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Supplementary Materials for

BIG1/Arfgef1 and Arf1 regulate the initiation of myelination by Schwann cells in mice

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Published 4 April 2018, *Sci. Adv.* **4**, eaar4471 (2018)
DOI: 10.1126/sciadv.aar4471

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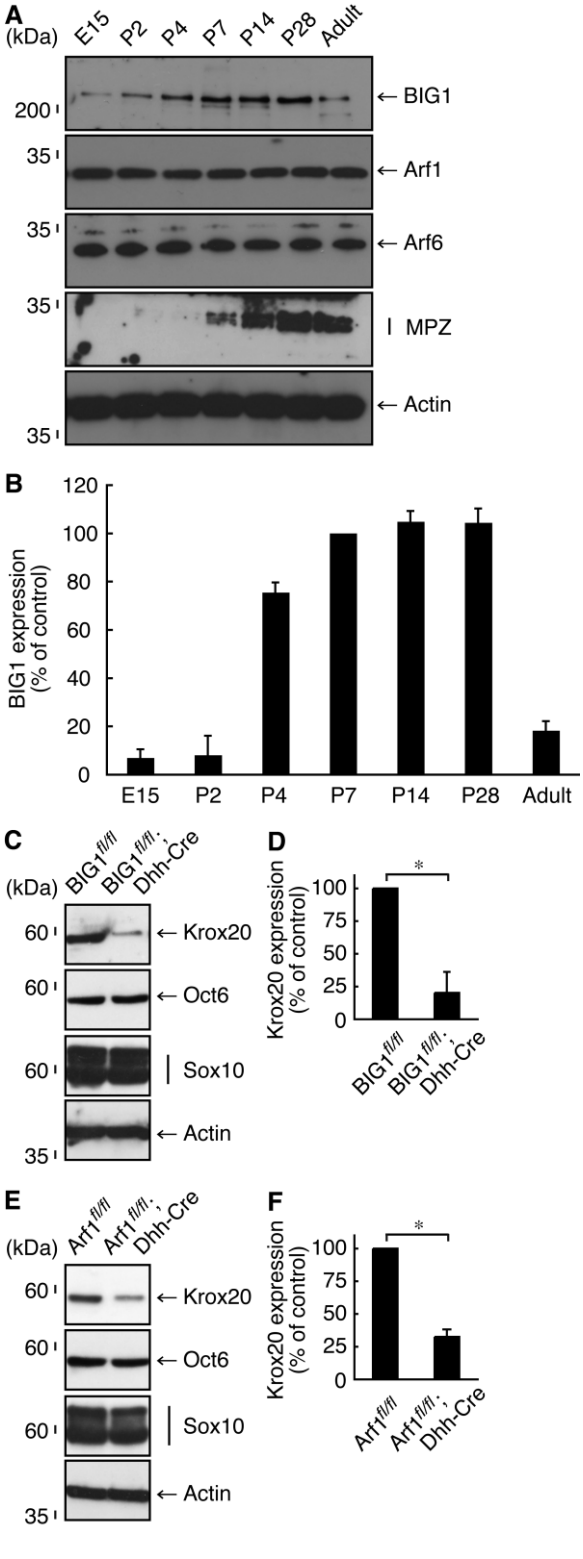
A
(kDa)
E15
P2
P4
P7
P14
P28
Adult
200
35
35
35
35
← BIG1
← Arf1
← Arf6
MPZ
← Actin
B
BIG1 expression (% of control)
120
100
80
60
40
20
0
E15
P2
P4
P7
P14
P28
Adult
C
(kDa)
BIG1fl/fl
BIG1fl/fl.; Dhh-Cre
60
60
60
35
← Krox20
← Oct6
Sox10
← Actin
D
Krox20 expression (% of control)
100
75
50
25
0
*
BIG1fl/fl
BIG1fl/fl.; Dhh-Cre
E
(kDa)
Arf1fl/fl
Arf1fl/fl.; Dhh-Cre
60
60
60
35
← Krox20
← Oct6
Sox10
← Actin
F
Krox20 expression (% of control)
100
75
50
25
0
*
Arf1fl/fl
Arf1fl/fl.; Dhh-Cre

fig. S1. Changes in expression levels of BIG1, the related proteins, and myelin marker protein MPZ during sciatic nerve development and the effect of BIG1 or Arf1 knockout on myelination-related transcription factor Krox20 expression. (**A, B**) Sciatic nerve tissues from embryonic day 15 (E15) to postnatal days 2 to 28 (P2 to P28) and adult stages were isolated, lysed, and used for immunoblotting with the respective antibodies for BIG1, Arf1, Arf6, MPZ, and control actin. BIG1 expression levels are statistically shown (n=3 blots). (**C, D**) Sciatic nerve tissue lysates of conditional knockout (*BIG1$^{fl/fl}$; Dhh-Cre*) and control mice at 7 days postnatal were used for immunoblotting with the respective antibodies for Krox20, Oct6, Sox10, and actin. Krox20 expression levels are statistically shown (*, $p<0.01$; n=3 blots). (**E, F**) Sciatic nerve tissue lysates of conditional knockout (*Arf1$^{fl/fl}$; Dhh-Cre*) and control mice at 7 days postnatal were used for immunoblotting with the respective antibodies for Krox20, Oct6, Sox10, and actin. Krox20 expression levels are statistically shown (*, $p<0.01$; n=3 blots).

