# Supplementary Material and Methods

## RT-PCR

Total RNA was isolated from Wild Type (WT) and $Fgd4^{SC-/-}$ mouse sciatic nerves using the Purelink RNA Minikit (#12183018A, Thermofisher Scientific, USA), following the manufacturer's protocol. cDNA was generated using the SuperScript III one-step RT-PCR (#12574018, ThermoFisher Scientific, USA) and random hexamers (#48190011, Thermofisher Scientific, USA). The deletion of exon 4 was detected by the amplification, from cDNA, of a fragment between exons 3 and 7 of the Fgd4 transcript, using the following primers (Fgd4-mouse-3F: 5'- GAGTCTAATCCGGCCCCTAC-3'and Fgd4-mouse-7R: 5'-AAGGAATGGCGCCAACTTTT-3').

## Behavioural gait test

For the gait experiment, sex and age-matched $Fgd4^{SC-/-}$ and WT littermates' mice were monitored at 6 (n=9 WT and n=9 $Fgd4^{SC-/-}$), 12 (n=12 WT and n=13 $Fgd4^{SC-/-}$) and 18 (n=11 WT and n=13 $Fgd4^{SC-/-}$) months old. Gait was analyzed during spontaneous walk using an automated gait analysis system (Gaitlab, Viewpoint, France). Before recording footprints, mice were acclimated and trained to walk on the Gait system for two days. During the testing session, a minimum of 2-3 completed runs were collected. We focused the analysis on intensity-based parameters, paw-size as well as gait/posture, as previously described in[1]. Animals that did not complete at least 2 successful runs (stalling or reversing during gait,…) were removed from the study.

## Electron microscopy and morphometric analysis

The sciatic nerves were dissected and fixed in 2% ParaFormaldehyde (PFA) and 2.5% Glutaraldehyde in 0.1M cacodylate buffer for 2 hours. The next day, the nerves were washed three times in 0.1M cacodylate buffer and post-fixed in buffered 1% OsO4 for one hour. After washes in distilled water, the samples were contrasted in aqueous 1% uranyl acetate. Samples were then dehydrated in graded series of ethanol baths (30 minutes each) and infiltrated with epon resin in ethanol (1:3, 2:2, 3:1) for 2 hours for each, and finally in pure resin overnight. The next day the nerves were embedded in fresh pure epon resin and cured for 48h at 60°C. 500 nm semi-thin and 70 nm ultra-thin sections were performed on a Leica UCT

33  Ultramicrotome (Leica, Austria). Semi-thin sections were stained with toluidine blue and

34  ultrathin sections were deposited on formvar-coated slot grids. The grids were contrasted

35  using uranyl acetate (10 minutes) and lead citrate (5 minutes) and observed using an FEI

36  Tecnai G2 at 200 KeV. The acquisition was performed on a Veleta camera (Olympus, Japan).

37  The proportion of fibers having out- and infoldings was counted on ten fields corresponding

38  to a range of 600-800 fibers for each sciatic nerve of at least three animals.

39  To perform g-ratio analysis, digitalized images of fiber semithin sections of the sciatic nerves

40  were obtained with a 100× objective of a phase-contrast microscope (BX59, Olympus). At

41  least ten images from three different animals per genotype at 3, 6, and 18 months old were

42  acquired. g-ratio analysis was performed on micrographs using Image J (National Institutes of

43  Health, Bethesda, MD) plug-in (g-ratio calculator) developed in collaboration with the

44  cellular imaging facility of the University of Lausanne and available at http://cifweb.unil.ch,

45  as previously described in Arnaud et al.2009 [2].

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48  **Immunohistochemistry**

49  Mice were sacrificed with an overdose of $CO_2$. Gastrocnemius muscles were dissected out

50  and fixed in 4 % PFA for 30 min. Samples were then incubated in 25 % sucrose solution at 4

51  °C for 24 h. Tissues were embedded in the Optimal Cutting Temperature compound

52  (#3801480,  Leica, USA) and stored at -80 °C before processing. Embedded muscles were

53  then cut to twenty-five μm-thick sections, mounted on slides coated with Superfrost Plus

54  (#LSFPLUS, THERMO SCIENTIFIC MENZEL, USA). Muscle sections were incubated

55  overnight at 4 °C in blocking solution (2 % BSA, 10 % Normal Goat Serum, 0.1 % Triton and

56  PBS) with chicken anti-NFM (#822701, BioLegend, USA, previously #PCK-593P, Covance,

57  USA). Sections were next incubated with Goat anti-Chicken IgY (H+L) Secondary Antibody,

58  Alexa Fluor 488, (#ab150169, Abcam, UK) and α-Bungarotoxin, Alexa Fluor™ 555

59  conjugate (#B35451, thermofisher Scientific, USA), (1:500), and mounted in Duolink

60  mounting medium (#DU082040, Sigma-Aldrich, USA) . Neuromuscular junctions were

61  imaged on a Zeiss ApoTome.2 microscope (Zeiss, Germany) equipped with an AxioCam

62  MRm camera.

63

64  **Lentivirus infection**

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66 Fgd4/FRABIN was either overexpressed or downregulated in WT DRG/SC cocultures or in
67 rat primary SC using Lentivirus vectors (Lv) designed and produced by the Vector builder
68 company (https://en.vectorbuilder.com, USA). For overexpression, we designed control Lv
69 (Lv-control) expressing EGFP under the CMV promoter and Lv allowing the expression of
70 mouse Fgd4 (Lv-Fgd4) under the CMV promoter. For the knockdown experiments, we used
71 an Lv expressing a shRNA against rat *Fgd4* previously validated in Horn et al. 2012[3]
72 (targeted sequence: 5'-GAAGAAGAGGATATTGTA-3') referred to as LvSHFgd4-GFP. Lv-
73 Shcontrol-GFP expressing a shRNA scramble, randomly produced, was provided by
74 Vectorbuilder (#VB151023-10034, Vectorbuilder, USA). Both Lv vectors express EGFP
75 under the CMV promoter, in addition to the shRNA, allowing tracing of the infected cells.
76 We used the same strategy to knockdown the *Snx3* gene in DRG/SC cocultures or in primary
77 SCs. Here, we used Lv expressing two shRNAs targeting *Snx3* (Sh-0: 5'-
78 GCCCAGAATGAACGTTGTCTT-3'; Sh-3: 5'-AGAGAGAGCAAGGTTGTAGTT-3'),
79 both provided by Sigma-Aldrich (USA). Cells (DRG/SC cocultures or primary SCs) were
80 infected with the described Lv at a dose of 15 TU/cell 24 h after plating.

81

## Transferrin assay

83 Rat primary SCs were plated at a density of 50 000 cells per well and infected 24h later with
84 Lv-SHcontrol-GFP or SHFgd4-GFP (15 TU/cell). Two days after the infection, cells were
85 incubated with pHrodo-red Transferrin conjugate (#P35376, Thermofischer Scientific, USA)
86 at a dilution of 50µg/ml, for 30 min on ice, followed by 15, 30 or 45 min of incubation at 37
87 °C. After one PBS wash, cells were fixed in 4% PFA for 15 min. Samples were then mounted
88 in Duolink mounting medium (#DU082040, Sigma-Aldrich, USA) and pHrodo-TF
89 fluorescence intensities were visualized and captured on a Zeiss ApoTome.2 microscope
90 (Zeiss, Germany) equipped with an AxioCam MRm camera, with a 20 X objective. The
91 iintegrated density of fluorescence signal was analyzed only in the infected cells (*i.e.*, GFP+)
92 using ImageJ software.

