

Supporting Information

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SI Materials and Methods

Automated Analysis of Cell Death. Cells were grown in 96-well plates in 100 μ L culture medium. Three days after indicated transfections, wells were stained by adding PI and Hoechst to the culture medium. Image acquisition was performed using a Cellomics ArrayScan VTI (Thermo Scientific) using a 20 \times 0.50 NA objective and eight images were acquired per well, which contained around 4,000 cells in total. Image analysis was performed using Cellomics ArrayScan HCS Reader (Thermo Scientific). The percentage of cell death was calculated by the amount of PI positive cells over the total Hoechst positive cells.

Flow Cytometry. Flow cytometry samples were harvested and fixed using 70% ethanol. α -MPM2 was incubated for 1 h in PBS-2% BSA-0.1% Tween and α -Mouse Cy5 for 1 h in PBS-0.1% Tween. Stained cells were collected in PBS containing RNase and Propidium Iodide. Fluorescence was measured on the FACSCalibur and analyzed with Cell Quest Pro software (BD Biosciences).

Immunofluorescence Microscopy. Cells plated on 12-mm coverslips were harvested 2 days after siRNA transfection in the presence

or absence of doxycycline. Fixation was done using 4% PFA in PBS. CREST and α -CENP-E were incubated O/N in PBS 3% BSA. Anti-human Alexafluor568, Anti-rabbitAlexafluor488, and DAPI were incubated in PBS 0.1% Tween. Stained coverslips were mounted with Vectashield Mounting Medium (Vector). Images were acquired on a Zeiss 510 Meta confocal laser scanning microscope with a 63 \times /1.4NA Plan-ApoChromat objective using the Zeiss LSM software. Intensity quantifications were performed using MetaMorph software.

Chromosome Spreads. Nocodazole was added for 4 h to the medium to enrich for mitotic cells. Cells were treated with 0.75 M KCl at 37 $^{\circ}$ C for 10 min, centrifuged at 2,000 rpm and fixed for 20 min with methanol:acetic acid (3:1). Fixation procedure was repeated three times. Samples were collected in methanol and DAPI to stain for DNA. Chromosome spreads were created by allowing the drops to fall from 30 cm height onto glass slides. Images were acquired as described above for immunofluorescence microscopy.

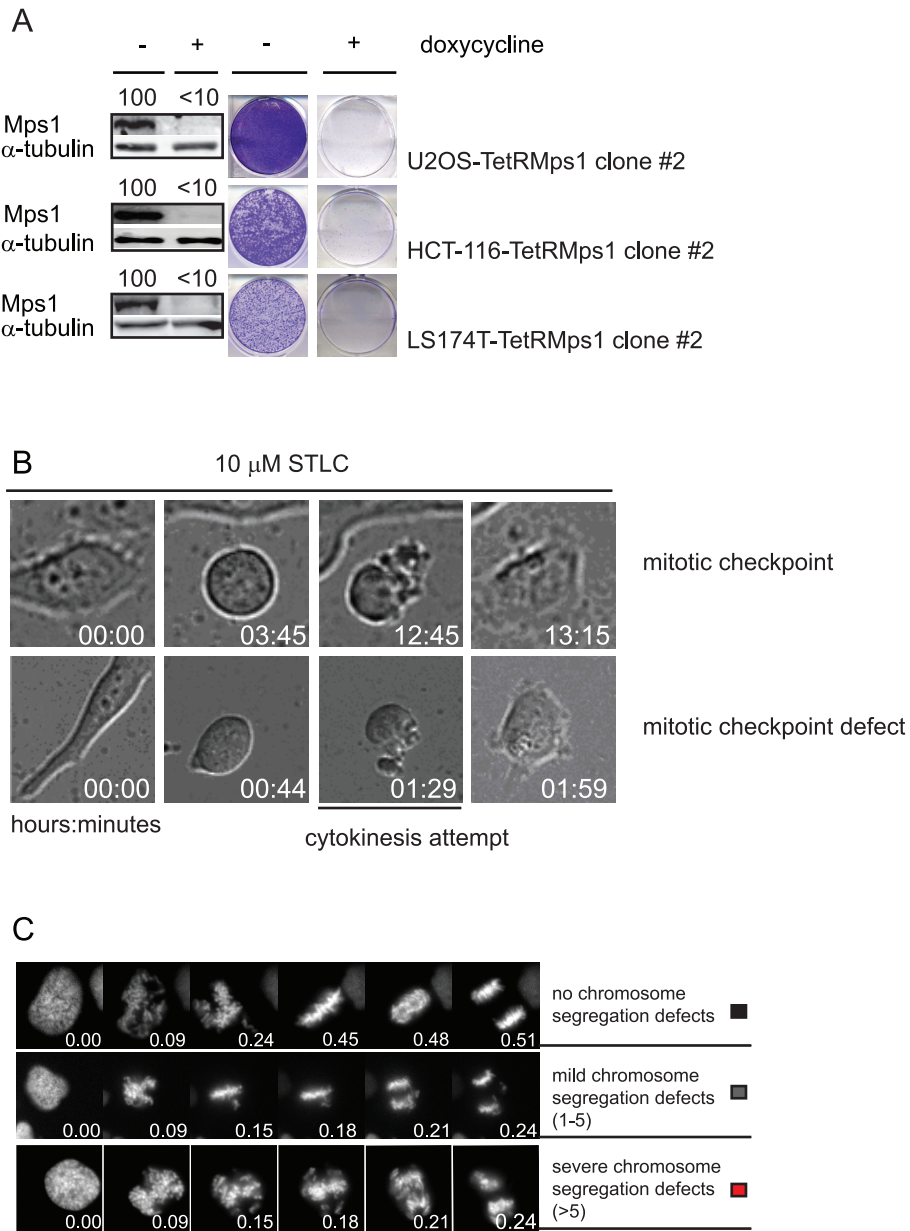


Fig. S1. (A) Indicated cell lines were treated with and without doxycycline and immunoblotted for Mps1 and α -tubulin. Values indicate relative percentage of Mps1 levels. (B) Representative image stacks (DIC) of cells treated with 10 μ M STLC in the presence or absence of a functional mitotic checkpoint. (C) Representative images of cells transfected with H2B-GFP and filmed using time-lapse microscopy showing three phenotypes: anaphase without mis-segregations, mild mis-segregations (1–5 chromosomes), and severe mis-segregations (5 chromosomes). Time indicates minutes after chromosome condensation.