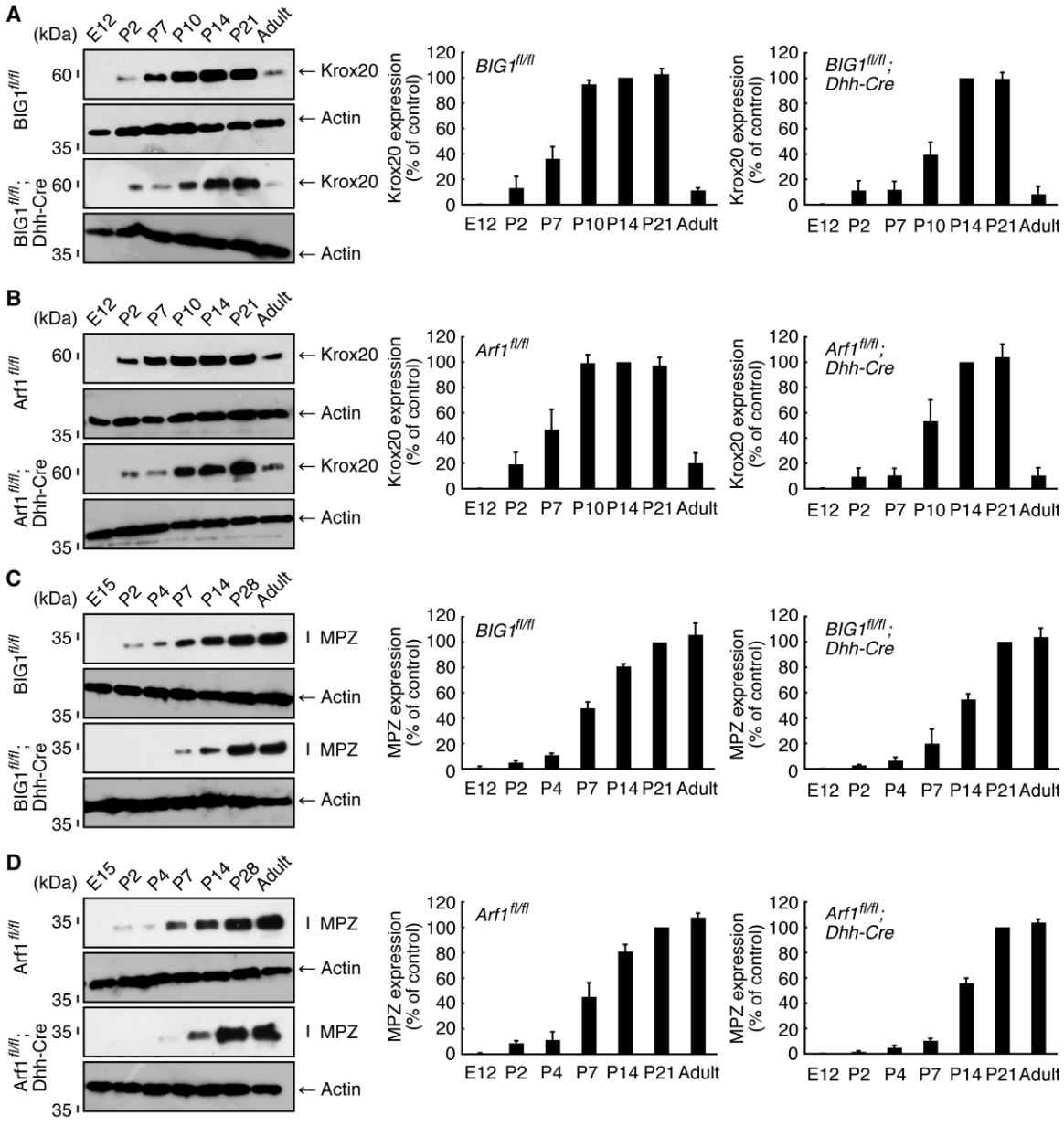

fig. S2. Effects of knockout of BIG1 or Arf1 on Krox20 or MPZ expression during development. (**A, B**) Sciatic nerve tissues from embryonic day 12 (E12) to adult stages in conditional knockout (*BIG1$^{fl/fl}$; Dhh-Cre* or *Arf1$^{fl/fl}$; Dhh-Cre*) and control mice were

isolated, lysed, and used for immunoblotting with the respective antibodies for Krox20 and actin. The blots in similar experiments were exposed at the same time. Krox20 expression levels are statistically shown (n=3 blots). (**C, D**) Sciatic nerve tissues from embryonic day 15 (E15) to adult stages in conditional knockout (*BIG1*$^{fl/fl}$*; Dhh-Cre* or *Arfl*$^{fl/fl}$*; Dhh-Cre*) and control mice were isolated, lysed, and used for immunoblotting with the respective antibodies for MPZ and actin. The blots in similar experiments were exposed at the same time. MPZ expression levels are statistically shown (n=3 blots).

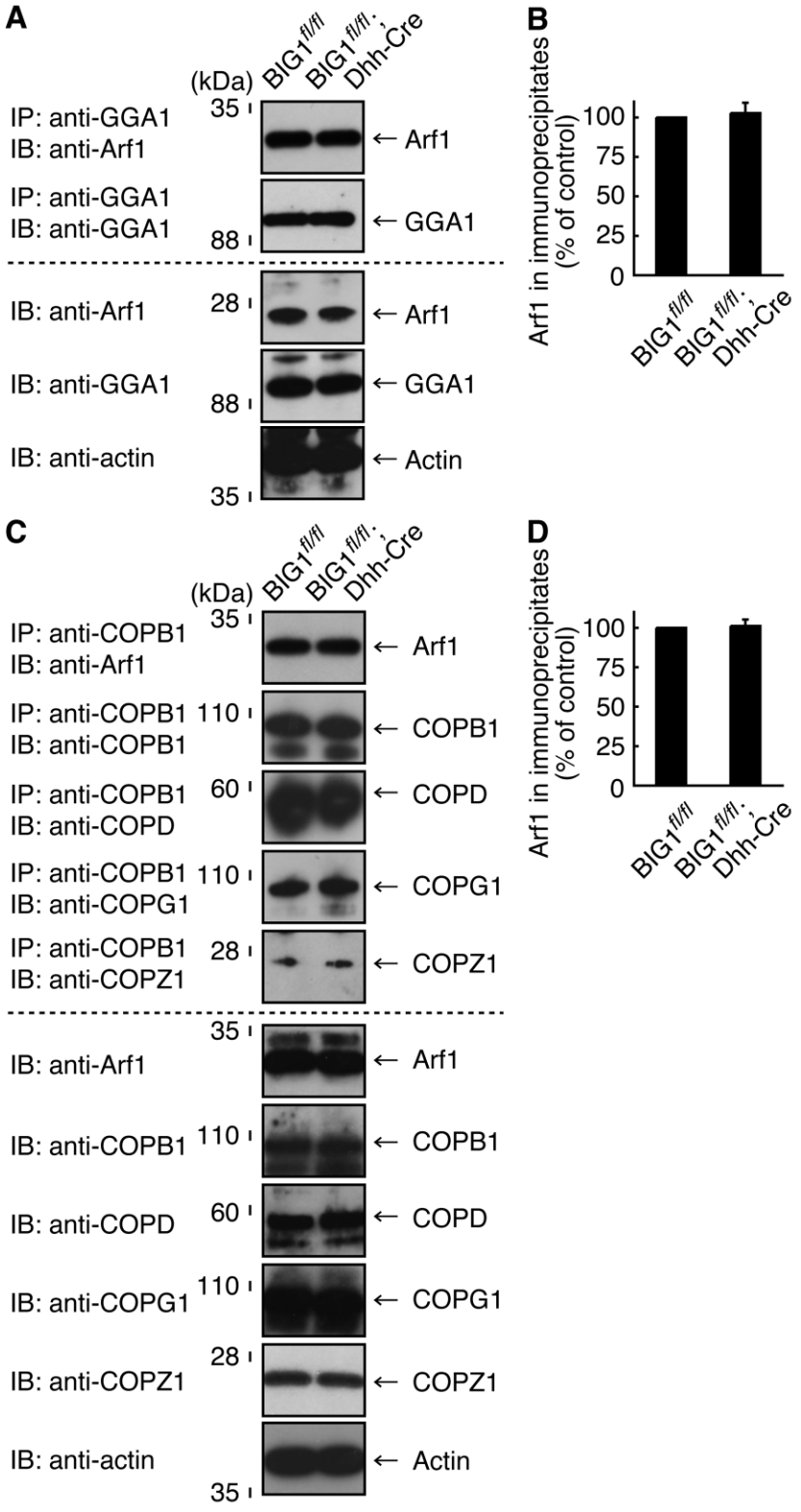
A
BIG1fl/fl
BIG1fl/fl; Dhh-Cre
(kDa)
IP: anti-GGA1
IB: anti-Arf1
35
← Arf1
IP: anti-GGA1
IB: anti-GGA1
88
← GGA1
IB: anti-Arf1
28
← Arf1
IB: anti-GGA1
88
← GGA1
IB: anti-actin
35
← Actin
B
Arf1 in immunoprecipitates (% of control)
100
75
50
25
0
BIG1fl/fl
BIG1fl/fl; Dhh-Cre
C
BIG1fl/fl
BIG1fl/fl; Dhh-Cre
(kDa)
IP: anti-COPB1
IB: anti-Arf1
35
← Arf1
IP: anti-COPB1
IB: anti-COPB1
110
← COPB1
IP: anti-COPB1
IB: anti-COPD
60
← COPD
IP: anti-COPB1
IB: anti-COPG1
110
← COPG1
IP: anti-COPB1
IB: anti-COPZ1
28
← COPZ1
IB: anti-Arf1
35
← Arf1
IB: anti-COPB1
110
← COPB1
IB: anti-COPD
60
← COPD
IB: anti-COPG1
110
← COPG1
IB: anti-COPZ1
28
← COPZ1
IB: anti-actin
35
← Actin
D
Arf1 in immunoprecipitates (% of control)
100
75
50
25
0
BIG1fl/fl
BIG1fl/fl; Dhh-Cre

