Jurisch-Yaksi et al., http://www.jcb.org/cgi/content/full/jcb.201208175/DC1

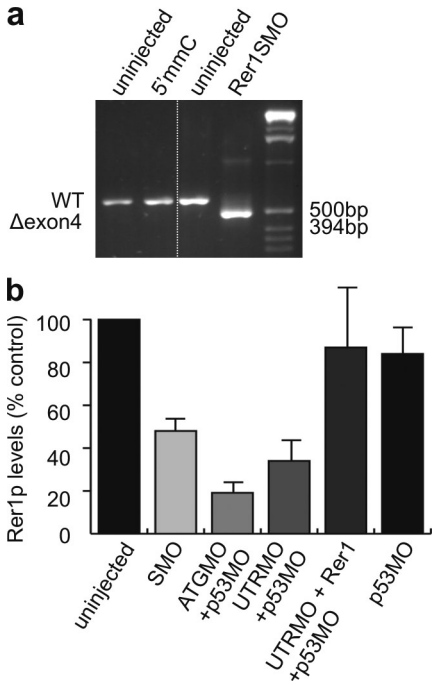
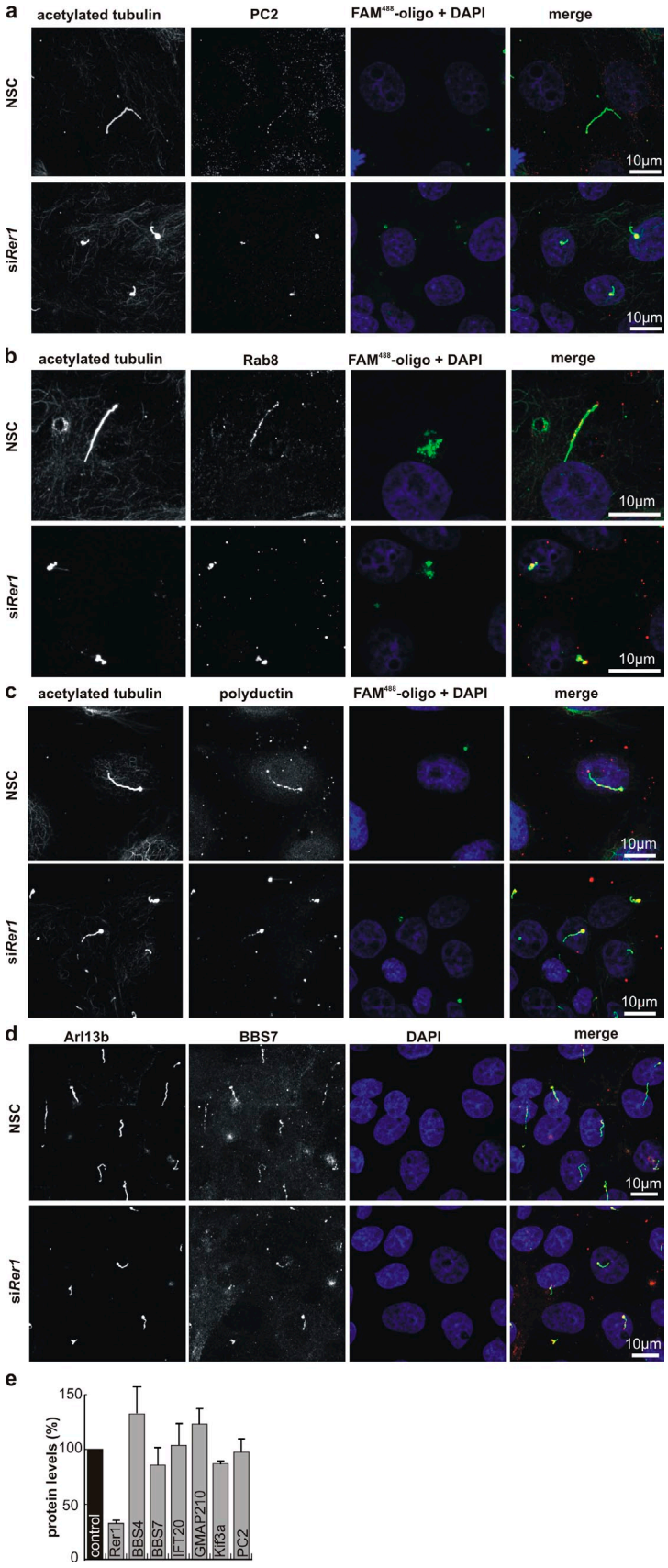
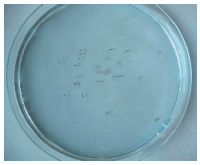


