Supplementary Note

Kidney-related ciliopathies

We found that few of the NPHP-associated genes were hits in our screen, particularly for those genes mutated in NPHP only and not other ciliopathies. In particular, the NPHP genes that are associated with the Inversin compartment (*INVS*, *NPHP3*, *ANKS6*, *NEK8*) and the polycystic kidney disease genes *PKD1*, *PKD2*, and *PKHD1* are all non-hits. These genes are unlikely to be false negatives in our screen, as NPHP4 mouse mutants have been shown to have an intact Hh response[1], and Inversin compartment-related ciliopathies present with kidney cysts and laterality defects but not symptoms typically linked to the Hh pathway, such as polydactyly and craniofacial abnormalities[2]. This finding suggests that these disorders likely arise independently of Hh signaling and possibly even involve pathomechanisms independent of cilia[3].

Previously unrecognized ciliopathies

The peptidyl-prolyl isomerase *Cwc27* was a hit in our screen and was recently identified as a retinitis pigmentosa (RP) gene[4]. Although this syndrome was not reported as a ciliopathy, the spectrum of reported *CWC27* pathologies includes canonical ciliopathy symptoms (craniofacial abnormalities, short stature, brachydactyly, and developmental delay), and we therefore suggest that *CWC27*-associated disease is a ciliopathy. Similarly, mutations in genes encoding the INTS1 and INTS8 subunits of the Integrator complex were recently described in individuals with a neurodevelopmental disorder and facial and skeletal malformations commonly seen in ciliopathies[5]. As the Integrator genes *Ints6* and *Ints10* are hits in our screen, disorders due to defects in Integrator complex function may also stem from altered ciliary signaling.

Screen performance and CRISPR-based screening

Several factors likely contributed to the success of our screen. Given the strong influence of cell confluence on cilium assembly and Hh signaling, the homogeneous growth conditions afforded by pooled screening were likely a major advantage. In an arrayed format, perturbations causing growth defects can indirectly affect ciliogenesis by decreasing cell density, thus generating false positives that need to be filtered out[6]. Pooled screens achieve confluence regardless of genotype,

and thus we can successfully identify hits that have moderate proliferation defects, including the four components of the TED complex.

Another key feature of our screen is the use of CRISPR-based gene disruption. The strong phenotypes produced by CRISPR/Cas9 likely made it possible to detect hit genes in cases where partial knockdown by RNAi or CRISPR interference (CRISPRi) might have failed to produce a detectable phenotype. A potential caveat of our approach is a decreased ability to detect hits among genes that are strictly required for cell viability. However, because ciliary signaling is dispensable for growth of cultured cells, this issue likely had a limited impact on our screen. It is also important to note that the allelic series achievable with RNAi and CRISPRi may be preferable when screening for phenotypic modifiers, as in chemical genetic or genetic interaction analyses.

The strong performance of CRISPR-based screening may also be attributable to our use of an sgRNA library comprised of many highly active sgRNAs with few off-target effects. High on-target activity is especially important for detecting hits in dropout-based screens (in which hits become depleted) and was achieved by using 10 sgRNAs per gene and by optimizing the stability of Cas9 expression. A second benefit of using 10 sgRNAs per gene is the increased statistical power achieved when multiple effective sgRNAs are found targeting a single gene. Indeed, for hit genes such as *Dync2h1*, *Tmem107*, *Ift80*, *B9d1*, and *Grk2*, at least 7 out of 10 sgRNAs were depleted more than 4-fold (and up to 45-fold), leading to high statistical confidence. While other genes may not have been targeted as efficiently, the use of 10 elements per gene strongly increases the statistical power of hit gene detection.

An additional benefit of our screening strategy is that it readily identifies effective sgRNAs against hit genes that can be used in follow-up studies. These sgRNAs can be combined with sgRNA-resistant cDNAs to enable rigorous validation of hits and functional testing of mutant alleles, as demonstrated for the candidate disease-causing mutation in *Txndc15*. We note however that the cDNA transfection and luciferase assay readout we used for *Fam92a* and *Txndc15* may not work in all cases, such as when mutant phenotypes are mild or when over-expression of the rescue transgene interferes with function. These circumstances likely led to inconclusive results when we attempted rescue experiments for *Armc9* and *Ttc23* (data not shown). In such cases, observing concordant phenotypes for multiple independent sgRNAs provides an effective means to minimize possible off-target effects.

