SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice, Plasmids and *In utero* Electroporation

Sdccag8$^{LacZ/+}$ mice were generated with the embryonic stem cell line (OST40418) from Texas A&M Institute of Genetic Medicine (Texas, USA), which contained the retroviral gene-trap vector VICTR24 in the intron 1 of *Sdccag8*. VICTR24 was described previously (Zambrowicz et al., 1998). *Sdccag8*$^{-/-}$ mice were generated with the embryonic stem cell line (EPD0581-2-D04) from the International Knockout Mice Consortium (IKMC/EUCOMM). This mouse line contained a targeted, non-conditional gene trapping cassette (Skarnes et al., 2011) in intron 6 of the *Sdccag8* gene, which resulted in a spliced insertion of 115 base pairs of *Engrailed2* exon of the trapping cassette into *Sdccag8* mRNA between exons 6 and 7. Chimeric mice were prepared by blastocyst microinjection and bred with C57BL/6J mice to obtain germ line transmission.

Four *Sdccag8* shRNA sequences were designed as follows: -1: tcaagttctgcaaggatgaa; -2: actgcagctgtcagtcac; -3 caaagtctcacaaggatc; and -4 atagcattcagcaaagct. All sense and anti-sense oligos were purchased from Sigma, annealed, and cloned into the HpaI and XhoI sites of the lentiviral vector pLL-3.7-EGFP or pLL-3.7-DsRedex (Rubinson et al., 2003), which contains a separate CMV promoter that drives the expression of the fluorescent reporter, as previously described (Bultje et al., 2009). DsRedex-CETN1 was described previously (Wang et al., 2009). EGFP-SDCCAG8 was produced by PCR cloning the coding sequence of mouse SDCCAG8 into the XhoI and SacII sites of pEGFP-C1 (Clonetech). Human *SDCCAG8* cDNA (*hSDCCAG8*) was PCR cloned into the NheI and XhoI sites of pEGFP-C1 to replace EGFP for shRNA rescue experiments. Human *PCM1* (PCM1) cDNA was obtained from ThermoScientific and PCR cloned into the XhoI and BamHI sites of pEGFP-N1 (Clonetech). To make the PCM1ΔC

construct, human *PCM1* coding for amino acids 1-1569 was PCR cloned into the BamHI and XhoI sites of pEGFP-C1 (Clonetech). Both full length mouse *Sdccag8*, full length or truncated human *SDCCAG8* and *PCM1ΔC* were PCR cloned into the SacII site of the pDcx-IRES-EGFP plasmid with a *Doublecortin (Dcx)* promoter (provided by Dr. Mueller at Scripps). Human truncation mutants of *SDCCAG8* were PCR cloned into the XhoI and SacII sites of pEGFP-C1 (Clonetech), and 3xFLAG tagged p.Glu447fsX463 hSDCCAG8 was cloned into the NheI and XhoI sites of pEGFP-C1 (Clonetech) to replace EGFP. The EGFP-EMTB plasmid was provided by Dr. Bulinski at Columbia University. The plasmid expressing γ-TUB-EGFP was provided by Dr. Khodjakov at the Wadsworth Center (New York, USA).

For *in utero electroporation*, timed pregnancies of CD-1 mice were obtained with the night of conception designated as E0.5. At 13.5 days of gestation (E13.5) the pregnant dam was anesthetized, and the uterine horns were exposed. Approximately 1 μL of DNA solution (1–5 μg/μL) mixed with fast green (Sigma) was injected through the uterus into the lateral ventricle of the embryos using a beveled and calibrated glass micropipette (Drummond Scientific). After injection, five 50 ms pulses of 40–50 mV with a 950 ms interval were delivered across the uterus to the head of the embryo with two 9-mm electrode paddles positioned on either side of the head (BTX, ECM830). Throughout the surgery, the embryos were bathed with warm phosphate-buffered saline (PBS, pH 7.4). After electroporation, the uterus was placed back in the abdominal cavity of the pregnant dam and the wound was surgically sutured and clipped. After surgery, the animal was placed in a warmed recovery incubator under close monitoring until the mouse fully recovered and resumed normal activity.

Brain Sections, Immunohistochemistry and Antibodies

Mouse embryos were removed and transcardially perfused with ice-cold phosphate buffered saline (PBS, pH 7.4), followed by cold 4% paraformaldehyde (PFA) in PBS. Brains were dissected out, postfixed and sectioned using a vibratome or a cryostat (Leica Microsystems). For the majority of immunohistochemical staining, tissue sections were incubated for 1 hour at room temperature in blocking buffer (10% horse serum in PBS with 0.5% Triton X-100), followed by incubation in primary antibodies diluted in the blocking buffer at 4°C overnight. After multiple washes with PBS with 0.1% Triton X-100, sections were incubated with appropriate fluorescence-conjugated secondary antibodies diluted in the blocking buffer for 1.5 hours at room temperature.

