Mark2 phosphorylation of KIF13A at 14-3-3 binding site is required for maintaining TfR vesicle transport dendritically in neuron

Yue Han[1,2], Min li[1,2], Bingqing zhao[1,2], Huichao wang[1,2], Yan liu[1,2], Zhijun Liu[3*], Jiaxi Xu[2*], Rui Yang[1,2,4*]

1. Institute of Neuroscience, Translational Medicine Institute, Health Science Center, Xi'an Jiaotong University, 710061 Xi'an, China.

2. Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Health Science Center, Xi'an Jiaotong University, 710061 Xi'an, China.

3. Shaanxi Provincial Center for Regenerative Medicine and Surgical Engineering, First Affiliated Hospital of Xi'an Jiaotong University, 710061 Xi'an, China.

4. Department of Anesthesiology, The First Affiliated Hospital of Xi'an Jiaotong University, 710061 Xi'an, China.

* Corresponding author: Rui Yang, Institute of Neuroscience, Translational Medicine Institute, Health Science Center, Xi'an Jiaotong University, 710061 Xi'an, China.

rui.yang@xjtu.edu.cn

Abstract

Microtubule based vesicle transport is an essential way of protein delivery. Selective transport of vesicles to soma-dendritic area or to axon is critical for maintaining neuronal polarity. Although neuronal axon is full-filled with plus-end-out microtubules, vesicles containing dendritic protein, like transferrin receptor (TfR) labeled vesicles, barely been transported to the axon. How does neuron maintain the transport of TfR labeled vesicles dendritically? Using live cell imaging and loss of function assay, we found that KIF13A is a major transporter for TfR vesicles at soma-dendritic area. Overexpression of KIF13A drives dendritic TfR vesicles to axon. We further found that MARK2 binds to and phosphorylates KIF13A at 14-3-3 binding motif inhibiting its kinesin activity at the axon initial segment (AIS), which is critical for maintaining TfR vesicle at the soma-dendritic area. Overall, our results suggested a novel kinesin-based mechanism for dendritic-selective transport.

Keywords: vesicle transportation; TfR vesicles, Kinesin 2, KIF13A, Mark2, neuron polarity

Conflict of interest statement

The authors declare no competing financial interests.

Introduction

Microtubule based vesicle transportation is an essential way for protein delivery in most types of cells. Neurons are highly polarized cell contains two distinct regions:

axon and soma-dendritic area. The high polarized morphology of neurons is critical for its unidirectional processing of information. The establishment and maintaining of this highly polarized neuron morphology require the correct trafficking of proteins to either axonal or soma-dendritic area.

Microtubule based vesicles transportation was mediated by motor protein kinesin (to the plus end of microtubules) or dynein (to the minus end of microtubules). Although, both the axon and dendrite of the neuron are full-filled with plus-end-out microtubules (1), which allows kinesin to free move to axon or dendrite, the actual movement of kinesin motors showed a clear preference(2, 3). For example, vesicles containing dendritic cargoes, like TfR labeled vesicles, are transported exclusively in dendrites and barely entering the axon with the fact that the axon are fulfilled with plus-end out microtubules. On the contrast, BDNF and NgCAM cargos show a clear bias to the axon. How cargos are transported differently has been a key question in the field.

Neuron expresses one dynein and some 15-kinesin family of motor proteins (2, 4). Among them, Kinesin-3 family member: KIF13A was found as potential dendritic vesicle transporter (2, 5). In neurons, KIF13A transports mannnose-6-phosphate receptor (M6PR) (6), viral proteins, early endosomes (5), the serotonin type 1A receptor (7) and so on. KIF13A also mediates AMPA receptor transportation in *c.elegant* (8) as well as dendritic spine-surface translocation of AMPA receptor during long-term potentiation (9). A recent live cell imaging study showed that KIF13A tail labeled vesicles was transported solely in soma-dendritic area of neuron and highly overlapped with transferrin receptor (TFR) (2). These evidence all suggested an important function

of KIF13A in maininging dendritic transport. What is unclear is if KIF13A were actively transporting TfR vesicles or passively hanging on TfR vesicles. Move over, it is unclear how KIF13A's movement is restricted to the soma-dendritic area?

In this study, using live cell imaging combining Crispr-Cas9 gene editing method, we investigated the role of KIF13A in dendritic vesicles transportation. Knockout of KIF13A with Crispr-Cas9 in cultured hippocampal neurons largely impairs the movement of TfR vesicles. Overexpression of full-length KIF13A causes dendritic TfR vesicles moving to the axon. Using BioID to screen the potential binding partner of KIF13A, we found that microtubule affinity-regulating kinase 2 (MARK2/Par1) bind to and phosphorylate KIF13A-tail at the 14-3-3 binding motif. Mark2 was involved in regulating the polarity of hippocampal neurons(10, 11), cortical neuron migrating (12) and axon formation (13, 14). We found that MARK2 is highly enriched at the AIS. Notably, phosphorylation of KIF13A at 14-3-3 binding motif by Mark2 is required for maintaining TfR vesicles stay dendritically. Knocking down of Mark2 decreased the dendrite to axon ratio of TfR vesicles. Together, in this study, we found that MARK2 phosphorylation of KIF13A at 14-3-3 binding site inhibits the kinase activity of KIF13A to prevent TfR vesicles from entering the axon.

Result

1. Kinesin-3 family member KIF13A mediates transporting of dendritic polarized TfR vesicles

TfR labeled vesicles are highly polarized to at soma-dendritic area in neurons. Previous studies showed that kinesin-3 family member, KIF13A, is a potential transporter for dendritic polarized vesicles (2). To test if KIF13A transports TfR labeled

vesicles, we co-expressed GFP fused KIF13A-tail with Halo tagged TfR in cultured hippocampal neurons and tracked the vesicles lively. KIF13A-tail labeled vesicles were polarized to soma-dendritic area and largely overlapped with TfR labeled vesicles (Fig 1A, B) (Movie1). Very few vesicles were observed moving into the axon.

We next used Crispr-Cas9 to knock out KIF13A in neurons and recorded the trafficking of TfR vesicles lively (Fig S1A). For a short period of time (1 day after transfection), we did not see an obviously change of TfR trafficking. After 3 days, we observed a decrease of TfR vesicle trafficking (Fig1C, D). Although lacking KIF13A largely impairs the transportation of TfR vesicles, it did not change the soma-dendritic distribution of TfR vesicles at all stages (Fig1C, E).

2. Inhibiting dynein or ectopic expressing KIF13A causes TfR vesicles moving to the axon

Previous study have shown that dynein is critical for maintaining vesicles dendritically. To confirm the function of dynein in TfR vesicle transportation, we overexpressed CC1 in neurons to inhibit dynein activity, then tracked the movement of TfR vesicle lively. When CC1 was expressed, TfR vesicles were ectopically moving anterogradely into the axon (Fig2A, Movie2) and changed the polarization of TfR vesicles (Fig2B). The kymograph showed TfR vesicles were majority anterograde transported into the axon. As mentioned before, axon is full-filled with plus-end out microtubules. The TfR vesicles moving to the axon must be mediated by kinesin.

If inhibiting dynein causes TfR vesicles moving into the axon, would ectopic expression of KIF13A do the same? We overexpressed KIF13A and lively tracked the

movement of TfR vesicles. In control neurons, TfR vesicles are mainly transported at the soma-dendritic area. Ectopic expression of KIF13A causes TfR vesicles moves to the axon (Fig2C, Movie 3), which decreased the dendrite to axon ratio of TfR (Fig 2D). Noticing that majority of the TfR vesicles in the axon are anterograde transported and co-labeled with KIF13A (Fig 2E). Interestingly, we found that overexpressing of KIF13A blocked the transferrin uptake (FigS-2A), which may be a consequence of the miss localization of TfR vesicles.

