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Title:

Absence of both thyroid hormone transporters MCT8 and OATP1C1 impairs neural stem cell fate in the adult mouse subventricular zone.

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19 **Summary**

20 Adult neural stem cell (NSC) generation in vertebrate brains requires thyroid hormones (THs).
21 How THs enter the NSC population is unknown, though TH availability determines
22 proliferation and neuronal versus glial progenitor determination in murine subventricular zone
23 (SVZ) NSCs. Mice display neurological signs of the severely disabling human disease, Allan
24 Herndon Dudley Syndrome (AHDS), if they lack both MCT8 and OATP1C1 transporters, or
25 MCT8 and deiodinase type 2. We analyzed the distribution of MCT8 and OATP1C1 in adult
26 mouse SVZ. Both are strongly expressed in NSCs and at a lower level in neuronal cell
27 precursors but not in oligodendrocyte progenitors. Next, we analyzed Mct8/Oatp1c1 double-
28 knockout (DKO) mice, where brain uptake of THs is strongly reduced. NSC proliferation and
29 determination to neuronal fates were severely affected, but not SVZ-oligodendroglial
30 progenitor generation. This work highlights how tight controls of TH availability determines
31 NSC function and glial-neuron cell-fate choice in adult brains.

32

33 **Key words**

34 Adult neural stem cell, Subventricular Zone, Thyroid hormone, Thyroid hormone transporters,
35 MCT8, OATP1C1, Olfaction

36 **Introduction**

37 In the adult mammalian brain, a pool of neural stem cells (NSCs) persists in two well-
38 defined neurogenic regions, the subgranular zone (SGZ) of the hippocampus and the
39 subventricular zone (SVZ) lining the lateral ventricles. Adult neurogenesis and
40 oligodendrogenesis are complex processes that involve proliferation and differentiation of
41 both neuronal and oligodendrocyte progenitors derived from NSCs. In rodents, newly-
42 generated SVZ-neuroblasts migrate tangentially towards the olfactory bulbs (OBs) where they
43 differentiate into interneurons that participate in olfaction (Breton-Provencher et al., 2009;
44 Enwere et al., 2004). SVZ-derived oligodendrocyte precursor cells (OPCs) migrate radially
45 into the overlying white matter (e.g. the corpus callosum).

46 Under physiological conditions, SVZ-NSCs generate principally neuronal precursor
47 cells (Menn et al., 2006). However, SVZ-NSCs can also generate few oligodendrocytes *in*
48 *vitro* and *in vivo* (Menn et al., 2006). The balance between NSC-derived neurons and
49 oligodendrocytes is modulated by several physiological and pathological stimuli. For example,
50 after a demyelinating lesion (characterized by a loss of myelinating oligodendrocytes, a
51 hallmark of several white matter diseases such as multiple sclerosis), the generation of new
52 SVZ-derived OPCs is favoured to stimulate to myelin repair (Nait-Oumesmar et al., 2007;
53 Remaud et al., 2017; Xing et al., 2014). Thus, understanding how NSC fate decision (neurons
54 *versus* glia) is modulated in the adult brain could have significant implications for
55 neurodegenerative diseases when new neurons or glia are needed to promote brain repair.

56 T₃, the biologically active form of THs, is essential for brain development in all
57 vertebrates, including humans. In the murine brain, it has recently been established that T₃
58 also regulates adult neurogenesis and especially NSC fate decision between neurons and glia.
59 In the SGZ, T₃ increases neuronal differentiation by enhancing proneural gene expression
60 (Fanibunda et al., 2018; Kapoor et al., 2012, 2015). In the SVZ, T₃ drives NSC fate

61 preferentially towards a neuronal fate while a transient lack of T₃ favours the generation of
62 new OPCs capable of functional remyelination (Remaud et al., 2017).

63 Local control of TH availability is crucial for spatiotemporal control of TH action, in
64 particular within the NSC niches (Mayerl et al., 2020; Remaud et al., 2017). Numerous factors
65 control TH availability in the brain from TH-transporters (THTs) (Friesema et al., 2003, 2008;
66 Pizzagalli et al., 2002;), to inactivation/activation by deiodinases (Bianco and Kim, 2006;
67 Luongo et al., 2019), presence of receptors for TH (Flamant and Gauthier, 2013) and even co-
68 activators/corepressors (Astapova and Hollenberg, 2013; Suzuki et al., 2007). SVZ-derived
69 OPCs are protected from the neuralizing effects of T₃ through the expression of the T₃-
70 inactivating enzyme deiodinase type 3 (*Dio3*) and the absence of the T₃-receptor TRα1. In
71 contrast, TRα1 was strongly expressed in neuronal progenitors whereas *Dio3* was not
72 (Remaud et al., 2017). However, cell-membrane transporters facilitating TH uptake represent
73 an additional level controlling intracellular TH availability, potentially influencing NSC fate
74 decision too, but their involvement is as yet unknown.

75 THTs facilitate TH uptake into different cell types, including glial and neuronal cells.
76 The importance of THTs for brain function is illustrated by patients carrying inactivating
77 mutations in the *Slc16a2* gene encoding the monocarboxylate transporter 8 (*Mct8*). These
78 patients suffer from a severe form of psychomotor retardation (Allan-Herndon-Dudley
79 syndrome or AHDS) combined with abnormal serum TH levels (high T₃, low T₄) (Dumitrescu
80 et al., 2004; Friesema et al., 2004; Groeneweg et al., 2019). Recently, it has been shown that,
81 in humans, mutations in OATP1C1 decrease T₄ uptake and are associated with developmental
82 impairments that later evolve to neurodegenerative disease (Strømme et al., 2018). Mice
83 deficient in MCT8 (*Mct8* KO) faithfully recapitulate the peripheral altered TH serum levels
84 observed in humans, but do not display neurobehavioral deficits, while they suffer from mild
85 brain hypothyroidism (Trajkovic et al., 2007a). It is well established that the organic anion

86 transporting polypeptide 1C1 (OATP1C1) cooperates with MCT8 to facilitate TH transport
87 across the blood brain and/or the blood-cerebrospinal fluid barriers into the rodent brain. Mice
88 deficient for both MCT8 and OATP1C1 (*Mct8/Oatp1c1* or DKO) exhibit central TH
89 deficiency associated with locomotor abnormalities as MCT8-deficient patients, showing that
90 this DKO mouse is a suitable model especially for the neurological component of AHDS
91 (Mayerl et al., 2014). To be more precise, only DKO mice display a strong decrease of both
92 T3 and T4 contents in the brain as a consequence of a highly reduced T4 and T3 transport
93 across brain barriers (Mayerl et al., 2014). This allowed us to compare the cellular effects
94 underlying the absence of both transporters with our previous well-established data obtained
95 after induction of adult onset hypothyroidism (Gothié et al., 2017a; Remaud et al., 2017;
96 Vancamp et al., 2019).

97 Here, we determined how the lack of MCT8 and/or OATP1C1 affects NSC fate in the
98 adult mouse brain. We demonstrate that both THTs are strongly expressed in NSCs and in
99 committed neuronal precursors but not in oligodendroglial lineage cells. Furthermore, using
100 DKO mice, we show that THTs regulate neuronal lineage progression, and that their absence
101 leads to impairments in olfactory function. In contrast, the generation of new OPCs derived
102 from SVZ-NSCs is unaffected by the absence of THTs.