93

## Cell lines and transfection

95 HEK293 (Human embryonic kidney, # CRL-1573) and S16 rat SC (#CRL-2941) lines were
96 both provided by ATCC (Manassas, Virginie, USA). HEK293 and S16 cells were cultured in
97 DMEM (#41965, Thermofisher Scientific, USA) complemented with 10 % of FBS (#15000-
98 036, Thermofisher, USA) and 1 % of penicillin/streptomycin (#15070063, Thermofisher

99   Scientific, USA). To overexpress hFGD4/FRABIN in HEK293 cells, the pEx-FGD4-His-V5

100  vector was generated by Gateway cloning technology (ThermoFischer Scientific, USA).

101  Transfection experiments were performed using promofectin reagent (#PK-CT-2000-50,

102  PromoCell GmbH, Germany). Briefly, 300000 cells were seeded and transfected with 4µg of

103  plasmid following the manufacturer's recommendation. For immunoprecipitation, cells were

104  harvested 72 h post-transfection.

105

## 106  Plasmids

107  The plasmids used for the yeast-two hybrid experiment (pENTR-FL-hFGD4) and transfection

108  studies (pEx-FGD4-His-V5) were generated using Invitrogen Gateway cloning technology

109  (ThermoFischer Scientific, USA) following the manufacturer's instructions. Briefly, the entry

110  plasmid (i.e., pENTR-FL-hFGD4) was generated after the BP recombination reaction between

111  the *att*B-flanked hFGD4 fragment and the *att*P-containing donor vector pDONR221

112  (#12536017, ThermoScientific, USA) using the BP clonase enzyme kit (#11789013,

113  ThermoScientific, USA). The *att*B-flanked hFGD4 fragment was produced by PCR using the

114  "Expand High Fidelity Plus PCR System" (#3300226001, Roche, Switzerland) and the

115  following                          primers:                         FGD4-GATEWAY-

116  1F:5'GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAGGAAATTAAACCTGCCTC

117  TGC3'                            and                          FGD4-GATEWAY-1R:

118  5'GGGGACCACTTTGTACAAGAAAGCTGGGTTCAGCATTCTGATTTTTTCTTAGG3'.

119  The expression vector pEx-FGD4-His-V5 was generated following an LR recombination

120  between the attL containing entry vector (pENTR-FL-hFGD4) and the attR destination vector

121  (pDEST40, #12274015, ThermoScientific, USA) using the LR Clonase enzyme mix

122  (#11791019, ThermoScientific, USA).

123

## 124  Immunocytochemistry

125  DRG/SC co-cultures were fixed with 4% PFA, washed in PBS, and then permeabilized for 5

126  minutes with methanol. After two PBS washes, cells were incubated 1-2 h in a blocking

127  solution (20% fetal bovine serum, 1% bovine serum albumin, 0.01% Triton in 1 X PBS) at

128  room temperature. Cells were then incubated with the following primary antibodies diluted in

129  the incubation solution overnight: chicken anti-neurofilament NF-M (1:1000) (#822701,

130  BioLegend, USA, previously #PCK-593P, Covance), rat anti-MBP (1:300) (#MAB386,

131  Merck-Millipore, Germany). After two washes in PBS, cells were incubated with one of the

132    following secondary antibodies: Donkey Anti-Rat IgG H&L (#150154, Alexa Fluor 555,

133    abcam, UK) (1:1000) and (#A21449, Goat Anti-Chicken IgY H&L (Alexa Fluor 647,

134    Invitrogen, USA) (1: 1000). Coverslips were then rinsed twice in PBS and mounted in a

135    duolink mounting medium (#DU082040, Sigma-Aldrich, USA) for microscope analysis.

136    Fluorescence images were captured with a Zeiss ApoTome.2 microscope (Zeiss, Germany)

137    equipped with an AxioCam MRm camera. Images were captured and merged with the ZEN

138    software (Zeiss, Germany) and were treated using ImageJ software.

139

## Protein extraction and Immunoblotting

141    DRG/SC co-cultures or nerve samples were lysed in RIPA buffer supplemented with a

142    protease/phosphatase inhibitor cocktail (#78442, ThermoFisher Scientific, USA). The lysate

143    was passed through an 18–21-gauge needle and submitted to sonication using the Bioruptor

144    VCD-200 (Diagenode, Belgium). After centrifugation for 10 min at 20,000 x g at 4 °C, the

145    supernatant was removed and protein concentration was measured by use of a Bicinchoninic

146    acid (BCA) solution (#B9643-1LSigma-aldrich, USA) coupled to copper II sulfate solution

147    (#C2284-25ml, Sigma-aldrich, USA) following the manufacturer's recommandations. 40 μg

148    of samples' proteins were loaded on a Precast NuPage 4-12 % Bis-Tris gels (Thermofisher,

149    USA) and transferred onto a nitrocellulose membrane (GE healthcare life science, Germany).

150    The membrane was then blocked by incubation in blocking buffer (Intercept-blocking buffer,

151    Licor). The membrane was then incubated overnight with the following primary antibodies:

152    mouse anti-Neuregulin1α/β1/2 (D10) (1:500) (#sc-393009; Santa Cruz Biotechnology, USA),

153    rabbit anti-phospho-Akt (Ser473) (D9E) (1:2000) (#4060, Cell Signaling Technology, USA),

154    rabbit anti-Akt(pan) (C67E7) (1:1000) (#4691, Cell Signaling Technology, USA), rabbit anti-

155    HER2/ERBB2 (29D8) (1:1000) (#2165, Cell Signaling Technology, USA), rabbit anti-

156    phospho-HER2/ERBB2 (Tyr1248) (1:1000) (#2247, Cell Signaling Technology, USA), rabbit

157    anti-HER3/ERBB3 (1B2) (1:1000) (#4754, Cell Signaling Technology, USA), rabbit anti-

158    phospho-HER3/ERBB3 (Tyr1289) (D1B5) 1:1000) (#2842, Cell Signaling Technology,

159    USA), rabbit anti-rab11a (1:1000) (#ab65200, Abcam, UK), rabbit anti-rab5 (1:1000)

160    (#ab18211, Abcam, UK), rabbit anti-mTOR (7C10) (1:1000) (#2983T, Cell Signaling

161    Technology, USA), rabbit anti-SNX3 (1:300) (#ab56078, Abcam, UK), goat anti-gapdh

162    (1:1000) (#sc-48167, Santa Cruz Biotechnology, USA), rabbit anti-c-MAF (1:100)

163    (#ab77071, abcam, UK), rabbit anti-ERBIN (1:500) (#LSC47097, LSBio, USA), mouse anti-

tubulin (1:4000) (#T6074, Sigma-Aldrich, USA). After three final washes in 0.1 % PBS-Tween 20, the membranes were incubated with secondary antibodies: IRDye® 800CW Donkey anti-Rabbit IgG (H + L), IRDye® 680RD Donkey anti-Goat IgG (H + L), and IRDye® 680RD Donkey anti-Mouse IgG (H + L) from Li-Cor Biosciences( USA), diluted at 1:10000. Membranes were then developed using the ChemiDoc imaging system from Biorad( USA). Intensities of the bands were then analyzed using the ImageJ gel analyzer tool (ImageJ Software, National Institute of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij). A plot profile of western blots was established, and the intensities of all bands were measured. For each sample, the peak intensity was calculated for each target protein by dividing the target protein intensity with the intensity of the loading control protein. Data were then normalized to the control sample.

