During cell division, mitotic spindles are assembled by microtubule-based motor proteins. The bipolar organization of spindles is essential for proper segregation of chromosomes, and requires plus-end-directed homotetrameric motor proteins of the widely conserved kinesin-5 (BimC) family. Hypotheses for bipolar spindle formation include the 'push–pull mitotic muscle' model, in which kinesin-5 and opposing motor proteins act between overlapping microtubules. However, the precise roles of kinesin-5 during this process are unknown. Here we show that the vertebrate kinesin-5 Eg5 drives the sliding of microtubules depending on their relative orientation. We found in controlled in vitro assays that Eg5 has the remarkable capability of simultaneously moving at ∼20 nm s\(^{-1}\) towards the plus-ends of each of the two microtubules it crosslinks. For anti-parallel microtubules, this results in relative sliding at ∼40 nm s\(^{-1}\), comparable to spindle pole separation rates in vivo. Furthermore, we found that Eg5 can tether microtubule plus-ends, suggesting an additional microtubule-binding mode for Eg5. Our results demonstrate how members of the kinesin-5 family are likely to function in mitosis, pushing apart interpolar microtubules as well as recruiting microtubules into bundles that are subsequently polarized by relative sliding. We anticipate our assay to be a starting point for more sophisticated in vitro models of mitotic spindles. For example, the individual and combined action of multiple mitotic motors could be tested, including minus-end-directed motors opposing Eg5 motility. Furthermore, Eg5 inhibition is a major target of anti-cancer drug development, and a well-defined and quantitative assay for motor function will be relevant for such developments.