103

104 **Results**

105 **MCT8 and OATP1C1 are both expressed in NSCs and are retained mainly by** 106 **committed neuronal precursors within the adult SVZ.**

107 Local TH availability is tightly regulated, permitting precise temporal and cell-specific
108 control of TH action in the central nervous system. Cell specific TH availability is determined
109 by (i) a balance between TH-activating (mainly *Dio2* in the brain) and inactivating (*Dio3*)

110 deiodinases enzymes, (ii) cell specific membrane THTs, (iii) ligand (T3)-dependent nuclear
111 receptors (TR) forms (mainly *Thra1* within the adult SVZ, (López-Juárez et al., 2012)).

112 To elucidate the roles of MCT8 and OATP1C1 in adult SVZ neurogenesis, we first
113 determined which SVZ cell types express these THTs by IHC (Figure 1). To detect OATP1C1
114 expressing cells *in vivo*, we used the OATP1C1:YFP reporter mouse (see Material and
115 methods) that exhibits a stronger signal (fluorescence in the cytosol) than the antibody
116 directed against OATP1C1 (fluorescence of membrane-bound OATP1C1). Alternatively, we
117 used this antibody to detect endogenous expression of OATP1C1 *in vitro* using neurosphere
118 cultures. We observed that MCT8 (Figure 1A) and OATP1C1 (YFP+, Figure 1B) were highly
119 expressed in NSCs co-expressing GFAP (pink) and SOX2 (red) in the adult SVZ, as was also
120 confirmed in single cells dissociated from *in vitro* neurospheres (see the dotted signal in
121 Figure S1A). MCT8 staining was absent in both the dorsal and lateral part of the SVZ in Mct8
122 KO mice (Figure S1B), confirming the specificity of the MCT8 antibody. The specificity of
123 the OATP1C1 antibody was previously demonstrated (Mayerl et al., 2012). Moreover, and as
124 expected, MCT8 and OATP1C1 were mainly expressed on the plasma membrane as shown *in*
125 *vitro* by the colocalization with the plasma membrane marker pan-Cadherin and by the three-
126 D reconstruction (Figure S1C).

127 Second, we observed that both MCT8 (Figure 1C) and OATP1C1 (Figure 1D) signals
128 were detected at a low level in cells that expressed the neuronal markers DLX2 and DCX,
129 respectively labelling early neuronal precursors and mature neuroblasts, whereas few (5%)
130 oligodendroglial precursors (OLIG2+ SVZ-OPCs) expressed them. Altogether, these data
131 indicate that MCT8 and OATP1C1 transporters are strongly expressed in NSCs, and are
132 retained by neuronal lineage cells, while largely absent from precursors of the
133 oligodendroglial lineage.

134

135 **The TH signalling pathway is dynamically regulated during neurogenesis**

136 To further characterize the mRNA expression patterns of THTs and other TH signal
137 pathway components in the different adult SVZ cell types, we performed RTqPCR on FACS-
138 purified SVZ populations at each stage of the neuronal lineage (Figure 2A). To this end, we
139 purified NSCs (quiescent NSCs CD133+EGFR- and activated NSCs CD133+EGFR+),
140 transient amplifying cells or TACs (EGFR+), immature and migrating neuroblasts
141 (EGFR+CD24+ and EGFR-CD24+, respectively). The purity of each FACS-purified SVZ cell
142 population was confirmed by the expression of cell type-specific marker genes by RTqPCR
143 (Figure S2). Statistical results are presented in Table 1. As expected, *Gfap* (Figure S2A) and
144 *Nestin* (Figure S2B) expression were limited to NSCs, whereas *Egfr* (Figure S2C) was mainly
145 expressed in highly proliferative TAC progenitors. Early and late neuronal lineage cell
146 markers (*Dlx2* (Figure S2D) and *Dcx* (Figure S2E), respectively) were detected in young and
147 mature neuroblasts. Then, we assessed the gene expression profiles of several components of
148 the TH signalling pathway in each isolated cell population. *Mct8* (*Slc16a2*, Figure 2B) and
149 *Oatp1c1* (*Slc1c1*, Figure 2C) mRNA expression were strongly expressed in NSCs and
150 almost undetectable in TACs and committed neuronal precursors. This discrepancy between
151 mRNA and protein expressions can be explained by a longer half-life of both THT protein, as
152 previously observed in rats (Wittmann et al., 2015). Similarly, we previously demonstrated
153 that *Ttr* transcripts are expressed in the SVZ of adult mice whereas the protein is not detected
154 by IHC (Vancamp et al., 2019).

155 Two others key THTs, *Mct10* (*Slc16a10*, Figure 2D) and *Lat2* (*Slc7a8*, Figure 2F),
156 had the opposite profile with a higher expression in young and mature neuroblasts compared
157 to low expression in both NSCs and TACs. The moderate expression of *Lat1* (*Slc7a5*, Figure
158 2E) was unchanged in the different SVZ cell types. *Thra*, both $\alpha 1$ (Figure 2G) and $\alpha 2$ (Figure

159 2H) isoforms were highly expressed in NSCs, significantly decreased in proliferating TACs
160 and then increased again in neuroblasts.

161 To assess in which cell type the TH signalling is preferentially activated, we quantified
162 the expression of *Klf9* (Figure 2I), a well-known TH target gene (Hu et al., 2016). We found
163 that *Klf9* was highly expressed in NSCs and decreased in more committed cell types.

164 Taken together, the data show a dynamic expression pattern of several TH components
165 during neuronal lineage specification indicating a high TH responsiveness, especially in NSCs.

166

167 **The absence of MCT8 and OATP1C1 impairs NSC density and self-renewal potential**

168 As the above results suggest that both THTs are strongly expressed in adult NSCs, we
169 next examined the functional properties of DKO derived NSCs (Figure 3). A first observation
170 was that the density of SOX2+ cells (Figure 3A), a pluripotency marker of both NSCs and
171 progenitors, was reduced in the lateral SVZ of DKO mice compared to wild-type littermates
172 (WT: 1156±95.07; DKO: 39.26±4.99; t test: p<0.0001, t=11.73, df=16; Figure 3B). Then, we
173 tested if the proliferative potential of SOX2+ cells was affected in DKO mice. To this end, we
174 performed IHC for SOX2 and KI67 (a cell proliferative associated marker, Figure 3A) to
175 identify cycling progenitors. The density of SOX2+KI67+ cells was reduced in the SVZ of
176 DKO mice compared to wild-type littermates (WT: 350.9±29.5; DKO: 175.6±24.32; t test:
177 p=0.0003, t=4.59, df=16; Figure 3C), suggesting that proliferative neural stem/progenitor cell
178 pool of the SVZ is impaired in the absence of both THTs. Accordingly, one day after plating
179 neurosphere-dissociated cells (Figure 3D), we observed a two-fold reduction of
180 SOX2+KI67+GFAP+ NSCs, showing that there were less cycling SVZ-NSCs in DKO mice
181 (WT: 62.45±4.68; DKO: 32.68±5.59; Mann-Whitney test: p=0.0002, U=203.5, Figure 3E).
182 The alteration observed in the SOX2+ population could be a consequence of a decreased self-
183 renewal potential.

