nature portfolio

Peer Review File

Glia-to-neuron mitochondrial transfer protects peripheral neuropathy

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
To Authors: This nice study proposes a new phenomenon to explain the pathophysiology of neuropathic pain. SGCs attempt to transfer mitochondria to adjacent neurons via tunneling nanotubes to prevent the neuronal hyperactivity. But in disease conditions, this process is impaired thus potentially explaining the development of pain. There are a lot of data and powerful methods are used. The significance may also be high since this phenomenon may provide new therapeutic targets for a difficult clinical problem. However, there are some issues that may require more consideration; more data and discussion may help.

1. The authors propose that SGCs transfer mitochondria to neurons via a process mediated by MYO10. However, the underlying mechanism of how SGCs drive mitochondrial migration via MYO10 to the adjacent neurons remains slightly unclear.
2. Moreover, does MYO10 affect mitochondrial biogenesis in SGCs? How do SGCs maintain their own mitochondrial count/homeostasis if they are “donating” a significant number of mitochondria?
3. The authors suggest that mitochondria transfer may be mediated by TNT. In Figure 1, it seems that over 80% of neurons uptake SGC-derived mitochondria, whereas about 20% of recipient neurons are TNT-positive. Are these estimates correct? If so, does this mean that other processes besides nanotubes play a key role, for example release and uptake of exosomes? Furthermore, TNT-mediated mitochondria transfer is assessed by a loss-of-function by CytoB. Because CytoB strongly inhibits network formation of actin filaments, this may also affect exosome release. Would some data using blockade of endocytosis be useful?
4. Importantly, how SGC-mediated mitochondria transfer to neurons is mitigating neuropathic pain remains to be fully addressed. What is the molecular mechanism? Is this because healthy mitochondrial transfer decreases neuronal hyperactivity; if so how?
5. Single cell analysis in mouse DRG shows Myo10 expression in SGCs in Figure 3. For human data in Figure 4, Myo10 expression may appear in both SGCs and neurons. How would this affect the hypothesis of Myo10-mediated mitochondria transfer from SGCs to neurons? Does this mean that in human cells, the mitochondria transfer can occur in both directions, i.e. also from neurons back to SGCs?
6. Furthermore, snRNA seq shows multiple SGC populations. Clusters 1 and 2 strongly express FABP7 and MYO10 but not other SGC clusters. The authors should provide more details regarding cell type. Are these known subsets from previous literature or are these novel subsets?
7. This paper also includes data suggesting that FABP7 overexpression reduces mechanical pain and protects neurons against chemotherapy-induced peripheral neuropathy. How mitochondria transfer compares to FABP7 in terms of therapeutic effect should be carefully validated and/or discussed.
8. Would it be helpful to look for Mito+Neurons as well as TNT+ neuros in the human cells in Figure 4? Just like what was done in Figures 1 and 3?
9. In Figure 5, xenogeneic human derived mitochondria reduce pain behavior in mice. It has reported that mouse and human cells do not retain functional compatibility of mtDNA (e.g. Yoon et al, Mitochondrion 2007). It may be helpful for authors to

consider these ideas: (i) is mitochondrial DNA not required for mitigating neuropathic pain but only mitochondrial components are sufficient; (ii) if so, then what are they? (iii) can transferred human mitochondria upregulate endogenous Myo10 in mouse SGCs, thus increasing mitochondrial transfer? (iv) if human and mouse mitochondria do not “work together”, then mitochondrial transfer is in fact not required to resolving neuropathic pain? The authors should address or discuss these issues.
10. Mitochondria from diabetic human SGCs appear to decrease the ability to resolve the pain compared to healthy mitochondria. The authors should consider verifying the differences in mitochondrial quality between one from normal vs those from diabetic sources.
11. Directionality may also be relevant for the arrows in Figure 5. Would adopted mitochondria transfer only target neurons? Or these may go into other cells like neurons directly as well?

Referee #2

(Remarks to the Author)
The topic of this MS is tunneling nanotubes (TNT), which are presumed to connect neurons and satellite glial cells (SGCs) in dorsal root ganglia of mice and humans. The authors propose that TNT allow the passage of mitochondria from neurons to SGCs, and present evidence for the importance of this mechanism to chronic pain. The authors used an impressive array of methods, and present a large amount of data. My impression is that in this case, the quantity is not an advantage. Some important conclusions are not well supported, and the Discussion is not satisfactory.
Originality - High
Statistics - OK
Methods - State of the art, but interpretation not always satisfactory.
References – Not always accurate, need to be checked.
Conclusions – Weak, see below for specifics.
Writing – good.

Major comments
1. Line 102, "SGC marker GFAP". GFAP is not an SGC marker. The cited paper (Woodham et al., 1989) states: "… the great majority of satellite cells within the fourth and fifth lumbar dorsal root ganglia on the unoperated side did not react with the polyclonal antisera to GFAP (Figs. 1 and 3). The proportion of neurons surrounded by fluorescent labelled satellite cells ranged from 3% 30% with a mean of 15.5%. Unoperated animals also gave results within that range." Therefore, the identity of the GFAP+ cells in the cultures is not clear, but they cannot be reliably called SGCs. There is evidence that SGCs from sensory ganglia change their phenotype in culture (doi: 10.1017/S1740925X1100007X.).
Thus, the images in Extended Data Fig. 1a probably do not represent SGCs. Also, in this figure it's impossible to find cells other than GFAP+ ones. Each cell type should be indicated. Also, in 5-day cultures neurons should have long processes, which are not visible in the images. Therefore, the experiments based in these cultures are doubtful. In the rest of the paper, SGCs were identified by staining for FABP7, and it's not clear why this was not done for the presumed SGCs in culture. These comments also hold for the results in Fig. 3, Extended Data Fig. 8, and the results on isolated mitochondria from cultures.
2. Line 110. "A quantitative analysis revealed mitochondrial transfer in 84.4% of DRG neurons,
and 27.5% of these neurons exhibited TNTs". How do you explain these numbers, which are apparently not in agreement?

3. Fig. 1e. How do you know that you injected to MitoTracker into an SGC? The images are not very convincing. The bright spot at 1h could be in a neuron.
4. Line 112, concerning mitochondrial transport via TNT. The citation from Ref 20 is incorrect. In Ref 20, P. 92, it is stated: "However, mitochondrial transport between pericytes was not observed, consistent with the closed-ended nature of IP-TNTs". The experiments with the gap junction blocker CBX make no sense, as gap junctions are channers that are too small to allow the passage of mitochondria. The role of gap junctions in mitochondrial transport is still possible, but not through the channels. You did not rule out a role for gap junctions in mitochondrial transport.
5. Extended Data Fig. 1 f,g. It is not clear what is "whole mount DRG". Is this a whole intact ganglion? It is not clear what are the cells that were labeled. The green labeling suggest that these are neurons, but the cellular details are fuzzy. DRG are covered with a connective tissue capsule, can cells connect through the capsule?
6. Fig 1 f, Fig. 4 k. How was it determined that: 1. the fibers are nanotubes (they might be neurites, connective tissue…, 2. That they originate in an SGC? 3. That the bulges (vesicles) are mitochondria? 4. How do you know that there is a vesicle inside the presumed TNT (line 901)?
7. The authors rely heavily ("critically", line 160) on the experiments where CytoB was used. CytoB is an inhibitor of actin polymerization, and can have numerous cellular effects, for example it inhibits protein trafficking in astrocytes, see doi: 10.1523/JNEUROSCI.19-23-10193.1999.
8. It is not clear why observations on the spinal cord were included, as they appear not to be related to the main theme of this work. This, and many other issues are not addressed in the discussion.
9. Line 172. ".. suggesting that transient (hours) tissue injury [by CFA] and inflammation may be insufficient to promote the transfer of mitochondria". However, the effects of a single CFA injection can last for days and even weeks; see 10.1016/s0304-3959(03)00201-x,
10.1016/j.jpain.2004.04.005

10. Fig 2b the boxed area in Tam-10d does not correspond to the magnified images below.
11. Fig. 3. The structure labeled "TNT" contains green (MYOTO) and red (Mitotracker), but the red-stained regions don't look like mitochondria, and there are no bulges/vesicles in it.

12. Lines 241-2, "Myo10 siRNA treatment also resulted in a moderate reduction in paw withdrawal threshold (Extended Data Fig. 6a-b)". But this figure shows no difference under von Frey test.
13. Lines 371-403 and Fig. 5i, You propose that the transferred SGCs formed TNT in the mouse DRG. Also do you propose that the transferred mitochondria entered the DRG cells, thereby exerting a beneficial effect. Is there direct evidence for this?
14. Discussion The discussion is not satisfactory. It is mostly about the potential clinical implications of this work, but not about the results themselves, and their shortcomings. The comments above need to be addressed and in particular comments 1,4,6,8,
Why did you use four different pain models (SNI, PTX, db/db, STZ), and what do we learn from that?
The results support previous reports on the essential roles of SGCs in chronic pain. However, the authors did not discuss their results within the wide picture of neuron-SGC interactions.

Minor comments
1. It is not clear how DRG neurons were purified.
2. Extended Fig. 1f. What does 'in vivo mean'. Scanning EM is not done in vivo.
3. Line 162, Ref 15 is not on CytoB.
4. Lines 301-323. How are these results related to the main topic?
5. Line 138, the gap shown in Fig. 1k appears to be about 500 nm, not <100 nm.
6. Line 380. Who "…displayed significant reduction of mechanical pain"?
7. Fig. 5h. How long after mitochondria transfer was the paw skin tested?

Referee #3

(Remarks to the Author)
This is a striking study by Xu et al. that presents evidence for a novel mechanism of sensory neuron support via mitochondrial transfer from satellite glial cells in the dorsal root ganglion to the neurons they envelop. The study is a tour-de-force that covers an enormous range of approaches to interrogate this process from multiple angles. The authors implicate tunnelling nanotubes (TNTs) and Myosin10 in the transfer process and illustrate changes in this process in the settings of injury, diabetes, and chemotherapy induced neuropathic pain. Overall, the studies are impressive and well-described. The findings are provocative and of substantial potential translational relevance, especially given the demonstration of similar phenomena in human DRGs and the demonstration that adoptive transfer of mouse or human SGCs into mouse DRG can revert some features of neuropathic pain. There are, however, a few issues that warrant further consideration.

1) In Fig 1b, the morphology of the SGC shown is amorphous. How are SGC cells distinguished from TRPV1 negative neurons that have somehow taken up mitochondria?

2) Is MitoTracker membrane impermeant? If not, in Fig 1d, how can the authors be sure that the spread to neurons is not by simple diffusion of the dye, rather than via mitochondria?

3) Higher resolution fluorescence imaging to resolve individual mitochondria would be helpful in the fluorescence microscopy images.

4) Page 5 line 117 - Do the authors mean between SGC and DRG neurons? "DRG" refers to the structure that includes SGCs, not solely to the neurons.

5) Differences between top and bottom panels in Ext Fig 1e are not well defined and don't seeem to be consistent among the three columns.

6) Low magnification of Ext Fig 1g makes it difficult to interpret

7) In Ext Fig 1g, there appear to be fewer macrophages labeled with MitoTracker. Therefore the interpretation of differential transfer of mitochondria between different cellular sources is confounded.

8) Page 5, Lin 141 – the authors suggest that cleavage of TNTs by trypsin is "expected". Are TNTs membrane bound or exposed proteinaceous structures?

9) Page 5, Line 140 - Are the authors indicating that these vesicular swellings were NOT oberved in TNTs from SGCs to other cell types or one another? The language is unclear.

10) Page 6, line 145 - Cilia-like structures not clear from image. They should be highlighted somehow.

11) Figure 2k-m – In the SNI model, not all neurons are injured. Is mito transfer preferentially into injured or spared neurons or both?

12) In figure 3B, a lower power image would be helpful to establish specificity of the Myo10 immuno signal.

13) Figure 3i-j - Does Myo10 knockdown reduce FABP7 expression in SGCs? It appears it might, from the images.

14) Result of 7c-d is confusing. If mitochondrial transfer were not disrupted by CytoD, abundance of healthy mitochondria in neurons should still be low after PTX.

15) The fate of TNTs in the PTX model is unclear. In Fig 2, it is suggested that the TNTs become more prominent after PTX. In Ext Data Fig 8, the authors imply that TNTs are reduced in this setting. This seems contradictory.

16) While intriguing, the implication of a MaR1-FABP7 binding and its role in the phenomena under study seems premature. Computational implication of MaR1-FABP7 interaction raises a possibility but is not directly tested experimentally. It is possible that numerous lipids bind FABP7. MaR1 effects on PTX allodynia could be through a different mechanism. This series of experiments seems underdeveloped, and the manuscript would not suffer from their omission.

17) In Fig 5c, the interpretation of an increase in OCR by human SGCs is confusing, as there is not a no-SGC control for comparison. It is clear that siRNA of Myo10 reduces the OCR, but not that the control SGCs increase it compared to untreated mouse DRGs.

18) How do the authors suspect that in vivo adoptively transferred mitochondria enter neurons? Use of CytoB or endocytosis inhibitors would be valuable here.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
No further comments regarding the data, analyses and interpretation. Only 1 minor suggestion. Perhaps the summary schematic in Fig 5K should include an endocytosis element?

Referee #2

(Remarks to the Author)
The authors have addressed some of the comments satisfactorily, but several concerns remain, in particular on the TEM and SEM data. The numbers refer to the original comments:
3. Fig. 1e. How do you know that you injected to MitoTracker into an SGC? The images are not very convincing. The bright spot at 1h could be in a neuron.
Response: We appreciate the reviewer's insight and conducted additional experiments of single-SGC MitoTracker microinjection in whole-mount DRG of Aldh1l1:eGFP reporter mice (Response Fig. 22a) to confirm the cells we patched are indeed SGCs 23. The new images showed that MitoTracker labeled mitochondria in SGC transferred to the adjacent neuron 30 min after the MitoTracker injection (Response Fig. 22b). Due to the space limitation, we moved this result from Fig. 1d-e to Extended Data Fig. 2a-b. Notably, compared to 1h imaging, 30-min imaging revealed local transfer of mitochondria to a portion of the DRG neuron adjacent to the end feet of the SGC (Response Fig. 22b). This result argues against the mitochondrial diffusion hypothesis.

Follow-up comment
In Extended Data Fig. 2b the authors claim that the red labeling is due to transfer of MitoTracker from SGC S1 to neuron N1. However, as the neurons are completely covered with SGCs, the MitoTracker may have moved within the same SGC (S1), and is not inside N1. The dotted arrow actually shows how the MitoTracker apparently moved inside S1. Also, it would have been much more likely that the MitoTracker be present in N1 close to the injection site, rather than at a distance of 10-20 um away from it.

4. Line 112, concerning mitochondrial transport via TNT. The citation from Ref 20 is incorrect. In Ref 20, P.92, it is stated: "However, mitochondrial transport between pericytes was not observed, consistent with the closed-ended nature of IP-TNTs". The experiments with the gap junction blocker CBX make no sense, as gap junctions are channels that are too small to allow the passage of mitochondria. The role of gap junctions in mitochondrial transport is still possible, but not through the channels. You did not rule out a role for gap junctions in mitochondrial transport.
Response: We apologize for our inaccurate citation of the reference. We corrected the description as "nanotubes capable of transferring mitochondria have been identified in the mouse retina, connecting two bona fide pericytes on separate capillary networks".
We agree that gap junction hemi-channels are too small for the passage of mitochondria, but an assisting role of gap junctions in mitochondrial transport is still possible. Our new results showed that gap junction blocker CBX treatment showed a partial but significant inhibition ($P < 0.0001$) of mitochondrial transfer in SGC-neuron co-cultures (Response Fig. 23a-c). Furthermore, CBX treatment reduced the proportion of the TNT formation between SGCs and neurons (Response Fig. 23d). Notably, the TNT and endocytosis inhibitors (CytoB and Pitstop 2) showed almost complete inhibition of the transfer (Response Fig. 23). Therefore, gap junction communication may be indirectly involved in the mitochondrial transfer between SGCs to neurons. We add this quantification data to Extended Data Fig. 1i-k.
Notably, SGCs highly express the gap junction protein Connexin43 (Cx43) 17,24,25, and Cx43-mediated gap junction communication plays a crucial role in SGC-neuron interactions in the DRG 26. Interestingly, Cx43 was proposed to function as a stabilizer, adhering to the docked membrane structures of two cells 27,28. Furthermore, Cx43 may increase the probability of successful formation of TNTs 29-33.

Follow-up comment
These data are valid, but add little to the main message of the paper, because the mechanisms involved are not known. This point is only cursorily mentioned in the discussion. This also holds for the other data in Extended Data Fig. 1.
6. Fig 1 f, Fig. 4 k. How was it determined that: 1. the fibers are nanotubes (they might be neurites, connective tissue…, 2. That they originate in an SGC? 3. That the bulges (vesicles) are mitochondria? 4. How do you know that there is a vesicle inside the presumed TNT (line 901)?
Response: We thank the reviewer for these important questions regarding the identification of tunneling nanotubes (TNTs) and mitochondrial transfer. We address each point below:
6.1. Identification of TNTs vs. neurites or connective tissue:
To reveal more details about TNTs and distinguish them from other cellular structures such as neurites or connective tissue, we conducted transmission electron microscopy (TEM) imaging on mouse and human DRG using 70 nm sections (Response Fig. 5-7, above).
Notably, TNTs were found to contain mitochondria and vesicles but lacked microtubules and myelin sheaths (Response Fig. 25b, below). In contrast, axons contain microtubules and, in the case of A-fibers, are also wrapped by myelin sheath 34 (Response Fig. 25b, below).
Connective tissue is characterized by the presence of a dense extracellular matrix (ECM) 35. However, ECM-like structures were found to be associated with neurons and SGCs but not TNTs.
Taken together, these morphological features allow us to clearly distinguish TNTs from axons and connective tissue.

Follow-up comments
I. Line 175, "with extracellular matrix (ECM) fibers associated with SGCs but not TNTs". This is not supported by the images, as collagen fibrils are present near presumed TNT. The extracellular space in DRG is packed with collagen fibrils, and if this claim is correct, it would mean that growing TNTs “dissolve” these fibers, all within minutes.
II. In the TEM images the TNT were identified by exclusion, but not by positive means. The processes shown in the TEM images could belong connective tissue cells or SGCs; see Pannese 2018 https://doi.org/10.1007/978-3-319-60140-3, Figs 1.11 and 1.30
The dotted lines in Extended Data Fig. 13 indicate foldings of SGCs membranes, and are unlikely to be TNTs.
III. The SEM Images in Fig 1, what is the evidence that the fibers indicated with arrows are TNTs? The surface of the presumed SGC is covered with numerous fibers. What are these? DRG neurons send fine processes into the SGC cover, see Pannese 2002 doi: 10.1016/s0074-7696(02)20002-9. These processes might be mistaken for TNTs.
6.2. Determining the origin from SGCs:
In the physiological conditions, most SGCs close-wrap the DRG neuron 17, which makes it hard to observe the TNT between SGC and neuron by scanning EM (SEM) imaging. However, we found the enlarged gaps between SGC and neuron after chemotherapy and diabetic peripheral neuropathy conditions, which made the TNT between SGC and neuron easier to detect; the images showed the TNT originating from SGC connected to the neuron (Main Fig. 1l-m). Importantly, our in vitro and ex vivo experiments strongly suggest the existence of TNTs formed between SGCs and neurons of DRG co-cultures (Fig. 1b and Extended Data Fig. 2c-e). However, we acknowledge that high-resolution immunoelectron microscopy is required to definitively confirm the presence of mitochondria within TNTs between SGC and neuron in DRG tissue.

Follow-up comment
The SEM images (Figs 1,3) show numerous tangled fibers. What is the evidence that these are TNTs? Is there any evidence in the literature for such structures in DRG?
The presumed TNT in Fig. 1f connects two SGCs, not SGC with neuron.
6.3. Confirmation that the bulges are mitochondria:
We used MitoTracker staining and SGC-specific mitochondrial labeling to track mitochondrial movement. Live-cell imaging and confocal microscopy showed the co-localization of these mitochondrial signals within the bulges along TNTs (Extended Data Fig. 1d). Furthermore, transmission electron microscopy (TEM) revealed double-membraned organelles with high-density and internal structures consistent with mitochondria inside TNTs, supporting the mitochondrial identity of the transported cargo (Fig. 1n-r).

Follow-up comment
See above concerning the validity of TEM data.
6.4. Evidence of vesicles inside TNTs:
TEM provided direct ultrastructural evidence of vesicular structures within TNTs of mouse and human DRG (Response Fig. 5-7, above). We added these TEM data to Fig. 1n-r, Extended Data Fig. 4, and Extended Data Fig. 13i-j.

Follow-up comment
See above concerning the validity of TEM data.
In summary, the TEM and SEM studies are not convincing.

14. Discussion The discussion is not satisfactory. It is mostly about the potential clinical implications of this work, but not about the results themselves, and their shortcomings. The comments above need to be addressed and in particular comments 1,4,6,8, Why did you use four different pain models (SNI, PTX, db/db, STZ), and what do we learn from that? The results support previous reports on the essential roles of SGCs in chronic pain. However, the authors did not discuss their results within the wide picture of neuron-SGC interactions.
Response: We appreciate your brief summary of the key points, which were highlighted in the Discussion Section. We apologize that we were not able to add extensive discussion of each question due to the space constraints of the journal. We have dressed these key comments in great detail in the previous responses. Also see below:

Follow-up comment
The discussion will have to be modified after the new corrections are made.
Additional Comment. Extended Data Fig. 17
The figure describes "inner layer TNT" and "out layer TNT". What is the evidence for this?"
"TNT from SGC to neurons" Do you have the evidence for this arrangement?

Typo
Fig 1, “myline”

Referee #3

(Remarks to the Author)
The authors have adequately addressed all of this reviewer’s criticisms

Version 2:

Reviewer comments:

Referee #2

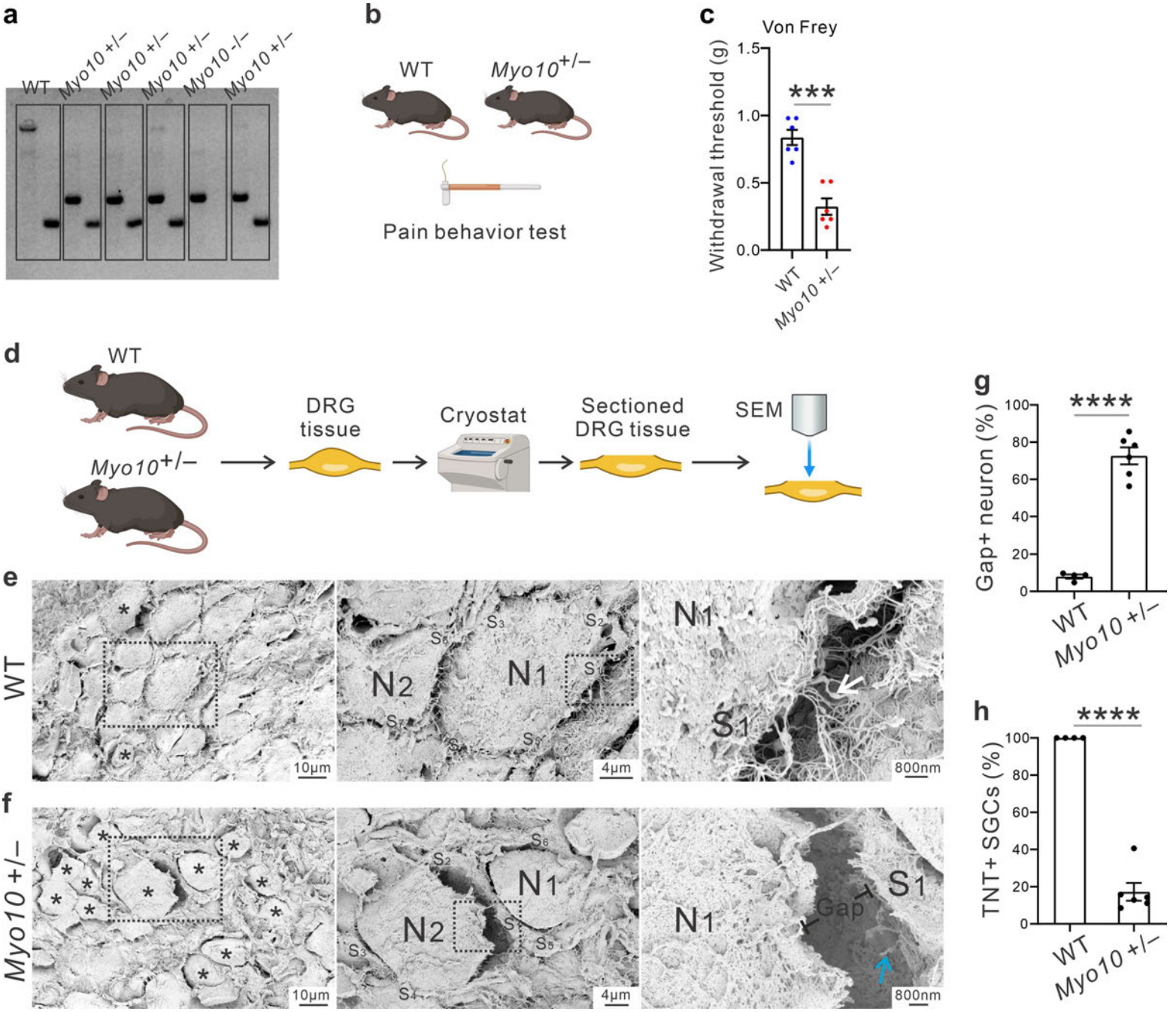
(Remarks to the Author)

[Redacted]

To editor and all reviewers:

Over the past six months, we have made the following new findings that support the major conclusions of our paper.

1. MYO10 is required for TNT formation and maintaining SGC-neuron interaction in mouse DRG.
Our siRNA knockdown experiment revealed that MYO10 plays a crucial role in TNT formation in the DRG. To further define the contribution of MYO10 to TNT formation and pain control, we obtained *Myo10* mutant mice from Dr. Richard Cheney of University of North Carolina. Due to developmental defects of homozygous mice[1], we utilized *Myo10* heterozygous (*Myo10*$^{+/-}$) mice (**Response Fig. 1a**) for behavioral and scanning EM studies. Compared to WT mice, *Myo10*$^{+/-}$ mice exhibited greater mechanical sensitivity (**Response Fig. 1b-c,**), suggesting an important role of MYO10 in maintaining physiological pain under normal conditions. Scanning EM (SEM) analysis revealed that partial loss of MYO10 in heterozygous mice resulted in enlarged gaps between SGCs and neurons and loss of TNTs in mouse DRG (**Response Fig. 1d-h**). We added them to **Main Fig 3m-r**.



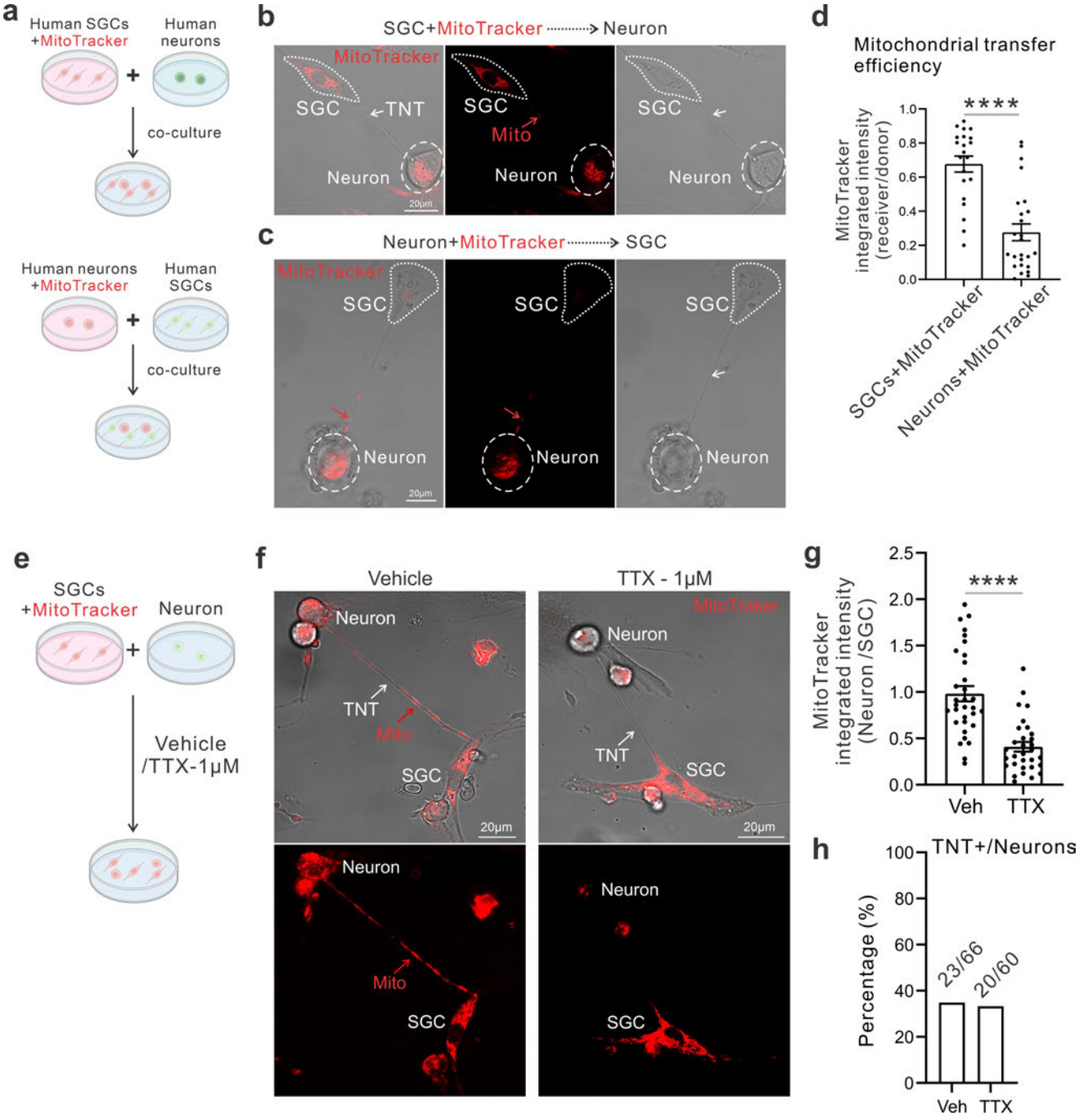
Response Fig. 1 / Main Fig 3m-r. Behavioral and DRG scanning EM studies in *Myo10* $^{+/-}$ and WT mice.

2. Bi-directional mitochondrial transfer and the role of neuronal activity

Reviewer #1 raised the question of whether mitochondrial transfer between SGCs and neurons is bidirectional. To address this question, we separately labeled human DRG SGCs and DRG neurons with MitoTracker (**Response Fig. 2a**). Although bidirectional mitochondrial transfer was observed (**Response Fig. 2b–c**), the rate of SGC-to-neuron transfer was significantly higher than that of neuron-to-SGC transfer (67.7% vs. 27.6%, $p < 0.0001$; **Response Fig. 2d**).

To examine whether SGC-to-neuron mitochondrial transfer depends on neuronal activity, we treated the co-culture with the sodium channel blocker tetrodotoxin (TTX; 1 μM, 24 h; **Response Fig. 2e**). TTX significantly reduced the SGC-to-neuron transfer rate ($p < 0.0001$; **Response Fig. 2f–g**), without affecting the proportion of TNT-forming neurons (**Response Fig. 2h**).

