A genetic compensatory mechanism regulated by *Jun* and *Mef2d* modulates the expression of distinct class IIa *Hdacs* to ensure peripheral nerve myelination and repair

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ABSTRACT

The class IIa histone deacetylases (HDACs) have pivotal roles in the development of different tissues. Of this family, Schwann cells express *Hdac4*, 5 and 7 but not *Hdac9*. Here we show that a transcription factor regulated genetic compensatory mechanism within this family of proteins, blocks negative regulators of myelination ensuring peripheral nerve developmental myelination and remyelination after injury. Thus, when *Hdac4* and 5 are knocked-out from Schwann cells in mice, a JUN-dependent mechanism induces the compensatory overexpression of *Hdac7* permitting, although with a delay, the formation of the myelin sheath. When *Hdac4,5* and 7 are simultaneously removed, the Myocyte-specific enhancer-factor d (MEF2D) binds to the promoter and induces the *de novo* expression of *Hdac9*, and although several melanocytic lineage genes are misexpressed and Remak bundle structure is disrupted, myelination proceeds after a long delay. Thus, our data unveil a finely tuned compensatory mechanism within the class IIa *Hdac* family, coordinated by distinct transcription factors, that guarantees the ability of Schwann cells to myelinate during development and remyelinate after nerve injury.

INTRODUCTION

During the postnatal development of the peripheral nervous system (PNS), immature Schwann cells ensheath large caliber axons of sensory and motor neurons and differentiate, forming myelin, a highly specialized plasma membrane that increases nerve impulse velocity by allowing saltatory conduction (Kristjan R. Jessen & Mirsky, 2005). Immature Schwann cells downregulate the transcription factor *Jun* (which negatively regulates myelination) and upregulate the expression of transcriptional regulators of myelination such as *Krox-20* and *Yy1* (Fazal et al., 2017; Monk et al., 2015; Parkinson et al., 2008). *Jun* is strongly re-expressed after nerve injury enabling trans-differentiation of Schwann cells into a repair phenotype that promotes axon regeneration and functional nerve repair (Arthur-Farraj et al., 2012; Gomez-Sanchez et al., 2015). After axon regeneration Schwann cells reestablish contact with them and downregulate *Jun*. This allows re-expression of *Krox-20* and the consequent reactivation of a gene expression program aimed at remyelination of axons and reestablishment of nerve function (Stassart & Woodhoo, 2020). Activation of Gpr126, a G-protein–coupled receptor that increases intracellular levels of cAMP, is required for Schwann cell myelination and remyelination (Monk et al., 2009, 2011). We have recently shown that the pro-differentiating activity of cAMP is in part mediated by its ability to shuttle HDAC4 into the nucleus of Schwann cells (Gomis-Coloma et al., 2018). Nuclear HDAC4 recruits the complex NcoR1/HDAC3 and deacetylates histone 3 on the promoter of *Jun*, repressing its expression. At the same time HDAC4 promotes *Krox-20* expression and activation of the myelination program (Velasco-Aviles et al., 2018). *In vivo*, *Hdac5* is able to partially compensate for the loss of *Hdac4* expression in Schwann cells and only the removal of both *Hdac4* and *Hdac5* from Schwann cells leads to an obvious myelination delay. Surprisingly by postnatal day 8, myelination in *Hdac4/5* double knockout mice proceeds at the same pace as in wild-type nerves, suggesting that there is an additional compensatory mechanism permitting nerve myelination (Gomis-Coloma et al., 2018). Here we show that the *in vivo* elimination of *Hdac4* and *Hdac5* from Schwann cells induces the overexpression of *Hdac7* through a mechanism

mediated by the transcription factor JUN. Notably, the removal of *Hdac7* from Schwann cells in the absence of *Hdac4* and *Hdac5* produces a much longer delay in myelin development. This demonstrates that overexpressed *Hdac7* can partially compensate for the absence of both *Hdac4* and *Hdac5* in myelinating Schwann cells. Interestingly, non-myelin forming Schwann cells in these triple knock-outs (KOs) mis-express melanocytic lineage genes and fail to properly segregate small caliber axons in the Remak bundles. We show that genetic compensation also plays a pivotal role during remyelination after nerve injury. Thus, and akin to what happens during development, remyelination is delayed when *Hdac4* and *Hdac5* are removed from Schwann cells. This delay is longer when *Hdac7* is also removed, which has a profound impact on nerve impulse conduction during nerve regeneration. Importantly, remyelination in the *Hdac4/5/7* triple KO also catches up, supporting the idea that an additional mechanism compensates for the absence of class IIa *Hdacs*. Strikingly, *Hdac9*, the only class IIa *Hdac* that is not normally expressed by Schwann cells, is *de novo* expressed in the nerves of the *Hdac4/5/7* triple KO mice, induced by the transcription factor MEF2D. These genetic compensatory mechanisms, centering around transcription factors, allow Schwann cells to retain a class IIa *Hdac* gene dosage high enough to permit eventual myelination during development and remyelination after injury.

RESULTS

Upregulation of *Hdac7* permits developmental myelination in the absence of *Hdac4* and *Hdac5*. We have previously shown that *Hdac4* and *Hdac5* redundantly contribute to activate the myelin transcriptional program in Schwann cells *in vivo*. However, although during postnatal development *Jun* levels remain high in the PNS of the *Hdac4/5* double conditional knock out mice (*Mpz-Cre*^{+/-}; *Hdac4*^{flx/flx}; *Hdac5*^{-/-}, hereafter called dKO), myelination proceeds normally after P8 and adult nerves are morphologically and functionally indistinguishable from those of wild type mice (Gomis-Coloma et al., 2018). In muscle development class IIa *Hdacs* can compensate for each other (Potthoff, Olson, et al., 2007). In addition to *Hdac4* and *Hdac5*, Schwann cells also

express *Hdac7* (Gomis-Coloma et al., 2018). To test if it can functionally compensate for the absence of *Hdac4* and *Hdac5*, we measured the expression levels of *Hdac7* in the nerves of dKO. As shown in Figure 1A, the expression of *Hdac7* was substantially induced in the sciatic nerve of the dKO mice at P60 ($325,1 \pm 48,1$ %; $p=0,0034$, $n=4$), while *Hdac9* expression remained residual. This is specific for the dKO, as minor or no changes at all were found in the single KOs (Figure1-figure supplement 1A and B). Importantly, *Hdac7* overexpression can be detected early during development (Figure1-figure supplement 1C). These results suggest that the simultaneous elimination of *Hdac4* and *Hdac5* from Schwann cells activates a mechanism aimed to compensate for the drop in the gene dose of class IIa *Hdacs* that upregulates 3-fold the expression of *Hdac7*. To test whether *Hdac7* can functionally compensate to allow myelination in the absence of *Hdac4/5*, we generated a *Hdac4/5/7* triple Schwann cell specific conditional KO (genotype *Mpz-Cre*^{+/-}; *Hdac4*^{flx/flx}; *Hdac5*^{-/-}; *Hdac7*^{flx/flx}, hereafter called tKO; Figure1-figure supplement 1D-F). To study myelin development in these mice we evaluated a number of morphological parameters of sciatic nerves at P2, P8 and P21 using transmission electron microscopy (TEM) images. We also quantified the mRNA and protein levels for a number of negative and positive regulators of myelination. We previously separately analyzed Schwann cell gene expression in sciatic nerves of *Hdac4* conditional KO mice (genotype *Mpz-Cre*^{+/-}; *Hdac4*^{flx/flx}, referred to as cKO4) mice and global *Hdac5* KO mice (referred to as KO5) (Gomis-Coloma et al., 2018). As additional controls, here we also performed a detailed morphological analysis of developing nerves in cKO4, KO5, dKO and *Hdac7* Schwann cell specific conditional KO mice (*Mpz-Cre*^{+/-}; *Hdac7*^{flx/flx} referred to as cKO7) (Figure1-figure supplement 2 to 5). As is shown in Figure1-figure supplement 2 to 4, morphological quantification showed that within the single mutants, only cKO4 showed a subtle, but consistent, delay in myelin development. In line with our previous results (Gomis-Coloma et al., 2018), the simultaneous elimination of *Hdac4* and *Hdac5* from Schwann cells produced a greater decrease in the percentage of myelinated axons at P2 that was almost normalized by P8 (Figure1-figure supplement 5). Strikingly, the simultaneous elimination of *Hdac4,5* and 7 from Schwann cells produced a much more pronounced delay in myelin development (Figures 1B-J).

144 Interestingly, expression of the negative regulators of myelination (including
145 *Jun*) was notably increased from P2 to P21, which can explain the delay in the
146 expression of myelin genes (Figure 1K-M) and in morphological parameters of
147 myelin development in the tKO mice. Thus, our data demonstrates that *Hdac7*
148 upregulation can compensate for the absence of *Hdac4* and *Hdac5* allowing
149 myelination to proceed, although with some delay. Interestingly, and although
150 the coordinated removal of *Hdac4*, *Hdac5* and *Hdac7* produces a long delay in
151 myelination, myelin is finally formed and adult tKO nerves show almost normal
152 myelination parameters (Figures 2A-D).

153
154 **Defects in Remak Schwann cell differentiation in the tKO.** Despite PNS
155 myelination looks normal in the adult (p60) tKO mice, we found the Remak
156 bundles profoundly altered in these nerves, with many axons not properly
157 segregated (Figure 2E). Thus, there is a significant increase in the number of
158 pockets with 2-5 axons and, although it is very rare to find pockets with more
159 than 5 axons in the control (2,3%), an important number of axons are grouped
160 together in packs of more than 5 in the tKO (16,5%), with some of them being in
161 pockets of more than 30 axons. These defects are specific of the tKO, as no
162 major changes were observed in the single neither the dKO nerves (*Figure 2-*
163 *figure supplement 1A*).

164 Whole genome-wide transcriptome analysis showed 654 upregulated and 616
165 downregulated genes in the nerves of adult tKO (Figure 2F and Source data file
166 2 online (RNA-seq source data)). Volcano plot shows that genes tended
167 towards being more strongly upregulated than downregulated. Surprisingly, the
168 most robustly upregulated gene is the Tyrosinase-related protein 1 encoding
169 gene (*Tyrp1*; Log FC= 6,03; FDR=0), a melanocyte lineage specific gene
170 (Figures 2G and 2H). Additionally, the melanoma cell adhesion molecule *Mcam*
171 and *Ngfr* genes are also highly induced in the sciatic nerves of the tKO.
172 RTqPCR confirmed the strong induction of *Tyrp1* and *Mcam* in the sciatic
173 nerves of the tKO (P60), but not in the single KOs neither dKO nerves (Figures
174 3A and 3B). Interestingly we also found increased the expression of
175 Microphthalmia-associated transcription factor (*Mitf*) and the Endothelin B
176 receptor (*Ednrb*), two other genes of the melanocytic lineage (Figures 3C and
177 3D). Importantly, the expression of all these genes increased from early in

postnatal development (*Figure 2-figure supplement 1B and C*). Western blot analysis (*Figures 3E and 3F*) and confocal microscopy confirmed these findings and showed that the mis-expression of melanocytic lineage markers is confined to the non-myelin forming Schwann cells of the Remak bundles (*Figures 3G-K*). Thus, our data suggest that class IIa HDACs are necessary to allow Schwann cell precursors (SCP) to differentiate into Remak Schwann cells and properly segregate small size axons.

Remyelination kinetics after nerve injury depends on class IIa *Hdac* gene dose. The molecular mechanisms of Schwann cell remyelination share similarities to myelination during development, however there are also notable differences (Stassart & Woodhoo, 2020). Given the role of class IIa HDACs in myelination during development we asked whether they are also involved in remyelination after nerve injury. To address this, we first performed crush experiments in the sciatic nerves of 8-week-old cKO4 mice. As controls, we used *Mpz-Cre^{-/-};Hdac4^{flx/flx}* littermates (*Figure 4-figure supplement 1A-K*). At 10 days post injury (dpi), we found a small decrease in the percentage of myelinated axons, with an increase in the number of unmyelinated axons with a diameter > 1,5 µm in a 1:1 relationship with the Schwann cells (*Figure 4-figure supplement 1F and K*). A small increase in the number of Schwann cell nuclei was also found at 20 dpi and 30 dpi (*Figure 4-figure supplement 1I*). Also, subtle but significant changes in myelin protein gene expression were found in the cKO4 nerves (*Figure 4-figure supplement 1L-N*). Notably, myelin clearance was normal ruling out this as the cause of remyelination delay (*Figure 4-figure supplement 1O*). Thus, *Hdac4* removal has as small impact on remyelination that is compensated for after 10 dpi.

Morphological analysis of remyelination in KO5 mice and wild type littermates (*Hdac5^{+/+}*) showed no differences (*Figure 4-figure supplement 2*). Also, no notable differences in myelin protein gene expression (*Figure 4-figure supplement 1L-N and Figure 4-figure supplement 2L-M*) nor myelin clearance were found between both genotypes (*Figure 4-figure supplement 1O and Figure 4-figure supplement 2N*).

To explore whether there is also genetic compensation within class Iia *Hdacs* in Schwann cells after injury, we analysed remyelination in the dKO crushed

nerve. In this case, the percentage of myelinated axons at 10 days after crush (10 dpi) was notably decreased ($15,5 \pm 2,3$ % in the dKO versus $60,4 \pm 4,8$ % in the control; $p \leq 0,0001$) (Figures 4A and 4K). Total axon counts were similar between genotypes suggesting that this difference was not due to an axon regeneration defect (Figure 4G). At 20 dpi the difference between both genotypes was reduced and normalized at 30 dpi. A notable increase in the number of unmyelinated axons with a diameter $> 1,5 \mu\text{m}$ in a 1:1 relationship with the Schwann cells was found at 10 dpi and at 20 dpi that almost normalized at 30 dpi (Figure 4F). Furthermore, *g-ratio* was increased at 10 dpi and at 20 dpi but showed no difference between control and dKO nerves at 30dpi (Figure 4D). Quantifying gene expression at 10 dpi demonstrated that mRNA for *Jun* remains higher in the dKO injured nerves, as does *Gdnf* (Figure 4L). In the same line, we found a significant decrease in the mRNA for *Krox-20*, *Periaxin*, *Mpz*, and *Mbp*. We did not find changes in the expression of *Runx2* or *Pou3f1* (Figures 4L and 4M). To substantiate these results, we looked at protein levels, and found JUN protein remained higher in the dKO at 10 dpi and was unchanged at 21 dpi. Though there was no difference in KROX-20, MPZ protein levels were decreased at 21 dpi. Together, our data supports the view that the simultaneous removal of *Hdac4* and *Hdac5* from Schwann cells produces a more pronounced delay in remyelination after nerve injury.

This remyelination delay could be caused by an intrinsic problem in the capacity of Schwann cells to reactivate the myelination program, but could also be secondary to a failure in the ability of myelinating Schwann cells to acquire the repair phenotype and clear myelin debris in the distal stump. However, we did not favor the second explanation because markers of the Schwann cell repair phenotype, such as *Bdnf* and *Olig1*, in addition to *Jun*, are highly expressed in the dKO at 10 dpi (Figure 4L and 4N). Furthermore, we did not find any change in the number of intact myelin sheaths at 4 days after cut in the dKO, or in clearance of MPZ protein, suggesting no effect on the rate of demyelination (Figure 4-figure supplement 3A and B). Finally, repair program genes were normally upregulated in the dKO (Figure 4-figure supplement 3C). Together, our data show that the delay in remyelination of the dKO is due to an intrinsic defect of Schwann cells to activate the myelin transcriptional program and not a

consequence of an altered reprogramming capacity to the repair phenotype or to delayed myelin clearance.

Since remyelination is only moderately delayed in the dKO, we asked whether *Hdac7* was able to functionally compensate in a similar way as in development (previously, we established that cKO7 mice have no defects in myelin clearance, injury induced gene expression or remyelination after an injury (Figure 5-figure supplement 1). First, we found that tKO Schwann cells upregulate repair program genes after a cut injury (Figure 5-figure supplement 2) and interestingly some of them (*Olig1* and *Shh*) appear to be overexpressed at some time points (Figure 5-figure supplement 2B and C), suggesting that the class IIa HDACs act as a brake on the initial induction of the Schwann cell repair phenotype. In line with this observation, myelin was more rapidly cleared in these mutants (Figures 5A-C). Surprisingly, we could not find changes in autophagy markers neither macrophages numbers (Figure 5-figure supplement 2G-I), suggesting these mechanisms are not responsible of the observed increased myelin clearance.

On assessment of tKO nerves after a crush injury (Figures 5D-N), strikingly, we could not find any myelinated axon profile in the four tKO sciatic nerves analyzed at 10 dpi. This is in contrast to the controls which showed myelin profiles in $73 \pm 3,1$ % of axons ($p \leq 0,0001$) (Figure 5N). At 20 dpi, the tKO still had only $19,2 \pm 5,5$ % of the axons myelinated, whereas almost all large caliber axons were myelinated in the control ($98,1 \pm 0,2$ %; $p = 0,0001$). Moreover, at 30 dpi only $60,3 \pm 6,1$ % of the axons were myelinated ($p = 0,0031$) in the tKO mice. At 60 dpi, myelination was only slightly delayed in the tKO (Figure 5N). Differences in *g-ratios* followed the same pattern (Figure 5G). In the same line we found a notable increase in the number of unmyelinated axons $>1,5 \mu\text{m}$ at 10 dpi, that decreases slowly but progressively up to 60 dpi, when it approaches a similar number to the control (Figure 5H). Interestingly most of these unmyelinated axons are in a 1:1 relationship with Schwann cells (Figure 5I), suggesting that the delay is in the transition from the pro-myelinating to the myelinating Schwann cell stage. We also found a notable increase in the number of Schwann cells per nerve section that was maintained after 60 dpi (Figure 5L), and an increase in the nerve area (Figure 5E). The increased

number of Schwann cells is probably consequence of over-proliferation, as suggested by Ki67 staining (*Figure 5-figure supplement 3*). We also observed a decrease in the number of axons $>1,5 \mu\text{m}$ at 20 and 30 dpi (*Figure 5J*), probably because the smaller diameter of the unmyelinated axons. Finally, the delay in remyelination was substantiated by Western blot. As shown in *Figure 5O*, JUN protein is clearly more abundant in the nerves of the tKO than in either control littermates or wild types, both at 10 dpi and 21 dpi. Conversely, the amount of MPZ protein is lower at 10 and 21dpi. KROX-20 is also lower at 10 dpi in the tKO, but levels had recovered by 21 dpi.

To gain insight into the functional consequences of the remyelination delay in the tKO we performed nerve impulse conduction studies of the sciatic nerves after crush injury (see Material and methods). In uninjured nerves, we found no differences in voltage amplitude or nerve conduction velocity between tKO and controls (*Figure 6A-D*) (curiously we observed a smaller amplitude and slower nerve conduction velocity for all genotypes when compared with wild type nerves (*Figure 6-figure supplement 1*), probably due to the absence of *Hdac5* in neurons). By contrast, at 40 dpi, whereas 6 of 9 sciatic nerves of control mice showed a response when electrically stimulated at 8V, only 1 of 8 tKO responded (*Figures 6E and 6F*). The same distribution was found at 10 V. At 15V, 8 of 9 control mice responded while only 4 of 8 tKO responded. In the same line, the amplitude of the A-fiber component of the compound action potential was decreased for 8, 10 and 15V stimuli (*Figure 6G*). Regarding the component corresponding to C fibers, amplitude was also decreased for 8 and 10V stimulation (*Figure 6H*). Moreover, nerve conduction velocity showed a statistically significant decrease when using 15V stimuli (*Figure 6I*).

All together our data demonstrate that the kinetics of remyelination after nerve injury is directly correlated with class IIa *Hdac* gene dose.

