Assembly and stability of IFT-B complex and its function in BBSome trafficking

**Highlights**

- IFT38 mediates anterograde IFT and retrograde trafficking of BBSome
- Subunit mutations cause differential instability of IFT-B
- IFT-B1 or IFT-B2 alone can be assembled but is unstable in vivo
- IFT-B is assembled in a modular pathway
Assembly and stability of IFT-B complex and its function in BBSome trafficking

Jieling Wang, Xin Zhu, Zhengmao Wang, Xuecheng Li, Hui Tao, and Junmin Pan

SUMMARY
The machinery of intraflagellar transport (IFT) consists of IFT motors and the ciliary cargo adaptors including IFT-A and IFT-B complexes and BBSome. IFT-B, which is composed of IFT-B1 and IFT-B2 subcomplexes, interacts with IFT motors and IFT-A during anterograde IFT and IFT-A during retrograde IFT while it is also implicated in BBSome trafficking. However, the assembly and stability of IFT-B and its regulation of anterograde IFT and BBSome trafficking remain not clear. Here, we show that IFT38 functions in the regulation of anterograde IFT and retrograde trafficking of BBSome. Deletion of IFT-B1 or IFT-B2 subunits results in differential instability of IFT-B1 and IFT-B2. The stability of IFT-B1 and IFT-B2 is mutually dependent and mediated by the connecting tetramer IFT38/IFT57/IFT52. The formation of an intact IFT-B1 and IFT-B2 is not altered by the deletion of IFT38 of IFT-B2 and IFT52 of IFT-B1, respectively. Further analysis suggests a modular pathway for IFT-B assembly.

INTRODUCTION
Cilia and eukaryotic flagella (exchangeable terms) are conserved cellular organelles with function in cell motility and signaling, defects which may cause a variety of human diseases and/or disorders – ciliopathies. The assembly and maintenance of cilia require a bidirectional transport between the ciliary base and tip termed intraflagellar transport (IFT). The IFT machinery consists of anterograde motor kinesin-II, retrograde motor IFT dynein, and large protein complexes as cargo adaptors including IFT-A, IFT-B, and BBSome. Mutations in individual IFT-B subunits typically cause severe ciliogenesis defects, which underscores the necessity of IFT-B in IFT and ciliogenesis. In addition to binding ciliary cargoes, IFT-B also ensures proper IFT. IFT-B physically interacts with kinesin-II, IFT dynein, and IFT-A during anterograde IFT while it interacts with IFT-A during retrograde IFT. IFT-B is a complex of 16 proteins that can be separated into core IFT-B1 (10 subunits) and peripheral IFT-B2 (6 subunits) subcomplexes. The interaction map of IFT-B subunits has been established although the complete structure of IFT-B has not been determined. IFT-B1 and IFT-B2 interact via a tetramer IFT38/IFT52/IFT57/IFT38. Loss of an IFT-B subunit may lead to the instability of IFT-B as shown in a few studies. How each IFT-B subunit contributes to the stability of IFT-B has not been systematically explored. In addition, the IFT-B1 or IFT-B2 subcomplex has been reconstituted in vitro. However, how IFT-B1 and IFT-B2 are assembled in vivo remains not clear.

IFT-B interacts with kinesin-II to initiate anterograde IFT. IFT54 interacts with kinesin-II and its partial deletion interferes with anterograde IFT. In addition, the connecting tetramer IFT88/IFT52/IFT57/IFT38 that bridges IFT-B1 and IFT-B2 has been shown to interact with kinesin-II. However, physiological data to support this finding are still lacking. BBSome is transported by IFT. Loss of IFT27 or its interactor LZTFL1 disturbs BBSome trafficking. It has been proposed that they mediate BBSome remodeling at the ciliary tip for its retrograde transport. IFT38 has been shown to interact directly with BBSome components. However, it is unclear whether IFT38 functions in anterograde or retrograde transport of BBSome.

To vigorously assess the function and assembly of IFT-B, we started out with the characterization of IFT38 in Chlamydomonas. We found that IFT38 is an anchor of cellular localization of IFT-B2 but not IFT-B1 at the basal body and is critical in organizing the IFT-B complex via its coiled-coil domain. In addition, the C-terminus of IFT38 regulates anterograde IFT and is also involved in the retrograde trafficking of BBSome.

1MOE Key Laboratory of Protein Sciences, Tsinghua-Peking Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China
2Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266000, China
3Lead contact
*Correspondence: panjunmin@tsinghua.edu.cn
https://doi.org/10.1016/j.isci.2022.105493

© 2022 The Author(s). This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
To systematically explore the contribution of an IFT-B subunit to the stability of IFT-B, we analyzed mutants from six subunits of IFT-B1 that critically affect ciliogenesis and from all subunits of IFT-B2. We found that the contribution of each subunit to the stability of IFT-B varies. In particular, the stability of IFT-B1 and IFT-B2 is mutually dependent and regulated by the connecting tetramer. Furthermore, intact IFT-B1 and IFT-B2 can be assembled independently of each other although with reduced stability. Through further analysis, we propose that IFT-B is assembled in a modular pathway. IFT-B1 and IFT-B2 are respectively assembled from small modules before an intact IFT-B1 or IFT-B2 is assembled, followed by assembly into a stable IFT-B complex.

RESULTS

Isolation and characterization of an ift38-1 mutant in Chlamydomonas

We have isolated an ift38-1 mutant (ift38:aphVIII) that was generated by insertional mutagenesis using aphVIII expressing cassette in Chlamydomonas (Figure 1A). The mutant cells were palmelloid (Figure 1B), an indication that the cells may not have cilia because cilia formation is required for hatching. No cilia were visible in the cells as examined by DIC microscopy when the mother cell walls were removed by autolysin treatment (Figure 1B). Transmission electron microscopy showed that the axoneme was barely formed beyond the “H” shaped transition zone (Figure 1C), similar to other IFT-B mutants such as ift52::NIT1 and ift54::aphVIII. IFT38 was not detected in ift38-1 by immunoblotting with an IFT38 antibody (Figure 1D). Transformants of ift38-1 expressing HA-tagged IFT38 rescued the ciliary phenotypes of the mutant (Figures 1B and 1D). IFT38-HA showed a typical cellular localization of IFT proteins with enrichment at the ciliary base and distribution in cilia (Figure 1E). Taken together, we obtained an ift38-1 mutant and showed that IFT38 in Chlamydomonas as in other organisms including worm, zebrafish, and mammal is critical for ciliogenesis.  

Figure 1. Characterization of an ift38-1 mutant in Chlamydomonas

(A) A diagram showing DNA insertions in the IFT38 gene in ift38-1. Black box, exon; Line, intron.
(B) DIC images of WT (wild-type), ift38-1, and ift38-1:IFT38-HA cells. Bar 5 μm.
(C) TEM images showing cilia of ift38-1 and WT cells. Bar 100 nm.
(D) Immunoblot of cell lysates from WT, ift38-1, and the rescued strains with the indicated antibodies. 10 μg proteins were loaded for each lane. Asterisk, non-specific band.
(E) IFT38-HA is distributed in cilia and enriched at the basal body region. WT and rescued cells were immunostained with HA and α-tubulin antibodies, respectively. Bar 5 μm.
The coiled-coil domain of IFT38 is critical for its association with IFT80, IFT57, IFT20 and the heterodimer IFT88/52 of IFT-B1

