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Supplementary Information for

A complex of distal appendage-associated kinases linked to human disease regulates ciliary trafficking and stability

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Supplementary Information Text

Materials and methods

Generation of *Csnk2A1* knockout cell lines

To deliver sgRNAs into cells, we produced lentiviruses using pLentiCrisprV2 plasmid (52961, Addgene) (1). This lentiviral plasmid briefly includes a U6 promoter controlling the expression of the cloned sgRNA followed by an EF-1α core promotor promoting the expression of Cas9. For each sgRNA, primers were designed, annealed, and inserted in the BsmBI site. For pLentiCrisprV2-non-targeting gRNA, the sgRNA sequence was 5'-ACCCATCGGGTGCGATATGG-3'. The CSNK2A1 sgRNA 5'-GGATTATGAATCACATGTGG-3' was utilized to generate the pLentiCrisprV2-*Csnk2a1* sgRNA. The reference sequence of the mouse *Csnk2a1* cDNA was found in NCBI (NM_007788.3). Wildtype and *Ttbk2*$^{null/gt}$ MEFs were transduced with control or *Csnk2a1* sgRNA lentiviruses. At 24 hours post-infection, cells were selected with 5 µg/ml puromycin-containing media. Clonal cell lines were generated by limiting dilution. Clones were picked and grown in 24-well plates. For each clone, we prepared a total cell lysate for western blot analysis. We only retained *Csnk2a1*-depleted clonal cell lines that showed a complete knockout. We then identified by sequencing the genomic mutations expected to occur within the 5' region of *Csnk2a1* gene. Genomic DNA was purified for each of the cell lines. We used the following primers to amplify the 5' sequence of Csnk2a1 gene: for *Csnk2a1* KO cell lines in WT Mefs, the forward primer is 5'- ccaagcagggccagagtttac -3' and the reverse is 5'- CATGGCCTCAGCTTCTCAGATTTC -3'. The extremities of the amplified sequence were flanked by EcorI/KpnI and inserted into pEGFP-N3. For *Csnk2a1* KO cell line *Ttbk2*$^{null/gt}$ MEFs, the forward primer is: 5'- ACCTTGAAATCCTGGGCCAC -3' and the reverse is 5'- CAGCAGCCTACTTTCCCTTCAA -3'. The extremities of the amplified sequence were flanked by EcorI and inserted into pEGFP-N3. XL1-Blue Competent Cells were transformed and individual colonies were sequenced by Sanger sequencing using a universal CMV primer 5'- CGCAAATGGGCGGTAGGCGTG- 3'. Sequences were aligned to chromosome 2 sequence and *Csnk2a1* gene using SnapGene 3.2.1. Two distinct mutations were identified from each KO cell line. All observed indels disrupt the *Csnk2a1* gene's reading frame leading to a premature stop codon (**Fig S3A, B**).

Generation of *Csnk2a1*-knockdown transgenic ARL13B-mCherry MEFs

Similar to the generation of knockout cell lines of *Csnk2a1*, we delivered control or *Csnk2a1* sgRNAs into cells with lentiviruses using pLentiCrisprV2 plasmid (52961, Addgene) (1). The sgRNA sequences and cloning were the same as previously described. MEFs that were isolated from a transgenic mouse model expressing endogenous ARL13B-mCherry (2) were transduced with control or *Csnk2a1* sgRNA lentiviruses. At 24 hours post-infection, cells were selected with 5 µg/ml puromycin-containing media for 10 days. We confirmed by immunofluorescence a 60% depletion for CSNK2A1. These cells were used for live imaging to characterize the ciliary tip breaks.

BioID of TTBK2

Generation of stable cell lines for biotin proximity labeling

HEK293T cells stably expressing a single copy of N-terminally tagged TTBK2- or GFP-BirA* were generated using Flp-IN T-REx cells (ThermoFisher K6500-01), Positive clones were selected with hygromycin resistance, and maintained in media with tetracycline-free FBS. For the proximity labeling experiments, cells were cultured in serum-free media for 48 hours to induce ciliogenesis, with tetracycline (1µg/ml) added to induce expression of the BirA* fusion constructs. Biotin (50µM) was then added to the cultures for an additional 24 hours to induce labeling. Cell were lysed in 50mM Tris pH 7.5, 100mM NaCl, 0.5% NP-40, and 10% glycerol, and briefly sonicated. Cleared lysates were incubated with washed Neutravidin beads, 4°C rotating, for 4 hours. Beads were then washed in 50mM ammonium bicarbonate (pH8.3) and submitted to the Duke University Proteomics and Metabolomics Core Facility for further processing and Mass spectrometry.

Sample Preparation

6 samples were submitted to the Duke Proteomics Core Facility (DPCF) (3 replicates of both TTBK2 and GFP). Samples were supplemented with 40µL 10% SDS in 50 mM TEAB, then reduced with 10 mM dithiolthreitol for 30min at 80C and alkylated with 20 mM iodoacetamide for 30min at room temperature. Next, they were supplemented with a final concentration of 1.2% phosphoric acid and 683 µL of S-Trap (Protifi) binding buffer (90% MeOH/100mM TEAB). Proteins were trapped on the S-Trap, digested using 20 ng/µl sequencing grade trypsin (Promega) for 1 hr at 47C, and eluted using 50 mM TEAB, followed by 0.2% FA, and lastly using 50% ACN/0.2% FA. All samples were then lyophilized to dryness and resuspended in 12 µL 1%TFA/2% acetonitrile containing 12.5 fmol/µL yeast alcohol dehydrogenase (ADH_YEAST). From each sample, 3 µL was removed to create a QC Pool sample which was run periodically throughout the acquisition period.

