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# Msps Governs Acentrosomal Microtubule Assembly and Reactivation of Quiescent Neural Stem Cells

Qiannan Deng, Ye Sing Tan, Liang Yuh Chew, and Hongyan Wang
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*Corresponding author(s): Hongyan Wang (hongyan.wang@duke-nus.edu.sg)*

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## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

## 1st Editorial Decision 28th Jan 2020

Dear Hongyan,

Thank you for submitting your manuscript to The EMBO Journal. I have now had a chance to read it carefully and to discuss it with my colleagues, and I am sorry to say that we cannot offer publication in The EMBO Journal.

Your analysis reports on Drosophila qNSCs that extend a MT-based protrusion towards the neuropil. The findings show that MT growth in qNSCs is predominantly acentrosomal. The microtubule polymerase Msps is expressed in qNSCs, promotes plus-end-out MT growth and is needed for qNSC activation. The findings further show that Msps is needed for E-cadherin localization to NSC-neuropil contact sites and that E-cadherin expression in NSCs is needed for their re-activation. I appreciate that the analysis adds new insight into the role of Msps in MT dynamics in qNSCs and their re-activation. However, the analysis also provides limited further insight into how Msps regulates E-cadherin localization or how the targeting of E-cadherin to the NSC-neuropil contact point regulates qNSC activation. We find that some further insight along these lines would be needed for consideration here.

I thank you for the opportunity to consider your manuscript for publication here and I am sorry that I can't be more positive on this occasion.

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Karin Dumstrei, PhD

Senior Editor

The EMBO Journal

16th November 2020

Dear Ediror,

Following our initial submission of a manuscript entitled "**Msps Governs Acentrosomal Microtubule Assembly and Reactivation of Quiescent Neural Stem Cells**", to ***The EMBO Journal*** in January 2020, we have performed numerous additional experiments to improve the manuscript and specifically addressed the following concerns raised in your letter.

*"However, the analysis also provides limited further insight into how Msps regulates E-cadherin localization or how the targeting of E-cadherin to the NSC-neuropil contact point regulates qNSC activation. We find that some further insight along these lines would be needed for consideration here."*

In this revised manuscript, we have shown a novel mechanism by which microtubules are required for E-cadherin localization at the quiescent neural stem cell (qNSC)-neuropil contact sites via Kinesin-2, a microtubule plus-end directed motor protein. The summary of these new findings are as the following.

To investigate the mechanism by which microtubule dynamics regulates E-Cad localization in the protrusion of qNSCs, we sought out to identify the motor protein that might be involved in localizing E-cad localization in qNSCs. As E-cad localizes to the tip of the plus-end microtubule oriented protrusion, we reasoned that kinesin motor proteins that move their cargos toward the plus-end microtubules is likely involved in E-cad localization and NSC reactivation. Toward this end, we performed a small-scale RNAi screen on major types of kinesins. Among 13 kinesin genes, we have identified *klp64D/kif3A, klp68D, and kap3*, encoding heterotrimeric kinesin-2, for their potential role in NSC reactivation. We found that knockdown of all three subunits of Kin-2 resulted in NSC reactivation defects (Figure S7).

To confirm the role of Kinesin-2 during NSC reactivation, we examined loss-of-function alleles of kinesin-2. First, we found that strong defects in NSC reactivation (reduced EdU incorporation and mitosis; retaining the primary protrusions) in $klp64D^{k5h}$ loss-of-function allele and a transheterozygote $klp64D^{k5h}$*/Klp64D deficiency* (Figure 6E-H). Second, we show that NSCs from $kap3^{V5}$ and $kap3^{V6}$, two loss-of-function *kap3* alleles, displayed strong defects in exiting quiescence, compared with the control (Figure 6E-H). Third, severe NSC reactivation defects seen in the stronger allele $kap3^{V6}$ was completely rescued by a wild-type *kap3* transgene (Figure 6E-H). Finally, we showed that Kinsin-2 is required for E-cad localization at the NSC-neuropil contact sites (Figure 6I-J). Therefore, we conclude that Kinesin-2, a microtubule plus-end directed motor protein, promotes NSC reactivation and is required for E-cad localization at the NSC-neuropil contact sites.

To further validate the localization of E-cad at the NSC-neuropil contact sites, we took the advantage of targeted GFP Reconstitution Across Synaptic Partners (t-GRASP), a method that specifically detects cell-cell interactions including those in synapse formation (Harold K. Shearin et al., J. Neuro. Methods 2018). In our t-GRASP experiment, if qNSCs have direct membrane contact with the neuropil, the full-length GFP can be reconstituted at the extracellular space between the two cell types, marking the membrane contact sites between qNSCs and the neuropil. Remarkably, only when these two split-GFP fragments were

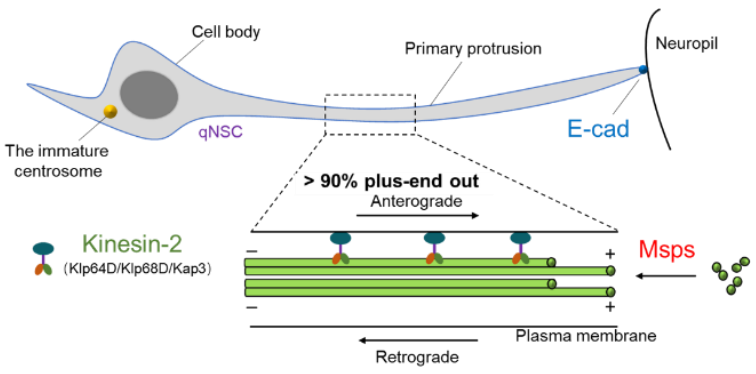
simultaneously expressed in both qNSCs and neuropil at early larval stages, specific reconstitution of GFP signal was observed at NSC-neuropil contact sites (Figure 5H). Moreover, Quantification of pixel intensity suggested that GFP was strongest at the tip of the protrusion, overlapping with E-cad at NSC-neuropil contact sites (Fig 5I). GFP signal was absent when expressing either of single split-GFP fragments by *grh*-Gal4 or *nSyb*-QF2 driver (Figure 5H), suggesting the specificity of t-GRASP. Therefore, E-cad is localized to the NSC-neuropil contact sites shown by the reconstituted GFP in t-GRASP. For the first time in our field, it demonstrates direct membrane contacts between qNSC and the neuropil.

These new data provide novel mechanisms by which Msps-dependent acentrosomal MTs activate NSCs reactivation by targeting E-cad to the NSC-neuropil contact sites via plus-end directed motor Kinesin-2.

