Supplementary materials for

Shh and Olig2 sequentially regulate oligodendrocyte differentiation from hiPSCs for the treatment of ischemic stroke

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### Table S1. Detailed composition of culture medium

| Media | Components | Provider | Final con. |
|-------|------------|----------|------------|
| NIM1  | DMEM/F12   | Life Technologies | 50%        |
|       | Neurobasal | Gibco     | 50%        |
|       | B27 Supplement (50X) | Stem cell technology | 1X        |
|       | N2 Supplement (100X) | Stem cell technology | 1X        |
|       | Recombinant hLIF | PEPROTech | 10 ng/ml   |
|       | SB431542 | Med Chem Express | 2 μM        |
|       | CHIR99021 | Med Chem Express | 3 μM        |
|       | GlutaMAX | Life Technologies | 2 mM        |
|       | Compound E | Med Chem Express | 0.1 μM      |
|       | Dorsomorphin | Med Chem Express | 2 μM        |
| NIM2  | DMEM/F12   | Life Technologies | 50%        |
|       | Neurobasal | Gibco     | 50%        |
|       | B27 Supplement (50X) | Stem cell technology | 1X        |
|       | N2 Supplement (100X) | Stem cell technology | 1X        |
|       | Recombinant hLIF | PEPROTech | 10 ng/ml   |
|       | SB431542 | Med Chem Express | 2 μM        |
|       | CHIR99021 | Med Chem Express | 3 μM        |
|       | GlutaMAX | Life Technologies | 2 mM        |
|       | Compound E | Med Chem Express | 0.1 μM      |
| NSMM  | DMEM/F12   | Life Technologies | 50%        |
|       | Neurobasal | Gibco     | 50%        |
|       | B27 Supplement (50X) | Stem cell technology | 1X        |
|       | N2 Supplement (100X) | Stem cell technology | 1X        |
|       | Recombinant hLIF | PEPROTech | 10 ng/ml   |
|       | SB431542 | Med Chem Express | 2 μM        |
|       | CHIR99021 | Med Chem Express | 3 μM        |
|       | GlutaMAX | Life Technologies | 2 mM        |
| GIM          | DMEM/F12                  | Gibco         |
|--------------|---------------------------|---------------|
| B27 supplement lacking vitamin A (50X) | Stem cell technologies | 1X            |
| N2 Supplement (100X) | Stem cell technologies | 1X            |
| penicillin/streptomycin   | Gibco                     | 1%            |
| SAG           | Med Chem Express          | 1 μM          |
| PDGF-AA       | R&D Systems               | 10 ng/ml      |
| NT-3          | Millipore                 | 10 ng/ml      |
| IGF-I         | R&D Systems               | 10 ng/ml      |
| AA            | Sigma–Aldrich             | 200 μM        |
| T3            | Sigma–Aldrich             | 60 ng/ml      |
| DM            | DMEM/F12                  | Gibco         |
| B27 supplement lacking vitamin A (50X) | Stem cell technologies | 1X            |
| N2 Supplement (100X) | Stem cell technologies | 1X            |
| penicillin/streptomycin   | Gibco                     | 1%            |
| NT-3          | Millipore                 | 10 ng/ml      |
| IGF-I         | R&D Systems               | 10 ng/ml      |
| AA            | Sigma–Aldrich             | 200 μM        |
| T3            | Sigma–Aldrich             | 60 ng/ml      |
| dbcAMP        | Sigma–Aldrich             | 100 μM        |
Table S2. Primary antibodies used for immunostainings