Quantitative Analysis, Methods

Quantitative LC/MS/MS was performed on 4 µL of each sample, using a nanoAcquity UPLC system (Waters Corp) coupled to a Thermo QExactive HF-X high-resolution accurate mass tandem mass spectrometer (Thermo) via a nanoelectrospray ionization source. Briefly, the sample was first trapped on a Symmetry C18 20 mm × 180 µm trapping column (5µl/min at 99.9/0.1 v/v water/acetonitrile), after which the analytical separation was performed using a1.8 µm Acquity HSS T3 C18 75 µm × 250 mm column (Waters Corp.) with a 90-min linear gradient of 5 to 30% acetonitrile with 0.1% formic acid at a flow rate of 400 nanoliters/minute (nL/min) with a column temperature of 55C. Data collection on the QExactive HF mass spectrometer was performed in a data-dependent acquisition (DDA) mode of acquisition with a r=120,000 (@ m/z 200) full MS scan from m/z 375 – 1600 with a target AGC value of 3e6 ions followed by 30 MS/MS scans at r=15,000 (@ m/z 200) at a target AGC value of 5e4 ions and 45 ms. A 20s dynamic exclusion was employed to increase depth of coverage. The total analysis cycle time for each sample injection was approximately 2 hours. Following 8 total UPLC-MS/MS analyses, data was imported into Proteome Discoverer 2.2 (Thermo Scientific Inc.), and analyses were aligned based on the accurate mass and retention time of detected ions using Minora Feature Detector algorithm in Proteome Discoverer. Relative peptide abundance was calculated based on area-under-the-curve (AUC) of the selected ion chromatograms of the aligned features across all runs. The MS/MS data was searched against the SwissProt H. sapiens database (downloaded in Nov 2017) with additional proteins, including yeast ADH1, bovine serum albumin, as well as an equal number of reversed-sequence “decoys”) false discovery rate determination. Mascot Distiller and Mascot Server (v 2.5, Matrix Sciences) were utilized to produce fragment ion spectra and to perform the database searches. Database search parameters included fixed modification on Cys (carbamidomethyl) and variable modifications on Meth (oxidation) and Asn and Gln (deamidation). Peptide Validator and Protein FDR Validator nodes in Proteome Discoverer were used to annotate the data at a maximum 1% protein false discovery rate.

Kinome CRISPR-Cas9 suppressor screen of TTBK2

Lentiviral production

In this screen, we used a mouse pooled kinome CRISPR-Cas9 pooled library (#75316, Addgene) (3), which contains 2851 unique sgRNAs targeting 713 mouse kinase genes. It also includes 100 non-targeting sgRNAs, as an internal control.

The lentiviral library was produced in HEK293T cells, grown in 15cm plates. We transfected the following plasmids sgRNA-Cas9 pLentiCrisprV2 (the library constructs), psPAX2, and pCMV-VSVg (3:2:1, respectively) for 20h using polyethylenimine (7.5mM). The transfection medium was removed and replaced by growth media. 48h later, virus-containing supernatants were collected and filtered using 0.45 µm filters. The viral titer was evaluated by transducing cells with a range of viral dilutions. Cells were then selected with 5 µg/ml puromycin for 48h. We employed the CellTiter-Glo Luminescent Cell Viability Assay (Promega, G7570) to determine the infectious units per ml (IFU/ml).

Kinome library in SHH-BlastR MEFs
This screen is based on the response of the Sonic hedgehog pathway. We introduced the transcriptional SHH-BlastR reporter (pGL-8xGli-Bsd-T2A-GFP-Hyg, (4)) in WT and *Ttbk2*$^{null/gt}$ MEFs. The reporter was transfected in these cells that were selected and grown in media containing 200 µg/ml of hygromycin. The next step was to integrate the lentiviral library in SHH-BlastR MEFs. Cells were grown in 6-well plates and transduced at a multiplicity of infection of 0.2 in sufficient numbers such a manner that there was a 650:1 ratio of transduced cells per sgRNA. Cell infection was facilitated by centrifugation at 2200 rpm for 45 min at 25°C. The viral supernatant was incubated for 24h in the presence of 8 µg/ml polybrene. Cells were selected for 13 days in growth 10% FBS media with 200 µg/ml of hygromycin, and 5 µg/ml puromycin.

Kinome-Blasticidin reporter screening
SHH-BlastR MEFs were seeded in 15cm plates at a confluence of about 90%. For the screen, we maintained a 1700:1 ratio of cells to sgRNAs. Each experimental condition was achieved in duplicate for each cell line. Cells were serum-starved for 30h with or without 200nM SAG in 0.5% FBS media supplemented with 200 µg/ml of hygromycin, and 5 µg/ml puromycin. At this point, T0 cells were collected, and corresponding pellets were frozen at -20°C. The remaining plates were treated or not with 2.25 µg/ml blasticidin for 16h. Cells were then washed with 1x PBS, and growth media was added with or without 200nM of SAG. Media was changed every 2-3 days up until most cells in control plates (-SAG, +Blasticidin) stop cycling and die. WT and *Ttbk2*$^{null/gt}$ SHH-BlastR cell lines were collected at 4 days and 11 days, respectively, post-blasticidin treatment.

Sample preparation and Illumina sequencing
From the collected pellets, genomic DNA was extracted and purified using the DNeasy Blood and tissue kit (Qiagen). We amplified by PCR the integrated sgRNAs using P5/P7 primers, following the CRISPR library-related instructions (#75316, Addgene). All PCRs were pooled and sequenced by Illumina sequencing (Duke Center for Genomic and Computational Biology (GCB), Duke University).

Analysis of data from Illumina sequencing
Raw data from Illumina sequencing were converted to the number of reads for each individual sgRNA. A read was assigned to a specific target sequence if it matched the reference sequence with at most one mismatch (GCB, Duke University).

