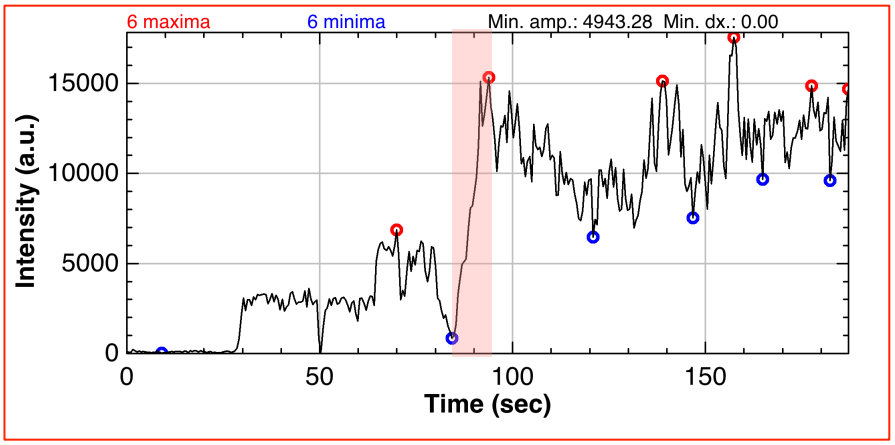
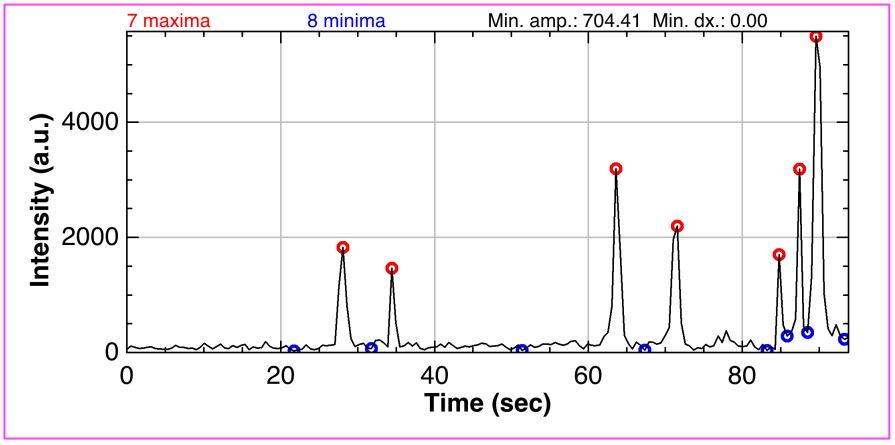
A

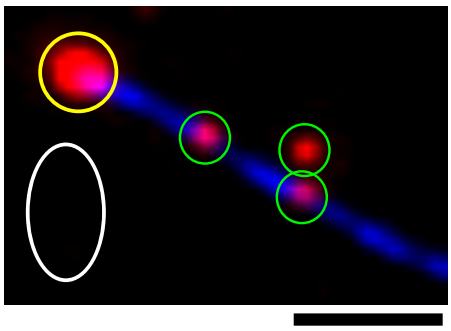
B


6 maxima
6 minima
Min. amp.: 4943.28 Min. dx.: 0.00
Intensity (a.u.)
15000
10000
5000
0
0
50
100
150
Time (sec)

C


7 maxima
8 minima
Min. amp.: 704.41 Min. dx.: 0.00
Intensity (a.u.)
4000
2000
0
0
20
40
60
80
Time (sec)