| Antigen         | Dilution | Reference                  |
|-----------------|----------|----------------------------|
| Olig2           | 1/100    | Rabbit IgG ab254043        |
| NESTIN          | 1/3200   | Mouse IgG CST#33475        |
| SOX2            | 1/400    | Mouse IgG CST#3579         |
| O4              | 1/500    | Mouse IgM R&D MAB1326      |
| O4-APC          | 1/50     | Mouse IgG Miltenyi Biotec 130-118-978 |
| NG2             | 1/500    | Rabbit IgG ab129051        |
| NG2-PE          | 1/200    | Rabbit IgG C06035P         |
| PDGFRa          | 1/1000   | Rabbit IgG CST#3174        |
| PDGFRa-APC      | 1/50     | Mouse IgG1 BioLegend #323512 |
| MBP             | 1/50     | Rabbit IgG CST#78896       |
| GFAP            | 1/400    | Mouse IgG G-3893           |
| Islet1          | 1/50     | Rabbit IgG ab20607         |
| beta III Tubulin(TUJ1) | 1/1000 | Rabbit IgG ab18207          |
| hNA(HuNu)       | 1/200    | Mouse IgG MAB1281          |
| NeuN            | 1/3200   | Rabbit IgG CST#24307       |
| A2B5            | 1/500    | Mouse IgM Invitrogen#433110 |
| SOX10           | 1/1000   | Rabbit IgG ab264405        |
| p-OLIG2 (S147)  | 1/500    | Rabbit IgG Bioworld AP0734 |
| CEPT1           | 1/500    | Rabbit IgG 20496-1-AP      |
| GAPDH           | 1/5000   | Mouse IgG ab8245           |
| β-actin         | 1/2000   | Rabbit IgG ab8227          |
| Gene name | Species | Sequence of primers |
|-----------|---------|---------------------|
| β-actin   | Human   | Forward: CCAGAGCCCCGTAGACCTTTTT<br>Reserve: CACTGCCCTCTAGCTTGCTCC |
| Olig2     | Human   | Forward: GCCCTGTATGTGAGGCAA<br>Reserve: TCATCAAGAAATGTCGACG |
| CSPG4     | Human   | Forward: CTTTGACCCTGACTATGGTG<br>Reserve: TGCAGGCGTCAGAGTATA |
| PDGFRα    | Human   | Forward: TGGCAAGTACCCCATGTCTGAA<br>Reserve: CCAAGACGTCACAAAGAGGC |
| ST8SIA1   | Human   | Forward: GTCTCTGTTGCGCTACATCT<br>Reserve: CCCCCTCATACCAACTGCTC |
| SOX10     | Human   | Forward: CTTTGACCCTGACTATGGTG<br>Reserve: TGCAGGCGTCAGAGTATA |
| NKK2.2    | Human   | Forward: TGGCAAGTACCCCATGTCTGAA<br>Reserve: CCAAGACGTCACAAAGAGGC |
| PAX6      | Human   | Forward: GCCTCGTATGTGAGGCAA<br>Reserve: TCATCAAGAAATGTCGACG |
| NESTIN    | Human   | Forward: GGGCTCTGATCTTCATCCTAC<br>Reserve: CCAAGACGTCACAAAGAGGC |
| NANOG     | Human   | Forward: TTGTGCTGCTGAAGAAACT<br>Reserve: CACCCCTCATATACCAACTG |
| POU5F1    | Human   | Forward: CTGGTTGATCTCCTGACCT<br>Reserve: CCAAGACGTCACAAAGAGGC |
| PLP1      | Human   | Forward: ACCTATGCCCTGAGCCTTG<br>Reserve: TGGGAGGAGGGCAATAGACT |
| NNG2      | Human   | Forward: AGGAAGAGGACGTGTTATG<br>Reserve: GCAATCTGATACAGACCCAG |
| SOX9      | Human   | Forward: AGCGAAGCGCACAATCAAGAC<br>Reserve: CCCTAAGGGGAAACAAGATTTG |
| HB9       | Human   | Forward: CTCTACTCTCTGCAAGGAG<br>Reserve: TGGGGTCCTAGAACACAAAG |
| SMARCA4   | Human   | Forward: GCCAGCAACTCCCAAGTTAC<br>Reserve: CCAAGGAGGGCAATAGACT |
| CEPT1     | Human   | Forward: ATGTTGGAGATCTCTACCCGGA<br>Reserve: TCTCTCTGCCCTTTGTTG |
| CHPT1     | Human   | Forward: CACCCAGAAGGGCCACATATG<br>Reserve: GGGGTTGCTGCTGTAAGT |
| PPARG     | Human   | Forward: GGGATCGCTCTGATGAA<br>Reserve: CGATGGGTACGCAGAAAATCCTG |
| PPARD     | Human   | Forward: GGGGGTGTGGATGAGG<br>Reserve: CCAAGCAGGAATGTGAGG |
| PPARA     | Human   | Forward: AGGCAAGCGACACATCAAGAC<br>Reserve: CCCTAAGGGGAAACAAGATTTG |
| MBP       | Human   | Forward: GGGGAGGAGGGCAATAGACT<br>Reserve: TGGGGTCCTAGAACACAAAG |
| MOG       | Human   | Forward: GCCAGCAATGGAATTTGGAAGG<br>Reserve: TGGGGTCTTGAACAACCAG |
| MAG       | Human   | Forward: GGTGTCCTGACCTCCATGCC<br>Reserve: TGGGGTCTTGAACAACCAG |
| BDNF      | Rat     | Forward: GCTGCTGATGAGGACCAG<br>Reserve: GCTGCTGATGAGGACCAG |
| β-actin   | Rat     | Forward: GCCCTCTCTTGGATAT<br>Reserve: GGGCATAGAGGTCCTTACCG |

