TITLE: Single-nuclei transcriptomics of dog hippocampus reveals the distinct cellular mechanism of domestication

AUTHORS

Qi-Jun Zhou^{1, #}, Xingyan Liu^{2,3, #}, Longlong Zhang^{1,5, #}, Rong Wang^{1,4,5}, Tingting Yin¹, Xiaolu Li⁶, Guimei Li¹, Yuqi He⁶, Zhaoli Ding⁶, Pengcheng Ma¹, Shi-Zhi Wang¹, Bingyu Mao^{1,4,5, *}, Shihua Zhang^{2,3,7,8, *}, and Guo-Dong Wang^{1,4,5,7,9, *}

AFFILIATIONS

¹State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223, China

²NCMIS, CEMS, RCSDS, Academy of Mathematics and Systems Science, Chinese Academy of Sciences, Beijing, 100190, China

³School of Mathematical Sciences, University of Chinese Academy of Sciences, Beijing, 100049, China

⁴College of Life Science, University of Chinese Academy of Sciences, Beijing, 100049, China

⁵Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, 650223, China

⁶Genomic Center of Biodiversity, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223, China

⁷Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, 650223, China

⁸Key Laboratory of Systems Biology, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Hangzhou, 310024, China

⁹Lead Contact

[#]These authors contributed equally

*Correspondence: wanggd@mail.kiz.ac.cn (G.-D. W.), zsh@amss.ac.cn (S.Z.), and mao@mail.kiz.ac.cn (B.M.)

30 **Abstract**

31 The process of dog domestication leads to dramatic differences in behavioral traits compared
 32 to grey wolves. A class of putative positively selected genes is related to learning and memory,
 33 for instance, long-term potentiation and long-term depression. In this study, we constructed a
 34 single-nuclei transcriptomic atlas of the dog hippocampus to illustrate its cell types, cell
 35 lineage, and molecular features. Using the transcriptomes of 105,057 single-nuclei from the
 36 hippocampus of a Beagle dog, we identified 26 cell clusters and a putative trajectory of
 37 oligodendrocyte development. Comparative analysis revealed a significant convergence
 38 between dog differentially expressed genes (DEGs) and putative positively selected genes
 39 (PSGs). 40 putative PSGs were DEGs in the glutamatergic neurons, especially in the cluster
 40 14, which is related to the regulation of nervous system development. In summary, this study
 41 provided a blueprint to understand the cellular mechanism of dog domestication.

42

43 **Keywords:** dog, snRNA-seq, hippocampus, domestication.

44 **Introduction**

45 The process of animal domestication leads to dramatic differences in behavioral and
 46 morphological traits compared to their wild ancestors (Plassais, et al. 2019; Wang, et al. 2020).
 47 Shared with the human being for parallel evolution and convergence evolution in the history
 48 of early hunting-gathering time and the recent living environment change from agrarian
 49 societies to modern urban lifestyle (Wang, et al. 2013; Wang, et al. 2016; Cao, et al. 2021; Liu,
 50 et al. 2021), domestic dogs have been undergoing strong artificial selection and resulted in
 51 approximately 450 globally recognized breeds, which makes them the most variable
 52 mammalian species on Earth (Hajeski 2016). Genome-wide scans for positive selection
 53 revealed that the behavioral and neurological traits are likely changed subsequently with the
 54 process of domestication. For instance, putative positively selected genes (PSGs) in dogs
 55 were reported linked to neural crest and central nervous system development (Pendleton, et al.
 56 2018). The gene expression in brains showed a strong difference of putative PSGs in neurons
 57 on learning, memory, and behavior between domestic animals and their wild relatives (Li, et
 58 al. 2013; Li, et al. 2014).

59
 60 The hippocampus is an important part of the limbic system in the brain that has an essential
 61 role in the consolidation of memory (Nadel and Moscovitch 1997; Bird and Burgess 2008). A
 62 class of domesticated genes is related to hippocampus synaptic long-term potentiation and
 63 long-term depression (Wang, et al. 2016). For instance, *DKK1* is expressed in the ventral
 64 hippocampus and is associated with increased susceptibility to social defeat stress in mice
 65 (Bagot, et al. 2016). The single-cell RNA sequencing (scRNA-seq) has been used to identify
 66 the cell type atlas (Zeisel, et al. 2015), revealed cellular and molecular dynamics in
 67 neurogenesis (Artegiani, et al. 2017; Hochgerner, et al. 2018), and decoded the hippocampus
 68 development and evolution (Tosches, et al. 2018; Zhong, et al. 2020). It is worth noting that
 69 dozens of putative PSGs were related to cell types of both excitatory and inhibitory neurons,
 70 contributed to the connectivity and development of synapses and dendrites (Li, et al. 2014;
 71 Pendleton, et al. 2018).

72

In the present study, we collected single nuclei from the hippocampus of a Beagle dog and performed the comparative analysis of gene expression profiles of 105,057 nuclei. We defined 26 cell clusters and identified a set of marker genes for each cell type. Interestingly, 86 differentially expressed genes (DEGs) were putative PSGs during dog domestication. The comprehensive cell landscape of the hippocampus could help us establish correspondence between cell types in nervous system and putative PSGs in dogs and facilitate the understanding of molecular features of cells during domestication.

Results

Single-nuclei transcriptomics of the dog hippocampus constructed using SPLiT-Seq

A standardized snRNA-seq pipeline was built using the SPLiT-seq (Rosenberg, et al. 2018). The scRNA-seq data of single nuclei from the hippocampus of a 5-month-old Beagle dog were obtained with the random and oligo(dT) primers. After detailed preprocessing and filtering (**Methods**), we created a digital expression matrix of 105,057 single nuclei with a median of 804 genes and 1,109 counts per nucleus. To remove the potential batch effects, partial principal component analysis (partial-PCA, **Methods**) was performed instead of the classical PCA and then uniform manifold approximation and projection (UMAP) was applied to project these two batches of transcripts into the common comparable two-dimensional (2D) space (**Figure S1A**). Furthermore, the Leiden community detection algorithm was employed to group these cells into 26 cell clusters (**Figure 1A**).

The DEGs (p-value < 0.001 and log2fold-change > 0.25) of each cell cluster were used to assign most of cells to specific cell types, based on the known markers in mouse and human (Zeisel, et al. 2015; Rosenberg, et al. 2018; Zhang, et al. 2019) (**Table S1** and **Figure S1B**). Glutamatergic neurons, GABAergic neurons, Cajal-Retzius cell, oligodendrocyte precursor cells (OPCs), myelinating oligodendrocytes, endothelial cells, astrocytes, and microglial cells were detected in the Beagle dog hippocampus (**Figure 1B, Figure S1C, D**). They are also typical cells in the mammalian hippocampus.

102 Among the clusters, 12 ones (0, 3, 5, 6, 7, 10, 11, 13, 14, 15, 17 and 20) were identified as the
 103 glutamatergic neurons, which produce the most common excitatory neurotransmitter in the
 104 central nervous system (Baude, et al. 2009). Although cluster 3, 5 and 6 have a similar
 105 expression pattern, there were unique DEGs expressed in each cluster. For instance, both
 106 cluster 3 and 6 have high expression of *GRM7*, *TENM2*, and *CACNA1E*, while cluster 3 has
 107 no expression of *STMN2* and *NEFL* (**Figure 1B**). The *CCBE1* is a marker gene specifically in
 108 cluster 0 (**Figure 1C**). It marked the canine hippocampus CA3, SP (**Figure 1D**) the same as
 109 that in the mouse (© 2020 Allen Institute for Brain Science. Allen Brain map. Available from:
 110 <http://mouse.brain-map.org/experiment/show/74513995>). Eight glutamatergic neurons
 111 clusters (cluster 0, 5, 6, 7, 10, 13, 14, 20) were involved in the regulation of trans-synaptic
 112 signaling (GO:0099177, **Table S2**). Seven clusters (cluster 5, 6, 7, 13, 14, 17, 20) were
 113 involved in the plasma membrane bounded cell projection morphogenesis (GO:0120039,
 114 **Table S2**). The GO enrichment analysis showed that cluster 7 and 13 were enriched in
 115 cognition (GO:0050890, **Table S2**), cluster 11 and 20 were enriched in behavior
 116 (GO:0007610, **Table S2**), and cluster 13 were enriched in adult locomotory behavior
 117 (GO:0008344, **Table S2**).

118
 119 Four clusters (cluster 4, 8, 12, and 21) are GABAergic neurons relating to a kind of inhibitory
 120 neurotransmitters in the central nervous system, indicated by the common marker gene *GAD2*
 121 (Zhang, et al. 2019) (**Figure 1E**). Immunofluorescence (IF) analysis showed that the *GAD2*
 122 was highly expressed in the GCL and ML of DG area, and in SLM and SP of CA area (**Figure**
 123 **1 F**). The GO enrichment analysis illustrated that four GABAergic neurons clusters enriched
 124 in the behavior (GO:0007610, **Table S2**) and the anterograde trans-synaptic signaling
 125 (GO:0098916, **Table S2**). Cluster 25 was identified as the Cajal-Retzius cell which is the
 126 project manager in the cerebral cortex and played a major role in the cortical development
 127 (Ogawa, et al. 1995; Villar-Cervino and Marin 2012; Meyer, et al. 2019). The marker gene of
 128 the cell type is *NDNF* (**Figure 1G, H**), which is also expressed in the Cajal-Retzius cells in
 129 human and mouse (Fan, et al. 2018; Rosenberg, et al. 2018). Beyond neuron cell clusters
 130 described above, another five clusters were representing non-neuron cells. Cluster 19 and 22

131 are two types of non-neuron cells. Cluster 1, 23, and 24 were identified as the astrocytes,
132 endothelial cells, and microglial cells, respectively.