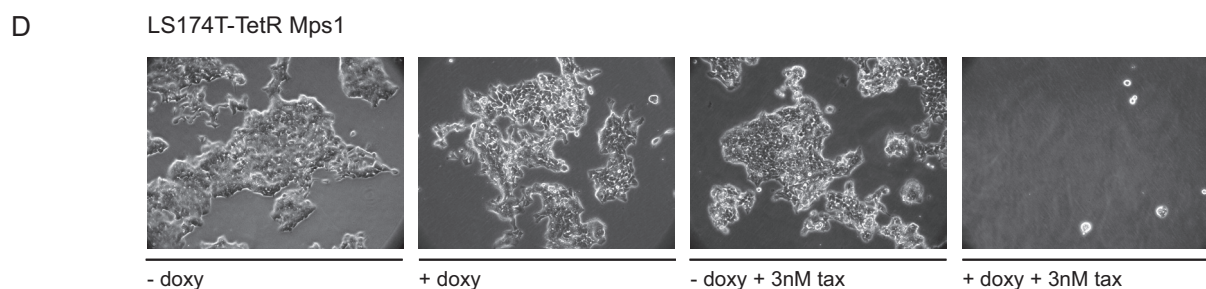
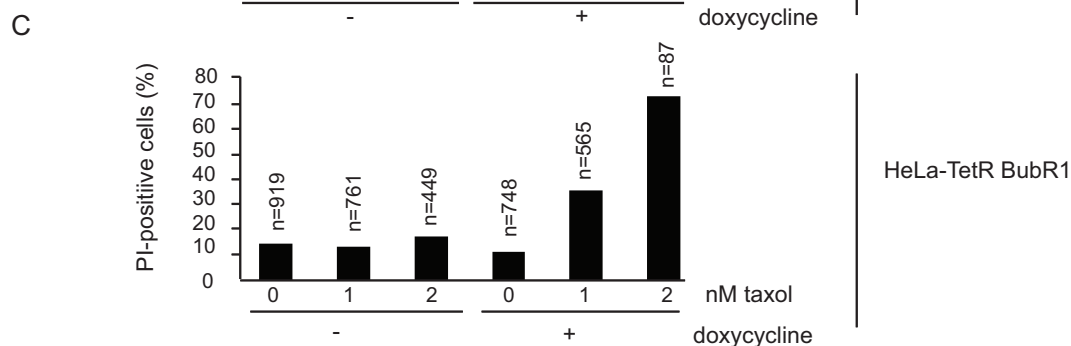
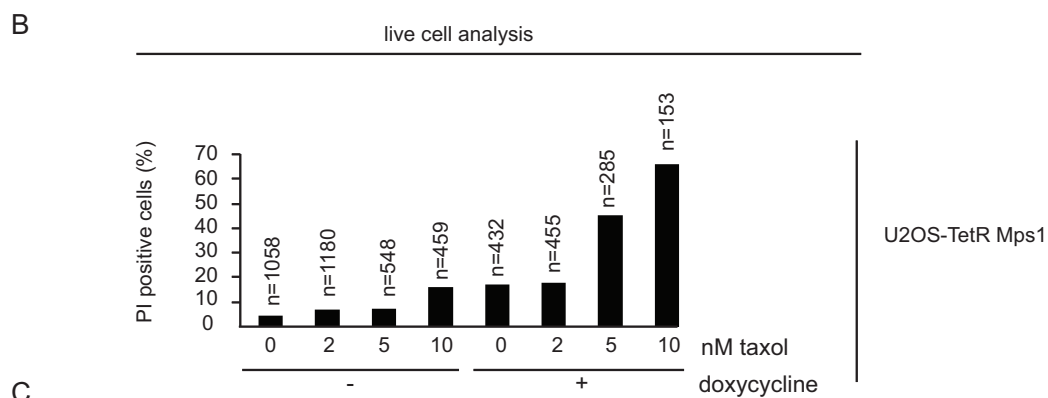
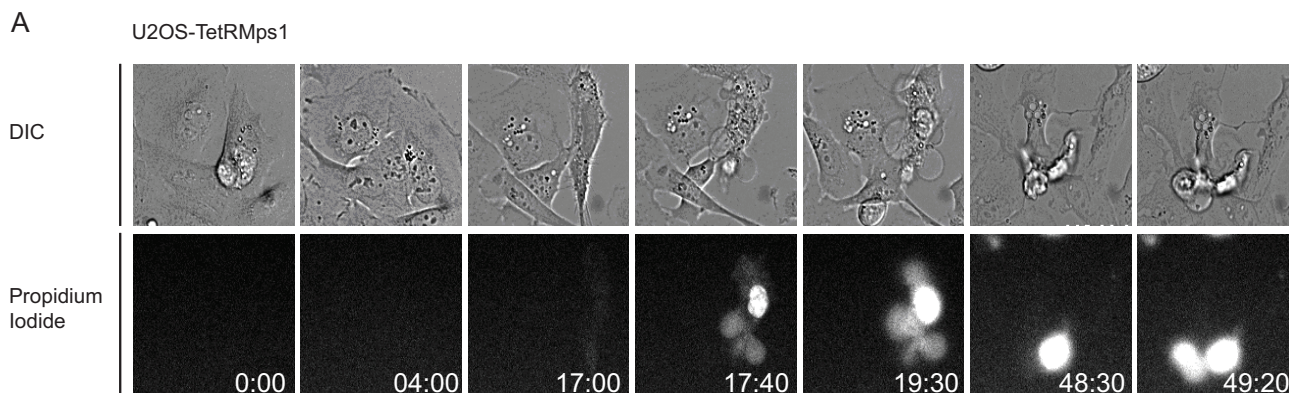


