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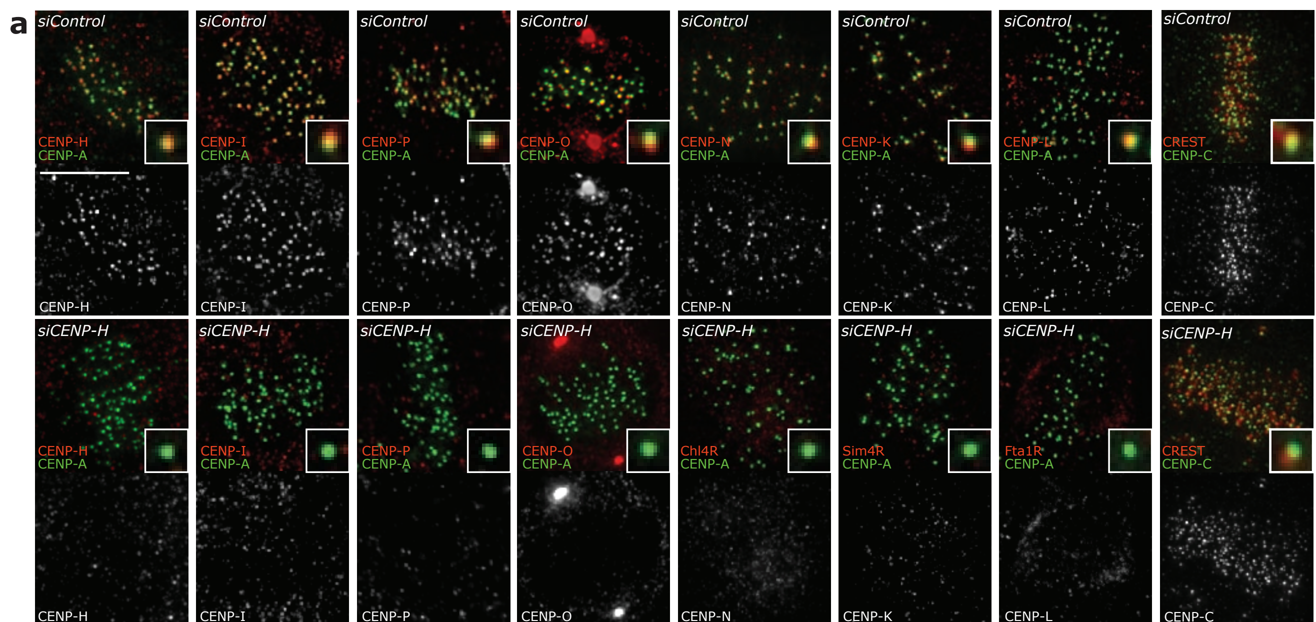

Figure S1 CENP-H depletion impairs the localization of the whole CENP-A NAC/CAD complex. (**a**) Representative images of cells treated with *siControl* or *siCENP-H* RNAs stained with CENP-A (green) or CREST antisera (red) and CENP-H, CENP-I, CENP-P, CENP-O, CENP-N, CENP-K or CENP-L antisera (red) or CENP-C (green). Insets show higher magnification views of a single kinetochore. Scale bar = 10 μm

