Supplementary Information

Quiescence of adult oligodendrocyte precursor cells requires thyroid hormone and hypoxia to activate Runx1

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Supplementary Figure S1  Immunocytochemistry of P7 rat OPCs cultured with TH in 20%, 3% or 1.5% O₂.

Cells cultured with TH in 20%, 3% or 1.5% O₂ for 10 days were labeled with DAPI (blue), anti-GC antibody (green), and A2B5 antibody (red). Scale bar: 100 µm.
Supplementary Figure S2  The delayed timing of the TH-dependent OL differentiation in 3% O₂ culture.
(a) Clonal analysis of P7 OPCs cultured at clonal-density in PDGF in 3% O₂, with TH (black bars) or without TH (white bars). After 11 days, the number of cells in each clone was counted and the number of cell divisions was estimated (n = 3). (b) P7 OPCs were cultured with TH in 3% O₂ for 10 days and then removed and re-cultured at clonal-density in the same conditions for another 10 days; the clones were then assessed as in (a-c) (n = 3). (c) Cells in (b) were stained with the nuclear stain DAPI (blue) and a monoclonal anti-GC (green) or A2B5 (red) antibody (Scale bar: 100 µm), and (d) the % of the DAPI⁺ cells that were GC⁺ or A2B5⁺ was determined. White bars; without TH (-TH), Black bars; with TH (+TH). P* < 0.01, P** < 0.01 (unpaired Student’s t-test, n = 3). Note that most of the cells cultured in TH become GC⁺ and A2B5⁺ OLs, whereas most of those cultured without TH remained GC⁻, A2B5⁺ OPCs.
Supplementary Figure S3  Confirmation of TH-dependent gene up-regulations and TH-dependent protein accumulations in adult-like OPCs.

a. To confirm the TH-dependent gene up-regulation of Klf9, Hif2α, Runx1 and p15/INK4b, a qRT-PCR assay was carried out. Total RNA was prepared from P7 rat optic nerve OPCs cultured with or without TH in 1.5% O₂ for 15 days. The resulting values were normalized to the endogenous control gene GAPDH. Results of TH-stimulated cells are presented as the relative expression to those of the non-stimulated cells. Hif1α and beta-actin are also shown as negative controls. b. To confirm the TH-dependent accumulation of Runx1 protein and p15/INK4b protein in OPCs, P7 rat optic nerve OPCs were cultured with or without TH in 1.5% O₂ for 15 days, and a western blot analysis was carried out. The detecting primary antibodies were anti-Runx1 mouse monoclonal antibody (sc-365644, Santa Cruz) and anti-p15/INK4b (M-20) goat polyclonal antibodies (sc-1429, Santa Cruz). Same membrane was re-blotted with anti-GAPDH rabbit monoclonal antibody (#2118, Cell Signaling Technology) for internal control. -TH; non-stimulated OPCs, +TH; TH-stimulated OPCs. By the addition of TH, Runx1 protein increased to 1.4 folds and p15/INK4b protein increased to 9.8 folds. The methods: The imaging of the western blotting sheets was carried out by using an Image Quant LAS 4000(GE Health Care). The quantification of each band was carried out by using ImageQuant TL software (GE
Health Care) and with the sequential procedures as mentioned below. (1) Draw a rectangle box around the band of interest (Box A). (2) Copy the rectangle box and paste it on just above or below the area where without any bands in the correspond lane (Box B). (3) Measure the area intensities of both Box A and Box B. (4) To calculate the intensity of the band of interest, subtract the value of Box B (as the background) from that of Box A.
Supplementary Figure S4  The gene silencing efficiencies of anti-Runx1 siRNA, anti-Hif1α siRNA and anti-Hif2α siRNA, and anti-p15/INK4b siRNA.

a. Human HEK293 cells were co-transfected with either 5 µg of target gene expressing
vector DNA (recombinant retrovirus vector plasmid DNA containing cDNA of each gene described in b) or 5 µg of empty vector DNA (pRetroX-IRES-ZsGreen1 retrovirus vector plasmid DNA, Clontech) and 200 pmol of siRNA against to each gene or non-target siRNA (shown in Supplementary Table 4). The cells were then cultured for 20 hours in 20% O₂. After harvesting, cell lysates were prepared and a series of western blotting analysis were carried out. The detecting primary antibodies were anti-Runx1 mouse monoclonal antibody (sc-365644, Santa Cruz), anti-p15/INK4b (M-20) goat polyclonal antibodies (sc-1429, Santa Cruz), anti-Hif1α rabbit polyclonal antibodies (NB100-479, Novus Biologicals) and anti-Hif2α rabbit polyclonal antibodies (NB100-122, Novus Biologicals). Same membrane was re-blotted with anti-tubulin rabbit polyclonal antibodies (3708-100, BioVision) for internal control. Lane 1; cells transfected with empty vector DNA, lane 2; with target gene expressing vector DNA, lane 3; with siRNA for each target genes and target gene expressing vector DNA, lane 4; with non-target siRNA and target gene expressing vector DNA. Black arrows show the positions of the band of the protein of target genes. Note; because of the cross reactivities of primary antibodies against the human homologues, the bands of Hif1α and Hif2α proteins appeared in the samples prepared from cells transfected with empty vector DNA (lane 1). The inhibitory efficiencies of protein expressions of each siRNA were; 52.6 ± 11.3% (n = 3) for anti-Hif1α siRNA, 53.7 ± 16.9% (n = 3) for anti-Hif2α siRNA, 55.8 ± 9.6% (n = 3) for anti-Runx1 siRNA, 68.6 ± 15.9% (n = 3) for anti-p15/INK4b siRNA. b. Structure of pINK4b-IRES-ZsGreen recombinant plasmid DNA and the efficiency of siRNA mediated gene silencing of p15/INK4b. Full length cDNA of rat p15/INK4b was cloned into NotI-ClaI site of the multiple cloning sites in pRetroX-IRES-ZsGreen1 vector plasmid DNA. The target sites of anti-p15/INK4b siRNA (Supplementary Table 4) are shown. LTR: long terminal repeat, SD: splice donor site, SA: splice acceptor site, Ψ: packaging signal, MCS: multiple cloning site, IRES: encephalomyocarditis virus derived internal ribosome entry site, ORF: open reading frame.

For the Construction of retrovirus vector plasmid DNA, 1.5 kb of p15/INK4b cDNA fragment, 0.8 kb of Klf9 cDNA fragment and 1.4 kb of Runx1b isoform cDNA fragment were amplified by PCR from rat OPC cDNA library. The forward primer for p15/INK4b was 5'-GGGGCGGCCGCATGTTGGGCGGCGAG-3', and the reverse primer for p15/INK4b was 5'-TATATCGATTTAAAAAGGACAAGCACTGTAATAC-3'.
The forward primer for Runx1b isoform cDNA was 5'-GGGGCGGCCGCTGATCCTCCCCGCATGATGC-3', and the reverse primer for Runx1b isoform was 5'-CCATCGATTCAGTTAGGGCCGCCGAC-3'. The forward primer for Hif1α was 5'-GCCGCGGCCGACCCAGCACTCGGATC-3', and the reverse primer for Hif1α was 5'-CCATCGATGTAGTTAGTTAGGACCCACACTAC-3'. The forward primer for Hif2α was 5'-GTAGCGCGCCCGACGGGTAGAAATGACCCAC-3', and the reverse primer for Hif2α was 5'-CCATCGATCCCTGGCTCAGGTGG-3'. The forward primer for Klf9 was 5'-CCCGCGGCCGCAAATGTCACGCGGC-3', and the reverse primer for Klf9 was 5'-CCATCGATCTGACAGGAAACCAACTAC-3'. The PCR reaction condition was same as RT-PCR. To construct pINK4b-IRES-ZsGreen plasmid DNA, the double strand PCR product was digested with restriction enzymes NotI and ClaI and then subcloned into the NotI-ClaI site in the multiple cloning sites of the pRetroX-IRES-ZsGreen1 retrovirus vector plasmid DNA. TOP10 chemically competent E. coli cells (Invitrogen) were transformed with recombinant plasmid DNA and cultured in LB medium containing 50 µg/ml of ampicillin at 37°C. Purification of plasmid DNA was carried out using an EndoFree Plasmid Maxi kit (QIAGEN) following the supplier's instructions. The nucleotide sequence was confirmed using a BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems).
Supplementary Figure S5  Overexpression of Klf9 in P7 rat OPCs under hypoxia.
Freshly prepared P7 rat OPCs were infected with a Klf9-IRES-ZsGreen expressing retrovirus vector were cultured at clonal-density in 1.5% O₂ and PDGF without TH for 7 days. The ZsGreen⁺ cells are bipolar. Left panel; phase contrast, right panel; ZsGreen⁺ (green). Scale bar: 50 µm.
**Supplementary Figure S6  Klf9 or Runx1 over expressing cells are A2B5⁺.**
P7 rat OPCs infected with retrovirus vector Klf9-IRES-ZsGreen (a) or Runx1-IRES-ZsGreen (b) were cultured without TH in 1.5% O₂ for 2 days and stained with A2B5 monoclonal antibody (red) and DAPI (blue) for nuclear staining. ZsGreen protein expressing retrovirus vector infected cells are shown in green. Scale bars: 100 µm.
Supplementary Figure S7  TH activates the P2 promoter of Runx1 gene in hypoxic OPCs.

