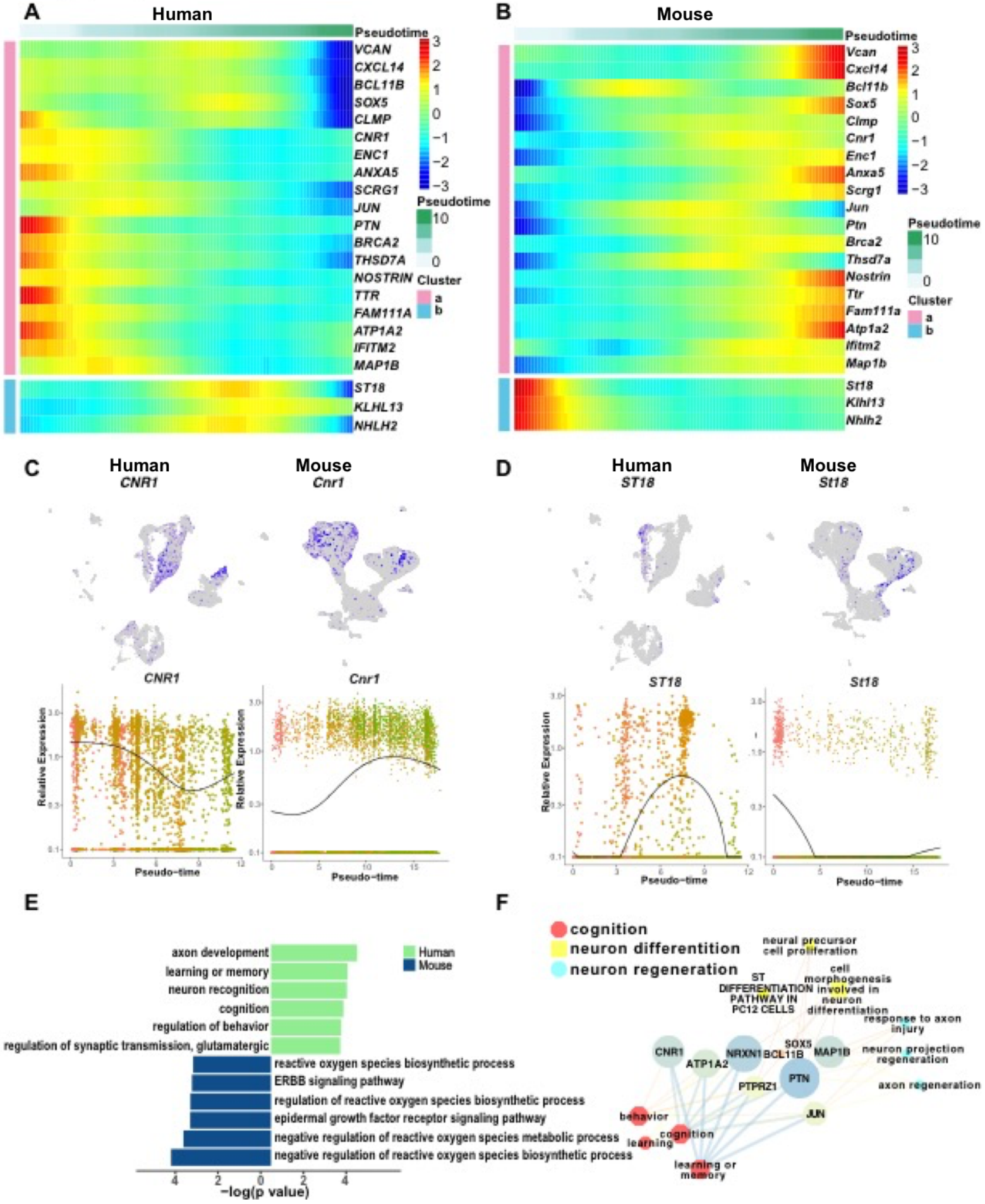

A
Human
Pseudotime
VCAN
CXCL14
BCL11B
SOX5
CLMP
CNR1
ENC1
ANXA5
SCRG1
JUN
PTN
BRCA2
THSD7A
NOSTRIN
TTR
FAM111A
ATP1A2
IFITM2
MAP1B
ST18
KLHL13
NHLH2
Cluster
a
b
B
Mouse
Vcan
Cxcl14
Bcl11b
Sox5
Clmp
Cnr1
Enc1
Anxa5
Scrg1
Jun
Ptn
Brca2
Thsd7a
Nostrin
Ttr
Fam111a
Atp1a2
Ifitm2
Map1b
St18
Klhl13
Nhlh2
C
Human
CNR1
Mouse
Cnr1
Relative Expression
Pseudo-time
D
Human
ST18
Mouse
St18
E
axon development
learning or memory
neuron recognition
cognition
regulation of behavior
regulation of synaptic transmission, glutamatergic
reactive oxygen species biosynthetic process
ERBB signaling pathway
regulation of reactive oxygen species biosynthetic process
epidermal growth factor receptor signaling pathway
negative regulation of reactive oxygen species metabolic process
negative regulation of reactive oxygen species biosynthetic process
Human
Mouse
−log(p value)
F
cognition
neuron differentition
neuron regeneration
neural precursor cell proliferation
ST DIFFERENTIATION PATHWAY IN PC12 CELLS
cell morphogenesis involved in neuron differentiation
response to axon injury
neuron projection regeneration
axon regeneration
CNR1
ATP1A2
NRXN1
SOX5
BCL11B
MAP1B
PTPRZ1
PTN
JUN
behavior
cognition
learning
learning or memory