Analysis of sgRNA reads
All the counts per sgRNA were normalized with the total number of counts per condition using R studio. We then generated the ratio +blasticidin(+SAG): -Blasticidin(+SAG) for each sgRNA. Four sgRNAs represented each gene in the library. The average of the two median values for each gene was calculated to evaluate the gene depletion's overall effect on the SHH response.

Antibodies
Antibodies and their condition of use are detailed as follows: Anti-acetylated tubulin (Immunofluorescence (IF): 1:1000; Sigma, T6793); Anti-γ-Tubulin (IF: 1:500; Sigma, SAB4600239): Anti-Centrin2 (IF: 1:500; Millipore, 04-1624); Smoothened (IF: 1:500; Pocono Rabbit Farm and Laboratory, Inc.); Anti-ARL13B (IF:1/500, western blot (WB): 1/1000; NeuroMab, 11000053); Anti- CSNK2A1 (IF and western blot (WB): 1:500; Proteintech, 10992-1-AP); Anti-IFT88 (IF and WB: 1:500, Proteintech, 13967-1-AP); Anti- IFT81 (IF: 1:300, Proteintech, WB: 1:500, 11744-1-AP,); Anti- IFT140 (IF: 1:200, Proteintech, 17460-1-AP), ; Anti-Flotillin-1 (WB: 1:1000, ABclonal, A6220); Anti-KIF7 (IF: 1/500; a gift from Dr. Mu He, (5)); Anti-GLI2 (IF: 1:500, a gift from J. T. Eggenschwiler); Anti-GLI2 (WB: 1:500, Proteintech, 18989-1-AP); Anti-CEP164 (IF: 1:200, Sigma); Anti-FLAG M2 (IF: 1:75, WB: 1:4000, Sigma, F1804); Anti-GFP (IF: 1:500, WB: 1:1000, Proteintech, 66002-1-Ig); Anti-Vinculin (WB: 1/10000, Sigma, V4505); Anti-PLEKHO1 (IF: 1:100, Proteintech, 24883-1-AP); Anti-CAPZB (IF: 1:200, Proteintech, 25043-1-AP).

Lattice SIM
WT MEFs were plated on high precision type 1.5 coverslips and incubated in 0.5% serum media for 48h. Cells were fixed with cold methanol for 4.5 min. For a better signal, we incubated primary antibodies for 20h at 4°C. We used a combination of three secondary antibodies for each staining (IF: 1:500; Alexa Fluor 488, Alexa Fluor 568, Alexa Fluor 647; Molecular probes, Thermo Fisher Scientific). These were incubated for 2h at room temperature. Coverslips were then mounted on glass slides using ProLong Gold mounting media (Molecular probes, Thermo Fisher Scientific). SIM acquisitions were performed on a Zeiss Elyra 7 system equipped with lattice SIM technology. The signal was collected using a Zeiss Plan-Apochromat 63/1.4 Oil objective. The laser power and exposure times were kept at a minimum. In ZEN imaging software, the SIM mode, in combination with the tiling functionality, was used to process the acquired images.

STED
Cells were prepared similarly to the Lattice SIM protocol. Instead, with two-colored STED, we labeled cells with Alexa Fluor 594 (Thermo Fisher Scientific) and ATTO 647N Antibodies (Sigma). STED was performed on a Leica DMi8 motorized inverted microscope coupled with a STED depletion laser (775 nm) and a pulsed white light laser (470nm-670nm). A Leica 100x/1.4 HCX PL APO oil objective was used to image ciliary and centrosomal labeling. 3D z-stacks were sequentially collected for each fluorophore using high sensitivity GaAsP Hybrid Detectors (HyDs) with gating capabilities for STED. Images were displayed and analyzed on the LAS X software. An additional STED deconvolution step was implemented in Huygens deconvolution software, linked to the LAS X software, to reach the optimal resolution.

Immunofluorescence microscopy
Cells were plated on glass coverslips with an 80% confluence, followed by a serum starvation step of 48h to induce ciliogenesis. Fixation was carried out with 4% paraformaldehyde for 5 min and permeabilized with 100% cold methanol for 5 min. Fixed cells were blocked in blocking buffer (2% FBS, 0.1% Triton X-100 in PBS) for 1h and then incubated with primary antibodies for 3h at room temperature, followed by 2h incubation with secondary antibodies (1:500; Alexa Fluor 488, Alexa Fluor 568, Alexa Fluor 647; Molecular probes, Thermo Fisher Scientific). DNA was stained with DAPI for 1 min. Coverslips were mounted on glass slides using ProLong Gold mounting media (Molecular probes, Thermo Fisher Scientific). Cells were imaged with a Zeiss Axio Observer-Z1 widefield microscope equipped with a Zeiss Plan-Apochromat 63x / 1.4 Oil objective, an Axiocam 506 monochrome camera, and an Apotome setting. To accurately quantify the fluorescence intensities of ciliary markers, we acquired a Z-stack and created a maximum intensity projection for each image using Fiji software.

Ciliary vesicle purification
CRISPR-engineered cell lines and ttbk2^{null} MEFs were plated on 15cm dishes to reach a 90% confluence. Cells were serum-starved for 24h and subsequently washed 3 times with PBS to remove non-ciliary vesicles present in the media. Fresh 0.5% FBS media was added and incubated for 24h. Vesicles-containing supernatant was transferred to a 50ml conical tube. Cells were washed with 10ml PBS, then added to the same conical. We also prepared total cell lysates by lysing the cells directly in a 2X sample buffer. The conical tubes were centrifuged at 300 RCF for 10 min at 4°C. The supernatant was then transferred to a new 50ml conical and centrifuged for 20 min at 2000 RCF (4°C). The resulting supernatant was centrifuged for 40 min at 7500 RPM in an sw32 ti Rotor at 4°C. The supernatant was finally ultracentrifuged for 90 min at 25000 RPM in the same rotor at 4°C. The pellet was washed with 40 ml PBS and ultracentrifuged for 90min at 25000 RPM at 4°C. Most of the supernatant was then aspirated, and the remaining volumes were carefully equalized between samples. The sample buffer was finally added for western blotting analysis.