Analysis of the interaction map of IFT-B shows that IFT38 is critical in organizing IFT-B1,10,11 (Figure 2A). IFT38 has three structural regions: an N-terminal calponin homology (CH) domain, two coiled-coil (CC) domains and a C-terminal region (Figure 2B). IFT38 interacts with IFT80 via the CH domain10,11 and with IFT20 via the CC domain.11 The CC domain has also been shown to be involved in the interaction with IFT57,10 however, an interaction between IFT38 and IFT57 was not found in another study.11 To test the functions of the CC domains in ciliogenesis and in the interactions of IFT38 with its partners in vivo, HA-tagged IFT38 with CC domain deletions were expressed in ift38-1 cells, respectively (Figures 2A, 2B, and 2C). Deletion of either CC1 or CC2 domain led to cells without cilia (Figure 2D), suggesting they are critical for IFT38 in mediating ciliogenesis. Immunoprecipitation with anti-HA antibody showed that the deletion of either CC1 or CC2 domain reduced the interaction between IFT38 and IFT20 (Figure 2E). Deletion of CC2 but not CC1 severely reduced the interactions of IFT38 with IFT57, IFT80 as well as IFT88/52. The reduced interactions of IFT38^CC1 with IFT20 and IFT57 support interactions of IFT38 with IFT20 and IFT57 via the CC domain.10,11 The reduced interaction of IFT38^CC2 with IFT80 is intriguing because it has been shown that IFT38 interacts with IFT80 via its CH domain.10,11 Nevertheless, our data showed that the CC domain of IFT38 is critical in its association with IFT20, IFT57, IFT80 and IFT88/52.
A patient with retinal degeneration carries a homozygous nonsynonymous mutation in IFT38/Cluap1,29 resulting in a single amino acid substitution (L273F). This residue is conserved in IFT38 of Chlamydomonas (L269) and localized in the CC2 domain (Figures S1A and S2). We examined the physiological consequence of this mutation by expressing HA-tagged IFT38L269F in ift38-1 cells (Figure S1B). No significant defects were found regarding ciliary length, ciliary assembly and disassembly, and ciliary maintenance (Figures S1C–S1G), suggesting this residue is not critical for ciliogenesis. This result is consistent with the fact that the patient does not have other systematic abnormality.29 It is likely that this residue is only important for the function of IFT38 in the signaling of the photoreceptor cilium.

The C-terminus of IFT38 is involved in the regulation of anterograde intraflagellar transport

The connecting tetramer IFT88/52/57/38 has been shown to link IFT-B to kinesin-II.8 Loss of either of these subunits strongly inhibits ciliogenesis,11,13,25,30 thus precluding the examination of their function in regulating IFT in vivo. The fact that the deletion of IFT38 C-terminus allows cilia formation in mammalian cells provides us an opportunity for this examination.11,22 A C-terminal truncation constructs IFT38CT tagged with HA or YFP was expressed in ift38-1 cells (Figures 2B, 3A and 3B). The transforms exhibited normal ciliary length (Figures S2A and S2B), ciliary assembly, and disassembly (Figures S2C and S2D). Interestingly, immunoblot analysis showed that IFT38CT-HA or -YFP was substantially increased in cilia relative to the control, although their protein levels in whole cells were comparable (Figures 3A and 3B). Representative subunits of IFT-A, IFT-B, and IFT motors were similarly increased in cilia (Figure 3C). This data is supported by immunostaining analysis of IFT38CT-YFP, IFT43, and IFT74 (Figures 3D, S2E, and S2F).

Next, we examined the IFT kinetics of IFT38CT-YFP by using total internal reflection fluorescence (TIRF) (Figure 3E). The velocity and frequency of anterograde IFT were reduced relative to the control (Figure 3F) while the retrograde IFT appeared to be normal (Figures S2G and S2H). Interestingly, the incidence of stalled trains per cilium in ift38-1:IFT38CT-YFP cells was significantly increased relative to the control (Figures 3E and 3G), which may account for the increase of IFT materials in cilia.

The disruption of anterograde IFT by the loss of the IFT38 C-terminus may be caused by interference with IFT-B complex formation or interaction with kinesin-II. Immunoprecipitation analysis showed that IFT-B proteins associated with IFT38-HA and IFT38CT-HA were at similar levels (Figure S2I), indicating that the formation of IFT-B complex is not altered, consistent with previous studies.22 Thus, the loss of IFT38 C-terminus may interfere with its interaction with kinesin-II. The C-terminus might not be critical in linking IFT-B and kinesin-II as its loss only moderately affects IFT. Nevertheless, we have provided the first physiological data to support the finding that IFT38 C-terminus is important for the function of IFT38 in the signaling of the photoreceptor cilium.

The C-terminus of IFT38 participates in the retrograde transport of BBSome

How BBSome links with the IFT trains during BBSome trafficking remains not well understood. Biochemical assays have shown that IFT38 interacts with BBSome via the C-terminal region while the CC domain may also be involved.22,23 Immunoblotting revealed that BBS8 was relatively increased in cilia of ift38-1:IFT38CT-YFP cells while its cellular level was similar to the control (Figure 4A). The ciliary increase of BBS8 was more than 2-fold that of IFT38CT-YFP, indicating it is not due to an overall increase of IFT proteins. Ciliary increase but not decrease of BBS8 suggests that IFT38 is not involved in the anterograde trafficking of BBSome.

We next examined the distribution of BBS8 in cilia by immunostaining. To this end, we expressed BBS8-YFP in ift38-1:IFT38-HA or ift38-1:IFT38CT-HA cells respectively because the BBS8 antibody was not suitable for immunostaining. BBS8-YFP was accumulated at the ciliary tip of ift38-1:IFT38CT-HA cells (Figure 4B). Immunoprecipitation analysis showed that the interaction of IFT38CT with BBS8-YFP was substantially reduced relative to the control (Figure 4C). Taken together, these data suggest that IFT38 C-terminus is involved in the interaction with BBSome in vivo and functions in the ciliary exit of BBSome. Based on the in vivo functional analysis of the domains and/or regions of IFT38, we propose a model for the critical function of IFT38 in regulating IFT and BBSome trafficking (Figure 4D).

Function of IFT38 in stability and basal body localization of IFT-B

We next examined the stability of IFT-B regulated by IFT38 because the loss of an IFT-B subunit often causes instability of the other subunits.13,15,31 Immunoblotting of whole cells showed that the abundance of IFT54 and IFT20 was severely reduced and that of IFT57 moderately reduced in ift38-1 cells relative to the control while...
the protein levels of IFT172 and IFT80 were apparently not affected (Figure 5A). Interestingly, the cellular abundance of the IFT88/52 dimer of IFT-B1 was also substantially reduced in the mutant (Figure 5A). Next, examination found that the abundance of other subunits of IFT-B1 including IFT81, IFT74, and IFT46 was also relatively reduced (Figure 5B). IFT-A levels, however, were slightly increased, consistently as seen in other IFT-B mutants.13,14 Thus, IFT38 affects the stability of both IFT-B1 and IFT-B2 but not IFT-A.
IFT complexes are enriched at the basal body region as reported elsewhere. It is not clear whether IFT38 has a role in this process. We examined the cellular localization of IFT172, IFT80, and IFT57 in ift38-1 cells as there are considerable amounts of these proteins (Figure 5A). They were not visible at the basal body in ift38-1 cells compared to the control (Figures 5C-5E), indicating that IFT38 is involved in their cellular localization. IFT38 forms a dimer with IFT57. We then examined IFT38 localization in an ift57-2 mutant, in which the stability of IFT38 was only modestly affected (see later in discussion for Figure 6A). Interestingly, the cellular localization of IFT38 was not affected (Figure 5F) and so was that of IFT80 (Figure 5G). Thus, IFT38 appears to be an anchor for the cellular localization of IFT-B2.

Next we examined whether IFT38 affected the cellular localization of IFT-B1. Immunostaining analysis showed that IFT46 and IFT74 localized to the basal body region in ift38-1 cells compared to the control (Figures 5H and 5I), suggesting that IFT38 is not involved in the basal body localization of IFT-B1. Taken together, IFT38 is critical for the basal body localization of IFT-B2 but not IFT-B1.

