

Deep visual proteomics uncovers nociceptor diversity and pain targets

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The richness of our somatosensory experience is reflected in the functional diversity of somatic sensory neurons. Single-cell RNA sequencing of sensory neurons has revealed a molecular basis for such diversity^{1–3}. However, sensory neuron diversity has yet to be captured at the level of the proteome. Here, we combined electrophysiology with deep visual proteomics⁴ to quantify over 6000 proteins from phenotypically-defined sensory neurons in mice and identified proteomic markers of sensory neuron subtypes. Comparative analysis revealed both concordance and meaningful divergence between transcriptomes and proteomes. We further show that up to 3000 proteins can be quantified from one-fourth of a single neuron, demonstrating subset-specific protein signatures. In culture, nociceptive neurons can be acutely sensitized to mechanical stimuli by nerve growth factor (NGF) which normally drives inflammatory pain *in vivo*⁵. Indeed, overnight exposure of peptidergic nociceptors to NGF and a protein kinase C (PKC) activator produced functional sensitization associated with proteome changes. Functional knockdown experiments identified the up-regulated B3GNT2 enzyme as a potential effector of nociceptor sensitization. In summary, we present a high-resolution proteomic resource linking molecular identity to function, enabling the discovery of mechanisms underlying somatic sensation and pain sensitization.

Somatic sensation involves a rich diversity of perceptions, ranging from light touch, limb position, coolness, and warmth. However, sensations can also be acutely or chronically painful with distinct qualitative features depending on the precipitating insult, such as burns or traumatic injury. Such qualitatively different sensations are initiated by activation of sensory neurons with their cell bodies in the dorsal root ganglia (DRG). The DRG was the first neuronal tissue to be analyzed using single-cell RNA sequencing (scRNA-seq), and the results revealed a rich diversity of sensory neuron sub-types that can be identified across species^{1–3,6,7}. Such data have enabled a deeper understanding of the sensory neuron diversity that contributes to the richness of

somatic sensation. However, while scRNA-seq data can be used to describe cellular phenotypes and developmental trajectories⁸ it cannot, by itself, predict the cellular proteome, which determines functional phenotypes^{9,10}. Independent of transcription, multiple mechanisms exist to dynamically regulate the proteome, with time-scales ranging from minutes to days^{11,12}. Bulk proteomics has been used to characterize protein changes in sensory tissue involved in inflammatory and neuropathic pain^{13–16}. However, such data cannot be directly mapped onto different cell types, which are equipped with their own specific signaling modules that mediate functionally relevant plasticity. Cell-specific signaling modules can only be accessed by

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obtaining high-resolution proteomes of single cell types. Recent breakthroughs in sample processing, ultrasensitive mass spectrometry, and computational proteomics have now made this feasible^{4,17}.

Here, we used deep visual proteomics (DVP)⁴, which combines artificial intelligence (AI)-guided image analysis, automated laser microdissection (LMD), and ultrasensitive mass spectrometry to obtain the proteomes of distinct sensory neuron cell types. We describe a quantitative proteomic comparison of mouse sensory neuron subsets, quantifying 5–6 thousand proteins per cell type. Sensory neurons that detect noxious stimuli are termed nociceptors, and the activation and sensitization of these neurons is necessary for acute and chronic pain¹⁸. There is considerable functional diversity amongst nociceptors, and one classic example is the distinction between peptidergic and non-peptidergic nociceptors^{19–21}. These two nociceptor subsets possess distinct functional properties^{21,22}, developmental trajectories^{23,24} and even regenerative capacities^{25,26}. Importantly, peptidergic nociceptors, but not non-peptidergic nociceptors, are sensitive to nerve growth factor (NGF), which is a key mediator of chronic inflammatory pain in animals and humans^{27–29}. We first focused our approach on electrophysiologically characterized peptidergic and non-peptidergic mouse neurons in culture. Deep proteomes for each cell type were obtained from 30–50 cells with high biological reproducibility across animals. We found high concordance between proteomes obtained from cultured neurons and corresponding neuronal cell bodies taken from DRG tissue sections. Using a cellular model of acute NGF and phorbol ester mediated sensitization, we show how only the peptidergic nociceptors display a rapid increase in their mechanosensitivity. This cellular model mimics nociceptor sensitization mediated by NGF in vivo, which has a rapid onset, but is also long-lasting⁵. We went on to identify dynamic protein changes associated with nociceptor sensitization and identified the glycosyltransferase enzyme B3GNT2 as being involved in nociceptor sensitization. Thus, our methodology was validated as a powerful tool that complements and extends the existing scRNA-seq datasets of neuronal diversity. Moreover, we show how deep neuronal subtype-specific proteomics can identify molecular drivers of pain hypersensitivity.

Results

Sensory neuron-subtype specific proteomes

Many, but not all, cultured sensory neurons possess mechanically-gated currents^{22,30–32}. Patch clamp electrophysiology shows that almost all large diameter mouse sensory neurons (> 30 μ m), which have an electrophysiological signature of mechanoreceptors, possess mechanically-gated currents to indentation stimuli^{30–33}. In contrast, small-medium diameter (< 30 μ m) sensory neurons with a nociceptor-like electrophysiological signature exhibit mechanically gated currents with slower kinetics and reduced frequency compared to mechanoreceptors^{30,32,34}. Peptidergic and non-peptidergic nociceptors exhibit distinctive biophysical properties, including responsiveness to mechanical stimuli^{21,22}. We used isolectin-B4 coupled to AlexaFluor-594, which binds to α -D-galactose carbohydrate residues on the surface of non-peptidergic nociceptors, to live label these cells in culture^{21,35} (Fig. 1a, b). The incidence of mechanically gated currents evoked by cell membrane indentation was significantly lower in IB4⁺ nociceptors (10%) than in IB4⁻ nociceptors (~ 50%) (IB4⁻, 12/23 vs. IB4⁺, 2/18, $p = 0.005$, chi-sq test Fig. 1c, d and Supplementary Fig. 1a). Approximately half of the mechanically evoked currents in nociceptors were rapidly adapting as has been described before^{32,34} (Supplementary Fig 1b). IB4⁺ nociceptors also showed a tendency to have broader action potentials (half-peak duration $p = 0.14$, unpaired t test with Welch's correction) with a larger ($p = 0.04$, unpaired t test) afterhyperpolarization peak amplitude (Fig. 1e). The two IB4⁺ neurons with a mechanosensitive current had action potential properties typical for this neurochemical sub-type (Fig. 1e). Therefore, IB4⁺ non-

peptidergic nociceptors are functionally distinct from IB4⁻ peptidergic nociceptors in mice, as previously observed in rats^{21,35}.

To profile the global proteomes of these functionally distinct nociceptor subsets, we utilized our recently developed ultra-low-input proteomics workflow, integrating imaging and laser microdissection¹⁷. A portion of the mouse sensory neurons assessed with electrophysiology was also cultured overnight on LMD-compatible slides, fixed, and immunolabeled with antibodies against the cell surface tyrosine kinase receptor, TrkA, and IB4, two markers that largely discriminate between these two nociceptor populations, peptidergic (TrkA⁺, IB4⁻) and non-peptidergic (IB4⁺, TrkA⁻)²³ (Fig. 1f). From each of the three mice, we collected 50 TrkA⁺ and 50 IB4⁺ neurons using LMD. We additionally included 30 large-diameter mechanoreceptors that do not normally stain positive for nociceptor markers, such as IB4 and TrkA. Proteomic measurements were conducted using a trapped ion mobility mass spectrometer (Bruker timsTOF SCP/Ultra) operated in data-independent (diaPASEF) acquisition mode. We identified more than 40,000 precursors and 6000 proteins across all three subsets of sensory neurons, with biological replicates closely grouping together in principal component analyses (Fig. 1g, h). Among the quantified proteins were ~ 400 transmembrane proteins, including many ion channels associated with nociception, such as Trpv1, Trpa1, and Scn9a (Supplementary Data 1). Hierarchical clustering of all 821 ANOVA-significant proteins revealed clearly demarcated clusters with unique protein signatures among the three subsets, enabling us to define a proteomics-based signature of peptidergic and non-peptidergic nociceptors, as well as mechanoreceptors (Supplementary Fig. 1c and Supplementary Data 2). Interestingly, among the top markers (Table 1) were proteins such as Hpcal1 (a neuron-specific, calcium-binding protein) and Armcx1 (a mitochondrial protein), which we found to be subtype-specific markers for peptidergic and non-peptidergic neurons, respectively.

The most abundant proteins quantified were Tubb3, Prph, and classical markers of sensory neurons³⁶, implying minimal admixing with non-neuronal cell types, such as satellite cells³⁷ (Fig. 1i). Furthermore, many well-known markers of the three different subsets were quantified and distributed over a dynamic range spanning more than four orders of magnitude (highlighted in Fig. 1i and Supplementary Fig. 1d). The proteome of IB4⁺ neurons differed significantly from that of TrkA⁺ neurons, with the largest differences in proteins encoded by well-established marker genes³, such as *Mrgprd* (in IB4⁺ population), *Ntrk1* (in TRKA⁺ population), and *Nefh/Ntrk3* (in mechanoreceptors) (Fig. 1j and Supplementary Fig. 1e). Notably, Tmem45b³⁸, a recently identified membrane protein implicated in mechanical pain hypersensitivity in the non-peptidergic population, was one of the most highly expressed proteins in IB4⁺ neurons in our dataset. Our subset-specific proteome analysis also revealed differential protein abundances between peptidergic and non-peptidergic clusters for genes with very low mRNA abundance, such as Stoml3 (a regulator of sensory mechanotransduction³⁹) and the synaptic remodeling protein Nptx1⁴⁰. Pathway enrichment analysis identified terms associated with “metabolism” and “biosynthesis” processes as significantly associated with the TrkA⁺ neuronal proteome (FDR < 0.05), potentially reflecting their superior capacity to sprout centrally in the dorsal horn²⁵ (Fig. 1k). Similar gene ontology-based enrichment analysis between IB4⁺ nociceptors and mechanoreceptors revealed “C-fiber” as the most significantly enriched term in the IB4⁺ neuronal proteome, validating the premise of such an analysis (Supplementary Fig 1f). In conclusion, our approach shows that it is possible to uncover deep proteomes from sensory neuron subtypes, a rich resource for the discovery of nociceptor-specific pain targets.

