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Supplemental information

**Mild hypothermia promotes neuronal
differentiation of human neural stem
cells via RBM3-SOX11 signaling pathway**

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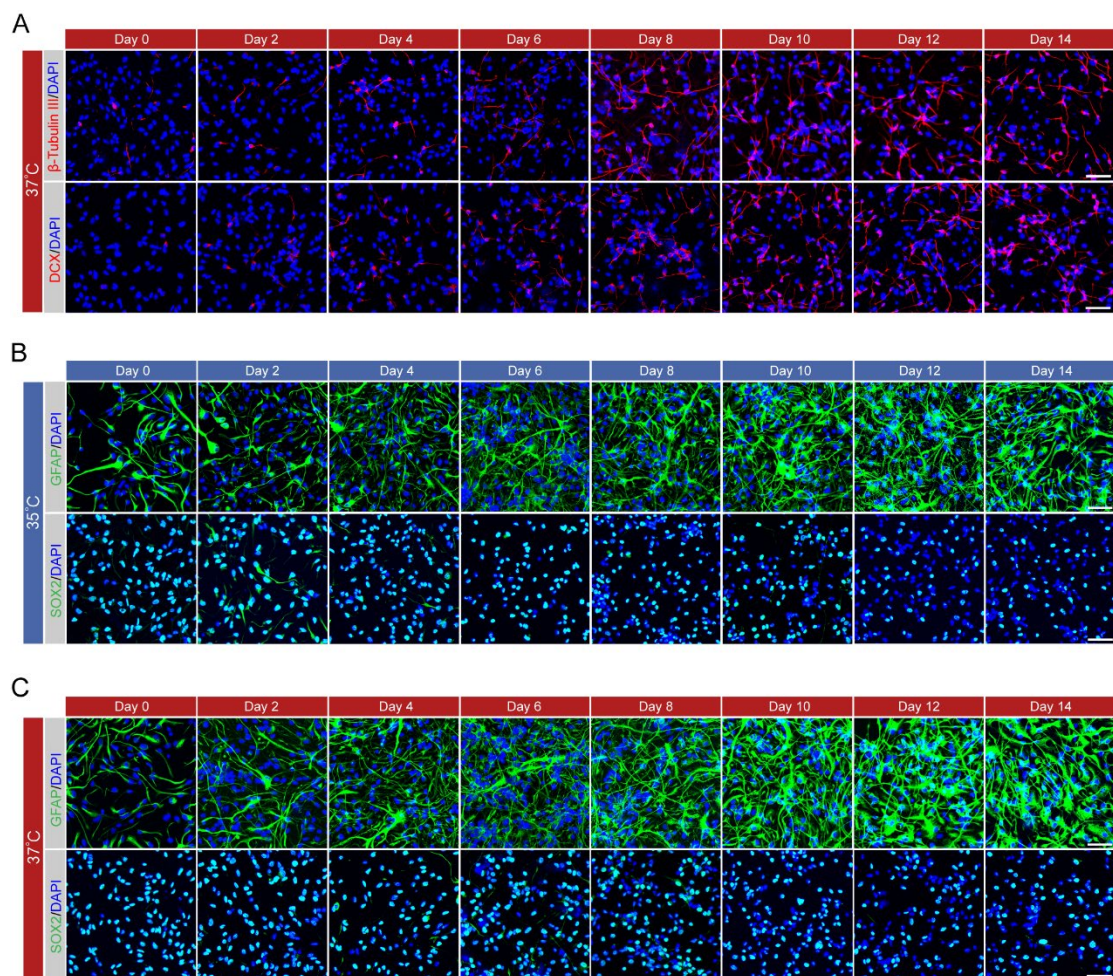


Figure S1. The timeline of neuronal and glial differentiation of cultured hNSCs with 35°C- and 37°C-treatment, related to Figure 1.

(A) Representative immunofluorescence images showing β -Tubulin III and DCX positive hNSCs from Day 0 to Day 14 every two days with 37°C-treatment. (B-C) Representative immunofluorescence images showing GFAP and SOX2 positive hNSCs from Day 0 to Day 14 every two days with 35°C- and 37°C-treatment. Scale bars represent 50 μ m in (A-C).

