A kinesin-based approach for inducing chromosome-specific mis-segregation in human cells

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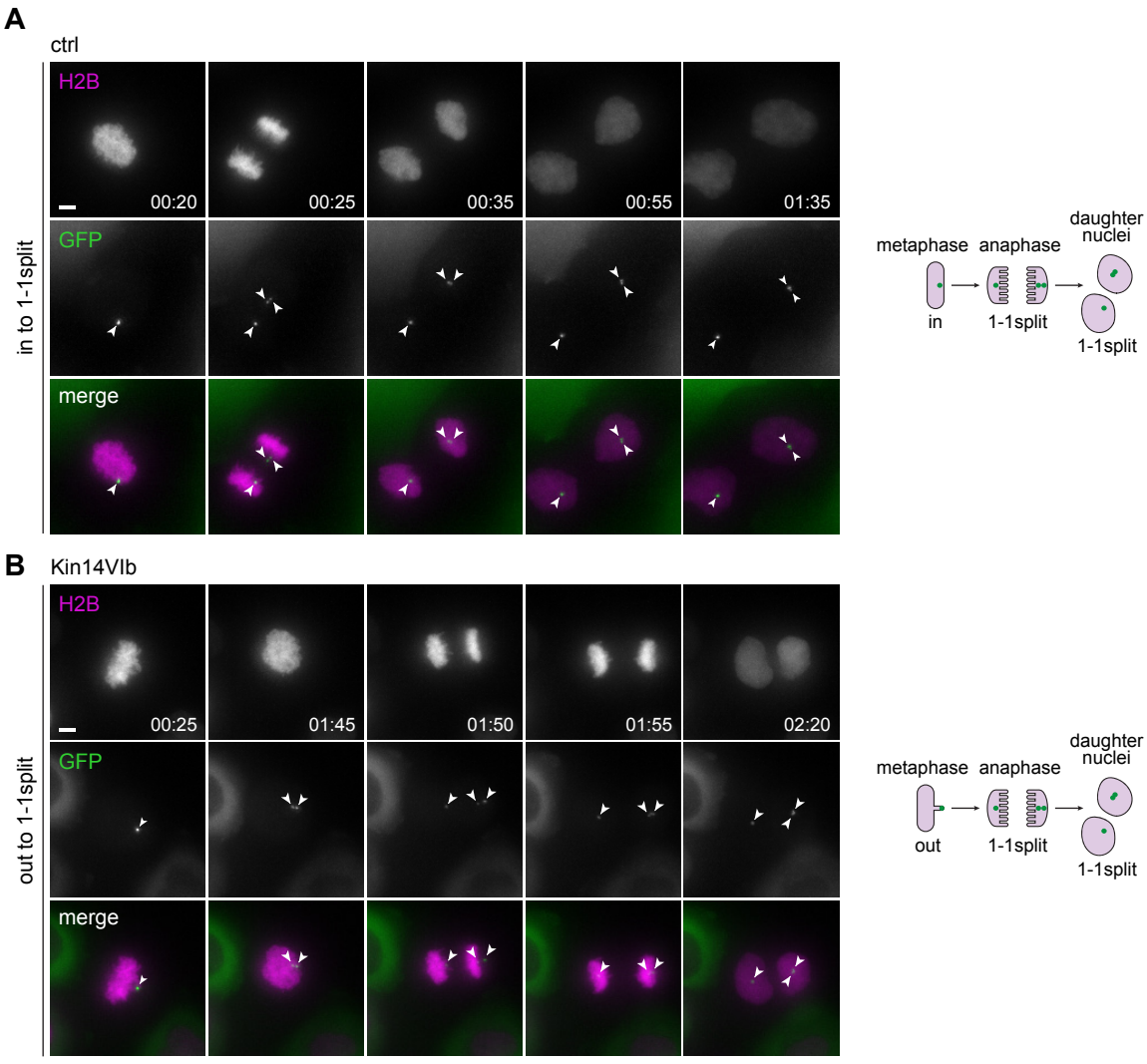
Appendix Figure S1

