1 Timely Schwann cell division during migration drives peripheral myelination *in vivo* via 2 Laminin/cAMP pathway

3 **Keywords:** *sil*; Schwann cells; peripheral nervous system; mitotic spindle; MCPH; zebrafish;
4 myelin; PLLn; mitosis; Laminin; cAMP.

5 Aya Mikdache^{1,#,λ}, Marie-José Boueid^{1,λ}, Emilie Lesport¹, Brigitte Delespierre¹, Julien Loisel-
6 Duwattez¹, Cindy Degerny^{1,*}, Marcel Tawk^{1,*}

7 * To whom correspondence should be addressed, e-mail: [cindy.degerny@universite-paris-](mailto:cindy.degerny@universite-paris-saclay.fr)
8 [saclay.fr](mailto:marcel.tawk@inserm.fr); marcel.tawk@inserm.fr

9 ¹ U1195, Inserm, University Paris-Saclay, 94276 Le Kremlin Bicêtre, France,

10 [#] A Mikdache present address: UMR 3215 – U934, Institut Curie, 75005 Paris, France

11 ^λ These authors contributed equally

12

13 Summary

14 Schwann cells (SC) migrate along peripheral axons and divide intensively to generate the
15 right number of cells prior to axonal ensheathment; however, little is known regarding the
16 temporal and molecular control of their division, particularly during migration, and its impact
17 on myelination. We report that *Sil*, a spindle pole protein associated with autosomal recessive
18 primary microcephaly (MCPH), is required for temporal mitotic exit of SC. In *sil*-deficient
19 *cassiopeia* (*csp*^{-/-}) mutants, SC fail to radially sort and myelinate peripheral axons. Elevation
20 of cAMP, but not Rac1 activity in *csp*^{-/-} restores myelin ensheathment. Most importantly, we
21 show a significant decrease in Laminin expression within *csp*^{-/-} posterior lateral line nerve and
22 that forcing Laminin2 expression in *csp*^{-/-} fully restores SC ability to myelinate. We also
23 discovered that SC have a restricted time window during which they have to divide, while
24 migrating, in order to trigger myelination. Thus, we unravel a novel and essential role for

25 timely SC division during migration in mediating Laminin expression to orchestrate radial
26 sorting and peripheral myelination *in vivo*.

27

28 **Introduction**

29 Schwann cells are the myelinating glia that ensure efficient nerve impulse conduction along
30 the nerves of the peripheral nervous system (PNS) (Boucanova & Chrast, 2020, Herbert &
31 Monk, 2017, Jessen & Mirsky, 2005, Pereira, Lebrun-Julien et al., 2012, Raphael, Lyons et
32 al., 2011, Sherman & Brophy, 2005, Woodhoo & Sommer, 2008). In order to myelinate,
33 Schwann cells go through a series of developmental changes that include i) migration and
34 division along peripheral axons ii) intensive proliferation prior to axonal ensheathment given
35 that one Schwann cell myelinates one axonal segment and iii) substantial cytoskeletal
36 rearrangements and changes in cell shape that allow a Schwann cell to radially sort an axon in
37 a 1:1 ratio, a process termed radial sorting (Feltri, Poitelon et al., 2016, Monk, Feltri et al.,
38 2015, Raphael & Talbot, 2011). The latter enables Schwann cells to extend their processes
39 along a specific and unique abaxonal-adaxonal polarity axis in order to select and sort an axon
40 to myelinate (Tricaud, 2017). It has been shown that *in vitro* Schwann cells first secrete and
41 assemble their own basal lamina (BL) (Colognato & Tzvetanova, 2011, Eldridge, Bunge et
42 al., 1989) that would later on regulate, through receptor interactions, different signals required
43 for Schwann cell proliferation, survival and differentiation (Chen & Strickland, 2003, Court,
44 Hewitt et al., 2009, Nodari, Previtali et al., 2008, Nodari, Zambroni et al., 2007, Yamada,
45 Denzer et al., 1996, Yu, Feltri et al., 2005). Of particular interest are the extracellular matrix
46 (ECM) proteins Laminins and Collagens that play essential roles in Schwann cell
47 development (Chernousov, Yu et al., 2008).

48 However, several issues regarding the characteristics of timely Schwann cell division during
49 migration and radial sorting, as well as the coupling of division with ECM proteins and
50 myelination *in vivo* remain unresolved.

51 The mitotic spindle is a bipolar array of microtubules that mediates chromosome separation
52 during cell division (Hara & Fukagawa, 2020, Petry, 2016). The organization and temporal
53 assembly of the spindle are all important features that dictate the outcome of division (Lu &
54 Johnston, 2013). One critical aspect of cell division is the temporal control of mitosis; this is
55 highlighted by the existence of a mitotic spindle checkpoint that ensures accurate mitotic
56 spindle organization prior to anaphase. It generates a wait signal until mitotic checkpoint
57 proteins are removed from the kinetochore during metaphase by dynein/dynactin, clearing the
58 way for accurate segregation of chromosomes into daughter cells (Lara-Gonzalez, Westhorpe
59 et al., 2012). A great number of proteins is involved in spindle organization, one such major
60 player is Sil that is ubiquitously expressed and specifically localizes to the poles of the mitotic
61 spindle in metaphase cells (Campaner, Kaldis et al., 2005, Pfaff, Straub et al., 2007). The *sil*
62 gene was originally cloned from leukemia-associated chromosomal translocation and is
63 overexpressed in several tumor types such as melanoma and lung carcinomas (Aplan,
64 Lombardi et al., 1990, Aplan, Lombardi et al., 1991). It is associated with autosomal recessive
65 primary microcephaly (MCPH), a neurogenic mitotic disorder that results in significantly
66 reduced brain size (Naveed, Kazmi et al., 2018, Zaqout, Morris-Rosendahl et al., 2017).
67 Studies of zebrafish *cassiopeia* mutant (*csp*^{-/-}), which has a nonsense mutation and a loss of
68 function in zebrafish *sil*, show abnormalities in prometaphase progression within retinal
69 neuroepithelium (Novorol, Burkhardt et al., 2013). Thus, *sil* represents an ideal candidate to
70 study for a role in mitotic synchronization and division during Schwann cells' development.
71 Here, we used live imaging, genetics and pharmacological tools in zebrafish to monitor
72 Schwann cell behavior during its division and unraveled for the first time an essential role for

early Schwann division during migration in peripheral myelination. We identified *Sil* as a critical regulator of Schwann cell radial sorting and myelination. Time-lapse imaging revealed an important role for *sil* in Schwann cell metaphase progression that delayed mitotic exit and caused a significant decrease of peripheral myelin markers, *krox20* and *mbp* in *csp*^{-/-} embryos. This coincided with a complete loss of axonal ensheathment as revealed by transmission electron microscopy (TEM). Treating *csp*^{-/-} with forskolin that binds to adenylyl cyclase and elevates the levels of cAMP, restored radial sorting, myelin-associated genes expression and axonal wrapping. Moreover, *csp*^{-/-} mutants failed to express Laminin within the posterior lateral line nerve (PLLn) and forcing Laminin2 expression was sufficient to fully restore peripheral myelination. These data unraveled an essential role for the timely process of mitotic exit within Schwann cells, specifically during migration, in radial sorting and myelination via Laminin2/cAMP dependent pathway. We provide evidence that Schwann cells have a limited window of time in which they have to divide during their migration in order to initiate radial sorting and myelination.

Results

Schwann cells show a regular pattern of division during radial sorting along the PLLn

In order to monitor Schwann cell division, we took advantage of the *Tg(foxd3:gfp)* (Gilmour, Maischein et al., 2002) and first imaged Schwann cells along the PLLn between 48 and 60 hours post-fertilization (hpf). During this time, Schwann cells divide intensively and start to radially sort axons that precedes axonal ensheathment (Figure S1) (Lyons, Pogoda et al., 2005, Raphael et al., 2011). Schwann cells were generally elongated along the axons until they went through mitotic rounding that led to division and then re-elongated again (Figure 1A). Cells divided along the Anterior-Posterior (AP) axis of the embryo and it took an average of 9.30±0.61 min for mitosis to complete (Figure 1B and Movie S1). To assess the

importance of this pattern of division in Schwann cell myelination, we decided to monitor their behavior in *cassiopeia* mutant (*csp*^{-/-}). Sil is a ubiquitously expressed protein that specifically localizes to the mitotic spindle in metaphase cells and plays a critical role in its organization (Novorol et al., 2013, Pfaff et al., 2007). Schwann cells' mitosis in *csp*^{-/-} took much longer to complete with an average of 90.10±14.13 min in comparison to 9.30±0.61 min in controls (Figure 1A,B and Movie S2). However, re-elongation of Schwann cells occurred normally after division in both mutants and controls (Figure 1A). This result suggests that Sil deficiency leads to a significant delay in Schwann cell mitosis.

Sil is essential for Schwann cell radial sorting and myelination

Having identified a delay in progression through mitosis in *csp*^{-/-} Schwann cells, we wondered whether this defect could have any impact on the ability of Schwann cells to ensheath axons. Transmitted Electron Microscopy (TEM) analysis showed a dramatic decrease in the number of myelinated axons in *csp*^{-/-} in comparison to controls. We observed an average of 5.6±0.55 myelinated axons per nerve in controls in comparison to 0 myelinated axon per nerve in *csp*^{-/-} at 72 hpf (or 3 days post-fertilization (dpf)) (Figure 2A,B,E). In addition, we observed a significant decrease in the total number of axons in these mutants with an average of 55±2.85 axons in controls and 28.63±2.67 in *csp*^{-/-} PLLn (Figure 2F). In order to test whether this defect is maintained at later stages, we analyzed axonal wrapping at 4 dpf. The myelination defect in *csp*^{-/-} embryos detected at 3 dpf was also observed at 4 dpf (Figure 2C-F). This analysis could not be extended to later stages since *csp*^{-/-} embryos died between 4 and 5 dpf. Given the drastic decrease in total number of axons in these mutants, we therefore calculated the ratio of myelinated axons relative to the total number of axons in the PLLn. While we counted an average of 10.46±1.14% of myelinated axons in controls, we observed 0% in *csp*^{-/-} at 3 and 4 dpf (Figure 2G). This myelination defect was not the result of a decrease in the

number of large caliber axons that are supposed to be myelinated (Figure 2H). Finally, the myelination defect coincided with a significant decrease in the number and the percentage of radially sorted axons per nerve (Figure 2I,J).

These results show that *sil* is required for Schwann cell radial sorting and myelination.

***Sil* is required within Schwann cells for axonal wrapping and normal Mbp expression and regulates the number of neurons within the PLLg**

Sil is ubiquitously expressed but highly enriched in neural cells and *csp*^{-/-} embryos show major neuronal defects such as an increase in the percentage of mitotically arrested neuroepithelial cells in the retina that coincides with an increase in apoptosis (Novorol et al., 2013). To test whether the myelination defect observed here is specific to Schwann cell or secondary to neuronal defects, we first examined several aspects of posterior lateral line ganglia (PLLg) and PLLn development in these mutants. *Csp*^{-/-} embryos presented no major defects in PLLn growth and Schwann cell migration at 48 hpf along the PLLn (Figure S2A). A few *csp*^{-/-} embryos (< 30%, n=7/24) showed an axonal growth arrest but posterior to the yolk extension and Schwann cells were found at the tip end of these axons. We next analyzed axonal transport along the PLLn using *mito:gfp* construct that labels mitochondria and we monitored their behavior by time-lapse imaging. We observed no significant difference in the average speed of mitochondria along the PLLn with an average of 1.92±0.14 μm/s and 1.85±0.06 μm/s in controls and *csp*^{-/-} respectively (Figure S2B,C and Movies S3, S4). We next looked at the PLLg and counted the number of neurons within the ganglia using the neuronal marker HuC. We observed a significant decrease in the number of neurons in *csp*^{-/-} embryos at 48 and 72 hpf in comparison to controls (Figure S2D,E) suggesting a role for *sil* in the development of PLLg that coincides with the reduction in the number of axons in the PLLn.

