Electronic Supplementary Information (ESI)

Spatiotemporal Regulation of CENP-E-guided Chromosomes Using A Fast-relaxing Arylazopyrazole Photoswitch

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Supporting information

1. General Methods

All reagents and solvents were purchased from commercial sources (TCI Chemical, Merck, Kanto Chemical, Fujifilm Wako Pure Chemical Corporation, Amatek Chemical Co., Ltd., and Cytoskeleton Inc.) and used without further purification. Thin layer chromatography was conducted on silica gel 60 F_{254}-precoated aluminum sheets (Merck). Purification of the synthesized compound was performed using a reverse phase high performance liquid chromatography (RP-HPLC) system (Shimadzu). The product was identified using ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy, and mass spectroscopy. ^{1}H-NMR spectra were recorded on a JEOL JNM-ECX 400 spectrometer. All chemical shifts are cited on the δ-scale in ppm relative to the signal of solvent (DMSO-d_6). The coupling constants (J) are reported in Hz. The proton-decoupled ^{13}C NMR spectra were also recorded on a JEOL JNM-ECX 400 spectrometer, and all chemical shifts were reported in ppm using solvent as the internal standard (DMSO-d_6). High resolution mass spectrum (HR-MS) was recorded by electrospray ionization (ESI) method using Thermo Scientific Exactive mass spectrometer.

Experimental Methods

Photophysical experiments

The UV-Vis absorption spectra of **2** (20 μM) in BRB80 buffer were recorded using a Shimadzu UV-2600 spectrophotometer.

Laser flash photolysis experiments

Laser flash photolysis experiments to measure the lifetime of unstable *cis*-**2** (20 μM) in BRB80 buffer was conducted with the apparatus according to a previous report[S1] equipped with a solid-state laser (355 nm). The time-dependent absorbance changes at 405 nm were recorded using a laser flash excitation. The curve fitting was calculated using the exponential form of the first-order rate equation.

In vitro ATPase Assay of CENP-E

In vitro ATPase assays were carried out using the ADP-Glo Max system (Promega) for the detection of ATP consumption by CENP-E. CENP-E (62.5 ng/mL, Cytoskeleton, Inc.) was added to the solution containing inhibitors, 2μM microtubules, 10 μM taxol, 1 mM DTT, 0.2% BSA, and 0.01% Brij-35 in BRB80 buffer. ATPase reactions in 384 well plates were initiated by the addition of ATP (1.25 μM). The reaction mixture was incubated for 60 min at 25 ºC with or without 405 nm light illumination (CL-1503 with CL-H1-405-9-1, ASAHI SPECTRA). Chemiluminescent intensities in each well were detected using

a plate reader (Infinite 200 Pro M Plex, Tecan) as per the manufacturer's protocol. IC_{50} values were calculated using a nonlinear regression analysis (four-parameter logistic model), where log[inhibitor] was plotted as a percentage of activity.

Immunofluorescence experiments

To visualize the localization of chromosomes, CENP-E and mitotic microtubules, immunofluorescence imaging was conducted using the same method as in the our previous report[S2]. Briefly, HeLa-Kyoto cells (1×10^5 cells) on a 35 mm glass bottom dish were treated with **2** (0.30 μM) and 10 μM MG-132 with/without the continuous illumination of 405 nm light (5 mW/cm^2) for 1 hour. After methanol fixation, the nuclei were stained with 1.0 μg/mL 4′,6-diamidino-2-phenylindole (DAPI, Dojindo). CENP-E and microtubules were also stained with an anti–α-tubulin antibody (YOL1/34; Abcam) with Alexa Fluor 568-conjugated secondary antibody and the anti-CENP-E antibody (1H12, ab5093, Abcam) with Alexa Fluor 488 secondary antibody. Images were taken with a Ti2 microscope (Nikon) equipped with a Plan Apo VC ×60 objective lens (NA 1.40, Nikon) or an Axio Observer 7 microscope (Carl Zeiss) equipped with an Objective Plan-Apochromat 20x/0.8 (Carl Zeiss).

