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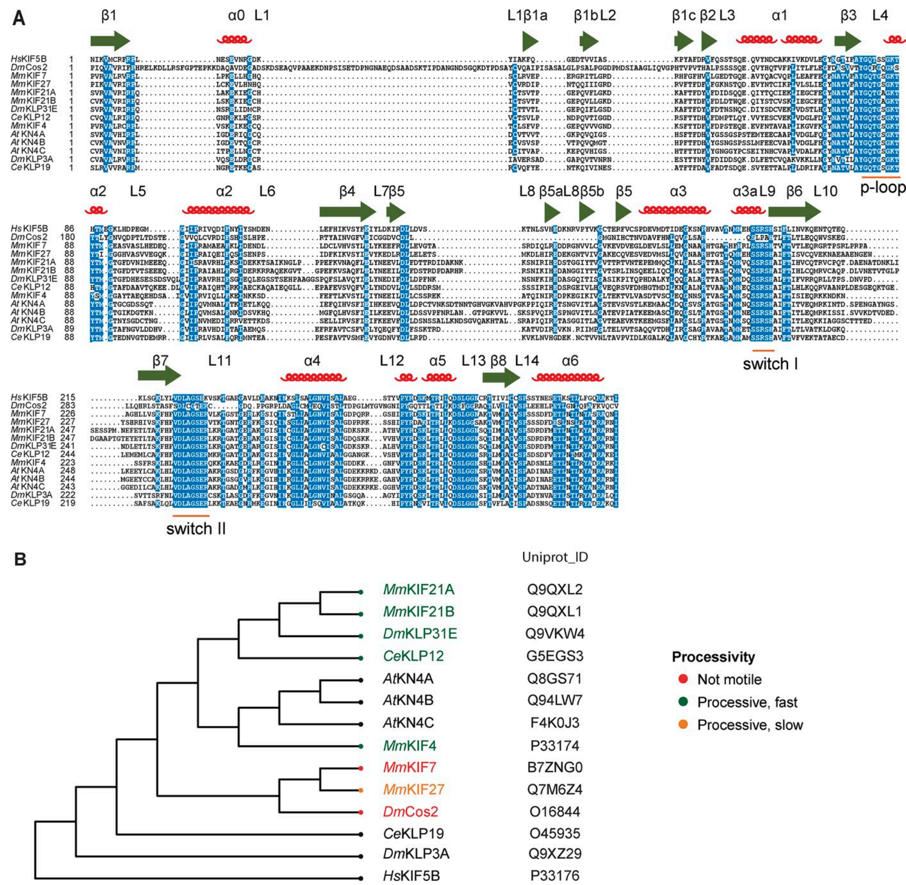

Figure S1. **Sequence alignment of the motor domains of kinesin-4 family motors. (A)** Sequence alignment of the motor domains of kinesin-4 family motors. The P loop, switch I, and switch II regions are labeled. Identical residues are colored in white on a blue background. Secondary structure elements based on the crystal structure of the KIF5B (PDB: 1BG2; Kull et al., 1996) are indicated above the sequence where red loops indicate α helices and green arrows indicate β sheets. **(B)** Phylogenetic tree of select kinesin-4 family members compared with and rooted at the kinesin-1 motor KIF5B.

