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## Cell metabolic alterations due to Mcph1 mutation in microcephaly

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1 **SUMMARY**

2

3 A distinctive feature of the neocortical development is the highly coordinated production of  
4 different progenitor cell subtypes, which are critical for ensuring adequate neurogenic outcome and  
5 the development of normal neocortical size. To further understand the mechanisms that underlie  
6 neocortical growth, we focused our studies on the microcephaly gene *Mcp1*, and we report here that  
7 *Mcp1* 1) exerts its functions in rapidly dividing apical radial glial cells (aRGCs) during mouse  
8 neocortical development stages that precede indirect neurogenesis, 2) is expressed at mitochondria;  
9 and 3) controls RGC proper proliferation and survival, potentially through crosstalk with cellular  
10 metabolic pathways, involving stimulation of mitochondrial activity via VDAC1/GRP75,  
11 AKT/HK2/VDAC1 and of glutaminolysis via ATF4/PCK2. We thus currently report the description of  
12 a MCPH gene implication in the interplay between bioenergetic pathways and neocortical growth,  
13 thus pointing to alterations of cellular metabolic pathways, in particular of glutaminolysis, as possible  
14 cause of microcephalic pathogenesis.

15

16

# 1 INTRODUCTION

2

3         The evolutionary expansion of the mammalian neocortex (nctx) underlies the development of  
4 sophisticated cognitive functions, culminating in humans. This evolutionary hallmark has raised  
5 interesting issues with regard to the mechanisms that determine neocortical size and how these  
6 mechanisms were adapted to drive neocortical expansion. Nctx size is determined by the neuronal  
7 output, which is governed by the proliferative capacity of neural progenitor cells. Three cellular  
8 subtypes of neural progenitors emerge progressively in tight coordination with neocortical  
9 development. The primary neuroepithelial cells amplify by symmetric divisions and give rise to apical  
10 radial glial cells (aRGCs), which divide at the ventricular border and constitute the ventricular zone  
11 (VZ). Basal progenitors, including intermediate progenitor cells (IPCs) and basal RGCs (bRGCs),  
12 arise from aRGC by asymmetric divisions and populate the subventricular zone (SVZ), in which they  
13 divide to generate neurons (Dehay et al., 2015; Florio and Huttner, 2014; Lui et al., 2011). Hence, all  
14 neuronal cell types arising in the nctx belong to the aRGC lineage and any alteration of aRGC  
15 production is expected to have a profound impact on the neocortical size. The greater neurogenic  
16 output seen in the gyrencephalic nctx of humans and non-human primates, compared to lissencephalic  
17 nctx of lower species like rodents, is thought to rely on the formation of a further germinative layer in  
18 the SVZ, called the outer SVZ (OSVZ), in which neurogenesis is extended by further proliferation of  
19 neural progenitors (Betizeau et al., 2013; Kalebic et al., 2019; Martinez-Martinez et al., 2016;  
20 Nowakowski et al., 2017).

21         Despite advances in the characterization of the molecular cues governing intrinsic functions in  
22 specific neural progenitor subtypes (Florio et al., 2015; Kalebic et al., 2019; Nowakowski et al., 2017),  
23 the molecular pathways controlling the transition and proper balance between these subtypes remain  
24 unclear. Recently, several lines of evidence have implied bioenergetics pathways in the fate  
25 determination of neural progenitor. During neocortical development, the actively proliferating aRGCs  
26 stimulate glycolysis, whereas neuronal differentiation by indirect neurogenesis requires a switch to  
27 oxidative phosphorylation (Lange et al., 2016; Wagenfuhr et al., 2015; Zheng et al., 2016). The fate of

1 early aRGCs is also influenced by the PERK (Protein kinase RNA-like endoplasmic reticulum  
2 Kinase)/ATF4 arm of UPR (Unfolded Protein Response), which promotes the birth of pioneer neurons  
3 by direct neurogenesis, and inhibits indirect neurogenesis (Frank et al., 2010; Laguesse et al., 2015).  
4 How bioenergetic pathways mechanistically control progenitor behavior with respect to proliferation  
5 rate, mode of division and survival, is not clear.

6 In this context, non-syndromic genetic autosomal recessive primary microcephaly (MCPH)  
7 has proved to be a powerful research model to investigate fundamental mechanisms that maintain  
8 neural progenitor homeostasis in the developing nctx. All of the hitherto identified MCPH genes  
9 (MCPH1-25) play a role in cell division (Duerinckx and Abramowicz, 2018; Jayaraman et al., 2018).  
10 To further elucidate the mechanisms responsible for microcephaly pathogenesis, which in turn may  
11 provide insight into the mechanisms regulating the size of the nctx, we focused our research on  
12 *MCPH1*, a MCPH gene with multifaceted functions (Jackson et al., 1998; Liang et al., 2010; Peng et  
13 al., 2009; Pulvers et al., 2015). In mouse neural progenitors, *McpH1* controls the coupling between the  
14 centrosome cycle and the entry into M-phase through the Cdc25b/Chk1 pathway (Gruber et al., 2011).  
15 Here, we show that MCPH1 is expressed at mitochondria and directly influences mitochondrial  
16 functions by interfering with the VDAC1/GRP75 pathway and notably, by stimulating glutaminolysis  
17 via the ATF4/PCK2 pathway. We thus reveal that *McpH1* is involved in the crosstalk between  
18 bioenergetic pathways, an essential function for the proliferation and survival of neocortical  
19 progenitors.

20

## 1 RESULTS

2

### 3 **McpH1 expression in aRGCs declines with the onset of indirect neurogenesis**

4 First, we determined the dynamic of Mcph1 expression in progenitors during mouse  
5 neocortical development. Immunofluorescence (IF) performed between embryonic day (E) 10.5 and  
6 E16.5 illustrated clear Mcph1 expression in the cytoplasm of aRGCs in the VZ (**Figures 1A-G**). More  
7 concentrated at the apical side of these cells, as indicated by co-localization with apical proteins, such  
8 as Par3 (Bultje et al., 2009) and Dock7 (Yang et al., 2012), this expression remained weak at more  
9 basal positions where APC staining extended (**Figure 1G**). Of note, no expression was detected in the  
10 nucleus or at the centrosome (**Figure 1D**). Furthermore, Mcph1 expression overlapped with that of  
11 RC2, a post-translationally modified nestin isoform specifically expressed in mouse aRGCs (Park et  
12 al., 2009) (**Figure 1E**), not with that of Tbr2 (**Figure 1F**). This discrimination between Mcph1 and  
13 Tbr2 expression was further supported by the decrease in Mcph1 expression from E10.5 to E13.5, and  
14 no detection of this expression on E14.5 (**Figures S1A-B**) and E16.5 (**Figure S1D**) nctx sections or in  
15 E14.5 whole-mount nctx (**Figure S1C**) further stained with  $\gamma$ Tubulin antibodies for the better  
16 visualization of mitotic cells at the ventricular border. This limited time-window of *McpH1* expression  
17 was confirmed at the RNA level by RNA *in situ* hybridization, as no signal was detected in E14.5 and  
18 E16.5 nctx (**Figure S1E**).

19 Taken together, these results indicate that Mcph1 is primarily expressed in aRGCs in a  
20 decreasing manner from E10.5 to E13.5. The discordance with a previous study showing Mcph1  
21 expression at E15.5 (Gruber et al., 2011) can be explained by the very sensitive method that was used,  
22 indicating an extremely lower level of Mcph1 expression compared to earlier stages that we examined  
23 here.

24 In contrast to our findings in mice, MCPH1 expression was observed at later stages of human  
25 neocortical development, such as post-conception weeks (PCW) 11 (**Figures 1H-K**) and PCW13 (data  
26 not shown). This expression was primarily detected in a subset of RGCs in both the VZ and OSVZ, as  
27 illustrated by its prominent overlapping with PAX6 expression compared to TBR2 (**Figures 1J** and  
28 **1L**). Of note, in contrast to mice, MPCH1 was expressed in nuclei in addition to the cytoplasm.

1 To consolidate our expression data in human, we analyzed transcriptomic data from the  
2 Human BrainSpan Atlas (Kang et al., 2011; Pletikos et al., 2014) and observed that MCPH1  
3 expression is highest during the earliest stages of neocortical development, before the PCW16 stage,  
4 and then progressively declines (**Figure S1F**).

5 Collectively, these expression data show that MCPH1 expression peaks at the early stages of  
6 neocortical development and is primarily associated with RGCs, both in humans and mice. They raise  
7 the question of a specific role of *MCPH1* in RGCs.

8

### 9 ***McpH1* conditional deletion in the mouse nctx induces microcephaly.**

10 To answer this question, we generated *McpH1<sup>lox/lox</sup>; Emx1<sup>kiCre/+</sup>* mice (cKO) with specific  
11 *McpH1* inactivation in neocortical progenitors, and we phenotypically compared these mutant mice to  
12 *McpH1<sup>lox/lox</sup>* (Ct) mice. No *McpH1* protein expression was detected in the lateral nctx of E12.5 cKO  
13 embryos, which gives rise to the neocortical neurons, whereas *McpH1* expression was seen in the  
14 medial nctx (**Figure S2A**), probably due to lower efficiency of *McpH1* excision in medial area. The  
15 cKO mice developed microcephaly, underlined by the reduction of brain weight and of longitudinal  
16 and lateral neocortical size (**Figures 2A and 2B**). To further characterize the morphogenic defects of  
17 the cKO nctx, we used two approaches for neuron BrdU birthdating: first, the double injection of  
18 pregnant mice with BrdU at E12.5 and E13.5 to trace the earliest layer VI neurons *Tbr1+* and analyze  
19 their outcome at post-natal day (P) 0; and second, the BrdU injection at E14.5 to trace neurons of  
20 layers V to II and analyze them at P5, when their migration is complete. DAPI-staining coupled with  
21 layer neuron marker and BrdU double IF allowed us to determine: 1) the global number of neurons  
22 and BrdU distribution within the cortical plate; 2) the number of neurons and BrdU distribution in  
23 each neuron layer.

24 Following BrdU injection at E12.5 and E13.5, P0 nctx displayed a decrease in the total  
25 number of neurons in cKO versus Ct nctx (**Figures S2B and S2C**), but no significant change in the  
26 total number of BrdU positive nuclei and the distribution of *Tbr1+* and *Tbr1+BrdU+* neurons (**Figure**  
27 **S2C**). After BrdU injection at E14.5, the significant decrease in the total number of neurons in the P5  
28 cKO versus Ct nctx was now associated with a global reduction of BrdU incorporation (**Figures 2C**



1 and **2D**). Again, the number of Tbr1+ neurons was similar in Ct and cKO nctx and, as expected, these  
2 neurons were barely traced by BrdU (**Figures 2C and 2D**). In contrast, the neuronal outcome in the  
3 layers V to II and the global BrdU distribution were significantly decreased (**Figures 2C and 2D**),  
4 whereas BrdU incorporation in each layer was not impacted (**Figure 2D**). This result can be explained  
5 by dilution of the global BrdU decrease in the different layers.

6 Collectively, these data suggested that neurogenesis proceeds normally between E12.5 and  
7 E13.5, whereas neuron arising is impaired later on. They are consistent with the decrease in upper  
8 neurons reported in the *Mcp1* full knock out nctx (Gruber et al., 2011), and suggest a possible  
9 premature exhaustion of progenitors.

10

### 11 ***Mcp1* mutation induces early mitotic anomalies associated with progenitor cell death**

12 In regard with these results, we examined progenitors in E12.5 Ct and cKO nctx. Double IF of  
13 Tpx2, a protein interacting with spindle microtubules (Gruss et al., 2002), and Aurora kinase B  
14 (AurkB), a cell cycle kinase that localizes to microtubules near kinetochores (Terada et al., 1998),  
15 evidenced frequent disorganized mitotic spindles at the ventricular border of *Mcp1* cKO nctx (**Figure**  
16 **S2D**): 24 abnormal mitoses among 53 counted in cKO (66%) versus 2 among 35 in Ct nctx (6%).  
17 Phospho-Histone H3 (PH3) and cleaved Caspase-3 (CC3) double IF indicated that these defects were  
18 associated with ab-ventricular mitoses and cell death (**Figure 2E**). As shown in **Figure 2F**, CC3 was  
19 expressed in long cellular processes evoking RGCs, not in Tbr2+ IPCs, which suggested that cell  
20 death impacted primarily RGCs. Further analysis at E11.5, E14.5 and E16.5 established that cell death  
21 in cKO nctx was largely prominent at E12.5 (**Figure 2G**), which was corroborated with a decrease in  
22 ventricular mitoses (PH3+), specific at E12.5 (**Figure 2H**). These results were consistent with the  
23 time-window of *Mcp1* expression.

24 Next, to assess the impact of these mitotic defects on progenitor production, we examined the  
25 distribution of BrdU acute incorporation in aRGCs (Pax6+) and of Tbr2+ IPCs newly born in the VZ  
26 (delineated by Pax6 expression) or settled in the SVZ (no Pax6 expression), as indicated in **Figure**  
27 **S2E**. The distribution of Pax6+ and Pax6+ BrdU+ cells was comparable in E12.5 Ct and cKO VZ  
28 (**Figure S2F**), but, in contrast, decreased significantly at E14.5 and E16.5 (**Figures S2G and S2H**).

1 Similar in E12.5 Ct and cKO VZ/SVZ (**Figure S2F**), the IPC population was reduced in the E14.5  
2 cKO VZ (**Figure S2G**). This defect seemed to be compensated in the E16.5 cKO VZ, but was  
3 associated with a decrease in IPCs in the E16.5 cKO SVZ (**Figure S2H**).

4 Collectively, these results indicate that the progenitor cell death peaking in the E12.5 cKO VZ  
5 is related to mitotic anomalies, progressively alters the aRGC pool and results in lower production of  
6 IPCs, which can account for the impaired upper layer neuron production afore described.