Besides the above experiments, we have also performed the following additional two major sets of experiments to strengthen our manuscript. We performed additional experiments to support our conclusion that Msps regulates microtubule polarity in qNSCs. We painstakingly performed live imaging on 41 and 26 individual quiescent NSCs from $msps^{P18}$ and control, respectively at 6h ALH, and found that 50% of EB1-GFP comets move in the retrograde direction in $msps^{P18}$ qNSCs, compared with 7.8% in the control quantified the percentage of retrograde EB1-GFP comets (page 16). In addition, we have examined microtubule minus-end marker Nod-β-Gal localization in qNSCs in $msps^{810}$ at 24 h ALH and found its delocalization (Figure 4C, M and page 16-17).

We have performed spinning disc super-resolution microscopy to show that robust γ-tubulin was detected as a “doughnut”-like pattern surrounding the centrioles labelled by Asl cycling Mushroom body NSCs, while γ-Tub in quiescent NSCs was localized to the centrioles, but not the pericentriolar material. This data strongly support our conclusion that the centrosomes in quiescent NSCs are immature; therefore, the microtubule network in qNSCs is acentrosomal (Figure 1C-D).

Taken together, we believe that we have a much improved and super exciting manuscript that first reports acentrosomal microtubule organization in quiescent neural stem cells (NSCs) and critical microtubule regulators during NSC reactivation (see model image below). We have discovered that microtubule arrays in the primary protrusion of quiescent NSCs are predominantly acentrosomal and are oriented with their plus-end-out. We have identified Msps as the first microtubule regulator in quiescent NSCs that governs NSC reactivation via regulating acentrosomal microtubule growth and plus-end-out orientation. We also show for the first time that quiescent NSCs form membrane contact with the neuropil and E-cadherin is targeted to this contact by Msps-dependent microtubules and plus-end directed motor protein Kinesin-2. This study opens a new direction of research in microtubule-based cargo transport and cell-to-cell signalling via the primary protrusion in quiescent NSCs.



I would like to thank you for your time and effort and I hope that you will find our manuscript of sufficient novelty, significance and general interest for consideration in ***The EMBO Journal***.

Sincerely yours,

Hongyan Wang, Ph.D.

## 2nd Editorial Decision 26th Feb 2021

Dear Hongyan,

Thank you for submitting your manuscript to The EMBO Journal. This is a resubmission of MS 104549 that was previously editorial rejection. The revised version addressed some of the editorial concerns raised initially. I appreciate the added data and did send the manuscript out for full review. I have now received the comments from two referees. I had also asked a third referee for input, but I haven't heard back from the referee and at this stage don't think that I will receive the last report. This is also what lead to some delays in the decision, which I would like to apologise for. So we will just go with the two reports on hand.

As you can see below, the referees appreciate the analysis and supports publication here. They both raise a number of points that would be good to address in a revised version. I think it is a good idea to discuss the revisions further and we can do so via email or video, whatever works best for you.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Thank you for the opportunity to consider your work for publication. I look forward to discussing the revisions further with you

best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
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IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.

- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 27th May 2021.

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Referee #1:

In this manuscript, Deng et al. identify the microtubule regulator Msps, the motor protein kinesin-2 and the cell adhesion molecule E-Cadherin as important factors during neuroblast reactivation after quiescence in Drosophila. They show that Msps is required for microtubule polymerization in quiescent neuroblasts and in particular in their prominent, but still largely uncharacterized, cellular protrusion. They further show that E- Cadherin accumulates at the tip of this protrusion adjacent to the neuropil, and that this accumulation is abolished in Msps and Kinesin-2 mutants. Based on these data, they propose that Msps and Kinesin-2 are required for E-Cadherin transport to this site, and that neuropil contact via the protrusion is necessary for reactivation.

The mechanism of neuroblast reactivation is of great interest in the stem cell field, and only relatively few factors are known to be involved. The function of the neuroblast cellular protrusion has recently garnered some interest but its potential significance for neuroblast activation is still unknown. The current study adds several new factors to this process and the characterization seems very solid. The study also puts forward a novel characterization of the cellular protrusion and an interesting hypothesis for its function. This study is therefore valuable and a significant advance in the field. However, the interplay between Msps, Kin-2 and ECad is not sufficiently characterized, and the conclusions regarding the role of the protrusion are still highly speculative. I would recommend publication once these issues are addressed.

Major criticisms:
1. In the proposed model, Msps, Kin-2 and ECad are epistatically linked, but this is only based on correlative data, and they could actually regulate reactivation via completely distinct mechanisms. It is therefore most important to assess their relationship by genetic interaction analysis. It would be

particularly helpful to address the following questions: do heterozygous or hypomorphic mutants interact synergistically? Does overexpression of ECad suppress the defects in Msps or Kin-2 mutants? It would be particularly helpful to establish genetic interactions between the MT regulators and ECad.

2. The observed ECad localization and the effects of Msps and Kin-2 are intriguing but not sufficiently characterized. For one, were the staining intensities normalized and if yes, how? For example, the loss of ECad at the protrusion could reflect overall reduced ECad expression, so it would be helpful to quantitatively compare ECad levels in the protrusion with those in the cell body. Because these stainings are important for the model, I would also suggest to repeat the localization by independent means, e. g., by using ECad::GFP and staining with GFP antibodies.

3. The proposed role for the protrusion is intriguing and likely very important for the field. Yet it is very hard to link the mutant phenotypes directly to the protrusion. The model therefore remains quite speculative. I think it is important to acknowledge that more clearly in the manuscript.

Smaller technical issues:
1. Especially in the reactivation assays, what are the exact genotypes of the "controls"? This is not precisely specified. In the case where a mutant over a deficiency was used (Klp64D), it would actually be helpful to test the deficiency alone.

2. p9, Jupiter::GFP fluorescence is taken as proxy for MT polymerization. That is an overstatement, it likely only reflects Jupiter expression levels. Also, was this properly quantified?

3. Can examples of the EB1::GFP kymographs be shown without the added coloured lines? They completely block the actual fluorescence image.

4. None of the arrows in the figures are explained in the legends.

Recommended text changes, typos
1. In the discussion, first paragraph, it is stated that the new mutants result in a "failure of reactivation". But Klp64D mutants are adult viable, so they must be able to reactivate eventually. Similarly, msps mutant neuroblasts can go on to divide (albeit with asymmetry defects). So I recommend rephrasing to "delay of" or "defects during reactivation". (Alternatively, please, assess reactivation also at 36 and 48 h ALH)

2. In the last sentence of the summary, the neuropil is proposed as a new niche in neuroblast activation. As the paper really does not address the role of the neuropil, this statement should be moved to the discussion.

