Supplemental Materials

Molecular Biology of the Cell

Dai et al.

Supplementary figures

Fig. S1) Behavior of IC2-NG in oda3 flagella

A) Kymograms from two-color imaging of IC2-NG and IFT20-mC in the *oda3 oda6* ift20 background. Shown are the single kymograms,the merged kymogram, and the IFT20-mC kymogram with a tracing of the IC2-NG trajectories.

B) Kymograms showing the behavior of IC2-NG at the flagellar tip. Typically, IC2-NG paused only briefly at the flagella tip.

C) Kymograms showing the presence of two IC2-NG particles on the same IFT train.

Bars (A-C) = 2μm and 2s; arrowheads, anterograde IFT; arrows, retrograde IFT; asterisks, stationary phases.

Fig. S2) Intact ODAs have a low diffusional mobility

A) Compilation of kymograms comparing diffusion of tagged PLD (PLD-NG), end binding protein 1 (EB1-NG,), the radial spoke subunits RSP4 (RSP4-sfGFP) and IC2 (IC2-NG).PLD-NG is alipidated membrane-anchored protein predicted to be ~50 kD (including the NG), EB1-NG is predicted to form a dimer of ~100 kDa, RSP4-sfGFP is part of a 12S transport complex approximately corresponding to ~400 kD, and IC2-NG is part of the 20S ~2 MD ODAs. The dashed red line serves as a fiduciary mark to facilitate comparison of the lateral displacement of IC2-NG particles.

B) Comparison of IC2-NG diffusion in the *oda*mutants*oda2, oda3, oda8* and *oda16*. The diffusional mobility of IC2-NG in oda8 was variable; the mobility of the fast diffusing particles ranked below those observed in *oda2* but above those in control and *oda3.* Occasionally, very fast diffusing particles (arrowheads) were observed in both wild-type and the mutants; examples are shown in *oda3* and *oda16*. The high mobility of these particles suggests that they are smaller than those in *oda2* and probably represent IC2-NG alone or IC2-NG fragments. In panel A, the IC2-NG Kymograms are based on recordings at 5 fps, all other kymograms are based on 10 fps recordings. Arrowheads, IFT; arrows, very fast diffusion; asterisk, stationary IC2-NG. Bars = 2μm and 2s (for all panels).

Fig. S3) Analysis of IC2-NG in the transport mutants oda16 and ift46 IFT46ΔN

A, B) TIRF, BF and merged images of IC2-NG in *oda16oda6* mutants with full-length (A) and regenerating (B) flagella. Note that the distribution of IC2-NG in regenerating *oda16 oda6* IC2-NG flagella resembles that of *oda6* IC2-NG *ift46IFT46ΔN* flagella and *oda3* flagella during early rescue. Bar = 2 μm.

C) TIRF images of *ift46IFT46ΔNoda6* IC2-NG cells. Bar = 2 μm.

D) TIRF images of *oda16 ift46IFT46ΔNoda6* IC2-NG cells. Bar = 2 μm.

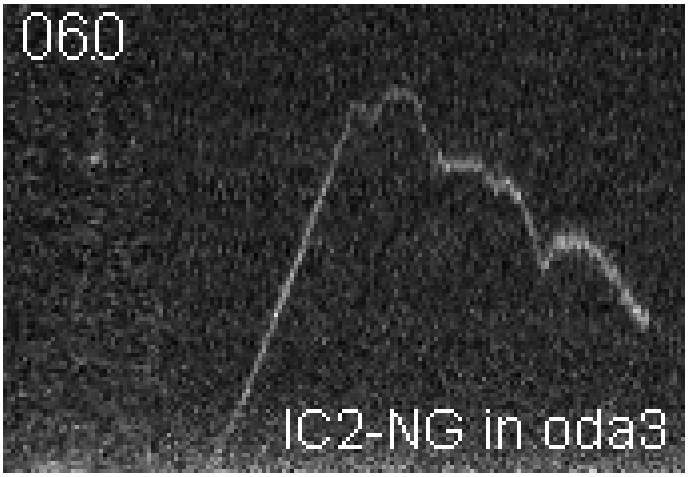
E) Western blot of flagella isolated from the strains indicated. For the IC2-NG quantification, the anti-IC2 signals were corrected for the anti-IFT81 signals. The faster migrating anti-IC2 positive band in the *oda6* IC2-NG flagella did not react with anti-NG.