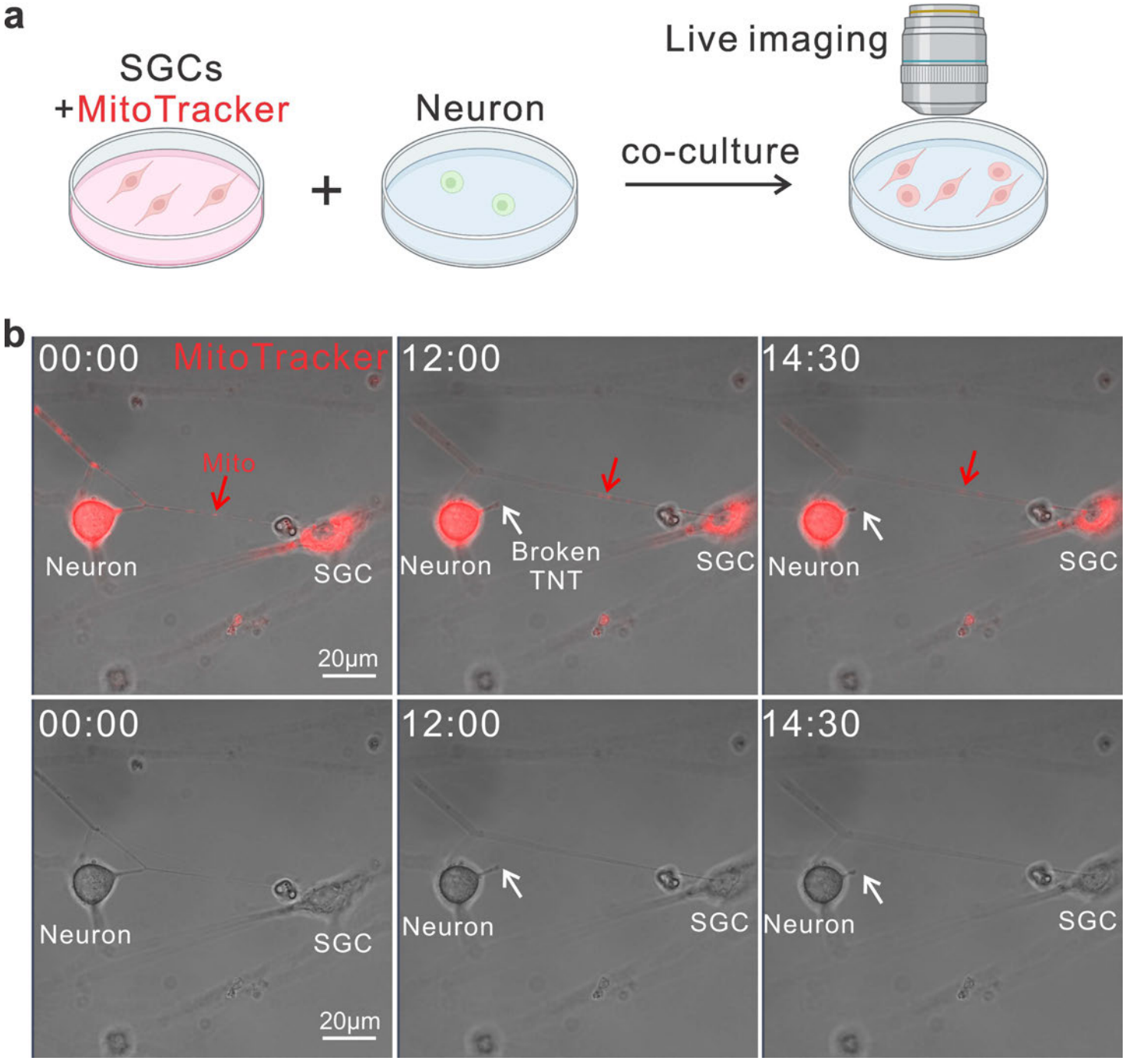
Taken together, these results indicate that: a) mitochondrial transfer occurs predominantly from SGCs to neurons and b) this process is dependent on neuronal activity. These results have been added to **Extended Data Fig. 1 and Extended Data Fig. 15**.



Response Fig. 2 / Extended Data Fig. 1 and Extended Data Fig. 15. (a-d) SGC and neuron co-cultures from human DRG showing bi-directional mitochondrial transfer (SGC-to-neuron vs. neuron-to-SGC). (e-h) Effects of TTX on mitochondrial transfer in mouse DRG co-cultures of SGCs and neurons.

3. TNT formation in mouse SGC-neuron cocultures is transient.

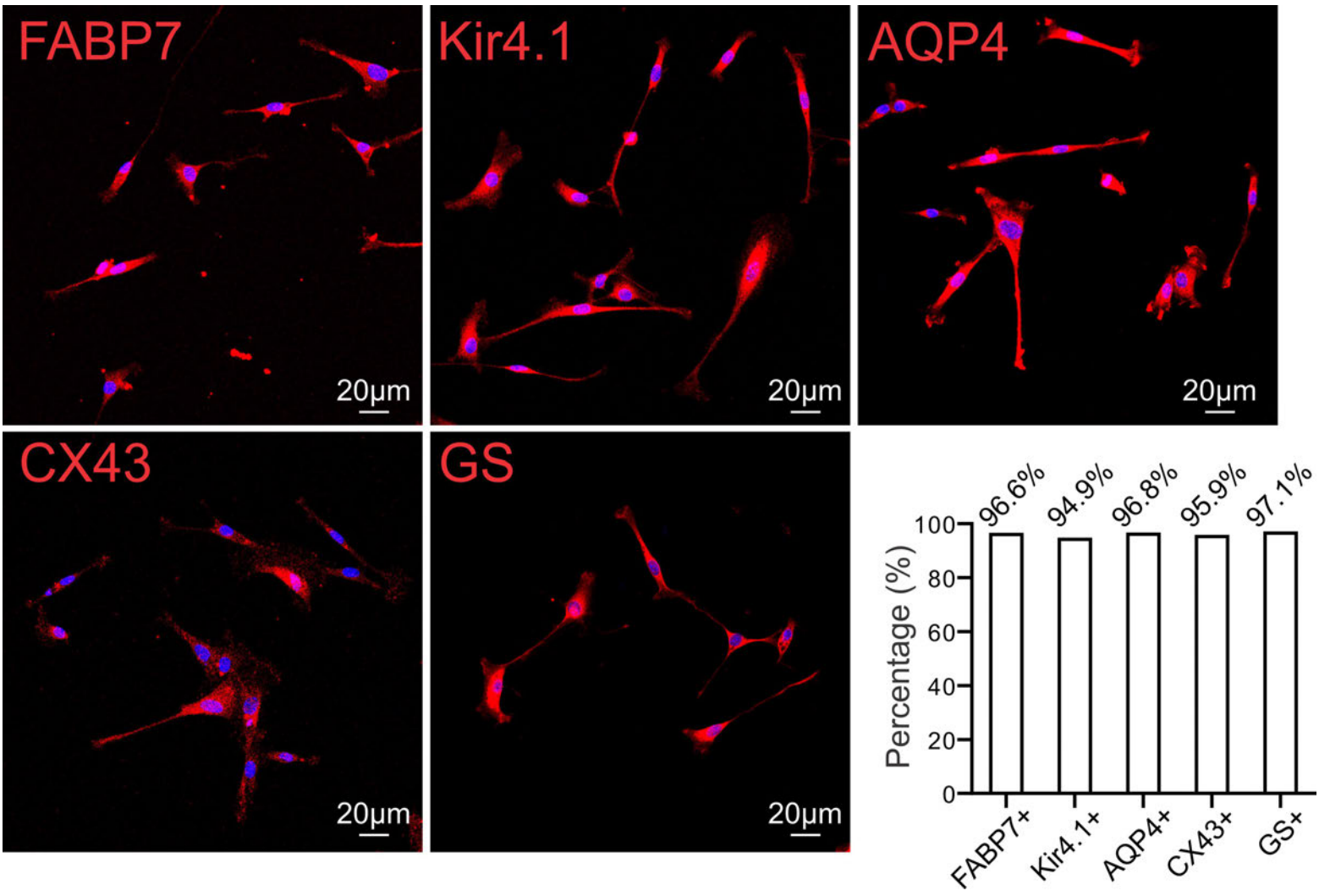
We only observed TNTs in 27.5% of neurons in SGC-neuron co-cultures (**Main Text Fig 1c**), raising the question of whether TNTs are consistently present in culture. To address this, we performed live-cell imaging in SGC-neuron co-cultures (**Response Fig. 3a**). We found that a TNT structure was disassembled within 12 minutes (Response Fig. 3b), indicating the highly transient nature of TNT formation. This observation is consistent with previous reports showing that TNTs can form within as little as 25 min[2]. These results have been added to **Extended Data Fig. 1c-d**.



Response Fig. 3 / Extended Data Fig. 1c-d. Live-cell imaging in mouse SGC-neuron co-culture showing the transient formation of TNTs.

4. Validation of SGC identity via immunostaining of different SGC markers.

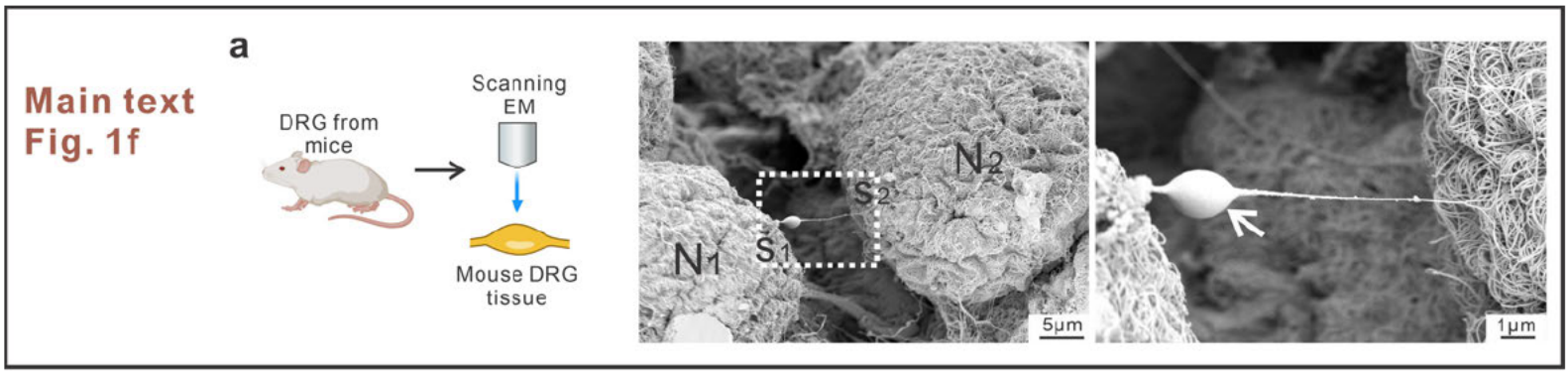
To address the concern of **Reviewer #2** and **#3**, we validated the identification and purity of our mouse SGC cultures using immunostaining for five SGC markers, including FABP7, Kir4.1, AQP4, Cx43, and GS. Further quantitative analysis revealed that 94.9-97.1% of SGCs express these markers (**Response Fig. 4**), validating the identity and purity of our SGC cultures. These results have been added to **Extended Data Fig. 1a**.



Response Fig. 4 / Extended Data Fig. 1a. Immunostaining for SGC markers in mouse SGC cultures and quantification for the percentage of SGCs positive for each marker. n=3-4 cultures per group.

5. Transmission Electron Microscopy (TEM) showing *in vivo* evidence of mitochondria-containing TNTs in mouse and human DRG.

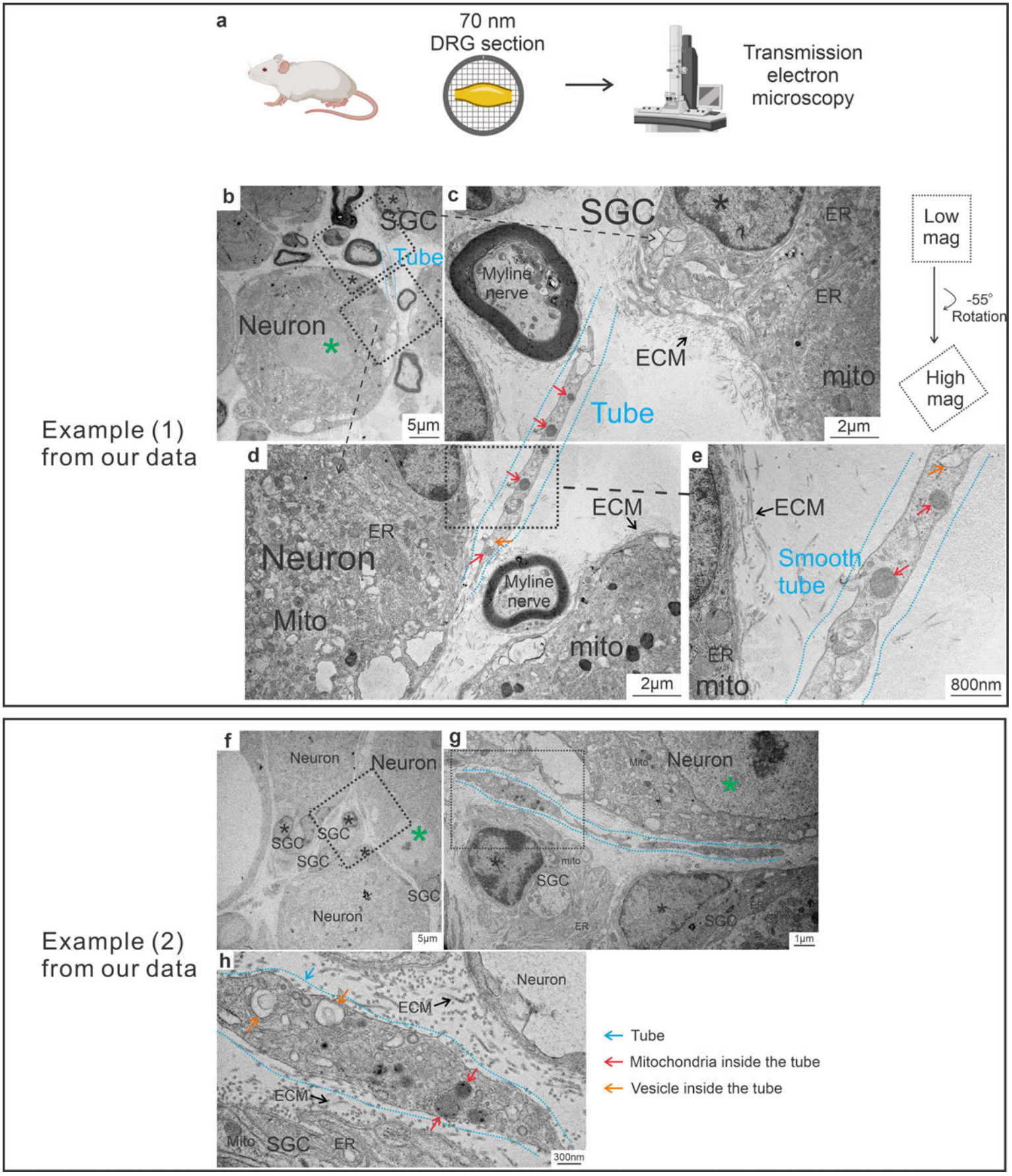
Our scanning EM (SEM) study revealed mitochondria-bearing TNTs in mouse DRG (**Response Fig. 5a**, **Main text Fig. 1f**) and human DRG (**Main text Fig. 4c**). To validate the presence of mitochondria-sized bulge in TNT, we searched DRG Transmission EM (TEM) images from public database (**Response Fig. 5**). We also conducted TEM experiments in mouse and human DRG samples (**Response Fig. 5-7**). We found smooth TNT-like tubes between SGCs and neurons in both mouse DRG (**Response Fig. 5-7**, below) and human DRG (**Response Fig. 7**, below). Importantly, we found mitochondria within these TNTs (**Response Fig. 5-7**). These results have been added to **Main Fig. 1, Extended Data Fig. 4, and Extended Data Fig. 13**.



[Redaction]

Response Fig. 5. Transmission Electron Microscopy (TEM) of mouse DRG from a public database showing two examples of TNTs between SGCs and neurons. Notably, these TNTs contain mitochondria and vesicles. TEM images were obtained from a public database:
https://rdr.ucl.ac.uk/articles/dataset/Transmission_electron_micrographs_dorsal_root_ganglia/21334356

[redacted]

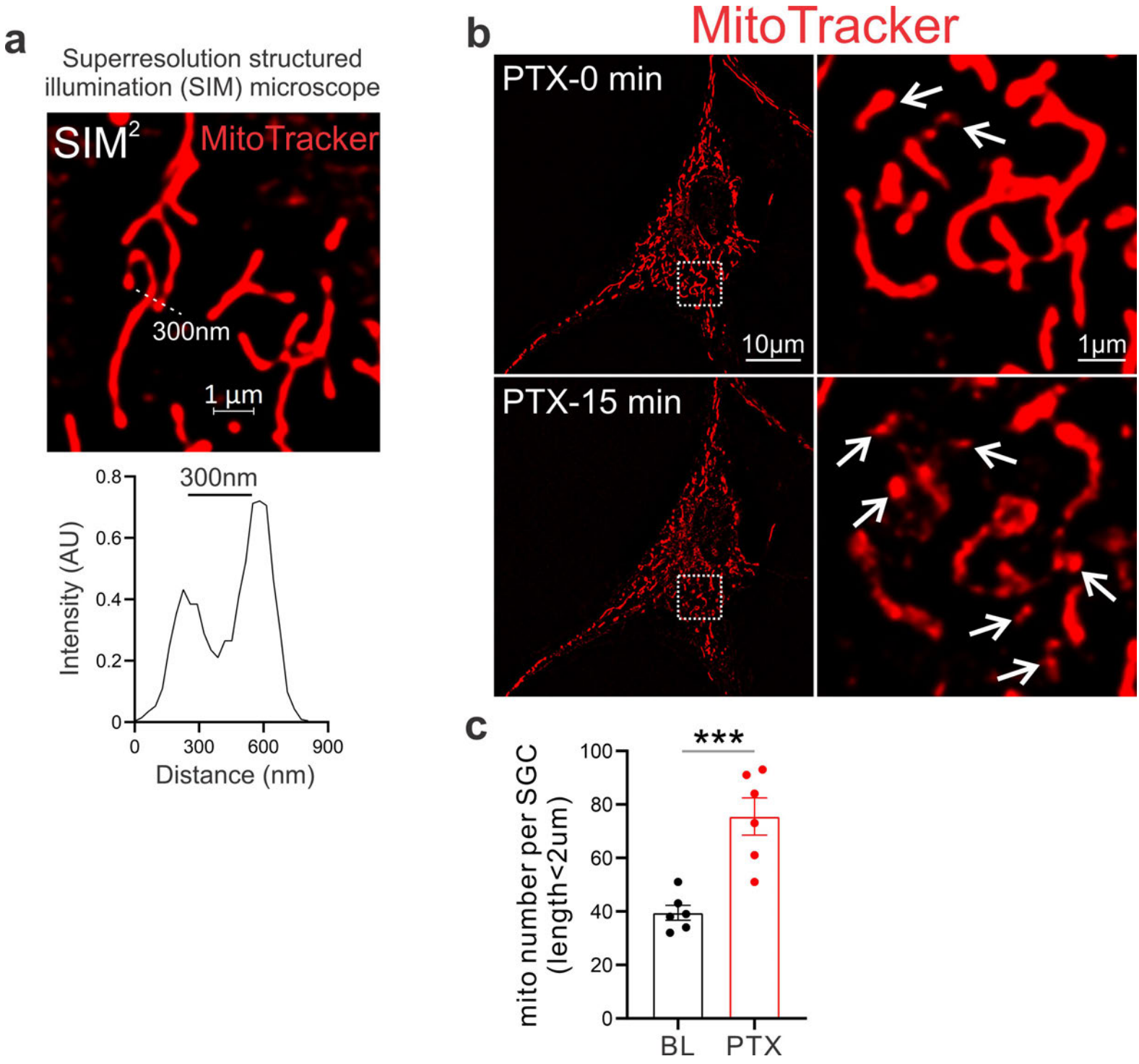


Response Fig. 6 / Main Fig. 1m-r and Extended Data Fig. 4. Transmission Electron Microscopy (TEM) images showing two examples of TNTs between SGCs and neurons in mouse DRG. Notably, these TNTs contain mitochondria and vesicles. TNTs are smooth and do not contain extracellular matrix (ECM), which are associated with SGCs and neurons. Also see additional annotation, blue arrows: smooth TNT in blue color without ECM; red arrows: mitochondria inside the TNT; orange arrows: vesicle inside the TNT; "Mito" in black color: mitochondria in cells; ER: endoplasmic reticulum.

[Redacted]

6. High-resolution imaging of individual mitochondria in mouse SGC cultures.

We conducted Structured Illumination Microscopy (SIM) to reveal the structure of individual mitochondria with high resolution (**Response Fig. 8a**). Our new results showed that chemotherapy treatment with PTX shortened the length of mitochondria and increased the number of short mitochondria (< 2 μm, **Response Fig. 8b-c**). **Extended Data Fig. 3**.



Response Fig. 8 / Extended Data Fig. 3g-i. SIM imaging of mitochondrial morphology and quantification of short mitochondria in SGC cultures. (a) Representative SIM images of MitoTracker-labeled mitochondria in SGCs (top), with the corresponding intensity profile plot (bottom) demonstrating SIM² resolution down to 300 nm. (b) Representative images before and 15 min after PTX treatment; arrows indicate mitochondria shorter than 2 μm. (c) Quantification shows that PTX treatment significantly increases the number of mitochondria shorter than 2 μm (*** $P< 0.001$, unpaired t-test).

Referee #1:

To Authors: This nice study proposes a new phenomenon to explain the pathophysiology of neuropathic pain. SGCs attempt to transfer mitochondria to adjacent neurons via tunneling nanotubes to prevent the neuronal hyperactivity. But in disease conditions, this process is impaired thus potentially explaining the development of pain. There are a lot of data and powerful methods are used. The significance may also be high since this phenomenon may provide new therapeutic targets for a difficult clinical problem. However, there are some issues that may require more consideration; more data and discussion may help.

Response: We appreciate the reviewer's insightful and constructive comments and suggestions, which we believe have greatly improved the manuscript. We have performed many new experiments in the last six months and provided additional mechanistic insights.

a) We obtained *Myo10* mutant mice from Dr. Richard Cheney of University of North Carolina. Due to developmental defects of homozygous mice, we utilized *Myo10* heterozygous ($Myo10^{+/-}$) mice (**Response Fig. 1a**) for behavioral and scanning EM studies. Compared to WT mice, $Myo10^{+/-}$ mice exhibited heightened pain sensitivity (**Response Fig. 1b-c, and Response Fig. 9a-c**), suggesting an important role of MYO10 in maintaining physiological pain under normal conditions. Further scanning EM (SEM) analysis revealed that partial loss of MYO10 in $Myo10^{+/-}$ mice resulted in greater gaps between SGCs and neurons and loss of TNTs (**Response Fig. 1d-h, and Response Fig. 9d-f**).

b) To address the question of whether mitochondrial transfer between SGCs and neurons is bidirectional, we separately labeled human DRG SGCs and DRG neurons with MitoTracker (**Response Fig. 2a**). Although the bidirectional mitochondrial transfer was observed **(Response Fig. 2b–c**), the rate of SGC-to-neuron transfer was significantly higher than that of neuron-to-SGC transfer (**Response Fig. 2d**). To examine whether SGC-to-neuron mitochondrial transfer depends on neuronal activity, we treated the co-culture with the sodium channel blocker tetrodotoxin (TTX, **Response Fig. 2e**). The result showed that TTX significantly reduced the SGC-to-neuron transfer rate (**Response Fig. 2f–g**), without affecting the proportion of TNT-forming neurons (**Response Fig. 2h**). Taken together, these results indicate that mitochondrial transfer occurs predominantly from SGCs to neurons and this process is dependent on neuronal activity.

c) To address whether TNTs are present all the time in cultures, we conducted live cell imaging in SGC-neuron co-cultures (**Response Fig. 3a**). We observed that a TNT is broken within 12 min (**Response Fig. 3b**), indicating a very transient nature of TNT formation.

Please also find our point-by-point response to each of Reviewer #1's comments below.

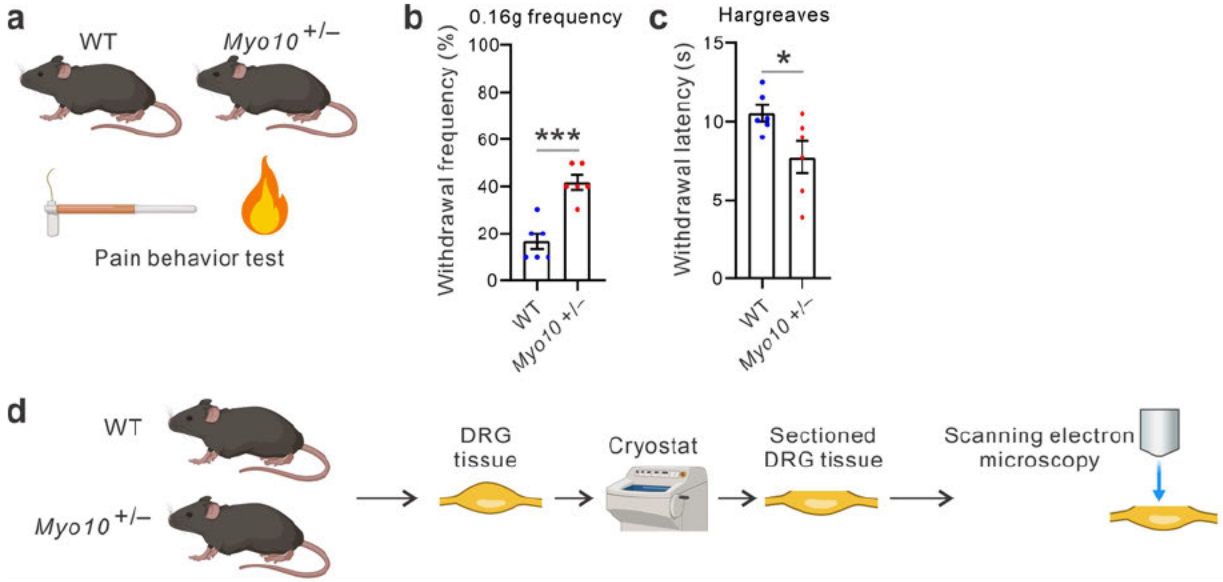
1. The authors propose that SGCs transfer mitochondria to neurons via a process mediated by MYO10. However, the underlying mechanism of how SGCs drive mitochondrial migration via MYO10 to the adjacent neurons remains slightly unclear.

Response: Thank you for this insightful comment. We conducted several experiments to address this comment.

a) SGC-to-neuron vs. neuron-to-SGC mitochondrial transfer: To determine the directionality of mitochondrial transfer between SGCs and neurons, we labeled either human SGCs or human neurons with MitoTracker (**Response Fig. 2a, above**). Notably, we found that mitochondrial transfer rate from SGCs to neurons was significantly higher than from neurons to SGCs (**Response Fig. 2b–d, above**). This suggests that DRG neurons have a higher demand for mitochondrial support from SGCs, likely due to their high metabolic needs driven by neuronal activity, including action potential firing, and the energy required to maintain long axonal projections and prevent degeneration. These findings have been added to **Extended Data Fig. 15**.

b) Neuronal activity dependence of mitochondrial transfer: To test this, we treated DRG neuron–SGC co-cultures with 1 μM TTX to block action potential firing (**Response Fig. 2e, above**). In the vehicle group, we observed TNTs containing mitochondria between SGCs and neurons. Notably, in the TTX-treated group, TNTs still formed between SGCs and neurons, but no mitochondria were present within the TNTs (**Response Fig. 2f, above**). Quantification showed a significant decrease in the MitoTracker fluorescence ratio between neurons and SGCs (from 0.98 ± 0.08 to 0.41 ± 0.05, $P < 0.0001$, **Response Fig. 2f-g, above**), whereas the percentage of TNT-positive neurons remained unchanged (**Response Fig. 2h, above**). These results indicate that while TNT formation is independent of neuronal activity, mitochondrial transfer through TNTs is activity-dependent. This supports the idea that neuronal activity is a key driver for mitochondrial acquisition. These findings have been included in **Extended Data Fig. 1e–h**. This in vitro finding is also in agreement with our in vivo finding showing that nerve blockade with bupivacaine-loaded film blocked nerve-injury induced mitochondrial transfer in the early phase (SNI, 5 days, **Main Fig. 2h–i**).

c) Role of MYO10 in TNT formation and SGC-neuron interaction: To further investigate the role of MYO10, we used *Myo10* mutant mice obtained from Dr. Richard Cheney (UNC). Homozygous knockout ($Myo10^{-/-}$) is semi-lethal, with many embryos exhibiting exencephaly[4]. Therefore, we used heterozygous $Myo10^{+/-}$ mice and confirmed genotypes as previously described (**Response Fig. 1a, above**). Behavioral tests revealed that $Myo10^{+/-}$ mice displayed reduced paw withdrawal thresholds in the von Frey test, increased withdrawal frequency in the 0.16 g von Frey test, and decreased thermal withdrawal latency in the Hargreaves test compared to wild-type (WT) mice (**Response Fig. 1b–c, above, and Response Fig. 9a–c, below**). These results showed that partial MYO10 deficiency leads to heightened mechanical and thermal sensitivity, supporting a functional role of MYO10 in pain modulation. These data were added to **Main Fig. 3m–n** and **Extended Data Fig. 9g–i**.



[Redaction]

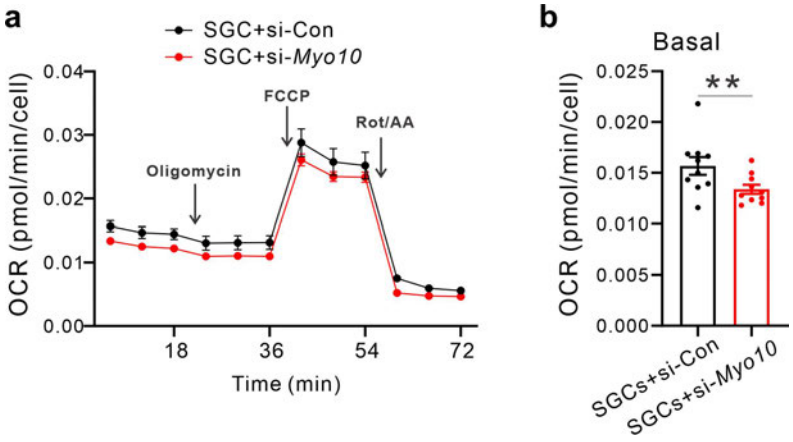
Response Fig. 9. Additional behavioral and DRG scanning EM studies in *Myo10* $^{+/-}$ and WT mice.

[redacted]

To further determine whether MYO10 deficiency affects TNT formation in vivo, we performed scanning electron microscopy (SEM) on DRG sections from WT and $Myo10^{+/-}$ mice (**Response Fig. 1d, above**). In WT DRG, abundant TNTs were observed around SGCs, with most neurons tightly enwrapped by SGCs (**Response Fig. 1e, above**). In contrast, $Myo10^{+/-}$ DRGs exhibited more neurons with expanded gaps between SGCs and significantly fewer TNT-positive SGCs (**Response Fig. 1f, above**). These results are presented in **Main Fig. 3o–r**. Additionally, representative low- and high-magnification images of DRG morphology highlight these structural alterations (**Response Fig. 9d–f, above**). Together, these findings demonstrate that MYO10 is essential for TNT formation and maintenance of close physical association of SGCs with neurons in the DRG. These data have been added to **Main Fig. 3o–r** and **Extended Data Fig. 9j–k**.

2. Moreover, does MYO10 affect mitochondrial biogenesis in SGCs? How do SGCs maintain their own mitochondrial count/homeostasis if they are "donating" a significant number of mitochondria?

Response: To determine whether MYO10 influences mitochondrial function in SGCs, we performed Seahorse oxygen consumption rate (OCR) measurements in human SGCs treated with either control siRNA or siRNA targeting *Myo10*. Knockdown of *Myo10* partially but significantly reduced both basal and maximal OCR (**Response Fig. 10a-b**, $P < 0.01$), suggesting impaired mitochondrial function. These findings indicate that MYO10 may play a role in mitochondrial biogenesis or maintenance, possibly by regulating mitochondrial content.



