PROJECT FINAL REPORT

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4.1 Final publishable summary report

A. Executive summary

During the past five years, SYSCILIA has delivered several large scale datasets to finally describe the first landscape of the ciliary system in health and disease. High resolution localization studies to define the sub-ciliary localization and transport add to the information on the connectivity within the ciliary protein network, and the developed models have generated important insights into disease mechanisms and pinpointed potential therapeutic avenues.

WP1 has generated a solid data framework that serves as a template for the final definition of the ciliary protein landscape as well as for the systems biology- and systems medicine-based analysis of cilia and ciliopathies.

WP2 operated as the hub for data storage and processing in SYSCILIA, which drove the analysis and integration of proteomics, mutation and public domain data for different modeling approaches. The developed tools for accessing the project's datasets will remain operational on EMBL servers.

WP3 applied different modelling approaches and computational tools to SYSCILIA ciliary datasets and, as needed, to externally generated data. A cross-species analysis has revealed the order in which the IFT-A, -B and BBSome components of the ciliary machinery evolved. The SYSCILIA Gold Standard protein set was defined which is adopted and used by the ciliary research field., and a ciliary Gene Ontology with 50 new terms has been developed in collaboration with the GO consortium.

In **WP4,** the integrative (Bayesian) analysis of diverse types of data (proteomic, disease/phenotype annotations, and resequencing datasets) has provided important insights into the function and behavior of cilia and identified novel ciliopathy genes and ciliopathy-associated modules. Mapping of pathway annotation data on identified disease networks allowed us to identify biological pathways that may be responsible for specific phenotypes, while others may be related to several ciliopathy phenotypes.

WP5 uncovered principle mechanisms of ciliary polarization, organ development, and signalling. The interaction between ciliopathy-associated modules and the actin cytoskeleton was detailed, and it was shown that cilia are involved in a highly conserved program termed rosette formation, that is utilized in the vertebrate kidney to extend tubules thereby creating the final geometry of mature nephrons. SYSCILIA also found that blocking one up-regulated signalling pathway (e.g. mTOR signalling) rapidly results in activation of other (bypass) signalling pathways, a manner otherwise observed in the therapy of cancers. This essentially has to be taken into account when designing ciliotherapies.

In **WP6** genes of interest were systematically disrupted using knockdown and knockout technologies, and the effects subsequently analysed using a wide range of cilium structure/function assays. This yielded many new (ciliopathy) genes required for normal cilium formation and function. These included vesicle transport regulators, components of the ciliary transition zone diffusion barrier and cilium-based signalling mediators. Also, SYSCILIA gained important new insights into the mechanisms of action of known ciliary disease genes, including new paradigms linking ciliopathy proteins with DNA damage response, chromatin remodelling and proteasome activity.

The whole genome siRNA-based reverse genetics screen for defects in ciliogenesis of **WP7** yielded a global resource for investigation into essential ciliary processes, have provided new insights into ciliogenesis complexity, and have identified roles for unanticipated pathways in human genetic disease.

WP8 assembled a cohort of both motile and primary ciliopathy cases that represent the full spectrum of phenotypic severity (mild, moderate and severe). Targeted resequencing and copy number variant (CNV) analysis of 785 ciliary genes was followed by functional validation in physiologically relevant zebrafish models to test allele pathogenicity. The genetic and functional data from the ciliopathy cohort were merged with experimental systems biology networks established by SYSCILIA.

WP9 treated patient cells successfully with known drugs and identified novel potential drugs to modulate cilia through the drug screen. To compensate for genetic heterogeneity in ciliopathies, we have also investigated the effectiveness of translational read-through inducing drugs (TRIDs) as therapy for several

genetic diseases caused by in-frame nonsense mutations. Modified aminoglycosides and PTC124 were found to hold great potential in treating the 10-20% of patients harbouring nonsense mutations. Although we are still quite far from entirely ameliorating the disease process in ciliopathies, SYSCILIA has laid a solid foundation for further work in this field.

B. Summary of project context and objectives

SYSCILIA aims to apply systems approaches to understand the basic biological processes underlying the role of cilia in human disease, and to develop models capable of predicting the effects of discrete perturbations or mutations in those protein networks that underpin cilia function. Cilia are ideal organelles for systems biology as they can be regarded as semi-closed systems being both largely spatially and biologically separated from other cellular structures and processes.

WP 1 aimed at the generation of a solid framework for the systems biology-based analysis of ciliary functions in health and disease. To this end, it was necessary to generate several types of data. The definition of the ciliary proteome of different types of cilia was a first step which was to be used, together with further resources to define the baits for systematic protein complex analysis and to define the ciliary protein interaction network by affinity purification in combination with mass spectrometry but also by yeast-two-hybrid screens. To understand principle mechanisms of disease, it was the goal of WP1 to identify alterations within the network due to different cellular states and or due to ciliopathy-associated mutations. For biochemical validation and testing of hypotheses but also for the analysis of distinct localization patterns of well-known as well as novel ciliary proteins, the development of new reagents and approaches was envisioned. Taken together, WP1 was designed to deliver data and tools, necessary to conduct the SYSCILIA approach to understand ciliary function and dys-function.

WP2 served as the central resource of the whole project. It aimed to provide a data storing and exchange system including Wiki-pages for internal documents, a consortium website for publications, a data server for feeding and querying of scientific data and analysis. To provide system biology ready inputs and facilitate the modelling approaches, raw data from experimental WPs were combined, incorporated, and embellished with public data sources. This includes protein annotations, sequencing data, interactions, phenotypes, and diseases related information. WP2 also revised and applied the *socio-affinity algorithm* to analyse the ciliary interactome, which results in a graphical protein-protein-interaction (PPI) network featured with GeneOntology annotation, pathogenic mutations, and results of phenotype screening. This PPI network provided a solid basis for subsequent cluster and modelling analysis in WP3 and WP4. Project related data from literature was also collected and maintained in the *SysWiz* software tool, a combined ciliary database and viewer.

WP3 was the middle of three computational workpackages, WP2 building and delivering resources and WP4 focusing on the network modelling. Data generated in experimental WPs would also feed into the WP3 work. A major objective was to generate large scale systems biology ready information to feed into systems modelling which was mainly conducted in WP4. A related objective was to assemble ciliary interactomes, functional networks, and pathways. These formed the basis to integrate ciliopathy mutations, siRNA knockdown ciliary phenotypes, worm and fish knockdown or knockout phenotypes etc. New methods to assess the networks have been developed for application in WP4. As needed, statistical analyses and bioinformatics methods development were applied to the siRNA datasets and to EPASIS proteomic datasets. Another objective was to apply comparative evolutionary analysis to ciliary function and disease. New insight has been provided into the evolution of the ciliary transport apparatus and it's integration into the networks allowed disease predictions to be made using the Ciliocarta system. An objective of the WP was also to provide predictions for experimental testing. These have included microtubule end-binding proteins, new IFT proteins and new components of motor protein complexes and connections between ciliary complexes and new candidate ciliopathy disease proteins.

The main objective of WP4 was to integrate the datasets generated by the consortium into a network view of core ciliary processes and to exploit the data to make predictions about the function of cilium and the pathogenesis of ciliopathies. The first step was to construct a large, annotated network of ciliary function, with known or predicted interactions based on the output from WP2 and WP3. In the beginning of the project the majority of the data came from public databases and consortium data were integrated during the

project. One of the first milestones of WP4 was to partition the constructed networks into protein complexes/functional modules. As the project proceeded and more and more data became available predictive methods were built to make use of the available data in the absence of detailed knowledge. The methods were designed so as to make predictions that not only would improve our knowledge but would also be possible to validate them through experiments. WP4 provided the appropriate tools for the integration and the analysis of the consortium data, aiming at the improvement of our understanding about ciliary function and ciliopathies.

It is currently unknown how cilia control the multi-cellular assembly of organs and tissues. Thus, the primary focus of WP5 was the analysis of basic characteristics that establish cilia as a secluded organelle, including 1) the recruitment of cargo to the cilium and transport and assembly of protein complexes within the ciliary compartment, 2) the mechanistic properties that enable motile cilia to generate fluid and particles flows (i.e. ciliary polarization), and 3) principle ciliary outputs such as Wnt and mTOR signalling. The consortium focused on establishing methods to measure ciliary transport in at least three different assay systems: cultured mammalian cells that are amendable to genetic manipulation, photoreceptor cells that are the target of several ciliopathies, and *C. elegans*, a powerful model organism suitable for genetic screens. Ciliary transport was successfully monitored by high resolution localization studies of molecules related to transport modules and fluorescence recovery after photobleaching (FRAP) assays in these models. Ciliary polarization was successfully analyzed using fluorescent markers that label the basal body and the basal rootlet in a *Xenopus* epidermis model. Fluid flow speed across the ciliated-epidermal skin was measured by tracking fluorescent particles added to the culture media of *Xenopus* embryos. Time-lapse imaging *in vivo* revealed a functional crosstalk between ciliopathy-associated modules (CAMs), and the actin cytoskeleton. The membrane-cytoskeletal linker protein complexes were found to regulate centriole/basal body migration, docking and spacing, a crucial step during ciliogenesis in multiciliated cells. Cilia have evolved as signalling hubs that control important pathways such as Shh and Wnt during organogenesis. In the developing kidney, ciliary defects typically disrupt the normal organ architecture and cause cystic kidney disease during the second half of embryogenesis. During this phase of kidney development, immature nephrons undergo rapid elongation to form the tubular structures of the mature organ. The consortium has introduced and systematically implemented suitable *in vivo* systems to assess ciliary signalling. Time-lapse movies in embryonic kidneys of *Xenopus*, the assessment of ciliary signalling in cell culture, and the characterization of different ciliopathy mouse models resulted in substantial progress towards the decipherment of the roles of different ciliary/ciliopathy-associated proteins particularly in the mTOR, the Wnt and the Shh signaling pathways.

The overall goal of WP6 was to validate and assess the functions of known and candidate CAM components derived from other workpackages within SYSCILIA. This was to be acheived by disrupting genes of interest in knock-down (siRNA, morpholinos) or knockout cell and animal-based (*in vivo*) systems, including ciliated mammalian cell-culture (siRNA), *C. elegans* mutant alleles, zebrafish mutant alleles and morphants, Xenopus morphants, and knock-out mice. Using a variety of assays, including those developed and presented in WP5, this workpackage assessed the effects of gene disruption on cilium structure, ciliary transport, ciliary polarization and cilium-based signalling. WP6 had four principle objectives. Objective 6.1 was to employ a semi high-throughput approach in mammalian cell culture and *C. elegans* to validate and further interrogate the roles of candidate CAM components (informed from WP1-4, 7 and 8) in cilium formation, maintenance, function, signaling and trafficking. Objective 6.2 strived to establish and functionally assess animal models (zebrafish, Xenopus, mice) of individual candidate and known CAM components, employing systematic integrated and strategic methodologies (e.g., ciliary transport, polarity, signalling) developed in WP5 and elsewhere. Objective 6.3 was to use animal models (*C. elegans*, zebrafish, mice) to determine the genetic interrelationships between known/candidate components of individual and different CAMs. Objective 6.4 sought to integrate all phenotypic profiling data into a public database of CAM constituents, as a component of the central SYSCILIA resource.

WP7 describes high-throughput functional genomics approaches to overcome bottlenecks in the molecular description of the cilium. We performed an integrated series of reverse genetics RNAi screens to validate, identify and confirm genes important in functional ciliary modules and complexes implicated in ciliopathy

phenotypes (ciliopathy-associated modules; CAMs). The outcome of this effort will be a public database of CAM genes that will have considerable use in several ways:

- identify new candidate CAM genes involved in ciliogenesis, ciliary maintenance and resorbtion
- integrate this knowledge in an interactive database to output from WP1 “Mapping the ciliome”, and to inform the selection of potential functional components in ciliary interactomes in WP3 “Construction, comparison and application of ciliary interactomes”, WP4 “Integrative modelling and predictions of ciliary system behaviour” and WP6.1 “Perturbation of CAMs in ciliated mammalian cell culture models”
- inform the choice of candidate and other ciliopathy genes for mutation analysis by high-throughput medical resequencing in WP8
- identify potential novel targets for rapid validation and testing of ciliotherapeutics in WP9

RNAi screening is a key relevant strategy for systems biology approaches for basic biological processes relevant to health and disease. In this context, RNAi screening is a powerful and rapid strategy for collecting quantitative data for hypothesis generation. The integrated approach of SYSCILIA allowed identified candidate CAM genes to be rapidly evaluated in functional assays (WP5). Successful screens for CAM genes have powerful synergies when integrated with previous and on-going research in ciliary function, and the molecular genetics (WP8) and clinical handling of ciliopathies.

WP8 is focused on the systematic, saturated identification of the total mutational load of the ciliary proteome in a large patient cohort whose diversity can capture the breadth of clinical manifestations of ciliary dysfunction in humans. This goal was achieved by the pooling of diverse clinical cohorts onto a single resource, followed by deep resequencing of the ciliary proteome as defined previously and expanded upon through the work of other WPs and the follow-up functional evaluation of candidate mutations through a shared, coordinated work environment. This WP had four objectives. 1) To establish a cohort of ~450 well-characterized patients with diverse types of ciliopathies ranging from mild to severe and create a database with relevant clinical and genetic information. 2) To perform next generation sequencing of this cohort on a large set of genes (~800) and determine total mutational load, followed by validations and development of a ciliary mutational database. 3) To functionally validate sequence variants to discern pathogenic from benign variants in a robust and physiologically relevant (*in vivo*) zebrafish system. And 4) To integrate data on functional sequence variants and clinical information with information on ciliary associated modules (CAM)s to come to a system model for explaining and predicting phenotypes and their variability.

WP9 aimed at translational systems biology approaches to counter ciliary dysfunction in the disease setting. The first two objectives of WP9, ‘assaying functional ciliary reconstitution: knowledge-based approach’ and ‘assaying functional ciliary reconstitution: compound screens’ outlined parallel strategies to investigate pharmaceutical interventions aimed at retarding progression of the degenerative processes associated with ciliopathies, namely in the kidney and the retina.The last objective, ‘lead compound characterization’ aimed at taking candidate drugs forward into lead compound testing in mice and/or humans.

To ensure more efficient collaborations and transfer of skills among the consortium partners WP10 coordinated training activities – workshops, webinars, etc. - inside the project. The second objective of WP10 was to raise public participation and awareness towards translation of the specific knowledge gained through this project to the scientific community as well as to enable translation of project results into medical and industrial applications and products. Specifically, this WP will strive to raise public awareness towards its activities via its public website, press releases, and the cooperation with PR-departments at the different partner institutions. The procedures, models, knowledge, reagents and skills shall be transferred and made available to the scientific community as well as to the pharmaceutical and chemical industry. The long-term effect will be to make the European industry more competitive with the latest technologies.

SYSCILIA was a large project, both in terms of manpower and number of parties involved, with ambitious interdisciplinary objectives. Thus, effectiveness of the overall coordination and management structure was key ensuring the overall coordination and the management of interfaces between WPs and components.

C. Main S&T results/foregrounds

Work Package 1; Mapping the Ciliome.

WP1 aimed at generating a solid basis for the analysis and integration of SYSCILIA results into an interaction network of ciliary proteins. The fact that knowledge about protein interactions within the cilium as well as of cilia-associated proteins was sparse, it was necessary to generate robust data on ciliary complexes and interactions. To achieve this, affinity-based methods in combination with mass spectrometry, quantitative and qualitative, were supplemented with yeast-2-hybrid (Y2H) as well as with the determination of the ciliary protein inventory by quantitative proteomic methods. The definition of the ciliary protein inventory could be achieved by the establishment of protocols for the isolation of cilia from bovine photoreceptors and from hTERT-RP1 cells. Both approaches were combined with quantitative mass spectrometry to determine the content of the cilia-enriched fraction by subtracting contaminants that were equally abundant in a crude fraction. This resulted in a robust set of ciliary proteins for both cell types. Together with a bioinformatics study to generate a count for all studies which defined the ciliary inventory by various methods as well as expert knowledge and the ciliary gold standard this served as the basis for selection of baits to identify the ciliary protein network.

To analyse the ciliary interaction network but also to validate complexes and interactions identified, the development of novel tools and approaches was necessary. One of these approaches was the “Elution profile analysis of SDS-induced sub-complexes” (EPASIS). It was developed in close collaboration of wet lab scientists and bio-informaticians and was applied to the identification of sub-complexes within 18 protein complexes. In addition to the information about sub-complexes, data on relative binding affinities were generated for all these complexes by the EPASIS approach (**figure 1**). The identification of protein complexes within tissue is a further important step for validation. The ICPL-IP was specifically established for this purpose. It combines immunoprecipitation from tissue samples with quantitative mass spectrometry and enables the identification and validation of interactions within their native tissue.

217 proteins were then selected and constructs were generated to express these proteins, fused to the SF-TAP tag in cells and to purify the protein complexes. The contents of each purification were identified by mass spectrometry and integrated into an in-house database for accession, annotation and storage.

To identify direct protein-protein interaction, we have employed the yeast two-hybrid (Y2H) technique. We have performed yeast two-hybrid screens (68 in total) in the adult kidney library (5 different bait proteins), fetal brain (8 different bait proteins) and in human and bovine retinal libraries (26 different bait proteins). This resulted in a Y2H library screen network of in total 417 proteins and 523 unique protein-protein interactions. In addition we employed the targeted screen (ciliary Y2H grid), including more than 200 clones, expressing ciliary and centrosomal proteins, to test for binary ciliary interactions with 52 different bait proteins. This resulted in a Y2H targeted screen network of in total 114 proteins and 355 unique protein-protein interactions. All Y2H data was processed and integrated into an in-house database and submitted to WP2 partners for further analysis. The final data consisting of 41,352 unique interactions between 4,701 proteins were then submitted to WP2 Partners for bio-informatic analysis by socio-affinity and hierarchical clique determination to identify remove false positive interaction and to determine the significance levels for interactions as well as to assemble proteins into complexes. By this approach, we could identify 43 high confidence complexes with a defined core and 71 attachments. The whole network, including proteins and their interactions which are not assigned to complexes, consists of 1,181 proteins and 4,318 interactions (false positive rate < 0.005). Approximately half of the identified complexes were previously described at least in parts, and the rest are novel. Even for several of the previously described complexes, we could achieve a more comprehensive description of the composition. For IFT complex B for example, we could show the interaction with the BLOC complex involved in vesicle formation and cargo loading as well as the interaction with a fraction of proteins that are highly enriched in membrane proteins. Together with the BLOC interaction, this provides a potential new functional link of the IFT-B complex to membrane structures. Further complexes and interactions within the network were subjected to validation by various methods. The interaction of the IFT-A complex with Lebercilin as well as with Tulp3 was validated extensively by

targeted proteomic methods. Furthermore, the stoichiometry of this complex was determined. We could demonstrate that it is composed of a highly repetitive assembly of the six proteins, resulting in a complex size of at least 4.2 MDa.

