LETTERS

A stomatin-domain protein essential for touch sensation in the mouse

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Touch and mechanical pain are first detected at our largest sensory surface, the skin. The cell bodies of sensory neurons that detect such stimuli are located in the dorsal root ganglia, and subtypes of these neurons are specialized to detect specific modalities of mechanical stimuli. Molecules have been identified that are necessary for mechanosensation in invertebrates but so far not in mammals. In *Caenorhabditis elegans*, *mec-2* is one of several genes identified in a screen for touch insensitivity and encodes an integral membrane protein with a stomatin homology domain[1]. Here we show that about 35% of skin mechanoreceptors do not respond to mechanical stimuli in mice with a mutation in stomatin-like protein 3 (SLP3, also called Stoml3), a mammalian *mec-2* homologue that is expressed in sensory neurons. In addition, mechanosensitive ion channels found in many sensory neurons do not function without SLP3. Tactile-driven behaviours are also impaired in *SLP3* mutant mice, including touch-evoked pain caused by neuropathic injury. SLP3 is therefore indispensable for the function of a subset of cutaneous mechanoreceptors, and our data support the idea that this protein is an essential subunit of a mammalian mechanotransducer.

Cutaneous sensory neurons detect mechanical stimuli in the periphery through specialized mechanotransduction ion channels that may form a part of a larger complex of proteins[2,3]. Studies in *C. elegans* have shown that the *mec-2* gene, which encodes a stomatin-domain-containing protein, is required for touch receptor function[1,4]. The MEC-2 protein is postulated to participate in a complex that includes the Deg/ENaC channels, MEC-4 and MEC-10, as well as the MEC-6 protein[2,5,6]. Stomatin (band 7.2b), first isolated from human red blood cells[7,8], is the prototypical stomatin-domain protein[9,10] expressed in mouse dorsal root ganglion (DRG) neurons[9]. Electrophysiological recordings in stomatin mutant mice indicate a mild impairment but not a complete loss of sensory neuron mechanosensitivity (C. Martinez-Salgado, A.B., C.W. and G.R.L., unpublished observations). We identified the complementary DNA of SLP3 (ref. 11), an integral membrane protein, as being expressed by DRG neurons. SLP3 and stomatin have similar degrees of sequence identity to MEC-2 (Supplementary Fig. 1). *In situ* hybridization and quantitative reverse transcriptase-mediated polymerase chain reaction (RT–PCR) showed *SLP3* messenger RNA to be expressed by all DRG neurons and to be selectively expressed in neuronal tissues (Supplementary Fig. 2).

We generated *SLP3* mutant mice (Supplementary Fig. 3) that were viable and fertile. To test mechanoreceptor function we used an *in vitro* skin nerve preparation to record from single cutaneous sensory neurons in the saphenous nerve (single-unit recording)[12]. Normally more than 90% of sensory fibres innervating the skin are mechanoreceptors. Mechano-insensitive fibres can be identified only with an electrical search technique (Fig. 1a)[13] (see Methods). With this technique the conduction velocity of single units was measured, permitting their classification as Aβ fibres, Aδ fibres or unmyelinated C fibres. For each single unit an extensive search was made for its mechanosensitive receptive field. In contrast with wild-type littermates, 30 and 40% of Aδ and Aβ fibres, respectively, lacked all mechanosensitivity in $SLP3^{-/-}$ mice (Fig. 1a). Among C fibres we observed no change in mechanoreceptive properties (Fig. 1a, d). Sensory axons without mechanosensitivity appeared otherwise normal (Supplementary Fig. 4a).