## Immunoprecipitation

Co-immunoprecipitation (co-IP) experiments were performed using the Dynabeads Protein G Immunoprecipitation Kit (#10007D, Thermofisher Scientific, USA) following the manufacturer's protocol. Briefly, HEK293 cells overexpressing His-V5-tagged human FRABIN were washed with PBS 72 h post-transfection (with plasmid pEx-FGD4-His-V5), and then incubated in a home-made lysis buffer (HEPES 50 mM, NaCl 150 mM, $MgCl_2$ 1.5 mM, EGTA 1 mM, Glycerol 10 %, Triton X100 0.1 %, protease inhibitor). First, 10ug of primary mouse anti-V5 antibody (#ab27671, Abcam, UK) diluted in PBS-Tween 0.01% were incubated with magnetic beads for 1h. The antibody-magnetic beads complex was then incubated with the lysate containing 1 mg of proteins, overnight with end over end rotation. The flow-through is then discarded after placing the tube containing the beads-antigen-antibody lysate, on the magnet. Bound proteins were then eluted with elution buffer and dissociated in 10 µl of NuPAGE LDS Sample Buffer (#NP0007, ThermoFisher Scientific, USA). Samples were then boiled at 70 °C for 10 min before loading on a NuPage Bis-Tris gel.

## Yeast two-hybrid (Y2H)

GAL4-based yeast two-hybrid assay was performed, using a commercial human fetal brain cDNA library containing cDNAs fused to the gal4 activation domain of pEXP-AD502 (ProQuest™, ThermoFisher Scientific, USA), as prey, and full length human *FGD4* cDNA as

197    a bait. To this purpose, the full-length coding sequence of *FGD4* (NM_139241) was

198    subcloned from the pENTR-FL-hFGD4, into a pDBA vector, using the Gateway technology

199    (Thermofisher Scientific, USA). The bait plasmid was transformed in MAV03 yeast strain

200    (MATα;    leu2-3,112;    trp1-901;    his3Δ200;    ade2-101;    gal4    Δ;    gal80    Δ;

201    SPAL10UASGAL1::URA3, GAL1::lacZ, GAL1::His3@LYS2, can1R, cyh2R) following the

202    previously described transformation protocol[4]. This bait did not show self-activation and was

203    further used for screening. MAV203 cells were then transformed with the prey cDNA library

204    as described[4]. Following transformation with the cDNA library, yeasts were plated onto

205    synthetic complete (SC) medium minus leucine (−L), minus tryptophane (−W), minus

206    histidine (−H) +25 mM 3-amino-1,2,4-triazole (3-AT) and, incubated at 30°C for 4–5 days.

207    Positive clones were patched onto SC-WHL + 3-AT in 96-well plates, incubated for 3 days at

208    30°C and transferred in liquid SC-WL for 3 days at 30°C with agitation to normalize the yeast

209    cell concentration used for the phenotypic assay. Cells were then diluted 1/20 in water,

210    spotted onto a selective medium (-WHL+25 mM 3-AT or -WUL), and incubated at 30°C for 4

211    to 5 days. To perform the β-galactosidase assay, undiluted yeast cells were spotted onto YPD

212    (yeast extract peptone dextrose) medium plates with nitrocellulose filters, and β-galactosidase

213    activity was evaluated one day after. Positive clones were sequenced by Sanger sequencing,

214    after    PCR    amplification    using    the    following    primers:    forward:    5'-

215    CGCGTTTGGAATCACTACAGGG-3'    and    reverse:    5'-

216    GGAGACTTGACCAAACCTCTGGCG-3'). The clones were identified by using BLAST.

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218

219    **Legends to Supplementary Figures and Table**

220

221    **Supplementary Figure 1. *Fgd4^SC-/-* animals display normal myelination thickness of**

222    **sciatic nerves, but a late muscle denervation** (**A**) G-ratio analysis revealed no statistical

223    differences in myelin thickness in the sciatic nerves of 3, 6 and 18 mo WT and *Fgd4^SC-/-* mice.

224    A total of 500-1000 axons of diameter between 0.5 and 6 µm were analysed (n=3 animals per

225    genotype). Data are represented as scatter plots of individual axons as a function of their

226    respective diameters determined at 3, 6 and 18 months old. Each point corresponds to one

227    fiber (gray points: *Fgd4^SC-/-* animals; black points: WT animals). (**B-C**) Late muscle

228    denervation observed in the gastrocnemius of *Fgd4^SC-/-* animals. Level of innervation of

229    gastrocnemius muscle evaluated by the colocalization of the neurofilament marker (NF-M)

230    and the acetylcholine receptor marker α-bungarotoxin in 12 months old WT (n=3) and
231    *Fgd4$^{SC-/-}$* (n=3) animals. Yellow asterisk indicate innervated NMJ, red asterisk indicate
232    denervated NMJ. Scale bar: 50 µm. Statistical analysis: two-way repeated-measures ANOVA
233    (genotype*type of NMJs) with Sidak post-hoc test. Two-way ANOVA revealed a significant
234    difference on the proportion of the type of NMJs between WT and *Fgd4$^{SC-/-}$* conditions
235    (p=0.001). Sidak post hoc test show a significant increase in the proportion of denervated
236    NMJs in *Fgd4$^{SC-/-}$* compared to WT (*p*:0,01). (**D-E**) Levels of expression of cleaved and full-
237    length Neuregulin 1-type III (named respectively cNRG1 and fNRG1) were assessed by
238    western-blot analysis in *Fgd4$^{SC-/-}$* cocultures compared to control. (**D**) Data are expressed as
239    mean ± sem (n=3-4 cocultures). Statistical analysis: unpaired Student's t-test. (**E**) Western
240    blot pictures illustrating the expression of the markers described in (D). (**F-G**) Levels of
241    expression of cNRG1 and fNRG1 were assessed by western-blot analysis in the sciatic nerves
242    of *Fgd4$^{-/-}$* mice compared to WT mice. (**F**) Data are expressed as mean ± sem (n=3 animals
243    per genotype). Statistical analysis: unpaired Student's t-test. (**G**) Western blot pictures
244    illustrating the expression of the markers described in (E). * p<0.05, ** p<0.01, ***p<0.001.
245

246    **Supplementary Figure 2. Protein levels of ERBIN, but not MAF-1, are upregulated in**
247    **sciatic nerves from *Fgd4$^{-/-}$* knock-out mice .**
248     (**A-B**) Levels of expression of MAF-1 and ERBIN were assessed by western-blot analysis in
249    the sciatic nerves of 1 year old *Fgd4$^{-/-}$* mice. (**A**) Data are expressed as mean ± sem (n=3
250    control and n=5 *Fgd4$^{-/-}$* animals). (**B**) Western blot pictures illustrating the expression of the
251    markers described in (**A**).

252

253    **Supplementary Figure 3. Effective knock-down of *Snx3* and *Fgd4/FRABIN in vitro*. (A-**
254    **B**) Lentivirus (Lv) expressing shRNA targeting *Snx3* lead to an efficient knock-down of *Snx3*
255    in primary rat SCs. Primary SCs were infected 1 day after plating with either Lv-SHcontrol,
256    LV-SH-SNX3-1 or 2, and harvested 7 days post-infection. Level of expression of SNX3 in
257    those conditions was evaluated by western-blot. (**C**) Knock-down of *Fgd4/FRABIN* in
258    primary SCs following Lv-shRNA targeting *Fgd4* and expressing GFP tag SHFgd4. Primary
259    SCs were infected 1 day after plating and fixed 3 days post-infection. Infected cells were
260    identified by GFP expression. Expression levels of FRABIN were evaluated by
261    immunofluorescence (in red). In contrast to non-infected cells (GFP negative, blue asterisks)

262    which express FRABIN, infected cells (GFP positive cells, yellow asterisks) are negative for
263    FRABIN.