### 8 ***Mcp1* interacts with cellular metabolic pathways and is localized at mitochondria.**

9 Next, to gain insight into how *Mcp1* inactivation affects the cell division and survival of  
10 neocortical progenitors at the molecular level, we took advantage of primary cultures of mouse neural  
11 progenitor cells (NPCs) that we derived from nctx dissected from E12.5 wild type (WTNPCs) and  
12 *Mcp1* full knock out (KONPCs) embryos, to ensure *Mcp1* deletion in all cells. Following RNA  
13 sequencing, gene expression profiling analyses revealed differential expression of genes controlling  
14 the cell cycle in KONPCs. For instance *Cdk1* and *Chek1*, genes already known as being regulated by  
15 *Mcp1* (Gruber et al., 2011), were significantly down regulated (fold change FC=5.13, adjusted *p*-  
16 value=8.53x10<sup>-3</sup> for *Cdk1*; FC=5.08 adjusted *p*-value=1.03x10<sup>-2</sup> for *Chek1*). Moreover, genes related  
17 to metabolic pathways, including 93 nuclear genes encoding mitochondrial proteins, also stood out  
18 among differentially expressed genes, highlighting oxidative phosphorylation as one of the most  
19 affected pathway (**Figure 3A; Table S1**). This unexpected finding indicated a large impact of the  
20 *Mcp1* mutation on bioenergetics pathways and was an incentive to explore the interplay between  
21 *Mcp1* and such pathways.

22 First, the sub-cellular distribution of the Mcp1 protein in WTNPCs established by IF  
23 suggested a possible localization of Mcp1 at the mitochondria or near the outer membrane (for  
24 simplicity, we will keep mentioning mitochondrial localization to refer to both situations). This was  
25 confirmed by 1) the overlapping distribution of Mcp1 and either SDHA (a protein of the respiratory  
26 chain complex II) or MitoTracker Deep Red (MitoT), a mitochondrial fluorescent probe (**Figure 3B**);  
27 2) importantly, evidence for this localization in aRGCs provided by gold-immunostaining and  
28 electronic microscopy analysis (**Figure 3C**); and 3) mitochondrial targeting of the transiently

1 expressed HA-tagged human MCPH1 protein in mouse NPCs (**Figure 3D**). Of note, MCPH1  
2 mitochondrial localization was also observed in human NPC (hNPC) (**Figure 3E**).

3

#### 4 ***McpH1* inactivation impacts mitochondrial functionality**

5 Altogether, these results raised the hypothesis of a link between *McpH1* and mitochondria  
6 functionality. With this regard, we first checked the impact of the *McpH1* deletion on mitochondrial  
7 integrity by comparing the morphology of the mitochondrial network in WTNPCs and KONPCs. When  
8 labeled with MitoT and a SDHA antibody, mitochondria appeared smaller and more fragmented in  
9 KONPCs (**Figure 3F**). This higher fragmentation was not related to a loss in mitochondrial membrane  
10 amount, as indicated by 1) ELISA quantification of the complex I of the respiratory chain (**Figure**  
11 **3G**); 2) similar expression levels of VDAC1, a major mitochondrial protein, analyzed by Western  
12 blotting (**Figure 3H**); and 3) morphometric analyses by electronic microscopy, indicating no  
13 significant change of the total mitochondrial surface relative to the cytoplasm, but an increase in  
14 mitochondria number (**Figure 3I**).

15 To determine whether the higher mitochondrial fragmentation in KONPCs was a sign of  
16 mitochondrial activity alteration, we measured the activity of respiratory chain enzymes in NPCs  
17 maintained either in complete medium with Glucose, Pyruvate and Glutamine (GPGln), or in medium  
18 deprived of either Glucose (PGln) or both Glucose and Pyruvate (Gln) to challenge mitochondrial  
19 activity. We thus measured the activity of the rotenone-sensitive NADH quinone reductase (CI), the  
20 malonate-sensitive succinate cytochrome *c* reductase (SCCR, CII+CIII), the glycerol cytochrome *c*  
21 reductase (GCCR, G3PDH+CIII), the Decylubiquinol cytochrome *c* reductase (QCCR, CIII), the  
22 cyanide-sensitive cytochrome *c* oxidase (Cox, CIV) and the oligomycine-sensitive ATPase (CV). In  
23 GPGln medium, each activity was comparable between KONPCs and WTNPCs (**Figures 4A-F**). In  
24 PGln medium compared to GPGln medium, the tested activities, except for SCCR in WTNPCs, tended  
25 towards general decrease, although not significant, in both WTNPCs and KONPCs (**Figure 4A-F**).  
26 Comparing activities between WTNPCs and KONPCs in either PGln or Gln medium, we found a  
27 significant decrease in ATPase activity (**Figure 4F**) in KONPCs, whereas other activities remained

1 unchanged (**Figures 4A-E**). The significant increase in SCCR enzymatic activity observed in Gln  
2 medium (**Figure 4A**) suggested attempt of cells to enhance mitochondrial biogenesis.

3 Overall these results indicate that the *Mcp1* deletion significantly affects ATPase activity,  
4 underlying mitochondrial dysfunction. This dysfunction is masked in presence of glucose, a well-  
5 known phenomenon (Benit et al., 2019).

6 Since mitochondrial activity is modulated by calcium concentration (Cardenas et al., 2010),  
7 we then questioned whether the observed mitochondrial deficiency was related to reduced calcium  
8 availability. We thus transiently transfected glucose-deprived NPCs with mito-GEM-GECO1, a  
9 fluorescent protein reporter of mitochondrial calcium (Zhao et al., 2011) (**Figure 4G**) and measured  
10 emission ratios. We thus found significantly lower calcium amount in KONPCs (**Figure 4H**),  
11 underlining alteration of the mitochondrial activity.

12 Next, we addressed whether the mitochondrial defects evidenced in KONPCs upon low carbon  
13 source availability were related to compromised cell survival. By CC3 IF and counting positive cells,  
14 WTNPCs displayed elevated cell death in Gln medium only (**Figure 4I**). In KONPCs, cell death was  
15 clearly more pronounced in both PGLn and Gln medium (**Figure 4I**). Notably, analysis of ROS  
16 (Reactive Oxygen Species) production by FACS (Fluorescence Activated Cell Sorting) did not show  
17 any difference between KONPCs and in PGLn (**Figures S3A and S3B**), suggesting that cell death in  
18 KONPCs was not related to higher ROS production.

19 Together these results indicated that exposure to low carbon source availability was more  
20 critical for KONPCs than WTNPCs. To determine whether deficiency of key metabolic enzymes  
21 involved in replenishing the tricarboxylic acid (TCA) cycle could account for this difference, we next  
22 assessed the activities of the following enzymes: 1) Hexokinases I-IV and lactate dehydrogenase  
23 (LDH), key enzymes of glycolysis; 2) Glucose-6-phosphate dehydrogenase (G6PDH), a rate-limiting  
24 enzyme in the pentose-phosphate pathway and the synthesis of nucleic acids; and 3) IDH3 and  
25 IDH1/2, respectively NAD<sup>+</sup>- and NADP<sup>+</sup>-dependent IDH (isocitrate dehydrogenase), enzymatic  
26 isoforms catalyzing the oxidative decarboxylation of isocitrate to produce  $\alpha$ -ketoglutarate. These  
27 enzymatic activities did not vary significantly between WTNPCs and KONPCs (**Figures 4J, 4K**) and

1 **S3C-E)**, and the mitochondrial defects of *Mcp1* KO NPCs could not be explained by deficiency of  
2 these enzymes.

3

#### 4 ***Mcp1* influences mitochondrial activity via glutaminolysis.**

5 We next considered another key pathway often solicited to sustain the TCA cycle and  
6 mitochondrial activity upon low glucose availability or high energetic demand, which is  
7 phosphoenolpyruvate (PEP) production from glutamine through the activity of mitochondrial  
8 phosphoenolpyruvate carboxykinase, PEPCCK-M or PCK2. In addition to driving the TCA cycle,  
9 Glutamine-derived PEP or glutaminolysis can fuel multiple biosynthetic processes, critically  
10 contributing to cell growth and survival during stress (Mendez-Lucas et al., 2014; Vincent et al.,  
11 2015). With this regard, we demonstrated PCK2 expression in WTNPCs (**Figure 5A**) and down  
12 regulation of this expression in KONPCs, consistent with our transcriptomic data (FC=2,72, adjusted  
13  $p$ -value=5,65x10<sup>-3</sup>). Importantly, PCK2 expression was markedly increased in WTNPCs upon PGI<sub>n</sub> or  
14 Gln medium, but remained very low in KONPCs (**Figure 5B**). Furthermore, our IF analysis indicated  
15 that PCK2 expression decreased between E10.5 and E14.5 (**Figures 5C-E**), and, of note, was down  
16 regulated in E10.5 KO nctx (**Figure 5C**). These results uncover that mouse neural progenitors solicit  
17 PCK2 and *a fortiori* glutaminolysis, and that *Mcp1* is involved in PCK2 regulation. In addition,  
18 illustration of PCK2 expression in human neocortical progenitors, in hNPCs and in the VZ of a  
19 PCW11 fetal nctx, (**Figures S4A and S4B**, respectively), suggests that this regulation may occur in  
20 Human as well. To strengthen this finding, we addressed whether ATF4, a transcription factor able to  
21 activate *PCK2* transcription (Mendez-Lucas et al., 2014), was also misregulated in KONPCs. In line  
22 with transcriptomic analyses indicating a 2.32 fold decrease in *ATF4* expression ( $p$ -value=2,11x10<sup>-2</sup>),  
23 the basal expression of the ATF4 protein was lower in KONPCs compared to WTNPCs, and was not  
24 stimulated in KONPCs in PGI<sub>n</sub> and Gln medium, as in the cas of PCK2 (**Figure 5F**).

25 Previous studies have reported that ATF4 is expressed, like *Mcp1*, in aRGCs before the bulk  
26 of indirect neurogenesis (Franck et al. 2010; Laguesse et al. 2015) and regulates progenitor  
27 proliferation and the stimulation of the PERK arm of the Unfolded Protein Response (UPR), (Frank et  
28 al., 2010; Godin et al., 2016; Laguesse et al., 2015). Since HSPA5/Bip1/GRP78 is the upstream

1 regulator of UPR, we examined the expression of this protein and found a significant increase in  
2 KONPCs upon Gln versus GPGLn medium (**Figure 5G**), indicating a possible stimulation of UPR.

3 Altogether our results show that *McpH1* may participate in glutaminolysis stimulation via the  
4 ATF4/PCK2 pathway.

5

### 6 **Glutaminolysis contributes with glycolysis to fuel the cell cycle of NPCs.**

7 In this context, we next assessed the respective influence of glycolysis and glutaminolysis on  
8 the proliferation of WTNPCs and KONPCs. By FACS analysis of the cell cycle, we examined the  
9 impact of the following metabolic inhibitors: 2-DG (2-Deoxyglucose), a glucose competitor that  
10 inhibits the first step of glycolysis by blocking Hexokinases; UK5099 (2-Cyano-3-(1-phenyl-1H-indol-  
11 3-yl)-2-propenoic acid), an inhibitor of the mitochondrial pyruvate carrier; BPTES (Bis-2-(5-  
12 phenylacetamido-1,3,4-thiadiazol-2-yl) ethyl sulfide), a glutaminase inhibitor (**Figure S5A**). Analyzing  
13 cellular DNA content and EdU incorporation during the S phase allowed us to determine the  
14 distribution of NPCs in G0/G1, S and G2/M phase. BPTES and UK5099 alone had no effect on the on  
15 both WTNPCs and KONPCs (data not shown). 2-DG, alone or coupled to BPTES did not impact the  
16 percentage of WTNPCs and KONPCs in G0/G1, but caused an increase in this percentage in KONPCs  
17 when it was coupled to UK5099 (**Figure 6A** and **6B**). In contrast, 2-DG caused a decrease in the  
18 percentage of NPCs in the S phase, potentially more significant in KONPCs compared to WTNPCs  
19 (based on *p* values; **Figures 6A** and **6C**). Interestingly, addition of BPTES or UK5099 accentuated  
20 this decrease in WTNPCs, not in KONPCs. In counterpart, 2-DG caused an increase in cells in G2/M,  
21 more pronounced in WTNPCs (**Figure 6D**).

22 These results suggest that glutaminolysis and mitochondrial activity may participate, in  
23 addition to glycolysis, in energetic supply during the S phase, and support the notion that these two  
24 functions are altered in KONPCs.

25 To further investigate the impact of metabolic inhibitors in WTNPCs and KONPCs, we  
26 compared the levels of transcriptional activation of *PCK2*, *ATF4*, *GLS1* (Glutaminase 1, expressed in  
27 the brain and converting glutamine to glutamate) (Olalla et al., 2002), and *GLUD1* (glutamate  
28 dehydrogenase, converting glutamate to  $\alpha$ -ketoglutarate) (Shashidharan et al., 1994). 2-DG alone

1 induced an increase in *PCK2* expression in WTNPCs, not in KONPCs (**Figure S5C**), consistently with  
2 the observed protein expression variations and with the hypothesis of glutaminolysis stimulation upon  
3 glucose and pyruvate deprivation in WTNPCs. This increase in *PCK2* activation was maintained by  
4 addition of BPTES or UK5099, indicating that these inhibitors do not impact *PCK2* transactivation. In  
5 contrast to *PCK2* and to the increase in the ATF4 protein expression observed upon glucose and  
6 pyruvate deprivation, 2-DG did not affect *ATF4* RNA expression (**Figure S5B**), which can be  
7 explained by the important role of post-translational modifications in the regulation of ATF4  
8 expression (Lassot et al., 2001). Furthermore, 2-DG caused a decrease in *GLS1* (**Figure S5D**) and  
9 *GLUD1* (**Figure S5E**) expression, indicating that the transcriptional activation of *PCK2* is regulated  
10 independently of *GLS1* and *GLUD1*.

11

## 12 **McpH1 interacts with VDAC1 and modulates its activity via GRP75 and HK2**

13 To gain insight into the modalities of McpH1 recruitment at mitochondria and into how this  
14 localization impacts mitochondrial activity, we searched for potential MCPH1 mitochondrial  
15 molecular partners by mining data obtained by co-immunoprecipitation (Co-IP) experiments (Liu et al.,  
16 2017). Following identification of HSPA9/GRP75 as a potential partner of MCPH1, McpH1 and  
17 GRP75 co-localization was observed in mNPCs by double IF (**Figure S6A**). Importantly, we  
18 demonstrated that the two MCPH1 C-terminal (C-ter) BRCT domains mediated the MCPH1 and  
19 GRP75 interaction by performing Co-IP with transiently expressed HA-tagged complete or deleted  
20 MCPH1 proteins and Flag-tagged GRP75 (**Figure 7A**). In addition, KONPCs displayed a lower  
21 GRP75 expression than WTNPCs (**Figure 7B**). Altogether these results indicate a strong interplay  
22 between McpH1 and GRP75.