Aβ and Aδ fibres can be classified into four major mechanoreceptor types[14], three of which are low-threshold mechanoreceptors (Supplementary Table 1). Low-threshold mechanoreceptors include slowly adapting mechanoreceptors (SAMs) and rapidly adapting mechanoreceptors (RAMs) and D-hair mechanoreceptors. The fourth group, high-threshold mechanoreceptors, are often referred to as Aδ-fibre mechanonociceptors. We went on to characterize the remaining functional mechanoreceptors in the *SLP3* mutant (Fig. 1b, and Supplementary Table 1). If any one of the above receptors selectively lost mechanosensitivity, a shift in the proportion of RAMs and SAMs in the Aβ range or Aδ-fibre mechanonociceptors, and D hairs in the Aδ range, would be expected. In fact the relative incidence of these mechanoreceptors was unchanged (Fig. 1b, Supplementary Table 1). Thus, SLP3 seems to be required for the function of 30–40% of the myelinated mechanoreceptors regardless of receptor type. It is still possible that mechanoreceptors affected by the *SLP3* mutation represent undetected subpopulations of receptors. For example, in rodents some RAMs are 'field' receptors not driven by hair movement[14,15], and these cannot be distinguished from other RAMs in the *in vitro* skin nerve preparation.

In $SLP3^{-/-}$ mice a large proportion (about 45%) of putative RAM fibres were unresponsive to even very fast movements (1.2–2.9 $\mu m\,ms^{-1}$). These units responded only to brisk tapping of the receptive field with a blunt probe and were designated 'tap' units. Less than 5% of RAM fibres were tap units in control mice (Fig. 1c). The conduction velocities of the more frequent tap fibres (about 40%) encountered in $SLP3^{-/-}$ mice were no different from those of normal mechanosensitive Aβ fibres (tap conduction velocity $15.8 \pm 0.8\,m\,s^{-1}$, compared with $15.7 \pm 1.0\,m\,s^{-1}$ for normal RAM fibres in the $SLP3^{-/-}$ mice; see Supplementary Table 1). The stimulus response properties of SAM fibres in mutant mice were also mildly but significantly impaired (Supplementary Fig. 4c–e). The remaining 'non-tap' RAMs and all D-hair receptors recorded in $SLP3^{-/-}$ mice

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had normal mechanosensitivity (Supplementary Fig. 4). Aδ-fibre mechanonociceptors and C-fibre nociceptors both displayed normal mechanosensitivity in *SLP3* mutant mice (Fig. 1d, and data not shown).

We tested whether the lack of mechanosensitivity in *SLP3* mutants is caused by a loss of sensory neurons, by a withdrawal of axons from the peripheral nerve or by changes in skin innervation. An anatomical analysis using light and electron microscopy revealed no loss of sensory fibres or their terminal endings in the skin of *SLP3* mutant mice (Supplementary Fig. 5).

We next tested whether SLP3, like *C. elegans* MEC-2 (ref. 4), is required for the generation of mechanosensitive currents. The neurites and cell soma of acutely isolated mouse sensory neurons possess mechanosensitive channels[16–18], and neuritic currents are activated by displacements of less than 750 nm with latencies of about 600 μs (ref. 16). With the use of such stimuli, more than 95% of isolated sensory neurons possessed one of three mechanically activated currents, rapidly adapting (inactivation in less than 2 ms), intermediate-adapting (inactivation in less than 50 ms) and slowly adapting (no adaptation during a 230-ms stimulus) (Fig. 2a). About 36% of sensory neurons from $SLP3^{-/-}$ mice had no current response to mechanical stimulation of their neurites, in contrast with less than 5% in control cultures (Fig. 2b). The proportion of cells that displayed slowly adapting or rapidly adapting mechanically activated currents was slightly reduced, indicating that SLP3 might be required for both types of current. The amplitude and kinetics of the mechanosensitive current in the remaining $SLP3^{-/-}$ neurons were indistinguishable from those of control neurons (Supplementary Table 2). Next we transfected *SLP3* mutant neurons with a mouse *SLP3* cDNA tagged at the carboxy terminus with green fluorescent protein (SLP3-C–EGFP). Fluorescently labelled SLP3 was observed in a punctate pattern in the newly grown neurites starting at 15 h in culture, and almost all labelled neurons had a mechanosensitive current (17 out of 18 cells; Fig. 2b, c, and Supplementary Table 2). Control EGFP constructs (Fig. 2c) had no effect on the incidence of mechanosensitive currents (four out of nine labelled cells had no mechanosensitive current).

