Edinburgh Research Explorer

Proteome profile of peripheral myelin in healthy mice and in a neuropathy model

Proteome profile of peripheral myelin in healthy mice and in a neuropathy model

Tracking no: 27-08-2019-TR-eLife-51406R2

Impact statement: Comprehensive compendium of myelin proteins in the peripheral nervous system and method to address molecular diversity of myelin sheaths in health and disease.

Competing interests: No competing interests declared

Author contributions:
Sophie Siems: Sophie B. Siems performed all experiments not specified otherwise, conducted statistical analysis, contributed to analysis and interpretation of data. Olaf Jahn: Olaf Jahn performed proteome analysis, contributed to analysis and interpretation of data. Maria Eichel: Maria A. Eichel performed teased fiber labeling and microscopy. Nirmal Kannaiyan: Nirmal Kannaiyan performed bioinformatic analysis of RNA-Seq data. Lai Man Wu: Lai Man N. Wu performed histological analysis. Diane Sherman: Diane L. Sherman performed histological analysis. Kathrin Kusch: Kathrin Kusch provided unpublished reagents. Dörte Hesse: Dörte Hesse contributed to proteome analysis. Ramona Jung: Ramona B. Jung performed biochemical purification of myelin. Robert Fledrich: Robert Fledrich provided an unpublished RNA-Seq dataset. Michael Sereda: Michael W. Sereda provided an unpublished RNA-Seq dataset. Moritz Rossner: Moritz J. Rossner supervised bioinformatic analysis of RNA-Seq data. Peter Brophy: Peter J. Brophy supervised histological analysis. Hauke Werner: Hauke B. Werner conceived, designed and directed the study, analyzed and interpreted data and wrote the article.

Data Availability:
All data generated or analysed during this study are included in the manuscript and supporting files. This includes the mass spectrometry proteomics data. Source data files have been provided for Figures 1, 3 and 5. Additional to being provided in the source data files, mass spectrometry proteomics data have been deposited to the PRIDE/ProteomeXchange Consortium with dataset identifier PXD015960 and are available to the reviewers. Please use username: reviewer94825@ebi.ac.uk and password: C5r5SVMi. Upon acceptance of the manuscript, we will notify PRIDE support to make the dataset publicly available.
N/A

Ethics:
Human Subjects: No Animal Subjects: Yes Ethics Statement: All animal work conformed to United Kingdom legislation (Scientific Procedures) Act 1986 and to the University of Edinburgh Ethical Review Committee policy; Home Office project license No. P0F4A25E9.

Information for reviewers (full submissions):

eLife aims to publish work of the highest scientific standards and importance in all areas of the life and biomedical sciences, from the most basic and theoretical work through to translational, applied and clinical research. Articles must be methodologically and scientifically rigorous, ethically conducted, and objectively presented according to the appropriate community standards.

You will be asked for a general assessment and a summary of any major concerns (ideally in fewer than 500 words), as well as a list of any minor comments (optional). You will also have the opportunity to comment on the statistical rigour of the work (optional).

In your general assessment, please articulate what is exciting and whether the work represents a significant contribution. Please note our guidelines about requests for additional work:

1. We will only request new work, such as experiments, analyses, or data collection, if the new data are essential to support the major conclusions. The authors must be able to do any new work in a reasonable time frame (additional work should be conducted and written up within two months); otherwise, we will usually reject the manuscript.
2. Any requests for new work must fall within the scope of the current submission and the technical expertise of the authors.

Our goal is to make peer review constructive and collaborative: after the reviews have been submitted independently, there is an online discussion between the reviewers in which each reviewer will see the identity of the other reviewers.

Proteome profile of peripheral myelin in healthy mice and in a neuropathy model

Sophie B. Siems[1,7], Olaf Jahn[2,7], Maria A. Eichel[1], Nirmal Kannaiyan[3], Lai Man N. Wu[4], Diane L. Sherman[4], Kathrin Kusch[1], Dörte Hesse[2], Ramona B. Jung[1], Robert Fledrich[1,5], Michael W. Sereda[1,6], Moritz J. Rossner[3], Peter J. Brophy[4], Hauke B. Werner[1,*]

Affiliations
[1]Department of Neurogenetics, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
[2]Proteomics Group, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
[3]Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, 80336 Munich, Germany
[4]Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, EH16 4SB, UK
[5]Institute of Anatomy, University of Leipzig, 04103 Leipzig, Germany
[6]Department of Clinical Neurophysiology, University Medical Center, 37075 Göttingen, Germany
[7]These authors contributed equally to this work

* Corresponding author
Dr. Hauke Werner
Max Planck Institute of Experimental Medicine
Department of Neurogenetics
Hermann-Rein-Str. 3
D-37075 Göttingen, Germany
Tel.: +49 (551) 3899-759; Fax.: +49 (551) 3899-758
E-mail: Hauke@em.mpg.de

Key words
Schwann cell, peripheral nervous system (PNS), myelin proteome, neuropathy, Charcot-Marie-Tooth disease (CMT4F), periaxin (PRX), MCT1/SLC16A1, demyelination, axon degeneration, transcriptome

Word and figure count
Abstract 149 words; Introduction/results/discussion 4786 words
6 main figures; 2 figure supplements; 2 main tables; 4 source data files

49 **ABSTRACT**

50

51 Proteome and transcriptome analyses aim at comprehending the molecular profiles of the

52 brain, its cell-types and subcellular compartments including myelin. Despite the relevance of

53 the peripheral nervous system for normal sensory and motor capabilities, analogous

54 approaches to peripheral nerves and peripheral myelin have fallen behind evolving technical

55 standards. Here we assess the peripheral myelin proteome by gel-free, label-free mass-

56 spectrometry for deep quantitative coverage. Integration with RNA-Sequencing-based

57 developmental mRNA-abundance profiles and neuropathy disease genes illustrates the utility

58 of this resource. Notably, the periaxin-deficient mouse model of the neuropathy Charcot-

59 Marie-Tooth 4F displays a highly pathological myelin proteome profile, exemplified by the

60 discovery of reduced levels of the monocarboxylate transporter MCT1/SLC16A1 as a novel

61 facet of the neuropathology. This work provides the most comprehensive proteome resource

62 thus far to approach development, function and pathology of peripheral myelin, and a

63 straightforward, accurate and sensitive workflow to address myelin diversity in health and

64 disease.

65

66

INTRODUCTION

The ensheathment of axons with myelin enables rapid impulse propagation, a prerequisite for normal motor and sensory capabilities of vertebrates (1,2). This is illustrated by demyelinating neuropathies of the Charcot-Marie-Tooth (CMT) spectrum, in which mutations affecting myelin genes as *MPZ, PMP22, GJB1* and *PRX* impair myelin integrity and reduce the velocity of nerve conduction in the peripheral nervous system (PNS) (3). Developmentally, myelination by Schwann cells in peripheral nerves is regulated by axonal neuregulin-1 (4,5) and the basal lamina (6–8) that is molecularly linked to the abaxonal Schwann cell membrane via integrins and the dystroglycan complex (9–12). In adulthood, the basal lamina continues to enclose all axon/myelin-units (13), probably to maintain myelin. Beyond regulation by extracellular cues, myelination involves multiple proteins mediating radial sorting of axons out of Remak bundles, myelin membrane growth and layer compaction (14–18). For example, the Ig-domain containing myelin protein zero (MPZ; also termed P0) mediates adhesion between adjacent extracellular membrane surfaces in compact myelin (19). At their intracellular surfaces, myelin membranes are compacted by the cytosolic domain of MPZ/P0 together with myelin basic protein (MBP; previously termed P1) (20,21). Not surprisingly, MPZ/P0 and MBP were early identified as the most abundant peripheral myelin proteins (22,23).

A system of cytoplasmic channels through the otherwise compacted myelin sheath remains non-compacted throughout life, i.e. the adaxonal myelin layer, paranodal loops, Schmidt-Lanterman incisures (SLI), and abaxonal longitudinal and transverse bands of cytoplasm termed bands of Cajal (14,24,25). Non-compacted myelin comprises cytoplasm, cytoskeletal elements, vesicles and lipid-modifying enzymes, and thus numerous proteins involved in maintaining the myelin sheath. The cytosolic channels probably also represent transport routes toward Schwann cell-dependent metabolic support of myelinated axons (26–31).

Considering that Schwann cells constitute a major proportion of the cells in the PNS, oligonucleotide microarray analyses have been used for mRNA abundance profiling of total sciatic nerves (32,33). Indeed, these systematic approaches allowed the identification of novel myelin constituents including non-compact myelin-associated protein (NCMAP/MP11) (34). Notwithstanding that the number of known peripheral myelin proteins has grown in recent years, a comprehensive molecular inventory has been difficult to achieve because applications of systematic ('omics') approaches specifically to Schwann cells and peripheral myelin remained comparatively scarce, different from studies addressing oligodendrocytes and CNS myelin (35–40). One main reason may be that the available techniques were not

104 sufficiently straightforward for general application. For example, the protein composition of
105 peripheral myelin was previously assessed by proteome analysis (41). However, at that time
106 the workflow of sample preparation and data acquisition (schematically depicted in **Figure**
107 **1A**) was very labor-intense and required a substantial amount of input material; yet the depth
108 of the resulting datasets remained limited. In particular, differential myelin proteome analysis
109 by 2-dimensional fluorescence intensity gel electrophoresis (2D-DIGE) requires considerable
110 hands-on-time and technical expertise (41,42). While this method is powerful for the
111 separation of proteoforms (43), it typically suffers from under-representation of highly basic
112 and transmembrane proteins. It thus allows comparing the abundance of only few myelin
113 proteins rather than quantitatively covering the entire myelin proteome. Because of these
114 limitations and an only modest sample-to-sample reproducibility, 2D-DIGE analysis of myelin,
115 although unbiased, has not been commonly applied beyond specialized laboratories.
116
117 The aim of the present study was to establish a straightforward and readily applicable
118 workflow to facilitate both comprehensive knowledge about the protein composition of
119 peripheral myelin and systematic assessment of differences between two states, e.g.,
120 pathological alterations in a neuropathy model. The major prerequisites were the biochemical
121 purification of myelin, its solubilization with the detergent ASB-14 and the subsequent
122 automated digestion with trypsin during filter-aided sample preparation (FASP). The tryptic
123 peptides were fractionated by liquid chromatography and analyzed by mass spectrometry for
124 gel-free, label-free quantitative proteome analysis. More specifically, we used nano-flow
125 ultra-performance liquid chromatography (nanoUPLC) coupled to an electrospray-ionization
126 quadrupole time-of-flight (ESI-QTOF) mass spectrometer with ion mobility option, providing
127 an orthogonal dimension of peptide separation. The utilized data-independent acquisition
128 (DIA) strategy relies on collecting data in an alternating low and elevated energy mode
129 (MS^E); it enables simultaneous sequencing and quantification of all peptides entering the
130 mass spectrometer without prior precursor selection (reviewed in (44,45)). With their high-
131 duty cycle utilized for the acquisition of precursor ions, MS^E-type methods are ideally suited
132 to reliably quantify proteins based on peptide intensities. Notably, these methods do not
133 involve the use of spectral libraries in the identification of proteins, different from other DIA
134 strategies. Instead, the achieved high-complexity fragmentation spectra are deconvoluted
135 before submission to dedicated search engines for peptide and protein identification (46,47).
136 In the MS^E mode, this deconvolution involves precursor-fragment ion alignment solely on the
137 basis of chromatographic elution profiles; on top, drift times of ion mobility-separated
138 precursors are used in the high-definition (HD)MS^E mode. An expansion of the latter referred
139 to as the ultra-definition (UD)MS^E mode, additionally implements drift time-dependent
140 collision energy profiles for more effective precursor fragmentation (48,49).

141

142   Indeed, compared to the previously used manual handling and in-gel digestion, the current

143   workflow (schematically depicted in **Figure 1A)** is considerably less labor-intense, and

144   automated FASP increases sample-to-sample reproducibility. Moreover, differential analysis

145   by quantitative mass spectrometry (MS) facilitates reproducible quantification of hundreds

146   rather than a few distinct myelin proteins. Together, the present workflow increases the

147   efficacy of assessing the peripheral myelin proteome while shifting the main workload from

148   manual sample preparation and gel-separation to automated acquisition and processing of

149   data. We propose that comprehending the expression profiles of all myelin proteins in the

150   healthy PNS and in myelin-related disorders can contribute to advancing our understanding

151   of the physiology and pathophysiology of peripheral nerves.

152

RESULTS

Purification of peripheral myelin

We biochemically enriched myelin as a light-weight membrane fraction from pools of sciatic nerves dissected from mice at postnatal day 21 (P21) using an established protocol of discontinuous sucrose density gradient centrifugation (41,50), in which myelin membranes accumulate at the interface between 0.29 and 0.85 M sucrose. By immunoblotting, proteins specific for both compact (MPZ/P0, MBP, PMP2) and non-compact (PRX) myelin were substantially enriched in the myelin fraction compared to nerve lysates (**Figure 1B**). Conversely, axonal (NEFH, KCNA1) and mitochondrial (VDAC) proteins and a marker for the Schwann cell nucleus (KROX20/EGR2) were strongly reduced in purified myelin. Together, these results imply that biochemically purified peripheral myelin is suitable for systematic analysis of its protein composition.

Proteome analysis of peripheral myelin

It has long been difficult to accurately quantify the most abundant myelin proteins both in the CNS (PLP, MBP, CNP (51)) and the PNS (MPZ/P0, MBP, PRX; this work), probably owing to their exceptionally high relative abundance. For example, the major CNS myelin constituents PLP, MBP and CNP comprise 17, 8 and 4% of the total myelin protein, respectively (51). We have recently provided proof of principle (52) that the mass spectrometric quantification of these high-abundant myelin proteins is accurate and precise when data are acquired in the MS^E data acquisition mode and proteins are quantified according to the TOP3 method, i.e. if their abundance values are obtained based on the proven correlation between the average intensity of the three peptides exhibiting the most intense mass spectrometry response and the absolute amount of their source protein (53,54). Using data acquisition by MS^E we confirmed that CNP constitutes about 4% of the total CNS myelin proteome and that the abundance of CNP in myelin from mice heterozygous for the *Cnp* gene (*Cnp*^*WT/null*^) compared to wild-type mice is 50.7% ($\pm$0.4%), in agreement with the halved gene dosage and gel-based quantification by silver staining or immunoblotting (52).

When applying the MS^E mode to PNS myelin, we quantified 351 proteins with a false discovery rate (FDR) of <1% at peptide and protein level and an average sequence coverage of 35.5% (**Figure 1-source data 1**). While MS^E (labeled in orange in **Figure 1C**) indeed provided a dynamic range of more than four orders of magnitude and thus quantitatively covered the exceptionally abundant myelin proteins MPZ/P0, MBP and PRX, the number of quantified proteins appeared limited when spectral complexity was deconvoluted solely on the basis of chromatographic elution profiles. Accordingly, by using the $UDMS^E$ mode, which

190 comprises ion mobility for additional peptide separation as well as drift time-specific collision
191 energies for peptide fragmentation, proteome coverage was increased about three-fold (1078
192 proteins quantified; average sequence coverage 34.3%; **Figure 1-source data 1**). However,
193 the dynamic range of UDMS^E (labeled in blue in **Figure 1C**) was found to be somewhat
194 compressed compared to that of MS^E, which can be considered an expectable feature of
195 traveling wave ion mobility devices (55), where the analysis of pulsed ion packages leads to
196 a temporal and spatial binning of peptides during ion mobility separation. Indeed, this
197 manifests as a ceiling effect for the detection of exceptionally intense peptide signals, which
198 results in an underestimation of the relative abundance of MPZ/P0, MBP and PRX by
199 UDMS^E.

200

201 The complementary nature of the MS^E and UDMS^E data acquisition modes led us to
202 conclude that a comprehensive analysis of the myelin proteome that facilitates both correct
203 quantification of the most abundant proteins and deep quantitative coverage of the proteome
204 would require analyzing the same set of samples with two different instrument settings for
205 MS^E and UDMS^E, respectively. Considering that instrument time is a bottleneck for the
206 routine differential proteome analysis of myelin from mutant mice, we aimed to combine the
207 strengths of MS^E and UDMS^E into a single data acquisition mode. Based on a gene ontology
208 enrichment analysis for cellular components of the 200 proteins of highest and lowest
209 abundance from the UDMS^E dataset, we realized that the 'bottom' of the quantified proteome
210 is probably largely unrelated to myelin but dominated by contaminants from other subcellular
211 sources including mitochondria. We thus reasoned that for a myelin-directed data acquisition
212 mode, proteome depth may be traded in for a gain in dynamic range and devised a novel
213 method referred to as dynamic range enhancement (DRE)-UDMS^E, in which a deflection lens
214 is used to cycle between full and reduced ion transmission during mass spectrometric
215 scanning. Indeed, DRE-UDMS^E quantified an intermediate number of proteins in PNS myelin
216 (554 proteins; average sequence coverage 30.6%; **Figure 1-source data 1**) while providing
217 an intermediate dynamic range (labeled in green in **Figure 1C**). We thus consider DRE-
218 UDMS^E as the data acquisition mode of choice most suitable for routine differential myelin
219 proteome profiling (see below).

220

221 Overall, we found a high reproducibility between replicates and even among the different
222 data acquisition modes as indicated by Pearson's correlation coefficients for protein
223 abundance in the range of 0.765-0.997 (**Figure 1-supplement 1**). When comparing the
224 proteins identified in PNS myelin using the three data acquisition modes, we found a very
225 high overlap (**Figure 1D**). We also found a high overlap (**Figure 1E**) between the proteins
226 identified in the present study by UDMS^E and those detected in previous proteomic

227 approaches to PNS myelin (41,42), thus allowing a high level of confidence. Together, the
228 three data acquisition modes exhibit distinct strengths in the efficient quantification of
229 exceptionally abundant proteins (MS^E), establishing a comprehensive inventory (UDMS^E)
230 and gel-free, label-free differential analysis of hundreds of distinct proteins (DRE-UDMS^E) in
231 peripheral myelin (see **Figure 1A**). Yet, analyzing the same set of samples by different
232 modes may not always be feasible in all routine applications when considering required
233 instrument time.
234
235 **Relative abundance of peripheral myelin proteins**
236 Considering that MS^E provides the high dynamic range required for the quantification of the
237 most abundant myelin proteins, we calculated the relative abundance of the 351 proteins
238 identified in myelin by MS^E (**Figure 1-source data 1**). According to quantitative assessment
239 of this dataset, the most abundant PNS myelin protein, myelin protein zero (MPZ/P0),
240 constitutes 44% (+/-4% relative standard deviation (RSD)) of the total myelin protein (**Figure
241 2**). Myelin basic protein (MBP), periaxin (PRX) and tetraspanin-29 (CD9) constitute 18% (+/-
242 1% RSD), 15% (+/-1%) and 1% (+/-0.2%) of the total myelin protein, respectively (**Figure 2**).
243 For MPZ/P0 and MBP, our quantification by MS^E is in agreement with but specifies prior
244 estimations upon gel-separation and protein labeling by Sudan-Black, Fast-Green or
245 Coomassie-Blue, in which they were judged to constitute 45–70% and 2–26% of the total
246 myelin protein, respectively (22,56–58). However, gel-based estimates of the relative
247 abundance of myelin proteins were not very precise with respect to many other proteins,
248 including those of high molecular weight. Indeed, periaxin was identified as a constituent of
249 peripheral myelin after the advent of gradient SDS-PAGE gels (59), which allowed improved
250 migration of large proteins into gels. The present MS^E-based quantification of myelin proteins
251 also extends beyond and partially adjusts an earlier mass spectrometric approach (41).
252 Indeed, the current approach identified and quantified more myelin proteins, probably owing
253 to improved protein solubilization during sample preparation and higher dynamic range of the
254 used mass spectrometer. By MS^E, known myelin proteins (**Table 1**) collectively constitute
255 over 85% of the total myelin protein (**Figure 2**) while proteins not yet associated with myelin
256 account for the remaining 15% of the total myelin protein.
257
258 **Comprehensive compendium and comparison to the transcriptome**
259 To systematically elucidate the developmental abundance profiles of the transcripts that
260 encode peripheral myelin proteins (**Figure 3**), we used our combined proteome inventory of
261 peripheral myelin (**Figure 1-source data 1**) to filter mRNA abundance data of all genes
262 expressed in sciatic nerves. By this strategy, **Figure 3** displays only those transcripts of
263 which the protein product was identified in peripheral myelin rather than all transcripts in the

264   nerve, thereby discriminating myelin-related mRNAs from other mRNAs such as those
265   present in peripheral axons, fibroblasts, immune cells etc. In this assessment we additionally
266   included PMP22 although it was not detected by MS as well as 45 proteins exclusively
267   identified by LC-MS of myelin separated by SDS-PAGE (**Figure 1-source data 1**). For
268   mRNA abundance profiles, we exploited a recently established RNA sequencing analysis
269   (RNA-Seq; platform Illumina HiSeq 2000) of sciatic nerves dissected form wild type Sprague
270   Dawley rats at embryonic day 21 (E21), P6, P18 and 6 months (60). RNA-Seq provides
271   reliable information about the relative abundance of all significantly expressed genes and is
272   thus not limited to those represented on the previously used oligonucleotide microarrays
273   (41). The raw data (accessible under GEO accession number GSE115930) were normalized
274   (**Figure 3-source data 1**) and standardized. When comparing the proteome and
275   transcriptome datasets, significant mRNA abundance was detected for all 1046 transcripts
276   for which an unambiguous unique gene identifier was found (**Figure 3**). 126 transcripts
277   displayed developmentally unchanged abundance levels, i.e., abundance changes below a
278   threshold of 10% coefficient of variation (**Figure 3B; Figure 3-source data 1**).

279

280   By fuzzy c-means clustering, those 920 transcripts that showed developmental abundance
281   changes were grouped into 5 clusters (**Figure 3A; Figure 3-source data 1**). Among those,
282   one cluster corresponds to an mRNA-abundance peak coinciding with an early phase of
283   myelin biogenesis (cluster 'P6-UP'), which includes the highest proportion of known myelin
284   proteins (**Table 1**) such as MPZ/P0, MBP, PRX, cyclic nucleotide phosphodiesterase (CNP),
285   fatty acid synthase (FASN), myelin-associated glycoprotein (MAG), proteolipid protein
286   (PLP/DM20), cell adhesion molecule-4 (CADM4/NECL4), connexin-29 (GJC3), claudin-19
287   (CLDN19) and CKLF-like MARVEL-transmembrane domain containing protein-5 (CMTM5).
288   However, many known myelin proteins clustered together according to their mRNA-
289   abundance peak coinciding with a later phase of myelination (cluster 'P18-UP'), including
290   peripheral myelin protein 2 (PMP2), tetraspanin-29 (CD9), tetraspanin-28 (CD81), connexin-
291   32 (GJB1), plasmolipin (PLLP), junctional adhesion molecule-3 (JAM3), CD59 and
292   dystrophin-related protein-2 (DRP2). The proportion of known myelin proteins was lower in
293   the clusters corresponding to mRNA-abundance peaks in adulthood (clusters 'late-UP', 'U-
294   shaped'). Yet, a considerable number of transcripts displayed abundance peaks at the
295   embryonic time-point (cluster 'Descending'), including carbonic anhydrase 2 (CA2), cofilin-1
296   CFL1), tubulin beta-4 (TUBB4b) and band 4.1-protein B (EPB41L3). Generalized, the
297   clusters were roughly similar when comparing previous oligonucleotide microarray analysis
298   of mouse sciatic nerves (41) and the RNA-Seq analysis of rat sciatic nerves (this study); yet,
299   the latter provides information on a larger number of genes and with a higher level of

300 confidence. Together, clustering of mRNA abundance profiles allows categorizing peripheral
301 myelin proteins into developmentally co-regulated groups.
302
303 When systematically assessing the proteins identified in myelin by gene ontology (GO)-term
304 analysis, the functional categories over-represented in the entire myelin proteome included
305 cell adhesion, cytoskeleton and extracellular matrix (labeled in turquoise in **Figure 4**). When
306 analyzing the clusters of developmentally co-expressed transcripts (from **Figure 3**), proteins
307 associated with the lipid metabolism were particularly enriched in the P6-UP and P18-UP
308 clusters, while those associated with the extracellular matrix (ECM) were over-represented in
309 the U-shaped and Descending clusters (**Figure 4**). For comparison, known myelin proteins
310 (**Table 1**) were over-represented in the P6-UP and P18-UP clusters (**Figure 4**). Together,
311 our proteome dataset provides comprehensive in-depth coverage of the protein constituents
312 of peripheral myelin purified from the sciatic nerves of wild type mice, and comparison to the
313 transcriptome allows identifying developmentally co-regulated and functional groups of
314 myelin proteins. Our data thus supply a solid resource for the molecular characterization of
315 myelin and for discovering functionally relevant myelin proteins.
316

317 **Neuropathy genes encoding myelin proteins**
318 Heritable neuropathies can be caused by mutations affecting genes preferentially expressed
319 in neurons, Schwann cells or both (3,61–63). To systematically assess which neuropathy-
320 causing genes encode peripheral myelin proteins, we compared our myelin proteome
321 dataset with a current overview about disease genes at the NIH National Library of Medicine
322 at https://ghr.nlm.nih.gov/condition/charcot-marie-tooth-disease#genes. Indeed, 31 myelin
323 proteins were identified to be encoded by a proven neuropathy gene (**Table 2**), a
324 considerable increase compared to eight disease genes found in a similar previous approach
325 (41). Notably, this increase is owing to both the larger size of the current myelin proteome
326 dataset (**Figure 1E**) and the recent discovery of numerous neuropathy genes by the
327 widespread application of next generation sequencing.
328

329 **Pathological proteomic profile of peripheral myelin in a neuropathy model**
330 The results presented thus far were based on analyzing myelin of healthy wild type mice; yet
331 we also sought to establish a straightforward method to systematically assess myelin
332 diversity, as exemplified by alterations in a pathological situation. As a model we chose mice
333 carrying a homozygous deletion of the periaxin gene ($Prx^{-/-}$) (26,64). Periaxin (PRX) is the
334 third-most abundant peripheral myelin protein (**Figure 2**) and scaffolds the dystroglycan
335 complex in Schwann cells. $Prx^{-/-}$ mice represent an established model of Charcot-Marie-
336 Tooth disease type 4F (65–67). Aiming to assess the myelin proteome, we purified myelin

337 from pools of sciatic nerves dissected from *Prx*^{-/-} and control mice at P21. Upon SDS-PAGE

338 separation and silver staining the band patterns appeared roughly similar (**Figure 5A**), with

339 the most obvious exception of the absence of the high-molecular weight band constituted by

340 periaxin in *Prx*^{-/-} myelin. Yet, several other bands also displayed genotype-dependent

341 differences in intensity. As expected, PRX was also undetectable by MS[E] in *Prx*^{-/-} myelin, in

342 which most of the total myelin protein was constituted by MPZ/P0 and MBP (**Figure 5B;**

343 **Figure 5-source data 1**).

344

345 Upon differential analysis by DRE-UDMS[E] (**Figure 5-source data 2**), multiple proteins

346 displayed genotype-dependent differences as visualized in a heatmap displaying those 40

347 proteins of which the abundance was reduced or increased with the highest statistical

348 significance in *Prx*^{-/-} compared to control myelin (**Figure 5C**). For example, the abundance of

349 the periaxin-associated dystrophin-related protein 2 (DRP2) was strongly reduced in *Prx*^{-/-}

350 myelin, as previously shown by immunoblotting (9). Notably, the abundance of multiple other

351 proteins was also significantly reduced in *Prx*^{-/-} myelin, including the extracellular matrix

352 protein laminin C1 (LAMC1; previously termed LAMB2), the laminin-associated protein

353 nidogen (NID1), Ig-like cell adhesion molecules (CADM4, MAG), the desmosomal junction

354 protein desmin (DES), cytoskeletal and cytoskeleton-associated proteins (EPB41L3, MAP1A,

355 CORO1A, SPTBN1, various microtubular and intermediate filament monomers), the

356 monocarboxylate transporter MCT1 (also termed SLC16A1) and the MCT1-associated (68)

357 immunoglobulin superfamily protein basigin (BSG, also termed CD147). On the other hand,

358 proteins displaying the strongest abundance increase in *Prx*^{-/-} myelin included immune-

359 related proteins (LGALS3, LYZ2, CTSD), cytoskeletal and cytoskeleton-associated proteins

360 (CAPG, CORO1C, CNN3, several myosin heavy chain subunits), peroxisomal enzymes

361 (CAT, HSD17B4, MDH1) and known myelin proteins (PLLP/plasmolipin, CRYAB,

362 GJB1/CX32). For comparison, the abundance of the marker proteolipid protein (PLP/DM20)

363 (69) and the periaxin-associated integrin beta-4 (ITGB4) (12) in myelin was unaltered in *Prx*^{-/-}

364 myelin. Together, differential proteome analysis finds considerably more proteins and protein

365 groups to be altered in *Prx*^{-/-} myelin than previously known (**Figure 5C, D-D‴**), probably

366 reflecting the complex pathology observed in this model (26,64).

367

368 The monocarboxylate transporter MCT1/SLC16A1 expressed by myelinating

369 oligodendrocytes (70,71) and Schwann cells (28,72) has been proposed to supply lactate or

370 other glucose breakdown products to axons, in which they may serve as substrate for the

371 mitochondrial production of ATP (73–75). In this respect it was striking to find the abundance

372 of MCT1 significantly reduced in peripheral myelin when PRX is lacking (**Figure 5C**), a result

373 that we were able to confirm by immunoblotting (**Figure 5E**) and immunolabeling of teased

374   fiber preparations of sciatic nerves (**Figure 5F**). Notably, reduced expression of MCT1 in

375   *Slc16a1$^{+/-}$* mice impairs axonal integrity at least in the CNS (70,76). The reduced abundance

376   of MCT1 thus represents an interesting novel facet of the complex pathology in *Prx$^{-/-}$* mice.

377   Considering that the integrity of peripheral axons may be impaired in *Prx$^{-/-}$* mice, we

378   assessed their quadriceps nerves. Indeed, *Prx$^{-/-}$* mice displayed reduced axonal diameters, a

379   progressively reduced total number of axons and a considerable number of myelin whorls

380   lacking a visible axon (**Figure 6**), indicative of impaired axonal integrity (77). Yet we note that

381   molecular or neuropathological features other than the reduced abundance of MCT1

382   probably also contribute to the axonopathy in *Prx$^{-/-}$* mice.

383

384   Together, gel-free, label free proteome analysis provides a cost- and time-efficient method

385   that provides an accurate, sensitive tool to gain systematic insight into the protein

386   composition of healthy peripheral myelin and its alterations in pathological situations. Indeed,

387   gel-free proteome analysis is particularly powerful and comprehensive compared to 2D-

388   DIGE; the workflow presented here appears readily applicable to other neuropathy models,

389   thereby promising discovery of relevant novel features of their neuropathology.

390

391

392 **DISCUSSION**

393

394 We used gel-free, label-free quantitative mass spectrometry to assess the protein
395 composition of myelin biochemically purified from the sciatic nerves of wild-type mice,
396 thereby establishing a straightforward and readily applicable workflow to approach the
397 peripheral myelin proteome. The key to comprehensiveness was to combine the strengths of
398 three data acquisition modes, i.e., MS^E for correct quantification of high-abundant proteins,
399 UDMS^E for deep quantitative proteome coverage including low-abundant proteins and DRE-
400 UDMS^E for differential analysis. We suggest that DRE-UDMS^E provides a good compromise
401 between dynamic range, identification rate and instrument run time for routine differential
402 myelin proteome profiling as a prerequisite for a molecular understanding of myelin
403 (patho)biology. We have also integrated the resulting compendium with RNA-Seq-based
404 mRNA abundance profiles in peripheral nerves and neuropathy disease loci. Beyond
405 providing the largest peripheral myelin proteome dataset thus far, the workflow is appropriate
406 to serve as starting point for assessing relevant variations of myelin protein composition, e.g.,
407 in different nerves, ages, species and in pathological conditions. The identification of
408 numerous pathological alterations of myelin protein composition in the *Prx$^{-/-}$* neuropathy
409 model indicates that the method is well suited to assess such diversity.

410

411 Aiming to understand nervous system function at the molecular level, multiple 'omics'-scale
412 projects assess the spatio-temporal expression profiles of all mRNAs and proteins in the
413 CNS including oligodendrocytes and myelin (35–39). Yet, peripheral nerves are also
414 essential for normal sensory and motor capabilities. Prior approaches to the molecular
415 profiles of Schwann cells and PNS myelin thus far, however, were performed >8 years ago
416 (32–34,41,78–80), and the techniques have considerably advanced since. For example,
417 current gel-free, label-free mass spectrometry can simultaneously identify and quantify the
418 vast majority of proteins in a sample, thereby providing comprehensive in depth-information.
419 Moreover, RNA-Seq technology has overcome limitations of the previously used microarrays
420 for characterizing mRNA abundance profiles with respect to the number of represented
421 genes and the suitability of the oligonucleotide probes. The present compendium thus
422 provides high confidence with respect to the identification of myelin proteins, their relative
423 abundance and their developmental mRNA expression profiles. This view is supported by the
424 finding that over 80% of the total myelin proteome is constituted by approximately 50
425 previously known myelin proteins. We believe that the majority of the other identified proteins
426 represent low-abundant myelin-associated constituents in line with the high efficiency of
427 biochemical myelin purification. Doubtless, however, the myelin proteome also comprises

428   contaminants from other cellular sources, underscoring the need of independent validation

429   for establishing newly identified constituents as true myelin proteins.

