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Supplementary Information for

Intracellular cargo transport by single-headed kinesin motors

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Other supplementary materials for this manuscript include the following:

Movies S1 to S4

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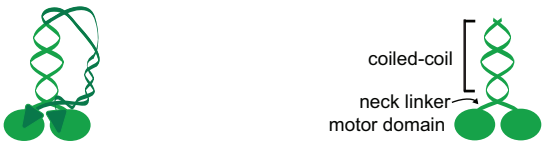

		full-length dimer, autoinhibited	truncated dimer, constitutively active	truncated, artificial monomer
Kinesin-1	KIF5A		aa1-560 (Ref. 2)	aa1-338 (Ref. 5,6)
	KIF5B		aa1-560 (Ref. 2)	aa1-336 (Ref. 5,6)
	KIF5C		aa1-559 (Ref. 1)	aa1-339 (Ref. 5,6)
Kinesin-2	KIF3A		aa1-419 (Ref. 2)	aa1-355 (Ref. 7,8)
	KIF3B		aa1-414 (Ref. 2)	aa1-350 (Ref. 7,8)
	KIF17		aa1-738 (Ref. 3)	aa1-349 (Ref. 3)
Kinesin-3	KIF1A		aa1-393 (Ref. 4,9)	aa1-369 (Ref. 4,9)
	KIF13B		aa1-412 (Ref. 4)	aa1-367 (Ref. 4)
	KIF16B		aa1-400 (Ref. 4)	aa1-372 (Ref. 4)

Fig. S1. Design of dimeric and monomeric kinesin constructs. Schematic of the domain organization of wild-type (full-length dimer, autoinhibited) and truncated (dimer and monomer) kinesin motors. The table lists the amino acid (aa) residues contained in each kinesin construct based on the following: *Rn*KIF5C(1-559) (Ref. 1), *Mm*KIF3A(1-419) and *Mm*KIF3B(1-414) (Ref. 2), *Hs*KIF17(1-738) (Ref. 3), *Rn*KIF1A(1-393)-LZ, *Hs*KIF13B(1-412ΔP), and *Hs*KIF16B(1-400) (Ref. 4). Dimeric versions of *Hs*KIF5A(1-560) and *Hs*KIF5B(1-560) were generated by PCR based on (Ref. 2). Monomeric versions were designed based on identification of coiled-coil neck segments using the COILS program and the following studies: kinesin-1 (Ref. 5, Ref. 6), kinesin-2 (Ref. 3, Ref. 7, Ref. 8), and kinesin-3 (Ref. 4, Ref. 9). In general, monomeric motors are truncated immediately before the start of the coiled-coil so that they contain only the catalytic motor domain and the neck linker.