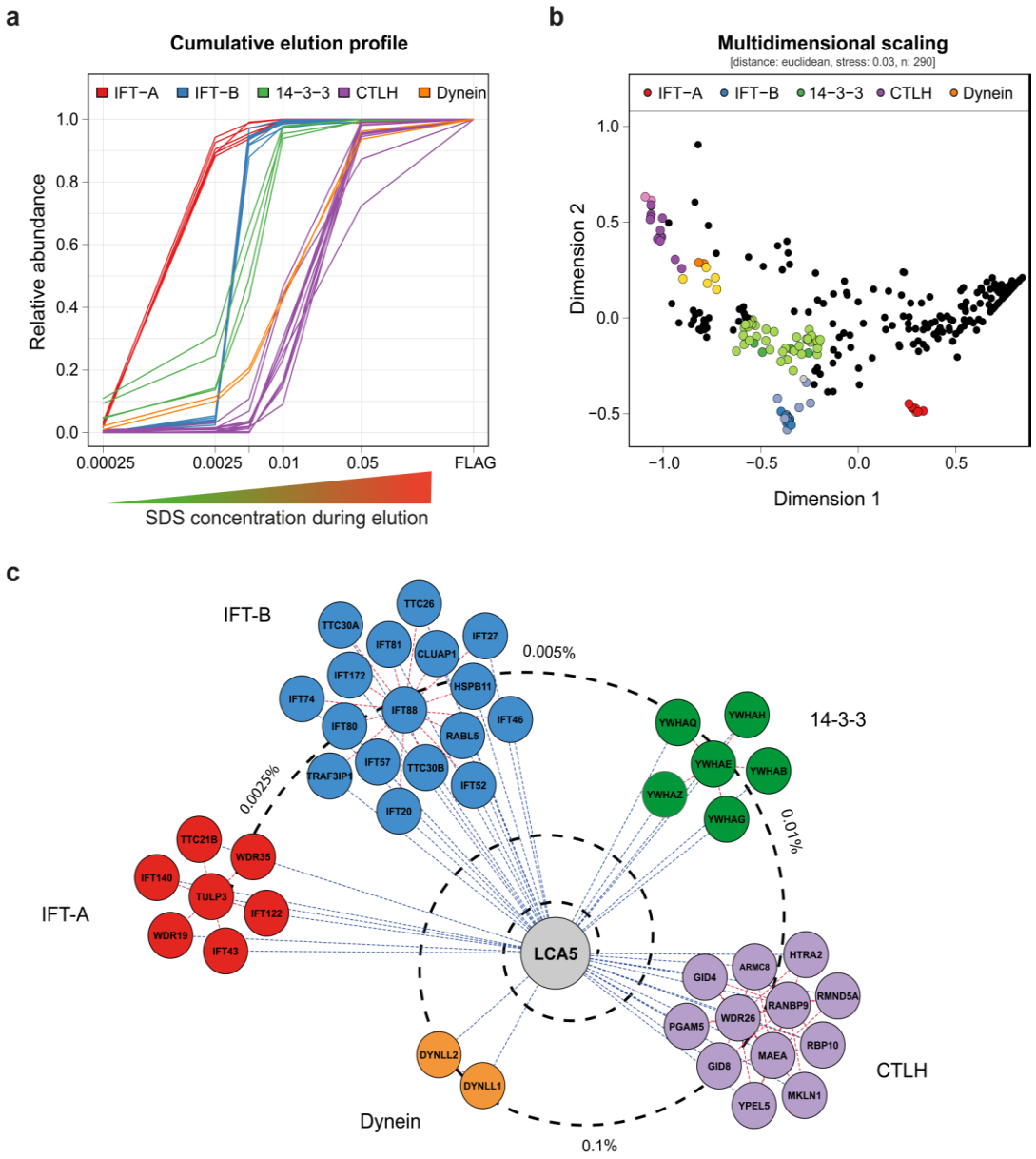

Figure 1: SDS destabilization of the IFT/Lebercilin protein complex. (a) Visualization of the elution profiles after analyzing the eluted fractions by LC-MS/MS and label-free quantification. The cumulative relative abundance (y-axis) is plotted against increasing SDS concentrations (x-axis). For each sub-module, consensus profiles were generated from known members. (b) Non-metric multidimensional scaling ordination plot based on Euclidean distances of elution profiles (stress 0.03). Data points (n=386) present the average of replicated data (n=8). (c) The predicted network of the interactome of Lebercilin. The closer they are to Lebercilin the higher the SDS-concentration that is needed for their complete dissociation. The dashed spiral line symbolizes the different SDS concentrations. The interactions shown by blue, dashed lines were determined by EPASIS, the red, dashed lines by SF-TAP.

In addition to the identification and validation of the ciliary protein network, the perturbations induced by selected mutations were investigated to understand mechanisms of disease. This necessitates the application of quantitative methods (SILAC) in combination with affinity purification to directly compare the

complexes formed by the WT form and the mutant form of a protein. The application of this approach is nicely illustrated by its application to the Lebercilin complex. We could detect the interaction of Lebercilin with both, the IFT-A and the IFT-B complex as well as with Dynein, the GID and the 14-3-3 complex. While Leber congenital amaurosis-associated mutations did not affect the binding of Lebercilin to the 14-3-3 and the Dynein complex, the GID complex as well as both IFT complexes are not able to interact with both Leber congenital amaurosis-associated mutants tested.

A final step and proof of principle of the integrated Component 1 work programme was to validate predictions and interactions that resulted from bio-informatic analysis of the interaction network. In terms of interactions, we attempted the validation of 34 interactions by targeted proteomics approaches. 25 of these interactions could be successfully validated. In addition, two predictions were tested. First, network analysis predicted that the IFT-B complex consists of two sub-modules. We could validate this prediction by the use of sucrose density centrifugation to separate the modules as well as by EPASIS to define the sub-modules. Second, structural analysis of IFT-B variants predicted an effect on the structure for several variants, identified in patients. 5 variants were tested and for 3 of those, we could show that the alterations induced by these variants affect one of the two IFT-B sub-modules specifically.

We have identified multiple proteins which directly interact with the Usher syndrome proteins SANS (USH1G), Harmonin (USH1C) and Vlgr1b/GPR98 (USH2C) by using large-scale yeast-2-hybrid (Y2H) screening and cell culture-based tandem affinity purification (SF-TAP using HEK293 cells) of protein complexes followed by mass-spectrometry. We have also generated rAAV viruses that are suitable to express SF-TAP-transgenes under the control of the strong mouse Opsin promoter. After the rAAV transfer via subretinal injection into mouse eyes and the retinal expression of the transgene, isolated retinas were subjected to TAPs and the composition of the purified protein complexes were be determined by MS analyses. Numerous of the distinct hits derived from the Y2H screen and TAP analyses were validated with complementary interaction assays to confirm these novel ciliary protein complexes. Via pull-down analyses, co-immunoprecipitation assays, membrane targeting assays and proximity ligation assays, we continuously built up a protein interactome related to Usher syndrome and other ciliopathies.

Additionally, we have examined the subcellular and subciliary locations of these proteins in murine, macaque and human retinas using correlative high-resolution immunofluorescence and immunoelectron microscopy. These include the Usher proteins myosin VIIa, Harmonin, Proto-Cadherin 15, SANS, Whirlin, Vlgr1 and USH2A. Using the same techniques, we have also defined the spatial distribution of IFT proteins, BBS proteins and numerous other ciliopathy-related proteins such as Myomegalin TOPORS, SPAG5, ARL2BP, TUB protein, FAM161A, and Magi2 in retinal sections. The co-distribution of SANS and Magi2 in the periciliary region of primary cilia prompted us to functionally test whether the complex partners contribute to ciliogenesis and/or maintenance of primary cilia. We showed that the SANS-Magi2-complex assembly is regulated by phosphorylation. In addition, we demonstrated that phosphorylated SANS tightly regulates Magi2-mediated endocytosis. Furthermore, our data indicate that the presence of Magi2 in the periciliary compartment and during endocytosis processes seem to be essential for ciliogenesis.

We selected two modules revolving around the Ninein-like protein (NINL) to a detailed investigation. In the first project, we identify NINL as a physical and genetic interaction partner of CC2D2A. Using a zebrafish model, we demonstrate that absence of Ninl or Cc2d2a results in similar retinal phenotypes, including short outer segments, Opsin mislocalization and accumulation of vesicles/vacuoles. Affinity proteomics identifies MICAL3, a protein previously shown to bind RAB8A, as an important NINL interaction partner. We propose a model where CC2D2A provides a docking point at the photoreceptor ciliary base, allowing RAB8A-positive vesicles to bind through a series of interactions involving CC2D2A-NINL-MICAL3-RAB8. In the second project we scrutinize the function of NINL with combined proteomic and zebrafish knockdown approaches. We identify the protein Double zinc ribbon and ankyrin repeat domains 1 (DZANK1) as a novel interaction partner of NINL^{isoB} and show that loss of function of *ninl*, *dzank1* or a genetic interaction leads to dysmorphic photoreceptor outer segments, accumulation of trans-Golgi-derived vesicles, mislocalization of Rhodopsin and USH2a in the zebrafish model. We further demonstrate that NINL and DZANK1 associate specifically with complementary subunits of the cytoplasmic Dynein1 motor complex and that retrograde Dynein-based transport is impaired in zebrafish morphants.

Work Package 2; Central Resource for Data Integration.

The most significant output of WP2 has been the final SYSCILIA landscape of protein modules involved in ciliary function and dysfunction. This was derived by a modified socioaffinity metric and by processing data particularly from WP1 and WP8, but also from many other WPs. The findings from this final network have led to important insights into ciliary transport, such as the resolution of sub-complexes in IFT-B, and roughly 30 novel complexes linked to ciliary function and disease. This, and the databases and interfaces to analyse these data, will be a significant boost to the global ciliary and ciliopathy research community.

A bioinformatics tool was developed by the SYSCILIA-IT group for the construction, maintenance and querying of a relational database using properly annotated tab delimited data files. The tool was developed using SQLalchemy, a set of Python tools for working with databases. Database construction was facilitated by a tool developed by partner 13/18 that creates the tables of the database and their relationships from a set of tab delimited files. Such software tools makes the integration of new data easier when they become available, as the correct formatting of the data files is sufficient for their aggregation to the database. The use of Python and SQLalchemy also facilitated the development of a web application, to make the database accessible for all partners. Flask, a tool for web application development using Python, was used for this purpose. To enable graphical representations of network information the cytoscape.js graph library was integrated. The data integration system, BDT (Big Data Table), and the graphical query/analysis interface, Quest, together form an application entity facilitating the data update integration analysis query workflow. Apart from SYSCILIA the developed tool can be used to facilitate the creation of relational databases in other projects that require the existence of a central data resource.

Work Package 3; Construction, comparison and application of ciliary interactomes.

The integrative databases of WP2 were also crucial for the accomplishment of several WP3 project tasks. Combination of protein interaction data with disease/phenotype annotations allowed the identification of graph areas enriched in disease causing genes or the prediction of modules associated with a specific phenotype. Using a graph-based probabilistic algorithm developed in WP4 and integrating the PPI network constructed from the SYSCILIA TAP data and phenotype data from Human Phenotype Ontology, novel ciliopathic genes and biological mechanisms responsible for known ciliopathy phenotypes were predicted. The inclusion of predicted genes to currently known disease genes can improve our current knowledge about phenotype mechanisms by providing a more complete picture of the underlying pathways from which hypotheses about the genesis of the phenotypes may be drawn. Mapping pathway annotations (from Reactome and Kegg data) to the predicted phenotype modules we identified pathways enriched in different ciliopathic phenotypes. The result is a phenotype-pathway network in which a phenotype is connected to all pathways that are enriched in this module. The pathway-phenotype network can be used not only for the prediction of pathways responsible for a specific ciliopathic phenotype but also to explain the genotypic and phenotypic complexity of ciliopathies by identifying pathways that may be responsible for several phenotypes. Similarly, disease-phenotype annotations were used for the identification of ciliary related diseases that are not currently considered ciliopathies.

The intraflagellar transport (IFT) complex is an integral component of the cilium, a quintessential organelle of the eukaryotic cell. The IFT system consists of three sub complexes [i.e., intraflagellar transport (IFT)-A, IFT-B, and the BBSome], which together transport proteins and other molecules along the cilium. The evolution of the IFT has been under heated discussion in the public debate on creationism versus evolution, specifically it has been suggested that IFT belongs to a special class of molecular machines that show irreducible complexity ergo it must have been created. On the other side it has been proposed that the IFT complexes originated from vesicle coats similar to coat protein complex I (COPI), COPII, and clathrin. Although the irreducible complexity viewpoint has no scientific basis and has been thoroughly debunked, phylogenetic evidence for the coatomer origin of IFT was lacking. SYSCILIA provided phylogenetic evidence for common ancestry of IFT subunits and α, β', and ε subunits of COPI, and trace the origins of the IFT-A, IFT-B, and the BBSome sub complexes. We find that IFT-A and the BBSome likely arose from an IFT-B-like complex by intracomplex subunit duplication. The distribution of IFT proteins across eukaryotes identifies the BBSome as

a frequently lost, modular component of the IFT. Significantly, loss of the BBSome from a taxon is a frequent precursor to complete cilium loss in related taxa. Given the inferred late origin of the BBSome in cilium evolution and its frequent loss, the IFT complex behaves as a "last-in, first-out" system. The protocoatomer origin of the IFT complex corroborates involvement of IFT components in vesicle transport. Expansion of IFT subunits by duplication and their subsequent independent loss supports the idea of modularity and structural independence of the IFT sub complexes. This work has been published in the Proceedings of the National Academy of Sciences (PNAS).

Evolutionary signatures of the cilium were used to predict novel ciliary components by way of co-evolution. A specially created database of orthologous genes spanning over 50 diverse eukaryotic genomes was used to calculate phylogenetic profiles for individual gene families, which were then compared to the phylogenetic profile of the cilium. These predictions were combined into the Ciliacarta (manuscript in preparation) and used by various partners to filter candidate genes in WP1 and WP 4. Gene expression screening combined with our phylogenetic profile approach has resulted in the identification of a novel transition zone protein TMEM107. Phylogenetic and sequence analysis of the gene family has guided experiments. The construction of co-evolving sub modules has provided additional evidence for a distinct sub complex in the transitionzone in which TMEM107 plays a central role. The manuscript describing TMEM107 is under revision at Nature Cell Biology.

By reconstructing the evolutionary paths of all gene families involved in cilia in human but also in many other organisms we now have an evolutionary model for how the ciliome has changed in evolution. We have created a detailed account of when duplications, gene losses and gene inventions took place and are able to observe events that pinpoint the emergence of novel ciliary specialisations, such as the photoreceptor cells at the origin of the vertebrates.

WP3 contributed the bioinformatics tool development and statistical analyses for the EPASIS method of protein complex decomposition by SDS concentration. The method has been applied to multiple protein complexes, in each case given informative subcomplex information and appears to be very generally applicable as part of the proteomics tool suite.

In an external collaboration, Partner 3 helped identify ~50 SxIP novel motif instances. SxIP is the key contact module for microtubule plus tip regulators and thus is a key module for cilia growth. Given the SYSCILIA protein networks and gold standard, we are now in position to apply th eSxIP pipeline for a more fine grained analysis of ciliary proteins.

Whole mRNA transcriptomic sequencing datasets have been obtained for growing cells versus, starved, ciliated cells. There are remarkable differences between the expressed mRNA isoforms for these different cell stages. These data are still being processed after the end of the grant period, but may have a substantial impact on the understanding of how gene expression is modulated to generate cell cycle-specific alternative transcripts.

In order to objectively evaluate the quality of SYSCILIA datasets and bioinformatic analyses we developed a gold standard of ciliary genes. A carefully compiled list of genes was generated by combining multiple public datasets. This list was then manually curated to remove false positives. Genes were added from literature and genes where annotated by ciliary localisation as well as disease. This list has been published as a resource to the community in the Cilia journal. The SYSCILIA gold standard was a necessity since it was observed that Gene Ontology, a annotation resource usually applied for dataset evaluation, contained many significant errors in annotation and definitions. We have since then put effort to improve Gene Ontology by collaborating with the Gene Ontology consortium and have added over 50 new Ontology terms as well as provided annotation for a significant part of our earlier defined Gold Standard. This work is directly beneficial for all SYSCILIA partners, the cilia community and the whole scientific community as the aim of the Gene Ontology consortium is to standardise the representation of gene and gene product attributes across species and databases. A manuscript on improving the Gene Ontology is in preparation.

Work Package 4; Integrative modelling and predictions of ciliary system behavior

For the identification of ciliary complexes from the Tandem Affinity Purification data collected in WP1, a new clustering algorithm was developed. Existing algorithms introduce several parameters that are not biologically supported and cannot be directly extracted from the data. For this reason in most cases a set of reference complexes is used to train the algorithm and identify the optimal parameters. The proposed algorithm does not suffer from this issue, as it is non-parametric. Despite its simplicity the algorithm is very efficient in predicting protein complexes from high-throughput TAP data, which are characterized by an increased level of noise. We evaluated its performance using three AP-MS datasets: two from *Saccharomyces cerevisiae* that have been extensively used as benchmarks, and a more recent one from *Drosophila melanogaster*. The results indicate that the algorithm performed better or equally well with existing parametric methods without introducing unnecessary parameters. Due to the lack of parameters, there is no need to identify optimal parameter values, easing its use by non-experts and increasing its reliability in the absence of reference datasets. Applying the algorithm to the SYSCILIA data we have predicted ~100 complexes from which ~70 are considered to be related to the function of cilia. Almost half of the 70 complexes are novel, while for several previously described complexes new components were identifies or a more detailed description of their structure was achieved. A manuscript describing the method is currently being revised to be resubmitted to a bioinformatics journal.