430

431   Do myelin proteins exist that escape identification by standard proteomic approaches?

432   Indeed, some proteins display atypically distributed lysine and arginine residues, which

433   represent the cleavage sites of the commonly used protease trypsin. The tryptic digest of

434   these proteins leads to peptides that are not well suited for chromatographic separation

435   and/or mass spectrometric detection/sequencing, as exemplified by the small hydrophobic

436   tetraspan-transmembrane myelin proteins MAL (81) and PMP22 (82). We can thus not

437   exclude that additional proteins with atypical tryptic digest patterns exist in peripheral myelin,

438   which would need to be addressed by the use of alternative proteases. Moreover, potent

439   signaling molecules including erbB receptor tyrosine kinases (83,84) and G-protein coupled

440   receptors (GPRs) (85–87) display exceptionally low abundance. Such proteins may be

441   identified when applying less stringent identification criteria, e.g., by requiring the sequencing

442   of only one unique peptide per protein. However, lower stringency would also result in

443   identifying false-positive proteins, which we wished to avoid for the purpose of the present

444   compendium. We note that a truly comprehensive spatio-temporally resolved myelin

445   proteome should preferentially also include systematic information about protein isoforms

446   and post-translational modifications, which still poses technical challenges.

447

448   Mutations affecting the periaxin (*PRX*) gene in humans cause CMT type 4F (65,88–90); the

449   neuropathology resulting from mutations affecting periaxin has been mainly investigated in

450   the *Prx$^{-/-}$* mouse model. Indeed, *Prx$^{-/-}$* mice display a progressive peripheral neuropathy

451   including axon/myelin-units with abnormal myelin thickness, demyelination, tomaculae, onion

452   bulbs, reduced nerve conduction velocity (64), reduced abundance and mislocalization of the

453   periaxin-associated DRP2 (9) and reduced internode length (26). Absence of SLIs (64) and

454   bands of Cajal (26) imply that the non-compact myelin compartments are impaired when

455   PRX is lacking. In the differential analysis of myelin purified from *Prx$^{-/-}$* and control mice we

456   find that the previously reported reduced abundance of DRP2 (9) represents one of the

457   strongest molecular changes in the myelin proteome when PRX is lacking. Notably, the

458   reported morphological changes in this neuropathy model (9,26,64) go along with alterations

459   affecting the abundance of multiple other myelin-associated proteins, including junctional,

460   cytoskeletal, extracellular matrix and immune-related proteins as well as lipid-modifying

461   enzymes. Thus, the neuropathology in *Prx$^{-/-}$* mice at the molecular level is more complex

462   than previously anticipated. It is striking that the abundance of the monocarboxylate

463   transporter MCT1/SLC16A1 that may contribute to the metabolic supply of lactate from

464   myelinating cells to axons (27–31) is strongly reduced in *Prx$^{-/-}$* myelin. Considering that

465 MCT1 in Schwann cells mainly localizes to Schmidt Lanterman incisures (SLI) (28) and that
466 SLI are largely absent from myelin when PRX is lacking (64), the reduced abundance of
467 MCT1 in $Prx^{-/-}$ myelin may be a consequence of the impaired myelin ultrastructure. Yet,
468 considering that SLI are part of the cytosolic channels that may represent transport routes
469 toward Schwann cell-dependent metabolic support of myelinated axons, the diminishment of
470 MCT1 may contribute to reduced axonal diameters or axonal loss in $Prx^{-/-}$ mice, probably in
471 conjunction with other molecular or morphological defects. Together, the in depth-analysis of
472 proteins altered in neuropathy models can contribute to an improved understanding of nerve
473 pathophysiology.

474

475 Compared to a previous approach (41), the number of proven neuropathy genes of which the
476 encoded protein is mass spectrometrically identified in peripheral myelin has increased four-
477 fold from eight to 32 in the present study. This reflects both that the number of proteins
478 identified in myelin has approximately doubled and that more neuropathy genes are known
479 due to the common use of genome sequencing. We note that our compendium comprises
480 not only myelin-associated proteins causing (when mutated) demyelinating CMT1 (e.g.,
481 MPZ/P0, NEFL, PMP2) or intermediate CMT4 (GDAP1, NDRG1, PRX) but also axonal
482 CMT2 (RAB7, GARS, HSPB1). Yet, the expression of genes causative of CMT2 is not
483 necessarily limited to neurons, as exemplified by the classical myelin protein MPZ/P0.
484 Indeed, a subset of *MPZ*-mutations causes axonal CMT2I or CMT2J (91–95), probably
485 reflecting impaired axonal integrity as consequence of a mutation primarily affecting
486 Schwann cells. We also note that the nuclear EGR2/KROX20 causative of demyelinating
487 CMT1D has not been mass spectrometrically identified in myelin, reflecting that Schwann cell
488 nuclei are efficiently removed during myelin purification.

489

490 While morphological analysis of peripheral nerves by light and electron microscopy is routine
491 in numerous laboratories, systematic molecular analysis has been less straightforward.
492 Using the sciatic nerve as a model, we show that systematic assessment of the myelin
493 proteome and the total nerve transcriptome are suited to determine comprehensive
494 molecular profiles in healthy nerves and in myelin-related disorders. Myelin proteome
495 analysis can thus complement transcriptome analysis in assessing development, function
496 and pathophysiology of peripheral nerves.

497

MATERIALS AND METHODS

Mouse models

Prx^{-/-} mice (64) were kept on c57Bl/6 background in the animal facility of the University of Edinburgh (United Kingdom). Genotyping was by PCR on genomic DNA using the forward primers 5'-CAGATTTGCT CTGCCCAAGT and 5'-CGCCTTCTAT CGCCTTCTTGAC in combination with reverse primer 5'-ATGCCCTCAC CCACTAACAG. The PCR yielded a 0.5 kb fragment for the wildtype allele and a 0.75 kb product for the mutant allele. The age of experimental animals is given in the figure legends. All animal work conformed to United Kingdom legislation (Scientific Procedures) Act 1986 and to the University of Edinburgh Ethical Review Committee policy; Home Office project license No. P0F4A25E9.

Myelin purification

A light-weight membrane fraction enriched for myelin was purified from sciatic nerves of mice by sucrose density centrifugation and osmotic shocks as described (41,52). Myelin accumulates at the interface between 0.29 and 0.85 M sucrose. *Prx*^{-/-} and wild type control C57Bl/6 mice were sacrificed by cervical dislocation at postnatal day 21 (P21). For each genotype, myelin was purified as three biological replicates (n=3); each biological replicate representing a pool of 20 sciatic nerves dissected from 10 mice. Protein concentration was determined using the DC Protein Assay Kit (Bio-Rad).

Filter-aided sample preparation for proteome analysis

Protein fractions corresponding to 10 µg myelin protein were dissolved and processed according to a filter-aided sample preparation (FASP) protocol essentially as previously described for synaptic protein fractions (96) and as adapted to CNS myelin (52,97). Unless stated otherwise, all steps were automated on a liquid-handling workstation equipped with a vacuum manifold (Freedom EVO 150, Tecan) by using an adaptor device constructed in-house. Briefly, myelin protein samples were lysed and reduced in lysis buffer (7 M urea, 2 M thiourea, 10 mM DTT, 0.1 M Tris pH 8.5) containing 1% ASB-14 by shaking for 30 min at 37°C. Subsequently, the sample was diluted with ~10 volumes lysis buffer containing 2% CHAPS to reduce the ASB-14 concentration and loaded on centrifugal filter units (30 kDa MWCO, Merck Millipore). After removal of the detergents by washing twice with wash buffer (8 M urea, 10 mM DTT, 0.1 M Tris pH 8.5), proteins were alkylated with 50 mM iodoacetamide in 8 M urea, 0.1 M Tris pH 8.5 (20 min at RT), followed by two washes with wash buffer to remove excess reagent. Buffer was exchanged by washing three times with 50 mM ammonium bicarbonate (ABC) containing 10 % acetonitrile. After three additional washes with 50 mM ABC/10% acetonitrile, which were performed by centrifugation to ensure

535 quantitative removal of liquids potentially remaining underneath the ultrafiltration membrane,
536 proteins were digested overnight at 37°C with 400 ng trypsin in 40 µl of the same buffer.
537 Tryptic peptides were recovered by centrifugation followed by two additional extraction steps
538 with 40 µl of 50 mM ABC and 40 µl of 1% trifluoroacetic acid (TFA), respectively. Aliquots of
539 the combined flow-throughs were spiked with 10 fmol/µl of yeast enolase-1 tryptic digest
540 standard (Waters Corporation) for quantification purposes and directly subjected to analysis
541 by liquid chromatography coupled to electrospray mass spectrometry (LC-MS). A pool of all
542 samples was injected at least before and after any sample set to monitor stability of
543 instrument performance.
544
545 **Mass spectrometry**
546 Nanoscale reversed-phase UPLC separation of tryptic peptides was performed with a
547 nanoAcquity UPLC system equipped with a Symmetry C18 5 µm, 180 µm × 20 mm trap
548 column and a HSS T3 C18 1.8 µm, 75 µm × 250 mm analytical column (Waters Corporation)
549 maintained at 45°C. Injected peptides were trapped for 4 min at a flow rate of 8 µl/min 0.1%
550 TFA and then separated over 120 min at a flow rate of 300 nl/min with a gradient comprising
551 two linear steps of 3-35% mobile phase B in 105 min and 35-60% mobile phase B in 15 min,
552 respectively. Mobile phase A was water containing 0.1% formic acid while mobile phase B
553 was acetonitrile containing 0.1% formic acid. Mass spectrometric analysis of tryptic peptides
554 was performed using a Synapt G2-S quadrupole time-of-flight mass spectrometer equipped
555 with ion mobility option (Waters Corporation). Positive ions in the mass range m/z 50 to 2000
556 were acquired with a typical resolution of at least 20.000 FWHM (full width at half maximum)
557 and data were lock mass corrected post-acquisition. UDMS[E] and DRE-UDMS[E] analyses
558 were performed in the ion mobility-enhanced data-independent acquisition mode with drift
559 time-specific collision energies as described in detail by Distler et al. (48,49). Specifically, for
560 DRE-UDMS[E] a deflection device (DRE lens) localized between the quadrupole and the ion
561 mobility cell of the mass spectrometer was cycled between full (100% for 0.4 sec) and
562 reduced (5% for 0.4 sec) ion transmission during one 0.8 sec full scan. Continuum LC-MS
563 data were processed for signal detection, peak picking, and isotope and charge state
564 deconvolution using Waters ProteinLynx Global Server (PLGS) version 3.0.2 (47). For
565 protein identification, a custom database was compiled by adding the sequence information
566 for yeast enolase 1 and porcine trypsin to the UniProtKB/Swiss-Prot mouse proteome and by
567 appending the reversed sequence of each entry to enable the determination of false
568 discovery rate (FDR). Precursor and fragment ion mass tolerances were automatically
569 determined by PLGS 3.0.2 and were typically below 5 ppm for precursor ions and below 10
570 ppm (root mean square) for fragment ions. Carbamidomethylation of cysteine was specified
571 as fixed and oxidation of methionine as variable modification. One missed trypsin cleavage

572   was allowed. Minimal ion matching requirements were two fragments per peptide, five

573   fragments per protein, and one peptide per protein. The FDR for protein identification was set

574   to 1% threshold.

575

576   **Analysis of proteomic data**

577   For each genotype (*Prx*$^{-/-}$ and wild type control mice sacrificed at P21), biochemical fractions

578   enriched for PNS myelin were analyzed as three biological replicates (n=3 per condition);

579   each biological replicate representing a pool of 20 sciatic nerves dissected from 10 mice. The

580   samples were processed with replicate digestion and injection, resulting in four technical

581   replicates per biological replicate and thus a total of 12 LC-MS runs per condition to be

582   compared, essentially as previously reported for CNS myelin (36,97). The freely available

583   software ISOQuant (www.isoquant.net) was used for post-identification analysis including

584   retention time alignment, exact mass and retention time (EMRT) and ion mobility clustering,

585   peak intensity normalization, isoform/homology filtering and calculation of absolute in-sample

586   amounts for each detected protein (48,49,98) according to the TOP3 quantification approach

587   (53,54). Only peptides with a minimum length of seven amino acids that were identified with

588   scores above or equal to 5.5 in at least two runs were considered. FDR for both peptides and

589   proteins was set to 1% threshold and only proteins reported by at least two peptides (one of

590   which unique) were quantified using the TOP3 method. The parts per million (ppm)

591   abundance values (i.e. the relative amount (w/w) of each protein in respect to the sum over

592   all detected proteins) were log2-transformed and normalized by subtraction of the median

593   derived from all data points for the given protein. Significant changes in protein abundance

594   were detected by moderated t-statistics essentially as described (96,97) across all technical

595   replicates using an empirical Bayes approach and false discovery (FDR)-based correction for

596   multiple comparisons (100). For this purpose, the Bioconductor R packages "limma" (101)

597   and "q-value" (102) were used in RStudio, an integrated development environment for the

598   open source programming language R. Proteins identified as contaminants (e.g. components

599   of blood or hair cells) were removed from the analysis. Proteins with ppm values below 100

600   which were not identified in one genotype were considered as just above detection level and

601   also removed from the analysis. The relative abundance of a protein in myelin was accepted

602   as altered if both statistically significant (q-value <0.05). Pie charts, heatmaps and volcano

603   plots were prepared in Microsoft Excel 2013 and GraphPad Prism 7. Pearson's correlation

604   coefficients derived from log2-transformed ppm abundance values were clustered and

605   visualized with the tool heatmap.2 contained in the R package gplots (CRAN.R-

606   project.org/package=gplots). Only pairwise complete observations were considered to

607   reduce the influence of missing values on clustering behavior. The mass spectrometry

608   proteomics data have been deposited to the ProteomeXchange Consortium

609 (proteomecentral.proteomexchange.org) via the PRIDE partner repository (103) with the
610 dataset identifier PXD015960.

611

612 **Gel electrophoresis and silver staining of gels**
613 Protein concentration was determined using the DC Protein Assay kit (BioRad). Samples
614 were separated on a 12% SDS-PAGE for 1 h at 200 V using the BioRad system, fixated
615 overnight in 10% [v/v] acetic acid and 40 % [v/v] ethanol and then washed in 30% ethanol (2x
616 20 min) and ddH$_2$O (1x 20 min). For sensitization, gels were incubated 1 min in 0.012% [v/v]
617 Na$_2$S$_2$O$_3$ and subsequently washed with ddH$_2$O (3x 20 sec). For silver staining, gels were
618 impregnated for 20 min in 0.2 % [w/v] AgNO$_3$ / 0.04% formaldehyde, washed with ddH$_2$O (3x
619 20 sec) and developed in 3% [w/v] Na$_2$CO$_3$ / 0.02% [w/v] formaldehyde. The reaction was
620 stopped by exchanging the solution with 5% [v/v] acetic acid.

621

622 **Immunoblotting**
623 Immunoblotting was performed as described (104,105). Primary antibodies were specific for
624 dystrophin-related-protein 2 (DRP2; Sigma; 1:1000), peripheral myelin protein 2 (PMP2;
625 ProteinTech Group 12717-1-AP; 1:1000), proteolipid protein (PLP/DM20; A431 (106);
626 1:5000), Monocarboxylate transporter 1 (MCT1/SLC16A1; (107); 1:1000), periaxin (PRX;
627 (59); 1:1000), sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1; 1:2000;
628 Abcam #13736-1-AP), myelin protein zero (MPZ/P0; (108); kind gift by J. Archelos-Garcia;
629 1:10.000), voltage-dependent anion-selective channel protein (VDAC; Abcam #ab15895;
630 1:1000), basigin (BSG/CD147; ProteinTech Group #ab64616; 1:1000), neurofilament H
631 (NEFH/NF-H; Covance #SMI-32P; 1:1000), voltage-gated potassium channel subunit A
632 member 1 (KCNA1; Neuromab #73-007; 1:1000), EGR2/KROX20 ((109); kind gift by D.
633 Meijer, Edinburgh; 1:1000) and myelin basic protein (MBP; 1:2000). To generate the latter
634 antisera, rabbits were immunized (Pineda Antikörper Service, Berlin, Germany) with the
635 KLH-coupled peptide CQDENPVVHFFK corresponding to amino acids 212-222 of mouse
636 MBP isoform 1 (Swisprot/Uniprot-identifier P04370-1). Anti-MBP antisera were purified by
637 affinity chromatography and extensively tested for specificity by immunoblot analysis of
638 homogenate of brains dissected from wild-type mice compared to *Mbp$^{shiverer/shiverer}$* mice that
639 lack expression of MBP. Appropriate secondary anti-mouse or -rabbit antibodies conjugated
640 to HRP were from dianova. Immunoblots were developed using the Enhanced
641 Chemiluminescence Detection kit (Western Lightning® Plus, Perkin Elmer) and detected with
642 the Intas ChemoCam system (INTAS Science Imaging Instruments GmbH, Göttingen,
643 Germany).

644

645 **Immunolabelling of teased fibers**

646 Teased fibers were prepared as previously described (9,110). For each genotype, one male
647 mouse was sacrificed by cervical dislocation at P17. Immunolabelling of teased fibers was
648 performed as described (69). Briefly, teased fibers were fixed for 5 min in 4%
649 paraformaldehyde, permeabilized 5 min with ice-cold methanol, washed in PBS (3x 5 min)
650 and blocked for 1 h at 21°C in blocking buffer (10% horse serum, 0.25% Triton X-100, 1%
651 bovine serum albumin in PBS). Primary antibodies were applied overnight at 4°C in
652 incubation buffer (1.5% horse serum, 0.25% Triton X-100 in PBS). Samples were washed in
653 PBS (3x 5 min) and secondary antibodies were applied in incubation buffer (1 h, RT).
654 Samples were again washed in PBS (2x 5 min), and 4',6-diamidino-2-phenylindole (DAPI;
655 1:50 000 in PBS) was applied for 10 min at RT. Samples were briefly washed 2x with ddH$_2$O
656 and mounted using Aqua-Poly/Mount (Polysciences, Eppelheim, Germany). Antibodies were
657 specific for myelin-associated glycoprotein (MAG clone 513; Chemicon MAB1567; 1:50) and
658 MCT1/SLC16A1 (107). Secondary antibodies were donkey α-rabbit-Alexa488 (Invitrogen
659 A21206; 1:1000) and donkey α-mouse-Alexa555 (Invitrogen A21202; 1:1000). Labeled
660 teased fibers were imaged using the confocal microscope Leica SP5. The signal was
661 collected with the objective HCX PL APO lambda blue 63.0.x1.20. DAPI staining was excited
662 with 405 nm and collected between 417 nm - 480 nm. To excite the Alexa488 fluorophore an
663 Argon laser with the excitation of 488 nm was used and the emission was set to 500 nm -
664 560 nm. Alexa555 was excited by using the DPSS561 laser at an excitation of 561 nm and
665 the emission was set to 573 nm - 630 nm. To export and process the images LAS AF lite and
666 Adobe Photoshop were used.
667

668 **mRNA abundance profiles**
669 Raw data were previously established (60) from the sciatic nerves of wild type Sprague
670 Dawley rats at the indicated ages (E21, P6, P18; n=4 per time point). Briefly, sciatic nerves
671 were dissected, the epineurium was removed, total RNA was extracted with the RNeasy Kit
672 (Qiagen), concentration and quality (ratio of absorption at 260/280 nm) of RNA samples were
673 determined using the NanoDrop spectrophotometer (ThermoScientific), integrity of the
674 extracted RNA was determined with the Agilent 2100 Bioanalyser (Agilent Technologies) and
675 RNA-Seq was performed using the Illumina HiSeq2000 platform. RNA-Seq raw data are
676 available under the GEO accession number GSE115930 (60). For the present analysis, the
677 fastqfiles were mapped to *rattus norvegicus* rn6 using Tophat Aligner and then quantified
678 based on the Ensemble Transcripts release v96. The raw read counts were then normalized
679 using the R package DESeq2. The normalized gene expression data was then standardized
680 to a mean of zero and a standard deviation of one, therefore genes with similar changes in
681 expression are close in the euclidian space. Clustering was performed on the standardized

682   data using the R package mfuzz. Transcripts displaying abundance differences of less than
683   10% coefficient of variation were considered developmentally unchanged.
684

685   **Venn diagrams**
686   Area-proportional Venn diagrams were prepared using BioVenn (111) at www.biovenn.nl/.
687

688   **GO-term**
689   For functional categorization of the myelin proteome the associated gene ontology terms
690   were systematically analyzed on the mRNA abundance cluster using the Database for
691   Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov). For
692   comparison known myelin proteins according to literature were added.
693

694   **Histological analysis**
695   *Prx$^{-/-}$* and control mice were perfused at the indicated ages intravascularly with fixative
696   solution (2.5% glutaraldehyde, 4% paraformaldehyde, 0.1 M sodium cacodylate buffer, pH
697   7.4). Quadriceps nerves were removed, fixed for 2 h at room temperature, followed by 18 h
698   at 4°C in the same fixative, postfixed in OsO_4, dehydrated a graded series of ethanol,
699   followed by propylene oxide and embedded in Araldite. All axons not associated with a
700   Remak bundle were counted and categorized as myelinated or non-myelinated. All myelin
701   profiles lacking a recognizable axon were counted. The total number of axons were counted
702   on micrographs of toluidine blue stained Araldite sections (0.5 μm) of quadriceps nerves.
703   Precise p-values for the quantitative comparison between Ctrl and *Prx$^{-/-}$* mice were: Total
704   number of axons (**Figure 6B**; Student's unpaired t-test): 2 mo p=0.01734; 4 mo p=2.1E-05; 9
705   mo p=0.007625; Number of myelinated axons (**Figure 6C**; Student's unpaired t-test): 2 mo
706   p=0.00444; 4 mo p=2.12E-05; 9 mo p=0.005766; Number of empty myelin profiles (**Figure
707   6D**; Student's unpaired t-test): 2 mo p=0.004445; 4 mo p=0.001461; 9 mo p=0.000695;
708   Axonal diameters (**Figure 6E-G**; two-sided Kolmogorow-Smirnow test): 2 mo p=2.20E-16; 4
709   mo p=2.20E-16; 9 mo p=2.20E-16.
710
711

724

725

726 **CONFLICT OF INTEREST STATEMENT**

727 The authors declare no conflict of interest.

728

729

730

REFERENCES

1. Weil M-T, Heibeck S, Töpperwien M, tom Dieck S, Ruhwedel T, Salditt T, et al. Axonal Ensheathment in the Nervous System of Lamprey: Implications for the Evolution of Myelinating Glia. J Neurosci [Internet]. 2018 Jul 18;38(29):6586–96. Available from: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.1034-18.2018

2. Hartline DK, Colman DR. Rapid Conduction and the Evolution of Giant Axons and Myelinated Fibers. Curr Biol [Internet]. 2007 Jan;17(1):R29–35. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960982206025231

3. Rossor AM, Polke JM, Houlden H, Reilly MM. Clinical implications of genetic advances in Charcot–Marie–Tooth disease. Nat Rev Neurol [Internet]. 2013 Oct 10;9(10):562–71. Available from: http://www.nature.com/articles/nrneurol.2013.179

4. Michailov G V. Axonal Neuregulin-1 Regulates Myelin Sheath Thickness. Science (80-) [Internet]. 2004 Apr 30;304(5671):700–3. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1095862

5. Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, et al. Neuregulin-1 Type III Determines the Ensheathment Fate of Axons. Neuron [Internet]. 2005 Sep;47(5):681–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627305006926

6. Chernousov MA, Yu W-M, Chen Z-L, Carey DJ, Strickland S. Regulation of Schwann cell function by the extracellular matrix. Glia [Internet]. 2008 Nov 1;56(14):1498–507. Available from: http://doi.wiley.com/10.1002/glia.20740

7. Petersen SC, Luo R, Liebscher I, Giera S, Jeong S-J, Mogha A, et al. The Adhesion GPCR GPR126 Has Distinct, Domain-Dependent Functions in Schwann Cell Development Mediated by Interaction with Laminin-211. Neuron [Internet]. 2015 Feb;85(4):755–69. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627314011660

8. Ghidinelli M, Poitelon Y, Shin YK, Ameroso D, Williamson C, Ferri C, et al. Laminin 211 inhibits protein kinase A in Schwann cells to modulate neuregulin 1 type III-driven myelination. Emery B, editor. PLOS Biol [Internet]. 2017 Jun 21;15(6):e2001408. Available from: https://dx.plos.org/10.1371/journal.pbio.2001408

9. Sherman DL, Fabrizi C, Gillespie CS, Brophy PJ. Specific Disruption of a Schwann Cell Dystrophin-Related Protein Complex in a Demyelinating Neuropathy. Neuron [Internet]. 2001 May;30(3):677–87. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627301003270

10. Masaki T, Matsumura K, Hirata A, Yamada H, Hase A, Arai K, et al. Expression of Dystroglycan and the Laminin-α2 Chain in the Rat Peripheral Nerve during Development. Exp Neurol [Internet]. 2002 Mar;174(1):109–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0014488601978562

11. Nodari A, Previtali SC, Dati G, Occhi S, Court FA, Colombelli C, et al. 6 4 Integrin and Dystroglycan Cooperate to Stabilize the Myelin Sheath. J Neurosci [Internet]. 2008 Jun 25;28(26):6714–9. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.0326-08.2008

772   12.   Raasakka A, Linxweiler H, Brophy PJ, Sherman DL, Kursula P. Direct Binding of the Flexible
773   C-Terminal Segment of Periaxin to β4 Integrin Suggests a Molecular Basis for CMT4F. Front
774   Mol Neurosci [Internet]. 2019 Apr 9;12. Available from:
775   https://www.frontiersin.org/article/10.3389/fnmol.2019.00084/full

776   13.   Hess A, Lansing AI. The fine structure of peripheral nerve fibers. Anat Rec. 1953;

777   14.   Sherman DL, Brophy PJ. Mechanisms of axon ensheathment and myelin growth. Nat Rev
778   Neurosci [Internet]. 2005 Sep;6(9):683–90. Available from:
779   http://www.nature.com/articles/nrn1743

780   15.   Pereira JA, Lebrun-Julien F, Suter U. Molecular mechanisms regulating myelination in the
781   peripheral nervous system. Trends Neurosci [Internet]. 2012 Feb;35(2):123–34. Available from:
782   https://linkinghub.elsevier.com/retrieve/pii/S0166223611001937

783   16.   Grove M, Brophy PJ. FAK Is Required for Schwann Cell Spreading on Immature Basal Lamina
784   to Coordinate the Radial Sorting of Peripheral Axons with Myelination. J Neurosci [Internet].
785   2014 Oct 1;34(40):13422–34. Available from:
786   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1764-14.2014

787   17.   Monk KR, Feltri ML, Taveggia C. New insights on schwann cell development. Glia [Internet].
788   2015 Aug;63(8):1376–93. Available from: http://doi.wiley.com/10.1002/glia.22852

789   18.   Feltri ML, Poitelon Y, Previtali SC. How Schwann Cells Sort Axons. Neurosci [Internet]. 2016
790   Jun 16;22(3):252–65. Available from:
791   http://journals.sagepub.com/doi/10.1177/1073858415572361

792   19.   Giese KP, Martini R, Lemke G, Soriano P, Schachner M. Mouse P0 gene disruption leads to
793   hypomyelination, abnormal expression of recognition molecules, and degeneration of myelin
794   and axons. Cell [Internet]. 1992 Nov;71(4):565–76. Available from:
795   https://linkinghub.elsevier.com/retrieve/pii/009286749290591Y

796   20.   Martini R, Mohajeri MH, Kasper S, Giese KP, Schachner M. Mice doubly deficient in the genes
797   for P0 and myelin basic protein show that both proteins contribute to the formation of the major
798   dense line in peripheral nerve myelin. J Neurosci. 1995;

799   21.   Nawaz S, Schweitzer J, Jahn O, Werner HB. Molecular evolution of myelin basic protein, an
800   abundant structural myelin component. Glia [Internet]. 2013 Aug;61(8):1364–77. Available
801   from: http://doi.wiley.com/10.1002/glia.22520

802   22.   Greenfield S, Brostoff S, Eylar EH, Morell P. PROTEIN COMPOSITION OF MYELIN OF THE
803   PERIPHERAL NERVOUS SYSTEM. J Neurochem [Internet]. 1973 Apr;20(4):1207–16.
804   Available from: http://doi.wiley.com/10.1111/j.1471-4159.1973.tb00089.x

805   23.   Brostoff SW, Karkhanis YD, Carlo DJ, Reuter W, Eylar EH. Isolation and partial
806   characterization of the major proteins of rabbit sciatic nerve myelin. Brain Res [Internet]. 1975
807   Mar;86(3):449–58. Available from:
808   https://linkinghub.elsevier.com/retrieve/pii/0006899375908951

809   24.   Nave K-A, Werner HB. Myelination of the Nervous System: Mechanisms and Functions. Annu
810   Rev Cell Dev Biol [Internet]. 2014 Oct 11;30(1):503–33. Available from:
811   http://www.annualreviews.org/doi/10.1146/annurev-cellbio-100913-013101

812   25.   Kleopa KA, Sargiannidou I. Connexins, gap junctions and peripheral neuropathy. Neurosci Lett

813   [Internet]. 2015 Jun;596:27–32. Available from:

814   https://linkinghub.elsevier.com/retrieve/pii/S0304394014008453

815   26.   Court FA, Sherman DL, Pratt T, Garry EM, Ribchester RR, Cottrell DF, et al. Restricted growth

816   of Schwann cells lacking Cajal bands slows conduction in myelinated nerves. Nature [Internet].

817   2004 Sep;431(7005):191–5. Available from: http://www.nature.com/articles/nature02841

818   27.   Beirowski B, Babetto E, Golden JP, Chen Y-J, Yang K, Gross RW, et al. Metabolic regulator

819   LKB1 is crucial for Schwann cell–mediated axon maintenance. Nat Neurosci [Internet]. 2014

820   Oct 7;17(10):1351–61. Available from: http://www.nature.com/articles/nn.3809

821   28.   Domenech-Estevez E, Baloui H, Repond C, Rosafio K, Medard J-J, Tricaud N, et al.

822   Distribution of Monocarboxylate Transporters in the Peripheral Nervous System Suggests

823   Putative Roles in Lactate Shuttling and Myelination. J Neurosci [Internet]. 2015 Mar

824   11;35(10):4151–6. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3534-

825   14.2015

826   29.   Kim S, Maynard JC, Sasaki Y, Strickland A, Sherman DL, Brophy PJ, et al. Schwann Cell O-

827   GlcNAc Glycosylation Is Required for Myelin Maintenance and Axon Integrity. J Neurosci

828   [Internet]. 2016 Sep 14;36(37):9633–46. Available from:

829   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1235-16.2016

830   30.   Gonçalves NP, Vægter CB, Andersen H, Østergaard L, Calcutt NA, Jensen TS. Schwann cell

831   interactions with axons and microvessels in diabetic neuropathy. Nat Rev Neurol [Internet].

832   2017 Mar 30;13(3):135–47. Available from: http://www.nature.com/articles/nrneurol.2016.201

833   31.   Stassart RM, Möbius W, Nave K-A, Edgar JM. The Axon-Myelin Unit in Development and

834   Degenerative Disease. Front Neurosci [Internet]. 2018 Jul 11;12. Available from:

835   https://www.frontiersin.org/article/10.3389/fnins.2018.00467/full

836   32.   Nagarajan R, Le N, Mahoney H, Araki T, Milbrandt J. Deciphering peripheral nerve myelination

837   by using Schwann cell expression profiling. Proc Natl Acad Sci [Internet]. 2002 Jun

838   25;99(13):8998–9003. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.132080999

839   33.   Le N, Nagarajan R, Wang JYT, Araki T, Schmidt RE, Milbrandt J. Analysis of congenital

840   hypomyelinating Egr2Lo/Lo nerves identifies Sox2 as an inhibitor of Schwann cell

841   differentiation and myelination. Proc Natl Acad Sci [Internet]. 2005 Feb 15;102(7):2596–601.

