TALPID3 and ANKRD26 selectively orchestrate FBF1 localization and cilia gating

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Transition fibers (TFs) regulate cilia gating and make the primary cilium a distinct functional entity. However, molecular insights into the biogenesis of a functional cilia gate remain elusive. In a forward genetic screen in Caenorhabditis elegans, we uncover that TALP-3, a homolog of the Joubert syndrome protein TALPID3, is a TF-associated component. Genetic analysis reveals that TALP-3 coordinates with ANKR-26, the homolog of ANKRD26, to orchestrate proper cilia gating. Mechanistically, TALP-3 and ANKR-26 form a complex with key gating component DYF-19, the homolog of FBF1. Co-depletion of TALP-3 and ANKR-26 specifically impairs the recruitment of DYF-19 to TFs. Interestingly, in mammalian cells, TALPID3 and ANKRD26 also play a conserved role in coordinating the recruitment of FBF1 to TFs. We thus report a conserved protein module that specifically regulates the functional component of the ciliary gate and suggest a correlation between defective gating and ciliopathy pathogenesis.
Cilia are microtubule-based subcellular organelles that arise from the basal body (derived from the mother centriole) and protrude from the surfaces of most eukaryotic cells\(^1,2\). Intracellular transport (IFT) machinery, which consists of IFT-A and IFT-B subcomplexes and mediates the bidirectional movement of IFT cargos along the axoneme, is required for the biogenesis and maintenance of all cilia across species\(^3,4\). Based on their motility, cilia are divided into motile cilia and immotile cilia (also called primary cilia). Motile cilia function as motile devices to propel cells or generate flow across cell surfaces\(^5\). Primary cilia act as cellular “antennae”, and participate in responses to various environmental stimuli (thermal, mechanical, or chemical) and various signal transduction pathways critical for the normal development and homeostasis of organs, including the sonic Hedgehog (Shh), Wnt, and various G-protein-coupled receptor signaling pathways\(^6-10\). Consistent with the ubiquitous presence of the cilia in most cell types in the human body, cilia dysfunctions cause dozens of syndromic disorders, such as Joubert syndrome (JBS), Meckel syndrome (MKS), nephronophthisis (NPHP), Bardet–Biedl syndrome (BBS), collectively termed ciliopathies\(^11,12\).

As a unique cellular organelle that is not membrane-enclosed, like the Golgi or lysosome, the primary cilium needs a stringent gating mechanism at the ciliary base to control the trafficking of various membrane and soluble proteins between the cytoplasm and ciliary compartment\(^13-16\). Transition fibers (TFs), which originate from transformation of the distal appendages (DAs) of the mother centriole during ciliogenesis, anchor the basal body to the membrane and represent the boundary between the apical membrane and the ciliary membrane\(^2,17\). TFs, together with the adjacent transition zone (TZ, the proximal part of the axoneme that contains highly organized Y-links), have been suggested to be key subdomains of the proposed ciliary gate\(^18,19\). Consistent with the proposed importance of TFs in the context of cilia, mutations in TF structural components CEP164, SLT1, and CEP83 or TF-related proteins OFD1 and C2CD3 have been linked to various ciliopathies\(^20-24\). We previously reported that FBF1 specifically localizes to TFs, where it acts as a functional component of the ciliary gate\(^25\). The fact that among the first six TF components (CEP164, CEP83, CEP89, SLT1, FBF1, and LRRRC45) identified\(^25-32\), FBF1 is the only one conserved from C. elegans to humans suggests that the FBF1 pathway likely represents a central part of TF-regulated cilia gating. Thus, understanding how the FBF1 pathway is regulated will reveal more key players of the ciliary gate.

Human KIAA0586, also known as TALPID3, is a ciliopathy protein. The TALPID3 gene was originally identified in an embryonic lethal mutant of chicken with defective Shh signaling\(^33-35\). Subsequent studies revealed that TALPID3 encodes a conserved centriole distal-end protein and that its roles in ciliogenesis are conserved across vertebrates, including zebrafish, mice, and humans\(^36-38\). Recently, mutations in TALPID3 were found to cause the ciliopathy JBS\(^39-43\). It has been postulated that TALPID3 regulates the removal of daughter centriole-specific/enriched proteins (DCPs) and promote basal body docking and ciliary vesicle formation\(^36,42,44-47\).