Western blot analysis
Cells were plated in a 6-well plate to reach a 90% confluence. Cells were directly lysed in 2x Laemmli sample buffer (50 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 1% 2-mercaptoethanol, 0.1% bromophenol blue) on ice. Whole-cell extracts were incubated at 95°C for 5 min to denature proteins. Total cellular proteins were separated by SDS-PAGE and transferred onto a PVDF

membrane. The membrane was saturated in 5% milk PBST (5% dry powdered milk, 0.1% Tween-20, 1X PBS) and incubated in primary antibodies followed by peroxidase-conjugated affinipure secondary antibodies. Membranes were developed with an ECL reagent kit (BioRad). Western blots were quantified using Fiji software ("Gels" command).

Pulldowns

In the HEK293T cells, we transfected the pDEST40-TTBK2-V5 construct with pEGFPN3 or pFLAP-DEST- CSNK2A1^{WT}-EGFP plasmids using polyethylenimine (7.5mM) for 16h. Transfection media was replaced with pre-warmed 10% serum media. We then serum-starved the cells for 72h in 0.5% serum media. Cells were collected on ice in 0.5% NP40 lysis buffer (50 mM tris-Hcl pH=8. 100 mM NaCl, 10% glycerol, EDTA-free protease inhibitor cocktail (Sigma), 10 µM of MG-132 (Sigma, M7449), 1mM EDTA, 10 mM N-Ethylmaleimide (Sigma, E3876) and 25 mM β-Glycerol phosphate). The cell lysate was incubated on ice for 10 min and then sonicated twice for 3 seconds with a 25% amplitude. We centrifuged the lysates for 20 min at 15000 rpm and recovered the supernatant. To pull down GFP-recombinant proteins, we incubated the GFP-Trap agarose beads (Chromotek, gta-10) with the total cell lysates for 3h at 4°C. These beads were previously blocked in 200 mg/ml BSA for 10 min at 4°C. The beads were then collected by centrifugation (2500 rpm, 1 min) and washed five times with cold lysis buffer. After adding the sample buffer, samples were resolved by western blot. The transfer onto PVDF membrane was carried out for 5h at 4°C and at a current power of 80 Volt.

Virus production and cell transduction

Lentiviruses were generated using a combination of three vectors. The sgRNA-Cas9 pLentiCrisprV2 construct, the packaging plasmid psPAX2, and the envelope plasmid pCMV-VSVg were transfected using polyethylenimine (7.5mM) in HEK293T cells for 20h. The transfection medium was removed and replaced with growth media. Virus-containing supernatant was collected 24h later and filtered through a 0.45 µm filter.

For the production of retroviruses, we transfected pDEST-FLAP constructs with pCMV-VSVg in Phoenix cells, which stably express packaging proteins of Moloney Murine Leukemia Virus. Cells were transduced by the addition of viral supernatant in the growth medium in the presence of 8 µg/ml of polybrene. Following 24h incubation, the viral medium was replaced by the appropriate antibiotic-containing growth medium (5 µg/ml of Puromycin, 500 µg/ml of Geneticin). The selection of infected cells was carried out for 10 days.