These results suggest a role for *sil* in the development of PLLg but not in axonal growth or transport.

To directly test whether *sil* has an autonomous function in Schwann cell myelination, we forced the expression of *sil* specifically in Schwann cells under the control of the *sox10* promoter. *Csp*^{-/-} embryos were injected with pTol2-*sox10:sil-P2A-mcherry-CaaX* and *tol2* transposase mRNA at 1 cell stage and selected at 3 dpf for *mcherry* expression in Schwann cells to be later fixed for TEM analysis. By forcing the expression of *sil* specifically in Schwann cells, we significantly increased the number of myelinated axons in *csp*^{-/-} embryos at 3 dpf (Figure 3A-B''). The percentage of myelinated axons per nerve in *csp*^{-/-} injected embryos was similar to the one observed in controls (Figure 3C).

We also processed control and *csp*^{-/-} larvae for immunohistochemistry to detect Mbp at 3 dpf. We observed a sharp decrease in Mbp expression in *csp*^{-/-} embryos in comparison to controls (Figure 3D-E''). We then injected *csp*^{-/-} embryos with pTol2-*sox10:sil-P2A-mcherry-CaaX* and *tol2* transposase mRNA at 1 cell stage and selected embryos for mosaic *mcherry*⁺ clonal analysis. These embryos were then processed for immunohistochemistry to detect Mbp. We analyzed 4 mutant larvae that had *sox10:mcherry*⁺ Schwann cells along PLLn; the *mcherry*⁺ clones appeared to express normal levels of Mbp (Figure 3F-G'').

Altogether, these results suggest a specific requirement for *sil* within Schwann cells to initiate axonal wrapping and Mbp expression.

Schwann cells in *csp*^{-/-} show delays in mitotic progression but exit mitosis with no significant increase in apoptosis

Given that *sil* deficiency leads to a decrease in the number of neurons with significant apoptosis in the retina, it is possible that the myelination defects observed in the PLLn of *csp*^{-/-} embryos could be related to a defect in the number of Schwann cells and/or their survival.

To address this question, we first labeled Schwann cells using the M phase marker PH3 in *Tg(foxd3:gfp)* at 48 and 72 hpf. As seen in the retina (Novorol et al., 2013), we observed a significant increase in the fraction of proliferating PH3+/Schwann cells at 48 hpf. This coincided with a decrease in the total number of Schwann cells and an increase in the ratio of PH3+ Schwann cells relative to the total number of Schwann cells counted within a region of 8 somites (Figure 4A-D). However, results were different at 72 hpf, as the total number of Schwann cells, the number of PH3+/Schwann cells and the ratio of PH3+/Schwann cells relative to the total number of Schwann cells showed no significant difference between controls and *csp*^{-/-} embryos (Figure 4A-D). Moreover, time-lapse analysis using the nuclear marker *h2b:gfp* pointed to a delay in prometaphase progression (Figure 4E and Movies S5, S6). However, in contrast to the neuronal phenotype in retina, most of Schwann cells exited mitosis and we detected no apoptotic Schwann cells in these mutants along the PLLn (Figure 4F,G). We also observed a significant increase in the number of acridine orange (AO)+ cells within the spinal cord that is comparable to the defect observed in the zebrafish retina suggesting a role for *sil* in cell survival outside the brain (Figure 4F,H).

These data suggest that *sil* is required in Schwann cells for the temporal control of mitotic exit, nevertheless, Schwann cells devoid of *sil* do exit mitosis following a significant delay and are not apoptotic.

Cell division during radial sorting is not required *per se* for Schwann cell myelination

Since Schwann cells in *csp*^{-/-} embryos show a delay in their mitotic progression, it is possible that there is a window of time during radial sorting in which Schwann cell have to divide in order to myelinate. Moreover, it has been shown that blocking cell division during radial sorting can alter their ability to radially sort axons and myelinate (Raphael et al., 2011). To test this hypothesis, we treated embryos with aphidicolin that blocks cell division *in vivo* in

zebrafish (Lyons et al., 2005, Raphael et al., 2011). First, we incubated embryos in aphidicolin between 45 and 54 hpf (see Materials and Methods for aphidicolin treatment/efficacy) to temporarily inhibit cell division during the initial phase of radial sorting. The medium was then washed and embryos were allowed to develop until 72 hpf (or 3 dpf) to be fixed for TEM. Another group of embryos was incubated from 45 hpf until 72 hpf to block Schwann cell division during the whole period of radial sorting. TEM analysis showed a significant decrease in the number of myelinated axons in both groups of treated embryos but also, as expected, in the total number of axons within the PLLn (Figure 5A-E). However, some large caliber axons were myelinated. Most importantly, the ratios of myelinated axons as well as the ratios of axons per diameter relative to the total number of axons showed no significant difference between the different groups (Figure 5F-H). This result suggests that cell division during radial sorting is not required *per se* for peripheral myelination.

Sil is required for mitotic temporal exit during Schwann cell migration

Given that cell division during radial sorting is not required *per se* for Schwann cell myelination, we therefore tested whether *sil* is also implicated in early Schwann cell division during migration. For this, we monitored Schwann cell behavior during axonal growth when Schwann cells migrate and divide along the axons of the PLLn. Schwann cells were generally elongated during migration too until they went through mitotic rounding, divided and then re-elongated (Figure 6A, Movie S7). Schwann cells divided along the AP axis of the embryo and it took an average of 9.81 ± 0.63 min for them to complete mitosis, showing a very similar behavior to the one observed during radial sorting. However, Schwann cells struggled to exit mitosis in *csp*^{-/-} with an average of 87.20 ± 4.72 min for mitosis to be completed (Figure 6A,B, Movie S8).

This result shows that *sil* is also required for the temporal control of mitosis in Schwann cell during migration similar to what we observed during radial sorting.

Early Schwann cell division during migration is essential for peripheral myelination

Since *sil* is also required for the temporal control of Schwann cell mitosis during migration, it is possible that the myelination defects observed in *csp*^{-/-} result from a defective division during these earlier events. It has been shown that Schwann cell division during migration is not required for migration *per se* (Lyons et al., 2005), however whether it is required for myelination has not been tested yet. We therefore treated embryos with aphidicolin to block cell division specifically during Schwann cell migration (between 22 and 40 hpf) and before radial sorting. The medium was then washed and embryos were allowed to develop until 3 dpf to be fixed for TEM. As expected, there was a sharp decrease in the total number of axons per nerve, however, we counted an average of only 0.125 myelinated axon in aphidicolin treated embryos at 3 dpf (Figure 7A-E). To test whether Schwann cells were able to divide again during radial sorting once the medium is washed, we analyzed their division and numbers during radial sorting, here at 52 hpf. We observed a significant decrease in the number of Schwann cells in treated embryos at 52 hpf but the number of Schwann cells that are PH3+ and the ratio of PH3+ Schwann cells relative to the total number of Schwann cells were similar between controls and treated embryos (Figure 7F-H). This result shows that Schwann cells were able to divide again during radial sorting following the temporal cell division block during migration.

We also monitored Schwann cell division during radial sorting using live imaging in controls and treated embryos. Our results showed a normal pattern of division during radial sorting in treated embryos similar to controls (Figure 7I,J, Movies S9,10).

Altogether, these results point to an essential role for Schwann cell division during migration in myelination.

***Sil* is required to initiate radial sorting, myelin gene expression and axonal wrapping by Schwann cells via cAMP pathway**

M phase is characterized by chromatin condensation and temporal ejection of transcription factors (Ma, Kanakousaki et al., 2015). Since *sil* is implicated in spindle checkpoint and mitotic progression during M phase, it is possible that the delayed mitotic exit observed in *csp*^{-/-} embryos could result in prolonged ejection of transcription factors required for myelination in Schwann cells. Alternatively, it is possible that transcriptional activity is effective after mitosis but Schwann cells in *csp*^{-/-} embryos present defective upstream signaling activity. To distinguish between these possibilities, we first analyzed the expression of *krox20* and *mbp* in controls and *csp*^{-/-} embryos at 52 hpf and 3 dpf respectively. *csp*^{-/-} embryos showed a sharp decrease in the expression of peripheral myelin genes (Figure 8B,E) in comparison to controls (Figure 8A,D) confirming the role of *sil* in this process. This coincided, as shown before, with a total lack of radial sorting and axonal ensheathment (Figure 8H). Second, we decided to treat embryos with forskolin that interacts with adenyl cyclase and elevates the levels of cAMP. It has been shown that elevation of cAMP *in vivo* in zebrafish is able to re-initiate the defective expression of myelin transcription factors in *gpr126* mutant (Monk, Naylor et al., 2009). Indeed, when *csp*^{-/-} embryos were treated with forskolin during radial sorting, the expression of *krox20* and *mbp* was restored (Figure 8C,F) as well as the ratio of myelinated axons relative to the total number of axons (Figure 8I,J). This result suggests that the myelin defects observed in *csp*^{-/-} embryos result from a defective cAMP signaling pathway and not from a prolonged ejection of transcription factors following a particularly lengthy M phase.

Forcing Rac1 activity does not rescue radial sorting and myelination defects in *csp*^{-/-}

The radial sorting defect and lack of axonal ensheathment by Schwann cell observed in this mutant might also be linked to a defective cytoskeletal re-arrangement mediated by Rac1. To test this hypothesis, we forced the expression of Rac1, a small GTPase protein that interacts with Schwann cell cytoskeleton (Nodari et al., 2007) and is able to restore peripheral radial sorting defects in zebrafish (Boueid, Mikdache et al., 2020, Mikdache, Fontenas et al., 2020). *csp*^{-/-} embryos injected with a constitutive active form of Rac1 were comparable to non-injected *csp*^{-/-} embryos and showed a total lack of radial sorting and axonal ensheathment (Figure 8K,L).

This result suggests that *csp*^{-/-} myelin defects do not ensue from a defective Rac1 activity.

Forcing Laminin α 2 expression fully rescues myelination defects in *csp*^{-/-}

Previous studies have shown that Laminin211 promotes myelination via GPR126/cAMP dependent pathway (Petersen, Luo et al., 2015), and since forskolin treatment of *csp*^{-/-} embryos is sufficient to re-establish myelination in these mutants, we hypothesized that Laminin 211 expression and/or function might be impaired in these mutants. To test this, we first analyzed Laminin expression at 48 hpf in controls and *csp*^{-/-} embryos using immunostaining. Indeed, Laminin expression was significantly reduced along the PLLn of *csp*^{-/-} embryos in comparison to controls (Figure 9A-C). We next wondered whether increasing extracellular Lama2 levels would rescue the myelination defect observed in *csp*^{-/-}. To this end, we took advantage of a *lama2* overexpression construct that expresses membrane EGFP as well as secreted mCherry-tagged Lama2 under the control of muscle-specific *skeletal-alpha actin* (*acta1*) promoter (Sztal, Sonntag et al., 2012) (Petersen et al., 2015) and injected 20 pg of this construct into *csp*^{-/-} mutants. A wide and important amount of Laminin

is secreted from muscle cells within the vicinity of PLLn in this case. Embryos that showed significant expression of EFGP within PLLn surrounding muscles and mCherry-tagged within PLLn (Figure 9D,E) were then selected and analyzed by TEM at 3 dpf. *csp*^{-/-} injected embryos showed a significant increase in the number of myelinated axons in comparison to non-injected *csp*^{-/-} larvae and were comparable to controls (WT *lama2* injected embryos) for the percentage of myelinated axons relative to the total number of axons (Figure 9F-J).

