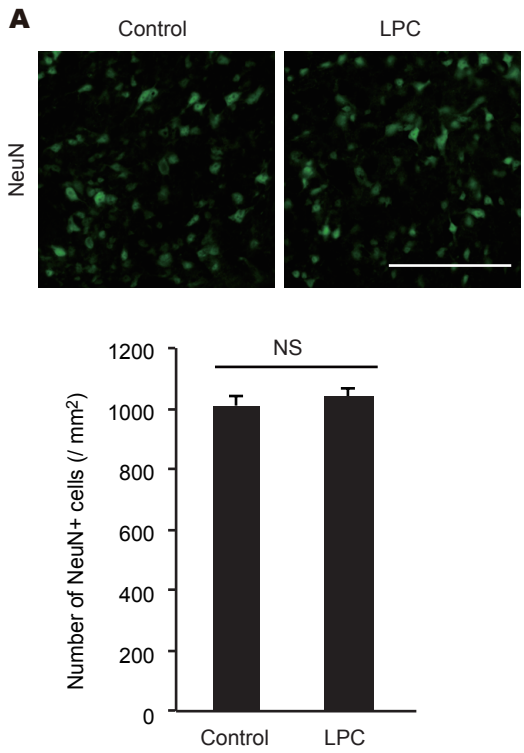
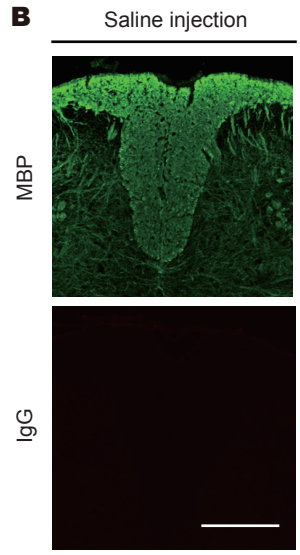
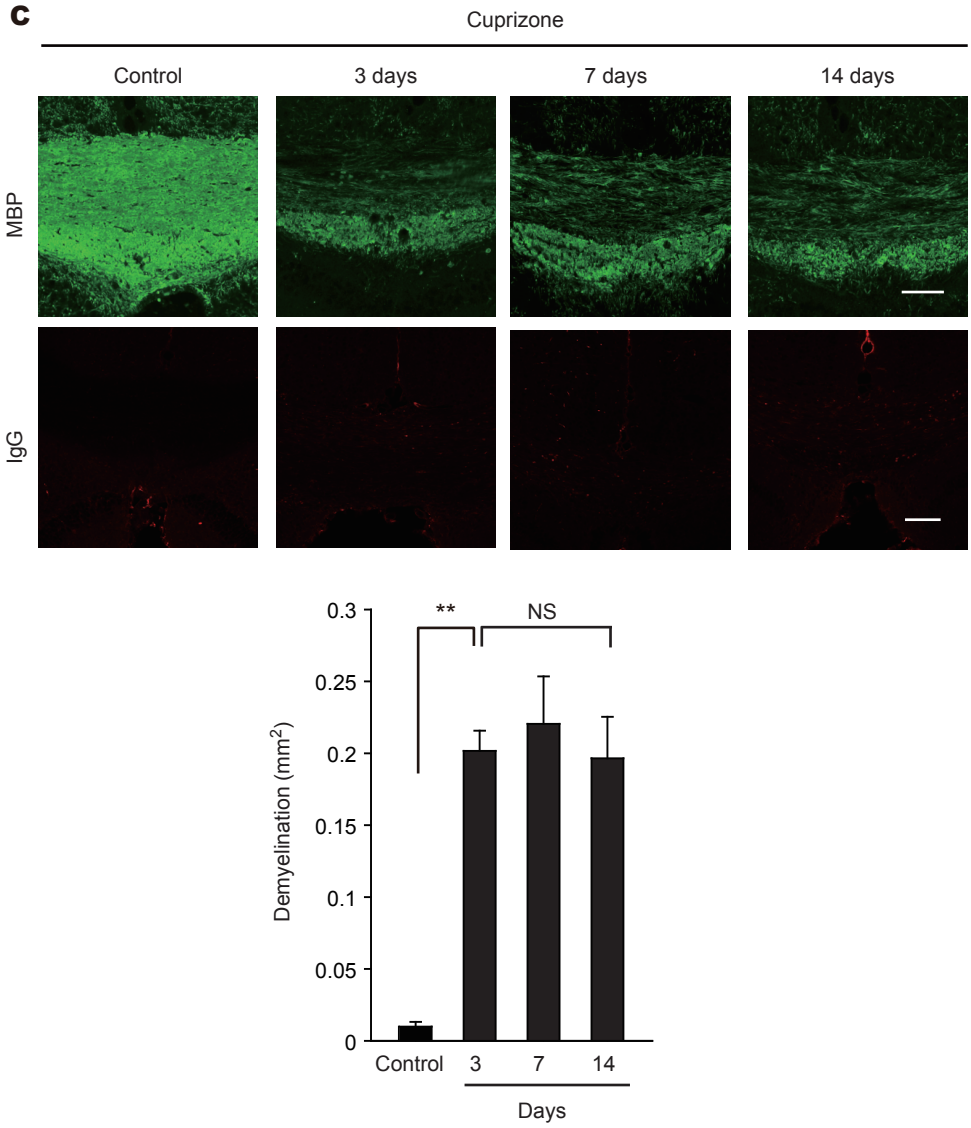
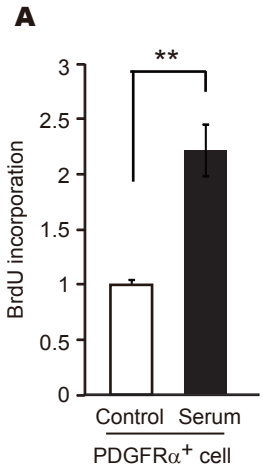

A
Control
LPC
NeuN
NS
Number of NeuN+ cells (/ mm2)
1200
1000
800
600
400
200
0
Control
LPC

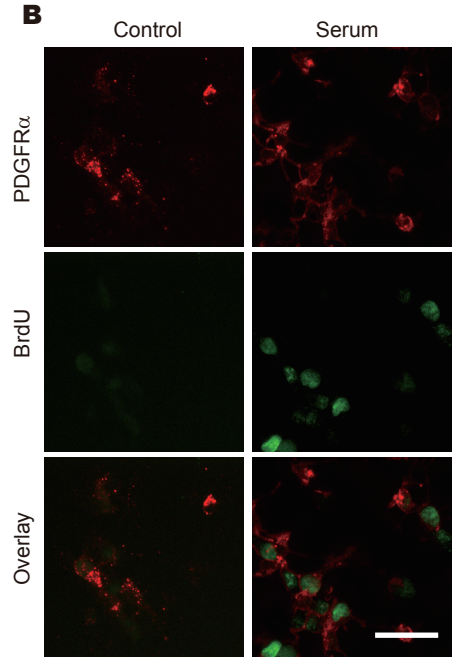

B
Saline injection
MBP
IgG

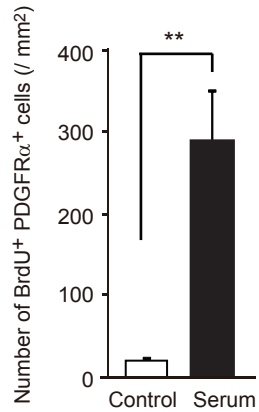

C
Cuprizone
Control
3 days
7 days
14 days
MBP
IgG
**
NS
Demyelination (mm2)
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
3
7
14
Days