184 To test this hypothesis, we performed a neurosphere clonal assay. We determined self-
185 renewal capacity by counting the number of neurospheres plated at a clonal density of 10,000
186 cells/ml over serial clonal passaging (from passages II to IX; Figure 3F). The number of
187 neurospheres from WT and DKO SVZ were similar at passage II (DKO-NS II: 0.93 ± 0.04 ;
188 Bonferroni's test following two-way ANOVA: $p > 0.99$, $t = 1.02$; Figure 3G). However, after
189 nine passages, WT *versus* DKO neurospheres revealed distinct NSC behaviors (Bonferroni's
190 test: $p = 0.0003$, $t = 7.75$). As expected (Yoshioka et al., 2015), neurosphere numbers derived
191 from WT mice drop strongly beyond the ninth passage (WT-NS IX: 0.24 ± 0.08 ; Bonferroni's
192 test: $p < 0.0001$, $t = 10.37$). In contrast, DKO mice generated a large number of neurospheres
193 forming-NSC that could be passaged extensively (DKO-NS II: 0.93 ± 0.04 ; DKO-NS IX:
194 0.81 ± 0.05 ; Bonferroni's test: $p = 0.89$, $t = 1.6$; Figure 3G). Therefore, neurospheres derived
195 from DKO mice had an enhanced self-renewal capacity, continuing to propagate beyond the
196 ninth passage. These results show that MCT8 and/or OATP1C1 exert key roles in self-
197 renewal and maintenance of SVZ-NSCs in adult mice.

198

199 **Loss of MCT8 and OATP1C1 impairs regulation of NSC lineage determination and**
200 **olfactory behavior**

201 Then, we determined consequences of the combined absence of both THTs on the
202 generation of new neuronal *versus* oligodendroglial precursors derived from SVZ-NSCs *in*
203 *vivo*. First, we quantified the density of uncommitted (DLX2+, DCX-) and committed
204 (DLX2+, DCX+) NPCs and mature neuroblasts (DLX2-, DCX+) in the SVZ of WT and DKO
205 mice by IHC on coronal sections (Figure 4A). In DKO mice, the density of uncommitted and
206 committed NPCs was significantly decreased (DLX2: WT: 130.7 ± 15.55 cells/mm²; DKO:
207 53.03 ± 8.24 cells/mm²; t test: $p = 0.002$, $t = 3.46$, $df = 30$; Figure 4B; DLX2-DCX: WT:
208 213.4 ± 20.04 cells/mm²; DKO: 132.6 ± 20.39 cells/mm²; t test: $p = 0.02$, $t = 2.57$, $df = 30$; Figure

209 4C) as compared to WT mice. Surprisingly, the density of DCX+ mature neuroblasts is higher
210 in DKO mice in comparison to controls (WT: 256.8 ± 32.14 cells/mm²; DKO: 401 ± 55.81
211 cells/mm²; t test: $p=0.02$, $t=2.41$, $df=30$; Figure 4D).

212 The accumulation of neuroblasts in the SVZ of DKO mice may reflect hampered or
213 delayed migration of mature neuroblasts, and so we investigated whether the loss of MCT8
214 and OATP1C1 affected SVZ-NPC migration. To this end, neurospheres generated from SVZ
215 of WT and DKO mice were plated on 8-well glass slides (Millicell) coated with Poly-D-
216 Lysine (Sigma). Their migration behavior was determined after 3 days in vitro without EGF
217 and FGF by measuring radial migration away from adherent neurospheres (Figure 4E). For
218 this, we measured the distance of each cell that migrated from the neurosphere, and averaged
219 the distances per neurosphere. We found that on average, cells migrated from DKO
220 neurospheres covered a significantly shorter distance than those migrated from WT
221 neurospheres (WT: 259.9 ± 21.79 ; DKO: 181.4 ± 22.21 ; Mann-Whitney test: $p=0.03$, $U=2$;
222 Figure 4F). Thus, ablation of *Mct8* and *Oatp1c1* reduces the migration of neurosphere-derived
223 NPCs.

224 Given that the absence of THTs strongly affects SVZ-neurogenesis, we investigated
225 possible effects on olfactory behavior by performing the short-term olfactory memory test (as
226 described in (Lazarini et al., 2009)). WT *versus* DKO mice were exposed four times to the
227 same non-social odor (almond, lemon or orange blossom) at different intervals (2 min, 30 min
228 and 1 hour). We observed that the decrease in investigation time of the cotton swab after two
229 consecutive exposures to a similar odor was comparable in DKO and WT mice (Habituation:
230 WT: 0.46 ± 0.04 ; DKO: 0.55 ± 0.03 ; Bonferroni's test following two-way ANOVA: WT
231 $p < 0.0001$, $t=10.15$, DKO $p < 0.0001$, $t=10.95$; WT vs. DKO $p=0.46$, $t=1.59$; Figure 4G),
232 suggesting that DKO mice are able to discriminate simple odors. In contrast, after a longer
233 interval of 30 minutes and one hour, DKO mice spent significantly more time investigating

234 the odor compared to control animals (Memory: 30min: WT: 0.28 ± 0.03 ; DKO: 0.82 ± 0.04 ;
235 Memory 1 hour: WT: 0.16 ± 0.05 ; DKO: 0.97 ± 0.05 ; WT vs. DKO : Memory 30 min $p<0.0001$,
236 $t=9.61$; Memory 1 hour $p<0.0001$, $t=14.46$; Figure 4G). Interestingly, the investigation time of
237 DKO mice during the last presentation (after an interval of one hour) was not statistically
238 different from that of the first presentation of the odor (Bonferroni's test: WT $p<0.0001$,
239 $t=15.76$; DKO $p>0.99$, $t=0.81$). This suggests that DKO mice are unable to memorize odors.
240 Thus, our findings show that short-term olfactory memory is affected by the absence of THTs,
241 associating reduced adult SVZ-neurogenesis with altered olfactory memory.

242

243 **The absence of MCT8 and OATP1C1 does not affect SVZ-oligodendrogenesis**

244 Lastly, we investigated whether the generation of new SVZ-OPCs was affected in
245 DKO mice by performing an IHC for the oligodendroglia lineage markers OLIG2 and SOX10
246 (Figure 5A). We observed that neither the OLIG2+ nor the SOX10+ SVZ-OPC density was
247 affected in DKO mice as compared to control animals (OLIG2: WT: 0.33 ± 0.04 cells/mm²;
248 DKO: 0.22 ± 0.03 cells/mm²; Mann-Whitney test, $p=0.098$, $U=24.5$; Figure 5B; SOX10: WT:
249 0.64 ± 0.1 ; DKO: 0.76 ± 0.12 ; Mann-Whitney test, $p=0.41$, $U=34.5$; Figure 5C), suggesting that
250 the generation of OPCs derived from adult SVZ-NSCs is not impacted by the absence of both
251 MCT8 and OATP1C1.

