Suppl. Figure 1. Localization of endogenous TopBP1 and ATRIP in U2OS cells 4 hours (h) after exposure to IR (9 Gy) or after mock-irradiation (0 Gy), as determined by immunofluorescence.

Suppl. Figure 2. Cell cycle dependency of IR-induced TopBP1 foci.

(A) Example of the images used to generate the histogram plot shown in Figure 1C correlating the behavior of TopBP1 to genomic DNA content. For each nucleus the DAPI staining was integrated and the values used to generate DNA contents; the final values for each cell are shown. Values of 2N and 4N correspond to cells in G1 and G2, respectively. In the two cells with 1.89N and 1.98N DNA contents, the TopBP1 and 53BP1 IR-induced foci colocalize.

(B) Example of the images used to generate the histogram plot shown in Figure 1D correlating the behavior of TopBP1 according to Cyclin B1 staining. The cell on the left stains positive for Cyclin B1 in the cytoplasm and its TopBP1 foci do not colocalize with the 53BP1 foci. The cell on the right stains negative for Cyclin B1 and its TopBP1 and 53BP1 IR-induced foci colocalize.

(C) Example of the images used to generate the histogram plot shown in Figure 1E correlating the behavior of TopBP1 to EdU staining and genomic DNA content. The cell on the left stains positive for EdU, has a DNA content of >2N and its TopBP1 foci do not colocalize with the 53BP1 foci. The cell on the right stains negative for Edu, has a 2N DNA content and its TopBP1 and 53BP1 IR-induced foci colocalize.

Suppl. Figure 3. Mapping the domains that mediate recruitment of TopBP1 to IR-induced 53BP1 or RPA foci.

(A-B) Examples of the intracellular localization of selected GFP-tagged TopBP1 mutants in U2OS cells 4 hours after exposure to IR (9 Gy). The intracellular localization of endogenous 53BP1 (A) or RPA (B) is also shown. The numbers refer to the residues present in the TopBP1 mutants. Δ, deletion.

Suppl. Figure 4. Different pairs of TopBP1 BRCT domains have distinct properties for recruitment to sites of DNA damage in cells exposed to ionizing radiation (9 Gy).

(A) Expression levels of GFP or GFP-tagged tetramers (TZp) of TopBP1 BRCT domains 1-2 (residues 2-300), 4-5 (residues 531-755) and 7-8 (residues 1258-1522) in transiently-transfected U2OS cells, as determined by immunoblotting with an antibody specific for GFP. Amino acid substitutions of selected lysine residues with alanine (A) are indicated. A154/5, double substitution of lysines 154 and 155 with alanine; wt, wild-type.

(B) Recruitment of BRCT domains 1-2 (residues 2-300) with the indicated lysine to alanine substitutions as tetramers (TZp) to sites of DNA damage.

(C) Recruitment of BRCT domains 4-5 (residues 531-755) with the indicated lysine to alanine substitutions as tetramers (TZp) to sites of DNA damage.

Suppl. Figure 5. Different pairs of TopBP1 BRCT domains have distinct properties for recruitment to sites of DNA replication stress in cells exposed to hydroxyurea (HU).

Recruitment of BRCT domains 1-2 (residues 2-300), 4-5 (residues 531-755) and 7-8 (residues 1258-1522) with the indicated lysine to alanine substitutions as tetramers (TZp) to sites of DNA replication stress in transiently-transfected U2OS cells 16 h after treatment with 2 mM HU.

Suppl. Figure 6. Analysis of the 53BP1-, ATM- and RPA70-dependency of TopBP1 recruitment to DNA damage sites.

(A) TopBP1 recruitment to DNA DSBs in G1 is dependent on 53BP1. Analysis of the experiments shown in Figures 4A (endogenous TopBP1; left graph) and 4B (stably-expressed HA-ZZ-TZp-TopBP1 BRCT domains 4-5; right graph). More than 100 cells were counted for each condition. Colocalization of TopBP1 with phosphorylated histone H2AX (γ-H2AX) is characteristic of DNA DSBs in G1 cells; colocalization of TopBP1 with RPA indicates sites of DNA replication stress/resected DSBs. The TopBP1 protein containing only BRCT domains 4-5 does not localize to sites of DNA replication stress/resected DNA DBSs (Figure 3) and therefore its recruitment to foci is not shown for Edu-positive cells.