Supplemental figures

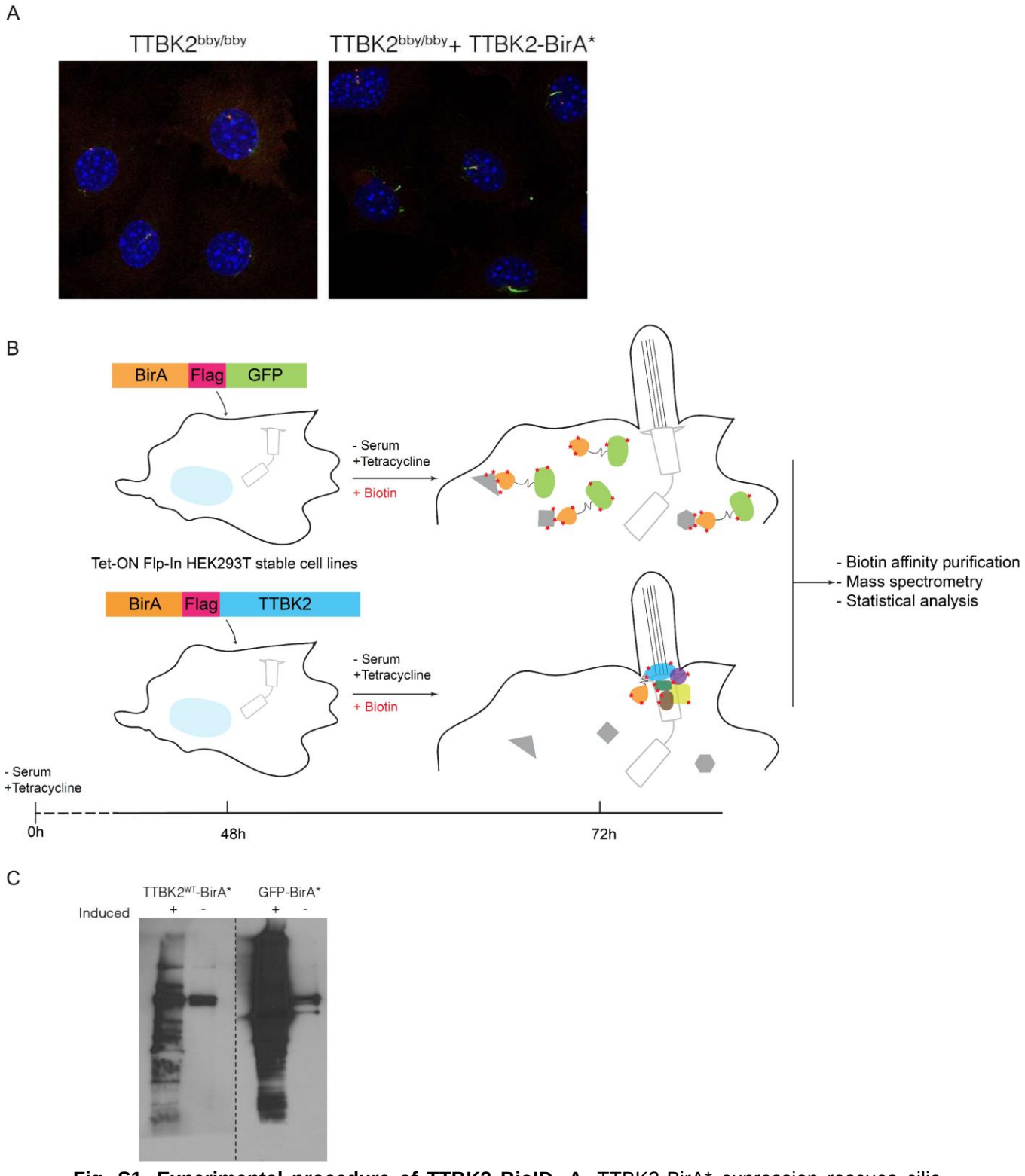
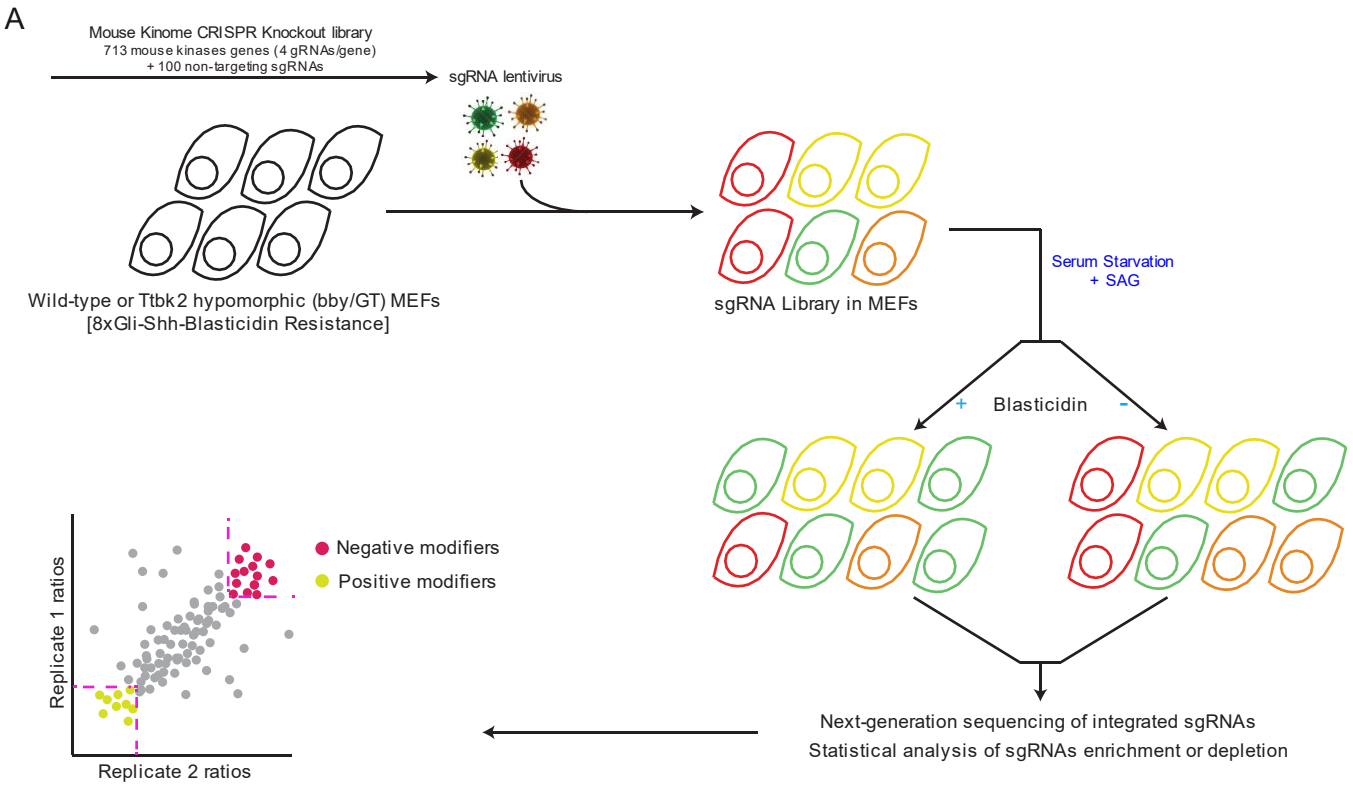


Fig. S1. Experimental procedure of TTBK2 BioID. A. TTBK2-BirA* expression rescues cilia defects in *Ttbk2*$^{bby/bby}$ MEFs (a null, ENU-induced allele of *Ttbk2*). Representative immunofluorescence of cilia labeled with ARL13B (green) and γ-tubulin (red). DNA was stained with DAPI. **B**. BirA*-GFP or BirA*-TTBK2 were introduced and stably expressed in Tet-ON Flp-In

HEK293T cells. While BirA-GFP was expressed throughout the cell, BirA*-TTBK2 localized to the basal body in ciliated cells. **C**. Purification of biotinylated proteins from inducible cell lines for GFP-BirA* or TTBK2^{WT}-BirA2*. Cells were incubated with biotin and induced or not with Tetracycline. Pulldowns were performed with streptavidin beads in whole-cell extracts. Dashed line marks where the western blot image was cut to remove lanes not relevant to this study.



