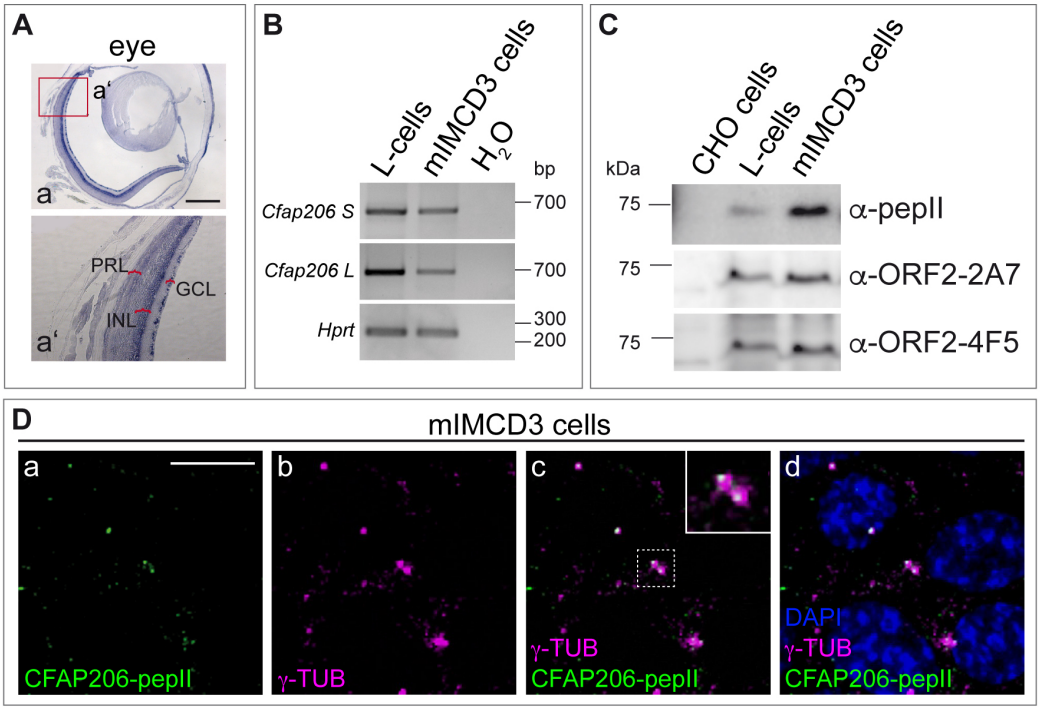

Fig. S1. Cfap206 expression in mouse cells or cell lines forming primary cilia. (A) *In situ* hybridisation on adult mouse eye section. Boxed area in (a) indicates the region shown at higher magnification in (a'). PRL: photoreceptor layer; INL: inner nuclear layer; GCL: ganglion cell layer. (B) RT-PCR on RNA isolated from murine L- and mIMCD3 cells, respectively, indicating that both transcripts were present. The full-size agarose gel is shown in Fig. S8. (C) Detection of endogenous CFAP206 protein by western blot analyses of lysates of murine L- and mIMCD3 cells. Lysates of CHO cells were used as negative control. (D) Localisation of endogenous CFAP206 (α-pepII) protein to basal bodies/centrosomes of mIMCD3 cells co-stained with gamma-Tubulin (γ-TUB) (a-d). Box with dotted line in c is magnified in the inset. Scale bars: A = 500 µm; D = 10 µm.

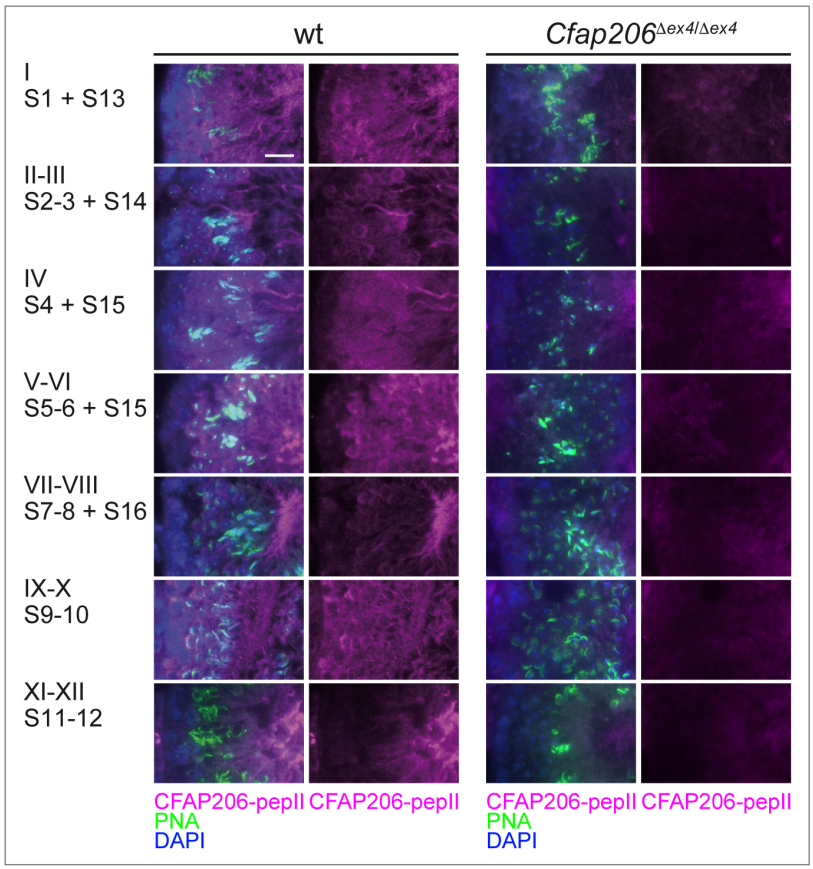

Fig. S2. CFAP206 distribution in wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ mouse spermatids.
Sections of methanol fixed wild type and mutant testis, stained for acrosomes (PNA; green), nuclei (DAPI; blue) and CFAP206 (α-pepII; magenta). S1-S16 indicate the stage of spermatogenesis, I-XII the stages of the epithelial cycle. Lumen of the sectioned seminiferous tubules is oriented to the right. Scale bar: 20 µm.

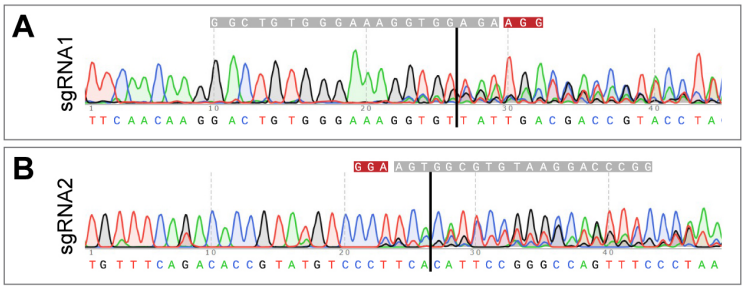

Fig. S3. Genome editing of the *cfap206* locus in *Xenopus*. (A) Sequence of *cfap206* L-allele of genome edited specimens injected with sgRNA1, which targeted exon 5. (B) Sequence of *cfap206* L-allele of genome edited specimens injected with sgRNA2, which targeted exon 2.

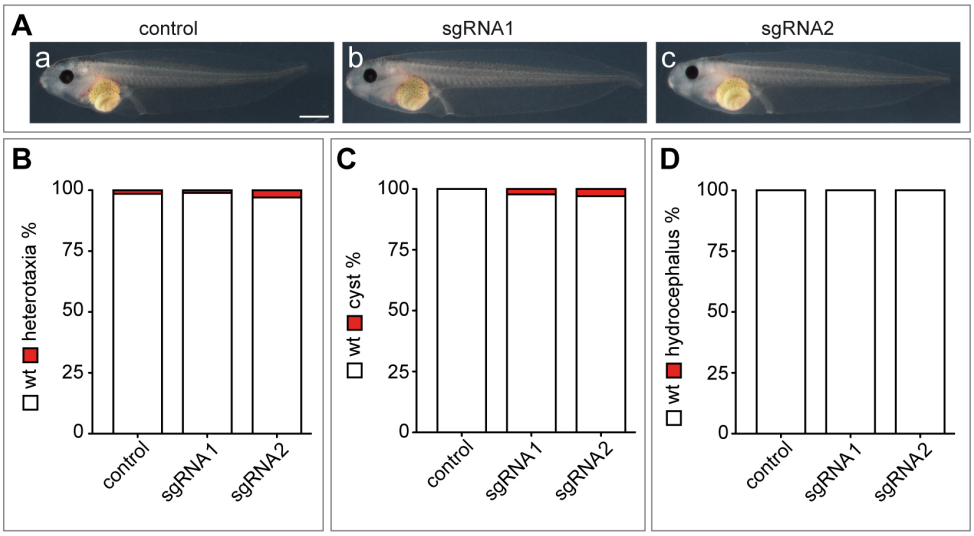

Fig. S4. Analysis of organ situs, cysts and hydrocephalus in stage 45 wild type and *cfap206* crispant tadpoles.
(A) Representative wild type (a), sgRNA1 crispant (b) and sgRNA2 crispant (c) tadpoles at stage 45. (B) Organ situs. (C) Cysts. (D) Hydrocephalus. Note that no significant deviations from wild type specimens were recorded for either potential ciliary phenotype. Scale bar: 1 mm.