P7 rat OPCs were cultured in PDGF, with or without TH in 1.5% O₂ for 12 days. Total RNA was prepared from these cells and a qRT-PCR assay was carried out. The resulting values were normalized to the endogenous control gene Gapdh. Results of TH-stimulated cells are presented as the relative expression to those of the non-stimulated cells using the ∆∆Ct methods. GAPDH; Gapdh specific PCR primer set, P1; Runx1 transcripts derived from P1 promoter (Runx1c) specific PCR primer set, P2; Runx1 transcripts derived from P2 promoter (Runx1b) specific primer set, Runx1; Common PCR primer set for Runx1c and Runx1b transcripts. P* <0.005, P** < 0.001 (ANOVA with Fisher’s LSD test, n = 3).

Supplementary Figure S8  Hifα inhibitors chetomin and CAY10585 induce cell death of OPCs under hypoxia.

P7 rat OPCs were cultured in PDGF without TH in 1.5% O₂ for 8 days. Then the medium was replaced to the TH containing medium. Cells were administrated with chetomin (2 μM) or CAY10585 (10 μM). For a control, 0.1% of DMSO was also added to the cultures. Cells were cultured for 24 hours and stained with nuclear staining dyes PI (2.5 μg/ml) and Hoechst 33342 (5 μg/ml). The number of cells was counted and PI⁺/Hoechst 33342⁺ double positive cells were considered as dead. In chetomin or CAY10585 containing culture, over 90% of OPCs died within 24 hours. P* < 0.01, P** < 0.001 (ANOVA with Fisher’s LSD test, n = 3).
**Supplementary Figure S9** *In vivo* labeling of optic nerve OPCs with pimonidazole.

(a) Pimonidazole was injected intraperitoneally into P7 rats or P14 rats. And after 2 hours, OPCs were purified from the optic nerves and labeled with a mouse monoclonal anti-pimonidazole antibody (*green*), rabbit anti-Ki-67 polyclonal antibodies (*red*) and DAPI (*blue*). Panels show the staining of P14 OPCs, white arrows show Pimo⁺/Ki-67⁻ cells; scale bar: 100 µm. (b) Cell count data for **Figure 7d**. Pim⁺: anti-pimonidazole antibody positive, Ki-67⁺: anti-Ki-67 antibody positive.
Supplementary Figure S10  The deceleration of cell cycle of OPCs in PDGF in 1.5% O₂ depends on in either TH or RA.

(a and b) P7 rat OPCs purified from optic nerves were cultured at clonal-density in PDGF, with either TH (black bars) or RA (1 ng/ml; gray bars) in either 20% O₂ (a) or 1.5% O₂ (b) for 13 days, and the number of cell divisions in each clone was estimated. (c) The phase-contrast images of P7 rat OPCs cultured for 13 days in 1.5% O₂ with TH (+TH; left panel) or RA (+RA; right panel) are shown. Scale bar: 100 µm.
Supplementary Figure S11 Thyroid hormone did not increase the cell death of OPCs under hypoxia (a TUNEL assay).