These results strongly suggest a defective Laminin expression and function within the PLLn of *csp*^{-/-} mutant that is responsible for its radial sorting and myelination defect.

Laminin2 is known to control several aspects of Schwann cell development such as proliferation and differentiation (Chernousov et al., 2008). We therefore wondered whether Lama2 overexpression impacts on Schwann cell timely division and/or proliferation to mediate myelination or it just triggers the radial sorting/myelination process regardless of the number of Schwann cells. Indeed, Schwann cells numbers at 48 hpf were comparable between *csp*^{-/-} and *csp*^{-/-}-*pact1*-lama2 injected embryos and showed a delay in mitotic exit similar to the one observed in *csp*^{-/-} embryos during migration and prior to axonal ensheathment (Figure 9K-M). This result strongly suggests that the myelination defect observed in *csp*^{-/-} is due to defective Laminin/cAMP pathway and that the temporal reduction in Schwann cells' numbers is not essential for myelination to proceed.

Schwann cells have a limited window of time to divide during migration in order to trigger myelination

Our findings have revealed an essential role for the temporal control of Schwann cell division during migration, mediated by Sil, in promoting radial sorting and myelination *in vivo* via Laminin2/cAMP pathway. This led us to hypothesize that Schwann cells might have a limited window of time during which they have to divide while migrating in order to trigger radial

sorting and myelination once migration is completed. First, we tested whether blocking cell division during migration would alter Laminin expression within the PLLn as observed in *sil* mutants. Embryos incubated in aphidicolin during Schwann cell migration showed a significant decrease in the expression of Laminin along the PLLn at 48 hpf (Figure 10A-C). Second, we treated embryos with aphidicolin for different periods of time during migration and our results showed that Schwann cells have a limited time window of around 8 h in which they have to divide in order to myelinate (Figure 10F and S3; see Materials and Methods for aphidicolin treatment/efficacy). To test if this temporal delay in division that results in radial sorting and myelination defects is related to a defective Laminin expression/function, we injected embryos with 20 pg of *lama2* overexpression construct and treated embryos with aphidicolin between 22 and 30 hpf. The group of *lama2* injected embryos and treated with aphidicolin showed a significant increase in the number of myelinated axons in comparison to treated non-injected embryos (Figure 10D-H) and the percentage of myelinated axons relative to the total number of axons was similar to controls (Figure 10I). This result strongly suggests a restricted time window for Schwann cells to divide while migrating during which Laminin expression is triggered.

Schwann cells are a major source of Laminin within the PLL nerve and forcing Laminin α 2 expression within Schwann cells rescues Mbp expression in *csp*^{-/-} and aphidicolin treated embryos

Results so far have shown a strong correlation between timely Schwann cell division during migration, Laminin expression and subsequent myelination. However, it remained important to determine the source of Laminin within the PLLn and whether Schwann cell specific expression of Laminin would rescue myelination autonomously in *sil* mutant and aphidicolin treated embryos. To do so, we first incubated embryos with AG1478 that specifically blocks

ErbB signaling in zebrafish and inhibits Schwann cell migration along the PLLn (Lyons et al., 2005). Embryos treated with AG1478 between 24 and 48 hpf presented no Schwann cells along the PLLn and showed a significant decrease in Laminin expression at 48 hpf (Figure 11A-C).

This result establishes Schwann cells as a major source of Laminin within the PLLn.

Second, we designed a *pUAS-lama2-EGFP-mcherry* construct and took advantage of the *tg(sox10:Gal4VP16)* to allow a specific expression of Lama2 within Schwann cells (EGFP+ labeling); Lama2 is also found secreted within the PLLn (mCherry+ labeling) (Figure 11D-E’’). We injected 20 pg of plasmid in *sox10:Gal4VP16* and *csp^{-/-}/sox10:Gal4VP16* at one cell stage and proceeded for EGFP and Mbp immunostaining for clonal analysis at 3 dpf. Injected *csp^{-/-}* embryos that showed an expression of EGFP in Schwann cells along the PLLn expressed normal levels of Mbp while those that were negative to EGFP labeling showed a significant decrease in Mbp expression similar to non-injected *csp^{-/-}* larvae (Figure 11F-H’’).

Moreover, we injected *pUAS-lama2-EGFP-mcherry* construct in *sox10:Gal4VP16* and incubated embryos in aphidicolin during Schwann cell migration (22-34 hpf). Aphidicolin treated embryos that showed an expression of EGFP in Schwann cells along the PLLn expressed normal levels of Mbp while those that were negative to EGFP labeling showed a significant decrease in Mbp expression at 3 dpf (Figure 11I-J’’).

These results strongly suggest that Schwann cell division during migration is largely responsible of early Laminin expression within the PLLn. This constitutes a crucial step for timely myelination.

Discussion

Schwann cells migrate and divide along peripheral axons and the intimate contact between the two contributes to Schwann cell proliferation, mainly through the neuregulin/ErbB axis. Other

signals provided by ECM proteins that Schwann cells secrete, are also important for their proliferation (Bunge, Clark et al., 1990, Bunge, Williams et al., 1982, Lyons et al., 2005, Michailov, Sereda et al., 2004, Nave & Salzer, 2006). Here, we investigate the characteristics of Schwann cell division during migration and radial sorting and their impact on myelination. We show that the temporal control of mitotic exit within Schwann cells is tightly controlled by the spindle pole protein Sil. We provide evidence that timely division of Schwann cells during migration is essential for radial sorting and myelination. Schwann cells must regulate their numbers in order to myelinate or ensheath all axons that are supposed to be myelinated by the end of radial sorting (Jessen & Mirsky, 2005), so one would predict that reducing the number of Schwann cells in the nerve would result in a reduction of myelinated axons. Indeed, several molecules that control Schwann cell proliferation have been identified such as Cdc42, focal adhesion kinase and several extracellular matrix proteins of the laminin family such as laminin-2, 8 and $\gamma 1$ (Benninger, Thurnherr et al., 2007, Grove, Komiyama et al., 2007, Yang, Bierman et al., 2005). Specific ablation of these molecules in Schwann cells causes a reduction in the number of myelinated axons. However, how important is the timely division of Schwann cells? If Schwann cells divide intensively during radial sorting to generate the right numbers prior to axonal ensheathment, then how would early division during migration contribute to myelination? Are insufficient Schwann cell numbers *per se* a cause for radial sorting and myelination defects? The importance of Schwann cell division during radial sorting has been examined *in vivo* (Raphael et al., 2011), showing that blockade of cell division leads to significant defects in radial sorting and myelination by Schwann cells. Several parameters should be considered in order to assess the role of division in myelination following aphidicolin treatment, that is obviously the drastic decrease in the total number of axons in the PLLn and the reduction in the number of Schwann cells. Our results show that Schwann cell division during radial sorting is not

required for myelination *per se* since blocking cell division reduces the number of myelinated axons in the PLLn but not the ratio of myelinated axons relative to the total number of axons resulting from this treatment. While aphidicolin-treated embryos during radial sorting show a normal percentage of myelinated axons, *csp*^{-/-} ones are totally devoid of radial sorting and myelination. However, when analyzing earlier events, we discovered that early Schwann cell division during migration, as well as the temporal control of its mitotic exit are essential in this process. These findings demonstrate that Schwann cells have a restricted time window during which they have to divide, specifically while migrating, in order to set up and initiate myelin wrapping at later stages.

How might Sil control radial sorting and myelination in the PNS?

Since *csp*^{-/-} embryos show a radial sorting defect, it remains possible that the myelination defects result, at least partially, from a defective cytoskeletal re-arrangement mediated by Rac1. Indeed, Schwann cells require a drastic process extension of their membrane in order to sort individual axons from within a bundle. A group of genes that regulate this aspect of Schwann cell behavior has been identified and includes *elmo1*, *dock1*, *β1 integrin* and *ILK* (Cunningham, Herbert et al., 2018, Feltri, Graus Porta et al., 2002, Mikdache et al., 2020). Some evidence points towards a role for these molecules in activating the small GTPase Rac1 that acts upon Schwann cells cytoskeleton during radial sorting (Guo, Moon et al., 2012, Nodari et al., 2007). Our analysis strongly suggests that the myelination defects observed in *csp*^{-/-} do not result from a defective Rac1 activity since forcing the expression of Rac1 does not rescue, even partially, this defect.

Schwann cells in *csp*^{-/-} struggle to progress through the M phase of the cell cycle and show a severe reduction in the expression of *krox20*. This phase is characterized by chromatin condensation and by ejection of transcription factors and chromatin binding proteins (Ma et al., 2015). Schwann cells are no exception since major chromatin remodeling complexes are

present and play an important role in temporal Schwann cell development (Frob & Wegner, 2019, Gomis-Coloma, Velasco-Aviles et al., 2018). It is possible that Schwann cell transcription factors required for the initiation of myelination are permanently ejected in *csp*^{-/-} following the temporal defect in M phase progression and therefore unable to bind the DNA. Another possibility is a defective upstream signaling activity in *csp*^{-/-} embryos that blocks Schwann cell differentiation, assuming that chromatin accessibility is re-established after division. Our analysis suggests that it is rather a defective signaling activity that is responsible for the peripheral lack of myelin observed in *csp*^{-/-} embryos and that transcription activity is responsive to upstream signaling cues.

Two major signaling pathways are required for several aspects of Schwann cell development, that are the Neuregulin/ErbB and the Gpr126/cAMP signaling pathways (Monk et al., 2009, Newbern & Birchmeier, 2010). The neuregulin/ErbB plays a major role in migration, survival, proliferation, radial sorting and myelination by Schwann cells through a plethora of downstream factors such as PI3K/Akt, MEK/ERK and Cdc42 (Glenn & Talbot, 2013, Newbern & Birchmeier, 2010). The Gpr126 pathway signals through cAMP and activates myelination by elevating the levels of cAMP so that a forskolin treatment of *gpr126* mutants fully restores myelin ensheathment (Monk et al., 2009). However, elevating cAMP in zebrafish *erbb2* mutants does not rescue its myelin defects (Glenn & Talbot, 2013). A forskolin treatment of *csp*^{-/-} embryos fully rescues radial sorting defects, restores *krox20* and *mbp* expression as well as axonal ensheathment, suggesting a defective cAMP pathway activity in these mutants.

