Touch receptor end-organ innervation and function requires sensory expression of the transcription factor Meis2.

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Short title: Meis2 in DRG sensory neurons

Abstract:

Touch sensation is primarily encoded by mechanoreceptors, sometimes called Low-Threshold Mechanoreceptors (LTMRs), with their cell bodies in the Dorsal Root Ganglia (DRG). LTMRs make up no more that 20% of all sensory neurons and exhibit great diversity in terms of molecular signature, terminal ending morphology and electrophysiological properties, mirroring the complexity of tactile experience. LTMRs are an interesting model to study the molecular cues controlling neuronal diversification in terms of both molecular specification and target-field innervation. The morphological specialization of the sensory end-organ of LTMRs exhibits striking diversity between different mechanoreceptor types and whether it occurs in the glabrous or hairy skin. Much has been learnt about transcriptional codes that define different LTMR subtypes, but the identification of molecular players that participate in their late maturation has not been extensively addressed. Here we identified the TALE homeodomain transcription factor Meis2 as a key regulator of LTMR target-field innervation. *Meis2* is specifically expressed in cutaneous LTMRs and its expression depends on target-derived signals. *Meis2* gene inactivation in mouse sensory neurons precursors or early post-mitotic neurons allows normal survival and specification of LTMRs. However, LTMRs lacking *Meis2* show pronounced defects in end-organ innervation which was accompanied by severely impaired receptor properties and behavioral responses. These results establish Meis2 as a major transcriptional regulator controlling the orderly formation of peripheral end-organs required for touch.

Key words:

Dorsal root ganglion, Meis2, sensory neurons, ASD, transcription factor, TALE homeodomain.

Introduction

Tactile stimuli like brush, light pressure, or roughness engage highly specialized, but also diverse arrays of mechanoreceptors in the hairy and glabrous skin (Delmas, Hao and Rodat-Despoix, 2011; Li *et al.*, 2011; Abraira and Ginty, 2013; Lechner and Lewin, 2013; Zimmerman, Bai and Ginty, 2014; Handler and Ginty, 2021; Wu *et al.*, 2021). Sensory neurons within the DRG can be broadly classified as nociceptors, mechanoreceptors or proprioceptors and each group is characterized by the expression of specific combination of genes, have distinctive physiological properties and projections within the spinal cord and periphery (Marmigère and Ernfors, 2007; Lallemend and Ernfors, 2012; Vermeiren, Bellefroid and Desiderio, 2020). Recent advances in combining single cell transcriptomic and genetic tracing have tremendously extended the classical subtypes repertoire (Bai *et al.*, 2015; Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Sharma *et al.*, 2020; Wu *et al.*, 2021).

Cutaneous mechanoreceptors or Low Threshold Mechanoreceptors (LMTRs) exhibit a variety of specialized terminal endings in the hairy and glabrous skin with strikingly unique morphologies (Delmas, Hao and Rodat-Despoix, 2011; Li *et al.*, 2011; Abraira and Ginty, 2013; Lechner and Lewin, 2013; Zimmerman, Bai and Ginty, 2014; Sharma *et al.*, 2020; Handler and Ginty, 2021; Schwaller *et al.*, 2021). LTMRs projecting to the glabrous skin innervate Merkel cell complexes or Meissner corpuscles at the dermal-epidermal border. LTMRs innervating Merkel cells in the glabrous or hairy skin have large thickly myelinated axons (Aβ-fibers) and are characterized as slowly-adapting mechanoreceptors responding to skin movement and static displacement (also referred to as Aβ-SAIs). Meissner corpuscles are mechanoreceptors which are only sensitive to skin movement or vibration (rapidly-adapting mechanoreceptors) and are referred to as Aβ-RAs. LTMRs innervating hair follicles in the hairy skin can form lanceolate endings or circumferential endings. Virtually all mechanoreceptors innervating hairs show rapidly-adapting properties and respond only to hair movement and not static displacement (Lechner and Lewin, 2013). LTMRs with large myelinated axons innervating hairy skin are characterized as Aβ-RAs, and a specialized population of slowly conducting myelinated fibers called D-hair mechanoreceptors (or Aδ-RAs) also form lanceolate endings on small hairs. D-hair mechanoreceptors are most sensitive to low velocity stroking have large receptive fields and are directionally tuned(Li *et al.*, 2011; Walcher *et al.*, 2018). A small number of LTMRs in the hairy skin are not activated by hair movement but show properties of rapidly-adapting mechanoreceptors (Lewin and McMahon, 1991). These were originally characterized as so-called field receptors (Burgess and Horch, 1973; Lewin and McMahon, 1991) and were recently shown to form circumferential endings around hair follicles (Bai *et al.*, 2015). LTMRs tuned to high frequency vibration are called Aβ-RAII and innervate Pacinian corpuscles deep in the skin or on the bone (Schwaller *et al.*, 2021). Ruffini endings that are thought to be innervated by

stretch sensitive mechanoreceptors (Aβ-SAII) are poorly characterized in mice (Handler and Ginty, 2021). Whereas genetic tools allow to identify at least 5 LTM neurons populations (Zimmerman, Bai and Ginty, 2014; Bai *et al.*, 2015), a recent update combining these tools with deep RNA sequencing clustered at least 20 different subtypes of LTM neurons (Zheng *et al.*, 2019).

Understanding how sensory neurons diversity is generated from an apparently generic progenitor and cracking the transcriptional codes supporting this diversification has been the object of tremendous efforts in the last decades (Marmigère and Ernfors, 2007; Lallemend and Ernfors, 2012; Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Sharma *et al.*, 2020; Wu *et al.*, 2021). Although the repertoire of transcription factor (TFs) of each subpopulation is largely established, their specific functions remain largely unexplored. The functions of specification factors or terminal selectors (Hobert, 2016; Hobert and Kratsios, 2019), like Maf, Shox2, Runx3, Pea3 and ER81 have been functionally implicated in LTMR segregation (Arber *et al.*, 2000; Marmigère and Ernfors, 2007; Abdo *et al.*, 2011; Scott *et al.*, 2011; Hu *et al.*, 2012; Lallemend and Ernfors, 2012). Less is understood about the transcriptional control of target cell innervation within the skin and the establishment of specialized peripheral end-organ complexes. Interestingly, recent advances in the understanding of Autism Spectrum Disorder (ASD) suggest that centrally affected neurons in ASD and LTMRs share specific transcriptional programs regulating late neuronal differentiation (Orefice *et al.*, 2016, 2019). Genes known to be involved in (ASD) are expressed by LTMRs, and mouse models for ASD exhibit sensory deficits (Orefice *et al.*, 2016, 2019). Furthermore, DRG specific ablation of some of these genes causes ASD-like behaviors. Meis2, is another TF in which mutations are associated with severe ASD (Gangfuß *et al.*, 2021) in humans, and is also specifically expressed in LTMRs (Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Sharma *et al.*, 2020). Thus, because ASD is a highly genetically and phenotypically heterogeneous neurodevelopmental disorder in turn involving neurogenesis, neurite outgrowth, synaptogenesis and synaptic plasticity (McFadden and Minshew, 2013; Gilbert and Man, 2017), Meis2 appears as a pertinent candidate to regulate late sensory neurons differentiation.

Meis2 belongs to a highly conserved homeodomain family of TFs containing three members in mammals, Meis1, Meis2 and Meis3 (Geerts *et al.*, 2003; Longobardi *et al.*, 2014). We previously showed that Meis1 plays a role in target-field innervation of sympathetic peripheral neurons (Bouilloux *et al.*, 2016). Here, we have asked whether Meis2 could have a role in regulating the innervation of specialized cutaneous end-organs important for LTMR function. We confirmed that *Meis2* expression is restricted to LTMR subclasses at late developmental stages compatible with a function in specification and/or target field innervation. Mice carrying *Meis2* gene inactivation in post-mitotic sensory neurons are healthy and viable and do not exhibit any neuronal loss, but display

severe tactile sensory defects as demonstrated physiologically and in behavioral assays. Consistent with these findings we found major morphological alterations in LTMR end-organ structures.

Results:

***Meis2* is expressed by cutaneous LTMRs.**

We analyzed *Meis2* expression using *in situ* hybridization (*ISH*) at various developmental stages in both mouse and chick lumbar DRG, combined with well-established molecular markers of sensory neuron subclasses (Figures 1A; Figures 1 Supplementary 1 and 2). In mouse, *Meis2* mRNA was first detected at embryonic day (E) 11.5 in a restricted group of large DRG neurons. This restricted expression pattern was maintained at E14.5, E18.5 and adult stages (Figure 1A). In chick, *Meis2* was expressed in most DRG neurons at Hamburger-Hamilton stage (HH) 24, but later become restricted to a well-defined subpopulation in the ventro-lateral part of the DRG where LTMRs and proprioceptors are located(Rifkin *et al.*, 2000) (Figure 1 Supplementary 2A). In both species, *Meis2*-positive cells also expressed the pan-neuronal marker Islet1, indicating that they are post-mitotic neurons. In chick, we estimated that *Meis2*-positive cells represented 16.4 ± *1.1* and 14.4 ± *1.2*% (mean ± *SEM*; n = 3) of Islet1-positive DRG neurons at HH29 and HH36 respectively, suggesting a stable expression in given neuronal populations during embryonic development. Double *ISH* for *Meis2* and *Ntrk2*, *Ntrk3* or *c-Ret* mRNAs in E14.5 and E18.5 mouse embryonic DRG (Figure 1 Supplementary 1A and B) showed a large co-expression in *Ntrk2*- and *Ntrk3*-positive neurons confirming that *Meis2*-positive neurons belong to the LTMR and proprioceptive subpopulations. We estimated that 16.0 ± *1.2*, 28.7 ± *2.5* and 30.5 ± *3.5*% (mean ± *SEM*; n = 3) of *Meis2*-positive neurons co-expressed *Ntrk2* at E14.5, E18.5 and P8 respectively, and that 43.7 ± *2.1*, 57.8 ± *5.1* and 39.5 ± *5.4*% (mean ± *SEM*; n = 3) co-expressed *Ntrk3* at these stages. Conversely, *Meis2* was co-expressed in 55.9 ± *3.1*, 52.1 ± *4.5* and 53.6 ± 9.4% (mean ± *SEM*; n = 3) of *Ntrk2*-positive neurons at E14.5, E18.5 and P8 respectively, and in 79.1 ± *3.4*, 63.8 ± *5.1* and 78.5 ± *5.0*% (mean ± *SEM*; n = 3) of *Ntrk3*-positive neurons. Finally, double *ISH* for *Meis2* and *c-Ret* in E14.5 mouse DRG showed that virtually all large *c-Ret*-positive neurons representing part of the LTMR pool co-expressed *Meis2* at this stage before the emergence of the small nociceptive *Ret*-positive population. Similar results were found in chick at HH29 (Figure 1 Supplementary 2B) where we estimated that 38.4 ± *7.7* and 41.7 ± *3.0*% (mean ± *SEM*; n = 3) of *Meis2*-positive neurons co-expressed *Ntrk2* or *Ntrk3* respectively, and inversely, that 62.1 ± *3.8* and 45.1 ± *4.7*% (mean ± *SEM*; n = 3) of *Ntrk2*- and *Ntrk3*-positive neurons co-expressed *Meis2* mRNA respectively.

