Supporting Information

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SI Materials and Methods

Antibodies. Anti-PIPKα (-phosphatidylinositol 4-phosphate 5-kinase alpha), anti-PIPKβ, anti-PIPKγ, and anti-PI4K antibodies were purchased from Santa Cruz Biotechnology, whereas anti-PI3K, anti-tyrosinated tubulin (TUB-1A2), and anti-T7 tag mAbs were obtained from Millipore, Sigma-Aldrich Japan, and Merck, respectively. Anti–Rac-1 and anti-EB1 mAbs were purchased from BD Bioscience Pharmingen Japan. The anti-KIF2A (kinesin superfamily protein 2A) mAb has been described previously (1).

Immunoprecipitation, Western Blotting, and Mass Spectrometry. Postnatal day 7 ICR mouse cerebellum was homogenized in a buffer composed of 20 mM Tris·HCl (pH 8.0), 0.32 M sucrose, 2 mM EGTA, 2 mM $MgCl_2$, 150 mM potassium acetate, and a protease inhibitor mixture (GE Healthcare Japan). After successive centrifugations at 1,000 × *g* for 10 min and 10,000 × *g* for 15 min, the S2 fraction was incubated with anti-KIF2A antibody or normal mouse IgG1 and protein A-conjugated MACS beads (Miltenyi Biotec) for 1 h at 4 °C in the presence or absence of 1% Triton X-100. Fractions eluted by 1% SDS were analyzed by Western blotting. For mass spectrometric analysis, the eluted fraction corresponding to 30 μg of antibody or Ig was separated by SDS/PAGE. Bands were eluted from the gel, trypsinized, and loaded on a 4700 proteomic TOF/TOF analyzer (Applied Biosystems). MS/MS spectra were analyzed with the MASCOT search database (http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=MIS).

Expression and Purification of Recombinant KIF2A, PIPKα, and PIPKγ. A human KIF2A clone (IMAGE clone no. 4932771) was obtained from Thermo Scientific. Mouse PIPKα and PIPKγ clones were obtained from the RIKEN fantom cDNA library (DNAFORM). The ORFs of KIF2A, PIPKα, and PIPKγ were amplified by PCR using KOD plus (TOYOBO) and ligated into pGEX4T-1 vector (GE Healthcare Japan) in the case of GST-fusion proteins, or pET21a (Merck) in the case of His tag-fusion proteins. The vector was transformed into BL21 codon plus RIL (Stratagene) in the case of pGEX vectors, or Rosetta (DE3) (Merck Japan) in the case of pET21a vectors. One colony was picked up and cultured in 2 mL of LB medium supplemented with carbenicillin (Wako) at 37 °C overnight. The next morning, the medium was transferred to 1,000 mL of 2xYT medium and cultured in a gyratory shaker (New Brunswick Scientific). When OD_{600} of cultured medium reached 0.6, the temperature was reduced to 18 °C and protein expression was induced by 0.5 mM isopropyl-β-D-thiogalactopyranoside (Wako). After 48 h of culture, cells were harvested by centrifugation and stocked at −80 °C until the purification process.

For His tag-fused KIF2A, PIPKα, and PIPKγ purification, frozen cells were resuspended in TBS (10 mM Tris, pH 7.4, 150 mM NaCl) and lysed twice using a French press (Ohtake Works, Tokyo, Japan). Cell debris and inclusion bodies were pelleted by ultracentrifugation at 35,000 rpm for 30 min at 4 °C using an XL100K ultracentrifuge and a type 45 Ti rotor (Beckman Coulter). TALON Co^{2+} beads (Takara Bio) were added to the lysate and incubated for 1 h. Then, beads were washed with TBS three times. His tag-fused proteins were eluted with TBS supplemented with 1 M imidazole (Nacalai Tesque). After the elution, imidazole was removed by PD-10 columns (GE Healthcare Japan). Fractions were tested and quantified by SDS/PAGE and Coomassie brilliant blue (CBB) staining using BSA as a standard.

For GST-PIPKα and -PIPKγ, frozen cells were resuspended in TBS (10 mM Tris, pH 7.4, 150 mM NaCl) and lysed as above. Glutathione Sepharose beads (GE Healthcare Japan) were added to the lysate and incubated for 3 h. Beads were washed with TBS three times and proteins were eluted with TBS supplemented with 20 mM glutathione.