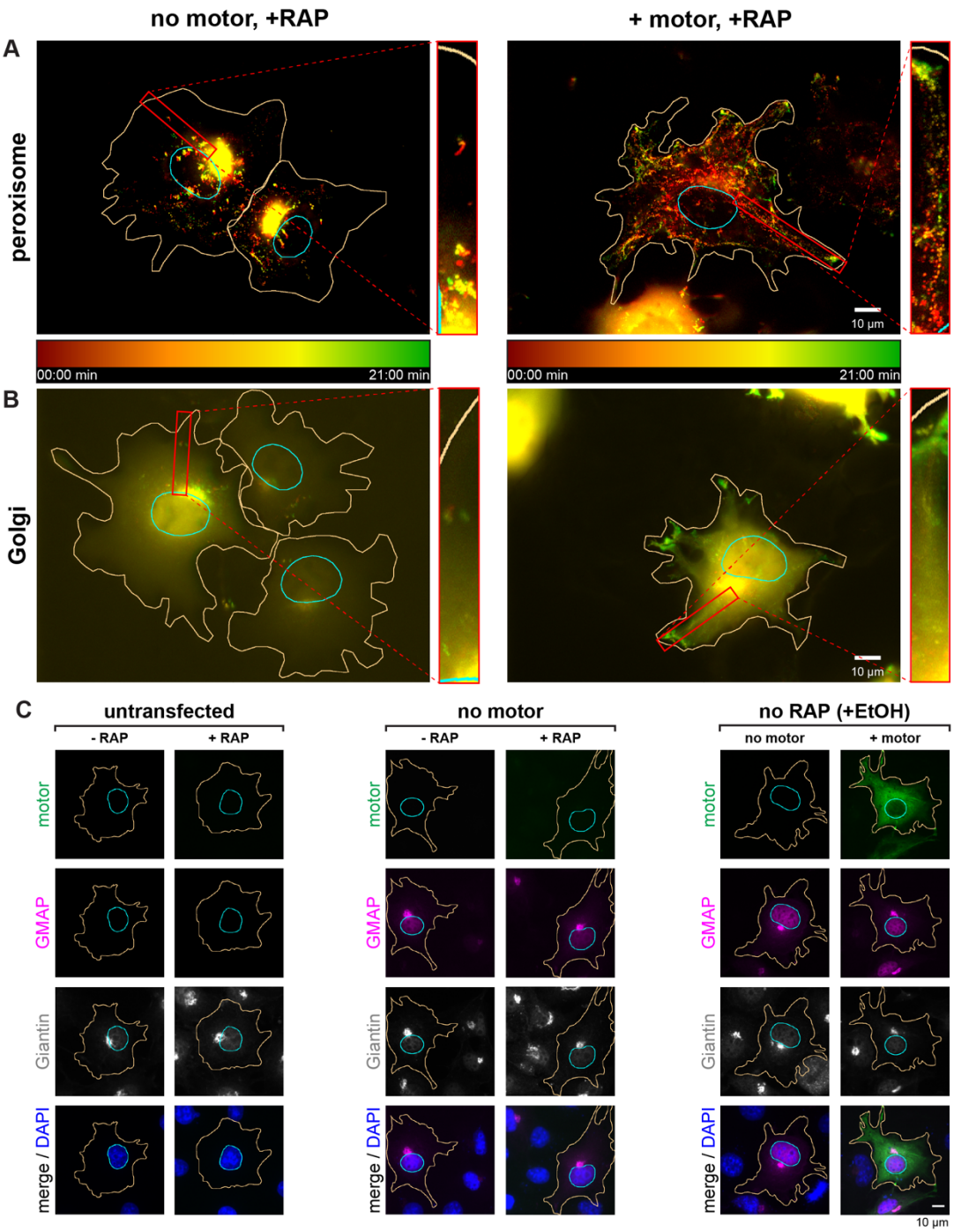

Fig. S2. Peroxisome and Golgi dispersion assays. (A,B) Live-cell imaging in the absence and presence of exogenous kinesin motor. COS-7 cells expressing (left) the cargo markers alone or (right) co-expressed with (A) KIF13B(1-367)-mNG-FRB or (B) KIF5C(1-559)-mNG-FRB were imaged for 20-45 min after rapamycin addition (+RAP) (see Videos S1-S4). The organelle localization for the first 21 minutes of each movie is shown with the indicated temporal-color coding. The cyan line indicates the nucleus and the tan line indicates the periphery of the cell. The red boxed regions are magnified to the right of each image. Scale bar: 10 µm. (C) Control experiments for Golgi dispersion. Cells were fixed and stained with an antibody to the Golgi marker giantin. (Left) Representative images of Golgi localization in untransfected cells untreated (-RAP) or treated (+RAP) for 30 min. (Middle) Representative images of Golgi localization in cells transfected with the Golgi-targeted GMAP210-mRFP-FKBP construct and then untreated (-RAP) or treated (+RAP) for 30 min. (Right) Representative images of cells transfected with the Golgi-targeted GMAP210-mRFP-FKBP construct alone or co-transfected with KIF5C(1-559)-mNG-FRB and treated with ethanol vehicle for 30 min.