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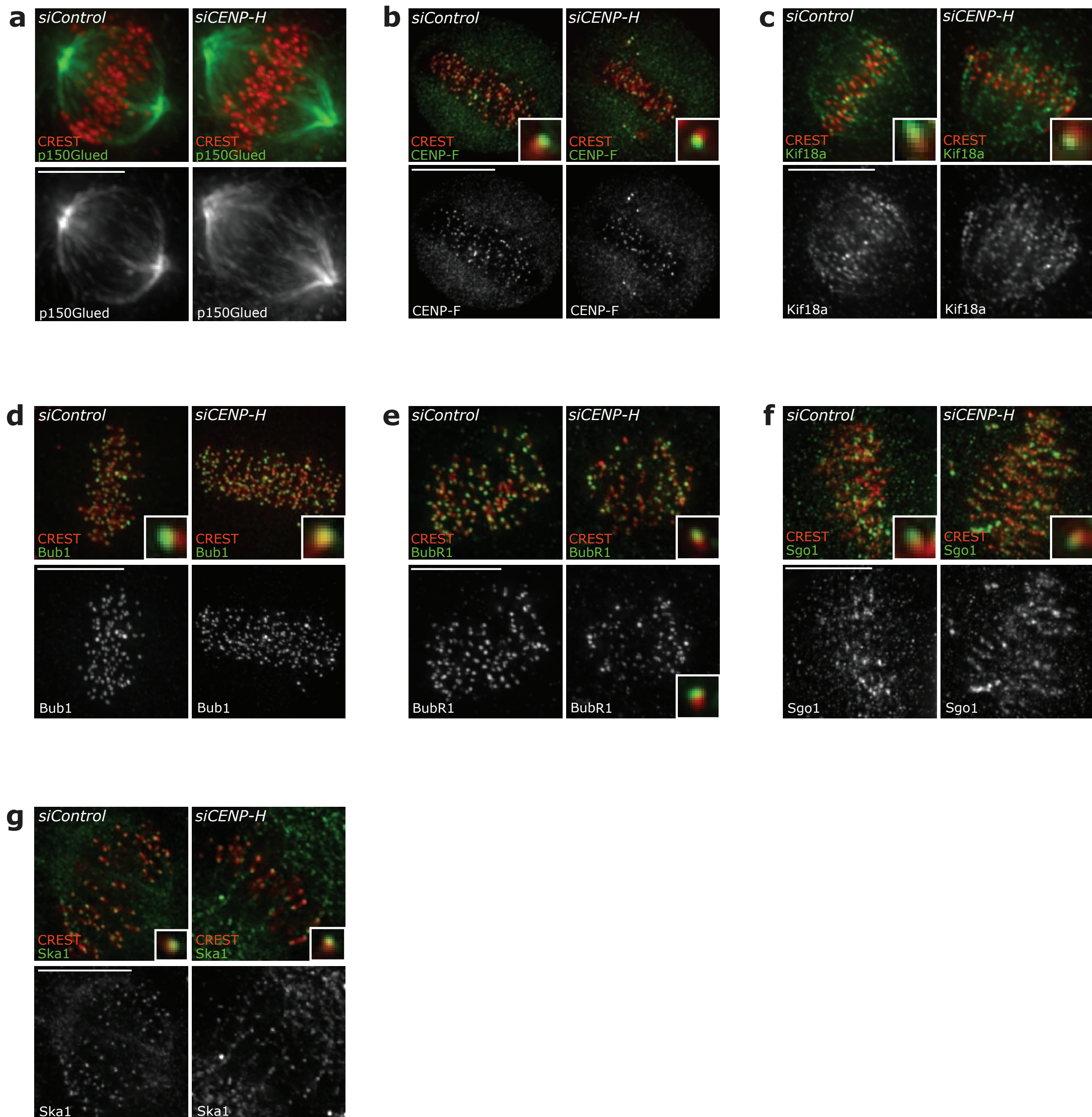

Figure S2 CENP-H depletion does not have affect the binding of other key kinetochore proteins. (**a-g**) Representative images of *siControl* or *siCENP-H* RNA-treated cells stained with CREST (red) and p150Glued (**a**), CENP-F (**b**), Kif18a (**c**), Bub1 (**d**), BubR1 (**e**), Sgo1 (**f**) or Ska1 (**g**) antisera (green). Insets show higher magnification views of a single kinetochore. Scale bars = 10 μm

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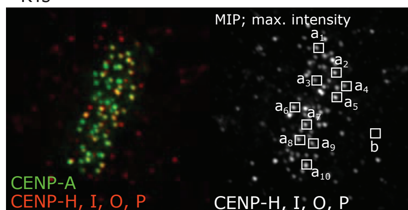
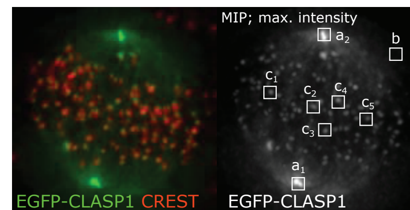
a CENP-H, CENP-I, CENP-O and CENP-P kinetochore intensities

