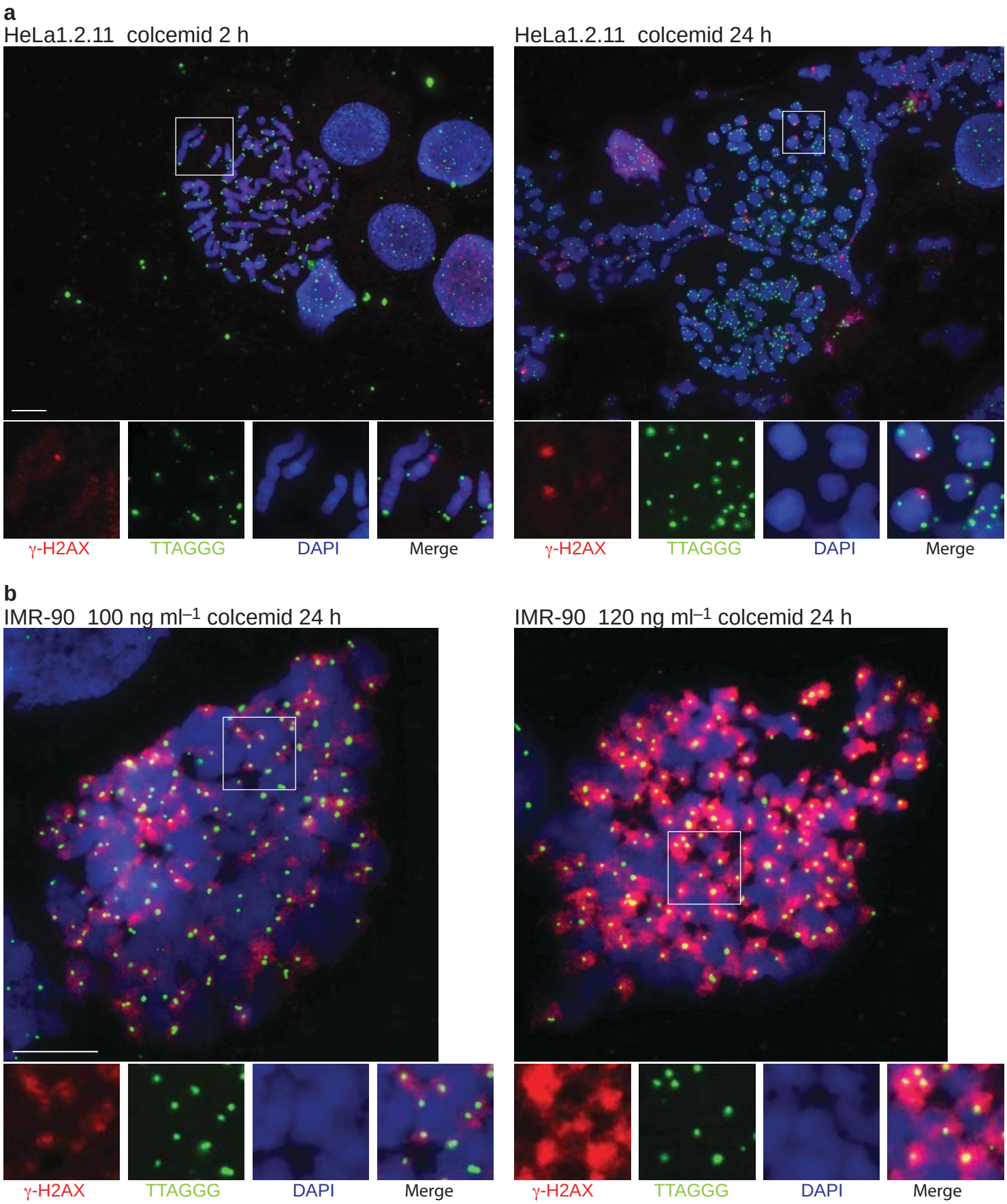