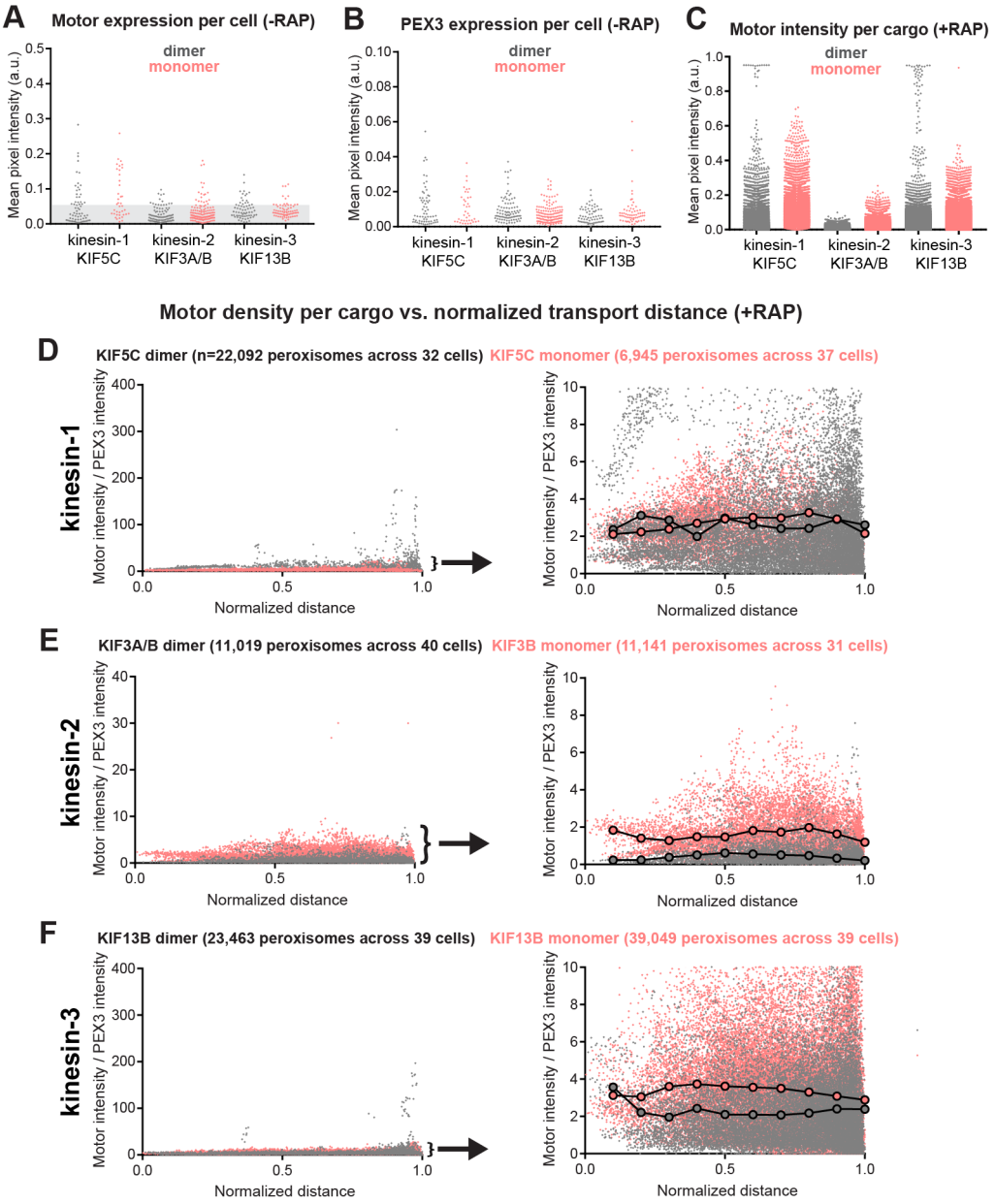

Fig. S3. Quantification of motor expression. COS-7 cells were transfected with plasmids for expressing the peroxisome-targeting PEX3-mRFP-FKBP construct together with the indicated motor-mNG-FRB constructs. (A,B) The mean fluorescence intensity was measured on a per-cell basis. Each spot represents the mean fluorescence intensity of one cell. The gray shaded region indicates cells with low to medium expression levels that were selected for analysis. (C-F) The cells were treated with rapamycin (+RAP) for 7.5 min to achieve partial peroxisome dispersion. The mean fluorescence intensity was measured on a per-cargo basis. Note that because only the KIF3B subunit is tagged with mNG in the dimeric kinesin-2 motor KIF3A/KIF3B, the fluorescence intensities are roughly half those of the other dimeric motors. (C) Each spot represents the mean motor fluorescence intensity on an individual peroxisome. (D-F) The distance traveled by each peroxisome was normalized against the distance to the cell periphery where 0.0 indicates the centroid of the cell and 1.0 indicates the edge of the cell. Each spot represents the mean fluorescence intensity of the motor-mNG-FRB divided by the mean fluorescence intensity of the PEX3-mRFP-FKBP on an individual peroxisome. The outliers in each population represent spots where motors that failed to attach to cargoes accumulated at the edge of the cell. The presence of these outlier values causes the majority (>99.9%) of the data to be constrained along the y-axis and thus these data are replotted on the right where the spots with black outline indicate the mean fluorescence values for every binned 0.1 unit of distance.