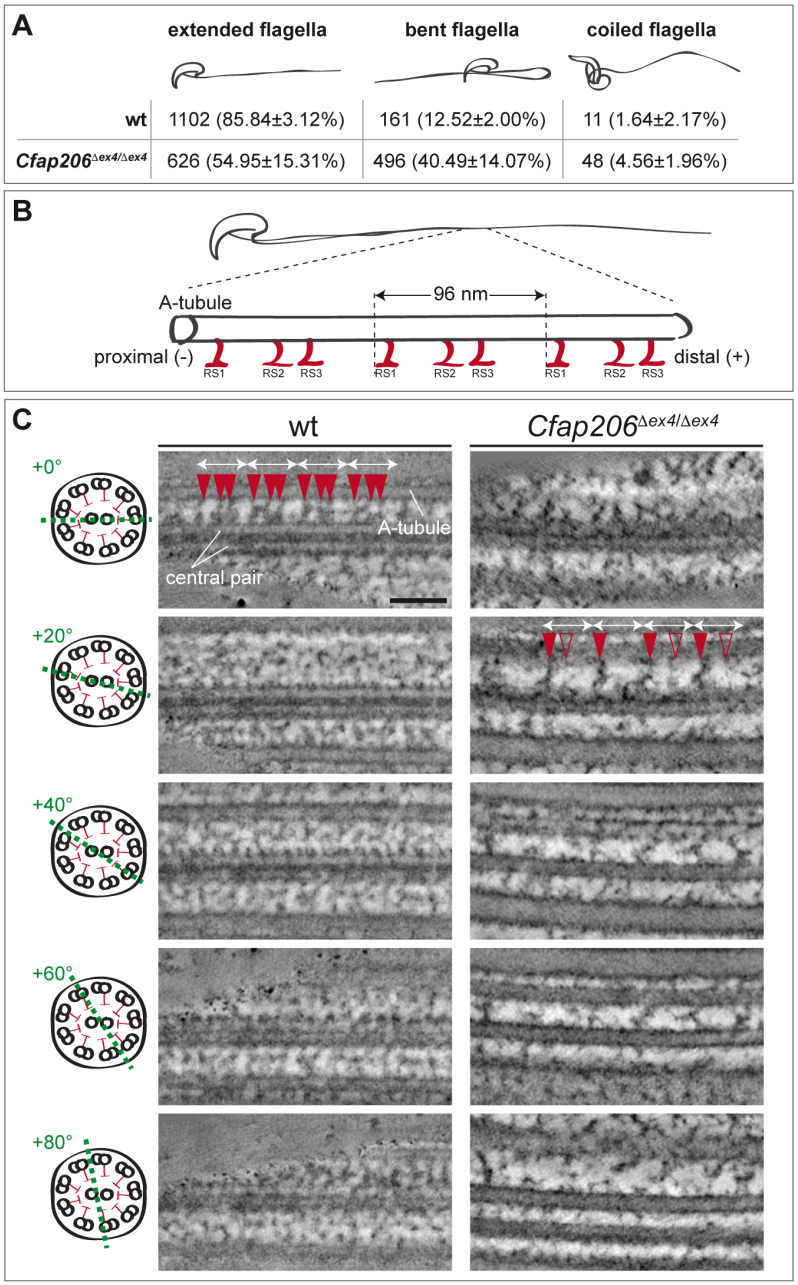

Fig. S5. Phenotypic and electro tomographic analysis of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ mutant sperm.
(A) Distribution of sperm phenotypes in wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ mutants. (B) Schematic representation of mouse sperm and localisation of the radial spokes (RS1, 2, 3; red hooks) at the A-tubule of axonemal tubulin doublets. (C) Electron tomography revealed RS in wild type (left) at A-tubule of outer doublets pointing to inner central pair of microtubules. Rotation of section plane along long axis of inner central pair of microtubules revealed alternating appearance of RS above and below inner central pair of microtubules at every 20° in wild type. Note absence of regular RS pattern in *Cfap206*$^{\Delta ex4/\Delta ex4}$ mutant (right). Scale bar: 100 nm.

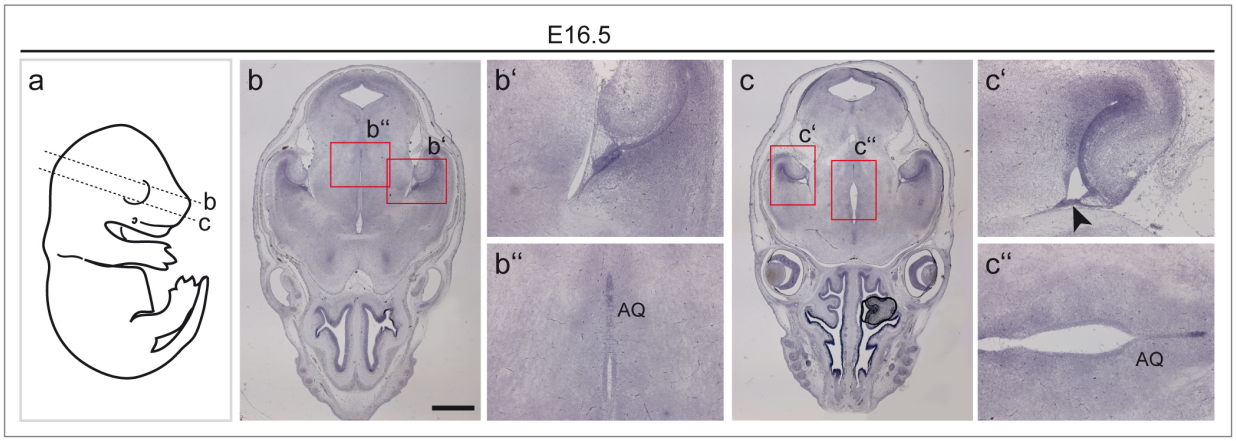

Fig. S6. *Cfap206* expression in mouse E16.5 embryonic brain.
(a) Schematic representation of section planes used for SISH in (b) and (c). Red rectangles in (b) and (c) indicate the areas shown enlarged in (b') and (b''), and (c') and (c''). *Cfap206* was expressed in the aqueduct (AQ) and in cells lining the medial wall of the lateral ventricles (arrow head).
Scale bar: 1 mm.

