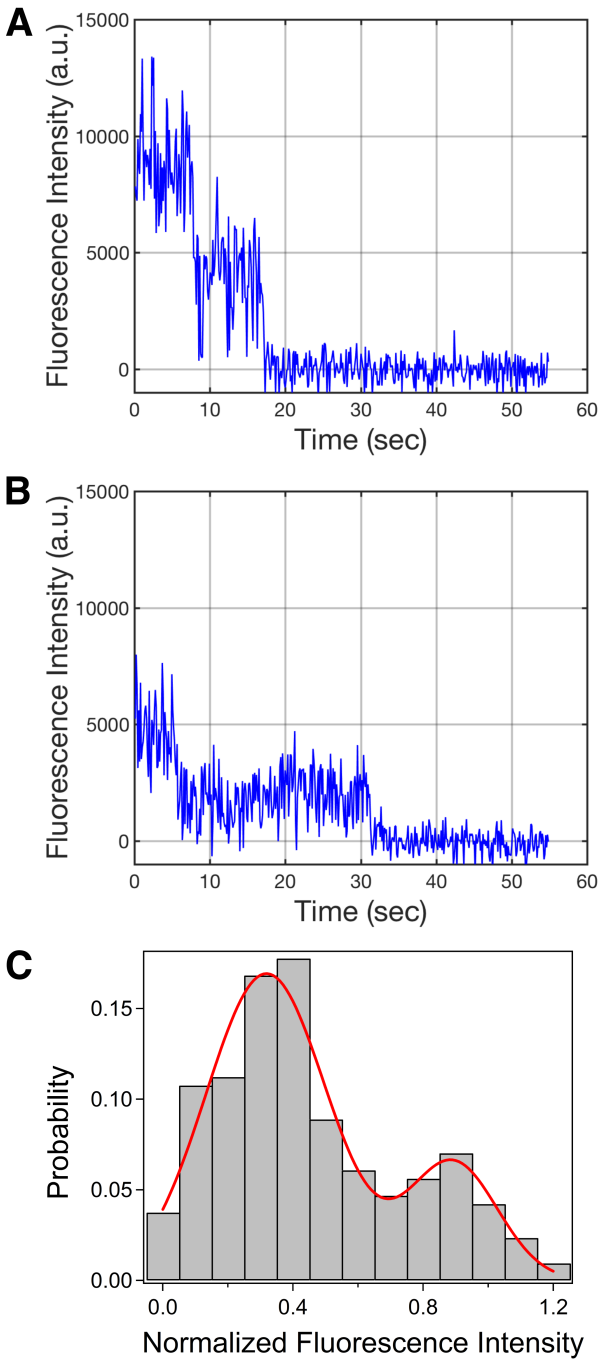

Figure S1 I Oligomerization State of Kif15, Related to Figure 1. The oligomerization state of FL-Kif15-GFP was confirmed to be a dimer through single molecule photobleaching. Figures S1-A and -B are representative fluorescence intensity traces versus time in seconds of a single FL-Kif15-GFP molecule non-specifically adsorbed to an etched coverslip showing sequential photobleaching in two steps. Figure S1-C shows a normalized fluorescence intensity distribution of the single FL-Kif15-GFP molecules. The two peaks indicate there are primarily two steps of photobleaching, which coincides with the construct being a dimer.

