Solid tumours are innervated by nerve fibres that arise from the autonomic and sensory peripheral nervous systems. Whether the neo-innervation of tumours by pain-initiating sensory neurons affects cancer immunosurveillance remains unclear. Here we show that melanoma cells interact with nociceptor neurons, leading to increases in their neurite outgrowth, responsiveness to noxious ligands and neuropeptide release. Calcitonin gene-related peptide (CGRP)—one such nociceptor-produced neuropeptide—directly increases the exhaustion of cytotoxic CD8+ T cells, which limits their capacity to eliminate melanoma. Genetic ablation of the TRPV1 lineage, local pharmacological silencing of nociceptors and antagonism of the CGRP receptor RAMP1 all reduced the exhaustion of tumour-infiltrating leukocytes and decreased the growth of tumours, nearly tripling the survival rate of mice that were inoculated with B16F10 melanoma cells. Conversely, CD8+ T cell exhaustion was rescued in sensory-neuron-depleted mice that were treated with local recombinant CGRP. As compared with wild-type CD8+ T cells, Ramp1−/− CD8+ T cells were protected against exhaustion when co-transplanted into tumour-bearing Rag1-deficient mice. Single-cell RNA sequencing of biopsies from patients with melanoma revealed that intratumoral RAMP1-expressing CD8+ T cells were more exhausted than their RAMP1-negative counterparts, whereas overexpression of RAMP1 correlated with a poorer clinical prognosis. Overall, our results suggest that reducing the release of CGRP from tumour-innervating nociceptors could be a strategy to improve anti-tumour immunity by eliminating the immunomodulatory effects of CGRP on cytotoxic CD8+ T cells.

Cytotoxic T cells express a variety of receptors, including PD-1 (programmed cell death protein 1), LAG3 (lymphocyte activation gene-3 protein) and TIM3 (T cell immunoglobulin and mucin domain-containing protein 3) RAMP1, which inhibit the function of CD8+ T cells after being activated by their cognate ligands. These checkpoint receptors ensure that immune responses to damage or infection are kept in check, thus preventing overly intense responses that might damage healthy cells. Tumour cells express ligands for these immune checkpoints, which, when activated, block the cytolytic functions of T cells, thereby favouring the survival of cancer cells.

In prostate cancer, doublecortin-expressing neural progenitors initiate autonomic adrenergic neurogenesis, which facilitates the development and dissemination of tumours. In head and neck tumours, a loss of TP53 drives the reprogramming of tumour-innervating sensory nerves into adrenergic neurons that promote tumour growth. The presence of such neo-innervation in cancer, together with the diverse actions of neuropeptides on immune cells, led us to examine whether the local release of neuropeptides from activated nociceptors could favour cancer growth by suppressing immune surveillance.
Melanomas are innervated

Although the expression of genes of neuronal origin is not detected by RNA-sequencing approaches in human malignant cells or immune cells (Extended Data Fig. 1a–c), we observed a significant increase in their expression in biopsies from patients with melanoma19–22 (Extended Data Fig. 1d). As these clinical data suggested increased innervation of melanomas, we tested for the presence of nociceptor neurons by assessing TRPV1+ neurons in biopsies from patients with melanoma. TRPV1 immunolabelling was increased by around twofold in the tumour compared to adjacent healthy tissue in each of the ten biopsies examined. The numbers of tumour-infiltrating lymphocytes (TILs) correlated ($R^2 = 0.63$) with increased TRPV1 immunolabelling (Extended Data Fig. 2). These data indicate that melanomas are innervated by sensory neurons and that these neurons may affect the intratumoral numbers of immune cells.

To investigate this in more detail, we inoculated a GFP-expressing melanoma (B16F10-eGFP) cell line into NaᵢL.8S reporter mice (Extended Data Fig. 1e). Twenty-two days after implantation, we found abundant NaᵢL.8S nociceptor neurons around and within the tumour (Fig. 1a). RNA sequencing of samples from B16F10-bearing mice revealed that malignant and melanoma-infiltrating immune cells had no detectable levels of neuronal markers (NaᵢL.8 or Trpv1), indicating that the NaᵢL.8 signal could be ascribed to tumour-infiltrating nerves (Extended Data Fig. 3). We next used an in vitro co-culture approach to assess whether malignant cells modulate the function of nociceptor neurons. When co-cultured, TRPV1+ nociceptors directly extended neurites towards the B16F10-eGFP melanoma cells, and the average length of neurites increased, whereas the overall neuronal arborization of neurites remained similar (Extended Data Fig. 4a–c). Together, these data indicate that nociceptor outgrowth is enhanced when in proximity to melanoma cells and that skin sensory neuron collaterals sprout directly into the tumour bed. Such tumour neo-innervation may be akin to cancer's neoangiogenesis.

Melanoma cells sensitize nociceptors

Given that melanoma promotes axonogenesis, leading to tumour innervation (Fig. 1a and Extended Data Fig. 2), we examined whether this physical proximity allows melanomas to modulate the sensitivity of the nociceptor. As nociceptor neurons detect signals from the local environment, we measured changes in calcium flux in response to sub-threshold concentrations of various noxious ligands. When nociceptors were cultured without melanoma cells, few responded...
to the ligands at the concentrations selected. However, the number of responsive neurons increased when they were co-cultured with B16F10 cells (Fig. 1b). Similarly, the amplitude of calcium flux responses to the ligands was greater in lumbar DRG neurons (L3–L5) that were collected ipsilaterally to a 14-day tumour inoculation in mice, as compared to those collected from mice that were injected with non-tumorigenic keratinocytes (Extended Data Fig. 4d). Signals released from melanoma, therefore, heighten nociceptor sensitivity.

We next tested whether this neuronal hypersensitivity would lead to an increased release of immunomodulatory neuropeptides. In contrast to B16F10 cells alone, DRG neurons co-cultured with B16F10 cells (5 × 10^4 cells, 96 h) actively release CGRP in the medium (Fig. 1c).

These data prompted us to test whether exposure to melanoma alters the transcriptome of nociceptor neurons. To do so, we cultured naïve DRG neurons (Trpv1cre::CheRiff-eGFP^WT) alone or in combination with B16F10-mCherry-OVA cells. After 48 h, TRPV1 nociceptors were purified by fluorescence-activated cell sorting (FACS) and RNA sequenced. Differentially expressed genes (DEGs) were calculated, and Calca—the gene that encodes CGRP—and the NGF receptor Trka (also known as Ntrk1) were found to be overexpressed in nociceptors that were exposed to cancer (Fig. 1d–e and Extended Data Fig. 4e). Overexpression of Trka may help to drive melanoma-induced hypersensitivity to pain, whereas CGRP, when released from activated nociceptors, may immunomodulate TILs.

To identify the mechanism through which melanoma sensitizes nociceptor neurons, we used a co-culture system designed to mimic the interactions that take place in the melanoma microenvironment. Type 1 (T1)-stimulated (ex-vivo-activated by CD3 and CD28, IL-12 and anti-IL-4 for 48 h) OVA-specific cytotoxic CD8^+ T cells (OT-I mice), naïve DRG neurons (Trpv1cre::CheRiff-eGFP^WT) and B16F10-mCherry-OVA melanoma cancer cells were cultured alone or in combination. After 48 h, the cells were collected, purified by FACS and RNA sequenced, and DEGs were calculated. Among others, we found that Slpi (secretory leukocyte protease inhibitor) was overexpressed in the melanoma cancer cells when co-cultured with either DRG neurons (around 3.6-fold) or OVA-specific cytotoxic CD8^+ T cells (around 270-fold), and when exposed to both populations (around 150-fold) (Fig. 2a, b and Extended Data Fig. 5a–e). We also found that B16F10-mCherry-OVA cells, when co-cultured with naïve DRG neurons and OVA-specific cytotoxic CD8^+ T cells, increased the secretion of SLPI into the culture medium, with this effect being maximal after 48 h (around 200-fold; Fig. 2c).

In addition to protecting epithelial cells from the activity of serine proteases, SLPI enhances the regeneration of transected retinal ganglion cell axons and the proliferation of neural stem cells. Although these data provide evidence of the effect of SLPI on neurons, its role in nociception is unclear. To address this, we measured whether SLPI directly activates cultured DRG neurons using calcium microscopy. We found that SLPI (0.01–10 μg ml^-1) activates around 20% of DRG neurons and that—consistent with these neurons being nociceptors—SLPI-sensitive neurons were mostly small (with a mean area of 15 μm²) capsaicin-responsive (around 90%) neurons (Fig. 2d, e and Extended Data Fig. 5f–l). Given that SLPI triggered calcium influx, we investigated whether this is the means by which B16F10 cells drive the release of CGRP from neurons (Fig. 1c). SLPI, when used at a concentration similar to that secreted by melanoma cells (Fig. 2c), induced the release of CGRP from cultured naïve DRG neurons (Fig. 2f). Finally, we sought to test whether SLPI can drive pain hypersensitivity in vivo. When administered into the right hindpaw of naive mice, SLPI generated transient thermal hypersensitivity (Extended Data Fig. 5j).

Melanoma-secreted SLPI acts on nociceptors to trigger calcium influx, neuropeptide release and thermal hypersensitivity, which indicates that these sensory neurons detect and react to the presence of cancer cells. Whether this gives the malignant cells a functional advantage over the host cells remains unknown. To assess this, we implanted B16F10-mCherry-OVA cells (intradermally i.d., 2 × 10^5 cells; i.d.) into the hindpaw of eight-week-old male and female mice. We found that mice with larger tumours had a higher proportion of intratumoral PD-1^+LAG3^+TIM3^+ CD8^+ T cells and greater hypersensitivity to thermal pain (not shown). Notably, heightened sensitivity to thermal pain positively correlated (n = 60; R^2 = 0.55, P < 0.0001) with increased frequency in intratumoral PD-1^+LAG3^+TIM3^+ CD8^+ T cells (Fig. 3a; measured on day 13 after implantation).

Melanoma-innervating nociceptors control tumour growth

The expression of adrenergic and cholinergic axon markers in tumours correlates with poor clinical outcome. Gastric tumour denervation limits growth and patients who have undergone vagotomy have lower rates of mortality from intestinal cancer. To test whether the presence of SLPI (Trpv1cre::DTA^WT) or intact (littermate control; Trpv1cre::DTA^WT) mice. In nociceptor-ablated male and female mice, the median length of survival increased by 2.5-fold (evaluated until day 22; Fig. 3b). In another set of mice that were analysed 16 days after tumour inoculation, we found that genetic ablation of nociceptors reduced tumour growth (Fig. 3c). In addition, nociceptor-ablated mice showed an increase in the total number and relative frequency of cytotoxic (IFNγ^+), TNF or IL-2 tumour-infiltrating CD8^+ T cells, but a reduced proportion of PD-1^+LAG3^+TIM3^+ CD8^+ T cells (Fig. 3d, e and Extended Data Fig. 6a, b).

Up to this point, our data suggest that nociceptor neurons are an upstream driver of intratumoral PD-1^+LAG3^+TIM3^+ CD8^+ T cells. To assess whether this is indeed the case, we mapped out the kinetics of thermal pain hypersensitivity, increased frequency in intratumoral PD-1^+LAG3^+TIM3^+ CD8^+ T cells and tumour growth. When compared to their baseline threshold and to that of sensory-neuron-ablated mice (Trpv1cre::DTA^WT; n = 19), eight-week-old littermate control mice (Trpv1WT::DTA^WT; n = 96) that were inoculated with B16F10-mCherry-OVA (left hindpaw, i.d., 2 × 10^5 cells) showed significant thermal hypersensitivity on day 7, an effect that peaked on day 21 (Extended Data Fig. 6c). In these mice, the intratumoral frequency of PD-1^+LAG3^+TIM3^+ (Extended Data Fig. 6d) or IFNγ^+ (Extended Data Fig. 6e) CD8^+ T cells was significantly increased 12 days after tumour inoculation and peaked on day 19. Finally, B16F10-mCherry-OVA tumour volume peaked on day 22 (Extended Data Fig. 6f). Altogether, these data show that thermal hypersensitivity precedes any significant exhaustion of intratumoral CD8^+ T cells by around five days and that pain hypersensitivity develops before the tumour is measurable using a digital caliper (Extended Data Fig. 6g).

Blocking the activity of immune checkpoint proteins releases a cancer-cell-induced ‘brake’ on the immune system, thereby increasing its ability to eliminate tumours. Immune checkpoint inhibitors (ICIs), including those that target PD-L1, improve clinical outcomes in patients with metastatic melanoma; however, the efficacy of ICIs varies considerably among patients, half of whom will not benefit. We set out to assess whether the presence (Trpv1cre::DTA^WT) or absence (Trpv1cre::DTA^WT) of tumour-innervating nociceptor neurons would affect responsiveness to treatment with anti-PD-L1. Anti-PD-L1 (intra-peritoneally i.p., days 7, 10, 13 and 16) was given either to mice whose tumour cells (B16F10-mCherry-OVA, i.d., 5 × 10^5 cells) were inoculated on the same day, or to mice with established tumours (around 85 mm^3; achieved by inoculating Trpv1cre::DTA^WT around 3 days before). In both scenarios, ablation of nociceptors increased the anti-PD-L1-mediated reduction in tumours and the infiltration of tumour-specific CD8^+ T cells (Extended Data Fig. 6h–k).

To test whether the reduction in tumour growth that was observed in the absence of nociceptor neurons depends on their action on...
immune cells, we compared the respective effects of nociceptors on the growth of an immunogenic and a non-immunogenic isogenic melanoma model. YUMMER1.7 is a highly immunogenic derivative of the BrafV600ECdkn2a-/- cell line modified by ultraviolet (UV) exposure, and provides a clinically relevant model of melanoma 28.

Next, we assessed whether these differences were due to nociceptor neurons directly modulating intratumoral T cells. We observed no major changes in tumour growth between nociceptor-intact and nociceptor-ablated mice after systemic depletion of CD8+ T cells (Fig. 3f). When BoNT/A (25 pg μl−1, 50 μl, five i.d. sites) was administered one and three days before the B16F10-OVA cell inoculation, it reduced the tumour volume was also linked to an increase in the intratumoral levels of CGRP, confirming the engagement of pain-transmitting neurons. Ablation of nociceptors decreased the slower tumour growth found in Trpelp<sup>noc</sup>−/−;Dta<sup>WT</sup> and RTX-exposed mice depends on the modulation of CD8+ T cells by nociceptors.

Optogenetic activation of skin nociceptor neurons triggers the antidromic release of neuropeptides that mediate anticipatory immunity against microorganisms 30 and potentiate skin immunity 31. We used transdermal illumination to stimulate tumour-innervating Na V1.8+ channelrhodopsin-expressing neurons (Trpv1cre:: DTAfl/WT) daily 24 h before BoNT/A administration and laser exposure had no effect on tumour growth (Extended Data Fig. 6q). Laser exposure had no effect on tumour growth (Extended Data Fig. 6p). This increase in tumour volume was also linked to an increase in the intratumoral levels of CGRP, confirming the engagement of pain-transmitting neurons (Extended Data Fig. 6q). Laser exposure had no effect on tumour growth in light-insensitive mice (Naul1.8<sup>−/−</sup>;ChR2<sup>WT</sup>); not shown).

The neonatal or embryonic ablation of neuronal subsets may lead to compensatory changes. To circumvent this possibility, we silenced neurons using botulinum neurotoxin A (BoNT/A), a neurotoxic protein produced by Clostridium botulinum, which acts by cleaving SNAP25 (ref. 2). BoNT/A causes a long-lasting (20 days) abolition of neurotransmitter release from skin-innervating neurons 30. BoNT/A reduces tumour growth in prostate cancer 3 and blocks nociceptor-mediated modulation of neutrophils during skin infection 30. BoNT/A does not affect the function of cultured B16F10 or CD8+ T cells in vitro (Extended Data Fig. 7a–f).

When BoNT/A (25 pg μl−1, 50 μl, five i.d. sites) was administered one and three days before the B16F10-OVA cell inoculation, it reduced...
Fig. 3 | Genetic ablation of nociceptors safeguards anti-tumour immunity.

a, Orthotopic B16F10-mCherry-OVA cells (2 × 10^5 cells, i.d.) were injected into the left hindpaw of wild-type mice. As measured on day 13 after tumour inoculation, intratumoral CD8^+ T cell exhaustion positively correlated with thermal hypersensitivity (R^2 = 0.55, P<0.0001). The thermal pain hypersensitivity represents the withdrawal latency ratio of the ipsilateral paw (tumour inoculated) to the contralateral paw.

b, Orthotopic B16F10-mCherry-OVA (5 × 10^5 cells, i.d.) were inoculated into the flank of eight-week-old male and female mice with sensory neurons intact (Trpv1^WT::DTAfl/WT) or ablated (Trpv1^cre::DTAfl/WT). The median length of survival was increased by around 250% in nociceptor-ablated mice (measured until 22 days after inoculation).

c, Sixteen days after tumour inoculation, sensory-neuron-ablated mice have reduced tumour growth (measured until 22 days after inoculation). d-f, Sixteen days after tumour inoculation, sensory-neuron-ablated mice have reduced tumour growth (e) and increased tumour infiltration of IFNγ^+ CD8^+ T cells (d), and the proportion of PD-1^+LAG3^+TIM3^+ CD8^+ T cells is decreased (e). This reduction in B16F10-mCherry-OVA (5 × 10^5 cells, i.d.) tumour volume was absent in nociceptor-ablated mice whose CD8^+ T cells were systemically depleted (f; assessed until day 14; anti-CD8, 200 μg per mouse, i.p., every 3 days).

d, g, h. To chemically deplete their nociceptor neurons, Rag1^−/− mice were injected with RTX. Twenty-eight days later, the mice were inoculated with B16F10-mCherry-OVA (5 × 10^5 cells, i.d.). RTX-injected mice that were adoptively transferred with naive OVA-specific CD8^+ T cells (i.v., 1 × 10^6 cells, when tumour reached around 500 mm^3) showed reduced tumour growth (g; assessed until day 19) and exhaustion (h) compared to vehicle-exposed Rag1^−/− mice. Data are shown as a linear regression analysis ± s.e. (a), as a Mantel–Cox regression (b), as mean ± s.e.m. (c,f,g) or as box-and-whisker plots (as defined in Fig. 1b,c), for which individual data points are given (d, e, h). n as follows: a: n = 60; b: intact (n = 62), ablated (n = 73); c: intact (n = 20), ablated (n = 25); d: intact (n = 24), ablated (n = 23); e: intact (n = 23), ablated (n = 26); f: intact + anti-CD8 (n = 10), ablated + anti-CD8 (n = 8); g: vehicle (n = 12), RTX (n = 10); h: vehicle (n = 11), RTX (n = 10). Experiments were independently repeated two (a,f–h) or six (b–e) times with similar results. P values were determined by simple linear regression analysis (a), Mantel–Cox regression (b), two-way ANOVA with post-hoc Bonferroni (c,f,g) or two-sided unpaired Student’s t test (d,e,h).