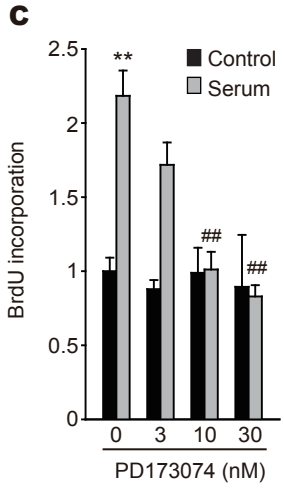
**Supplemental Figure 1 Time course of vascular barrier disruption and remyelination.**

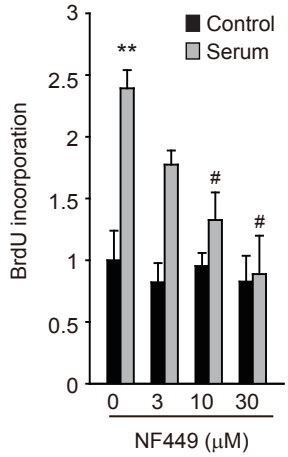
**(A)** Representative image of spinal cord sections labeled with NeuN. Sections were prepared 3 days after LPC injection. Graph show $NeuN^+$ cell density in the spinal cord section (n= 4 for Control, n= 6 for LPC); $P$ = 0.70913. **(B)** Representative images of spinal cord sections visualized with MBP (upper panel) and endogenous IgG (lower panel). Sections were prepared 3 days after saline injection. **(C)** Representative images of brain sections visualized with MBP (upper panel) and endogenous IgG (lower panel) the indicated number of days after removal of the cuprizone diet. Graphs show the time course of demyelination the indicated number of days after removal of the cuprizone diet (n = 3 each); $P$ = 0.0017 as determined by Student's *t*-test or ANOVA with Tukey's test. Error bars represent s.e.m. NS indicates no significant difference. Scale bars: 100 μm for **A** and **B**, 50 μm for **C**.

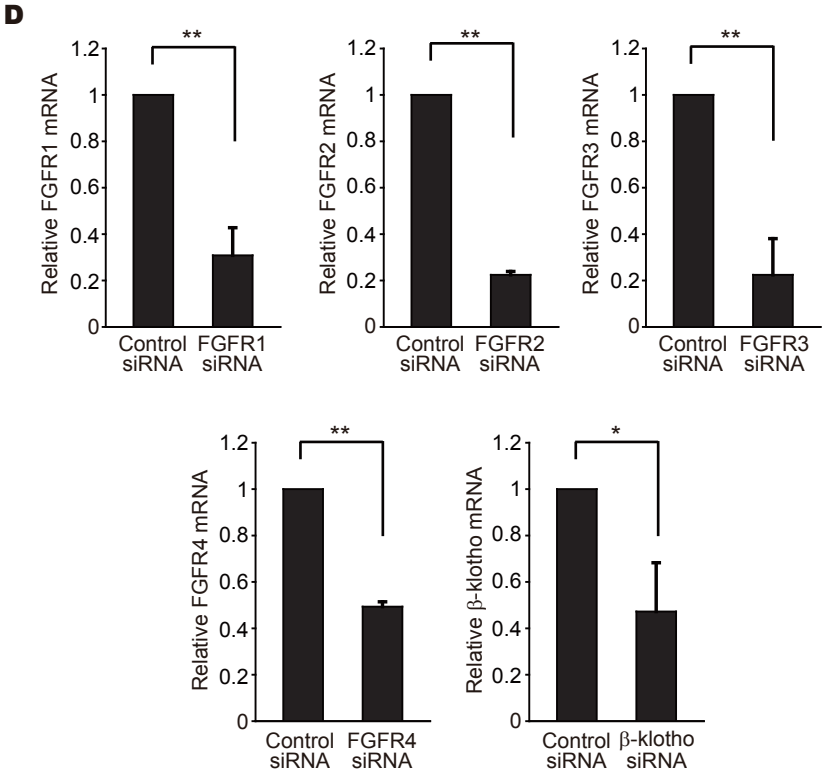

A
**
BrdU incorporation
3
2.5
2
1.5
1
0.5
0
Control
Serum
PDGFRα+ cell


B
Control
Serum
PDGFRα
BrdU
Overlay


**
Number of BrdU+ PDGFRα+ cells (/ mm2)
400
300
200
100
0
Control
Serum

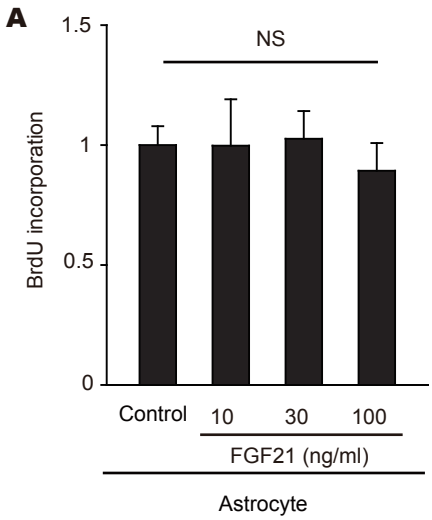

C
**
Control
Serum
BrdU incorporation
2.5
2
1.5
1
0.5
0
##
##
0
3
10
30
PD173074 (nM)

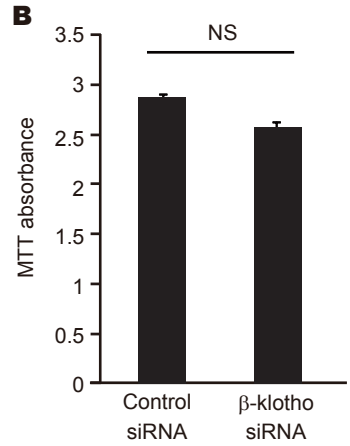

**
Control
Serum
BrdU incorporation
3
2.5
2
1.5
1
0.5
0
#
#
0
3
10
30
NF449 (μM)

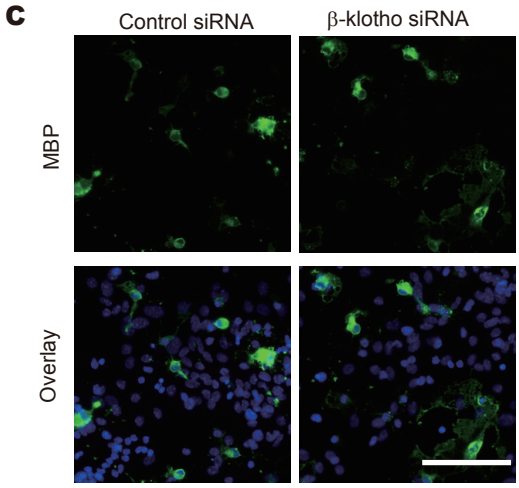

D
**
Relative FGFR1 mRNA
Control siRNA
FGFR1 siRNA
**
Relative FGFR2 mRNA
Control siRNA
FGFR2 siRNA
**
Relative FGFR3 mRNA
Control siRNA
FGFR3 siRNA
**
Relative FGFR4 mRNA
Control siRNA
FGFR4 siRNA
*
Relative β-klotho mRNA
Control siRNA
β-klotho siRNA
1.2
1
0.8
0.6
0.4
0.2
0