252 Two distinct populations of OPCs are present in the adult mammalian brain. Contrary
253 to SVZ-OPCs that are continuously generated from NSCs in the adult SVZ, another source of
254 oligodendrocytes is located in the parenchyma (pOPCs), notably in the corpus callosum.
255 Since, these parenchymal resident pOPCs are generated earlier during development and
256 remain quiescent in the adult under steady-state conditions, we hypothesised that pOPCs were
257 affected during development of DKO mice (Figure 5D). Although the SVZ area of DKO mice
258 is unchanged compared to control SVZ (data not shown), the corpus callosum area is

259 significantly thinner in DKO than in controls ($619 \pm 17.24 \text{ mm}^2$ in DKO mice vs. $838.01 \pm$
260 53.01 mm^2 in WT), as previously observed in (Mayerl et al., 2014). Using IHC, we found that
261 the density of either OLIG2+ or SOX10+ pOPCs was strongly reduced in DKO mice as
262 compared to WT mice (OLIG2: WT: 32.64 ± 3.05 ; DKO: 12.95 ± 1.05 ; t test, $p < 0.0001$, $t = 5.96$,
263 $df = 31$; Figure 5E; SOX10: WT: 17.96 ± 1.7 ; DKO: 7.31 ± 0.78 ; t test, $p < 0.0001$, $t = 5.57$, $df = 31$;
264 Figure 5F). These findings show that pOPCs are highly sensitive to the absence of MCT8
265 and/or OATP1C1 whereas newly generated SVZ-OPCs are unaffected.

266

267 **Discussion**

268 The SVZ niche is a well-established TH target tissue: TH preferentially drives SVZ-
269 derived NSC fate toward a neuronal fate (Gothié et al., 2017b; López-Juárez et al., 2012;
270 Remaud et al., 2017). In contrast, a T_3 -free window due to the presence of *Dio3* and the
271 absence of $TR\alpha 1$ is required for OPC generation. However, how intracellular TH levels are
272 regulated within the different SVZ-cell types remains unknown. In this study, we determined
273 how the different components of the TH signalling pathway are modulated during NSC
274 determination to a neuronal fate. In particular, we provide new insights into the functional
275 role of two key THTs, MCT8 and OATP1C1, in NSC fate choice to a neuronal or a glial
276 progenitor within the adult mouse brain. Our work shows that cellular uptake/efflux of TH
277 through membrane transporters is involved in NSC function, impacting the generation of new
278 neuronal and glial cells within the adult mouse brain.

279

280 **Stem cell TH status**

281 Our data support a key role for MCT8 and/or OATP1C1 in NSC function. First, we
282 observed by RTqPCR and IHC that both THTs MCT8 and OATP1C1, are highly expressed in
283 NSCs within the adult SVZ. Second, in adult DKO mice, the density of SOX2+ NSCs and

284 progenitors is decreased. This result was confirmed *in vitro*: the number of cycling
285 SOX2+GFAP+ NSCs was reduced when neurospheres were grown from NSC dissected from
286 the SVZ of DKO mice, suggesting altered stem/progenitor cell behavior in absence of THTs.
287 Further, using the neurosphere assay, we determined that the absence of THTs does not
288 exhaust SVZ-NSC self-renewal after IX passages, contrary to WT NSCs.

289 Two subpopulations of NSCs co-exist in the adult SVZ: highly proliferative (activated,
290 aNSC) and quiescent (qNSCs) stem cells (Codega et al., 2014; Morshead et al., 1994). The
291 mechanisms regulating the transition from a quiescent to an activated state are still poorly
292 known. The TH signalling pathway could be a key regulator of this cell transition, especially
293 by controlling exit of NSCs from quiescence. The two-fold depletion of cycling NSCs in
294 DKO mice could indicate a return to quiescence in the absence of intracellular T₃ signalling.
295 qNSCs contribute to the generation of neurospheres when the pool of aNSC has been
296 experimentally depleted (Morshead et al., 1994). Hence, the challenging *in vitro* environment
297 could reactivate the expected accumulation of qNSCs due to the absence of a functional TH
298 signalling in DKO mice. In the absence of intracellular T₃, reactivated NSCs could proliferate
299 indefinitely, thus maintaining the neurosphere-forming potential that we still observed after
300 passage IX. Similarly, in another NSC niche, the subgranular zone (SGZ) of the hippocampus,
301 Mayerl et al. (Mayerl et al., 2020) have recently demonstrated that activation of NSCs is
302 impaired in a global Mct8 KO adult mouse model. Furthermore, Mayerl et al. (Mayerl et al.,
303 2018) demonstrated that the activation of muscle stem cells is reduced to about 50% in DKO
304 mice, impairing the myogenic program. They also found that a higher expression of both
305 *Mct8* and *Oatp1c1* is associated with the activation of quiescent satellite stem cells. These
306 data support our hypothesis that the absence of both THTs and intracellular T₃ induce the
307 transition of SVZ-NSCs to a quiescent state. Altogether, our work combined with Mayerl et
308 al.'s studies, underline a crucial function of MCT8 and/or OATP1C1 transporters in stem cell

309 quiescence and reactivation. However, the relative contribution of MCT8 and/or OATP1C1 to
310 maintain NSC identity could be further assessed by analysing NSC behaviour in simple KO
311 mice or following inhibition using commercially available inhibitors (Mayerl et al., 2020).

312 Furthermore, our *in vitro* experiments were done in presence of T₃ (0.3 nM, present in
313 the B27 supplement), and in this experimental condition, we still observed a reduction in the
314 numbers of NSCs and also impaired cell migration. Therefore, these results strongly suggest
315 that alterations in NSCs behaviour observed *in vivo* could be largely due to a local
316 intracellular hypothyroidism. However, we cannot exclude an additional impact due to (i) the
317 lack of MCT8 in both choroid plexus and endothelial cells of the BBB (Dumitrescu et al.,
318 2006; Heuer et al., 2005; Trajkovic et al., 2007) and (ii) OATP1C1 that facilitates T₄ uptake
319 across the BBB and/or the CSF (Roberts et al., 2008; Mayerl et al., 2014). Furthermore, since
320 MCT8 and OATP1C1 are also found in radial glia cells in the prenatal human brain (López-
321 Espíndola et al., 2019), we cannot rule out a developmental impact of the absence of both
322 transporters contributing to the adult phenotype that we observed in the SVZ of DKO mice.
323 Further analysis is needed to assess whether a reduction of TH availability within radial glial
324 cells of the mouse prenatal brain long-lasting influences NSC proliferation and cell fate in the
325 adult SVZ.

326 A decrease of intracellular T₃ could preserve/maintain the NSC pool that can be
327 reactivated under more favourable environmental conditions. We previously demonstrated
328 that SVZ-NSCs are blocked in G₂-phase, the phase before mitosis, following an adult-onset
329 hypothyroidism (Lemkine et al., 2005). In the *Drosophila* brain, G₂-quiescent cells are
330 capable of reactivating faster than G₀-stem cells (Otsuki and Brand, 2018). Interestingly, the
331 localization of NSCs in their niche, i.e. dorsal vs. ventral, would determine the enter in phase
332 G₀ or G₂: under the control of patterning factors, dorsal NSCs enter G₀ quiescence while
333 ventral NSCs undergo G₂ quiescence (Otsuki and Brand, 2019). Elucidating whether SVZ-

334 NSCs are preferentially arrested either in G0 or G2 phase following a decrease of TH
335 availability could help to adapt therapeutic purposes so as to manipulate NSC physiology and
336 promote generation of new neural cells.