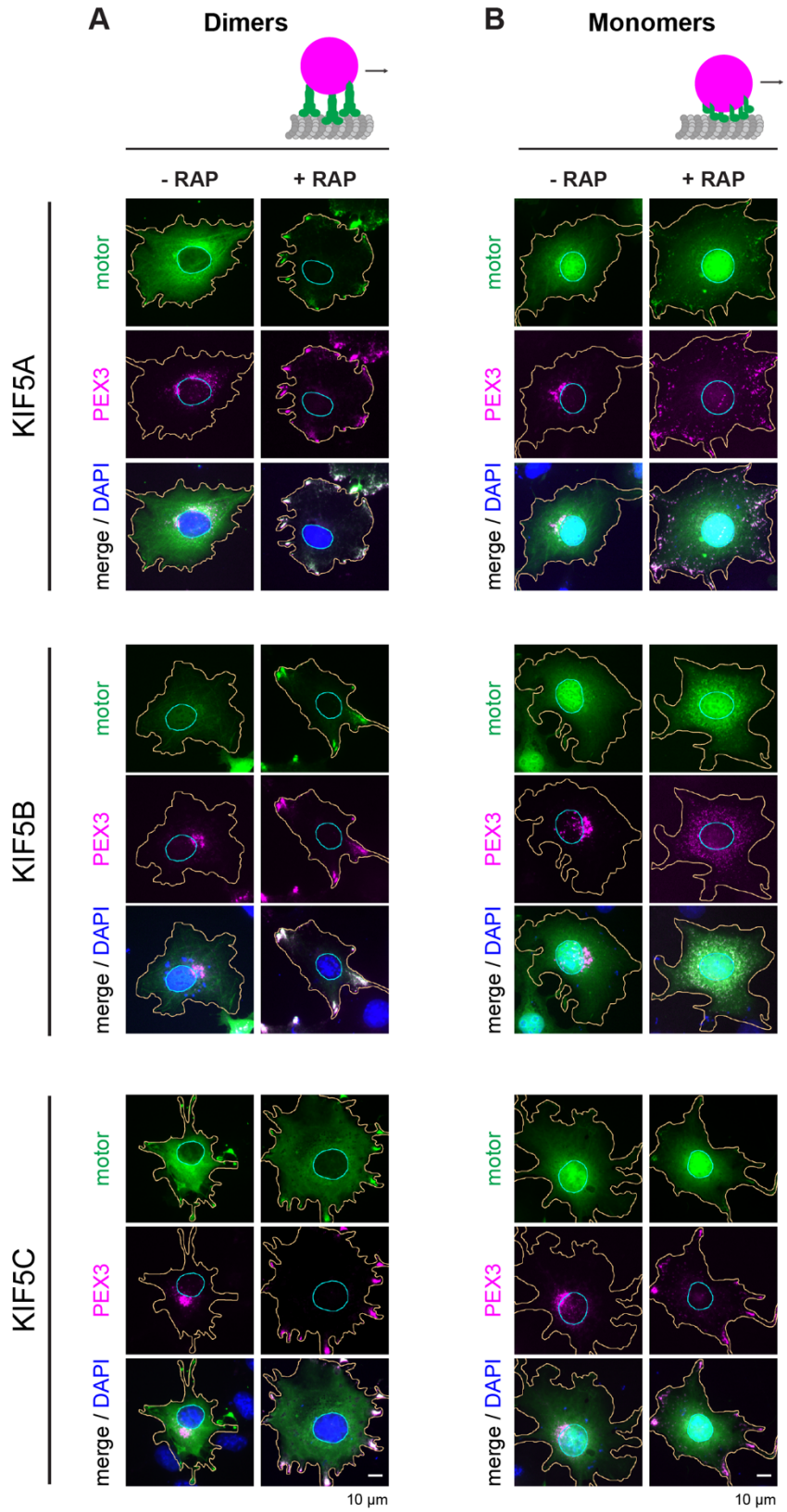

Fig. S4. Peroxisome dispersion assay with kinesin-1 motors (extension of Fig. 2B-C showing individual fluorescence channels). Representative images of fixed COS-7 cells co-expressing PEX3-mRFP-FKBP (magenta) and (A) dimeric or (B) monomeric motor-mNG-FRB (green). Left column: no RAP; right column: 30 min. after RAP addition. Bar: 10 µm.