Figure 6

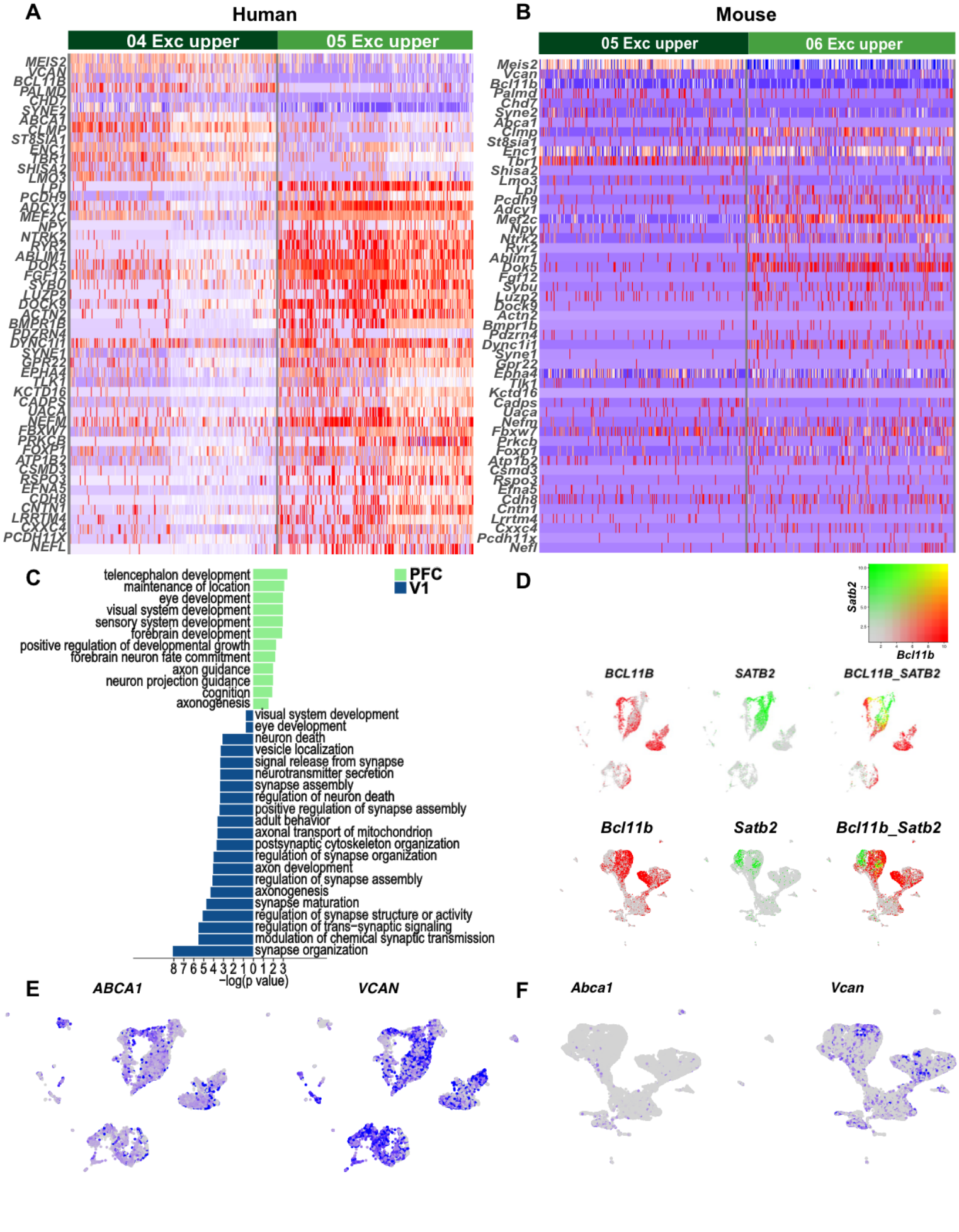

A
Human
04 Exc upper
05 Exc upper
MEIS2
VCAN
BCL11B
PALMD
CHD7
SYNE2
ABCA1
CLMP
ST8SIA1
ENC1
TBR1
SHISA2
LMO3
LPL
PCDH9
ADCY1
MEF2C
NPY
NTRK2
RYR2
ABLIM1
DOK5
FGF12
SYBU
LUZP2
DOCK9
ACTN2
BMPR1B
PDZRN4
DYNC1I1
SYNE1
GPR22
EPHA4
TLK1
KCTD16
CADPS
UACA
NEFM
FBXW7
PRKCB
FOXP1
ATP1B2
CSMD3
RSPO3
EFNA5
CDH8
CNTN1
LRRTM4
CXXC4
PCDH11X
NEFL
B
Mouse
05 Exc upper
06 Exc upper
Meis2
Vcan
Bcl11b
Palmd
Chd7
Syne2
Abca1
Clmp
St8sia1
Enc1
Tbr1
Shisa2
Lmo3
Lpl
Pcdh9
Adcy1
Mef2c
Npy
Ntrk2
Ryr2
Ablim1
Dok5
Fgf12
Sybu
Luzp2
Dock9
Actn2
Bmpr1b
Pdzrn4
Dync1i1
Syne1
Gpr22
Epha4
Tlk1
Kctd16
Cadps
Uaca
Nefm
Fbxw7
Prkcb
Foxp1
Atp1b2
Csmd3
Rspo3
Efna5
Cdh8
Cntn1
Lrrtm4
Cxxc4
Pcdh11x
Nefl
C
PFC
V1
telencephalon development
maintenance of location
eye development
visual system development
sensory system development
forebrain development
positive regulation of developmental growth
forebrain neuron fate commitment
axon guidance
neuron projection guidance
cognition
axonogenesis
visual system development
eye development
neuron death
vesicle localization
signal release from synapse
neurotransmitter secretion
synapse assembly
regulation of neuron death
positive regulation of synapse assembly
adult behavior
axonal transport of mitochondrion
postsynaptic cytoskeleton organization
regulation of synapse organization
axon development
regulation of synapse assembly
axonogenesis
synapse maturation
regulation of synapse structure or activity
regulation of trans-synaptic signaling
modulation of chemical synaptic transmission
synapse organization
8 7 6 5 4 3 2 1 0 1 2 3
−log(p value)
D
Satb2
Bcl11b
BCL11B
SATB2
BCL11B_SATB2
Bcl11b
Satb2
Bcl11b_Satb2
E
ABCA1
VCAN
F
Abca1
Vcan

