Rpgrip11 controls ciliary gating by ensuring the proper amount of Cep290 at the vertebrate transition zone

Antonia Wiegering¹, Renate Dildrop^{1,§}, Christine Vesque², Sylvie Schneider-Maunoury² and Christoph Gerhardt^{1,*}

¹Institute for Animal Developmental and Molecular Biology, Heinrich Heine University, 40225 Düsseldorf, Germany.

²Sorbonne Université, CNRS UMR7622, INSERM U1156, Institut de Biologie Paris Seine (IBPS) – Developmental Biology Unit, 75005 Paris, France.

§Present address: Institute of Genetics, Heinrich Heine University, 40225 Düsseldorf, Germany.

*Correspondence: Christoph Gerhardt, e-mail: Christoph.Gerhardt@hhu.de

Short Title: Rpgrip11 governs ciliary gating by Cep290

Abstract

A range of severe human diseases called ciliopathies are caused by the dysfunction of primary cilia. Primary cilia are cytoplasmic protrusions consisting of the basal body (BB), the axoneme and the transition zone (TZ). The BB is a modified mother centriole from which the axoneme, the microtubule-based ciliary scaffold, is formed. At the proximal end of the axoneme, the TZ functions as the ciliary gate governing ciliary protein entry and exit. Since ciliopathies often develop due to mutations in genes encoding proteins that localise to the TZ, the understanding of the mechanisms underlying TZ function is of eminent importance. Here, we show that the ciliopathy protein Rpgrip11 governs ciliary gating by ensuring the proper amount of Cep290 at the vertebrate TZ. Further, we identified the flavonoid eupatilin as a potential agent to tackle ciliopathies caused by mutations in *RPGRIP1L* as it rescues ciliary gating in the absence of Rpgrip11.

Introduction

The spatiotemporal regulation of cellular processes such as proliferation, apoptosis, migration, specification and differentiation depends on the cells' ability to transduce signals from the environment into the cell's interior. In nearly all mammalian cells, the primary cilium is dedicated to signal reception and transduction. Consequently, dysfunctional primary cilia result in severe often deadly human diseases, collectively called ciliopathies [1]. The current treatment of ciliopathies is restricted to symptomatic therapies and a curative medication against ciliopathies is missing [2]. In many cases, ciliopathies are caused by mutations in genes encoding TZ proteins [3, 4]. As the TZ functions as the ciliary gatekeeper governing ciliary protein import and export [4-12], a defective TZ can affect the proper formation of cilia and alter signalling pathways transduction [13-24]. Thus, the investigation of the molecular mechanisms underlying TZ function is an important prerequisite for the development of curative ciliopathy therapies.

In this study, we shed light on the role of Rpgrip11 in regulating the ciliary gating function of the TZ. Our previous investigations revealed that mutations in *RPGRIP1L* cause deadly ciliopathies [25], that Rpgrip11 localises to the vertebrate TZ [26] and that it is a decisive factor in vertebrate TZ assembly [27]. Our current work demonstrates that Rpgrip11 deficiency results in an altered ciliary protein composition and that Rpgrip11 governs ciliary gating by ensuring the proper amount of Cep290 at the vertebrate TZ. Further, we revealed that the flavonoid eupatilin rescues ciliary gating in the absence of Rpgrip11. Consequently, eupatilin might represent a potential agent for the development of therapies against ciliopathies caused by mutations in *RPGRIP1L*.

Results

Rpgrip11 and Cep290 but not Nphp1, Nphp4 and Invs function as ciliary gatekeepers in mouse embryonic fibroblasts

Among the proteins allowed to cross the TZ are receptors and mediators of signalling pathways essential for proper development. Examples for such proteins are ADP Ribosylation Factor Like GTPase 13B (Arl13b), Somatostatin Receptor 3 (Sstr3), Smoothened (Smo), Polycystin 2 (Pkd2) or Adenylate Cyclase 3 (Ac3) which are often used as indicators to evaluate whether the gate function of the TZ is impaired [13, 14, 17, 19, 28-31]. In a former study, we showed that Rpgrip11 deficiency leads to a reduction of the ciliary Arl13b amount in all analysed mouse cells *in vitro* and *in vivo* [in mouse embryonic fibroblasts (MEFs), in mouse embryonic kidneys and in mouse limb buds][27]. However, we could not detect an alteration of the ciliary Smo amount in *Rpgrip11*^{-/-} MEFs [26] raising the question whether the effect of Rpgrip11 is Arl13b-specific or whether it functions as a more general ciliary gatekeeper at the vertebrate TZ. In order to answer this question, we analysed the ciliary Sstr3 amount. It has been known for a long time that Sstr3 localises to cilia of neuronal cells [32]. More recently, it was shown that Sstr3 is also present in cilia of retinal pigment epithelial (RPE-1) cells [33]. We were now able to visualise endogenous Sstr3 in cilia of MEFs. Importantly, the amount of Sstr3 was decreased in *Rpgrip11*^{-/-} MEFs (Figure 1A) demonstrating that Rpgrip11 exerts a TZ gatekeeper function. We next aimed to investigate how Rpgrip11 implements this function. Several proteins function as ciliary gatekeepers and it is an important task to unveil possible relationships between these gatekeeper proteins in order to understand the mechanisms which govern ciliary protein composition. In this context, Centrosome And Spindle Pole-Associated Protein 1 (Cspp1) was an interesting object of investigation since its truncation in humans results in a reduced ciliary amount of Arl13b and Ac3 [34] and since it interacts with Rpgrip11 [35]. Previously, we and others reported that Rpgrip11 ensures the proper amount of many proteins at the base of vertebrate cilia [19, 27] suggesting that it serves as a vital scaffold protein. Thus, we quantified

