Supplementary Material for

High levels of TopBP1 induce ATR-dependent shut-down of rRNA transcription and nucleolar segregation

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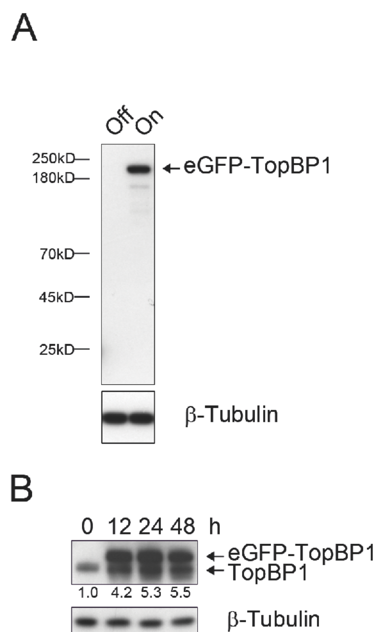
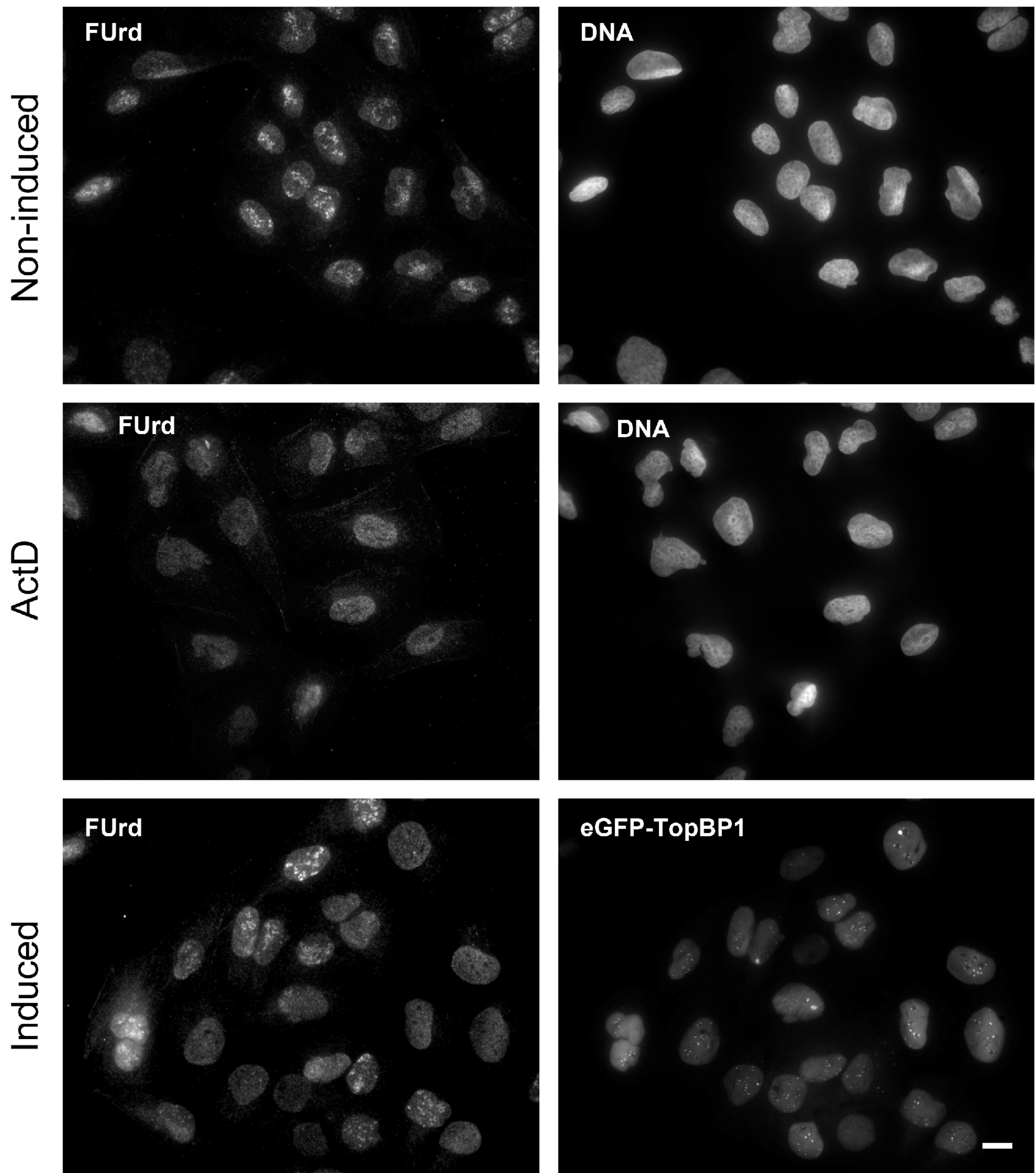
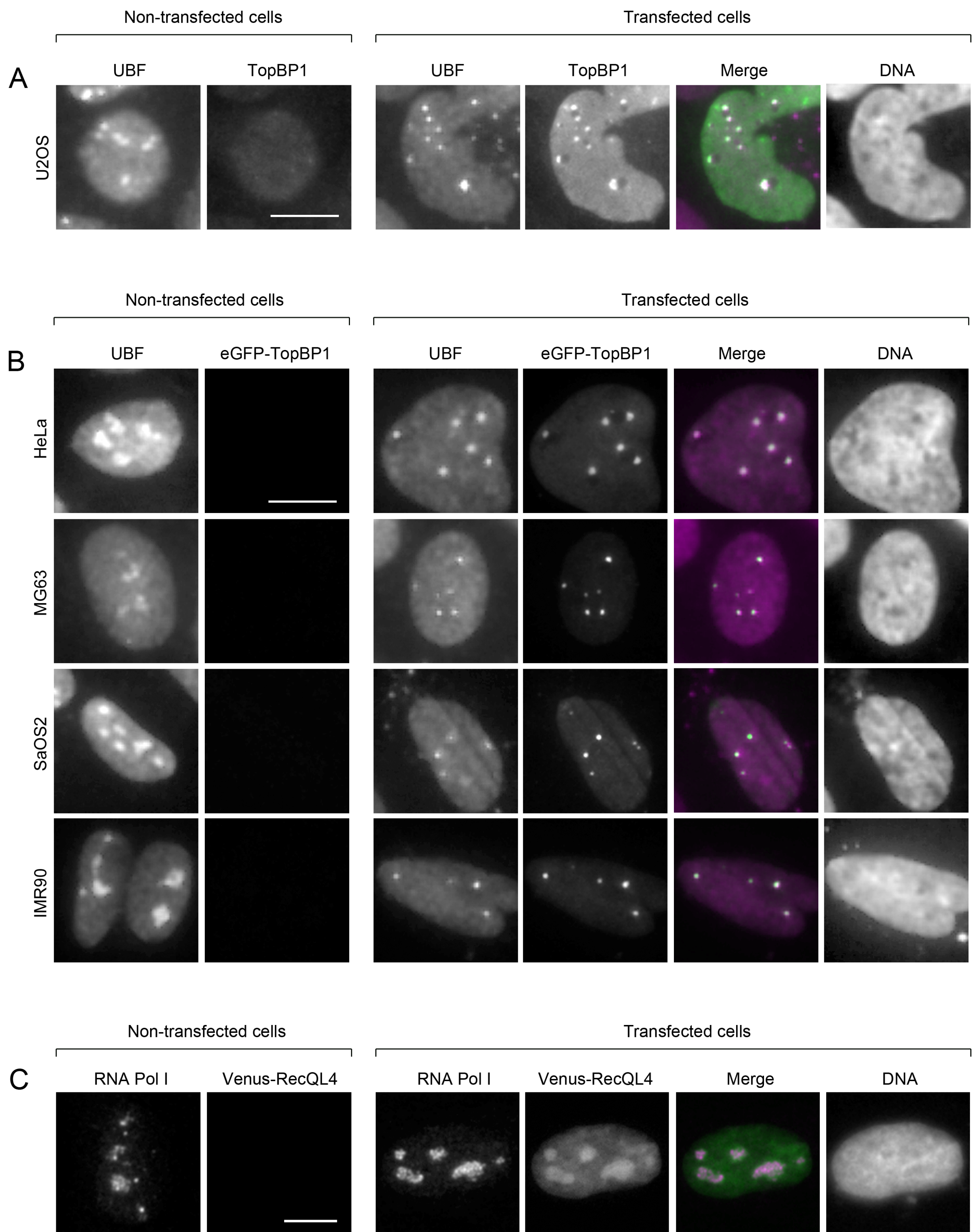