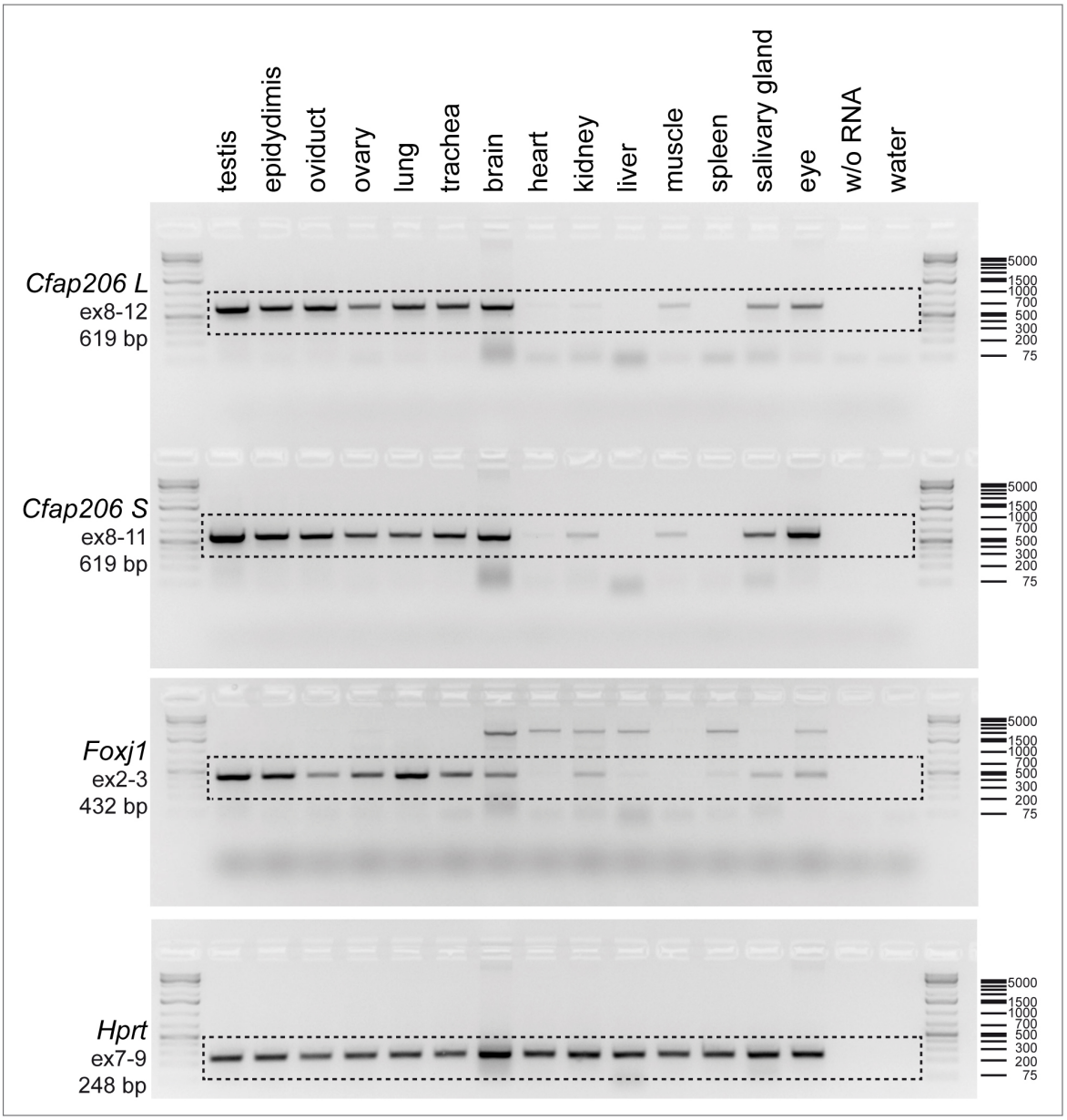

Fig. S7. Full size agarose gel of the RT-PCR analysis of mouse adult tissues.
Black dashed boxes indicate the gel areas shown in Fig. 1B. GeneRuler 1kb Plus was used as standard.

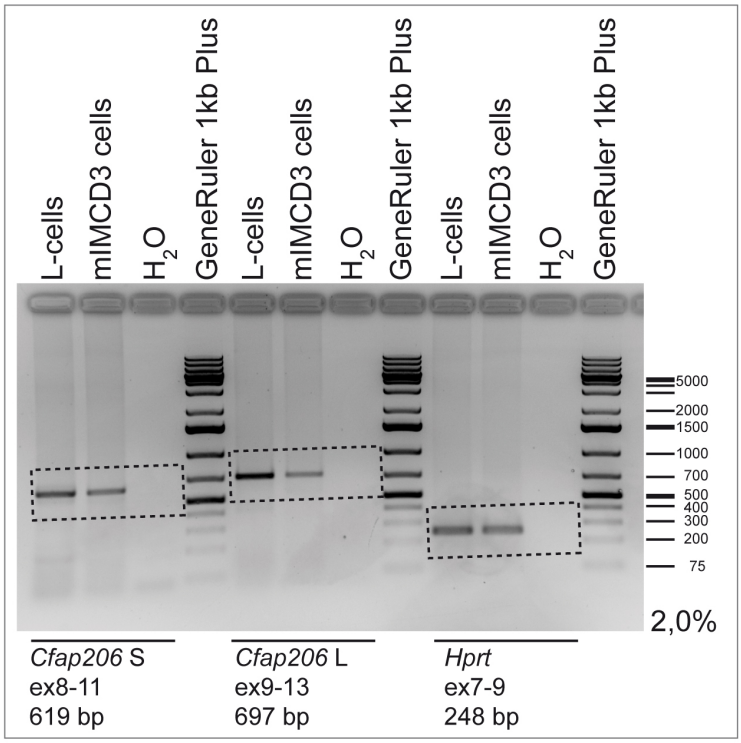

Fig. S8. Full size agarose gel of the RT-PCR analysis of L- and mIMCD3 cells.
Black dashed boxes indicate the gel areas shown in Fig. S1B. GeneRuler 1kb Plus was used as standard.

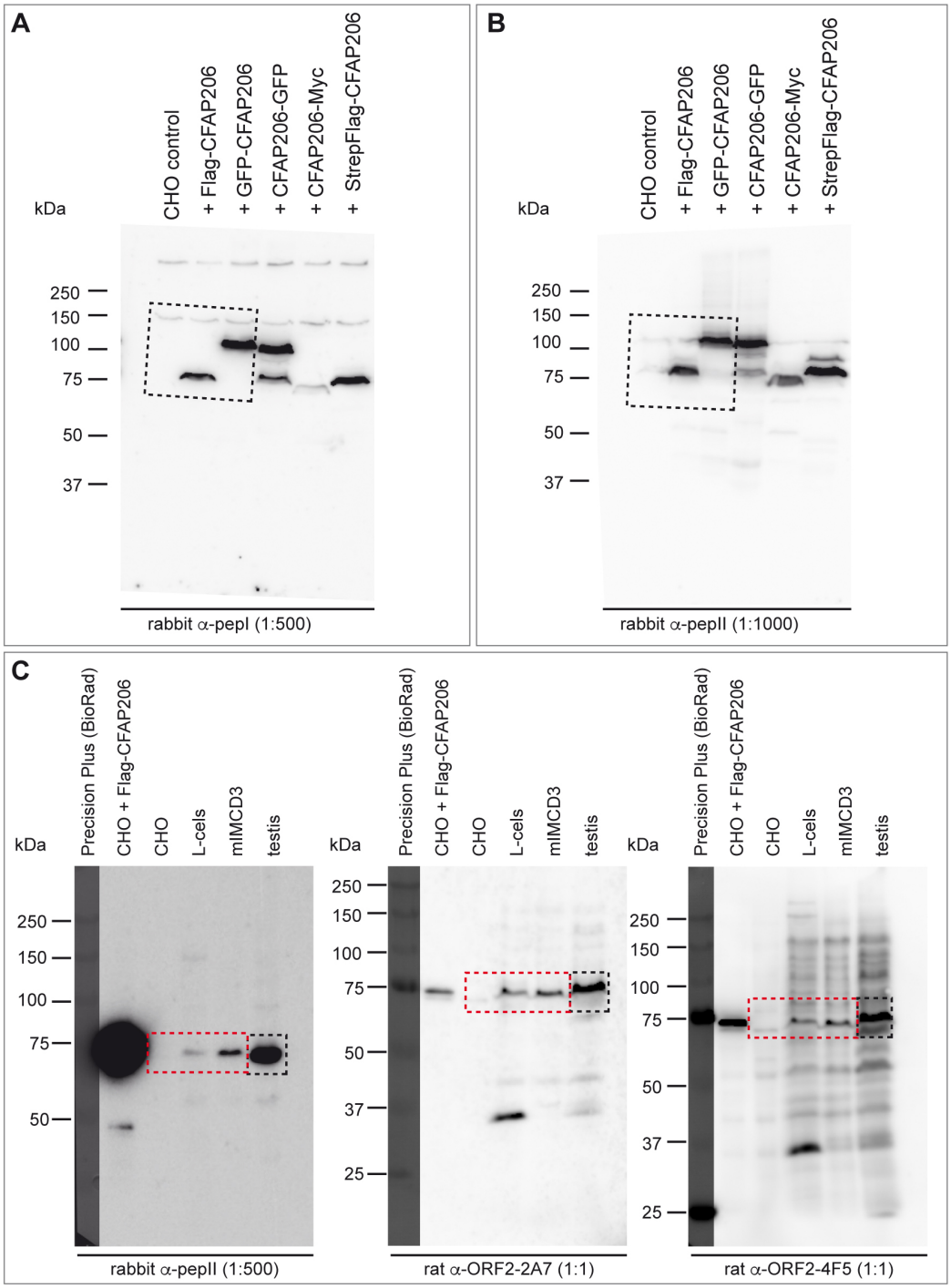

Fig. S9. Full size Western blots of CHO, L- and mIMCD3 cell lysates as well as wild type mouse testis lysates.

Black dashed boxes indicate membrane areas shown in Fig. 3A. Red dashed boxes indicate membrane areas shown in Fig. S1C. Precision Plus Dual Color (BioRad) was used as protein size standard.

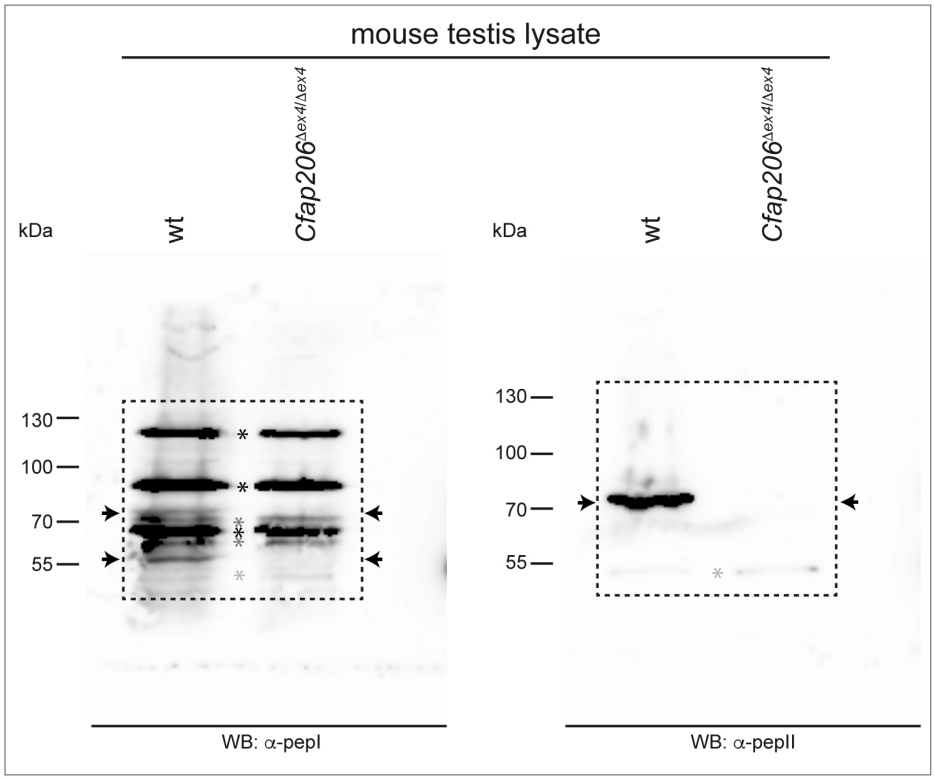

Fig. S10. Full size Western blots of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ mutant mouse testis lysates.
Black dashed boxes indicate membrane areas shown in Fig. 5B. Arrows mark the CFAP206 specific bands, disappearing in the mutant. Asteriks mark the unspecific bands seen in wild type and mutant lysates. PageRuler Prestained Protein Ladder (ThermoFisher) was used as protein size standard.