Common proteomic signatures of intact and cultured neurons

Cultured sensory neurons are amenable to a host of electrophysiological, biochemical, and functional manipulations and are an

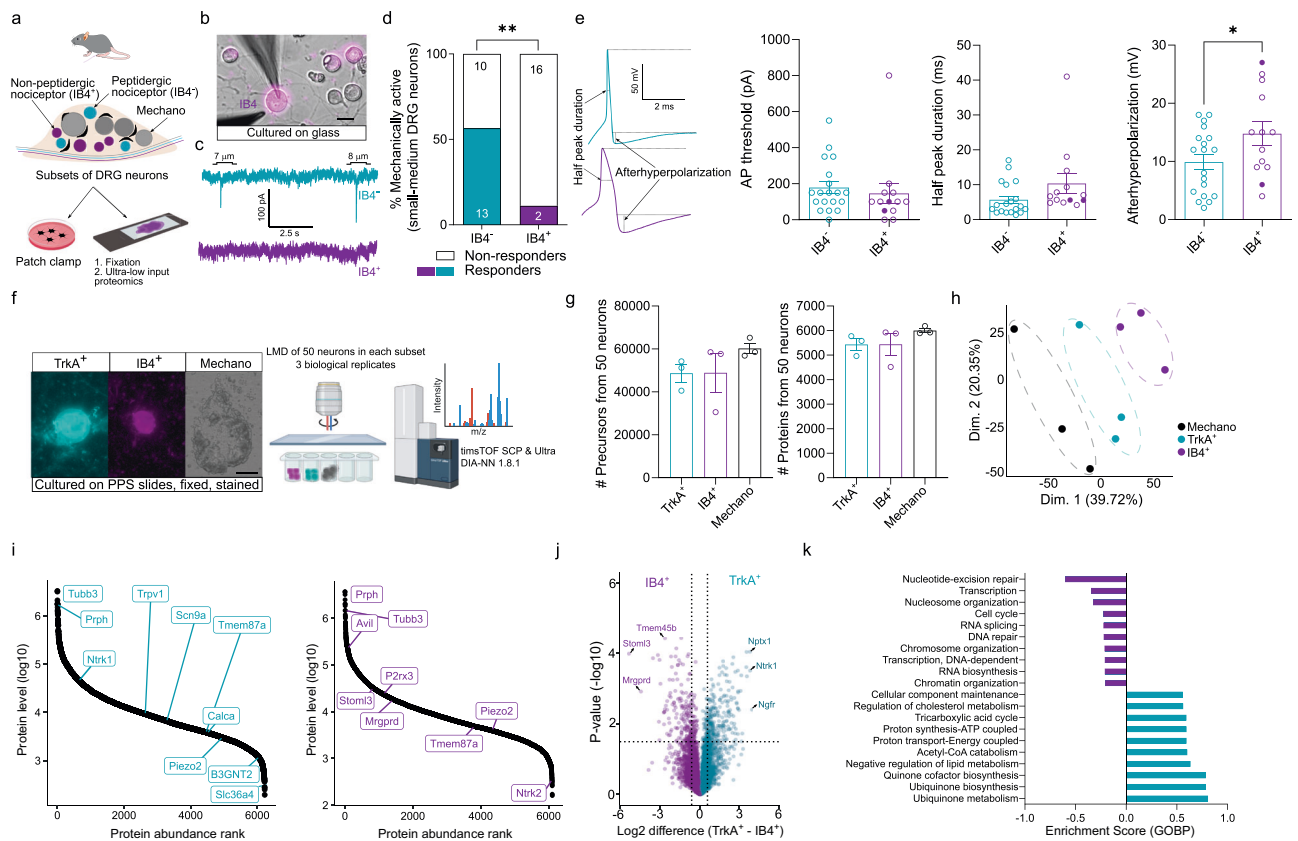


Fig. 1 | Electrophysiology-guided, subset-specific proteome of cultured sensory neurons. **a** Schematic workflow showing patch clamp electrophysiology and ultra-low input proteomics of cultured sensory neurons. **b** IB4 labeling in magenta (scale = 20 μ m). Independently repeated in at least three cultures from three different mice with similar results. **c** Representative traces of indentation-evoked, mechanically-activated currents in an IB4⁻ peptidergic nociceptor and the absence of such currents in an IB4⁺ non-peptidergic nociceptor. **d** Percentage of mechanically sensitive IB4⁻ and IB4⁺ neurons. The number of neurons is indicated in the bars. ** represents $p = 0.0027$, two-sided chi-sq test. **e** Representative action potential (AP) traces recorded from an IB4⁻ and an IB4⁺ nociceptor, with corresponding quantification of AP threshold, half peak duration, and afterhyperpolarization of the measured APs. IB4⁻: $n = 19$, IB4⁺: $n = 13$. Each dot represents a single neuron. Filled dots in the IB4⁺ group represent the two mechanosensitive non-peptidergic neurons. Data were obtained from at least three biological replicates, both male and female mice. * represents $p = 0.04$, unpaired two-tailed Student's *t* test. **f** Representative images of sensory neurons cultured and stained on

laser microdissection (LMD)-compatible membrane slides prior to LMD and mass spectrometry analysis (scale = 20 μ m). **g** Numbers of precursors and proteins identified from peptidergic (IB4⁻/TrkA⁺) and non-peptidergic (IB4⁺/TrkA⁻) nociceptors and mechanoreceptors. $N = 3$. Each dot represents a biological replicate from a male mouse. **h** Principal component analysis of peptidergic and non-peptidergic nociceptors and mechanoreceptors. **i** Dynamic range of protein abundance in the two nociceptor subsets. **j** Volcano plot showing pairwise proteomic comparison between nociceptor subsets with marker proteins highlighted (two-sided Student's *t* test). **k** Pathway enrichment analysis based on *t* test differences between nociceptor subsets. The top ten pathways with a Benjamini-Hochberg false discovery rate (FDR) < 0.05 are shown. Cyan = peptidergic, magenta = non-peptidergic, black = mechanoreceptor. Error bars in all panels represent s.e.m with the mean as the center. Source Data are provided as a Source Data file. Parts of Fig. 1a, f created in BioRender. Coscia, F. (2026) <https://BioRender.com/a39426f>.

important model for pain research⁴¹. However, questions remain on how the culturing process changes gene expression and protein levels when compared to intact DRG neurons. A comparison between cultured and intact DRGs using bulk RNA sequencing revealed an overall similarity between these conditions, but such an analysis at the protein level is missing⁴². To address this, we placed 5 μ m thick cryosections from intact mouse lumbar DRGs onto LMD-compatible membrane slides and immunostained them for calcitonin gene-related peptide (CGRP) and P2X purinoceptor 3 (P2X3) (Fig. 2a). These two markers, which largely overlap with TrkA⁺ and IB4⁺ cells, respectively, show little species-specific variability and are good markers for peptidergic and non-peptidergic neurons^{43–45}. To improve the efficiency of sample throughput, we used a custom-trained deep learning-based Cellpose 2.0⁴⁶ model to segment CGRP⁺ and P2X3⁺ neurons. The generated segmentation masks were dilated radially using the image analysis software QuPath⁴⁷, as shown in representative images, to prevent laser-induced damage of cell bodies. These contours were visually inspected to ensure no overlap between adjacent neurons, followed by

automated LMD, as per our DVP workflow¹⁷. Neurons double-positive for CGRP and P2X3 were excluded. We collected 100 CGRP⁺ and 100 P2X3⁺ nociceptor sections pooled from each of the three mice to quantify ~25000 precursors and ~3500 proteins from each subset (Supplementary Fig. 2a and Supplementary Data 3). A similar proteome depth was obtained from large-diameter mechanoreceptors in a separate experiment (Supplementary Fig. 2b, c and Supplementary Data 4). Importantly, we found that over 90% of the proteins quantified in both peptidergic and non-peptidergic groups in intact sensory neurons were also detected in cultured sensory neurons (Fig. 2b). The lower depth of proteome compared to cultured neurons is likely due to the lower volume of samples collected from tissue slices. A plot of the differentially regulated proteins in peptidergic vs. non-peptidergic neurons from tissue (CGRP⁺ vs. P2X3⁺) and cell culture (TRKA⁺ vs. IB4⁺) showed excellent correlation with *Calca* (gene name for CGRP) being the top-regulated protein in the peptidergic cluster (Pearson correlation = 0.73, Fig. 2c). This finding confirms that the proteomic identity of peptidergic and non-peptidergic neurons remains consistent,

Table 1 | Top 50 marker proteins based on subset-specific sensory neuron proteomics

Peptidergic Markers		Non-Peptidergic Markers		Mechanoreceptor Markers	
Gene	Trka Marker Avg. z-score	Gene	IB4 Marker Avg. z-score	Gene	Mechano Marker Avg. z-score
Hpcal1	1.2809	Armxc1	1.3190	Ntrk3	1.2889
Rigi	1.2801	Phf24	1.3171	Gphn	1.2856
Htr3a	1.2732	Cyp4f39	1.3156	Myh10	1.2774
Tanc1	1.2675	Arhgap26	1.3138	Lancl3	1.2748
Hk1	1.2547	Actn1	1.3136	Pcp4	1.2693
Nrbp2	1.2349	Adk	1.3070	Sptbn2	1.2665
Pak1ip1	1.2277	Stoml3	1.3068	Stk39	1.2622
Prep	1.2131	Lxn	1.3046	Mrpl12	1.2598
Sqle	1.2083	Calcoco1	1.3032	Gramd1b	1.2591
Eya3	1.2062	Prkca	1.2964	Haghl	1.2567
Serp1b1b	1.2053	Gpd1l	1.2934	Vamp1	1.2561
Ptk2b	1.2024	Atg7	1.2934	Extl2	1.2552
Cdk14	1.2003	Mmp25	1.2868	Rem2	1.2481
Letmd1	1.1973	Mvk	1.2824	Cox5a	1.2465
Ntrk1	1.1960	Ckap5	1.2800	Cox7a2l	1.2426
Sec24d	1.1950	Mrgprd	1.2767	Calb1	1.2422
Efh2	1.1945	Ldb2	1.2767	Mrps9	1.2417
Pls3	1.1898	Dgki	1.2751	Mrpl15	1.2415
Sytl3	1.1882	Magi1	1.2745	Timm13	1.2392
Plxna4	1.1831	Rgs10	1.2708	Nek9	1.2386
Epn2	1.1817	Mb21d2	1.2655	Mrs2	1.2361
Rps6ka5	1.1799	Myo1e	1.2639	Ermp1	1.2354
Dab2ip	1.1778	Snx10	1.2629	Atp8a2	1.2348
Wdr44	1.1751	Fam98b	1.2619	Ube3c	1.2314
Atp2b4	1.1681	Kif5a	1.2600	Fam20b	1.2302
Itm2b	1.1672	Kcnt1	1.2598	Ehd3	1.2286
Ugp2	1.1593	Gna14	1.2580	Oxr1	1.2263
Calca	1.1557	Txn1	1.2565	Pls1	1.2260
Plcb1	1.1549	Scn11a	1.2545	Miga2	1.2239
Hmgcr	1.1423	Plcx3	1.2531	Stxbp6	1.2237
Pde2a	1.1406	Ftl1	1.2506	Ndufa8	1.2224
Pde1c	1.1381	Tax1bp1	1.2466	Arap2	1.2212
Ampd2	1.1376	Arfip1	1.2462	Cpeb3	1.2205
Chp2	1.1270	Mink1	1.2446	Osbpl6	1.2126
Slc25a23	1.1266	Rasgrp1	1.2436	Acs16	1.2100
Eps15	1.1227	Apc2	1.2413	Slc35b2	1.2079
Klhl22	1.1149	Bag2	1.2401	Uqcrb	1.2078
Pde3b	1.1036	Arfgef2	1.2359	Ndufb3	1.2075
Snx25	1.0818	Arhgap36	1.2353	Ndufb8	1.2069
Nt5dc3	1.0799	Ctnna2	1.2345	Mtmr10	1.2068
Mia3	1.0731	Ubr1	1.2339	Ndufb11	1.2063
Abcg2	1.0709	Dusp26	1.2337	Mrpl13	1.2057
Acs13	1.0617	Stom	1.2333	Ndufb4	1.2036
Cdhr1	1.0614	Prex1	1.2330	Atp5me	1.2029
Raly1	1.0533	Arhgef11	1.2326	Lgi2	1.1998
Dcl1	1.0462	Tp53bp1	1.2322	Mrpl41	1.1978
Srcin1	1.0459	Thop1	1.2320	Hecw1	1.1960
Cebpz	1.0406	Nt5e	1.2318	Ptcd3	1.1936
Fgf12	1.0333	Evi5l	1.2311	Ndufb5	1.1922
Serp1b1a	1.0316	Apba1	1.2306	Myo18a	1.1898

regardless of the markers used for classification. This also suggests that the culturing procedure had only a minimal influence on the proteomic identity of the neurons. Gene ontology (GO) enrichment analysis of the 315 and 261 unique proteins in the CGRP⁺ and P2X3⁺ subsets of sensory neurons showed “extracellular matrix structure” as a highly significant term, which is consistent with intact sensory neurons being embedded in their native matrix (Fig. 2d). In contrast, the GO terms associated with the unique proteins identified from cultured nociceptors (Supplementary Fig. 2d) were “membrane coat” as well as metabolism related terms in the peptidergic subset similar to Fig. 1k. “Extracellular matrix” was also amongst the most significant enrichment term for the 650 unique proteins in the intact NF200⁺ mechanoreceptors (Supplementary Fig. 2e).