fig. S3. Effects of knockout of BIG1 on the amounts of Arf1 in transporting protein complexes. (**A, B**) Sciatic nerve tissue lysates were used for immunoblotting with the respective antibodies for Arf1 and GGA1, following immunoprecipitation with an anti-GGA1 antibody. Total proteins and Arf1 co-immunoprecipitation's statistical data (*, $p<0.01$; n=3 blots) are also shown. (**C, D**) Tissue lysates were used for immunoblotting with the respective antibodies for Arf1, COPB1, COPD, COPG1, and COPZ1, following immunoprecipitation with an anti-COPB1 antibody. Total proteins and Arf1 co-immunoprecipitation's statistical data (*, $p<0.01$; n=3 blots) are also shown.

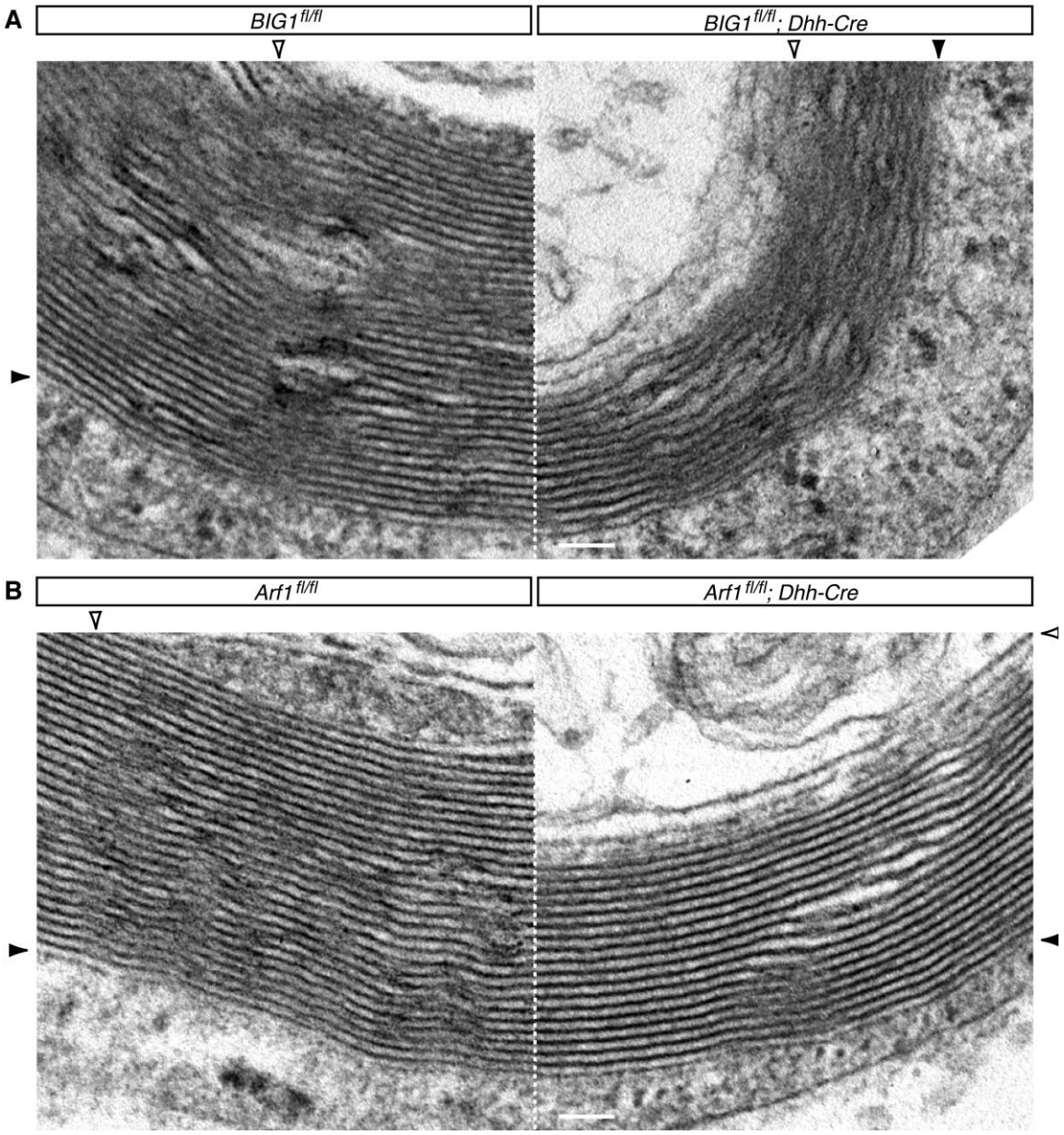

fig. S4. Comparison of thickness between the myelin sheath and the neighboring myelin sheath in Dhh-Cre–mediated BIG1 or Arf1 knockout mice and controls. (**A**) Comparison of thickness between the myelin sheath and the neighboring sheath in

