Auckland et al., https://doi.org/10.1083/jcb.201607096

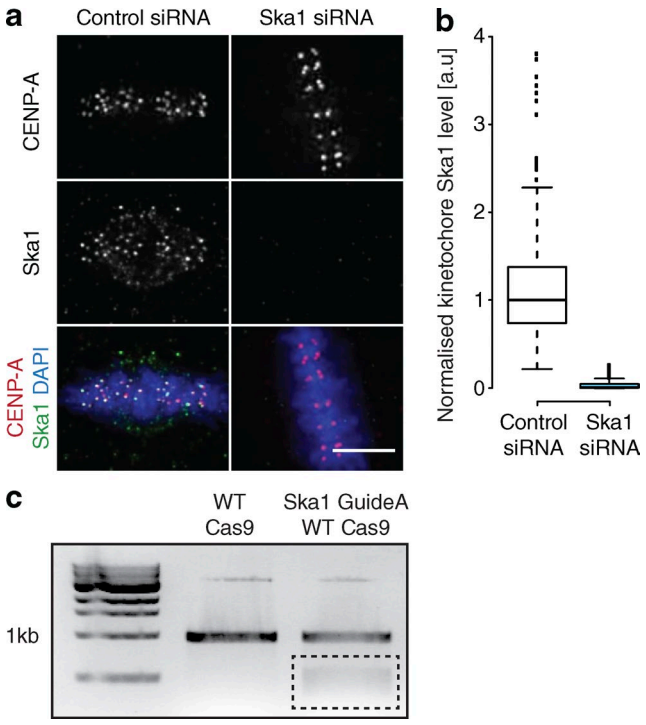

Figure S1. **Effectiveness of siRNA and CRISPR/Cas9 targeting Ska1.** (a) Immunofluorescence images of HeLa K cells treated with either control or Ska1 siRNA for 48 h and stained with DAPI and antibodies against CENP-A and Ska1. Bar, 5 µm. (b) Quantification of Ska1 kinetochore staining intensity relative to CENP-A in HeLa cells treated with either control or Ska1 siRNA. Bar, 5 µm. *n* = 200 KT from 20 cells per condition. (c) Inverted image of the T7 nuclease digest products (ran on 2% agarose gel) produced during the Surveyor assay to confirm guide-induced indel mutation. See Materials and methods for further details.

