# A WDR47 homolog facilitates ciliogenesis by modulating intraflagellar transport

Chun-Xue Song, Xian-Ting Zeng, Wan-Xin Zeng, Rong Liu, Xia-Jing Tong and Qian Li
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**Original submission**

First decision letter

MS ID#: JOCES/2022/260303

MS TITLE: WDR47 facilitates ciliogenesis by modulating intraflagellar transport

AUTHORS: Chun-Xue Song, Xian-Ting Zeng, Wan-Xin Zeng, Xia-Jing Tong, and Qian Li
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

*Advance summary and potential significance to field*

The manuscript by Song et al. explored the ciliary function of WDR47/NMTN-1 in C. elegans. WDR47 has been proposed to indirectly bind to microtubules and play an essential role in mammalian motile cilia and neuronal axon guidance. In this study, Song et al. demonstrated that WDR47 is expressed explicitly in AWB chemosensory neurons and localizes at the basal body of the AWB cilia. Mutation of WDR47 causes abnormal morphology and structural integrity of the AWB cilia and induces aberrant AWB-mediated aversive behaviours. Further studies showed that WDR47 affects the velocity of IFT components and the ciliary localization of TAX-4, the cyclic nucleotide-gated channel protein, but not the ultrastructure of AWB cilia. Overall, this study provides insightful information for our understanding of how the morphology and function of cilia are regulated in a cell type-specific manner.

*Comments for the author*

The manuscript by Song et al. explored the ciliary function of WDR47/NMTN-1 in *C. elegans*. WDR47 has been proposed to indirectly bind to microtubules and play an essential role in mammalian motile cilia and neuronal axon guidance. In this study, Song et al. demonstrated that WDR47 is expressed explicitly in AWB chemosensory neurons and localizes at the basal body of the AWB cilia. Mutation of WDR47 causes abnormal morphology and structural integrity of the AWB cilia and induces aberrant AWB-mediated aversive behaviours. Further studies showed that WDR47 affects the velocity of IFT components and the ciliary localization of TAX-4, the cyclic nucleotide-gated channel protein, but not the ultrastructure of AWB cilia. Overall, this study provides insightful information for our understanding of how the morphology and function of cilia are regulated in a cell type-specific manner. Therefore, the reviewer would recommend its publication when the authors address the following concerns properly:

1. In Fig. 1F, WDR47/NMTN-1 is clearly localized at the cilia. The authors need to comment on this. For instance, how often was it detected?

2. It is surprising that the velocity of OSM-3 is increased while the one of OSM-6 is decreased in the AWB cilia middle segments of *wdr47/nmtn-1* mutants. The authors should further discuss or clarify this phenotype. Besides, a detailed description of how the velocities of IFT components are analyzed should be provided.

3. Line 273-274, the description of the relationship of IFT-A and IFT-B with OSM-3 and kinesin-II is incorrect (Ishida et al., Mol Biol Cell 2022; Zhu et al., EMBO J 2021).

4. In Fig. 4B and 4C, quantifying the fluorescence intensity at the basal body, transition zone, and cilia would be appreciated.

Reviewer 2

*Advance summary and potential significance to field*

This work studies how WDR47/NMTN-1 contributes to cell-specific ciliogenesis and chemosensation of the AWB neurons in C.elegans. The data showed that WDR47/NMTN-1 is expressed in AWB neurons and located at ciliary base. In addition, the wdr47/nmtn-1 mutants showed altered ciliary morphology and structural integrity. Furthermore, the wdr47/nmtn-1 mutant also showed altered aversion behaviors. Finally, the authors provided data that suggest WDR47/NMTN-1 contributes to the structure of the AWB cilia by regulating the IFT mechanism. I concur with the authors that the molecular mechanisms underlying cell type-specific ciliogenesis is an interesting issue. Moreover, the role of WDR47 in the genesis of primary cilia remain unclear. Overall, I think the topic is suitable for potential publication in JCS. Moreover, I think the structural and functional phenotypes are clear (but see some minor suggestions). However, I think the major weakness of the current manuscript is that the data and conclusions on the underlying mechanisms (e.g. on how WDR47 regulates IFT) are still weak. The authors should make more efforts in solving this major issue. The authors have raised some good ideas in the Discussion, for example the loss of WDR47 may affect the microtubule network in the cilia or the assembly of IFT complexes at ciliary base. I would encourage the authors to further test these ideas. Finally, my suggestion to the Editor is to offer a major revision opportunity.

