Published in final edited form as:
Neuron. 2011 January 13; 69(1): 91–105. doi:10.1016/j.neuron.2010.12.003.

Specific functions for ERK/MAPK signaling during PNS development

Jason M. Newbern[1], **Xiaoyan Li**[1], **Sarah E. Shoemaker**[1], **Jiang Zhou**[1], **Jian Zhong**[1], **Yaohong Wu**[1], **Daniel Bonder**[1], **Steven Hollenback**[1], **Giovanni Coppola**[3], **Daniel H. Geschwind**[3], **Gary E. Landreth**[2], and **William D. Snider**[1,*]

[1]UNC Neuroscience Center, University of North Carolina, Chapel Hill, NC, USA

[2]Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, OH, USA

[3]Program in Neurogenetics, Department of Neurology, David Geffen School of Medicine, University of California, Los Angeles, CA, USA

Abstract

We have established functions of the stimulus dependent MAPKs, ERK1/2 and ERK5 in DRG, motor neuron, and Schwann cell development. Surprisingly, many aspects of early DRG and motor neuron development were found to be ERK1/2 independent and *Erk5* deletion had no obvious effect on embryonic PNS. In contrast, *Erk1/2* deletion in developing neural crest resulted in peripheral nerves that were devoid of Schwann cell progenitors, and deletion of *Erk1/2* in Schwann cell precursors caused disrupted differentiation and marked hypomyelination of axons. The Schwann cell phenotypes are similar to those reported in *neuregulin-1* and *ErbB* mutant mice and neuregulin effects could not be elicited in glial precursors lacking *Erk1/2*. ERK/MAPK regulation of myelination was specific to Schwann cells, as deletion in oligodendrocyte precursors did not impair myelin formation, but reduced precursor proliferation. Our data suggest a tight linkage between developmental functions of ERK/MAPK signaling and biological actions of specific RTK-activating factors.

Introduction

Nervous system development and function is dependent upon a variety of soluble and membrane bound trophic stimuli, many of which act through receptor tyrosine kinases (RTKs). Even though more than 50 RTKs are present in the human genome, a handful of signaling pathways are repeatedly implicated in downstream cellular responses, particularly the MAPK, PI-3 kinase, JAK-STAT, and PLC pathways. Surprisingly, there has been little progress in assigning specific developmental functions to individual pathways (Lemmon and Schlessinger, 2010). Indeed, the many *in vitro* studies carried out with pharmacological inhibitors clearly predict that these signaling cascades integrate the effects of multiple

[*]**Address Correspondence to:** UNC Neuroscience Center - Campus Box #7250 University of North Carolina School of Medicine 115 Mason Farm Rd Chapel Hill, NC 27599-7250 william_snider@med.unc.edu.

extracellular signals and that elimination of even a single pathway *in vivo* would result in complex, difficult to interpret phenotypes.

MAPK signaling generally refers to four cascades, each defined by the final tier of the pathway: Extracellular signal-Regulated Kinases 1 & 2 (ERK1 & 2), ERK5, c-Jun N-terminal Kinases (JNK), and p38 (Raman et al., 2007). ERK1 and ERK2 (ERK1/2) exhibit a high degree of similarity and are considered functionally equivalent, although isoform specific effects have been described. In the nervous system, ERK1/2 and ERK5 are the primary MAPK cascades activated by trophic stimuli and have been shown to mediate proliferation, growth, and/or survival in specific contexts (Nishimoto and Nishida, 2006). Aberrant ERK1/2 signaling plays a primary role in a range of human syndromes that affect the nervous system, particularly the family of “neuro-cardio-facial-cutaneous” (NCFC) syndromes (Bentires-Alj et al., 2006). The precise role of ERK1/2 in the neurodevelopmental abnormalities that characterize these disorders is only now being investigated (Newbern et al., 2008; Samuels et al., 2008; Samuels et al., 2009). Indeed, most of our understanding of ERK function is derived from *in vitro* models in the context of isolated trophic stimuli. Such studies provide support for involvement of ERK/MAPK signaling in almost every aspect of neural development and function. However, the physiological relevance of many *in vitro* findings has not been adequately tested, and much less is known about ERK functions in the context of multiple extracellular signals, as occurs *in vivo*.

The PNS has been the standard model system for defining biological actions of many neurotrophic molecules. The PNS principally derives from the neural crest, which generates sensory and sympathetic neurons, satellite glia within the DRG, and Schwann cells within the peripheral nerve. Peripheral neuron development requires trophic signaling via Neurotrophins and GDNF family members, which act via RTKs that activate ERK1/2 (Marmigere and Ernfors, 2007). Analyses of PNS neuronal development *in vitro* have shown that ERK1/2 signaling is important for differentiation and neurite outgrowth in response to neurotrophins, other trophic factors, and ECM molecules (Atwal et al., 2000; Klesse et al., 1999; Kolkova et al., 2000; Markus et al., 2002). ERK1/2 activation by some of these same molecules has been implicated in regulating neurite outgrowth from motor neurons, which also extend axons in peripheral nerves (Soundararajan et al., 2010). Many trophic stimuli also activate ERK5, which appears to be involved in neuronal survival in the PNS, particularly in the context of NGF mediated retrograde signaling (Finegan et al., 2009; Watson et al., 2001). These findings plainly predict that disruption of either the ERK1/2 or ERK5 pathway *in vivo* would result in profound defects in neuronal differentiation, survival, and/or developmental axon growth.

Schwann cell development is also critically dependent on extracellular factors that act through RTKs, including neuregulins, PDGF, IGFs, FGFs, and ECM components (Jessen and Mirsky, 2005). These factors are capable of activating PI3K, PLC, ERK1/2, and ERK5 signaling (Lemmon and Schlessinger, 2010). However, the role of ERK5 in glia has not been assessed and the data regarding ERK1/2 function in Schwann cell development are controversial. ERK1/2 has been shown to regulate the survival of early Schwann cell progenitors (SCPs) and mature Schwann cells *in vitro*, however, other research has not supported these findings (Dong et al., 1999; Li et al., 2001; Parkinson et al., 2002). Some evidence suggests ERK1/2 signaling regulates Schwann cell myelination, yet other careful studies found little effect and argue that PI3K/Akt signaling plays a more central role (Harrisingh et al., 2004; Hu et al., 2006; Maurel and Salzer, 2000; Ogata et al., 2004). The reasons for these discrepancies are unclear and the precise role of ERK1/2 signaling in Schwann cell development remains unresolved.

Here, we have defined the roles of ERK1/2 and ERK5 in neuronal and glial development *in vivo*. *In vivo* analyses have previously been hampered by the strong redundancy between ERK1 and ERK2 and the early embryonic lethality of *Erk2* and *Erk5* knockouts (Nishimoto and Nishida, 2006). To circumvent these issues we have utilized a combination of *Erk2* conditional and *Erk1* null alleles to eliminate ERK1/2 signaling *in vivo*, and have generated an *Erk5* conditional allele. We demonstrate that *Erk1/2* and *Erk5* are surprisingly dispensable for many aspects of prenatal DRG and motor neuron development *in vivo*, although *Erk1/2* deletion in DRG neurons compromises sensory axon innervation in NGF-expressing target fields. In contrast, *Erk1/2* signaling is essential at multiple stages of Schwann cell development and is required for PNS myelination. Our data constrain interpretation of the many prior *in vitro* studies and suggest tight linkage between ERK/MAPK functions *in vivo* and biological actions of specific RTK activating factors.

Results

ERK1/2, but not ERK5, deletion in the embryonic PNS results in DRG degeneration

To establish the role of ERK1/2 in embryonic PNS development, we generated *Erk1*$^{-/-}$ *Erk2*$^{fl/fl}$ *Wnt1:Cre* (hereafter referred to as ***Erk1/2***$^{\boldsymbol{CKO(Wnt1)}}$) and *Mek1*$^{fl/fl}$ *Mek2*$^{-/-}$ *Wnt1:Cre* (***Mek1/2***$^{\boldsymbol{CKO(Wnt1)}}$) mice. The *Wnt1:Cre* driver induces recombination at ~E8.5 in pluripotent neural crest cells, which generate both neuronal and glial components of the PNS (Danielian et al., 1998). We have previously characterized major defects in craniofacial and cardiac neural crest derived structures by E10.5 and embryonic lethality between E18-19 in *Erk1/2*$^{CKO(Wnt1)}$ and *Mek1/2*$^{CKO(Wnt1)}$ mice (Newbern et al., 2008). However, DRGs in E10.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos appear to be morphologically intact (Figure S1A-B). ERK1/2 expression is significantly reduced in the DRG by E10.5 and Western blotting of E12.5 *Erk1/2*$^{CKO(Wnt1)}$ or *Mek1/2*$^{CKO(Wnt1)}$ DRG lysates shows a near complete loss of ERK1/2 or MEK1/2 protein, respectively (Figure S1C-E). RSK3, a downstream substrate of ERK1/2, showed significantly reduced phosphorylation further indicating functional inactivation of ERK1/2 signaling (Figure S1E).

We therefore utilized *Erk1/2*$^{CKO(Wnt1)}$ mice to ask whether the loss of *Erk1/2* disrupts PNS development *in vivo*. Compared to controls (Figure 1A,C), massive cell loss was observed at both brachial and lumber levels in E17.5 *Erk1/2*$^{CKO(Wnt1)}$ DRG's (Figure 1B,D;Figure S1F-J). We found that homozygous deletion of both genes was necessary for the decreased neuronal number in the DRG (data not shown). E17.5 *Mek1/2*$^{CKO(Wnt1)}$ embryos show a qualitatively similar, though more severe phenotype, than in stage matched *Erk1/2*$^{CKO(Wnt1)}$ embryos (Figure S1F-H). Endogenous levels of MEK1/2 protein are reported to be lower than ERK1/2, likely resulting in more rapid protein clearance following recombination and a relatively accelerated phenotypic onset (Ferrell, 1996). Whole mount neurofilament immunolabeling of E15.5 control and *Erk1/2*$^{CKO(Wnt1)}$ forelimbs revealed that nearly all peripheral projections are absent in mutant forelimbs (Figure 1E-F). It is notable that motor neurons do not undergo recombination in the *Wnt1:Cre* line, yet their projections totally degenerate. Overall, these data demonstrate that inactivation of *Erk1/2* in the PNS results in the loss of all peripheral projections and massive DRG neuron death.

