Supporting Information

Endothelial cells mediated by UCP2 control the neurogenic-to-astrogenic neural stem cells fate switch during brain development

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### Supplementary Table 1: QPCR Primers

| Name              | Sequence(5’-3’)                      |
|-------------------|--------------------------------------|
| MouseUCP2-RT-F    | TGTTGTAAAGGTCCGCTTCC                 |
| MouseUCP2-RT-R    | TCCCTTCCTCTCGTGCAATG                 |
| β-Actin-RT-F      | GGCTGTATTCCCCCTCCATCG                |
| β-Actin-RT-R      | CCAGTTGGTAACAATGCCATGT               |
| CMA1-RT-F         | AGCTAACCTAGGTGTGGGA                  |
| CMA1-RT-R         | GGCTGGCTCATTACGTTTG                 |
| OMA1-RT-F         | GCCGAAGCTGACAAAGTTGG                 |
| OMA1-RT-R         | CTGGCAACTTGGGATAGCCA                 |
| POLG2-RT-F        | CTCTGGAAGAAGGTGGCTGG                 |
| POLG2-RT-F        | TTCTCGGAGGAGGGCATTTG                |
| TWNK-RT-F         | TGCAATCCCTCCGGATCT                  |
| TWNK-RT-R         | TTTCACTCGTAGTCACCGGC                |
| Dguok-RT-F        | GGCTTCACCCCAGGTTTGA                 |
| Dguok-RT-R        | CTGCAGAGCTTGAAGTGGGA                |
**Figure S1. Endothelial UCP2 deletion reduces the blood vessel diameter.**

**(A)** Confocal immunofluorescence image of IB4 and GFAP at E18. The right is higher magnification images and 3D reconstructions of z-stacks of brain vessels and astrocytes. Scale bars, 100μm (left), 10μm (right).

**(B)** RT-PCR was performed to detect the mRNA levels of UCP2 in endothelial cells, NPC, neurons and astrocytes, ***P < 0.001 (mean ± SEM, unpaired two-tailed Student’s t test, n = 3 independent experiments).

**(C)** Confocal immunofluorescence image of IB4 and UCP2 in the UCP2<sup>WT</sup> and UCP2<sup>ECKO</sup> isolated brain endothelial cells. Scale bar, 50μm.

**(D)** Quantification of UCP2 relative expression and showing the expression of UCP2 was depleted in UCP2<sup>ECKO</sup> brain endothelial cells.***P<0.001(mean ± SEM, unpaired two-tailed Student’s t test, n =5 each group from 3 independent experiments).

**(E)** Western blot analysis of UCP2 expression levels in the UCP2<sup>WT</sup> and UCP2<sup>ECKO</sup> isolated brain endothelial cells. β-actin was detected as loading control.

**(F)** Confocal immunofluorescence image of IB4 and PDGFRβ in the UCP2<sup>WT</sup> and UCP2<sup>ECKO</sup> cortical sections at P2. The right magnification images showing a decreased mean diameter in UCP2<sup>ECKO</sup> cortical sections. Scale bars, 100μm (left),10μm (right).

**(G-I)** Quantification of the mean diameter (G), vessel length(H) and branch points(I), and showing a decreased mean diameter in UCP2<sup>ECKO</sup> cortical sections. *P<0.05, n.s., not significant (mean ± SEM, unpaired two-tailed Student’s t test, UCP2<sup>WT</sup> n =6 mice; UCP2<sup>ECKO</sup> n=7 mice).

**(J)** Quantification of the PDGFRβ<sup>+</sup>pericytes coverage showing no changes between
UCP2

\textit{UCP2}^{WT} and \textit{UCP2}^{ECKO} mice. n.s., not significant (mean ± SEM, unpaired two-tailed Student’s \textit{t} test, n = 4 mice each group).

Data are represented as means ± SEM. unpaired two-tailed Student’s \textit{t} test; At least three biological replicates are shown. n.s., not significant. *P<0.05, ***P<0.001.
Figure S2. Endothelial UCP2 deletion does not affect blood-brain barrier integrity.