337 We cannot exclude that the decreased density of cycling NSCs/progenitors might be
338 related to a developmental issue. THs are crucial for brain development: congenital
339 hypothyroidism and AHDS (*Mct8* deficiency) are two pathological conditions where low
340 brain TH content incurs intellectual disability (López-Espíndola et al., 2014; Schwartz and
341 Stevenson, 2007; Vatine et al., 2017). Here, we showed that NSCs express high levels of
342 several components of the TH signalling pathway in mice.

343 We speculate that high intracellular T3 levels in NSCs (high expression of both *THTs*,
344 *Thra1*, *Klf9*) could be acquired during the transition from embryonic/post-natal NSCs to adult
345 NSCs. The absence of both THTs could prevent sufficient uptake of THs and therefore an
346 intracellular lack of TH to determine the features of adult NSCs, consequently decreasing the
347 density of proliferating NSCs, as we observed in the adult SVZ of DKO mice. Further
348 investigation is necessary to determine whether T₃ is a key molecular signal controlling the
349 transition from qNSC to aNSC and inversely.

350 Once activated, NSCs express the epidermal growth factor receptor (*Egfr*), a key
351 proliferation-activating factor that also regulates NSC fate by blocking neurogenesis and
352 promoting glial fate (Ayuso-Sacido et al., 2010). Interestingly, high *Egfr* expression in
353 activated NSC and their progeny (TACs) associates with reduced intracellular TH levels due
354 to high expression of *Dio3* and absence of TR α 1 expression (Remaud et al., 2017). Similarly,
355 we detected a low mRNA expression of both *Mct8* and *Oatp1c1* and others THTs as *Mct10*,
356 *Lat1* and *Lat2* in TACs, suggesting that absence of THTs and TR α 1, combined with elevated
357 *Dio3* expression (Remaud et al., 2017), associate with actively proliferating progenitors
358 derived from SVZ-NSCs.

359 **MCT8 and/or OATP1C1 absence impairs SVZ-neurogenesis**

360 We demonstrated that the absence of MCT8 and/or OATP1C1 impairs SVZ-
361 neurogenesis whereas the generation of new SVZ-OPCs is unaffected. This is consistent with
362 our previous studies showing that TH signalling acts as a neurogenic switch in SVZ-
363 progenitors (Gothié et al., 2017b; López-Juárez et al., 2012) whereas a T₃-free window is
364 necessary to promote SVZ-oligodendrogenesis (Remaud et al., 2017). However, we did not
365 expect to observe an accumulation of DCX+ neuroblasts along the lateral ventricles of DKO
366 mice, suggesting a potential migration failure. Similarly, radial migration of newly-generated
367 SVZ-neural precursors was impaired *in vitro*.

368 Thus, the absence of MCT8 and/or OATP1C1 could alter the molecular and cellular
369 machinery necessary for the phosphorylation of doublecortin and the migration of neuroblasts,
370 inducing the accumulation of newly immature neurons along the lateral ventricles. The role of
371 TH signalling on neuroblast migration through the Rostral Migratory Stream (RMS) remains
372 unknown, though the role of TH in optimal cell migration has been shown in other brain
373 regions, notably during development in the cerebellum (Morte et al., 2002; Portella et al.,
374 2010) and neocortex (Pathak et al., 2010). Misregulation of two well-established TH target
375 genes involved in neural migration, *reelin* (Alvarez-Dolado et al., 1999) and NCAM (Iglesias
376 et al., 1996) blocked radial neuroblast migration, resulting in loss of cortical layering and
377 behavioral defects. Similarly, depletion of key components of the Reelin pathway (as
378 ApoER2/VLDLR or Dab1) resulted in accumulation of neuroblasts within the adult SVZ
379 (Andrade et al., 2007), phenocopying the results found in our DKO mice. Thus, the
380 interaction of TH with the Reelin signalling pathway deserves further analysis in the context
381 of adult SVZ-neurogenesis.

382 Using RTqPCR analysis on isolated cell types by FACS, we observed that both *Mct8*
383 and *Oatp1c1* mRNA were expressed at low levels in young and mature neuroblasts. In

384 contrast, IHC allowed us to detect high expression of both MCT8 and OATP1C1 proteins in
385 these cell types. To our knowledge, this is the first study highlighting a functional role of
386 these transporters in the neuronal lineage derived from adult SVZ-NSCs. However, further
387 work is needed to analyse the relative contribution of MCT8 and OATP1C1 during lineage
388 progression of neural progenitors in the SVZ of adult mice. Recently, using similar
389 approaches, Mayerl (Mayerl et al., 2020) detected high levels of MCT8 in neuroblasts located
390 within the hippocampus at both the mRNA and protein levels. Furthermore, they
391 demonstrated that the density of differentiated neuroblasts is decreased in Mct8 KO animals,
392 showing that a global inactivation of MCT8 impaired adult neurogenesis in the mouse SGZ
393 too. However, they found that the density of TACs and proliferating neuroblasts is unchanged
394 in Mct8 KO, strongly suggesting that MCT8 acts at later stages during SGZ-NSC lineage
395 progression, during cell transition from neuroblast to immature neurons. This is consistent
396 with previous work by Kapoor et al. (Kapoor et al., 2012) showing that TH signalling targets
397 predominantly post-mitotic committed neural progenitors and that THs accelerate neuronal
398 differentiation both *in vivo* and *in vitro*.

399 These studies demonstrate that TH action on adult neurogenesis is NSC niche-specific
400 and depends on the developmental window. Contrary to the adult SGZ, our study shows that
401 MCT8 and/or OATP1C1 are involved in the proliferation of SOX2+ TACs and DLX2+
402 young neuroblasts, indicating that THT-dependent TH availability regulates lineage
403 progression at multiple steps, from NSC/progenitor proliferation (Lemkine et al., 2005) to cell
404 fate determination to a neuronal fate (Gothié et al., 2017b; López-Juárez et al., 2012; Remaud
405 et al., 2017) in the adult SVZ. Our findings echo a recent study showing that MCT8
406 knockdown reduced the pool of progenitors, and impaired neurogenesis in the chicken optic
407 tectum, thus demonstrating that MCT8-dependent TH supply is crucial for early
408 corticogenesis (Vancamp et al., 2017).

409 Compared to *Mct8* and *Oatp1c1* mRNA, mRNA levels for others THTs (*Mct10* and
410 *Lat2*) are higher in the neuronal lineage. Further analysis is needed to study the function of
411 these secondary THTs, *Lat2* and *Mct10*, during neuronal lineage progression. In contrast to
412 the SVZ, Mayerl (Mayerl et al., 2020) detected high expression of *Mct10* (and *Mct8*, as
413 mentioned before) in SGZ-neuroblasts whereas *Lat1* and *Lat2* are expressed in NSCs and
414 TACs, showing distinct expression patterns of these THTs in the two neurogenic niches. This
415 recent finding is consistent with many others showing a complementary distribution pattern of
416 both *Mct8* and *Mct10* in several brain areas such as in the cerebral cortex (Müller and Heuer,
417 2014), strongly suggesting a compensatory function of *Mct10* in absence of *Mct8*. Due to the
418 mutually exclusive distribution of *Mct8* and *Mct10* mRNA in the SVZ, this region SVZ can
419 provide a key model to assess the physiological significance of *Mct10* as a specific THT in
420 SVZ-derived neuroblasts. Moreover, the contribution of all these THTs to SVZ-
421 oligodendrogenesis should also be addressed. In particular, a high *Mct10* signal has been
422 observed in white matter regions of the adult mouse brain (Müller and Heuer, 2014), raising
423 the question of whether *Mct10* provides TH during migration of SVZ-derived
424 oligodendrocytes towards the corpus callosum, thereby participating in axonal myelination in
425 physiological or demyelinated conditions (Menn et al., 2006).