133

134 *Putative trajectory analysis reveals oligodendrocyte development in dogs*

135 With the defined cell-type markers, cluster 2 and cluster 9 were inferred as myelinating
136 oligodendrocytes and OPCs respectively (**Figure 2A**). Consistent with the classification,
137 *CNP*, a myelin-related marker gene of cluster 2 (Yu, et al. 1994; Darmanis, et al. 2015), was
138 highly expressed in the Hilus, ML, SLM, F and A areas (**Figure 2B**). The *AGAPI* was a DEG in
139 cluster 9 and could be used as a new marker of OPCs (**Figure 2B**). Cluster 16 was linked in
140 cluster 2 and 9 (**Figure 1A**), with few specifically expressed genes to assign its identity.
141 Therefore, we inferred that cluster 2, 9, 16 might be a development trajectory from OPCs to
142 myelinating oligodendrocytes as the Alexander B. Rosenberg et al. 2018 found in the mouse
143 hippocampus (Rosenberg, et al. 2018).

144

145 To validate the hypothesis, we constructed the single-nuclei trajectories using the Slingshot
146 (Street, et al. 2018) based on the re-calculated UMAP embedding and the original cluster
147 labels (**Figure 2C**). As result, *PTPRZI*, the marker gene for precursor cells that maintained
148 OPCs in an undifferentiated state (Kuboyama, et al. 2012), was expressed in cluster 9; *SOX6*,
149 was expressed in cluster 16 and repressed oligodendrocyte differentiation (Stolt, et al. 2006);
150 *PLP1* is another myelin-related gene expressed in cluster 2 (**Figure S2A**). The GO
151 enrichment analysis showed that oligodendrocytes (cluster 2, 9 and 16) were involved in
152 different biological pathways (**Figure 2D**). The DEGs in cluster 9 (OPCs) were related to
153 neuron differentiation (GO:0030182, **Table S2**) and regulation of neurogenesis (GO:0050767,
154 **Table S2**). Those in cluster 16 were related to cell projection morphogenesis (GO:0048858,
155 **Table S2**) and neuron projection development (GO:0031175, **Table S2**). Those in cluster 2
156 were enriched in ensheathment of neurons (GO:0007272, **Table S2**) and axon ensheathment
157 (GO:0008366, **Table S2**). The DEGs of the putative trajectory were consistent with the
158 differentiation process of oligodendrocyte development, and we also verified this result on
159 mouse data (**Figure 2E, Figure S2B-E**).

160

161 *Significant convergence between DEGs and putative PSGs in domestication*

162 Previous studies showed that dog putative PSGs were enriched in the neurological process
163 such as learning and memory (Freedman, et al. 2016; Wang, et al. 2016) and expressed
164 specifically in brain tissues (Li, et al. 2013). To identify which type of cell plays an important
165 role during domestication, we gathered the DEGs in the 26 clusters (with p-value < 0.001 and
166 log2fold-change > 0.25) and compared them with the putative PSGs from published studies
167 (vonHoldt, et al. 2010; Axelsson, et al. 2013; Freedman, et al. 2016; Wang, et al. 2016;
168 Pendleton, et al. 2018). The genes expressed in more than three cells on the dog hippocampus
169 were used as the background (**Methods**). The results showed that 630 of 841 putative PSGs
170 were detected in the hippocampus single nuclei transcriptomes (**Table S3, Figure S3**). 86 of
171 630 detected putative PSGs occurred in the 1,628 DEGs with a statistical significance of
172 5.10E-09 (**Table 1**). However, the 1,000 random gene sets with equal size are not significant
173 with the p-value of 0.47. Since Freedman et al. 2016 did a statistical analysis to determine the
174 likelihood that specific genes were under selection, we used their putative PSGs to verify the
175 statistical significance. As result, 16 genes occurred in both DEGs and putative PSGs with the
176 p-value of 6.99E-03. Furthermore, there is also a significant overlap between DEGs and the
177 putative PSGs reported at least in two published studies (p-value of 2.45E-07, **Table S4**).

178

179 To explore whether putative PSGs were of higher or lower specificity across clusters or cell
180 types, we calculated the entropy of putative PSGs. The higher entropy-specificity score for a
181 gene implies its uniqueness in specific clusters or cell types (Martinez and Reyes-Valdes
182 2008). We found that the putative PSGs occurred in more than one literature have
183 significantly higher entropy specificity across both clusters and cell types compared with the
184 background (**Table 2**). To figure out which cluster is highly enriched with putative PSGs, we
185 did enrichment analysis for each cluster-specific in the putative PSG set (**Table 3**). Four
186 clusters (cluster 2, 9, 14, 24) were significantly enriched in three putative PSGs sets, six
187 clusters (cluster 0, 8, 11, 16, 17, 20) were enriched in two putative PSGs sets (with p-value <
188 0.05).

189

190 Glutamatergic receptors constitute major excitatory transmitter system, and dog excitatory

191 synaptic plasticity increased in the domestication process (Li, et al. 2014). 40 of 86 putative

192 PSGs were expressed in the glutamatergic neurons, 28 in cluster 0, 11, 14, 17 and 20. The GO

193 enrichment analysis showed that DEGs of cluster 0 enriched in the regulation of cell

194 migration (GO:0030334, **Table S2**) and regulation of cell motility (GO:2000145, **Table S2**),

195 those in cluster 11 and cluster 14 involved in neuron differentiation (GO:0030182, **Table S2**),

196 and those in cluster 17 and cluster 20 enriched in axon guidance (GO:0007411, **Table S2**) and

197 neuron projection guidance (GO:0097485, **Table S2**). *CUX2* was highly expressed in DG

198 granular cells as glutamatergic neurons DEG in cluster 11 and 20, and it was also expressed

199 in CA granular cells, the same expression pattern in the mouse (**Figure 3** and **Figure S4B**)

200 (Yamada, et al. 2015). Another putative PSG, *GRIK3* in cluster 14 was detected mainly in

201 GCL of DG and SP of CA area (**Figure 3**). It is worth noting that putative PSGs were

202 significantly enriched in cluster 14 (**Table 3**), and the DEGs in cluster 14 were enriched in

203 synapse organization (GO:0050808, **Table S2**), neuron differentiation (GO:0030182, **Table**

204 **S2**), synaptic signaling (GO:0099536, **Table S2**), and synapse assembly (GO:0007416, **Table**

205 **S2**). It suggests that these clusters may involve in stress responses (O'Rourke and Boeckx

206 2020).

207

208 11 putative PSGs were expressed in the GABAergic neuron, and seven of them belong to

209 cluster 8. GO enrichment analysis suggested DEGs in cluster 8 were involved in behavior

210 (GO:0007610, **Table S2**), forebrain development (GO:0030900, **Table S2**) and adult

211 locomotory behavior (GO:0008344, **Table S2**). *GAD2* as a candidate gene (**Figure 3**), to

212 regulate brain development and behavior during domestication (Pendleton, et al. 2018), and it

213 was expressed in cluster 4, 8, 12 and 21 (**Figure 1E**). Moreover, DEGs in cluster 4, 8, 12 and

214 21 enriched with behavior (GO:0007610, **Table S2**) and anterograde trans-synaptic signaling

215 (GO:0098916, **Table S2**). Previous studies suggested that ablation *GAD2*, mouse reduced

216 freezing and increased flight and escape behavior (Stork, et al. 2003). *NRXN3* (cluster 4 and 8)

217 encodes a receptor and cell adhesion molecule in the nervous system, and it was highly

expressed in the SP of the hippocampus CA area (**Figure 3**). GO enrichment analysis showed DEGs in cluster 4 and 8 were relevant to behavior (GO:0007610, **Table S2**), regulation of synapse organization (GO:0050807, **Table S2**), and adult locomotory behavior (GO:0008344, **Table S2**). A previous study also confirmed that putative PSGs in the Chinese native dogs enriched in locomotory behavior (Li, et al. 2013).

24 putative PSGs occurred in the oligodendrocytes including cluster 2, 9, 16, 18. Three of them are related to OPC differentiation. Oligodendrocytes are a kind of cells that primarily form the central nervous system (CNS) myelin. Myelination is critical for the normal functioning of the CNS (Fancy and Miller 2020). One of the overlapped genes *COL11A1* (cluster 9, 16) marked OPCs and was expressed in the partial area of hippocampus CA (**Figure 3**). Previous studies showed that the OPC was important for human white matter expansion and myelination (Huang, et al. 2020). Electron microscopic analysis revealed that glutamatergic synapses connected OPCs and neurons (Bergles, et al. 2000). Myelinating oligodendrocyte was the result of OPC differentiation and made saltatory conduction of nerve impulses possible (Rivers, et al. 2008; Freeman and Rowitch 2013). *MBP* was a myelinating oligodendrocyte cell marker, expressed in cluster 2, 16 and 18, and highly expressed in the Hilus, ML, SLM and other hippocampus regions (**Figure 3**). As a domestication gene, *MBP* encoded myelin basic protein. Domestication influenced myelination and/or axonal diameter in rabbits (Brusini, et al. 2018), and affected neurological function in the dogs (Freedman, et al. 2016). The relevant functions on these clusters were ensheathment of neurons (cluster 2, 16, GO:0007272, **Table S2**), plasma membrane bounded cell projection morphogenesis (cluster 16, 18, GO:0120039, **Table S2**), and axon ensheathment (cluster 2, 16, GO:0008366, **Table S2**).