Subsequent tumour growth and preserved the cytotoxic potential of intratumoral CD8^+ T cells (Extended Data Fig. 7g–n; as measured 18 days after inoculation). Pre-treatment with BoNT/A also reduced the growth of YUMMER1.7 tumours and enhanced anti-PD-L1-mediated tumour regression (Extended Data Fig. 7o,p). When administered to mice with established tumours (around 200 mm^3), BoNT/A had limited efficacy (Extended Data Fig. 7g–n). BoNT/A also did not affect tumour growth when given to mice in which TRPV1^+ nociceptor neurons were genetically ablated (Extended Data Fig. 7o), which suggests that its anti-tumour effectiveness depends on the presence of tumour-innervating nociceptor neurons.

We next tested the anti-tumour efficacy of a proven nociceptor-selective silencing strategy4. This protocol uses large-pore ion channels (TRPV1) as cell-specific drug-entry ports to deliver QX-314—a charged and membrane-impermeable form of lidocaine—to block voltage-gated sodium (NaV) channels. During inflammation, similar to what we observed in tumour microenvironments, these large-pore ion channels open, which allows QX-314 to permeate the neurons and results in a long-lasting electrical blockade. Although QX-314 did not affect cultured B16F10-mCherry-OVA cells or CD8^+ T cell function in vitro (Extended Data Fig. S8a–f), we confirmed that it silences tumour-innervating nociceptors in vivo, as shown by reduced B16F10-induced release of CGRP and pain hypersensitivity (Extended Data Fig. 8g–i). We found that vehicle-exposed B16F10-mCherry-OVA-bearing mice succumbed at a 2.7-fold higher rate (P<0.02) than QX-314-exposed mice (Extended Data Fig. S8j; measured until day 19). As observed 17 days after tumour inoculation, QX-314-mediated silencing of sensory neurons (0.3%; daily administration) reduced melanoma growth and limited the subsequent tumour growth (Extended Data Fig. 8k–r), suggesting that it could be used as a therapeutic agent in cancer.

CGRP attenuates the activity of RAMP1^+ CD8^+ T cells

In breast cancer, tumour-specific sympathetic denervation downregulates the expression of PD-L1, PD-1 and FOXP3 in TILs54. Human and
B16F10-mCherry-OVA cells (5 × 10^5 cells, i.d.) were inoculated into eight-week-old male and female mice. Starting one day after inoculation (defined as prophylactic, (48 h), CGRP (100 nM; once daily) also reduced the ability of OT-I cytotoxic CD8+ T cells to eliminate B16F10-mCherry-OVA cancer cells. In nociceptor-ablated mice, peritumoral recombinant CGRP injection (100 nM, i.d., once daily) rescues B16F10 growth (assessed until day 12). In mice with sensory-neuron-ablated mice (Trpv1cre::DTAfl/WT), CGRP (100 nM) was administered systemically (i.p.) once every two days. In another group of mice, BIBN4096 (5 mg kg⁻¹, i.p., every two days) injections were started once the tumour reached a volume of around 200 mm³ (defined as therapeutic). Prophylactic or therapeutic BIBN4096 treatments decreased tumour growth and reduced the proportion of intratumoral PD-1+LAG3+TIM3+ CD8+ T cells; assessed until day 13. Data are shown as box-and-whisker plots (as defined in Fig. 1b, c), for which individual data points are given (a, b, e), or as mean ± s.e.m. (c, d), as follows:

- a: Ramp1WT CD8+ vehicle (n = 9), Ramp1WT CD8+ + CGRP (n = 10), Ramp1WT CD8+ + vehicle (n = 10); Ramp1−/− CD8+ +CGRP (n = 9); b: n = 4 per group; c: intact + vehicle (n = 15), ablated + CGRP (n = 11); d: vehicle (n = 13), BIBN prophylactic (n = 16), BIBN therapeutic (n = 18); e: vehicle (n = 10), BIBN prophylactic (n = 13), BIBN therapeutic (n = 16). Experiments were independently repeated three times with similar results. P values were determined by one-way ANOVA with post-hoc Bonferroni (a, e) or two-way ANOVA with post-hoc Bonferroni (c, d).

Mouse cytotoxic CD8+ T cells express multiple neuropeptide receptors (10 or more), including the CGRP receptor RAMP1 (Extended Data Figs. 1b and 3b). Given that nociceptors readily interact with CD8+ T cells in culture and that the neuropeptides they release block anti-tumour responses of cytotoxic CD8+ T cells, we aimed to test whether these mediators drive the expression of immune checkpoint receptors in CD8+ T cells. First, splenocyte-isolated CD8+ T cells were cultured under type I (Tc1) cytotoxic CD8+ T cell-stimulating conditions for two days and then co-cultured with DRG neurons for an additional four days. We found that nociceptor stimulation with capsaicin increased the proportion of PD-1+LAG3+TIM3+ cytotoxic CD8+ T cells but decreased the levels of IFNγ, TNFα and IL-2. Capsaicin had no measurable effect on CD8+ T cells in the absence of DRG neurons (Extended Data Fig. 9a, b). When Tc1-activated CD8+ T cells were exposed to fresh conditioned medium (1:2 dilution) collected from KCl-induced conditioned medium from BoNT/A-silenced sensory neurons intact or ablated. In nociceptor-ablated mice, peritumoral recombinant CGRP injection (100 nM, i.d., once daily) rescues B16F10 growth (assessed until day 12). In mice with sensory-neuron-ablated mice (Trpv1cre::DTAfl/WT), CGRP (100 nM) was administered systemically (i.p.) once every two days. In another group of mice, BIBN4096 (5 mg kg⁻¹, i.p., every two days) injections were started once the tumour reached a volume of around 200 mm³ (defined as therapeutic). Prophylactic or therapeutic BIBN4096 treatments decreased tumour growth and reduced the proportion of intratumoral PD-1+LAG3+TIM3+ CD8+ T cells; assessed until day 13. Data are shown as box-and-whisker plots (as defined in Fig. 1b, c), for which individual data points are given (a, b, e), or as mean ± s.e.m. (c, d), as follows:

- a: Ramp1WT CD8+ vehicle (n = 9), Ramp1WT CD8+ + CGRP (n = 10), Ramp1WT CD8+ + vehicle (n = 10); Ramp1−/− CD8+ + CGRP (n = 9); b: n = 4 per group; c: intact + vehicle (n = 15), ablated + CGRP (n = 11); d: vehicle (n = 13), BIBN prophylactic (n = 16), BIBN therapeutic (n = 18); e: vehicle (n = 10), BIBN prophylactic (n = 13), BIBN therapeutic (n = 16). Experiments were independently repeated three times with similar results. P values were determined by one-way ANOVA with post-hoc Bonferroni (a, e) or two-way ANOVA with post-hoc Bonferroni (c, d).

Nociceptor-produced neuropeptides reduce immunity against bacterial and fungal activity, and promote cytotoxic CD8+ T cell exhaustion (Fig. 4a, b and Extended Data Fig. 9). Given that nociceptor-released CGRP is increased when cultured with B16F10 cells (Fig. 1c) or exposed to SLPI (Fig. 2), and that tumour-infiltrating nociceptor neurons suppress the antitumour response of T cells (Calca (1–20), (1, e), we next sought to test whether the intratumoral levels of CGRP correlate with CD8+ T cell exhaustion. To do this we used an Naul1.8−/− driver to ablate most mechano- and thermosensitive nociceptors with diphtheria toxin (Naul1.8−/−::DTAfl/WT). When compared with melanoma-bearing littermate controls (Naul1.8−/−::DTAfl/WT), the ablation of Naul1.8 sensory neurons preserved the functionality of intratumoral CD8+ T cells (Extended Data Fig. 10a–d). In both groups of mice, the proportion of intratumoral CGRP directly correlated with the frequency of PD-1+LAG3+TIM3+ CD8+ T cells (Extended Data Fig. 10e).

We then set out to rescue CGRP levels (by daily intratumoral injection) in sensory-neuron-ablated mice and measured the effect on tumour growth and TIL exhaustion. At 11 days after inoculation, CGRP-treated sensory-neuron-ablated mice (Trpv1−/−::DTAfl/WT) showed similar tumour growth and CD8+ T cell exhaustion to that of nociceptor-intact mice (Fig. 4c and Extended Data Fig. 10f). Next, we treated tumour-bearing mice with the selective RAMP1 antagonist BIBN4096 (5 mg kg⁻¹, i.p., once every two days). The latter was previously found to block neuro-immune interactions during murine Charcot-Marie-Tooth infections and rescues host antibacterial activity (BIBN4096-exposed mice succumb at a rate 2.6-fold lower (P < 0.02) than that of vehicle-exposed B16F10-bearing mice (Extended Data Fig. 10g; measured until day 19). As measured...
RAMP1 (primary biopsy bulk RNA sequencing). In comparison to patients with low RAMP1 expression, higher RAMP1 levels correlate with decreased patient survival. In silico analysis of single-cell RNA sequencing of human melanoma reveals that intratumoral RAMP1-expressing CD8+ T cells strongly overexpress several immune checkpoint receptors (PD-1 [also known as PDCD1], TIM3, LAG3, CTLA4) in comparison to RAMP1-negative CD8+ T cells. Data are shown as mean ± s.e.m. (a), slope graph (b), as a heatmap showing normalized gene expression (log10(1 + (TPM/10))) as a violin plot (c). As a Mantel–Cox regression (d) or as a violin plot (e). n as follows: a–c: n = 5 per group; d: high (n = 45), low (n = 68); e: RAMP1 CD8 (n = 1,732), RAMP1 CD8 (n = 25). Experiments were independently repeated two (a, b) times with similar results. The sequencing experiment was not repeated (c). P values were determined by two-way ANOVA with post-hoc Bonferroni (a), two-sided unpaired Student’s t-test (b) or Mantel-Cox regression (d).

To directly address whether RAMP1 is the main driver of CD8+ T cell exhaustion, we transplanted Rag1−/− mice with Ramp1−/− or Ramp1 wild-type (Ramp1++) CD8+ T cells (intravenously, i.v., 2.5 × 10⁶) or a 1:1 mixture of both. Although we retrieved similar numbers of CD8+ T cells across all three groups (Extended Data Fig. 10u), limited B16F10-OVA tumour growth (Fig. 5a) was found in mice that received the Ramp1−/− CD8+ T cells—which are not responsive to CGRP. The relative proportion of intratumoral PD-1+LAG3+TIM3+ CD8+ T cells was also lower in Ramp1−/−-transplanted Rag1−/− mice (Extended Data Fig. 10v). In Rag1−/− mice co-transplanted with RAMP1-expressing and -non-expressing CD8+ T cells, we found that within the same tumour, the relative proportion of intratumoral PD-1+LAG3+TIM3+ CD8+ T cells was lower in Ramp1−/− CD8+ T cells (Fig. 5b and Extended Data Fig. 10w). Next, we RNA sequenced FACS-purified Ramp1WT and Ramp1−/− CD8+ T cells from these tumours. Compared to their Ramp1WT counterparts, we found that intratumoral Ramp1−/− CD8+ T cells expressed fewer pro-exhaustion transcription factors (Tox and Eomes) and markers (Pdcd1 [encoding PD-1], Lag3 and Tim3 [also known as Havcr2]; Fig. 5c). Overall, CGRP-unresponsive Ramp1−/− CD8+ T cells are protected against undergoing nociceptor-induced exhaustion, which safeguards their anti-tumour responses.
modelling in mice, human data imply that RAMP1-expressing CD8+ T cells are more prone to exhaustion and are associated with lower responsiveness to ICIs.

Tumour-innervating nociceptors dampen the immune response to melanoma by upregulating multiple immune checkpoint receptors on cytotoxic CD8+ T cells. Blocking the CGRP–RAMP1 axis attenuates this immunomodulatory action of the nervous system on CD8+ T cells, thereby safeguarding the anti-tumour immunity of the host (Extended Data Fig. 12) and providing potential therapeutic opportunities by interrupting pro-cancerous neuro-immune links.

Online content
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**Methods**

**Secondary use of biopsies as research specimens**

The ten melanoma samples used in this study were collected by Sanford Health and classified by a board-certified pathologist. Their secondary use as research specimens (fully de-identified formalin-fixed, paraffin-embedded (FFPE) blocks) was approved under Sanford Health IRB protocol 640 (title: Understanding and improving cancer treatment of solid tumours). As part of this Institutional Review Board (IRB)-approved retrospective tissue analysis, and in accordance with the US Department of Health and Human Services (HHS) secretary’s advisory committee on human research protections, no patient consent was necessary as these secondary use specimens were free of linkers or identifiers and posed no more than minimal risk to the human individuals.

**Immunohistochemistry and scoring**

In compliance with all the relevant ethical regulations and as approved by Sanford Health IRB protocol 640, ten fully de-identified FFPE melanoma blocks were randomly selected for secondary use as research specimens. The notes of a board-certified pathologist on these specimens are provided in Supplementary Table 1. The specimens were stained using a BenchMark XT slide staining system (Ventana Medical Systems). The Ventana iView DAB detection kit was used as the chromogen, and slides were counterstained with haematoxylin and anti-TRPV1 (Alo- mone Labs, ACC-030; 1:100). Haematoxylin and eosin (H&E) staining followed standard procedures. TRPV1 immunohistochemistry-stained specimens were analysed on an Olympus BX51 bright-field microscope. Sections were viewed under 20× magnification. Five random fields per sample for both tumour and adjacent normal tissue were analysed and scored on a scale from 0 to 3. Scores were averaged. A score of 0 indicates no appreciated nerve fibres in the evaluated field; +1 indicates sparse nerve fibres; +2 indicates 5–20 nerve fibres; +3 indicates more than 20 nerve fibres.

**IACUC approval**

The Institutional Animal Care and Use Committee (IACUC) of Boston Children’s Hospital and of the Université de Montréal (Comité de Déontologie de l’Expérimentation sur les Animaux; #21046; 21047) approved all animal procedures.

**Housing of mice**

Mice were housed in standard environmental conditions (12-h light–dark cycle; 23 °C; food and water ad libitum) at facilities accredited by the Canadian Council of Animal Care (Université de Montréal) or the Association for Assessment and Accreditation of Laboratory Animal Care (Boston Children’s Hospital).

**IACUC end-points**

As per our IACUC-approved protocol, the following end-points were used in all of the experiments and were not exceeded. Along with standard procedures, TRPV1 immunohistochemistry-stained specimens were analysed on an Olympus BX51 bright-field microscope. Sections were viewed under 20× magnification. Five random fields per sample for both tumour and adjacent normal tissue were analysed and scored on a scale from 0 to 3. Scores were averaged. A score of 0 indicates no appreciated nerve fibres in the evaluated field; +1 indicates sparse nerve fibres; +2 indicates 5–20 nerve fibres; +3 indicates more than 20 nerve fibres.

**Mouse lines**

Six-to-twenty-week-old male and female C57BL6J (Jax, 000664); CD45.1 C57BL6J (Jax, 002014), Ramp1+ (Jax, 031560), Rag1- (Jax, 002216), OT-I (Jax, 003831), Trp1+ (Jax, 017769), ChR2fl/WT (Jax, 012567), tdTomatofl/WT (Jax, 007908), DTAt/WT (Jax, 009669) or DTAt/WT (Jax, 010527), QuasAr2-dark mOrange2-CheRiff-eGFPfl/WT (referred to in the text as CheRiff-eGFPfl/WT; Jax, 028678) mice were purchased from the Jackson Laboratory. NaisL8−/− mice were supplied by R. Kuner and J. Wood. Excluding CD45.1+ mice, all other lines were backcrossed for more than six generations on a C57BL6/J background (H-2Kb). Although Capecci’s DTAt/WT (Jax, 010527) was created on a mixed C57BL6J/129 background, both haplotypes are H-2Kb. All these lines are therefore fully compatible with being transplanted with B16F10-derived cells (C57BL6/J background (H-2Kb)).

We used the Cre–loxP toolbox to engineer the various mice lines used (Trpv1cre::DTAt/WT, Trp1cre::CheRiff-eGFPfl/WT, Trp1cre::tdTomatofl/WT, Nav1.8−/−::DTAt/WT, NaisL8−/−::tdTomatofl/WT, NaisL8−/−::CheRiff-eGFPfl/WT and Iltter control) by crossing heterozygote Cre mice with homozygous loxP mice. Mice of both sexes were used for these crosses. All Cre driver lines used were viable and fertile, and abnormal phenotypes were not detected. Offspring were tail-clipped and tissue was used to assess the presence of the transgene by standard PCR, as described by The Jackson Laboratory or the donating investigators. Offspring of both sexes were used at 6–20 weeks of age.

**Cell lines**

B16F010 (ATCC, CRL-6322), B16F1011 (ATCC, CRL-6475), B16F10-mCherry-OVA2 (M. F. Krummel, UCSF), B16F10-eGFP (Imanis, CL053), YUMM1.729 (ATCC, CRL-3362), and non-tumorigenic keratinocytes (CellnTEC, MPEK-BL6100) were cultured in complete Dulbecco’s modified Eagle’s medium high glucose (DMEM; Corning, 10-013-CV) supplemented with 10% fetal bovine serum (FBS; Seradigm, 3100) and 1% penicillin–streptomycin (Corning, MT-3001-CI), and maintained at 37 °C in a humidified incubator with 5% CO2. YUMMER1.728 (M. Bosenberg, Yale University) cells were cultured in DMEM/F12 (Gibco, 11320033) supplemented with 10% FBS, 1% penicillin–streptomycin (Corning, MT-3001-CI) and MEM nonessential amino acids (Corning, 25-025C1), and maintained at 37 °C in a humidified incubator with 5% CO2.

All the cell lines tested negative for mycoplasma, and none are listed by the International Cell Line Authentication Committee registry (v.11). Non-commercial cell lines (B16F10-OVA, B16F10-OVA-mCherry and B16F10-eGFP) were authenticated using antibodies (against OVA, eGFP and mCherry) and/or imaging as well as morphology and growth properties. Commercial cell lines were not further authenticated.

**Cancer inoculation and volume measurement**

Cancer cells were resuspended in phosphate buffered saline (PBS; Corning, 21040-CV) and injected into the mouse’s skin in the right flank (5 × 105 cells, i.d., 100 μl) or hindpaw (2 × 105 cells, i.d., 50 μl). Growth was assessed daily using a handheld digital caliper and tumour volume was determined by the formula (L × W2 × 0.52) (ref. 38), in which L = length and W = width.

**BoNT/A**

BoNT/A18 (List Biological Labs, 130B) was injected (25 pg μl−1, i.d., five neighbouring sites injected with 20 μl) into the skin three days and one day before tumour inoculation (defined as prophylactic). BoNT/A (25 pg μl−1; i.d., five neighbouring sites injected with 20 μl) was injected around the tumour one day and three days after the tumour reached a volume of around 200 mm3 (defined as therapeutic) in other groups of C57BL6J mice.