2,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated T25 flasks. These cells were pre-cultured in BS medium containing PDGF without TH for 14 days in 3% O₂ at 37°C. After then, OPCs dissociated and harvested. 10,000 of OPCs were re-inoculated on PDL/fibronectin coated 12 mm glass bottom dishes and cultured in BS medium containing PDGF without TH (white bar) or with TH (black bar) in 1.5% O₂ for 48 hours at 37°C. A TUNEL assay was carried out by using an Insite Apoptosis Detection Kit (MK500; Takara Bio.) The percentage of terminal deoxynucleotidyl transferase (TdT)-labeled cells (dead cells) are shown (n = 3).
| Symbol   | Raw data | Fold  | Description                                                                 |
|----------|----------|-------|----------------------------------------------------------------------------|
| Abca7    | 392      | 5.27  | ATP-binding cassette sub-family A member 7                                  |
| Adams1   | 508      | 8.72  | ADAM metallopeptidase with thrombospondin type 1 motif, 1                  |
| Apbb1ip  | 176      | 9.58  | Rap1-GTP adapter molecule                                                   |
| Apln     | 117      | 23.92 | Apelin, ligand for the G protein coupled receptor APJ                      |
| Apod     | 176      | 48.25 | Apolipoprotein D                                                            |
| Arhge28  | 773      | 5.12  | Rho guanine nucleotide exchange factor 28                                  |
| Bgn      | 220      | 13.96 | Biglycan                                                                   |
| Bmp4     | 3        | 119.81| Bone morphogenetic protein 4                                               |
| Car2     | 26       | 37.53 | Carbonic anhydrase 2                                                        |
| C1qtnf5  | 1,130    | 10.05 | C1q and tumor necrosis factor related protein 5                            |
| Cacna1e  | 307      | 7.10  | Voltage-dependent R-type calcium channel subunit alpha-1E                  |
| Caly     | 1,881    | 9.06  | Calcyon, neuron-specific vesicular protein                                  |
| Cav1     | 623      | 14.52 | Caveolin 1                                                                  |
| Cd9      | 202      | 12.20 | CD9                                                                        |
| Cdkn2b   | 1,177    | 7.72  | Cyclin dependent kinase inhibitor p15/INK4b                                 |
| Chn2     | 127      | 12.69 | Chimerin 2                                                                  |
| Chst5    | 218      | 12.12 | Carbohydrate Sulfotransferase 5                                            |
| Cldn11   | 54       | 28.49 | Claudin 1                                                                   |
| Clu      | 756      | 6.26  | Clusterin (Apolipoprotein J)                                                |
| Col9a2   | 612      | 5.44  | Collagen α2 (IX)                                                            |
| Col11a2  | 65       | 29.74 | Collagen α2 (XI)                                                            |
| Coro2b   | 121      | 42.08 | Coronin, actin binding protein, 2B                                           |
| Cryab    | 1,811    | 8.29  | Crystallin, alpha B                                                          |
| Crym     | 185      | 436.66| Crystallin, mu, (NADP-regulated thyroid-hormone-binding protein)            |
| Csrp1    | 222      | 17.92 | Transcription factor, Csrp1                                                 |
| Gene   | Expression | Fold Change | Description                                      |
|--------|------------|-------------|--------------------------------------------------|
| Ctsl1  | 1,627      | 8.34        | Cathepsin L                                      |
| Dbndd2 | 143        | 14.69       | Dysbindin domain containing 2                    |
| Dbp    | 3,201      | 6.68        | Transcription factor, Dbp                        |
| Dbx2   | 140        | 30.80       | Transcription factor, Dbx2                       |
| Derl3  | 199        | 6.23        | Derlin 3                                         |
| Dynlt3 | 1,373      | 5.65        | Dynein, light chain, Tctex-type 3                 |
| Epas1  | 132        | 14.82       | Transcription factor, Hif2α                       |
| Espn   | 100        | 18.47       | Espin, actin-bundling protein                     |
| Evl2a  | 731        | 6.27        | Ecotropic viral integration site 2A              |
| Fa2h   | 455        | 8.75        | Fatty acid 2-hydroxylase                         |
| Fam134b| 578        | 6.25        | Family with sequence similarity 134, member B    |
| Fbxa2  | 113        | 15.63       | F-box protein 2                                  |
| Fgfr2  | 130        | 44.39       | FGF receptor 2                                   |
| Galnt14| 82         | 19.56       | Polypeptide N-acetylgalactosaminyltransferase 14 |
| Gfra2  | 237        | 21.52       | GDNF family receptor alpha 2                     |
| Glul   | 1,622      | 8.55        | Glutamate-ammonia ligase                         |
| Gpd1   | 70         | 91.66       | Glycerol-3-phosphate dehydrogenase 1             |
| Gpld1  | 119        | 9.44        | Glycosylphosphatidylinositol specific phospholipase D1 |
| Gpnmb  | 146        | 12.48       | Glycoprotein (transmembrane) nmb                 |
| Gpr37  | 10         | 100.69      | G protein-coupled receptor 37 (Pael-R)           |
| Gpr37l1| 8,348      | 6.93        | G protein-coupled receptor 37 like 1             |
| Gramd3 | 432        | 13.53       | GRAM domain containing 3                         |
| Gsg1l  | 282        | 15.76       | Germ cell associated 1-like                      |
| Gsn    | 2,037      | 5.09        | Gelsolin                                         |
| Hapln2 | 59         | 55.10       | Hyaluronan and proteoglycan link protein 2       |
| Hdac11 | 2,370      | 7.66        | Histone deacetylase 11                           |
| Hist2h2aa3| 2,002   | 5.17        | Histone cluster 2, H2aa3                         |
| Hmox1  | 396        | 17.79       | Heme oxygenase 1                                 |
| Htra1  | 83         | 31.31       | HtrA serine peptidase 1                          |
| Ifi27l2b| 322        | 9.47        | Interferon, alpha-inducible protein 27 like 2B  |
| Gene  | FPKM | FPKM Percent | Description |
|--------|------|--------------|-------------|
| Igf11  | 162  | 30.25        | Immunoglobulin superfamily, member 11 |
| Kcna1  | 917  | 7.84         | Potassium channel, voltage gated shaker related subfamily A, member 1 |
| Klf9   | 265  | 14.73        | Transcription factor, Klf9 |
| Lgals3 | 674  | 11.66        | Lectin, galactoside-binding, soluble, 3 |
| Lgi3   | 602  | 21.14        | Leucine-rich repeat LGI family, member 3 |
| Lhfp   | 2,017| 9.72         | Lipoma HMGIC fusion partner |
| Lhfpl2 | 341  | 19.89        | Lipoma HMGIC fusion partner-like 2 |
| Lppr5  | 356  | 6.19         | Lipid phosphate phosphatase-related protein type 5 |
| Mag    | 86   | 95.74        | Myelin associated glycoprotein |
| Marc2  | 256  | 6.95         | Mitochondrial amidoxime reducing component 2 |
| Mbp    | 5,773| 45.22        | Myelin basic protein |
| Mel1   | 7,802| 8.89         | Malic enzyme 1, NADP (+)-dependent |
| Mettl7a| 248  | 9.44         | Methyltransferase like 7A |
| Mfsd6  | 1,487| 5.48         | Major facilitator superfamily domain containing 6 |
| Mobp   | 18   | 192.83       | Myelin-associated oligodendrocyte basic protein |
| Mog    | 10   | 163.83       | Myelin oligodendrocyte glycoprotein |
| Mrf    | 91   | 6.61         | Transcription factor, Mrf |
| Mro    | 173  | 10.14        | Transcription factor, Mro |
| Myo1d  | 516  | 9.80         | Myosin 1D |
| Ndr1   | 16   | 59.35        | N-myc downstream regulated 1 |
| Neu4   | 894  | 5.13         | Sialidase 4 |
| Nfasc  | 419  | 8.75         | Neurofascin |
| Ninj2  | 2,780| 5.24         | Ninjurin 2 |
| Nkd1   | 280  | 8.20         | Naked cuticle 1, Wnt antagonist |
| Nkx6-2 | 29   | 113.59       | Transcription factor, Nkx6-2 |
| Nr1d1  | 63   | 5.73         | Transcription factor, Rev-erbα |
| Nrnx3  | 403  | 11.25        | Neurexin 3 |
| Oaf    | 134  | 14.25        | Out at first homolog |
| Gene       | MQSA | Log2 Fold Change | Description                                                                 |
|------------|------|------------------|------------------------------------------------------------------------------|
| Opalin     | 3    | 384.72           | Oligodendrocytic myelin paranodal and inner loop protein                     |
| Pgfl       | 88   | 102.47           | Placental growth factor                                                      |
| Plcd4      | 203  | 12.09            | Phospholipase C, delta 4                                                    |
| PLEC       | 190  | 8.06             | Plectin                                                                     |
| Plekha1    | 3,504| 5.43             | Pleckstrin homology domain containing, family A member 1                    |
| Pllp       | 842  | 11.03            | Plasmolipin                                                                 |
| Plp1       | 221  | 146.97           | Proteolipid protein 1                                                       |
| Ppp1r14a   | 22   | 58.95            | Protein phosphatase 1, regulatory (inhibitor) subunit 14A                   |
| Ptcchd2    | 191  | 8.67             | Patched domain containing 2                                                 |
| Ptgsd      | 18   | 256.55           | Prostaglandin D2 synthase (brain)                                           |
| Ptpre      | 248  | 7.20             | Protein tyrosine phosphatase, receptor type, E                              |
| Rhp1       | 611  | 5.13             | Retinol binding protein 1, cellular                                          |
| Rgs7bp     | 973  | 9.38             | Regulator of G-protein signaling 7 binding protein                          |
| Rpe65      | 3    | 244.49           | Retinal pigment epithelium-specific protein 65kDa                           |
| Runx1      | 112  | 24.79            | Transcription factor, Runx1                                                 |
| Sat1       | 1,033| 7.96             | Spermidine/spermine N1-acetyltransferase                                    |
| Scg2       | 122  | 11.98            | Secretogranin II, neuroendocrine secretory proteins                         |
| Scg5       | 372  | 6.23             | Secretogranin V, neuroendocrine secretory proteins                          |
| Sds1       | 1,271| 10.73            | Solanesyl diphosphate synthase                                              |
| Sema5a     | 50   | 22.96            | Sema domain, seven thrombospondin repeats, transmembrane domain and short cytoplasmic domain, (semaphorin) 5A |
| Sept4      | 367  | 82.35            | Septin 4, nucleotide binding protein                                        |
| Serinc5    | 2,292| 6.02             | Serine incorporator 5                                                       |
| Shroom2    | 2,515| 10.99            | Shroom family member 2                                                      |
| Symbol   | Raw data | Fold | Shown as                                         |
|----------|----------|------|-------------------------------------------------|
| **Sirt2** | 1321     | 5.36 | Sirtuin 2                                       |
| **Slc1a2** | 298      | 9.60 | Excitatory amino-acid transporter 2             |
| **Slc6a3** | 488      | 11.81| Dopamine transporter                            |
| **Slc7a3** | 7,801    | 5.35 | Cationic amino acid transporter 3               |
| **Slc48a1** | 287      | 5.02 | Heme transporter                                |
| **Sparc11** | 3,433    | 6.84 | SPARC-like 1 (hevin)                           |
| **Slc28a1** | 698      | 8.26 | SplA/Ryanodine receptor domain and SOCS box containing 1 |
| **Srpk3** | 504      | 6.32 | SRSF protein kinase 3                          |
| **Srtn1** | 312      | 6.43 | Sulfiredoxin 1                                 |
| **Syt4** | 618      | 18.91| Synaptotagmin-4, Ca$^{2+}$ sensor              |
| **Tf** | 72       | 29.44| Transferrin                                    |
| **Tmcc2** | 1,838    | 6.51 | Transmembrane and coiled-coil domain family 2   |
| **Tmef2** | 276      | 7.68 | Transmembrane protein with EGF-like and two follistatin-like domains 2 |
| **Tmem100** | 179      | 9.86 | Transmembrane protein 100                      |
| **Tnr** | 260      | 80.08| Tenascin R                                     |
| **Tppp3** | 46       | 99.75| Tubulin polymerization-promoting protein family member 3 |
| **Tprn** | 582      | 5.65 | Taperin                                        |
| **Tspan2** | 1,457    | 12.86| Tetraspinan-2                                  |
| **Tri1** | 3,254    | 5.08 | TLR4 interactor with leucine-rich repeat        |
| **Ttyh2** | 364      | 7.86 | Tweety family member 2, calcium(2+)-activated large conductance chloride(-) channel |
| **Tuba4a** | 79       | 21.27| Tubulin, alpha 4a                              |
| **Ugt8** | 262      | 11.44| UDP glycosyltransferase 8                      |
| **Vega2** | 2,997    | 8.90 | Vascular endothelial growth factor A           |