Fig. S2. (A) Representative DIC images of U2OS-TetRMps1 cells undergoing cell death after doxycycline and 5 nM taxol treatment. Propidium Iodide was added to the culture medium to determine uptake by dying cells. (B and C) Quantification of time-lapse analysis performed as in (A) of cell death induction in U2OS-TetRMps1 and HeLa-TetRBubR1 cells treated with or without doxycycline and indicated taxol concentrations on days 4, 5, and 6. DIC and PI images were acquired every 15 min. The percentage of cell death was measured as the fraction of PI-positive cells over the total amount of cells (n) at the end of the experiment. (D) Representative images of LS174T-TetRMps1 colonies 11 days after indicated treatments.

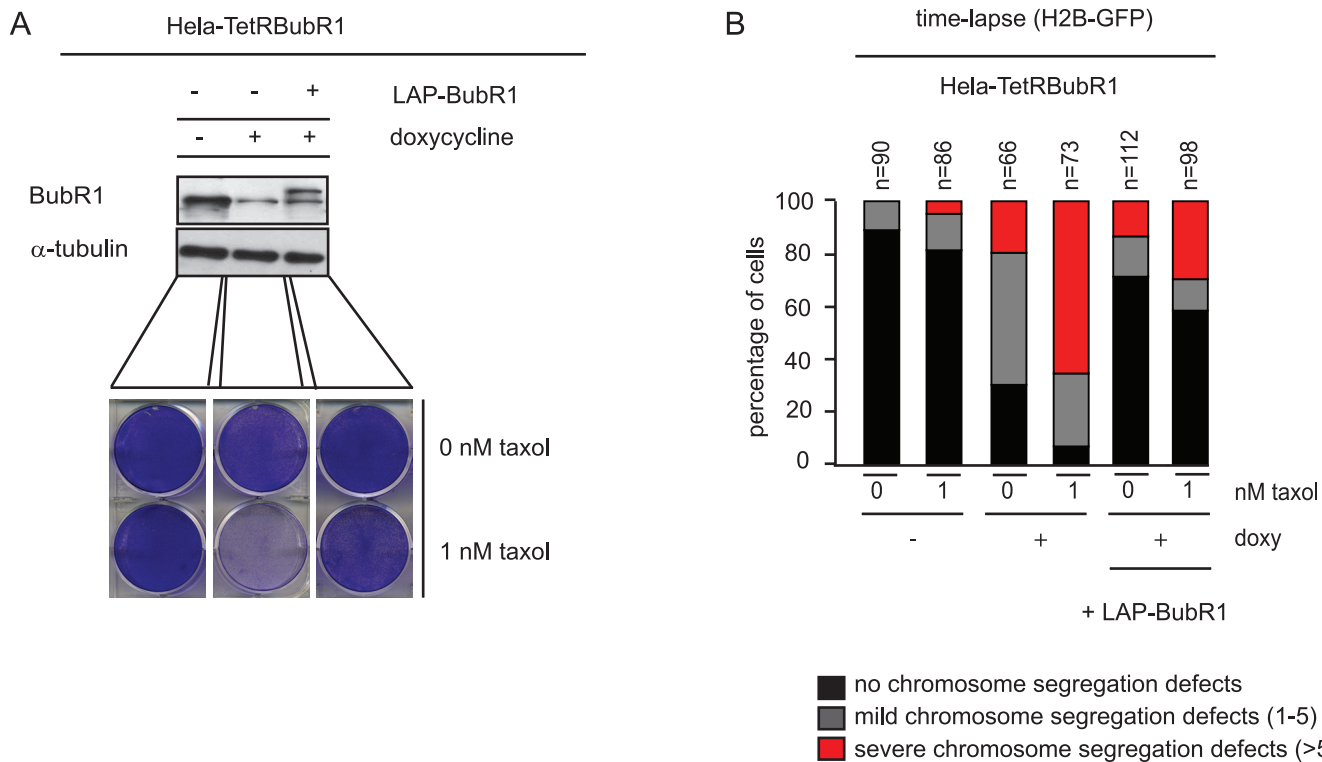


Fig. S3. (A) Top: HeLa-TetRBubR1 cells with and without stable expression of RNAi insensitive Lap-BubR1 were immunoblotted for BubR1 and α -tubulin. Bottom: Representative pictures of colony formations treated with or without 1 nM taxol. (B) Quantification of time-lapse analysis of HeLa-TetRBubR1 with or without stable BubR1 expression and doxycycline as in Fig. 1E. n = amount of cells filmed.