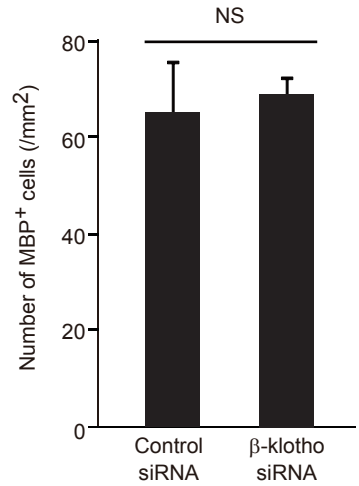
**Supplemental Figure 2 FGF21 signaling is involved in circulating factor–mediated OPC proliferation.**

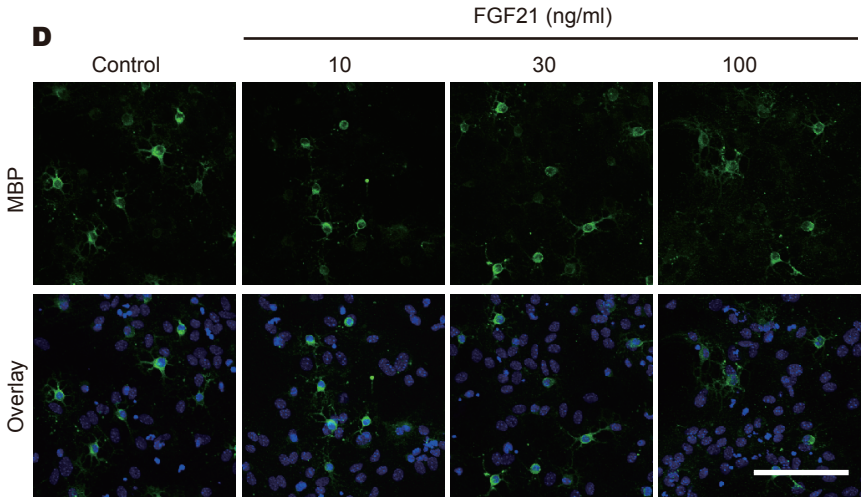
**(A)** Concentration-dependence of BrdU incorporation in PDGFRα$^+$ OPCs cultured with adult mouse serum (n = 6); $P < 0.0001$. **(B)** Representative images of OPC culture isolated using A2B5-positive microbeads. Cells were cultured with adult mouse serum, and then double-labeled for PDGFRα and BrdU. No cells that were double-labeled for GFAP and BrdU were detected in the culture. Graphs show quantitation as indicated in each images. (n = 9); $P < 0.0001$. **(C)** BrdU incorporation in OPCs after serum stimulation with PD173074 (left, $n = 4$) and NF449 (right, $n = 4$); $P < 0.0001$ (left graph), $P = 0.0009$, 0.0231, 0.0013 (right graph, left to right). #, ## compared with serum treatment. **(D)** Relative expression of FGFRs mRNA in OPCs after transfection with siRNAs against the indicated FGFRs. A2B5$^+$ cells were isolated 48 h after transfection (n = 3 for FGFR1, n = 4 for FGFR2, n = 5 for FGFR3, n = 3 for FGFR4, n = 4 for β-klotho); $P = 0.0022$, 0.0001, 0.001, 0.0001, 0.023 (left to right) as determined by Student's t-test, ANOVA with Tukey's post-hoc test. Error bars represent s.e.m. NS indicates not significant. Scale bars: 50 μm.