Response Fig. 10

[redacted]

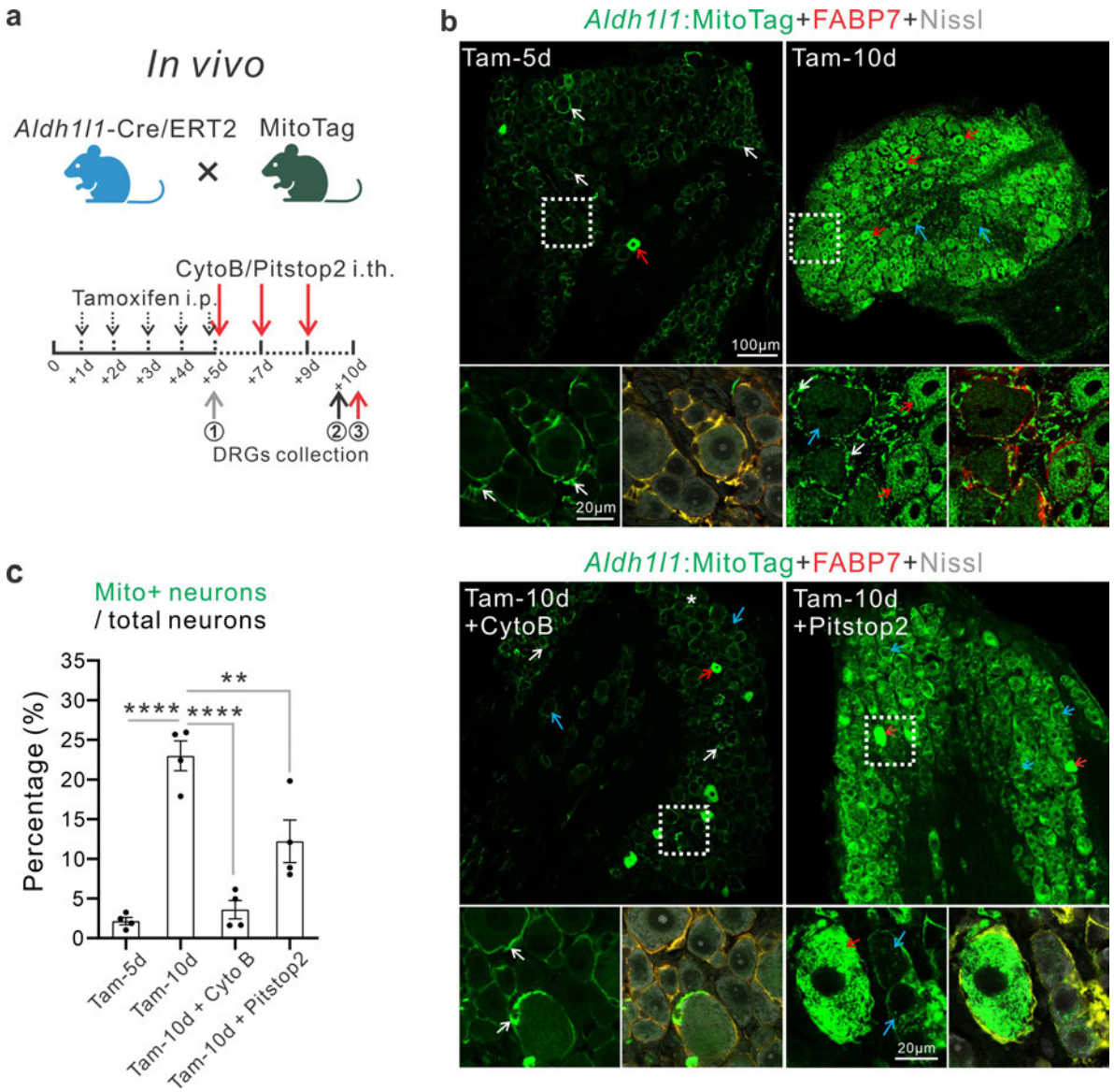
[Redaction]

Response Figure. 11

3. The authors suggest that mitochondria transfer may be mediated by TNT. In Figure 1, it seems that over 80% of neurons uptake SGC-derived mitochondria, whereas about 20% of recipient neurons are TNT-positive. Are these estimates correct? If so, does this mean that other processes besides nanotubes play a key role, for example release and uptake of exosomes? Furthermore, TNT-mediated mitochondria transfer is assessed by a loss-of-function by CytoB. Because CytoB strongly inhibits network formation of actin filaments, this may also affect exosome release. Would some data using blockade of endocytosis be useful?

Response: The reviewer is correct: we only observed TNTs in 27.5% of neurons in SGC-neuron co-cultures (**Main Fig 1c**), raising the question of whether TNTs are consistently present in culture. To address this, we performed live-cell imaging in SGC-neuron co-cultures (**Response Fig. 3a, above**). We found that a TNT structure disassembled within 12 minutes (**Response Fig. 3b, above**), indicating the highly transient nature of TNT formation. This observation is consistent with previous reports showing that TNTs can form within as little as 25 min[2]. Thus, most neurons may form TNTs but we can only detect TNTs in a subset of neurons at any given time due to their transient and dynamic nature.

To test whether endocytosis contributes to mitochondrial transfer from SGCs to neurons in the DRG, we performed intrathecal injection of 120 ng Pitstop2, an endocytosis inhibitor, in *Ald*:Mito mice to block endocytic activity in the DRG (**Response Fig. 12a**). Imaging of DRG sections and quantification of Mito$^+$ neurons revealed that Pitstop2 treatment partially but significantly reduced mitochondrial transfer from SGCs to neurons ($P < 0.01$, **Response Fig. 12b-c**). Together with our results using Cytochalasin B (CytoB), a TNT formation inhibitor, these findings suggest that both TNT and endocytosis mechanisms contribute to mitochondrial transfer from SGCs to neurons in vivo. These data have been included in **Extended Data Fig. 5f**.



Response Fig. 12 / Extended Data Fig. 5f

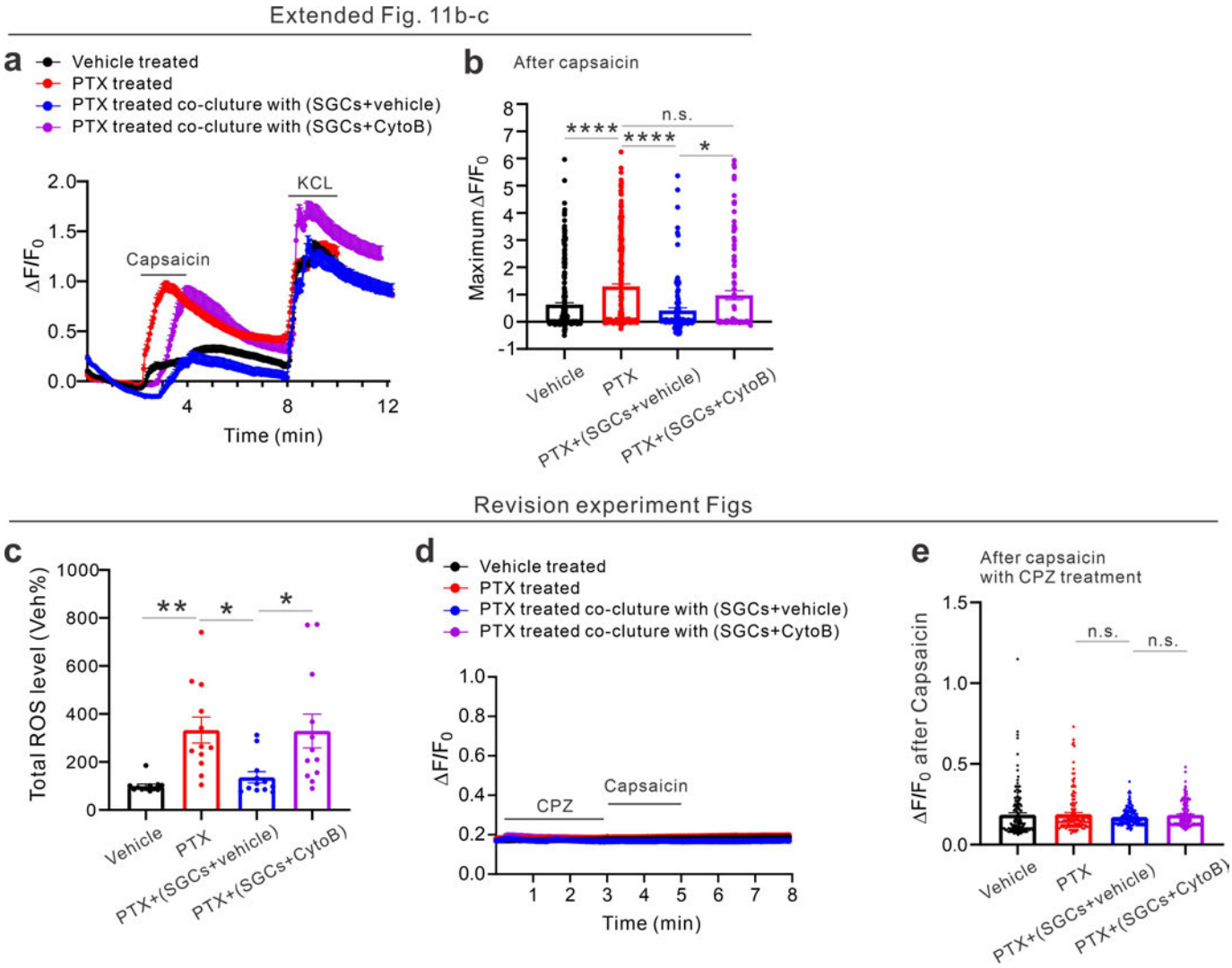
4. Importantly, how SGC-mediated mitochondria transfer to neurons is mitigating neuropathic pain remains to be fully addressed. What is the molecular mechanism? Is this because healthy mitochondrial transfer decreases neuronal hyperactivity; if so how?

Response: In primary cultures of DRG neurons, we observed that paclitaxel (PTX) treatment enhanced calcium responses to capsaicin, indicating abnormal sensory neuronal hyperactivity induced by chemotherapy. Notably, co-culture with SGCs significantly attenuated this hyperactivity (**Response Fig. 13a-b**; **Extended Data Fig. 11b-c**).

To further investigate the molecular mechanisms underlying this effect, we measured reactive oxygen species (ROS) levels using a commercial ROS assay kit (Thermo, 88-5930-74). Consistent with the calcium imaging data, co-culture with SGCs suppressed the PTX-induced ROS increase. However, this protective effect was abolished by Cytochalasin B (CytoB), an inhibitor of TNT formation (**Response Fig. 13c**; **Extended Data Fig. 11d**).

Since ROS is known to modulate TRPV1 activity, we next examined calcium responses to capsaicin in the presence of capsazepine (CPZ, 100 μM), a TRPV1 antagonist. CPZ treatment effectively blocked the enhanced calcium responses in both PTX-treated neurons and PTX-treated neurons co-cultured with SGCs (**Response Fig. 13d-e**; **Extended Data Fig. 11e-f**).

Taken together, our results suggest that PTX induces ROS production in sensory neurons, which in turn drives TRPV1-dependent neuronal hyperactivity. Mitochondrial transfer from SGCs to neurons reduces ROS levels, thereby inhibiting this pathological hyperexcitability. These findings have been incorporated into **Extended Data Fig. 11**.



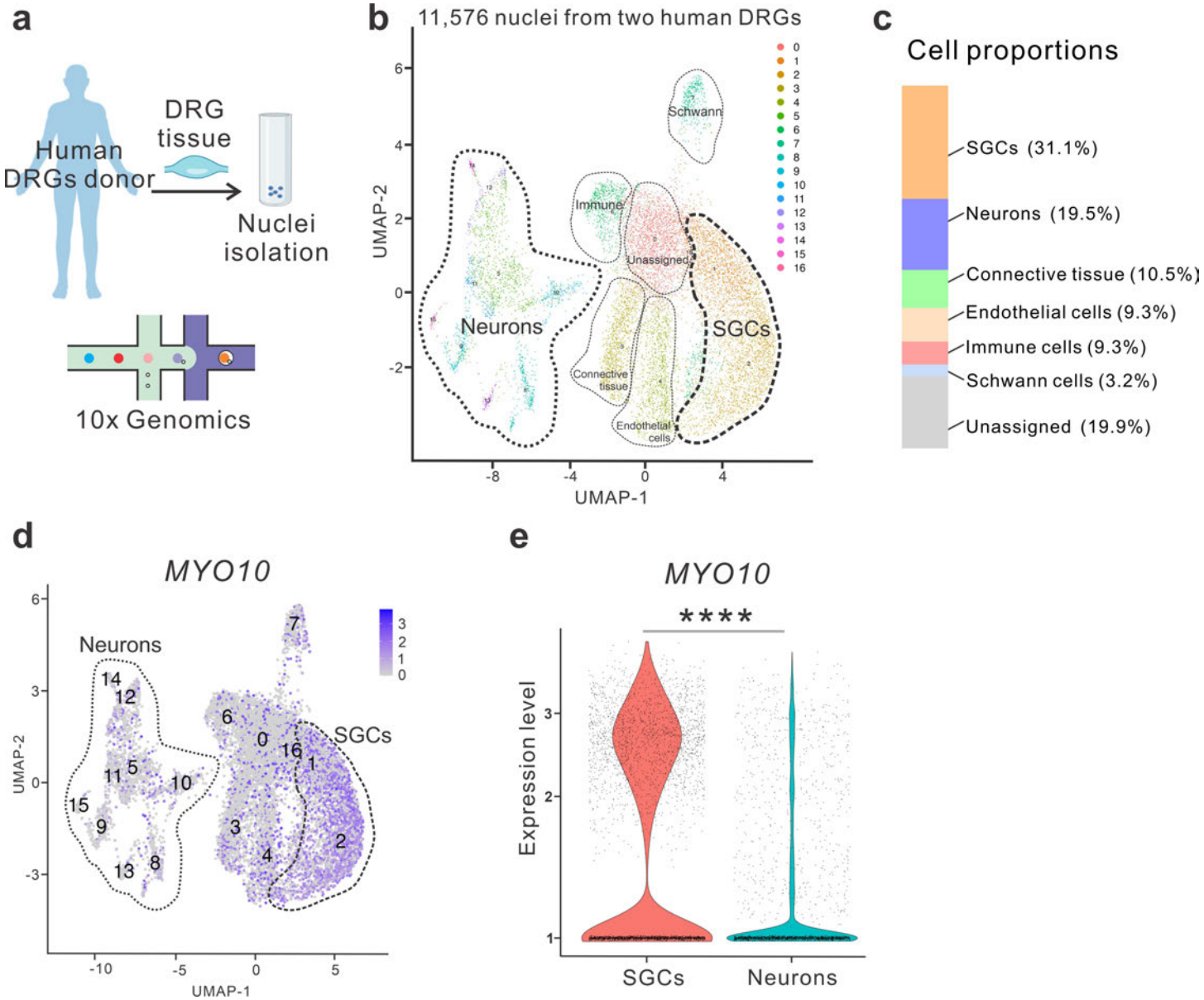
Response Figure. 13 / Extended Data Fig. 11

5. Single cell analysis in mouse DRG shows Myo10 expression in SGCs in Figure 3. For human data in Figure 4, Myo10 expression may appear in both SGCs and neurons. How would this affect the hypothesis of Myo10-mediated mitochondria transfer from SGCs to neurons? Does this mean that in human cells, the mitochondria transfer can occur in both directions, i.e. also from neurons back to SGCs?

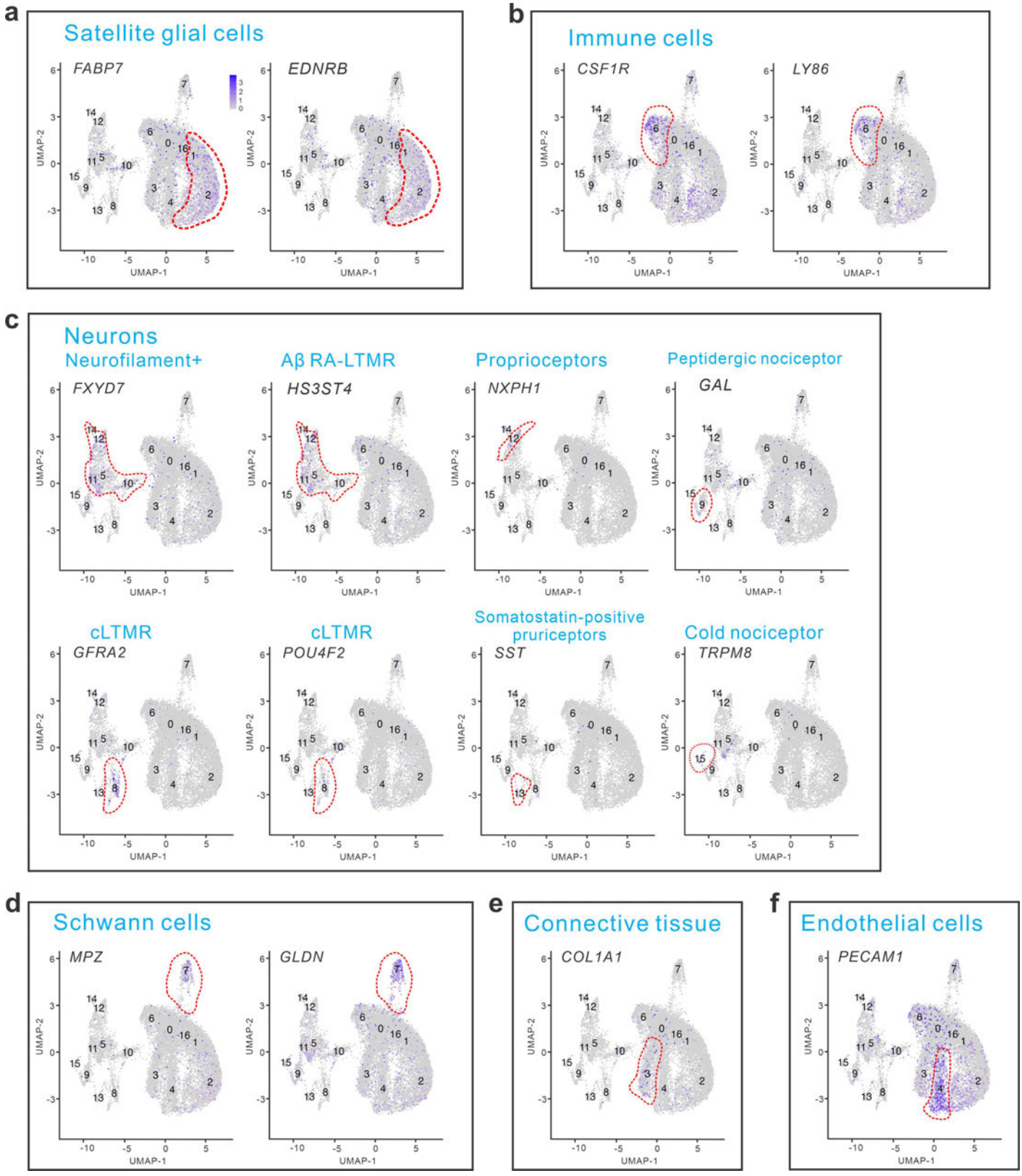
Response: We appreciate this important question regarding MYO10 expression in the human DRG and its implications for mitochondrial transfer. To address this, we performed additional analysis using single-nucleus RNA-seq (snRNA-seq) data from human DRG. We re-annotated cell clusters based on an expanded set of cell type-specific markers and identified differentially expressed genes for each cluster using the Seurat function FindAllMarkers (**Response Figs. 14 and 15**).

In our updated analysis, we examined multiple cell types, including SGCs, neurons, connective tissue cells, endothelial cells, immune cells, and Schwann cells (**Response Fig. 14a–c, Response Fig. 15**). Importantly, the violin plot analysis showed that *MYO10* expression is significantly higher in the SGC cluster compared to the neuronal cluster. Notably, the median (Q2) expression level of MYO10 was 0.99 in SGCs and 0 in neurons, while the third quartile (Q3) was 1.70 in SGCs versus 0.25 in neurons (**Response Fig. 14d–e**).

These results demonstrate that *MYO10* expression in human DRG is predominantly enriched in SGCs, consistent with our in situ hybridization result in human DRG (**Main Fig. 4i-j**). Therefore, although low-level expression may be detectable in some human neurons, the primary role of MYO10 in regulating TNT formation and mitochondrial transfer likely resides in SGCs. We have incorporated these findings into **Main Figure 4d–h** (**Response Fig. 14**) and **Extended Data Fig. 14** (**Response Fig. 15**).



Response figure 14/ Fig. 4d-4h



Response Figure. 15 / Extended Data Fig. 14

To address the question of whether mitochondrial transfer between SGCs and neurons is bidirectional, we separately labeled human DRG SGCs and DRG neurons with MitoTracker (**Response Fig. 2a, above**). Although bidirectional mitochondrial transfer was observed (**Response Fig. 2b–c, above**), the rate of SGC-to-neuron transfer was significantly higher than that of neuron-to-SGC transfer (67.7% vs. 27.6%, $p < 0.0001$; **Response Fig. 2d, above**). This result suggests that neurons acquire more mitochondria from SGCs, as neurons activated during action potential firing require large amount of energy which comes from mitochondria. We added this result to **Extended Data Fig. 15a-d**.

6. Furthermore, snRNA seq shows multiple SGC populations. Clusters 1 and 2 strongly express FABP7 and MYO10 but not other SGC clusters. The authors should provide more details regarding cell type. Are these known subsets from previous literature or are these novel subsets?

Response: We thank the reviewer for this insightful comment. To further address this point, we re-analyzed the single-nucleus RNA-seq data using additional cell-type markers and improved clustering resolution. This analysis identified multiple cell populations in the human DRG, including neurons, SGCs, Schwann cells, endothelial cells, immune cells, and connective tissue cells (**Response Fig. 14a–c and Response Fig. 15**). Notably, in this new analysis we only identified two distinct SGC clusters—Cluster 1 and Cluster 2.

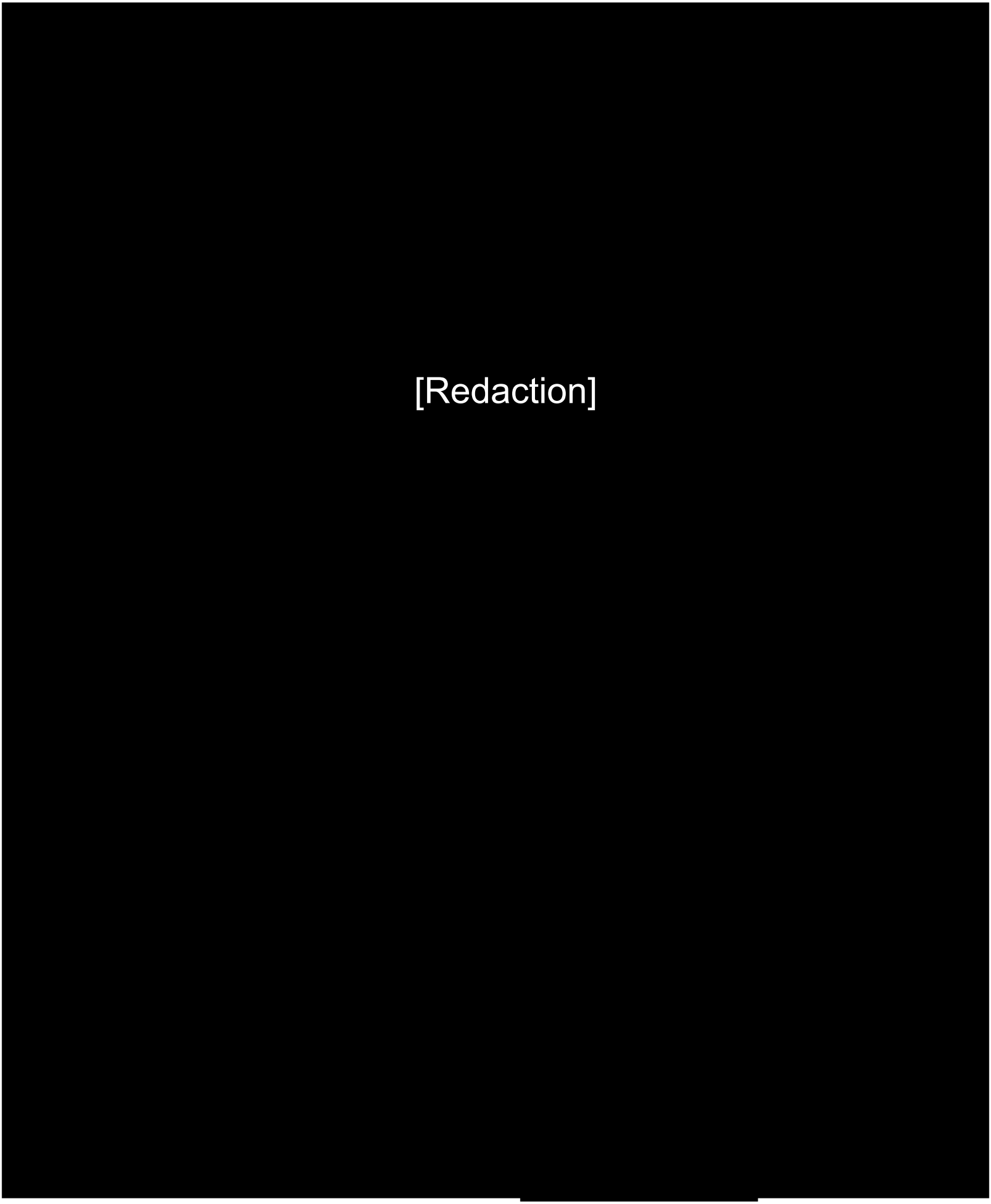
Both SGC clusters expressed canonical and functionally relevant markers, including *NCAM1* (encoding a cell adhesion molecule [3]), *HMGCS1* (a marker gene expressed in SGCs and regulated by peripheral nerve injury [4]), *CDH19* (a gene involved in SGC development and function [5]). Cluster 1 expressed *APOE* (a marker gene in SGCs involved in lipid metabolism [6]), while Cluster 2 expressed *BCAN* (coding a cell adhesion molecule [7]) and *S100B* (encoding a calcium-binding protein and a canonical marker of mature SGCs[8]). Please find more marker genes in **Extended Table 3**, including the enriched gene list in Cluster 1 and 2.

While both clusters share core SGC features, the differential gene expression suggests functionally distinct subtypes. Although some of these markers (e.g., APOE and S100B) have been reported in SGCs, the specific combination of genes in each cluster may represent previously uncharacterized SGC subsets.

7. This paper also includes data suggesting that FABP7 overexpression reduces mechanical pain and protects neurons against chemotherapy-induced peripheral neuropathy. How mitochondria transfer compares to FABP7 in terms of therapeutic effect should be carefully validated and/or discussed.

Response: [redacted]

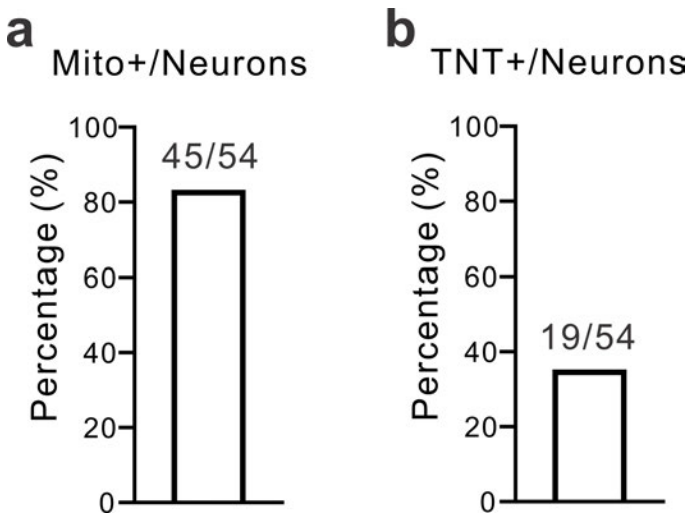
[redacted] To improve clarity and maintain focus on mitochondrial transfer as a therapeutic strategy, we have removed the original Extended Data Figs. 9 and 10 related to FABP7 and MaR1 from the revised manuscript. [redacted]



Response Figure 16

8. Would it be helpful to look for Mito+Neurons as well as TNT+ neuros in the human cells in Figure 4? Just like what was done in Figures 1 and 3?

Response: Thank you for the suggestion and we did a quantitative analysis in human cell co-culture. The result revealed mitochondrial transfer in 83.3% of human DRG neurons, and 35.3% of these neurons exhibited TNTs (**Response Fig. 17**). We added these results in **Extended Data Fig. 15e-f**.

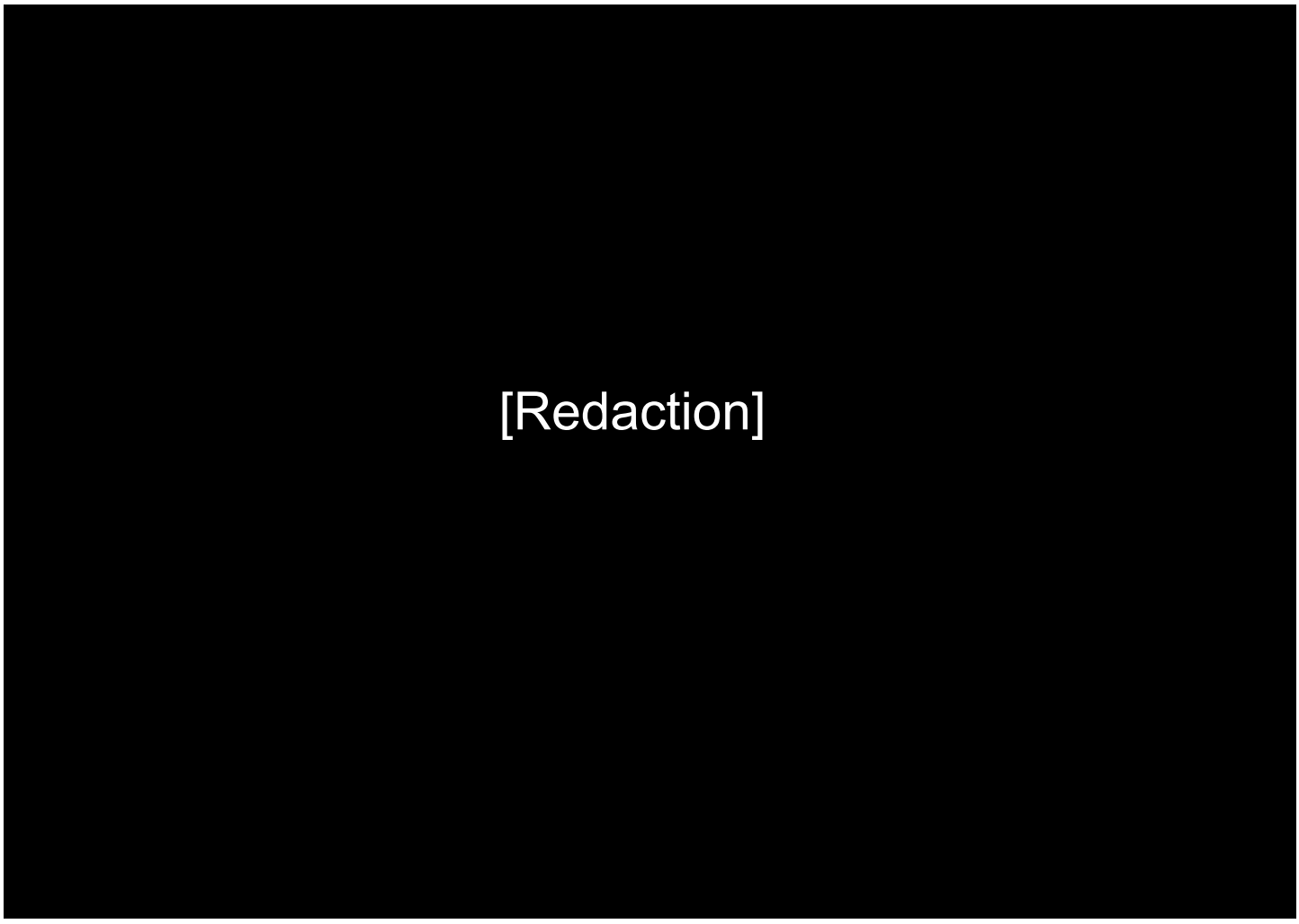


Response Fig. 17 / Extended Data Fig. 15e-f

9. In Figure 5, xenogeneic human derived mitochondria reduce pain behavior in mice. It has reported that mouse and human cells do not retain functional compatibility of mtDNA (e.g. Yoon et al, Mitochondrion 2007). It may be helpful for authors to consider these ideas: (i) is mitochondrial DNA not required for mitigating neuropathic pain but only mitochondrial components are sufficient; (ii) if so, then what are they? (iii) can transferred human mitochondria upregulate endogenous Myo10 in mouse SGCs, thus increasing mitochondrial transfer? (iv) if human and mouse mitochondria do not "work together", then mitochondrial transfer is in fact not required to resolving neuropathic pain? The authors should address or discuss these issues.

Response: Thank you for these insightful points. During mitochondrial transfer, various mitochondrial components—such as DNA, RNA, and proteins—can be exchanged between cells [9]. We have to acknowledge that mitochondrial DNA (mtDNA) editing faces significant challenges due to the unique mitochondrial environment and the lack of efficient RNA import [10].