For GST-KIF2A purification, cells were resuspended in HEM (10 mM Hepes, pH 7.4, 1 mM EGTA, 1 mM $MgCl_2$) supplemented with 150 mM KCl. Lysate was prepared as described above. Glutathione Sepharose beads were added to the lysate and incubated for 3 h. Beads were washed three times with HEM supplemented with 100 mM KCl, and GST-KIF2A was eluted with elution buffer (100 mM Hepes, pH 7.4, 100 mM KCl, 20 mM glutathione). Buffer was changed to BRB80 (80 mM Pipes, pH 6.9, 1 mM MgCl, 1 mM EGTA) supplemented with 100 mM KCl using a PD-10 column. The eluted fraction was loaded on an SP Sepharose column (GE Healthcare Japan), washed three times with BRB80 supplemented with 100 mM KCl, and eluted by KCl gradient. Fractions were tested and quantified by SDS/PAGE and CBB staining using BSA as a standard. Peak fractions were divided into small aliquots in PCR tubes, frozen in liquid nitrogen, and kept at −80 °C.

Direct Binding Assays. GST-PIPKα and purified KIF2A-$(His)_6$ were prepared as described above. Fifty microliters of GST-KIF2A beads was resuspended in 1 mL of TBS and 10 μg of PIPKα was added. Beads were incubated for 1 h and washed with TBS three times. Beads were eluted by boiling in SDS sample buffer (62.5 mM Tris, pH 6.5, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, 0.002% bromophenol blue).

Yeast Two-Hybrid Assay. Three fragments of KIF2A corresponding to amino acids 1–193, 194–331, and 526–717 were inserted into the bait vector pGBKT7 (Takara Bio). PIPKα and PIPKγ were inserted into the prey vector pGADT7 (Takara Bio). Each pair of bait and prey vectors was cotransformed, and the interaction between the inserted fragments was measured using α-galactosidase (Takara Bio).

Immunofluorescent Microscopy. Primary cultured hippocampal neurons were cultivated on coverslips coated with polylysine. Three day in vitro (div) neurons were fixed in 4% paraformaldehyde, permeabilized by PBS supplemented with 0.1% Triton X-100 (Wako), and labeled with primary antibodies and then Alexa 488- or Alexa 568-conjugated secondary antibodies. We observed the neurons under an LSM 510 confocal microscope (Carl Zeiss).

Photoactivated Localization Microscopy. Anti-KIF2A antibody was directly labeled with Atto 488 NHS ester (Enzo Life Sciences). Unconjugated fluorophores were removed by dialysis. Cells were fixed and prepared for immunocytochemistry. Alexa 647-labeled secondary antibody was used to visualize PIPKα staining. Cells were observed in PBS supplemented with 10% sucrose (Wako), catalase (Sigma), glucose oxidase (Sigma), and 10 mM glutathione (Sigma). photoactivated localization microscopy (PALM) observation was conducted using the ELYRA P.1 prototype system (Carl Zeiss) as described (2). We used an Alpha Plan-Apochromat 100× N.A. 1.46 (Carl Zeiss) objective lens. In PALM, each signal (dot) represents each molecule theoretically. As both KIF2A and PIPKα signals (dots) showed clustered patterns, we considered each cluster as a unit and calculated the ratio of colocalization.

Preparation of Tubulin and GMPCPP-Stabilized Microtubules. Cycled tubulin was purified from porcine brains as described before (3). Tubulin was mixed with ice-cold BRB80 (final tubulin concentration: 1 mg/mL, 0.1 μM) and clarified by ultracentrifugation for

5 min at 2 °C in a TLA110 rotor (Beckman Coulter). The supernatant was incubated at 37 °C for 30 min in the presence of 0.5 mM guanosine 5′-[(α,β)-methileno] triphosphate (GMPCPP) (Jena Biosciences) and 1 mM DTT. Microtubules (MTs) were pelleted for 5 min in a TLA120.2 rotor (Beckman Coulter) at 22 °C and resuspended in BRB80, 50 mM KCl, 1 mM DTT, and 2 mM MgATP. Tubulin labeling by tetramethylrhodamine (TMR) was performed as described (4). For TMR labeling of MTs, 0.01 μM TMR-tubulin was added to the above reaction.