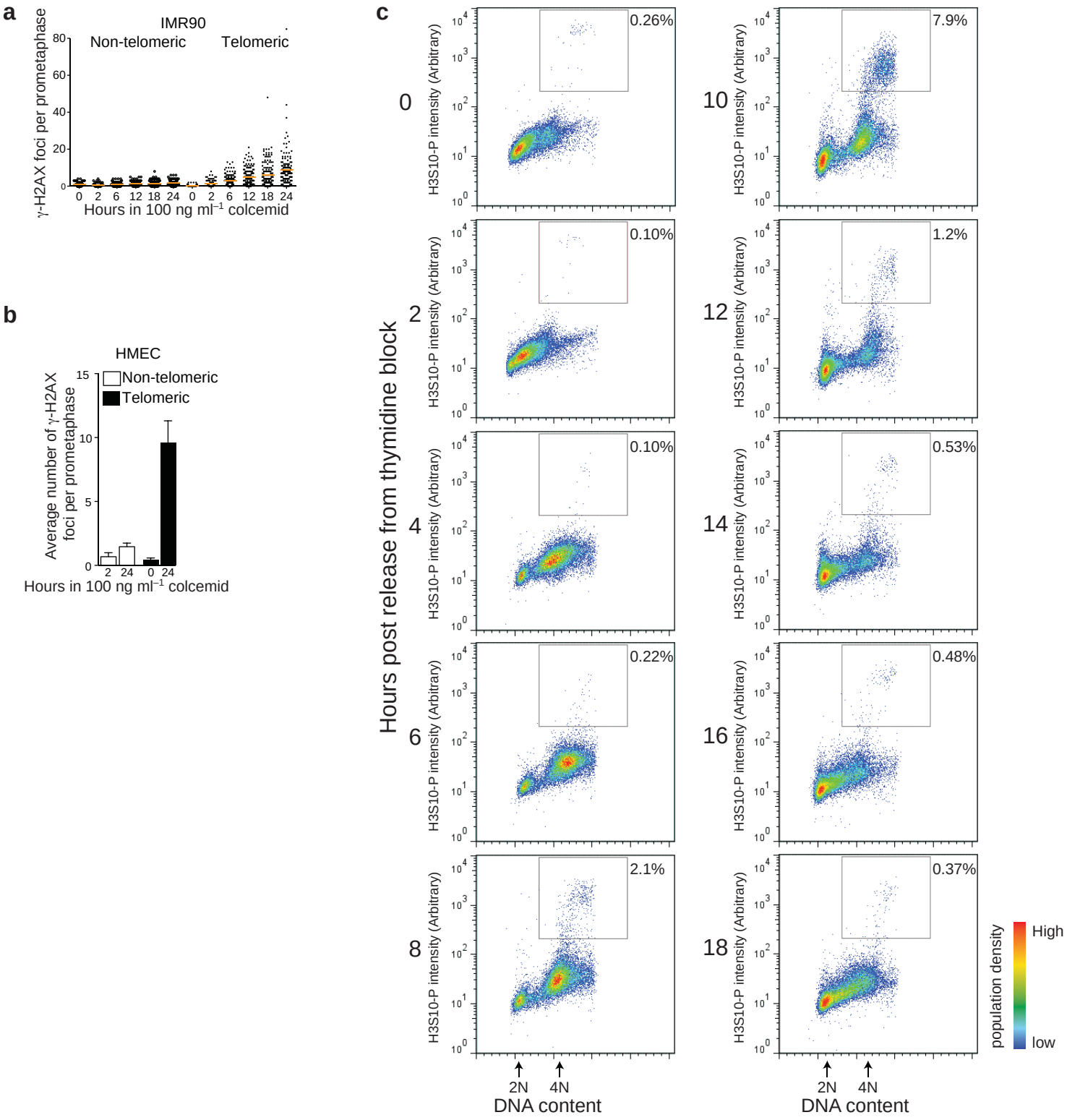
Supplemental Figure 1
Hayashi et al.