In mouse, comparison of *Meis2* mRNA expression to *Ntrk1*, a well-established marker for early nociceptive and thermo-sensitive neurons, showed that only few *Meis2*-positive neurons co-expressed *Ntrk1* at E11.5 and E18.5 (Figure 1 Supplementary 1C). In chick HH29 embryos, *Meis2* expression was fully excluded from the *Ntrk1* subpopulation (Figure 1 Supplementary 2C). In adult

mouse DRG, comparison of *Meis2* mRNA to Ntrk1, Calca and TrpV1 immunostaining confirmed that *Meis2*-expressing neurons are largely excluded from the nociceptive and thermo-sensitive populations of DRG neurons. Instead, a large proportion of *Meis2*-positive neurons co-expressed *Nefh*, a marker for large myelinated neurons including LTMR and proprioceptors, and Pvalb, a specific marker for proprioceptors (Figure 1 Supplementary 1D). Finally, *Meis2* expression in LTMRs projecting to the skin was confirmed by retrograde-tracing experiments using Cholera toxin B subunit (CTB) coupled with a fluorochrome injected into hind paw pads of P5 newborn mice. Analyses of CTB expression in lumbar DRG three days later at P8 showed that many retrogradely labelled sensory neurons were also immuno-positive for Meis2, Maf, Ntrk2 and Ntrk3 (Figure 1B).

Altogether, our results on Meis2 co-localization with Nfeh, NtrK2, Ntrk3, Ret, Pvalb and Maf at different embryonic and postnatal stages are consistent with previous report on restricted Meis2 expression to the Aβ-field, Aβ-SA1 and Aβ-RA subclasses of LTMR neurons and proprioceptive neurons (Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Sharma *et al.*, 2020; Shin, Catela and Dasen, 2020). The relatively lower co-incidence of Meis2 and Ntrk2 expressions compared to Ntrk3 is consistent with Meis2 being excluded from the Aδ-LTMRs (D-hair mechanoreceptors). The lack of co-expression with Ntrk1 and TrpV1 also confirmed Meis2 exclusion from peptidergic and non-peptidergic subpopulations.

The maintenance of *Meis2* expression depends on target-derived signals.

The requirement for extrinsic signals provided by limb mesenchyme and muscles for proprioceptor and LTMR development has been documented (Arber *et al.*, 2000; Patel *et al.*, 2003; de Nooij, Doobar and Jessell, 2013; Poliak *et al.*, 2016; Wang *et al.*, 2019; Sharma *et al.*, 2020; Shin, Catela and Dasen, 2020). Whereas *Hox* gene expression in sensory neurons does not depend on limb derived signals (Shin, Catela and Dasen, 2020), *Pea3*, *ER81* and *Runx3* expressions are lost when limb-derived signals are removed (Lin *et al.*, 1998; Lallemend *et al.*, 2012). *ER81* and *Runx3* expressions are regulated by *Ntf3*/*Ntrk3* signaling and in this model, different levels of *Ntf3* expression provided by each muscle seem to contribute to proprioceptive neurons diversity (Patel *et al.*, 2003; de Nooij, Doobar and Jessell, 2013; Wang *et al.*, 2019). Early after limb ablation, sensory neuron differentiation and Ntrk receptors expression was unaffected, but later survival of ventro-lateral LTMRs and proprioceptors neurons is compromised and can be rescued by addition of BDNF and NT3 (Oakley *et al.*, 1995; Calderó *et al.*, 1998). To test the influence of target-derived signals on *Meis2* expression in sensory neurons, limb buds were unilaterally ablated in HH18 chick embryos. Embryos were harvested at HH27 and HH36, before and after ventro-lateral neurons are lost respectively (Oakley *et al.*, 1995, 1997; Calderó *et al.*, 1998) (Figure 1C and D; Figure 1 Supplementary 2D). As previously

reported, about 30% of all sensory DRG neurons represented by the pan-neuronal marker Islet1 were lost in HH36 embryos on the ablated side compared to the contralateral side (Figure 1C). About 50% of Ntrk2 and 65% of Ntrk3 positive VL-neurons were lost, but Ntrk2-positive DL neurons were not significantly affected (Figure 1 Supplementary 2D). Consistently, at this stage, about 65% of Meis2-positive neurons were lost (Figure 1C). In HH27 embryos, whereas no significant loss of Islet1-positive neurons was detected following limb ablation, about 40% of Meis2-positive neurons were lost, and remaining *Meis2*-positive neurons expressed very low levels of *Meis2* mRNAs (Figure 1D). These results indicate that target-derived signals are necessary for the maintenance, but not the induction, of Meis2 expression in sensory neurons.

***Meis2* gene inactivation in post-mitotic sensory neurons induces severe behavioral defects.**

We next asked whether inactivation of the ASD-associated *Meis2* gene would induce changes in LTMR structure and function. We generated a conditional mouse mutant strain for *Meis2* (*Meis2*$^{LoxP/LoxP}$) in which the first coding exon for the homeodomain was flanked by *LoxP* sites (Figure 1 Supplementary 3A). This strategy was previously successfully used to specifically disrupt *Meis1* function in the developing sympathetic nervous system (Bouilloux *et al.*, 2016). This strain was independently crossed with the *Wnt1*^{Cre} or the *Isl1*$^{Cre/+}$ strains to address *Meis2* function in neural crest derivatives including sensory neurons precursors or in post-mitotic sensory neurons, respectively. *Wnt1*^{Cre}*::Meis2*$^{LoxP/LoxP}$ new-born pups were smaller in size and unable to stand compared to WT littermates (Figure 1 Supplementary 3B). As previously reported in human (Crowley *et al.*, 2010; Johansson *et al.*, 2014; Louw *et al.*, 2015; Conte *et al.*, 2016; Fujita *et al.*, 2016; Giliberti *et al.*, 2020; Gangfuß *et al.*, 2021) and in another conditional *Meis2* mouse strain (Machon *et al.*, 2015), specific *Meis2* inactivation in neural crest cells produced a cleft palate (Figure 1 Supplementary 3B). New-born pups from the *Wnt1*^{Cre}*::Meis2*$^{LoxP/LoxP}$ strain were however not viable, therefore precluding functional and anatomical analyses at mature stages. To bypass this neural crest phenotype and to more specifically address *Meis2* function in post-mitotic neurons, we focused our analysis on the *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ strain. Mutant pups were viable, appeared healthy and displayed a normal palate although they were variable in size at birth. At adult stages, WT and *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mutant mice were almost indistinguishable from each other. Although *Meis2* and *Isl1* are both expressed by spinal motor neurons and proprioceptors (Ericson *et al.*, 1992; Dasen *et al.*, 2005; Catela *et al.*, 2016), we did not observe obvious motor deficits in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mice. Thus in a catwalk analysis we found no differences in any of the gait parameters measured between WT and mutant mice (Figure 1 Supplementary Table 1).

We next monitored tactile evoked behaviors in adult WT, *Isl1*$^{Cre/+}$ and *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mice, using stimuli applied to both glabrous and hairy skin. We used von Frey filaments to apply a series of low forces ranging from 0.16 to 0.6g to the hind paw and found the frequency of withdrawal responses to be significantly decreased in *Isl1*$^{+/Cre}$*::Meis2*$^{LoxP/LoxP}$ mice compared to control WT and *Isl1*$^{+/Cre}$ mice (Figure 1E), indicating that mutant mice are less responsive to light touch. No differences were observed between *WT* and *Isl1*$^{+/Cre}$ mice for any of the stimuli used. Behaviors evoked from stimulation of the glabrous skin were next assessed using the dynamic touch assay "cotton swab" test (Bourane *et al.*, 2015). Here, responses were significantly decreased in Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice compared to control WT and Isl1$^{+/Cre}$ littermates (Figure 1F). In the von Frey test, the thresholds for paw withdrawal were similar between all genotypes when using filaments exerting forces ranging from 1 to 1.4g, which likely reflects the activation of mechanical nociception suggesting that *Meis2* gene inactivation did not affect nociceptor function. We also used hot plate assay to assess noxious heat evoked behaviors and found no difference in jump latencies between WT, *Isl1*$^{Cre/+}$ and *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mice (Figure 1G). Finally, we compared the sensitivity of mice to stimuli applied to the hairy skin using the sticky tape test. Placing sticky tape on the back skin evoked attempts to remove the stimulus in a defined time window and we found that such bouts of behavior were significantly reduced in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mice compared to WT and *Isl1*$^{Cre/+}$ control mice (Figure 1H).

Overall, these behavior analyses indicate that *Meis2* gene inactivation specifically affects light touch sensation both in the glabrous and the hairy skin while sparing other somatosensory behaviors. The impaired behavioral response to light touch in *Meis2* mutant suggested that *Meis2* gene activity is necessary for the anatomical and functional maturation of LTMRs.

Meis2 is dispensable for LTMR specification and survival, but is necessary for normal end-organ innervation.

To investigate whether *Meis2* gene inactivation interfered with LTMR survival during embryonic development, we performed histological analysis of the *Wnt1*^{Cre}*::Meis2*$^{LoxP/LoxP}$ and *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ strains (Figure 2). Quantification of DRG neuron populations at P0 in *Wnt1*^{Cre}*::Meis2*$^{LoxP/LoxP}$ mice showed no differences in the number of Ntrk2 and Ntrk3-positive neurons (Figure 2A), indicating that *Meis2* inactivation does not compromise LTMR and proprioceptor survival or specification during embryogenesis. Similar to the *Wnt1*^{Cre}*::Meis2*$^{LoxP/LoxP}$ strain, there was no difference in the size of the DRGs between E16.5 WT and *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ embryos as well as in the number of Ntrk2 and Ntrk3-positive neurons (Figure 2B) suggesting no cell loss. In E18.5 embryonic DRGs, the number of LTMR and proprioceptors identified as positive for

Ntrk2, Ntrk3, Ret and Maf were unchanged following *Meis2* inactivation (Figure 2C). Consistent with the lack of *Meis2* expression in nociceptors, the number of Ntrk1-positive neurons was also unaffected (Figure 2C). At this stage, phosphoCreb (pCreb) expression in Ntrk2 and Ntrk3-positive neurons was similar in WT and mutants (Figure 2 Supplementary 2), suggesting that Ntrk signaling is not affected. Altogether, these results show that *Meis2* is dispensable for LTMR and proprioceptor survival and specification.

Our assays in chick limb ablation indicated that like other TFs involved in target field innervation, the maintenance of *Meis2* expression also depends on target-derived signals. In the developing sympathetic nervous system, we previously demonstrated that another member of the Meis family, *Meis1*, is involved in distal target-field innervation of sympathetic neurons but was dispensable for their specification (Bouilloux *et al.*, 2016). In P0 $Wnt1^{Cre}::Meis2^{LoxP/LoxP}$, Nefh staining in the hind paws showed strong innervation deficits as reflected by a paucity of neurofilament-positive myelinated branches in both the glabrous and hairy skin (Figure 2D). LTMRs form specialized sensory endings in a variety of end-organs specialized to shape the mechanoreceptor properties. We next used the $Isl1^{+/Cre}::Meis2^{LoxP/LoxP}$ mice to assess the effects of late loss of *Meis2* on LTMR structure and function.

Meis2 gene inactivation compromises SA-LTMR morphology and function in the glabrous, but not the hairy skin.

We next investigated if post mitotic *Meis2* inactivation impacts terminal morphologies and physiological properties of LTMRs. We made recordings from single mechanoreceptors and probed their responses to defined mechanical stimuli in adult WT, $Isl1^{+/Cre}$ and $Isl1^{+/Cre}::Meis2^{LoxP/LoxP}$ mice using *ex vivo* skin nerve preparations as previously described (Wetzel *et al.*, 2007; Walcher *et al.*, 2018; Schwaller *et al.*, 2021).