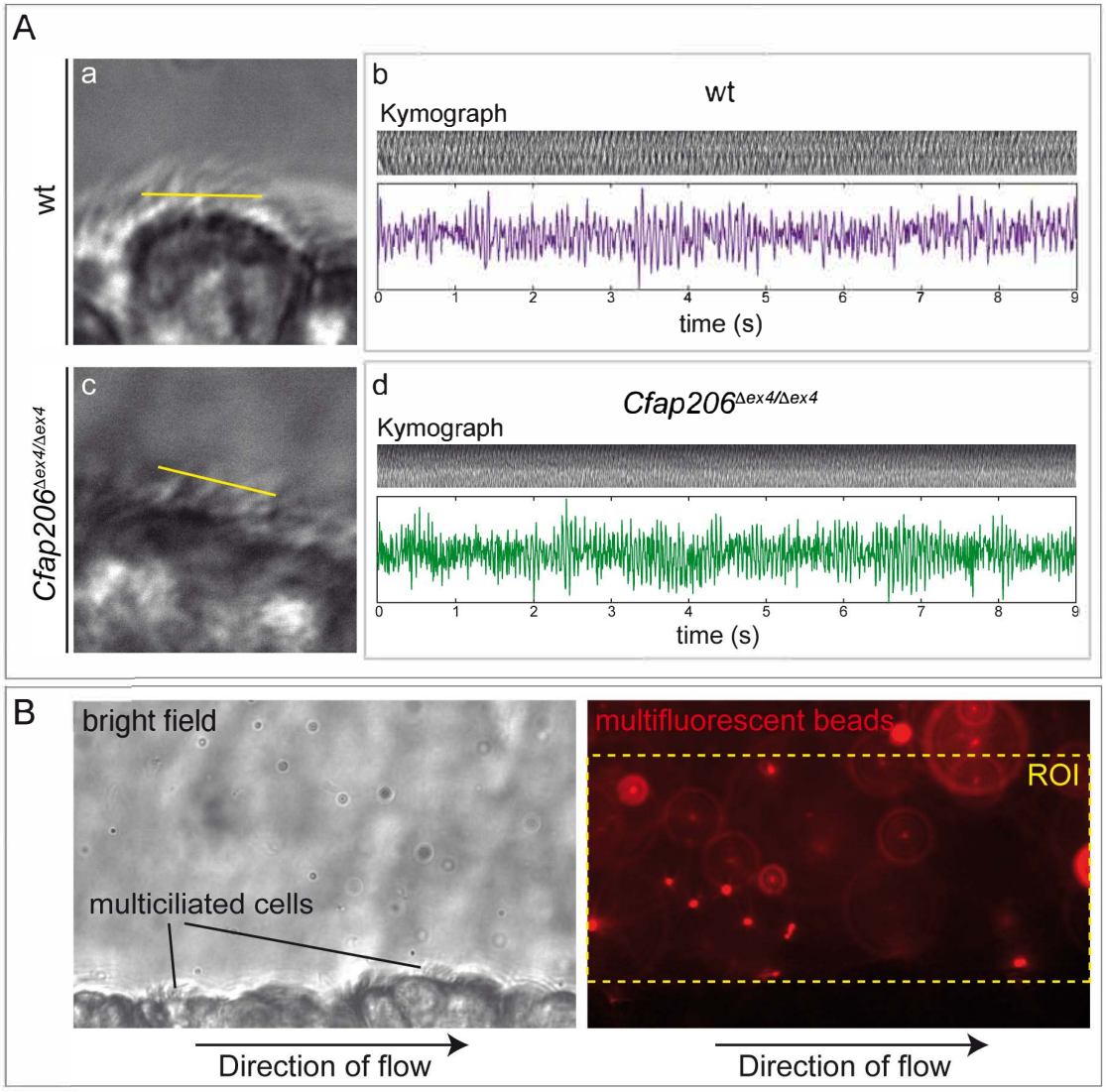

Fig. S11. Ciliary beat frequency (CBF) and cilia generated flow in wild type and *Cfap206* mutant multiciliated tracheal cells.

(A) Representative single frames (a,c) of movies (Movie S4) show the region of interest (ROI; yellow line), set parallel to the surface of the cell of interest using FIJI (Schindelin et al., 2012). Representative kymographs (b,d) for the ROI and plotted graphs whose values were used for analysis. (B) A representative bright field image (left) shows ciliated cells of the trachea explant. The single frame of the recorded movie displays multiple fluorescent beads that were tracked in defined ROIs (yellow dashed box) to determine the average bead velocity in µm/s using IMARIS (bitplane).

Table S1. Similarity matrix of CFAP206 amino acid sequences from different species.

		Identity Scores (%)								
		H. sapiens	***M. musculus***	***G. gallus***	***X. laevis***	***D. rerio***	***B. lanceolatum***	***C. intestinalis***	***T. thermophila***	***C. reinhardtii***
H. sapiens	Similarity Scores (%)	100.0	79.9	57.9	57.5	47.0	60.5	59.2	24.0	19.3
M. musculus		90.2	100.0	58.4	58.7	48.1	62.1	62.4	24.0	19.9
G. gallus		76.5	77.0	100.0	53.1	45.6	55.7	55.7	22.9	19.2
X. laevis		72.9	74.2	70.4	100.0	43.2	58.8	57.7	22.7	18.7
D. rerio		67.3	69.0	65.3	63.6	100.0	51.1	49.5	22.2	19.9
B. lanceolatum		76.5	77.5	73.7	73.8	70.4	100.0	74.1	25.0	21.1
C. intestinalis		77.2	79.0	74.5	73.7	70.6	86.8	100.0	24.1	19.7
T. thermophila		43.2	43.5	43.4	40.9	42.4	44.1	44.6	100.0	17.6
C. reinhardtii		35.2	34.5	34.3	32.6	35.6	36.6	35.9	32.9	100.0

Table S2. Raw data of cilia generated flow (CGF in µm/s) on *Xenopus* epidermal MCCs.

	wt	sgRNA 1	sgRNA 2
cilia generated flow (speed in µm/s)	510.97	364.19	511.00
	519.58	488.97	411.28
	619.95	569.18	407.27
	567.34	268.31	326.99
	584.10	435.80	493.77
	431.44	473.26	481.41
	627.71	443.64	459.87
	547.50	267.90	140.77
	518.58	460.94	382.41
	221.29	472.62	358.17
	637.57	627.06	333.65
	640.22	377.69	283.87
	549.52	596.60	389.40
	531.09	539.60	446.79
	738.97	413.98	505.09
mean	**549.72**	**453.32**	**395.45**
s.d.	**116.44**	**106.20**	**98.93**

Table S3. Raw data of ciliary beat frequency (CBF in Hz) on *Xenopus* epidermal MCCs.