the amount of Cspp1 at the ciliary base of *Rpgrip11*^{-/-} MEFs but we could not detect any alteration (Figure S1A) indicating that Rpgrip11 exerts its ciliary gatekeeper function independently of Cspp1. Recently, a potential link between Rpgrip11 and the septin proteins was discussed [36]. Septins are known ciliary gatekeepers in vertebrates localising at the proximal end of the axoneme in MEFs [28]. We measured the ciliary amount of two different septins, Septin 2 (Sept2) and Septin 7 (Sept7), in *Rpgrip11*^{-/-} MEFs. Neither Sept2 nor Sept7 was altered by the loss of Rpgrip11 (Figure S1B and C). We then turned to the TZ proteins Nephrocystin 4 (Nphp4), Nephrocystin 1 (Nphp1), Centrosomal Protein 290 (Cep290) and Inversin (Invs) whose amount at the vertebrate TZ is decreased in the absence of Rpgrip11 [27]. Previous studies in the invertebrates *Chlamydomonas reinhardtii* and/or *Caenorhabditis elegans* demonstrated that ciliary gating is regulated by the TZ proteins Nphp4, Nphp1, Cep290 and Invs [12, 17, 21, 23, 37] raising the possibility that Rpgrip11 might govern ciliary gating by controlling the amount of one of these proteins at the TZ. However, it is unknown whether these proteins function as ciliary gatekeepers in vertebrates and hence we quantified the ciliary amount of Arl13b and Sstr3 in *Nphp4*^{-/-}, *Nphp1*^{-/-}, *Cep290*^{-/-} and *Invs*^{-/-} mouse cells. The ciliary amount of both Arl13b and Sstr3 was unaffected in *Nphp4*^{-/-} MEFs (Figure 1B). To analyse TZ assembly, we had previously inactivated *Nphp1*, *Cep290* and *Invs* in NIH3T3 cells (immortalised MEFs) [27]. In the current study, we used these cells to investigate a possible involvement of these proteins in ciliary gating. To be able to perform comparative analyses in this context, we also inactivated Rpgrip11 in NIH3T3 cells (Figure S2A-C). As observed in MEFs [26], ciliary length was increased in *Rpgrip11*^{-/-} NIH3T3 (Figure S2D). Moreover, the ciliary amount of Arl13b and Sstr3 was decreased in *Rpgrip11*^{-/-} NIH3T3 (Figure 2A and B). In contrast to these cells, *Nphp1*^{-/-} and *Invs*^{-/-} NIH3T3 cells did not show an altered ciliary amount of Arl13b and Sstr3 (Figure 2A and B) indicating that the decreased amount of Nphp1 and Invs in *Rpgrip11*^{-/-} MEFs is not the reason for the reduced ciliary Arl13b and Sstr3 amount. Importantly, Cep290 deficiency causes a decrease of the ciliary Arl13b and Sstr3 amount

(Figure 2A and B) making it conceivable that the reduced amount of Cep290 might provoke the diminished amount of Arl13b and Sstr3 in the absence of Rpgrip11.

Restoration of the Cep290 amount at the TZ of *RPGRIP1L*^{-/-} HEK293 cells rescues the ciliary Arl13b amount

To test this hypothesis, we tried to enhance the amount of Cep290 at the TZ of *Rpgrip1l*^{-/-} MEFs and NIH3T3 cells by transfecting a plasmid that encodes a Flag-mCep290 fusion protein. Since the transfection failed in these cells, we transfected the plasmid into *RPGRIP1L*^{-/-} HEK293 cells. In a previous study, we revealed a reduced amount of Cep290 at the TZ of *RPGRIP1L*^{-/-} HEK293 cells [27]. The HEK293 cell line is an easily transfectable cell line [38] and, indeed, we detected the Flag-mCep290 fusion protein in *RPGRIP1L*^{-/-} HEK293 cells after transfection (Figure 3A). Remarkably, the fusion protein was located at the TZ (Figure 3B). In contrast to the Flag-mCep290 fusion protein, we could not detect a transfected Myc-mNphp1 fusion protein at the TZ of *RPGRIP1L*^{-/-} HEK293 cells (Figure S3A and B). Most likely, Rpgrip11 functions as a TZ scaffold for Nphp1 but not for Cep290.

The transfection of the plasmid encoding the Flag-mCep290 fusion protein into *RPGRIP1L*^{-/-} HEK293 cells, rescued the amount of Cep290 at the TZ (Figure 3C). Importantly, this rescue restored the ciliary amount of Arl13b in *RPGRIP1L*^{-/-} HEK293 cells (Figure 3D) indicating that the decreased amount of Cep290 in *RPGRIP1L*^{-/-} HEK293 cells causes an impaired ciliary gating. Cilia of HEK293 cells are elongated by the loss of Rpgrip11 [27]. Interestingly, the increased cilia length in the absence of Rpgrip11 was not rescued by the expression of the Flag-mCep290 fusion protein (Figure 3E).

Eupatilin treatment rescues ciliary gating in *Rpgrip11*-negative mouse embryonic fibroblasts

HEK293 cells lack *Sstr3*, preventing the analysis of its localisation in the absence of *Rpgrip11* and its rescue by *Cep290* overexpression [39]. For this reason, we performed another approach by using the flavonoid eupatilin. A recent report showed that eupatilin rescues the ciliary gating in *CEP290*^{-/-} human cells ⁶. The treatment of *Rpgrip11*^{-/-} NIH3T3 cells with eupatilin restored the amount of both *Arl13b* and *Sstr3* (Figure 4A and B) indicating that *Rpgrip11* controls ciliary gating via ensuring the proper amount of *Cep290* at the vertebrate TZ. The enhanced cilia length in *Rpgrip11*^{-/-} NIH3T3 cells was not rescued by eupatilin (Figure 4C).

Discussion

Primary cilia mediate numerous signalling pathways thereby ensuring proper development and homeostasis. In this context, the intraciliary concentration of proteins involved in these signalling pathways is of enormous importance. Consequently, ciliary import and export and hence ciliary protein composition has to be tightly controlled. This control is implemented by the TZ. Since mutations in genes encoding TZ proteins result in ciliopathies [1, 3, 4], current cilia research aims to uncover mechanisms underlying TZ assembly and function. However, little is known about these mechanisms in vertebrates. Recently, we described Rpgrip11 as a decisive factor in vertebrate TZ assembly [27]. In this context, Rpgrip11 deficiency leads to a reduced amount of Cep290, Nphp1, Nphp4 and Invs at the TZ [27]. Rpgrip11, Cep290, Nphp1, Nphp4 and Invs were previously shown to govern ciliary gating in *Chlamydomonas reinhardtii* and/or *Caenorhabditis elegans* [12, 17, 21, 23, 37, 40]. However, loss of Nphp1, Nphp4 and Invs did not alter the ciliary amount of Arl13b or Sstr3 in MEFs and NIH3T3 cells (Figure 1B and Figure 2) indicating that they are not involved in gating these proteins in vertebrate primary cilia. Remarkably, several reports point to cell type-specific functions of some TZ proteins [14, 16, 27, 41, 42] making a potential regulation of ciliary gating by Nphp1, Nphp4 and Invs in other vertebrate cell types conceivable. *Rpgrip11*^{-/-} and *Cep290*^{-/-} mice have a much more severe phenotype than *Nphp1*^{-/-}, *Nphp4*^{-/-} and *Invs*^{-/-} mice [20, 25, 27, 42-59]. Moreover, mutations in *RPGRIP1L* and *CEP290* result in more severe human ciliopathies than mutations in *NPHP1*, *NPHP4* or *INVS* [60-63]. On the one hand, these differences might reflect that Rpgrip11 and Cep290 function as ciliary gatekeepers in vertebrates while Nphp1, Nphp4 and Invs do not. On the other hand, these differences might be based on the fact that Rpgrip11 and Cep290 exert additional functions in the cytoplasm, e.g. the regulation of protein degradation systems [26, 64] or the organisation of the cytoplasmic microtubule network [65].