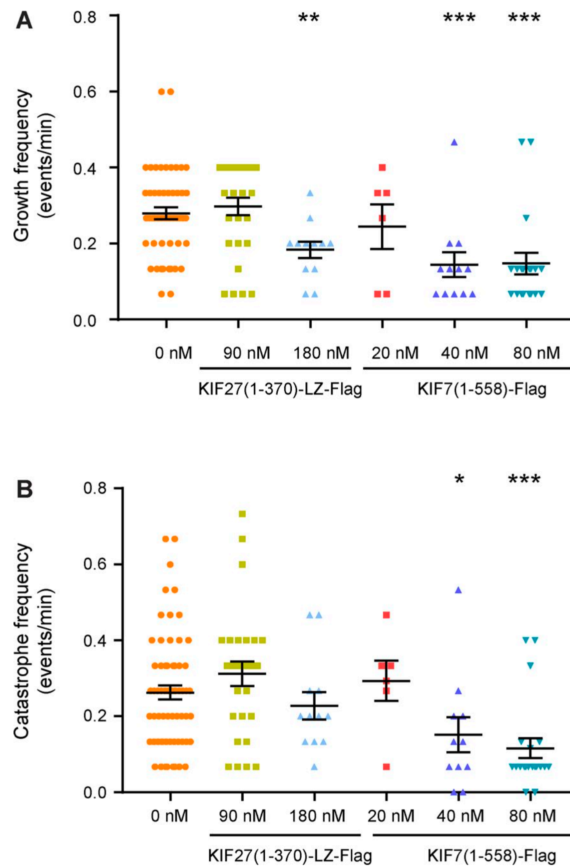

Figure S2. **The frequencies of microtubule growth and catastrophe in the absence or presence of indicated concentrations of KIF27 and KIF7 in vitro.** **(A)** Plots of frequencies of microtubule growth events measured in the absence (0 nM) or presence of indicated concentrations of KIF27 and KIF7 in vitro. **(B)** Plots of catastrophe frequencies of microtubules measured in the absence (0 nM) or presence of indicated concentrations of KIF27 and KIF7 in vitro. Scatterplots display means ± SEM (*n* = 6–64 for each condition) from two or three independent experiments. *, P < 0.05; **, P < 0.01; ***, P < 0.001 as compared with the frequencies in the absence of motors (two-tailed *t* test).

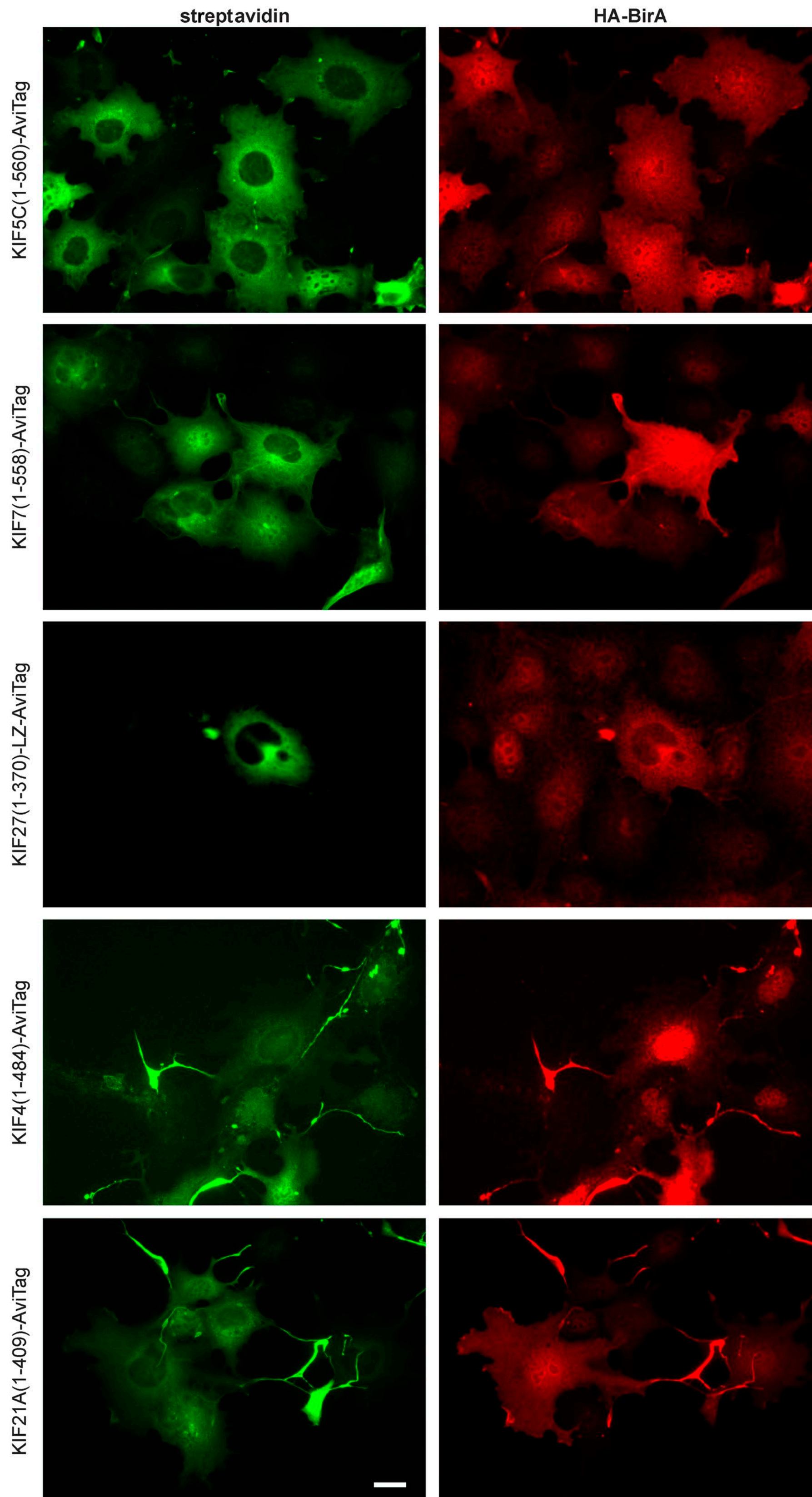

Figure S3. **Biotinylation of AviTag motors in cells.** COS-7 cells were transfected with plasmids for coexpression of AviTagged-kinesin motors and HA-BirA. After 24 h, the cells were fixed and stained with fluorescent streptavidin (green) and an antibody to the HA tag (red). Bar, 15 µm.