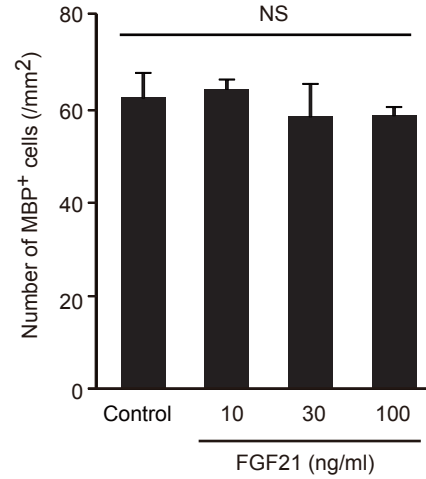

A
NS
BrdU incorporation
1.5
1
0.5
0
Control
10
30
100
FGF21 (ng/ml)
Astrocyte

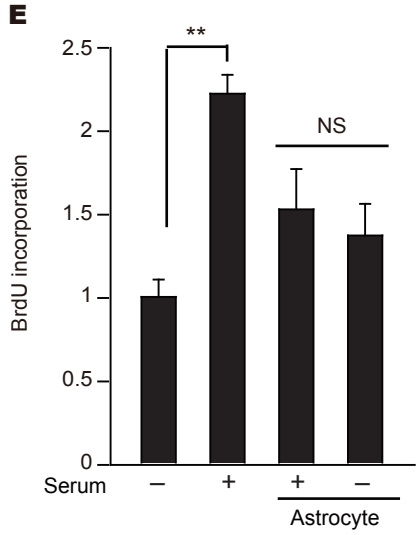

B
NS
MTT absorbance
3.5
3
2.5
2
1.5
1
0.5
0
Control siRNA
β-klotho siRNA


C
Control siRNA
β-klotho siRNA
MBP
Overlay


NS
Number of MBP+ cells (/mm2)
80
60
40
20
0
Control siRNA
β-klotho siRNA

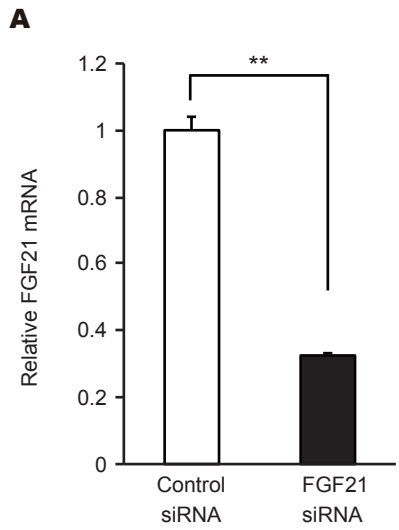

D
FGF21 (ng/ml)
Control
10
30
100
MBP
Overlay

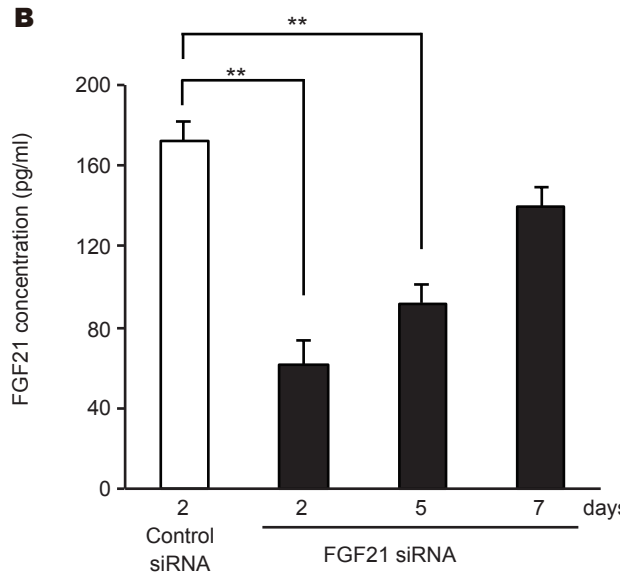

NS
Number of MBP+ cells (/mm2)
80
60
40
20
0
Control
10
30
100
FGF21 (ng/ml)


E
**
NS
BrdU incorporation
2.5
2
1.5
1
0.5
0
Serum
−
+
+
−
Astrocyte

**Supplemental Figure 3 Astrocyte is not involved in FGF21-mediated OPC proliferation.**

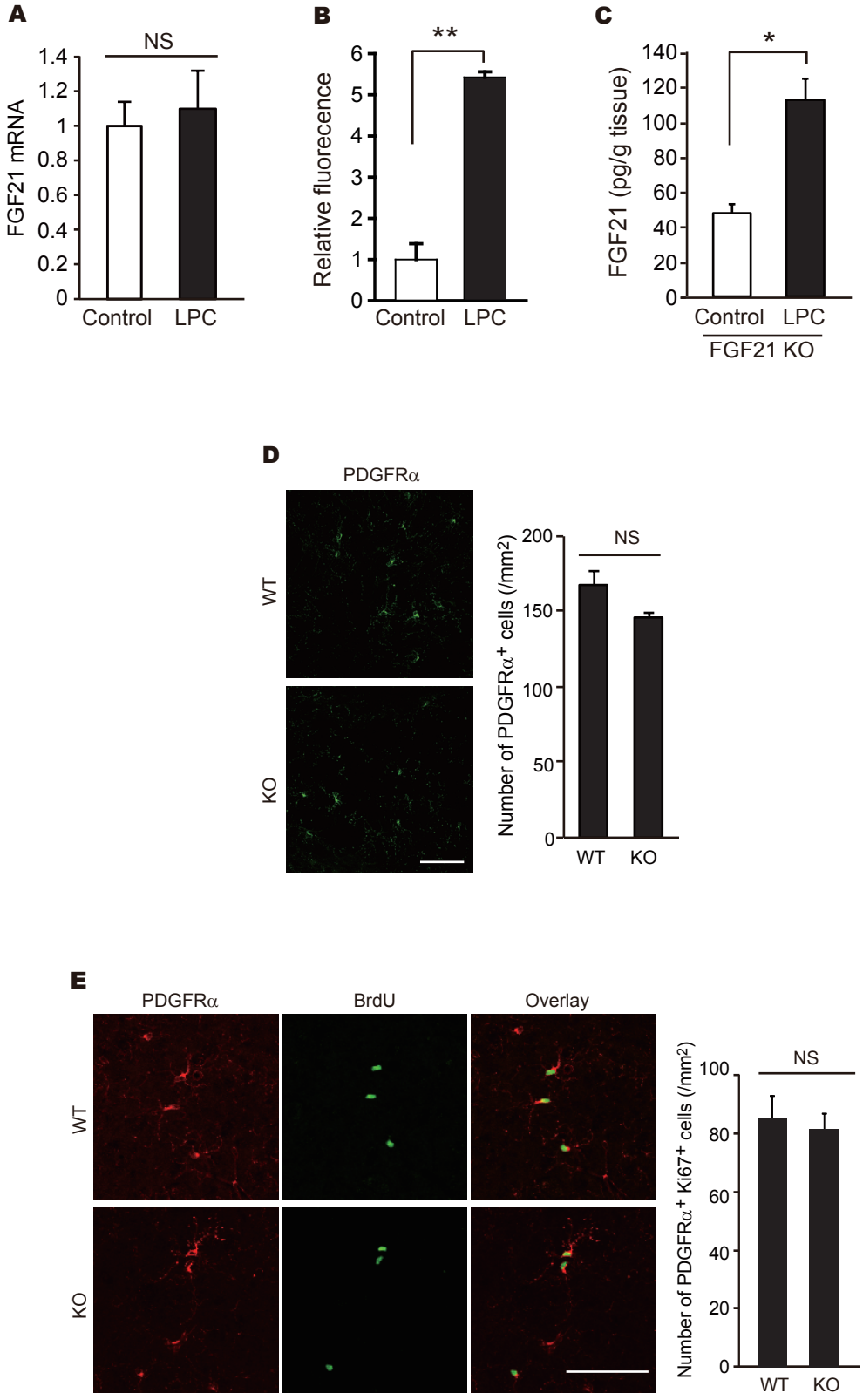
**(A)** BrdU incorporation in astrocyte 3 days after stimulation with the indicated concentrations of FGF21 (n = 4), *P* =0.8956. **(B)** Change in MTT reduction in OPCs 48 h after β-klotho siRNA transfection (n = 6); *P* = 0.7936. **(C)** Representative images of cultures labeled for MBP and DAPI. OPCs were transfected with β-klotho siRNA. Graphs show quantitation as indicated in each image (n = 4); *P* = 0.4044. **(D)** Representative images of culture labeled for MBP and DAPI. OPCs were treated with recombinant FGF21. Graphs show quantitation as indicated in each images (n = 3); *P* = 0.9321, 0.6148, 0.6744 (left to right). **(E)** BrdU incorporation in $A2B5^+$ OPCs 3 days after stimulation with astrocyte supernatant. Astrocytes were cultured for 1 day with or without stimulation with adult mouse serum. After culture, the astrocyte supernatant was collected and added into the $A2B5^+$ cell culture (n = 6); *P* = 0.0002 as determined by Student's *t*-test, ANOVA with Tukey's post-hoc test. Error bars represent s.e.m. NS indicates not significant. Scale bars: 50 μm.