23 GRP75 is regulated by glucose and interacts with the Voltage-dependent anion channel 1  
24 (VDAC1), a protein found in the outer mitochondrial membrane and important for the exchange of  
25 metabolites between mitochondria and the intracellular environment (Betz et al., 2013; Flachbartova  
26 and Kovacech, 2013; Szabadkai et al., 2006). In particular, GRP75 bridges VDAC1 to Inositol-  
27 triphosphate receptor (IP3R) to allow calcium flux from endoplasmic reticulum into mitochondria and  
28 stimulation of the TCA cycle (Betz et al., 2013; Szabadkai et al., 2006). This raised the hypothesis

1 of possible interaction between MCPH1 and VDAC1 too, which we assessed by expressing HA-  
2 tagged MCPH1, either full length or deleted of the two C-ter BRCT domains, in HEK293T cells, and  
3 by performing Co-IP. Considering the amounts of the co-immunoprecipitated VDAC1 and the input of  
4 each MCPH1 protein, VDAC1 interaction seemed stronger with the full-length MCPH1 protein  
5 (**Figure S6B**), suggesting that the two C-ter BRCT domains are important for interaction with VDAC1  
6 as with GRP75. We then examined the modalities of Mcph1/VDAC1/GRP75 interactions by  
7 performing series of Proximity Ligation Assays (PLA) in NPCs. In WTNPCs, we found that Mcph1  
8 interacts more robustly with VDAC1 than with GRP75 in PGLn medium, and that Mcph1 interaction  
9 with VDAC1 is markedly enhanced in both PGLn and Gln medium, whereas interaction with GRP75  
10 increases significantly in Gln medium only (**Figures 7C and 7D**). These increases were not merely  
11 due to higher expression levels of GRP75 and VDAC1, since protein expression did not change  
12 between conditions (**Figure S6C**). Second, these interactions were detected *in vivo* on sections of  
13 mouse E10.5 nctx, again with a higher dot distribution in the case of Mcph1 and VDAC1 than Mcph1  
14 and GRP75 (**Figure 7H**). Third, MCPH1 interaction with VDAC1/GRP75 is similarly modulated in  
15 hNPCs (**Figures 7E and 7F**). Importantly, interaction between GRP75 and VDAC1 was mainly  
16 detected in WTNPCs maintained in Gln medium, and was not observed in KONPCs (**Figure 7G**),  
17 despite higher expression of two proteins in PGLn and Gln medium (**Figure S6C**). Interestingly, these  
18 data indicated that Mcph1 influences VDAC1/GRP75 interaction.

19         Altogether, these results uncovered that Mcph1 interacts with the VDAC1/GRP75 pathway.  
20 They prompted us to address whether Mcph1 also interferes with the Hexokinase-2 (HK2) pathway,  
21 which attenuates VDAC1 activity by stimulating glycolysis via Glucose-6-Phosphate (G6P)  
22 production (Betz et al., 2013; Hou et al., 2015). Interestingly, by performing PLA, we provided  
23 evidence for HK2/VDAC1 interaction in KONPCs, not in WTNPCs (**Figure S6D**). This difference was  
24 not related to higher expression of HK2 in KONPCs (**Figure S6E**). Two main mechanisms control  
25 HK2 binding to VDAC1: 1) primary HK2 phosphorylation by Akt (Roberts et al., 2013); and 2)  
26 negative feedback exerted by high levels of G6P (Hou et al., 2015). Hence, stimulation of HK2  
27 binding in KONPCs raised the question of a possible higher Akt activation in these cells. Akt is  
28 activated by phosphorylation of two residues, Serine (S) 473 and Threonine (T) 308, by mTOR2 and



1 PDPK1 (3-Phosphoinositide Dependent Protein Kinase 1), respectively (Beg et al., 2017). By Western  
2 blotting, we found that 1) the expression of total Akt did not significantly vary between KONPCs and  
3 WTNPCs, whatever the culture condition; 2) the expression of pAkt S473 appeared to be increased,  
4 although not significantly, in KONPCs in PGI<sub>n</sub> and Gln medium; and 3) pAkt T308 expression was in  
5 contrast markedly elevated in KONPCs, not in WTNPCs, upon PGI<sub>n</sub> and Gln medium. Importantly, the  
6 pAkt T308 stimulation in KONPCs was corroborated with that of PDPK1 (**Figure S6F**).

7 Taken together our results indicate that Mcph1 can influence VDAC1 activity via two  
8 pathways: stimulation of GRP75 binding and constraint of HK2 binding via inhibition of Akt  
9 activation.

10

## 1 DISCUSSION

2

3 *MCPHI* has been involved in a variety of basic functions related to the cell cycle, such as  
4 chromosome segregation, double strand DNA repair, and DNA recombination during meiosis.  
5 Altogether, these functions are essential for the maintenance of genomic integrity, and their alteration  
6 can promote tumorigenesis or infertility (Liang et al., 2015; Liang et al., 2010). Despite the clear  
7 implication of *MCPHI* mutations in human microcephaly (Jackson et al., 1998; Pulvers et al., 2015),  
8 studies exploring the functions of the gene during neocortical development and explaining how  
9 *MCPHI* deficiency can lead to microcephaly remain limited. A previous study analyzing an *McpH1*  
10 full knock out mouse line indicated that *McpH1* promotes the symmetric division of neuroprogenitor  
11 cells via the Chk1/Cdc25b pathway, which controls the coupling of the centrosome cycle with the  
12 entry into the M-phase of the cell cycle (Gruber et al, 2011). Here, we provide information in regard  
13 with the temporality of *McpH1* implication in neocortical development and the mechanisms underlying  
14 this implication.

15 In this respect, we present a detailed expression pattern of *McpH1* in the mouse developing  
16 nctx, showing that *McpH1* expression is restricted to aRGCs during a time-window corresponding to  
17 the prevalence of the symmetric division of these cells, and thereby strengthening the notion that  
18 *McpH1* promotes this cell division mode. These data and the lack of expression of *McpH1* at the  
19 centrosome distinguish *McpH1* from other MCPH genes, in particular such as *Wdr62* (*MCPH2*) (Chen  
20 et al., 2014), *Cdk5Rap2* (*MCPH3*) (Issa et al., 2013), *Aspm* (*MCPH5*) (Fish et al., 2006), *Cenpj*  
21 (*MCPH6*) (Garcez et al., 2015), whose expression at the centrosome is maintained in the mouse VZ  
22 and/or SVZ during all stages of mouse neocortical development.

23 Interestingly, a comprehensive analysis of expression data from the Human BrainSpan Atlas  
24 (Kang et al., 2011; Pletikos et al., 2014) also emphasized a prominent *MCPHI* expression during the  
25 earliest stages of the human neocortical development, and furthermore indicated that, in contrast to  
26 mice, this expression is maintained at later developmental stages, albeit at lower levels. According to  
27 our data obtained at PCW11 and 13, this later expression is related to a subset of aRGCs and bRGCs  
28 in the VZ and OSVZ respectively. At these stages indirect neurogenesis is well underway, however

1 aRGCs and bRGCs continue to undergo frequent symmetric divisions (Betizeau et al., 2013; Dehay et  
2 al., 2015; Florio and Huttner, 2014; Lui et al., 2011; Martinez-Martinez et al., 2016). Altogether, these  
3 data raise the challenging question of whether MCPH1 expression in human bRGCs promotes their  
4 symmetric division, thus facilitating their expansion in the OSVZ as well. Furthermore, the expression  
5 of MCPH1 in the nucleus of human neural progenitors suggests that the human protein assumes  
6 further functions that may also participate in their expansion. This nuclear localization in humans may  
7 rely on amino acid substitutions existing in the three characterized nuclear localization sequences of  
8 the murine and human protein (Gavvovidis et al., 2012) or on specific post-translational modifications  
9 that would selectively control translocation of the human protein to the nucleus. This hypothesis is  
10 supported by the poor cross-reaction that we observe between antibodies raised against the human and  
11 the mouse MCPH1 proteins (data not shown). The molecular bases of this differential subcellular  
12 localization will be an interesting issue to address.

13         Despite the short time window of *McpH1* expression, its deletion in neocortical progenitors  
14 was sufficient to induce microcephaly. Neuron birth dating experiments showed that the primary layer  
15 VI Tbr1 neurons are produced to a similar extent in cKO and Ct nctx, whereas the outcome of the  
16 upper neurons is affected, which corroborates with data obtained with the *McpH1* full knock-out mice  
17 (Gruber et al., 2011). This situation can be explained by primary loss of progenitors due to the cell  
18 death (this study), and by an imbalance in asymmetric neurogenic divisions (Gruber et al., 2011)  
19 contributing to the generation of Tbr1 neurons, both defects finally leading to exhaustion of the  
20 progenitor pool. These hypotheses are supported by several lines of data: 1) a peak of cell death  
21 observed in the E12.5 cKO VZ; 2) a reduction of the longitudinal size of cKO newborn nctx, such  
22 reduction being supposed to rely on impaired expansion of RGCs (Florio et al., 2014); 3) a decrease in  
23 Pax6+ progenitors proliferating in the E14.5 and E16.5 cKO VZ, as demonstrated by acute BrdU  
24 incorporation; 4) a diminution of IPCs arising in the VZ at E14.5 and settled in the SVZ at E16.5; 5) a  
25 decreased distribution of the cortical neurons traced by BrdU injection at E14.5.

26         Unexpectedly for an MCPH gene, comparative gene expression profiling between WTNPCs  
27 and KONPCs uncovered a crosstalk between *McpH1* and metabolic pathways. The emergence of  
28 oxidative phosphorylation as one of the most affected pathways in KONPCs and the uncovering of the

1 MCPH1 localization at mitochondria solidified the notion that this crosstalk involves mitochondria,  
2 which our study supports in different ways.

3         The fact that higher mitochondrial segmentation in KONPCs was not associated with loss of  
4 global mitochondrial amount indicated that this fragmentation is not related to mitophagy (Chen and  
5 Chan, 2009). Based on ATPase deficiency evidenced in PGI<sub>n</sub> and Gln medium, this fragmentation is  
6 rather associated with mitochondrial activity alteration. The lack of significant impact of the *McpH1*  
7 mutation on key glycolytic enzymes, such as HK2 and LDH, indicates that insufficient replenishing of  
8 the TCA cycle due to glycolysis impairment cannot account for the mitochondrial activity alteration.  
9 Instead, our data have related this alteration to a deficiency of the ATF4/PCK2 pathway, which  
10 triggers glutaminolysis, a further pathway participating in replenishing the TCA cycle and essential for  
11 active cell proliferation or survival in the context of metabolic stress (Colombo et al., 2011; Mendez-  
12 Lucas et al., 2014; Vincent et al., 2015). Several lines of data emphasize this implication of  
13 glutaminolysis in neocortical progenitors. First, during mouse neocortical development, the functions  
14 of the ATF4 protein, *PCK2* transactivator (Mendez-Lucas et al., 2014), take place during the time-  
15 window of *McpH1* and *PCK2* expression, before the launch of indirect neurogenesis (Frank et al.,  
16 2010; Laguesse et al., 2015), which is consistent with a possible crosstalk between the three genes.  
17 The clear down-regulation of both ATF4 and *PCK2* in KONPCs and of *PCK2* in the E10.5 KO nctx  
18 strongly supports that *McpH1* regulates the ATF4/*PCK2* pathway. Furthermore, our data indicate that  
19 the mis-regulation of this pathway in KONPCs is associated with impaired glutaminolysis. This notion  
20 is based on the differential impact of BPTES addition to 2-DG on the cell cycle between WTNPCs and  
21 KONPCs, and the higher cell death induced in KONPCs in Gln medium. On the basis of data reporting  
22 glutaminolysis solicitation for normal progression of cell division from late G1 (Colombo et al., 2011)  
23 and of our present data indicating that glutaminolysis inhibition strengthens the effect of 2-DG on the  
24 S phase, we suggest that glutaminolysis is required in addition to glycolysis for normal cycle  
25 progression in neuroprogenitors.

26         MCPH1 interaction with VDAC1/GRP75 appears to be an important clue for understanding  
27 the interplay between MCPH1, glycolysis, mitochondria and glutaminolysis. The prominent MCPH1  
28 interaction with VDAC1 compared to GRP75 can result from the larger distribution of VDAC1 at

1 mitochondria and indicates that this interaction can occur independently of GRP75. According to our  
2 data, Mcph1 stimulates VDAC1 activity in two ways. First, the disruption of GRP75 and VDAC1  
3 interaction in absence of Mcph1 infers that Mcph1 is required for this interaction to occur in neural  
4 progenitors, either by facilitating GRP75 binding to VDAC1, or by a positive control of GRP75  
5 expression, as suggested by the decrease in this expression in KONPCs. The lower mitochondrial  
6 calcium uptake associated with this disruption reflects functional alteration of VDAC1, compromising  
7 cell survival, as underlined by the higher cell death observed in KONPCs in PGI<sub>n</sub> and GIn medium and  
8 consistently with previous studies involving VDAC1 in cell survival (Szabadkai et al., 2006). Second,  
9 Mcph1 participates in sustaining VDAC1 activity by inhibiting the PDK1/Akt/HK2 pathway, thus  
10 preventing VDAC1 inhibition by HK2 binding (Hou et al., 2015; Roberts et al., 2013). Of note,  
11 activated Akt is also supposed to disrupt the IP3R/GRP75/VDAC1 complex (Betz et al., 2013), and  
12 Mcph1 may facilitate GRP75 and VDAC1 interaction by constraining Akt activation.

13         Altogether, our studies have uncovered a role of MCPH1 at the intersection of bioenergetics  
14 pathways that participate in the homeostasis of early neocortical neural progenitor cells, which, due to  
15 intense proliferation, sustain active biogenesis and high energetic demand. To fulfill this demand,  
16 aRGCs have been shown to take advantage of glycolysis (Lange et al. 2016). Collectively, our data  
17 indicate that glutaminolysis may be critically solicited in addition to glycolysis to respond to high  
18 metabolic challenges that they are exposed to *in vivo* during rapid periods of proliferation. The impact  
19 of low glucose availability or glutaminolysis deficiency on microcephaly etiology is not known. In this  
20 regard, our study opens new windows to explore the mechanisms underlying microcephalic  
21 pathogenesis and will help attract the attention of clinicians and geneticists on possible implication of  
22 bioenergetics alterations in such etiology.