F) Analysis of the presence and type of IC2-NG accumulation near the basal bodies in various strains. n, number of cells analyzed.

Fig. S4) Behavior of IC2-NG in oda mutants

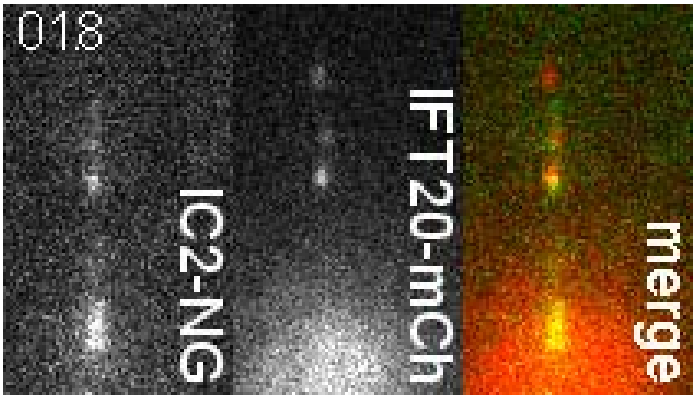
Compilation of kymograms showing the behavior of IC2-NG in wild-type (*oda6* IC2-NG) and *oda*mutant flagella. Kymograms of *oda6* IC2-NG, *oda6*, *oda3*, *oda8*, and *oda10* were acquired from zygotes. The flagellaof *oda6* x *oda6* IC2-NG zygotes were photobleached prior to document IC2-NG behavior in *oda6* IC2-NG-derived and in *oda6*-derived flagella. Kymograms of *oda2* and *oda16* were acquired from vegetative cells. Most of the kymograms are identical with the ones shown in the main figures. Bars = 2 μm and 2 s.

Supplementary videos



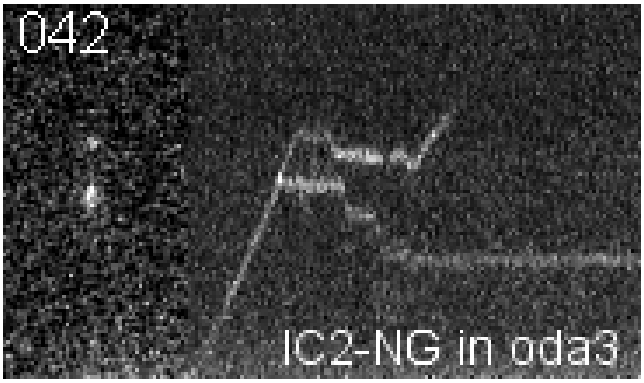
Video S1) IFT of IC2-NG in oda3 flagella

TIRF video and kymogram showing IFT transport and diffusion of IC2-NG in *oda3* flagella. Images were acquired at 10 fps and the timer counts seconds. The movie corresponds to Fig. 2A.



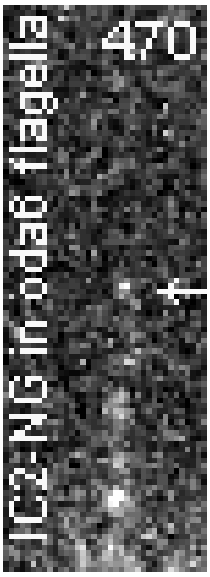
Video S2) IC2-NG co-migrates with IFT20-mC

Two-color TIRF video showing IC2-NG and IFT20-mC in the corresponding *oda6 ift20-1* double mutant background. Images were acquired at 10 fps and timer counts seconds.