(A) Confocal immunofluorescence image of IB4 and Claudin-5 in the $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 20μm.

(B) Quantification of the percent of Claudin-5$^+$ IB4$^+$ vessels showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, $n=4$ mice each group).

(C) Confocal immunofluorescence image of IB4 and ZO-1 in the $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 20μm.

(D) Quantification of the percent of ZO-1$^+$ IB4$^+$ vessels showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, $n=6$ mice each group).

(E) Confocal images of IB4 and Cad-A555 showing no cadaverine extravasation in $UCP2^{WT}$ and $UCP2^{ECKO}$ brain cortex from the blood vessels. Scale bar, 100μm.

(F) Confocal images of Col IV showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ brain cortex. Scale bar, 100μm.

(G) Quantification of the fluorescence intensity of Col IV staining showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, $n=4$ mice each group).

Data are represented as means ± SEM. unpaired two-tailed Student’s $t$ test; At least three biological replicates are shown. n.s., not significant. ***P<0.001.
Figure S3. Endothelial UCP2 deletion promotes astrocyte progenitor production.

(A) Confocal immunofluorescence image of SOX2$^+$ cells in E13 $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 50μm.

(B) Quantification showing that number of SOX2$^+$ precursor cells have comparable levels between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, $UCP2^{WT}$ n =6 mice; $UCP2^{ECKO}$ n=5 mice).

(C) Confocal immunofluorescence image of SOX2$^+$ cells in E16 $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 100μm.

(D) Quantification showing that number of SOX2$^+$ precursor cells have comparable levels between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, n=4 mice each group).

(E) Confocal immunofluorescence image of pH3$^+$ cells in E13 $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 50μm.

(F) Quantification showing that number of pH3$^+$ cells had comparable levels between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, n=4 mice each group).

(G) Confocal immunofluorescence image of BLBP$^+$ cells in E16 $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bars, 100μm.

(H) Quantification showing the increased number of BLBP$^+$ cells in $UCP2^{ECKO}$ mice. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s $t$ test, $UCP2^{WT}$ n =3 mice; $UCP2^{ECKO}$ n=4 mice).

(I) Western blot analysis of the expression levels of astrocyte progenitor marker
GLAST and BLBP. β-actin was detected as loading control.

(J) Statistics of relative intensity of GLAST and BLBP showing the increased expression of GLAST⁺ and BLBP⁺ cells in UCP²ECKO mice. *P<0.05, **P<0.01 (mean ± SEM, unpaired two-tailed Student’s t test, n = 4 mice each group).

Data are represented as means ± SEM. unpaired two-tailed Student’s t test; At least three biological replicates are shown. n.s., not significant. *P<0.05, **P<0.01, ***P<0.001.
Figure S4. Endothelial UCP2 deletion affects neuronal development.

(A) Confocal immunofluorescence image of NEUN⁺ and GFAP⁺ cells in E18 UCP2WT and UCP2ECKO mice. Scale bar, 100μm.

(B) Quantification showing decreased number of NEUN⁺ cells in UCP2ECKO mice. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, n=4 mice each group).

(C) Quantification showing increased number of GFAP⁺ cells in UCP2ECKO mice. **P<0.01 (mean ± SEM, unpaired two-tailed Student’s t test, UCP2WT n =5 mice; UCP2ECKO n=4 mice).

(D) Confocal immunofluorescence image of Olig2⁺ cells in E18 UCP2WT and UCP2ECKO mice. Scale bar, 100μm.

(E) Quantification of the number of Olig2⁺ cells showing no statistical difference between UCP2WT and UCP2ECKO mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s t test, n =4 independent experiments).

(F) Confocal immunofluorescence image of SATB2⁺ and CTIP2⁺ cells in E18 UCP2WT and UCP2ECKO mice. Scale bar, 100μm.

(G) Quantification showing decreased number of SATB2⁺ cells in UCP2ECKO mice. **P<0.01 (mean ± SEM, unpaired two-tailed Student’s t test, n=5 mice each group).

(H) Quantification showing decreased number of CTIP2⁺ cells in UCP2ECKO mice. **P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, UCP2WT n =6 mice; UCP2ECKO n=5 mice).