3. The tail domain of KIF13A is critical for moving TfR vesicles to the axon

How does overexpressing of KIF13A drives TfR vesicles to the axon? We first looked into if ectopic expression of KIF13A miss sorted dendritic proteins to axonal vesicles. We co-expressed NgCAM and TfR with/without KIF13A. NgCAM labeled vesicles showed a higher preference to the axon (Fig3A, Movie 4). In control neurons, TfR vesicles were soma-dendritic polarized and barely entering the axon. NgCAM vesicles were presented in the soma-dendritic area, but more of them are moving to the axon. We did not see any obvious overlapping between these two populations. When KIF13A was overexpressed, TfR vesicles started moving towards the axon. But we still did not see overlapping between TfR vesicles and NgCAM vesicles in the axon or in the dendrite (Fig3A). This result indicated that KIF13A did not disturb the protein sorting.

We next tested if the motor activity is required for driving TfR vesicles to the axon. We introduced the KIF13A motor mutation to the motor domain (KIF13A-mm) to impair its motor activity. Co-expressing KIF13A-mm with TfR vesicles did not cause

TfR vesicles to move to the axon (Fig. 3B). On the contrast, it increased the dendrite/axon ratio of TfR vesicles, which may due to a dominant negative effect of KIF13A-mm. Another kinesin-3 family member, KIF13B, were previously reported as a potential transporter for dendritic vesicles. In a live cell imaging study, KIF13B showed much less overlapping with TfR vesicles compared to KIF13A(2). We generated two chimera constructs - one containing KIF13A-tail and KIF13B-motor (KIF13A-B), and the other containing KIF13A-motor and KIF13B-tail (KIF13B-A) (Fig. 3B). When co-expressed these chimera constructs with TfR in neurons, the construct containing KIF13A-B drove TfR vesicles to the axon. On the contrast, the one containinf KIF13A motor and the KIF13B tail did not change the dendrite/axon ratio of TfR (Fig. 3B).

To this end, our results show that KIF13A is involved in TfR vesicle transportation. Knockdown of KIF13A impairs the transporting of TfR vesicles. Overexpressing KIF13A drives TfR vesicles to the axon, which is KIF13A tail-specific and kinesin motor activity-dependent.

4. Mark2 binds to and phosphorylates KIF13A-tail at the 14-3-3 binding site

To understand how KIF13A transport of TfR is regulated, we used BioID assay to screen possible protein binding partners for KIF13A. BioID was fused to KIF13A-tail and transfected HEK293T cells. After adding biotin, KIF13A's binding partners were tagged by biotin. The biotinylated proteins were then collected by streptavidin magnetic beads and analyzed using MS/MS (FigS3A, table 1). Among all trapped proteins, microtubule affinity-regulating kinase 2 (MARK2/Par1) was previously reported to be

involved in modulation of neuron polarity (10, 11). In addition, Par1b/MARK2 regulates axonal formation via phosphorylation on another Kineisn-3 family member: KIF13B at 14-3-3 binding motif (13). When expressed in neurons, Mark2-GFP showed an enrichment at AIS (Fig 4A).

To identify the critical binding region for Mark2, we divided KIF13A-tail into 4 fragments (F1, F2, F3, F2+3) based on its primary sequence (Fig 4B). Then we performed co-immunoprecipitation (Co-IP) assay to test the interaction between Mark2 and different KIF13A tail regions, where F2+3 showed the strongest interaction with Mark2 (Fig 4B). Accordingly, these fragments were expressed in neurons, and F1 and F2+3 both showed clear labeling of vesicles. However, only F2+3 fragment labeled vesicles co-localized with TfR vesicles (Fig4C, D). F1 fragment labeled vesicles are not soma-dendritic polarized, and it showed no overlap with TfR vesicles (Fig4C, D).

It was reported previously that KIF13B-tail contained a 14-3-3 binding site (RXXXSXP), which was phosphorylated by Mark2. The 14-3-3 binding site is conserved in KIF13A tail region (Fig 5A). We replaced the serine on the 14-3-3 binding site (S1371) with alanine (S1371A) or aspartic (S1371D) to mimic the non-phosphorylable or continuously phosphorylation of KIF13A, to test how the phosphorylation of KIF13A by Mark2 influence the 14-3-3 binding activity. Accordingly, we expressed 14-3-3-β/ε/ζ with wildtype KIF13A-S1371D in HEK293T cells and performed Co-IP assay. We found that 14-3-3-ε binds to continuously phosphorylated KIF13A mutation (S1371D) much stronger than wildtype (Fig 5C). Taken together, these results show that Mark2 phosphorylated KIF13A at the 14-3-3

binding site recruits 14-3-3-ε to KIF13A.

5. Mark2 phosphorylation of KIF13A-tail is required for its association with TfR vesicles.

To determine if Mark2 phosphorylation of KIF13A-tail regulates its cargo binding ability, KIF13A with or without Mark2 were co-expressed in rat embryonic fibroblasts (REF) cells (Fig5D). Compared to neurons, REF cells are flat, making the kinesin/vesicle colocalization easier to be imaged. Also, the microtubules of REF cells are orientated as plus-end-out from the centrosome to the cell periphery, which is more convenient to observe the direction of kinesin movement. In non-transfected REF cells, TfR vesicles were evenly distributed (Fig5D). When KIF13A was expressed, KIF13A was colocalized with TfR vesicles and drove TfR vesicles to the periphery of cell, reflecting the movement of KIF13A and TfR vesicles on the microtubule (Fig5D). When Mark2 was co-expressed, the colocalization between KIF13A and TfR vesicles were reduced. In REF cell, evenly distributed TfR vesicles further suggest that the kinesin activity of KIF13A is inhibited by Mark2. Co-expressing of mark2-Kinase dead mutation (Mark2-KD) did not change the puncta localization of KIF13A or the distribution of TfR vesicles (Fig5D). When KIF13A-S1371A mutant and Mark2 were co-expressed, TfR vesicles still showed an accumulation at the periphery of REF cell and an overlap of KIF13A and TfR vesicles, suggesting that Mark2 regulates the KIF13A activity by phosphorylating KIF13A at the 14-3-3 bind site.

The motor and tail domain of kinesin could bind to each other and formed an auto-inhibition conformation to inactivate kinesin (15). To find out how phosphorylation of

KIF13A at the 14-3-3 binding motif regulate the kinesin activity of KIF13A. We co-expressed KIF13A-tail (WT) containing the phosphor-mimic (S1371D) or non-phosphorylatable mutation (S1371A) with the motor region in HEK293T cell and performed a Co-IP assay. The results showed that KIF13A-tail-WT, SA and SD could all bind to motor region (Fig5F). By contrast, the non-phosphorylatable mutation (S1371A) showed a reduced binding affinity with the motor, suggesting that phosphorylation of KIF13A tail by Mark2 enhanced the motor-tail interaction and inactivated KIF13A. Reducing kinase activity of Mark2 benefits the association of KIF13A to its cargos.

Taken together, our results show that Mark2 phosphorylation of KIF13A at the 14-3-3 binding site inhibits KIF13A's kinesin activity and causes a detachment of KIF13A from vesicles.

