

APPENDIX

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Appendix Figure S1. Efficiency of Rab35 depletion and location of RAB35 mutations in knockout NIH3T3 cell lines

A, B hTERT-RPE1 (A) or IMCD3 (B) cells were transfected with two independent siRNAs targeting human RAB35 (RAB35-1, RAB35-2) or a pool of siRNAs targeting mouse Rab35, respectively. Knockdown efficiency was determined by qPCR. Graphs show relative mRNA levels remaining compared to cells transfected with non-targeting siRNA control (Neg). Data points are means \pm S.E.M. of 3 independent experiments.

C Sequence analysis of the Rab35 knockout (KO) NIH3T3 cell lines. The position of the target and PAM sequences of the 2 different sgRNA guides are indicated. Note that for both Rab35 KO cell lines 4 different alleles were detected. This is in agreement with the near tetraploid karyotype of the NIH3T3 cell line (Leibiger et al. 2013), which contains 4 copies of chromosome 5, on which the Rab35 gene is located.

Appendix Figure S2. Depletion of Rab35 GEF and GAP regulators

A hTERT-RPE1 cells were transfected with siRNAs targeting DENND1A, DENND1B, TBC1D10A or TBC1D10B. Depletion efficiency was determined by qPCR. Graphs show relative mRNA levels remaining compared to cells transfected with non-targeting siRNA control (Neg). Data points are means \pm S.E.M. of 3 independent experiments.

B Representative images of hTERT-RPE1 cells transfected with indicated siRNAs that were serum-starved for 48 h and stained for acetylated tubulin (acetyl. tub.) and DNA. Regions within white boxes shown at higher magnifications in smaller panels. Scale bars; 10 μ m.

C, D Localisation of transiently expressed GFP-DENND1A (C) or GFP-TBC1D10B (D) in hTERT-RPE1 cells after 24 h serum starvation and staining for DNA and polyglutamylated

tubulin (polyglu. tub.) or acetylated tubulin (acetyl. tub.), respectively. Higher magnification images of the cilia region are shown in smaller panels. Scale bars; 10 μ m.

Appendix Figure S3. Dose dependence effect of Rab35 MO in zebrafish development and KV cilia number in Rab35 morphants

A Death rate of embryos injected with 140 or 280 μ M of Rab35 Morpholino (Rab35 MO), compared with wild type non-injected embryos (WT).

B, C Effects of Rab35 MO on the heart and liver positioning of zebrafish embryos treated with 140 or 280 μ M of MO, compared with wild type non-injected embryos (WT), scored at 30 hpf and 53 hpf respectively. Values are expressed as percentages (n = 3).

D Organ situs determined by observing the heart position at 30 hpf, and the liver position at 53 hpf, by *in situ* hybridisation with a probe for *foxa3*, in non-injected (WT) controls or Rab35 MO. *Situs solitus* means left heart and liver; *Situs inversus* means right heart and liver. Heterotaxia means any other possible combination of the heart and liver position.

E Quantification of the number of cilia per KV in Rab35 MO at 8 somite stage from confocal images of Fig. 4D, compared with the non-injected (WT) or mismatch MO controls. Data shown as box-and-whisker plot of n > 20 embryos per condition from 3 independent experiments.

Appendix Table S1. Plasmid vectors used in this study

No.	Vectors	Inserts
1	pEGFP-C1	Human RAB35
2	pEGFP-C1	Human RAB35 (rescue construct resistant to siRNA s21707 and s21708)
3	pEGFP-C1	Human RAB35-S22N
4	pEGFP-C1	Human RAB35-Q67L
5	pENTR-D-TOPO-C1	Mouse RAB35
6	pENTR-D-TOPO-C1	Mouse RAB35-Q67L
7	pENTR-D-TOPO-C1	Mouse RAB35-S22N
8	pEGFP-C1	Human DENND1A
9	pEGFP-C1	Human DENND1B
10	pEGFP-C1	Human TBC1D10A
11	pEGFP-C1	Human TBC1D10B
12	pmCherry-C1	Human RAB35
14	p3XFLAG-CMV-14	Human ARL13B
15	pEGFP-N1	Human ARL13B
16	pEGFP-N1	Human ARL13B (C8S, C9S)
19	pEGFP-N1	Human ARL13B (V358A)
20	pEGFP-N1	Human ARL13B (1-197 aa)
21	pEGFP-N1	Human ARL13B (1-250 aa)