Formerly, we demonstrated that Rpgrip11 deficiency does not affect the overall cellular amount of Cep290 but its localisation at the vertebrate TZ [27]. There is a perennial debate about the

function(s) of Rpgrip11. Does it predominantly serve as a structural TZ anchor or scaffold protein and interacts with other proteins thereby ensuring their localisation and proper amount at the TZ or does it control the TZ localisation and amount of proteins by exerting additional functions e.g. regulating protein degradation systems, functioning as a TZ assembly factor, establishing a ciliary zone of exclusion (CIZE) that excludes signal transduction proteins etc. [15, 19, 23, 26, 27, 64, 66-68]? In this study, the Flag-mCep290 protein was able to localise to the TZ in the absence of Rpgrip11 (Figure 3B) indicating that Rpgrip11 does not function as a structural scaffold for the TZ presence of Cep290. In line with this assumption, it was not shown yet that Rpgrip11 interacts with Cep290. To stress this point, we also transfected a plasmid encoding a Myc-mNphp1 fusion protein into *RPGRIP1L*^{-/-} HEK293 cells. It was reported before that Nphp1 interacts with Rpgrip11 [69]. Since Myc-mNphp1 was not present at the TZ in the absence of Rpgrip11 (Figure S3B), we suggest that Rpgrip11 functions as structural anchor for Nphp1 but not for Cep290. Dissecting the mechanism by which Rpgrip11 regulates the amount of Cep290 at the vertebrate TZ will be a thrilling future project.

Here, we show that Rpgrip11 controls ciliary gating via ensuring the proper amount of Cep290 at the TZ (Figure 3D). Cep290 governs ciliary protein composition by interacting with Nphp5 [70]. Consequently, Rpgrip11, Cep290 and Nphp5 can be defined as ciliary gatekeepers. Cep290 binds to Nphp5 thereby preventing the binding of calmodulin to Nphp5 and promoting the recruitment of Nphp5 to the TZ [71]. In the absence of Cep290, Nphp5 is not present at the TZ. However, the treatment of *CEP290*^{-/-} cells with eupatilin rescues the TZ amount of Nphp5 and ciliary gating since eupatilin inhibits the binding between Nphp5 and calmodulin [71]. In the current study, eupatilin treatment rescued ciliary gating in the *Rpgrip11* deficient state (Figure 4A and B). For this reason, we hypothesise that Rpgrip11 controls the proper TZ amount of Nphp5 by its effect on Cep290. In turn, Nphp5 ensures ciliary gating (Figure 4D). Thus, we propose eupatilin as a potential agent for the treatment of ciliopathies caused by mutations in *RPGRIP1L*. Interestingly, Rpgrip11 was not required for the ciliary localisation of Csppl

(Figure S1A) but the proper amount of Rpgrip11 at the TZ depends on Cspp1 [35]. Moreover, mutations in *CSPP1* disturb ciliary protein composition (e.g. reduced ciliary Arl13b amount) [34] and cause Joubert syndrome and Meckel syndrome [72]. Based on these facts, we place Cspp1 at the top of the “ciliary gating hierarchy” involving Rpgrip11, Cep290 and Nphp5 (Figure 4D).

Interestingly, Garcia-Gonzalo et al. revealed that the loss of the TZ gatekeeper protein Tmem67 diminishes the ciliary amount of Arl13b and Ac3 but the ciliary amount of Smo remains normal [14] demonstrating the existence of a specificity between the gatekeeper proteins and the proteins which are allowed to cross the TZ. In *Rpgrip11*^{-/-} MEFs, the ciliary amount of Smo is also unaltered [26]. The lack of Cep290 and Nphp5 in RPE-1 cells results in a reduced ciliary amount of Smo [70]. Since the amount of Cep290 is reduced in *Rpgrip11*^{-/-} MEFs [27], the expectation would be that the amount of Smo was decreased in these MEFs. However, these findings have been made in different cell types making it possible that the ciliary gating function of these proteins might be cell type-specific. The analysis of this hypothesis is an exciting subject of future studies which would shed further light on the ciliary gating function of the TZ.

Many ciliopathies can be attributed to mutations in genes encoding TZ proteins [3, 4]. For this reason, the assembly and function of the TZ is a hot topic in biomedical research. A lot of proteins participate in TZ assembly and/or function as ciliary gatekeepers at the TZ [12-14, 16, 17, 19, 23, 24, 27, 29, 30, 33, 34, 37, 41, 69, 70, 73-90]. However, the relationships between these proteins and hence the mechanisms underlying ciliary gating at the TZ remain largely elusive. Recently, we showed that Rpgrip11 represents a central factor in vertebrate TZ assembly [27]. Our current study reveals that Rpgrip11 also regulates ciliary gating by ensuring the proper amount of Cep290 at the vertebrate TZ. Combining our results with previous findings, we suggest a protein hierarchy regulating ciliary gating in which Cspp1, Rpgrip11, Cep290 and Nphp5 are involved. Our work is an important piece of a puzzle depicting this

fundamental ciliary process. The completion of this puzzle will be one of the most important tasks of cilia research in the next few years.

Materials and Methods

Cell lines

We used two different cell lines in this study. NIH3T3 cells (#ACC59) and HEK293 cells (#ACC35), both purchased by the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). Cells were grown in DMEM supplemented with 10% fetal calf serum (FCS), 1/100 (v/v) L-glutamine (Gibco), 1/100 (v/v) sodium pyruvate (Gibco), 1/100 (v/v) non-essential amino acids (Gibco) and 1/100 (v/v) pen/strep (Gibco) at 37 °C and 5% CO₂. The following clones were used: *Rpgrip1l*^{-/-} NIH3T3 cells (clone 10-61), *Cep290*^{-/-} NIH3T3 cells (clones 39-10, 39-51, 39-53) [27], *Nphp1*^{-/-} NIH3T3 cells (clone 21-21) [27], *Invs*^{-/-} NIH3T3 cells (clones 48-7, 48-20) [27] and *RPGRIP1L*^{-/-} HEK293 cells (clone 1-7) [27].