$I_{KTs} = \bar{a} - b$



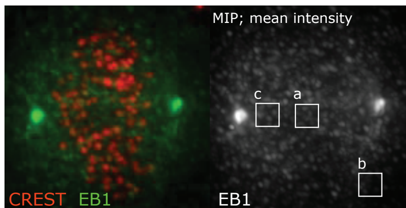
b EGFP-CLASP1 intensity at the KTs relative to the spindle poles

$I_{KTs} = (\bar{c} - b) / (\bar{a} - b)$



c EB1 intensity at the metaphase plate relative to the spindle

$I_{PLATE} = (a - b) / (c - b)$



d Acetylated tubulin intensity relative to α-tubulin (based on mean intensity projections; mean intensity)

$I = a / b$

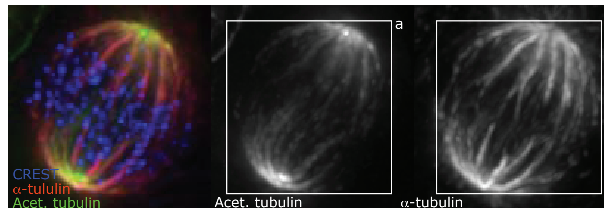


Figure S3 Immunofluorescence quantifications**.** (**a**) Scheme for the quantification of the intensity of CENP-H, CENP-I, CENP-P, CENP-O, CENP-L, MCAK and Nnf1R levels at kinetochores. (**b**) Scheme for the quantification of the intensity of EGFP-CLASP1 at the kinetochores. Intensities were normalized with the poles for each cell. (**c**) Scheme for the quantification of the intensity of EB1 at kinetochores. Intensities were normalized with the spindle intensity. (**d**) Scheme for the quantification of the intensity of acetylated α-tubulin. The intensities were normalized with the total α-tubulin intensity. Scale bar = 10 μm

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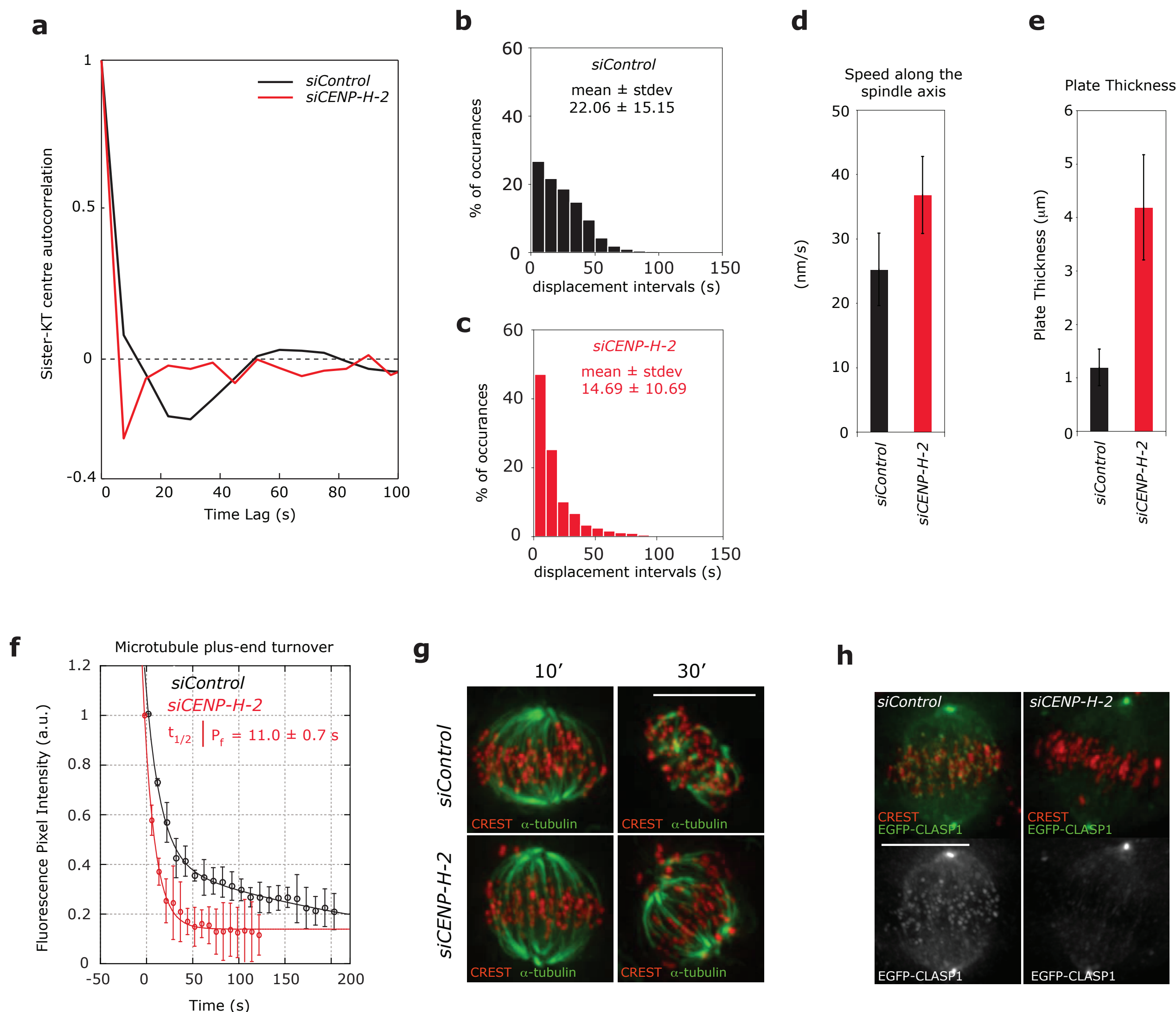