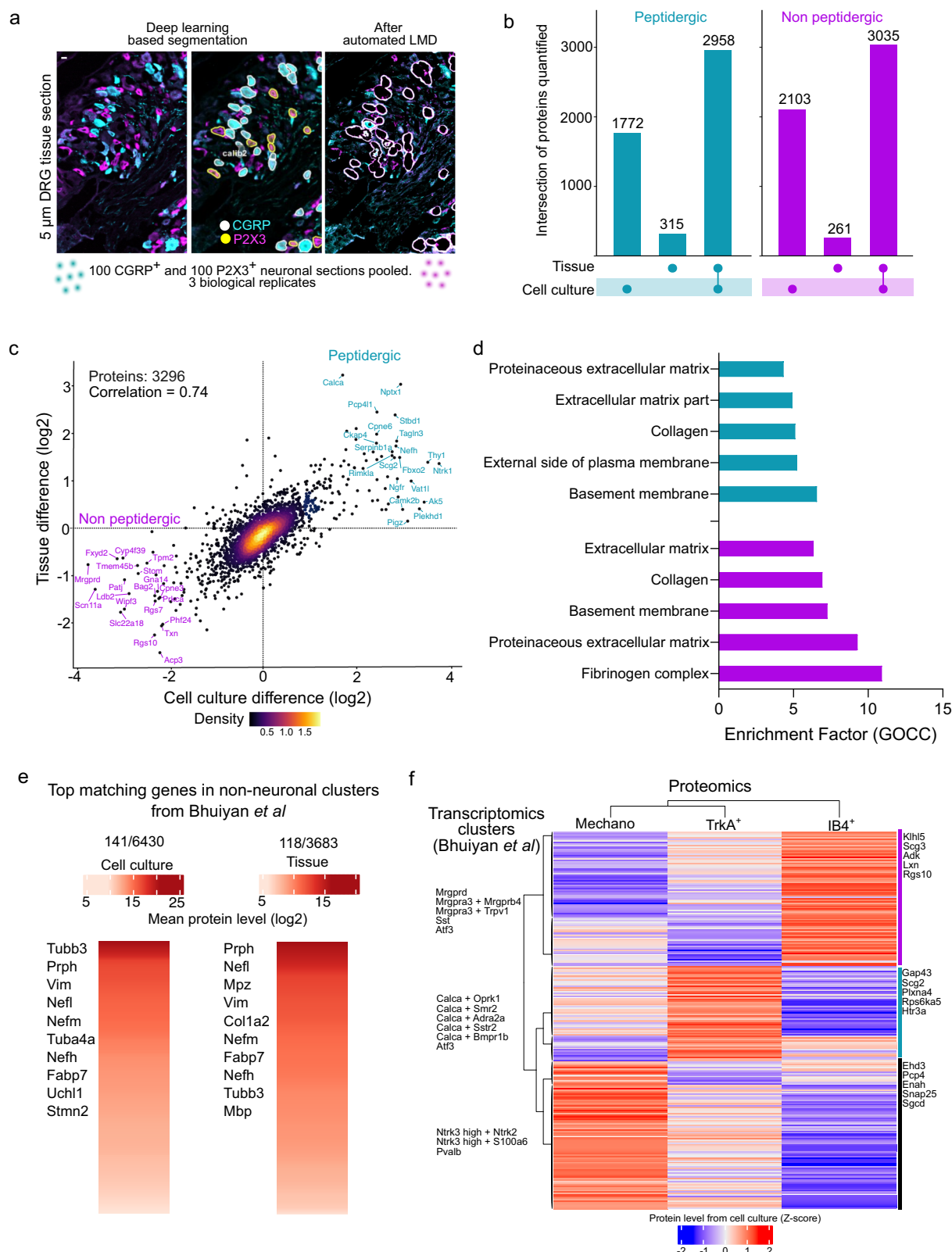
Next, we compared our subset-specific proteomes to existing scRNA-seq-based sensory neuronal clusters. For this analysis, we utilized a recently published database from Bhuiyan and colleagues³, which harmonized existing sensory neuron and associated non-neuronal single-cell databases comprising both single-cell and single-nucleus RNA-seq data (referred to hereon as scRNA-seq data). Among the 1556 unique genes quantified in non-neuronal cells via scRNA-seq, only ~150 genes matched our proteome dataset. Therefore, we estimated that ~3% of our measured proteomes may have been derived from non-neuronal cells, such as satellite and Schwann cells, which are tightly attached to neurons in sections or in culture (Fig. 2e). Notably, genes like *Tubb3* and *Prph* are detected in both neurons and Schwann cells and therefore 3% is probably an overestimate of the non-neuronal contribution.

Next, a compiled list of the top 50 marker genes from the 18 sensory neuronal subclusters in the harmonized scRNA-seq database was matched to our deep proteome from cultured neurons. Among the 554 unique genes present in the transcriptomic marker list, 255 matched our proteomes, revealing well matching clusters for cell type-specific transcriptomes and proteomes (Fig. 2f). For example, transcriptomics based peptidergic markers, *Gap43* and *Scg2*, were also highly expressed at the protein level in our TrkA⁺ peptidergic group. Taken together, our ultra-low input, subset-specific proteome closely aligns with the scRNA-seq-derived transcriptomic signatures of sensory neuron subsets.

We additionally compared the transcript/protein detectability between the ~15000 gene deep scRNA-seq dataset of Sharma and colleagues² and our ~6000 protein deep cultured neuron data. Proteins with higher transcript counts in the scRNA-seq study were more likely to be detected by proteomics (Supplementary Fig. 3a). For example, we detected 80% of genes with a transcript read count 30 or higher, which compared to 60% of the genes with ~5 average transcript reads. However, even genes with low transcript levels (e.g., *Mrgprd*) could be identified by proteomics as nociceptor subset-specific markers (Supplementary Fig. 3b).

Single neuron proteomes delineate nociceptor subsets

Next, we investigated whether our method is capable of quantifying single intact sensory neuron proteomes and whether these data capture subset specificity. We laser microdissected single CGRP⁺ and P2X3⁺ nociceptor sections from DRGs of each of the three mice (Fig. 3a). After quality control, 20 CGRP⁺ peptidergic neurons and 22 P2X3⁺ non-peptidergic single neurons remained in the dataset, with peptidergic and non-peptidergic nociceptors forming distinct clusters (Fig. 3b). The highest number of proteins quantified from a single nociceptor was 1691, from an average of ~3000 precursors (Supplementary Fig. 4a). A comparison of differentially regulated proteins between peptidergic and non-peptidergic nociceptors from 100 pooled contours *versus* a single contour revealed similar protein level differences, with CGRP (*Calca*) being one of the highest regulated proteins in the peptidergic subset (Pearson correlation = 0.6, Fig. 3c). As expected, the quantified proteins from single neuron slices were the



most abundant proteins and were part of the stable neuronal core proteome⁴⁸. We demonstrated the existence of such a stable core proteome in a single section of a neuron, while preserving subset specificity. Comparing peptidergic and non-peptidergic single neurons revealed distinct differentially abundant proteins, with 32 significantly upregulated proteins in peptidergic neurons and 25 in non-peptidergic neurons (Fig. 3d and Supplementary Data 5). By searching

the open-source drug-gene interaction database (DGIdb, v.5.0.8) against upregulated proteins in the single peptidergic neuron proteome, we found interactions between *Hspb1* and two approved analgesics: the non-steroidal anti-inflammatory drug (NSAID) rofecoxib and argipressin. Additionally, *Eno2*, which is upregulated in the peptidergic cluster, interacts with gabapentin, a widely used analgesic, and indomethacin, an NSAID. Similarly, among the differentially

Fig. 2 | Subset-specific intact nociceptor proteomics from tissue sections.

a Representative images of a 5 μ m intact dorsal root ganglion (DRG) tissue section stained with CGRP and P2X3, segmented using a Cellpose 2.0 model and processed by semi-supervised, automated LMD. “calib2” denotes one of the calibration points used to transfer masks from Cellpose to LMD. **b** Upset plot showing common and unique proteins quantified from nociceptor subsets in intact tissue and culture. **c** Correlation of differences in protein levels between nociceptor subsets for common proteins identified in both cultured and intact sensory neurons, along with a color gradient of the relative point density. **d** Pathway enrichment analysis (Fischer’s exact test) of uniquely identified proteins from intact tissue-derived nociceptors. The top five pathways with a Benjamini-Hochberg false discovery rate

(FDR) < 0.05 are shown. **e** Heat map of non-neuronal protein levels quantified in cultured and intact sensory neurons, corresponding to genes identified in non-neuronal clusters in the harmonized single-cell transcriptomics atlas (Bhuiyan et al.³). Each row represents the mean Log2 protein level across all three sensory neuron subsets of a given matched gene. **f** Heat map showing overlap between the combined top 50 marker genes from all subsets of the harmonized single-cell transcriptomics atlas and our subset-specific proteome from cultured neurons. Each row represents the z-score of the protein level of a matched gene. Cyan = peptidergic, magenta = non-peptidergic. Source Data are provided as a Source Data file.