D



E

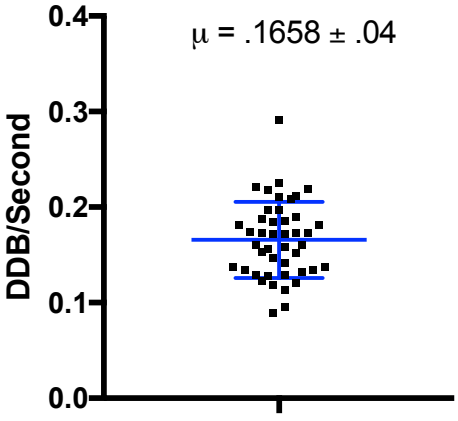
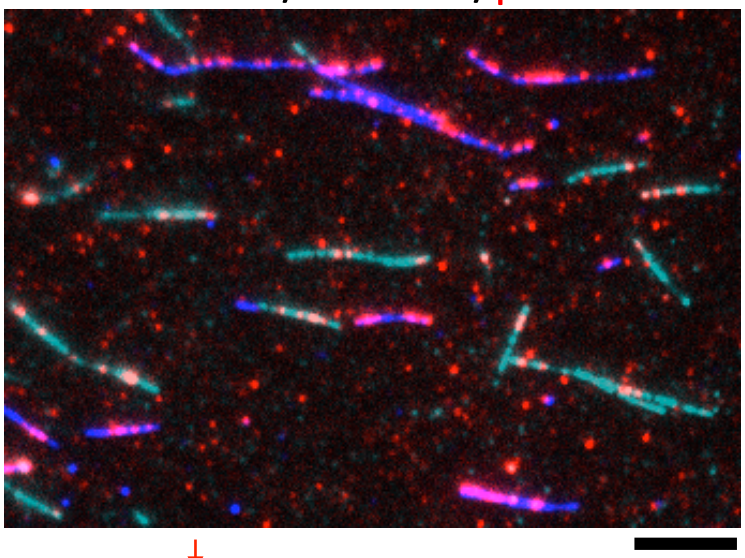
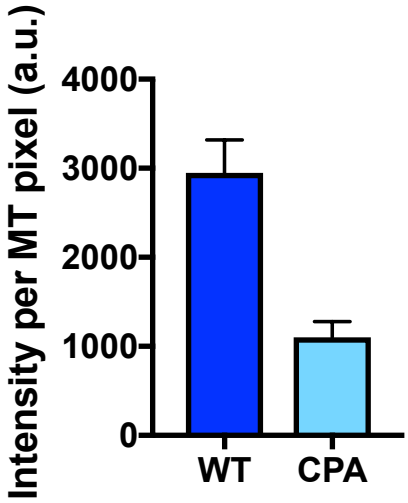

μ = .1658 ± .04
DDB/Second
0.4
0.3
0.2
0.1
0.0

Figure S1: Determination of Numbers of DDB Molecules at MT Minus-Ends, related to Figure 1. **(A)** A kymograph of a microtubule accumulating DDB at its minus-end (reproduced from Figure 1B). A line scan was drawn along the region corresponding to the MT minus-end (red). The earliest peak of the fluorescence intensity plateau along this line scan was used as a fiducial for when the minus-end accumulation reaches saturation. A second line scan (pink) extending as far as the DDB intensity plateau fiducial in the Y-axis was drawn further into the MT as close to the minus-end as possible without interference from the growing minus-end accumulation. **(B)** Intensity plot of the microtubule minus-end (red line in kymograph (**A**)). The earliest peak of the fluorescence intensity plateau as indicated by the red highlight was used as a fiducial for when a minus-end reaches saturation. **(C)** Intensity plot in the microtubule lattice used to count the number of DDB that enter the minus-end accumulation until saturation. Every peak intensity on this new line scan corresponds to a single DDB crossing the point in space. Red circles indicate peaks and correspond to individual DDB. **(D)** Example of intensity analysis for numbers of DDB at MT ends. Yellow, white, and green circles indicate ROIs for minus-end accumulations, background fluorescence normalization, and single molecules, respectively. Scale bar: 2 μm. **(E)** Plot of the rate at which DDB enter the minus-end. $\mu = .1658 \pm .04$. $N = 44$.