Reconstructed gene regulatory networks of dog hippocampus

We reconstructed dog hippocampus gene regulatory networks by GENIE3 software (Huynh-Thu, et al. 2010). To attenuate the effects of noise and outliers, we used 4,523 genes and 4,281 pseudo cells (**Methods** about WGCNA), which contained all the cell types in this study.

247 More than two million interactions were found between the putative regulatory genes and
248 target genes with 2,239 putative regulatory genes were in the top 1% (**Table S5**). The
249 transcription factors in dogs (*Canis familiaris*) from AnimalTFDB3.0 (Hu, et al. 2019) were
250 used to verify these putative regulatory genes. As the results shown, there were 118 putative
251 regulatory genes, and 11 of those are both PSGs and DEGs, including Cut Like Homeobox 2
252 (*CUX2*) (Freedman, et al. 2016; Pendleton, et al. 2018) and Regulatory Factor X3 (*RFX3*)
253 (Wang, et al. 2016) in the glutamatergic neurons. GO enrichment analysis showed that target
254 genes of *CUX2* were related to glutamatergic synaptic transmission (GO:0035249, **Table S6**).
255 The transcription factor *CUX2* is involved in early neocortical circuits, cellular fate selection
256 and mechanosensation development (Bachy, et al. 2011; Miskic, et al. 2021). Another
257 regulatory gene *RFX3* was enriched in neurogenesis (GO:0022008, **Table S6**), neuron
258 development (GO:0048666, **Table S6**) and locomotion (GO:0040011, **Table S6**). Taken
259 together, results above implied that glutamatergic neurons may involve in dog behaviors
260 adaptive evolution.

261

262 **Discussion**

263 In this study, we presented the single-nuclei transcriptomics of the dog hippocampus and
264 identified 26 cell clusters based on 105,057 single-nuclei transcriptomes, such as
265 glutamatergic neurons, GABAergic neurons, Cajal-Retzius cells, oligodendrocyte precursor
266 cells (OPCs), myelinating oligodendrocytes, endothelial cells, astrocytes, and microglial cells.
267 Besides, our study demonstrated the trajectory of oligodendrocytes differentiation based on
268 the re-calculated UMAP embedding analysis.

269

270 As a subtype of inhibitory neurons, the GABAergic neuron has been linked to the response to
271 learning and fear memory (Harris and Westbrook 1998; Stork, et al. 2002). Reduced fear and
272 aggression are important traits selected by humans in the first step of animal domestication,
273 which help animals not only live commensally with humans, but also stay in a crowded
274 environment (de Kloet, et al. 2005; Price 2008). Especially, *GAD2* could influence fear
275 behavior and relieve sensitized pain behavior (Stork, et al. 2003; Zhang, et al. 2011), *KCNC2*

276 (Kv3.2) expressed in the GABAergic neurons synaptic which had been related to diurnal and
 277 circadian rhythms of wheel-running behavior (Chow, et al. 1999; Kudo, et al. 2011).

278

279 Glutamate receptors play an important role in the central nervous system, responded for basal
 280 excitatory synaptic transmission, some of them are involved in learning and memory, and
 281 there were great changes of glutamate receptors in animal domestication and modern human
 282 evolution (O'Rourke and Boeckx 2020). *FOXP2* is a DEG in cluster 14. A previous study
 283 illustrated that laboratory rats are good at learning than wild rats because of the selected
 284 *FOXP2* (Zeng, et al. 2017). The *FOXP2* protein in humans and mice is extremely conserved
 285 (Enard, et al. 2002). Putative target genes of the *FOXP2* showed these genes play roles in the
 286 CNS patterning, development, and function (Fisher and Scharff 2009). The absence of
 287 *FOXP2* protein could lead to developmental delays, severe motor dysfunction, and neural
 288 abnormalities in mice (French, et al. 2007). Besides *FOXP2*, another two putative PSGs in
 289 glutamatergic neurons also need to note. One is *GRIK3*. Genome wide studies showed that
 290 signals of selection exist in the dog (Axelsson, et al. 2013; Freedman, et al. 2016), cattle
 291 (Qanbari, et al. 2014) and sheep (Naval-Sanchez, et al. 2018). *GRIK3* may play important
 292 roles in reducing stress responses between domesticated animals and humans, leading to more
 293 prone to human custody (O'Rourke and Boeckx 2020). Another is *GRM8* (mGluR8) as one of
 294 the DEGs in cluster 14 and one of the parallel evolutionary genes between humans and dogs
 295 (Wang, et al. 2013). As a member of the Group III receptor subunit, mice lacking *GRM8* are
 296 heavier, reduce locomotory behavior, increase anxiety-like behaviors in the open field
 297 (Duvoisin, et al. 2005; Duvoisin, et al. 2010).

298

299 Furthermore, DEGs in cluster 14 were found enriched in regulation of synapse structure or
 300 activity (GO:0050803, **Table S2**) and regulation of nervous system development
 301 (GO:0051960, **Table S2**). It revealed that cells in cluster 14 may play an important role in a
 302 decreased stress response not only in domesticated animals, but also in humans (O'Rourke
 303 and Boeckx 2020). These references could verify the hypothesis that glutamatergic neurons
 304 changed the behavior during domestication and reduce fear response for dogs through

305 regulating cells in the hippocampus. It suggested that cluster 14 is probably an important
306 cluster in dog domestication.

307

308 Genome scan for selection signature in domestic animals also revealed that putative PSGs
309 enriched in the nervous system development (Montague, et al. 2014; Qanbari, et al. 2014;
310 Schubert, et al. 2014; Qiu, et al. 2015). Here we found the putative PSGs in domestication
311 were involved in the development of oligodendrocytes. For instance, *MBP*, a putative PSG
312 that may lead to behavioral differences between dogs and wolves (Cagan and Blass 2016),
313 was also one of the marker genes of myelinating oligodendrocytes. The function is to encode
314 the myelin basic protein, a component of the myelin sheath, considered to be essential for
315 saltatory impulse propagation (chiron and Miron 2007; Simons and Nave 2016). In addition,
316 domestication may reduce myelination level, compromise neural conduction, and change the
317 size of brain structure relevant for memory, reflexes, and fear processing (Brusini, et al. 2018).
318 The lower reactive level is a component of domestication syndrome that is a collection of
319 common traits in domestic animals (Wilkins, et al. 2014).

320

321 It is worth to note that the PSGs from the published studies were described as putative PSGs
322 in the present study. Wang et al., vonHoldt et al., Axelsson et al., and Pendleton et al. reported
323 205, 29, 122 and 429 PSGs through the whole genome scan for selection, respectively
324 (vonHoldt, et al. 2010; Axelsson, et al. 2013; Wang, et al. 2016; Pendleton, et al. 2018). None
325 of those were validated as the PSGs by the additional statistical analysis nor the experimental
326 assays. Freedman et al. reported 145 PSGs and performed the statistical analysis to determine
327 the likelihood under selection (Freedman, et al. 2016). Thus, we performed statistical analysis
328 using the putative PSGs in Freedman et al 2016 only, and those reported at least in the two
329 references, leading to the same significant overlap. Interestingly, *GRIK3* in cluster 14 was
330 reported in four published studies, except vonHoldt, et al. 2010. Glutamate plays a major role
331 in the excitatory transmitter system, responded for basal excitatory synaptic transmission
332 which is involved in learning and memory, hypothalamic-pituitary-adrenal activity, and
333 tameness and the reduction of reactive aggression (Herman, et al. 2004; Baude, et al. 2009;

O'Rourke and Boeckx 2020). Nevertheless, further statistics or experiment assays should be done in the future to verify the status of the PSGs.

In conclusion, 105,057 single-nuclei transcriptomes were classified into 26 clusters in this study and were defined with distinct identities. Further exploration revealed 86 genes that were overlapped between putative PSGs and DEGs. Besides, we illustrated the OPC differentiation trajectory based on the UMAP embedding. Our results contributed to defining the cell types and revealing the development of oligodendrocyte in the dog hippocampus, illustrating the difference between the sub-cell types and connecting the gap of our understanding between the molecular and cellular mechanisms of animal domestication.

Materials and Methods

Ethics approval and consent to participate

Hippocampus tissue was collected from a five-month-old Beagle ordered from the department of laboratory animal science, Kunming Medical University. All the animal processing procedures and experiments performed in the present study were approved by the Animal Ethics Committee of Kunming Institute of Zoology, Chinese Academy of Sciences (SMKX-20160301-01).

Tissue dissection and nucleus extraction

Broader dissections (no layer enrichment or multiple layers combined) were used to facilitate isolation of sufficient number of cells. Nucleus extraction protocol was adapted from Rosenberg et al. 2018 (Rosenberg, et al. 2018). Briefly, a dounce homogenizer (Wheaton, cat. no. 357538) was used for nucleus extraction. Hippocampus was homogenized in the dounce homogenizer (Kimble) 10X with loose pestle and 20X with tight pestle. A homogenization buffer (4.845 mL of NIM1 buffer (250 mM sucrose, 25 mM KCl, 5 mM MgCl₂, 10 mM Tris pH=8.0), 5 µL of 1 mM DTT, 50 µL of Enzymatics RNase Inhibitor (40U/µL), 50 µL of SupraseIn RNase Inhibitor (20U/µL), 50 µL of 10% Triton-X100) was used to make a homogeneous nucleus solution and protect the RNA from degradation. Nuclei were collected

363 by the centrifugation and filtered through sterile 40µm cell strainer (Corning) into 1.5ml tubes.
364 The nucleus concentration was checked by the hemocytometer to ensure it was within
365 1,000,000 nuclei/ml.