conditional knockout (*BIG1*$^{fl/fl}$*; Dhh-Cre*) and control mice at 7 days postnatal in high-magnification electron microscopy. Closed and open triangles indicate outside and inside positions of myelin sheaths. Scale bar indicates 25 nm. (**B**) Comparison of thickness between the myelin sheath and the neighboring sheath in conditional knockout (*Arf1*$^{fl/fl}$*; Dhh-Cre*) and control mice at 7 days postnatal in high-magnification electron microscopy. Closed and open triangles indicate outside and inside positions of myelin sheaths. Scale bar indicates 25 nm.

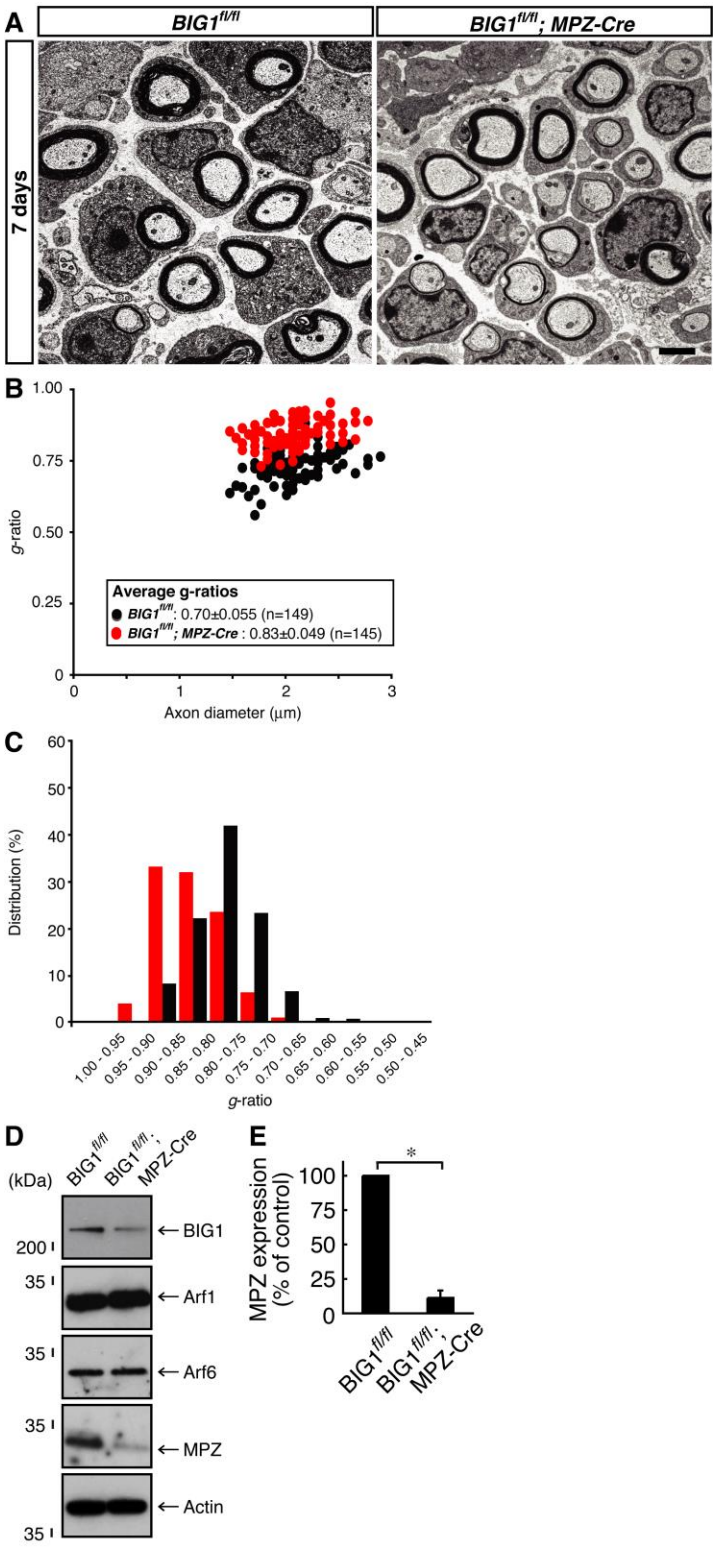
A
BIG1fl/fl
BIG1fl/fl; MPZ-Cre
7 days
B
1.00
0.75
0.50
0.25
0
g-ratio
Average g-ratios
BIG1fl/fl: 0.70±0.055 (n=149)
BIG1fl/fl; MPZ-Cre : 0.83±0.049 (n=145)
0
1
2
3
Axon diameter (μm)
C
60
50
40
30
20
10
0
Distribution (%)
1.00 - 0.95
0.95 - 0.90
0.90 - 0.85
0.85 - 0.80
0.80 - 0.75
0.75 - 0.70
0.70 - 0.65
0.65 - 0.60
0.60 - 0.55
0.55 - 0.50
0.50 - 0.45
g-ratio
D
(kDa)
BIG1fl/fl
BIG1fl/fl ; MPZ-Cre
200
35
35
35
35
← BIG1
← Arf1
← Arf6
← MPZ
← Actin
E
*
100
75
50
25
0
MPZ expression (% of control)
BIG1fl/fl
BIG1fl/fl ; MPZ-Cre

fig. S5 MPZ-Cre–mediated BIG1 knockout mice exhibit decreased myelin thickness. (**A**) Electron microscopic images of the sciatic nerve cross sections of conditional knockout (*BIG1$^{fl/fl}$; MPZ-Cre*) and control mice at 7 days postnatal are shown. Scale bar indicates 1 μm. (**B, C**) Graph of g-ratios of myelinated axons for axon diameters, as well as their distributions, is shown (n=145 nerves for knockout mice and n=149 nerves for controls; 3 independent mice). (**D, E**) Sciatic nerve tissue lysates of conditional knockout (*BIG1$^{fl/fl}$; MPZ-Cre*) mice and their littermate controls were used for immunoblotting with the respective antibodies for BIG1, Arf1, Arf6, MPZ, and control actin. MPZ expression levels are statistically shown (*, $p<0.01$; n=3 blots).