3. The first section of the results is very long and could use an additional headline

4. Typos in Figure 4, "Localization"

5. References: on p.11, is Morshead et al. really the right reference for Nod-betaGal localization? Otsuki and Brand 2018 is found twice in the references section. The Bostock et al. reference from biorxiv has now been published

Referee #3:

Deng et al., EMBO Journal; 2021

Stem cells switch between quiescence and proliferation but the regulatory mechanisms underlying these transition states are still incompletely understood. Here, Deng et al., use Drosophila neural stem cells as a model to investigate the mechanisms underlying exit of quiescence. They base their findings on previous reports, showing that quiescent neural stem cells contain a cellular protrusion from the cell body prior to their reactivation. Deng et al., show that this protrusion appears to establish membrane contacts with the neuropil through the cell adhesion molecule E-cadherin. E-Cadherin targeting to the NSC-neuropil junction appears to be dependent on Mini Spindles (Msps), a key microtubule regulator, and the plus-end directed motor protein Kinesin-2. The authors propose that Msps, Kinesin-2 and E-cadherin are required for NSCs to exit quiescence and that Msps acts as a key regulator of acentrosomal microtubule assembly in the cellular extension.

Overall, this is an interesting story, addressing the relevant question of how stem cells regulate exit from stem cell quiescence. Drosophila neuroblasts are a well understood system and ideally suited for this question because it provides the opportunity to use several different orthogonal tests and assays. Deng et al., provide a compelling molecular model contributing to an enhanced understanding of how neuroblasts exit quiescence, which is to a large extend supported by the data. However, I strongly recommend that the authors address the concerns specified below.

Major comments:

(1) Several figure panels lack appropriate quantification to allow comparing different conditions. For instance, for Figure 1C, D and FigS1F-H, would greatly benefit from quantifying intensity differences of cnn, g-Tub or Msps between the different Nb populations or time points with intensity ratios (e.g g-Tub intensity at 6h/g-Tub at 0h).

(2) The centrosome characterization is very interesting, and I appreciate the higher resolution images. However, the doughnut-like pattern (e.g Figure 1C) is very difficult to see. The quality of the data would vastly improve for this figure if another, higher resolution method could be used (e.g 3D-SIM).

(3) Deng et al., investigate Jupiter-GFP signal, a MT-binding protein under nutritional restrictions. Because the Jupiter signal is reduced, the authors conclude that microtubule growth could be enhanced in the presence of nutrition. An alternative interpretation could be that because of reduced larval growth, brain size is altered, affecting Jupiter-GFP accumulation and intensity. A more refined analysis would be necessary to exclude this possibility.

(4) The authors conclude that acentrosomal microtubule growth, rather than centrosomal microtubule growth, likely plays a major role in microtubule assembly in the cellular extension of qNSCs. Is it possible that centrosomes are responsible for microtubule growth during embryogenesis and that the established extension is a remnant of the embryo? The images do not support the notion that these microtubules are growing during quiescence. The authors conclusion is partially supported by their analysis of Arl2- and Ana2 mutants but it is not known whether these alleles already affect centrosome biogenesis in embryos. It would be good to remove other genes essential for centrosome function (e.g centriole duplication factors and/or PCM component

proteins), or using more acute centrosome perturbation methods to remove centrosomes in the embryo, or during quiescence specifically (e.g colcemid treatment in larvae?).

(5) My biggest concern is related to the role of Msps and microtubule growth in qNCSs' exit from quiescence. For instance, Msps mutants fail to exit quiescence but the question is whether this is a direct phenotype or due to embryonic defects. Msps mutants have additional defects (Mira localization) and it needs to be ruled out that failed exit from quiescence is a consequence of a general microtubule growth defect, afflicting later stages of embryogenesis and neuroblast biology.

(6) I commend the authors for the careful genetic analysis using different alleles for the investigated genes. However, in some instances, the authors should simplify the figures and manuscript by sticking to one or two alleles only. For instance, in Figure 4 & 5, the authors switch between RNAi and different alleles for msps. It would be much easier to correlate the different phenotypes if a group of alleles would be used consistently for the different assays.

Minor comments:

Page 18: There appears to be a missing number in the figure calling. '(Fig ?C; In control, E-cad intensity normalized to 1).

**Point-to-point response to reviewer comments**

Referee #1:

We thank the reviewer for the strong endorsement of our manuscript and constructive comments.

Main points:

In the proposed model, Msps, Kin-2 and ECad are epistatically linked, but this is only based on correlative data, and they could actually regulate reactivation via completely distinct mechanisms. It is therefore most important to assess their relationship by genetic interaction analysis. It would be particularly helpful to address the following questions: do heterozygous or hypomorphic mutants interact synergistically? Does overexpression of ECad suppress the defects in Msps or Kin-2 mutants? It would be particularly helpful to establish genetic interactions between the MT regulators and ECad.

We have now performed many additional experiments to prove the epistatic links among Msps, Kins-2 and E-cad.

1) We overexpressed *klp64D* and found that NSC reactivation phenotypes were significantly suppressed in *msps* RNAi. At 24h ALH, in *msps* knockdown with *klp64D* expression under the control of *insc*-Gal4, the number of EdU-negative NSCs was significantly reduced to 35.6% (Fig 7A, B; n=20 BL) compared with 45.0% in *msps* RNAi (Fig 7A, B; n=13 BL). Moreover, at 24h ALH, the NSC diameter in *msps* RNAi with *klp64D* overexpression was significantly increased to 6.6 ± 1.0 µm in comparison with 6.3 ± 1.0 µm in *msps* knockdown alone (Fig 7C; n=457 and n=494, respectively).

2) Re-introduction of the genomic construct of *kap3* (*g-kap3*) to *msps* RNAi led to a partial suppression of NSC reactivation defects. 34.3% of EdU-negative NSCs were observed in *msps* RNAi with *g-kap3* expression (Figure 7D, E; n=19 BL), which was significantly lower than 45.1% in *msps* RNAi (Fig 7D, E; n=15 BL), but higher than 1.8% in RNAi control (Fig 7D, E; n=13 BL). In addition, the NSC diameter was increased to 6.5 ± 1.1 µm in *msps* RNAi with *g-kap3* expression (Fig 7F; n=409), which was significantly larger than *msps* knockdown alone (Fig 7F; 6.1 ± 1.1 µm, n=357), but it was still smaller than that in control (Fig 7F; 8.7 ± 1.5 µm, n=339).

These genetic data strongly support our conclusion that Kin-2 functions downstream of Msps to promote NSC reactivation.