6. Mark2 phosphorylation of KIF13A is required for maintaining TfR vesicles dendritically.

So far, we have shown that Mark2 phosphorylation of KIF13A at 14-3-3 binding site regulates the kinesin activity of KIF13A. To figure out how Mark2 influence TfR polarization in neurons, we use Crispr-Cas9 to knock out Mark2. By measuring the dendrite to axon ratio, we found that the enrichment of TfR in the soma-dendritic area was decreased (Fig 6A, B). However, this change might due to Mark2 phosphorylation of other substrates, like microtubule associated protein (11, 16). To rule out this possibility, we co-transfected Mark2 with KIF13A and live imaged the trafficking of TfR vesicles. Normally, TfR vesicles are restrict to the soma dendritic area. When

KIF13A was overexpressed, TfR vesicles was driven to the axon due to extra force generated by KIF13A (Fig 6C, D). When we co-express Mark2 on top of KIF13A, TfR vesicles in the axon were highly reversed. We interpret this change comes from Mark2 phosphorylation and inhibition of the kinesin activity of KIF13A. The reverse of axonal TfR vesicles is Mark2 kinase activity-dependent. Since Mark2 kinase dead mutation failed to reduce TfR vesicles in the axon (Fig 6C, D). We further confirm that Mark2 phosphorylation of KIF13A at 14-3-3 binding site is required for regulating the kinase activity of KIF13A. Because Mark2 did not reverse the axonal TfR vesicles caused by overexpression of KIF13A-S1371A non-phosphorylatable mutation (Fig 6C,D).

Discussion

The selective transport of dendritic protein is a long-lasting question in the field. Neuron expresses 15 some kinesin to move cargos along the microtubule cytoskeletons. Previously studies suggest that different kinesin are responsible for transport of different vesicle populations, which contribute to the diversity of delivering various cargos. In this study, we identify that kinesin-3 family member KIF13A associates and transports dendritic polarized TfR vesicles. KIF13A tail is found to associate to the TfR vesicles at soma-dendritic area. Knocking out KIF13A impairs the transportation of TfR vesicles. Overexpression of KIF13A in neurons alters the movement of TfR vesicles in axon. Using BioID to screen the potential regulator for KIF13A, we further find that MARK2 binds to and phosphorylates KIF13A-tail domain at 14-3-3 binding motif, thus inhibiting the kinesin activity. Knocking out of Mark2 induces leakage of TfR vesicles to the axon. Low expression of Mark2 exhibits a clear enrichment at AIS.

Mark2 phosphorylation of KIF13A at 14-3-3 binding motif enhances the interaction between motor and tail domain and inhibits the kinesin activity. Taken together, we proposed that KIF13A mediates TfR vesicles transportation at the soma-dendritic area. MARK2 phosphorylation of KIF13A at the AIS inhibits the kinesin activity of KIF13A and prevents TfR vesicles entering the axon (Fig7A).

Multiple research groups have brought thoughts of how the dendritic vesicles are limited to the soma-dendritic area from different aspects. Mu-ming Poo and colleagues propose that the high density of actin-network at the axon initial segment work as a molecular filter to block the traffic of some dendritic vesicles (17). Another study based on the actin-network at AIS is from Arnold DB and colleagues, where they showed that actin-based motor protein MyosinV catches dendritic vesicles at AIS to prevent the dendritic vesicles entering the axon(18). Hoogenraad CCand colleagues showed that dynein and NDEL1 steers dendritic cargos back to soma-dendritic area at the AIS (19, 20). On the same line, CDK5 is found to regulate dynein and affect dendritic transport of TfR vesicles (21).

There are studies also focusing on kinesin's role in selective transport. The kinesin-2 family protein KIF17 has been reported as a dendrite-specific motor protein and shown to interact with several dendritic cargos (21-23). Kinesin motors reflect the orientation of microtubules and show some level of preference depending on the surface modification of microtubes, which explain some aspects of the selective transport (24-26). On the other hand, the tail of kinesin with different adaptors displays specificity in cargo-association and vesicle-transportation (27, 28). Our study provides new evidence

showing how kinesin tail is regulated in modulation of the dendritic transportation.

KIF13A and KIF13B are both kinesin-3 family members. In a split-kinesin assay, both KIF13A and KIF13B bind early endosome (29). However, KIF13A-tail and KIF13B-tail labeled vesicles are differently distributed. KIF13B-tail labeled vesicles move to the axon, whereas KIF13A-tail labeled vesicle barely enter the axon (2). This difference is also confirmed in this study, where KIF13A overexpression drove TfR vesicles to the axon. But KIF13B overexpression fails to change the movement of TfR vesicles obviously. In addition, when we tested different regions of KIF13A-tail, F1 was found to label distinct vesicle populations compare the F2+3. F1 labeled vesicles could be PH domain-dependent.

Microtubule affinity-regulating kinase (MARK) family is originally discovered for their ability to phosphorylate tau. The kinase activity of MARK2 is very often reported in regulating cell polarization and the growth of neurites (30-32). It is also reported that MARK2 phosphorylation of another kinesin-3 family member: KIF13B is critical for axon formation (13). Hirokawa N and colleagues found Mark2 regulates the transportation of TRIM46 by motor KIF3A/B/KAP3 at the AIS (33). Studies about Mark2 implies that Mark2's kinase activity could function at a broad area in the cell. We have tested multiple commercial antibodies for Mark2, and none of them gives an ideal staining in fixed cells. But we did see a clear enrichment of overexpressed Mark2 at the AIS.

Dynein is reported to mediate the transportation of dendritic vesicles, and activated dynein at the AIS drives vesicles back to the soma (19, 20). We have confirmed the

function of dynein in dendritic vesicle transportation. In addition, we find that KIF13A is also involved in transporting dendritic vesicles. To our understanding, transportation of dendritic vesicles involves co-working of multiple motors. For the portion transported by KIF13A, due to the low abundance of Mark2 kinase in the soma-dendritic area, KIF13A binds to and actively transports TfR. When the dendritic vesicles are delivered to the AIS, the high concentration of Mark2 inhibits the kinesin activity of KIF13A. In the meantime, dynein is activated and reverse the vesicle back to the soma.

There are still questions left unsolved. How Mark2 is anchored at the AIS is not clear. Is it ankyrin G related or not? How the kinase activity of Mark2 is regulated differently at different regions in the neuron? Further investigation should be carried out to address these questions.

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Author contribution

Yue Han: Design and conduct the experiments, data analysis, manuscript writing; Min li, Bingqing zhao, Huichao wang, Yan liu: design and conduct the experiments; Rui Yang; Jiaxi Xu and Zhijun Liu: Project design; Manuscript writing and Editing.