22	pEGFP-N1	Human ARL13B (245-425aa)
23	pEGFP-N1	Human ARL13B (Δ RVEP)
24	pEGFP-N1	Human ARL13B (Δ PR)
25	pCS2 (+)	mCherry
26	pCS2 (+)-mCherry-N1	Mouse RAB35 WT
27	pCS2 (+)-mCherry-N1	Mouse RAB35-Q67L
28	pCS2 (+)-mCherry-N1	Mouse RAB35-S22N
29	PX459 V2	Mouse Rab35 guide 1
30	PX459 V2	Mouse Rab35 guide 2
31	pDEST-tRFP7	Human HTR6
32	pEGFP-N1	PH-PLC δ

Appendix Table S2. Antibodies used in this study

Antibodies	Source	Clone or Catalog number	Dilution (purpose)
Monoclonal mouse anti-acetyl- α -tubulin	Sigma-Aldrich	6-11B-1	1:5000 (IF)
Monoclonal mouse anti-acetyl- α -tubulin	Gift from G. Pereira	C3B9	1:250 - 500 (IF)
Monoclonal mouse anti- β -tubulin	CST	86298T	1:1000 (IB)
Monoclonal mouse anti-EHD	Santa Cruz	sc-390513	1:200 (IF)
Monoclonal mouse anti-FLAG	Sigma-Aldrich	F3165	1:5000 (IB)
Monoclonal mouse anti- γ -tubulin	Sigma-Aldrich	GTU88	1:1000 (IF)
Monoclonal mouse anti-mCherry/RFP	Chromotek	6G6	1:1000 (IB)
Monoclonal mouse anti-polyglu. tubulin	Adipogen	GT335	1:1000 (IF)
Monoclonal mouse anti-SMO	Santa Cruz	sc-166685	1:100 (IF)
Monoclonal rat anti-ARL13B	Gift from K. Kontani	1E8	1:100 (IF)
Monoclonal rat anti-GFP	Santa Cruz	sc-101536	1:500 (IF)
Polyclonal goat anti-GAPDH	SicGen	AB0049-200	1:5000 (IB)
Polyclonal guinea pig anti-IFT88	Gift from G. Pereira (Kurtulmus et al. 2016)		1:500 (IF)
Polyclonal rabbit anti-ARL13B	Proteintech	17711-1-AP	1:3000 (IF) 1:2500 (IB)
Polyclonal rabbit Anti-Arl13b	Gift from T.Caspary		1:800 (IF)
Polyclonal rabbit anti-DsRed-mCherry	Clontech	632496	1:300 (IF)
Polyclonal rabbit anti-GFP	Life Technology	A11122	1:800 (IF)
Polyclonal rabbit anti-GFP	Santa Cruz	sc-8334	1:500 (IB)
Polyclonal rabbit anti-INPP5E	Proteintech	17797-1-AP	1:500 (IF)
Polyclonal rabbit anti-RAB35	Proteintech	11329-2-AP	1:500 (IB)
Polyclonal rabbit anti-Rab35	From A.Echard (Kouranti et al. 2006)		1:500 (IB)
Cy3-conjugated anti-acetyl- α -tubulin	Gift from G. Pereira (Kuhns et al. 2013)	Cy3-C3B9	1:500 (IF)
Atto488-conjugated anti-GFP	Chromotek	gba488-10	1:500 (IF)

AlexaFluor-conjugated secondary	Molecular Probes	A11029, A11031, A11034, A11036, A11075, A21208, A21127, A21236, A21240, A21245	1:500 - 1000 (IF)
DyLight 650-conjugated secondary	Invitrogen	SA5-10029	1:500 (IF)
HRP-conjugated secondary	CST	7074, 7076	1:2000 (IB)
HRP-conjugated secondary	GE		1:5000 (IB)

Appendix Table S3. Oligo DNAs used in this study

No.	Names	Sequence
1	mCherry-Zebrafish-FW	tcaagcttcgaaatggcaaaagcaacaactattaagaggc
2	mCherry-Zebrafish-Rev	cgctggatccttaactctcccctcagttcc
3	Rab35- FW	gcgcccggccctcgaggccccgcccgcct
4	Rab35-Rev	agtgggggtacctctagcacacaggtatgagg
5	qPCR mGAPDH-F	aacttggcattgtggaagg
6	qPCR mGAPDH-R	acacattggggtaggaaca
7	qPCR hGAPDH-F	agccacatcgctcagacac
8	qPCR hGAPDH-R	gcccaatacgaccaatcc
9	qPCR mRAB35-F	ttcaagattcggactgtggag
10	qPCR mRAB35-R	tcgtaaaccacaatgacccc
11	qPCR hRAB35-F	aagctgcagatctgggaca
12	qPCR hRAB35-R	gggtccccgataatacg
13	qPCR DENND1A-F	agaactcccagcatacctgag
14	qPCR DENND1A-R	ctggcgtacagatgcaacat
15	qPCR DENND1B-F	gcgtgatgaaacagttcctg
16	qPCR DENND1B-R	ccagtcgaccatcgataaact
17	qPCR TBC1D10A-F	gatcatgcaggaggccttt