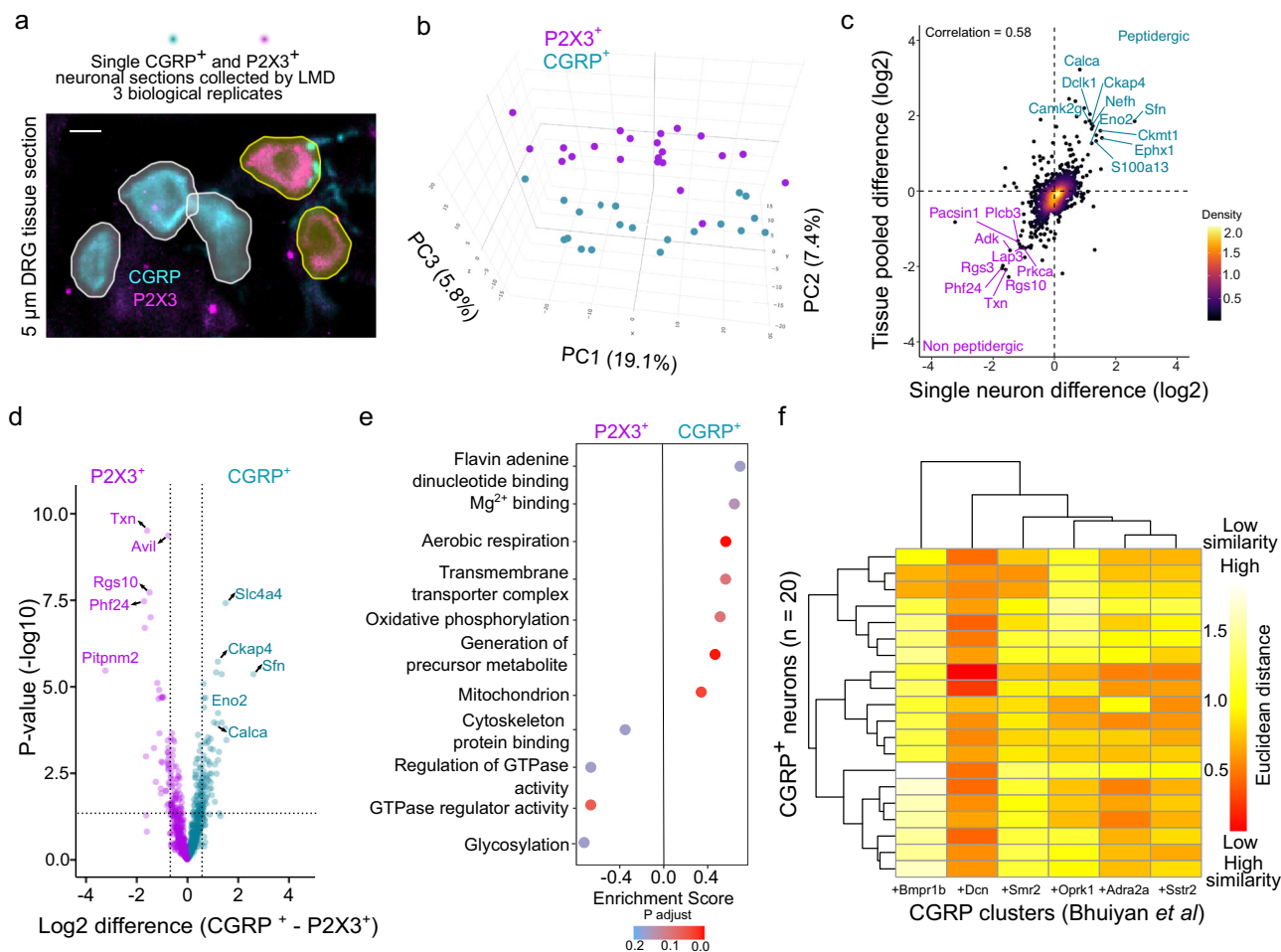


Fig. 3 | Proteome from a section of a single nociceptor. **a** Representative image of a dorsal root ganglion (DRG) section stained with CGRP (peptidergic) and P2X3 (non-peptidergic) and the corresponding Cellpose derived segmentation masks. Scale bar = 10 μ m. **b** A 3D principal component analysis of individual CGRP⁺ and P2X3⁺ nociceptors. **c** Correlation of differences in protein abundance between nociceptor subsets for common proteins identified in both pooled and single sensory neuron sections, along with a color gradient of the relative point density. **d** Volcano plot showing pairwise proteomic comparison between single nociceptor

proteomes, with marker proteins of each subset highlighted (two-sided Student’s *t* test). **e** Pathway enrichment analysis based on *t* test differences between nociceptor subsets. The top pathways enriched in the peptidergic and non-peptidergic neurons are shown. **f** Similarity plot based on Euclidean distance between enriched genes in CGRP (peptidergic) subclusters (columns) identified by single-cell transcriptomics and proteomics of individual CGRP⁺ nociceptors (rows). Red = high similarity; white = low similarity. Cyan = peptidergic, magenta = non-peptidergic. Source Data are provided as a Source Data file.

upregulated proteins in the non-peptidergic cluster, *Clgn* interacts with the anti-rheumatic agent leflunomide. Gene ontology analysis further showed that the proteome of single CGRP⁺ peptidergic nociceptors were enriched in “metabolism”-related terms like those observed with our deep proteome from 50 cultured nociceptors, while “glycosylation” was associated with non-peptidergic neurons in agreement with their IB4 (lectin) binding capacity (Fig. 3e). Previous scRNA-seq studies identified six subclusters of CGRP⁺ neurons, prompting us to explore whether we could probabilistically map single

CGRP⁺ neuronal proteomes to these clusters. Based on the Euclidean distance between normalized positive fold change values from transcriptomics and protein levels of the matched marker genes, we found that our pooled 50 cultured peptidergic nociceptor proteome best matched the “*Calca*+*Dcn*” transcriptome cluster, with undefined function³ (Supplementary Fig. 4b). A similar comparison of single CGRP⁺ neurons with transcriptomic clusters revealed that some neurons also had a probability of belonging to other clusters, such as “*Calca* + *Adra2a*” or “*Calca* + *Smr2*”. Single neurons showed the lowest

likelihood of belonging to the rare cell type “*Calca + Oprk1*” or Aδ-nociceptors “*Calca + Bmpr1b*”. Therefore, our analysis suggests that single neuron proteomes can, in principle, be used to generate unbiased clustering of sensory neurons, and their function can be probed directly using electrophysiology to determine molecular determinants of function at a single neuron level.

Proteome coverage was significantly higher in large diameter mechanoreceptors ($n = 36$), with a range of 1404–3615 identified proteins (Supplementary Fig. 4c and Supplementary Data 3). We expect that advances in sample preparation techniques and higher-sensitivity MS will further enhance proteome depth from single neurons. Our analysis shows that, at present, a pooled approach of 50 phenotype-matched cultured neurons yields the best proteomic depth and workflow robustness, which may be leveraged to identify pain targets.

A cellular model of mechanical pain hypersensitivity

NGF is an important mediator of painful mechanical hypersensitivity in humans and rodents and acts by downstream signaling via its receptor TrkA⁵. We next hypothesized that our neuronal subtype-resolved proteomics approach could identify downstream proteins involved in NGF-dependent mechanical hypersensitivity. We used an in vitro “inflammation” model in which isolated nociceptors were exposed to nerve growth factor (NGF) and the PKC activator phorbol 12-myristate 13-acetate (PMA) (Fig. 4a). As previously reported in rat sensory neurons²², overnight treatment with 100 ng/ml NGF and 10 nM PMA doubled the proportion of neurons with indentation-induced currents (control, 12/23 vs. NGF-PMA, 19/22, $p = 0.01$, chi-sq test, Fig. 4b). A non-significant trend towards increased peak current amplitude was also observed when compared to the control ($p = 0.2$, unpaired t test with Welch’s correction, Fig. 4b and Supplementary Fig. 5a). The proportion of rapidly, intermediately and slow-adapting currents were similar in control and sensitized states (Supplementary Fig. 5a). Notably, the proportion of neurons in which residual non-adapting inward current was observed after removal of indentation increased from 16.6 % in control to 52.6 % in inflamed neurons ($p = 0.04$, chi-sq test, Fig. 4c). No changes in the action potential firing threshold or threshold for mechanically activated currents were observed (Supplementary Fig. 5b). The sensitizing effects of NGF and PMA were restricted to small-medium diameter IB4⁺, peptidergic neurons, consistent with their expression of the TrkA receptor (Fig. 4d and Supplementary Fig. 5c). Therefore, we hypothesized that our phenotype-resolved discovery proteomics pipeline of inflamed versus control TrkA⁺ nociceptors might reveal regulated proteins with a causal role in nociceptor sensitization.

The enzyme B3GNT2 is a putative effector of nociceptor sensitization

Differential analysis of NGF-PMA-treated versus control neurons identified nine upregulated proteins (Fig. 4e) in IB4⁺, seven in Mechano (Supplementary Fig. 5d) and 18 in TrkA⁺ neurons (Fig. 4f) (Supplementary Data 6). All upregulated proteins were unique to each of the three sensory neuron subsets. We focused on the most significantly upregulated proteins in TrkA⁺ peptidergic nociceptors. The sequence information available for the candidate protein with the most significant p -value, *Gm8011* (predicted gene locus in Chromosome 14), appeared to be incomplete in NCBI, and it was thus not selected for functional validation. The second most significant candidate was Rrad, a Ras-related glycolysis inhibitor and a calcium channel regulator. Recent studies have suggested that Rrad increases cell surface tension, which could enhance the activity of mechanosensitive channels, such as Piezo1 and Trekl1^{49,50}. To evaluate the role of Rrad in inflammation-induced mechanical hypersensitivity in peptidergic neurons, we used siRNA directed against *Rrad* prior to nociceptor sensitization with NGF and PMA treatment (Supplementary Fig. 5e). Non-targeting siRNA was used as a control, and here the effects of NGF and PMA on

mechanically gated currents were similar to controls, with 25/26 “inflamed” neurons showing whole cell currents to cell indentation (Fig. 4a–c and Supplementary Fig. 5f). However, this NGF-PMA-induced mechanical hypersensitivity was not decreased in sensitized neurons after *Rrad* knockdown (Supplementary Fig. 5f, g). This could be explained by a comparatively small number of sensory neurons that express Rrad, as assessed from scRNA seq studies^{2,3}.

Our second candidate, B3GNT2⁵¹, an enzyme involved in the synthesis of poly-N-acetyllactosamine, was the third most upregulated protein in peptidergic-sensitized neurons. B3GNT2 was consistently detected in all three biological replicates of TrkA⁺ sensitized neurons, showing an estimated two-fold increase in protein abundance compared to TrkA⁺ control neurons (only detected in one of the three samples) (Fig. 4g). We transfected sensory neurons with siRNA targeting *B3gnt2*, followed by NGF-PMA treatment (Supplementary Fig. 5e and Fig. 4h). Compared to sensitized IB4⁺ peptidergic neurons transfected with control siRNA, *B3gnt2* knockdown reduced the proportion of neurons with mechanically activated currents to levels seen in untreated control nociceptors (11/21 responded to mechanical indentation, $p = 0.0003$, Chi-sq test, Fig. 4i). No difference was found between control siRNA transfected and *B3gnt2* siRNA transfected IB4⁺ treated neurons in peak mechanically activated current amplitude or in the proportion of neurons with slowly inactivating residual currents, suggesting the role of other unknown proteins in NGF-induced mechanical hypersensitivity (Fig. 4j). Nevertheless, our data identify a specific role for the B3GNT2 enzyme in the appearance of newly mechanosensitive peptidergic nociceptors following inflammatory stimuli. We did not investigate the role of *B3gnt2* in IB4⁺ non-peptidergic neurons, since the protein was not differentially enriched in NGF-PMA-treated IB4⁺ neurons. Our findings provide in vitro proof-of-concept validation for using ultra-low input proteomics to identify cell-type-specific proteomic changes directly relevant to pain physiology.

Discussion

It has long been known that transcriptional signatures can significantly diverge from the proteome signature^{10,12}. Here, we demonstrate how deep visual proteomics can be leveraged to obtain sensory neuron subtype-specific proteomes with depths equivalent to that of previous whole tissue proteomes¹⁴. This powerful technique allowed us to align cell-type-specific proteomes to sensory neuron scRNA seq data.