*Comments for the author*

Summary

This work studies how WDR47/NMTN-1 contributes to cell-specific ciliogenesis and chemosensation of the AWB neurons in C.elegans. The data showed that WDR47/NMTN-1 is expressed in AWB neurons and located at ciliary base. In addition, the wdr47/nmtn-1 mutants showed altered ciliary morphology and structural integrity. Furthermore, the wdr47/nmtn-1 mutant also showed altered aversion behaviors. Finally, the authors provided data that suggest WDR47/NMTN-1 contributes to the structure of the AWB cilia by regulating the IFT mechanism. I concur with the authors that the molecular mechanisms underlying cell type-specific ciliogenesis is an interesting issue. Moreover, the role of WDR47 in the genesis of primary cilia remain unclear. Overall, I think the topic is suitable for potential publication in JCS. Moreover, I think the structural and functional phenotypes are clear (but see some minor suggestions). However, I think the major weakness of the current manuscript is that the data and conclusions on the underlying mechanisms (e.g. on how WDR47 regulates IFT) are still weak. The authors should make more efforts in solving this major issue. The authors have raised some good ideas in the Discussion, for example the loss of WDR47 may affect the microtubule network in the cilia or the assembly of IFT complexes at ciliary base. I would encourage the authors to further test these ideas. Finally, my suggestion to the Editor is to offer a major revision opportunity.

Major

1. Fig. 1A-B: The authors described the expression of WDR47/NMTN-1 in the AWB chemosensory neurons. What are the other cells that showed positive signal for Pnmtn-1::GFP? I suggest to give a complete summary on the expression pattern of WDR47/NMTN-1.
2. Fig. 1D-F: There are punctate signals for MNG::NMTN-1 in the dendrite. What are they? I suggest to add a short description about how the authors think of these dendritic signals.
3. Fig. s5: How did the authors determine which cells are AWB in the TEM data? In addition, this set of TEM data needs to be quantified, in order to better support the conclusion of “suggesting no disruption of the overall ultrastructure of AWB cilia (Line 264).”
4. Fig. 3C: I suggest to add the data of the rescue strains.
5. Fig. 4A: The conclusion on the changes in the IFT velocity is interesting but there are two major issues with the data. First, the velocity of OSM-3 in WT showed roughly a two-peak distribution, while the one in the mutant showed a single-peak distribution. Is that due to a smaller sample size in WT or does that suggest that OSM-3 moves in different ways in WT and mutant? Second, because the sample size is larger than 100, I suggest to use Bonferroni correction in addition to the standard t-test to check whether the difference between WT and mutant velocities are of real statistical significance.
6. Fig. 4B-C: Based on the presented images, the differences are quite subtle, which makes this conclusion weak. I suggest to re-consider this conclusion.

Minor

1. Fig. s2A-B: The images for the localization of both MKS-5 and DYF-19 are not intuitive. Why are there multiple signal puncta? I suggest to add cartoon schematics in the figure and also further descriptions in the caption.
2. Fig. 2C: It is hard to see where the mean is in this plot. I suggest to use another color
3. Sample sizes are generally missing, for example in Fig.1G, Fig.2C, 2E, 2F, 3B-E….
4. Fig 3C: ‘nmtn-1’, Italic.
5. For a better reading experience and resentation, it would be better to present all the cilia images in the same orientation (both diagram and fluorescence images).

**First revision**

Author response to reviewers' comments

**REVIEWER COMMENTS**

Reviewer 1
The manuscript by Song et al. explored the ciliary function of WDR47/NMTN-1 in *C. elegans*. WDR47 has been proposed to indirectly bind to microtubules and play an essential role in mammalian motile cilia and neuronal axon guidance. In this study, Song et al. demonstrated that WDR47 is expressed explicitly in AWB chemosensory neurons and localizes at the basal body of the AWB cilia. Mutation of WDR47 causes abnormal morphology and structural integrity of the AWB cilia and induces aberrant AWB-mediated aversive behaviours. Further studies showed that WDR47 affects the velocity of IFT components and the ciliary localization of TAX-4, the cyclic nucleotide- gated channel protein, but not the ultrastructure of AWB cilia. Overall, this study provides insightful information for our understanding of how the morphology and function of cilia are regulated in a cell type-specific manner. Therefore, the reviewer would recommend its publication when the authors address the following concerns properly:

We thank the reviewer for the positive remarks. Please see our responses to the reviewer's concerns.