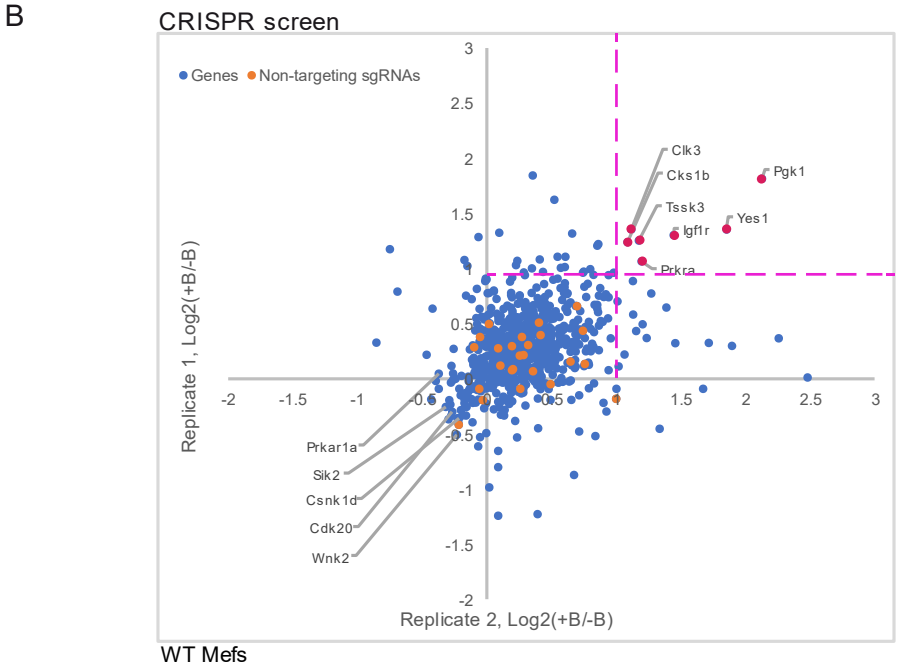


Fig. S2. Experimental procedure of the CRISPR-Cas9 screen and results from WT Mefs. **A**. Illustration of the experimental procedure of the kinome CRISPR-Cas9 screen of TTBK2. The detailed description is found in the “materials and methods” section. **B**. The CRISPR screen plot for WT MEFs, as in Fig 1B.

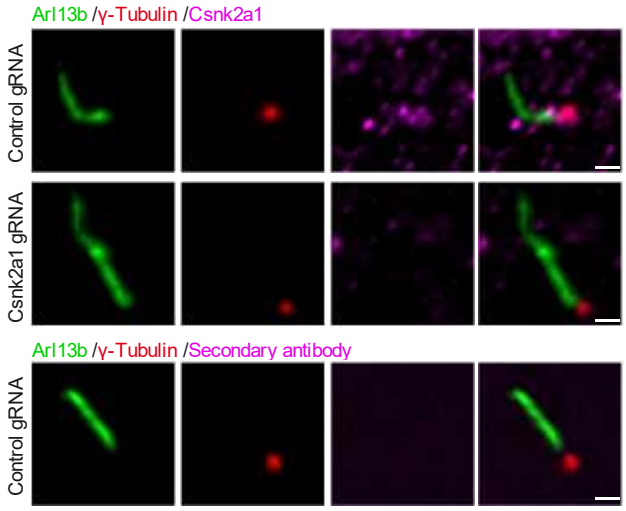


Fig. S3. CSNK2A1 antibody specifically labels endogenous CSNK2A1 at the centrosome. Representative immunofluorescence images, labeled with CSNK2A1 (magenta, upper panels) or only secondary antibodies (magenta, lower panel); co-labeled for ARL13B (green), γ-Tubulin (red), from control and *Csnk2a1* KO-1 cells. Scale bars: 1 µm.