Targets of class IIa HDACs in Schwann cells. To try to identify the genes regulated by class IIa HDACs we first performed a genome-wide transcriptomic analysis of the tKO remyelinating sciatic nerves after a crush injury and control littermates (Source data file 2 online (RNA-seq source data)). At 1 dpi, 395 genes were up regulated and 274 downregulated in the tKO (*Figure 5-figure supplement 4A* and *Figure 5-figure supplement 5A*). Similar to the uninjured

nerve analysis, the 10 most robustly changed genes were all upregulated. Interestingly *Tyrp1* and *Mcam* were also among the most upregulated genes. At 10 dpi the number of dysregulated genes was notably increased, with 1227 transcripts upregulated and 1550 downregulated (*Figure 5-figure supplement 4B* and *Figure 5-figure supplement 5A*). Among the most robustly upregulated genes was the repair cell marker *Bdnf* (Arthur-Farraj et al., 2017; Kristjan R. Jessen & Mirsky, 2019), possibly reflecting impairment of the tKO repair Schwann cells to fully re-differentiate into myelin and non-myelin forming Schwann cells. Interestingly, in contrast with previous time points, 8 of the 10 most robustly changed genes were downregulated, including many myelin-related genes (*Mal*, *Prx*, *Mag*, *Ncmamp*, *Pmp22*, *Mbp* and *Mpz*), which is expected given the dramatic delay in myelin sheath development in the tKO at 10 dpi (*Figure 5N*). At 20 dpi, we identified 1895 transcripts upregulated and 2450 downregulated in the tKO (*Figure 5-figure supplement 4C* and *Figure 5-figure supplement 5A*). As before, the 10 most robustly changed genes were upregulated and among them we found again *Tyrp1*, *Mcam* and *Ednrb* (*Figure 5-figure supplement 4C*). The negative regulator of myelination *Jun* was upregulated in the tKO from 1 dpi and remained increased up to 20 dpi (*Figure 5-figure supplement 5B*). A similar profile was shown for *Runx2*, *Gdnf*, *Ngfr* and *Sox2* (*Figure 5-figure supplement 5C-F*). *Pou3f1* was induced up to 10 dpi in both control and tKO nerves, to later (20 dpi) be downregulated in the control (20 dpi) but not in the tKO nerves (*Figure 5-figure supplement 5G*). By contrast, the master transcriptional regulator of myelination *Krox-20*, was downregulated at 10 and 20 dpi in the tKO, when remyelination was highly active in the controls (*Figure 5-figure supplement 5H*). Probably as a consequence, early myelin genes such as *Drp2* and *Prx* were downregulated (*Figure 5-figure supplement 5I* and *J*). Other myelin protein genes that are expressed later, such as *Mpz*, *Mbp*, *Mag*, *Pmp22* and *Plp1* were also consistently downregulated (*Figure 5-figure supplement 5K-O*). Myelin is a specialized plasma membrane with a distinctive lipid composition particularly rich in cholesterol (Poitelon et al., 2020). During remyelination of the tKO nerves we found several genes of the sterol branch of the mevalonate pathway downregulated (*Hmgcs*, *Lss*, *Dhcr24*) (*Figure 5-figure supplement 5P-R*). We also found downregulated genes encoding for enzymes involved in the elongation (*Elovl1*), transport (*Pmp2*) and

insertion of double bonds (*Scd2*, and *Fads1*) into fatty acids (Figure 5-figure supplement 5S-V). Interestingly, *Cers2* and *Ugt8a* were also downregulated. These genes are involved in the synthesis of sphingomyelin and galactosylceramide respectively (Figure 5-figure supplement 5W and X), both abundant lipids in myelin (Poitelon et al., 2020). Together our data shows that class IIa HDACs are necessary to block negative regulators of myelination and induce the expression of genes encoding for myelin proteins and key enzymes for the biosynthesis of myelin lipids.

To learn which genes are direct targets of class IIa HDACs and which are regulated indirectly, we performed a chromatin immunoprecipitation assay with anti-HDAC4 coupled to massive sequencing (ChIP-Seq) in dbcAMP differentiated Schwann cells. We found 3,932 peaks, 67,27% of which were located in the proximal promoter regions of genes (≤ 1 Kb from the transcription start site (TSS)) (Figure 7A). The localization of these peaks in the rat genome is shown in the Source data file 3 online (ChIP-Seq peaks source data). Importantly, ChIP-Seq analysis confirmed our previous results (Gomis-Coloma et al., 2018) showing that HDAC4 binds to the promoters of *Jun*, *Gdnf* and *Runx2* (Figure 7B-E). Interestingly, HDAC4 also binds to the promoter region of *Sox2* (Figure 7B and 7F), another negative regulator of myelination. We found also peaks for *Id2* and *Hey2* (Figure 7B and Source data file 3 online (ChIP-Seq peaks source data)).

Here we show that *Pou3f1* is overexpressed in the PNS of the tKO during development (Figure 1K-M), and that it is not properly downregulated during remyelination (Figure 5-figure supplement 5G). Interestingly, we found three peaks of HDAC4 bound near the TSS of *Pou3f1* (Figures 7B and 7G), a result that was confirmed by ChIP-qPCR (Figure 7H).

Regarding the melanocyte lineage, we found a clear peak of HDAC4 close the TSS of *Mcam* (Figure 7B), however, we did not detect peaks in *Tyrp1* and *Ednrb*, suggesting that, while HDAC4 directly represses the expression of *Mcam* it likely indirectly leads to *Tyrp1* and *Ednrb* repression. However, an alternative explanation is that it achieves repression of these genes by using alternative promoters or enhancers.

Surprisingly, we also found peaks in the promoter regions of *Mbp* and *Hmgcr* (Fig 7B), two genes highly expressed during myelination. Although it could

seem contradictory as HDACs have mainly been described as transcriptional repressors, HDACs have been shown to be bound to the promoter regions of highly expressed genes in other tissues (Wang et al., 2009).

JUN binds to the promoter and induces the expression of *Hdac7* gene in

the PNS. As we have shown, the simultaneous elimination of *Hdac4* and *Hdac5* activates a mechanism to compensate for the drop in class IIa *Hdac* gene-dose in Schwann cells. This mechanism multiplies by 3-fold the expression of *Hdac7*, the other member of this family expressed in these cells (Figure 1A). But what mechanism is involved? We have shown before that *Jun* is highly expressed in the developing PNS of the dKO mice (Gomis-Coloma et al., 2018). Here we show that *Jun* expression remains high during remyelination in the dKO sciatic nerves (Figures 4L,4N and Figure 5-figure supplement 5B). Interestingly, we found in ENCODE (<https://www.encodeproject.org/>) that JUN binds to the promoter region of *Hdac7* in A549 cells. Thus, the increased JUN might bind to the promoter of *Hdac7* inducing its compensatory overexpression in the dKO nerves. To test this, we used ChIP-qPCR and found that, indeed, in cultured Schwann cells JUN binds to the *Hdac7* promoter (Figure 8A).

We then identified an evolutionarily conserved JUN consensus binding motif in the proximal promoter region of the mouse *Hdac7* gene (Figure 8-figure supplement 1). To test it functionally, a fragment of 1.189 bp containing this region was amplified by PCR and cloned into the pGL3-luciferase reporter vector (see Materials and methods). The 1.189-promoter-*Hdac7*-pGL3 luciferase construct was transfected into HEK293 cells together with a pcDNA3 plasmid encoding for *Jun*, and luciferase activity measured 12h post-transfection. As shown in Figure 8B, this promoter fragment responded to JUN by increasing the luciferase activity by 3,4 fold over the control, which supports the idea that *Hdac7* expression is regulated by JUN.

To further test this hypothesis *in vivo*, we utilized mouse transgenic lines (*Mpz-Cre^{+/-}/Rosa26^{flox-stop-Jun/+}* mice) that either overexpresses *Jun* in Schwann cells, referred to as Jun_OE mice (Fazal et al., 2017) or lack *Jun* expression in Schwann cells (*Mpz-Cre^{+/-}/Jun^{flox/flox}* mice, referred to as Jun_cKO mice; Parkinson et al., 2008) (Figure 8C). *Jun* overexpression in Schwann cells induced *Hdac7* expression by almost two-fold (Figure 8D). This was a specific

effect as no changes were found for *Hdac4* and *Hdac5* mRNA (Figures 8E and 8F). By contrast, *Jun* removal produced no changes in the expression of any class IIa *Hdacs*, suggesting it is not necessary for the basal expression of these *Hdacs* (Figures 8D-F). Thus, our data clearly show that JUN induces the expression of *Hdac7* by Schwann cells *in vivo*. Further supporting this tenet, we found that *Hdac7* is not overexpressed in the nerves of the dKO that lack *Jun* in Schwann cells (dKO;Jun_cKO) (Figure 8G).

Interestingly, we detected a peak of HDAC4 bound to the promoter of *Hdac7* (Figures 7B and 8H) in the ChIP-Seq experiment, a result that we confirmed by ChIP-qPCR (Figure 8I). This suggests that, in differentiated Schwann cells, other class IIa HDACs contribute to maintaining *Hdac7* expression at the basal level. The simultaneous loss of *Hdac4* and *Hdac5* allows the expression of *Jun*, which can bind to the promoter of *Hdac7*, now free of the repression by class IIa HDACs, increasing the transcription of this deacetylase.

***Hdac9* is expressed *de novo* in the sciatic nerve of the tKO.** Despite substantial delay, Schwann cells in tKO nerves are still able to eventually myelinate axons in development and during nerve repair. One possible explanation is that *Hdac9*, the remaining class IIa *Hdac* left, could compensate for the loss of the other three class IIa *Hdacs* in Schwann cells. However we have consistently found extremely low or undetectable levels of the mRNA for this protein in the C57BL/6 mouse sciatic nerves and cultured rat Schwann cells (Gomis-Coloma et al., 2018), a result that has been recently confirmed in the Sciatic Nerve ATlas (SNAT,<https://www.snat.ethz.ch>) (Gerber et al., 2021). Surprisingly, we found *Hdac9* among the most robustly upregulated genes by the tKO nerves in the RNA-seq analysis (Figure 2H). To confirm this result, we measured the mRNA for this gene in the sciatic nerves of the tKO and control adult mice (P60) by RTqPCR. As is shown in Figure 9A, the mRNA for *Hdac9* was increased by 4,4-fold in the tKO mice ($p<0,0001$). This increase was not found in the dKO and was smaller in the cKO7 (2,4 fold; $p=0,0057$) and cKO4 (1,3 fold; $p=0,01$) mice. These results suggest that *Hdac9* is strongly expressed in the sciatic nerves of the tKO mice to compensate for the absence of other class IIa HDACs. Indeed, we found that *Hdac9* is already induced early in development in the tKO (P2) whereas it was practically undetectable in the

nerves of control animals (Figure 9B). At this time, myelin sheaths are present, although sparse, in mutant nerves (Figure 1B-J). At P8, *Hdac9* expression remains extremely low in control nerves, but it is robustly expressed in the tKO mice (an increase of 5,3-fold; $p=0,0117$), correlating with an increase in the number of myelinated axons (Figure 1B-J). Robust *Hdac9* gene expression is maintained in the sciatic nerve of the tKO at P60 (Figure 9A). Interestingly, we found that in the tKO nerves, the lysine 9 of histone 3 associated to the *Hdac9* promoter is more acetylated (Figure 9C), suggesting that the absence of class IIa HDACs allows the expression of this gene. Importantly, *Hdac9* gene is furtherly upregulated in the injured sciatic nerves of tKO mice, as is shown in Figure 9D, where we show the alignment of the reads of the RNA-seq from three individual sciatic nerves from control and tKO mice UI and at 20 days post crush (20 dpi), and in Figure 9E, where we followed the mRNA levels of *Hdac9* (as Fragments Per Kilobase Million, FPKMs) at 0, 1, 10 and 20 days post crush in the RNA-seq experiment. Thus far, our data suggested that in the absence of class IIa *Hdacs*, repression is lost and Schwann cells start to express *de novo Hdac9*.

To investigate which transcription factor is responsible of *Hdac9* expression in the tKO we again looked at JUN. However we couldn't detect JUN bound to the promoter region of *Hdac9* in A549 cells in the ENCODE database. Also, *Hdac9* mRNA levels were not increased in the sciatic nerves of the Jun_OE mice (Figure 9-figure supplement 1).

Interestingly, it has been described that MEF2D binds to the promoter and regulates the expression of *Hdac9* during muscle differentiation, and in leiomyosarcoma cells (Di Giorgio et al., 2020; Haberland et al., 2007). We therefore reasoned that *Mef2d* might be involved in the upregulation of *Hdac9* in Schwann cells. Indeed, we have previously shown that *Mef2* family members are expressed in cultured Schwann cells and adult sciatic nerves (Gomis-Coloma et al., 2018). Importantly, RTqPCR shows that *Mef2d* mRNA is induced in the nerves of the tKO during development (Figure 9F). Also, RNA-seq data showed that *Mef2d* is upregulated in the sciatic nerves of the tKO mice at 10 and 20 dpi (Figure 9G). Western blot supported this idea (Figures 9H and 9I) and immunofluorescence studies (Figure 9J) showed that *Mef2d* is expressed

by Schwann cells (SOX10⁺). Importantly, we detected MEF2D bound to the promoter of *Hdac9* in the tKO nerves at 20 dpi (Figure 9K).

Altogether our data support the view that in the tKO nerve, *Mef2d* is induced to activate the *de novo* expression of *Hdac9* and maintain a sufficient class IIa *Hdac* gene dose to allow myelin formation during development and after nerve injury.

DISCUSSION

Functional redundancies, the consequence of gene duplications, are found in most genomes, and are postulated to give robustness to organisms against mutations. However, it has been also predicted that redundancies are evolutionarily unstable and have only a transient lifetime. Despite of this, numerous examples exist of gene redundancies that have been conserved throughout dilated evolutionary periods (Kafri et al., 2006; Peng, 2019).

Fluctuations in gene expression (noise) is a well-known phenomenon that has been described from bacteria to mammalian cells, and may have dramatic effects on fitness if they persist long enough (Raser, 2010). It has been suggested that some gene redundancies have been evolutionarily selected because they can reduce the harmful effects of gene expression noise (Kafri et al., 2006). Thus, the deleterious effect of the eventual decrease in the expression of a noisy gene pivotal for a determined biological process (such as differentiation) can theoretically be buffered by the expression of a redundant gene controlled by a different promoter.

Redundancies have been shown to be particularly relevant during development. One example is the couple *Myod/Myf-5*, which are master regulators of skeletal muscle development (Sabourin & Rudnicki, 2000). Similar to what happens with other redundant couples, *Myf-5* expression has a linear response that strictly depends on the *Myod* gene expression dosage, and can likely contribute to reduce gene expression noise allowing skeletal muscle differentiation (Kafri et al., 2006).

It has been shown that slow oxidative fiber gene expression in skeletal muscles depends on gene redundancy between class IIa *Hdacs* (Potthoff, Wu, et al., 2007). Here we show that the activation of the myelin gene expression program

by Schwann cells is also ensured by *class IIa Hdacs* gene redundancy. Although the physiological role of genetic compensation within this family of proteins remains unknown, it is tempting to speculate that it could avoid potential fluctuations in *class IIa Hdac* gene dose ensuring Schwann cell differentiation and the proper myelination of the peripheral nervous system.

But how is gene compensation regulated? Despite being documented many times in different organisms, our understanding of the underlying molecular mechanisms that control this process still remains limited (El-Brolosy & Stainier, 2017). Thus, genetic compensation of *class I Hdacs* has been previously described during myelin development (Jacob et al., 2011), although the mechanisms regulating this process have not been investigated. Here we show that removal of *Hdac4* and *Hdac5* upregulates the compensatory overexpression of *Hdac7* in Schwann cells allowing, although with delay, myelin formation both during development and after nerve injury. Our data strongly suggests that this compensatory overexpression is regulated by the transcription factor JUN. In support of this tenet, we show that JUN binds to and induces the overexpression of *Hdac7* both *in vitro* and *in vivo*. Moreover, we also show *Hdac7* is not overexpressed in the nerves of dKO mice that lack *Jun* expression in Schwann cells. Interestingly, we found that HDAC4 binds to the promoter of *Hdac7* in differentiated Schwann cells, suggesting that other *class IIa Hdacs* contribute to maintain the expression of this gene at basal levels in normal nerves. In this scenario, the absence of *Hdac4* and *Hdac5* in the dKO nerves might allow JUN to bind and stimulate the compensatory expression of *Hdac7* in Schwann cells (Figure 10).

We show that although *Hdac9* is normally not expressed by Schwann cells it is robustly upregulated in the nerves of the tKO. *Hdac9* is also upregulated, although much less, in the cKO7 and cKO4 mice. This support the idea that *Hdac9* gene is *de novo* expressed in response to the drop of *class IIa Hdacs* gene dose to allow myelination. Interestingly, *Hdac9* is also induced in the nerves dKO;Jun_cKO (Figure 8G), probably as a response to the drop of *class IIa Hdacs* in Schwann cells that cannot overexpress *Hdac7* because of the absence of *Jun*.

To investigate the mechanisms that activate the expression of *Hdac9* in the tKO nerves we focused our attention in the MEF2 family of transcription factors, as they regulate *Hdac9* expression in other tissues (Di Giorgio et al., 2020; Haberland et al., 2007). Interestingly, we found MEF2D overexpressed and bound to the *HDCA9* promoter in the tKO nerves.

It has been shown that MEF2 transcriptional activity is blocked by class IIa HDACs (Haberland et al., 2007). Thus, other class IIa HDACs can theoretically block the expression of *Hdac9* in control nerves. However, in the tKO, no class IIa *Hdac* is expressed in Schwann cells and a free of repression MEF2D might be able to induce *Hdac9* gene expression. Supporting this view, we found much more H3K9Ac associated with the *Hdac9* promoter in the tKO nerves.

Taken together, our data suggest that, the overexpressed and unrepressed promoter-bound MEF2D transcription factor, induces the *de novo* expression of *Hdac9* in the Schwann cells of the tKO mice (Figure 10).

Although adult tKO nerves have morphologically normal myelin, RNA-seq analysis showed 1270 genes differently expressed, the most robustly changed genes being upregulated. This suggests a predominantly gene repressive function for class IIa HDACs in Schwann cells, as is the case for other cell types (Parra, 2015; Parra & Verdin, 2010). The most robustly upregulated gene in these mice was *Tyrp1*, a gene involved in the stabilization of tyrosinase and the synthesis of melanin. Strikingly, we did not find expression of the tyrosinase gene in these nerves, suggesting a different role for *Tyrp1*. In fact, our data shows that *Tyrp1* mRNA is not translated into protein (Figure 2-figure supplement 1D). It has been shown that *Tyrp1* mRNA indirectly promotes melanoma cell proliferation by sequestering miR-16 (Gautron et al., 2020; Gilot et al., 2017). Whether *Tyrp1* mRNA is also responsible for the increased cell proliferation of Schwann cells in the tKO nerve is something that needs to be clarified in the future.

It has been previously shown that a subgroup of melanocytes are formed from Schwann cell precursor cells (Adameyko et al., 2009). Because *Mpz-Cre* is already expressed by SCPs, our data points towards a role for class IIa HDACs in the repression of genes of the melanocytic lineage in these cells. Interestingly, it has been previously suggested that axonal derived signals

repress SCP from going into the melanocytic lineage (Graham, 2009). Thus, our data suggest that these signals are not properly interpreted by tKO Schwann cells, precluding them to repress several melanocytic lineage genes. Notably, we found that melanocyte lineage genes are still expressed by the Remak Schwann cells of the adult tKO nerves. This mis-expression could be in the origin of the alterations in the segregation of small size axons at the Remak bundles, a defect that remains during the whole life of the animal.

We also show that class IIa *Hdacs* removal delays remyelination after a nerve crush injury. Importantly, tKO Schwann cells are efficiently reprogrammed into the repair phenotype and myelin clearance is even more efficient than in control nerves, ruling out a problem in debris removal as the cause of remyelination delay. Although we don't know why myelin clearance is accelerated, it is worthy to mention that no increased autophagy markers neither macrophage numbers could be found in the tKO nerves. If myelin clearance is accelerated because changes in the rate axon degeneration or in ovoid formation is something that needs to be clarified in future experiments.

