

Description of Additional Supplementary Files

File name: Supplementary Data 1

Description: Numerical values of the represented data.

File name: Supplementary Movie 1

Description: Mitosis in cells expressing GFP-CENP-E T422A. Spinning-disk confocal live-cell imaging of stable U2OS cell lines with inducible expression of GFP-CENP-E WT or T422A (grey and green), infected with AV-H2B-RFP for DNA visualization (blue) and tubulin stained using SiR-tubulin (red). Time: hour:min.

File name: Supplementary Movie 2

Description: CENP-E localization upon Aurora A inhibition in STLC- induced monopolar spindles. Spinning-disk confocal live-cell imaging of U2OS GFP CENP-E WT and T422A cells (CENP-E in grey and green), infected with AV-H2B-RFP for DNA visualization (blue) and stained for tubulin using SiR-tubulin (red), undergoing the indicated treatments. Time: hour:min.

File name: Supplementary Movie 3

Description: CENP-E T422A stripping from kinetochores. High temporal resolution spinning-disk confocal live-cell imaging of early mitosis U2OS GFP-CENP-E T422A cells (CENP-E T422A in green), infected with AV-H2B-RFP for DNA visualization (magenta). Arrowheads highlight poleward transport of a CENP-E T422A comet. Time: min:sec.

File name: Supplementary Movie 4

Description: CENP-E WT and T422A localization in nocodazole-treated cells entering mitosis. Spinning-disk confocal live-cell imaging of stable U2OS cell lines with inducible expression of GFP-CENP-E WT or T422A (grey and green), infected with AV-H2B-RFP for DNA visualization (blue) and stained for tubulin using SiR-tubulin (red), entering mitosis in presence of nocodazole. Time: hour:min.

File name: Supplementary Movie 5

Description: Mitosis in Spindly-depleted GFP-CENP-E T422A cells. Spinning-disk confocal live-cell imaging of control and Spindly-depleted stable U2OS cell lines with inducible expression of GFP-CENP-E WT or T422A (grey and green), infected with AV-H2B-RFP for DNA visualization (blue) and stained for tubulin using SiR-tubulin (red). Time: hour:min.

File name: Supplementary Movie 6

Description: Mitosis in CENP-E-depleted DHC-GFP cells. Spinning-disk confocal live-cell imaging of HeLa cells stably expressing DHC-GFP (left) and CENP-A-mCherry (center), with SiR-DNA used for DNA staining (right). Time: hour:min.

File name: Supplementary Movie 7

Description: CENP-E localization in metaphase with perturbed MT-flux. Spinning-disk confocal live-cell imaging of stable U2OS cell line with inducible expression of GFP-CENP-E WT (grey and green), infected with AV-H2B-RFP for DNA visualization (blue) and stained for tubulin using SiR-tubulin (red), undergoing the indicated treatments. Time: hour:min.

File name: Supplementary Movie 8

Description: Mitosis in GFP-CENP-E WT and T422A cells upon CENP-E- inhibition by Cmpd-A. Spinning-disk confocal live-cell imaging of stable U2OS cell lines with inducible expression of GFP-

CENP-E WT or T422A (grey and green), infected with AV- H2B-RFP for DNA visualization (blue) and stained for tubulin using SiR-tubulin (red), undergoing the indicated treatments. Time: hour:min.

File name: Supplementary Movie 9

Description: Mitosis in GFP-CENP-E WT and T422A cells upon CENP-E- inhibition. Spinning-disk confocal live-cell imaging of stable U2OS cell lines with inducible expression of GFP-CENP-E WT or T422A (grey and green), infected with AV-H2B-RFP for DNA visualization (blue) and stained for tubulin using SiR-tubulin (red), undergoing the indicated treatments. Time: hour:min.