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RECRUITMENT OF DNA REPAIR PROTEINS TO DNA LESIONS AND FORMATION OF RADIATION-INDUCED FOCI DURING THE CELL CYCLE

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The DNA damage response is a fundamental, well-regulated process that occurs in the genome in order to recognise DNA lesions. Here, we studied kinetics of proteins involved in DNA repair pathways and their recruitment to DNA lesions during the cell cycle. In non-irradiated and irradiated cells, we analysed the distribution pattern and spatiotemporal dynamics of γ H2AX, 53BP1, BMI1, MDC1, NBS1, PCNA, coilin and BRCA1 proteins.

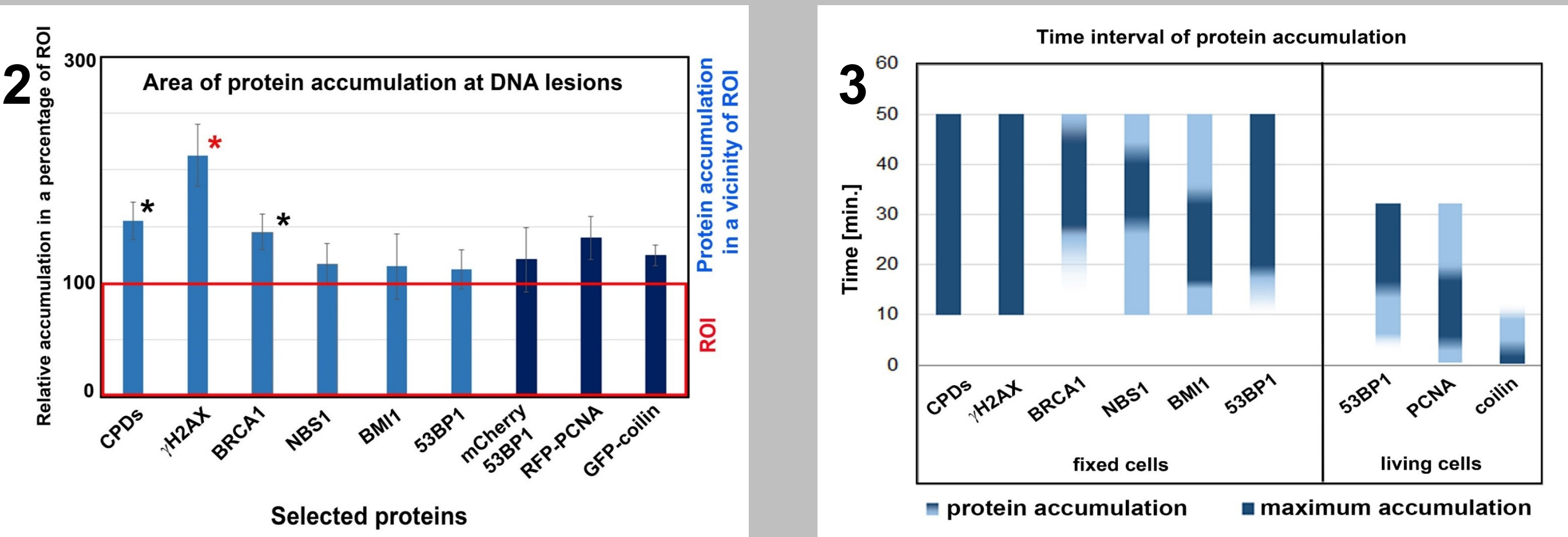
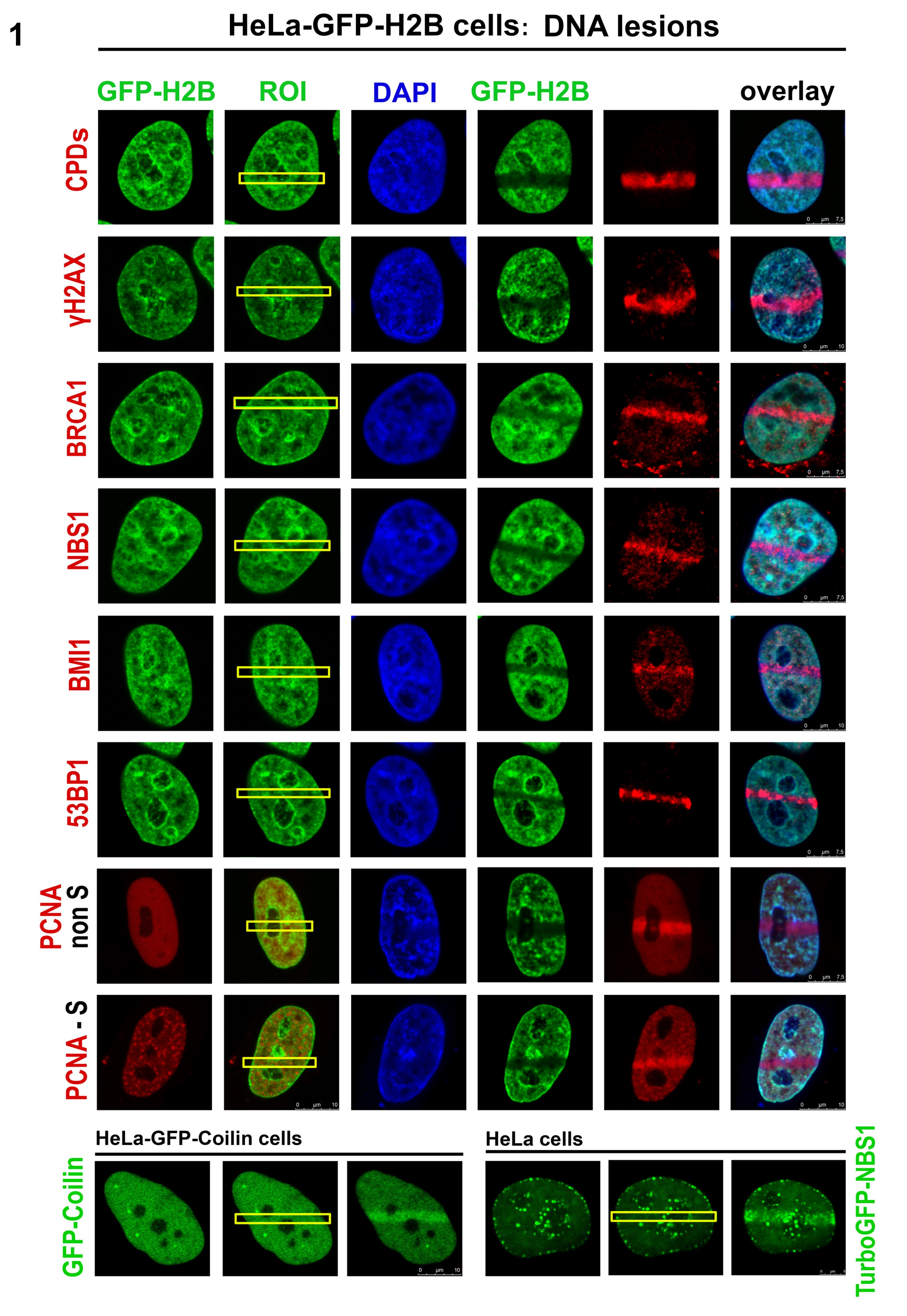


Fig. 1: Distribution patterns of selected proteins in UVA-irradiated regions. Proteins of interest were detected in irradiated ROIs by immunofluorescence or we studied GFP/mCherry/RFP-tagged protein recruitment to DNA lesions in live cells.

Fig. 2: Analysis of areas of protein accumulation at irradiated regions. CPDs, γ H2AX and BRCA1 proteins were spread around UVA radiation-induced DNA lesions. The quantification of the area of protein accumulation is expressed as a percentage of the area of the irradiated ROI [red rectangle; 100 %].

Fig. 3: Analysis of protein accumulation kinetics at irradiated regions. BMI1, PCNA and coilin were rapidly recruited to the lesions, 10–15 s after UVA-irradiation, whereas among the other proteins studied, BRCA1 demonstrated the slowest recruitment. Maximum fluorescence intensity is shown by dark-blue colour; shades of pale-blue colour show protein appearance/disappearance.