MT-Depolymerizing Assays. MT-depolymerizing assays were performed using a previously described method (5, 6). The amounts of PIPKα were estimated by quantification of the corresponding nondegraded bands, because these fractions contained constant amounts of degraded bands of PIPKα. Depolymerization assays were performed using GMPCPP-stabilized MTs. Assays were performed in BRB80 supplemented with 50 mM KCl, 1 mM DTT, and 2 mM MgATP. To determine the EC_{50}, 0–1,000 nM GST-KIF2A was incubated with 1 μM MTs (equivalent to 0.1 mg/mL MTs) in the presence of 100 nM BSA, PIPKα, or PIPKγ. Protein mixtures were reacted for 15 min at 22 °C and centrifuged in a Beckman TLA 120.2 rotor for 5 min at 90,000 rpm at 22 °C. The pellet was resuspended in BRB80 buffer. The supernatant and resuspended pellet were analyzed by SDS/PAGE and CBB staining. The data were plotted and fit to the four-parameter logistic equation (Eq. **S1**) and the EC_{50} was calculated using KaleidaGraph 4.0 software (Synergy), where Response is the amount of tubulin in the supernatant fraction, R_{min} is the baseline, R_{max} is the maximal response, X is the enzyme concentration, and H is the Hill slope:

$$\text{Response} = R_{min} + (R_{max} - R_{min})/[1 + 10^{H*\log(EC50/X)}]. \quad \textbf{[S1]}$$

Transfection. When transfection was conducted when cells were plated (Fig. 4), vectors were electroporated with Neon as described in the manufacturer's protocol (Invitrogen). In other experiments, hippocampal neurons were transfected by the high-efficiency Ca^{2+}-phosphate method at 2 div as described (7). Ca^{2+}-phosphate reagent was obtained from Takara Clontech. N1E-115 cells were transfected with Lipofectamine 2000 (Invitrogen).

MT Observation. Human EB3 was amplified by PCR using an IMAGE clone encoding full-length human EB3 (IMAGE clone no. 4561018) as a template. The PCR product was inserted into pEGFP-N1 vector (Takara Clontech). Vectors were prepared by an Endo-free Plasmid Maxi Kit (Qiagen). Cells were transfected as described above and incubated for 24 h. Time-lapse observation was conducted using an LSM 710 confocal microscope system (Carl Zeiss) equipped with αPlan Fluor (100×, N.A. 1.45) as described (8).

1. Noda Y, Sato-Yoshitake R, Kondo S, Nangaku M, Hirokawa N (1995) KIF2 is a new microtubule-based anterograde motor that transports membranous organelles distinct from those carried by kinesin heavy chain or KIF3A/B. *J Cell Biol* 129(1):157–167.
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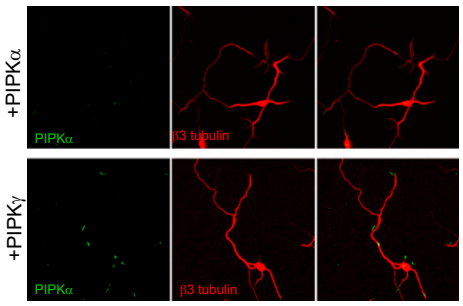


Fig. S1. Blocking of PIPKα staining by purified PIPKs. Cells were fixed and incubated with anti-β3 tubulin antibody, anti-PIPKα antibody, and either purified PIPKα or PIPKγ. Then, secondary antibodies were incubated.

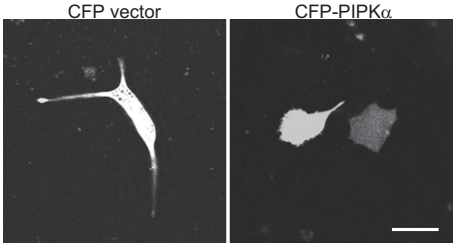


Fig. S2. Comparison of CFP- and CFP-PIPKα–overexpressing cells. Undifferentiated N1E-115 cells were transfected with GFP and either CFP or CFP-PIPKα and differentiated for 24 h. Cell shapes were observed by GFP signals. Note that differentiation was inhibited in PIPKα-expressing cells. (Scale bar, 50 μm.)