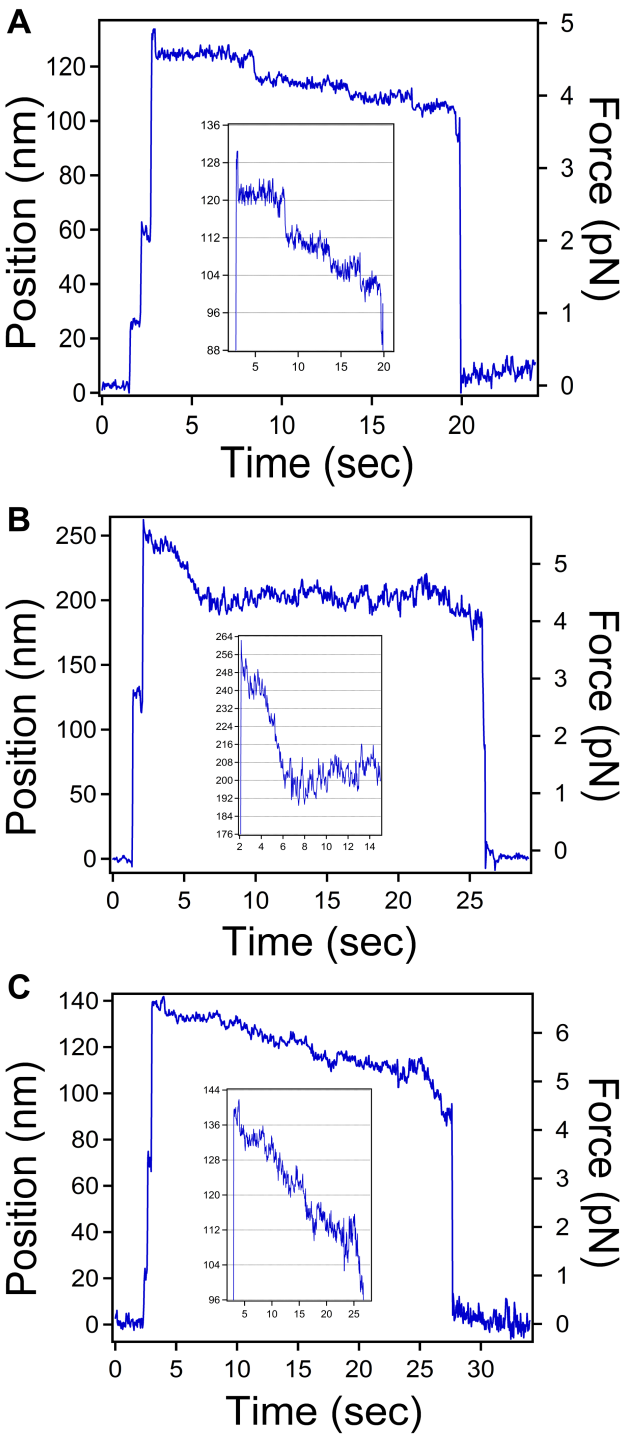

Figure S2 I Structured Movement of Coil-1 Under Force, Related to Figure 3. Figures S2 A-C are examples of Coil-1 rupture traces that exhibit structured movement while Coil-1 is under tension. The insets reveal discrete transitions while Coil-1 is being pulled, which may potentially be due to the ability of Coil-1 to readjust its position and accommodate for the induced force by binding to the next sequential E-hook position. Lines at 8 nm periodicity are provided as a guide.