We showed that the kinetics of the accumulation of selected DNA repair-related proteins is protein specific at locally induced DNA lesions, and also γ H2AX is the most striking protein present not only at DNA lesions, but also spreading out in their vicinity. The formation of γ H2AX-, but not 53BP1-, positive nuclear bodies (foci), is cell cycle dependent after γ -irradiation. However, after local UVA irradiation both γ H2AX and 53BP1 proteins recruited identically to DNA lesions in G1, S and G2 phases of the cell cycle. Our conclusions highlight the significant role of the spatiotemporal dynamics of DNA repair-related proteins and their specific assembly/disassembly at DNA lesions, which can be cell type- and cell cycle specific.

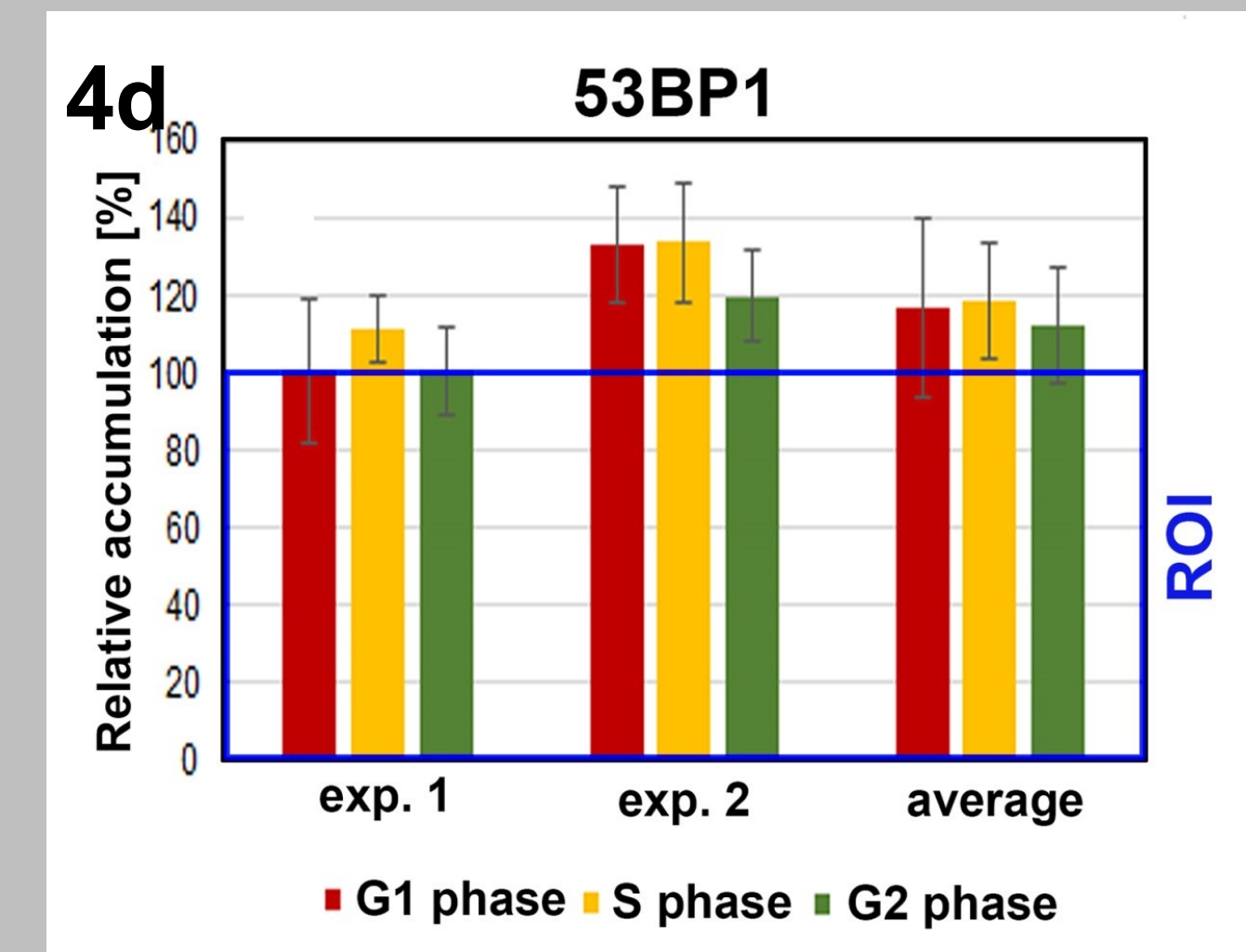
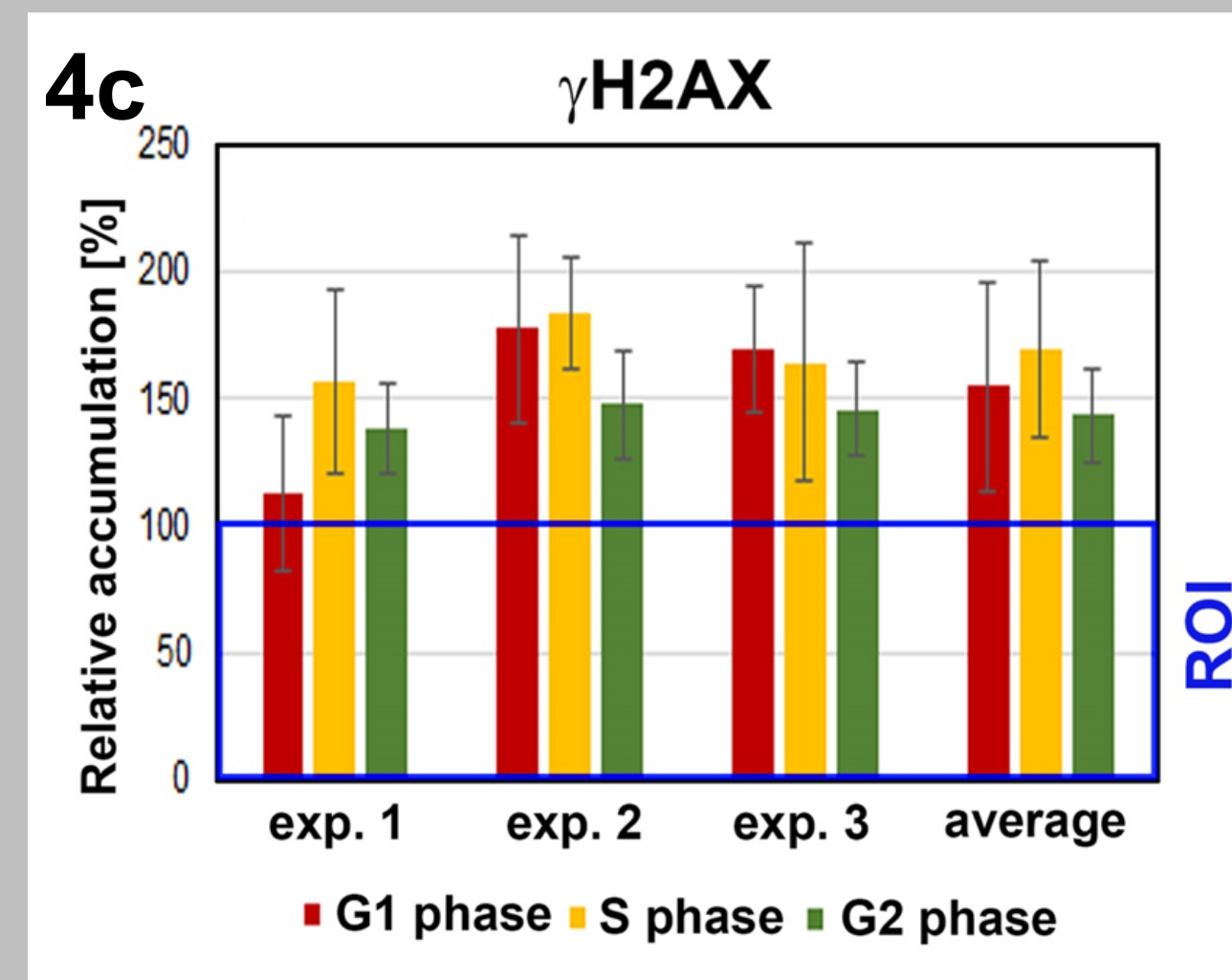
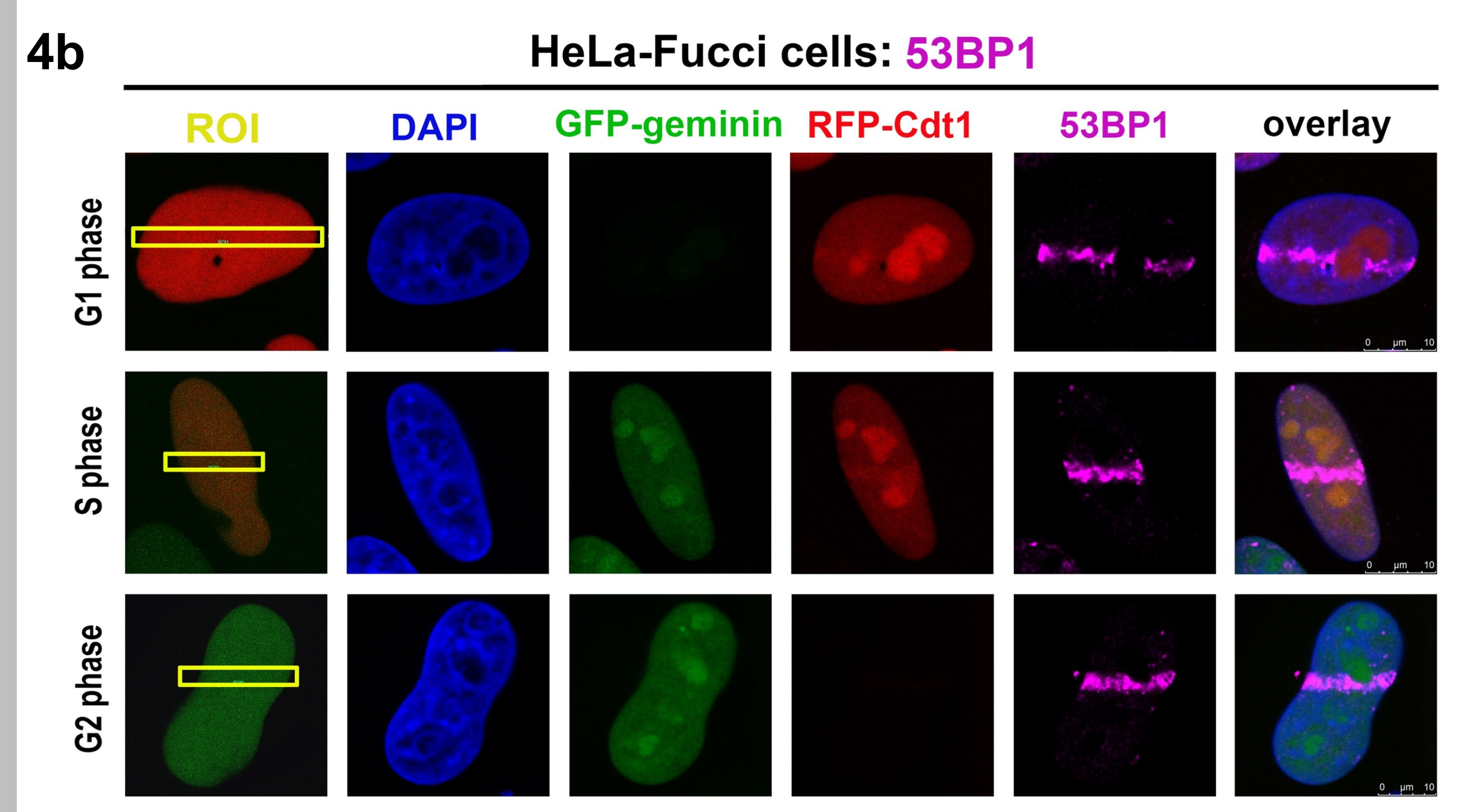
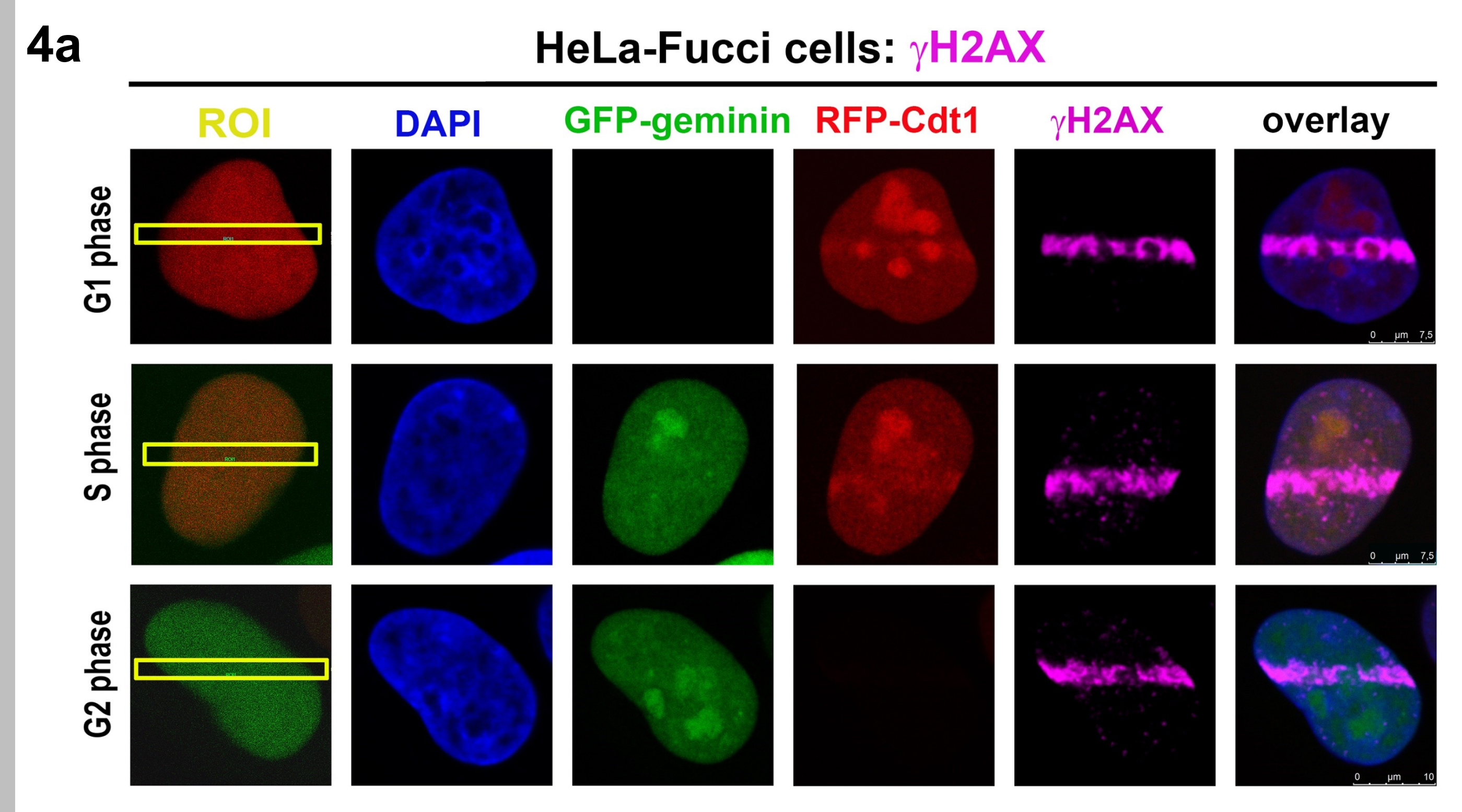


Fig. 4: Recruitment of γ H2AX (Fig. 4a, 4c) and 53BP1 (Fig. 4b, 4d) to DNA lesions in individual cell cycle phases. Both γ H2AX and 53BP1 proteins were identically recruited to DNA lesions in G1- (red), S- (orange) and G2-phases (green) of the cell cycle, studied in Fucci-HeLa cells.

