

Nerve-to-cancer transfer of mitochondria during cancer metastasis

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The nervous system has a pivotal role in cancer biology, and pathological investigations have linked intratumoural nerve density to metastasis¹. However, the precise impact of cancer-associated neurons and the communication channels at the nerve–cancer interface remain poorly understood. Previous cancer denervation models in rodents and humans have highlighted robust cancer dependency on nerves, but the underlying mechanisms that drive nerve-mediated cancer aggressivity remain unknown^{2,3}. Here we show that cancer-associated neurons enhance cancer metabolic plasticity by transferring mitochondria to cancer cells. Breast cancer denervation and nerve–cancer coculture models confirmed that neurons significantly improve tumour energetics. Neurons cocultured with cancer cells undergo metabolic reprogramming, resulting in increased mitochondrial mass and subsequent transfer of mitochondria to adjacent cancer cells. To precisely track the fate of recipient cells, we developed MitoTRACER, a reporter of cell-to-cell mitochondrial transfer that permanently labels recipient cancer cells and their progeny. Lineage tracing and fate mapping of cancer cells acquiring neuronal mitochondria in primary tumours revealed their selective enrichment at metastatic sites following dissemination. Collectively, our data highlight the enhanced metastatic capabilities of cancer cells that receive mitochondria from neurons in primary tumours, shedding new light on how the nervous system supports cancer metabolism and metastatic dissemination.

Cancer plasticity has a pivotal role in cancer heterogeneity and the emergence of adaptive phenotypes⁴. Although cancer cells often rely on glycolysis for energy, numerous studies indicate that they adapt their metabolism, including oxidative phosphorylation (OXPHOS), to changing environments. The metabolic plasticity of cancer cells is becoming increasingly recognized as essential for cancer progression by enabling cancer cells to efficiently navigate through the metastatic cascade^{5,6}. Studies on cancer metabolic plasticity have primarily focused on cell-autonomous mechanisms, in which cells modify their metabolic program and perform metabolic rewiring by regulating metabolic enzymes and adapting alternative substrates. The non-autonomous mechanisms of metabolic plasticity are less well understood and can involve complex interactions within the cancer microenvironment⁷. For instance, stromal cells, including fibroblasts and immune cells, can share a variety of metabolites, growth factors and cytokines, contributing to the metabolic reprogramming of cancer⁸. Overall, non-cell-autonomous mechanisms of the cancer cells' metabolic plasticity are still unclear and may represent promising targets for preventing metastatic dissemination.

Many cancers are innervated, and in recent years, cancer neurobiology has garnered considerable attention¹. Studies scrutinizing the impact of the nervous system in various cancers have established a critical role of cancer-infiltrating neurons in controlling cancer aggressivity, including supporting cancer cell metabolism through direct metabolic interactions^{1,9}. Pathological analyses in patients with cancer consistently associated cancer innervation with negative clinical outcomes, and targeted ablation of intratumoural nerves can suppress cancer growth in a tissue-specific manner^{1,10–12}. The neuronal component of the cancer microenvironment can arise from the recruitment of pre-existing nerves through perineural invasion or the de novo generation of nerves within the cancer stroma through cancer neurogenesis^{13,14}. Nerve withdrawal in human prostate cancer demonstrated impaired tumour growth and metabolism, and metabolomic analysis of denervated cancers across rodent and human species revealed disrupted metabolic efficiency that is characterized by their downregulated mitochondrial metabolism and transition of the cancer cells into a more glycolysis-dependent production of energy². Overall, converging evidence indicates that denervated cancer actively suffers from nerve withdrawal, with a predominant effect on their energetic metabolism^{1,15}.

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These studies demonstrated the cancer metabolic dependency on nerves and suggested the existence of metabolic support mechanisms acting at the nerve–cancer interface. However, their nature remains unknown, and characterizing these mechanisms, which represent a critical gap in the understanding of the metabolic support provided by nerves to cancer cells, is the focus of this study.

Here we demonstrate the metabolic dependency of breast cancer cells on nerves. Using orthogonal *in vitro* and *in vivo* methods, we observed the prevalence of transfers of mitochondria between neurons and cancer cells. To trace the fate of the recipient cells, we developed MitoTRACER, a genetic reporter that permanently marks cancer cells receiving mitochondria from donor cells. Our approach revealed that neuron-derived mitochondria enhance cancer cell metabolic capacity, stemness and resistance to metastatic stressors. Fate mapping of the recipient cells *in vivo* has shown their increased metastatic capacities. In the human context, multispectral imaging with machine-learning deconvolution showed increased mitochondrial mass in metastatic cells, and perineural invasion was associated with higher mitochondrial content in cancer cells near nerves. Finally, cancer chemically denervated with botulinum neurotoxin type A (BoNT/A) confirmed reduced mitochondrial load in cancer cells. These findings highlight nerve-driven metabolic support as essential to cancer metabolic plasticity and metastatic potential.

Nerve withdrawal impairs tumour bioenergetics

Previous studies on the effects of BoNT/A-mediated prostate denervation conducted in humans and rats uncovered the cancer dependency on nerves and the metabolic reprogramming of prostate cancer cells after nerve withdrawal². Similarly, breast cancer denervation resulted in a marked decrease in cancer growth¹⁵. However, the impact of cancer-infiltrating nerves in breast cancer metabolism has not been explored. We investigated the consequences of nerve withdrawal in breast cancer using two complementary mouse models. We first adapted a BoNT/A-mediated denervation protocol to the 4T1 model of triple-negative breast cancer (TNBC; Fig. 1a,b) and to a human ductal carcinoma *in situ* xenograft model¹⁶ (Extended Data Fig. 1a–d). In both settings, transcriptomic profiling of cancer cells from control and denervated tumours revealed distinct expression signatures following denervation (Fig. 1b and Extended Data Fig. 1a,b), and Gene Ontology analyses have underscored the prevalent downregulation of metabolic processes (Extended Data Fig. 1b–d and Supplementary Tables 1–3). Gene set enrichment analysis of the ductal carcinoma *in situ* model identified the tricarboxylic acid cycle as the most suppressed pathway overall (Extended Data Fig. 1c and Supplementary Table 2). Histopathological examination of this model has shown a reduction in the incidence of invasive lesions from 55% in control mice to 12% in denervated mice (Extended Data Fig. 1e), underscoring the functional importance of innervation for breast cancer progression.

Cancer boosts neuronal mitochondria

To explore in more detail the mechanisms associated with cancer metabolic dependencies on nerves in breast cancer progression, we developed an *in vitro* nerve–cancer coculture. We mixed the 4T1 aggressive mouse breast carcinoma cells with neuronal stem cells (NSCs) from the mouse subventricular zone (SVZ) (Extended Data Fig. 2a–d), as they have been proved to be a source of cancer innervation¹⁴. After stimulation by 4T1 cancer cells, SVZ-NSCs rapidly undergo differentiation, evidenced by their morphological transition from round, neural progenitor cells to more elongated cells with neurite extensions and forming close contact with the cancer cell (Fig. 1c and Extended Data Fig. 2e). SVZ-NSCs can differentiate into neurons, astrocytes or oligodendrocyte glial cells. Using our coculture model, we confirmed the neuronal commitment of NSCs, as shown by microscopy through the

expression of the neuronal marker tubulin $\beta 3$ (TUBB3) (Extended Data Fig. 2f). Absolute quantification by flow cytometry showed that more than 90% of progenitor cells differentiated into TUBB3⁺ and MAP2⁺ neurons but remained negative for the glial cell markers O4 and ALDH1L1 (Extended Data Fig. 2g–i). SVZ-NSCs also exhibited neuronal functional characteristics, including calcium pulsatile activity (Extended Data Fig. 2j and Supplementary Video 2) and the ability to generate action potentials in response to depolarizing current injection, with an action potential threshold averaging $-46 \text{ mV} \pm 5 \text{ mV}$ as observed through whole-cell electrophysiology recordings (Extended Data Fig. 2k,l).

After establishing the biological relevance of the nerve–cancer coculture *in vitro*, we next confirmed that the cancer's metabolic dependency on nerves, observed in mouse xenografts, could also be replicated in a more simplistic *in vitro* model. Cancer cells in monoculture or cultivated in the presence of neurons were isolated before their metabolic analysis. In comparison to those cultivated alone, the cancer cells isolated from the nerve–cancer coculture exhibited upregulated mitochondrial respiration (Fig. 1d), characterized by significant increases in their basal and maximal mitochondrial respiration as well as enhanced spare respiratory capacities (Extended Data Fig. 3a). This reflects the establishment of the nerve–cancer metabolic dependencies in the coculture model *in vitro*.

As previously described, cancer-induced neuronal progenitor differentiation is integral to the nerve–cancer cross-talk established during cancer innervation¹. For instance, we and others have previously demonstrated how the cancer-driven expression of axon guidance molecules such as semaphorin 4F controls the cancer-induced neuronal precursor differentiation into the cancer stroma and increases intratumoural nerve density and cancer aggressivity^{17–19}. Physiologically, differentiating neuronal progenitors must undergo a metabolic shift to increase their mitochondrial metabolism and meet their specific energetic demands during physiological differentiation²⁰ (Extended Data Fig. 3b,c). We examined whether similar reprogramming also occurs during cancer-driven neuronal differentiation and confirmed a robust increase in the mitochondrial mass of SVZ-NSCs exposed to 4T1 cancer cells (Extended Data Fig. 3d), with absolute quantification of mitochondrial DNA (mtDNA) showing an increasing mtDNA load from about 16 to 226 mtDNA/nuclear DNA copies per neuron after their cancer-induced differentiation (Extended Data Fig. 3e). Finally, genetic fluorescent labelling of SVZ-NSC mitochondria confirmed morphological changes of the SVZ-NSCs exposed to the cancer cells and the development of an extended mitochondrial network across the nerve–cancer coculture, transitioning from globular structures in NSC monoculture to thin and elongated tubular structures extending throughout the NSCs in coculture with breast cancer cells (Extended Data Fig. 3f). Such changes represent typical hallmarks of metabolic reprogramming, in which maturing neurons transition from glycolytic to mitochondrial oxidative metabolism^{20,21}.

Neuron-to-cancer mitochondria transfer

The establishment of close nerve–cancer cross-talk in our *in vitro* cocultures, the significant increase in neuronal mitochondria abundance and the enhancement of mitochondrial metabolism in isolated cancer cells following exposure to neuronal cells in coculture suggested that metabolism-related collaboration mechanisms initiated at the nerve–cancer interface. The cell–cell transfer of mitochondria has recently become a subject of investigation as it has crucial functions in health and disease^{22–24}, and was recently shown to have profound effects on cancer progression^{25,26}. Moreover, research has demonstrated that astrocytes can provide metabolic support to glioblastoma cells by mitochondria transfer in the central nervous system²⁷.

We therefore investigated whether mitochondrial transfer could occur within the peripheral nervous system and assessed whether breast cancer cells exposed to neurons could acquire neuron-derived

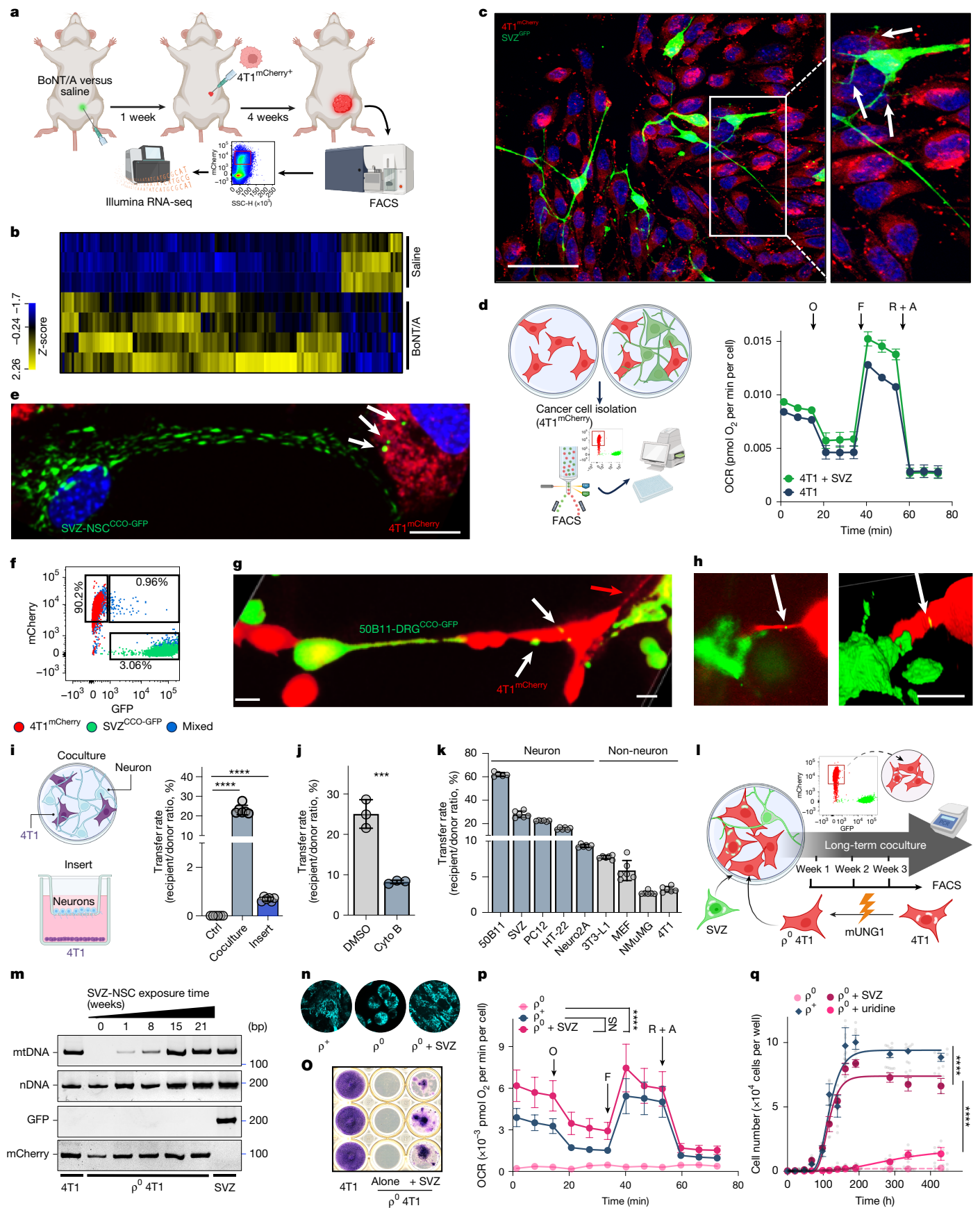


Fig. 1 | See next page for caption.

Fig. 1 | Cancer metabolic dependency on nerves and intercellular transfer of functional mitochondria at the nerve–cancer interface. **a, b**, Pre-denervation breast cancer model using BoNT/A injections in BALB/c mice followed by implantation of 4T1^{mCherry} cells. Transcriptomic analysis of 4T1^{mCherry} cells revealed a distinct transcriptomic signature and downregulation of metabolic processes. SSC-H, side scatter height; RNA-seq, RNA sequencing. **c**, Confocal micrograph of SVZ-NSCs^{GFP} mixed with 4T1^{mCherry} cells. White arrows show the establishment of neuron–cancer contacts. Scale bar, 50 μ m. **d**, 4T1 cells FACS-isolated from coculture (left) showed increased OXPHOS capacities (Seahorse assay; mean $\pm$ s.e.m.; representative profile ($n = 3$); right). O, oligomycin; F, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; R + A, rotenone and antimycin A; OCR, oxygen consumption rate. **e, f**, Time-lapse confocal microscopy (**e**) and flow cytometry (**f**) highlighted mitochondrial transfer from SVZ-NSCs^{CCO-GFP} to 4T1^{mCherry} cells ($n = 6$ independent cocultures). Scale bar, 10 μ m. The white arrows show transferred mitochondria. **g, h**, 3D reconstruction shows transfer through tunnelling nanotubes between peripheral nervous system-derived 50B11-DRG^{CCO-GFP} cells and 4T1^{mCherry} cells. Red arrow shows the tunnelling nanotube structure, and white arrows show transferred mitochondria. Scale bars, 20 μ m. **i**, Quantification of direct cell–cell contact (23.04%) versus distant (0.59%) transfers using Transwell inserts. Normalized transfer rate is calculated as the percentage of 4T1^{mCherry}+/GFP+ cells among the eGFP+ cells in the coculture. Mean $\pm$ s.d., Student's two-tailed unpaired *t*-test; *****P* < 0.0001

mitochondria. To test this hypothesis, we established nerve–cancer cocultures by combining central nervous system-derived neurons (SVZ-NSCs) or dorsal root ganglia-derived neurons (50B11-DRG) with 4T1 breast cancer cells expressing the mCherry fluorophore (4T1^{mCherry}). Neurons were genetically modified to express enhanced green fluorescent protein (eGFP)-labelled mitochondria (SVZ-NSC^{CCO-GFP} and 50B11-DRG^{CCO-GFP}) (Fig. 1e–h and Supplementary Video 1). Confocal microscopy and flow cytometry analysis of the coculture confirmed mitochondrial transfer from neurons to cancer cells (Fig. 1e–h and Extended Data Fig. 4a) and the formation of tunnelling nanotube-like structures, facilitating organelle transfer (Fig. 1g, red arrow), as confirmed by three-dimensional (3D) reconstruction (Fig. 1h, white arrows). Flow cytometry (Fig. 1f and Extended Data Fig. 4a) showed acquisition of a double-positive 4T1^{GFP+/mCherry+} subpopulation of cells in the cocultures that reflects the acquisition of eGFP-labelled mitochondria into the recipient 4T1^{mCherry} cells. Although this population accounts for an average of 0.96% of the total cells in the coculture (Fig. 1f), it represents only a snapshot of the current mitochondria transfers, and the SVZ-NSCs providing the mitochondria represent, on average, only 3.06% of the coculture. When normalized to the population of eGFP-labelled cells in the coculture, the double-positive (4T1^{mCherry}+/GFP+) fraction accounted for an equivalent of 31.4% of the donor population. This normalization method was used to standardize the transfer rate from donor to recipient cells for different experimental conditions. Beyond the direct cell–cell contact-mediated transfer of mitochondria, we investigated whether distant mechanisms, such as microvesicles, could be involved. Distant coculture using Transwell inserts confirmed that cell–cell contact is the primary route of mitochondrial transfer, although distant transfers also occurred (Fig. 1i). Inhibition of tunnelling nanotube formation further validated the role of these structures in the transfer process without a significant impact on cell viability (Fig. 1j and Extended Data Fig. 4b), as previously shown²⁷. Using different cell lines, we next tested the capacities of cells with diverse origins (50B11-DRG neurons, SVZ neurons, PC12 pheochromocytoma, HT-22 hippocampal, Neuro2A neuroblastoma, 3T3-L1 pre-adipocytes, mouse embryonic fibroblasts, NMuMG normal mouse mammary gland and 4T1 breast cancer cells) in transferring their mitochondria to cancer cells (Fig. 1k). Every tested donor significantly transferred mitochondria to the recipient 4T1 cells. Notably, cell lines of neuronal origin exhibited higher mitochondrial transfer rates. In addition, we confirmed mitochondria transfer in nerve–cancer cocultures using human cancer cell lines (Extended Data Fig. 4c).