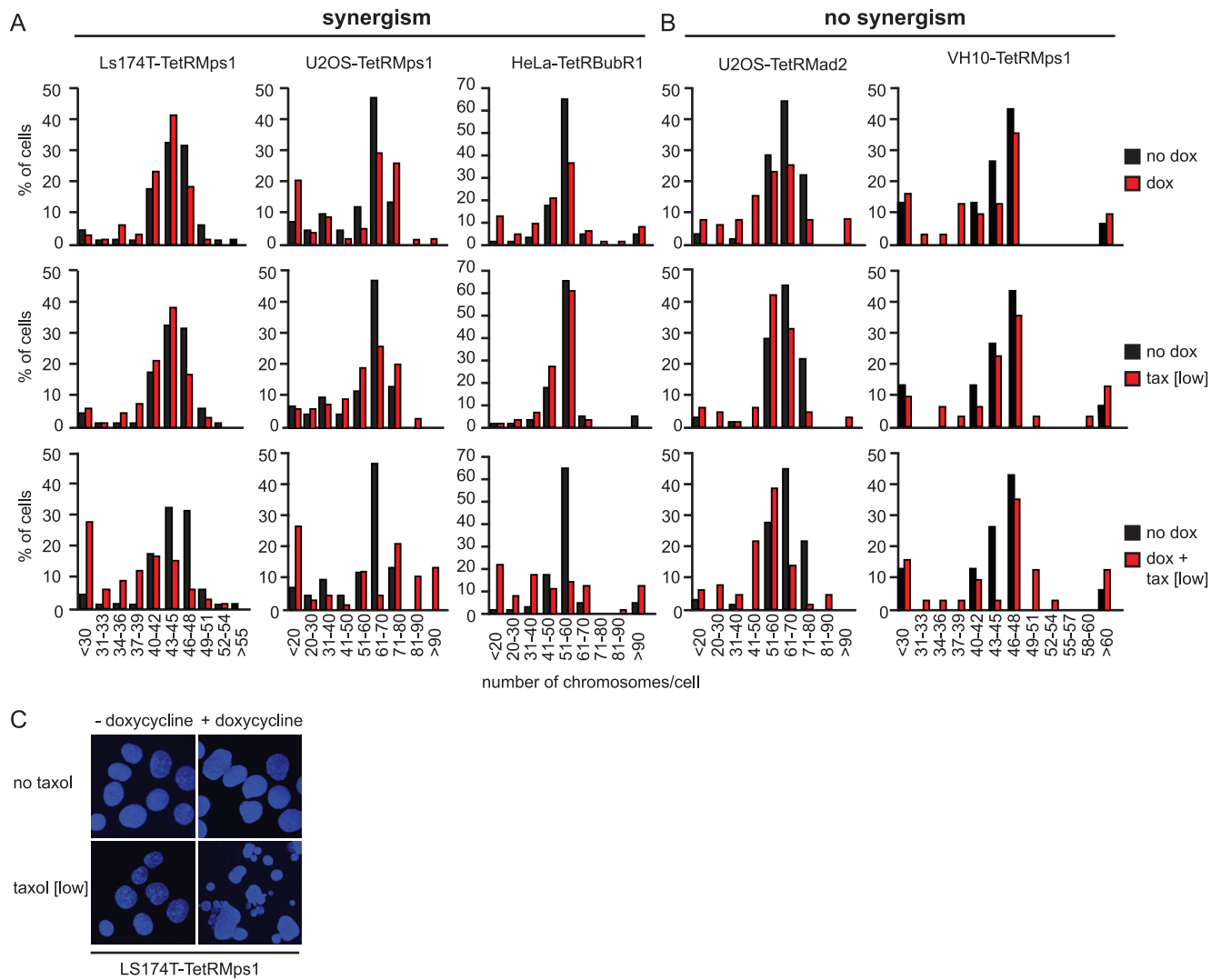


Fig. S4. Taxol-induced synergy correlates with enhanced aneuploidy. (A, B) Chromosome spreads were performed on cells after 4 days of treatment with or without dox. At least 60 chromosome-spreads were counted per condition. Where indicated, taxol was added the last 2 days. Taxol [low] concentrations are: LS174T, 3 nM; U2OS, 5 nM; HeLa, 1 nM; VH10, 2 nM. (C) Representative pictures of nuclei of LS174T-TetRMps1 cells after indicated treatments.