(B) Defective recruitment of endogenous TopBP1 to sites of DNA DSBs in ataxia-telangiectasia fibroblasts (A-TF) in the G1 phase of the cell cycle 4 hours after exposure to IR (9 Gy). NHF; normal human fibroblasts.

(C) RPA70 is not required for formation of TopBP1 IR-induced foci in G1 cells. The intracellular localization of endogenous RPA70 and TopBP1 was monitored by immunofluorescence in U2OS cells 4 hours after exposure to IR (9 Gy). The cells were previously treated with control (Ctl) siRNAor siRNA targeting *rpa70*.

Suppl. Figure 7. Efficiency of depletion of endogenous TopBP1, 53BP1, ATM, ATRIP and RPA70 in U2OS cells by siRNA, as determined by immunoblotting analysis 48 hours after transfection.

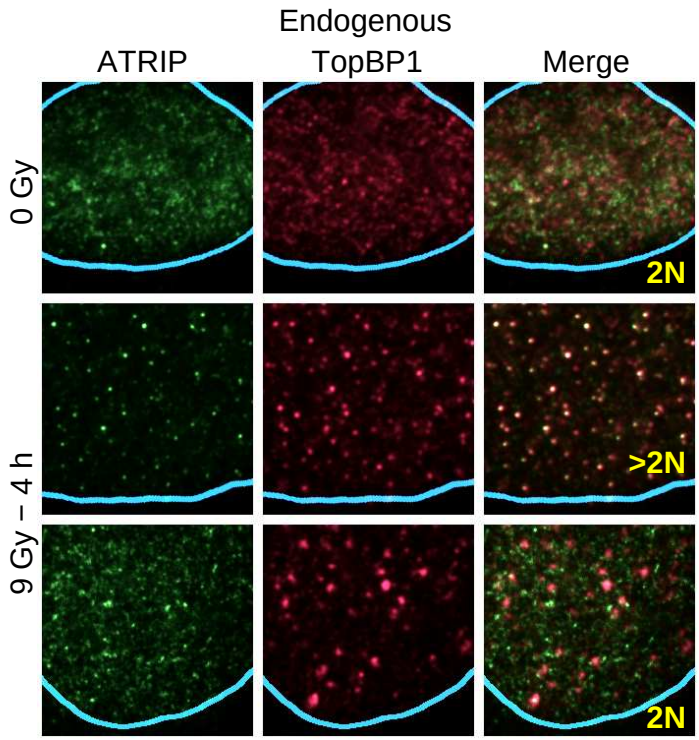
Suppl. Figure 8. Analysis of the histone H2AX-, MDC1- and RNF8-dependency of TopBP1 recruitment to DNA damage sites.

(A) Wild-type (wt), *h2ax*-/-, *mdc1*-/-, *rnf8*-/- mouse embryo fibroblasts were exposed to 9 Gy IR and examined by immunofluorescence 4 hours later. Representative G1 cells (2N DNA content; Edu-) were examined for colocalization of endogenous 53BP1 and TopBP1 with phosphorylated histone H2AX (γ-H2AX).