Video S3) IFT trains carrying two IC2-NG particles

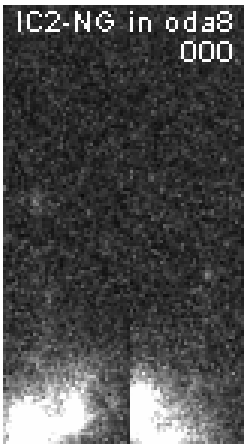
TIRF video and kymogram showing transport and unloading of IC2-NG from IFT. One of the two particles was photobleached during the recording. Images were acquired at 10 fps and the timer counts seconds. The movie corresponds to Fig. S3 C.



Video S4) Docking of IC2-NG particles in oda6 *flagella*

TIRF video showing transport, unloading and rapid immobilization of IC2-NG in an *oda6*-derived flagellum of an *oda6* x *oda6* IC2-NG zygote. The arrows mark the

positions of the immobilized IC2-NG particles. Images were acquired at 10 fps and the timer counts seconds. The movie corresponds to Fig. 3C top panel.



Video S5) Diffusion and IFT of IC2-NG in zygotic oda8 flagella.

TIRF video showing IC2-NG in two flagella of an *oda8 oda6* x *oda8 oda6* IC2-NG zygote. Note long-lasting diffusion of IC2-NG particles compared to Video S6 showing zygotic oda3 flagella. Images were acquired at 10 fps and the timer counts ss:mm. The movie corresponds to Fig. 5B.

Video S6) Transport of IC2-NG in flagella of an oda3 oda6 x oda3 oda6 IC2-NG zygote.

TIRF video showing IC2-NG behavior in the absence of the DC. Note particles cycling between IFT, diffusion, and transient docking. Images were acquired at 10 fps and the timer counts seconds. A corresponding kymogram is shown in Fig. S3B top row, right.

Figure S1 (1 column)

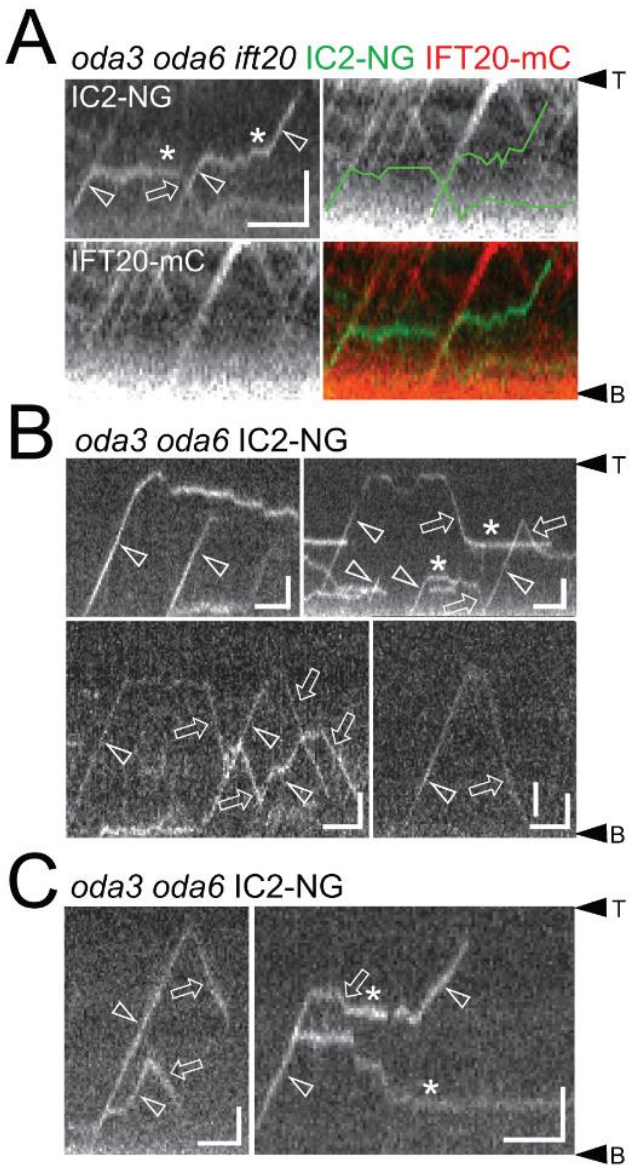


Figure S2 (2 columns)