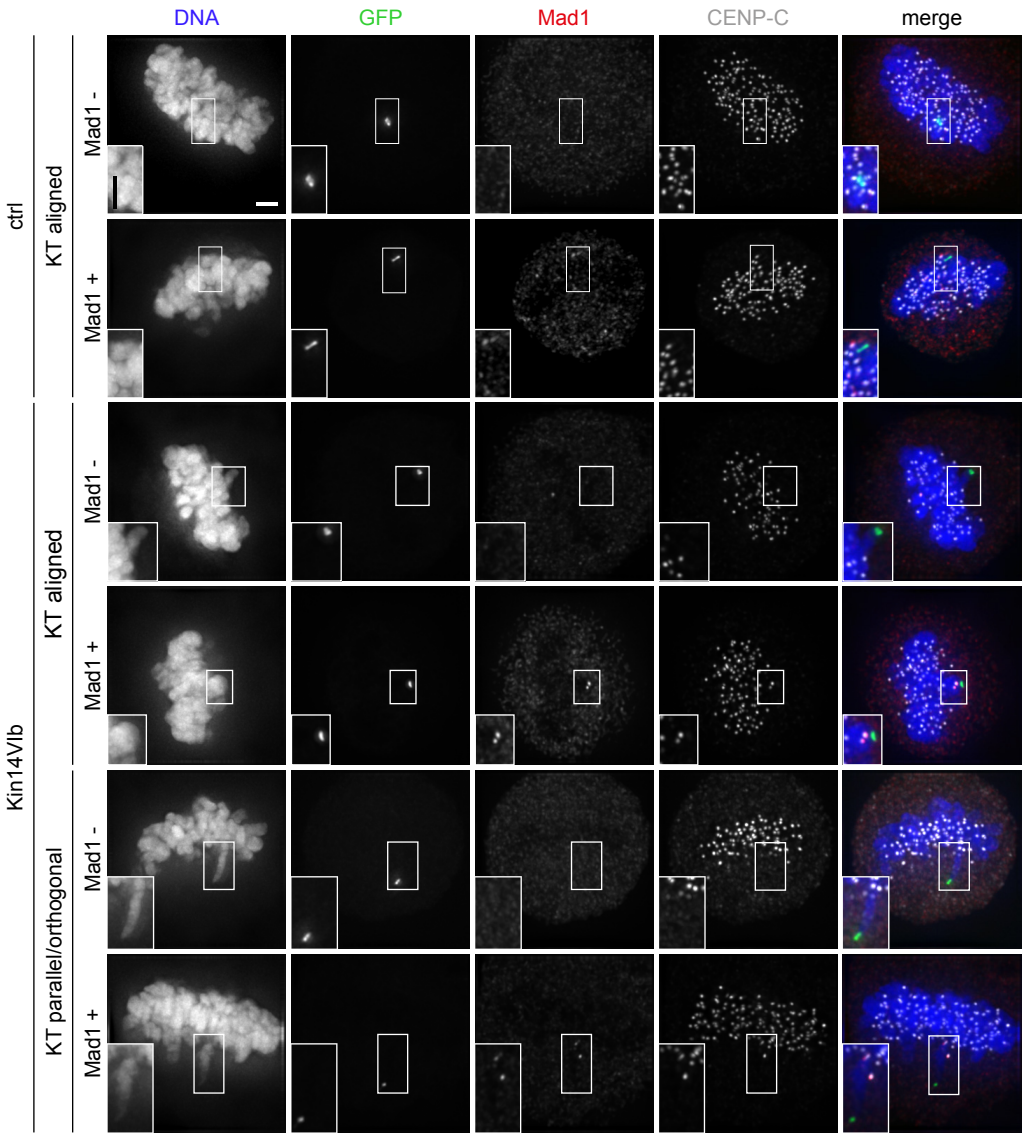


Appendix Figure S1: Examples of U-2 OS TetO cells displaying a 1-1split TetO locus distribution

A, B. Still images from the live microscopy experiment shown in Fig 1. Cells expressing either rTetR-GFP (ctrl, A) or rTetR-GFP-Kin14VIb (Kin14VIb, B), displaying a 1-1split distribution of the TetO locus (arrowheads). Schemes illustrating the metaphase localization, anaphase segregation, and daughter cell distribution of the TetO locus in each case are shown on the right. Scale bar = 5 μm. Time = h:min.