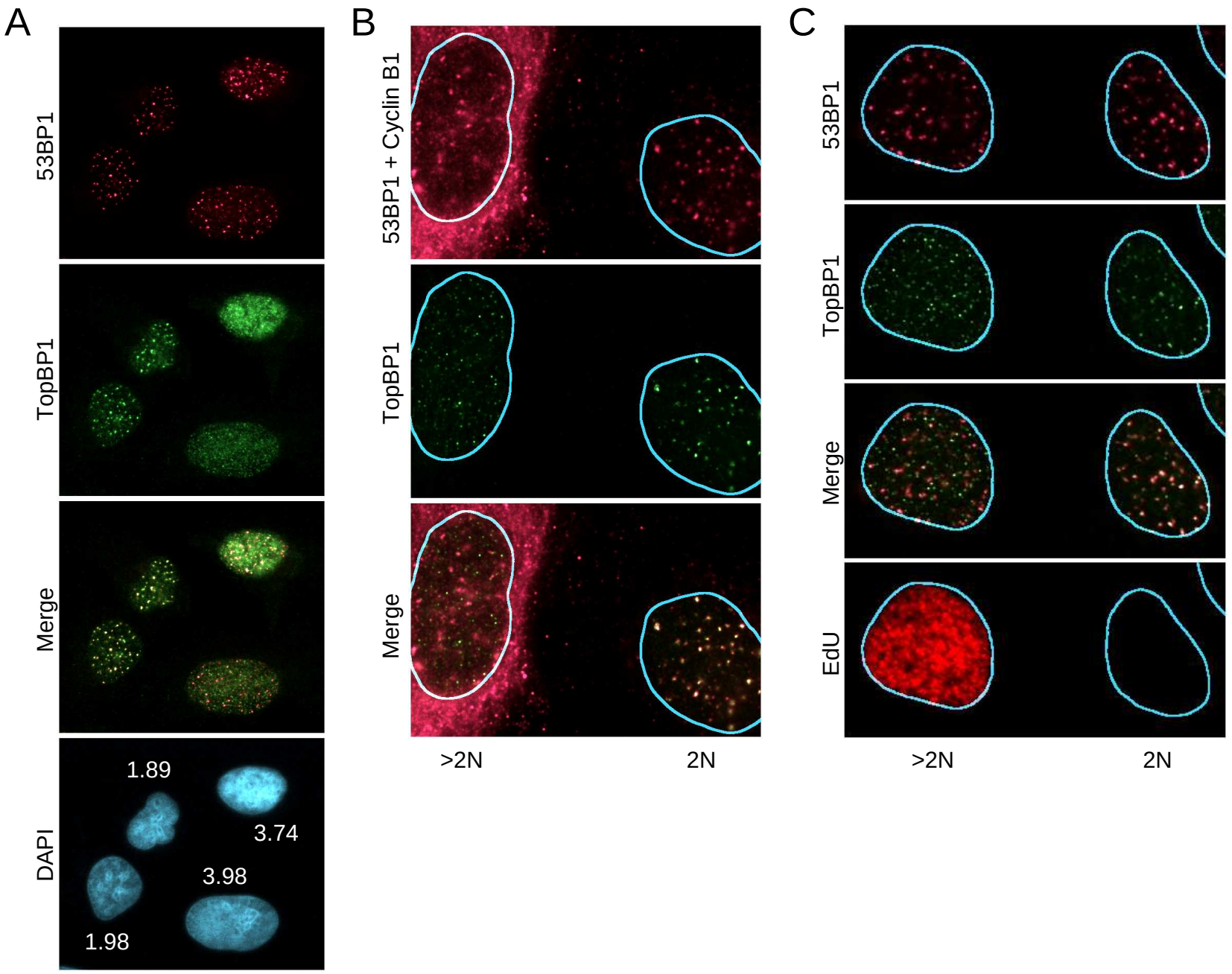
(B) Wild-type (wt), *h2ax*-/-, *mdc1*-/-, *rnf8*-/- mouse embryo fibroblasts were exposed to 2 mM hydroxyurea and examined by immunofluorescence 16 hours later. Representative S phase cells (>2N DNA content; Edu+) were examined for colocalization of endogenous TopBP1 with ATRIP.

Suppl. Figure 9. Model for the checkpoint function of 53BP1 and TopBP1 at sites of DNA DSBs in G1 cells. Initially, MDC1, Nbs1, 53BP1 and ATM are recruited to sites of DNA DSBs. Nbs1 physically interacts with ATM and can facilitate its activation, possibly also aided by 53BP1. In a second phase of recruitment TopBP1 is recruited by interacting via BRCT domains 1-2 with Nbs1 and via BRCT domains 4-5 with 53BP1. Once recruited, TopBP1 may activate ATM or ATR (see text for more details). The BRCT domains of TopBP1 are colored green; those domains that harbor lysines that are critical for recruitment of TopBP1 to sites of DNA damage (1, 2, 5 and 7) are demarcated by red lines. AD, ATR activation domain of TopBP1.

