Supporting Online Material for

The Transcription Factor c-Maf Controls Touch Receptor Development and Function
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Materials and Methods

Immunohistochemistry, in situ hybridization and semi-thin sections
For immunostaining, embryos were fixed in 2% PFA or with Zamboni fixative for 30 min, and DRGs were fixed in Zamboni fixative or 2% PFA for 10 min and embedded in OCT compound. 12 μm frozen sections were blocked and stained as previously described (32). Skin samples were fixed with PFA or Zamboni fixative for 1h or overnight, embedded in gelatin and postfixed for 5h or overnight in 2% PFA. 50 μm vibratome sections were blocked with 1% horse serum in PBX, and incubated for 2-3 days with the primary antibodies diluted in blocking solution supplemented with 0.02% Na-Azide, washed with PBX, incubated with secondary antibodies for 1-2 days, washed with PBX and mounted in Immu-Mount (Thermo Scientific). To visualize Pacinian corpuscles, the fibula with associated corpuscles was dissected freshly and then fixed overnight in 4% PFA. Whole mount immunostaining was performed as described (33). Muscle spindles were identified by staining of intrafusal fibers with anti-Egr3 and with anti-Calbindin antibodies in P0 and P15 animals, respectively. Muscle spindle size was assessed at the equatorial plane by measuring the inner perimeter (periaxial space of Sherrington, delineated by Collagen IV staining) using ImageJ 1.45.

Antibodies used were: rabbit anti-Calbindin (Swant, 1:3000), rabbit anti-Calretinin (Swant, 1:3000), rabbit anti-CGRP (Sigma, 1:5000), mouse anti-Cytokeratin 20 (Santa Cruz), goat anti-Collagen IV (Millipore, 1:1000), rabbit anti-Egr3 (Santa-Cruz, 1:1000), rabbit anti-Fabp7 (Thomas Müller, MDC, 1:5000), guinea pig anti-Isl1/2 (Susan Morton, Tom Jessell, Columbia University, 1:20000), rabbit anti-c-Maf (1:10000), guinea pig anti-c-Maf (1:10000), rabbit anti-MafA (1:10000), mouse anti-Neurofilament (2H3, DSHB, 1:50), mouse anti-Neurofilament (RT97, Abcam, 1:100), chick anti-NF200 (Millipore, 1:20000), rabbit anti-PGP9.5 (Dako, 1:1000), rabbit anti-PV (Swant, 1:3000), goat anti-Ret (R&D Systems, 1:500), rabbit anti-S100 (Dako, 1:1000), rabbit anti-TrkA (Louis Reichardt, UCSF, 1:10000), goat anti-TrkB (R&D Systems, 1:500), goat anti-
TrkC (R&D Systems, 1:500). Cy2-, Cy3- and Cy5-conjugated secondary antibodies were obtained from Dianova. Fluorescence was visualized by laser-scanning microscopy (LSM 700, Carl-Zeiss), using Zen 2009 software. Figures were assembled using Adobe Photoshop and Adobe Illustrator.

For in situ hybridization P15 DRG were freshly embedded into OCT compound, 20 μm frozen sections were processed as previously described (32). Axon and axon diameter quantifications were performed on semi-thin sections of saphenous and interosseous nerves (33). Axon diameter quantifications were performed using Adobe Photoshop and ImageJ.

