Supplementary Materials and methods

Animal care

All procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, were approved by the Institutional Safety Committee on Recombinant DNA Experiments and the Animal Research Committee of Osaka Bioscience Institute, and by the Recombinant DNA and Animal Experimental Committees of Institute for Protein Research, Osaka University, and were performed in compliance with the Institutional guidelines. Mice were housed in a temperature-controlled room at 22 °C with a 12 h light/dark cycle. Fresh water and rodent diet were available at all times.

In situ hybridization

In situ hybridization was performed as described previously (Muranishi *et al.*, 2011). Digoxigenin-labeled riboprobes for mouse *ICK* were generated by *in vitro* transcription using 11-digoxigenin UTPs (Roche). An *ICK* cDNA fragment for an *in situ* hybridization probe was obtained by PCR using the RIKEN full-length enriched library clone (E130304P20, Genbank accession number AK087484) as a template. The following primers were used: 5'-AAAAATCGATATGAATAGATACACAACGATC AAGC-3' (*ICK-ClaI-F*) and 5'-AAAAGCGGCCGCTCATGATGTAAGGAGGAGGG CCTGTCT-3' (*ICK-NotI-R2*).

Northern blot analysis

Northern blot analysis was performed as described previously (Sanuki *et al.*, 2011).

Total RNAs were extracted from the mouse brain at several developmental stages. Ten micrograms of total RNA were electrophoresed on a 1.0% agarose formaldehyde gel and transferred to a nylon membrane (Pall). The *ICK* cDNA fragment for the *in situ* hybridization probe was used to synthesize radiolabeled probes.

Immunohistochemistry

For immunostaining of the retina, mouse eyes were fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) from 15 s to overnight. For immunostaining of brain sections, brains were fixed by immersion (P4 brains) in 4% PFA in PBS at 4 °C for 16 h or by perfusion (adult brains) in the same fixative. Adult brains were postfixed overnight at 4 °C. For immunostaining of ependymal cilia, brains were not fixed. For immunostaining of E10.5 or E15.5 tissues, embryos were fixed in 4% PFA in PBS for 1h at 4 °C. The tissues were then rinsed in PBS, cryoprotected with 30% sucrose in PBS, embedded in TissueTec OCT compound 4583 (Sakura), frozen, and sectioned. Frozen 20 μm sections on slides were dried for 30 min at room temperature, rehydrated in PBS for 5 min, incubated with blocking buffer (5% normal goat serum, and 0.5% Triton X-100 in PBS) for 1 h, and then with primary antibodies for 4 h at room temperature. Slides were washed with PBS three times for 10 min each time and incubated with secondary antibodies for 2 h at room temperature. The specimens were observed under a laser confocal microscope (LSM700, Carl Zeiss). Zebrafish larvae were fixed in 4% PFA in PBS for 1 h. Whole mount immunostaining of zebrafish larvae was performed using an anti-acetylated α-tubulin antibody (1:2000), phalloidin (1:250), and DAPI as described previously (Omori *et al.*, 2008).

Scanning electron microscopy

For observation of neural tube cilia, samples were washed with PBS, and fixed 1 h on ice with 1% glutaraldehyde in PBS. They were then incubated for 30 min on ice with 1% osmium tetroxide in PBS, dehydrated with ethanol, freeze-dried in t-butyl octanol, and exposed to osmium tetroxide before observation with a scanning electron microscope (S4800, Hitachi).

Immunohistochemical analysis of cilia in the neural tube

Double staining of cilia in the neural tube by antibodies against IFT88 and Arl13b was performed using Zenon Labeling Kit (Lifetechnologies). Each of approximately 1 μg of antibody was labeled with different fluorochromes according to the manufacturer's protocol and used for immunostaining.

Cell culture and transfection

NIH3T3 cells were cultured in DMEM (Sigma) with 10% fetal calf serum and 2 mg/L L-glutamine. Wild-type and *ICK*$^{-/-}$ primary mouse embryonic fibroblasts (MEFs) were generated from embryonic day 13.5 embryos and cultured in DMEM with 10% fetal bovine serum and 2 mg/L L-glutamine. To induce ciliogenesis in MEFs, cells were grown to 80-90% confluency and serum-starved for 48 h. Transfection was performed using Lipofectamine-LTX (Invitrogen) for both NIH3T3 cells and MEFs according to the manufacturer’s instructions. After 24 h, the medium was replaced by serum-free medium. Cells were cultured for from 24 h to 72 h in serum-free medium to induce cilia. For immunostaining, cells were washed with PBS, fixed with 4% PFA in PBS for 5 min at room temperature, and subsequently incubated with blocking buffer for 30

min. Cells were immunostained with a primary antibody in the blocking buffer for 4 h at room temperature and subsequently incubated with the secondary antibody solution for 2 h at room temperature.

***In vitro* kinase assay**

The fusion protein was expressed in *Escherichia coli* strain *DH5a* and purified using glutathione Sepharose 4B (GE healthcare) as previously described (Omori *et al.*, 2010). Purified recombinant GST-tagged full-length human ICK (Abnova) was used for the kinase assay. The kinase reaction was performed with kinase buffer (50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 5 mM DTT, 20 μM ATP, 10 μCi of [γ-^{32}P]ATP) supplemented with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Roche) at 30 °C for 60 min. Reaction products were resolved by SDS-PAGE. The gels were dried and exposed to x-ray films.