1. In Fig. 1F, WDR47/NMTN-1 is clearly localized at the cilia. The authors need to comment on this. For instance, how often was it detected?

WDR47/NMTN-1 is indeed localized at the cilia, yet with much lower expression level. We revised the text in page 6-7 line 158-161 following the reviewer's suggestion. The revised text is shown below:

"The mNeonGreen signals were observed at the cilia and cell body of AWB neurons. Further, although WDR47/NMTN-1 was found throughout the cilia, it was more enriched at the base of the cilia (the revised Fig. 1D, 1F-G). This localization pattern was observed in all of the analyzed animals."

2. It is surprising that the velocity of OSM-3 is increased while the one of OSM-6 is decreased in the AWB cilia middle segments of *wdr47/nmtn-1* mutants. The authors should further discuss or clarify this phenotype. Besides, a detailed description of how the velocities of IFT components are analyzed should be provided.

In most of the amphid channel cilia, kinesin-II and OSM-3 act together to transport IFT particles containing OSM-6. The velocities of kinesin-II, OSM-3, and OSM-6 are similar. However, it has been reported that a few OSM-3 moves independently with kinesin- II/OSM-6 in the AWB cilia. Therefore, OSM-3 moves a bit faster than kinesin-II/OSM-6 (Mukhopadhyay et al., 2007). In *wdr47/nmtn-1* mutants, we observed increased velocity of OSM-3 and decreased velocity of OSM-6. We hypothesized that some kinesin-II/OSM-3/OSM-6 complexes are dissociated in *wdr47/nmtn-1* mutants. As a result, more separate OSM-3 is released to cause increased average velocity of OSM-6 On the other hand, OSM-6 is retained with kinesin-II to cause decreased average velocity of OSM-6.

To further test the hypothesis, we generated 3 double mutant animals and performed time-lapse imaging experiments on OSM-6 movement (the revised Fig. 4B). KAP-1 is a subunit of kinesin-II. In *kap-1;nmtn-1* double mutants, the velocity of OSM-6 was increased to 1.09 μm/s, which is similar to the velocity of OSM-3 alone. In *osm-3;nmtn- 1* double mutants, OSM-6 moved at 0.55 μm/s, which is similar to the velocity of kinesin-II alone. In *bbs-8;nmtn-1* double mutants, kinesin-II and OSM-3 were dissociated as previously reported (Ou et al., 2005). Now OSM-6 is transported by kinesin-II and OSM-3 separately, which is consistent with a mean movement speed of 0.70 μm/s.

Those results together support our model that WDR47/NMTN-1 may assist the coupling of OSM-3 and OSM-6. We made a cartoon illustration in the revised Fig. 4C to better interpret the IFT data.

We also added more details on how the velocities of IFT components are analyzed in page 16 line 433-444 of the revised manuscript.

3. Line 273-274, the description of the relationship of IFT-A and IFT-B with OSM-3 and kinesin-II is incorrect (Ishida et al., Mol Biol Cell 2022; Zhu et al., EMBO J 2021).

We are sorry for the ambiguity of our previous description. In mammals and Chlamydomonas, IFT-B is powered by heterotrimeric kinesin-2 (kinesin-II) for anterograde trafficking, while IFT-A is powered by dynein-2 for retrograde trafficking (Zhu et al., 2021). However, an additional homodimeric kinesin-2 (OSM-3) coordinates with kinesin-II to drive anterograde transport in *C. elegans* (Pan et al., 2006; Liang et al., 2014; Prevo et al., 2017).

Please see Figure 3B from Pan et al., 2006 (https://rupress.org/jcb/article/174/7/1035/44594/Mechanism-of-transport-of-IFT-particles-in-C) for details.

To be more precise, we changed the text in page 10-11 line 276-282 of the revised manuscript. The revised text is shown below:

"IFT particles involve two sub-complexes: IFT-A and IFT-B. In *C. elegans*, IFT-A associates with kinesin-II to form IFT-A sub-complex, and IFT-B associates with OSM-3 to form IFT-B sub-complex. In addition, the two sub-complexes are coupled by BBS proteins to move together during anterograde transport. In BBSome mutants, IFT-A/kinesin-II and IFT-B/OSM-3 sub-complexes are dissociated and move separately (Hao and Scholey, 2009; Liang et al., 2014; Prevo et al., 2017)."