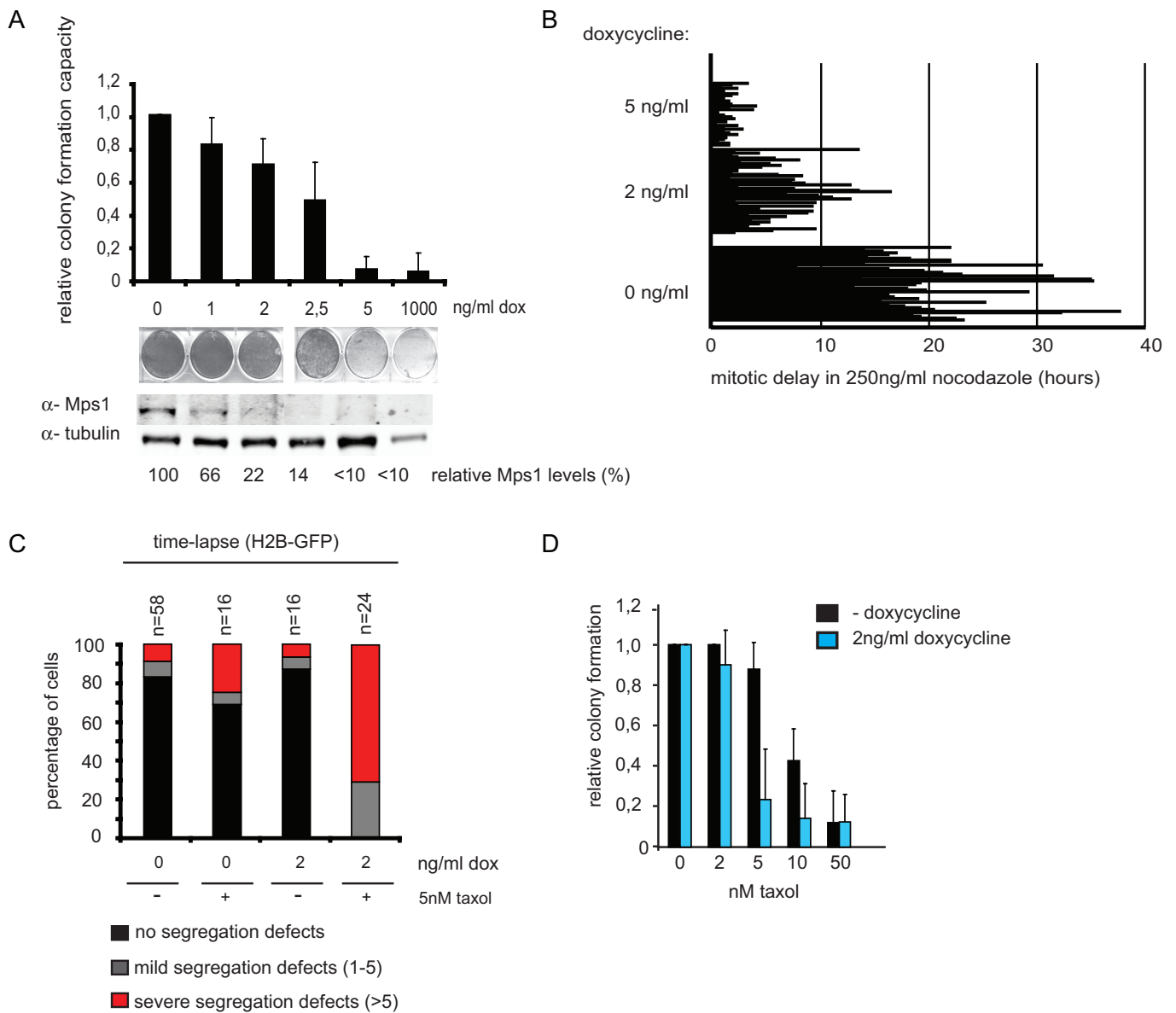


Fig. 55. (A) Top: Quantification of colony formations of U2OS-TetRMps1 clone#2 cells that were treated with indicated doxycycline concentrations. Colony formation capacity of non-treated cells (0 μ g/mL doxycycline) was set at 1. Bars represent three independent experiments (+SD). Bottom: Immunoblots of Mps1 and α -tubulin of cells that were treated with indicated doxycycline concentrations for 3 days. Values below immunoblots represent relative amount of Mps1 protein levels. (B) Time-lapse analysis of the duration of the mitotic delay in cells treated with indicated doxycycline concentrations for 3 days and 250 ng/mL nocodazole. DIC images were acquired every 15 min for 64 h. Each bar represents a single cell. (C) Quantification of anaphase progression as in Fig. 1E of cells that were treated with indicated doxycycline concentrations for 3 days. n = amount of cells filmed. Indicated taxol concentrations were added 1 h before filming. (D) Quantification of colony formations as in Fig. 2.

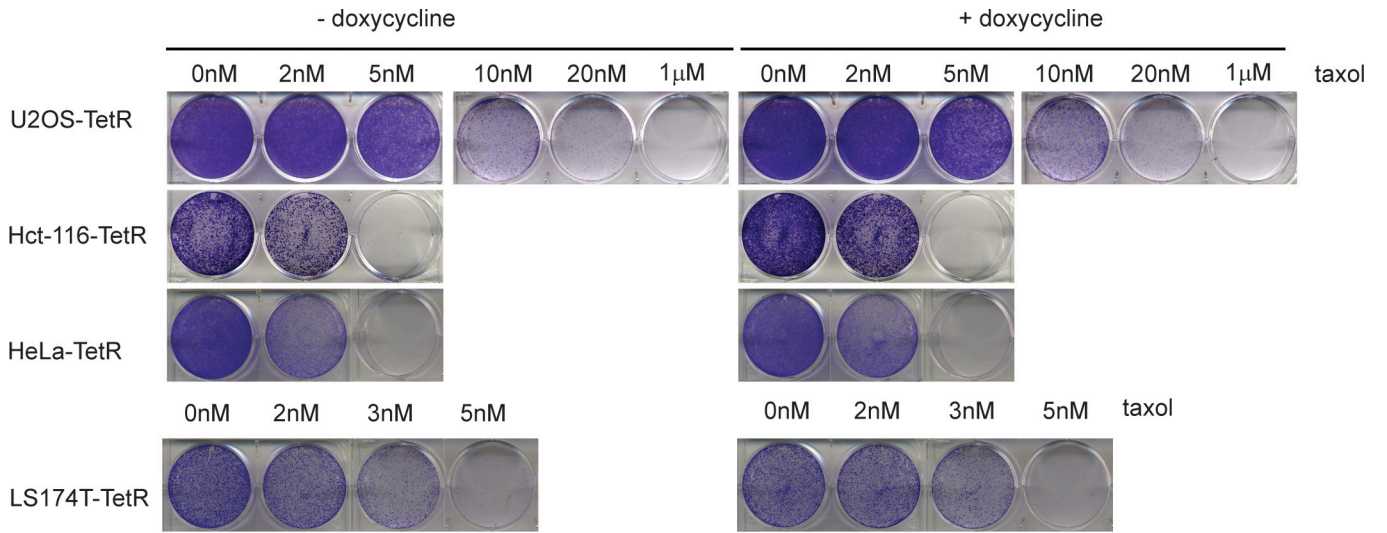


Fig. S6. Colony formations of indicated parental cell lines treated with and without doxycycline (day 0) and indicated taxol concentrations (day 1). Representative pictures are shown.

