Supplemental Materials

Molecular Biology of the Cell

Harris et al.

Supplementary Figure Legends

Figure S1

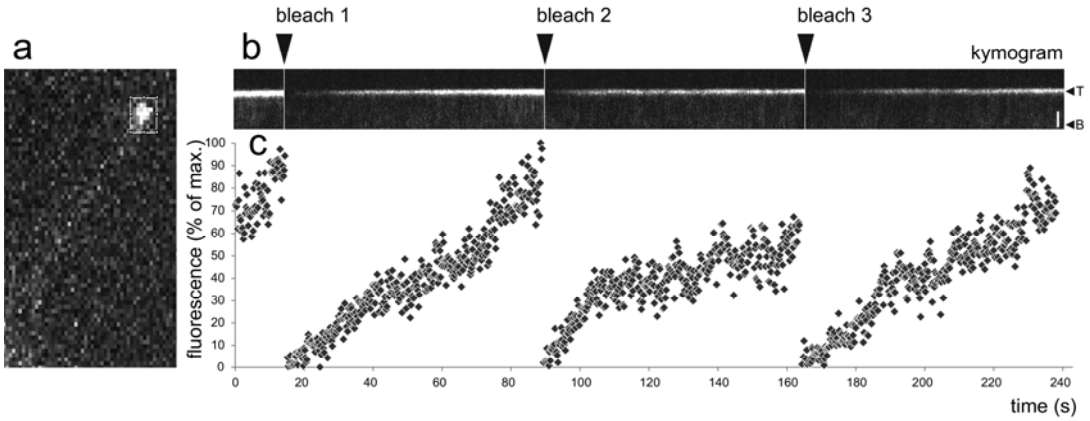

Figure S1) The EB1-FP signal recovers repeatedly after photobleaching.

A) TIRF image of a flagellum from a cell expressing EB1-GFP. The white box outlines the region used for FRAP analysis. B) Kymogram from the flagellum shown in (A); the orientation and the bleaching steps are indicated. Scale bar, 2μm. C) Measurement of the fluorescence intensity (as a percentage of the maximum intensity prior to bleaching) following repeated photobleaching of the entire flagellum.