ERK5 is another well-known stimulus dependent MAPK under trophic control during PNS development (Watson et al., 2001). We tested the role of this pathway in *Erk5*$^{fl/fl}$ *Wnt1:Cre* (*Erk5*$^{CKO(Wnt1)}$) mice (Figure S1K-N). In contrast to *Erk1/2*$^{CKO(Wnt1)}$ mice, *Erk5*$^{CKO(Wnt1)}$ mice are viable, and able to breed. However, *Erk5*$^{CKO(Wnt1)}$ adult mice are smaller than controls and exhibit external ear truncation and mandibular shortening, likely due to an alteration in the development of the craniofacial neural crest (Figure S1M). Perhaps surprisingly, markers for proprioceptive (Parvalbumin) and nociceptive (CGRP and TrkA) sensory neurons, exhibited relatively normal expression in P1 *Erk5*$^{CKO(Wnt1)}$ DRG's (Figure

1G-J and data not shown). Whole mount neurofilament immunolabeling did not reveal any deficit in the peripheral projections of E14.5 *Erk5*$^{CKO(Wnt1)}$ forelimbs compared to controls (Figure 1K-L). Both CGRP and Parvalbumin positive central afferents within the spinal cord appeared intact as well (Figure 1G-J). Overall, these data suggest that ERK5 does not play a primary role in early aspects of PNS morphogenesis *in vivo*.

Erk1/2 is required for Schwann cell progenitor development

In order to determine the precise developmental defect underlying the massive decrease in DRG size observed in E17.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos, we assessed the expression of neuronal and glial markers at earlier stages of development. Within the DRG of E10.5-12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos, appropriate neuronal (neurofilament, NeuN, TrkA, Brn3a, Tau, and Islet1/2) markers are expressed (Figure 2A-D, 3;Figure S3), suggesting that early stages of neuronal specification are intact in these embryos. The pattern of two markers of glial differentiation, Sox2 and BFABP, *within* the DRG of E10.5-E12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos also appeared normal suggesting satellite glia are appropriately specified (Figure 2A-D, Figure S2C-D).

In striking contrast, we noted a marked loss of Sox2 and BFABP labeled Schwann cell progenitors (SCPs) within the peripheral nerve of E11.5-12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos (Figure 2E-F, Figure S2A-D). Generic labeling of all cells with Hoechst (Figure 2E-F) or *Rosa26*^{LacZ} (Figure S2E-F) shows a similar pattern demonstrating loss of cells rather than changes in the expression levels of these specific glial markers. These data indicate that ERK1/2 is required for SCP colonization of the peripheral nerve *in vivo*.

SCPs are heavily reliant upon neuregulin/ErbB signaling; a potent activator of the ERK1/2 pathway (Birchmeier and Nave, 2008). Mice lacking *Nrg1*, *ErbB2*, or *ErbB3* exhibit an absence of SCPs in the developing nerve (Birchmeier and Nave, 2008). *Nrg-1* or *ErbB2* gene expression was not decreased in E12.5 *Erk1/2*$^{CKO(Wnt1)}$ DRGs (Figure S2G). We tested whether the disruption of SCP development was due to a glial cell autonomous requirement for ERK1/2 in neuregulin/ErbB signaling. Glial progenitors from E11.5 *Erk1/2*$^{CKO(Wnt1)}$ DRGs were cultured in the presence of neuregulin-1. The loss of *Erk1/2* clearly abolished the survival promoting effect of neuregulin-1 *in vitro* (Figure 2G-I). These data indicate that ERK1/2 is required for glial responses to neuregulin-1, which likely contributes to the failure of SCP development *in vivo*.

It has been previously shown that the neural crest derived, boundary cap (BC) generates SCPs and establishes ECM boundaries that prevent the migration of neuronal cell bodies into the peripheral nerve (Bron et al., 2007; Maro et al., 2004). We examined this gliogenic niche in *Erk1/2*$^{CKO(Wnt1)}$ embryos by immunostaining for Egr2/Krox-20, which is expressed by the BC. Interestingly, the proximal ventral root of E12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos exhibited a near complete absence of Egr2/Krox-20 expressing BC cells (Figure 2J-K). We also noted Islet1/2 positive neuronal bodies in the ventral root of E11.5-12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos, further indicating a failure in function (Figure 2L-M). Overall, these data suggest that the defect in SCP development is due in part to a disruption in a gliogenic niche.

Initial stages of sensory axon outgrowth do not require Erk1/2

A key consequence of the loss of SCPs in *Nrg-1/ErbB* mutant mice is nerve defasciculation, loss of all peripheral projections, and neuron death (Meyer and Birchmeier, 1995; Morris et al., 1999; Riethmacher et al., 1997). Indeed, the absence of SCPs likely contributes to the complete loss of motor projections in E15.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos, since recombination in motor neurons is not induced by *Wnt1:Cre* (Figure 1E-F). However, the ERK1/2

signaling pathway plays a central role in the response to numerous axon growth promoting stimuli. We predicted that DRG neuron outgrowth in *Erk1/2*$^{CKO(Wnt1)}$ embryos would be disrupted, prior to the point when the loss of SCPs would effect neuronal development. Thus, we examined the temporal dynamics of axon outgrowth and DRG neuron number in *Erk1/2*$^{CKO(Wnt1)}$ embryos.

We first examined changes in neuron number in these embryos. At E11.5, when SCP number in the peripheral nerve is reduced, no pyknotic nuclei were detected in the DRG. By E12.5, occasional pyknotic nuclei and increased caspase-3 activity were detected in *Erk1/2*$^{CKO(Wnt)}$ rostral DRGs (Figure S3A-B,F). However, relative counts of Islet1/2 positive neurons in brachial DRGs at E12.5 did not reveal a statistically significant difference (Figure S3E). We examined neuronal number with a *Tau*$^{loxp\text{-}STOP\text{-}loxp\text{-}mGFP\text{-}IRES\text{-}nlsLacZ}$ (*Tau*^{STOP}) reporter line in E15.5 mutants and found only 19.6±4.1% of nls-LacZ expressing neurons remained (Hippenmeyer et al., 2005)(Figure S3C-E). The time course of neuronal death closely mirrors that reported in *ErbB-2* or *-3* null mice (Morris et al., 1999; Riethmacher et al., 1997). Thus, neurogenesis is relatively unaffected by the loss *Erk1/2*, however, neuronal death is initiated after E12.5, likely an indirect effect resulting from disruption of the SCP pool.

The pattern of early peripheral nerve growth in *Erk1/2*$^{CKO(Wnt1)}$ embryos was evaluated with whole mount neurofilament immunolabeling, which labels all peripheral projections of sensory, motor, or sympathetic origin. In contrast to our predictions, the extent of initial axonal outgrowth in *Erk1/2*$^{CKO(Wnt1)}$ embryos appeared normal at E10.5 and E12.5 (Figure 3A-D). At E12.5, nerves were disorganized and defasciculated in the forelimb, similar to what has been observed in *Nrg-1/ErbB* mutant mice (Figure 3C-D). Comparable results were obtained with *Mek1/2*$^{CKO(Wnt1)}$ embryos (Figure 3E-F), though again deficits appeared slightly earlier when compared to *Erk1/2*$^{CKO(Wnt1)}$ embryos. A specific defect in sensory neuron outgrowth could be masked by neurofilament expression in motor axons. This possibility was excluded by analyzing two sensory neuron specific reporter lines, the *Tau*^{STOP} reporter line, which does not label motor neurons in *Wnt1:Cre* mice, and the *Brn3a*^{TauLacZ} mouse (Eng et al., 2001; Hippenmeyer et al., 2005). Both reporter lines revealed that sensory neuron outgrowth in E12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos is of relatively normal extent, but defasciculated (Figure S3G-J). In summary, these data indicate that in the absence of ERK1/2, neuronal number and the extent of axon outgrowth are intact up to E12.5. Following this stage a number of neuronal defects are evident, which are consistent with the loss of SCPs in this model, namely, defasciculation, the overt loss of all peripheral projections, and sensory neuron death.

Erk1/2 is required for NGF-dependent cutaneous innervation

The requirement of SCPs for ERK1/2 and the potential for complex neuron-glial interactions in the context of neural crest *Erk1/2* deletion, limited our analysis of neuronal roles for ERK1/2 signaling. To better understand neuronal ERK1/2, we employed two additional Cre lines. *Nestin:Cre* induces recombination in progenitors throughout the CNS and leads to gene deletion in both neuronal and glial populations. However, recombination in the DRG occurs beginning at ~E10.5 resulting in gene deletion in most DRG neurons, but not in Schwann cells (Kao et al., 2009; Tronche et al., 1999; Zhong et al., 2007). The *Advillin:Cre* line induces recombination in virtually all DRG and trigeminal ganglion neurons beginning at ~E12.5 and is almost exclusive for these populations (Hasegawa et al., 2007).

Mek1/2$^{CKO(Nes)}$ mice die shortly after birth and major reductions in MEK1/2 expression and ERK1/2 activation were noted in the *Mek1/2*$^{CKO(Nes)}$ DRG by E14 (Figure S4A). Whole mount neurofilament labeling at mid-embryonic stages revealed a normal pattern of early peripheral nerve development in the absence of *Mek1/2* (Figure 4A-B). DRG morphology is

grossly normal at birth and the expression of nociceptive markers, P2×3 and TrkA, and the proprioceptive marker, Parvalbumin, are relatively unchanged (Figure 4C-D and data not shown). In the target field, the main nerve trunks of P0 *Mek1/2*$^{CKO(Nes)}$ peripheral nerves were relatively normal in size, however, we noted a reduction in the innervation of the subepidermal plexus and the number of cutaneous fibers entering the epidermal field (Figure 4E-H). These data show that the early loss of ERK1/2 signaling in DRG neurons does not modify initial stages of axon outgrowth, but inhibits axon innervation of the cutaneous fields by birth.