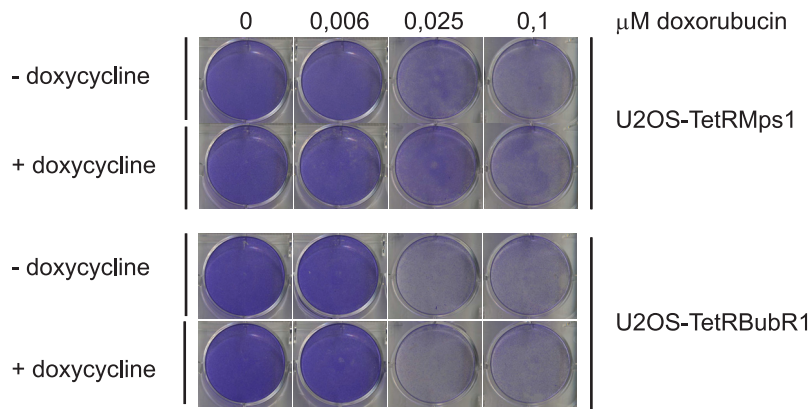


Fig. S7. Representative pictures of colony formations of U2OS-TetRMps1 or BubR1 cells treated with or without doxycycline for 11 days and indicated doxorubicin concentrations. Doxorubicin was added 1 day after doxycycline addition.