4. In Fig. 4B and 4C, quantifying the fluorescence intensity at the basal body, transition zone, and cilia would be appreciated.

We performed the colocalization analyses of P*str-1*::TAX-4::sfGFP with the cilia (the revised Supplementary Fig. 5A), the basal body (the revised Supplementary Fig. 5B), and the transition zone (the revised Supplementary Fig. 5C). We further quantified the fluorescence intensities of TAX-4::sfGFP at the cilia, the basal body, and the transition zone in the revised Supplementary Fig. 5D. We found that the fluorescence intensities of TAX-4::sfGFP at the transition zone were higher in *wdr47/nmtn-1* mutants having mislocalized TAX-4 than those in WT, suggesting that *wdr47/nmtn-1* mutation may alter TAX-4 localization.

Reviewer 2

This work studies how WDR47/NMTN-1 contributes to cell-specific ciliogenesis and chemosensation of the AWB neurons in *C. elegans*. The data showed that WDR47/NMTN-1 is expressed in AWB neurons and located at ciliary base. In addition, the *wdr47/nmtn-1* mutants showed altered ciliary morphology and structural integrity. Furthermore, the *wdr47/nmtn-1* mutant also showed altered aversion behaviors. Finally, the authors provided data that suggest WDR47/NMTN-1 contributes to the structure of the AWB cilia by regulating the IFT mechanism. I concur with the authors that the molecular mechanisms underlying cell type-specific ciliogenesis is an interesting issue. Moreover, the role of WDR47 in the genesis of primary cilia remain unclear. Overall, I think the topic is suitable for potential publication in JCS. Moreover, I think the structural and functional phenotypes are clear (but see some minor suggestions). However, I think the major weakness of the current manuscript is that the data and conclusions on the underlying mechanisms (e.g. on how WDR47 regulates IFT) are still weak. The authors should make more efforts in solving this major issue. The authors have raised some good ideas in the Discussion, for example the loss of WDR47 may affect the microtubule network in the cilia or the assembly of IFT complexes at ciliary base. I would encourage the authors to further test these ideas. Finally, my suggestion to the Editor is to offer a major revision opportunity.

We want to thank the reviewer for the positive comments on our findings. We also appreciate his/her critical thinking when evaluating our paper. We have performed new experiments and rewritten some sections to address the reviewer's concerns. We think these changes together

significantly improve the quality of our work, and hope that the reviewer will agree.

We agree with the reviewer that the mechanisms underlying IFT regulation by WDR47/NMTN-1 are weak. In the revised manuscript, we added additional IFT analyses on several double mutant animals to further elucidate the potential role of WDR47/NMTN-1 (the revised Fig. 4B-C, and please also see our response to Reviewer 1 point 2). Our data suggest that WDR47/NMTN-1 may assist the coupling of OSM-3 and OSM-6. However, how does WDR47/NMTN-1 that is enriched in the basal body contribute to association between OSM-3 and OSM-6 is unknown. We suspect that WDR47/NMTN-1 could gate the entry of other proteins into the cilia and indirectly assist the coupling of OSM-3 and OSM-6. To test this hypothesis, we performed screening of potential WDR47/NMTN-1 interacting proteins by the yeast two-hybrid system, which is listed below. Unfortunately, we failed to identify any targets. It could be due to the false negative results of the screening method. Alternatively, the interacting proteins may not be included in our screening list. We agree that the molecular mechanisms on how WDR47/NMTN-1 regulates IFT require our further investigation in the future studies. We wrote a paragraph in Discussion to discuss about this point.