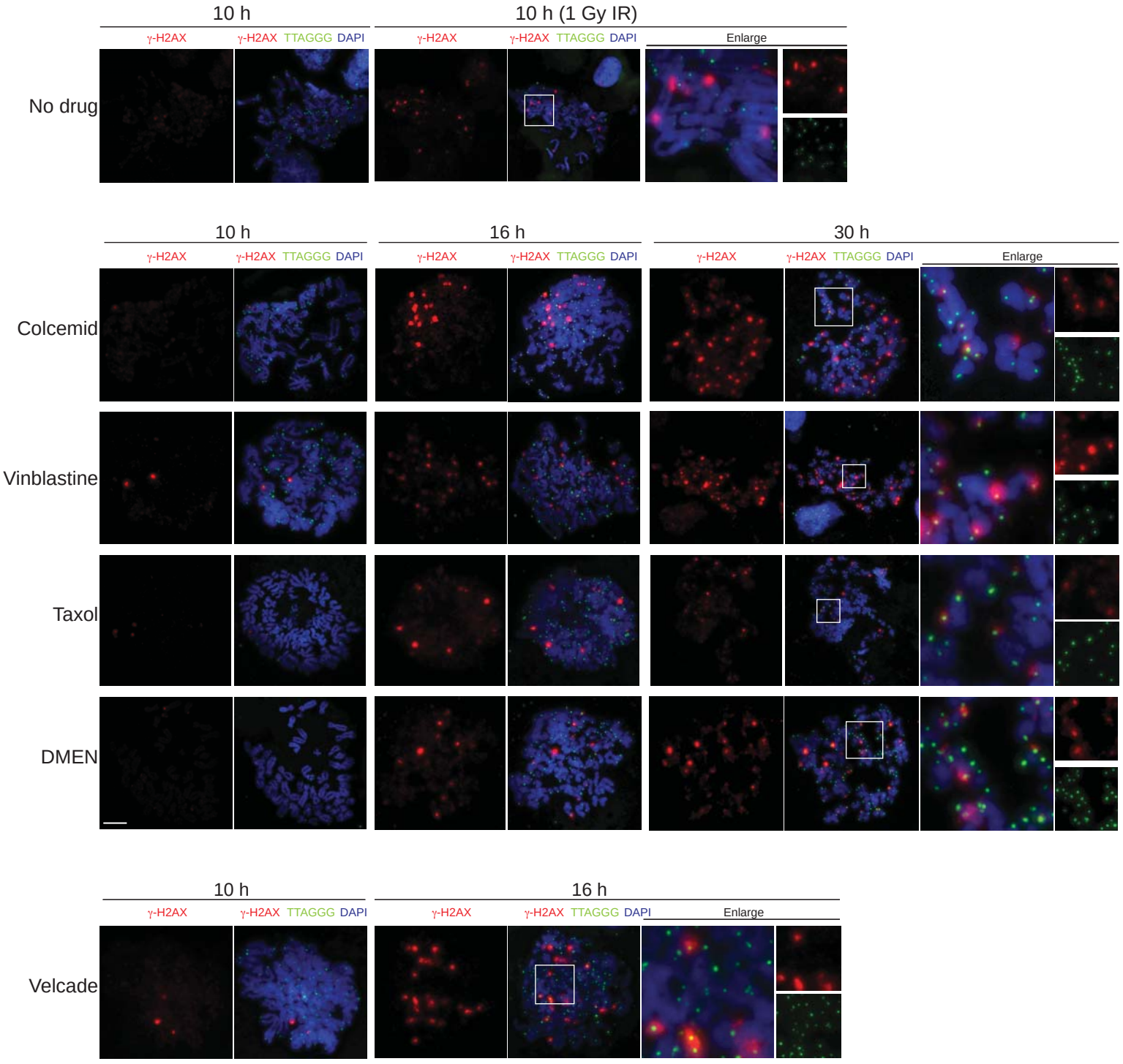
Supplementary Figure 1. Colcemid exposure leads to TIF formation in transformed and primary cells. (**a**) Immunofluorescence staining of metaphase chromosomes of HeLa1.2.11 cells treated with 100 ng ml-1 colcemid for 0 or 24 hrs. γ-H2AX is shown in the red channel, TTAGGG repeats in green and the DNA has been stained with DAPI in blue. Magnifications of indicated regions are shown on the bottom. (**b**) Immunofluorescence staining of metaphase chromosomes of IMR90 primary fibroblasts treated with 100 or 120 ng ml-1 colcemid for 24 hrs. γ-H2AX is shown in the red channel, TTAGGG repeats in green and the DNA has been stained with DAPI in blue. Magnifications of indicated regions are shown on the bottom. Scale bar, 10 μm.