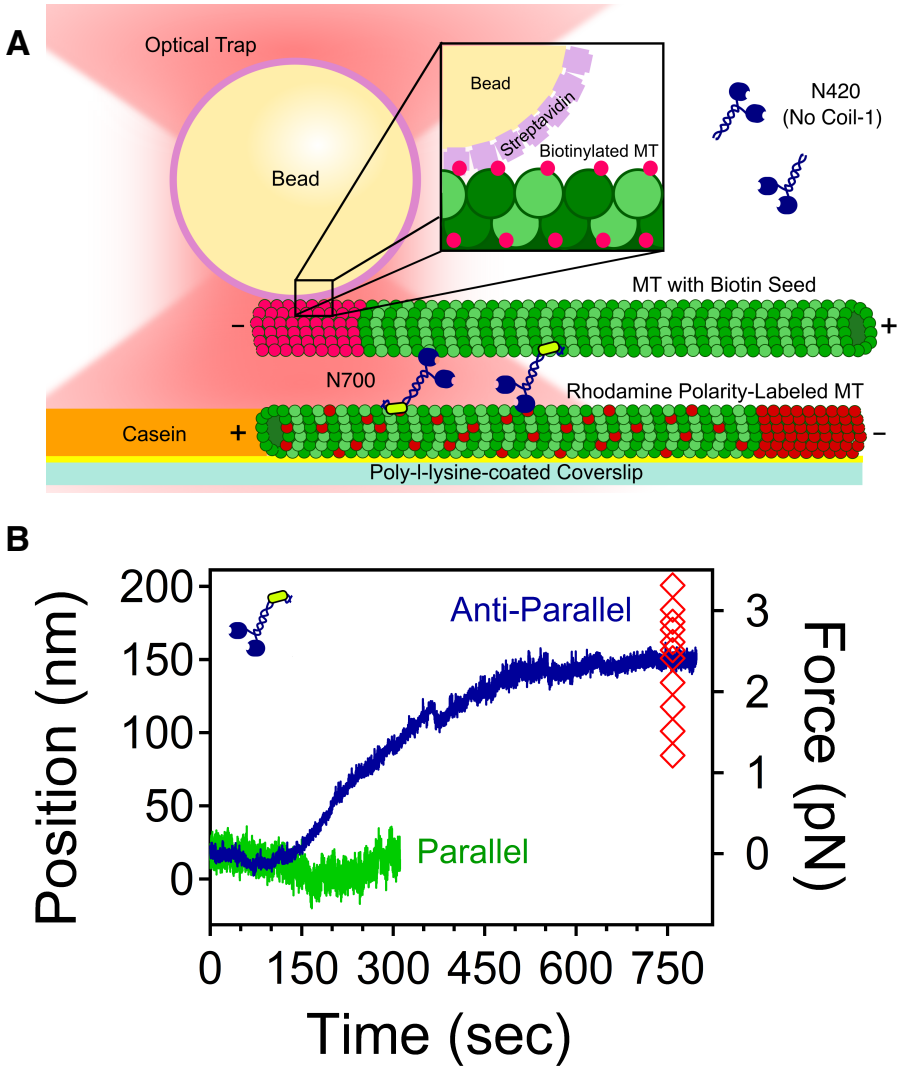

Figure S3 I N700 Bundle Assay, Related to Figure 4. (A) Schematic of N700 bundle assay that is assembled in the same manner as the Kif15 bundle assay in Figure 4. This assay was also performed with N420 with results shown in Figure S4. (B) N700 exhibits a ramp/plateau pattern (representative trace in blue) when sliding anti-parallel bundles with an average plateau force of 2.4 pN ± 0.6 pN (red diamonds are individual plateau forces, N=11). N700 does not generate force in parallel bundles (representative trace, green, N=25) yet exhibits a 69% bundling bias for this orientation. These results are consistent with Kif15 in bundles.