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Figure

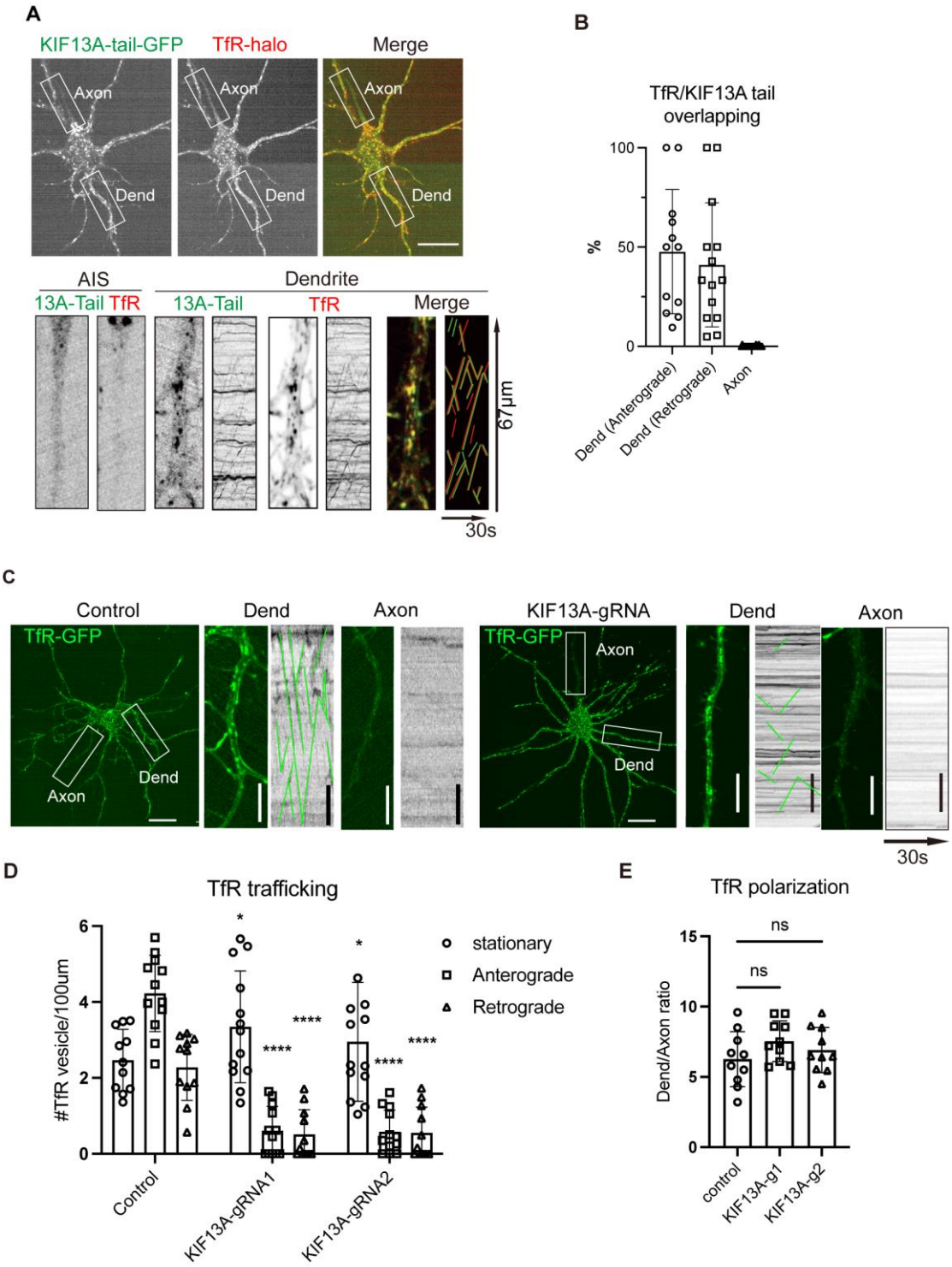

Figure 1. KIF13A targets and transports dendritic polarized TfR vesicles.

(A) div5 rat hippocampal neurons were co-transfected with KIF13A-tail-GFP and TfR-halo constructs. Time-lapse images were collected in 6 hours after transfection (30 seconds, 2 frames/sec). JF594 dye was used to label halo. Single frame of the time-lapse images was displayed to show the distribution of KIF13A-tail-GFP and TfR-halo.

Scale bar is 25 μm. Axon and dendrite of the neuron is marked with white boxes and enlarged. Kymograph of vesicles in the dendrite/axon was displayed (Green line for GFP vesicles. Red line for Halo). **(B)** Quantification of the overlapping between fragments of KIF13A-GFP tail and TfR. **(C)** div5 rat hippocampal neurons were co-transfected with CRISPR-Cas9 with scrambled sequence or with KIF13A specific guide RNA and TfR-GFP. Axon and dendrite of the neuron is marked with white boxes and enlarged. Single frame of the time-lapse images was displayed to show the distribution of TfR-GFP in Dendrite and axon. Scale bar is 25 μm, zoom magnified pics scale bar is 5μm. **(D)** The movement of TfR vesicles is quantified. **(E)** The polarization of TfR vesicles were quantified. (Student t-test, ns $P > 0.05$, *$P < 0.05$, ****$P < 0.0001$)

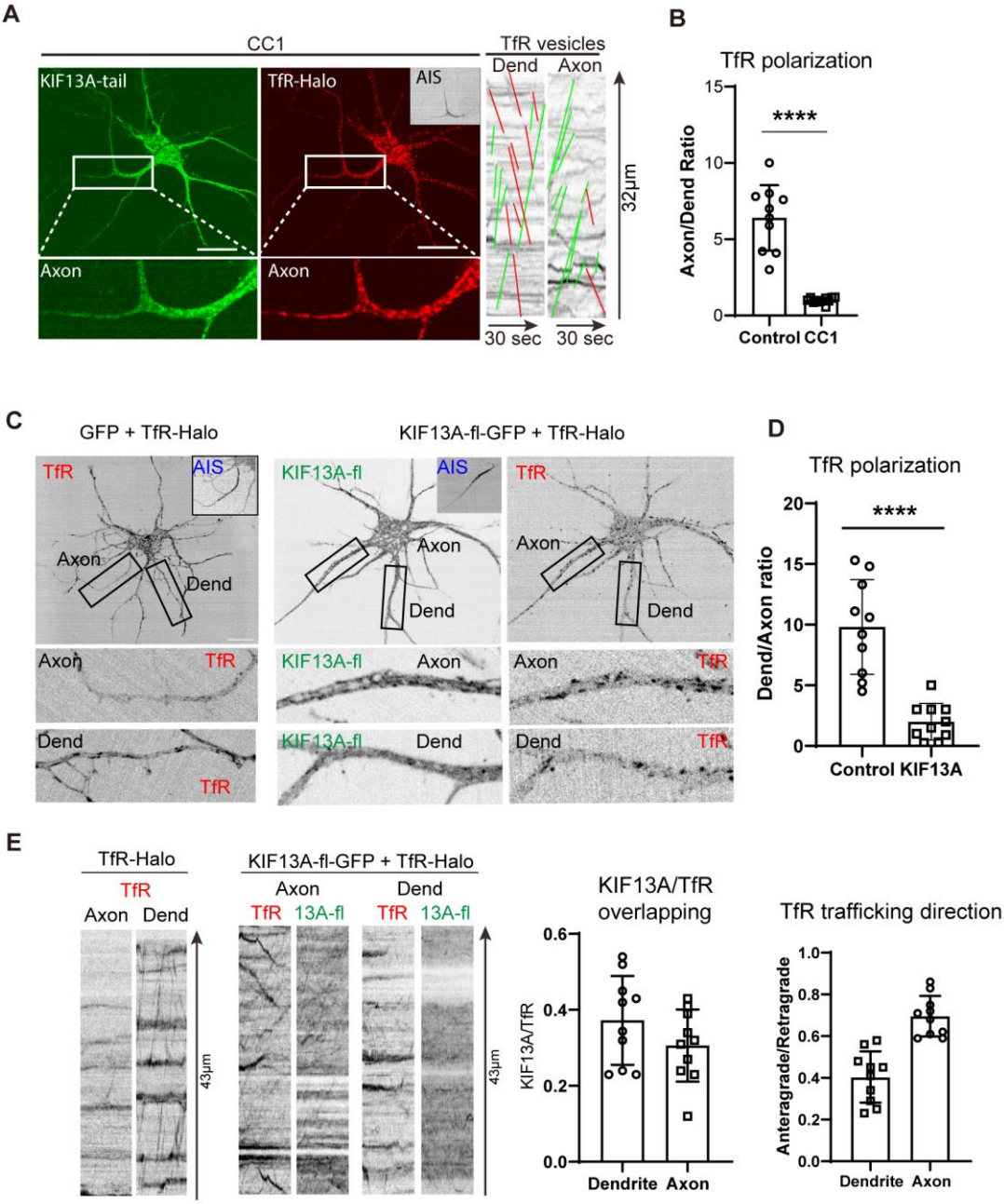

Figure 2. Inhibiting dynein or ectopic expressing KIF13A causes TfR vesicles moving to the axon