Table S3. Sequence of primers for qPCR analysis.
Table S4. Sequences of shRNA assays.

| RNAi Name     | Species Specificity | Target sequences          |
|---------------|---------------------|---------------------------|
| CEPT1 shRNA1  | Human               | 5’- ACTGTAGCAGGGACCATATTT-3’ |
| CEPT1 shRNA2  | Human               | 5’- GGCACTCTCTGTGGGCATATAT-3’ |
| CEPT1 shRNA3  | Human               | 5’- TGGTAACACGCCCTAACTATC-3’ |
| Scramble shRNA| Human               | 5’-GATCTCGCTTGGGCGAGGATGAA-3’ |
Supplementary Figures

Figure S1 Establishment of hiPSC cell lines with induced expression of Olig2. A Schematic diagram of Tet-inducible Olig2 expression in hiPSCs. B qPCR analysis of Olig2 mRNA expression levels with treated or untreated doxycycline after 24 h (1 µg/mL) (n = 3, *** p < 0.001, by a two-tailed Student’s t test). C Western blot for Olig2 expression with treated or untreated doxycycline after 24 h. mRNA and protein levels were normalized to the housekeeping gene β-actin. Graphs represent the individual data points and the mean ± SEM of three independent experiments.
**Figure S2** A Diagram of hiPSCs differentiated into NPCs and OPCs/OLs with untreated or treated GANT61, followed by induction of Olig2 expression. B qPCR analysis of *SOX10* mRNA expression at day 14 of differentiation (n = 3, *** p < 0.001). Graphs represent the individual data points and the mean ± SEM of three independent experiments.
**Figure S3** Adherent hiPSC (A) colonies treated with NIM1 for 2 days, followed by NIM2 for another 5 days, generated neural tube-like structures (B). The cultured cells were dissociated as single cells in six-well plates precoated with Matrigel. Confluent GANT61-NPCs (passage 2) were cultivated in adherent monoculture and maintained typical neural crest morphology during *in vitro* culture (C). **D** Representative immunofluorescent staining images of Olig2, NG2, SOX10, PDGFRα, and A2B5 in Olig2 cultures at d4 after Olig2 induction (scale bar, 50 μm). **E** Representative immunofluorescent staining images of PDGFRα in Olig2 cultures at d7 (scale bar, 100 μm).
Figure S4 A The relative mRNA expression of GANT61-NPCs makers (PAX6, NESTIN) and pluripotency genes (OCT4, NANOG) in undifferentiated hiPSCs were detected by qPCR (n = 3, ** p < 0.01, *** p < 0.001, by a two-tailed Student’s t test). 
B qPCR analysis for the OL-specific lineage maker genes SOX10, NKX2.2 and SOX9 mRNA expression level (ns, not significant, p > 0.05).
Figure S5  

A Representative images for Olig2-induced OLs (scale bar, left, 100μm; right, 50 μm).  

B by d21, the Olig2-induced OLs coexpressed O4-epitope, the more mature OL marker, MBP (scale bars, 50 μm).  

C Quantification of O4+ mature OL yields at d14 and d28 after Olig2 transduction (n = 3, *** p < 0.001).
Figure S6. A KEGG pathway enrichment analysis of the Olig2/control-OPC-related mRNA-based RNA-seq data. The PPAR signaling pathway was highly enriched in Olig2-OPCs, and \( p < 0.05 \) was used as the threshold to select KEGG terms. B RNA-seq data indicated that the \( GPR17 \) mRNA expression level was significantly downregulated in Olig2 OPCs \((n = 6, \ * p < 0.05, \) by a two-tailed Student’s t test).
Figure S7  A qPCR analysis of *CEPT1* knockdown efficiency (n = 3, *** p < 0.001, by a two-tailed Student’s *t* test).  B Western blot analysis of *CEPT1* knockdown efficiency. Proteins were normalized to GAPDH.
Figure S8  A Gating strategies to purify NG2+ OPCs for RNA-seq and cell transplantation. B Gating strategies to analyze the expression of the OPC-specific surface protein markers NG2, PDGFRa, and O4 in control-OPCs and Olig2-OPCs. C Gating strategies to analyze the coexpression of OPC-specific surface markers NG2 or PDGFRa in control-OPCs and Olig2-OPCs.
Figure S9 qPCR analysis for the mRNA expression level of *OLIG1* at 1 weeks after Olig2 induction. Olig2 overexpression did not affect the mRNA expression of *OLIG1* (n = 3, ns p > 0.05, by a two-tailed Student’s t test).