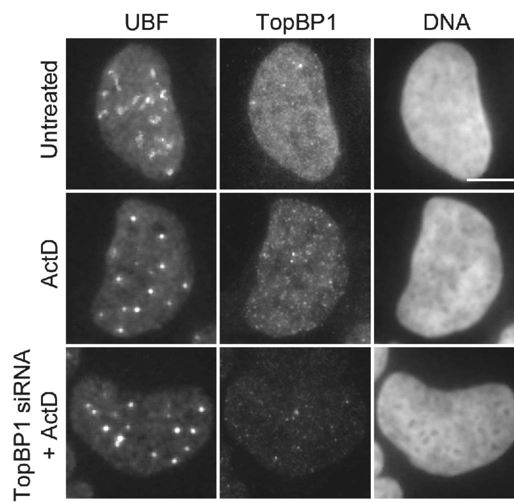
Supplementary Figure S1. Protein expression profile of the eGFP-TopBP1 cell line. (**A**) Immunoblot of whole-cell extracts non-induced (Off) or induced (On) to express eGFP-TopBP1. Anti-GFP antibody was used to detect the fusion protein. (**B**) Immunoblot of whole-cell extracts left non-induced (0) or induced for 12, 24 or 48 hours to express eGFP-TopBP1. Both endogenous and ectopic TopBP1 were detected with anti-TopBP1 antibody. β-Tubulin served as a control of protein loading. Quantification of the total TopBP1 (eGFP-TopBP1 plus endogenous TopBP1) signal relative to non-induced is shown below the blot.