(I) Western blot analysis of the expression levels of neuron marker TUJ1 and NEUN. β-actin was detected as loading control.
(J) Statistics of relative intensity of TUJ1 and NEUN showing decreased the expression of TUJ1+ and NEUN+ cells in UCP2<sup>ECKO</sup> mice. *P<0.05, **P<0.01 (mean ± SEM, unpaired two-tailed Student’s t test, TUJ1 n =4 independent experiments; NEUN n =4 independent experiments).

(K) Western blot analysis of the expression levels of synaptic marker p-CREB and synaptophysin at P2. β-actin was detected as loading control.

(L) Statistics of relative intensity of p-CREB and synaptophysin. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s t test, p-CREB n =3 mice each group; NEUN n =4 mice each group).

(M) Western blot analysis of the expression levels of synaptic marker p-CREB and synaptophysin at P8. β-actin was detected as loading control.

(N) Statistics of relative intensity of p-CREB and synaptophysin. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s t test, p-CREB n =4 mice each group; NEUN n =4 mice each group).

Data are represented as means ± SEM. At least three biological replicates are shown. unpaired two-tailed Student’s t test; n.s., not significant. *P<0.05, **P<0.01.
**Figure S5. Endothelial UCP2 deletion does not affect inflammatory response.**

(A) Confocal immunofluorescence image of TUNEL$^+$ cells in $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 100μm.

(B) Quantification of the number of TUNEL$^+$ cells showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, n =4 mice each group).

(C) Confocal immunofluorescence image of IBA1$^+$ and CD68$^+$ cells in $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. Scale bar, 100μm.

(D and E) Quantification of the number of IBA1$^+$ and CD68$^+$ cells showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, $UCP2^{WT}$ n =4 mice; $UCP2^{ECKO}$ n =5 mice).

(F) Western blot analysis of the expression levels of IL6 and IL1$\beta$. β-actin was detected as loading control.

(G) Statistics of relative intensity of IL6 and IL1$\beta$ showing no statistical difference between $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. n.s., not significant. (mean ± SEM, unpaired two-tailed Student’s $t$ test, n =4 mice each group).

Data are represented as means ± SEM. unpaired two-tailed Student’s $t$ test; n.s., not significant. n.s., not significant.
Figure S6. Deletion of UCP2 in endotheliocyte upregulates ERK1/2 signaling by ROS.

(A) Graphs showing oxygen consumption rate (OCR) in primary endothelial cells of $UCP^2_{WT}$ and $UCP^2_{ECKO}$. n.s., not significant. ***P<0.001 (mean ± SEM, unpaired two-tailed Student’s t test, n = 3 mice each group, Basal Res P=0.0002; ATP Production P=0.0009; Maximal Res P=0.001).

(B) Graphs showing extracellular acidification rate (EACR) in primary endothelial cells of $UCP^2_{WT}$ and $UCP^2_{ECKO}$. *P<0.05. (mean ± SEM, unpaired two-tailed Student’s t test, n = 3 mice each group).

(C) Confocal immunofluorescence image of ROS levels in primary endothelial cells treated with different concentration hydrogen peroxide (H$_2$O$_2$). Scale bar, 50µm.

(D) Quantification of the fluorescence intensity of ROS showing that ROS production also tends to increase with the increasing concentration of H$_2$O$_2$. (mean ± SEM, n = 3 independent experiments).

(E) Confocal immunofluorescence image of ROS levels in primary endothelial cells treated with different concentration UCP2 selective inhibitor genipin. Scale bar, 50µm.

(F) Quantification of the fluorescence intensity of ROS showing that ROS production also tends to increase with the increasing concentration of genipin. (mean ± SEM, n = 3 independent experiments).

(G) Representative images of EC mitochondrial morphology of $UCP^2_{WT}$ and $UCP^2_{ECKO}$ mice by Mito tracker red staining. A and B are higher magnification images. Scale bar, 5µm.
(H) Graphs showing mitochondrial length in EC. P=0.0089 (mean ± SEM, unpaired two-tailed Student’s t test, n = 6 from 3 independent experiments).

