

SUPPLEMENTAL MATERIALS

Supplemental Table 1. Effects of pH on myelin stability in sciatic nerves for S34C transgenic mice.

Supplemental Figure 1. X-ray diffraction data from unembedded, glutaraldehyde-fixed sciatic nerves from WT and S63C mice. The nerves were fixed by immersion in 2% glutaraldehyde in 0.12 M phosphate buffer at pH 7.4. X-ray exposure times were 1 hour. **(A)** X-ray patterns from WT (black) and S63C (red) show that the peaks superpose closely, but are weaker in the latter, indicating less relative amount of myelin. **(B)** Scatterplot of $M/(M+B)$ vs. periodicity d indicates consistent hypomyelination in the S63C myelin.

Supplemental Figure 2. X-ray diffraction data from unfixed sciatic nerves at physiological pH for the genotypes examined in this paper, expressed as a scatterplot of $M/(M+B)$ vs. periodicity d . The data have been assembled from Tables 1-4, and include only the major diffracting phase. The error bars are standard deviations. Note that myelin with P0S34C is more adhesive than P0+/-, as indicated by the smaller period. At higher concentrations of P0S34C, the *trans* disulfide bonds form, resulting in a vastly greater periodicity than either P0+/- or P0-/- myelin. The inset indicates the p-values for significance levels comparing different pairs of the genotypes. Values above and below the diagonal line pertain, respectively, to myelin periodicity (d) and to the relative amount of myelin ($M/M+B$).

Supplemental Figure 3. The 50 kDa band was P0-specific. Protein extracts from WT, S63C(+/+) and P0-/- sciatic nerves were subjected to SDS-PAGE under reducing or non-reducing conditions. Neither the 27-30 nor the 50 kDa (*arrow*) bands were detected in the samples from P0-/- mice, suggesting that these bands are specific for P0.

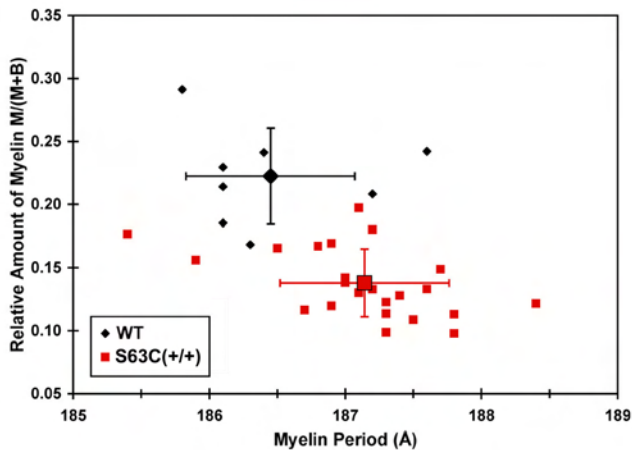
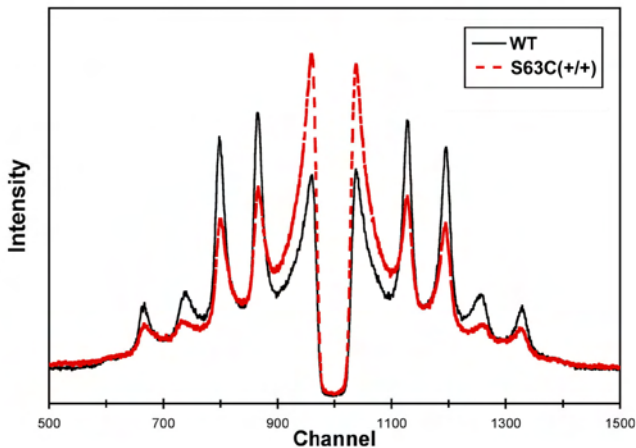
Supplemental Figure 4. MALDI-TOF analysis of the P0wt and dimer bands that were excised as indicated in Figure 5C. Datasets and spectra for the analysis are included here.