264


265    **Supplementary Figure 4. Schematic figure summarizing the biochemical findings and**
266    **the proposed pathomechanisms underlying CMT4H pathology.** (**A**) Description of the
267    role of FRABIN in the regulation of the NRG1-type III-ERBB2/3 pathway and endocytic
268    trafficking in control conditions. The NRG1-type III-ERBB2/3/AKT/mTOR pathway is one
269    of the main pathways regulating myelination thickness through the expression of myelin and
270    lipid genes. ERBB2/3 levels are mainly regulated through endocytic trafficking. Through its
271    two phosphoinositides binding domains, FRABIN interacts and/or regulates key factors
272    involved in endosomal trafficking such as SNX3, RAB11 and, RAB11FIP2, promoting either
273    ERBB2/3 degradation through lysosomes or its recycling back to the membrane through
274    recycling endosomes. (**B**) Loss of FRABIN impairs NRG1-type III-ERBB2/3/AKT/mTOR
275    and endocytic trafficking, leading to hypermyelination (i.e generation of outfoldings). Loss of
276    FRABIN leads to an increase of key proteins regulating endosomal trafficking (SNX3,
277    RAB11 and, RAB11FIP2), consequently accelerating endosomal recycling and the presence
278    of ERBB2/3 receptors at the membrane surface. The increase of ERBB2/3 receptors leads to
279    an increase in AKT/mTOR pathway and may finally promote the expression of genes
280    controlling lipid production, a key constituent of the myelin sheet, as well as othersgenes such
281    as RAB11FIP2, a regulator of endosomal trafficking, as well as ERBIN, an adaptor protein
282    for ERBB2 receptor, ensuring its proper localization on the membrane.


283    **Supplementary Figure 5. Full western-blot membranes related to Figures 2 and 4.**
284    **(A-D)** Western blot pictures illustrating the expression of P-ERBB2, P-AKT, AKT, and the
285    related protein control GAPDH in $Fgd4^{SC-/-}$ and control cocultures conditions (see **Figure 2**).
286    The yellow rectangle represents the western blot part used in each corresponding Figure. **(E-**
287    **G)** Western blot pictures illustrating the expression of ERRB2, mTOR and the related protein
288    control GAPDH in $Fgd4^{SC-/-}$ and control cocultures conditions (see **Figure 2**). The yellow
289    rectangle represents the western blot part used in each corresponding Figure.  **(H-J)** Western
290    blot pictures illustrating the expression of mTOR, ERBB2, and the related protein control
291    GAPDH, in the sciatic nerves of $Fgd4^{SC-/-}$ and control animals (see **Figure 2**). The yellow
292    rectangle represents the western blot part used in each corresponding Figure. **K-M)** Western
293    blot pictures illustrating the expression of P-AKT, AKT, and the related protein control

294    GAPDH, in the sciatic nerves of $Fgd4^{SC-/-}$ and control animals (see **Figure 2**). The yellow

295    rectangle represents the western blot part used in each corresponding Figure **(N-O)** Western

296    blot pictures illustrating the expression of RAB11 as well as the related protein control

297    GAPDH in $Fgd4^{SC-/-}$ and control cocultures conditions (see **Figure 4**). The yellow rectangle

298    represents the western blot part used in each corresponding Figure. **(P-Q)** Western blot

299    pictures illustrating the expression of RAB5 as well as the related protein control GAPDH in

300    $Fgd4^{SC-/-}$ and control cocultures conditions (see **Figure 4**). The yellow rectangle represents

301    the western blot part used in each corresponding Figure.

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304    **Supplementary Figure 6. Full western-blot membranes related to Figures 5, 6 and**

305    **Supplementary Figures 1 and 2.**

306    **(A)** Western blot picture illustrating the immunoprecipitation of SNX3 and FRABIN in

307    HEK293 cells overexpressing V5-tagged FRABIN. SNX3 was detected using an anti-SNX3

308    antibody after immunoprecipitation of V5-FRABIN using an anti-V5 antibody (see **Figure 5**).

309    The yellow rectangle represents the western blot part used in the corresponding Figure. **(B-C)**

310    Western blot pictures illustrating the expression of SNX3 and the related protein control

311    Tubulin in $Fgd4^{SC-/-}$ and control cocultures conditions (see **Figure 4**). **(D-E)** Full western blot

312    pictures illustrating the expression of ERBIN, MAF1 (see **Supplementary Figure 2**), SNX3

313    (see **Figure 5**), and the related protein control GAPDH, in the sciatic nerves of $Fgd4^{SC-/-}$ and

314    control animals. The yellow rectangle represents the western blot part used in each

315    corresponding Figure. **(F-H)** Western blot pictures illustrating the expression of P-ERBB2,

316    ERBB2 and the related protein control GAPDH in $Fgd4^{SC-/-}$ and control cocultures conditions

317    (see **Figure 6**). The yellow rectangle represents the western blot part used in each

318    corresponding Figure. **(I-J)** Western blot pictures illustrating the expression of cleaved and

319    full-length Neuregulin 1-type III and the related protein control Tubulin in $Fgd4^{SC-/-}$ and

320    control cocultures conditions (see **Supplementary Figure 1**). The yellow rectangle represents

321    the western blot part used in each corresponding Figure. **(K-L)** Full western blot pictures

322    illustrating the expression of cleaved and full-length Neuregulin 1-type III and the related

323    protein control GAPDH in the sciatic nerves of $Fgd4^{SC-/-}$ and control animals (see

324    **Supplementary Figure 1**). **(M)** Western blot pictures illustrating the expression of SNX3 in

325    primary rat SCs infected with Lentivirus-SHcontrol, LV-SH-SNX3-1 or 2 (see

326    **Supplementary Figure 3).** The yellow rectangle represents the western blot part used in each

327    corresponding Figure.

328

**Supplementary Table1. Results of Differential Gene Expression analysis for a selected list of genes involved in pathways relevant to PNS myelination.**

Genes with significant deregulation are highlighted in bold (padj value<0.05). A Fold Change (FC) threshold of 1.5 and 0.5 has been chosen for up- and down-regulation respectively.