($n = 6$ independent cocultures). Ctrl, control. j, Cytochalasin B (Cyto B) reduces mitochondrial transfer. DMSO, dimethylsulfoxide. Mean $\pm$ s.d., Student's two-tailed unpaired *t*-test, ****P* = 0.001 ($n = 3$ independent cocultures). **k**, Mitochondrial transfer rates vary with donor cells from different origins. MEF, mouse embryonic fibroblast. Mean $\pm$ s.d. ($n = 5$ independent cocultures). **l**, 4T1^{mCherry} cells rendered devoid of mtDNA (ρ^0) were cocultured with ρ^+ SVZ-NSCs^{GFP} and isolated by FACS at various times to monitor the transfers. mUNG1, Y147A mutant of uracil-*N*-glycosylase. **m**, PCR of mtDNA content in FACS-sorted ρ^0 4T1^{mCherry} cells showed progressive reacquisition of mtDNA. Expression of GFP and mCherry confirmed the purity of the 4T1 cells. Nuclear DNA (nDNA) was used as the loading control. **n**, MitoTracker microscopy imaging of ρ^+ 4T1, ρ^0 4T1 and ρ^0 4T1 cells FACS-sorted from coculture shows rescue in their mitochondrial morphology. **o**, Reacquisition of SVZ-derived mtDNA in ρ^0 4T1 cells restores uridine-independent growth. **p, q**, FACS-isolated ρ^0 4T1 cells rescued by mitochondrial transfer regained OXPHOS (**p**; Seahorse assay, mean $\pm$ s.e.m.; representative profile ($n = 3$)) and proliferative capacities (**q**; direct cell counting, mean $\pm$ s.d., $n = 6$ independent cultures); two-way analysis of variance (ANOVA), *****P* < 0.0001; NS, not significant. **a**, Created in BioRender. S. Grelet (2025) <https://biorender.com/0j8zovf>. **d**, Created in BioRender. S. Grelet (2025) <https://biorender.com/oxxilq>. **i**, Created in BioRender. S. Grelet (2025) <https://biorender.com/8tdz09x>. **l**, Created in BioRender. S. Grelet (2025) <https://biorender.com/pm5yh64>.

Transferred mitochondria are functional

We subsequently validated the transfer of neuronal mitochondria to cancer cells through an additional orthogonal validation approach. We generated rho-zero (ρ^0) 4T1 cancer cells lacking mtDNA and cocultured them with rho-plus (ρ^+) SVZ-NSCs²⁸ (Fig. 1l). PCR amplification of mtDNA from the ρ^0 4T1^{mCherry} cells confirmed a complete loss of mtDNA and revealed their gradual reacquisition of mtDNA through coculture with SVZ-NSCs (Fig. 1m and Extended Data Fig. 4d). The mitochondrial morphology was altered in ρ^0 cells, with the mitochondria exhibiting a globular and fragmented appearance as described previously²⁹. This morphology was restored following the mtDNA restoration through nerve–cancer coculture (Fig. 1n). ρ^0 cells are also phenotypically characterized by the complete loss of OXPHOS capacities and auxotrophy for uridine^{30,31}. Therefore, ρ^0 cells require uridine complementation to grow in culture in vitro. We tested whether transferred mitochondria are functional in recipient cells. We grew ρ^0 4T1 cells in the presence of uridine either alone or in coculture with ρ^+ SVZ-NSCs. After 5 days of coculture, 4T1^{mCherry} cells were sorted by fluorescence-activated cell sorting (FACS) and subcultured without uridine. In the absence of uridine, ρ^0 4T1 cells did not form colonies, but a subset of the ρ^0 4T1 cells enriched from coculture with ρ^+ neurons formed viable colonies (Fig. 1o), reflecting rescue of uridine synthesis, therefore demonstrating the functionality of the transferred mitochondria. The SVZ-NSC-derived mitochondria transferred to the ρ^0 4T1 cells also rescued their mitochondrial respiration and proliferative capacities as observed by Seahorse extracellular flux metabolic analysis (Fig. 1p, ρ^0 + SVZ) and proliferation assays, respectively (Fig. 1q, ρ^0 + SVZ).

Transfers are prevalent in vivo

We next tested the occurrence of nerve–cancer transfers of mitochondria in vivo. Clinically, human prostate cancer samples showed an increased mitochondrial load in cancer cells associated with perineural invasion (Fig. 2a). High-throughput multispectral imaging quantification based on machine learning³² highlighted that cancer cells closer to nerves had a significantly higher mitochondrial load than cells farther from nerves (Fig. 2b and Supplementary Tables 4 and 5). To assess whether this increased mitochondrial load may result from mitochondrial transfer, we analysed prostate tissue from a clinical trial (NCT01520441), in which human prostate cancer was chemically denervated. Multispectral imaging revealed that cancer cells of the

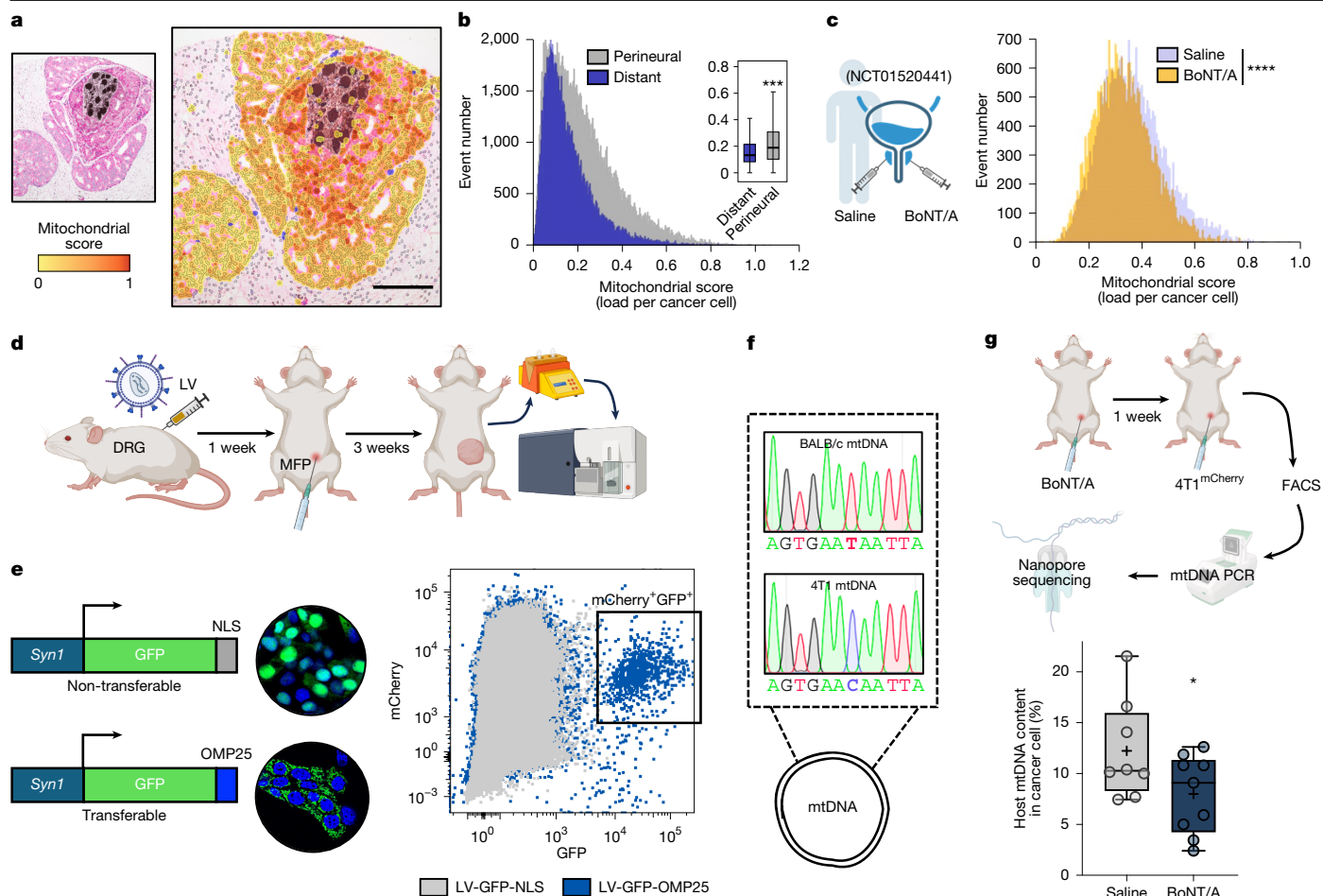


Fig. 2 | Inter cellular transfer of mitochondria between host neuron and cancer. **a**, Histopathology of human prostate cancer with perineural invasion shows increased mitochondrial content near nerves (mitochondria are visualized by periodic acid–Schiff staining (magenta); nerves are visualized by diaminobenzidine staining (brown)). Representative profile ($n = 72$ patients). Scale bar, 180 μm . **b**, Multispectral imaging combined with machine learning-based image deconvolution, spatial correlation, and quantification indicate significantly higher mitochondrial loads in prostate cancer cells near nerves (perineural; $n = 72$, 40,007 cells) compared to distant cancer cells (distant; $n = 58$, 20,766 cells). The line shows the median, the box boundaries show the 25th and 75th percentiles, and the whiskers show the minimum and maximum values. Two-sided Welch's t -test, $***P < 0.001$. **c**, BoNT/A-mediated denervation reduces mitochondrial load in human prostate cancer cells (paired analysis: saline versus BoNT/A; saline: $n = 10,918$ cells, BoNT/A: $n = 14,186$ cells). Two-sided Welch's t -test, $****P = 1.463 \times 10^{-113}$. Clinical trial (NCT01520441). **d**, Mouse DRG neurons innervating the mammary gland were labelled with lentivirus (LV) to tag neuronal mitochondria before injection of 4T1^{mCherry} cells into mammary fat pads (MFPs). Cancer cells were isolated post-tumour growth for mitochondrial

transfer analysis to detect host-derived mitochondrial transfer. **e**, Neuronal mitochondria were labelled using lentiviruses expressing either nuclear-localized (GFP-NLS, non-transferable) or mitochondria-localized (GFP-OMP25, transferable) eGFP under the synaptin1 (*Syn1*) promoter. Flow cytometry of cancer cells identified eGFP⁺ subpopulations, indicating neuronal mitochondrial transfer between mouse host neurons and cancer xenografts. **f**, Sanger sequencing enabled the detection of mtDNA polymorphisms between host BALB/c cell and 4T1 cancer cell mtDNA. **g**, Oxford Nanopore sequencing analysis of mtDNA heteroplasmy in FACS-isolated cancer cells demonstrated host-to-cancer mitochondrial transfer. BoNT/A-mediated pre-denervation at the xenograft site significantly reduced mitochondrial transfer to cancer cells (saline $n = 8$, BoNT/A $n = 9$; median values indicated). The line shows the median, the box boundaries show the 25th and 75th percentiles, and the whiskers show the minimum and maximum values. One-tailed unpaired Student's t -test $*P = 0.0316$ ($n = 8$ saline, $n = 9$ BoNT/A mice). **c**, Created in BioRender. S. Grelet (2025) <https://biorender.com/mpdr95w>. **d**, Created in BioRender. S. Grelet (2025) <https://biorender.com/98cu18d>. **g**, Created in BioRender. S. Grelet (2025) <https://biorender.com/xapzg43>.

BoNT/A-denervated side of the prostate had a lower mitochondrial load than the saline-injected side, supporting the hypothesis that nerves promote mitochondrial load in cancer cells, with mitochondrial transfer as a contributing factor (Fig. 2c and Supplementary Tables 6 and 7).

Using a BALB/c mouse xenograft model, we evaluated mitochondrial transfer from mouse host neurons to 4T1 breast cancer cells in vivo (Fig. 2d). We designed a lentiviral construct encoding a neuron-specific, *Syn1*-GFP-OMP25, mitochondria-anchored eGFP reporter to label the mitochondria of host mammary fat pad neurons (Fig. 2e and Extended Data Fig. 5a–c). After genetic modification of the mouse DRG innervating the lower mammary fat pads, 4T1^{mCherry} cancer cells were injected into the corresponding fat pads, and flow

cytometry analysis of the primary cancer showed a subpopulation of cancer cells exhibiting the green signal of eGFP, consistent with mitochondrial transfer from mouse neurons to cancer cells in situ (Fig. 2e). We developed a similar lentiviral construct as a control, in which eGFP was targeted to the nucleus (GFP-NLS); this construct was non-transferable and showed no signal transfer between mouse nerves and 4T1^{mCherry} cells (Fig. 2e).