In a whole-genome genetic screen of C. elegans mutants with disrupted ciliogenesis, we retrieved and cloned talp-3, a homolog of mammalian TALPID3. In C. elegans, TALP-3 colocalizes with DYS-19 (a homolog of the TF protein FBF1) and ANKR-26 (a C-terminal homolog of the TF protein ANKRD26) at the basal body. Although single talp-3 or ankr-26 mutants have subtle or no defects in cilia formation and gating, talp-3; ankr-26 double mutants show severely disrupted ciliogenesis and cilia gating. Remarkably, co-depletion of TALP-3 and ANKR-26 completely abolishes the recruitment of DYS-19 to TFs. We further discovered that TALP-3, ANKR-26, and DYS-19 associate in vitro and in vivo. Depletion of TALP-3 or ANKR-26 alone could compromise the in vivo association of the remaining two proteins. Furthermore, we show that human TALPID3 and ANKRD26 share conserved functions with their worm counterparts in orchestrating FBF1 recruitment, ciliogenesis, and cilia gating. Collectively, our findings demonstrate that a highly conserved functional module containing TALPID3-ANKRD26-FBF1 is essential for the proper formation of a functional cilia gate.

### Results

**TALP-3 is a TF-associated protein involved in ciliogenesis.** Among all identified TF components so far, only FBF1 and ANKRD26 are evolutionarily conserved between C. elegans and humans\(^22,48\). DYS-19 is the C. elegans homolog of FBF1, which plays an essential role in cilia gating\(^25\). ANK-26 is encoded by k10g6.4 and is homologous to human ANKRD26 C-terminus, which alone is sufficient for TF localization of human ANKRD26 (Supplementary Fig. 1a–d). More conserved TF or TF-associated components may await identification. We previously performed a genome-wide ethyl methanesulfonate (EMS) mutagenesis screen in C. elegans to search for mutant nematodes with ciliogenesis defects\(^39\). In C. elegans, mutants with abnormal ciliogenesis cannot take up fluorescent dye and are thus called dye-filling defective (Dyf)\(^50\). We retrieved hundreds of Dyf alleles, and have been actively mapping the causal loci. One allele, jhus11, was mapped to y57g11c.32 (Fig. 1a; Supplementary Fig. 2a). Protein blast homolog searches against the mouse database revealed that the Y57G11C.32 protein contains a region homologous to the highly conserved region of mouse TALPID3\(^38\) (Supplementary Fig. 1b, c). Based on sequence similarity (Supplementary Fig. 2b–d), subcellular localization, and functional data (see below), we believe that the Y57G11C.32 protein is homologous to mammalian TALPID3; hereafter, we refer to y57g11c.32 as talp-3. jhus11 is a G-A point mutation that alters the splicing donor site of the 2nd intron of the talp-3 gene and creates a putative null allele that encodes a truncated TALP-3 protein with the majority of its amino acid sequence deleted (Supplementary Fig. 2a, e). Surprisingly, contrary to the assumed importance of TALPID3 in mammalian ciliogenesis\(^46\), talp-3 (jhus11) mutants showed only mild ciliogenesis defects with ~20% amphid cilia and ~40% phasmid cilia shortened (Fig. 1b, c) and a subtle reduction in the ciliary IFT machinery (Fig. 1d), suggesting functional redundancy for TALP-3 in the context of cilia. Introduction of the wild-type talp-3 gene rescued the ciliogenesis defect of the jhus11 allele (Fig. 1c). talp-3 (tm78833), an independent allele obtained from the Japanese National BioResource Project (NBRP), encodes a truncated TALP-3, showing similarly mild ciliogenesis defects (Supplementary Fig. 2a, f–h).

Mammalian TALPID3 localizes to the distal end of centrioles\(^47\). Mutations in TALPID3 lead to defective ciliogenesis invertebrates\(^36-38\) and cause human JBS\(^39-43\). Promoter expression analysis demonstrated that talp-3 is exclusively expressed in ciliated cells in C. elegans (Supplementary Fig. 3a). Consistent with the localization of TALPID3 on the distal end of the centriole in mammalian cells, TALP-3::GFP was found immediately below the TZ marker MKS-5, colocalized with the TF markers DYS-19 and ANKR-26, and partially overlapped with the TF-adjacent protein GASR-8 (a putative homolog of human GAS8) (Fig. 1e; Supplementary Fig. 3b). Interestingly, the highly conserved region in TALP-3 is required for its localization at basal bodies and for its function in ciliogenesis (Supplementary Fig. 3c, d). These results indicate that TALP-3 is located and functions specifically on TFs in C. elegans.
HYLS-1 regulates the localization of TALP-3 to TFs. We previously showed that HYLS-1, the ortholog of hydrolethalus syndrome protein 1, is required for TF integrity in *C. elegans* [48]. The identified worm TF components DYF-19 and ANKR-26 and the TF-adjacent protein GASR-8 lost their ability to target the cilia base in *hyls-1* mutants [48]. Accordingly, GFP-tagged TALP-3 lost its specific enrichment in TFs in *hyls-1* mutants, and was dispersed at the periciliary membrane compartment (PCMC), a
subcellular compartment below TFs\(^5\) (Fig. 1f). In contrast, loss of GASR-8, ANKR-26, or DYF-19 did not perturb the localization of TALP-3 (Supplementary Fig. 3c). Notably, TALP-3 deficiency did not affect the localization of ANKR-26 and DYF-19 (Supplementary Fig. 3d), suggesting that although TALP-3 is an exclusive TF-associated protein, it is dispensable for the structural integrity of TFs.