**Mouse lines**
The c-Maf null and floxed c-Maf alleles were generated by homologous recombination in embryonic stem (ES) cells as described (34). In the c-Maf null allele the c-Maf coding region was replaced by a cassette containing a gap43-LacZ and a self-excision neo cassette (35). In the floxed c-Maf allele, one loxP site was introduced 1547 bp upstream of the ATG, and the second together with a FRT flanked neomycin resistance cassette 411bp downstream of the termination codon (Fig. S4A). Mutant ES cells were injected into blastocysts to generate the mutant mouse strains. The neomycin cassette of the floxed c-Maf allele was removed by crossing F1 mice with FLPe deleter mice (36). Homologous recombination and removal of the neomycin resistance cassette were verified by Southern blot analysis (Fig. S4B). Isl1cre was used to introduce conditional mutation into the c-Maf locus; the Isl1cre mouse strain was kindly provided by Tom Jessell (37). Isl1cre-mediated recombination interfered with c-Maf expression in DRG neurons, but did not affect c-Maf expression in the dorsal horn of the spinal cord or in peripheral glial cells (Fig. S4C-H). Isl1cre c-Maf^flox/- mice survived to adulthood, they were apparently healthy and fertile. Their body weight was mildly (17%) reduced compared to control littermates (Fig. S4I). Compared to their littermates, Isl1cre c-Maf^flox/- mice did, however, perform poorly on a Rotorod test (Fig. S4J). Deficits in mechanosensory function might result in impaired motor-coordination. In addition, c-Maf is also expressed in other neurons and tissues, for instance in a motoneuron subpopulation.
and in neurons of the ventral spinal cord. As yet uncharacterized phenotypes in such cells might contribute to the behavioral deficits in conditional c-Maf mutant mice.

**Quantifications and statistical analysis**
All neuron counts were performed on at least 10 sections of three or more animals/genotype. Other quantifications were done using at least three animals of each genotype. Unpaired Student’s two-tailed t-tests were performed using Graphpad Prism 5 to determine significance. Average and SEM are displayed.

**In vitro skin-nerve preparation**
The skin-nerve preparation was used as described (20). Single units were isolated with a mechanical search stimulus applied with a glass rod and classified by conduction velocity, von Frey hair thresholds and adaptation properties to suprathreshold stimuli (20). Mechanical ramp-and-hold stimuli were applied with a computer-controlled nanomotor® (Kleindieck). The probe was a stainless steel metal rod with a flat circular contact area of 0.8 mm. To determine the proportion of mechanoinsensitive fibers, the receptive fields of randomly chosen nerve fibers were first located with an electrical stimulus and then probed for mechanosensitivity using a glass rod. To test the effect of linopirdine (Invitrogen), standardized supra-threshold displacement stimuli were applied to the receptive field. Stimuli duration was 2 seconds, separated by 58 seconds interruption. The overall stimulation period was 12 minutes. Linopirdine was added to a stainless steel ring that isolated receptive fields from the surrounding bath and prevented washout of the drug during the experiment.

The signal driving the movement of the mechanical stimulators and raw electrophysiological data were recorded with a Powerlab 4/30 system and Labchart 7.1 software (AD Instruments), and spikes were discriminated off-line using the spike histogram extension of the software.

**Analysis of paw hair morphology and mechanosensory innervation**
Electrophysiological analysis was performed on neurons innervating the lower leg and the paw. Hair of the dorsal aspect of the paw skin is distinct from other hair types. To
define this more closely, hair was plugged and analyzed by dark field microscopy (Fig. S6D). This showed that hair from the paw is shorter than guard,awl or zigzag hair in the back and is structurally similar to tail hair. Only one hair type is present in the paw that is innervated by circumferential and type I-III lanceolate endings.

**Human psychophysics**

The limits of spatial resolution in the fingertip were determined with a two-interval forced-choice tactile grating orientation test which was performed with the Tactile Acuity Cube™ and TAG JVP Domes™ (Med-Core TAG™) using a transformed-rule up and down trial design. Subjects had to detect whether a grating was pressed in a longitudinal or transverse orientation against their finger. Thresholds (79% probability) were calculated from the last ten of 15 turning points. Tactile acuity, i.e. the limits of spatial resolution in the fingertip, was not significantly different in c-MAF carriers and healthy subjects (Fig. S6J).

Vibrotactile thresholds at different frequencies were determined with an ascending method of limits approach (Fig. 3D) using a custom-made device. Briefly, mechanical vibration stimuli of increasing amplitude (0-45 µm within 30 seconds) were delivered to the nail bed of the little finger with a piezo actuator (Physik Instrumente PI) controlled by Powerlab 4/30 and LabChart 7.1 software (both AD-Instruments). The probe was made of glass and had a flat circular contact area with a diameter of 5 mm. Subjects signaled the detection of vibratory stimuli by pressing a button (Fig. 3D, top). The little fingers of both hands were tested over a range of frequencies (5, 10, 20, 40, 80, 160, and 240 Hz) and each frequency was tested in triplicates. To avoid acoustic perception of vibratory stimuli white noise was played to the subjects via headphones during the time of testing.