A particularly interesting observation is the difference in *sil* deficiency phenotype between retina and Schwann cells. Sil is a ubiquitously expressed protein and is involved in the fundamental process of mitosis but is highly enriched in the nervous system. Neurons might in this case be more sensitive to Sil defects leading to massive apoptosis within the central

nervous system (CNS); however, it is Schwann cell differentiation that is impaired in *csp*^{-/-} with no increase in apoptosis. It is therefore possible that different cells might respond differently to *sil* defects and our results suggest that MCPH genes have a broader requirement during development that is not solely confined to neuronal cells in the CNS. It is also important to note that zebrafish dynein cytoplasmic 1 heavy chain 1 (*dync1h1*) mutant shows comparable myelin defects to *csp*^{-/-} (Langworthy & Appel, 2012). Dynein is involved in spindle organization and checkpoint (Grava, Schaerer et al., 2006, Kiyomitsu & Cheeseman, 2012, Silva, Reis et al., 2014) similar to Sil and therefore it is highly possible that spindle checkpoints proteins are fundamentally required for the temporal control of mitosis within Schwann cells in order to initiate myelination.

One important question remained to be solved: what is the molecular link between Schwann cell division, its temporal control and cAMP pathway? Our analyses have shown an essential role for Sil in Schwann cell differentiation, this added to the fact that cAMP stimulation rescued the myelination defects prompted us to test for upstream signals that mediates cAMP activity. Several studies have now established an essential role for the Gpr126 in driving cAMP activity in Schwann cells through G proteins (Mogha, Benesh et al., 2013) and this has recently been attributed to Gpr126 interaction with ECM, more specifically with Laminin211 (Petersen et al., 2015). It is therefore possible that *sil* deficient Schwann cells are incapable of secreting extracellular proteins and/or responding to them to drive the ECM/Gpr126/cAMP pathway (Paavola, Sidik et al., 2014, Petersen et al., 2015). Our results showed that *csp*^{-/-} larvae presented a significant decrease in Laminin expression in the PLLn and forcing Laminin2 expression within the vicinity of the nerve was sufficient to activate the myelination program in Schwann cells, drive radial sorting and re-establish axonal wrapping.

Based on all these findings, it was therefore interesting to assess the temporal setting and the mechanism by which Schwann cell division triggers myelination. This study and others have

shown that Schwann cells go through extensive cell proliferation prior to myelination at different timepoints of their development: (i) during migration and (ii) during radial sorting once they finished migrating in zebrafish (Lyons et al., 2005) and rodents (Stewart, Morgan et al., 1993). To understand how timely division impact on Schwann cell differentiation, we used the cell division inhibitor, aphidicolin, to temporally block cell division at these two essential timepoints of intensive Schwann cell proliferation and analyzed its impact on myelination. To our surprise, it was Schwann cell division during migration that dictated the radial sorting and myelination events. In addition to characterizing how temporal control of mitotic exit impacts myelination, our study also provided documentation of the existence of a restricted window of time (around 8 hours) during which Schwann cells have to divide while migrating in order to initiate radial sorting and myelination *in vivo*. Several studies have pointed to a role for Laminin in controlling Schwann cell proliferation but this is the first study that implicates Schwann cell division, particularly during migration, in Laminin expression and subsequent myelination. The temporal control of this particular division is therefore an essential step towards initiating the myelination program through Laminin/cAMP pathway, a role that stretches beyond simply increasing the pool of Schwann cells required to continuously engulf the nerve as it grows.

To come back to the question of numbers, is it important to generate enough Schwann cells to myelinate? Our findings reveal that the myelination defects observed in *csp*^{-/-} and aphidicolin-treated embryos do not ensue from reducing Schwann cells numbers *per se*, since Laminin overexpression can fully rescue the percentage of myelinated axons within the PLLn even if Schwann cell numbers remain significantly reduced. It would be interesting to know in the future to what extent we can reduce the number of Schwann cells before they fully stretch along the axons and whether they would still be able to myelinate in presence of Laminin under acute mechanical strain.

Finally, we show that Schwann cells are a major source of Laminin within peripheral nerves in zebrafish. Our clonal analysis shows that forcing Laminin expression autonomously within Schwann cells is enough to secrete Laminin in the extracellular space and restore normal Mbp expression within the vicinity of these cells in *csp*^{-/-} and aphidicolin treated embryos. In summary, our work provides evidence of a novel ON/OFF mechanism that links early temporal control of Schwann cell division during migration to radial sorting and axonal wrapping via Laminin/cAMP pathway *in vivo*.

Materials and Methods

Embryo care

Embryos were staged and cared for according to standard protocols (https://zfin.org/zf_info/zfbook/cont.html). *Tg(foxd3:gfp)* stable transgenic line, that labels SCs was used in this study (Gilmour et al., 2002). *Tg(sox10:Gal4VP16)^{el159}* was kindly provided by Gage Crump. *Csp*^{+/-} embryos (*stil*^{c265}) were purchased from ZIRC. All animal experiments were conducted with approved protocols at Inserm by DDPP Val de Marne, France under license number F 94-043-013.

Plasmid constructs

pTol2-Sox10:Sil-P2A-mCherry: The sil-P2A-mCherry cassette allowing simultaneous expression of sil and membrane-localized mCherry separated by the self-cleaving P2A peptide was generated by PCR amplification.

The 5'-CGATTCACCTAGTATGAACCGTGTACAAGTGGATTTTAAAGG-3' forward and 5'-GAAGTTCGTGGCTCCGGATCCAAAGAGCTTGGGGAGCTGCCGTAACC - 3' reverse primers were used onto the pDNR-lib-STIL cDNA bought from addgene and the 5'-GGTTACGGCAGCTCCCCAAGCTCTTTGGATCCGGAGCCACGAAGTTC-3' forward

and 5'-CTATGACCATGATTACGCCAAG-3' reverse primers onto the pCS2-mCherry-CAAX plasmid.

The resulting PCR fragment was digested by SpeI and NotI before sub-cloning into the -4725Sox10-cre vector (a gift from Robert Kelsh) to obtain the *pSox10:sil-P2A-mCherry* plasmid.

p5UAS: lama2-mcherry-T2A-EGFPcaax : The pT2i5uasbiH2bChe-GFP (a gift from Michel Volovitch) was used to amplify the 5 UAS sequence using forward and reverse primers. Then, in order to replace *Acta1* promoter, the PCR product was subcloned into the *pacta1:lama2-mcherry-T2A-EGFPcaax* vector (a gift from Peter Currie and Kelly Monk) digested by ApaI and NdeI enzymes.

Microinjections

Mito:gfp (a gift from Dominik Paquet) and *h2b::gfp* (a gift from Jon Clarke) mRNAs were synthesized using SP6 mMessage mMachine System after linearization with NotI and injected at 200 pg per embryo. *pTol2:sox10-sil-2A-mcherry-Caax* was injected at 10 pg per embryo along with 50 pg of Tol2 transposase mRNA (a gift from David Lyons). *Rac1V12* (constitutively active Rac1, kindly provided by Nicolas David) mRNA were synthesized using SP6 mMessage mMachine System after linearization with NotI and injected at 2-10 pg per embryo. *pacta1-lama2* (kindly provided by Kelly Monk and Peter Currie) was injected at 20 pg per embryo along with 100 pg of Tol2 transposase mRNA. *pUAS-lama2* was injected at 20 pg per embryo.

In situ hybridization

In situ hybridization was performed following standard protocols using *krox20* and *mbp* probes (Fontenas, De Santis et al., 2016). Embryos were raised in egg water with PTU (0.003%) to avoid pigmentation and were fixed overnight in 4% paraformaldehyde at 52 hpf and 3 dpf. Embryos were dehydrated the next day and kept in methanol at -20°C. Embryos were then rehydrated and treated with proteinase K and incubated overnight with the corresponding probe. Embryos were then washed and the staining was revealed using anti-digoxigenin antibody.

Immunofluorescence

The following antibodies and dilutions were used: mouse anti-acetylated tubulin (Sigma; 1/500; T7451), mouse anti-HuC/HuD neuronal protein (Invitrogen; 1/500; REF A21271), rabbit anti-laminin (Sigma, 1/200; L9393), mouse anti-phospho Histone 3 (Ser 10) (Millipore; 1/500; Cat # 06-570), rabbit anti-myelin basic protein (Custom produced (Tingaud-Sequeira Angèle, Anja Knoll-Gellida et al., 2017)), gift from Patrick Babin, 1/500), mouse anti-EGFP (Millipore; 1/500; REF MAB3580). Primary antibodies were detected with appropriate secondary antibodies conjugated to either Alexa 488, Alexa 568 or Alexa 647 (Invitrogen) at a 1:500 dilution. For immunostaining, embryos were fixed in 4% paraformaldehyde 1X PBS overnight at 4°C, then washed with 1xPBS to be dehydrated in Methanol for at least 6 hours at -20°C. Samples were then rehydrated, digested with proteinase K and blocked with 0.5% triton in PBS and 10% sheep serum and then incubated with primary antibody overnight at 4°C (diluted in PBS+2% sheep serum). Larvae were then washed with PBS for a few hours and then incubated with secondary antibody in 0.5% triton in PBS and 2% sheep serum overnight at 4°C. Stained larvae were then imaged with a Leica SP8 confocal microscope. For Laminin and Mbp staining, embryos were fixed in 4% PFA for 30 min and treated for 7 min with acetone at -20°C. Samples were then blocked with 0.5% triton in PBS and 10% sheep

serum and then incubated with primary antibody overnight at 4°C (diluted in PBS+2% sheep serum). Larvae were then washed with PBS for a few hours and then incubated with secondary antibody in 0.5% triton in PBS and 2% sheep serum for 3 hours at RT. For Laminin fluorescence intensity quantification, the same parameters (Excitation/emission, gain for detectors, lasers intensity) were applied for images' acquisition in controls, *csp*^{-/-} and aphidicolin treated embryos and fluorescence intensity was measured using image J (Analyze/measure).

Aphidicolin, forskolin and AG1478 treatment

Embryos were incubated in fish water containing 150 µM aphidicolin diluted in DMSO between 45 and 54 hpf or 45 and 72 hpf and with 100 µM between 22 and 40 hpf, 22 and 30 hpf or 22 and 34 hpf. Controls were incubated in equivalent amount of DMSO (0.5 and 0.33%) solution during the same periods. It took around 2 to 3 h for the aphidicolin to become fully efficient following incubation.

Embryos were incubated in fish water containing 20 µM forskolin diluted in DMSO between 45 and 54 hpf. Controls were incubated in equivalent amount of DMSO solution (0.1%) during the same period.

Embryos were incubated in fish water containing 4 µM AG1478 diluted in DMSO between 24 and 48 hpf. Controls were incubated in equivalent amount of DMSO solution (0.1%) during the same period.

Acridine orange staining

Embryos were anesthetized with 0.03% tricaine and incubated in fish water containing 5µM acridine orange for 20 minutes in the dark. After wash, they were embedded in 1.5% low melting point agarose, and imaged with a Leica SP8 confocal microscope.

597

598 **Live imaging**

599 Embryos were anesthetized with 0.03% tricaine and embedded in 1.5% low melting point
600 agarose. For mito:GFP tracking experiments, PLLn was examined at 48 hpf from a lateral
601 view. A series of 10 minutes time-lapses were recorded. Recordings were performed at 27°C
602 using a Leica SP8 confocal microscope. For *Tg(foxd3:gfp)*, *Tg(foxd3:gfp)/csp^{-/-}* as well as
603 controls and *csp^{-/-}* injected with *h2b:gfp*, PLLg was examined at 28 hpf and 48 hpf and
604 recordings were acquired for up to 12h.