366

367 Library preparation

368 SPLiT-seq (split-pool ligation-based transcriptome sequencing) was used to generate the
369 libraries with Uniquely Barcoded Cells (UBC) (Rosenberg, et al. 2018). Briefly, nuclei were
370 distributed into 48 individual wells in two 96-well plates. Each well loaded about 5,000-
371 8,000 nuclei. The random hexamer and the anchored poly(dT)15 barcoded RT primers were
372 respectively distributed into the 48 individual wells with nuclei of the 96-well. The links
373 between well ID and barcode were recorded for downstream data processing. For the other
374 protocol of the reverse transcription, ligation barcoding, lysis, cDNA amplification,
375 tagmentation and Illumina amplicon generation were processed according to the SPLiT-seq
376 protocol Version 3.0. The libraries were processed on the Illumina platform for sequencing of
377 150 bp pair-end reads.

378

379 Processing of raw scRNA-seq data

380 According to the procedure of SPLiT-seq, the site of read 11-18 bp that did not match the
381 barcode list were removed. The sequencing data was processed with the Drop-seq core
382 computational protocol ("<http://mccarrolllab.org/dropseq/>") to align, filter, and count unique
383 molecular identifiers (UMIs) per sample by default. Data were mapped to the dog reference
384 genome CanFam3.1 (GCA_000002285.2) and the transcriptome annotation from the Ensembl
385 database, Canis_familiaris. CanFam3.1.92. Cells with more than 350 and no more than 5,000
386 expressed genes were retained. After that, genes expressed in less than three cells were
387 filtered out. At the same time, cells that were expressed in a high proportion of mitochondrial
388 genes (> 0.03) were also removed. As a result, 18,039 cells from the random group and
389 87,018 cells from the poly-T group were used for further analysis.

390

391 Data normalization and HVG selection

392 The gene-by-cell matrices were adjusted by a total-count normalization. Formally, denote x_{ij}
 393 as the raw count of gene i in cell j . For each cell j , counts were divided by its total count
 394 $c_j = \sum_i x_{ij}$ of this cell and then multiplied by the median of all the total counts $m =$
 395 $\text{median}\{c_j\}$. The total-size normalized expressions $\hat{x}_{ij} = mx_{ij}/c_j$ were then log-transformed
 396 to $\hat{x}_{ij} \leftarrow \log(\hat{x}_{ij} + 1)$ for downstream analysis. Highly variable genes (HVGs) were selected
 397 separately within random-group and poly-T-group, using the build-in function
 398 “scanpy.pp.highly_variable_genes” with the Python package ScanPy (Wolf, et al. 2018).
 399 Genes with a (log-normalized) mean expression above 0.025 and a normalized dispersion
 400 higher than 0.25 were identified as highly variable ones in each group. Finally, we took those
 401 genes that were highly variable in both groups and those with top dispersion as final HVGs
 402 for downstream analysis. This process resulted in 2,000 HVGs for downstream analysis.

403

404 Dimensionality reduction and clustering

405 Before performing dimensionality reduction, the gene-by-cell expression matrix was
 406 centralized and scaled within each group of the same RNA capturing primer, for elimination
 407 of the batch effect. The partial-PCA was performed to combine the random and poly-T groups.
 408 That was calculating the principal components on the polyT-group first, and projecting the
 409 random-group onto the same PC space of the polyT-group. We selected the top 50 principal
 410 components (PCs) with the highest explained variances. We also tried different numbers of
 411 PCs and got similar results. UMAP (McInnes and Healy 2018) was used to embed each cell
 412 from the reduced PC space into a 2D space. It first computed the approximate k nearest
 413 neighbors (KNNs) for each data point, built a weighted mutual-KNN graph with each node
 414 representing each cell, and embedded each node of the graph into the low-dimensional space.
 415 We computed 30 approximate nearest neighbors for each single cell based on cosine distance
 416 in the PC space. Leiden community detection algorithm (Traag, et al. 2019) was applied (with
 417 resolution=0.8, which resulted a fine grained clustering) onto the weighted KNN graph built
 418 by UMAP to cluster single cells into distinct groups.

419

420 Identification of DEGs and enrichment analysis

We compared the transcriptomic profile of each cluster versus the others using three differential analysis methods to get reliable DEG sets (Student's t-test, Wilcox (Wilcoxin 1947), MAST (Finak, et al. 2015)) with the same thresholds (p-values less than 0.001 and log2fold-changes more than 0.25). The intersection of them were 64 to 233 DEGs for each cluster (**Table S7**). Each detected cluster was mapped to cell types or intermediate states by matching their corresponding DEGs to Allen Brain Atlas database and consulting published literatures. GO enrichment analysis of these DEGs was performed by the R package "clusterProfiler" (Yu, et al. 2012) with the reference database "org.Cf.eg.db" (Carlson 2019). Instead of the genes in whole genome of the dog, we used the genes that were detected in the present hippocampus data as the background.

431

432 Categorization of genes using WGCNA

We adopted WGCNA (Weighted gene correlation network analysis) (Langfelder and Horvath 2008) analysis to detect gene modules with the R package "WGCNA" (R version 3.6.3, <https://cran.r-project.org/web/packages/WGCNA/>; package version 1.69), which was initially designed for the bulk RNA-seq data. For the convenience of calculation, we used 4,523 genes expressed in more than 25% of populations in at least one cluster. Considering the large number of single-nuclei and the sparsity of the expression profiles, samples used in the WGCNA were created by aggregating and averaging each of the small clusters (< 100 cells) of single cells of similar transcripts, resulting 4,281 pseudo cells. The soft power value (power = 4) was determined by inspecting the soft-threshold-mean-connectivity curve plot. Modules with distance less than 0.25 (i.e., correlation more than 0.75) were merged. The module-cluster relationships were evaluated with the Pearson correlation coefficients between the module-memberships (MMs, a gene-by-module matrix) for genes and the gene-significances (GSs, a gene-by-cluster matrix) for clusters. The MM was defined as the Pearson correlation coefficients between the pseudo cell expressions and the eigengenes of the modules (computed by the WGCNA), while the GS was defined as the Pearson correlation coefficients between the pseudo cell expressions and the one-hot coded cluster labels.

450

451 Putative trajectory analysis for a subset of hippocampus cells

452 To validate our hypothesis of the trajectory, we separated these clusters of cells, and redid
453 dimensionality reduction using PCA and UMAP. Moreover, we analyzed the polyT group
454 only for that they hold the majority (15,791 cells, nearly 90% of the entire trajectory) of the
455 transcriptomes that formed the trajectory. Therefore, we can keep the biological information
456 from being lost without introducing additional technical noise. In detail, we selected the top
457 20 PCs according to the PC-elbow plot, found 10 nearest neighbors for each cell using
458 Euclidean distance and performed UMAP with the parameter “min_dist = 0.2”. After that, the
459 pseudo-time of each cell on the trajectory was inferred using Slingshot, based on the re-
460 calculated UMAP embeddings and the original cluster labels. The student’s t-test was
461 performed to find out the DEGs within the three clusters that formed the trajectory.

462

463 Cross-species integration of oligodendrocyte

464 Integration of single-cell data from mouse and domestic dogs was processed with LIGER
465 (liger) (Welch, et al. 2019), which is an R package for integrating and analyzing multiple
466 single-cell datasets. We downloaded 5,072 transcriptomes of single cells from the mouse
467 oligodendrocyte lineage obtained at GEO (GSE75330) and separated cells of correlative
468 clusters to do integrated analysis. We used LIGER with the following processing and
469 parameters. First, we normalized the data to account for differences in sequencing depth and
470 capture efficiency across cells, selected variable genes, and scaled the data with “var.thresh =
471 0.1”. Next, we performed integrative non-negative matrix factorization to identify shared and
472 distinct metagenes across the datasets using the LIGER function “optimizeALS (k = 25)”. We
473 performed a quantile alignment step “quantileAlignSNF” with the default settings and used
474 UMAP to visualize the integrated data.

475

476 Enrichment analysis of DEGs on putative PSG sets

477 The domesticated gene list was obtained from five studies (**Table S8**) (vonHoldt, et al. 2010;
478 Axelsson, et al. 2013; Freedman, et al. 2016; Wang, et al. 2016; Pendleton, et al. 2018). We

479 pooled the DEGs from all clusters for the enrichment analysis based on the hypergeometric
480 test. The hypergeometric distribution, which describes the probability of k successes (random
481 draws for which the object drawn has a specified feature) in n draws, without replacement,
482 from a finite population of size N that contains exactly K objects with that feature, where
483 each draw is either a success or a failure. The probability distribution function is given
484 by $P(X = k) = \frac{\binom{K}{k} \binom{N-K}{n-k}}{\binom{N}{n}}$, where $\binom{n}{m}$ is the binomial coefficient. And the probability of over-
485 representation is $\sum_{k \leq i \leq n} P(X = i)$. In our case, we take a total of $N=22,639$ genes expressed
486 in more than three cells as the gene universe instead of using the whole genome of the dog. K
487 is the number of intersects between a given putative PSG set and the gene universe. We used
488 the method to calculate not only all putative PSGs but also those only reported in Freedman et
489 al. (2016) and in at least 2 references.