Erk1/2$^{CKO(Advillin)}$ mice are indistinguishable from controls in the days following birth. However, by the end of the first postnatal week, mutant mice are noticeably smaller and the mice do not survive past three weeks of age. Importantly, the number of fibers innervating the epidermis in P3 *Erk1/2*$^{CKO(Advillin)}$ hindlimbs was significantly decreased relative to controls (Figure 4I-J,O). At this time point, a relatively normal number of DRG neurons were present, which exhibit a typical pattern of TrkA and CGRP expression and complete loss of ERK2 expression (Figure 4M-N, data not shown). CGRP labeled central afferents also appeared intact in the dorsal spinal cord of mutant mice (Figure 4K-L). In P18 mutants, DRG neuron number was 41.5±3.0% (n=2) of littermate controls. This is likely an indirect phenotype related to the decrease in cutaneous innervation, which diminishes trophic support. Taken together, these data demonstrate that DRG neuron specific inactivation of ERK1/2 signaling is not necessary for initial stages of axon outgrowth *in vivo* but is required for superficial cutaneous innervation.

Arborization within superficial cutaneous target fields is known to be dependent upon NGF/TrkA signaling (Patel et al., 2000). The link between ERK1/2 and NGF was further assessed *in vitro*. Indeed, both dissociated and explanted DRG neurons from *Erk1/2*$^{CKO(Wnt)}$ and *Mek1/2*$^{CKO(Nes)}$ embryos exhibit decreased axon outgrowth in response to NGF (Figure 4P-R and data not shown). Thus, the deficit in cutaneous innervation observed *in vivo* is likely mediated by a disruption of NGF/TrkA signaling.

Though cutaneous innervation was clearly deficient, various aspects of proprioceptive morphological development appeared qualitatively normal in P3 *Erk1/2*$^{CKO(Advillin)}$ mice, including central projections into the spinal cord, and the innervation of muscle spindles within the soleus (Figure S4E-L). However, these finding should be interpreted with caution as the proprioceptive system develops relatively early related to the recombination induced by *Advillin:Cre*. Indeed, the mice develop an abnormality of spindle innervation and exhibit a hindlimb clasping phenotype when raised by the tail beginning in the second postnatal week (Figure S4B-D). These findings suggest that ERK/MAPK signaling is required for some aspects of proprioceptive development, possibly downstream of NT-3.

The ERK1/2 and ERK5 signaling pathways have been shown to modify similar substrates (Nishimoto and Nishida, 2006). Thus, compensatory interactions between ERK1/2 and ERK5 might mask a requirement for the other pathway in *Erk1/2* or *Erk5* mutants. In order to test whether *Erk1/2* and *Erk5* exhibit compensatory interactions in DRG neurons, we generated *Erk1*$^{-/-}$ *Erk2*$^{fl/fl}$ *Erk5*$^{fl/fl}$ *Advillin:Cre* mice. The added deletion of *Erk5* does not appear to strongly modify the *Erk1/2* deletion phenotypes. Thus, *Erk1/2/5* triple mutants exhibit a hindlimb clasping phenotype and die at the same postnatal ages as *Erk1/2* double mutants. These data suggest that the ERK1/2 and ERK5 pathways do not significantly compensate for one another during DRG neuron development.

Peripheral myelination requires Erk1/2 signaling

The effects of ERK1/2 on the establishment of the SCP pool precluded analyses of later stages of Schwann cell development, particularly myelination. To this end, we utilized the

Desert hedgehog:Cre knockin mouse, which induces recombination at ~E13, almost exclusively in the Schwann cell lineage (Jaegle et al., 2003). Loss of ERK1/2 occurs after the specification of the SCP pool and during the transition into immature Schwann cells (Jessen and Mirsky, 2005). *Erk1/2*$^{CKO(Dhh)}$ mice were born at normal Mendelian ratios, but exhibited tremor and hindlimb paresis within two weeks of birth and do not survive past the fourth postnatal week. Western blotting of mutant P1 sciatic nerve lysates revealed a significant decrease in the expression of ERK1/2 (Figure S5A). Whole mount LacZ staining of control embryos crossed with *Rosa26*^{LacZ} demonstrates recombination within the proximal peripheral nerve by E13.5 and most of the sciatic nerve by E14.5 (data not shown). E15.5 *Erk1/2*$^{CKO(Dhh)}$ *Rosa26*^{LacZ} embryos showed a morphologically similar pattern of recombination and peripheral nerve patterning as littermate controls (Figure 5A-B). The distribution of Schwann cells in the mature, P20 *Erk1/2*$^{CKO(Dhh)}$ *Z/EG* phrenic nerve projections appeared similar to controls, with Schwann cells present up to the NMJ (Figure S5B-C). These finding suggest Schwann cells take up relatively normal positions in *Erk1/2*$^{CKO(Dhh)}$ embryos.

Although Schwann cells appeared to be present within the nerve, gross dissection of P18, *Erk1/2*$^{CKO(Dhh)}$ sciatic nerves revealed markedly decreased nerve caliber and increased translucency (Figure 5C). Electron microscopy revealed a clear, striking reduction in the number of myelinated axons and an increase in the number of un-myelinated axons in *Erk1/2*$^{CKO(Dhh)}$ mice (Figure 5D-F). There was no change in the number of Schwann cell nuclei within the sciatic nerve and dying, pyknotic nuclei were not detected at this stage (Figure 5G). These data show that loss of ERK1/2 in Schwann cell progenitors clearly inhibits myelination.

The development of myelinating Schwann cells involves the upregulation of numerous factors, including Egr2/Krox-20, S100β, and various myelin components (Jessen and Mirsky, 2005). Immunohistochemical analysis of P18 *Erk1/2*$^{CKO(Dhh)}$ sciatic nerves revealed a 77.8±8.1% decrease in the number of Egr2/Krox-20 positive cells and the expression of S100β was nearly absent (Figure 5H-K). GFAP immunolabeling of non-myelinating Schwann cells appeared normal at this stage (data not shown). These findings suggest that ERK1/2 is required for the progression of the myelinating Schwann cell lineage after initial specification.

ERK1/2 regulates a transcriptional network critical to Schwann cell development

In order to better understand the potentially diverse developmental mechanisms underlying ERK1/2 regulation of PNS development, we performed microarray analysis on RNA extracts derived from E12.5 *Erk1/2*$^{CKO(Wnt1)}$ and wildtype DRG's. We did not detect overt changes in DRG neuron number at this developmental stage, suggesting the profile is a reflection of ERK1/2 regulated genes and not the loss of any particular cell type. 209 distinct genes met our inclusion criteria, which included 98 down-regulated and 111 up-regulated genes in *Erk1/2*$^{CKO(Wnt1)}$ samples (Figure 6A, Figure S6). Functional annotation of regulated genes revealed significant changes in mediators of transcriptional regulation, cell-cell signaling, intracellular signaling, and cell-cycle/division (Figure 6A, Figure S6).

A number of genes involved in transcriptional regulation were modified that have been shown to regulate glial development (Figure 6B). Microarray changes were validated by qPCR of DRG samples from E12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos (Figure 6C and data not shown). Significantly decreased expression was observed for *Egr2*/*Krox-20*, *Id4*, *Id2*, and *Etv1/Er81*, all of which have been shown to be required for or modify myelinating glia differentiation (Marin-Husstege et al., 2006; Topilko et al., 1994). Surprisingly, mutant DRGs exhibited increased mRNA levels of the myelin components, *MBP* and *MAG*. The

increase in *MBP* and *MAG* suggest that the loss of ERK1/2 signaling may have triggered, in part, a molecular program of premature differentiation.

Requirement for Erk1/2 in motor neuron and oligodendrocyte development

In order to explore ERK1/2 regulation of another class of peripherally projecting neuron and to assess regulation of another type of myelinating cell, we utilized an *Olig2:Cre* mouse to induce recombination by E9.5 in the spinal cord progenitor domain that produces motor neurons and oligodendrocytes (Dessaud et al., 2007; Novitch et al., 2001). We first examined the development of spinal motor neurons. *Erk1/2*$^{CKO(Olig2)}$ mice do not survive past the first day of birth. Cre dependent reporter line expression and a decrease in ERK1/2 expression were noted in E14.5 motor neurons and the progenitor domain from which they arise (Figure 7A-B, Figure S7A-B). Whole mount immunolabeling of the E14.5 mutant forelimbs revealed a normal pattern of motor neuron outgrowth (Figure 7A-B). Motor innervation of neuromuscular junctions (NMJs) in the soleus and diaphragm also appeared intact in P1 *Erk1/2*$^{CKO(Olig2)}$ mice (Figure 7C-F). Thus, motor neuron axon development does not appear to be at all dependent on ERK1/2 signaling during embryonic development.

Given the profound effects on peripheral glial following the loss *Erk1/2* we analyzed the development of oligodendrocytes within the spinal cord of *Erk1/2*$^{CKO(Olig2)}$ mice. A significant decrease in the number of oligodendrocyte progenitors in the spinal cord white matter was evident by E14.5. Quantification in the white matter at E14.5 revealed that 51.1±4.9% of PDGF-Rα positive cells remained in the mutants while the number of S100β positive cells at P1 was 41.2±6.5% of controls (Figure 8A-C and Figure S8A-B). The total number of nuclei in the white matter was similarly decreased in *Erk1/2*$^{CKO(Olig2)}$ embryos indicating that the defect is not due to altered expression of glial markers (Figure 8A-C). The number of oligodendrocytes thus appears to be regulated by ERK1/2 signaling *in vivo*.

Oligodendrocyte proliferation *in vivo* is strongly regulated by PDGF acting through the receptor tyrosine kinase, PDGF-Rα, a known ERK1/2 activator (Calver et al., 1998). In exploring the mechanism underlying the reduction in white matter glia, we noted a significant decrease in the proportion of PDGF-Rα cells co-labeled with BrdU in E14.5 *Erk1/2*$^{CKO(Olig2)}$ white matter (Figure 8D). In contrast, we did not detect changes in activated caspase-3 expression in the embryonic spinal cord (data not shown). Together these results suggest that ERK1/2 is critical for the proliferation, but not the survival of oligodendrocyte progenitors.

In striking contrast to ERK1/2 regulation of the Schwann cell lineage, *Erk1/2* deleted oligodendrocytes were capable of myelination. The early lethality of *Erk1/2*$^{CKO(Olig2)}$ mice limited our analysis to only the initial stages of myelination, however, a clear increase in MBP labeling is apparent in P1 *Erk1/2*$^{CKO(Olig2)}$ ventral spinal cords (Figure 8E-F). S100β labeled oligodendrocytes in the white matter of mutant embryos exhibited a more ramified, complex morphology than controls, further suggesting that loss of *Erk1/2* triggered premature differentiation (Figure 8A-B, data not shown). Co-immunostaining of MBP positive cells with an ERK2 antibody confirmed that myelinating oligodendrocytes were truly ERK1/2 deficient in mutants (Figure S8C-D). These data show that, in contrast to Schwann cells, myelination by oligodendrocytes can proceed in the absence of *Erk1/2*.