23

24

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7

## 8 **AUTHOR CONTRIBUTIONS**

9 J.N., P.G. and P.R. designed the research; N.J, S.C, P.B, J.G-J, X.L., S. J, V.F, E.M, T.H, A.D-D, Z.C,  
10 S.L, C.V-P, H.A-B, P.R, J.N performed the research, analyzed and formatted data; J-F.O provided  
11 human tissues; Z-Q.W provided information on MCPH1 partners screening, J.N, P.G, P.R and S.M  
12 wrote the manuscript.

13

## 14 **DECLARATION OF INTERESTS**

15 The authors declare no competing interests.

16

1 **FIGURE LEGENDS**

2

3 **Figure 1: MCPH1 expression in RGCs in the developing nctx diverges between mouse and**  
4 **human**

5 (A-F) *McpH1* IF (red) on mouse nctx at E10.5 (A, B) and E12.5 (C-G), combined with (A-D) DAPI  
6 nuclear staining (blue) and with (D)  $\gamma$ -Tubulin (green; yellow stars indicate  $\gamma$ -Tubulin, not *McpH1*,  
7 expression at centrosomes), (E) RC2 (green) and (F) *Tbrce* IF; right panel is a higher-magnification  
8 image of the area boxed in the right panel.

9 (G) Single plan sections extracted from 3D reconstructions of confocal Z-stack pictures obtained from  
10 whole-mount E12.5 nctx labeled with mouse monoclonal *McpH1* antibody (green) and (upper panel)  
11 *Dock7* and Phalloidin Deep Red (respectively red and grey); (middle panel) *Par3* (red); and (lower  
12 panel) *APC* (red). Yellow arrows indicate orientation from the apical (a) to the basal (b) side.

13 (H-L) MCPH1 (red) co-IF with (H, J) *PAX6* (green), (K) *SOX2* (green), (L) *TBR2* (green), combined  
14 with (H) DAPI staining (blue) on the VZ/SVZ (left panels) and OSVZ (right panels) of a PCW11  
15 human fetal cortex.

16 Bars, 50  $\mu$ m in A, C, H-L; 20  $\mu$ m in F; 10  $\mu$ m in B, D and E;

17 See also Figure S1

18

19 **Figure 2: The conditional deletion of *McpH1* induces microrcephaly in the mouse neocortex**  
20 **(nctx).**

21 A) Cresyl violet staining on p5 control (Ct) and *McpH1* conditional knock out (cKO) nctx; black lines  
22 delineate the neocortical area, red lines indicate the neocortical radial thickness. Bar, 400  $\mu$ m.

23 (B) Microcephaly in *McpH1* cKO embryos is underlined by a decrease in the brain weight (Ct n=12;  
24 cKO n=9;  $p < 0.05$ ), in the neocortical surface (Ct n=4, cKO n=3;  $p < 0.01$ ), in both the lateral (Ct n=4,  
25 cKO n=4,  $p < 0.05$ ) and the radial (Ct n=4, cKO n=4  $p < 0.01$ ) extent; Unpaired Student's *t* test; Error  
26 bars, SEM. Red and blue bars represent Ct and cKO, respectively.

1 (C) Birth date tracing of layer (L) neurons analyzed after BrdU injection at E14.5 by co-IF BrdU (red)  
2 and either Tbr1 (LVI), Ctbp2 (LV), Satb2 (LIV-II) or Cux1 (LII-III), all green. Yellow lines delineate  
3 cortical layers; DAPI staining (blue) in upper left panels. Bar, 100  $\mu$ m.  
4 (D) Quantification of the total number of neurons in the cortical plate (DAPI-stained nuclei; Ct n=4,  
5 cKO n=3) and of the BrdU distribution in Tbr1+ (LVI; Ct n=3, cKO n=3); Ctbp2+ (LV; Ct n=3, cKO  
6 n=3); Satb2+ (LIV-II; Ct n=3, cKO n=3) or Cux1 (LII-III; Ct n=4, cKO n=3) neurons, all green; *ns*,  
7 not statistically significant; \* $p$ <0.05; Unpaired Student's *t* tests; Error bars, SEM.  
8 (E) DAPI-staining (blue) combined with phospho-Histone H3 (PH3, grey) and cleaved Caspase3  
9 (CC3, green) double IF on E12.5 Ct and cKO nctx. Bar, 100  $\mu$ m.  
10 (F) DAPI-staining (blue) combined with CC3 (green) and Tbr2 (red) co-IF on E12.5 cKO nctx. Bars,  
11 left panel 20  $\mu$ m, right panel 5  $\mu$ m.  
12 (G) Quantification of CC3 positive cells in Ct and cKO cortices at E11.5 (Ct n=4, cKO n=3;  $p$ <0.05),  
13 E12.5 (Ct n=3, cKO n=3;  $p$ <0.001), E14.5 (Ct n=3, cKO n=4;  $p$ <0.01) and E16.5 (Ct n=3, cKO n=3,  
14  $p$ <0.05); *ns*, not statistically significant; Unpaired Student's *t* test; Error bars, SEM.  
15 (H) Quantification of PH3 positive cells in the VZ (green bars) and SVZ (purple bars) of Ct  
16 (n=4) and cKO (n=4) nctx at E11.5, E12.5 ( $p$ <0.05), E14.5 and E16.5; *ns*, not statistically  
17 significant; Unpaired Student's *t* test; Error bars, SEM.  
18 See also Figure S2

19

### 20 **Figure 3: Mcph1 interacts with mitochondria and impacts the mitochondrial network.**

21 (A) Gene enrichment tested using Gene set enrichment analysis (GSEA) for the Molecular Signature  
22 database (MSigDB) hallmark gene sets following transcriptome analysis from WTNPCs and KONPCs.  
23 The most significantly enriched ( $p$ <10<sup>-4</sup>) pathways regulated negatively and positively are presented  
24 with green and orange bars, respectively.  
25 (B) DAPI (blue) and MitoTracker Deep Red (MitoT, grey) staining combined with Mcph1 (red) and  
26 SDHA (green) co-IF on WTNPCs; lower panels, higher magnification images of the area boxed in the  
27 upper panels. Bars, 10  $\mu$ m upper panels, 3  $\mu$ m lower panels.



1 (C) Mcph1 gold immunostaining on E12.5 mouse nctx analyzed by electronic microscopy pointed by  
2 black arrows; n, nucleus. Bar, 5 nm.

3 (D) DAPI (blue) and MitoT (grey) staining combined with GFP (green) and HA (red) co-IF in  
4 WTNPCs transiently transfected with pCAGG-MCPH1-HA-IRES-GFP.

5 (E) DAPI (blue) and MitoT staining (grey) combined with MCPH1 IF (red) in human NPCs (hNPCs).

6 (F) DAPI (blue) and MitoT (grey) staining combined with SDHA (green) IF in WTNPCs and KONPCs.

7 (G) Quantification of the mitochondrial respiratory chain complex I by ELISA in WTNPCs (red bars,  
8 n=7) and KONPCs (blue bars, n=6); *ns*, not statistically significant; Error bars, SEM; Unpaired  
9 Student's *t* test.

10 (H) Comparison of VDAC1 expression in WTNPCs (red bars; n=3) and KONPCs (blue bars; n=3) by  
11 Western blotting; *ns*, not statistically significant; error bars, SEM; Unpaired Student's *t* test.

12 (I) Electron microscopy comparative analysis of the mitochondrial network (black arrows) in WTNPCs  
13 and KONPCs and quantification of the mitochondria mean surface (*ns*, not statistically significant),  
14 total number ( $p<0.01$ ) and mean parameter ( $p<0.05$ ) between WTNPCs (red bars) and KONPCs (blue  
15 bars). Data represent the mean of 3 independent experiments including 6 cells of each genotype; Non-  
16 parametric Mann-Whitney U test; Error bars, SEM.

17 Bars, 10  $\mu$ m in C, D, E and 20  $\mu$ m in F, 500 nm in I

18 See also Table S1

19

#### 20 **Figure 4: The *Mcph1* mutation alters mitochondrial functionality**

21 (A-F) Activity of the various segments of the respiratory chain in WTNPCs and KONPCs maintained  
22 in GPGln (purple signs), PGln (red signs) and Gln (blue signs) medium: (A), Malonate-sensitive  
23 succinate reductase (SCCR, CII+CIII); (B) Glycerol cytochrome *c* reductase (GCCR, G3PDH+CIII);  
24 (C) Decylubiquinol cytochrome *c* reductase (QCCR, CIII); (D) Cyanide-sensitive cytochrome *c*  
25 oxidase (Cox, CIV); (E) Rotenone-sensitive NADH quinone reductase (CI); (F) Oligomycine-sensitive  
26 ATPase (CV). Enzymatic activities were measured in two separate sets of experiments, each including  
27 a group of NPCs in GPGln (control) and a second group of NPCs maintained either in PGln or in Gln  
28 medium. For statistical analyses, the values obtained in the control GPGln condition from the two sets

1 were pooled (WTNPCs n=14 for all enzymes except for ATPase, n=10; KONPCs n=14 and 10,  
2 respectively); in PGI<sub>n</sub> medium, n=4 for WTNPCs and n=5 for KONPCs for all enzyme, except for  
3 ATPase, n=3 and n=5 respectively; in Gln medium, samples of WTNPCs and KONPCs correspond,  
4 respectively, to n=8 and n=6 for SCCR, GCCR and Cox, n=8 and n=10, for ATPase, n=8 in each  
5 group for NADH reductase. *ns*, not statistically significant; \**p*<0.05, \*\**p*<0.001; One way ANOVA  
6 Tukey-Kramer multiple comparison test; Error bars, SD.

7 (G) Transient expression of the fluorescent mitochondrial calcium reporter protein mito-GEM-GECO1  
8 in WTNPCs and KONPCs. Signal of the protein free of Ca<sup>2+</sup> (- Ca<sup>2+</sup>) and bound to Ca<sup>2+</sup> (+Ca<sup>2+</sup>) is  
9 illustrated in red and blue, respectively. Bar, 10 μm.

10 (H) Heat map indicates the fluorescence scale ratio. Quantification of fluorescence ratios in WTNPCs  
11 (n=24 cells) and KONPCs (n=30 cells) maintained in PGI<sub>n</sub> and from 3 different experiments; *p*<0.001;  
12 Two tailed Mann-Whitney U-test; Error bars, SEM.

13 (I) Quantification of cell death in WTNPCs (purple signs; n=4) and KONPCs (orange signs; n=4)  
14 maintained in GPLN, PGI<sub>n</sub> and Gln medium. Total cells (DAPI stained nuclei) and cells expressing  
15 cleaved caspase-3 were counted on 5 images per sample. Data are reported as percentage of cleaved  
16 caspase-3 positive cells. *ns*, non statistically significant; \**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001; One way  
17 Anova Tukey-Kramer Multiple Comparison Test; Error bars, SEM.

18 (J) Hexokinase I-IV (HK) and (K) Lactate dehydrogenase (LDH) activities measured in protein  
19 extracts of WTNPCs (n=4) and KONPCs (n=3) maintained in GPGln (black signs), PGI<sub>n</sub> (red signs) or  
20 Gln medium (blue signs). *p*<0.01; *ns*, not statistically significant; one way ANOVA Tukey-Kramer  
21 Multiple Comparison Test; Error bars, SD.

22 See also Figure S3

23

24 **Figure 5: Mcph1 controls the ATF4/PCK2 pathway in mouse NPCs.**

25 (A) DAPI-staining (blue) and PCK2 IF on WTNPCs. Bar, 10 μm.

1 (B) Western blot analysis of PCK2 expression in WTNPCs and KONPCs in GPGln, PGln or Gln  
2 medium (n=3;  $p<0.01$ ); expression level in KONPCs was too low for quantification; *ns*, not statistically  
3 significant; One way ANOVA Tukey-Kramer Multiple Comparison Test; Error bars, SEM.  
4 (C) DAPI-staining (blue) and PCK2 IF (red) on E10.5 WT (upper panels) and KO (lower panels)  
5 mouse nctx. Confocal pictures were monitored with identical laser setting. Bar, 20  $\mu$ m.  
6 (D) Tile-scan of an E12.5 WT mouse nctx section immunostained for PCK2 and illustrating ventro-  
7 dorsally increasing PCK2 expression. Bar, 100  $\mu$ m.  
8 (E) DAPI-staining (blue) and PCK2 IF (red) on E14.5 WT nctx. Bar, 50  $\mu$ m.  
9 (F) Western blot analysis of ATF4 expression in WTNPCs and KONPCs in GPGln, PGln or Gln  
10 medium (n=3 for each group); expression level in KONPCs was too low for quantification; *ns*, not  
11 statistically significant; \* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ); One way ANOVA Tukey-Kramer Multiple  
12 Comparison Test; Error bars, SEM.  
13 (G) Western blot analysis of GRP78 expression in WTNPCs (n=6) and KONPCs (n=5) in GPGln, PGln  
14 or Gln medium; *ns*, not statistically significant; One way ANOVA Tukey-Kramer Multiple  
15 Comparison Test; Error bars, SEM.  
16 See also Figure S4

17

18 **Figure 6: Comparative effects of 2-DG, alone or coupled to BPTES or UK5099, on the**  
19 **proliferation of WTNPCs and KONPCs**

20 (A) Dot plots of EdU and PI signal analysis by flow cytometry in representative WTNPC and KONPC  
21 samples, non-treated control (Ct) or upon 2-DG, 2-DG+BPTES and 2-DG+UK5099 exposure. The  
22 cell distribution in the cell cycle phases « G0/G1 », « S », and « G2/M » is expressed in percentages.  
23 (B) Quantitative analysis of the effects of metabolic inhibitors on the proliferation of WTNPCs (red  
24 signs) and KONPCs (blue signs). Ct, control untreated cells; 2DG (2-DG); 2DG+B (2-DG+BPTES)  
25 and 2DG+U (2DG+UK5099). The distribution of cells in G0/G1 (B), S (C) and G2/M (D) phase is  
26 expressed as percentages relative to the control condition; *ns*, not statistically significant; \* $p<0,05$ ,

1 \*\* $p < 0,01$ , \*\*\* $p < 0,001$ ; One way Anova Tukey-Kramer Multiple Comparison Test; Error bars,  
2 SD.  
3 See also Figure S5  
4  
5 **Figure 7: MCPH1 interacts with the VDAC1/GRP75 pathway**  
6 (A) Co-immunoprecipitation (Co-IP) of GRP75-FLAG and HA-MCPH1 proteins, full length or  
7 deleted of BRCT domains (BR1, N-terminal; BR2 and BR3, C-terminal;  $\Delta$ , deletion) co-expressed in  
8 HEK293T cells; Left panel, input for each protein, 10% of the protein amount used for Co-IP; Right  
9 panel, Co-IP with FLAG antibodies followed by immunoblotting (IB) with HA and FLAG antibodies.  
10 (B) Western blot quantification of GRP75 expression in WTNPCs (n=6; red bars) and KONPCs (n=5,  
11 blue bars);  $p < 0.05$ ; Unpaired Student's *t* test; Error bars, SEM.  
12 (C) DAPI-staining (blue) and Proximity Ligation Assay (PLA, red dots) on WTNPCs (WT) in GPGln,  
13 PGln or Gln medium performed with Mcph1 antibodies coupled to either GRP75 (upper panel) or  
14 VDAC1 (lower panels) antibodies.  
15 (D) For each group, dot count was collected on an average of 30 cells from 3 independent  
16 experiments; *ns*, not statistically significant; \* $p < 0.05$ ; \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; One way ANOVA non-  
17 parametric Kruskal-Wallis test followed by Dunn's Multiple; Error bars, SEM.  
18 (E) DAPI-staining (blue) and PLA (red dots) performed on human NPCs (hNPCs) in GPGln, PGln or  
19 Gln medium by coupling MCPH1 antibodies to either GRP75 (upper panels) or VDAC1 (lower  
20 panels) antibodies.  
21 (F) Dot counts grouped from two independent experiments on two individual hNPC primary cultures;  
22 *ns*, not statistically significant; \* $p < 0.05$ ; One way ANOVA non-parametric Kruskal-Wallis test  
23 followed by Dunn's Multiple; Error bars, SEM.  
24 (G) DAPI-staining (blue) and PLA (red) with VDAC1 and GRP75 antibodies on WTNPCs and  
25 KONPCs. The data were validated in 5 individual cultures of WTNPCs and KONPCs in two distinct  
26 experiments.