Figure S2

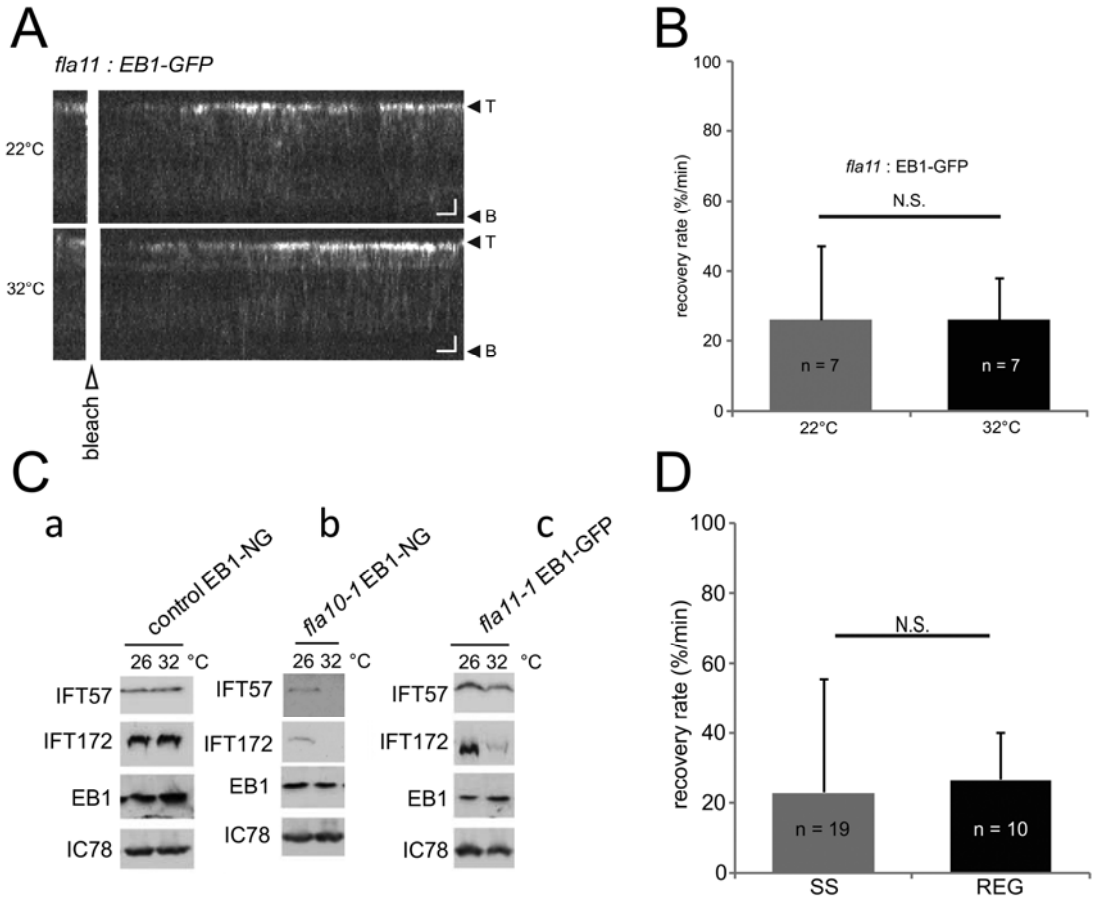

Figure S2) EB1-GFP presence and exchange at the flagellar tip are not affected in *fla11-1*

A) Kymograms depicting the presence and recovery of EB1-GFP in *fla11* cells at the permissive (22°C) or restrictive (32°C) temperature. The flagellar orientation and bleaching events are indicated. Scale bars, 1µm and 1 s. B) Mean recovery rates from *fla11-1* cells expressing EB1-GFP at the permissive (22°C) and restrictive (32°C) temperatures for IFT172 function. Error bars indicate the SD. C) Western blot analysis of flagellar preparations obtained at the permissive (26 °C) and restrictive (32 °C) temperature from wild type (a), and the conditional IFT mutants *fla10-1* (b) and *fla11-1* (c). Western blots were stained with the antibodies indicated. Note the presence of endogenous EB1 and reduction of IFT proteins including IFT172 in cilia harvested from

cells maintained at the restrictive temperature. D) The rate of EB1-NG exchange at flagellar tip is not affected by the growth state of the flagellum. Mean recovery rates of EB1-NG at the tip of steady-state (SS) and regenerating (REG) flagella in wild-type cells. Error bars indicate SD. No statically significant difference in recovery rate was observed under the two growth conditions.