SYSCILIA data output is integrated into a single dataset to predict novel ciliary genes by applying a naïve Bayesian integration approach. Tandem affinity purification, SILAC and Yeast-2-Hybrid screens from WP1 has fed directly into this analysis, as well as expression-, transcription factor binding-, and evolutionary datasets that where generated by bioinformatics specifically for this project. By integrating these datasets using a Bayesian probability scheme we have generated a single set of high quality predictions for which we can control the false discovery rate (FDR). A final list of candidate ciliary genes of 283 has been produced at a theoretical FDR of 25%. Over the last year many SYSCILIA partners have evaluated a subset of these predictions using a wide range of experimental procedures including: dye uptake response and roaming essays in worm, fluorescence microscopy of eCFP fused constructs in human RPE1 cells, immunofluorescence microscopy in human lung epithelial cells and mouse retina, immunoelectron microscopy in mouse retina, and morpholino studies in zebrafish. Of 36 genes tested, 25 were verified as being novel ciliary genes and amounts to an observed FDR of 0.69, which is within statistical range of our theoretical FDR validating our method.

Altogether our set of Bayesian predictions of novel ciliary genes is one of the most comprehensive datasets on ciliary genes. We have combined our predictions with our Gold Standard (Van Dam et al., Cilia 2013) and Gene Ontology annotations to create a ‘compendium’ of 801 ciliary genes, which we call the “Ciliacarta”. The Ciliacarta expands the currently known set of ciliary genes by as much as 240 new genes and will be of great benefit to the cilia research community as a resource. A manuscript is currently being drafted and will be submitted to a high impact journal in the Fall of 2015.

Integration of protein-protein interaction and clinical data was also used to predict disease genes. The ciliary interactome constructed for TAP data was annotated with known gene-diseases associations for Human Phenotype Ontology. A graph-based statistical algorithm was developed to predict disease-causing genes from the annotated network. The algorithm is based on the well-known Gene Set Enrichment Analysis method that was properly transformed to be applicable to protein-protein interaction networks. For a given protein in the network, the method predicts the clinical phenotypes that may appear upon protein alteration based on the phenotypes associated with the neighbours of the protein. Applying the algorithm to the SYSCILIA data we constructed sub-networks associated with all ciliary phenotypes. Specifically, for each phenotype in Human Phenotype Ontology the statistical method was used to predict genes associated with the phenotype and the corresponding network was constructed based on the protein-protein interactions from the TAP data. In this way we managed to build a more complete picture (**figure 2**) of the mechanisms underlying ciliopathic features and draw new hypotheses about their pathogenesis.

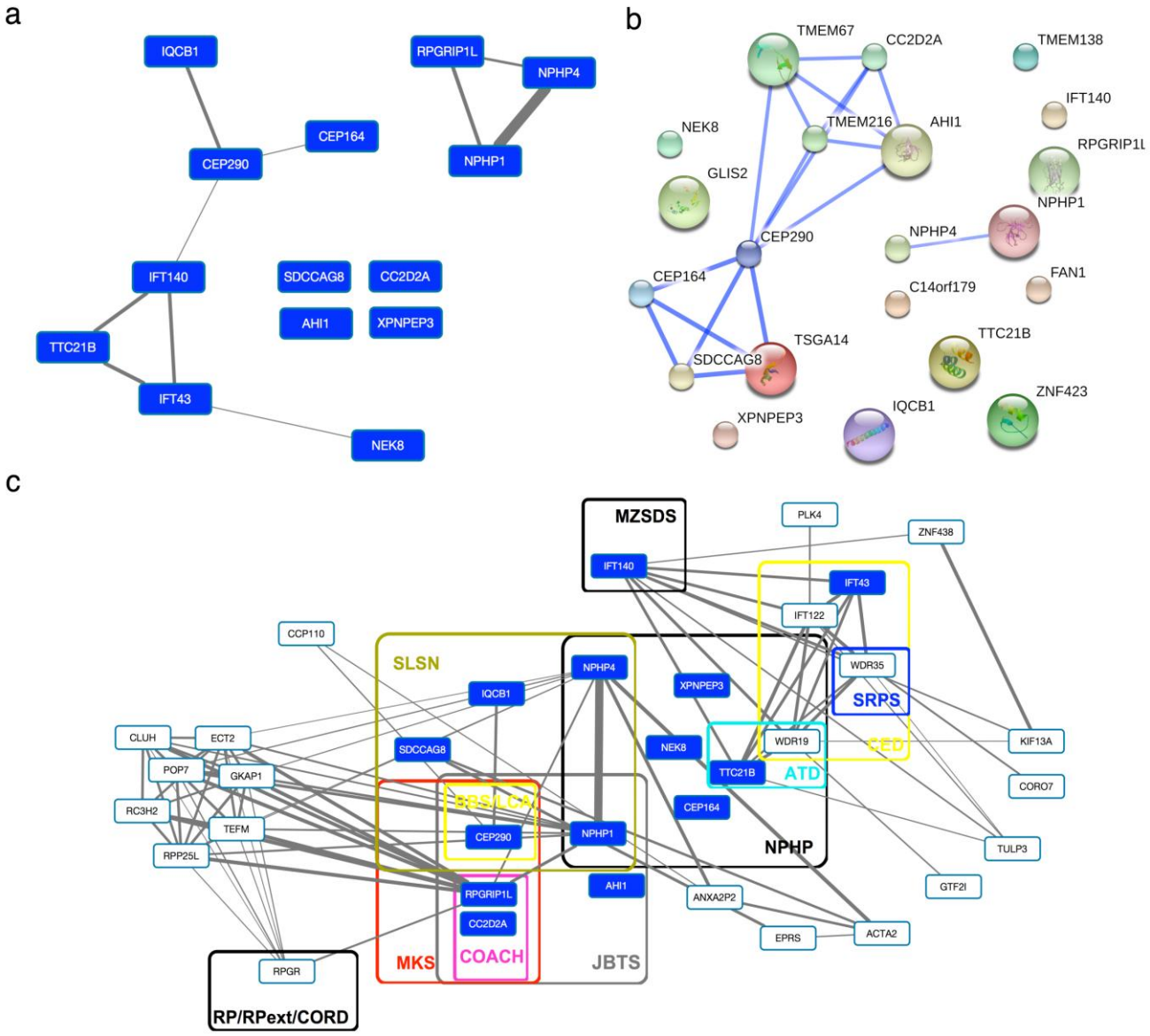

Figure 2: Known and predicted Nephronophthisis PPI networks. (a) Protein interactions in Syscilia TAP data connecting proteins known to be associated with Nephronophthisis (blue nodes). Edge thickness is analogous to the Socio-Affinity Index (SAI), with thicker edges corresponding to higher SAI scores (only interactions with SAI above 4 are shown). (b) Protein interactions in String-DB between proteins related to Nephronophthisis. Only medium confidence interactions (score above 0.4) are shown. The score is computed using all evidences except for text-mining. (c) As in (a) but including proteins predicted to be associated with Nephronophthisis (white color). The coloured boxes indicate the proteins associated with the same ciliopathy.

Work Package 5; Assay systems to study functional ciliary modules

The involvement of cilia in cell migration and nephron extension

The consequences of ciliary defects are particularly well characterized in the vertebrate kidney: depletion of structural or functional cilia-associated molecules (CAMs), for example *PKD1*, *PKD2*, nephronophthisis (*NPHP*) or Bardet-Biedl syndrome (*BBS*) gene families almost universally cause cyst formation. In well-studied mouse models, cyst formation typically is observed in the second half of embryogenesis, leading to organ failure and perinatal death. This particular time period is characterized by rapid extension of the tubular structures to form the final geometry of the nephron. Two programs, oriented cell division and cell intercalation drive this extension (**figure 3**); members of the SYSCILIA consortium have previously

demonstrated that the genetic elimination of CAMs appear to interfere with either or both cellular programs. We observed that depletion of the nephronophthisis family member Inversin/ NPHP2 abrogates the collective cell migration of the proximal pronephros in *Xenopus* embryos, which typically originates in the dorsal region of the embryo and extends the tubule ventrally while forming an extensive convolution. Since the Xenopus pronephros is accessible by confocal microscopy and time-lapse video-microscopy, we decided to utilize this animal model to study the basic cellular migration programs underlying the tubular extension that shapes the vertebrate nephron. As previously proposed, tubular extension is driven by extensive cell intercalation. Unexpectedly, cell intercalation was not driven by lateral-to-medial cell migration but by a multicellular rosette-based mechanism previously only described in Drosophila germ band extension. This process entails the formation of a rosette involving 5-7 cells, the rotation of the rosette, and the resolution of the rosette in a perpendicular direction. This process required Dishevelled, and was blocked by a Dishevelled mutant that specifically blocks the planar cell polarity pathway. The involvement of non-canonical Wnt signalling in rosette formation and tubular extension in the mammalian kidney was further supported by the observation of rosettes in the developing mouse kidney.

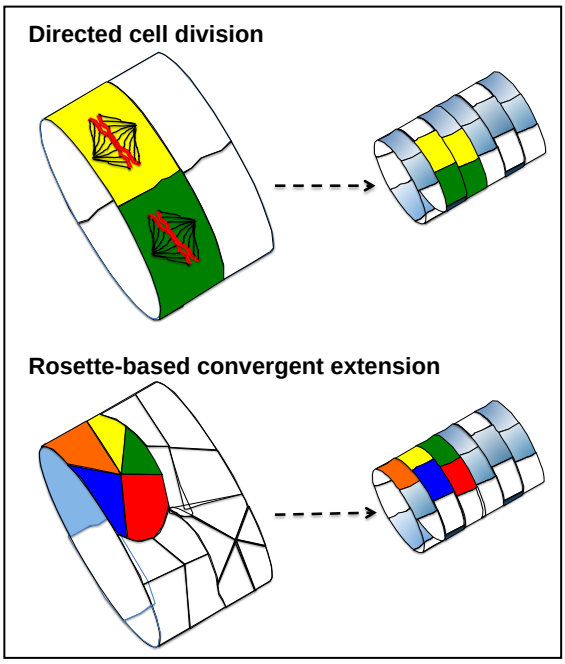

Figure 3: Cilia have been implicated in two complex cellular programs that promote tubular elongation to establish the final geometry of kidney tubules, directed cell division and convergent extension movements.

Furthermore, analysis of Wnt9b-deficient mice revealed that a hypomorphic mutation of this non-canonical Wnt member not only resulted in cystic kidney disease, but also abrogated the formation of higher order rosette formation. Thus, cilia and cilia-associated molecules appear to contribute to programs that ultimately define the localization of individual cells in a larger context to shape a tissue into an organ of defined geometry during development.

Polarization of motile cilia

Motile cilia generate a directional fluid or particle flow. This important ciliary function is required to establish a normal body axis, to promote fluid flow between the cerebral ventricles, or to clear airways from pollution. Defective motile cilia are associated with situs inversus and cardiac malformation, hydrocephalus and recurrent respiratory infections among other manifestations. In SYSCILA, we utilized the *Xenopus* epidermis as a model system to study how cilia are instructed to assume a coordinated beating pattern. The epidermis contains isolated multi-ciliated cells that are polarized during early development to create a fluid flow along the anterior-posterior body axis of the *Xenopus* embryo. While early patterning events (stage 22) cause a directional bias, the ensuing fluid flow re-enforces the final direction (stage 29) of the beating pattern that can be followed by confocal microscopy, monitoring the localization of the basal body rootlet relative to the basal body. An important component of the normal ciliary polarization is the apical actin cytoskeleton that

consists of two layers, a dense apical layer surrounding the basal bodies, and a second sub-apical layer with short actin filaments that connect neighbouring basal bodies (**figure 4**). Disruption of the apical actin cytoskeleton typical causes basal body docking defects, abnormal ciliogenesis and defective fluid flow.

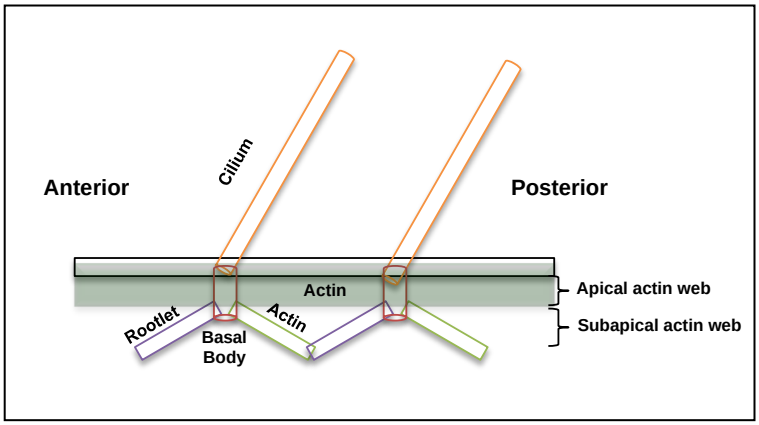

Figure 4: Polarization of motile cilia requires an intact apical actin cytoskeleton, consisting of two layers, a dense apical actin web, and short subapical actin filaments that connect neighbouring basal bodies

We observed that depletion of cilia-associated molecules (CAMs) of the nephronophthisis (NPHP) gene family reduced fluid speed generated by the motile cilia of the *Xenopus* epidermis without disrupting the dense apical actin layer, providing an opportunity to study the underlying mechanisms of ciliary polarization. Using the systems biology approach of SYSCILIA, we found that several NPHP proteins can directly or indirectly interact with actin-nucleating proteins of the formin gene family. Inturned, a molecule previously implicated in planar cell polarity signalling, can function as an adaptor molecules that links formin proteins to NPHP molecules. One example is the assembly of the NPHP4/Inturned/Daam1 protein complex: depletion in the *Xenopus* epidermis of either component of the complex disrupts the actin filaments of the subapical actin web and destroys the interaction between neighbouring basal bodies (**figure 5**).

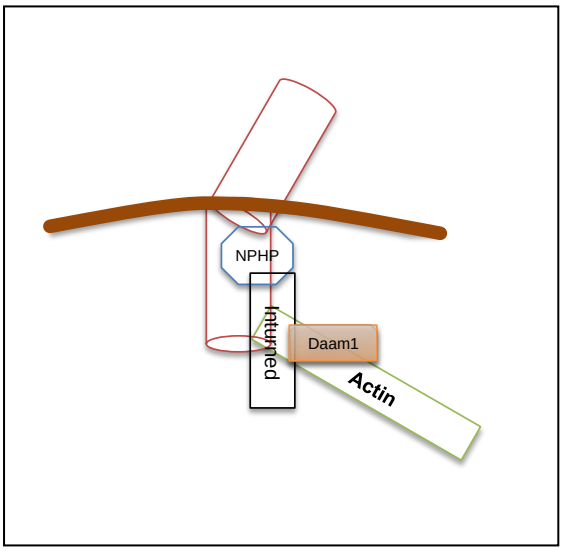

Figure 5: Nephrocystin family members (NPHP) can recruit actin-modifying proteins such as the formin family member Daam1 through interaction with Inturned to configure the subapical actin web.

In SYSCILA, we further determined that the actin-modifying protein complex consisting of the Dock180/ELMO/Ezrin represents a crucial component for basal body docking and polarization of motile cilia in zebrafish and Xenopus embryos. ELMO1 binds to DOCK180 to release the self-inhibition of DOCK180, which can now bind Rac1. This small GTPase has previously been shown to remodel the actin cytoskeleton during phagocytosis and cell migration; SYSCILIA now uncovered its role in ciliogenesis and ciliary polarization. DOCK180/ELMO additionally recruit Arl4a and Ezrin; both binding partners are required for normal basal body polarization.

Control of the mTORC1 signalling cascade by cilia

Ciliary defects lead to an almost universal activation of the mTOR signalling cascade, in particular mTORC1, a protein kinase complex entailing the adaptor protein Raptor (**figure 6**). To study the importance of mTORC1 as potential target for ciliopathy-associated disease manifestations, a KspCRE KIF3A$^{fl/fl}$ mouse was crossed with a Raptor$^{fl/fl}$ mouse. The loss of cilia due excision of KIF3A and abrogation of ciliogenesis in the distal segments of the nephron results in rapid cyst formation and renal failure within the first 8 postnatal weeks. The simultaneous excision of Raptor and inactivation of mTORC1 prolonged the survival of these mice by about 3-fold. However, eventually, even mTORC1-deficient kidneys developed cysts. A detailed expression profiling revealed that within few weeks other growth promoting pathways as well as inflammatory response drive cyst formation in this mTORC1-deficient mouse model of cystic kidney disease.