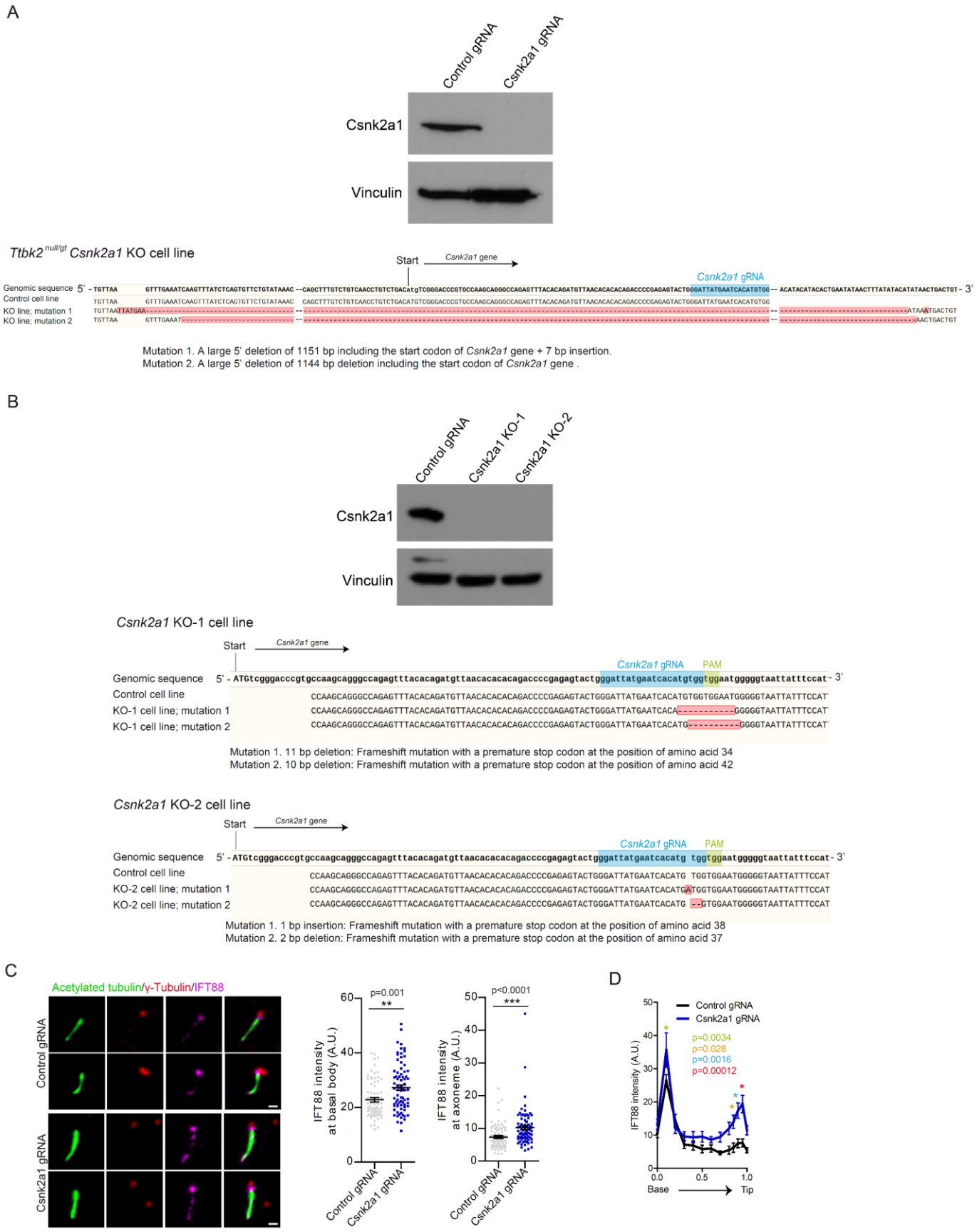


Fig. S4. Validation of *Csnk2a1* KO cell lines and IFT88 accumulation in mutant cilia.
A. Control or *Csnk2a1* gRNAs were used to generate clonal cell lines in *Ttbk2*$^{null/gt}$ MEFs. *Csnk2a1* depletion was evaluated in total cell lysates from control and *Csnk2a1* KO cells, using western blot analysis. Vinculin was used as a loading control. Alignment of genomic sequences of *Csnk2a1*

from control and KO cell lines. *Csnk2a1* gRNA, without the PAM sequence, is highlighted in cyan. Dashes highlighted in red display deletion mutations. **B**. Western blots labeled with antibodies to CSNK2A1 and VINCULIN, shown as a loading control. Lower panels display the alignment of *Csnk2a1* genomic sequences from control and both KO cell lines at the 5' flanking region of the gene. Dashes or nucleotides highlighted in red display respectively deletion and insertion mutations. **C**. IFT88 accumulates at the basal body and cilia in *Csnk2a1* KO-1 cells. Representative immunofluorescence images labeled with ARL13B (green), γ-tubulin (red), and IFT88 (magenta). Graphs show the mean intensity of IFT88 ± SEM at the basal body and the axoneme in control and *Csnk2a1* KO-1 cells. Statistical comparison was performed using the nonparametric Mann-Whitney test. P-values are shown on the graph. Basal body: control gRNA n=73 cells, *Csnk2a1* gRNA n=77 cells; Axoneme: control gRNA n=73 cells, *Csnk2a1* gRNA n=77 cells. Scale bars: 1 µm. **D**. IFT88 exhibits a distal accumulation in *Csnk2a1* KO-1 mutant cilia. The mean intensity of IFT88 ± SEM is profiled along the cilium. All cilia lengths were normalized to 1 and displayed on the X-axis. Statistical comparison was executed by multiple t-tests. P-values are shown on the graph. Control gRNA n=20 cells and *Csnk2a1* gRNA n=20 cells.