Primary Cell Culture

We isolated MEFs from single mouse embryos (male and female) at embryonic stage (E) 12.5 after standard procedures. MEFs were grown in DMEM supplemented with 10% fetal calf serum (FCS), 1/100 (v/v) L-glutamine (Gibco), 1/100 (v/v) sodium pyruvate (Gibco), 1/100 (v/v) non-essential amino acids (Gibco) and 1/100 (v/v) pen/strep (Gibco) at 37 °C and 5% CO₂. The following mutant mice were used: *Rpgrip1l*-mutant mice on a C3H-background [20] and *Nphp4*-mutant mice on a C57BL/6J-background [27].

Method Details

Cell culture, transfection and drug treatment

Ciliogenesis in confluent grown MEFs and NIH3T3 cells was induced by serum-starvation (0.5% FCS) for at least 24 hours. For DNA transfection, Lipofectamin 3000 (Invitrogen) was

used following the manufactures guidelines. NIH3T3 cells were treated with 20 μ M eupatilin (#SML1689; Sigma-Aldrich) or DMSO as a solvent control for 24 h.

Antibodies and Plasmids

Cells were immunolabeled with primary antibodies targeting actin (#A2066; Sigma-Aldrich), Arl13b (#17711-1-AP; Proteintech), Cep290 (#ab84870; Abcam), Cspp1 (#11931-1-AP; Proteintech), Flag (#F7425; Sigma-Aldrich), Myc (#sc-789; Santa Cruz Biotechnology, Inc.), Sept2 (#11397-1-AP; Proteintech), Sept7 (#13818-1-AP; Proteintech), Sstr3 (#20696-1-AP; Proteintech and #PA3-207; Pierce Biotechnology), acetylated α -tubulin (#sc-23950; Santa Cruz Biotechnology, Inc.), and γ -tubulin (#sc-7396; Santa Cruz Biotechnology, Inc.). The generation of the polyclonal antibody against Rpgrip11 was described formerly [20].

The following plasmids were used: pMyc-mNphp1 (kindly provided by Sophie Saunier) and pFlag-mCep290 (#27381; Addgene). pMyc-mNphp1 encodes for the murine full-length Nphp1 protein fused to a myc-tag (vector: CMV) and pFlag-mCep290 encodes for the murine full-length Cep290 protein fused to a flag-tag (vector: CMV2).

CRISPR/Cas9-mediated Gene Inactivation

Inactivation of mouse *Rpgrip11* in NIH3T3 cells was performed as previously described [27]. We choose a target site which is located in exon3 of the gene (Figure S2). After inactivation and single-cell cloning, 8 clones, which upon RFLP analysis appeared to have lost the diagnostic *EagI* recognition sequence, were further analysed. To establish the genotype, individual alleles were cloned and sequenced (Figure S2).

Sequences of the target site and primer pairs used to amplify the targeted region are as follows:

Rpgrip11 –T3: CTCGAGTTAACACCGGCCCGCCGG

Rpgrip11-T3b-for: GAATGGCCACCAAGTTAATACGGCTAG

Rpgrip11-T3b-rev: CTTCAGGATCTGACAGAGAGCAAGCCTC

Off-target Analyses

Off-target analyses were performed by RFLP analyses as previously described [27]. For the on-target Rpgr11-T3 there exist no off-targets carrying either one, two or three mismatches. From the remaining 4-mismatch off-targets we tested the top-3 ranking sites [91] (crispr.mit.edu/) on the DNAs from the same 8-set-clones analysed for targeting of the on-target. In summary, we did not detect any mutations (data not shown).

Sequences of the off-target sites and primer pairs used for amplification are as follows:

T3 offtarget-1: CACGAGTCAGCACCGGCCACTGG

T3 off-1 for: CTGTCAGGTTTCCCAGTGTGCAG

T3 off-1 rev: CTCTCAGCTCCTTTTAGGTCTCCAG

T3 offtarget-2: CTCTACTGAACAACGGCCGCAGG

T3 off-2 for: ATCCAGCCAAACCCTGCCTGTTC

T3 off-2 rev: GGTTTGTCTCTGTCCTGACATGTCAC

T3 offtarget-3: ATCCAGTTGACACCGGCCTCTGG

T3 off-3 for: GTCTCCTTCAGACCCACTGAAGTG

T3 off-3 rev: GTCCCAGGAAGCCAGGCTGTTG

The following restriction enzymes were used: *Bs*II (T3 offtarget-1), *Eag*I (T3 offtarget-2), *Bs*II (T3 offtarget-3).

Image Processing

Image acquisition and data analysis were carried out at room temperature using a Zeiss Imager.A2 microscope, 100x, NA 1.46 oil immersion objective lens (Carl Zeiss AG), a monochrome charge-coupled device camera (AxioCam MRm, Carl Zeiss AG), and the AxioVision Rel. 4.8 software package (Carl Zeiss AG). Appropriate anti-mouse, anti-rabbit and anti-goat Alexa405, Cy3, and Alexa488 antibodies were used as fluorochromes.

Immunofluorescence

For immunofluorescence on MEFs, NIH3T3 cells and HEK293 cells, cells were plated on coverslips until confluency. MEFs and NIH3T3 cells were serum-starved for at least 24 hours. Cells were fixed with 4% PFA (for stainings with the antibodies to Cep290, Rpgrip11, Flag, Myc, Sept7 and Arl13b) or methanol (for stainings with the antibodies to Cspp1, Sept2, Sstr3). Fixed cells were rinsed three times with PBS, followed by a permeabilisation step with PBS/0.5% Triton-X-100 for 10 minutes. The samples were rinsed three times with PBS. Samples were incubated for at least 10 minutes at room temperature in PBST (PBS/0.1% Triton-X-100) containing 10% donkey serum. Diluted primary antibodies (in blocking solution) were incubated overnight at 4 °C. After three washing steps with PBST, incubation with fluorescent secondary antibody (diluted in blocking solution) was performed at room temperature for 1 hour followed by several washing steps and subsequent embedding with Mowiol.

Western Blotting

Whole-cell lysates were obtained by lysis with radioimmunoprecipitation buffer (150 mM sodium chloride, 50 mM Tris-HCl, pH 7.4, 0.1% sodium deoxycholate, and 1 mM EDTA). Protein content was measured by the Bradford method, and samples were normalized. 20 mg of total protein was separated by SDS-PAGE on polyacrylamide gels (10%) and transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories, Inc.). The membrane was probed with antibodies against Flag (#F7425; Sigma-Aldrich) and Myc (#sc-789; Santa Cruz Biotechnology, Inc.). Anti-actin (#A2066; Sigma-Aldrich) antibody was used as loading control. Proteins were detected with secondary antibodies conjugated to horseradish peroxidase (RPN4201 and RPN4301) and the ECL detection kit (both GE Healthcare). Visualization of

protein bands was realized by LAS-4000 mini (Fujifilm). Band intensities were measured by using ImageJ (National Institutes of Health).