For SDCCAG8 staining in the embryonic brain, tissue was prepared as previous described (Patil et al., 2012). Freshly dissected E16.5 brains were embedded in optimum cutting temperature (OCT) and flash frozen. Brains were sectioned on a cryostat (Leica Microsystems). Sections (10 μm) were washed in PBS then exposed to the primary antibodies diluted in 0.1% saponin (Sigma) in PBS for 2 hours at room temperature, washed with PBS, and then exposed to the secondary antibodies for 1 hour at room temperature. After extensive washing, sections were fixed with 4% PFA for 20 minutes at room temperature to preserve staining, and mounted with a coverslip.

A specific fixation protocol was used to maintain MTs, as previously described (Lechler and Fuchs, 2007). Briefly, embryonic brains were removed and immediately fixed at 37°C in a fixative solution containing 80 mM PIPES, 50 mM NaCl, 2 mM $MgCl_2$, 0.4 mM $CaCl_2$, 1%

glutaraldehyde, 3% PFA, and 0.2% Triton X-100 (pH 6.9). After extensive washing, brains were treated with sodium borohydride (0.1%) in PBS for 30 minutes, and then cryoprotected in 30% sucrose, embedded in OCT and frozen. After sectioning on a cryostat (Leica Microsystems), sections were again treated with sodium borohydride (0.1%) in PBS for 10 minutes at room temperature before staining.

For LacZ staining, fresh embryonic brains were removed and fixed in ice-cold PFA for 20 minutes, followed by washing and cryoprotection in 30% sucrose in PBS. After cryosectioning, sections were washed twice in a coplin jar with X-gal washing buffer (2 mM $MgCl_2$, 0.1% NP-40, 0.05% deoxycholate in PBS) for 10 minutes and then incubated in X-gal reaction buffer (0.106g potassium ferrocyanide, 0.082 g potassium ferricyanide, 2 mL X-gal substrate stock solution at 25 mg/mL, 48 mL X-gal washing buffer) for 12-48 hours at 37°C. Sections were counterstained with Nuclear Fast Red (Sigma-Aldrich).

Primary cilia at the VZ surface and in the choroid plexus were identified as a single ARL13B-positive cilium emanating from a single basal body (PCNT focus), and their length was measured. Motile cilia in the choroid plexus, defined more than one cilia emanating from a basal body or a group of basal bodies, were excluded from the analysis due to a high internal variability in the length.

Primary antibodies used with corresponding dilutions for immunofluorescence staining (IF) and western blots (WB): rabbit anti-SDCCAG8 (Proteintech; 1:1000 IF, 1:2000 WB), rabbit anti-SDCCAG8 (Abcam, 1:2000 WB), mouse anti-PCTN (Millipore, 1:500 IF), mouse anti-TUJ1

(Covance, 1:1000 IF), rat anti-EGFP (Nacalai, 1:1000 IF), rabbit anti-EGFP (Life Technologies, 1:1000 IF, 1:5000 WB), rabbit anti-ARL13B (IF 1:2000), rabbit anti-PCTN (Covance, 1:500 IF, 1:1000 WB), mouse anti-β-ACTIN (Sigma, 1:5000 WB), rabbit anti-RFP/DsRedex/mCherry (Rockland, 1:500 IF), mouse anti-α-TUB (Sigma, 1:2000 IF), rabbit anti-PCM1 (a generous gift from Dr. A Merdes, 1:2000 IF), rabbit anti-PCM1 (Santa Cruz, 1:1000 WB), rabbit anti-γ-TUB (Sigma, 1:1000 IF), mouse anti-γ-TUB (Santa Cruz, 1:1000 IF), mouse anti-γ-TUB (Sigma, 1:2000 WB), rabbit anti-PAX6 (Covance, 1:500 IF), rabbit anti-TBR2 (Abcam, 1:500 IF), chicken anti-EGFP (Aves Laboratory, 1:2000 IF), rabbit anti-C-MYC (Covance, 1:1000 WB), rabbit and mouse anti-FLAG (Sigma, 1:1000 WB), mouse anti-FLAG (Rockland, 1:1000 IF), goat anti-BRN2 (Santa Cruz, 1:500 IF), rabbit anti-TBR1 (Abcam, 1:500 IF), rabbit anti-CUX1 (Santa Cruz, 1:100 IF), rat anti-CTIP2 (Abcam, 1:500 IF), mouse anti-N-CADHERIN (Life Technologies, 1:500 IF), mouse anti-TAU1 (Millipore 1:1000 IF), rabbit anti-MAP2 (Millipore, 1:1000 IF). Appropriately conjugated (goat or donkey anti mouse/rat/rabbit/chicken) Alexafluor (488, 546, 647) secondary antibodies were used at 1:500 dilution. DNA was stained with 4',6-diamidino-2-phenylindole (DAPI).