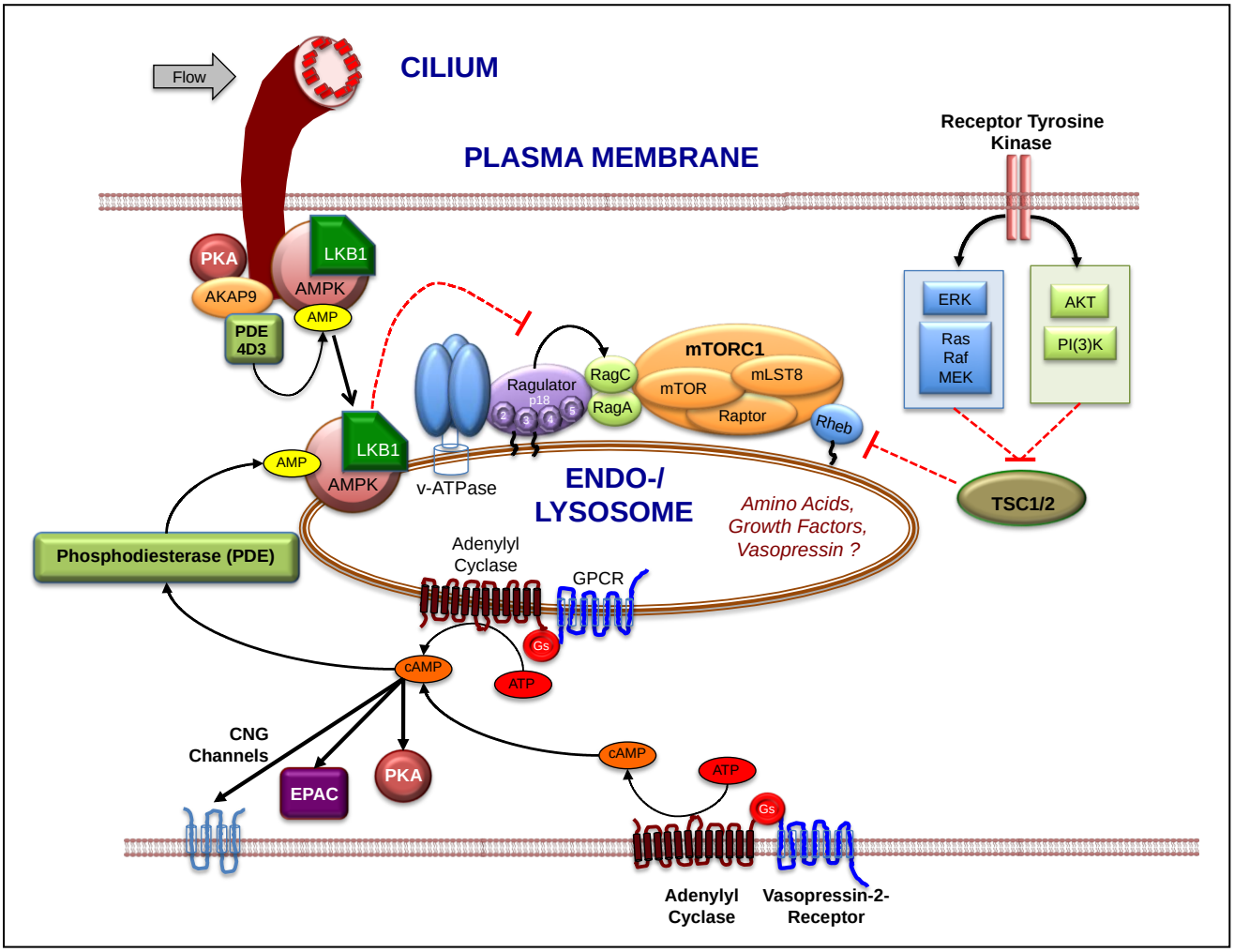

Figure 6: Ciliary defects are commonly associated with increased mTORC1 activation and cAMP production. Recently, the site for mTORC1 activation was localized to the membrane of late endosomes and lysosomes. Seven membrane-spanning receptors (GPCR) can undergo endocytosis and continue to signal at endosomal compartments. Thus, trafficking defects and accumulation of receptor-bearing vesicles could cause activation of both signalling cascades.

Ciliary transport

It has become clear over the past 15 years that ciliary protein transport underlies central aspects of cilium formation, function, and the establishment of a unique molecular identity within the organelle. Also, many of the genes associated with human ciliary disease are thought to encode proteins with transport functions. Thus, by understanding ciliary transport, we can understand better disease pathogenesis. SYSCILIA employed

two models to study different aspects of ciliary transport: vertebrate photoreceptor cells and the small roundworm, *C. elegans*.

Vertebrate photoreceptor cells are highly polarized and compartmentalized neurons characterized by a photosensitive outer segment which resembles a modified primary sensory cilium. Since the outer segment disks are continually renewed, extraordinary high rates of biosynthesis and translocation from the inner segment are necessary, not only during ciliogenesis but also for maintenance of photoreceptor cells, providing an excellent model system for studying intracellular protein trafficking. Trafficking of ciliary cargo occurs in several sub-modular events. In cooperation with WP1, we applied complementary, independent assays to investigate the different modules, e.g. different interaction assays in cell culture and correlative immunofluorescence and -electron microscopy analyses of photoreceptor inner segments. Our focus was the different trafficking routes from the Golgi apparatus through the photoreceptor inner segment towards the photoreceptor cilium. We investigated the role of intraflagellar transport (IFT) proteins and components of the supramolecular complex of the BBSome in the ciliary transport of photoreceptor cells. We showed that IFT proteins and BBSome components are associated with cargo vesicles in the target compartment of the inner segmental transport, namely the periciliary compartment of the apical inner segment and at the basal body and the adjacent centriole of photoreceptor cells. Furthermore, we identified IFT20 as a resident of the Golgi-apparatus and associated with the post-Golgi transport vesicles released from the trans-Golgi network (TGN). Additionally, we could show co-localization of the Golgi associated protein myomegalin, the components of cytoplasmic dynein motor complex and SANS (USH1G), a protein related to the human Usher syndrome (USH) in close association with microtubules, the known transport routes for rhodopsin cargo vesicles from the trans-Golgi network to the base of the connecting cilium. Fluorescence recovery after photobleaching (FRAP) experiments elucidate the transport of the ciliary cargo rhodopsin through the photoreceptor inner segments of rhodopsin-GFP knock-in mice *in vivo* and the role of cytoskeletal elements, as well as USH proteins, for inner segment transport.

C. elegans was used as an *in vivo* model to discover how proteins – including those associated with ciliary disease – are normally transported into and out of the ciliary organelle. In one project, we investigated how the nematode equivalent of human Arl13b, which is mutated in Joubert syndrome, is transported to and restricted at the ciliary membrane. This work has shown that nematode Arl13 has two modes of transport; membrane diffusion and active movement via IFT. IFT is required to get Arl13b into cilia, where the diffusing protein is prevented from leaving by a membrane diffusion barrier at the ciliary base (transition zone). This work also demonstrated that additional ciliary disease proteins associated with Meckel-Gruber syndrome (MKS) and Nephronopthisis are essential for building and maintaining the diffusion barrier. In another project, we used advanced imaging approaches such as fluorescence recovery after photobleaching (FRAP) and super resolution microscopy (STED) to investigate architectural features of the *C. elegans* membrane diffusion barrier. This work revealed that MKS disease proteins which make up the barrier are physically anchored and immobile, with periodic localisations. Thus, the transition zone transport barrier has a defined molecular architecture and substructure. Additionally, we used protein localisation assays to identify new proteins - including novel ciliopathy proteins and endocytic machinery - that function at the base of the cilium. Furthermore, live transport assays revealed that a G-protein transport regulator is docked to and moves with IFT machinery.

In summary, SYSCILIA's work using *C. elegans* has provided important insight into ciliary protein transport mechanisms and discovered new ciliary associations for ciliopathy proteins and transport regulators that also operate elsewhere in the cell.

Work Package 6; Assays to distort Ciliopathy-Associated Modules (CAMs).

The *C. elegans in vivo* model was also employed to identify and characterise new and novel CAM components, by using worms with loss-of-function mutations in genes of interest, together with assays for cilium structure, function and transport. Because mutant alleles are readily available, and ciliary assays relatively easy to perform, nematodes were a primary *in vivo* validation model for candidate ciliary genes arising from the 'omics' workpackages (WP1/3/7). We identified and validated novel conserved ciliary

components regulating cilium structure and function. These included a new ciliopathy-associated transition zone protein that associates with the MKS (Meckel Gruber syndrome) module at the ciliary base, where it facilitates the formation of a membrane diffusion barrier. Using electron microscopy, we also identified functional interactions between MKS and NPHP (Nephronphthisis) module genes in regulating the establishment of the ciliary transition zone, and specifically the formation of the elusive Y-link connectors. Related to this, SYSCILIA determined that the transition zone membrane diffusion barrier restricting Joubert-Syndrome-associated ARL-13 in cilia is dependent on NPHP and MKS modules, but not IFT modules. Additionally, we discovered new conserved basal body proteins, including a novel kinase and a new protein associated with Joubert syndrome. Finally, the *C. elegans* studies uncovered new roles for endocytic transport machinery at the ciliary base in regulating ciliary membrane homeostasis.

In summary, SYSCILIA has expanded the repertoire of proteins with cilia and ciliopathy associations, and provided new insight into ciliary protein transport, especially in relation to the transition zone membrane diffusion barrier.

Next to the C. elegans model, SYSCILIA provided a morphological characterization of **different mouse models** with *OFD1* inactivation. The OFD1 protein is located at the ciliary basal body and associated with the ciliopathies Orofaciodigital Syndrome 1 and Joubert syndrome (JBST10). The characterization of the mouse models provided *in vivo* evidence that the role of cilia-associated transcripts in cilium development is context and stage dependent. This is consistent with our observations that Ofd1 inactivation from early stages of development abrogates cilium formation in early embryonic structures such as the node, whilst inactivation at later stages affects cilium formation after - but not before - renal cysts appear. In addition, analysis of the tole of OFD1 transcripts also revealed unexpected functions for cilia-associated transcripts in DNA binding and chromatin remodelling. Previously, we demonstrated that OFD1 interacts with the TIP60 complex, involved in transcription and chromatin remodelling. Unexpectedly, mass spec experiments revealed that the largest category of OFD1 interactors were proteins linked to processes controlling cellular protein content such as RNA processing, protein synthesis, protein folding and degradation. Furthermore, using a network-based approach to ciliary genes, we identified functionally relevant “communities” significantly specialized for the same processes. Moreover, we unveiled a role for OFD1 and members of the BBSome in the regulation of proteasomal activity and composition at the centrosome. Finally, the characterization of different conditional Ofd1 mutants generated intriguing data on the role of the mTORC complex, Shh signalling, and NFκβ pathways in the OFD1-associated pathomechanisms.

Different mouse models were also used in SYSCILIA to investigate complete or partial loss of ciliary proteins. Initial experiments showed that deficiencies of different proteins related to human Usher syndrome (USH) did not cause retinal phenotypes, unlike in humans, where the retina degenerates. In contrast, we showed that mutations in Fam161a cause RP28, an autosomal recessive form of retinitis pigmentosa. Fam161a was also shown to be part of the ciliary apparatus of photoreceptor cells. To further characterize the cellular role of Fam161a in the retina, we analyzed loss of Fam161a in a gene trap Fam161aGT/GT mouse and found significantly reduced connecting cilium lengths, spreading of ciliary microtubule doublets, and expansion of the outer segment (OS) base, followed by disorganization of OS membranous disks. Also, targeting of Fam161a interacting proteins lebercilin and Cep290 was disturbed, and centrin3, a protein found in the connecting cilium, was reduced and mislocalized. Mislocalization of rhodopsin and peripherin to the inner segment, outer nuclear layer and synapses indicated further defects in ciliary transport. In close connection to WP1, we identified POC1B as a binary interaction partner of FAM161A. Mutations in *POC1B* are associated with autosomal recessive cone-rod dystrophy, and these mutations also disturb the interaction of FAM161A and POC1B, indicating that this partnership is essential for the retinal function of this module. In SYSCILIA, we also examined mice deficient for Centrin1-/-, and found no retinal degeneration and normal retinal function. As Cetn1-/- males are sterile, we conducted in-depth immunofluorescent and immunoelectron analyses of spermatogenesis and found that Cetn1-/- spermatids do not develop flagella as the last step of spermogenesis is altered. Also, our investigations revealed failures in centriole rearrangement during basal body maturation and in the basal-body–nucleus connection of the sperm cell.

SYSCILIA also used mouse models to investigate the ciliary associations of pulmonary hypoplasia, which is a poorly described feature of Meckel Gruber syndrome and short rib polydactyly syndromes, and which may

be a generally under-reported clinical feature of ciliopathies. To determine a possible disease mechanism for pulmonary hypoplasia in ciliopathies, we characterized the Tmem67-/- knock-out mouse model for Meckel-Gruber syndrome and the function of the TMEM67 protein. Pulmonary hypoplasia is a nearly consistent finding in Tmem67-/- embryos and pups. Our study showed that TMEM67 is a receptor of non-canonical Wnt signalling that preferentially binds Wnt5a and mediated downstream signalling through ROR2 as a co-receptor. Loss or mutation of any component in the Wnt5a-TMEM67-ROR2 axis contributed to pulmonary hypoplasia, condensed mesenchyme and impaired development of the alveolar system observed in the ciliopathy disease state. Lung branching morphogenesis in Tmem67-/- ex vivo cultured lungs was rescued by treatment with calpeptin, an activator of RhoA (a downstream effector of the non-canonical Wnt signalling pathway)

In SYSCILIA, we also **employed the zebrafish model to investigate genes associated with the ciliopathies Usher syndrome (inherited deaf-blindness), short Rib Polydactyly disorders (SRPD), Bardet-Biedl syndrome (BBS) and Alström syndrome**.

One major focus has been the functional characterisation of NINL and this research has resulted into two major pieces of work. In the first study, we show that DZANK1 interacts with NINLisoB, and loss of function of ninl, dzank1 or a genetic interaction lead to dysmorphic photoreceptor outer segments, accumulation of trans-Golgi-derived vesicles, mislocalization of rhodopsin and ush2a. Furthermore, NINL and DZANK1 associate with subunits of the cytoplasmic dynein1 motor complex, and retrograde dynein-based transport is impaired in zebrafish ninl and dzank1 morphants. In the second study, we describes CC2D2A as an interaction partner of NINL. We also demonstrate that loss of Ninl or Cc2d2a results in similar retinal phenotypes, including short outer segments, opsin mislocalization and accumulation of vesicles/vacuoles. In addition, affinity proteomics identified MICAL3, a protein previously shown to bind RAB8A, as an important NINL interaction partner. From this work, SYSCILIA proposes a model where CC2D2A provides a docking point at the photoreceptor ciliary base, allowing RAB8A-positive vesicles to bind through a series of interactions involving CC2D2A-NINL-MICAL3-RAB8.

SYSCILIA's work on SRPD employed zebrafish models to investigate known and novel causative genes including *ift80, ift172, cep120* and *tctex1d2*. The data from this work indicates that the skeletal phenotypes result from imbalances in hedgehog signaling stemming from defective intraflagellar transport.

In SYSCILIA, we have also used zebrafish as a key tool to model the loss of disease genes in Bardet-Biedl syndrome and Alström syndrome. We showed that Bbs8 and the core PCP protein Vangl2 interact and are required to establish and maintain left–right asymmetry during early embryogenesis. Furthermore, we identified a genetic and physical interaction between BBS9 and the Alstrom syndrome gene ALMS1, which may help explain the close phenotypic overlap between the two diseases. In addition, we investigated the previously described obesity modifier gene Fto and found that knockdown in zebrafish led to short, absent or disorganised cilia. This suggests Fto is a good target for more study to help understand the obesity that is prevalent across the ciliopathy spectrum.

Work Package 7; Systematic RNAi screens to distort and identify ciliopathy-associated modules.

WP7 has successfully implemented a whole genome siRNA-based functional genomics screen to identify genes whose activity is required for ciliogenesis and/or cilia maintenance. By interrogating every gene for a role in these processes, we have obtained a global resource for investigation and interventions into the processes that are critical for the ciliary system. Our visual assay (**figure 7**) was primarily designed to test the presence or absence of cilia in the primary screen, and we have provided all numerical features for this cellular phenotype as a functional resource to investigate aspects of cilia biology (see: http://syscilia.org). In addition, we also imaged other cellular phenotypes during data acquisition (cilia intensity and nuclear morphology) and include these other numerical features to enable the scientific community to interrogate and further annotate our screen data-set.

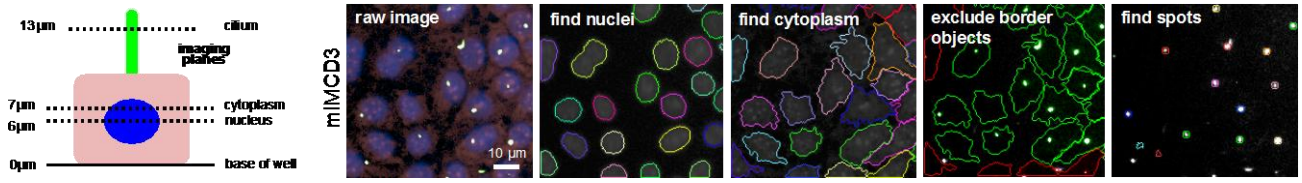

Figure 7. Automated high content imaging of primary cilia. Left panel: schematic of a polarised mIMCD3 cell showing the focal planes used to image nuclei (blue), cytoplasm (pink) and ciliary axonemes (green). Right panel: mIMCD3 cells imaged using an Operetta high-content imaging system, with representative images from Harmony/Columbus software of cilia recognition ("find spots"). Scale bar = 10 µm.

To minimise the false positive rate, we extensively characterised a widely used cellular model system (ciliated mIMCD3 cells) by arrayCGH, and our siRNA library was filtered for off-target effects, partial on-target effects and microRNA-like effects. Using stringent filters and robust statistical cut-offs, we identified 194 genes which significantly affected ciliogenesis and were of particular functional interest, and took these on for validation by further knock down experiments in mIMCD3 and hTERT-RPE1 cells.

In total, we have identified a high confidence list of 68 validated genes that play significant roles in ciliogenesis in mIMCD3 cells, of which 37 were further found to affect ciliogenesis in hTERT-RPE1 cells. A parallel secondary screen validated a further 44 genes encoding components of the ubiquitin-proteasome system (UPS). Validated hits include *CEP120*, *KCNQ1*, several G-protein-coupled receptors (GPCRs; *CRHR2*, *HTR1B* and *OPRL1*) and pre-mRNA splicing factors (PRPFs; *PRPF6, PRPF8, PRPF31* and *PRPF38A*). The specificity of the screen for targeting mediators of ciliogenesis is demonstrated by the number of genes that have been independently implicated in this process. These include genes encoding known centriolar or centrosomal proteins, including *CCDC41*, *OFD1*, CEP120 and *PIBF1*. *C21orf2* has been suggested to have a role in ciliogenesis from a previous small-scale siRNA screen. In a complementary functional genomics study, *PLK4*, *CEP120*, *PRPF8* and *PRPF38A* have all been implicated in the process of centriolar under-duplication. Furthermore, *PLK4*, *OFD1*, *CDC27*, *PRPF6* and *PRPF8* were identified in the Mitocheck screen as important effectors of cell division. Shared hits across multiple independent functional genomics studies indicate that our screen is a useful resource for the identification of proteins and functional modules that are essential for cilia biology and related cell biological processes.