Discussion

We have assessed the functions of ERK1/2 and ERK5 in distinct cell types during PNS development *in vivo*. Our data lead to several clear conclusions. First, many aspects of embryonic sensory and motor neuron development occur normally in the setting of *Erk1/2* deletion, although sensory axons do not invade NGF-expressing target fields. Second, ERK5

does not appear to strongly regulate embryonic PNS development. Third, ERK1/2 is critical for fundamental aspects of Schwann cell development. *Erk1/2* deletion phenotypes resemble those of *Nrg-1* and *ErbB* mutants, and *Erk1/2* deleted Schwann cell progenitors do not respond to neuregulin-1. Finally, the requirement of ERK1/2 for myelination is specific to Schwann cells, as myelination by oligodendrocytes can proceed in the absence of *Erk1/2*. Overall, our findings tightly link *in vivo* functions of ERK/MAPK signaling to biological actions of specific RTK activating factors.

ERK/MAPK signaling is dispensable for many aspects of PNS neuron development

Gene targeting studies have defined roles for numerous trophic factors, ECM molecules, and axon guidance cues in directing PNS neuron development (Marmigere and Ernfors, 2007). However, the signaling pathways mediating these effects *in vivo* have not been defined. Many growth promoting cues appear to converge upon ERK1/2, and combinations of trophic stimuli, such as integrins and growth factors, trigger synergistic ERK1/2 activation (Perron and Bixby, 1999). Overall, these data predict that ERK1/2 is a central regulator of neuronal morphology and development *in vivo*.

In spite of a wealth of *in vitro* data, our *in vivo* findings provide surprisingly little support for a broad and essential role for ERK1/2 for the acquisition of neuronal phenotypes, survival, or initial axon outgrowth. Our results instead show that ERK1/2 signaling is required for NGF-mediated cutaneous sensory neuron innervation at late embryonic and early postnatal stages. These results are generally consistent with previous findings in *B-Raf/C-Raf* and *SRF* conditional knockout mice (Wickramasinghe et al., 2008; Zhong et al., 2007) and in line with the established role for NGF/TrkA signaling in promoting the formation and maintenance of these afferents (Patel et al., 2000). The peripheral nerve defect is not as severe as that reported in *TrkA/Bax* mutant mice, suggesting a contribution to morphological development by other pathways, such as PI3K and PLC (Patel et al., 2000). Overall, our results establish that ERK1/2 signaling *in vivo* is required to transduce the morphological effects of skin derived NGF. However, neuronal ERK/MAPK signaling is surprisingly dispensable for early phases of neuronal differentiation, neuronal survival, long range axon growth, and formation of the neuromuscular junction.

Another surprise relates to the apparently limited role of ERK5. ERK5 has been convincingly established as a retrograde survival signal for NGF-stimulated DRG and sympathetic ganglion neurons *in vitro* (Finegan et al., 2009; Watson et al., 2001). The reason for the discrepancy between *in vitro* and *in vivo* findings related to ERK5 mediated survival functions remains elusive. ERK5 and ERK1/2 exhibit some overlap in downstream targets, opening the possibility of a compensatory interaction. However, the drastically different phenotypes and mechanisms leading to lethality in $Erk2^{-/-}$ vs. $Erk5^{-/-}$ embryos demonstrate that ERK1/2 and ERK5 possess many unique, independent functions (Nishimoto and Nishida, 2006). Our results with $Erk1^{-/-}$ $Erk2^{fl/fl}$ $Erk5^{fl/fl}$ *Advillin:Cre* mutants suggests that compensatory interactions between these two cascades are minimal in sensory neurons.

ERK1/2 mediates effects of neuregulin-1 on Schwann cell development

Although numerous extracellular factors that activate ERK1/2 are known to regulate Schwann cell development, the requirement for ERK1/2 signaling in mediating Schwann cell responses has been controversial. Instead the PI3K/Akt pathway appears to play a particularly prominent role (Harrisingh et al., 2004; Hu et al., 2006; Li et al., 2001; Maurel and Salzer, 2000; Ogata et al., 2004). An important caveat is that much of this prior work regarding ERK1/2 signaling has relied upon varying *in vitro* models, likely contributing to disparate conclusions. Our data help resolve a long-standing debate in establishing that ERK1/2 is absolutely necessary for multiple stages of Schwann cell development *in vivo*.

The neuregulin/ErbB axis is critical for Schwann cell development (Birchmeier and Nave, 2008). The signaling pathways required to mediate neuregulin functions have been of intense interest, particularly in relation to the control of myelination (Grossmann et al., 2009;; Kao et al., 2009;). Our data, taken together with other lines of evidence, strongly suggest that ERK1/2 is a key signaling pathway necessary to transduce effects of neuregulin-1 on Schwann cells *in vivo*. The phenotypes in *Erk1/2* and *Nrg-1/ErbB* mutant mice are remarkably similar. The *Erk1/2*$^{CKO(Wnt1)}$ mice that we report here, and *ErbB2*$^{-/-}$, *ErbB3*$^{-/-}$, and *NRG-1*$^{-/-}$ mice previously reported, all exhibit a near complete absence of SCPs in the peripheral nerve by E12.5, yet satellite glia appear to be relatively unaffected (Meyer and Birchmeier, 1995; Morris et al., 1999; Riethmacher et al., 1997; Woldeyesus et al., 1999). The loss of SCPs in developing peripheral nerve results in axon defasciculation, the subsequent loss of all peripheral axon projections, and neuronal death, a phenotype observed in other mouse models lacking SCPs, such as *Sox10*$^{-/-}$ (Britsch et al., 2001). Inhibition of Schwann cell myelination is also present in both *Erk1/2*$^{CKO(Dhh)}$ and conditional *ErbB* mutant mice, where gene inactivation occurs after SCP's have been specified in immature Schwann cells (Garratt et al., 2000). Inactivation of *Shp2*, an ERK1/2 pathway activator recruited by ErbB, results in similar disruptions in Schwann cell development (Grossmann et al., 2009). Indeed, the defects in *Shp2* mutant Schwann cells *in vitro* correlated with decreased sustained ERK1/2, but not PI3K/Akt, activity (Grossmann et al., 2009). Finally, we demonstrate here that loss of ERK1/2 in glial progenitors blocks the effects of neuregulin-1 *in vitro*. These data establish that ERK1/2 is necessary to transduce neuregulin-1/ErbB signals during the development of the Schwann cell lineage *in vivo*.

Mechanisms and specificity of the ERK1/2 requirement for myelination

The precise mechanisms underlying the failed development of Schwann cells in *Erk1/2* mutant mice are likely complex given the extensive repertoire of ERK1/2 substrates and downstream targets (Yoon and Seger, 2006). The loss of the gliogenic boundary cap in *Erk1/2*$^{CKO(Wnt1)}$ mice presumably leads to a reduction in SCPs in the peripheral nerve. This phenotype may result from a direct defect in survival as demonstrated *in vitro*, but may also involve aberrant differentiation. Expression profiling of early glial progenitors in the *Erk1/2*$^{CKO(Wnt1)}$ DRG demonstrates that ERK1/2 promotes the expression of *Id2* and *Id4*, genes that maintain pluripotency and regulate glial differentiation (Marin-Husstege et al., 2006). Additionally, ERK1/2 signaling suppresses the expression of markers of mature glia, *MBP* and *MAG*. One interpretation of these data is that loss of *Erk1/2* leads to premature differentiation. Thus, SCPs or the BC may have lost the ability to maintain the progenitor state, which contributes to their loss in *Erk1/2*$^{CKO(Wnt1)}$ embryos. Interestingly, *Erk1/2* deletion at a later stage of Schwann cell development with *Dhh:Cre* did not result in a significant change in Schwann cell number in the sciatic nerves. This stage dependent difference in the regulation of Schwann cell development mirrors the increasingly limited effects of *ErbB2* deletion as development proceeds (Atanasoski et al., 2006; Garratt et al., 2000), and presumably results from an uncoupling of ERK1/2 from specific cellular functions.

How is it that ERK1/2 regulates myelination? *Erk1/2*$^{CKO(Dhh)}$ peripheral nerves fail to express markers of mature Schwann cells, such as S100β, and myelination is severely inhibited. ERK1/2 regulation of *Egr2/Krox-20* might underlie this Schwann cell specific phenotype. *Egr2/Krox-20* is a critical gene for promoting Schwann cell myelination and is mutated in a subset of patients with Charcot-Marie-Tooth (CMT) disease (Topilko et al., 1994; Warner et al., 1998). Mice expressing CMT-related, *Egr2/Krox-20* mutations exhibit hypomyelination and a temporal progression of behavioral dysfunction and death that closely phenocopies the *Erk1/2*$^{CKO(Dhh)}$ mice we report here (Baloh et al., 2009). *In vitro* evidence demonstrates that *Egr2/Krox-20* induction is downstream of ERK1/2 and

neuregulin signaling (Murphy et al., 1996; Parkinson et al., 2002). Our findings suggest this interaction is relevant *in vivo* as *Egr2/Krox-20* expression is strongly reduced in *Erk1/2* mutant mice.

An important and surprising finding is that the cellular mechanisms regulated by ERK1/2 vary depending on the type of myelinating glia. We demonstrate that *Erk1/2* deletion strongly reduces oligodendrocyte progenitor proliferation. It is well established that oligodendrocyte progenitor proliferation is regulated by PDGF *in vivo* (Calver et al., 1998). PDGF acts through the RTK, PDGF-Rα and we hypothesize that in oligodendrocytes, PDGF effects on proliferation require ERK1/2 signaling. Interestingly, some oligodendrocytes were generated and indeed initiated the expression of MBP earlier than controls and expressed markers of mature oligodendrocytes. The premature differentiation observed in *Erk1/2* deleted oligodendrocytes is consistent with data showing that oligodendrocyte differentiation requires a down-regulation of PDGF-Rα, and a down-regulation of ERK1/2 activity (Chew et al., 2010; Dugas et al., 2010). Thus, in contrast to Schwann cells, *Erk1/2* deleted oligodendrocytes are able to myelinate axons. The conditional ablation of the ERK1/2 upstream regulator, *B-Raf*, has been reported to result in a strong reduction in the number of myelinated fibers in the postnatal brain (Galabova-Kovacs et al., 2008). However, ERK1/2 activation was not completely abolished in these mice due to compensation by other Raf family members and the connection between oligodendrocyte precursor proliferation and the number of myelinated fibers was not clearly drawn.