426 Thus, analysing the function of the dynamic expression pattern of *Mct8/Oatp1c1* and
427 others THTs in the different SVZ cell populations will open new avenues on how cell-
428 autonomous function of the TH signalling could regulate NSC fate commitment to a neuronal
429 fate.

430

431 **Olfactory function is affected by the absence of both MCT8 and OATP1C1**

432 In rodents, newly-generated neuroblasts derived from NSCs migrate through the
433 RMS to the OBs where they differentiate into mature interneurons throughout life. This

434 continuous integration of new neurons into the pre-existing neuronal circuits has been
435 implicated in olfactory behavior, including odor memory and discrimination.

436 We showed a decreased generation of new migrating neuroblasts in DKO mice, that
437 together with a neuroblast migration defect, may affect the recruitment of new neurons in the
438 adult OBs. Newly generated neurons in the olfactory network play a key role in short-term
439 olfactory memory (Breton-Provencher et al., 2009). In particular, reduced levels of SVZ
440 neurogenesis following antimitotic drug AraC treatment caused a depletion of SVZ cells
441 derived from proliferating cells (Doetsch et al., 1999), moreover, a loss of olfactory memory
442 is seen, without affecting odor discrimination. Similarly, an upregulation of SVZ
443 neurogenesis by an olfactory-enriched environment increased olfactory memory (Rochefort et
444 al., 2002). These data strongly suggest that olfactory memory is dependent on the rate of adult
445 SVZ neurogenesis and that newly-generated neurons play a crucial role in establishing short-
446 term olfactory memory, but not odor discrimination (Breton-Provencher et al., 2009; Enwere
447 et al., 2004). Finally, absence of MCT8 and/or OATP1C1 impairs (i) generation and
448 migration of SVZ-neuroblasts (ii) olfactory capacities, suggesting that SVZ neurogenesis in
449 DKO mice is functionally impaired. However, the discriminative capacity of WT and DKO
450 mice was tested using simple, easily discernable odors. Some studies have previously
451 demonstrated that discrimination between two simple different odors is not impaired in mice
452 with reduced SVZ neurogenesis (Breton-Provencher et al., 2009; Enwere et al., 2004). We
453 cannot rule out that more difficult discrimination tasks using very similar odorants would
454 have been more challenging for DKO mice, as it has been recently demonstrated in other
455 mouse models aiming to analyse the impact of aging (Enwere et al., 2004) and consequences
456 of a transitory increase of the NSC pool on olfactory discrimination (Bragado Alonso et al.,
457 2019).

458

459 **Myelination status**

460 TH deficiency is usually linked to impaired myelogenesis during development. It is
461 well established that TH controls the timing of oligodendrocyte differentiation (Barres et al.,
462 1994; Billon et al., 2002; Kim and Petratos, 2019). Our work showed that DKO mice have a
463 significant lower density of resident pOPCs localised in the corpus callosum, above the lateral
464 ventricle, corresponding to the reduced myelination observed in the corpus callosum of DKO
465 brains with a silver staining technique (Mayerl et al., 2014). This hypomyelination state
466 associates with neurological and behavioral impairments described in DKO mice as well as in
467 AHDS patients (Mayerl et al., 2014). Post-mortem brain tissue analysis of an AHDS patient
468 strongly suggest that MCT8 drives OPCs T₃-dependent differentiation into mature and
469 functional myelinating oligodendrocytes (López-Espíndola et al., 2014). These resident
470 pOPCs, widely distributed in the parenchyma throughout the adult brain, are generated during
471 development and can remain quiescent throughout adult life (Tokumoto et al., 2017). The
472 significant reduction of resident parenchymal pOPCs in the corpus callosum could reflect
473 neurodevelopmental and behavioral consequences of *Mct8* and *Oatp1c1* depletion in the
474 developmental DKO mouse model. A conditional DKO mouse line would permit to better
475 distinguish effects generated early during development from those affected later during the
476 adulthood. Lastly, the crucial role of MCT8 in the differentiation of OPCs is known in
477 humans: MCT8 downregulation in hESCs-derived cells induces oligodendrocyte apoptosis
478 and myelin damage (Lee et al., 2017). These data suggest that the decrease in resident OPCs
479 in the corpus callosum of DKO mice could be due to OPCs apoptosis.

480 In contrast to resident pOPCs, our work shows that newly generated OPCs derived
481 from adult SVZ-NSCs express neither MCT8 nor OATP1C1, with generation of new OPCs in
482 the SVZ of DKO mice being unaffected. Our previous results showed that a transient
483 hypothyroidism promotes the generation of new SVZ-OPCs and not resident pOPCs,

484 demonstrating that both OPC populations differentially respond to a TH-free window (Gothié
485 et al., 2017a; Remaud et al., 2017). The discrepancy observed in the number of the SVZ-
486 OPCs compared to our previously published data could depend on the timing of when the
487 hypothyroidism was induced. In Remaud et al., 2017 and Gothié et al., 2017a, we induced a
488 systemic transient hypothyroidism in adult mice, whereas in the present work, brain TH
489 deficiency already occurs during developmental stages. Thus, the absence of an increase in
490 OPCs in the DKO mice could be due to a developmental defect. Several studies underline
491 that SVZ-OPCs are more capable of functional repair in demyelinating lesions than pOPCs
492 (Brousse et al., 2015; Remaud et al., 2017; Xing et al., 2014). Thus, how TH signalling
493 preferentially regulates SVZ-OPCs, an efficient source of remyelinating cells, will provide
494 new therapeutic options to favour myelin repair in multiple sclerosis and others demyelinating
495 diseases. More precisely, understanding how local intracellular TH levels differentially
496 regulate SVZ-OPCs and resident-OPCs could overcome the gap of knowledge on the distinct
497 behavior of these two kinds of OPCs after a myelin insult.

498

499 **Conclusion.**

500 This work highlights first, a new role of MCT8 and/or OATP1C1 in adult NSC
501 physiology, second, a tight and dynamic regulation of TH signalling during neuronal lineage
502 progression and third, an impairment of olfactory behavior and memory. Our data could shed
503 new lights on potential therapeutic purposes for AHDS patients and more generally for
504 demyelinating diseases where new remyelinating cells are needed. The neurological
505 phenotype of DKO mice and AHDS patients could be due to a developmental defect
506 worsened by an impaired neurogenesis in the adult.