**Systematic analysis of IFT-B stability**

To learn how each subunit of IFT-B contributes to its stability, we performed a systematic study. We analyzed mutants from all IFT-B2 subunits and six IFT-B1 subunits (IFT88/81/74/70/52/46) that are critical for...
ciliogenesis.25,30,31,33,34 ift80 and ift54 mutants of IFT-B2 and ift88, ift81, ift70, ift52 and ift46 mutants of IFT-B1 are from previous studies.15,25 The mutants of ift172, ift57 and ift20 of IFT-B2 and ift74 of IFT-B1 were generated in this study (Figure S3A and see STAR Methods). ift172-3 and ift20 mutant cells were aciliated while the majority of cells of ift57-2 and ift74-3 were also aciliated, similar to previous reports13,14,35 (Figures S3B and S3C).

Figure 5. Function of IFT38 in the stability and cellular localization of IFT-B
(A) Loss of IFT38 reduces the abundance of IFT20/54/57 but not IFT172/80 as examined by immunoblotting (10 μg of proteins loaded for each lane). Please note, the abundance of IFT88/52 of IFT-B1 that interacts with IFT38/57 was also reduced. Asterisk, non-specific band.
(B) The abundance of IFT-B1 subunits IFT81/74/46 but not that of IFT-A subunits is also reduced due to loss of IFT38 as examined by immunoblotting (10 μg of proteins loaded for each lane).
(C–E) IFT38 is necessary for the basal body localization of IFT172/80/57. ift38-1 cells were immunostained with anti-α-tubulin and anti-IFT172 (C), anti-IFT80 (D), or anti-IFT57 (E) antibodies with WT and rescued cells as controls. Bar 5 μm.
(F–G) Loss of IFT57 does not alter the basal body localization of IFT38 and IFT80. WT and ift57-2 cells were immunostained with anti-α-tubulin and anti-IFT38 (F) or anti-IFT80 (G) antibodies. Bar 5 μm.
(H–I) Loss of IFT38 does not alter the basal body localization of the IFT-B1 subunits IFT46 and IFT74. Cells as indicated were immunostained with anti-α-tubulin and anti-IFT46 (H) or anti-IFT74 (I) antibodies. Bar 5 μm.
Immunoblot analysis of whole cells from IFT-B2 mutants was performed to examine the stability of IFT-B2 and IFT88/52 dimer of IFT-B1 that connects IFT-B2 (Figure 6A). The abundance of each subunit due to the loss of the other subunits was differentially affected. IFT172 did not regulate the stability of the other subunits while its stability was affected by its physical interactor IFT80 and IFT57. Loss of IFT80 reduced the stability of the other subunits but not vice versa. The stability of IFT54 and IFT20 depended on each other, and also required IFT57, IFT38, and IFT80, indicating that IFT54 and IFT20 are the most vulnerable. IFT57 and IFT38 form a dimer, how-ever, the stability of IFT57 depended on IFT38 but not vice versa. Interestingly, the loss of either IFT57 or IFT38 influenced the stability of IFT88 and IFT52 of IFT-B1. However, the rest of the IFT-B2 subunits except IFT80 and IFT88 mutants.

Next, we examined how IFT-B1 influenced the stability of IFT-B2. Loss of either IFT52 or IFT88 substantially reduced the abundance of the IFT-B2 subunits except for IFT80 (Figure 6B). However, the loss of IFT81, IFT74, IFT70, or IFT46 had no or slight effects on the stability of IFT-B2 compared to ift88-2 and ift52-1 mutants (Figure 6C). Thus, the regulation of the stability of IFT-B2 by IFT-B1 is mainly mediated by the IFT88/52 dimer that interacts with IFT-B2.10,11

Figure 6. Systematic analysis of the stability of IFT-B

(A) Stability of IFT-B2 influenced by its own subunits. Cell lysates from IFT-B2 mutants as indicated were analyzed by immunoblotting. 10 µg of proteins were loaded for each lane in this and the following immunoblot analysis. IC2, loading control. Asterisk, non-specific band.

(B) Stability of IFT-B2 affected by IFT88/52 of IFT-B1. Immunoblots of cell lysates of ift52-1, ift88-2, and WT cells were probed with the indicated antibodies.

(C) Regulation of the stability of IFT-B1 and IFT-B2 by IFT-B1 subunits. Immunoblots of cell lysates from WT and IFT-B1 mutants as indicated were probed with the indicated antibodies. Please note, the abundance of IFT-B1 was differentially affected in the IFT-B1 mutants. The abundance of IFT-B2 was not substantially reduced in the IFT-B1 mutants except for ift52 and ift88 mutants.

(D) A model describing mutually dependent stability of the IFT-B subunits after the loss of one subunit. Single-headed arrows indicate a one-sided relationship while double-headed arrows show a mutual relationship.
How about the stability of IFT-B1 influenced by its own subunits? The abundance of IFT88 was reduced in ift52-1 and vice versa, consistent with the formation of a heterodimer. Loss of either subunit of the IFT88/52 dimer, IFT81, IFT74, IFT70, or IFT46 induced differential instability of the other IFT-B1 subunits (Figure 6C). IFT52 appeared to be most critical as its loss severely reduced the abundance of the other IFT-B1 subunits, which is consistent with the role of IFT52 in organizing IFT88, IFT70, and IFT46, a module that links with the other IFT-B1 subunits.

Taken together, we have revealed the contribution of each subunit to the stability of IFT-B. In particular, we have found that IFT-B1 and IFT-B2 are mutually dependent on stability, which is mediated by the connecting tetramer. Based on the interaction map of IFT-B and the above analysis, we have proposed a model where how the loss of one subunit influences the stability of the other subunits (Figure 6D).

**Intact complexes of IFT-B1 and IFT-B2 can be assembled independently of each other in vivo**

IFT-B complex can be separated into IFT-B1 and IFT-B2 by increasing ionic strength. Furthermore, IFT-B1 and IFT-B2 can be in vitro reconstituted, respectively and assembled into an IFT-B complex. It is curious how IFT-B complex is assembled in vivo. Analyzing IFT-B mutants may provide insights into IFT-B complex assembly. First, we examined whether the loss of IFT80 would allow the rest subunits to assemble a complex from the residual amount of IFT proteins. Immunoprecipitation with anti-IFT38 antibody using cell lysates of ift80-2 mutant pulled down subunits of both IFT-B2 and IFT-B1 though with less amount compared to the control (Figure S4). Thus, the residual amount of IFT proteins may assemble into complexes in the absence of one subunit.

The tetramer IFT88/52/57/38 bridges IFT-B1 and IFT-B2. It is thus possible that the disruption of this tetramer may separate IFT-B into IFT-B1 and IFT-B2. We thus analyzed mutants ift38-1 and ift52-1 with wild-type cells as a control by sucrose gradient. In wild-type cells, both IFT-B1 and IFT-B2 subunits migrated together and peaked around fractions #16 and #17 (Figures S5 and 7A), consistent with previous studies. In ift38-1 mutant, IFT-B2 was disrupted as evidenced by the separation of IFT57 from IFT172 and IFT80 (Figure 7A). In contrast, IFT-B1 components IFT88 and IFT52 migrated together and peaked at fractions #13 and #14, which is similar to a previous report, indicating the formation of an intact IFT-B1 complex.

Similarly, by analyzing the ift52-1 mutant, it showed that IFT-B1 appeared to be separated into two subcomplexes represented by IFT88/81/74, and by IFT70/46 (Figure 7B). However, IFT-B2 components migrated together and peaked around fractions #11-12, indicative of the formation of an intact IFT-B2. Taken together, we have provided in vivo data supporting that the IFT88/52/57/38 tetramer is critical in organizing IFT-B1 and IFT-B2 into an IFT-B complex. More importantly, we showed that IFT-B1 or IFT-B2 can be assembled independently of each other, suggesting that IFT-B1 and IFT-B2 are assembled separately in vivo before they assemble into an IFT-B complex.