Phylogenetic analysis of TED complex genes

Turk et al.[7] recently observed a phylogenetic pattern in which the presence or absence of ε-tubulin in a given species predicts whether δ- or ζ-tubulin is also present. The evolutionary co-occurrence of these centriolar tubulins supports a functional link between these proteins and prompted us to ask whether *Tedc1* and *Tedc2* also share a similar phylogenetic distribution. We found *Tedc1* and *Tedc2* homologs in annelids and sea urchin and *Tedc1* homologs in evolutionarily distant species such as *Paramecium tetraurelia* and *Tetrahymena thermophila* (Supplementary Table 8). Consistent with a conserved functional relationship among TED complex components, all of these species also have ε-, δ- and/or ζ- tubulins; conversely, we did not detect *Tedc1* or *Tedc2* homologs in any species lacking ε-, δ- and ζ- tubulins. The scope of this analysis was limited by the more rapid divergence seen for *Tedc1* and *Tedc2* sequences than for ε-, δ- and ζ- tubulins (Supplementary Fig. 7), but this phylogenetic distribution further supports a shared function and echoes what is seen for γ-tubulin and subunits of the γ-tubulin ring complex.

Supplementary Table 9. List of oligonucleotides and recombinant DNA.

REAGENT	SOURCE	IDENTIFIER
Oligonucleotides		
sgRNA library oligos (genome-wide and cilia/Hh pathway-focused libraries)	Agilent	N/A
siGenome Smartpool Iqce siRNA, targeting sequences: GAAAGAAGCCCAUGGUGGA, GAAAGGA–UCGGCAGAUAGA, GCAUUGCCAUGGAAACAUA, AC–ACCUAGCUCGUUCGAAG	Dharmacon, Pusapati, et al. [8]	Cat#M-059692-01-0005
siGenome Non-targeting Smartpool #1, targeting sequences: UAGCGACUAAACACAUCAA, UAAGGCU–AUGAAGAGAUAC, AUGUAUUGGCCUGUAUUAG, AU–GAACGUGAAUUGCUCAA	Dharmacon	Cat#D-001206-13-05
siRNA Evc2, targeting sequence: GAUGGAAUCCAGACUUUCA	Sigma Aldrich, Pusapati, et al. [8]	Cat#SASI_Mm01_00106977
Mision siRNA universal negative control #1	Sigma Aldrich	Cat#SIC001
Primer: sgRNA_Amp_F1: AGGCTTGGATTTCTATAACTTCGTATAGCATACATTATAC	Deans, et al. [9]	N/A
Primer: sgRNA_Amp_R1: ACATGCATGGCGGTAATACGGTTATC	Deans, et al. [9]	N/A
Primer: sgRNA_Amp_F2: CAAGCAGAAGACGGCATACGAGATGCACAAAAGGAAACTCACCCT	Deans, et al. [9]	N/A
Primer: sgRNA_Amp_R2: AATGATACGGCGACCACCGAGATCTACACGATCGGAAGAGCACACGTCTGAACTCCAGTCACNNNNNNCGACTCGGTGCCACTTTTTC	Deans, et al. [9]	N/A
Primer: sgRNA_Seq: GCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTAAACTTGCTATGCTGTTTCCAGCTTAGCTCTTAAAC	This paper	N/A
Recombinant DNA		
Plasmid: pGL-8xGli-Bsd-T2A-GFP-Hyg	This paper	N/A
Plasmid: pGL3-8xGli-Firefly-luciferase	Philip Beachy	N/A
Plasmid: pGL3-SV40-Renilla-luciferase	Philip Beachy	N/A
Plasmid: pHR-Pgk-Cas9-BFP	This paper	N/A
Plasmid: pMCB320-mU6-sgRNA-mCherry-Puro (see Supplementary Table 1 for sgRNA sequences)	Han, et al. [10]	Addgene #89359
Plasmid: pMCB306-mU6-sgRNA-GFP-Puro	Han, et al. [10]	Addgene #89360
Plasmid library: mouse CRISPR KO	Morgens, et al. [11]	N/A
Plasmid library: mouse cilia/Hh sgRNAs	This paper	N/A
pDONR-221	Thermo Fisher	Cat#12536017
pENTR-4	Thermo Fisher	Cat#A10465
pMD2.G	Michael Bassik	Addgene #12259
pRSV-Rev	Michael Bassik	Addgene #12253
pMDLg/RRE	Michael Bassik	Addgene #12251
pCMV-ΔR-8.91	Bob Weinberg	N/A
pCMV-VSVG	Bob Weinberg	Addgene #8454