Supplemental Figure 2
Hayashi et al.



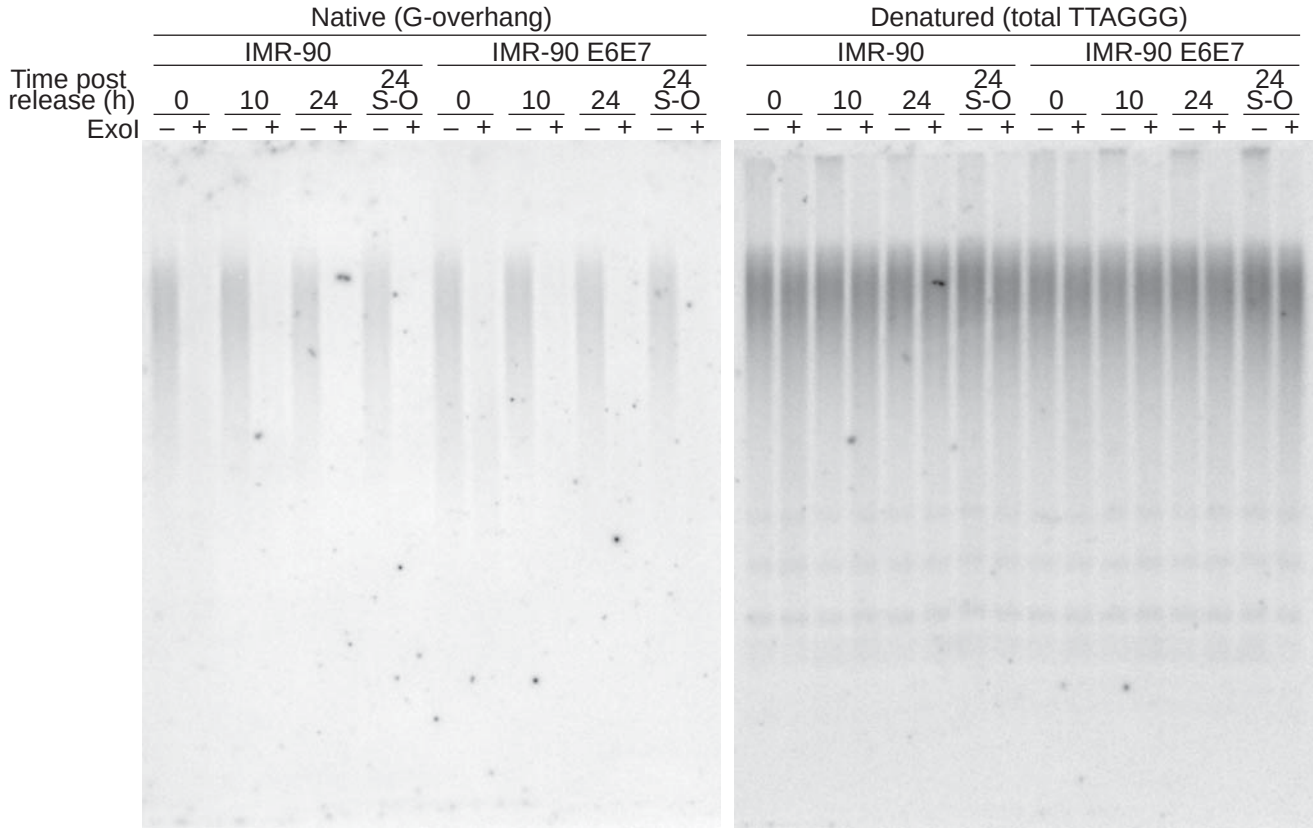
Supplementary Figure 2. Time dependence of TIF formation. (**a**) Quantification of γ-H2AX foci in IMR90 fibroblast, where individual quantifications from three independent experiments are shown. Each dot represents a different image and the orange bar represents the mean value. (**b**) Quantification of γ-H2AX foci in HMEC prometaphases following the indicated treatment. The mean and standard deviation of three experiments quantifying at least 25 prometaphases is shown. (**c**) Cell cycle progression of IMR90 cells post release from a G1/S arrest. Hrs post release, H3-Ser10P and DNA content have been indicated.