**QX-314**

Starting one day after tumour inoculation (defined as prophylactic), QX-314 (ref. 31; Tocris, 2313; 0.3% w/v) was injected (i.d., 100 μl) daily at five points around the tumour. In another group of mice, QX-314 daily injection started once the tumour reached a volume of around 200 mm3 (defined as therapeutic).

**BIBN4096**

Starting one day after tumour inoculation, BIBN4096 (ref. 33; Tocris, 4561; 5 mg kg−1) was administered systematically (i.p., 50 μl) on alternate days to eight-week-old male and female mice (defined as prophylactic).
In another group of mice, BIBN4096 (5 mg kg\(^{-1}\)) was administered systemically (i.p., 50 μl) on alternate days once the tumour reached a volume of around 200 mm\(^3\) (defined as therapeutic).

**RTX**

RTX (ref. 33; Alomone Labs, R-400) was injected (subcutaneously; s.c.) in three dosages (30, 70 and 100 μg kg\(^{-1}\)) into the right flank of Rag1\(^{-/-}\) and C57BL/6J mice of around three weeks of age. Denervation was confirmed 28 days after RTX by an absence of pain withdrawal reflex (paw flinching) when exposed to heat (see 'Thermal hypersensitivity' for details on the test).

**Survival**

In specific groups of mice, orthotopic B16F10-mCherry-OVA (5 × 10\(^5\) cells, i.d.) cells were administered to intact and nociceptor-ablated mice and survival was measured until day 22 and determined by the tumour reaching a volume of 1,000 mm\(^3\) or greater, or according to the ethical end-points described above. In B16F10-mCherry-OVA-inoculated mice treated with QX-314 or BIBN4096, survival was measured until day 19 and determined by the tumour reaching a volume of 800 mm\(^3\) or greater, or according to the ethical end-points described above. As the survival analysis of vehicle-injected, QX-314-treated and BIBN4096-treated mice was performed simultaneously, the same group of vehicle-injected mice is shown in the respective panels for QX-314 and BIBN4096.

**iDISCO imaging**

Whole-mount immunohistochemistry of tumours was performed using an iDISCO protocol with methanol pre-treatment optimized for tumours. In brief, adult mice (eight weeks old) were perfused with 25 ml of PBS (HyClone) and 25 ml of 4% paraformaldehyde (PFA; Sigma) sequentially at room temperature. Tumours were post-fixed with 4% PFA for 6 h at 4 °C. For methanol pre-treatment, fixed tumours were washed sequentially in 50% methanol (in PBS) for 1 h and 100% methanol for 1 h, and then bleached in 5% H\(_2\)O\(_2\) in 20% DMSO and methanol overnight at 4 °C. Tumours were subsequently rehydrated in 100% methanol for 1 h twice, 20% DMSO and methanol for 1 h twice, 50% methanol in PBS for 1 h, PBS for 1 h twice and PBS. 0.2% Triton X-100 for 1 h twice at room temperature.

Tumours were then left in PBS, 0.2% Triton X-100, 20% DMSO and 0.3 M glycine (Sigma) overnight at room temperature and blocked in PBS, 0.2% Triton X-100, 10% DMSO, 6% donkey serum (Jackson ImmunoResearch) and anti-CD16/CD32 (Fc block; Bio X Cell) overnight at room temperature. Tumours were subsequently washed in PBS, 0.2% Tween-20 and 10 mg ml\(^{-1}\) heparin (PTwH, Sigma-Aldrich) for 1 h at room temperature, and a mixture of one part benzyl alcohol and PBS and 0.2% Triton X-100 for 1 h twice at room temperature. Tumours were subsequently rehydrated in 100% methanol for 1 h twice, 20% DMSO and methanol for 1 h twice, 50% methanol in PBS for 1 h, PBS for 1 h twice and PBS. 0.2% Triton X-100 for 1 h twice at room temperature. Tumours were washed in the same way as after primary antibody incubation for one day, and tumour-labelled tumours were then processed for clearing, which included sequential incubation with 50% methanol for 1 h at room temperature, 100% methanol for 1 h and PBS for 1 h twice at room temperature. Tumours were further incubated with a secondary panel of species-specific anti-IgG (H+L) Alexa Fluor 488 or 546-conjugated antibodies (Invitrogen or Jackson ImmunoResearch), all at 1:500, in PTwH, 5% DMSO, 3% donkey serum and Fc block 1:100 for four days at room temperature. Tumours were permeabilized using PFA and a mixture of one part benzyl alcohol (Sigma): two parts benzyl benzoates (Sigma) overnight at 4 °C. For tdTomato and GFP immunolabelling, mCherry and GFP antibodies were preabsorbed against tumours from tdTomato mice overnight at room temperature before use. Cleared whole-mount tissues were imaged in BABB between two cover glasses using Olympus FX3000 confocal imaging system.

**Tumour and tumour-draining lymph node digestion**

Mice were euthanized when the tumour reached a volume of 800–1,500 mm\(^3\) (refs. 50,51,56). Tumours and their draining lymph nodes were collected. Tumours were enzymatically digested in DMEM + 5% FBS (Seradigm, 3100) + 2 mg ml\(^{-1}\) collagenase D (Sigma, 11088866001) + 1 mg ml\(^{-1}\) collagenase IV (Sigma, C5138-1G) + 40 μg ml\(^{-1}\) DNAse I (Sigma, 10104159001) under constant shaking (40 min, 37 °C). The cell suspension was centrifuged at 400 g for 5 min. The pellet was resuspended in 70% Percoll gradient (GE Healthcare), overlaid with 40% Percoll and centrifuged at 500 g for 20 min at room temperature with acceleration and deceleration at 1. The cells were aspirated from the Percoll interface and passed through a 70-μm cell strainer. Tumour-draining lymph nodes were dissected in PBS + 5% FBS, mechanically dissociated using a plunger, strained (70 μm) and washed with PBS.

**Immunophenotyping**

Single cells were resuspended in FACS buffer (PBS, 2% fetal calf serum and EDTA), and stained with ZombieAqua (15 min, room temperature; BioLegend, 423102) or a Viability Dye eFluor 780 (15 min, 4 °C; eBioscience, 65-0865-14). The cells were washed and Fc-blocked (0.5 mg ml\(^{-1}\), 15 min, 4 °C; BD Biosciences, 553141). Finally, the cells were stained (30 min, 4 °C) with one of anti-CD45–BV421 (1:100, BioLegend, 103134), anti-CD45.1–BV421 (1:100, BioLegend, 110732), anti-CD45.2–BV560 (1:100, BioLegend, 109836), anti-CD45-Alexa Fluor 700 (1:100, BioLegend, 103128), anti-CD11b–APC/Cy7 (1:100, BioLegend, 101226), anti-CD8–AF700 (1:100, BioLegend, 100730), anti-CD8–BV421 (1:100, BioLegend, 100753), anti-CD8–PerCP/Cy5.5 (1:100, BioLegend, 100734), anti-CD8–Pacific Blue (1:100, BioLegend, 100725), anti-CD4–PerCP/Cy5.5 (1:100, BioLegend, 100540), anti-CD4–FITC (1:100, BioLegend, 100406), anti-CD4–PE (1:200, BioLegend, 109110), anti-LAG3–PE (1:100, BioLegend, 125208), anti-LAG3–PerCP/Cy5.5 (1:100, BioLegend, 125212) or anti-4–APC (1:100, BioLegend, 119706), washed and analysed using a LSRFortessa or FACSCanto II (Becton Dickinson). Antigen-specific CD8\(^+\) T cells were stained with H-2Kb/OVA257-264 (15 min, 37 °C; NIH tetramer core facility), washed and stained with surface markers. Cytokine expression was analysed after in vitro stimulation (PMA–ionomycin; see ‘Intracellular cytokine staining’).

**Intracellular cytokine staining**

Cells were stimulated (3 h) with phorbol-12-myristate 13-acetate (PMA; 50 ng ml\(^{-1}\), Sigma-Aldrich, P585), ionomycin (1 μg ml\(^{-1}\), Sigma-Aldrich, I3909) and GolgiStop and stained with anti-IFNγ–APC (1:100, BioLegend, 554724). The cells were then fixed and permeabilized (1:100, BioLegend, 554714) and stained with anti-IFNy–APC (1:100, BioLegend, 505810), anti-IFNγ–FITC (1:100, BioLegend, 505806), anti-TNF–BV510 (1:100, BioLegend, 506339), anti-TNF–BV571 (1:100, BioLegend, 506349), anti-TNF–PE (1:100, BioLegend, 506306), anti-IL-2–PE-Cy7 (1:100, BioLegend, 503812), anti-IL-2–Pacific Blue (1:100, BioLegend, 503820), anti-IL-2–VB510 (1:100, BioLegend, 503833), and analysed using a LSRFortessa or FACSCanto II (Becton Dickinson).

**In vivo depletion of CD3 or CD8**

Anti-mouse CD3 (200 μg per mouse, Bio X Cell, BE0001-1) or anti-mouse CD8 (200 μg per mouse, Bio X Cell, BP0061) were injected (i.p.) three days before B16F10-mCherry-OVA inoculation (5 × 10\(^5\) cells; i.d.) and continued every three days. Blood samples were taken twice weekly to confirm depletion, and tumour growth was measured daily.

**In vivo CGRP rescue experiment**

Trp1\(^{-/-}\) ablated mice were injected (i.d.) once daily with recombinant CGRP (100 nm) at five points around the tumour (treatment began once the tumour was visible), and tumour growth was measured daily by a handheld digital caliper. Mice were euthanized, and tumour-infiltrating
CD8+ cell exhaustion was immunophenotyped by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson).

**Anti-PD-L1 treatment**

Orthotopic B16F10-mCherry-OVA cells (5 × 10^5 cells, i.d.) were inoculated into eight-week-old male and female sensory-neuron-intact or -ablated mice. On days 7, 10, 13 and 16 after tumour inoculations, the mice were treated with anti-PD-L1 (Bio X Cell, BE0101, 6 mg kg^-1; i.p., 50 μl) or isotype control. Nineteen days after tumour inoculation, the effect of anti-PD-L1 on tumour growth was analysed and TIL exhaustion was immunophenotyped using an LSRFortessa or a FACSCanto II (Becton Dickinson).

**Anti-PD-L1 treatment in mice with similar tumour sizes**

Orthotopic B16F10-mCherry-OVA cells (5 × 10^5 cells; i.d.) were injected into a cohort of nociceptor neuron-ablated mice three days before nociceptor-intact mice were injected. Mice from each group with a similar tumour size (around 85 mm^3) were selected and exposed to anti-PD-L1 (Bio X Cell, BE0101, 6 mg kg^-1; i.p., 50 μl) or isotype control once every three days for a total of nine days. The effect of anti-PD-L1 treatment on tumour growth was analysed until day 18.

One and three days before tumour inoculation, the skin of eight-week-old male and female mice was injected with BoNT/A (25 pg μl^-1; i.d., five neighbouring sites injected with 20 μl) or vehicle. On day seven after the last injection, orthotopic B16F10-mCherry-OVA cells (5 × 10^5 cells, i.d.) were inoculated into the area pre-exposed to BoNT/A. On days 7, 10, 13 and 16 after tumour inoculation, the mice were exposed to anti-PD-L1 (6 μg kg^-1, i.p.) or isotype control. Eighteen days after tumour inoculation, we found that neuron silencing using BoNT/A potentiated anti-PD-L1-mediated tumour reduction.

Orthotopic B16F10-mCherry-OVA cells (5 × 10^5 cells; i.d.) were injected into mice treated with QX-314 (0.3%, i.d.) two to three days before tumour inoculation. We then presented these data as percentages of the maximum (100%).

**Adoptive transfer of Ramp1WT or Ramp1−/− CD8 T cells**

Total CD8+ T cells were isolated from the spleen of wild-type (CD45.1+) or Ramp1−/− (CD45.2+) mice, expanded and stimulated in vitro using a mouse T cell Activation/Expansion Kit (Miltenyi Biotec. #130-093-100). CD8+ cells from Ramp1−/− and Ramp1WT−/− were injected separately or 1:1 mix through tail vein of Rag1−/− mice. One week after the mix, the mice were inoculated with B16F10-mCherry-OVA cancer cells (5 × 10^5 cells; i.d.), and tumour growth was measured daily using a handheld digital caliper. On day 10, tumours were collected and Ramp1−/− (CD45.2+) and Ramp1WT−/− (CD45.1+) CD8+ T cells were immunophenotyped using a FACSCanto II (Becton Dickinson) or FACS purified using a FACS Aria Ilu cell sorter (Becton Dickinson).

**RNA sequencing of adoptive transferred Ramp1WT or Ramp1−/− CD8 T cells**

For FACS-purified cells, Ramp1WT−/− and Ramp1WT−/− CD8+ T cell RNA sequencing libraries were constructed using KAPA Hyperprep RNA (1 × 75 bp) following the manufacturer’s instructions. Nextseq500 (0.5 Flowcell High Output; 200 M degragments; 75 cycles single-end read) sequencing was performed on site at the Institute for Research in Immunology and Cancer (IRIC) genomics centre. Sequences were trimmed for sequencing adapters and low-quality 3’ bases using Trimmomatic v.0.35 and aligned to the reference mouse genome version GRCm38 (gene annotation from Gencode v.M23, based on Ensembl 98) using STAR v.2.5.1b (ref. 59). Gene expression levels were obtained both as a read count directly from STAR and computed using RSEM to obtain normalized gene and transcript level expression, in TPM values, for these stranded RNA libraries. DESeq2 v.1.18.1 (ref. 60) was then used to normalize gene read counts. Individual cell data are shown as a log2 (TPM × 1000).

These data have been deposited in the National Center for Biotechnology Information (NCBI)’s Gene Expression Omnibus (GEO) (GSE205863).

**Adoptive T cell transfer in mice treated with RTX**

CD8+ T cells were isolated from OT-I mice spleens and magnet sorted (StemCell; 19858). Naive CD8+ T cells (CD8+CD45.1−CD62L−) were then purified by FACS using an FACS Aria Ilu cell sorter (Becton Dickinson) and injected (1 × 10^6 cells, i.v., tail vein) into vehicle- or RTX-exposed Rag1−/− mice.

**Mechanical hypersensitivity**

B16F10-mCherry-OVA (2 × 10^5 cells, i.d.) or non-cancerous keratinocytes (MPEK-BL6; 2 × 10^5 cells, i.d.) were inoculated intradermally in the left hindpaw of the mice. On alternate days, mechanical sensitivity was evaluated using von Frey filaments (Ugo Basile, 52-37450-275). To do so, the mice were placed in a test cage with a wire mesh floor and allowed to acclimatize (three consecutive days: 1 h per session). Von Frey filaments of increasing size (0.028–2 g) were applied to the plantar surface and the response rate was evaluated using the up-down test paradigm (61).

**Thermal hypersensitivity**

To measure thermal sensitivity, the mice were placed on a glass plate of a Hargreaves’s apparatus (Ugo Basile) and stimulated using radiant heat (infrared beam). The infrared beam intensity was set at 44 and calibrated to result in a withdrawal time of around 12 seconds in acclimatized wild-type mice. An automatic cut-off was set to 25 s to avoid tissue damage. The radiant heat source was applied to the dorsal surface of the hindpaw and latency was measured as the time for the mouse to lift, lick or withdraw the paw.

Before any treatment, the mice were allowed to acclimatize in the apparatus (minimum of three consecutive days; 1 h per session) and three baseline measurements were taken on the following day. In some instances, B16F10-mCherry-OVA (2 × 10^5 cells, i.d.) or non-cancerous keratinocytes (MPEK-BL6; 2 × 10^5 cells, i.d.) were inoculated intradermally to the mouse’s left hindpaw and thermal pain hypersensitivity was measured on alternate days (10:00). In other instances, SLPI (1 μg per 20 μl) or saline (20 μl) were injected in the left and right hindpaw, respectively, and thermal hypersensitivity was measured in both hindpaws at 1, 3 and 6 h after treatment.

**Kinetics of pain and intratumoral CD8 T cell exhaustion**

We implanted B16F10-mCherry-OVA (2 × 10^5 cells, i.d.) in several groups of littermate control (Trpv1WT−/−:DTA expressing; n = 96) and nociceptor-ablated (Trpv1WT−/−:DTA expressing; n = 18) mice. We then evaluated the level of thermal hypersensitivity (daily), tumour size (handheld digital caliper), and intratumoral CD8+ T cell exhaustion (flow cytometry) at the time of euthanasia (days 1, 4, 7, 8, 12, 13, 14, 19 and 22). We processed these data by determining the percentage change of each data point to the maximal value obtained in the pain, CD8+ T cell exhaustion and tumour size datasets, and then presented these data as percentages of the maximum (100%).

**Optogenetic stimulation**

Orthotopic B16F10-mCherry-OVA cells (5 × 10^5 cells, i.d.) were inoculated into the left flank of eight-week-old transgenic male mice expressing the light-sensitive protein channelrhodopsin 2 under the control of the Naiv.L promoter (Nav1.8::ChR2 (wt)). Optogenetic stimulation (3.5 ms, 10 Hz, 478 nm, 60 mW, in a 0.39-NA fibre placed 5–10 mm from the skin, for 20 min) started either when the tumour was visible (around 20 mm^3; 5 days after inoculation) or when it reached a volume of 200 mm^3 (8 days after inoculation) and lasted up to 14 days after tumour inoculation. The control mice (Nav1.8::ChR2 (wt)) were tumour-injected but
Cells were cultured with Neurobasal-A medium (Gibco, 21103-049) at 37 °C. Ganglia were triturated with glass Pasteur pipettes of decreasing size and incubated for 80 min at 37°C (Sigma, 51558) completed with 1 mg ml⁻¹ collagenase IV (Sigma, C0130) + cillin and 50 μg ml⁻¹ streptomycin (Corning, MT-3001-Cl) and 10% FBS.

Mice were euthanized, and dorsal root ganglia were dissected out into 24-well plates and cultured in DMEM containing 1 μl ml⁻¹ protease inhibitor and 1% penicillin–streptomycin, 10% FBS, 1% penicillin–streptomycin, 1 μl ml⁻¹ protease inhibitor, and capsaicin (1 μM, Sigma, M2028).

After stimulation, the supernatant was collected and CGRP levels were subsequently stimulated (3 h) with vehicle or SLPI (0.1–5.0 ng ml⁻¹).

1 × 10⁴ naive DRG neurons were cultured for 24 h in complete DMEM (10% FBS, 1% penicillin–streptomycin, 1 μl ml⁻¹ protease inhibitor) and capsaicin (1 μM, Sigma, M2028) into 24-well plates and cultured in DMEM containing 1 μl ml⁻¹ of protease inhibitor (Sigma, P1860) and capsaicin (1 μM, Sigma, M2028).

After a 30-min incubation (37 °C), the supernatant was collected and the release of CGRP was analysed using a commercial enzyme-linked immunosorbent assay (ELISA) (Cayman Chemical, S98001).