18	qPCR TBC1D10A-R	ctcaatctggcgctctgtc
19	qPCR TBC1D10B-F	acctcccagggttactacagtgc
20	qPCR TBC1D10B-R	aaaaagatctccccgtccag
21	Rab35S22N-F	gcggtgtgggcaagaacagttactgttgcg
22	Rab35S22N-R	cgcaacagtaaaactgttcttggcccacaccgc
23	Rab35Q67L-F	cacagcggggctggagcgttcc
24	Rab35Q67L-F	ggaagcgtccagccccgctgtg

Appendix Table S4. siRNAs used in this study

Name	siRNA ID	Sense siRNA Sequence
Neg	s813	UAACGACGCGACGACGUAAtt
RAB35-1	s21708	GAAGAGAUGUUCAACUGCAtt
RAB35-2	s21707	GCAGUUUACUGUUGCGUUUtt
ARL13B	s47283	GAGCUGAACGAGUGCGAAAtt
DENND1A-1	s33637	GCAGUUUAUUGAUGGUCGAtt
DENND1A-2	s33638	GGUUCGAGGUAUUUUUAUAAtt
DENND1B-1	s46460	GUGUAUUACAAGCUUCUAAtt
DENND1B-2	s46461	GAGAUGCACUGAGAUACAAtt
TBC1D10A-1	s38253	GUAGUGGAGUUGCCCGUGAtt
TBC1D10A-2	s38254	GGCUAUCUUGGAUGCAGAAtt
TBC1D10B-1	s24905	CCUGUCUAAUAGCAAGGAAtt
TBC1D10B-2	s24906	CGGCGAUUCCAGAAGGUGAtt
mRab35 pool	M-042604-00-0005	siGENOME mouse RAB35 siRNA
Neg Control	D-001206-14-05	siGENOME Non-Targeting siRNA Pool #2

Appendix Table S5. Morpholinos used in this study

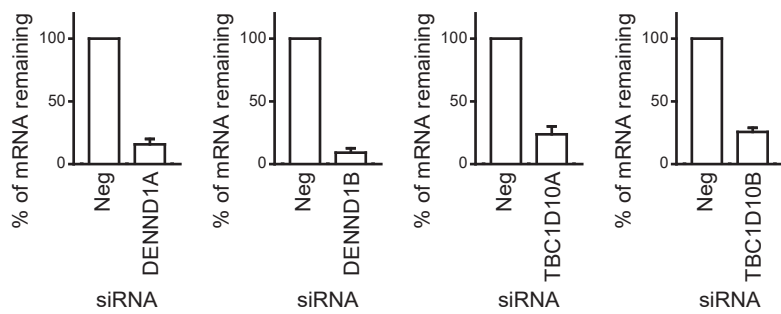
Mismatch MO	Genetools	TGgAcCTTgACcCCTCTCTCCAcCA
Rab35 MO	Genetools	TGCAGCTTCACGCCTCTCTCCAGCA

Appendix References

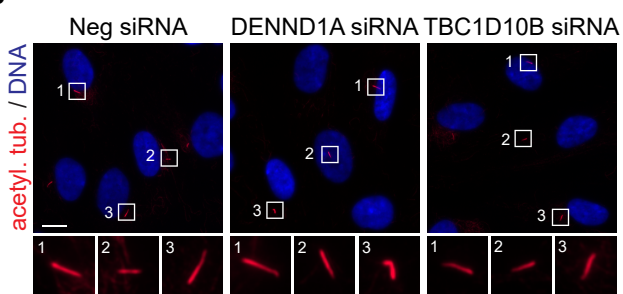
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Appendix Figure S2

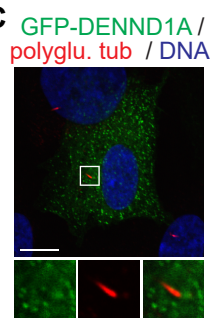
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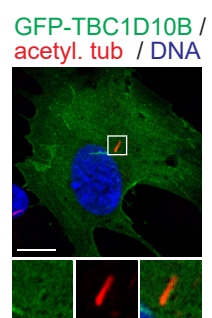
B



C



D



Appendix Figure S3