A
**
1.2
1
0.8
0.6
0.4
0.2
0
Relative FGF21 mRNA
Control siRNA
FGF21 siRNA


B
**
**
200
160
120
80
40
0
FGF21 concentration (pg/ml)
2
2
5
7
days
Control siRNA
FGF21 siRNA

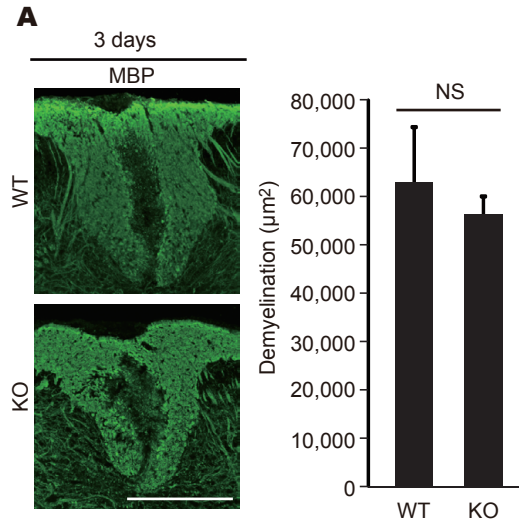
**Supplemental Figure 4 FGF21 in the CNS from the circulation.**

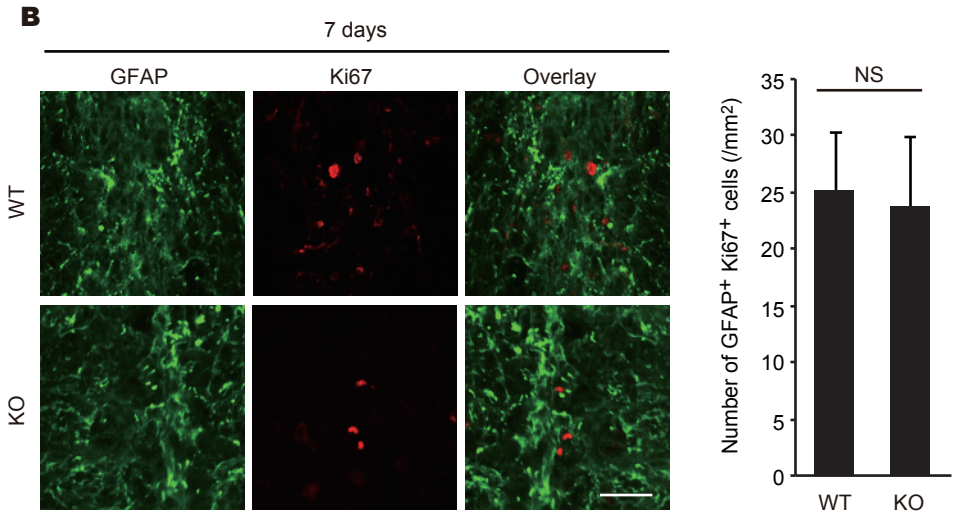
**(A)** Relative expression of FGF21 mRNA in pancreas 2 days after siRNA transfection (n = 3); $P$ = 0.0001. **(B)** FGF21 content in serum of mice that received FGF21 siRNA transfection (n = 3 for control siRNA, 5 for other groups). Serum was prepared the indicated number of days after siRNA injection; $P$ = 0.0004, 0.0047 (left to right) as determined by Student's *t*-test or ANOVA with Dunnett's test. Error bars represent s.e.m.


A
NS
FGF21 mRNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Control
LPC
B
**
Relative fluorecence
6
5
4
3
2
1
0
Control
LPC
C
*
FGF21 (pg/g tissue)
140
120
100
80
60
40
20
0
Control
LPC
FGF21 KO
D
PDGFRα
WT
KO
NS
Number of PDGFRα+ cells (/mm2)
200
150
100
50
0
WT
KO
E
PDGFRα
BrdU
Overlay
WT
KO
NS
Number of PDGFRα+ Ki67+ cells (/mm2)
100
80
60
40
20
0
WT
KO

**Supplemental Figure 5 Peripheral Fgf21 promotes OPC proliferation and remyelination.**

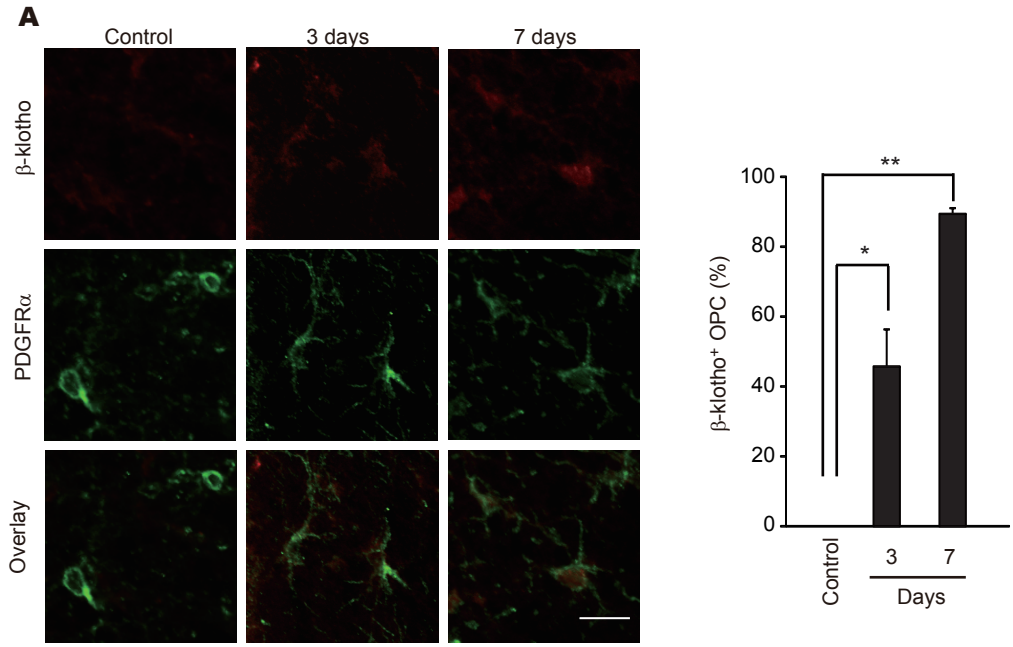
**(A)** Quantitations of *Fgf21* mRNA in the spinal cord 3 days after LPC injection ($n = 4$); $P = 0.0353$. NS indicates not significant. **(B)** Relative intensity of fluorescence in the spinal cord 3 days after LPC injection. WT mice intravenously received recombinant FGF21 which is labeled with HiLyte Fluor 555 (n =3); $P = 0.004$. **(C)** Measurement of FGF21 in spinal cord (pg/g) of *Fgf21*-knockout mice ($n = 3$) infused subcutaneously with FGF21 for 7 days; $P = 0.0191$. **(D)** Representative images of spinal cord sections labeled for PDGFRα. Spinal cord sections were obtained from *Fgf21*-knockout mice and control littermates. Graphs show quantitations as indicated in the images (n = 4); $P = 0.1091$. NS indicates not significant. **(E)** Representative images of spinal cord sections double-labeled for PDGFRα and BrdU. Spinal cord sections were obtained from *Fgf21*-knockout mice and control littermates. Graphs show quantitations as indicated in the images (n = 3); $P = 0.7953$. **(F)** Representative images of spinal cord sections labeled for MBP. Spinal cord sections were obtained from *Fgf21*-knockout mice and control littermates. Graphs show quantitations as indicated in the images (n = 4); $P = 0.5106$ as determined by Student's *t*-test. Error bars represent s.e.m. NS indicates not significant. Scale bars: 50 μm for **D** and **E**, 200 μm for **F**.