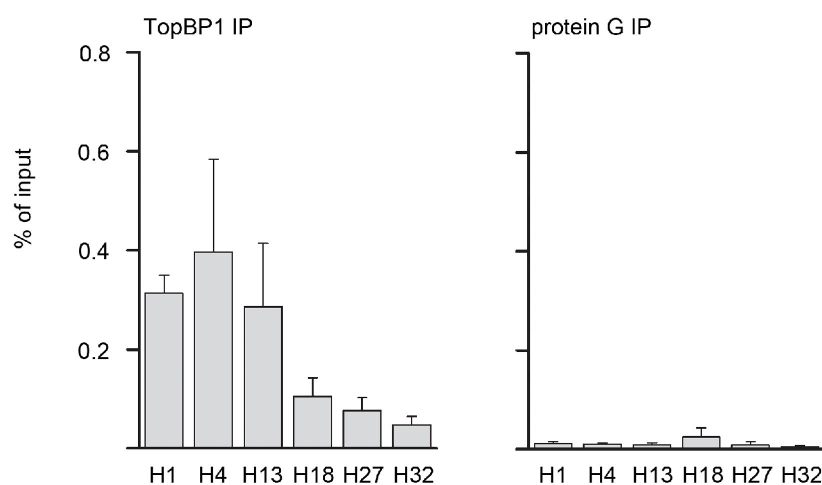
Supplementary Figure S2. Most cells that display high eGFP-TopBP1 expression levels and the nucleolar segregation phenotype show little or no nucleolar FUrd incorporation (bottom panel). Cells were left non-induced and non-treated or treated with Actinomycin D or induced to express eGFP-TopBP1 as indicated. Cells were labeled with a 30 min pulse of fluorouridine (FUrd) to label nascent RNA. DNA was stained with Hoechst. Scale bar is 20 µm.



