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Supplemental Information

Absence of Both Thyroid Hormone Transporters MCT8 and OATP1C1 Impairs Neural Stem Cell Fate in the Adult Mouse Subventricular Zone

Cristina Luongo, Lucile Butruille, Anthony Sébillot, Karine Le Blay, Markus Schwaninger, Heike Heuer, Barbara A. Demeneix, and Sylvie Remaud

Fig. S1

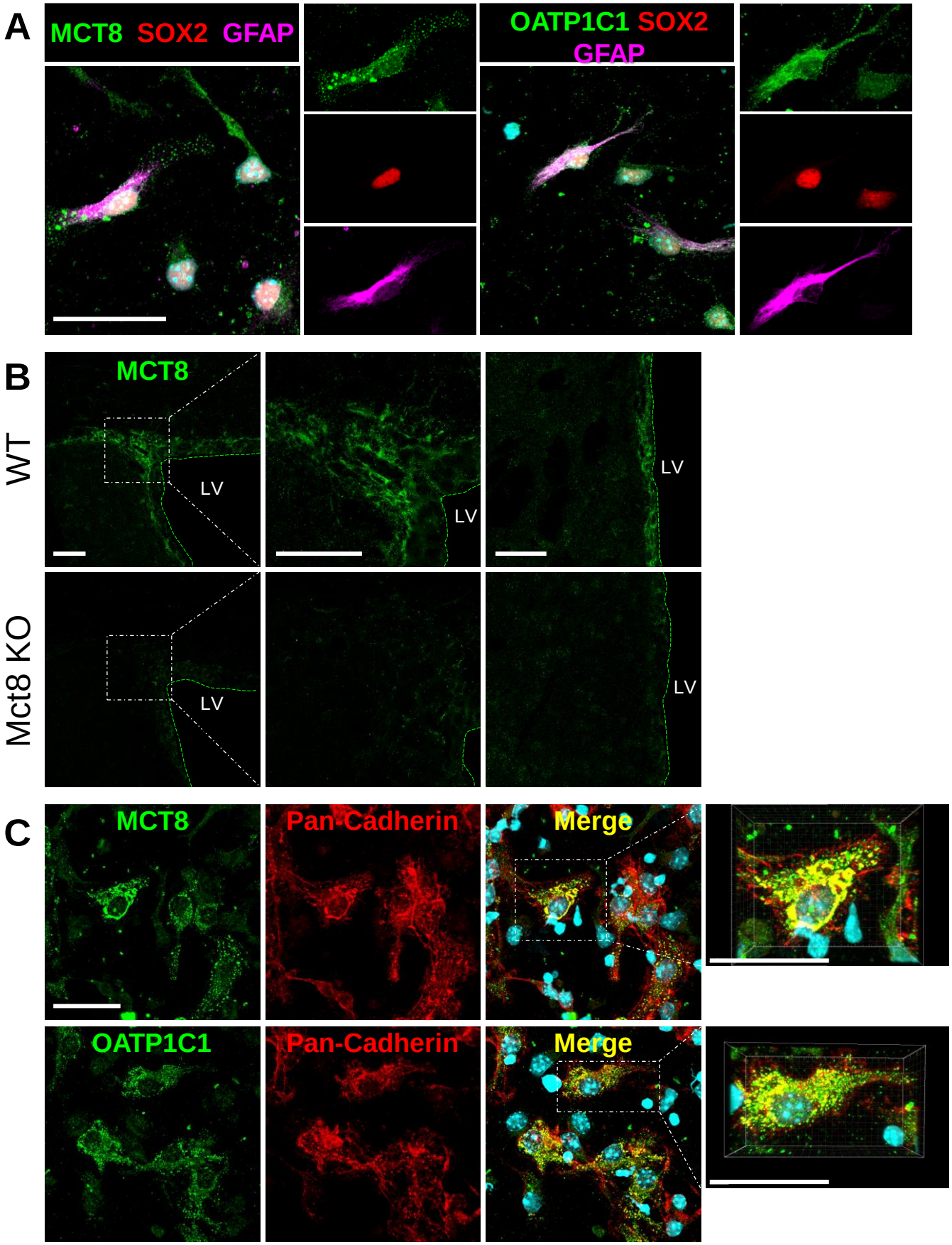
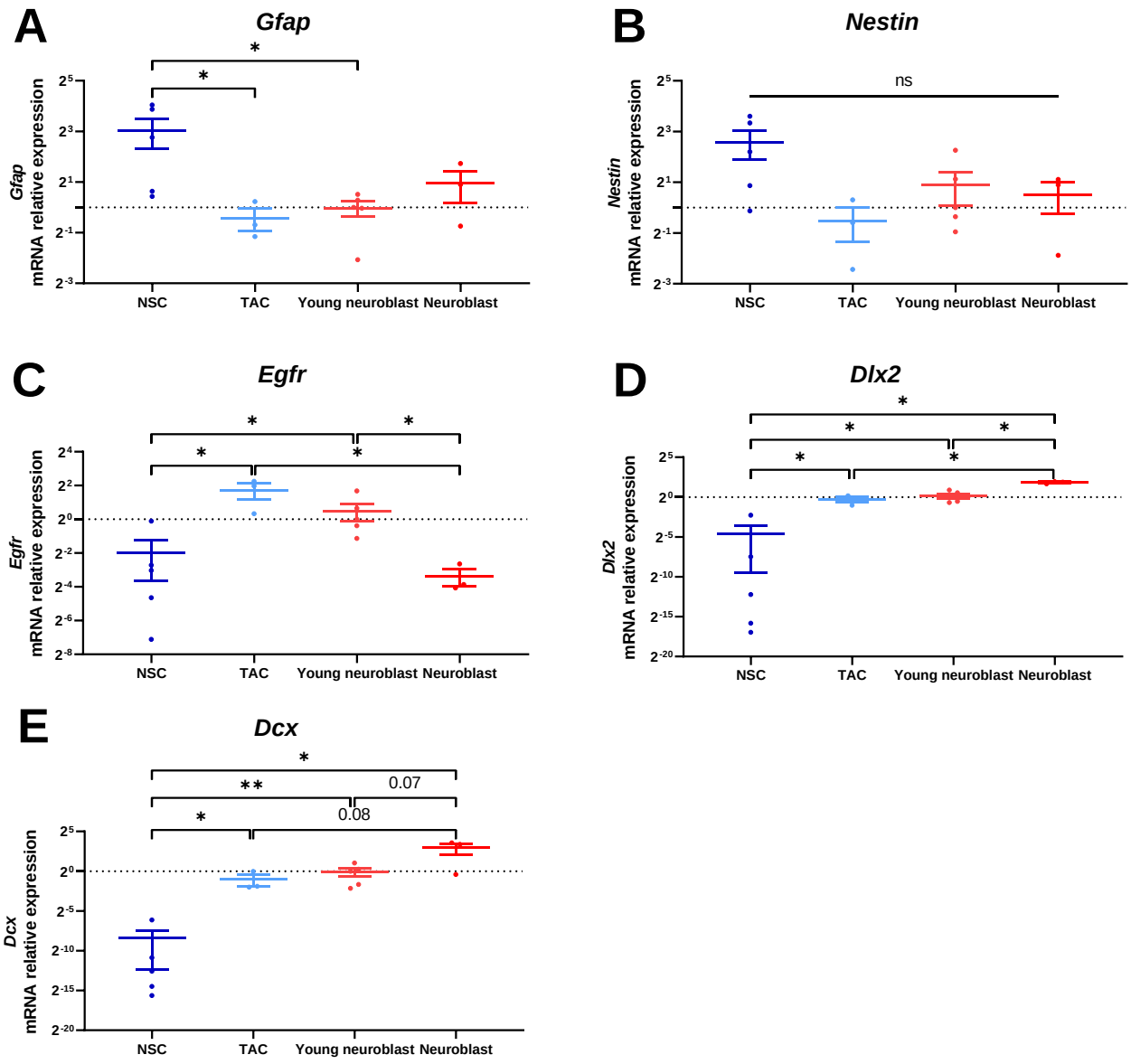