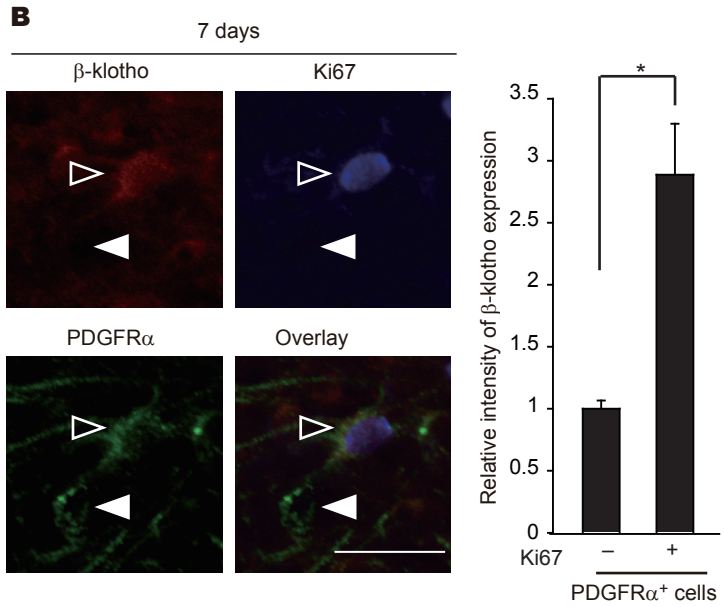

A
3 days
MBP
WT
KO
NS
Demyelination (μm²)
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
0
WT
KO

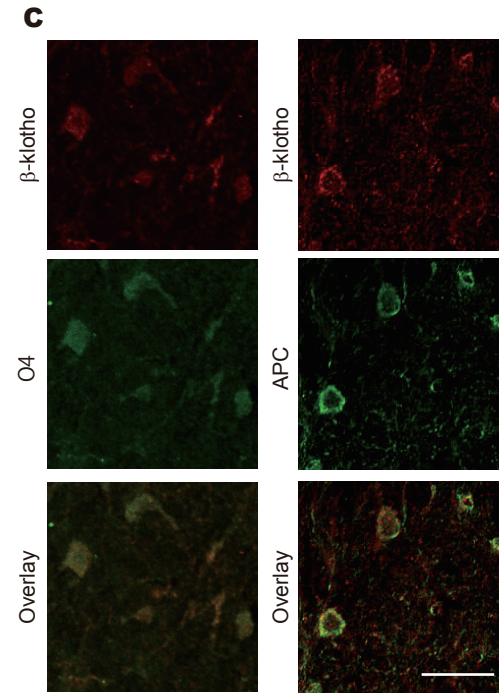

B
7 days
GFAP
Ki67
Overlay
WT
KO
NS
Number of GFAP+ Ki67+ cells (/mm²)
35
30
25
20
15
10
5
0
WT
KO

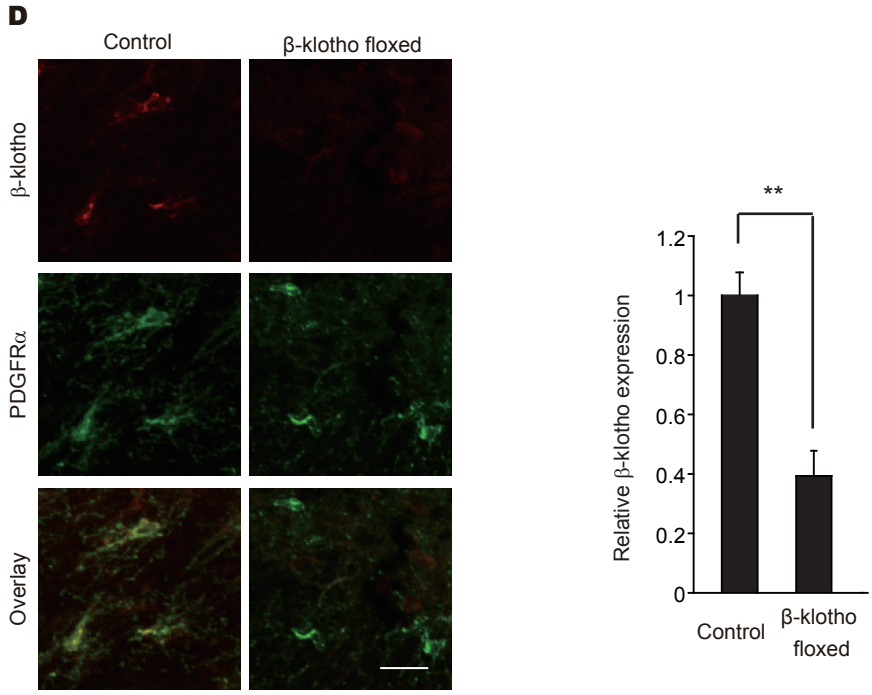
**Supplemental Figure 6 Peripheral Fgf21 does not associate with demyelination.**

**(A)** Representative images of spinal cord sections labeled for MBP 3 days after LPC injection. Spinal cord sections were obtained from *Fgf21*-knockout mice and control littermates. Graphs show quantitations as indicated in the images (n = 3); $P = 0.45144$. **(B)** Representative images of spinal cord sections labeled for GFAP 7 days after LPC injection. Spinal cord sections were obtained from *Fgf21*-knockout mice and control littermates. Graphs show quantitations as indicated in the images (n = 3); $P = 0.8851$ as determined by Student's *t*-test. Error bars represent s.e.m. NS indicates not significant. Scale bars: 200 μm for **A**, 50 μm for **B**.


A
Control
3 days
7 days
β-klotho
PDGFRα
Overlay
β-klotho+ OPC (%)
100
80
60
40
20
0
**
*
Control
3
7
Days

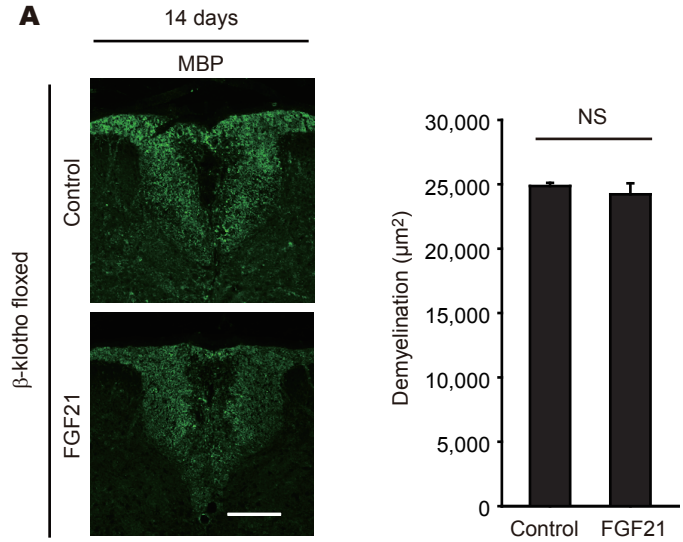

B
7 days
β-klotho
Ki67
PDGFRα
Overlay
Relative intensity of β-klotho expression
3.5
3
2.5
2
1.5
1
0.5
0
*
Ki67
–
+
PDGFRα+ cells