Supplemental Figure 3
Hayashi et al.



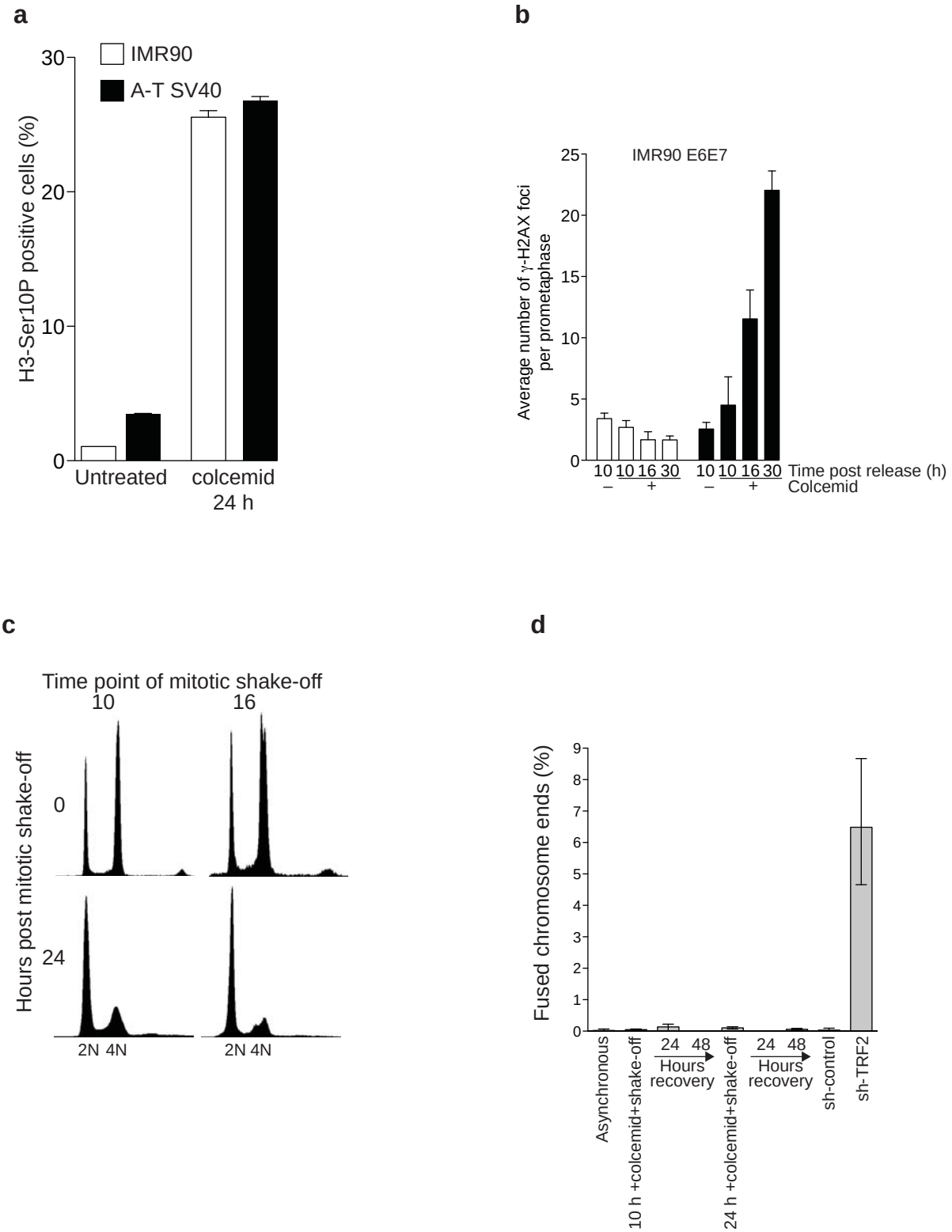
Supplementary Figure 3. TIF accumulation upon exposure of IMR90 cells to mitotic inhibitors. Immunofluorescence staining of metaphase chromosomes of IMR90 primary fibroblasts treated with 100 ng ml-1 of colcemid, 100 nM vinblastine, 500 nM taxol, 1 μM dimethylenastron (DMEN) or 1 μM velcade. γ-H2AX is shown in the red channel, TTAGGG repeats in green and the DNA has been stained with DAPI in blue. Magnifications of indicated regions are shown on the right. Time points of harvest after G1/S release are indicated above the panels. No-drug and γ-irradiation controls are shown as the top panels. Scale bar, 10 μm.

Supplemental Figure 4
Hayashi et al.



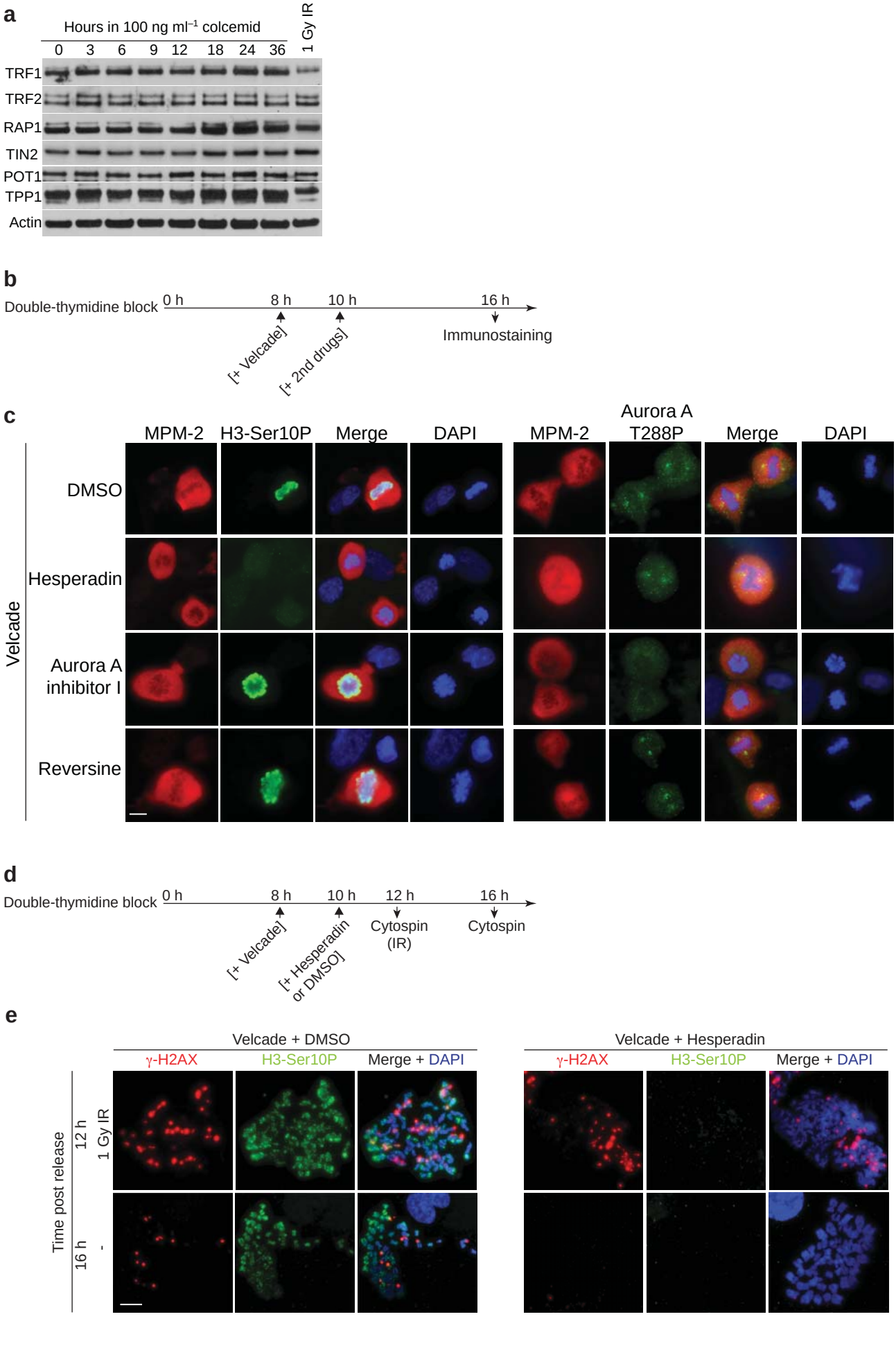
Supplementary Figure 4. Exonuclease I treatment of DNA isolated from IMR90 and IMR90 E6E7 cells. Example of native (left panel) and denatured (right panel) signals from telomeric southern analysis of IMR90 cells or IMR90 E6E7 cells. Time post release is indicated and refers to the schematic in (Fig. 2a).

Supplemental Figure 5
Hayashi et al.