**TALP-3 and ANKR-26 orchestrate cilia gating for IFT proteins.** Considering the mild ciliogenesis defects observed in mutants of both *talp-3* alleles, we hypothesized that another TF protein complements the role of TALP-3 in ciliogenesis. We thus explored the genetic interaction between *talp-3* and other TF genes *dyf-19* and *ankr-26*. Intriguingly, *talp-3; ankr-26* double mutants, but not *talp-3; dyf-19* double mutants, showed synergetic defects in ciliogenesis (Fig. 2a, b; Supplementary Fig. 4a–c). The *ankr-26* single mutant showed completely normal ciliogenesis, and the *talp-3* single mutant had slight defective ciliogenesis; however, all cilia in *talp-3; ankr-26* double mutants were severely truncated, as demonstrated by both the IFT marker OSM-6::GFP and the axonemal tubulin marker TBB-4::mCherry (Fig. 2a, b). Consistent with these results, transmission electron microscopy (TEM) analysis further confirmed that the cilia in *talp-3; ankr-26* double mutants lacked distal segments, but contained partial middle segments (Supplementary Fig. 4b, c). These results suggest that TALP-3 genetically interacts with ANKR-26 to orchestrate ciliogenesis.

Interestingly, we observed that the IFT-B component OSM-6 tended to accumulate at the tips of truncated cilia in *talp-3; ankr-26* double-mutant worms (Fig. 2a; Supplementary Fig. 4d). Gating defects in *dyf-19* mutants lead to defects in the ciliary import of most IFT components, except for IFT-B components, which abnormally accumulate at the tip of truncated cilia\(^25\). Considering the exclusive localization of TALP-3 and ANKR-26 on TFs, we hypothesized that *talp-3* and *ankr-26* genetically interact to regulate the DYF-19 pathway in cilia gating. To address this hypothesis, we introduced a battery of IFT markers into *talp-3* single, *ankr-26* single, and *talp-3; ankr-26* double mutants, and examined their localization. All IFT components examined showed either normal ciliary signal in *ankr-26* single mutants or slightly reduced cilia signal in *talp-3* single mutants (Fig. 2c).

Of note, in *talp-3; ankr-26* double mutants, the IFT-B components OSM-5/IFT88 and IFT-20 also tended to accumulate at the tip of residual cilia (Supplementary Fig. 4d), whereas the IFT-A-associated kinesin-II motor KAP-1, the IFT-A component CHE-11/IFT140, the IFT retrograde motor dynein light chain XBX-1, and the BBSome component BBS-7 were restricted below the ciliary base and failed to enter the cilia (Fig. 2c; Supplementary Fig. 4e). These observations indicated that *talp-3; ankr-26* double mutants completely recapitulate the phenotypes of *dyf-19* mutants, supporting the conclusion that TALP-3 and ANKR-26 function on TFs to orchestrate the proper gating of IFT machinery and control ciliogenesis.

**The TALP-3-ANKR-26 module recruits DYF-19 to TFs.** Interestingly, *talp-3; ankr-26* double mutant exhibits gating defects similar to those observed in *dyf-19* single mutants\(^25\). We thus aimed to explore the functional relationship between DYF-19 and TALP-3/ANKR-26. The localization of TALP-3 or ANKR-26 was not disrupted in *dyf-19* mutant cilia (Supplementary Fig. 4f). In contrast, GFP-tagged DYF-19 completely lost its ability to target TFs in *talp-3; ankr-26* double mutants (Fig. 3a, b), but not in *talp-3* or *ankr-26* single mutants. This suggests that TALP-3 and ANKR-26 cooperate to recruit DYF-19 to TFs to constitute a functional cilia gate. Since these three proteins exhibit similar localizations in *C. elegans* cilia, we speculated that they physically associate with each other. Indeed, GST pull-down assays showed that TALP-3, ANKR-26, and DYF-19 directly interact with each other (Fig. 3c–e). Specifically, the C-terminus of TALP-3 directly interacts with ANKR-26 (Fig. 3c), whereas its N-terminus binds DYF-19 (Fig. 3d).