We further assessed mitochondrial transfer by analysing mtDNA heteroplasmy arising from a mtDNA polymorphism identified between the mouse host and cancer cells, as identified by Sanger sequencing (Fig. 2f), with this analysis serving as an additional independent approach. 4T1^{mCherry} cells from cancer xenografts were FACS-sorted

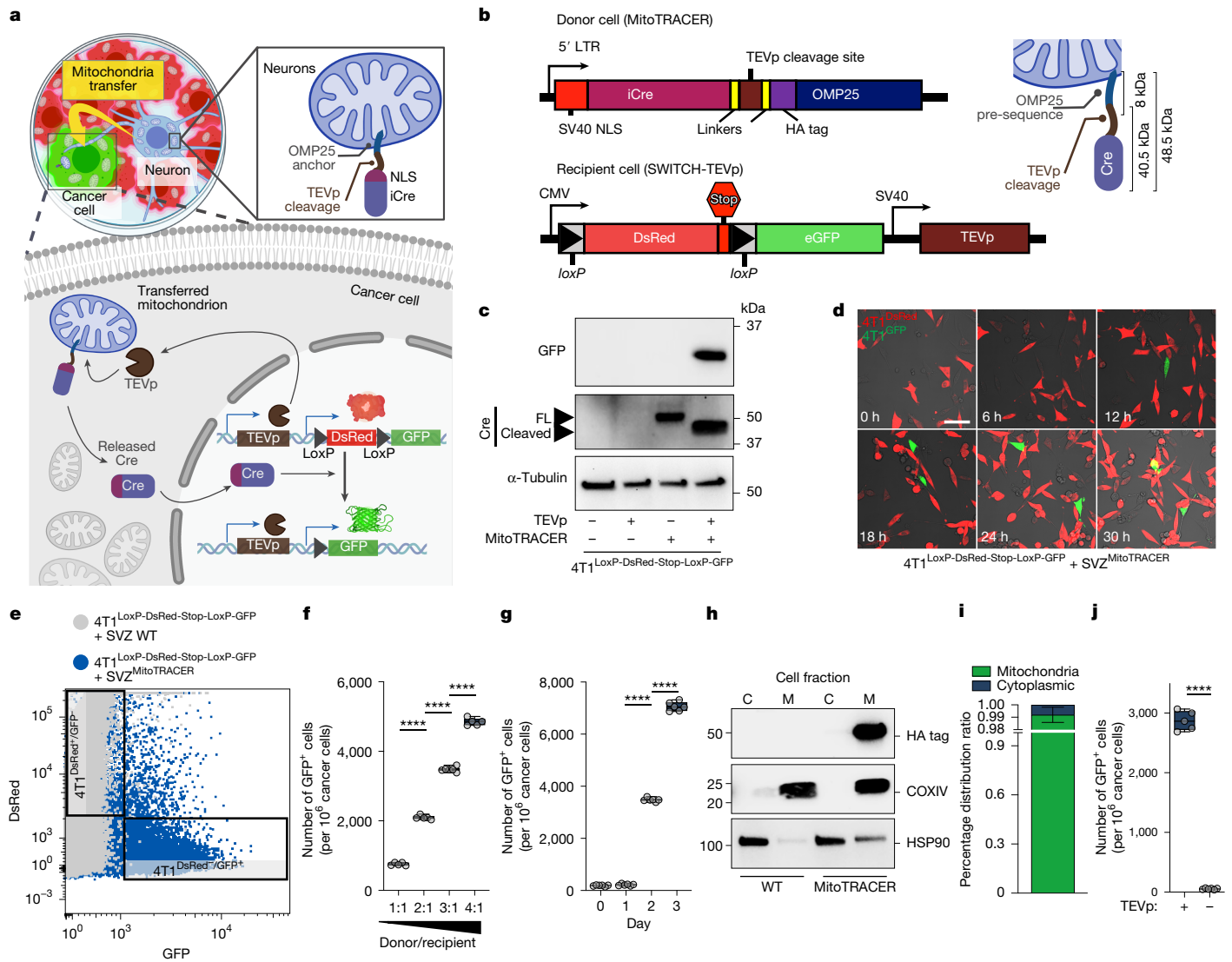


Fig. 3 | MitoTRACER for lineage tracing of cell–cell transfer of mitochondria. **a, b**, MitoTRACER strategy. Donor neurons express mitochondria-targeted Cre recombinase (iCre) with an SV40 nuclear localization signal (NLS-iCre), linked to the OMP25 mitochondrial outer membrane domain. Recipient cells express both a loxP-DsRed-Express2-Stop-loxP-eGFP switch and the TEVp. After transfer, TEVp cleaves NLS-iCre, enabling nuclear localization and excision of DsRed-Express2, resulting in a permanent change from DsRed (red) to eGFP (green) expression. LTR, long terminal repeat. **c**, 4T1^{loxP-DsRed-Express2-Stop-loxP-eGFP} co-expressing both MitoTRACER and TEVp shows efficient NLS-iCre cleavage and eGFP expression activation. No unintended cleavage was detected in the absence of TEVp expression. The same sample extracts were loaded in different gels. Representative experiment ($n = 3$). FL, full length. **d, e**, Confocal microscopy (**d**) and flow cytometry (**e**) of SVZ neuron–4T1 coculture confirmed red-to-green conversion, confirming the transfers and suitability of the approach for high-throughput analysis and collection of recipient cells. WT, wild type. Scale bar, 50 μm . **f**, Dose-dependent increase of mitochondrial transfer with donor-to-recipient ratios (1:1 to 4:1). The centre line shows the median, the box boundaries show the 25th and 75th percentiles, and the whiskers show the minimum and

maximum values. Student's two-tailed unpaired t -test, **** $P < 0.0001$ ($n = 5$ independent cocultures). **g**, Time-dependent and cumulative increase of mitochondrial transfer from day 1 to day 3. The centre line shows the median, the box boundaries show the 25th and 75th percentiles, and the whiskers show the minimum and maximum values. Student's two-tailed unpaired t -test, **** $P < 0.0001$ ($n = 6$ independent cocultures). **h, i**, Western blot (**h**) and densitometry analysis (**i**) of the MitoTRACER subcellular localization through the HA tag expression on the construct confirmed its mitochondrial localization. Expression of COXIV and HSP90 validated the subcellular fraction purity. The same sample extracts were loaded in different gels. Representative experiment ($n = 3$) (mean $\pm$ s.d.; $n = 4$ independent experiments). C, cytoplasmic fraction; M, mitochondrial fraction. **j**, Coculture using recipient cells lacking TEVp confirmed the signal's specificity. The line shows the median, the box boundaries show the 25th–75th percentiles, and the whiskers show the minimum and maximum values. Student's two-tailed unpaired t -test, $P = 2.16463 \times 10^{-10}$, **** $P < 0.0001$ ($n = 5$ independent cocultures). **a**, Created in BioRender. S. Grelet (2025) <https://biorender.com/aa3gxf0>. **b**, Created in BioRender. S. Grelet (2025) <https://biorender.com/ytn18rx>.

and analysed for their mtDNA content through Nanopore sequencing, which confirmed the overall acquisition of mouse-derived mtDNA by cancer cells (Fig. 2g). The neuronal origin of these transfers was further validated through BoNT/A-mediated chemical denervation, which demonstrated that neurons accounted for approximately 35% of the total mitochondrial transfers between host neurons and cancer cells (Fig. 2g).

Permanent labelling of recipient cells

As the previous results established the biological relevance of nerve-to-cancer mitochondrial transfer in vitro and in vivo, and suggested its clinical relevance, it was important to investigate the effects of mitochondrial transfer on cancer cell biology. However, the p0 4T1 cell model (Fig. 1l–q) was limited in biological relevance for studying the

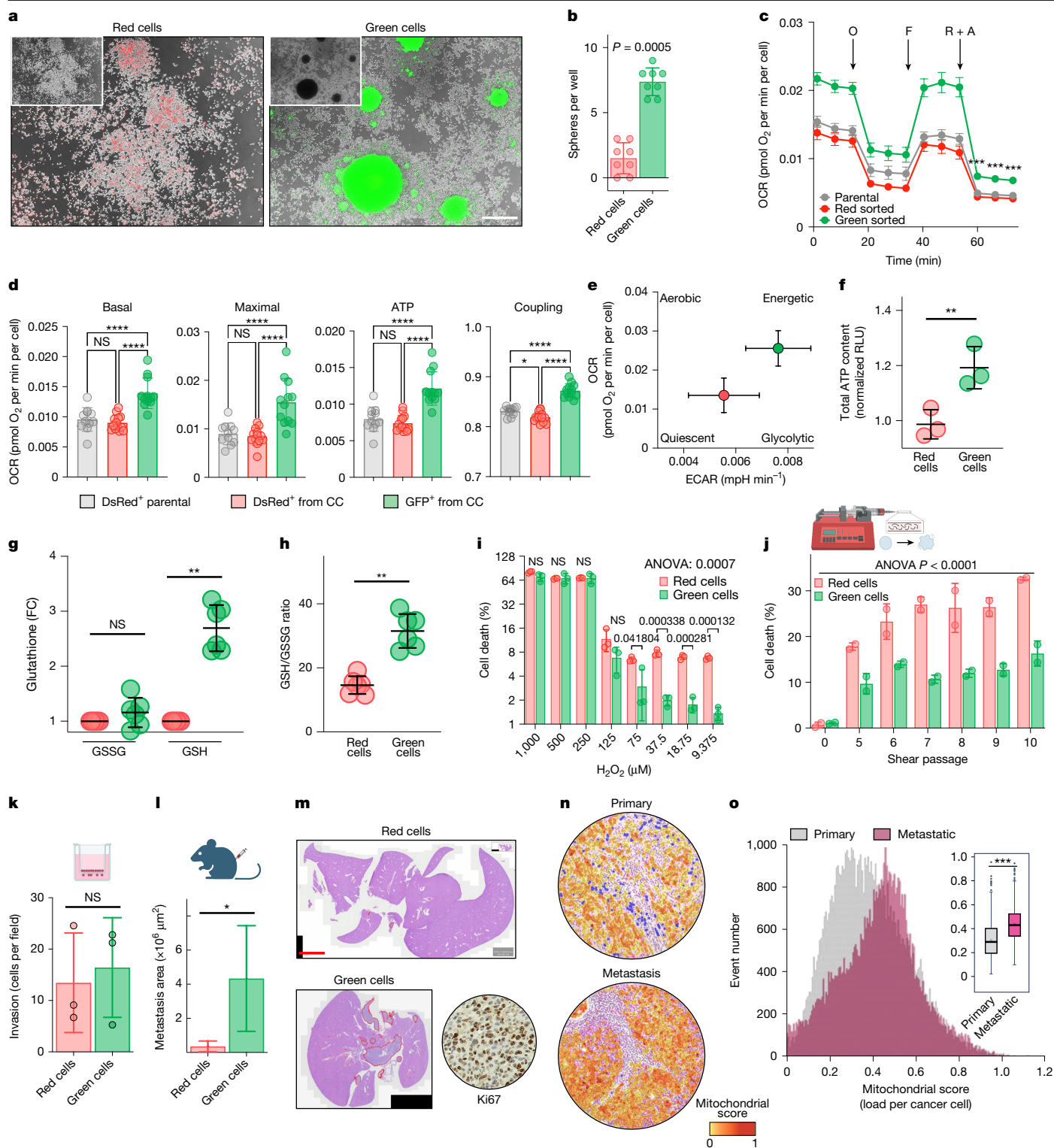


Fig. 4 | See next page for caption.

functional consequences of mitochondrial transfer in recipient cells. In addition, the mitochondrial-bound methods used in vitro (Fig. 1e–k) and in vivo (Fig. 2d,e) were limited to mitochondrial transfer at the time of analysis and could not evaluate previous transfers. Furthermore, after an eGFP-labelled mitochondrion entered a recipient cell, the eGFP signal faded quickly because the recipient cells did not express the mitochondrial genetic reporter. Therefore, these methods do not allow for distinguishing between cells in the same culture that received mitochondria versus those that did not, nor do they enable lineage tracing of recipient

cells and their progeny. To address this limitation, we designed a new genetic reporter strategy known as MitoTRACER to permanently label the recipient cancer cells after mitochondrial transfer from neurons and distinguish them from cells that did not receive mitochondria.

In the MitoTRACER method, the recipient cells constitutively express a red (DsRed-Express2) fluorophore (hereafter referred to as red cells) until they receive mitochondria from the donor cells, which triggers the removal of the red fluorescence expression and activates the permanent expression of the eGFP green fluorophore

Fig. 4 | Neuron-to-cancer mitochondrial transfer enhances cancer OXPHOS, stemness and resistance to metastatic stressors. **a**, 4T1 recipient (green) cells sorted after MitoTRACER coculture showed spontaneous sphere formation capacities. Scale bar, 500 μ m. **b**, Mammosphere formation assay confirmed increased stemness potential in green cells ($***P = 0.0005$, $n = 8$ independent cultures). Student's unpaired two-tailed t -test. **c,d**, Recipient green cells show enhanced mitochondrial OXPHOS capacities. CC, coculture. Representative profile ($n = 3$); mean $\pm$ s.e.m., parental: $n = 11$, red: $n = 13$, green: $n = 12$ cell cultures; Student's unpaired two-tailed t -test, $*P = 0.037$, $***P < 0.001$, $****P < 0.0001$. **e**, Energy map shows a more aerobic and energetic phenotype in green cells compared to the red counterpart (mean $\pm$ s.d. $n = 24$). ECAR, extracellular acidification rate. **f**, Luminescence-based assay of total cellular ATP content showed significantly higher levels in green cells ($**P = 0.0044$, $n = 3$ independent cultures). RLU, relative light units normalized per cell. Mean $\pm$ s.d., Student's two-tailed paired t -test. **g,h**, Green cells exhibited increased GSH ($***P = 0.0002$, $n = 6$ independent cultures) and overall improved GSH/GSSG ratios ($**P = 0.0028$ (**g**), $*P < 0.01$ (**h**), $n = 6$ independent cultures). Mean $\pm$ s.d., Student's two-tailed paired t -test. FC, fold change. **i,j**, Green cells exhibited higher tolerance to H_2O_2 -induced oxidative stress (**i**; mean $\pm$ s.d., Student's

two-tailed paired t -test, NS, not significant; two-way ANOVA, $P = 0.0007$) and greater resistance to shear stress (**j**; two-way ANOVA, $P < 0.001$). Representative profile ($n = 3$). **k**, Modified Boyden chamber assay revealed no change in intrinsic invasion capacity between green and red cells in vitro (NS, not significant; Student's two-tailed t -test, $n = 3$ independent cultures). **l**, In vivo, metastatic progression was enhanced in green versus red cells in mouse mammary fat pad xenografts, as observed by increased liver metastasis (mean $\pm$ s.d.; Student's two-tailed unpaired t -test, $*P = 0.01997$, $n = 8$ mice). **m**, Haematoxylin and eosin liver sections showed metastatic lesions, and Ki67 immunostaining confirmed the cancerous character of the lesions. Scale bar, 4 mm. **n,o**, Multispectral imaging in human breast cancer and matched metastatic sites revealed increased mitochondrial content at metastatic versus primary sites. Representative image from patient breast cancer samples (**n**) and matching metastasis and mitochondrial score quantification curve in the samples set (**o**). The line shows the median, the box boundaries show the 25th and 75th percentiles, and the whiskers show the minimum and maximum values (two-sided Welch's t -test, $***P < 0.001$; $n = 8$ patients). **k,l**, Created in BioRender. S. Grelet (2025) <https://biorender.com/35rzyib>.

in these cells (hereafter referred to as green cells; Fig. 3a). In brief, the SVZ-NSC^{MitoTRACER} donor cells were genetically labelled with a mitochondrial-anchored Cre recombinase, and recipient 4T1 cancer cells were equipped with a loxP-DsRed-Express2-Stop-loxP-eGFP switch (Fig. 3b). The addition of a specific tobacco etch virus protease (TEVp) proteolytic cleavage site to the donor construct was required for the release of the mitochondria-bound Cre recombinase and its nuclear translocation into the recipient cells (Fig. 3c and Extended Data Fig. 6a). In the MitoTRACER coculture, following the transfer of nerve-derived mitochondria to the recipient cancer cell, the Cre recombinase triggers the red-to-green switch of the recipient cells, as observed by using time-lapse fluorescence microscopy and flow cytometry analyses of the nerve–cancer coculture (Fig. 3d,e). Time-lapse microscopy of the MitoTRACER coculture revealed the development of a green signal in recipient cells following the establishment of tunnelling nanotube connections, further supporting the role of these structures in mitochondrial transfer (Extended Data Fig. 6b and Supplementary Video 3). The use of various donor cell types, such as SVZ (Supplementary Video 4), mouse embryonic fibroblasts (Supplementary Video 5) and 3T3-L1 pre-adipocytes (Supplementary Video 6), demonstrated successful mitochondrial tracking through time-lapse imaging.

No unintended signal activation was observed in recipient cells until donor cells were introduced into the coculture. Dose–response (Fig. 3f) and time-course (Fig. 3g) analyses of the coculture demonstrated the sensitivity of the MitoTRACER approach in capturing the dynamics of mitochondrial transfer and its cumulative capability. Subcellular fractionation of MitoTRACER expression confirmed that nearly all of the construct is anchored to mitochondrial organelles (Fig. 3h,i). Finally, MitoTRACER coculture with recipient cells lacking TEV protease showed no signal, indicating that no unintended self-cleavage and further transfer through non-mitochondrial routes, such as secretory pathways, occurred (Fig. 3j). Together, these findings demonstrate the efficacy of this system in allowing real-time observation of mitochondrial transfer to recipient cells and its permanent nature, enabling lineage tracing of recipient cells.

Functional imprint on recipient cells

We used MitoTRACER to examine the biological impact of the nerve–cancer transfer of mitochondria in the recipient cancer cells. We also examined their fate during cancer progression, both in vitro and in vivo. From the MitoTRACER coculture, we FACS-isolated cells that have received mitochondria from the neurons (4T1^{MitoTRACERGreen}) and those that have not (4T1^{MitoTRACERRed}). Separate subcultures of the red

versus green cells obtained from the MitoTRACER coculture revealed distinct growth patterns. Green cells having received mitochondria had a higher propensity for anchorage-independent growth patterns and exhibited the development of spheres throughout the culture (Fig. 4a). Anchorage-independent growth capacities of cancer cells are associated with their stemness potential and are usually tied to specific metabolic profiles driven by mitochondrial metabolism^{33–35}. We confirmed the increased stemness of the recipient cancer cells by a mammosphere formation assay (Fig. 4b). Metabolic profiling of mitochondrial metabolism of the recipient cells indicated enhanced respiratory capacities of the green recipient cancer cells compared to red cells exposed to the neurons but not having received mitochondria and to parental 4T1 cells that were not exposed to neurons (Fig. 4c,d). Energetic mapping of the red versus green cells indicated a shift towards a more energetic status, corresponding to increases in both the oxygen consumption rate and the extracellular acidification rate (Fig. 4e), and this metabolic shift was associated with a significant increase in basal and maximal respiration capacities of the cancer cells, increased coupling and increased ATP production (Fig. 4d,f). Analysis of the functional outcomes of mitochondrial transfer revealed improved redox balance in the recipient (green) cancer cells, as indicated by their higher levels of reduced glutathione (GSH) (Fig. 4g). This increase translated into an improved GSH to oxidized glutathione (GSSG) ratio (Fig. 4h), a key marker of cellular redox status³⁶. We next confirmed that enhanced redox balance was associated with an increased capacity of recipient cancer cells to withstand oxidative stress (Fig. 4i) at physiological doses (9–75 μ M) and greater resistance to shear stress (Fig. 4j).