3) Overexpression of E-cad partially suppressed NSC reactivation defects caused by *msps* depletion. At 24h ALH, in *msps* RNAi with *E-cad* overexpression driven by *grh*-Gal4, the number of EdU-negative NSCs (Fig 7G, H; 21.5%, n=22 BL) was significantly fewer than that in *msps* RNAi alone (Fig 7G, H; 32.7% n=21 BL), but higher than 6.5% in control (Fig 7G, H; n=21 BL). In addition, the NSC diameter in *msps* RNAi with *E-cad over*expression was increased to 6.3 ± 1.3 µm (n=210) from 5.7 ± 1.2 µm (n=230) in *msps* knockdown (Fig 7I), but it was smaller than 7.3 ± 1.4 µm in control (Fig 7I; n=127).

4) E-cad overexpression significantly suppressed NSC reactivation defects in $klp64D^{k5h}$ brains. The number of EdU-negative NSCs was reduced to 9.8% in $klp64D^{k5h}$ with the overexpression of $E\text{-}cad^{7}$ (Fig 7J, K; n=10 BL) from 21.1% in $klp64D^{k5h}$ (Fig 7J, K; n=12 BL), and was close to 6.8% in RNAi control (Fig 7J, K; n=10 BL).

These new data strongly support our conclusion that E-cad functions downstream of Msps and Kin-2 in NSC reactivation.

5) Simultaneously knockdown of *kap3* (*kin-2* subunit) and *msps* by RNAi enhanced the NSC reactivation defects in single knockdowns. At 24h ALH, *msps kap3* double knockdown under the control of *grh*-Gal4 had more qNSCs that failed to incorporate EdU (Fig EV5D, E; 43.5% of NSCs, n=17 BL) than either of the single knockdown (Fig EV5D, E; *msps* RNAi, 31.3%, n=21 BL; *kap3* RNAi, 16.1%, n=11 BL). Further, at 24h ALH, the average NSC diameter in *msps* and *kap3* double knockdown (Fig EV5F; 5.9 ± 1.2 µm, n=247) was significantly decreased, compared with either *msps* RNAi (6.6 ± 1.3 µm, n=201) or *kap3* RNAi (7.3 ± 1.4 µm, n=162).

6) *msps klp64D* double knockdown resulted in significant more NSCs with failed EdU incorporation (Fig EV5D, E; 40.3%, n=14 BL), compared to single knockdown (Fig EV5D, E; *msps* RNAi, 31.3%, n=21 BL; *klp64D* RNAi, 12.3%, n=9 BL) or RNAi control (Fig EV5D, E; 3.8%, n=16). In addition, NSC diameter of *msps klp64D* double knockdown (Fig EV5F; 5.7 ± 1.2 µm, n=130) was significantly smaller than that in *msps* RNAi (Fig EV5F; 6.6 ± 1.3 µm, n=201), *klp64D* RNAi (7.3 ± 1.4 µm, n=170), or RNAi control (8.1 ± 1.3 µm, n=192).

7) In double knockdown of *msps* and *E-cad,* significantly more EdU-negative NSCs were observed (Fig EV5G, H; 37.7%, n=12 BL) compared with 30.7% in *msps* knockdown, 15.0% in *E-cad* knockdown, and 4.7% in RNAi control (Fig EV5G, H; n=13, n=12 and n=11, respectively) at 24h ALH. Further, at 24h ALH, the NSC diameter of *msps* and *E-cad* double knockdown was significantly dropped to 5.7 ± 1.1 µm (Fig EV5I; n=216), compared with *msps* or *E-cad* knockdown alone (Fig EV5I; 6.5 ± 1.3 µm, n=117 and 7.2 ± 1.5 µm, n=103, respectively).

Taken together, all the above new data on p25-27 of the revised manuscript strongly support epistatic links among Msps, Kins-2, and E-cad during NSC reactivation.

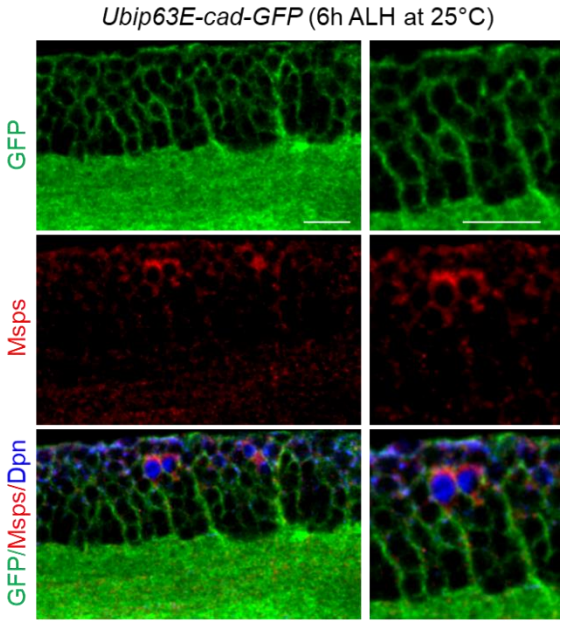
The observed ECad localization and the effects of Msps and Kin-2 are intriguing but not sufficiently characterized. For one, were the staining intensities normalized and if yes, how? For example, the loss of ECad at the protrusion could reflect overall reduced ECad expression, so it would be helpful to quantitatively compare ECad levels in the protrusion with those in the cell body. Because these stainings are important for the model, I would also suggest to repeat the localization by independent means, e. g., by using ECad::GFP and staining with GFP antibodies.

We observed similar E-cad intensity in the cell body in $msps^{924}$ qNSCs (Appendix Fig S2H, 0.35-fold, n=37) to that of control qNSCs (*vs* 0.32-fold, n=60), when normalized against Dpn. Therefore, the loss of E-cad at the protrusion tips upon *msps* depletion is unlikely due to overall reduction of E-cad expression. In contrast, E-Cad intensity at protrusion tips normalized against Dpn intensity was decreased from “0.56” in control qNSCs (Fig 5D, F; n=60) to “0.35” in $msps^{924}$ qNSCs (Fig 5D, F; n=37). This fold change (0.35/0.56=0.63) after normalizing against Dpn intensity was very similar to 0.69-fold in $msps^{924}$ qNSCs before the normalization (Fig 5F in our previous manuscript). Similarly, after normalizing against Dpn intensity, we found the relative fluorescence intensity of E-cad at protrusion tip in $msps^{810}$ qNSCs was significantly reduced to 0.45-fold (n=40) in contrast to 0.74-fold in control qNSCs (Fig 5A, C; n=34).

In addition, the relative protein levels of E-cad at the protrusion tip of qNSCs from $klp64D^{k5h/Df}$ and $kap3^{V5}$ were 0.37-fold and 0.42-fold, respectively (Fig I, J; n=40 and n=48, respectively), significantly lower than 0.67-fold from control (Fig I, J; n=85).