Counting of cells containing aligned/misaligned chromosomes using microscopic imaging

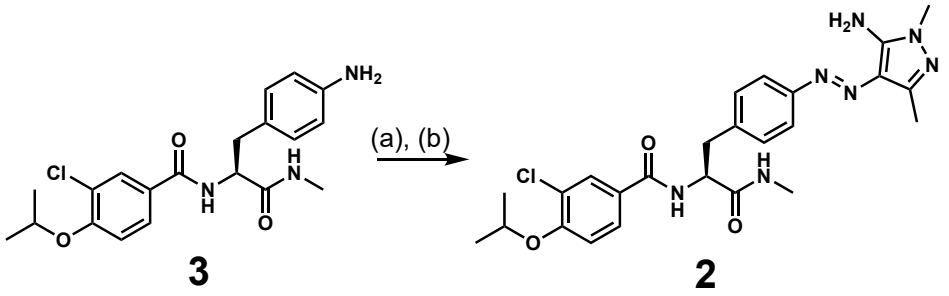
After two hours of incubation of HeLa cells (1×10^5 cells) with **2** (0.30 μM) and MG-132 (10 μM)

with/without the continuous light illumination of 405 nm light (5 mW/cm^2), cells were fixed with 4% PFA (paraformaldehyde) in PBS (phosphate-buffered saline) for 10 min at room temperature. Chromosomes were stained using 1.0 μg/mL 4′,6-diamidino-2-phenylindole (DAPI, Dojindo), overnight at 4 ºC. After washing, the cells were visualized with a Ti2 microscope (Nikon) equipped with a Plan Apo VC ×20 objective lens (NA 0.75, Nikon) or an Axio Observer 7 microscope (Carl Zeiss) equipped with an Objective Plan-Apochromat 20x/0.8 (Carl Zeiss).

Live cell experiments for the photocontrol of mitotic chromosomes

For live cell experiments, chromosome in HeLa cells (1×10^5 cells) was stained with 1.0 μM SiR-DNA (Cytoskeleton, inc.) in DMEM (supplemented with 10% FBS, w/o phenol red) for 1 h. Cells were treated with 0.30 μM **2** without illumination for 1 hour. The fluorescent images of cells were taken with CLSM system (Nikon A1 and Ti-E or Ti2) equipped with a Plan Apo VC ×60 objective lens (NA 1.40, Nikon) or an Axio Observer 7 microscope (Carl Zeiss) equipped with an Objective Plan-Apochromat 20x/0.8 (Carl Zeiss). The system was humidified with a microscope stage incubator (TOKAI HIT) with 5% CO_2 at 37 ºC. A laser of 405 nm with 2% intensity was used for photoactivation of **2**. A 633 nm laser with 1% intensity was used for excitation of SiR-DNA.

Synthesis of 2



Scheme S1. Synthetic route of **2**. Conditions: (a) sodium nitrite, HClaq, EtOH, 1 hour, 0 °C. (b) 1,3-dimethyl-5-aminopyrazole, sodium acetate, EtOH, acetic acid, 2 h, room temperature.

Sodium nitrite (138 mg, 2.0 mmol) in water (1.0 mL) was added to the solution of **3**[S2] (390 mg, 1.0 mmol) in water (0.5 mL), EtOH (2.5 mL) and 12N HCl (1 mL) over 1 hour at 0 °C. To this solution, 1,3-dimethyl-5-aminopyrazole (250 mg, 2.2 mmol) in EtOH (2 mL) and acetic acid (2 mL) and sodium acetate (500 mg, 6.1 mmol) were added. The reaction mixture was stirred for 2 h at room temperature. AcOEt and brine were added to the solution. The organic phase was extracted, washed with sat. $NaHCO_3$aq and brine, dried over $MgSO_4$, and filtered. The resulting solution was dried *in vacuo*. The crude product was purified with reverse phase HPLC system to afford compound **2** (89 mg, 17%) as a yellow solid.

^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.60 (d, J = 8.8 Hz, 1H), 8.03 (q, J = 4.4 Hz, 1H), 7.94(d, J = 2.0 Hz, 1H), 7.76 (dd, J = 8.8, 2.4 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.8 Hz,

1H), 4.76 (septet, J = 6.0 Hz, 1H), 4.67-4.61 (m, 1H), 3.50 (s, 3H), 3.14-2.95 (m, 1H), 2.61 (d, J = 4.8 Hz, 3H), 2.27 (s, 3H), 1.29 (d, J = 6.0 Hz, 6H).

^{13}C-NMR (100 MHz, DMSO-d_6) δ 172.13, 165.05, 155.75, 152.06, 140.71, 138.81, 130.17, 129.84, 128.51, 127.28, 122.60, 122.23, 120.94, 114.80, 109.00, 71.75, 55.50, 37.55, 34.22, 26.19, 22.20, 11.90.