Figure 7

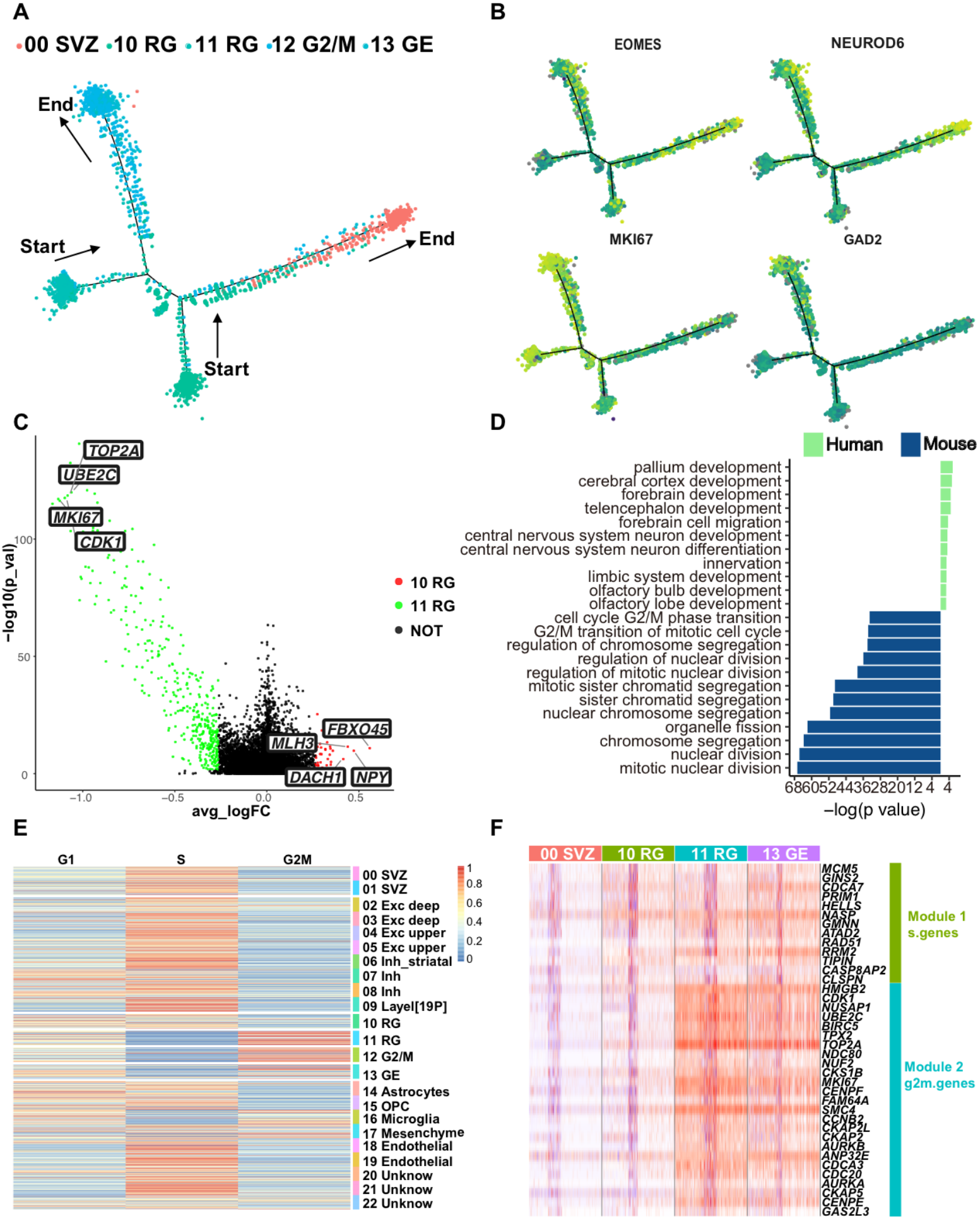