605

606 **Transmission electron microscopy**

607 At 3 and 4 dpf, embryos were fixed in a solution of 2% glutaraldehyde, 2% paraformaldehyde
608 and 0.1M sodium cacodylate pH 7.3 overnight at 4°C. This was followed by a post-fixation
609 step in cacodylate-buffered 1% osmium tetroxide (OsO₄, Serva) for 1h at 4°C and in 2%
610 uranyl acetate for 1h at room temperature. The tissue was then dehydrated and embedded in
611 epoxy resin. Sections were contrasted with saturated uranyl acetate solution and were
612 examined with a 1010 electron microscope (JEOL) and a digital camera (Gatan).

613

614 **Statistical analysis**

615 Means and standard deviations were calculated with Graph Pad Prism 7. All data were first
616 tested for normal distribution using the ShapiroWilk normality test combined with D'Agostino
617 & Pearson normality test. All experiments with only two groups and one dependent variable
618 were compared using an unpaired *t*-test with Welch's correction if they passed normality test;
619 if not, groups were compared with the nonparametric Mann-Whitney test. Statistically
620 significant differences were determined using one-way ANOVA for all experiments with
621 more than two groups but only one dependent variable. Error bars depict standard errors of

the mean (SEM). ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$. n represents the number of embryos.

Acknowledgments

We would like to thank Peter Currie and Kelly Monk for the *pact1-lama2* plasmid, Michel Volovitch and Sophie Vriz for *pUAS* construct and discussing cloning strategy, Robert Kelsh for *sox10* promoter, Gage Crump for *Tg(sox10:Gal4VP16)^{el159}* embryos, Patrick Babin for providing Mbp antibody and Jon Clarke for his critical reading of the manuscript. This work was funded by Inserm and University Paris-Saclay. Emilie Lesport is funded by “Institut Professeur Baulieu”.

Figure legends

Figure 1. Schwann cells show a regular pattern of division during radial sorting

(A) Still images of time-lapse imaging in *Tg(foxd3:gfp)* control and *Tg(foxd3:gfp)/csp^{-/-}* embryos. Arrows indicate Schwann cells along the PLLn at different timepoints prior to and after division. Scale bars = 25µm.

(B) Quantification of the time required for control (20 cells, n= 6 embryos) and *csp^{-/-}* (20 cells, n= 5 embryos) Schwann cells to successfully complete mitotic division. (****, $p \leq 0.0001$). m or min, minutes.

Figure 2. Sil is essential for radial sorting and myelination by Schwann cells

Schematic illustration of a zebrafish larvae. PLLn is shown in red. Dotted line represents the anterior-posterior position of cross section analysis in TEM. PLLn, posterior lateral line nerve.

TEM of a cross section of the PLLn at 3 dpf in control (A) and *csp*^{-/-} embryo (B) and at 4 dpf in control (C) and *csp*^{-/-} (D). Magenta asterisks highlight some large caliber myelinated axons (some shown at higher magnification in A', scale bar = 0.2 μm) and blue asterisks show some large caliber non-myelinated axons. Scale bars = 0.5 μm. SC, Schwann cell. (A'') Example of a 1:1 association between a Schwann cell and an axon, i.e. a radially sorted axon in control embryo at 3 dpf. Scale bar = 0.5 μm. a, axon. Axons remained bundled in *csp*^{-/-} whereby one Schwann cell is associated with a bundle of axons, delimited in white in B.

(E) Quantification of the number of myelinated axons per nerve at 3 dpf in controls (9 nerves, n= 5 embryos) and *csp*^{-/-} (8 nerves, n= 6 embryos) and at 4 dpf in controls (average of 8.3±0.57 myelinated axons; 10 nerves, n= 6 embryos) and *csp*^{-/-} (0 myelinated axon; 3 nerves, n= 3 embryos) (**, p= 0.0035 at 3 dpf; **, p= 0.005 at 4 dpf).

(F) Quantification of the total number of axons per nerve at 3 dpf in controls and *csp*^{-/-} and at 4 dpf in controls (54±3.16 axons) and *csp*^{-/-} (23±1 axons) (**, p= 0.003; ****, p≤0.0001).

(G) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls and *csp*^{-/-} and at 4 dpf in controls (15.44±0.87%) and *csp*^{-/-} (0%) (****, p≤0.0001; ****, p≤0.0001).

(H) Quantification of the number of axons relative to their diameter at 3 dpf in controls and *csp*^{-/-} (**, p= 0.0013; ns, p= 0.14).

(I) Quantification of the number of sorted axons per nerve at 3 dpf in controls (average of 7.00±0.52) and *csp*^{-/-} (0.37±0.26) (****, p≤0.0001).

(J) Quantification of the percentage of sorted axons relative to the total number of axons at 3 dpf in controls (12.97±1.21) and *csp*^{-/-} (1.95±1.55) (**, p=0.0019).

Figure 3. Sil is required within Schwann cells for axonal wrapping

TEM of a cross section of the PLLn at 3 dpf in *csp*^{-/-} (A) and *csp*^{-/-} embryos injected with *psox10-sil-P2A-mcherry* (B). Magenta asterisks represent some large caliber myelinated axons (some shown at higher magnification in B', B'', scale bars = 0.2 μm). Scale bars = 0.5 μm.

(C) Quantification of the percentage of myelinated axons relative to the total number of axons in control (average of 10.46±1.14%, 9 nerves, n= 5 embryos), *csp*^{-/-} (0%, 8 nerves, n= 6 embryos) and *csp*^{-/-} injected with *psox10-sil-P2A-mcherry* (average of 8.64±1.20%, 3 nerves, n= 3 embryos) (****, p≤0.0001; ns, p= 0.4871). Acetylated tubulin (Ac-tub) and Mbp immuno-labeling in control (D-D''), *csp*^{-/-} (E-E'') and *csp*^{-/-}+*psox10-sil-P2A-mcherry* (F-G'') embryos at 3 dpf. Arrows indicate the PLLn in D and E, the myelin sheaths in D', mcherry+ Schwann cells along the PLLn in F and G and the corresponding Mbp expression in F' and G'. D'', E'', F'' and G'' are the corresponding merge images of D and D', E and E', F and F', G and G' respectively. Scale bars = 20 μm.

Figure 4. Schwann cells in *csp*^{-/-} show delays in mitotic progression but exit mitosis with no significant increase in apoptosis

(A) PH3 immuno-labeling in *Tg(foxd3:gfp)* and *Tg(foxd3:gfp)/csp*^{-/-} at 48 hpf and 72 hpf. Arrows indicate Schwann cells that are GFP and PH3 positives. Scale bar = 25 μm.

(B) Quantification of the number of Schwann cells within a defined region of the PLLn at 48 and 72 hpf in control (average of 80.56±3.76 cells at 48 hpf, n= 9 embryos; average of 60±1.78 cells at 72 hpf, n= 11 embryos) and *csp*^{-/-} (average of 53.57±2.50 at 48 hpf, n= 9 embryos; average of 56.42±2.75 cells at 72 hpf, n= 12 embryos) (****, p≤ 0.0001; ns, p= 0.2897).

(C) Quantification of the number of PH3⁺/Schwann cells within a defined region of the PLLn at 48 and 72 hpf in control (average of 1.41±0.37, n=12 embryos at 48 hpf; average of

0.38±0.23, n= 13 embryos at 72hpf) and *csp*^{-/-} (average of 10.56±1.34, n=9 embryos at 48 hpf; average of 1.11±0.35, n= 9 embryos at 72 hpf) (****, p≤ 0.0001; ns, p= 0.064).

(D) Quantification of the ratio of PH3⁺ Schwann cells relative to the total number of Schwann cells within a defined region of the PLLn at 48 and 72 hpf in control (average of 1.68±0.48, n= 9 embryos at 48 hpf; average of 0.75±0.41, n= 11 embryos at 72hpf) and *csp*^{-/-} (average of 20.13±2.82, n= 9 embryos at 48 hpf; average of 1.59±0.64, n= 12 embryos at 72 hpf) (***, p= 0.0002; ns, p= 0.3636).

(E) Still images of time-lapse imaging in control (average of 16.67±1.22 min, 6 nuclei, n= 3 embryos) and *csp*^{-/-} (average of 98.67±4.55 min, 6 nuclei, n= 3 embryos) embryos injected with *h2b:gfp*. Arrows indicate Schwann cells nuclei from the beginning of M phase (time 0) when mitotic rounding takes place until the two nuclei split. Scale bars = 10 μm. m, minutes.

(F) Acridine orange (AO) staining at 50 hpf in control and *csp*^{-/-} embryos within a defined region of the PLLn and spinal cord. Scale bar = 25 μm.

(G) Quantification of the number of AO positive cells in control (average of 0.18±0.08, n= 11 embryos) and *csp*^{-/-} (average of 0.27±0.09, n= 11 embryos) within a defined region of the PLLn (ns, p= 0.72).

(H) Quantification of the number of AO positive cells in control (average of 0.66±0.33, n= 6 embryos) and *csp*^{-/-} (average of 47.83±2.05, n= 6 embryos) within a defined region of the spinal cord (****, p≤0.0001).

Figure 5. Schwann cell division during radial sorting is not required *per se* for myelination by Schwann cells

TEM of a cross section of the PLLn at 3 dpf in control (A), embryo treated with aphidicolin between 45 and 54 hpf (B) and embryo treated with aphidicolin between 45 and 72 hpf (C).

Magenta asterisks represent some large caliber myelinated axons, (some shown at higher magnification in B' and C', scale bars = 0.2 μ m). SC, Schwann cell. Scale bars = 0.5 μ m.

(D) Quantification of the number of myelinated axons per nerve at 3 dpf in controls (average of 5.7 ± 0.56 , 9 nerves, n= 6 embryos), embryos treated with aphidicolin between 45 and 54 hpf (average of 2.11 ± 0.31 , 5 nerves, n= 5 embryos), and embryos treated with aphidicolin between 45 and 72 hpf (average of 2.00 ± 0.32 , 9 nerves, n= 5 embryos) (****, $p \leq 0.0001$; ns, $p = 0.9790$).

(E) Quantification of the total number of axons per nerve at 3 dpf in controls (average of 57 ± 2.9), embryos treated with aphidicolin between 45 and 54 hpf (average of 25.11 ± 2.04) and embryos treated with aphidicolin between 45 and 72 hpf (average of 28.00 ± 1.98) (****, $p \leq 0.0001$; ns, $p = 0.6574$).

(F) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.5 ± 1.2), embryos treated with aphidicolin between 45 and 54 hpf (average of 8.76 ± 1.42) and embryos treated with aphidicolin between 45 and 72 hpf (average of 7.46 ± 1.66). ns, $p > 0.05$ in all cases.

(G) Quantification of the percentage of axons according to their diameter relative to the total number of axons per nerve at 3 dpf in controls (average of 68.12 for 0-0.4 μ m; average of 31.88 for >0.4 μ m) and aphidicolin treated embryos between 45 and 54 hpf (average of 68.62 for 0-0.4 μ m; average of 31.38 for >0.4 μ m). ns, $p = 0.9607$.

(H) Quantification of the percentage of axons according to their diameter relative to the total number of axons per nerve at 3 dpf in controls (average of 68.12 for 0-0.4 μ m; average of 31.88 for >0.4 μ m) and aphidicolin treated embryos between 45 and 72 hpf (average of 70.76 for 0-0.4 μ m; average of 29.24 for >0.4 μ m). ns, $p = 0.7545$.

Figure 6. *Sil* is required for Schwann cell temporal mitotic exit during migration

(A) Still images of time-lapse imaging in *Tg(foxd3:gfp)* control and *Tg(foxd3:gfp)/csp^{-/-}* embryos at around 30 hpf. Arrows indicate Schwann cells along the PLLn at different timepoints prior to and after division. Scale bar = 20 μ m.