**Genes with padj<0.05 and FC >1.5 or <0.5 are in highlighted in bold blue**.
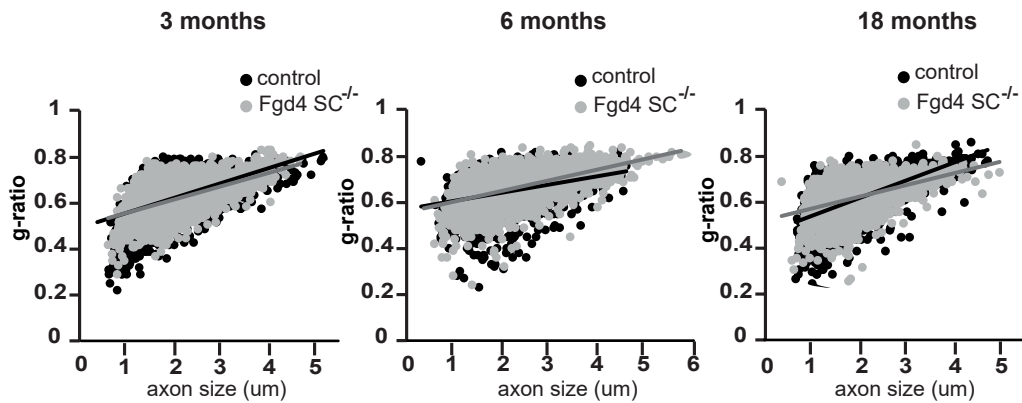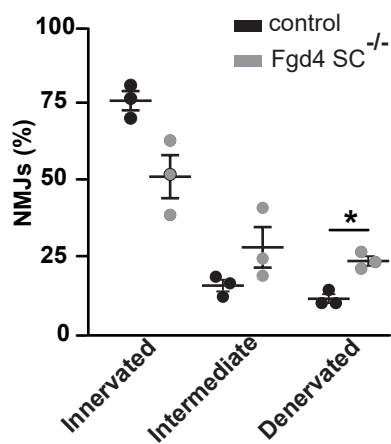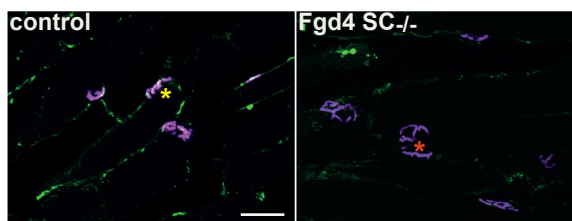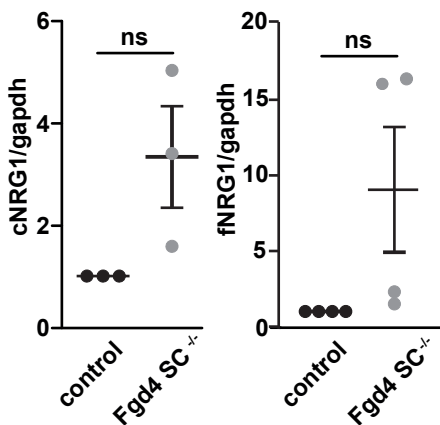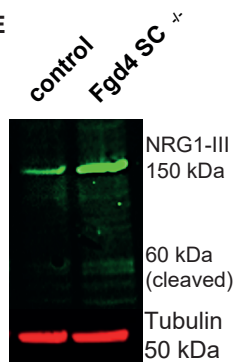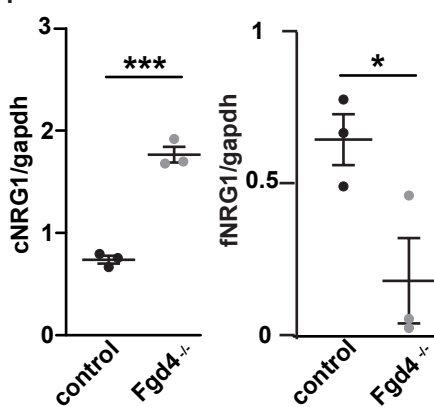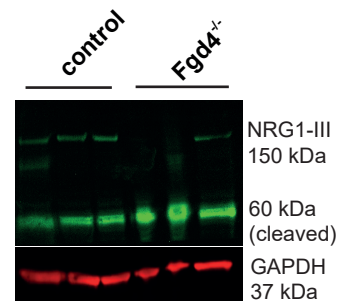
*FC=Fold Change; padj=padjusted value. Basemean represents the average of the normalized count values, dividing by size factors, taken over all samples. Pvalue=P-value of the test for the gene or transcript. padj=Adjusted P-value for multiple testing for the gene or transcript.NA=Not Assessed*

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**A**

3 months　　　6 months　　　18 months

● control
● Fgd4 SC$^{-/-}$

g-ratio / axon size (um)

**B**



NMJs (%)

■ control
■ Fgd4 SC$^{-/-}$

Innervated　Intermediate　Denervated

*

**C**



control　　Fgd4 SC-/-

**D**



cNRG1/gapdh

ns

control　Fgd4 SC$^{-/-}$

fNRG1/gapdh

ns

control　Fgd4 SC$^{-/-}$

**E**



control　Fgd4 SC$^{-/-}$

NRG1-III
150 kDa

60 kDa
(cleaved)

Tubulin
50 kDa

**F**



cNRG1/gapdh

***

control　Fgd4$^{-/-}$

fNRG1/gapdh

*

control　Fgd4$^{-/-}$

**G**



control　　Fgd4$^{-/-}$

NRG1-III
150 kDa

60 kDa
(cleaved)

GAPDH
37 kDa

**Supplementary Figure 1**

**A**



**B**



**Supplementary Figure 2**

**A**



**B**



SNX3
19 kDa

Tubulin
50 kDa

**C**

**Lv-SHFgd4-EGFP**



GFP

FRABIN

merge

**Supplementary Figure 3**

**A. control**

**B. Fgd4^SC-/-**

Supplementary Figure 4

**Supplementary Figure 5**

**A**

kDa

Total lysate
Flow-through
IP lysate

140
100
50
35
25
15
10

FRABIN
(105 kDa)

SNX3
(19 kDa)

**B**

kDa

control
Fgd4 SC⁻ʼ⁻

38
25
8

SNX3
(19 kDa)

**C**

kDa

control
Fgd4 SC⁻ʼ⁻

160
125
90
50

Tubulin
(50 kDa)

**D**

kDa

control          Fgd4 SC⁻ʼ⁻

260
160
125
90
50
30
15
8

ERBIN
(158kDa)

MAF-1
(58kDa)

SNX3
(19 kDa)

**E**

kDa

control          Fgd4 SC

50
30

GAPDH
(37 kDa)

**F**

kDa

Fgd4 SC⁻ʼ⁻
Fgd4 SC⁻ʼ⁻ +
Niacin

160

P-ERBB2
(185 kDa)

**G**

kDa

Fgd4 SC⁻ʼ⁻
Fgd4 SC⁻ʼ⁻ +
Niacin

260
90

ERBB2
(185 kDa)

**H**

kDa

Fgd4 SC⁻ʼ⁻
Fgd4 SC⁻ʼ⁻ +
Niacin

38

GAPDH
(37 Kka)

**I**

kDa

control
Fgd4 SC⁻ʼ⁻

260
160
125
70

NRG1-III
150kDa

60kDa
(cleaved)

**J**

kDa

control
Fgd4 SC⁻ʼ⁻

160
125
90
50

Tubulin
(50 kDa)

**K**

kDa

control          Fgd4 SC⁻ʼ⁻

260
125
70

NRG1-III
150kDa

60kDa
(cleaved)

**L**

kDa

control          Fgd4 SC⁻ʼ⁻

50
30

GAPDH
(37 kDa)

**M**

kDa

SHcontrol
SH-SNX3-1
SH-SNX3-2

260
160
125
90
70
50
38
30
15

Tubulin
(50 kDa)

SNX3
(19 kDa)

