Supplemental Materials

Molecular Biology of the Cell

Wagenbach et al.

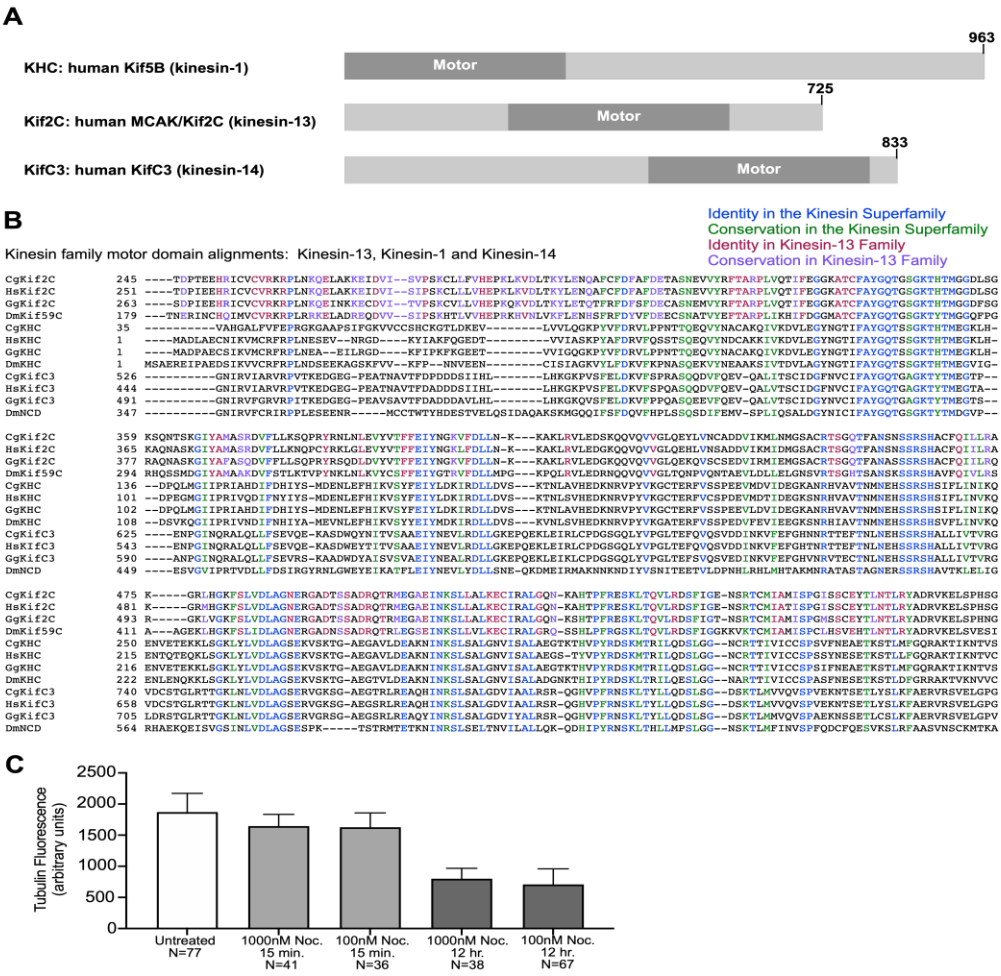


Figure S1: A. Three families of kinesin-related proteins and their basic sequence arrangement. **B.** Alignment of kinesin-13 depolymerizers with kinesin-1 and kinesin-14 motile kinesins. **C.** Depolymerization of cellular microtubules with nocodazole results in lower tubulin fluorescence over 12 hours relative to 15 minutes.