Injection of antisense morpholino and *in vitro* synthesized mRNA into zebrafish embryos

The breeding and maintenance of zebrafish strains and staging of embryonic development were performed as described previously (Kimmel *et al.*, 1995; Malicki *et al.*, 2002). To rescue morphant phenotypes, Kif3a-WT, Kif3a-CA, and Kif3a-dC were cloned into the *pXT7* vector and transcribed using mMessage mMachine kit (Ambion), according to manufacturer's instructions. 1.0 ng of morpholino and 100 pg of RNA were injected into embryos at the one-cell stage. The morpholino sequence is as follows; Kif3a-ATG, GTTTGTCCAGCTTATTGCTCGGCAT.

Nissl staining

Coronal or sagittal sections from frozen mouse brains were fixed in 4% PFA in PBS for 10 min. After washing in de-ionized water, sections were stained by 0.1% (w/v) cresyl violet for 5 min, washed in 100% ethanol, and incubated in xylene. Slides were coverslipped with Permount (Fisher Scientific).

Antibody production

A cDNA fragment encoding a middle portion of mouse ICK (residues 346-412) was amplified by PCR (Primers: forward 5'-AAAGAATTCCACCCCTACAAAGGCGATG TCTCT-3', reverse 5'-AAAGCGGCCGCTCAAGAACCCTTTGTCGACCTGGAGAT -3'), and subcloned into EcoRI and NotI sites of *pGEX4T-1* (GE Healthcare). The glutathione S-transferase (GST)-tagged fusion protein of ICK was expressed in *Escherichia coli* strain *DH5α* and purified with glutathione sepharose 4B (GE Healthcare). An antiserum against ICK was raised by immunizing guinea pigs with the purified fusion protein (MBL, Nagano, Japan). Then, the antisera were reacted with the fusion protein-coupled CNBr-activated Sepharose 4B (GE healthcare). This sepharose 4B was washed with PBS and eluted with 0.1 M glycine buffer (pH 2.5) to obtain a purified antibody against ICK. The eluted antibody was neutralized by 20 x PBS, and further dialyzed in PBS at 4 °C. An antibody against phosphorylated ($pThr^{674}$) Kif3a was obtained by immunizing rabbits with a phosphorylated peptide ERPRpTSKGKA (MBL, Nagano, Japan).

Staining of the bone and cartilage

Euthanized mice were skinned, eviscerated, and fixed in 95% ethanol for 3-5 days. All specimens were then stained with 0.15% Alcian Blue (Sigma A 3157) dissolved in glacial acetic acid plus 75% ethanol and 0.5% Alizarin Red (Sigma A 5533) dissolved in 2% KOH.

Quantitative real-time PCR (Q-PCR)

Q-PCR was performed using SYBR Green ER Q-PCR Super Mix (Invitrogen) and Thermal Cycler Dice Real Time System Single MRQ TP870 (Takara) according to the manufacturer's protocols. Quantification was performed by Thermal Cycler Dice Real Time System software Ver. 2.0 (Takara). Primer sequences are as follows (Wen *et al.*, 2010) (Omori *et al.*, 2010): for *ICK*, forward (F), 5'-TGGGAAGAATGCATGAACCT TCG-3' (*ICK-Q3*), and reverse (R), 5'-ATGGTCGTTTTCCCTGATAACCTC-3' (*ICK-Q4*); *Gli1*, F, 5'-GCAGTGGGTAACATGAGTGTCT-3 (*Gli1-Q3*), and R, 5'-AG GCACTAGAGTTGAGGAATTGT-3' (*Gli1-Q4*); *Mak*, F, 5'-TATCCAGATGGTGTG CAGAAGAGCC-3′ (*Mak-Q2*) and R, 5'-GAGATGCTGAACTGGGATCCAAAG-3′ (*Mak-P5*); *Ccrk*, F, 5'-CTGGAGGATGGTATTCCT AACCAG-3' (*Ccrk-Q1*), and R, 5'-GGTCCGACAGCATAAATTCGAAAG-3' (*Ccrk-Q2*); *GAPDH*, F, 5'-ACTGGC ATGGCCTTCCGTGTTCCTA-3' (*GAPDH-P1*), and R, 5'-TCAGTGTAGCCCAAGA TGCCCTTC-3' (*GAPDH -P2*).

Western blot analysis

MEFs were washed by PBS twice and lysed in a SDS-sample buffer. P0 retinas and P4 brains were lysed in a lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 1 mM EDTA) supplemented with protease inhibitors (1 mM PMSF, 2

μg/ml leupeptin, 5 μg/ml aprotinin, and 3 μg/ml pepstatin A). Samples were resolved by SDS-PAGE and transferred to PVDF membranes. We used the following primary antibodies: mouse monoclonal anti-FLAG-M2 (Sigma, F1804, 1:6000) and anti-α-tubulin (Cell Signaling, DM1A, 1:6000); rabbit polyclonal anti-GFP (MBL, 598, 1:5000); guinea pig polyclonal anti-ICK (1:250) antibodies.

Supplementary References

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