Genome-wide transcriptomic analysis of the injured tKO nerves showed that the number of differentially expressed genes increases after crush, and is maximum at 20 dpi. Many genes are robustly upregulated, particularly at 1 and 20 dpi, supporting further the idea that the main role of class IIa HDACs in Schwann cells is to repress gene expression. This agrees with the role of this family of deacetylases in other contexts (Chang et al., 2004, 2006; Parra & Verdin, 2010) where they work mainly as co-repressors of transcription factors such as MEF2 and RUNX2 (Bialek et al., 2004; Potthoff & Olson, 2007; Vega et al., 2004). Importantly, among the upregulated genes in the tKO injured nerves we found *Jun*, *Runx2*, *Gdnf*, *Ngfr* and *Sox2*, all expressed by non-myelinating and repair Schwann cells. We also found *Pou3f1* overexpressed in the tKO nerves. It has been shown that *Pou3f1* overexpression delays PNS myelination (Ryu et al., 2007). Thus, *Pou3f1* mis-expression may also contribute to the delayed myelination of the tKO nerve both during development and after injury.

In a simplistic model, the failure of class IIa HDACs to downregulate *Jun* could indirectly induce the expression of other negative regulators of myelination controlled by this transcription factor. However, class IIa HDACs could also

directly repress the expression of other negative regulators of myelination. To explore this idea, we performed a genome-wide mapping of genes that are direct targets of HDAC4. Importantly, we confirmed our previous results showing that HDAC4 binds to the promoters of *Jun*, *Runx2* and *Gdnf* in Schwann cells (Gomis-Coloma et al., 2018). We found that HDAC4 also binds to the promoters of many other genes including other negative regulators of myelination such as *Sox2*, *Id2* and *Hey2*. Interestingly, HDAC4 also binds to the promoter of *Pou3f1*. Thus, the direct repressive effect of class IIa HDACs is not circumscribed to *Jun* but is much wider, supporting the view that they work as a cAMP-regulated blocking hub for repressors of myelination.

Surprisingly, we found that HDAC4 is also bound to the promoter of *Mbp* and *Hmgcr*, two genes that are actively expressed during myelination. HDACs are usually bound to repressed genes and replaced by histone acetyl transferases (HATs) upon gene activation (Lan et al., 1999). However, it has been shown that class I HDACs are also bound, together with HAT, to the promoter regions of actively transcribed genes (Wang et al., 2009). Interestingly, the histones associated with these promoters are heavily acetylated, what makes these authors to propose that one function of HDACs is to remove the acetyl groups added by HATs in active genes to reset chromatin modification after gene activation. Although class IIa HDACs have no deacetylase activity, they recruit class I HDACs by forming a complex with NCOR1 and SMRT. Thus, the possibility exists that class IIa and class I HDACs have a similar role when bound to the promoter of highly active genes in myelinating Schwann cells.

In summary, the data presented in this manuscript unveil responsive back-up circuits mediated by the transcription factors JUN and MEF2D, that coordinate genetic compensatory mechanisms within class IIa *Hdacs*, aimed at repressing the expression of negative regulators of myelination to ensure differentiation of Schwann cells in response to cAMP, and the generation of the myelin sheath during development and after nerve injury.

MATERIALS AND METHODS

Animal studies: All animal work was conducted according to European Union guidelines and with protocols approved by the Comité de Bioética y Bioseguridad del Instituto de Neurociencias de Alicante, Universidad Miguel Hernández de Elche and Consejo Superior de Investigaciones Científicas (<http://in.umh-csic.es/>). Reference number for the approved protocol was 2017/VSC/PEA/00022 tipo 2.

To avoid suffering, animals were sacrificed by cervical dislocation. The *Mpz-cre* mouse line is described in Feltri et al., 1999. *Mpz-cre*^{-/-} littermates were used as controls. *Hdac4* floxed mice are described in Lehmann et al., 2018 and Potthoff et al., 2007. The *Hdac5* KO mouse line is described in Chang et al., 2004. *Hdac7* floxed mice are described in (Chang et al., 2006). The Jun_OE and Jun_cKO mouse lines are described in Fazal et al., 2017 and Parkinson et al., 2008. MGI ID can be found online (Key Resources Table). Experiments used mice of either sex on the C57BL/6 background.

Plasmids: The luciferase reporter plasmid was generated by cloning the mouse *Hdac7* promoter into the NheI site of pGL4 Luciferase reporter plasmid (Promega). The mouse *Hdac7* promoter was amplified using Platinum SuperFi II DNA Polymerase (12361010, Thermo Fisher Scientific) and primers described online (Key Resources Table). The plasmid pCMV-Jun was a gift of Dr Marta Giralt (Universitat de Barcelona).

Reporter activity assays: HEK293 cells were transfected with the indicated constructs and then lysed. Their luciferase activity was determined with the Luciferase Assay System (Promega) using the manufacturer's recommendations.

Cell cultures: Schwann cells were cultured from sciatic nerves of neonatal rats as described previously (Brockes et al., 1979) with minor modifications. We used P3-P4 Wistar rat pups. The sciatic nerves were cut out from just below the dorsal root ganglia and at the knee area. During the extraction and cleaning, the nerves were introduced into a 35-mm cell culture dish containing 2 ml of cold Leibovitz's F-15 medium (Gibco) placed on ice. The nerves were cleaned,

desheathed, and placed in a new 35-mm cell culture dish containing DMEM GlutaMAX, 4.5 g/l glucose (Gibco) with 1 mg/ml of collagenase A (Roche). Subsequently they were cut into very small pieces using a scalpel and left in the incubator for 2 h. Nerve pieces were homogenized using a 1 ml pipette, digestion reaction stopped with complete medium, and the homogenate poured through a 40- μ m Falcon Cell Strainer (Thermo Fisher Scientific). We then centrifuged the homogenate at 210 g for 10 min at room temperature and resuspended the pellet in complete medium supplemented with 10 μ M of cytosine- β -D-arabinofuranoside (Sigma-Aldrich) to prevent fibroblast growth. The resuspended cells were then introduced into the poly-L-lysine-coated 35-mm cell culture dishes. After 72 h, the medium was removed and cell cultures expanded in DMEM supplemented with 3% FBS, 5 μ M forskolin, and 10 ng/ml recombinant NRG1 (R&D Systems). Where indicated, cells were incubated in SATO medium (composed of a 1:1 mixture of DMEM and Ham's F12 medium (Gibco) supplemented with ITS (1:100; Gibco), 0.1 mM putrescine, and 20 nM of progesterone (Bottenstein & Sato, 1979). HEK 293 cells were obtained from Sigma-Aldrich (Cat# 85120602). The cells were grown in non-coated flasks with DMEM GlutaMAX, 4.5 g/l glucose (Gibco) supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10% bovine fetal serum. Cells were transfected with plasmid DNA using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's recommendations.

Nerve injury: Mice were anesthetized with 2 % isoflurane. To study nerve regeneration and remyelination (axonal regrowth inside the distal stump) we performed a nerve crush injury model. Briefly, the sciatic nerve was exposed at the sciatic notch and crushed 3 times for 15s with 3 different rotation angles using angled forceps. To study the repair Schwann cell phenotype activation and myelin clearance, we performed a nerve cut to avoid nerve regeneration inside the distal stump. In this case, the sciatic nerve was exposed and cut at the sciatic notch using scissors. The wound was closed using veterinary autoclips (AutoClip® System). The nerve distal to the cut or crush was excised for analysis at various time points after euthanasia. Contralateral uninjured sciatic nerves were used as controls. For Western blotting and mRNA extraction we used the first 8 mm of the distal stump of crush injured nerves, and 1 cm or

3 cm of the control nerve, respectively. For electron microscopy we used the first 5,5 mm of the distal stump of crush or cut injured nerves.

Myelin clearance: Intact myelin sheaths were counted using transverse toluidine blue stained semithin sections (2µm) of sciatic nerve at 5 mm from the nerve cut injury site. Whole nerve merged images were taken with a x63 objective using a Leica Thunder Tissue Imager and quantified with ImageJ software.

mRNA detection and quantification by RT-qPCR: Total mRNA from uninjured or injured sciatic nerves was extracted using TRI reagent / chloroform (Sigma-Aldrich) and the mRNA was purified using a NucleoSpin RNA mini kit (Macherey-Nagel), following manufacturer's recommendations. RNA quality and concentration were determined using a nanodrop 2000 machine (Thermo). Genomic DNA was removed by incubation with RNase free DNase I (Thermo Fisher Scientific), and 500 ng RNA was primed with random hexamers (Invitrogen) and retrotranscribed to cDNA with Super Script II Reverse transcriptase (Invitrogen). Control reactions were performed omitting retrotranscriptase. qPCR was performed using an Applied Biosystems QuantStudio 3 Real Time PCR System and 5x PyroTaq EvaGreen qPCR Mix Plus (CMB). To avoid genomic amplification, PCR primers were designed to fall into separate exons flanking a large intron wherever possible. A list of the primers used can be found online (Key Resources Table). Reactions were performed in duplicates of three different dilutions, and threshold cycle values were normalized to the housekeeping gene 18S. The specificity of the products was determined by melting curve analysis. The ratio of the relative expression for each gene to 18S was calculated by using the $2^{-\Delta\Delta CT}$ formula. Amplicons were of similar size (≈100 bp) and melting points (≈85°C). Amplification efficiency for each product was confirmed by using duplicates of three dilutions for each sample.

RNA sequencing analysis: Total RNA was isolated using the NucleoSpin RNA, Mini kit for RNA purification (Macherey-Nagel). The purified mRNA was

fragmented and primed with random hexamers. Strand-specific first-strand cDNA was generated using reverse transcriptase in the presence of actinomycin D. The second cDNA strand was synthesized using dUTP in place of dTTP to mark the second strand. The resultant cDNA was then “A-tailed” at the 3' end to prevent self-ligation and adapter dimerization. Truncated adaptors containing a T overhang were ligated to the A-tailed cDNA. Successfully ligated cDNA molecules were then enriched with limited cycle PCR (10–14 cycles). Libraries to be multiplexed in the same run were pooled in equimolar quantities. Samples were sequenced on the NextSeq 500 instrument (Illumina). Run data were demultiplexed and converted to fastq files using Illumina's bcl2fastq Conversion Software version 2.18 on BaseSpace. Fastq files were aligned to the reference genome (Mouse (GRCm38/Ensembl release 95) and analyzed with Artificial Intelligence RNA-SEQ (A.I.R) software from Sequentia Biotech (<https://www.sequentiabiotech.com/>).

Antibodies: Immunofluorescence antibodies: JUN (Cell Signaling Technology, rabbit 1:800), Ki67 (Abcam, rabbit 1:100), L1 (Chemicon International, rat 1:50), MCAM (Origene, rabbit 1:200), MPZ (AvesLab, chicken 1:1000), NGFR (Thermo Fisher Scientific, mouse 1:100), SOX10 (R and D Systems, goat 1:100), donkey anti-goat IgG (H+L) Alexa Fluor 555 conjugate (Molecular Probes, 1:1000), donkey anti-rabbit IgG (H+L) Alexa Fluor 488 conjugate (Molecular Probes, 1:1000), donkey anti-chicken IgG (H+L) Alexa Fluor 488 conjugate (Jackson ImmunoResearch Labs, 1:1000), goat anti-rat IgG (H+L) Alexa Fluor 555 conjugate (Molecular Probes, 1:1000), Cy3 donkey anti-mouse IgG (H+L) (Jackson Immunoresearch, 1:500) and Cy3 donkey anti-rabbit IgG (H+L) (Jackson Immunoresearch, 1:500).

Antibodies used for western blotting: CALNEXIN (Enzo Life Sciences, rabbit 1:1000), JUN (Cell Signaling Technology, rabbit 1:1000), GAPDH (Sigma-Aldrich, rabbit 1:5000), HDAC5 (Santa Cruz, mouse 1:500), KROX-20 (Millipore, rabbit 1:500), MCAM (Origene, rabbit 1:1000), MPZ (AvesLab, chicken 1:1000), NGFR (Covance, rabbit 1:1000), TYRP1 (Sigma-Aldrich, rabbit 1:1000), IgY anti-chicken HRP-linked (Sigma-Aldrich, 1:2000), IgG anti-mouse and IgG anti-rabbit HRP-linked (Cell Signaling Technology, 1:2000). A list of the antibodies used can be found online (Key Resources Table).

786

787 **Immunofluorescence:** For immunofluorescence, mice were sacrificed by
788 cervical dislocation and fresh frozen tissue was embedded in OCT (Sakura,
789 4583). Cryosections were cut at 10 μ m on Superfrost Plus slides (Thermo
790 Scientific, J1800AMNZ). Sections were thawed and fixed with 4% PFA for 5 min
791 at room temperature. Then, samples were washed 3x in PBS 1x and immersed
792 in 50% acetone, 100% acetone and 50% acetone for 2 min each. Then samples
793 were washed 3x in PBS 1x and blocked in 5% donkey serum 0.1% BSA in PBS
794 for 1h. Samples were incubated with the appropriate primary antibodies diluted
795 in blocking solution overnight at room temperature. A list of the antibodies used
796 can be found online (Key Resources Table). Samples were washed and
797 incubated with secondary antibodies and DAPI in blocking solution for 1h at
798 room temperature. Samples were mounted in Fluoromont G. Images were
799 obtained at room temperature using a confocal ultraspectral microscope
800 (Vertical Confocal Microscope Leica SPEll) with a 63x Leica objective and
801 using Leica LAS X software. Images were analyzed with ImageJ software.

802

803 **EM studies:** Mice were sacrificed by cervical dislocation and sciatic nerve were
804 exposed and fixed by adding fixative solution (2% PFA (15710, Electron
805 Microscopy Sciences), 2.5% Glutaraldehyde (16220, Electron Microscopy
806 Sciences), 0.1M Cacodylate buffer pH=7.3 (12300, Electron Microscopy
807 Sciences) for 15 minutes. Afterwards, the nerve was removed and placed in
808 same fixative solution overnight at 4°C. Then, the nerve was washed in 0.1M
809 cacodylate buffer 3x for 15 minutes each. Then, the nerve was osmicated by
810 adding 1% osmium tetroxide, 0.1M cacodylate buffer pH=7.3 for 1 and half hour
811 at 4°C. Then, the nerve was washed 2x with dd H₂O for 15 minutes each.
812 Samples were dehydrated by washing progressively in: 25% ethanol for 5
813 minutes, 50% ethanol for 5 minutes, 70% ethanol for 5 minutes, 90% ethanol
814 for 10 minutes, 100% ethanol for 10 minutes (x4), propylene oxide for 10
815 minutes (x3). They were then changed into a 50:50 mixture of Agar 100
816 resin:propylene oxide for 1 hour at RT. The final change was into a 75:25
817 mixture of Agar 100 resin:propylene for 2 hours at RT. Nerves were blocked in
818 resin and left shaking O/N at RT. These nerves were re-blocked the following
819 day with fresh resin for 2 hours at RT. The nerves were finally embedded in

fresh resin and left in the oven for 24 hours at 65°C. Transverse ultrathin sections from nerves were taken 5 mm from the sciatic notch and mounted on film (no grid bars). Images were taken using a Jeol 1010 electron microscope with a Gatan camera analyzed with ImageJ software.

SDS-PAGE and immunoblotting: Sciatic nerves were homogenized at 4°C in RIPA buffer (PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 5 mM EGTA) containing protease inhibitors (Mini Protease Inhibitor Cocktail; Sigma-Aldrich) and phosphatase inhibitors (Phosphatase Inhibitor Mini Tablets; Fisher Scientific). We homogenized the tissue using Bullet Blender Homogenizer BBX24-CE (Next Advance) and then sonicated for 4 minutes (30 sec on / off) using a Biorruptor Pico (Diagenode). Protein concentrations were determined by the BCA method (Thermo Scientific). 10 µg of total protein was subjected to SDS-PAGE and blotted on to Protran nitrocellulose membrane (Amersham Biosciences). Membranes were blocked using 5 % milk (Sigma-Aldrich) in TBS 1 % and incubated for 16 hours at 4°C with the indicated primary antibody, washed and incubated with secondary antibodies, and developed with ECL Prime (Amersham). Antibodies used can be found online (Key Resources Table). We used an Amersham Imager 680 machine (Amersham) for visualization. Measurements from the proteins of interest were normalized to loading control GAPDH and/or CALNEXIN. When normalized to both loading controls, a mean between the normalization with GAPDH and the normalization with CALNEXIN was used for analysis. The whole membrane Western blot images are shown in Source data file 4 online.

Chromatin immunoprecipitation assays (ChIP): Chromatin immunoprecipitation assays (ChIP): The ChIP assay was a modification of the method described by Jang et al (Jang et al., 2006). Schwann cell cultures were incubated in PBS/1% PFA for 10 min at room temperature, harvested by centrifugation (1,000 g, 5min, 4°C) and washed with PBS. The pellet was resuspended in 1 ml of buffer A (50 mM Hepes-KOH, pH 8.1, 1 mM EDTA, 0.5 mM EGTA, 140 mM NaCl, 10 % glycerol, 0.5 % NP40, 0.25 % Triton X-100 and protease inhibitors), homogenized, and sonicated (15 pulses of 30 s separated) in a Biorruptor Pico (diagenode). Chromatin was clarified by centrifugation at

17,000 g for 3 min at room temperature. Protein concentration in the supernatant was quantified by the BCA method (Thermo Scientific). An aliquot was saved as input. The volume corresponding to 60–100 µg of protein was incubated with the corresponding antibody and Dynabeads Protein G (Life Technologies) overnight at 4°C to form immunocomplexes. For in vivo ChIP, freshly dissected uninjured and injured nerves were incubated in PBS/1% PFA for 10 min at room temperature and then quenched for 5 min with glycine 0.125 M. Nerves were washed in PBS for 10 min at 4°C and then lysed in 200 µl of buffer A, using Bullet Blender Homogenizer BBX24-CE (Next Advance). Nuclei were harvested by centrifugation (10,000 g, 5min, 4°C) and washed with 1 ml of buffer B (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 200 mM NaCl and protease inhibitors), and sonicated (15 pulses of 30 s separated) in a Biorruptor Pico (diagenode). Chromatin was clarified by centrifugation at 17,000 g for 3 min at room temperature. Protein concentration in the supernatant was quantified by the BCA method (Thermo Scientific). An aliquot was saved as input. The volume corresponding to 200–300 µg of protein was incubated with the corresponding antibody and Dynabeads Protein G (Life Technologies) overnight at 4°C to form immunocomplexes. In both cases, immune complexes were centrifuged (500 g, 3 min) and washed twice with 1 ml of “low-salt buffer” (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris, pH 8.1, 150 mM NaCl, and protease inhibitors; Roche), and then washed once with 1 ml of “high-salt buffer” (the same but with 500 mM NaCl) and washed three times with 1 ml of LiCl buffer (0.25 M LiCl, 1% IGEPAL, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris, pH 8.1, and protease inhibitors). Chromatin from immunocomplexes and input was eluted with 200 µl of 1% SDS, 0.1 M NaHCO₃, and 200 mM NaCl and incubated at 65°C for 6 h (to break the DNA–protein complexes). DNA was purified using a column purification kit (GE Healthcare) and submitted to 5× PyroTaq EvaGreen qPCR Mix Plus (CMB) qPCR with the indicated primers.

The ChIP-Seq experiment was performed following a single-end sequencing strategy. High quality reads were aligned against the reference genome (*Rattus norvegicus* (Rnor_6.0)) with Minimap2 (<https://github.com/lh3/minimap2>). Read duplicates from the PCR amplification step in the sequencing process were removed with Picard tools (<https://broadinstitute.github.io/picard/>) and only

uniquely mapped reads were kept in the alignments. The uniquely mapped reads were obtained with SAMtools (<http://www.htslib.org/>) filtering by mapping quality ≥ 30 . MACS2 (<https://github.com/macs3-project/MACS> , version 2.2.4) was used for peak calling and the results were filtered by $-\log_{10} \text{FDR} > 3$. To enable a more informative functional interpretation of experimental data, we identified genes close to or having ChIP-Seq tags on their sequence. ChIPseeker (<http://bioconductor.org/packages/release/bioc/html/ChIPseeker.html>) was used for this step. *Rattus norvegicus* (Rnor_6.0) was selected as annotation database (<https://bioconductor.org/packages/release/data/annotation/html/TxDb.Rnorvegicus.UCSC.rn6.refGene.html>).