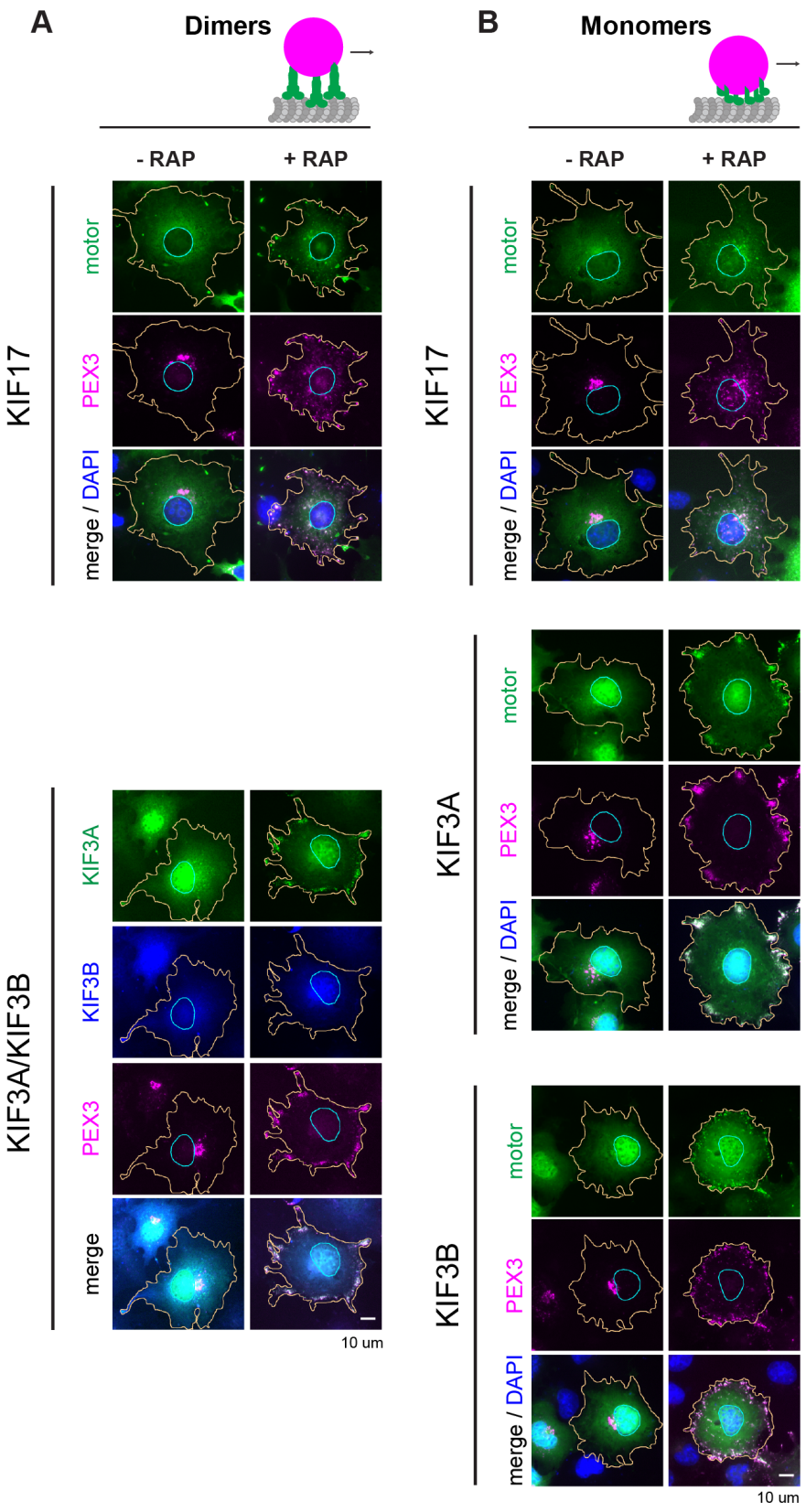

Fig. S5. Peroxisome dispersion assay with kinesin-2 motors (extension of Fig. 2B-C showing individual fluorescence channels). Representative images of fixed COS-7 cells co-expressing PEX3-mRFP-FKBP (magenta) and (A) dimeric or (B) monomeric motor-mNG-FRB (green). Left column: no RAP; right column: 30 min. after RAP addition. Bar: 10 µm.