842   Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0407836102

843   34.   Ryu EJ, Yang M, Gustin JA, Chang L-W, Freimuth RR, Nagarajan R, et al. Analysis of

844   Peripheral Nerve Expression Profiles Identifies a Novel Myelin Glycoprotein, MP11. J Neurosci

845   [Internet]. 2008 Jul 23;28(30):7563–73. Available from:

846   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1659-08.2008

847   35.   Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, et al. An RNA-Sequencing

848   Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral

849   Cortex. J Neurosci [Internet]. 2014 Sep 3;34(36):11929–47. Available from:

850   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1860-14.2014

851   36.   Patzig J, Erwig MS, Tenzer S, Kusch K, Dibaj P, Möbius W, et al. Septin/anillin filaments

852   scaffold central nervous system myelin to accelerate nerve conduction. Elife. 2016;5:e17119.

853   37.   Sharma K, Schmitt S, Bergner CG, Tyanova S, Kannaiyan N, Manrique-Hoyos N, et al. Cell

854   type– and brain region–resolved mouse brain proteome. Nat Neurosci [Internet]. 2015 Dec

855   2;18(12):1819–31. Available from: http://www.nature.com/articles/nn.4160

856   38.   Thakurela S, Garding A, Jung RB, Müller C, Goebbels S, White R, et al. The transcriptome of

857   mouse central nervous system myelin. Sci Rep [Internet]. 2016 May 13;6(1):25828. Available

858   from: http://www.nature.com/articles/srep25828

859   39.   Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcao A, Xiao L, et al.

860   Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system.

861   Science (80-) [Internet]. 2016 Jun 10;352(6291):1326–9. Available from:

862   http://www.sciencemag.org/cgi/doi/10.1126/science.aaf6463

863   40.   De Monasterio-Schrader P, Jahn O, Tenzer S, Wichert SP, Patzig J, Werner HB. Systematic

864   approaches to central nervous system myelin. Cellular and Molecular Life Sciences. 2012.

865   41.   Patzig J, Jahn O, Tenzer S, Wichert SP, de Monasterio-Schrader P, Rosfa S, et al.

866   Quantitative and Integrative Proteome Analysis of Peripheral Nerve Myelin Identifies Novel

867   Myelin Proteins and Candidate Neuropathy Loci. J Neurosci [Internet]. 2011 Nov

868   9;31(45):16369–86. Available from:

869   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4016-11.2011

870   42.   Kangas SM, Ohlmeier S, Sormunen R, Jouhilahti E-M, Peltonen S, Peltonen J, et al. An

871   approach to comprehensive genome and proteome expression analyses in Schwann cells and

872   neurons during peripheral nerve myelin formation. J Neurochem [Internet]. 2016

873   Sep;138(6):830–44. Available from: http://doi.wiley.com/10.1111/jnc.13722

874   43.   Kusch K, Uecker M, Liepold T, Möbius W, Hoffmann C, Neumann H, et al. Partial

875   Immunoblotting of 2D-Gels: A Novel Method to Identify Post-Translationally Modified Proteins

876   Exemplified for the Myelin Acetylome. Proteomes [Internet]. 2017 Jan 12;5(4):3. Available

877   from: http://www.mdpi.com/2227-7382/5/1/3

878   44.   Neilson KA, Ali NA, Muralidharan S, Mirzaei M, Mariani M, Assadourian G, et al. Less label,

879   more free: Approaches in label-free quantitative mass spectrometry. Proteomics. 2011.

880   45.   Distler U, Kuharev J, Tenzer S. Biomedical applications of ion mobility-enhanced data-

881   independent acquisition-based label-free quantitative proteomics. Expert Rev Proteomics.

882   2014;

883   46.   Geromanos SJ, Vissers JPC, Silva JC, Dorschel CA, Li GZ, Gorenstein M V., et al. The

884   detection, correlation, and comparison of peptide precursor and product ions from data

885   independent LC-MS with data dependant LC-MS/MS. Proteomics. 2009;

886   47.   Li GZ, Vissers JPC, Silva JC, Golick D, Gorenstein M V., Geromanos SJ. Database searching

887   and accounting of multiplexed precursor and product ion spectra from the data independent

888   analysis of simple and complex peptide mixtures. Proteomics. 2009;

889   48.   Distler U, Kuharev J, Navarro P, Tenzer S. Label-free quantification in ion mobility–enhanced

890   data-independent acquisition proteomics. Nat Protoc [Internet]. 2016 Apr 24;11(4):795–812.

891   Available from: http://www.nature.com/articles/nprot.2016.042

892   49.   Distler U, Kuharev J, Navarro P, Levin Y, Schild H, Tenzer S. Drift time-specific collision

893   energies enable deep-coverage data-independent acquisition proteomics. Nat Methods

894   [Internet]. 2014 Feb 15;11(2):167–70. Available from:

895   http://www.nature.com/articles/nmeth.2767

896   50.   Larocca JN, Norton WT. Isolation of Myelin. Curr Protoc Cell Biol [Internet]. 2006
897   Dec;33(1):3.25.1-3.25.19. Available from: http://doi.wiley.com/10.1002/0471143030.cb0325s33

898   51.   Jahn O, Tenzer S, Werner HB. Myelin proteomics: Molecular anatomy of an insulating sheath.
899   Molecular Neurobiology. 2009.

900   52.   Erwig MS, Hesse D, Jung RB, Uecker M, Kusch K, Tenzer S, et al. Myelin: Methods for
901   Purification and Proteome Analysis. In: Methods in Molecular Biology [Internet]. 2019. p. 37–
902   63. Available from: http://link.springer.com/10.1007/978-1-4939-9072-6_3

903   53.   Silva JC, Gorenstein M V., Li G-Z, Vissers JPC, Geromanos SJ. Absolute Quantification of
904   Proteins by LCMS E. Mol Cell Proteomics [Internet]. 2006 Jan;5(1):144–56. Available from:
905   http://www.mcponline.org/lookup/doi/10.1074/mcp.M500230-MCP200

906   54.   Ahrné E, Molzahn L, Glatter T, Schmidt A. Critical assessment of proteome-wide label-free
907   absolute abundance estimation strategies. Proteomics. 2013;

908   55.   Dodds JN, Baker ES. Ion Mobility Spectrometry: Fundamental Concepts, Instrumentation,
909   Applications, and the Road Ahead. J Am Soc Mass Spectrom. 2019;

910   56.   Micko S, Schlaepfer WW. PROTEIN COMPOSITION OF AXONS and MYELIN FROM RAT
911   and HUMAN PERIPHERAL NERVES. J Neurochem [Internet]. 1978 May;30(5):1041–9.
912   Available from: http://doi.wiley.com/10.1111/j.1471-4159.1978.tb12397.x

913   57.   Smith ME, Curtis BM. FROG SCIATIC NERVE MYELIN: A CHEMICAL CHARACTERIZATION.
914   J Neurochem [Internet]. 1979 Aug;33(2):447–52. Available from:
915   http://doi.wiley.com/10.1111/j.1471-4159.1979.tb05174.x

916   58.   Whitaker JN. The protein antigens of peripheral nerve myelin. Ann Neurol [Internet].
917   1981;9(S1):56–64. Available from: http://doi.wiley.com/10.1002/ana.410090710

918   59.   Gillespie CS, Sherman DL, Blair GE, Brophy PJ. Periaxin, a novel protein of myelinating
919   schwann cells with a possible role in axonal ensheathment. Neuron [Internet]. 1994
920   Mar;12(3):497–508. Available from:
921   https://linkinghub.elsevier.com/retrieve/pii/0896627394902089

922   60.   Fledrich R, Abdelaal T, Rasch L, Bansal V, Schütza V, Brügger B, et al. Targeting myelin lipid
923   metabolism as a potential therapeutic strategy in a model of CMT1A neuropathy. Nat Commun
924   [Internet]. 2018 Dec 2;9(1):3025. Available from: http://www.nature.com/articles/s41467-018-
925   05420-0

926   61.   Pareyson D, Marchesi C. Diagnosis, natural history, and management of Charcot–Marie–Tooth
927   disease. Lancet Neurol [Internet]. 2009 Jul;8(7):654–67. Available from:
928   https://linkinghub.elsevier.com/retrieve/pii/S1474442209701103

929   62.   Baets J, De Jonghe P, Timmerman V. Recent advances in Charcot–Marie–Tooth disease. Curr
930   Opin Neurol [Internet]. 2014 Oct;27(5):532–40. Available from:
931   http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00019052-
932   201410000-00006

933   63.   Brennan KM, Bai Y, Shy ME. Demyelinating CMT–what's known, what's new and what's in
934   store? Neurosci Lett [Internet]. 2015 Jun;596:14–26. Available from:
935   https://linkinghub.elsevier.com/retrieve/pii/S0304394015000725

936   64.   Gillespie CS, Sherman DL, Fleetwood-Walker SM, Cottrell DF, Tait S, Garry EM, et al.
937   Peripheral Demyelination and Neuropathic Pain Behavior in Periaxin-Deficient Mice. Neuron
938   [Internet]. 2000 May;26(2):523–31. Available from:
939   https://linkinghub.elsevier.com/retrieve/pii/S0896627300811848

940   65.   Guilbot A. A mutation in periaxin is responsible for CMT4F, an autosomal recessive form of
941   Charcot-Marie-Tooth disease. Hum Mol Genet [Internet]. 2001 Feb 1;10(4):415–21. Available
942   from: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/10.4.415

943   66.   Berger P, Niemann A, Suter U. Schwann cells and the pathogenesis of inherited motor and
944   sensory neuropathies (Charcot-Marie-Tooth disease). Glia [Internet]. 2006 Sep;54(4):243–57.
945   Available from: http://doi.wiley.com/10.1002/glia.20386

946   67.   Marchesi C, Milani M, Morbin M, Cesani M, Lauria G, Scaioli V, et al. Four novel cases of
947   periaxin-related neuropathy and review of the literature. Neurology [Internet]. 2010 Nov
948   16;75(20):1830–8. Available from:
949   http://www.neurology.org/cgi/doi/10.1212/WNL.0b013e3181fd6314

950   68.   Philp NJ, Ochrietor JD, Rudoy C, Muramatsu T, Linser PJ. Loss of MCT1, MCT3, and MCT4
951   Expression in the Retinal Pigment Epithelium and Neural Retina of the 5A11/Basigin-Null
952   Mouse. Investig Opthalmology Vis Sci [Internet]. 2003 Mar 1;44(3):1305. Available from:
953   http://iovs.arvojournals.org/article.aspx?doi=10.1167/iovs.02-0552

954   69.   Patzig J, Kusch K, Fledrich R, Eichel MA, Lüders KA, Möbius W, et al. Proteolipid protein
955   modulates preservation of peripheral axons and premature death when myelin protein zero is
956   lacking. Glia [Internet]. 2016 Jan;64(1):155–74. Available from:
957   http://doi.wiley.com/10.1002/glia.22922

958   70.   Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, et al. Oligodendroglia
959   metabolically support axons and contribute to neurodegeneration. Nature [Internet]. 2012 Jul
960   11;487(7408):443–8. Available from: http://www.nature.com/articles/nature11314

961   71.   Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, et al. Glycolytic
962   oligodendrocytes maintain myelin and long-term axonal integrity. Nature [Internet]. 2012 May
963   29;485(7399):517–21. Available from: http://www.nature.com/articles/nature11007

964   72.   Morrison BM, Tsingalia A, Vidensky S, Lee Y, Jin L, Farah MH, et al. Deficiency in
965   monocarboxylate transporter 1 (MCT1) in mice delays regeneration of peripheral nerves
966   following sciatic nerve crush. Exp Neurol [Internet]. 2015 Jan;263:325–38. Available from:
967   https://linkinghub.elsevier.com/retrieve/pii/S0014488614003549

968   73.   Morrison BM, Lee Y, Rothstein JD. Oligodendroglia: Metabolic supporters of axons. Trends in
969   Cell Biology. 2013.

970   74.   Saab AS, Tzvetanova ID, Nave K-A. The role of myelin and oligodendrocytes in axonal energy
971   metabolism. Curr Opin Neurobiol [Internet]. 2013 Dec;23(6):1065–72. Available from:
972   https://linkinghub.elsevier.com/retrieve/pii/S0959438813001888

973   75.   Rinholm JE, Bergersen LH. White matter lactate – Does it matter? Neuroscience [Internet].
974   2014 Sep;276:109–16. Available from:
975   https://linkinghub.elsevier.com/retrieve/pii/S0306452213008464

976   76.   Jha MK, Lee Y, Russell KA, Yang F, Dastgheyb RM, Deme P, et al. Monocarboxylate

977   transporter 1 in Schwann cells contributes to maintenance of sensory nerve myelination during
978   aging. Glia. 2019;

979   77.   Edgar JM, McLaughlin M, Werner HB, McCulloch MC, Barrie JA, Brown A, et al. Early
980   ultrastructural defects of axons and axon-glia junctions in mice lacking expression of Cnp1.
981   Glia. 2009;

982   78.   Verheijen MHG. Local regulation of fat metabolism in peripheral nerves. Genes Dev [Internet].
983   2003 Oct 1;17(19):2450–64. Available from:
984   http://www.genesdev.org/cgi/doi/10.1101/gad.1116203

985   79.   Buchstaller J. Efficient Isolation and Gene Expression Profiling of Small Numbers of Neural
986   Crest Stem Cells and Developing Schwann Cells. J Neurosci [Internet]. 2004 Mar
987   10;24(10):2357–65. Available from:
988   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4083-03.2004

989   80.   D'antonio M, Michalovich D, Paterson M, Droggiti A, Woodhoo A, Mirsky R, et al. Gene
990   profiling and bioinformatic analysis of Schwann cell embryonic development and myelination.
991   Glia [Internet]. 2006 Apr 1;53(5):501–15. Available from:
992   http://doi.wiley.com/10.1002/glia.20309

993   81.   Schaeren-Wiemers N, Bonnet A, Erb M, Erne B, Bartsch U, Kern F, et al. The raft-associated
994   protein MAL is required for maintenance of proper axon–glia interactions in the central nervous
995   system. J Cell Biol [Internet]. 2004 Aug 30;166(5):731–42. Available from:
996   http://www.jcb.org/lookup/doi/10.1083/jcb.200406092

997   82.   Adlkofer K, Martini R, Aguzzi A, Zielasek J, Toyka K V., Suter U. Hypermyelination and
998   demyelinating peripheral neuropathy in Pmp22-deficient mice. Nat Genet [Internet]. 1995
999   Nov;11(3):274–80. Available from: http://www.nature.com/articles/ng1195-274

1000   83.   Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier
1001   C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature
1002   [Internet]. 1997 Oct;389(6652):725–30. Available from: http://www.nature.com/articles/39593

1003   84.   Woldeyesus MT, Britsch S, Riethmacher D, Xu L, Sonnenberg-Riethmacher E, Abou-Rebyeh
1004   F, et al. Peripheral nervous system defects in erbB2 mutants following genetic rescue of heart
1005   development. Genes Dev [Internet]. 1999 Oct 1;13(19):2538–48. Available from:
1006   http://www.genesdev.org/cgi/doi/10.1101/gad.13.19.2538

1007   85.   Ackerman SD, Luo R, Poitelon Y, Mogha A, Harty BL, D'Rozario M, et al. GPR56/ADGRG1
1008   regulates development and maintenance of peripheral myelin. J Exp Med [Internet]. 2018 Mar
1009   5;215(3):941–61. Available from: http://www.jem.org/lookup/doi/10.1084/jem.20161714

1010   86.   Monk KR, Oshima K, Jors S, Heller S, Talbot WS. Gpr126 is essential for peripheral nerve
1011   development and myelination in mammals. Development [Internet]. 2011 Jul 1;138(13):2673–
1012   80. Available from: http://dev.biologists.org/cgi/doi/10.1242/dev.062224

1013   87.   Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, et al. A G Protein-
1014   Coupled Receptor Is Essential for Schwann Cells to Initiate Myelination. Science (80-)
1015   [Internet]. 2009 Sep 11;325(5946):1402–5. Available from:
1016   http://www.sciencemag.org/cgi/doi/10.1126/science.1173474

1017   88.   Kabzinska D, Drac H, Sherman DL, Kostera-Pruszczyk A, Brophy PJ, Kochanski A, et al.

Charcot-Marie-Tooth type 4F disease caused by S399fsx410 mutation in the PRX gene. Neurology [Internet]. 2006 Mar 14;66(5):745–7. Available from: http://www.neurology.org/cgi/doi/10.1212/01.wnl.0000201269.46071.35

89. Baránková L, Šišková D, Hühne K, Vyhnálková E, Sakmaryová I, Bojar M, et al. A 71-nucleotide deletion in the periaxin gene in a Romani patient with early-onset slowly progressive demyelinating CMT. Eur J Neurol [Internet]. 2008 Jun;15(6):548–51. Available from: http://doi.wiley.com/10.1111/j.1468-1331.2008.02104.x

90. Tokunaga S, Hashiguchi A, Yoshimura A, Maeda K, Suzuki T, Haruki H, et al. Late-onset Charcot–Marie–Tooth disease 4F caused by periaxin gene mutation. Neurogenetics [Internet]. 2012 Nov 1;13(4):359–65. Available from: http://link.springer.com/10.1007/s10048-012-0338-5

91. Gallardo E, García A, Ramón C, Maraví E, Infante J, Gastón I, et al. Charcot–Marie–Tooth disease type 2J with MPZ Thr124Met mutation: clinico-electrophysiological and MRI study of a family. J Neurol [Internet]. 2009 Dec 22;256(12):2061–71. Available from: http://link.springer.com/10.1007/s00415-009-5251-y

92. Leal A, Berghoff C, Berghoff M, Rojas-Araya M, Ortiz C, Heuss D, et al. A Costa Rican family affected with Charcot-Marie-Tooth disease due to the myelin protein zero (MPZ) p.Thr124Met mutation shares the Belgian haplotype. Rev Biol Trop [Internet]. 2014 Dec 1;62(4):1285. Available from: http://revistas.ucr.ac.cr/index.php/rbt/article/view/13473

93. Tokuda N, Noto Y, Kitani-Morii F, Hamano A, Kasai T, Shiga K, et al. Parasympathetic Dominant Autonomic Dysfunction in Charcot-Marie-Tooth Disease Type 2J with the *MPZ* Thr124Met Mutation. Intern Med [Internet]. 2015;54(15):1919–22. Available from: https://www.jstage.jst.go.jp/article/internalmedicine/54/15/54_54.4259/_article

94. Duan X, Gu W, Hao Y, Wang R, Wen H, Sun S, et al. A Novel Asp121Asn Mutation of Myelin Protein Zero Is Associated with Late-Onset Axonal Charcot-Marie-Tooth Disease, Hearing Loss and Pupil Abnormalities. Front Aging Neurosci [Internet]. 2016 Sep 22;8. Available from: http://journal.frontiersin.org/Article/10.3389/fnagi.2016.00222/abstract

95. Fabrizi GM, Tamburin S, Cavallaro T, Cabrini I, Ferrarini M, Taioli F, et al. The spectrum of Charcot-Marie-Tooth disease due to myelin protein zero: An electrodiagnostic, nerve ultrasound and histological study. Clin Neurophysiol [Internet]. 2018 Jan;129(1):21–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1388245717310854

96. Ambrozkiewicz MC, Schwark M, Kishimoto-Suga M, Borisova E, Hori K, Salazar-Lázaro A, et al. Polarity Acquisition in Cortical Neurons Is Driven by Synergistic Action of Sox9-Regulated Wwp1 and Wwp2 E3 Ubiquitin Ligases and Intronic miR-140. Neuron [Internet]. 2018 Dec;100(5):1097-1115.e15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627318308961

97. Erwig MS, Patzig J, Steyer AM, Dibaj P, Heilmann M, Heilmann I, et al. Anillin facilitates septin assembly to prevent pathological outfoldings of central nervous system myelin. Elife [Internet]. 2019 Jan 23;8. Available from: https://elifesciences.org/articles/43888

98. Kuharev J, Navarro P, Distler U, Jahn O, Tenzer S. In-depth evaluation of software tools for data-independent acquisition based label-free quantification. Proteomics [Internet]. 2015 Sep;15(18):3140–51. Available from: http://doi.wiley.com/10.1002/pmic.201400396

1059   99.   Silva JC, Denny R, Dorschel C, Gorenstein M V., Li G-Z, Richardson K, et al. Simultaneous
1060   Qualitative and Quantitative Analysis of the Escherichia coli Proteome. Mol Cell Proteomics
1061   [Internet]. 2006 Apr;5(4):589–607. Available from:
1062   http://www.mcponline.org/lookup/doi/10.1074/mcp.M500321-MCP200

1063   100.   Kammers K, Cole RN, Tiengwe C, Ruczinski I. Detecting significant changes in protein
1064   abundance. EuPA Open Proteomics [Internet]. 2015 Jun;7:11–9. Available from:
1065   https://linkinghub.elsevier.com/retrieve/pii/S2212968515000069

1066   101.   Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential
1067   expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res [Internet].
1068   2015 Apr 20;43(7):e47–e47. Available from:
1069   http://academic.oup.com/nar/article/43/7/e47/2414268/limma-powers-differential-expression-
1070   analyses-for

1071   102.   Storey JD. The positive false discovery rate: a Bayesian interpretation and the q -value. Ann
1072   Stat [Internet]. 2003 Dec;31(6):2013–35. Available from:
1073   http://projecteuclid.org/euclid.aos/1074290335

1074   103.   Vizcaíno JA, Csordas A, Del-Toro N, Dianes JA, Griss J, Lavidas I, et al. 2016 update of the
1075   PRIDE database and its related tools. Nucleic Acids Res [Internet]. 2016 Jan 4;44(D1):D447–
1076   56. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkv1145

1077   104.   Schardt A, Brinkmann BG, Mitkovski M, Sereda MW, Werner HB, Nave K-A. The SNARE
1078   protein SNAP-29 interacts with the GTPase Rab3A: Implications for membrane trafficking in
1079   myelinating glia. J Neurosci Res [Internet]. 2009 Nov 15;87(15):3465–79. Available from:
1080   http://doi.wiley.com/10.1002/jnr.22005

1081   105.   de Monasterio-Schrader P, Patzig J, Möbius W, Barrette B, Wagner TL, Kusch K, et al.
1082   Uncoupling of neuroinflammation from axonal degeneration in mice lacking the myelin protein
1083   tetraspanin-2. Glia. 2013;

1084   106.   Jung M, Sommer I, Schachner M, Nave KA. Monoclonal antibody O10 defines a
1085   conformationally sensitive cell- surface epitope of proteolipid protein (PLP): Evidence that PLP
1086   misfolding underlies dysmyelination in mutant mice. J Neurosci. 1996;16(24):7920–9.

1087   107.   Stumpf SK, Berghoff SA, Trevisiol A, Spieth L, Düking T, Schneider L V., et al. Ketogenic diet
1088   ameliorates axonal defects and promotes myelination in Pelizaeus–Merzbacher disease. Acta
1089   Neuropathol [Internet]. 2019 Jul 27;138(1):147–61. Available from:
1090   http://link.springer.com/10.1007/s00401-019-01985-2

1091   108.   Archelos JJ, Roggenbuck K, Scheider-Schaulies J, Linington C, Toyka K V., Hartung H-P.
1092   Production and characterization of monoclonal antibodies to the extracellular domain of P0. J
1093   Neurosci Res [Internet]. 1993 May 1;35(1):46–53. Available from:
1094   http://doi.wiley.com/10.1002/jnr.490350107

1095   109.   Darbas A, Jaegle M, Walbeehm E, van den Burg H, Driegen S, Broos L, et al. Cell autonomy of
1096   the mouse claw paw mutation. Dev Biol [Internet]. 2004 Aug;272(2):470–82. Available from:
1097   https://linkinghub.elsevier.com/retrieve/pii/S0012160604003616

1098   110.   Catenaccio A, Court FA. Teased Fiber Preparation of Myelinated Nerve Fibers from Peripheral
1099   Nerves for Vital Dye Staining and Immunofluorescence Analysis. In: Methods in Molecular

1100   Biology [Internet]. 2018. p. 329–37. Available from: http://link.springer.com/10.1007/978-1-
1101   4939-7649-2_21
1102 111. Hulsen T, de Vlieg J, Alkema W. BioVenn – a web application for the comparison and
1103   visualization of biological lists using area-proportional Venn diagrams. BMC Genomics
1104   [Internet]. 2008;9(1):488. Available from:
1105   http://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-488
1106
1107

FIGURE LEGENDS

Figure 1. Proteome analysis of peripheral myelin

(A) Schematic illustration of a previous approach to the peripheral myelin proteome (41)
compared with the present workflow. Note that the current workflow allows largely automated
sample processing and omits labor-intense 2-dimensional differential gel-electrophoresis,
thereby considerably reducing hands-on time. Nano LC-MS analysis by data-independent
acquisition (DIA) using three different data acquisition modes provides efficient identification
and quantification of abundant myelin proteins (MS^E; see **Figure 2**), a comprehensive
inventory (UDMS^E; see **Figures 3-4**) and gel-free differential analysis of hundreds of distinct
proteins (DRE-UDMS^E; see **Figure 5**). Samples were analyzed in three biological replicates.
(B) Immunoblot of myelin biochemically enriched from sciatic nerves of wild-type mice at
postnatal day 21 (P21). Equal amounts of corresponding nerve lysate were loaded to
compare the abundance of marker proteins for compact myelin (MPZ/P0, MBP, PMP2), non-
compact myelin (PRX), the Schwann cell nucleus (KROX20/EGR2), axons (NEFH, KCNA1)
and mitochondria (VDAC). Blots show n=2 biological replicates representative of n=3
biological replicates. Note that myelin markers are enriched in purified myelin; other cellular
markers are reduced.
(C) Number and relative abundance of proteins identified in myelin purified from the sciatic
nerves of wild-type mice using three different data acquisition modes (MS^E, UDMS^E, DRE-
UDMS^E). Note that MS^E (orange) provides the best information about the relative abundance
of high-abundant myelin proteins (dynamic range of more than four orders of magnitude) but
identifies comparatively fewer proteins in purified myelin. UDMS^E (blue) identifies the largest
number of proteins but provides only a lower dynamic range of about three orders of
magnitude. DRE-UDMS^E (green) identifies an intermediate number of proteins with an
intermediate dynamic range of about four orders of magnitude. Note that MS^E with very high
dynamic range is required for the quantification of the exceptionally abundant myelin protein
zero (MPZ/P0), myelin basic protein (MBP) and periaxin (PRX). ppm, parts per million.
(D) Venn diagram comparing the number of proteins identified in PNS myelin by MS^E,
UDMS^E and DRE-UDMS^E. Note the high overlap of identified proteins.
(E) Venn diagram of the proteins identified in PNS myelin by UDMS^E in this study compared
with those identified in two previous approaches (41,42).

Figure 1-supplement 1. Clustered heatmap of Pearson's correlation coefficients for
protein abundance comparing data acquisition modes.

1145 The heatmap compares the $\log_2$ transformed ppm protein abundance values to assess
1146 peripheral myelin purified from wild type mice using three data acquisition modes (MS^E,
1147 UDMS^E, DRE-UDMS^E). The inset shows the color key and the histogram for the values of the
1148 correlation coefficients. Note that the runs cluster with a high overall correlation (>0.75) into
1149 three conditions defined by the acquisition mode, in agreement with the experimental design.
1150 Among the samples analyzed by different acquisition modes, DRE-UDMS^E similarly
1151 correlates with both MS^E and UDMS^E, reflecting its intermediate nature.

1152
1153

1154 **Figure 2. Relative abundance of peripheral myelin proteins**
1155 MS^E was used to identify and quantify proteins in myelin purified from the sciatic nerves of
1156 wild-type mice at P21; their relative abundance is given as percent with relative standard
1157 deviation (% +/- RSD). Note that known myelin proteins constitute >80% of the total myelin
1158 protein; proteins not previously associated with myelin constitute <20%. Mass spectrometric
1159 quantification based on 3 biological replicates per genotype with 4 technical replicates each
1160 (see **Figure 1-source data 1**).

1161
1162

1163 **Figure 3. Developmental mRNA abundance profiles of myelin-associated genes**
1164 **(A)** K-means clustering was performed for the mRNA profiles of those 1046 proteins in our
1165 myelin proteome inventory for which significant mRNA expression was found by RNA-Seq in
1166 the sciatic nerve of rats dissected at ages E21, P6, P18 and 6 months (M6). Note that this
1167 filtering strategy allows to selectively display the developmental abundance profiles of those
1168 transcripts that encode myelin-associated proteins rather than of all transcripts present in the
1169 nerve. Standardized mRNA abundance profiles are shown (n=4 biological replicates per
1170 age). Known myelin genes are displayed in red. For comparison, *Pmp22* mRNA was
1171 included although the small tetraspan protein PMP22 was not mass spectrometrically
1172 identified due to its unfavorable distribution of tryptic cleavage sites. Normalized counts for all
1173 mRNAs including those displaying developmentally unchanged abundance are provided in
1174 **Figure 3-Source data 1**.
1175 **(B)** Numbers of mRNAs per cluster.

1176
1177

1178 **Figure 4. Categorization of annotated protein functions**
1179 All proteins identified in peripheral myelin by UDMS^E (turquoise) and the respective
1180 developmental expression clusters (**Figure 3**; shades of red) were analyzed for
1181 overrepresented functional annotations using gene ontology (GO) terms. The graph displays

1182 the percentage of proteins in each cluster that were annotated with a particular function. For

1183 comparison, known myelin proteins were annotated. n.o., not over-represented.

1184

1185

1186 **Figure 5. Molecular analysis of myelin in the *Prx*^{-/-} mouse model of CMT4F**

1187 **(A)** Myelin purified from sciatic nerves dissected from *Prx*^{-/-} and control mice at P21 was

1188 separated by SDS-PAGE (0.5 µg protein load) and proteins were visualized by silver

1189 staining. Bands constituted by the most abundant myelin proteins (MPZ/P0, MBP, PRX) are

1190 annotated. Note that no band constituted by PRX was detected in *Prx*^{-/-} myelin and that

1191 several other bands also display genotype-dependent differences in intensity. Gel shows n=2

1192 biological replicates representative of n=3 biological replicates.

1193 **(B)** The relative abundance of proteins in myelin purified from *Prx*^{-/-} sciatic nerves as

1194 quantified by MS^E is given as percent with relative standard deviation (% +/- RSD). Note the

1195 increased relative abundance of MPZ/P0 and MBP compared to wild-type myelin (see

1196 **Figure 2**) when PRX is lacking. Mass spectrometric quantification based on 3 biological

1197 replicates with 4 technical replicates each (see **Figure 5-source data 1**).

1198 **(C,D)** Differential proteome analysis by DRE-UDMS^E of myelin purified from *Prx*^{-/-} and wild-

1199 type mice. Mass spectrometric quantification based on 3 biological replicates per genotype

1200 with 4 technical replicates each (see **Figure 5-source data 2**). **(C)** Top 40 proteins of which

1201 the abundance is reduced (blue) or increased (red) in peripheral myelin purified from *Prx*^{-/-}

1202 compared to wild-type mice with the highest level of significance according to the -$\log_{10}$

1203 transformed q-value (green). In the heatmaps, each horizontal line corresponds to the fold-

1204 change (FC) of a distinct protein compared to its average abundance in wild-type myelin

1205 plotted on a $\log_2$ color scale. Heatmaps display 12 replicates, i.e. 3 biological replicates per

1206 genotype with 4 technical replicates each. **(D-D''')** Volcano plots representing genotype-

1207 dependent quantitative myelin proteome analysis. Data points represent quantified proteins

1208 in *Prx*^{-/-} compared to wild-type myelin and are plotted as the log2-transformed fold-change

1209 (FC) on the x-axis against the -log10-transformed q-value on the y-axis. Stippled lines mark a

1210 -log10-transformed q-value of 1.301, reflecting a q-value of 0.05 as significance threshold.

1211 Highlighted are the datapoints representing the Top 10 proteins displaying highest zdist

1212 values (Euclidean distance between the two points (0,0) and (x,y) with x = log2(FC) and y = -

1213 log10(q-value) (red circles in **D**), immune-related proteins (purple circles in **D'**), proteins of

1214 the extracellular matrix (ECM; yellow circles in **D''**) and known myelin proteins (blue circles in

1215 **D'''**). n.d., not detected; n.q., no q-value computable due to protein identification in one

1216 genotype only. Also see **Figure 5-supplement 1.**

1217 **(E)** Immunoblot of myelin purified from *Prx*^{-/-} and control sciatic nerves confirms the reduced

1218 abundance of DRP2, SLC16A1/MCT1, BSG and PMP2 in *Prx*^{-/-} myelin, as found by

1219   differential DRE-UDMS^E analysis (in **Figure 5C,D**). PRX was detected as genotype control;

1220   PLP/DM20 and ATP1A1 serve as markers. Blot shows n=2 biological replicates per

1221   genotype.