We have replaced the quantifications of E-cad with the new ones after normalization against Dpn intensity (Fig 5C, F, Fig 6J and p19-20, 24-25 of the revised manuscript).

To confirm the E-cad localization at the NSC-neuropil contact sites, we overexpressed E-cad-GFP, which was under the control of ubiquitous promoter Ubiquitin-63E. However, E-cad-GFP signal was dramatically enhanced at the neuropil and its localization at the protrusion tip was completely masked by strong signals from the neuropil.




We then examined the localization of *UASp-E-cad-GFP* line under the control of *grh*-Gal4. We stained 16h ALH larval brains expressing *UASp-E-cad-GFP* with anti-GFP and anti-E-cad antibodies. We could detect the co-localization of GFP with E-cad and distinct E-cad-GFP at the tip of the protrusion in 30.7% (Fig EV4M, n=13) of the qNSCs. E-cad-GFP localization at the tip of the protrusion was faint but visible in the remaining 69.3% (Fig EV4M, n=13) of qNSCs, presumably due to weak GFP signal in these cells.

We have included this data in Fig EV4M and p20 of the revised manuscript.

The proposed role for the protrusion is intriguing and likely very important for the field. Yet it is very hard to link the mutant phenotypes directly to the protrusion. The model therefore remains quite speculative. I think it is important to acknowledge that more clearly in the manuscript. Especially in the reactivation assays, what are the exact genotypes of the "controls"? This is not precisely specified. In the case where a mutant over a deficiency was used (Klp64D), it would actually be helpful to test the deficiency alone.

We have specified the exact genotypes of “controls” in figure legends in the revised manuscript (p42-53).

The *klp64D* deficiency (*klp64D*$^{df}$*:* Df(3L)BSC371/TM6B, Sb) was homozygous lethal at 24h ALH, so we examined hemizygous *klp64D*$^{df/+}$, and found only 7.3% of NSCs did not incorporate EdU, which was indistinguishable from 8.4% in control (Fig EV4I, J; n=16 BL and n=15 BL, respectively).

To further validate the role of Klp64D in NSC reactivation, we performed rescue experiment in *klp64D*$^{k5h}$ with the expression of *UASp-klp64D* construct driven by *grh*-Gal4 at 24h ALH and found a near complete rescue. Only 6.1% of NSC remained cellular protrusion in the rescued animals, which was significantly reduced in contrast to 14.5% in *klp64D*$^{k5h}$ (Fig

EV4F; n=14 BL and n=10 BL), and similar to the wild-type control (Fig EV4F; 5.3%, n=10 BL). In addition, EdU-negative NSCs was reduced to 10.5% in rescue (Fig EV4E, G; n=11 BL), which was apparently lower than 25.2% in *klp64D*$^{k5h}$ (Fig EV4E, G; n=13 BL), and indistinguishable from the wild-type control (Fig EV4H; 11.4%, n=13 BL). Moreover, the mitotic NSCs positive for PH3 in rescue were completely restored in contrast to *klp64D*$^{k5h}$ as well as control (Fig EV4H; rescue, 19.7%, n=14 BL; *klp64D*$^{k5h}$, 9.9%, n=11 BL; control, 20.7%, n=10 BL). Taken together, our data indicate that Klp64D is intrinsically required for NSC reactivation. These new data have been included in the revised manuscript on p24.

p9, Jupiter::GFP fluorescence is taken as proxy for MT polymerization. That is an overstatement, it likely only reflects Jupiter expression levels. Also, was this properly quantified?

We have quantified the overall intensity of Jupiter-GFP in non-Mushroom (MB) NSCs central brains and normalized against that in presumptive MB NSCs where Jupiter-GFP had highest expression. The Jupiter-GFP intensity ratio was 0.78-fold in larval brains under fed condition (Fig 1F, G; n=10 BL) but dropped to 0.12-fold upon nutritional restriction (Fig 1F, G; n=14 BL). We have moderated the conclusion to “This result suggests that the expression of Jupiter-GFP in qNSCs is dependent on nutrition” on p10 of the revised manuscript.

Can examples of the EB1::GFP kymographs be shown without the added coloured lines? They completely block the actual fluorescence image.

We have shown the kymographs without the lines and the ones with thinner coloured lines in Figure 2C-D, Figure 4A, and Figure S4C. Note that in Fig 4A, we have replaced the Kymograph with a new one that is representative.

None of the arrows in the figures are explained in the legends.

We have added description of arrows in the figure legends in the revised manuscript (p40-52) and appendix (p1-2).

Recommended text changes, typos

1. In the discussion, first paragraph, it is stated that the new mutants result in a "failure of reactivation". But Klp64D mutants are adult viable, so they must be able to reactivate eventually. Similarly, msps mutant neuroblasts can go on to divide (albeit with asymmetry defects). So I recommend rephrasing to "delay of" or "defects during reactivation". (Alternatively, please, assess reactivation also at 36 and 48 h ALH)

We have changed the phrases to “defects in NSC reactivation” in the first paragraph according to the review’s suggestion on p28.

2. In the last sentence of the summary, the neuropil is proposed as a new niche in neuroblast activation. As the paper really does not address the role of the neuropil, this statement should be moved to the discussion.

We have removed the last sentence of the summary.

3. The first section of the results is very long and could use an additional headline

We have included additional headlines for the first section of the results on p5, 7, 9, and 10.

4. Typos in Figure 4, "Localization"

We have corrected the misspelling in Figure 4G.

5. References: on p.11, is Morshead et al. really the right reference for Nod-betaGal localization? Otsuki and Brand 2018 is found twice in the references section. The Bostock et al. reference from biorxiv has now been published

Reference Morshead et al. on page 11 was a mistake and has been removed in the revised manuscript. We have removed the repeated reference Otsuki and Brand 2018 (on p6 and p29) and updated the Bostock et al. reference (on p29).

Referee #3:

We thank the reviewer for the constructive comments that are tremendously helpful to improve our manuscript.

Major comments:

Several figure panels lack appropriate quantification to allow comparing different conditions. For instance, for Figure 1C, D and FigS1F-H, would greatly benefit from quantifying intensity differences of cnn, g-Tub or Msps between the different Nb populations or time points with intensity ratios (e.g g-Tub intensity at 6h/g-Tub at 0h).