We observed no change in the properties of voltage-gated channels in *SLP3* mutant neurons (data not shown). However, *SLP3* mutant neurons had a slightly more depolarized resting membrane potential than wild-type neurons (-56.6 ± 1.5 mV in $SLP3^{-/-}$ neurons in comparison with -62.0 ± 1.0 mV in $SLP3^{+/+}$ neurons; $P < 0.005$, Student's *t*-test). However, the resting membrane potential of

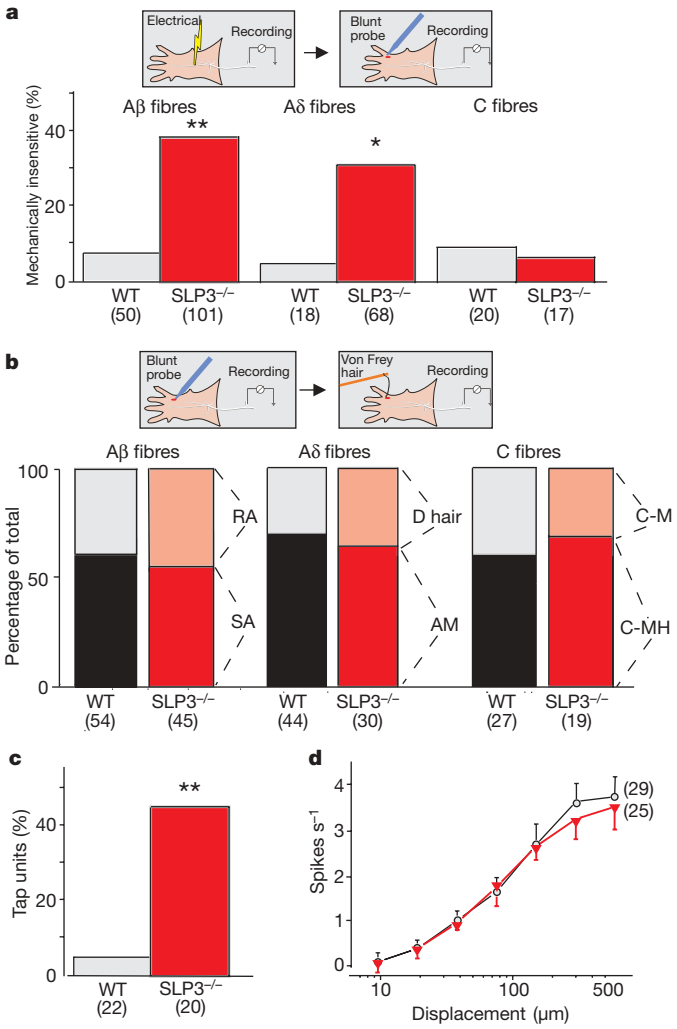

Figure 1 | Mechanically insensitive primary afferents. **a**, The electrical search protocol is illustrated in the insets. Note the increase in mechanically insensitive A fibres in $SLP3^{-/-}$ mice (asterisk, $P < 0.05$ (Aδ); two asterisks, $P < 0.005$ (Aβ); χ^2 test), but no change in mechanosensitive C fibres; $n = 18$–31 mice per genotype. WT, wild-type. **b**, Using the illustrated mechanical search protocol no significant difference ($P > 0.05$; χ^2 test) in the proportions of mechanoreceptor types in Aβ, Aδ (AM) and C fibres was found; $n = 8$–10 mice per genotype. RA, rapidly adapting; SA, slowly adapting; C-M, C-mechanonociceptor; C-MH, C-mechanoheat nociceptor. **c**, Increase in the proportion of RAM fibres in $SLP3^{-/-}$ with a tap response (two asterisks, $P < 0.005$; χ^2 test). **d**, Normal stimulus response functions of C-fibre nociceptors in $SLP3^{-/-}$ mice. Black circles, $SLP3^{+/+}$; red triangles, $SLP3^{-/-}$. The number of neurons recorded is indicated in parentheses in each panel. Error bars indicate s.e.m.