**Microarray and data analysis**

Total RNA was isolated from P15 L3, L4, L5 and L6 DRGs using TRIzol (Invitrogen) and the RNeasy kit (Qiagen). Total RNA was labeled using the Illumina TotalPrep RNA Amplification Kit (Ambion) and hybridized to MouseRef-8 v2 Sentrix BeadChip arrays
(Illumina) as specified by the manufacturer. RNA of a single animal was used to generate cRNA for one chip. Quantile normalization and background subtraction were applied using Illumina Genome Studio, and microarray data was analyzed using the Partek Genomics Suite. Among the significantly deregulated genes (Benjamini Hochberg false discovery rate (FDR) <0.05), we identified c-Maf and others listed in Supporting Table 1. However, even among the non-significant changes (FDR >0.05, ANOVA p-value <0.001), we identified several genes that were indeed deregulated, as verified by RT-PCR or in situ hybridization (Supporting Table 1). MIAME compliant data was submitted to Gene Expression Omnibus (GEO).

**Animal behavior**

The Rotarod test was performed essentially as described (38). In brief, the experiment was performed on Ugo Basile Rota-Rod 47600. Acceleration was set to ramp from 4 to 40 rpm in 500s. The ridges on the plastic drums have been covered with bicycle inner tubing. Mice remaining on the apparatus after 500s were removed. Four trials per day were performed on 4 consecutive days, with 10 min breaks between trials. The data was analyzed by two-way ANOVA with Bonferroni post-test.

**Real Time PCR analysis**

RNA from P15 DRGs was isolated using TRIzol (Invitrogen) in accordance with the manufacturer’s instructions. cDNA was synthetized using SuperScript III (Invitrogen). Real time PCR was performed with Absolute qPCR SYBR Green mix (AbGene) on a Biorad C1000 Thermal cycler. Ct-values were normalized against Ube2l3.

Primer sequences:

- **qRet_up**: AATTGAGTCCCTTTTCGATCACA
- **qRet_lw**: TGCCTCCAGGTCACAATCT
- **qTrkB_up**: GAGAGACAGATCTCCGCTCACT
- **qTrkB_lw**: CGTGGAGGGGATTTCATTACTT
- **qCav32_up**: GCGACCCCCTTTGTGC
- **qCav32_lw**: CCTTCCAGGGACTCTGCGTGC
- **qPiez2_up**: TCTCAACCTGACCGGAAGTAG
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<tr>
<td>qMafA_up</td>
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<tr>
<td>qMafA_lw</td>
<td>CGCTTCTGTTTCAGTGGATG</td>
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<td>qKcnh5_up</td>
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<td>qKcnh4_up</td>
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<td>qKcna1_up</td>
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<td>qNefh_lw</td>
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<td>ATGGAGTTGCCAGCGACTAGGTA</td>
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<tr>
<td>Ube2l3_up</td>
<td>GGTCTGCTGCCCAGTCATTAGTGC</td>
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<tr>
<td>Ube2l3_lw</td>
<td>GGGTCTACCACTGCTATGAG</td>
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Table S1. Microarray expression analysis of control and c-Maf mutant DRGs. Gene expression analysis was performed using Illumina bead arrays and RNA obtained from entire P15 DRGs of control and c-Maf mutant mice that contain in addition to mechanoreceptors other neuronal and non-neuronal cells. Displayed are gene symbols and definitions, ANOVA p-values, false discovery rates (FDR) and fold changes (FC). We verified the deregulated expression of a number of genes by qPCR and/or in situ hybridization. To quantify qPCR and in situ hybridization results, fold change differences (qPCR FC) and the number of neurons per section in P15 control and c-Maf mutant DRGs were determined, respectively. n.d., not determined.