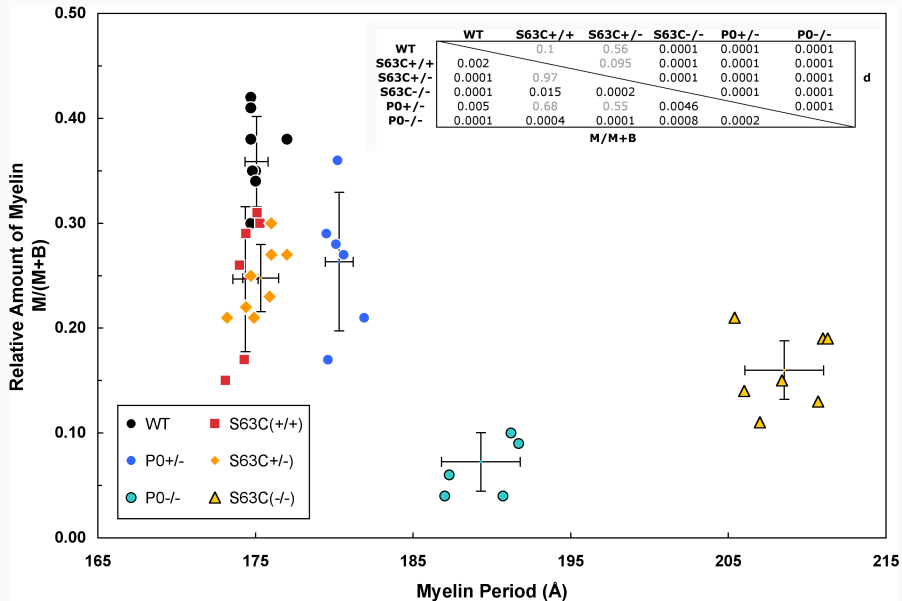
Supplemental Table 1. Effects of pH on myelin stability in sciatic nerves for S63C transgenic mice

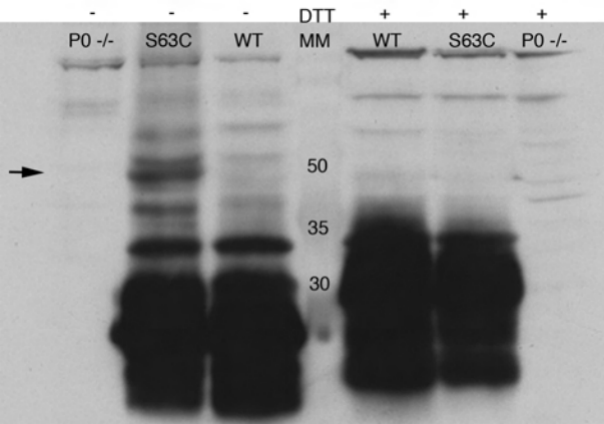
pH	Dimension (Å)				% myelin	M/(M+B)
	<i>d</i>	<i>cyt</i>	<i>lpg</i>	<i>ext</i>		
WT						
6	202.6	32	46	79		0.33
6.2	173.1	–	–	–	62%	0.34
	203.9	–	–	–	38%	
6.5	174.5	31	48	48	97%	0.34
	212.4	–	–	–	3%	
6.6	176.1	32	48	48	99%	0.28
	213.0	–	–	–	1%	
7	175.9	31	50	45		0.27
7.2	175.7	31	49	47		0.27
7.5	174.7	31	49	46		0.30
7.6	177.5	31	49	47		0.30
8	202.6	32	49	48		0.16
S63C(+/+)						
6	202.3	–	–	–	71%	0.18
	172.5	–	–	–	29%	
6.2	200.0	–	–	–	75%	0.25
	172.4	–	–	–	25%	
6.5	172.1	34	45	48	53%	0.25
	204.4	–	–	–	57%	
6.5	173.8	32	48	46		0.20
6.6	173.5	31	48	47	96%	0.27
	212.4	–	–	–	4%	
7	173.9	32	47	48	97%	0.21
	216.0	–	–	–	3%	
7.2	173.6	31	48	47		0.28
7.5	174.0	31	48	47		0.26
7.6	174.8	31	48	48		0.22
8	173.6	32	47	48		0.22
S63C(+/-)						
6	204.9	32	48	77	93%	0.23
	172.2	–	–	–	7%	
6.2	200.0	–	–	–	64%	0.26
	172.9	–	–	–	36%	
6.5	174.1	33	46	49	83%	0.15
	207.1	–	–	–	17%	
6.6	174.6	32	47	49	74%	0.23
	204.2	–	–	–	26%	
7	173.2	33	46	48	74%	0.23
	216.0	–	–	–	26%	
7.2	175.0	32	48	47	81%	0.25
	197.7	–	–	–	5%	
	234.1	–	–	–	14%	
7.5	174.4	32	47	48	96%	0.22
	232.0	–	–	–	4%	
7.6	176.0	32	48	48	85%	0.20
	197.0	–	–	–	4%	