**DISCUSSION**

**IFT38 functions in organizing intraflagellar transport complex, localizing IFT-B, and linking intraflagellar transport trains to kinesin-II**

We have used in vivo deletions to decipher the critical function of the CC domain. Our data support the interaction of IFT38 via its CC domain with IFT57 and IFT20 and confirm the role of the CC domain in the interaction of IFT38 with IFT88/52 of IFT-B1, complementing the previous studies. It has been shown that an IFT38/57 dimer is required for the interaction with IFT88/52 and the CC-domain of IFT38 is involved. Our data indicate that the interaction of IFT38 with IFT57 via the CC domain enables the formation of a tetramer. Our finding that loss of the CC domain of IFT38 leads to IFT80 dissociation is intriguing because the CH domain has been shown to interact with IFT80. One possibility is that IFT80-IFT38 interaction may require the interaction of IFT80 with IFT20/54 that binds IFT38.

The tetramer IFT88/52/57/38 has been shown to interact with kinesin-II biochemically. However, the physiological data that support this has not been available. We found that the deletion of the IFT38 C-terminus does not affect IFT complex formation but induces ciliary accumulation of IFT proteins and motors. The velocity and frequency of anterograde IFT are reduced with increasing stalled IFT trains while retrograde IFT is not affected. These data suggest that the interaction of kinesin-II with IFT-B is likely impaired. However, the alteration in anterograde IFT is mild though statistically significant, which suggests that the C-terminus of IFT38 is
involved but not critical in the possible interaction of IFT-B with kinesin-II. Nevertheless, it provides first physiological evidence to support the interaction of kinesin-II with an IFT-B1/IFT-B2 connecting tetramer that includes IFT38.

IFT complexes are enriched at the basal bodies and are anchored likely to the transition fibers. How IFT proteins are associated with the basal body is not clear. We showed that the localization of IFT-B2 but not IFT-B1 depends on IFT38. Furthermore, the cellular localization of IFT38 is not dependent on its interacting partner IFT57, suggesting that IFT38 is a critical component in localizing IFT-B2. The cellular localization of IFT38 may result from its direct interaction with the structural components of the basal body or from its interaction with the IFT-B1 components.

**IFT38 mediates the retrograde trafficking of BBSome**

The BBSome is a heterooctameric protein complex and involved in the ciliary exit of membrane proteins by association with the IFT trains. How IFT proteins are associated with the basal body is not clear. We showed that the localization of IFT-B2 but not IFT-B1 depends on IFT38. Furthermore, the cellular localization of IFT38 is not dependent on its interacting partner IFT57, suggesting that IFT38 is a critical component in localizing IFT-B2. The cellular localization of IFT38 may result from its direct interaction with the structural components of the basal body or from its interaction with the IFT-B1 components.
C1orf74 (aka IFTAP) interacts with IFT-A via IFT122 and loss of which impairs the ciliary entry of BBSome. However, trafficking of membrane proteins Smoothened or GPR161 that are known to depend on BBSome is not affected, suggesting that C1orf74 is not critical for BBSome trafficking. Depletion of IFT27 of IFT-B1 in murine cells induces the ciliary accumulation of BBSome. It has been proposed that IFT27 may be involved in the ciliary exit of BBSome by interacting with LZTFL1 that further interacts with BBS9 or in BBSome remodeling at the ciliary tip. In Chlamydomonas, it has been shown that IFT27 and LZTFL1 are involved in the reassembly of the BBSome at the ciliary tip. Thus, it is not clear whether LZTFL1 functions as an IFT adaptor for retrograde trafficking of BBSome.

IFT38 interacts with BBSome where both the CC domain and C-terminus are required. Interestingly, loss of the IFT38 C-terminus does not alter the ciliary distribution of BBSome while inducing ciliary accumulation of GPR161 upon the stimulation of hedgehog signaling. In another study, it is proposed that IFT38 C-terminus is necessary and sufficient for BBSome binding. We showed that the deletion of IFT38 C-terminus reduces the interaction with BBS8 around 2-fold. However, a reduction but not the abolishment of the interaction indicates that the C-terminus of IFT38 is required but not sufficient. Given that the CC domain is involved in the interaction, we propose that the CC-domain and the C-terminus together ensure a proper binding of BBSome to IFT38. In addition, we found that BBS8 is increased in cilia and enriched at the ciliary tip in the C-terminus deletion mutant of IFT38, suggesting that IFT38 is involved in retrograde but not anterograde trafficking of BBSome. Taken together, we propose that IFT38 mediates retrograde trafficking of BBSome with the involvement of both the C-terminus and the CC domains.

The stability and assembly of IFT-B in vivo

We have systematically studied the stability of IFT-B by using mutants from all IFT-B subunits and six IFT-B subunits that critically affect ciliogenesis. The reduced abundance of IFT-B subunits in an IFT-B mutant is due to protein degradation rather than defects in transcription. The stability of IFT-B1 or IFT-B2 is differentially affected by the loss of one of its subunits. In particular, IFT-B1 and IFT-B2 are mutually dependent for stability, which is regulated by the connecting tetramer IFT88/52/57/38. Our systematic studies provide a panoramic view of the stability of IFT-B. How this stability is controlled is not clear. Induction of the instability of a protein complex after the loss of one of its subunits has been widely observed. It has been proposed that excessed proteins due to the change in the stoichiometry of a protein complex or incompletely assembled intermediates were prone to form cytotoxic aggregates that were degraded by the “assembly quality control” (AQC) system to safeguard proteostasis.

The order of the de novo assembly of IFT-B is not clear. There are two pathways for complex assembly: linear and modular. Many classic complexes such as the proteasome are undergoing modular assembly, in which small modules are first assembled, followed by sequential assembly into a large complex. Previous data showed that different modules of IFT-B can be formed in vitro, for example, IFT88/70/52/46 and IFT81/74/27/25/22 of IFT-B1. In addition, IFT-B1 and IFT-B2 can be reconstituted in vitro and assembled into a full IFT-B complex. We showed that in vivo intact IFT-B1 and IFT-B2 can be assembled the independent of each other. Furthermore, small modules can be formed. For example, co-migration of IFT80/172 in sucrose gradient in the absence of IFT38; separation of IFT-B1 into two peaks containing IFT88/81/74 and IFT70/46 in the absence of IFT52. Formation of small IFT-B modules has also been found in other IFT-B mutants. Importantly, the connecting tetramer is important for the stability of IFT-B1 and IFT-B2, indicating that IFT-B is only stable when IFT-B1 and IFT-B2 assemble into a complete IFT-B complex. Thus, we propose that the IFT-B complex is assembled in a modular pathway (Figure 7C). Following different module formations within IFT-B1 and IFT-B2, IFT-B1 and IFT-B2 are assembled individually and associate with each other via the connecting tetramer to form an IFT-B complex. The exact order of a sequential assembly of different modules into an IFT-B complex remains to be experimentally verified. Quantitative mass spectrometry combined with the complexome profiling of the IFT-B complex would be an attracting approach.

Limitations of the study

We have shown that the deletion of the IFT38 C-terminus affected the velocity and frequency of anterograde transport and caused an enrichment of BBSome at the ciliary tip, indicating that the C-terminus of IFT38 is engaged in regulating the anterograde IFT and retrograde trafficking of BBSome. However, it remains unclear how IFT38 C-terminus functions in these processes. Future in vitro biochemical assays and protein structural studies may reveal the detailed mechanisms. We have proposed a modular pathway...
for the assembly of the IFT-B complex. The hypothesis is based on the results of sucrose gradient analysis using IFT-B mutants as reported here as well as evidence provided elsewhere in the literature. However, our results are only from studies in Chlamydomonas though the IFT machinery is highly conserved across different species. In addition, the exact assembly order of the IFT-B complex is unknown. Quantitative mass spectrometry combined with complexome profiling would likely answer this question.

**STAR+ METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Insertional mutagenesis, gene cloning, and transformation
  - Generation of knockout strains using CRISPR/Cas9
  - Cilia isolation, cilia assembly and disassembly, ciliary length measurement and DIC cell imaging
  - Primary antibodies
  - SDS-PAGE, immunoblotting, immunoprecipitation and sucrose gradient analysis
  - Immunofluorescence (IF) and transmission electron microscopy (TEM)
  - Living imaging of IFT by total internal reflection fluorescence (TIRF) microscopy
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105493.