(B) Quantification of the time required for control (11 cells, n= 3 embryos) and *csp^{-/-}* (10 cells, n= 3 embryos) Schwann cells to successfully complete mitotic division during migration. (****, $p \leq 0.0001$). m or min, minutes.

Figure 7. Early Schwann cell division during migration is essential for myelination

(A) TEM of a cross section of the PLLn at 3 dpf in control and embryo treated with aphidicolin between 22 and 40 hpf. Magenta asterisks represent some large caliber myelinated axons. SC, Schwann cell. Scale bars = 0.5 μ m.

(B) Quantification of the number of myelinated axons per nerve at 3 dpf in controls (average of 5.63 ± 0.45 , 11 nerves, n= 7 embryos) and embryos treated with aphidicolin between 22 and 40 hpf (average of 0.125 ± 0.125 , 8 nerves, n= 6 embryos) (****, $p \leq 0.0001$).

(C) Quantification of the total number of axons per nerve at 3 dpf in controls (average of 55.09 ± 2.32) and embryos treated with aphidicolin between 22 and 40 hpf (average of 20 ± 3.04) (****, $p \leq 0.0001$).

(D) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.36 ± 0.93) and embryos treated with aphidicolin between 22 and 40 hpf (average of 0.46 ± 0.46) (****, $p \leq 0.0001$).

(E) Quantification of the percentage of axons according to their diameter relative to the total number of axons per nerve at 3 dpf in controls (average of 68.12 for 0-0.4 μ m; average of 31.88 for >0.4 μ m) and aphidicolin treated embryos between 22 and 40 hpf (average of 53.69 for 0-0.4 μ m; average of 46.31 for >0.4 μ m). ns, $p=0.2045$.

(F) Quantification of the number of Schwann cells within a defined region of the PLLn at 52 hpf in control (average of 59.10 ± 2.44 cells, $n = 10$ embryos) and embryos treated with aphidicolin between 22 and 40 hpf (average of 18 ± 3.27 cells, $n = 11$ embryos) (****, $p \leq 0.0001$).

(G) Quantification of the number of PH3^+ /Schwann cells within a defined region of the PLLn at 52 hpf in control (average of 0.87 ± 0.47 , $n = 8$ embryos) and embryos treated with aphidicolin between 22 and 40 hpf (average of 0.18 ± 0.12 , $n = 11$ embryos) (ns, $p = 0.3265$).

(H) Quantification of the ratio of PH3^+ Schwann cells relative to the total number of Schwann cells within a defined region of the PLLn at 52 hpf in control (average of 2.54 ± 0.95 , $n = 10$ embryos) and embryos treated with aphidicolin between 22 and 40 hpf (average of 2.34 ± 1.65 , $n = 11$ embryos) (ns, $p = 0.3274$).

(I) Still images of time-lapse imaging in *Tg(foxd3:gfp)* control and *Tg(foxd3:gfp)* treated with aphidicolin between 22 and 40 hpf embryos at around 52 hpf. Arrows indicate Schwann cells along the PLLn at different timepoints prior to and after division. Scale bars = 20 μm .

(J) Quantification of the time required for control (average of 8.70 ± 0.41 min, 20 cells, $n = 5$ embryos) and embryos treated with aphidicolin between 22 and 40 hpf (average of 8.14 ± 0.67 min, 7 cells, $n = 4$ embryos) Schwann cells to successfully complete mitotic division between 48 and 60 hpf. (ns, $p = 0.1796$).

Figure 8. *Sil* is required to initiate myelin gene expression and axonal wrapping by Schwann cells via cAMP dependent pathway

Lateral views of *krox20* expression at 52 hpf revealed by in situ hybridization along the PLLn in control (A) showing a robust expression ($n = 29$), *csp*^{-/-} embryo (B) showing a sharp decrease in *krox20* expression ($n = 22$) and in *csp*^{-/-} treated with forskolin (FSK) (C) showing a

robust expression similar to controls (n=30). Arrows indicate Schwann cells expressing *krox20* along the PLLn.

Lateral views of *mbp* expression at 3 dpf revealed by in situ hybridization along the PLLn in control (D) showing a robust expression (n=31), *csp*^{-/-} embryo (E) showing a sharp decrease in *mbp* expression (n=24) and in *csp*^{-/-} treated with forskolin (F) showing a robust expression similar to controls (n=28). Arrows indicate Schwann cells expressing *mbp* along the PLLn. Scale bar = 200 μm.

TEM of a cross section of the PLLn at 3 dpf in control (G), *csp*^{-/-} (H) and *csp*^{-/-} treated with forskolin between 45 and 54 hpf (I). Magenta asterisks represent some large caliber myelinated axons. SC, Schwann cell. Scale bars = 0.5 μm.

(J) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.6±1.17), *csp*^{-/-} (average of 0) and *csp*^{-/-} treated with forskolin (average of 8.74±1.77) (****, p≤0.0001; ns, p= 0.4846; ****, p≤0.0001).

(K) TEM of a cross section of the PLLn at 3 dpf in *csp*^{-/-} embryo injected with a constitutive active form of Rac1 (*carac1*). Scale bar = 0.5 μm.

(L) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.5±1.13), *csp*^{-/-} (average of 0) and *csp*^{-/-} injected with *carac1* (average of 0) (****, p≤0.0001, ns, p≥ 0.999).

Figure 9. Laminin expression is significantly reduced in *csp*^{-/-} and Lamininα2 over-expression rescues radial sorting and myelination defects in *csp*^{-/-} embryos without increasing the number of Schwann cells

Laminin expression in control (A, n=15) and *csp*^{-/-} (B, n=18) embryos at 48 hpf showing the PLLn nerve (arrows). Scale bar = 20 μm. (C) Quantification of Laminin fluorescence intensity along the PLLn in controls (average of 35.95±1.39) and *csp*^{-/-} (average of

23.37±0.90) at 48 hpf (****, $p \leq 0.0001$), a.u., arbitrary unit. (D) Lateral view of EGFP expression in muscles surrounding the PLLn following *pactal-lama2* injection. Scale bar = 20 μ m. (E) Lateral view of mCherry tagged secreted Laminin in muscles and within the PLLn (white arrows). TEM of a cross section of the PLLn at 3 dpf in control (F), *csp*^{-/-} (G) and *csp*^{-/-} + *pactal-lama2* (H) embryos. Magenta asterisks represent some large caliber myelinated axons, some are shown at higher magnification in H'. Scale bars = 0.5 μ m. (I) Quantification of total number of axons per nerve at 3 dpf in controls (average of 55.20±2.56, 10 nerves, n= 6 embryos), *csp*^{-/-} (average of 29.56±2.55, 9 nerves, n= 7 embryos) and *csp*^{-/-} + *pactal-lama2* (average of 34.78±1.84, 9 nerves, n= 7 embryos) (****, $p \leq 0.0001$; ns, $p=0.1190$). (J) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.82±1.085), *csp*^{-/-} (average of 0±0) and *csp*^{-/-} + *pactal-lama2* (average of 8.28±0.77) (****, $p \leq 0.0001$; ns, $p=0.075$). (K) Quantification of the time required for control (average of 9.66±0.39, 12 cells, n= 4 embryos), *csp*^{-/-} (average of 86.80±4.77, 10 cells, n= 4 embryos) and *csp*^{-/-} injected with *pactal-lama2* (average of 88.60±4.31, 10 cells, n= 4 embryos) Schwann cells to successfully complete mitotic division during migration (****, $p \leq 0.0001$; ns, $p=0.7828$). min, minutes. (L) Quantification of the time required for control (average of 9.77±0.61, 9 cells, n= 4 embryos), *csp*^{-/-} (average of 94.42±14.18, 19 cells, n= 4 embryos) and *csp*^{-/-} injected with *pactal-lama2* (average of 100.2±10.01, 12 cells, n= 4 embryos) Schwann cells to successfully complete mitotic division during radial sorting (****, $p \leq 0.0001$; ns, $p=0.1424$). (M) Quantification of the number of Schwann cells within a defined region of the PLLn at 48 hpf in controls (average of 77.45±2.05 cells, n= 20 embryos), *csp*^{-/-} (average of 51.60±1.40, n= 20 embryos) and *csp*^{-/-} injected with *pactal-lama2* (average of 48.18±1.74 cells, n= 11 embryos), (****, $p \leq 0.0001$; ns, $p=0.1402$).

Figure 10. Laminin α 2 overexpression rescues defective myelination in embryos with delayed Schwann cell division during migration

Laminin expression in a control embryo (A, n=15) and aphidicolin treated embryo (B, n=15) at 48 hpf showing the PLLn nerve (arrows). Scale bar = 20 μ m. (C) Quantification of Laminin fluorescence intensity along the PLLn in controls (average of 36 ± 1.4) and aphidicolin treated embryos (average of 26.80 ± 0.92) at 48 hpf (****, $p \leq 0.0001$), a.u, arbitrary unit. (D) Lateral view of EGFP expression in muscles surrounding the PLLn following *pacta1-lama2* injection. Scale bar = 20 μ m. (E) Lateral view of mCherry-tagged secreted Laminin in muscles and within the PLLn (white arrows). TEM of a cross section of the PLLn at 3 dpf in aphidicolin treated embryo (F) and aphidicolin treated embryo injected with *pacta1-lama2* (G). Magenta asterisks represent some large caliber myelinated axons, (some are shown at higher magnification in G',G'', scale bars = 0.2 μ m). Blue asterisks represent some large caliber non myelinated axons. Scale bars = 0.5 μ m. (H) Quantification of the total number of axons per nerve at 3 dpf in controls (average of 55.56 ± 2.7), aphidicolin treated embryos (average of 30.53 ± 1.75) and aphidicolin treated embryos + *pacta1-lama2* (average of 26.00 ± 2.32) (****, $p \leq 0.0001$; ns, $p = 0.1401$). (I) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 3 dpf in controls (average of 10.73 ± 1.4 , 9 nerves, n= 6 embryos), aphidicolin treated embryos (average of 1.77 ± 0.96 , 17 nerves, n=13 embryos) and aphidicolin treated embryos + *pacta-lama2* (average of 15.53 ± 3.61 , 8 nerves, n=9 embryos) (****, $p \leq 0.0001$; **, $p = 0.0063$; ns, $p = 0.2166$).