To evaluate whether transferred mitochondrial components are sufficient to enhance mitochondrial function, we applied specific inhibitors targeting different complexes of the electron transport chain in SGCs. We used rotenone (0.5 μM) to inhibit mitochondrial complex I [11], oxaloacetate (OAA, 10 μM) to inhibit mitochondrial complex II [12], antimycin A (0.5 μM) to inhibit mitochondrial complex III [13], and Cyanide (10 μM) to inhibit inhibitor of mitochondrial complex IV [14] in SGC cultures. [redacted]



Response Fig. 18

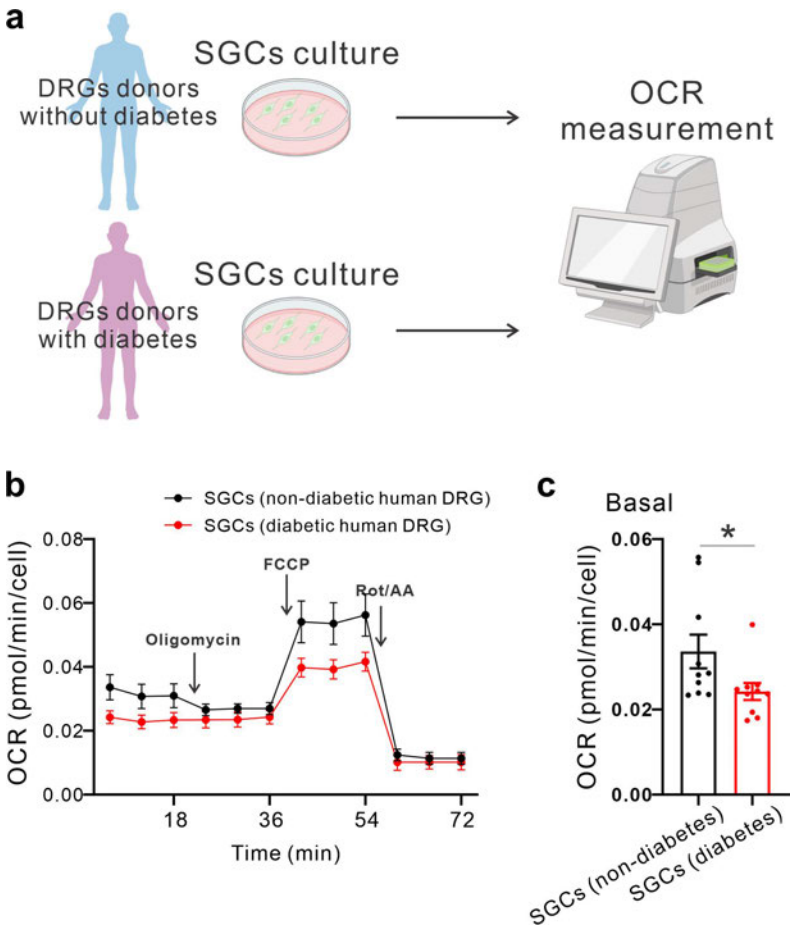
[redacted]

Response Fig. 19

We also identified a previous study demonstrating mitochondrial fusion between mouse and human cells[15], indicating that human and mouse mitochondria may function together. We have included this description in the Discussion section. Future studies are warranted to further investigate their compatibility and potential additive effects.

10. Mitochondria from diabetic human SGCs appear to decrease the ability to resolve the pain compared to healthy mitochondria. The authors should consider verifying the differences in mitochondrial quality between one from normal vs those from diabetic sources.

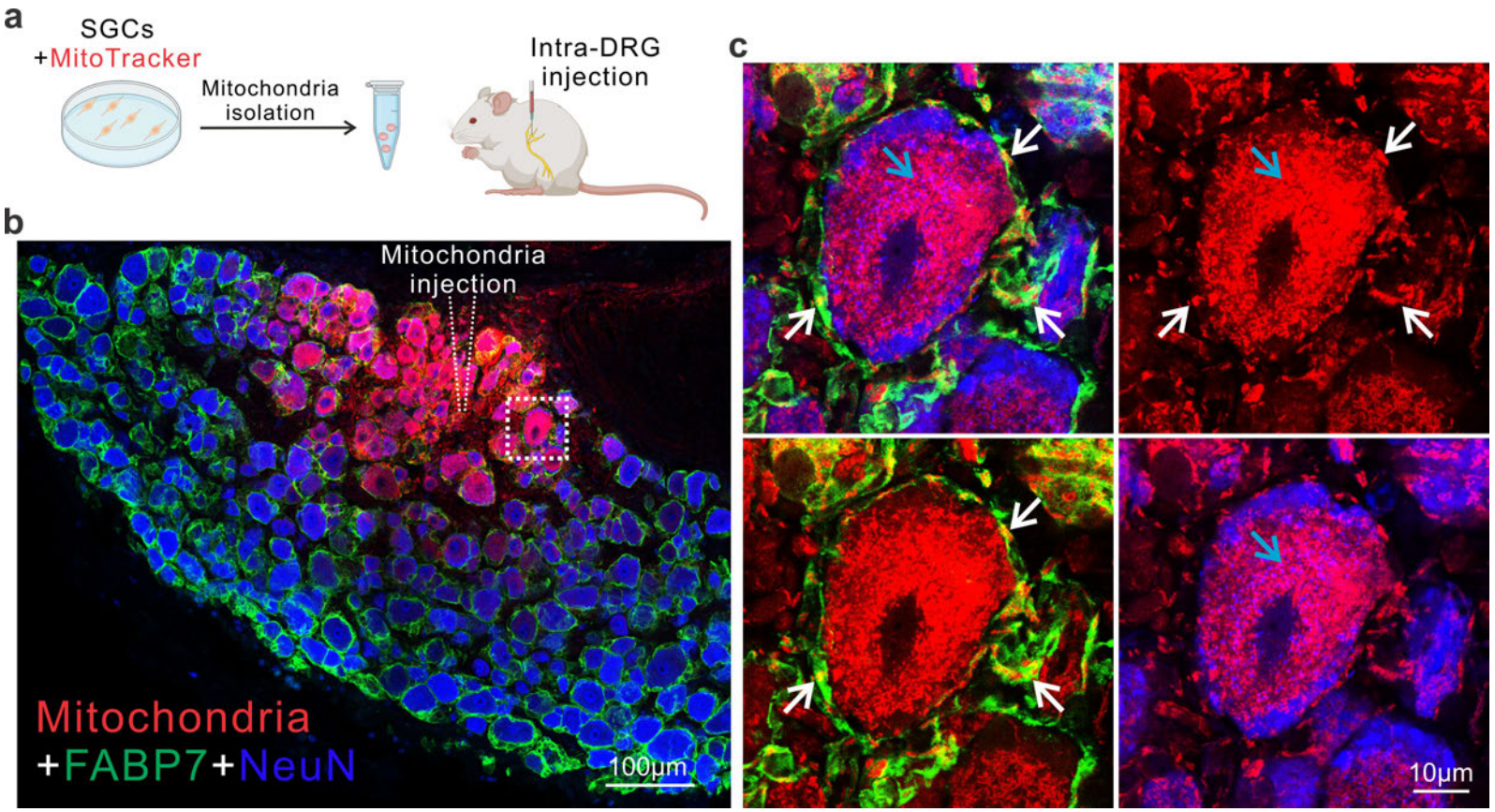
Response: Thank you for these insightful points. To test whether diabetes would affect the mitochondrial quality of SGCs, we measured mitochondrial function of SGCs from human DRG donors with and without diabetes. Our Seahorse OCR measurement revealed that the basal oxygen consumption of the diabetic SGCs was significantly decreased compared to non-diabetic SGCs (0.033 ± 0.004 vs. 0.024 ± 0.002, $P < 0.05$. **Response Fig. 20**). This data suggests that diabetes would cause dysfunctional mitochondria in human SGCs, which results in to decrease in the ability to resolve the pain compared to healthy mitochondria in SGCs adopted transfer experiment. We added this quantification data to **Extended Data Fig. 16k**.



Response Fig. 20 / Extended Data Fig. 16k

11. Directionality may also be relevant for the arrows in Figure 5. Would adopted mitochondria transfer only target neurons? Or these may go into other cells like neurons directly as well?

Response: To determine whether adoptively transferred mitochondria specifically target neurons or can also enter other cell types within the DRG, we labeled mitochondria in cultured human SGCs using MitoTracker and isolated them as previously described[16]. We then performed intra-DRG injection of isolated mitochondria derived from 10,000 SGCs into the L5 DRG (**Response Fig. 21a**). Immunostaining for FABP7 and NeuN revealed that the adoptively transferred mitochondria targeted not only neurons but also SGCs within the DRG (**Response Fig. 21b-c**). This data has been added to **Extended Data Fig. 16a-c**.



Response Fig. 21 / Extended Data Fig. 16a-c

Referee #2:

The topic of this MS is tunneling nanotubes (TNT), which are presumed to connect neurons and satellite glial cells (SGCs) in dorsal root ganglia of mice and humans. The authors propose that TNT allow the passage of mitochondria from neurons to SGCs, and present evidence for the importance of this mechanism to chronic pain. The authors used an impressive array of methods, and present a large amount of data. My impression is that in this case, the quantity is not an advantage. Some important conclusions are not well supported, and the Discussion is not satisfactory.
Originality - High
Statistics - OK
Methods - State of the art, but interpretation not always satisfactory.
References – Not always accurate, need to be checked.
Conclusions – Weak, see below for specifics.
Writing – good.

Response: We thank the reviewer for their extensive expertise in this area and careful reading of our manuscript. We appreciate their insightful, critical, and constructive feedback. Accordingly, we performed many new experiments in the last six months and revised the manuscript to consolidate the conclusions and improve scientific rigor of this study.

a) We included the statistical information of all the figures of this study in **Extended Table 4**. We have included more details in the methods section and updated the references. We revised the discussion section to highlight the major findings of this study.

b) We have performed many new experiments to address all the comments, including validation of SGC cultures with 5 different antibodies (**Response Fig. 4**), [redacted] transmission electron microscopy (TEM) of mouse and human DRG to detect TNTs with mitochondria and vesicles (**Response Fig. 5 - Response Fig. 7**).

c) We have improved the resolution and quality of several images (**Main text: Fig. 3d, Extended Data Fig. 1, and 2**).

Please also find our point-by-point response to each of Reviewer #2's comments below.

Major comments

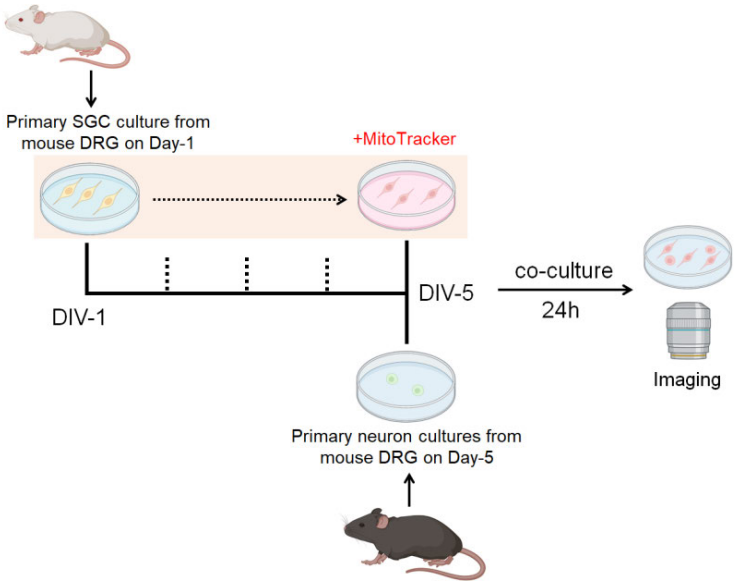
1. Line 102, "SGC marker GFAP". GFAP is not an SGC marker. The cited paper (Woodham et al., 1989) states: "… the great majority of satellite cells within the fourth and fifth lumbar dorsal root ganglia on the unoperated side did not react with the polyclonal antisera to GFAP (Figs. 1 and 3). The proportion of neurons surrounded by fluorescent labelled satellite cells ranged from 3% 30% with a mean of 15.5%. Unoperated animals also gave results within that range." Therefore, the identity of the GFAP+ cells in the cultures is not clear, but they cannot be reliably called SGCs. There is evidence that SGCs from sensory ganglia change their phenotype in culture (doi: 10.1017/S1740925X1100007X.).

Thus, the images in Extended Data Fig. 1a probably do not represent SGCs. Also, in this figure it's impossible to find cells other than GFAP+ ones. Each cell type should be indicated. Also, in 5-day cultures neurons should have long processes, which are not visible in the images. Therefore, the experiments based in these cultures are doubtful. In the rest of the paper, SGCs were identified by staining for FABP7, and it's not clear why this was not done for the presumed SGCs in culture. These comments also hold for the results in Fig. 3, Extended Data Fig. 8, and the results on isolated mitochondria from cultures.

Response: Thank you for raising this important question. We acknowledge that GFAP may not be an ideal marker for SGCs. A snRNA-seq study showed that SGC shares gene transcripts with astrocytes, including *Gfap* and *Fabp7* [6]. Many studies have shown GFAP immunoreactivity in DRG SGCs, and its upregulation in SGCs has been reported in different injury and pain conditions[17-19].

We agree that sole verification of SGCs by GFAP is not sufficient. We conducted immunostaining with additional SGC markers, including FABP7 [6], Kir4.1 (potassium channel 4.1) [20], AQP4 [7], CX43 (connexin 43) [21], and GS (glutamine synthetase)[22]. Our quantitative analysis revealed that 94.9-97.1% of SGCs express these markers (**Response Fig. 4**), validating the identity and purity of our SGC cultures (**Response Fig. 5)**. We also added the data to **Extended Data Fig. 1a.**

We apologize for the confusion due to the lack of experimental details. Indeed, we cultured SGCs for 5 days and DRG neurons for 1 day, and then mixed two cultures. Please find the inserted co-culture schematic below (**Main Fig. 1A**).



The reviewer is correct, DRG neurons exhibit extensive neurites 5 days in culture (previous Extended Data Fig. 8e, now **Extended Data Fig. 12f**). However, we did not see obvious axonal outgrowth of mouse DRG neurons 1 day in culture. We seeded primary DRG neuron on coverslips coated with 0.1 mg/mL poly-D-lysine and labeled neurons via βIII-tub staining. We add more details of primary culture of SGCs and neurons in the Method section (page 21).

2. Line 110. "A quantitative analysis revealed mitochondrial transfer in 84.4% of DRG neurons, and 27.5% of these neurons exhibited TNTs". How do you explain these numbers, which are apparently not in agreement?

Response: We only observed TNTs in 27.5% of neurons in SGC-neuron co-cultures (**Main Fig. 1c**), raising the question of whether TNTs are consistently present in culture. To address this, we performed live-cell imaging in SGC-neuron co-cultures (**Response Fig. 3a, above**). We found that a TNT structure disassembled within 12 minutes (**Response Fig. 3b, above**), indicating the highly transient nature of TNT formation. This observation is consistent with previous reports showing that TNTs can form within as little as 25 min[2]. We added the new data to **Extended Data Fig. 1c-d.**

3. Fig. 1e. How do you know that you injected to MitoTracker into an SGC? The images are not very convincing. The bright spot at 1h could be in a neuron.

Response: We appreciate the reviewer's insight and conducted additional experiments of single-SGC MitoTracker microinjection [redacted] SGCs [23]. [redacted]

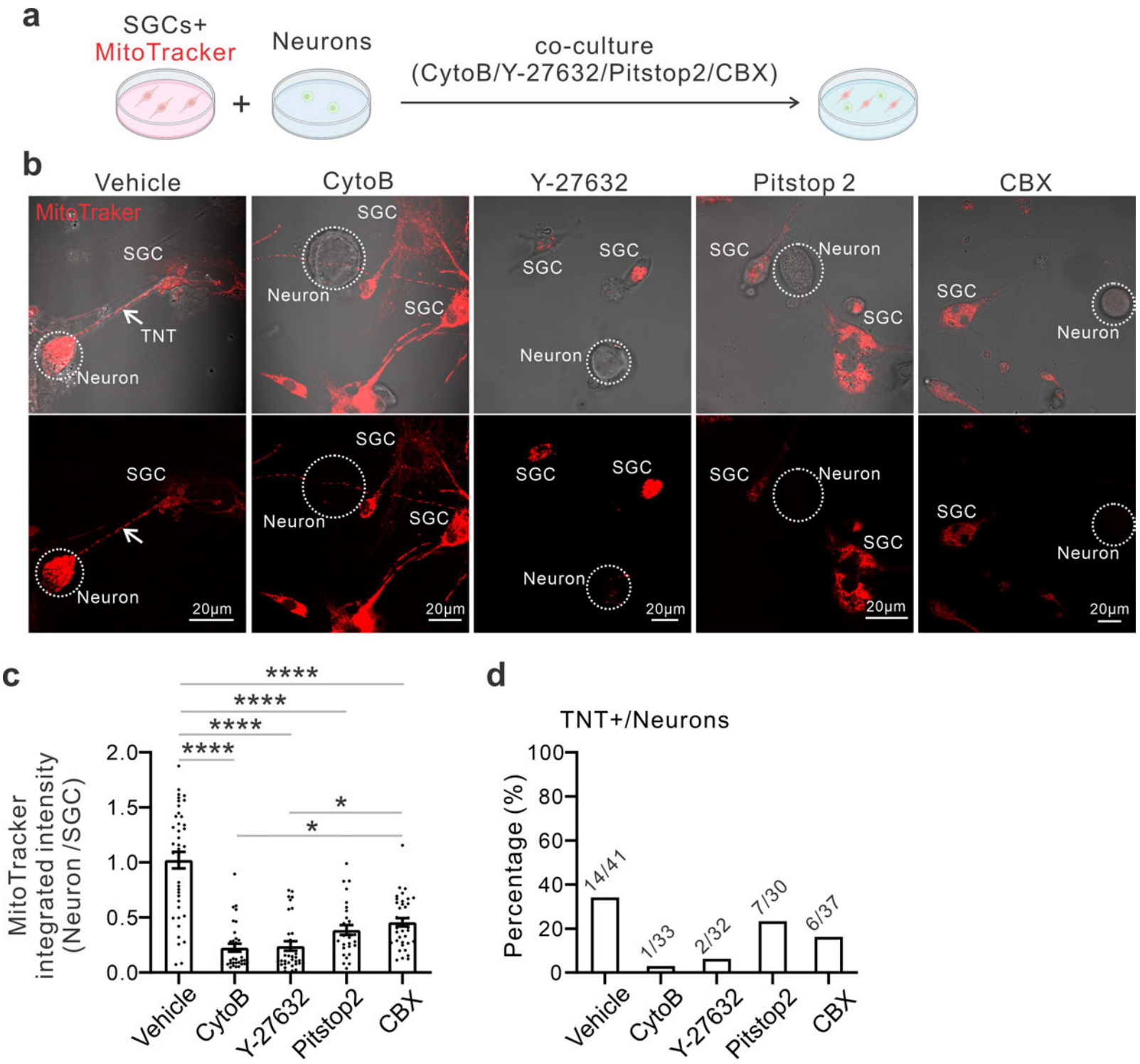
[Redaction]

4. Line 112, concerning mitochondrial transport via TNT. The citation from Ref 20 is incorrect. In Ref 20, P. 92, it is stated: "However, mitochondrial transport between pericytes was not observed, consistent with the closed-ended nature of IP-TNTs". The experiments with the gap junction blocker CBX make no sense, as gap junctions are channers that are too small to allow the passage of mitochondria. The role of gap junctions in mitochondrial transport is still possible, but not through the channels. You did not rule out a role for gap junctions in mitochondrial transport.

Response: We apologize for our inaccurate citation of the reference. We corrected the description as “nanotubes capable of transferring mitochondria have been identified in the mouse retina, connecting two bona fide pericytes on separate capillary networks”.

We agree that gap junction hemi-channels are too small for the passage of mitochondria, but an assisting role of gap junctions in mitochondrial transport is still possible. Our new results showed that gap junction blocker CBX treatment showed a partial but significant inhibition ($P < 0.0001$) of mitochondrial transfer in SGC-neuron co-cultures (**Response Fig. 23a-c**). Furthermore, CBX treatment reduced the proportion of the TNT formation between SGCs and neurons (**Response Fig. 23d**). Notably, the TNT and endocytosis inhibitors (CytoB and Pitstop 2) showed almost complete inhibition of the transfer (**Response Fig. 23**). Therefore, gap junction communication may be indirectly involved in the mitochondrial transfer between SGCs to neurons. We add this quantification data to **Extended Data Fig. 1i-k**.

Notably, SGCs highly express the gap junction protein Connexin43 (Cx43) [17,24,25], and Cx43-mediated gap junction communication plays a crucial role in SGC-neuron interactions in the DRG [26]. Interestingly, Cx43 was proposed to function as a stabilizer, adhering to the docked membrane structures of two cells [27,28]. Furthermore, Cx43 may increase the probability of successful formation of TNTs [29-33].

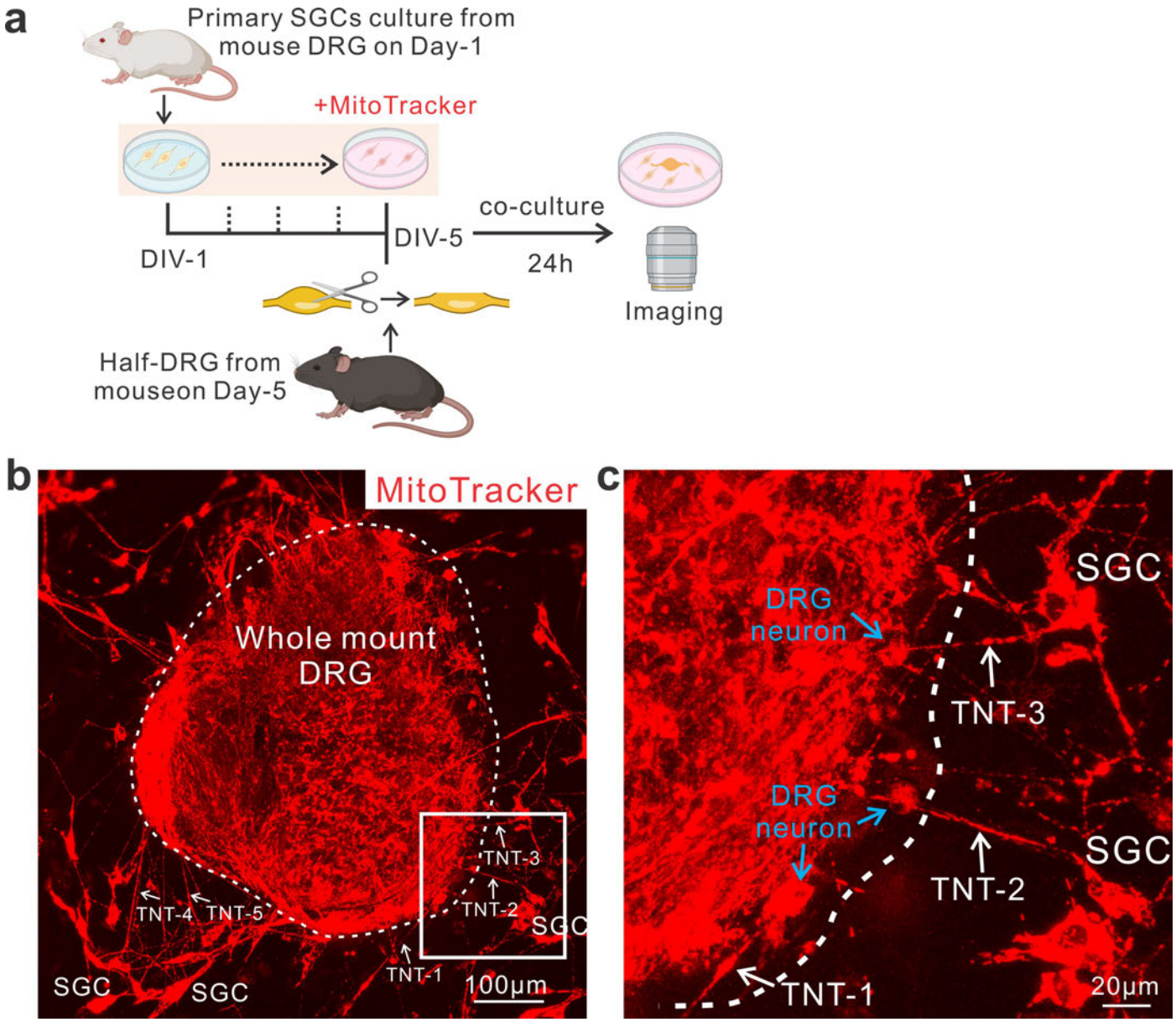


Response Fig. 23 / Extended Data Fig. 1i-k

5. Extended Data Fig. 1 f,g. It is not clear what is "whole mount DRG". Is this a whole intact ganglion? It is not clear what are the cells that were labeled. The green labeling suggest that these are neurons, but the cellular details are fuzzy. DRG are covered with a connective tissue capsule, can cells connect through the capsule?

Response: We apologize for the confusion. In this experiment, “whole mount DRG” refers to half of the DRG obtained via longitudinal sectioning. The same preparation was used for the scanning EM experiment, in which a flat surface of the DRG tissue was exposed by cutting with Vannas spring scissors (FST, 15019-10) to disrupt the connective tissue capsule. The half-DRG were then cultured with MitoTracker-labeled SGCs (**Response Fig. 24a**). We have added this clarification to both the Result and Method Sections.

We also included high-magnification images of the sectioned DRG co-cultured with SGCs (**Response Fig. 24b-c**), showing the TNTs formed between the cultured SGCs and DRG tissue (indicated by arrowheads). Moreover, MitoTracker labeling was observed in neurons at the edge of DRG (indicated by arrows, **Response Fig. 24c**).



Response Fig. 24 / Extended Data Fig. 2c-e

Representative images of SGC co-culture with the whole-mount DRG with longitudinal sectioning. (a) Schematic of co-culture. (b) Low-magnification image showing the TNTs formed between the cultured SGCs and DRG tissue. (c) High-magnification image showing the TNTs formed between the cultured SGCs and DRG tissue. White arrows indicate TNTs, and blue arrows indicate the DRG neurons. Scale bars: 100 μm (left), 20 μm (right).

6. Fig 1 f, Fig. 4 k. How was it determined that: 1. the fibers are nanotubes (they might be neurites, connective tissue…, 2. That they originate in an SGC? 3. That the bulges (vesicles) are mitochondria? 4. How do you know that there is a vesicle inside the presumed TNT (line 901)?

Response: We thank the reviewer for these important questions regarding the identification of tunneling nanotubes (TNTs) and mitochondrial transfer. We address each point below:

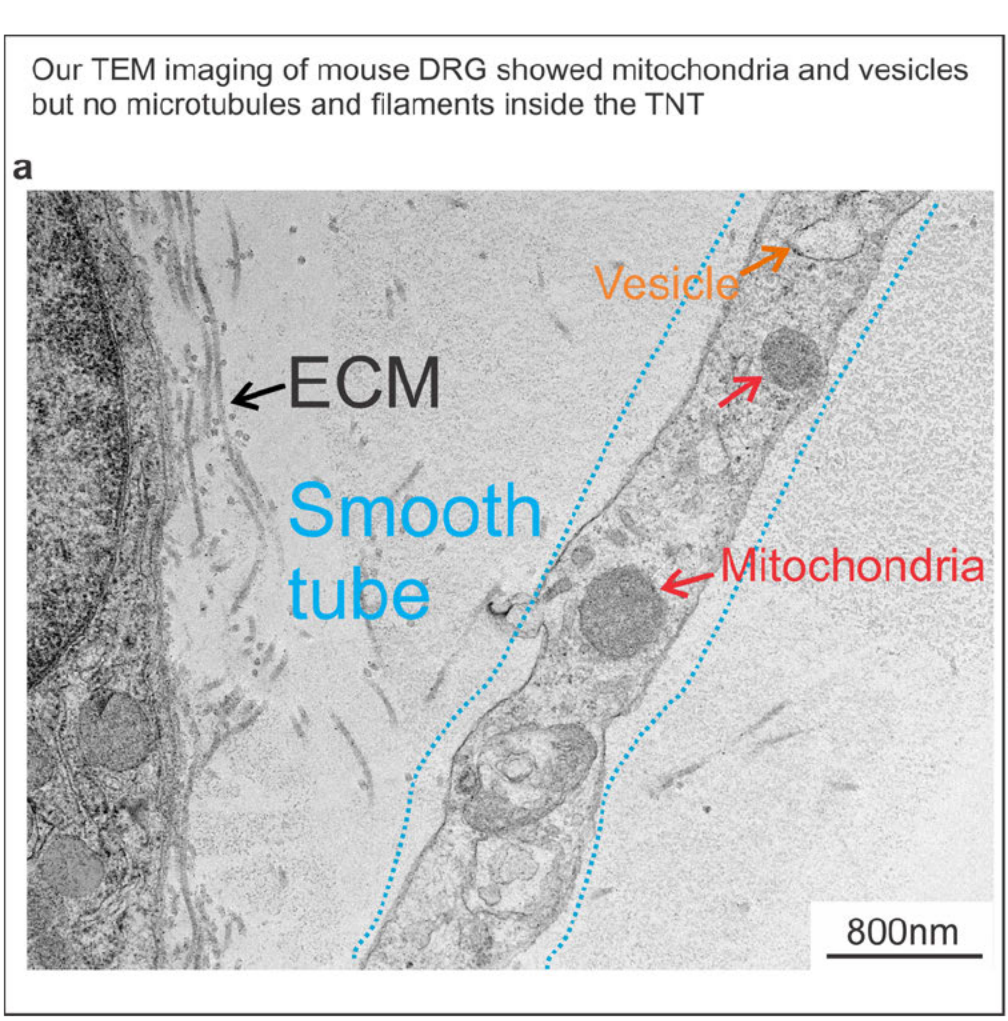
6.1. Identification of TNTs vs. neurites or connective tissue:

To reveal more details about TNTs and distinguish them from other cellular structures such as neurites or connective tissue, we conducted transmission electron microscopy (TEM) imaging on mouse and human DRG using 70 nm sections (**Response Fig. 5-7, above**).

Notably, TNTs were found to contain mitochondria and vesicles but lacked microtubules and myelin sheaths (**Response Fig. 25b, below**). In contrast, axons contain microtubules and, in the case of A-fibers, are also wrapped by myelin sheath [34] [redacted]

[redacted]

[redacted]



[Redaction]

Response Fig. 25

6.2. Determining the origin from SGCs:

In the physiological conditions, most SGCs close-wrap the DRG neuron [17], which makes it hard to observe the TNT between SGC and neuron by scanning EM (SEM) imaging. However, we found the enlarged gaps between SGC and neuron after chemotherapy and diabetic peripheral neuropathy conditions, which made the TNT between SGC and neuron easier to detect; the images showed the TNT originating from SGC connected to the neuron (**Main Fig. 1l-m**). Importantly, our in vitro and ex vivo experiments strongly suggest the existence of TNTs formed between SGCs and neurons of DRG co-cultures (**Fig. 1b and Extended Data Fig. 2c-e**). However, we acknowledge that high-resolution immunoelectron microscopy is required to definitively confirm the presence of mitochondria within TNTs between SGC and neuron in DRG tissue.

6.3. Confirmation that the bulges are mitochondria:

We used MitoTracker staining and SGC-specific mitochondrial labeling to track mitochondrial movement. Live-cell imaging and confocal microscopy showed the co-localization of these mitochondrial signals within the bulges along TNTs (**Extended Data Fig. 1d**). Furthermore, transmission electron microscopy (TEM) revealed double-membraned organelles with high-density and internal structures consistent with mitochondria inside TNTs, supporting the mitochondrial identity of the transported cargo (**Fig. 1n-r**).