**ACKNOWLEDGMENTS**

We are grateful to Drs. Guoliang Xu and Hui Chen (Shanghai Institutes for Biological Sciences, CAS) for the assistance in gene editing. We also thank the Core Facility Center of Biomedical Analysis (Tsinghua University) for assistance with cell imaging analysis. This work was supported by the National Natural Science Foundation of China (31991191, 31972888) to JP.

**AUTHOR CONTRIBUTIONS**

All authors designed the studies. J.W. carried out most of the experiments, X.Z. identified the ift38-1 mutant and generated the HA-tagged IFT38 rescue strains, Z.W. and X.L helped to generate the ift57-2 and ift20-2/-3 mutants by gene editing and H.T. isolated the ift172-3 mutant. J.P. provided resources. J.P. and J.W. wrote the article.

**DECLARATION OF INTERESTS**

The authors declare that they have no conflict of interest.

Received: August 12, 2022  
Revised: October 13, 2022  
Accepted: November 1, 2022  
Published: December 22, 2022

**REFERENCES**

1. Reiter, J. F., and Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. Nat. Rev. Mol. Cell Biol. 18, 533–547. https://doi.org/10.1038/nrm.2017.60.
2. Rosenbaum, J. L., and Witman, G. B. (2002). Intraflagellar transport. Nat. Rev. Mol. Cell Biol. 3, 813–825.
3. Taschner, M., and Lorentzen, E. (2016). The intraflagellar transport machinery. Cold Spring Harb. Perspect. Biol. 8, a028092. https://doi.org/10.1101/cshperspect.a028092.
4. Ishikawa, H., and Marshall, W. F. (2017). Intraflagellar transport and ciliary dynamics. Cold Spring Harb. Perspect. Biol. 9, a021998. https://doi.org/10.1101/cshperspect.a021998.
5. Klena, N., and Pigino, G. (2022). Structural biology of cilia and intraflagellar transport. Annu. Rev. Cell Dev. Biol. 38, 103–123. https://doi.org/10.1146/annurev-cellbio-120219-034938.


6. Lechtreck, K.F. (2015). IFT-cargo interactions and transport protein in cilia. Trends Biochem. Sci. 40, 765–778. https://doi.org/10.1016/j.tibs.2015.09.003.

7. Jordan, M.A., Diener, D.R., Stepanek, L., and Pigno, G. (2018). The cyto-EM structure of intraflagellar transport trains reveals how dynein is inactivated to ensure unidirectional anterograde movement in cilia. Nat. Cell Biol. 20, 1250–1255. https://doi.org/10.1038/s41556-018-0213-1.

8. Funabashi, T., Katoh, Y., Okazaki, M., Sugawa, M., and Nakayama, K. (2018). Interaction of heterotrimeric kinesin-II with IFT-B-connecting tetramer is crucial for ciliogenesis. Cell Biol. Int. 42, 217–226. https://doi.org/10.1002/cbin.101803.

9. Lucker, B.F., Behal, R.H., Qin, H., Siron, L.C., Taggart, W.V., Rosenbaum, J.L., and Cole, D.G. (2005). Characterization of the intraflagellar transport complex B: direct interaction of the IFT81 and IFT74/72 subunits. J. Biol. Chem. 280, 27686–27696. https://doi.org/10.1074/jbc.M505062200.

10. Taschner, M., Weber, K., Mourao, A., Vetter, M., Awasthi, M., Stiegl, M., Bhogaraju, S., and Lorentzen, E. (2016). Intraflagellar transport protein 172, 80, 57, 54, 38, and 20 form a stable tubulin-binding IFT-BZ complex. EMBO J. 35, 773–790. https://doi.org/10.15252/emby.201593164.

11. Katoh, Y., Terada, M., Nishijima, Y., Takei, R., Nozaki, S., Hamada, H., and Nakayama, K. (2016). Overall architecture of the intraflagellar transport (IFT)-B complex containing Cluap1/IFT38 as an essential component of the IFT-B peripheral subcomplex. J. Biol. Chem. 291, 10962–10975. https://doi.org/10.1074/jbc.M116.713883.

12. Taschner, M., Kotis, F., Braue, P., Kuehn, E.W., and Lorentzen, E. (2014). Crystal structures of IFT70/52 and IFT52/46 provide insight into intraflagellar transport B complex core complex assembly. J. Cell Biol. 207, 269–282. https://doi.org/10.1083/jcb.201408002.

13. Jiang, X., Hernandez, D., Hernandez, C., Pang, Y., Wu, Q., Hu, Z., Han, X., Xu, Y., Deng, H., and Pan, J. (2014). FLAB/KIF3B phosphorylation regulates kinesin-II interaction with IFT-B to control IFT entry and turnaround. Dev. Cell 30, 585–597. https://doi.org/10.1016/j.devcel.2014.07.019.

14. Brown, J.M., Cochran, D.A., Craige, B., Kubo, T., and Witman, G.B. (2015). Assembly of IFT trains at the ciliary base depends on IFT70. Curr. Biol. 25, 1583–1593. https://doi.org/10.1016/j.cub.2015.04.060.

15. Zhu, X., Wang, J., Li, S., Lechtreck, K., and Pan, J. (2021). IFT54 directly interacts with kinesin-II and dynein to regulate anterograde intraflagellar transport. EMBO J. 40, e105781. https://doi.org/10.15252/embj.2020105781.

16. Funabashi, T., Katoh, Y., Okazaki, M., Sugawa, M., and Nakayama, K. (2018). Interaction of heterotrimeric kinesin-II with IFT-B-connecting tetramer is crucial for ciliogenesis. Cell Biol. Int. 42, 217–226. https://doi.org/10.1002/cbin.101803.

17. Zhao, Q., Li, S., Shao, S., Wang, Z., and Pan, J. (2020). FL2SL2 is a CDK-like kinase that directly binds IFT70 and is required for proper ciliary disassembly in Chlamydomonas. PLoS Genet. 16, e1008651. https://doi.org/10.1371/journal.pgen.1008651.

18. Lin, H., Guo, S., and Dutcher, S.K. (2018). RPPGRPP1L helps to establish the ciliary gate for entry of proteins. J. Cell Sci. 131, jcs220905. https://doi.org/10.1242/jcs.220905.

19. Cole, D.G., Diener, D.R., Himmelblau, A.L., Beech, P.L., Fuster, J.C., and Rosenbaum, J.L. (1998). Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT). IFT particles contain proteins required for ciliary assembly in Caenorhabditis elegans sensory neurons. J. Cell Biol. 141, 993–1008.

20. Pettman, N.A., Loureiro-López, M., Taschner, M., Zacharia, N.K., Georgieva, M.M., Boegholm, N., Mourão, A., Russell, R.B., Andersson, J.S., and Lorentzen, E. (2022). Biochemically validated structural model of the 15-subunit IFT-B complex. Preprint at bioRxiv. https://doi.org/10.1101/2022.08.20.504624.
proteins of HeLa cells are synthesized normally and degraded rapidly. J. Mol. Biol. 115, 315–333.

49. Pla-Prats, C., and Thomas, N. H. (2022). Quality control of protein complex assembly by the ubiquitin-proteasome system. Trends Cell Biol. 32, 696–706. https://doi.org/10.1016/j.tcb.2022.02.005.

50. Huttlin, E. L., Bruckner, R. J., Paulo, J. A., Cannon, J. R., Ting, L., Baltier, K., Colby, G., Gebreab, F., Gygi, M. P., Parzen, H., et al. (2017). Architecture of the human interactome defines protein communities and disease networks. Nature 545, 505–509. https://doi.org/10.1038/nature22366.