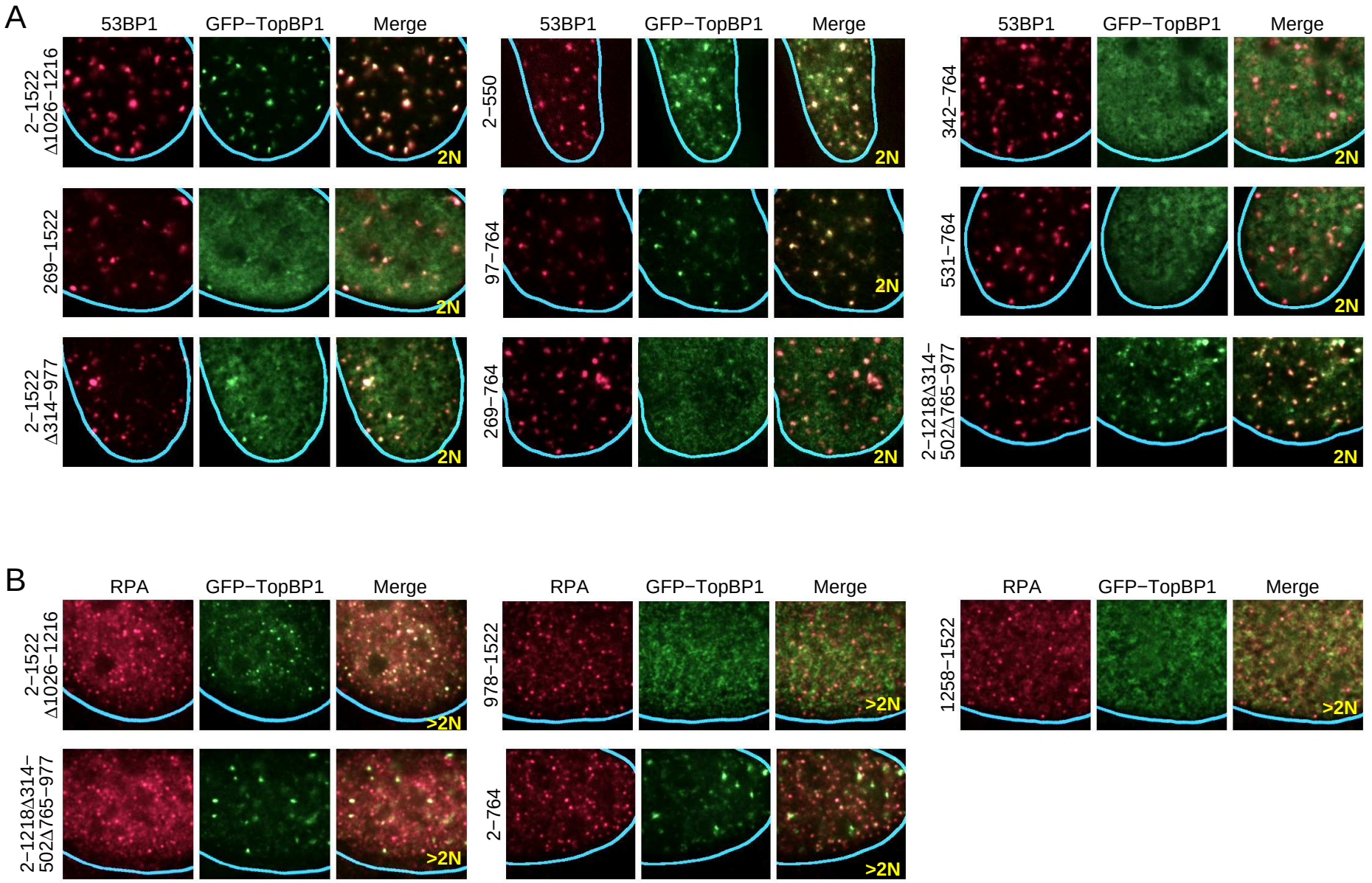
Suppl. Figure 1



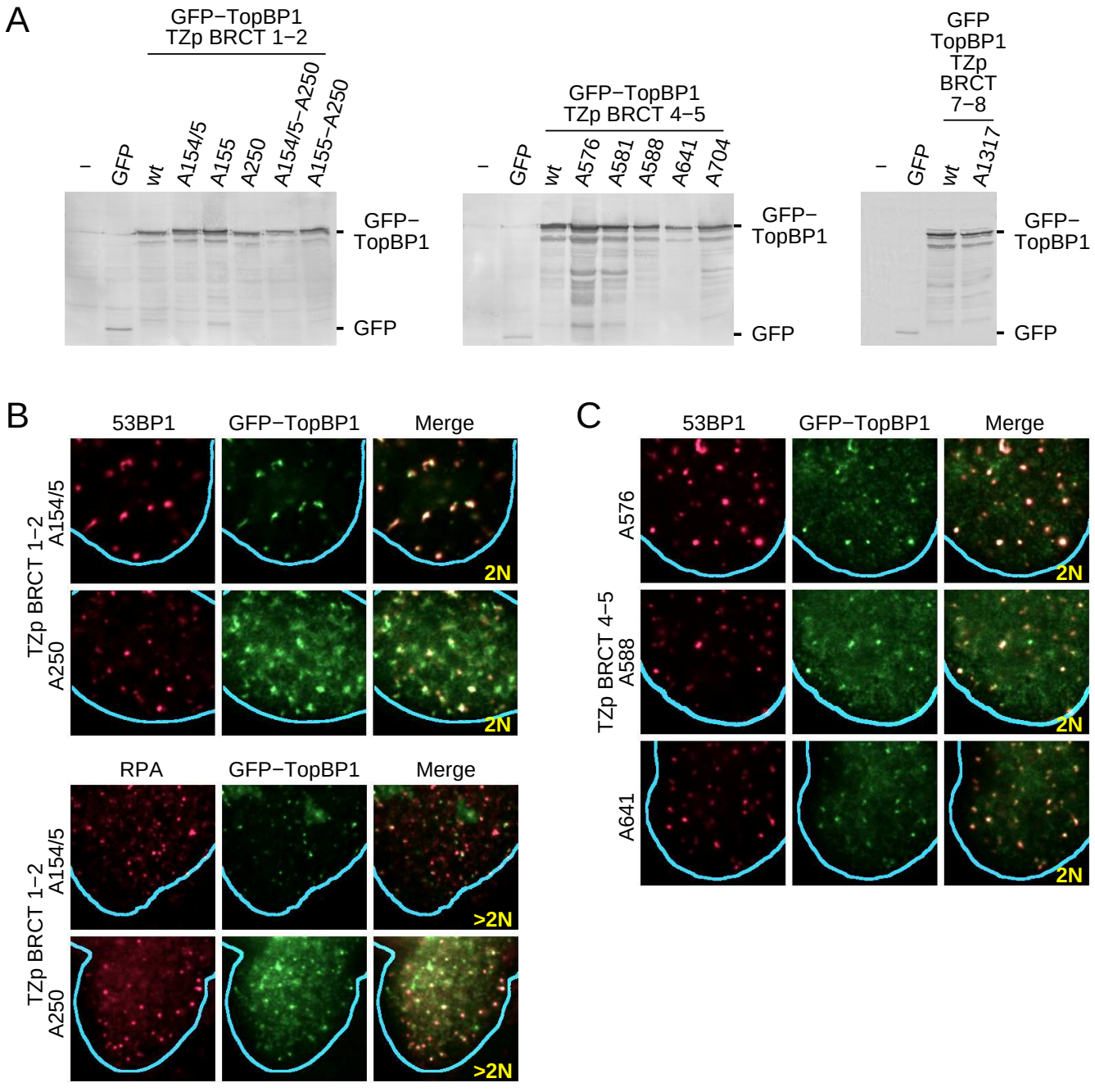
Suppl. Figure 2