**Genes Down-regulated (60)**

| Symbol   | Raw data | Fold | Shown as                  |
|----------|----------|------|---------------------------|
| **Actg2** | 1,021    | -8.54| Actin, gamma 2            |
| Gene         | Log2 FC | Description                                              |
|--------------|---------|----------------------------------------------------------|
| Aif1l        | -7.22   | Allograft inflammatory factor 1-like                    |
| Arhgap11a    | -6.01   | Rho GTPase activating protein 11A                        |
| Aumip        | -5.93   | Aurora kinase A and ninein interacting protein           |
| Aurkb        | -6.41   | Aurora kinase B                                          |
| Brip1        | -5.41   | BRCA1-interacting protein 1                             |
| Bub1b        | -5.19   | BUB1 mitotic checkpoint serine/threonine kinase B       |
| Cbx2         | -5.20   | Transcription factor, Cbx2                               |
| Ccna2        | -6.08   | Cyclin A2                                                |
| Ccnb1        | -6.25   | Cyclin B1                                                |
| Ccnb2        | -5.21   | Cyclin B2                                                |
| Cdc6         | -7.37   | Cell division cycle 6                                    |
| Cdc45        | -6.21   | Cell division cycle 45                                   |
| Cdc6a        | -6.25   | Cell division cycle associated protein 2                 |
| Cdc6a7       | -14.51  | Cell division cycle-associated protein 7                 |
| Cenpa        | -6.94   | Centromere protein A                                     |
| Cenpm        | -5.23   | Centromere protein M                                     |
| Cep55        | -5.15   | Centrosomal protein 55 kDa                               |
| Csrp2        | -11.06  | Transcription factor, Csrp2                              |
| Dll3         | -14.34  | Delta-like 3                                             |
| Dsn1         | -5.52   | DSN1 homolog, MIS12 kinetochore complex component        |
| Fam64a       | -5.64   | Family with sequence similarity 64, member A             |
| Fgf13        | -5.13   | Fibroblast growth factor 13                              |
| Foxm1        | -5.10   | Transcription factor, Foxm1                              |
| Gins1        | -6.34   | GINS complex subunit 1 (Psf1 homolog)                   |
| Hist1h1b     | -6.84   | Histone cluster 1, H1b                                   |
| Hmgb2        | -5.77   | High mobility group box 2                                |
| Igfhp4       | -12.68  | Insulin-like growth factor binding protein 4             |
| Kif2C        | -5.55   | Kinesin family member 2C                                 |
| Kif15        | -5.17   | Kinesin family member 15                                 |
| Kif18a       | -5.85   | Kinesin family member 18A                                |
| Gene   | Start | End   | Description                                                  |
|--------|-------|-------|--------------------------------------------------------------|
| Klf14  | 617   | -5.20 | Transcription factor, Klf14                                  |
| Kntrc1 | 1,345 | -5.22 | Kinetochoore associated 1                                   |
| Mcm3   | 11,644| -6.37 | Minichromosome maintenance complex component 3              |
| Mcm5   | 2,353 | -6.55 | Minichromosome maintenance complex component 5              |
| Mcm6   | 3,608 | -8.58 | Minichromosome maintenance complex component 6              |
| Melk   | 2,341 | -5.27 | Maternal embryonic leucine zipper kinase                     |
| Mis18a | 2,879 | -5.56 | MIS18 kinetochore protein A                                  |
| Mms22l | 1,387 | -6.33 | MMS22-like, DNA repair protein                               |
| Mybl2  | 3,618 | -6.00 | Transcription factor, Mybl2                                  |
| NcapH  | 8,547 | -5.06 | Non-SMC condensin I complex, subunit H                       |
| Ns5atp9| 2,547 | -5.55 | PCNA-associated factor (KIAA0101)                           |
| Prim1  | 4,030 | -5.89 | Primase, DNA, polypeptide 1                                 |
| Racgap1| 2,355 | -5.42 | Rac GTPase activating protein 1                              |
| Rad51  | 3,451 | -5.28 | RAD51 recombinase                                            |
| Rasl11a| 1,482 | -5.53 | RAS-like, family 11, member A                                |
| Rps6ka6| 617   | -5.47 | Ribosomal protein S6 kinase, 90kDa, polypeptide 6            |
| Rrm2   | 1,217 | -7.71 | Ribonucleotide reductase M2                                  |
| Sdcl   | 6,488 | -6.42 | Syndecan 1                                                  |
| Sept6  | 666   | -9.05 | Septin 6, GTPase                                            |
| Tac1   | 3,918 | -5.27 | Tachykinin, precursor 1, peptide hormone                     |
| Ticrr  | 744   | -7.91 | TOPBP1-interacting checkpoint and replication regulator      |
| Timeless| 1,843 | -5.17 | Timeless circadian clock                                    |
| Tmopo  | 4,748 | -5.28 | Thymopoietin                                                |
| Top2a  | 6,453 | -5.81 | Topoisomerase (DNA) II alpha                                 |
| Tp53i11| 693   | -5.67 | Tumor protein p53 inducible protein 11 (PIG11)              |
| Tpx2   | 2,006 | -5.28 | TPX2, microtubule-associated                                 |
Supplementary Table 1   The list of differentially expressed genes with TH treatment.

Gene symbols are shown in italic. Average raw data of the P7 OPCs cultured without TH in 1.5% O₂ for 15 days are shown (n = 3). 129 Genes up-regulated with TH treatment are shown in (+) fold, and 60 genes down-regulated are shown in (-) hold. The moderate genes described in the text (11 genes) are also shown. For calculating probability (P value), Student’s t test was carried out. The P value of every gene is P < 0.05 (t-test, n=3).

The TH-dependent up-regulations of the major genes described in the text were confirmed by qRT-PCR (Supplementary Figure 3a). And the TH-dependent up-regulations of protein expression of Runx1 and p15/INK4b were confirmed by western blot analysis (Supplementary Figure 3b).
| Name of Gene | PCR Primer | Sequence (5’-3’) | Length (mer) |
|--------------|------------|------------------|--------------|
| p15/INK4b    | FW         | GCGGCAGTGACGCGGG | 16           |
|              | REV        | AGCGTGTCAGGAAGCC | 17           |
| p16/INK4a    | FW         | TCACAAACGGCCCGAAC | 18          |
|              | REV        | ATCGCGCACATCCAGCC | 17          |
| p19ARF       | FW         | TGGTCGCAAGTTCTGGA | 18          |
|              | REV        | ATCGCGCACATCCAGCC | 18          |
| p18/INK4c    | FW         | GCAACTTACTAGTTTGTCG | 20         |
|              | REV        | AGGCTGTGTGCTCCATTAG | 19       |
| p19/INK4d    | FW         | AGCTGGTGCATCCTGAC | 17          |
|              | REV        | GATGGAGATCAGATTCAG | 18          |
| p21/Cip1     | FW         | CAACTACGTCTGGGAGC | 17          |
|              | REV        | CTTGCAGAAGACCAATCG | 18          |
| p27/Kip1     | FW         | AGCTTGCCCGAGTTCTAC | 18         |
|              | REV        | TCCACAGTGCCCAGCATTTC | 18       |
| p57/Kip2     | FW         | CAGCCCAAGGTTTTGTGG | 18          |
|              | REV        | AGAGGGATCCACGATTCG | 18          |
| Cyclin A2    | FW         | TTGCTGGAGCTGCTCTTC | 18          |
|              | REV        | ACAGCATGAACCTGAGCG | 19          |
| Cyclin B1    | FW         | AACTCTGCAGCCTACCTG | 19          |
|              | REV        | AACTGCATCTGCAGACAG | 19          |
| Cyclin B2    | FW         | AGAACAATATGCCAGCAG | 20          |
|              | REV        | TCTTCAGTACAGACAGC | 19          |
| Cyclin D1    | FW         | GGAAGTTTTTGTTGCTG | 19          |
|              | REV        | TCAGACATGGCCCTAAACC | 19        |
| Cyclin D2    | FW         | GCACAACGGCTCAAGTC | 18          |
|              | REV        | CTGGAATTGAACAGGTACC | 20        |
| Cyclin D3    | FW         | CAGTGAACCCACACACC | 19          |
|              | REV        | CCATCAGACCCCTCAAGAC | 19        |
| Cyclin E1    | FW         | TGTCAGAACAAATAGGATTTC | 22       |
|              | REV        | TGTTGTCTGAGGTGTCG | 18          |
| Gene Name | Length (bp) | Forward Primer Sequence | Reverse Primer Sequence | Length |
|-----------|-------------|--------------------------|--------------------------|--------|
| Cyclin E2 | 329 bp      | ATGACACCACCCAAGAGCA      | TTGGCTTCCATCCACTATTCC    | 19     |
| Csrf1     | 270 bp      | GGGTAGAGCTGTGCTTAG       | TGATAAATCAAGCTTCACAGGC  | 19     |
| Dbp       | 616 bp      | CTGAGGAGCCTTCTGCAAG      | TCAATTCCTCCTCTGAGAAG     | 18     |
| Dbx2      | 538 bp      | GGAAGAGTTTCTGATCG        | TCTGGAACCAATCTTCACC      | 20     |
| Hif1α     | 309 bp      | TGCTCATCAGTTGCCACCTCC    | TCCTCATGGTACATGGATG      | 20     |
| Hif1β     | 384 bp      | GTCAAGGGGCTGATTTTGATG    | GCTGCTCCAAAAATCAAATG     | 20     |
| Hif2α     | 405 bp      | TGGAATCAGGAGGCCAG        | TCACCTCACAGCTATCTG       | 18     |
| Klf9      | 376 bp      | GAGGAGAGACAGATTCTG       | TGATCTCCTGTATCG          | 20     |
| Mrf       | 334 bp      | TACACTGGACGCTGCTGC       | ACTCTGCACTGGACTATGG      | 18     |
| Mro       | 260 bp      | TGTTCCTAAGGGCTGCACCC    | TGAGAAAGGGAGGGATGG       | 19     |
| Nkx6-2    | 356 bp      | CTCAACGGGCTGCCCCTC       | GCACGATTTCGGAACCAC       | 18     |
| Rev-erbA  | 368 bp      | TTCACGGCAGTGTTACCTG      | GCCAGGGCTCATCTTGG        | 19     |
| Runx1     | 316 bp      | AGCAACTCGCCACCAAC        | GCAATCAATAAGGTCGCGGA     | 18     |
| GAPDH     | 452 bp      | ACCACAGTCCATGCGACAC      | TCCACACCCCTGTGTGCATGA    | 20     |