Together, metabolic plasticity, improved redox balance and increased oxidative and shear stress resistance are hallmarks of metastatic cancer cells^{37–39}. We therefore investigated whether mitochondrial transfer could enhance metastatic behaviours in cancer cells. In vitro analysis of the recipient cancer cells' invasive potential revealed no increase in intrinsic invasiveness (Fig. 4k). However, in vivo xenografts with recipient cells demonstrated significantly higher metastatic potential than their 'non-recipient' counterparts (Fig. 4l,m), suggesting that mitochondrial transfer may contribute to the metastatic cascade beyond the invasion process.

Pathological analysis of human breast cancer samples further underscored the role of mitochondria in tumour dissemination, with metastatic cells exhibiting a significant increase in mitochondrial load (Fig. 4n,o and Supplementary Tables 8 and 9). Together, our findings clearly suggest that mitochondrial transfer from neurons to cancer cells may enhance metastatic behaviour by strengthening the resilience of cells against metastatic stressors, such as oxidative and shear

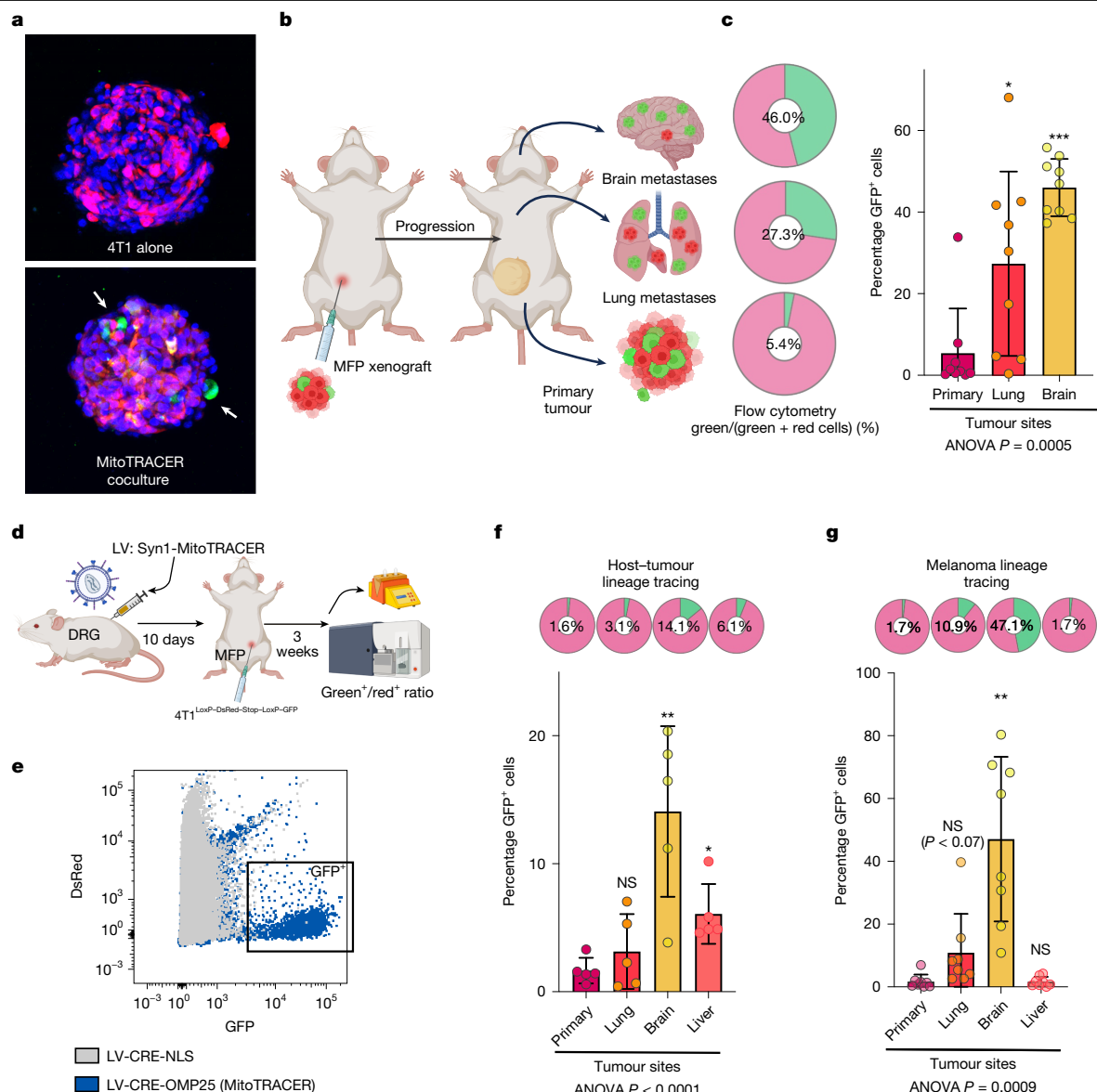


Fig. 5 | Lineage tracing of the intercellular transfer of mitochondria from neuron to cancer cell during breast cancer dissemination in vivo. **a**, 3D spheroids of SVZ-NSCs^{MitoTRACER} mixed with 4T1 recipient cancer cells confirmed mitochondrial transfer, evidenced by red-to-green fluorescence conversion in 4T1 cells (white arrows). **b,c**, MitoTRACER spheroids were transplanted into mammary fat pads of BALB/c mice (**b**); after cancer progression, flow cytometry of cells isolated from primary tumours, lungs and brains revealed selective enrichment of green fluorescent cells (eGFP⁺) in lung and brain metastases compared to the primary tumour (**c**). Mean $\pm$ s.d.; Student's two-tailed paired *t*-test, lung: $^{*}P = 0.018$, brain: $^{***}P = 1.2275 \times 10^{-6}$; $n = 9$ mice; ANOVA, $P = 0.0005$. **d**, Schematic of the procedure for lineage tracing of mitochondrial transfer between mouse host mammary neurons and cancer cells in vivo. Lentivirus expressing Syn1-MitoTRACER construct was injected into the DRG area, innervating the lower mammary fat pads. After 10 days, the SWITCH-TEVp-expressing 4T1 recipient cells were injected. Following tumour growth, tissues (primary, lung, brain and liver) were analysed for mitochondrial transfer by

flow cytometry. **e**, Flow cytometry of primary tumours showed eGFP⁺ cells, confirming mitochondrial transfer. Control lentivirus expressing Syn1-driven nuclear-localized Cre (LV-CRE-NLS) showed no green signal, validating the specificity of our approach. **f**, Ex vivo quantification of mitochondrial transfer in the primary tumour and lineage tracing of metastatic development in the lung, brain and liver showed significant enrichment of eGFP⁺ cells in metastatic sites compared to the primary tumour (ANOVA, $P < 0.0001$), with significant enrichment in brain and liver metastases (mean $\pm$ s.d., Student's one-tailed paired *t*-test values, brain: $^{**}P = 0.0096$, liver: $^{*}P = 0.0215$; $n = 5$ mice). Top panel shows distribution of red and green cells. **g**, Validation using syngeneic B16-F1 melanoma cells co-injected with SVZ-NSCs^{MitoTRACER} as mixed-cell spheroids in C57BL/6 mice showed significant mitochondrial transfer enrichment in brain metastases (mean $\pm$ s.d., ANOVA, $P = 0.0009$; Student's one-tailed paired *t*-test, brain: $^{**}P = 0.0017$; $n = 8$ mice). Top panel shows distribution of red and green cells. **b**, Created in BioRender. S. Grelet (2025) <https://biorender.com/culfgzj>. **d**, Created in BioRender. S. Grelet (2025) <https://biorender.com/2w707rr>.

stress, thereby augmenting their metastatic potential through adaptive mechanisms.

Recipient cell fate during metastasis

The observed differences in mitochondrial load between primary and metastatic cancer suggested that mitochondria-recipient cancer

cells in primary tumours may possess enhanced metastatic potential. The metastatic cascade is inefficient, with cancer cells encountering multiple stressors that impede their successful dissemination and growth at secondary sites⁴⁰. Metabolic reprogramming and plasticity have emerged as crucial adaptive mechanisms for the successful metastatic dissemination of cancer cells^{5,41–43}. Thus, we reasoned that neuron-derived mitochondria confer enhanced metabolic adaptability

and resilience, enabling recipient cancer cells to better spread and ultimately survive and proliferate at distant sites. To test this hypothesis, we performed lineage tracing to follow the fate of primary cancer cells receiving mitochondria from nerves.

We developed a preclinical model of the nerve–cancer transfer of mitochondria (Fig. 5). We combined the MitoTRACER coculture approach with the 4T1 mammary fat pad xenograft model that can fully recapitulate the breast cancer progression steps and metastasis of TNBC^{44,45}. Mixed-cell spheroids of the MitoTRACER coculture (Fig. 5a) were transplanted into mammary fat pads. When the xenograft reached appropriate size, cancer cells were collected from the primary tumour and both lung and brain tissues, which are prevalent metastatic sites in TNBC. The cells were then analysed by flow cytometry to probe the ratio between 4T1^{Red+} versus 4T1^{Green+} cells (Fig. 5b). We observed the development of an average of about 5.4% 4T1^{GFP+} cells within the primary tumour. This proportion was significantly enriched in both lung and brain tissues to reach 27.3% and 46.0% of the total cancer cells in those sites, respectively (Fig. 5c). This demonstrates that cells that acquired mitochondria from the SVZ-NSCs within the primary tumour, or their progeny, are more likely to form distant metastases successfully. To extend the observations with implanted mixed-cell spheroids to a model of host-mediated neuronal mitochondrial transfer, we genetically modified mouse DRG *in vivo* using a lentivirus encoding a MitoTRACER construct driven by the synaptin1 promoter (LV-CRE-OMP25) or its non-transferable, nuclear-targeted variant (LV-CRE-NLS; Fig. 5d). At 10 days after lentiviral transduction, SWITCH-TEVP-expressing recipient cells were injected into the mammary fat pad, and tumours were subsequently excised to assess eGFP fluorophore expression, indicative of mouse-derived neuronal mitochondrial transfer into cancer cells (Fig. 5e). We observed only 1.6% eGFP⁺ cells in the primary tumour, which was less than the 5.4% observed with the mixed-cell spheroids, probably owing to the lower nerve density in the tumour *in situ*, when compared to the spheroid model. Lineage tracing reproduced by labelling the endogenous nerve of the house mouse using lentiviruses injected into the DRG area also demonstrated a marked enrichment of eGFP⁺ recipient cells in metastatic sites, with significant increases in the brain and liver compared to the primary tumour (Fig. 5f).

We tested whether nerve-mediated mitochondrial transfer may affect distant metastasis in another cancer progression model using the B16-F10 melanoma xenograft. The observations about transferred mitochondria in primary and metastatic tumours were similar in the melanoma and breast cancer models, but the rate of mitochondrial transfer was lower in the melanoma than in the 4T1 breast cancer model. Furthermore, the melanoma model showed no significant enrichment of green eGFP⁺ cells in the lungs or liver but marked enrichment in the brain, corroborating findings from the breast cancer model and providing evidence that neuronal mitochondrial transfer may promote brain metastases with various cancer types (Fig. 5g).

Discussion

The nerve–cancer interplay was initially discovered in prostate cancer, in which cancer cells can promote cancer innervation by expressing axon guidance molecules, such as semaphorins, promoting neuronal progenitor differentiation in the cancer stroma and the establishment of the nerve–cancer interface^{17,46–48}. Initial observations in prostate cancer have shown how the aberrant expression of semaphorin 4F by cancer cells promotes increased prostate nerve density and cancer aggressiveness^{18,49}. Later studies supported the relevance of semaphorin 4F in other biological contexts such as gastric cancer¹⁹ and breast cancer¹⁷, in which cancer cell plasticity triggers the expression of semaphorin 4F to promote cancer innervation and metastasis⁵⁰. Our previous studies demonstrated how cancer innervation and cancer cell plasticity are intimately linked^{17,50}, and although progress has been

made in understanding the mechanisms leading to cancer-mediated neuronal differentiation and the establishment of the nerve–cancer interface, the mechanisms and functional effects of neurons on breast cancer progression and metastatic potential remain incompletely understood. Our study demonstrates how cancer-induced neuron differentiation leads to marked neuronal metabolic reprogramming with clear functional consequences. We show that cancer-associated neurons are a significant source of mitochondria transferred to the cancer cell to induce their metabolic reprogramming and increase stemness potential.

Pathological analysis and animal denervation models, including the breast carcinoma denervation model presented herein, have consistently associated cancer nerve density with cancer invasion and metastasis^{51–54}. Metabolic plasticity and stemness potential are essential hallmarks of cancer metastasis⁵⁵. To test whether the nerve–cancer transfers of mitochondria relate to the development of distant metastasis, we developed the MitoTRACER genetic reporter capable of permanently marking recipient cells. We used this approach to create a preclinical model of nerve–cancer transfer of mitochondria *in vivo*. The fate mapping experiment revealed enrichment of mitochondria-recipient cancer cells or their progeny at metastatic tumour sites relative to the primary tumour, reflecting their increased capacities to achieve the metastatic colonization steps successfully. Although the exact molecular mechanisms remain unclear, our study shows that cancer cells acquiring mitochondria from neurons gain adaptive advantages, enabling resilience against metastatic stressors such as oxidative³⁹ and shear stress^{37,38,56}, which are well-known key barriers to metastasis. From a probabilistic point of view, cancer cells that energetically outperform others have a greater probability of moving and seeding. It is probably a selection process by which the incorporation of these neuronal mitochondria into the cancer cells provides an increased capacity to survive the metastatic process.

We noted selective enrichment of mitochondria-recipient cancer cells in brain metastases in both breast cancer and melanoma models. This suggests that mitochondrial acquisition from neurons may prime cancer cells to adapt more effectively to the brain's unique microenvironment. Previous research supports this idea, as brain-metastatic cancer cells must metabolically adapt to survive in the brain's nutrient-poor environment⁵⁷ due to the high energy demands of neurons⁵⁸.

Neuronal mitochondria are renowned for their highly efficient metabolic potential, which contributes to the superior metabolic efficiency of neurons compared to epithelial cells⁵⁹. Therefore, cancer cell acquisition of neuron-derived mitochondria may provide them with an adequate metabolic arsenal to survive and successfully proliferate in the brain environment. Our findings also suggest that neurons may be particularly effective donors of mitochondria, probably owing to their abundant mitochondrial content and the establishment of strong contacts at the nerve–cancer interface. Further studies are needed to clarify whether the impact of neuronal mitochondria is primarily due to their intrinsic metabolic efficiency or simply their highest transfer frequency.

Collectively, our findings provide a compelling metabolic explanation for the observed dependency between cancer cells and nerves, potentially extending to broader contexts. These results advocate for more in-depth studies into underlying mechanisms and therapeutic strategies targeting nerve–cancer mitochondrial transfers to prevent metastatic disease.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-025-09176-8>.

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Methods

Cell culture

The 4T1 (CRL-2539), Neuro2A (CCL-131) and NMuMG (CRL-1636) cell lines were purchased from ATCC. HT-22 (ESA111) and 3T3-L1 (EF3001) were purchased from Keratfast. The 50B11 rat DRG neuronal cells were obtained from the GRCF Biorepository & Cell Center (Johns Hopkins University). MEFs were provided by S. Lloyd. Primary SVZ-NSCs were isolated from fresh BALB/c mouse brains as described previously⁶⁰ (Extended Data Fig. 2a–d). The cell lines were routinely checked for mycoplasma contamination by using the MycoStrip Test Kit (InvivoGen), and through Hoechst-33342 staining. The cell lines used on this study has been authenticated by genomic profiling. Cancer cells were cultured using Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 5% fetal bovine serum (FBS; Fisher Scientific), 5% bovine calf serum (Fisher Scientific), antimicrobial solution (penicillin, streptomycin and amphotericin B; 1% antibiotic–antimycotic, Life Technologies) and antimycoplasma solution (1.25 mg l⁻¹ plasmodin prophylactic, InvivoGen). SVZ-NSCs were cultured in specialized medium (Neurobasal, Life Technologies) supplemented with serum-free supplement (2% B-27, Life Technologies), 1 mg l⁻¹ heparin (STEMCELL Technologies), L-alanyl-L-glutamine dipeptide supplement (1% GlutaMAX, Life Technologies), antimicrobial solution (1% antibiotic–antimycotic, Life Technologies), 10 ng ml⁻¹ recombinant human epidermal growth factor (Bio-Techne) and 10 ng ml⁻¹ of recombinant human fibroblast growth factor basic (Bio-Techne). 50B11 cells were cultured in specialized medium (Neurobasal, Life Technologies) supplemented with both 10% serum and the serum-free supplement (2% B-27, Life Technologies), L-alanyl-L-glutamine dipeptide supplement (0.27% GlutaMAX, Life Technologies), antimicrobial solution (1% antibiotic–antimycotic, Life Technologies) and 0.22% glucose. SVZ-NSC adherence in vitro was aided by adding 0.25 µl ml⁻¹ ECMatrix-511 Silk E8 Laminin Substrate (MilliporeSigma). For serum-free experiments, FBS and bovine calf serum were substituted with a serum-free medium (10% Knockout Serum Replacement (KSR), Life Technologies). Dialysed FBS (Thermo Fisher Scientific) was substituted for FBS in uridine depletion assays. p⁰ cells were cultured in medium supplemented with 50 µg ml⁻¹ uridine (Sigma-Aldrich) and 1 mM sodium pyruvate (Fisher Scientific). Antibiotic selection pressure was applied with combinations of 10–20 µg ml⁻¹ puromycin (Fisher Scientific), 100–200 µg ml⁻¹ phleomycin D1 (Zeocin, InvivoGen) and 10–20 µg ml⁻¹ Blastidicin (InvivoGen) to maintain transgene expression. Cells were cultured in an incubator at 37 °C and 5% carbon dioxide and were tested routinely for mycoplasma contamination.