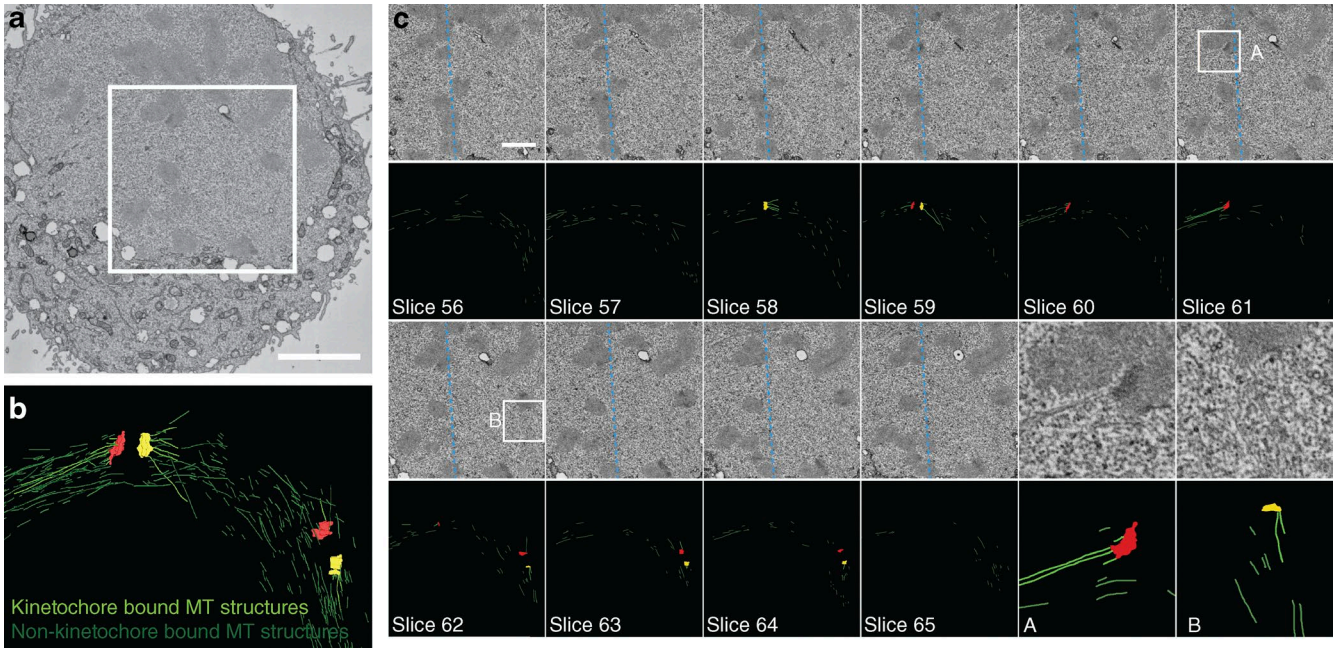

Figure S2. **Slice by slice representation of the EM and its associated render for the kinetochore pairs shown in Fig. 6.** (a) A single slice from a SBF-SEM image of a prometaphase HeLa cell. White box, region used to render microtubule attachment at the aligned bioriented and unaligned bioriented kinetochore pairs shown in panels b and c and Fig. 6. Bar, 5 µm. (b) *z*-Projection (slices 56–65) of the kinetochore and microtubule render generated from an aligned bioriented and unaligned bioriented kinetochore pair in the cell depicted in a. Kinetochore-attached and non–kinetochore-attached microtubule structures are indicated in light green and dark green, respectively. (c) Slice-by-slice representation of the SBF-SEM image and its associated render for the kinetochore pairs depicted in b and Fig. 6. Zoom boxes show microtubule structures terminating at the kinetochores. Kinetochore-attached and non–kinetochore-attached microtubule structures are indicated in light green and dark green, respectively. Bar, 1 µm.