Cultured sensory neurons are a robust model for investigating nociceptive mechanisms and screening for drug targets due to their relative ease of culture and functional manipulation⁴¹. However, concerns remain regarding whether culturing alters their fundamental cell state. While bulk sequencing data demonstrates minimal changes due to culturing⁵², these effects have not been thoroughly investigated at the protein level or in a neuronal subtype-specific manner. In the present study, we demonstrate that the proteome of peptidergic and non-peptidergic nociceptors, as well as mechanoreceptors derived from cultured or intact DRG sections exhibit considerable overlap and quantitative similarity. The small number of unique proteins present in tissue sections are mostly derived from the extracellular matrix since these neurons are still embedded in their native matrix. Therefore, intact DRG neurons are preferable for studying neuronal interactions with endogenous extracellular matrix, while subset-specific mechanisms can be investigated in both cultured and intact neurons.

We found that sensory neuron identity is ingrained in their proteomes, which can be defined by our ~2000 protein deep single neuron dataset obtained from tissue sections. This proteome coverage was comparable to that of cultured single-neuron proteomes⁵³. The purpose of our single neuron proteome data was to demonstrate that, in principle, single-cell proteomics can complement scRNA seq classifications. Through this exploratory study, we demonstrate that obtaining meaningful electrophysiological and proteome data from

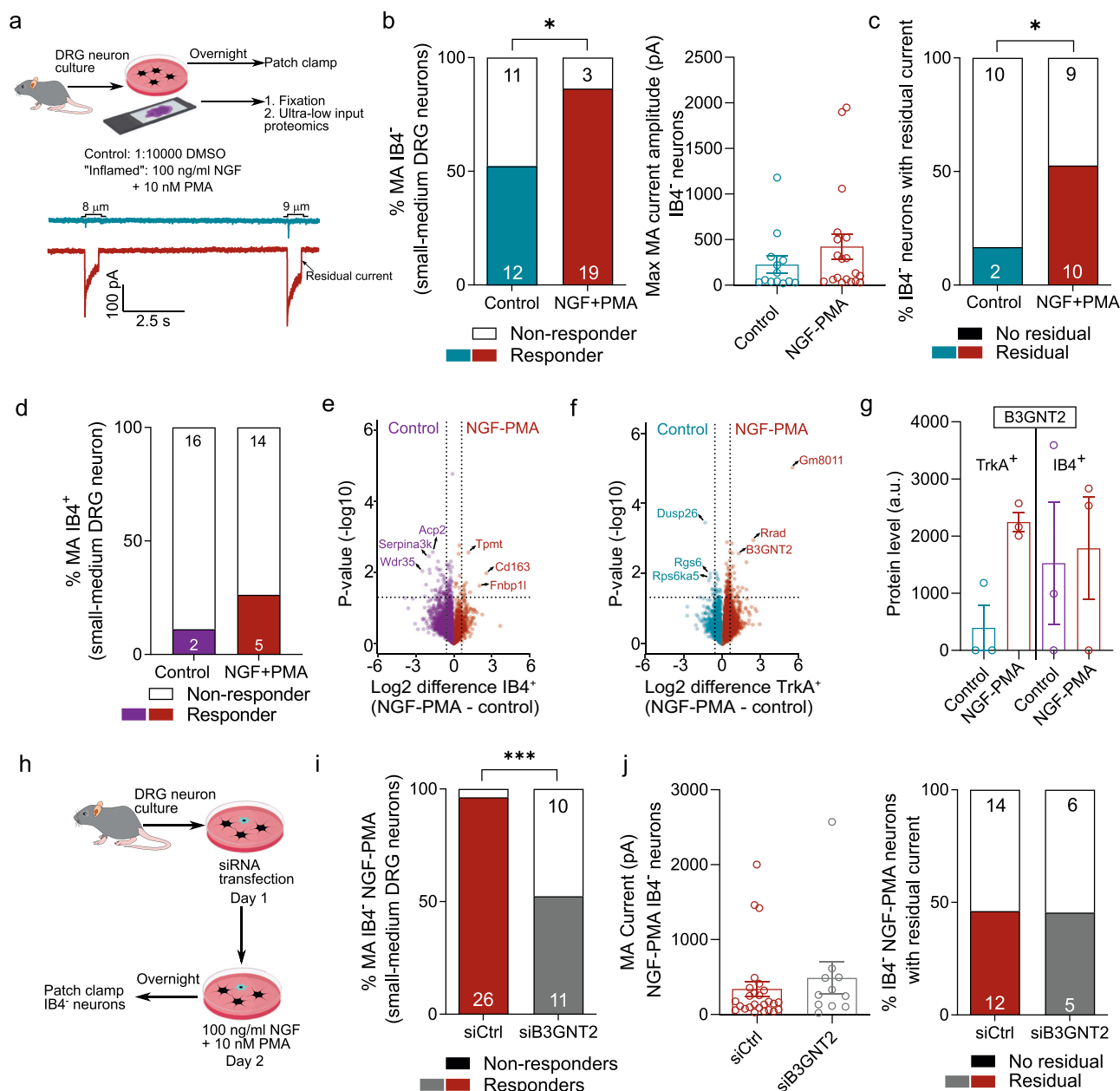


Fig. 4 | B3GNT2 is identified as a putative driver of peptidergic neuron sensitization. **a** Schematic workflow of sensory neurons cultured in an “inflammatory soup” containing nerve growth factor, NGF, and phorbol 12-myristate 13-acetate, PMA (top), along with representative indentation-evoked currents from a control (cyan) and an inflamed (red) peptidergic neuron. **b** Percentage of mechanically sensitive control and inflamed peptidergic nociceptors (left). * represents $p = 0.027$, two-sided chi-sq test. The number of neurons is indicated in the bars, along with their corresponding current amplitude (right). **c** Percentage of control and inflamed peptidergic nociceptors with residual current. * represents $p = 0.045$, two-sided chi-sq test. **d** Percentage of mechanically sensitive control (magenta) and inflamed (red) non-peptidergic nociceptors. The number of neurons is indicated in the bars. Volcano plots showing the pairwise proteomic comparison between control and inflamed neurons in non-peptidergic (e) and peptidergic (f) nociceptors with top regulated proteins highlighted (two-sided Student’s t test). **g** Bar plots

showing raw protein levels of B3GNT2 in the control and inflamed groups of peptidergic (TrkA⁺, $n = 3$) and non-peptidergic (IB4⁺, $n = 3$) nociceptors. Each dot represents a single mouse. **h** Schematic workflow of the validation experiment by knockdown of *B3gnt2* using siRNA prior to inflammation and patch-clamp electrophysiology of peptidergic neurons. **i** Percentage of mechanically sensitive inflamed peptidergic nociceptors transfected with control siRNA (red) and B3gnt2-targeting siRNA (gray). The number of neurons is indicated in the bars. *** represents $p = 0.0003$, two-sided chi-sq test. **j** Current amplitudes (left) and percentage of neurons with residual currents in inflamed peptidergic nociceptors transfected with control siRNA (red, $n = 26$) and B3gnt2-targeting siRNA (gray, $n = 11$). Error bars in all panels represent s.e.m with the mean as the center. Source Data are provided as a Source Data file. Parts of Fig. 4a were created in BioRender. Coscia, F. (2026) <https://BioRender.com/a39426f>.

the same sensory neuron is feasible, with future applications in matching proteomes to single-cell physiology. The high sensitivity of our system also implies that quantifying proteins from the fine nerve endings is a future possibility.

Our ~ 6400-protein dataset for three subtypes of sensory neurons was largely consistent with transcriptionally defined gene sets from the

harmonized single-cell transcriptomics atlas³. Importantly, we define proteome-based markers of sensory neuron subsets, which complement single-cell transcriptomics-derived markers. For example, only six genes (*Htr3a*, *Calca*, *Ntrk1*, *Atp2b4*, *Fgf12*, and *Dcl1*) in our top 50 proteome-defined peptidergic markers matched a compiled list of all top 50 genes across six Calca-expressing transcriptomic clusters. This

highlights the importance of measuring cell type-specific proteomes that are not predictable from transcriptomes.

Here, we show how peptidergic and non-peptidergic nociceptive neurons have highly distinctive proteome signatures. Our data shed light on how the molecular make-up of these distinct nociceptor populations may determine their distinct anatomical connectivity and physiological properties^{21–23,54}. The rapid appearance of mechanosensitivity in nociceptors that were previously unresponsive to mechanical stimuli is termed “unsilencing” and can be initiated by inflammatory mediators, such as NGF^{5,55–57}. Nociceptor “unsilencing” is long known to be rapid in onset and long-lasting in a wide variety of nociceptors⁵⁸, making it likely that changes in protein abundance or localization underlie this phenomenon. To illustrate the utility of our proteomics approach, we asked whether agents that produce nociceptor “unsilencing” change protein abundance in defined sensory neuron sub-populations (Fig. 4). We showed that NGF combined with activation of protein kinase C (PKC) produced subtype-specific nociceptor “unsilencing” in vitro²² (Fig. 4). Peptidergic sensory neurons, which predominantly express the NGF receptor *Ntrk1* (TRKA), were specifically sensitized to mechanical stimuli²². Following functional phenotyping, we identified significant changes in the proteome of stimulated peptidergic neurons that could, in principle, be effectors of inflammatory sensitization. We found that the beta-1,3-N-acetylglucosaminyltransferase enzyme B3GNT2⁵⁹ was one such upregulated protein in sensitized nociceptors⁵¹. Using siRNA-mediated gene knockdown of *B3gnt2*, we could demonstrate reversal of inflammation-induced “unsilencing” of peptidergic neurons. Although the role of B3gnt2 in inflammatory acute and chronic pain requires further in vivo validation, our data implicate sugar modification of proteins as a necessary step in the acute mechanical sensitization of nociceptors. The loss of B3GNT2 activity in the mouse has been associated with mislocalization of axonal guidance factors in the olfactory system⁶⁰, therefore, we speculate that the activity of this enzyme is necessary for the transport of mechanically gated ion channels or their regulators to the plasma membrane to mediate “unsilencing” in nociceptors. Interestingly, genetic variation at the *B3GNT2* locus in humans has been associated with more severe symptoms in patients with ankylosing spondylitis (a painful inflammatory arthropathy⁶¹). In contrast, knockdown of another candidate protein upregulated in sensitized nociceptors, *Rrad*, did not reverse inflammation-induced mechanical hypersensitivity, highlighting the importance of combining electrophysiology with ultra-sensitive proteomics in validation studies.

In summary, we provide a high-resolution, subset-specific proteome of functionally distinct sensory neurons. Importantly, we show that understanding the molecular pathways involved in nociceptor sensitization requires cell type-specific proteomes. Our dataset offers opportunities to explore the molecular mechanisms underlying nociception, further advancing our understanding of how chronic pain develops and can be prevented.

Methods

Animals

Wildtype C57BL/6 N adult (10–30 weeks) mice of both sexes were used in the study for electrophysiology and only male in proteomics experiments. $N = 4$ male and $n = 3$ female mice were used in patch clamp experiments in Fig. 1. $N = 3$ male mice were used for both electrophysiology and cultured neuron proteomics. $N = 2$ female and $n = 4$ male mice were used for siCtrl patch clamp experiments. siB3gnt2 and siRrad patch clamp data was derived from $n = 2$ male and $n = 1$ female mice in each condition. Mice were housed in groups of 5 with food, water, and enrichment available ad libitum at the animal house of the Max Delbrück Center for Molecular Medicine (Berlin, Germany). The animals were kept at $22 \pm 2^\circ\text{C}$, with a humidity of $55 \pm 10\%$ and a light regime of 12 h light and 12 h dark. All animal protocols were approved by the German federal authorities (State of Berlin). Before the

dissection of dorsal root ganglia, all animals were euthanized by cervical dislocation.