***In vivo* recording of compound action potential from mouse sciatic nerves:** Mice were deeply anesthetized by intraperitoneal injection of 40 mg/kg ketamine and 30 mg/kg xylazine. The whole length of the right sciatic nerve was then exposed from its proximal projection into the L4 spinal cord to its distal branches innervating gastrocnemius muscles: tibial, sural and common peroneal. Extracellular recording of compound action potentials (CAPs) was carried out by placing the proximal part of the sciatic nerve on an Ag/AgCl recording electrode with respect to a reference electrode (Ag/AgCl) placed inside the contralateral paw of the animal. For electrical stimulation, another electrode was placed in the distal part of the sciatic nerve just before its trifurcation. To avoid nerve desiccation and the consequent axonal death, the nerve was continuously lubricated with paraffin oil (Panreac). To selectively activate A β -, A δ -, and C-fibers, we recorded CAPs evoked by graded electrical stimulations (5, 8, 10 and 15 V intensity, 0.03 ms pulse duration, Grass Instruments S88, A-M Systems) using both normal and inverse polarity. CAPs were amplified (x1000) and filtered (high pass 0.1 Hz, low pass 10kHz) with an AC amplifier (DAM 50, World Precision Instruments) and digitalized and stored at 25 kHz in a computer using a CED micro-1401interface and Spike2 v.7.01 software (both from Cambridge Electronic Design). In the CAPs, different waveform components corresponding to A- (β and δ), and C-fiber activation were easily distinguished by latency. The amplitude of the different components

and the mean nerve conduction velocity (NCV) were measured. The amplitude of each component was measured from the maximum negative to the maximum positive deflection (Sdrulla et al., 2015). For NCV measurement, we divided the distance between the stimulating and recording electrodes by the latency to the CAP component with the biggest amplitude (Vleggeert-Lankamp et al., 2004). The distance between the stimulating and recording electrodes was measured for each experiment using an 8/0 suture thread.

Statistics: Values are given as means $\pm$ standard error (SE). Statistical significance was estimated with the Student's t test with or without Welch's correction, one-way ANOVA with Tukey's multiple comparisons test, mixed ANOVA with Bonferroni's multiple comparisons test, Chi-Squared test and the Mann-Whitney U test. A p-value <0.05 was considered statistically significant. For the parametric tests (t test and ANOVA), data distribution was assumed to be normal (Gaussian), but this was not formally tested. Analysis was performed using GraphPad software (version 6.0). Statistics for each experiment are described in more detail in the legends to figures.

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LEGENDS TO FIGURES

Figure 1.- Myelin development is notably delayed in the tKO mice. A) A 325,1 ± 48,1 % (p=0,0034) increase in the amount of mRNA for *Hdac7* was found in the dKO nerves. No changes in the expression of HDAC9 were found. RT-qPCR with mouse-specific primers for *Hdac7* was performed and normalized to 18S rRNA. The scatter plot, which include also the mean ± SE, shows the fold change in mRNA normalized to control littermates. 4 to 8 mice per genotype were used. Data were analyzed with the unpaired t-test. **B)** Representative transmission TEM mages of P2, P8 and P21 sciatic nerves of tKO mice (*Mpz-Cre^{+/-};Hdac4^{flx/flx};Hdac5^{-/-};Hdac7^{flx/flx}*) and the control (*Mpz-Cre^{-/-};Hdac4^{flx/flx}; Hdac5^{-/-};Hdac7^{flx/flx}*) littermates. Scale bars 5 μm **C)** No statistically significant differences were observed between the area of the tKO nerves and control littermates (P2: p=0,5234; P8: p=0,9279; P21: p=0,9009). **D)** The number of myelinated axons is notably decreased at P2 (208 ± 24 in tKO versus 1.160 ± 29 in controls; p≤0,0001) and P8 (1.487 ± 179 in tKO versus 4.235 ± 129 in controls; p≤0,0001). **E)** *g-ratio* was increased at P8 (0,80 ± 0,01 in the tKO versus 0,76 ± 0,01 in control; p=0,0045). **F)** The number of unmyelinated axons in a 1:1 relationship with Schwann cells was notably increased at P8 (3.187 ± 111 in the tKO versus 628 ± 21 in controls; p≤0,0001) **G)** The total number of sorted axons in a 1:1 relationship with Schwann cells is decreased at P2 (1.128 ± 90 in the tKO versus 2.131 ± 95 in the control; p=0007). **H)** The total number of Schwann cells (counted as nuclei) is increased at P8 (823 ± 37 in the tKO versus 476 ± 20 in controls; p≤0,0001) and at P21 (503 ± 31 in the tKO versus 337 ± 32 in controls; p≤0,0152). **I)** In contrast, the number of myelinating Schwann cells is decreased at P2 (22 ± 1 in the tKO versus 134 ± 8 in controls; p≤0,0001) and at P8 (153 ± 25 in the tKO versus 309 ± 11 in controls; p=0,0013). **J)** The percentage of myelinated axons is decreased at P2 (18,5 ± 3,7 % in the tKO versus 54,6 ± 1,1 % in controls; p≤0,0001), P8 (31,6 ± 2,9% in the tKO versus 54,6 ± 1,1 % in controls; p≤0,0001) and, although much less, at P21 (97,9 ± 0,4 % in the tKO versus 99,9 ± 0,0 % in controls; p=0,0135). For these experiments 3 to 4 animals per

genotype were used; Unpaired t-test was applied for statistical analysis. **K)** Markers of non-myelin forming Schwann cells are upregulated whereas those of myelin-forming Schwann cells are downregulated in the tKO. P2, sciatic nerves were removed and total RNA extracted. RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. Graph shows the percentage of mRNA for each gene in the tKO normalized to the control littermates. A scatter plot is shown with the results obtained, which include also the mean $\pm$ SE. **L)** The same for P8. **M)** The same for P21. For these experiments, 4 to 5 mice per genotype and age were used. Data were analyzed with the unpaired t-test. **N)** A representative WB of protein extracts from tKO, control, and wild type P8 nerves is shown. In the quantification, JUN protein increased in the tKO ($2,88 \pm 0,19$ in the tKO versus $1,12 \pm 0,071$ in the control nerve; $p=0,004$). Mpz protein was found decreased ($0,55 \pm 0,03$ in the tKO versus $1,21 \pm 0,09$ in the control nerve; $p=0,0115$) as was Mbp ($0,62 \pm 0,045$ in the tKO versus $1,31 \pm 0,100$ in the control nerve; $p=0,012$) We couldn't find changes in KROX-20. Densitometric analysis was done for 7 to 9 WB from the same number of mice and normalized to the WT. Data were analyzed with the One-way ANOVA Tukey's test (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 2.- Characterization of the tKO. A) Representative transmission TEM image of the sciatic nerve of an adult (P60) tKO mouse and a control littermate. Scale bars 5 μ m. **B)** Scatter plot of *g-ratio* versus axon diameter. 1100 axons of 4 different mice per genotype were used. No changes in *g-ratio* were detected. **C)** mRNA for *Jun* remains increased in the tKO by 2,6-fold ($1,88 \pm 0,19 \times 10^{-4}$ au in the tKO versus $0,72 \pm 0,05 \times 10^{-4}$ au in controls; $p=0,003$) whereas *Mpz* was slightly decreased ($1,57 \pm 0,13 \times 10^{-2}$ au in the tKO versus $2,13 \pm 0,05 \times 10^{-2}$ au in controls; $p=0,027$). RTqPCR with mouse-specific primers for the indicated genes was performed. Graph shows a scatter plot for the Δ Ct (which include also the mean $\pm$ SE) of the gene normalized to the housekeeping 18S. 5 mice per genotype and age were used. Data were analyzed with the unpaired t-test). **D)** JUN and MPZ protein levels. A representative Western blot of protein extracts from wild type (C57BL/6), control and tKO sciatic nerves is

shown. The densitometric analysis of 6 to 7 different experiments normalized to WT is also shown. Data were analyzed with the unpaired t-test. Only for JUN were detected consistent changes ($2,04 \pm 0,22$ in the tKO versus $1,05 \pm 0,04$ in controls; $p=0,0003$) at the protein level (** $p<0,001$). **E)** Failed segregation of the axons in the Remak bundles of the tKO. A representative high power TEM image is shown. Morphometric analysis shows that axon diameter distribution is preserved in the tKO, but the number of axons per Remak bundle and the distribution of axon per pocket is changed. 500 axons from 4 animals per genotype were counted. Mixed model ANOVA with Bonferroni post-hoc test was used for comparisons. Scale bars 1 μm . **F)** Pie chart and DEG heatmap of the RNA-seq analysis of P60 showing the distribution of changed genes in the tKO. **G)** Volcano plot shows that the most robustly changed genes were upregulated. ENSEMBL identification numbers for the 10 most robustly changed genes is shown. **H)** List of the 35 most upregulated genes in the adult (P60) tKO classified by FDR. **I)** List of the 35 most downregulated genes in the adult (P60) tKO classified by FDR. (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 3.- Melanocyte lineage markers are expressed by non-myelinating Schwann cells of the Remak bundles in the sciatic nerves of the tKO. A)

mRNA for *Tyrp1* is dramatically increased by 1.081-fold in the tKO ($3,35 \pm 0,71 \times 10^{-4}$ au in the tKO versus $0,11 \pm 0,05 \times 10^{-6}$ au in controls; $p=0,0092$) whereas no changes were found in the cKO4, cKO7 neither dKO sciatic nerves. **B)** mRNA for *Mcam* is also upregulated (5,13-fold) in the tKO ($7,39 \pm 0,79 \times 10^{-4}$ au in the tKO versus $1,44 \pm 0,06 \times 10^{-4}$ au in controls) with only minor or no changes at all for the other genotypes. The same although less marked (1,74-fold) for *Mitf* ($0,87 \pm 0,09 \times 10^{-5}$ au in the tKO versus $0,50 \pm 0,02 \times 10^{-5}$ au in controls; $p=0,0128$) (**C**) and *Ednrb* (4,1-fold; $4,23 \pm 0,52 \times 10^{-5}$ au in the tKO versus $1,04 \pm 0,09 \times 10^{-5}$ au in controls; $p=0,0032$) (**D**). RTqPCR with mouse-specific primers for the indicated genes was performed. Graph shows a scatter plot for the ΔCt (which include also the mean $\pm$ SE) of the gene normalized to the housekeeping 18S. 4 to 5 mice per genotype were used. Data were analyzed with the unpaired t-test with Welch's correlation. **E)** MCAM protein levels in the sciatic

nerves of the tKO. A representative Western blot of protein extracts from wild type (C57BL/6), control and tKO sciatic nerves is shown. MCAM protein was increased by 7,6-fold in the tKO ($9,93 \pm 1,75$ au in the tKO versus $1,30 \pm 0,13$ in controls; $p=0,0003$). **F)** NGFR protein was increased by 2,15-fold ($2,16 \pm 0,29$ in the tKO versus $1,005 \pm 0,09$ in controls; $p=0,0003$). 4 to 8 WB of the same number of animals per genotype were quantified. Data were analyzed with the One-way ANOVA Tukey's test. **G)** MCAM signal colocalizes with SOX10. **H)** MCAM signal colocalizes with NGFR. **I)** MCAM is not expressed by myelin forming Schwann cells (MPZ⁺). **J)** Same happens with NGFR. **K)** MCAM signal colocalizes with L1cam, a marker of the non-myelin forming Schwann cells of the Remak bundles. P60 sciatic nerves were fixed and submitted to immunofluorescence with the indicated antibodies. Nuclei were counterstained with Hoechst. Representative confocal images of sections obtained from the sciatic nerves of wild type (WT), control and tKO mice are shown. Scale bars= 20 μ m (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 4.- Remyelination is delayed in the nerves of the dKO mice. A) Representative transmission TEM images of P60 sciatic nerves uninjured (UI) and 10, 20 and 30 days post crush (dpi) of dKO (*Mpz-Cre*^{+/-}; *Hdac4*^{flx/flx}; *Hdac5*^{-/-}) and the control (*Mpz-Cre*^{-/-}; *Hdac4*^{flx/flx}; *Hdac5*^{-/-}) littermates are shown. Scale bars 5 μ m. **B)** No statistically significant differences were observed in the area of the dKO nerves and control littermates (UI: $p=0,804$; 10 dpi: $p=0,195$; 20 dpi: $p=0,559$; 30 dpi: $p=0,0594$). **C)** The number of myelinated axons is notably decreased at 10 dpi (388 ± 55 in the dKO versus 1.889 ± 330 in the control; $p=0,0005$). **D)** *g-ratio* was increased at 10 dpi ($0,989 \pm 0,003$ in the dKO versus $0,934 \pm 0,015$ in control ($p=0,002$)) and at P21 ($0,776 \pm 0,003$ in the dKO versus $0,767 \pm 0,003$ in control ($p=0,043$)). **E)** The number of unmyelinated axons in a 1:1 relationship with Schwann cells was notably increased at 10 dpi (2.969 ± 203 in the dKO versus 1.512 ± 119 in controls; $p=0,0007$) and at 20 dpi (224 ± 25 in the dKO versus 88 ± 14 in controls; $p=0,0016$). **F)** The total number of unmyelinated axons in a 1:1 relationship with Schwann cells is increased at

1285 10 dpi (2.148 ± 155 in the dKO versus 1.158 ± 56 in the control; $p=0.011$) at 20
 1286 dpi (175 ± 20 in the dKO versus 68 ± 12 in the control; $p=0.002$) and at 30 dpi (63
 1287 ± 17 in the dKO versus 22 ± 5 in the control; $p=0.043$). **G)** No changes in the
 1288 total axon number was found (UI: $p=0.157$; 10 dpi: $p=0.910$; 20 dpi: $p=0.349$; 30
 1289 dpi: $p=0.666$). **H)** neither in the total sorted axon number (UI: $p=0.193$; 10 dpi:
 1290 $p=0.169$; 20 dpi: $p=0.294$; 30 dpi: $p=0.682$). **I)** The total number of Schwann
 1291 cells (counted as nuclei) was increased at 20 dpi (861 ± 34 in the dKO versus
 1292 630 ± 53 in controls; $p=0.0041$). **J)** In contrast, the number of myelinating
 1293 Schwann cells was found decreased at 10 dpi (35 ± 8 in the dKO versus $164 \pm$
 1294 37 in controls; $p=0.0032$). **K)** The percentage of myelinated axons is decreased
 1295 at 10 dpi (15.5 ± 2.3 % in the dKO versus 60.4 ± 4.8 % in controls; $p<0.0001$),
 1296 20 dpi (96.6 ± 0.4 % in the dKO versus 98.8 ± 0.2 % in controls; $p=0.0016$) and,
 1297 although much less, at P21 (98.9 ± 0.3 % in the dKO versus 99.6 ± 0.1 % in
 1298 controls; $p=0.0482$). For these experiment 3 to 6 animals per genotype were
 1299 used; Unpaired t-test was applied for statistical analysis. **L)** Expression of
 1300 several negative regulators of myelination and repair Schwann cell markers is
 1301 enhanced at 10 dpi in the sciatic nerves of the dKO: *Jun* (1,51 -fold; $p=0.0056$),
 1302 *Gdnf* (1,85-fold; $p=0.0025$), *Bdnf* (2,60-fold; $p=0.001$) and *Olig1* (1,60-fold;
 1303 $p=0.008$). **M)** Expression of positive regulators and myelin genes is decreased
 1304 at 10 dpi in the sciatic nerves of the dKO: *Krox-20* (0,47-fold; $p=0.0068$), *Prx*
 1305 (0,45-fold; $p=0.001$), *Mpz* (0,33-fold; $p=0.005$) and *Mbp* (0,33-fold; $p=0.012$).
 1306 RTqPCR with mouse-specific primers for the indicated genes was performed
 1307 and normalized to 18S rRNA. The scatter plot, which include also the mean $\pm$
 1308 SE, shows the fold change of mRNA for each gene at 10 dpi normalized to the
 1309 uninjured nerve. 5 to 8 mice per genotype were used. Data were analyzed with
 1310 the unpaired t-test with Welch's correlation. **N)** A representative WB of protein
 1311 extracts from dKO, control, KO5^{-/-} and wild type nerves is shown. In the
 1312 quantification, JUN protein remains higher in the dKO at 10 dpi (1.72 ± 0.17 -
 1313 fold; $p=0.012$) and tend to equalize at 21 dpi. MPZ protein was found decreased
 1314 by 0.32 ± 0.02 -fold at 21 dpi ($p=0.02$), however we couldn't find changes in
 1315 *KROX-20* (KO5^{-/-} mice were used to compare with the wild types littermates).
 1316 Densitometric analysis was done on 7 to 9 WB from the same number of mice
 1317 and normalized to 10 dpi WT. Data were analyzed with the unpaired t-test. (*)

p<0,05; ** p<0,01; *** p<0,001; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 5.- Remyelination is dramatically delayed in the tKO. A) Myelin clearance is accelerated in the sciatic nerves of the tKO. A representative toluidine blue staining image of 4 days-cut sciatic nerve of tKO and control mice is shown. The quantification of intact myelin sheaths shows a 0,55-fold change (p<0,0001) in the tKO (150 ± 7 intact myelin sheaths in the dKO versus 274 ± 8 in controls; p<0,0001). 7 to 8 animals per genotype were used for the experiment. Data were analyzed with the unpaired t-test. Scale bars 10 μ m. **B)** WB supports an accelerated myelin clearance in the tKO. As is shown, MPZ protein is decreased (0,7-fold; p=0,0062) in the tKO. As expected, JUN was increased. 4 mice per genotype were used. **C)** At 7 days post-cut the decrease in the MPZ protein was more marked (0,43-fold; p=0,012). 3 to 4 mice per genotype were used. CALNEXIN and GAPDH were used as housekeepings. Densitometric analysis was preformed and normalized to the controls. Data were analyzed with the unpaired t-test. **D)** Representative transmission TEM images of P60 sciatic nerves uninjured (UI) and 10, 20, 30 and 60 days post crush (dpi) of tKO and the control littermates are shown. Scale bars 5 μ m. **E)** No statistically significant differences were observed for the area of the tKO nerves and control littermates in the UI (p=0,897), at 10 dpi (p=0,456) neither at 20 dpi (p=0,602). The nerve area of the tKO was increased at 30 dpi (1,26-fold; p=0,0002) and at 60 dpi (1,30-fold; p=0,0008). **F)** No myelinated axons in the tKO were found at 10 dpi whereas the control had 2.383 ± 112 axons myelin (p<0,0001). Myelinated axons were decreased at 20 dpi (606 ± 200 in the tKO versus 5.525 ± 222 in the control; p<0,0001), at 30 dpi (2.659 ± 323 in the tKO versus 6.003 ± 125 in the control; p<0,0001), to catch up at 60 dpi (6.458 ± 240 in the tKO versus 6.689 ± 212 in the control: p=0,491). **G)** *g-ratio* was increased in the tKO at 10 dpi (1 ± 0 in the tKO versus $0,92 \pm 0,003$ in control; p<0,0001) at 20 dpi ($0,94 \pm 0,027$ in the tKO versus $0,77 \pm 0,006$ in control; p=0,0023) and at 30 dpi ($0,82 \pm 0,008$ in the tKO versus $0,75 \pm 0,002$ in control; p=0,0009). **H)** The number of unmyelinated axons >1,5 was increased at 10 dpi (3.477 ± 236