We made recordings from single myelinated afferents in the saphenous nerve which innervates the hairy skin of the foot or from the tibial nerve that innervates the glabrous skin of the foot (Walcher *et al.*, 2018; Schwaller *et al.*, 2021). In control nerves all the single units (n=78) with conduction velocities in the Aβ-fiber range (>10 m/s) could be easily classified as either a rapidly-adapting mechanoreceptor or a slowly-adapting mechanoreceptor (RA-LTMR or SA-LTMRs respectively), using a set of standard quantitative mechanical stimuli. However, in the $Isl1^{+/Cre}::Meis2^{LoxP/LoxP}$ mice about 10 and 18% of Aβ fibers in the hairy and glabrous skin respectively could not be reliably activated by any of the quantitative mechanical stimuli used. Sensory neurons that could not be activated by our standard array of mechanical stimuli, but could still be activated by rapid manual application of force with a glass rod were classified as so called “Tap” units. Such “tap” units have been found in several mice with deficits in sensory mechanotransduction (Wetzel *et al.*, 2007; Ranade *et al.*, 2014).

In both the glabrous and the hairy skin, Merkel cells are innervated by Slowly-Adapting Mechanoreceptor type I (SAI-LTMR) neurons responding to both static skin indentation and moving stimuli such as vibration. In the glabrous skin, Merkel cells form clusters in the basal layer of the epidermis, and in the hairy skin, similar clusters of Merkel cells called touch-domes are located at the bulge region of guard hairs. Histological analysis indicated that in the forepaw glabrous skin of *Isl1*$^{+/Cre}$*::Meis2*$^{LoxP/LoxP}$ adult mice, the number of Merkel cells contacted by Nefh-positive fibers was strongly decreased compared to WT (Figure 3B and E). However, in contrast to the glabrous skin, Merkel cell innervation by Nefh-positive fibers appeared largely unaffected in the hairy skin of *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mice (Figure 3D and E). Whole mount analysis of CK8-positive Merkel cells in the hairy back skin of E18.5 embryos showed that the overall number of touch domes and of Merkel cells per touch dome were unchanged in mutant animals compared to WT (Figure 3C).

We made recordings from SA-LTMRs from both glabrous and hairy skin, but decided to pool the data as there was an insufficient sample size from either skin area alone. We reasoned that electrophysiological recordings would pick up primarily receptors that had successfully innervated Merkel cells and miss those fibers that had failed to innervate end-organs and would likely not be activated by mechanical stimuli. In this mixed sample of SA-LTMRs the mean vibration threshold was significantly elevated in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$mice, but it was clear that many fibers in this sample had mechanical thresholds similar to those in the wild type (Figure 3F). The response of the same SA-LTMRs to a 25 Hz sinusoidal stimulus was unchanged in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mice compared to controls (Figure 3F). The response of these fibers to ramp stimuli of increasing velocities or to increasing amplitudes of ramp and hold stimuli were also not significantly different in mutant mice compared to controls (Figure 3F). Finally, consistent with the lack of neuronal loss in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$, the number of recorded fibers were identical in WT and *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ (Figure 4 Supplementary 1E). Altogether, these data indicate that Meis2 is necessary for Merkel cells innervation in the glabrous, but not in the hairy skin. There was some indication that amongst SA-LTMRs with mechanosensitivity there was a light loss of sensitivity that could be associated with poor innervation of Merkel cells in the glabrous skin.

***Meis2* gene inactivation compromises structure and function of RA-LTMRs.**

In the glabrous skin, Meissner corpuscles are located in the dermal papillae and are innervated by Rapidly-Adapting type LTMR (RA-LTMR) that detect small-amplitude skin vibrations <80 Hz.

Histological analysis of the glabrous skin showed that Nefh-positive innervation of the Meissner corpuscles was strongly disorganized (Figure 4A, Figure 4 Supplementary video 1-2). However recordings from RA-LTMRs innervating these structures in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ animals showed

largely normal physiological properties (Figure 4B). Thus RA-LTMRs recorded from *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ displayed normal vibration sensitivity in terms of absolute threshold and their ability to follow 25 sinusoids. There was a tendency for RA-LTMRs in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ mutant mice to fire fewer action potentials to sinusoids and to the ramp phase of a series 2 second duration ramp and hold stimuli, but these differences were not statistically significant (Figure 4B).

In the hairy skin, RA-LTMRs form longitudinal lanceolate endings parallel to the hair shaft of guard and awl/auchene hairs and respond to hair deflection only during hair movement, but not during maintained displacement (Lechner and Lewin, 2013). Similar to Meissner corpuscles, they are tuned to frequencies between 10 and 50 Hz (Schwaller *et al.*, 2021). Whole mount analysis of Nefh-positive fibers in the adult back skin showed an overall decrease in the innervation density of hairs in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$ animals compared to WT (Figure 4C). Our analysis revealed significant decreases in both the number of plexus branch points and in the number of innervated hair follicles (Figure 4D).

Consistent with the hypo innervation of hair follicle in *Isl1*$^{Cre/+}$*::Meis2*$^{LoxP/LoxP}$, we observed robust deficits in the mechanosensitivity of RA-LTMRs in the hairy skin (Figure 4E). Thus we needed sinusoids of significantly larger amplitudes to evoke the first (threshold) spike in RA-LTMRs. We measured the total number of spikes evoked by a sinusoid stimulus (25 Hz) of gradually increasing amplitude and here too RA-LTMRs in the in *Isl1*$^{+/Cre}$*::Meis2*$^{LoxP/LoxP}$ mutant mice fired considerably less than in controls. This finding was confirmed using a series of vibration steps of increasing amplitudes again demonstrating decreased firing to 25 Hz vibration stimuli (Figure 4E).

D-Hair mechanoreceptors or Aδ–LTMRs are the most sensitive skin mechanoreceptors with very large receptive fields (Shin *et al.*, 2003; Li *et al.*, 2011; Walcher *et al.*, 2018). They form lanceolate endings, are thinly myelinated and are activated by movement of the smaller zigzag hairs (Lechner and Lewin, 2013). Consistent with the lack of Meis2 expression in this population reported by single-cell RNA-seq databases, Aδ fibers D-hair in the hairy skin showed similar vibration responses in WT and *Isl1*$^{+/Cre}$*::Meis2*$^{LoxP/LoxP}$ mice (Supplementary 1).

The functional deficits in RA-LTMRs correlate well with the defects in LTMR cutaneous projections we observed in *Isl1*$^{+/Cre}$*::Meis2*$^{LoxP/LoxP}$ mutant mice.

Discussion:

The function of the Meis family of TFs in post-mitotic neurons has only been marginally addressed (Agoston *et al.*, 2014; Jakovcevski *et al.*, 2015; Bouilloux *et al.*, 2016). In agreement with previous reports on *Meis2* expression in DRG sensory neurons (Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Sharma *et al.*, 2020; Shin, Catela and Dasen, 2020), we showed that *Meis2* is selectively expressed by subpopulations of early post-mitotic cutaneous LTMR and proprioceptive neurons during development of both mouse and chick, highlighting the conserved Meis2 expression across vertebrates species in those neurons. We unambiguously demonstrate that *Meis2* differentially regulates target-field innervation and function of post-mitotic LTMR neurons by using different recombination strategies: *Meis2* gene inactivation prior to birth in migratory and dividing neural crest cells only (Wnt1^{Cre}) or in early Isl1-positive post-mitotic sensory neurons. In addition, *Meis2* inactivation in post-mitotic sensory neurons differentially impaired LTMRs projections and functions without affecting their survival and molecular identity.

Combined single cell RNAseq (scRNAseq) analysis and genetic tracing have accurately resolved Meis2 expression (Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Sharma *et al.*, 2020). A scRNAseq unbiased classification of adult mouse sensory neurons identified more than 11 molecularly distinct subtypes of neurons and *Meis2* is expressed in four of the five clusters of Nefh- and Pvalb-positive myelinated neurons representing LTMR and proprioceptive neurons. *Meis2* expression is mostly excluded from the C-LTMR population and the five other peptidergic and non-peptidergic nociceptive populations (Usoskin *et al.*, 2015). In a combined subclasses-specific genetic labelling and RNA bulk deep-sequencing strategy (Zheng *et al.*, 2019), the transcriptomes of five functionally defined LTMR subtypes (C-LTMRs, Aδ-LTMRs, Aβ RALTMRs, Aβ SA1-LTMRs, Aβ field-LTMRs) and proprioceptors were established. These analyses confirmed *Meis2* expression in proprioceptive neurons, Aβ field-LTMR and Aβ-SA1-LTMR, and a weaker expression in Aβ-RA-LTMR. This study also confirmed the lack of *Meis2* expression in C-LTMR, Aδ-LTMR, peptidergic and non-peptidergic neurons.

The morphological and functional phenotypes we report following specific *Meis2* gene inactivation in all post-mitotic sensory neurons are consistent with *Meis2* expression pattern, and ultimately, both defective morphological and electrophysiological responses resumed in specifically impaired behavioral responses to light touch mechanical stimuli. In these mutants, the decreased innervation of Merkel cells in the glabrous skin and the decreased sensitivity in SA-LTMR electrophysiological responses to mechanical stimuli is consistent with *Meis2* being expressed by Aβ-SA1-LTMR neurons. Interestingly, *Meis2* gene inactivation compromise Merkel cells innervation and electrophysiological responses in the glabrous skin but not in touch domes of the hairy skin where innervation appeared

unchanged. This difference support previous work suggesting that the primary afferents innervating Merkel cells in the glabrous and the hairy skin maybe different (Niu, Vysochan and Luo, 2014; Olson *et al*., 2016). Whereas Merkel cells of the glabrous skin are exclusively contacted by large Ntrk3/Nefh-positive Aβ afferents, neonatal mouse touch domes receive innervation of two types of neuronal populations, a Ret/Ntrk1-positive one that depend on Ntrk1 for survival and innervation, and another Ntrk3/Nefh-positive that do not depend on Ntrk1 signaling during development (Niu, Vysochan and Luo, 2014). However, the functional significance of these different innervations is unknown. Denervation in rat also pointed at differences between Merkel cells of the glabrous and the hairy skin. Following denervation, Merkel cells of the touch dome almost fully disappear, whereas in the footpad, Merkel cells developed normally (Mills, Nurse and Diamond, 1989). Thus to our best knowledge, Meis2 may represent the first TF which inactivation differentially affect Merkel cells innervation in the glabrous skin and hairy touch domes.

Because touch domes innervation and Aδ fibers D-hair vibration responses were unaffected in *Isl1*$^{+/Cre}$*::Meis2*$^{LoxP/LoxP}$ mice, we postulate that the innervation defects we observed in the hairy skin is supported by defects in lanceolate endings with RA-LTMR electrophysiological properties. However, the increased number of "tap" units both in the hairy and glabrous skin is compatible with wilder deficits also including Aβ-field LTMRs peripheral projections. Similarly, although the severely disorganized Meissner corpuscle architecture did not result in significant consequences on RAM fibers electrophysiological responses in the glabrous skin, it is possible that the large increase in the number of "tap units" within Aβ fibers of the glabrous skin represent Meissner corpuscles whose normal electrophysiological responses are abolished. In agreement, challenging sensory responses in the glabrous skin with either von Frey filament application or cotton swab stroking clearly showed a dramatic loss of mechanical sensitivity specifically within the range of gentle touch neurons. Recent work reported that the von Frey filaments test performed within low forces and challenging light touch sensation could distinguish Merkel cells from Meissner corpuscles dysfunctions: mice depleted from Merkel cells performed normally on this test while mice mutated at the *Ntrk2* locus that display Meissner corpuscle innervation deficits showed decreased hind paw withdrawal threshold in response to filament within the 0.02-0.6 g range (Neubarth *et al.*, 2020). Thus, our result in the von Frey filament test possibly involves Meissner corpuscles functioning. Finally, the unaltered D-hair fibers electrophysiological responses, the normal noxious responses in the hot plate setting and the normal responses to high forces Von Frey filaments are consistent with the absence of *Meis2* expression in Aδ-LTMR, peptidergic and non-peptidergic neurons. Surprisingly, although *Meis2* is expressed in proprioceptive neurons (Usoskin *et al.*, 2015; Zheng *et al.*, 2019; Shin, Catela and Dasen, 2020), their function appeared not to be dramatically affected as seen by normal gait behavior in

catwalk analysis. This is in agreement with studies in which HoxC8 inactivation, a classical Meis TF co-factor expressed by proprioceptive neurons from E11.5 to postnatal stages, did not affect proprioceptive neurons early molecular identity or survival (Shin, Catela and Dasen, 2020). In the HoxC8 mutant, proprioceptive neurons normally innervate their peripheral target and extend axons in the central nervous system, but instead exhibit ectopic synapses.