Figure 11. Laminin expression is significantly reduced in AG1478 treated embryos and Laminin α 2 over-expression within Schwann cells rescues Mbp expression defects in *csp*^{-/-} and aphidicolin treated embryos

Laminin immuno-staining in *foxd3:gfp* (A-A'', n=6) and *foxd3:gfp* embryos treated with AG1478 (B-B'', n=8) at 48 hpf. Arrows indicate GFP+ Schwann cells in A and Laminin expression along the PLLn in A'. Scale bar = 20 μ m. A'' and B'' are the merge images of A and A' and of B and B' respectively. (C) Quantification of Laminin fluorescence intensity along the PLLn in *foxd3:gfp* (average of 33.63 $\pm$ 1.52) and *foxd3:gfp*/AG1478 treated embryos (average of 21.67 $\pm$ 1.44) (**, p=0.0013), a.u, arbitrary unit. (D,E) Lateral views of EGFP expression in Schwann cells of the PLLn following *pUAS-lama2* injection in *sox10:Gal4VP16*. Scale bars = 20 μ m. (D',E') Lateral views of mCherry-tagged secreted Laminin in Schwann cells and within the PLLn. (D'',E'') merge of D and D' and of E and E' respectively. mCherry tagged secreted Laminin is highlighted in E''' at higher magnification; white arrows in D'' and E''' indicate extracellular Laminin within the PLLn and white arrowheads in E'' and E''' indicate mCherry tagged Laminin within Schwann cells. (F) Lateral view of Mbp immuno-labeling in control embryo at 3 dpf. Scale bar = 50 μ m. (G, control, n=24) Lateral view showing the absence of EGFP expression in Schwann cells of the PLLn that correlates with a significant decrease in Mbp immuno-labeling (G') following *pUAS-lama2* injection but with no EGFP/mCherry expression in *csp*^{-/-}/*sox10:Gal4VP16*. Scale bar = 50 μ m. (G'') merge of G and G'. (H, n=8) Lateral view showing EGFP expression in Schwann cells of the PLLn that correlates with a normal Mbp immuno-labeling (H') following *pUAS-lama2* injection with positive clones of EGFP/mCherry in *csp*^{-/-}/*sox10:Gal4VP16*. Scale bar = 20 μ m. (H'') merge of H and H'. (I, control, n=26) Lateral view showing the absence of EGFP expression in Schwann cells of the PLLn that correlates with a significant decrease in Mbp immuno-labeling (I') following *pUAS-lama2* injection but

with no EGFP/mCherry expression in *sox10:Gal4VP16* treated with aphidicolin between 22 and 34 hpf. Scale bar = 50 μ m. (I'') merge of I and I'. (J, n=9) Lateral view showing EGFP expression in Schwann cells of the PLLn that correlates with a normal Mbp immuno-labeling (J') following *pUAS-lama2* injection with positive clones of EGFP/mCherry in *sox10:Gal4VP16* treated with aphidicolin between 22 and 34 hpf. Scale bar = 20 μ m. (J'') merge of J and J'.

Supplemental information

Figure S1. Timeline of Schwann cell behavior and myelination in zebrafish. Schwann cells migrate and divide along growing axons between 24 hpf and 48 hpf. They start to radially sort axons of the PLLn at around 48 hpf and divide intensively. Myelin sheath is analyzed starting from 3 dpf (or 72 hpf). hpf, hours post fertilization; dpf, days post fertilization; PLLn, posterior lateral line nerve.

Figure S2. Sil is not required for axonal growth nor for mitochondrial axonal transport along the PLLn but regulates the number of neurons within the PLLg

(A) Acetylated tubulin expression in a control embryo (n=12) and *csp*^{-/-} embryo (n=13) at 48 hpf showing the PLLn nerve (arrows). Lateral views of a control (n=16) and *csp*^{-/-} embryo (n=11) at 48 hpf showing PLLn GFP-expressing Schwann cells (arrows). Scale bars = 50 μ m.

(B) Still images from time-lapse imaging in control and *csp*^{-/-} embryos injected with *mito:gfp*. Arrows point to the same mitochondria followed through time in control and *csp*^{-/-}. Scale bar = 5 μ m. s, seconds.

(C) Quantification of the average speed of mitochondria along the PLLn at 50 hpf in controls (302 mitochondria, n= 8 embryos) and *csp*^{-/-} embryos (93 mitochondria, n= 6 embryos) (ns, p=0.6976).

(D) HuC immuno-labeling of the PLLg at 48 and 72 hpf in control and *csp*^{-/-} embryos. Scale bar = 5 μ m.

(E) Quantification of the number of neurons in the PLLg at 48 hpf in control (average of 54.58 $\pm$ 5.99 neurons, n= 12) and *csp*^{-/-} (average of 31.71 $\pm$ 5.31 neurons, n= 7) embryos and at 72 hpf in control (average of 74.33 $\pm$ 5.88 neurons, n= 12) and *csp*^{-/-} (40.29 $\pm$ 1.60 neurons n= 7) embryos (****, p $\leq$ 0.0001; ****, p $\leq$ 0.0001).

Figure S3. Schwann cells have a limited window of time to divide during migration in order to radially sort axons and myelinate

TEM of a cross section of the PLLn at 5 dpf in control (A) and embryo treated with aphidicolin between 22 and 34 hpf (B). Magenta asterisks highlight some large caliber myelinated axons (some shown at higher magnification in A', scale bar = 0.2 μ m) and blue asterisks show some large caliber non-myelinated axons (some shown at higher magnification in B', scale bar = 0.2 μ m). Scale bars = 0.5 μ m. (C) Quantification of the number of myelinated axons per nerve at 5 dpf in controls (average of 9 $\pm$ 1.29, 4 nerves, n= 4 embryos) and embryos treated with aphidicolin between 22 and 34 hpf (average of 0, 4 nerves, n= 4 embryos) (**, p=0.0061).

(D) Quantification of the total number of axons per nerve at 5 dpf in controls (average of 49.75 $\pm$ 3.32) and embryos treated with aphidicolin between 22 and 34 hpf (average of 28.75 $\pm$ 3.27) (**, p=0.0041).

(E) Quantification of the percentage of myelinated axons relative to the total number of axons per nerve at 5 dpf in controls (average of 17.94 $\pm$ 1.92) and embryos treated with aphidicolin between 22 and 34 hpf (average of 0) (**, p=0.0021).

(F) Quantification of the percentage of axons according to their diameter relative to the total number of axons per nerve at 5 dpf in controls (average of 66.66 $\pm$ 5.26 for 0-0.4 μ m; average

of 33.34 ± 5.27 for $>0.4 \mu\text{m}$) and aphidicolin treated embryos between 22 and 34 hpf (average of 54.34 ± 6.7 for $0-0.4 \mu\text{m}$; average of 45.66 ± 6.75 for $>0.4 \mu\text{m}$). ns, $p=0.2209$.

Movie S1. Real-time imaging of Schwann cells in *Tg(foxd3:gfp)* at 48 hpf.

A 48 hpf embryo expressing GFP in Schwann cells; the control embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents four and a half hours of continuous real-time imaging.

Movie S2. Real-time imaging of Schwann cells in *Tg(foxd3:gfp)/csp^{-/-}* at 48 hpf.

A 48 hpf embryo expressing GFP in Schwann cells; the *csp^{-/-}* embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents four and a half hours of continuous real-time imaging.

Movie S3. Real-time imaging of mitochondria in a control PLLn at 48 hpf.

A 48 hpf control embryo expressing GFP in mitochondria after *mito-gfp* mRNA injection; the embryo was imaged every 120 milliseconds for several minutes by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 18 seconds of real-time continuous imaging.

Movie S4. Real-time imaging of mitochondria in a *csp^{-/-}* PLLn at 48 hpf.

A 48 hpf *csp^{-/-}* embryo expressing GFP in mitochondria after *mito-gfp* mRNA injection; the embryo was imaged every 120 milliseconds for several minutes by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 36 seconds of continuous real-time imaging.

Movie S5. Real-time imaging of Schwann cell nuclei in control at 48 hpf.

A 48 hpf control embryo expressing GFP in Schwann cell nuclei after *h2b-gfp* mRNA injection; the embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 40 minutes of continuous real-time imaging.

Movie S6. Real-time imaging of Schwann cell nuclei in *csp*^{-/-} at 48 hpf.

A 48 hpf *csp*^{-/-} embryo expressing GFP in Schwann cell nuclei after *h2b-gfp* mRNA injection; the embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents 100 minutes of continuous real-time imaging.

Movie S7. Real-time imaging of Schwann cells in *Tg(foxd3:gfp)* at 28 hpf

A 28 hpf embryo expressing GFP in Schwann cells; the control embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents two hours of continuous real-time imaging.

Movie S8. Real-time imaging of Schwann cells in *Tg(foxd3:gfp)/csp*^{-/-} at 28 hpf.

A 28 hpf embryo expressing GFP in Schwann cells; the *csp*^{-/-} embryo was imaged every 4 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents four hours of continuous real-time imaging.

Movie S9. Real-time imaging of Schwann cells in *Tg(foxd3:gfp)* at 48 hpf.

A 48 hpf embryo expressing GFP in Schwann cells; the control embryo was imaged every 3 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents three hours of continuous real-time imaging.

Movie S10. Real-time imaging of Schwann cells in *Tg(foxd3:gfp)* treated with aphidicolin between 22 and 40 hpf at 48 hpf.

A 48 hpf embryo expressing GFP in Schwann cells; the aphidicolin treated embryo was imaged every 3.5 minutes for several hours by confocal microscopy. Lateral view; anterior to the left and dorsal to the top. This video represents six hours of continuous real-time imaging.

References

- Aplan PD, Lombardi DP, Ginsberg AM, Cossman J, Bertness VL, Kirsch IR (1990) Disruption of the human SCL locus by "illegitimate" V-(D)-J recombinase activity. *Science* 250: 1426-9
- Aplan PD, Lombardi DP, Kirsch IR (1991) Structural characterization of SIL, a gene frequently disrupted in T-cell acute lymphoblastic leukemia. *Mol Cell Biol* 11: 5462-9
- Benninger Y, Thurnherr T, Pereira JA, Krause S, Wu X, Chrostek-Grashoff A, Herzog D, Nave KA, Franklin RJ, Meijer D, Brakebusch C, Suter U, Relvas JB (2007) Essential and distinct roles for cdc42 and rac1 in the regulation of Schwann cell biology during peripheral nervous system development. *J Cell Biol* 177: 1051-61
- Boucanova F, Chrast R (2020) Metabolic Interaction Between Schwann Cells and Axons Under Physiological and Disease Conditions. *Front Cell Neurosci* 14: 148
- Boueid MJ, Mikdache A, Lesport E, Degerny C, Tawk M (2020) Rho GTPases Signaling in Zebrafish Development and Disease. *Cells* 9
- Bunge MB, Clark MB, Dean AC, Eldridge CF, Bunge RP (1990) Schwann cell function depends upon axonal signals and basal lamina components. *Ann N Y Acad Sci* 580: 281-7
- Bunge MB, Williams AK, Wood PM (1982) Neuron-Schwann cell interaction in basal lamina formation. *Dev Biol* 92: 449-60
- Campaner S, Kaldis P, Izraeli S, Kirsch IR (2005) Sil phosphorylation in a Pin1 binding domain affects the duration of the spindle checkpoint. *Mol Cell Biol* 25: 6660-72
- Chen Z-L, Strickland S (2003) Laminin γ 1 is critical for Schwann cell differentiation, axon myelination, and regeneration in the peripheral nerve. *Journal of Cell Biology* 163: 889-899
- Chernousov MA, Yu WM, Chen ZL, Carey DJ, Strickland S (2008) Regulation of Schwann cell function by the extracellular matrix. *Glia* 56: 1498-507
- Colognato H, Tzvetanova ID (2011) Glia unglued: how signals from the extracellular matrix regulate the development of myelinating glia. *Dev Neurobiol* 71: 924-55

1024 Court FA, Hewitt JE, Davies K, Patton BL, Uncini A, Wrabetz L, Feltri ML (2009) A laminin-2,
1025 dystroglycan, utrophin axis is required for compartmentalization and elongation of myelin
1026 segments. *J Neurosci* 29: 3908-19

1027 Cunningham RL, Herbert AL, Harty BL, Ackerman SD, Monk KR (2018) Mutations in dock1
1028 disrupt early Schwann cell development. *Neural Dev* 13: 17

1029 Eldridge CF, Bunge MB, Bunge RP (1989) Differentiation of Axon-Related Schwann Cells in
1030 V&O:II. Control of Myelin Formation by Basal Lamina.