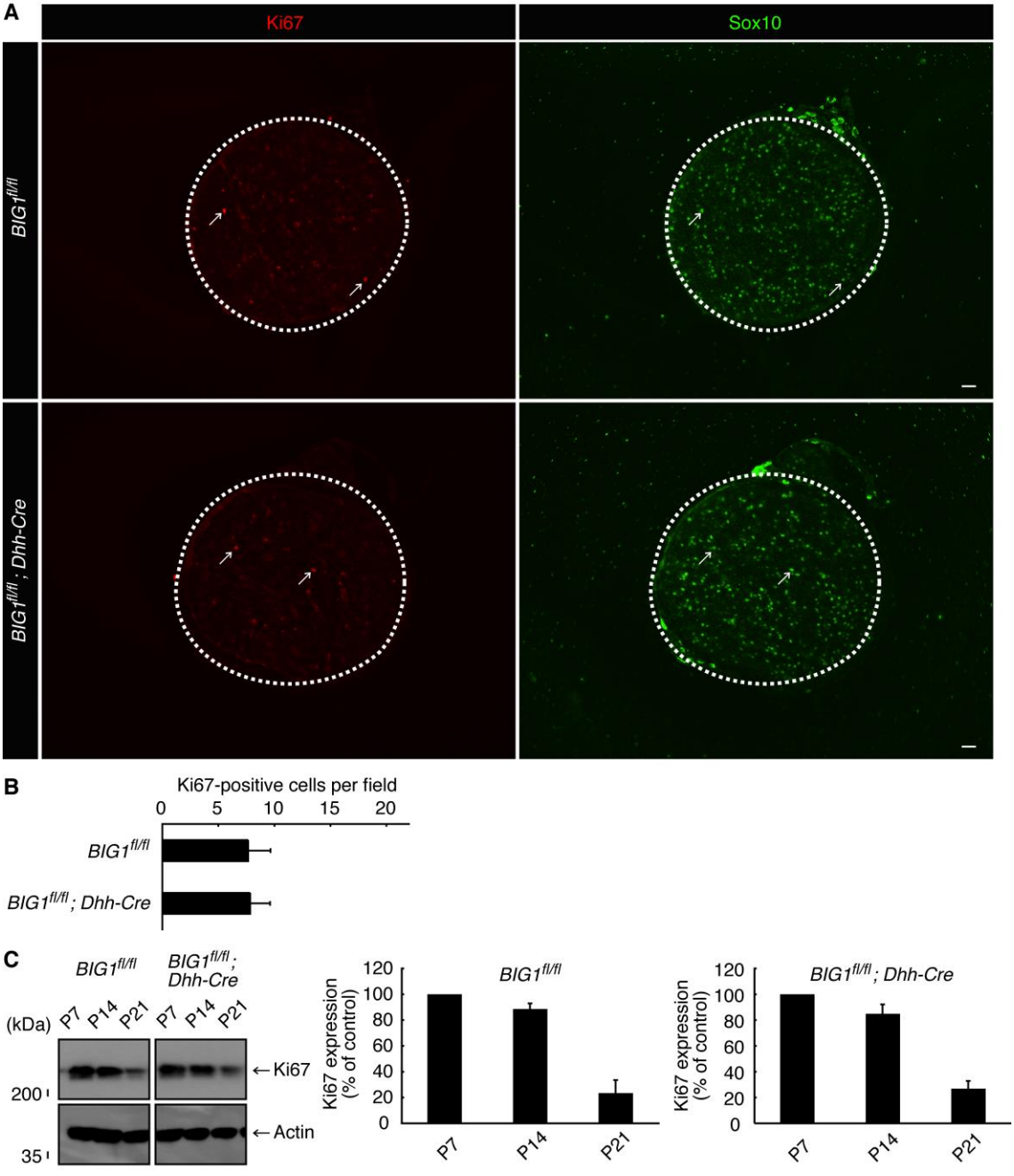

fig. S6. Staining for the proliferating cell marker (Ki67) in Dhh-Cre–mediated BIG1 knockout and control mouse nerve cross sections and Ki67 expression during

development. (**A**) Sciatic nerve cross sections of conditional knockout (*BIG1$^{fl/fl}$; Dhh-Cre*) and control mice at 8 days postnatal were stained with antibodies against Ki67 (red) and Sox10 (Schwann cell lineage cell marker, green). Each arrow indicates the representative Ki67-positive cell, as well as the corresponding Sox10-positive cell. Some Ki67-positive cells were detected in both sections. White dot circles indicate outlines of sciatic nerve cross sections. Scale bar indicates 100 μm. (**B**) The statistical data for Ki67-staining positive cell number per sciatic nerve cross section are shown (n=3 independent mice). (**C**) Sciatic nerve tissues in conditional knockout (*BIG1$^{fl/fl}$; Dhh-Cre*) and control mice were isolated, lysed, and used for immunoblotting with the respective antibodies for Ki67 and actin. The levels of Ki67 are statistically shown (n=3 blots).