1222   **(F)** Teased fiber preparations of sciatic nerves dissected from *Prx*$^{-/-}$ and control mice

1223   immunolabelled for MAG (red) and SLC16A1 (green). Note that SLC16A1 co-distributes with

1224   MAG in Schmidt-Lanterman incisures (SLI) in control but not in *Prx*$^{-/-}$ nerves, in accordance

1225   with the reduced abundance of SLC16A1 in *Prx*$^{-/-}$ myelin (**Figure 5C-E**). Also note that, in

1226   *Prx*$^{-/-}$ myelin, SLI were largely undetectable by MAG immunolabeling.

1227

1228

1229   **Figure 5-supplement 1. Clustered heatmap of Pearson's correlation coefficients for**

1230   **protein abundance comparing genotypes.**

1231   **(A)** The heatmap compares the log$_2$ transformed ppm protein abundance values from the

1232   DRE-UDMS^E runs to assess peripheral myelin purified from wild type and *Prx*$^{-/-}$ mice. The

1233   inset shows the color key and the histogram for the values of the correlation coefficients.

1234   Note that the runs cluster with a high overall correlation (>0.85) into two conditions defined

1235   by the genotype, in agreement with the experimental design.

1236   **(B)** Volcano plot representing genotype-dependent quantitative myelin proteome analysis.

1237   Data points represent quantified proteins in *Prx*$^{-/-}$ compared to wild-type myelin plotted as the

1238   log2-transformed fold-change (FC) on the x-axis against the -log10-transformed q-value on

1239   the y-axis. Note the different axis scale compared to **Figure 5D**. Stippled line marks a -log10-

1240   transformed q-value of 1.301, reflecting a q-value of 0.05 as significance threshold.

1241   Highlighted is the datapoint for PRX to illustrate that only trace amounts of PRX were

1242   detected when assessing *Prx*$^{-/-}$ myelin. ATP2A1, ATP1A4 and PLCD1 were not detected in

1243   *Prx*$^{-/-}$ myelin.

1244

1245

1246   **Figure 6. Progressive loss and reduced diameters of peripheral axons in *Prx*$^{-/-}$ mice**

1247   **(A-D)** Genotype-dependent quantitative assessment of light micrographs of toluidine-stained

1248   semi-thin sectioned quadriceps nerve dissected at 2, 4 and 9 months of age reveals

1249   progressive loss of peripheral axons in *Prx*$^{-/-}$ compared to control mice.

1250   **(A)** Representative micrographs. Arrows point at myelinated axons; asterisk denotes an

1251   unmyelinated axon; arrowhead points at a myelin whorl lacking a recognizable axon. Scale

1252   bars, 10 µm.

1253   **(B)** Total number of axons per nerve that are not associated with a Remak bundle.

1254   **(C)** Total number of myelinated axons per nerve.

1255   **(D)** Total number per nerve of myelin whorls that lack a recognizable axon.

1256 Mean +/ SD, n=3-4 mice per genotype and age; *P<0.05, **P<0.01, ***P<0.001 by Student's

1257 unpaired t-test.

1258 (**E-G**) Genotype-dependent assessment of myelinated axons shows a shift toward reduced

1259 axonal diameters in quadriceps nerves of *Prx*^{-/-} compared to control mice at 2 months (**E**), 4

1260 months (**F**) and 9 months (**G**) of age. Data are presented as frequency distribution with 0.5

1261 µm bin width. ***, p<0.001 by two-sided Kolmogorow-Smirnow test. For precise p-values see

1262 methods section.

1263

1264

1265 **Figure 1-source data 1. Label-free quantification of proteins in wild-type PNS myelin**

1266 **fractions by three different data acquisition modes**

1267 Identification and quantification data of detected myelin-associated proteins. Tryptic peptides

1268 derived from four technical replicates (replicate digestion and replicate injection) per three

1269 biological replicate (20 sciatic nerves pooled from 10 animals) were analyzed by LC-MS (12

1270 runs in total). Proteins (FDR < 1%; 2 peptides/protein) and peptides (FDR < 1%; ≥7 amino

1271 acids) were identified by database search against the UniprotKB/SwissProt mouse database

1272 using PLGS. Data were post-processed with the software package ISOQuant to calculate

1273 absolute in-sample amounts for each detected protein based on the TOP3 approach.

1274 Reported abundance values are defined as the relative amount of each protein in respect to

1275 the sum over all detected proteins (ppm: parts per million (w/w) of total protein). Typical

1276 contaminant proteins like keratins were filtered.

1277 → sheet 1: protein identification details

1278 → sheet 2: WT myelin proteome by MS^E

1279 → sheet 3: WT myelin proteome by UD-MS^E

1280 → sheet 4: WT myelin proteome by DRE UD-MS^E

1281 → sheet 5: 45 proteins additionally identified in WT myelin by 1D-gel-LC-MS

1282

1283

1284 **Figure 3-source data 1. Normalized developmental mRNA abundance data**

1285 → sheet 1: normalized values for all individual 4 biological replicates per age

1286 → sheet 2: normalized values for biological replicates averaged to give mean per age

1287

1288

1289 **Figure 5-source data 1. Label-free quantification of proteins in PNS myelin fractions**

1290 **from *Prx*^{-/-} mice by MSe**

1291 Identification and quantification data of detected myelin-associated proteins. Tryptic peptides

1292 derived from four technical replicates (replicate digestion and replicate injection) per three

1293 biological replicate (20 sciatic nerves pooled from 10 animals) were analyzed by LC-MS (12

1294 runs in total). Proteins (FDR < 1%; 2 peptides/protein) and peptides (FDR < 1%; ≥7 amino

1295 acids) were identified by database search against the UniprotKB/SwissProt mouse database

1296 using PLGS. Data were post-processed with the software package ISOQuant to calculate

1297 absolute in-sample amounts for each detected protein based on the TOP3 approach.

1298 Reported abundance values are defined as the relative amount of each protein in respect to

1299 the sum over all detected proteins (ppm: parts per million (w/w) of total protein). Typical

1300 contaminant proteins like keratins were filtered.

1301 → sheet 1: protein identification details

1302 → sheet 2: *Prx*^{-/-} myelin proteome by MS[E]

1303

1304

1305 **Figure 5-source data 2. Label-free quantification of proteins in PNS myelin fractions**

1306 **from WT and *Prx*^{-/-} mice by DRE-UDMSe**

1307 Identification and quantification data of detected myelin-associated proteins by DRE-UDMSe.

1308 For each genotype, tryptic peptides derived from four technical replicates (replicate digestion

1309 and replicate injection) per three biological replicate (20 sciatic nerves pooled from 10

1310 animals) were analyzed by LC-MS (24 runs in total). Proteins (FDR < 1%; 2 peptides/protein)

1311 and peptides (FDR < 1%; ≥7 amino acids) were identified by database search against the

1312 UniprotKB/SwissProt mouse database using PLGS. Data were post-processed with the

1313 software package ISOQuant to calculate absolute in-sample amounts for each detected

1314 protein based on the TOP3 approach. Reported abundance values are defined as the

1315 relative amount of each protein in respect to the sum over all detected proteins (ppm: parts

1316 per million (w/w) of total protein). Typical contaminant proteins like keratins were filtered. The

1317 -log10-transformed q-value was plotted against the log2-transformed fold change to obtain

1318 the volcano plot shown in Figure 5D. As no imputation of missing values was performed,

1319 proteins exclusive for only one of the conditions do not appear in the volcano plot, but are

1320 appended at the end of the list. Criteria for statistically significant regulation were as follows:

1321 fold change of at least 1.5 and q-value below 0.05.

1322 → sheet 1: protein identification details

1323 → sheet 2: comparison of WT vs. *Prx*^{-/-} myelin proteome by DRE-UDMS[E]

1324

1325

| Protein name	Gene	Reference	TMD	Cluster
2-hydroxyacylsphingosine 1-beta-galactosyltransferase	*Ugt8*	Bosio et al., 1996	2	P6-up
Syntrophin α1	*Snta1*	Fuhrmann-Stroissnigg et al., 2012	-	P18-up
Annexin A2	*Anxa2*	Hayashi et al., 2007	-	Descending
Band 4.1 protein B / 4.1B	*Epb41l3*	Ivanovic et al., 2012	-	Descending
Band 4.1 protein G / 4.1G	*Epb41l2*	Ohno et al., 2006	-	P6-up
Breast carcinoma-amplified sequence 1	*Bcas1*	Ishimoto et al., 2017	-	P6-up
Cadherin 1/ E-Cadherin	*Cdh1*	Fannon et al., 1995	1	P18-up
Carbonic anhydrase 2	*Ca2*	Cammer et al., 1987	-	Descending
Catenin α1	*Ctnna1*	Murata et al., 2006	-	U-shaped
Catenin ß1	*Ctnnb1*	Fannon et al., 1995	-	Descending
Caveolin 1	*Cav1*	Mikol et al., 2002	1	P18-up
CD9, tetraspanin 29	*Cd9*	Ishibashi et al., 2004	4	P18-p
CD59A	*Cd59a*	Funabashi et al., 1994	1	P18-up
CD47, integrin-associated signal transducer	*Cd47*	Gitik et al., 2011	5	P6-up
CD81, tetraspanin 28	*Cd81*	Ishibashi et al., 2004	4	P18-up
CD82, tetraspanin 27	*Cd82*	Chernousov et al., 2013	4	P18-up
CD151, tetraspanin 24	*Cd151*	Patzig et al., 2011	4	P18-up
Cell adhesion molecule 4/ NECL4	*Cadm4*	Spiegel et al., 2007	1	P6-up
Cell division control protein 42	*Cdc42*	Benninger et al., 2007	-	P6-up
Cell surface glycoprotein MUC18	*Mcam*	Shi et al., 1998	1	Descending
Ciliary neurotrophic factor	*Cntf*	Rende et al., 1992	-	Late-up
CKLF-like MARVEL TMD-containing 5	*Cmtm5*	Patzig et al., 2011	4	P6-up
Claudin-19	*Cldn19*	Miyamoto et al., 2005	4	P6-up
Cofilin 1	*Cfl1*	Sparrow et al., 2012	-	Descending
Crystallin α2	*Cryab*	d'Antonio et al., 2006	-	P18-up
Cyclic nucleotide phosphodiesterase	*Cnp*	Matthieu et al., 1980	-	P6-up
Sarcoglycan δ	*Sgcd*	Cai et al., 2007	1	Late-up
Dihydropyrimidinase related protein 1	*Crmp1*	d'Antonio et al., 2006	-	Descending
Disks large homolog 1	*Dlg1*	Cotter et al., 2010	-	Descending
Dynein light chain 1	*Dynll1*	Myllykoski et al. 2018	-	P6-up
Dystroglycan	*Dag1*	Yamada et al, 1994	1	P6-up
Dystrophin/DP116	*Dmd*	Cai et al., 2007	-	P6-up
Dystrophin-related protein 2	*Drp2*	Sherman et al., 2001	-	P18-up
E3 ubiquitin-protein ligase NEDD4	*Nedd4*	Liu et al., 2009	-	Descending
Ezrin	*Ezr*	Scherer et al., 2001	-	P6-up
Fatty acid synthase	*Fasn*	Salles et al., 2002	-	P6-up
Flotillin 1	*Flot1*	Lee et al., 2014	-	P18-up
Gap junction ß1 protein / Cx32	*Gjb1*	Li et al., 2002	4	P18-up
Gap junction γ3 protein / Cx29	*Gjc3*	Li et al., 2002	1	P6-up
Gelsolin	*Gsn*	Gonçalves et al., 2010	-	Late-up
Glycogen synthase kinase 3ß	*Gsk3b*	Ogata et al., 2004	-	P6-up
Integrin α6	*Itga6*	Nodari et al., 2008	1	P6-up
Integrin αV	*Itgav*	Chernousov & Carey, 2003	1	Descending
Integrin ß1	*Itgb1*	Feltri et al., 2002	1	Descending
Integrin ß4	*Itgb4*	Quattrini et al., 1996	2	P18-up
Junctional adhesion molecule C	*Jam3*	Scheiermann et al., 2007	1	P18-up
Laminin α2	*Lama2*	Yang et al., 2005	-	P6-up
Laminin α4	*Lama4*	Yang et al., 2005	-	Descending
Laminin ß1	*Lamb1*	LeBeau et al., 1994	-	Descending
Laminin ß2	*Lamb2*	LeBeau et al., 1994	-	P18-up
Laminin γ1	*Lamc1*	Chen & Strickland, 2003	-	Descending
Membrane Palmitoylated Protein 6	*Mpp6*	Saitoh et al., 2019	-	P6-up
Microtubule-associated protein 1A	*Map1a*	Fuhrmann-Stroissnigg et al., 2012	-	P18-up
Microtubule-associated protein 1B	*Map1b*	Fuhrmann-Stroissnigg et al., 2012	-	P6-up
Mitogen-activated protein kinase 1/ ERK2	*Mapk1*	Mantuano et al., 2015	-	Descending
Mitogen-activated protein kinase 3/ ERK1	*Mapk3*	Mantuano et al., 2015	-	P18-up
Moesin	*Msn*	Scherer et al., 2001	-	Unchanged
Monocarboxylate transporter 1	*Slc16a1*	Domenech-Estevez et al., 2015	11	P18-up

Myelin associated glycoprotein	*Mag*	Figlewicz et al., 1981	1	P6-up
Myelin basic protein	*Mbp*	Boggs, 2006	-	P6-up
Myelin protein 2	*Pmp2*	Trapp et al., 1984	-	P18-up
Myelin protein zero/ P0	*Mpz*	Giese et al., 1992	1	P6-up
Myelin proteolipid protein	*Plp1*	Garbern et al., 1997	4	P6-up
Myotubularin-related protein 2	*Mtmr2*	Bolino et al., 2004	-	P6-up
Noncompact myelin-associated protein	*Ncmap*	Ryu et al., 2008	1	P18-up
NDRG1, N-myc downstream regulated	*Ndrg1*	Berger et al., 2004	-	P18-uP
Neurofascin	*Nfasc*	Tait et al., 2000	2	P18-up
Nidogen 1	*Nid1*	Lee et al., 2007	-	Descending
P2X purinoceptor 7	*P2rx7*	Faroni et al., 2014	-	P6-up
Paxillin	*Pxn*	Fernandez-Valle et al., 2002	-	P6-up
Periaxin	*Prx*	Gillespie et al., 1994	-	P6-up
Plasmolipin	*Pllp*	Bosse et al., 2003	4	P18-up
Profilin 1	*Pfn1*	Montani et al., 2014	-	Descending
Lin-7 homolog C	*Lin7c*	Saitoh et al., 2017	-	P6-up
Rac1	*Rac1*	Benninger et al., 2007	-	U-Shaped
Radixin	*Rdx*	Scherer et al., 2001	-	Descending
RhoA	*Rhoa*	Brancolini et al., 1999	-	U-Shaped
Septin 2	*Sept 2*	Buser et al., 2009	-	Descending
Septin 7	*Sept 7*	Buser et al., 2009	-	U-Shaped
Septin 8	*Sept 8*	Patzig et al., 2011	-	P18-up
Septin 9	*Sept 9*	Patzig et al., 2011	-	P6-up
Septin 11	*Sept 11*	Buser et al., 2009	-	Descending
Sirtuin 2, NAD-dependent deacetylase	*Sirt2*	Werner et al., 2007	-	P18-up
Spectrin alpha chain, non-erythrocytic 1	*Sptan1*	Susuki et al., 2018	-	P18-up
Spectrin beta chain, non-erythrocytic 1	*Sptbn1*	Susuki et al., 2018	-	P18-up
Tight junction protein ZO-1	*Tjp1*	Poliak et al., 2007	-	P6-up
Tight junction protein ZO-2	*Tjp2*	Poliak et al., 2007	-	P6-up
Transferrin	*Tf*	Liu et al., 1990	2	Late-up
Vimentin	*Vim*	Triolo et al., 2012	-	Unchanged
Vinculin	*Vcl*	Beppu et al., 2015	-	Descending

Table 1. Known myelin proteins in the myelin proteome. Proteins mass-spectrometrically identified in peripheral myelin are compiled according to availability of prior references as myelin proteins. Given are the official gene name, one selected reference, the number of transmembrane domains (TMD) and the mRNA abundance profile cluster (see **Figure 3**).

1332

Protein name	Gene name	OMIM#	Gene Locus	Neuropathy
Monoacylglycerol lipase ABHD12	*ABHD12*	613599	20p11.21	PHARC
Apoptosis-inducing factor 1	*AIFM1*	300169	Xq26.1	CMTX4, DFNX5
Na+/K+ -transporting ATPase α1	*ATP1A1*	182310	1p13.1	CMT2DD
Cytochrome c oxidase subunit 6A1	*COX6A1*	602072	12q24.31	CMTRID
Dystrophin-related protein 2	*DRP2*	300052	Xq22.1	CMTX
Dynactin subunit 1	*DCTN1*	601143	2p13.1	DHMN7B
Dynamin 2	*DNM2*	602378	19p13.2	CMT2M, CMTDIB
Cytoplasmic dynein 1 heavy chain 1	*DYNC1H1*	600112	14q32.31	CMT20, SMALED1
E3 SUMO-protein ligase	*EGR2*	129010	10q21.3	CMT1D, CMT3, CMT4E
Glycine-tRNA ligase	*GARS* (Gart)	600287	7p14.3	CMT2D, HMN5A
Gap junction ß1 protein / Cx32	*GJB1*	304040	Xq13.1	CMTX1
Guanine nucleotide-binding protein ß4	*GNB4*	610863	3q26.33	CMTDIF
Histidine triad nucleotide-binding protein 1	*HINT1*	601314	5q23.3	NMAN
Hexokinase 1	*HK1*	142600	10q22.1	CMT4G
Heat shock protein ß1	*HSPB1*	602195	7q11.23	CMT2F, DHMN2B
Kinesin heavy chain isoform 5A	*KIF5A*	602821	12q13.3	SPG10
Prelamin A/C	*LMNA*	150330	1q22	CMT2B1
Neprilysin	*MME*	120520	3q25.2	CMT2T, SCA43
Myelin protein zero/ P0	*MPZ*	159440	1q23.3	CHN2,CMT1B, CMT2I, CMT2J,CMT3, CMTDID, Roussy-Levy syndrome
Myotubularin-related protein 2	*MTMR2*	603557	11q21	CMT4B1
Alpha-N-acetylglucosaminidase	*NAGLU* (NAGA)	609701	17q21.2	CMT2V
NDRG1, N-myc downstream regulated	*NDRG1*	605262	8q24.22	CMT4D
Neurofilament heavy polypeptide	*NEFH*	162230	22q12.2	CMT2CC
Neurofilament light polypeptide	*NEFL*	162280	8p21.2	CMT2E, CMT1F, CMTDIG
Peripheral myelin protein 2	*PMP2*	170715	8q21.13	CMT1G
Peripheral myelin protein 22	*PMP22*	601907	17p12	CMT1A, CMT1E, CMT3, HNPP, Roussy-Levy syndrome
Ribose-phosphate pyrophosphokinase 1	*PRPS1*	311850	Xq22.3	Arts syndrome, CMTX5, DFNX1
Periaxin	*PRX*	605725	19q13.2	CMT4F, CMT3
Ras-related protein Rab 7a	*RAB7A*	602298	3q21.3	CMT2B
Septin 9	*SEPT9*	604061	17q25.3	HNA
Transitional ER-ATPase	*VCP*	601023	9p13.3	CMT2Y
Tryptophan-tRNA ligase, cytoplasmic	*WARS*	191050	14q32.32	HMN9
Tyrosine-tRNA ligase, cytoplasmic	*YARS*	603623	1p35.1	DI-CMTC

1333 **Table 2. Peripheral myelin proteins identified in PNS myelin involved in neuropathological diseases.** Proteins
1334 massspectrometically identified in peripheral myelin were analyzed regarding the involvement of the ortholog human gene
1335 in neuropathological diseases. PMP22 was added, though it was not identified by MS analyses due to its unfavorable
1336 distribution of tryptic cleavage sites. CMT, Charcot-Marie-Tooth disease; DHMN, distal hereditary motor neuropathy; DI-
1337 CMTC, dominant intermediate CMTC; DFN, X-linked deafness; HMN, hereditary motor neuropathy; HSAN, hereditary
1338 sensory and autonomic neuropathy; HNA, hereditary sensory and autonomic neuropathy; OMIM, Online Mendelian
1339 Inheritance in Man; PHARC, polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract; SCA, spinocerebellar
1340 ataxia; SPG, spastic paraplegia.

1341
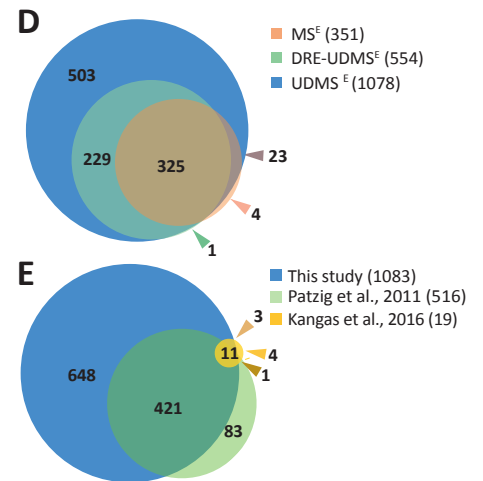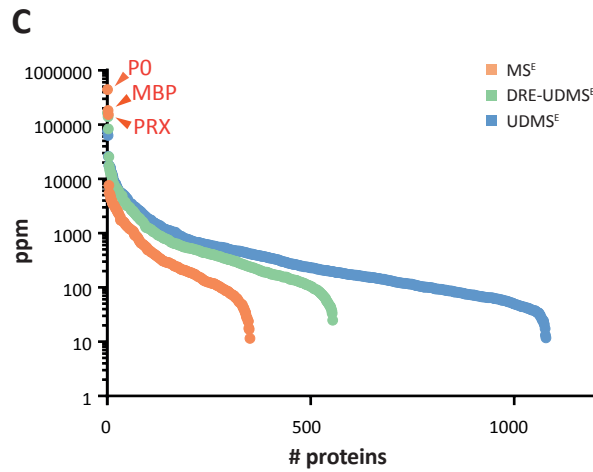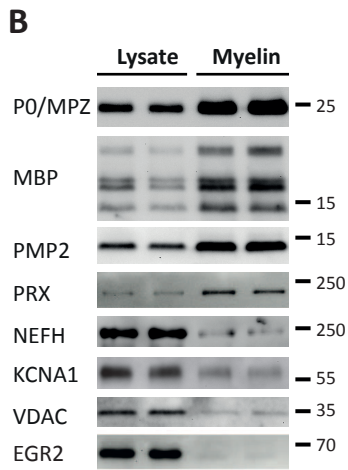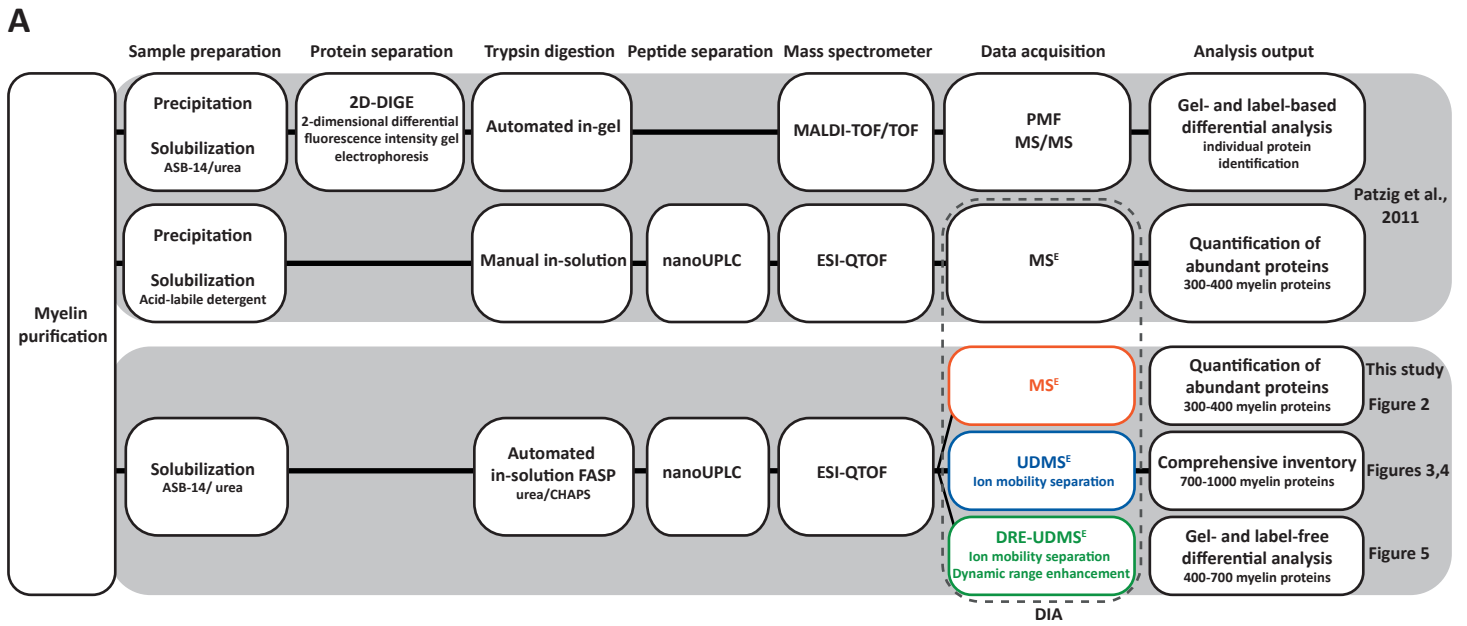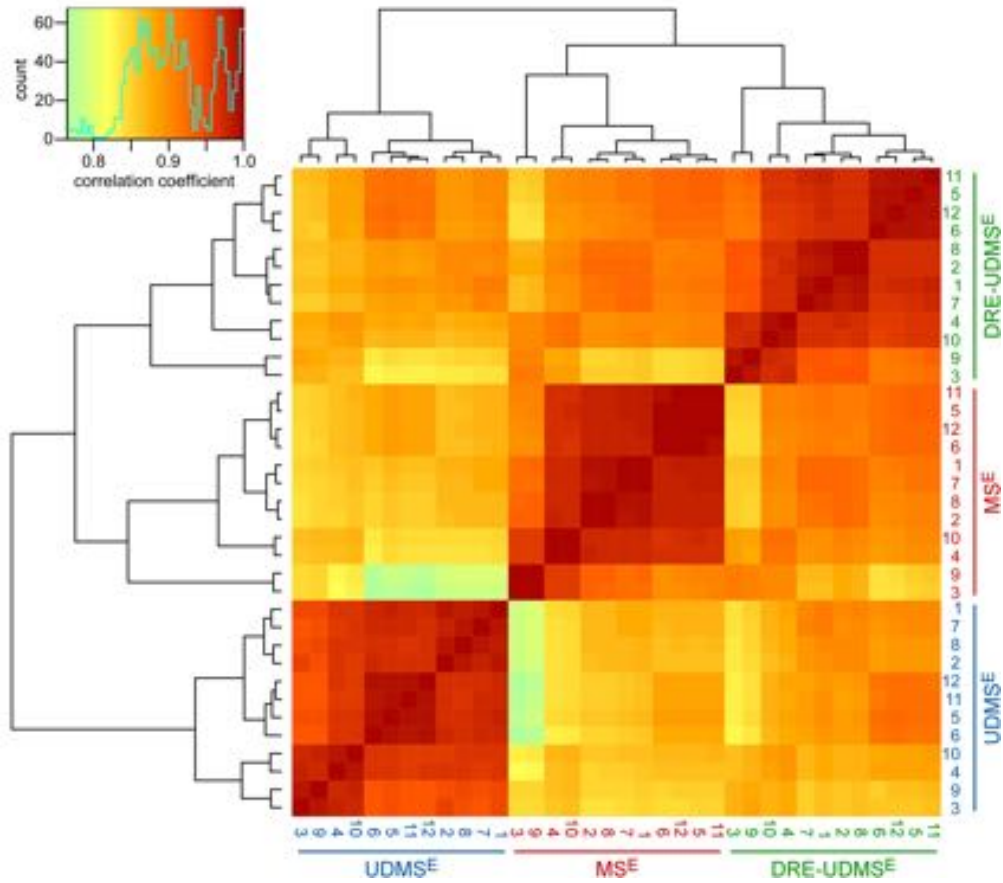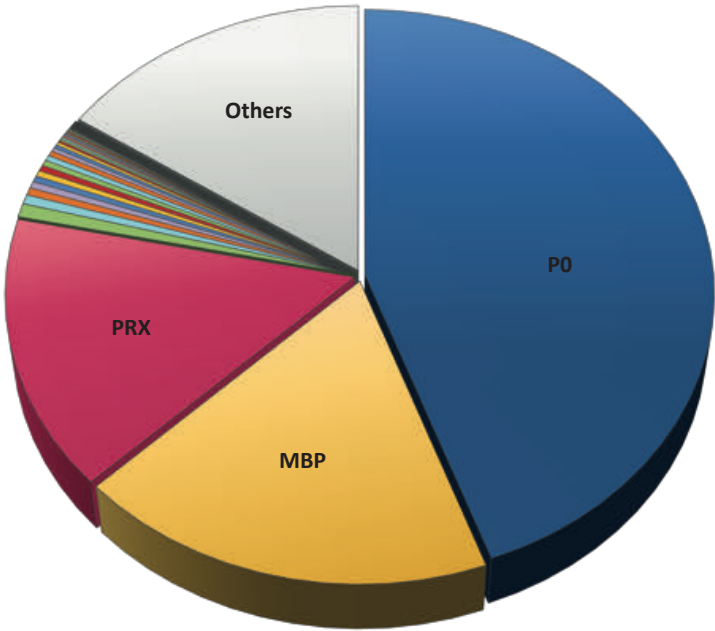
Protein	% (+/-	RSD)	Protein	% (+/-	RSD)
P0/ MPZ	44.48	4.15	LAMC1	0.06	0.01
MBP	18.35	1.15	RAC1	0.05	0.01
PRX	15.48	1.46	VCL	0.04	0.00
CD9	0.77	0.15	CA2	0.04	0.01
SPTBN1	0.52	0.06	SEPT2	0.04	0.00
SPTAN1	0.42	0.07	CFL1	0.04	0.01
EPB41L2	0.38	0.05	CRYAB	0.03	0.00
VIM	0.34	0.07	MPP6	0.03	0.00
CNP	0.33	0.06	CAV1	0.03	0.00
TF	0.33	0.03	SEPT7	0.02	0.00
PMP2	0.32	0.03	GJC3	0.02	0.00
MAP1B	0.31	0.06	RDX	0.02	0.00
FASN	0.29	0.04	CDC42	0.02	0.01
ANXA2	0.22	0.03	PFN1	0.02	0.00
MAG	0.20	0.02	SEPT11	0.02	0.00
PLP1	0.17	0.02	CRMP1	0.02	0.00
CD81	0.12	0.01	RHOA	0.02	0.00
GSN	0.11	0.02	MAPK3	0.02	0.00
PLLP	0.11	0.01	MAPK1	0.01	0.00
BCAS1	0.09	0.00	CMTM5	0.01	0.00
NDRG1	0.09	0.03	JAM3	0.01	0.00
MSN	0.09	0.01	DYNLL1	0.01	0.03
EPB41L3	0.07	0.01	EZR	0.01	0.00
NID1	0.07	0.01	CD59A	0.01	0.00
CADM4	0.07	0.01	Others	15.39	

A

P6-up

mRNA-abundance (normalized) vs E21, P6, P18, M6
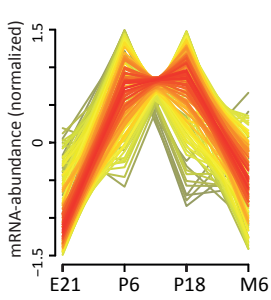
Aacs, **Cd47**, Cpne3, Eml1, Gmfb, **Lama2**, **Mpp6**, Osbpl1a, Pygb, **Tjp1**, Abcd3, **Cdc42**, Cpt1a, Csnk2a1, Gna11, Letm1, **Mtmr2**, Pdhb, Rab8b, Rcn1, **Tjp2**, Acaca, Cfl2, Cyp51, Fam177a1, Gnaq, Lss, Mvk, Pgls, Rer1, Tspan15, Acat2, Ckb, Dhcr7, Far1, Gng2, M6pr, Myh14, Plg, Rpl13, Tubb2a, Adam10, **Cldn19**, **Dag1**, Fdft1, Gsk3b, Mag, Myo18a, Plp1, Rps16, Ubl3, Agps, **Cmtm5**, Dhrs1, Fdps, Gypc, **Map1b**, Naga, **Pmp22**, Rps2, **Ugt8**, Appl1, **Cnp**, **Dmd**, Fmnl2, Hmgcs1, Map4, Napb, Ppa2, **Sept9**, Art3, Col15a1, Dusp15, Fscn1, Hsd17b7, Map6, Ndufb7, Prkacb, Slc25a10, **Bcas1**, Col1a1, Dync1h1, Fut8, Idi1, Mapre3, Ndufs8, **Prx**, Slc25a5, Bdh1, Col1a2, **Dynll1**, Gamt, **Itga6**, **Mbp**, Nefl, Psmb6, Slc44a1, Brox, Col28a1, Eci1, Gapdhs, Itpr3, Mical1, Nefm, Psmc3, Snap91, Cab39l, Col4a2, Ehd3, **Gjc3**, Kif5b, Mlec, Nsdhl, Pura, Sorbs1, **Cadm4**, Col6a1, Elovl1, **Epb41l2**, Klc2, Mme, Opa1, **Pxn**, Stk39, Camk2b, Col6a2, **Ezr**, **Fasn**, **Mpz**, **P2rx7**, **Plp1**