	wt														
animal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ciliary beat frequenzy (Hz)	10.5	19	19.5	21	21	24.5	23.5	27.5	27	25	23	26.5	26	26.5	23.5
	10.5	18.5	15.5	19.5	19.5	27.5	26.5	23.5	28	19.5	23.5	25	22	25.5	22.5
	12	19.5	15.5	21.5	21	27.5	24.5	21	28	26	23.5	23	21	24	23
	11	17.5	12.5	22	20.5	22	26	23	32	26	22	22.5	21.5	26	20.5
	11	17.5	14.50	18.5	21	26.5	24.5	23	21	24.5	20	26	20.5	24.5	18.5
mean	**11**	**18.4**	**15.5**	**20.5**	**20.6**	**25.6**	**25**	**23.6**	**27.2**	**24.2**	**22.4**	**24.6**	**22.2**	**25.3**	**21.6**
s.d.	**0.61**	**0.89**	**2.55**	**1.46**	**0.65**	**2.36**	**1.22**	**2.38**	**3.96**	**2.71**	**1.47**	**1.78**	**2.20**	**1.04**	**2.07**

	sgRNA 1														
animal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ciliary beat frequenzy (Hz)	23	23.5	22.5	24	22.5	29.5	28.5	27	26.5	27.5	31.5	23.5	29	29	28.5
	23	22.5	22	26	22	31	23	29	31.5	32	27	30.5	29.5	29.5	24
	23	23	23	23.5	26.5	32.5	23	25.5	27.5	27.5	28	24	28	30	25
	21.5	19	20	22.5	23.5	30.5	14.5	23	26.5	22.5	23.5	31	21	28.5	24.5
	20.5	15	19	24	18.5	22	18.5	21.5	26	22	25	26.5	32.5	26	24.5
mean	**22.2**	**20.6**	**21.3**	**24**	**22.6**	**29.1**	**21.5**	**25.2**	**27.6**	**26.3**	**27**	**27.1**	**28**	**28.6**	**25.3**
s.d.	**1.15**	**3.60**	**1.72**	**1.27**	**2.88**	**4.11**	**5.28**	**3.01**	**2.25**	**4.13**	**3.06**	**3.52**	**4.26**	**1.56**	**1.82**

	sgRNA 2														
animal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ciliary beat frequenzy (Hz)	23.5	20.5	26.5	23	23.5	31	32.5	26	26.5	27	28.5	28.5	27	25	26
	22	23.5	28.5	30	23	30	29	29	24.5	30.5	28	29.5	27.5	27	27.5
	24	27	25.5	29	28	30.5	27	26	22	28.5	27	28.5	24	28	25.5
	22.5	22	25	24	27	28	28	27	24.5	24	30.5	27	22.5	26.5	20.5
	19	24	24	25.5	25.5	23.5	25.5	23	19	26	29.5	29	21.5	26.5	26
mean	**22.2**	**23.4**	**25.9**	**26.3**	**25.4**	**28.6**	**28.4**	**26.2**	**23.3**	**27.2**	**28.7**	**28.5**	**24.5**	**26.6**	**25.1**
s.d.	**1.96**	**2.43**	**1.71**	**3.07**	**2.16**	**3.07**	**2.63**	**2.17**	**2.89**	**2.46**	**1.35**	**0.94**	**2.67**	**1.08**	**2.68**

Development: doi:10.1242/dev.188052: Supplementary information

Table S4. Length of cilia determined in isolated mouse tracheal epithelial cells (mTECs).

		wt						*Cfap206*$^{\Delta ex4/\Delta ex4}$					
animal		**1**		**2**		**3**		**1**		**2**		**3**	
cell	**ROI #**	**length**	**average/ cell**	**length**	**average/ cell**	**length**	**average/ cell**	**length**	**average/ cell**	**length**	**average/ cell**	**length**	**average/ cell**
	1	4.138		4.142		3.883		4.057		3.999		3.639	
1	2	4.872	4.151	3.393	3.640	3.389	3.690	3.805	3.773	3.810	3.883	3.886	3.905
	3	3.444		3.384		3.797		3.457		3.841		4.189	
	1	4.243		3.506		3.329		3.572		3.597		4.888	
2	2	3.506	3.803	3.755	3.564	3.194	3.505	3.500	3.514	3.311	3.421	4.150	4.283
	3	3.660		3.432		3.991		3.469		3.355		3.810	
	1	4.103		4.258		3.957		3.289		4.150		3.809	
3	2	3.668	3.818	3.985	4.195	3.341	3.513	3.730	3.586	4.250	4.037	3.706	3.680
	3	3.684		4.343		3.241		3.738		3.711		3.525	
	1	3.090		4.126		4.671		5.025		3.255		2.894	
4	2	4.005	3.668	3.522	3.645	3.202	3.672	4.069	4.325	2.914	3.308	2.582	2.746
	3	3.908		3.286		3.144		3.880		3.755		2.762	
	1	4.236		3.706		4.571		3.803		3.969		3.055	
5	2	3.818	4.173	4.244	3.996	4.215	4.352	3.486	3.886	3.711	3.880	3.109	3.077
	3	4.466		4.037		4.270		4.369		3.960		3.067	
	1	3.691		3.658		4.468		3.997		3.793		2.936	
6	2	3.704	3.942	4.387	4.054	3.758	4.276	3.901	4.033	3.271	3.534	4.034	3.498
	3	4.430		4.118		4.603		4.200		3.539		3.525	
	1	3.136		3.574		3.626		3.282		4.090		3.771	
7	2	3.899	3.391	2.727	3.122	4.229	3.913	3.173	3.273	4.204	4.250	3.206	3.363
	3	3.139		3.065		3.883		3.364		4.457		3.113	
	1	4.936		3.577		4.460		3.580		4.090		3.755	
8	2	4.372	4.513	3.088	3.265	3.701	4.201	3.750	3.705	4.204	4.250	4.154	3.897
	3	4.230		3.129		4.441		3.786		4.457		3.781	
	1	3.835		3.832		4.182		4.187		4.757		3.810	
9	2	3.997	3.702	3.908	3.675	4.117	4.137	4.061	4.108	4.939	4.604	4.797	4.231
	3	3.274		3.286		4.111		4.075		4.117		4.087	
	1	4.524		4.282		4.731		3.822		3.417		3.793	
10	2	3.792	3.882	4.514	4.507	3.380	3.938	3.786	3.881	3.919	3.409	3.604	3.679
	3	3.329		4.724		3.702		4.036		2.890		3.639	
	1	3.786		4.037		2.952		3.911		3.330		3.192	
11	2	3.899	3.846	4.498	4.215	3.639	3.600	3.350	3.415	4.643	3.896	3.389	3.181
	3	3.852		4.111		4.208		2.985		3.714		2.961	
	1	3.692		3.849		3.341		4.183		2.820		3.006	
12	2	3.551	3.743	3.521	3.609	3.711	3.562	4.187	3.993	4.157	3.539	3.976	3.480
	3	3.987		3.457		3.634		3.608		3.639		3.457	
	1	2.858		3.506		3.035		3.837		2.939		3.771	
13	2	4.150	3.359	3.485	3.482	2.762	3.201	4.201	3.842	3.153	3.154	3.430	3.635
	3	3.069		3.455		3.806		3.489		3.371		3.705	
	1	3.090		2.746		3.329		3.155		3.561		3.604	
14	2	3.931	3.531	3.295	3.543	3.531	3.552	3.341	3.414	3.215	3.487	4.022	3.875
	3	3.572		4.588		3.796		3.745		3.684		3.999	
	1	3.845		3.468		3.878		4.096		3.706		4.393	
15	2	4.041	3.993	3.656	3.681	3.577	3.647	4.304	4.127	3.639	3.666	4.348	4.122
	3	4.094		3.919		3.485		3.981		3.653		3.626	
	1	4.595		3.199		4.055		2.626		3.071		3.745	
16	2	3.395	4.008	3.081	3.243	3.635	3.949	3.271	3.296	3.153	3.020	3.770	3.758
	3	4.033		3.449		4.157		3.991		2.835		3.758	
	1	3.917		4.020		4.307		3.359		3.580		5.574	
17	2	3.796	3.783	3.177	3.400	3.745	3.867	4.304	3.620	3.088	3.308	5.236	5.018
	3	3.635		3.003		3.550		3.196		3.255		4.244	
	1	3.446		3.244		4.515		4.012		3.906		4.730	
18	2	4.100	3.739	3.129	3.147	3.906	3.886	4.011	3.909	3.899	4.029	4.562	4.513
	3	3.671		3.068		3.236		3.704		4.282		4.247	
	1	3.902		4.200		3.916		2.896		2.990		3.969	
19	2	3.878	3.804	4.342	4.235	3.521	3.651	3.361	3.155	3.575	3.245	3.891	3.925
	3	3.632		4.162		3.516		3.209		3.171		3.916	
	1	3.320		3.595		4.037		4.259		4.258		4.660	
20	2	4.273	3.927	3.286	3.546	3.457	3.651	3.792	3.906	5.097	4.571	3.449	3.855
	3	4.188		3.758		3.460		3.668		4.357		3.455	
mean ± s.d.		**3.839 ± 0.265**		**3.688 ± 0.392**		**3.788 ± 0.295**		**3.738 ± 0.320**		**3.725 ± 0.461**		**3.786 ± 0.514**	

Table S5. Raw data of cilia generated flow (CGF) in mouse lateral ventricles of P7 wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ individuals.

	wt	*Cfap206*$^{\Delta ex4/\Delta ex4}$
cilia generated flow (speed in µm/s)	22.84113	41.91978
	34.19332	37.88292
	49.32148	38.26470
	88.13414	35.77974
	48.16223	52.51180
	55.40595	46.23798
	67.78381	56.25151
	36.17910	66.49065
		76.26575
		57.4969
		58.78702
		69.65802
mean	50.25	53.13
s.d.	20.64	13.40

Table S6. Raw data of ciliary beat frequencies (CBF in Hz) of tracheal cilia determined for four wild type and three *Cfap206*$^{\Delta ex4/\Delta ex4}$ individuals.

