Supplemental data

Supplemental figure legends

**Figure S1 Purification of full-length CrIFT25/27 and truncated CrIFT25ΔC/27**

(A) The left panel shows the elution profile of CrIFT25/27 from a Superdex 75 size exclusion column. The IFT25/27 complex is seen to elute as a heterodimer whereas excess monomeric IFT25 elutes later. The calculated molecular weights (Mw) based on the amino acid sequences as well as experimentally determined values by static light scattering (SLS) are indicated. The somewhat lower Mw(SLS) value of 35 kDa vs. a Mw(calc) of 43 kDa for full length IFT25/27 could be due to the presence of degradation fragments. The right panel shows an SDS PAGE gel of the two peaks from the SEC experiment. (B) As (A) but with the CrIFT25ΔC/27 truncated complex.

**Figure S2 Multiple sequence alignment of IFT25**

Secondary structure elements derived from the CrIFT25 structure are indicated above the sequences. IFT25 residues that interact directly with residues from IFT27 are colored green and the calcium-ion coordinating residues are red. CrIFT25 is seen to have a long glycine-rich C-terminal extension not found in other IFT25 orthologues. Hs: *Homo sapiens*, Mm: *Mus musculus*, Xl: *Xenopus laevis*, Dr: *Danio rerio*, Cr: *Chlamydomonas reinhardtii*.

**Figure S3 Structural comparison with HsIFT25 and sialidases**

(A) The structure of human IFT25 (in salmon) has been superimposed on the *Chlamydomonas* IFT25/27 complex. HsIFT25 and CrIFT25 show high structural similarity and share a conserved calcium-binding site. (B) Structural comparison of a bacterial sialidase with the IFT25/27 complex. The overall structure of the sialidase is
similar to IFT25 and the two proteins share a common calcium-binding site. However, the galactose-binding site of sialidases is not conserved in IFT25 and is seen to overlap with the C-terminal helix of IFT27 in the IFT25/27 complex structure.

**Figure S4 Effect of different buffer conditions on the IFT25ΔC/27 complex**

(A) SEC profile of a standard IFT25ΔC/27 purification using a Superdex 75 column. The IFT25ΔC/27 complex is seen to elute around 60 mL whereas excess 25ΔC elutes at around 80 mL. (B) The SEC purified IFT25ΔC/27 complex (peak 1 from panel (A)) was incubated with 10 mM of the strong calcium chelator EGTA for 30 min at 20°C and subjected to another round of SEC where 1 mM of EGTA (and no divalent metal ions) was included in the buffer to assess the role of calcium in complex formation. The IFT25ΔC/27 complex is not disrupted indicating that calcium-binding is not a requirement for complex stability. (C) The effect of salt on IFT25ΔC/27 complex stability. SEC purified IFT25ΔC/27 complex (peak 1 from panel (A)) was incubated with a buffer containing 300 mM NaCl for 30 min at 20°C and subjected to SEC run in a 300 mM NaCl-containing buffer. The higher salt concentration does not have any apparent effect on the IFT25ΔC/27 complex stability.

**Figure S5 IFT27 binds IFT25 regardless of its nucleotide state**

(A) The effect of the nucleotide bound to IFT27 on the ability to bind IFT25 was tested experimentally in pull-down assays. GST-tagged IFT25ΔC can pull down IFT27 regardless of the nucleotide-bound state of IFT27. The proteins were pre-incubated for 30 min at 20°C with 5 mM GDP, 5 mM GTP or with 10 mM of EDTA before incubating for 1h with GSH-beads. After washing the beads 3 times, the protein was eluted with 30 mM glutathione (B) Pull-down of WT IFT27 using CBP-
tagged WT (CBP-25ΔCWT) or mutant (CBP-25ΔCmut, V38R, T40R and T125E triple mutant) IFT25. Since the proteins are co-expressed and IFT25 is expressed to a much higher degree than IFT27, the band for IFT25 is stronger than that for IFT27. Whereas WT IFT25 can pull-down IFT27, the interface mutant of IFT25 no longer precipitates IFT27 consistent with the results shown in Figure 2B.

**Figure S6 IFT25ΔC/27 does not bind ATP**

To test if the IFT25ΔC/27 complex binds ATP, 50mM of the complex was titrated with 500 μM of ATP in an ITC experiment. The titration curve demonstrates that IFT25ΔC/27 does not bind ATP.
Table S1 Putative GAPs for CrIFT27

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Since IFT27 has very low intrinsic GTPase activity and belongs to the Rab family of small GTPases it may be regulated by a TBC-domain GAP as seen for other Rabs. To find putative candidates, the TBC-domain Rab-GAP Gyp1p from yeast was blasted against the *Chlamydomonas* genome and significant hits (E value>10^-3) listed in the table above. One of the candidates (code: XP_001699577.1) was found in the cilium in a previous study (Pazour et al., 2005) but does not contain the classical catalytic sequence motifs of Rab-GAPs.
Figure S1

A

IFT25/27
Mw(calc)=43kDa
Mw(SLS)=59kDa
peak 1

IFT25
Mw(calc)=21kDa
Mw(SLS)=21kDa
peak 2

B

IFT25ΔC/27
Mw(calc)=38kDa
Mw(SLS)=37kDa
peak 1

IFT25ΔC
Mw(calc)=16kDa
Mw(SLS)=16kDa
peak 2

IFT25/27 (peak 1)  IFT25 (peak 2)
IFT25ΔC/27 (peak 1)  IFT25ΔC (peak 2)
Figure S4

A

25ΔC/27 (38kDa) peak 1

25ΔC (15 kDa) peak 2

elution volume (ml)

A_{280} (mAU)

10 mM Tris\textsuperscript{+}HCl pH=7.5
150 mM NaCl
1 mM CuCl\textsubscript{2}
5 mM MgSO\textsubscript{4}

B

25ΔC/27 (38kDa) peak 1

elution volume (ml)

A_{280} (mAU)

10 mM Tris\textsuperscript{+}HCl pH=7.5
150 mM NaCl
1 mM EGTA

C

25ΔC/27 (38kDa) peak 1

elution volume (ml)

A_{280} (mAU)

10 mM Tris\textsuperscript{+}HCl pH=7.5
300 mM NaCl
1 mM CuCl\textsubscript{2}
5 mM MgSO\textsubscript{4}
Figure S5
Figure S6