**TALP-3, ANKR-26, and DYF-19 associate on TFs in vivo.** To determine whether TALP-3, ANKR-26, and DYF-19 form a complex in vivo, we employed the bimolecular fluorescence complementation (BiFC) assay, which directly visualizes the interactions of proteins in the same macromolecular complex in their natural environment\(^35\). As expected, strong fluorescence complementation among TALP-3, ANKR-26, and DYF-19 was observed specifically on TFs in live animals (Fig. 3f). Notably, TALP-3 and ANKR-26 BiFC signals could be observed in the periciliary membrane compartment below TFs (Fig. 3f), suggesting that they likely form a complex before being targeted to TFs. The TALP-3-ANKR-26 association did not require the presence of DYF-19, whereas depletion of TALP-3 or ANKR-26 partially affected the stability of the complex formed by the remaining two components (Fig. 4a–c). Consistent with the finding that *hyls-1* is required for TF formation, all BiFC signals disappeared in *hyls-1* mutants (Supplementary Fig. 5). Collectively, our data suggest that TALP-3 and ANKR-26 first form a complex and then localize to TFs, where they recruit DYF-19 to form the TALP-3-ANKR-26-DYF-19 module, which primes TFs into a functional ciliary gate for both membrane and soluble proteins. Interestingly, although TALP-3 and ANKR-26 do not have similar protein domains or structures, they show functional redundancy in recruiting DYF-19 to TFs.

**A conserved TALPID3-ANKRD26-FBF1 module in mammalian cilia.** Human TALPID3 mutations cause JBTS, and *Talpid3*\(^−/−\) mice show typical ciliopathy phenotypes\(^36\). *Ankrd26*\(^−/−\) mice show defective cilia signaling and develop obesity, a manifestation associated with ciliopathies\(^38\). Gaining mechanistic insights into the function of mammalian TALPID3.
and ANKRD26 would be critical for understanding the pathogenesis underlying corresponding ciliopathies. We first investigated whether TALPID3 and ANKRD26 show any correlation with TFs in mammalian cilia. Both endogenous TALPID3 and overexpressed TALPID3 strongly labeled both centrioles, and ANKRD26 specifically labeled mother centrioles and basal bodies (Fig. 5a, b). To accurately define their localization on the centriole, we performed super-resolution structured illumination (SIM) microscopy. By co-labeling with the subdistal marker ODF2, we confirmed that TALPID3 and ANKRD26 localize above subdistal appendages and on the same focal plane as TFs (Fig. 5c). TALPID3 localizes to the distal end of both mother and
daughter centrioles, where it forms a “rim” around the centriolar barrel close to the distal appendages. In agreement with this, by using an antibody specifically recognizing the C-terminus of endogenous TALPID3, we observed a “ring” with an ~350 nm diameter that was smaller than the ~450 nm “ring” formed by FBFI. By using FBFI as a TF marker, we confirmed that both endogenous ANKRD26 and overexpressed Flag-tagged ANKRD26 exclusively labeled TFs (Fig. 5a–d), in agreement with very recent data. SIM microscopy revealed that ANKRD26 forms a ring with a diameter of ~450 nm on TFs similar to the ring formed by FBFI (Fig. 5d). As expected, ANKRD26, but not TALPID3, lost its localization at the cilia base in CEP83-deficient cells, in which distal appendage formation was disrupted (Fig. 5e). We conclude that ANKRD26 and FBFI completely colocalize on TFs, whereas TALPID3 localizes more proximal to the centriolar wall (Fig. 5f). Notably, from our SIM analysis, although the mean diameter of TALPID3 rings (~350 nm) was smaller than that of FBFI or ANKRD26 rings (~450 nm), the rings partially overlapped (Fig. 5d). Interestingly, recent high-resolution characterization of DA organization by stochastic optical reconstruction microscopy revealed that ANKRD26 forms a toroid with an inner diameter ~314 nm and an outer diameter of ~578 nm, but FBFI forms a toroid with inner and outer diameters of ~269 and ~496 nm, respectively, supporting the notion that TALPID3 partially overlaps FBFI and ANKRD26. As expected, endogenous communoprecipitation experiments and in vitro GST pull-down assays confirmed that TALPID3, ANKRD26, and FBFI associate with each other in mammals (Fig. 5g; Supplementary Fig. 6).