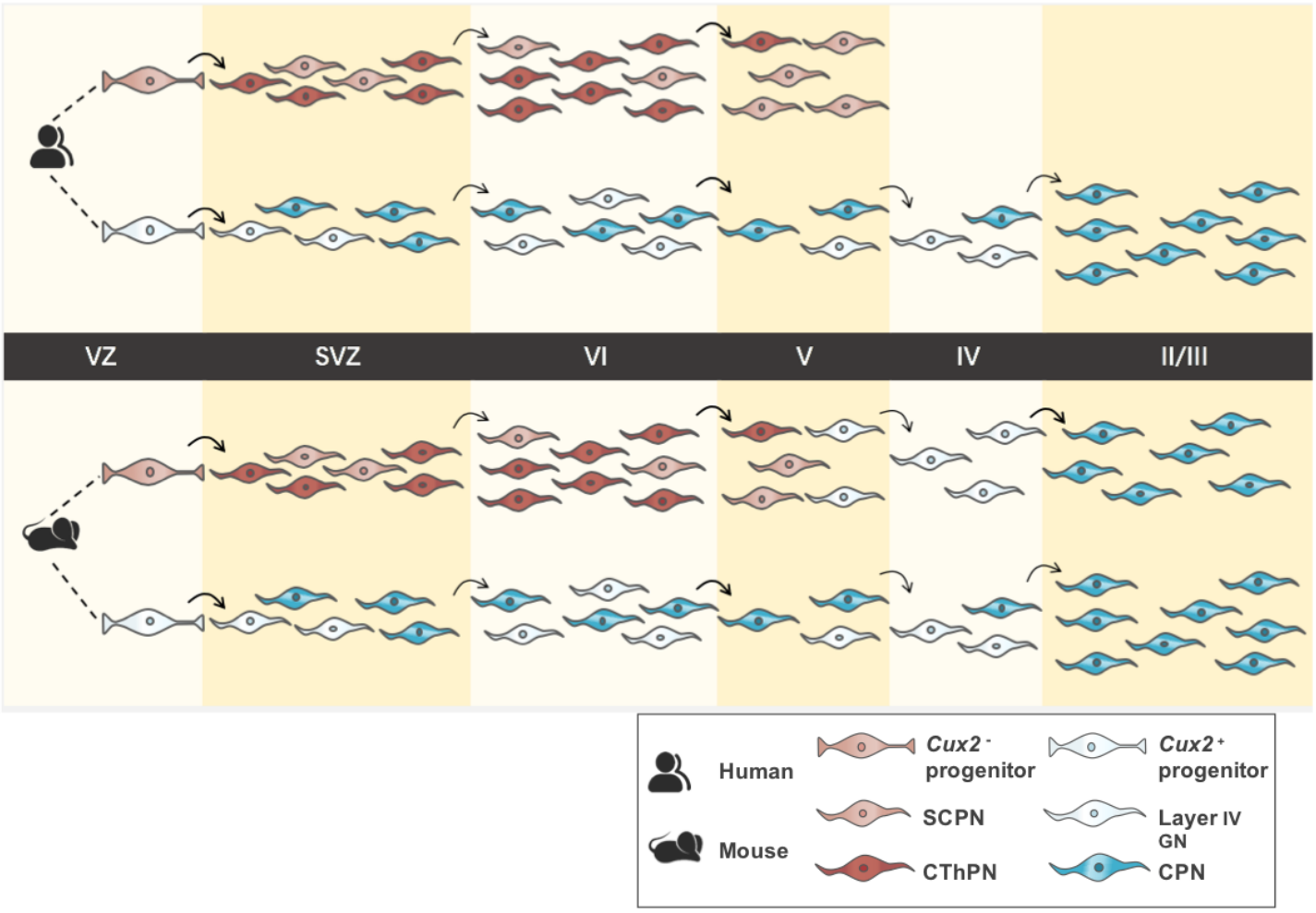