Figure S1. **Efficiency of Rer1p down-regulation in zebrafish upon MO injection.** (a) Binding of SMO to the splice-acceptor site of exon 4 leads to exon skipping and a frameshift, creating a 37–amino acid nonsense sequence after the first transmembrane domain, at the end of which is a premature stop codon. This truncated product is predicted to be nonfunctional. RT-PCR at 30-hpf stage shows a 630-bp RT-PCR product representing the full-length transcript and detected in both uninjected and 5′mmC-injected embryos. The shorter RT-PCR product (530 bp) is a new transcript that lacks exon 4 ($Rer1_{\Delta exon4}$) and is present only in embryos injected with Rer1 SMO. The white line between the second and third lanes shows where intervening lanes were removed for presentation purposes. (b) Quantification of Western blot showing 50% down-regulation of Rer1p using SMO, 80% down-regulation using combined ATGMO and p53MO, and 70% down-regulation using combined UTRMO and p53MO. Coinjection of 75pg Rer1 mRNA with the UTRMO rescued Rer1p expression to 80% of control. p53MO was coinjected with the ATGMO or UTRMO (or separately as a control) to prevent nonspecific apoptosis in the brain, often caused by ATGMO injections (Robu et al., 2007). ATGMO binds to the translation initiation site, whereas UTRMO binds upstream in the 5′UTR region. Rer1p levels were normalized to actin. Error bars are SDs. WT, wild type.

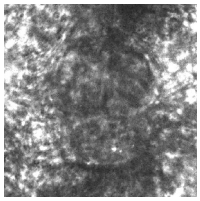
Figure S2. ***Rer1* down-regulation in CL4 cells does not affect the ciliary localization of proteins important for cilia biogenesis and function.** (a–d) Confocal images depict the localization of PC2 (a), Rab8 (b), polyductin (c), and Arl13b and BBS7 (d) within cilia (marked by acetylated tubulin), both in nonspecific control and Rer1p down-regulated CL4 cells. The down-regulated cells are identified by the presence of the FAM-labeled siRNA oligonucleotides (green in third column), with DAPI-labeled nuclei in blue. In the merge images, the FAM signal has been omitted. (e) Western blot quantification analysis of several major ciliary proteins after *rer1* down-regulation. Represented are total protein levels in CL4 cells (calculated in relation to internal nonspecific control [NSC] for each protein and normalized to GAPDH).



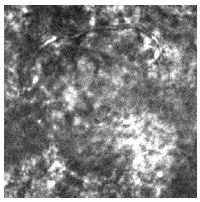


Video 1. **Video recording of the acoustic startle reflex in uninjected zebrafish embryos.** At 80 hpf, embryos were stimulated with a series of taps or vibrational stimuli (Sidi et al., 2003). Control animals exhibited a normal startle reflex. The videos were acquired with a digital camera (Powershot A640; Canon).

Video 2. **Video recording of the acoustic startle reflex in Rer1p morphant zebrafish embryos.** At 80 hpf, embryos were stimulated with a series of taps or vibrational stimuli (Sidi et al., 2003). In contrast to the control animals, Rer1p morphants did not respond to the acoustic stimuli. However, they did respond to touch stimuli (not depicted). The videos were acquired with a digital camera (Powershot A640; Canon).



Video 3. **Video recording fluid flow in KV of uninjected embryos.** Tail bud of control 12-hpf zebrafish embryos (dorsal view) after injection with fluorescent beads (Essner et al., 2005; Kramer-Zucker et al., 2005). Control embryos showed a counterclockwise flow of beads within the KV. Images were acquired on a compound microscope (DMRA using a 40× Plan Apochromat objective with a digital camera [CoolSNAP HQ]) at 10 frames/s. Beads were tracked for 25 frames.



Video 4. **Video recording of fluid flow in KV of Rer1p morphants.** Tail bud of Rer1p morphant 12-hpf zebrafish embryos (dorsal view) after injection with fluorescent beads (Essner et al., 2005; Kramer-Zucker et al., 2005). Rer1p morphants show no persistent directional flow of beads. Images were acquired on a compound microscope (DMRA using a 40× Plan Apochromat objective with a digital camera [CoolSNAP HQ]) at 10 frames/s. Beads were tracked for 25 frames.