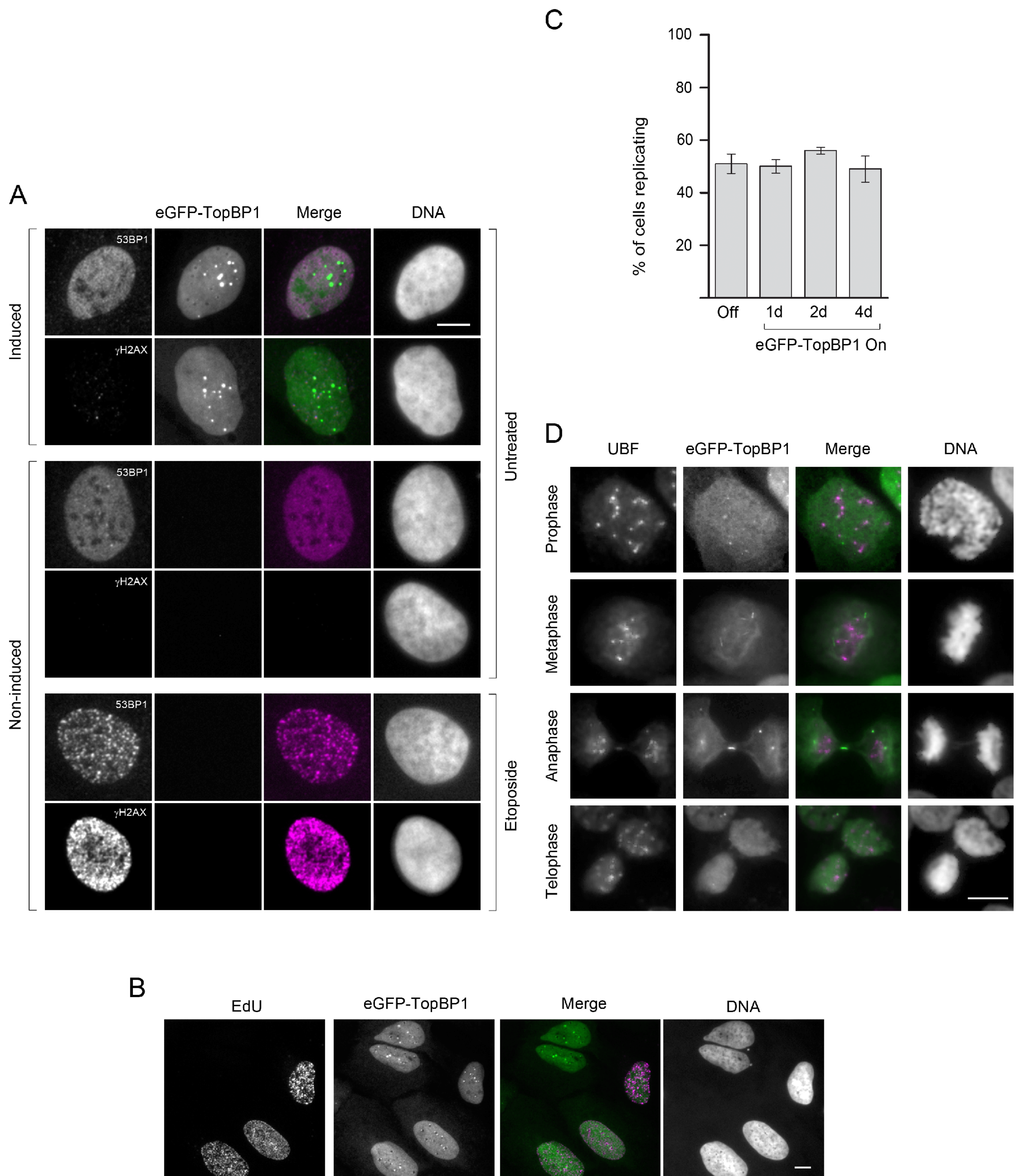
Supplementary Figure S3. Controls for eGFP-TopBP1 expression. (**A**) U2OS cells were transfected with plasmids encoding TopBP1 without the eGFP tag. TopPB1 expression and endogenous UBF were detected by immunostaining. (**B**) Different cell lines were transiently transfected with plasmids encoding eGFP-TopBP1. Endogenous UBF was indirectly immunostained. (**C**) U2OS cells were transiently transfected with plasmids encoding Venus-RecQL4 (Ven-RecQL4). Endogenous RNA Pol I (RPA194) was indirectly immunostained. DNA was stained with Hoechst 33258. Scale bars are 10 µm. The panels on the left present non-transfected cells from the same sample as the transfected cells.



Supplementary Figure S4. TopBP1 knock-down does not prevent ActD-induced nucleolar segregation. U2OS cells were left non-transfected or transfected with TopBP1 siRNA. Endogenous UBF and TopBP1 was indirectly immunostained. DNA was stained with Hoechst 33258. Scale bars represent 10 μm .



Supplementary Figure S5. Endogenous TopBP1 associates with transcribed rDNA. Percentages of input material precipitated with anti-TopBP1 (left) and protein G beads only (right) in chIP assay using nuclear material from U2OS cells. DNA was quantitated with qPCR using primer pairs as indicated in Figure 5C. Results of three independent experiments are shown with standard deviations.



Supplementary Figure S6. The nucleolar eGFP-TopBP1 foci are not sites of DNA damage. (**A**) Cells left non-induced or induced to express eGFP-TopBP1 or non-induced cells treated with Etoposide (50μM, 1h) were immunostained for endogenous 53BP1 or γH2AX, as indicated. (**B**) Nascent DNA was labeled with a 30min EdU pulse in cells induced to express eGFP-TopBP1. (**C**) Cells were left non-induced (Off) or induced for the indicated days. Replicating cells were counted from cells labeled with a 15min BrdU-pulse. Means of three independent experiments are shown with standard deviations. (**D**) UBF was immunolabeled in eGFP-TopBP1 expressing cells. Cells undergoing different phases of mitosis are shown. Scale bars are 10 μm.

SUPPLEMENTARY MATERIAL AND METHODS

Cell Culture

SaOS2 cells (ATCC) were cultivated at 37°C and 5% CO_2 in McCoy's 5a medium (Sigma) including 10% fetal bovine serum. MCF7 (ATCC), MG63 (ECACC), HeLa (ECACC) and IMR90 (ATCC) cells were cultivated at 37°C and 5% CO_2 in minimum essential medium including 10% fetal bovine serum.

Transfections

IMR90 cells were transfected using Fugene HD (Promega) non-liposomal transfection reagent. All other DNA transfections were done with Effectene (Qiagen) transfection reagent. TopBP1 siRNA (Ambion, s21824) was transfected as described in for ATR siRNA in the main text.

Quantification of Immunoblots

Amount of endogenous and ectopic TopBP1 was quantified with ImageJ (1.48v). Western blot band intensities were normalized to relative intensities of β-Tubulin.