P18-up

mRNA-abundance (normalized) vs E21, P6, P18, M6

Abat, Aldh9a1, Atp6v1e1, **Cd59**, **Cryab**, Fkbp1a, Hibch, **Lamb2**, Mpc1, Ndufs4, **Pmp2**, Rab5b, Serpinf1, Steap3, Tst, Abhd12, Anxa11, Atp6v1h, **Cd81**, Ctsd, Flnb, Hk1, Lamp1, Mpp2, Ndufs7, Por, Rab7a, Sft2d2, Stx12, Tubb4a, Acaa1a, Anxa4, Auh, **Cd82**, Cyb5a, Gatm, Hras, Lamp2, Mpst, Ndufv2, Prdx5, Rdh11, Sfxn3, Stxbp1, Tubb4a, Acaa2, Arl3, Bcap31, **Cd9**, Cyb5r3, Gdi1, Hsd17b10, Lancl1, Myh9, Nes, Prelp, Reep5, **Sirt2**, Sucla2, Vps26a, Acadm, Arl8b, Bgn, **Cdh1**, Ddah1, **Gjb1**, Hsd17b11, Ldhb, Myo1b, **Nfasc**, Prkca, Rida, **Slc16a1**, Sult1a1, Vps29, Acads, Asah1, Bphl, Cdipt, Decr1, Glod4, Hsd17b12, Limch1, Myo1c, Nptn, Psmf1, Rtkn, Slc25a1, Susd2, Vsnl1, Acadvl, Asl, **Bsg**, Cers2, Dpysl2, Glul, Hspa12a, Map1lc3a, Ncdn, Ostf1, Rab11b, Rtn4, Slc3a2, Svip, Ywhaq, Acsl1, Atp1a1, **Cadm3**, Chchd3, **Drp2**, Gnao1, Hspa1a, **Map1a**, Ndcn, Pcolce, Rab12, Rufy3, Slc44a2, Sypl1, Acss2, Atp1a2, Canx, Clip2, Ech1, Gpi, Hspa2, Map1lc3b, **Ncmap**, Pdcd6ip, Rab21, S100a6, **Snta1**, Taldo1, Ak3, Atp1a4, Capn1, Cmtm6, Eno2, Hadh, Hspa12a, **Ndrg1**, Pfkm, Rab2a, Sacm1l, Sod2, Tecr, Aldh1a1, Atp1b3, **Cav1**, Cndp2, Etfb, Hagh, **Itgb4**, Marc2, Ndrg2, Plec, Rab3a, Sbds, Sparc, Tkt, Aldh1a7, Atp6v0c, Cavin3, Comt, Fam213a, Hepacam, **Jam3**, **Mapk3**, Ndrg4, Plekhb1, Rab43, Scarb2, **Sptan1**, Tln1, Aldh3a2, Atp6v1c1, **Cd151**, Copg2, Fis1, Hibadh, Jup, Mgll, Ndufa13, **Pllp**, Rab4b, Scp2, **Sptbn1**, Tns3, Aldh6a1, Atp6v1d, Crip2, Kit, Mgst3, Ndufa7, **Sept8**, Tom1l2, Ndufb4

Late-up

mRNA-abundance (normalized) vs E21, P6, P18, M6
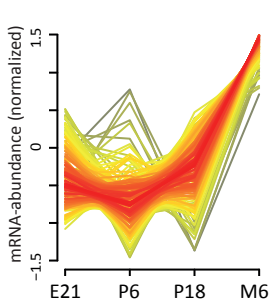
Actn3, Arpc4, Cab39, Cfh, Des, Esyt1, Got2, Idh3g, Myl6, Pdha1, Rab18, Rras, Stx7, Uqcrb, Ahcy, Aspa, Camk2g, Cisd1, Dnajb4, Etfa, Gpd1l, Myl9, Pdlim5, Rab5c, Rras2, Tagln, Vat1, Akr1b1, Atp12a, Capg, Ckm, Dnpep, F13a1, Gpd1, Lap3, Ndufa6, Pebp1, Rhob, Rtn1, Tagln3, Vwa5a, Aldh2, Atp1a3, Capns1, Ckmt1, Dstn, Fabp4, Gpx3, Lcp1, Ndufa8, Pfkp, Rhog, S100b, **Tf**, Zc2hc1a, Aldoa, Atp1b1, Capzb, **Cntf**, Dusp3, Fam129b, Gpx4, Lypla2, Ndufb10, Pgam2, Rpl12, Sbspon, Tomm34, Aldoc, Atp2b2, Car3, **Cntn1**, Dynll2, Fbxo2, **Gsn**, Lyz2, Ndufb3, Phb, Rpl13a, Sdha, Tpi1, Anpep, Atp5f1d, Cast, Coro1a, Echs1, Fgg, Gstk1, Macrod1, Ndufb6, Picalm, Rpl19, Serpinb6a, Tpm1, Anxa1, Atp5mg, Cat, Cox4i1, Eef1a2, Flot2, Gstm1, Mapt, Ndufc2, Pkm, Rpl26, Serping1, Tppp3, Anxa3, Atp6v1g1, Cavin1, Cox6c, Ehd2, Fn3krp, Gstm2, Mdh1, Npc2, Plin1, Rpl29, Sfn, Tsg101, Anxa7, Bag3, Cavin2, Cp, Ehd4, Gfap, Gstm3, Me1, Ogn, Plin3, Rpl30, **Sgcd**, Tuba1c, Aoc3, Bag5, Cd36, Csrp1, Eif4a2, Glg1, Gstp1, Mrc1, Pacsin2, Pls3, Rps14, Slc25a20, Tuba8, Apoe, Bcam, Ces1d, Cst3, Eno3, Glud1, Hadhb, Myh11, Pc, Prnp, Rps15, Sncg, Twf2, Apoh, Blvrb, Cfb, Dcn, Entpd2, Gnai2, Hspa1l, Myh4, Pcmt1, Prph, Rps25, Sord, Uchl1, Arl6ip5, C3, Dctn2, Ephx1, Gng3, Hspb1, Myl12b, Pygm, Psmc6, Sri, Ugp2

U-shaped

mRNA-abundance (normalized) vs E21, P6, P18, M6
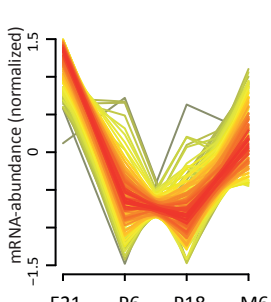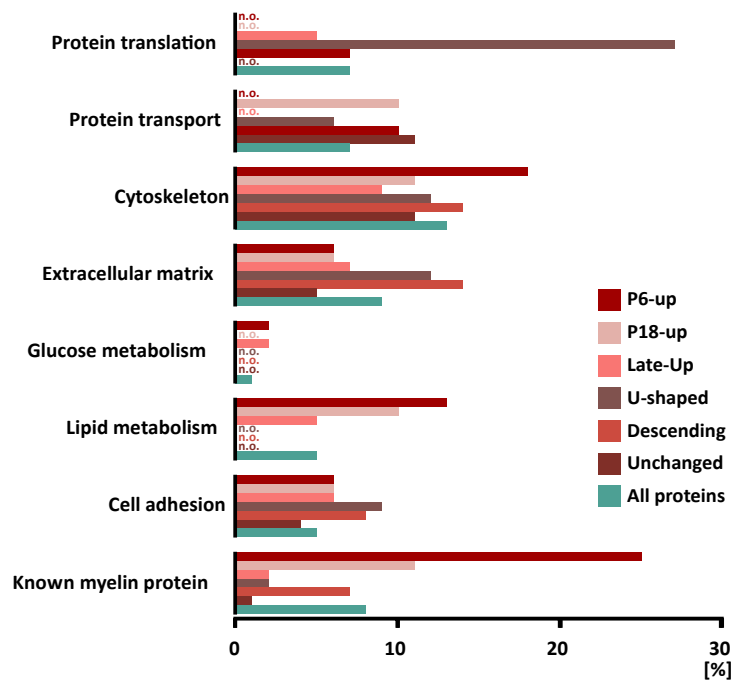
Acadl, Atp5f1b, Cycs, Gclm, Mat2a, Ppia, Rack1, Rpl23, Rpl9, Rps8, Tomm70, Acot7, Atp5mf, Dnm3, Gnb1, Mcts1, Ppid, Ralb, Rpl23a, Rpn2, Rpsa, Tpm3, Actb, Avil, Dpysl4, Gng12, Ndufa12, Ppp1cb, Rap1a, Rpl24, Rps11, **Sept7**, Tuba4a, Actn1, Cacybp, Dsp, Gpx1, Ndufa3, Ppp2r1b, Rap1b, Rpl27, Rps15a, S100a10, Tubb3, Actn4, Calm1, Dync1i2, Hpcal1, Ndufa4, Ppp2r2a, **Rhoa**, Rpl27a, Rps20, Skp1, Ube2d1, Adsl, Camk2d, Eef1a1, Hsp90b1, Ndufs1, Prdx1, Rhoc, Rpl28, Rps23, Slc25a24, Uqcrc1, Ahsa1, Capza2, Ehd1, Hspa12b, Nxn, Prdx3, Rpl10, Rpl3, Rps24, Slc25a3, Vapa, Akr1a1, Cct4, Emc1, Hspa8, Pacsin1, Prps1, Rpl11, Rpl32, Rps27a, Slc25a4, Vdac3, Anp32a, Cnn2, Eno1, Ina, Paics, Psma6, Rpl14, Rpl35, Rps3, Snx1, Wdr1, Anxa6, Cnn3, Erp29, Kpna4, Pcbp2, Ptges3, Rpl15, Rpl4, Rps3a, Snx2, Ybx3, Ap2b1, Cox6a1, Etf1, Lasp1, Pdia4, Rab39a, Rpl17, Rpl5, Rps4x, Ssr4, Ywhab, Arhgap1, Cpne6, Fbn1, Ldha, Pgam1, Rab6b, Rpl18, Rpl6, Rps5, Stoml2, Ywhag, Arpc1b, Cs, Fh, Lman2, Pgk1, Rab8a, Rpl21, Rpl7, Rps6, Tagln2, Ywhah, Arpc5, **Ctnna1**, Fhl1, Lxn, Phgdh, **Rac1**, Rpl22, Rpl7a, Rps6ka3, Thy1, Zyx

Descending

mRNA-abundance (normalized) vs E21, P6, P18, M6
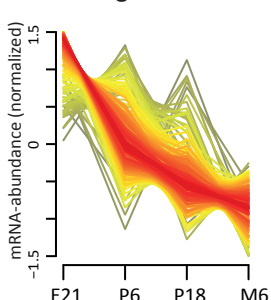
Abce1, Apex1, Calu, Col18a1, Dnajb11, Fermt2, Hist1h1a, Ilk, Lrrc59, Nid2, Pdia3, Psmb2, Rps17, Snd1, Tomm40, Ybx1, Acly, Apoa1, Cand1, Copa, Dnm1l, Flna, Hist1h1b, Immt, Lum, Nipsnap1, Pdia6, Psmd13, Rps18, Snx6, Tubb1, Ykt6, Actr1b, Arcn1, Car2, Copb1, Dpysl3, Flnc, Hist1h1e, Ipo5, **Mapk1**, Nipsnap3b, Phb2, Psmd5, Rps19, Ssr1, Tubb2b, Ywhae, Actr2, Arf4, **Car2**, Cope, Dpysl5, Fn1, Hist1h3a, Iqgap1, Mapre1, Nme1, Plcd1, Psmd7, Rrbp1, Ssr3, Tubb4b, Ywhaz, Add3, Arf5, Capza1, Copg1, Dync1li2, Ganab, Hist1h3b, Islr, Marcks, Nme2, Postn, Ptpa, Sars, Stip1, Tubb5, Zc3hav1l, Aifm1, Arf6, Cct2, Coro1c, Eef1d, Gars, Hmgb1, Isoc1, **Mcam**, Nnt, Ppib, Ran, Sec22b, Stx1b, Tubb6, Ak2, Aspn, Cct3, Crmp1, Eef1g, Glipr2, Hnrnpa2b1, **Itgav**, Mthfd1, Npm1, Ppic, Rap2a, Sec23a, Stxbp6, Tufm, Akap12, Atp2a2, Cct5, **Ctnnb1**, Eif2s1, Glrx3, Hnrnpa3, **Itgb1**, Myadm, Nras, Ppp1ca, Rap2b, Sec31a, Surf4, Txndc5, Alad, Atp4a, Cct6a, Cttn, Eif3b, Gnai3, Hnrnpd, Kif5a, Myh10, Nsf, Ppp1cc, Rars, Sec61a1, Syncrip, Uba1, Alcam, Atp5f1a, Cct7, Dad1, Eif4a1, Gnas, Hnrnpk, Kif5c, Nap1l1, Ola1, Prdx2, Rcn3, Sec61b, Tcp1, Ube2i, Aldh7a1, Bax, Cct8, Ddost, Eif4h, Gnb2, Hsp90aa1, Kpnb1, Nap1l4, Otub1, Prdx4, **Rdx**, **Sept2**, Tgfbi, Ube2n, Anp32b, Basp1, **Cfl1**, Ddx1, Eif5a, Gnb4, Hsp90ab1, Kras, Ncam1, P4hb, Prkaca, Rpl10a, **Sept11**, Tln2, Usp5, **Anxa2**, Bin1, Ckap4, **Dlg1**, **Epb41l3**, Gpd2, Hspa5, **Lama4**, Ncl, Pa2g4, Prdx2, Rpl31, Sept6, Tm9sf3, **Vcl**, Ap2a2, Bzw1, Ckap5, Dlst, Eprs, Gpx7, Hspd1, **Lamb1**, **Nedd4**, Pabpc1, Psma1, Rpl8, Serpinh1, Tmed2, Vcp, Ap2s1, C1qbp, Cnrip1, Dnaja1, Fabp5, Hdlbp, Idh2, **Lamc1**, Nefh, Pafah1b2, Psma5, Rpn1, Set, Tmed9, Vdac1, Calr, Col14a1, Dnaja2, Fam129a, Ikbip, Lpp, **Nid1**, Pcyox1, Psma7, Rps10, Slk, Tmx1, Yars, **Pfn1**

B

Cluster	#mRNA
1 P6-up	132
2 P18-up	203
3 Late-up	186
4 U-shaped	154
5 Descending	245
6 Unchanged	126
All	1046

A

B

A

Quadriceps nerve 9mo

Ctrl

Prx⁻/⁻

B

Total axons (n)

2mo   *
4mo   ***
9mo   **

C

Myelinated axons (n)

2mo   **
4mo   ***
9mo   **

D

Myelin whorls (n)

2mo   **
4mo   **
9mo   ***

Ctrl
Prx⁻/⁻

E

2 mo

$***p=2.20e^{-16}$

Relative frequency [%]
Axonal diameter [μm]

F

4 mo

$***p=2.20e^{-16}$

Relative frequency [%]
Axonal diameter [μm]

G

9 mo

$***p=2.20e^{-16}$

Relative frequency [%]
Axonal diameter [μm]

Ctrl
Prx⁻/⁻

Proteome profile of peripheral myelin in healthy mice and in a neuropathy model

Sophie B. Siems[1,7], Olaf Jahn[2,7], Maria A. Eichel[1], Nirmal Kannaiyan[3], Lai Man N. Wu[4],
Diane L. Sherman[4], Kathrin Kusch[1], Dörte Hesse[2], Ramona B. Jung[1], Robert Fledrich[1,5],
Michael W. Sereda[1,6], Moritz J. Rossner[3], Peter J. Brophy[4], Hauke B. Werner[1,*]

Affiliations
[1]Department of Neurogenetics, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
[2]Proteomics Group, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
[3]Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, 80336 Munich, Germany
[4]Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, EH16 4SB, UK
[5]Institute of Anatomy, University of Leipzig, 04103 Leipzig, Germany
[6]Department of Clinical Neurophysiology, University Medical Center, 37075 Göttingen, Germany
[7]These authors contributed equally to this work

* Corresponding author
Dr. Hauke Werner
Max Planck Institute of Experimental Medicine
Department of Neurogenetics
Hermann-Rein-Str. 3
D-37075 Göttingen, Germany
Tel.: +49 (551) 3899-759; Fax.: +49 (551) 3899-758
E-mail: Hauke@em.mpg.de

Key words
Schwann cell, peripheral nervous system (PNS), myelin proteome, neuropathy, Charcot-Marie-Tooth disease (CMT4F), periaxin (PRX), MCT1/SLC16A1, demyelination, axon degeneration, transcriptome

Word and figure count
Abstract 149 words; Introduction/results/discussion 4786 words
6 main figures; 2 figure supplements; 2 main tables; 4 source data files

49 **ABSTRACT**

50

51 Proteome and transcriptome analyses aim at comprehending the molecular profiles of the

52 brain, its cell-types and subcellular compartments including myelin. Despite the relevance of

53 the peripheral nervous system for normal sensory and motor capabilities, analogous

54 approaches to peripheral nerves and peripheral myelin have fallen behind evolving technical

55 standards. Here we assess the peripheral myelin proteome by gel-free, label-free mass-

56 spectrometry for deep quantitative coverage. Integration with RNA-Sequencing-based

57 developmental mRNA-abundance profiles and neuropathy disease genes illustrates the utility

58 of this resource. Notably, the periaxin-deficient mouse model of the neuropathy Charcot-

59 Marie-Tooth 4F displays a highly pathological myelin proteome profile, exemplified by the

60 discovery of reduced levels of the monocarboxylate transporter MCT1/SLC16A1 as a novel

61 facet of the neuropathology. This work provides the most comprehensive proteome resource

62 thus far to approach development, function and pathology of peripheral myelin, and a

63 straightforward, accurate and sensitive workflow to address myelin diversity in health and

64 disease.

65

66

INTRODUCTION

The ensheathment of axons with myelin enables rapid impulse propagation, a prerequisite for normal motor and sensory capabilities of vertebrates (1,2). This is illustrated by demyelinating neuropathies of the Charcot-Marie-Tooth (CMT) spectrum, in which mutations affecting myelin genes as *MPZ, PMP22, GJB1* and *PRX* impair myelin integrity and reduce the velocity of nerve conduction in the peripheral nervous system (PNS) (3). Developmentally, myelination by Schwann cells in peripheral nerves is regulated by axonal neuregulin-1 (4,5) and the basal lamina (6–8) that is molecularly linked to the abaxonal Schwann cell membrane via integrins and the dystroglycan complex (9–12). In adulthood, the basal lamina continues to enclose all axon/myelin-units (13), probably to maintain myelin. Beyond regulation by extracellular cues, myelination involves multiple proteins mediating radial sorting of axons out of Remak bundles, myelin membrane growth and layer compaction (14–18). For example, the Ig-domain containing myelin protein zero (MPZ; also termed P0) mediates adhesion between adjacent extracellular membrane surfaces in compact myelin (19). At their intracellular surfaces, myelin membranes are compacted by the cytosolic domain of MPZ/P0 together with myelin basic protein (MBP; previously termed P1) (20,21). Not surprisingly, MPZ/P0 and MBP were early identified as the most abundant peripheral myelin proteins (22,23).

A system of cytoplasmic channels through the otherwise compacted myelin sheath remains non-compacted throughout life, i.e. the adaxonal myelin layer, paranodal loops, Schmidt-Lanterman incisures (SLI), and abaxonal longitudinal and transverse bands of cytoplasm termed bands of Cajal (14,24,25). Non-compacted myelin comprises cytoplasm, cytoskeletal elements, vesicles and lipid-modifying enzymes, and thus numerous proteins involved in maintaining the myelin sheath. The cytosolic channels probably also represent transport routes toward Schwann cell-dependent metabolic support of myelinated axons (26–31).

Considering that Schwann cells constitute a major proportion of the cells in the PNS, oligonucleotide microarray analyses have been used for mRNA abundance profiling of total sciatic nerves (32,33). Indeed, these systematic approaches allowed the identification of novel myelin constituents including non-compact myelin-associated protein (NCMAP/MP11) (34). Notwithstanding that the number of known peripheral myelin proteins has grown in recent years, a comprehensive molecular inventory has been difficult to achieve because applications of systematic ('omics') approaches specifically to Schwann cells and peripheral myelin remained comparatively scarce, different from studies addressing oligodendrocytes and CNS myelin (35–40). One main reason may be that the available techniques were not

104   sufficiently straightforward for general application. For example, the protein composition of
105   peripheral myelin was previously assessed by proteome analysis (41). However, at that time
106   the workflow of sample preparation and data acquisition (schematically depicted in **Figure**
107   **1A**) was very labor-intense and required a substantial amount of input material; yet the depth
108   of the resulting datasets remained limited. In particular, differential myelin proteome analysis
109   by 2-dimensional fluorescence intensity gel electrophoresis (2D-DIGE) requires considerable
110   hands-on-time and technical expertise (41,42). While this method is powerful for the
111   separation of proteoforms (43), it typically suffers from under-representation of highly basic
112   and transmembrane proteins. It thus allows comparing the abundance of only few myelin
113   proteins rather than quantitatively covering the entire myelin proteome. Because of these
114   limitations and an only modest sample-to-sample reproducibility, 2D-DIGE analysis of myelin,
115   although unbiased, has not been commonly applied beyond specialized laboratories.
116
117   The aim of the present study was to establish a straightforward and readily applicable
118   workflow to facilitate both comprehensive knowledge about the protein composition of
119   peripheral myelin and systematic assessment of differences between two states, e.g.,
120   pathological alterations in a neuropathy model. The major prerequisites were the biochemical
121   purification of myelin, its solubilization with the detergent ASB-14 and the subsequent
122   automated digestion with trypsin during filter-aided sample preparation (FASP). The tryptic
123   peptides were fractionated by liquid chromatography and analyzed by mass spectrometry for
124   gel-free, label-free quantitative proteome analysis. More specifically, we used nano-flow
125   ultra-performance liquid chromatography (nanoUPLC) coupled to an electrospray-ionization
126   quadrupole time-of-flight (ESI-QTOF) mass spectrometer with ion mobility option, providing
127   an orthogonal dimension of peptide separation. The utilized data-independent acquisition
128   (DIA) strategy relies on collecting data in an alternating low and elevated energy mode
129   (MS^E); it enables simultaneous sequencing and quantification of all peptides entering the
130   mass spectrometer without prior precursor selection (reviewed in (44,45)). With their high-
131   duty cycle utilized for the acquisition of precursor ions, MS^E-type methods are ideally suited
132   to reliably quantify proteins based on peptide intensities. Notably, these methods do not
133   involve the use of spectral libraries in the identification of proteins, different from other DIA
134   strategies. Instead, the achieved high-complexity fragmentation spectra are deconvoluted
135   before submission to dedicated search engines for peptide and protein identification (46,47).
136   In the MS^E mode, this deconvolution involves precursor-fragment ion alignment solely on the
137   basis of chromatographic elution profiles; on top, drift times of ion mobility-separated
138   precursors are used in the high-definition (HD)MS^E mode. An expansion of the latter referred
139   to as the ultra-definition (UD)MS^E mode, additionally implements drift time-dependent
140   collision energy profiles for more effective precursor fragmentation (48,49).

141

142   Indeed, compared to the previously used manual handling and in-gel digestion, the current

143   workflow (schematically depicted in **Figure 1A)** is considerably less labor-intense, and

144   automated FASP increases sample-to-sample reproducibility. Moreover, differential analysis

145   by quantitative mass spectrometry (MS) facilitates reproducible quantification of hundreds

146   rather than a few distinct myelin proteins. Together, the present workflow increases the

147   efficacy of assessing the peripheral myelin proteome while shifting the main workload from

148   manual sample preparation and gel-separation to automated acquisition and processing of

149   data. We propose that comprehending the expression profiles of all myelin proteins in the

150   healthy PNS and in myelin-related disorders can contribute to advancing our understanding

151   of the physiology and pathophysiology of peripheral nerves.

152

153   **RESULTS**

154

155   **Purification of peripheral myelin**

156   We biochemically enriched myelin as a light-weight membrane fraction from pools of sciatic

157   nerves dissected from mice at postnatal day 21 (P21) using an established protocol of

158   discontinuous sucrose density gradient centrifugation (41,50), in which myelin membranes

159   accumulate at the interface between 0.29 and 0.85 M sucrose. By immunoblotting, proteins

160   specific for both compact (MPZ/P0, MBP, PMP2) and non-compact (PRX) myelin were

161   substantially enriched in the myelin fraction compared to nerve lysates (**Figure 1B**).

162   Conversely, axonal (NEFH, KCNA1) and mitochondrial (VDAC) proteins and a marker for the

163   Schwann cell nucleus (KROX20/EGR2) were strongly reduced in purified myelin. Together,

164   these results imply that biochemically purified peripheral myelin is suitable for systematic

165   analysis of its protein composition.

166

167   **Proteome analysis of peripheral myelin**

168   It has long been difficult to accurately quantify the most abundant myelin proteins both in the

169   CNS (PLP, MBP, CNP (51)) and the PNS (MPZ/P0, MBP, PRX; this work), probably owing to

170   their exceptionally high relative abundance. For example, the major CNS myelin constituents

171   PLP, MBP and CNP comprise 17, 8 and 4% of the total myelin protein, respectively (51). We

172   have recently provided proof of principle (52) that the mass spectrometric quantification of

173   these high-abundant myelin proteins is accurate and precise when data are acquired in the

174   MS^E data acquisition mode and proteins are quantified according to the TOP3 method, i.e. if

175   their abundance values are obtained based on the proven correlation between the average

176   intensity of the three peptides exhibiting the most intense mass spectrometry response and

177   the absolute amount of their source protein (53,54). Using data acquisition by MS^E we

178   confirmed that CNP constitutes about 4% of the total CNS myelin proteome and that the

179   abundance of CNP in myelin from mice heterozygous for the *Cnp* gene (*Cnp$^{WT/null}$*) compared

180   to wild-type mice is 50.7% (±0.4%), in agreement with the halved gene dosage and gel-

181   based quantification by silver staining or immunoblotting (52).

182

183   When applying the MS^E mode to PNS myelin, we quantified 351 proteins with a false

184   discovery rate (FDR) of <1% at peptide and protein level and an average sequence coverage

185   of 35.5% (**Figure 1-source data 1**). While MS^E (labeled in orange in **Figure 1C**) indeed

186   provided a dynamic range of more than four orders of magnitude and thus quantitatively

187   covered the exceptionally abundant myelin proteins MPZ/P0, MBP and PRX, the number of

188   quantified proteins appeared limited when spectral complexity was deconvoluted solely on

189   the basis of chromatographic elution profiles. Accordingly, by using the UDMS^E mode, which

190   comprises ion mobility for additional peptide separation as well as drift time-specific collision
191   energies for peptide fragmentation, proteome coverage was increased about three-fold (1078
192   proteins quantified; average sequence coverage 34.3%; **Figure 1-source data 1**). However,
193   the dynamic range of UDMS^E (labeled in blue in **Figure 1C**) was found to be somewhat
194   compressed compared to that of MS^E, which can be considered an expectable feature of
195   traveling wave ion mobility devices (55), where the analysis of pulsed ion packages leads to
196   a temporal and spatial binning of peptides during ion mobility separation. Indeed, this
197   manifests as a ceiling effect for the detection of exceptionally intense peptide signals, which
198   results in an underestimation of the relative abundance of MPZ/P0, MBP and PRX by
199   UDMS^E.
200
201   The complementary nature of the MS^E and UDMS^E data acquisition modes led us to
202   conclude that a comprehensive analysis of the myelin proteome that facilitates both correct
203   quantification of the most abundant proteins and deep quantitative coverage of the proteome
204   would require analyzing the same set of samples with two different instrument settings for
205   MS^E and UDMS^E, respectively. Considering that instrument time is a bottleneck for the
206   routine differential proteome analysis of myelin from mutant mice, we aimed to combine the
207   strengths of MS^E and UDMS^E into a single data acquisition mode. Based on a gene ontology
208   enrichment analysis for cellular components of the 200 proteins of highest and lowest
209   abundance from the UDMS^E dataset, we realized that the 'bottom' of the quantified proteome
210   is probably largely unrelated to myelin but dominated by contaminants from other subcellular
211   sources including mitochondria. We thus reasoned that for a myelin-directed data acquisition
212   mode, proteome depth may be traded in for a gain in dynamic range and devised a novel
213   method referred to as dynamic range enhancement (DRE)-UDMS^E, in which a deflection lens
214   is used to cycle between full and reduced ion transmission during mass spectrometric
215   scanning. Indeed, DRE-UDMS^E quantified an intermediate number of proteins in PNS myelin
216   (554 proteins; average sequence coverage 30.6%; **Figure 1-source data 1**) while providing
217   an intermediate dynamic range (labeled in green in **Figure 1C**). We thus consider DRE-
218   UDMS^E as the data acquisition mode of choice most suitable for routine differential myelin
219   proteome profiling (see below).
220
221   Overall, we found a high reproducibility between replicates and even among the different
222   data acquisition modes as indicated by Pearson's correlation coefficients for protein
223   abundance in the range of 0.765-0.997 (**Figure 1-supplement 1**). When comparing the
224   proteins identified in PNS myelin using the three data acquisition modes, we found a very
225   high overlap (**Figure 1D**). We also found a high overlap (**Figure 1E**) between the proteins
226   identified in the present study by UDMS^E and those detected in previous proteomic

227 approaches to PNS myelin (41,42), thus allowing a high level of confidence. Together, the
228 three data acquisition modes exhibit distinct strengths in the efficient quantification of
229 exceptionally abundant proteins (MS^E), establishing a comprehensive inventory ($UDMS^E$)
230 and gel-free, label-free differential analysis of hundreds of distinct proteins ($DRE-UDMS^E$) in
231 peripheral myelin (see **Figure 1A**). Yet, analyzing the same set of samples by different
232 modes may not always be feasible in all routine applications when considering required
233 instrument time.

234

235 **Relative abundance of peripheral myelin proteins**
236 Considering that MS^E provides the high dynamic range required for the quantification of the
237 most abundant myelin proteins, we calculated the relative abundance of the 351 proteins
238 identified in myelin by MS^E (**Figure 1-source data 1**). According to quantitative assessment
239 of this dataset, the most abundant PNS myelin protein, myelin protein zero (MPZ/P0),
240 constitutes 44% (+/-4% relative standard deviation (RSD)) of the total myelin protein (**Figure
241 2**). Myelin basic protein (MBP), periaxin (PRX) and tetraspanin-29 (CD9) constitute 18% (+/-
242 1% RSD), 15% (+/-1%) and 1% (+/-0.2%) of the total myelin protein, respectively (**Figure 2**).
243 For MPZ/P0 and MBP, our quantification by MS^E is in agreement with but specifies prior
244 estimations upon gel-separation and protein labeling by Sudan-Black, Fast-Green or
245 Coomassie-Blue, in which they were judged to constitute 45–70% and 2–26% of the total
246 myelin protein, respectively (22,56–58). However, gel-based estimates of the relative
247 abundance of myelin proteins were not very precise with respect to many other proteins,
248 including those of high molecular weight. Indeed, periaxin was identified as a constituent of
249 peripheral myelin after the advent of gradient SDS-PAGE gels (59), which allowed improved
250 migration of large proteins into gels. The present MS^E-based quantification of myelin proteins
251 also extends beyond and partially adjusts an earlier mass spectrometric approach (41).
252 Indeed, the current approach identified and quantified more myelin proteins, probably owing
253 to improved protein solubilization during sample preparation and higher dynamic range of the
254 used mass spectrometer. By MS^E, known myelin proteins (**Table 1**) collectively constitute
255 over 85% of the total myelin protein (**Figure 2**) while proteins not yet associated with myelin
256 account for the remaining 15% of the total myelin protein.