In short-term nerve–cancer coculture experiments (≤5 days), 4T1 cells were initially seeded in DMEM and allowed to adhere. After 1 day, the medium was changed to KSR medium, and SVZ-NSCs were added the following day. For long-term coculture experiments, 4T1 cells were maintained in DMEM, with SVZ-NSCs added weekly and 4T1 cells passaged twice a week. SVZ-NSCs were introduced into the 4T1 cell cultures at 2–4% of the total cell population, matching the percentage observed in primary cancers formed from 4T1 cell xenografts⁶¹. For the 3D coculture and reconstruction imaging experiment, a total of 1.2×10^5 50B11^{CCO-GFP} cells and 1.2×10^5 4T1^{mCherry} cells (both in 10 µl PBS) were mixed with 60 µl of Growth Factor Reduced Matrigel (Corning, catalogue number 356231) and seeded onto the glass area of uncoated glass-bottomed 35-mm dishes (MatTek). The dishes were incubated at 37 °C in a humidified atmosphere of 5% CO₂ in air for 1 h. Following incubation, 2 ml of Neurobasal complete medium containing 75 µM forskolin (MilliporeSigma) was added. Confocal images were captured 2 days later using a Nikon A1 confocal laser microscope.

Virus production and cell line generation

Lenti-X 293T cells (Takara Bio USA) were used for lentivirus generation, and Phoenix-AMPHO cells (ATCC) were used for retrovirus production.

Cells were plated in complete DMEM 1 day before transfection using a transfection reagent (FuGENE, 3 µl µg⁻¹ of plasmid; Promega).

For lentivirus production, Lenti-X 293T cells were transfected by lipofection with the following components: 1,000 ng of transfer plasmid, 750 ng of packaging plasmid (psPAX2; Addgene) and 250 ng of vesicular stomatitis virus G envelope-expressing plasmid (pMD2.G; Addgene). Both psPAX2 and pMD2.G were gifts from D. Trono, École Polytechnique Fédérale de Lausanne (Addgene plasmid numbers 12260 and 12259). For in vivo work, the lentivirus was further concentrated in a concentrator solution (40% PEG-8000; 1.2 M NaCl in PBS) added at a 1:3 ratio of solution/supernatant, gently shaken for 2 days at 4 °C, collected through 1,600g centrifugation and resuspended in a 1:100 volume of PBS and stored at –80 °C.

For retrovirus production, Phoenix-AMPHO cells were transfected with 1,000 ng of plasmid DNA. The medium was replaced with KSR-containing medium 1 day after transfection, and the viral supernatant was collected over a period of 2 days. For viral transduction, target cells were plated at 60% confluence and transduced in the presence of 10 µg ml⁻¹ Polybrene (MilliporeSigma) in KSR medium. SVZ-NSCs were cultured in Neurobasal medium without Polybrene to maintain cell viability. The pLenti-CMV-GFP-puro plasmid was provided by E. Campeau (Addgene plasmid number 17448). The pLVX-EF1a-CCO-IRES-puromycin plasmid was provided by D. Andrews (Addgene plasmid number 134861). The pFUGW mCherry-KASH plasmid was a gift from H. MacGillavry (Addgene plasmid number 131505). The CMV-loxP-DsRed-loxP-eGFP plasmid was provided by D. Gilkes (Addgene plasmid number 141148). The pLenti-CMV-puro-2A-TEVp plasmid was a gift from M. Tripodi (Addgene plasmid number 99610).

p⁰ 4T1 cell lines were developed as described previously²⁸. The complete and permanent loss of mtDNA in 4T1 cells was achieved by transient co-overexpression of a mUNG1 and UL12.SM185 herpesvirus protein. 4T1^{mCherry} cells were cultured on 6-cm plates. At 1 day after seeding, cells were lipofected using 15 µl of FuGENE transfection reagent and 2,500 ng each of pMA4008 and pMA3790 plasmids provided by M. Alexeyev⁶². Lipofection was performed on medium with uridine and sodium pyruvate and without antibiotics, and medium was changed after 1 day to fresh medium with uridine, sodium pyruvate and antibiotics. Single-cell sorting based on strong positive eGFP expression (top 3%) was performed after 3 days into 96-well plates supplemented with uridine, sodium pyruvate and 20% FBS. The medium was changed weekly and twice weekly when colonies were observed.

The MitoTRACER donor cells were prepared using retroviral or lentiviral transduction, followed by selection with 20 µg ml⁻¹ Blastidicin. To further purify cells with the highest MitoTRACER expression, we incorporated a GFP-11 strand into the MitoTRACER construct, allowing for additional selection by FACS. If necessary, the MitoTRACER cells were further purified to ensure consistent and robust expression by transiently transfecting them with 5,000 ng of GFP1-10 plasmid and sorting based on GFP signal 3 days later. The plasmid pQCXIP-GFP1-10 was provided by Y. Hata (Addgene plasmid number 68715). Loss of GFP signal following transient selection was confirmed by flow cytometry before utilizing the cells in MitoTRACER coculture experiments.

DNA extraction and PCR

DNA was extracted from cells using a kit (GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific). Reaction mixes were used for end-point PCR (DreamTaq Hot Start Green PCR Master Mix, Thermo Fisher Scientific) and quantitative PCR (PowerTrack SYBR Green Master Mix, Thermo Fisher Scientific). The quantitative PCR was performed with a thermocycler (QuantStudio 7 Pro, Thermo Fisher Scientific). Sample concentration was adjusted to 10 ng µl⁻¹, and PCR was performed with 20 ng of DNA template. The end-point PCR products were separated on acrylamide/bis-acrylamide (29:1) 10% gels (Thermo Fisher Scientific) and stained with ethidium bromide (Thermo Fisher Scientific). Mouse mtDNA amplification was performed as described

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previously⁶³ using the mMitoF1 5'-CTAGAAACCCCGAAACAAA-3' and mMitoR1 5'-CCAGTATCACCAAGCTCGT-3' primers. The mtDNA copy numbers were normalized to the nuclear copy numbers using the B2MF1 5'-ATGGGAAGCCGAACATACTG-3' and B2M-R1 5'-CA GTCTCAGTGGGGGTGAAT-3' primers. Uncropped gels are provided in Supplementary Fig. 1.

Western blot

Western blot was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Whole-cell lysates were prepared from $2-5 \times 10^6$ cells in 300 μ l of lysis buffer (20 mM Tris, pH 7.4, 1% Triton X-100, 10% glycerol, 137 mM sodium chloride, 2 mM ethylene diamine tetraacetic acid, 1 mM sodium orthovanadate) and protease inhibitors (Thermo Fisher Scientific). Subcellular fractionation was performed using the Mitochondria Isolation Kit for Cultured Cells (Life Technologies) according to the manufacturer's instructions. With mitochondrial-bound proteins, sonication (30 pulses, each 20 s) was performed instead of centrifugation of excess material to ensure that mitochondrial proteins remained in suspension. Lysates were clarified by centrifugation at 4 °C for 30 min at 17,000g. Typically, whole-cell lysates (5–20 μ g) were separated on 10% or 12% acrylamide minigels and transferred to a membrane (Immuno-Blot, Bio-Rad).

Samples were separated with electrophoresis (Any kD gels and Mini-PROTEAN apparatus; Bio-Rad). The membrane was blocked for 30 min in wash buffer (0.1% Tween 20 in PBS) containing 5% nonfat dry milk and incubated overnight with primary antibody (Cre recombinase (1:1,000, number 15036, lot 2), mCherry (1:1,000, number 43590, lot 2) or α -tubulin (1:1,000 number 2144, lot 5), Cell Signaling Technology; or GFP (1:200, number sc-9996, lot E2521) or HA tag (1:1,000, number sc-7392 lot 10992), Santa Cruz Biotechnology) that was diluted in the same buffer. After extensive washing, the blot was incubated with secondary antibody (1:1,000, Thermo Fisher PI31430 or PI31460) for 30 min in blocking buffer, washed and processed with a western blot detection system (ChemiDoc MP Imaging System, Bio-Rad). Uncropped blots are provided in Supplementary Fig. 1.

Imaging and flow cytometry analysis

Cell imaging was performed with a fluorescence (BZ-X810, Keyence) or inverted confocal microscope (A1R, Nikon). Cell sorting was performed using FACS (FACSARIA II (BD Biosciences) with analysis of GFP (laser excitation, 488 nm; detection, 525/30 band-pass filter width (BP)) and DsRed-Express2 and mCherry Red fluorophores (561 nm; 610/20 BP). The gating strategy is provided in Supplementary Fig. 2.

Dye staining (MitoTracker, Invitrogen) was performed according to the protocol from the manufacturer. Sorting was performed with 1 cell per well in 96-well plates (single-cell sorting) or 5,000 cells per well in 6-well plates containing 2 ml of DMEM with 10% FBS. Immunostaining and cytometry were performed as follows: cells were collected by trypsinization, fixed in 4% paraformaldehyde for 15 min at room temperature, and permeabilized with 0.1% Triton X-100 diluted in 0.5% bovine serum albumin. Fixed cells were incubated for 1 h at room temperature with primary antibodies, washed in PBS and incubated for 30 min at room temperature with an Alexa Fluor 647 secondary antibody (1:10,000, Thermo Fisher A21235, lot 2836809; A21244, lot 2674387). For animal xenograft samples, deparaffinized slides were microwave-boiled (heat-induced epitope retrieval) with 10 mM sodium citrate, blocked in 1% bovine serum albumin, incubated in primary antibodies overnight, washed, incubated in secondary antibodies, Alexa 647 and Alexa 568, for 40–60 min (1:250, Thermo Fisher A78952; lot 3034155, A11004, lot 2198584) and counterstained with 4',6-diamidino-2-phenylindole (DAPI). The following primary antibodies were used: O4 (1:10,000, R&D Systems MAB1326, lot HWW143051), ALDH1L1 (1:10,000, Origene TA501868S, lot A01), Map2 (1:1,000 Proteintech 17490-I-AP, lot 00126726), GFP (1:200, Invitrogen A10262, lot 2738237) and β 3 Tubb3 (1:400, Santa Cruz sc80005, lot A1821).

Whole-cell current clamp recordings

Whole-cell current clamp recordings were performed on neurons differentiated from cocultures of SVZ-neural progenitor cell mixed with 4T1 cancer cells using an Axopatch 200B amplifier and Digidata 1322A, with data acquisition through pClamp 8 software (Molecular Devices). A Zeiss Axiovert microscope with epifluorescence was used to identify and record neural cells expressing eGFP in the nerve–cancer coculture. Cells were clamped at -80 to -90 mV, and depolarizing currents were injected using 10 current ramp increments from 20 to 200 pA (800 ms duration), interleaved by 5-s intervals. Membrane voltages were sampled at 5 kHz and filtered at 2 kHz. The action potential threshold was determined from the dV/dt derivative calculated using IgorPro 6 (WaveMetrics)^{64,65}.

The external bath solution consisted of 120 mM NaCl, 6 mM KCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 10 mM HEPES and 20 mM glucose (pH 7.4). Patch pipettes were filled with an internal solution containing 125 mM K^+ gluconate, 4 mM KCl, 4 mM NaCl, 1 mM MgCl_2 , 10 mM HEPES, 4 mM MgATP, 0.3 mM Na_2GTP and 10 mM phosphocreatine (pH 7.26). Osmolarity of all solutions was verified with an Osmette III osmometer (Precision Systems). Series resistance and membrane capacitance were not compensated but were measured at the beginning and end of each recording to ensure quality. Electrophysiology figures were prepared using IgorPro and GraphPad Prism 9.

Uridine auxotrophy assay

The p^0 4T1^{mCherry+} cells were cocultured with SVZ-NSCs^{CCO-GFP} and sorted on the basis of mCherry and eGFP expression using flow cytometry. The sorted cells were seeded on DMEM with dialysed FBS, with or without uridine and sodium pyruvate, and stained with 2% crystal violet in 20% methanol. The rescued cells were cultured in the presence of antibiotic (Zeocin), associated with the cancer cell expression of the mCherry fluorophore. 4T1 cells were checked for the presence of mCherry and the absence of eGFP expression to confirm the 4T1 nature of the collected cells and exclude contamination with SVZ-NSCs during FACS.

Seahorse metabolic assay

Cells were plated at 4×10^4 cells per well (Seahorse XFe96 plate, Agilent) on the day before assay, and the assay tools and calibrant solution (Seahorse FluxPak, Agilent) were acclimated in an incubator without carbon dioxide on the night before assay according to the protocol from the manufacturer. An assay kit was used (Seahorse XF Mito Stress Test Kit, Agilent) with oligomycin (1.5 μ M), FCCP (1.0 μ M), and rotenone and antimycin A (0.5 μ M). On the assay day, the medium was changed (Seahorse XF DMEM, Agilent) and supplemented with 10 mM glucose (Agilent), 1 mM pyruvate (Agilent) and 2 mM glutamine (Agilent). Uridine and sodium pyruvate were added for assays with p^0 cells. Assays were performed according to the protocol from the manufacturer, and wells were normalized by direct cell counting to determine the absolute number of cells per well. Cells were stained with Hoechst dye and counted directly with an automatic microscope reader (Celigo S, Nexcelom Bioscience). The absence of cross-contamination following the sorting of coculture was confirmed by the analysis of the presence of eGFP⁺ cells in the mCherry-FACS-sorted cancer cell samples.

Mitochondria transfer assays

In cases in which inserts were used to physically block contact between cell types, 100,000 4T1^{mCherry} cells were seeded in the bottom well of a 6-well Transwell system (Corning, 3412) or 6-well plate, and the following day, the medium was changed to 10% KSR-containing DMEM, and 200,000 SVZ-NSCs^{CCO-GFP} were added either to the insert or directly to the well. For the dose–response and time–response assays, 4T1^{mCherry} cells were seeded at 100,000 cells per well and changed to KSR-containing medium the next day. For the dose–response assay, between 25,000 and 400,000 SVZ-NSCs^{MitoTRACER} were then added to

the culture. For the time–response assay, 200,000 SVZ-NSCs^{MitoTRACER} were added daily until the time of FACS analysis. FACS analysis was carried out on the third day after adding SVZ-NSCs^{MitoTRACER}.

Donor cell cloning

The MitoTRACER construct was cloned into the pMXs-IRES-Blasticidin retroviral vector RTV-016. We used the HA-MITO plasmid pMXs-3XHA-EGFP-OMP25 as a template. pMXs-3XHA-EGFP-OMP25 was a gift from D. Sabatini (Addgene plasmid number 83356). The OMP25 mitochondrial tagging element and attached 3' linker (Pro-Arg-His) were conserved. The HA-MITO plasmid was digested to insert our construct design consisting of the assembly of GFP-11–HA tag–TEVp cleavage recognition site–linker–iCre recombinase–SV40-NLS. The GFP-11 fragment was added between the OMP25 and TEVp cleavage site for convenience to generate cells having the desired amount of reporter using GFP1-10 fluorescence complementation and FACS. The construct also included a Blasticidin S deaminase gene separated by an internal ribosome entry site (IRES), enabling the selection of cells that were transduced successfully by the retroviruses. All constructs were validated by Nanopore sequencing. For in situ labelling, the MitoTRACER construct was transferred into a lentiviral construct with the *SynI* promoter (Addgene plasmid number 71427). The CRE-NLS control was generated through site-directed mutagenesis (Q5 Site-Directed Mutagenesis E0554S, New England Biolabs) by removing the OMP25 section of MitoTRACER. Similar constructs were generated by replacing the MitoTRACER with the eGFP fluorophore instead to generate the lentiviral *SynI*-GFP-OMP25 and *SynI*-GFP-NLS constructs.

Recipient cell cloning

The recipient cell construct for the MitoTRACER method was a DsRED and eGFP *loxP* switch with TEVp expression under the control of an SV40 promoter and tied to a BleoR gene by a 2A self-cleaving peptide. This construct was derived from the CMV-*loxP*-DsRed-*loxP*-eGFP plasmid (Addgene number 141148) that was digested with *DraIII* and *SwaI* restriction enzymes to insert the F2A-TEVp at the C_t end of BleoR with a DNA assembly kit (NEBuilder HiFi DNA Assembly, New England Biolabs).