TALPID3 and ANKRD26 coordinate FBFI centrosomal recruitment. To assess the function of TALPID3 and ANKRD26 in the context of cilia, we used siRNA to knockdown the TALPID3 and ANKRD26 genes in human retinal pigment epithelial (RPE) cells. Knockdown efficiencies were validated by both immunofluorescence staining and immunoblotting (Supplementary Fig. 7a–d). Similar to what was observed in C. elegans, depletion of TALPID3 led to truncated cilia and a reduced ciliation ratio, whereas depletion of ANKRD26 did not affect ciliogenesis (Fig. 6a). Consistently, co-depletion of TALPID3 and ANKRD26 indeed exacerbated ciliogenesis defects, compromised ciliary import of the IFT component IFT140 (Fig. 6a), and significantly reduced FBFI intensity on TFs (Fig. 6b). We also examined newly synthesized DAs on daughter centrioles. During the cell cycle, the daughter centriole transforms into a new mother centriole and assembles distal and subdistal appendages. FBFI and other DA proteins are recruited to newly synthesized DAs at this stage. By carefully examining dividing cells, we discovered that simultaneous depletion of TALPID3 and ANKRD26, but not their individual depletion, significantly disrupted FBFI recruitment to the newly formed mother centriole (Fig. 6c). These data indicate that TALPID3 and ANKRD26 indeed coordinate the recruitment of FBFI to the distal appendages of newly formed mother centrioles. Once FBFI is associated with DAs/TFs, it is likely very stable and does not require TALPID3 and ANKRD26 to maintain its DA/TF localization. Remarkably, none of the other examined DA components (CEP164, CEP89, and SCLT1), proteins localized to the distal part of the mother centriole (ODF2), or TZ components (TCTN1 and CEP290) were affected by co-depletion of TALPID3 and ANKRD26 (Fig. 6b, c; Supplementary Fig. 8a, b). These results demonstrate that TALPID3 and ANKRD26 specifically regulate the recruitment of FBFI, but do not affect the overall integrity of DAs/TFs, the TZ, and the distal centriole.

**TALPID3 and ANKRD26 orchestrate cilia entry of receptors.** Various signaling receptors are anchored on the cilia surface, a spatial arrangement that is crucial for the function of cilia as sensory organelles. Although much is known about the critical role of cilia in sensory transduction, little is known about the mechanisms of the proper ciliary import of sensory receptors. For example, the localization mechanism of polycystins mutated in the most common monogenic human disease, autosomal poly-cystic kidney disease (ADPKD), remains poorly understood. Upon TALPID3 knockdown, there was a subtle reduction in the percentage of cells with positive PKD2 signal on cilia, while the intensity of PKD2 on cilia decreased about 50%. ANKRD26 deficiency had no effect on either the ratio of PKD2-positive cells or the ciliary intensity of endogenous PKD2. Co-depletion of TALPID3 and ANKRD26 dramatically decreased the ciliary entry of PKD2 (Fig. 7a). Similarly, the ciliary localization of endogenous Smoothened (Smo) receptor or the signaling molecule Gli3 was also compromised upon co-depletion of TALPID3 and ANKRD26, but not affected by the depletion of either of the two genes (Fig. 7b, c). Collectively, these data indicate that the essential role of the TALPID3-ANKRD26 module in regulating the cilia gating for sensory receptors and signaling molecules is highly conserved from C. elegans to humans.

**Discussion.** TFs anchor basal bodies to the apical membrane and constitute the first visible physical barrier between the cytoplasm and the ciliary lumen. TFs and the adjacent TZ are highly conserved subdomains across ciliated species, and have been proposed as central functional compartments of the proposed ciliary gate1,18,19. However, how the ciliary gate forms and controls selective cilia import/export remains unclear. Our previous studies suggested that the TF protein FBFI is a central player in cilia gating25. Here, by using both the C. elegans genetic model and mammalian cells, we uncovered that the ciliopathy protein
**Fig. 3 TALP-3 and ANKR-26 cooperate to recruit the transition fiber protein DYF-19.**