51. Timón-Gómez, A., Nyítová, E., Abrilta, L. A., Vila, A. J., Hosler, J., and Barrientos, A. (2018). Mitochondrial cytochrome c oxidase biogenesis requires the TEP1-SEM1 complex. Cell Dev. Biol. 76, 163–178. https://doi.org/10.1007/s10199-017-0585-1.

52. Vercellino, I., and Sazanov, L. A. (2022). The assembly, regulation and function of the mitochondrial respiratory chain. Nat. Rev. Mol. Cell Biol. 23, 141–161. https://doi.org/10.1038/s41580-021-0015-0.

53. Onischchenko, E., Noor, E., Fischer, J. S., Gillet, L., Woytyniec, M., Vallotton, P., and Weis, K. (2020). Maturation kinetics of a multiprotein complex revealed by metabolic labeling. Cell 183, 1785–1800.e26. https://doi.org/10.1016/j.cell.2020.11.001.

54. Gu, Z. C., and Erenkel, C. (2014). Proteasome assembly. Cell. Mol. Life Sci. 71, 4729–4745. https://doi.org/10.1007/s00018-014-1699-8.

55. Wittig, I., and Malacarne, P. F. (2021). Complexome profiling: assembly and remodeling of protein complexes. Int. J. Mol. Sci. 22, 7809. https://doi.org/10.3390/ijms22157809.

56. Liang, Y., and Pan, J. (2013). Regulation of flagellar biogenesis by a calcium dependent protein kinase in Chlamydomonas reinhardtii. PLoS One 8, e69902.

57. Zhu, B., Zhu, X., Wang, L., Liang, Y., Feng, Q., and Pan, J. (2017). Functional exploration of the IFT-A complex in intraflagellar transport and ciliogenesis. PLoS Genet. 13, e1006627. https://doi.org/10.1371/journal.pgen.1006627.

58. Li, S., Wan, K. Y., Chen, W., Tao, H., Liang, X., and Pan, J. (2020). Functional exploration of heterotetrameric kinesin-II in IFT and ciliary length control in Chlamydomonas. Elife 9, e58868. https://doi.org/10.7554/eLife.58868.

59. Wu, Q., Gao, K., Zheng, S., Zhu, X., Liang, Y., and Pan, J. (2018). Calmodulin regulates a TRP channel (ADF1) and phospholipase C (PLC) to mediate elevation of cytosolic calcium during acidic stress that induces deflagellation in Chlamydomonas. FASEB J. 32, 3689–3699. https://doi.org/10.1096/fj.201701366R.

60. Lv, B., Wan, L., Taschner, M., Cheng, X., Lorentzen, E., and Huang, K. (2017). Intraflagellar transport protein IFT52 recruits IFT64 to the basal body and flagella. J. Cell Sci. 130, 1662–1674. https://doi.org/10.1242/jcs.200758.

61. Sager, R., and Granick, S. (1953). Nutritional studies with Chlamydomonas reinhardtii. Ann. N. Y. Acad. Sci. 56, 831–838.

62. Sizova, I., Fuhrmann, M., and Hegemann, P. (2001). A Streptomyces rimosus aphVIII gene coding for a new type phosphotransferase provides stable antibiotic resistance in Chlamydomonas reinhardtii. Gene 277, 221–229.

63. González-Ballester, D., de Montagut, A., Galván, A., and Fernández, E. (2005). Restriction enzyme site-directed amplification PCR: a tool to identify regions flanking a marker DNA. Anal. Biochem. 340, 330–335.

64. Meng, D., Cao, M., Oda, T., and Pan, J. (2014). The conserved cilary protein Bug22 controls planar beating of Chlamydomonas flagella. J. Cell Sci. 127, 281–287.

65. Xue, J. H., Chen, G. D., Hao, F., Chen, H., Pan, Z., Chen, F. F., Pang, B., Yang, Q. L., Wei, X., Fan, Q. Q., et al. (2019). A vitamin-C-derived DNA modification catalysed by an algal TET homologue. Nature 569, 581–585. https://doi.org/10.1038/s41586-019-1160-0.

66. Pan, J., and Snell, W. J. (2000). Regulated targeting of a protein kinase into an intact flagellum. An aurora/Ipl1p-like protein kinase translocates from the cell body into the flagella during gamete activation in chlamydomonas. J. Biol. Chem. 275, 24106–24114.

67. Pan, J., and Snell, W. J. (2003). Kinesin II and regulated intraflagellar transport of Chlamydomonas aurora protein kinase. J. Cell Sci. 116, 2179–2186.

68. Craigie, B., Tsao, C. C., Diener, D. R., Hou, Y., Lechtreck, K. F., Rosenbaum, J. L., and Witman, G. B. (2010). CEP290 tethers flagellar transition zone microtubules to the membrane and regulates flagellar protein content. J. Cell Biol. 190, 927–940.

69. Wren, K. N., Craft, J. M., Tritschler, D., Schauer, A., Patel, D. K., Smith, E. F., Porter, M. E., Kner, P., and Lechtreck, K. F. (2013). A differential cargo-loading model of cilary length regulation by IFT. Curr. Biol. 23, 2463–2471. https://doi.org/10.1016/j.cub.2013.10.044.

70. Wingfield, J. L., Mengoni, I., Bomberger, H., Jiang, Y. Y., Walsh, J. D., Brown, J. M., Picciolo, T., Cochran, D. A., Zhu, B., Pan, J., et al. (2017). IFT trains in different stages of assembly queue at the ciliary base for consecutive release into the cilium. Elife 6, e26609. https://doi.org/10.7554/eLife.26609.
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Rat anti-HA, monoclonal (1:2000 for IB, 1:200 for IF) | Roche | Cat# 11867431001, RRID:AB_390919 |
| Mouse anti-α-tubulin, monoclonal (1:2000 for IB, 1:100 for IF) | Sigma | Cat# T6199, RRID:AB_477583 |
| Mouse anti-IC2, monoclonal (1:10000 for IB) | Sigma | Cat# D6168, RRID:AB_259231 |
| Mouse anti-GFP, monoclonal (1:2000 for IB) | Abmart | Cat# M20004S, RRID:AB_2619674 |
| Rabbit anti-GFP, polyclonal (1:200 for IF) | MBL | Cat# 598 |
| Rabbit anti-CDPK3, polyclonal (1:5000 for IB) | Liang and Pan, 2013 | N/A |
| Mouse anti-IFT139, monoclonal (1:10000 for IB) | Cole et al., 1998 | N/A |
| Mouse anti-IFT144, polyclonal (1:2000 for IB) | Zhu et al., 2017 | N/A |
| Rabbit anti-IFT121, polyclonal (1:2000 for IB) | Zhu et al., 2017 | N/A |
| Rabbit anti-IFT143, polyclonal (1:2000 for IB, 1:50 for IF) | Zhu et al., 2017 | N/A |
| Rabbit anti-IFT172, polyclonal (1:1000 for IB, 1:100 for IF) | Zhu et al., 2021 | N/A |
| Rabbit anti-IFT57, polyclonal (1:1000 for IB, 1:50 for IF) | Li et al., 2020 | N/A |
| Rabbit anti-IFT54, polyclonal (1:2000 for IB, 1:100 for IF) | Zhu et al., 2017 | N/A |
| Rabbit anti-IFT38, polyclonal (1:2000 for IB, 1:100 for IF) | Wu et al., 2018 | N/A |
| Rabbit anti-IFT20, polyclonal (1:250 for IB) | This study | N/A |
| Rabbit anti-IFT88, polyclonal (1:2000 for IB, 1:100 for IF) | This study | N/A |
| Mouse anti-IFT81, monoclonal (1:2000 for IB) | Cole et al., 1998 | N/A |
| Rabbit anti-IFT74, polyclonal (1:2000 for IB, 1:100 for IF) | This study | N/A |
| Rabbit anti-IFT70, polyclonal (1:2000 for IB) | Zhao et al., 2020 | N/A |
| Rabbit anti-IFT52, polyclonal (1:2000 for IB, 1:100 for IF) | This study | N/A |
| Rabbit anti-IFT46, polyclonal (1:2000 for IB) | Lv et al., 2017 | N/A |
| Rabbit anti-BBS8, polyclonal (1:250 for IB) | This study | N/A |
| Rabbit anti-D1bLIC, polyclonal (1:2000 for IB) | Zhu et al., 2017 | N/A |
| Rabbit anti-FLA8, polyclonal (1:2000 for IB) | Liang et al., 2014 | N/A |
| Goat Anti-Mouse IgG (H&L)-HRP Conjugated (1:5000 for IB) | EASYBIO | Cat# BE0102, RRID:AB_2923205 |
| Goat Anti-Rabbit IgG (H&L)-HRP Conjugated (1:5000 for IB) | EASYBIO | Cat# BE0101 |
| Goat anti-Rat IgG (H + L), HRP (1:5000 for IB) | EASYBIO | Cat# BE0108 |
| Alexa Fluor 594 goat anti-mouse IgG (H + L) (1:200 for IF) | Invitrogen | Cat# A11005, RRID:AB_2534073 |
| Alexa Fluor 488 goat anti-rat IgG (H + L) (1:200 for IF) | Invitrogen | Cat# A11006, RRID:AB_141373 |
## REAGENT or RESOURCE SOURCE IDENTIFIER