Laser-capture microdissection, RNA extraction and cDNA microarray analysis

Laser-capture microdissection, RNA extraction and cDNA microarray analysis were conducted as previously detailed^{2,66}. Cancer cells from BoNT/A and saline groups were procured through laser-capture microdissection from 8- μ m frozen sections cut using a cryostat from the cancerous areas of fresh, unfixed xenograft specimens. The sections were mounted on non-charged glass slides and stored at -80°C until analysis. Following staining and dehydration as per the manufacturer's protocol, a laser-capture microdissection was performed before cDNA preparation, and microarray analyses as previously described². After performing quantile normalization, we carried out principal component analysis as it was noted that samples from the BoNT/A and saline groups have clear distinct expression patterns. A total of 116 genes (135 probes) were identified as differently regulated by an empirical Bayes test with a false discovery rate cutoff <0.30 and a twofold change as an exploratory approach. To identify the biological processes and pathways associated with the differentially expressed genes, we performed a GATHER analysis⁶⁷ and used gene set enrichment analysis⁶⁸ to score association of BoNT/A treatment with Kyoto Encyclopedia of Genes and Genomes (KEGG)⁶⁹ pathways.

RNA-seq of mouse mammary fat pad denervation model

Reads were trimmed for quality control to remove low-quality bases and adaptor sequences. Samples failing quality assurance and quality control thresholds (based on read quality, length or count) were excluded. Quality-trimmed reads were aligned to the *Mus musculus*

mm39 genome using Bowtie2, in paired-end mode with a 500-bp fragment length. SAM and BAM files were processed with SAMtools and Sambamba, and alignment quality was assessed using SAMtools *idxstats*, retaining only reads aligning to mm39. Read counts were normalized with DESeq2's median-of-ratios method, and genes with expression below 1.0 (geometric mean) were filtered out. Gene set ANOVA was performed on gene sets after excluding low expression levels (geometric mean <1.0) on sets containing between 2 and 50 genes. ANOVA was applied using a log-normal model with the criterion Akaike information criterion (AIC), followed by false discovery rate (FDR) correction for multiple comparisons. KEGG pathways were analysed to identify differentially regulated gene sets.

Nanopore mtDNA sequencing and classification of mtDNA heteroplasmy

To analyse mtDNA transfer in situ, cancer cells were FACS-sorted to isolate mCherry⁺ 4T1 cancer cells from tumour tissues. DNA was extracted from collected cells using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific). The mtDNA region containing the mutation site was amplified using PCR with Q5 High-Fidelity DNA Polymerase (New England Biolabs) with primers designed to amplify a 420-base-pair product around the variation point (forward: 5'-CTAGAAACCCCGAAACCAAA-3', reverse: 5'-TCATACTAACAGTGTTCATC-3').

The PCR reaction was column-purified and analysed using Oxford Nanopore Technology sequencing (Plasmidsaurus). A custom Python script (available via GitHub at <https://github.com/GreletLab/mtDNA-heteroplasmy>) was developed specifically for this project to process the obtained raw reads, align them to the target sequence, classify each read as wild type (mouse host-derived) or mutated (cancer cell-derived) and generate statistical output. The script was validated using mtDNA sequence from pure 4T1 cell extract or BALB/c mouse tissue, as well as through the manual counting of some samples to ensure the accuracy of the output.

Intraductal human-in-mouse transplantation model

Intraductal transplantation of human ductal carcinoma in situ cells was performed as previously described¹³. In brief, before transplantation into 6-week-old virgin female SCID-beige mice, DCIS.COM cells were resuspended as single cells in PBS and counted. A 30-gauge Hamilton syringe, 50- μ l capacity, with a blunt-ended 1/2-inch needle, was used to deliver the cells. The mice were anaesthetized, and a Y-incision was made on the abdomen to allow the skin covering the inguinal mammary fat pads to be peeled back to expose the inguinal gland. The nipple of the inguinal gland was snipped so that the needle could be directly inserted through the nipple. Two microlitres of cell culture medium (with 0.1% trypan blue) containing cells at a concentration of 2,500 to 5,000 cells per microlitre was injected; the injected liquid can be visually detected in the duct. The skin flaps were repositioned normally and held together with wound clips.

Chemical denervation

BoNT/A denervation was performed as previously described⁸, adapting the procedure through intraductal delivery of the toxin 4 weeks after the intraductal transplantation of the cells, and the experiment was concluded at 10 weeks. As previously described, the loss of nerve function, whether through physical or chemical denervation procedures, resulted in axonal atrophy and decreased intratumoural nerve density with an effect akin to Wallerian degeneration^{6,16,70,71}. BoNT/A was reconstituted in 0.1 ml of 0.9% saline without preservatives to achieve a concentration of $1\text{ U }\mu\text{l}^{-1}$ and was utilized within 2 h of reconstitution. For the intraductal model, 15 U kg^{-1} of BoNT/A per xenograft was carefully injected using a 30-gauge needle and a precision glass syringe, with meticulous attention to avoid extra ductal leakage. For the mammary fat pad injection model, 0.3 units of BoNT/A in $100\text{ }\mu\text{l}$ PBS was injected

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into the mammary fat pad 1 week before the injection. One million 4T1 cancer cells (100 μ l of a suspension of 10 million cells per ml) were injected into the same mammary fat pad. Using calipers, the tumour's height and width were subsequently estimated.

Mammosphere assay

Cancer cells isolated from the MitoTRACER coculture and established as long-term subcultures were seeded at 5,000 cells per ml in 96-well ultralow-attachment plates. Mammospheres were collected on the seventh day of culture, washed with PBS, and sedimented for 30 min. All mammospheres with diameters greater than 50 μ m were counted with a fluorescence microscope (BZ-X810, Keyence).

Invasion assay

Cell invasion was assessed using the Corning BioCoat Matrigel Invasion Chamber (Fisher Scientific), following the manufacturer's instructions. In brief, the Matrigel-coated chambers were rehydrated with serum-free medium for 2 h at 37 °C in a humidified incubator with 5% CO₂. After rehydration, 50,000 cells were seeded in the upper chamber in a serum-free DMEM medium; the lower chamber contained DMEM supplemented with 20% FBS as a chemoattractant. The plates were incubated for 24 h at 37 °C. Non-invaded cells and Matrigel were removed from the upper membrane surface using a cotton swab. The invaded cells on the bottom side of the membrane were fixed and stained with DAPI. Fluorescence microscopy was used to image the stained cells, and a custom image analysis script (available via GitHub at <https://github.com/GreletLab/DAPI-count>) quantified the number of cells that migrated through the membrane. The script was previously validated by traditional manual counting to compare to the output data and ensure its accuracy. All assays were performed in independent triplicate.

Shear stress assay

The shear stress assay was performed as previously described³⁸. In brief, cancer cells were resuspended at a concentration of 1×10^5 cells per ml and loaded into the injection pump using a 30-ml syringe. The suspension was dispensed through a 26-gauge, 150-mm blunt tip Luer lock needle at a controlled flow rate of 0.25 ml s⁻¹. The cells were subjected to 10 shear cycles, after which cell viability and quantity were assessed using a Celigo image cytometer. Cell viability was determined with Hoechst–propidium iodide staining to evaluate cell quantity and cell death.

Oxidative stress assay

To induce oxidative stress, cancer cells were cultured in vitro and treated with increasing concentrations of hydrogen peroxide. Cells were seeded at a density of 50×10^4 cells per well in a 96-well plate and allowed to adhere overnight. The following day, cells were exposed to a range of hydrogen peroxide concentrations from 100 mM to 9.375 μ M in serum-free medium for 24 h. After treatment, cells were washed with PBS and analysed for recovery before Hoechst–propidium iodide staining to evaluate cell quantity and cell death using the Celigo image cytometer.

Calcium flux imaging

Nerve–cancer cocultures were grown in Nunc Lab-Tek II chambered coverglass slides (Roskilde) at 37 °C with 5% CO₂. Cells were stained with Fluo-4AM calcium indicator dye and Hoechst 33342 (Invitrogen) just before imaging, following the manufacturer's instructions. Time-lapse videos were acquired using a Nikon A1r confocal microscope equipped with a stage-top incubator for temperature and gas control, using a 20 $\times$ objective lens (numerical aperture 0.8). Images were captured every 15 s over a duration of 10 min. Calcium fluorescence intensity was quantified by defining a region of interest around the cell to be measured and calculating the mean fluorescence

intensity of the green channel within the region of interest for each time point.

Mouse xenograft, lineage tracing and fate-mapping experiments

Mixed-cell MitoTRACER spheroids were injected into the mammary fat pads of 6-week-old female BALB/c mice. In accordance with the Institutional Animal Care and Use Committee (IACUC) protocol for this study, tumour volumes were monitored every 2–3 days using digital calipers, and humane end points were applied if tumours exceeded 2 cm³ or signs of distress or ulceration appeared. Tumour volume was estimated through (short side $\times$ short side $\times$ long side)/2. The maximal size set by IACUC was 2 cm³ and was not exceeded in any experiment.

At end point, primary tumours, lungs and brains were collected, rinsed in PBS, and processed for dissociation, cell culture and flow cytometry to quantify red-to-green cell ratios in both primary and metastatic sites. Dissociated cell suspensions were filtered through 40- μ m strainers, centrifuged at 300g for 5 min, and seeded in DMEM for further analysis. To confirm metastasis at distant sites, a subset of cells was cultured in vitro with Blasticidin selection for 2–4 weeks before re-examination of red and green fluorescence signals.

Tumour dissociation was performed using the Tumor Dissociation Kit (Miltenyi Biotec 130-096-730); tumours or organs weighing between 0.04 and 1 g were cut into 2–4-mm pieces and placed in 1–2.5 ml of enzyme medium. Tumours weighing more than 0.2 g were placed in C-tubes and processed using the gentleMACS Dissociator both before and after incubation. Samples not placed in C-tubes were manually separated after incubation using a filter. All samples were then strained through a 40- μ m or 70- μ m filter into a 50-ml tube and rinsed with 10–20 ml of RPMI or DMEM. The samples were centrifuged at 500g for 7 min, after which the supernatant was discarded, and flow buffer was added.

Dual immunohistochemistry and inForm analysis

The human breast cancer tissue microarray was purchased from Bio-Techne. The prostate cancer perineural array and controls were obtained at Baylor College of Medicine. Tissue sections from four patients with prostate cancer were obtained from the NCT01520441 phase 1/2 trial designed as a proof of principle that nerves affect the biology of cancer in humans. Patients served as their own controls by receiving BoNT/A injections into the right peripheral and transition zones and sham saline injections into the left peripheral and transition zones². A paired treated cancer and control was available for only one patient and was used for this study.

Tissue microarray slides and clinical trial slides were dual-stained with antibodies to mitochondria (NeoBiotechnologies, catalogue number MSM2-740-PIABX) and PGP 9.5 (Invitrogen, PA5-29012) using the Dako Omnis instrument with Envision Flex HRP and high-pH reagents (Agilent, Dako Omnis, GV800). Antigen retrieval was performed using EnV FLEX TRS at high pH (97 °C for 30 min). The PGP 9.5 antibody was used at a dilution of 1:1,500 with a 30-min room-temperature incubation, and the mitochondria antibody was used at 1:5,000 with a 30-min room-temperature incubation. Diaminobenzidine and magenta were used as chromogens for PGP 9.5 and mitochondria, respectively.

Slides with dual immunohistochemical staining were converted to high-resolution digital images through multispectral imaging using a Nuance Multispectral Microscope (PerkinElmer). The images were analysed using inForm analysis software (Akoya Biosciences), as previously described³². The system was trained to recognize and segment cancer, stroma and empty spaces. After segmentation of image compartments, each cell within the identified compartments was analysed separately for nuclear and cytoplasmic regions. Mitochondrial load was quantified by measuring the optical density of magenta staining specifically in the cytoplasm of cancer cells.

Statistics and reproducibility

Statistical analyses were conducted using Prism 10 for macOS (GraphPad) and Excel for MacOS (Microsoft). Unless otherwise specified in the figure legends, all in vitro data analyses were performed using unpaired *t*-tests for pairwise comparisons. For clinical data interpretation, high-throughput analysis of mitochondrial load in cancer cells from clinical histology samples was conducted. The equality of variances was first assessed using Levene's test. A standard Student's *t*-test was used for comparisons when variances were equal, and Welch's *t*-test was applied when variances were unequal. For animal studies, unless specified otherwise, data were analysed using ANOVA with Tukey's multiple comparison test. Two-tailed *t*-tests were used for exploratory experiments, and one-tailed *t*-tests were used when a directional effect was supported by prior evidence presented earlier in the study. The number of independent animals used is provided in the figure legends. Shear stress and Seahorse metabolic assays were conducted independently three times, with multiple culture replicates per assay. For Seahorse measurements, 8 to 16 technical replicates were included per assay, depending on the number of biological conditions tested in the 96-well plate. The experiments were analysed using the Wave Controller software (Agilent Technologies) and Prism 10 for MacOS, and graphs shown represent a typical experiment. Microscopy, western blot and PCR experiments were performed at least in triplicate, and representative images are provided. Uncropped western blots and PCR gels are provided in the Supplementary Information. *P* values are reported as exact values or symbolically as follows: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. A *P* value of less than 0.05 was considered to indicate nominal statistical significance.

Animals and housing

Six-week-old virgin female SCID-beige mice were used for the intraductal breast cancer model. The mammary fat pad injection model was performed on BALB/c female mice (age, 5 weeks) and flank injection of B16-F10 in C57BL/6 male mice (age, 6 weeks). Mice were housed under a 12 h/12 h light/dark cycle in a barrier vivarium. Room temperature (22.8 °C ± 1.7 °C) and relative humidity (30–70%) were continuously monitored and maintained. Animals were housed in cages of four, and each experimental condition was assigned to one cage. For each experiment, one cage was randomly selected from a pool of pre-assigned condition-specific cages. Although allocation was not randomized at the individual animal level, random selection at the cage level ensured unbiased assignment. All mice were age- and sex-matched, and housed under identical environmental and handling conditions to minimize potential covariates.

Institutional Review Board statement

The study was conducted according to the guidelines of the Declaration of Helsinki. All experiments and procedures were conducted in accordance with the guidelines described in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). Approvals for animal work were obtained from the Baylor College of Medicine Animal Care and Use and Human Subjects Committee and the University of South Alabama IACUC. Informed consent was obtained from all human participants involved in the study.

Reporting summary

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

Data availability

Plasmids generated for this study have been deposited in the Addgene database (greletlab; https://www.addgene.org/Simon_Grelet/). The bioinformatic datasets for the RNA-seq and RNA microarray experiments

are available in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession numbers GSE288069 and GSE292157, respectively. Source data are provided with this paper.

Code availability

Custom code developed for this study is available via GitHub at <https://github.com/GreletLab/mtDNA-heteroplasmy> and <https://github.com/GreletLab/DAPI-count>.

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Author contributions S. Grelet supervised the study, designed the study and conceived methodological development, and acquired funding. G.A. collaborated in study design and methodological development and supervised pathological interpretations; G.H., S. Gilbert and O.C. assisted in developing the cell-genetic models and other methodological development throughout the study; S. Gilbert, O.C., C.O. and S. Grelet performed the TNBC and melanoma animal work; C.O. and S. Grelet developed the SVZ-NSC cultures and the nerve-cancer coculture model; G.H., Y.D., P.B., G.A. and S. Grelet developed the DRG-based nerve-cancer coculture model; J.F.A., G.A. and S. Grelet assisted with microscopy experiments; G.H., M.F.A.

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and S. Grelet assisted in the generation of ρ^0 cells; S. Grelet performed RNA-seq and Nanopore bioinformatics interpretation and wrote the code presented in the study; Y.D. and P.B. assisted in the processing and interpretation of pathology samples; J.T.C. designed and performed microarray bioinformatic and statistical gene-expression analyses in the Botox denervation model of ductal carcinoma in situ; F.B. and D.M. developed the intraductal breast carcinoma model; G.H., M.T.L., G.A. and S. Grelet performed and interpreted electrophysiology experiments and assisted in manuscript editing; and W.H. and T.W.P. assisted in experimental development and troubleshooting. S. Grelet wrote the manuscript, and S. Grelet and G.A. validated it. All authors reviewed the manuscript and approved the final version for publication.

Competing interests The MitoTRACER technology developed in this manuscript is covered under a pending patent application titled Methods and applications for monitoring

mitochondrial transfer between donor and receiver cells, US Patent and Trademark Office, application number 18/885,864. S. Grelet and G.H. are listed as inventors of the technology.