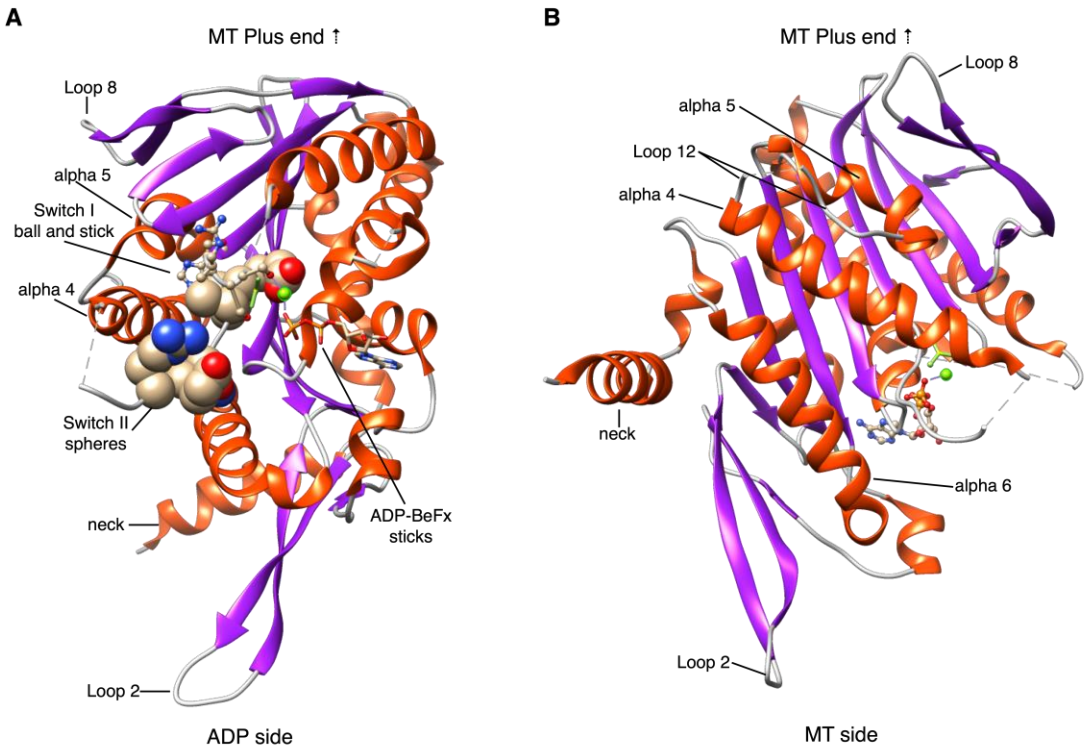
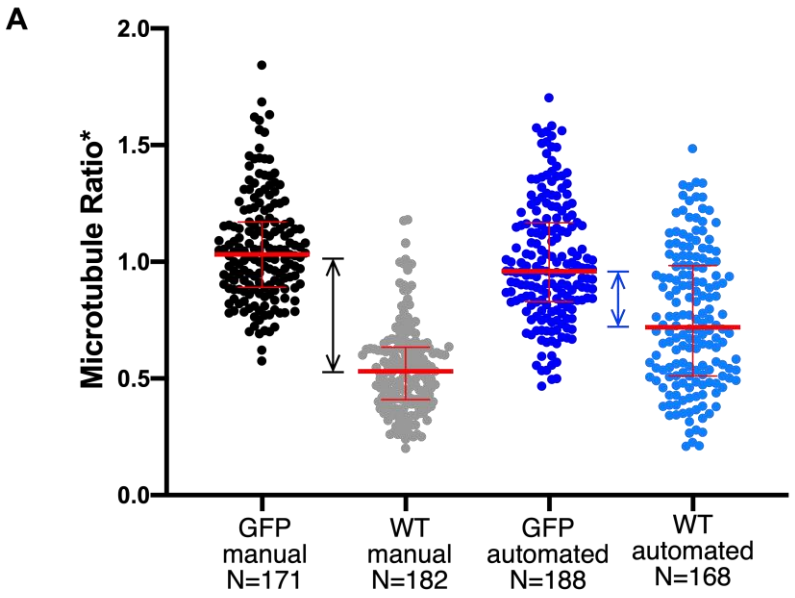


Figure S2: Positions of structural elements of the MCAK/Kif2C motor domain in Kif2C BeFx structure (5XJB). **A.** ADP side. **B.** MT binding elements side.



N = number of individual cells (manual, black and grey); number of fields of cells (automated, blue and light blue). Each field contained between 1-3 transfected cells.
Microtubule Ratio: manual MR = tubulin flr. GFP cell/tubulin flr. of untransfected cell in the same field; automated MR = arbitrary tubulin flr. GFP cell/mean of negative control tubulin fluorescence

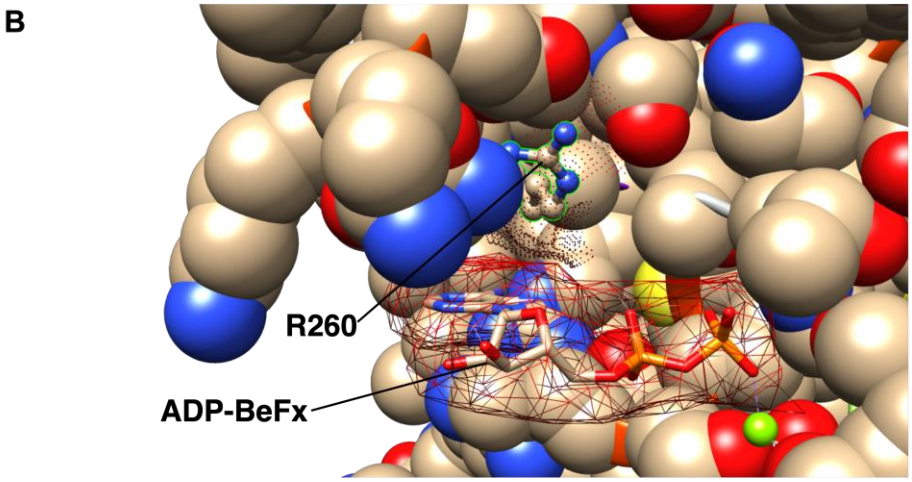


Figure S3: A. Direct comparison of tubulin fluorescence measurements for negative (GFP-transfected) and positive (GFP-MCAK/Kif2C transfected) controls measured manually (black, grey) or using automated analysis (blue, lt. blue). **B.** Structural models predict that the R260H mutant would sterically interfere with ATP. Close-up of the ATP binding pocket showing ADP-

BeFx (wireframe) and R260 (dot surface). ADP-BeFx mimics the pre-hydrolysis state of ATP. Note the close apposition of the Van Der Waals surfaces in these two moieties. Substitution of arginine by histidine would be unlikely to be compatible with ATP binding due to steric clashing of the imidazole ring of histidine and the adenine ring.