1031 Feltri ML, Graus Porta D, Previtali SC, Nodari A, Migliavacca B, Casseti A, Littlewood-Evans A,
1032 Reichardt LF, Messing A, Quattrini A, Mueller U, Wrabetz L (2002) Conditional disruption of
1033 beta 1 integrin in Schwann cells impedes interactions with axons. *J Cell Biol* 156: 199-209

1034 Feltri ML, Poitelon Y, Previtali SC (2016) How Schwann Cells Sort Axons: New Concepts.
1035 *Neuroscientist* 22: 252-65

1036 Fontenas L, De Santis F, Di Donato V, Degerny C, Chambraud B, Del Bene F, Tawk M (2016)
1037 Neuronal Ndr4 Is Essential for Nodes of Ranvier Organization in Zebrafish. *PLoS Genet* 12:
1038 e1006459

1039 Frob F, Wegner M (2019) The role of chromatin remodeling complexes in Schwann cell
1040 development. *Glia*

1041 Gilmour DT, Maischein HM, Nusslein-Volhard C (2002) Migration and function of a glial
1042 subtype in the vertebrate peripheral nervous system. *Neuron* 34: 577-88

1043 Glenn TD, Talbot WS (2013) Analysis of Gpr126 function defines distinct mechanisms
1044 controlling the initiation and maturation of myelin. *Development* 140: 3167-75

1045 Gomis-Coloma C, Velasco-Aviles S, Gomez-Sanchez JA, Casillas-Bajo A, Backs J, Cabedo H
1046 (2018) Class IIa histone deacetylases link cAMP signaling to the myelin transcriptional
1047 program of Schwann cells. *J Cell Biol* 217: 1249-1268

1048 Grava S, Schaerer F, Faty M, Philippsen P, Barral Y (2006) Asymmetric recruitment of dynein
1049 to spindle poles and microtubules promotes proper spindle orientation in yeast. *Dev Cell* 10:
1050 425-39

1051 Grove M, Komiyama NH, Nave KA, Grant SG, Sherman DL, Brophy PJ (2007) FAK is required
1052 for axonal sorting by Schwann cells. *J Cell Biol* 176: 277-82

1053 Guo L, Moon C, Niehaus K, Zheng Y, Ratner N (2012) Rac1 controls Schwann cell myelination
1054 through cAMP and NF2/merlin. *J Neurosci* 32: 17251-61

1055 Hara M, Fukagawa T (2020) Dynamics of kinetochore structure and its regulations during
1056 mitotic progression. *Cell Mol Life Sci*

1057 Herbert AL, Monk KR (2017) Advances in myelinating glial cell development. *Curr Opin*
1058 *Neurobiol* 42: 53-60

1059 Jessen KR, Mirsky R (2005) The origin and development of glial cells in peripheral nerves. *Nat*
1060 *Rev Neurosci* 6: 671-82

1061 Kiyomitsu T, Cheeseman IM (2012) Chromosome- and spindle-pole-derived signals generate
1062 an intrinsic code for spindle position and orientation. *Nat Cell Biol* 14: 311-7

1063 Langworthy MM, Appel B (2012) Schwann cell myelination requires Dynein function. *Neural*
1064 *Dev* 7: 37

1065 Lara-Gonzalez P, Westhorpe FG, Taylor SS (2012) The spindle assembly checkpoint. *Curr Biol*
1066 22: R966-80

1067 Lu MS, Johnston CA (2013) Molecular pathways regulating mitotic spindle orientation in
1068 animal cells. *Development* 140: 1843-56

1069 Lyons DA, Pogoda HM, Voas MG, Woods IG, Diamond B, Nix R, Arana N, Jacobs J, Talbot WS
1070 (2005) *erbb3* and *erbb2* are essential for schwann cell migration and myelination in
1071 zebrafish. *Curr Biol* 15: 513-24
1072 Ma Y, Kanakousaki K, Buttitta L (2015) How the cell cycle impacts chromatin architecture and
1073 influences cell fate. *Front Genet* 6: 19
1074 Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C,
1075 Schwab MH, Nave KA (2004) Axonal neuregulin-1 regulates myelin sheath thickness. *Science*
1076 304: 700-3
1077 Mikdache A, Fontenas L, Albadri S, Revenu C, Loisel-Duwattez J, Lesport E, Degerny C, Del
1078 Bene F, Tawk M (2020) Elmo1 function, linked to Rac1 activity, regulates peripheral neuronal
1079 numbers and myelination in zebrafish. *Cell Mol Life Sci* 77: 161-177
1080 Mogha A, Benesh AE, Patra C, Engel FB, Schoneberg T, Liebscher I, Monk KR (2013) Gpr126
1081 Functions in Schwann Cells to Control Differentiation and Myelination via G-Protein
1082 Activation. *Journal of Neuroscience* 33: 17976-17985
1083 Monk KR, Feltri ML, Taveggia C (2015) New insights on Schwann cell development. *Glia* 63:
1084 1376-93
1085 Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, Moens CB, Talbot WS
1086 (2009) A G protein-coupled receptor is essential for Schwann cells to initiate myelination.
1087 *Science* 325: 1402-5
1088 Nave KA, Salzer JL (2006) Axonal regulation of myelination by neuregulin 1. *Curr Opin*
1089 *Neurobiol* 16: 492-500
1090 Naveed M, Kazmi SK, Amin M, Asif Z, Islam U, Shahid K, Tehreem S (2018) Comprehensive
1091 review on the molecular genetics of autosomal recessive primary microcephaly (MCPH). In
1092 *Genet Res (Camb)*,
1093 Newbern J, Birchmeier C (2010) Nrg1/ErbB signaling networks in Schwann cell development
1094 and myelination. *Semin Cell Dev Biol* 21: 922-8
1095 Nodari A, Previtali SC, Dati G, Occhi S, Court FA, Colombelli C, Zambroni D, Dina G, Del Carro
1096 U, Campbell KP, Quattrini A, Wrabetz L, Feltri ML (2008) Alpha6beta4 integrin and
1097 dystroglycan cooperate to stabilize the myelin sheath. *J Neurosci* 28: 6714-9
1098 Nodari A, Zambroni D, Quattrini A, Court FA, D'Urso A, Recchia A, Tybulewicz VL, Wrabetz L,
1099 Feltri ML (2007) Beta1 integrin activates Rac1 in Schwann cells to generate radial lamellae
1100 during axonal sorting and myelination. *J Cell Biol* 177: 1063-75
1101 Novorol C, Burkhardt J, Wood KJ, Iqbal A, Roque C, Coutts N, Almeida AD, He J, Wilkinson CJ,
1102 Harris WA (2013) Microcephaly models in the developing zebrafish retinal neuroepithelium
1103 point to an underlying defect in metaphase progression. *Open Biol* 3: 130065
1104 Paavola KJ, Sidik H, Zuchero JB, Eckart M, Talbot WS (2014) Type IV collagen is an activating
1105 ligand for the adhesion G protein-coupled receptor GPR126. *Sci Signal* 7: ra76
1106 Pereira JA, Lebrun-Julien F, Suter U (2012) Molecular mechanisms regulating myelination in
1107 the peripheral nervous system. *Trends Neurosci* 35: 123-34
1108 Petersen SC, Luo R, Liebscher I, Giera S, Jeong SJ, Mogha A, Ghidinelli M, Feltri ML,
1109 Schoneberg T, Piao X, Monk KR (2015) The adhesion GPCR GPR126 has distinct, domain-
1110 dependent functions in Schwann cell development mediated by interaction with laminin-
1111 211. *Neuron* 85: 755-69
1112 Petry S (2016) Mechanisms of Mitotic Spindle Assembly. *Annu Rev Biochem* 85: 659-83
1113 Pfaff KL, Straub CT, Chiang K, Bear DM, Zhou Y, Zon LI (2007) The zebra fish *cassiopeia*
1114 mutant reveals that SIL is required for mitotic spindle organization. *Mol Cell Biol* 27: 5887-97

1115 Raphael AR, Lyons DA, Talbot WS (2011) ErbB signaling has a role in radial sorting
1116 independent of Schwann cell number. *Glia* 59: 1047-55

1117 Raphael AR, Talbot WS (2011) New insights into signaling during myelination in zebrafish.
1118 *Curr Top Dev Biol* 97: 1-19

1119 Sherman DL, Brophy PJ (2005) Mechanisms of axon ensheathment and myelin growth. *Nat*
1120 *Rev Neurosci* 6: 683-90

1121 Silva PM, Reis RM, Bolanos-Garcia VM, Florindo C, Tavares AA, Bousbaa H (2014) Dynein-
1122 dependent transport of spindle assembly checkpoint proteins off kinetochores toward
1123 spindle poles. *FEBS Lett* 588: 3265-73

1124 Stewart HJS, Morgan L, Jessen KR, Mirsky R (1993) Changes in DNA Synthesis Rate in the
1125 Schwann Cell Lineage In Vivo Are Correlated With the Precursor - Schwann Cell Transition
1126 and Myelination.

1127 Sztal TE, Sonntag C, Hall TE, Currie PD (2012) Epistatic dissection of laminin-receptor
1128 interactions in dystrophic zebrafish muscle. *Hum Mol Genet* 21: 4718-31

1129 Tingaud-Sequeira Angèle DRb, Julie Lavie a, Guilaine Mathieu a, Magali Bordier a,c,, Anja
1130 Knoll-Gellida a PR, Isabelle Coupry a, Michèle André a, EvaMalmd, Claes Möller e,, Sten
1131 Andreasson d NDRf, Lisbeth Tranebjærg f, Michel Koenig g, Didier Lacombe a,c,, Cyril Goizet
1132 a c, Patrick J. Babin a, (2017) Functional validation of ABHD12 mutations in the
1133 neurodegenerative disease PHARC. *Neurobiology of Disease* 36-51

1134 Tricaud N (2017) Myelinating Schwann Cell Polarity and Mechanically-Driven Myelin Sheath
1135 Elongation. *Front Cell Neurosci* 11: 414

1136 Woodhoo A, Sommer L (2008) Development of the Schwann cell lineage: from the neural
1137 crest to the myelinated nerve. *Glia* 56: 1481-90

1138 Yamada H, Denzer AJ, Hori H, Tanaka T, Anderson LVB, Fujita S, Fukuta-Ohi H, Shimizu T,
1139 Rueff MA, Matasumura K (1996) Dystroglycan Is a Dual Receptor for Agrin and Laminin-2 in
1140 Schwann Cell Membrane.

1141 Yang D, Bierman J, Tarumi YS, Zhong YP, Rangwala R, Proctor TM, Miyagoe-Suzuki Y, Takeda
1142 S, Miner JH, Sherman LS, Gold BG, Patton BL (2005) Coordinate control of axon
1143 defasciculation and myelination by laminin-2 and -8. *J Cell Biol* 168: 655-66

1144 Yu WM, Feltri ML, Wrabetz L, Strickland S, Chen ZL (2005) Schwann Cell-Specific Ablation of
1145 Laminin 1 Causes Apoptosis and Prevents Proliferation.

1146 Zaqout S, Morris-Rosendahl D, Kaindl AM (2017) Autosomal Recessive Primary Microcephaly
1147 (MCPH): An Update. *Neuropediatrics* 48: 135-142

1148

1149

1150

Control

csp^{-/-}

A Control

Aphi.22-40 hpf