6.4. Evidence of vesicles inside TNTs:

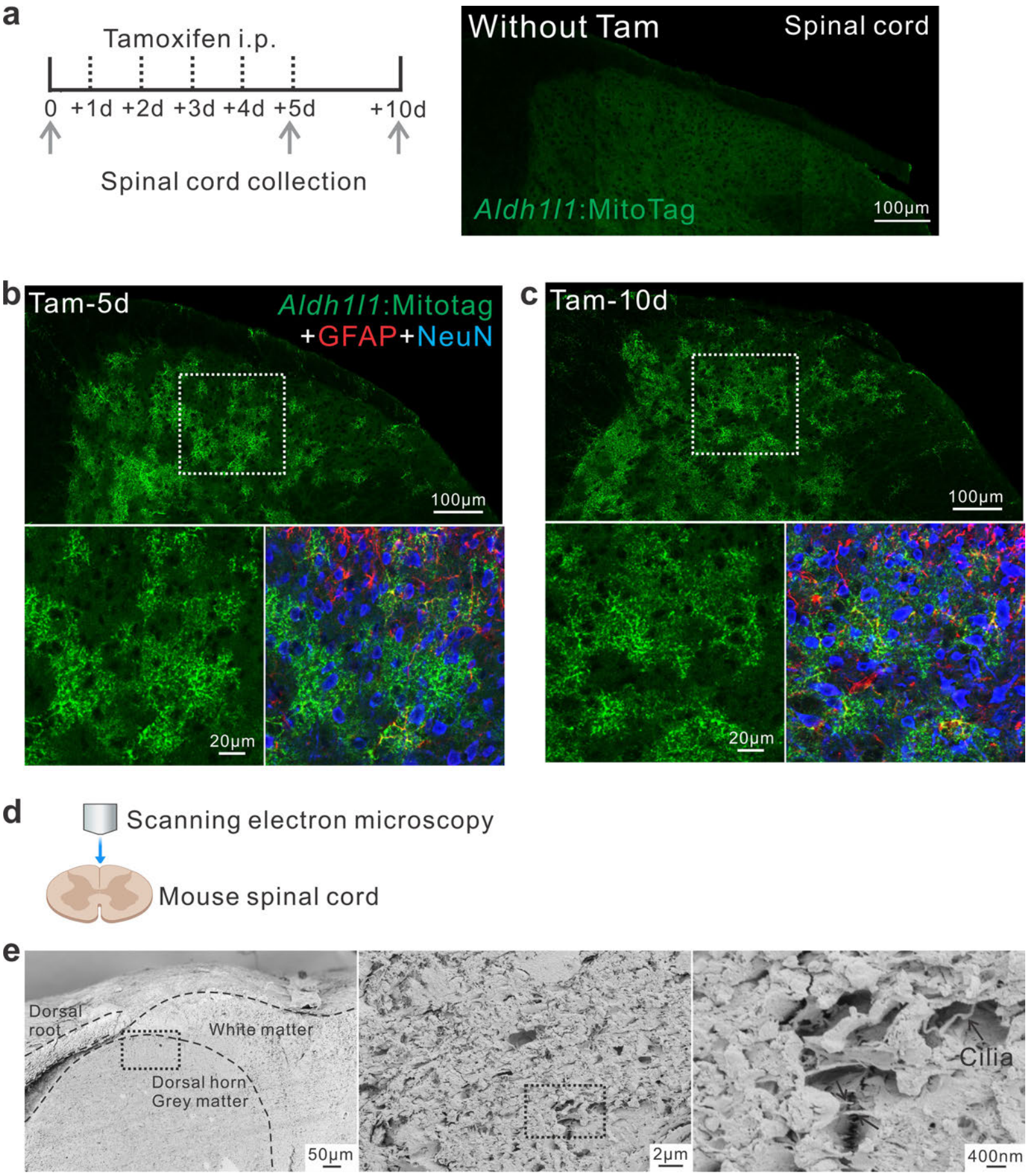
TEM provided direct ultrastructural evidence of vesicular structures within TNTs of mouse and human DRG (**Response Fig. 5-7, above**). We added these TEM data to **Fig. 1n-r, Extended Data Fig. 4, and Extended Data Fig. 13i-j.**

7. The authors rely heavily ("critically", line 160) on the experiments where CytoB was used. CytoB is an inhibitor of actin polymerization, and can have numerous cellular effects, for example it inhibits protein trafficking in astrocytes, see doi: 10.1523/JNEUROSCI.19-23-10193.1999.

Response: We tested additional TNT inhibitor, Y-27632, a ROCK inhibitor known to block uropod formation [36]. Similar to the effect observed with CytoB, treatment of 10 μM Y-27632 in SGC-neuron co-culture inhibited both TNT formation and mitochondrial transfer from SGCs to neurons (**Response Fig. 23**). We added this result in **Extended Data Fig. 1i-k**.

8. It is not clear why observations on the spinal cord were included, as they appear not to be related to the main theme of this work. This, and many other issues are not addressed in the discussion.

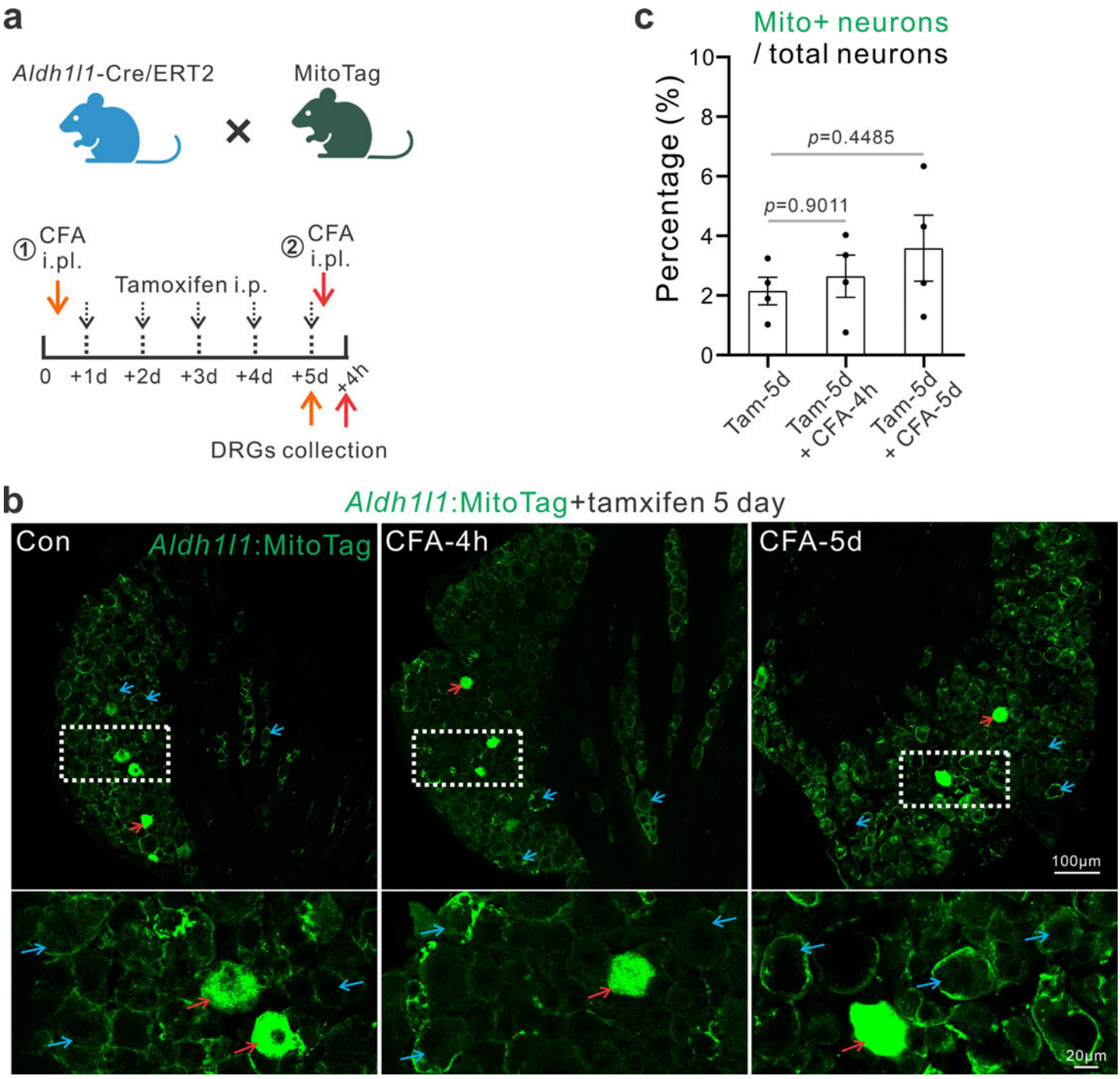
Response: Spinal cord astrocytes share many molecular markers with SGCs [24], and mitochondrial transfer from astrocytes to neurons has been reported in the brain as a protective mechanism against stroke [37]. Surprisingly, in our study using *Aldh1l1*:MitoTag mice, we failed to see mitochondrial transfer from astrocytes to neurons in the spinal cord. Instead of TNTs, we observed **cilia-like structures** in spinal cord astrocytes. As suggested by the reviewer, we removed the data from the main text to sharpen the focus of this study on SGCs. The spinal cord data from Aldh1l1:MitoTag mice, along with the corresponding scanning EM images, are now included in **Extended Fig. 6 (see below)**. We have also addressed this important issue in the discussion section "Although spinal cord astrocytes share many molecular markers with SGCs, we did not observe mitochondrial transfer from astrocytes to neurons in the spinal cord. Instead of TNTs, we observed cilia-like structures in spinal cord astrocytes, consistent with previous research in brain astrocyte."



Extended Data Fig. 6

9. Line 172. ".. suggesting that transient (hours) tissue injury [by CFA] and inflammation may be insufficient to promote the transfer of mitochondria". However, the effects of a single CFA injection can last for days and even weeks; see 10.1016/s0304-3959(03)00201-x, 10.1016/j.jpain.2004.04.005

Response: We thank the reviewer for the constructive comment. To address this issue, we tested mitochondrial transfer in Aldh1l1-MitoTag mice 5 days after a single CFA injection, matching the time point used in the SNI model (**Response Fig. 26a; Main Fig. 2e-f**). The new results did not show significant increase in the percentage of MitoTag-labeled neurons (**Response Fig. 26b-c**), suggesting that, unlike nerve injury, CFA-induced inflammation may be insufficient to trigger SGC-neuron mitochondrial transfer. These results have been added to **Extended Data Fig. 5g-h**.



Response Fig. 26 / Extended Data Fig. 5g-h

Representative DRG images from CFA-treated *Aldh1l1*-MitoTag. Red arrows indicate MitoTag+ neurons, and blue arrows indicate MitoTag-negative neurons.

10. Fig 2b the boxed area in Tam-10d does not correspond to the magnified images below.

Response: Thank you for your careful review. We have replaced the box with the corrected images (**Fig. 2b**).

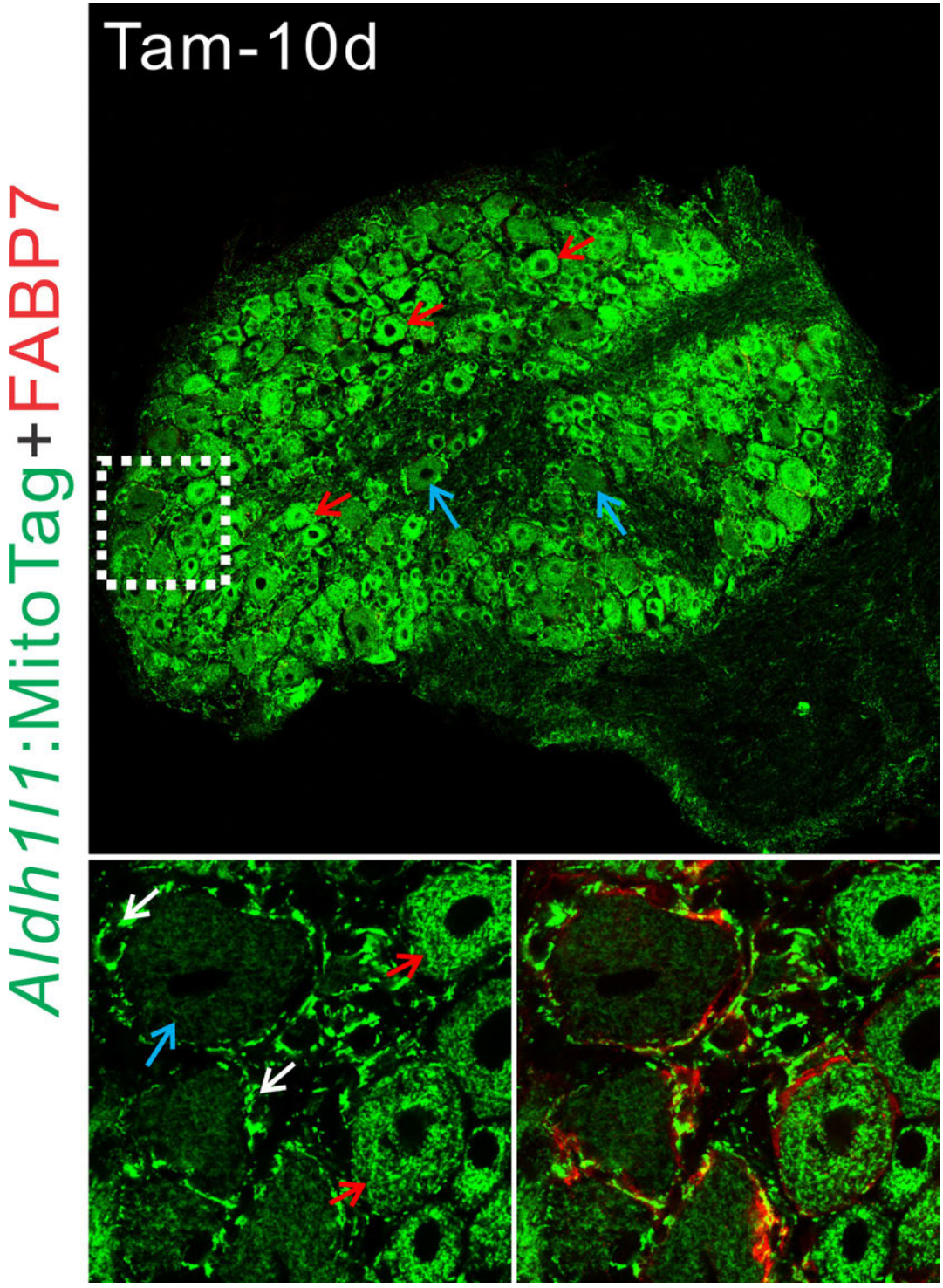
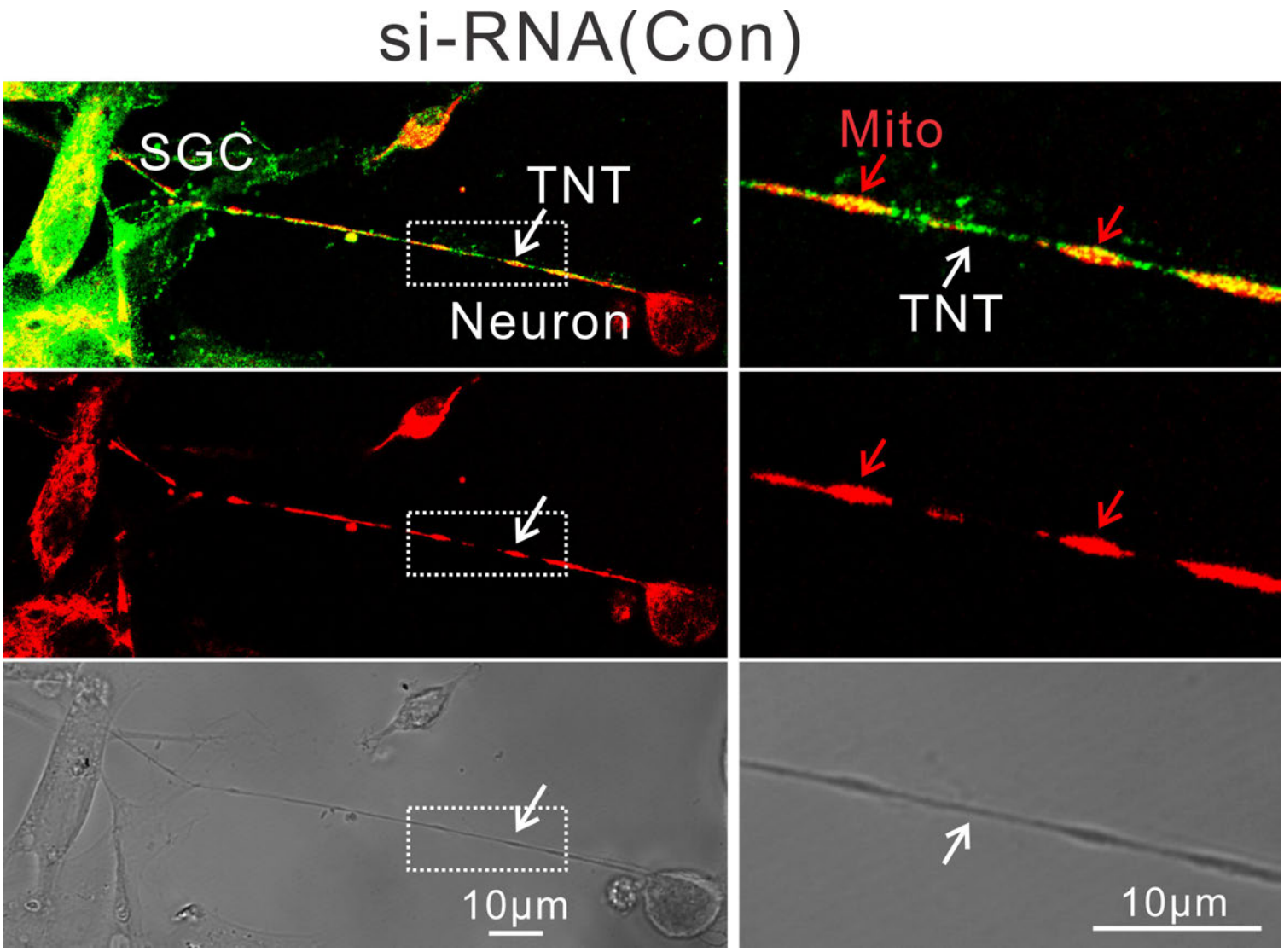


Fig. 2b

11. Fig. 3. The structure labeled "TNT" contains green (MYO10) and red (Mitotracker), but the red-stained regions don't look like mitochondria, and there are no bulges/vesicles in it.

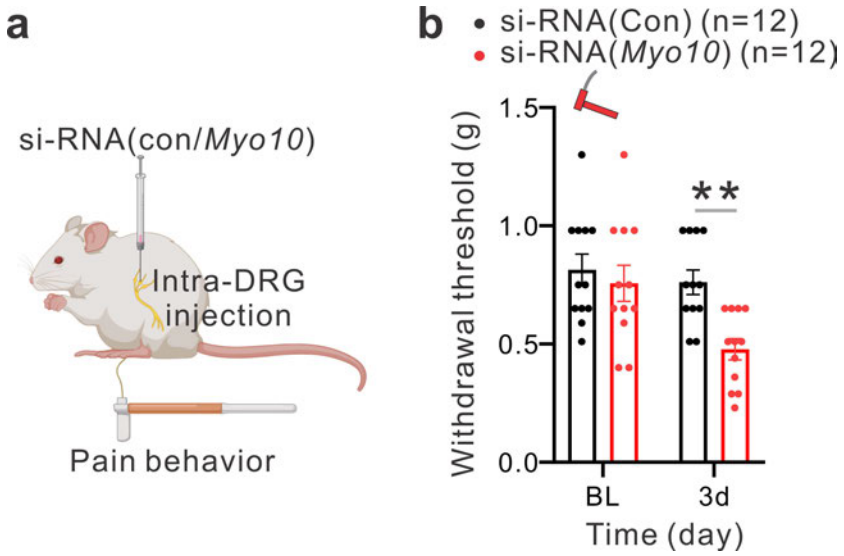
Response: We thank the reviewer for their careful review. We replaced the images in **Fig. 3d** and included high-magnification images to highlight the bulged mitochondria within the TNTs (**Response Fig. 27 / Fig. 3d**).



Response Fig. 27 / Fig. 3d

12. Lines 241-2, "Myo10 siRNA treatment also resulted in a moderate reduction in paw withdrawal threshold (Extended Data Fig. 6a-b)". But this figure shows no difference under von Frey test.

Response: We increased the sample sizes of animals to n=12 in each group (6 male mice and 6 female mice). The new von Frey test result showed the si-RNA (*Myo10*) treatment via intra-DRG injection resulted in a significant decrease in paw withdrawal threshold at day 3 ($P < 0.01$, **Response Fig. 28**). This new result has been added to **Extended Data Fig. 9b-c**.

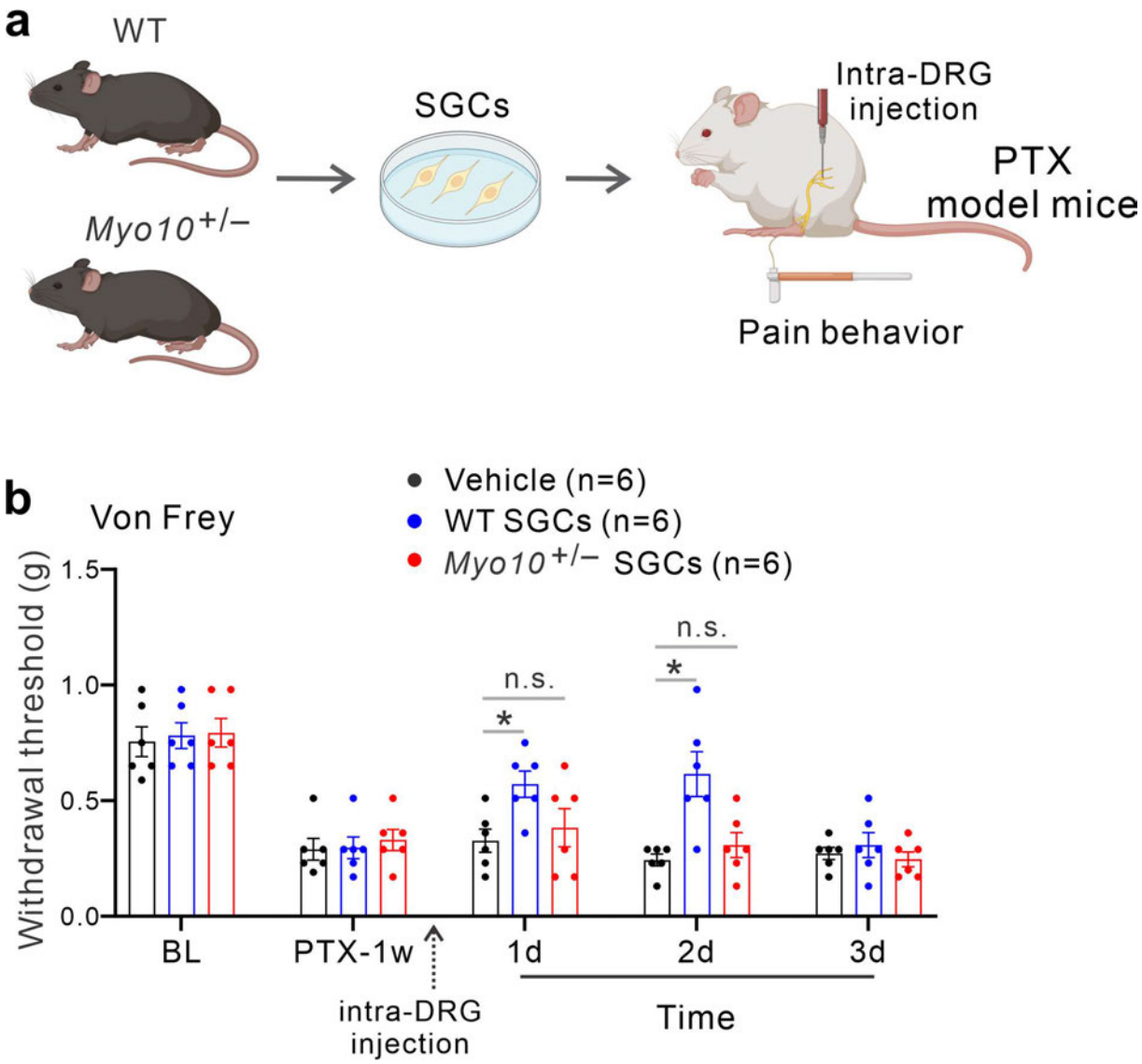


Response Fig. 28 / Extended Data Fig. 9b-c

Furthermore, we obtained *Myo10* mutant mice from Dr. Richard Cheney of University of North Carolina. Due to developmental defects observed in homozygous mice [1]. we utilized *Myo10* heterozygous ($Myo^{+/-}$) mice for our experiment (**Response Fig. 1a, above**). We tested mechanical and thermal responses in $Myo10^{+/-}$mice and WT mice. Compared to WT controls, $Myo^{+/-}$ mice exhibited heightened mechanical and heat sensitivity (**Response Fig. 1b-c, and Response Fig. 2a-c, above**). We have added these results to **Fig. 3m-n and Extended Data Fig. 9g-i**.

13. Lines 371-403 and Fig. 5i, You propose that the transferred SGCs formed TNT in the mouse DRG. Also do you propose that the transferred mitochondria entered the DRG cells, thereby exerting a beneficial effect. Is there direct evidence for this?

Response: Since MYO10 in SGCs plays a crucial role in TNT formation in the DRG, we examined whether adoptive transfer of SGCs could form TNTs and alleviate pain. We cultured SGCs from the DRG of WT and *Myo10*$^{+/-}$ mice, and then performed intra-DRG injection of these SGCs into PTX-treated mice, followed by behavioral testing (**Response Fig. 29a**). The new results showed that adoptive transfer of WT SGCs reduced mechanical pain on day 1 and 2; however, SGCs from *Myo10*$^{+/-}$ mice did not have any analgesic effect (**Response Fig. 29b**). These findings suggest that adoptively transferred SGCs can alleviate chemotherapy-induced pain and that MYO10 is essential for mitochondrial transfer from SGCs to DRG neurons via TNTs. These results have been added to **Fig. 5d-e**.



Response Fig. 29 / Fig. 5d-e

14. Discussion The discussion is not satisfactory. It is mostly about the potential clinical implications of this work, but not about the results themselves, and their shortcomings. The comments above need to be addressed and in particular comments 1,4,6,8,
Why did you use four different pain models (SNI, PTX, db/db, STZ), and what do we learn from that?
The results support previous reports on the essential roles of SGCs in chronic pain. However, the authors did not discuss their results within the wide picture of neuron-SGC interactions.

Response: We appreciate your brief summary of the key points, which were highlighted in the Discussion Section. We apologize that we were not able to add extensive discussion of each question due to the space constraints of the journal. We have dressed these key comments in great detail in the previous responses. Also see below:

Re: Comments 1: We tested five additional markers to validate our SGC cultures (**Extended Data Fig. 1a**). One limitation is Aldh1l1 also expressed by other glial cell types, such as astrocytes. To improve specificity, we plan to test additional mouse lines for SGCs, when they become available to us. (page 18, line 520-5231). (also see Response to Comment 1).

Re: Comments 4: We tested the effects of gap-junction blockers (CBX) and found that CBX significantly inhibited mitochondrial transfer ($P < 0.0001$) from SGCs to neurons in the DRG and further reduced TNT formation ration (**Extended Data Fig. 1i-j**). (page 17, line 488-490, also see Response to Comment 4).

Re: Comments 6: We conducted transmission electron microscopy (TEM) to address the comment (also see Response to Comment 6).

Re: Comments 8: We moved the spinal cord data out of main text (**Extended Data Fig. 6**). (page 17, Discussion, line 479-481).

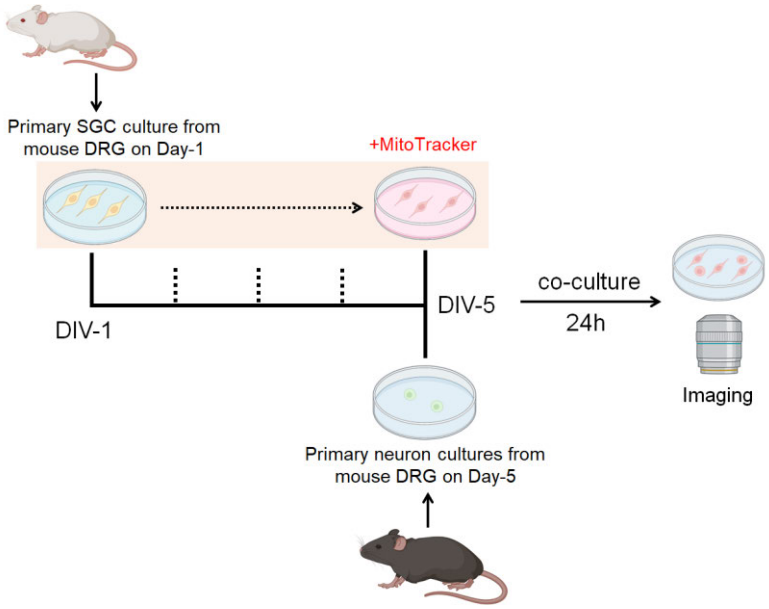
We appreciate the comment regarding the four different pain models included in this manuscript. We tested four different models of neuropathic pain: the SNI (nerve injury) model, PTX (chemotherapy-induced peripheral neuropathy, CIPN) model, genetic model of diabetes in db/db mice, and STZ-induced diabetic peripheral neuropathy (DPN) model. These are all established models of neuropathic pain and represent diverse etiologies of neuropathic pain. We believe that mitochondrial dysfunction represents a common mechanism underlying neuropathic pain across these models. Furthermore, the use of these models enables us to investigate the role of mitochondrial transfer in the DRG under various pathological conditions (page 18, line 501-509).

Finally, we provide a broader perspective on neuron–SGC interactions. For example, SGCs communicate with neurons through ion channels such as the potassium channel Kir4.1 [20], gap junctions modulated by Cx43 [25], and ATP receptors including P2X3 and P2RY12 [38], as well as cytokine signaling[24]. In our study, under both physiological and neuropathic pain conditions, we demonstrate that SGCs can transfer mitochondria to neurons in the DRG. This finding reveals a novel mechanism by which SGC–neuron interactions can regulate neuronal activity and promote nerve regeneration.

We highlighted these mechanisms in the Discussion Section. “In summary, multiple mechanisms have been implicated in SGC-mediated pain regulation through neuroglial interactions involving ATP, cytokines, potassium channels, and gap junction signaling in the DRG [17,24]. In this study, we reveal a previously unrecognized mechanism by which SGCs transfer mitochondria to surrounding neurons, contributing to neuropathic pain protection” (page: 18-19, line 524-531).

Minor comments

1. It is not clear how DRG neurons were purified.
Response: We apologize for the confusion due to the lack of experimental details. Indeed, we cultured SGCs for 5 days and DRG neurons for 1 day, and then mixed two cultures. Please find the inserted co-culture schematic below.



2. Extended Fig. 1f. What does 'in vivo mean'. Scanning EM is not done in vivo.
Response: We used intact whole DRG or half-cut DRG for SEM imaging without any prior culture. To avoid confusion, we have described it simply as whole or half-cut DRG used for SEM imaging (Page 6, line 153).

3. Line 162, Ref 15 is not on CytoB.
Response: We apologize for this confusion; Ref 15 is about the intrathecal (i.t.) route.

4. Lines 301-323. How are these results related to the main topic?
Response: We agree that the effects of FABP7 loss- and gain-of-function on pain are not the central focus of this manuscript. In alignment with comments from two other reviewers, we have removed the original Extended Figures 9 and 10 (related to FABP7 and MaR1) to maintain a clearer focus on mitochondrial transfer and its role in pain therapy.

5. Line 138, the gap shown in Fig. 1k appears to be about 500 nm, not <100 nm.
Response: We changed to < 500 nm.

6. Line 380. Who "…displayed significant reduction of mechanical pain"?
Response: We apologize for this confusion. We included a vehicle intra-DRG injection as a control. Human SGCs treated with control siRNA significantly reduced mechanical pain two days after transfer compared to the vehicle-treated group. ($P < 0.01$, **Main Fig. 5a-b**).

7. Fig. 5h. How long after mitochondria transfer was the paw skin tested?
Response: Hind paw skin samples were collected three days after adoptive mitochondrial transfer; this detail has been added to the Results section (Page 16, line 463).

Referee #3:

This is a striking study by Xu et al. that presents evidence for a novel mechanism of sensory neuron support via mitochondrial transfer from satellite glial cells in the dorsal root ganglion to the neurons they envelop. The study is a tour-de-force that covers an enormous range of approaches to interrogate this process from multiple angles. The authors implicate tunnelling nanotubes (TNTs) and Myosin10 in the transfer process and illustrate changes in this process in the settings of injury, diabetes, and chemotherapy induced neuropathic pain. Overall, the studies are impressive and well-described. The findings are provocative and of substantial potential translational relevance, especially given the demonstration of similar phenomena in human DRGs and the demonstration that adoptive transfer of mouse or human SGCs into mouse DRG can revert some features of neuropathic pain. There are, however, a few issues that warrant further consideration.

Response: We thank the reviewer for the positive evaluation of our manuscript and the constructive comments. All the comments raised are insightful. We have performed many new experiments to address these comments.

a) We conducted Structured illumination microscopy (SIM) imaging using the Zeiss Elyra 7 system with SIM^2 processing to resolve individual mitochondria in mouse SGC (**Response Fig. 9**).

[redacted]

[redacted]

d) We performed intra-DRG injection of MitoTracker-labeled isolated mitochondria and performed imaging to examine whether adoptively transferred mitochondria targeted neurons and SGCs in the DRG (**Response Fig. 21 and 39**).