ERK/MAPK functions are linked to specific RTK systems

Evidence from numerous *in vitro* systems has suggested that ERK1/2 is activated or functionally required in response to a very wide range of extracellular cues, including netrins, semaphorins, GPCR's, trophic factors, hormones, and ECM molecules (Raman et al., 2007). Thus, we might have predicted that early *Erk1/2* deletion in the developing nervous system would lead to drastic phenotypes that reflect the inhibition of nearly all receptor systems. Instead, our findings suggest ERK1/2 mediates effects of specific RTKs *in vivo*, including invasion of cutaneous target fields (NGF/TrkA), Schwann cell development (Neuregulin/ErbB), and oligodendrocyte proliferation (PDGF/PDGF-Rα). Although this initial analysis is of necessity superficial and many subtle roles for ERK1/2 signaling *in vivo* will certainly be uncovered, our results do suggest caution in interpreting *in vitro* results. At the very least, our work suggests that the ability to induce transient ERK1/2 activation or block cellular functions pharmacologically in a strongly reduced *in vitro* system is not a strong predictor of the biological requirement for ERK/MAPK signaling *in vivo*.

Implications for human neurodevelopmental syndromes

Aberrant ERK1/2 signaling plays a primary role in a range of human syndromes that affect the nervous system, particularly the family of "neuro-cardio-facial-cutaneous" (NCFC) syndromes (Bentires-Alj et al., 2006; Samuels et al., 2009). We have previously demonstrated that ERK1/2 signaling is a critical regulator of neural crest contributions to craniofacial and great vessel development helping to explain the pathogenesis of these disorders (Newbern et al., 2008). The NCFC spectrum includes neurofibromatosis (NF), which exhibits an increased propensity to peripheral nerve tumors of Schwann cell precursor origin. Importantly, NF type 1 is caused by mutations in the Ras-GAP, neurofibromin, which leads to increased signaling through the ERK1/2 pathway. Our data demonstrating a requirement for ERK/MAPK signaling at multiple stages of Schwann cell development are consistent with abnormalities in the intricate balance between differentiation and proliferation that leads to tumors in these patients.

Experimental Procedures

Mutant mice

Animal experiments were performed in accordance with protocols approved by IACUC at UNCCH. *Erk1*$^{-/-}$ mice possess a neo insertion in exons 1 through 6 (Nekrasova et al., 2005). *Erk2*$^{fl/fl}$ mice contain loxp sites around exon 3 (Samuels et al., 2008). *Mek1*$^{fl/fl}$ mice possess a loxp flanked exon 3 while *Mek2*$^{-/-}$ mice contain a neo insertion in exons 4-6 and were kindly provided by Dr. J. Charron (Belanger et al., 2003; Bissonauth et al., 2006). The *Wnt1:Cre* and *Nestin:Cre* mice were purchased from Jackson Laboratories. The *Dhh:Cre* mouse was generously provided by Dr. D. Meijer and the *Olig2:Cre* mouse by Dr. T. Jessell. For the *Erk5* floxed allele, a targeting vector was generated based on an *Erk5* fragment derived from a 129sv genomic BAC library and incorporated loxp sites around Exon 3 (Figure S1K-L). All mice were of mixed genetic background. For BrdU injections, timed pregnant dams were injected at E14.5 with 75 mg/kg BrdU and sacrificed 4 hours later. For timed breedings, the appearance of a vaginal plug was considered to be E0.5 on the day of detection. All experiments were replicated at least three times with mice derived from independent litters. *Cre* expressing littermates were utilized as control embryos for most experiments. Further detail regarding the *Erk5* floxed mouse and genotyping is listed in Supplemental Experimental Procedures.

Western Blotting

Western blotting was performed according to a standard protocol outlined in Supplemental Experimental Procedures. The primary antibodies used were anti-phospho or pan ERK1/2, anti-ERK5, anti-phospho-RSK3, anti-cleaved Caspase-3, anti-MEK1/2, and anti-GAPDH (all from Cell Signaling Technologies).

Immunohistochemistry

Paraformaldehyde fixed, cryopreserved specimens were immunostained according to standard procedures and imaged with a Zeiss LSM 510 NLO confocal microscope. Further detail regarding immunohistochemistry, primary antibodies, whole mount staining, and counting methods are described in Supplemental Experimental Procedures.

Electron Microscopy

Mice were anesthetized and perfused with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer. A 2-3 mm section of the sciatic nerve, proximal to the division into the cutaneous and tibial nerve, was dissected, rinsed, and postfixed overnight at 4°C and prepared for EM as described in Supplemental Experimental Procedures.

Tissue Culture

Embryonic DRG's were cultured as previously described with minor modifications (Markus et al., 2002) as described in Supplemental Experimental Procedures.

RNA Extraction and Analysis

E12.5 DRGs from control and *Erk1/2*$^{CKO(Wnt1)}$ embryos derived from three independent litters were dissected in PBS supplemented with 10% RNAlater (Qiagen) and frozen on dry ice. Total RNA was extracted with Trizol (Invitrogen) and a Qiagen RNeasy Mini kit per manufacturer's instructions. RNA samples for were assayed for quality and quantity with an Agilent 2100 Bioanalyzer and a Nanodrop spectrophotometer. Total RNA was amplified, labeled, and hybridized on Illumina arrays (MouseRef-8 V2 Expression BeadChip, Illumina). Slides were processed and scanned in an Illumina BeadStation platform according to the manufacturer protocol. Data was further processed using quantile normalization.

Log_2ratios and p values are calculated in R from a Bayesian moderated t-test using the LIMMA package. Regulated transcripts were defined by a greater than 1.5 fold change and a p-value less than 0.05. Functional annotation of differentially expressed genes was obtained through the use of The Ingenuity Pathways Knowledge Base (Ingenuity Systems, http://www.ingenuity.com), The Database for Annotation, Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov), the Gene Ontology Project (http://www.geneontology.org), and extensive literature review. Further detail regarding qPCR is listed in Supplemental Experimental Procedures.

Highlights

1. *Erk1/2* is dispensable for much of embryonic sensory and motor neuron development.
2. *Erk5* is largely dispensable for PNS neuronal and glial development.
3. *Erk1/2* mediates neuregulin-1 effects on Schwann cell development.
4. *Erk1/2* is required for myelination by Schwann cells, but not oligodendrocytes

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are extremely grateful to D. Meijer, T. Jessell, and J. Charron for providing transgenic mice; Ben Novitch, Barbara Han, and Monica Mendelsohn for the generation of the *Olig2:Cre* line in the laboratory of T. Jessell; T. Müller and C. Birchmeier for the generous gift of the BFABP antibody and helpful advice; E. Anton and J. Weimer for kindly providing antibodies and guidance; Dr. Louis Reichardt for kindly providing the TrkA antibody; and A. McKell and L. Goins for assistance with mouse breeding and genotyping. This work is supported by NIH grant NS031768 to WDS; NSF grant IBN97–23147 to GEL; and a NRSA award F32NS061591 to JMN. Generation of mice and imaging were supported by Cores 3 and 5 respectively of NINDS Center Grant P30 NS04892.

References

Atanasoski S, Scherer SS, Sirkowski E, Leone D, Garratt AN, Birchmeier C, Suter U. ErbB2 signaling in Schwann cells is mostly dispensable for maintenance of myelinated peripheral nerves and proliferation of adult Schwann cells after injury. J Neurosci. 2006; 26:2124–2131. [PubMed: 16481445]

Atwal JK, Massie B, Miller FD, Kaplan DR. The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase. Neuron. 2000; 27:265–277. [PubMed: 10985347]

Baloh RH, Strickland A, Ryu E, Le N, Fahrner T, Yang M, Nagarajan R, Milbrandt J. Congenital hypomyelinating neuropathy with lethal conduction failure in mice carrying the Egr2 I268N mutation. J Neurosci. 2009; 29:2312–2321. [PubMed: 19244508]

Belanger L-F, Roy S, Tremblay M, Brott B, Steff A-M, Mourad W, Hugo P, Erikson R, Charron J. Mek2 Is Dispensable for Mouse Growth and Development. Mol Cell Biol. 2003; 23:4778–4787. [PubMed: 12832465]

Bentires-Alj M, Kontaridis MI, Neel BG. Stops along the RAS pathway in human genetic disease. Nat Med. 2006; 12:283–285. [PubMed: 16520774]

Birchmeier C, Nave KA. Neuregulin-1, a key axonal signal that drives Schwann cell growth and differentiation. Glia. 2008; 56:1491–1497. [PubMed: 18803318]

Bissonauth V, Roy S, Gravel M, Guillemette S, Charron J. Requirement for Map2k1 (Mek1) in extra-embryonic ectoderm during placentogenesis. Development. 2006; 133:3429–3440. [PubMed: 16887817]

Britsch S, Goerich DE, Riethmacher D, Peirano RI, Rossner M, Nave KA, Birchmeier C, Wegner M. The transcription factor Sox10 is a key regulator of peripheral glial development. Genes Dev. 2001; 15:66–78. [PubMed: 11156606]

Bron R, Vermeren M, Kokot N, Andrews W, Little GE, Mitchell KJ, Cohen J. Boundary cap cells constrain spinal motor neuron somal migration at motor exit points by a semaphorin-plexin mechanism. Neural Dev. 2007; 2:21. [PubMed: 17971221]

Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD. Oligodendrocyte population dynamics and the role of PDGF in vivo. Neuron. 1998; 20:869–882. [PubMed: 9620692]

Chew L-J, Coley W, Cheng Y, Gallo V. Mechanisms of Regulation of Oligodendrocyte Development by p38 Mitogen-Activated Protein Kinase. J Neurosci. 2010; 30:11011–11027. [PubMed: 20720108]

Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP. Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. Curr Biol. 1998; 8:1323–1326. [PubMed: 9843687]

Dessaud E, Yang LL, Hill K, Cox B, Ulloa F, Ribeiro A, Mynett A, Novitch BG, Briscoe J. Interpretation of the sonic hedgehog morphogen gradient by a temporal adaptation mechanism. Nature. 2007; 450:717–720. [PubMed: 18046410]

