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Supplemental Material and Methods

Preparation of the transfection solutions

Antibodies:

GM130 Alexa Fluor® 647 (#558712, BD Biosciences)

2 µg (1 mg/mL) antibody were complexed together with 1.25 µL Lipofectamine®3000 (Invitrogen) transfection reagent in 2.50 µL OptiMEM, containing 1.82 M trehalose dihydrate, for 5 minutes. Then 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to get a total volume of the transfection solution of 9.375 µL.

alpha tubulin (#3873, Cell Signaling Technology); KIF11 (#ABT149, Merck Millipore); KIF11 (#AP9097a, Abgent); negative control: Anti-rabbit IgG Cy3® (#A10520; Thermo Fisher Scientific*)*

2 µg (1 mg/mL) antibody were complexed together with 2.25 µL Lipofectamine®RNAiMAX (Invitrogen) transfection reagent in 1.50 µL OptiMEM, containing 1.82 M trehalose dihydrate, for 5 minutes. Then 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to get a total volume of the transfection solution of 9.375 µL.

GFP:

Green fluorescent protein (#ab84191, abcam)

1 µg (1 mg/mL) GFP was complexed together with 0.50 µL Lipofectamine®RNAiMAX (Invitrogen) transfection reagent in 4.25 µL OptiMEM, containing 1.82 M trehalose dihydrate, for 5 minutes. Then 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to get a total volume of the transfection solution of 9.375 µL.

siRNA:

KIF11 Silencer Select siRNA (#S7903, ambion), Silencer® Select Negative Control No. 1 (#4390844, ambion)

7.5 pmol (30 µM) siRNA were complexed together with 1.25 µL Lipofectamine®RNAiMAX (Invitrogen) transfection reagent in 2.00 µL OptiMEM, containing 1.82 M trehalose dihydrate, for 5 minutes. Then 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to get a total volume of the transfection solution of 9.375 µL.

cDNA:

CRISPR all-in-one cDNA (Sigma-Aldrich): PLK1 (HS0000306668); KIF11 (HS0000296443); INCENP (HS0000074011)

350 ng cDNA (350 ng/µL) were complexed together with 1.25 µL TransIT-X2® (Mirus Bio) transfection reagent in 3.50 µL OptiMEM, containing 1.82 M trehalose dihydrate, for 5 minutes. Then 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to get a total volume of the transfection solution of 9.375 µL.

Simultaneous co-transfection of Cas9 recombinant protein together with gRNA:

CRISPR gRNA (Sigma-Aldrich): PLK1 (HS0000306668); KIF11 (HS0000296443); INCENP (HS0000074011); non-targeting control (no sequence information available; Gene: CRISPR06, lot number: 06251514MN); recombinant Cas9 nuclease NLS (S. pyogenes; #M0641; New England Biolabs)

40 ng gRNA (40 ng/µL) were pre-incubated with 160 ng (160 ng/µL) of recombinant Cas9 nuclease at RT for 5 minutes. This mix was complexed together with 0.75 µL Lipofectamine®3000 (Invitrogen) transfection reagent in 3.00 µL OptiMEM, containing 1.82 M trehalose dihydrate, for 5 minutes. Then 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to get a total volume of the transfection solution of 9.375 µL.

Mock-coating:

1.50 µL OptiMEM, containing 1.82 M trehalose dihydrate, 4.25 µL $_{dd}H_2O$ and 3.625 µL of a 1 mg/mL collagen type IV solution in $_{dd}H_2O$, (#C5533, Sigma-Aldrich) were added to a well of a 384-multiwell plate to get a total volume of 9.375 µL.

Cell numbers for transfection and incubation times (37°C, 5% CO_2)

	HeLa	HEK293T
Antibodies		
GM130 Alexa Fluor® 647	3.200 cells/80 µL; 20 hours/4 hours	
alpha tubulin	3.200 cells/80 µL; 2 hours/4 hours	
KIF11 (IF) *KIF11* Alexa Fluor® 647 (IF)	3.200 cells/80 µL; 2 hours/4 hours	
KIF11 (phenotype experiment)	1.400 cells/80 µL; 24 hours	
Anti-rabbit IgG Cy3® (negative control)	1.400 cells/80 µL; 24 hours	
Protein		
GFP	1.400 cells/80 µL; 24 hours	
siRNA		
KIF11, negative control	1.400 cells/80 µL; 24 hours	
cDNA		
PLK1/*KIF11*/*INCENP*	800 cells/80 µL; 50 hours	3.000 cells/80 µL; 50 hours
rec. Cas9 + gRNA		
PLK1/*KIF11*/*INCENP*	800 cells/80 µL; 50 hours	3.000 cells/80 µL; 50 hours
Lentivral transduction		
PLK1/*KIF11*/*INCENP*	300 cells/80 µL; 72 hours post transduction	

gRNA target sequences and PCR primer sequences

Gene	Target sequence	Primer sequences
PLK1 (HS0000306668)	TCGGACGCGGACACCAAGGAGG	fw: GCTTTTGTAACGTTCCCAGC rv: TAGCGTCCATTACCAGCACC
KIF11 (HS0000296443)	CCTAATGAAGAGTATACCTGGG	fw: ACTGTGCCTGGCTTGTTTTTTG rv: CTTCAATACAAAGCCCTCCC
INCENP (HS0000074011)	CCGACGGAAGAAGAGACGGATT	fw: GGGCTCCCTGCTGTATACTG rv: CAAAGCCGTACTGCTCTGCT
Non-targeting negative control #	*no sequence information available; Gene: CRISPR06, lot number: 06251514MN*	

Antibodies/GFP

Antibody	Species	#	Manufacturer
GM130 Alexa Fluor® 647	mouse	558712; Custom formulation without BSA	BD Biosciences
alpha tubulin	mouse	3873	Cell Signaling Technology
KIF11	rabbit	ABT149; Lot: 2606356; Lot: 2702413 (Labeled to Alexa Fluor® 647); Lot: 2851238	Merck Millipore
KIF11	rabbit	AP9097a	Abgent
EEA1	mouse	610456	BD Biosciences
Anti-mouse IgG AlexaFluor® 488	goat	A11029	Thermo Fisher Scientific
Anti-rabbit IgG Cy3®	goat	A10520	Thermo Fisher Scientific
Green fluorescent protein	Aequorea victoria; produced in E.coli	ab84191	Abcam