Dorsal root ganglion (DRG) neuron culture

Lumbar DRGs (L2–L5) were collected from mice into plating medium (DMEM-F12 (Gibco) supplemented with 10% fetal horse serum (FHS, Life Technologies) and 1% penicillin and streptomycin (P/S, Sigma-Aldrich)). These DRGs then underwent enzymatic digestion in 1.25% Collagenase IV (1 mg/ml, Sigma-Aldrich) for 1 h at 37°C and 2.5% Trypsin (Sigma-Aldrich) for 15 min at 37°C , followed by mechanical trituration with a P1000 pipette tip and purification in a 15% fraction V BSA column. The neurons were plated on glass-bottomed plates (for indentation assays) coated with poly-L-lysine and laminin for electrophysiology and in UV-sterilized (30 min) polyphenylene sulfide (PPS, Leica microsystems) membrane slides for neuroproteomics. Neurons were cultured at 37°C in a 5% CO_2 incubator.

For experiments involving in vitro inflammation, neurons were incubated overnight in 100 ng/ml nerve growth factor (NGF- β human, N1408, Sigma, 0.1 mg/ml stock in water) and 10 nM phorbol 12-myristate 13-acetate (PMA, P1585, Sigma, diluted from a 1 mM stock in DMSO) before whole cell electrophysiology.

Patch clamp electrophysiology

Whole-cell patch clamp electrophysiology was performed using heat-polished borosilicate glass pipettes (Harvard apparatus, 1.17 mm $\times$ 0.87 mm) with a resistance of 4–7 M Ω . The pipettes (pulled using a DMZ puller) were filled with a solution containing (in mM): 110 KCl, 10 NaCl, 1 MgCl₂, 1 EGTA, and 10 HEPES, pH adjusted to 7.3 with KOH. The extracellular solution contained (ECS, in mM): 140 NaCl, 4 KCl, 2 CaCl₂, 1 MgCl₂, 4 Glucose, and 10 HEPES, pH adjusted to 7.4 with NaOH. To elicit action potentials, neurons were recorded in current-clamp configuration while being subjected to a series of incremental current injections. Each sweep began with a 50 ms baseline at 0 pA, followed by a hyperpolarizing pulse of -100 pA for 100 ms. Subsequently, depolarizing current steps were applied in 50 pA increments (ranging from 0 to 950 pA) for 100 ms, after which the holding current was returned to 0 pA.

For indentation experiments, neuronal cell membranes were displaced ten times within a range of 1 – 9 μm (empirically measured membrane displacements were 0.6, 1.6, 2.6, 4, 4.6, 5.6, 6.6, 7.2, 8, 9 μm) using a heat-polished borosilicate glass pipette (mechanical stimulator) driven by an MM3A micromanipulator (Kleindiek Nanotechnik, Germany)³². All dishes were incubated in 1:400 Isolectin-GS-IB4-AlexaFluor 594 conjugate (I21413, Thermo Fischer Scientific) for 10 min followed by a 3 min wash in ECS. Labeled IB4⁺ neurons were identified under a Zeiss 200 upright epifluorescence microscope (40x objective) connected to a CoolSnapEZ camera (Photometrics). For a neuron to be considered “responder” to mechanical indentation, it had to respond to all subsequent force stimuli after its first response to an indentation. A “non-responder” neuron did not show currents between the 1–9 μm of indentation tested. The first force at which the neurons responded was their mechanical threshold. Mechanically evoked currents were further characterized according to time constant to inactivation into rapidly adapting (RA, $T_{\text{inact}} < 10$ ms), intermediately adapting (IA, $T_{\text{inact}} > 10 < 50$ ms) or slowly adapting (SA, $T_{\text{inact}} > 50$ ms) currents.

Neurons were first tested for action potential properties, then switched to voltage-clamp mode for indentation experiments. Current-clamp and voltage-clamp traces were analyzed using FitMaster (HEKA, Elektronik GmbH, Germany).

siRNA transfection experimental design

Two hours after plating the DRG neurons in glass-bottomed dishes, siRNA transfection was carried out using DharmaFECT (Horizon) reagents according to the manufacturers’ guidelines. Briefly, siRNAs

were resuspended in serum- and antibiotic-free DRG neuron plating medium in a tube (total volume of 100 μ L per dish), and 4.5 μ L of DharmaFECT 4 transfection reagent was mixed with serum- and antibiotic-free DRG neuron plating medium (total volume of 100 μ L) in another tube. Each tube was incubated separately for 5 min at RT and then mixed and incubated for an additional 20 min. Antibiotic-free complete medium (800 μ L) was added to the transfection mix before being added to the neurons. Experimental dishes were transfected with a final concentration of 50 nM ON-TARGETplus SMARTpool mouse *B3gnt2* or mouse *Rrad* and 50 nM of siGLO green transfection indicator, and control dishes were transfected with 50 nM of ON-TARGETplus Non-targeting Pool and 50 nM of siGLO green transfection indicator. Transfected neurons that were not live-stained for IB4 were patched 48 h after transfection with siRNA.

Immunocytochemistry

Cultured DRG neurons in PPS membrane slides were washed once for 5 min with PBS-Tween and fixed with Zamboni's fixative for 10 minutes at room temperature ($\sim$ 22 deg C). Post-fixation, slides were washed three times for 5 min with PBS-Tween, permeabilized with 0.05% Triton X-100 for 5 min and washed again three times for 5 min each. Before staining, slides were blocked in antibody diluent solution: 0.2% (v/v) Triton X-100, 5% (v/v) donkey serum and 1% (v/v) bovine serum albumin in PBS for 1 h at room temperature before overnight incubation at 4 °C with 1:500 polyclonal anti-goat TrkA (R&D systems, AF1056) primary antibody. The next day, slides were washed three times using PBS-Tween and incubated with anti-goat conjugated secondary antibody (1:500 Alexa Fluor 488, Jackson Immuno Research Labs 705545147) and 1:200 Isolectin-GS-IB4-AlexaFluor 594 conjugate (I21413, Thermo Fischer Scientific) for 2 h and then washed three times with PBS-Tween. Finally, slides were washed once with distilled water, air dried, and stored at 4 °C until laser microdissection (LMD).

Immunohistochemistry of sections

L3 and L4 DRG were collected from male mice, postfixed in Zamboni's fixative for 1 h, and incubated overnight in 30% (w/v) sucrose (in PBS) at 4 °C for cryoprotection. DRG were next embedded in Shandon M-1 Embedding Matrix (Thermo Fisher Scientific), snap frozen on dry ice, and stored at -80 °C. Embedded DRG were sectioned (5 μ m) using a Leica Cryostat (CM3000; Nussloch) and mounted on polyethylene naphthalate (PEN) glass frame membrane slides (Leica microsystems). Consecutive sections were collected on different slides to prevent sampling the same neuronal cell body multiple times. During staining, slides were washed with PBS-Tween, blocked in antibody diluent solution for 1 h, and then incubated with primary antibodies at 4 °C. The following primary antibodies were used: anti-P2X3 (host: guinea pig, Alomone Labs APR-016-GP, 1:200), anti-CGRP (host: rabbit, Immunostar 24112, 1:1000), anti-NF200 (host: chicken, Abcam AB72996, 1:1000). After washing the unbound primary antibodies as described for immunocytochemistry, slides were incubated for 2 h at room temperature with the following species-specific conjugated secondary antibodies at 1:500: Alexa Fluor 488 anti-rabbit (A21206), Alexa Fluor 568 anti-guinea pig (A11075), and Alexa Fluor 488 (A11039). The unbound secondary antibodies were then thoroughly washed with PBS-Tween three times, and then once with distilled water and air-dried. The sections were imaged using an Olympus CSU-WI spinning disk microscope at 20x magnification. Exposure levels were kept constant for each slide, and the same contrast enhancements were made for all slides. Stained and imaged slides were stored at 4 °C until LMD.

Image segmentation

The images obtained from DRG tissue sections were imported to the QuPath software with an integrated Cellpose extension (as detailed in <https://github.com/BIOP/qupath-extension-cellpose>) for further

analysis. We previously trained our Cellpose 2.0⁴⁶ model ("trk_test") based on the cyto2 model using a human-in-the-loop approach with a mean diameter of 30. The human-in-the-loop approach meant that each contour was verified visually before and during laser microdissection. This model was integrated into QuPath⁴⁷ to segment neurons positive for specific antibody staining. The contours thus obtained were dilated radially to prevent accidental laser ablation and converted to an LMD-compatible format for automated LMD¹⁷ (as detailed in https://github.com/CosciaLab/Qupath_to_LMD).

Laser microdissection

We collected cultured cells and tissue samples using the Leica LMD 7 system and Leica Laser Microdissection software (version 8.3.0.08259). Cell culture and tissue samples were cut using a 40x objective in fluorescence or brightfield mode. For tissue samples, we used the following laser settings: power 60, aperture 1, speed 26, head current 45%, pulse frequency 2110. For the cell culture samples, we used the following laser settings: power 60, aperture 1, speed 20, head current 37%, pulse frequency 1537. Low-binding 384-well plates (Eppendorf, cat.no. 0030129547) were used for sample collection and configured over the "universal holder" function with one empty well between the samples. For pooled sample analysis we cut $\sim$ 100 neuron contours from tissue and $\sim$ 50 cultured neurons of each of the three subsets from each of the three mice. Different cohorts of mice were used for tissue and cell culture experiments. For single-neuron analysis, 18 tissue sections were taken from each of three mice in each of the three neuron subsets.

Sample preparation for LC-MS analysis

Sample preparation was performed as previously described¹⁷. Briefly, before sample preparation, 15 μ L of acetonitrile (HPLC grade, VWR, cat. 83640.290) was added to each well, vortexed, and dried using speedvac. This drying process ensured that the sample contours were concentrated at the bottom of each well. Pipetting was performed either manually or using the MANTIS Liquid Dispenser (Formulatrix, V3.3 ACC RFID, software version 4.7.5) and high-volume diaphragm chips (Formulatrix, cat.no. 233128).

For cell culture, samples were first incubated in 4 μ L 60 mM Triethylammonium bicarbonate pH 8.5 (TEAB; Merck, cat.no. T7408-100ML) at 95 °C for 60 min. Then, the samples were cooled, and 1 μ L of 100% ACN (HPLC-grade, VWR, cat. 83640.290) was added and incubated for 60 min at 75 °C. Next, 1 μ L of LysC (Promega, cat. no. VA1170) (2 ng/ μ L) was added, followed by a 2 h digestion at 37 °C. Then, 1 μ L of trypsin (Promega, cat.no. V5117) (2 ng/ μ L) was added with overnight incubation at 37 °C in the thermal cycler. Digestion was stopped the next day by the addition of trifluoroacetic acid (TFA, final concentration 1% v/v) (cat. no. 96924-250ML-F). The samples were vacuum dried before peptide clean-up.