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<th>SYMBOL</th>
<th>DEFINITION</th>
<th>p-value</th>
<th>FDR</th>
<th>FC</th>
<th>qPCR FC</th>
<th>Neuron # in control DRG</th>
<th>Neuron # in c-Maf mutant DRG</th>
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<td>c-Maf</td>
<td>avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog</td>
<td>4.06E-09</td>
<td>0.0001</td>
<td>-8.99</td>
<td>n.d.</td>
<td>4.6 ± 0.8</td>
<td>7.9 ± 0.4</td>
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<td>Kcnq4</td>
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<td>2.75E-08</td>
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<td>Lgi2</td>
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<td>0.0053</td>
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<td>n.d.</td>
<td>11.6 ± 1.3</td>
<td>5.9 ± 0.8</td>
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<td>n.d.</td>
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<td>-6.09±1.70</td>
<td>6.8 ± 0.9</td>
<td>1.3 ± 0.3</td>
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<td>netrin G2 (Nntg2), transcript variant b</td>
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<td>0.0237</td>
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<td>n.d.</td>
<td>8.9 ± 0.9</td>
<td>6.0 ± 0.6</td>
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<td>Nudt7</td>
<td>nudix (nucleoside diphosphate linked moiety X)-type motif 7</td>
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<td>0.0481</td>
<td>-1.86</td>
<td>n.d.</td>
<td>8.9 ± 0.9</td>
<td>6.0 ± 0.6</td>
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<td>Cpne6</td>
<td>copine VI</td>
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<td>0.0481</td>
<td>-1.36</td>
<td>n.d.</td>
<td>8.9 ± 0.9</td>
<td>6.0 ± 0.6</td>
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<td>Gdap111</td>
<td>ganglioside-induced differentiation-associated protein 1-like 1</td>
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<td>0.0481</td>
<td>-1.13</td>
<td>n.d.</td>
<td>8.9 ± 0.9</td>
<td>6.0 ± 0.6</td>
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<td>Pvr4</td>
<td>poliovirus receptor-related 4</td>
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<td>0.0481</td>
<td>-1.33</td>
<td>n.d.</td>
<td>8.9 ± 0.9</td>
<td>6.0 ± 0.6</td>
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<td>Slc35c1</td>
<td>GDP-fucose transporter 1</td>
<td>2.06E-05</td>
<td>0.0481</td>
<td>-1.19</td>
<td>n.d.</td>
<td>8.9 ± 0.9</td>
<td>6.0 ± 0.6</td>
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<td>Rgs6</td>
<td>regulator of G-protein signaling 6 (Rgs6)</td>
<td>4.95E-05</td>
<td>0.0772</td>
<td>-1.58</td>
<td>n.d.</td>
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<td>Calb2</td>
<td>calbindin 2</td>
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<td>0.0889</td>
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<td>n.d.</td>
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<td>6.0 ± 0.6</td>
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<td>Nefh</td>
<td>Neurofilament heavy</td>
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<td>0.0889</td>
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<td>netrin G1</td>
<td>0.000119</td>
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<td>Crygs</td>
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<td>0.000685</td>
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<td>-1.86±0.06</td>
<td>12.1 ± 0.3</td>
<td>5.1 ± 1.3</td>
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Fig. S1.

c-Maf is expressed in spinal and DRG neurons. Spinal (A) and DRG neurons (B,C) in mice express c-Maf. In the dorsal spinal cord, neurons co-expressing c-Maf and MafA are particularly abundant in layer III. (B,C) MafA$^+$ neurons co-express c-Maf and Ret at E13.5 and P0. (D) Quantification demonstrates that the vast majority of MafA$^+$ neurons co-express c-Maf and Ret at all developmental stages. (E) Summary of the proportions of early and late c-Maf$^+$ DRG neurons co-expressing Ret. (F) Expression of c-Maf in chick DRGs. (G) Expression of c-Maf in human DRGs obtained from two donors. Lower panels show higher magnification of the upper panels. In all analyzed organisms, c-Maf was expressed in large neurons, as well as in very small cells that appear to correspond to satellite glia. Bars (A,G) 100 μm, (B,C) 30 μm, (F) 50 μm.

Fig. S2.