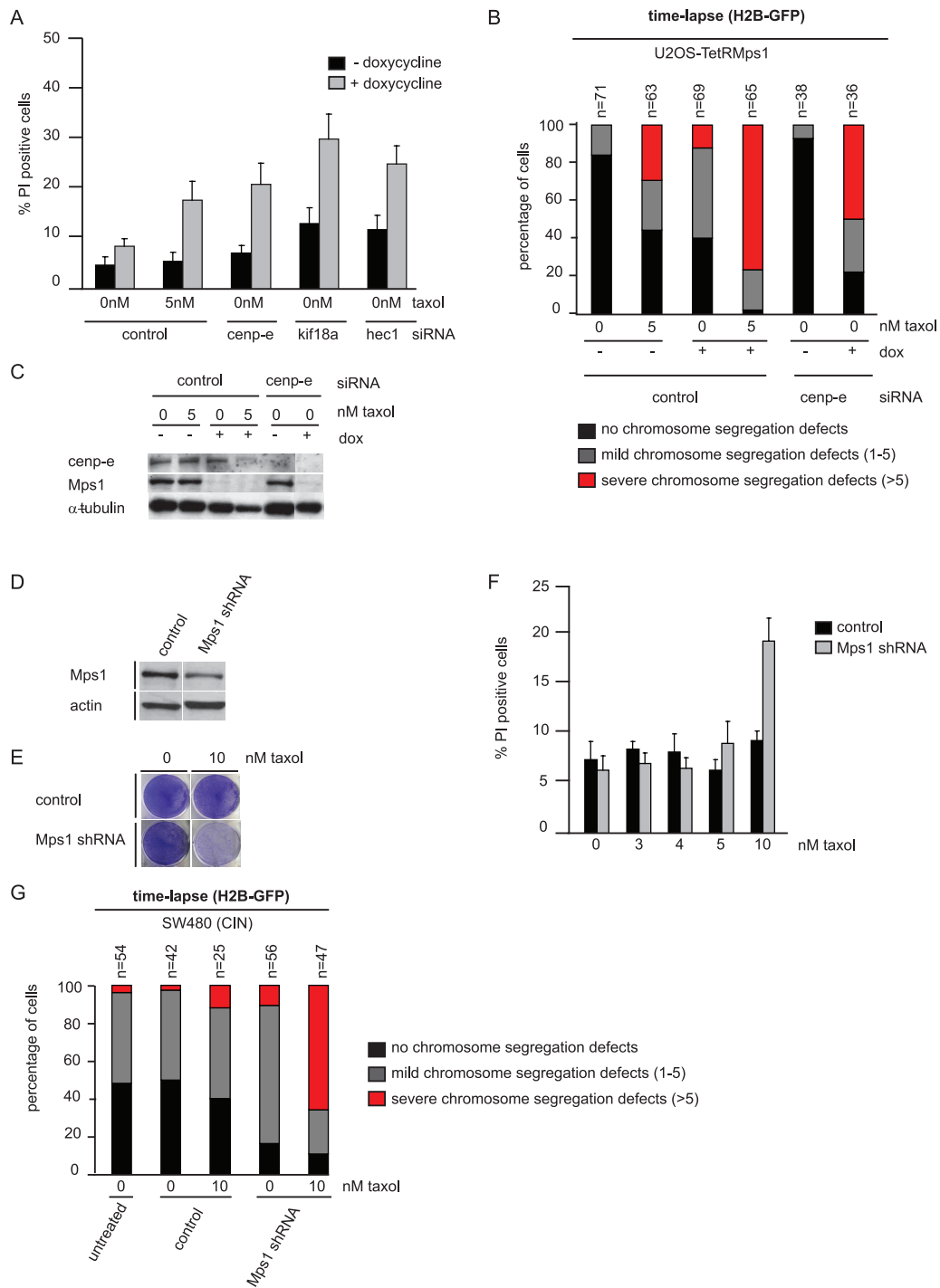


Fig. 58. (A) Automated analysis of the percentage of PI positive over Hoechst positive U2OS-TetRMps1 cells on day 6, treated with or without doxycycline (day 0), indicated siRNAs (day 2) and 0 or 5 nM taxol (day 3). Bars represent the average of five independent experiments (+SEM). (B) Quantification of time-lapse analysis of U2OS-TetRMps1 cells as in Fig. 1E. siRNA transfections were performed 36 h before filming. Indicated taxol concentrations were added 1 h before filming. (C) Immunoblots of Cenp-E, Mps1 and α -tubulin of U2OS-TetRMps1 cells collected after time-lapse acquisition. (D) Immunoblots of Mps1 and actin of SW480 cells transfected with empty pSuperior (control) and pSuperiorMps1 (Mps1 shRNA) and selected for 2 days with puromycin. (E) Representative pictures of colony formations of SW480 cells on day 8. SW480 cells were transfected with pSuperior (control) and pSuperiorMps1 (Mps1 shRNA) (day 0). Cells were selected with puromycin (day 1), replated in 6-well plates (day 2), and indicated taxol concentrations were added on day 3. (F) Automated analysis of the percentage of PI positive over Hoechst positive SW480 cells on day 6 after transfection as in (E). Bars represent the average of four independent experiments (+SEM). (G) Quantification of time-lapse analysis of SW480 cells as in Fig. 1E. SW480 cells were transfected with H2B-GFP (untreated) or H2B-GFP combined with empty pSuperior (control) or pSuperiorMps1 (Mps1 shRNA). Cells were filmed 2 days after transfection and indicated taxol concentrations were added 1 h before filming. n = amount of cells filmed.

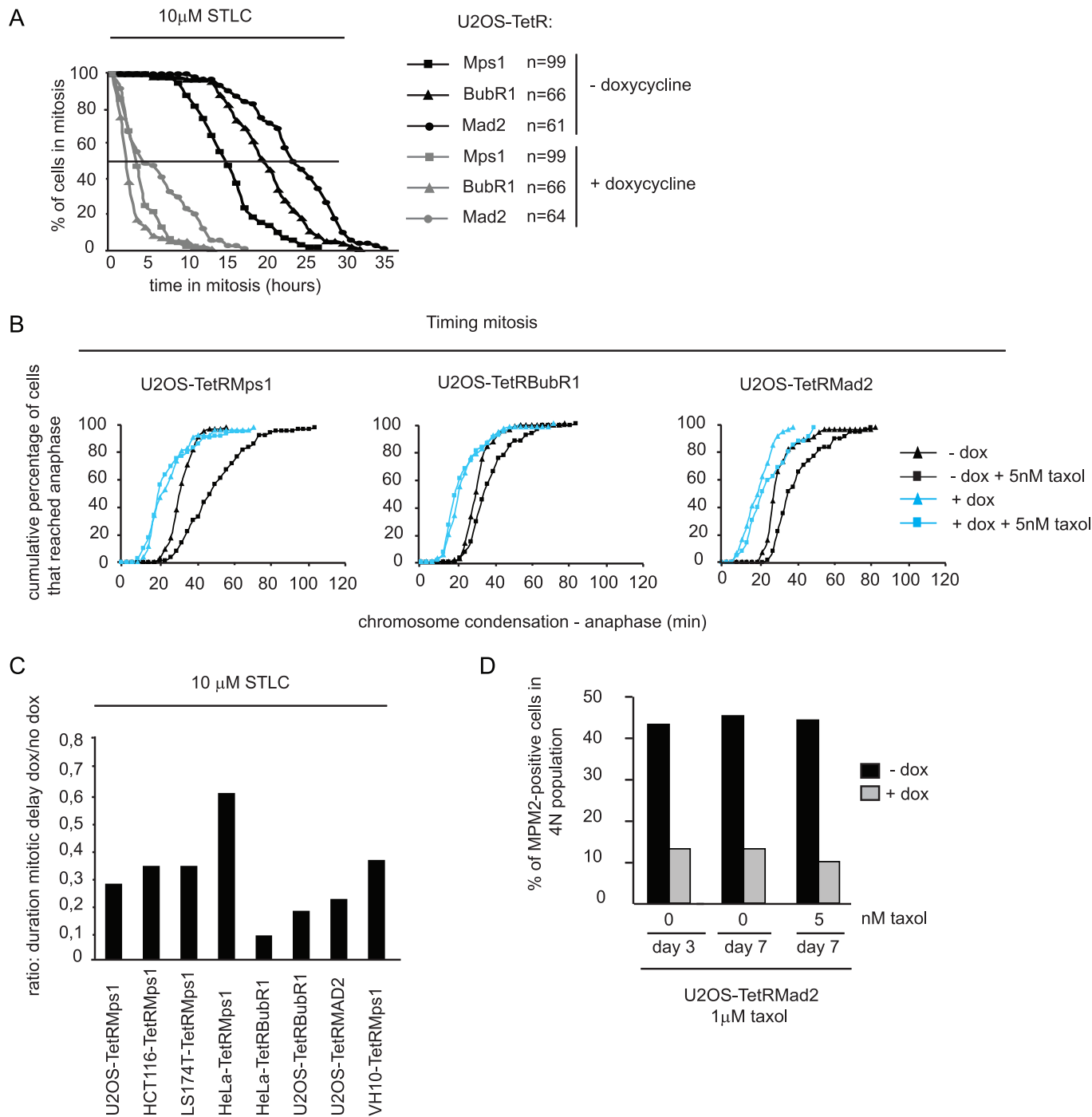


Fig. 59. (A) Live cell imaging (DIC) of indicated U2OS cell lines in the presence of 10 μ M STLC as in Fig. 1B. (B) Time-lapse analysis of the duration of mitosis from chromosome condensation until the start of anaphase. Indicated cell lines were treated with and without dox for 2 days and 5 nM taxol was added 1 h before filming. At least 30 cells were filmed per condition. (C) Ratio of the average mitotic duration of dox treated cell lines divided by the average mitotic duration of control treated cell lines in the presence of 10 μ M STLC. At least 40 cells were filmed per condition. (D) FACS analysis of U2OS-TetRMad2 cells after doxycycline addition for 3 or 7 days. Five nanomolar taxol was added 1 day after doxycycline administration. After the various treatments cells were treated with 1 μ M taxol for 18 h to determine the mitotic checkpoint efficiency. The percentage of mitotic cells was measured as the fraction of cells with 4N DNA content that were positive for MPM2.