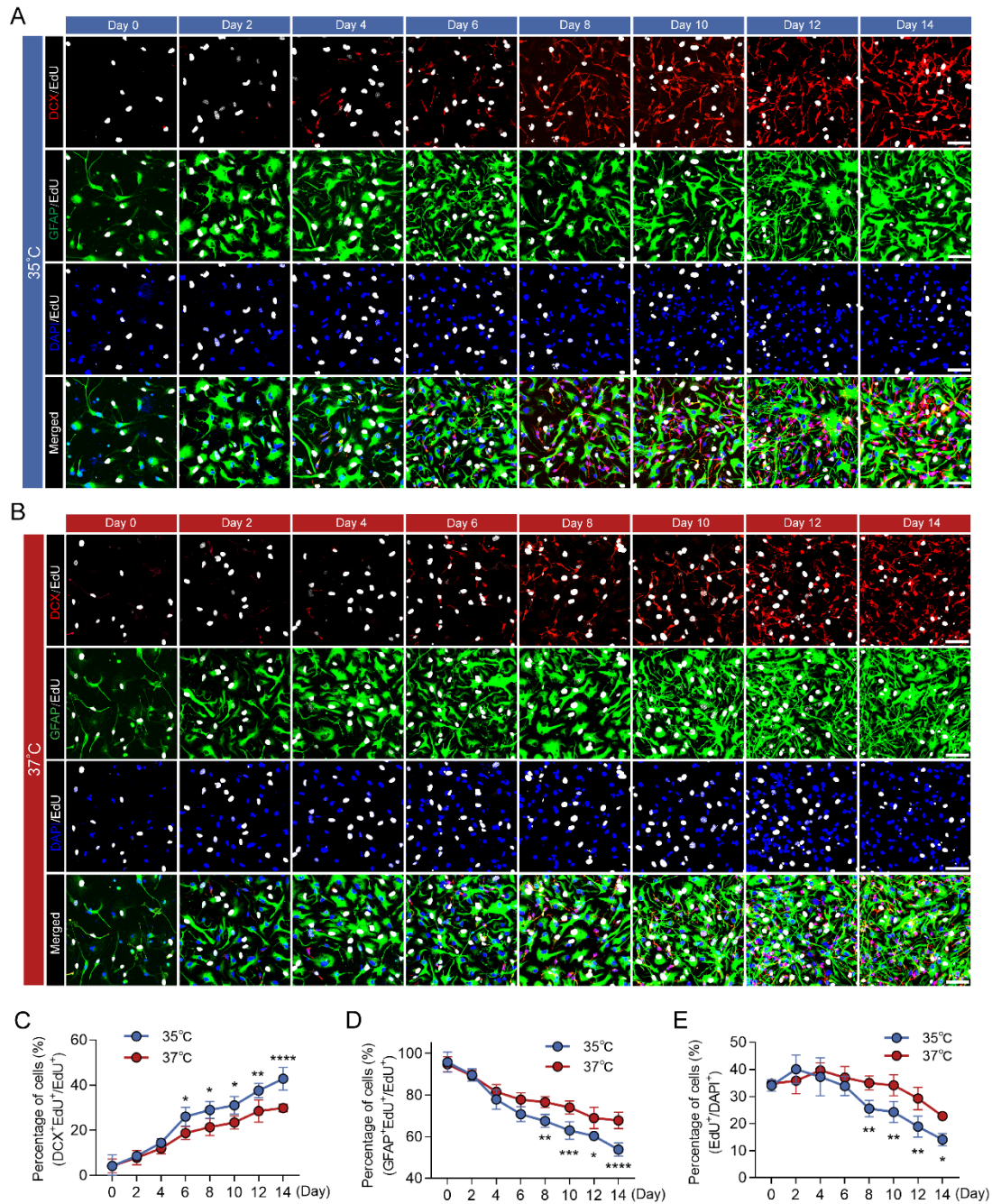


Figure S2. Mild hypothermia of 35°C induced newborn neurons and repressed the proliferation of differentiated hNSCs, related to Figure 1.