Dong Z, Sinanan A, Parkinson D, Parmantier E, Mirsky R, Jessen KR. Schwann cell development in embryonic mouse nerves. J Neurosci Res. 1999; 56:334–348. [PubMed: 10340742]

Dugas JC, Cuellar TL, Scholze A, Ason B, Ibrahim A, Emery B, Zamanian JL, Foo LC, McManus MT, Barres BA. Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. Neuron. 2010; 65:597–611. [PubMed: 20223197]

Eng SR, Gratwick K, Rhee JM, Fedtsova N, Gan L, Turner EE. Defects in sensory axon growth precede neuronal death in Brn3a-deficient mice. J Neurosci. 2001; 21:541–549. [PubMed: 11160433]

Ferrell JE Jr. Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. Trends Biochem Sci. 1996; 21:460–466. [PubMed: 9009826]

Finegan KG, Wang X, Lee EJ, Robinson AC, Tournier C. Regulation of neuronal survival by the extracellular signal-regulated protein kinase 5. Cell Death Differ. 2009; 16:674–683. [PubMed: 19148185]

Galabova-Kovacs G, Catalanotti F, Matzen D, Reyes GX, Zezula J, Herbst R, Silva A, Walter I, Baccarini M. Essential role of B-Raf in oligodendrocyte maturation and myelination during postnatal central nervous system development. J Cell Biol. 2008; 180:947–955. [PubMed: 18332218]

Garratt AN, Voiculescu O, Topilko P, Charnay P, Birchmeier C. A dual role of erbB2 in myelination and in expansion of the schwann cell precursor pool. J Cell Biol. 2000; 148:1035–1046. [PubMed: 10704452]

Grossmann KS, Wende H, Paul FE, Cheret C, Garratt AN, Zurborg S, Feinberg K, Besser D, Schulz H, Peles E, et al. The tyrosine phosphatase Shp2 (PTPN11) directs Neuregulin-1/ErbB signaling throughout Schwann cell development. Proc Natl Acad Sci U S A. 2009; 106:16704–16709. [PubMed: 19805360]

Harrisingh MC, Perez-Nadales E, Parkinson DB, Malcolm DS, Mudge AW, Lloyd AC. The Ras/Raf/ERK signalling pathway drives Schwann cell dedifferentiation. Embo J. 2004; 23:3061–3071. [PubMed: 15241478]

Hasegawa H, Abbott S, Han BX, Qi Y, Wang F. Analyzing somatosensory axon projections with the sensory neuron-specific Advillin gene. J Neurosci. 2007; 27:14404–14414. [PubMed: 18160648]

Hippenmeyer S, Vrieseling E, Sigrist M, Portmann T, Laengle C, Ladle DR, Arber S. A developmental switch in the response of DRG neurons to ETS transcription factor signaling. PLoS Biol. 2005; 3:e159. [PubMed: 15836427]

Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, Yan R. Bace1 modulates myelination in the central and peripheral nervous system. Nat Neurosci. 2006; 9:1520–1525. [PubMed: 17099708]

Jaegle M, Ghazvini M, Mandemakers W, Piirsoo M, Driegen S, Levavasseur F, Raghoenath S, Grosveld F, Meijer D. The POU proteins Brn-2 and Oct-6 share important functions in Schwann cell development. Genes Dev. 2003; 17:1380–1391. [PubMed: 12782656]

Jessen KR, Mirsky R. The origin and development of glial cells in peripheral nerves. Nat Rev Neurosci. 2005; 6:671–682. [PubMed: 16136171]

Kao SC, Wu H, Xie J, Chang CP, Ranish JA, Graef IA, Crabtree GR. Calcineurin/NFAT signaling is required for neuregulin-regulated Schwann cell differentiation. Science. 2009; 323:651–654. [PubMed: 19179536]

Klesse LJ, Meyers KA, Marshall CJ, Parada LF. Nerve growth factor induces survival and differentiation through two distinct signaling cascades in PC12 cells. Oncogene. 1999; 18:2055–2068. [PubMed: 10321730]

Kolkova K, Novitskaya V, Pedersen N, Berezin V, Bock E. Neural Cell Adhesion Molecule-Stimulated Neurite Outgrowth Depends on Activation of Protein Kinase C and the Ras-Mitogen-Activated Protein Kinase Pathway. J Neurosci. 2000; 20:2238–2246. [PubMed: 10704499]

Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010; 141:1117–1134. [PubMed: 20602996]

Li Y, Tennekoon GI, Birnbaum M, Marchionni MA, Rutkowski JL. Neuregulin signaling through a PI3K/Akt/Bad pathway in Schwann cell survival. Mol Cell Neurosci. 2001; 17:761–767. [PubMed: 11312610]

Marin-Husstege M, He Y, Li J, Kondo T, Sablitzky F, Casaccia-Bonnefil P. Multiple roles of Id4 in developmental myelination: predicted outcomes and unexpected findings. Glia. 2006; 54:285–296. [PubMed: 16862533]

Markus A, Zhong J, Snider WD. Raf and akt mediate distinct aspects of sensory axon growth. Neuron. 2002; 35:65–76. [PubMed: 12123609]

Marmigere F, Ernfors P. Specification and connectivity of neuronal subtypes in the sensory lineage. Nat Rev Neurosci. 2007; 8:114–127. [PubMed: 17237804]

Maro GS, Vermeren M, Voiculescu O, Melton L, Cohen J, Charnay P, Topilko P. Neural crest boundary cap cells constitute a source of neuronal and glial cells of the PNS. Nat Neurosci. 2004; 7:930–938. [PubMed: 15322547]

Maurel P, Salzer JL. Axonal regulation of Schwann cell proliferation and survival and the initial events of myelination requires PI 3-kinase activity. J Neurosci. 2000; 20:4635–4645. [PubMed: 10844033]

Meyer D, Birchmeier C. Multiple essential functions of neuregulin in development. Nature. 1995; 378:386–390. [PubMed: 7477375]

Morris JK, Lin W, Hauser C, Marchuk Y, Getman D, Lee KF. Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development. Neuron. 1999; 23:273–283. [PubMed: 10399934]

Murphy P, Topilko P, Schneider-Maunoury S, Seitanidou T, Baron-Van Evercooren A, Charnay P. The regulation of Krox-20 expression reveals important steps in the control of peripheral glial cell development. Development. 1996; 122:2847–2857. [PubMed: 8787758]

Nekrasova T, Shive C, Gao Y, Kawamura K, Guardia R, Landreth G, Forsthuber TG. ERK1-Deficient Mice Show Normal T Cell Effector Function and Are Highly Susceptible to Experimental Autoimmune Encephalomyelitis. J Immunol. 2005; 175:2374–2380. [PubMed: 16081808]

Newbern J, Zhong J, Wickramasinghe RS, Li X, Wu Y, Samuels I, Cherosky N, Karlo JC, O'Loughlin B, Wikenheiser J, et al. Mouse and human phenotypes indicate a critical conserved role for ERK2 signaling in neural crest development. Proc Natl Acad Sci U S A. 2008; 105:17115–17120. [PubMed: 18952847]

Nishimoto S, Nishida E. MAPK signalling: ERK5 versus ERK1/2. EMBO Rep. 2006; 7:782–786. [PubMed: 16880823]

Novitch BG, Chen AI, Jessell TM. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron. 2001; 31:773–789. [PubMed: 11567616]

Ogata T, Iijima S, Hoshikawa S, Miura T, Yamamoto S, Oda H, Nakamura K, Tanaka S. Opposing extracellular signal-regulated kinase and Akt pathways control Schwann cell myelination. J Neurosci. 2004; 24:6724–6732. [PubMed: 15282275]

Parkinson DB, Langner K, Namini SS, Jessen KR, Mirsky R. beta-Neuregulin and autocrine mediated survival of Schwann cells requires activity of Ets family transcription factors. Mol Cell Neurosci. 2002; 20:154–167. [PubMed: 12056846]

Patel TD, Jackman A, Rice FL, Kucera J, Snider WD. Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. Neuron. 2000; 25:345–357. [PubMed: 10719890]

Perron JC, Bixby JL. Distinct neurite outgrowth signaling pathways converge on ERK activation. Mol Cell Neurosci. 1999; 13:362–378. [PubMed: 10356298]

Raman M, Chen W, Cobb MH. Differential regulation and properties of MAPKs. Oncogene. 2007; 26:3100–3112. [PubMed: 17496909]

Riethmacher D, Sonnenberg-Riethmacher E, Brinkmann V, Yamaai T, Lewin GR, Birchmeier C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. Nature. 1997; 389:725–730. [PubMed: 9338783]

Samuels IS, Karlo JC, Faruzzi AN, Pickering K, Herrup K, Sweatt JD, Saitta SC, Landreth GE. Deletion of ERK2 mitogen-activated protein kinase identifies its key roles in cortical neurogenesis and cognitive function. J Neurosci. 2008; 28:6983–6995. [PubMed: 18596172]

Samuels IS, Saitta SC, Landreth GE. MAP'ing CNS development and cognition: an ERKsome process. Neuron. 2009; 61:160–167. [PubMed: 19186160]

Soundararajan P, Fawcett JP, Rafuse VF. Guidance of postural motoneurons requires MAPK/ERK signaling downstream of fibroblast growth factor receptor 1. J Neurosci. 2010; 30:6595–6606. [PubMed: 20463222]

Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi AB, Seitanidou T, Babinet C, Charnay P. Krox-20 controls myelination in the peripheral nervous system. Nature. 1994; 371:796–799. [PubMed: 7935840]

Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R, Schutz G. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. Nat Genet. 1999; 23:99–103. [PubMed: 10471508]

Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR. Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. Nat Genet. 1998; 18:382–384. [PubMed: 9537424]

Watson FL, Heerssen HM, Bhattacharyya A, Klesse L, Lin MZ, Segal RA. Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. Nat Neurosci. 2001; 4:981–988. [PubMed: 11544482]

Wickramasinghe SR, Alvania RS, Ramanan N, Wood JN, Mandai K, Ginty DD. Serum response factor mediates NGF-dependent target innervation by embryonic DRG sensory neurons. Neuron. 2008; 58:532–545. [PubMed: 18498735]

Woldeyesus MT, Britsch S, Riethmacher D, Xu L, Sonnenberg-Riethmacher E, Abou-Rebyeh F, Harvey R, Caroni P, Birchmeier C. Peripheral nervous system defects in erbB2 mutants following genetic rescue of heart development. Genes Dev. 1999; 13:2538–2548. [PubMed: 10521398]