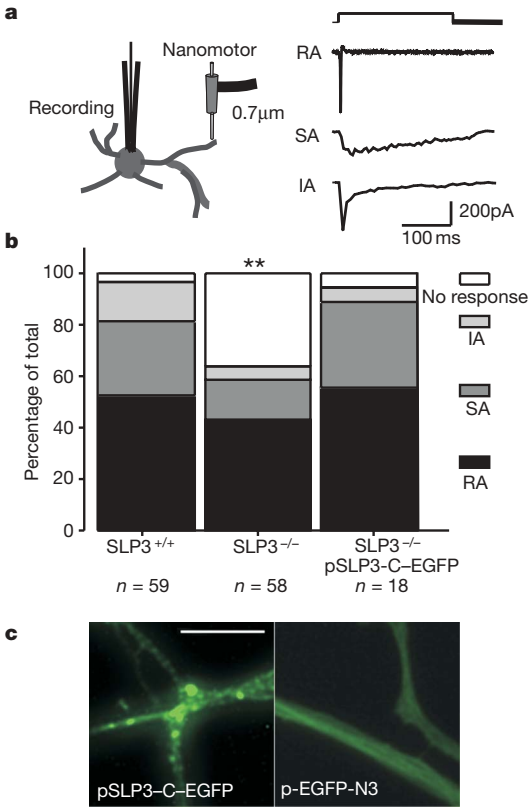

Figure 2 | SLP3 is required for the expression of mechanosensitive currents in subsets of DRG neurons. **a**, Three types of mechanically activated current, designated rapidly adapting (RA), slowly adapting (SA) or intermediate-adapting (IA), observed by using displacement stimuli (750 nm) of the neurite. **b**, Stacked histogram showing proportions of sensory neurons with the different mechanically activated currents from $SLP3^{+/+}$, $SLP3^{-/-}$ and $SLP3^{-/-}$ mice transfected with an EGFP-tagged *SLP3* cDNA. Note a significant increase in the proportion of neurons lacking a mechanosensitive current in $SLP3^{-/-}$ neurons (two asterisks, $P < 0.005$; χ^2 test). Mechanically activated currents were restored to normal proportions in cells expressing SLP3-C–EGFP protein. The numbers of cells tested are indicated. **c**, Left: cells transfected with pSLP3-C–EGFP showed labelled SLP3 in puncta throughout the neurites. Right: expression of EGFP in sensory neurites. Scale bar, 5 μm.

SLP3$^{-/-}$ neurons with or without mechanosensitive current was not different (Supplementary Table 2).

Mutations in genes that encode the acid-sensitive ion channels ASIC2a, ASIC2b and ASIC3 alter mechanoreceptor sensitivity *in vivo*[19–22]. We therefore tested whether the SLP3 protein can also interact with members of the ASIC family. We showed that ASIC2a, ASIC2b and ASIC3 are immunoprecipitated with SLP3 when overexpressed in HEK-293 cells (Fig. 3a, b). TRP-V1, a thermosensitive member of the *trp* channel family[23], did not immunoprecipitate with SLP3 (data not shown). HEK-293 cells possess amiloride-sensitive proton-gated currents with properties similar to those described for heteromeric ASIC channels[24] (Fig. 3c, and Supplementary Fig. 6). When rat *SLP3* was expressed in HEK-293 cells, there was a clear inhibition of proton gating of the endogenously expressed ASICs that was similar to that reported for stomatin[25] (Fig. 3c, d). We also found that sustained proton-gated currents were larger in *SLP3* mutant sensory neurons (Fig. 3e). The proton-gated currents in the large and medium-sized cells tested were sensitive to benzamil, an agent that blocks ASICs, and the cells were insensitive to capsaicin (data not shown). The increased amplitude of proton-gated currents in *SLP3*$^{-/-}$ neurons was observed in cells with and without mechanosensitive currents (Fig. 3f). Thus, SLP3 can physically associate and modulate channels that have been implicated in mechanotransduction.