**a** Localization of the transition fiber protein DYF-19 to the basal body is disrupted in the *talp-3; ankr-26* double mutant. **b** Quantification of relative DYF-19 fluorescence intensity in WT, *talp-3* single mutant, *ankr-26* single-mutant, and *talp-2; ankr-26* double-mutant worms. Data are presented as the mean value ± s.d. *n* = 36. **,** P < 0.001 by two-tailed unpaired Student’s *t*-test. **c** TALP-3 directly interacts with ANKR-26. A GST pulldown assay was used to detect the interaction between His-fused ANKR-26 and GST-fused full-length TALP-3 and truncated TALP-3 constructs (N-terminus, amino acids (aa) 1–450; C-terminus, aa 354 to the C-terminal end). Upper panel, blotted with anti-His antibody; lower panel, loading of the GST and GST-TALP-3 proteins shown by Ponceau S staining. **d** TALP-3 directly interacts with DYF-19 in a GST pull-down assay. **e** ANKR-26 directly interacts with DYF-19 in vitro. **f** TALP-3, ANKR-26 and DYF-19 interact in vivo on TFs. The BiFC assay was performed to visualize the in vivo interaction between TALP-3, ANKR-26, and DYF-19 in living worms. Stable fluorescence complementation between the ANKR-26::VN and TALP-3::VC pair, the ANKR-26::VN and DYF-19::VC pair, and the TALP-3::VN and DYF-19::VC pair was observed on TFs. Notably, strong BiFC signals for ANKR-26::VN and TALP-3::VC are observed in the PCMC region. Scale bars = 1 μm. Source data are provided as a Source Data file.
TALPID3 associates with TFs and characterized ANKRD26 as a TF component. In both *C. elegans* and human cilia, TALPID3/TALP-3, ANKRD26/ANKR-26, and FBF1/DYF-19 physically associate with each other to presumably form a TF-specific protein module. The pathway by which TALPID3 coordinates ANKRD26 to promote the TF recruitment of FBF1 and the proper formation of ciliary gate for either soluble or membrane proteins is highly conserved from *C. elegans* to humans. Notably, the TALPID3-ANKRD26-FBF1 protein module specifically localizes on TFs, but not on the TZ, and is essential for the selective import of both membrane and soluble cilia cargoes. Simultaneous depletion of TALPID3 and ANKRD26 or depletion of FBF1 alone, disrupt cilia gating but not affect the overall structure of either TFs or the TZ (Fig. 7d). These data emphasize...

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**Fig. 4** In vivo interactions among TALP-3, ANKR-26 and DYF-19 in the corresponding mutants. **a** BiFC signal between ANKR-26::VN and TALP-3::VC in amphid cilia and phasmid cilia and relative fluorescence intensity in WT and dyf-19 mutant worms. Stable fluorescence complementation between ANKR-26::VN and TALP-3::VC is observed in WT worms, and the signal is not impaired in dyf-19 mutants. **b** BiFC signal between ANKR-26::VN and DYF-19::VC in amphid cilia and phasmid cilia and the relative fluorescence intensity in WT and talp-3 mutant worms. Stable fluorescence complementation between ANKR-26::VN and DYF-19::VC is observed in WT worms, and the BiFC fluorescence intensity is dramatically decreased in the talp-3 mutant, suggesting that TALP-3 is required for the proper spatial association between ANKR-26 and DYF-19. **c** BiFC signal between TALP-3::VN and DYF-19::VC in amphid and phasmid cilia, and the relative fluorescence intensity in WT and ankr-26 mutant worms. Stable fluorescence complementation between TALP-3::VN and DYF-19::VC is observed, and the BiFC fluorescence intensity is decreased in the ankr-26 mutant, suggesting that ANKR-26 also affects the proper spatial association between TALP-3 and DYF-19 in vivo. All data are presented as the mean ± s.d. Numbers of cilia analyzed are indicated in the bars. NS, *P* > 0.05; ***, *P* < 0.001 by two-tailed unpaired Student’s *t* test. Scale bars = 5 μm. Source data are provided as a Source Data file.
the notion that the TALPID3-ANKRD26-FBF1 module is likely the central functional component of the ciliary gate, and support the idea that TFs, independent of the TZ, could constitute a functional gate for both membrane and soluble proteins\cite{13,18,19}.

ANKRD26 was characterized as a centrosome protein required for the cilia import of signaling molecules/receptors in the central nervous system\cite{56}. Our discoveries that ANKRD26 colocalizes with FBF1 and coordinates with TALPID3 to form a functional cilia gate may explain the pathogenesis of mutant phenotypes observed in \textit{Ankrd26}−/− mice. Mutations in ANKRD26 have been linked to thrombocytopenia and myeloid malignancies in humans\cite{59-61}. Although primary cilia were believed to be absent from hematopoietic cells based on conjecture, IFT proteins have been implicated as having important roles in immune cells\cite{62,63}. 
proteins (DCPs), a prerequisite for DA assembly, centriole docking32,65, FBF1 is dispensable for ciliogenesis initiation but SCLT1, and CEP89, which are essential for basal body maturation since TALPID3 might not evolve as a central player in centriole formation, and ciliogenesis. From an evolutionary perspective, to support its postulated functions in centriole maturation, DA proteins across ciliated species and their tight correlation with ciliopathies suggest that FBF1-related TF is likely the most important subdomain required for cilia gating. Future studies of conserved TF or TF-associated proteins in C. elegans have great potential to provide insights into the core function of TFs in the context of cilia and ciliopathies.