Quantification and Statistical Analysis

Quantification

Quantifications of ciliary protein staining and protein bands intensity were quantified using ImageJ (National Institutes of Health). Intensity measurement of proteins based on immunofluorescence staining was performed as described before [14, 24, 26, 27, 29, 64, 92]. The ciliary length has to be taken into account while quantifying the ciliary amount of Arl13b and Sstr3 in different genotypes. Therefore we used the area marked by acetylated α -tubulin as a reference and quantified the average pixel intensity of the Arl13 and Sstr3 staining. For all other ciliary protein intensities (Cep290, Flag, Csppl, Myc, Rpgrip11, Sept2 and Sept7), we selected the region labelled by γ -tubulin (for BB proteins) or the area in-between the γ -tubulin staining and the proximal part of the acetylated α -tubulin staining and measured the total pixel intensity. To exclude unspecific staining from the measurements, we subtracted the mean value of the average pixel intensity (in the case of Arl13b and Sstr3) or of the total pixel intensity (all other ciliary proteins) of three neighboring regions free from specific staining.

Statistical Analysis

Data are presented as mean $\pm$ standard error of mean (SEM). Two-tailed *t*-test with Welch's correction was performed for all data in which two datasets were compared. Analysis of variance (ANOVA) and Tukey honest significance difference (HSD) tests were used for all data in which more than two datasets were compared. A P-value <0.05 was considered to be statistically significant (one asterisk), a P-value <0.01 was defined as statistically very significant (two asterisks) and a P-value <0.001 was noted as statistically high significant (three

asterisks). All statistical data analysis and graph illustrations were performed by using Graphpad Prism (Graphpad Software Inc). Sample sizes are indicated in the figure legends.

Acknowledgments

The authors thank Matias Zurbriggen and Leonie-Alexa Koch for their generous help to enable the continuation of the study. Moreover, we are grateful to Sophie Saunier for providing the Myc-mNphp1 construct. This work was funded by the Fondation ARC pour la Recherche sur le Cancer (Project ARC PJA 20171206591 to S.S.M.) and the Fondation pour la Recherche Médicale (Equipe FRM EQU201903007943 to S.S.M.).

Author contributions

Conceptualisation, C.G.; Methodology, R.D.; Validation, A.W., R.D. and C.G.; Formal Analysis, A.W.; Investigation, A.W., R.D. and C.G.; Resources, C.V. and S.S.M.; Writing – Original Draft, C.G.; Visualisation, A.W.; Supervision, C.G.; Project Administration, C.G.; Funding Acquisition, S.S.M.

Declaration of Interests

The authors declare no competing interests.

References

1. Reiter J, Leroux M. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol.* 2017;18(9):533-47.
2. McIntyre J, Williams C, Martens J. Smelling the roses and seeing the light: gene therapy for ciliopathies. *Trends Biotechnol.* 2013;31(6):355-63.
3. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. *N Engl J Med.* 2011;364(16):1533-43.
4. Czarnecki PG, Shah JV. The ciliary transition zone: from morphology and molecules to medicine. *Trends in Cell Biol.* 2012;22(4):201-10.
5. Reiter J, Blacque O, Leroux M. The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep.* 2012;13(7):608-18.
6. Garcia-Gonzalo F, Reiter J. Scoring a backstage pass: Mechanisms of ciliogenesis and ciliary access. *J Cell Biol.* 2012;197(6):697-709.
7. Omran H. NPHP proteins: gatekeepers of the ciliary compartment. *J Cell Biol.* 2010;190(5):715-7.
8. Benzing T, Schermer B. Transition zone proteins and cilia dynamics. *Nat Genet.* 2011;43(8):723-4.
9. Garcia-Gonzalo F, Reiter J. Open Sesame: How Transition Fibers and the Transition Zone Control Ciliary Composition. *Cold Spring Harb Perspect Biol.* 2017;9(2):pii: a028134.
10. Jensen V, Leroux M. Gates for soluble and membrane proteins, and two trafficking systems (IFT and LIFT), establish a dynamic ciliary signaling compartment. *Curr Opin Cell Biol.* 2017;47:83-91.
11. Betleja E, Cole D. Ciliary trafficking: CEP290 guards a gated community. *Curr Biol.* 2010;20(21):R928-31.

12. Craig B, Tsao C, Diener D, Hou Y, Lehtreck K, Rosenbaum J, et al. CEP290 tethers flagellar transition zone microtubules to the membrane and regulates flagellar protein content. *J Cell Biol.* 2010;190(5):927-40.
13. Chih B, Liu P, Chinn Y, Chalouni C, Komuves L, Hass P, et al. A ciliopathy complex at the transition zone protects the cilia as a privileged membrane domain. *Nat Cell Biol.* 2011;14(1):61-72.
14. Garcia-Gonzalo F, Corbit K, Sirerol-Piquer M, Ramaswami G, Otto E, Noriega T, et al. A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nat Genet.* 2011;43(8):776-84.
15. Jensen V, Li C, Bowie R, Clarke L, Mohan S, Blacque O, et al. Formation of the transition zone by Mks5/Rpgrip1L establishes a ciliary zone of exclusion (CIZE) that compartmentalises ciliary signalling proteins and controls PIP2 ciliary abundance. *EMBO J.* 2015;34(20):2537-56.
16. Lewis W, Bales K, Revell D, Croyle M, Engle S, Song C, et al. Mks6 mutations reveal tissue- and cell type-specific roles for the cilia transition zone. *FASEB J.* 2019;33(1):1440-55.
17. Li C, Jensen V, Park K, Kennedy J, Garcia-Gonzalo F, Romani M, et al. MKS5 and CEP290 Dependent Assembly Pathway of the Ciliary Transition Zone. *PLoS Biol.* 2016;14(3):e1002416.
18. Reiter J, Skarnes W. Tectonic, a novel regulator of the Hedgehog pathway required for both activation and inhibition. *Genes Dev.* 2006;20(1):22-7.
19. Shi X, Garcia Gr, Van De Weghe J, McGorty R, Pazour G, Doherty D, et al. Super-resolution microscopy reveals that disruption of ciliary transition-zone architecture causes Joubert syndrome. *Nat Cell Biol.* 2017;19(10):1178-88.
20. Vierkotten J, Dildrop R, Peters T, Wang B, Rther U. Ftm is a novel basal body protein of cilia involved in Shh signalling. *Development.* 2007;134(14):2569-77.