This study also demonstrated the power of an unbiased screen to discover cellular or molecular processes that play a role in cilium biology. To illustrate this point, our screen unveiled neuroactive GPCRs as one of the functionally enriched groups of proteins affecting ciliogenesis. GPCRs may play sensory or signalling roles in the ciliary vesicle or procilium at the earliest stages of ciliogenesis both in the developing brain and in other tissues. In support of this hypothesis, adenylyl cyclase III co-localises with GPCRs at proximal ciliary regions in the developing neocortex, and over-expression of specific ciliary GPCRs, including the 5-HT_6 serotonin receptor, in cortical neurons causes cilia elongation.

Although the role of the PRPFs in the spliceosome is well-known and well-studied, we identified a group of seven splicing factors including PRPF6, PRPF8, PRPF31 and PRPF38a that are required for ciliogenesis. We demonstrated that PRPFs mutated in retinitis pigmentosa localise specifically to the base of the primary cilium in several different cell-types, and to the basal body complex and connecting cilium in photoreceptors. We suggest that some of the splicing factors may fulfil an additional ciliary function independent of their nuclear role in splicing. In support of this notion, two previous studies of the centrosomal proteome have suggested that splicing factors, including two of the splicing factors identified in our screen (PRPF6 and PRPF8), may be true centrosomal proteins. However, it seems less speculative to hypothesise that these proteins may be required for the correct splicing of an unidentified subset of genes that are involved in cilia formation. In support of the possible involvement of pre-mRNA processing factors in ciliogenesis, previous screens have identified that several splicing factor hits are important in related cellular processes of microtubule formation and regulation, namely centriolar biogenesis (PRPF8, PRPF38A) and cell division (PRPF6, PRPF8).

Several hits in our screen have a known function in the ubiquitin-proteasome system (UPS), reflecting an importance of specific proteostasis mechanisms in mediating or regulating ciliogenesis which could be mediated through, for example, ubiquitination or SUMOylation of ciliary transcription factors or chaperoning of ciliary proteins to the base of the cilium. Remarkably, 5/7 of our splicing factor hits (LSM2, PRPF6, PRPF8, PRPF31 and USP39) are implicated in the ubiquitin-dependent regulation of the spliceosome. It is interesting to note that the interaction of PRPF8 with ubiquitinated PRPF3 is regulated by the deubiquitinating enzyme USP4, and that loss of USP4 prevents the correct splicing of mRNAs including those for α-tubulin. In support of this mechanism, we observed that a viable *C. elegans* strain possessing a splice site mutation in *PRPF8*/*prp-8* has structural and numerical defects of axonemal microtubules in the primary cilia of amphid sensory neurons. Thus, UPS and/or PRPF proteins could act as multifunctional "nexus molecules" that are involved in multiple aspects of proteostasis of ciliary proteins or their trafficking (for example, the ubiquitin-mediated internalisation and endosomal sorting of GPCRs). Alternatively, or additionally, these specific PRPFs could also ensure the correct splicing of transcripts encoding proteins important for ciliogenesis, including structural components of the cilium such as α-tubulin.

We also demonstrated the specificity and clinical utility of the screen as a tool for disease-gene discovery. When combined with large variant datasets, for example WES of ciliopathy patients, the functional data from our siRNA screen allowed the filtering and prioritisation of variants to identify pathogenic mutations. Although previous studies have indicated both *PIBF1* and *C21orf2* as functional candidate genes for ciliopathies, we formally demonstrated the utility and validity of a systems biology approach by the identification of mutations in these genes in the ciliopathies Joubert syndrome (JBTS) and Jeune asphyxiating thoracic dystrophy (JATD), respectively. Furthermore, complementary affinity proteomics data provided an explanation of the mechanism for the variable phenotype in the C21orf2 form of JATD. We proposed that the retinal phenotype observed in *C21orf2*-mutated patients may result from a dysfunctional SPATA7-C21orf2-containing module in the photoreceptors of these patients. Conversely, the skeletal phenotype observed in both *C21orf2*- and *NEK1*-mutated human patients probably has a common origin in the disruption of the C21orf2-NEK1 interaction. NEK1 is a serine-threonine kinase and two serines (positions 136 and 177) in C21orf2 have been previously found to be phosphorylated. In light of this, our affinity proteomics findings, C21orf2 might represent a substrate of NEK1.

In conclusion, SYSCILIA highlights the utility of combining different systems biology approaches, namely high-content functional genomic screening, WES, and affinity proteomics. Our approach has identified ciliary roles for well-studied proteins, identified disease-causing genes, allowed the refinement of patient phenotypes, and highlighted potential disease pathways that could provide deeper insights into cilium biology. This work demonstrates the utility of a systems biology approach in providing a compendium of candidate genes and offering insights into pathogenic mechanisms for an increasingly important group of Mendelian conditions, the ciliopathies.

Work Package 8; Assessment of the involvement of the predicted ciliary molecular machines in the pathogenesis of ciliopathies.

Cilia are nearly ubiquitous cellular structures in vertebrates that have emerged in recent years as key controllers of a multitude of developmental processes, including the establishment of left-right asymmetry, limb and kidney morphogenesis, closure of the neural tube, various aspects of neurodevelopment, differentiation of photoreceptors and mechanosensory hair cells, olfaction, and sperm motility. Consistent with the broad roles of these organelles, ciliary and basal body defects have been causally linked with at least 15 discrete disorders in humans, caused by mutations in upwards of 100 causal genes; as many as 100 additional independent clinical entities having been proposed as ciliopathies. However, the vexing problem of the limited ability of the genotype to predict phenotype persists; numerous examples are now documented in which variation at a causal locus cannot explain differences in penetrance and expressivity. Among the models proposed to explain these common phenomena, one attractive hypothesis is that of mutational load, in which the amount and distribution of alleles in a discrete cellular module can influence variability. In WP8, we use genetic, *in vivo* (mouse and zebrafish), and *in vitro* (ciliated mammalian cell lines)

approaches to **a)** identify novel primary drivers of ciliary disease; **b)** discover novel second-site modulators that influence penetrance and expressivity; and **c)** understand how the cumulative amount and distribution of mutational burden in discrete biochemical complexes (intraflagellar transport; BBSome; transition zone complexes) gives rise to discrete endophenotypes in ciliopathies.

Establishment of a ciliopathy cohort for genetic and functional analysis.

Since the discovery of mutations in *Ift88* in the *orpk* mouse renal cystic mutant and the identification of recessive mutations in basal body components in Bardet-Biedl syndrome (BBS) patients just over one decade ago, the ciliopathies have been the subject of intense genetic and biochemical study. Among several important paradigms that have emerged has been the recognition that allelism at a single locus cannot fully explain phenotypic variability, and that ciliopathy loci can contribute causal and epistatic modifiers across the ciliopathy spectrum. For example, recessive loss of function, mutations in *CEP290* have been reported to cause a range of ciliopathy phenotypes including Meckel-Gruber Syndrome (MKS), Bardet-Biedl syndrome (BBS), Joubert syndrome (JBTS), isolated nephronopthisis (NPHP), and Leber congenital amaurosis (LCA). Therefore, we hypothesized that mutational load at the cilium, in addition to the recessive locus, is driving this phenotypic variability. To begin to test this posit, we assembled a cohort of 457 ciliopathy samples representative of a diverse set of clinical entities including Primary Ciliary Dyskinesia (PCD), Bardet-Biedl syndrome (BBS), Meckel-Gruber syndrome (MKS), Orofacialdigital 1 syndrome (OFD), Usher syndrome (USH), and Jeune Asphyxiating Thoracic Dystrophy (JATD). In addition to the aggregation of patient samples (DNA), we also aggregated patient phenotype data into locally-hosted phenotype database (CHDM phenoDB) with a web interface.

Targeted next generation sequencing of ciliopathy cases and controls determine total mutational load of the ciliary proteome.

Next generation sequencing.

We prioritized 785 genes from the ciliary proteome (www.ciliaproteome.org) identified by at least two different ciliary proteomics studies, as well as genes with a published or putative association with ciliary disease that were not in the ciliary proteome database. In collaboration with the Human Genome Sequencing Center at Baylor College of Medicine (BCM-HGSC), we conducted a targeted exon capture followed by next-generation sequencing on an Illumina platform. Specifically, we conducted pre-capture pooling of 23 samples/pool, NimbleGen targeted liquid capture of 785 ciliary genes (12,000 exons; 1.9 Mb of target) and next-gen sequencing using the Illumina HiSeq2000 (paired-end 100bp reads with two pools/lane; 10 lanes total). In collaboration with the Genome Centers at Baylor College of Medicine and University of Washington, we have accrued detailed, individual data from ~1,000 controls (exome data from the Atherosclerosis in Communities (ARIC) and NHLBI Exome Sequencing Project Exome Variant Server (EVS)). In parallel, and on the same set of 457 ciliopathy samples, we conducted high resolution array comparative genomic hybridization (a-CGH) to identify copy number variants that are undetectable by traditional sequencing approaches, but which also fall below the detection thresholds of traditional microarray platforms to detect large structural variants in the genome.

Bioinformatic filtering and secondary confirmation.

We proceeded with data filtering for all variants that had >10x coverage and >30% of alternate allele reads. Next, we retained all functional variants (nonsense, frameshift, splice site (+/- 5bp from the exon-intron junction), and missense variants that had a minor allele frequency of <1% in control datasets from ARIC and ESP controls. To identify novel causal ciliopathy genes, we identified samples with either multiple variants in the same gene (compound heterozygous paradigm) or homozygous variants that may be putative causal variants since the ciliopathies are primarily recessive disorders. For each of the candidate genes (as defined by the presence of a rare functional homozygous or compound heterozygous variant) we generated lists for segregation by first evaluating the known causal ciliary genes, and secondly, evaluating the candidacy of

putative novel ciliopathy loci. Where possible (we do not have DNAs from full pedigrees from all of the samples included in this study), we have conducted Sanger sequencing to confirm putative variants by a secondary method and confirm that they segregate appropriately with disease in the pedigree. In parallel, Partners from WP8 have also, in part, determined the mutational load of known causal ciliopathy genes in their cohorts (particularly MKS and JATD). These data were integrated into WP8, and used to bolster the data in favor of a high sensitivity low false positive rate for the sequencing data.

Functional evaluation of variants using in vivo zebrafish complementation assays and integration with the ciliary systems model.

In vivo assays to determine variant pathogenicity.

Zebrafish are an experimentally tractable model to evaluate the physiological relevance and direction of allele effect for variants of unclear significance (primarily missense and copy number variants) due to: **a)** optic transparency for easy visualization; **b)** high level of conservation with the human genome (~70% of genes); **c)** conserved developmental pathways and rapid early development; and **d)** ability to genetically manipulate zebrafish embryos to induce gene overexpression or suppression. To test the functional significance of the missense mutations identified in this project, we have conducted *in vivo* complementation assays according to standard procedures according to the following tiered strategy (**figure 8**):

1. For each gene, we designed two MOs (splice-blocking (sbMO) and translation-blocking (tbMO)) targeting the orthologous *D. rerio* gene. Next, we generated titration curves for each MO (typically 3ng, 6ng, and 9ng; embryo batches of 50-100 embryos; masked scoring; repeated at least twice); a dose-dependent response is indicative of specific knockdown. For splice blocking MOs, we evaluated MO efficiency by harvesting total RNA from MO injected embryos, generating oligo dT primed cDNA, PCR amplification using primers encompassing the MO target site, and sequencing of the resulting bands to determine the MO effect (such as exonic retention/removal). Once we have determined that MOs are efficient, we rescue morphant phenotypes (gastrulation defects or renal defects) by injecting wild-type (WT) capped human mRNA.
2. Next, we introduced each test allele into the WT construct by site-directed mutagenesis according to standard procedures. We screened resulting clones by Sanger sequencing to ensure that the mutation has been introduced, and to ensure the sequence integrity of the entire open reading frame (ORF). Using this process we have a >90% success rate, including genes >6kb or of high GC content.
3. All Sequence confirmed WT and mutant constructs are linearized and we transcribed capped mRNA (mMessage mMachine) using a cost-efficient robotic method.
4. For each test allele, we injected WT zebrafish embryos (n=100) with a) sham; b) sbMO (repeated with tbMO); c) sbMO+wt human mRNA; d) sbMO+mutant mRNA; e) wt mRNA; f) mutant mRNA (total 600 embryos, masked scoring) at the one to four cell stage.
5. We scored embryos using quantitative criteria (e.g. anatomical landmarks in midsomite embryos). For certain genes/alleles, we have assessed gastrulation defects quantitatively in midsomitic embryos (using *in situ* hybridization of a riboprobe cocktail of *krox20, pax2a, and myod*) or renal morphology at 4 days post fertilization (dpf; using anti-Na/K ATPase antibody staining). Images were acquired using medium-throughput imaging on a Nikon AZ100 stereomacroscope to expedite this process.
6. Scoring data were compared using standard χ^2 tests: mutant rescue indistinguishable from WT, benign; mutant rescue improved from MO, but worse than WT rescue, hypomorphic (partial loss of function); mutant rescue indistinguishable from MO, functional null; mutant mRNA alone indistinguishable from MO but can be rescued by titration with WT mRNA, dominant negative; or mutant mRNA gives rise to a phenotype that cannot be titrated by WT mRNA, gain of function. All results were replicated 3 times.

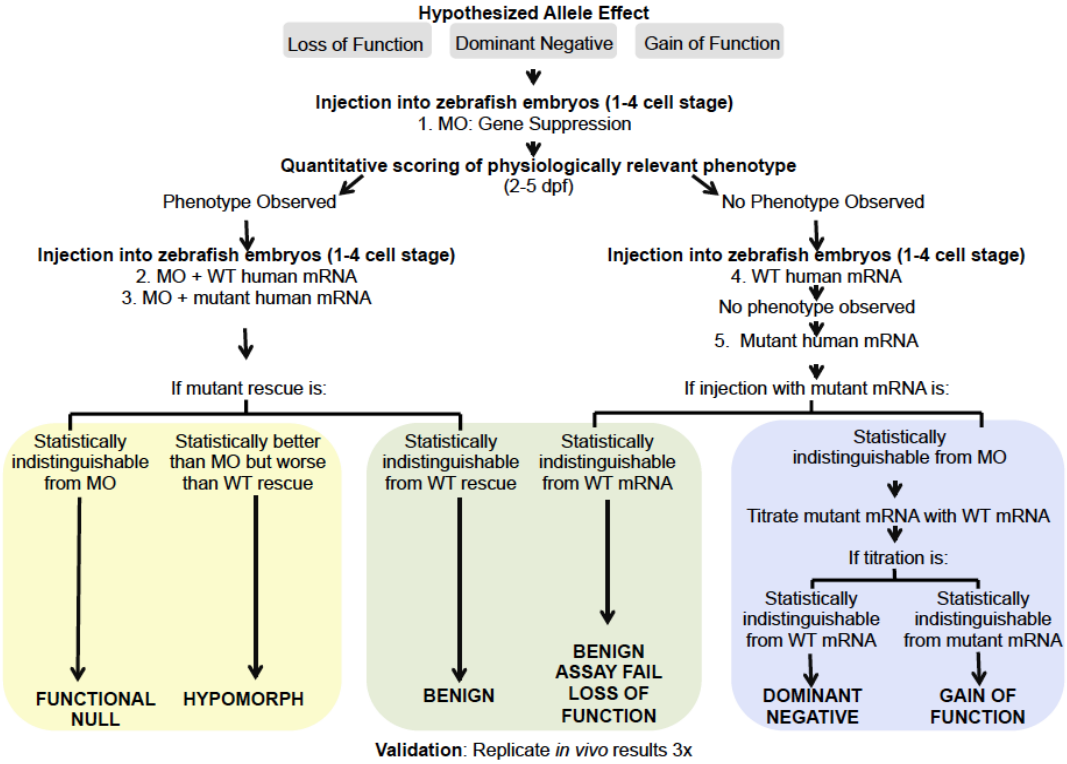

Figure. 8. *In vivo* complementation strategy and interpretation of results

Key results.

Through our combined genetic (targeted resequencing combined with high resolution a-CGH) and functional studies, SYSCILIA has been successful at accelerating the following discoveries:

1. Novel ciliopathy genes: This workpackage has contributed significantly to the discovery of novel ciliopathy genes that have a) increased our knowledge of the ciliary mutational load (for example, novel PCD gene *ARMC4*, JATD gene *IFT172;* MKS gene *TMEM231*); b) demonstrated further that recessive mutations in single ciliary genes can contribute to multiple clinical phenotypes (examples include mutations in *TTC21B* as a causal locus for each of JATD and NPHP; the common *NPHP1* whole gene deletion causes each of NPHP, as well as BBS).

2. Ciliary mutational load is enriched in specific cases: Systematic analysis of the mutational burden in ciliopathies in our targeted gene set has demonstrated that there is an enrichment of variation, often changes in the heterozygous state, that are present in addition to the primary causal ciliopathy locus. Our work on *TTC21B* demonstrated specifically that up to 5% of cases harbor pathogenic variation at this locus encoding a retrograde intraflagellar transport complex protein. Broader analysis of the entire dataset suggests this to be true on a global level for cases in comparison to controls; further studies are required to refine the extent of this enrichment with respect to the ciliary systems model (work will be ongoing after the SYSCILIA funding period).

Work Package 9; Translational systems biology: ciliotherapeutics.