**Supplementary Table 2**  The nucleotide sequences of RT-PCR primers.

The nucleotide sequences of PCR primers are shown from 5’ (left) to 3’ (right) direction. The numbers shown below each gene name are the length of predicted PCR products (bp). FW; forward primer, REV; reverse primer.
**Primers for qRT-PCR**

| Gene      | Length (bp) | Forward (5’ to 3’) | Reverse (5’ to 3’) | Length |
|-----------|-------------|---------------------|-------------------|--------|
| GAPDH     | 108         | TGGCATTGCTCTCAATGAC | TCCTTGGAGGCCATGTAG | 19     |
| Actb      | 63          | TAAGGCCAACCGTGAAAAG | TACATGGCTGGGTGTGG | 18     |
| Runx1     | 105         | AGAAAGATTTTATACCCCTTGACTTG | AAGCATGCAGGCTGC | 25     |
| Ldha      | 126         | TCCAGTGTGAAGTAAGCCATCG | CATCATCCATATGCAGATCTTTAC | 20     |
| Pgk1      | 115         | AGCAGCTGCACAGCATC | AGATGCCTCCTACATAATGCAC | 17     |
| Vegfa     | 106         | GTGTGTGTGTATGAAATCTGTG | GGCAGAGCTGAGTTAGC | 22     |
| Klf9      | 108         | CAACCAGGCAGAGATGG | AAAGAGCAGTGACCTCTCG | 19     |
| Hif1a     | 88          | TGGACACTGGTGCTGC | CTAAACTATCTGAGTTGCTGCAG | 16     |
| Hif2a     | 109         | GGATAACTTGTACCTGAAAGCC | CTGGGTGAACTTTGGACATGAAC | 22     |
| Runx1 P1  | 127         | CACAGTGTCAGCCAGGCAG | AAGCCATCGTTTCTTTCTGA | 19     |
| Runx1 P2  | 120         | CCTCCGGTAGTAATAAAGGCTCCTG | CCGATTTAGTAAGGACCCTGAA | 25     |

**Supplementary Table 3**  **The nucleotide sequences of qRT-PCR primers.**

The nucleotide sequences of PCR primers are shown from 5’ (left) to 3’ (right) direction. The numbers shown below each gene name are the length of predicted PCR products (bp). FW; forward primer, REV; reverse primer.
| Name of target gene | Name of siRNA | Target sequence |
|---------------------|---------------|-----------------|
| Non target control  | Non-target 1   | 5’UGGUUUACAUGUCGACUAA3’ |
|                     | Non-target 2   | 5’UGGUUUACAUGUUGUGUA3’ |
|                     | Non-target 3   | 5’UGGUUUACAUGUUUUCUGA3’ |
|                     | Non-target 4   | 5’UGGUUUACAUGUUUUCCUA3’ |
| p15/INK4b (CDKN2B)  | J-093997-09    | 5’GAGCAGGGCCACCGUGAUA3’ |
|                     | J-093997-10    | 5’CCGCCUGCCGGUAGACUUA3’ |
|                     | J-093997-12    | 5’CUGUAAACGUGAGAGGAU3’ |
|                     | J-093997-17    | 5’GAUCUCUGCUAAACGGUGA3’ |
| Csrp1 (Csrp1)       | J-090904-08    | 5’UGAGAUCUAUUGCAAAGGA3’ |
|                     | J-090904-07    | 5’CGACCACCCUGGCAGACAA3’ |
|                     | J-090904-06    | 5’CAAGUCAUGUUAUGGCAAG3’ |
|                     | J-090904-05    | 5’GCCGGGAAGUCCGGCAU3’ |
| Dbp (Dbp)           | J-089811-12    | 5’GCUAAUGACCUUUGAACCU3’ |
|                     | J-089811-11    | 5’GGAAAGUCCAGGUGCCCGA3’ |
|                     | J-089811-10    | 5’UGAAGGAAAAGGAGCCGAA3’ |
|                     | J-089811-09    | 5’GUGCUGUGCUUUCACGCUA3’ |
| Dbx2 (Dbx2)         | J-108084-12    | 5’GAGCUGUGUUCUCGGAGGA3’ |
|                     | J-108084-11    | 5’CUGUAAUAUAUUCUCGUU3’ |
|                     | J-108084-10    | 5’AGGGAAGAGUCUCGGAU3’ |
|                     | J-108084-09    | 5’ACUAAGAUAAGUGUAA3’ |
| Hif1a (Hif1a)       | J-091718-08    | 5’CUGUAUAACGUGAACAUA3’ |
|                     | J-091718-07    | 5’UUACUGAGUUGAGGUUA3’ |
|                     | J-091718-06    | 5’GGAACAGUGAAAGGAU3’ |
|                     | J-091718-05    | 5’UGAGAGAAUUGCUUACACAA3’ |
| Hif2a (Epas1)       | J-091647-12    | 5’AGGAUGAAGUGUACCACU3’ |
|                     | J-091647-11    | 5’CCAUGAGACCCAGGCGAA3’ |
|                     | J-091647-10    | 5’AGAUAGAGAAGAUGACGU3’ |
|                     | J-091647-09    | 5’UUGAUGAUAACGUCACUAA3’ |
| Klf9 (Klf9)         | J-091801-08    | 5’GGAAAAUCCCUCUCUUA3’ |
|                     | J-091801-07    | 5’GAAGGAUAUUGCAACUCUG3’ |
| Gene   | Accession   | Sequence                      |
|--------|-------------|-------------------------------|
| Mrf (Myrf) | J-091801-06 | 5’GAACAAAUACCGACCCAUC3’       |
|         | J-091801-05 | 5’GGGAAACACGCCUCCCGAAA3’      |
| Mro (Mro) | J-085053-12 | 5’AAGAGGAAGCACUCGAAU3’        |
|         | J-085053-11 | 5’GAAACAACAAACACGGCAU3’       |
|         | J-085053-09 | 5’UAGUGAACAAGGAGCGGAU3’       |
|         | J-085053-18 | 5’GAGCUUUGUUGGAGCGGAAG3’      |
| Nkx6.2 (Nkx6-2) | J-092502-11 | 5’GAGUUUCCAAAGUGAAGAA3’       |
|         | J-092502-10 | 5’CUGCAUUGCGUGCCGGA3’         |
|         | J-092502-09 | 5’UGUCUUAUUAUUUCGGAUGA3’      |
|         | J-092502-18 | 5’ACCCUGGAGUCGACGCAA3’        |
| Rev-erbA (Nr1d1) | J-085148-12 | 5’AGCACAACCCUCGAACUU3’        |
|         | J-085148-11 | 5’CGGAGGAUGAUGACGAGUA3’       |
|         | J-085148-10 | 5’AACUGGGACUCGCGGCAUA3’       |
|         | J-085148-09 | 5’CAGAGAUGGCGUCGCAUA3’        |
| Runx1 (Runx1) | J-097573-09 | 5’GCUGUAUGUUAACGUGA3’         |
|         | J-097573-10 | 5’GCCAGGGCCAAUCGAGAAA3’       |
|         | J-097573-11 | 5’GCUGAAGAUCGACGCAA3’         |
|         | J-097573-12 | 5’UAACCAAGCUCUAACGGAU3’       |
|         | J-092238-08 | 5’AGAACCAAGUGGCCGAGAU3’       |
|         | J-092238-07 | 5’GCGACGUGGGAACCGCA3’         |
|         | J-092238-06 | 5’GACCAUCACCGUUCUUCACA3’      |
|         | J-092238-05 | 5’UGACCAGUCUACCGUACGC3’       |