HR-MS (ESI) for $[M + H]^+$ calcd. 512.2171, found 512.2172.

Supplementary Figures

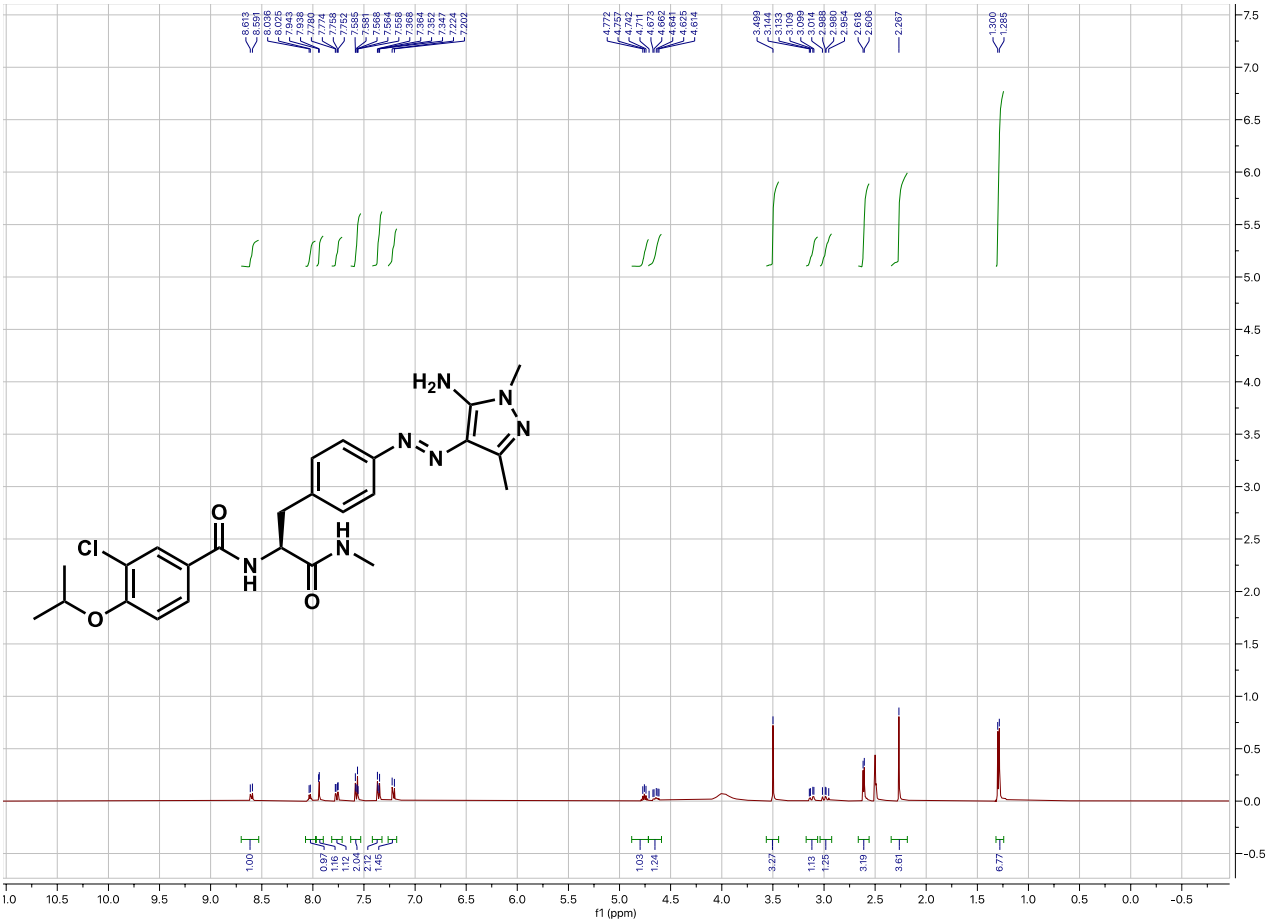


Figure S1. ^{1}H-NMR of **2**.