The versatility of using zebrafish to generate reproducible gene knockdown in hundreds of embryos relatively quickly has lent this model well to a semi-high throughput method for compound screening. In SYSCILIA, we characterised the knockdown of many cilia related genes including, IFT complex genes and ciliopathy genes, including members of the BBSome, via the morpholino approach. In most models

characteristic ciliopathy phenotypes were observed, including small eyes, curved body axis, hydrocephalus, and pronephric cysts.

We examined these models for a reliable phenotype to score for a high throughput compound screen and chose renal cystogenesis due to its ease of scoring but more importantly due to its major clinical relevance to ciliopathy patients. The morpholino model chosen to show the most reliable cystogenesis was that for the intraflagellar transport gene *ift80,* so we implemented a semi-high throughput compound screen to identify candidate drugs that could ameliorate the pronephric cyst formation present in this model. We initially used the Prestwick Library of 880 FDA approved small molecules. The re-profiling of already FDA approved drugs means that any candidates to come out of the screen can be used immediately on patients without extensive clinical trials. Fourty compounds were picked from the initial screen based on reduction in cyst growth and selected for further analysis. More stringent testing eliminated a number of these. The rest were taken on to a second stage of testing in a murine kidney explant model. In this more relevant model three of the drugs, primaquine, chloraquine and 17-DMAG were still found to ameliorate cyst growth and as such are good candidates for more extensive testing in murine models.

As this number of candidates is somewhat lower than hoped however, we decided to take on a secondary screen to try and find more candidates for further testing. This time retinal degeneration was chosen as the clinically relevant phenotype of interest. We used a panel of 640 FDA approved compounds with limited overlap to the Prestwick library that had been previously screened. A morpholino against BBS6 was used to induce a phenotype which included small eyes, reduction in photoreceptor number and disorganisation of the photoreceptor cell layer in rhodopsin::GFP fish. After stringent testing two of the drugs mitomycin C and capecitabine were confirmed to reliably rescue the small eye phenotype however this rescue did not extend to the reduced number and altered morphology of the photoreceptors and as such is unlikely to be of clinical relevance. As such we have three possible drugs with which to move forward from screening over 1500 across the two large screens. Looking at the literature for other repurposing screens carried out by external groups over the timescale of SYSCILIA, hit rates are always extremely low so our findings are not unusual. During the SYSCILIA project, our aim was to test the efficiency, as well as the toxicity of translational read-through inducing drugs (TRIDs) on premature termination codons causing retinal degenerations related to ciliopathies in cell-based systems (**figure 9**). We demonstrated a significant, dose dependent read-through on nonsense mutations mediated by designer aminoglycosides and the structurally not related PTC124 in fluorescence microscopy and Western blot analyses. We also assessed the efficiency and retinal biocompatibility of the TRIDs in organotypic retina cultures, which serve as reliable tools to monitor drug effects on the retina (**figure 10**). Last but not least, we also demonstrated the read-through ability of PTC124 *in vivo* (**figure 11**). Present data prove in principle that PTC124 and designer aminoglycoside-mediated read-through of nonsense mutations is suitable to restore retinal protein expression in the organism. However, our studies indicate that the efficiency of read-through for different genes varies, depending on several factors, including the nature of the stop mutation, the neighboring DNA sequence context, and last but not least the chosen read-through drug. Therefore, the examination of read-through rate of each specific nonsense mutation and chosen drug is necessary. The results for translational read-through of a nonsense mutation causing Usher type 1C have been published (Goldmann et al. 2010, 2011, 2012), while our lab is continuing to further evaluate the efficiency, as well as the biocompatibility, of different TRIDS on different ciliopathy-causing mutations, including redesigned aminoglycosides with even better read-through activity and reduced toxicity.

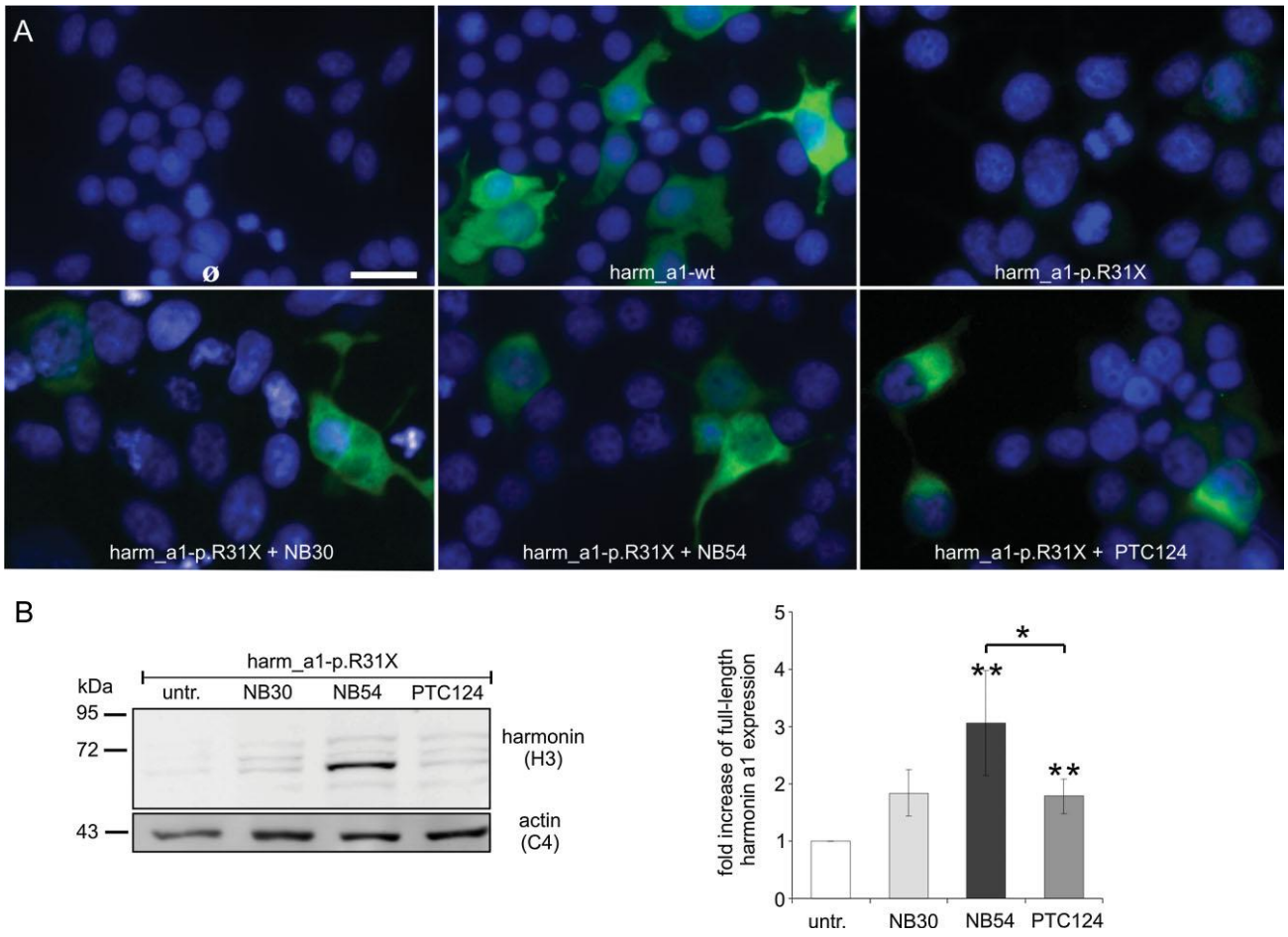

Figure 9. Indirect immunofluorescence and Western blot analyses of TRID-mediated read-through in HEK293T cells. A. Read-through in transient transfected HEK293T cells analyzed by indirect immunofluorescence with anti-harmonin antibodies. Harmonin staining was detected in harm_a1-transfected cells whereas no staining was visible in untransfected cells and harm_a1-p.R31X controls. NB30 (2 mg/ml), NB54 (0.5 mg/ml) or PTC124 (10 mg/ml) treatment restored harmonin a1 (green) in p.R31X-transfected cells. Nuclear DNA was stained by DAPI (blue). **B**. Read-through in transiently transfected HEK293T cells analyzed by Western blot with anti-harmonin antibodies. Treatment with NB30, NB54 or PTC124 restored full-length harmonin a1 (~80 kDa) in p.R31X-transfected cells. Actin staining (~42 kDa) was used as loading control. For quantification of TRID-mediated read-through of the p.R31X mutation, the optical densities of harmonin a1 bands, stained by anti-harmonin antibodies, were measured and normalized to the appropriate loading control. The increase of read-through is shown as fold increase over untreated (untr.) cells. Quantitative data resulted from three to five independent repeats of the experiments, Error bars represent SD, _p<0.05, __p<0.01, scale bar 10mm.

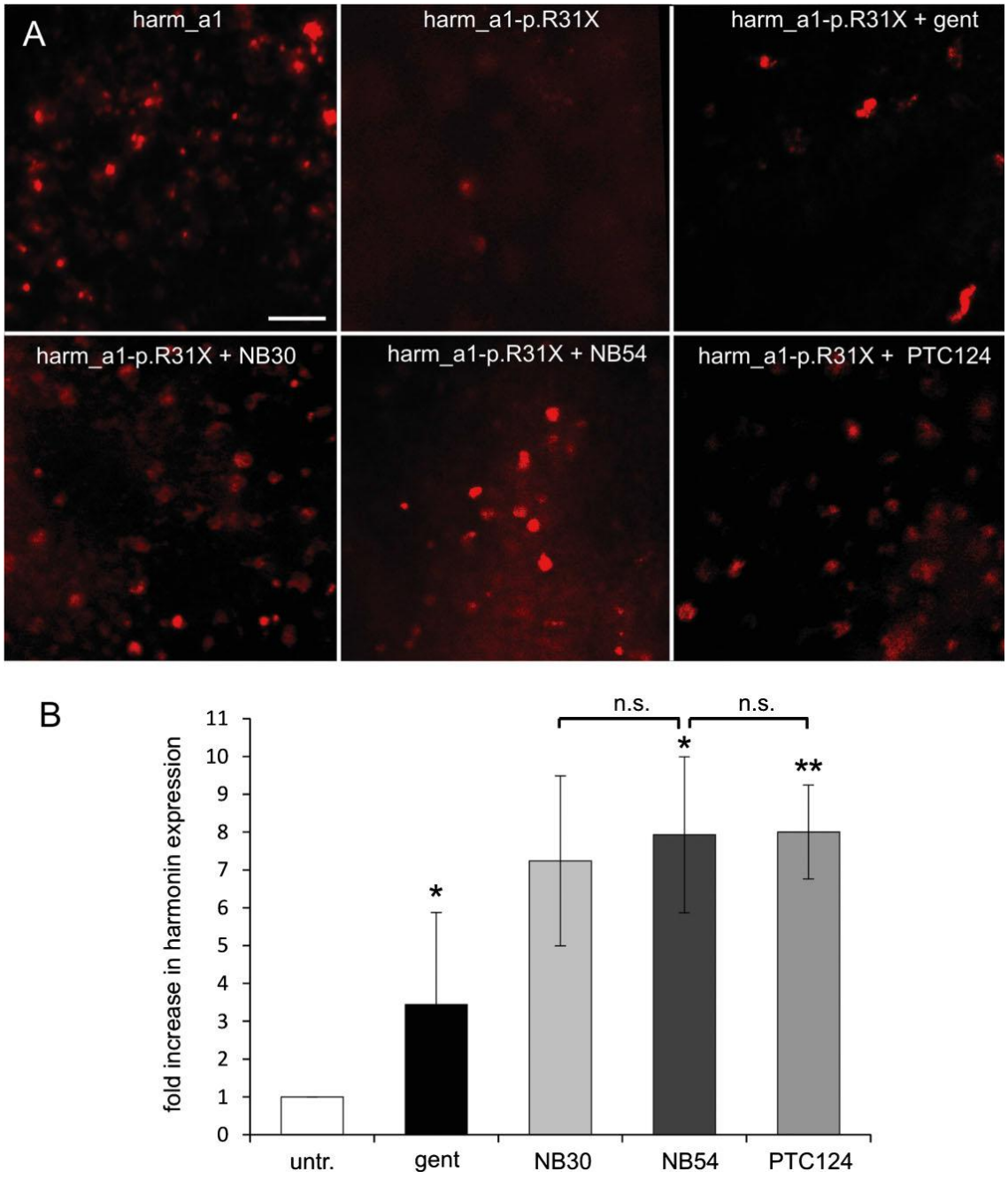

Figure 10. TRID-mediated read-through of the p.R31X nonsense mutation in organotypic retina cultures. **A**. TRID-mediated read-through in p.R31X transfected murine retinal cultures. Retinas were transfected with harm_a1-p.R31X-mRFP, subsequently cultured in the presence of NB30, NB54 or PTC124, the harm_a1 transfection was used as control. Retinal whole mounts were analyzed by fluorescence microscopy. Expression of the red fluorescent marker indicates read-through of the p.R31X nonsense mutation. **B**. Quantification of mRFP-positive cells revealed an increase of harmonin expression after TRIDs treatment. Quantification was the result of two to four independent experiments, error bars represent SD, *p<0.05, **p<0.01, scale bar: 100 µm.

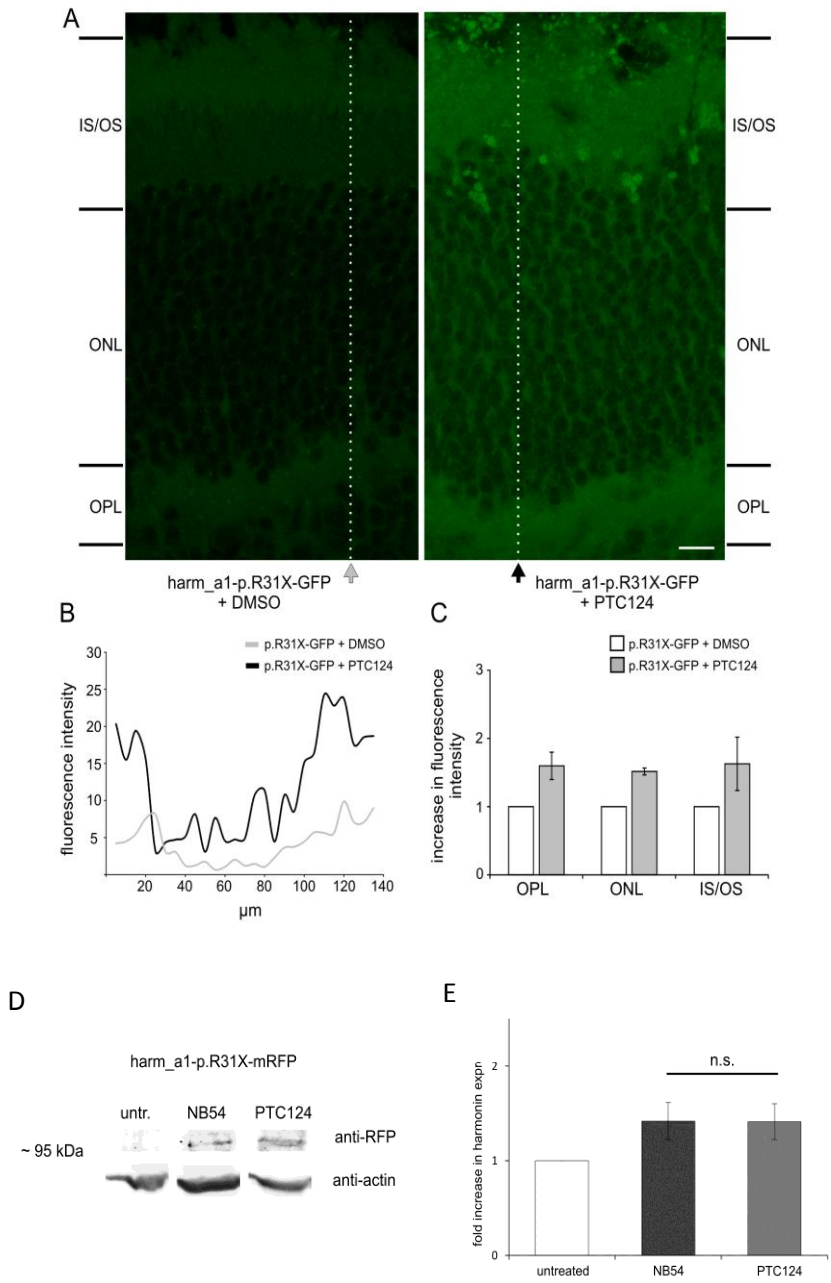

Figure 11. Confocal analyses of PTC124-mediated read-through in retinas *in vivo*: retinas of harm_a1-p.R31X-GFP-electroporated mice. (A) Comparison of representative cryosections of DMSO- and PTC124-treated retinas revealed harmonin a1–GFP expression in photoreceptor cells of PTC124-treated retinas. (B) Intensity profiles of GFP fluorescence at the dotted lines in the images of the retinas in (A). DMSO- and PTC124-treated retinas are represented by gray and black lines, respectively. (C) Quantification of GFP fluorescence intensity in selected regions of photoreceptor cells, namely the outer plexiform layer (OPL), outer nuclear layer (ONL), and inner and outer segment (IS/OS) in DMSO-treated (gray columns) and PTC124-treated (solid columns) retinas, respectively. GFP fluorescence intensity was higher in all compartments of photoreceptor cells in PTC124-treated retinas compared with DMSO-treated retinas, indicating that PTC124 induced read-through of the p.R31X mutation in vivo. Scale bar in (A): 10 µm. (D) Western blot analyses of NB54 or PTC124 mediated read-through in the retina of harm_a1-p.R31X-RFP electroporated mice. Subretinal injections of PTC124 or NB54 restored full-length harmonin a1-mRFP expression. Actin-staining (42 kDa) was used as loading control. (E) For quantification of read-through of the p.R31X mutation, the optical densities of harmonin a1-mRFP bands, stained by anti-RFP antibodies, were ascertained and normalized to the appropriate loading control. The increase of read-through is shown as fold increase over control mice. Quantitative data resulted from two to three independent experiments; error bars represent SD, n.s. not significant.

Work Package 10; Dissemination and translation of knowledge.