A
MCT8 SOX2 GFAP
OATP1C1 SOX2 GFAP
B
WT
Mct8 KO
MCT8
LV
C
MCT8
Pan-Cadherin
Merge
OATP1C1

Figure S1. Expression and localization of MCT8 and OATP1C1 transporters. Related to Figure 1
(A) *In vitro* expression of MCT8 and OATP1C1 THTs in single cells dissociated from neurospheres. MCT8 and OATP1C1 are strongly expressed in SOX2+ and GFAP+ NSCs. (B) Specificity of the MCT8 antibody. MCT8 is totally absent in the dorsal and lateral SVZ of Mct8 KO mice. (C) MCT8 and OATP1C1 colocalization with the plasma membrane marker pan-Cadherin. Three-D reconstruction demonstrates the *in vitro* membrane localization of both THTs. Scale bars: 30µm (A), 50µm (B) and 30µm (C).

Fig. S2


A
Gfap
Gfap mRNA relative expression
NSC
TAC
Young neuroblast
Neuroblast
B
Nestin
Nestin mRNA relative expression
ns
C
Egfr
Egfr mRNA relative expression
D
Dlx2
Dlx2 mRNA relative expression
E
Dcx
Dcx mRNA relative expression
0.07
0.08

Figures S2. Expression of cell type-specific marker genes at each stage of the neuronal lineage. Related to Figure 2

(A-B) Detection of NSCs mRNA expression levels *Gfap* (A) and *Nestin* (B) in FACS-sorted SVZ cells of five adult WT male mice. (C) Gene expression analysis of transient amplifying cells mRNA *Egfr* in SVZ. (D-E) Detection of neuronal cells mRNA expression *Dlx2* (D) and *Dcx* (E) in FACS-sorted SVZ cells. n = 3–5 samples per cell population, Kruskal-Wallis test followed by permutation test, *: $p<0.05$, **: $p<0.01$. Data are presented in boxplots with medians, minimum and maximum values.

Table 1: Statistical results of qPCR analysis. Related to Figure 2 and Figure S2.
NSC: neural stem cell, TAC: transient amplifying cell

Genes	Kruskal Wallis test	Permutation post test
Gfap	p = 0.02	NSC vs. TAC p = 0.05 NSC vs. Young neuroblast p = 0.03
Nestin	p = 0.19	
Egfr	p = 0.002	NSC vs. TAC p = 0.05 NSC vs. Young neuroblast p = 0.03 TAC vs. Neuroblast p = 0.032 Young neuroblast vs. Neuroblast p = 0.017
Dlx2	p < 0.0001	NSC vs. TAC p = 0.043 NSC vs. Young neuroblast p = 0.014 NSC vs. Neuroblast p = 0.03 TAC vs. Neuroblast p = 0.034 Young neuroblast vs. Neuroblast p = 0.02
Dcx	p = 0.0008	NSC vs. TAC p = 0.019 NSC vs. Young neuroblast p = 0.006 NSC vs. Neuroblast p = 0.015 TAC vs. Neuroblast p = 0.08 Young neuroblast vs. Neuroblast p = 0.07
Scl16a2/Mct8	p = 0.0033	NSC vs. TAC p = 0.024 NSC vs. Young neuroblast p = 0.007 NSC vs. Neuroblast p = 0.019
Sclo1c1/Oatp1c1	p = 0.0047	NSC vs. TAC p = 0.012 NSC vs. Young neuroblast p = 0.004 NSC vs. Neuroblast p = 0.011
Scl16a10/Mct10	p = 0.012	NSC vs. Neuroblast p = 0.032 TAC vs. Neuroblast p = 0.029
Slc7a5/Lat1	p = 0.39	
Slc7a8/Lat2	p = 0.06	
Thra1	p = 0.017	NSC vs. TAC p = 0.015 NSC vs. Young neuroblast p = 0.07 TAC vs. Neuroblast p = 0.036
Thra2	p = 0.021	NSC vs. TAC p = 0.022 NSC vs. Young neuroblast p = 0.014
Klf9	p = 0.0032	NSC vs. TAC p = 0.014 NSC vs. Young neuroblast p = 0.0067 NSC vs. Neuroblast p = 0.022

Supplementary experimental procedures

Olfactory function

The short-term olfactory memory test was performed as previously described (Lazarini et al., 2009). Three WT and five DKO mice were first habituated to a cotton swab and a fresh cage 15 min before the test. Animals were then exposed four times to the same odor for 5 min presentations separated by 2, 30, 60 min intervals. The second presentation is considered as the habituation phase to the odors, and the third and fourth presentations are the memorization phases. This experiment was carried out three times and three different non-social odors were presented to the mice on the cotton swab: almond (vahiné, 1/100), orange blossom (Sainte Lucie, 1/50) and lemon (Sainte Lucie, 1/200). The odor investigation time was measured for each presentation and compared to the first presentation.

Immunohistochemistry

Three to five sections per mice separated by 150 µm along the antero-posterior axis were incubated for 30 min in a blocking solution (10% donkey serum (Sigma), 1% BSA (Sigma), 1X PBS with 0.2% Triton) at room temperature (RT), and then incubated with primary antibodies diluted in the same blocking solution overnight at 4°C. After 3x10 min washes in 1X PBS at RT, sections were incubated with fluorescent secondary antibodies (1/500, Invitrogen) diluted in 1% donkey serum, 1% BSA, 1X PBS with 0.2% Triton. Following 3x10 min washes, sections were incubated with DAPI for 5 min at RT and mounted on glass slides with Prolong Gold antifade reagent (Invitrogen). Antibodies and their dilution factors were as follows: Goat anti-SOX2 (sc-17320), 1:300, Santa Cruz; Rabbit anti-KI67 (AB15580), 1:800, Abcam; Chicken anti-GFAP (AB4674), 1:300, Abcam; Goat anti-DCX (sc-8066), 1:300, Santa Cruz; Guinea Pig anti-DLX2, 1/3000, gift from K. Yoshikawa laboratory; Mouse anti-OLIG2 (MABN50), 1:300, Millipore; Guinea Pig anti-SOX10, 1:300, gift from Mickael Wegner laboratory; Rabbit anti-GFP (sab4301138), 1/300, Sigma; Anti-pan Cadherin antibody [CH-19] (AB6528), 1/500, Abcam; Rabbit anti-MCT8 and anti-OATP1C1, 1:300, gift from T.J. Visser laboratory.