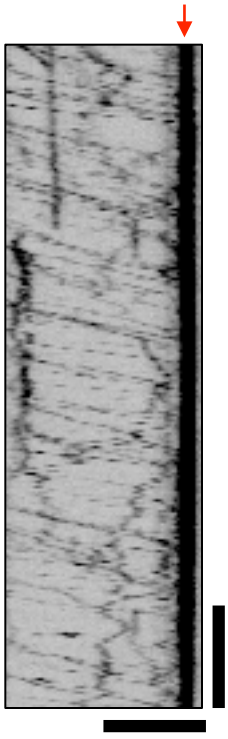
A



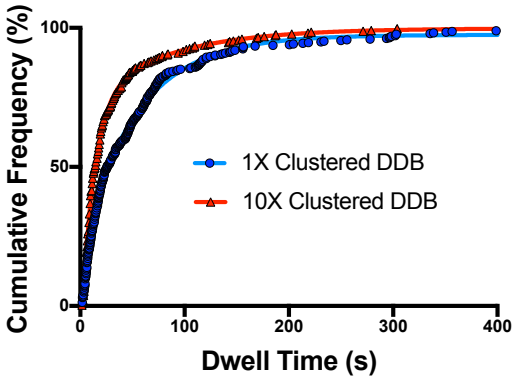
B



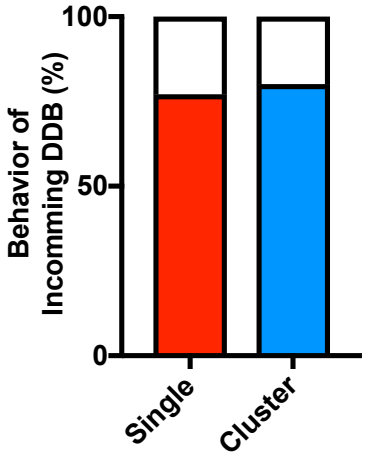
C



D



F



E

	τ_1 (s)	τ_2 (s)	%τ_1	%τ_2	N
1X Clustered DDB	8.4 (7.5 - 9.4)	54.1 (52.2 - 56.2)	28.8 (27 – 30.7)	71.2 (69.3 – 73)	397
10X Clustered DDB	11.8 (11.2 – 12.5)	78.5 (49.9 – 96.5)	76.3 (73.7 – 78.5)	23.7 (21.5 – 26.3)	320

Figure S2: The p150-MT Interaction is Not Required for Cooperative Accumulation of DDB at Minus-ends, related to Figure 2. (A) Representative image of TMR-labeled p150-SNAP (red) binding affinity for WT MTs (blue) compared to carboxypeptidase A (CPA)-treated MT (cyan). Scale bars: 5 µm, 30 sec. **(B)** Quantification of integrated p150 intensity on either WT or CPA-treated MT. Data represented as intensity minus background per pixel of p150 signal along a microtubule. Average WT and CPA-treated intensity are 2950 a.u. ± 370 and 1100 a.u. ± 176, respectively. P=.0001 by Student's T-test. Error bars represent SEM. N = 90 and 64 MTs, from two independent experiments. **(C)** Kymograph showing that DDB accumulation at a MT minus-end (red arrow) is constant in size even at much higher flux of incoming DDB molecules. Scale bars: 5 µm, 30 sec. **(D)** Plot of cumulative frequency comparing single DDB dwell times within minus-end DDB clusters at 1X and 10X DDB concentration. Note 1X DDB concentration data is reproduced from Figure 2E. (**E)** Table summarizing the parameters of cumulative frequency graphs comparing 1X and 10X DDB concentrations, including the characteristic dwell time (τ) of short (τ_1) and long (τ_2) populations, percentage of molecules in each population, and number of molecules measured (N) are given. 95% confidence intervals given in parentheses. All regressions have a goodness of fit (R^2) greater than .99. All data from 2-3 independent experiments. (**F)** Plot of the percent of incoming DDB molecules to the MT minus-end that either dwell (filled) or fall off immediately (empty) on bare minus-ends (red) and minus-ends with DDB clusters (blue). Percent of molecules that dwell are 77% and 83% for vacant and clustered minus ends, respectively. P = .2734 by Fischer's exact test.