**CGRP release triggered by SLPI**

1 × 10⁴ naive DRG neurons were cultured for 24 h in complete DMEM (10% FBS, 1% penicillin–streptomycin, 1 μl ml⁻¹ protease inhibitor) and subsequently stimulated (3 h) with vehicle or SLPI (0.1–5 ng ml⁻¹).

After stimulation, the supernatant was collected and CGRP levels were measured using a commercial ELISA kit (Cayman Chemical, S98001).

**Neuron culture**

Mice were euthanized, and dorsal root ganglia were dissected out into DMEM medium (Corning, 10-013-CV), completed with 50 U ml⁻¹ penicillin and 50 μg ml⁻¹ streptomycin (Corning, MT-3001-Cl) and 10% FBS (Seradigm, 3100). Cells were then dissociated in HEPES buffered saline (Sigma, S158) completed with 1 mg ml⁻¹ collagenase IV (Sigma, C0130) + 2.4 U ml⁻¹ dispase II (Sigma, 04942078001) and incubated for 80 min at 37°C. Ganglia were triturated with glass Pasteur pipettes of decreasing size in supplemented DMEM medium, then centrifuged over a 10% BSA gradient and plated on laminin (Sigma, L2020)-coated culture plates. Cells were cultured with Neurobasal-A medium (Gibco, 21103-049) completed with 0.05 ng μl⁻¹ NGF (Life Technologies, 13257-019), 0.002 ng μl⁻¹ GDNF (PeproTech, 450-51-10), 0.01 mM AraC (Sigma, C6645) and 200 mM L-glutamine (VWR, 02-0131) and B-27 supplement (Gibco, #17504-044).

**Calcium imaging**

L3–L5 DRG neurons were collected and co-cultured with B16F10, B16F0 or MPEK-BL6 for 24–48 h. The cells were then loaded with 5 mM Fura-2 AM (BioVision, 2243) in complete Neurobasal-A medium for 30 min at 37 °C, washed in Standard Extracellular Solution (SES, 145 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose and 10 mM HEPES, pH 7.5), and the response to noxious ligands (100 nM capsaicin, 100 μM AITC or 1 μM ATP) was analysed at room temperature. Ligands were flowed (15 s) directly onto neurons using perfusion barrels followed by buffer washout (105 s minimum). Cells were illuminated by a UV light source (Xenon lamp, 75 watts, Nikon), 340-nm and 380-nm excitation alternated by an LEP MAC 5000 filter wheel (Spectra services), and fluorescence emission was captured by a Cool SNAP ES camera (Princeton Instruments). The 340/380 ratiometric images were processed, background-corrected and analysed (IPLab software, Scientific Analytics), and Microsoft Excel was used for post-hoc analyses. Responsiveness to a particular ligand was determined by an increase in fluorescence (F₃₄₀/F₃₈₀) of at least 5–10% above baseline recording (SES). To test neuronal sensitivity in mice inoculated with B16F10 or non-tumorigenic keratinocytes, the mice were euthanized two weeks after inoculation (left hindpaw, i.d.), and L3–L5 DRG neurons were collected and cultured (3 h). Calcium flux to noxious ligands (1 μM capsaicin or 10 μM ATP) was subsequently tested. For SLPI, the DRG neurons were cultured for 24 h, loaded with 5 mM Fura-2 AM in complete Neurobasal-A medium for 45 min at 37 °C and washed into SES, and the responses to noxious ligands (0–10 ng ml⁻¹ of mouse recombinant SLPI (LifeSpan BioSciences, LS-G13637-10), 1 μM capsaicin or 50 mM KCl) were analysed at room temperature.

**Immunofluorescence**

A total of 2 × 10⁴ DRG neurons were co-cultured with 2 × 10⁴ B16F10-mCherry-OVA cells for 24–48 h. The cells were fixed (4% PFA; 30 min), permeabilized (0.1% Triton X-100; 20 min), and blocked (PBS, 0.1% Triton X-100 and 5% BSA; 30 min). The cells were rinsed (PBS), stained, and mounted with vechtashield containing DAPI (Vector Laboratories, H-1000). Images were acquired using a TI2 Nikon fluorescent microscope (IS-Elements Advanced Research v.4.5).

**Neurite length and ramification index**

TRPV1+ nociceptors (Trpvl1+::tdTomato⁶⁷) were cultured alone (2 × 10³ cells) or co-cultured (2 × 10⁴ cells) with B16F10-GFP, B16F0 or non-tumorigenic keratinocytes (MPEK-BL6). After 48 h, cells were fixed (see ‘Immunofluorescence’), and images were acquired using a TI2 Nikon fluorescent microscope. The neurite length of TRPV1+ (tdTomato) neurons was measured using a neurite tracer macro in ImageJ (Fiji, v.1.53c) developed by the Fournier laboratory⁶³, and the Schoenen ramification index (SRI) was measured by a Sholl analysis⁶⁴ macro in ImageJ (Fiji, v.1.53c).

**Isolation of CD8⁺ T cells**

Six-to-eight-week-old male and female mice were euthanized, and their spleens were collected in ice-cold PBS (5% FBS) and mechanically dissociated. The cells were strained (70 μm), RBC lysed (Life Technologies, A1049201; 2 min), and counted using a haemocytometer. Total CD8⁺ T cells were magnet sorted (Stem Cell, 19853A) and cultured (DMEM + 10% FBS, 1% penicillin–streptomycin, 1 μl ml⁻¹ nonessential amino acids (Corning, 25-025-CI) + vitamin + β-mercaptoethanol (Gibco, 21985-023) + l-glutamine (VWR, 02-0131) + sodium pyruvate (Corning, 25-000-CI). Cell purity was systematically confirmed after magnet sorting and the numbers of CD8⁺ C6de2L⁺ were immunophenotyped by flow cytometry.

To generate cytotoxic T lymphocytes, 2 × 10⁴ CD8⁺ T cells were seeded and stimulated for 48 h under Tc₁, inflammatory conditions (2 μg ml⁻¹ plate bound anti-CD3 and anti-CD28 (Bio X Cell, BE00011, BE00151) + 10 ng ml⁻¹ IL-12 (BioLegend, S77008) + 10 μg ml⁻¹ of anti-IL-4 (Bio X Cell, BE0045).

**In vitro stimulation of cytotoxic CD8⁺ T cells with neuron-conditioned medium**

Naive or ablated DRG neurons were cultured (48 h) in Neurobasal-A medium supplemented with 0.05 ng μl⁻¹ NGF (Life Technologies, 13257-019) and 0.002 ng μl⁻¹ GDNF (PeproTech, 450-51-10). After 48 h, the neurobasal medium was removed, neurons were washed with PBS and 200 μl per well of T cell medium supplemented with 1 μl ml⁻¹ peptidase inhibitor (Sigma, P1860) and, in certain cases, capsaicin (1 μM) or KCl (50 mM) was added to DRG neurons. The conditioned medium or vehicle were collected after 30 min and added to T cells for another 96 h. The expression of exhaustion markers (PD-1, LAG3 and TIM3) and cytokines (IFNγ, TNF and IL-2) by CD8⁺ T cells was analysed by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson). Cytokine expression levels were analysed after in vitro stimulation (PMA–ionomycin; see ‘Intracellular cytokine staining’).

**In vitro stimulation of cytotoxic CD8⁺ T cells with CGRP**

CDS⁺ T cells were isolated and stimulated under Tc₁ conditions in a 96-well plate. After 48 h, cells were treated with either CGRP (0.1 μM) or PBS in the presence of peptidase inhibitor (1 μM) for another 96 h. The expression of PD-1, LAG3 and TIM3, as well as IFNγ, TNF and IL-2, was immunophenotyped by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson). Cytokine expression levels were analysed after in vitro stimulation (PMA–ionomycin; see ‘Intracellular cytokine staining’).

**In vitro silencing of DRG neurons with BoNT/A**

Naive DRG neurons (2 × 10⁴) were seeded in a 96-well plate with neurobasal medium supplemented with NGF and GDNF. Neurons were
pre-treated with 50 pg ml⁻¹ of BoNT/A for 24 h. After 24 h, the culture medium was removed, neurons were washed with PBS and 200 μl per well of T cell medium supplemented with 1 μl ml⁻¹ peptidase inhibitor, and KCl (50 mM) was added to DRG neurons. The conditioned medium or vehicle were collected after 30 min and added to T₈ CD8⁺ T cells for another 96 h.

In vitro RAMP1 blockade
CD8⁺ T cells were treated with CGRP₈₋₉₋₋ (Tocris, 1169) 6 h before being exposed to the neuron-conditioned medium. In other instances, the neuron-conditioned medium was incubated for 1 h with 2 μg ml⁻¹ of CGRP₈₋₉₋₋ before being added to the CD8⁺ T cells.

Co-culture of CD8⁺ T cells and DRG neurons
Naïve DRG neurons (2 × 10⁵) were seeded in a 96-well-plate with T cell medium (supplemented with 0.05 ng μl⁻¹ NGF (Life Technologies, 13257-019) and 0.002 ng μl⁻¹ GDNF (PeproTech, 450-51-10)). One day after, T₈ CD8⁺ cells (1 × 10⁵) were added to the neurons in the presence of IL-2 (BioLegend, 575408). In some instances, co-cultures were stimulated with either capsaicin (1 μM) or KCl (50 mM). After 96 h, the cells were collected by centrifugation (5 min at 1,300 rpm), stained and immunophenotyped by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson). Cytokine expression levels were analysed after in vitro stimulation (PMA–ionomycin; see 'Intracellular cytokine staining').

RNA sequencing of triple co-cultures and data processing
A total of 1 × 10⁴ naïve Trpv1cre::CheRiff-eGFPfl/WT DRG neurons were co-cultured with 1 × 10⁵ B16F10-mCherry-OVA overnight in T cell medium (supplemented with 0.05 ng μl⁻¹ NGF (Life Technologies, 13257-019), 0.002 ng μl⁻¹ GDNF (PeproTech, 450-51-10)). One day after, 4 × 10⁵ stimulated OVA-specific CD8⁺ T cells under Tc1 conditions were added to the co-culture. After 48 h, the cells were detached and TRPV1 neurons (CD45⁺ eGFP⁺ mCherry⁻), B16F10-mCherry-OVA (CD45⁺ eGFP⁻ mCherry⁺) and OVA-specific CD8⁺ T cells (eGFP⁺ mCherry⁺ CD45⁺ CD3⁺ CD8⁺) were FACs purified using a FACSaria Ilu cell sorter (Becton Dickinson), and the cell supernatant was collected for ELISAs.

RNA sequencing libraries were constructed using the Illumina TruSeq Stranded RNA LT Kit (Illumina) following the manufacturer’s instructions. Illumina sequencing was performed at Fulgent Genetics. Reads were aligned to the Mouse mm10 (Genbank assembly accession GCA_000001635.2) using STAR v.2.7 (ref. 58). Aligned reads were assigned to genes using the featureCounts function from subread v.1.6.4 (ref. 66). Gene expression levels were represented by TPM. Hierarchical clustering was computed using the heatmap.2 function (ward.D2 method) from the gplots R package (v.3.1.3). Differential gene expression analysis was performed using DeSeq2 v.1.28.1 (ref. 59). These data have been deposited in the NCBI’s GEO (ref. 60) (GSE205864).

RNA sequencing of cancer and neuron co-cultures and data processing
A total of 1 × 10⁴ naïve Trpv1cre::CheRiff-eGFPfl/WT DRG neurons were co-cultured with 5 × 10⁴ B16F10-mCherry-OVA cells overnight in complete DMEM (Corning, 10-013-CV) supplemented with 10% FBS (Seradigm, 3100), 1% penicillin–streptomycin (Corning, MT-3001-CI), 0.05 ng μl⁻¹ NGF (Life Technologies, 13257-019), 0.002 ng μl⁻¹ GDNF (PeproTech, 450-51-10). After 48 h, the cells were detached and TRPV1 neurons (eGFP⁺ mCherry⁻) and B16F10-mCherry-OVA (eGFP⁺ mCherry⁺) were FACs purified using a FACSaria Ilu cell sorter (Becton Dickinson), and the cell supernatant was collected for ELISAs.

RNA sequencing libraries were constructed using the Illumina TruSeq Stranded RNA LT Kit (Illumina) following the manufacturer’s instructions. Illumina sequencing was performed at Fulgent Genetics. Reads were aligned to the mouse mm10 reference genome (GenBank assembly accession GCA_000001635.2) using STAR v.2.7 (ref. 58). Aligned reads were assigned to genes using the featureCounts function from subread v.1.6.4 (ref. 66). Gene expression levels were represented by TPM. Hierarchical clustering was computed using the heatmap.2 function (ward.D2 method) from the gplots R package (v.3.1.3). Differential gene expression analysis was performed using DeSeq2 v.1.28.1 (ref. 59). These data have been deposited in the NCBI’s GEO (ref. 60) (GSE205865).

ELISA on co-cultures of B16F10 cells and DRG neurons
A total of 1 × 10⁴ naïve DRG neurons were cultured (96 h) with and without 5 × 10⁴ B16F10 cells in complete DMEM (10% FBS, 1% penicillin–streptomycin, 1 μl ml⁻¹ protease inhibitor). The cells were then challenged (30 min) with sterile PBS or KCl (40 mM) and the supernatant was collected. Neuropeptide releases were measured using commercial ELISAs for VIP (Antibodies Online, ABIN6974414), SP (Cayman Chemical, S83751) and CGRP (Cayman Chemical, S89001).

ELISA on co-cultures of B16F10 cells, CD8⁺ T cells and DRG neurons
Levels of SLPI (R&D Systems, DY1735-05) were measured in the cells’ supernatant using a commercial ELISA.

OT-1 CD8⁺ T cell-induced B16F10 elimination
A total of 2 × 10⁴ naïve Trpv1cre::CheRiff-eGFPfl/WT DRG neurons were co-cultured with 1 × 10⁵ B16F10-mCherry-OVA cells overnight in T cell medium (supplemented with 0.05 ng μl⁻¹ NGF (Life Technologies, 13257-019) and 0.002 ng μl⁻¹ GDNF (PeproTech, 450-51-10)). One day after, 4 × 10⁵ stimulated OVA-specific CD8⁺ T cells under Tc1 conditions were added to the co-culture. After 48 h, the cells were detached by trypsin (Gibco, 2062476) and collected by centrifugation (5 min at 1,300 rpm), stained using anti-Annexin V, 7-AAD (BioLegend, 640930) and anti-CD8 for 20 min at 4 °C, and immunophenotyped by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson). Cytokine expression levels were analysed after in vitro stimulation (PMA–ionomycin; see ‘Intracellular cytokine staining’).

Effect of neuron-conditioned medium on OT-1 CD8⁺ T cell-induced B16F10 elimination
A total of 4 × 10⁵ stimulated OVA-specific CD8⁺ T cells were added to 1 × 10⁵ B16F10-mCherry-OVA and treated with fresh condition medium (1:2 dilution). After 48 h, cells were stained using anti-Annexin V, 7-AAD (BioLegend, 640930) and anti-CD8 for 20 min at 4 °C, and were immunophenotyped by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson). For CGRP, 4 × 10⁵ stimulated OVA-specific CD8⁺ T cells were added to 1 × 10⁵ B16F10-mCherry-OVA and treated with CGRP (100 ng ml⁻¹) for 24 h. After 24 h, the cells were stained using Annexin V, 7-AAD (BioLegend, 640930) and anti-CD8 for 20 min at 4 °C, and were immunophenotyped by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson). Cytokine expression levels were analysed after in vitro stimulation (PMA–ionomycin; see ‘Intracellular cytokine staining’).

Survival of B16F10 cells
A total of 1 × 10⁵ B16F10 cells were cultured in six-well plates and challenged with BoNT/A (0–50 pg μl⁻¹) for 24 h, QX-314 (0–1%) for 72 h, BBN4096 (1–8 μM) for 24 h or their vehicle. The survival of B16F10 cells was assessed using anti-Annexin V staining and measured by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson), or counted using a haemocytometer.

Effect of drugs on the function of CD8⁺ T cells
Splenocyte-isolated CD8⁺ T cells from naïve C57BL/6J mice were cultured under Tc1-stimulating conditions (ex-vivo-activated by CD3 and CD28, IL-12 and anti-IL4) in 24-well plates for 48 h. The cells were then exposed to QX-314 (50–150 μM), BoNT/A (10–50 pg μl⁻¹) or BBN4096 (1–4 μM) for 24 h. Apoptosis, exhaustion and activation levels were measured by flow cytometry using an LSRFortessa or a FACSCanto II (Becton Dickinson).
In silico analysis of neuronal expression profiles using RNA-seq and microarray datasets

Publicly available RNA gene expression data from seven datasets were downloaded from the NCBI GEO portal. RNA gene expression values of genes of interest were extracted. Expression values from single-cell sequencing were averaged for all cells. To be able to compare expression from datasets that were generated using different techniques (single-cell RNA sequencing, bulk RNA sequencing and microarrays) and normalization methods (TPM, RPKM (reads per kilobase per million mapped reads), RNA (robust multiarray analysis) and UMI (unique molecular identifiers)), all genes of interest were ratioed over TRPV1 expression, then multiplied by 100, and the log_{10} values of these values were plotted as a heat map.

Kupari et al. used single-cell RNA sequencing of JNC neurons, whereas Usoskin et al. and Li et al. used single-cell RNA sequencing of lumbar neurons. Chiu et al. measured mRNA expression by microarrays of whole and FACS-sorted NaL.8 lumbar neurons. Goswami et al. performed RNA sequencing of TRPV1 lumbar neurons, whereas Ray et al. performed RNA sequencing of human lumbar neurons.

In silico analysis of tumour expression profiles of patients with melanoma using single-cell RNA sequencing

Using the publicly available Broad Institute single-cell bioportal, we performed an in silico analysis of single-cell RNA sequencing of human melanoma biopsies. We assessed the gene profile of RAMP1-expressing and RAMP1-negative T cells in the tumours of patients with metastatic melanoma. Similarly, we assessed the genetic program of RAMP1-expressing and RAMP1-negative CD8+ T cells in patients with melanoma. The latter dataset was also used to analyse the genetic profile of CD8+ T cells in patients who were responsive to immune checkpoint blockers or unresponsive to such treatment, as well as the genetic profile of malignant melanoma cells (defined as CD90+ CD45−) from the biopsies of ten different patients. Individual cell data are shown as a log_{10}-transformed 1 + (TPM/10). Experimental details and cell clustering have been defined in previous studies.

In silico analysis of the expression profiles of human immune cells

Publicly available RNA gene expression data from a previous study were downloaded from the NCBI GEO portal. Read counts normalized to transcripts per million protein-coding genes (pTPM) values for genes of interest were extracted. Expression values from single-cell sequencing were averaged for all cells. Experimental details and cell clustering have been defined in previous studies.