Supplementary Figure 5. Telomeric consequences of mitotic TIF formation. (**a**) Analysis of the dynamics of H3-Ser10P in IMR90 cells (white bars) and A-T SV40 fibroblasts (black bars). Untreated cells or cells exposed to 100 ng ml-1 of colcemid for 24 hrs were analyzed. (**b**) Quantification of γ-H2AX foci in prometaphase IMR90 E6E7 cells over time, treated with 100 ng ml-1 of colcemid. Time points of harvest after G1/S release are indicated on the bottom. Non-telomeric foci are shown as white bars and telomeric foci as black bars. Error bars represent the standard deviation of three independent experiments, where at least 25 prometaphases have been analyzed per treatment and time point. (**c**) Analysis of DNA content of colcemid treated IMR90 cells at the 10 and 16 hr time points and 24 hrs after release from the arrest as described in Fig. 4D. (**d**) Quantification of metaphase chromosomes with fused ends in IMR90 E6E7 cells that were synchronized, exposed to colcemid and allowed to recover as described in Fig. 4B. IMR90 E6E7 cells expressing a control shRNA served as negative control, and IMR90 E6E7 cells expressing a shRNA targeting TRF2 served as positive control. Error bars represent the standard deviation of three independent experiments, where 30 metaphases have been analyzed per time point or shRNA treatment.

Supplemental Figure 6
Hayashi et al.



Supplementary Figure 6. Aurora B dependence of TIF formation. (**a**) Western analysis of shelterin components during mitotic arrest. Asynchronous IMR90 cells were exposed to 100ng ml-1 colcemid for the indicated times and expression of the six shelterin components was analyzed in whole cell extracts. Actin served as a loading control and exposure of cells to 1 GY of γ irradiation as damage-control. (**b**) Schematic of the timing of experiments in panel **c**. (**c**) IF staining of IMR90 cells treated with Velcade/Hesperadin/Aurora A kinase inhibitor I/Reversine. MPM-2 is shown in red, Aurora A Thr288P in green and the DNA in blue (DAPI). (**d**) Schematic of the timing of experiments in panel **e**. (**e**) γ-H2AX focus formation in IMR90 cells subjected to ionizing irradiation. Cells were treated with Velcade and DMSO or Velcade and Hesperadin where indicated. Scale bar, 10 μm.