

**Supplemental movie 1:** Example of a single, fluorescently labeled NK433 motor moving along a native microtubule at 1.60  $\mu\text{m/s}$ . The pixel size corresponds to 160 nm x 160 nm. Data were acquired at 128ms/frame.

**Supplemental movie 2:** Examples of a single, fluorescently labeled NK433 motor moving along a digested microtubule at 1.12  $\mu\text{m/s}$  and 1.33  $\mu\text{m/s}$ . Pixels represent areas of 160 nm x 160 nm. Data were acquired at 128ms/frame.

**Supplemental movie 3:** Multiple motor gliding assay of biotinylated NK433 using brightly labeled, native microtubules and dimly labeled, digested microtubules. Two microtubules move in the same direction but at different speed. The native microtubule moves at 1.82  $\mu\text{m/s}$ , while the digested microtubule moves significantly slower at a speed of 1.29  $\mu\text{m/s}$ . Individual pixels represent areas of 260 nm x 260 nm, data were acquired at an exposure time of 95.2ms/frame.