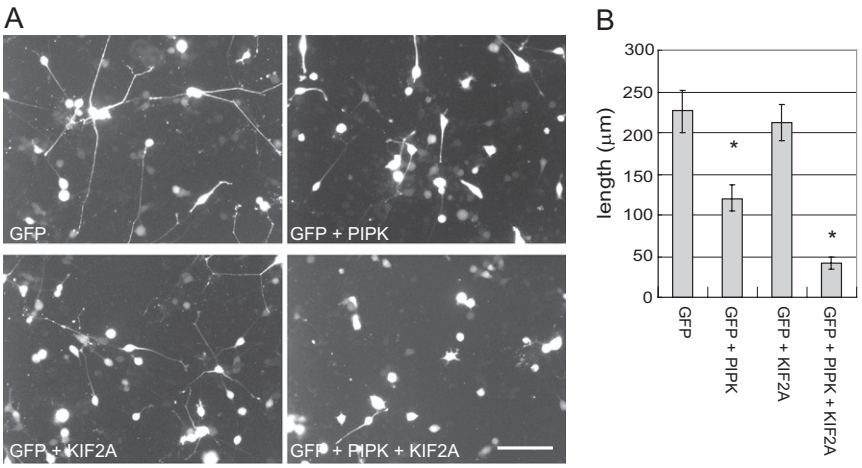
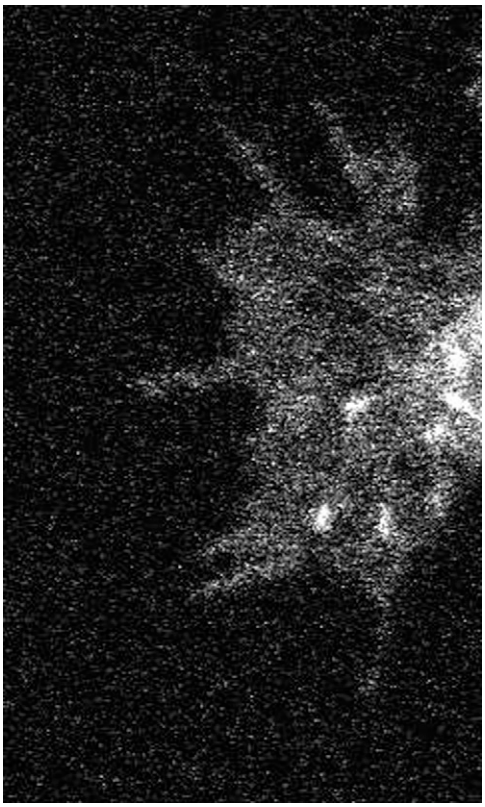


Fig. S3. The cooperative effect of PIPKα and KIF2A. (*A*) Representative images of control cells, PIPKα-overexpressing cells, KIF2A-overexpressing cells, or both PIPKα- and KIF2A-overexpressing cells. (Scale bar, 200 μm.) (*B*) Statistical analysis of *A*. Overexpression of KIF2A only did not significantly change neurite length compared with that in control cells; overexpression of both PIPKα and KIF2A affected the neurites more significantly than overexpression of PIPKα only. Data are shown as mean ± SEM; $*P < 0.01$, Student's *t* test, compared with control; $n = 50$ from five independent samples.

Movie S1. Dynamics of plus end of MTs in a growth cone of WT cells. Hippocampal neurons were transfected with EB3-GFP. Images were analyzed by time-lapse microscopy using a confocal microscope (LSM; Carl Zeiss) equipped with a 100× objective lens (N.A. 1.45). Frames were taken every 3 s for 3 min with 3 s of exposure. The movie is repeated twice.

Movie S1

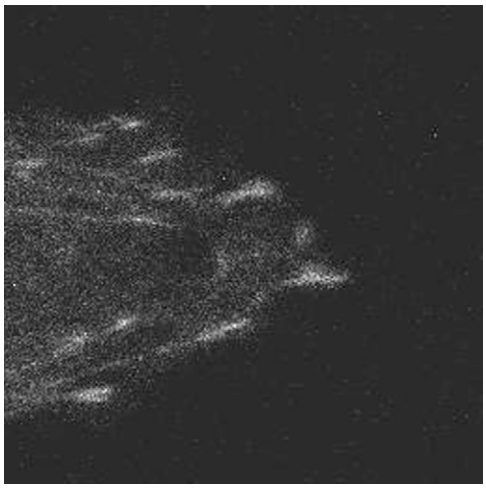


Movie S2. Dynamics of plus end of MTs in a growth cone of PIPKα-overexpressing cells. Hippocampal neurons were cotransfected with both CFP-PIPKα and EB3-GFP, and GFP signal was observed. Images were analyzed by time-lapse microscopy using a confocal microscope (LSM; Carl Zeiss) equipped with a 100× objective lens (N.A. 1.45). Frames were taken every 3 s for 3 min with 3 s of exposure. The movie is repeated twice.