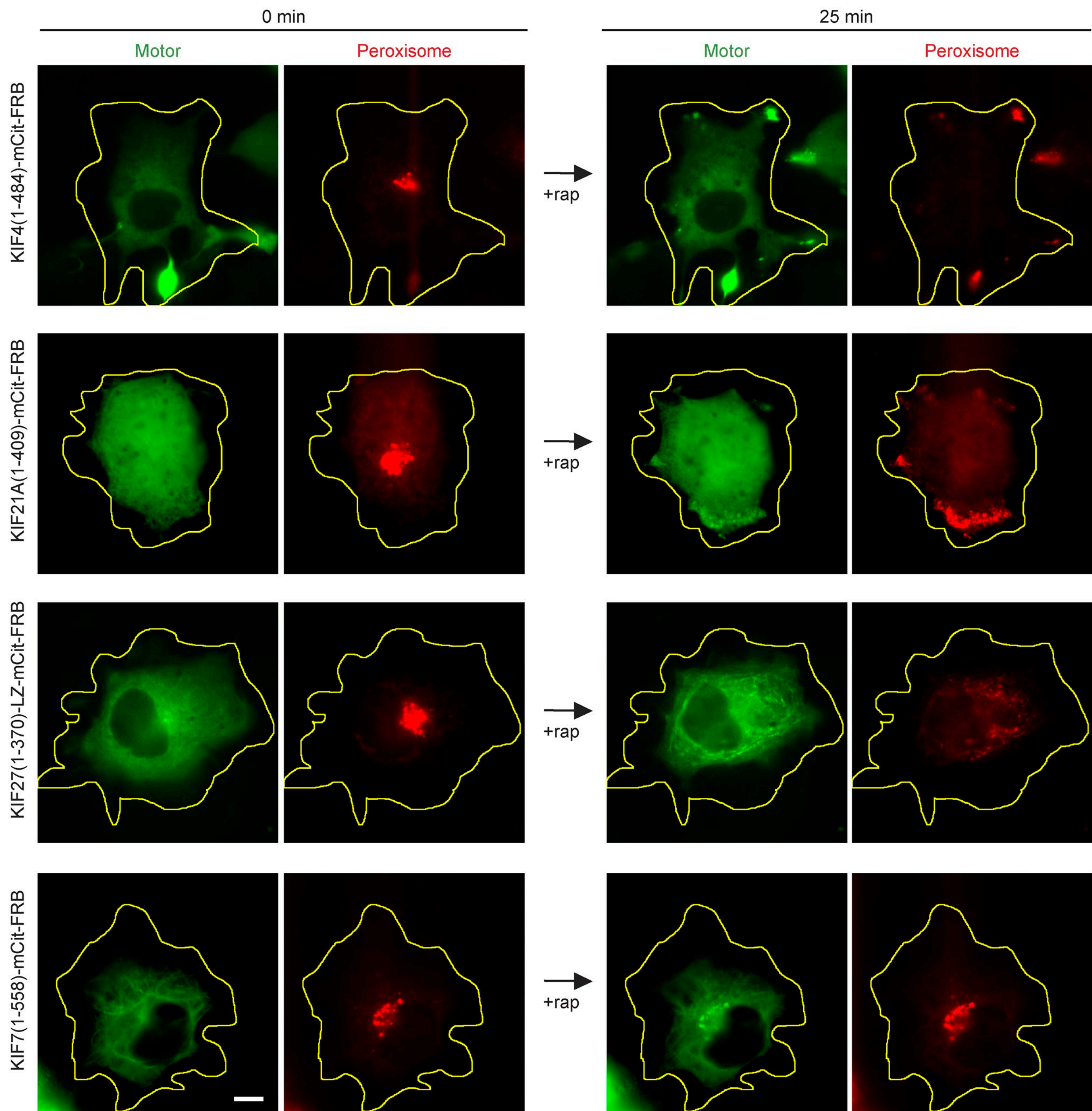

Figure S4. **Representative images of motors and peroxisomes in the peroxisome dispersion assay.** Representative images of kinesin motor-mCit-FRB (green) and peroxisome (red) localization before (0 min) and after (25 min) addition of rapamycin (rap) to recruit the motors to the peroxisome surface. The yellow line indicates the cell periphery. Bar, 10 µm.