- 1 (H) DAPI-staining (blue) and PLA (red) on E10.5 mouse nctx with Mcph1 antibodies coupled to
- 2 VDAC1 (left panels) or GRP75 (right panels) antibodies; lower-panels, higher magnification images
- 3 of white boxes.
- 4 Bars, 10  $\mu\text{m}$  in C, E, G; 20  $\mu\text{m}$  in H upper panels and 10  $\mu\text{m}$  in H lower panels.
- 5 See also Figure S6
- 6

## 1 STAR METHODS

2 Detailed methods are provided in the online version of this paper and include the following:

3 • **KEY RESSOURCES TABLE**

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5 • **EXPERIMENTAL MODEL AND SUBJECTS DETAILS**

6 ○ Mouse lines

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27

1 **SUPPLEMENTAL INFORMATION**

2 Supplementary Table S1 related to Figure 3: Enrichment for differentially expressed genes  
 3 in *McpH1* KO mouse versus wild type NPCs for several sets of genes.

4

5 **KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
MCPH1 (rabbit)	Abcam	Cat # ab2612
MCPH1 mouse exon 8, aa221-598 (rabbit)	Proteintech Group Inc customer service	N/A
MCPH1 mouse exon 9-14 aa600-822 (rabbit)	Proteintech Group Inc customer service	N/A
MCPH1 (mouse)	Abgent	Cat# AT2823a
Vimentin phospho S55 (mouse)	Abcam	Cat # ab22651 RRID AB_447222
gamma Tubulin (mouse)	Abcam	Cat # ab27074 RRID AB_2211240
SATB2 (mouse)	Abcam	Cat # ab51502 RRID AB_822455
Tpx2 (rabbit)	Santa Cruz	Cat # sc-32683
SDHA (rabbit)	Abcam	Cat # ab14715 RRID AB_301433
VDAC1 (mouse)	Abcam	Cat # ab14734 RRID AB_443084
VDAC1 (rabbit)	Abcam	Cat # ab15895 RRID AB_2214787
HSPA9/GRP75 (mouse)	Santa Cruz Biotechnology	Cat # sc-133137 RRID AB_2120468
HSPA5/GRP78 (mouse)	Millipore	Cat # MAB C675 RRID
Pax6 (mouse)	DSHB	PAX6 RRID AB_528427
TBR2 (rabbit)	Abcam	Cat # ab15894 RRID AB_10615604
TBR2 (chick)	Millipore	Cat # AB15894 RRID AB_10615604
Tbr2 (rat)	eBioscience	Cat # 14-4875-82 RRID AB_11042577
TBR1 (rabbit)	Abcam	Cat # ab31940 RRID AB_2200219
Cux1 (rabbit)	Santa Cruz Biotechnology	Cat # sc-13024
cleaved Caspase 3 (rabbit)	Cell Signalling	Cat # 9664 RRID AB_2070042
Par3 (rabbit)	Millipore	Cat # 07-330 RRID AB_11213581
APC (rabbit)	Santa Cruz Biotechnology	Cat # sc-896
PCK2 (rabbit)	Cell Signalling	Cat # 6924
HA (rat)	Roche	Cat # 11867423 RRID AB_2314621
CTIP2 (rat)	Abcam	Cat # ab18465 RRID AB_2064130
Phospho-Histone H3 (rat)	Abcam	Cat # ab10543 RRID AB_2295065
BrdU (rat)	Abcam	Cat # ab6326 RRID AB_305426
DOCK7 (goat)	Santa Cruz Biotechnology	Cat # sc-104220 RRID
HK2 (goat)	Santa Cruz Biotechnology	Cat # sc-6521
GFP (chick)	Aves	Cat # GFP-1020 RRID AB_10000240
AKT total (rabbit)	Cell Signalling	Cat # 4691 RRID AB_915783
pAKT S473 (mouse)	Cell Signalling	Cat # 4051 RRID AB_331158
pAKT T308 (mouse)	Santa Cruz Biotechnology	Cat # sc-271966 RRID AB_10715102

PDPK1 (rabbit)	Abcam	Cat # ab109253
ATF4 (rabbit)	Cell Signalling	Cat # 11815 RRID AB_2616025
Beta-Actin (mouse)	Millipore	Cat # MABT826
FLAG M2 (mouse)	Sigma-Aldrich	Cat # F1804 RRID AB_262044
Nanogold®-conjugated goat anti rabbit IgG	Nanoprobes	Cat # 2001-1 mL
<b>Biological Samples</b>		
Human tissues	Gynecology-Obstetric Department at Robert Debre Hospital, Paris	N/A
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
UK-5099	SSIGMA Aldrich	Cat # PZ0160
BPTES	SIGMA Aldrich	Cat # SML0601
2-Deoxyglucose	SIGMA Aldrich	
MitoTracker Deep Red	Invitrogen	Cat # M7514
Phalloidin	Invitrogen	Cat # A22284
Phosphatase and protease cocktail inhibitor	Thermofisher	Cat # 815-968-0747
Fluoromount-G mounting medium	SouthernBiotech	Cat #0 Cat # 100-01
DAPI (4', 6-diamidino-2-phenylindole)	Sigma Aldrich	Cat # D9542
Entellan	Millipore	Cat #107961
Durcupan ACM	Fluka	Cat # 44610
Protease inhibitor	Roche	Cat # 04693159001
<b>Critical Commercial Assays</b>		
DUOLINK kit	SIGMA Aldrich	Cat # DUO92101
ELISA Complex I Rodent profiling kit	Abcam	Cat # ab136809
HA-Tag Magnetic Co-IP kit	Pierce	Cat # 88838
CellROX® Deep Red Flow Cytometry Assay Kit	Invitrogen	Cat # C10493
647 EdU Click Proliferation Kit	Invitrogen.	Cat # C10491
Cy3 TSA amplification kit	BD Biosciences	Cat # 565456
QIAquick PCR purification Kit	Perkin Elmer	Cat # SAT704A001EA
Sybr Green Supermix	Bio-Rad	Cat #1708880
Quickchange XL Site-Directed Mutagenesis Kit	Qiagen	Cat # 28104
ScriptSeq V2 kit	Stratagene/Agilent Technologies	Cat # 200516
Ribo-Zero kit	Epicentre	Cat # SSV21106, #SSV21124
ExGen500	Epicentre	Cat # MRZH11124, #MRZH116
RNeasy Mini Kit	Euromedex	Cat # R0511
iScript cDNA Synthesis Kit	Qiagen	Cat # 74104, #2012
<b>Deposited Data</b>		
WT and Mch1 KO NPC Transcriptomes	GSE140976	
Mouse FAST DB	omicX	V2013,1
Molecular Signatures Database		V5.2
<b>Experimental Models: Cell Lines</b>		
HEK293T	ATCC	CRL-11268
<b>Experimental Models: Organisms/Strains</b>		



Mouse: B6.129S2-Emx1 <sup>tm1(cre)</sup> Krj/J (Emx1 <sup>Kicre/+</sup> )	The Jackson Laboratory	Emx1- kilRES cre
Mouse: Mcph1 <sup>tm1a</sup> (EUCOMM) <sup>Wtsi</sup>	EUCOMM	244329
Mouse: B6;SJL-Tg(ACTFLPe) <sup>9205Dym/J</sup> (beta-Actin-FLP)	The Jackson Laboratory	ACTB:FLPe B6;SJL
Mouse: Mcph1 <sup>lox/+</sup> ; Emx1 <sup>kicre/+</sup>	This paper	N/A
Mouse : Mcph1 <sup>tm1.2Kali</sup>	Liang et al., PLoS Genetics, 2010, 6(1)e1000826	N/A
Mouse: C57BL/6J	Charles River	C57BL/6J
<b>Oligonucleotides</b>		
mATF4-F: 5'CCTATAAAGGCTTGCGCCCA3'	This paper	N/A
mATF4-R: 5'GCTGGATTCCTGAACAGCG3'	This paper	N/A
mPCK2-F: 5'TGTCACCATAACCTCGTGCT3'	This paper	N/A
mPCK2-R: 5'TAGGGACACCTTCTGGTGCT3'	This paper	N/A
mGLS1-F: 5'AGGCAGTTCGGAATACACTGA3'	This paper	N/A
mGLS1-R: 5'AAGAATACCCCGCAACTCC3'	This paper	N/A
mGLUD1-R: 5'CTACGGCCGATTGACCTTCA3'	This paper	N/A
mGLUD1-R: 5'GCACCCGATATCCTGTCTG3'	This paper	N/A
mRpl13a-F : 5'ACA GCC ACT CTG GAG GAG AA3'	This paper	N/A
mRpl13a-R 5'GAG TCC GTT GGT CTT GAG GA3'	This paper	N/A
<b>Recombinant DNA</b>		
Mouse <i>Mcph1</i> cDNA	IMAGE	#5697978
pCAGGS-MCPH1FL-HA	This paper	N/A
pCAGGS-MCPH1 $\Delta$ BR2/3-HA	This paper	N/A
pCAGGS-ires-EGFP	Magnus et al Science. 2011 Sep 2;333(6047):1292-6.	Addgene #32482
pCMV-mito-GEM-GECO1	Zhao et al Science. 2011 Sep 8.	Addgene #32461

pcDNA3-MCPH1 full-length, $\Delta$ □□1, $\Delta$ BR2, $\Delta$ BR2-3	This paper	N/A
pcDNA3-GRP75	This paper	N/A
<b>Software and Algorithms</b>		
FIJI/ImageJ	Schneider et al., 2012	<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
Photoshop C3 Adobe Systems		<a href="https://www.adobe.com/products/photoshop.html">https://www.adobe.com/products/photoshop.html</a>
Excel	Microsoft	<a href="https://products.office.com">https://products.office.com</a>
GENEsys V1.5.4.0 software	Syngene	V1.5.4.0
STAR (v2.3.0)	Dobin et al., 2013	<a href="http://code.google.com/p/rna-star/">http://code.google.com/p/rna-star/</a>
FastQC	Babraham Bioinformatics	V0.10.1
Rseqc	Github	V2.4
Picard-Tools	Broad Institute	V1.119
Samtools	Source Forge. Github	V0.1.19
Primer3 plus Software	Primer3	<a href="http://primer3plus.com">http://primer3plus.com</a>
Morada Soft Imaging System	Olympus Soft Imaging Solutions	<a href="http://www.soft-imaging.net">www.soft-imaging.net</a>
GraphPadPrism 5.0 software	GraphPad Prism Software Inc.	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a>
And Deseq	Bioconductor	V.1.12.1
Bio-Rad CFX Manager	Bio-Rad	3.0
BioMaRt Bioconductor R package	Naro et al., 2007; Noli et al., 2015	V2.40.4

1

## 2 LEAD CONTACT AND MATERIALS AVAILABILITY

3 Further information and requests for resources and reagents generated in this study should be directed  
4 to and will be fulfilled by the Lead Contact, Jeannette Nardelli ([jeannette.nardelli@inserm.fr](mailto:jeannette.nardelli@inserm.fr)). All  
5 unique/stable reagents generated in this study are available from the Lead Contact with a completed  
6 Materials Transfer Agreement.

7

## 8 EXPERIMENTAL MODEL AND SUBJECTS DETAILS

### 9 Mouse lines.

10 All mouse lines were maintained in the C57Bl/6J genetic background. All animals were  
11 handled in strict accordance with good national animal practice as defined by the national rules, and  
12 all the mouse work was approved (# B75-19-01) by the Paris DDPP (Département De Protection des  
13 Populations).

1           The *Emx1*<sup>tm1<sup>(cre)</sup>Krj/J</sup> (Jackson laboratory #005628) and the *McpH1*<sup>McpH1<sup><tm1.2Kali></sup></sup> full knock out  
2 (KO) mouse lines were respectively described in Gorski et al., 2002 and in Liang et al., 2010. To  
3 generate *McpH1*<sup>lox/lox</sup> control and *McpH1*<sup>lox/lox</sup>; *Emx1*<sup>Kicre/+</sup> conditional knock out (cKO) mice, *McpH1*  
4 <sup>lox/+</sup> were derived from the *McpH1*<sup>tm1a (Eucomm)wtsi</sup> mice after crossing with beta-Actin-FLP mice  
5 (Rodriguez et al., 2000) for excision of the lacZ-neo cassette, then crossed with the *Emx1*<sup>kicre/+</sup> mice to  
6 produce *McpH1*<sup>lox/+</sup>; *Emx1*<sup>kicre/+</sup> mice, which were finally mated with *McpH1*<sup>lox/lox</sup> mice. The *McpH1*  
7 mutation in the KO and the cKO mice consists in the deletion of exon2 or exon 4 respectively, leading  
8 to out of reading frame mutation of *McpH1* in both cases. Midday of the vaginal plug observation was  
9 considered as embryonic day (E) 0.5. Pregnant females received intra-peritoneal BrdU injection (50  
10 mg/kg) at relevant stages of gestation and sacrificed 1 hour after injection for proliferation studies at  
11 E12, E14.5 and 2h at E16.5, while pups were sacrificed at P0 or P5 for neuron tracing. Animals and  
12 embryos were genotyped by PCR on DNA extracted from tail and yolk salk, respectively.