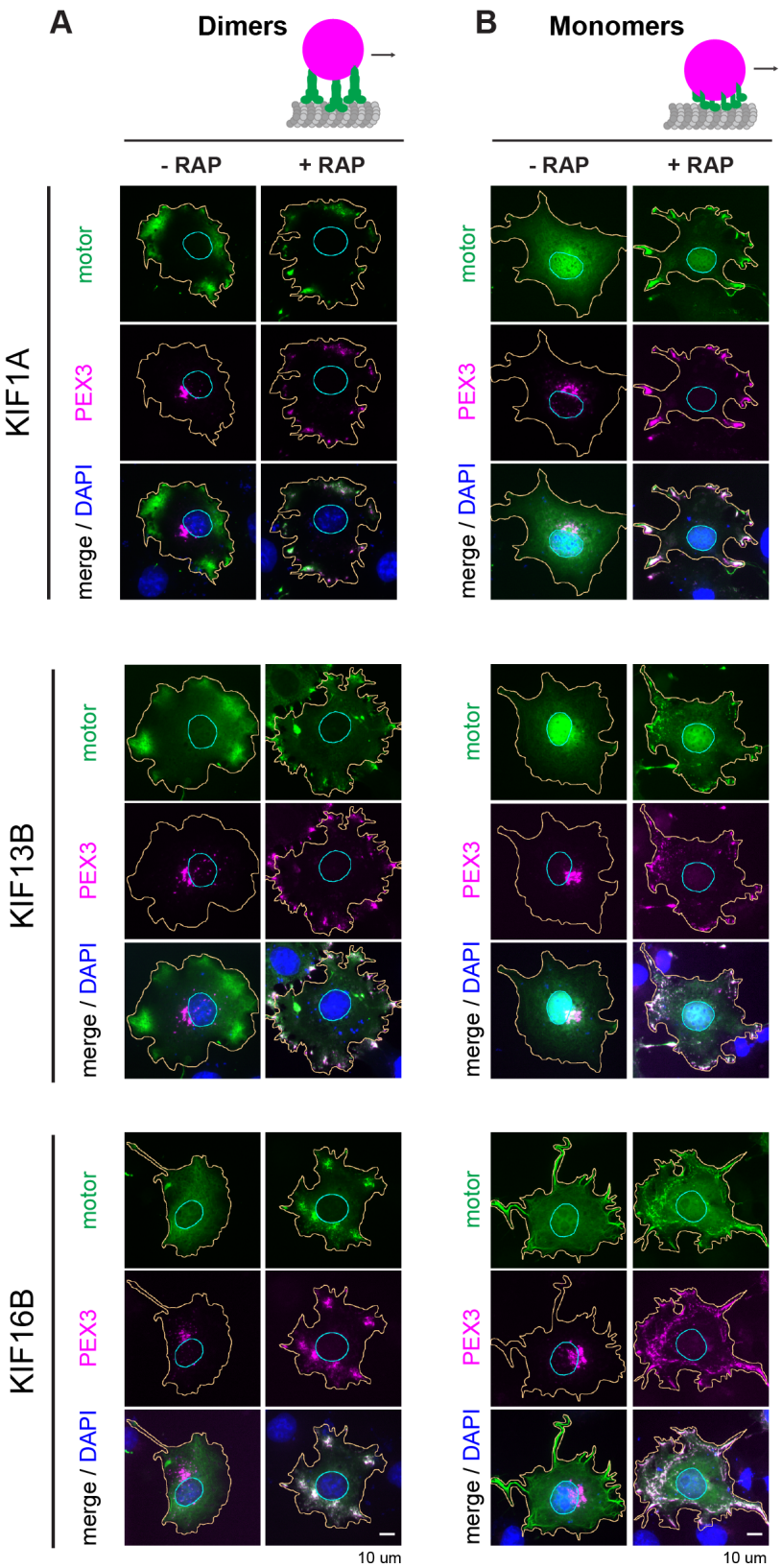

Fig. S6. Peroxisome dispersion assay with kinesin-3 motors (extension of Fig. 2B-C showing individual fluorescence channels). Representative images of fixed COS-7 cells co-expressing PEX3-mRFP-FKBP (magenta) and (A) dimeric or (B) monomeric motor-mNG-FRB (green). Left column: no RAP; right column: 30 min. after RAP addition. Bar: 10 µm.

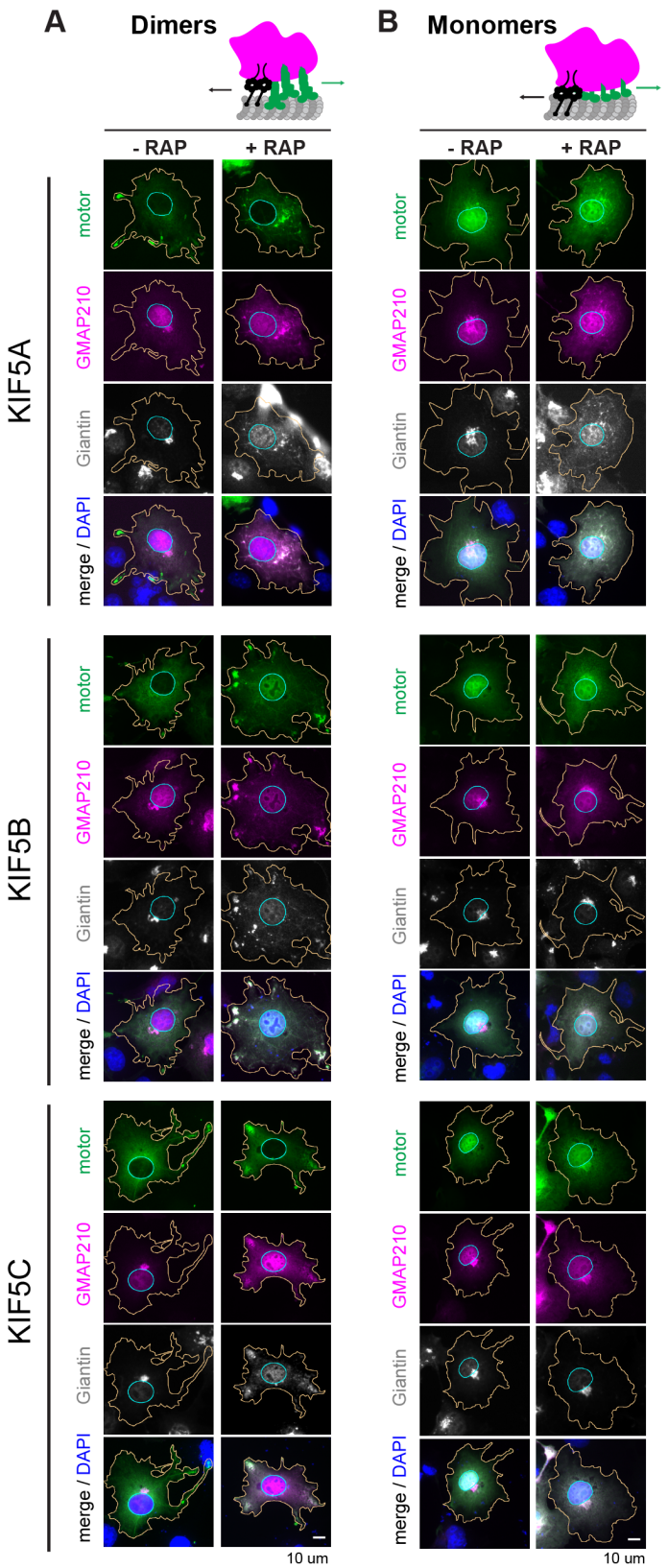

Fig. S7. Golgi dispersion assay with kinesin-1 motors (extension of Fig. 4B-C showing individual fluorescence channels). Representative images of fixed COS-7 cells co-expressing GMAP210-mRFP-FKBP (magenta) and (A) dimeric or (B) monomeric motor-mNG-FRB (green). Left column: no RAP; right column: 30 min. after RAP addition. Bar: 10 µm.