In silico analysis of the expression profiles of cultured B16F10 cells

Publicly available RNA gene expression data from a previous study were downloaded from the NCBI GEO portal. Read counts normalized to TPM for genes of interest were extracted. Experimental details and cell clustering have been defined in previous studies.

In silico analysis of the expression profiles of mouse immune cells using the ImmGen database

Using the publicly available ImmGen database, we performed an in silico analysis of RNA-sequencing data (DESeq2 data) of various mouse immune cells. As per ImmGen protocol, RNA-sequencing reads were aligned to the mouse genome GENCODE GRCm38/mm10 primary assembly (GenBank assembly accession GCA_000001635.2) and gene annotations vM16 with STAR 2.5.4a. The ribosomal RNA gene annotations were removed from the general transfer format file. The gene-level quantification was calculated by featureCounts. Raw read count tables were normalized by the median of ratios method with the DESeq2 package from Bioconductor and then converted to GCT and CLS format. Samples with fewer than 1 million uniquely mapped reads were automatically excluded from normalization. Experimental details can be found at https://www.immgen.org/Protocols/ImmGenULI-RNAseq_methods.pdf.

Oncomine

In silico analysis of the expression profiles of biopsies from patients with melanoma using bulk microarray sequencing. As described previously, samples from 45 cutaneous melanomas and 18 benign melanocytic skin nevi biopsies (around 5–20 μm) were collected and amplified, and their transcriptomes were profiled using Affymetrix U133A microarrays. Data were downloaded from the Oncomine database (https://www.oncomine.com/) as log_{10}-transformed (median centred intensity) and genes of interest were shown as heat maps. Experimental details and cell clustering have been defined before.

Survival analysis of patients with melanoma

OncoLnc (http://www.oncolnc.org/) contains survival data for 8,647 patients from 21 cancer studies performed by TCGA. Using OncoLnc, we assessed the transcript expression of a user-defined list of 333 neuronal-enriched genes (neuronal membrane proteins, neural stem cell markers, transcription factors, ion channel receptors and neuro-peptides) in 459 skin cancer (SKCM) tumour biopsies from the TCGA database. Of these genes, 206 were expressed, and 108 were selected on the basis of their negative Cox coefficient value, indicating a link between lower gene expression and improved patient survival. Kaplan–Meier curves show the survival of the patients after segregation into two groups defined by their low or high expression of a gene of interest. Details of patients can be found in TCGA and computational analyses can be found at https://doi.org/10.7717/peerj-cs.67.

Data collection and analysis

GraphPad Prism (v.9.0) and Microsoft Excel (v.2019) were used for data entry, graph construction and data analysis. Image analysis (neurite length and ramification index) was performed using ImageJ macros (Fiji, v.1.53c). Flow cytometry data were analysed using FlowJo (v.10.0.0). Calcium microscopy analysis was performed using a Nikon Eclipse Ti2 microscope (NIS-Elements Advanced Research v.4.5). Patient biopsy images were collected using an Olympus BX51 bright-field microscope and mouse tumour innervation images were acquired using an Olympus FV3000 confocal imaging system. For RNA sequencing, the reads were aligned to the mouse reference genome GRCm38/mm10 (GenBank assembly accession GCA_000001635.2) using STAR (versions used: 2.5.4a, 2.5.1b and 2.7). Aligned reads were assigned to genomic regions using the featureCounts function from Subread (v.1.6.42). Hierarchical clustering was computed using the heatmap.2 function (ward.D2 method) from Gplots R package (v.3.1.3). Differential gene expression analysis was carried out by DESeq2 (versions used: 1.18.1 and 1.28.1). TCGA data were accessed using Oncomine (https://www.oncomine.com/ for gene expression) and OncoLnc (http://www.oncolnc.org/ for survival). Single-cell RNA sequencing was analysed using the Broad Single-Cell Portal (https://singlecell.broadinstitute.org). Human and mouse immune cell gene profiles were respectively analysed using the Human Protein Atlas (https://www.proteinatlas.org/humanproteome/immune+cell) and Immunological Genome Project (https://www.immgen.org/).

Sample size

Statistical methods were not used to predetermine sample size. The size of the cohort, based on similar studies in the field, was validated by pilot studies. All sample sizes are indicated in the figures and/or figure legends. All n values are indicated within the figure legends. In the only case in which a range is used (Fig. 2a,b), exact n values are provided in the source data files. For in vivo experiments, we used n > 8 mice. For in vitro experiments in which replicate samples were used, we repeated the experiments at least three independent times to confirm the findings. For other mouse experiments a minimum of five mice were used.
to ensure that proper statistics could be used. We determined this to be sufficient as per our pilot data, use of internal controls and/or the observed variability between within experimental groups.

Replication

The number of replicates is indicated in the figures, figure legends and/or methods. On the graphs, individual dots represent individual samples or mice used. For each experiment, all attempts at replication were successful and our findings showed comparable results.

Randomization

Breeding pairs and their offspring (nociceptor-intact and -ablated mice) were co-housed and, in respect with the ARRIVE guidelines, were randomly allocated into each experimental group. For in vitro experiments, randomization was used for treatment selection. In some calcium microscopy experiments, the investigators performing the data collection were tasked to select all ligand-responsive cells for downstream analysis. In these rare cases, randomization was not used for cell selection.

Blinding

Double blinding was used for all in vivo treatments. In calcium microscopy experiments involving co-culture (for example, nociceptors and cancer cells), the differences in cell morphology are obvious and, therefore, the investigator performing the experiment was not blind. However, this investigator was always blinded to the treatment being applied to the cells and a second blinded investigator performed the downstream data analysis.

Data exclusions

No data were excluded.

Statistics

Statistical significance was determined using GraphPad Prism (Dottics, v.9) and calculated using simple linear regression analysis, Mantel–Cox regression, one-way or two-way ANOVA for multiple comparisons and two-sided unpaired Student’s t-test for single variable comparison. In calcium imaging experiments, the P-value is calculated on ligand-responsive neurons (calcium flux ≥ 5–10%). P-values < 0.05 were considered significant.

Antibodies

All of the antibodies used in this study are also listed in Supplementary Table 2.

Reporting summary

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

Data availability

All data are readily available online (https://www.talbotlab.com/nature) and from the corresponding author. The RNA-sequencing datasets have been deposited in the NCBI’s GEO (GSE205863, GSE205864 and GSE205865). Source data are provided with this paper.

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3a,b,d,e,h, 4a,b,e and 5a–c and Extended Data Figs. 4e, 5a–e, 6a–g,k,q, 7a–f,h–n, 8a–e,l–r, 9 and 10a–f,h–w. M.A. performed the experiments in Figs. 1d,e, 2a,b, 3a–c, 4a–d and 5a and Extended Data Figs. 4a–c,e, 5a–e,h–j, 6l–n, 7g,o,p, 8g,j,k,s,t and 10e,g. T.E. performed the experiments in Fig. 2c–f and Extended Data Fig. 5f–j and did the in silico analysis for Fig. 5d,e and Extended Data Figs. 1a–d, 3 and 11. A.A. performed the experiments in Fig. 3g,h and Extended Data Figs. 6o and 9. A.M. performed the experiments in Fig. 3f. Karine Roversi performed the experiments in Fig. 3a and Extended Data Fig. 6c–g.p. Katiane Roversi performed the experiments in Fig. 3a. C.T.L. performed the experiments in Extended Data Fig. 2. A.C.R. performed the experiments in Extended Data Fig. 2. S.H. performed the experiments in Fig. 1a. L.J. did the bioinformatic analysis of the experiments in Figs. 1d,e and 2a,b and Extended Data Figs. 4e and 5a–e. D.W.V. performed the experiments in Extended Data Fig. 2. S.T. performed the experiments in Fig. 1b,c and Extended Data Figs. 4d and 8f,hi.

Competing interests S.T. and C.J.W. have an equity stake in Nocion Therapeutics. S.T. and C.J.W. have deposited a provisional patent (WO 2021/173916) on the use of charged sodium channel blockers to silence nociceptor neurons as a means to safeguard host anti-tumour immunity.

Additional information

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Extended Data Fig. 1 | TRPV1, NAV1.8, SNAP25 or RAMP1 transcripts are expressed in patient melanoma biopsies but are not detected in human immune cells or malignant cells. (a–b) In silico analysis of single-cell RNA sequencing of human melanoma-infiltrating cells revealed that Trpv1, Nav1.8 (Scn10a), Snap25 (the molecular target of BoNT/A), Calca (gene encoding for CGRP) transcripts are not detected in malignant melanoma cells (defined as CD90−CD45−) from ten different patients’ biopsies (a) nor in cancer-associated fibroblasts, macrophage, endothelial, natural killer, T, and B cells (b). Individual cell data are shown as a log2 of 1 + (transcript per million / 10). Experimental details and cell clustering were defined in Jerby-Arnon et al. N are defined in the figures. (c) In silico analysis of human immune cells revealed their basal expression of Cd45. Using RNA sequencing approaches, Calca, Snap25, Trpv1 or NaV1.8 are not detected in these cells. Heat maps show the read counts normalized to transcripts per million protein-coding genes (pTPM) for each of the single-cell clusters. Experimental details and cell clustering were defined in Monaco et al. (d) Forty-five cutaneous melanomas and 18 benign melanocytic skin nevus biopsies transcriptomes were profiled using Affymetrix U133A microarrays. In silico analysis of this dataset revealed that cutaneous melanoma heightened expression levels of Calca (1.4-fold), Pouf4f1 (2-fold), Eno2 (1.4-fold), and Tubb3 (1.1-fold), as well as other neuronal-enriched genes. Heat map data are shown as log2 (median centred intensity); two-sided unpaired Student’s t-test; p-values and n are shown in the figure. Experimental details were defined in Haqq et al.
Extended Data Fig. 2 | TRPV1+ neurons innervate patient melanomas. Patients' melanoma sections were stained with hematoxylin eosin (a–f), and the presence of TRPV1 (d–f; brown) neurons was analysed by immunohistochemistry. Increased levels of TRPV1+ neurons (g) were found in the tumour (delimited by red square; a–b, d–e) compared to adjacent healthy skin (delimited by blue square; a, c, d, f). Increased TRPV1 immunolabelling in tumour sections primarily correlated with enhanced levels of tumour-infiltrating leukocytes (h) as scored from a retrospective correlation analysis performed on the patients' pathology reports. Data are shown as representative immunohistochemistry images (a–f), box-and-whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median), for which individual data points are given (g) or as a heat map (h) displaying Pearson's correlation ($R^2$). N are as follows: a–f: n = 10, g: intact (n = 8), tumour (n = 10), h: n = 10. Slides were scored blindly by two experienced medical pathologists. P-values are shown in the figure and determined by two-sided unpaired Student's t-test (g). Scale = 100 μm (a, d), 50 μm (b, c, e, f).
Extended Data Fig. 3 | Trpv1, Nav1.8, Snap25 or Ramp1 transcripts are not detected in B16F10 cancer cells or mouse immune cells. (a) In silico analysis of three different B16F10 cells cultures (labelled as i, ii, iii) revealed their basal expression of Braf and Pten. In contrast, Calca, Snap25, Trpv1 or Nav1.8 transcripts are not detected in B16F10 cells. Heat map data are shown as transcript per million (TPM) on a linear scale. Experimental details were defined in Castle et al. N=3/group. (b) ImmGen RNA sequencing of leukocyte subpopulations reveals their basal expression of Cd45 and Ramp1. In contrast, Snap25, Trpv1, or Nav1.8 transcripts are not detected in mouse immune cells. Heat map data are shown as DESeq, on a logarithmic scale. (c) A meta-analysis of seven published nociceptor neuron expression profiling datasets revealed the basal expression of sensory neuron markers (Trpv1, Trpa1) and neuropeptides (Sp, Vip, Nmu, Calca). Expression across datasets was ratioed over Trpv1 and multiplied by 100. The log10 of these values is presented as a heat map. i) RNA sequencing of human lumbar neurons; ii) microarrays of mouse FACS-sorted NaV1.8+ neurons; iii) and iv) single-cell RNA sequencing of mouse lumbar neurons; v) microarray profiling of mouse NaV1.8+ DRG neurons; vi) performed RNA sequencing of mouse TRPV1+ neurons; and vii) single-cell RNA sequencing of mouse vagal ganglia.
Extended Data Fig. 4 | B16F10 cells interact with nociceptor neurons.

(a–c) When co-cultured with B16F10-eGFP cells (green), TRPV1+ nociceptor (Trpvlcre::tdTomato<sup>fl/WT</sup>; orange) neurons form neuro-neoplasmic contacts (a), show longer neurites (b), and exhibit reduced arborization (c) than when cultured alone or with non-tumorigenic keratinocytes (b–c).

(d) L3–L5 DRG neurons were collected from mice 2-weeks after they were inoculated (left hindpaw; i.d.) with B16F10- or non-tumorigenic keratinocytes, cultured and calcium flux to ligands tested (ATP (10 μM), and capsaicin (1 μM)). Compared to neurons from keratinocytes-injected mice, the one from tumour-bearing mice showed increased sensitivity to capsaicin.

(e) Naive DRG neurons (Trpvlcre::CheRiff-eGFP<sup>fl/WT</sup>) were cultured alone or in combination with B16F10-mCherry-OVA. After 48h, the cells were collected, FACS purified, and RNA sequenced. Hierarchical clustering of sorted neuron DEG show distinct groups of transcripts enriched in TRPV1+ neuron vs cancer-exposed TRPV1+ neuron populations. Pairwise comparison of naive TRPV1+ neuron vs cancer-exposed TRPV1+ neuron populations showing differentially expressed transcripts as a volcano plot (p<0.05). Among others, Calca (gene encoding for CGRP) was overexpressed in TRPV1+ (FACS-purified eGFP-expressing cells) neurons when co-cultured with B16F10-mCherry-OVA. Data are shown as representative image (a), mean ± S.E.M (b–d), or volcano plot (e). N are as follows: a: n = 4, b: neuron (n=8), neuron + keratinocytes (n = 7), neuron + B16F0 (n = 7), neuron + B16F10 (n = 7), c: n = 15/groups, d: keratinocytes inj. + ATP (n=5), B16F10 inj. + ATP (n=36), Keratinocytes inj. + caps (n = 6), B16F10 inj. + caps (n = 44), e: n = 4/groups. Experiments were independently repeated two (d) or three (a–c) times with similar results. Sequencing experiment was not repeated (e). P-values are shown in the figure and determined by one-way ANOVA post-hoc Bonferroni (b–c) or two-sided unpaired Student’s t-test (d). Scale bar = 100 μm (a).
Extended Data Fig. 5 | B16F10-secreted SLPI activates nociceptor neurons.

(a–e) Naive DRG neurons (TrpV1Cre::CheRiff-eGFP fl/WT), B16F10-mCherry-OVA, and OVA-specific cytotoxic CD8+ T cells were cultured alone or in combination. After 48h, the cells were collected, FACS purified, and RNA sequenced. DEGs were calculated, and Fgfr1 (fibroblast growth factor receptor 1) was found to be overexpressed in OVA-specific cytotoxic CD8+ T cells when co-cultured with cancer cells and DRG neurons (a). Conversely, OVA-specific cytotoxic CD8+ T cells downregulates the expression of the pro-nociceptive factor Hmgb1 (High–mobility group box 1; b), Braf (c), as well as Fgfr3 (d) when co-cultured with B16F10-mCherry-OVA and DRG neurons. Tslp expression level was not affected in any of tested groups (e).