507

508 **Experimental procedures**

509 **Animals**

510 Generation and genotyping of *Mct8* KO and *Mct8/Oatp1c1* double-knockout (DKO)
511 male mice were reported elsewhere (Mayerl et al., 2014; Trajkovic et al., 2007). For
512 visualising OATP1C1 expressing cells, transgenic animals were used that express YFP
513 reporter and a tamoxifen inducible cre-recombinase under the control of the *Slco1c1* promoter
514 (Ridder et al., 2011). Four weeks prior to immunofluorescence analysis, these OATP1C1:YFP
515 reporter mice were treated with tamoxifen (40 µg/g i.p.) for five consecutive days in order to
516 induce YFP expression specifically in OATP1C1 expressing cells.

517 Only male mice on a C57BL/6 background at the age of 2 months (WT, DKO;
518 OATP1C1:YFP) or 4 months (*Mct8* KO) were used in the study. *Mct8* KO, DKO,
519 OATP1C1:YFP mice and age-matched wildtype mice on the same genetic background were
520 obtained from the Heuer lab. C57BL/6 WT mice purchased from Janvier Labs (Le Genest St.
521 Isle, France) were used for qRT-PCR experiment (see protocols below). Mice were left
522 undisturbed for at least ten days after arrival in the animal facility to limit environmental
523 stress due to transport and to allow a better acclimatization to their new environment. Mice
524 were housed with an enriched environment to ameliorate anxious behaviour. Mice were kept
525 under standard conditions (22°C, 50% humidity, 12h light/dark cycle) and food and water
526 were available *ad libitum*. Animals were handled and cared for in accordance with the EU
527 Directive of 22 September 2010 (2010/63/EU). All procedures were conducted according to
528 the principles and procedures in Guidelines for Care and Use of Laboratory Animals and
529 validated by local and national ethical committees (CEEA Cuvier n°68; AZ 84-
530 02.04.2017.A219 LANUV NRW Recklinghausen Germany).

531

532 **Olfactory function**

533 Procedure is described in the Supplemental Information.

534 **Tissue preparation and immunohistochemistry (IHC)**

535 Three WT and three DKO mice were anesthetized with Pentobarbital (130 mg/kg,
536 Centravet) and perfused rapidly through the left heart ventricle with 1X PBS followed by 4%
537 paraformaldehyde in PBS (0.1 M, pH 7.4). Brains were harvested and post-fixed at 4°C in the
538 same fixative solution overnight. Brains were cryoprotected in 30% sucrose in 1X PBS at 4°C
539 and embedded in OCT (Sakura) and stored at -80°C. Brain coronal sections (30 µm thick)
540 were performed using a cryostat and were rigorously selected from bregma +0.9 mm to -0.1
541 mm and then, collected in sterile 1X PBS. The IHC procedure is described in the
542 Supplemental Information.

543

544 **Imaging and quantification**

545 A Leica TCS-SP5 confocal microscope was used to acquire fluorescence images
546 (1024x1024 pixels; 400X magnification). Images were taken on 3-5 sections per mouse.
547 Three detailed images were taken lining the lateral ventricles corresponding to the lateral SVZ
548 of both sides to quantify NSCs, NPCs and OPCs. In addition, three images were taken of the
549 corpus callosum at each side to quantify the density of parenchymal OPCs (pOPCs). Cell
550 counting was performed in the freely available FIJI software. For each image, the region of
551 interest (i.e. the lateral ventricular border or the corpus callosum) has been drawn. Moreover,
552 the immunopositive cells were quantified using the cell counter plugin. The results were
553 expressed as the number of immunopositive cells per mm². For MCT8 and OATP1C1
554 colocalization with the plasma membrane marker pan-Cadherin, Z-stack images were
555 acquired using 1024X1024 pixels; stack images: 0.34 µm; 630X magnification zoom: 2.5,
556 visualisations were done on Max Intensity Z projections using FIJI software and 3D
557 reconstitution were processed using Imaris software.

558

559 **Adult neurosphere assay**

560 Three WT and five DKO animals were sacrificed by cervical dislocation and the
561 region containing the lateral SVZ was dissected. Tissues were incubated in digestion medium
562 (papain (Worthington), DNase (Worthington), L-cysteine (Sigma-Aldrich)) for 30 min at
563 37°C and mechanically dissociated to obtain a single cell suspension. Cells were collected by
564 centrifugation at 1,000 rcf for 5 min and then cultured in a tissue culture flask 25 (TPP)
565 containing DMEM-F12 (Gibco), 40 mg/ml insulin (Sigma), 1/200 B-27 supplement (Gibco)
566 which contains 0.3 nM T3, 1/100 N-2 supplement (Gibco), 0.3% glucose (Sigma-Aldrich), 5
567 nM HEPES (Thermo Fisher), 100 U/ml penicillin/streptomycin (Thermo Fisher), 20 ng/ml
568 EGF (Peprotech) and 20 ng/ml FGF2 (Peprotech) to obtain primary neurospheres. The
569 cultures were maintained in a 5% CO₂ environment at 37 °C for 7 days and the growing
570 medium was supplemented with EGF and FGF2 every two days.

571 Primary neurospheres were first used for cell migration (procedure described in the
572 Supplemental Information). To analyse cell differentiation, primary neurospheres were
573 dissociated and 50x10³ cells were plated in 8-well glass slides (PEZGS0816, Millicell) coated
574 with Poly-D-Lysine (0.1 mg/ml, Sigma-Aldrich). Differentiation was induced by EGF and
575 FGF2 withdrawal. After 24 hours of differentiation, cells were fixed with 4%
576 paraformaldehyde in PBS (0.1 M, pH 7.4). IHC was performed as described above.
577 Quantifications were done on Max Intensity Z projections of 4 µm stack images using FIJI
578 software. Results are presented as the number of immunopositive cells respect to the total
579 number of cells.

580

581 **Clonality neurosphere assay**

582 Primary neurospheres (NS I), obtained as indicated above, were dissociated and then,
583 plated at 10,000 cells/ml concentration in 24-well tissue culture test plate (TPP) as indicated

584 above (n = 5-10 wells per passage, n = 1 experimental replicate). Three wells of floating
585 neurospheres (NS II to NS IX) were imaged with EVOS XL Core cell imaging system
586 microscope (x10) and the number of neurospheres per well was determined using FIJI
587 software.

588

589 **Flow Cytometry and Real-time PCR**

590 Procedure is described in the Supplemental Information.

591

592 **Statistical analysis**

593 Statistical analyses were performed with GraphPad Prism8 software (GraphPad
594 Software, San Diego, CA) and StatXact8 (Cytel Inc.). A Kruskal-Wallis test followed by
595 permutation test were used for the qPCR analyses. For *in vivo* and *in vitro* quantifications,
596 means from two groups were compared using a two-tailed Student's t test or a Mann-Whitney
597 U test, depending on whether data were normally distributed or not, respectively. A two-way
598 ANOVA test followed by a Bonferroni post-test was used to compare the number of
599 neurospheres and the short-term olfactory memory in CTL and DKO mice. Differences were
600 considered statistically significant for a p-value<0.05. Data are presented in the histograms
601 and expressed as mean \pm standard error of the mean (S.E.M.) or in the boxplots with medians,
602 minimum and maximum values.