21. Warburton-Pitt S, Jauregui A, Li C, Wang J, Leroux M, Barr M. Ciliogenesis in *Caenorhabditis elegans* requires genetic interactions between ciliary middle segment localized NPHP-2 (inversin) and transition zone-associated proteins. *J Cell Sci.* 2012;125(Pt 11):2592-603.
22. Weng R, Yang T, Huang C, Chang C, Wang W, Liao J. Super-Resolution Imaging Reveals TCTN2 Depletion-Induced IFT88 Lumen Leakage and Ciliary Weakening. *Biophys J.* 2018;115(2):263-75.
23. Williams C, Li C, Kida K, Inglis P, Mohan S, Semenec L, et al. MKS and NPHP modules cooperate to establish basal body/transition zone membrane associations and ciliary gate function during ciliogenesis. *J Cell Biol.* 2011;192(6):1023-41.
24. Yee L, Garcia-Gonzalo F, Bowie R, Li C, Kennedy J, Ashrafi K, et al. Conserved Genetic Interactions between Ciliopathy Complexes Cooperatively Support Ciliogenesis and Ciliary Signaling. *PLoS Genet.* 2015;11(11):e1005627.
25. Delous M, Baala L, Salomon R, Laclef C, Vierkotten J, Tory K, et al. The ciliary gene *RPGRIP1L* is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet.* 2007;39(7):875-81.
26. Gerhardt C, Lier J, Burmühl S, Struchtrup A, Deutschmann K, Vetter M, et al. The transition zone protein *Rpgrip1l* regulates proteasomal activity at the primary cilium. *J Cell Biol.* 2015;210(1):115-33.
27. Wiegering A, Dildrop R, Kalfhues L, Spychala A, Kuschel S, Lier J, et al. Cell type-specific regulation of ciliary transition zone assembly in vertebrates. *EMBO J.* 2018;37(10):pii: e97791.
28. Hu Q, Milenkovic L, Jin H, Scott M, Nachury M, Spiliotis E, et al. A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. *Science.* 2010;329(5990):436-9.

29. Roberson E, Dowdle W, Ozanturk A, Garcia-Gonzalo F, Li C, Halbritter J, et al. TMEM231, mutated in orofacioidigital and Meckel syndromes, organizes the ciliary transition zone. *J Cell Biol.* 2015;209(1):129-42.
30. Takao D, Wang L, Boss A, Verhey K. Protein Interaction Analysis Provides a Map of the Spatial and Temporal Organization of the Ciliary Gating Zone. *Curr Biol.* 2017;27(15):2296-306.e3.
31. Ye F, Nager A, Nachury M. BBSome trains remove activated GPCRs from cilia by enabling passage through the transition zone. *J Cell Biol.* 2018;217(5):1847-68.
32. Händel M, Schulz S, Stanarius A, Schreff M, Erdtmann-Vourliotis M, Schmidt H, et al. Selective targeting of somatostatin receptor 3 to neuronal cilia. *Neuroscience.* 1999;89(3):909-26.
33. Klinger M, Wang W, Kuhns S, Bärenz F, Dräger-Meurer S, Pereira G, et al. The novel centriolar satellite protein SSX2IP targets Cep290 to the ciliary transition zone. *Mol Biol Cell.* 2014;25(4):495-507.
34. Tuz K, Bachmann-Gagescu R, O'Day D, Hua K, Isabella C, Phelps I, et al. Mutations in CSPP1 cause primary cilia abnormalities and Joubert syndrome with or without Jeune asphyxiating thoracic dystrophy. *Am J Hum Genet.* 2014;94(1):62-72.
35. Patzke S, Redick S, Warsame A, Murga-Zamalloa C, Khanna H, Doxsey S, et al. CSPP is a ciliary protein interacting with Nephrocystin 8 and required for cilia formation. *Mol Biol Cell.* 2010;21(15):2555-67.
36. Patnaik S, Zhang X, Biswas L, Akhtar S, Zhou X, Kusuluri D, et al. RPGR protein complex regulates proteasome activity and mediates store-operated calcium entry. *Oncotarget.* 2018;9(33):23183-97.
37. Awata J, Takada S, Standley C, Lechtreck K, Bellvé K, Pazour G, et al. NPHP4 controls ciliary trafficking of membrane proteins and large soluble proteins at the transition zone. *J Cell Sci.* 2014;127(Pt 21):4714-27.

38. Thomas P, Smart T. HEK293 cell line: a vehicle for the expression of recombinant proteins. *J Pharmacol Toxicol Methods*. 2005;51(3):187-200.
39. War S, Kumar U. Coexpression of human somatostatin receptor-2 (SSTR2) and SSTR3 modulates antiproliferative signaling and apoptosis. *J Mol Signal*. 2012;7(1):5.
40. Lin H, Guo S, Dutcher S. RPGRIP1L helps to establish the ciliary gate for entry of proteins. *J Cell Sci*. 2018;131(20):pii: jcs220905. doi: 10.1242/jcs.220905.
41. Lambacher N, Bruel A, van Dam T, Szymańska K, Slaats G, Kuhns S, et al. TMEM107 recruits ciliopathy proteins to subdomains of the ciliary transition zone and causes Joubert syndrome. *Nat Cell Biol*. 2016;18(1):122-31.
42. Rachel R, Yamamoto E, Dewanjee M, May-Simera H, Sergeev Y, Hackett A, et al. CEP290 alleles in mice disrupt tissue-specific cilia biogenesis and recapitulate features of syndromic ciliopathies. *Hum Mol Genet*. 2015;24(13):3775-91.
43. Chang B, Khanna H, Hawes N, Jimeno D, He S, Lillo C, et al. In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. *Hum Mol Genet*. 2006;15(11):1847-57.
44. Lancaster M, Gopal D, Kim J, Saleem S, Silhavy J, Louie C, et al. Defective Wnt-dependent cerebellar midline fusion in a mouse model of Joubert syndrome. *Nat Med*. 2011;17(6):726-31.
45. Li Y, Klena N, Gabriel G, Liu X, Kim A, Lemke K, et al. Global genetic analysis in mice unveils central role for cilia in congenital heart disease. *Nature*. 2015;521(7553):520-4.
46. McEwen D, Koenekoop R, Khanna H, Jenkins P, Lopez I, Swaroop A, et al. Hypomorphic CEP290/NPHP6 mutations result in anosmia caused by the selective loss of G proteins in cilia of olfactory sensory neurons. *Proc Natl Acad Sci U S A*. 2007;104(40):15917-22.