Movie S2

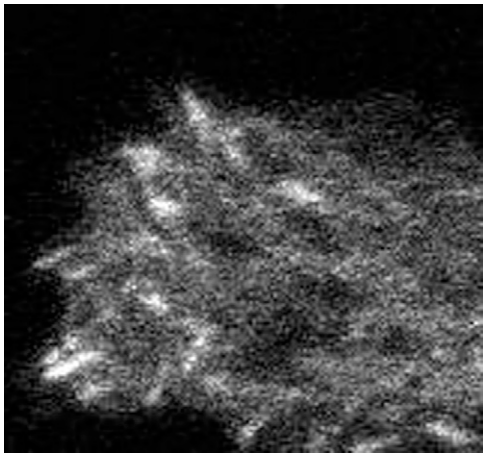
Movie S3. Dynamics of plus end of MTs in a growth cone of PIPKα-knockdown cells. Hippocampal neurons were cotransfected with both PIPKα-miRNA and EB3-GFP, and GFP signal was observed. Images were analyzed by time-lapse microscopy using a confocal microscope (LSM; Carl Zeiss) equipped with a 100× objective lens (N.A. 1.45). Frames were taken every 3 s for 3 min with 3 s of exposure. The movie is repeated twice.

Movie S3



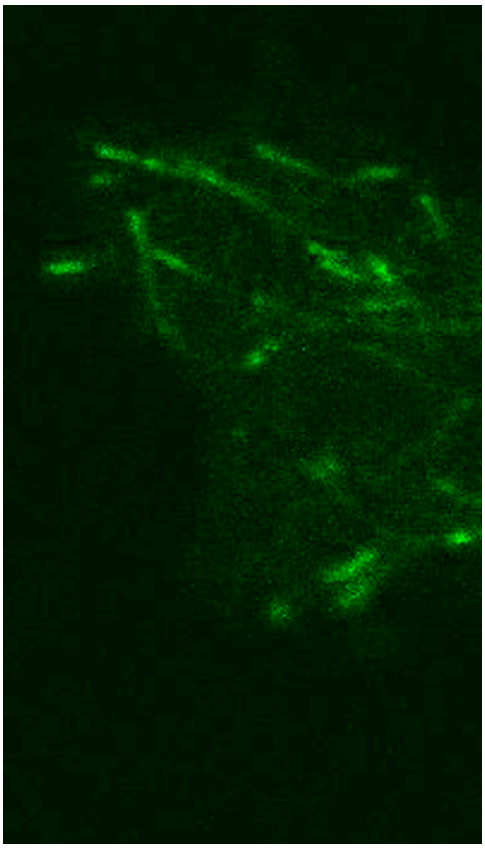
Movie S4. Dynamics of plus end of MTs in a growth cone of *Kif2a*$^{-/-}$ cells. Hippocampal neurons derived from *Kif2a*$^{-/-}$ mice were transfected with EB3-GFP. Images were analyzed by time-lapse microscopy using a confocal microscope (LSM; Carl Zeiss) equipped with a 100× objective lens (N.A. 1.45). Frames were taken every 3 s for 3 min with 3 s of exposure. The movie is repeated twice.

Movie S4



Movie S5. Dynamics of plus end of MTs in a growth cone of PIPKα-overexpressing *Kif2a*$^{-/-}$ cells. Hippocampal neurons derived from *Kif2a*$^{-/-}$ mice were cotransfected with ECFP-PIPKα and EB3-GFP, and GFP signal was observed. Images were analyzed by time-lapse microscopy using a confocal microscope (LSM; Carl Zeiss) equipped with a 100× objective lens (N.A. 1.45). Frames were taken every 3 s for 3 min with 3 s of exposure. The movie is repeated twice.

Movie S5



Movie S6. Dynamics of plus end of MTs in a growth cone of PIPKα-knockdown *Kif2a*$^{-/-}$ cells. Hippocampal neurons derived from *Kif2a*$^{-/-}$ mice were transfected with PIPKα-miRNA and EB3-GFP, and GFP signal was observed. Images were analyzed by time-lapse microscopy using a confocal microscope (LSM; Carl Zeiss) equipped with a 100× objective lens (N.A. 1.45). Frames were taken every 3 s for 3 min with 3 s of exposure. The movie is repeated twice.

Movie S6