cDNA: Armc9 (mouse, IMAGE clone 6406321)	Dharmacon (GE Healthcare)	Cat# MMM1013-202859176
cDNA: Tedc2 (mouse, IMAGE clone 6414405)	Dharmacon (GE Healthcare)	Cat#MMM1013-202859268
cDNA: Ttc23 (rat, IMAGE clone 7745883)	Dharmacon (GE Healthcare)	Cat#MRN1768-202784317
cDNA: Fam92a-sgResist (mouse)	This paper	N/A
cDNA: Txndc15-sgResist (human)	Dharmacon (GE Healthcare)	Cat#MHS6278-202801775
Plasmid: pENTR-Txndc15-mut	This paper	N/A
Plasmid: pENTR-Tedc1(mouse)-sgResist	This paper	N/A
Plasmid: pENTR-Cby1 (human)	This paper	N/A
Plasmid: pEF5B-FRT-DEST-LAP	Liew, et al. [12]	N/A
Plasmid: pEF5B-FRT-DEST-3xFlag	This paper	N/A
Plasmid: pEF5B-FRT-DEST-6xMyc	This paper	N/A

Supplementary Table 10. List of primary antibodies.

REAGENT	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-acetylated tubulin (6-11B-1)	Sigma Aldrich	Cat#T6793; RRID: AB_477585
Mouse anti-gamma tubulin (GTU-88)	Sigma-Aldrich	Cat#T6557; RRID: AB_477584
Mouse anti-centrin2 (clone 20H5)	EMD Millipore	Cat#04-1624; RRID: AB_10563501
Mouse anti-centrin3 (clone 3E6)	Novus Biologicals	Cat#H00001070-M01; RRID: AB_537701
Mouse anti-Arl13b (clone N295B/66)	UC Davis/NIH NeuroMab	Cat#73-287; RRID: AB_11000053
Mouse anti-Cby1 (clone 8-2)	Santa Cruz Biotechnology	Cat#sc-101551; RRID: AB_1561972
Mouse anti-Gli1 (clone L42B10)	Cell Signaling Technology	Cat#2643S; RRID: AB_2294746
Mouse anti-polyglutamylated tubulin (clone GT335)	Carsten Janke	
Rabbit anti-GFP	Maxence Nachury	N/A
Chicken anti-GFP	Thermo Fisher	Cat#A10262; RRID: AB_2534023
Goat anti-Gli2	R&D Systems	Cat#AF3635; RRID: AB_2111902
Goat anti-Gli3	R&D Systems	Cat#AF3690; RRID: AB_2232499
Goat anti-Sufu	Santa Cruz Biotechnology	Cat#sc-10933; RRID: AB_671172
Rabbit anti-IFT88	Proteintech	Cat#13967-1-AP; RRID:AB_2121979
Rabbit anti-Smo	Ocbina, et al. [13]	N/A
Rabbit anti-Importin Beta	Santa Cruz Biotechnology	Cat#sc-11367; RRID: AB_2265549
Rabbit anti-Fam92a1	Proteintech	Cat#24803-1-AP
Rabbit anti-Ninein	Michel Bornens	N/A
Rabbit anti-Iqce	Pusapati, et al. [8]	N/A
Rabbit anti-Evc	Dorn, et al. [14]	N/A
Rabbit anti-Tube1	Sigma Aldrich	Cat#HPA032074; RRID: AB_10601216
Rabbit anti-Tubd1	Sigma Aldrich	Cat#HPA027090; RRID: AB_1858457
Mouse anti-Flag M2	Sigma Aldrich	Cat#F1804; RRID: AB_262044
Rabbit anti-Flag	Sigma Aldrich	Cat#F7425; RRID: AB_439687
Mouse anti-Myc (9E10)	ATCC for hybridoma	Cat#CRL-1729; RRID: CVCL_G671
Rabbit anti-Myc	Santa Cruz Biotechnology	Cat#sc-789; RRID: AB_631274

Supplementary References

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