**Supplementary Table 4  The sequences of siRNA target sites.**

The symbol of gene is shown in *Italic*. For the gene silencing experiments, a mixture of four siRNAs (1 µM each) specific to each target gene was used.
Legend of videos

Video 1. Perinatal OPCs without TH in 1.5% O\textsubscript{2} condition (as control).
OPCs purified from P7 rat optic nerves were cultured in the hypoxic condition without TH. These cells passaged once on day 14. On day 21, time-lapse differential interference microscopic images of cells were recoded every 15 minutes for 24 hours.

Video 2. Adult-like OPC with TH in 1.5% O\textsubscript{2} condition.
OPCs purified from P7 rat optic nerves were cultured in the hypoxic condition with TH. On day 21, time-lapse differential interference microscopic images of cells were recoded every 15 minutes for 24 hours.

Video 3. Adult-like OPCs differentiated into OLs in 1.5% O\textsubscript{2} condition.
300 of OPCs purified from P7 rat optic nerve were plated in BS medium containing PDGF with TH in 1.5% O\textsubscript{2} on PDL/fibronectin coated 12 mm glass-bottom dishes and pre-cultured at 37°C. On the culture day 10, the culture medium was replaced to PDGF free BS medium, after which (recording time 0) they were followed by time-lapse video microscopy. Images of cells were captured at every 15 minutes for 96 hours.

Video 4. Adult-like OPCs differentiated into type-2 astrocytes in 1.5% O\textsubscript{2} condition.
300 of OPCs purified from P7 rat optic nerve were plated in BS medium containing PDGF with TH in 1.5% O\textsubscript{2} on PDL/fibronectin coated 12 mm glass-bottom dishes and pre-cultured at 37°C. On the culture day 14, the culture medium was replaced to BS medium containing 10% FBS, after which (recording time 0) they were followed by time-lapse video microscopy. Images of cells were captured at every 20 minutes for 120 hours.
Supplementary Figure legends of Main Figures

Supplementary Figure Legend for Figure 1
(a) OPCs were purified from P7 rat optic nerve by immunopanning. 2,000 of OPCs were inoculated in each PDL-coated T25 flask. These cells were cultured in BS medium containing both PDGF and TH in 1.0~20% O₂ condition at 37°C for 10 days. Then the cells were dissociated and harvested and the number of cells was counted. The P value of the cell number compared that of in 20% O₂ conditions are shown *P < 0.05 (ANOVA with Fisher’s LSD test, n = 3), and compared that of in 2% O₂ conditions are shown ∨P < 0.05 (ANOVA, n = 3).

(b) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask. These cells were cultured in BS medium containing both PDGF and TH in 1.0~20% O₂ condition at 37°C for 10 days. After then, the cells were fixed and stained with anti-GC or A2B5 monoclonal antibody, and the percentage of GC⁺ cells (white bars) and A2B5⁺ cells (black bars) was determined (upper panel). The ratios of A2B5⁺ versus GC⁺ are shown in the lower panel. *P < 0.05 (ANOVA with Fisher’s LSD test, n = 3).

(c) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated T25 flask. These cells were cultured in BS medium containing PDGF with TH (black bars) or without TH (white bars) in 1.5 or 20% O₂ condition at 37°C for 11 days. The number of cells in each clone was counted and the number of cell divisions was estimated (n = 3).

(d) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated T25 flask. These cells were cultured in BS medium containing PDGF without TH in either 20% O₂ (closed squares) or 1.5% O₂ (open squares) at 37°C and were passaged at day 10, day 20, day 30 and at day 40 (1,000 cells per T25 flask). Cell numbers estimated from the total cell number at the last passage multiplied by the proliferation rate (n = 3).

Supplementary Figure Legend for Figure 2
(a) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated T25 flask. These cells were cultured in BS medium containing both PDGF and TH in
either 20% O$_2$ (closed squares) or 1.5% O$_2$ (open squares) condition at 37°C. These cells were stained for GC after 3, 6 and 12 days, and the % of GC$^+$ cells were counted at each time point. *P < 0.05 (unpaired Student’s t-test, n = 3).

(b) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask. These cells were were cultured in BS medium containing PDGF, without TH (-TH, white bar) or with TH (+TH, black bar), in 1.5% O$_2$ at 37°C. At culture day 15, PI (2.5 µg/ml) and Hoechst 33342 (5 µg/ml) were added to the culture for 90 minutes. Then, the percentage of dead cells (PI$^+$, Hoechst 33342$^+$ double positive cells) was determined (n = 3).

(c) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL/fibronectin-coated 12 mm glass bottom dish. Cells were cultured in both PDGF and TH in 1.5% O$_2$ at 37°C for 20 days and stained for SA-βGal (left panel); as a positive control, 1,000 of OPCs purified from P7 rat optic nerve were cultured in 15% FBS in 20% O$_2$ for 20 days to induce replicative senescence were also stained for SA-βGal (right panel). Scale bar: 50 µm.

(d) 10,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask and cultured without PDGF at 37°C for 3 days, in either 20% O$_2$ (upper right panel) or 1.5% O$_2$ (lower right panel). After then, cells were labeled with anti-GC antibody (green) and DAPI (blue). Scale bar: 100 µm. Percentages of GC$^+$ cells in 20% O$_2$ culture and 1.5% O$_2$ culture are shown in the graph on the left (n = 3). Note that rat OL differentiation obeying withdrawal of PDGF is not inhibited in 1.5% O$_2$, the extensions of GC$^+$ membrane-like structure around the cell body in 1.5% O$_2$ were comparable with those in 20% O$_2$.

**Supplementary Figure Legend for Figure 3**

(a) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask and cultured in BS medium containing PDGF with TH (+TH; black bars) or without TH (-TH; white bars) in 1.5% O$_2$ at 37°C. After 2 or 15 days, cells were treated with 5 µM of BrdU for 20 hours and then the % of BrdU$^+$ cells was determined. *P < 0.001 (unpaired Student’s t-test, n = 3).

(b) 300 of OPCs purified from P7 rat optic nerve were plated in BS medium containing PDGF, with TH (closed circles) or without TH (open squares) and in either 1.5% or 3% O$_2$ on PDL/fibronectin coated 12 mm glass-bottom dishes and pre-cultured for 24 hours
at 37°C, after which (recording time 0) they were followed by time-lapse video microscopy. Images of cells were captured at every 30 minutes for 120 hours. The average time between M-phases was estimated on day 1 (recording time 0-23.5 hours), day 2 (24-47.5 hours), and day 3 (48-71.5 hours). At the recording time 0, the following number of cells were analyzed in each condition: 42 in 1.5% O₂ with TH; 48 in 1.5% O₂ without TH; 131 in 3% O₂ with TH; 89 in 3% O₂ without TH. *P < 0.05 (unpaired Student’s t-test).

(c) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask and cultured in BS medium containing PDGF and TH in 1.5% O₂ at 37°C. At culture day 11, 20 or 30, the cells were fixed and labeled with anti-GC and A2B5 monoclonal antibodies and the percentage of each type of labeled cells was determined (n = 3).

(d) 500 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask and cultured in BS medium containing PDGF and TH in 1.5% O₂ at 37°C. After 15 or 30 days, the representative fields were examined by phase-contrast microscopy. Scale bar: 50 μm.