Cell culture, Western Blotting and Immunoprecipitation

COS7 cells were maintained in culture media (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) in 37°C humidified incubator with 5% CO_2. Cells were transfected using FuGene6 (Roche) following the manufacturer's protocol. Immunostaining after methanol fixation was carried out as described above, but with a different blocking/antibody dilution buffer (5% goat serum in PBS with 0.1% Triton X-100). Cells on coverslips were mounted on glass slides using Fluoromount G mounting media (Electron Microscopy Sciences), and imaged

using an inverted microscope equipped with epifluorescent illumination and a cooled CCD camera (Axio Observer, Zeiss). Images were analyzed with Axiovision (Zeiss) and Photoshop.

For western blotting, COS7 cells were washed with ice-cold PBS, collected and lysed using NP-40 lysis buffer (50 mM Tris, 150 mM NaCl, 1.0% NP-40 with protease inhibitors tablets (Roche), pH 8.0) at 4°C with constant agitation for 20-60 minutes. Lysates were cleared by centrifugation at 12,000 rpm at 4°C for 10 minutes. Protein concentrations were determined using the Coomassie Protein Assay Reagent (Pierce). Protein lysates were prepped for loading by addition of Laemmli buffer and denaturization at 70°C for 10 minutes. Equal amounts of protein were loaded onto polyacrylamide gels (typically 8%), run to completion and transferred to PVDF membranes (Millipore). Membranes were blocked using 5% non-fat evaporated milk in Tris-buffered saline with Tween 20 (TBS-T, 50 mM Tris, 150 mM NaCl, and 0.05% Tween 20), and incubated with primary antibodies (indicated above) diluted in 2% non-fat evaporated milk in TBS-T. Signal was detected with the appropriate HRP-conjugated secondary antibodies in combination with ECL detection reagent (Pierce).

For immunoprecipitation (IP) experiments, cells were collected 16-18 hours post transfection. Lysates were pre-cleared using normal mouse IgG and beads and 750 μg of total lysate was used for IP reactions. Monoclonal mouse anti-EGFP (Roche) or mouse anti-FLAG (Sigma) antibody was used for IP, along with normal mouse IgG as a control in combination with agarose beads conjugated to protein A/G plus (Santa Cruz). Beads were washed three times with PBS following IP, denatured in Laemmli buffer, and proteins were analyzed using SDS-PAGE.

Organotypic Cortical Slice Culture

Embryonic brains were dissected in L15 culture media (Life Technologies). Whole embryonic brains were embedded in 4% low-melt agarose in PBS and sectioned in the coronal plane using a vibratome (~250 μm, Leica Microsystems) while being maintained in ice-cold oxygenated ACSF (125 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, and 20 mM glucose; pH 7.4, 310 mOsm/L). Sections containing transfected cells were chosen using a stereoscope equipped with epifluorescence (Olympus), and embedded in collagen gel (Millipore) in a glass-bottom petri dish (MatTek Corporation). After the collagen gel solidified, non-serum slice media was added (neurobasal media supplemented with 1X B-27 supplement, 25 μM L-glutamine, 0.5% (w/v) D-glucose, 1X penicillin/streptomycin) and the slices were allowed to recover for 1-3 hours before imaging.

Cortical Neuronal Culture and Neuronal Polarization Assay

In utero electroporation was performed at E13.5 and the electroporated areas of E16 cortices were isolated using a fluorescent dissection microscope (Olympus). Tissues were dissociated and cultured as previously described (Shi et al., 2003). Neurons cultured *in vitro* for ~2-3 days were fixed in 4% PFA, and stained. The percentage of EGFP- and MAP2-expressing neurons that possessed a single TAU1-positive axon was analyzed as previously described (Shi et al., 2003).

SUPPLEMENTAL REFERENCES

Lechler, T., and Fuchs, E. (2007). Desmoplakin: an unexpected regulator of microtubule organization in the epidermis. J Cell Biol *176*, 147-154.

Patil, H., Tserentsoodol, N., Saha, A., Hao, Y., Webb, M., and Ferreira, P.A. (2012). Selective loss of RPGRIP1-dependent ciliary targeting of NPHP4, RPGR and SDCCAG8 underlies the degeneration of photoreceptor neurons. Cell death & disease *3*, e355.

Rubinson, D.A., Dillon, C.P., Kwiatkowski, A.V., Sievers, C., Yang, L., Kopinja, J., Rooney, D.L., Zhang, M., Ihrig, M.M., McManus, M.T., *et al.* (2003). A lentivirus-based system to functionally silence genes in primary mammalian cells, stem cells and transgenic mice by RNA interference. Nat Genet *33*, 401-406.

Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., *et al.* (2011). A conditional knockout resource for the genome-wide study of mouse gene function. Nature *474*, 337-342.

Zambrowicz, B.P., Friedrich, G.A., Buxton, E.C., Lilleberg, S.L., Person, C., and Sands, A.T. (1998). Disruption and sequence identification of 2,000 genes in mouse embryonic stem cells. Nature *392*, 608-611.