490

491 Entropy specificities of putative PSG sets across clusters and cell-types

492 We followed (Martinez and Reyes-Valdes 2008) and computed the entropy specificity for
493 each gene, based on the normalized average expressions, grouped by those 26 clusters and 10
494 major cell types. The computation was done by the build-in function ‘entropySpecificity’ of
495 BioQC (Zhang, et al. 2017). To avoid the noises from the lowly expressed genes, we took the
496 genes expressed in more than 10% of populations in at least one cluster as the background.
497 The entropy-specificity scores were normalized and averaged for each putative PSG set and
498 the backgrounds.

499

500 Genetic regulatory networks (GRN) analysis by the GENIE3

501 Inferable regulatory links for each gene were predicted by the GENIE3 software (version
502 1.16.0, Huynh-Thu, et al. 2010) with the random forest machine learning algorithm. The links
503 ranked by weight and only top 1% regulatory genes were reserved for subsequent analysis.
504 Transcription factors of the dog were downloaded from the AnimalTFDB 3.0 (Hu, et al.
505 2019). GO enrichment analysis for target genes was performed using the g:Profiler (version
506 e104_eg51_p15_3922dba) by the dog annotation (Raudvere, et al. 2019).

507

508 Immunofluorescent (IF) and immunohistochemistry (IHC) staining

509 Formalin-fixed and paraffin-embedded hippocampus tissue specimens were sliced into 9 μm
510 sections and then deparaffinized in turpentine oil (TO), rehydrated through graded ethanol
511 solutions and antigen retrieval was performed by placing the slides at 95°C for 40 min in a
512 microwave oven and allowed to cool at room temperature. The slides were washed three
513 times with PBS, and then the slides were permeabilized with 1% Triton X solution for 10 min.
514 After nonspecific binding was blocked by 5% Bovine serum albumin (BSA) for 1h at room
515 temperature, the slides were incubated with primary antibodies overnight at 4 °C. For
516 immunofluorescent staining, DAPI (4-6-diamidino-2-phenylindole) and DyLight 488- or
517 DyLight 555- labeled secondary antibodies (1:400, Thermo Fisher Scientific, Waltham, MA)
518 were added for 1.5h at room temperature. For immunohistochemistry staining, the slides were
519 treated with HRP-labeled second antibody (Thermo Fisher Scientific) for 30 min. The slides
520 were incubated with DAB until desired stain intensity is observed and then followed by slight
521 hematoxylin counterstaining. The slides were finally dehydrated and mounted with a cover
522 glass. Stained slides were visualized and imaged using a laser scanning confocal microscope
523 (Olympus, Tokyo, Japan). Primary antibodies used in this study are shown in **Table S9**.

524

525 **Availability of data and materials**

526 All raw sequence data are also available in the Genome Sequence Archive in the BIG Data
527 Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession
528 number PRJCA004294. We also constructed the dog hippocampus atlas website at
529 <https://ngdc.cnca.ac.cn/idog/> (Single cell module).

530

531 **Declaration of Interests**

532 The authors declare no competing interests.

533

534 **Author contributions**

535 G.-D. W., S. Z. and B. M. conceived of the study, Q.-J. Z., X. L. and R. W. analyzed the data,
536 Q.-J. Z. and L. Z collected the data, L. Z. performed experiment, X. L., G. L., Y. H., Z. D. and

537 P. M. performed scRNA-seq library, T. Y helped anatomy experiment, S.-Z. W revised the
538 manuscript. All authors wrote, revised, and approved the manuscript.

539

540 **Acknowledgement**

541 This work was supported by the National Key R&D Program of China (2019YFA0707101,
542 2019YFA0709501), Key Research Program of Frontier Sciences of the CAS (ZDBS-LY-
543 SM011, QYZDB-SSW-SYS008), National Natural Science Foundation of China (61621003),
544 National Ten Thousand Talent Program for Young Top-notch Talents, and Innovative
545 Research Team (in Science and Technology) of Yunnan Province (202005AE160012).
546 G.D.W. is supported by the Youth Innovation Promotion Association of CAS. This work was
547 supported by the Animal Branch of the Germplasm Bank of Wild Species, Chinese Academy
548 of Sciences (the Large Research Infrastructure Funding).

Table 1. Overlapped genes between putative PSGs and DEGs.

¹indicates this gene appears in different cell types, i.e., *CUX2* in Glutamatergic neurons, GABAergic neurons and non-neurons.

Cell type	cluster	gene
glutamatergic neurons	0, 3, 5, 6, 7,	<i>ADCY8, ARHGAP32, ASAP1¹, CACNA1A, CADM2¹,</i>
	10, 11, 13,	<i>CADPS2¹, CCBE1, CNTN5, CUX2¹, DGKI, EEF1A1¹,</i>
	14, 15, 17,	<i>EML6, FOXP2¹, FSTL4, GLIS1, GRIK3, HAPLN4,</i>
	20	<i>KCNJ3, KCNMA1¹, KLF12¹, LRRTM3¹, NRXN3¹, NTNG1, PIK3R1, PPM1E, PRKCA¹, PRRC2B, RFX3¹, RIMS2¹, RYR3, SATB2, SBF2, SCN2A, SYN2¹, TCF4¹, TIAM1, TMEM132D¹, TMEM59L, YWHAH, ZMAT4¹</i>
GABAergic neuron	4, 8, 12, 21	<i>CADPS2, CUX2, GABRA4, GAD2, KCNC2¹, NRXN3, PRKCA, ROR1, SYN2, TCF4, ZMAT4</i>
Oligodendrocytes	2, 9, 16, 18	<i>AGAP1, ANKRD44¹, ASAP1, BAZ2B¹, CADM2, CCSE2, CLIC4, COL11A1, ENSCAFG00000031463, FAM107B, HIPK2¹, ITCH, KLF12, LIMCH1, LRRTM3, MAP3K1, MBP, PLXDC2¹, PRKCA, RALGAP2¹, REEP3, RIMS2, SAMD12, TMEM132D</i>
Microglia	24	<i>ANKRD44, BAZ2B, DOCK2, ENTPD1, IKZF1, KLF12, MRC2, PLXDC2, SHB, SLCO2B1¹, SSH2, TBXAS1</i>
Other	1, 19, 22, 23, 25	<i>AHCYL2, ATXN2, CAPS2, CUX2, DNAH3, EEF1A1, FHOD3, FOXP2, GNA12, HIPK2, HYDIN, ITGB1, KCNC2, KCNMA1, KIAA1328, MYOF, PDE7B, PPM1H, PSD2, RALGAP2, RBPMS, RFTN2, RFX3, SLCO2B1, TOM1L2, WDR66</i>

Table 2. The entropy of putative PSGs in different cell types and cluster.

²bg: background genes that were expressed by at least a proportion of “proportion-cut” in any cell cluster to filter out those genes having low expression frequencies. PSG-841: putative PSGs in five articles; PSG-78: putative PSGs occurred in no less than two articles; PS-145: putative PSGs occurred in Freedman et al. 2016.

		Gene-specificity based on the average expressions of cluster			Gene-specificity based on the average expressions in a cell type		
	proportion-cut	PSG-841	PSG-78	PSG-145	PSG-841	PSG-78	PSG-145
PSG	0	1.57E-01	1.46E-01	1.85E-01	2.11E-01	1.94E-01	2.36E-01
bg	0	2.47E-01	2.45E-01	2.45E-01	3.31E-01	3.28E-01	3.28E-01
PSG	0.1	7.96E-02	1.26E-01	1.05E-01	1.01E-01	1.52E-01	1.27E-01
bg	0.1	9.36E-02	9.28E-02	9.29E-02	1.20E-01	1.19E-01	1.19E-01
PSG	0.2	7.43E-02	1.23E-01	8.97E-02	9.21E-02	1.52E-01	1.10E-01
bg	0.2	8.97E-02	8.87E-02	8.89E-02	1.14E-01	1.13E-01	1.13E-01
PSG	0.25	7.00E-02	1.07E-01	9.32E-02	8.68E-02	1.34E-01	1.15E-01
bg	0.25	9.00E-02	8.88E-02	8.89E-02	1.14E-01	1.13E-01	1.13E-01

566

567

568

569

570

571

572

573

574

575

576

577

578

579

580

581

582

583

584

585

Table 3. Enrichment analysis for each cluster-specific genes in putative PSG sets.