(A) CC1 and KIF13A-tail/TfR-Halo were co-expressed in div5 rat hippocampal neurons. The The axon initial segment (AIS) label is shown in the upper right embedded box. Axon of the neuron is marked with white basket and enlarged. Time-lapse images were collected (30 seconds ,2 frames/sec). Kymograph of TfR vesicles in the axon or dendrite were displayed on the right (Green line for KIF13A tail, red line for TfR vesicles). Scale bar is 25 μm. **(B)** The polarization of TfR vesicles between dendrite and axon were quantified. **(C)** div5 rat hippocampal neurons were co-transfected with

full-length KIF13A construct (KIF13A-fl-GFP) and TfR-Halo. The AIS label is shown in the upper right embedded box. Axon and dendrite of the neuron is marked with black basket and enlarged. Scale bar is 25 μm. **(D)** The polarization of TfR vesicles between control and full-length KIF13A groups were quantified. **(E)** Time-lapse images were collected after 6 hours of KIF13A-fl-GFP and TfR-Halo transfection for 30 seconds, with 200ms of exposure. Single frame of the time-lapse images was displayed to show the distribution of KIF13A and TfR. The overlapping of KIF13A and TfR vesicles, TfR trafficking direction are quantified. (Student t-test, ****$P < 0.0001$)

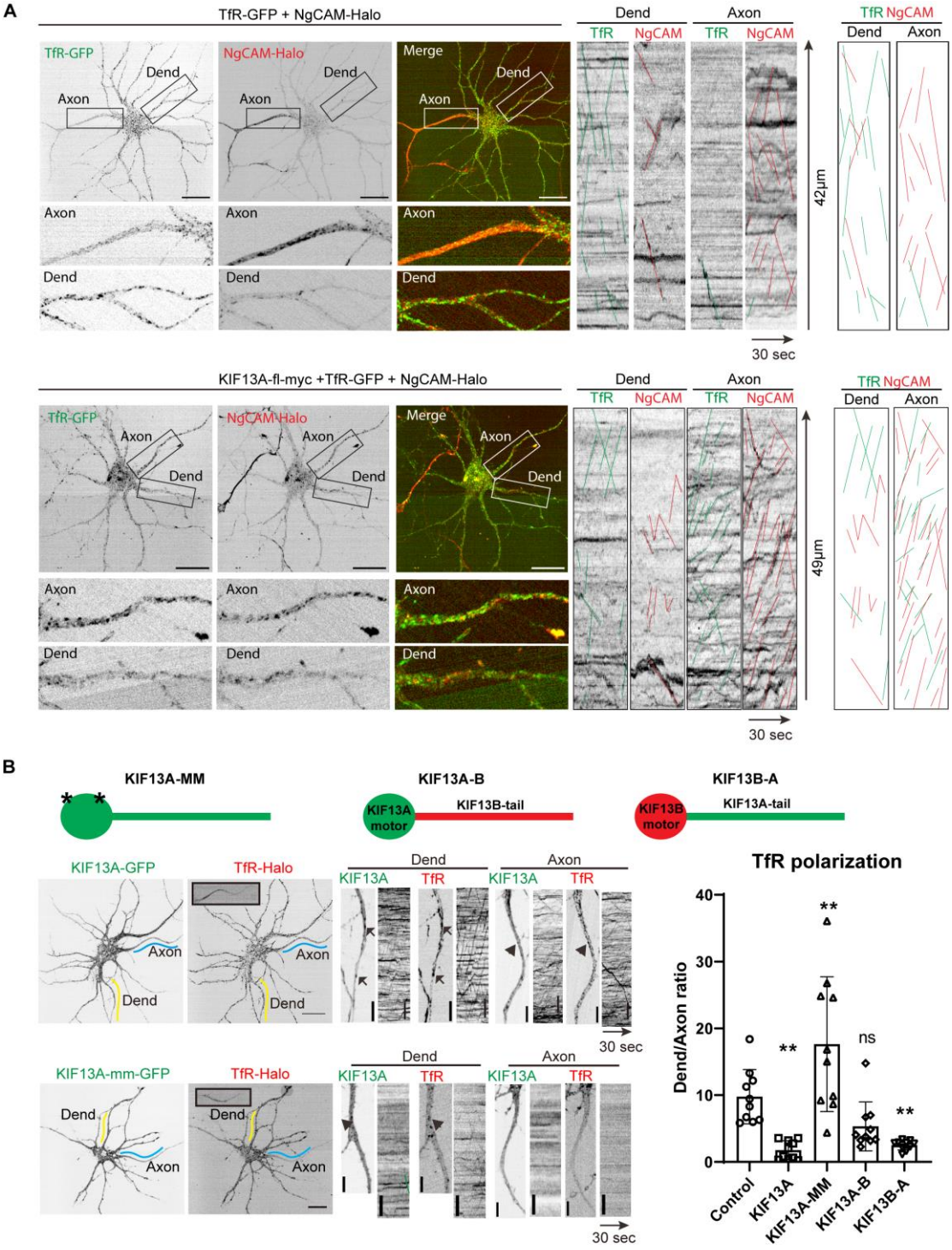

Figure 3. The tail domain of KIF13A is critical for moving TfR vesicles to the axon

(A) div5 rat hippocampal neurons were co-transfected with TFR-GFP and NgCAM-halo constructs (up), and co-transfected with full-length KIF13A-fl-myc, TFR-GFP and NgCAM-halo constructs (down). Axon and dendrite of the neuron is marked with black (white in merge graph) basket and enlarged. Scale bar is 25 μm. Time-lapse images

were collected in 6 hours after transfection (30 seconds, 2 frames/sec). JF594 dye was used to label halo. Kymograph of vesicles in the dendrite/axon was displayed on the right (Green line for TfR vesicles. Red line for NgCAM vesicles). **(B)** div5 rat hippocampal neurons were co-transfected with motor mutant KIF13A (KIF13A-mm, diagram on upper left) and TFR-halo, KIF13A motor and KIF13B tail fusion protein (KIF13A-B, diagram on upper middle) and TFR-halo, KIF13B motor and KIF13A tail fusion protein (KIF13B-A, diagram on upper right) and TFR-halo, full-length KIF13A construct (KIF13A-GFP) TFR-halo. Typically graph of neurons which transfected with KIF13A-GFP and KIF13A-mm-GFP were shown below. The AIS label is shown in the upper left embedded box. Axon and dendrite of the neuron is marked with blue and yellow curve, enlarged on the right. Scale bar is 25 μm. zoom magnified pics Scale bar is 5μm. Time-lapse images were collected (30 seconds ,2 frames/sec). The polarization of TfR vesicles under different isoform of KIF13A were quantified.