**Supplementary Figure 6**

| padj | pvalue | Ensembl ID | HGNC Gene symbol | baseMean | log2(FC) | FC |
|---|---|---|---|---|---|---|
| **NRG1-EBB2-3 pathway** | | | | | | |
| 0.43819 | 0.180994 | ENSMUSG00000062991.7|Nrg1 | *Nrg1* | 118.948 | -0.54732 | 0.68429 |
| 0.28716 | 0.088773 | ENSMUSG00000032311.17|Nrg4 | *Nrg4* | 26.50165 | -1.31886 | 0.400852 |
| 0.141851 | 0.030578 | ENSMUSG00000062312.5|Erbb2 | *Erbb2* | 3855.963 | 0.366331 | 1.28907 |
| **0.006345** | **0.000445** | **ENSMUSG00000018166.8|Erbb3** | **Erbb3** | **7345.251** | **0.487969** | **1.402469** |
| 0.472484 | 0.206585 | ENSMUSG00000062209.15|Erbb4 | *Erbb4* | 40.39399 | -0.86434 | 0.549299 |
| **0.000342** | **1.18E-05** | **ENSMUSG00000021709.14|Erbin** | **Erbin** | **10892.24** | **0.72783** | **1.656146** |
| 0.086574 | 0.015001 | ENSMUSG00000013663.7|Pten | *Pten* | 12586.44 | 0.300629 | 1.231681 |
| 0.041169 | 0.005329 | ENSMUSG00000022770.16|Dlg1 | *Dlg1* | 7211.615 | 0.456034 | 1.371765 |
| 0.124126 | 0.025167 | ENSMUSG00000041417.15|Pik3r1 | *Pik3r1* | 12356 | 0.292736 | 1.224961 |
| 0.162919 | 0.037558 | ENSMUSG00000034614.14|Pik3ip1 | *Pik3ip1* | 2432.501 | -0.32628 | 0.797592 |
| 0.374588 | 0.138018 | ENSMUSG00000028698.13|Pik3r3 | *Pik3r3* | 2474.129 | 0.228918 | 1.171955 |
| 0.435797 | 0.179052 | ENSMUSG00000033628.15|Pik3c3 | *Pik3c3* | 1632.136 | 0.217502 | 1.162719 |
| 0.485684 | 0.216219 | ENSMUSG00000032571.14|Pik3r4 | *Pik3r4* | 2075.567 | 0.17431 | 1.128425 |
| 0.711939 | 0.44546 | ENSMUSG00000031834.15|Pik3r2 | *Pik3r2* | 3950.073 | 0.105829 | 1.076112 |
| 0.721107 | 0.456363 | ENSMUSG00000032462.14|Pik3cb | *Pik3cb* | 433.0512 | 0.217911 | 1.163048 |
| 0.996264 | 0.989948 | ENSMUSG00000026447.16|Pik3c2b | *Pik3c2b* | 276.4396 | -0.00377 | 0.99739 |
| NA | 0.015473 | ENSMUSG00000046207.14|Pik3r6 | *Pik3r6* | 5.920665 | 5.729172 | 53.04601 |
| **6.29E-06** | **9.37E-08** | **ENSMUSG00000030660.9|Pik3c2a** | **Pik3c2a** | **3826.552** | **0.977572** | **1.969149** |
| **1.34E-05** | **2.35E-07** | **ENSMUSG00000025017.9|Pik3ap1** | **Pik3ap1** | **461.9112** | **2.25651** | **4.778342** |
| **7.35E-05** | **1.79E-06** | **ENSMUSG00000020573.17|Pik3cg** | **Pik3cg** | **117.1629** | **3.574393** | **11.91241** |
| **0.009386** | **0.000736** | **ENSMUSG00000039936.18|Pik3cd** | **Pik3cd** | **674.6376** | **0.635754** | **1.553749** |
| **0.020476** | **0.002102** | **ENSMUSG00000020901.13|Pik3r5** | **Pik3r5** | **378.2187** | **1.176062** | **2.259592** |
| **0.03711** | **0.004613** | **ENSMUSG00000027665.13|Pik3ca** | **Pik3ca** | **5124.153** | **0.377022** | **1.298659** |
| 0.891783 | 0.741992 | ENSMUSG00000001729.14|Akt1 | *Akt1* | 9988.497 | 0.039302 | 1.027616 |
| 0.96188 | 0.905924 | ENSMUSG00000004056.15|Akt2 | *Akt2* | 7633.627 | 0.016005 | 1.011156 |
| 0.039456 | 0.005015 | ENSMUSG00000019699.16|Akt3 | *Akt3* | 3501.403 | 0.387865 | 1.308455 |
| 0.580322 | 0.297197 | ENSMUSG00000028161.17|Ppp3ca | *Ppp3ca* | 8459.122 | 0.12355 | 1.089412 |