(A-B) Representative immunofluorescence images showing DCX, GFAP and EdU positive hNSCs from Day 0 to Day 14 every two days with 35°C- and 37°C-treatment. (C) The percentage of DCX and EdU positive cells to EdU positive cells in (A-B). (D) The percentage of GFAP and EdU positive cells to EdU positive cells in (A-B). (E) The percentage of EdU positive cells in (A-B). All data presented as mean \pm SD. Two-way

ANOVA tests were used in (C-E). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.
Scale bars represent 50 μm in (A-B).

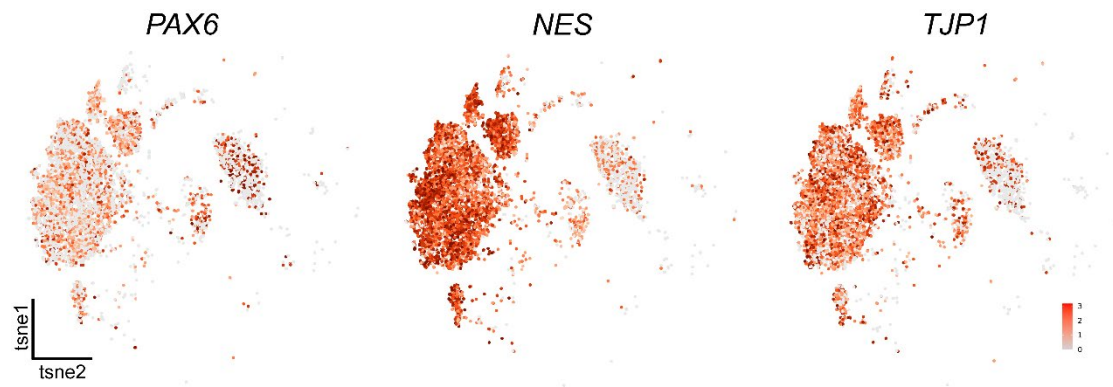


Figure S3. Single-cell RNA sequencing results of hNSCs at Day 0, related to Figure 2.

The tSNE plots of single-cell RNA sequencing showing the *PAX6*, *NES*, *TJP1* genes expression pattern.

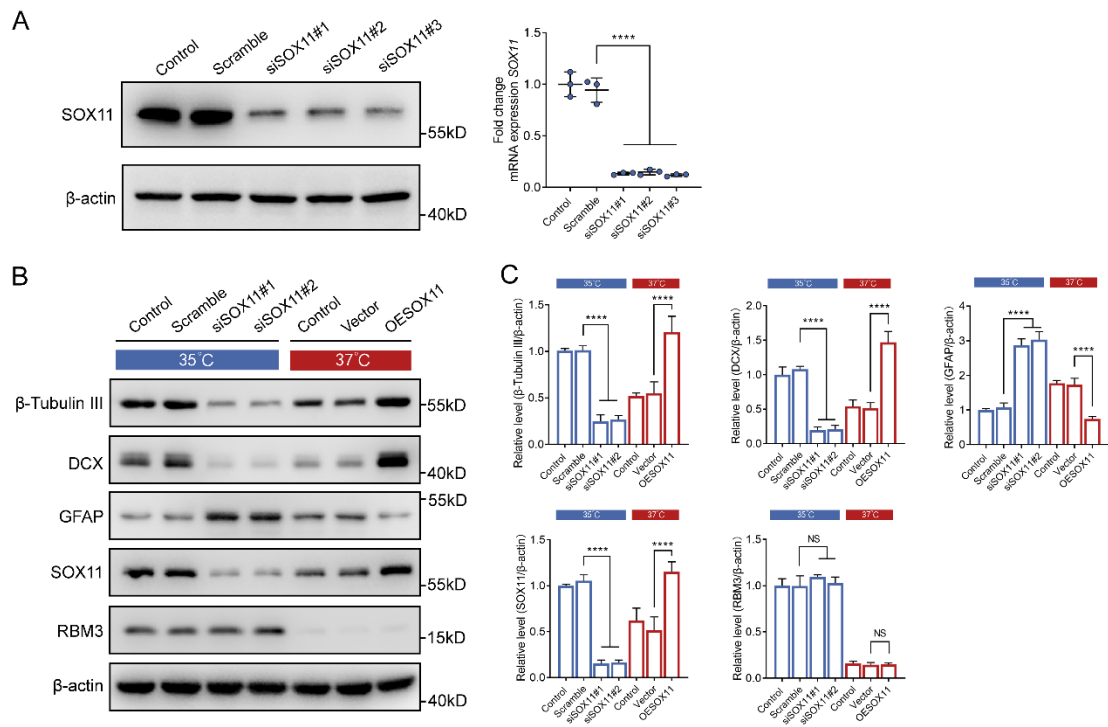


Figure S4. SOX11 promoted neuronal differentiation of hNSCs, related to Figure 4.