For tissue samples, the lysis buffer for sample preparation consisted of 0.1% n-dodecyl-beta-maltoside (DDM; Sigma-Aldrich, cat.no. D4641-500MG), 5 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP; Sigma-Aldrich, cat.no. C4706-2G), 20 mM 2-chloroacetamide (CAA; Sigma-Aldrich, cat.no. C0267-100G and 0.1M TEAB in water. 2 μ L of lysis buffer were added to wells containing samples and incubated for 60 min at 95 °C. Subsequently, 1 μ L of LysC (Promega, cat.no. VA1170) (2 ng/ μ L) was added and digested for 2 h at 37 °C, followed by the addition of 1 μ L of trypsin (Promega, cat.no. V5117) (2 ng/ μ L) and overnight digestion at 37 °C in a thermal cycler. After digestion, the reaction was stopped by adding trifluoroacetic acid (TFA, final concentration 1% v/v) (cat. 96924-250ML-F). The samples were then vacuum-dried before peptide clean-up.

Peptide clean-up with Evotips

Peptide clean-up was performed using the Evotip Pure protocol, as recommended by the manufacturer. Briefly, Evotips (EV2013, Evotip

Pure, Evosep, cat.no. EV2013) were first washed with 20 μ L of buffer B (99.9% ACN, 0.1% FA) and centrifuged (800 rcf for 1 min). Then, 20 μ L of buffer A (99.9% water, 0.1% FA) was added to each evotip, activated in isopropanol for 20 s, and centrifuged (800 rcf for 1 min). Digested tissue or cell culture samples were then loaded onto Evotips by centrifuging (800 rcf for 1 min), washed once with 20 μ L buffer A (800 rcf for 1 min), and finally eluted with 20 μ L buffer B in a 96-well plate (Thermo Fisher Scientific, cat. no. ABI300), and vacuum dried (15 min at 60 °C). The samples were stored at 20 °C until liquid chromatography–mass spectrometry (LC–MS) analysis. For LC–MS analysis, 4.2 μ L of MS loading buffer (3% acetonitrile, 0.1% TFA in water) was added, the plate was vortexed for 10 s, and centrifuged for 1 min at 700 $\times$ g. Four microliters were injected into the mass spectrometer for LC–MS analysis.

Liquid chromatography–mass spectrometry (LC – MS) analysis

LC–MS analysis was performed with an EASYnLC-1200 system (Thermo Fisher Scientific) connected to a trapped ion mobility spectrometry quadrupole time-of-flight mass spectrometer (timsTOF SCP (cell culture samples), timsTOF Ultra (tissue samples), Bruker Daltonik) with a nano-electrospray ion source (CaptiveSpray, Bruker Daltonik). An autosampler was configured to pick samples from 96-well plates. Peptides were loaded onto a 20 cm home-packed HPLC column (75 mm inner diameter packed with 1.9 mm ReproSil-Pur C18-AQ silica beads, Dr. Maisch).

For tissue samples, peptides were separated using a linear gradient from 7–30% buffer B (0.1% formic acid and 90% ACN in LC–MS grade water) for 14 min, followed by an increase to 60% for 1 min and a 1.5 min wash in 90% buffer B at 250 nL/min. Buffer A consisted of 0.1% formic acid in liquid chromatography–mass spectrometry (LC–MS) grade water. The total gradient length was 21 min. A column oven was used to maintain the column temperature at 40 °C.

For cell culture analysis, peptides were separated using a linear gradient from 4–30% buffer B (0.1% formic acid and 90% ACN in LC–MS grade water) for 47 min, followed by an increase to 60% for 3 min and a 3 min wash in 90% buffer B, followed by a gradual decrease to 50% buffer B over 7 min. The flow rate was 250 nL/min.

For tissue samples, we used a dia-PASEF method with 8 dia-PASEF scans separated into 3 ion mobility windows per scan covering a 400–1000 m/z range by 25 Th windows and an ion mobility range from 0.64 to 1.37 Vs cm². The mass spectrometer was operated in high sensitivity mode, with an accumulation and ramp time at 100 ms, capillary voltage set to 1750 V and the collision energy as a linear ramp from 20 eV at 1/k₀ = 0.6 Vs cm² to 59 eV at 1/k₀ = 1.6 Vs cm².

For cell culture analysis, we used a dia-PASEF long method with 16 dia-PASEF scans separated into 2 ion mobility windows per scan covering a 400–1000 m/z range by 26 Th windows and an ion mobility window range from 0.60 to 1.60 cm². The mass spectrometer was operated in high sensitivity mode, with an accumulation and ramp time at 100 ms, capillary voltage set to 1600 V and the collision energy as a linear ramp from 20 eV at 1/k₀ = 0.6 Vs cm² to 59 eV at 1/k₀ = 1.6 Vs cm².

Mass spectrometry raw file analysis

We used DIA-NN⁶ (version 1.8.1) for the dia-PASEF raw file analysis and spectral library generation. For spectral library generation to analyze dia-PASEF data, the mouse FASTA file was downloaded from Uniprot (2023 release, UP000000589_10090 *Mus Musculus*, downloaded on August 2nd). DIA-NN in silico predicted libraries were generated using the mouse FASTA file, and frequently found contaminants⁶² (mouse + mouse tissue contaminants). Deep learning-based spectra, RTs, and IMs prediction were enabled for the appropriate mass range of 300–1000 m/z. N-terminal M excision and cysteine carbamidomethylation were enabled as fixed modifications. A maximum of two missed cleavages were allowed, and the precursor charge was set to 2–4. The

library consisted of 22092 protein isoforms, 34222 protein groups, and 6776851 precursors in 3121869 elution groups.

DIA-NN⁶³ was operated in the default mode with minor adjustments. Briefly, MS1 and MS2 accuracies were set to 15.0, scan windows to 0 (assignment by DIA-NN), isotopologues were enabled, no MBR for project-specific DIA-NN-refined libraries, heuristic protein inference, and no shared spectra. Proteins were inferred from genes, a neural network classifier was set to single-pass mode, quantification strategy as 'Robust LC (high precision)'. Cross-run normalization was set to 'RT-dependent', library generation as 'smart profiling', speed and RAM usage as 'optimal results'.

Quantification and statistical analysis

Proteomics data analysis was performed with Perseus⁶⁴ (version 1.6.15.0) and within the R environment (<https://www.r-project.org/>) with the following packages: tidyverse (version 2.0.0), UpSetR (version 1.4.0), FactoMineR (version 2.11), factoextra (version 1.0.7), ComplexHeatmap (version 2.18.0), RColorBrewer (version 1.1.3), circlize (version 0.4.16), dendsort (version 0.3.4), ggrepel (version 0.9.5), viridis (version 0.6.5) and clusterProfiler (version 4.6.2). Before statistical analysis, data were filtered to retain only proteins with 70% non-missing data in at least one group (if not stated otherwise in the figure legend). Before statistical testing, missing values were imputed independently for each sample (column-wise) in the Perseus software based on a normal distribution drawn from the measured values using a downshift of 1.8 standard deviations and a width of 0.3. For single-neuron proteomics (Fig. 3), two outlier samples (ids .6944 and .7041) were removed, and samples only kept for further analysis with a minimum of 700 protein groups, resulting in 20 CGRP⁺ peptidergic and 22 P2X3⁺ non-peptidergic neuron proteomes. For multiple group comparisons, analysis of variance (ANOVA) was used. For both tests, a permutation-based FDR of 5% was applied to correct multiple hypothesis testing. Pathway enrichment analysis was performed in Perseus based on Fisher's exact test (categorical data) or 1D pathway enrichment analysis⁶⁵. Hallmark gene sets, WikiPathways, and Reactome pathways were enriched terms filtered using a Benjamini-Hochberg FDR cut-off of 0.05. The minimum category size was set to five.

To estimate transcriptome vs proteome depth in Supplementary Fig. 3, we downloaded the scRNA seq GEO dataset from Sharma et al. (GSM4130750_WT_1.csv)². CGRP and Non-peptidergic neurons were then grouped to calculate the average transcript reads from all genes in each subset. These genes were then matched by name to our proteomics dataset. Data were plotted as a histogram across the average transcript reads, showing the proportion of gene counts detected in proteomics over transcriptomics. Furthermore, we selected some genes with low (<100 transcripts) and high (>1000 transcripts) average copy numbers to demonstrate the robust detection of proteins from genes with low transcript reads.

For proteomic-transcriptomic comparisons in Fig. 2, we used data from Bhuiyan et al.³. Briefly, the "Marker Genes" file was downloaded from the accompanying website (https://painseq.shinyapps.io/harmonized_painseq_v1/), and DRG neuron and non-neuron tabs were used for further analysis in R or Perseus. To match non-neuronal gene names between transcriptomics and proteomics data, we compiled all non-neuronal associated genes from all types of DRG-associated non-neuronal cells (Fig. 2e). These were matched with our proteome dataset to calculate the number of non-neuronal proteins. The mean values of the matched proteins were plotted in a sequential color map.

To compare neuronal marker genes (Fig. 2f), we compiled a list of the top 50 transcriptomics-related genes from all 18 subsets of sensory neurons (with all positive log fold change values), matched them against our proteomics data, and performed z-scoring based on spearman distance for hierarchical clustering. For single CGRP⁺ neuron

comparisons, all genes with a positive log fold change value (obtained from the Marker Genes file in Bhuiyan et al.) in each of the CGRP-related transcriptomic clusters were normalized between 0 and 1 using the `preProcess()` function of the `caret` library in R using the range method. Similar normalization was carried out for the log₂ intensity of proteins quantified in each of the CGRP⁺ single neurons. The names of the genes were matched to get a normalized value from transcriptomics (Gtn) and a normalized value from proteomics (Gpn) for each matching gene. For each transcriptomics cluster we then calculated a Euclidean distance score = $\sqrt{\sum (Gtn - Gpn)^2}$ (resulting in a 20 × 6 matrix) to estimate the probability that our single neuron belongs to a specific transcriptomics-defined CGRP cluster and visualized using a heatmap.

Statistical tests for electrophysiological data are detailed in the figure legends. Briefly, comparisons between two groups were carried out using Student's *t* test (with Welch's correction if the variances were significantly different between groups), and proportions were compared using the chi-squared test in GraphPad Prism 9. Figures were created using Inkscape.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository⁶⁶ with the dataset identifier PXD070495 (<https://www.ebi.ac.uk/pride/archive>). Source data are provided in this paper.

Code availability

The code used for analysis and plots were adapted from standard R packages as detailed in the Methods section. More detailed information is available upon request.