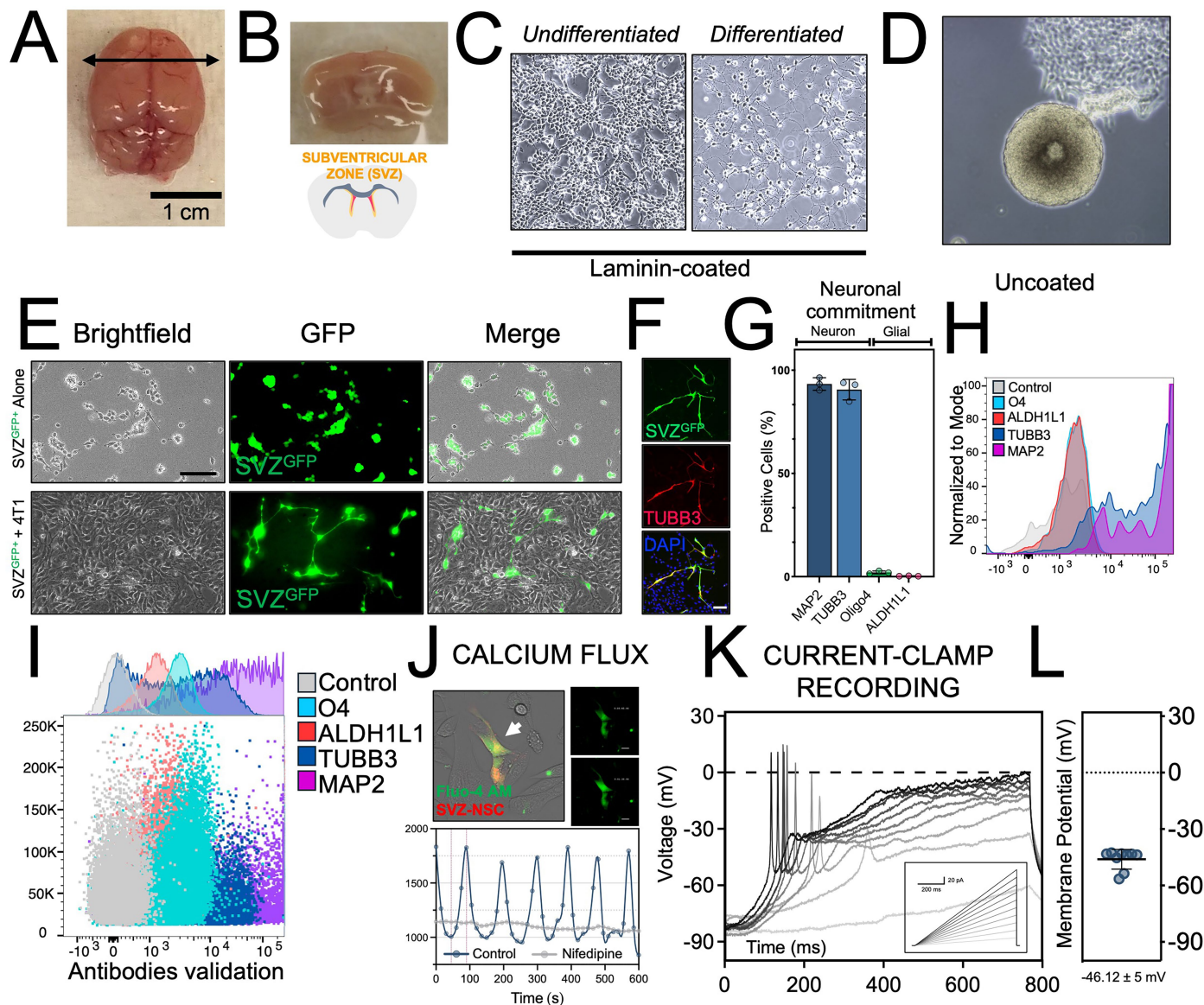
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-025-09176-8>.

Correspondence and requests for materials should be addressed to Gustavo Ayala or Simon Grelet.

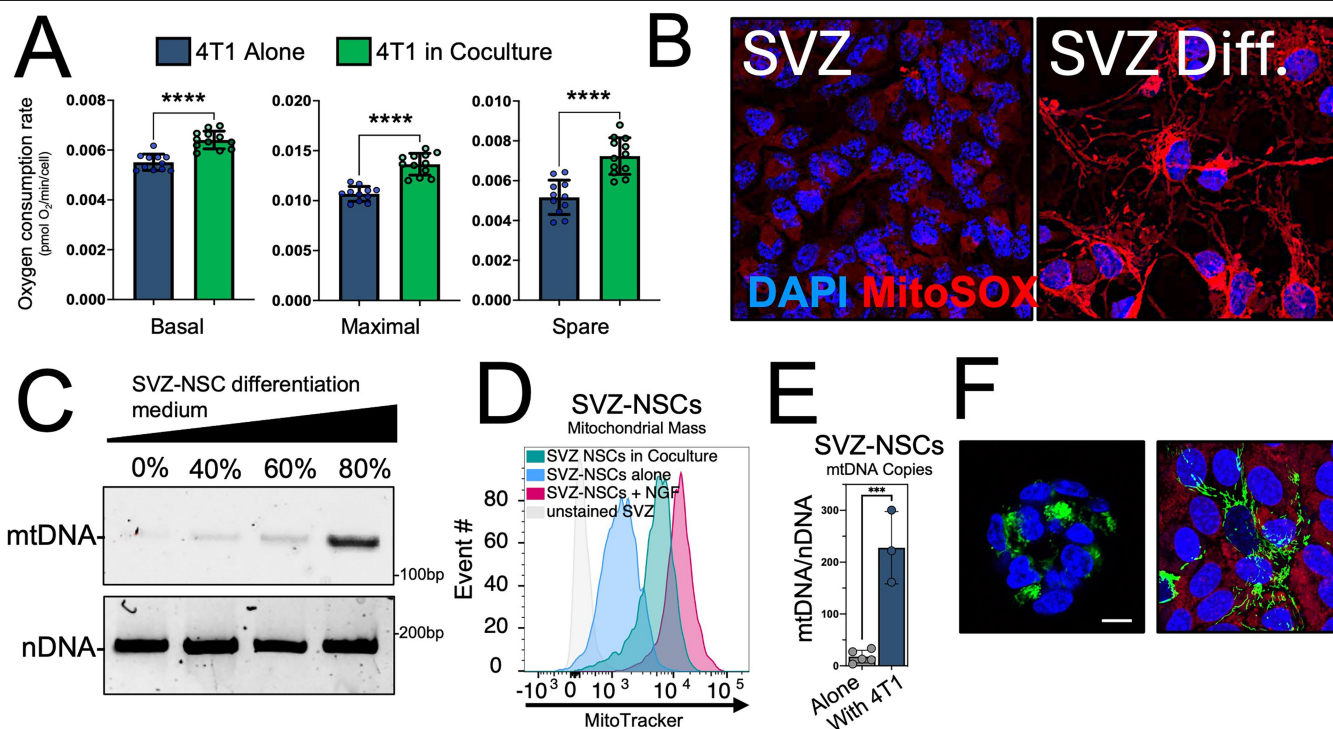
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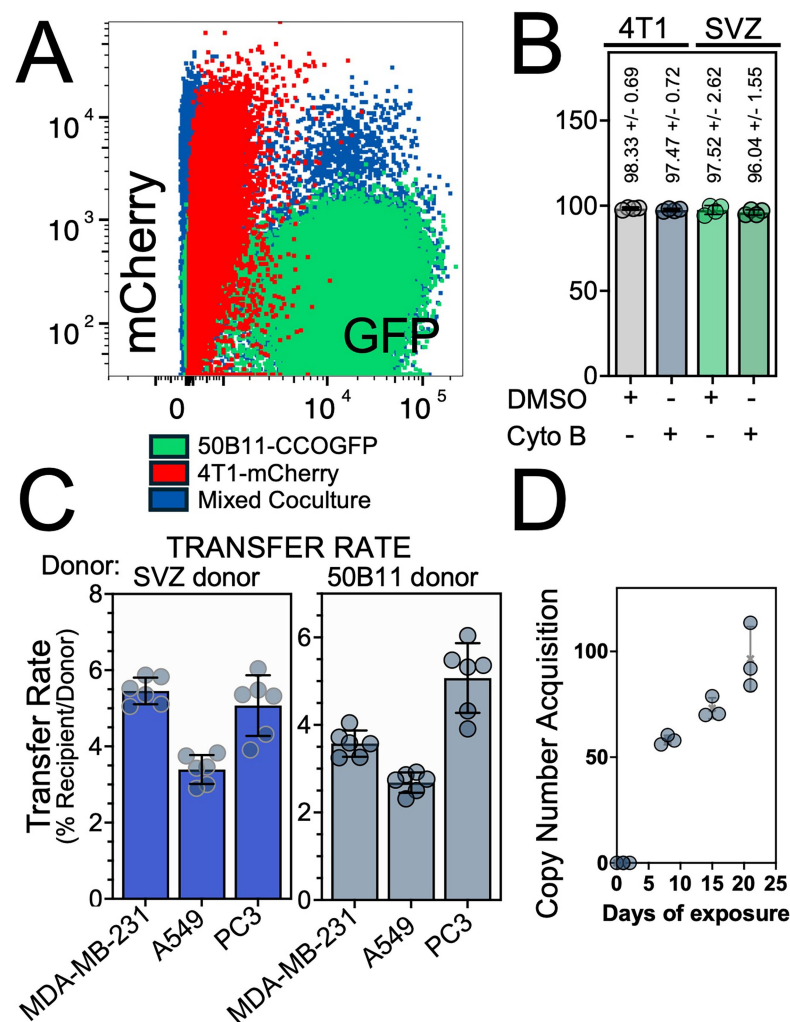
Extended Data Fig. 2 | Related to Fig. 1 - Nerve-cancer crosstalk model in vitro. (A) Subventricular zone neuronal stem cells (SVZ-NSCs) were isolated from a fresh 6-week-old female BALB/c mouse brain. The dorsal view of the dissected mouse brain is shown (scale bar, 1 cm). (B) Lateral view of the mouse brain showing the translucent SVZ region. The diagram shows the area (orange) from which SVZ-NSCs were extracted. (C) Isolated SVZ-NSCs were cultured on laminin-coated plates. The cells differentiated into mature neurons using a differentiation medium. (D) The SVZ-NSCs were maintained as neurospheres on uncoated plates, confirming stemness (self-renewal). (E) Fluorescence microscopy of SVZ-NSCs transduced with GFP-expressing lentiviruses and cultured independently (SVZ^{GFP} Alone) showed moderate neurite development, evidenced by the absence of neurite extensions. SVZ-NSCs cocultured with 4T1 cells (SVZ-NSC^{GFP} + 4T1) showed a marked increase in SVZ-NSC differentiation, evidenced by extensive neurite outgrowth traversing the coculture. Scale: 100 μm. (F) Immunohistochemistry of SVZ-NSC^{GFP} cocultured with 4T1 cells shows SVZ-NSCs expressing the neuronal marker Tubulin β-3 (Tubb3), confirming neuronal differentiation. Scale: 100 μm. (G) Flow cytometry analysis of nerve-cancer cocultures indicates the commitment of neuronal precursors to neurons. Nearly all neurons expressed MAP2 and Tubulin β-3 (TUBB3) but were negative for O4 and ALDH1L1, confirming that these cells were not oligodendrocytes or astrocytes. (Mean ± S.D., Student's two-tailed unpaired t-test, n = 3). (H) Histogram plot from flow cytometry illustrating the neuronal commitment of SVZ-NSCs in the nerve-cancer coculture. EGFP-expressing SVZ-NSCs, introduced into the coculture and comprising

approximately 1.6% of the total cell population in the series, were gated based on eGFP expression to assess SVZ marker expression using antibodies against O4, ALDH1L1, TUBB3, and MAP2. The omission of the primary antibodies in coculture consisting of eGFP-expressing SVZ-NSCs mixed with 4T1 cancer cells was used as control. (I) Validation of antibodies used to quantify the neuronal commitment of SVZ-NSCs in the nerve-cancer coculture. SVZ-NSCs were treated with triiodothyronine (T3) to induce oligodendrocyte commitment and stained with the O4 antibody. To induce astrocyte differentiation, cells were treated with fetal bovine serum (FBS) and stained with the ALDH1L1 antibody. For neuronal differentiation, cells were treated with differentiation medium and stained with Tubulin β3 (TUBB3) or Microtubule-Associated Protein 2 (MAP2) antibodies. The omission of primary antibodies on SVZ-NSCs culture was used as control. (J) Calcium flux analysis in nerve-cancer coculture using Fluo-4 AM staining. (Top) Time-lapse imaging showed pulsatile calcium activity in neurons. (Bottom) Signal dynamics with 10 μM nifedipine treatment demonstrated a blockade of calcium flux, abolishing pulsatile activity. (K) Current-clamp recording of neurons in nerve-cancer coculture. Whole-cell recordings were used to record the membrane potentials of neurons. Cells clamped at -80 mV, were depolarized with incremental current ramp injections ranging from 20–200 pA, as shown in the inset. Cocultured neurons showed action potential activity. (L) Distribution of action potential threshold measurements between neurons analyzed in the nerve-cancer coculture. Action potentials were elicited in all recorded neurons (mean ± SEM n = 9 neuron cells acquisition; 100% response).



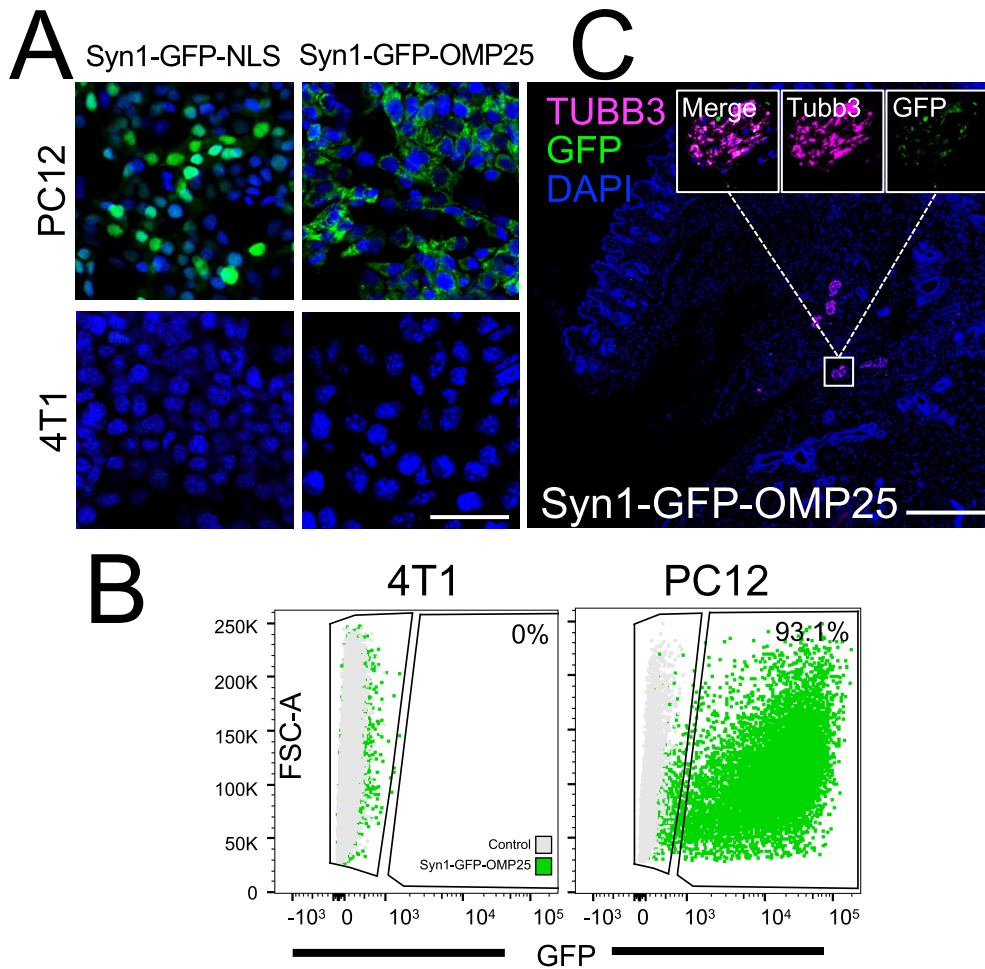
Extended Data Fig. 3 | Related to Fig. 1 - Nerve-cancer crosstalk and mitochondrial metabolism. (A) Related to Fig. 1d - Oxygen consumption rate (OCR) analysis comparing basal, maximal, and spare respiratory capacities of 4T1 cells alone versus coculture with SVZ-NSCs. Coculture led to a significant increase in all three parameters, suggesting enhanced mitochondrial function (Representative Profile (n = 3); Mean $\pm$ SEM; Student's two-tailed unpaired t-test **** $P < 0.0001$). (B) MitoSOX mitochondrial indicator staining (red) of SVZ-NSCs before (SVZ-NSC) and after differentiation (NSC Diff.). There was a significant increase in mitochondrial activity observed after differentiation, evidenced by an increase in MitoSOX staining. (C) Cancer-driven neuronal differentiation markedly upregulated mitochondrial load in SVZ-NSCs. PCR of mtDNA content in SVZ-NSCs under varied concentrations of differentiation medium (0% to 80%) showed a progressive increase in mtDNA content throughout differentiation (nDNA, nuclear DNA loading control). (D) FACS analysis following mitochondrial

staining (MitoTracker) shows SVZ-NSCs in three conditions: undifferentiated and cultured alone (blue, SVZ-NSCs alone), differentiated with nerve growth factor (red, SVZ-NSCs + NGF), and differentiated by coculture with 4T1 cancer cells (green, SVZ-NSCs in coculture). Representative Profile (n = 3 independent cell cultures). (E) FACS and quantitative polymerase chain reaction (qPCR) of mitochondrial DNA (mtDNA) to nuclear DNA (nDNA) ratio in SVZ-NSCs alone (Alone) or in coculture with 4T1^{mCherry} cells (With 4T1) (mean $\pm$ S.D.). There was increased mitochondrial mass in SVZ-NSCs after exposure to 4T1 cells. (F) Fluorescence microscopy of SVZ-NSCs having eGFP-labeled mitochondria (SVZ-NSC^{CCO-GFP}). The mitochondria had globular morphology and were localized around the nucleus. In contrast, SVZ-NSC^{CCO-GFP} cocultured with 4T1^{mCherry} cells had mitochondria with elongated morphology and were dispersed throughout the cell cytoplasm. Scale: 10 μ m.