603

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610

611 **Author contributions**

612 C.L, L.B, A.S and K.L conducted the experiments. C.L, L.B, A.S and K.L did cell counting
613 and analysed data. M.S and H.H provided the transgenic mice. C.L, L.B, A.S, S.R and B.D
614 designed the experiments and wrote the paper. All authors were involved in commenting on
615 the paper.

616

617 **Declaration of Interests**

618 The authors declare no competing interests

619

620 **References**

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701

702 **Figure legends**

703 **Figure 1. MCT8 and OATP1C1 are strongly expressed in NSCs and are maintained**
704 **principally in committed neuronal cells within the adult SVZ**

705 (A-B) Expression of MCT8 and OATP1C1 THTs in NSCs. MCT8 (A) and OATP1C1 (B)
706 staining's on coronal section of adult dorsal SVZ show strong expression of both THTs in
707 SOX2+ and GFAP+ NSCs. (C-D) Expression of THTs in neuronal (DLX2+, DCX+) and

708 oligodendroglia (OLIG2+) differentiation pathways. MCT8 (C) and OATP1C1-YFP (D) are
709 maintained in neuronal differentiation pathway but are not expressed in oligodendroglia
710 differentiation pathway. Scale bar: 30 μ m.

711

712 **Figure 2. TH signalling components are dynamically expressed in neuronal lineage cells**
713 **within the adult SVZ**

714 (A) Schematic representation of the markers used to quantify TH signaling components
715 mRNA expression levels in quiescent and activated NSCs (CD133+EGFR), TACs (EGFR+),
716 NPCs (EGFR+CD24+) and neuroblasts (CD24+) from FACS-sorted SVZ cells of five adult
717 WT male mice. (B-F) Detection of TH signaling components mRNA expression levels in FACS-sorted SVZ cells:
718 *Slc16a2/Mct8* (B), *Slco1c1/Oatp1c1* (C), *Slc16a10/Mct10* (D), *Slc7a5/Lat1* (E), *Slc7a8/Lat2*
719 (F). (G-H) Detection of TH receptors mRNA expression levels in FACS-sorted SVZ cells:
720 *Thra1* (G) and *Thra2* (H). (I) Gene expression analysis of TH responsive gene *Klf9*. n = 3–5
721 samples per cell population, Kruskal-Wallis test followed by permutation test, *: p<0.05, **:
722 p<0.01. Data are presented in boxplots with medians, minimum and maximum values.

723

724 **Figure 3. DKO adult mice differentially affects NSC and progenitor proliferation within**
725 **the lateral SVZ**

726 (A) Representative images of proliferative NSCs (Sox2+KI67+) within the lateral SVZ from
727 WT and DKO adult male mice. Less proliferative NSCs are observable in DKO mice. (B-C)
728 Statistical analysis revealed a significantly decreased SOX2+ NSCs (B) and SOX2+KI67+
729 proliferative NSCs (C) in the lateral SVZ of DKO mice as compared to WT mice (n= 3 mice
730 per group and n=2-5 sections per mice, t test, ***: p<0.001). (D) Representative images of
731 proliferative NSCs (SOX2+KI67+GFAP+) after plating primary neurospheres following a
732 24h period under a low growth factor condition. DKO mice show less proliferative NSCs. (E)

733 DKO mice display a significant decreased SOX2+KI67+GFAP+ proliferative NSCs as
734 compared to WT mice (n= 3-5 mice per group and n=30 images per condition, Mann-Whitney
735 test, ***: p<0.001). (F) Representative images of floating neurospheres II (NS II) and
736 neurospheres IX (NS IX) derived from lateral SVZ of adult male WT and DKO mice. (G) The
737 neurospheres formation in WT mice significantly decreased after nine passages (NS IX)
738 whereas the number of neurospheres is maintained in DKO mice (n= 3 wells per condition, 2-
739 way ANOVA followed by Bonferroni's test, ***: p<0.0001). Bars represent mean \pm SEM.
740 Scale bars: 30 μ m (A, D) and 250 μ m (F). LV: lateral ventricle, NS II: neurospheres II, NS IX :
741 neurospheres IX.

742

743 **Figure 4. DKO adult mice display reduced SVZ-derived NPCs and impaired neuroblast**
744 **migration within the SVZ**

745 (A) Representative images of NPCs (DLX2+) and neuroblasts (DCX+) within the lateral SVZ
746 from WT and DKO adult male mice. (B-D) Quantification of uncommitted (DLX2+ cells)
747 and committed (DLX2+DCX+ cells) neural progenitors and mature neuroblasts (DCX+ cells)
748 within the lateral SVZ of WT and DKO male mice. DKO mice display reduced density of
749 uncommitted (B) and committed (C) neural progenitors as compared to WT mice. In contrast,
750 the density of mature neuroblasts (D) increased in DKO mice as compared to WT mice (n= 3
751 mice per group and n=2-5 sections per mice, t test, *: p<0.05, **: p<0.01). (E) Representative
752 images of the migration calculation method. Migrating cells can be seen as mixed cell
753 outgrowth constituted by both individual and chain cells that migrate from neurospheres. The
754 migration distance is determined using a line drawing tool under FIJI software. The lines are
755 drawn from the outline of the neurospheres to the center of the migrating cells. All cells that
756 migrate from the neurospheres are considered. The two others images represented plated SVZ
757 neurospheres generated from WT and DKO mice following a 3 days period under a low

758 growth factor condition. (F) *In vitro* measurement of neural precursors migration away from
759 plated primary neurospheres show reduced migration distance per neurosphere in DKO mice
760 (n= 5 neurospheres per condition, Mann-Whitney test, *: p<0.05). (G) Short-term olfactory
761 memory behavioral test. The graph represents the time spent by WT and DKO mice to
762 investigate the odor during the first presentation, the habituation phase (2-min later) and the
763 two-memory phase (after 30-min and 1-hour rest period). The short-term olfactory memory is
764 impaired in DKO mice as shown by the increase of the investigation time 30 min and one
765 hour after the first exposure (n= 3-5 mice per group, n= 3 experiments, Two-way ANOVA
766 followed by Bonferroni's multiple comparisons test to compare the four presentations and to
767 compared WT and DKO mice, ***: p<0.001). Bars represent mean \pm SEM. Scale bars: 30 μ m
768 (A) and 250 μ m (E). LV: lateral ventricle.

769

770 **Figure 5. DKO adult mice display reduced SVZ-derived OPCs within the corpus**
771 **callosum but not within the SVZ**

772 (A) Representative images of OPCs (OLIG2+ and SOX10+) within the lateral SVZ from WT
773 and DKO adult male mice. (B-C) Quantification of OLIG2+ (B) and SOX10+ (C) OPCs into
774 the lateral SVZ demonstrate no density difference between WT and DKO mice (n= 3 mice per
775 group and n=2-5 sections per mice, Mann-Whitney test, ns: p>0.05). (D) Representative
776 images of OPCs (OLIG2+ and SOX10+) within the corpus callosum from WT and DKO adult
777 male mice. (E-F) DKO mice display a significant decreased OLIG2+ (E) and SOX10+ (F)
778 OPCs density in the corpus callosum as compared to WT mice (n= 3 mice per group and n=2-
779 5 sections per mice, t test, ***: p<0.001). Bars represent mean \pm SEM. Scale bars: 30 μ m (A)
780 and 60 μ m (D). LV: lateral ventricle.