Understanding the transcriptional programs controlling each step in the generation of a given fully differentiated and specified neuron is an extensive research field in developmental neurobiology. Basic studies in model organisms led to a functional classification of TFs. Proneural TFs such as *Neurogenins* control the expression of generic pan-neuronal genes and are able to reprogram nearly any cell type into immature neurons (Guillemot and Hassan, 2017; Baker and Brown, 2018). Terminal selectors are TFs mastering the initiation and maintenance of terminal identity programs through direct regulation of neuron type-specific effector genes critical for neuronal identity and function such as genes involved in neurotransmitters synthesis and transport, ion channels, receptors, synaptic connectivity or neuropeptide content (Hobert, 2016; Hobert and Kratsios, 2019). The proneural function of *Ngn1* and *Ngn2* genes in neural crest cells, the precursors of DRG sensory neurons is well demonstrated (Ma *et al.*, 1999; Marmigère and Ernfors, 2007), and several terminal selector genes shaping the different DRG sensory subpopulations have also been clearly identified including for cutaneous LTMRs (Marmigère and Ernfors, 2007; Lallemend and Ernfors, 2012; Olson *et al.*, 2016). *Maf*, *Runx3*, *Shox2*, *ER81* and *Pea3* are part of this regulatory transcriptional network regulating cutaneous LTMR neurons diversification through intermingled crossed activation and/or repression of subclasses specific effector genes.

In *Shox2* mutant mice, lanceolate endings in the hairy skin and Nefh-positive innervation of Merkel cells both in the glabrous skin and in touch domes are defective (Abdo *et al.*, 2011). Meissner corpuscles are also poorly innervated in these mice (Abdo *et al.*, 2011). *Maf* is important for Aβ RA-LTMR both in the hairy and the glabrous skin, and in *Maf* mutant mice, the number of Meissner corpuscles are greatly reduced and circumferential and lanceolate endings are affected while Merkel cells neurites complexes remain intact although Aβ-SAI LTMR have higher mechanical thresholds (Wende *et al.*, 2012). In *Runx3* mutants, Ntrk3-positive proprioceptive neurons failed to extend peripherally and die (Inoue *et al.*, 2002), but *Runx3* expression by some LTMR suggests that it play additional functions in these populations (Yoshikawa *et al.*, 2013). In addition to their functions as terminal selectors for the proprioceptive subclasses (Lin *et al.*, 1998), ER81 and Pea3, two members of the Ets family of TF are also involved in Pacinian corpuscles formation. In *ER81* mutant mice, Pacinian corpuscles are absent and in *Pea3* mutant as well but to a lesser extent.

Despite the strong implication of Meis TFs in many human neurological disorders (Hammerschlag *et al.*, 2017; Lane *et al.*, 2017; Jiménez-Jiménez *et al.*, 2018; Sarayloo, Dion and Rouleau, 2019), their mechanisms of action in particular in post-mitotic neurons are poorly understood. In humans, at least 17 different mutations in the *Meis2* gene have been associated to neurodevelopmental delay and ASD (Giliberti *et al.*, 2020; Gangfuß *et al.*, 2021), emphasizing its essential function in neuronal differentiation. The *Meis2* function in late differentiation of post-mitotic peripheral sensory neurons add to the wide actions of this TF in the developing and adult nervous system in number of regions of the mouse nervous system. Its expression both in dividing neural progenitors, in immature neurons and in discrete populations of mature neurons (Toresson *et al.*, 1999; Toresson, Parmar and Campbell, 2000; Allen *et al.*, 2007; Bumsted-O'Brien *et al.*, 2007; Jakovcevski *et al.*, 2015; Chang and Parrilla, 2016; Frazer *et al.*, 2017; Yan *et al.*, 2020; Yang *et al.*, 2021) argues for diverse functions ranging from regulation of neuroblasts cell-cycle exit, to cell-fate decision, neurogenesis, neuronal specification, neurites outgrowth and mature neurons maintenance. In DRG LTMRs, *Meis2* fulfils some but not all of the criteria defining terminal selector genes. Its inactivation in neural crest cells does not affect sensory neurons generation nor pan-neuronal features, clearly excluding it from a proneural TF function. Although *Meis2* expression is continuously maintained in defined sensory neurons subtypes starting from early post-mitotic neurons throughout life, its expression is not restricted to a unique neuronal identity and its early or late inactivation in either sensory neurons progenitors or post-mitotic neurons does not influence neuronal subtypes identity nor survival as seen by the normal numbers of Ntrk2, Ntrk3 or c-Ret positive neurons. It is however possible that in LTMRs, Meis2 regulate other type of terminal effector genes such as genes participating in neurotransmitters machinery specification and/or recognition, establishment and maintenance of physical interactions between LTMRs and their peripheral target.

To conclude, together with studies on Meis2 function in the SVZ where Meis2 is necessary to maintain the neurogenic effect of Pax6 in neural progenitors and is later expressed in their mature progenies (Agoston *et al.*, 2014), our results raise the possibility that this TF sets up a lineage specific platform on which various specific co-factors in turn participate in different steps of the neuronal differentiation program. Sensory neurons subtypes specification is the consequence of intermingled repressive and activator activities of identified terminal selector TFs that activate subclasses specific effector genes while repressing those specific to other subclasses. Thus, in this scenario, Meis2 could be a central hub dynamically interacting with different TFs belonging to distinct functional classes including terminal selector genes.

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Contribution:

Design of experiments, data analysis and writing: GL, PC, FM

Electrophysiology experiments: GL and FS

Histology and behavior: SD and FM

Figure legends:

Figure 1: *Meis2* is expressed in subclasses of DRG cutaneous mechanoreceptive neurons in mouse embryos (A-B). A) *ISH* for *Meis2* mRNA showed expression in a subpopulation of DRG sensory neurons at embryonic stages E11.5, E14.5 and E18.5, at P0 and at adult stages. Dashed lines delineate the DRG. Scale bar=50µm. B) IF for Meis2 (red) and c-Maf, Ntrk2 or Ntrk3 (blue) at P7 following injection of cholera toxin B (CTB in green) in the skin of new-born pups. Note that Meis2^{+}/CTB^{+} retro-traced sensory neurons co-expressed c-Maf, Ntrk2 or Ntrk3 (arrows). Scale bar=50µm. ***Meis2* expression depends on target-derived signals (C-D).** C) Representative images of *Meis2* mRNA expression (blue or pseudo-colored in red) and islet1 (green) in DRGs of Hamburger-Hamilton stage (HH) 36 chick embryos on the ablated and contralateral sides. Box plots showing the number of Islet1^{+}/Meis2^{+} DRG neurons per section at stage HH36 following limb bud ablation. For Islet1-positive neurons, the contralateral side was taken as 100%. For Meis2, values represent the percentage of Meis2^{+} over Islet1^{+} neurons. D) Representative images of *Meis2* mRNA expression (blue or pseudo-colored in red) and islet1 (green) in DRGs of HH29 chick embryos on the ablated and contralateral sides. Box plots showing the quantification of Islet1^{+}/Meis2^{+} neurons number per section at stage HH29 on the contralateral and ablated sides. Arrow heads point at remaining Meis2-positive VL neurons. Dashed lines encircle the DRGs. ***$p\leq0.005$ *** $p\leq0.0005$; *ns*= not significant following Student t-test. n=3 chick embryos. Scale bar=100µm. **Altered touch perception in *Meis2* mutant mice (E-H).** E) Box plots showing the responses following application of Von Frey filaments of different forces. Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice exhibited a significantly reduced sensitivity to the 0.16, 0.4 and 0.6 g Von Frey filaments but not to higher forces filaments compared to WT and Isl1$^{+/CRE}$ littermates. * $p\leq0.05$; ** $p\leq0.005$; *** $p\leq0.001$ following Kruskal-Wallis statistical analysis. F) Box plots showing the dynamic touch responses when the hind paw palms of individual mice were stroked with a tapered cotton-swab. Analysis showed that Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice were less responsive to the stimulus than WT and Isl1$^{+/Cre}$ littermates. *** $p\leq0.0001$ following a one-way Anova statistical analysis. G) Box plots indicating that the latency to the first signs of aversive behavior in the hot plate test is similar in all groups of mice. WT, n=19; Isl1$^{+/Cre}$, n=16; Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$, n=9. H) Box plots showing the number of bouts when a sticky paper tape was applied on the back skin of mice. Analysis indicated a significant decrease in the number of bouts in Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice compared to WT and Isl1$^{+/Cre}$ littermates. * $p\leq0.05$ following a one-way Anova statistical analysis.

Figure 2: *Meis2* is necessary for LTM neurons peripheral projections but dispensable for their survival and specification. A) Representatives images showing IF for Ntrk2 or Ntrk3 (green) with Pvalb or Maf (red) in P0 WT and Wnt1^{Cre}::Meis2$^{LoxP/LoxP}$ DRGs. Box plots showing that the number of

Ntrk2^{+} and Ntrk3^{+} neurons are unchanged in P0 WT and Wnt1^{Cre}::Meis2$^{LoxP/LoxP}$ DRGs. n=3, *n.s.* = not significant. Scale bar = 20µm. B) Box plots showing that the DRGs volumes along the rostro-caudal axis are similar in E16.5 WT and Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$ embryos. IF for Ntrk2 or Ntrk3 (red) and Islet1 (blue) and box plots analysis indicating that the percentage of Ntrk2^{+} and Ntrk3^{+} neurons are not affected in E16.5 Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$. Dashed lines encircle the DRGs. n=4; *n.s.* = not significant. Scale bar = 20µm. C) Representative images showing IF for Ntrk1 (red), Ret, Ntrk2, Ntrk3 and Maf in E18.5 WT and Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$ DRGs. Box plots showing that the number of Ret^{+}, Ntrk2^{+}, Ntrk3^{+} and Maf^{+} LTM neurons and of Ntrk1^{+} nociceptive neurons are similar in E18.5 WT and Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$ DRGs. n=3; *n.s.* = not significant. Scale bar = 100µm. D) Representative images showing a strong overall deficits in Nfeh^{+} (red) sensory projections in the hairy and the glabrous skin of P0 Wnt1^{Cre}::Meis2$^{LoxP/LoxP}$ neonates forepaw compared to WT littermates. Dashed lines delineate the hair follicle and the epidermis. Scale bar = 50µm.