1351 in the tKO versus 950 ± 116 in controls; $p < 0,0001$), at 20 dpi (2.885 ± 209 in the
 1352 tKO versus 184 ± 15 in controls; $p = 0,0002$), at 30 dpi (1.925 ± 319 in the tKO
 1353 versus 76 ± 16 in controls; $p = 0,0044$), and at 60 dpi (257 ± 43 in the tKO versus
 1354 69 ± 11 in controls; $p = 0,010$). **I)** The number of unmyelinated axons $>1,5$ in a
 1355 1:1 relationship with Schwann was notably increased at 10 dpi (2.405 ± 209 in
 1356 the tKO versus 864 ± 102 in controls; $p = 0,0006$), at 20 dpi (2.487 ± 170 in the
 1357 tKO versus 110 ± 13 in controls; $p = 0,0001$), at 30 dpi (1.728 ± 250 in the tKO
 1358 versus 43 ± 8 in controls; $p = 0,0025$), and at 60 dpi (224 ± 43 in the tKO versus
 1359 28 ± 5 in controls; $p = 0,010$). **J)** In contrast, the total number of axons $>1,5 \mu\text{m}$
 1360 was decreased at 20 dpi (3.492 ± 184 in the tKO versus 5.790 ± 228 in the
 1361 control; $p < 0,0001$) and at 30 dpi (4.584 ± 184 in the tKO versus 6.080 ± 131 in
 1362 the control; $p = 0,0002$) to finally catch up at 60 dpi (6.716 ± 198 in the tKO
 1363 versus 6.758 ± 221 in the control; $p = 0,889$). **K)** The total number of axons $>1,5$
 1364 μm sorted (in a 1:1 relationship with Schwann cells) was decreased at 10 dpi
 1365 (2.405 ± 209 in the tKO versus 3.247 ± 60 in the control; $p < 0,0082$), at 20 dpi
 1366 (3.093 ± 147 in the tKO versus 5.653 ± 233 in the control; $p < 0,0001$) and at 30
 1367 dpi (4.387 ± 158 in the tKO versus 6.046 ± 127 in the control; $p < 0,0001$). **L)** The
 1368 number of Schwann cells (counted as nuclei) was increased at 10 dpi ($1.017 \pm$
 1369 95 in the tKO versus 609 ± 33 in the control; $p < 0,0001$), at 20 dpi (1.576 ± 100
 1370 in the tKO versus 779 ± 54 in the control; $p < 0,0001$) and at 30 dpi (1.618 ± 116
 1371 in the tKO versus 723 ± 47 in the control; $p < 0,0001$). **M)** The number of
 1372 myelinating Schwann cells was also decreased at 10 dpi (0 ± 0 in the tKO
 1373 versus 212 ± 18 in the control; $p < 0,0001$), at 20 dpi (48 ± 12 in the tKO versus
 1374 326 ± 33 in the control; $p < 0,0001$) and at 30 dpi (181 ± 24 in the tKO versus
 1375 325 ± 17 in the control; $p = 0,0011$). **N)** The percentage of myelinated axons is
 1376 decreased at 10 dpi (0 ± 0 % in the tKO versus $73,1 \pm 3,1$ % in controls;
 1377 $p < 0,0001$), 20 dpi ($19,2 \pm 5,5$ % in the tKO versus $98,1 \pm 0,2$ % in controls;
 1378 $p < 0,0001$), 30 dpi ($60,3 \pm 6,1$ % in the tKO versus $99,3 \pm 0,1$ % in controls;
 1379 $p = 0,0031$) and at 60 dpi ($96,6 \pm 0,7$ % in the tKO versus $99,6 \pm 0,1$ % in
 1380 controls; $p < 0,0001$). For these experiments 4 to 5 animals per genotype were
 1381 used; Unpaired t-test was applied for statistical analysis. **O)** A representative
 1382 WB of protein extracts from tKO, control and wild type nerves is shown. In the

quantification, JUN protein remains higher in the tKO at 10 dpi ($3,24 \pm 0,35$ -fold; $p < 0,0001$) and at 21 dpi ($2,25 \pm 0,38$ -fold; $p = 0,031$). *KROX-20* was found decreased at 10 dpi ($0,61 \pm 0,08$ -fold; $p = 0,011$). MPZ protein was found decreased at 21 dpi by $0,15 \pm 0,04$ -fold dpi ($p = 0,0091$). Densitometric analysis was done for 7 to 9 WB from the same number of mice and normalized to 10 dpi WT. Data were analyzed with the unpaired t-test. (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 6.- Remyelination failure in the tKO hampered nerve impulse conduction. **A)** Sample recordings of compound action potential (CAP) in uninjured sciatic nerves of control and tKO mice showing the waveform components corresponding to myelinated (A fibers) and unmyelinated (C) fibers. **B-C)** Waveform component of A fibers (**B**) and C-fibers (**C**) showed similar amplitude in both genotypes for stimulation with pulses of increasing intensity (5, 8, 10 and 15 V). **D)** Nerve conduction velocity was also preserved in the tKO mice. **E)** Sample recordings of CAPs obtained in a control and a tKO sciatic nerve 40 days after nerve crushing. **F)** Number of nerves that responded with a detectable CAP after the stimulation with increasing intensity. **G)** Amplitude of CAP corresponding to A fibers was significantly smaller in tKO than in control nerves for stimulation with 8, 10 and 15 V. **H)** Amplitude of C fiber component was significantly smaller in tKO than in control nerves for stimulation with 8 and 10 V. **I)** Nerve conduction velocity was significantly reduced after injury in both genotypes, being significantly lower in tKO than in control nerves for stimulation at 15 V. In this set of experiments, the whole length of a sciatic nerve was exposed from its proximal projection (L4 spinal cord) to its distal branches in deeply anesthetized mice. Compound action potentials (CAPs) were evoked by electrical stimulation of increasing intensity (5, 8, 10 and 15 V, 0.03 ms pulse duration). The maximum amplitude of the A-fiber and C-fiber components of CAP electrical signal, and their mean nerve conduction velocity were measured. Seven to 18 animals per genotype and condition were used. Mann-Whitney's U were used for non-parametric paired

comparisons and Chi-Squared test were used for statistical comparisons. (*
p<0,05; ** p<0,01; *** p<0,001; ns: no significant). See Source data file 1 online
(graphs source data) for more details.

Figure 7.- HDAC4 binds to the promoter of pivotal genes for myelin development. Cultured rat Schwann cells were incubated with 1 mM dbcAMP to shuttle HDAC4 into the nucleus, and ChIP-seq analysis performed on the crosslinked chromatin, with anti-HADC4 antibody. **A)** 3.932 HDAC4 peaks were found in the rat genome. Most of these peaks (67,27%) were located in the promoter regions ($\leq$ 1Kb from the TSS). **B)** A table with the localization of some of these peaks (a complete list can be found in Source data file 3 online (ChIP-seq peaks source data)). **C-E)**, ChIP-seq signal analysis confirmed our previous results (Gomis-Coloma et al., 2018) showing that HDAC4 binds to the promoters of *Jun*, *Gdnf* and *Runx2*. **F)** HDAC4 was also found bound to the promoter region of *Sox2*. **G)** Three peaks (NA_peaks 2916,2917 and 2918) near the TSS of *Pou3f1* were also found. **H)** The binding of HDAC4 to *Pou3f1* gene was confirmed by ChIP-qPCR. 4 different experiments from 4 distinct cultures were used. Data were analyzed with the Mann-Whitney test. (* p<0,05; ** p<0,01; *** p<0,001; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 8.- HDAC7 compensatory overexpression is induced by JUN. **A)** ChIP-qPCR of dbcAMP treated rat Schwann cells with anti-JUN antibody showed that this transcription factor is bound to the promoter of *Hdac7*. 4 different experiments from 4 distinct cultures were used. Data were analyzed with the Mann-Whitney test. **B)** A 1.189 fragment of the mouse *Hdac7* promoter containing a conserved JUN binding consensus sequence was PCR amplified and cloned into the pGL3 luciferase reporter vector. HEK293 cells were transfected with this construct and the pcDNA3 (empty vector) or pcDNA3 *Jun*. As is shown JUN induced the luciferase activity by $3,4 \pm 0,22$ fold (p<0,0001; n=10). Unpaired t-test with Welch's correlation was used for statistical comparison. **C)** Levels of mRNA for *Jun* in the nerves of Jun_OE and Jun_cKO

mice. **D)** *Hdac7* expression is enhanced in the sciatic nerves of the Jun_OE mice ($5,16 \pm 0,46 \times 10^{-5}$ in the Jun_OE versus $3,09 \pm 0,29 \times 10^{-5}$ in the WT; $p=0,005$) but doesn't change in the Jun_cKO mice. **E)** *Hdac4* expression in sciatic nerves doesn't change in Jun_OE and Jun_cKO mice. **F)** *Hdac5* expression in sciatic nerves doesn't change in Jun_OE and Jun_cKO mice. **G)** Removal of *Jun* from Schwann cells in the dKO (dKO;Jun_cKO genotype) prevents *Hdac7* compensatory overexpression. Interestingly, *Hdac9* expression is induced in these mice (see discussion). RTqPCR with mouse-specific primers for the indicated genes was performed. The scatter plot, which include also the mean $\pm$ SE, shows the expression of each gene normalized to the housekeeping 18S. 4 to 5 mice per genotype were used. Data were analyzed with the unpaired t-test with Welch's correlation. **H)** A peak of HDAC4 (NA_peak 3487) was found on the *Hdac7* promoter in the ChIP-seq experimnt. **I)** ChIP-qPCR confirmed that HDAC4 is bound to the promoter of *Hdac7*. 4 different experiments from 4 distinct cultures were used. Data were analyzed with the Mann-Whitney test. (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 9.- MEF2D mediates *Hdac9* de novo expression in the tKO. A) *Hdac9* expression is notably induced in the sciatic nerves of the adult (P60) tKO mice ($6,48 \pm 0,53 \times 10^{-6}$ au the tKO versus $1,46 \pm 0,28 \times 10^{-6}$ in the control; $p<0,0001$). Only minor changes were observed in the cKO4 and cKO7. 4 to 8 mice per genotype were used. Unpaired t-test was used for comparisons. **B)** *Hdac9* expression is increased from early postnatal development of the tKO nerve. At P2 we found $1,67 \pm 0,13 \times 10^{-6}$ au in the tKO versus $0,39 \pm 0,03 \times 10^{-6}$ in the controls ($p<0,0001$) and at P8 we found $3,43 \pm 0,52 \times 10^{-6}$ au in the tKO versus $0,65 \pm 0,09 \times 10^{-6}$ in the controls ($p<0,0001$). RTqPCR with mouse-specific primers for *Hdac9* was performed. The scatter plot, which include also the mean $\pm$ SE, shows the expression of *Hdac9* normalized to the housekeeping 18S. 4 to 5 mice per genotype were used. Data were analyzed with the unpaired t-test with Welch's correlation. **C)** ChIP-qPCR with anti-H3K9Ac of adult (P60) sciatic nerves of tKO and control mice. Three different

experiments of 4-5 animals per genotype is shown. Data were normalized to the IgG value as shown as relative enrichment. Unpaired t-test was used for comparisons. **D)** Alignment of the reads of the RNA-seq from 3 individual sciatic nerves of control and 3 tKO mice, both uninjured and at 20 days post crush (20 dpi). *Hdac9* gene is transcribed at detectable levels in the sciatic nerve of the uninjured tKO mice, whereas it is almost nondetectable in the control sciatic nerves. The tKO mice (but not the controls) increase additionally the expression of *Hdac9* gene during remyelination (20 dpi). **E)** mRNA levels of *Hdac9* (as FPKMs) at 0, 1, 10 and 20 days post crush (dpi) in the RNA-seq experiment. Two-way ANOVA was used for statistical comparison. **F)** *Mef2d* expression is increased early in development (P8) in tKO nerve ($1,65 \pm 0,18$ in the tKO versus $0,97 \pm 0,10$ in controls; $p=0,025$). RTqPCR with mouse-specific primers for *Mef2d* was performed. The scatter plot, which include also the mean $\pm$ SE, shows the expression normalized to the housekeeping 18S. 4 to 5 mice per genotype were used. Data were analyzed with the unpaired t-test with Welch's correlation. **G)** mRNA levels of *Mef2d* (as FPKMs) at 0, 1, 10 and 20 days post crush (dpi) in the RNA-seq experiment. **H)** A representative WB of protein extracts from tKO, control and wild type nerves at 10 dpi is shown. In the quantification, MEF2D protein was increased in the tKO nerves ($2,31 \pm 0,19$ au in the tKO versus $1,33 \pm 0,19$ in controls; $p<0,0069$) **(I)** Same for 20 dpi ($2,19 \pm 0,03$ au in the tKO versus $1,33 \pm 0,11$ in controls; $p<0,0073$). Densitometric analysis was done for 3 to 4 WB from the same number of mice and normalized to the control 20 dpi. Data were analyzed with the unpaired t-test. **J)** MEF2D colocalizes with the transcription factor SOX10⁺, suggesting that it is expressed by Schwann cells. P60 sciatic nerves were fixed and submitted to immunofluorescence with the indicated antibodies. Nuclei were counterstained with Hoechst. Representative confocal images of sections obtained from the sciatic nerves of control and tKO mice are shown. Scale bar 20 μ m. **K)** MEF2D binds to the *Hdac9* promoter in the tKO. ChIP-qPCR of 20 dpi nerves of tKO mice was performed using an anti-MEF2D specific antibody. 5 different experiments from 4 to 5 mice per genotype were performed. Data were analyzed with the unpaired t-test. (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

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1518 **Figure 10.- A graphical summary of the proposed model:** In wild type
1519 nerves (WT) the expression of *Hdac4*, *Hdac5* and *Hdac7* allows myelin
1520 formation and blocks the expression of *Hdac9*. The removal of *Hdac5* (control)
1521 has no effects on myelination neither *Hdac9* expression. In the nerves of the
1522 dKO, JUN induces the compensatory overexpression of *HDac7*, allowing
1523 delayed myelination but having no effect on *Hdac9* gene expression. The
1524 simultaneous elimination of *Hdac4*, *Hdac5* and *Hdac7* (tKO) induces the
1525 overexpression of *Mef2d*, which binds to the promoter and induce the
1526 compensatory expression of *Hdac9*, which after a long delay induces
1527 myelination.

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LEGENDS TO SUPPLEMENTARY FIGURES

Figure 1-figure supplement 1: Class IIa HDAC gene expression and removal from Schwann cells. **A)** Only minor or no changes were found for in the expression of *Hdac5*, *Hdac7* or *Hdac9* in the nerves of the cKO4 . **B)** No changes in the expression of *Hdac4*, *Hdac5* and *Hdac9* were found in the nerves of the KO5. **C)** *Hdac7* compensatory overexpression is already detected in the nerves of the dKO during postnatal development (P2 and P8). RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. The scatter plot, which includes also the mean $\pm$ SE, shows the fold change in mRNA normalized to control littermates. 4 to 8 mice per genotype were used. Data were analyzed with the unpaired t-test. **D)** To generate a Schwann cell specific cKO4 mice, the *Mpz-Cre^{+/-}* mouse was crossed with a knock-in mouse with the exon 6 of *Hdac4* floxed. RTqPCR with specific primers for exon 6 (F and R) demonstrates a decrease by more than 50% in the amount of mRNA in the sciatic nerve. The absence of a complete elimination is probably due to the contribution of mRNA from other cell types. **E)** For HDAC5 we used a complete HDAC5 KO mouse that has no apparent PNS phenotype. In the sciatic nerves of these mice HDAC5 was undetectable both with RT-qPCR and WB. **F)** To generate a Schwann cell specific cKO7 mice, the *Mpz-Cre^{+/-}* mouse was crossed with a knock-in mouse with the exons 2 to 10 of *Hdac7* floxed. RTqPCR with specific primers (F and R) demonstrates a decrease of about 50% in the amount of mRNA for this HDAC in the sciatic nerve. As in the case of *Hdac4*, the absence of a complete elimination is probably due to the contribution of mRNA from the axons and other cell types. Primer sequences are described online (Key Resources Table). (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 1-figure supplement 2: Myelin development in the cKO4 mice sciatic nerves: Representative transmission TEM images of P2 and P8 sciatic nerves of cKO4 mice (*Mpz-Cre^{+/-};Hdac4^{flx/flx}*) and the control (*Mpz-Cre^{-/-};Hdac4^{flx/flx}*) littermates. Scale bars 5 μ m. **B)** No statistically significant differences were

observed between the area of the *cKO4* nerves and control littermates. **C)** The number of myelinated axons was slightly decreased at P2 (912 ± 25 in the *cKO4* versus 1363 ± 29 in the control; $p < 0,0001$) but not at P8. **D)** *g-ratio* was not changed at P8. **E)** The number of unmyelinated axons in a 1:1 relationship with Schwann cells was slightly increased at P8 (845 ± 57 in the *cKO4* versus 625 ± 45 in controls; $p = 0,023$). **F)** The total number of sorted axons in a 1:1 relationship with Schwann cells was not changed. **G)** The total number of Schwann cells (counted as nucleus) is slightly increased at P2 (421 ± 12 in the *cKO4* versus 333 ± 25 in controls; $p = 0,013$). **H)** The number of myelinating Schwann cells was not changed. **I)** The percentage of myelinated axons is slightly decreased at P2 ($41,17 \pm 1,68$ % in the *cKO4* versus $56,29 \pm 3,39$ % in controls; $p = 0,0037$) and P8 ($85,37 \pm 0,4$ % in the *cKO4* versus $88,35 \pm 0,41$ % in controls; $p = 0,002$). For these experiments 4 to 5 animals per genotype were used; Unpaired t-test was used for statistical analysis. (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details. Primer sequences and antibodies are listed online (Key Resources Table).

Figure 1-figure supplement 3: Myelin development in the KO5 mice sciatic nerves: Representative transmission TEM images of P2 and P8 sciatic nerves of KO5 mice (*HDAC5*^{-/-}) and the control (*HDAC5*^{+/+}) littermates. Scale bars 5 μ m. **B)** No statistically significant differences were observed between the area of the *cKO4* nerves and control littermates **C)** The number of myelinated axons was not changed at P2 neither at P8. **D)** *g-ratio* was not changed at P2 neither at P8. **E)** The number of unmyelinated axons in a 1:1 relationship with Schwann cells was slightly increased at P8 (632 ± 19 in the KO5 versus 503 ± 57 in controls; $p = 0,044$). **F)** The total number of sorted axons in a 1:1 relationship with Schwann cells was not changed. **G)** The total number of Schwann cells (counted as nucleus) was not changed. **H)** The number of myelinating Schwann cells was not changed **I)** The percentage of myelinated axons was not changed. For these experiments 3 animals per genotype were used; Unpaired t-test was used for statistical analysis. (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 1-figure supplement 4: Myelin development in the cKO7 mice sciatic nerves: Representative transmission TEM images of P2 and P8 sciatic nerves of cKO7 mice ($Mpz-Cre^{+/-};Hdac7^{flx/flx}$) and the control littermates ($Mpz-Cre^{-/-};Hdac7^{flx/flx}$). Scale bars 5 μ m. No statistically significant differences were observed between the area of the cKO4 nerves and control littermates **(B)**, number of myelinated axons **(C)**, *g-ratio* **(D)**, number of unmyelinated axons in a 1:1 relationship with Schwann cells **(E)**, the total number of sorted axons in a 1:1 relationship with Schwann cells **(F)**, the total number of Schwann cells (counted as nucleus) **(G)**, and number of myelinating Schwann **(H)**. The percentage of myelinated axons was not changed **(I)**. For these experiments 3 to 5 animals per genotype were used; Unpaired t-test was used for statistical analysis. (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 1-figure supplement 5: Myelin development in the dKO mice sciatic nerves: Representative transmission TEM images of P2 and P8 sciatic nerves of dKO mice ($Mpz-Cre^{+/-};Hdac4^{flx/flx};Hdac5^{-/-}$) and the control littermates ($Mpz-Cre^{-/-};Hdac4^{flx/flx};Hdac5^{-/-}$). Scale bars 5 μ m. **B)** No statistically significant differences were observed between the area of the cKO4 nerves and control littermates **C)** The number of myelinated axons is decreased at P2 (743 ± 115 in the dKO versus 1645 ± 269 in the control; $p=0,0012$) but not at P8. **D)** *g-ratio* was not changed at P8. **E)** The number of unmyelinated axons in a 1:1 relationship with Schwann cells was slightly increased at P8 (721 ± 32 in the dKO versus 550 ± 15 in controls; $p=0,003$). **F)** The total number of sorted axons in a 1:1 relationship with Schwann cells was not changed. **G)** The total number of Schwann cells (counted as nucleus) was slightly increased at P8 (543 ± 18 in the dKO versus 461 ± 29 in controls; $p=0,038$). **H)** The number of myelinating Schwann cells was not changed. **I)** The percentage of myelinated axons is decreased at P2 ($34,89 \pm 1,75$ % in the dKO versus $59,29 \pm 1,97$ % in controls; $p<0,0001$) and P8 ($86,27 \pm 0,62$ % in the dKO versus $89,47 \pm 0,49$ % in

controls; $p=0,0061$). For these experiments 4 to 5 animals per genotype were used; Unpaired t-test was used for statistical analysis. (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 2-figure supplement 1: Characterizing the tKO mice: A) Representative TEM images and morphometric analysis of the Remak bundles of single KO and dKO show no major changes in the segregation of the small size axons in these mice. 800-1000 axons from 3 to 4 animals per genotype were counted. Mixed model ANOVA with Bonferroni post-hoc test was used for comparisons. Scale bars 1 μm . **B)** Melanocyte lineage genes are upregulated during early development. P2, P8 and P21 mouse sciatic nerves were removed and total RNA extracted. RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. Graph shows the percentage of mRNA for each gene in the tKO normalized to the control littermates. A scatter plot is shown with the results obtained, which include also the mean $\pm$ SE. 4/5 mice per genotype and age were used. Data were analyzed with the unpaired t-test. **C)** A representative Western blot of protein extracts obtained from the sciatic nerves of P8 WT, control and tKO mice is shown. CALNEXIN was used as a protein loading control. Densitometric analysis was done for 4 WB and normalized the WT. Data were analyzed with the One-way ANOVA Tukey's test. **D)** The mRNA for *Tyrp1* is not translated to protein. A representative Western blot of protein extracts obtained from the sciatic nerves of adult WT, control and tKO mice is shown. Iris was used as a positive control. CALNEXIN and GAPDH were used as protein loading controls. Because GAPDH is a doublet in iris, normalization was performed exclusively to CALNEXIN. Primer sequences and antibodies are listed online (Key Resources Table). (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 4-figure supplement 1: Remyelination in the cKO4 mice: A) Representative transmission TEM images of P60 sciatic nerves uninjured (UI)