Characterization of c-Maf expression in murine DRG neurons. Analysis of c-Maf expression in DRG neurons at P15 using the indicated antibodies. (A) All c-Maf$^+$ neurons co-express NF200. (B-F) Note that c-Maf$^+$ neurons can co-express Ret, TrkB, TrkC, CB, and CR, and combinations thereof. See also Fig. 1J for the summary of c-Maf$^+$ neuron subtypes. Bars 30 μm.

Fig. S3.

Characterization of c-Maf expression in neurons and glia of the mouse DRG. Analysis of c-Maf expression in DRG neurons at P15 using the indicated antibodies. (A-E) Note that c-Maf$^+$ neurons can co-express TrkC, PV, Ret, TrkA, CGRP but not IB4, TH and peripherin. See also Fig. 1J for the summary of c-Maf$^+$ neuron subtypes. (F) In addition, the Fabp7$^+$ satellite glia also expresses c-Maf. Bars 30 μm.

Fig. S4.

Targeted mutation of c-Maf and characterization of conditional c-Maf mutant mice. (A) Schematic display of the wildtype c-Maf locus, and of the null and floxed c-Maf alleles generated by homologous recombination in embryonic stem cells. In the null allele, c-Maf sequences are replaced by a cDNA encoding membrane-associated β-
galactosidase. Expression of β-galactosidase in glial membranes ensheathing all DRG neurons interferes with an unambiguous identification of β-galactosidase+ neuronal membranes. (B) Southern blot analysis of EcoRV digested DNA from mice carrying c-Maf<sup>flox/flox</sup>, c-Maf<sup>flox/+</sup> and c-Maf<sup>lacZ/+</sup> alleles. (C-H) Immunohistological analysis of DRG neurons and satellite glia (C-F) and spinal neurons (G,H) from conditional c-Maf mutants using antibodies against c-Maf, neurofilament (2H3), and Isl1/2. This experiment shows that Isl1<sup>cre</sup>-mediated recombination of the c-Maf locus occurs prior to E11.5 in DRG neurons, but the locus is not recombined in the dorsal spinal cord or in glial cells. (I) Isl1<sup>cre</sup> c-Maf<sup>flox/lacZ</sup> mice survive to adulthood and are fertile. Adult mutants displayed a 17% reduction in body weight compared to their control littermates, but no difference in body weight was apparent at P15. (J) Rotarod test of control and adult Isl1<sup>cre</sup> c-Maf<sup>flox/lacZ</sup> mice demonstrates behavioral changes of mutant mice compared to their control littermates. Bars (C-F) 50 μm, (G,H) 100 μm.

**Fig. S5.**

Electrophysiological properties of RAMs, SAMs, D-hair mechanoreceptors and Aδ-nociceptors in c-Maf mutant mice. Quantification of (A) NF200<sup>+</sup> DRG neurons and (B) of myelinated axons in the saphenous nerve of control and c-Maf mutant mice. (C) Von Frey thresholds (median with interquartile range) of various mechanoreceptor subtypes (** p<0.01, Mann-Whitney test). (D,E) Spike trains of mechanically evoked responses from RAMs, SAMs, D-hairs (DH) and Aδ-nociceptors (AM) from control and c-Maf mutant mice. The blue line at the top shows the time course of the mechanical stimulus. The light grey area indicates the movement phase of the mechanical stimulus. For RAMs and SAMs, spikes were grouped into 50ms bins; the mean spike counts per bin from all tested fibers are shown below the example traces. Spike train adaptation time constants (τ) for the static stimulus phase were determined with a single exponential fit (red trace). Although mutant RAMs fired spikes during the static phase of the stimulus, their adaptation rate (τ=108.9 ms) was distinct from the ones observed in control (317.7 ms) or mutant (339.9 ms) SAMs. (F-H) The number of spikes evoked in SAMs, DHs and AMs by a 2 second skin indentation are plotted as a function of displacement amplitudes.
**Fig. S6.**