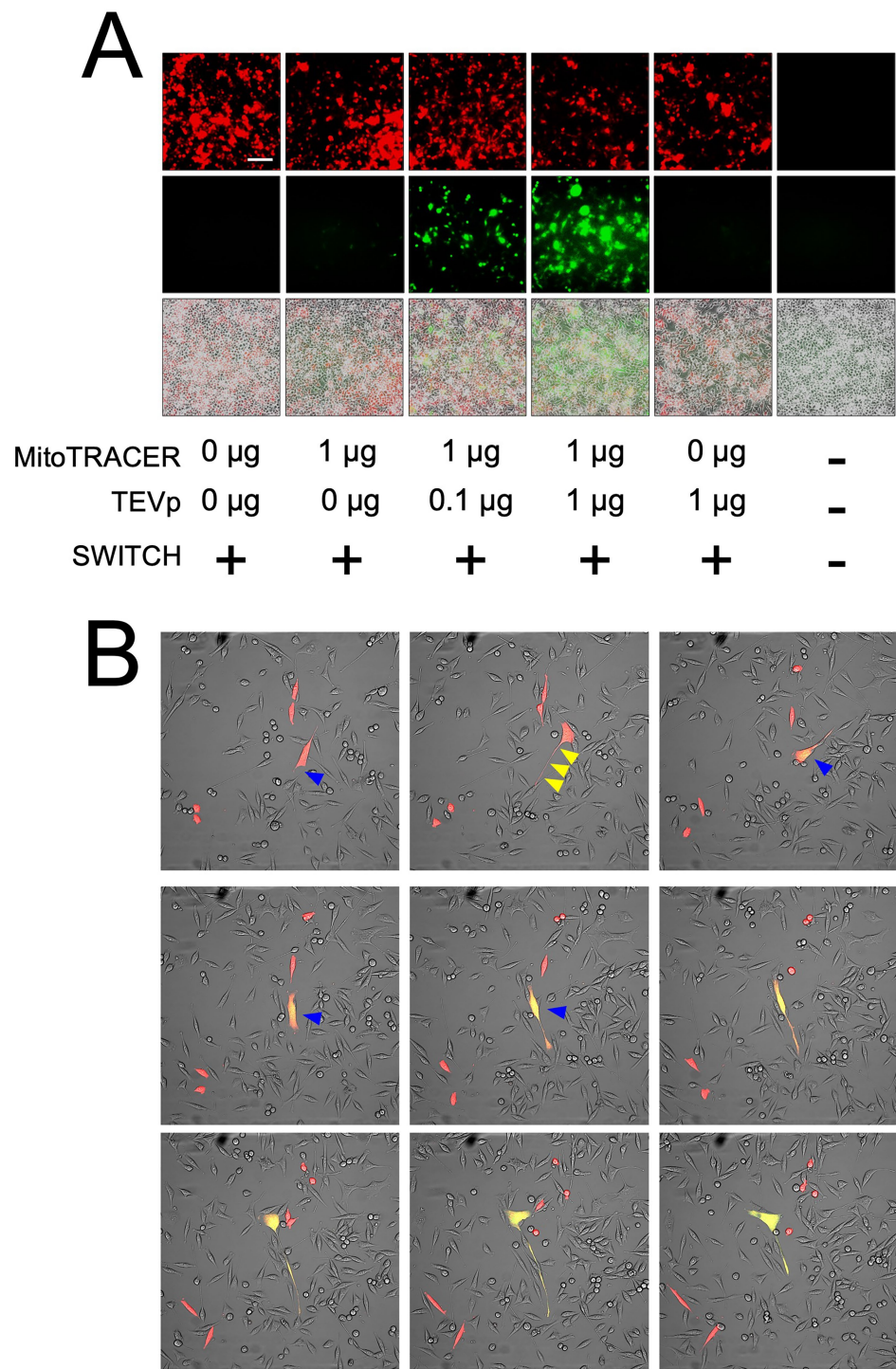
Extended Data Fig. 4 | Related to Fig. 1 - Nerve-cancer transfer of mitochondria in vitro. (A) Quantification of mitochondrial transfer. Flow cytometry confirmed strong eGFP fluorescence in 50B11 DRG-derived cells (50B11^{CCO-GFP}). When cocultured with 4T1^{mCherry+} cells, mitochondrial transfer was indicated by the acquisition of eGFP fluorescence in the 4T1^{mCherry+} cells. The gating strategy was established using 50B11^{CCO-GFP} and 4T1-mCherry cells cultured independently. (B) Cell viability of cultures treated with cytochalasin B (Cyto B) for 24 h. The percentage of live cells was calculated using Hoechst/PI staining and automated counting with a fluorescence cytometer (Celigo).

No significant change in cell viability was observed after treatment in both SVZ-NSC and 4T1 cancer cells. (C) Quantification of mitochondrial transfer from (Left) SVZ-NSC^{CCO-GFP} or (Right) 50B11 mitochondrial donor cells to cancer cell lines derived from human breast (MDA-MB-231), lung (A549), and prostate (PC3) carcinomas. The normalized transfer rate was calculated as the percentage of recipient cells within the eGFP+ population in the coculture, standardized across biological conditions. Mean ± S.D. (n = 6). (D) Related to Fig. 1m. Quantitative PCR analysis of mtDNA acquisition in p⁰ 4T1 cancer cells exposed to SVZ-NSCs and FACS isolated from the co-culture. Mean ± S.D. (n = 3).



Extended Data Fig. 5 | Related to Fig. 2 - Nerve-cancer transfer of mitochondria in vivo. (A) Microscopy validation of the Syn1-GFP-NLS and Syn1-GFP-OMP25 lentiviral constructs in PC12 neuronal cells and 4T1 breast cancer cells. Only neurons displayed expression of the synapsin 1-driven constructs, with eGFP localized to the nucleus (localization signal, NLS) or mitochondria (OMP25). DAPI was used as a counterstain for the nuclei. Scale: 50 μ m. (B) Flow cytometry validated the Syn1-GFP-OMP25 construct and confirmed neuron-specific expression by showing strong expression in

PC12 neuronal cells and no activity in 4T1 cancer cells. This analysis also determined the titer of the production batch. (C) Immunohistochemical staining of mouse mammary fat pad xenograft tissue nerves using tubulin beta-3 (Tubb3) and labeling of the eGFP fluorophore confirms the expression of the transgene in the mouse host nerves. 4X magnification of the xenograft area. Zoom: Nerve fiber at 40X magnification. Magenta: tubulin beta-3, Green: eGFP, Blue: DAPI. Scale: 500 μ m.



Extended Data Fig. 6 | Related to Fig. 3 – MitoTRACER. (A) Validation of the cloning constructs. We tested the plasmid constructs by co-transfection of MitoTRACER with increasing amounts of a separated TEVp-encoding plasmid into 4T1 cells stably expressing the LoxP-DsRed Express2-STOP-LoxP-eGFP recipient genetic switch but lacking the TEVp construct. Fluorescence microscopy showed activation of the Red-to-Green fluorophore switch when all components of the system were expressed in the cells by co-transfection with the TEVp. This confirmed the Red-to-Green switch and the requirement

of TEVp to ensure feasibility. Scale: 100 μ m. (B) Time-lapse fluorescence microscopy over 12 h of SVZ-MitoTRACER mitochondrial donor cells cocultured with 4T1 SWITCH-TEVp recipient cells, illustrating the dynamics of mitochondrial transfer. The process included the establishment of close cell-cell contacts, the development of tunneling nanotube structures, and subsequent red-to-green fluorescence transition in recipient cells. Arrowheads: blue recipient cells; yellow, tunneling nanotube structure. Captures from Video S3.

Reporting Summary

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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.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

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Software and code

Policy information about [availability of computer code](#)

Data collection	Data was collected using Excel for MacOS (v16.91 ; Microsoft), FACS Diva (v8.0.1 build 2014 07 03 11 47; BD Biosciences), ImageLab (v6.1.0 build 7 ; Bio-Rad Laboratories), Wave Controller (Agilent Technologies), NIS Element Advanced Research (Nikon), Celigo (Nexcelom), and BZX800 Viewer Capture Still Images (Keyence).
Data analysis	Data were analyzed using Excel for macOS (v16.91; Microsoft), Prism 10 for macOS (v10.4.2; GraphPad), FlowJo (v10.8.1; BD Biosciences), and ImageJ (v2.1.0/1.53c; NIH, USA). Gene expression data were analyzed using R for macOS (v4.1.1 GUI 1.77; R Foundation for Statistical Computing) with Bioconductor libraries (v3.14) DESeq2 (v1.46.0) "marray" and "samr", Sambamba (v1.0.1), SAMtools (v1.21), , and Bowtie2 (v2.5.4). inForm analysis software (Akoya Biosciences) was used for pathology scoring. pClamp software (v8.0 ; Molecular Devices) have been used for electro-physiology analysis. Custom Codes are available in the following links: - https://github.com/GreletLab/DAPI-count - https://github.com/GreletLab/mtDNA-heteroplasmy

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All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
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The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

The bioinformatic datasets for the RNA sequencing experiment is available in the NCBI SRA under the accession number "GSE288069":

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE288069>

The RNA microarray dataset is deposited in the NCBI SRA under the accession number "GSE292157":

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE292157>

Plasmids generated for this study are deposited in the Addgene database (greletlab).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	This is a pilot study examining biological endpoints in men with localized PCa who are scheduled to have radical prostatectomies. Four patients with bilateral localized PCa were recruited to a Phase I/II clinical trial: Neoadjuvant Botox injection prior to radical prostatectomy (NCT01520441), (IRB: H-25362).
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	The trial was designed as a proof of principle that nerves affect the biology of PCa in humans. The inclusion criteria were as follows: male and at least 50 years of age; biopsy-proven, clinically localized PCa; low risk for recurrence defined as a Kattan nomogram score of less than 115, serum PSA<10ng/mL, individual Gleason grade of 7 or lower, or clinical stage T2b or below; must have agreed to radical prostatectomy and signed an informed consent; and the patient must be able to complete the study protocol in the opinion of the investigator.
Recruitment	The inclusion criteria were as follows: male and at least 50 years of age; biopsy-proven, clinically localized PCa; low risk for recurrence defined as a Kattan nomogram score of less than 115, serum PSA<10ng/mL, individual Gleason grade of 7 or lower, or clinical stage T2b or below; must have agreed to radical prostatectomy
Ethics oversight	The study was approved IRB: H-25362. Approvals were obtained from both the Baylor College of Medicine Animal Care and Use and Human Subjects Committee. Informed consent was obtained from all human participants involved in the study.

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

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Life sciences study design

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

Sample size	The experiments were independently repeated at least three times, each performed in triplicate. The exact number of mice used in each experiment is detailed in the corresponding figure legend. Similarly, the number of patient samples included in the clinical data is specified in the figure legend. While no formal prospective sample size calculation was performed, sample sizes were chosen based on prior experimental experience and reproducibility of outcomes in comparable studies. Previous analyses allowed us to estimate effect sizes using Cohen's d, which informed the selection of sample sizes that provide sufficient statistical power to detect biologically relevant differences using appropriate statistical tests, as specified in each figure legend.
Data exclusions	For the transcriptomic dataset, the samples not meeting quality requirements were not included in the analysis. No inclusion/exclusion criteria were used for other samples or animals.
Replication	Experiments were replicated at least three times and repeated independently. Attempts of replication were successful.
Randomization	Animals were housed in cages of four, and each experimental condition was assigned to one cage. For each experiment, one cage was

Randomization	randomly selected from a pool of pre-assigned condition-specific cages. Although allocation was not randomized at the individual animal level, random selection at the cage level ensured unbiased assignment. All mice were age- and sex-matched, and housed under identical environmental and handling conditions to minimize potential covariates.
Blinding	Blinding was not performed in this study because the experimental procedures, data acquisition, and analysis involved objective measurements that are not influenced by operator bias. All data processing and statistical analyses were conducted using standardized, automated pipelines, minimizing the potential for subjective interpretation.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

α -Tubulin - Cell Signaling (#2144s, Lot5)
 Cre Recombinase - Cell Signaling (#15036, Lot2 clone D7L7L)
 GFP - Santa Cruz Biotechnology (#sc-9996, LotE2521, Clone B-2)
 HA Tag - Santa Cruz (#sc-7392, LotI0922, clone F-7)
 mCherry - Cell Signaling (#43590S, Lot2, clone E5D8F)
 ALDH1L1 (OriGene TA501868, Lot A01, Clone OT17G8)
 O4 (R&D Systems MAB1326, Lot: HWW143051 Clone O4)
 Map2 (Proteintech 17490-I-AP, Lot: 00126726)
 Tubb3 (Santa Cruz sc80005, Lot: A1821, Clone D-10)
 Secondary Mouse or Rabbit HRP (ThermoFisher PI31430 or PI31460)
 Alexa Fluor™ 647 secondary antibody (ThermoFisher A21235, Lot 2836809; A21244 Lot 2674387).

Validation

Antibodies against Cre, mCherry, GFP, and the HA tag were validated in this study using negative (wild-type cells) and/or positive (overexpression) controls. The α -tubulin antibody (Cell Signaling Technology, #2144) used as a loading control was validated by the manufacturer for Western blotting, and its reliability has been extensively confirmed in the literature. For flow cytometry, antibodies targeting TUBB3, Oligo4, MAP2, and ALDH1L1 were validated by the manufacturers. In addition, their specificity was confirmed in this study by demonstrating their ability to detect the corresponding cell types following subventricular zone (SVZ) cell differentiation, as shown in Figure S2I.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

4T1, 50B11, PC-12, HT22, Neuro2A, and NMuMG cells were obtained from the American Type Culture Collection (ATCC). 3T3-L1 cells were purchased from Kerastat, Inc. Mouse embryonic fibroblasts (MEFs) were a gift from R. Stephen Lloyd's laboratory and were generated from C57BL/6 mice. DCIS.COM cells were purchased from Asterand, Inc. Primary SVZ neural stem cells (SVZ-NSCs) were obtained by dissection from fresh brains of BALB/c mice purchased from the Jackson Laboratory.

Authentication

The cell lines used on this study has been authenticated by genomic profiling.

Mycoplasma contamination

The cell lines were routinely checked for mycoplasma contamination by using the MycoStrip Test Kit (Invivogen), and through Hoechst-33342 staining. Plasmocin was used as a prophylactic treatment to prevent mycoplasma contamination. All cells were negative for mycoplasma. Cells were tested by IMPACT testing (Idexx Bioanalytics) prior to animal work.

Commonly misidentified lines (See [ICLAC](#) register)

No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	6 week-old virgin female SCID-beige mice were used for the intraductal breast cancer model. Mammary fat pad injection of mixed-cell MitoTRACER spheroids was performed on BALB/c female mice (age, 6 wk). Mice were housed under a 12 h:12 h light:dark cycle in a barrier vivarium. Room temperature ($22.8^{\circ}\text{C} \pm 1.7^{\circ}\text{C}$) and relative humidity (30 %–70 %) were continuously monitored and maintained.
Wild animals	No wild animals used in the study
Reporting on sex	Female animals were used to align with the origin of the breast cancer cell lines and SVZ NSCs.
Field-collected samples	No field-collected samples used in the study
Ethics oversight	All experiments and procedures were conducted in accordance with the guidelines described in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD, USA). Approvals were obtained from both the Baylor College of Medicine Animal Care and Use and Human Subjects Committee and the University of South Alabama Institutional Animal Care and Use Committees.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Plants

Seed stocks	No seeds associated to the study.
Novel plant genotypes	No plants associated to the study.
Authentication	No plants associated to the study.

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	Cells were detached using 0.25% trypsin with EDTA, then resuspended in culture media. After centrifugation and supernatant removed, the cell pellet was gently broken up and prevented from clumping by tapping the tube. It was then resuspended in
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	PBS containing 2% serum and kept on ice until processing
Instrument	BD FACSAria II Cell Sorter and BD FACSCanto II were used in this study.
Software	BD FACSDiva Software for cell collection and analysis. The .fcs data were analyzed using FlowJo10 software.
Cell population abundance	100,000 to 8,000,000 cells were analyzed. Sorting was performed with 1 cell per well in 96-well plates (single-cell sorting) or a minimum of 5000 cells per well in 6-well plates containing 2 ml of DMEM with 10% FBS.
Gating strategy	Cells were gated based on their mCherry/GFP fluorescence expression. Before this gating process, doublets and debris were removed from the analysis using FSC-A/FSC-H & SSC-H/SSC-A gating. Cells that did not express (WT) or that overexpressed fluorescent proteins served as negative and positive controls, respectively. These controls were used to define the boundaries of the gating strategy and accurately distinguish between positive and negative populations. In the nerve-cancer co-culture experiments, monocultures were used as negative controls.

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