Taken together, these results indicated that *SLP3* mutant mice might have reduced tactile acuity, but no suitable behavioural tests were available to test this idea in mice. We therefore developed new behavioural tests to examine this possibility (Fig. 4a, and Supplementary Fig. 7). We tested the ability of mice to detect and respond to a grating cue placed on the floor of an arena in complete darkness (see Methods for details). Control C57BL/6N mice showed significant interest in all three grids tested, as reflected by a positive ratio of distance travelled in the area of the cue compared with no cue (Fig. 4b). *SLP3*$^{-/-}$ mice (C57BL/6N genetic background) did not show any significant tendency ($P > 0.05$, paired *t*-test) to prefer the finest grids used in the task (250 and 500 μm), but *SLP3*$^{-/-}$ mice did respond significantly to the coarsest grid used (750 μm) (Fig. 4b, c). Thus, the loss of mechanoreceptor function in *SLP3*$^{-/-}$ mice correlates with a reduction in tactile discrimination capability. *SLP3*$^{-/-}$ mice performed largely normally in a motor task (the accelerating rotorod test), which indicates that proprioception is not severely affected in these mice (Supplementary Fig. 8).

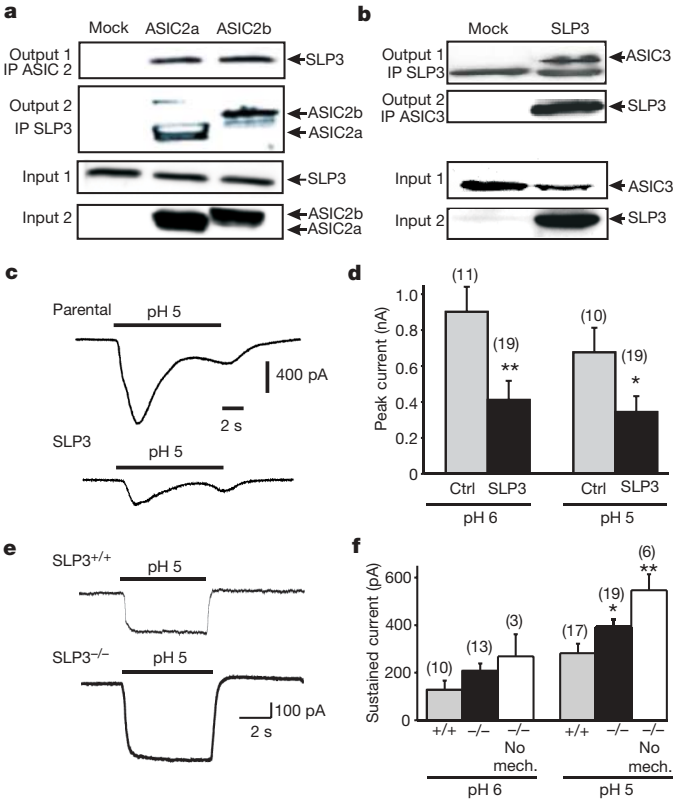

Figure 3 | Association of SLP3 with ASIC ion channels. **a**, Interaction of SLP3 with both ASIC2a and ASIC2b. HEK-293 cells expressing Myc-tagged SLP3 (input 1) were transfected with control (mock), Flag-tagged ASIC2a or Flag-tagged ASIC2b (input 2). Immunoprecipitates containing ASIC2a or ASIC2b were blotted and probed to detect SLP3 (output 1). Both ASIC2a and ASIC2b were detected in lysates immunoprecipitated with anti-Myc (output 2). IP, immunoprecipitation. **b**, HEK-293 cells expressing a Flag-tagged ASIC3 (input 1) were transfected with control (mock) or Myc-tagged SLP3 (input 2). Immunoprecipitates containing ASIC3 and SLP3 were detected in western blot (output 1). ASIC3 is detected in immunoprecipitates containing SLP3 (output 2). **c**, Proton-gated inward currents shown in parental HEK-293 cells and HEK-293 cells stably expressing SLP3. **d**, Mean amplitude of proton-gated current in control and SLP3-expressing HEK-293 cells. **e**, Proton gated currents in large DRG neurons from *SLP3*$^{+/+}$ and *SLP3*$^{-/-}$ mice. Amplitude of sustained current at pH 5 was clearly larger in *SLP3*$^{-/-}$ neurons than in wild-type neurons. **f**, All neurons, with a mechanosensitive current or without one (no mech.), had larger proton-gated currents at pH 5 and pH 6. Asterisk, $P < 0.05$; two asterisks, $P < 0.01$; Mann–Whitney *U*-test. Error bars indicate s.e.m.