(f–i) Using calcium microscopy, we probed whether SLPI directly activates cultured DRG neurons. We found that SLPI (0.01-10 ng/mL) induces a significant calcium influx in DRG neurons (f). SLPI-responsive neurons are mostly small-sized neurons (g,h; mean area = 151 μm²) and largely capsaicin-responsive (i; ~42%). (j) The right hindpaw of naive mice was injected with saline (20 μL) or SLPI (i.d., 1 μg/20 μL), and the mice’s noxious thermal nociceptive threshold was measured (0–6h). The ipsilateral paw injected with SLPI showed thermal hypersensitivity in contrast with the contralateral paw. Saline had no effect on the mice’s thermal sensitivity. Data are shown as box and whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median), for which individual data points are given (a–f, h, j), stacked bar graph on a logarithmic scale (g), and Venn Diagram (i). N are as follows: a–e: n = 2–4/group, f: vehicle (n = 28), 10pg/ml (n = 28), 100 pg/ml (n = 132), 1,000 pg/ml (n = 191), 10 ng/ml (n = 260), capsaicin (n = 613), KCl (n = 1,139); g: 0-100 (SLPI=19; KCl=177), 100-200 (SLPI = 45; KCl = 390), 200-300 (SLPI = 16; KCl = 216), 300-400 (SLPI=11; KCl = 138), 400-500 (SLPI = 5; KCl = 68), 500-600 (SLPI = 2, KCl = 18), 600-700 (SLPI = 0; KCl = 10), 700-800 (SLPI = 0; KCl = 13), 800+ (SLPI = 0; KCl = 12), h: n = 98, i: KCl+=1139, KCl+Caps+=614, KCl+Caps’SLPI+=261, KCl’Caps SLPI+=29; j: 0h (n = 9), SLPI at 1h (n = 6), saline at 1h (n = 3), SLPI at 3h (n = 6), saline at 3h (n = 3), SLPI at 6h (n = 6), saline at 6h (n = 3). Experiments were independently repeated two (j) or three (f–i) times with similar results. Sequencing experiment was not repeated (a–e). P-values were determined by one-way ANOVA post-hoc Bonferroni (a–f); or two-sided unpaired Student’s t-test (j). P-values are shown in the figure or indicated by * for p ≤ 0.05; ** for p ≤ 0.01; *** for p ≤ 0.001.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Nociceptor ablation reduces the exhaustion of intratumoral CD8+ T cells. (a-b) Orthotopic B16F10-mCherry-OVA (5x10^5 cells; i.d.) cells were injected to nociceptor intact (Trpv1WT::DTA^WT^) and ablated (Trpv1Cre::DTA^Cre^) mice. Sixteen days post-B16F10-mCherry-OVA cells inoculation (5x10^5 cells; i.d.), tumour-infiltrating CD8+ T cells were immunophenotyped (a) and were found to be more numerous in sensory neuron depleted mice (b). (c-g) Orthotopic B16F10-mCherry-OVA (2x10^5 cells; i.d.) cells were injected into the left hindpaw of nociceptor intact (n = 96; Trpv1WT::DTA^WT^) or ablated (n = 18; Trpv1Cre::DTA^Cre^) mice. When compared to their baseline threshold, littersmate control mice showed significant thermal hypersensitivity on day 7, an effect that peaks on day 21 (c). In these mice, intratumoral frequency of PD-1+LAG3+TIM3+ CD8+ T cells (day 12), and significant tumour growth (day 12; p < 0.05). Thermal pain hypersensitivity (day 7) preceded the increase in intratumoral (~85 mm^3) were selected and exposed to αPD-L1 (6 mg/kg, i.p.) once every 3 days post tumour inoculation, an effect that peaked on day 19. Finally, B16F10 tumour volume peaked on day 22 (f). When compared with littersmate control mice, sensory neuron ablated mice inoculated with B16F10 cells showed no thermal pain hypersensitivity (c), reduced intratumoral frequency of PD-1+LAG3+TIM3+ CD8+ T cells (d) and tumour volume (f). In littersmate control mice, thermal pain hypersensitivity (day 7) precedes the increase in intratumoral frequency of PD-1+LAG3+TIM3+ CD8+ T cells (day 12), and significant tumour growth (day 12; g). (h) Orthotopic B16F10-mCherry-OVA cells (5x10^5 cells; i.d.) were inoculated into 8-week-old male and female sensory neuron intact or ablated mice. The mice were treated with αPD-L1 (6 mg/kg, i.p.; days 7, 10, 13, 16 post tumour inoculation) or its isotype control. On day 19, αPD-L1 potentiated the nociceptor ablation mediated reduction in B16F10-OVA tumour volume. (i-k) Orthotopic B16F10-mCherry-OVA cells (5x10^5 cells, i.d.) were injected into a cohort of nociceptor neuron-ablated mice 3 days prior to the injection given to nociceptor intact mice. Mice from each group with similar tumour size (~85 mm^3) were selected and exposed to αPD-L1 (6 mg/kg, i.p.) once every 3 days for a total of 9 days. Eighteen days post tumour inoculation, we found that αPD-L1 reduced tumour growth was higher (~47%) in nociceptor-ablated mice than in nociceptor-intact mice (~32%; i-j). In addition, nociceptor ablation increased the proportion of intratumoral tumour-specific (k) defined as H-2Kb+ CD8+ T cells. These differences were further enhanced by αPD-L1 treatment (i-k). (l-m) Sensory neurons ablation (Trpv1WT::DTA^WT^) decreased growth of YUMMER1.7 cells (5x10^5 cells; i.d.), an immunogenic version of a Braf^V600E;Cdkn2a−/−Pten−/− melanoma cell line (I; assessed until day 12). The non-immunogenic YUMML1.7 cell line (5x10^5 cells; i.d.; assessed until day 14) cells were injected to nociceptor intact (Trpv1WT::DTA^WT^) and ablated mice (Trpv1Cre::DTA^Cre^). Nociceptor ablation had no effect on YUMML1.7 growth (m). (n) Orthotopic B16F10-mCherry-OVA (5x10^5 cells; i.d.) cells were injected to nociceptor intact (Trpv1WT::DTA^WT^) and ablated mice (Trpv1Cre::DTA^Cre^). The reduction in B16F10-mCherry-OVA (5x10^5 cells; i.d.) tumour growth observed in nociceptors ablated mice was absent following systemic CD3 depletion (asessed until day 15; αCD3, 200 μg/mouse; i.p.; every 3 days). (o) To deplete their nociceptor neurons, C57BL6J mice were injected with RTX (c., s.c., 30, 70, 100 μg/kg) and were subsequently (28 days later) inoculated with B16F10-mCherry-OVA (2x10^5 cells). RTX-injected mice showed reduced tumour growth when compared to vehicle-exposed mice (assessed until day 13). (p-q) Orthotopic B16F10-mCherry-OVA (5x10^5 cells; i.d.) cells were injected to light-sensitive mice (Nav1.8^Cre::ChR2^Fl/WT^). As opposed to unstimulated mice, the optogenetic activation (3.5 ms, 10Hz, 478nm, 60 mW, giving approx. 2.6 mW/mm^2 with a 0.39-NA fibre placed 5–10 mm from the skin. 20 min) of tumour-innervating nociceptor neurons, when started once B16F10 tumours were visible (~20 mm^3) or well established (~200 mm^3), resulted in enhanced tumour growth (p, as measured until day 14) and intratumoral CGRP release (q). Data are shown as FACS plot (a, b, k, q), scatter dot plot (c-f, j-k), box-and-whisker plots (i, o), as box and whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median) for which individual data points are given (b, k, q), scatter dot plot (c-f), percentage change from maximal thermal hypersensitivity, intratumoral frequency of PD-1+LAG3+TIM3+ CD8+ T cells and tumour volume (g), or mean ± S.E.M (h, j, i-p). N are as follows: a-b: intact (n = 29), ablated (n = 33), c: intact (n = 96), ablated (n = 19), d: intact (n = 92), ablated (n = 15), e: intact (n = 96), ablated (n = 15), f: intact (n = 96), ablated (n = 16), g: n = 96, h: intact (n = 9), ablated (n = 10), intact+αPD-L1 (n = 9), ablated+αPD-L1 (n = 8), i: intact (n = 14), ablated (n = 4), j: intact+αPD-L1 (n = 12), ablated+αPD-L1 (n = 12), k: intact (n = 5), ablated (n = 6), intact+αPD-L1 (n = 5), ablated+αPD-L1 (n = 5), l: intact (n = 8), ablated (n = 11), m: intact (n = 6), ablated (n = 13), n: intact (n = 5), ablated (n = 5), intact+αCD3 (n = 6), ablated+αCD3 (n = 5), o: vehicle (n = 11), RTX (n = 10), p: Nav1.8^Cre::ChR2^Fl/WT^ (n = 12), Nav1.8^Cre::ChR2^Fl/WT^ + Light (vol. -200 mm^3) (n = 8), Nav1.8^Cre::ChR2^Fl/WT^ + Light (vol. -20 mm^3) (n = 8), q: Nav1.8^Cre::ChR2^Fl/WT^ (n = 12), Nav1.8^Cre::ChR2^Fl/WT^ + Light (vol. -200 mm^3) (n = 7), Nav1.8^Cre::ChR2^Fl/WT^ + Light (vol. -20 mm^3) (n = 9). Experiments were independently repeated two (c-g), three (h-q) or six (a,b) times with similar results. P values are shown in the figure and determined by two-sided unpaired Student’s t-test (b-f, k, q), or two-way ANOVA post-hoc Bonferroni (h-j, l-p).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | BoNT/A silencing of B16F10-innervating neurons decreases tumour growth. (a–e) Splenocytes-isolated CD8+ T cells from naive C57BL6j mice were cultured under Tc1-stimulating conditions ((ex vivo) activated by CD3 and CD28, IL-12, and anti-IL4) for 48 h. The cells were then exposed to BoNT/A (10–50 pg/μL) for 24 h; effects on apoptosis, exhaustion, and activation were measured by flow cytometry. When compared to vehicle-exposed cells, BoNT/A did not affect the survival (a) of cultured cytotoxic CD8+ T cells, nor their relative expression of IFNγ (b), TNF (c), IL-2 (d) and PD-1/LAG3/TIM3 (e). (f) B16F10 (1x10⁵ cells) were cultured for 24 h and subsequently exposed to BoNT/A (1.6–50 pg/μL) or its vehicle for an additional 24 h. BoNT/A did not trigger B16F10 cells apoptosis, as measured by the mean fluorescence intensity of Annexin V. (g–n) One and three days prior to tumour inoculation (defined as prophylactic), the skin of 8-week-old male and female mice was injected with BoNT/A (25 pg/μL; i.d.) or its vehicle. One day after the last injection, orthotopic B16F10-mCherry-OVA (5x10⁵ cells; i.d.) were inoculated into the area pre-exposed to BoNT/A. In another group of mice, BoNT/A was administered (25 pg/μL; i.d.) one and three days after the tumour reached a volume of ~200 mm³ (defined as therapeutic). The effect of neuron silencing on tumour size and tumour-infiltrating CD8+ T cell exhaustion was measured. Nineteen days post tumour inoculation, we found that the tumour volume (g, h) and weight (i) were reduced in mice treated with BoNT/A (Prophylactic group). In parallel, we found that silencing tumour-innervating neurons increased the proportion of IFNγ (k), TNF (l), and IL-2 (m) CD8+ T cells. BoNT/A had no effect on the total number of intratumoral CD8 T cells (j) or the relative proportion of PD-1/LAG3/TIM3 (n) CD8+ T cells. (o) One and three days prior to tumour inoculation, the skin of 8-week-old male and female sensory neuron-intact or ablated mice was injected with BoNT/A (25 pg/μL; i.d.) or its vehicle. One day following the last injection, orthotopic YUMMER1.7 cells (5x10⁵ cells; i.d.) were inoculated into the area pre-exposed to BoNT/A. The effects of nociceptor neuron ablation on tumour size and volume were measured. Thirteen days post tumour inoculation, we found that the tumour growth was lower in mice treated with BoNT/A or in sensory neuron-ablated mice. BoNT/A had no additive effects when administered to sensory neuron-ablated mice. (p) One and three days prior to tumour inoculation, the skin of 8-week-old male and female mice was inoculated with BoNT/A (25 pg/μL; i.d.) or its vehicle. One day following the last injection, orthotopic B16F10-mCherry-OVA cells (5x10⁵ cells; i.d.) were inoculated into the area pre-exposed to BoNT/A. On days 7, 10, 13 and 16 post tumour inoculation, the mice were exposed to αPD-L1 (6 mg/kg, i.p.) or its isotype control. Eighteen days post tumour inoculation, we found that neuron silencing using BoNT/A potentiated αPD-L1-mediated tumour reduction. Data are shown as box-and-whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median), for which individual data points are given (a–f; h–n) or as mean ± S.E.M (g, o, p). N are as follows: a–e: n = 5/ groups, f: n = 3/ groups, g–l: vehicle (n = 12), BoNT/A therapeutic (n = 12), BoNT/A prophylactic (n = 10); j: vehicle (n = 11), BoNT/A therapeutic (n = 12), BoNT/A prophylactic (n = 8), k–n: vehicle (n = 10), BoNT/A therapeutic (n = 12), BoNT/A prophylactic (n = 8), o: intact + vehicle (n = 9), ablated + vehicle (n = 8), intact + BoNT/A (n = 10), ablated + BoNT/A (n = 8), p: vehicle (n = 7), αPD-L1 (n = 8), αPD-L1 + BoNT/A (n = 7). Experiments were independently repeated two (a–f, o–p) or four (g–n) times with similar results. P-values are shown in the figure and determined by one-way ANOVA posthoc Bonferroni (a–f, h–n) or two-way ANOVA post-hoc Bonferroni (g, o, p).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | QX-314 silencing of B16F10-innervating neurons reduces tumour growth. (a–e) Splenocytes-isolated CD8+ T cells from naïve C57BL/6J mice were cultured under Tc1-stimulating conditions (ex vivo activated by CD3 and CD28, IL-12, and anti-IL-4) for 48h. The cells were then exposed to QX-314 (0.3–150 μM) for 24h, effects on apoptosis, exhaustion and activation were measured by flow cytometry. When compared to vehicle-exposed cells, QX-314 did not affect the survival of cultured cytotoxic CD8+ T cells (a), nor their relative expression of PD-1+LAG3+TIM3+ (b), IFNγ+ (c), TNF+ (d) and IL-2+ (e). (f) B16F10 (1x105 cells) were cultured for 24h. The cells were then exposed or not to QX-314 (0.1–1%) for an additional 24–72h, and cell count was analysed by bright-field microscopy. QX-314 did not affect B16F10 cells’ survival, as measured by relative cell count changes (at each time point) in comparison to vehicle-exposed cells. (g–i) One and three days prior to tumour inoculation, 8-week-old male and female wild-type mice’s right hindpaws or flanks were injected with BoNT/A (25 pg/μL, i.d.) or its vehicle. On the following day, orthotopic B16F10 cells (g; 5x105 cells; i.d.; h: 2x105 cells; i.d.) were inoculated into the area pre-exposed to BoNT/A. Starting one day post inoculation, QX-314 (0.3%; i.d.) was administered (i.d.) once daily in another group of mice. The effects of sensory neuron silencing were tested on neuropeptide release (g), as well as mechanical (h) and thermal pain hypersensitivity (i). First, CGRP levels were increased in B16F10 tumour surrounding skin explant (assessed on day 15) in comparison to control skin; an effect further enhanced by capsaicin (1 μM; h) but was absent in skin pre-treated with BoNT/A (25 pg/μL) or QX-314 (0.3%; g). We also found that B16F10 injection induced mechanical (h) and thermal pain hypersensitivities (i) fourteen days post tumour inoculation. These effects were stopped by sensory neuron silencing with QX-314 or BoNT/A (h–i). (j) Orthotopic B16F10-mCherry-OVA cells (5x105 cells; i.d.) were inoculated into 8-week-old male and female mice. Starting one day post inoculation, QX-314 (0.3%; i.d.; 5 sites) was injected once daily around the inoculated site. As measured seventeen days post tumour inoculation, silencing tumour innervation also decreased tumour volume (k, l) and weight (m), as well as the relative proportion of PD-1+LAG3+TIM3+(n) CD8+ T cells. QX-314 treatment also increased the total number of intratumoral CD8+ T cells (o), as well as relative proportion of IFNγ+ (p), TNF+ (q), and IL-2+ (r) CD8+ T cells. Orthotopic B16F10-mCherry-OVA cells (5x105 cells, i.d.) were injected into mice treated with QX-314 (0.3%; i.d.) 2–3 days prior to being injected into vehicle-exposed mice. Mice from each group with similar tumour size (~100mm3) were selected and exposed to αPD-L1 (6 mg/kg, i.p.) once every 3 days for a total of 9 days. Eighteen days post tumour inoculation, we found that αPD-L1-reduced tumour growth was higher (~61%) in nociceptor silenced mice than was observed in isotype vehicle-exposed mice (~49%; s–t). Data are shown as box-and-whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median), for which individual data points are given (a–e, g–l–r), as mean ± S.E.M. (f, h, i, k, s, t), or as Mantel–Cox regression analysis (j). N are as follows: a–n = 4/groups, b–e = n = 5/groups, f = n = 3/groups, g: naïve (n = 4), vehicle (n = 7), B16F10+vehicle (n = 5), B16F10+BoNT/A (n = 5), B16F10+QX-314 (n = 5), h–i: n = 6/groups, j: vehicle (n = 89), QX-314 (n = 12), k: vehicle (n = 21), QX-314 prophylactic (n = 21), QX-314 therapeutic (n = 17), l: vehicle (n = 26), QX-314 therapeutic (n = 26), QX-314 prophylactic (n = 28), m: vehicle (n = 25), QX-314 therapeutic (n = 22), QX-314 prophylactic (n = 25), n: vehicle (n = 31), QX-314 therapeutic (n = 29), QX-314 prophylactic (n = 28), o: n = 30/groups, p–r: vehicle (n = 24), QX-314 therapeutic (n = 23), QX-314 prophylactic (n = 25), s: vehicle (n = 9), QX-314 (n = 13), t: vehicle + αPD-L1 (n = 18), QX-314 + αPLDI (n = 13). Experiments were independently repeated two (a–l, s–t) or four (j–r) times with similar results. P values are shown in the figure and determined by one-way ANOVA posthoc Bonferroni (a–g, l–r), two-sided unpaired Student’s t-test (h–i), Mantel–Cox regression (j), or two-way ANOVA posthoc Bonferroni (k, s–t).
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Nociceptor-released CGRP increases cytotoxic CD8+ T cell exhaustion. (a–b) Splenocytes-isolated CD8+ T cells were cultured under Tc1-stimulating condition (ex vivo activated by CD3 and CD28, IL-12, and anti-IL4) for 48h. The cells were then cultured or not with wild-type DRG neurons and exposed to capsaicin (1 μM, challenged once every two days) or its vehicle. As measured after 4 days stimulation, capsaicin-stimulated intact neuron increased the proportion of PD-1+LAG3+TIM3+ (a) cytotoxic CD8+ T cells, while it decreased the one of IFNγ+ (b). (c–d) Splenocytes-isolated CD8+ T cells were cultured under Tc1-stimulating conditions (ex vivo activated by CD3 and CD28, IL-12, and anti-IL4) for 48h. In the presence of peptidase inhibitors (1 μL/mL), naive DRG neurons were cultured in the presence of BoNT/A (50 pg/mL) or its vehicle for 24h. The cells were then washed, stimulated (30 min) with KCl (50mM), and the conditioned medium collected. On alternate days for 4 days, the cytotoxic CD8+ T cells were exposed or not to a RAMP1 blocker (CGRP 8–37; 2 μg/mL) and challenge (1:2 dilution) with fresh KCl-induced conditioned medium from naive, or BoNT/A-silenced neurons. As measured after 4 days stimulation, KCl-stimulated neuron-conditioned medium increased the proportion of PD-1+LAG3+TIM3+ (c) cytotoxic CD8+ T cells, while it decreased the one of IFNγ+ (d). Such effect was absent when the cells were co-exposed to the RAMP1 blocker CGRP 8–37 (2 μg/mL; measured after 24h). CGRP (0.1 μM) challenges also reduced OT-I cytotoxic CD8+ T cells elimination of B16F10-OVA cell (i; measured after 24h). Data are shown as box-and-whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median), for which individual data points are given (a–e, g–h), or representative FACS plot (f, i). N are as follows: a: CD8 + vehicle (n = 4), CD8 + capsaicin (n = 9), CD8 + neuron + capsaicin (n = 9), b: n = 5/groups, c: CD8 (n = 6), CD8 + KCl-induced neurons CM (n = 5), CD8 + KCl-induced neurons CM + CGRP 8–37 (n = 6), d: n = 5/groups, e: RampWT CD8 + vehicle (n = 7), RampWT CD8 + CGRP (n = 8), Ramp−/− CD8 + vehicle (n = 6), Ramp−/− CD8 + CGRP (n = 6), g: B16F10 (n = 3), B16F10 + OT-I CD8 (n = 4), B16F10 + OT-I CD8 + KCl-induced neuron CM (n = 4), h: B16F10 + OT-I CD8 (n = 4), B16F10 + OT-I CD8 + KCl-induced neuron CM (n = 4), B16F10 + OT-I CD8 + KCl-induced neuron CM + CGRP 8–37 (n = 5). Experiments were repeated a minimum of three independent times with similar results. P-values are shown in the figure and determined by one-way ANOVA posthoc Bonferroni (a–e, g–h).
Extended Data Fig. 10 | See next page for caption.
Orthotopic B16F10-mCherry-OVA cells (5x10^5 cells; i.d.) were inoculated into 8-week-old T cell exhaustion. When administered as therapeutic, BIBN4096 reduced tumour volume. Intratumoral CD8+ T cells exhaustion (PD-1+LAG3+TIM3+) was also collected and measured by ELISA. Intratumoral CGRP levels positively correlated with the proportion of PD-1+LAG3+TIM3+ CD8+ T cells. When administered prophylactically, BIBN4096 decreased tumour volume (~200 mm^3) of mice transplanted with B16F10-mCherry-OVA cells (5x10^5 cells; i.d.), and naïve C57BL6J mice were cultured under Tc1-stimulating conditions (ex vivo activated by CD3 and CD28, IL-12, and anti-IL-4) for 48h. The cells were then exposed to BIBN4096 (1–4 μM) for 24h; effects on apoptosis, exhaustion and activation were measured by flow cytometry. When compared to vehicle-exposed cells, BIBN4096 did not affect the survival (o) of cultured CD8+ T cells, nor their relative expression of PD-1+LAG3+TIM3+ (p), IFNγ+ (q), TNF+ (r), and IL-2+ (s). T cells were then exposed (or not) to BIBN4096 (1-8 μM) for an additional 24h; effects on apoptosis were measured by flow cytometry. BIBN4096 did not trigger B16F10 cells apoptosis, as measured by the mean fluorescence intensity of Annexin V. Naïve splenocyte CD8+ T cells were FACS purified from Rag1−/− mice, expanded and stimulated in vitro. 8-week-old female Rag1−/− mice were transplanted with either Ramp1−/− or Ramp1WT CD8+ T cells or a 1:1 mix of Ramp1−/− and Ramp1WT CD8+ T cells. The effect of nociceptor neuron-silencing on tumour size and tumour-infiltrating CD8+ T cells was measured on day 19. Experiments were independently repeated twice (a–f, h–m; o–u) or four (g–m) times with similar results. P-values are shown in the figure and determined by two-sided unpaired Student's t-test (a–d, f, h–m, o–v), simple linear regression analysis (e), Mantel–Cox regression (g), mean ± S.E.M (n, o), or as FACS plot (w). N are as follows: a–c: Naul.8WT::DTA+/− (n = 18), Naul.8WT::DTA+/− (n = 10), f: Trpv1WT::DTA+/− (n = 16), Trpv1WT::DTA+/− (n = 9), Trpv1WT::DTA+/− (n = 7), Trpv1WT::DTA+/− (n = 9), g: vehicle (n = 89), BIBN4096 (n = 16), h–m: Vehicle (n = 13), BIBN4096 therapeutic (n = 18), BIBN4096 prophylactic (n = 16), n: Trpv1WT::DTA+/− (n = 8), Trpv1WT::DTA+/− (n = 9), Trpv1WT::DTA+/− (n = 9), Trpv1WT::DTA+/− (n = 7), vehicle (n = 3), p: s: n = 5/group, t: n = 4/group, u–w: n = 5/group. Experiments were independently repeated twice (a–f, n–w) or four (g–m) times with similar results. As measured fifteen days post inoculation, Na V1.8+ and ablated mice (CD45.2+) mice, expanded and stimulated (CD3 and CD28 + IL-2) in vitro, 8-week-old female Rag1−/− mice were transplanted with either Ramp1−/− or Ramp1WT CD8+ T cells or a 1:1 mix of Ramp1−/− and Ramp1WT CD8+ T cells. One week post transplantation, the mice were inoculated with B16F10-mCherry-OVA cells (5x10^5 cells; i.d.). Ten days post tumour inoculation, we retrieved a similar number of CD8+ T cells across the three tested groups (w). The relative proportion of intra-tumour PD-1+LAG3+TIM3+ CD8+ T cells was lower in Ramp1−/− transplanted mice (w). Within the same tumour, intratumoral CD8+ T cell exhaustion was immunophenotyped by flow cytometry (representative panel shown inw) and showed that the relative proportion of PD-1+LAG3+TIM3+ CD8+ T cells was 3-fold lower in Ramp1−/− CD8+ T cells than in Ramp1WT CD8+ T cells (w). Data are shown as box- and whisker plots (runs from minimal to maximal values; the box extends from 25th to 75th percentile and the middle line indicates the median), for which individual data points are given (a–d, f, h–m, o–v), linear regression (e), Mantel–Cox regression (g), mean ± S.E.M (n, o), or as FACS plot (w).
Extended Data Fig. 11 | RAMP1 expression in patient melanoma-infiltrating T cells correlates with worsened survival and poor responsiveness to ICIs.