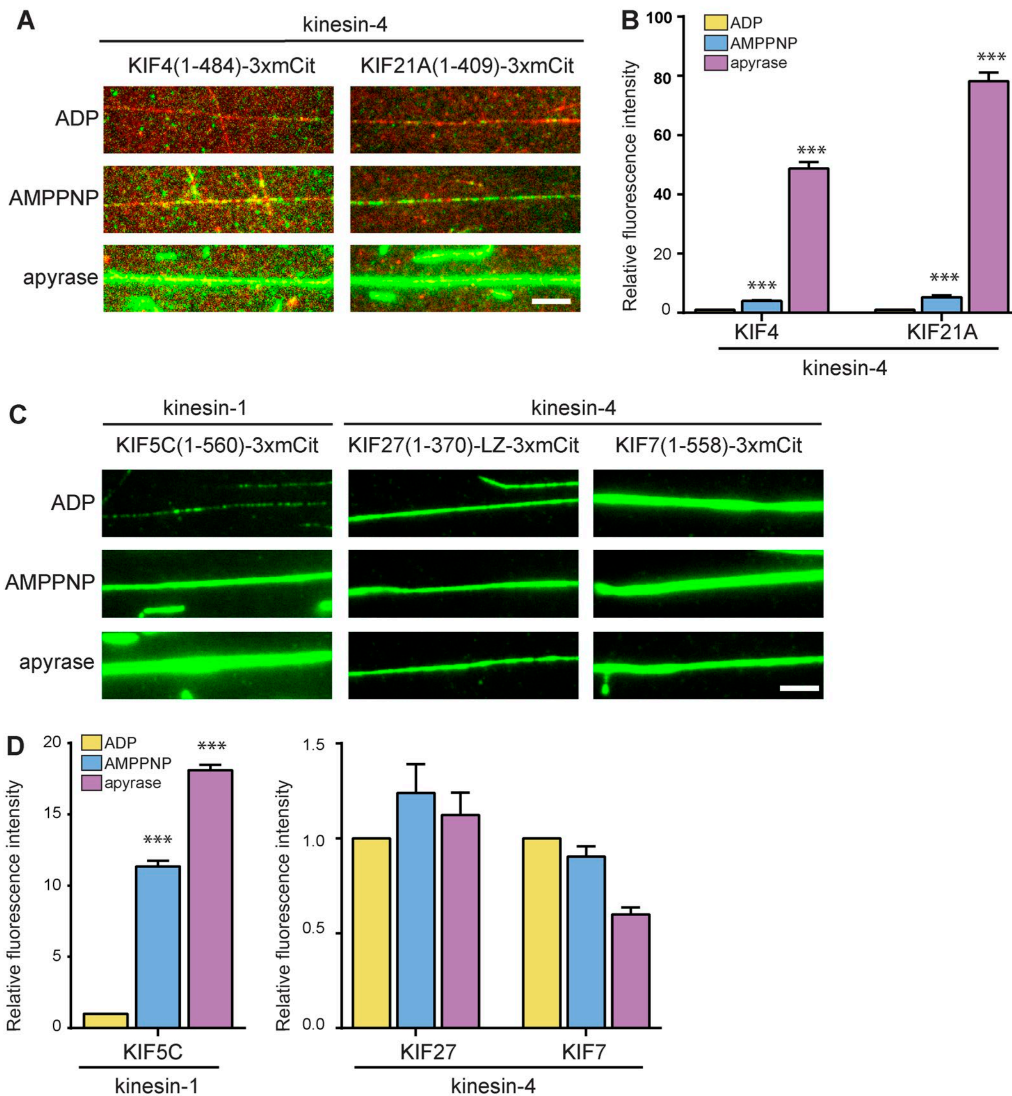

Figure S5. **Chemomechanical coupling of the kinesin-4 motors. (A and B)** Truncated dimeric kinesin-4 motors KIF4(1–484) or KIF21A(1–409) were tagged at their C termini with 3×mCit. Cell lysates containing equivalent amounts of motors were added to flow cells containing taxol-stabilized microtubules in the presence of the indicated nucleotides. **(A)** Representative images of motor (green) binding to microtubules (red) in the presence of ADP, AMPPNP, or apyrase. **(B)** The fluorescence intensity of each motor along microtubules was quantified for each nucleotide condition. The mean fluorescence intensity of KIF4 or KIF21 in AMPPNP or apyrase was normalized to the mean fluorescence intensity in the ADP state. **(C)** Representative images of a replicative experiment of KIF27(1–370)-LZ and KIF7(1–558) motors (green) binding to microtubules in the presence of ADP, AMPPNP, or apyrase. Bars, 5 µm. **(D)** The fluorescence intensity of each motor along microtubules was quantified for each nucleotide condition. The mean fluorescence intensity of each motor in AMPPNP and apyrase was normalized to the mean fluorescence intensity in the ADP state. Data indicate means ± SEM of more than five microtubules from one representative experiment. ***, P < 0.001 as compared with the ADP state (two-tailed *t* test).

Reference

Kull, F.J., E.P. Sablin, R. Lau, R.J. Fletterick, and R.D. Vale. 1996. Crystal Structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature.* 380:550–555.