Figure S3

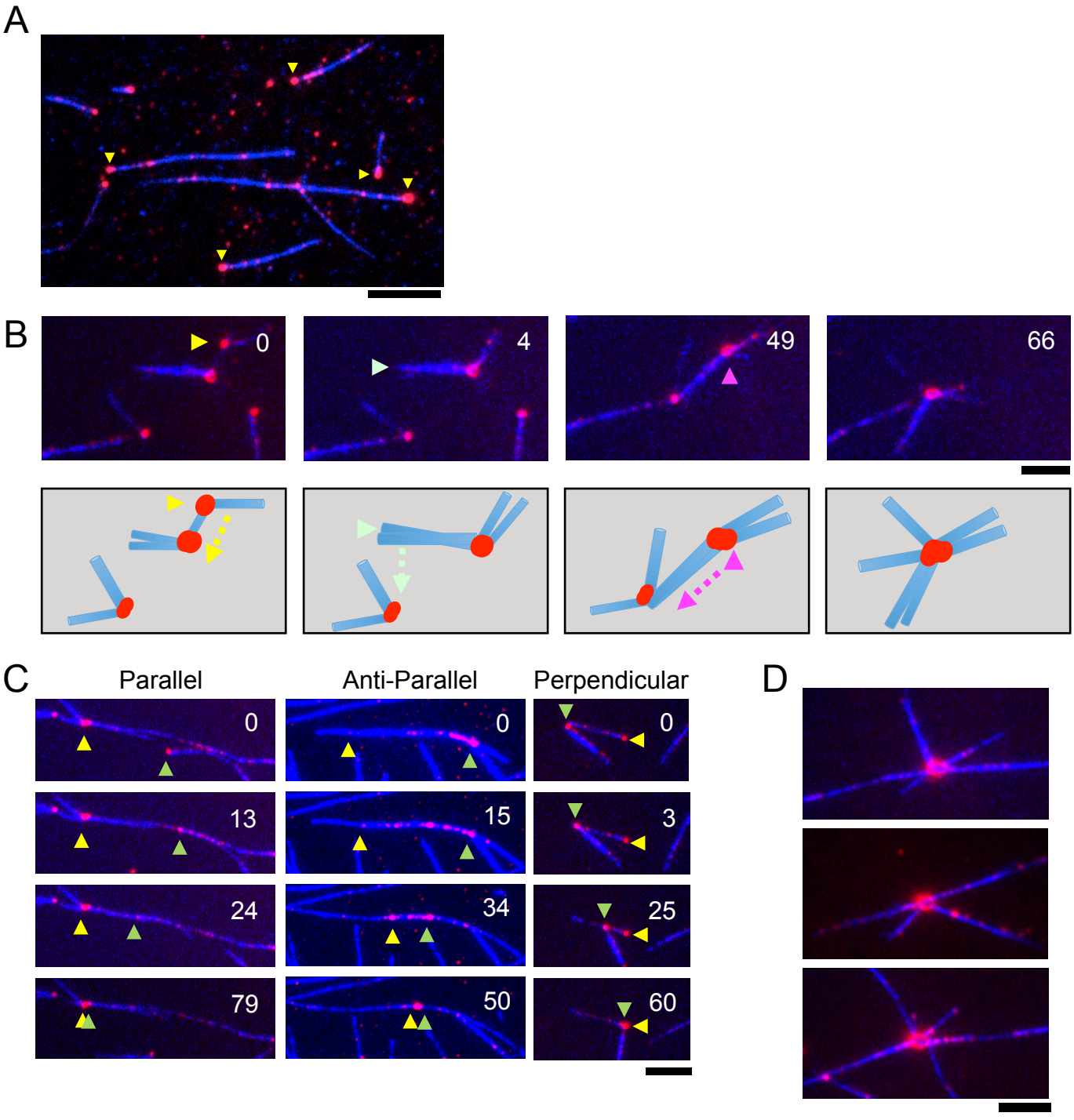

A
B
0
4
49
66
C
Parallel
Anti-Parallel
Perpendicular
0
0
0
13
15
3
24
34
25
79
50
60
D

Figure S3: Minus-End Accumulations of DDH Drive MT Reorganization Similar To DDB, related to Figure 3. (A) TIRF-M image of DDH (red) forming clusters (yellow arrows) at MT minus-ends. Scale bar: 5 μm. **(B)** Top: Image frames showing steps during MT reorganization into a mini-aster. Arrows highlight minus-end accumulations that drive sliding in that frame. Bottom: Schematic depicting the DDB-driven movements of MTs. Red dots indicate DDB clusters at minus-ends. Arrows highlight the direction of sliding MTs. Scale bar: 2 μm, time is in sec. **(C)** Examples of parallel, anti-parallel, and oblique sliding driven by minus-end accumulations of DDH. Yellow and green arrows indicate minus-ends of sliding microtubules inferred from DDH accumulation. Scale bar: 5 μm, time is in sec. **(D)** Examples of mini-asters formed through DDH-driven MT-MT sliding. Scale bars: 5 μm.