(A) Western blots and qRT-PCR analysis showing the protein and mRNA expressions of SOX11 after scramble or RBM3 siRNA transfection. β -actin was used as the loading control. (B) Western blots analysis showing β -Tubulin III, DCX, GFAP, SOX11 and RBM3 protein expression at Day 14. (left 4 lanes) hNSCs were transfected with scramble or RBM3 siRNA with 35°C-treatment. (right 3 lanes) hNSCs were transfected with vector or RBM3 by lentivirus with 37°C-treatment. β -actin was used as the loading control. (C) The quantification of Western blots of (B). Normalized β -Tubulin III, DCX, GFAP, SOX11 and RBM3 to corresponding loading control were summarized for three independent trials. All data presented as mean \pm SD. One-way ANOVA tests were used in (A) and (C). NS, not significant; **** $p < 0.0001$.

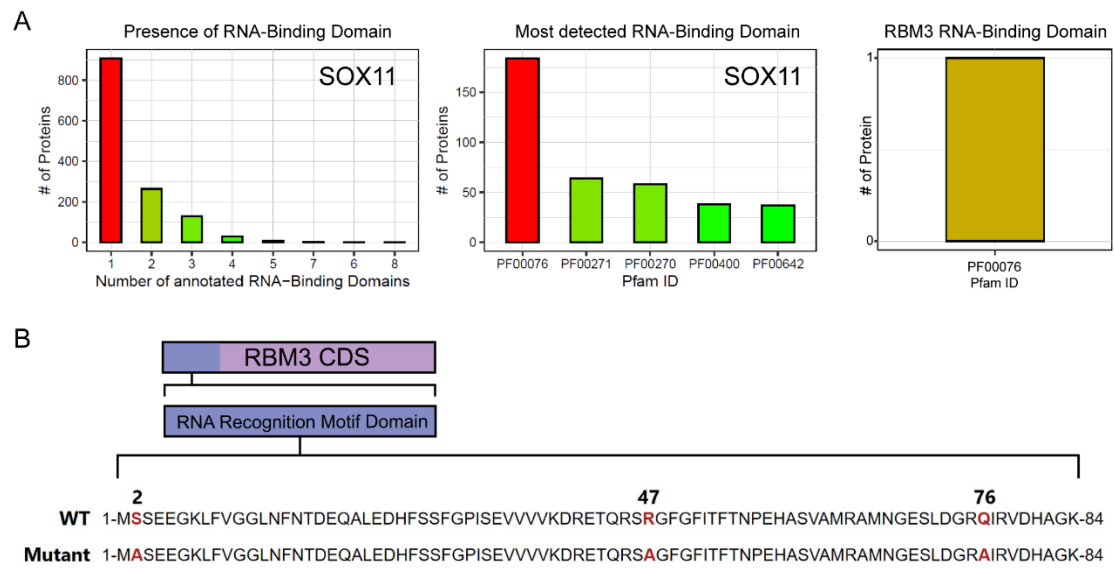


Figure S5. RBM3 binds to mRNA through RNA recognition motif domain, related to Figure 5.

(A) The predicted binding domain to SOX11 mRNA and of RBM3 by transcripts VS RNA-binding proteome analysis. (B) The amino acid sequence and mutation sites of RNA recognition motif domain of RBM3.