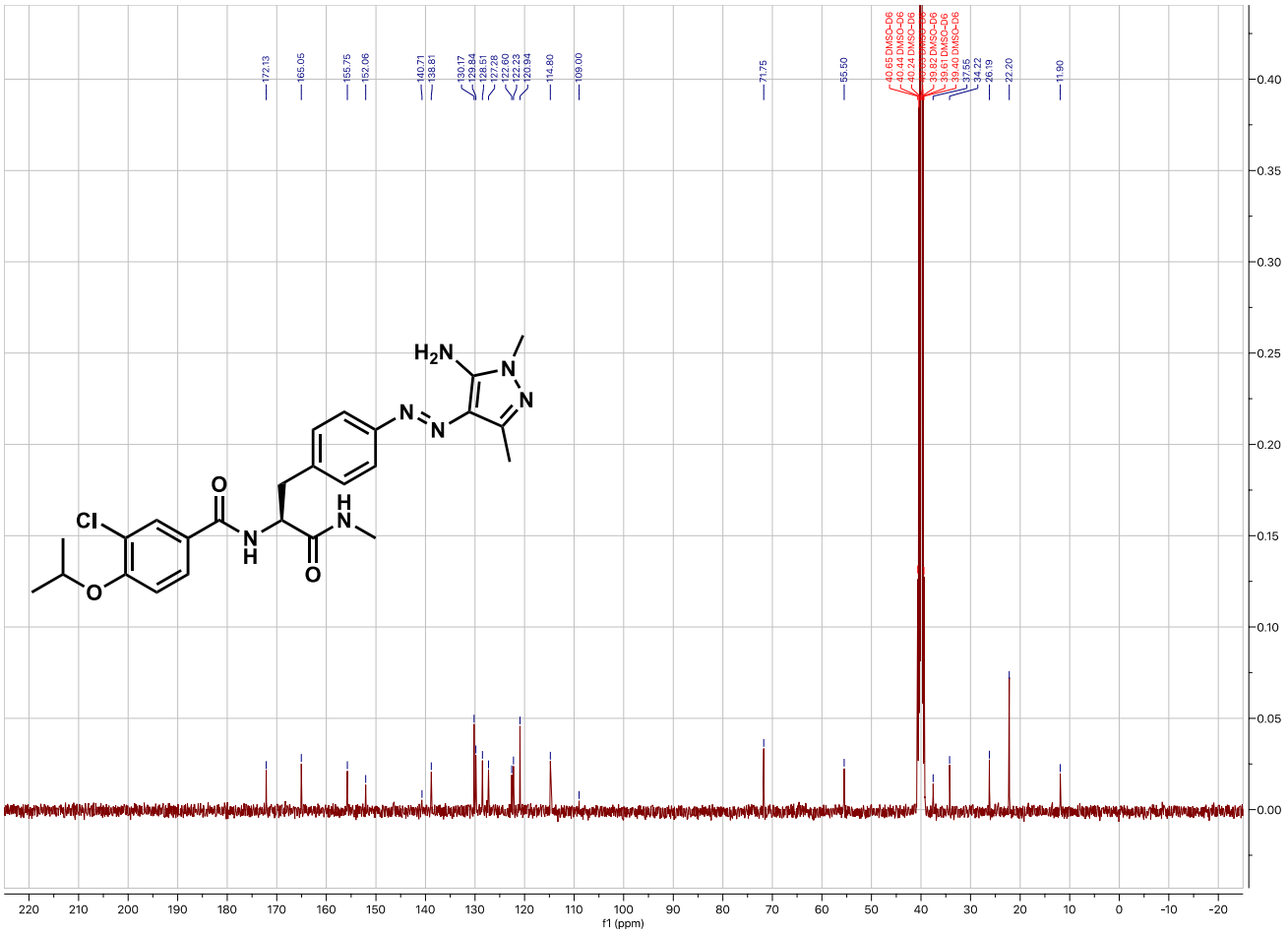


Figure S2. ^{13}C-NMR of **2**.

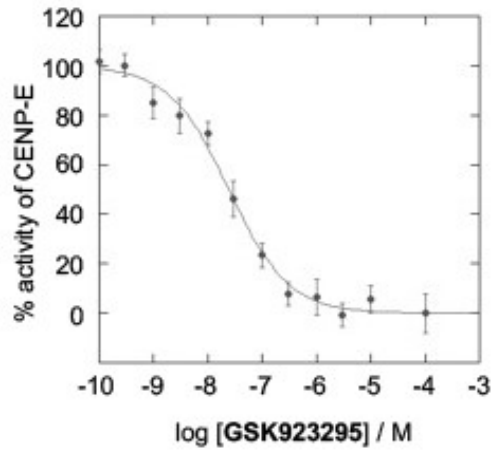


Figure S3. Inhibition profile of **GSK923295** with *in vitro* ATPase assay (IC_{50} = 24 nM).