(I) Confocal immunofluorescence image of p-ERK1/2 in primary endothelial cells from $UCP2^{WT}$ and $UCP2^{ECKO}$ mice.

(J) Quantification of the fluorescence intensity of p-ERK1/2 showing increased p-ERK1/2 cells in primary endothelial cells from $UCP2^{WT}$ and $UCP2^{ECKO}$ mice. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, n =6 from 3 independent experiments).

(K) ELISA analysis by collecting supernatant in primary endothelial cells infected with control and CMA1 showing increased Ang II in primary endothelial cells infected with CMA1. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, n =5 each group from 3 independent experiments).

Data are represented as means ± SEM. unpaired two-tailed Student’s t test; At least three biological replicates are shown. n.s., not significant. *P<0.05, **P<0.01, ***P<0.001.
**Figure S7. CMA1 is downstream target of UCP2.**

(A) Confocal immunofluorescence image of CMA1 and IB4 showing that CMA1 was expressed in IB4-labeled brain vessels. Scale bar, 100μm.

(B) Confocal immunofluorescence image of CMA1 and IB4 showing that CMA1 was expressed in IB4-labeled brain endothelial cells. Scale bar, 50μm.

(C) Confocal immunofluorescence image of GFAP+ and MAP2+ cells of primary neural precursor cells co-cultured with endothelial cells infected with CMA1 and control. Scale bar, 50μm.

(D and E) Quantification of the percent of MAP2+ neurons and GFAP+ astrocytes showing decreased MAP2+ neurons and increased GFAP+ astrocytes in primary neural precursor cells co-cultured with endothelial cells infected with CMA1. **P<0.01 (mean ± SEM, unpaired two-tailed Student’s t test, n =5 each group from 3 independent experiments).**

(F) Western blot analysis of the expression levels of TUJ1, GFAP, p-STAT3 and STAT3. β-actin was detected as loading control.

(G) Statistics of relative intensity of TUJ1 and GFAP showing decreased the expression of TUJ1+ and the increased expression GFAP+ cells in primary neural precursor cells co-cultured with endothelial cells infected with CMA1. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, n =3 independent experiments).

(H) Statistics showing the ratio of p-STAT3 and STAT3 in primary neural precursor cells co-cultured with endothelial cells infected with CMA1. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, n =3 independent experiments).
(I) Confocal immunofluorescence images of GFAP and MAP2 of neural precursor cells after H2O or Ang II treatments. Scale bars, 50μm.

(J and K) Quantification of the percent of MAP2⁺ neurons and GFAP⁺ astrocytes showing decreased MAP2⁺ neurons and increased GFAP⁺ astrocytes in primary neural precursor cells after H2O or Ang II treatments. **P<0.01, ***P<0.001 (mean ± SEM, unpaired two-tailed Student’s t test, control n =5, Ang II n=4 from 3 independent experiments).

Data are represented as means ± SEM. unpaired two-tailed Student’s t test; At least three biological replicates are shown. *P<0.05, **P<0.01, ***P<0.001.
Figure S8. Ang II accelerates human NPCs differentiation toward astrocytes.

(A) Confocal immunofluorescence images of MAP2 and GFAP of human NPCs after H₂O or Ang II treatments. Scale bar, 50μm.

(B) Quantification of the percent of MAP2⁺ neurons showing decreased MAP2⁺ neurons in human NPCs after H₂O or Ang II treatments. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, control n = 7, Ang II n=4 from 3 independent experiments).

(C) Quantification of the percent of GFAP⁺ astrocytes showing increased GFAP⁺ astrocytes in human NPCs after H₂O or Ang II treatments. *P<0.05 (mean ± SEM, unpaired two-tailed Student’s t test, n = 7 each group from 3 independent experiments).

(D) Model showing how endothelial UCP2 regulates the neurogenic-to-astrogenic fate switch through Ang II in the developing cortex.

Data are represented as means ± SEM. unpaired two-tailed Student’s t test; At least three biological replicates are shown. *P<0.05.