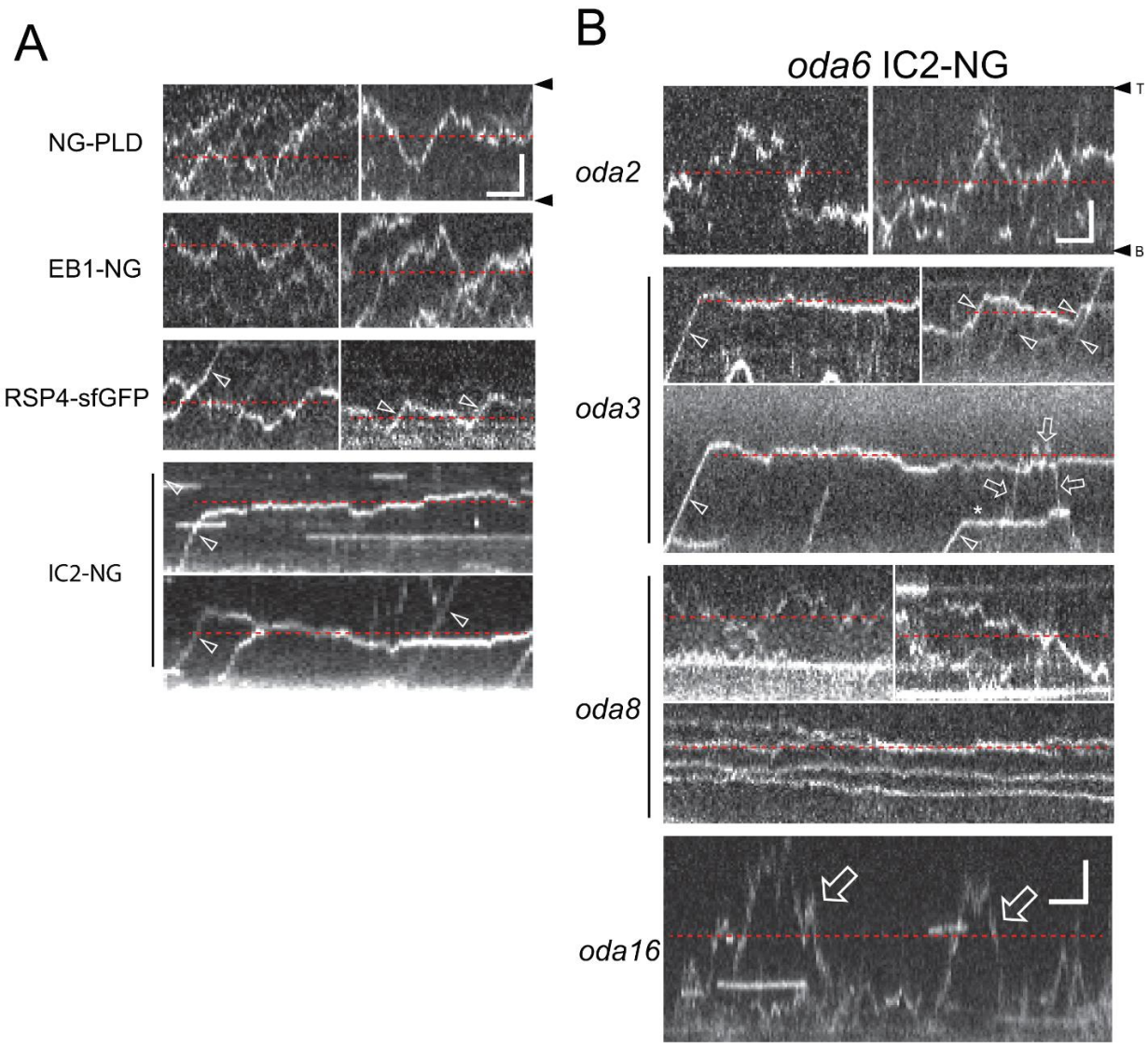


Figure S3 (2 columns)