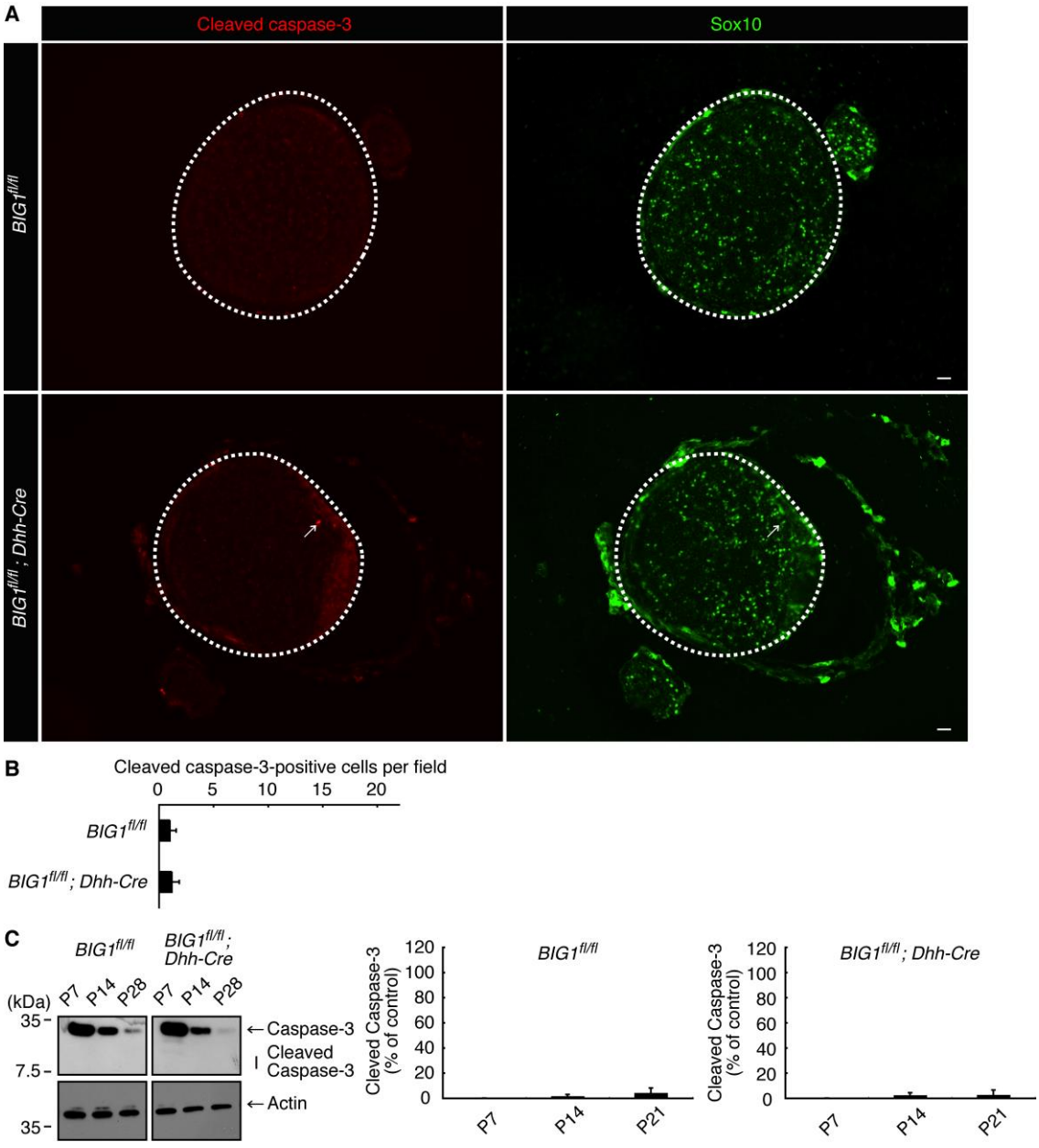

fig. S7. Staining for the apoptotic cell marker (cleaved caspase-3) in Dhh-Cre–mediated BIG1 knockout and control mouse nerve cross sections and caspase-3 expression during development. (**A**) Sciatic nerve cross sections of conditional

knockout (*BIG1*$^{fl/fl}$*; Dhh-Cre*) and control mice at 8 days postnatal were stained with antibodies against cleaved caspase-3 (red) and Sox10 (Schwann cell lineage cell marker, green). Each arrow indicates the cleaved caspase-3-positive cell, as well as the corresponding Sox10-positive cell. The cleaved caspase-3-positive cell was hardly detected in sections. White dot circles indicate outlines of sciatic nerve cross sections. Scale bar indicates 100 μm. (**B**) The statistical data for cleaved caspase-3-staining positive cell number per sciatic nerve cross section are shown (n=3 independent mice). (**C**) Sciatic nerve tissues in conditional knockout (*BIG1*$^{fl/fl}$*; Dhh-Cre*) and control mice were isolated, lysed, and used for immunoblotting with the respective antibodies for caspase-3 and actin. Right line indicates the positions of the cleaved caspase-3, which are hardly detected at the immunoblotting level. The levels of caspase-3 are statistically shown (n=3 blots).

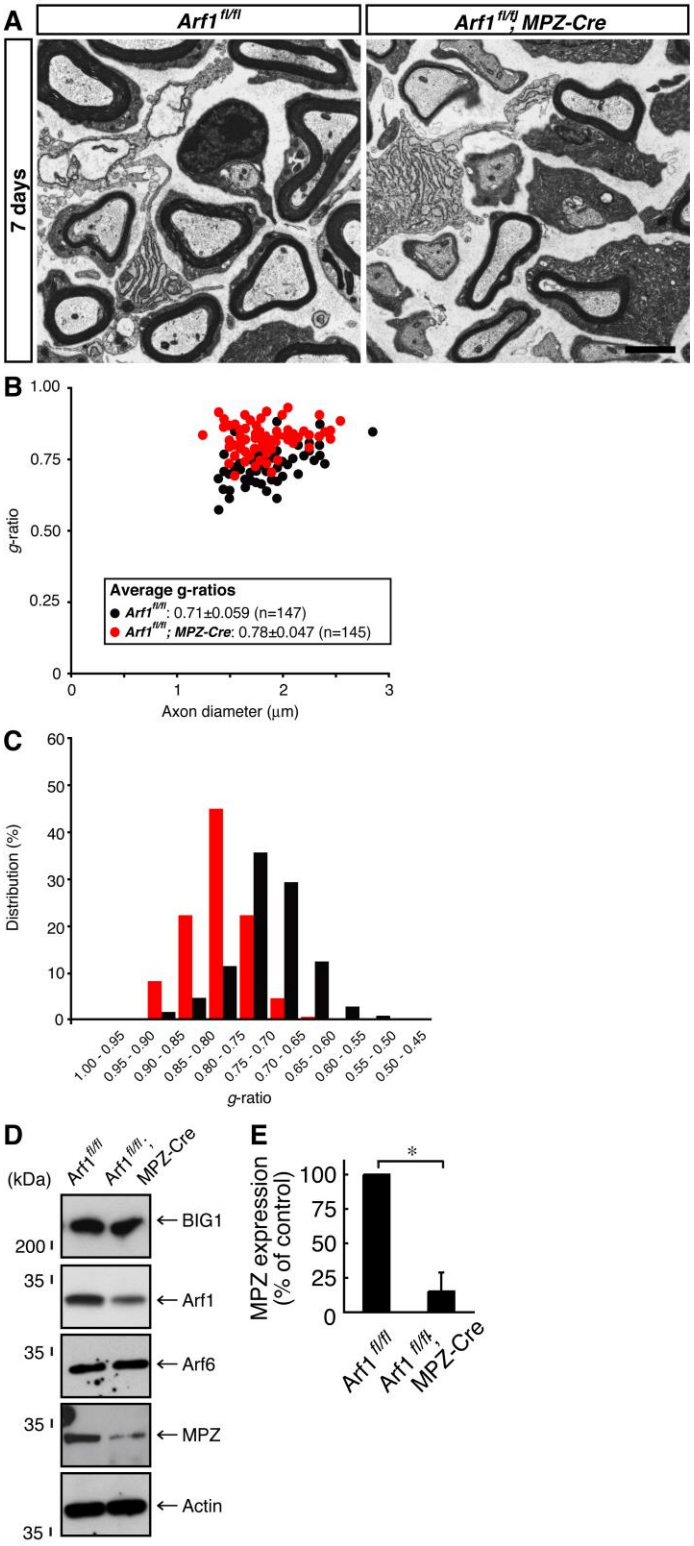
A
Arf1 fl/fl
Arf1 fl/fl; MPZ-Cre
7 days
B
1.00
0.75
0.50
0.25
0
g-ratio
Average g-ratios
Arf1 fl/fl: 0.71±0.059 (n=147)
Arf1 fl/fl; MPZ-Cre: 0.78±0.047 (n=145)
0
1
2
3
Axon diameter (μm)
C
60
50
40
30
20
10
0
Distribution (%)
1.00 - 0.95
0.95 - 0.90
0.90 - 0.85
0.85 - 0.80
0.80 - 0.75
0.75 - 0.70
0.70 - 0.65
0.65 - 0.60
0.60 - 0.55
0.55 - 0.50
0.50 - 0.45
g-ratio
D
(kDa)
Arf1 fl/fl
Arf1 fl/fl; MPZ-Cre
200
35
35
35
35
← BIG1
← Arf1
← Arf6
← MPZ
← Actin
E
*
MPZ expression (% of control)
100
75
50
25
0
Arf1 fl/fl
Arf1 fl/fl; MPZ-Cre