Yoon S, Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors. 2006; 24:21–44. [PubMed: 16393692]

Zhong J, Li X, McNamee C, Chen AP, Baccarini M, Snider WD. Raf kinase signaling functions in sensory neuron differentiation and axon growth in vivo. Nat Neurosci. 2007; 10:598–607. [PubMed: 17396120]

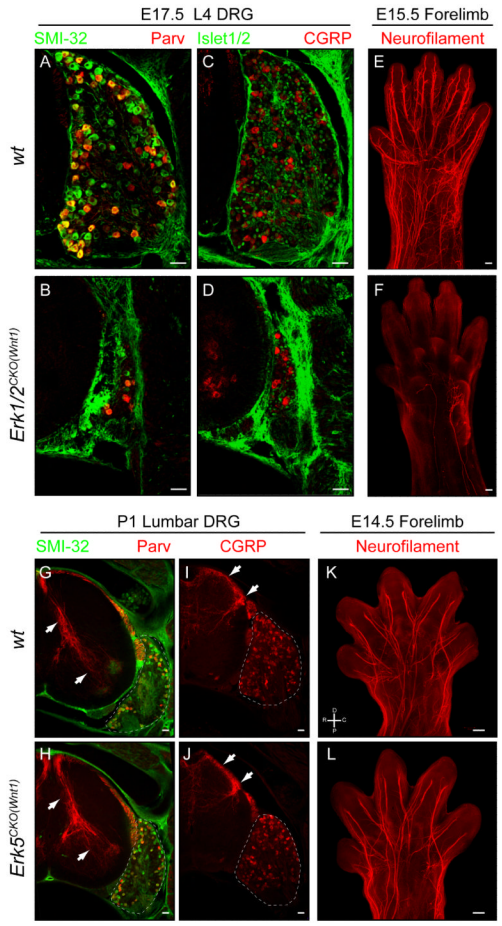


Figure 1. Deletion of *Erk1/2*, but not *Erk5*, in the PNS results in DRG neuron loss and the absence of all peripheral projections

A-D. Compared to wild-type (A,C), E17.5 *Erk1/2*$^{CKO(Wnt1)}$ (B,D) L4 DRG cross sections revealed a massive decrease in DRG size as indicated by the gross morphology and decreased number of cells expressing neuronal markers. (Scale bar=50μM)

E-F. Whole mount neurofilament labeling of E15.5 control (E) and mutant (F) embryos revealed only sparse labeling in *Erk1/2*$^{CKO(Wnt1)}$ forelimbs, demonstrating that both motor and sensory projections are absent. (Scale bar=100μM)

G-J. Analysis of the expression of Parvalbumin and CGRP in P1 control (G,I) and *Erk5*$^{CKO(Wnt1)}$ (H,J) lumbar DRG cross sections did not show any gross change in DRG sensory neurons or in central projections within the spinal cord (arrows). (Scale bar=50μM)

K-L. Whole mount neurofilament immunolabeling of E14.5 control (K) and *Erk5*$^{CKO(Wnt1)}$ (L) forelimbs showed no differences in peripheral axon growth. (Scale bar = 100 μm) (See also Figure S1)

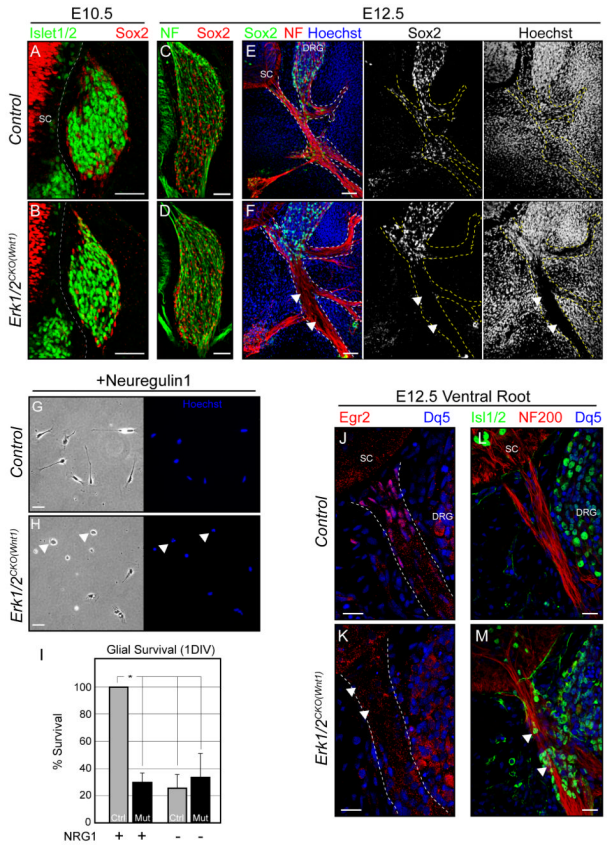


Figure 2. ERK1/2 is required for the development of SCPs *in vivo* and mediates neuregulin-1 signaling *in vitro*

A-D. The expression of neuronal and glial markers is grossly normal within control (A,C) and *Erk1/2*$^{CKO(Wnt1)}$ (B,D) brachial DRGs at E10.5 and E12.5.

E-F. Sox2 positive glial progenitors entering the neurofilament stained peripheral nerve are readily detectable in cross sections of the brachial region of E12.5 wildtype embryos (E). However, E12.5 *Erk1/2*$^{CKO(Wnt1)}$ embryos exhibit a near complete absence of glial progenitors in the peripheral nerve (F, arrowheads). (Scale bar=50μM)

G-I. Glial progenitors cultured from E11.5 control DRGs (G,I) can be sustained by Type III neuregulin-1 *in vitro*. The effect of neuregulin-1 on glial progenitor survival is essentially absent in *Erk1/2*$^{CKO(Wnt1)}$ (H, I) cultures after 1 DIV (mean ± SEM, *=p<0.001). DNA staining revealed a large number of pyknotic, presumably dying glial progenitors in mutant cultures at this time point (arrowheads).

J-M. Egr2/Krox-20 immunolabeling labels the BC in the proximal segments of control (J) E12.5 peripheral nerves. Egr2/Krox-20 expressing cells are nearly absent in *Erk1/2*$^{CKO(Wnt1)}$ (K) ventral roots (arrowheads). A number of Islet1/2 positive neurons are present within the ventral root and proximal peripheral nerve of E12.5 *Erk1/2*$^{CKO(Wnt1)}$ (M) embryos (arrowheads). (See also Figure S2)

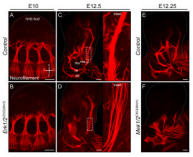


Figure 3. Axons extend normally following *Erk1/2* or *Mek1/2* inactivation in the PNS but are defasciculated

A-D. Whole mount neurofilament immunolabeling was utilized to examine peripheral nerve outgrowth in the forelimb. Compared to littermate controls (A,C), *Erk1/2*$^{CKO(Wnt1)}$ (B,D) embryos demonstrated a normal extent of axon outgrowth up to E12.5, however, the mutant nerves were defasciculated (inset in D).

E-F. Similar results were obtained in E12.25 *Mek1/2*$^{CKO(Wnt1)}$ (F) embryos. (Scale bar= 100μM, ax=axillary n., ra=radial n., mu=musculocutaneous n., me=median n., ul=ulnar n.) (See also Figure S3)

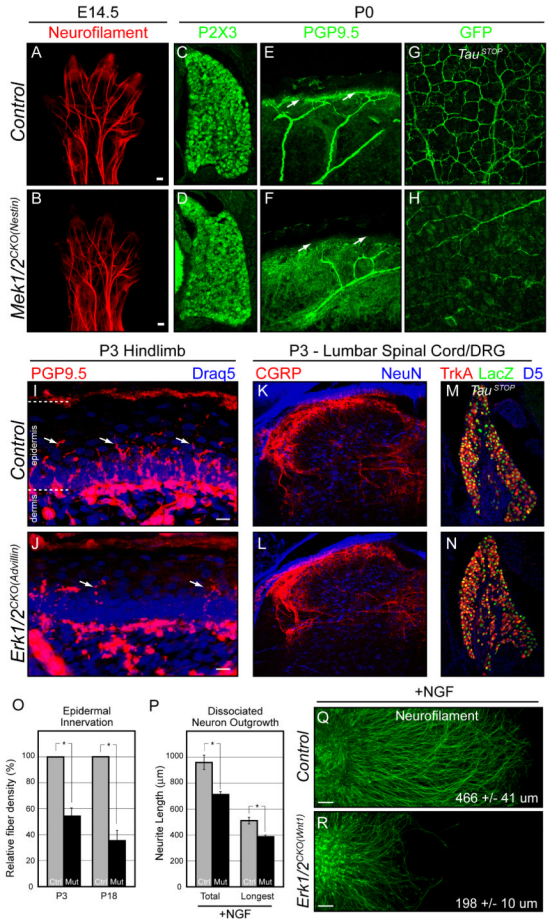


Figure 4. Neuron specific deletion of *Erk1/2* or *Mek1/2* does not alter initial axon extension, but disrupts NGF dependent cutaneous innervation

A-B. Compared to littermate controls (A), E14.5 *Mek1/2*$^{CKO(Nestin)}$ (B) forelimbs exhibit normal growth of major peripheral nerve trunks as detected by whole mount neurofilament immunolabeling.

C-D. P0 DRGs in *Mek1/2*$^{CKO(Nestin)}$ (D) mice appear morphologically similar to controls (C) and exhibit relatively normal expression of the nociceptive marker P2×3.

E-H. Compared to controls (E,G), cutaneous innervation is reduced in P0 *Mek1/2*$^{CKO(Nestin)}$ (F,H) mice. PGP9.5 labeling of cross sections from P0 forepaws show a reduction in cutaneous afferents in the epidermis of mutant (F) mice. Whole mount GFP immunolabeling of skin derived from the trunk of P0 *Tau*^{STOP} controls (G) in flat mount readily labels the subepidermal plexus. *Mek1/2*$^{CKO(Nestin)}$ (H) mice exhibit significant reductions in innervation.

I-O. Relative to controls (I), a significant and persistent reduction in the number of PGP9.5 labeled epidermal afferents was detected in the hindlimb of P3 *Erk1/2*$^{CKO(Advillin)}$ (J) mice, which is quantitated in O (mean ± SEM, *=p<0.001). However, CGRP labeled central afferents in the dorsal spinal cord (K-L) and the number of sensory neurons in the DRG were not significantly altered in the P3 *Erk1/2*$^{CKO(Advillin)}$ dorsal spinal cord (M-N).