Methods

C. elegans strains. All worm strains used in this study are listed in Supplementary Table 1. Standard procedures for the culture and maintenance of C. elegans were used67. Transgenic animals were generated by microinjection. Standard genetic crossing was used to introduce reporter transgenes from wild-type worms into mutant worms. Polymerase chain reaction (PCR) or sequencing was used to generate mutant genotypes. Primers used are listed in Supplementary Table 2. dyf-19 (jhu455) was used as described before25. gasr-8 (gk1001), and k106k4 (gk567) were obtained from the Japanese National Bioresource Project (NBRP). We isolated the jha511 mutant during an EMS screen for Dyf mutants as described49. The mutation was mapped to chromosome IV using standard single-nucleotide polymorphism mapping techniques. Sequencing of jha511 mutants identified a G to A mutation at the 2nd intron donor site in Y57G11C.32, and ciliary phenotypes of the jha511 mutant were rescued upon injection of the Y57G11.32 CDS. Before phenotypic analyses, the jha511 mutant was outcrossed six times to the wild-type (N2) strain.

Dye-filling assay. Worms were washed with M9 buffer, and then incubated in the fluorescent lipophilic carbocyanine dye DiI (Sigma-Aldrich, 24364) for 2 h at room temperature. DiI was prepared as a 2 mg/ml stock solution in DMSO and then diluted 1:200 in M9 buffer for use. After incubation with DiI, the worms were washed three times with M9 buffer, and then observed using a fluorescence microscope. Dye filling in amphid was observed with a Nikon SMZ18 stereomicroscope, and dye filling in phasmid was scored under a Nikon Eclipse Ti microscope with a Plan Apochromat ×100 1.49 numerical aperture oil-immersion objective.

Microscopy and imaging. Worms were mounted onto 5% agarose pads and anesthetized using 20 mM levamisole. Images were acquired using either an fluorescent microscope (Nikon Eclipse Ti or TE 2000-U) with a Plan Apochromat ×1.49 oil objective or Olympus FV1000s confocal microscope.

For centrosome staining, cells were fixed with methanol at −20 °C. For cilia staining, cells were fixed with paraformaldehyde at room temperature for 20 min and then permeabilized in 0.2% Triton X-100 for 10 min. After fixation, cells were blocked in 3% BSA and treated with the indicated antibodies. Images were acquired using a Nikon TE2000-U. Three-dimensional structured illumination microscopy (3D-SIM) was performed following a standard protocol.

GST pull-down assay. The pET28a and pGEX-4T-1 vectors were used as backbone to construct plasmids for His- and GST-tagged protein expression, respectively. Primers used are listed in Supplementary Table 2. His- and GST-tagged recombinant proteins were expressed in Escherichia coli strain BL21 (DE3), and purified by using Ni-Sepharose beads (GE Healthcare) and GST Sepharose beads (GE Healthcare), respectively. Purified GST or GST fusion protein was immobilized on glutathione Sepharose beads in binding buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 1 mM dithiothreitol, 10% glycerol, protease inhibitors),
and then incubated with His fusion protein for 4 h at 4 °C. After incubation, the beads were washed with binding buffer five times, loading buffer was added, and the beads were boiled for 10 min. The samples were then subjected to SDS-PAGE and analyzed by western blotting with monoclonal anti-His antibody.

Bimolecular fluorescence complementation (BiFC) assay. The Venus-based BiFC assay was used to examine protein interactions in living worms as described. To detect the interactions between TALP-3, ANKR-26 and DYF-19, the following BiFC pairs were used: TALP-3:VC155 and ANKR-26:VN173, DYF-19:VC155 and TALP-3:VN173, and DYF-19:VC155 and ANKR-26:VN173. The paired BiFC plasmids were co-injected along with the co-injection marker pRF4 [rol-6 (su1006)] and the TZ marker MKS-5::mCherry into wild-type animals (a 15 ng/μL concentration for each BiFC plasmid and MKS-5 plasmid and a 100 ng/μL concentration for the pRF4 plasmid). Fluorescent signals were checked using a YFP filter.

Cell culture and siRNA. Human retinal pigment epithelial (hTERT RPE-1) and human embryonic kidney (HEK293T) cells were obtained from American Type Culture Collection (ATCC). RPE cells were cultured in the DMEM/F12 medium and then incubated with His fusion protein for 4 h at 4 °C. After incubation, the beads were washed with binding buffer five times, loading buffer was added, and the beads were boiled for 10 min. The samples were then subjected to SDS-PAGE and analyzed by western blotting with monoclonal anti-His antibody.