(e) 500 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated slide flask and cultured in BS medium containing PDGF and TH in 1.5% O₂ at 37°C for 15 days. Before the differentiation inducing treatments, colonies in these flasks were examined by a phase-contrast microscopy. The positions of the colonies of which contained only OPCs with characteristic bipolar bodies were marked and labeled on the bottom of the slide flasks. Cultures were then either deprived of PDGF for 5 days and labeled for MBP or treated with 10% FBS for 5 days and labeled for GFAP. The morphologies of the cells and the expressions of MBP or GFAP in the cells of the marked colonies were examined. Scale bar: 100 μm.

(f) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. These cells were cultured in BS medium containing both PDGF and TH in 1.5% O₂ for 15 days at 37°C. Cells were dissociated with trypsin and re-cultured at clonal-density (2,000 cells/PDL-coated T25 flask) for another 7 days at 37°C in PDGF in 20% O₂ — without TH (white bars), with TH (black bars), or without TH in the presence of NRG1 (50 ng/ml) and IBMX (100 μM) (gray bars). Then cells were fixed and the number of cell divisions in each clone was estimated. The average numbers of cell divisions are show in the inset (1.24 ± 0.18 without TH; 0.24 ± 0.60 with TH; 1.95
± 0.60 without TH, but with NRG1 and IMBX). *P < 0.001 (unpaired Student’s t-test, n = 3).

**Supplementary Figure Legend for Figure 4**

(a and b) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in each PDL-coated T25 flask. These cells were were cultured in BS medium containing PDGF, without TH (-TH) or with TH (+TH), in 1.5% O₂ at 37°C. At culture day 15, total RNA was prepared from these cells and and assayed by RT-PCR. The PCR products were detected by 2% agarose gel electrophoresis. GAPDH was used as an internal control. (a) In Cyclin genes, TH decreased the mRNA for G2/M Cyclins A2, B1 and B2. (b) In CKI genes, TH strongly increased the mRNA for p15/INK4b.

(c) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated slide flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 1.5% O₂ for 10 days at 37°C. Then TH (40 ng/ml) was added to some cultures (+TH) but not to others (-TH) for 2 days. The cells were then labeled with rabbit anti-p15/INK4b antibodies (green) and DAPI (blue). Note that p15/INK4b protein increased in the nucleus of the TH-treated cells. Scale bar: 100 µm.

(d) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. OPCs were pre-cultured in BS medium containing PDGF without TH in 3% O₂ for 12 days at 37°C. Cells were dissociated and harvested. These cells were co-transfected with anti-p15/INK4b siRNA (si-p15) and GFP expressing transfection-reporter plasmid DNA (pMaxGFP); a non-targeting siRNA pool (si-NT) served as a negative control. Cells were re-cultured in PDL-coated slide flasks at clonal-density with BS medium containing PDGF with TH in 1.5% O₂ for 5 days at 37°C. The number of GFP⁺ cells in each clone was counted. Note that the anti-p15/INK4b siRNA prevented the deceleration of cell cycle that occurs in 1.5% O₂ in the presence of TH. *P < 0.001 (unpaired Student’s t-test, n = 3).

(e) 20,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 3% O₂ for 12 hours at 37°C. Then cells were infected with either a p15/INK4b-IRES-ZsGreen expressing retrovirus vector (p15) or the ZsGreen expressing empty vector as a negative control (Cont) for 4 hours at 37°C. Cells were dissociated with trypsin and re-inoculated at clonal density in PDL-coated slide flasks (500 cells/slide flask). Cells were cultured
in BS medium containing PDGF with TH in 3% O₂ for 7 days at 37°C. The number of ZsGreen⁺ cells in each clone was counted, and the results are shown in the graph on left. Note that the cells over expressing p15/INK4b proliferated much less than control. *P < 0.001 (unpaired Student’s t-test, n = 3). The p15INK4b-IRES-ZsGreen expressing cells were also labeled with A2B5 antibody (red) and DAPI (blue); as shown in the panels on right, the ZsGreen⁺ OPCs (green) are bipolar and A2B5⁺. Scale bar: 100 µm.

Supplementary Figure Legend for Figure 5

(a) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 1.5% O₂ for 12 days at 37°C. Cells were dissociated and harvested. These cells were co-transfected with anti-p15/INK4b siRNA (si-p15/INK4b) or siRNA against the gene of each transcription factor and pMaxGFP. Cells were re-cultured in PDL-coated slide flasks at clonal-density with BS medium containing PDGF with TH in 1.5% O₂ for 4 days at 37°C. The number of GFP⁺ cells in each clone was counted. Data was normalized against the average number of negative control (cells transfected with non-target siRNA; si-NT). P* <0.05, P** < 0.01, P***< 0.001 (unpaired Student’s t-test, n = 3).

(b) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 1.5% O₂ for 10 days at 37°C. To investigate the immediate early responses of the genes of the deceleration of cell cycle related transcription factors, these cells were pre-treated with cycloheximide (50 µg/ml) for 6 hours, after then TH was added to the medium. Cells were harvested at 0, 0.5, 1 and 2 hours later. Total RNA was prepared from these cells and a RT-PCR assay was carried out. Gapdh (GAPDH) was used as a negative control. The PCR products were detected by 2% agarose gel electrophoresis.

(c) 20,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 3% O₂ for 12 hours at 37°C. Then cells were infected with either Klf9-IRES-ZsGreen expressing retrovirus vector or the ZsGreen expressing empty vector as a negative control for 4 hours in 3% O₂ at 37°C. Cells were dissociated with trypsin and re-inoculated at clonal density in PDL-coated slide flasks (500 cells/slide flask). Cells were cultured in BS medium containing PDGF without TH (-TH) in 1.5% or 3% O₂
conditions for 7 days at 37°C. The number of ZsGreen+ cells in each clone was counted, and the results are shown in the graphs. Cont; empty vector RetroX-IRES-ZsGreen 1 infected cells, Klf9; Klf9 over expressing cells. P* < 0.001 (unpaired Student’s t-test, n = 3).

(d) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were cultured in BS medium containing PDGF without TH (-TH) or with TH (+TH) in 1.5% O₂ for 1, 4 and 15 days at 37°C, or in 3% O₂ for 15 days at 37°C. Total RNA was prepared from those cells and a RT-PCR assay was carried out. Gapdh (GAPDH) was used as a negative control. The PCR products were detected by 2% agarose gel electrophoresis.

(e) 20,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 3% O₂ for 12 hours at 37°C. Then cells were infected with Runx1 (Runx1b-IRES-ZsGreen) over-expressing retrovirus vector (Runx1) or ZsGreen expressing empty vector (Control) for 4 hours in 3% O₂ at 37°C. Cells were dissociated with trypsin and re-inoculated at clonal density in PDL-coated slide flasks (500 cells/slide flask). These cells were cultured in BS medium containing PDGF with TH (+TH) or without TH (-TH) in 3% O₂ conditions for 7 days at 37°C. The number of ZsGreen+ cells in each clone was counted, and the results are shown in the graphs. Control; empty vector RetroX-IRES-ZsGreen 1 infected cells, Runx1; Runx1 over expressing cells. The number of ZsGreen+ cells in each clone was counted. P* <0.005, P** < 0.001 (unpaired Student’s t-test, n = 3).

(f) OPCs overexpressing Runx1 (green) in (d) were stained with the nuclear stain DAPI (blue) and rabbit anti-p15/INK4b antibodies (red). Scale bar: 100 μm. By using the BZ-II Analyzer software (Keyence), the fluorescent intensities of the staining of anti-p15/INK4b antibodies in each cells were estimated. The average staining intensity of Runx1-overexpressing cells was 22,743 ± 11,425 pxl (n = 29), that was 11-folds higher than that of the non-transformed cells (2,032 ± 3,862 pxl; n = 49).

**Supplementary Figure Legend for Figure 6**

(a) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were cultured in BS medium containing PDGF without TH (-TH) or with
TH (+TH) in 1.5% O2 for 15 days at 37°C. Total RNA was prepared from these cells and a RT-PCR assay for the gene expressions of Hif1α (Hif1α), Epas1 (Hif2α) and Arnt (Hif1β) was carried out. Gapdh (GAPDH) was used as a negative control. The PCR products were detected by 2% agarose gel electrophoresis.