To confirm the localization of MCT8 and OATP1C1 at the cellular membrane, we performed IHC against MCT8 and OATP1C1 together with pan-cadherin on dissociated cells from WT primary neurospheres cultivated as described below. Cells at 24 hours of differentiation were fixed in 100% methanol and blocked with 10% donkey serum and 1% BSA (Sigma Aldrich) in 1X PBS for 30 min at RT. IHC was performed as described above.

Cell migration analysis

To analyse cell migration, primary neurospheres were plated in 8-well glass slides (PEZGS0816, Millicell) coated with Poly-D-Lysine (0.1 mg/ml; Sigma-Aldrich) in growing culture medium without EGF/FGF2 for 3 days. Brightfield images were taken with the EVOS XL Core cell imaging system microscope (x20) and migration distance per neurosphere (µm) was determined using a line drawing tool under FIJI software. The lines are drawn from the outline of the neurospheres to the center of the migrating cells. All cells that migrate from the neurospheres are considered. Migration distances were averaged per neurospheres.

Flow Cytometry and Real-time PCR

The lateral SVZs of five adult WT male mice were dissected and incubated at 37°C in digestion medium containing papain (Worthington), DNase (Worthington), L-cysteine (Sigma-Aldrich) with mechanically dissociation to obtain a single-cell suspension. After the dissociation and resuspension, the cell debris was removed using the Debris Removal Solution (Miltenyi Biotec). Cells were then incubated with a combination of the following antibodies: an Alexa488-conjugated Rat anti-mouse antibody directed against CD133 (Clone 13A4, Thermo Fisher Scientific), a Brilliant Violet 421-conjugated Rat anti-mouse antibody against CD24 (BV421-CD24, BD Biosciences), and an APC-conjugated antibody against EGFR (E13345, Thermo Fisher Scientific) for 30 min at 4°C. Cell sorting was performed on a BD FACS AriaTM III. After 10 min centrifugation at 2,000 rpm, cell pellets of CD133+ EGFR− quiescent NSCs (qNSCs), CD133+ EGFR+ activated NSCs (aNSCs), EGFR+ CD133−CD24− transiently amplifying cells (TACs), EGFR+ CD24+ immature neuroblasts (NPCs), and CD24+ EGFR− migrating neuroblasts were frozen at −20 °C until RNA extraction.

RNA extraction and reverse-transcription were done using the RNAqueous-Micro kit with DNase treatment (ThermoFisher) and the Reverse Transcription Master Mix from Fluidigm, following the manufacturer's instructions. Pre-amplifications were performed for genes of interest using the Taqman Preamp Master Mix kit (ThermoFisher), with primers from Taqman. Gene expression assays: *Slc16a2/Mct8* (mm00486204_m1); *Slco1c1/Oatp1c1* (mm00451845_m1); *Slc16a10/Mct10* (mm00661045_m1); *Slc7a5/Lat1* (mm00441516_m1); *Slc7a8/Lat2* (mm01196249-m1); *Thra1* (Nm178060.3); *Thra2* (BC046795.1); *Klf9* (Mm00495172_m1); *Gfap* (mm01253033_m1); *Nestin* (Mm01223404_g1); *Egfr* (mm01187858_m1); *Dlx2* (mm00438427_m1); *Dcx* (mm00438400_m1); *Gapdh* (mm99999915_g1); *Actb* (mm00607939_s1). RTqPCR reactions were performed in triplicate for each sample using Reverse Transcription Master Mix (Fluidigm) on a QuantStudioTM6 Flex Real-Time PCR System (Applied Biosystems), following manufacturer recommendations. The Ct's were averaged and

normalised (ΔCt) against the geometric mean of two reference genes (Gapdh and Actin beta). Variations of expression were quantified by the ΔΔCt method (Livak KJ, Schmittgen TD (2001)). Analysis of relative gene expression data was done using real-time quantitative PCR and the 2(-ΔΔCt) Method.