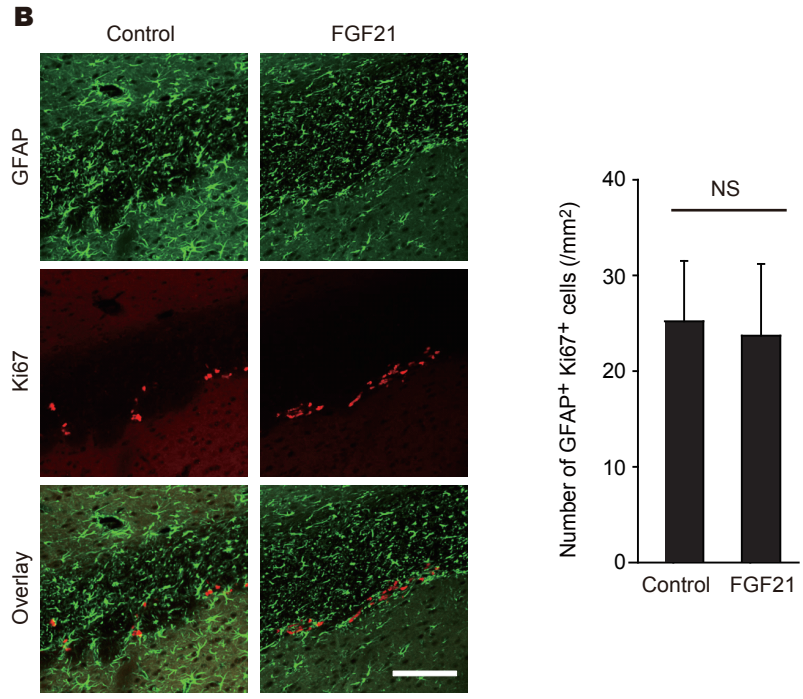

C
β-klotho
β-klotho
O4
APC
Overlay
Overlay


D
Control
β-klotho floxed
β-klotho
PDGFRα
Overlay
Relative β-klotho expression
1.2
1
0.8
0.6
0.4
0.2
0
**
Control
β-klotho floxed

**Supplemental Figure 7 β-klotho expression is involved in OPC proliferation after demyelination.**

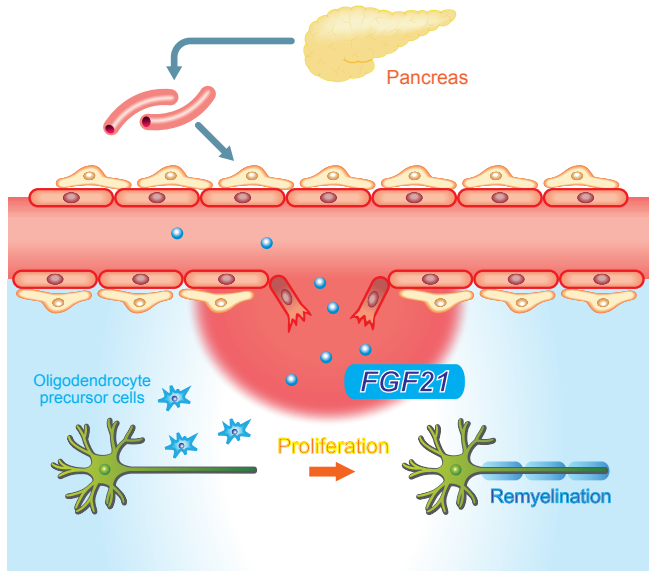
**(A)** Representative images of spinal cord sections double-labeled for PDFGRα and β-klotho. Graphs show quantitation as indicated in the images (n = 3); $P$ = 0.0145, 0.0005 (left to right). **(B)** Representative images of spinal cord sections triple-labeled for PDFGRα, Ki67, and β-klotho. Spinal cord sections were obtained 7 days after LPC injection. Graphs show quantitation as indicated in the images (n = 3); $P$ = 0.011. Open arrowhead indicates a Ki67-positive cell labeled with PDGFRα. White arrowhead indicates a Ki67-negative cell labeled with PDGFRα. **(C)** Representative image of spinal cord sections double-labeled for O4 (left) or APC (right) with β-klotho. Spinal cords were obtained 3 days after LPC injection. **(D)** Relative intensity of β-klotho protein expression in PDGFRα-positive OPCs obtained from spinal cords of β-klotho-conditional knockout mice 7 days after LPC injection ($n$ = 3 each); $P$ = 0.0062 as determined by Student's $t$-test or ANOVA with Dunnett's test. Error bars represent s.e.m. NS indicates not significant. Scale bars: 20 μm for **A**, **B** and **D**, 50 μm for **C**.


A
14 days
MBP
β-klotho floxed
Control
FGF21
NS
Demyelination (μm²)
30,000
25,000
20,000
15,000
10,000
5,000
0
Control
FGF21


B
Control
FGF21
GFAP
Ki67
Overlay
NS
Number of GFAP⁺ Ki67⁺ cells (/mm²)
40
30
20
10
0
Control
FGF21

**Supplemental Figure 8 β-klotho expression in OPC is essential for remyelination by peripheral hormone.**

**(A)** Representative images of spinal cord sections labeled for MBP. Spinal cord sections were obtained from β-klotho-conditional knockout mice with or without FGF21 treatment. Graphs show quantitations as indicated in the images (n = 4); $P = 0.4997$. **(B)** Representative images of brain sections double-labeled for GFAP and Ki67. Brain sections were obtained 7 days after injury. Graphs show quantitations as indicated in the images (n = 3); $P = 0.8851$ as determined by Student's *t*-test. Error bars represent s.e.m. NS indicates not significant. Scale bars: 20 μm for 200 μm for **A**, 100 μm for **B**.