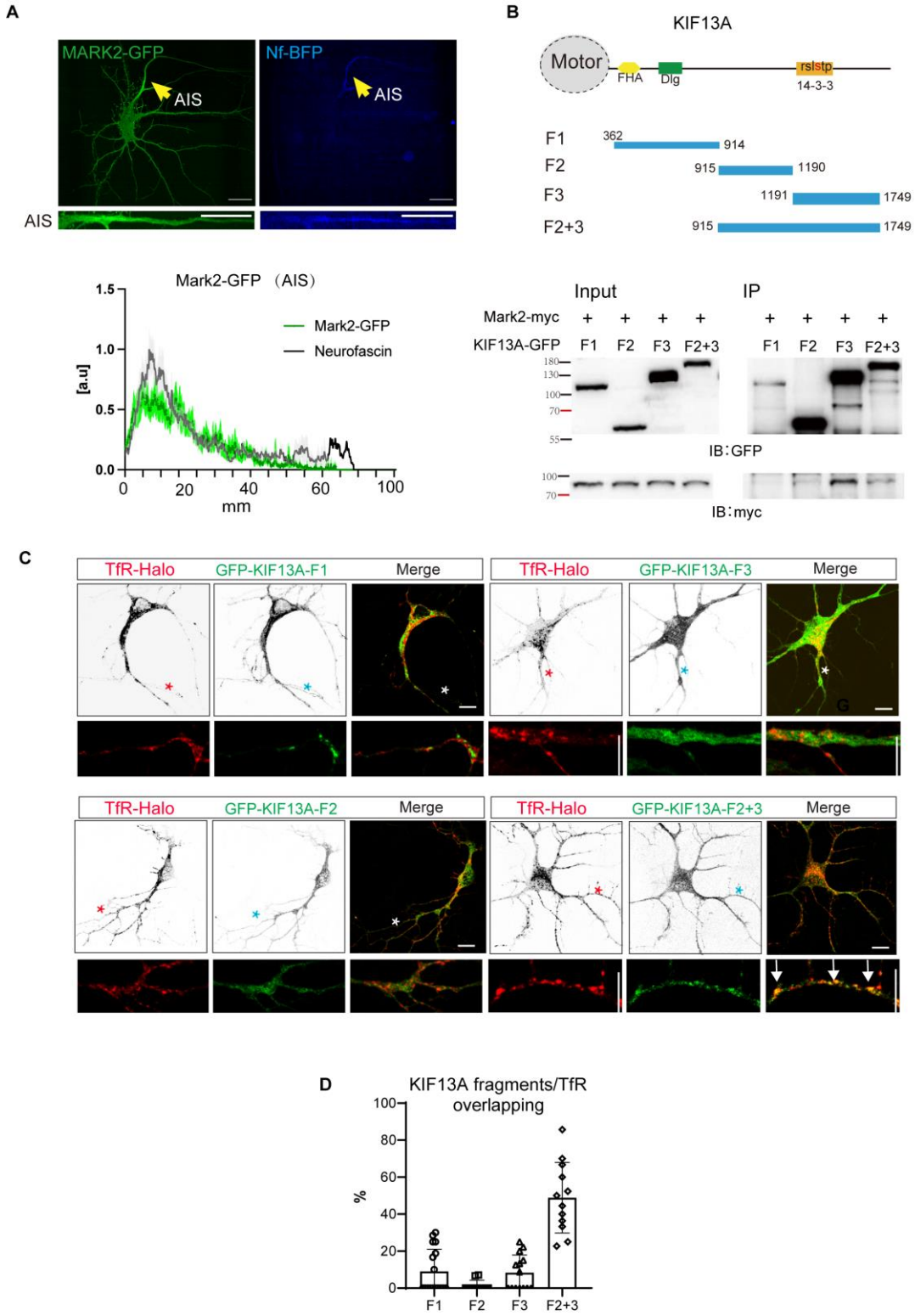

Figure 4. Mark2 binds to and phosphorylates KIF13A-tail

(A) MARK2-GFP construct was transfected with AIS marker neurofascin-BFP in cultured hippocampal neurons. Scale bar is 25 μm, zoom magnified pics scale bar is 5μm. Average intensity of Mark2 and neurofascin staining at the AIS is plotted and

aligned below. **(B)** Top: Schematic of fragments of KIF13A tail. Bottom: Fragments KIF13A-tail were co-expressed with MARK2-myc constructs in HEK293T cells. KIF13A fragments were immunoprecipitated with anti-GFP antibody. The binding of MAKR2 were detected with anti-myc antibody. **(C)** Plasmids for fragments of KIF13A-GFP tail were co-transfected with TfR-halo into div5 cultured hippocampal neurons. A high magnification picture of asterisk marked dendrite fragment was displayed on the bottom, the white arrow The white arrow points the KIF13A tail and TfR co-localization. Scale bar is 25 μm, zoom magnified pics scale bar is 5μm. **(D)** The overlapping between fragments of KIF13A-GFP tail and TfR was quantified.

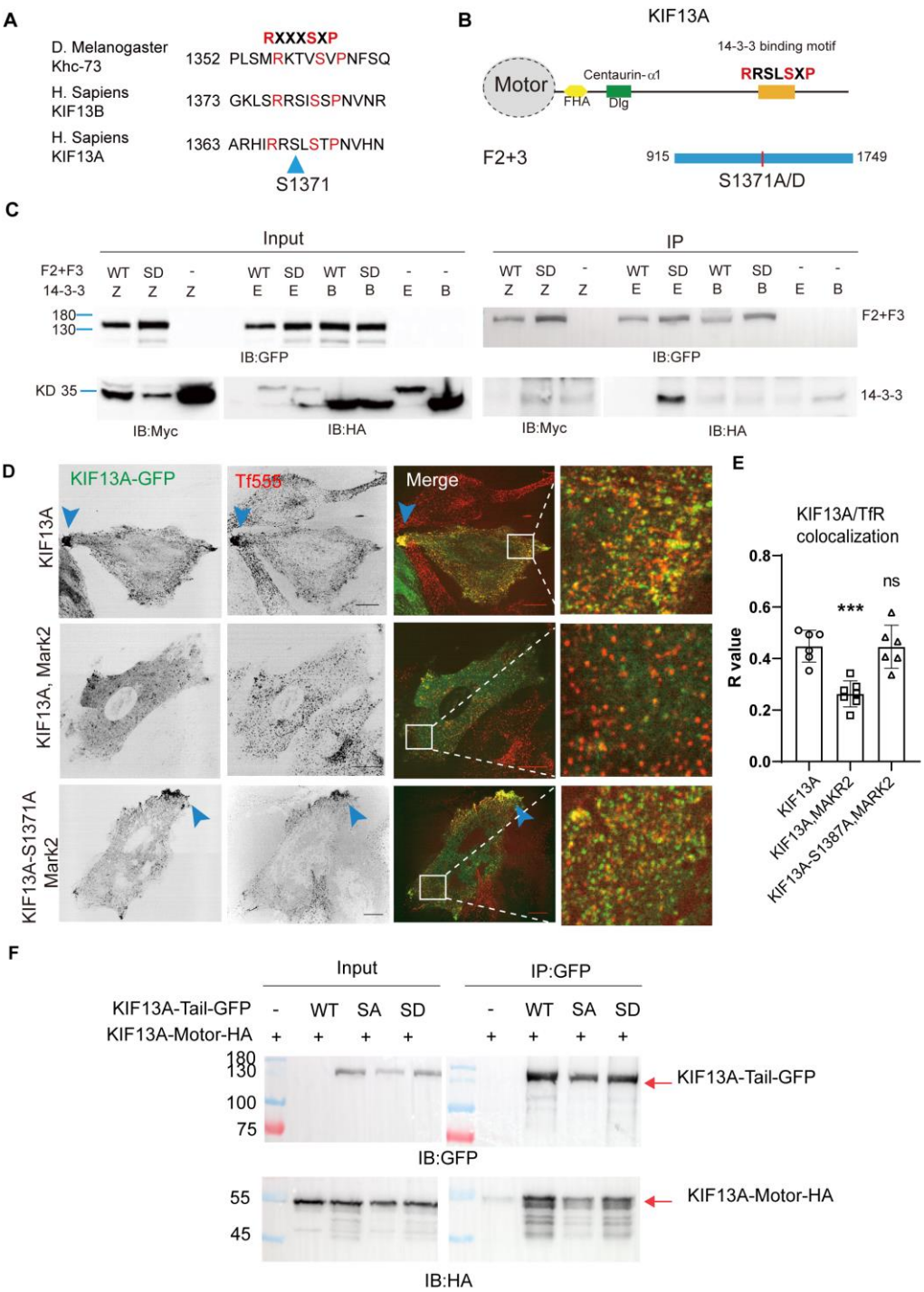

Figure 5. Mark2 phosphorylation of KIF13A-tail on S1371 site is required for its association with TfR vesicles.