1665 and 10, 20 and 30 days post crush (dpi) of dKO (*Mpz-Cre*^{+/-}; *Hdac4*^{flx/flx}) and the
 1666 control (*Mpz-Cre*^{-/-}; *Hdac4*^{flx/flx}) littermates are shown. Scale bars 5 μ m. **B)** Only
 1667 slight significant statistically differences were observed in the area of the cKO4
 1668 at 10 dpi (p=0.0372). **C)** The number of myelinated axons was not changed at
 1669 any point. **D)** the same for *g-ratio*. **E)** The number of unmyelinated axons >1,5
 1670 μ m was increased at 10 dpi in the cKO4 (1.730 $\pm$ 175 in the control versus
 1671 2.266 $\pm$ 116 in the cKO4; p=0,024). **F)** The total number of unmyelinated axons
 1672 in a 1:1 relationship with Schwann cells is increased at 10 dpi (1.869 $\pm$ 115 in
 1673 the cKO4 versus 1.472 $\pm$ 130 in the control; p=0047). No changes in the total
 1674 axon number was found (**G)** neither in the sorted total axon number (**H)**. **I)** The
 1675 total number of Schwann cells (counted as nuclei) was increased at 20 dpi (765
 1676 $\pm$ 29 in the cKO4 versus 643 $\pm$ 41 in controls; p=0,0041) and at 30 dpi (752 $\pm$ 34
 1677 in the cKO4 versus 575 $\pm$ 32 in controls; p=0,0066). **J)** The number of
 1678 myelinating Schwann cells was slightly increased at 20 dpi (368 $\pm$ 15 in the
 1679 cKO4 versus 319 $\pm$ 14 in controls; p=0,0045). **K)** The percentage of myelinated
 1680 axons was decreased at 10 dpi (29,0 $\pm$ 3,2 % in the cKO4 versus 42,8 $\pm$ 5,4 %
 1681 in controls; p=0,0474). For these experiments 4 to 7 animals per genotype were
 1682 used; Unpaired t-test was applied for statistical analysis. **L)** A representative
 1683 WB of protein extracts from cKO4 and control nerves is shown. In the
 1684 quantification, JUN protein was higher in the cKO4 at 10 dpi and tended to
 1685 equalize at 21 dpi. No changes were found in KROX-20 or MPZ. Densitometric
 1686 analysis was done on 4 to 6 WB from the same number of mice. Data were
 1687 analyzed with the unpaired t-test. To decrease the variability of standardizing for
 1688 a condition with low expression, normalization was done for conditions with
 1689 higher protein expression. **M)** Expression of *Jun*, *Runx2* and *Gdnf* at 10 dpi was
 1690 not changed in the sciatic nerves of the cKO4. **N)** Expression of *Krox-20* and
 1691 *Pou3f1* was not changed, whereas *Prx*, *Mpz* and *Mbp* expression was
 1692 decreased in the cKO4 sciatic nerve at 10 dpi. RTqPCR with mouse-specific
 1693 primers for the indicated genes was performed and normalized to 18S rRNA.
 1694 The scatter plot, which include also the mean $\pm$ SE, shows the fold change of
 1695 mRNA for each gene at 10 dpi normalized to the uninjured nerve. 4 to 6 mice
 1696 per genotype were used. Data were analyzed with the unpaired t-test. **O)** A
 1697 representative toluidine blue staining image of 4 days-cut sciatic nerve of cKO4

and control mice is shown. The quantification of intact myelin sheaths showed no changes in the cKO4. 6 animals were used for the experiment. Scale bar 10 μ m. Data were analyzed with the unpaired t-test. Primer sequences and antibodies are listed online (Key Resources Table). (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 4-figure supplement 2: Remyelination in the KO5 mice: A) Representative transmission TEM images of P60 sciatic nerves uninjured (UI) and 10, 20, 30 and 60 days post crush (dpi) of KO5 (*HDAC5*^{-/-}) and the WT control littermates are shown. Scale bars 5 μ m. **B)** A slight increase in the area of the KO5 at 60 dpi ($p=0.0419$) was found. **C)** The number of myelinated axons was not changed at any point. **D)** The *g-ratio* was found slightly decreased in at 20 dpi in the KO5 ($0,75 \pm 0,004$ in the KO5 versus $0,78 \pm 0,007$ in the control; $p=0,0154$). **E)** The number of unmyelinated axons $>1,5 \mu$ m was slightly increased at 60 dpi in the KO5 (52 ± 10 in the control versus 22 ± 6 in the KO5; $p=0,0352$). The total number of unmyelinated axons in a 1:1 relationship with Schwann cells **(F)**, the total axon number **(G)**, the total number of sorted axons **(H)**, the total number of Schwann cells (counted as nuclei) **(I)**, the number of myelinating Schwann cells **(J)** neither the percentage of myelinated axons **(K)** were found changed at any point. For these experiments 3 to 5 animals per genotype were used. Unpaired t-test was applied for statistical analysis. **L)** Expression of *Jun*, *Runx2* and *Gdnf* at 10 dpi was not changed in the sciatic nerves of the KO5. **M)** Expression of *Krox-20*, *Pou3f1*, *Prx* and *Mpz* was not changed, whereas *Mbp* expression was slightly decreased in the KO5 sciatic nerve at 10 dpi. RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. The scatter plot, which include also the mean $\pm$ SE, shows the fold change of mRNA for each gene at 10 dpi normalized to the uninjured nerve. 4 to 6 mice per genotype were used. Data were analyzed with the unpaired t-test. **N)** A representative toluidine blue staining image of 4 days-cut sciatic nerve of KO5 and control mice is shown. The quantification of intact myelin sheaths showed

no changes in the KO5. Scale bar 10 μ m. 6 animals were used for the experiment. Data were analyzed with the unpaired t-test. Primer sequences are listed in Source data section online (Key Resources Table). (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 4-figure supplement 3: Myelin clearance and repair phenotype activation in the dKO mice. A) A representative toluidine blue staining image of 4 days-cut sciatic nerve of dKO and control mice is shown. The quantification of intact myelin sheaths showed no changes. Scale bar 10 μ m. 4 to 5 animals were used for the experiment. Data were analyzed with the unpaired t-test. **B)** WB against JUN and MPZ supports that myelin clearance is normal in the dKO nerves. CALNEXIN and GAPDH were used as housekeeping. Three mice per genotype were analyzed independently by densitometry. To decrease the variability of standardizing for a condition with low expression, normalization was done for conditions with higher protein expression. Data were analyzed with the One-way ANOVA Tukey's test. **C)** Repair phenotype activation was determined by measuring the expression of marker genes and comparing with the uninjured control nerve. As is shown only a slight increase in the expression of *Jun* at 1 day after cut in the dKO was found ($3,71 \pm 0,24$ in the dKO versus $2,35 \pm 0,22$ in the control; $p=0,0102$). RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. Graph shows the percentage of mRNA for each gene in the injured nerve normalized to the uninjured controls. A scatter plot is shown with the results obtained, which include also the mean $\pm$ SE. 3 to 4 mice per genotype were used. Data were analyzed with the unpaired t-test. Primer sequences and antibodies are listed online (Key Resources Table). (* $p<0,05$ ** $p<0,01$ *** $p<0,001$; ns: not significant). See Source data file 1 online (graphs source data) for more details.

Figure 5-figure supplement 1: Remyelination in the cKO7 mice: A) Representative transmission TEM images of P60 sciatic nerves uninjured (UI)

and 10 and 20 days post crush (dpi) of cKO7 (*Mpz-Cre^{+/-};Hdac7^{flx/flx}*) and the WT control littermates (*Mpz-Cre^{-/-};Hdac7^{flx/flx}*) are shown. Scale bars 5 μ m. No changes were found at any point in the nerve area (**B**), the number of myelinated axons(**C**), *g-ratios* (**D**), number of unmyelinated axons >1,5 μ m (**E**),the total number of unmyelinated axons >1,5 μ m in a 1:1 relationship with Schwann cells (**F**), the total axon number (>1,5 μ m) (**G**), the total number of sorted axons (**H**), the total number of Schwann cells (counted as nuclei) (**I**) , the number of myelinating Schwann cells (**J**) neither the percentage of myelinated axons (**K**). For these experiments 3 to 5 animals per genotype were used. Unpaired t-test was applied for statistical analysis. **L**) A representative Western blot of protein extracts obtained from sciatic nerves UI, 10 and 21 days post crush (pdi) is shown. Densitometric quantification shows no differences between phenotypes.6 to 9 mice were used for quantification. To decrease the variability of standardizing for a condition with low expression, normalization was done for conditions with higher protein expression. **M**) No changes were found in the mRNA for *Jun* and *Gdnf* at 10 dpi. **N**) No changes were found for *Krox-20*, *Prx* and *Mpz* at 10 dpi. RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. Graph shows the percentage of mRNA for each gene in the crush injured (10 dpi) nerve normalized to the uninjured controls. A scatter plot is shown with the results obtained, which include also the mean $\pm$ SE. 4 mice per genotype were used. Data were analyzed with the unpaired t-test. **O**) A representative toluidine blue staining image of 4 days-cut sciatic nerve of cKO7 and control mice is shown. The quantification of intact myelin sheaths shows no changes in the cKO7. Scale bar 10 μ m. 3 to 5 animals were used for the experiment. Data were analyzed with the unpaired t-test. Primer sequences and antibodies are listed online (Key Resources Table). (* $p<0,05$ ** $p<0,01$ *** $p<0,001$; ns: not significant). See Source data file 1 online (graphs source data) for more details.

Figure 5-figure supplement 2: Repair Schwann cell phenotype and myelin removal in the injured tKO. tKO Schwann cells express efficiently markers of the repair phenotype after a cut experiment. In some time-points, *Jun* (**A**), *Olig1*

(B), *Shh* (C) and *Ngfr* (D) were even more expressed in the tKO than in the control nerves. *Mpz* was decreased in the UI and at 4 days post cut (E). No changes were found in *Gdnf* (F). G) Autophagy gene expression at 4 days after cut was not modified in the tKO nerves. A cut experiment was performed (P60) and sciatic nerves removed at 1, 4 and 7 days postinjury. RTqPCR with mouse-specific primers for the indicated genes was performed and normalized to 18S rRNA. Graph shows the percentage of mRNA for each gene in the cut injured (4d cut) nerve normalized to the uninjured controls. A scatter plot is shown with the results obtained, which include also the mean $\pm$ SE. 3 to 5 animals per time point and genotype were used, except for uninjured (10-11 nerves). H) We also found no changes in the activation of LC3B in the tKO nerves by WB during myelin clearance. Data were analyzed with the One-way ANOVA Tukey's test. I) No changes were found in the number of macrophages in the tKO nerves. Representative confocal images of sections obtained from the sciatic nerves of control and tKO mice 4 days after cut are shown. Macrophages were stained with anti F4/80 antibody. Scale bar 20 μ m. For the quantification 4 animals per genotype were used. Antibodies and primers used are listed in Source data section online (Key Resources Table). Data were analyzed with the unpaired t-test. (* $p < 0,05$ ** $p < 0,01$ *** $p < 0,001$; ns: not significant). See Source data file 1 online (graphs source data) for more details.

Figure 5-figure supplement 3: Increased Jun and Schwann cell proliferation in the tKO sciatic nerve. A) tKO mice sciatic nerves showed increased numbers of Schwann cells (SOX10⁺) expressing JUN both before and 20 days post crush. B) Most of the JUN positive cells not colocalize with MPZ (arrowheads), suggesting they are Remak Schwann cells. However, some of them colocalize with MPZ, showing JUN is also expressed by subgroup of myelinating Schwann cells (arrow). C) tKO mice sciatic nerves show increased numbers of Schwann cell (SOX10⁺) proliferating (Ki67⁺) at 20 dpi. Uninjured and 20 dpi crushed sciatic nerves (P60) were fixed and submitted to immunofluorescence with the indicated antibodies. Nuclei were counterstained with Hoechst. Representative confocal images of sections obtained from the sciatic nerves of wild type (WT), control and tKO mice are shown. Scale bars=

40 μ m. For the quantification 4 animals per genotype were used. Data were analyzed with the One-way ANOVA Tukey's test. Antibodies used are listed online (Key Resources Table). (* $p < 0,05$ ** $p < 0'01$ *** $p < 0'001$; ns: not significant). See Source data file 1 online (graphs source data) for more details.

Figure 5-figure supplement 4: Evolution of sciatic nerve gene expression profile at 1, 10 and 20 days post crush (dpi). The Pie chart, DEG heatmap, volcano plot and list of the 35 most upregulated and downregulated genes in the tKO classified by FDR at 1 dpi (**A**), 10 dpi (**B**) and 20 dpi (**C**) is shown. Data obtained from the RNA-seq analysis of 3 animals per condition. See Source data file 2 online (RNA-seq source data) for more details.

Figure 5-figure supplement 5: Time course analysis of myelination-relevant gene expression during nerve regeneration from RNA-seq. **A)** Bar chart showing an increase in the number of DE genes at different time points after nerve crush. In the injured tKO nerve 1.270 genes are DE being 390 upregulated and 274 downregulated. At 1 dpi, the number of genes DE genes decreased to 664, being 390 upregulated and 274 downregulated. At 10 dpi, there were 2.777 genes DE, being 1.227 upregulated and 1.550 downregulated. The bigger differences were found at 20 dpi, with up to 4.345 DE genes, being 1.895 upregulated and 2.450 downregulated. **B-F)** The expression of markers of non-myelin forming Schwann cells, such as *Jun*, *Runx2*, *Gdnf*, *Ngfr* and *Sox2*, is enhanced in the sciatic nerves of the tKO mice **G)** *Pou3f1* is induced in the control and tKO animals by more than 10-fold at 10 dpi. Then, at 20 dpi it drops in the control nerves but still grows up in the tKO (up to 15-fold). **H-O)** *Krox-20* and myelin protein genes are downregulated in the tKO nerves. **P-R)** Different genes of the sterol branch of the mevalonate pathway (*Hmgcs*, *Lss*, *Dhcr24*) are downregulated in the tKO. We also found downregulated genes encoding for enzymes involved in the elongation, (*Elovl1*) (**S**), transport (*Pmp2*) (**T**) and the insertion of double bonds (*Scd2*, and *Fads1*) (**U** and **V**) into fatty acids. Finally, we found downregulated *Cers2* and *Ugt8a*, genes involved in the synthesis of sphingomyelin and galactosyl-ceramide respectively (**W** and **X**).

Level of mRNA for each gene (as FPKM) and genotype was normalized to the level of the uninjured control nerve and expressed as fold change. The sciatic nerve of 3 mice per genotype and timepoint were used to perform the RNA-seq (see Material and methods). Two-way ANOVA test was used for the analysis. (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 6-figure supplement 1: HDAC5 elimination decreases nerve conduction velocity and voltage amplitude. A) Sample recordings of compound action potential (CAP) in uninjured sciatic nerves of WT, KO5,dKO and tKO mice showing the waveform components corresponding to myelinated (A fibers) and unmyelinated (C) fibers. **B)** Waveform component of A fibers showed a decreased amplitude for all genotypes (compared to WT) at pulses of 8, 10 and 15 V. **C)** The same for nerve conduction velocity. In this set of experiments, the whole length of a sciatic nerve was exposed from its proximal projection (L4 spinal cord) to its distal branches in deeply anesthetized mice. Compound action potentials (CAPs) were evoked by electrical stimulation of increasing intensity (5, 8, 10 and 15 V, 0.03 ms pulse duration). The maximum amplitude of the A-fiber components of CAP electrical signal, and their mean nerve conduction velocity were measured. Seven to 18 animals per genotype were used. Mann-Whitney's U were used for non-parametric paired comparisons. (* $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Figure 8-figure supplement 1: The proximal promoter region of the *Hdac7* has a JUN consensus binding sequence. A) The alignment of the proximal promoter region for human, mouse and rat is shown. A JUN consensus binding sequence in the promoter of the mouse *Hdac7* gene was identified (yellow). The consensus sequence is conserved in rat and humans. The transcription starting site defined for rat is shown in a red box. In blue, the sequence of the primers used to amplify and clone the mouse *Hdac7* promoter region are shown. In purple are the sequences of the primers used for the ChIPqPCR with

anti-JUN and anti-HDAC4. **B)** Density of reads in the ChIP-seq and mapping of the JUN consensus sequence and primer position in the *Hdac7* gene.

Figure 9-figure supplement 1: *Hdac9* gene expression regulation. **A)** The *in vivo* overexpression of JUN in Schwann cells (Jun_OE mice) did not modify the expression of *Hdac9* gene in the PNS. RTqPCR with mouse-specific primers for *Hdac9* was performed and normalized to 18S rRNA. Graph shows the relative expression of the mRNA normalized to the expression of 18S. A scatter plot is shown with the results obtained, which include also the mean $\pm$ SE. 5 mice per genotype were used. Data were analyzed with the unpaired t-test. Primer sequences are listed online (Key Resources Table). (* $p<0,05$; ** $p<0,01$; *** $p<0,001$; ns: no significant). See Source data file 1 online (graphs source data) for more details.