Suppl. Figure 3



Suppl. Figure 4



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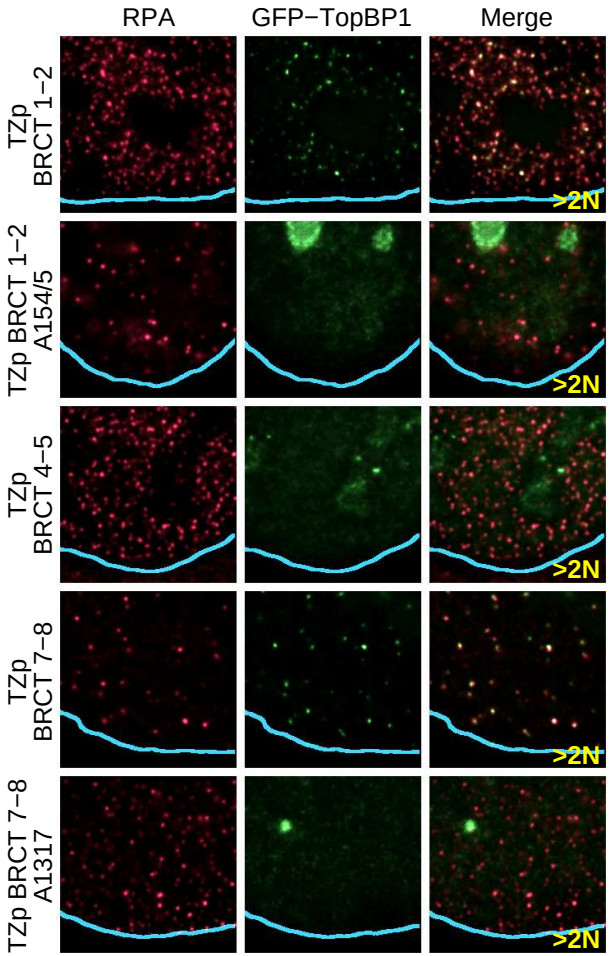