Screening the potential WDR47/NMTN-1 binding proteins by yeast two-hybrid

| AD fusion protein | BD fusion proteins | | |
|---|---|---|---|
| | IFT proteins | Microtubule associated proteins | Basal body proteins |
| $^{AD}$NMTN-1 | $^{BD}$KLP-11 | $^{BD}$MAPH-1.1 | $^{BD}$DYF-19 |
| | $^{BD}$KLP-20 | $^{BD}$MAPH-1.2 | $^{BD}$GASR-8 |
| | $^{BD}$KAP-1 | $^{BD}$MAPH-1.3 | |
| | $^{BD}$OSM-3 | $^{BD}$MAPH-1.1 NLC* | |
| | $^{BD}$OSM-6 | $^{BD}$MAPH-1.2 NLC* | |
| | $^{BD}$BBS-7 | $^{BD}$MAPH-1.3 NLC* | |
| | $^{BD}$DYF-1 | $^{BD}$PTRN-1 | |
| | $^{BD}$DYF-13 | $^{BD}$EEF-1A.1 | |
| | | $^{BD}$EEF-1A.2 | |
| | | $^{BD}$CCT-1 | |
| | | $^{BD}$CCT-2 | |
| | | $^{BD}$CCT-3 | |
| | | $^{BD}$CCT-4 | |
| | | $^{BD}$CCT-5 | |
| | | $^{BD}$CCT-6 | |
| | | $^{BD}$CCT-7 | |
| | | $^{BD}$CCT-8 | |

| BD fusion protein | AD fusion proteins | | |
|---|---|---|---|
| | IFT proteins | Microtubule associated proteins | Basal body proteins |
| $^{BD}$NMTN-1 | $^{AD}$KLP-11 | $^{AD}$MAPH-1.3 | $^{AD}$DYF-19 |
| | $^{AD}$KLP-20 | $^{AD}$MAPH-1.1 NLC* | $^{AD}$GASR-8 |
| | $^{AD}$KAP-1 | $^{AD}$MAPH-1.2 NLC* | |
| | $^{AD}$OSM-3 | $^{AD}$MAPH-1.3 NLC* | |
| | $^{AD}$OSM-6 | $^{AD}$PTRN-1 | |
| | $^{AD}$BBS-7 | $^{AD}$EEF-1A.1 | |
| | $^{AD}$BBS-8 | $^{AD}$EEF-1A.2 | |
| | $^{AD}$DYF-1 | $^{AD}$CCT-1 | |
| | $^{AD}$DYF-3 | $^{AD}$CCT-2 | |
| | $^{AD}$DYF-13 | $^{AD}$CCT-3 | |
| | | $^{AD}$CCT-4 | |
| | | $^{AD}$CCT-5 | |
| | | $^{AD}$CCT-6 | |
| | | $^{AD}$CCT-7 | |
| | | $^{AD}$CCT-8 | |

* NLC: N terminal of light chain

Major

1. Fig. 1A-B: The authors described the expression of WDR47/NMTN-1 in the AWB chemosensory neurons. What are the other cells that showed positive signal for Pnmtn-1::GFP? I suggest to give a complete summary on the expression pattern of WDR47/NMTN-1.

As suggested by the reviewer, we added a complete summary on neurons showing positive WDR47/NMTN-1 signals based on the neuron morphology and position in Wormatlas. In addition, we integrated the expression level (TPM values) from the single cell RNAseq data in the table (Taylor et al., 2021). The table was generated as the revised Table 1.

2. Fig. 1D-F: There are punctate signals for MNG::NMTN-1 in the dendrite. What are they? I suggest to add a short description about how the authors think of these dendritic signals.

We firstly investigated the possibility that the WDR47/NMTN-1 puncta in the dendrites represent the vesicles trafficking along the dendrites. We performed time-lapse imaging analysis on WDR47/NMTN-1 in the dendrites. We found that WDR47/NMTN-1 is immobile in the dendrites (the revised Supplementary Fig. 4C). Thus, we hypothesized that WDR47/NMTN-1 may function as microtubule associated proteins to facilitate vesicle trafficking in the dendrites, in analogy to ODR-4 forming puncta in the dendrites that could be involved in targeting ODR-10-containing vesicles to the cilia (Dwyer et al., 1998). However, we do not have direct evidence for the hypothesis. Following the reviewer's suggestion, we added a short description of the puncta signals in the dendrites in page 7 line 162 of the revised manuscript.

3. Fig. s5: How did the authors determine which cells are AWB in the TEM data? In addition, this set of TEM data needs to be quantified, in order to better support the conclusion of "suggesting no disruption of the overall ultrastructure of AWB cilia (Line 264)."

The Nicastro and Sengupta groups have collaborated to generate the high-resolution morphological and ultrastructural TEM map of the sensory cilia in the adult *C. elegans* (Doroquez et al., 2014). This great cilia atlas provides us the reference to identify the AWB cilia based on the morphology and position across serial TEM sections.