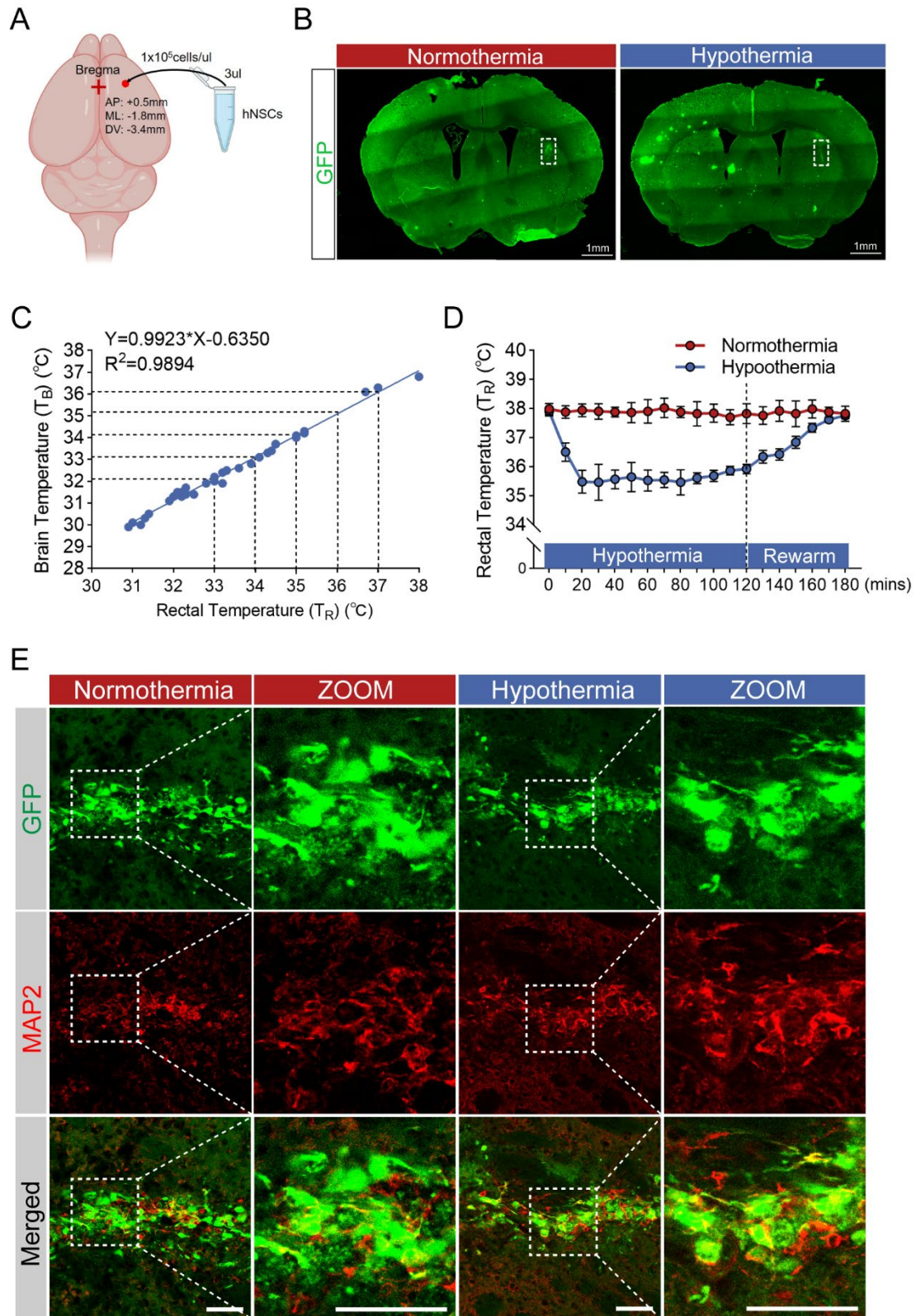


Figure S6. Mild hypothermia of 35°C regulated transplanted hNSCs differentiation into neurons *in vivo*, related to Figure 6.

(A) The transplantation schematic of hNSCs to the mouse brain. **(B)** The transplanted

region of GFP-labeled hNSCs. The GFP-labeled hNSCs that were transplanted are located in the rectangle region. **(C)** The relationship of mouse brain temperature and rectal temperature. **(D)** The rectal temperature during different period of normothermia and mild hypothermia. **(E)** Representative immunofluorescence images of MAP2 positive GFP-labeled cells at Day 14 with normothermia and mild hypothermia treatments. All data presented as mean \pm SD. Scale bars represent 1mm in **(B)** and 50 μ m in **(E)**.