	wt				*Cfap206*$^{\Delta ex4/\Delta ex4}$		
animal	1	2	3	4	1	2	3
ciliary beat frequency (Hz)	17.10042	13.53376	10.54963	12.11111	11.51071	22.14208	20.17208
	15.22329	17.11192	11.20486	21.86727	11.66274	13.65449	10.32187
	19.10983	12.74828	13.99043	13.57240	14.60921	17.32688	12.75823
	11.44053	12.71578	11.65500	12.56809	14.91413	17.86742	13.08916
	7.77512	12.27657	9.87756	11.33721	20.71800	18.89366	22.74314
	16.33107	13.19257	9.43851	4.17744	14.09273	14.31953	21.32114
	8.66607	14.61920		17.03413	18.18981	15.56058	12.42436
	7.66300	17.53703		13.85719	23.52483	30.79967	10.54057
	14.98737	10.51841		10.01698	16.56446	11.44320	23.54256
	13.94269	15.65004		13.57124	13.31436	14.87500	19.75199
		13.10887		12.40749	17.55519	19.95191	21.87758
		11.55611		13.32692			24.39150
		10.76463		8.55988			20.84165
		18.53723		18.73473			
		9.87364		13.43923			
		16.39303		7.59564			
				7.98897			
				17.30027			
				15.97561			
				9.31289			
				8.44739			
				5.10061			
				5.76986			
				10.90554			
				21.56941			
mean	13.22	13.76	11.12	12.26	16.06	17.89	17.98
s.d.	4.10	2.64	1.63	4.72	3.73	5.26	5.27

Table S7. Raw data of cilia generated flow (CGF) in mouse tracheal explants determined for four wild type and three *Cfap206*$^{\Delta ex4/\Delta ex4}$ individuals.

	wt				*Cfap206*$^{\Delta ex4/\Delta ex4}$		
animal	1	2	3	4	1	2	3
cilia generated flow (speed in µm/s)	9.54661	7.06188	4.54716	4.97708	8.60762	3.96560	5.69169
	5.87100	8.69106	8.05653	6.32160	5.40686	3.50495	4.24066
	9.22160	10.68202	9.16563	9.97790	12.00591	4.29557	4.13021
		4.75734	9.65208	5.54395	10.98393	5.89312	4.29781
		7.80637	4.81116	6.30942	11.99785	7.78172	11.11388
			3.05738	6.53277	12.00956	4.89477	9.69348
				5.76499	10.62464		10.21132
					12.00196		
mean	8.21	7.80	6.55	6.49	10.45	5.06	7.05
s.d.	2.04	2.17	2.76	1.63	2.35	1.57	3.15

Table S8. Computer-assisted sperm analysis (CASA) of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ sperm.

	animal ID	genotype	age (months)	sample_measurement	sperm conc. (mio/ml)	progressiveness (%)	motile sperm (%)	immotile sperm (%)	velocity (µm/s)
	1	wt	2	epididymis_M1	58.2	77.6	83	17	17
				epididymis_M2	53.6	77.4	82.1	17.9	18
				epididymis_M3	39.8	76	77.9	22.1	15
				epididymis_M4	32.4	66.8	69	31	15
mean					**46**	**74.45**	**78**	**22**	**16.25**
	2	wt	2	epididymis_M1	47.3	68.9	73.9	26.1	18.5
				epididymis_M2	38.6	56.8	63	37	17
				epididymis_M3	53.4	81.1	84.9	15.1	19
				epididymis_M4	55	80.8	84	16	19
				epididymis_M5	83	87.6	90	10	16
				epididymis_M6	73.7	84.8	86.9	13.1	16
				epididymis_M7	102.6	93.3	94.1	5.9	15
				epididymis_M8	109.2	92.6	93.1	6.9	15
mean					**70.4**	**80.7**	**83.7**	**16.3**	**16.9**
	3	wt	3	epididymis_M1	48.6	95.1	96	4	18
				epididymis_M2	29.6	90	91	9	18
				epididymis_M3	67.8	98	98.1	1.9	23
				epididymis_M4	58.8	96.1	97	3	22
				epididymis_M5	57.2	88.8	90	10	18
				epididymis_M6	46.8	86.1	87	13	17
				epididymis_M7	52.2	86.2	86.9	13.1	16
				epididymis_M8	48.6	72.8	78	22	18
mean					**51.2**	**89.1**	**90.5**	**9.5**	**18.8**
	4	wt	3	epididymis_M1	40.2	69.1	71	29	13
				epididymis_M2	47.8	68.4	72	28	15
				epididymis_M3	54.8	67.4	71	29	14
				epididymis_M4	41	67.4	71	29	14
				epididymis_M5	47.6	44.5	49	51	15
				epididymis_M6	47.4	52.8	57.1	42.9	14
				epididymis_M7	52.6	61.3	67	33	14
				epididymis_M8	34.2	67.3	73.9	26.1	15
mean					**45.7**	**62.3**	**66.5**	**33.5**	**14.3**
	5	*Cfap206*$^{\Delta ex4/\Delta ex4}$	2	epididymis_M1	41.2	11.5	16	84	11
				epididymis_M2	36.4	12	17.1	82.9	10
				epididymis_M3	42.6	7.7	10	90	10
				epididymis_M4	43.4	9	11	89	10
mean					**40.9**	**10.05**	**13.5**	**86.5**	**10.25**
	6	*Cfap206*$^{\Delta ex4/\Delta ex4}$	2	epididymis_M1	34.9	21.2	23.5	76.5	12
				epididymis_M2	36.4	14.6	20	80	11
				epididymis_M3	36.1	19.1	22	78	10.5
				epididymis_M4	40.6	14.3	19	81	12
				epididymis_M5	42.2	28.8	31	69	12.5
				epididymis_M6	39.4	24.2	30	70	13
				epididymis_M7	49.2	23.9	30.9	69.1	12
				epididymis_M8	56.4	31	37.1	62.9	12
mean					**41.9**	**22.1**	**26.7**	**73.3**	**11.9**
	7	*Cfap206*$^{\Delta ex4/\Delta ex4}$	3	epididymis_M1	55.4	48.3	51	49	12
				epididymis_M2	49.8	50.7	56	44	14
				epididymis_M3	59.6	52	56.9	43.1	11.5
				epididymis_M4	49.4	41.3	49	51	13
				epididymis_M5	54.6	57.5	60	40	11
				epididymis_M6	43.8	42.7	46	54	13
				epididymis_M7	42	39.5	46	54	15
				epididymis_M8	43.8	46.9	53.9	46.1	14
				epididymis_M9	51.8	35.4	40.9	59.1	13
				epididymis_M10	45	31.5	38	62	15
				epididymis_M11	47.4	39.7	46	54	14
				epididymis_M12	49.8	30.3	36	64	14
mean					**49.4**	**43.0**	**48.3**	**51.7**	**13.3**
	8	*Cfap206*$^{\Delta ex4/\Delta ex4}$	3	epididymis_M1	79.6	25.8	32.0	68.0	12
				epididymis_M2	79.2	23.8	31.0	69.0	11
				epididymis_M3	80	34.2	37.0	63.0	12
				epididymis_M4	60	13	19.0	81.0	12
				epididymis_M5	63.2	20.3	27.0	73.0	11
				epididymis_M6	58.4	22.4	29.0	71.0	12
				epididymis_M7	46.4	10.4	18.0	82.0	12
				epididymis_M8	52.4	15.7	23.0	77.0	13
				epididymis_M9	50	13.2	16.0	84.0	10
				epididymis_M10	83.6	30.2	33.0	67.0	11
				epididymis_M11	71.2	24.1	28.0	72.0	12
				epididymis_M12	64	20	28.0	72.0	12
mean					**65.7**	**21.1**	**26.75**	**73.25**	**11.7**

Table S9. Blastocyst development in IVF of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ sperm.

genotype	eggs	eggs/experiment (mean ± s.d.)	blastocysts after IVF	blastocysts/ experiment	developed blastocysts (% ± s.d.)
wt	1132	377 ± 53	656	219 ± 39	57,76 ± 2,78
Cfap206$^{\Delta ex4/\Delta ex4}$	1657	552 ± 87	11	3,7 ± 4,0	0,70 ± 0,69

Table S10. Phenotypes of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ sperm.

	wt							*Cfap206*$^{\Delta ex4/\Delta ex4}$						
phenotype	normal		bent		coiled		total	normal		bent		coiled		total
	count	%	count	%	count	%	count	count	%	count	%	count	%	count
sperm preparation 1	68	81.928	11	13.253	4	4.819	83	43	56.579	28	36.842	5	6.579	76
sperm preparation 2	309	89.049	34	9.798	4	1.153	347	110	41.667	140	53.03	14	5.303	264
sperm preparation 3	287	87.5	41	12.5	0	0	328	236	75.884	69	22.186	6	1.929	311
sperm preparation 4	438	84.884	75	14.535	3	0.581	516	237	45.665	259	49.904	23	4.432	519
	1102		161		11		1274	626		496		48		1170
mean %		85.84		12.522		1.638			54.949		40.491		4.561	
s.d.		3.124		2		2.172			15.314		14.074		1.963	

Table S11. Potential interaction partners of CFAP206 identified by mass spectrometry. CFAP206 (C6orf165 homolog) is marked in yellow