We have now quantified the ration of protein intensity between qNSCs and MB (dividing) NSCs. At 0h ALH, the γ-tub protein levels at the centrosomes in qNSCs were only 0.14-fold of that in the dividing MB NSCs (Fig EV1H, I; n=84). At 6h ALH, fluorescence intensity of γ-tub at the centrosomes in qNSCs was increased to 0.34-fold (Fig EV1H, I; n=63). At 0h ALH, CNN was barely detectable at the centrosomes, 0.006-fold of that of MB NSCs (Fig EV1B, D, F; n=40) and was significantly increased at 6h ALH (Fig EV1B, D, F; 0.09-fold, n=99). These quantifications further support our conclusion that the centrosomes in qNSCs are immature, but PCM protein levels increase over time. Since Msps is detected in the cytoplasm and not at the centrosomes in qNSCs, we quantified overall Msps intensity in qNSCs. Msps intensity was 0.19-fold of that in MB NSCs at 0h ALH (Fig 1I; n=45) and was increased to 0.26-fold of that in MB NSCs at 6 h ALH (Fig 1I; n=81). These quantifications were updated in the revised manuscript on p7-8, 10.

(2) The centrosome characterization is very interesting, and I appreciate the higher resolution images. However, the doughnut-like pattern (e.g Figure 1C) is very difficult to see. The quality of the data would vastly improve for this figure if another, higher resolution method could be used (e.g 3D-SIM).

The blurry of the centrosomal proteins was presumably due to the compression of files in the earlier submission, as we could clearly visualize the doughnut-like pattern. We have included high-resolution images of Figure 1C, D along with the revision submission.

(3) Deng et al., investigate Jupiter-GFP signal, a MT-binding protein under nutritional restrictions. Because the Jupiter signal is reduced, the authors conclude that microtubule growth could be enhanced in the presence of nutrition. An alternative interpretation could be that because of reduced larval growth, brain size is altered, affecting Jupiter-GFP accumulation and intensity. A more refined analysis would be necessary to exclude this possibility.

We have quantified the overall intensity of Jupiter-GFP in non-Mushroom (MB) NSCs central brains and normalized it against that in presumptive MB NSCs where Jupiter-GFP had highest expression. The Jupiter-GFP intensity ratio was 0.78-fold in larval brains under fed condition (Fig 1F, G; n=10 BL) but dropped to 0.12-fold upon nutritional restriction (Fig 1F, G; n=14 BL). This result suggests that the expression of Jupiter-GFP in qNSCs is dependent on the nutrition (on p9-10 of the revised manuscript).

(4) The authors conclude that acentrosomal microtubule growth, rather than centrosomal

microtubule growth, likely plays a major role in microtubule assembly in the cellular extension of qNSCs. Is it possible that centrosomes are responsible for microtubule growth during embryogenesis and that the established extension is a remnant of the embryo? The images do not support the notion that these microtubules are growing during quiescence. The authors conclusion is partially supported by their analysis of Arl2- and Ana2 mutants but it is not known whether these alleles already affect centrosome biogenesis in embryos. It would be good to remove other genes essential for centrosome function (e.g centriole duplication factors and/or PCM component proteins), or using more acute centrosome perturbation methods to remove centrosomes in the embryo, or during quiescence specifically (e.g colcemid treatment in larvae?).

The reviewer has raised a good point, as we could not rule out the possibility that the centrosomes are responsible for microtubule assembly in the protrusion of qNSCs during embryogenesis. We have included the following discussion on this point on p30 of the revised manuscript.

"Was the assembly of the primary protrusion in late embryonic stages dependent on the centrosomes? Although not been tested directly, this was unlikely, as depletion of a centrosomal protein γ-tubulin throughout embryonic stages and early larval stages did not disturb NSC cell cycle re-entry. Although we cannot formally rule out the possibility that microtubule growth in qNSCs requires the centrosomes at the embryonic stages, all our evidence point at acentrosomal microtubule organization in qNSCs during the larval stages."

To further exclude the possibility that the centrosomes might contribute to microtubule growth in qNSCs, we examined two independent RNAi lines targeting *γ-tub23C*, a major centrosomal (PCM) protein that is required for microtubule nucleation and anchoring in dividing NSCs. Under the control of *insc*-Gal4 at 24h ALH, 5.3% of NSCs in *γ-tub23C* RNAi I and 5.4% of NSCs in *γ-tub23C* RNAi II had no EdU incorporation (Fig EV3J, K; n=11BL and n=14 BL, respectively), which was indistinguishable from the wild-type control (Fig EV3J, K; 4.9%, n=12 BL). These two *γ-tub23C* RNAi lines worked effectively, as 75.6% of *γ-tub23C* RNAi I and 62.5% of *γ-tub23C* RNAi II, γ-tub protein levels were lost or dramatically reduced at the centrosomes marked by Msps (Fig EV3L; n=180 and n=136, respectively), in contrast to a strong expression of γ-tub at the centrosomes in the majority of the control NSCs (Fig EV3L; 96.4%, n=139). Therefore, the centrosomes are non-essential for NSC reactivation. The new data were shown on p14 of the revised manuscript.

We have attempted Colchicine treatment in early larvae with various concentrations up to 1mg/ml, the highest concentration reported in previous studies (Forkosh *et al*, 2020; Minestrini *et al*, 2002). It efficiently depolymerized microtubules in dividing NSCs, but not in qNSCs. As shown in the figure on the next page of this letter, in the untreated control NSCs, interphase microtubule aster and mitotic spindle were clearly marked by α-tub (1$^{st}$ column, arrow). Upon Colchicine treatment, microtubules were efficiently depolymerized and only faint signal of α-tub was detected in the cytoplasm or cell cortex (3$^{rd}$ column, arrow). However, microtubules marked by α-tub in the cell body and the primary protrusion were similarly abundant in both untreated control (2$^{nd}$ column) and Colchicine-treated (4$^{th}$ column) qNSCs. Probably microtubules in qNSCs are relatively resistant to drug treatment.

*Figure for reviewers removed*

(5) My biggest concern is related to the role of Msps and microtubule growth in qNCSs' exit from quiescence. For instance, Msps mutants fail to exit quiescence but the question is whether this is a direct phenotype or due to embryonic defects. Msps mutants have additional defects (Mira localization) and it needs to be ruled out that failed exit from quiescence is a consequence of a general microtubule growth defect, afflicting later stages of embryogenesis and neuroblast biology.

To exclude the possibility that NSC reactivation defects in *msps* loss was due to embryonic defects, we took advantage of Gal80$^{ts}$ to temporarily knock down *msps* only in larval stages. Briefly, we incubated eggs at 18°C to turn off *msps* knockdown during embryonic stage in the presence of Gal80$^{ts}$ for 44 hours until larval hatching, followed by a shift to 29°C to induce *msps* knockdown. At this condition, we still detected strong NSC reactivation defects, as 32.4% of NSCs were negative for EdU at 24h ALH (Fig EV4K, L; n=15 BL), which was dramatically higher than 9.0% in control (Fig EV4K, L; n=13 BL). This result indicates that *msps* deletion during larval stages is sufficient to result in NSC reactivation defects. We have included this new result on p16 of the revised manuscript

(6) I commend the authors for the careful genetic analysis using different alleles for the investigated genes. However, in some instances, the authors should simplify the figures and manuscript by sticking to one or two alleles only. For instance, in Figure 4 & 5, the authors switch between RNAi and different alleles for msps. It would be much easier to correlate the different phenotypes if a group of alleles would be used consistently for the different assays.