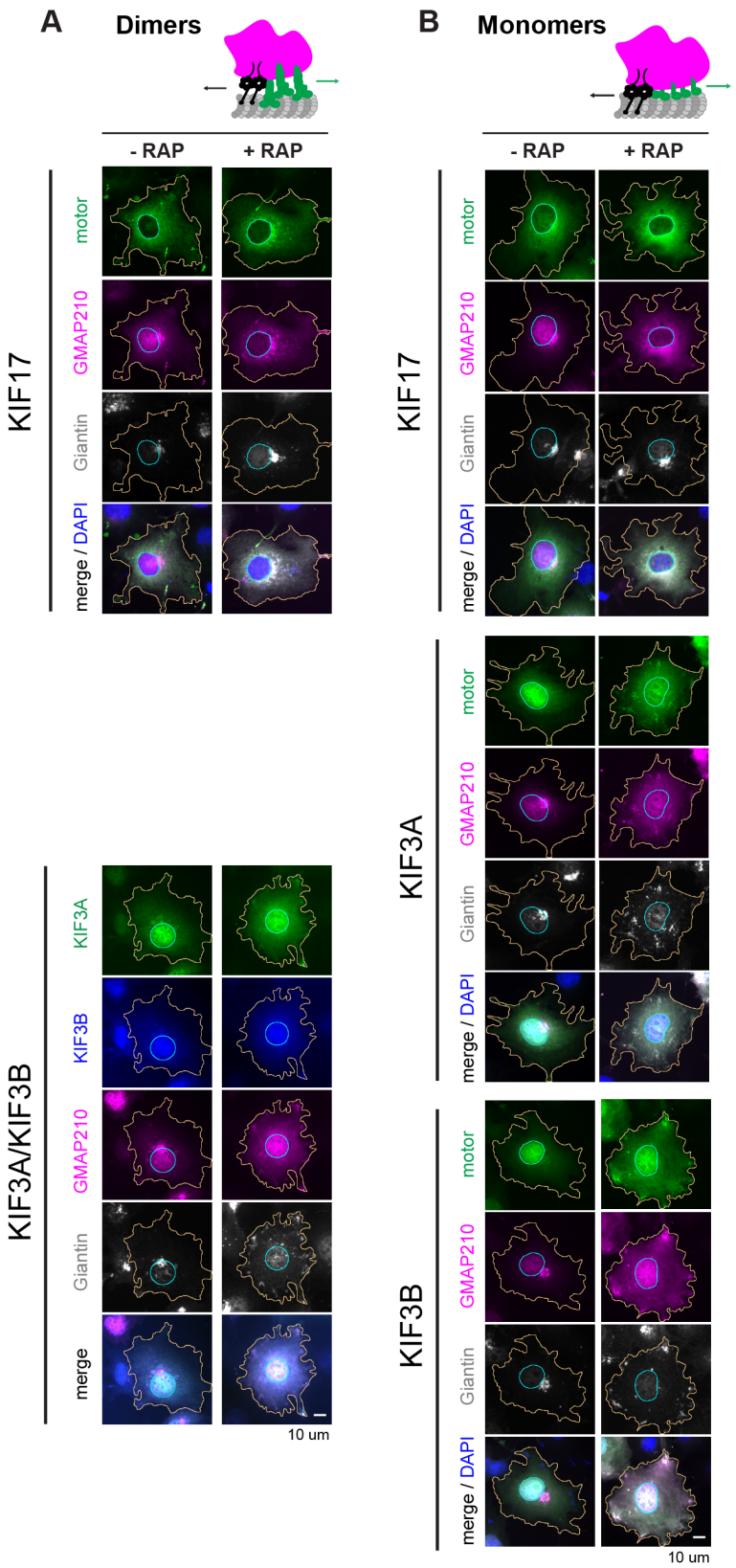

Fig. S8. Golgi dispersion assay with kinesin-2 motors (extension of Fig. 4B-C showing individual fluorescence channels). Representative images of fixed COS-7 cells co-expressing GMAP210-mRFP-FKBP (magenta) and (A) dimeric or (B) monomeric motor-mNG-FRB (green). Left column: no RAP; right column: 30 min. after RAP addition. Bar: 10 µm.