Pancreas
FGF21
Oligodendrocyte
precursor cells
Proliferation
Remyelination

**Supplemental Figure 9 Diagrammatic illustration of this study.**

CNS is isolated from peripheral milieu by the vascular barrier. Therefore, CNS regeneration has been thought to be controlled by the CNS microenvironment *in situ*. However, CNS injury disrupts the vascular barrier, leading to leakage of circulating factors into the CNS. One of these factors, FGF21 expressed in pancreas, promotes OPC proliferation and subsequent remyelination. We propose that peripheral milieu regulates CNS regeneration.

| Inhibitor | Inhibition rate (%) |
|---|---|
| ATM Kinase | 118.33 |
| ATM/ ATR Kinase Inhibitor | -51.37 |
| AG 1024 | 94.95 |
| AG L 2043 | 135.01 |
| Akt Inhibitor IV | -27.87 |
| Akt Inhibitor V, Triciribine | 127.38 |
| Akt Inhibitor VIII, Isozyme-Selective, | -27.53 |
| Akt Inhibitor X | -52.15 |
| Aminopurvalanol A | 131.09 |
| AMPK Inhibitor, Compound C | 134.93 |
| Aurora Kinase Inhibitor II | 117.20 |
| Aurora Kinase Inhibitor III | 116.92 |
| Aurora Kinase/Cdk Inhibitor | 112.71 |
| BAY11-7082 | 134.01 |
| Bcr-abl Inhibitor | 2.25 |
| Bisindolylmaleimide I | 135.82 |
| Bisindolylmaleimide IV | 119.54 |
| Bohemine | 148.06 |
| BPIQ-I | 117.06 |
| Cdk1 Inhibitor | 133.30 |
| Cdk1 Inhibitor, CGP74514A | 82.28 |
| Cdk1/2 Inhibitor III | 79.87 |
| Cdk1/5 Inhibitor | 93.16 |
| Casein Kinase I Inhibitor, D4476 | 109.62 |
| Casein Kinase II Inhibitor III, TBCA | 101.96 |
| Cdk4 Inhibitor II, NSC 625987 | 102.37 |
| Cdk4 Inhibitor III | -21.85 |
| Cdc2-Like Kinase Inhibitor, TG003 | 100.06 |
| Cdk/Crk Inhibitor | 95.33 |
| Chelerythrine Chloride | -38.82 |
| Chk2 Inhibitor II | 73.83 |
| Compound 52 | 127.38 |
| Compound 56 | 105.07 |
| Cdk2 Inhibitor III | 133.70 |
| Cdk2 Inhibitor IV, NU6140 | -18.97 |
| DNA-PK Inhibitor II | 95.87 |
| DNA-PK Inhibitor III | 87.55 |
| DNA-PK Inhibitor V | 88.99 |
| Diacylglycerol Kinase Inhibitor II | -36.30 |
| DMBI | 96.66 |
| EGFR Inhibitor | -38.06 |
| EGFR/ErbB-2/ErbB-4 Inhibitor | 152.83 |
| ERK Inhibitor II, FR180204 | 105.79 |
| ERK Inhibitor II, Negative control | 125.28 |
| ERK Inhibitor III | 95.03 |
| Fascaplysin, Synthetic | -32.44 |
| Flt-3 Inhibitor | 108.46 |
| Flt-3 Inhibitor II | 104.42 |
| Flt-3 Inhibitor III | 87.06 |
| GSK-3b Inhibitor I | -44.49 |
| GSK-3b Inhibitor II | 103.53 |
| GSK-3b Inhibitor VIII | 150.74 |
| GSK-3b Inhibitor XI | 175.07 |
| GSK3b Inhibitor XII, TWS 119 | 156.94 |
| GSK-3 Inhibitor XIII | 191.45 |
| Gö 6983 | 111.18 |
| GTP-14564 | 107.52 |
| Isogranulatimide | 121.86 |
| H-89, Dihydrochloride | 105.79 |
| HA 1077, Dihydrochloride | 113.76 |
| Herbimycin A, Streptomyces sp. | 56.24 |
| IC261 | 126.31 |
| IKK-2 Inhibitor IV | 124.55 |
| Indirubin Derivative E804 | -40.71 |
| JAK3 Inhibitor II | 120.53 |

| Inhibitor | Inhibition rate (%) |
|---|---|
| JNK Inhibitor II | 49.98 |
| JAK3 Inhibitor IV | -20.49 |
| JNK Inhibitor, Negative Control | -10.53 |
| IGF-1R Inhibitor II | -40.63 |
| JNK Inhibitor V | -22.59 |
| JNK Inhibitor VIII | 90.00 |
| K-252a, Nocardiopsis sp. | -21.99 |
| KN-93 | -21.09 |
| Lck Inhibitor | -21.96 |
| LY 294002 | 8.32 |
| LY 303511- Negative control | 117.54 |
| MEK Inhibitor I | -12.97 |
| MEK Inhibitor II | 121.32 |
| MEK1/2 Inhibitor | 37.69 |
| MNK1 Inhibitor | 29.47 |
| MK2a Inhibitor | -0.45 |
| NF449 | 26.31 |
| NF-kB Activation Inhibitor | -6.45 |
| p38 MAP Kinase Inhibitor III | -13.76 |
| p38 MAP Kinase Inhibitor | 29.46 |
| PD 98059 | 52.55 |
| PD 169316 | 12.03 |
| PD 158780 | 8.99 |
| PD 173074 | 19.49 |
| PD 174265 | -16.49 |
| PDGF Receptor Tyrosine Kinase | -73.53 |
| PDGF Receptor Tyrosine Kinase | -24.1 |
| PDGF RTK Inhibitor | -67.15 |
| PDK1/Akt/Flt Dual Pathway Inhibitor | -31.40 |
| PKR Inhibitor | -85.61 |
| PKR Inhibitor, Negative Control | -28.16 |
| PI-103 | -68.06 |
| PI 3-Kg Inhibitor | 53.20 |
| PI 3-Kg Inhibitor II | 19.57 |
| SB 218078 | -34.31 |
| SC-68376 | 131.22 |
| SKF-86002 | 56.82 |
| Sphingosine Kinase Inhibitor | -32.77 |
| Src Kinase Inhibitor I | -51.58 |
| Staurosporine, Streptomyces sp. | -33.01 |
| STO-609 | 133.24 |
| SU6656 | 84.14 |
| SU9516 | 37.45 |
| SU 11652 | -32.69 |
| Syk Inhibitor | 110.96 |
| Syk Inhibitor II | 40.63 |
| Syk Inhibitor III | 91.39 |
| Tpl2 Kinase Inhibitor | 123.67 |
| TGF-b RI Kinase Inhibitor | 68.65 |
| TGF-b RI Inhibitor III | -28.28 |
| AG 9 | 63.04 |
| AG 490 | -27.77 |
| AG 112 | 89.44 |
| AG 1295 | 96.15 |
| AG 1296 | 63.69 |
| AG 1478 | 64.42 |
| VEGF Receptor 2 Kinase Inhibitor I | 59.23 |
| VEGF Receptor Tyrosine Kinase | 30.61 |
| VEGFR Tyrosine Kinase Inhibitor IV | -24.64 |
| VEGF Receptor 2 Kinase Inhibitor II | 95.16 |
| VEGF Receptor 2 Kinase Inhibitor III | 42.94 |
| VEGF Receptor 2 Kinase Inhibitor IV | 82.36 |
| Wortmannin | 62.02 |
| ROCK Inhibitor, Y-27632 | 134.25 |

**Supplemental Table 1 Pharmacological screening of BrdU incorporation into OPCs after serum treatment.**

Inhibitors, except for PD173074 (10 nM), were used at a final concentration of 10 μM. Inhibition ratios of BrdU incorporation was calculated by the following formula: Inhibition ratio = (Absorbance of the sample treated with serum and indicated inhibitor − Absorbance of the sample treated indicated inhibitor) / (Absorbance of the sample treated with serum − Absorbance of the control) × 100. A lower value indicates more inhibition.