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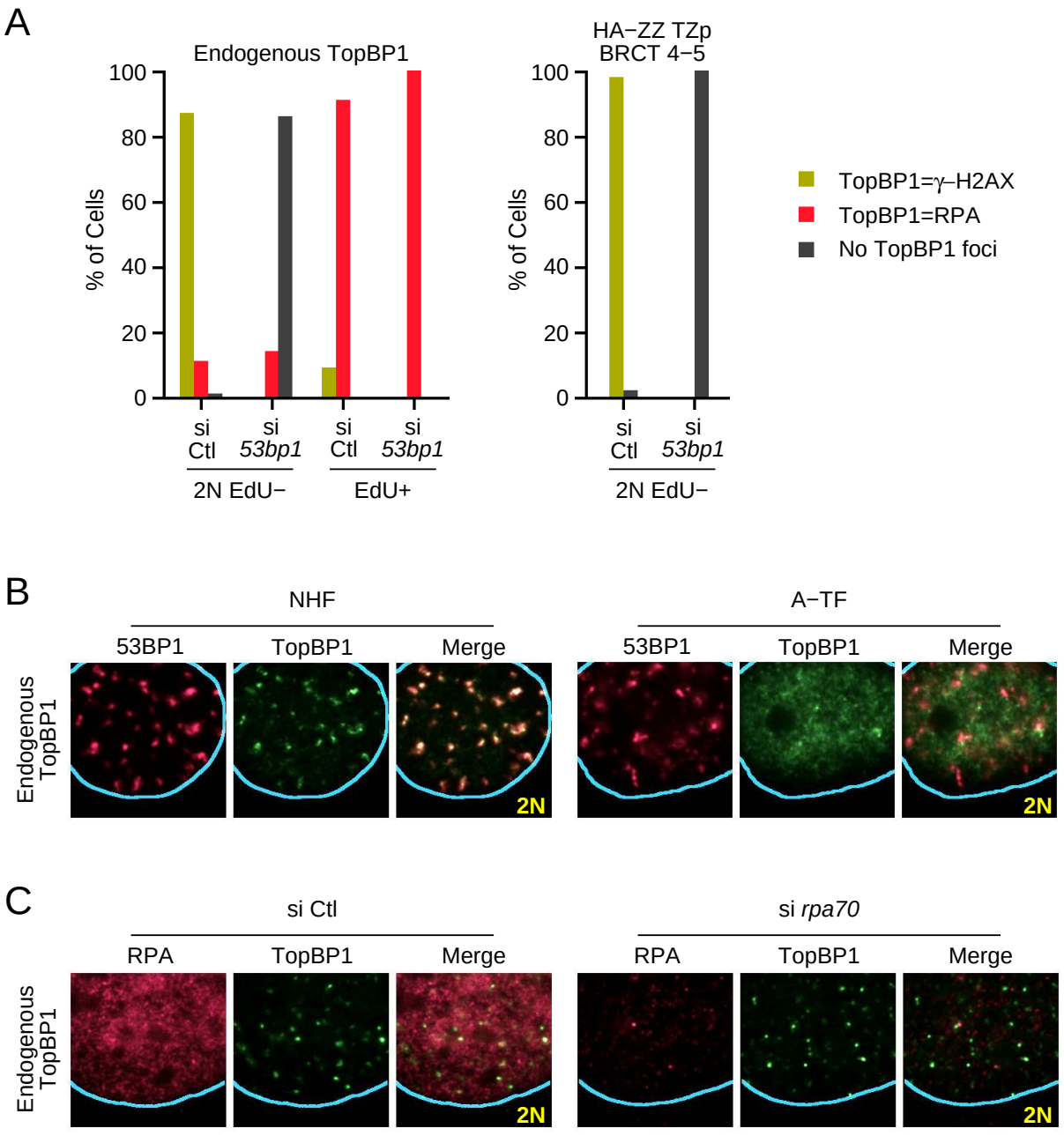