| | | | | | |
|---|---|---|---|---|---|
| 0.791934 | 0.558113 | ENSMUSG00000022092.10|Ppp3cc | Ppp3cc | 681.3545 | -0.11055 | 0.926235 |
| 0.876171 | 0.708198 | ENSMUSG00000028310.2|Ppp3r2 | Ppp3r2 | 13.4879 | -0.40122 | 0.757217 |
| 0.070239 | 0.011147 | ENSMUSG00000021816.11|Ppp3cb | Ppp3cb | 4763.976 | 0.345142 | 1.270276 |
| 0.654851 | 0.372752 | ENSMUSG00000059923.13|Grb2 | Grb2 | 3646.282 | 0.114613 | 1.082685 |
| 0.749643 | 0.497543 | ENSMUSG00000016933.17|Plcg1 | Plcg1 | 4356.898 | 0.090765 | 1.064935 |
| **0.015597** | **0.001445** | **ENSMUSG00000034330.10|Plcg2** | **Plcg2** | **146.1442** | **1.944124** | **3.848041** |
| 0.30888 | 0.100147 | ENSMUSG00000042626.13|Shc1 | Shc1 | 9074.716 | 0.216848 | 1.162192 |
| 0.745429 | 0.492031 | ENSMUSG00000020312.12|Shc2 | Shc2 | 1858.432 | 0.103934 | 1.0747 |
| 0.553667 | 0.272862 | ENSMUSG00000035109.14|Shc4 | Shc4 | 977.9834 | 0.233873 | 1.175988 |
| 0.808209 | 0.583182 | ENSMUSG00000021448.7|Shc3 | Shc3 | 69.73623 | 0.336288 | 1.262504 |
| 0.899781 | 0.757653 | ENSMUSG00000022322.8|Shcbp1 | Shcbp1 | 367.8136 | -0.09946 | 0.933385 |
| **0.020802** | **0.00215** | **ENSMUSG00000031714.9|Gab1** | **Gab1** | **10659.16** | **0.390408** | **1.310764** |
| 0.894073 | 0.746486 | ENSMUSG00000001847.14|Rac1 | Rac1 | 16699.4 | 0.043314 | 1.030478 |
| 0.850086 | 0.658274 | ENSMUSG00000006699.17|Cdc42 | Cdc42 | 21798.67 | -0.05597 | 0.961945 |
| **0.000462** | **1.73E-05** | **ENSMUSG00000024241.6|Sos1** | **Sos1** | **2598.422** | **0.628269** | **1.545709** |
| 0.955817 | 0.89115 | ENSMUSG00000025225.14|Nfkb2 | Nfkb2 | 1618.804 | 0.023708 | 1.016569 |
| 0.970434 | 0.924565 | ENSMUSG00000030595.15|Nfkbib | Nfkbib | 416.7245 | 0.022398 | 1.015646 |
| 0.340651 | 0.118227 | ENSMUSG00000023947.7|Nfkbie | Nfkbie | 489.3592 | -0.3758 | 0.770676 |
| 0.343576 | 0.119708 | ENSMUSG00000028163.17|Nfkb1 | Nfkb1 | 3895.871 | 0.206704 | 1.154049 |
| 0.351699 | 0.12434 | ENSMUSG00000035356.16|Nfkbiz | Nfkbiz | 722.8807 | 0.386129 | 1.306882 |
| 0.624303 | 0.33987 | ENSMUSG00000042419.8|Nfkbil1 | Nfkbil1 | 329.5072 | -0.25265 | 0.839354 |
| 0.631793 | 0.346968 | ENSMUSG00000021025.8|Nfkbia | Nfkbia | 1390.241 | 0.16228 | 1.119054 |
| 0.830571 | 0.621747 | ENSMUSG00000036931.15|Nfkbid | Nfkbid | 21.94257 | 0.447472 | 1.363649 |
| 0.916137 | 0.79385 | ENSMUSG00000063065.13|Mapk3 | Mapk3 | 7246.235 | 0.041078 | 1.028883 |
| 0.372786 | 0.137078 | ENSMUSG00000063358.15|Mapk1 | Mapk1 | 7794.191 | 0.188154 | 1.139305 |
| **0.019785** | **0.001995** | **ENSMUSG00000021754.17|Map3k1** | **Map3k1** | **1746.545** | **0.56038** | **1.474657** |
| 0.546057 | 0.266104 | ENSMUSG00000035027.18|Map2k2 | Map2k2 | 2303.944 | -0.20246 | 0.869068 |
| 0.566792 | 0.284138 | ENSMUSG00000052837.6|Junb | Junb | 1418.494 | -0.18866 | 0.877423 |
| 0.835999 | 0.632831 | ENSMUSG00000071076.6|Jund | Jund | 4019.524 | -0.09702 | 0.934961 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.96498 | 0.910985 | ENSMUSG00000052684.4\|Jun | *Jun* | 9504.418 | 0.013414 | 1.009341 |
| 0.243296 | 0.068482 | ENSMUSG00000020516.15\|Rps6kb1 | *Rps6kb1* | 2809.235 | 0.277378 | 1.21199 |
| 0.279578 | 0.085021 | ENSMUSG00000028991.15\|Mtor | *Mtor* | 2649.592 | 0.235803 | 1.177562 |
| 0.247636 | 0.070246 | ENSMUSG00000026812.16\|Tsc1 | *Tsc1* | 1431.294 | 0.297643 | 1.229135 |
| 0.611303 | 0.327886 | ENSMUSG00000002496.9\|Tsc2 | *Tsc2* | 3582.222 | 0.127857 | 1.092669 |
| **0.00173** | **8.68E-05** | **ENSMUSG00000042406.7\|Atf4** | ***Atf4*** | **7319.911** | **-0.57001** | **0.673613** |
| **0.001872** | **9.61E-05** | **ENSMUSG00000005667.8\|Mthfd2** | ***Mthfd2*** | **1109.636** | **-0.90232** | **0.535026** |
| **0.011004** | **0.000912** | **ENSMUSG00000031490.6\|Eif4ebp1** | ***Eif4ebp1*** | **1254.967** | **-0.66088** | **0.632493** |
| 0.762307 | 0.515451 | ENSMUSG00000028156.12\|Eif4e | *Eif4e* | 3268.458 | 0.08441 | 1.060254 |
| **8.91E-05** | **2.27E-06** | **ENSMUSG00000003847.16\|Nfat5** | ***Nfat5*** | **6856.594** | **0.709679** | **1.63544** |
| **0.023569** | **0.002554** | **ENSMUSG00000031902.10\|Nfatc3** | ***Nfatc3*** | **4338.228** | **0.461474** | **1.376948** |
| 0.253156 | 0.072699 | ENSMUSG00000023411.11\|Nfatc4 | *Nfatc4* | 1795.49 | -0.29686 | 0.814023 |
| **Endocytic trafficking** | | | | | | |
| 0.293808 | 0.092099 | ENSMUSG00000019804.12\|Snx3 | *Snx3* | 4878.23 | -0.24198 | 0.845583 |
| **0.002403** | **0.00013** | **ENSMUSG00000071669.14\|Snx29** | ***Snx29*** | **406.6079** | **0.919201** | **1.891068** |
| 0.128409 | 0.026519 | ENSMUSG00000022500.14\|Litaf | *Litaf* | 4149.401 | -0.37776 | 0.769633 |
| 0.296879 | 0.093789 | ENSMUSG00000017831.7\|Rab5a | *Rab5a* | 5292.429 | 0.225821 | 1.169442 |
| 0.57979 | 0.296661 | ENSMUSG00000027637.3\|1110008F13Rik | *Rab5if* | 1993.569 | -0.17004 | 0.88882 |
| 0.642659 | 0.359081 | ENSMUSG00000000711.3\|Rab5b | *Rab5b* | 6268.775 | 0.119281 | 1.086193 |
| 0.998431 | 0.995078 | ENSMUSG00000019173.11\|Rab5c | *Rab5c* | 5851.344 | -0.00077 | 0.999463 |
| **0.000378** | **1.35E-05** | **ENSMUSG00000040022.14\|Rab11fip2** | ***Rab11fip2*** | **1744.883** | **0.764147** | **1.698365** |
| 0.41397 | 0.163463 | ENSMUSG00000017639.13\|Rab11fip4 | *Rab11fip4* | 277.7209 | -0.57684 | 0.67043 |
| 0.886834 | 0.730381 | ENSMUSG00000037098.17\|Rab11fip3 | *Rab11fip3* | 2391.225 | 0.048893 | 1.034471 |
| 0.901869 | 0.763519 | ENSMUSG00000031488.14\|Rab11fip1 | *Rab11fip1* | 99.6553 | 0.146736 | 1.107062 |
| 0.997491 | 0.992665 | ENSMUSG00000004771.12\|Rab11a | *Rab11a* | 3994.743 | -0.00141 | 0.999025 |
| 0.908187 | 0.780765 | ENSMUSG00000077450.12\|Rab11b | *Rab11b* | 5658.079 | -0.03805 | 0.973969 |
| 0.941931 | 0.851932 | ENSMUSG00000051343.11\|Rab11fip5 | *Rab11fip5* | 2767.754 | -0.02548 | 0.982492 |
| **Myelin genes** | | | | | | |
| 0.898169 | 0.754411 | ENSMUSG00000056569.10\|Mpz | *Mpz* | 21482.08 | -0.22693 | 0.854452 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.144957 | 0.031642 | ENSMUSG00000018217.12\|Pmp22 | *Pmp22* | 16422.81 | -0.49145 | 0.71131 |
| 0.680595 | 0.403483 | ENSMUSG00000041607.16\|Mbp | *Mbp* | 10741.13 | -0.6543 | 0.635384 |
| 0.552567 | 0.271691 | ENSMUSG00000036634.15\|Mag | *Mag* | 382.9271 | -1.24442 | 0.422078 |
| 0.377353 | 0.139799 | ENSMUSG00000047797.14\|Gjb1 | *Gjb1* | 13.37527 | 1.909331 | 3.756348 |
| 0.762179 | 0.515278 | ENSMUSG00000037868.15\|Egr2 | *Egr2* | 1131.125 | 0.163869 | 1.120288 |
| 0.622139 | 0.337443 | ENSMUSG00000033006.9\|Sox10 | *Sox10* | 7814.012 | 0.17023 | 1.125238 |