**Peripheral mechanoreceptive endings in c-Maf mutant mice.** (A) Double fluorescent in situ hybridization of P15 DRG neurons shows that neurons expressing high levels of TrkB (TrkB$^{\text{high}}$) co-express Ca$_{v}$3.2 (arrow) and therefore represent D-hair mechanoreceptors. Conversely large diameter TrkB$^{\text{low}}$ neurons do not express Ca$_{v}$3.2 (arrowhead). (B,C) Lanceolate and circumferential endings associated with non-tylotrich hair follicles of the back skin in control and c-Maf mutant mice visualized by the use of antibodies against CB, NF200, TrkB and S100. Arrowheads, arrows and asterisks point towards type I, II and III lanceolate endings, respectively. (D) Morphology of tylotrich, awl and zigzag hair in the back skin, and tail and paw hair. Note that hair in the dorsal hindpaw is shorter than the hair in back skin, and it resembles hair of the tail. (E) Neurons innervating Meissner corpuscles co-express CB, NF200 and TrkB. (F) Merkel cell-neurite complexes, lanceolate and circumferential endings of tylotrich hair follicles of the back skin of control and c-Maf mutants were visualized by the use of antibodies against cytokeratin 20 (CK20) and NF200. Insets show NF200 staining; note that the contrast in the insets was increased to show the highly branched and thin lanceolate endings in the mutants. The numbers of Merkel cells/cluster (16 ±1 and 14 ±1 of control and c-Maf mutant mice, respectively) and Merkel cell clusters per mm$^2$ of back skin (1.68 ±0.21 and 1.78 ±0.13 control and c-Maf mutant mice, respectively) were comparable. (G) Muscle spindles (arrows) in control and c-Maf mutant mice were visualized by the use of antibodies against CB, NF200 and Collagen IV. Spindle numbers were similar, i.e. 14.3 ±1.9, and 14.9 ±0.9 spindles/section in lower hind limb muscles of control and c-Maf mutant mice, respectively. (H,I) Nociceptive endings in the glabrous skin of the hind paw of control and c-Maf mutant mice were analyzed using PGP9.5 and NF200 (H) and CGRP and NF200 (I). We observed no difference in skin innervation by PGP9.5$^+/NF200^-$ or CGRP$^+/NF200^-$ fibers between control and c-Maf mutant mice. (J) Detection of tactile spatial information (grating orientation) in healthy subjects and family members that carry dominant c-MAF mutations. Bars (A,E,F) 50 μm, (B,C) 12.5 μm, (D) 1 mm and 100 μm, (G) 20 μm, (H,I) 50 μm.
Fig. S7.

Gene expression changes in DRG neurons of c-Maf mutant mice. Analysis of Ret⁺/TrkA⁻ neurons (A,B) and their quantification (C) in control and c-Maf mutant mice at E13.5. Analysis of MafA⁺ neurons (D,E) and their quantification (F) in control and c-Maf mutant mice at E13.5. Ret mutant mice were reported to display similar phenotypes as the one observed in c-Maf mutants; we therefore also analyzed (G,H) and quantified (I) c-Maf⁺ neurons in control and Ret mutant mice at E13.5 and P0. Analysis of MafA⁺ neurons (J,K) and their quantification (L) in control and Ret mutant mice at E13.5 and P0. (M) Results of quantitative RT-PCR. mRNA levels of the Ret and TrkB receptors, MafA, the Ca v3.2, Piezo2, Kcng4, Kcnh5, Kcnq4, Kcna1 ion channels, neurofilament Nefh, and crystallins Cryba2 and CrygS were measured using P15 DRGs from littermate control (black bars) and c-Maf mutant mice (white bars). Among these, Ca v3.2 and Piezo2 are known to be expressed in D-hairs and in mechanosensory neurons, respectively (39, 40). Quantitative RT-PCR data were normalized with Ube2l3. Bars (A-K) 30 μm.
A

- **c-Maf locus**
  - EcoRV site
  - ORF
  - 3' probe
  - 20.2 kb

- **c-Maf null allele**
  - EcoRV site
  - 3' probe
  - 7.5 kb

- **floxed c-Maf allele**
  - EcoRV site
  - 3' probe
  - 8.3 kb

B

- **NF (2H3) c-Maf**
- **Isli1/2 c-Maf**
- **c-Maf**

C

- **control**
- **Isl1cre c-Maf-flox**

D

- **E11.5**

E

- **P0**

F

- **P0**

G

- **Rotarod**

I

- **body weight**

J

- **Rotarod**

**Trials**

**time on rotarod [s]**

**control (n=15)**

**c-Maf^-/- (n=14)**

**P15**

**P15**

**adult**

**control**

**c-Maf^-/-**

**control**

**c-Maf^-/-**

**control**

**c-Maf^-/-**
Figure A: Comparison of % NF200+ neurons and # myelinated axons between control and c-Maf−/−.

Figure B: Graph showing von Frey thresholds for RAM, SAM, DH, and AM.