References

- Usoskin, D. et al. Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat. Neurosci.* **18**, 145–153 (2015).
- Sharma, N. et al. The emergence of transcriptional identity in somatosensory neurons. *Nature* **577**, 392–398 (2020).
- Bhuiyan, S. A. et al. Harmonized cross-species cell atlases of trigeminal and dorsal root ganglia. *Sci. Adv.* **10**, ead9173 (2024).
- Mund, A. et al. Deep visual proteomics defines single-cell identity and heterogeneity. *Nat. Biotechnol.* **40**, 1231–1240 (2022).
- Lewin, G. R., Lechner, S. G. & Smith, E. S. J. Nerve growth factor and nociception: from experimental embryology to new analgesic therapy. *Handb. Exp. Pharm.* **220**, 251–282 (2014).
- Yu, H. et al. Leveraging deep single-soma RNA sequencing to explore the neural basis of human somatosensation. *Nat. Neurosci.* **1**, 15 (2024).
- Zeisel, A. et al. Molecular architecture of the mouse nervous system. *Cell* **174**, 999–1014 (2018).
- Adameyko, I. et al. Applying single-cell and single-nucleus genomics to studies of cellular heterogeneity and cell fate transitions in the nervous system. *Nat. Neurosci.* **27**, 2278–2291 (2024).
- Guo, T., Steen, J. A. & Mann, M. Mass-spectrometry-based proteomics: from single cells to clinical applications. *Nature* **638**, 901–911 (2025).
- Slavov, N. Unpicking the proteome in single cells. *Science* **367**, 512–513 (2020).
- Liu, Y., Beyer, A. & Aebersold, R. On the dependency of cellular protein levels on mRNA abundance. *Cell* **165**, 535–550 (2016).
- Buccitelli, C. & Selbach, M. mRNAs, proteins and the emerging principles of gene expression control. *Nat. Rev. Genet.* **21**, 630–644 (2020).
- Barry, A. M., Sonderrmann, J. R., Sonderrmann, J.-H., Gomez-Varela, D. & Schmidt, M. Region-resolved quantitative proteome profiling reveals molecular dynamics associated with chronic pain in the PNS and spinal cord. *Front. Mol. Neurosci.* **11**, <https://doi.org/10.3389/fnmol.2018.00259> (2018).
- Grundtner, S. et al. Deep proteomics and network pharmacology reveal sex- and age-shared neuropathic pain signatures in mouse dorsal root ganglia. *Pharmacol. Res.* **211**, 107552 (2025).
- Pogatzki-Zahn, E. M. et al. A proteome signature for acute incisional pain in dorsal root ganglia of mice. *Pain* **162**, 2070–2086 (2021).
- Leal-Julia, M. et al. Proteomic quantitative study of dorsal root ganglia and sciatic nerve in type 2 diabetic mice. *Mol. Metab.* **55**, 101408 (2022).
- Makhmut, A. et al. A framework for ultra-low-input spatial tissue proteomics. *Cell Syst.* **14**, 1002–1014 (2023).
- Lewin, G. R. & Moshourab, R. Mechanosensation and pain. *J. Neurobiol.* **61**, 30–44 (2004).
- Nagy, J. I. & Hunt, S. P. Fluoride-resistant acid phosphatase-containing neurones in dorsal root ganglia are separate from those containing substance P or somatostatin. *Neuroscience* **7**, 89–97 (1982).
- Silverman, J. D. & Kruger, L. Selective neuronal glycoconjugate expression in sensory and autonomic ganglia: relation of lectin reactivity to peptide and enzyme markers. *J. Neurocytol.* **19**, 789–801 (1990).
- Stucky, C. L. & Lewin, G. R. Isolectin B(4)-positive and -negative nociceptors are functionally distinct. *J. Neurosci.* **19**, 6497–6505 (1999).
- Di Castro, A., Drew, L. J., Wood, J. N. & Cesare, P. Modulation of sensory neuron mechanotransduction by PKC- and nerve growth factor-dependent pathways. *Proc. Natl. Acad. Sci. USA* **103**, 4699–4704 (2006).
- Molliver, D. C. et al. IB4-Binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron* **19**, 849–861 (1997).
- Marmigere, F. & Ernfor, P. Specification and connectivity of neuronal subtypes in the sensory lineage. *Nat. Rev. Neurosci.* **8**, 114–127 (2007).
- Belyantseva, I. A. & Lewin, G. R. Stability and plasticity of primary afferent projections following nerve regeneration and central degeneration. *Eur. J. Neurosci.* **11**, 457–468 (1999).
- McNeill, D. L., Carlton, S. M., Coggeshall, R. E. & Hulsebosch, C. E. Denervation-induced intraspinal synaptogenesis of calcitonin gene-related peptide containing primary afferent terminals. *J. Comp. Neurol.* **296**, 263–268 (1990).
- Lewin, G. R. William D. Willis, Jr, MD, PhD Memorial Lecture: The evolutionary history of nerve growth factor and nociception. *Pain* **161**, S36–S47 (2020).
- Lane, N. E. et al. Tanezumab for the treatment of pain from osteoarthritis of the knee. *N. Engl. J. Med.* **363**, 1521–1531 (2010).
- Reid, J., Gildea, E., Davies, V., Thompson, J. & Scott, M. Measuring the effect of the anti-nerve growth factor antibodies bedinvetmab and frunevetmab on quality of life in dogs and cats with osteoarthritis using a validated health-related quality of life outcome measure: an observational real-world study. *Front. Vet. Sci.* **11**, 1395360 (2024).
- Hu, J. & Lewin, G. R. Mechanosensitive currents in the neurites of cultured mouse sensory neurones. *J. Physiol.* **577**, 815–828 (2006).

31. Coste, B., Crest, M. & Delmas, P. Pharmacological dissection and distribution of Na_v1.9, T-type Ca²⁺ currents, and mechanically activated cation currents in different populations of DRG neurons. *J. Gen. Physiol.* **129**, 57–77 (2007).
32. Chakrabarti, S. et al. Touch sensation requires the mechanically gated ion channel ELKIN1. *Science* **383**, 992–998 (2024).
33. Lechner, S. G., Frenzel, H., Wang, R. & Lewin, G. R. Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development. *EMBO J.* **28**, 1479–1491 (2009).
34. Ranade, S. S. et al. Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature* **516**, 121–125 (2014).
35. Fang, X. et al. Intense isolectin-B4 binding in rat dorsal root ganglion neurons distinguishes C-fiber nociceptors with broad action potentials and high Nav1.9 expression. *J. Neurosci.* **26**, 7281 (2006).
36. Cantor, E. L. et al. Passage number affects differentiation of sensory neurons from human induced pluripotent stem cells. *Sci. Rep.* **12**, 15869 (2022).
37. Hanani, M. & Spray, D. C. Emerging importance of satellite glia in nervous system function and dysfunction. *Nat. Rev. Neurosci.* **21**, 485–498 (2020).
38. Tanioku, T. et al. Tmem45b is essential for inflammation- and tissue injury-induced mechanical pain hypersensitivity. *Proc. Natl. Acad. Sci. USA* **119**, e2121989119 (2022).
39. Wetzel, C. et al. A stomatin-domain protein essential for touch sensation in the mouse. *Nature* **445**, 206–209 (2007).
40. Warth Perez Arias, C. C. et al. Proteomic analysis of the human hippocampus identifies neuronal pentraxin 1 (NPTX1) as synaptosomal target in late-stage Parkinson's disease. *J. Neurochem.* **166**, 862–874 (2023).
41. Melli, G. & Höke, A. Dorsal root ganglia sensory neuronal cultures: a tool for drug discovery for peripheral neuropathies. *Expert. Opin. Drug Discov.* **4**, 1035–1045 (2009).
42. Wangzhou, A. et al. Pharmacological target-focused transcriptomic analysis of native vs cultured human and mouse dorsal root ganglia. *Pain* **161**, 1497–1517 (2020).
43. Shiers, S., Klein, R. M. & Price, T. J. Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. *Pain* **161**, 2410–2424 (2020).
44. Chakrabarti, S. et al. Functional characterization of ovine dorsal root ganglion neurons reveal peripheral sensitization after osteochondral defect. *eNeuro* **8**, <https://doi.org/10.1523/eneuro.0237-21.2021> (2021).
45. Shiers, S. I. et al. Convergence of peptidergic and non-peptidergic protein markers in the human dorsal root ganglion and spinal dorsal horn. *J. Comp. Neurol.* **529**, 2771–2788 (2021).
46. Pachitariu, M. & Stringer, C. Cellpose 2.0: how to train your own model. *Nat. Methods* **19**, 1634–1641 (2022).
47. Bankhead, P. et al. QuPath: Open source software for digital pathology image analysis. *Sci. Rep.* **7**, 16878 (2017).
48. Brunner, A. et al. Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation. *Mol. Syst. Biol.* **18**, e10798 (2022).
49. Shiomi, A. et al. High-throughput mechanical phenotyping and transcriptomics of single cells. *Nat. Commun.* **15**, 3812 (2024).
50. Bavi, N., Cox, C. D., Bavi, O. & Martinac, B. Perturbation of bilayer surface tension differentially modulates mechanosensitive ion channels. *Biophys. J.* **112**, 416a (2017).
51. Xie, A., Wang, J., Liu, Y., Li, G. & Yang, N. Impacts of β -1, 3-N-acetylglucosaminyltransferases (B3GNTs) in human diseases. *Mol. Biol. Rep.* **51**, 476 (2024).
52. Wangzhou, A. et al. Pharmacological target-focused transcriptomic analysis of native versus cultured human and mouse dorsal root ganglia. *Pain* **161**, 1497–1517 (2020).
53. Ghatak, S. et al. Single-cell patch-clamp/proteomics of human Alzheimer's disease iPSC-derived excitatory neurons versus isogenic wild-type controls suggests novel causation and therapeutic targets. *Adv. Sci.* **11**, 2400545 (2024).
54. Braz, J. M., Nassar, M. A., Wood, J. N. & Basbaum, A. I. Parallel “pain” pathways arise from subpopulations of primary afferent nociceptor. *Neuron* **47**, 787–793 (2005).
55. Prato, V. et al. Functional and molecular characterization of mechanosensitive “Silent” nociceptors. *Cell Rep.* **21**, 3102–3115 (2017).
56. Schmelz, M. et al. Nerve growth factor antibody for the treatment of osteoarthritis pain and chronic low-back pain: mechanism of action in the context of efficacy and safety. *Pain* **160**, 2210–2220 (2019).
57. Gruen, M. E., Myers, J. A. & Lascelles, B. D. X. Efficacy and safety of an anti-nerve growth factor antibody (frunvetmab) for the treatment of degenerative joint disease-associated chronic pain in cats: a multisite pilot field study. *Front. Vet. Sci.* **8**, 610028 (2021).
58. McMahon, S. B. & Koltzenburg, M. Novel classes of nociceptors: beyond Sherrington. *Trends Neurosci.* **13**, 199–201 (1990).
59. Zhou, D. et al. A beta-1,3-N-acetylglucosaminyltransferase with poly-N-acetyllactosamine synthase activity is structurally related to beta-1,3-galactosyltransferases. *Proc. Natl. Acad. Sci. USA* **96**, 406–411 (1999).
60. Henion, T. R., Madany, P. A., Faden, A. A. & Schwarting, G. A. β 3GnT2 null mice exhibit defective accessory olfactory bulb innervation. *Mol. Cell. Neurosci.* **52**, 73–86 (2013).
61. Wang, C.-M. et al. Genetic effects of B3GNT2 on ankylosing spondylitis susceptibility and clinical manifestations in Taiwanese. *J. Formos. Med. Assoc.* **121**, 1283–1294 (2022).
62. Frankenfield, A. M., Ni, J., Ahmed, M. & Hao, L. Protein contaminants matter: building universal protein contaminant libraries for DDA and DIA proteomics. *J. Proteome Res.* **21**, 2104–2113 (2022).
63. Demichev, V., Messner, C. B., Vernardis, S. I., Lilley, K. S. & Ralser, M. DIA-NN: neural networks and interference correction enable deep proteome coverage in high throughput. *Nat. Methods* **17**, 41–44 (2020).
64. Tyanova, S. et al. The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat. Methods* **13**, 731–740 (2016).
65. Cox, J. & Mann, M. 1D and 2D annotation enrichment: a statistical method integrating quantitative proteomics with complementary high-throughput data. *BMC Bioinform.* **13**, S12 (2012).
66. Perez-Riverol, Y. et al. The PRIDE database at 20 years: 2025 update. *Nucleic Acids Res.* **53**, D543–D553 (2025).

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Competing interests

S.C., G.R.L., and F.C. are inventors in an EU patent filed based on this work. The authors declare no other competing interests.

Additional information

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