### Alexa Fluor 488 goat anti-rabbit IgG (H + L) (1:200 for IF)
Invitrogen Cat# A11008; RRID:AB_143165

### Bacterial and virus strains

| Strain (Species) | Source | Identifier |
|------------------|--------|------------|
| DH5a Competent Cell (E. coli) | TIANGEN | Cat# CB101-01 |
| Rosetta (DE3) Competent Cell (E. coli) | CWBIO | Cat# CW0811S |

### Chemicals, peptides, and recombinant proteins

| Chemical | Source | Identifier |
|----------|--------|------------|
| Rat anti-HA Affinity Matrix | Roche | Cat# 1867423001 |
| Anti-GFP tag Rabbit mAb conjugated Agarose Beads | Engibody | Cat# AT0083 |
| Ni-NTA Agarose | Qiagen | Cat# 30210 |
| Mini Complete (EDTA free) | Roche | Cat# 4693132001 |
| MG-132 | Selleck | Cat# S2619 |
| MG-101 (ALLN) | Selleck | Cat# S7386 |
| Poly-L-lysine | Sigma | Cat# P8920 |

### Critical commercial assays

| Assay | Source | Identifier |
|-------|--------|------------|
| HiScribe™ Quick T7 High Yield RNA Synthesis Kit | NEB | Cat# E2050 |
| RNAclean Miniquick RNA Purification Kit | ZOMANBIO | Cat# ZP417 |

### Experimental models: Organisms/strains

| Strain (Species) | Source | Identifier |
|------------------|--------|------------|
| C. reinhardtii: 21gr, mt+ | Chlamydomonas Resource Center | CC-1690 |
| C. reinhardtii: ift38-1, mt+ | Zhu et al., 2017 | N/A |
| C. reinhardtii: ift38-1::IFT38-HA, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT384.Ct1-HA, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT384.Ct2-HA, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT384.Ct-HA, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT3812.YFP-HA, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT38-YFP, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT384.Ct-YFP, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT38-HA;BBS8-YFP, mt+ | This study | N/A |
| C. reinhardtii: ift38-1::IFT384.Ct-HA;BBS8-YFP, mt+ | This study | N/A |
| C. reinhardtii: ift172-3, mt+ | This study | N/A |
| C. reinhardtii: ift80-1, mt+ | Zhu et al., 2017 | N/A |
| C. reinhardtii: ift57-2, mt+ | This study | N/A |
| C. reinhardtii: ift54-1, mt+ | Zhu et al., 2017 | N/A |
| C. reinhardtii: ift20-2, mt+ | This study | N/A |
| C. reinhardtii: ift20-3, mt+ | This study | N/A |
| C. reinhardtii: ift81-2, mt+ | Zhu et al., 2017 | N/A |
| C. reinhardtii: ift74-3, mt+ | This study | N/A |
| C. reinhardtii: ift88-2, mt+ | Zhu et al., 2017 | N/A |
| C. reinhardtii: bld1-1, mt+ | Brazelton et al., 2001 | CC-477 |
| C. reinhardtii: ift70-1, mt+ | Zhu et al., 2017 | N/A |
| C. reinhardtii: ift46-3, mt+ | Zhu et al., 2017 | N/A |

### Oligonucleotides

| sgRNA target sequence of IFT57: | This study | http://www.rgenome.net/cas-designer/ |
|-------------------------------|-------------|-----------------------------------|
| CCCGCAGATACAAACAGCGC | | |

(Continued on next page)
## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Junmin Pan (panjunmin@tsinghua.edu.cn).

### Materials availability

The materials reported in this study will be available upon request.

### Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
EXPERIMENTAL MODEL AND SUBJECT DETAILS

C. reinhardtii wild-type strain 21gr (mt+; CC-1690) was used and is available from the Chlamydomonas Resource Center (University of Minnesota, St. Paul, MN). All the other strains used are from previous studies or generated from this study and the detailed information is described in Table S1. The methods for generating mutants or transgenic strains are described in the sections below. The cells were cultured on 1.5% agar plates or in liquid M medium at 23°C with aeration under 14/10 h light/dark cycle. For transformation, cells were grown in liquid tris-acetate-phosphate (TAP) medium in constant light.

METHOD DETAILS

Insertional mutagenesis, gene cloning, and transformation

Chlamydomonas insertional mutants were generated by transformation of wild-type strain 21gr via electroporation of a cassette expressing aphVIII from plasmid pIS103m, a derivative of pIS103. The genomic sequences flanking the insertion sites were determined by restriction enzyme site-directed amplification PCR (RESDA-PCR) followed by DNA sequencing.

To generate a rescue construct for ift38-1, the full-length IFT38 gene was cloned by PCR from the genomic DNA of the wild-type strain 21gr. The DNA fragment was subcloned by replacing IFT54 sequence into the pKH-IFT54-HA vector, termed pKH-IFT38-HA. The deletion constructs of IFT38 were made by overlapping PCR and conventional molecular methods and were cloned into pKH-IFT38-HA. To obtain constructs expressing YFP-tagged IFT38 or BBS8, IFT38 or BBS8 replaced the IFT43 gene of IFT43-YFP in a construct, and these constructs were termed pJP-IFT38-YFP or pJP-BBS8-YFP. All constructs were verified by sequencing. The final constructs were linearized with ScaI or AclII and transformed into the indicated strains by electroporation.

Generation of knockout strains using CRISPR/Cas9

Chlamydomonas gene-editing mutants were generated following a published procedure with modifications. Single-guide RNAs (sgRNAs) of IFT57 and IFT20 were designed using CRISPR RGEN Tools (http://www.rgenome.net/cas-designer/). The sgRNA includes the guide RNA sequences, the common sequence containing the crRNA, tetraloop, and tracrRNA (Table S2). The sgRNAs were prepared in vitro with a DNA template containing a T7 promotor and corresponding sgRNA sequences by the HiScribe™ Quick T7 High Yield RNA Synthesis Kit (NEB, USA, #E2050) and RNAclean Miniquick RNA Purification Kit (Zoman Biotech, #ZP417, China). SpCas9 was expressed in bacteria, purified and stored at −80°C before use. The efficacy of sgRNAs for cleavage of corresponding DNA fragments was tested in vitro.