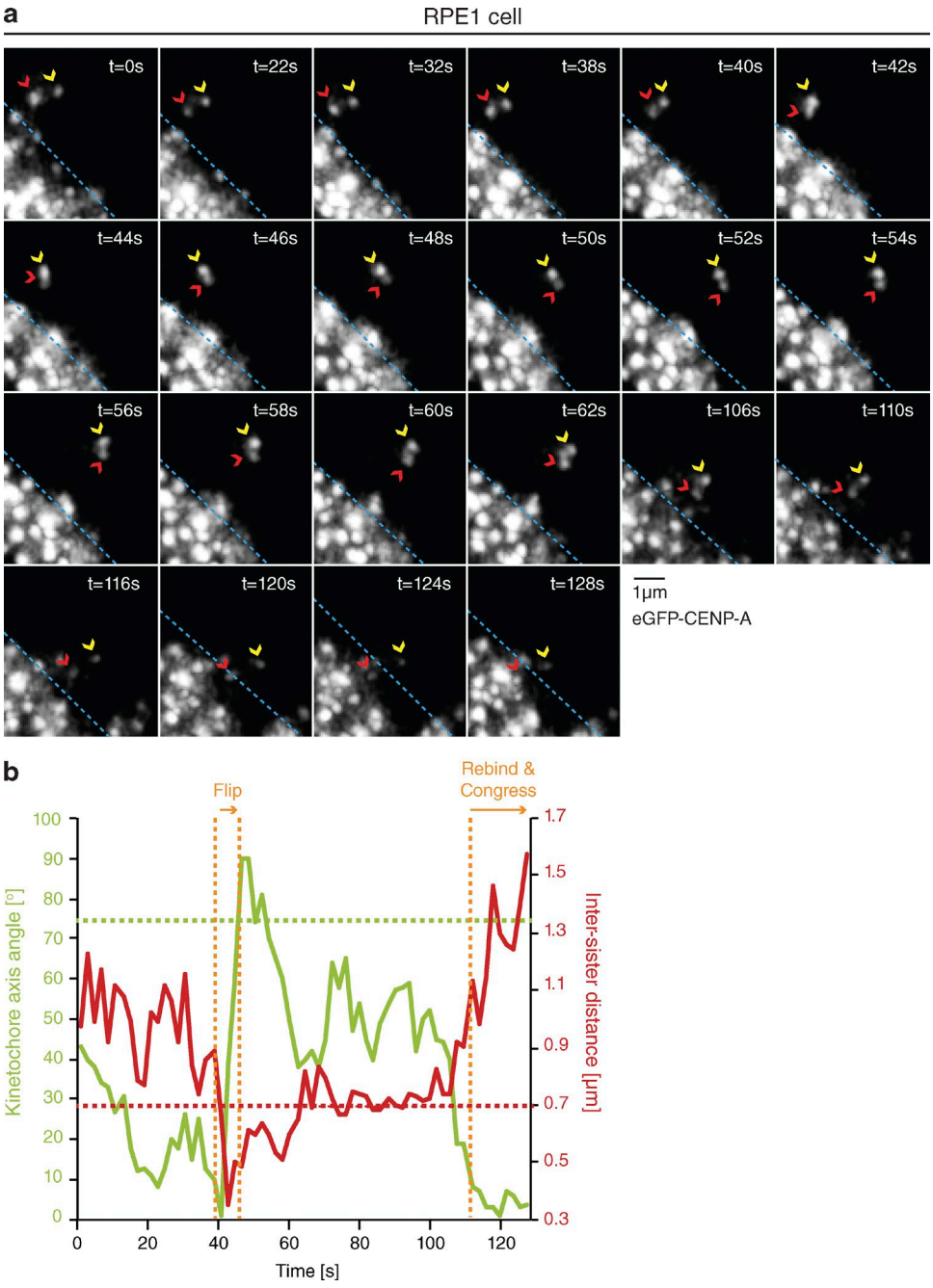

Figure S3. **Example flip event from an untreated RPE1 cell.** (a) Stills of an unaligned kinetochore pair undergoing a flip event in an hTERT RPE1 cell expressing eGFP-CENP-A. Red and yellow arrows, P and AP kinetochores; dotted blue line, metaphase plate periphery. Bar, 1 µm. (b) Track of K–K axis angle and 3D intersister distance over time for the flipping kinetochore pair depicted in a. See Fig. 7 h for a full description of this tracking. Dotted green line, K–K axis angle of 75° (where the sister pair is in a nonoriented state); dotted red line, resting intersister distance of 700 nm.