PSG-841: putative PSGs in five articles; PSG-78: putative PSGs occurred in no less than 2 articles, PS-145: putative PSGs occurred in Freedman et al. 2016.

cluster	PSG-841	PSG-78	PSG-145
0 Glutamatergic neurons	4.06E-05	6.18E-03	1.42E-01
0 Glutamatergic neurons(random)	4.33E-01	2.32E-01	3.00E-01
3 Glutamatergic neurons	2.38E-03	5.35E-02	4.86E-01
3 Glutamatergic neurons(random)	4.30E-01	2.25E-01	3.04E-01
5 Glutamatergic neurons	4.37E-01	3.76E-01	2.04E-01
5 Glutamatergic neurons(random)	4.27E-01	2.63E-01	3.26E-01
6 Glutamatergic neurons	9.51E-02	2.31E-01	3.75E-01
6 Glutamatergic neurons(random)	4.01E-01	1.87E-01	2.63E-01
7 Glutamatergic neurons	1.38E-01	2.57E-01	4.11E-01
7 Glutamatergic neurons(random)	4.06E-01	1.94E-01	2.66E-01
10 Glutamatergic neurons	1.02E-02	2.77E-01	4.39E-01
10 Glutamatergic neurons(random)	4.10E-01	2.14E-01	2.89E-01
11 Glutamatergic neurons	7.28E-05	2.43E-02	6.88E-02
11 Glutamatergic neurons(random)	4.22E-01	1.74E-01	2.43E-01
13 Glutamatergic neurons	1.83E-01	2.15E-01	3.50E-01
13 Glutamatergic neurons(random)	4.08E-01	1.74E-01	2.52E-01
14 Glutamatergic neurons	1.77E-02	2.04E-04	1.51E-02
14 Glutamatergic neurons(random)	4.04E-01	1.99E-01	2.69E-01
15 Glutamatergic neurons	5.16E-02	2.52E-01	4.05E-01
15 Glutamatergic neurons(random)	4.11E-01	1.95E-01	2.79E-01
17 Glutamatergic neurons	1.54E-03	3.47E-02	4.08E-01
17 Glutamatergic neurons(random)	4.05E-01	1.98E-01	2.81E-01
20 Glutamatergic neurons	8.16E-02	4.23E-02	2.06E-02
20 Glutamatergic neurons(random)	4.33E-01	2.12E-01	2.79E-01
4 GABAergic neurons	7.88E-02	4.18E-03	1.13E-01
4 GABAergic neurons(random)	4.05E-01	2.14E-01	2.91E-01
8 GABAergic neurons	2.32E-03	2.09E-03	3.64E-01
8 GABAergic neurons(random)	3.99E-01	1.80E-01	2.53E-01
12 GABAergic neurons	2.08E-01	4.87E-03	4.58E-01
12 GABAergic neurons(random)	4.18E-01	2.21E-01	2.99E-01

21 GABAergic neurons	1.57E-01	5.90E-02	5.06E-01
21 GABAergic neurons(random)	4.27E-01	2.39E-01	3.05E-01
25 Cajal-Retzius	2.69E-01	1.78E-01	2.95E-01
25 Cajal-Retzius(random)	3.79E-01	1.48E-01	2.19E-01
1 Astrocytes	1.46E-01	1.07E-01	2.62E-01
1 Astrocytes(random)	4.26E-01	2.81E-01	3.50E-01
24 Microglia	1.09E-05	3.34E-04	3.11E-04
24 Microglia(random)	4.15E-01	2.13E-01	2.82E-01
9 Oligodendrocyte precursor cells	2.38E-03	5.30E-04	5.45E-04
9 Oligodendrocyte precursor cells(random)	4.42E-01	2.21E-01	3.07E-01
16 Unknown	8.92E-02	2.72E-03	3.84E-03
16 Unknown(random)	4.42E-01	2.84E-01	3.55E-01
2 Myelinating oligodendrocytes	6.22E-03	2.57E-02	5.62E-03
2 Myelinating oligodendrocytes(random)	4.42E-01	2.96E-01	3.53E-01
18 Myelinating oligodendrocytes	3.81E-01	4.52E-02	1.22E-01
18 Myelinating oligodendrocytes(random)	4.05E-01	2.18E-01	2.88E-01
19 Non-neuron	1.46E-01	2.37E-03	2.62E-01
19 Non-neuron(random)	4.45E-01	2.87E-01	3.46E-01
22 Non-neuron	2.12E-01	1.59E-01	7.21E-01
22 Non-neuron(random)	4.43E-01	3.11E-01	3.71E-01
23 Endothelial cells	2.28E-01	1.01E-01	7.12E-02
23 Endothelial cells(random)	4.26E-01	2.77E-01	3.45E-01

590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606

607 **Figure Legends**

608 **Figure 1. Single-nuclei transcriptomic landscape of dog hippocampus.** (A) Clustering and
 609 visualization of transcriptomes of 105,057 single cells by UMAP. In this article, “cluster”
 610 means group of cells, is related with cluster analysis result. “Cell type” is group of clusters,
 611 we defined these clusters were same cell types. (B) Dot plot showing the averaged DEG
 612 expressions (z-cores) in different clusters (**Table S10**). (C) Visualization of *CCBE1* by
 613 UMAP. (D) *CCBE1* expressed in dog hippocampus CA3 SP region revealed by
 614 immunohistochemical staining. (E) Visualization of *GAD2* by UMAP. (F) *GAD2* expression
 615 in dog hippocampus revealed by immunohistochemical staining. Red: *GAD2*; Blue: DAPI;
 616 White square indicates higher magnification view of top region boxed. (G) Visualization of
 617 *NDNF* by UMAP. (H) *NDNF* expression in dog hippocampus revealed by
 618 immunohistochemical staining. Red point means cells were found differentially express
 619 DEGs. DG: Dentate Gyrus, GCL: Granule Cell Layer, SGZ: Subgranular Zone, ML:
 620 Molecular Layer, CA: Cornu Amonis, SO: Stratum Oriens, SP: Stratum Pyramidale, SR:
 621 Stratum Radiatum, SLM: stratum lacunosum-moleculare, Sub: Subiculum, HF: Hippocampus
 622 Fissure, A: Alveus.

623 **Figure 2. Putative trajectory analysis and cross-species transcriptome comparison**
 624 **between dog and mouse oligodendrocyte.** (A) UMAP embedding of oligodendrocyte
 625 subclusters. Red indicates cells of cluster 2, 16, and 9. (B) Staining of AGAP1 and CNP of
 626 dog brain at hippocampus. Red: AGAP1 (top) and CNP (bottom), Blue: DAPI; White square
 627 indicates higher magnification view of top region boxed. (C) UMAP embedding of OPCs
 628 putative trajectory. Cells are colored by pseudotime, with dark colors representing mature cell
 629 stages and light colors representing immature cell stages. (D) GO enrichment analysis of
 630 OPCs differentiation clusters. Color bar means adjust p-value; dot size means gene ratio. (E)
 631 UMAP visualization of 20,989 single cells (15,920 from dog and 5,069 from mouse) analyzed
 632 by LIGER, color-coded by cell types.

633 **Figure 3. The mean expression of DEGs in different cell types.** The values of each gene
 634 (row) were its original ones divided by its maximum. Six genes on the right of the picture
 635 were differentially expressed in different cell types and the GO enrichment analysis showed

636 their relevant gene functions. It is worth to note that *CUX2* is a DEG in glutamatergic
637 neuron as compared to other cells although highly expressed both in glutamatergic
638 neuron and non-neurons. High-definition immunohistochemical staining is in the **Figure S4A**.
639

Reference

- © 2015 Allen Institute for Brain Science. Allen Cell Types Database. Available from: https://celltypes.brain-map.org/rnaseq/human_m1_10x. [Internet].
- © 2015 Allen Institute for Brain Science. Allen Cell Types Database. Available from: https://celltypes.brain-map.org/rnaseq/mouse_ctx-hip_10x. [Internet].
- © 2020 Allen Institute for Brain Science. Allen Brain map. Available from: <http://mouse.brain-map.org/experiment/show/74513995>.
- Artegiani B, Lyubimova A, Muraro M, van Es JH, van Oudenaarden A, Clevers H. 2017. A Single-Cell RNA Sequencing Study Reveals Cellular and Molecular Dynamics of the Hippocampal Neurogenic Niche. *Cell Reports* 21:3271-3284.
- Axelsson E, Ratnakumar A, Arendt ML, Maqbool K, Webster MT, Perloski M, Liberg O, Arnemo JM, Hedhammar A, Lindblad-Toh K. 2013. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495:360-364.
- Bachy I, Franck MCM, Li LL, Abdo H, Pattyn A, Ernfors P. 2011. The transcription factor Cux2 marks development of an A-delta sublineage of TrkA sensory neurons. *Developmental Biology* 360:77-86.
- Bagot RC, Cates HM, Purushothaman I, Lorsch ZS, Walker DM, Wang JS, Huang XJ, Schluter OM, Maze I, Pena CJ, et al. 2016. Circuit-wide Transcriptional Profiling Reveals Brain Region-Specific Gene Networks Regulating Depression Susceptibility. *Neuron* 90:969-983.
- Baude A, Strube C, Tell F, Kessler JP. 2009. Glutamatergic neurotransmission in the nucleus tractus solitarius: Structural and functional characteristics. *Journal of Chemical Neuroanatomy* 38:145-153.
- Bergles DE, Roberts JDB, Somogyi P, Jahr CE. 2000. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* 405:187-191.
- Bird CM, Burgess N. 2008. The hippocampus and memory: insights from spatial processing. *Nature Reviews Neuroscience* 9:182-194.
- Brusini I, Carneiro M, Wang CL, Rubin CJ, Ring H, Afonso S, Blanco-Aguilar JA, Ferrand N, Rafati N, Villafuerte R, et al. 2018. Changes in brain architecture are consistent with altered

669 fear processing in domestic rabbits. *Proceedings of the National Academy of Sciences of the*
670 *United States of America* 115:7380-7385.

671 Cagan A, Blass T. 2016. Identification of genomic variants putatively targeted by selection
672 during dog domestication. *Bmc Evolutionary Biology* 16.