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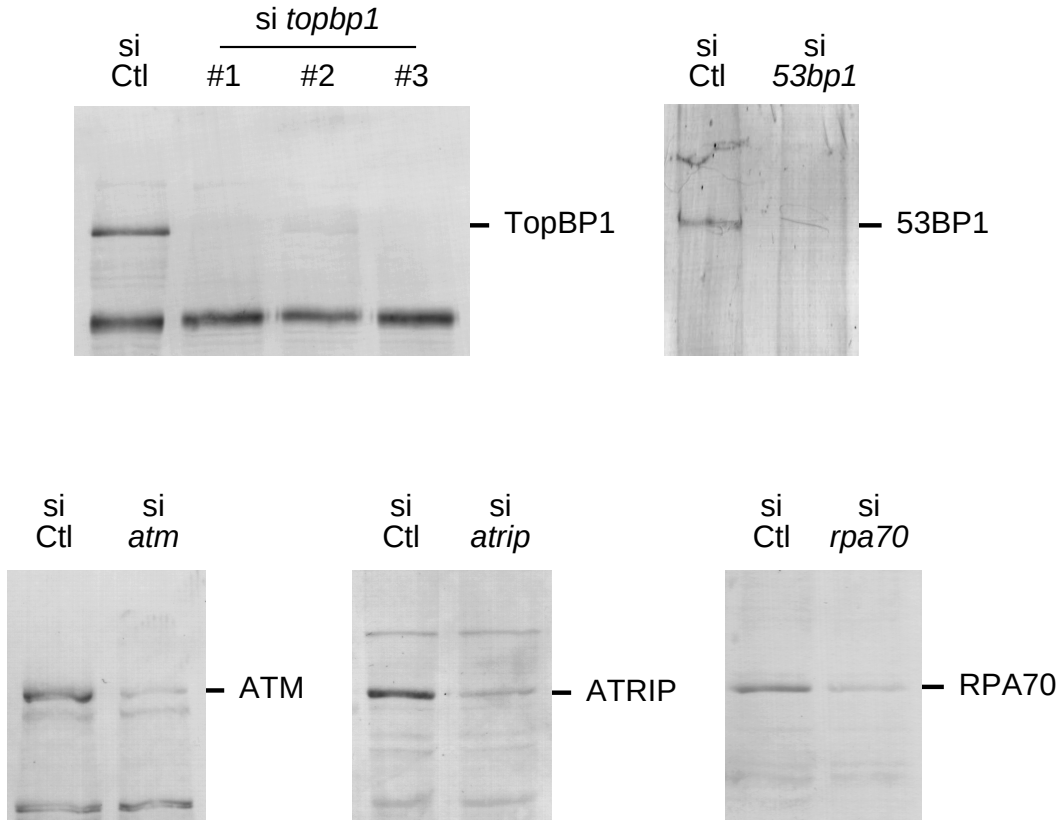