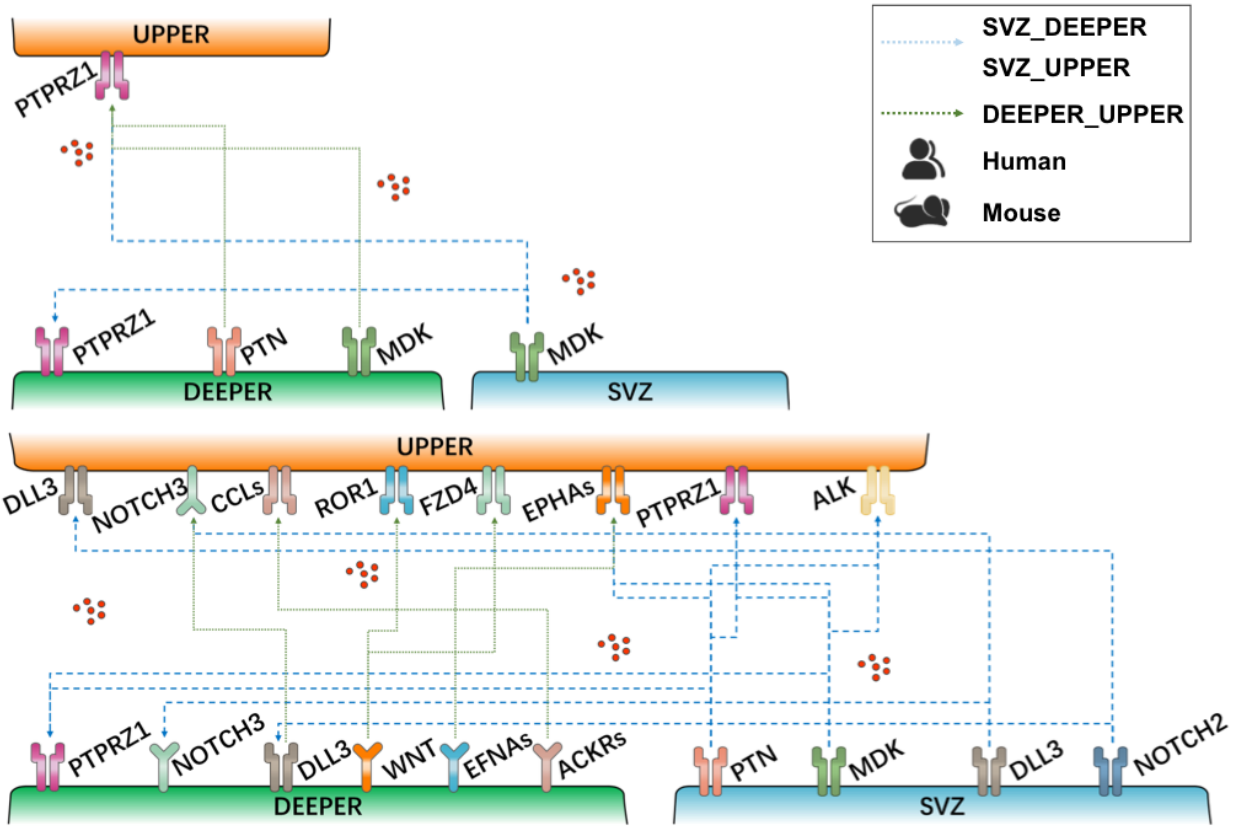