Figure 3: *Meis2* gene inactivation compromised Merkel cells innervation in the glabrous skin and increased SAM vibration threshold. A) Percentage of tap units among Aβ fibers in the hairy and glabrous skin detected by electrophysiological recording in the nerve-skin preparation. Note that Tap-units are only present in both the hairy and glabrous skin of adult Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice but not in WT and Isl1$^{Cre/+}$ littermates. B) Confocal images of Nefh^{+} innervation (green) of CK8^{+} Merkel cells (red) in the forepaw glabrous skin of WT and Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$ adult mice. Dotted white squares indicate the close-up on CK8^{+} Merkel cells. Note the lack of Nefh^{+} fibers innervating Merkel cells in mutant mice. Scale bar = 10µm. C) Representative images of whole mount staining for CK8 in the hairy back skin of adult WT and Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$ E18.5 embryos showing no difference in the number of touch dome between genotypes. Box plots show the number of touch domes per surface area and the number of Merkel cells per guard hair. No significant differences were found between both genotype in Mann-Whitney test. n=5 (WT) and 4 (Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$). D) Confocal images of Nefh^{+} innervation (green) and CK8^{+} Merkel cells (red) of guard hairs in the hairy back skin of WT and Isl1$^{Cre/+}$::Meis2$^{LoxP/LoxP}$ adult mice. Dotted white squares indicate the close-up on CK8^{+} Merkel cells with apparently normal Nefh^{+} innervation. Scale bar = 10µm. E) Box plot indicating the percentage of Merkel cells in contact with Nfeh^{+} fibers in the forepaw glabrous and in the back hairy skin of adult WT and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice. n=4; * $p \leq 0.05$ in Mann-Whitney test. F) In the hairy and glabrous skins, SAMs in Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice (n = 22 from 6 mice) had significantly increased vibration threshold compared to WT and Isl1$^{+/Cre}$ mice (n = 29 from 6 mice), but normal firing activity to a 25-Hz vibration. Trace shows the stimulation and red square indicate the time frame during when the number of spikes was calculated. G) SAM response to a ramp of 50 Hz vibration with increasing

amplitude are similar in WT, Isl1$^{+/Cre}$ and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice. SAM responses to ramp stimuli and their static force responses were also identical in the different genotypes. * *p≤0.05*; ** *p≤0.005*.

Figure 4: *Meis2* gene inactivation affects Meissner corpuscles morphology, hair follicle innervation and RAM fibers electrophysiological responses in the hairy but not the glabrous skin. A) Representative images showing S100β^{+} Meissner corpuscles (red) and their innervation by Nefh^{+} fibers (green) in the glabrous skin of WT and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ adult mice. Scale bar=10µm. B) RAMs of the glabrous skin exhibited similar vibration threshold and firing activity to a 25-Hz vibration in WT (n = 16 from 4 mice) and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice (n = 21 from 6 mice). Glabrous RAMs showed a non-significant decrease in firing activity to a ramp of 50 Hz vibration with increasing amplitude in Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ compared to WT littermates, but their response to ramp stimuli was similar in both genotypes. Traces indicate the type of stimulation and red squares the time frame during which the number of spikes was calculated. C) Representative images of whole mount immunostaining for Nfeh^{+} sensory projections in the hairy skin of adult WT and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ embryos. Scale bar=100µm. D) Box plots showing the quantification for the number of branch points in the innervation network and for the number of innervated hair follicles. n=3; * *p≤0.05*. E) RAMs in the hairy skin of Isl1^{Cre}::Meis2$^{LoxP/LoxP}$ mice (n = 24 from 3 mice) exhibited significantly increased vibration threshold and reduced firing activity to a 25-Hz vibration compared to WT and Isl1$^{+/Cre}$ mice (n = 20 from 3 mice). Traces show the stimulation and red square indicate the time frame when the numbers of spikes were calculated. RAMs in the hairy skin of Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice also showed a reduced firing activity in response to a ramp of 50 Hz vibration with increasing amplitude compared to WT and Isl1$^{+/Cre}$ animals. * *p≤0.05*; ** *p≤0.005*.

Figure 1 supplementary 1: *Meis2* mRNA expression in LTM neurons of mouse DRG. A) Double *ISH* for *Meis2* (blue) and *Ntrk2*, *Ntrk3* or *c-Ret* (red) showed that *Meis2* mRNA partly colocalises with mRNA for *Ntrk2*, *Ntrk3* and *c-Ret* in E14.5 mouse embryos. Arrows point at double positive neurons. Arrowheads point at only Meis2^{+} neurons. Stars indicate Meis2^{-}/Ntrk2^{+} or Ntrk3^{+} neurons. Note that all large c-Ret-positive neurons (pseudo-color in green) express *Meis2* mRNA. Scale bar=25µm. B) Double ISH for *Meis2* (blue) and *Ntrk2* or *Ntrk3* (red) on E18.5 embryos. Arrows point at double positive neurons. Arrowheads point at only Meis2^{+} neurons. Stars indicate Meis2^{-}/Ntrk2^{+} or Ntrk3^{+} neurons. Scale bar=25µm. C) Combined *ISH* for *Meis2* mRNA with IF for Ntrk1 (red) and Islet1 (green) showed that *Meis2* is expressed by Islet1-positive post-mitotic neurons and is mostly excluded from the Ntrk1-positive subpopulation of DRG sensory neurons at E11.5 and E18.5. Arrowheads point at Meis2^{+}/Ntrk1^{-} neurons, arrows point at Meis2^{+}/Ntrk1^{+} neurons. Note that the level of *Meis2* mRNA expression in Ntrk1^{+}/Meis2^{+} neurons is very low at the limit of detection. Dashed lines delineate the

DRG. Scale bar=50µm. D) *ISH* for *Meis2* (blue) combined with IF against Ntrk1, Calca, Trpv1, Pvalb (red) and Islet1 or Nefh (green) in adult mouse lumbar DRG. Arrows point at Meis2$^+$ neurons. Arrowheads point at Ntrk1$^+$, Calca$^+$, Trpv1$^+$ or Pvalb$^+$ neurons. Stars indicate neurons that are both positive for Meis2 and Ntrk1, Calca, Trpv1 or Pvalb. Graphs showing the percentage of Meis2$^+$ neurons showing immunoreactivity for Ntrk1, Trpv1, Calca, Pvalb and Nefh, and the percentage of Isl1$^+$, Ntrk1$^+$, Calca$^+$, Trpv1$^+$, Pvalb$^+$ or Nefh$^+$ neurons co-expressing Meis2. Scale bar=50µm.

Figure 1 Supplementary 2: *Meis2* is expressed in a subset of chick ventro-lateral DRG sensory neurons during embryogenesis. A) Developmental expression of *Meis2* visualized by *ISH* in chick DRG at HH24, HH29 and HH36. Dashed lines delineate the DRGs split into the ventro-lateral (VL) and dorso-medial (DM) parts. At early stages after DRG condensation, *Meis2* showed a broad expression in chick DRGs neurons. As differentiation progresses (HH29 and HH36), *Meis2* expression became progressively restricted to the VL population of sensory neurons which represents the Ntrk2$^+$ and Ntrk3$^+$ populations of mechano- and proprioceptive neurons. B) Combined *ISH* for *Meis2* (blue) with IF against Islet1 (green) and Ntrk2 or Ntrk3 (red) in chick lumbar DRGs at HH29 showed that Meis2 expression is shared between the Ntrk2$^+$ and Ntrk3$^+$ subpopulations of sensory neurons. Arrowheads point at Meis2$^+$/Islet1$^+$/Ntrk2$^-$ and Meis2$^+$/Islet1$^+$/Ntrk3$^-$ neurons; arrows point at Meis2$^+$/Islet1$^+$/Ntrk2$^+$ and Meis2$^+$/Islet1$^+$/Ntrk3$^+$ neurons; stars indicate Meis2$^-$/Islet1$^+$/Ntrk2$^+$ and Meis2$^-$/Islet1$^+$/Ntrk3$^+$ neurons. C) Combined *ISH* for *Meis2* (blue) with IF against Islet1 (green) and Ntrk1 (red) in chick lumbar DRGs at HH29 showed that Meis2 is excluded from the Ntrk1$^+$ population of sensory neurons. Arrowheads point at Meis2$^+$/Islet1$^+$/Ntrk1$^-$ neurons. Enlargement is indicated by a dashed square. D) Representative images showing immunostaining for Ntrk2 or Ntrk3 (red) and Islet1 (green) on HH36 chick embryos DRG sections following limb ablation at HH18. Dashed lines delineate the DRGs. Arrows point at Ntrk2-positive VL neurons. Arrow heads point at Ntrk2-positive DL neurons. C) Box plots showing the percentage of Ntrk2 and Ntrk3 VL neurons and of Ntrk2 DL neurons. *** $p \leq 0.0005$; *ns*= not significant following Student t-test. n=3 chick embryos.

Figure 1 Supplementary 3: Mice with a conditional deletion of *Meis2* gene in neural crest derivatives (Wnt1^{Cre}) exhibited cleft-palate and died at birth. A) Targeting vector used for the generation of a conditional knockout mouse strain for Meis2 (Meis2$^{LoxP/LoxP}$). The Exon 8 (first coding exon of the DNA binding Homeodomain) is flanked by LoxP sites allowing deletion of Meis2 DNA binding domain. B) Representative images showing that new-born Wnt1^{Cre}::Meis2$^{LoxP/LoxP}$ pups mutant pups are smaller at birth compared to WT littermates, unable to stand on their legs and exhibit a cleft palate phenotype. Representatives images showing coronal sections of new-born WT

and Wnt1^{Cre}::Meis2$^{LoxP/LoxP}$ heads stained by eosin/hematoxylin treatment. Black arrows indicate the cleft palate.

Figure 1 Supplementary Table 1: Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ adult mice exhibit normal locomotion. Table recapitulating different Catwalk two-paw analysis parameters in 3 months old female mice. Several recordings were performed for each mice. Only sequence when mice showed a constant and straight locomotion with an average speed between 25 and 55 cm/sec were selected for analysis. Student-t-test analysis showed no significant differences for any of the parameters.

Figure 2 Supplementary 1: *Meis2* gene inactivation does not affect phospho-Creb expression. Representative images showing phosphor-Creb (pCreb in green), Ntrk2 or Ntrk3 (red) and Isl1 (blue) expression in WT and Isl$^{1+/Cre}$::Meis2$^{LoxP/LoxP}$ E16.5 DRG embryos.

Figure 3 Supplementary 1: WT and Isl1$^{+/Cre}$ mice exhibit similar electrophysiological responses. Graphs show the same results than in Figure 3 and 4 but including Isl1$^{+/Cre}$ mice (n=5 from 2 mice) as additional control for SAMs (A) and RAMs of the hairy skin (B).

Figure 4 Supplementary video 1: Meissner corpuscle in WT. 3D visualization of Meissner corpuscles in adult WT glabrous skin visualized by IF against Nefh and S100β.

Figure 4 Supplementary video 2: Meissner corpuscles in Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$. 3D visualization of Meissner corpuscles in adult Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ glabrous skin visualized by IF against Nefh and S100β.

Figure 4 Supplementary 1: Normal electrophysiological responses of D-Hair following *Meis2* gene inactivation. A) D-Hair of the hairy skin exhibited similar vibration threshold and firing activity to a 25-Hz vibration in WT (n=8 from 3 mice) and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ mice (n = 10 from 3 mice). B) D-Hair showed similar responses to a ramp of 50 Hz vibration with increasing amplitude and to a ramp stimuli in WT and Isl1$^{+/Cre}$::Meis2$^{LoxP/LoxP}$ animals. Traces showed the type of stimulation and red squares indicate the time frame during when the number of spikes was calculated.

Animals.

All procedures involving animals and their care were conducted according to European Parliament Directive 2010/63/EU and the 22 September 2010 Council on the protection of animals, and were approved by the French Ministry of research (APAFIS#17869-2018112914501928 v2, June the 4th fo 2020).