13

#### 14 **Human tissues**

15 All human tissues were obtained from the Gynecology-Obstetric Department at Robert Debre  
16 Hospital, Paris, according to the rules established by the French Bioethics Agency and with parental  
17 consent. 9-10 or 11 and 13 Post Conception Weeks (PCW) fetal brains were obtained after volunteer  
18 abortion or spontaneous pregnancy termination, respectively.

19

#### 20 **Cell lines**

21 MCPH1 and GRP75 Expression was obtained in HEK293T cells by transient transfection.

22

### 23 **METHODS DETAILS**

24

#### 25 **Tissue processing**

26           Whole E10.5 to E12.5 embryos were fixed in 4% paraformaldehyde (PFA, Sigma Aldrich),  
27 whereas E14.5 to P5 brains were dissected before fixation in the same solution. Tissues were cryo-  
28 protected in 30% sucrose in Phosphate buffered saline (PBS), embedded in gelatin before freezing and

1 kept at -80°C until use. Serial 12 micron-thick sections were cut on a Leica CM 3050 cryostat.  
2 Sections of P5 cortices were stained in a 0.3% cresyl violet solution, dehydrated and mounted in  
3 Entellan (Millipore) for brain morphometric analysis. For whole mount staining, fixed brains were  
4 rinsed in PBS and dorsal telencephalon was dissected before immunostaining.

5 After fixation in PFA, human fetal brains were processed as described for mouse brains.

6

### 7 **Embryonic neocortical neuroprogenitor cell (NPC) primary culture.**

8 Mouse dorsal telencephalon was dissected from E12.5 brains in DMEM/F12 medium  
9 (Invitrogen). Cell suspension was obtained by mechanical dissociation. NPCs from each telencephalon  
10 were grown individually as neurospheres in 6 well plates and in DMEM/F12 medium supplemented  
11 with 1xN2 and 0.5xB27, 10 ng/ml FGF (Invitrogen) and bFGF (Sigma Aldrich) and 10 µg/ml Insulin  
12 (Sigma Aldrich). Two days after the culture start, wild type (WTNPCs) or *Mcp1* KO (KONPCs)  
13 primary cultures were pooled by two or three in a T75 flask for further expansion during two to three  
14 days. At this stage, neurospheres were pelleted for protein or RNA extraction, or mechanically  
15 dissociated for seeding on gelatin-coated dishes, glass coverslips in 24-well plates or IBIDI slides  
16 (IBIDI). Attached cells were cultured in DMEM medium (Invitrogen) supplemented with 10% fetal  
17 bovine serum and either i) 4,5 g/L Glucose, 1mM sodium pyruvate and 1mM Glutamine (GPGln  
18 complete medium); ii) 2mM sodium pyruvate and 1mM Glutamine (PGln medium); iii) 1mM  
19 Glutamine only (Gln medium). Cells were then maintained in culture for four days.

20 For exposure to 2-DG (1mM), BPTES (1µM) or UK5099 (1µM), NPCs were primarily seeded  
21 in DMEM medium containing 1 g/L Glucose, 1mM sodium pyruvate and 1mM Glutamine for 24h.  
22 Inhibitors were added into the medium and cells left in contact with inhibitors for 24 hours.

23 Primary cultures of human embryonic neocortical NPCs (hNPCs) were generated from dorsal  
24 telencephalons dissected on embryos collected at 9-10 PCW by following the procedure afore-  
25 described for mouse NPCs.

26

### 27 **Immunofluorescence**

1 Two rabbit antibodies were raised against the mouse Mcph1 protein by the customer service  
2 of the Proteintech Group Inc. One antibody was raised to a synthetic internal polypeptide encoded by  
3 exon 8 (aa221-598), the second antibody to a synthetic peptide including the two C-ter BRCT domains  
4 encoded by exons 9-14 (aa600-822). The specificity of the two antibodies was established in  
5 transiently transfected NPCs expressing HA-tagged Mcph1 proteins, either complete or deleted of the  
6 two C-Ter BRCT domains. Both Mcph1 antibodies were diluted 1:2000 for immunofluorescence, to  
7 conduct expression studies from E10.5 to E16.5, along with a commercial mouse monoclonal MCPH1  
8 antibody directed against the human MCPH1 protein (Abgent). The three antibodies produced  
9 matching results on not frozen tissues, such as whole mount cortices. On frozen sections, only our  
10 custom-made antibodies gave specific and matching results, but required the use of a Cy3 TSA  
11 amplification kit (Perkin Elmer). Unless specified the data presented here were obtained with the  
12 Mcph1-Cter antibodies. Human MCPH1 protein expression was detected using the same procedure  
13 and Abcam MCPH1 antibodies (ab2612).

14 Immunodetection of other molecular markers was performed as described (El Wakil et al.,  
15 2006). To follow with BrdU co-staining, immuno-stained sections were post-fixed in PFA 4%, treated  
16 with HCl 2N at room temperature for 30 mn, rinsed in PBS and processed for BrdU staining. Whole  
17 dorsal telencephalon samples were permeabilized in PBS 0.3% triton X100, then saturated in PBS  
18 containing 10% normal goat serum (NGS) and 0.1% triton (PBGT) for at least two hours. Samples  
19 were then incubated at 4°C with primary antibodies diluted in PBGT either overnight (E12.5) or for  
20 two days (E14.5). After several washes in PBT, tissues were incubated for 2 hours (E12.5) or  
21 overnight (E14.5) in secondary antibodies diluted in PBGT containing 1 µg/ml DAPI (SIGMA  
22 Aldrich), washed in PBT and flat-mounted in Fluoromount-G (SouthernBiotech), and sealed with  
23 Entellan (Millipore).

24 NPCs were fixed in 4% PFA, rinsed in PBS, permeabilized in PBT during 15 minutes, and  
25 saturated with PBS containing 10% NGS (PBG) for one hour. Before fixation, NPCs were eventually  
26 incubated with 200 nM MitoTracker Deep red (Invitrogen) for 30 minutes. Primary antibodies diluted  
27 in PBG were incubated overnight and rinsed in PBS. Secondary antibodies diluted in PBG containing

1 1 µg/ml DAPI (4',6-diamidino-2-phénylindole) were applied for 30 minutes at room temperature,  
2 washed in PBS and coverslips were mounted in Fluoromount-G (SouthernBiotech).

3 The commercial antibodies used in our studies were: as mouse antibodies, MCPH1 (1:1000,  
4 Abgent #2823a), phospho-Vimentin-S55 (1:1000, Abcam ab22651), γ-tubulin (1:2000, Abcam  
5 ab27074), Satb2 (1:500, Abcam ab51502), SDHA (1:500; Abcam ab14715), GRP75 (1:250; Santa-  
6 Cruz Biotechnology sc-133137), GRP78 (1:500, Millipore #MABC675), PAX6 (1:20, DSHB PAX6),  
7 as rabbit antibodies, MCPH1 (1:2000, Abcam ab2612), Tbr2 (1:250, Abcam ab15894), Tpx2 (1:500;  
8 Santa Cruz Biotechnology sc-32683), Tbr1 (1:500, Abcam ab31940), Cux1 (1:500, Santa Cruz  
9 Biotechnology sc-13024), cleaved Caspase3 (1:2000, Cell Signaling #9664), Par3 (1:1000, Millipore  
10 #07-330), APC (1:1000, Santa Cruz Biotechnology sc-896), PCK2 (1:1000, Cell Signaling #6924), as  
11 rat antibodies, HA (1:2000, Roche #11867423), Ctip2 (1:1000, Abcam ab18465), phospho-Histone H3  
12 (1:2000; Abcam ab10543), anti BrdU (1:1000, ab6326), as goat antibodies Dock7 (1:100, Santa Cruz  
13 Biotechnology sc-104220), HK2 (1:500, Santa Cruz Biotechnology sc-6521 ), as chick antibodies,  
14 anti GFP (1:2000, Aves GFP-1020), anti TBR2 (1:500, Millipore #15891). Co-staining was eventually  
15 performed with Phalloidin Deep Red (1/1000) and MitoTracker Deep Red (Invitrogen). Secondary  
16 Goat or Donkey antibodies were coupled to Alexa488, Alexa633 (Invitrogen), or to Cy3, Cy5  
17 (Jackson ImmunoResearch). All were used diluted 1:2000.

18

### 19 ***In situ* hybridization**

20 To analyze *McpH1* expression by *in situ* hybridization, a cDNA fragment including  
21 nucleotides +1 to + 850 of the coding sequence was amplified from the IMAGE clone # 5697978 by  
22 PCR with a reverse primer including the T3 promoter sequence. The PCR product was checked on  
23 agarose gel and purified using the QIAquick PCR purification kit (Qiagen). A digoxigenin anti-sense  
24 RNA probe was used to performed *in situ* hybridization on sections of E10.5, E12.5, E14.5 and E16.5  
25 embryonic brains, as described in (El Wakil et al., 2006).

26

### 27 **Imaging and cell counts**

1           Imaging was performed using a Leica TCS SP8 confocal scanning system (Leica  
2 Microsystems) equipped with 405-nm Diode, 488-nm Ar, 561-nm DPSS and 633-nm HeNe lasers.  
3 Eight-bit digital images were collected in a sequential mode from a single optical plane using a 63x  
4 HC PL APO CS2 oil-immersion Leica objective (numerical aperture 1.40) for cell analyzes. For brain  
5 sections and whole mounts samples, a 20x and a 40x HC PL APO oil-immersion objectives were used  
6 to take single plan, tiles or Z-stack images in a sequential mode. Pictures with composite colors and  
7 the corresponding Tiff files were generated on ImageJ and further processed on Photoshop C3 for  
8 sizing and eventually for improving color contrast. 3D reconstructions were obtained from Z-stacks  
9 images taken within 40-90  $\mu$ m from the ventricle surface using the Leica 3D software (Leica  
10 Microsystems). Single plan images were sequentially extracted at different levels to examine the  
11 distribution of marker expression.

12           At P5, morphometric analyses were performed on ImageJ (Schneider et al., 2012) on  
13 transverse cortical sections stained with cresyl violet and selected at the level of the somato-sensorial  
14 area. The cortical thickness was measured by drawing lines perpendicular to the ventricle surface and  
15 extending within the whole cortical wall. The lateral size of the neocortex was measured by drawing a  
16 curved line following the cortical surface, from the boundary with the cingulate cortex to the rhinal  
17 fissure. Four brains of each group, conditional knock out and control, 4 to 8 sections from each brain,  
18 were included in the studies.

19           Cells were counted on cortical transverse sections obtained with a x20 objective and  
20 1024x1024 resolution and surface of 500 x 700  $\mu$ m (P0) and 600  $\mu$ m x 1400  $\mu$ m (P5) (width x height)  
21 was delineated on tile-scan images from the cortical surface in the S1 cortical area and using ImageJ  
22 (Schindelin et al., 2012). Neurons and BrdU were counted with the ImageJ cell counter plugin in each  
23 layer as defined by their respective specific markers: Tbr1 for layer VI, Ctip2 for layer V, Satb2 for  
24 layers IV-II, and Cux1 for layer II-III. Five brains of each P0 group and three brains of each P5 group,  
25 control and *Mcp1* cKO, with 2 to 5 sections per brain, were used in the study.

1 BrdU and Tbr2 positive cells count was performed on images cropped to a width of 250  $\mu\text{m}$  at  
2 E12.5 (3 brains for each genotype), 400  $\mu\text{m}$  at 14.5 (4 brains for each genotype) and E16.5 (3 control  
3 and 4 cKO brains). Two to five sections of each brain were included in the study.

4 The number of PH3+ dividing cells at the ventricle border or in the SVZ of E11.5 (3 control  
5 and 4 cKO brains), E12.5 (4 brains of each group), E14.5 (3 control and 4 cKO brains) and E16.5 (4  
6 control and 5 cKO brains) nctx was counted according to a ventricular length ( $\mu\text{m}$ ) calculated on  
7 Image J.

8 Activated caspase-3 positive NPCs were counted manually on 184  $\mu\text{m}$  x 184  $\mu\text{m}$  (1024 x 1024  
9 pixels) images taken with an x63 objective. The number of DAPI-stained nuclei per image was  
10 counted with the ImageJ cell counter plugin. Counts were obtained from 4 different cultures of  
11 WTNPCS and KONPCS processed in two independent experiments and from an average of 20 images  
12 per culture condition.

13

#### 14 **Electron microscopy**

15 NPCs were fixed for 20 min with 37°C pre-warmed 4% PFA with 0.1% glutaraldehyde in  
16 0.1M phosphate buffer (PB) containing 4% sucrose. After washing in PB, cells were post-fixed post-  
17 fixed in 1% glutaraldehyde in PBS for 10 min at RT. And washing in PB, they were post-fixed in 1%  
18 osmium tetroxide in Phosphate buffer (PB) for 10 min at RT, washed in PB, and dehydrated in an  
19 ascending series of ethanol, which included 1% uranyl acetate in 70% ethanol. Cultures were then  
20 embedded in Durcupan ACM (Fluka) resin. After polymerization at 60°C for 48 h, coverslips were  
21 separated from the embedded cells, and resin blocks were processed for ultrathin sectioning on a  
22 Reichert Ultracut S microtome.