Figure C: Graph showing the number of spikes per stimulus for control and c-Maf−/− at different skin displacement values.

Figure D: Activity histograms for control (left) and c-Maf−/− (right) at different times.

Figure E: Activity histograms for control (left) and c-Maf−/− (right) at different times.

Figure F: Graph showing the number of spikes per stimulus at different skin displacement values for control and c-Maf−/−.

Figure G: Graph showing the number of spikes per stimulus at different skin displacement values for control and c-Maf−/−.

Figure H: Graph showing the number of spikes per stimulus at different skin displacement values for control and c-Maf−/−.
Control c-Maf-/-

**Figure A**

**TrkB**

**Ca3.2 DAPI**

**P15 DRG**

**in situ**

**non-tylotrich hair**

**TrkB**

**c-Maf-/-**

**Figure B**

**CB NF200**

**Figure C**

**TrkB S100 NF200**

**D**

**non-tylotrich hair**

**Figure E**

**CB NF200**

**Figure F**

**CK20 NF200**

**TrkB CB NF200 TrkB**

**Figure G**

**Col IV**

**tylotrich hair**

**muscle spindle**

**Figure H**

**PGP9.5 NF200 DAPI**

**control**

**c-Maf-/-**

**Figure I**

**CGRP NF200 DAPI**

**control**

**c-Maf-/-**

**Figure J**

**tactile acuity 79% prob. threshold [mm]**

**n.s.**

(15) (4)
**Relative Expression**

- **TrkB**
- **Cav3.2**
- **Piezo2**
- **MafA**
- **Kcng4**
- **Kcnh5**
- **Kcnq4**
- **Kcna1**
- **Nefh**
- **Cryba2**
- **CrygS**

**Graphs and Images**

- **A** and **B**: Images showing Ret/TrkA expression in control and c-Maf mutant backgrounds.
- **C**: Bar graph showing Ret+/TrkA- neurons in E13.5.
- **D** and **E**: Images showing MafA and Is1/2 expression in control and c-Maf mutant backgrounds.
- **F**: Bar graph showing MafA+ neurons in E13.5.
- **G** and **H**: Images showing Is1/2 and NF200 expression in c-Maf and c-Maf mutant backgrounds.
- **I**: Bar graph showing the number of neurons in E13 P0.
- **J** and **K**: Images showing MafA expression in control and c-Maf backgrounds.
- **L**: Bar graph showing the number of neurons in E13 P0.

**M**: Bar graph showing relative expression of various genes in control and c-Maf mutant backgrounds.

- **Legend**:
  - **Black** for control
  - **White** for c-Maf mutant

The graphs and images illustrate the expression patterns and relative expression levels of various genes under different conditions.
References and Notes

1. Q. Ma, RETouching upon mechanoreceptors. Neuron 64, 773 (2009).
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21. Type II lanceolate endings are TrkB$^{\text{high}}$, and TrkB$^{\text{high}}$ neurons do not express c-Maf (<5%). TrkB$^{\text{high}}$ neurons co-express Ca$_{\text{v}}$.3.2 and correspond thus to D-hairs (Fig. S6A). Type III lanceolate endings are NF200$^+$, and NF200$^-$ neurons do not express c-Maf.

22. Heterogeneity of lanceolate endings was recently also noted by Li L, Rutlin M, Abraira VE, Cassidy C, Kus L, Gong S, Jankowski MP, Luo W, Heintz N, Koerber HR, Woodbury CJ, Ginty DD. Cell. 2011, 147(7):1615-27. They report that Aβ- Aδ- and C-low threshold mechanoreceptors terminate in lanceolate endings, and comparisons of marker expression indicate that these correspond to type I, II and III endings, respectively.


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