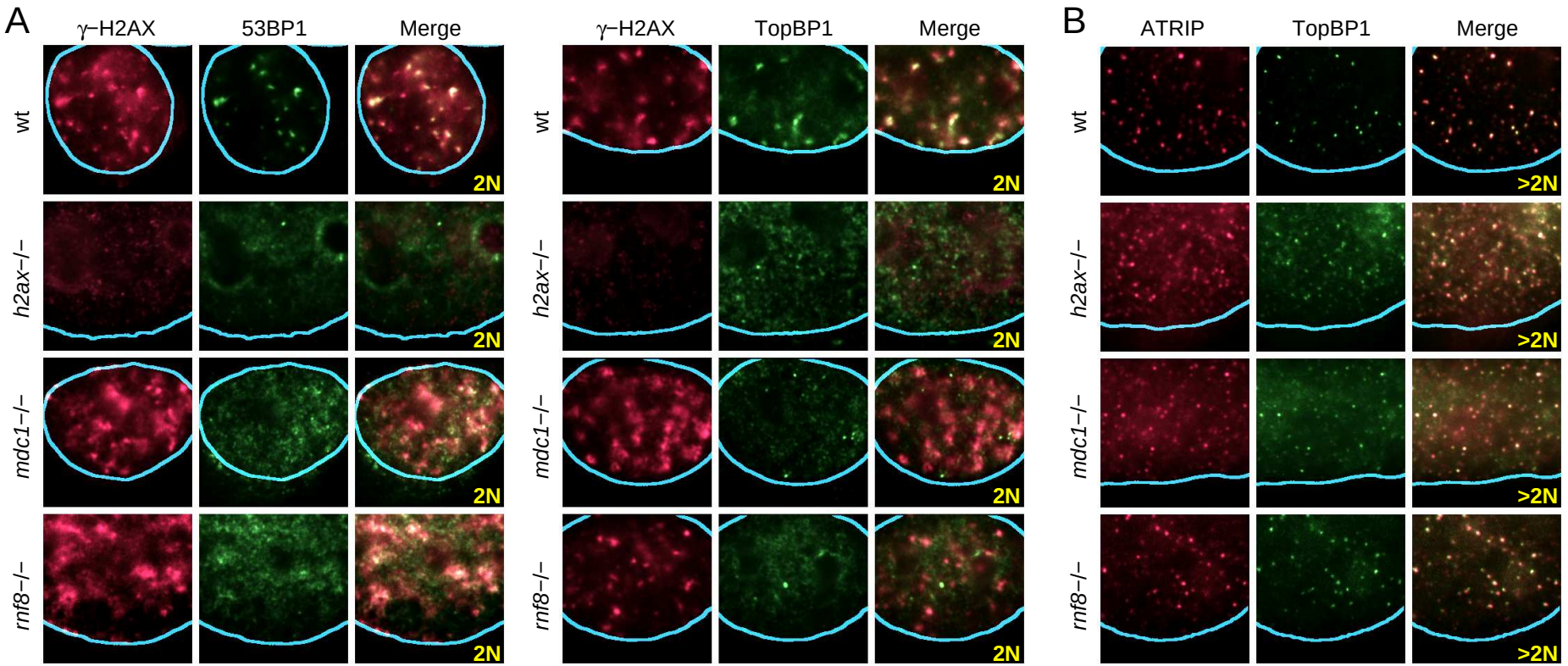


Suppl. Figure 8



Suppl. Figure 9