47. Besse L, Neti M, Anselme I, Gerhardt C, Rüther U, Laclef C, et al. Primary cilia control telencephalic patterning and morphogenesis via Gli3 proteolytic processing. *Development*. 2011;138(10):2079-88.
48. Gerhardt C, Lier J, Kuschel S, Rüther U. The ciliary protein Ftm is required for ventricular wall and septal development. *PLoS One*. 2013;8(2):e57545.
49. Laclef C, Anselme I, Besse L, Catala M, Palmyre A, Baas D, et al. The role of primary cilia in corpus callosum formation is mediated by production of the Gli3 repressor. *Hum Mol Genet*. 2015;24(17):4997-5014.
50. Andreu-Cervera A, Anselme I, Karam A, Laclef C, Catala M, Schneider-Maunoury S. The ciliopathy gene *ftm/rpgrip11* controls mouse forebrain patterning via region-specific modulation of hedgehog/gli signaling. *J Neurosci*. 2019;39(13):2398-415.
51. Choi Y, Laclef C, Yang N, Andreu-Cervera A, Lewis J, Mao X, et al. RPGRIP1L is required for stabilizing epidermal keratinocyte adhesion through regulating desmoglein endocytosis. *PLoS Genet*. 2019;15(1):e1007914.
52. Chen J, Laclef C, Moncayo A, Snedecor E, Yang N, Li L, et al. The ciliopathy gene *Rpgrip11* is essential for hair follicle development. *J Invest Dermatol*. 2015;135(3):701-9.
53. Jiang S, Chiou Y, Wang E, Lin H, Lee S, Lu H, et al. Targeted disruption of *Nphp1* causes male infertility due to defects in the later steps of sperm morphogenesis in mice. *Hum Mol Genet*. 2008;17(21):3368-79.
54. Jiang S, Chiou Y, Wang E, Chien Y, Ho H, Tsai F, et al. Essential role of nephrocystin in photoreceptor intraflagellar transport in mouse. *Hum Mol Genet*. 2009;18(9):1566-77.
55. Louie C, Caridi G, Lopes V, Brancati F, Kispert A, Lancaster M, et al. *AHI1* is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet*. 2010;42(2):175-80.

56. Mochizuki T, Saijoh Y, Tsuchiya K, Shirayoshi Y, Takai S, Taya C, et al. Cloning of *inv*, a gene that controls left/right asymmetry and kidney development. *Nature*. 1998;395(6698):177-81.
57. Morgan D, Turnpenny L, Goodship J, Dai W, Majumder K, Matthews L, et al. *Inversin*, a novel gene in the vertebrate left-right axis pathway, is partially deleted in the *inv* mouse. *Nat Genet*. 1998;20(2):149-56.
58. Won J, Marín de Evsikova C, Smith R, Hicks W, Edwards M, Longo-Guess C, et al. *NPHP4* is necessary for normal photoreceptor ribbon synapse maintenance and outer segment formation, and for sperm development. *Hum Mol Genet*. 2011;20(3):482-96.
59. Hynes A, Giles R, Srivastava S, Eley L, Whitehead J, Danilenko M, et al. Murine Joubert syndrome reveals Hedgehog signaling defects as a potential therapeutic target for nephronophthisis. *Proc Natl Acad Sci U S A*. 2014;111(27):9893-8.
60. Madhivanan K, Aguilar R. Ciliopathies: the trafficking connection. *Traffic*. 2014;15(10):1031-56.
61. Szymanska K, Johnson C. The transition zone: an essential functional compartment of cilia. *Cilia*. 2012;1(1):10.
62. Mitchison H, Valente E. Motile and non-motile cilia in human pathology: from function to phenotypes. *J Pathol*. 2017;241(2):294-309.
63. Zaghloul N, Katsanis N. Functional modules, mutational load and human genetic disease. *Trends Genet*. 2010;26(4):168-76.
64. Struchtrup A, Wiegering A, Stork B, Rüther U, Gerhardt C. The ciliary protein *RPGRIP1L* governs autophagy independently of its proteasome-regulating function at the ciliary base in mouse embryonic fibroblasts. *Autophagy*. 2018;14(4):567-83.
65. Kim J, Krishnaswami S, Gleeson J. *CEP290* interacts with the centriolar satellite component *PCM-1* and is required for *Rab8* localization to the primary cilium. *Hum Mol Genet*. 2008;17(23):3796-805.

66. Coene K, Mans D, Boldt K, Gloeckner C, van Reeuwijk J, Bolat E, et al. The ciliopathy-associated protein homologs RPGRIP1 and RPGRIP1L are linked to cilium integrity through interaction with Nek4 serine/threonine kinase. *Hum Mol Genet.* 2011;20(18):3592-605.
67. Wiegering A, Rüther U, Gerhardt C. The ciliary protein Rpgrip1l in development and disease. *Dev Biol.* 2018;442(1):60-8.
68. Assis L, Silva-Junior R, Dolce L, Alborghetti M, Honorato R, Nascimento A, et al. The molecular motor Myosin Va interacts with the cilia-centrosomal protein RPGRIP1L. *Sci Rep.* 2017;7:43692.
69. Sang L, Miller J, Corbit K, Giles R, Brauer M, Otto E, et al. Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. *Cell.* 2011;145(4):513-28.
70. Barbelanne M, Hossain D, Chan D, Peränen J, Tsang W. Nephrocystin proteins NPHP5 and Cep290 regulate BBSome integrity, ciliary trafficking and cargo delivery. *Hum Mol Genet.* 2015;24(8):2185-200.
71. Kim Y, Kim S, Jung Y, Jung E, Kwon H, Kim J. Eupatilin rescues ciliary transition zone defects to ameliorate ciliopathy-related phenotypes. *J Clin Invest.* 2018;128(8):3642-8.
72. Shaheen R, Shamseldin H, Loucks C, Seidahmed M, Ansari S, Ibrahim Khalil M, et al. Mutations in CSPP1, encoding a core centrosomal protein, cause a range of ciliopathy phenotypes in humans. *Am J Hum Genet.* 2014;94(1):73-9.
73. Lu H, Galeano M, Ott E, Kaeslin G, Kausalya P, Kramer C, et al. Mutations in DZIP1L, which encodes a ciliary-transition-zone protein, cause autosomal recessive polycystic kidney disease. *Nat Genet.* 2017;49(7):1025-34.
74. Lapart J, Gottardo M, Cortier E, Duteyrat J, Augière C, Mangé A, et al. Dzip1 and Fam92 form a ciliary transition zone complex with cell type specific roles in *Drosophila*. *Elife.* 2019;8. pii: e49307.