(a–l) In silico analysis of Cancer Genome Atlas (TCGA) data linked the survival rate among 459 patients with melanoma with their relative expression levels of various genes of interest (determined by bulk RNA sequencing of tumour biopsy). Kaplan–Meier curves show the patients’ survival after segregation in two groups defined by their low or high expression of a gene of interest. Increased gene expression (labelled as high; red curve) of TUBB3 (b), PGP9.5 (e), NaVL7 (E), SLPI (k) and RAMP1 (l) in biopsy correlate with decreased patient survival (p≤0.05). The mantel–Haenszel hazard ratio and number of patients included in each analysis are shown in the figure (a–l). Experimental details were defined in Cancer Genome Atlas (TCGA) 40. (m) In silico analysis of single-cell RNA sequencing of human melanoma-infiltrating T cells revealed that RAMP1 T cells downregulated IL-2 expression and strongly overexpressed several immune checkpoint receptors (PD-1, TIM3, LAG3, CTLA4, CD28, ICOS, BTLA, CD27) in comparison to RAMP1 T cells. Individual cell data are shown as a log_{10} (transcript per million / 10). Experimental details and cell clustering were defined in Tirosh et al 42. N are defined in each panel. (n–p) On the basis of the clinical response of patients with melanoma to immune checkpoint blocker, patients were clustered into two groups defined as ICI-responsive or ICI-resistant 41. In silico analysis of single-cell RNA sequencing of patients’ biopsies revealed that tumour-infiltrating CD8+ T cells from patients who were resistant to ICIs significantly overexpressed RAMP1 (2.0-fold), PD-1 (1.7-fold), LAG3 (1.6-fold), CTLA4 (1.6-fold), and TIM3 (1.7-fold; n–p). Individual cell data are shown as a log_{10} (transcript per million / 10). Experimental details and cell clustering were defined in Jerby-Arnon et al 41. P-values are shown in the figure and determined by two-sided unpaired Student’s t-test. N are defined in each panel (n–o).
Melanoma-innervating nociceptors attenuate cancer immunosurveillance. Melanoma growth sets off anti-tumour immune responses, including the infiltration of effector CD8 T cells and their subsequent release of cytotoxic cytokines (i.e., IFNγ, TNF, Granzyme B). By acting on tissue-resident nociceptor neurons, melanoma-produced SLPI promotes pain hypersensitivity, tweaks the neurons’ transcriptome, and drives neurite outgrowth. These effects culminate in dense melanoma innervation by nociceptors and abundant release of immunomodulatory neuropeptides. CGRP, one such peptide, acts on tumour-infiltrating effector CD8+ T cells that express the CGRP receptor RAMP1, increasing their expression of immune checkpoint receptors (i.e., PD-1, LAG3, TIM3). Therefore, along with the immunosuppressive environment present in the tumour, nociceptor-produced CGRP leads to the functional exhaustion of tumour-infiltrating CD8+ T cells, which opens the door to unchecked proliferation of melanoma cells. Genetically ablating (i.e., TRPV1 lineage) or pharmacologically silencing (i.e., QX-314, BoNT/A) nociceptor neurons as well as blocking the action of CGRP on RAMP1 using a selective antagonist (i.e., BIBN4096) prevents effector CD8+ T cells from undergoing exhaustion. Therefore, targeting melanoma-innervating nociceptor neurons constitutes a novel strategy to safeguard host anti-tumour immunity and stop tumour growth.
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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐ Give P values as exact values whenever suitable.
☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about: availability of computer code

Data collection

Cell were immunophenotyped using a LSRFortessa or FACSCanto II (Becton Dickinson).

Calium microscopy analysis was performed using Nikon eclipse Ti2 microscope (NIS-Elements Advanced Research version 4.5).

Patient biopsy images were collected using an Olympus BX51 bright-field microscope.

Mouse tumor innervation images were acquired using an Olympus FV3000 confocal imaging system.

GraphPad Prism v9 and Microsoft Excel (version 2019) was used for data entry, graph construction and data analysis.

Data analysis

GraphPad Prism [Version 9.0] and Microsoft Excel [version 2019] were used for data entry, graph construction, and data analysis.

Image analysis (neurite length, ramification index) was performed using ImageJ macros (Fiji; version 1.53c).

Calium microscopy analysis was performed using Nikon eclipse Ti2 microscope (NIS-Elements Advanced Research version 4.5).

Flow cytometry data were analyzed using FlowJo (version 10.0.0).

TCGA data were accessed via Oncomine [www.oncomine.com for gene expression] and OncoLnc [www.oncolnc.org for survival].

Single-cell RNA sequencing was analyzed using the Broad single-cell portal [https://singlecell.broadinstitute.org].

Human and mouse immune cell gene profiles were respectively analyzed using the human protein atlas [https://www.proteinatlas.org/humanproteome/immune-cell] and Immunological genome project [https://www.imagen.org/].
For RNA sequencing, the reads were aligned to the mouse reference genome GRCm38/mm10 (GenBank assembly accession GCA_000001635.2) using STAR (version used: 2.5.4a, 2.5.1b, 2.7).

Aligned reads were assigned to genic regions using the featureCounts function from Subread (version 1.6.4.22).

Hierarchical clustering was computed using the heatmap.2 function (ward.D2 method) from Gplots R package (version 3.1.3).

Differential gene expression analysis was carried out by DESeq2 (version used: 1.18.1 or 1.28.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Git-lab). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data are readily available online [https://www.talbotlab.com/nature], from the corresponding author (email: sebas.talbot@gmail.com) and within the paper and its Supplementary information files.

The RNA sequencing datasets have been deposited in the National Center for Biotechnology Information’s Gene Expression Omnibus (#GSE205863, #GSE205864, #GSE205865).

GSE205863 is available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205863

GSE205864 is available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205864

GSE205865 is available at: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE205865

Mouse reference genome GRCm38/mm10 is available at www.ncbi.nlm.nih.gov/assembly/GCF_000001635.20

Oncomine is available at: www.oncomine.com

Oncolnc is available at: www.oncolnc.org

Broad single-cell bioportal is available at: https://singlecell.broadinstitute.org

Tirosh et al., is available at: https://singlecell.broadinstitute.org/single_cell/study/SCP11/melanoma-intra-tumor-heterogeneity

Jerby-Aron is available at: https://singlecell.broadinstitute.org/single_cell/study/SCP109/melanoma-immunotherapy-resistance#study-summary

Human immune cell gene profiles are accessible at: www.proteinatlas.org/humanproteome/immune-cell

Mouse immune cell gene profiles are available at: www.immen.org/

Meta-analysis of receptor neurons gene profiles are available at: www.talbotlab.com/dataset

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Statistical methods were not used to predetermine sample size. The size of the cohort, based on similar studies in the field, was validated by pilot studies. All sample sizes are indicated in the figures and/or figure legends. All n values are clearly indicated within the figure legends. In the only case where a range is used (figure 2a/b), exact n values are provided in the source data files. For in vivo experiments we used n=8 animals. For in vitro experiments where replicate samples were used, we repeated the experiments at least 3 independent times to confirm the findings. For other mouse experiments a minimum of 5 mice were used to ensure proper statistics could be utilized. We determined this to be sufficient as per our pilot data, use of internal controls and/or the observed variability between within experimental groups.

Data exclusions

No data were excluded.
Replication: This is indicated in the figures, figure legends and/or methods. On the graphs individual dots represent individual samples/mice used. For each experiment, all attempts at replication were successful and our findings showed comparable results.

Randomization: Animals in a particular cohort were generated from one breeding pair and all offspring (e.g. nociceptor intact and ablated mice) were co-housed and, in respect with the ARRIVE guidelines, were randomly allocated into each experimental group. In vitro experiments, randomization was used for treatment selection. In some calcium microscopy experiments, the investigators performing the data collection were tasked to select all ligands responsive cells for downstream analysis. In these rare cases, randomization was not used for cell selection.

Blinding: Double blind was used for all in vivo treatments. In calcium microscopy involving co-culture (e.g. nociceptors and cancer cells), the differences in cell morphology are obvious and, therefore, the investigator performing the experiment was not blind. However, these investigators were always blinded to the treatment being applied to the cells and a second blinded investigator performed the downstream data analysis.

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| [ ] Animals and other organisms | |
| [ ] Human research participants | |
| [ ] Clinical data | |
| [ ] Dual use research of concern | |

### Antibodies

Antibodies used:

- Anti-Annexin V-APC and 7-AAD (BioLegend), cat no: 640930, Dilution: 1:100
- Anti-CD11b-APC/Cy7 (BioLegend), cat no: 101226, Dilution: 1:100
- Anti-CD16/32 (BioLegend), cat no: 156604, Dilution: 1:100
- Anti-CD28 (Bio X Cell), cat no: BE0015-5, Dilution: 1:4000
- Anti-CD3 (Bio X Cell), cat no: BE0001-1, Dilution: 200ng/mouse
- Anti-CD3 (Bio X Cell), cat no: BE00011, Dilution: 1:3000
- Anti-CD45.1-BV421 (BioLegend), cat no: 110732, Dilution: 1:100
- Anti-CD45.2-BV650 (BioLegend), cat no: 110986, Dilution: 1:100
- Anti-CD45-Alexa Fluor 700 (BioLegend), cat no: 103128, Dilution: 1:100
- Anti-CD45-BV421 (BioLegend), cat no: 103134, Dilution: 1:100
- Anti-CD4-FITC (BioLegend), cat no: 100406, Dilution: 1:100
- Anti-CD4-PerCP/Cy5.5 (BioLegend), cat no: 100540, Dilution: 1:100
- Anti-CD8 (Bio X Cell), cat no: B8061, Dilution: 200ng/mouse
- Anti-CD8-AF700 (BioLegend), cat no: 100730, Dilution: 1:100
- Anti-CD8-BV421 (BioLegend), cat no: 100753, Dilution: 1:100
- Anti-CD8-Pacific Blue (BioLegend), cat no: 100725, Dilution: 1:100
- Anti-CD8-PerCP/Cy5.5 (BioLegend), cat no: 100734, Dilution: 1:100
- Anti-GFP (Aves Labs), cat no: GFP-1020, Dilution: 1:500
- Anti-H-2Kb/OVA257-264 (NIH tetramer core facility), EDB ID: 58560, Dilution: 1:100
- Anti-IFN-γ-APC (BioLegend), cat no: 505810, Dilution: 1:100
- Anti-IFN-γ-FITC (BioLegend), cat no: 505806, Dilution: 1:100
- Anti-GLI(H-1)-AF488 (Invitrogen), cat no: A28175, Dilution: 1:500
- Anti-I-L-2-BV510 (BioLegend), cat no: 503833, Dilution: 1:100
- Anti-I-L-2-Pacific Blue (BioLegend), cat no: 503820, Dilution: 1:100
- Anti-I-L-2-Pecy7 (BioLegend), cat no: 503832, Dilution: 1:100
- Anti-Ig-4 (Bio X Cell), cat no: BE0045, Dilution: 0.1:1000 - 1:1000
- Anti-Ig3-PE (BioLegend), cat no: 125208, Dilution: 1:100
- Anti-Ig3-PerCP/Cy5.5 (BioLegend), cat no: 125212, Dilution: 1:100
- Anti-mCherry (Origene), cat no: A9040-200, Dilution: 1:500
- Anti-FD1-PE Cy7 (BioLegend), cat no: 329917, Dilution: 1:100
- Anti-FD11 (Bio X Cell), cat no: BF0101, Dilution: 1:500
- Anti-Tim3-APC (BioLegend), cat no: 119706, Dilution: 1:100
- Anti-TNFα-BV510 (BioLegend), cat no: 506339, Dilution: 1:100
- Anti-TNFα-BV711 (BioLegend), cat no: 506349, Dilution: 1:100
- Anti-TNFα-PE (BioLegend), cat no: 506360, Dilution: 1:100
- Anti-TRPV1 (Alomone Labs), cat no: ACC-080, Dilution: 1:100
- DAPI (Vector Laboratories), cat no: H-1000, Dilution: 1:2000
Validation

Anti-Annexin V-APC and 7-AAD was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 16 publications [Broggi A, et al. 2017. Nat Immunol. 18:1084]

Anti-CD11b-APC/Cy7 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 128 publications [Kiepper M et al. 2018. Cancer cell. 33(1):29-43]

Anti-CD16/32 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 12 publications [Opun Y, et al. 2020. Cell. 183(3):563-577.e26]

Anti-CD28 was validated for in vitro T cell stimulation/activation by supplier, and previously used in ≥ 9 publications [Vegran F, et al. 2014. Nat Immunology. 15(8):758-66]

Anti-CD3 was validated for in vitro T cell stimulation/activation by supplier, and previously used in ≥ 19 publications [Wendland K et al., 2018. J Immunol. 15:20(2):524-532]

Anti-CD3 was validated for in vivo T cell depletion in the mouse by supplier, and previously used in ≥ 19 publications [Peng B, et al., 2009. Blood; 12;114(20):4373-82]

Anti-CD45.1-BV421 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 22 publications [Phan TG, et al. 2007. Nature Immunol. 8:992]

Anti-CD45.2-BV650 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 11 publications [Kohlermeier JE, et al. 2008. Immunity, 29:101]

Anti-CD45-Alexa Fluor 700 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 96 publications [Radtke AJ, et al. 2012. Nat Protoc. 17:378-401]

Anti-CD45-BV421 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 50 publications [Haynes NM, et al. 2007. J. Immunol. 179:5099]

Anti-CD4-FITC was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 100 publications [Zheng B, et al. 1996. J. Exp. Med. 184:1083]

Anti-CD4-PerCP/Cy5.5 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 48 publications [Leon-Ponie M, et al. 2007. Blood 109:3139]

Anti-CD8 was validated for cell depletion by supplier, and previously used in ≥ 19 publications [Vegran, F., et al. (2014) Nat Immunol 15(8): 758-766]

Anti-CD8-AF700 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 54 publications [Shih FF, et al. 2006. J. Immunol. 176:3438]

Anti-CD8-BV421 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 43 publications [Bouwer HGA, et al. 2006. P. Natl. Acad. Sci. USA 103:5102]

Anti-CD8-Pacific Blue was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 38 publications [Ko SY, et al. 2005. J. Immunol. 175:3309]

Anti-CD8-PerCP/Cy5.5 was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 110 publications [Bankoti J, et al. 2010. Toxicol. Sci. 115:422]

Anti-CFP was validated for flow cytometry and for use in mouse cell lines by supplier, and previously used in ≥ 49 publications [Zimmerman A et al., 2019 Neuron. 162(2):420-434.e8]

Anti-H-2Kb/OVA257-264 was validated for flow cytometry and for use in the NIH tetramer core and previously used in Crittenden et al., 2018, Sci Rep. 3:811:7012]

Anti-IFN-γ-APC was validated for flow cytometry and for use in mouse cell lines by supplier and previously used in ≥ 142 publications [Ferrick D, et al. 1995. Nature 373:255]

Anti-IFN-γ-FITC was validated for flow cytometry and for use in mouse cell lines by supplier and previously used in ≥ 74 publications [Ko SY, et al. 2005. J. Immunol. 175:3309]

Anti-IgG1(H+L) AF488 was validated for immunofluorescence by supplier and previously used in ≥ 305 publications [Miao et al., 2022. J Exp Med. 5:2199:e20220214]

Anti-II-2-BV510 was validated for flow cytometry and for use in mouse cell lines by supplier and previously used in ≥ 2 publications [Dikiy S, et al. 2021. Immunity, 54(5):931-945.e11]

Anti-II-2-Pacific Blue was validated for flow cytometry and for use in mouse cell lines by supplier and previously used in Mohammed RN, et al. 2019. Sci Rep. 4:185:416667]
Eukaryotic cell lines

Policy information about: cell lines

Cell line source(s)
- B16F0 [ATCC, #CRL-6322]
- B16F10 [ATCC, #CRL-6475]
- B16F10-OVA (Matthew F. Krummel, UCSF)
- B16F10-OVA-mCherry (Matthew F. Krummel, UCSF)
- B16F10-EGFP (Imaris, #C053)
- YUMM1.7 [ATCC, #CRL-3362]
- YUMMER1.7 (Marcus Rosenberg, Yale U)
- Non-tumorigenic keratinocytes [CellInTEC, #MPEK-BL6100]

Authentication
- Non-commercial cell lines (B16F10-OVA, B16F10-OVA-mCherry and B16F10-EGFP) were authenticated using antibody (against OVA, eGFP, mCherry) and/or imaging as well as morphology and/or growth property. Commercial cell line (ATCC, Imaris, CellInTEC) provides a certificate of analysis in which they validate the cell lines with specific test and procedures such as growth property, morphology, mycoplasma detection, species determination, and sterility test.