Mice strains: Wnt1^{CRE} and Islet1$^{+/CRE}$ mice were previously described (Lewis et al., 2013; Srinivas et al., 2001). To generate a conditional mutant strain for Meis2 (Meis2$^{LoxP/LoxP}$), exon 8 of the Meis2 gene was flanked by the LoxP recognition elements for the Cre recombinase at the ITL (Ingenious Targeting Laboratory, NY, USA) using standard homologous recombination technology in mouse embryonic stem cells. FLP-FRT recombination was used to remove the neomycin selection cassette and the Meis2$^{LoxP/LoxP}$ mutant mice were backcrossed for at least 8 generations onto the C57BL/6 background before use. Primers used to genotype the different strains were: Meis2 sense 5'-TGT TGG GAT CTG GTG ACT TG-3'; Meis2 antisense 5'-ACT TCA TGG GCT CCT CAC AG-3'; CRE sense 5'-TGC CAG GAT CAG GGT TAA AG-3'; CRE antisense 5'-GCT TGC ATG ATC TCC GGT AT-3'. Mice were kept in an animal facility and gestational stages were determined according to the date of the vaginal plug.

For retro-tracing experiments, newborn pups were anesthetized on ice and cholera toxin B coupled to Cholera Toxin Subunit B conjugated with Alexa Fluo 488 (Thermofisher) was injected using a glass micropipette in several points of the glabrous and hairy forepaw. Mice were sacrificed 7 days after injection and L4 to L6 DRGs were collected for analysis.

For behavioral assays, skin-nerve preparation and electrophysiological recording, sex-matched 12-weeks old mutant and WT littermates mice were used.

Chick: Fertilized eggs were incubated at 37°C in a humidified incubator. For limb ablation experiments, eggs were opened on the third day of incubation (embryonic day 3, stage 17/18) (Hamburger and Hamilton, 1992) and the right hind limb bud was surgically removed as previously reported (Oakley *et al.*, 1995). Eggs were closed with tape and further grown in the incubator for 4 (HH27) or 7 (HH36) additional days before collection.

Tissue preparation.

Mouse and chick embryos were collected at different stages, fixed in 4% paraformaldehyde/PBS overnight at 4°C and incubated overnight at 4°C for cryopreservation in increasing sucrose/PBS solutions (10 to 30% sucrose). After snap freezing in TissueTek, embryos were sectioned at 14-µm thickness and stored at -20°C until use.

Cloning of mouse and chick Meis2 and probes preparation.

For preparation of digoxigenin- and fluorescein- labeled probes, RNA from whole mouse or chick embryos was extracted using Absolutely RNATM Nanoprep kit (Stratagene) following manufacturer's instruction. Reverse transcription (RT) was carried out 10 minutes at 65°C followed by 1 hour at 42°C and 15 minutes at 70°C in 20 µl reactions containing 0.5 mM dNTP each, 10 mM DTT, 0.5 µg oligod(T)15 (Promega) and 200 U of Super Script II RT (Gibco BRL Life Technologies). A 1206 bp long and a 1201 bp long Meis2 fragments were amplified from mouse and gallus cDNA respectively using the following primers: mMeis2 forward: 5'-ATGGCGCAAAGGTACGATGAGCT-3'; mMeis2 reverse: 5'-TTACATATAGTGCCACTGCCCATC-3'; gMeis2 forward: 5'-ATGGCGCAAAGGTACGATGAG-3'; gMeis2 reverse: 5'-TTACATGTAGTGCCATTGCCCAT-3'. PCR were conducted in 50 µl reactions containing 10% RT product, 200 µM each dNTP, 10 pmol of each primer (MWG-Biotech AG), 3 mM MgCl2, 6% DMSO and 2.5 U of Herculase hotstart DNA polymerase (Stratagene). cDNA was denatured 10 minutes at 98°C and amplified for 35 cycles in a three steps program as following: 1 minute denaturation at 98°C, 1 minute annealing at annealing temperature and then 1.5 minutes polymerization at 72°C. PCR products were separated on 2% agarose gels containing ethidium bromide. Bands at the expected size were excised, DNA was extracted, and the fragment was cloned into pCR4Blunt-TOPO vector (Invitrogen) and confirmed by sequencing. Other probes used for ISH have been described elsewhere (Bouilloux *et al.*, 2016).

***In situ* hybridization (ISH).**

Before hybridization, slides were air dried for 2-3 hours at room temperature. Plasmids containing probes were used to synthesize digoxigenin-labeled or fluorescein-labeled antisense riboprobes according to supplier's instructions (Roche) and purified by LiCl precipitation. Sections were hybridized overnight at 70°C with a solution containing 0.19 M NaCl, 10 mM Tris (pH 7.2), 5 mM NaH2PO4*2H2O/Na2HPO4 (pH 6.8), 50 mM EDTA, 50% formamide, 10% dextran sulphate, 1 mg/ml yeast tRNA, 1XDenhardt solution and 100 to 200 ng/ml of probe. Sections were then washed four times for 20 minutes at 65°C in 0.4X SSC pH 7.5, 50% formamide, 0.1% Tween 20 and three times for 20 minutes at room temperature in 0.1 M maleic acid, 0.15 M NaCl and 0.1% Tween 20 (pH 7.5). Sections were blocked 1 hour at room temperature in presence of 20% goat serum and 2% blocking agent (Roche) prior to incubation overnight with AP-conjugated anti-DIG-Fab-fragments (Roche, 1:2000). After extensive washing, hybridized riboprobes were revealed by performing a NBT/BCIP reaction in 0.1 M Tris HCl pH 9.5, 100 mM NaCl, 50 mM MgCl2 and 0.1% Tween 20.

For double *in situ* hybridization, the procedure was the same except that hybridization was conducted by incubation with 100-200 ng/ml of one digoxigenin-labeled probe and 100-200 ng/ml of

one fluorescein-labeled probe. Fluorescein-labeled probe was first revealed after overnight incubation with AP-conjugated anti-fluorescein-Fab-fragment (Roche, 1:2000) and further incubation with Fast Red tablets in 0.1 M Tris HCl pH 8.5, 100 mM NaCl, 50 mM MgCl2 and 0.1% Tween 20. Pictures of fluorescein alone were taken after mounting in glycerol/PBS (1:9). To reveal the digoxigenin-labeled probe, sections were unmounted, washed extensively in PBS and Alkalin Phosphatase was inhibited by incubation in a solution of 0.1M glycin pH2.2, 0.2% Tween 20 for 30 minutes at room temperature. After extensive washing in PBS, digoxigenin-labeled-probe was revealed as described using the AP-conjugated anti-DIG-Fab-fragments (Roche, 1:2000) and the NBT/BCIP reaction. Sections were mounted again in glycerol/PBS (1:9) and pictures of both fluorescein and digoxigenin were taken. For removing the Fast Red staining, sections were unmounted again, washed extensively in PBS and incubated in increasing solutions of ethanol/PBS solutions (20-100% ethanol). After extensive washing in PBS, sections were mounted in glycerol/PBS (1:9) and pictures of the digoxigenin staining alone. Wide field microscopy (Leica DMRB, Germany) was only used for ISH and ISH combined with immunochemistry.

Immunochemistry.

Immunochemistry was performed as previously described (Bouilloux *et al.*, 2016). *In situ* hybridized sections or new sections were washed 3x10 min with PBS, blocked with 4% normal goat serum, 1% bovine serum albumin and 0.1% Triton X100 in PBS and incubated overnight at 4°C with primary antibodies. After washing 3x10 min with PBS incubation occurred for 2-4 hours with secondary species and isotype-specific fluorescent antibodies (Alexa Fluor Secondary Antibodies, Molecular Probes). After repeated washing with PBS, slides were mounted in Glycerol/PBS (9/1) or Mowiol. Picture were taken using a confocal microscope (Leica SP5-SMD, Germany). Confocal images are presented as maximal projections.

The following antibodies were used for immunochemistry: mouse-anti-islet1 39.4D used for mouse and chick (diluted 1:100, Developmental Studies Hybridoma Bank); TrkB (1/2000; R and D Systems); TrkC (1/1000; R and D Systems); CGRP (1/500; Sigma-Aldrich); TrkA (1/500; Millipore); rabbit anti-parvalbumin antibody (1:500, Swant); guinea pig anti-calcitonin gene-related peptide (CGRP) antibody (1:500, Peninsula Laboratories); Nfh (Sigma N4142, rabbit 1:1000), c-maf (generous gift of C. Birchmeier, MDC, 1/1000), TrpV1 (Sigma V2764, rabbit 1:1000), S100β (Sigma S2532, mouse 1:1000), Phospho-CREB (Cell Signaling, 87G3, 1/200, Germany); Goat anti-c Ret (R and D Systems, Cat#AF482, 1/100) and Meis2 (Sigma Aldrich, WH0004212M1 or Abcam ab244267, 1/500). The chick TrkB and C antibodies were a generous gift from LF Reichardt, UCSF and have been previously reported.

Whole-mount immunohistochemistry.

Whole-mount immunohistochemistry of adult mice back hairy skin was performed a described elsewhere(Chang *et al.*, 2014). Briefly, mice were euthanized by CO_2 asphyxiation. The back skin was shaved and cleaned with commercial hair remover. The back skin was removed, carefully handled with curved forceps and fixated in 4% PFA at 4°C for 20'. The tissue was then washed with PBS containing 0.3% Triton X-100 (PBST) every 30 minutes for 3 to 5 hours and kept overnight in the washing solution. The next day, the skin was incubated for 5 days with primary antibodies diluted in PBST containing 5% donkey serum and 20% DMSO. The skin was washed the following day 8 to 10 times over a day before being incubated with secondary antibodies diluted in PBST containing 5% donkey serum and 20% DMSO. The skin was then washed every 30 minutes for 6 to 8 hours before being dehydrated in successive baths of 25%, 50%, 75% and 100% methanol. They were then incubated overnight in a 1:2 mixture of benzyl alcohol and benzyl benzoate before being mounted and sealed into chambers filled with the same medium.

Hematoxylin-Eosin staining.

As previously described(Bouilloux *et al.*, 2016), air dried frozen sections were washed in water then stained with hematoxylin for 1 min at room temperature and washed extensively with water. After dehydration in PBS/alcohol (70%), slides were stained with eosin for 30 sec at room temperature. After serial wash in water, sections were dehydrated in PBS solutions with increasing alcohol concentration (50%, 75%, 95%, and 100%), mounted and observed with a microscope (Leica DMRB, Germany).

Mouse skin-nerve preparation and sensory afferent recordings

Cutaneous sensory fiber recordings were performed using the ex vivo skin-nerve preparation as previously described(Schwaller *et al.*, 2021). Mice were euthanized by CO2 inhalation for 2–4 min followed by cervical dislocation. Three different preparations were performed in separate experiments using different paw regions: the saphenous nerve innervating the hairy hind paw skin; the tibial nerve innervating the glabrous hind paw skin; and the medial and ulnar nerves innervating the forepaw glabrous skin. In all preparations, the hairy skin of the limb was shaved and the skin and nerve were dissected free and transferred to the recording chamber, where muscle, bone and tendon tissues were removed from the skin to improve recording quality. The recording chamber was perfused with a 32 °C synthetic interstitial fluid: 123 mM NaCl, 3.5 mM KCl, 0.7 mM MgSO4, 1.7 mM NaH2PO4, 2.0 mM CaCl2, 9.5 mM sodium gluconate, 5.5 mM glucose, 7.5 mM sucrose and 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), pH 7.4. The skin was pinned out and stretched, such that the outside of the skin could be stimulated using stimulator

probes. The peripheral nerve was fed through to an adjacent chamber in mineral oil, where fine filaments were teased from the nerve and placed on a silver-wire recording electrode.