P-R. DRG neurons from E11.5 control and *Erk1/2*$^{CKO(Wnt1)}$ embryos were either dissociated (P) or explanted (Q-R), cultured in the presence of NGF, and stained with neurofilament after 2DIV. In dissociated cultures, the length of the total and longest neurites demonstrated a significant decrease in both parameters in *Erk1/2*$^{CKO(Wnt1)}$ neurons (P)

(mean ± SEM, n = >100 neurons in each condition from three independent cultures, *=p<0.001). *Erk1/2*$^{CKO(Wnt1)}$ explants exhibited a significant reduction ($p < 0.001$) in the extent of NGF induced neuronal outgrowth. The average hemidiameter length ± SEM of >20 explants from three independent cultures is reported. (See also Figure S4)

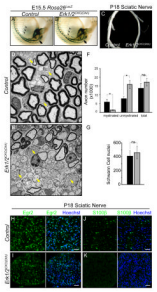


Figure 5. Schwann cell specific *Erk1/2* inactivation results in hypomyelination

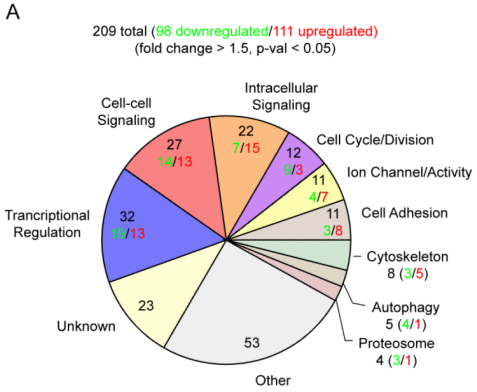
A-B. Whole mount LacZ staining of the trunk and hindlimb of E15.5 control (A) and *Erk1/2*$^{CKO(Dhh)}$ (B) mice crossed with *Rosa26*^{LacZ} show a similar pattern of staining in the peripheral nerve, including the intercostal (arrowheads) and sciatic (arrows) nerve.

C. Upon gross dissection, the sciatic nerves of P18 *Erk1/2*$^{CKO(Dhh)}$ mice were noticeably smaller in diameter and more transparent than control nerves.

D-G. EM analysis of P18 sciatic nerve cross sections from control (D) and *Erk1/2*$^{CKO(Dhh)}$ (E) mice show a dramatic reduction in the number of myelinated axons and an increase in unmyelinated axons (yellow arrows) in mutant mice. The total number of axons and Schwann cell nuclei in the nerve was not changed (F,G). (mean ± SEM, *=p<0.01)

H-K. Immunolabeling for markers of myelinating Schwann cell differentiation, Egr2/Krox-20 (H-I) and S100β (J-K), was performed in P18 control and *Erk1/2*$^{CKO(Dhh)}$ sciatic nerve cross-sections. A decrease in the number of Egr2/Krox-20 positive cells and a massive reduction in S100β labeling were found in mutant nerves. Note the decreased nerve caliber in mutants. Since Schwann cell number is not altered, the density of nuclei is increased. (See also Figure S5)

A



B

Candidate Glial Development Genes Downregulated in Erk1/2$^{CKO(Wnt1)}$ DRGs.

Transcript Name		Fold Change	p-value
Egr2	early growth response 2 (Krox-20)	**-2.23**	$2.5x10^{-8}$
Idb4	inhibitor of DNA binding 4	**-1.92**	$1.2x10^{-2}$
Etv1	ets variant gene 1 (Er81)	**-1.67**	$1.3x10^{-5}$
MBP	myelin basic protein	**2.30**	$8.3x10^{-4}$

C

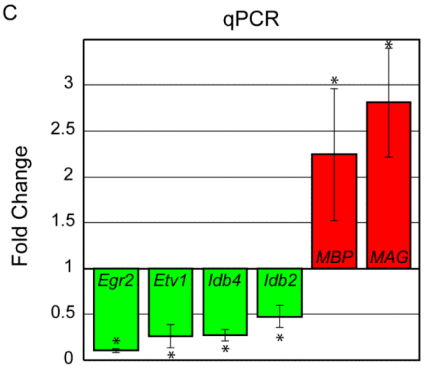


Figure 6. ERK1/2 regulates the expression of genes important for Schwann cell progenitor development and myelination

A. 209 genes met the criteria for differential expression in RNA samples derived from E12.5 *Erk1/2$^{CKO(Wnt1)}$* DRGs. The pie chart summarizes the major functional categories represented by the differentially expressed genes and the number downregulated (green) and upregulated (red) within each category.

B. Candidate genes with known roles in glial development are differentially expressed in the DRG of *Erk1/2$^{CKO(Wnt1)}$*embryos.

C. qPCR analysis of E12.5 *Erk1/2$^{CKO(Wnt1)}$* DRG samples validated the decrease in the expression of transcription factors thought to regulate glial development. Interestingly, the myelin components, *MBP* and *MAG*, show increased expression in mutant DRGs. (n = 4, * = p-value <0.05) (See also Figure S6)

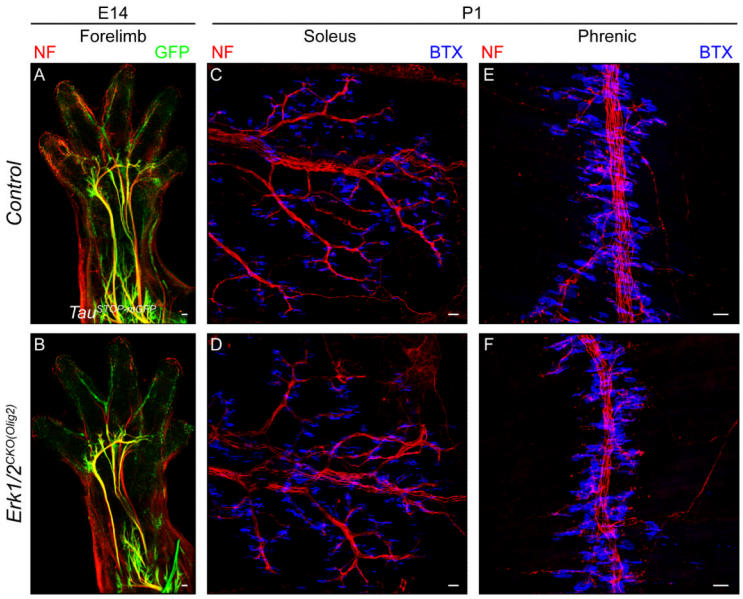


Figure 7. Spinal motor neuron peripheral innervation is not regulated by ERK1/2 signaling

A-B. Whole mount immunostaining of E14.5 *Tau*^{STOP} forelimbs from control (A) and *Erk1/2*$^{CKO(Olig2)}$ (B) embryos did not reveal any difference in the initial growth of motor neuron peripheral projections.

C-F. The pattern of motor innervation of NMJs in the P1 soleus (C-D) and diaphragm (E-F) was not altered in *Erk1/2*$^{CKO(Olig2)}$ (D,F) embryos relative to controls (C,E). (See also Figure S7)

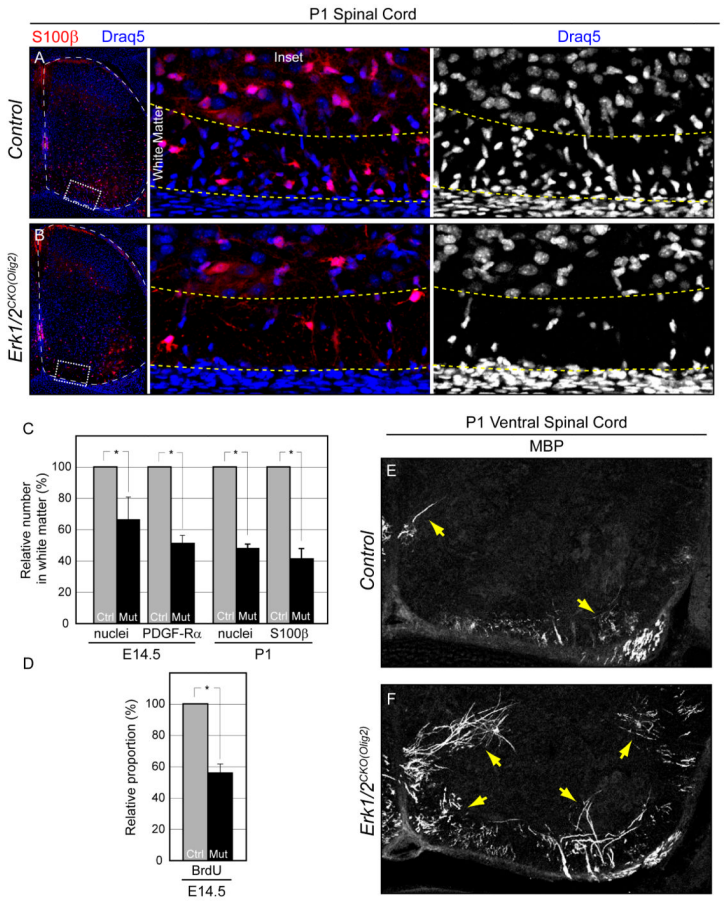


Figure 8. ERK1/2 signaling is necessary for oligodendrocyte precursor proliferation, but is not required for myelination

A-B. Relative to controls (A) the number of oligodendrocytes in the spinal cord ventral white matter of P1 *Erk1/2*$^{CKO(Olig2)}$ (B) embryos is strongly reduced.

C-D. Quantification of the relative decrease in the number of nuclei and labeled oligodendrocytes in E14.5 and P1 *Erk1/2*$^{CKO(Olig2)}$ white matter (C). The proportion of BrdU labeled oligodendrocytes is significantly decreased in *Erk1/2*$^{CKO(Olig2)}$ E14.5 embryos (D). (mean ± SEM, *=p<0.01)

E-F. At P1, MBP immunolabeling indicates that myelination has just initiated in a small proportion of oligodendrocytes in the ventral spinal cord of control mice (E) (arrows). In *Erk1/2*$^{CKO(Olig2)}$ (F) littermates, there is a significant increase in the number of oligodendrocytes that express MBP (E) (arrows). (See also Figure S8)