WP 10 has successfully achieved to create an efficient training & dissemination platform and ensure intensive training for consortium partners throughout the duration of the project. Well-structured online training sessions (Webinars) tackling scientific ciliopathy topics of partners' interest were successfully conveyed.

At the same time, partners paid attention on extensive translation of the know-how gained in this project towards applications in the life science area, pharmacology and medicine, with the aim of dissemination of relevant project results and gaining potential relevant inputs from industry, clinicians and academics.

Finally, the young scientists had the opportunity to gain clearer picture of career options in academia and industry, work-life balance and various requirements for each sector, especially SMEs and large companies.

In its final year, SYSCILIA has teamed up with other main European players in the ciliary research field, such as the Ciliopathy Alliance, the Nordic Cilia and Centrosome Network and the French Cilia and Flagella research network, to collaboratively organize the international CILIA 2014 conference, that was held in Paris (November 18-21). This main event succeeded the first international Cilia conference in Europe, organized by Ciliopathy Alliance UK together with SYSCILIA in London, 2012. An attractive hallmark of the Cilia conferences is the exchange of knowledge of high quality, mainly unpublished work in a friendly and open atmosphere. The conference highlights both scientific and clinical progress, uniquely integrating patient perspectives, and bringing together scientists from different disciplines.

D. The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

Potential impact

Work Package 1

Cilia are critical for many important functions such as vision, hearing, smelling, breathing, reproduction and more. Non-functional cilia cause a large group of diseases named ciliopathies. Gaining knowledge about the constituent components of the system, their localization and interactions is the underlying basis for understanding ciliary function. This is of critical importance to find treatment options. Taken together, the result obtained within WP1 shed light on the mechanisms of disease for several ciliopathies.

Furthermore, our proteomics experiments have significantly improved our knowledge about proteins involved in ciliopathies like Usher Syndrome (USH), Joubert syndrome, Sensenbrenner syndrome and several others. This is valuable knowledge to understand the underlying molecular mechanisms of disease pathogenesis. The interaction and complex analysis of Usher syndrome-associated proteins for example yielded intriguing insights and the intracellular mechanisms via which mutations in USH genes cause photoreceptor degeneration on the long term. These are the insights that will likely provide the basis for the development of successful therapeutic strategies in the future.

A further example is Lebercilin and mutations in Lebercilin which cause Leber congenital amaurosis. We demonstrated mechanistically that the mutations lead to the disruption of the intraflagellar transport in photoreceptors and thereby to degeneration and blindness. Defining the mechanism of disease is a first step towards therapy. This example demonstrates this nicely because we could show that it is a disruption of a process that can be rescued by approaches like gene therapy.

Thes are only two examples of many to show the potential impact of WP1. The amount of publications which made use of WP1 interaction data clearly demonstrates that the progress reached already is highly valuable for the biology of cilia in health and disease. Now that the dataset is completed and bio-informatic analysis is achieved, we expect a large impact. We deliver a vast amount of data with high significance to the community. We can demonstrate how this data can be used for systems biology and systems medicine approaches that aim to understand ciliary function in health and disease. The work performed in WP1 provides a framework that allows others to reproduce and amend our analysis and even apply this framework to other organelles or disease types.

Work Package 2

The data processed and distributed by WP2 will be of massive importance to the growing body of researchers studying ciliary diseases and ciliary function, and the more precise insights generated by collaboration with other workpackages will immediately drive research into the understanding of ciliary function and disease.

The final network has also provided many key links between known ciliary diseases and has suggested that several other conditions, notably 3M and Hermansky-Pudlak syndromes, might also be ciliopathies. This has important implications for methods to diagnose and potentially treat ciliary diseases, and will ultimately help improve the lives of the hundreds of thousands Europeans suffering from these conditions.

At a more general scientific level, this work has demonstrated a processes for integrating data from a disease-focussed systems medicine initiative, and the lessons learned here will greatly benefit future efforts not just in ciliary diseases, but in any focussed investigation into the molecular basis of diseases.

Work Package 3

The work on the Gold Standard and Gene Ontology is beneficial work not only for SYSCILIA but the research community at large, since these annotations feed into public services researchers use to evaluate their datasets, such as the Database for Annotation, Visualization and Integrated Discovery (DAVID) (http://david.abcc.ncifcrf.gov/).

WP3 has contributed to the understanding of cilia proteins and complexes. The scientific knowledge and the bioinformatics networks and resources that have been generated are basic research activities that will in time feed into our understanding of ciliopathies, and thus have biomedical relevance.

Work Package 4

On the methodological side several algorithms were developed for the analysis and the integration of diverse experimental data. The applicability of the algorithms is more general and not specific to the study of cilia. The graph-clustering and the Bayesian integration approaches can be used in different contexts, while the graph-based statistical approach could be used for the prediction of disease genes for several complex/multigenic diseases.

On the biological side the *in silico* predictions will significantly improve our knowledge about the biology of cilia and ciliopathies. The predicted protein complexes and genes expand the currently known sets of ciliary complexes and genes and we are confident that they will be the basis for the design of future experiments. Similarly, the predicted disease genes/mechanisms will be valuable to understand the underlying molecular mechanisms of disease pathogenesis. Understanding the molecular and cellular mechanisms of diseases is vital for dissecting the pathogenic process, identifying appropriate therapeutic targets and designing effective treatments.

Work Package 5

In WP5, we implemented assays to measure ciliary function quantitatively. These assays not only enlighten some of the numerous vital, but still enigmatic, features of cilia but also enable us to study ciliopathy associated modules (in WP6) and provide readout systems for therapeutic approaches (in WP9). One model system we used was the vertebrate photoreceptor cell characterized by photosensitive outer segment as a highly modified primary cilium. With extremely high rates of biosynthesis and efficient translocation of ciliary components, these highly compartmentalized cells provide an excellent model system for studying ciliary transport, not only during ciliogenesis and maintenance of photoreceptor cells, but for ciliary transport in general. *In vivo* analysis of photoreceptor trafficking provides a useful biomarker system to study the molecular mechanisms underlying (retinal) ciliopathies.

Data derived from work with *C. elegans* provide new testable models for understanding the function of ciliopathy proteins, especially those related to ciliary transport. Although our findings were made in relatively simple nematodes, there is universal acceptance that nematode cilium biology mirrors basic underpinning biology of human cilia. Therefore, the new knowledge gained by our efforts is directly relevant to cilia disease, and provides new avenues of exploration in more complex experimental systems.

Our findings with regards to ciliary signalling have significant implication for the therapy of ciliopathies. Frequently, mutations of cilia-associated molecules result in cystic kidney disease and end-stage renal disease. Currently, the only approved therapy (Europe, Canada, Japan) is the inhibition of cAMP using the vasopressin-2-receptor antagonist Tolvaptan®. Our findings have two significant implications: 1) mTORC1 is a

significant target to ameliorate ciliopathy-associated disease manifestations, and 2) selective inhibition of a single pathway is likely rapidly circumvented by other growth-promoting pathways. Thus, approaches to treat ciliopathies likely have to apply similar strategies utilized to treat cancer, i.e. combinatorial or sequential drug regimen.

Work Package 6

By examining the effect of gene loss on cilium structure and function, the results from WP6 have greatly expanded our knowledge of cilium biology and shed important insight into ciliary disease mechanisms. The ever increasing number of individuals being diagnosed with a ciliopathy underscores the wider societal impact of this work. Major WP6 discoveries were made in ciliary transport, signalling and disease. For example, experiments in mammalian cells provided the first evidence that TMEM67 is a receptor, operating within a Wnt5a-TMEM67-ROR2 developmental signalling pathway. Work in mice provided novel insights into the pathomechanism of retinal ciliopathies that affect the function and viability of photoreceptor cells. As there is no effective treatment available for non-syndromic and syndromic retinal dystrophies, understanding the physiology of photoreceptor cilia and the according disease mechanisms is critical for finding therapeutic strategies. Experiments in mammalian cells and mice also uncovered surprising new roles for ciliopathy proteins in the regulation of basic cellular processes such as proteasome activity, thus leading to new hypotheses. Research in *C. elegans* helped to lead to the discovery of new ciliary genes associated with ciliopathies, and uncovered new insight into ciliary transport mechanisms across the ciliary transition zone. Research in zebrafish deciphered functional relationships between ciliopathy genes, which is relevant to understanding the clinical spectrum in disease. Together, the WP6 work has pushed the boundaries of our knowledge of ciliary function in health and disease, and lead to important new avenues for future therapeutic exploration and exploitation.

Work Package 7

Ciliopathies remain a group of conditions that are both medically challenging and important to diverse fields of basic scientific research. The “systems medicine” strategy that we have implemented in this Work Package is therefore likely to be of wider, longer-term importance to patient care. This issue continues to provoke debate in the research community, yet the approach has significant challenges and the clinical utility of this approach remains unclear. We believe that a whole genome functional annotation, that evaluates the contribution of every human gene to ciliogenesis will, for example, enable better interpretation of clinical WES data. A fundamental challenge in analyzing WES data is distinguishing pathogenic mutations from benign polymorphisms, particularly in the context of genetically heterogeneous conditions such as the ciliopathies and missense variants of unknown significance. This challenge is likely to continue in the foreseeable future, since it is very probable that widespread adoption of WES will be used as a routine diagnostic strategy. However, this work is often limited by the extreme genetic heterogeneity of the ciliopathies, and the inability to interpret “private” mutations in single families as pathogenic or variants of unknown significance. Our ability to overcome this challenge will inform the debate on the eventual clinical utility and clinical validity of a “systems medicine” strategy. Finally, the functional annotation data from this project could provide outcome evidence for the health economic modelling of the impact of systems medicine approaches which are still unknown at the present time. We envisage that better interpretation of WES data will enable considerable potential cost savings for clinical service testing for mendelian conditions including the ciliopathies.

Work Package 8

WP8 has focused on the genetic and functional dissection of mutational burden in the ciliopathies. Ciliopathies are individually rare disorders, however, collectively they represent up to 1:1000 live births making them nearly as common as disorders such as Down syndrome. As such, our findings have the

greatest impact on improvement of diagnostics for patients and their families and also have the potential to influence eventual therapeutic development.

Improved diagnostics. The ciliopathies are genetically and phenotypically heterogeneous, meaning that they have been notoriously difficult to diagnose both on the clinical as well as the molecular levels. Moreover, these challenges are augmented by the observation that the vast majority of causal ciliopathy alleles are a) private alleles (not recurrent in the general population); and b) can often be ambiguous changes of uncertain significance (missense variants). Our work has expanded our knowledge of the mutational burden of this patient population and has also developed improved tools to predict the effect of variant pathogenicity. Moreover, our improved knowledge of the mutational burden with respect to ciliary systems networks and patient phenotypes may ultimately enable improved prediction of patient phenotype before the manifestation of disease. For example, our results, in some instances, may ultimately improve our ability to predict whether a child with a ciliopathy will eventually require kidney transplantation due to untreatable renal cystic disease; or whether, and at what age, a child might undergo retinal degeneration. Admittedly, we currently do not have the ability to predict such phenotypic outcomes with genotype information, however we have added to the body of knowledge that is leading us toward this objective in the future.

Broadened therapeutic targets. In addition to improved diagnostics, an obvious impact of our work on health care is that we have facilitated therapeutic discovery. During the course of our work, we have identified novel ciliopathy genes and elucidated their biological functions through the development of relevant *in vivo* models of disease. In doing so, we have developed models that can and should be used to identify novel drugs to ameliorate or inhibit progression of ciliary disease.

Work Package 9

One hallmark of many ciliopathies is vision loss due to degeneration of photoreceptor cells. Although not life-threatening, loss of sight has a great impact on the quality of life. Blindness is a substantial obstacle to leading an economically and socially independent life. Blindness has a huge impact on healthcare systems and the European society as a whole. One big problem in is the genetic heterogeneity of ciliopathies, and differential splicing and/or big protein sizes make genetic treatment very challenging. Approximately 10-20 % of ciliopathy patients have in-frame nonsense mutations. We have investigated the effectiveness of translational read-through inducing drugs (TRIDs) as therapy for several genetic diseases caused by in-frame nonsense mutations. Our results indicate that treatment through translational read-through is very promising, and we are confident that future clinical trials will result in treatment for some of the genetic disorders.

We generated novel techniques for quantitation of patient-derived cells in ciliopathies. For example, we have pioneered a standardized and novel methodology to extract ciliated cells from urine (urine-derived renal epithelial cells) and deciduous “milk” teeth (Giles et al, Nature Protocols, 2014; and Ajzenberg et al, Cilia, 2015), and have generated SOPs that have been implemented in several hospitals in the Netherlands. Because these cell sources are obtainable outside the clinical setting (eg the researcher can pick up the biomaterials at their home) we have observed a tremendously improved accrual for our studies. The non-invasive nature of these cell sources is of course also child- and parent-friendly and fosters a cooperative relationship.

Furthermore our studies directed attention to the medical treatment of the concomitant fibrosis accompanying renal cyst development in ciliopathies (nephronophthisis, NPHP). We demonstrated that the pro-fibrotic response is an early event after reduction of NPHP protein CEP164 in vitro and in vivo, and drives renal epithelial cells to undergo epithelial-to-mesenchymal transition (Slaats et al, PLoS Genetics, 2014). This could be a game-changer in the treatment of NPHP, where physicians are focussing on treating cysts instead of fibrosis.

Dissemination and exploitation

Work Package 10

WP10, "Training, dissemination and translation of knowledge", has successfully achieved to create an efficient training & dissemination platform and ensure intense trainings for consortium partners throughout the duration of the project. Well-structured online trainings tackling scientific ciliopathy topics of partners' interest were successfully conveyed.

Business oriented workshops offered discussions about the ways to provide detailed knowledge transfer from this project and subsequently how to conduct research into specific industrial needs in toxicology and pharmacology in order to address some of these needs. The main idea was to facilitate translation of research project results and know how into a technology and to deliver to the industry useful techniques - assays for therapeutic areas connected to ciliopathies, systems biology based knowledge extraction procedures with strict industrial quality controls and testing services

At the same time, partners paid attention on extensive translation of the know-how gained in this project towards applications in the life science area, pharmacology and medicine, with the aim of dissemination of relevant project results and gaining potential relevant inputs from industry, clinicians and academics.

Finally, the young scientists had the opportunity to gain clearer picture of career options in academia and industry, work-life balance and various requirements for each sector, especially SMEs and large companies.

The SYSCILIA project has provided a good training ground for the many PhD students and postdoctoral researchers. These junior scientists not only obtained technical experimental expertise, but also gained networking experience on account of the highly collaborative nature of the project.

Overall, SYSCILIA results have been published in leading scientific journals and presented at national and international conferences of cilium biology and human disease. This has led to a wide dissemination of our results and provided a platform for new ongoing collaboration within SYSCILIA and beyond. Also, results have been communicated through newsprint, radio channels, on television and the SYSCILIA website, thus providing the general public access to the findings. New insights into the disease mechanism for cystic kidney disease or retinal degeneration have led to local and national media coverage.

Furthermore, SYSCILIA results have been disseminated and communicated directly to ciliopathy patient groups such as the LMBBS society, a patient group for UK-based Bardet-Biedl syndrome patients and their families, as well as national and international patient foundations such as FAUN, ProRetina Germany, and Foundation Fighting Blindness (USA). We have used public engagement commitments to disseminate research findings to a lay audience, such as school-children as part of the "Researchers in Residence" scheme, and other patient-public involvement as part of the wider remit of research into recessive conditions. Partner 10 is an advisory member of the scientific panel for Ciliopathy Alliance UK. CAUK is an alliance of medical researchers, doctors and patient organisations representing patients and families suffering from ciliopathy diseases. The aims of CAUK are to share knowledge and understanding in the medical research community and encourage collaborative research, promote awareness of ciliopathy diseases and the respective patient organizations, and encourage patient involvement in research and clinical management. We have disseminated appropriate research findings through CAUK, since expected outcomes for the proposed research project will be relevant to basic research and to future translational research benefits to patients. Partner 10 presented this research at the "Joubert Syndrome Biennial Conference: Advancing Translational Ciliopathy Research, Enhancing Clinical Care" October 21-22, 2013 Boston, MA. This was an excellent opportunity to present to family members and supporters of The Joubert Syndrome & Related Disorders Foundation (JSRDF), the leading support group for patients with Joubert syndrome and allied ciliopathies (see http://www.jsrdf.org/). Partner 10 also attends meetings of the local family support group Joubert Syndrome UK and Alström Syndrome UK, and often speaks informally to parents in these groups about progress in research.

We also have very strong links with the LMBBS society a patient group for UK based BBS patients and their families. We attend their annual conference yearly to give updates on our progress, as well as to be available for patients to discuss any aspect of their experience directly with researchers. We are also one of the key teams involved in running UK wide nationally commissioned multidisciplinary clinics for BBS. These allow streamlining of care for the patients and clinicians and provide a wealth of data on disease pathology and progression that will be used to more fully inform clinical decision making and the information given to patients. As such we are in a prime position both to understand from the patients what their main clinical concerns are, allowing our research to be shaped accordingly and to disseminate our results back to our patients and families. Once any of the therapeutic strategies investigated during WP9 reach clinical trial stage we are also in an excellent position to find and assess possible candidates based on our strong existing links with the patients and on our thorough knowledge of their clinical presentation, genetics and history.

Our research findings have also been disseminated at suitable national and international meetings such as CILIA 2012 "Cilia in Development and Disease" (see http://www.ciliopathyalliance.org/events/past-events/104-cilia-conference-2012.html), the annual meeting of the American Society of Cell Biology and other, smaller Gordon or FASEB conferences with a focus on cilia biology, and at the Santorini conference focusing on Systems Medicine, personalized Health and Therapy (2014). We have maintained existing and fostered new collaborations with academic researchers both in the medical genetics and ciliary biology fields.

