

Description of Additional Supplementary Files:

Supplementary Movie 1: Lattice-light sheet microscopy (LLSM)-based assay for largescale live cell imaging.

Supplementary Movie 2: RPE1 cells stably expressing CENP-A-GFP and Centrin1-GFP in control and CENP-E reactivated conditions, imaged by LLSM.

Supplementary Movie 3: RPE1 cells stably expressing CENP-A-GFP and Centrin1-GFP in CENP-E depleted and CENP-E inhibited conditions, imaged by LLSM.

Supplementary Movie 4: RPE1 cells stably expressing CENP-A-mCerulean and Mad2- mRuby following reactivation of CENP-E activity, imaged by confocal microscopy.

Supplementary Movie 5: RPE1 cells stably expressing CENP-A-GFP and Centrin1-GFP in CENP-E inhibited condition following addition of inhibitors of Aurora kinases, and in CENP-E reactivated condition following depletion of BubR1 and addition of inhibitor of phosphatases, imaged by confocal microscopy.