The receptive fields of individual mechanoreceptors were identified by mechanically probing the surface of the skin with a blunt glass rod or blunt forceps. Analog output from a Neurolog amplifier was filtered and digitized using the Powerlab 4/30 system and Labchart 7.1 software (AD instruments). Spike-histogram extension for Labchart 7.1 was used to sort spikes of individual units. Electrical stimuli (1 Hz, square pulses of 50–500 ms) were delivered to single-unit receptive fields to measure conduction velocity and enable classification as C-fibers (velocity <1.2 m s−1), Aδ-fibers (1.2–10 m s−1) or Aβ-fibers (>10 m s−1). Mechanical stimulation of the receptive fields of neurons were performed using a piezo actuator (Physik Instrumente, catalog no. P-841.60) and a double-ended Nanomotor (Kleindiek Nanotechnik, catalog no. MM-NM3108) connected to a force measurement device (Kleindiek Nanotechnik, catalog no. PL-FMS-LS). Calibrated force measurements were acquired simultaneously using the Powerlab system and Labchart software during the experiment.

As different fiber types have different stimulus-tuning properties, different mechanical stimuli protocols were used based on the unit type. Low-threshold Aβ-fibers (RAMs and SAMs) and Aδ-fiber D-hairs were stimulated with the piezo actuator with three vibration stimuli (5 Hz, 25 Hz and 50 Hz, distortions introduced by the in-series force sensor precluded using frequencies >50 Hz) with increasing amplitude over six steps (peak-to-peak amplitudes of ~6–65 mN; 20 cycles per step), and a dynamic stimulus sequence with four ramp-and-hold waveforms with varying probe deflection velocities (3 s duration; 0.075, 0.15, 0.45 and 1.5 mm s−1; average amplitude 100 mN). Aβ-fiber SAMs and RAMs were classified by the presence or absence of firing during the static phase of a ramp-and-hold stimulus, respectively, as previously described. Single units were additionally stimulated with a series of five static mechanical stimuli with ramp-and-hold waveforms of increasing amplitude (3 s duration; ranging from ~10 mN to 260 mN). Low-threshold SAMs, high-threshold Aδ-fibers and C-fibers were also stimulated using the nanomotor with five ramp-and-hold stimuli with increasing amplitudes 20.

Behavioral assays.

Von Frey paw withdrawal test: Mice were placed on an elevated wire mesh grid into PVC chambers. Before the test, mice were habituated to the device for one hour for two consecutive days. On the day test, mice were placed in the chamber one hour before Von Frey filaments application. The test was performed as previously described(Neubarth *et al.*, 2020). During the test, withdrawal response following Von Frey filament application on the palm of the left hind paw was measured. Starting with

the lowest force, each filament ranging from 0.008 g to 1.4 g was applied ten times in a row with a break of 30 seconds following the fifth application. The number of paw withdrawals for each filament was counted.

Hot Plate test: Before starting the test, mice were habituated to the experimentation room for at least five minutes. Mice were individually placed on the hot plate set up at 53°C and removed at the first signs of aversive behavior (paw licking or shaking). The time to this first stimulus was recorded. A 30 sec cut off was applied to avoid skin damages. After 5 min recovery in their home cage, the test was repeated three times for each mouse and averaged. Data are shown as the average of these three measurements.

Sticky tape test: A two cm^2 of laboratory tape was placed on the upper back skin of mice just before they were placed on an elevated wire mesh grid into PVC chambers. The number of tape-directed reactions were then counted during 5 min. Considered responses were body shaking like a "wet-dog", hindlimb scratching directed to the tape, trying to reach the tape with the snout and grooming of the neck with forepaws.

Dynamic touch test: Mice were placed in the same conditions as described above for the Von Frey paw withdrawal test. Sensitivity to dynamic touch was performed by stroking hind paws with a tapered cotton-swab in a heel to toe direction. The stimulation was repeated 10 times by alternating left and right hind paws and the number of paw withdrawals was counted.

Gait analysis: Gait was analyzed using the Catwalk™ system (Noldus Information Technology, Netherlands) in a dark room with minimized light emission from the computer screen. Mice were allowed to voluntarily cross a 100-cm-long, 5-cm-wide walkway with a glass platform illuminated by green fluorescent light. An illuminated image is produced when a mouse paw touches the glass floor through dispersion of the green light, and footprints were captured by a high-speed camera placed under the glass floor. Data were analyzed using the CatWalk™ XT 10.1 software. For each mouse, several recordings were performed until at least 3 runs met criteria defined by a minimum of three consecutive complete step cycles of all four paws without stopping or hesitation and within the range of 25 to 50 $cm.s^{-1}$. Data are reported as the average of at least three runs per mouse.

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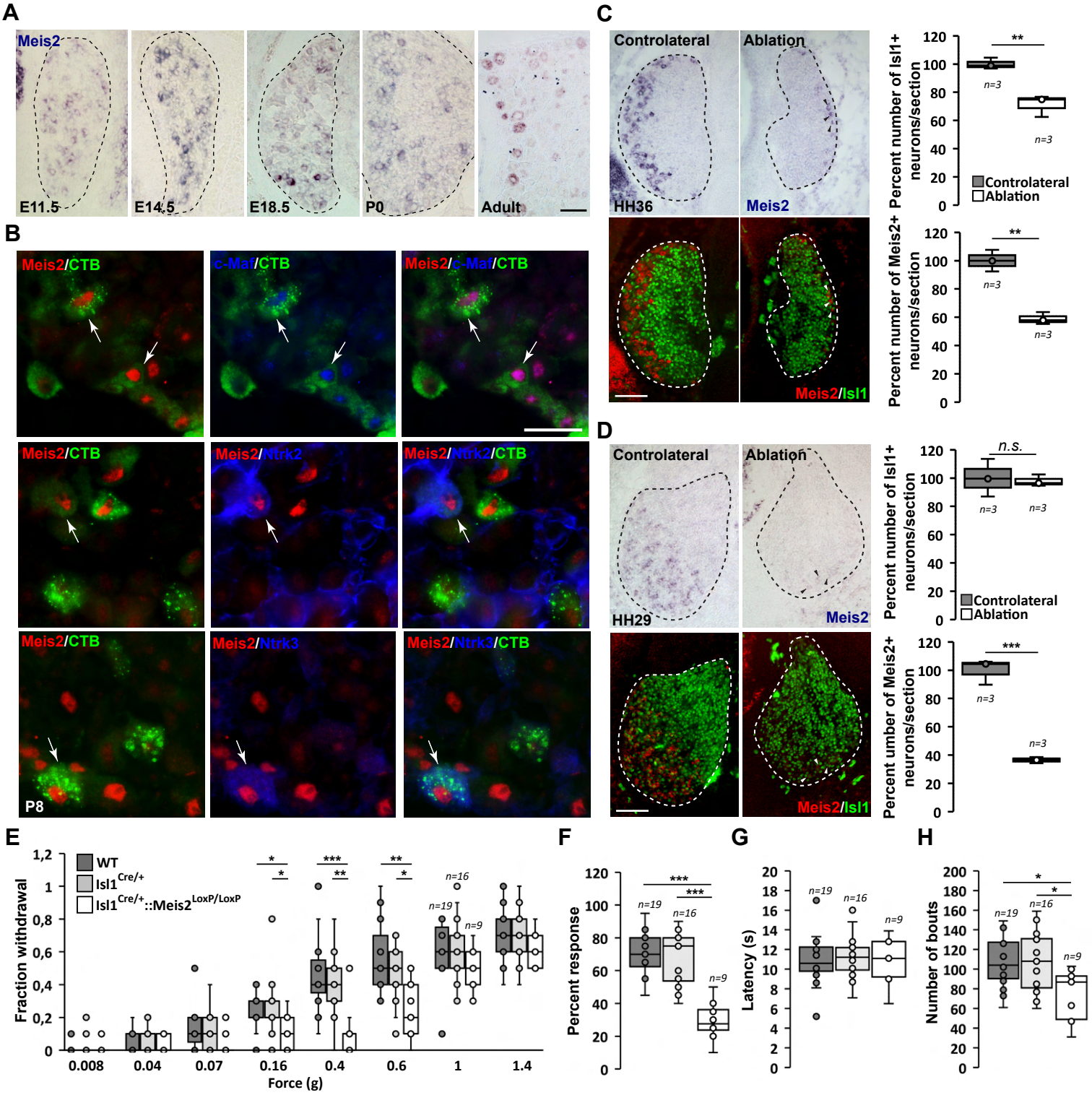
A
Meis2
E11.5
E14.5
E18.5
P0
Adult
B
Meis2/CTB
c-Maf/CTB
Meis2/c-Maf/CTB
Meis2/Ntrk2
Meis2/Ntrk2/CTB
Meis2/Ntrk3
Meis2/Ntrk3/CTB
P8
C
Controlateral
Ablation
HH36
Meis2
Meis2/Isl1
Percent number of Isl1+ neurons/section
Percent number of Meis2+ neurons/section
Controlateral
Ablation
n=3
D
HH29
n.s.
Percent umber of Meis2+ neurons/section
E
WT
Isl1Cre/+
Isl1Cre/+::Meis2LoxP/LoxP
Fraction withdrawal
Force (g)
0.008
0.04
0.07
0.16
0.4
0.6
1
1.4
n=19
n=16
n=9
F
Percent response
G
Latency (s)
H
Number of bouts

Figure 1

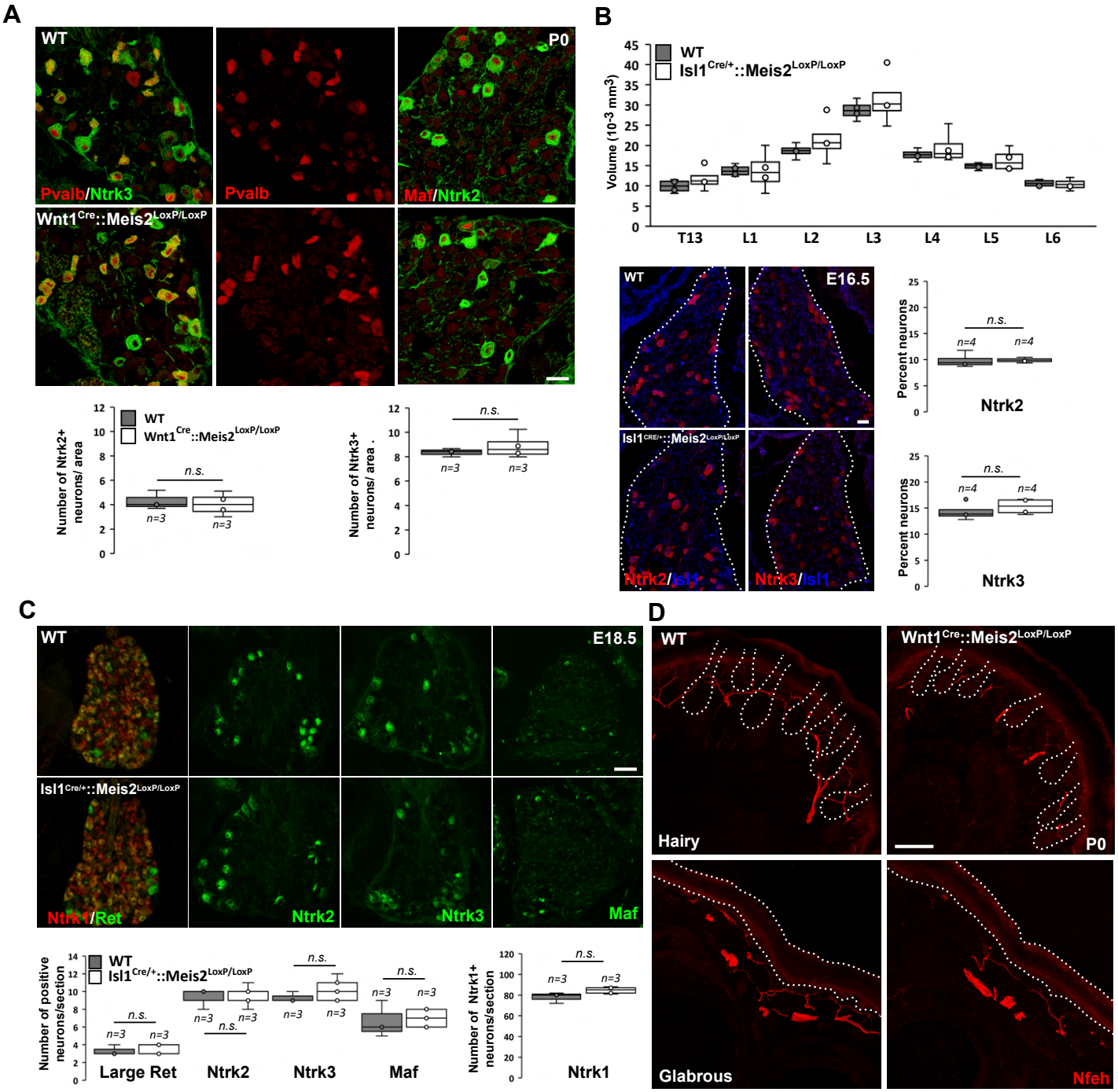
A
WT
P0
Pvalb/Ntrk3
Pvalb
Maf/Ntrk2
Wnt1Cre::Meis2LoxP/LoxP
WT
Wnt1Cre::Meis2LoxP/LoxP
Number of Ntrk2+ neurons/ area
Number of Ntrk3+ neurons/ area
n.s.
n=3
B
WT
Isl1Cre/+::Meis2LoxP/LoxP
Volume (10-3 mm3)
T13
L1
L2
L3
L4
L5
L6
WT
E16.5
Isl1CRE/+::Meis2LoxP/LoxP
Ntrk2/Isl1
Ntrk3/Isl1
Percent neurons
n=4
Ntrk2
Ntrk3
C
WT
E18.5
Isl1Cre/+::Meis2LoxP/LoxP
Ntrk1/Ret
Ntrk2
Ntrk3
Maf
Number of positive neurons/section
Large Ret
Ntrk2
Ntrk3
Maf
Number of Ntrk1+ neurons/section
Ntrk1
D
WT
Wnt1Cre::Meis2LoxP/LoxP
Hairy
P0
Glabrous
Nfeh