fig. S8. MPZ-Cre–mediated Arf1 knockout mice exhibit decreased myelin thickness.

(**A**) Electron microscopic images of the sciatic nerve cross sections of conditional knockout (*Arf1$^{fl/fl}$; MPZ-Cre*) and control mice at 7 days postnatal are shown. Scale bar indicates 1 μm. (**B, C**) Graph of g-ratios of myelinated axons for axon diameters, as well as their distributions, is shown (n=145 nerves for knockout mice and n=147 nerves for controls; 3 independent mice). (**D, E**) Sciatic nerve tissue lysates of conditional knockout (*Arf1$^{fl/fl}$; MPZ-Cre*) mice and their littermate controls were used for immunoblotting with the respective antibodies for BIG1, Arf1, Arf6, MPZ, and control actin. MPZ expression levels are statistically shown (*, $p<0.01$; n=3 blots).

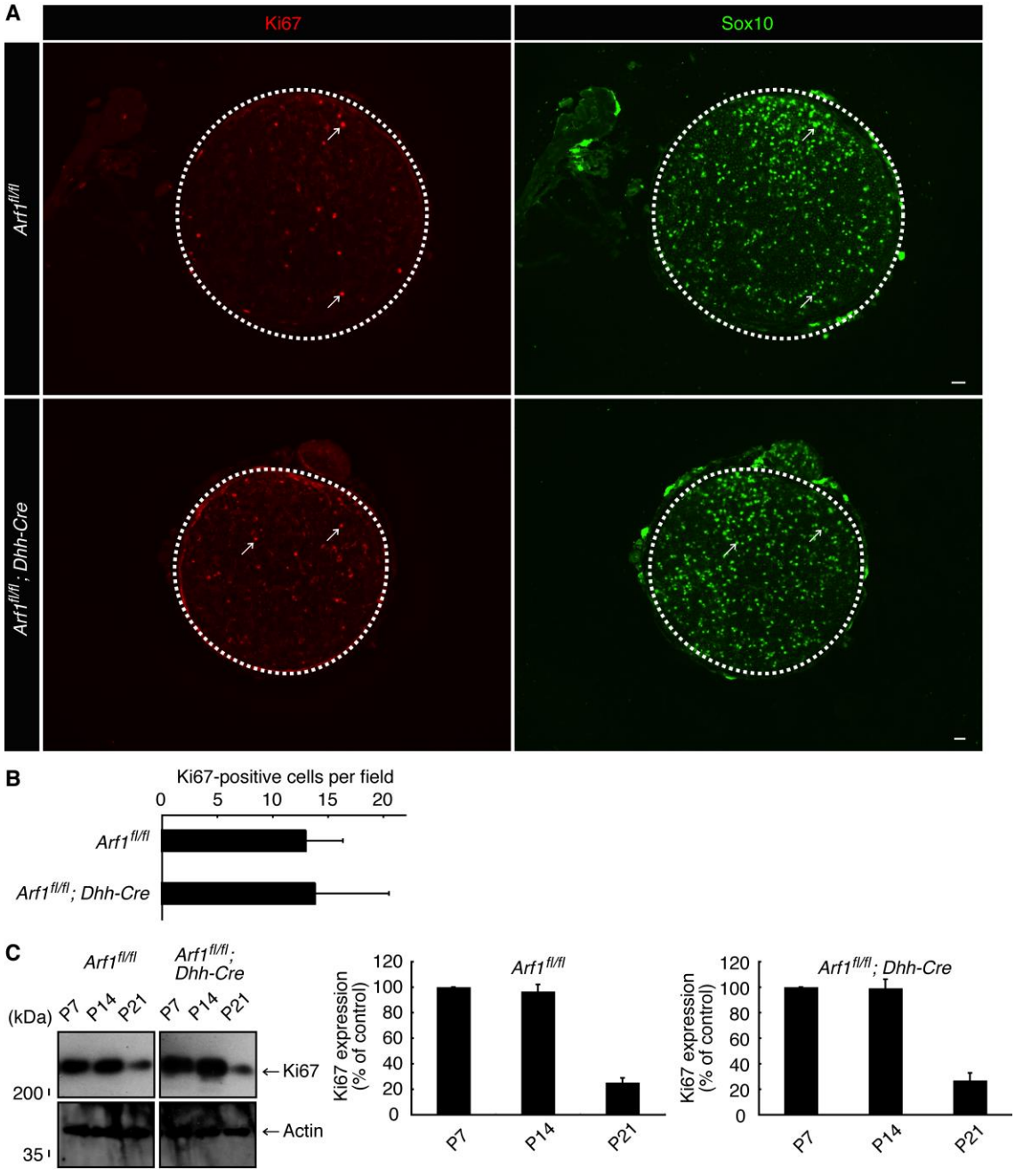

fig. S9. Staining for the proliferating cell marker (Ki67) in Dhh-Cre–mediated Arf1 knockout and control mouse nerve cross sections and Ki67 expression during