| 0.407887 | 0.159458 | ENSMUSG00000090125.3\|Pou3f1 | *Pou3f1* | 336.5669 | 0.577193 | 1.491943 |
| 0.210427 | 0.054811 | ENSMUSG00000095139.2\|Pou3f2 | *Pou3f2* | 137.4265 | -0.92968 | 0.524976 |
| 0.704889 | 0.436666 | ENSMUSG00000052468.7\|Pmp2 | *Pmp2* | 838.2964 | -0.32763 | 0.796846 |
| 0.530544 | 0.253464 | ENSMUSG00000053198.13\|Prx | *Prx* | 2837.423 | -0.36805 | 0.774827 |
| **Lipid metabolism and cholesterol synthesis** | | | | | | |
| **8.21E-05** | **2.06E-06** | **ENSMUSG00000055435.6\|Maf** | ***Maf*** | **6049.204** | **0.657916** | **1.577801** |
| 0.545362 | 0.265642 | ENSMUSG00000020538.15\|Srebf1 | *Srebf1* | 4318.675 | 0.190077 | 1.140825 |
| 0.765008 | 0.519542 | ENSMUSG00000022463.7\|Srebf2 | *Srebf2* | 8066.606 | 0.082783 | 1.059059 |
| **ECM/integrins** | | | | | | |
| **3.88E-05** | **8.31E-07** | **ENSMUSG00000027111.15\|Itga6** | ***Itga6*** | **12394.81** | **0.982093** | **1.97533** |
| **2.90E-09** | **1.70E-11** | **ENSMUSG00000039115.13\|Itga9** | ***Itga9*** | **7156.76** | **0.905284** | **1.872913** |
| **0.000474** | **1.79E-05** | **ENSMUSG00000030786.18\|Itgam** | ***Itgam*** | **507.6395** | **4.037302** | **16.41909** |
| **0.000492** | **1.88E-05** | **ENSMUSG00000032243.8\|Itga11** | ***Itga11*** | **1407.479** | **-0.81339** | **0.569043** |
| **0.001767** | **8.95E-05** | **ENSMUSG00000030830.18\|Itgal** | ***Itgal*** | **100.9593** | **3.273645** | **9.670868** |
| **0.003732** | **0.000229** | **ENSMUSG00000027087.11\|Itgav** | ***Itgav*** | **9771.51** | **0.43798** | **1.354706** |
| **0.012968** | **0.001124** | **ENSMUSG00000090210.7\|Itga10** | ***Itga10*** | **640.1986** | **-0.73403** | **0.601221** |
| 0.066913 | 0.010474 | ENSMUSG00000027009.18\|Itga4 | *Itga4* | 381.0249 | 0.63189 | 1.549593 |
| 0.127424 | 0.026148 | ENSMUSG00000026768.10\|Itga8 | *Itga8* | 5094.602 | 0.53972 | 1.453691 |
| 0.299509 | 0.095298 | ENSMUSG00000000555.7\|Itga5 | *Itga5* | 4157.454 | -0.2217 | 0.857554 |
| 0.354608 | 0.126038 | ENSMUSG00000034664.13\|Itga2b | *Itga2b* | 173.4799 | -0.57587 | 0.67088 |
| 0.598459 | 0.315172 | ENSMUSG00000001507.16\|Itga3 | *Itga3* | 1174.635 | 0.180398 | 1.133197 |
| 0.641351 | 0.357489 | ENSMUSG00000042284.10\|Itga1 | *Itga1* | 14742 | 0.142267 | 1.103638 |
| 0.943815 | 0.857184 | ENSMUSG00000015533.9\|Itga2 | *Itga2* | 1249.488 | 0.03837 | 1.026953 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.996754 | 0.991393 | ENSMUSG00000025348.9|Itga7 | *Itga7* | 8358.219 | -0.0015 | 0.99896 |
| **9.88E-20** | **1.07E-22** | **ENSMUSG00000025321.14|Itgb8** | ***Itgb8*** | **19569.38** | **1.176076** | **2.259613** |
| **7.32E-07** | **8.29E-09** | **ENSMUSG00000000290.13|Itgb2** | ***Itgb2*** | **228.669** | **3.307438** | **9.900066** |
| **0.0031** | **0.000179** | **ENSMUSG00000020689.4|Itgb3** | **Itgb3** | **2213.103** | **0.581636** | **1.496545** |
| 0.056372 | 0.008227 | ENSMUSG00000022817.14|Itgb5 | *Itgb5* | 6858.318 | -0.51889 | 0.697907 |
| 0.447299 | 0.186731 | ENSMUSG00000025809.15|Itgb1 | *Itgb1* | 64736.22 | 0.157445 | 1.11531 |
| 0.491626 | 0.220459 | ENSMUSG00000028549.17|Itgb3bp | *Itgb3bp* | 298.5605 | -0.35543 | 0.781634 |
| 0.653405 | 0.371103 | ENSMUSG00000020758.15|Itgb4 | *Itgb4* | 9286.396 | 0.160876 | 1.117965 |
| 0.785845 | 0.549528 | ENSMUSG00000062352.13|Itgb1bp1 | *Itgb1bp1* | 1146.963 | -0.09858 | 0.93395 |
| 0.853009 | 0.664619 | ENSMUSG00000001281.9|Itgb7 | *Itgb7* | 300.1425 | 0.1541 | 1.112727 |
| 0.856983 | 0.671372 | ENSMUSG00000032925.16|Itgbl1 | *Itgbl1* | 9900.586 | -0.07517 | 0.949233 |
| 0.747194 | 0.494004 | ENSMUSG00000019899.16|Lama2 | *Lama2* | 12565.15 | -0.09002 | 0.939512 |
| 0.937544 | 0.843337 | ENSMUSG00000002900.16|Lamb1 | *Lamb1* | 19794.36 | -0.03484 | 0.97614 |
| 0.400955 | 0.155105 | ENSMUSG00000026478.14|Lamc1 | *Lamc1* | 32910.12 | 0.177825 | 1.131177 |
| 0.918246 | 0.797716 | ENSMUSG00000022607.14|Ptk2 | *Ptk2* | 4506.624 | 0.031356 | 1.021972 |
| **GPCR signaling** | | | | | | |
| **9.82E-05** | **2.54E-06** | **ENSMUSG00000063234.4|Gpr84** | ***Gpr84*** | **28.17772** | **7.978702** | **252.2485** |
| **0.000138** | **3.93E-06** | **ENSMUSG00000040133.2|Gpr176** | ***Gpr176*** | **695.3308** | **-1.13642** | **0.454888** |
| **0.010094** | **0.000815** | **ENSMUSG00000021886.7|Gpr65** | ***Gpr65*** | **42.05601** | **3.398591** | **10.54576** |
| **0.022427** | **0.002378** | **ENSMUSG00000040836.15|Gpr161** | ***Gpr161*** | **1346.138** | **0.52714** | **1.441069** |
| **0.041099** | **0.005306** | **ENSMUSG00000046961.7|Gpr156** | ***Gpr156*** | **131.4803** | **-1.51648** | **0.349539** |
| **cAMP signaling** | | | | | | |
| **2.56E-08** | **1.85E-10** | **ENSMUSG00000022376.7|Adcy8** | ***Adcy8*** | **131.2815** | **-2.55334** | **0.17036** |
| **1.95E-05** | **3.72E-07** | **ENSMUSG00000005580.11|Adcy9** | ***Adcy9*** | **531.147** | **1.20252** | **2.301413** |
| **0.00027** | **8.96E-06** | **ENSMUSG00000031659.13|Adcy7** | ***Adcy7*** | **8184.577** | **0.688989** | **1.612153** |
| **0.007014** | **0.000502** | **ENSMUSG00000024256.6|Adcyap1** | ***Adcyap1*** | **418.6966** | **1.101277** | **2.145444** |
| **0.009386** | **0.000735** | **ENSMUSG00000022840.8|Adcy5** | ***Adcy5*** | **2227.753** | **0.540373** | **1.454348** |
| **9.38E-09** | **5.92E-11** | **ENSMUSG00000029778.12|Adcyap1r1** | ***Adcyap1r1*** | **1375.504** | **1.240339** | **2.36254** |
| **Other signaling pathways** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.003026 | 0.000172 | **ENSMUSG00000015340.10\|Cybb** | *Cybb* | 413.9266 | 4.116552 | 17.34625 |
| 0.011383 | 0.000949 | **ENSMUSG00000042286.13\|Stab1** | *Stab1* | 1920.658 | 2.782242 | 6.879205 |
| 0.005292 | 0.00035 | **ENSMUSG00000024401.14\|Tnf** | *Tnf* | 26.17281 | 4.397772 | 21.07954 |
| 0.005004 | 0.000328 | **ENSMUSG00000045382.6\|Cxcr4** | *Cxcr4* | 141.6688 | -1.44541 | 0.367187 |

**Supplementary Table1. Results of Differential Gene Expression analysis for a selected list of genes involved in pathways relevant to PNS myelination.**

Genes with significant deregulation are highlighted in bold (padj value<0.05). A Fold Change (FC) threshold of 1.5 and 0.5 has been chosen for up- and down-regulation respectively. **Genes with padj<0.05 and FC >1.5 or <0.5 are in highlighted in bold blue**.

*FC=Fold Change; padj=padjusted value. Basemean represents the average of the normalized count values, dividing by size factors, taken over all samples. Pvalue=P-value of the test for the gene or transcript. padj=Adjusted P-value for multiple testing for the gene or transcript.NA=Not Assessed*