257

258 **Comprehensive compendium and comparison to the transcriptome**
259 To systematically elucidate the developmental abundance profiles of the transcripts that
260 encode peripheral myelin proteins (**Figure 3**), we used our combined proteome inventory of
261 peripheral myelin (**Figure 1-source data 1**) to filter mRNA abundance data of all genes
262 expressed in sciatic nerves. By this strategy, **Figure 3** displays only those transcripts of
263 which the protein product was identified in peripheral myelin rather than all transcripts in the

264   nerve, thereby discriminating myelin-related mRNAs from other mRNAs such as those
265   present in peripheral axons, fibroblasts, immune cells etc. In this assessment we additionally
266   included PMP22 although it was not detected by MS as well as 45 proteins exclusively
267   identified by LC-MS of myelin separated by SDS-PAGE (**Figure 1-source data 1**). For
268   mRNA abundance profiles, we exploited a recently established RNA sequencing analysis
269   (RNA-Seq; platform Illumina HiSeq 2000) of sciatic nerves dissected form wild type Sprague
270   Dawley rats at embryonic day 21 (E21), P6, P18 and 6 months (60). RNA-Seq provides
271   reliable information about the relative abundance of all significantly expressed genes and is
272   thus not limited to those represented on the previously used oligonucleotide microarrays
273   (41). The raw data (accessible under GEO accession number GSE115930) were normalized
274   (**Figure 3-source data 1**) and standardized. When comparing the proteome and
275   transcriptome datasets, significant mRNA abundance was detected for all 1046 transcripts
276   for which an unambiguous unique gene identifier was found (**Figure 3**). 126 transcripts
277   displayed developmentally unchanged abundance levels, i.e., abundance changes below a
278   threshold of 10% coefficient of variation (**Figure 3B; Figure 3-source data 1**).
279
280   By fuzzy c-means clustering, those 920 transcripts that showed developmental abundance
281   changes were grouped into 5 clusters (**Figure 3A; Figure 3-source data 1**). Among those,
282   one cluster corresponds to an mRNA-abundance peak coinciding with an early phase of
283   myelin biogenesis (cluster 'P6-UP'), which includes the highest proportion of known myelin
284   proteins (**Table 1**) such as MPZ/P0, MBP, PRX, cyclic nucleotide phosphodiesterase (CNP),
285   fatty acid synthase (FASN), myelin-associated glycoprotein (MAG), proteolipid protein
286   (PLP/DM20), cell adhesion molecule-4 (CADM4/NECL4), connexin-29 (GJC3), claudin-19
287   (CLDN19) and CKLF-like MARVEL-transmembrane domain containing protein-5 (CMTM5).
288   However, many known myelin proteins clustered together according to their mRNA-
289   abundance peak coinciding with a later phase of myelination (cluster 'P18-UP'), including
290   peripheral myelin protein 2 (PMP2), tetraspanin-29 (CD9), tetraspanin-28 (CD81), connexin-
291   32 (GJB1), plasmolipin (PLLP), junctional adhesion molecule-3 (JAM3), CD59 and
292   dystrophin-related protein-2 (DRP2). The proportion of known myelin proteins was lower in
293   the clusters corresponding to mRNA-abundance peaks in adulthood (clusters 'late-UP', 'U-
294   shaped'). Yet, a considerable number of transcripts displayed abundance peaks at the
295   embryonic time-point (cluster 'Descending'), including carbonic anhydrase 2 (CA2), cofilin-1
296   CFL1), tubulin beta-4 (TUBB4b) and band 4.1-protein B (EPB41L3). Generalized, the
297   clusters were roughly similar when comparing previous oligonucleotide microarray analysis
298   of mouse sciatic nerves (41) and the RNA-Seq analysis of rat sciatic nerves (this study); yet,
299   the latter provides information on a larger number of genes and with a higher level of

300 confidence. Together, clustering of mRNA abundance profiles allows categorizing peripheral
301 myelin proteins into developmentally co-regulated groups.
302
303 When systematically assessing the proteins identified in myelin by gene ontology (GO)-term
304 analysis, the functional categories over-represented in the entire myelin proteome included
305 cell adhesion, cytoskeleton and extracellular matrix (labeled in turquoise in **Figure 4**). When
306 analyzing the clusters of developmentally co-expressed transcripts (from **Figure 3**), proteins
307 associated with the lipid metabolism were particularly enriched in the P6-UP and P18-UP
308 clusters, while those associated with the extracellular matrix (ECM) were over-represented in
309 the U-shaped and Descending clusters (**Figure 4**). For comparison, known myelin proteins
310 (**Table 1**) were over-represented in the P6-UP and P18-UP clusters (**Figure 4**). Together,
311 our proteome dataset provides comprehensive in-depth coverage of the protein constituents
312 of peripheral myelin purified from the sciatic nerves of wild type mice, and comparison to the
313 transcriptome allows identifying developmentally co-regulated and functional groups of
314 myelin proteins. Our data thus supply a solid resource for the molecular characterization of
315 myelin and for discovering functionally relevant myelin proteins.
316
317 **Neuropathy genes encoding myelin proteins**
318 Heritable neuropathies can be caused by mutations affecting genes preferentially expressed
319 in neurons, Schwann cells or both (3,61–63). To systematically assess which neuropathy-
320 causing genes encode peripheral myelin proteins, we compared our myelin proteome
321 dataset with a current overview about disease genes at the NIH National Library of Medicine
322 at https://ghr.nlm.nih.gov/condition/charcot-marie-tooth-disease#genes. Indeed, 31 myelin
323 proteins were identified to be encoded by a proven neuropathy gene (**Table 2**), a
324 considerable increase compared to eight disease genes found in a similar previous approach
325 (41). Notably, this increase is owing to both the larger size of the current myelin proteome
326 dataset (**Figure 1E**) and the recent discovery of numerous neuropathy genes by the
327 widespread application of next generation sequencing.
328
329 **Pathological proteomic profile of peripheral myelin in a neuropathy model**
330 The results presented thus far were based on analyzing myelin of healthy wild type mice; yet
331 we also sought to establish a straightforward method to systematically assess myelin
332 diversity, as exemplified by alterations in a pathological situation. As a model we chose mice
333 carrying a homozygous deletion of the periaxin gene ($Prx^{-/-}$) (26,64). Periaxin (PRX) is the
334 third-most abundant peripheral myelin protein (**Figure 2**) and scaffolds the dystroglycan
335 complex in Schwann cells. $Prx^{-/-}$ mice represent an established model of Charcot-Marie-
336 Tooth disease type 4F (65–67). Aiming to assess the myelin proteome, we purified myelin

337 from pools of sciatic nerves dissected from *Prx*^{-/-} and control mice at P21. Upon SDS-PAGE
338 separation and silver staining the band patterns appeared roughly similar (**Figure 5A**), with
339 the most obvious exception of the absence of the high-molecular weight band constituted by
340 periaxin in *Prx*^{-/-} myelin. Yet, several other bands also displayed genotype-dependent
341 differences in intensity. As expected, PRX was also undetectable by MS[E] in *Prx*^{-/-} myelin, in
342 which most of the total myelin protein was constituted by MPZ/P0 and MBP (**Figure 5B;**
343 **Figure 5-source data 1**).

344

345 Upon differential analysis by DRE-UDMS[E] (**Figure 5-source data 2**), multiple proteins
346 displayed genotype-dependent differences as visualized in a heatmap displaying those 40
347 proteins of which the abundance was reduced or increased with the highest statistical
348 significance in *Prx*^{-/-} compared to control myelin (**Figure 5C**). For example, the abundance of
349 the periaxin-associated dystrophin-related protein 2 (DRP2) was strongly reduced in *Prx*^{-/-}
350 myelin, as previously shown by immunoblotting (9). Notably, the abundance of multiple other
351 proteins was also significantly reduced in *Prx*^{-/-} myelin, including the extracellular matrix
352 protein laminin C1 (LAMC1; previously termed LAMB2), the laminin-associated protein
353 nidogen (NID1), Ig-like cell adhesion molecules (CADM4, MAG), the desmosomal junction
354 protein desmin (DES), cytoskeletal and cytoskeleton-associated proteins (EPB41L3, MAP1A,
355 CORO1A, SPTBN1, various microtubular and intermediate filament monomers), the
356 monocarboxylate transporter MCT1 (also termed SLC16A1) and the MCT1-associated (68)
357 immunoglobulin superfamily protein basigin (BSG, also termed CD147). On the other hand,
358 proteins displaying the strongest abundance increase in *Prx*^{-/-} myelin included immune-
359 related proteins (LGALS3, LYZ2, CTSD), cytoskeletal and cytoskeleton-associated proteins
360 (CAPG, CORO1C, CNN3, several myosin heavy chain subunits), peroxisomal enzymes
361 (CAT, HSD17B4, MDH1) and known myelin proteins (PLLP/plasmolipin, CRYAB,
362 GJB1/CX32). For comparison, the abundance of the marker proteolipid protein (PLP/DM20)
363 (69) and the periaxin-associated integrin beta-4 (ITGB4) (12) in myelin was unaltered in *Prx*^{-/-}
364 myelin. Together, differential proteome analysis finds considerably more proteins and protein
365 groups to be altered in *Prx*^{-/-} myelin than previously known (**Figure 5C, D-D'''**), probably
366 reflecting the complex pathology observed in this model (26,64).

367

368 The monocarboxylate transporter MCT1/SLC16A1 expressed by myelinating
369 oligodendrocytes (70,71) and Schwann cells (28,72) has been proposed to supply lactate or
370 other glucose breakdown products to axons, in which they may serve as substrate for the
371 mitochondrial production of ATP (73–75). In this respect it was striking to find the abundance
372 of MCT1 significantly reduced in peripheral myelin when PRX is lacking (**Figure 5C**), a result
373 that we were able to confirm by immunoblotting (**Figure 5E**) and immunolabeling of teased

374   fiber preparations of sciatic nerves (**Figure 5F**). Notably, reduced expression of MCT1 in

375   *Slc16a1$^{+/-}$* mice impairs axonal integrity at least in the CNS (70,76). The reduced abundance

376   of MCT1 thus represents an interesting novel facet of the complex pathology in *Prx$^{-/-}$* mice.

377   Considering that the integrity of peripheral axons may be impaired in *Prx$^{-/-}$* mice, we

378   assessed their quadriceps nerves. Indeed, *Prx$^{-/-}$* mice displayed reduced axonal diameters, a

379   progressively reduced total number of axons and a considerable number of myelin whorls

380   lacking a visible axon (**Figure 6**), indicative of impaired axonal integrity (77). Yet we note that

381   molecular or neuropathological features other than the reduced abundance of MCT1

382   probably also contribute to the axonopathy in *Prx$^{-/-}$* mice.

383

384   Together, gel-free, label free proteome analysis provides a cost- and time-efficient method

385   that provides an accurate, sensitive tool to gain systematic insight into the protein

386   composition of healthy peripheral myelin and its alterations in pathological situations. Indeed,

387   gel-free proteome analysis is particularly powerful and comprehensive compared to 2D-

388   DIGE; the workflow presented here appears readily applicable to other neuropathy models,

389   thereby promising discovery of relevant novel features of their neuropathology.

390

391

DISCUSSION

We used gel-free, label-free quantitative mass spectrometry to assess the protein composition of myelin biochemically purified from the sciatic nerves of wild-type mice, thereby establishing a straightforward and readily applicable workflow to approach the peripheral myelin proteome. The key to comprehensiveness was to combine the strengths of three data acquisition modes, i.e., MS^E for correct quantification of high-abundant proteins, UDMS^E for deep quantitative proteome coverage including low-abundant proteins and DRE-UDMS^E for differential analysis. We suggest that DRE-UDMS^E provides a good compromise between dynamic range, identification rate and instrument run time for routine differential myelin proteome profiling as a prerequisite for a molecular understanding of myelin (patho)biology. We have also integrated the resulting compendium with RNA-Seq-based mRNA abundance profiles in peripheral nerves and neuropathy disease loci. Beyond providing the largest peripheral myelin proteome dataset thus far, the workflow is appropriate to serve as starting point for assessing relevant variations of myelin protein composition, e.g., in different nerves, ages, species and in pathological conditions. The identification of numerous pathological alterations of myelin protein composition in the *Prx$^{-/-}$* neuropathy model indicates that the method is well suited to assess such diversity.

Aiming to understand nervous system function at the molecular level, multiple 'omics'-scale projects assess the spatio-temporal expression profiles of all mRNAs and proteins in the CNS including oligodendrocytes and myelin (35–39). Yet, peripheral nerves are also essential for normal sensory and motor capabilities. Prior approaches to the molecular profiles of Schwann cells and PNS myelin thus far, however, were performed >8 years ago (32–34,41,78–80), and the techniques have considerably advanced since. For example, current gel-free, label-free mass spectrometry can simultaneously identify and quantify the vast majority of proteins in a sample, thereby providing comprehensive in depth-information. Moreover, RNA-Seq technology has overcome limitations of the previously used microarrays for characterizing mRNA abundance profiles with respect to the number of represented genes and the suitability of the oligonucleotide probes. The present compendium thus provides high confidence with respect to the identification of myelin proteins, their relative abundance and their developmental mRNA expression profiles. This view is supported by the finding that over 80% of the total myelin proteome is constituted by approximately 50 previously known myelin proteins. We believe that the majority of the other identified proteins represent low-abundant myelin-associated constituents in line with the high efficiency of biochemical myelin purification. Doubtless, however, the myelin proteome also comprises

428   contaminants from other cellular sources, underscoring the need of independent validation
429   for establishing newly identified constituents as true myelin proteins.

430

431   Do myelin proteins exist that escape identification by standard proteomic approaches?
432   Indeed, some proteins display atypically distributed lysine and arginine residues, which
433   represent the cleavage sites of the commonly used protease trypsin. The tryptic digest of
434   these proteins leads to peptides that are not well suited for chromatographic separation
435   and/or mass spectrometric detection/sequencing, as exemplified by the small hydrophobic
436   tetraspan-transmembrane myelin proteins MAL (81) and PMP22 (82). We can thus not
437   exclude that additional proteins with atypical tryptic digest patterns exist in peripheral myelin,
438   which would need to be addressed by the use of alternative proteases. Moreover, potent
439   signaling molecules including erbB receptor tyrosine kinases (83,84) and G-protein coupled
440   receptors (GPRs) (85–87) display exceptionally low abundance. Such proteins may be
441   identified when applying less stringent identification criteria, e.g., by requiring the sequencing
442   of only one unique peptide per protein. However, lower stringency would also result in
443   identifying false-positive proteins, which we wished to avoid for the purpose of the present
444   compendium. We note that a truly comprehensive spatio-temporally resolved myelin
445   proteome should preferentially also include systematic information about protein isoforms
446   and post-translational modifications, which still poses technical challenges.

447

448   Mutations affecting the periaxin (*PRX*) gene in humans cause CMT type 4F (65,88–90); the
449   neuropathology resulting from mutations affecting periaxin has been mainly investigated in
450   the *Prx*$^{-/-}$ mouse model. Indeed, *Prx*$^{-/-}$ mice display a progressive peripheral neuropathy
451   including axon/myelin-units with abnormal myelin thickness, demyelination, tomaculae, onion
452   bulbs, reduced nerve conduction velocity (64), reduced abundance and mislocalization of the
453   periaxin-associated DRP2 (9) and reduced internode length (26). Absence of SLIs (64) and
454   bands of Cajal (26) imply that the non-compact myelin compartments are impaired when
455   PRX is lacking. In the differential analysis of myelin purified from *Prx*$^{-/-}$ and control mice we
456   find that the previously reported reduced abundance of DRP2 (9) represents one of the
457   strongest molecular changes in the myelin proteome when PRX is lacking. Notably, the
458   reported morphological changes in this neuropathy model (9,26,64) go along with alterations
459   affecting the abundance of multiple other myelin-associated proteins, including junctional,
460   cytoskeletal, extracellular matrix and immune-related proteins as well as lipid-modifying
461   enzymes. Thus, the neuropathology in *Prx*$^{-/-}$ mice at the molecular level is more complex
462   than previously anticipated. It is striking that the abundance of the monocarboxylate
463   transporter MCT1/SLC16A1 that may contribute to the metabolic supply of lactate from
464   myelinating cells to axons (27–31) is strongly reduced in *Prx*$^{-/-}$ myelin. Considering that

465   MCT1 in Schwann cells mainly localizes to Schmidt Lanterman incisures (SLI) (28) and that
466   SLI are largely absent from myelin when PRX is lacking (64), the reduced abundance of
467   MCT1 in *Prx*^{-/-} myelin may be a consequence of the impaired myelin ultrastructure. Yet,
468   considering that SLI are part of the cytosolic channels that may represent transport routes
469   toward Schwann cell-dependent metabolic support of myelinated axons, the diminishment of
470   MCT1 may contribute to reduced axonal diameters or axonal loss in *Prx*^{-/-} mice, probably in
471   conjunction with other molecular or morphological defects. Together, the in depth-analysis of
472   proteins altered in neuropathy models can contribute to an improved understanding of nerve
473   pathophysiology.
474
475   Compared to a previous approach (41), the number of proven neuropathy genes of which the
476   encoded protein is mass spectrometrically identified in peripheral myelin has increased four-
477   fold from eight to 32 in the present study. This reflects both that the number of proteins
478   identified in myelin has approximately doubled and that more neuropathy genes are known
479   due to the common use of genome sequencing. We note that our compendium comprises
480   not only myelin-associated proteins causing (when mutated) demyelinating CMT1 (e.g.,
481   MPZ/P0, NEFL, PMP2) or intermediate CMT4 (GDAP1, NDRG1, PRX) but also axonal
482   CMT2 (RAB7, GARS, HSPB1). Yet, the expression of genes causative of CMT2 is not
483   necessarily limited to neurons, as exemplified by the classical myelin protein MPZ/P0.
484   Indeed, a subset of *MPZ*-mutations causes axonal CMT2I or CMT2J (91–95), probably
485   reflecting impaired axonal integrity as consequence of a mutation primarily affecting
486   Schwann cells. We also note that the nuclear EGR2/KROX20 causative of demyelinating
487   CMT1D has not been mass spectrometrically identified in myelin, reflecting that Schwann cell
488   nuclei are efficiently removed during myelin purification.
489
490   While morphological analysis of peripheral nerves by light and electron microscopy is routine
491   in numerous laboratories, systematic molecular analysis has been less straightforward.
492   Using the sciatic nerve as a model, we show that systematic assessment of the myelin
493   proteome and the total nerve transcriptome are suited to determine comprehensive
494   molecular profiles in healthy nerves and in myelin-related disorders. Myelin proteome
495   analysis can thus complement transcriptome analysis in assessing development, function
496   and pathophysiology of peripheral nerves.
497

MATERIALS AND METHODS

Mouse models

Prx^{-/-} mice (64) were kept on c57Bl/6 background in the animal facility of the University of Edinburgh (United Kingdom). Genotyping was by PCR on genomic DNA using the forward primers 5'-CAGATTTGCT CTGCCCAAGT and 5'-CGCCTTCTAT CGCCTTCTTGAC in combination with reverse primer 5'-ATGCCCTCAC CCACTAACAG. The PCR yielded a 0.5 kb fragment for the wildtype allele and a 0.75 kb product for the mutant allele. The age of experimental animals is given in the figure legends. All animal work conformed to United Kingdom legislation (Scientific Procedures) Act 1986 and to the University of Edinburgh Ethical Review Committee policy; Home Office project license No. P0F4A25E9.

Myelin purification

A light-weight membrane fraction enriched for myelin was purified from sciatic nerves of mice by sucrose density centrifugation and osmotic shocks as described (41,52). Myelin accumulates at the interface between 0.29 and 0.85 M sucrose. *Prx^{-/-}* and wild type control C57Bl/6 mice were sacrificed by cervical dislocation at postnatal day 21 (P21). For each genotype, myelin was purified as three biological replicates (n=3); each biological replicate representing a pool of 20 sciatic nerves dissected from 10 mice. Protein concentration was determined using the DC Protein Assay Kit (Bio-Rad).

Filter-aided sample preparation for proteome analysis

Protein fractions corresponding to 10 µg myelin protein were dissolved and processed according to a filter-aided sample preparation (FASP) protocol essentially as previously described for synaptic protein fractions (96) and as adapted to CNS myelin (52,97). Unless stated otherwise, all steps were automated on a liquid-handling workstation equipped with a vacuum manifold (Freedom EVO 150, Tecan) by using an adaptor device constructed in-house. Briefly, myelin protein samples were lysed and reduced in lysis buffer (7 M urea, 2 M thiourea, 10 mM DTT, 0.1 M Tris pH 8.5) containing 1% ASB-14 by shaking for 30 min at 37°C. Subsequently, the sample was diluted with ~10 volumes lysis buffer containing 2% CHAPS to reduce the ASB-14 concentration and loaded on centrifugal filter units (30 kDa MWCO, Merck Millipore). After removal of the detergents by washing twice with wash buffer (8 M urea, 10 mM DTT, 0.1 M Tris pH 8.5), proteins were alkylated with 50 mM iodoacetamide in 8 M urea, 0.1 M Tris pH 8.5 (20 min at RT), followed by two washes with wash buffer to remove excess reagent. Buffer was exchanged by washing three times with 50 mM ammonium bicarbonate (ABC) containing 10 % acetonitrile. After three additional washes with 50 mM ABC/10% acetonitrile, which were performed by centrifugation to ensure

535   quantitative removal of liquids potentially remaining underneath the ultrafiltration membrane,

536   proteins were digested overnight at 37°C with 400 ng trypsin in 40 µl of the same buffer.

537   Tryptic peptides were recovered by centrifugation followed by two additional extraction steps

538   with 40 µl of 50 mM ABC and 40 µl of 1% trifluoroacetic acid (TFA), respectively. Aliquots of

539   the combined flow-throughs were spiked with 10 fmol/µl of yeast enolase-1 tryptic digest

540   standard (Waters Corporation) for quantification purposes and directly subjected to analysis

541   by liquid chromatography coupled to electrospray mass spectrometry (LC-MS). A pool of all

542   samples was injected at least before and after any sample set to monitor stability of

543   instrument performance.

544

545   **Mass spectrometry**

546   Nanoscale reversed-phase UPLC separation of tryptic peptides was performed with a

547   nanoAcquity UPLC system equipped with a Symmetry C18 5 µm, 180 µm × 20 mm trap

548   column and a HSS T3 C18 1.8 µm, 75 µm × 250 mm analytical column (Waters Corporation)

549   maintained at 45°C. Injected peptides were trapped for 4 min at a flow rate of 8 µl/min 0.1%

550   TFA and then separated over 120 min at a flow rate of 300 nl/min with a gradient comprising

551   two linear steps of 3-35% mobile phase B in 105 min and 35-60% mobile phase B in 15 min,

552   respectively. Mobile phase A was water containing 0.1% formic acid while mobile phase B

553   was acetonitrile containing 0.1% formic acid. Mass spectrometric analysis of tryptic peptides

554   was performed using a Synapt G2-S quadrupole time-of-flight mass spectrometer equipped

555   with ion mobility option (Waters Corporation). Positive ions in the mass range m/z 50 to 2000

556   were acquired with a typical resolution of at least 20.000 FWHM (full width at half maximum)

557   and data were lock mass corrected post-acquisition. UDMS^E and DRE-UDMS^E analyses

558   were performed in the ion mobility-enhanced data-independent acquisition mode with drift

559   time-specific collision energies as described in detail by Distler et al. (48,49). Specifically, for

560   DRE-UDMS^E a deflection device (DRE lens) localized between the quadrupole and the ion

561   mobility cell of the mass spectrometer was cycled between full (100% for 0.4 sec) and

562   reduced (5% for 0.4 sec) ion transmission during one 0.8 sec full scan. Continuum LC-MS

563   data were processed for signal detection, peak picking, and isotope and charge state

564   deconvolution using Waters ProteinLynx Global Server (PLGS) version 3.0.2 (47). For

565   protein identification, a custom database was compiled by adding the sequence information

566   for yeast enolase 1 and porcine trypsin to the UniProtKB/Swiss-Prot mouse proteome and by

567   appending the reversed sequence of each entry to enable the determination of false

568   discovery rate (FDR). Precursor and fragment ion mass tolerances were automatically

569   determined by PLGS 3.0.2 and were typically below 5 ppm for precursor ions and below 10

570   ppm (root mean square) for fragment ions. Carbamidomethylation of cysteine was specified

571   as fixed and oxidation of methionine as variable modification. One missed trypsin cleavage

572 was allowed. Minimal ion matching requirements were two fragments per peptide, five

573 fragments per protein, and one peptide per protein. The FDR for protein identification was set

574 to 1% threshold.

575

576 **Analysis of proteomic data**

577 For each genotype ($Prx^{-/-}$ and wild type control mice sacrificed at P21), biochemical fractions

578 enriched for PNS myelin were analyzed as three biological replicates (n=3 per condition);

579 each biological replicate representing a pool of 20 sciatic nerves dissected from 10 mice. The

580 samples were processed with replicate digestion and injection, resulting in four technical

581 replicates per biological replicate and thus a total of 12 LC-MS runs per condition to be

582 compared, essentially as previously reported for CNS myelin (36,97). The freely available

583 software ISOQuant (www.isoquant.net) was used for post-identification analysis including

584 retention time alignment, exact mass and retention time (EMRT) and ion mobility clustering,

585 peak intensity normalization, isoform/homology filtering and calculation of absolute in-sample

586 amounts for each detected protein (48,49,98) according to the TOP3 quantification approach

587 (53,54). Only peptides with a minimum length of seven amino acids that were identified with

588 scores above or equal to 5.5 in at least two runs were considered. FDR for both peptides and

589 proteins was set to 1% threshold and only proteins reported by at least two peptides (one of

590 which unique) were quantified using the TOP3 method. The parts per million (ppm)

591 abundance values (i.e. the relative amount (w/w) of each protein in respect to the sum over

592 all detected proteins) were log2-transformed and normalized by subtraction of the median

593 derived from all data points for the given protein. Significant changes in protein abundance

594 were detected by moderated t-statistics essentially as described (96,97) across all technical

595 replicates using an empirical Bayes approach and false discovery (FDR)-based correction for

596 multiple comparisons (100). For this purpose, the Bioconductor R packages "limma" (101)

597 and "q-value" (102) were used in RStudio, an integrated development environment for the

598 open source programming language R. Proteins identified as contaminants (e.g. components

599 of blood or hair cells) were removed from the analysis. Proteins with ppm values below 100

600 which were not identified in one genotype were considered as just above detection level and

601 also removed from the analysis. The relative abundance of a protein in myelin was accepted

602 as altered if both statistically significant (q-value <0.05). Pie charts, heatmaps and volcano

603 plots were prepared in Microsoft Excel 2013 and GraphPad Prism 7. Pearson's correlation

604 coefficients derived from log2-transformed ppm abundance values were clustered and

605 visualized with the tool heatmap.2 contained in the R package gplots (CRAN.R-

606 project.org/package=gplots). Only pairwise complete observations were considered to

607 reduce the influence of missing values on clustering behavior. The mass spectrometry

608 proteomics data have been deposited to the ProteomeXchange Consortium

609　(proteomecentral.proteomexchange.org) via the PRIDE partner repository (103) with the
610　dataset identifier PXD015960.
611

612　**Gel electrophoresis and silver staining of gels**
613　Protein concentration was determined using the DC Protein Assay kit (BioRad). Samples
614　were separated on a 12% SDS-PAGE for 1 h at 200 V using the BioRad system, fixated
615　overnight in 10% [v/v] acetic acid and 40 % [v/v] ethanol and then washed in 30% ethanol (2x
616　20 min) and ddH$_2$O (1x 20 min). For sensitization, gels were incubated 1 min in 0.012% [v/v]
617　Na$_2$S$_2$O$_3$ and subsequently washed with ddH$_2$O (3x 20 sec). For silver staining, gels were
618　impregnated for 20 min in 0.2 % [w/v] AgNO$_3$ / 0.04% formaldehyde, washed with ddH$_2$O (3x
619　20 sec) and developed in 3% [w/v] Na$_2$CO$_3$ / 0.02% [w/v] formaldehyde. The reaction was
620　stopped by exchanging the solution with 5% [v/v] acetic acid.
621

622　**Immunoblotting**
623　Immunoblotting was performed as described (104,105). Primary antibodies were specific for
624　dystrophin-related-protein 2 (DRP2; Sigma; 1:1000), peripheral myelin protein 2 (PMP2;
625　ProteinTech Group 12717-1-AP; 1:1000), proteolipid protein (PLP/DM20; A431 (106);
626　1:5000), Monocarboxylate transporter 1 (MCT1/SLC16A1; (107); 1:1000), periaxin (PRX;
627　(59); 1:1000), sodium/potassium-transporting ATPase subunit alpha-1 (ATP1A1; 1:2000;
628　Abcam #13736-1-AP), myelin protein zero (MPZ/P0; (108); kind gift by J. Archelos-Garcia;
629　1:10.000), voltage-dependent anion-selective channel protein (VDAC; Abcam #ab15895;
630　1:1000), basigin (BSG/CD147; ProteinTech Group #ab64616; 1:1000), neurofilament H
631　(NEFH/NF-H; Covance #SMI-32P; 1:1000), voltage-gated potassium channel subunit A
632　member 1 (KCNA1; Neuromab #73-007; 1:1000), EGR2/KROX20 ((109); kind gift by D.
633　Meijer, Edinburgh; 1:1000) and myelin basic protein (MBP; 1:2000). To generate the latter
634　antisera, rabbits were immunized (Pineda Antikörper Service, Berlin, Germany) with the
635　KLH-coupled peptide CQDENPVVHFFK corresponding to amino acids 212-222 of mouse
636　MBP isoform 1 (Swisprot/Uniprot-identifier P04370-1). Anti-MBP antisera were purified by
637　affinity chromatography and extensively tested for specificity by immunoblot analysis of
638　homogenate of brains dissected from wild-type mice compared to *Mbp^shiverer/shiverer* mice that
639　lack expression of MBP. Appropriate secondary anti-mouse or -rabbit antibodies conjugated
640　to HRP were from dianova. Immunoblots were developed using the Enhanced
641　Chemiluminescence Detection kit (Western Lightning® Plus, Perkin Elmer) and detected with
642　the Intas ChemoCam system (INTAS Science Imaging Instruments GmbH, Göttingen,
643　Germany).
644

645　**Immunolabelling of teased fibers**

646   Teased fibers were prepared as previously described (9,110). For each genotype, one male
647   mouse was sacrificed by cervical dislocation at P17. Immunolabelling of teased fibers was
648   performed as described (69). Briefly, teased fibers were fixed for 5 min in 4%
649   paraformaldehyde, permeabilized 5 min with ice-cold methanol, washed in PBS (3x 5 min)
650   and blocked for 1 h at 21°C in blocking buffer (10% horse serum, 0.25% Triton X-100, 1%
651   bovine serum albumin in PBS). Primary antibodies were applied overnight at 4°C in
652   incubation buffer (1.5% horse serum, 0.25% Triton X-100 in PBS). Samples were washed in
653   PBS (3x 5 min) and secondary antibodies were applied in incubation buffer (1 h, RT).
654   Samples were again washed in PBS (2x 5 min), and 4',6-diamidino-2-phenylindole (DAPI;
655   1:50 000 in PBS) was applied for 10 min at RT. Samples were briefly washed 2x with ddH$_2$O
656   and mounted using Aqua-Poly/Mount (Polysciences, Eppelheim, Germany). Antibodies were
657   specific for myelin-associated glycoprotein (MAG clone 513; Chemicon MAB1567; 1:50) and
658   MCT1/SLC16A1 (107). Secondary antibodies were donkey α-rabbit-Alexa488 (Invitrogen
659   A21206; 1:1000) and donkey α-mouse-Alexa555 (Invitrogen A21202; 1:1000). Labeled
660   teased fibers were imaged using the confocal microscope Leica SP5. The signal was
661   collected with the objective HCX PL APO lambda blue 63.0.x1.20. DAPI staining was excited
662   with 405 nm and collected between 417 nm - 480 nm. To excite the Alexa488 fluorophore an
663   Argon laser with the excitation of 488 nm was used and the emission was set to 500 nm -
664   560 nm. Alexa555 was excited by using the DPSS561 laser at an excitation of 561 nm and
665   the emission was set to 573 nm - 630 nm. To export and process the images LAS AF lite and
666   Adobe Photoshop were used.
667

mRNA abundance profiles
669   Raw data were previously established (60) from the sciatic nerves of wild type Sprague
670   Dawley rats at the indicated ages (E21, P6, P18; n=4 per time point). Briefly, sciatic nerves
671   were dissected, the epineurium was removed, total RNA was extracted with the RNeasy Kit
672   (Qiagen), concentration and quality (ratio of absorption at 260/280 nm) of RNA samples were
673   determined using the NanoDrop spectrophotometer (ThermoScientific), integrity of the
674   extracted RNA was determined with the Agilent 2100 Bioanalyser (Agilent Technologies) and
675   RNA-Seq was performed using the Illumina HiSeq2000 platform. RNA-Seq raw data are
676   available under the GEO accession number GSE115930 (60). For the present analysis, the
677   fastqfiles were mapped to *rattus norvegicus* rn6 using Tophat Aligner and then quantified
678   based on the Ensemble Transcripts release v96. The raw read counts were then normalized
679   using the R package DESeq2. The normalized gene expression data was then standardized
680   to a mean of zero and a standard deviation of one, therefore genes with similar changes in
681   expression are close in the euclidian space. Clustering was performed on the standardized

682   data using the R package mfuzz. Transcripts displaying abundance differences of less than
683   10% coefficient of variation were considered developmentally unchanged.
684