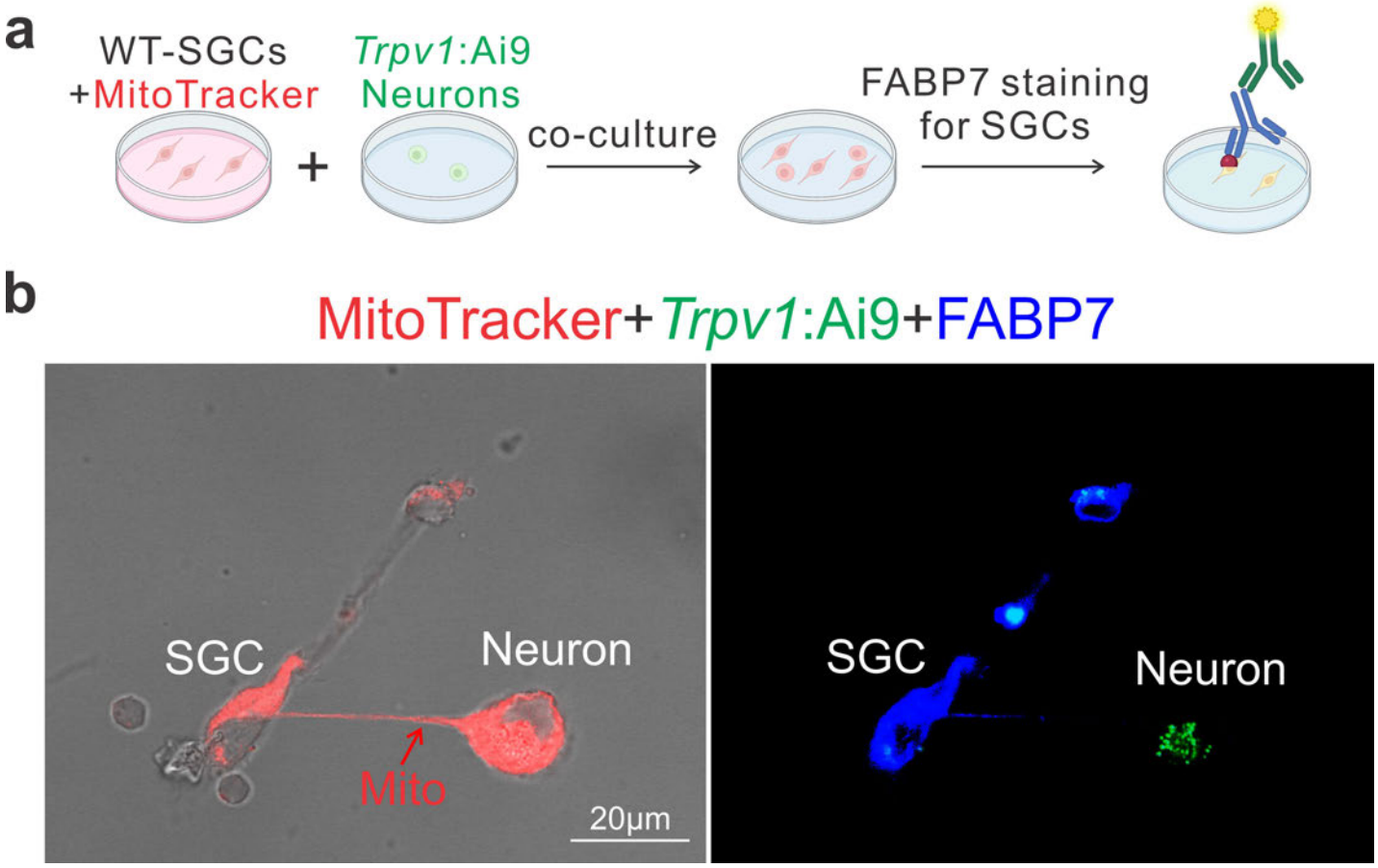
e) We improved the image quality in several figures (Main text: Fig. 3d, Extended Data Fig. 1, and 2).

Please also find our point-by-point response to each of Reviewer #3's comments below.

1) In Fig 1b, the morphology of the SGC shown is amorphous. How are SGC cells distinguished from TRPV1 negative neurons that have somehow taken up mitochondria?

Response: We conducted additional immunostaining for SGC markers, including FABP7 [6], Kir4.1 (potassium channel 4.1) [20], AQP4 (Aquaporin-4) [7], CX43 (connexin 43) [21], and GS (glutamine synthetase)[22]. The results validated the purity of SGCs (**Response Fig. 5**). We added this data to **Extended Data Fig. 1a**.

We conducted additional FABP7 staining in co-cultures of MitoTracker-labeled SGCs and TRPV1-labeled neurons from *Trpv1*:Ai9 reporter mice (**Response Figure. 30a**). The new results showed mitochondrial transport from FABP7-labeled SGC to *Trpv1*+ neuron (**Response Figure. 30b**). We added this data to **Extended Data Fig. 1b.**



Response Fig. 30 / Extended Fig. 1a-b

2) Is MitoTracker membrane impermeant? If not, in Fig 1d, how can the authors be sure that the spread to neurons is not by simple diffusion of the dye, rather than via mitochondria?

Response: MitoTracker is a membrane-permeant dye that readily enters live cells and selectively accumulates in active mitochondria when added to the culture medium in vitro.

[redacted]

3) Higher resolution fluorescence imaging to resolve individual mitochondria would be helpful in the fluorescence microscopy images.

Response: To obtain high-resolution fluorescence images, we performed structured illumination microscopy (SIM) using the Zeiss Elyra 7 microscope with SIM² processing, enabling us to resolve individual mitochondria in mouse SGCs. We analyzed mitochondrial length and found that the number of mitochondria shorter than 2 µm significantly increased 15 minutes after treatment with 1 µg/ml PTX (**Response Fig. 9a-c**). The increase in short mitochondria is indicative of enhanced mitochondrial fission, a cellular response to stress [39]. These findings suggest that PTX treatment directly induces mitochondrial dysfunction in SGCs. We have added these results to **Extended Fig. 3g-i**.

4) Page 5 line 117 - Do the authors mean between SGC and DRG neurons? "DRG" refers to the structure that includes SGCs, not solely to the neurons.

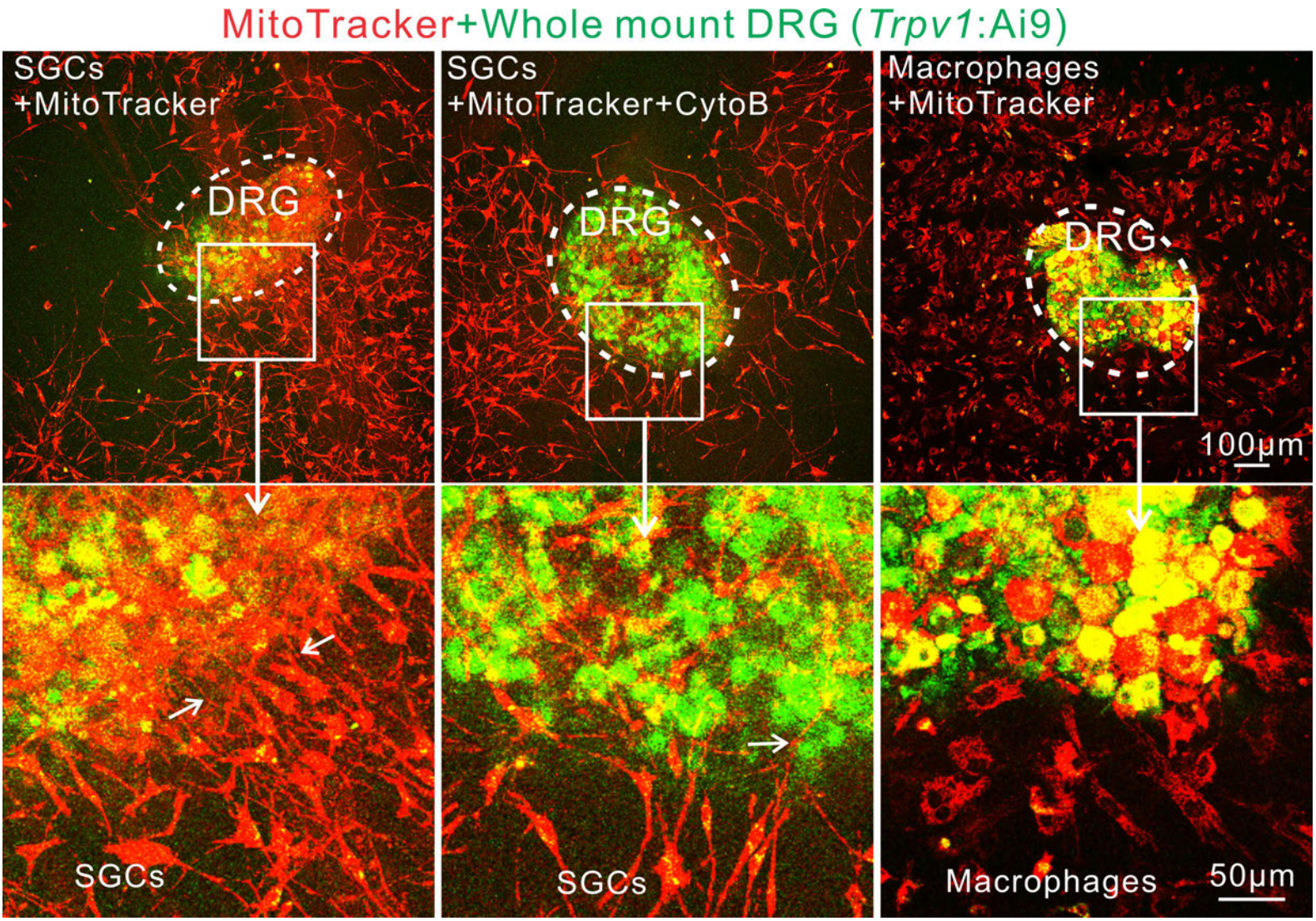
Response: We modified this statement to "We observed extensive TNTs formed between SGCs and DRG cells, including neurons and SGCs" (**Response Fig. 24**).

5) Differences between top and bottom panels in Ext Fig 1e are not well defined and don't seem to be consistent among the three columns.

Response: Thank you for raising this issue. We have re-organized these images to improve consistency (**Extended Fig. 1i**).

6) Low magnification of Ext Fig 1g makes it difficult to interpret

Response: We provided high-magnification images of whole-mount DRG co-culture with SGCs, SGCs treated with CytoB, and macrophages. These images revealed both MitoTracker-positive and MitoTracker-negative *Trpv1*:Ai9 neurons (**Response Fig. 31 / Extended Fig. 2g**). Note that only SGCs but not macrophages formed TNTs with TRPV1+ neurons.



Response Fig. 31 / Extended fig. 2g

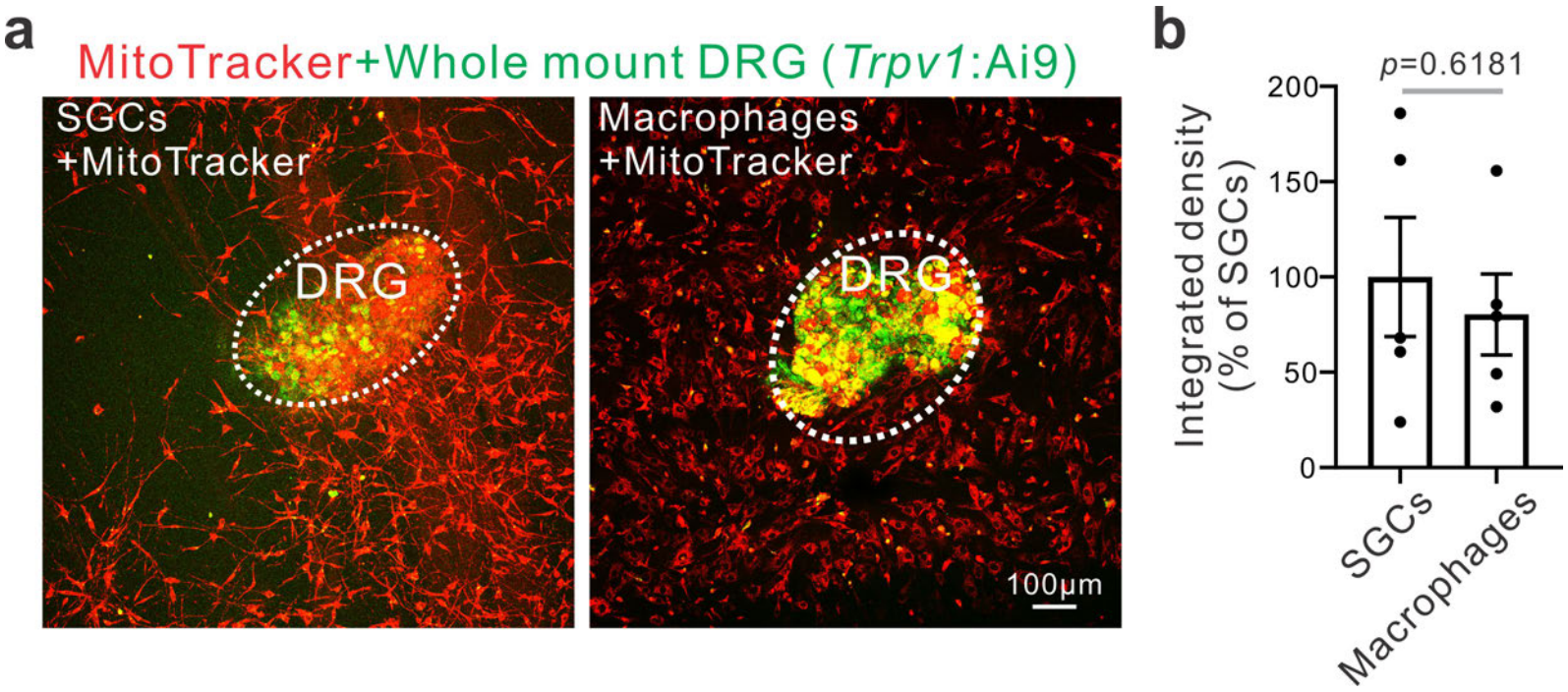
Representative images of SGCs co-culture with whole-mount DRG (previously sectioned). Arrows indicate TNTs. Scale bar: 100 μm (top), 50 μm (bottom).

7) In Ext Fig 1g, there appear to be fewer macrophages labeled with MitoTracker. Therefore the interpretation of differential transfer of mitochondria between different cellular sources is confounded.

Response: We repeated the macrophage co-culture experiment with whole-mount DRG, seeding macrophages at a high density of 200 cells/mm² to match the seeding density used for SGCs. This increased cell density improved the efficiency of mitochondrial transfer from macrophages to the DRG (**Response Fig. 32a-b**), supporting the conclusion that macrophages are also capable of transferring mitochondria to DRG neurons.

However, unlike SGCs, macrophages do not form TNTs with neurons, suggesting that the transfer occurs via an alternative mechanism, likely endocytosis [40].

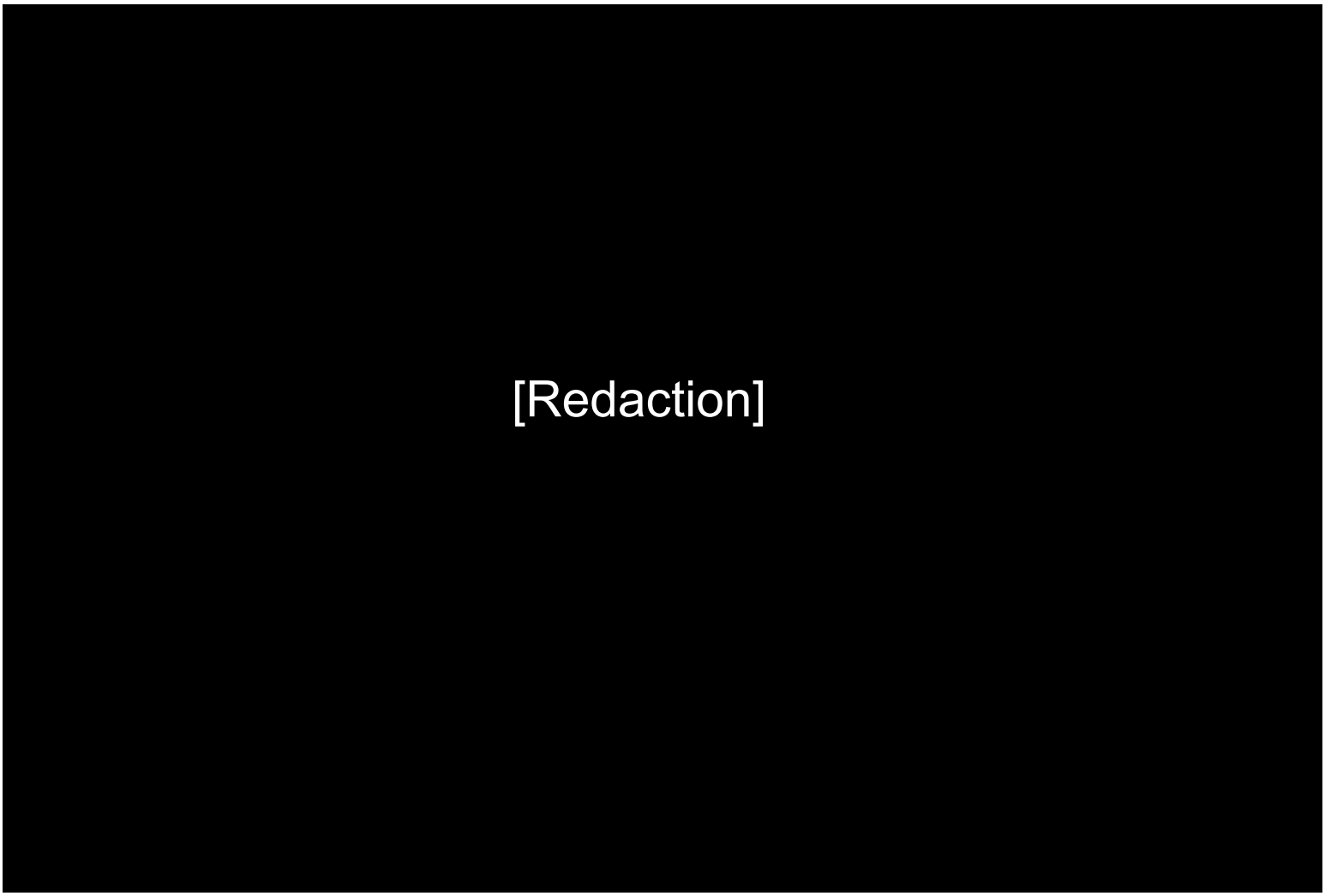
Accordingly, we have updated the macrophage image in the revised figure (now shown in **Extended Fig. 2g**).



Response Fig. 32 / Extended fig. 2e-g

8) Page 5, Lin 141 – the authors suggest that cleavage of TNTs by trypsin is “expected”. Are TNTs membrane bound or exposed proteinaceous structures?

Response: A previous publication using SEM imaging of adult frog, chick, rat, and rabbit DRG demonstrated the structural changes in DRG tissue following trypsin treatment [redacted] [41]. These images revealed a loss of extracellular matrix (ECM) components and the absence of TNTs in DRG cells post-treatment. Consistently, our experiment using 0.25% trypsin also resulted in a “naked” neuron–SGC unit, indicating that trypsin disrupts extracellular protein structures, including TNTs. This supports the notion that TNTs are protein-based structures. Our study further highlights the critical role of MYO10 in TNT formation. We have added this reference to the manuscript.

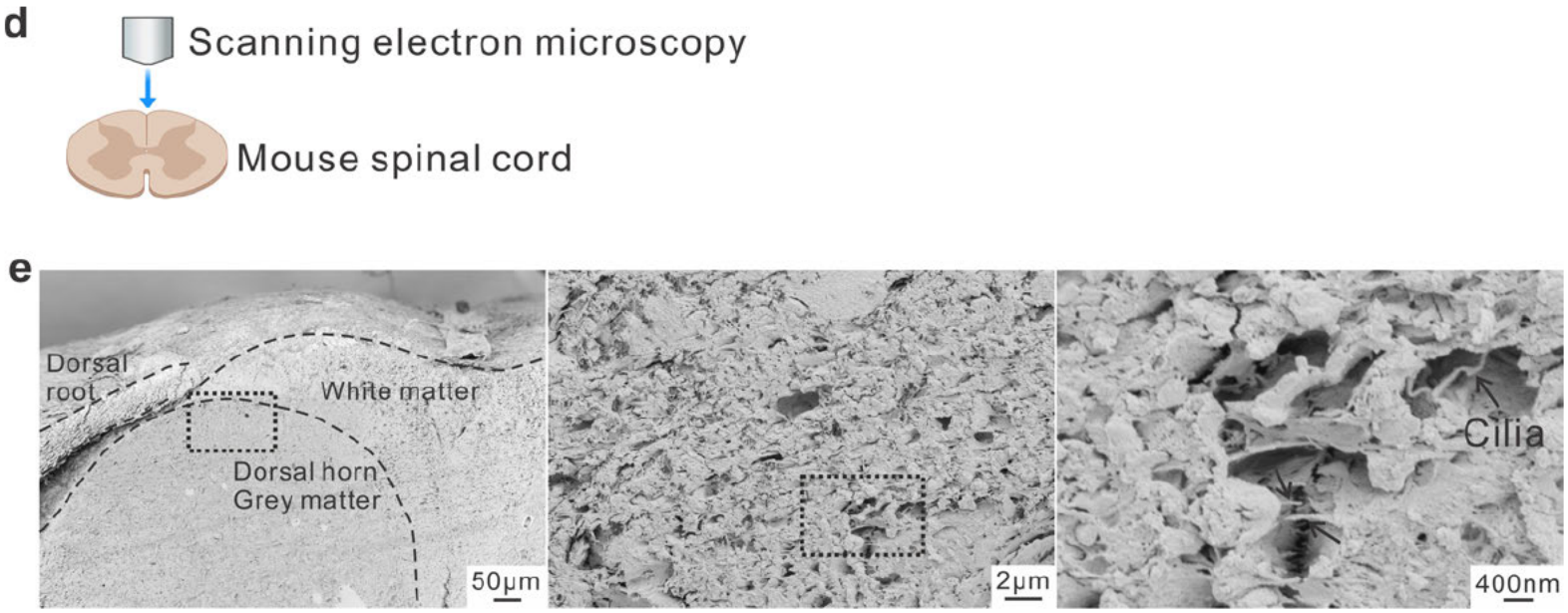


9) Page 5, Line 140 - Are the authors indicating that these vesicular swellings were NOT observed in TNTs from SGCs to other cell types or one another? The language is unclear.

Response: Thank you for pointing this out. These large bulges observed within TNTs can represent either mitochondria or vesicles. However, given their relatively large size (approximately 1 μm), they are more likely to be mitochondria (see **Main Fig. 1h-i**). This interpretation is further supported by our TEM analysis, which confirmed the presence of mitochondria within TNTs (**Main Fig. 1n-r**). To clarify this point, we have revised the text to read:
"Abundant TNTs were observed in mouse DRG, often containing bulges consistent in size with mitochondria (Fig. 1h-i)."

10) Page 6, line 145 - Cilia-like structures not clear from the image. They should be highlighted somehow.

Response: We added a high-magnification image of SEM to show the cilia-like structures in the spinal cord; we also indicated the cilia-like structures by arrows. We revised these images in **Extended Fig. 6c-f**. Consistent with SEM imaging result of brain cilia [42], [redacted] Moreover, these cilia are attached to a single cell and are not connected between two cells like the tubular structures."



Extended fig. 6d-e

[Redaction]

[redacted]

11) Figure 2k-m – In the SNI model, not all neurons are injured. Is mito transfer preferentially into injured or spared neurons or both?

Response: We appreciate this insightful question. [redacted]

[redacted]

[redacted]

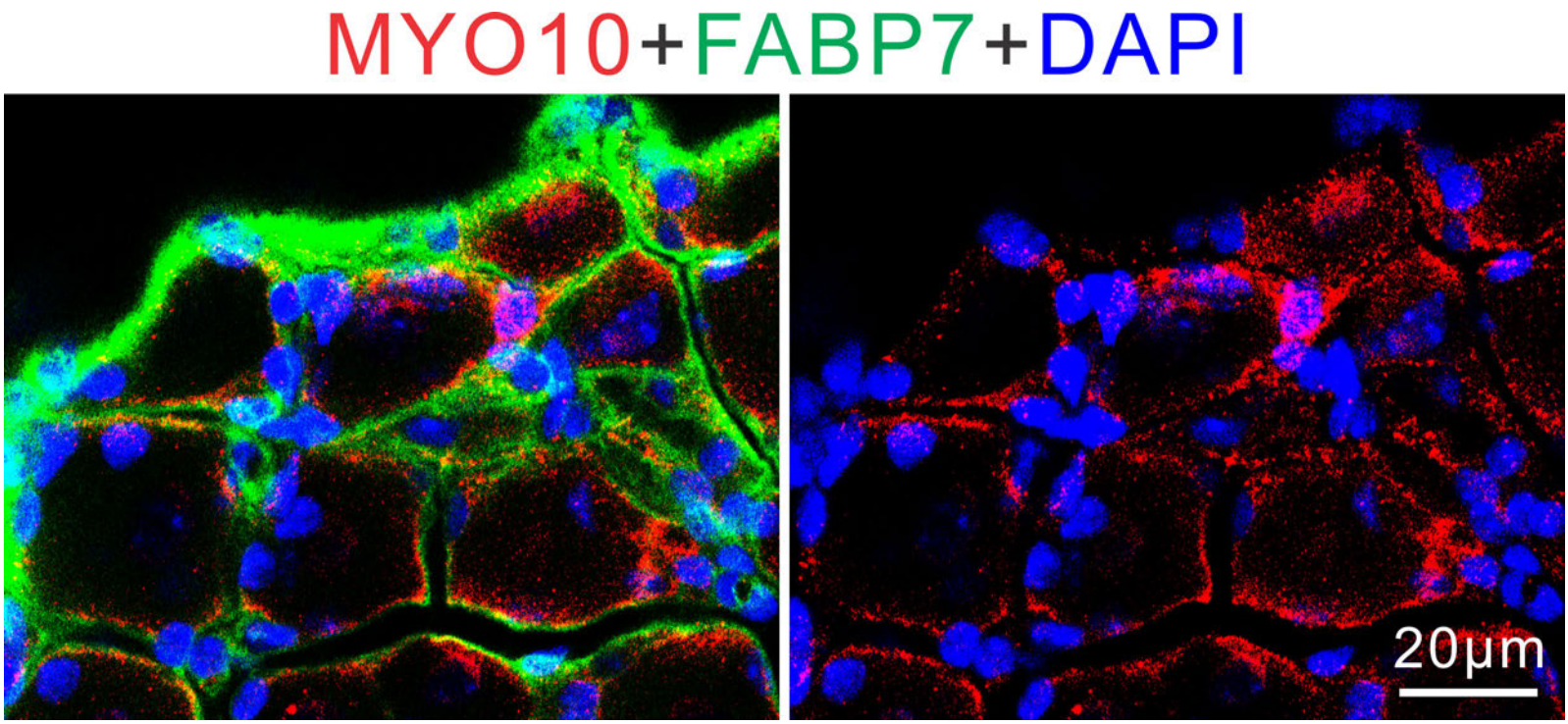
[redacted]

[Redaction]

[redacted]

12) In figure 3B, a lower power image would be helpful to establish specificity of the Myo10 immuno signal.

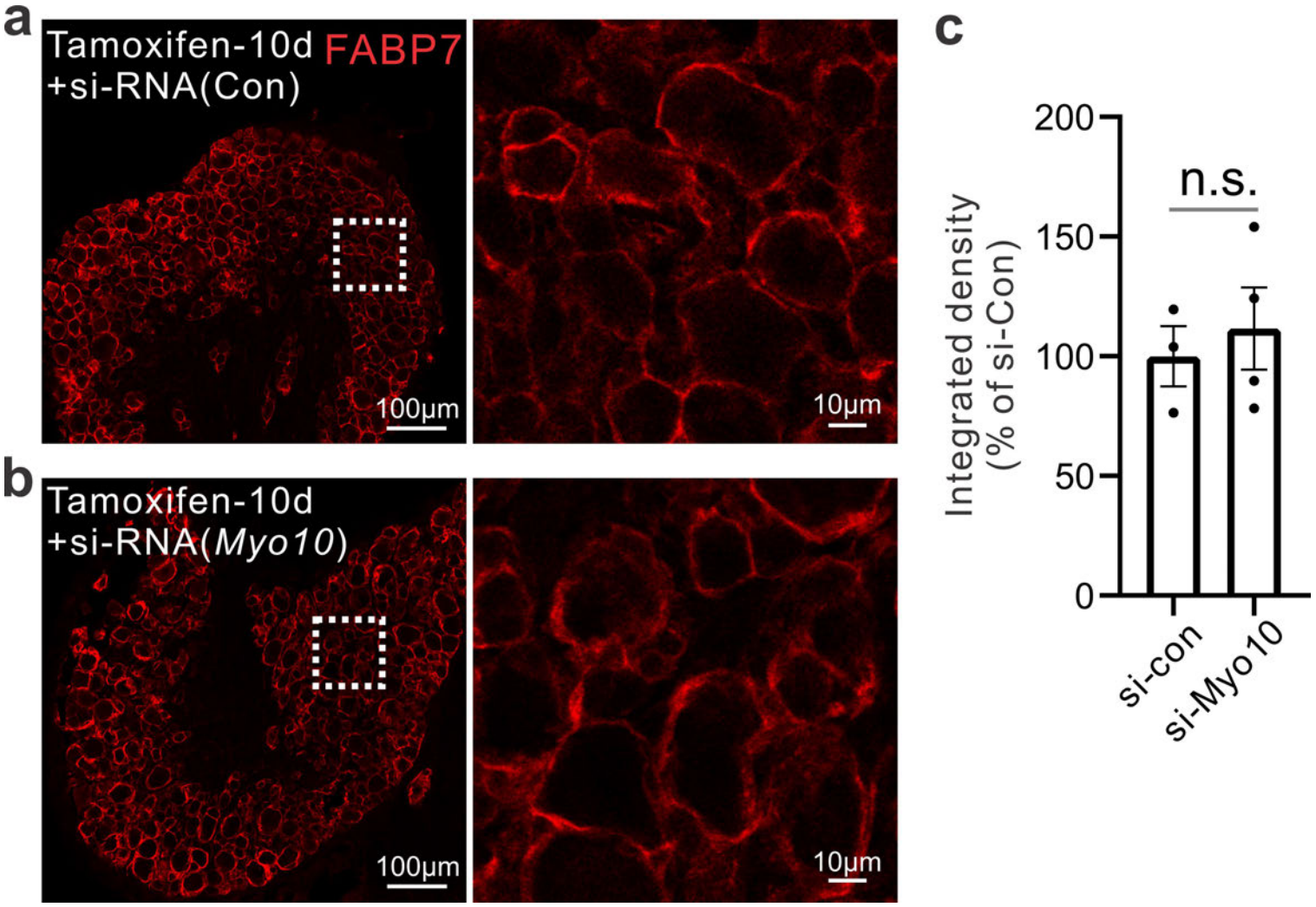
Response: As suggested, we have included low-magnification images of MYO10 and FABP7 double-staining in **Extended Fig. 9a**. These images showed the specific expression of MYO10 in SGC and further indicated that MYO10 is predominantly expressed in the cell bodies and extended processes of SGCs (**Response Fig. 36 / Extended Fig. 9a**).



Response Fig. 36 / Extended Fig. 9a

13) Figure 3i-j - Does Myo10 knockdown reduce FABP7 expression in SGCs? It appears it might, from the images.

Response: We quantified the integrated density of FABP7 immunostaining in both the si-control and si-*Myo10* groups. The analysis did not reveal a significant difference in FABP7 expression between the two groups (**Response Fig. 37**), indicating that MYO10 knockdown does not affect FABP7 levels in SGCs.