Figure S3

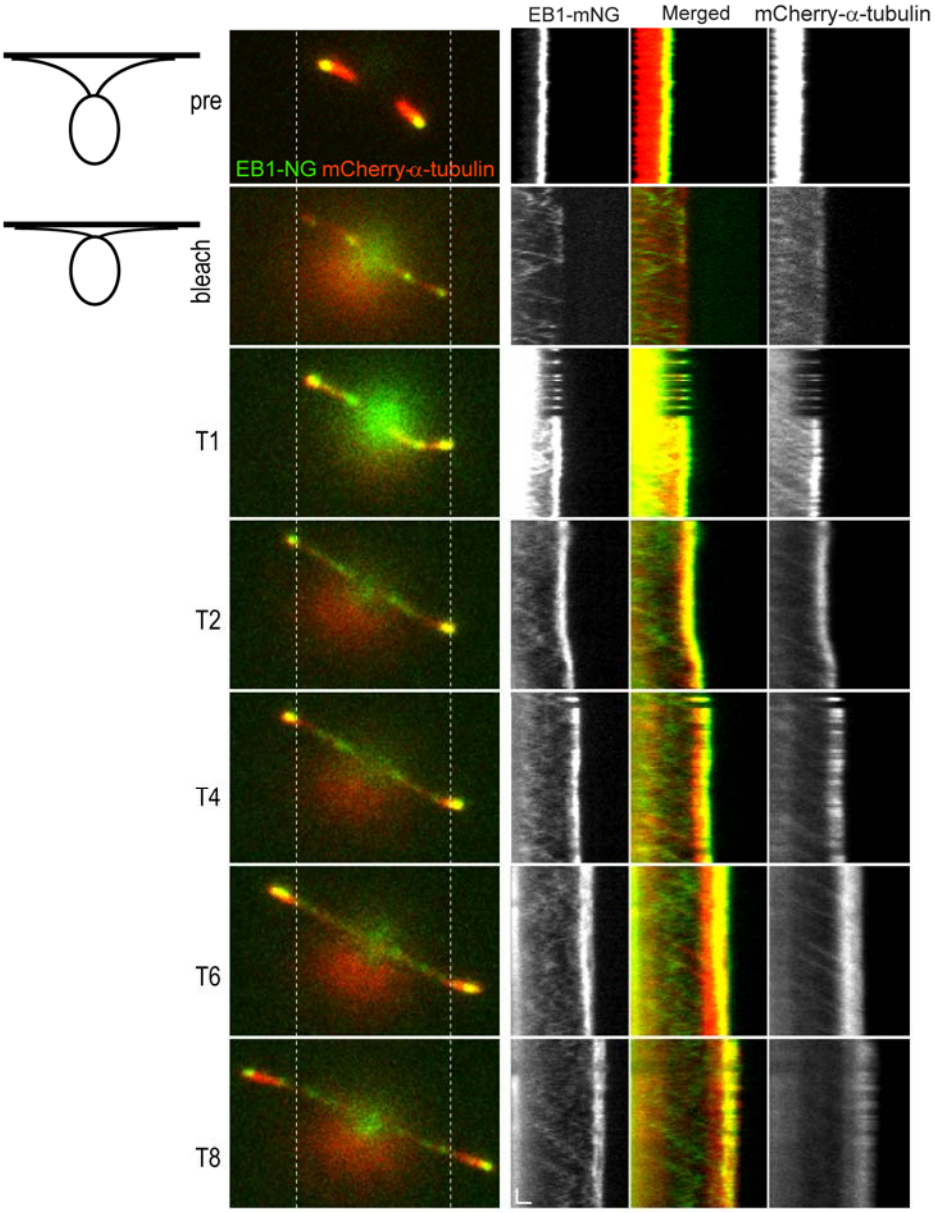

Figure S3. EB1-NG and mCherry-α-tubulin recover after FRAP of growing flagella. Cell expressing EB1-NG and mCherry-α-tubulin during flagellar regeneration. Shown are TIRF images with corresponding single and merged channel kymograms before (pre), during photobleaching of the entire flagellum (bleach) and at various time points (T1-T8 in minutes) after photobleaching. Dashed white lines serve as marks to better visualize

flagellar growth over the course of the analysis. Drawings illustrate the change in cell adherence to the coverslip between in the first two panels. Scale bar, 1μm and 1 s.