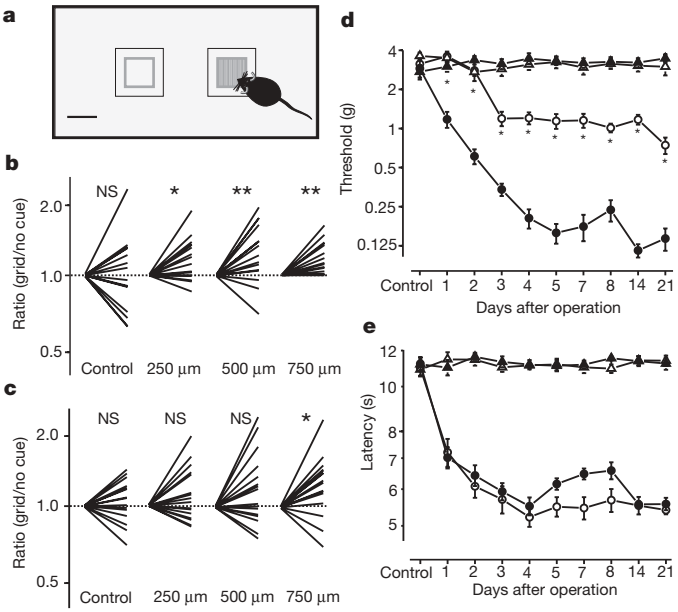

Figure 4 | Tactile driven behaviour is altered in *SLP3* mutant mice. **a**, Schematic drawing of the experimental arrangement, showing how the grid cue was placed in one of two spatially equivalent positions. Scale bar, 4 cm. **b**, The ratio of the distance travelled between no cue and grid cue (no cue set to 1.0) is shown as a single line for each of 16 *SLP3*$^{+/+}$ mice (more than 1.0 indicates more time on the grid; less than 1.0 indicates less time). Control mice spent more time in the vicinity of the cue for all grids tested (asterisk, $P < 0.05$; two asterisks, $P < 0.005$; paired *t*-test). **c**, *SLP3*$^{-/-}$ mice exhibited a significant preference only for the coarsest grid (750 μm). Tests with no cue indicated no preference for either location (control). **d**, Mechanical thresholds (von Frey test) for foot withdrawal plotted before and after the induction of CCI (circles) or sham surgery (triangles). *SLP3*$^{-/-}$ mice (open symbols) developed significantly less mechanical allodynia than controls throughout the entire time course after CCI (asterisk, $P < 0.05$; two-way repeated-measures ANOVA and Bonferroni *t*-test). Further, *SLP3*$^{-/-}$ mice developed mechanical allodynia later than *SLP3*$^{+/+}$ mice (filled symbols) ($P < 0.05$; one-way repeated-measures ANOVA and Dunnett's test). There were no significant changes in sham-operated mice of either genotype ($P > 0.05$; two-way repeated-measures ANOVA). **e**, No significant differences in thermal hyperalgesia between *SLP3*$^{-/-}$ and *SLP3*$^{+/+}$ mice (two-way repeated-measures ANOVA; $P > 0.05$). The symbols used are the same as those in **d**. Error bars in **d** and **e** indicate s.e.m.