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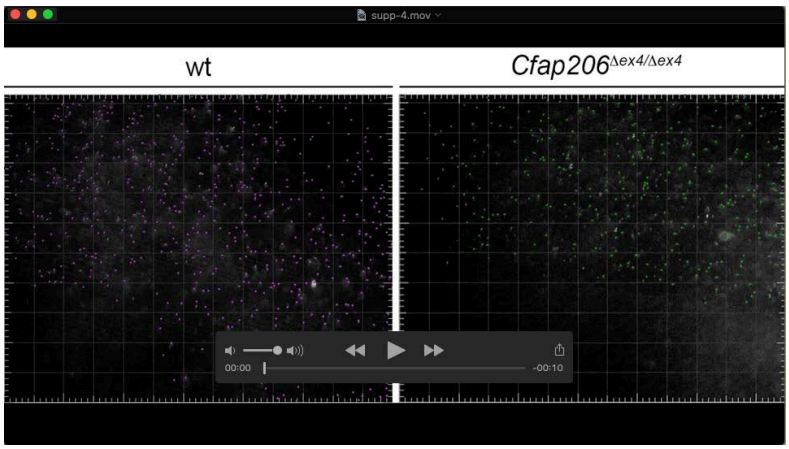
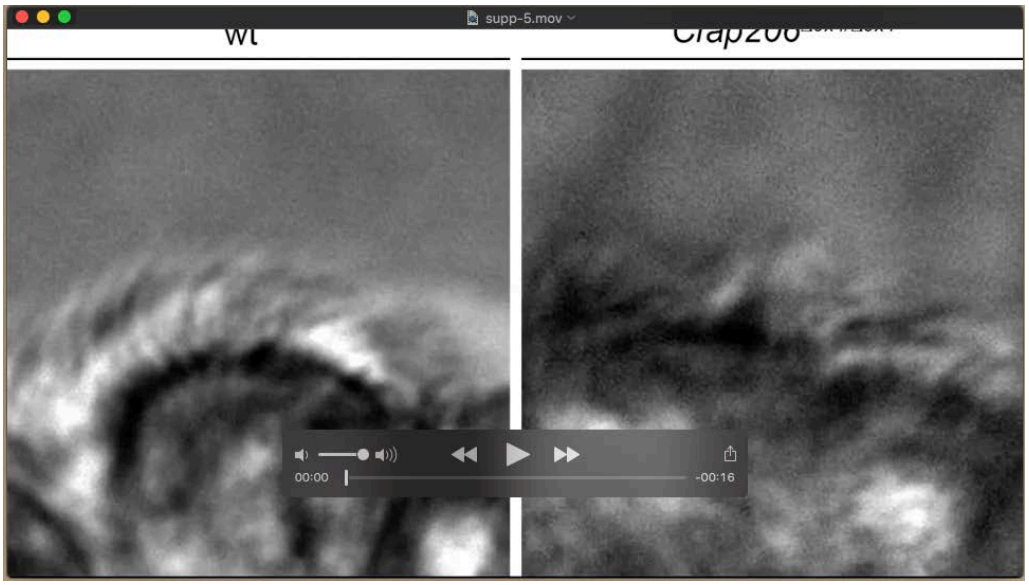
Movie 1. Cilia motility in wild type and *cfap206* crispant *Xenopus* larval skin.
High-speed (800 frames per second (fps)) videography of single MCCs at stage 32. Left: wild type (control); middle: sgRNA1 crispant; right: sgRNA2 crispant. Note that cilia were motile in all cases. Movie plays at 15 fps, i.e. at about 0.02x real time.

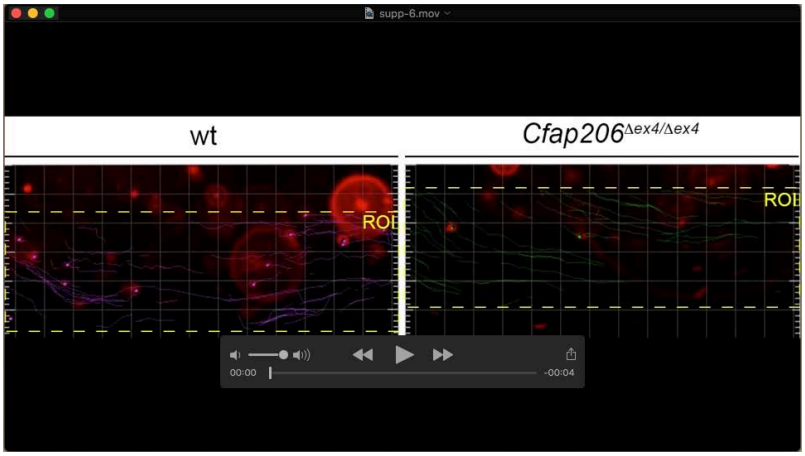
Movie 2. Bead transport in wild type and *cfap206* crispant *Xenopus* larval skin.
Fluorescent beads were added to wild type or crispant specimens at stage 32 and bead transport was recorded at 175 fps. Left: wild type; middle: sgRNA1 crispant; right: sgRNA2 crispant. Measured bead transport was slower in crispants. Movie plays at 50 fps, i.e. at about 0.3x real time.



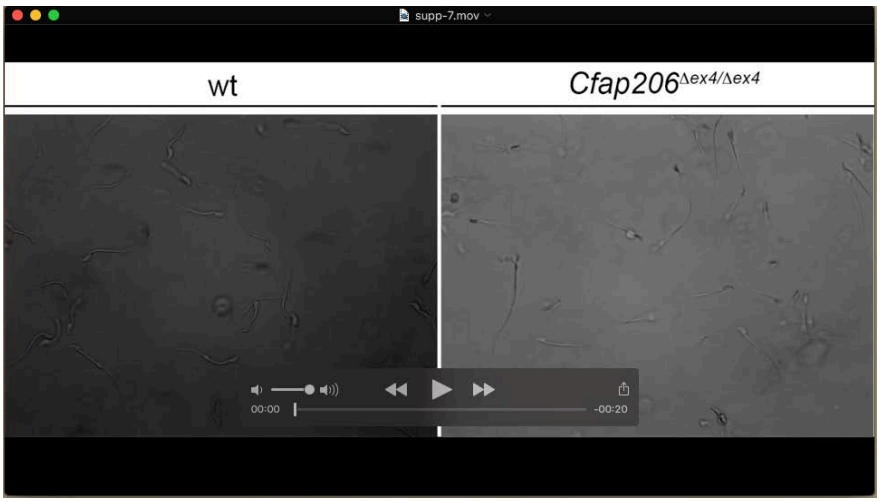
Movie 3. Tracking of fluorescent beads to determine cilia generated flow in mouse ependyma.
Representative movies showing tracked fluorescent beads in lateral ventricle explants wild type (left) and *Cfap206*$^{\Delta ex4/\Delta ex4}$ (right) P7 old mice. The speed of the fluorescent beads was determined by tracking using IMARIS (bitplane). Bullets represent the tracked beads (violet, wild type; green, *Cfap206*$^{\Delta ex4/\Delta ex4}$). Movies were recorded at a rate of 6.7 fps and played back at a rate of 10 fps.



Movie 4. Ciliary motility of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ murine tracheal epithelial cells.
Representative movies showing tracheal multiciliated cells of wild type (left) and *Cfap206*$^{\Delta ex4/\Delta ex4}$ (right) mice. The movies were recorded at a rate of 159.27 and 164.58 fps, respectively for 9 s and played back at a rate of 15 fps.

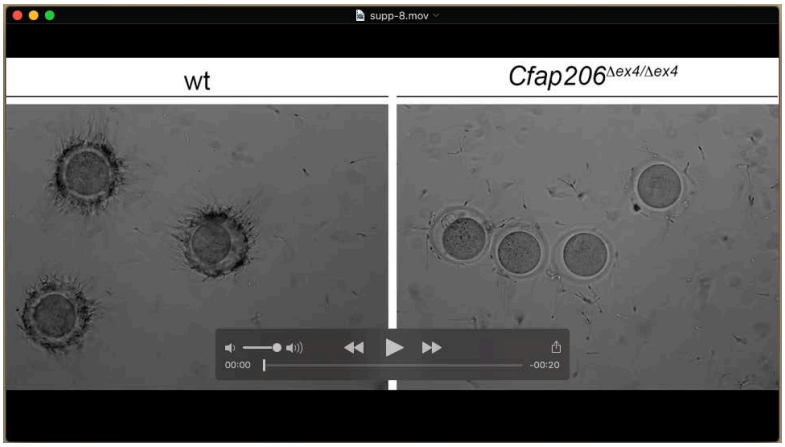


Movie 5. Tracking of fluorescent beads to determine cilia generated flow in mouse tracheas.
Representative movies showing tracked fluorescent beads in trachea explants of wild type (left) and *Cfap206*$^{\Delta ex4/\Delta ex4}$ (right) mice. The speed of the fluorescent beads was determined by tracking using IMARIS (bitplane). Bullets represent the tracked beads, lines trace the bead tracks (violet, wild type; green, *Cfap206*$^{\Delta ex4/\Delta ex4}$). Movies were recorded with 4.76 fps and played back at a rate of 10 fps.



Movie 6. Motility of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ mouse sperm cells.