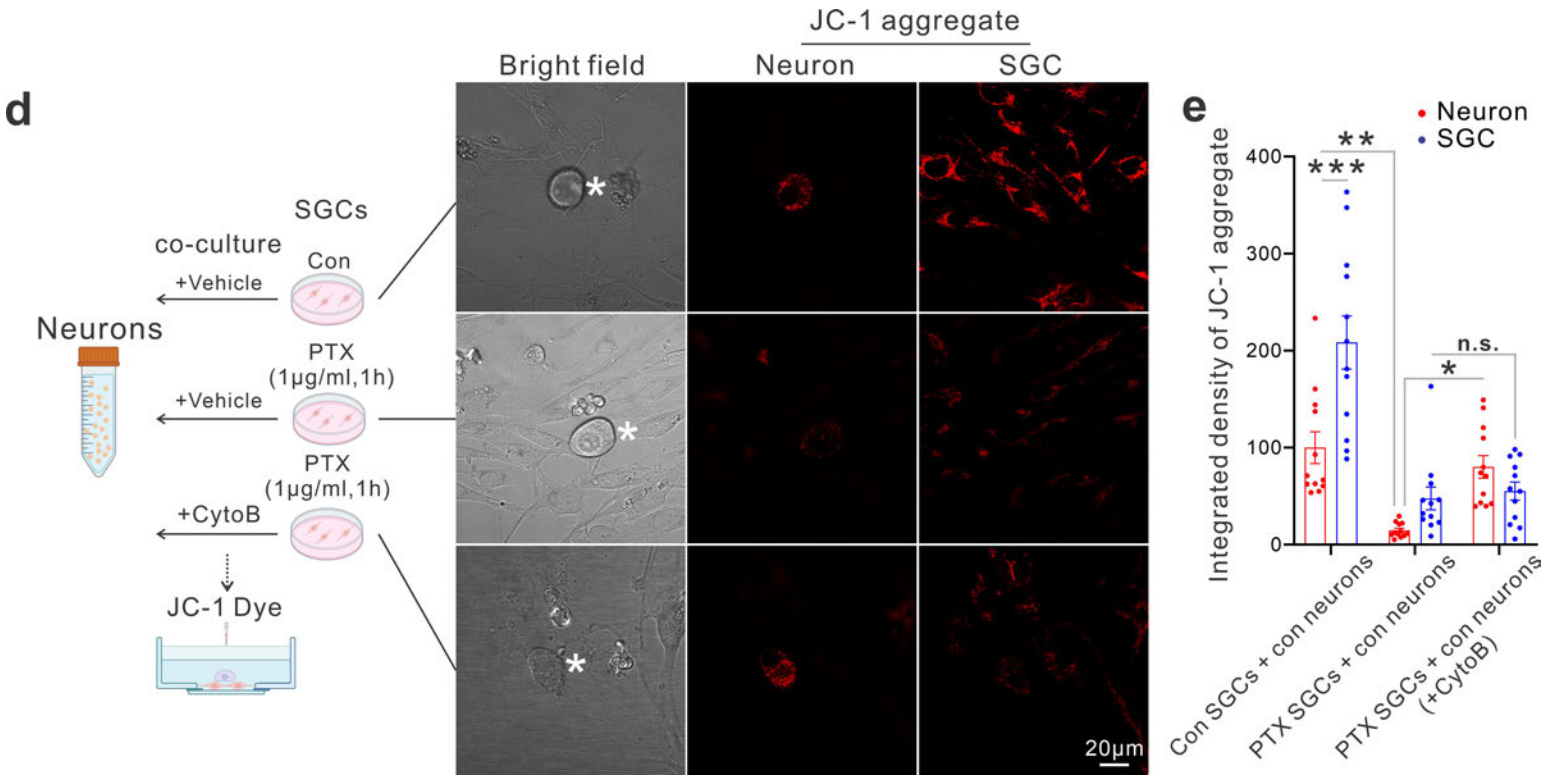
Response Fig. 37

14) Result of 7c-d is confusing. If mitochondrial transfer were not disrupted by CytoD, abundance of healthy mitochondria in neurons should still be low after PTX.

Response: We apologize for the confusion. In this experiment, only SGCs were treated with PTX and subsequently labeled with MitoTracker (**Extended Fig. 10d**). These PTX-treated SGCs were then co-cultured with healthy neurons.

Our results showed that dysfunctional mitochondria from PTX-treated SGCs were transferred to neurons, leading to a reduction in the density of aggregated JC-1 signals in neurons—an indicator of impaired mitochondrial function (**Extended Fig. 10e**).

Therefore, the reduced mitochondrial function observed in neurons was not due to a lack of transfer, but rather the transfer of dysfunctional mitochondria from PTX-treated SGCs."



Extended Fig. 10 d-e

15) The fate of TNTs in the PTX model is unclear. In Fig 2, it is suggested that the TNTs become more prominent after PTX. In Ext Data Fig 8, the authors imply that TNTs are reduced in this setting. This seems contradictory.

Response: We apologize for the confusion. Under normal conditions, SGCs tightly enwrap neurons, and TNTs extending from SGCs to neurons are typically curved and closely associated with the cell surface, making them difficult to visualize. After PTX treatment, gaps emerge between SGCs and neurons, which makes TNTs more apparent. However, these PTX-induced TNTs often appear irregular and morphologically altered, suggesting they may be non-functional (**Extended Data Fig. 3c-d**). Thus, while the visibility of TNTs increases after PTX, their structural integrity and function are likely compromised.

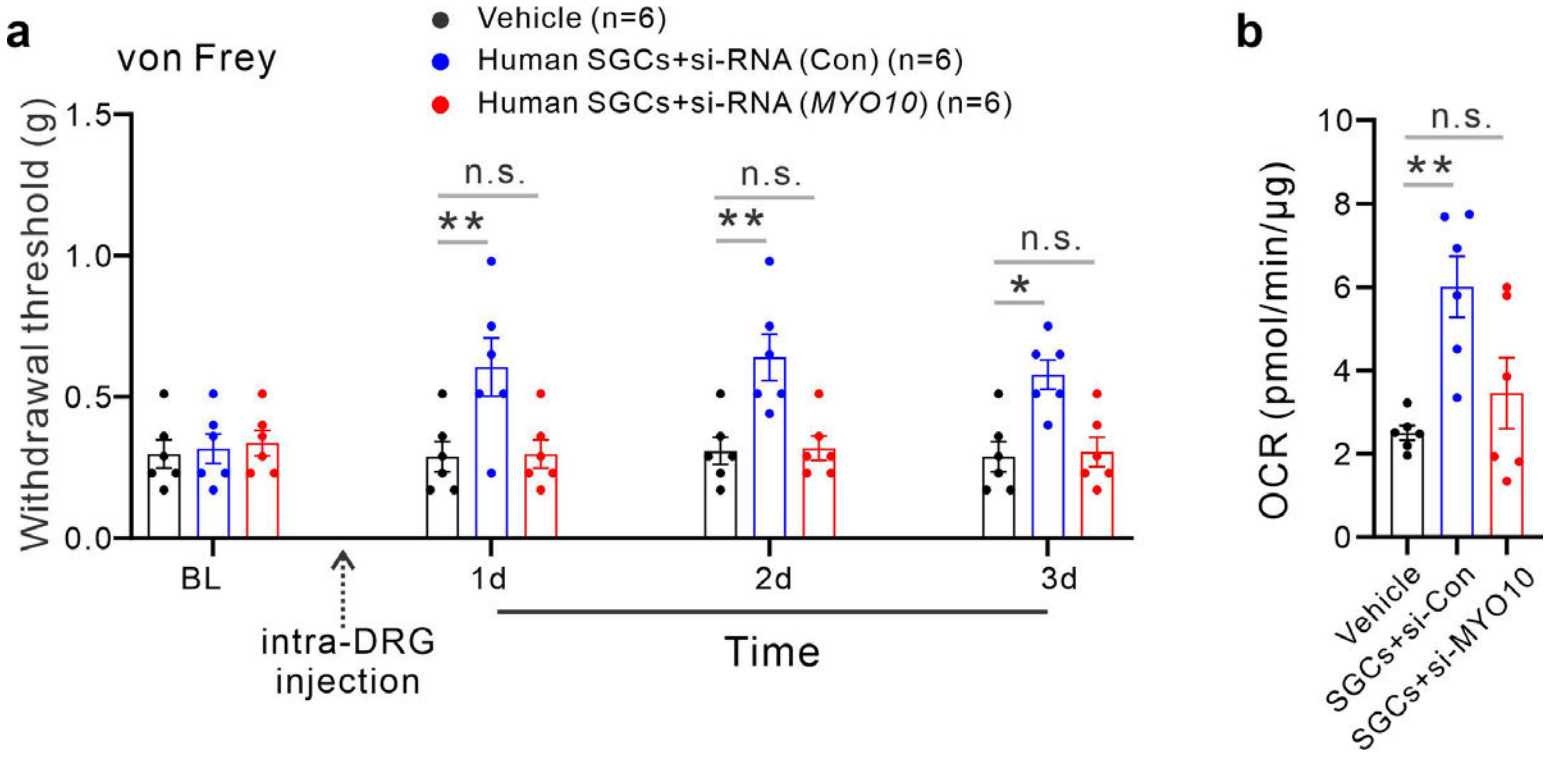
16) While intriguing, the implication of a MaR1-FABP7 binding and its role in the phenomena under study seems premature. Computational implication of MaR1-FABP7 interaction raises a possibility but is not directly tested experimentally. It is possible that numerous lipids bind FABP7. MaR1 effects on PTX allodynia could be through a different mechanism. This series of experiments seems underdeveloped, and the manuscript would not suffer from their omission.

Response: We agree that the computational modeling alone is insufficient to establish a definitive interaction between MaR1 and FABP7. Additionally, in light of comments from all three reviewers, we acknowledge that the effects of FABP7 loss- and gain-of-function on pain are not central to the main focus of this manuscript.

To maintain clarity and focus on the core findings—mitochondrial transfer and its therapeutic relevance to pain—we have removed the original Extended Data Figs. 9 and 10 related to FABP7 and MaR1 from the revised manuscript.

17) In Fig 5c, the interpretation of an increase in OCR by human SGCs is confusing, as there is not a no-SGC control for comparison. It is clear that siRNA of Myo10 reduces the OCR, but not that the control SGCs increase it compared to untreated mouse DRGs.

Response: We apologize for the confusion. To improve clarity and scientific rigor, we have now included a vehicle-treated group of *db/db* mice as a no-SGC control for both the pain behavior assay and OCR measurements. The von Frey test showed that adoptive transfer of human SGCs treated with control siRNA (si-Con), but not *MYO10* siRNA, significantly increased the paw withdrawal threshold compared to the vehicle group (**Response Fig. 38a**). Correspondingly, DRGs from db/db mice receiving si-Con-treated human SGCs showed increased basal OCR levels, whereas the si-MYO10 group did not (**Response Fig. 38b**). These new data have been incorporated into **Fig. 5b-c** to strengthen the interpretation.



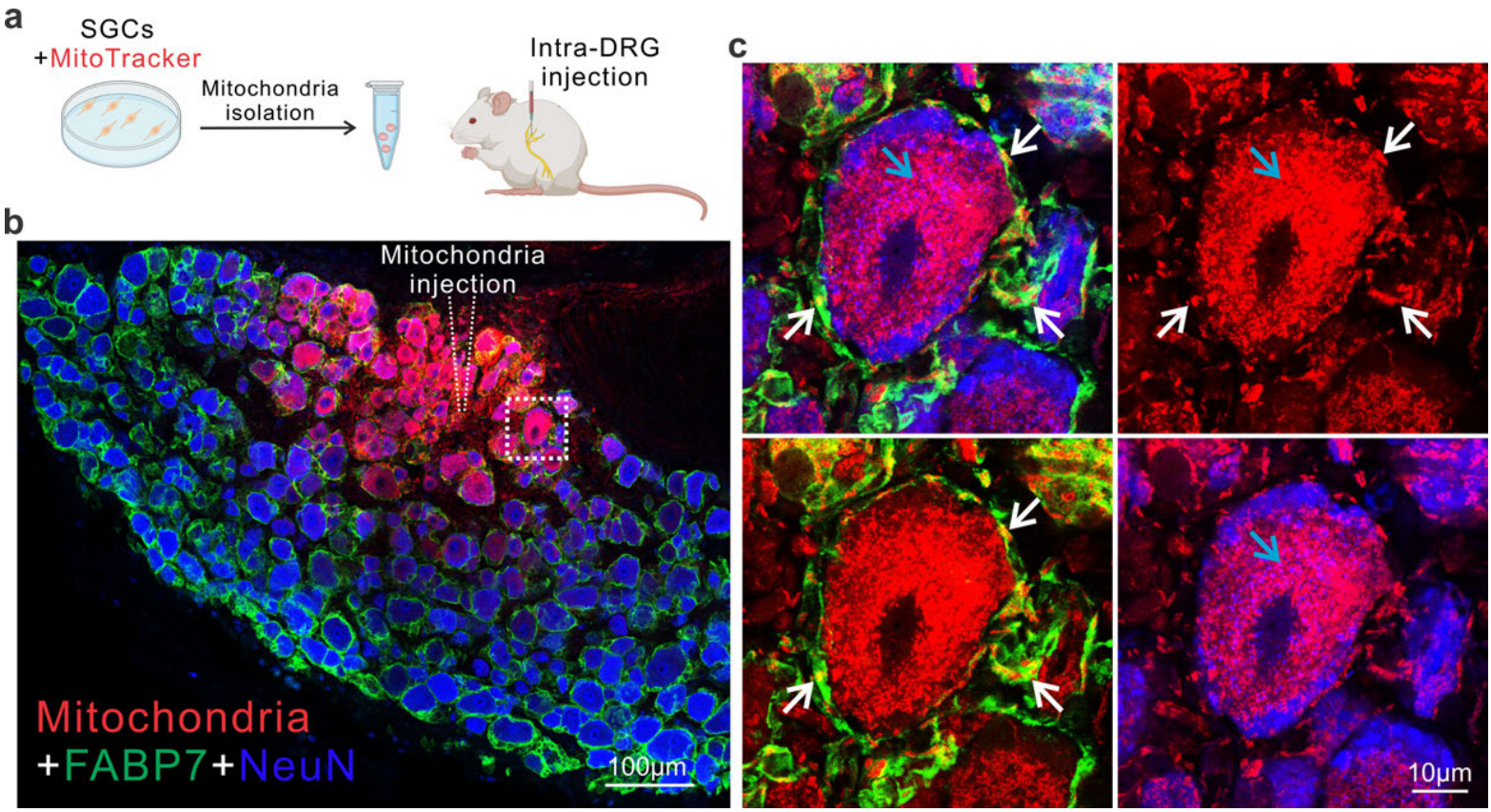
Response Fig. 38 / Fig 5b-c

18) How do the authors suspect that in vivo adoptively transferred mitochondria enter neurons? Use of CytoB or endocytosis inhibitors would be valuable here.

Response: To examine how adoptively transferred mitochondria enter neurons and SGCs *in vivo*, we first labeled mitochondria in cultured SGCs using MitoTracker and isolated mitochondria from 10,000 cells. These isolated mitochondria were then injected into the L5 DRG (**Response Fig. 21a**). Immunostaining for FABP7 and NeuN revealed that the transferred mitochondria localized to both neurons and SGCs within the DRG (**Response Fig. 21b-c**).

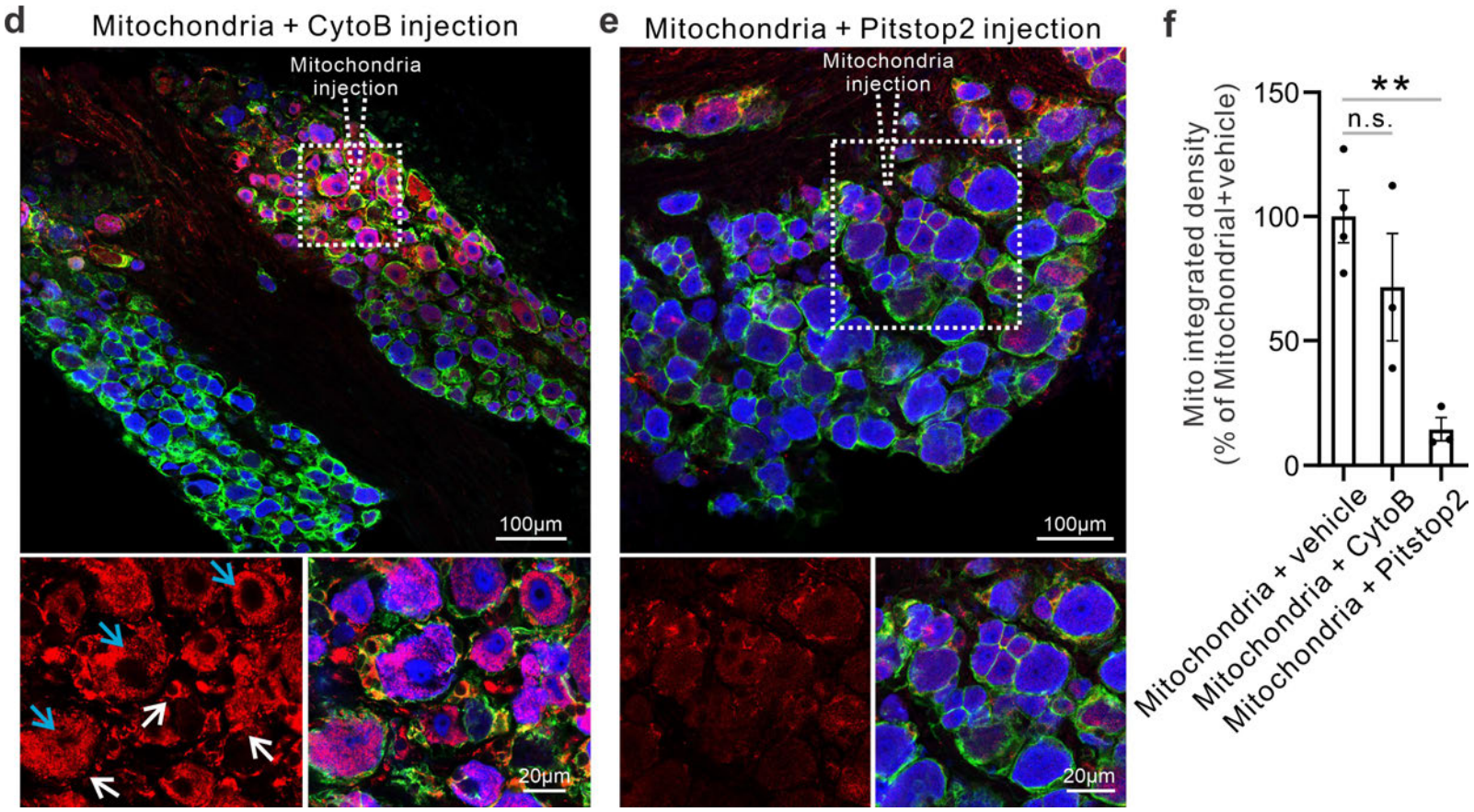
To investigate the entry mechanism, we performed intra-DRG injections of labeled mitochondria in the presence of either a TNT formation inhibitor (Cytochalasin B, 3.5 μM) or an endocytosis inhibitor (Pitstop 2, 20 μM). Quantification and imaging showed that CytoB treatment did not affect mitochondrial uptake by neurons or SGCs (**Response Fig. 39d-f**). In contrast, Pitstop 2 markedly reduced the MitoTracker signal, and mitochondria were rarely detected inside neurons.

These findings suggest that adoptively transferred mitochondria enter DRG neurons primarily through an endocytosis-dependent mechanism. Furthermore, mitochondria that fail to enter cells appear to have reduced viability. These results have been added to **Extended Fig. 16a-c**.



Response Fig. 21 / Extended Fig. 16a-c

Representative images of the DRG following intra-DRG injection of mitochondria. Blue arrows indicate mitochondria transferred into $NeuN^+$ neurons, and white arrows indicate mitochondria transferred into $FABP7^+$ SGCs. Scale bars: 100 μm (low magnification), 10 μm (high magnification).



Response Fig. 39 / Extended Fig. 16d-f

Representative images of the DRG following intra-DRG injection of mitochondria. Blue arrows indicate mitochondria transferred into NeuN$^+$ neurons, and white arrows indicate mitochondria transferred into FABP7$^+$ SGCs. Scale bars: 100 μm (low magnification), 10 μm (high magnification).

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Referees' comments:

Referee #1 (Remarks to the Author):

No further comments regarding the data, analyses and interpretation. Only 1 minor suggestion. Perhaps the summary schematic in Fig 5K should include an endocytosis element?

Repones: We thank the reviewer for their positive assessment of our revised manuscript. We added an endocytosis element in Fig, 5K.

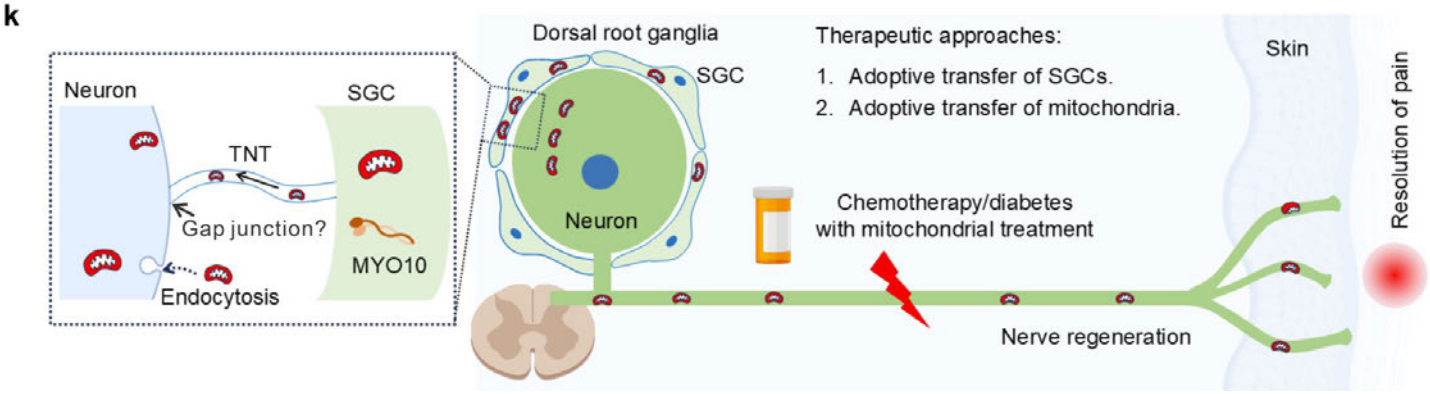


Fig. 5K

Referee #2 (Remarks to the Author):

The authors have addressed some of the comments satisfactorily, but several concerns remain, in particular on the TEM and SEM data. The numbers refer to the original

Repones: We thank the reviewer for additional comments. To address these concerns, we have included more SEM and TEM images (**Supplementary Figures 1-3)**. For clarity, we now present both the original and annotated versions of the SEM and TEM images, with certain regions color-coded. To ensure accurate interpretation, we describe the observed structures as "TNT-like structure (TNT-LS)" and "Extracellular matrix-like structure (ECM-LS)" in SEM and TEM images.

Comments:

3. Fig. 1e. How do you know that you injected to MitoTracker into an SGC? The images are not very convincing. The bright spot at 1h could be in a neuron.
Response: We appreciate the reviewer's insight and conducted additional experiments of single-SGC MitoTracker microinjection in whole-mount DRG of Aldh1l1:eGFP reporter mice (Response Fig. 22a) to confirm the cells we patched are indeed SGCs 23. The new images showed that MitoTracker labeled mitochondria in SGC transferred to the adjacent neuron 30 min after the MitoTracker injection (Response Fig. 22b). Due to the space limitation, we moved this result from Fig. 1d-e to Extended Data Fig. 2a-b. Notably, compared to 1h imaging, 30-min imaging revealed local transfer of mitochondria to a portion of the DRG neuron adjacent to the end feet of the SGC (Response Fig. 22b). This result argues against the mitochondrial diffusion hypothesis.

Follow-up comment
In Extended Data Fig. 2b the authors claim that the red labeling is due to transfer of MitoTracker from SGC S1 to neuron N1. However, as the neurons are completely covered with SGCs, the MitoTracker may have moved within the same SGC (S1), and is not inside N1. The dotted arrow actually shows how the MitoTracker apparently moved inside S1. Also, it would have been much more likely that the MitoTracker be present in N1 close to the injection site, rather than at a distance of 10-20 um away from it.

Repones: We thank the reviewer for this important observation. We agree that fluorescence microscopy, rather than confocal imaging, was used in this experiment, and therefore the resolution was insufficient to determine whether the MitoTracker signal was inside the neuron or remained within the SGC. To avoid overinterpretation, we have removed the data on single-cell SGC injection of MitoTracker from the revised manuscript. We plan to address this issue in future studies using advanced imaging and recording systems.

4. Line 112, concerning mitochondrial transport via TNT. The citation from Ref 20 is incorrect. In Ref 20, P.92, it is stated: "However, mitochondrial transport between pericytes was not observed, consistent with the closed-ended nature of IP-TNTs". The experiments with the gap junction blocker CBX make no sense, as gap junctions are channels that are too small to allow the passage of mitochondria. The role of gap junctions in mitochondrial transport is still possible, but not through the channels. You did not rule out a role for gap junctions in mitochondrial transport.
Response: We apologize for our inaccurate citation of the reference. We corrected the description as “nanotubes capable of transferring mitochondria have been identified in the mouse retina, connecting two bona fide pericytes on separate capillary networks”.
We agree that gap junction hemi-channels are too small for the passage of mitochondria, but an assisting role of gap junctions in mitochondrial transport is still possible. Our new results showed that gap junction blocker CBX treatment showed a partial but significant inhibition ($P < 0.0001$) of mitochondrial transfer in SGC-neuron co-cultures (Response Fig. 23a-c). Furthermore, CBX treatment reduced the proportion of the TNT formation between SGCs and neurons (Response Fig. 23d). Notably, the TNT and endocytosis inhibitors (CytoB and Pitstop 2) showed almost complete inhibition of the transfer (Response Fig. 23). Therefore, gap junction communication may be indirectly involved in the mitochondrial transfer between SGCs to neurons. We add this quantification data to Extended Data Fig. 1i-k.
Notably, SGCs highly express the gap junction protein Connexin43 (Cx43) 17,24,25, and Cx43-mediated gap junction communication plays a crucial role in SGC-neuron interactions in the DRG 26. Interestingly, Cx43 was proposed to function as a stabilizer, adhering to the docked membrane structures of two cells 27,28. Furthermore, Cx43 may increase the probability of successful formation of TNTs 29-33.

Follow-up comment
These data are valid, but add little to the main message of the paper, because the mechanisms involved are not known. This point is only cursorily mentioned in the discussion. This also holds for the other data in Extended Data Fig. 1.

Repones: We have expanded the discussion on the role of gap junction protein Cx43 in satellite glial cells, highlighting its contribution to pain and TNT formation (Page 13, line 371-375). In addition, we have incorporated the potential involvement of gap junctions in TNT formation into the summary schematic (Figure 5k).

6. Fig 1 f, Fig. 4 k. How was it determined that: 1. the fibers are nanotubes (they might be neurites, connective tissue…, 2. That they originate in an SGC? 3. That the bulges (vesicles) are mitochondria? 4. How do you know that there is a vesicle inside the presumed TNT (line 901)?
Response: We thank the reviewer for these important questions regarding the identification of tunneling nanotubes (TNTs) and mitochondrial transfer. We address each point below:
6.1. Identification of TNTs vs. neurites or connective tissue:
To reveal more details about TNTs and distinguish them from other cellular structures such as neurites or connective tissue, we conducted transmission electron microscopy (TEM) imaging on mouse and human DRG using 70 nm sections (Response Fig. 5-7, above).
Notably, TNTs were found to contain mitochondria and vesicles but lacked microtubules and myelin sheaths (Response Fig. 25b, below). In contrast, axons contain microtubules and, in the case of A-fibers, are also wrapped by myelin sheath 34 (Response Fig. 25b, below).
Connective tissue is characterized by the presence of a dense extracellular matrix (ECM) 35. However, ECM-like structures were found to be associated with neurons and SGCs but not TNTs.
Taken together, these morphological features allow us to clearly distinguish TNTs from axons and connective tissue.

Follow-up comments

I. Line 175, "with extracellular matrix (ECM) fibers associated with SGCs but not TNTs". This is not supported by the images, as collagen fibrils are present near presumed TNT. The extracellular space in DRG is packed with collagen fibrils, and if this claim is correct, it would mean that growing TNTs "dissolve" these fibers, all within minutes.

Repones: To avoid potential confusion, we have removed the description of extracellular matrix (ECM) from TEM images.

II. In the TEM images the TNT were identified by exclusion, but not by positive means. The processes shown in the TEM images could belong connective tissue cells or SGCs; see Pannese 2018 https://doi.org/10.1007/978-3-319-60140-3, Figs 1.11 and 1.30

The dotted lines in Extended Data Fig. 13 indicate foldings of SGCs membranes, and are unlikely to be TNTs.

Repones: To avoid potential confusion, we have removed TEM images of human DRG to focus this study on TEM images of mouse DRG.

III. The SEM Images in Fig 1, what is the evidence that the fibers indicated with arrows are TNTs? The surface of the presumed SGC is covered with numerous fibers. What are these? DRG neurons send fine processes into the SGC cover, see Pannese 2002 doi: 10.1016/s0074-7696(02)20002-9. These processes might be mistaken for TNTs.

Repones: We thank the reviewer for raising this important point. Notably, our co-author Dr. Richard Cheney is a cell biologist and a recognized leader in myosin research. We agree that distinguishing TNTs from ECM structures in SEM images is challenging. To ensure caution and accuracy, we have revised our description of the observed structures in SEM images, referring to them as TNT-like structures (TNT-LS) and ECM-like structures (ECM-LS). The ECM (e.g., collagen fibrils and elastin fibers, 10–500 nm in diameter) typically appears as tangled networks without clear endpoints, and small vesicles may attach externally to ECM fibers. In contrast, TNTs display distinct termini connecting to SGCs or neurons and often contain large bulges. TNTs have a wide range of diameters (20–700 nm) and lengths (a few microns to ~100 µm) and form highly elastic membranous protrusions that hover above the substrate to connect distant cells (PMID: 33646572, left image below) [1] (PMID: 33646572. [redacted]).

By comparison, in the normal nerve, fibers ranging from 1 to 20 µm in diameter are present, containing axons from ~0.5 µm in the smallest to ~15 µm in the largest (Sanders, *The thickness of the myelin sheaths of normal and regenerating peripheral nerve fibres*, 1947; right image below). Thus, TNTs are much thinner than DRG neuronal axons or perikaryal surface specializations of neurons (Pannese, 2002). However, we acknowledge that the largest TNTs and the smallest nerve axons may rarely overlap in size, and we cannot entirely exclude this possibility.

To improve clarity, we provided additional SEM images and labelled the nerves and TNT-like structure (TNT-LS) in several figures to highlight their differences in morphology and size (**Supplementary Figures 1 and 2**) and their disruption by MYO10 knockdown (**Supplementary Figure 4**). We also provided STM images to highlight the unique structures of TNTs (**Supplementary Figure 3**). Importantly, these thin tubular structures are observed to connect two adjacent cells, which is consistent with the established ultrastructural criteria for TNTs.

6.2. Determining the origin from SGCs:

In the physiological conditions, most SGCs close-wrap the DRG neuron 17, which makes it hard to observe the TNT between SGC and neuron by scanning EM (SEM) imaging. However, we found the enlarged gaps between SGC and neuron after chemotherapy and diabetic peripheral neuropathy conditions, which made the TNT between SGC and neuron easier to detect; the images showed the TNT originating from SGC connected to the neuron (Main Fig. 1l-m). Importantly, our in vitro and ex vivo experiments strongly suggest the existence of TNTs formed between SGCs and neurons of DRG co-cultures (Fig. 1b and Extended Data Fig. 2c-e). However, we acknowledge that high-resolution immunoelectron microscopy is required to definitively confirm the presence of mitochondria within TNTs between SGC and neuron in DRG tissue.

Follow-up comment

The SEM images (Figs 1,3) show numerous tangled fibers. What is the evidence that these are TNTs? Is there any evidence in the literature for such structures in DRG?

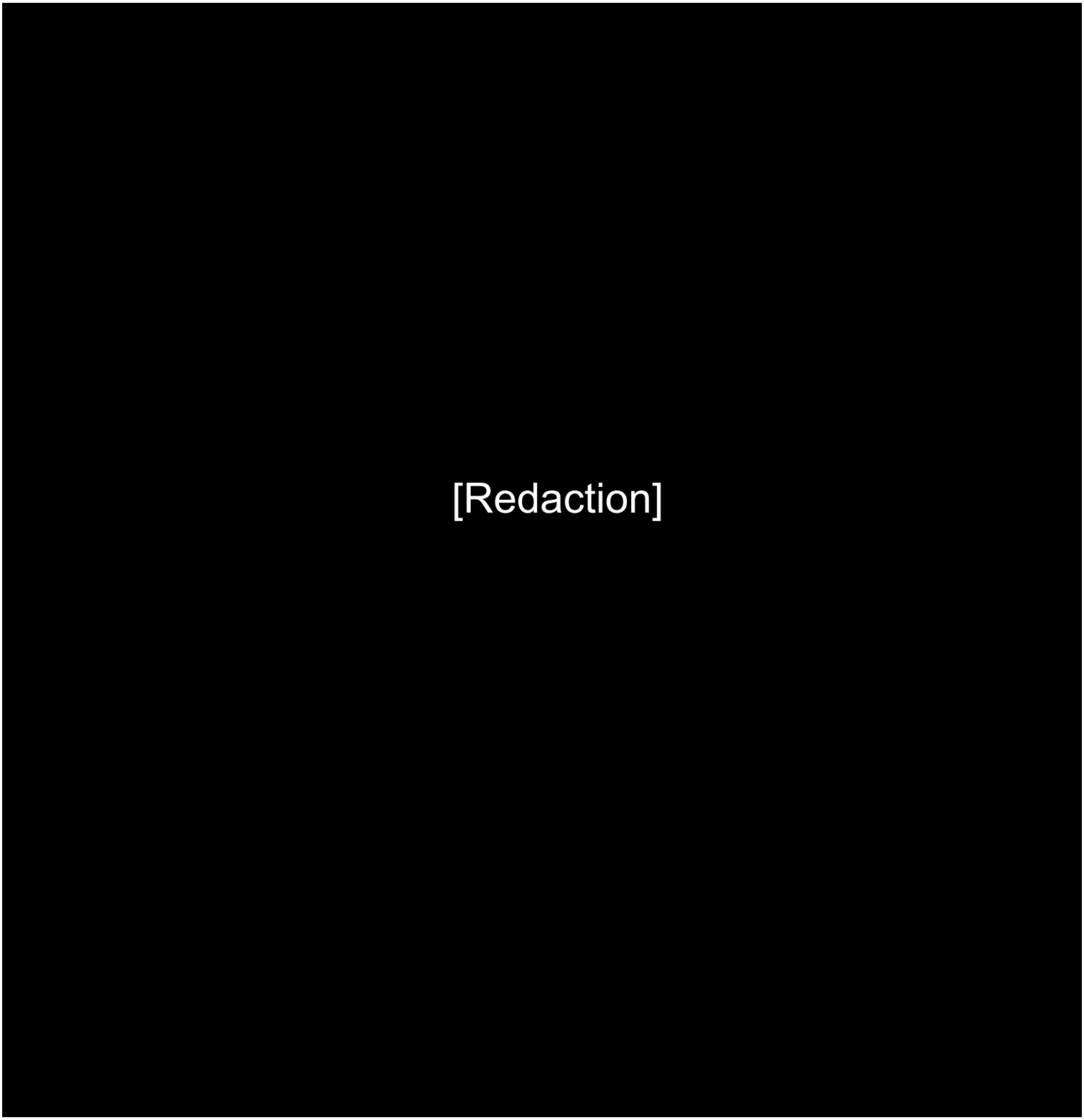
The presumed TNT in Fig. 1f connects two SGCs, not SGC with neuron.