Figure S4

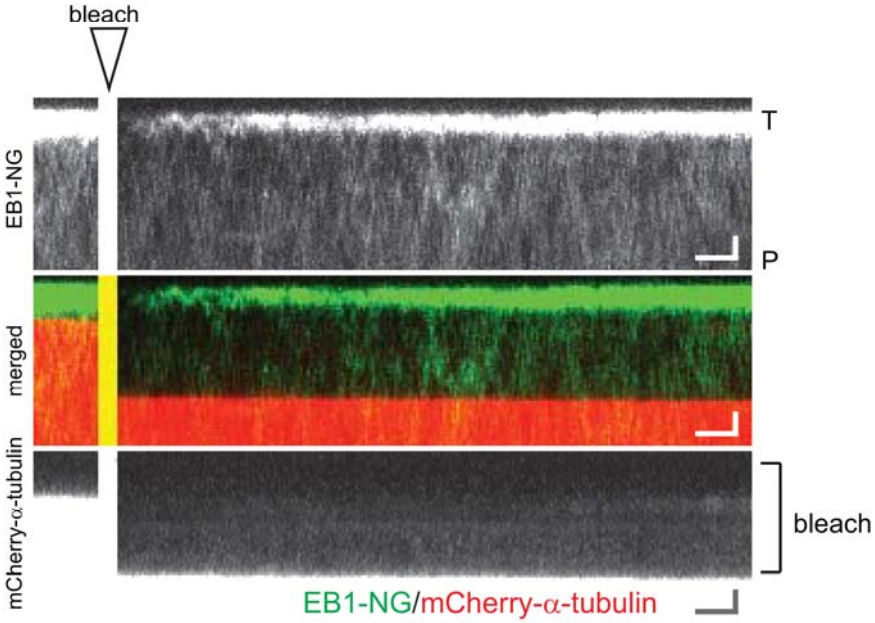

Figure S4. Comparison of EB1-NG and mCherry-α-tubulin exchange at the flagellar tip Single and merged channel kymograms illustrating a FRAP experiment using a cell expressing EB1-NG and mCherry-α-tubulin with steady-state flagella. Note that EB1-NG recovers rapidly following photobleaching of the distal portion of the flagellum while mCherry-α-tubulin fails to recover over the course of the recording. The flagellar orientation and bleaching event are indicated. Scales bars, 1μm and 2 s.

Figure S5

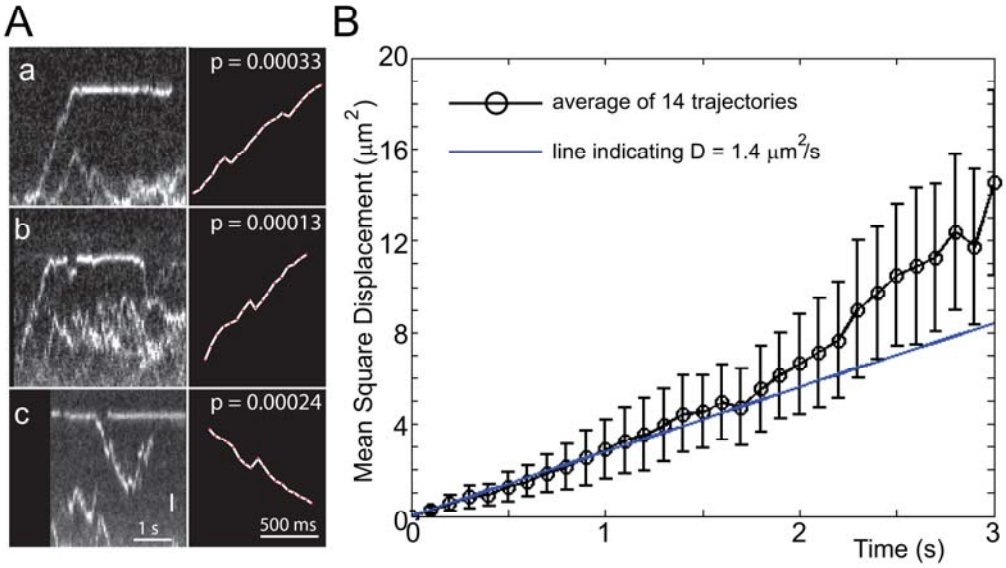

Fig. S5) Unusual diffusional properties of some EB1-NG particles in flagella

A) Kymograms (left) and manual tracings (right) of EB1-NG particles in flagella. Fast moving particles are marked by arrows and the binominal probabilities of the particle's path are indicated.

B) Mean square displacement vs. time plots for EB1-NG particles with a ratio of <4 for the total distance moved to the end-to-end distance. Note that data follow not a linear but a parabolic path indicative for directed movements.