Representative movies showing normal motility of wild type (left) and abnormal inefficient motility of *Cfap206*$^{\Delta ex4/\Delta ex4}$ (right) sperm. Movies were recorded at a rate of 5.18 fps and played back with 12 fps.



Movie 7. Motility of wild type and *Cfap206*$^{\Delta ex4/\Delta ex4}$ mouse sperm cells in presence of wild type mouse egg cells.

Representative movies showing sperm movement in presence of egg cells. While wild type sperm move directionally towards and attach to egg cells (left), *Cfap206*$^{\Delta ex4/\Delta ex4}$ sperm show abnormal and not directed movement when incubated with egg cells (right). Movies were recorded at a rate of 5.2 fps and played back with 12 fps.

KEY RESOURCES TABLE

Reagent or resource	Source	Identifier
Antibodies		
Mouse α–acetylated α-tubulin; clone 6-11B-1	Sigma Aldrich	Cat.#T6793; RRID:AB_477585
Rabbit α–AKAP3	Proteintech	Cat.#13907-1-AP; RRID:AB_2273887
Mouse α–gamma-Tubulin; clone GTU-88	Sigma Aldrich	Cat.#T5326 RRID:AB_532292
Rabbit α–SEPTIN7	IBL international	Cat.#18991; RRID:AB_10705434
Goat α–Mouse-Alexa555	Invitrogen	Cat.#A21424; RRID:AB_141780
Goat α–Mouse-Alexa488	Invitrogen	Cat.#A11029 RRID:AB_2534088
Goat α–Rabbit-Alexa555	Invitrogen	Cat.# A21429; RRID:AB_141761
Goat α–Rabbit-Alexa488	Invitrogen	Cat.#11034; RRID:AB_2576217
Donkey α–Rabbit-HRP	Amersham	Cat.#NA934; RRID:AB_772206
Goat α–Rat-HRP	Amersham	Cat.#NA935; RRID:AB_772207
Rabbit α–CoxIV [EPR9442(ABC)]	Abcam	Cat.#ab202554
Rat α–CFAP206 ORF2-2A7 (monoclonal)	This paper	N/A
Rat α–CFAP206 ORF2-4F5 (monoclonal)	This paper	N/A
Rabbit α–CFAP206 pepI (polyclonal)	This paper	N/A
Rabbit α–CFAP206 pepII (polyclonal)	This paper	N/A

Bacterial and Virus Strains		
E.coli XL-1 blue for cloning	Stratagene	
E.coli SCS110 for cloning	Stratagene	
Chemicals, Peptides, and Recombinant Proteins		
CFAP206 peptide IRLFNRDSGKGGEG (pepI: aa194-207; generation of polyclonal ABs)	This paper	N/A
CFAP206 peptide KEASTQSKREGSSR (pepII: aa576-589; generation of polyclonal ABs)	This paper	N/A
CFAP206 peptide PLKEASTQSKREG (ORF2: aa574-586; generation of monoclonal ABs)	This paper	N/A
DAPI	AppliChem	Cat.#A4099
Lectin PNA-Alexa 488	Molecular Probes	Cat.#L-21409; RRID:AB_2315178
Alexa Fluor Plus 405 Phalloidin	Invitrogen	A30104
Taq DNA Polymerase	Promega	M3001
SulfoLink coupling resin	Thermo Fisher Scientific	Cat.#20401
Fluoresbrite Multifluorescent 0.5 micron Microspheres	Polysciences	Cat.#24054
Halt Protease and Phosphatase Inhibitor Cocktail	Thermo Fisher Scientific	Cat.#78440
Pfu DNA Polymerase	Promega	M7741
FluoSpheres carboxylate 1.0 µm yellow-green (505/515)	Invitrogen	F8823
Cas9 protein from Streptococcus pyogenes with NLS	PNA Bio	CP01
Critical Commercial Assays		
Superscript II Reverse Trancriptase	Invitrogen	Cat.#18064-022
TriReagent	SigmaAldrich	Cat.#T9424
Direct-zol RNA Miniprep Plus	Zymo Research	Cat.#R2071
PAS staining kit	SigmaAldrich	Cat.#395B
DIG RNA labelling kit	Roche	Cat.#11175025910
PerFectin Transfection Reagent	Genlantis	Cat.#T303007
Pierce Crosslink IP Kit	Thermo Fisher Scientific	Cat.#26147
MEGAshortscrip T7 Transcription Kit	Invitrogen	AM1354

MEGAclear Transcription Clean-Up Kit	Invitrogen	AM1908
Experimental Models: Cell Lines		
Hamster: CHO cells	ATCC	CRL-11268
Mouse: L-cells	ATCC	CRL-2648
Mouse: mIMCD3 cells	ATCC	CRL-2123
Experimental Models: Organisms and Strains		
Mouse: 129Sv/CD1 hybrids	Own colony	N/A
Mouse: *Foxj1*^{lacZ}	Brody et al., 2000	MGI:2158221
Mouse: FLPe	Rodríguez et al., 2000	MGI:2448985
Mouse: ZP3:Cre	De Vries et al., 2000	MGI:2176187
Mouse: *Cfap206*^{loxP}	This paper	N/A
Mouse: *Cfap206*$^{\Delta ex4}$	This paper	N/A
Rat: LOU/C		
Xenopus laevis	Nasco	LM00715/LM00535
Oligonucleotides (5'-3')		
Cfap206-loxP-F1: ATCACGGAGTCAGGGCTAAGTTG	This paper	N/A
Cfap206-loxP-R1: GGCAAGCAGTCTACCAACTGAGG	This paper	N/A
Cfap206-R1: CCAACCAGCCCATACTATTC	This paper	N/A
Cfap206_Ex8for: TCCCAAGTCTTCCCCATCTTCG	This paper	N/A
Cfap206_Ex12rev: TGTGTGTATCTGTCTGTGTGCCG	This paper	N/A
Cfap206_RTex9for: CGATGGCGTCGTCGTGAAAAG	This paper	N/A
Cfap206_RTex13rev: CCCACGAAGGCCAGCTATGAA	This paper	N/A
Cfap206_Ex8for: AAAATCTAAGACGGCGGTCCC	This paper	N/A
Cfap206_Ex11rev: AGTCAGGAGTTACAAACCCAGGTG	This paper	N/A
Hprt_RT_for_ex7: GCTGGTGAAAAGGACCTCT	This paper	N/A
Hprt_RT_rev_ex9: CACAGGACTAGAACACCTGC	This paper	N/A
Foxj1_RT_for_ex2: CTTCTGCTACTTCCGCCATGC	This paper	N/A
Foxj1_RT_rev_ex3: TCCTCCTGGGTCAGCAGTAAGG	This paper	N/A

sgRNA reverse oligo: AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC	Merck	N/A
cfap206-sgRNA 1 forward oligo: GCAGCTAATACGACTCACTATAGGCTGTGGGAAAGGTGGAGAGTTTTAGAGCTAGAAATAGCAAG	Merck	N/A
cfap206-sgRNA 2 forward oligo: GCAGCTAATACGACTCACTATAGGCCCAGGAATGTGCGGTGAGTTTTAGAGCTAGAAATAGCAAG	Merck	N/A
foxj1-sgRNA forward oligo: GCAGCTAATACGACTCACTATAGGGATACATACCTGCCAGGTGTTTTAGAGCTAGAAATAGCAAG	Merck Rachev et al., 2019	N/A
cfap206 target site 1 forward primer: TCCCCCACACAGAACTTGGT	Merck	N/A
cfap206 target site 1 reverse primer: AGGATAGCTGGCACTACAATGA	Merck	N/A
cfap206 target site 2 forward primer: AGGTTTGAGCGAGCAATGATG	Merck	N/A
cfap206 target site 2 reverse primer: CCCCAAAGTAGGCACTCCAA	Merck	N/A
Recombinant DNA		
Cfap206 FANTOM plasmid (*in situ* probe generation)	Kawai and Hayashizaki, 2003	ZX00119D08
IRAV clone 6306249 (IRAV103H5)- 1700003M02Rik	ImaGenes	IRAVp968H05103D
EST clone IMAGp998J0615090Q (*in situ* probe generation)	Source BioScience Limited	7207016
pcDNA6.2 N-EmGFP-Cfap206 (mouse CFAP206 L expression plasmid)	This paper	N/A
pcDNA6.2 N-Flag-Cfap206 (mouse CFAP206 L expression plasmid)	This paper	N/A
pCS2+ Cetn4-RFP	Zhang and Mitchell, 2012	
Software and Algorithms		
FIJI (ImageJ)	Schindelin et al., 2012	RRID:SCR_002285
Prism	GraphPad	RRID:SCR_002798
Imaris	Bitplane	RRID:SCR_007370
MATLAB	MathWorks	RRID:SCR_001622
MacVector	MacVector	RRID:SCR_015700

Photoshop	Adobe	RRID:SCR_014199
Illustrator	Adobe	RRID:SCR_010279
Chi-square		http://www.physics.csbsju.edu/stats/contingency_NROW_NCOLUMN_form.html
Other		

Table S11. Potential interaction partners of CFAP206 identified by mass spectrometry. CFAP206 (C6orf165 homolog) is marked in yellow

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