Figure S4 Key experiments with an alternative CENP-H siRNA oligonucleotide (*siCENP-H-2*) confirm the results of this study (*siCENP-H*). (**a**) Autocorrelation function of translational sister-kinetochore movements along the spindle axis (kinetochore oscillation) of *siControl* (black line; same as in Figure 1b) and *siCENP-H-2* (red line). The autocorrelation function was calculated by combining all aligned sister-kinetochore pairs for each condition (see Supplementary Table 2). (**b-c**) Histograms of the mean interval time between directional switches of the sister-kinetochore pairs along the spindle axis of *siControl* (black bars, same as in Figure 1b) and *siCENP-H-2* (red bars). For each condition, the mean values and SDs are indicated. (**d**) Average sister-kinetochore pair speed along the spindle axis of *siControl* (black bar; same as in Figure 1b) and *siCENP-H-2* (red bar). Error bars represent SD based on n = independent experiments. (**e**) Width of the metaphase plate of *siControl* (black bar; same as in Figure 1b) and *siCENP-H-2* (red bar). Error bars represent SD based on n = independent experiments. (**f**) Representative images of *siControl* and *siCENP-H-2* cold-treated cells for 10 or 30 min stained with CREST antisera (kinetochores; red) and α-tubulin antibodies (MTs; green). (**g**) Fluorescence intensity decay of the photoactivated regions over time in *siControl* (black; same as in Figure 2) and *siCENP-H-2* (red) treated PAGFP-α-tubulin/H2B-mRFP cells. Curve fitting indicated that the decay in CENP-H-2 depleted cells fitted to a curve with single exponential with the indicated half-life. Error bars represent SD (**h**) Representative images of HeLa cells stably expressing EGFP-CLASP1 treated with *siControl* or *siCENP-H-2* RNAs and stained with CREST antisera (kinetochores; red). Scale bars = 10 μm