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
strain, strain background (Mus musculus, male and female)	cKO4 mouse <i>Mpz</i> <Cre/+>; <i>Hdac4</i> <f/f>	Potthoff et al. (2007)	<i>Hdac4</i> ^{tm2.1Eno}	C57BL/6 background, RRID:MGI:4418117
strain, strain background (Mus musculus, male and female)	KO5 mouse <i>Hdac5</i> (-/-)	Chang et al. (2004)	<i>Hdac5</i> ^{tm1Eno}	C57BL/6 background, RRID:MGI:3056065
strain, strain background (Mus musculus, male and female)	cKO7 mouse <i>Mpz</i> <Cre/+>; <i>Hdac7</i> <f/f>	Chang et al. (2006)	<i>Hdac7</i> ^{tm2Eno}	C57BL/6 background, RRID:MGI:3693628
strain, strain background (Mus musculus, male and female)	Jun_OE mouse <i>Mpz</i> <Cre/+>; <i>Rosa26</i> <flox-stop-Jun/+>	Fazal et al. (2017)	Gt(ROSA)26Sor ^{tm15(Jun)Rsky}	C57BL/6 background, RRID:MGI:6478892
strain, strain background (Mus musculus, male and female)	Jun_cKO mouse <i>Mpz</i> <Cre/+>; <i>Jun</i> <f/f>	Behrens et al. (2002)	<i>Jun</i> ^{tm4Wag}	C57BL/6 background, RRID:MGI:2445420
strain, strain background (Mus musculus, male and female)	<i>Mpz</i> <Cre/+>	Jackson Laboratory	B6N.FVB-Tg(<i>Mpz-cre</i>)26Mes/J	<i>Mpz</i> -Cre mouse C57BL/6J background, RRID:IMSR_JAX:017927
antibody	anti-CANELXIN (rabbit polyclonal)	Enzo Life Sciences	Cat# ADI-SPA-860-D; RRID:AB_2038898	WB (1:1000)
antibody	anti-JUN (rabbit monoclonal)	Cell Signaling	Cat #9165; RRID:AB_2130165	WB (1:1000) IF (1:800)

antibody	anti-KROX-20 (rabbit polyclonal)	Millipore	Cat# ABE1374; RRID:AB_2715555	WB (1:500)
antibody	anti-Ki67 (rabbit polyclonal)	Abcam	Cat# ab15580; RRID:AB_443209	IF (1:100)
antibody	anti-F4/80 (rat monoclonal)	Bio-Rad	Cat# MCA497GA; RRID:AB_323806	IF (1:100)
antibody	anti-GAPDH (rabbit polyclonal)	Sigma-Aldrich	Cat# G9545; RRID:AB_796208	WB (1:5000)
antibody	anti-HDAC4 (mouse monoclonal)	Sigma-Aldrich	Cat# H0163; RRID:AB_477042	ChIP (10µg)
antibody	anti-HDAC5 (mouse monoclonal)	Santa Cruz	Cat# sc-133106; RRID:AB_2116793	WB (1:1500)
antibody	anti-IgG2a (mouse monoclonal)	Sigma-Aldrich	Cat# M7769; RRID:AB_1163540	ChIP (10µg)
antibody	anti-L1 (rat monoclonal)	Chemicon International	Cat# MAB5272; RRID:AB_2133200	IF (1:50)
antibody	anti-LC3B (rabbit polyclonal)	Sigma-Aldrich	Cat# L7543; RRID:AB_796155	WB (1:1000)
antibody	anti-MCAM (rabbit monoclonal)	Origene	Cat# TA303592; RRID:AB_2143390	WB (1:1000) IF (1:200)

antibody	anti-MPZ (chicken polyclonal)	AvesLab	Cat# PZ0; RRID:AB_2313561	WB (1:1000) IF (1:1000)
antibody	anti-p75NTR (NGFR) (rabbit polyclonal)	Covance	Cat# PRB-602C; RRID:AB_291707	WB (1:1000)
antibody	anti-NGFR (mouse monoclonal)	Thermo Fisher Scientific	Cat# MA5-13314; RRID:AB_10982037	IF (1:100)
antibody	anti-SOX10 (goat polyclonal)	R&D Systems	Cat# AF2864; RRID:AB_442208	IF (1:100)
antibody	anti-TYRP1 (rabbit polyclonal)	Sigma-Aldrich	Cat# SAB2102617 RRID:AB_10611135	WB (1:1000)
antibody	anti-Rabbit IgG, HRP-linked (goat polyclonal)	Cell Signaling	Cat# 7074; RRID:AB_2099233	WB (1:2000)
antibody	anti-Mouse IgG, HRP-linked (horse polyclonal)	Cell Signaling	Cat# 7076; RRID:AB_330924	WB (1:2000)
antibody	anti-Chicken IgY (IgG) (rabbit polyclonal)	Sigma-Aldrich	Cat# A9046; RRID:AB_258432	WB (1:2000)
antibody	Cy3 anti-Rabbit IgG (H+L) (donkey polyclonal)	Jackson Immuno Research Labs	Cat# 711-165-152; RRID:AB_2307443	IF (1:500)
antibody	anti-Goat Alexa 555 Conjugated (donkey)	Molecular Probes - Thermo Fisher	Cat# A21432; RRID:AB_2535853	IF (1:1000)

	polyclonal)			
antibody	anti-Rabbit Alexa 488 Conjugated (donkey polyclonal)	Molecular Probes - Thermo Fisher	Cat# A21206; RRID:AB_253 5792	IF (1:1000)
antibody	anti-Chicken Alexa 488 Conjugated (donkey polyclonal)	Jackson Immuno Research Labs	Cat# 703- 545-155; RRID:AB_234 0375	IF (1:1000)
antibody	anti-Rat Alexa 488 Conjugated (donkey polyclonal)	Molecular Probes - Thermo Fisher	Cat# A21208; RRID:AB_141 709	IF (1:1000)
antibody	anti-Rat Alexa 555 Conjugated (goat polyclonal)	Molecular Probes - Thermo Fisher	Cat# A21434; RRID:AB_253 5855	IF (1:1000)
antibody	Cy3 anti- Mouse IgG (H+L) (donkey polyclonal)	Jackson Immuno Research Labs	Cat# 715- 165-151; RRID:AB_231 5777	IF (1:500)
sequence-based reagent	<i>18S_F</i>	Gomez- Sanchez et al. (2009)	PCR primers	CGGCTACCA CATCCAAGG AA
sequence-based reagent	<i>18S_R</i>	Gomez- Sanchez et al. (2009)	PCR primers	GCTGGAATT ACCGCGGCT
sequence-based reagent	<i>Bdnf_F</i>	Ma et al. (2016)	PCR primers	GGTATCCAA AGGCCAACT GA
sequence-based reagent	<i>Bdnf_R</i>	Ma et al. (2016)	PCR primers	GCAGCCTTC CTTGGTGTA AC

sequence-based reagent	<i>Jun_F</i>	Arthur-Farraj et al. (2012)	PCR primers	CCTTCTACG ACGATGCCC TC
sequence-based reagent	<i>Jun_R</i>	Arthur-Farraj et al. (2012)	PCR primers	GGTTCAAGG TCATGCTCT GTTT
sequence-based reagent	<i>Ednrb_F</i>	NM_007904.4	PCR primers	CTGGCTCTG GGAGACCTA CT
sequence-based reagent	<i>Ednrb_R</i>	NM_007904.4	PCR primers	GGGCACCAG CTTACACATC T
sequence-based reagent	<i>Gdnf_F</i>	Ma et al. (2016)	PCR primers	TCTCGAGCA GGTTCGAAT GG
sequence-based reagent	<i>Gdnf_R</i>	Ma et al. (2016)	PCR primers	AAGAACCGT CGCAAATT TACC
sequence-based reagent	<i>Hdac4_F</i>	NM_207225.2	PCR primers	GCGAGCAC AGAGGTGA AGAT
sequence-based reagent	<i>Hdac4_R</i>	NM_207225.2	PCR primers	CGCTGGAA ATGCAGTG GTTC
sequence-based reagent	<i>Hdac5_F</i>	NM_001077696.1	PCR primers	GGGGTGGA GGTGGAGG TAG
sequence-based reagent	<i>Hdac5_R</i>	NM_001077696.1 (20)	PCR primers	CCGTAGCG CAGGGTCC AT

sequence-based reagent	<i>Hdac7_F</i>	NM_001204 275.1	PCR primers	AGGAGCAA GAACTTCG GCAA
sequence-based reagent	<i>Hdac7_R</i>	NM_001204 275.1	PCR primers	ACTGTTCTC TCAAGGGC TGC
sequence-based reagent	<i>Hdac9_F</i>	NM_001271 386.1	PCR primers	CCCCTATG GGAGATGT TGAG
sequence-based reagent	<i>Hdac9_R</i>	NM_001271 386.1	PCR primers	CAATGCATC AAATCCAG CAG
sequence-based reagent	<i>Hmgcr_F</i>	Gomez-Sanchez et al. (2009)	PCR primers	TGGATCGA AGGACGAG GAAAG
sequence-based reagent	<i>Hmgcr_R</i>	Gomez-Sanchez et al. (2009)	PCR primers	GAATTACGT CAACCATA GCTTCCG
sequence-based reagent	<i>Krox-20_F</i>	NM_010118.3	PCR primers	ACCCCTGG ATCTCCCGT ATC
sequence-based reagent	<i>Krox-20_R</i>	NM_010118.3	PCR primers	CAGGGTAC TGTGGGTC AATGG
sequence-based reagent	<i>Mbp_F</i>	Gomez-Sanchez et al. (2009)	PCR primers	ATCCAAGTA CCTGGCCA CAG
sequence-based reagent	<i>Mbp_R</i>	Gomez-Sanchez et al. (2009)	PCR primers	CCTGTCAC CGCTAAAG AAGC

sequence-based reagent	<i>Mcam_F</i>	NM_023061.2	PCR primers	GAAACGGC TACCCATT CCT
sequence-based reagent	<i>Mcam_R</i>	NM_023061.2	PCR primers	AGCCACTG GACTCGAC AATC
sequence-based reagent	<i>Mef2a_F</i>	NM_001033713.2	PCR primers	AGTAGCGG AGACTCGG AATTG
sequence-based reagent	<i>Mef2a_R</i>	NM_001033713.2	PCR primers	ATGCATCGT ACACAGCT CCT
sequence-based reagent	<i>Mef2c_F</i>	Materna et al. (2019)	PCR primers	GTGCTGTG CGACTGTG AGAT
sequence-based reagent	<i>Mef2c_R</i>	Materna et al. (2019)	PCR primers	TCTGAGTTT GTCCGGCT CTC
sequence-based reagent	<i>Mef2d_F</i>	NM_001310587.1	PCR primers	GATCTGAA CAATGCCC AGCG
sequence-based reagent	<i>Mef2d_R</i>	NM_001310587.1	PCR primers	GGCAGCTG GTAATCTGT GTTG
sequence-based reagent	<i>Mitf_F</i>	NM_001113198.1	PCR primers	GGAGCTCA CAGCGTGT ATTT
sequence-based reagent	<i>Mitf_R</i>	NM_001113198.1	PCR primers	TCCTTAATG CGGTCGTT TATGT

sequence-based reagent	<i>Mpz_F</i>	Gomez-Sanchez et al. (2009)	PCR primers	ACCAGACA TAGTGGGC AAGACCTC
sequence-based reagent	<i>Mpz_R</i>	Gomez-Sanchez et al. (2009)	PCR primers	AAGAGCAA CAGCAGCA ACAGCACC
sequence-based reagent	<i>Ngfr_F</i>	Fontana et al. (2012)	PCR primers	TGATGGAG TCGGGCTA ATGTC
sequence-based reagent	<i>Ngfr_R</i>	Fontana et al. (2012)	PCR primers	AGATTCATC CCTCCACA AATGC
sequence-based reagent	<i>Olig1_F</i>	Ma et al. (2016)	PCR primers	AGCGATGT AGTTGCTTG GGAT
sequence-based reagent	<i>Olig1_R</i>	Ma et al. (2016)	PCR primers	CTGGCTCT AAACAGGT GGGAT
sequence-based reagent	<i>Pou3f1_F</i>	NM_011141.2	PCR primers	GAGCACTC GGACGAGG ATG
sequence-based reagent	<i>Pou3f1_R</i>	NM_011141.2	PCR primers	TGATGCGT CGTTGCTT GAAC
sequence-based reagent	<i>Prx_F</i>	NM_198048.2	PCR primers	AGTGGCCA AGCTGAAC ATCC
sequence-based reagent	<i>Prx_R</i>	NM_198048.2	PCR primers	AGAACTCG ACGTCAAC AGGG

sequence-based reagent	<i>Runx2_F</i>	NM_001146038.2	PCR primers	GTCTTCCAC ACGGGGCA C
sequence-based reagent	<i>Runx2_R</i>	NM_001146038.2	PCR primers	GCCAGAGG CAGAAGTC AGAG
sequence-based reagent	<i>Sox2_F</i>	Quintes et al. (2016)	PCR primers	TCCAAAAAC TAATCACAA CAATCG
sequence-based reagent	<i>Sox2_R</i>	Quintes et al. (2016)	PCR primers	GAAGTGCA ATTGGGAT GAAAA
sequence-based reagent	<i>Sox10_F</i>	NM_011437.1	PCR primers	GAGCAAGC CGCACGTC AAGA
sequence-based reagent	<i>Sox10_R</i>	NM_011437.1	PCR primers	GTGGAGGT GAGGGTAC TGGTC
sequence-based reagent	<i>Shh_F</i>	Ma et al. (2016)	PCR primers	CAGCGACT TCCTCACCT TCCT
sequence-based reagent	<i>Shh_R</i>	Ma et al. (2016)	PCR primers	AGCGTCTC GATCACGT AGAAGAC
sequence-based reagent	<i>Tyrp1_F</i>	NM_031202.3	PCR primers	CCGCTTTTC TCACATGG CAC
sequence-based reagent	<i>Tyrp1_R</i>	NM_031202.3	PCR primers	TCGCAGAC GTTTTTCCC AGT

sequence-based reagent	ChIP <i>Hdac7</i> Promoter _F	ID_84582	PCR primers	CCCTCCAC AATGACCCT CCTT
sequence-based reagent	ChIP <i>Hdac7</i> Promoter _R	ID_84582	PCR primers	GTGATCCG CTGTAATGC ACTG
sequence-based reagent	ChIP <i>Hdac9</i> Promoter _F	ID_687001	PCR primers	GCTGCAAT CACTCGGC CAT
sequence-based reagent	ChIP <i>Hdac9</i> Promoter _R	ID_687001	PCR primers	GCCACAG GCACAGAA ATAGA
sequence-based reagent	ChIP <i>Pou3f1</i> Promoter _F	ID_192110	PCR primers	CAGAAGGA GAAGCGCA TGAC
sequence-based reagent	ChIP <i>Pou3f1</i> Promoter _R	ID_192110	PCR primers	CTCCCCAG GCGCATAA ACG
sequence-based reagent	<i>Jun_Flox</i> P_OE_F	Fazal et al. (2017)	PCR primers	TGGCACAG CTTAAGCA GAAA
sequence-based reagent	<i>Jun_Flox</i> P_OE_R	Fazal et al. (2017)	PCR primers	GCAATATG GTGGAAAA TAAC
sequence-based reagent	<i>Jun_Flox</i> P_cKO_F	Arthur-Farraj et al. (2012)	PCR primers	CCGCTAGC ACTCACGTT GGTAGGC
sequence-based reagent	<i>Jun_Flox</i> P_cKO_F	Arthur-Farraj et al. (2012)	PCR primers	CTCATACCA GTTCGCAC AGGCGGC

sequence-based reagent	<i>Hdac4</i> _Fl oxP_F	Gomis-Coloma et al. (2018)	PCR primers	ATCTGCCC ACCAGAGT ATGTG
sequence-based reagent	<i>Hdac4</i> _Fl oxP_R	Gomis-Coloma et al. (2018)	PCR primers	CTTGTTGAG AACAACTC CTGCAGCT
sequence-based reagent	<i>Hdac5</i> _Fl oxP_F	Gomis-Coloma et al. (2018)	PCR primers	CAAGGCCT TGTGCATG CTGGGCTG G
sequence-based reagent	<i>Hdac5</i> _Fl oxP_R	Gomis-Coloma et al. (2018)	PCR primers	CTGCTCCC GTAGCGCA GGGTCCAT G
sequence-based reagent	<i>Hdac5</i> _Fl oxP_LacZ	Gomis-Coloma et al. (2018)	PCR primers	GCCCGTTT GAGGGGAC GACGACAG TATTCG
sequence-based reagent	<i>Hdac7</i> _Fl oxP_F	Chang et al. (2006)	PCR primers	GTTGCAGG GTCAGCAG CGCAGGCT CTG
sequence-based reagent	<i>Hdac7</i> _Fl oxP_R	Chang et al. (2006)	PCR primers	CCAGTGGA CGAGCATT CTGGAGAA AGG
sequence-based reagent	<i>Mpz</i> -Cre_F	Feltri et al. (2008)	PCR primers	CCACCACC TCTCTCCAT TGCAC
sequence-based reagent	<i>Mpz</i> -Cre_R	Feltri et al. (2008)	PCR primers	GCTGGCCCA AATGTTGCT GG
commercial assay or kit	QIAquick PCR Purification Kit	Qiagen	Cat# 28104	

commercial assay or kit	NucleoSpin RNA, Mini kit for RNA purification	Macherey-Nagel	Cat# 740955.50	
commercial assay or kit	Luciferase Assay System	Promega	Cat# E1500	
commercial assay or kit	Beta-Glo® Assay System	Promega	Cat# E4720	
commercial assay or kit	Pierce BCA Protein Assay Kit	Thermo Scientific	Cat# 23225	
commercial assay or kit	ECL Prime Western Blotting Detection Reagent	Amersham	Cat# RPN223	Highly sensitive chemiluminescent detection reagent for Western blotting
commercial assay or kit	ECL Western Blotting Analysis System	Amersham	Cat# RPN2109	Chemiluminescent detection reagent for Western blotting
commercial assay or kit	Invitrogen Dynabeads Protein G	Life Technologies	Cat# 10004D	Superparamagnetic beads with recombinant Protein G covalently coupled to surface for Immunoprecipitation
commercial assay or kit	MasterMix qPCR ROx PyroTaq EvaGreen 5x	CMB	Cat# 08-24-00001	

chemical compound, drug	Agar 100 Resin	Agar Scientific	Cat# R1043	Concentration: various, see methods
chemical compound, drug	Dodeceny l Succinic Anhydride - DDSA	Agar Scientific	Cat# R1051	Concentration: various, see methods
chemical compound, drug	Methyl Nadic Anhydride - MNA	Agar Scientific	Cat# R1082	Concentration: various, see methods
chemical compound, drug	Benzyl dim ethylamine - BDMA	Agar Scientific	Cat# R1062	Concentration: various, see methods
chemical compound, drug	Paraform aldehyde 16 % solution, EM grade	Electron Microscopy Sciences	Cat# 15710	Concentration: 2%
chemical compound, drug	Glutaraldehyde 25 % solution, EM grade	Electron Microscopy Sciences	Cat# 16220	Concentration: 2,5%
chemical compound, drug	Sodium Cacodylate Trihydrate	Electron Microscopy Sciences	Cat# 12300	Concentration: 0,1M
chemical compound, drug	Ethanol absolute	J.T.Baker	Cat# 8025	Concentration: various, see methods
chemical compound, drug	Propylene Oxide, ACS Reagent	Electron Microscopy Sciences	Cat# 20401	Concentration: various, see methods
chemical compound, drug	Leibovitz's L-15 Medium	Company	Cat# 11570396	Concentration: various, see methods

chemical compound, drug	DMEM GlutaMAX Medium	Gibco	Cat# 11574516	Concentration: various, see methods
chemical compound, drug	Forskolin	Gibco	Cat# F6886	Concentration: various, see methods
chemical compound, drug	rhNRG1-beta1	Sigma-Aldrich	Cat# RYD-396-HB-050	Concentration: various, see methods
chemical compound, drug	FBS - Fetal Bovine Serum	R&D Systems	Cat# 11550356	Concentration: various, see methods
chemical compound, drug	Penicillin-Streptomycin	Fisher	Cat# 11548876	Concentration: various, see methods
chemical compound, drug	DMEM Ham's F12 Medium	Gibco	Cat# 11520396	Concentration: various, see methods
chemical compound, drug	Insulin-Transferrin-Selenium	Gibco	Cat# 41400-045	Concentration: various, see methods
chemical compound, drug	Putrescine	Gibco	Cat# P5780	Concentration: various, see methods
chemical compound, drug	Progesterone	Sigma-Aldrich	Cat# P0130	Concentration: various, see methods
chemical compound, drug	dbcAMP - Dibutyryl cAMP sodium salt	Sigma-Aldrich	Cat# D0627	Concentration: various, see methods

chemical compound, drug	LipoD293 DNA In Vitro Transfection Reagent	Sigma-Aldrich	Cat# SL100668	Concentration: various, see methods
chemical compound, drug	Mini Protease Inhibitor Cocktail	Roche	Cat# 11836153001	Concentration: various, see methods
chemical compound, drug	Phosphatase Inhibitor Mini Tablets	Fisher Scientific	Cat# 15691759	Concentration: various, see methods
chemical compound, drug	RNase A	Fisher Scientific	Cat# 10618703	Concentration: various, see methods
chemical compound, drug	Proteinase K	Sigma-Aldrich	Cat# 3115836001	Concentration: various, see methods
chemical compound, drug	TRI Reagent	Sigma-Aldrich	Cat# T9424	Concentration: various, see methods
chemical compound, drug	Chloroform	Sigma-Aldrich	Cat# 319988	Concentration: various, see methods
chemical compound, drug	DNase I RNase Free	Thermo Fisher Scientific	Cat# EN0521	Concentration: various, see methods
chemical compound, drug	Deoxynucleotide Mix	Sigma-Aldrich	Cat# D7295	Concentration: various, see methods
chemical compound, drug	Random Primers	Invitrogen	Cat# 48190-011	Concentration: various, see methods

chemical compound, drug	DTT	Invitrogen	Cat# Y00147	Concentration: various, see methods
chemical compound, drug	RNaseOUT	Invitrogen	Cat# 10777-019	Concentration: various, see methods
chemical compound, drug	RevertAid Reverse Transcriptase	Thermo Fisher Scientific	Cat# EP0441	Concentration: various, see methods
software, algorithm	GraphPad Prism 9.0.0	GraphPad Prism	RRID:SCR_002798	
software, algorithm	ImageJ	Open Source	RRID:SCR_003070	
software, algorithm	QuantStudio 3 Real-Time PCR Systems Software	Applied Biosystems	RRID:SCR_018712	
other	QuantStudio 3 Real-Time PCR Systems	Applied Biosystems	Cat# A28131	Real Time PCR thermal cycler
other	Biorruptor Pico sonication device	Diagenode	Cat# B01060010	Sonication device
other	Bullet Blender Homogenizer BBX24-CE	Next Advance	Cat# BBX24-CE	Tissue Homogenizer
other	Zirconium Oxide Beads	Next Advance	Cat# ZrOB05	For the homogenization of medium-tough tissue and cells

other	Microplate Reader EZ Read 400	Biochrom	Cat# 12694795	96 well plate reader for Pierce BCA protein assay kit
other	Nitrocellulose Membrane	Amersham Hybond ECL	Cat# RPN203D	Protein blotting membrane (pore size: 0.45µm)
other	Amersham Imager 600	Amersham	Cat# Amersham Imager 600	Western blot (chemiluminescence) developer
other	4',6-diamidino-2-phenylindole (DAPI stain)	Thermo Fisher	Cat# D1306; RRID:AB_2629482	Blue-fluorescent nucleic acid stain IF (1:1000)