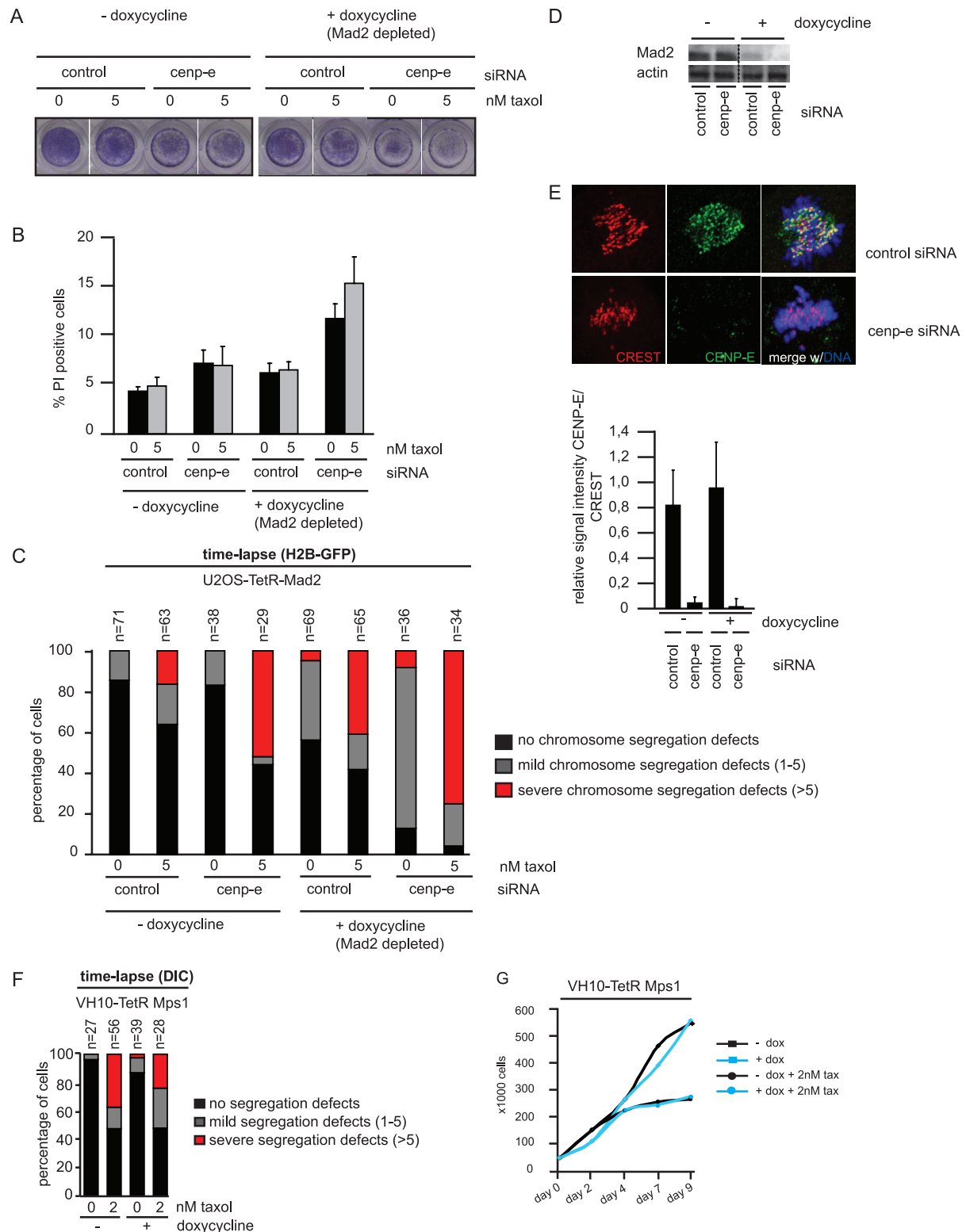


Fig. S10. (A) Representative pictures of colony formations of U2OS-TetRMad2 cells on day 6 treated with or without doxycycline (day 0), control or CENP-E siRNA (day 2), and 0 or 5 nM taxol (day 3). Luciferase or GAPDH siRNA were used as a control. (B) Automated analysis of the percentage of PI positive over Hoechst positive U2OS-TetRMad2 cells on day 6 after treatment as in (A). Bars represent the average of 4 independent experiments (+SEM). (C) Quantification of time-lapse analysis as in Fig. 1E. siRNA transfections were performed 36 h before filming. Indicated taxol concentrations were added 1 h before filming. n = amount of cells filmed. (D) Immunoblots of Mad2 and actin of U2OS-TetRMad2 cells treated with and without doxycycline and control or CENP-E siRNA. (E) Top: Immunolocalization of CENP-E (green) and CREST (red) in mitotic U2OS-TetRMad2 cells transfected with control or CENP-E siRNA. Bottom: Quantification of intensity of CENP-E staining over CREST. Average is shown of 35 kinetochores +SD of seven analyzed cells per condition. (F) Quantification of time-lapse analysis (DIC) of chromosome segregation in anaphase. n = amount of cells filmed. (G) Growth curve of VH10-TetR Mps1 cells after indicated treatments. On day 0, doxycycline was added; on day 2, 2 nM of taxol was added where indicated.