Switching among different alleles will be a little challenging for the readers, but we hope the reviewer will appreciate that we did this for valid reasons and for vigorous analysis. For Fig

4A-C, we chose two hypomorphic alleles *msps*$^{P18}$ and *msps*$^{P18/P}$, so that we could monitor the remaining EB1-GFP comets including retrograde comets. If stronger *msps* alleles were used for this experiments, likely we will not detect any EB1-GFP comets and won't able to determine the microtubule orientation in these mutants. For the rest of experiments in Fig 4 & 5, we normally focus on the null allele *msps*$^{810}$ and also analyzed at least another *msps* hypomorphic allele or *msps* RNAi to support the conclusion. We thought about moving some of the data in Fig 4 & 5 into supplementary figures, but even the supplementary figures are quite full. I hope the reviewer will allow us to keep Fig 4 & 5 as how they were in the manuscript.

Minor comments:

Page 18: There appears to be a missing number in the figure calling. '(Fig ?C; In control, E-cad intensity normalized to 1).

We have added the missing number "Fig 5C" on page 19 (the last line).

**References**

Forkosh E, Kenig A, Ilan Y (2020) Introducing variability in targeting the microtubules: Review of current mechanisms and future directions in colchicine therapy. *Pharmacol Res Perspect* 8: e00616-e00616

Minestrini G, Máthé E, Glover DM (2002) Domains of the Pavarotti kinesin-like protein that direct its subcellular distribution: effects of mislocalisation on the tubulin and actin cytoskeleton during Drosophila oogenesis. *J Cell Sci* 115: 725-736

## 1st Revision - Editorial Decision 30th Jun 2021

Dear Hongyan,

Thank you for submitting your manuscript to The EMBO Journal. Your study has been re-reviewed by the two referees. As you can see below, both referees appreciate the introduced changes and support publication here. Referee #1 has a few minor concerns that should be straight forward to respond to.

When you submit the revised version will you also take care of the following points:

- The appendix file needs a ToC

- Please remove Movie Legends from MS and zip together with the corresponding movie file. Please update Movie file names, nomenclature and callouts to Movie EV1 etc.

- We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.

- We also need a summary figure for the synopsis. The size should be 550 wide by [200-400] high (pixels). You can also use something from the figures if that is easier.

- I have asked our publisher to do their checks on the paper. They will send me the file within the next few days. Please wait to upload the revised version until you have received their comments.

Let me know if you have any further questions

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Please check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
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IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.

- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 28th Sep 2021.

https://emboj.msubmit.net/cgi-bin/main.plex

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Referee #1:

In my previous review, I had asked that the authors investigate genetic interactions between Kin-2, Msps, and ECad in order to support their pathway model which was at that point not supported. The new data provided by the authors, especially the amelioration of the msps phenotype by Kin-2 or ECad overexpression are in line with, or support, the proposed model. The enhancement between RNAis targeting two factors is probably a little less informative as it is not clear whether we are looking at hypomorphic conditions (enhancement) or additive effects.

ECad localization in the protrusion tip is now better quantified and also independently verified with ECad::GFP.

I also like the gammatub knockdown experiment suggested by the other reviewer. Supports non-centrosomal MT polymerization.

At this point, I can in principle recommend publication. Two things -

As it stands, the involvement of the protrusion tip is still speculative, and the authors are too bold in stating their model. For example, it says in the Discussion: "Our study, for the first time, demonstrates microtubule plus-end-out orientation in the primary protrusion of quiescent NSCs and a novel mechanism by which Msps governs NSC reactivation by targeting E-cad to NSC-neuropil contact sites via microtubule plus-end directed motor protein kinesin-2."
From the data, it is relatively clear that Msps, Kin-2 and Ecad are linked. The protrusion data are, strictly speaking, correlative. it is safer to state: "Our data are consistent with a model where Msps governs NSC reactivation by transporting E-cad via microtubule plus-end directed motor protein

kinesin-2. Based on our data, it is interesting to speculate that the target site and site of Ecad action are NSC-neuropil contact sites."

I would also still recommend working on the text and wording as the manuscript seems to have been written in a hasty manner. For example, several of the paragraphs end with two consecutive concluding sentences (Therefore,.... . Therefore,..../ Therefore,.... Taken together,.... .)

The source and identity of UAS-Ecad lines is not given in the methods section.

Referee #3:

The authors addressed my earlier concerns sufficiently.
I recommend this manuscript for publication in EMBO Journal.

**Point-to-point response to reviewer comments**

Referee #1:

As it stands, the involvement of the protrusion tip is still speculative, and the authors are too bold in stating their model. For example, it says in the Discussion: "Our study, for the first time, demonstrates microtubule plus-end-out orientation in the primary protrusion of quiescent NSCs and a novel mechanism by which Msps governs NSC reactivation by targeting E-cad to NSC-neuropil contact sites via microtubule plus-end directed motor protein kinesin-2."
From the data, it is relatively clear that Msps, Kin-2 and Ecad are linked. The protrusion data are, strictly speaking, correlative. it is safer to state: "Our data are consistent with a model where Msps governs NSC reactivation by transporting E-cad via microtubule plus-end directed motor protein kinesin-2. Based on our data, it is interesting to speculate that the target site and site of Ecad action are NSC-neuropil contact sites."

We have changed the statement on page 27 according to the review's suggestion.

I would also still recommend working on the text and wording as the manuscript seems to have been written in a hasty manner. For example, several of the paragraphs end with two consecutive concluding sentences (Therefore,.... . Therefore,..../ Therefore,.... Taken together,.... .)

We have removed the redundant conclusion sentences on page 6, 9, 20, 24, and 26.

The source and identity of UAS-Ecad lines is not given in the methods section.

We have included the source and identity of UAS-E-cad line in the materials and methods section on page 32.

## 2nd Revision - Editorial Decision 9th Jul 2021

Dear Hongyan,

Thanks for sending me the revised manuscript. I have now had a chance to take a careful look at everything and all looks good.

I am therefore very pleased to accept the manuscript for publication here.