(b) 1,000 of OPCs purified from P7 rat optic nerve were inoculated on PDL/fibronectin coated 12 mm glass-bottom dishes. Cells were cultured in BS medium containing PDGF without TH in 20% O2 for 7 days, and some were then changed to 1.5% O2 for 20 hours. The cells were labeled with rabbit anti-Hif1α antibodies (green) and PI (red). Scale bar: 50 μm.

(c) 1,000 of OPCs purified from P7 rat optic nerve were inoculated on PDL/fibronectin coated 12 mm glass-bottom dishes. Cells were cultured in BS medium containing PDGF, without TH (-TH) or with TH (+TH) in 1.5% O2 for 10 days. The cells were labeled with rabbit anti-Hif2α antibodies (green) and PI (red). To prevent the degradation of Hif2α protein, some cultures were treated with 0.2 mM of CoCl2 (Yuan et al., 2003) for last 7 hours. Scale bar: 50 μm.

(d) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF without TH in 1.5% O2 for 12 days at 37°C. Then the cells were dissociated and harvested. These cells were co-transfected with anti-Hif1α siRNA and pMaxGFP or anti-Hif2α siRNA and pMaxGFP. Cells were re-cultured in PDL-coated slide flasks at clonal-density with BS medium containing PDGF with TH in 1.5% O2 for 4 days at 37°C. The number of GFP+ cells in each clone was counted. si-NT; non-target siRNA, si-Hif1α; anti-Hif1α siRNA, si-Hif2α; anti-Hif2α siRNA. P* <0.05, P** < 0.01 (ANOVA with Fisher’s LSD test, n = 3).

(e) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were cultured in BS medium containing PDGF with TH in 3% O2 or 1.5% O2 conditions at 37°C. For several flasks in 3% O2, DMOG (1 mM) was added. After 7 days, the number of cells in each clone was counted. P* < 0.005, P** < 0.05 (ANOVA with Fisher’s LSD test, n = 3).

(f) 2,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were pre-cultured in BS medium containing PDGF with TH in 3% O2 for 10 days at 37°C. Then, some cells were treated with DMOG (1 mM) for 24 hours. Total RNA was prepared from the cells and a qRT-PCR analysis was carried out. Results
were presented as the relative amount of transcripts to that of the DMOG free culture using comparative ΔΔCt method. Actb is an endogenous negative control. Ldha, Pgk1 and Vegfa are HIFs-inducible positive control. The P values of these genes are P < 0.001 (ANOVA with Fisher’s LSD test, n = 3).

(g) 1,000 of OPCs purified from P7 rat optic nerve were inoculated in PDL-coated slide flasks. Cells were pre-cultured in BS medium containing PDGF with TH in 3% O₂ for 10 days at 37°C. Then, some cells were treated with DMOG (1 mM) for 24 hours. After then, cells were stained with rabbit anti-Runx1 antibodies (green) and PI (red). Scale bars; 50 μm. Note; in the DMOG treated cells (3% O₂ + DMOG), Runx1 protein increased and colocalized with nucleus (yellow).

**Supplementary Figure Legend for Figure 7**

(a) 2,000 of A2B5+/GC- OPCs purified from P14 rat optic nerve were inoculated in PDL-coated T25 flasks. Cells were cultured in BS medium containing PDGF with TH (black bars) or without TH (white bars) in 1.5% O₂ for 13 days at 37°C. After then, the number of cells in each clone was counted and the number of cell divisions was estimated (n = 3). Around 50% of clones contained the cells of which divided less than three times are shown in red square.

(b) P7 OPCs and P14 OPCs were purified from rat optic nerve and their total RNA was prepared immediately. qRT-PCR analysis was carried out. The resulting values were normalized to the endogenous control gene, β-actin (Actb). Results of P14 OPCs are presented as the relative expression to those of P7 OPCs using the ΔΔCt methods. P* <0.001, P** < 0.001 (ANOVA with Fisher’s LSD test, n = 3).

(c) GC-negative optic nerve cells prepared from P14 rat were sorted with A2B5 monoclonal antibody (right panel). These GC+/A2B5+ OPCs were stained for Runx1 and Ki-67 and examined in immunohistochemistry. The percentages of Runx1 expressing cells in Ki-67+ or Ki-67+ OPCs are shown (left graph). *P < 0.01 (unpaired Student’s t-test, n = 10).

(d) Pimonidazole was injected intraperitoneally into P7 rats or P14 rats. 2 hours later, OPCs were purified from the rat optic nerves. These cells were stained for pimonidazole and Ki-67. The percentages of Ki-67+ OPCs that were Pimo− (white bars) or Pimo+ (black bars) at P7 and P14 are shown. *P < 0.001 (unpaired Student’s t-test, n = 4).
Supplementary Figure Legend for Figure 8

(a) 2,000 of OPCs purified from P7 rat cerebral cortex were inoculated in PDL-coated T25 flasks. Cells were cultured in BS medium containing PDGF without TH (-TH; *white bars*) or with TH (+TH; *black bars*), in 5% O₂ or 1.5% O₂ for 11 days at 37°C. Cells were dissociated and the number of cells was counted. 0.01 < *P* < 0.02 (unpaired Student’s *t*-test, *n* = 3).

(b) 1,000 of OPCs purified from P7 rat cerebral cortex were inoculated in PDL-coated slide flasks. OPCs were pre-cultured in BS medium containing PDGF without TH (-TH; *white bars*) or with TH (+TH; *black bars*) in 1.5% O₂ for 10 days at 37°C. The cells were treated with BrdU for 20 hours and labeled with anti-BrdU antibody and Hoechst 33342 dye. The percentage of BrdU⁺ cells was determined. *P* < 0.01 (unpaired Student’s *t*-test, *n* = 3).

(c) 1,000 of OPCs purified from P7 rat cerebral cortex were inoculated in PDL-coated slide flasks. Cells were cultured in BS medium containing PDGF without TH (-TH; *white bars*) or with TH (+TH; *black bars*) 1.5% O₂ for 11 days at 37°C. They were then stained with the nuclear stain DAPI and a monoclonal anti-GC or A2B5 antibody, and the percentage of the DAPI⁺ cells that were GC⁺ or A2B5⁺ was determined (unpaired Student’s *t*-test, *n* = 3).

(d) 2,000 of OPCs purified from P7 mouse optic nerve were inoculated in PDL-coated T25 flasks. Cells were cultured in BS medium containing PDGF without TH (-TH; *white bars*) or with TH (+TH; *black bars*), in either 3% O₂ or 1% O₂ for 12 days at 37°C. The number of cell divisions was estimated from the cell numbers in each clone (*n* = 3).
Fig. 4a Original images

Cyclin D1, Cyclin D2
Cyclin D3
Cyclin E1, Cyclin E2
Cyclin A2
Cyclin B1
Cyclin B2
GAPDH

Fig. 4b Original images

p15/INK4b
p16/INK4a, p19ARF, p18/INK4c
p19/INK4d
p21/Cip1
P27/Kip1
P57/Kip2
GAPDH
Klf9 (1.5% O₂ at day 1 in Fig. 6d on the same gel)

Nkx6.2 (1.5% O₂ at day 1 in Fig. 6d on the same gel)

Rev-erbA

Runx1

Fig. 5b Original images

GAPDH 1.5% O₂ day 1
p15/INK4b 1.5% O₂ day 1
Csrp1 1.5% O₂ day 1
Hif2α 1.5% O₂ day 1
Nkx6.2 1.5% O₂ day 1
Rev-erbA 1.5% O₂ day 1

1.5% O₂ day 1 Runx1
1.5% O₂ day 4 GAPDH
1.5% O₂ day 4 + d15 Csrp1
1.5% O₂ day 4 + day 15 Hif2α

Fig. 5d Original images 1

40
1.5% O₂ day 4 + day 15  
Klf9

1.5% O₂ day 4  
Nkx6.2, Rev-erbA, Runx1

1.5% and 3% O₂ day 15  
GAPDH

1.5% O₂ day 15  
Nkx6.2

1.5% and 3% O₂ day 15  
Csrp1

1.5% O₂ day 15  
Rev-erbA, Runx1

3% O₂ day 15  
p15/INK4b, Hif2α

Fig. 5d Original images 2

3% O₂ day 15  
Klf9, Nkx6.2, Rev-erbA

3% O₂ day 15  
Runx1

Fig. 5d Original images 3

GAPDH  
Hif1α  
Hif1β  
Hif2α

Fig. 6a Original images
Original data of Supplementary Fig. S3b
Original image of Supplementary Fig. S4a.
Original data of Supplementary Fig. S4a
Original data of Supplementary Fig. S4a