	220.0	–	–	–	11%	
8	176.8	32	49	47		0.13
S63C(-/-)						
6	205.9	–	–	–		0.21
6.2	204.3	–	–	–		0.17
6.5	206.1	–	–	–		0.19
6.6	203.8	–	–	–		0.19
7	207.8	–	–	–		0.13
7.2	215.2	–	–	–		0.20
7.5	211.3	33	46	86		0.19
7.6	212.6	–	–	–		0.14
8	212.1	–	–	–		0.09
P0+/-						
6	205.8	–	–	–		0.27
6.2	205.0	–	–	–		0.27
6.5	204.1	–	–	–	85%	0.25
	180.4				15%	
6.6	182.5	33	48	54	76%	0.20
	202.5	–	–	–	22%	
7	211.3	–	–	–	83%	0.26
	178.9	–	–	–	17%	
7.2	179.7	33	48	51	68%	0.27
	209.3	–	–	–	32%	
7.5	179.6	33	48	51	98%	0.17
	216.1	–	–	–	2%	
7.6	179.8	32	49	50		0.25
8	179.9	32	48	52		0.22
P0-/-						
6	179.5	31	45	59		0.04
6.2	193.6	–	–	–		0.04
6.5	192.1	–	–	–		0.03
6.6	195.6	–	–	–		0.05
7	187.4	34	45	64		0.05
7.2	196.9	–	–	–		0.04
7.5	190.7	33	45	68		0.04
7.6	201.9	–	–	–		0.04
8	184.2	31	47	59		0.05

Myelin stability was assessed by XRD of nerves treated at physiological ionic strength, 154 mM NaCl, 5 mM phosphate buffer, and titrated to the various pHs as described in *Experimental Procedures*. See legend to Table 1 for details, and Fig. 3.







Supplementary Dataset

Below are the matched peptides and raw spectra from MALDI-TOF analysis of the P0wt and Dimers bands that were excised as shown in Figure 5C.

Sample wt P0

(see Figure 5C for image of Coumassie Blue-stained gel)

Mascot Search Results MALDI-TOF analysis

F002306.dat

Match to: [gi|162139829](#) Score: 102 Expect: 1.4e-005
myelin protein zero [Mus musculus]

Nominal mass (M_r): 27634; Calculated pI value: 9.50
NCBI BLAST search of [gi|162139829](#) against nr
Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Mus musculus](#)

Variable modifications: Carbamidomethyl (C), Oxidation (M), Nethylmaleimide (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 18
Number of mass values matched: 10
Sequence Coverage: 21%