A
00 SVZ
10 RG
11 RG
12 G2/M
13 GE
End
Start
End
Start
B
EOMES
NEUROD6
MKI67
GAD2
C
TOP2A
UBE2C
MKI67
CDK1
FBXO45
MLH3
DACH1
NPY
10 RG
11 RG
NOT
−log10(p_val)
avg_logFC
D
Human
Mouse
pallium development
cerebral cortex development
forebrain development
telencephalon development
forebrain cell migration
central nervous system neuron development
central nervous system neuron differentiation
innervation
limbic system development
olfactory bulb development
olfactory lobe development
cell cycle G2/M phase transition
G2/M transition of mitotic cell cycle
regulation of chromosome segregation
regulation of nuclear division
regulation of mitotic nuclear division
mitotic sister chromatid segregation
sister chromatid segregation
nuclear chromosome segregation
organelle fission
chromosome segregation
nuclear division
mitotic nuclear division
−log(p value)
E
G1
S
G2M
00 SVZ
01 SVZ
02 Exc deep
03 Exc deep
04 Exc upper
05 Exc upper
06 Inh_striatal
07 Inh
08 Inh
09 Layel[19P]
10 RG
11 RG
12 G2/M
13 GE
14 Astrocytes
15 OPC
16 Microglia
17 Mesenchyme
18 Endothelial
19 Endothelial
20 Unknow
21 Unknow
22 Unknow
F
00 SVZ
10 RG
11 RG
13 GE
MCM5
GINS2
CDCA7
PRIM1
HELLS
NASP
GMNN
ATAD2
RAD51
RRM2
TIPIN
CASP8AP2
CLSPN
HMGB2
CDK1
NUSAP1
UBE2C
BIRC5
TPX2
TOP2A
NDC80
NUF2
CKS1B
MKI67
CENPF
FAM64A
SMC4
CCNB2
CKAP2L
CKAP2
AURKB
ANP32E
CDCA3
CDC20
AURKA
CKAP5
CENPE
GAS2L3
Module 1
s.genes
Module 2
g2m.genes

Figure 8

A


VZ
SVZ
VI
V
IV
II/III
Human
Mouse
Cux2⁻ progenitor
Cux2⁺ progenitor
SCPN
Layer IV GN
CThPN
CPN

B


SVZ_DEEPER
SVZ_UPPER
DEEPER_UPPER
Human
Mouse
UPPER
PTPRZ1
PTPRZ1
PTN
MDK
MDK
DEEPER
SVZ
UPPER
DLL3
NOTCH3
CCLs
ROR1
FZD4
EPHAs
PTPRZ1
ALK
PTPRZ1
NOTCH3
DLL3
WNT
EFNAs
ACKRs
DEEPER
PTN
MDK
DLL3
NOTCH2
SVZ