Bimolecular fluorescence complementation (BiFC) assay. The Venus-based BiFC assay was used to examine protein interactions in living worms as described. To detect the interactions between TALP-3, ANKR-26 and DYF-19, the following BiFC pairs were used: TALP-3:VC155 and ANKR-26:VN173, DYF-19:VC155 and TALP-3:VN173, and DYF-19:VC155 and ANKR-26:VN173. The paired BiFC plasmids were co-injected along with the co-injection marker pRF4 [rol-6 (su1006)] and the TZ marker MKS-5::mCherry into wild-type animals (a 15 ng/μL concentration for each BiFC plasmid and MKS-5 plasmid and a 100 ng/μL concentration for the pRF4 plasmid). Fluorescent signals were checked using a YFP filter.

Cell culture and siRNA. Human retinal pigment epithelial (hTERT RPE-1) and human embryonic kidney (HEK293T) cells were obtained from American Type Culture Collection (ATCC). RPE cells were cultured in the DMEM/F12 medium
supplemented with 10% FBS, HEK293T cells were grown in DMEM containing 10% FBS. To induce cilia formation, RPE cells were starved in DMEM/F12 medium without FBS for 24 h.

All synthetic siRNAs were obtained from Invitrogen and transfected using Lipofectamine RNAiMAX (Invitrogen), according to the manufacturer’s instructions. The sequences of the siRNA used were as follows:

- siANKRD26-1: GAAUCAAGACUAUGAAUUUtt
- siANKRD26-2: CAAGGUUAAUGUACUACAtt
- siTALPID3-1: GUUAAAGGCACUAAGGUAAtt
- siTALPID3-2: GGGACUAGUUUGAAUGGAAtt.

Antibodies. The following primary antibodies were used: acetylated α-tubulin (T7451, diluted 1:2000 for immunofluorescence, Sigma), γ-tubulin (T6557, Sigma), FLAG (F1804, Sigma), HA (H3663, Sigma), CEP83 (HPA038161, Sigma), CEP164

(1:1) supplemented with 10% FBS; HEK293T cells were grown in DMEM containing 10% FBS. To induce cilia formation, RPE cells were starved in DMEM/F12 medium without FBS for 24 h.
Fig. 7 TALPID3 and ANKRD26 orchestrate the cilia gating for membrane receptors. The localizations of PKD2 and Shh signaling pathway-associated proteins were analyzed in control, TALPID3 single-knockdown, ANKRD26 single-knockdown and TALPID3 and ANKRD26 double-knockdown RPE cells. Cells were examined by IF staining after 24 h of serum starvation with the indicated antibodies. a Compared with WT, TALPID3 single-knockdown, and ANKRD26 single-knockdown cells, TALPID3 and ANKRD26 double-knockdown cells showed significantly reduced ciliary entry of PKD2. Right panel, both the percentages of cells with PKD2 ciliary signaling and the mean fluorescence intensity in each group were quantified. For PKD2-positive ratio, n = 300 cells over three independent experiments. For PKD2 intensity, numbers of cell examined are indicated above each dataset. b Cells treated without (+ SAG) or with (– SAG) SAG were compared to analyze Gli3 and Smothened (Smo) localization. c The percentages of cells with ciliary Gli3 and Smo were quantified. Results from a minimum of 100 cells in each group were averaged. The results from three independent experiments were statistically analyzed and plotted. n = 300 cells over three independent experiments. d A prospective model of the regulation of FBF1 by TALPID3 and ANKRD26. Recruitment of FBF1 to DAs/TFs or its stabilization on DAs/TFs requires coordination between the distal centriole wall and DA blades. Deletion of TALPID3 compromises the condition of the distal centriole wall, and deletion of ANKRD26 may affect the condition of the DA blade; therefore, co-depletion of TALPID3 and ANKRD26 exacerbates defects in ciliary localization. All data are presented as the mean ± s.d. Significant differences were identified by two-tailed unpaired Student’s t test. NS, P > 0.05; **, P < 0.001. No adjustments were made for multiple comparisons. Scale bars, 2 μm. Source data are provided as a Source Data file.
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Author contributions
Q.W. and J.H. initiate the project and designed experiments. H.Y. and C.C. designed and performed the experiments. H.Y., C.C., Q.W., and J.H. analyzed the data. H.Y., Q.W., and J.H. wrote the paper with the help of Y.H., H.C. and K.L.

Competing interests
The authors declare no competing interests.

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