Matched peptides shown in **Bold Red**

1 MAPGAPSSSP SPILAALLFS SLVLSPALAI VVYTDREIYG AVGSQVTLHC
51 SFWSSEWVSD DISFTWR**YQP EGGRDAISIF HYAKGQPYID EVGTFKERIQ**
101 **WVGDP**RWKDG SIVIHNLDYS DNGTFTCDVK NPPDIVGKTS QVTLYVFEKV
151 PTRYGVVLGA VIGGILGVVL LLLLLFYLR YCWLRRQAAL QRRLSAMEKG
201 RFHKSSKDSS KR**GRQTPVLY AMLDHSR**STK AASEKSKGL GESRKDKK

Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
68 - 74	806.3603	805.3530	805.3718	-0.0188	0	R.YQPEGGR.D
68 - 84	1951.8865	1950.8792	1950.9587	-0.0795	1	R.YQPEGGRDAISIFHYAK.G
75 - 84	1164.5716	1163.5643	1163.5975	-0.0331	0	R.DAISIFHYAK.G
85 - 96	1353.6243	1352.6170	1352.6612	-0.0442	0	K.GQPYIDVGTGTFK.E
85 - 98	1638.7536	1637.7463	1637.8049	-0.0585	1	K.GQPYIDVGTGTFKER.I
99 - 106	970.4862	969.4789	969.5032	-0.0243	0	R.IQWVGDP.R.W
213 - 227	1743.8278	1742.8205	1742.8886	-0.0681	1	R.GRQTPVLYAMLDSR.S
213 - 227	1759.8299	1758.8226	1758.8835	-0.0609	1	R.GRQTPVLYAMLDSR.S Oxidation (M)
215 - 227	1530.7194	1529.7121	1529.7660	-0.0539	0	R.QTPVLYAMLDSR.S
215 - 227	1546.7139	1545.7066	1545.7609	-0.0543	0	R.QTPVLYAMLDSR.S Oxidation (M)

No match to: 829.3810, 845.3715, 1323.6152, 1482.7115, 1513.6939, 1529.6948, 1562.7154, 1608.7438

Sample Dimers

(see Figure 5C for image of Coumassie Blue-stained gel)

Mascot Search Results MALDI-TOF analysis**F002308.dat**Match to: [gi|162139829](#) Score: **80** Expect: **0.002****myelin protein zero [Mus musculus]**Nominal mass (M_r): **27634**; Calculated pI value: **9.50**NCBI BLAST search of [gi|162139829](#) against nrUnformatted [sequence string](#) for pasting into other applicationsTaxonomy: [Mus musculus](#)Variable modifications: **Carbamidomethyl** (C), Oxidation (M), **Nethylmaleimide** (C)

Cleavage by Trypsin: cuts C-term side of KR unless next residue is P

Number of mass values searched: **12**Number of mass values matched: **7**Sequence Coverage: **18%**Matched peptides shown in **Bold Red**

1 MAPGAPSSSP SPILAALLFS SLVLSPALAI VVYTDREIYG AVGSQVTLHC
51 SFWSSEWVSD DISFTWRYQP EGGR**DAISIF HYAKGQPYID EVGTFKERIQ**
101 **WVGDP**RWKDG SIVIHNLDYS DNGTFTCDVK NPPDIVGKTS QVTLYVFEKV
151 PTRYGVVLGA VIGGILGVVL LLLLLFYLR YCWLRRQAAL QRRLSAMEKG
201 RFHKSSKDSS KR**GRQTPVLY AMLDHSR**STK AASEKSKGL GESRKDKK

Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
75 - 84	1164.6060	1163.5987	1163.5975	0.0013	0	R.DAISIFHYAK.G
85 - 96	1353.6637	1352.6564	1352.6612	-0.0048	0	K.GQPYIDEVGTFFK.E
85 - 98	1638.8014	1637.7941	1637.8049	-0.0107	1	K.GQPYIDEVGTFFKER.I
99 - 106	970.5164	969.5091	969.5032	0.0059	0	R.IQWVGDP.R.W
213 - 227	1759.8710	1758.8637	1758.8835	-0.0198	1	R.GRQTPVLYAMLDHSR.S Oxidation (M)
215 - 227	1530.7569	1529.7496	1529.7660	-0.0164	0	R.QTPVLYAMLDHSR.S
215 - 227	1546.7606	1545.7533	1545.7609	-0.0076	0	R.QTPVLYAMLDHSR.S Oxidation (M)