(A) The alignment of the conserve 14-3-3 binding motif. **(B)** Schematic of replaced the serine on the 14-3-3 binding site (S1371) to alanine (S1371A) or aspartic (S1371D). **(C)** GFP-KIF13A-tail(F2+3) phosphorylated mimic (SD) or wild type were co-expressed in HEK293T cells with myc-14-3-3ζ, 14-3-3ε-HA and 14-3-3β -HA. KIF13A fragments were immunoprecipitated with GFP antibody. The binding of 14-3-3ζ were

detected with myc antibody, and 14-3-3ε/β were detected with HA antibody. **(D)** Co-expressed KIF13A(WT)/ KIF13A(S1371A) with or without Mark2 in rat embryonic fibroblasts (REF) cells. Fluorescent images were collected and displayed to show the distribution of KIF13A and Tf555 (Alexa 555™ fluorescent dye conjugated transferrin). Scale bar is 25 μm. **(E)** The overlapping between fragments of KIF13A-GFP tail and TfR was quantified. **(F)** Co-expressed of KIF13A-tail (WT) and phosphomimic (S1371D) or non-phosphorylatable mutation (S1371A) with the motor region (KIF13A-motor-HA) in HEK293T cells. KIF13A fragments were immunoprecipitated with anti-GFP antibody. The binding of KIF13A-motor was detected with anti-HA antibody. (Student t test, ***$P < 0.001$)

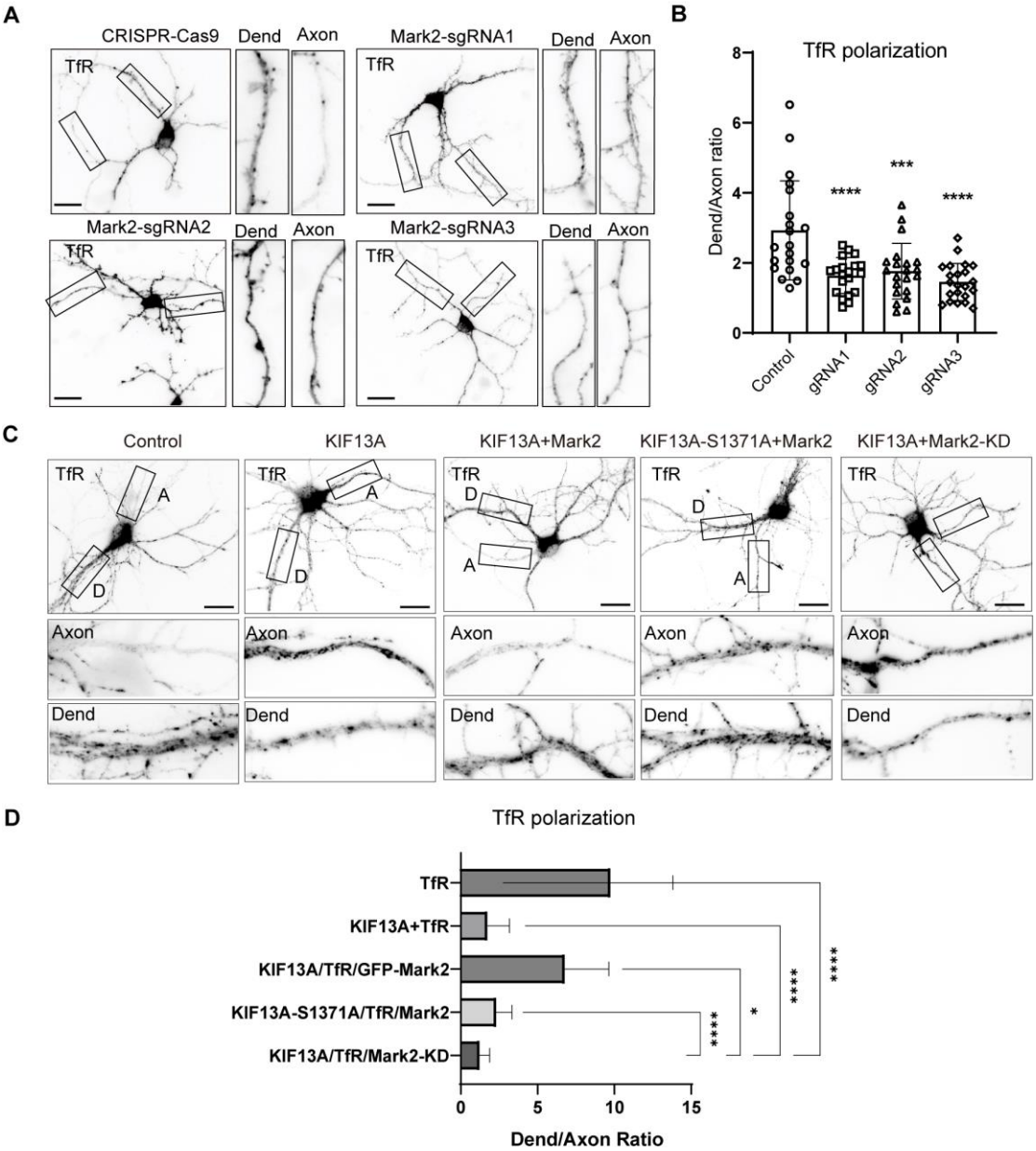

Figure 6. Mark2 phosphorylation of KIF13A is required for maintaining TfR vesicles dendritically

(A) Cultured hippocampal neurons were co-transfected with TfR-halo and Mark2-gRNA constructs as indicated. Scale bar is 25 μm. Axon and dendrite of the neuron is marked with black basket and enlarged on the right. **(B)** The polarization of TfR vesicles in the axon versus dendrites were quantified. **(C)** Cultured hippocampal neurons were co-transfected TfR-halo and GFP-KIF13A with or without Mark2, GFP-KIF13A-S1371A with Mark2, GFP-KIF13A with Mark2-KD. Axon and dendrite of the neuron is marked with black basket and enlarged on bottom. Scale bar is 25 μm. **(D)** The polarization of TfR vesicles in the axon versus dendrites were quantified. (Student

t-test, *P < 0.05, ***P < 0.001, ****P < 0.0001)

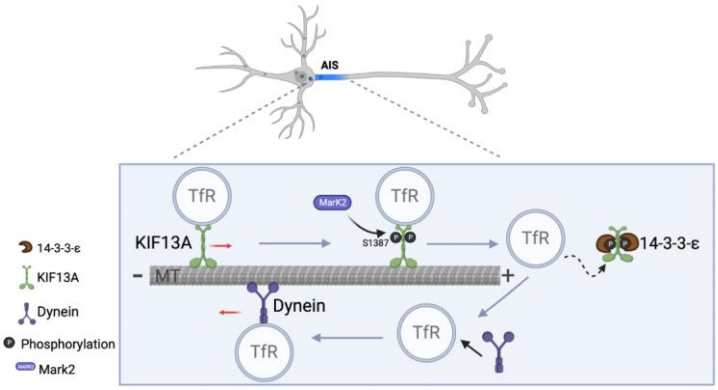

Figure 7. Diagram of guessed MARK2 phosphorylation of KIF13A at 14-3-3 binding motif in the axon initial segment (AIS)

S1A

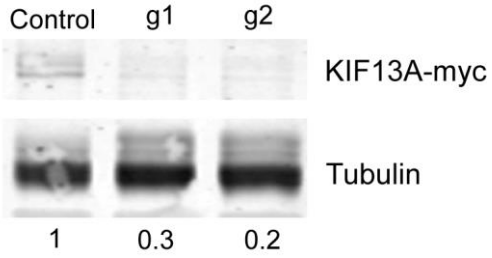

Figure S1. Western Blot of KIF13A-tail-myc after CRISPR-Cas9-sgRNA knockout in 293T cells.