development. (**A**) Sciatic nerve cross sections of conditional knockout Arf1$^{fl/fl}$*; Dhh-Cre*) and control mice at 8 days postnatal were stained with antibodies against Ki67 (red) and Sox10 (Schwann cell lineage cell marker, green). Each arrow indicates the representative Ki67-positive cell, as well as the corresponding Sox10-positive cell. Some Ki67-positive cells were detected in both sections. White dot circles indicate outlines of sciatic nerve cross sections. Scale bar indicates 100 μm. (**B**) The statistical data for Ki67-staining positive cell number per sciatic nerve cross section are shown (n=3 independent mice). (**C**) Sciatic nerve tissues in conditional knockout (*Arf1*$^{fl/fl}$*; Dhh-Cre*) and control mice were isolated, lysed, and used for immunoblotting with the respective antibodies for Ki67 and actin. The levels of Ki67 are statistically shown (n=3 blots).

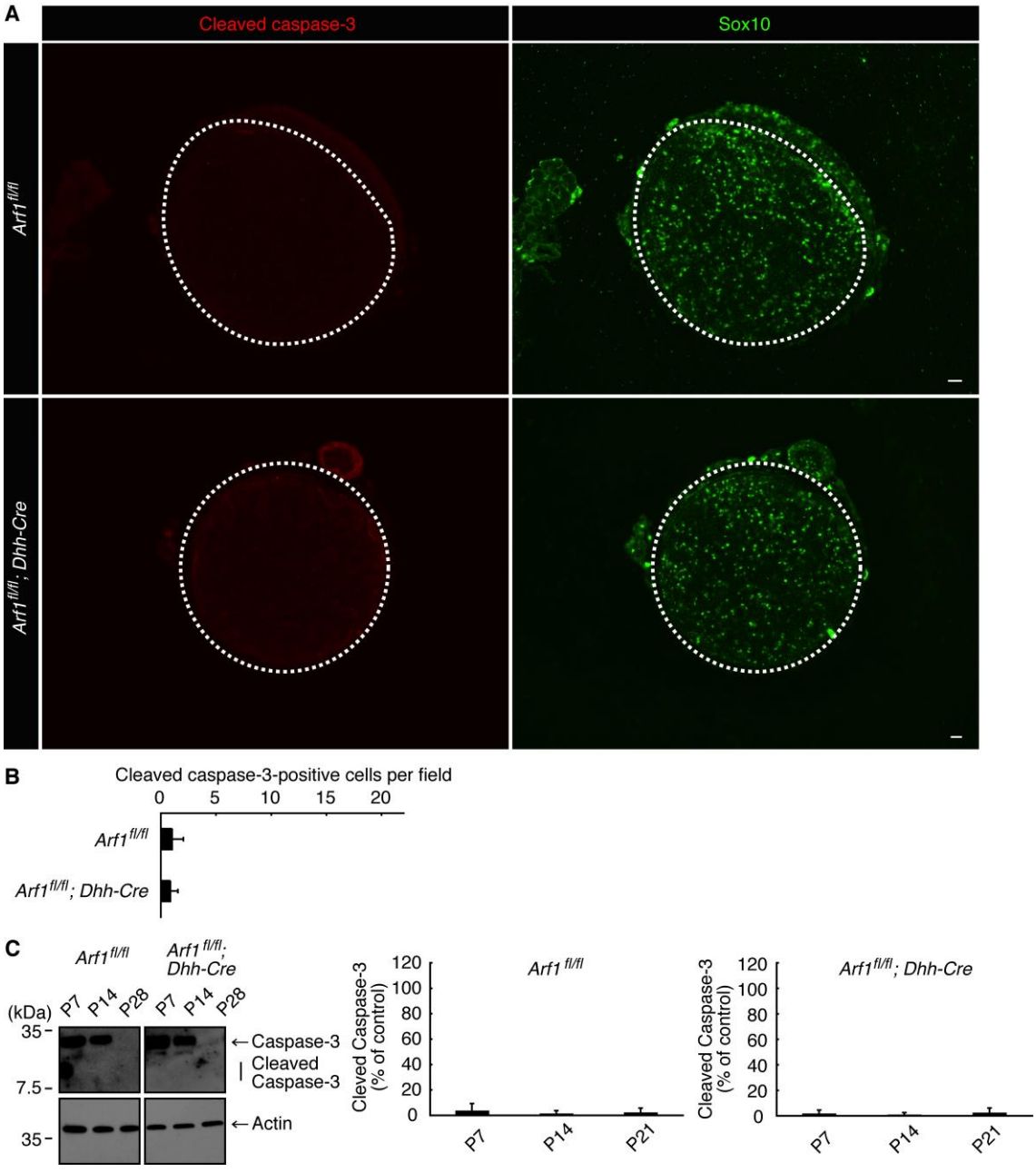

fig. S10. Staining for the apoptotic cell marker (cleaved caspase-3) in Dhh-Cre–mediated Arf1 knockout and control mouse nerve cross sections and caspase-3 expression during development. (**A**) Sciatic nerve cross sections of conditional

knockout (*Arf1*$^{fl/fl}$*; Dhh-Cre*) and control mice at 8 days postnatal were stained with antibodies against cleaved caspase-3 (red) and Sox10 (Schwann cell lineage cell marker, green). The cleaved caspase-3-positive cell was hardly detected in sections. White dot circles indicate outlines of sciatic nerve cross sections. Scale bar indicates 100 μm. (**B**) The statistical data for cleaved caspase-3-staining positive cell number per sciatic nerve cross section are shown (n=3 independent mice). (**C**) Sciatic nerve tissues in conditional knockout (*Arf1*$^{fl/fl}$*; Dhh-Cre*) and control mice were isolated, lysed, and used for immunoblotting with the respective antibodies for caspase-3 and actin. Right line indicates the positions of the cleaved caspase-3, which are hardly detected at the immunoblotting level. The levels of caspase-3 are statistically shown (n=3 blots).