No match to: 1375.6760, 1482.7115, 1529.7383, 1562.7652, 1804.9244

sample wt P0

Mascot Search Results MALDI-TOF analysis

F002595.dat

Match to: [gi|162139829](#) Score: 128 Expect: 3.5e-008

myelin protein zero [Mus musculus]

Nominal mass (M_r): 27634; Calculated pI value: 9.50

NCBI BLAST search of [gi|162139829](#) against nr

Unformatted [sequence string](#) for pasting into other applications

Taxonomy: [Mus musculus](#)

Variable modifications: Carbamidomethyl (C), Oxidation (M), Nethylmaleimide (C)

Cleavage by Trypsin+AspN, a mixture of enzymes:

cuts C-term side of KR unless next residue is P

cuts N-term side of DB

Number of mass values searched: 49

Number of mass values matched: 19

Sequence Coverage: 46%

Matched peptides shown in **Bold Red**

1 MAPGAPSSSP SPILAALLFS SLVLSPALAI VVYTDREIYG AVGSQVTLHC
 51 SFWSSEWVSD DISFTWRYP EGGRDAISIF HYAKGQPYID EVGTFKERIQ
 101 WVGDPWKDG SIVIHNL DYS DNGTFTCDVK NPPDIVGKTS QVTLYVFEKV
 151 PTRYGVV LGA VIGGILGVV LLLLLFYLR YCWLRRQAAL QRRLSAMEKG
 201 RFHKSSKDSS KRGRQTPVLY AMLDHSRSTK AASEKSKGL GESRKDKK

Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence	
35 - 59	2900.2744	2899.2671	2899.3235	-0.0563	1	T.DREIYGAVGSQVTLHCSFWSSEWVS.D	Carbamidomethyl
(C) 35 - 60	3015.3822	3014.3749	3014.3504	0.0245	2	T.DREIYGAVGSQVTLHCSFWSSEWVSD.D	Carbamidomethyl
(C) 61 - 67	924.4733	923.4660	923.4501	0.0159	0	D.DISFTWR.Y	
61 - 74	1711.8349	1710.8276	1710.8114	0.0163	1	D.DISFTWRYP EGGR.D	
68 - 74	806.3858	805.3785	805.3718	0.0067	0	R.YQPEGGR.D	
75 - 84	1164.6115	1163.6042	1163.5975	0.0068	0	R.DAISIFHYAK.G	
75 - 89	1722.8847	1721.8774	1721.8777	-0.0002	1	R.DAISIFHYAKGQPYI.D	
90 - 98	1080.5424	1079.5351	1079.5247	0.0104	1	I.DEVGTFFKER.I	
90 - 103	1663.8466	1662.8393	1662.8365	0.0028	2	I.DEVGTFFKERIQWVG.D	
97 - 103	887.4823	886.4750	886.4661	0.0090	1	K.ERIQWVG.D	
104 - 117	1649.8355	1648.8282	1648.8685	-0.0403	2	G.DPRWKDGSIVIHNL.D	
107 - 117	1281.6949	1280.6876	1280.6877	-0.0001	1	R.WKDG SIVIHNL.D	
109 - 117	967.5218	966.5145	966.5134	0.0011	0	K.DGSIVIHNL.D	
134 - 149	1826.9898	1825.9825	1825.9825	0.0000	1	P.DIVGKTSQVTLYVFEK.V	
212 - 223	1420.7867	1419.7794	1419.7656	0.0138	2	K.RGRQTPVLYAML.D	Oxidation (M)
213 - 223	1248.6858	1247.6785	1247.6696	0.0089	1	R.GRQTPVLYAML.D	
213 - 223	1264.6810	1263.6737	1263.6645	0.0092	1	R.GRQTPVLYAML.D	Oxidation (M)
215 - 227	1530.7668	1529.7595	1529.7660	-0.0065	1	R.QTPVLYAML DHSR.S	
215 - 227	1546.7778	1545.7705	1545.7609	0.0096	1	R.QTPVLYAML DHSR.S	Oxidation (M)