(A) HEK293T cells were transfected with KIF13A-tail-myc and CRISPR-Cas9-sgRNA targeting KIF13A-tail gene. The expression of KIF13A-tail was detected with myc tag antibody.

S2A

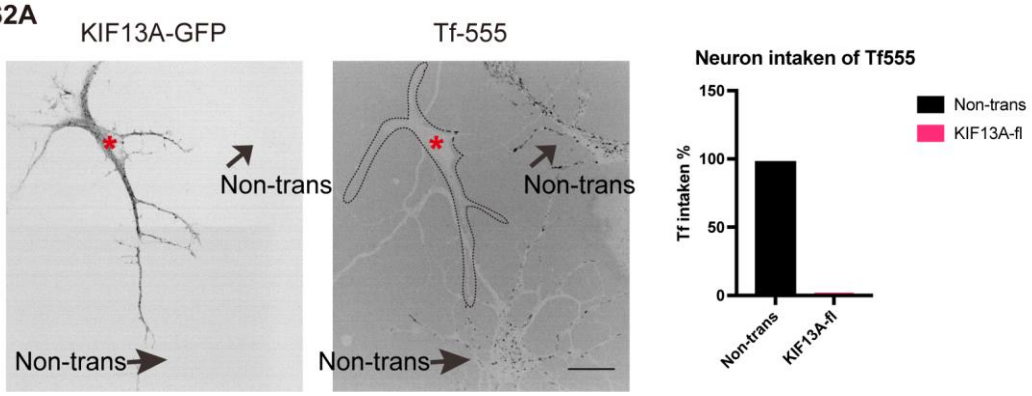

Figure S2. Overexpressing of KIF13A blocked the transferrin uptake

(A) div5 rat hippocampal neurons were transfected with full-length KIF13A construct

(KIF13A-GFP). 6 hours after transfection, Alexa 555™ fluorescent dye conjugated transferrin from human serum were added to medium. The black arrow points to neurons that were not transfected with KIF13A-GFP. The percentage of neurons taking transferrin is quantified. Scale bar is 25 μm.

S3A

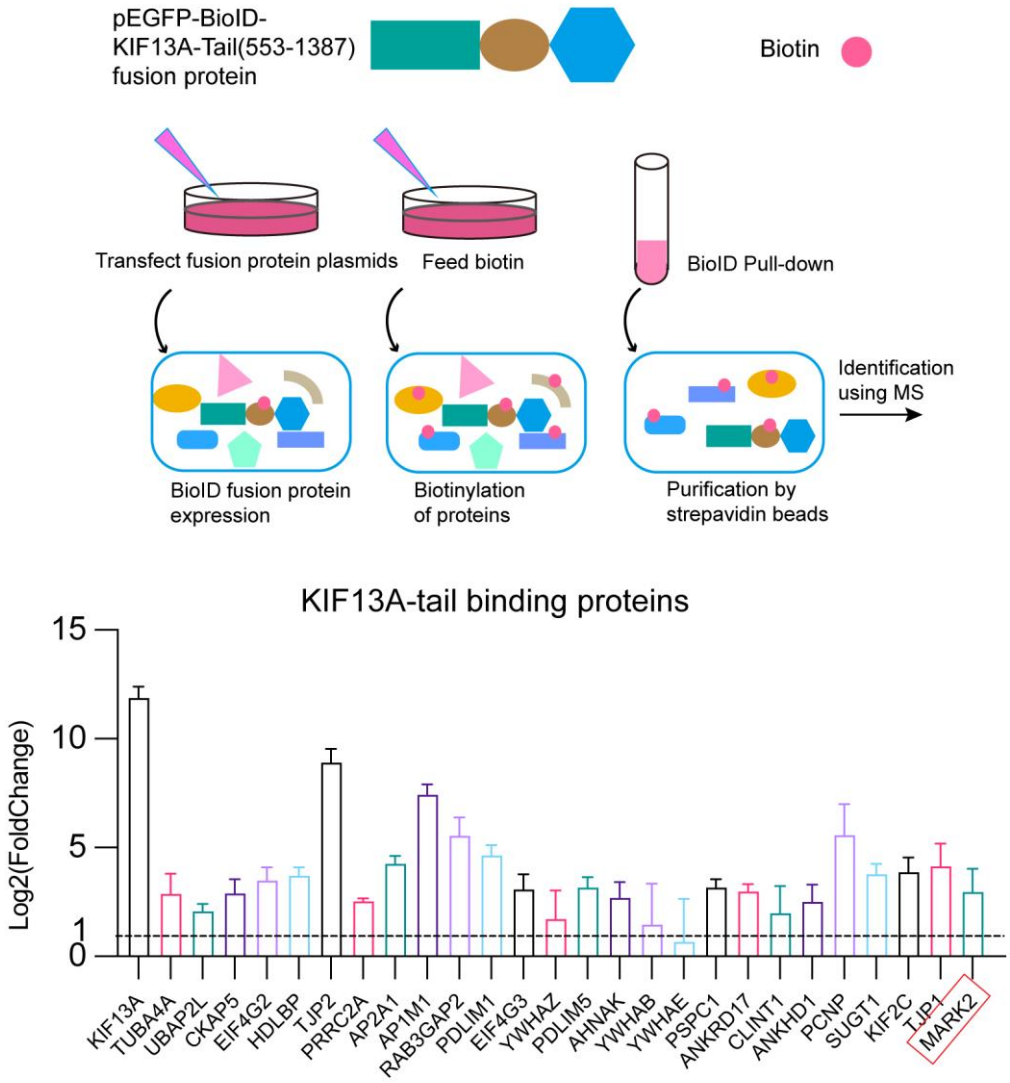

Figure S3. KIF13A tail-BioID-based proximity biotinylated proteins screening

(A) Top: Schematic of GFP-BioID-KIF13A Tail fusion plasmid transfection and biotin labeled proteins purification. KIF13A proteins would be labeled with biotin. Proteins was purified with streptavidin beads and used for mass spectrometry analysis. Bottom: Candidate KIF13A proteins screening according to the results of mass spectrometry,

the ratio (Log2) of abundance (area) of interest proteins in GFP-BioID-KIF13A and GFP-BioID expressed 293T cells were shown. Which confirmed mark2 as a potential kinase of KIF13A involved in the phosphorylation at site 1371.

S4A

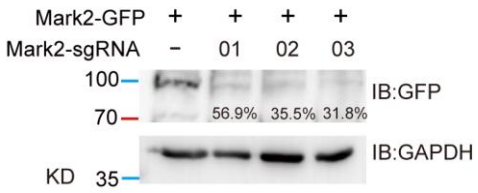

Figure S4. Western Blot of Mark2-GFP after CRISPR-Cas9-sgRNA knockout in 293T cells.

(A) HEK293T cells were co-transfected with MARK2-GFP and CRISPR-Cas9 containing the small guide RNA targeting MARK2 gene. The expression of MARK2 were detected with GFP.