In its final year, SYSCILIA has teamed up with other main European players in the ciliary research field, such as the Ciliopathy Alliance, the Nordic Cilia and Centrosome Network and the French Cilia and Flagella research network, to collaboratively organize the international CILIA 2014 conference, that was held in Paris (November 18-21). This main event successfully focused on recent developments of us and others in investigating cilia structure and function including trafficking, cilia and development, cilia in human genetic diseases and cilia in infectious microorganisms. An attractive hallmark of the Cilia conferences is the exchange of knowledge of high quality, mainly unpublished work in a friendly and open atmosphere. The conference highlights both scientific and clinical progress, uniquely integrate patient perspective , and brings together scientist from different disciplines.

Cilia2016 will be organized in Amsterdam, October 2016, as an EMBO conference series.

Since the start of the project **a total of 171 publications** have been published in scientific journals (~45% with IF>8; almost 10% with IF>30). 34 publications featured with more than 1 SYSCILIA partner (almost 20%)

E. Address of the project public website, relevant contact details.

http://syscilia.org

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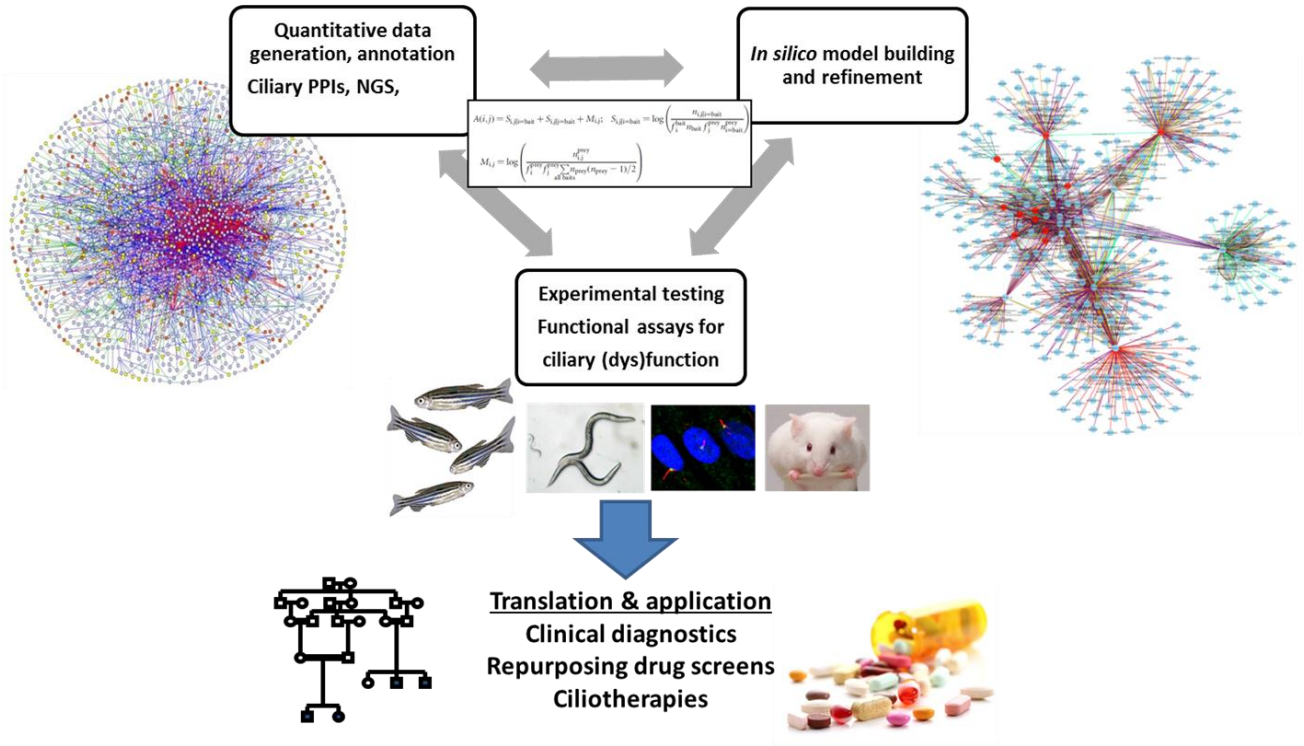
SYSCILIA approach:



SYSCILIA folder:

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Syscilia

Syscilia

Find out more, visit our website:

http://syscilia.org/

SYSCILIA

A systems biology approach to dissect cilia function and its disruption in human genetic disease.

The aim of SYSCILIA is to identify the molecular mechanisms characterizing cilium function, and the discrete perturbations associated with dysfunction caused by mutations in inherited ciliopathies, applying a systems biology approach.

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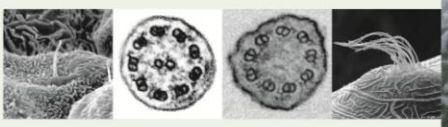
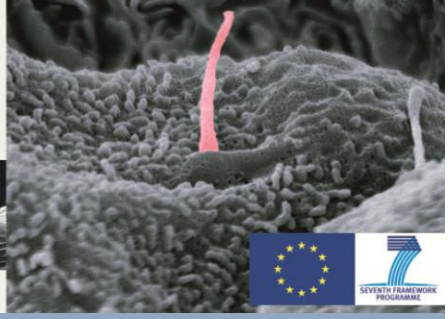


Design: © Final Design

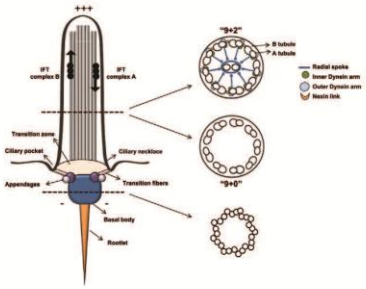
SYSCILIA is a large scale integrating project funded by the European Community's Seventh Framework Programme (FP7/2009/241955) under the Health Cooperation Programme.



Ciliopathies - *hereditary diseases caused by defects in the function of cilia*

The cilium

A cilium is a longitudinal projection from the surface of almost every vertebrate cell. Cilia are derived from the mature centriole of the centrosome, and perform essential cellular functions. Some cilia are motile, which are used by the cell to propel fluid. Most cilia however are non-motile, also named primary cilia. These were long thought to be vestigial organelles that had lost their crucial motile function throughout evolution. In the last decade however, they were found to harbour key receptors and other components of cellular signalling pathways that are vitally important in the regulation of many biological processes. They turned out to be essential signalling hubs, acting like the cell's antenna.



Ciliopathies

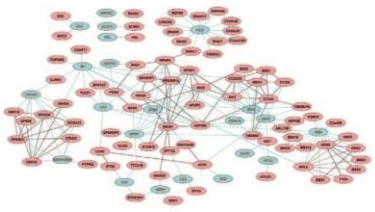
Consistent with their broad tissue and cellular distribution, it is now recognized that defects in cilia give rise to an equally broad but consistent range of phenotypes in mammals. They underlie a number of often chronically disabling and sometimes life-threatening genetic conditions, such as **blindness, deafness, chronic respiratory infections, kidney disease, heart disease, infertility, obesity, diabetes, and even some types of cancer**. What links these diverse individual disorders is that the proteins, known to cause the disease upon mutation of their cognate genes, are localized to the cilia or their anchoring structure, the basal body and the adjacent centriole and play some role in their function. Therefore, these hereditary diseases are called ciliopathies

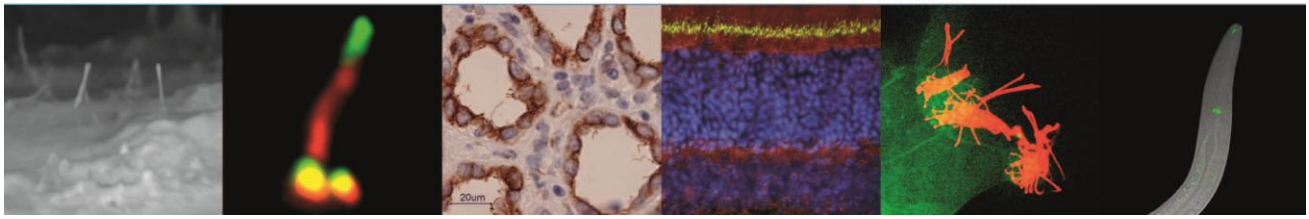
Scientific & Technical Objectives

Our overall objectives are to **establish a paradigm for studying and modelling complex eukaryotic systems**, to **understand how system perturbation contributes to the modulation of clinical phenotypes**, and to provide a **better understanding of ciliary processes in biology and their associated diseases**.
Our objectives focus on all critical components of the systems biology process, namely:

- assay development and application,
- data generation, handling and integration,
- model building and testing followed by refinement.

We also exploit the models to find new insights into biological mechanism and human disease.





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Radboud University Medical Centre, Nijmegen	Dr. Martijn Huijnen	The Netherlands
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Universitätsklinikum Freiburg	Prof. Dr. Gerd Walz	Germany
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Universitair Medisch Centrum Utrecht	Dr. Rachel Giles	The Netherlands
University College London	Prof. Dr. Phil Beales	United Kingdom
The Leeds Institute of Molecular Medicine	Prof. Dr. Colin Johnson	United Kingdom
University College Dublin	Dr. Oliver Blacque	Ireland
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Cambridge Cell Networks Ltd.	Dr. Gordana Apic	United Kingdom
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Westfaelische Wilhelms Universitaet Muenster	Prof. Dr. Heymut Omran	Germany

SYSCILIA poster 2012:

Syscilia

SEVENTH FRAMEWORK PROGRAMME

A systems biology approach to dissect cilia function and its disruption in human genetic disease

Ronald Roepman[1,2,3] Marius Ueffing[4,5] and the SYSCILIA consortium[6]

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Introduction

This project aims to apply systems approaches to cilia, long-known organelles of growing biomedical importance. A cilium can be regarded as a relatively small, experimentally tractable, isolated system that is ideally suited to such analyses. Primary cilia are basically signaling hubs (**Fig. 1**), and their disruption leads to striking developmental defects. Some ciliopathy-associated proteins have recently been revealed to be physically or functionally associated in several distinct groupings, with limited connections to other crucial biological processes. The project will focus on the core systems biology activities of data generation, integration, assay development, model building and model refinement. The models and associated discoveries will ultimately be employed to accurately diagnose and therapeutically target the growing number of human diseases associated with ciliary dysfunction.

Strategy

The Systems Biology workflow of SYSCILIA involves iterative cycles of quantitative data generation, model building, experimental testing and model refinement. The work is divided into four scientific components, and a fifth component involving project management, training, and dissemination of data, including translation to the industry (**Fig. 2 & 3**)

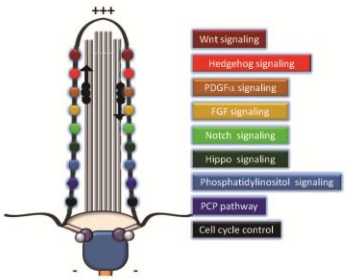

Figure 1 *Scheme of a cilium and its basic involvement in cellular and developmental processes*

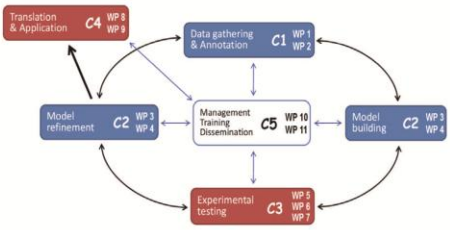

Figure 2 *The SYSCILIA workflow. The work is organized into five and 11 workpackages.*
C1 Defining the elements and variables of ciliary systems
C2 Modelling the variables of ciliary systems
C3 Assessing and manipulating the variables of ciliary systems
C4 Applying ciliary systems to human health towards improved diagnostics and therapy
C5 Project management, training, and dissemination of data, including translation to the industry

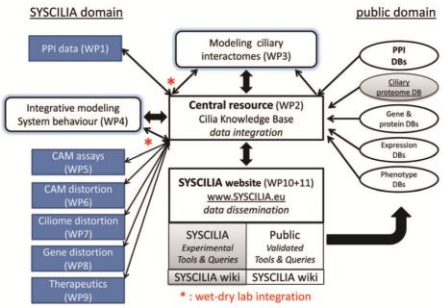

Figure 3 *SYSCILIA integrative knowledge management through the central resource. Boxes in blue indicate wet-lab science; clear or gray boxes indicate computational efforts/resources*

Figure 4 *Screenshot of the SYSCILIA website: www.SYSCILIA.org*

Systems biology… is about putting together rather than taking apart, integration rather than reduction. It requires that we develop ways of thinking about integration that are as rigorous as our reductionist paradigms, but different… It means changing our philosophy.
– Denis Noble, The Music of Life: Biology beyond the genome –

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Picture of consortium members taken during the annual meeting 2011

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For more information please visit: www.SYSCILIA.org

SYSCILIA poster 2014:

SYSTEMATIC EXPLORATION OF THE CILIARY PROTEIN LANDSCAPE BY LARGE-SCALE AFFINITY PROTEOMICS

K. Boldt[1]*, J. van Reeuwijk[2-3]*, Q. Lu[4], K. Koutroumpas[5], G. Toedt[8], T.J. van Dam[9], N. Horn[1], SE. van Beersum[2-3], Y. Texier[6], TM. Nguyen[2-3], JR. Willer[7], EU. The Syscilia Consortium[10], N. Katsanis[7], M.A. Huynen[9], T. J. Gibson[8], F. Kepes[5], RB. Russell[4], M. Ueffing[1], R. Roepman[2-3]

*equal contribution

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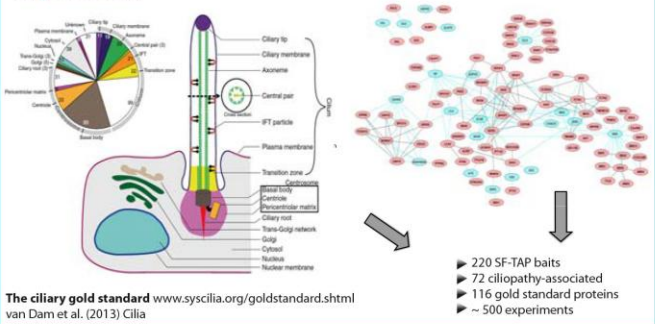
Introduction

Mutations in different ciliopathy-associated genes often result in overlapping clinical phenotypes, affecting only single organs or, in case of syndromic ciliopathies, several organs with different disease severity. Despite the phenotypic variability, the basic disease mechanisms causing ciliopathies show a major overlap for many forms of ciliopathies. To fully understand these mechanisms, an in-depth understanding of ciliary processes in health and disease is essential. To achieve this using a systems biology approach, we joined the forces of 18 research groups from Europe and the US in the SYSCILIA consortium (www.SYSCILIA.org). In the current study, we aimed to generate a comprehensive view of the ciliary protein interaction landscape by conducting large-scale affinity proteomics to boost insights into the assembly of these ciliary modules, and their connectivity in larger functional protein networks. This provides an important framework to deconvolute the pathways and processes that drive ciliopathies, and to understand the general importance of ciliary function for cellular homeostasis.

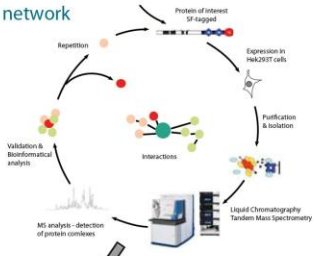
Target selection



The ciliary gold standard www.syscilia.org/goldstandard.shtml
van Dam et al. (2013) Cilia

- 220 SF-TAP baits
- 72 ciliopathy-associated
- 116 gold standard proteins
- ~ 500 experiments

Defining the ciliary protein network

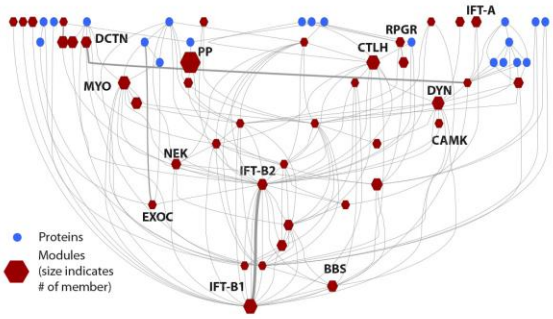


Raw network:

~ 4500 proteins
> 30,000 interactions

- Socio-affinity-index based filtering
- Hirarchical Clique determination
- PCA-based filtering of complexes

For details see Poster 211 by Q. Lu

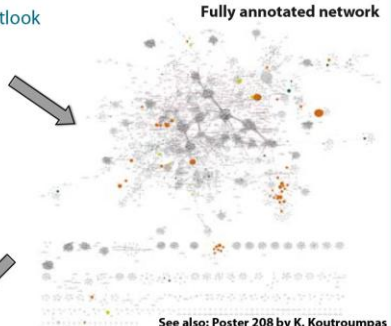


Integration, application and outlook

Integration of:
Public domain data
Syscilia data:
- disease associated variants
- siRNA screen
- ciliary gold standard
- Bayesian integration

--> ciliary proteome

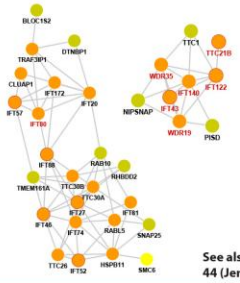
Fully annotated network



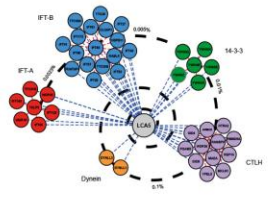
See also: Poster 208 by K. Koutroumpas

Current development: Data accessibility and sharing

Novel members of known modules



Sub-module detection



See also talks: 30 (Schmidts), 33 (Szymanska), 35 (Ueffing), 44 (Jensen); posters: 75 (De Vrieze), 106 (Beyer)

Conclusion

Our systems oriented approach, employing affinity proteomics to define the ciliary network, has resulted in a comprehensive description of known and candidate ciliary protein networks and modules. By using this as a resource for defining novel ciliary modules and novel components of well know ones, we could already identify numerous novel candidates that are likely linked to ciliary (dys-) function. The integration of additional datasets into this network, such as whole genome siRNA screening information and gene variants from ciliopathy patients, will make this a highly valuable and easily accessible resource to identify novel candidate ciliopathy proteins and to improve our understanding of pathogenic mechanisms underlying ciliopathies.

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