We also took more TEM images and quantified the TEM data, including the number of doublet and singlet microtubules in the channel cilia and AWB cilia, and the number of open B-tubules (the revised Supplementary Fig. 3B-D). We did not observe any significant differences between WT and *wdr47/nmtn-1* mutants.

4. Fig. 3C: I suggest to add the data of the rescue strains.

We added the data of the rescue strains in the revised Fig. 3C, showing that restoring WDR47/NMTN-1 expression in the WDR47/NMTN-1-expressing neurons (under the *wdr47/nmtn-1* promoter) rescued the chemotaxis defects to octanol.

5. Fig. 4A: The conclusion on the changes in the IFT velocity is interesting but there are two major issues with the data. First, the velocity of OSM-3 in WT showed roughly a two-peak distribution, while the one in the mutant showed a single-peak distribution. Is that due to a smaller sample size in WT or does that suggest that OSM-3 moves in different ways in WT and mutant? Second, because the sample size is larger than 100, I suggest to use Bonferroni correction in addition to the standard t-test to check whether the difference between WT and mutant velocities are of real statistical significance.

In the middle segments of most *C. elegans* amphid channel cilia, kinesin-II and OSM- 3 act together to transport IFT particles containing OSM-6 with similar velocities. However, it has been reported that a few OSM-3 moves independently with kinesin-II/OSM-6 in the AWB cilia (Mukhopadhyay et al., 2007). Therefore, there are two main populations of OSM-3: the slower moving kinesin-II/OSM-6/OSM-3 complex and the faster moving OSM-3 or OSM-3/OSM-6 complex. This could be the reason why OSM-3 showed roughly a two- peak distribution in WT. In our model of the revised Fig. 4C, the population of the faster moving OSM-3 increased in *wdr47/nmtn-1* mutants. As a result, OSM-3 moved faster and displayed a single-peak distribution in *wdr47/nmtn-1* mutants.

Please see Figure 8A from Mukhopadhyay et al., 2007 (https://www.embopress.org/doi/full/10.1038/sj.emboj.7601717) for details.

Following the reviewer's suggestion, we performed Bonferroni correction to analyze the differences of velocities between WT and mutants, and added an extra column to show the results in the revised Table 2. We obtained similar conclusions after performing Bonferroni correction analyses.

6. Fig. 4B-C: Based on the presented images, the differences are quite subtle, which makes this conclusion weak. I suggest to re-consider this conclusion.

We agree with the reviewer that the differences of TAX-4 localization between WT and *wdr47/nmtn-1* mutants are subtle. Therefore, we moved the data to the revised Supplementary Fig. 5 and rewrote the paragraph in a more conservative way.

Minor

1. Fig. s2A-B: The images for the localization of both MKS-5 and DYF-19 are not intuitive. Why are there multiple signal puncta? I suggest to add cartoon schematics in the figure and also further descriptions in the caption.

We previously constructed MKS-5::mCherry and DYF-19::mCherry fusion proteins driven by nonspecific promoters. Therefore, MKS-5 and DYF-19 were observed in all of the amphid cilia, and displayed multiple signal puncta. Following the reviewer's suggestion, we reconstructed MKS-5::mCherry and DYF-19::mCherry under the AWB-specific *str-1* promotor, and performed colocalization analyses between MNG::NMTN-1 and MKS-5::mCherry or DYF-19::mCherry. The data were presented in the revised Fig. 1F-G. In addition, we added the cartoon schematics in the revised Fig. 1H.

2. Fig. 2C: It is hard to see where the mean is in this plot. I suggest to use another color.

According to the reviewer's suggestion, we changed the color of *wdr47/nmtn-1* mutants to light green for better visualization in all of the related figures, including the revised Fig. 2C, 2E-F, 3B-E, Supplementary Fig. 2C, 2E, 3B-D, 5D.

3. Sample sizes are generally missing, for example in Fig.1G, Fig.2C, 2E, 2F, 3B-E….

We added the sample sizes in all of the related figures.

4. Fig 3C: 'nmtn-1', Italic.

We changed to Italic 'nmtn-1' in the revised Fig. 3C.

5. For a better reading experience and resentation, it would be better to present all the cilia images in the same orientation (both diagram and fluorescence images).

Thanks for the kind thought. We changed all the cilia images in the same orientation with cilia branches pointing to the lower right.