75. Jack B, Mueller D, Fee A, Tetlow A, Avasthi P. Partially Redundant Actin Genes in Chlamydomonas Control Transition Zone Organization and Flagellum-Directed Traffic. *Cell Rep.* 2019;27(8):2459-67.e3.
76. Jensen V, Lambacher N, Li C, Mohan S, Williams C, Inglis P, et al. Role for intraflagellar transport in building a functional transition zone. *EMBO Rep.* 2018;19(12):pii: e45862.
77. Scheidel N, Blacque O. Intraflagellar Transport Complex A Genes Differentially Regulate Cilium Formation and Transition Zone Gating. *Curr Biol.* 2018;28(20):3279-87.e2.
78. Schou K, Mogensen J, Morthorst S, Nielsen B, Aleliunaite A, Serra-Marques A, et al. KIF13B establishes a CAV1-enriched microdomain at the ciliary transition zone to promote Sonic hedgehog signalling. *Nat Commun.* 2017;8:14177.
79. Dyson J, Conduit S, Feeney S, Hakim S, DiTommaso T, Fulcher A, et al. INPP5E regulates phosphoinositide-dependent cilia transition zone function. *J Cell Biol.* 2017;216(1):247-63.
80. Vieillard J, Paschaki M, Duteyrat J, Augière C, Cortier E, Lapart J, et al. Transition zone assembly and its contribution to axoneme formation in Drosophila male germ cells. *J Cell Biol.* 2016;214(7):875-89.
81. Pratt M, Titlow J, Davis I, Barker A, Dawe H, Raff J, et al. Drosophila sensory cilia lacking MKS proteins exhibit striking defects in development but only subtle defects in adults. *J Cell Sci.* 2016;129(20):3732-43.
82. Wei Q, Zhang Y, Schouteden C, Zhang Y, Zhang Q, Dong J, et al. The hydroletharus syndrome protein HYLS-1 regulates formation of the ciliary gate. *Nat Commun.* 2016;7:12437.
83. Slaats G, Isabella C, Kroes H, Dempsey J, Gremmels H, Monroe G, et al. MKS1 regulates ciliary INPP5E levels in Joubert syndrome. *J Med Genet.* 2016;53(1):62-72.

84. Bachmann-Gagescu R, Dona M, Hetterschijt L, Tonnaer E, Peters T, de Vrieze E, et al. The Ciliopathy Protein CC2D2A Associates with NINL and Functions in RAB8-MICAL3-Regulated Vesicle Trafficking. *PLoS Genet.* 2015;11(10):e1005575.
85. Damerla R, Cui C, Gabriel G, Liu X, Craige B, Gibbs B, et al. Novel Jbts17 mutant mouse model of Joubert syndrome with cilia transition zone defects and cerebellar and other ciliopathy related anomalies. *Hum Mol Genet.* 2015;24(14):3994-4005.
86. Basiri M, Ha A, Chadha A, Clark N, Polyanovsky A, Cook B, et al. A migrating ciliary gate compartmentalizes the site of axoneme assembly in *Drosophila* spermatids. *Curr Biol.* 2014;24(22):2622-31.
87. Cevik S, Sanders A, Van Wijk E, Boldt K, Clarke L, van Reeuwijk J, et al. Active transport and diffusion barriers restrict Joubert Syndrome-associated ARL13B/ARL-13 to an Inv-like ciliary membrane subdomain. *PLoS Genet.* 2013;9(12):e1003977.
88. Wang W, Tay H, Soni R, Perumal G, Goll M, Macaluso F, et al. CEP162 is an axoneme-recognition protein promoting ciliary transition zone assembly at the cilia base. *Nat Cell Biol.* 2013;15(6):591-601.
89. Aubusson-Fleury A, Lemullos M, de Loubresse N, Laligné C, Cohen J, Rosnet O, et al. The conserved centrosomal protein FOR20 is required for assembly of the transition zone and basal body docking at the cell surface. *J Cell Sci.* 2012;125(Pt 18):4395-404.
90. Huang L, Szymanska K, Jensen V, Janecke A, Innes A, Davis E, et al. TMEM237 is mutated in individuals with a Joubert syndrome related disorder and expands the role of the TMEM family at the ciliary transition zone. *Am J Hum Genet.* 2011;89(6):713-30.
91. Hsu P, Scott D, Weinstein J, Ran F, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat Biotechnol.* 2013;31(9):827-32.
92. Garcia-Gonzalo F, Phua S, Roberson E, Garcia Gr, Abedin M, Schurmans S, et al. Phosphoinositides Regulate Ciliary Protein Trafficking to Modulate Hedgehog Signaling. *Dev Cell.* 2015;34(4):400-9.

Figures and Figure Legends

Figure 1

Figure 1: In mouse cells, ciliary gating is disturbed by the loss of *Rpgrip1* but not by the loss of *Nphp4*.

(A) Immunofluorescence on MEFs obtained from WT (n=5) and *Rpgrip1*^{-/-} (n=5) embryos. (B) Immunofluorescence on MEFs obtained from WT (Arl13b: n=4; Sstr3: n=3) and *Nphp4*^{-/-} (Arl13b: n=4; Sstr3: n=3) embryos. (A, B) The ciliary axoneme is stained in green by acetylated α -tubulin, the basal body is stained in blue by γ -tubulin. The scale bars represent a length of 0.5 μ m.

Figure 2

Figure 2: Loss of Cep290 but not of Nphp1 or Invs impairs ciliary gating in mouse cells.

(A, B) Immunofluorescence on NIH3T3 cells. The ciliary axoneme is stained in green by acetylated α -tubulin. The scale bars represent a length of 0.5 μ m.

Figure 3

Figure 3: Rescue of the Cep290 amount at the TZ of *RPGRIP1L*^{-/-} HEK293 cells restores the ciliary Arl13b amount.

(A) Western blot analysis with lysates obtained from *RPGRIP1L*^{-/-} HEK293 cells (clone 1-7). (B-D) Immunofluorescence on *RPGRIP1L*^{+/+} HEK293 cells and *RPGRIP1L*^{-/-} HEK293 cells (clone 1-7). The ciliary axoneme is stained in green by acetylated α -tubulin, the basal body is stained in blue by γ -tubulin (B, C). The scale bar represents a length of 1 μ m (B) or 0.5 μ m (C, D). (E) Ciliary length quantification.

Figure 4

Figure 4: Eupatilin treatment rescues ciliary gating in the absence of Rpgrip11.

(A, B) Immunofluorescence on *Rpgrip11*^{+/+} and *Rpgrip11*^{-/-} NIH3T3 cells. The ciliary axoneme is stained in green by acetylated α -tubulin. The scale bars represent a length of 0.5 μ m. (C) Ciliary length quantification. (D) Model of ciliary gating hierarchy. Interaction partners are depicted in circles.