Congratulations on a nice study

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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| Journal Submitted to: The EMBO Journal |
| Manuscript Number: EMBOJ-2020-104549R-Q |

**Reporting Checklist For Life Sciences Articles (Rev. June 2017)**

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

## A- Figures

### 1. Data

**The data shown in figures should satisfy the following conditions:**

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

### 2. Captions

**Each figure caption should contain the following information, for each panel where they are relevant:**

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

**In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.**

## B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

| | |
|---|---|
| 1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? | For immunochemistry studies, minimum six brain lobes were quantified for each sample. |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | For immunochemistry studies, minimum five brain lobes were quantified for each sample. For EB1-GFP tracking, minumum 20 qNSCs were analyzed. |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | NA |
| 3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe. | NA |
| For animal studies, include a statement about randomization even if no randomization was used. | No randomization was used in this study. |
| 4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe. | Some experiments were repeated by two different authors independently. |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | No blinding was done in this study. |
| 5. For every figure, are statistical tests justified as appropriate? | Yes |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | Statistical analysis between two groups was performed by two-tailed unpaired Student's t-test, and a value of $P < 0.05$ was considered as statistically significant. For three groups and above, one-way ANOVA was performed and p-values were calculated. For grouped graphs, two-way ANOVA was performed and p-values were generated. |
| Is there an estimate of variation within each group of data? | Yes |
| Is the variance similar between the groups that are being statistically compared? | Yes |

## C- Reagents

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http://www.antibodypedia.com
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http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-report

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http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumo

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jij.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

| | |
|---|---|
| 6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right). | guinea pig anti-Dpn (1:1000), mouse anti-Mira (1:50, F. Matsuzaki), rabbit anti-Mira (1:500, W. Chia), rabbit anti-GFP (1:3,000; F. Yu), mouse anti-GFP (1:5,000; F. Yu), guinea pig anti-Asl (1:200, C. Gonzalez), rabbit anti-Sas-4 (1:100, J. Raff), mouse anti-α-tubulin (1:200, Sigma, Cat#: T6199), mouse anti-γ-tubulin (1:200, Sigma, Cat#: T5326), rabbit anti-CNN (1:5000, E. Schejter and T. Megraw), rabbit anti-Msps (1:500), rabbit anti-Msps (1:1000, J. Raff), rabbit anti-PH3 (1:200, Sigma, Cat#: 06-570), rat anti-E-cadherin (1:20, DCAD2, DSHB), mouse anti-β-Gal (1:1000, Promega, Cat#: Z3781), rabbit anti-β-galactosidase (1:5000, Invitrogen, A-11132), mouse nc82 (1:20, DSHB) and mouse anti-synaptotagmin1 (1:50, DSHB, 3H2 2D7), rabbit anti-sas-4 (1:200, J. Raff), rabbit anti-Ana2 (Wang et al., 2011) (1:50), α-tubulin (1:200, Sigma, Cat#: T6199). |
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | NA |

* for all hyperlinks, please see the table at the top right of the document

## D- Animal Models

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| 8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals. | The following fly strains were used in this study: insc-Gal4 (BDSC#8751; 1407-Gal4), grh-Gal4 (A. Brand), insc-Gal4 tub-Gal80ts, msps924 (F. Yu), msps810 (F. Yu), msps810 UAS-Nod-β-gal (F. Yu), mspsP18 (Chen et al., 2016), mspsP (Cullen et al., 1999), g-msps (HN267) (Cullen et al., 1999), UAS-Kin-β-gal (Clark et al., 1997), UAS-arl2T30N/TM6B Tb (Chen et al., 2016), Jupiter-GFP (G147), UAS-β-tub-Venus/CyOβ, UAS-GFP-msps/TM6B Tb (F. Yu), kap3V5 (K. Ray), tacc74 UAS-Nod-β-gal (F. Yu), tacc59 (F. Yu), klp64Dk5h (Ray et al., 1999), kap3V6 (Sarpal et al, 2003b), kap3V6;; P{213 w+ 11} (labelled as kap3V6;; g-kap3#11 in this study) (Sarpal, 2003 #3), kap3V6;; P{213 w+ 31} (labelled as kap3V6;; g-kap3#31 in this study) (Sarpal, 2003 #3). The following stocks were obtained from Bloomington Drosophila Stock Center (BDSC): UAS-Gal RNAi (BDSC#50680; this stock is often used as a control UAS element to balance the total number of UAS elements), UAS-Nod-β-gal (BDSC#9912), FRT42D E-cadR69.Ubi-p63E-E-cad.GFP (BDSC#58742), UAS-E-cad RNAi (BDSC#32904), UAS-E-cad RNAi (BDSC#38207), nSyb-QF2 (BDSC#51955), 10XQUAS-post-t-GRASP.20XUAS-pre-t-GRASP (BDSC#79038), klp64D RNAi I (BDSC#40945), khc RNAi (BDSC#25898), khc RNAi RNAi (BDSC#35770), Df(3L)BSC371 (klp64D deficiency; BDSC#24395), UAS-klp64D (BDSC#32008), g-kap3#11 (separated from kap3V6;; g-kap3#11 in this study). |
| | The following stocks were obtained from Vienna Drosophila Resource Center (VDRC): msps RNAi (21982), arl2 RNAi (110627), sas-4 RNAi (106051), kap3 RNAi I (45400), kap3 RNAi I (103548), klp64D RNAi II (103358), klp68D RNAi (101058), Klp61F RNAi (52549), pav RNAi (1103330), klp67A RNAi (108852), Klp67A RNAi (52105), costa RNAi (108914), klp3A RNAi (104682), klp31E RNAi (110696), klp98A RNAi (40605), kif3C RNAi (108308), kif3C RNAi (43639). msps RNAi knockdown efficiency in larval brains was verified by immunostaining with an anti-Msps antibody. Various RNAi knockdown or overexpression constructs were induced using grh-Gal4 or insc-Gal4 unless otherwise stated.<br>All experiments were carried out at 25°C, except for RNAi knockdown or overexpression at 29°C. |
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. | NA |
| 10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance. | We confirm compliance. |

## E- Human Subjects

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| 11. Identify the committee(s) approving the study protocol. | NA |
| 12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. | NA |
| 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. | NA |
| 14. Report any restrictions on the availability (and/or on the use) of human data or samples. | NA |
| 15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. | NA |
| 16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list. | NA |
| 17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines. | NA |

## F- Data Accessibility

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| 18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'.<br><br>Data deposition in a public repository is mandatory for:<br>a. Protein, DNA and RNA sequences<br>b. Macromolecular structures<br>c. Crystallographic data for small molecules<br>d. Functional genomics data<br>e. Proteomics and molecular interactions | Done. |
| 19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right). | NA |
| 20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right). | NA |
| 21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information. | NA |

## G- Dual use research of concern

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| 22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could. | NA |