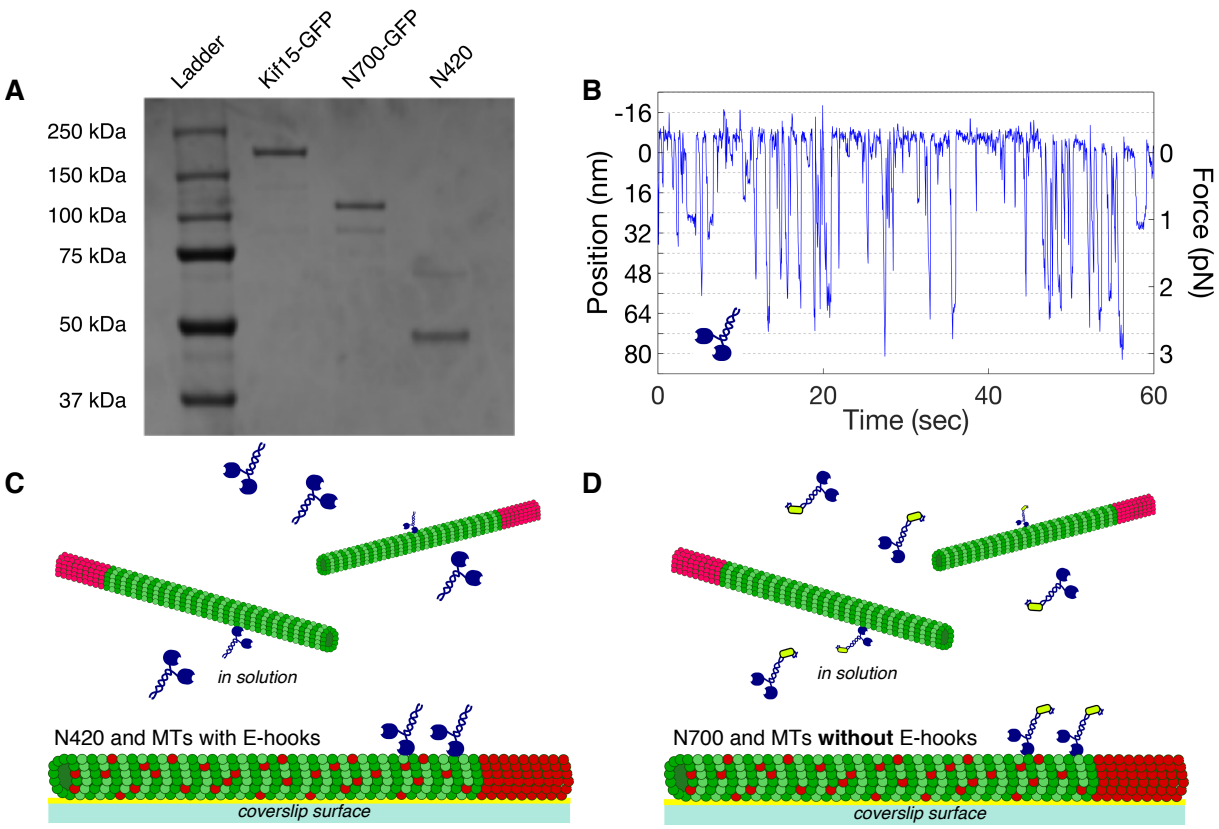

Figure S4 I Coil-1/E-hook interaction needed for bundling, Related to Figure 4. The bundle assay in Figure S3 was repeated with N420 which lacks Coil-1. (A) SDS-PAGE gel of insect purified Kif15-GFP, insect purified N700-GFP, and bacterially purified N420. Sizes of Kif15 and N700 with GFP at the C-terminus are consistent with [S1]. (B) Control experiment to show that N420 is active at the single molecule level attached to a bead. The motor reaches a maximum force of ~3pN before detaching from the microtubule, returning to the trap center, and starting to walking again. (C) No bundles were formed when N420 was present as 0/100 streptavidin beads bound biotinylated microtubules near the surface. (D) The bundle experiment was repeated with N700 but with digested polarity marked microtubules (rhodamine and biotin seed microtubules). As with N420, no bundles were formed as 0/100 streptavidin beads bound biotinylated microtubules near the surface. These experiments show that the Coil-1/E-hook interaction is necessary for microtubule bundling by Kif15.