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Second decision letter

MS ID#: JOCES/2022/260303

MS TITLE: WDR47 facilitates ciliogenesis by modulating intraflagellar transport

AUTHORS: Chun-Xue Song, Xian-Ting Zeng, Wan-Xin Zeng, Rong Liu, Xia-Jing Tong, and Qian Li
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

*Advance summary and potential significance to field*

The authors have nicely addressed all my concerns. After the revision, this study not only provides insights into the regulation of cilia morphology and function in a cell type-specific manner but also implies new regulatory mechanisms for IFT assembly. I believe that these findings are to be interesting to the readers of Journal of Cell Science and therefore recommend its publication.

*Comments for the author*

No more revision is needed.

Reviewer 2

*Advance summary and potential significance to field*

The authors have made efforts to address my questions. I feel the manuscript has improved. I agree with the authors that WDR47 contributes to ciliary function via regulating ciliary microtubules.

*Comments for the author*

However, the TEM data are still not of sufficient quality to support the conclusion of “no disruption of the overall ultrastructure of AWB cilia after deletion of wdr47/nmtn-1 (Line 272-273)”. This weakness unfortunately leaves the potential working mechanism of WDR47 undefined in this type of cilia. Therefore, I suggest the authors to be more cautious about the interpretation of this set of TEM data. The authors may consider to discuss the possible structural changes that might happen to the microtubules in the absence of wdr47, for example 3D organization of the MTs.

Minors:

Page7, line 162: I suggest to make it clear that the dendritic puncta are of unknown identity.
Fig. 2: the representative images for the cilia of nmtn-1 were not provided.
Fig. s3D: Open not “ppen”?

**Second revision**

Author response to reviewers' comments

**REVIEWER COMMENTS**

Reviewer 1 Advance Summary and Potential Significance to Field:

The authors have nicely addressed all my concerns. After the revision, this study not only provides insights into the regulation of cilia morphology and function in a cell type-specific manner but also implies new regulatory mechanisms for IFT assembly. I believe that these findings are to be interesting to the readers of Journal of Cell Science and therefore recommend its publication.

Reviewer 1 Comments for the Author:

No more revision is needed.

We are glad that the reviewer is satisfied with our revised manuscript. We thank the reviewer for his/her previous comments that greatly improve our manuscript.

Reviewer 2 Advance Summary and Potential Significance to Field:

The authors have made efforts to address my questions. I feel the manuscript has improved. I agree with the authors that WDR47 contributes to ciliary function via regulating ciliary microtubules.

Reviewer 2 Comments for the Author:

However, the TEM data are still not of sufficient quality to support the conclusion of “no disruption of the overall ultrastructure of AWB cilia after deletion of wdr47/nmtn-1 (Line 272-273)”. This weakness unfortunately leaves the potential working mechanism of WDR47 undefined in this type of cilia. Therefore, I suggest the authors to be more cautious about the interpretation of this set of TEM data. The authors may consider to discuss the possible structural changes that might happen to the microtubules in the absence of wdr47, for example 3D organization of the MTs.

We thank the reviewer for his/her further rigorous comments. Following the reviewer’s suggestion, we rewrote the paragraph in a more conservative way in page 10 line 270-272 of the revised manuscript as below:
“However, we could not completely rule out the possibility that WDR47/NMTN-1 regulates some aspects of the cilia structure, such as the 3D organization of microtubules as previously reported (Schouteden et al., 2015; Wei et al., 2013; Williams et al., 2011).”

Minors:
Page7, line 162: I suggest to make it clear that the dendritic puncta are of unknown identity.

We changed the text in page 7 line 162-163 of the revised manuscript following the reviewer’s suggestion. The revised text is shown below:
“Interestingly, MNG::NMTN-1 appears punctate in the dendrites with unknown identity (Fig. 1D).”

Fig. 2: the representative images for the cilia of nmtn-1 were not provided.

We added the representative images for the AWB cilia of *wdr47/nmtn-1* mutants in the revised Fig. 2A.

Fig. s3D: Open not “ppen”?

We corrected the typo in the revised Supplementary Fig. 3D.

Third decision letter

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MS TITLE: WDR47 facilitates ciliogenesis by modulating intraflagellar transport

AUTHORS: Chun-Xue Song, Xian-Ting Zeng, Wan-Xin Zeng, Rong Liu, Xia-Jing Tong, and Qian Li
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.