673 Cao X, Liu WP, Cheng LG, Li HJ, Wu H, Liu YH, Chen C, Xiao X, Li M, Wang GD, et al.
674 2021. Whole genome analyses reveal significant convergence in obsessive-compulsive
675 disorder between humans and dogs. *Science Bulletin* 66:187-196.

676 Carlson M. 2019. org.Cf.eg.db: Genome wide annotation for Canine. R package version
677 3.10.0. .

678 chiron AA, Miron S. 2007. Myelin associated antibodies.

679 Chow A, Erisir A, Farb C, Nadal MS, Ozaita A, Lau D, Welker E, Rudy B. 1999. K⁺ channel
680 expression distinguishes subpopulations of parvalbumin-and somatostatin-containing
681 neocortical interneurons. *Journal of Neuroscience* 19:9332-9345.

682 Darmanis S, Sloan SA, Zhang Y, Enge M, Caneda C, Shuer LM, Gephart MGH, Barres BA,
683 Quake SR. 2015. A survey of human brain transcriptome diversity at the single cell level.
684 *Proceedings of the National Academy of Sciences of the United States of America* 112:7285-
685 7290.

686 de Kloet ER, Joels M, Holsboer F. 2005. Stress and the brain: From adaptation to disease.
687 *Nature Reviews Neuroscience* 6:463-475.

688 Duvoisin RM, Villasana L, Pfankuch T, Raber J. 2010. Sex-dependent cognitive phenotype of
689 mice lacking mGluR8. *Behavioural Brain Research* 209:21-26.

690 Duvoisin RM, Zhang C, Pfankuch TF, O'Connor H, Gayet-Primo J, Quraishi S, Raber J. 2005.
691 Increased measures of anxiety and weight gain in mice lacking the group III metabotropic
692 glutamate receptor mGluR8. *European Journal of Neuroscience* 22:425-436.

693 Enard W, Przeworski M, Fisher SE, Lai CSL, Wiebe V, Kitano T, Monaco AP, Paabo S. 2002.
694 Molecular evolution of FOXP2, a gene involved in speech and language. *Nature* 418:869-872.

695 Fan XY, Dong J, Zhong SJ, Wei Y, Wu Q, Yan LY, Yong J, Sun L, Wang XY, Zhao YY, et al.
696 2018. Spatial transcriptomic survey of human embryonic cerebral cortex by single-cell RNA-
697 seq analysis. *Cell Research* 28:730-745.

698 Fancy SPJ, Miller RH. 2020. Chapter 35 - Signaling pathways that regulate glial development
699 and early migration—oligodendrocytes. In: Rubenstein J, Rakic P, Chen B, Kwan KY, editors.
700 Patterning and Cell Type Specification in the Developing CNS and PNS (Second Edition):
701 Academic Press. p. 867-890.

702 Finak G, McDavid A, Yajima M, Deng JY, Gersuk V, Shalek AK, Slichter CK, Miller HW,
703 McElrath MJ, Prlic M, et al. 2015. MAST: a flexible statistical framework for assessing
704 transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data.
705 Genome Biology 16.

706 Fisher SE, Scharff C. 2009. FOXP2 as a molecular window into speech and language. Trends
707 in Genetics 25:166-177.

708 Freedman AH, Schweizer RM, Ortega-Del Vecchyo D, Han EJ, Davis BW, Gronau I, Silva
709 PM, Galaverni M, Fan ZX, Marx P, et al. 2016. Demographically-Based Evaluation of
710 Genomic Regions under Selection in Domestic Dogs. Plos Genetics 12.

711 Freeman MR, Rowitch DH. 2013. Evolving Concepts of Gliogenesis: A Look Way Back and
712 Ahead to the Next 25 Years. Neuron 80:613-623.

713 French CA, Groszer M, Preece C, Coupe AM, Rajewsky K, Fisher SE. 2007. Generation of
714 mice with a conditional Foxp2 null allele. Genesis 45:440-446.

715 Hajeski NJ. 2016. Every dog : the ultimate guide to over 450 dog breeds. Buffalo, New York:
716 Firefly Books.

717 Harris JA, Westbrook RF. 1998. Evidence that GABA transmission mediates context specific
718 extinction of learned fear. Psychopharmacology 140:105-115.

719 Herman JP, Mueller NK, Figueiredo H. 2004. Role of GABA and glutamate circuitry in
720 hypothalamo-pituitary-adrenocortical stress integration. Stress: Current Neuroendocrine and
721 Genetic Approaches 1018:35-45.

722 Hochgerner H, Zeisel A, Lohnerberg P, Linnarsson S. 2018. Conserved properties of dentate
723 gyrus neurogenesis across postnatal development revealed by single-cell RNA sequencing.
724 Nature Neuroscience 21:290-299.

725 Hu H, Miao YR, Jia LH, Yu QY, Zhang Q, Guo AY. 2019. AnimalTFDB 3.0: a comprehensive
726 resource for annotation and prediction of animal transcription factors. Nucleic Acids Research

727 47:D33-D38.

728 Huang W, Bhaduri A, Velmeshev D, Wang SH, Wang L, Rottkamp CA, Alvarez-Buylla A,
729 Rowitch DH, Kriegstein AR. 2020. Origins and Proliferative States of Human
730 Oligodendrocyte Precursor Cells. *Cell* 182:594-608.

731 Huynh-Thu VA, Irrthum A, Wehenkel L, Geurts P. 2010. Inferring Regulatory Networks from
732 Expression Data Using Tree-Based Methods. *Plos One* 5.

733 Kuboyama K, Fujikawa A, Masumura M, Suzuki R, Matsumoto M, Noda M. 2012. Protein
734 Tyrosine Phosphatase Receptor Type Z Negatively Regulates Oligodendrocyte Differentiation
735 and Myelination. *Plos One* 7.

736 Kudo T, Loh DH, Kuljis D, Constance C, Colwell CS. 2011. Fast Delayed Rectifier
737 Potassium Current: Critical for Input and Output of the Circadian System. *Journal of*
738 *Neuroscience* 31:2746-2755.

739 Langfelder P, Horvath S. 2008. WGCNA: an R package for weighted correlation network
740 analysis. *Bmc Bioinformatics* 9.

741 Li Y, vonHoldt BM, Reynolds A, Boyko AR, Wayne RK, Wu DD, Zhang YP. 2013. Artificial
742 Selection on Brain-Expressed Genes during the Domestication of Dog. *Molecular Biology*
743 *and Evolution* 30:1867-1876.

744 Li Y, Wang GD, Wang MS, Irwin DM, Wu DD, Zhang YP. 2014. Domestication of the Dog
745 from the Wolf Was Promoted by Enhanced Excitatory Synaptic Plasticity: A Hypothesis.
746 *Genome Biology and Evolution* 6:3115-3121.

747 Liu YH, Wang L, Zhang ZG, Otecko NO, Khederzadeh S, Dai YQ, Liang B, Wang GD,
748 Zhang YP. 2021. Whole-Genome Sequencing Reveals Lactase Persistence Adaptation in
749 European Logs. *Molecular Biology and Evolution* 38:4884-4890.

750 Martinez O, Reyes-Valdes MH. 2008. Defining diversity, specialization, and gene specificity
751 in transcriptomes through information theory. *Proceedings of the National Academy of*
752 *Sciences of the United States of America* 105:9709-9714.

753 Mcinnes L, Healy J. 2018. UMAP: Uniform Manifold Approximation and Projection for
754 Dimension Reduction. *The Journal of Open Source Software* 3:861.

755 Meyer G, Gonzalez-Arnay E, Moll U, Nemajero A, Tissir F, Gonzalez-Gomez M. 2019.

756 Cajal-Retzius neurons are required for the development of the human hippocampal fissure.
757 *Journal of Anatomy* 235:569-589.

758 Miskic T, Kostovic I, Rasin MR, Krsnik Z. 2021. Adult Upper Cortical Layer Specific
759 Transcription Factor CUX2 Is Expressed in Transient Subplate and Marginal Zone Neurons of
760 the Developing Human Brain. *Cells* 10.

761 Montague MJ, Li G, Gandolfi B, Khan R, Aken BL, Searle SMJ, Minx P, Hillier LW, Koboldt
762 DC, Davis BW, et al. 2014. Comparative analysis of the domestic cat genome reveals genetic
763 signatures underlying feline biology and domestication. *Proceedings of the National Academy*
764 *of Sciences of the United States of America* 111:17230-17235.

765 Nadel L, Moscovitch M. 1997. Memory consolidation, retrograde amnesia and the
766 hippocampal complex. *Current Opinion in Neurobiology* 7:217-227.

767 Naval-Sanchez M, Nguyen Q, McWilliam S, Porto-Neto LR, Tellam R, Vuocolo T, Reverter
768 A, Perez-Enciso M, Brauning R, Clarke S, et al. 2018. Sheep genome functional annotation
769 reveals proximal regulatory elements contributed to the evolution of modern breeds. *Nature*
770 *Communications* 9.

771 O'Rourke T, Boeckx C. 2020. Glutamate receptors in domestication and modern human
772 evolution. *Neuroscience and Biobehavioral Reviews* 108:341-357.

773 Ogawa M, Miyata T, Nakajima K, Yagyu K, Seike M, Ikenaka K, Yamamoto H, Mikoshiba K.
774 1995. The Reeler Gene-Associated Antigen on Cajal-Retzius Neurons Is a Crucial Molecule
775 for Laminar Organization of Cortical-Neurons. *Neuron* 14:899-912.