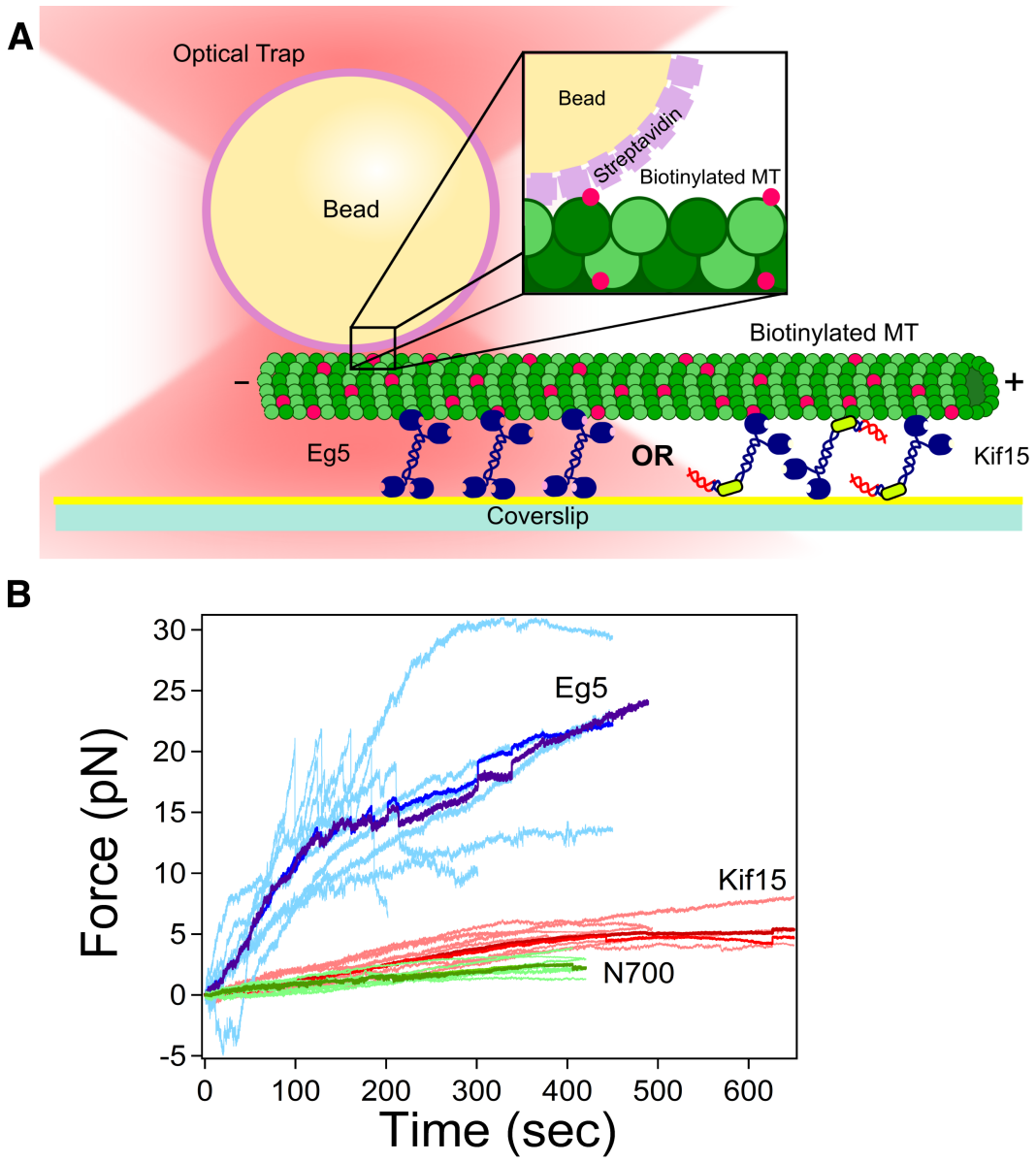

Figure S5 I N700 Gliding Assay, Related to Figure 5. (A) Schematic of gliding assay that is assembled in the same manner as the Kif15 and Eg5 gliding assay in Figure 5. (B) N700 exhibits a ramp/plateau pattern (green, N=7) in the same manner as Kif15 (red). The plateau force is somewhat smaller than that of Kif15; however, it is consistent with the N700 bundle plateau force in Figure S3. In the same way, the plateau force of Kif15 in this gliding assay is also consistent with its bundle assay plateau force in Figure 4. It is possible that the GFP on the C-terminus is interfering with Coil-1 binding and not allowing for as much force generation as Kif15. The median of the traces is shown in dark green.

Supplemental References

[S1] Sturgill, E.G., Das, D.K., Takizawa, Y., Shin, Y., Collier, S.E., Ohi, M.D., Hwang, W., Lang, M.J., and Ohi, R. (2014). Report Kinesin-12 Kif15 Targets Kinetochore Fibers through an Intrinsic Two-Step Mechanism. Curr. Biol. *24*, 2307–2313.