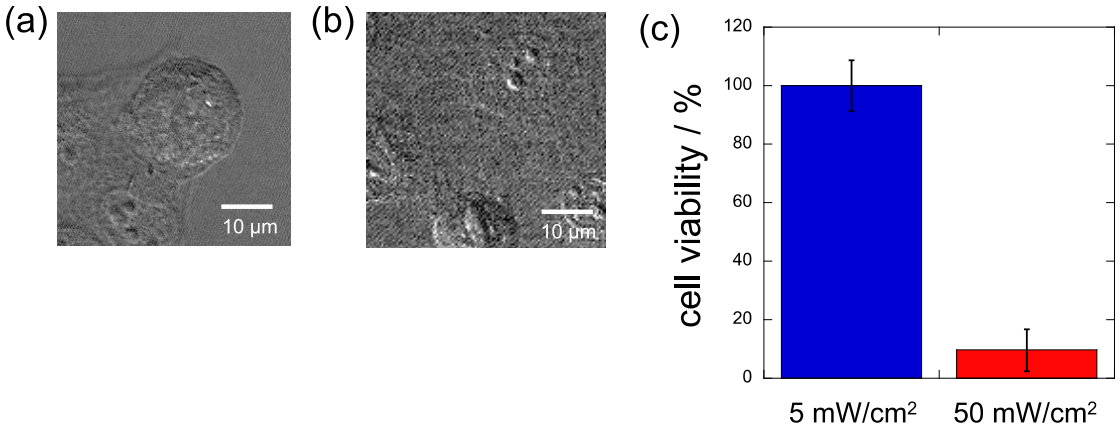


Figure S4. Phototoxic effects of 405 nm light illumination. (a) DMSO-treated cells with 405 nm light illumination (5 mW/cm^2) for 30 min. (b) DMSO-treated cells with 405 nm light illumination (50 mW/cm^2) for 30 min. The diameters of mitotic cells were calculated to be ca. 22 μm for (a) and ca. 14 μm for (b). (c) Cell viability data with 405 nm light illumination (5 or 50 mW/cm^2) for 30 min.

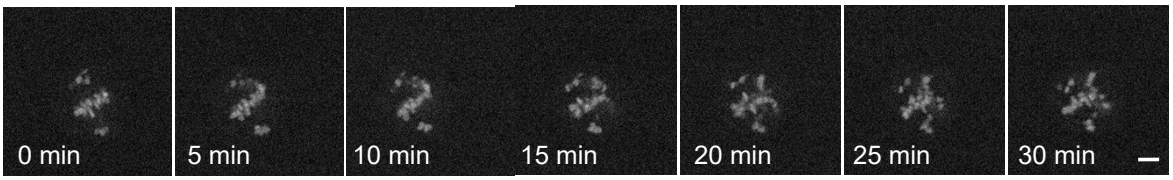


Figure S5. Live cell imaging of misaligned chromosomes in a mitotic cell in the presence of **GSK923295** (0.30 μM) with 405 nm laser illumination (0.2% intensity) for 30 min. Scale bar: 5 μm.

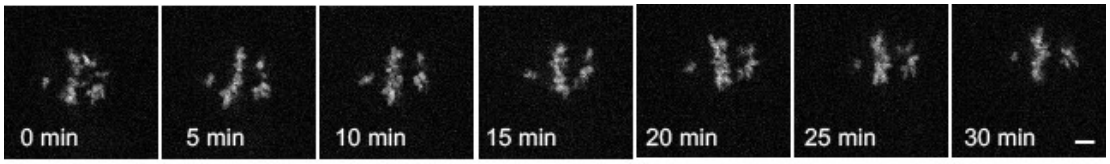


Figure S6. Live cell imaging of misaligned chromosomes in a mitotic cell in presence of **2** (0.30 μM) without light illumination for 30 min. Scale bar: 5 μm.