Stimulation of low-threshold mechanoreceptors by brushing the skin can, under neuropathic conditions (after nerve injury), lead to intense pain in humans and animals[14,26]. We subjected control C57BL/6N mice and *SLP3*$^{-/-}$ mice (C57BL/6N background) to a unilateral chronic constriction injury of the sciatic nerve (CCI)[26]. Within the first few days, control mice developed a full-blown allodynia with mechanical thresholds for paw withdrawal decreasing from 3 g to less than 0.25 g. In contrast, *SLP3*$^{-/-}$ mice did not show any hyperalgesia until the third day after the lesion, and mechanical thresholds decreased to just above 1 g until the end of the observation period (21 days) (Fig. 4d). No impairment of the CCI-induced thermal hyperalgesia was observed in *SLP3*$^{-/-}$ mice (Fig. 4e). Thus, targeting essential mechanotransduction molecules such as SLP3 might provide a novel peripheral means of neuropathic pain control.

We have shown that SLP3 is absolutely required for the function of many mechanoreceptors in the mouse. In contrast, polymodal nociceptors in the DRG are not significantly affected by the mutation of *SLP3* (Figs 1 and 2). Thus, many but not all sensory neurons require SLP3 for normal mechanoreceptor function and to maintain mechanosensitive channels (Fig. 2, and Supplementary Table 2). Our data are therefore consistent with a model in which about one-third of sensory neurons transduce mechanical stimuli by using mechanosensitive ion channels that depend on SLP3 for their function. SLP3 can physically interact with and modulate the gating of ASIC channels that have previously been implicated in the transduction of mechanical stimuli by sensory neurons[19,22,27]. Our data therefore indicate that SLP3 might act as an essential subunit in a complex that includes these channels or other, as yet unidentified, mechanosensitive channels.

METHODS

See Supplementary Information for detailed methods.

Molecular biology and electrophysiology. The rat *SLP3* cDNA was cloned from mRNA isolated from DRG with the use of similar methods to those described previously[9]. The *SLP3* mutant mouse was generated with standard gene-targeting methods[28]. Single-unit recordings with a skin nerve preparation and patch-clamp experiments with cultured DRG neurons were performed in accordance with previously published procedures[16,29].

Tactile acuity test. The MoTil system (TSE Systems) was used to monitor open-field behaviour of mice in complete darkness. After an acclimatization period of 10 min, the target area (Fig. 4a) was replaced with a tactile cue (grid) and the animals' behaviour was monitored for the next 90 min. The distance travelled in the vicinity of the grid and no cue area was expressed as a ratio with the control area set to a value of 1.0. More time spent in the vicinity of the grid is reflected as a ratio more than 1.0, and less time as less than 1.0. Control experiments in which no cue was placed in either area indicated that the time spent in either area was randomly distributed between ratios more than 1.0 and less than 1.0 (Fig. 4b). Each mouse was tested with one tactile cue, once per day, and the order was randomized. Equal numbers of male and female mice were used.

Sensory neuron transfection. Cells were transiently transfected with the use of the commercially available Nucleofector system (Amaxa Biosystems). In brief, neurons were suspended in 100 μl of Mouse Neuron Nucleofector Solution and 5 μg of plasmid DNA at 22–25 °C. The mixture was transferred to a cuvette for electroporation with program C-13. After electroporation the cell suspension was transferred to 500 μl of RPMI 1640 medium (Gibco) for 10 min at 37 °C. This suspension, supplemented with 10% horse serum, was used to plate the cells onto glass coverslips for recording. The RPMI medium was replaced with the standard DRG medium 3–4 h later.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions C.W., J.H. and G.R.L. performed electrophysiological experiments on *SLP3* mutants. D.R. and A.B. generated the *SLP3* mutant. C.W. characterized the *SLP3* mouse. L.H. and C.W. performed the behavioural experiments. A.E. and R.M. performed biochemical interaction studies, and O.C. and P.H. characterized SLP3 effects in HEK-293 cells. A.B. cloned the rat *SLP3* cDNA. J.H. and A.K. conducted rescue experiments. B.E. performed electron microscopy. H.M. and D.L. conducted neuropathic pain measurements in the *SLP3* mutant mice. G.R.L. conceived and planned experimental studies with help from C.W. and P.A.H. G.R.L. wrote the paper.

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