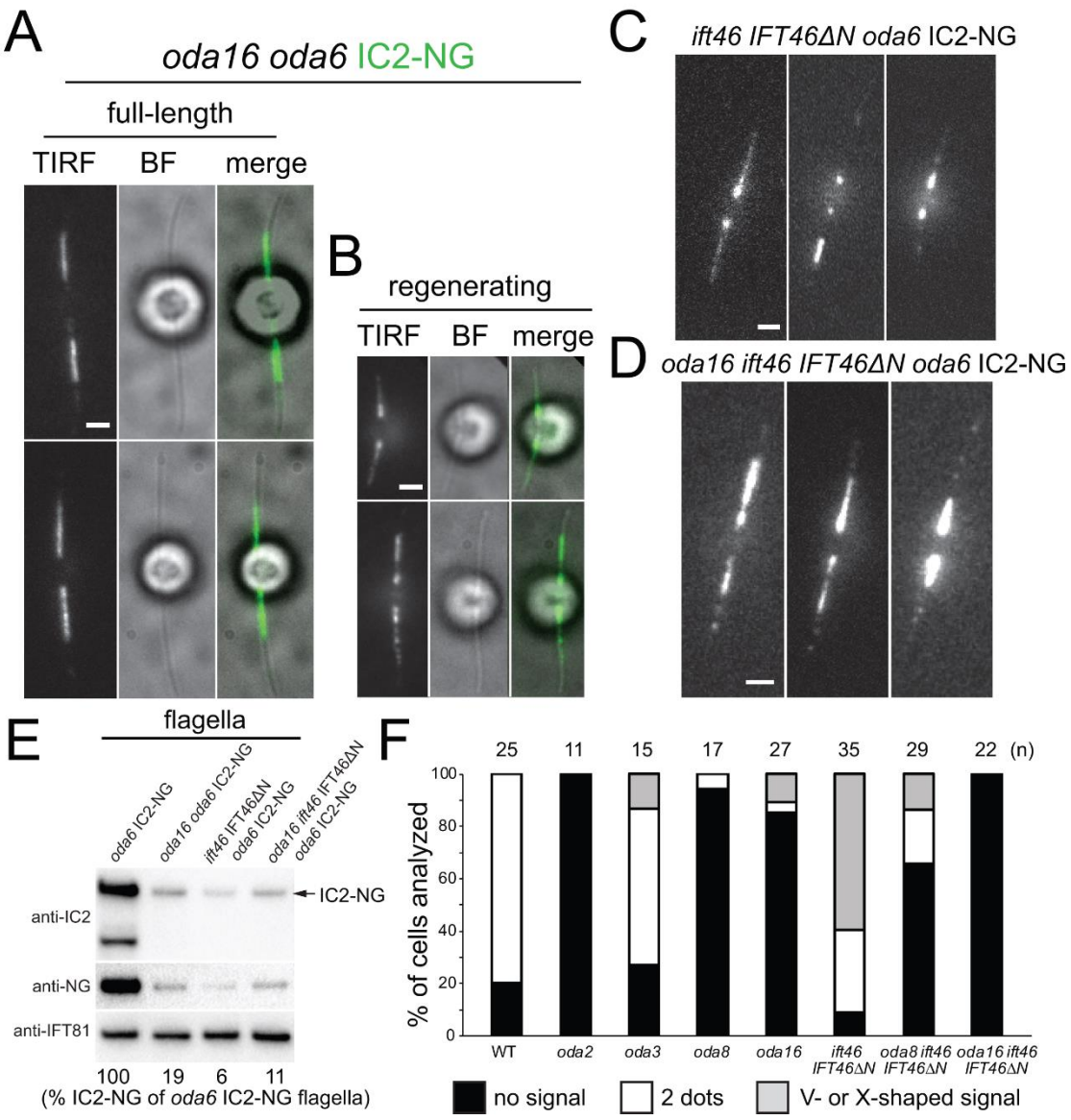


Figure S4 (1 column)