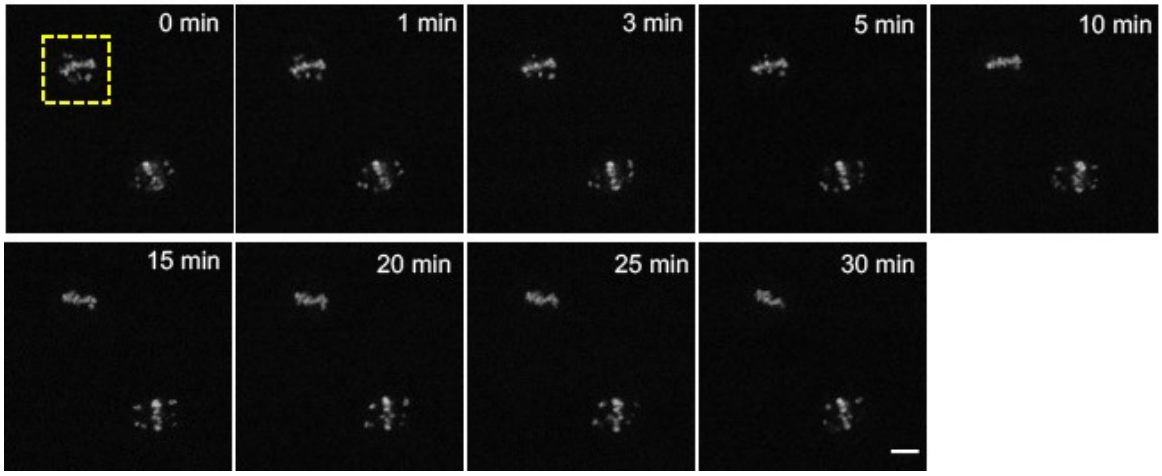


Figure S7. Photocontrol of the movement of mitotic chromosomes in one cell out of the two mitotic cells using 2% intensity of 405 nm laser light. Yellow square shows the ROI (region of interest) for photoactivation. Scale bar: 10 μm.

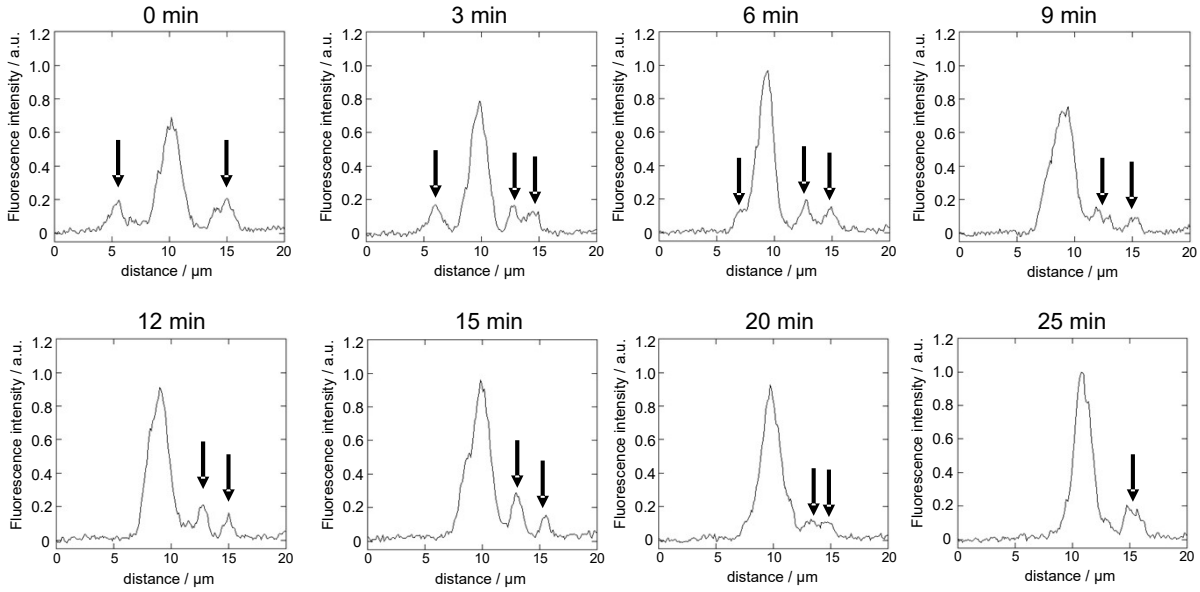


Figure S8. Line profiles of chromosomal fluorescence intensities in Figure 4b. Black arrows exhibits the misaligned chromosomes.

References

S1. K. I. Takao, T. Kikukawa, T. Araiso, N. Kamo, *Biophys. Chem.,* 1998, **73**, 145.

S2. N. N. Mafy, K. Matsuo, S. Hiruma, R. Uehara, N. Tamaoki, *J. Am. Chem. Soc.,* 2020, **142**, 1763.