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H Upregulated genes in the tKO

Alias	Gene ID	FDR	P-value	Log FC
1 Tyrp1	ENSMUSG00000005994	0	0	6.03
2 Gria2	ENSMUSG000000033981	5.19E-251	8.10E-255	5.21
3 Ngfr	ENSMUSG00000000120	9.21E-118	2.16E-121	2.25
4 Mcam	ENSMUSG000000032135	4.76E-105	1.49E-108	2.03
5 Pwll4	ENSMUSG000000036912	3.03E-101	1.37E-104	3.73
6 Ntm	ENSMUSG000000059974	3.03E-101	1.42E-104	3.12
7 Tenm1	ENSMUSG000000016150	2.00E-86	1.09E-89	3.04
8 EphA5	ENSMUSG000000029245	1.52E-58	1.07E-61	1.96
9 Tnfr	ENSMUSG000000027692	1.55E-58	1.21E-61	1.48
10 Zan	ENSMUSG000000079173	5.84E-58	5.02E-61	2.78
11 Hdac9	ENSMUSG000000004698	1.19E-56	1.11E-59	1.72
12 Pou3f1	ENSMUSG00000000125	8.15E-55	8.28E-58	2.05
13 Cadm1	ENSMUSG000000032075	8.70E-52	1.02E-54	1.45
14 Adbar2	ENSMUSG000000052551	6.08E-50	7.60E-53	2.63
15 Rcan2	ENSMUSG000000039601	1.39E-41	1.85E-52	1.34
16 Cdh6	ENSMUSG000000039385	1.08E-48	1.52E-51	2.03
17 Tenm3	ENSMUSG000000031561	1.92E-45	2.84E-48	1.29
18 Rab3c	ENSMUSG000000021700	1.22E-44	1.91E-47	2.55
19 Chd5	ENSMUSG000000005045	2.50E-41	4.10E-44	2.08
20 Grk2	ENSMUSG000000056073	4.30E-41	7.38E-44	1.38
21 Daam2	ENSMUSG000000040260	4.11E-40	7.38E-43	1.13
22 Adam23	ENSMUSG000000025964	1.64E-39	3.07E-42	1.22
23 Rgs8	ENSMUSG000000042671	3.28E-36	6.40E-39	2.37
24 Rgs16	ENSMUSG000000026475	5.67E-35	1.15E-37	1.94
25 Actg2	ENSMUSG0000000059430	2.79E-33	6.10E-36	2.23
26 Col27a1	ENSMUSG000000045672	3.20E-32	7.25E-35	1.11
27 Itga2	ENSMUSG000000015533	8.65E-32	2.03E-34	1.07
28 Sox4	ENSMUSG000000076431	9.30E-31	2.25E-33	1.43
29 Gm48261	ENSMUSG0000000113073	1.14E-30	2.84E-33	2.21
30 Pde4d	ENSMUSG000000021699	6.78E-29	1.75E-31	1.29
31 Itgb8	ENSMUSG000000025321	4.40E-28	1.17E-30	0.81
32 Btc	ENSMUSG000000082361	1.18E-27	3.22E-30	2.07
33 Jun	ENSMUSG000000052684	1.99E-27	5.59E-30	0.98
34 Plxn4	ENSMUSG000000029765	2.07E-26	5.98E-29	1.13
35 Tmem196	ENSMUSG000000048004	2.39E-26	7.10E-29	1.99

I Downregulated genes in the tKO

Alias	Gene ID	FDR	P-value	Log FC
1 Cdh2	ENSMUSG000000024304	2.10E-74	1.31E-77	-1.69
2 Cnmd	ENSMUSG000000022025	2.99E-52	3.27E-55	-2.06
3 Aldh1a1	ENSMUSG000000053279	2.17E-34	4.58E-37	-1.39
4 Slc9a9	ENSMUSG000000031129	7.78E-25	2.49E-27	-0.92
5 Ogn	ENSMUSG000000021390	2.23E-24	7.31E-27	-0.84
6 C4b	ENSMUSG000000073418	1.80E-23	6.19E-26	-0.76
7 Grid2	ENSMUSG000000071424	8.48E-23	3.05E-25	-1.57
8 Mmd2	ENSMUSG000000039533	5.47E-22	2.18E-24	-1.61
9 Mfap5	ENSMUSG000000030116	7.19E-21	3.03E-23	-0.77
10 Zdhhc2	ENSMUSG000000039470	8.94E-20	3.98E-22	-0.67
11 Serpind1	ENSMUSG000000022766	1.31E-19	5.95E-22	-1.02
12 C130071C03Rik	ENSMUSG000000050334	1.36E-19	6.26E-22	-1.79
13 Slc1a2	ENSMUSG000000005089	2.04E-19	9.54E-22	-1.67
14 Lctf	ENSMUSG000000032401	2.12E-19	1.01E-21	-0.85
15 Cntr1	ENSMUSG000000050222	2.18E-19	1.06E-21	-1.66
16 Plscr2	ENSMUSG000000032372	3.99E-19	2.09E-21	-1.41
17 Adam22	ENSMUSG000000040537	9.90E-19	5.26E-21	-0.97
18 Gpr3711	ENSMUSG000000026424	1.49E-18	8.03E-21	-0.65
19 Bmp7	ENSMUSG000000008999	1.63E-18	9.01E-21	-0.82
20 Chrd11	ENSMUSG000000031283	3.39E-17	1.98E-19	-0.73
21 Fads1	ENSMUSG000000010663	6.84E-17	4.11E-19	-0.57
22 Vstm4	ENSMUSG000000050666	7.41E-17	4.51E-19	-0.85
23 A730049H05Rik	ENSMUSG000000048636	1.76E-16	1.10E-18	-0.93
24 Gmfb	ENSMUSG000000062014	3.78E-15	2.69E-17	-0.64
25 Tmem28	ENSMUSG000000071719	9.39E-15	6.82E-17	-1.41
26 Fbln5	ENSMUSG000000021186	1.29E-14	9.50E-17	-0.60
27 Lrrc4c	ENSMUSG000000050587	1.05E-13	8.36E-16	-1.11
28 Lss	ENSMUSG000000033105	2.67E-13	2.19E-15	-0.47
29 Adamts5	ENSMUSG000000022894	3.01E-13	2.49E-15	-0.69
30 Mgl2	ENSMUSG000000040950	4.29E-13	3.59E-15	-0.89
31 Ptptr	ENSMUSG000000053141	3.50E-12	3.09E-14	-0.77
32 Myo7a	ENSMUSG000000030761	3.52E-12	3.13E-14	-0.99
33 Hmox1	ENSMUSG00000005413	3.65E-12	3.28E-14	-0.75
34 Rap1gds1	ENSMUSG000000028149	4.86E-12	4.40E-14	-0.42
35 Pex5l	ENSMUSG000000027674	8.64E-12	7.89E-14	-0.79

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Gene	Gene Id	Chr	Peak name	Distance TSS	Length	Gene strand	Fold_enrichment	-log10(pvalue)	Annotation
<i>Jun</i>	24516	5	NA_peak_2855	948	299	-	4,28816	5,43946	Promoter (<=1kb)
<i>Gdnf</i>	25453	2	NA_peak_1913	-6	340	+	7,93422	12,3698	Promoter (<=1kb)
<i>Runx2</i>	367218	9	NA_peak_3766	-235	642	+	6,28141	9,002	Promoter (<=1kb)
<i>Sox2</i>	499593	2	NA_peak_1959	51	502	+	8,37521	13,21818	Promoter (<=1kb)
<i>Id2</i>	25587	6	NA_peak_3105	-4570	433	-	11,16694	19,3168	Distal Intergenic
<i>Hey2</i>	155430	1	NA_peak_31	34022	396	+	6,43225	9,77868	Distal Intergenic
<i>Hey2</i>	155430	1	NA_peak_32	34506	374	+	6,61185	9,66529	Distal Intergenic
<i>Mcam</i>	78967	8	NA_peak_3564	214	313	+	6,61185	9,66529	Promoter (<=1kb)
<i>Pou3f1</i>	192110	5	NA_peak_2916	-394	372	+	7,67727	11,77405	Promoter (<=1kb)
<i>Pou3f1</i>	192110	5	NA_peak_2917	0	1209	+	7,32279	14,33382	Promoter (<=1kb)
<i>Pou3f1</i>	192110	5	NA_peak_2918	1165	341	+	5,4609	7,65931	Promoter (1-2kb)
<i>Mbp</i>	24547	18	NA_peak_1719	0	317	+	7,93422	12,3698	Promoter (<=1kb)
<i>Hmgcr</i>	25675	2	NA_peak_1882	0	401	-	6,28141	9,002	Promoter (<=1kb)
<i>Hdac7</i>	84582	7	NA_peak_3487	367	1064	+	14,46443	26,92004	Promoter (<=1kb)

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human	CGGCCGGGTAGGATGACAATGGAAGGAAATGCTTATCTGAGTCTGAGAGCGGGCCAAGG	60	human	CAGATTTTGAAAGTTTCTTTTTTAAAGAAATTTCTCAAGGGACTTCCTTCCTCCTCT	741
mouse	CGGCCGGGTAGGATGACAATGGAAGGAAATGCTTATCTGAGTCTGAGAGCGGGCCAAGG	60	mouse	GTGGTG-CAGTTTTCGAAACTTAAAAAATTTTCTCAAGGGATTTCCTTCATCCTCT	750
rat	CGGCCGGGTAGGATGACAATGGAAGGAAATGCTTATCTGAGTCTGAGAGCGGGCCAAGG	60	rat	ATGCAG-TTTTGAACCTTTTAAAAAAGAAATTTCTCAAGGGATTTCCTTCACCTCT	727

human	GGGAGGGAGGGAGGGGCGAGCGCTCGCGGCCCTGGGACACACACCCCGGACAGACT	120	human	CCTCTTCCTCTCTTCTCCCATTCCTCTCCCTCCACAACCCCTCTTTCCCTCCCGG	801
mouse	GGGAGGGAGGGAGGGGCGAGCGCTCGCGGCCCTGGGACACACACCCCGGCTTCGCTC	120	mouse	CCTCTTCCTCTTCCATTCCTCTCCCTCCACAATGACCTCTTGGCCCCCACTCCTGG	810
rat	GGGAGGGAGGGAGGGGCGAGCGCTCGCGGCCCTGGGACACACACCCCGGCTTTGCTC	120	rat	CCTCTTCCTCTTCCATTCCTCTCCCTCCACAATGACCTCTTGGCCCCCACTCCTGG	785

human	GCACCGCGCGGCGACAGCGCGGCGCGACACCGCGCGCGCCAGATCGCGCGGGGCG	180	human	TCCCTATGAGTCAAACTTCAGCAATGTGCCAGTGTCCCCAGTGCATTACAGCGGATCA	861
mouse	AGCAG-----CACACAGCTGGCGGAGACACCGA-----TCGCGCGCGGGGCG	163	mouse	ACCCTATGAGTCAAACTTCAGCAATGTGCCAGTGTCCCCAGTGCATTACAGCGGATCA	870
rat	AGCGG-----CACACAGGCTGGCGGACACCGA-----TCGCGCGCGGGGCG	163	rat	TCCCTATGAGTCAAACTTCAGCAATGTGCCAGTGTCCCCAGTGCATTACAGCGGATCA	845

human	CGGCGCACTGCGGGGAGGCGAGCGCG-----AACCGGGAACGCGG	222	human	CAGAAATGTTCCAGTCTGTGAGTCCGAATGCAGCGCCTCCAGCCCTCCTCAGTAATA	921
mouse	CGGCTCACGCGCGGGAGGCGAGCGCGCGAACCAGGACGCGGAGCGCGGACCGCGG	223	mouse	CAGAAATGTTCCAGTCTGTGAGTCCGAATGCAGCGCCTCCAGCCCTCCTCAGTAATA	930
rat	CGGCTCACGCGCGGGAGGCGAGCGCGCGAACCAGGACGCGGAGCGCGGACCGCGG	223	rat	AGAAATGTTCTAGTTTGTGAGTCCGAATGCAGCGCCTCCAGCCCTCCTCAGTAATA	905

human	CGCGGCTCCGAGCCCCAGCGCGCGAGGCGCTCCGAGCGGTGCACAAATTCGCGGAAC	282	human	AACTCAGCTCAGGCGCGCTTTAGGAGTGGCCCTCCCTGCCTCCTCCGCTT---TT	978
mouse	CGCGGCGCGGAAGCCCCAGCGCGCGCGCGCTGCGCGCGCGCACAAATTCGCGGAAC	283	mouse	AACTCAGCTCAGGAGTGGCTTTGAGTGAAGCCCTCCCTGCCTCCTCCGCTT---ATCT	984
rat	CGCGGCGCGGAAGCCCCAGCGCGCGCGCGCTGCGCGCGCGCACAAATTCGCGGAAC	283	rat	AACTCAGCTCAGGAGTGGCTTTGAGTGAAGCCCTCCCTGCCTCCTCCGCTT---ATCT	965

human	CCAGCTACCTGGGCGCAGCACCTCGCATACACAGCTACAGCAGCAGCATCCATGTT	342	human	AGCTGGAGAGAAGGTCAGATTACAGGGTTCAGGGGTCAGGACCTCGCCATTGTGCTTCCT	1038
mouse	CCAGATACTGAGTGCACGCAACCGTGCATACACAGCGTACAGGGCGTGCATCCATGTT	343	mouse	TAGACGGGAGGAGGTCAGATTACAGGGTTCAGGGGTCAGGACCTCGCCATTGTGCTTCCT	1044
rat	CCAGATACTGAGTGCACGCAACCGTGCATACACAGCGTACAGGCGTGCATCCATGTT	343	rat	TAGATGGGAGGAGGTCAGATTACAGGGTTCAGGGGTCAGGACCTCGCCATTGTGCTTCCT	1025

human	TACAAGGGGAGAATCATTACACAGCACACTGGGGCTTCGGGGTGGACCCCGTGCA	402	human	GGGTAGCCGGTGCAGCGGTGTCTGCCTCCAGCAATCTGGAGTGTCTCT---AGGAAGGG	1095
mouse	TACAAGCCAGGGTGCTTACGCTTCGGGGAGGAGTCTCTGTGCACACATCTACACACT	403	mouse	GGCTTGGCCTGTGAGTCTCTGTCTCCAGCAATTTGGCAGGTGCAGAAACTGGGAAGGG	1104
rat	TACAAGCCAGGGTGCTTACGCTTCGGGGAGGAGTCTCTGTGCACACATCTACACACT	403	rat	GGC-TGGCCTGTACGCCCTGCCTCCAGCAATCTGGCAGGTGCAGAAACTGGGACGGG	1084

human	CACACTACATACCCGTACAGCCCAAGCAGCAGCACACGCGGGCAGTGGGACAG	462	human	GAGAGGTCCACACGAGGAACACTGGGCTCTCTGCAGCGTGGAGGCCACCATTTGAAA	1155
mouse	TAAGTCAACGAGGGC-----AGCGCTACAC-ACAGGGAGAGCAGGCGACGCTTGGAGTCT	457	mouse	AGAGCGGTTCATCCACAGGAACACTATGCTCCCTGCCTTGGGTGCCGCCACTGAAA	1164
rat	TAAAGTCAACGAGGGC-----AGCGCTACACACAGGAGAGCAGGCGACGCTTGGAGTCC	460	rat	AGCAAGGTTCATCCACAGGAACACTGGGCTCTCTGCAGCGTGGAGGCCACTGAAA	1144

human	-----CGTGGTGTCCCATGTCCC-----ATACAAGGAATCCAGCAGGACA	503	human	CCAAGGAGCAGCTTCCCTGACCCCATCTCTATCTCCGCAATCCTAAGAAACCCCAAGAA	1215
mouse	CGAGCAAGGAATCCAGAGCGGACACCCCTGCCCCATCTTAACACACACACACACACA	517	mouse	GGC-----AGCTTCCTTCTGGTCCCAATCTACTCTCTGAGTC-CTCGAACTCCAAGAA	1217
rat	CGAGCAAGGAATCCAGAGCGGACACCCCTGCCCCATCTTAACACACACACACACACA	505	rat	GGC-----AGCTTCCTTCTGGTCCCAATCTACTCTCTGAGTC-CTAGGAACTCCAAGAA	1197

human	CCCCCACCACACACACACATAGCAGCACTTGCCCTCAGGAAGTTCCAGGATCTG	563	human	TCCAGAGAAGGAAAAATTGAGGAGCCCCAGAGGTGGAGTCATGCTGATTGGAAGCAAG	1275
mouse	CACACACACACACACACACACACACAGCACTCTCTCAGGAACACTTCAGGATCTG	577	mouse	CCTTTAGAGGGGAAAAATGAGGAGCACTAG-----AGATGTGATATCTTGAAGCCAG	1271
rat	-----TACACACTCAAGCGGACTCGCTCAGGAACGCTTCAGGGTCTG	550	rat	CCTTTAGAGGGGAAAAATGAGGAGCACTAG-----AGATGTGATATCTTGAAGCCAG	1251

human	ATTGGAATCACTGCCAGCCCTCCCACTGGCACCCCA--CACACACACCATGCTGCG	621	human	AGCCACAGTGGCTAGGAGGGCTTGG-CCATGCTGGCGGTGAACCTCAAACCATAGATT	1334
mouse	ATTGGAACCACTGCCAGTCCCAACCC-AGT-----GGCACCCCGCCCACTGCTGCG	631	mouse	AGTCTTGAGTGGCTAGAGGGAGCTTGGCCATGTGCTAGGTCACACACAGGTTAGACT	1331
rat	ATTGGAACCACTGCCAGTCCCAACCCCGTGGCACCCCTTCCCTCTCCTCCCACTGCTGCG	610	rat	AGTCAAGAGTAGCTAGAGAGATGTTGGCCATGCCACTGATGAACAGCTTGGCTCACAGG	1311

human	TGAGGGAGCAGGAAGGTCCAGCTGTTCTTATTAAGAAAAAAGGGTGTGGGGTGGTG	681	human	CTATTCCTTC	1345
mouse	TGAGGGAGCAGGAAGGTCCAGCTGTTCTTATTAAGAAAAAAGGGATGGGGTGGTG	691	mouse	-----	1331
rat	TGAGGGAGCAGGAAGGTCCAGCTGTTCTTATTAAGAAAAAAGGGGTGGGGT--G	668	rat	TTATGCTAAG	1322

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- JUN binding consensus sequence
- Region of the promoter cloned
- Primes used in ChIP-qPCR

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