Response: We appreciate the reviewer's comments. Relevant SEM work on DRG by Matsuda et al. reported that after treatment with trypsin (0.25%, 20–30 min at 37 °C) followed by hydrolysis with HCl, connective tissue was removed and neurons appeared "naked," making thick nerve fibers (~2 μm) readily distinguishable (blue color), while thinner structures (< 1 μm) persisted (red color, TNT-like structure) [2] [redacted] Moreover, Nascimento et al. described thin projections in DRG, referring to them as "Microvilli" and hypothesizing—based on earlier work by Pannese in 1999 [3]—that such structures may mediate molecular exchange between neurons and SGCs (PMID: 29729299. [redacted] [4].

In contrast, our study examined the ultrastructure of DRG without enzymatic or acid treatment, thereby preserving the native surface architecture. Moreover, advances in scanning electron microscopy have significantly improved resolution over the years. Across multiple preparations, we observed abundant ECM-like fibers on the SGC surface, as well as TNT-like structures with bulges that frequently bridged adjacent cells. To illustrate these findings, we prepared a schematic showing ECM-like fibers surrounding SGCs and TNT-like structures connecting SGC–neuron, and SGC–SGC (**Supplementary Fig. 5**). Importantly, these TNT-like structures were disrupted following *Myo10* knockdown in mouse DRG, indicating a critical role of *Myo10* in their formation (**Supplementary Fig. 4, below**). Meanwhile, studies also found that Myo10 knockdown led to a reduction in filopodia formation and ECM assembly [5,6]. Taken together, these results suggest that MYO10 plays a critical role in the formation of TNTs and filopodia, as well as in ECM assembly.

To strengthen our evidence, we now provide additional representative images: four SEM images from whole-mount (unsectioned) mouse DRG (**Supplementary Figure 1, below**) and from sectioned preparations (**Supplementary Figure 2a-d, below**), as well as four TEM images (**Supplementary Figure 3, below**). For clarity, we present both the original and annotated versions of the SEM and TEM images with some structures color-coded. Taken together, these features are consistent with TNT-like morphology described in the literature and support our interpretation that the observed thin tubes are distinct from neuronal fibers and ECM. We added these data into **Supplementary Figure 1-5**.

Regarding the reviewer's comment on Fig. 1f (now Fig. 1e): we agree that the highlighted structure connects two SGCs rather than an SGC and a neuron. We have revised the description accordingly and consistently use the term "TNT-like structure (TNT-LS)" throughout the manuscript of SEM and TEM images to accurately reflect the interpretation of these structures.



[Redaction]

6.3. Confirmation that the bulges are mitochondria:
We used MitoTracker staining and SGC-specific mitochondrial labeling to track mitochondrial movement. Live-cell imaging and confocal microscopy showed the co-localization of these mitochondrial signals within the bulges along TNTs (Extended Data Fig. 1d). Furthermore, transmission electron microscopy (TEM) revealed double-membraned organelles with high-density and internal structures consistent with mitochondria inside TNTs, supporting the mitochondrial identity of the transported cargo (Fig. 1n-r).
Follow-up comment
See above concerning the validity of TEM data.
6.4. Evidence of vesicles inside TNTs:
TEM provided direct ultrastructural evidence of vesicular structures within TNTs of mouse and human DRG (Response Fig. 5-7, above). We added these TEM data to Fig. 1n-r, Extended Data Fig. 4, and Extended Data Fig. 13i-j.
Follow-up comment
See above concerning the validity of TEM data.
In summary, the TEM and SEM studies are not convincing.

Response: We acknowledge the reviewer's concern about the interpretation of the TEM and SEM images. Here, we provided additional representative images: four SEM images from whole-mount (unsectioned) mouse DRG (**Supplementary Fig. 1, below**) and four SEM images from sectioned preparations (**Supplementary Fig. 2, below**), as well as four TEM images (**Supplementary Fig. 3, below**). For each image, the original (unannotated) file is included with key acquisition parameters visible (electron microscope model, magnification, and capture date/time). We also supply annotated versions in which TNT-like structure (TNT-LS) with bulges are marked in red, extracellular matrix-like structure (ECM-LS) in green, and nerve fibers in blue. Across different mice and experimental batches, the SGC surface consistently shows numerous tangled ECM-like fibers (green), from which thinner TNT-LS (red) extend; in contrast, nerve fibers (blue) are visibly thicker than TNT-LS. These additions clarify the morphology and help distinguish TNT-LS from ECM-LS and nerve fibers.

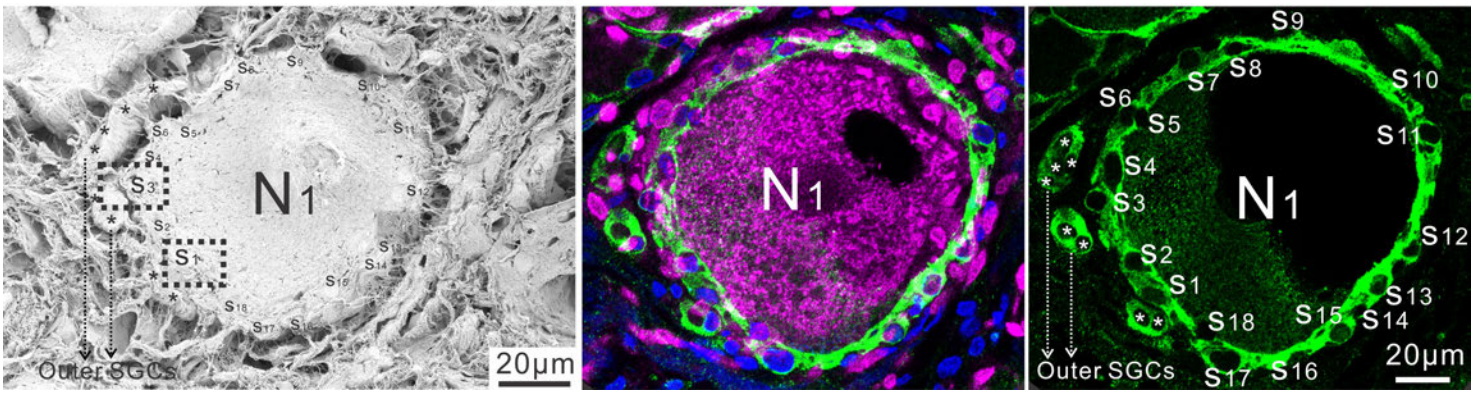
14. Discussion The discussion is not satisfactory. It is mostly about the potential clinical implications of this work, but not about the results themselves, and their shortcomings. The comments above need to be addressed and in particular comments 1,4,6,8, Why did you use four different pain models (SNI, PTX, db/db, STZ), and what do we learn from that? The results support previous reports on the essential roles of SGCs in chronic pain.

However, the authors did not discuss their results within the wide picture of neuron-SGC interactions.
Response: We appreciate your brief summary of the key points, which were highlighted in the Discussion Section. We apologize that we were not able to add extensive discussion of each question due to the space constraints of the journal. We have dressed these key comments in great detail in the previous responses. Also see below:

Follow-up comment

The discussion will have to be modified after the new corrections are made.
Additional Comment. Extended Data Fig. 17
The figure describes "inner layer TNT" and "out layer TNT". What is the evidence for this?"
"TNT from SGC to neurons" Do you have the evidence for this arrangement?

Repones: We thank the reviewer for these insight comments. Based on SEM imaging and FABP7 immunostaining in human DRG, we observed that some SGCs were positioned at a distance of ~10–20 μm from the neuron, while most other SGCs closely enwrapped neuronal soma (**Response Fig. 2).** However, we acknowledge that "outer-layer SGCs" is not accurate, as these outside SGCs do not forma a layer. Therefore, we changed to "outer SGCs" and labeled these cells with white asterisks. To avoid overinterpretation, we changed our description to "High-magnification SEM imaging showed the TNT-LS between SGCs and sensory neurons and between SGCs" in Fig. 4c. Moreover, we have removed Extended Data Fig. 17a and revised the text accordingly.



Response Fig. 2

Typo
Fig 1, "myline"

Repones: We corrected it to "myelinated nerve".

Referee #3 (Remarks to the Author):

The authors have adequately addressed all of this reviewer's criticisms

Repones: We thank reviewer for their positive assessment of our revised manuscript.

Reference List

1 Cordero Cervantes, D. & Zurzolo, C. Peering into tunneling nanotubes-The path forward. *EMBO J* **40**, e105789 (2021). https://doi.org:10.15252/embj.2020105789

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3 Nascimento, A. I., Mar, F. M. & Sousa, M. M. The intriguing nature of dorsal root ganglion neurons: Linking structure with polarity and function. *Prog Neurobiol* **168**, 86-103 (2018). https://doi.org:10.1016/j.pneurobio.2018.05.002

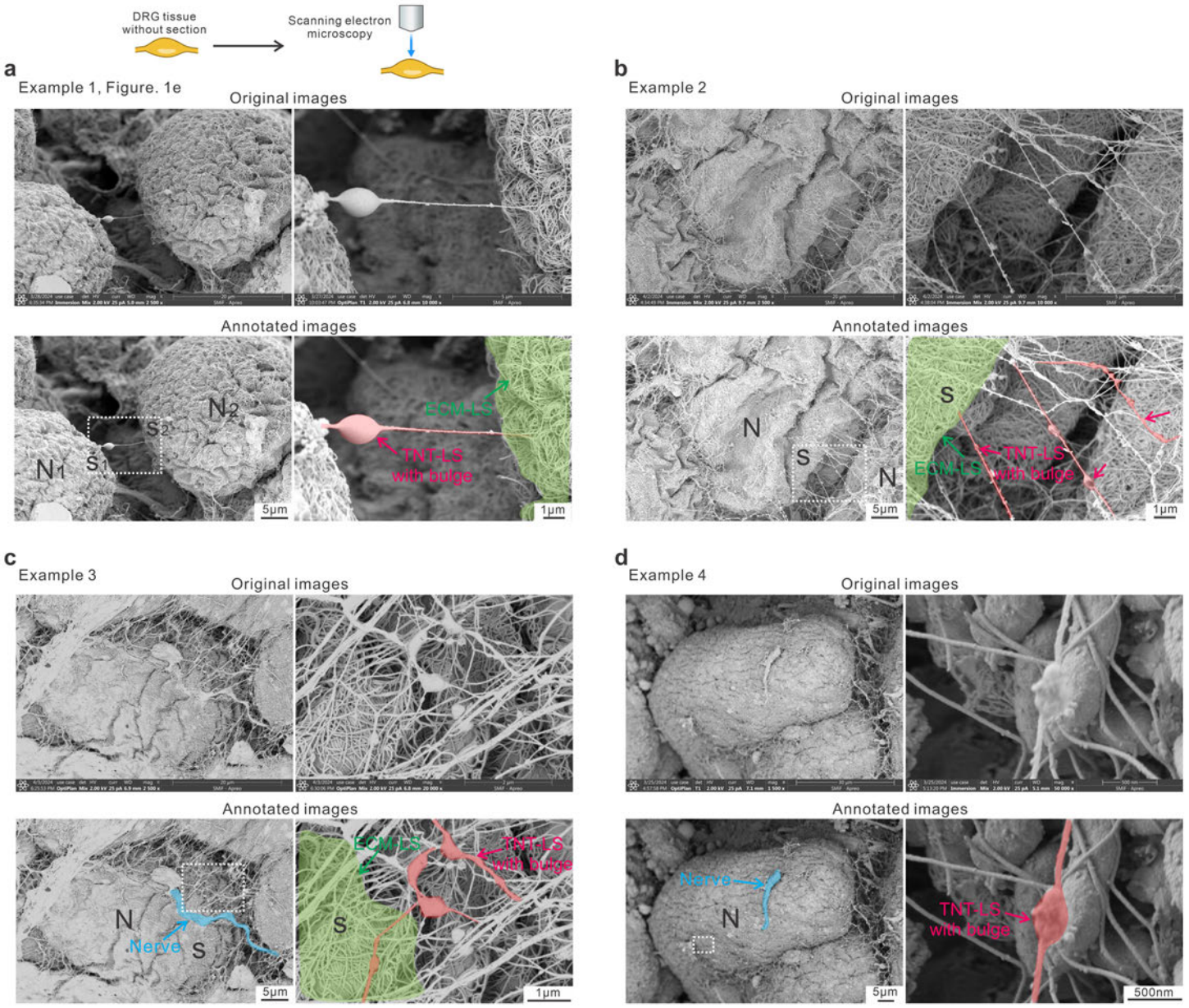
4 Pannese, E., Procacci, P., Berti, E. & Ledda, M. The perikaryal surface of spinal ganglion neurons: differences between domains in contact with satellite cells and in contact with the extracellular matrix. *Anat Embryol (Berl)* **199**, 199-206 (1999). https://doi.org:10.1007/s004290050220

5 Peuhu, E. *et al.* MYO10-filopodia support basement membranes at pre-invasive tumor boundaries. *Dev Cell* **57**, 2350-2364 e2357 (2022). https://doi.org:10.1016/j.devcel.2022.09.016

6 Summerbell, E. R. *et al.* Epigenetically heterogeneous tumor cells direct collective invasion through filopodia-driven fibronectin micropatterning. *Sci Adv* **6**, eaaz6197 (2020). https://doi.org:10.1126/sciadv.aaz6197

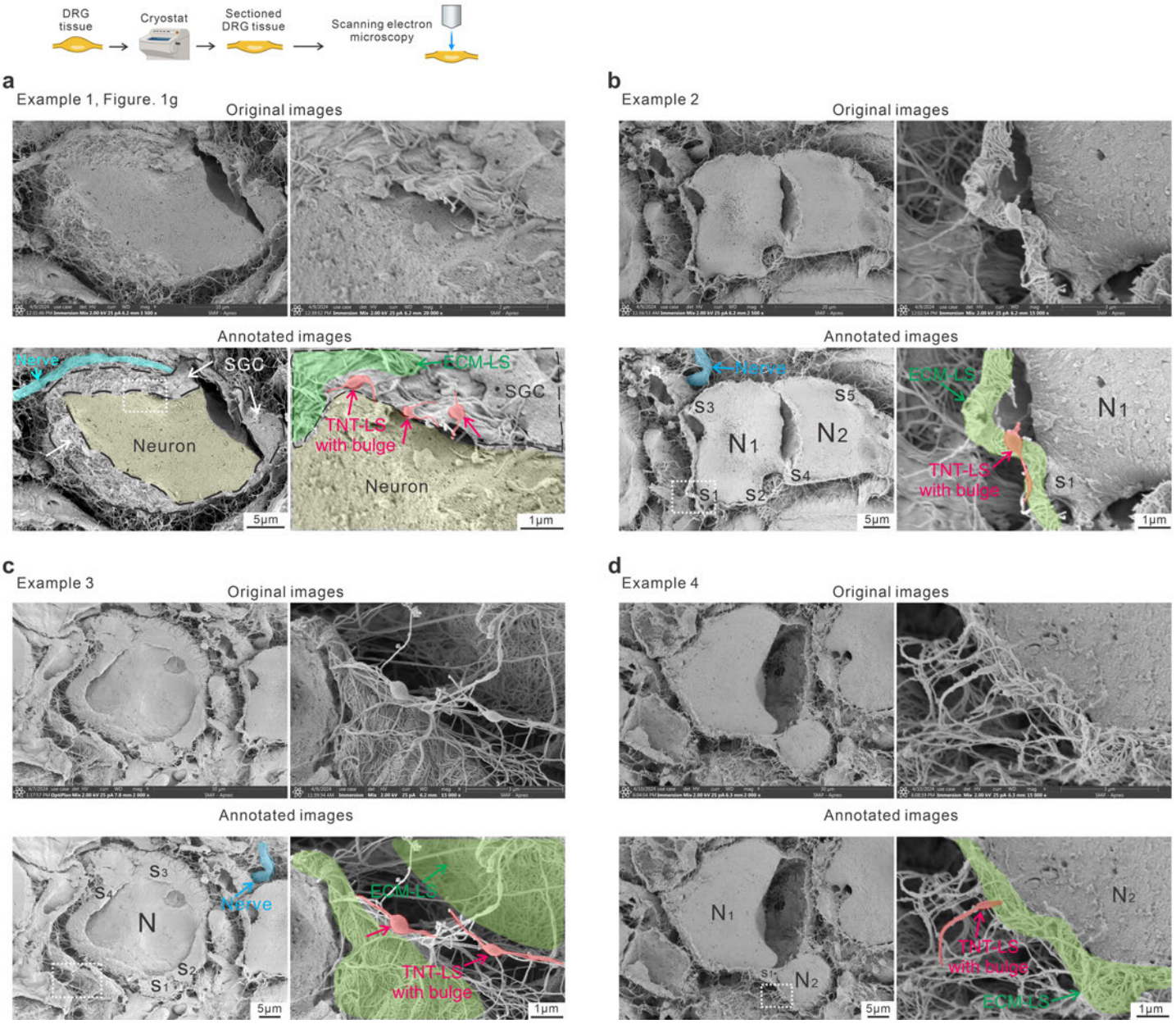
Below please see Supplementary Figures 1-5. Some images are color-coded for clarity.

Supplementary Figure 1



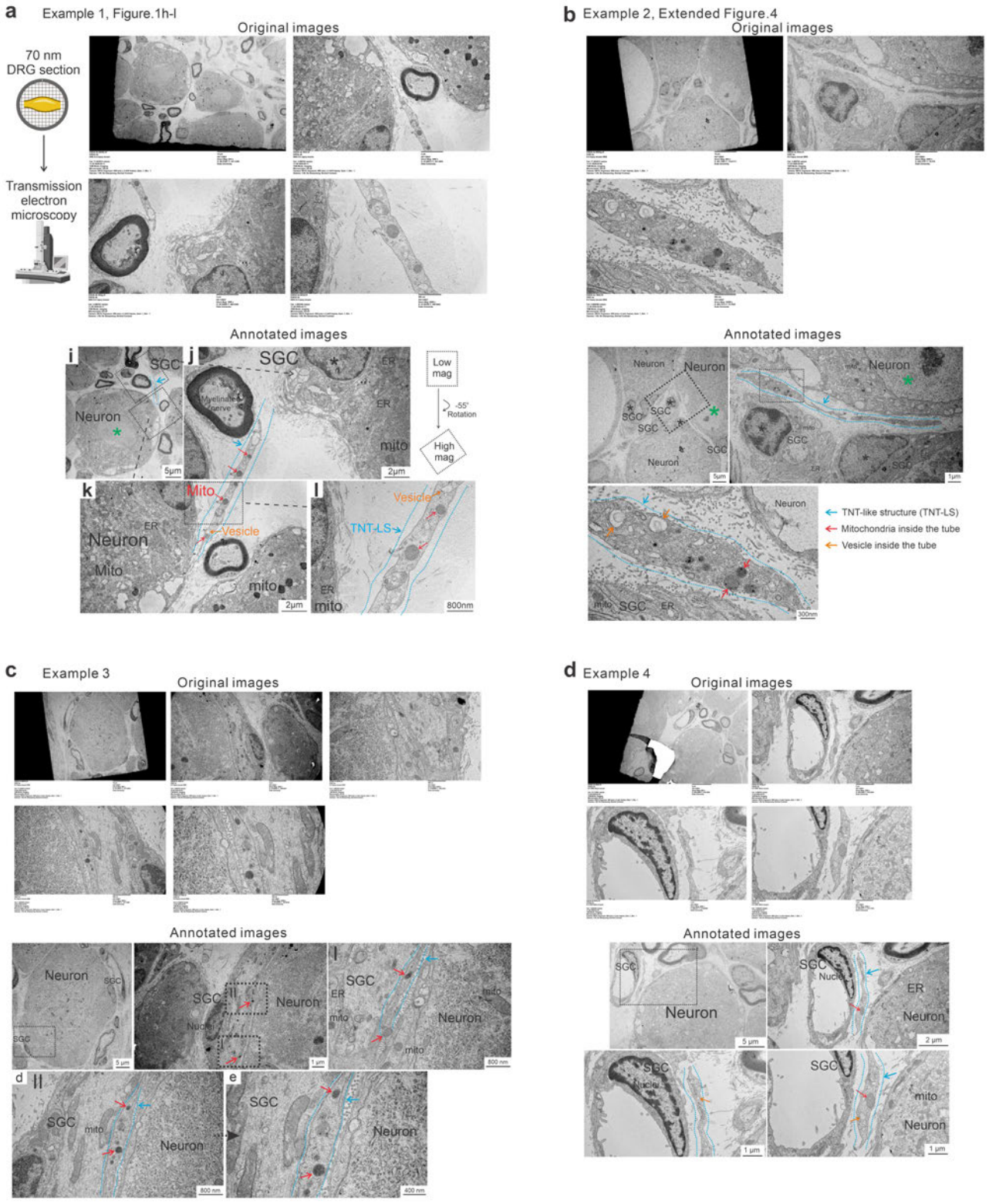
Supplementary Figure 1. a-d, Representative original and annotated SEM images of four examples: Example 1 (**a**, also shown in **Fig. 1e**), Example 2 (**b**), Example 3 (**c**), and Example 4 (**d**). "N" denotes sensory neurons; "S" denotes satellite glial cells (SGCs). In annotated images, boxes (left) are enlarged in the right panels. Blue, red, and green arrows indicate nerve fibers, TNT-like structures (TNT-LS) with bulges, and extracellular matrix-like structures (ECM-LS), respectively. Scale bars are as indicated. SEM images were prepared from whole-mount mouse DRG without sectioning.

Supplementary Figure 2



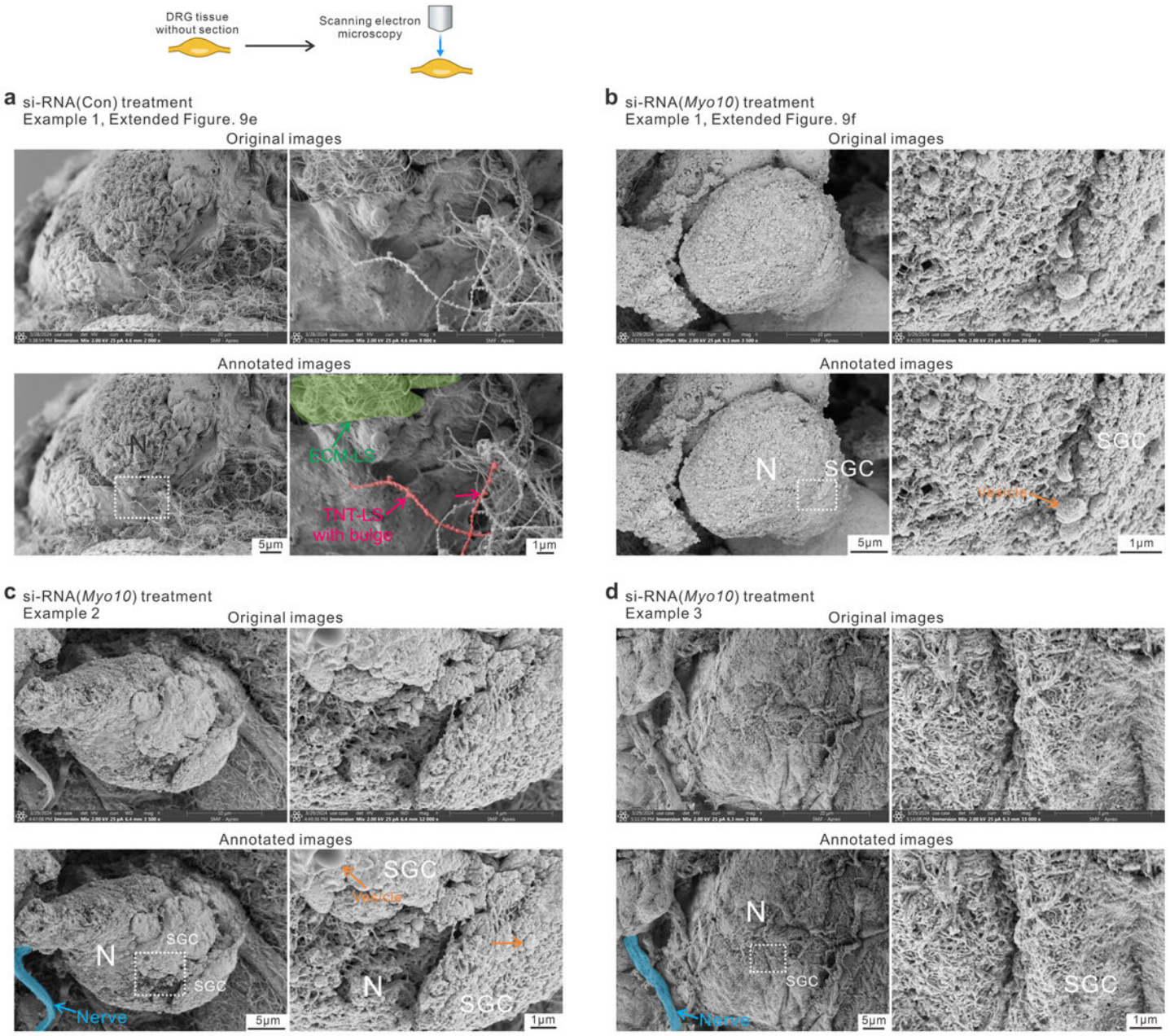
Supplementary Figure 2. a–d, Representative original and annotated SEM images of four examples: Example 1 (**a**, also shown in **Fig. 1g**), Example 2 (**b**), Example 3 (**c**), and Example 4 (**d**). "N" denotes sensory neurons; "S" denotes SGCs. In annotated images, boxes (left) are enlarged in the right panels. Blue, red, and green arrows indicate nerve fibers, TNT-LS with bulges, and ECM-LS, respectively. Scale bars are as indicated. SEM images were prepared from sectioned whole-mount mouse DRG. Notably, multiple TNT-LS are observed between a single SGC-neuron pair (**a**).

Supplementary Figure 3



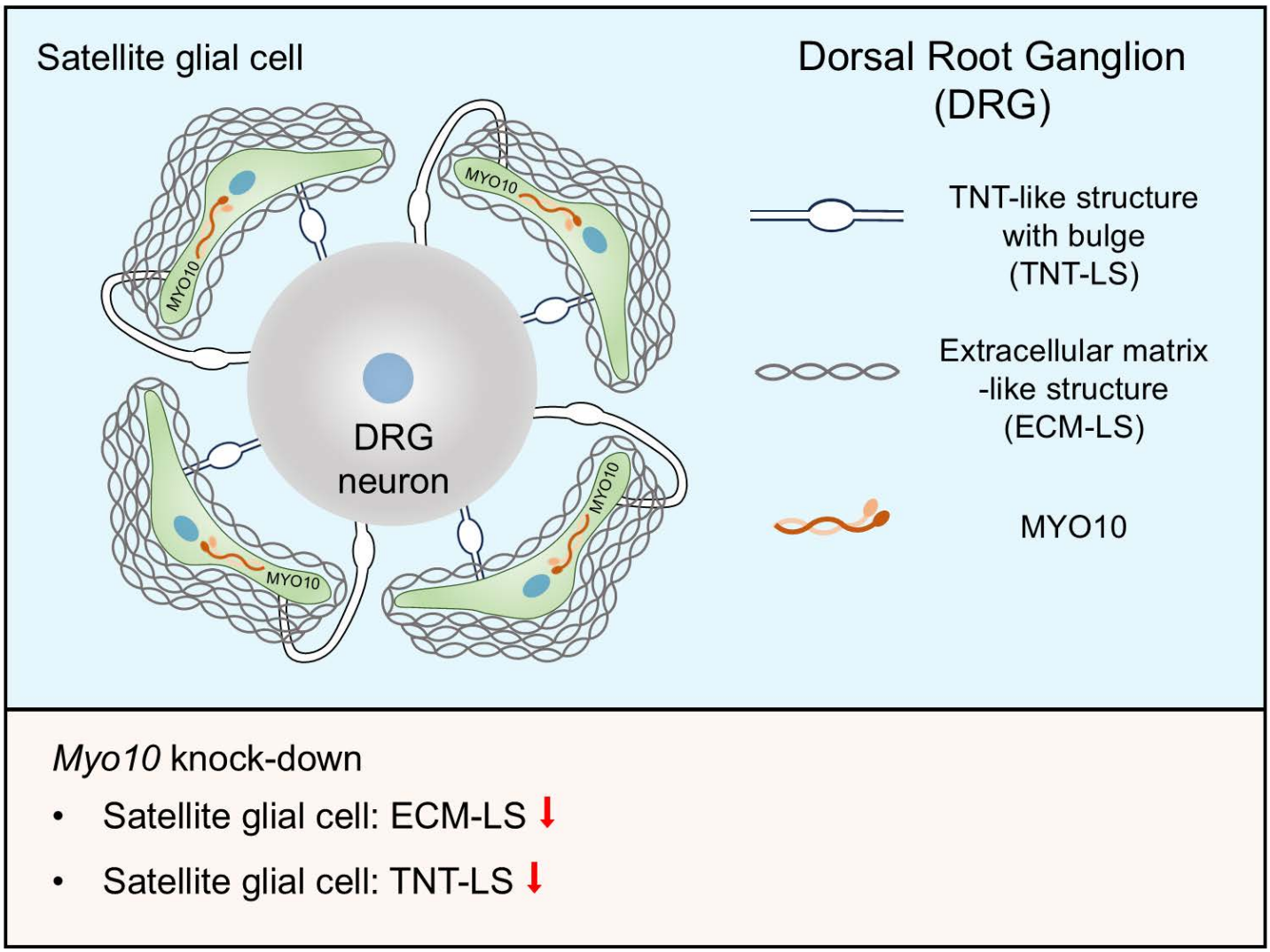
Supplementary Figure 3. a-d, Representative original and annotated TEM images of four examples in mouse DRG: Example 1 (**a**, also shown in **Fig. 1h–l**), Example 2 (**b**, also shown in **Extended Data Fig. 4**), Example 3 (**c**), and Example 4 (**d**). Blue, red, and orange arrows indicate TNT-LS, mitochondria within TNT-LS, and vesicles within TNT-LS, respectively. Mito (black) labels mitochondria inside cells, green asterisks (*) denote neuronal nuclei, and ER indicates endoplasmic reticulum. Scale bars are as indicated.

Supplementary Figure 4



Supplementary Figure 4. a-d, Representative original and annotated SEM images of four examples, treated with si-RNA(Con) or si-RNA(*Myo10*): Example 1 (**a**, also shown in **Extended Data Fig. 9e**), Example 2 (**b,** also shown in **Extended Data Fig. 9f**), Example 3 (**c**), and Example 4 (**d**). "N" indicates sensory neuron. Blue arrows mark nerve fibers, red arrows indicate TNT-LS with bulges, green arrows indicate ECM-LS. Scale bars are as indicated. SEM images were prepared from whole-mount mouse DRG without sectioning. Orange arrows indicate vesicles on the surface of SGCs, suggesting a possibility of impaired vesicle trafficking following *Myo10* knockdown.

Supplementary Figure 5



Supplementary Figure 5. Schematic illustration of MYO10 expression and SGC-neuron communication via TNT-LS. Knockdown of *Myo10* in DRG leads to disruption of TNT-LS between SGC and neuron. Furthermore, ECM at the outer layer of SGCs appears to be thicker than inner layer, providing additional structural support for sensory neurons. Notably, *Myo10* knockdown in DRG also disrupts ECM-LS on SGC.