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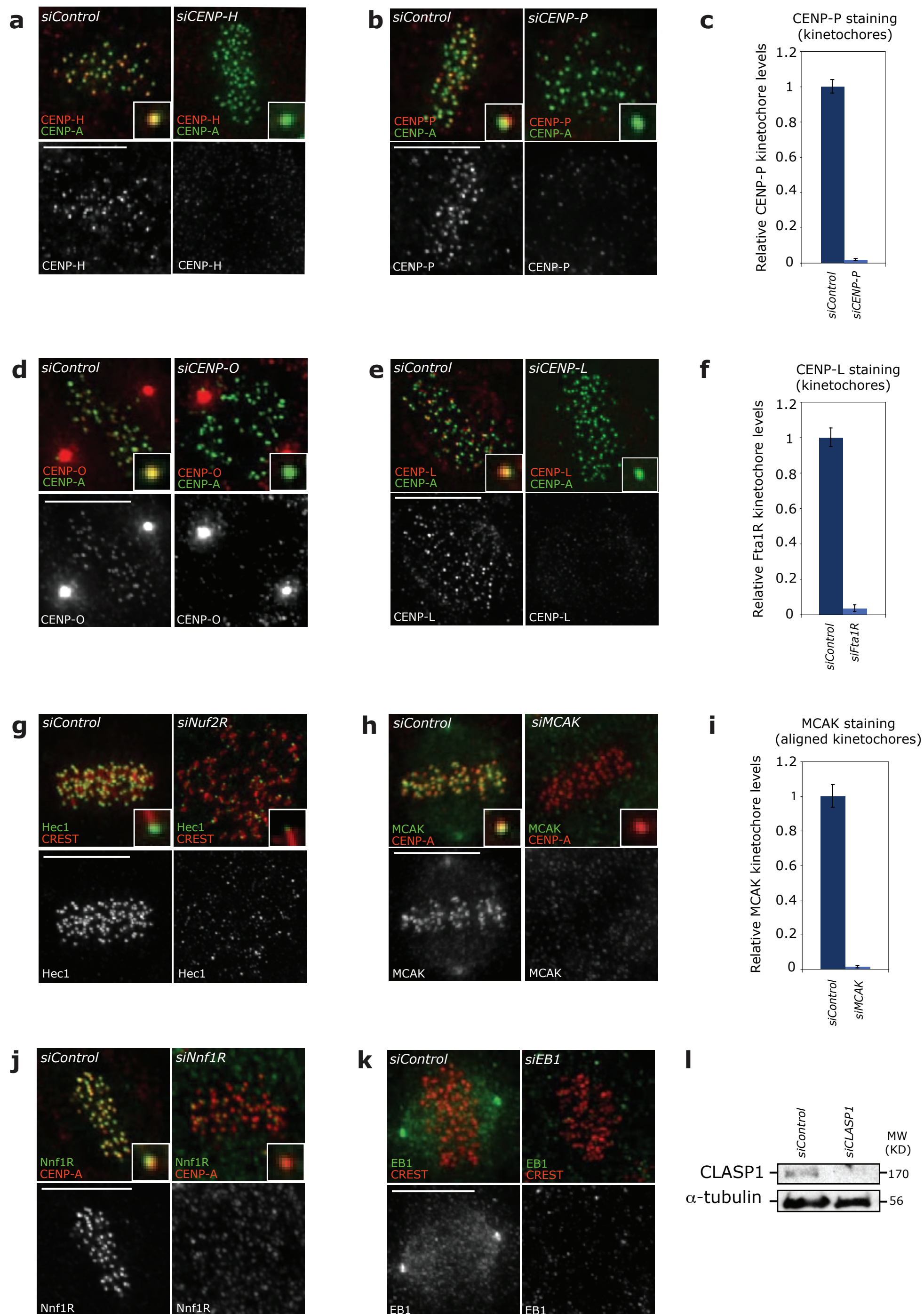

Figure S5 siRNA depletions and antibody validations (**a, b, d, e, g, h, j, k**) Representative images of *siControl* and *siCENP-H* (**a**), *siCENP-P* (**b**), *siCENP-O* (**d**), *siCENP-L* (**e**), *siNuf2R* (**g**), *siMCAK* (**h**), *siNnf1R* (**j**) or *siEB1* (**k**) RNA-treated cells stained with CREST or CENP-A (red) and CENP-H (**a**), CENP-P (**b**), CENP-O (**d**), CENP-L (**e**), Nuf2R (g), MCAK (**h**), Nnf1R (**j**) or EB1 (**k**) antisera (green). Insets show higher magnification views of a single kinetochore. (**c, f, i**) Immunofluorescence quantification of CENP-P (**c**), CENP-L (**f**) and MCAK (**i**) levels on aligned kinetochores after treatment with *siControl* and *siCENP-P*, *siCENP-L* or *siMCAK* RNAs from images such as shown in (b, e, h). Error bars indicate SEM (**l**) Validation of CLASP1 depletion. Immunoblots of whole cell lysates treated with siRNAs as indicated and probed with CLASP1 or α-tubulin antibodies are shown.