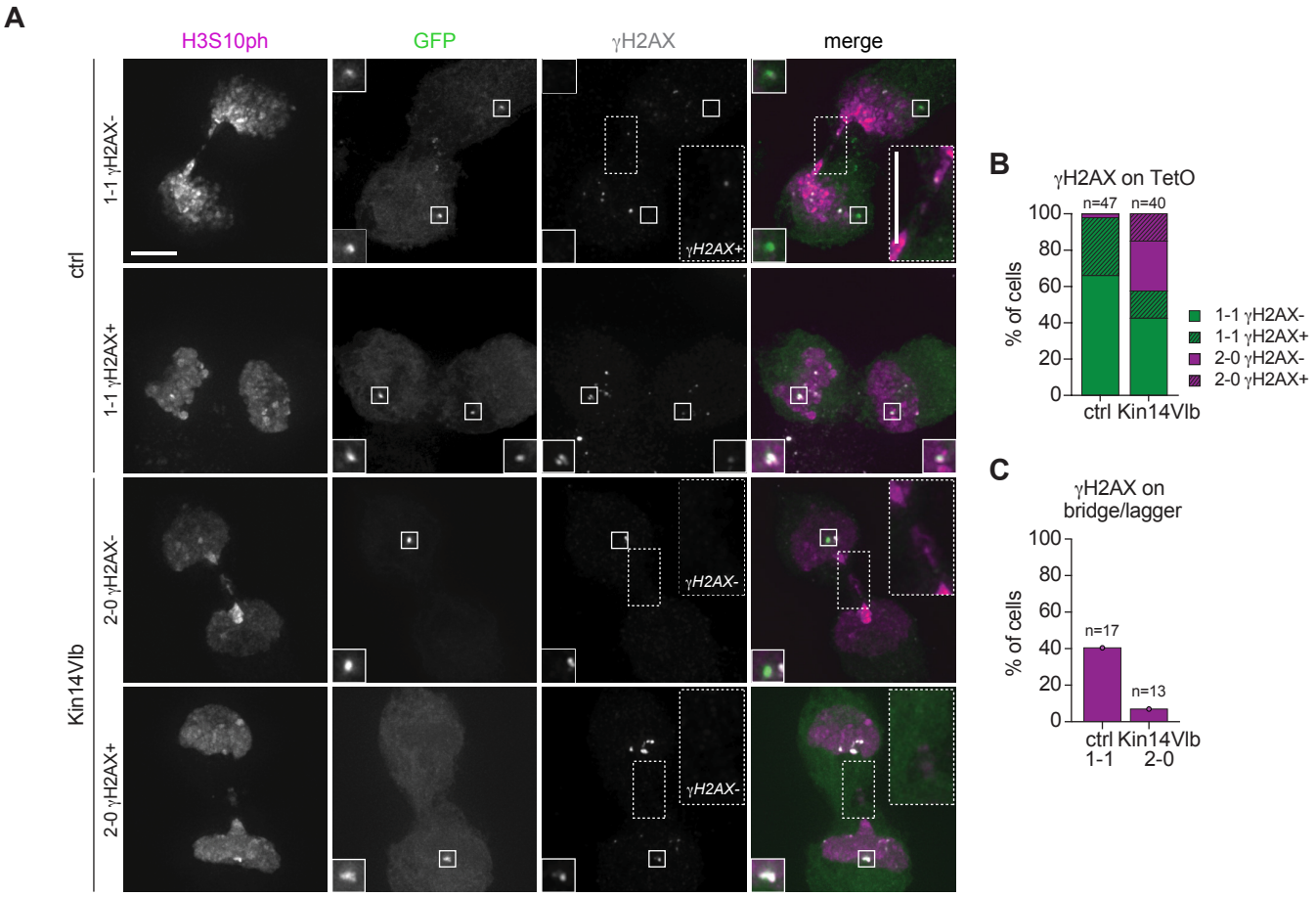
Appendix Figure S2

A



Appendix Figure S2: Mad1 kinetochore localization in U-2 OS TetO cells
IF for GFP, Mad1 and CENP-C of U-2 OS-TetO cells in metaphase expressing rTetR-GFP (ctrl) or rTetR-GFP-Kin14VIb (Kin14VIb). Representative images of the different orientations of the TetO chromosome or locus and the sister KTs are shown. Magnifications of the white boxed indicating the TetO chromosome are shown at the corner of the images. For clarity, the maximum intensity projection of a subset of z-stacks in which the TetO locus is in focus (9-15/50) is shown. Scale bars = 2 μm. For optimal visibility of CENP-C, the brightness and contrast for this channel were linearly adjusted for individual cells.