23 For preembedding immunocytochemical detection of Mcph1 at the ultra-structural level, the  
24 brains of the E12.5 embryos were quickly dissected and fixed in 4% PFA for at least 24 h at 4°C.  
25 Horizontal sections were cut on a vibratome at 70  $\mu\text{m}$  and collected in PBS, equilibrated in 25%  
26 sucrose and 10% glycerol in 0.05 M PB, frozen rapidly in iso-pentane cooled in liquid nitrogen, and  
27 thawed in PBS at RT. Sections were pre-incubated for 30 min in 5% NGS in PBS, and were then



1 incubated overnight at RT in rabbit anti-Mcph1 antibody (1:1000) diluted in PBS containing 1% NGS.  
2 After washing in PBS, sections were incubated for 120 min in Nanogold®-conjugated goat anti rabbit  
3 IgG (1:100; Nanoprobes) diluted in PBS containing 2% of bovine serum albumin-c and 0.2% of cold  
4 water fish gelatin. Sections were washed in PBS and post-fixed in 1% glutaraldehyde in PBS for 10  
5 min. After washings in PBS and 0.1 M sodium acetate buffer, pH 7.0, Mcph1 immuno-gold labeling  
6 was intensified using a silver enhancement kit (HQ Silver; Nanoprobes) for 5-10 min in the dark at  
7 RT. After washings in acetate buffer and in PB, sections were post-fixed in 1% osmium tetroxide in  
8 PB for 10 min at RT. After washings in PB, they were dehydrated in an ascending series of ethanol,  
9 which included 1% uranyl acetate in 70% ethanol. Sections were then treated with propylene oxide  
10 twice for 10 min, equilibrated overnight in Durcupan ACM (Fluka), mounted on glass slides and cured  
11 at 60°C for 48 h. Areas of interest including the dorsal telencephalon were cut out from the slides and  
12 glued to blank cylinders of resin. Blocks were cut in semithin sections (1 µm) and then in ultrathin  
13 sections on a Reichert Ultracut S microtome.

14 For both NPCs and sections of the dorsal telencephalon, ultrathin sections were collected on  
15 pioloform-coated single-slot grids, stained with lead citrate and examined with a Philips CM120  
16 electron microscope equipped with Morada Soft Imaging System (Olympus Soft Imaging Solutions).  
17 The acquired images were equally adjusted for brightness and contrast, and composite illustrations  
18 were built in Adobe Photoshop CS3 (Adobe Systems).

19 Mitochondrial profiles in NPCs were identified and morphologically characterized using the  
20 Measure command of FIJI/ImageJ software (Schindelin et al., 2012; Schneider et al., 2012). Results  
21 were expressed as number of mitochondrial profiles per µm<sup>2</sup> cytoplasmic surface and relative  
22 mitochondrial surface compared to cytoplasmic surface. An average of 6 cells/group was analyzed in 3  
23 independent experiments.

24

## 25 **Proximity Ligation assay (PLA)**

26 Proximity Ligation assays (PLA) were performed on NPCs seeded on glass-cover slips or  
27 E10.5 mouse cortical sections, formerly processed as for immunostaining. The assays were performed  
28 using a DUOLINK kit (SIGMA Aldrich), following the supplier instructions and with the following

1 antibody associations: rabbit Mcph1 C-ter/mouse GRP75 (1:250; Santa-Cruz sc-133137), rabbit  
2 Mcph1 C-ter/mouse VDAC1 (Abcam ab14734), mouse GRP75 (1:250; Santa-Cruz sc-133137)/rabbit  
3 VDAC1 (1/500, Abcam ab15895), goat HK2 (1/500; Santa Cruz Biotechnonology)/rabbit VDAC1  
4 (1/500).

5 Duolink signal was analyzed using a Leica TCS SP8 confocal scanning system (Leica  
6 Microsystems) and a 63x HC PL APO CS2 oil-immersion Leica objective (numerical aperture 1.40),  
7 as described before. Duolink particles and DAPI-stained nuclei were segmented and counted using the  
8 FIJI/ImageJ software (Schindelin et al., 2012; Schneider et al., 2012). Results were expressed as  
9 Duolink particles per nuclei and per 1000  $\mu\text{m}^2$  cytoplasmic surface. An average of 30 cells/group was  
10 analyzed in 3 independent experiments. Two sets of experiments, Duolink of Mcph1 and GRP75 and  
11 Duolink of Mcph1 and VDAC1 were analyzed, each set including 3 groups of cells: PGln, PGLn and  
12 Gln.

13

#### 14 **Ca<sup>2+</sup> imaging and quantification**

15 For ratiometric mito-GEM-GECO1 imaging of neuroepithelial cultures, the 405-nm Diode  
16 laser of a Leica TCS SP8 confocal scanning system (Leica Microsystems) was used. Emission  
17 windows were set to 427-467 nm (blue channel) and 502-537 nm (green channel). Eight-bit digital  
18 images were collected from a single optical plane using a 63x HC PL APO CS2 oil-immersion Leica  
19 objective (numerical aperture 1.40). Settings for laser intensity, pinhole (1 Airy unit), range property  
20 of emission window, electronic zoom, gain and offset of photomultiplier, field format, scanning  
21 speed were optimized initially and held constant throughout the study so that all cells were digitized  
22 under the same conditions. Mitochondrial signals of the blue and green channels were segmented;  
23 their ratio was calculated and measured using the Analyze Particles command of FIJI/ImageJ  
24 (Schindelin et al., 2012; Schneider et al., 2012). An average of 26 cells/group, two groups  
25 corresponding to WT (n=3) and *Mcph1* KO (n=3) NPCs in PGln medium, was analyzed in 2  
26 independent experiments. Mean ratio values for the experimental groups were calculated and  
27 statistically analyzed using the non-parametric Mann-Whitney U test.

28

## 1 **Western blotting and ELISA**

2 Pellets of NPCs cultured in GPGln, PGLn or Gln medium were homogenized in RIPA buffer  
3 (SIGMA) with phosphatase and protease inhibitor cocktails (Invitrogen) added. Protein concentration  
4 was measured by the BCA method and using the Paradigm spectrophotometer (Beckman). Proteins  
5 were separated on Mini-Protean TGX 4-15% gels (Bio-Rad), transferred to PVDF membranes with a  
6 Trans-Blot TurboTransfert apparatus (Bio-Rad), then immunoblotted for VDAC1 (1:1000, Rabbit  
7 Abcam ab15895), GRP75 (1:1000, Mouse Santa Cruz Biotechnology sc-133137), HK2 (1:1000, Goat  
8 Santa Cruz Biotechnology sc-6521), PCK2 (1:1000, Rabbit Cell Signaling #6924), AKT total (1:1000,  
9 Rabbit Cell Signaling #4691), pAKT S473 (1 :200, Mouse Cell Signalling #4051), pAKT T308  
10 (1 :200, Mouse Santa Cruz Biotechnology sc-271966), PDPK1 (1 :1000, Rabbit Abcam ab109253),  
11 ATF4 (1 :500, Rabbit Cell Signaling #11815), HA epitope (1 :1000, Rat Roche #11867423) and beta-  
12 Actin (1:20000, Mouse Millipore MABT826) as loading control. HRP conjugated secondary  
13 antibodies (Jackson Immunoresearch) were used diluted 1:20000 and bands were detected using the  
14 PXi multi-application image analysis system with the GENESys V1.5.4.0 software (Syngene) and  
15 densitometry analysis on each blot was calculated using GENESys-Tool (Syngene) or FIJI/ImageJ  
16 softwares. Densitometry values were normalized to beta-Actin. For each protein, the normalized  
17 expression ratio was established for each analyzed sample by calculating the marker/Actin  
18 densitometric ratio and dividing this ratio by the mean of the ratios obtained from the WT/GPGln  
19 control group. For quantification of phosphorylated Akt proteins, each ratio was normalized to the  
20 mean ratio obtained for total Akt in WTNPCs.

21 Mitochondria quantification was performed using the ELISA Complex I Rodent profiling kit  
22 (Abcam, ab136809), and following the supplier instructions. Optical densities were read at 450 nm  
23 using the Paradigm spectrophotometer (Beckman). Six *Mcp1* KO and 7 WT samples were included  
24 in the experiments.

25

## 26 **FACS analyzes**

27 Intracellular radical oxygen species (ROS) production and cell mortality were analyzed by

1 flow cytometry with the CellROX<sup>®</sup> Deep Red Flow Cytometry Assay Kit (Invitrogen, Thermo Fisher  
2 Scientific, Eugene, OR, USA). Cells were incubated with or without 400 $\mu$ M Tert-butyl hydroperoxyde  
3 (TBHP) for 2 hours, and with 1000nM CellRox and 1 $\mu$ M SYTOX respectively for the last 60 and 15  
4 minutes. After trypsinization, cells were analyzed by flow cytometry. The gating strategy selected  
5 FSC and SSC singlets, with exclusion of SYTOX<sup>high</sup> apoptotic cells.

6 Cell cycle was analyzed by EdU incorporation and DNA content measurement using 647 EdU  
7 Click Proliferation Kit (BD Biosciences) and propidium iodide (PI), respectively. EdU was added to  
8 cell cultures during the last 3 hours of incubation with metabolic inhibitors. After trypsinization, click  
9 reaction and PI staining were performed according to manufacturer recommendations. Cells were  
10 analyzed by flow cytometry. The gating strategy selected FSC and SSC singlets, followed by selection  
11 of cells in “G0/G1”, “S” and “G2/M” phases. For each cell cycle phase results were expressed as  
12 percentage relative to control condition.

13

#### 14 **Plasmids and cell transient transfections**

15 For transient expression in NPCs cells, the human full-length (FL) *MCPH1* cDNA was  
16 synthesized with the HA-tag sequence fused at the 3' end, and subcloned into the Xho/XbaI restriction  
17 sites of the pCAGGS-IRES-GFP (Addgene # 32482) plasmid by the customer service of the GenScript  
18 Biotech Corporation to generate the pCAGGS-MCPH1FL-HA. To produce the pCAGGS-  
19 MCPH1 $\Delta$ BR2-3-HA, the sequence overlapping exons 1 to 8 was amplified by PCR, sub-cloned into  
20 the same plasmid using the same strategy and checked by sequencing. pCMV-mito-GEM-GECO1 was  
21 a gift from Robert Campbell (Addgene plasmid #32461). Transient transfections were performed in  
22 NPCs using ExGen500 (5  $\mu$ l per 1  $\mu$ g of DNA and per well of a 24 well plate; Euromedex). NPCs  
23 were seeded either on glass coverslips for immunostaining or IBIDI chambers (IBIDI) for live  
24 imaging, and cells analyzed 24 hours after transfection. For live imaging, NPCs were placed in HBSS  
25 containing 15 mM HEPES, kept in a chamber à 37°C, and imaging was performed as described  
26 previously. For MCPH1 and GRP75 co-immunoprecipitation (co-IP), human full-length *MCPH1*

1 cDNA were obtained from HeLa mRNA and cloned into pcDNA-3HA vector using EcoRI/XhoI sites.  
2 *MCPH1* mutants ( $\Delta$ BR1: 94-835aa,  $\Delta$ BR2:  $\Delta$ 671-730aa,  $\Delta$ BR3: 1-730aa,  $\Delta$ BR2-3: 1-670aa) were  
3 produced using a Quickchange XL Site-Directed Mutagenesis Kit (Stratagene). Human *GRP75* cDNA  
4 was cloned into pcDNA-3FLAG vector using XhoI sites. The transfection of HEK293T cells was  
5 performed with polyethylenimine (PEI, Polyscience, Eppelheim, Germany) at a ratio of 5 $\mu$ g HA-  
6 MCPH1-FL or mutants plasmids and 5  $\mu$ g FLAG-GRP75 in 30  $\mu$ g PEI solutions. Co-IP was  
7 performed 48hr after transfection. For MCPH1 and VDAC1 Co-IP, the pCAGGS-ires-GFP MCPH1-  
8 HA constructs afore described were transfected in HEK293T cells using the same conditions than for  
9 NPCs.

10

### 11 **Co-immunoprecipitations**

12 For MCPH1 and GRP75 Co-IP, cell pellets were incubated in NETN lysis buffer (50mM Tris-  
13 HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA, 0.5%NP-40, and protease inhibitor cocktail (Roche,  
14 #04693159001) on ice for 30 min. For IP, 2  $\mu$ g of the anti-FLAG antibody (Sigma-Aldrich, F-1804)  
15 were incubated with 1mg of total lysate together with the protein G Sepharose<sup>TM</sup> 4 fast flow (GE  
16 Healthcare) at 4°C overnight. The precipitates were washed with the NETN buffer without protease  
17 inhibitors. MCPH1 and VDAC1 Co-IP were performed using the Perkin-Elmer HA magnetic beads,  
18 following manufacturer instruction for cell extraction and Co-IP.

19

### 20 **Mitochondrial respiratory chain and metabolic enzymes activities**

21 Cells were scraped in cold PBS, quickly pelleted at 4°C and kept frozen at -80°C until use.  
22 The activity of the various complexes of the mitochondrial respiratory chain and of Glucose-6-  
23 Phosphate Dehydrogenase (G6PDH), total hexokinase (Hexo I-IV), lactate dehydrogenase (LDH),  
24 NADP- dependant Isocytate dehydrogenase (IDH1-2) and NAD<sup>+</sup> dependant isocitrate dehydrogenase  
25 (IDH 3) activities were monitored on cell pellets, at 37°C and by spectro-photometry (double  
26 wavelength spectrophotometer (Cary 60, Varian, or Cary 50 spectrophotometer, Agilent Technologies,  
27 respectively) according to (Benit et al., 2006; Rustin et al., 1994). Protein content of cell pellets was  
28 estimated by the Bradford assay.

1

## 2 **Sample preparation for RNA-seq analysis**

3 Total RNA was extracted from NPCs obtained from WTNPCs and WTNPCs. Sample quality  
4 and quantification were assessed on Experion chips (Bio-Rad). Twin samples were pooled according  
5 to genotype and matching amounts. Library preparation and Illumina sequencing were performed on  
6 wild type and *Mcp1* KO samples, two of each, at the Genomic Platform of the Ecole Normale  
7 Superieure (Paris, France). Ribosomal RNA depletion was performed with the Ribo-Zero kit  
8 (Epicentre), using 1.5 µg of total RNA. Libraries were prepared using the strand specific RNA-Seq  
9 library preparation ScriptSeq V2 kit (Epicentre). Libraries were multiplexed by 4 on 1 flow cell lane.  
10 A 75bp read sequencing was performed on a HiSeq 1500 device (Illumina). A mean of  $39.3 \pm 7$   
11 million passing Illumina quality filter reads was obtained for each of the 4 samples.

12

## 13 **Gene expression analysis in mouse NPCs**

14 RNA-Seq data analysis was performed by GenoSplice technology ([www.genosplice.com](http://www.genosplice.com)).  
15 Sequencing, data quality, reads repartition (e.g., for potential ribosomal contamination), and insert size  
16 estimation were performed using FastQC (v0.10.1), Picard-Tools (v1.119), Samtools (v0.1.19) and  
17 rseqc (v2.4). Reads were mapped using STAR (v2.3.0) (Dobin et al., 2013). Gene expression was  
18 estimated as already described (Dobin et al., 2013; Naro et al., 2017) using Mouse FAST DB v2013\_1  
19 annotations. Only genes expressed in at least Mutated *Mcp1* cortex or WT cortex were further  
20 analyzed. Genes were considered as expressed if their rpkkm value was greater than 97.5% of the  
21 background rpkkm value based on intergenic regions. Raw count data normalization and differential  
22 expression analysis were then performed using Deseq (v1.12.1). Results were considered statistically  
23 significant for unadjusted  $p$ -values  $\leq 0.05$  and fold-changes  $\geq 1.5$ .