Table S1. **Overview of cell lines**

Cell lines	Source/reference
S12 (Gli luciferase C3H10T1/2 mouse osteoblasts)	Frank-Kamenetsky et al., 2002
LLC-PK1-Cl4	J. Bartles

Table S2. **Summary of siRNA sequences**

RNAi constructs	Target sequence/catalog no.	Source
mRer1[a]	5′-CCTGGTGATGTACTTCATCATGCTT-3′	Stealth, Invitrogen
Luciferase (nonspecific control)	5′-ACAUCACGUACGCGGAAUACUUCGA-3′	
hRer1	5′-AATATCAGTCCTGGCTAGACA-3′	Thermo Fisher Scientific
Smoothened	D-041026-01 to -04	
Ift88	D-050417-01 to -04	
NSC (nonspecific control for Hh signaling)	D-001810-10-20	

[a]Used for down-regulation in S12 and CL4 cells.

Table S3. **List of primers used in this study**

Primers	Sequence
ISH probe Rer1 5′ (1–360 bp)	Forward 5′-ATGCCAGAAGGAGACAGTGC-3′
	Reverse 5′-GAAATTAATACGACTCACTATAGGCTTGAACTCTGGCAACCTCC-3′
ISH probe Rer1 3′ (241–585 bp)	Forward 5′-GCTTTCCTCTCACCAAAAGTGG-3′
	Reverse 5′-GAAATTAATACGACTCACTATAGGAGCAAAGGTTTTCCCTGTGTCG-3′
Rer1 quantitative PCR primers	Forward 5′-TTATCAGTCCTGGCTAGACAAG-3′
	Reverse 5′-GCTCAGGCCCAGTGTCA-3′
Foxj1a quantitative PCR primers	Forward 5′-TACTTCCGCCACGCAGAT-3′
	Reverse 5′-GAAGCATTTGTTCAGGGACAG-3′
Actin quantitative PCR primers	Forward 5′-TGCCCCTCGTGCTGTTTT-3′
	Reverse 5′-TCCCATGCCAACCATCACT-3′

ISH, in situ hybridization.

Table S4. **Sequences of MOs**

MOs	Target sequence
ATGMO	5′-CCGGCACTGTCTCCTTCTGGCATTC-3′
SMO	5′-CCACCCCTAATACAAACAAACAAAC-3′
5′mmC	5′-CCAgCCgTAATACAAAgAAtCAtAC-3′
UTRMO	5′-TCCGGTTGTGCCGCTGTGTCACTTT-3′
p53	5′-GACCTCCTCTCCACTAAACTACGAT-3′

Lowercase letters represent the mismatches compared with SMO.

Table S5. **Overview and dilution of antibodies used for immunofluorescence and Western blotting**

Antibodies (α-)	Source/reference	Dilution	Assay
mRer1		1:1,000	WB
zfRer1		1:200	IF
hRer1	J. Füllekrug	1:2,000 or 1:150	WB or IF
Acetylated tubulin	Sigma-Aldrich (6-11B-1)	1:200	IF
γ-Tubulin	Sigma-Aldrich (GTU-88)	1 µg/ml	IF
F-actin	Sigma-Aldrich (AC-15)	1:10,000	WB
GAPDH	EMD Millipore (6C3)	1:1,000	WB
PC2	G. Pazour	1:100	IF
Polyductin	L.M. Guay-Woodford	1:100	IF
Rab8	K. Simons	1:100	IF
Arl13b	K. Kontani	1:100	IF
BBS4	Abcam	1:100	WB
BBS7	Abcam	1:100	IF
Kif3a	Sigma-Aldrich	1:150	WB
Gli3 (6F5)	S.J. Scales (Wen et al., 2010)	2 µg/ml	WB
Gli3 (2676A)	S.J. Scales (Wen et al., 2010)	3 µg/ml	IF
Ift88	Proteintech (13967-1-AP)	1:3,000	WB
Smoothened	S.J. Scales (Wen et al., 2010)	3 µg/ml	IF
Tubulin	Sigma-Aldrich (1A2)	1:10,000	WB
Zpr1	Zebrafish International Resource center (ZIRC)	1:200	IF
Zpr3	ZIRC	1:200	IF
c-Myc (A-14)	Santa Cruz Biotechnology, Inc. (sc789)	1:2,000	WB
Cleaved Notch1 (Val 1744)	Cell Signaling Technology	1:1,000	WB
N-cadherin	BD	1:1,000	WB
Ift20	Abcam	1:1,000	WB
GMAP210	BD	1:1,000	WB
HA (3F10)	Roche	1:1,000 or 1:100	WB or IF

WB, Western blot; IF, immunofluorescence.

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