Online supplemental material:

**Supplementary Figure 1. Co-immunoprecipitation of KLC1 by KHC-mCit in COS cells.** Cell lysate was prepared from COS cells transfected with KHC-mCit. Immunoprecipitation was performed with GFP antibodies or rabbit IgG as a negative control; western blots were probed with GFP to detect KHC-mCit and KLC1 antibodies to detect endogenous KLC in COS cells. A fraction of expressed KHC-mCit in COS cells interacts with endogenous KLC. Asterisk: IgG bands; arrows: specific KLC1 and KHC-mCit bands. A higher exposure was needed to reveal KLC1 in the input lane (1%).

**Supplementary video 1**: GFPsyd wt movement on rhodamine labeled microtubules with KHC. Pseudo-colored movie showing microtubules in red and GFPsyd wt molecules in green. Lysates containing KHC and GFPsyd wt were mixed prior to the motility assay. The closed arrowhead marks processive movement of a GFP syd wt molecule along a microtubule.

**Supplementary video 2**: GFPsyd wt movement on rhodamine labeled microtubules without KHC. Pseudo-colored movie showing microtubules in red and GFPsyd wt molecules in green. Non transfected lysates were mixed with lysate containing GFPsyd wt prior to the motility assay. The closed arrowhead marks processive movement of a GFP syd wt molecule along a microtubule.
**Supplementary video 3:** GFPsyd 3-239 movement on rhodamine labeled microtubules with KHC. Pseudo-colored movie showing microtubules in red and GFPsyd 3-239 molecules in green. Lysates containing KHC and GFPsyd 3-239 were mixed prior to the motility assay. The closed arrowhead marks processive movement of a GFPsyd 3-239 molecule along a microtubule.

**Supplementary video 4:** KHC-mCit movement on rhodamine labeled microtubules. Pseudo-colored movie showing microtubules in red and KHC-mCit molecules in green. Non transfected lysates was mixed with lysate containing KHC-mCit prior to the motility assay. The closed arrowhead marks processive movement of a KHC-mCit molecule along a microtubule.

**Supplementary video 5:** KHC-mCit movement on rhodamine labeled microtubules in the presence of Flag-syd wt. Pseudo-colored movie showing microtubules in red and KHC-mCit molecules in green. Lysates containing Flag syd wt was mixed with lysate containing KHC-mCit prior to the motility assay. The closed arrowhead marks processive movement of a KHC-mCit molecule along a microtubule.

**Supplementary video 6:** KHC-mCit movement on rhodamine labeled microtubules in the presence of Flag-sydΔKBD. Pseudo-colored movie showing microtubules in red and KHC-mCit molecules in green. Lysates containing Flag syd ΔKBD was mixed with lysate containing KHC-mCit prior to the motility assay. The closed arrowhead marks processive movement of a KHC-mCit molecule along a microtubule.
**Supplementary video 7**: KHC-mCit movement on rhodamine labeled microtubules in the presence of Flag-syd3-239. Pseudo-colored movie showing microtubules in red and KHC-mCit molecules in green. Lysates containing Flag syd3-239 was mixed with lysate containing KHC-mCit prior to the motility assay. The closed arrowhead marks processive movement of a KHC-mCit molecule along a microtubule.
Sun et al., Supplementary Figure 1