For cell transformation, an assembled ribonucleoprotein complex (RNP), 3.3 μg donor DNA and 2 μg of aphVIII expressing cassette (as a selection marker) were mixed with 80 μL of de-walled wild-type cells (1.25x10⁸ cells/ml) in a total volume of 110 μL. The cell walls were removed by treatment with autolysin. An RNP complex was assembled by mixing 50 μg SpCas9 and 75 μg sgRNA in a total volume of 20 μL and incubating at 37°C for 15 min. The donor DNA contains a common sequence with tandem stop codons and two 17 bp sequences flanking the cleavage site (Table S2). The cells were transformed in an electroporator (BTX, ECM630, Germany) with the following setting: 4 mm cuvette, 600 V, 50 μF, infinite resistance. The transformed cells were grown on agar plates containing 10 μg/mL paramomycin. The colonies were screened by PCR for genotyping and the identified mutants were further characterized by DNA sequencing.

Cilia isolation, cilia assembly and disassembly, ciliary length measurement and DIC cell imaging

Cilia isolation was performed following a previous report. Briefly, cells were deciliated by pH-shock and cilia were purified via a sucrose gradient. Isolated cilia were suspended in HMDEK buffer (50 mM HEPES, pH 7.2, 5 mM MgCl₂, 0.5 mM EDTA, 1 mM DTT, 25 mM KCl) containing various protease inhibitors including protease inhibitor cocktail (mini-complete EDTA-free, Roche, China), 20 μM MG-132 and 65 μM MG-101 (Selleck, China), and frozen in liquid nitrogen before storage at −80°C.

The assays for cilia assembly and disassembly were performed according to methods in a previous publication. Briefly, cells were deciliated by pH shock to allow cilia assembly. For cilia disassembly, cells were treated with 20 mM NaPPi. The cells were fixed with 1% glutaraldehyde at the indicated times and imaged.
by differential interference contrast (DIC) microscopy on a Zeiss Axio Observer Z1 microscope (Carl Zeiss, Germany) that is equipped with a CCD camera (QuantEM512SC, Photometric, USA). Ciliary length was measured using Image J (NIH, USA) and the data were plotted using GraphPad Prism 6.0 (GraphPad, USA). At least 50 cells were used for ciliary length measurement.

**Primary antibodies**

Details of the antibodies used are described in Table S3. Of particular, the antigens used for immunizing rabbits to generate polyclonal antibodies are 248–454 amino acids for IFT52, 123–324 amino acids for IFT88, 207–641 amino acids for IFT74, full-length protein for IFT20 and 16–193 amino acids for BBS8. All the antibodies were affinity purified (ABclonal, China). Monoclonal anti-IFT139 and IFT81 antibodies were kindly provided by Dr Joel Rosenbaum (Yale University, New Haven, CT).

**SDS-PAGE, immunoblotting, immunoprecipitation and sucrose gradient analysis**

SDS-PAGE and immunoblotting were carried out following a previous publication.66 In brief, frozen cells were dissolved in Buffer A (50 mM Tris, pH 7.5, 10 mM MgCl2, 1 mM EDTA, 1 mM DTT) containing protease inhibitors as described above on ice. The samples were then boiled in 1x SDS sample buffer for 10min, separated on SDS-PAGE, and analyzed by immunoblotting with the indicated antibodies.

A previous protocol was followed for immunoprecipitation analysis.17 In brief, ~1x10⁹ cells were lysed in IP buffer (20 mM HEPES, pH 7.2, 5 mM MgCl₂, 1 mM DTT, 1 mM EDTA, 150 mM NaCl, 5% glycerol) containing protease inhibitors as described above. The supernatant was incubated with pre-washed anti-HA affinity matrix (Roche) or anti-GFP rabbit mAb conjugated agarose beads (Engibody, #AT0083) for 2 h at 4°C. The beads/matrixes were washed three times with IP buffer containing 0.05% NP40 and 0.05% Triton X-100 and analyzed by immunoblotting.

Sucrose gradient analysis has been detailed previously.67 Briefly, frozen cells were dissolved in HMDEK buffer containing protease inhibitors as described above followed by centrifugation at 12000xg for 15 min at 4°C. About 200 μL supernatant (~1 mg total protein) was loaded onto a 2 mL 5–20% linear sucrose gradient by centrifugation at 200,000xg for 4 h at 4°C (Beckman Coulter Optima MAX-XP, rotor TLS-55). Typically, 25 fractions were collected from top to bottom for further immunoblot analysis.

**Immunofluorescence (IF) and transmission electron microscopy (TEM)**

For IF, a previous protocol was followed.15 Fresh cells were resuspended in MT buffer (30 mM HEPES, pH 7.2, 25 mM KCl, 3 mM EGTA and 1 mM MgSO₄) and fixed with an equal volume of 8% paraformaldehyde for 5 min. The fixed cells were permeabilized in MT buffer containing 0.5% NP40 for 2 min and attached to a 10 well slide previously coated with 0.01% poly-L-lysine for 10 min. The cells were further extracted with cold-methanol at −20°C for 10min followed by washing three times with PBS and blocking with 5% goat serum for 1 h at 37°C. The cells were then incubated for 2 h at 37°C with primary antibodies in G-blocking buffer (10 mM PBS, pH 7.4, 1% Gelatin, 0.04% Sodium Azide, 0.5% Goat Serum, 5% Glycerol). After washing three times with PBST buffer (PBS buffer containing 0.5% Tween-20), cells were incubated with the secondary antibodies for 1–2 h at 37°C followed by washing. The secondary antibodies used are the following: alexa Fluor 594 goat anti-mouse IgG, alexa Fluor 488 goat anti-rat IgG and alexa Fluor 488 goat anti-rabbit IgG from Invitrogen (USA) and all were used at 1:200. The samples were mounted with Fluoromount-G (SouthernBiotech, USA) and viewed on Zeiss LSM880 with Airyscan (Axio Observer 7) using a 63x lens. Images were acquired and processed by ZEN 2012 blue Edition (Zeiss) and Photoshop (Adobe). For fluorescent quantification of IFT proteins in cilia, the images were analyzed using Fiji (NIH, USA). The background fluorescence was subtracted from the total fluorescence in a cilium.

TEM was performed based on previous protocols.64,68 ift38-1 cells with mother cell wall being removed by autolysin treatment or wild-type cells were primarily fixed with 2% glutaraldehyde to a final concentration of 1% for 15 min at room temperature. The cells were collected by centrifugation for 3 min at 2,000 rpm on a table-top centrifuge (Eppendorf 5417R), and were fixed in a secondary fixative (1% glutaraldehyde in 100 mM sodium cacodylate, pH 7.2) for 1–2 h at room temperature. The cells were harvested by centrifugation, washed with 100 mM sodium cacodylate, pH 7.2 three times 15 min each time. The cells were transferred to a new eppendorf tube in 100 mM sodium cacodylate, pH 7.2. After centrifugation, 200 μL pre-warmed low-melting agarose was mixed with the cell pellet. The solidified cell/agarose was removed from the tube, cut into ~1 mm³ pieces and submerged in 100 mM sodium cacodylate, pH 7.2.
were further processed by the Center of Biomedical analysis, Tsinghua University. Cells were imaged on an electron microscope (H-7650B; Hitachi Limited) equipped with a digital camera (ATM Company).

Living imaging of IFT by total internal reflection fluorescence (TIRF) microscopy
Previous protocols were followed.69,70 Briefly, fresh cells were placed to a home-made 8-well observation chamber pre-coated with 0.1% poly-L-lysine, then imaged on an Olympus microscope (IX83) with a 100x TIRF objective (N.A. 1.49) and a cooled Photometrics Prime 95B camera. Images were captured at 10 fps, and analyzed with NIS-Elements Advanced Research software (Nikon, Japan). Kymographs were assembled from the obtained data using Fiji (NIH, USA) and the velocity and frequency of the IFT train were calculated as reported.

QUANTIFICATION AND STATISTICAL ANALYSIS
All the data were independently verified two or more times. Data were presented as mean ± SD. Statistical significance was performed by using unpaired two-tailed Student’s t test analysis. p < 0.01 was considered to be statistically significant. *, p < 0.01; **, p < 0.001; ***, p < 0.0001.