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**Microtubule defects influence kinesin-based transport in vitro.<sup>1</sup>**

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Microtubules are protein polymers that form “molecular highways” for long-range transport within living cells. Molecular motors actively step along microtubules to shuttle cellular materials between the nucleus and the cell periphery; this transport is critical for the survival and health of all eukaryotic cells. Structural defects in microtubules exist, but whether these defects impact molecular motor-based transport remains unknown. Here, we report a new, to our knowledge, approach that allowed us to directly investigate the impact of such defects. Using a modified optical-trapping method, we examined the group function of a major molecular motor, conventional kinesin, when transporting cargos along individual microtubules. We found that microtubule defects influence kinesin-based transport in vitro. The effects depend on motor number: cargos driven by a few motors tended to unbind prematurely from the microtubule, whereas cargos driven by more motors tended to pause. To our knowledge, our study provides the first direct link between microtubule defects and kinesin function. The effects uncovered in our study may have physiological relevance in vivo.

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