24 The mouse dataset was annotated with human Ensembl gene ID using BiomaRt Bioconductor  
25 R package (Naro et al., 2017; Noli et al., 2015) and selecting human genes that were ‘one-to-one’  
26 orthologues with mouse genes. As previously described (Delahaye-Duriez et al., 2016), we generated  
27 a gene-level score reflecting the significance and the intensity of differential expression (multiplying

1 the  $-\log_{10}$  of p value by the log-transformed fold change). This score was used as a metric to ‘rank’  
2 genes to test using Gene set enrichment analysis (GSEA) if gene sets defined in the Molecular  
3 Signatures database (MSigDB v5.2) occupy higher (or lower) positions in the ranked gene list than  
4 what it would be expected by chance (Subramanian et al., 2005). The enrichment was tested for  
5 several sets of genes of the MSigDB: Gene ontology (GO) biological process, cellular component and  
6 molecular functions, Reactome pathways, and Hallmark gene sets. The MSigDB hallmark gene sets is  
7 a collection of 50 gene sets that provide coherently expressed signatures derived by aggregating many  
8 MSigDB gene sets (Molecular Signatures Database v5.2) to represent well-defined biological states or  
9 processes (Liberzon et al., 2015). Gene set enrichment scores (NES, p value, FDR and FWER) were  
10 provided in the GSEA output format developed by Broad Institute of MIT and Harvard (permutations  
11 = 10,000).

12

### 13 **Analysis of MCPH1 expression in transcriptomic datasets from the Human BrainSpan Atlas.**

14 To determine the spatiotemporal expression dynamics of modules, we used quantile  
15 normalised gene level expression values ( $\log_2$  transformed) from GSE25219 (Kang et al., 2011).  
16 These transcriptome data were generated using Affymetrix Human Exon 1.0 ST array analysis of 16  
17 brain regions comprising the cerebellar cortex, mediodorsal nucleus of the thalamus, striatum,  
18 amygdala, hippocampus and 11 areas of the neocortex. The data were generated from 1263 samples  
19 collected from 53 clinically unremarkable post-mortem human brains, spanning embryonic  
20 development to late adulthood (from 10 weeks of post-conception to 82 years of age, which  
21 corresponded to periods 3–15, as previously designated) (Kang et al., 2011). The  $\log_2$ -transformed  
22 gene expression data follow a bimodal distribution contributed by low (likely non-functional) and high  
23 expressed genes (Hebenstreit et al. 2011). We used the expectation maximisation (EM) algorithm to  
24 model gene expression levels as a mixture of normal distributions and identify the underlying  
25 distributions of low and high expressed genes. Only the genes, with mean of  $\log_2$ -transformed  
26 expression values over the 95 % percentile of distribution of low-expressed genes (here  $> 5.61$ ) were  
27 considered for further analysis ( $n = 8704$ ). The EM algorithm was implemented using normalMixEM  
28 function from the mixtools R package. Spatio-temporal dynamics of MCPH1 across 16 brain regions

1 and 13 developmental time points were illustrated as a heatmap, as previously described (Pletikos et  
2 al., 2014). The resultant heatmap graphs illustrate the changes in expression of *MCPHI* across brain  
3 development and cortical regions.

4

## 5 **Data statistical analyzes**

6 Statistical analysis was performed with Excel (Microsoft, Richmond, WA) and GraphPad  
7 Prism 5.0 (GraphPad Prism Software Inc.). For all cell counts *in vivo*, results were obtained by using  
8 at least 3 embryos from at least 2 litters and 2-6 sections per embryo were used for counting. No data  
9 was excluded from further analyses. For experiments on NPCs, results were obtained from at least 3  
10 different primary cultures and at least two independent experiments. Sample sizes (n) and statistical  
11 methods are indicated in the Figure legends. n is the number of primary cultures or brains used in the  
12 experiments. Shapiro-Wilk normality test was used for testing normality of all samples. The two-tailed  
13 unpaired Student's *t* test was applied for comparison of two two groups: Ct and cKO brains at P5 and  
14 P0, counts of PH3+ and CC3+ cells in 11.5, E12.5, E14.5 and E16.5 Ct and cKO embryonic brains,  
15 and for counts of Pax6+, BrdU+ and Tbr2+ cells in E12.5, E14.5 and E16.5 Ct and cKO embryonic  
16 brains. The fluorescence ratiometric values of Mito-GEM-GECO1, morphometric measures by  
17 electronic microscopy and ROS production data were analyzed using the non-parametric Mann-  
18 Whitney U test. Duolink mean values for the experimental groups were calculated and statistically  
19 analyzed using the non-parametric Kruskal-Wallis test followed by Dunn's Multiple. Activities of  
20 respiratory chain enzymes, of LDH, HKI-IV, IDH and G6PDH, Western blots, cleaved caspase3 in  
21 GPGln, PGLn, and Gln and FACS cell cycle data were analyzed using one-way ANOVA with Tukey-  
22 Kramer Multiple Comparison Tests. In all cases, differences were considered as statistically  
23 significant at  $p < 0.05$ . The meaning of error bars, either SEM or SD, is indicated in the figure legends.

24

## 25 **DATA AND CODE AVAILABILITY**

26 The accession number of RNA-seq data generated in this study from WTNPCs and KONPCs  
27 is GSE140976.

28



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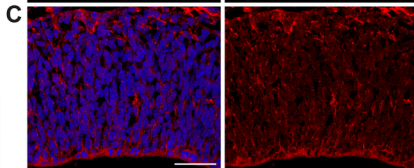
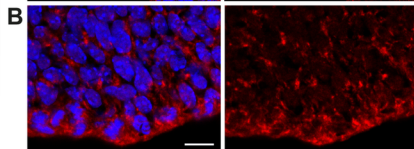
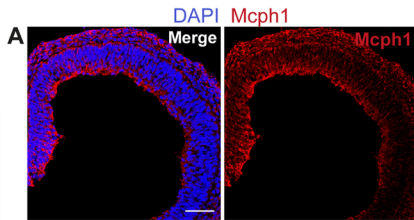
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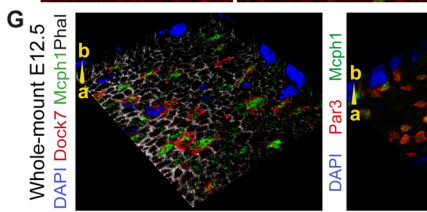
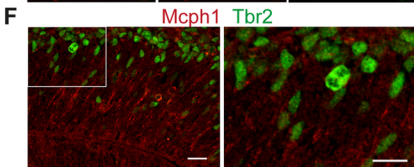
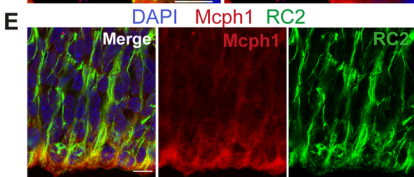
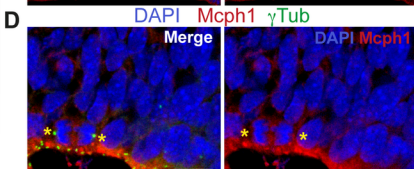
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Human fetal neocortex PCW11

E10.5

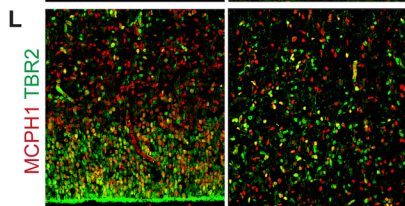
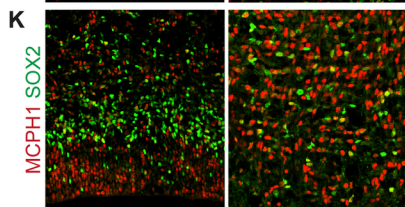
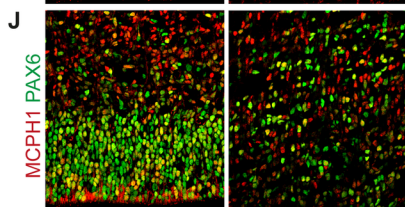
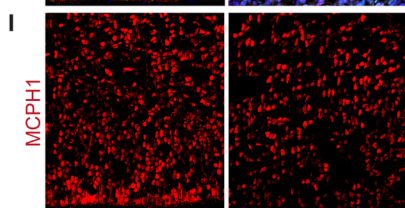
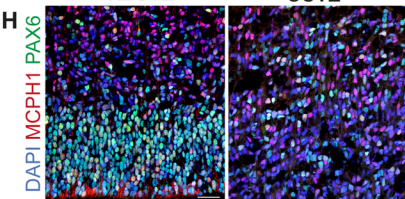


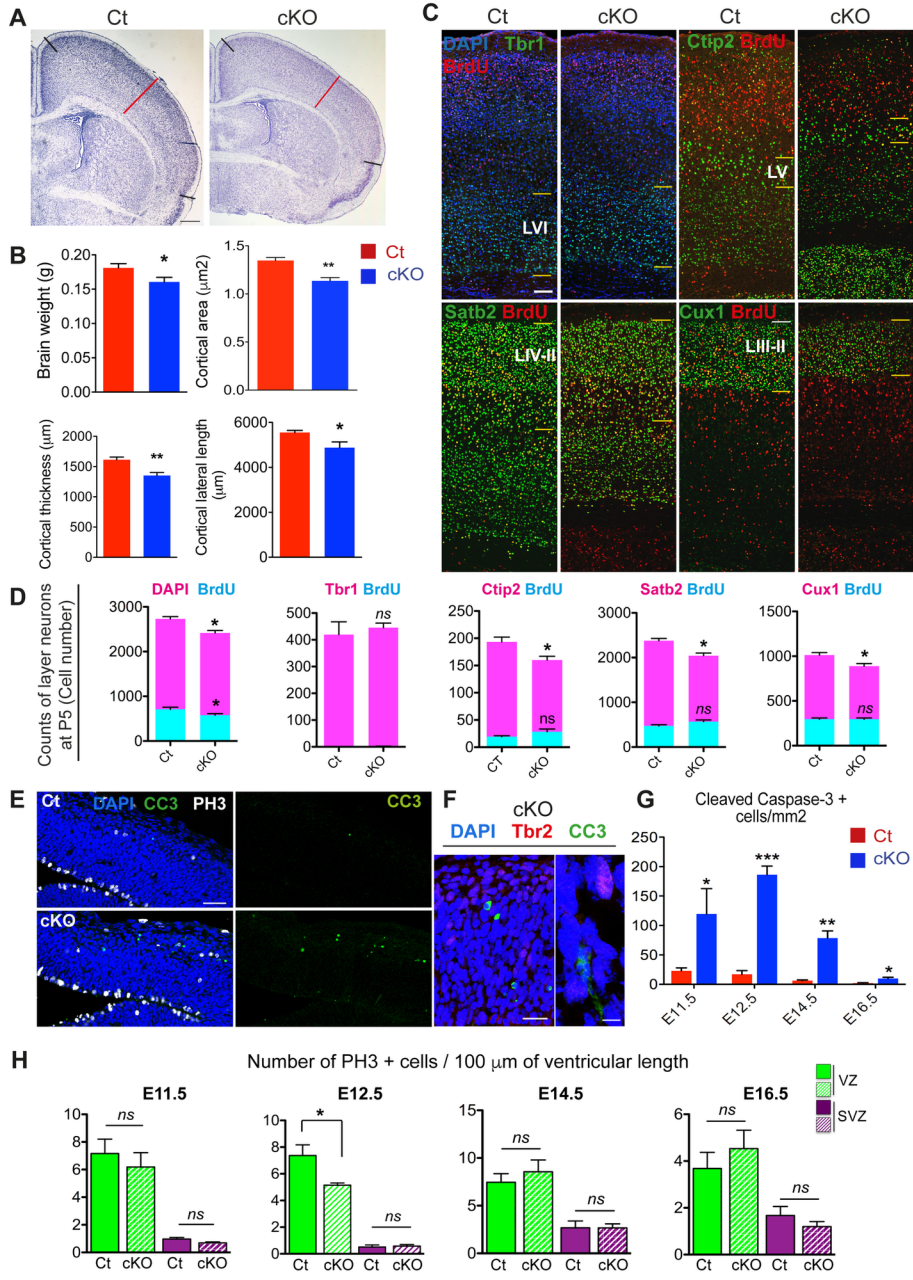
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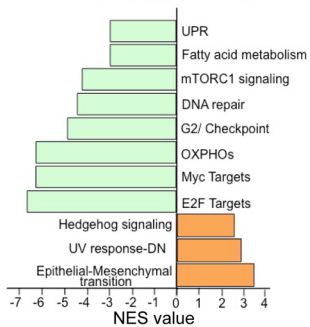
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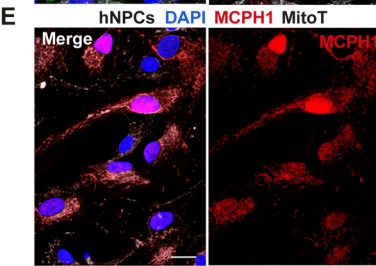
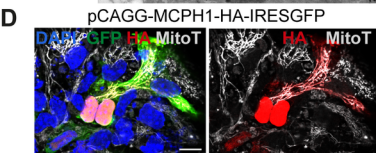
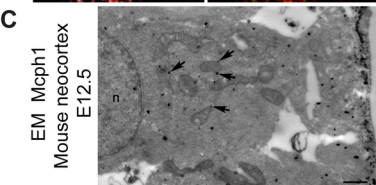
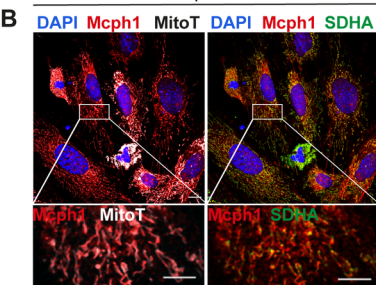




**A** Differentially regulated pathways  
KO versus WT NPCs



Mitochondrial expression of MCPH1



Alteration of the mitochondrial network  
KO mNPCs