776 Pendleton AL, Shen FC, Taravella AM, Emery S, Veeramah KR, Boyko AR, Kidd JM. 2018.
777 Comparison of village dog and wolf genomes highlights the role of the neural crest in dog
778 domestication. *Bmc Biology* 16.

779 Plassais J, Kim J, Davis BW, Karyadi DM, Hogan AN, Harris AC, Decker B, Parker HG,
780 Ostrander EA. 2019. Whole genome sequencing of canids reveals genomic regions under
781 selection and variants influencing morphology. *Nature Communications* 10.

782 Price EO. 2008. *Principles and Applications of Domestic Animal Behavior: Principles and*
783 *Applications of Domestic Animal Behavior*.

784 Qanbari S, Pausch H, Jansen S, Somel M, Strom TM, Fries R, Nielsen R, Simianer H. 2014.

785 Classic Selective Sweeps Revealed by Massive Sequencing in Cattle. *Plos Genetics* 10.

786 Qiu Q, Wang LZ, Wang K, Yang YZ, Ma T, Wang ZF, Zhang X, Ni ZQ, Hou FJ, Long RJ, et

787 al. 2015. Yak whole-genome resequencing reveals domestication signatures and prehistoric

788 population expansions. *Nature Communications* 6.

789 Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, Vilo J. 2019. g:Profiler: a

790 web server for functional enrichment analysis and conversions of gene lists (2019 update).

791 *Nucleic Acids Research* 47:W191-W198.

792 Rivers LE, Young KM, Rizzi M, Jamen F, Psachoulia K, Wade A, Kessaris N, Richardson

793 WD. 2008. PDGFRA/NG2 glia generate myelinating oligodendrocytes and piriform

794 projection neurons in adult mice. *Nature Neuroscience* 11:1392-1401.

795 Rosenberg AB, Roco CM, Muscat RA, Kuchina A, Sample P, Yao ZZ, Graybuck LT, Peeler

796 DJ, Mukherjee S, Chen W, et al. 2018. Single-cell profiling of the developing mouse brain

797 and spinal cord with split-pool barcoding. *Science* 360:176-182.

798 Schubert M, Jonsson H, Chang D, Sarkissian CD, Ermini L, Ginolhac A, Albrechtsen A,

799 Dupanloup I, Foucal A, Petersen B, et al. 2014. Prehistoric genomes reveal the genetic

800 foundation and cost of horse domestication. *Proceedings of the National Academy of*

801 *Sciences of the United States of America* 111:E5661-E5669.

802 Simons M, Nave KA. 2016. Oligodendrocytes: Myelination and Axonal Support. *Cold Spring*

803 *Harbor Perspectives in Biology*:a020479.

804 Stolt CC, Schlierf A, Lommes P, Hillgartner S, Werner T, Kosian T, Sock E, Kessaris N,

805 Richardson WD, Lefebvre V, et al. 2006. SoxD proteins influence multiple stages of

806 oligodendrocyte development and modulate SoxE protein function. *Developmental Cell*

807 11:697-709.

808 Stork O, Ji FY, Obata K. 2002. Reduction of extracellular GABA in the mouse amygdala

809 during and following confrontation with a conditioned fear stimulus. *Neuroscience Letters*

810 327:138-142.

811 Stork O, Yamanaka H, Stork S, Kume N, Obata K. 2003. Altered conditioned fear behavior in

812 glutamate decarboxylase 65 null mutant mice. *Genes Brain and Behavior* 2:65-70.

813 Street K, Risso D, Fletcher RB, Das D, Ngai J, Yosef N, Purdom E, Dudoit S. 2018. Slingshot:

814 cell lineage and pseudotime inference for single-cell transcriptomics. *Bmc Genomics* 19.

815 Tosches MA, Yamawaki TM, Naumann RK, Jacobi AA, Tushev G, Laurent G. 2018.

816 Evolution of pallium, hippocampus, and cortical cell types revealed by single-cell

817 transcriptomics in reptiles. *Science* 360:881-888.

818 Traag VA, Waltman L, van Eck NJ. 2019. From Louvain to Leiden: guaranteeing well-

819 connected communities. *Sci Rep* 9:5233.

820 Villar-Cervino V, Marin O. 2012. Cajal-Retzius cells. *Current Biology* 22:R179-R179.

821 vonHoldt BM, Pollinger JP, Lohmueller KE, Han EJ, Parker HG, Quignon P, Degenhardt JD,

822 Boyko AR, Earl DA, Auton A, et al. 2010. Genome-wide SNP and haplotype analyses reveal

823 a rich history underlying dog domestication. *Nature* 464:898-U109.

824 Wang GD, Zhai WW, Yang HC, Fan RX, Cao X, Zhong L, Wang L, Liu F, Wu H, Cheng LG,

825 et al. 2013. The genomics of selection in dogs and the parallel evolution between dogs and

826 humans. *Nature Communications* 4.

827 Wang GD, Zhai WW, Yang HC, Wang L, Zhong L, Liu YH, Fan RX, Yin TT, Zhu CL,

828 Poyarkov AD, et al. 2016. Out of southern East Asia: the natural history of domestic dogs

829 across the world. *Cell Research* 26:21-33.

830 Wang MS, Thakur M, Peng MS, Jiang Y, Frantz LAF, Li M, Zhang JJ, Wang S, Peters J,

831 Otecko NO, et al. 2020. 863 genomes reveal the origin and domestication of chicken. *Cell*

832 *Research* 30:693-701.

833 Welch JD, Kozareva V, Ferreira A, Vanderburg C, Martin C, Macosko EZ. 2019. Single-Cell

834 Multi-omic Integration Compares and Contrasts Features of Brain Cell Identity. *Cell*

835 177:1873-1887.

836 Wilcoxin F. 1947. Probability tables for individual comparisons by ranking methods.

837 *Biometrics* 3:119-122.

838 Wilkins AS, Wrangham RW, Fitch WT. 2014. The "Domestication Syndrome" in Mammals: A

839 Unified Explanation Based on Neural Crest Cell Behavior and Genetics. *Genetics* 197:795-

840 808.

841 Wolf FA, Angerer P, Theis FJ. 2018. SCANPY: large-scale single-cell gene expression data

842 analysis. *Genome Biology* 19.

843 Yamada M, Clark J, McClelland C, Capaldo E, Ray A, Iulianella A. 2015. Cux2 Activity
844 Defines a Subpopulation of Perinatal Neurogenic Progenitors in the Hippocampus.
845 Hippocampus 25:253-267.

846 Yu GC, Wang LG, Han YY, He QY. 2012. clusterProfiler: an R Package for Comparing
847 Biological Themes Among Gene Clusters. Omics-a Journal of Integrative Biology 16:284-287.

848 Yu WP, Collarini EJ, Pringle NP, Richardson WD. 1994. Embryonic Expression of Myelin
849 Genes - Evidence for a Focal Source of Oligodendrocyte Precursors in the Ventricular Zone
850 of the Neurol Tube. Neuron 12:1353-1362.

851 Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, Marques
852 S, Munguba H, He LQ, Betsholtz C, et al. 2015. Cell types in the mouse cortex and
853 hippocampus revealed by single-cell RNA-seq. Science 347:1138-1142.

854 Zeng L, Ming C, Li Y, Su LY, Su YH, Otecko NO, Liu HQ, Wang MS, Yao YG, Li HP, et al.
855 2017. Rapid Evolution of Genes Involved in Learning and Energy Metabolism for
856 Domestication of the Laboratory Rat. Molecular Biology and Evolution 34:3148-3153.

857 Zhang JTD, Hatje K, Sturm G, Broger C, Ebeling M, Burtin M, Terzi F, Pomposiello SI, Badi
858 L. 2017. Detect tissue heterogeneity in gene expression data with BioQC. BMC Genomics 18.

859 Zhang XX, Lan YJ, Xu JY, Quan F, Zhao EJ, Deng CY, Luo T, Xu LW, Liao GM, Yan M, et
860 al. 2019. CellMarker: a manually curated resource of cell markers in human and mouse.
861 Nucleic Acids Research 47:D721-D728.

862 Zhang Z, Cai YQ, Zou F, Bie BH, Pan ZZZ. 2011. Epigenetic suppression of GAD65
863 expression mediates persistent pain. Nature Medicine 17:1448-U1152.

864 Zhong SJ, Ding WY, Sun L, Lu YF, Dong H, Fan XY, Liu ZY, Chen RG, Zhang S, Ma Q, et al.
865 2020. Decoding the development of the human hippocampus. Nature 577:531-536.

866

867

868 **Supplemental Information**

869 **Table S1 DEG list.**

870 **Table S2 GO enrichment analysis.**

871 **Table S3 630 PSGs expression in each cell type.**

872 **Table S4 Putative PSGs and DEGs statistical analysis.**

873 **Table S5 Interaction by GENIE3**

874 **Table S6 GO enrichment analysis result for regulatory genes**

875 **Table S7 Three differential expression analyses.**

876 **Table S8 Domesticated gene list.**

877 **Table S9 Antibody used in IF and IHC of Canis Hippocampus.**

878 **Table S10 Gene expression average and proportion.**

879

880 **Figure S1 Cluster analysis detail information.**

881 **Figure S2 The gene expression pattern of oligodendrocyte and molecular diversity**
882 **between dog and mouse.**

883 **Figure S3 The mean expression of 630 putative PSGs in different cell types.**

884 **Figure S4 High-definition immunohistochemical staining.**

885

Figure 1

Figure 2

Figure 3