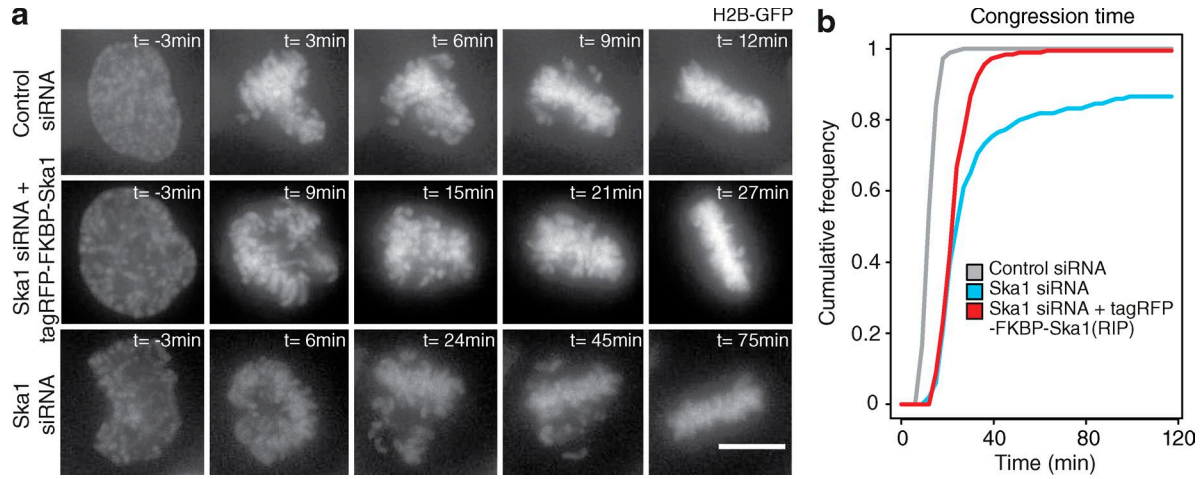

Figure S4. **Changes in eGFP-CENP-A signal do not contribute to the observed increase in Ska1 at congressed kinetochore pairs.** (a) Stills of congressing chromosomes labeling with H2B-GFP in cells treated with control siRNA, Ska1 siRNA, or Ska1 siRNA and rescued with a protected tagRFP-FKBP-Ska1 transgene. Bar, 10 µm. (b) Cumulative frequency plot of congression timing in cells treated with control siRNA, Ska1 siRNA, or Ska1 siRNA and rescued with a protected tagRFP-FKBP-Ska1 transgene.

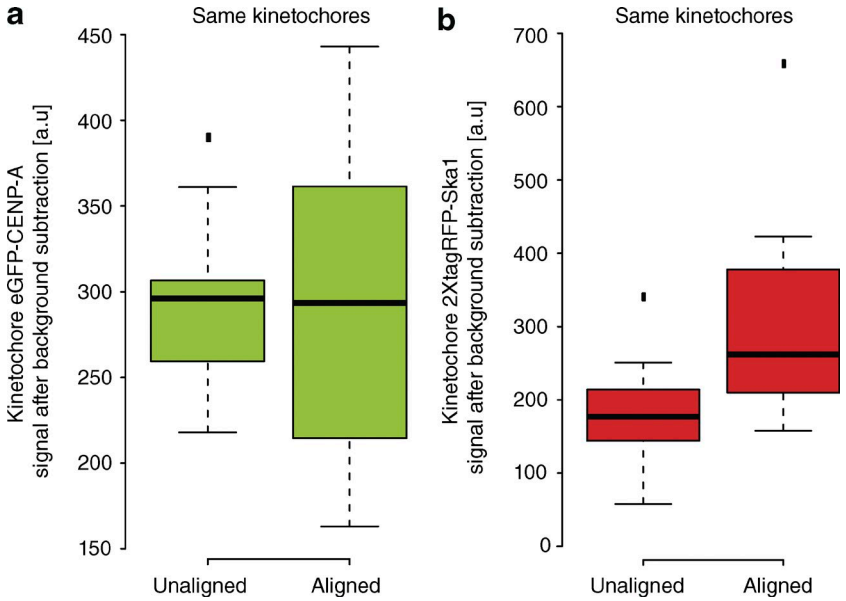

Figure S5. **Quantification of eGFP-CENP-A and tagRFP intensity.** (a) Quantification of eGFP-CENP-A intensity after background subtraction at the kinetochores quantified for Ska complex loading during congression in Fig. 8 f. (b) Quantification of tagRFP intensity after background subtraction at the kinetochores quantified for Ska complex loading during congression in Fig. 8 f. This, together with panel a, demonstrates that an increase in tagRFP intensity, and not changes in eGFP-CENP-A intensity, are responsible for the calculated increase in Ska complex at these kinetochores.