Venn diagrams

686   Area-proportional Venn diagrams were prepared using BioVenn (111) at www.biovenn.nl/.
687

GO-term

689   For functional categorization of the myelin proteome the associated gene ontology terms
690   were systematically analyzed on the mRNA abundance cluster using the Database for
691   Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov). For
692   comparison known myelin proteins according to literature were added.
693

Histological analysis

695   *Prx⁻/⁻* and control mice were perfused at the indicated ages intravascularly with fixative
696   solution (2.5% glutaraldehyde, 4% paraformaldehyde, 0.1 M sodium cacodylate buffer, pH
697   7.4). Quadriceps nerves were removed, fixed for 2 h at room temperature, followed by 18 h
698   at 4°C in the same fixative, postfixed in OsO_4, dehydrated a graded series of ethanol,
699   followed by propylene oxide and embedded in Araldite. All axons not associated with a
700   Remak bundle were counted and categorized as myelinated or non-myelinated. All myelin
701   profiles lacking a recognizable axon were counted. The total number of axons were counted
702   on micrographs of toluidine blue stained Araldite sections (0.5 µm) of quadriceps nerves.
703   Precise p-values for the quantitative comparison between Ctrl and *Prx⁻/⁻* mice were: Total
704   number of axons (**Figure 6B**; Student's unpaired t-test): 2 mo p=0.01734; 4 mo p=2.1E-05; 9
705   mo p=0.007625; Number of myelinated axons (**Figure 6C**; Student's unpaired t-test): 2 mo
706   p=0.00444; 4 mo p=2.12E-05; 9 mo p=0.005766; Number of empty myelin profiles (**Figure**
707   **6D**; Student's unpaired t-test): 2 mo p=0.004445; 4 mo p=0.001461; 9 mo p=0.000695;
708   Axonal diameters (**Figure 6E-G**; two-sided Kolmogorow-Smirnow test): 2 mo p=2.20E-16; 4
709   mo p=2.20E-16; 9 mo p=2.20E-16.
710
711

724

725

726 **CONFLICT OF INTEREST STATEMENT**

727 The authors declare no conflict of interest.

728

729

730

REFERENCES

1. Weil M-T, Heibeck S, Töpperwien M, tom Dieck S, Ruhwedel T, Salditt T, et al. Axonal Ensheathment in the Nervous System of Lamprey: Implications for the Evolution of Myelinating Glia. J Neurosci [Internet]. 2018 Jul 18;38(29):6586–96. Available from: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.1034-18.2018

2. Hartline DK, Colman DR. Rapid Conduction and the Evolution of Giant Axons and Myelinated Fibers. Curr Biol [Internet]. 2007 Jan;17(1):R29–35. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960982206025231

3. Rossor AM, Polke JM, Houlden H, Reilly MM. Clinical implications of genetic advances in Charcot–Marie–Tooth disease. Nat Rev Neurol [Internet]. 2013 Oct 10;9(10):562–71. Available from: http://www.nature.com/articles/nrneurol.2013.179

4. Michailov G V. Axonal Neuregulin-1 Regulates Myelin Sheath Thickness. Science (80-) [Internet]. 2004 Apr 30;304(5671):700–3. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1095862

5. Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, et al. Neuregulin-1 Type III Determines the Ensheathment Fate of Axons. Neuron [Internet]. 2005 Sep;47(5):681–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627305006926

6. Chernousov MA, Yu W-M, Chen Z-L, Carey DJ, Strickland S. Regulation of Schwann cell function by the extracellular matrix. Glia [Internet]. 2008 Nov 1;56(14):1498–507. Available from: http://doi.wiley.com/10.1002/glia.20740

7. Petersen SC, Luo R, Liebscher I, Giera S, Jeong S-J, Mogha A, et al. The Adhesion GPCR GPR126 Has Distinct, Domain-Dependent Functions in Schwann Cell Development Mediated by Interaction with Laminin-211. Neuron [Internet]. 2015 Feb;85(4):755–69. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627314011660

8. Ghidinelli M, Poitelon Y, Shin YK, Ameroso D, Williamson C, Ferri C, et al. Laminin 211 inhibits protein kinase A in Schwann cells to modulate neuregulin 1 type III-driven myelination. Emery B, editor. PLOS Biol [Internet]. 2017 Jun 21;15(6):e2001408. Available from: https://dx.plos.org/10.1371/journal.pbio.2001408

9. Sherman DL, Fabrizi C, Gillespie CS, Brophy PJ. Specific Disruption of a Schwann Cell Dystrophin-Related Protein Complex in a Demyelinating Neuropathy. Neuron [Internet]. 2001 May;30(3):677–87. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627301003270

10. Masaki T, Matsumura K, Hirata A, Yamada H, Hase A, Arai K, et al. Expression of Dystroglycan and the Laminin-α2 Chain in the Rat Peripheral Nerve during Development. Exp Neurol [Internet]. 2002 Mar;174(1):109–17. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0014488601978562

11. Nodari A, Previtali SC, Dati G, Occhi S, Court FA, Colombelli C, et al. 6 4 Integrin and Dystroglycan Cooperate to Stabilize the Myelin Sheath. J Neurosci [Internet]. 2008 Jun 25;28(26):6714–9. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.0326-08.2008

772   12.   Raasakka A, Linxweiler H, Brophy PJ, Sherman DL, Kursula P. Direct Binding of the Flexible
773   C-Terminal Segment of Periaxin to β4 Integrin Suggests a Molecular Basis for CMT4F. Front
774   Mol Neurosci [Internet]. 2019 Apr 9;12. Available from:
775   https://www.frontiersin.org/article/10.3389/fnmol.2019.00084/full

776   13.   Hess A, Lansing AI. The fine structure of peripheral nerve fibers. Anat Rec. 1953;

777   14.   Sherman DL, Brophy PJ. Mechanisms of axon ensheathment and myelin growth. Nat Rev
778   Neurosci [Internet]. 2005 Sep;6(9):683–90. Available from:
779   http://www.nature.com/articles/nrn1743

780   15.   Pereira JA, Lebrun-Julien F, Suter U. Molecular mechanisms regulating myelination in the
781   peripheral nervous system. Trends Neurosci [Internet]. 2012 Feb;35(2):123–34. Available from:
782   https://linkinghub.elsevier.com/retrieve/pii/S0166223611001937

783   16.   Grove M, Brophy PJ. FAK Is Required for Schwann Cell Spreading on Immature Basal Lamina
784   to Coordinate the Radial Sorting of Peripheral Axons with Myelination. J Neurosci [Internet].
785   2014 Oct 1;34(40):13422–34. Available from:
786   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1764-14.2014

787   17.   Monk KR, Feltri ML, Taveggia C. New insights on schwann cell development. Glia [Internet].
788   2015 Aug;63(8):1376–93. Available from: http://doi.wiley.com/10.1002/glia.22852

789   18.   Feltri ML, Poitelon Y, Previtali SC. How Schwann Cells Sort Axons. Neurosci [Internet]. 2016
790   Jun 16;22(3):252–65. Available from:
791   http://journals.sagepub.com/doi/10.1177/1073858415572361

792   19.   Giese KP, Martini R, Lemke G, Soriano P, Schachner M. Mouse P0 gene disruption leads to
793   hypomyelination, abnormal expression of recognition molecules, and degeneration of myelin
794   and axons. Cell [Internet]. 1992 Nov;71(4):565–76. Available from:
795   https://linkinghub.elsevier.com/retrieve/pii/009286749290591Y

796   20.   Martini R, Mohajeri MH, Kasper S, Giese KP, Schachner M. Mice doubly deficient in the genes
797   for P0 and myelin basic protein show that both proteins contribute to the formation of the major
798   dense line in peripheral nerve myelin. J Neurosci. 1995;

799   21.   Nawaz S, Schweitzer J, Jahn O, Werner HB. Molecular evolution of myelin basic protein, an
800   abundant structural myelin component. Glia [Internet]. 2013 Aug;61(8):1364–77. Available
801   from: http://doi.wiley.com/10.1002/glia.22520

802   22.   Greenfield S, Brostoff S, Eylar EH, Morell P. PROTEIN COMPOSITION OF MYELIN OF THE
803   PERIPHERAL NERVOUS SYSTEM. J Neurochem [Internet]. 1973 Apr;20(4):1207–16.
804   Available from: http://doi.wiley.com/10.1111/j.1471-4159.1973.tb00089.x

805   23.   Brostoff SW, Karkhanis YD, Carlo DJ, Reuter W, Eylar EH. Isolation and partial
806   characterization of the major proteins of rabbit sciatic nerve myelin. Brain Res [Internet]. 1975
807   Mar;86(3):449–58. Available from:
808   https://linkinghub.elsevier.com/retrieve/pii/0006899375908951

809   24.   Nave K-A, Werner HB. Myelination of the Nervous System: Mechanisms and Functions. Annu
810   Rev Cell Dev Biol [Internet]. 2014 Oct 11;30(1):503–33. Available from:
811   http://www.annualreviews.org/doi/10.1146/annurev-cellbio-100913-013101

812   25.   Kleopa KA, Sargiannidou I. Connexins, gap junctions and peripheral neuropathy. Neurosci Lett

813   [Internet]. 2015 Jun;596:27–32. Available from:

814   https://linkinghub.elsevier.com/retrieve/pii/S0304394014008453

815   26.   Court FA, Sherman DL, Pratt T, Garry EM, Ribchester RR, Cottrell DF, et al. Restricted growth

816   of Schwann cells lacking Cajal bands slows conduction in myelinated nerves. Nature [Internet].

817   2004 Sep;431(7005):191–5. Available from: http://www.nature.com/articles/nature02841

818   27.   Beirowski B, Babetto E, Golden JP, Chen Y-J, Yang K, Gross RW, et al. Metabolic regulator

819   LKB1 is crucial for Schwann cell–mediated axon maintenance. Nat Neurosci [Internet]. 2014

820   Oct 7;17(10):1351–61. Available from: http://www.nature.com/articles/nn.3809

821   28.   Domenech-Estevez E, Baloui H, Repond C, Rosafio K, Medard J-J, Tricaud N, et al.

822   Distribution of Monocarboxylate Transporters in the Peripheral Nervous System Suggests

823   Putative Roles in Lactate Shuttling and Myelination. J Neurosci [Internet]. 2015 Mar

824   11;35(10):4151–6. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3534-

825   14.2015

826   29.   Kim S, Maynard JC, Sasaki Y, Strickland A, Sherman DL, Brophy PJ, et al. Schwann Cell O-

827   GlcNAc Glycosylation Is Required for Myelin Maintenance and Axon Integrity. J Neurosci

828   [Internet]. 2016 Sep 14;36(37):9633–46. Available from:

829   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1235-16.2016

830   30.   Gonçalves NP, Vægter CB, Andersen H, Østergaard L, Calcutt NA, Jensen TS. Schwann cell

831   interactions with axons and microvessels in diabetic neuropathy. Nat Rev Neurol [Internet].

832   2017 Mar 30;13(3):135–47. Available from: http://www.nature.com/articles/nrneurol.2016.201

833   31.   Stassart RM, Möbius W, Nave K-A, Edgar JM. The Axon-Myelin Unit in Development and

834   Degenerative Disease. Front Neurosci [Internet]. 2018 Jul 11;12. Available from:

835   https://www.frontiersin.org/article/10.3389/fnins.2018.00467/full

836   32.   Nagarajan R, Le N, Mahoney H, Araki T, Milbrandt J. Deciphering peripheral nerve myelination

837   by using Schwann cell expression profiling. Proc Natl Acad Sci [Internet]. 2002 Jun

838   25;99(13):8998–9003. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.132080999

839   33.   Le N, Nagarajan R, Wang JYT, Araki T, Schmidt RE, Milbrandt J. Analysis of congenital

840   hypomyelinating Egr2Lo/Lo nerves identifies Sox2 as an inhibitor of Schwann cell

841   differentiation and myelination. Proc Natl Acad Sci [Internet]. 2005 Feb 15;102(7):2596–601.

842   Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0407836102

843   34.   Ryu EJ, Yang M, Gustin JA, Chang L-W, Freimuth RR, Nagarajan R, et al. Analysis of

844   Peripheral Nerve Expression Profiles Identifies a Novel Myelin Glycoprotein, MP11. J Neurosci

845   [Internet]. 2008 Jul 23;28(30):7563–73. Available from:

846   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1659-08.2008

847   35.   Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, et al. An RNA-Sequencing

848   Transcriptome and Splicing Database of Glia, Neurons, and Vascular Cells of the Cerebral

849   Cortex. J Neurosci [Internet]. 2014 Sep 3;34(36):11929–47. Available from:

850   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1860-14.2014

851   36.   Patzig J, Erwig MS, Tenzer S, Kusch K, Dibaj P, Möbius W, et al. Septin/anillin filaments

852   scaffold central nervous system myelin to accelerate nerve conduction. Elife. 2016;5:e17119.

853   37.   Sharma K, Schmitt S, Bergner CG, Tyanova S, Kannaiyan N, Manrique-Hoyos N, et al. Cell

854   type– and brain region–resolved mouse brain proteome. Nat Neurosci [Internet]. 2015 Dec

855   2;18(12):1819–31. Available from: http://www.nature.com/articles/nn.4160

856   38.   Thakurela S, Garding A, Jung RB, Müller C, Goebbels S, White R, et al. The transcriptome of

857   mouse central nervous system myelin. Sci Rep [Internet]. 2016 May 13;6(1):25828. Available

858   from: http://www.nature.com/articles/srep25828

859   39.   Marques S, Zeisel A, Codeluppi S, van Bruggen D, Mendanha Falcao A, Xiao L, et al.

860   Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system.

861   Science (80-) [Internet]. 2016 Jun 10;352(6291):1326–9. Available from:

862   http://www.sciencemag.org/cgi/doi/10.1126/science.aaf6463

863   40.   De Monasterio-Schrader P, Jahn O, Tenzer S, Wichert SP, Patzig J, Werner HB. Systematic

864   approaches to central nervous system myelin. Cellular and Molecular Life Sciences. 2012.

865   41.   Patzig J, Jahn O, Tenzer S, Wichert SP, de Monasterio-Schrader P, Rosfa S, et al.

866   Quantitative and Integrative Proteome Analysis of Peripheral Nerve Myelin Identifies Novel

867   Myelin Proteins and Candidate Neuropathy Loci. J Neurosci [Internet]. 2011 Nov

868   9;31(45):16369–86. Available from:

869   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4016-11.2011

870   42.   Kangas SM, Ohlmeier S, Sormunen R, Jouhilahti E-M, Peltonen S, Peltonen J, et al. An

871   approach to comprehensive genome and proteome expression analyses in Schwann cells and

872   neurons during peripheral nerve myelin formation. J Neurochem [Internet]. 2016

873   Sep;138(6):830–44. Available from: http://doi.wiley.com/10.1111/jnc.13722

874   43.   Kusch K, Uecker M, Liepold T, Möbius W, Hoffmann C, Neumann H, et al. Partial

875   Immunoblotting of 2D-Gels: A Novel Method to Identify Post-Translationally Modified Proteins

876   Exemplified for the Myelin Acetylome. Proteomes [Internet]. 2017 Jan 12;5(4):3. Available

877   from: http://www.mdpi.com/2227-7382/5/1/3

878   44.   Neilson KA, Ali NA, Muralidharan S, Mirzaei M, Mariani M, Assadourian G, et al. Less label,

879   more free: Approaches in label-free quantitative mass spectrometry. Proteomics. 2011.

880   45.   Distler U, Kuharev J, Tenzer S. Biomedical applications of ion mobility-enhanced data-

881   independent acquisition-based label-free quantitative proteomics. Expert Rev Proteomics.

882   2014;

883   46.   Geromanos SJ, Vissers JPC, Silva JC, Dorschel CA, Li GZ, Gorenstein M V., et al. The

884   detection, correlation, and comparison of peptide precursor and product ions from data

885   independent LC-MS with data dependant LC-MS/MS. Proteomics. 2009;

886   47.   Li GZ, Vissers JPC, Silva JC, Golick D, Gorenstein M V., Geromanos SJ. Database searching

887   and accounting of multiplexed precursor and product ion spectra from the data independent

888   analysis of simple and complex peptide mixtures. Proteomics. 2009;

889   48.   Distler U, Kuharev J, Navarro P, Tenzer S. Label-free quantification in ion mobility–enhanced

890   data-independent acquisition proteomics. Nat Protoc [Internet]. 2016 Apr 24;11(4):795–812.

891   Available from: http://www.nature.com/articles/nprot.2016.042

892   49.   Distler U, Kuharev J, Navarro P, Levin Y, Schild H, Tenzer S. Drift time-specific collision

893   energies enable deep-coverage data-independent acquisition proteomics. Nat Methods

894   [Internet]. 2014 Feb 15;11(2):167–70. Available from:

895   http://www.nature.com/articles/nmeth.2767

896   50.   Larocca JN, Norton WT. Isolation of Myelin. Curr Protoc Cell Biol [Internet]. 2006
897   Dec;33(1):3.25.1-3.25.19. Available from: http://doi.wiley.com/10.1002/0471143030.cb0325s33

898   51.   Jahn O, Tenzer S, Werner HB. Myelin proteomics: Molecular anatomy of an insulating sheath.
899   Molecular Neurobiology. 2009.

900   52.   Erwig MS, Hesse D, Jung RB, Uecker M, Kusch K, Tenzer S, et al. Myelin: Methods for
901   Purification and Proteome Analysis. In: Methods in Molecular Biology [Internet]. 2019. p. 37–
902   63. Available from: http://link.springer.com/10.1007/978-1-4939-9072-6_3

903   53.   Silva JC, Gorenstein M V., Li G-Z, Vissers JPC, Geromanos SJ. Absolute Quantification of
904   Proteins by LCMS E. Mol Cell Proteomics [Internet]. 2006 Jan;5(1):144–56. Available from:
905   http://www.mcponline.org/lookup/doi/10.1074/mcp.M500230-MCP200

906   54.   Ahrné E, Molzahn L, Glatter T, Schmidt A. Critical assessment of proteome-wide label-free
907   absolute abundance estimation strategies. Proteomics. 2013;

908   55.   Dodds JN, Baker ES. Ion Mobility Spectrometry: Fundamental Concepts, Instrumentation,
909   Applications, and the Road Ahead. J Am Soc Mass Spectrom. 2019;

910   56.   Micko S, Schlaepfer WW. PROTEIN COMPOSITION OF AXONS and MYELIN FROM RAT
911   and HUMAN PERIPHERAL NERVES. J Neurochem [Internet]. 1978 May;30(5):1041–9.
912   Available from: http://doi.wiley.com/10.1111/j.1471-4159.1978.tb12397.x

913   57.   Smith ME, Curtis BM. FROG SCIATIC NERVE MYELIN: A CHEMICAL CHARACTERIZATION.
914   J Neurochem [Internet]. 1979 Aug;33(2):447–52. Available from:
915   http://doi.wiley.com/10.1111/j.1471-4159.1979.tb05174.x

916   58.   Whitaker JN. The protein antigens of peripheral nerve myelin. Ann Neurol [Internet].
917   1981;9(S1):56–64. Available from: http://doi.wiley.com/10.1002/ana.410090710

918   59.   Gillespie CS, Sherman DL, Blair GE, Brophy PJ. Periaxin, a novel protein of myelinating
919   schwann cells with a possible role in axonal ensheathment. Neuron [Internet]. 1994
920   Mar;12(3):497–508. Available from:
921   https://linkinghub.elsevier.com/retrieve/pii/0896627394902089

922   60.   Fledrich R, Abdelaal T, Rasch L, Bansal V, Schütza V, Brügger B, et al. Targeting myelin lipid
923   metabolism as a potential therapeutic strategy in a model of CMT1A neuropathy. Nat Commun
924   [Internet]. 2018 Dec 2;9(1):3025. Available from: http://www.nature.com/articles/s41467-018-
925   05420-0

926   61.   Pareyson D, Marchesi C. Diagnosis, natural history, and management of Charcot–Marie–Tooth
927   disease. Lancet Neurol [Internet]. 2009 Jul;8(7):654–67. Available from:
928   https://linkinghub.elsevier.com/retrieve/pii/S1474442209701103

929   62.   Baets J, De Jonghe P, Timmerman V. Recent advances in Charcot–Marie–Tooth disease. Curr
930   Opin Neurol [Internet]. 2014 Oct;27(5):532–40. Available from:
931   http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00019052-
932   201410000-00006

933   63.   Brennan KM, Bai Y, Shy ME. Demyelinating CMT–what's known, what's new and what's in
934   store? Neurosci Lett [Internet]. 2015 Jun;596:14–26. Available from:
935   https://linkinghub.elsevier.com/retrieve/pii/S0304394015000725

936   64.   Gillespie CS, Sherman DL, Fleetwood-Walker SM, Cottrell DF, Tait S, Garry EM, et al.
937   Peripheral Demyelination and Neuropathic Pain Behavior in Periaxin-Deficient Mice. Neuron
938   [Internet]. 2000 May;26(2):523–31. Available from:
939   https://linkinghub.elsevier.com/retrieve/pii/S0896627300811848
940   65.   Guilbot A. A mutation in periaxin is responsible for CMT4F, an autosomal recessive form of
941   Charcot-Marie-Tooth disease. Hum Mol Genet [Internet]. 2001 Feb 1;10(4):415–21. Available
942   from: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/10.4.415
943   66.   Berger P, Niemann A, Suter U. Schwann cells and the pathogenesis of inherited motor and
944   sensory neuropathies (Charcot-Marie-Tooth disease). Glia [Internet]. 2006 Sep;54(4):243–57.
945   Available from: http://doi.wiley.com/10.1002/glia.20386
946   67.   Marchesi C, Milani M, Morbin M, Cesani M, Lauria G, Scaioli V, et al. Four novel cases of
947   periaxin-related neuropathy and review of the literature. Neurology [Internet]. 2010 Nov
948   16;75(20):1830–8. Available from:
949   http://www.neurology.org/cgi/doi/10.1212/WNL.0b013e3181fd6314
950   68.   Philp NJ, Ochrietor JD, Rudoy C, Muramatsu T, Linser PJ. Loss of MCT1, MCT3, and MCT4
951   Expression in the Retinal Pigment Epithelium and Neural Retina of the 5A11/Basigin-Null
952   Mouse. Investig Opthalmology Vis Sci [Internet]. 2003 Mar 1;44(3):1305. Available from:
953   http://iovs.arvojournals.org/article.aspx?doi=10.1167/iovs.02-0552
954   69.   Patzig J, Kusch K, Fledrich R, Eichel MA, Lüders KA, Möbius W, et al. Proteolipid protein
955   modulates preservation of peripheral axons and premature death when myelin protein zero is
956   lacking. Glia [Internet]. 2016 Jan;64(1):155–74. Available from:
957   http://doi.wiley.com/10.1002/glia.22922
958   70.   Lee Y, Morrison BM, Li Y, Lengacher S, Farah MH, Hoffman PN, et al. Oligodendroglia
959   metabolically support axons and contribute to neurodegeneration. Nature [Internet]. 2012 Jul
960   11;487(7408):443–8. Available from: http://www.nature.com/articles/nature11314
961   71.   Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, et al. Glycolytic
962   oligodendrocytes maintain myelin and long-term axonal integrity. Nature [Internet]. 2012 May
963   29;485(7399):517–21. Available from: http://www.nature.com/articles/nature11007
964   72.   Morrison BM, Tsingalia A, Vidensky S, Lee Y, Jin L, Farah MH, et al. Deficiency in
965   monocarboxylate transporter 1 (MCT1) in mice delays regeneration of peripheral nerves
966   following sciatic nerve crush. Exp Neurol [Internet]. 2015 Jan;263:325–38. Available from:
967   https://linkinghub.elsevier.com/retrieve/pii/S0014488614003549
968   73.   Morrison BM, Lee Y, Rothstein JD. Oligodendroglia: Metabolic supporters of axons. Trends in
969   Cell Biology. 2013.
970   74.   Saab AS, Tzvetanova ID, Nave K-A. The role of myelin and oligodendrocytes in axonal energy
971   metabolism. Curr Opin Neurobiol [Internet]. 2013 Dec;23(6):1065–72. Available from:
972   https://linkinghub.elsevier.com/retrieve/pii/S0959438813001888
973   75.   Rinholm JE, Bergersen LH. White matter lactate – Does it matter? Neuroscience [Internet].
974   2014 Sep;276:109–16. Available from:
975   https://linkinghub.elsevier.com/retrieve/pii/S0306452213008464
976   76.   Jha MK, Lee Y, Russell KA, Yang F, Dastgheyb RM, Deme P, et al. Monocarboxylate

977   transporter 1 in Schwann cells contributes to maintenance of sensory nerve myelination during
978   aging. Glia. 2019;

979   77.   Edgar JM, McLaughlin M, Werner HB, McCulloch MC, Barrie JA, Brown A, et al. Early
980   ultrastructural defects of axons and axon-glia junctions in mice lacking expression of Cnp1.
981   Glia. 2009;

982   78.   Verheijen MHG. Local regulation of fat metabolism in peripheral nerves. Genes Dev [Internet].
983   2003 Oct 1;17(19):2450–64. Available from:
984   http://www.genesdev.org/cgi/doi/10.1101/gad.1116203

985   79.   Buchstaller J. Efficient Isolation and Gene Expression Profiling of Small Numbers of Neural
986   Crest Stem Cells and Developing Schwann Cells. J Neurosci [Internet]. 2004 Mar
987   10;24(10):2357–65. Available from:
988   http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4083-03.2004

989   80.   D'antonio M, Michalovich D, Paterson M, Droggiti A, Woodhoo A, Mirsky R, et al. Gene
990   profiling and bioinformatic analysis of Schwann cell embryonic development and myelination.
991   Glia [Internet]. 2006 Apr 1;53(5):501–15. Available from:
992   http://doi.wiley.com/10.1002/glia.20309

993   81.   Schaeren-Wiemers N, Bonnet A, Erb M, Erne B, Bartsch U, Kern F, et al. The raft-associated
994   protein MAL is required for maintenance of proper axon–glia interactions in the central nervous
995   system. J Cell Biol [Internet]. 2004 Aug 30;166(5):731–42. Available from:
996   http://www.jcb.org/lookup/doi/10.1083/jcb.200406092

997   82.   Adlkofer K, Martini R, Aguzzi A, Zielasek J, Toyka K V., Suter U. Hypermyelination and
998   demyelinating peripheral neuropathy in Pmp22-deficient mice. Nat Genet [Internet]. 1995
999   Nov;11(3):274–80. Available from: http://www.nature.com/articles/ng1195-274

1000   83.   Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier
1001   C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature
1002   [Internet]. 1997 Oct;389(6652):725–30. Available from: http://www.nature.com/articles/39593

1003   84.   Woldeyesus MT, Britsch S, Riethmacher D, Xu L, Sonnenberg-Riethmacher E, Abou-Rebyeh
1004   F, et al. Peripheral nervous system defects in erbB2 mutants following genetic rescue of heart
1005   development. Genes Dev [Internet]. 1999 Oct 1;13(19):2538–48. Available from:
1006   http://www.genesdev.org/cgi/doi/10.1101/gad.13.19.2538

1007   85.   Ackerman SD, Luo R, Poitelon Y, Mogha A, Harty BL, D'Rozario M, et al. GPR56/ADGRG1
1008   regulates development and maintenance of peripheral myelin. J Exp Med [Internet]. 2018 Mar
1009   5;215(3):941–61. Available from: http://www.jem.org/lookup/doi/10.1084/jem.20161714

1010   86.   Monk KR, Oshima K, Jors S, Heller S, Talbot WS. Gpr126 is essential for peripheral nerve
1011   development and myelination in mammals. Development [Internet]. 2011 Jul 1;138(13):2673–
1012   80. Available from: http://dev.biologists.org/cgi/doi/10.1242/dev.062224

1013   87.   Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, et al. A G Protein-
1014   Coupled Receptor Is Essential for Schwann Cells to Initiate Myelination. Science (80-)
1015   [Internet]. 2009 Sep 11;325(5946):1402–5. Available from:
1016   http://www.sciencemag.org/cgi/doi/10.1126/science.1173474

1017   88.   Kabzinska D, Drac H, Sherman DL, Kostera-Pruszczyk A, Brophy PJ, Kochanski A, et al.

1018   Charcot-Marie-Tooth type 4F disease caused by S399fsx410 mutation in the PRX gene.

1019   Neurology [Internet]. 2006 Mar 14;66(5):745–7. Available from:

1020   http://www.neurology.org/cgi/doi/10.1212/01.wnl.0000201269.46071.35

1021   89.   Baránková L, Šišková D, Hühne K, Vyhnálková E, Sakmaryová I, Bojar M, et al. A 71-

1022   nucleotide deletion in the periaxin gene in a Romani patient with early-onset slowly progressive

1023   demyelinating CMT. Eur J Neurol [Internet]. 2008 Jun;15(6):548–51. Available from:

1024   http://doi.wiley.com/10.1111/j.1468-1331.2008.02104.x

1025   90.   Tokunaga S, Hashiguchi A, Yoshimura A, Maeda K, Suzuki T, Haruki H, et al. Late-onset

1026   Charcot–Marie–Tooth disease 4F caused by periaxin gene mutation. Neurogenetics [Internet].

1027   2012 Nov 1;13(4):359–65. Available from: http://link.springer.com/10.1007/s10048-012-0338-5

1028   91.   Gallardo E, García A, Ramón C, Maraví E, Infante J, Gastón I, et al. Charcot–Marie–Tooth

1029   disease type 2J with MPZ Thr124Met mutation: clinico-electrophysiological and MRI study of a

1030   family. J Neurol [Internet]. 2009 Dec 22;256(12):2061–71. Available from:

1031   http://link.springer.com/10.1007/s00415-009-5251-y

1032   92.   Leal A, Berghoff C, Berghoff M, Rojas-Araya M, Ortiz C, Heuss D, et al. A Costa Rican family

1033   affected with Charcot-Marie-Tooth disease due to the myelin protein zero (MPZ) p.Thr124Met

1034   mutation shares the Belgian haplotype. Rev Biol Trop [Internet]. 2014 Dec 1;62(4):1285.

1035   Available from: http://revistas.ucr.ac.cr/index.php/rbt/article/view/13473

1036   93.   Tokuda N, Noto Y, Kitani-Morii F, Hamano A, Kasai T, Shiga K, et al. Parasympathetic

1037   Dominant Autonomic Dysfunction in Charcot-Marie-Tooth Disease Type 2J with the *MPZ*

1038   Thr124Met Mutation. Intern Med [Internet]. 2015;54(15):1919–22. Available from:

1039   https://www.jstage.jst.go.jp/article/internalmedicine/54/15/54_54.4259/_article

1040   94.   Duan X, Gu W, Hao Y, Wang R, Wen H, Sun S, et al. A Novel Asp121Asn Mutation of Myelin

1041   Protein Zero Is Associated with Late-Onset Axonal Charcot-Marie-Tooth Disease, Hearing

1042   Loss and Pupil Abnormalities. Front Aging Neurosci [Internet]. 2016 Sep 22;8. Available from:

1043   http://journal.frontiersin.org/Article/10.3389/fnagi.2016.00222/abstract

1044   95.   Fabrizi GM, Tamburin S, Cavallaro T, Cabrini I, Ferrarini M, Taioli F, et al. The spectrum of

1045   Charcot-Marie-Tooth disease due to myelin protein zero: An electrodiagnostic, nerve

1046   ultrasound and histological study. Clin Neurophysiol [Internet]. 2018 Jan;129(1):21–32.

1047   Available from: https://linkinghub.elsevier.com/retrieve/pii/S1388245717310854

1048   96.   Ambrozkiewicz MC, Schwark M, Kishimoto-Suga M, Borisova E, Hori K, Salazar-Lázaro A, et

1049   al. Polarity Acquisition in Cortical Neurons Is Driven by Synergistic Action of Sox9-Regulated

1050   Wwp1 and Wwp2 E3 Ubiquitin Ligases and Intronic miR-140. Neuron [Internet]. 2018

1051   Dec;100(5):1097-1115.e15. Available from:

1052   https://linkinghub.elsevier.com/retrieve/pii/S0896627318308961

1053   97.   Erwig MS, Patzig J, Steyer AM, Dibaj P, Heilmann M, Heilmann I, et al. Anillin facilitates septin

1054   assembly to prevent pathological outfoldings of central nervous system myelin. Elife [Internet].