Mycoplasma contamination
- All the cell lines tested negative for mycoplasma

Commonly misidentified lines [See ITAG register]
- No commonly misidentified lines were used in this study.

Animals and other organisms

Policy information about: studies involving animals, ARRIVE guidelines, recommended for reporting animal research

Laboratory animals
- Both males and females mice (mus musculus) were used equally, and they were used at 6 weeks of age up to 20 weeks of age. Mice were housed in standard environmental conditions (12h light/dark cycle; 23°C, food and water ad libitum) at facilities accredited by the Canadian Council of Animal Care (UdeM) or Association for Assessment and Accreditation of Laboratory Animal Care (BCH).
C57BL/6J (Jax, #000664); CD45.1+ C57BL/6J (Jax, #002014), RAMP1-/- (Jax, #031560), Rag1-/- (Jax, #002216), OT1 (Jax, #003831), TRPV1cre (Jax, #017769), ChR2IRF (Jax, #012567), td-tomatoIRF (Jax, #007908), DTAIRF (Jax, #009669), QuASR2IRF (Jax, #028678) mice were purchased from Jackson Laboratory. NaVI.8cre mice were generously supplied by Professor Rohini Kuner (Heidelberg University) and Professor John Wood (UCL). Excluding CD45.1+ mice, all other lines were backcrossed >6 generations on C57BL/6J background.

We used the cre/lox toolbox to engineer the various mice lines used (TRPV1cre::DTAIRF/WT, TRPV1cre::QuASR2IRF/WT, TRPV1cre::td-tomatoIRF/WT, NaVI.8cre::DTAIRF/WT, NaVI.8cre::ChR2IRF/WT and littermate control) by crossing heterozygote Cre mice with homozygous flox mice. Mice of both sexes were used in the various crosses. All Cre driver lines used were viable and fertile, and abnormal phenotypes were not detected. Offspring were tail-clipped; tissue was used to assess the presence of transgene by standard PCR, as described by Jackson Laboratory or the donating investigator.

**Wild animals**

The study did not involve any wild animals.

**Field-collected samples**

The study did not involve any field-collected samples.

**Ethics oversight**

The Institutional Animal Care and Use Committees of Boston Children's Hospital and the Université de Montréal (CEFA: #21046; #21047) approved all animal procedures.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

### Human research participants

**Policy information about studies involving human research participants**

**Population characteristics**

In compliance with all the relevant ethical regulation and as approved by Sanford Health IRB protocol #640, ten fully de-identified FFPE melanoma blocks were randomly selected for secondary use research specimen. Described below is the clinical characteristics of these specimens:

- **DERM103** patient sample was from a malignant melanoma of the shoulder with evidence of dense lymphohistiocytic inflammatory infiltrate; mitotic index was low, staging was pT1a with negative lymph nodes
- **DERM105** patient sample was from a malignant melanoma of the posterior shoulder with slight to moderate inflammatory infiltrate; mitotic index was low, staging was pT2a with negative lymph nodes
- **DERM106** patient sample was a malignant melanoma of the thigh with negative lymph nodes. Breslow’s thickness 1.2mm and negative lymph nodes. Mitotic index was low, staging pT2a with negative lymph nodes
- **DERM107** patient sample was a malignant melanoma from the upper arm, Breslow’s thickness 1.6mm, Clark level IV, lymph nodes were negative, margins were clear, mitotic index was high, staging pT2a with negative lymph nodes
- **DERM110** patient sample was a malignant melanoma of the arm, Breslow's thickness 0.81 mm, Clark level IV, negative lymph nodes, staging pT1b, pNO, mitotic index high
- **DERM112** patient sample is a metastatic malignant melanoma which metastasized to the neck and liver, mitotic index high, staging pT4b with negative lymph nodes.
- **DERM113** patient sample is a metastatic malignant melanoma which metastasized to the vulva and liver, mitotic index high, staging pT4b with negative lymph nodes.
- **DERM114** patient sample is a metastatic malignant melanoma which metastasized to the shoulder, lung and liver. Mitotic index was high with positive lymph nodes. Staging pT4a.
- **DERM115** patient sample is a metastatic malignant melanoma which metastasized to the back and brain. Mitotic index was low with positive lymph nodes, staging pT2a.
- **HN480** patient sample is a malignant melanoma of the right temple, Breslow's thickness 7.0 mm, Clark level IV, negative lymph nodes, staging pT4a, mitotic index of 10

**Recruitment**

No patients were recruited for this study.

**Ethics oversight**

The ten melanoma samples used in this study were collected by Sanford Health and classified by a board-certified pathologist. Their secondary use as research specimen (fully de-identified FFPE blocks) was approved under Sanford Health IRB protocol #640 (Titled: understanding and improving cancer treatment of solid tumors). As part of this IRB-approved retrospective tissue analysis, and in accordance with US Department of Health and Human Services (DHHS) secretary's advisory committee on human research protections, no patient consent was necessary as these secondary use specimens were free of linkers/identifiers and posed no more than minimal risk to human subjects.

Note that full information on the approval of the study protocol must also be provided in the manuscript.
Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Immunophenotyping tumor and tumor-draining lymph node. Mice were euthanized when the tumor reached a volume of 800-1500 mm³. Tumors and their draining lymph nodes (tDLN) were harvested. Tumors were enzymatically digested in DMEM + 5% FBS (Seradigm, #3100) + 2 mg/ml collagenase D (Sigma, #1308866001) + 1 mg/ml Collagenase IV (Sigma, #C5138-1G) + 40 μg/ml DNase I (Sigma, #1010415001) under constant shaking (40 min, 37°C). The cell suspension was centrifuged at 400 g for 5 min. The pellet was resuspended in 70% Percoll gradient (GE Healthcare), overlaid with 40% Percoll, and centrifuged at 500g for 20 min at room temperature with acceleration and deceleration at 1. The cells were aspirated with the Percoll interface and passed through a 70-μm cell strainer.

Tumor-draining lymph nodes were disseminated in PBS + 5% FBS, mechanically dissociated with a plunger, strained (70μm), and washed with PBS.

Single cells were resuspended in FACS buffer (PBS, 2% FCS, EDTA), Fc blocked (0.5 mg/ml, 15 min; BD Biosciences, #553141) and stained (15 min, RT) with Zombie Aqua (Biolegend, #423102) or (15 min, RT) a Viability Dye eFluor 780 (eBioscience, #65-0865-14). The cells were then stained [30min, 4°C] with either of anti-CD45-BV421 (1:100, Biolegend, #103134), anti-CD45.1-BV421 (1:100, Biolegend, #121672), anti-CD45.2-BV650 (1:100, Biolegend, #109836), anti-CD45-Alexa Fluor 700 (1:100, Biolegend, #105128), anti-CD11b-APC/Cy7 (1:100, Biolegend, #101226), anti-CD8-AF700 (1:100, Biolegend, #100730), anti-CD8-BV421 (1:100, Biolegend, #100753), anti-CD8-PerCP/Cy5.5 (1:100, Biolegend, #100734), anti-CD8-Pacific Blue (1:100, Biolegend, #100725), anti-CD4-PerCP/Cy5.5 (1:100, Biolegend, #100540), anti-CD4-FITC (1:100, Biolegend, #100046), anti-CD3-PE-Cy7 (1:100, Biolegend, #109110), anti-Lag3-PE (1:100, Biolegend, #125208), anti-Lag3-PerCP/Cy5.5 (1:100, Biolegend, #125212), anti-CD3-APC (1:100, Biolegend, #119706), washed and analyzed using an LSRFortessa or FACSComp II (Becton Dickinson). Antibodies specific CD8+ T cells were stained with H-2Kb/OVA257-264 (NIH tetramer core facility) for 15 minutes at 37°C and were then stained with surface markers.

Intracellular cytokine staining. Cells were stimulated (3h) with phorbol-12-myristate 13-acetate (PMA; 50 ng/ml, Sigma-Aldrich, #P0583), ionomycin (1 μg/ml, Sigma-Aldrich, #I3909) and GolgiStop (1:1000, BD Biosciences, #554724). The cells were then fixed/permeabilized (1:100, BD Biosciences, #554714) and stained with anti-IFN-γ-APC (1:100, Biolegend, #505810), anti-IFN-γ-FITC (1:100, Biolegend, #505806), anti-IFN-γ-PE (1:100, Biolegend, #505804), anti-IFN-γ-APC (1:100, Biolegend, #505803), anti-IL-2-BV510 (1:100, Biolegend, #503820), anti-IL-2-BV510 (1:100, Biolegend, #503830), and analyzed using an LSRFortessa or FACSComp II (Becton Dickinson).

B16F10 survival. 1x105 B16F10 cells were cultured in 6-well-plate and challenged with BoNT/A (0.50 pg/μl) for 24h, QX-314 (0-1%) for 72h, B18N4096 (1-4 μM) for 24h or their vehicle. B16F10 cell survival was assessed using anti-annexin V staining and measured by flow cytometry using a LSRFortessa or FACSComp II (Becton Dickinson).

Drugs impact on CD8+ T-cells function. Splenocytes-isolated CD8+ T-cells from naive C57Bl/6 mice were cultured under Tc1-stimulating conditions (ex vivo activated by CD3/CD28, IL-12, and anti-IFN-γ) in 24-well plate for 48h. The cells were then exposed to QX-314 (0-150 μM), BoNT/A (10-50 pg/μl) or B18N4096 (1-4 μM) for 24h. Apoptosis, exhaustion and activation were measured by flow cytometry using a LSRFortessa or FACSComp II (Becton Dickinson).

In vitro cytotoxic CD8+ T-cell stimulation with GCRP. CD8+ T-cells were isolated and stimulated under Tc1 condition in 96-wells plate. After 48h, cells were treated with either GCRP (0.1 μM) or PBS in the presence of peptide inhibitor (1 μM) for another 96h. Expression of PD-1, Lag-3, and Tim-3, as well as IFN-γ, TNF-α, and IL-2, was immunophenotyped by flow cytometry using a LSRFortessa or FACSComp II (Becton Dickinson). Cytokines expression levels were analyzed after in vitro stimulation (PMA/ionomycin; see Intracellular cytokine staining).

In vitro cytotoxic CD8+ T-cell stimulation with neuron-conditioned media. Naive or ablated DRG neurons were cultured (72h) in Neurobasal-A medium supplemented with 0.05 ng/μl NGF (Life Technologies, #13257-019) and 0.002 ng/μl GDNF (PeproTech, #450-51-10). After 48h, the neurobasal medium was removed, neurons were washed with PBS, and 200 μl/well of T-cell media supplemented with 1 μl/ml peptide inhibitor (Sigma, #P1860) and, in certain cases, capsaicin (1 μM) or KCl (50 mM) was added to DRG neurons. The conditioned media or vehicle were collected after 30min and added to Tc1 CD8+ T-cells for another 96h. The CD8+ T-cells expression of exhaustion markers (PD-1, Lag-3, Tim-3) and cytokine (IFN-γ, TNF-α, IL-2) were analyzed by flow cytometry using a LSRFortessa or FACSComp II (Becton Dickinson). Cytokines expressions were analyzed after in vitro stimulation (PMA/ionomycin; see Intracellular cytokine staining).

CD8+ T-cell and DRG neurons co-culture. Naive DRG neurons (2×104) were seeded in a 96-well-plate with T-cell media (supplemented with 0.05 ng/μl NGF (Life Technologies, #13257-019), 0.002 ng/μl GDNF (PeproTech, #450-51-10). One day after, Tc1 CD8+ cells (1×105) were added to the neurons in the presence of IL-2 (Biolegend, #575408). In some instances, cocultures were stimulated with either capsaicin (1 μM) or KCl (50 mM). After 96h, the cells were collected by centrifugation (5
min at 1300 rpm), stained, and immunophenotyped by flow cytometry using a LSRFortessa or FACSCanto II (Becton Dickenson). Cytokines expression were analyzed after in vitro stimulation [PMA/IONOMycin; see Intracellular cytokine staining].

OT1 CD8+ T-cells induced B16F10 elimination. 2x104 naïve TRPV1Cre:QuaASR2-eGFPFl/wt DRG neurons were co-cultured with 1x105 B16F10-mCherry-OVA overnight in T-cell media supplemented with 0.05 ng/μL NGF [Life Technologies, #13257-019], 0.002 ng/μL GDNF [PeproTech, #450-51-10]. One day after, 4x105 stimulated OVA-specific CD8+ T-cells under Tc1 condition were added to the co-culture. After 48h, the cells were detached by trypsin [Gibco, #2062476] and collected by centrifugation (5 min at 1300 rpm), stained with anti-Annexin V, 7-AAD [Biolegend, #640930], and anti-CD8 for 20 minutes at 4°C, and were immunophenotyped by flow cytometry using a FACSCanto II (Becton Dickenson). Cytokines expression were analyzed after in vitro stimulation [PMA/IONOMycin; see Intracellular cytokine staining].

Neuron’s conditioned media impact on OT1 CD8+ T-cells induced B16F10 elimination. 4x105 stimulated OVA-specific CD8+ T-cells were added to 1x105 B16F10-mCherry-OVA and treated with fresh condition media [1:2 dilution]. After 48h, cells were stained using anti-Annexin V, 7-AAD [Biolegend, #640930], and anti-CD8 for 20 minutes at 4°C, and were immunophenotyped by flow cytometry using a LSRFortessa or FACSCanto II (Becton Dickenson). For CCRG, 4x105 stimulated OVA-specific CD8+ T-cells were added to 1x105 B16F10-mCherry-OVA and treated with CCRG (100nM). After 24h the cells were stained using anti-Annexin V, 7-AAD [Biolegend, #640930], and anti-CD8 for 20 minutes at 4°C, and were immunophenotyped by flow cytometry using a LSRFortessa or FACSCanto II (Becton Dickenson). Cytokines expression were analyzed after in vitro stimulation [PMA/IONOMycin; see Intracellular cytokine staining].

Adaptive transfer of RAMP1wt or RAMP1/- CD8 T-cells. Total CD8+ T-cells were isolated from the spleen of wild-type CD45.1+ or RAMP1/- (CD45.2+) mice, expanded and stimulated in vitro using a mouse T-cell Activation/Expansion Kit (Miltenyi cat #130-093-627). CD8+ cells from RAMP1/- and RAMP1wt were injected separately or 1:1 mix through tail vein of Rag1/- mice. One week after, the mice were inoculated with B16F10-mCherry-OVA cancer cells [5x105 cells; i.d.). On day 10, tumors were harvested and RAMP1/- (CD45.2+) and RAMP1wt (CD45.1+) CD8+ T-cells were immunophenotyped using a FACSCanto II (Becton Dickenson) or FACs purifying using a FACs Aria Iliu cell sorter (Becton Dickenson).

Adaptive T-cell transfer in RTX-exposed mice. CD8+ T-cells were isolated from OT-1 mice spleens and magnet-sorted (StemCell; #19858). Naïve CD8+ T-cells (CD8+CD44lowCD62Lhi) cells were then FACs-purified using a FACs Aria Iliu cell sorter (Becton Dickenson) and injected (1x106 cells; i.v. tail vein) to vehicle- or RTX-exposed RAG1/- mice.

RNA sequencing of triple co-culture and data processing. 1x104 naïve TRPV1Cre:QuaASR2/eGFPFl/wt DRG neurons were co-cultured with 1x105 B16F10-mCherry-OVA overnight in T-cell media supplemented with 0.05 ng/μL NGF [Life Technologies, #13257-019], 0.002 ng/μL GDNF [PeproTech, #450-51-10]. One day after, 4x105 stimulated OVA-specific CD8+ T-cells under Tc1 condition were added to the co-culture. After 48h, the cells were detached and TRPV1 neurons (CD45-eGFP-mCherry), B16F10-mCherry-OVA (CD45-eGFP-mCherry), and OVA-specific CD8+ T-cells (eGFP-mCherry; CD45+ CD3+ CD8+) were FACS-purified using a FACs Aria Iliu cell sorter (Becton Dickenson) prior to sequencing.

RNA sequencing of cancer and neurons co-culture and data processing. 1x104 naïve TRPV1Cre:QuaASR2-eGFPFl/wt DRG neurons were co-cultured with 5x104 B16F10-mCherry-OVA overnight in complete Dulbecco’s Modified Eagle’s Medium high glucose [DMEM, Corning, #10-013-CV] supplemented with 10% fetal bovine serum FBS, Seradigm, #31001, 1% penicillin/ streptomycin [Corning, #MT-3001-C], 0.05 ng/μL NGF [Life Technologies, #13257-019], 0.002 ng/μL GDNF [PeproTech, #450-51-10]. After 48h, the cells were detached and TRPV1 neurons (eGFP-mCherry); and B16F10-mCherry-OVA (eGFP-mCherry) were FACS-purified using a FACs Aria Iliu cell sorter (Becton Dickenson) prior to sequencing.

**Instrument**

Cells were immunophenotyped using a LSRFortessa (Becton Dickenson) or FACSCanto II (Becton Dickenson).

**Software**

Data were analyzed using FlowJo v10.0.0 software (Tree Star).

**Cell population abundance**

For sorting experiments (i.e. CD8, neurons or B16F10 cells), flow cytometry was used and >96% purity was achieved.

**Gating strategy**

Relevant gating strategy are provided as extended data figure 6A, 9F, 10W.

To gate samples for FACS analysis, cell were initially gated by FSC-A vs SSC for the exclusion of debris and identification of relevant population (lymphocytes) by size and granularity. For single cells, samples were further gated by FSC-A vs FSC-H. Live cells were finally gated and identified by using fixable dye or fluorescent reporters.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.