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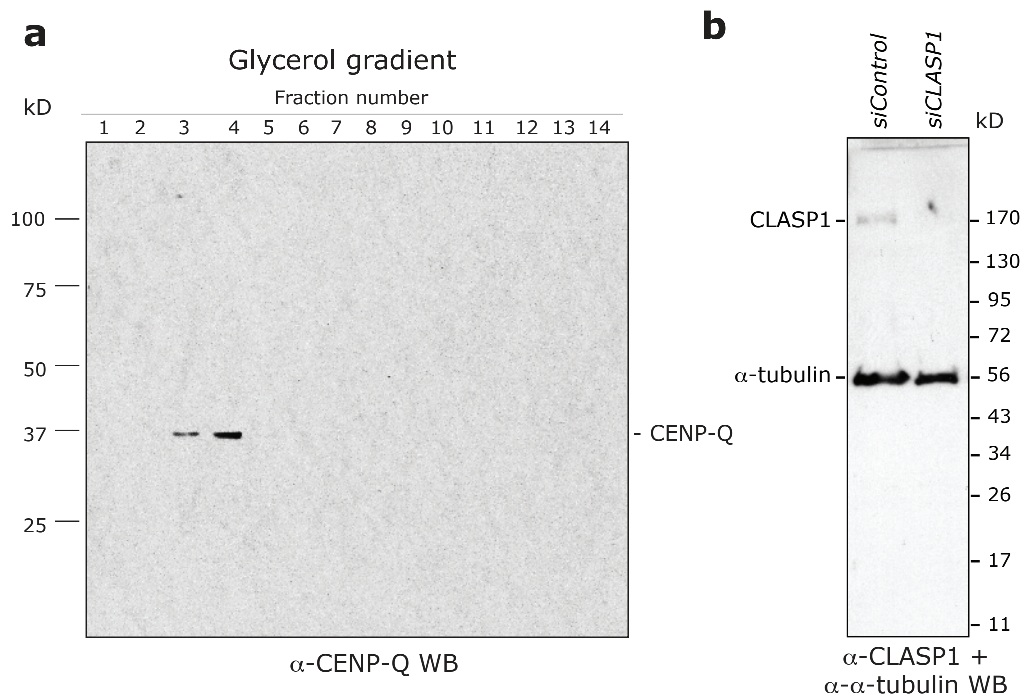

Figure S6 Top-bottom gels of cropped immunoblots (**a**) Immunoblot of 5-40% glycerol gradient shown in Figure 7d probed with anti-CENP-Q antibodies. (**b**) Validation of CLASP1 depletion. Immunoblots of whole cell lysates treated with siRNAs as indicated and probed with CLASP1 or α-tubulin antibodies are shown. The cropped version is shown in Supplementary Figure S5I.

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Supplementrary movie legends

Supplementary Video S1: Poleward MT flux and tubulin turnover following photoactivation of PA-GFP-α-tubulin in a Control-depleted cell.
Supplementary Video S2: Poleward MT flux and tubulin turnover following photoactivation of PA-GFP-α-tubulin in a CENP-H-depleted cell.
Supplementary Video S3: Oscillating sister-kinetochore pair in an EGFP-CENP-I stable cell line.

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Supplementary Tables

Supplementary Table 1. Qualitative levels of EB1 and CLASP1 at the kinetochores[a]

Protein/Condition	*siControl*	*siNuf2R*	*siNnf1R*	*siCENP-H*
EB1	+++[e]	+++	++[d]	++
CLASP1	+++	+++	++	-[b]

[a]as determined by immunofluorescence
[b]no signal
[c]weak signal
[d]reduced signal compared to control cells
[e]normal signal

Supplementary Table 2. Number of imaged cells and sister kinetochore pairs used for the kinetochore tracking analysis

Condition	**N^o of cells**	**N^o sister KT pairs**
siControl	95	2636
siCENP-H	112	3573
siCENP-H-2	41	1213
Fixed Cells	48	1533

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