Appendix Figure S3



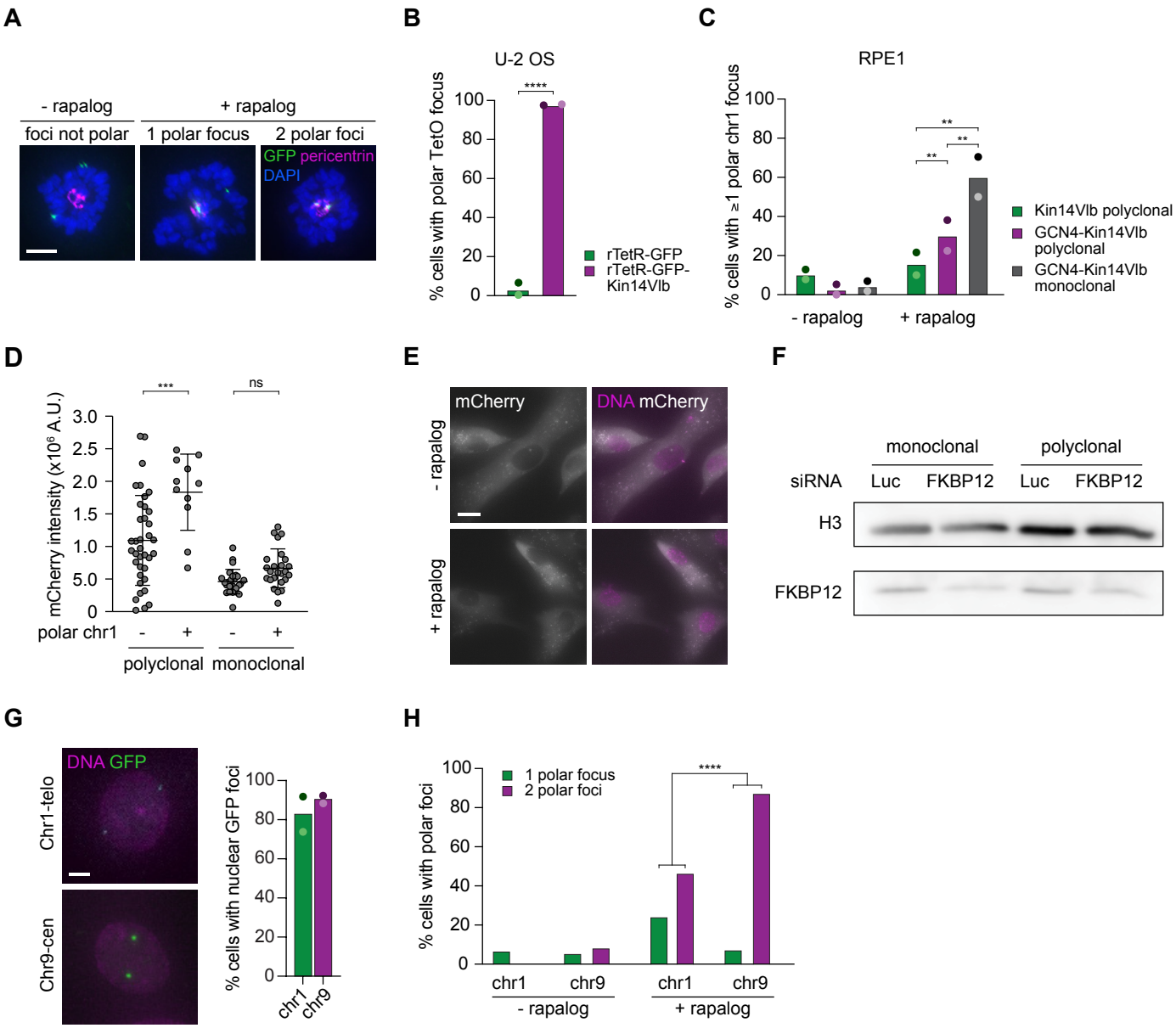
Appendix Figure S3. γH2AX is not detected on the TetO locus nor on the H3S10ph+ bridges in U-2 OS TetO cells expressing rTetR-GFP-Kin14VIb

