## **Supplementary materials**

Supplementary figures

Fig. S1

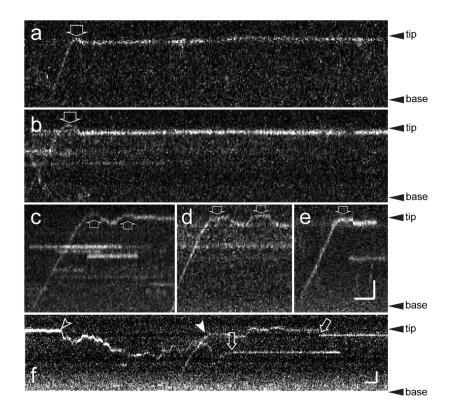


Figure S1) Behavior of RSP4-sfGFP in zygotic pf1-derived flagella

- a-e) Arrival of RSP4-sfGFP via IFT at the flagellar tip (open arrows). RSP4-sfGFP often dwelled for extended periods in a subdistal position near the tip.
- f) Putative docking events of RSP4-sfGFP (marked by open arrows) preceded by periods of diffusion. Arrowheads, onset of RSP4-sfGFP diffusion. Bars (in e for a e and in f) = 2s 2 $\mu$ m.

Fig. S2

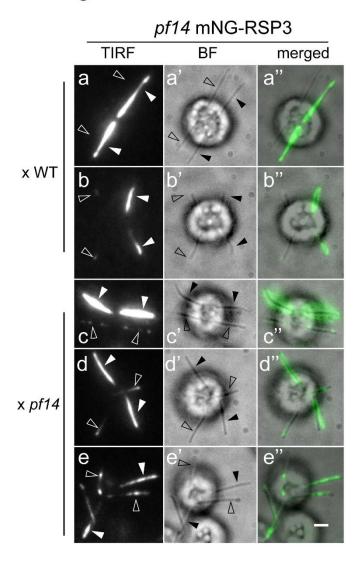


Fig. S2) Incorporation of mNG-RSP3 into wild-type and pf14-derived flagella

TIRF, bright field (BF), and merged images of zygotes obtained by mating wild-type or pf14 with the pf14 mNG-RSP3 rescue strain. Shown are mostly zygotes with shorter flagella indicating that extended time (1-3 hours) have passed since cell fusion. In wild-type derived flagella mNG-RSP3 was absent or limited to the very tip. pf14-derived flagella showed mNG-RSP3 incorportation along the length with a preferences for distal regions. Bar=  $2 \mu m$ .

Supplementary movies

Movie1) Zygotes obtained by mating pf1 RSP4-sfGFP and pf14 mC-RSP3

The resulting zygotes possess to green RSP4-sfGFP and two red mC-RSP3 flagella. Also, note presence of some RSP4-sfGFP in the pf14 mC-RSP3 derived flagella. The movie was recorded at 10 fps and playback is set to 2x. See figure 5C for still images.

Movie 2) Co-transport of mNG-RSP4 and mC-RSP3.

Co-transport of both proteins is visible in the regenerating flagellum pointing to the top. Images were acquired at 10 fps and playback is set at 15 fps. The timer counts mm:ss. A kymogram of the recording is shown in Fig. 7C.