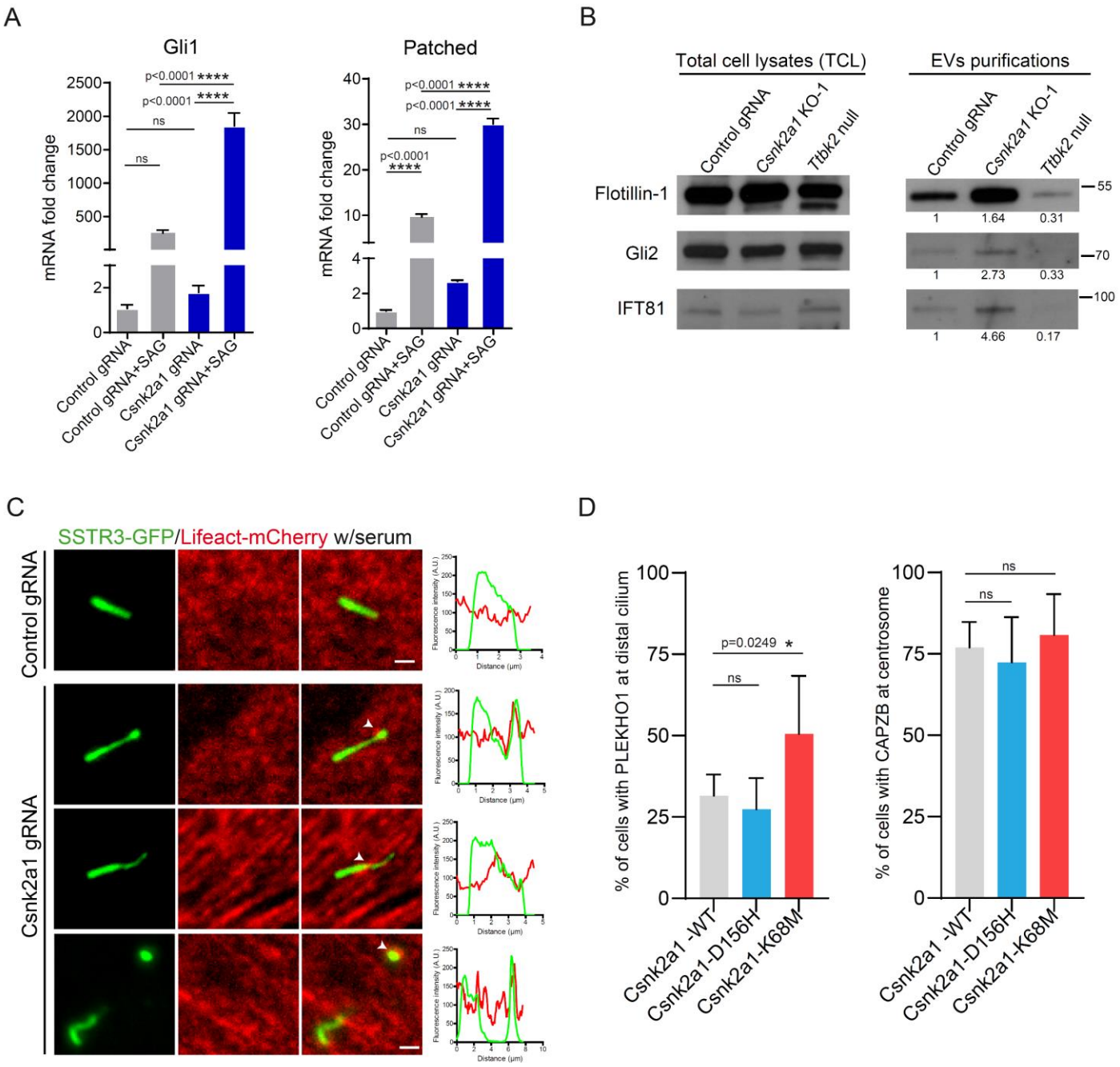


Fig. S5. *Csnk2a1*-knockout cells exhibit higher levels of SHH, EVs, and aberrant ciliary F-actin. A. Real-time quantitative PCR shows a significant increase in mRNA transcripts fold change ± SEM of *Gli1* and *Ptch* in *Csnk2a1* KO-1 cells. Graphs were produced from 3 replicates per condition from two independent experiments. Statistical comparison was performed by two-way ANOVA analysis with Tukey's multiple comparisons test. P-values are shown on the graph (ns: not significant). **B**. Western blots from total cell lysates (TCL, left blots) and EVs purifications (right blots) are probed with antibodies to FLOTILLIN-1, GLI2, and IFT81. Fold change values calculated as mentioned in Fig 5C. **C**. F-actin is enriched in mutant cilia during cilia disassembly. SSTR3-GFP and Lifeact-mCherry were transfected in control and *Csnk2a1* KO-1 cells. Serum starvation was performed for 48h followed by serum re-addition to disassemble cilia. Graphs show the intensity profiles of SSTR3-GFP (green) and Lifeact-mCherry (red), which display enrichment of Lifeact-mCherry in mutant cilia. Control cilia do not exhibit an apparent overlapping signal between the two markers. Scale bars: 1 µm. **D**. The kinase-dead variant of CSNK2A1 shows a dominant-negative effect on PLEKHO1 localization, but not on CAPZB. Left graph shows the mean percentage of cells

with PLEKHO1 at distal cilium in cells stably expressing CSNK2A1-WT, CSNK2A1-D156H, and CSNK2A1-K68M. Statistical comparison was performed by one-way ANOVA with Tukey's multiple comparisons test. P-values are shown on the graph. Cell number details: CSNK2A1-WT n=75, CSNK2A1- D156H n=69, CSNK2A1-K68M n=79 cells. Right graph shows the mean percentage of cells with CAPZB at the centrosome in cells stably expressing CSNK2A1-WT, CSNK2A1-D156H, and CSNK2A1-K68M. Statistical comparison was performed by one-way ANOVA with Tukey's multiple comparisons test. P-values are shown on the graph. Cell number details: CSNK2A1-WT n=44, CSNK2A1- D156H n=43 cells, CSNK2A1-K68M n=57 cells.

Movie S1 (separate file). Transgenic ARL13B-mCherry control MEFs show normal cilia movement behavior.
Movie S2 (separate file). Transgenic ARL13B-mCherry *Csnk2a1*-depleted MEFs exhibit a tip breakage event. For V1 and V2 videos, cells were serum-starved to induce cilia formation. Frames of cilia were acquired every minute. The time-series are shown at a speed of 24 frames per second (fps).

Movie S3 (separate file). F-actin is not enriched in control cilia
Movie S4 (separate file). F-actin transiently localizes to cilia tip prior to breakage event in *Csnk2a1* KO-1 cells. For V3 and V4 videos, control and *Csnk2a1* KO-1 cells were transfected with Lifeact-mCherry and SSTR3-GFP and serum-starved to induce ciliogenesis. Acquisitions were taken every 10 min. Videos are shown at a speed of 0.5 fps.

Dataset S1 (separate file). Results of TTBK2 BioID. Sheet 1 displays the raw data that was normalized to generate fold change and significance values. Sheet 2 shows the high confidence hits enriched in the TTBK2 condition in comparison to GFP.

Dataset S2 (separate file). CRISPR-Cas9 data sets. Sheet 1 shows the raw counts for each gRNA. Each gene was targeted by 4 different gRNAs. Sheet 2 displays the mean of the medians for each gene from WT MEFs. Sheet 3 shows the mean of the medians for each gene from *Ttbk2*$^{null/gt}$ MEFs.

SI References

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