A. Representative IF images of U-2 OS TetO cells in anaphase/telophase expressing rTetR-GFP (ctrl) or rTetR-GFP-Kin14VIb (Kin14VIb) and stained with the indicated antibodies. γH2AX was used as a marker for DNA damage. Magnifications of the white boxed regions are shown in the corners of the images. Solid lined boxes represent the GFP (TetO) foci, the dotted lined boxes chromatin bridges. Scale bars = 5 μm.

B. Quantification of γH2AX co-localization with the segregating TetO locus in U-2 OS cells expressing rTetR-GFP or rTetR-GFP-Kin14VIb.

C. Frequency of cells with γH2AX on chromatin bridges (incl. stretched arms) or lagging chromosomes during anaphase/telophase. N = number of cells with bridge/laggers.

Appendix Figure S4



Appendix Figure S4: Harnessing dCas9 to target Kin14Vlb to repetitive chromosomal loci in RPE1 cells

A. Representative IF images of STLC-treated mitotic RPE1 dCas9-Kin14Vlb cells displaying either 0, 1, or 2 polar Chr1-telo foci. Cells were transduced with lentivirus containing sgRNAs for 48 hours, followed by an overnight incubation with STLC and 1 μg/ml doxycyclin to prevent centrosome separation and to induce FRB-mCherry-GCN4-Kin14Vlb expression, respectively. Rapalog was added 4 h prior to fixation. Scale bar = 5μm.

B. Quantification of mitotic monopolar U-2 OS cells with a polar TetO focus. n ≥ 32 per condition, per experiment.

Appendix Figure S4: Harnessing dCas9 to target Kin14VIb to repetitive chromosomal loci in RPE1 cells

C. Quantification of mitotic monopolar RPE1 cells expressing dCas9-GFP-3xFKBP and FRB-mCherry-Kin14VIb dimers (polyclonal) or FRB-mCherry-GCN4-Kin14VIb tetramers (polyclonal or monoclonal), which display at least 1 polar Chr1-telo focus. n ≥ 31 per condition, per experiment.

D. Quantification of cytoplasmic mCherry fluorescence intensity levels in polyclonal and monoclonal RPE1 dCas9-Kin14VIb cells expressing FRB-mCherry-GCN4-Kin14VIb, and displaying either 0, or at least 1 polar Chr1-telo foci as shown in A and C.

E. Representative images of RPE1 dCas9-Kin14VIb cells from the experiments in Fig 5A, showing FRB-mCherry-GCN4-Kin14VIb outside the nucleus in interphase cells. Scale bar = 20 μm.

F. Western blot of FKBP12 protein in polyclonal and monoclonal RPE1 dCas9-Kin14VIb cells (expressing GCN4-Kin14VIb). Cells were transfected with siRNA Luciferase (Luc) or siRNA FKBP 72 hours before lysis.

G. Representative images of interphase RPE1 dCas9-Kin14VIb cells transduced with Chr1-telo (top), or Chr9-cen sgRNA (bottom) and quantification of lentiviral transduction efficiency for Chr1-telo and Chr9-cen sgRNAs, indicated by the fraction of cells displaying focal localization of dCas9 (= GFP). Scale bar = 5 μm. Interphase cells from the experiments shown in Figs 5D and E and 6B and C were analysed.

H. Quantification of mitotic monopolar RPE1 dCas9-Kin14VIb cells that are transduced with either Chr1-telo or Chr9-cen sgRNAs and displaying 1 or 2 polar loci in the presence or absence of rapalog. Percentages from a single experiment are shown. N ≥ 35 per condition.

Data information: (B, C, G, H): Bars represent the mean of 2 independent experiments, while dark- and light-colored dots represent the individual values of each experiment. **** P<0.0001; ** P< 0.01; * P< 0.05, Fisher's exact test. (D): Each dot represents an individual cell. *** p<0.001; ns not significant (p=0.4787), one-way anova test with multiple comparisons.