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No match to: 901.5081, 915.4839, 919.4718, 995.4939, 997.4688, 1050.5302, 1055.4847, 1065.5088, 1112.4938, 1180.5202, 1186.5956, 1195.6026, 1203.6705, 1217.6430, 1235.6543, 1633.8402, 1644.8368, 1661.8276, 1679.8348, 1779.8984, 1782.8508, 1784.8265, 1842.8328, 1858.8251, 1899.8455, 1915.8248, 1967.8691, 1985.8712, 2916.2503, 2931.2802

sample dimers

Mascot Search Results MALDI-TOF analysis

F002582.dat

Match to: [gi|162139829](#) Score: 64 Expect: 0.085

myelin protein zero [Mus musculus]

Nominal mass (M_r): 27634; Calculated pI value: 9.50NCBI BLAST search of [gi|162139829](#) against nrUnformatted [sequence string](#) for pasting into other applicationsTaxonomy: [Mus musculus](#)

Variable modifications: Carbamidomethyl (C), Oxidation (M), Nethylmaleimide (C)

Cleavage by Trypsin+AspN, a mixture of enzymes:

cuts C-term side of KR unless next residue is P

cuts N-term side of DB

Number of mass values searched: 27

Number of mass values matched: 9

Sequence Coverage: 23%

Matched peptides shown in **Bold Red**

1 MAPGAPSSSP SPILAALLFS SLVLSPALAI VVYTDREIYG AVGSQVTLHC
 51 SFWSSEWVSD DISFTWR**YQP EGGDAISIF HYAKGQPYID EVGTFKERIQ**
 101 **WVGDPRWKDG SIVIHNL**DYS DNGTFTCDVK NPPDIVGKTS QVTLYVFEKV
 151 PTRYGVVPGA VIGGILGVVL LLLLLFYLR YCWLRRQAAL QRRLSAMEKG
 201 RFHKSSK**DSS KRGRQTPVLY AML**DHSRSTK AASEKSKGL GESRKDKK

Start - End	Observed	Mr (expt)	Mr (calc)	Delta	Miss	Sequence
68 - 74	806.3907	805.3834	805.3718	0.0116	0	R.YQPEGGR.D
75 - 84	1164.6126	1163.6053	1163.5975	0.0079	0	R.DAISIFHYAK.G
90 - 98	1080.5429	1079.5356	1079.5247	0.0109	1	I.DEVGTFKER.I
90 - 103	1663.8522	1662.8449	1662.8365	0.0084	2	I.DEVGTFKERIQWVG.D
97 - 103	887.4859	886.4786	886.4661	0.0126	1	K.ERIQWVG.D
107 - 117	1281.7017	1280.6944	1280.6877	0.0067	1	R.WKDGSIIVHNL.D
109 - 117	967.5257	966.5184	966.5134	0.0050	0	K.DGSIIVHNL.D
208 - 214	805.4322	804.4249	804.4202	0.0047	2	K.DSSKRGR.Q
213 - 223	1264.6792	1263.6719	1263.6645	0.0074	1	R.GRQTPVLYAML.D Oxidation (M)

No match to: 901.5114, 974.4780, 997.4704, 1112.4943, 1128.4823, 1186.6548, 1203.6710, 1237.6376, 1513.6807, 1560.6874, 1637.7972, 1644.8345, 1661.8231, 1695.8316, 1784.8214, 1899.8443, 1915.8426, 2679.1182