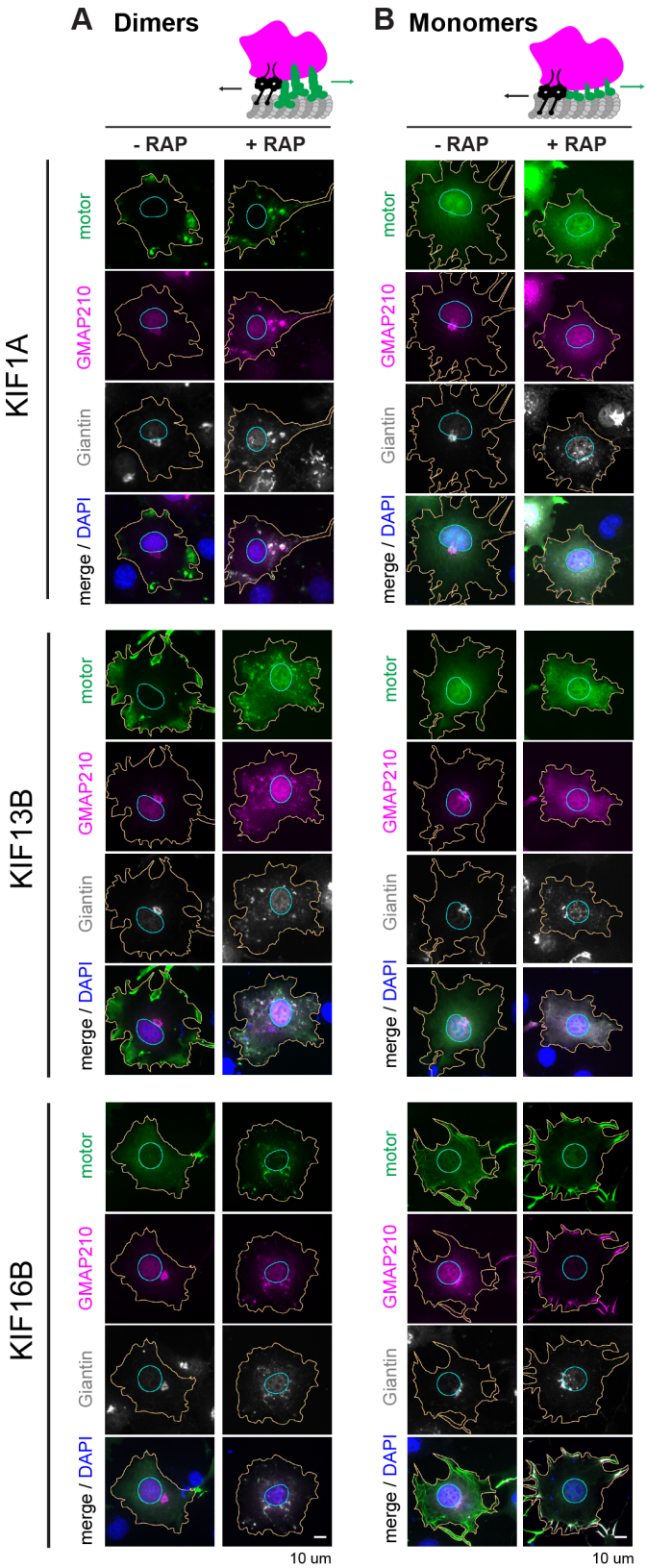

Fig. S9. Golgi dispersion assay with kinesin-3 motors (extension of Fig. 4B-C showing individual fluorescence channels). Representative images of fixed COS-7 cells co-expressing GMAP210-mRFP-FKBP (magenta) and (A) dimeric or (B) monomeric motor-mNG-FRB (green). Left column: no RAP; right column: 30 min. after RAP addition. Bar: 10 µm.

Captions for Movies S1 to S4

Movie S1. Live-cell imaging of peroxisomes in the absence of exogenous kinesin motor. COS-7 cells expressing the peroxisome-targeted module PEX3-mRFP-FKBP were imaged live for 1.5 min and then rapamycin was added (+RAP) and imaging was continued for 43.5 min. The cyan line indicates the nucleus and the tan line indicates the cell periphery. Scale bar: 10 μm.

Movie S2. Live-cell imaging of peroxisome dispersion driven by a monomeric kinesin-3 motor. COS-7 cells expressing the monomeric kinesin-3 motor KIF13B(1-367)-mNG-FRB (green) and the peroxisome-targeted module PEX3-mRFP-FKBP (red) were imaged live for 2 min and then rapamycin was added (+RAP) and imaging was continued for 23 min. Scale bar: 10 μm.

Movie S3. Live-cell imaging of Golgi in the absence of exogenous kinesin motor. COS-7 cells expressing the Golgi-targeted module GMAP210-mRFP-FKBP were imaged live for 15 min and then rapamycin was added (+RAP) and imaging was continued for 30 min. The cyan line indicates the nucleus and the tan line indicates the cell periphery. Scale bar: 10 μm.

Movie S4. Live-cell imaging of Golgi dispersion driven by a dimeric kinesin-1 motor. COS-7 cells expressing the dimeric kinesin-1 motor KIF5C(1-559)-mNG-FRB (green) and the peroxisome-targeted module PEX3-mRFP-FKBP (red) were imaged live for 2 min and then rapamycin was added (+RAP) and imaging was continued for 19 min. The cyan line indicates the nucleus and the tan line indicates the cell periphery. Scale bar: 10 μm.

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