Figure 2

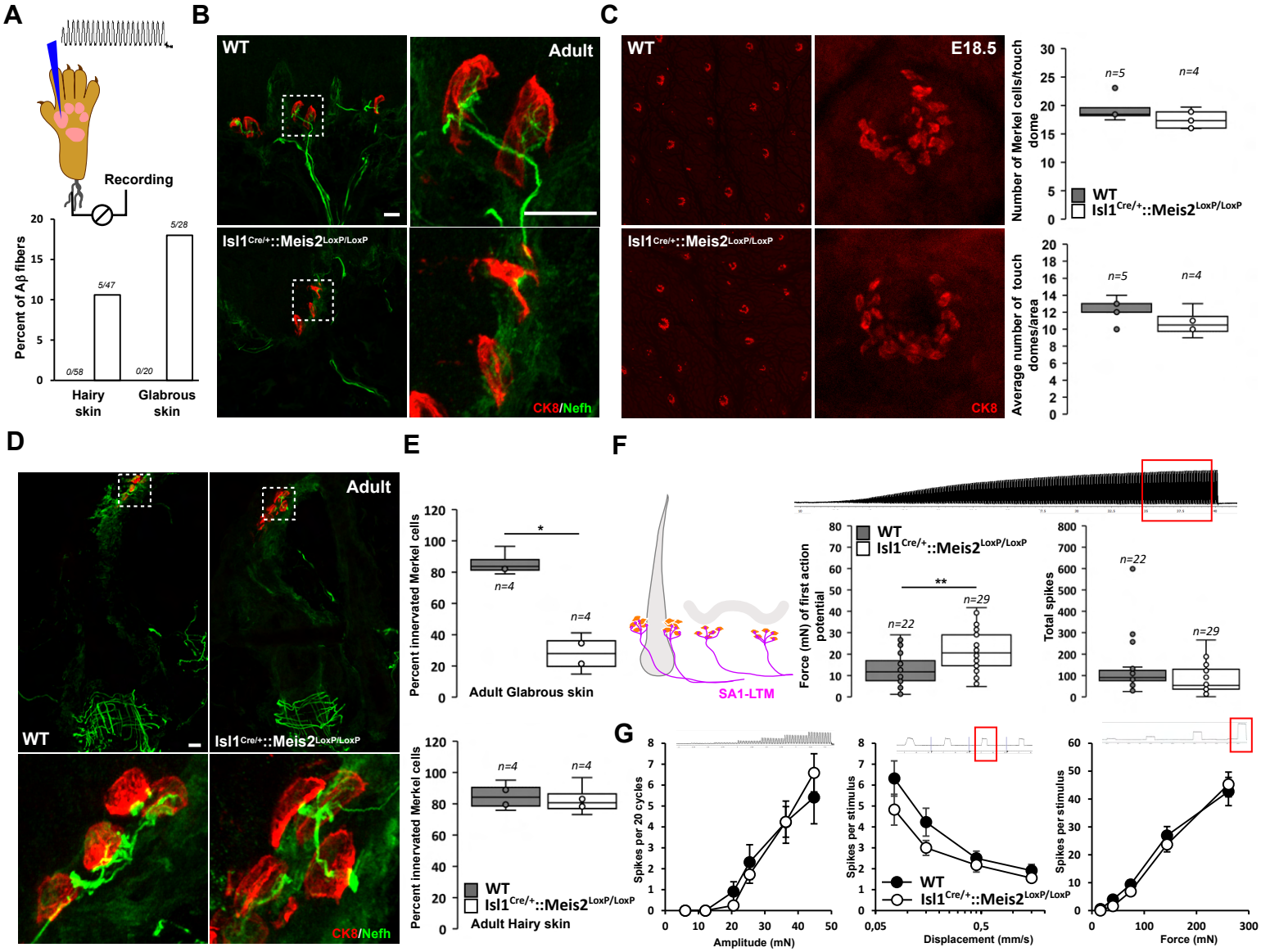
A
Recording
Percent of Aβ fibers
0/58
5/47
0/20
5/28
Hairy skin
Glabrous skin
B
WT
Adult
Isl1Cre/+::Meis2LoxP/LoxP
CK8/Nefh
C
WT
E18.5
Isl1Cre/+::Meis2LoxP/LoxP
CK8
Number of Merkel cells/touch dome
Average number of touch domes/area
n=5
n=4
WT
Isl1Cre/+::Meis2LoxP/LoxP
D
WT
Adult
Isl1Cre/+::Meis2LoxP/LoxP
CK8/Nefh
E
Percent innervated Merkel cells
Adult Glabrous skin
Adult Hairy skin
SA1-LTM
F
Force (mN) of first action potential
Total spikes
n=22
n=29
G
Spikes per 20 cycles
Amplitude (mN)
Spikes per stimulus
Displacement (mm/s)
Force (mN)

Figure 3

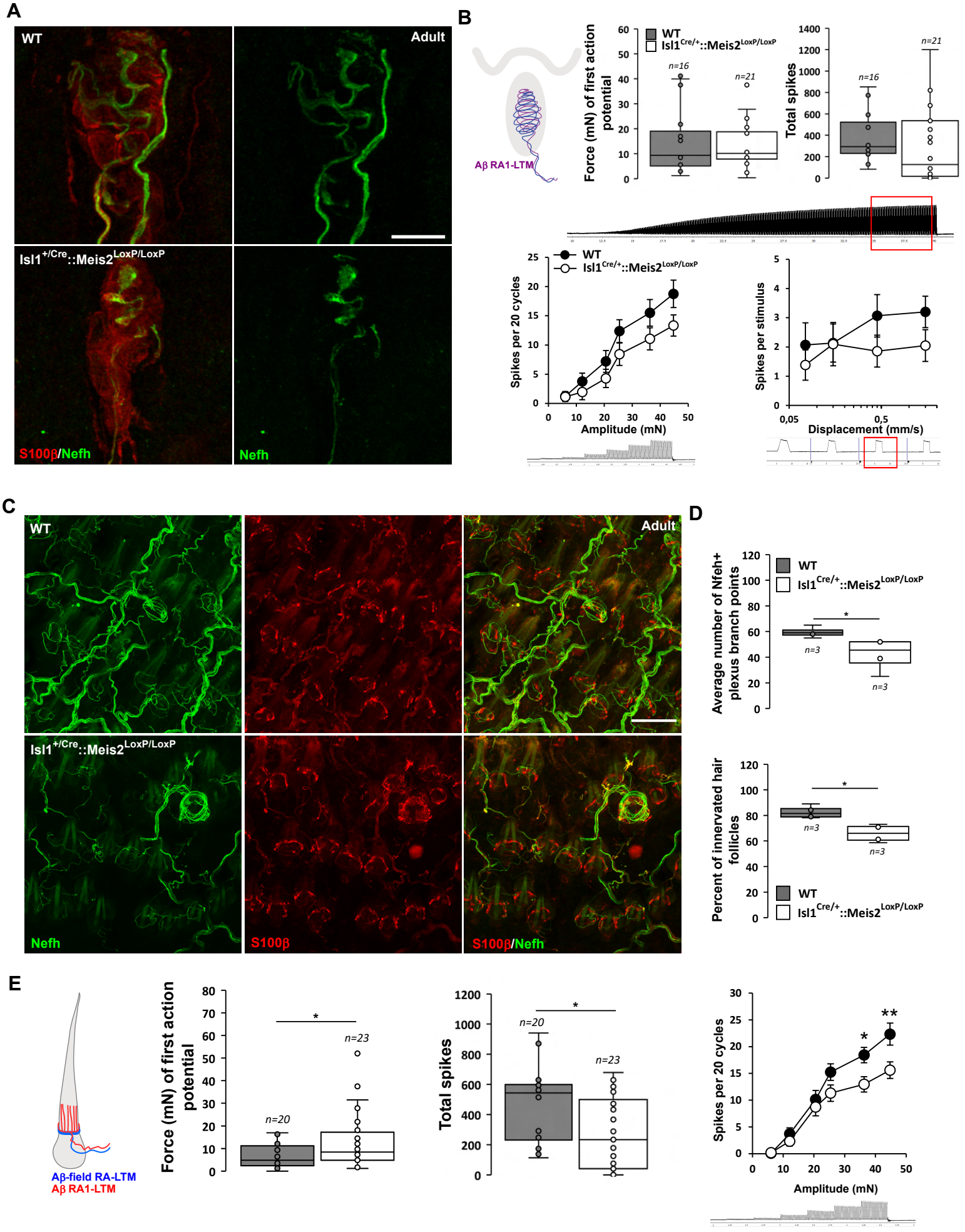
A
WT
Adult
Isl1+/Cre::Meis2LoxP/LoxP
S100β/Nefh
Nefh
B
Aβ RA1-LTM
Force (mN) of first action potential
WT
Isl1Cre/+::Meis2LoxP/LoxP
n=16
n=21
Total spikes
Spikes per 20 cycles
Amplitude (mN)
Spikes per stimulus
Displacement (mm/s)
C
WT
Adult
Isl1+/Cre::Meis2LoxP/LoxP
Nefh
S100β
S100β/Nefh
D
Average number of Nfeh+ plexus branch points
Percent of innervated hair follicles
n=3
E
Aβ-field RA-LTM
Aβ RA1-LTM
Force (mN) of first action potential
n=20
n=23
Total spikes
Spikes per 20 cycles
Amplitude (mN)

Figure 4