1055   2019 Jan 23;8. Available from: https://elifesciences.org/articles/43888

1056   98.   Kuharev J, Navarro P, Distler U, Jahn O, Tenzer S. In-depth evaluation of software tools for

1057   data-independent acquisition based label-free quantification. Proteomics [Internet]. 2015

1058   Sep;15(18):3140–51. Available from: http://doi.wiley.com/10.1002/pmic.201400396

1059   99.   Silva JC, Denny R, Dorschel C, Gorenstein M V., Li G-Z, Richardson K, et al. Simultaneous
1060   Qualitative and Quantitative Analysis of the Escherichia coli Proteome. Mol Cell Proteomics
1061   [Internet]. 2006 Apr;5(4):589–607. Available from:
1062   http://www.mcponline.org/lookup/doi/10.1074/mcp.M500321-MCP200

1063   100. Kammers K, Cole RN, Tiengwe C, Ruczinski I. Detecting significant changes in protein
1064   abundance. EuPA Open Proteomics [Internet]. 2015 Jun;7:11–9. Available from:
1065   https://linkinghub.elsevier.com/retrieve/pii/S2212968515000069

1066   101. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential
1067   expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res [Internet].
1068   2015 Apr 20;43(7):e47–e47. Available from:
1069   http://academic.oup.com/nar/article/43/7/e47/2414268/limma-powers-differential-expression-
1070   analyses-for

1071   102. Storey JD. The positive false discovery rate: a Bayesian interpretation and the q -value. Ann
1072   Stat [Internet]. 2003 Dec;31(6):2013–35. Available from:
1073   http://projecteuclid.org/euclid.aos/1074290335

1074   103. Vizcaíno JA, Csordas A, Del-Toro N, Dianes JA, Griss J, Lavidas I, et al. 2016 update of the
1075   PRIDE database and its related tools. Nucleic Acids Res [Internet]. 2016 Jan 4;44(D1):D447–
1076   56. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkv1145

1077   104. Schardt A, Brinkmann BG, Mitkovski M, Sereda MW, Werner HB, Nave K-A. The SNARE
1078   protein SNAP-29 interacts with the GTPase Rab3A: Implications for membrane trafficking in
1079   myelinating glia. J Neurosci Res [Internet]. 2009 Nov 15;87(15):3465–79. Available from:
1080   http://doi.wiley.com/10.1002/jnr.22005

1081   105. de Monasterio-Schrader P, Patzig J, Möbius W, Barrette B, Wagner TL, Kusch K, et al.
1082   Uncoupling of neuroinflammation from axonal degeneration in mice lacking the myelin protein
1083   tetraspanin-2. Glia. 2013;

1084   106. Jung M, Sommer I, Schachner M, Nave KA. Monoclonal antibody O10 defines a
1085   conformationally sensitive cell- surface epitope of proteolipid protein (PLP): Evidence that PLP
1086   misfolding underlies dysmyelination in mutant mice. J Neurosci. 1996;16(24):7920–9.

1087   107. Stumpf SK, Berghoff SA, Trevisiol A, Spieth L, Düking T, Schneider L V., et al. Ketogenic diet
1088   ameliorates axonal defects and promotes myelination in Pelizaeus–Merzbacher disease. Acta
1089   Neuropathol [Internet]. 2019 Jul 27;138(1):147–61. Available from:
1090   http://link.springer.com/10.1007/s00401-019-01985-2

1091   108. Archelos JJ, Roggenbuck K, Scheider-Schaulies J, Linington C, Toyka K V., Hartung H-P.
1092   Production and characterization of monoclonal antibodies to the extracellular domain of P0. J
1093   Neurosci Res [Internet]. 1993 May 1;35(1):46–53. Available from:
1094   http://doi.wiley.com/10.1002/jnr.490350107

1095   109. Darbas A, Jaegle M, Walbeehm E, van den Burg H, Driegen S, Broos L, et al. Cell autonomy of
1096   the mouse claw paw mutation. Dev Biol [Internet]. 2004 Aug;272(2):470–82. Available from:
1097   https://linkinghub.elsevier.com/retrieve/pii/S0012160604003616

1098   110. Catenaccio A, Court FA. Teased Fiber Preparation of Myelinated Nerve Fibers from Peripheral
1099   Nerves for Vital Dye Staining and Immunofluorescence Analysis. In: Methods in Molecular

1100   Biology [Internet]. 2018. p. 329–37. Available from: http://link.springer.com/10.1007/978-1-
1101   4939-7649-2_21
1102 111. Hulsen T, de Vlieg J, Alkema W. BioVenn – a web application for the comparison and
1103   visualization of biological lists using area-proportional Venn diagrams. BMC Genomics
1104   [Internet]. 2008;9(1):488. Available from:
1105   http://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-488
1106
1107

FIGURE LEGENDS

1109

1110

1111 **Figure 1. Proteome analysis of peripheral myelin**

1112 **(A)** Schematic illustration of a previous approach to the peripheral myelin proteome (41)

1113 compared with the present workflow. Note that the current workflow allows largely automated

1114 sample processing and omits labor-intense 2-dimensional differential gel-electrophoresis,

1115 thereby considerably reducing hands-on time. Nano LC-MS analysis by data-independent

1116 acquisition (DIA) using three different data acquisition modes provides efficient identification

1117 and quantification of abundant myelin proteins (MS^E; see **Figure 2**), a comprehensive

1118 inventory (UDMS^E; see **Figures 3-4**) and gel-free differential analysis of hundreds of distinct

1119 proteins (DRE-UDMS^E; see **Figure 5**). Samples were analyzed in three biological replicates.

1120 **(B)** Immunoblot of myelin biochemically enriched from sciatic nerves of wild-type mice at

1121 postnatal day 21 (P21). Equal amounts of corresponding nerve lysate were loaded to

1122 compare the abundance of marker proteins for compact myelin (MPZ/P0, MBP, PMP2), non-

1123 compact myelin (PRX), the Schwann cell nucleus (KROX20/EGR2), axons (NEFH, KCNA1)

1124 and mitochondria (VDAC). Blots show n=2 biological replicates representative of n=3

1125 biological replicates. Note that myelin markers are enriched in purified myelin; other cellular

1126 markers are reduced.

1127 **(C)** Number and relative abundance of proteins identified in myelin purified from the sciatic

1128 nerves of wild-type mice using three different data acquisition modes (MS^E, UDMS^E, DRE-

1129 UDMS^E). Note that MS^E (orange) provides the best information about the relative abundance

1130 of high-abundant myelin proteins (dynamic range of more than four orders of magnitude) but

1131 identifies comparatively fewer proteins in purified myelin. UDMS^E (blue) identifies the largest

1132 number of proteins but provides only a lower dynamic range of about three orders of

1133 magnitude. DRE-UDMS^E (green) identifies an intermediate number of proteins with an

1134 intermediate dynamic range of about four orders of magnitude. Note that MS^E with very high

1135 dynamic range is required for the quantification of the exceptionally abundant myelin protein

1136 zero (MPZ/P0), myelin basic protein (MBP) and periaxin (PRX). ppm, parts per million.

1137 **(D)** Venn diagram comparing the number of proteins identified in PNS myelin by MS^E,

1138 UDMS^E and DRE-UDMS^E. Note the high overlap of identified proteins.

1139 **(E)** Venn diagram of the proteins identified in PNS myelin by UDMS^E in this study compared

1140 with those identified in two previous approaches (41,42).

1141

1142

1143 **Figure 1-supplement 1. Clustered heatmap of Pearson's correlation coefficients for**

1144 **protein abundance comparing data acquisition modes.**

The heatmap compares the $\log_2$ transformed ppm protein abundance values to assess peripheral myelin purified from wild type mice using three data acquisition modes (MS^E, UDMS^E, DRE-UDMS^E). The inset shows the color key and the histogram for the values of the correlation coefficients. Note that the runs cluster with a high overall correlation (>0.75) into three conditions defined by the acquisition mode, in agreement with the experimental design. Among the samples analyzed by different acquisition modes, DRE-UDMS^E similarly correlates with both MS^E and UDMS^E, reflecting its intermediate nature.

Figure 2. Relative abundance of peripheral myelin proteins

MS^E was used to identify and quantify proteins in myelin purified from the sciatic nerves of wild-type mice at P21; their relative abundance is given as percent with relative standard deviation (% +/- RSD). Note that known myelin proteins constitute >80% of the total myelin protein; proteins not previously associated with myelin constitute <20%. Mass spectrometric quantification based on 3 biological replicates per genotype with 4 technical replicates each (see **Figure 1-source data 1**).

Figure 3. Developmental mRNA abundance profiles of myelin-associated genes

(A) K-means clustering was performed for the mRNA profiles of those 1046 proteins in our myelin proteome inventory for which significant mRNA expression was found by RNA-Seq in the sciatic nerve of rats dissected at ages E21, P6, P18 and 6 months (M6). Note that this filtering strategy allows to selectively display the developmental abundance profiles of those transcripts that encode myelin-associated proteins rather than of all transcripts present in the nerve. Standardized mRNA abundance profiles are shown (n=4 biological replicates per age). Known myelin genes are displayed in red. For comparison, *Pmp22* mRNA was included although the small tetraspan protein PMP22 was not mass spectrometrically identified due to its unfavorable distribution of tryptic cleavage sites. Normalized counts for all mRNAs including those displaying developmentally unchanged abundance are provided in **Figure 3-Source data 1**.

(B) Numbers of mRNAs per cluster.

Figure 4. Categorization of annotated protein functions

All proteins identified in peripheral myelin by UDMS^E (turquoise) and the respective developmental expression clusters (**Figure 3**; shades of red) were analyzed for overrepresented functional annotations using gene ontology (GO) terms. The graph displays

1182   the percentage of proteins in each cluster that were annotated with a particular function. For

1183   comparison, known myelin proteins were annotated. n.o., not over-represented.

1184

1185

1186   **Figure 5. Molecular analysis of myelin in the *Prx$^{-/-}$* mouse model of CMT4F**

1187   **(A)** Myelin purified from sciatic nerves dissected from *Prx$^{-/-}$* and control mice at P21 was

1188   separated by SDS-PAGE (0.5 µg protein load) and proteins were visualized by silver

1189   staining. Bands constituted by the most abundant myelin proteins (MPZ/P0, MBP, PRX) are

1190   annotated. Note that no band constituted by PRX was detected in *Prx$^{-/-}$* myelin and that

1191   several other bands also display genotype-dependent differences in intensity. Gel shows n=2

1192   biological replicates representative of n=3 biological replicates.

1193   **(B)** The relative abundance of proteins in myelin purified from *Prx$^{-/-}$* sciatic nerves as

1194   quantified by MS^{E} is given as percent with relative standard deviation (% +/- RSD). Note the

1195   increased relative abundance of MPZ/P0 and MBP compared to wild-type myelin (see

1196   **Figure 2**) when PRX is lacking. Mass spectrometric quantification based on 3 biological

1197   replicates with 4 technical replicates each (see **Figure 5-source data 1**).

1198   **(C,D)** Differential proteome analysis by DRE-UDMS^{E} of myelin purified from *Prx$^{-/-}$* and wild-

1199   type mice. Mass spectrometric quantification based on 3 biological replicates per genotype

1200   with 4 technical replicates each (see **Figure 5-source data 2**). **(C)** Top 40 proteins of which

1201   the abundance is reduced (blue) or increased (red) in peripheral myelin purified from *Prx$^{-/-}$*

1202   compared to wild-type mice with the highest level of significance according to the -log$_{10}$

1203   transformed q-value (green). In the heatmaps, each horizontal line corresponds to the fold-

1204   change (FC) of a distinct protein compared to its average abundance in wild-type myelin

1205   plotted on a log$_2$ color scale. Heatmaps display 12 replicates, i.e. 3 biological replicates per

1206   genotype with 4 technical replicates each. **(D-D''')** Volcano plots representing genotype-

1207   dependent quantitative myelin proteome analysis. Data points represent quantified proteins

1208   in *Prx$^{-/-}$* compared to wild-type myelin and are plotted as the log2-transformed fold-change

1209   (FC) on the x-axis against the -log10-transformed q-value on the y-axis. Stippled lines mark a

1210   -log10-transformed q-value of 1.301, reflecting a q-value of 0.05 as significance threshold.

1211   Highlighted are the datapoints representing the Top 10 proteins displaying highest zdist

1212   values (Euclidean distance between the two points (0,0) and (x,y) with x = log2(FC) and y = -

1213   log10(q-value) (red circles in **D**), immune-related proteins (purple circles in **D'**), proteins of

1214   the extracellular matrix (ECM; yellow circles in **D''**) and known myelin proteins (blue circles in

1215   **D'''**). n.d., not detected; n.q., no q-value computable due to protein identification in one

1216   genotype only. Also see **Figure 5-supplement 1.**

1217   **(E)** Immunoblot of myelin purified from *Prx$^{-/-}$* and control sciatic nerves confirms the reduced

1218   abundance of DRP2, SLC16A1/MCT1, BSG and PMP2 in *Prx$^{-/-}$* myelin, as found by

1219   differential DRE-UDMS^E analysis (in **Figure 5C,D**). PRX was detected as genotype control;

1220   PLP/DM20 and ATP1A1 serve as markers. Blot shows n=2 biological replicates per

1221   genotype.

1222   **(F)** Teased fiber preparations of sciatic nerves dissected from *Prx*$^{-/-}$ and control mice

1223   immunolabelled for MAG (red) and SLC16A1 (green). Note that SLC16A1 co-distributes with

1224   MAG in Schmidt-Lanterman incisures (SLI) in control but not in *Prx*$^{-/-}$ nerves, in accordance

1225   with the reduced abundance of SLC16A1 in *Prx*$^{-/-}$ myelin (**Figure 5C-E**). Also note that, in

1226   *Prx*$^{-/-}$ myelin, SLI were largely undetectable by MAG immunolabeling.

1227

1228

1229   **Figure 5-supplement 1. Clustered heatmap of Pearson's correlation coefficients for**

1230   **protein abundance comparing genotypes.**

1231   **(A)** The heatmap compares the log$_2$ transformed ppm protein abundance values from the

1232   DRE-UDMS^E runs to assess peripheral myelin purified from wild type and *Prx*$^{-/-}$ mice. The

1233   inset shows the color key and the histogram for the values of the correlation coefficients.

1234   Note that the runs cluster with a high overall correlation (>0.85) into two conditions defined

1235   by the genotype, in agreement with the experimental design.

1236   **(B)** Volcano plot representing genotype-dependent quantitative myelin proteome analysis.

1237   Data points represent quantified proteins in *Prx*$^{-/-}$ compared to wild-type myelin plotted as the

1238   log2-transformed fold-change (FC) on the x-axis against the -log10-transformed q-value on

1239   the y-axis. Note the different axis scale compared to **Figure 5D**. Stippled line marks a -log10-

1240   transformed q-value of 1.301, reflecting a q-value of 0.05 as significance threshold.

1241   Highlighted is the datapoint for PRX to illustrate that only trace amounts of PRX were

1242   detected when assessing *Prx*$^{-/-}$ myelin. ATP2A1, ATP1A4 and PLCD1 were not detected in

1243   *Prx*$^{-/-}$ myelin.

1244

1245

1246   **Figure 6. Progressive loss and reduced diameters of peripheral axons in *Prx*$^{-/-}$ mice**

1247   **(A-D)** Genotype-dependent quantitative assessment of light micrographs of toluidine-stained

1248   semi-thin sectioned quadriceps nerve dissected at 2, 4 and 9 months of age reveals

1249   progressive loss of peripheral axons in *Prx*$^{-/-}$ compared to control mice.

1250   **(A)** Representative micrographs. Arrows point at myelinated axons; asterisk denotes an

1251   unmyelinated axon; arrowhead points at a myelin whorl lacking a recognizable axon. Scale

1252   bars, 10 µm.

1253   **(B)** Total number of axons per nerve that are not associated with a Remak bundle.

1254   **(C)** Total number of myelinated axons per nerve.

1255   **(D)** Total number per nerve of myelin whorls that lack a recognizable axon.

1256   Mean +/ SD, n=3-4 mice per genotype and age; *P<0.05, **P<0.01, ***P<0.001 by Student's

1257   unpaired t-test.

1258   (**E-G**) Genotype-dependent assessment of myelinated axons shows a shift toward reduced

1259   axonal diameters in quadriceps nerves of *Prx*^{-/-} compared to control mice at 2 months (**E**), 4

1260   months (**F**) and 9 months (**G**) of age. Data are presented as frequency distribution with 0.5

1261   µm bin width. ***, p<0.001 by two-sided Kolmogorow-Smirnow test. For precise p-values see

1262   methods section.

1263

1264

1265   **Figure 1-source data 1. Label-free quantification of proteins in wild-type PNS myelin**

1266   **fractions by three different data acquisition modes**

1267   Identification and quantification data of detected myelin-associated proteins. Tryptic peptides

1268   derived from four technical replicates (replicate digestion and replicate injection) per three

1269   biological replicate (20 sciatic nerves pooled from 10 animals) were analyzed by LC-MS (12

1270   runs in total). Proteins (FDR < 1%; 2 peptides/protein) and peptides (FDR < 1%; ≥7 amino

1271   acids) were identified by database search against the UniprotKB/SwissProt mouse database

1272   using PLGS. Data were post-processed with the software package ISOQuant to calculate

1273   absolute in-sample amounts for each detected protein based on the TOP3 approach.

1274   Reported abundance values are defined as the relative amount of each protein in respect to

1275   the sum over all detected proteins (ppm: parts per million (w/w) of total protein). Typical

1276   contaminant proteins like keratins were filtered.

1277   → sheet 1: protein identification details

1278   → sheet 2: WT myelin proteome by MS[E]

1279   → sheet 3: WT myelin proteome by UD-MS[E]

1280   → sheet 4: WT myelin proteome by DRE UD-MS[E]

1281   → sheet 5: 45 proteins additionally identified in WT myelin by 1D-gel-LC-MS

1282

1283

1284   **Figure 3-source data 1. Normalized developmental mRNA abundance data**

1285   → sheet 1: normalized values for all individual 4 biological replicates per age

1286   → sheet 2: normalized values for biological replicates averaged to give mean per age

1287

1288

1289   **Figure 5-source data 1. Label-free quantification of proteins in PNS myelin fractions**

1290   **from *Prx*^{-/-} mice by MSe**

1291   Identification and quantification data of detected myelin-associated proteins. Tryptic peptides

1292   derived from four technical replicates (replicate digestion and replicate injection) per three

1293   biological replicate (20 sciatic nerves pooled from 10 animals) were analyzed by LC-MS (12
1294   runs in total). Proteins (FDR < 1%; 2 peptides/protein) and peptides (FDR < 1%; ≥7 amino
1295   acids) were identified by database search against the UniprotKB/SwissProt mouse database
1296   using PLGS. Data were post-processed with the software package ISOQuant to calculate
1297   absolute in-sample amounts for each detected protein based on the TOP3 approach.
1298   Reported abundance values are defined as the relative amount of each protein in respect to
1299   the sum over all detected proteins (ppm: parts per million (w/w) of total protein). Typical
1300   contaminant proteins like keratins were filtered.
1301   → sheet 1: protein identification details
1302   → sheet 2: *Prx*^{-/-} myelin proteome by MS[E]
1303
1304

1305   **Figure 5-source data 2. Label-free quantification of proteins in PNS myelin fractions**
1306   **from WT and *Prx*^{-/-} mice by DRE-UDMSe**
1307   Identification and quantification data of detected myelin-associated proteins by DRE-UDMSe.
1308   For each genotype, tryptic peptides derived from four technical replicates (replicate digestion
1309   and replicate injection) per three biological replicate (20 sciatic nerves pooled from 10
1310   animals) were analyzed by LC-MS (24 runs in total). Proteins (FDR < 1%; 2 peptides/protein)
1311   and peptides (FDR < 1%; ≥7 amino acids) were identified by database search against the
1312   UniprotKB/SwissProt mouse database using PLGS. Data were post-processed with the
1313   software package ISOQuant to calculate absolute in-sample amounts for each detected
1314   protein based on the TOP3 approach. Reported abundance values are defined as the
1315   relative amount of each protein in respect to the sum over all detected proteins (ppm: parts
1316   per million (w/w) of total protein). Typical contaminant proteins like keratins were filtered. The
1317   -log10-transformed q-value was plotted against the log2-transformed fold change to obtain
1318   the volcano plot shown in Figure 5D. As no imputation of missing values was performed,
1319   proteins exclusive for only one of the conditions do not appear in the volcano plot, but are
1320   appended at the end of the list. Criteria for statistically significant regulation were as follows:
1321   fold change of at least 1.5 and q-value below 0.05.
1322   → sheet 1: protein identification details
1323   → sheet 2: comparison of WT vs. *Prx*^{-/-} myelin proteome by DRE-UDMS[E]
1324
1325

Protein name	Gene	Reference	TMD	Cluster
2-hydroxyacylsphingosine 1-beta-galactosyltransferase	*Ugt8*	Bosio et al., 1996	2	P6-up
Syntrophin α1	*Snta1*	Fuhrmann-Stroissnigg et al., 2012	-	P18-up
Annexin A2	*Anxa2*	Hayashi et al., 2007	-	Descending
Band 4.1 protein B / 4.1B	*Epb41l3*	Ivanovic et al., 2012	-	Descending
Band 4.1 protein G / 4.1G	*Epb41l2*	Ohno et al., 2006	-	P6-up
Breast carcinoma-amplified sequence 1	*Bcas1*	Ishimoto et al., 2017	-	P6-up
Cadherin 1/ E-Cadherin	*Cdh1*	Fannon et al., 1995	1	P18-up
Carbonic anhydrase 2	*Ca2*	Cammer et al., 1987	-	Descending
Catenin α1	*Ctnna1*	Murata et al., 2006	-	U-shaped
Catenin ß1	*Ctnnb1*	Fannon et al., 1995	-	Descending
Caveolin 1	*Cav1*	Mikol et al., 2002	1	P18-up
CD9, tetraspanin 29	*Cd9*	Ishibashi et al., 2004	4	P18-p
CD59A	*Cd59a*	Funabashi et al., 1994	1	P18-up
CD47, integrin-associated signal transducer	*Cd47*	Gitik et al., 2011	5	P6-up
CD81, tetraspanin 28	*Cd81*	Ishibashi et al., 2004	4	P18-up
CD82, tetraspanin 27	*Cd82*	Chernousov et al., 2013	4	P18-up
CD151, tetraspanin 24	*Cd151*	Patzig et al., 2011	4	P18-up
Cell adhesion molecule 4/ NECL4	*Cadm4*	Spiegel et al., 2007	1	P6-up
Cell division control protein 42	*Cdc42*	Benninger et al., 2007	-	P6-up
Cell surface glycoprotein MUC18	*Mcam*	Shi et al., 1998	1	Descending
Ciliary neurotrophic factor	*Cntf*	Rende et al., 1992	-	Late-up
CKLF-like MARVEL TMD-containing 5	*Cmtm5*	Patzig et al., 2011	4	P6-up
Claudin-19	*Cldn19*	Miyamoto et al., 2005	4	P6-up
Cofilin 1	*Cfl1*	Sparrow et al., 2012	-	Descending
Crystallin α2	*Cryab*	d'Antonio et al., 2006	-	P18-up
Cyclic nucleotide phosphodiesterase	*Cnp*	Matthieu et al., 1980	-	P6-up
Sarcoglycan δ	*Sgcd*	Cai et al., 2007	1	Late-up
Dihydropyrimidinase related protein 1	*Crmp1*	d'Antonio et al., 2006	-	Descending
Disks large homolog 1	*Dlg1*	Cotter et al., 2010	-	Descending
Dynein light chain 1	*Dynll1*	Myllykoski et al. 2018	-	P6-up
Dystroglycan	*Dag1*	Yamada et al, 1994	1	P6-up
Dystrophin/DP116	*Dmd*	Cai et al., 2007	-	P6-up
Dystrophin-related protein 2	*Drp2*	Sherman et al., 2001	-	P18-up
E3 ubiquitin-protein ligase NEDD4	*Nedd4*	Liu et al., 2009	-	Descending
Ezrin	*Ezr*	Scherer et al., 2001	-	P6-up
Fatty acid synthase	*Fasn*	Salles et al., 2002	-	P6-up
Flotillin 1	*Flot1*	Lee et al., 2014	-	P18-up
Gap junction ß1 protein / Cx32	*Gjb1*	Li et al., 2002	4	P18-up
Gap junction γ3 protein / Cx29	*Gjc3*	Li et al., 2002	1	P6-up
Gelsolin	*Gsn*	Gonçalves et al., 2010	-	Late-up
Glycogen synthase kinase 3ß	*Gsk3b*	Ogata et al., 2004	-	P6-up
Integrin α6	*Itga6*	Nodari et al., 2008	1	P6-up
Integrin αV	*Itgav*	Chernousov & Carey, 2003	1	Descending
Integrin ß1	*Itgb1*	Feltri et al., 2002	1	Descending
Integrin ß4	*Itgb4*	Quattrini et al., 1996	2	P18-up
Junctional adhesion molecule C	*Jam3*	Scheiermann et al., 2007	1	P18-up
Laminin α2	*Lama2*	Yang et al., 2005	-	P6-up
Laminin α4	*Lama4*	Yang et al., 2005	-	Descending
Laminin ß1	*Lamb1*	LeBeau et al., 1994	-	Descending
Laminin ß2	*Lamb2*	LeBeau et al., 1994	-	P18-up
Laminin γ1	*Lamc1*	Chen & Strickland, 2003	-	Descending
Membrane Palmitoylated Protein 6	*Mpp6*	Saitoh et al., 2019	-	P6-up
Microtubule-associated protein 1A	*Map1a*	Fuhrmann-Stroissnigg et al., 2012	-	P18-up
Microtubule-associated protein 1B	*Map1b*	Fuhrmann-Stroissnigg et al., 2012	-	P6-up
Mitogen-activated protein kinase 1/ ERK2	*Mapk1*	Mantuano et al., 2015	-	Descending
Mitogen-activated protein kinase 3/ ERK1	*Mapk3*	Mantuano et al., 2015	-	P18-up
Moesin	*Msn*	Scherer et al., 2001	-	Unchanged
Monocarboxylate transporter 1	*Slc16a1*	Domenech-Estevez et al., 2015	11	P18-up

Myelin associated glycoprotein	*Mag*	Figlewicz et al., 1981	1	P6-up
Myelin basic protein	*Mbp*	Boggs, 2006	-	P6-up
Myelin protein 2	*Pmp2*	Trapp et al., 1984	-	P18-up
Myelin protein zero/ P0	*Mpz*	Giese et al., 1992	1	P6-up
Myelin proteolipid protein	*Plp1*	Garbern et al., 1997	4	P6-up
Myotubularin-related protein 2	*Mtmr2*	Bolino et al., 2004	-	P6-up
Noncompact myelin-associated protein	*Ncmap*	Ryu et al., 2008	1	P18-up
NDRG1, N-myc downstream regulated	*Ndrg1*	Berger et al., 2004	-	P18-uP
Neurofascin	*Nfasc*	Tait et al., 2000	2	P18-up
Nidogen 1	*Nid1*	Lee et al., 2007	-	Descending
P2X purinoceptor 7	*P2rx7*	Faroni et al., 2014	-	P6-up
Paxillin	*Pxn*	Fernandez-Valle et al., 2002	-	P6-up
Periaxin	*Prx*	Gillespie et al., 1994	-	P6-up
Plasmolipin	*Pllp*	Bosse et al., 2003	4	P18-up
Profilin 1	*Pfn1*	Montani et al., 2014	-	Descending
Lin-7 homolog C	*Lin7c*	Saitoh et al., 2017	-	P6-up
Rac1	*Rac1*	Benninger et al., 2007	-	U-Shaped
Radixin	*Rdx*	Scherer et al., 2001	-	Descending
RhoA	*Rhoa*	Brancolini et al., 1999	-	U-Shaped
Septin 2	*Sept 2*	Buser et al., 2009	-	Descending
Septin 7	*Sept 7*	Buser et al., 2009	-	U-Shaped
Septin 8	*Sept 8*	Patzig et al., 2011	-	P18-up
Septin 9	*Sept 9*	Patzig et al., 2011	-	P6-up
Septin 11	*Sept 11*	Buser et al., 2009	-	Descending
Sirtuin 2, NAD-dependent deacetylase	*Sirt2*	Werner et al., 2007	-	P18-up
Spectrin alpha chain, non-erythrocytic 1	*Sptan1*	Susuki et al., 2018	-	P18-up
Spectrin beta chain, non-erythrocytic 1	*Sptbn1*	Susuki et al., 2018	-	P18-up
Tight junction protein ZO-1	*Tjp1*	Poliak et al., 2007	-	P6-up
Tight junction protein ZO-2	*Tjp2*	Poliak et al., 2007	-	P6-up
Transferrin	*Tf*	Liu et al., 1990	2	Late-up
Vimentin	*Vim*	Triolo et al., 2012	-	Unchanged
Vinculin	*Vcl*	Beppu et al., 2015	-	Descending

Table 1. Known myelin proteins in the myelin proteome. Proteins mass-spectrometrically identified in peripheral myelin are compiled according to availability of prior references as myelin proteins. Given are the official gene name, one selected reference, the number of transmembrane domains (TMD) and the mRNA abundance profile cluster (see **Figure 3**).

Protein name	Gene name	OMIM#	Gene Locus	Neuropathy
Monoacylglycerol lipase ABHD12	*ABHD12*	613599	20p11.21	PHARC
Apoptosis-inducing factor 1	*AIFM1*	300169	Xq26.1	CMTX4, DFNX5
Na+/K+ -transporting ATPase α1	*ATP1A1*	182310	1p13.1	CMT2DD
Cytochrome c oxidase subunit 6A1	*COX6A1*	602072	12q24.31	CMTRID
Dystrophin-related protein 2	*DRP2*	300052	Xq22.1	CMTX
Dynactin subunit 1	*DCTN1*	601143	2p13.1	DHMN7B
Dynamin 2	*DNM2*	602378	19p13.2	CMT2M, CMTDIB
Cytoplasmic dynein 1 heavy chain 1	*DYNC1H1*	600112	14q32.31	CMT20, SMALED1
E3 SUMO-protein ligase	*EGR2*	129010	10q21.3	CMT1D, CMT3, CMT4E
Glycine-tRNA ligase	*GARS* (Gart)	600287	7p14.3	CMT2D, HMN5A
Gap junction ß1 protein / Cx32	*GJB1*	304040	Xq13.1	CMTX1
Guanine nucleotide-binding protein ß4	*GNB4*	610863	3q26.33	CMTDIF
Histidine triad nucleotide-binding protein 1	*HINT1*	601314	5q23.3	NMAN
Hexokinase 1	*HK1*	142600	10q22.1	CMT4G
Heat shock protein ß1	*HSPB1*	602195	7q11.23	CMT2F, DHMN2B
Kinesin heavy chain isoform 5A	*KIF5A*	602821	12q13.3	SPG10
Prelamin A/C	*LMNA*	150330	1q22	CMT2B1
Neprilysin	*MME*	120520	3q25.2	CMT2T, SCA43
Myelin protein zero/ P0	*MPZ*	159440	1q23.3	CHN2,CMT1B, CMT2I, CMT2J,CMT3, CMTDID, Roussy-Levy syndrome
Myotubularin-related protein 2	*MTMR2*	603557	11q21	CMT4B1
Alpha-N-acetylglucosaminidase	*NAGLU* (NAGA)	609701	17q21.2	CMT2V
NDRG1, N-myc downstream regulated	*NDRG1*	605262	8q24.22	CMT4D
Neurofilament heavy polypeptide	*NEFH*	162230	22q12.2	CMT2CC
Neurofilament light polypeptide	*NEFL*	162280	8p21.2	CMT2E, CMT1F, CMTDIG
Peripheral myelin protein 2	*PMP2*	170715	8q21.13	CMT1G
Peripheral myelin protein 22	*PMP22*	601907	17p12	CMT1A, CMT1E, CMT3, HNPP, Roussy-Levy syndrome
Ribose-phosphate pyrophosphokinase 1	*PRPS1*	311850	Xq22.3	Arts syndrome, CMTX5, DFNX1
Periaxin	*PRX*	605725	19q13.2	CMT4F, CMT3
Ras-related protein Rab 7a	*RAB7A*	602298	3q21.3	CMT2B
Septin 9	*SEPT9*	604061	17q25.3	HNA
Transitional ER-ATPase	*VCP*	601023	9p13.3	CMT2Y
Tryptophan-tRNA ligase, cytoplasmic	*WARS*	191050	14q32.32	HMN9
Tyrosine-tRNA ligase, cytoplasmic	*YARS*	603623	1p35.1	DI-CMTC

Table 2. Peripheral myelin proteins identified in PNS myelin involved in neuropathological diseases. Proteins massspectrometically identified in peripheral myelin were analyzed regarding the involvement of the ortholog human gene in neuropathological diseases. PMP22 was added, though it was not identified by MS analyses due to its unfavorable distribution of tryptic cleavage sites. CMT, Charcot-Marie-Tooth disease; DHMN, distal hereditary motor neuropathy; DI-CMTC, dominant intermediate CMTC; DFN, X-linked deafness; HMN, hereditary motor neuropathy; HSAN, hereditary sensory and autonomic neuropathy; HNA, hereditary sensory and autonomic neuropathy; OMIM, Online Mendelian Inheritance in Man; PHARC, polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract; SCA, spinocerebellar ataxia; SPG, spastic paraplegia.