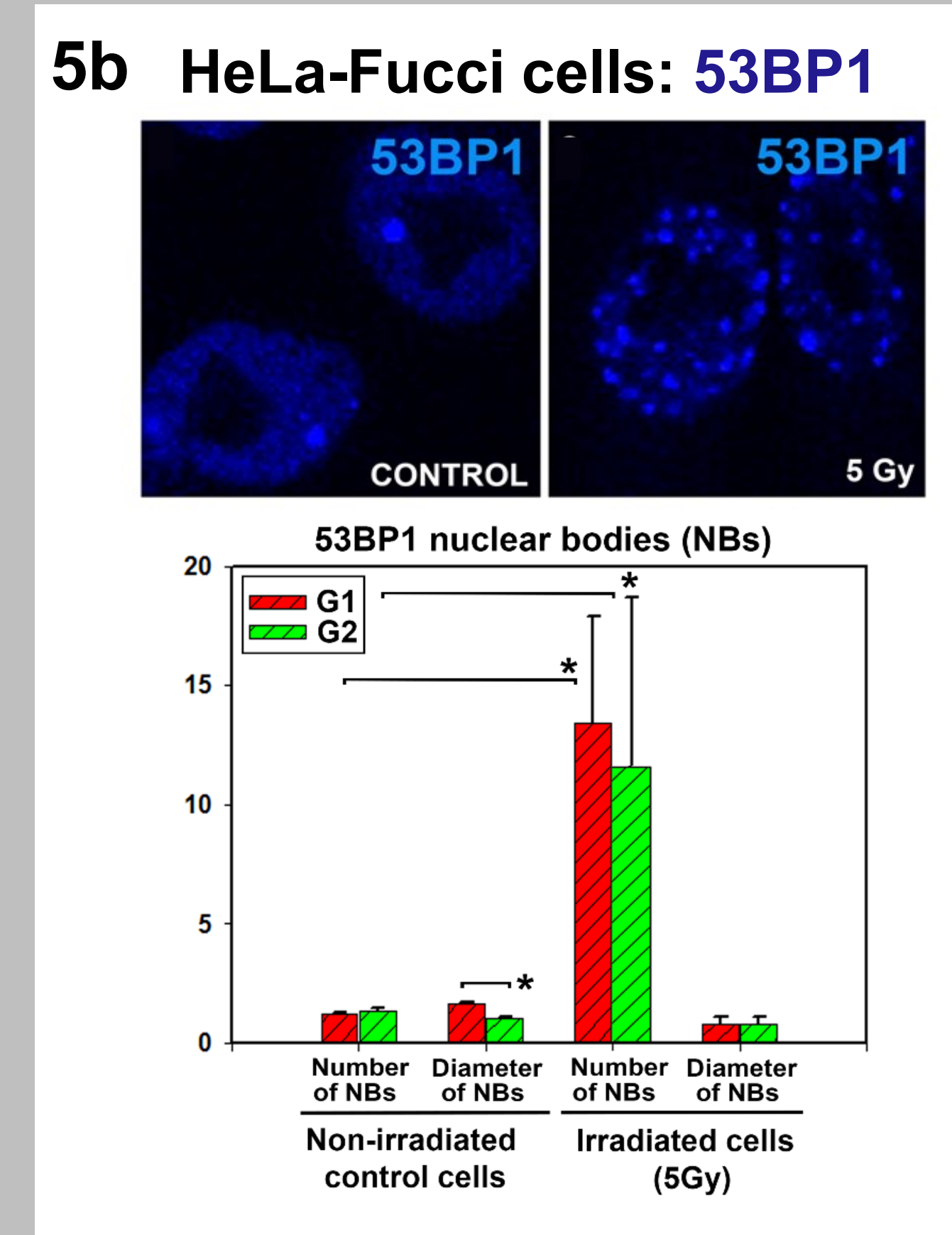
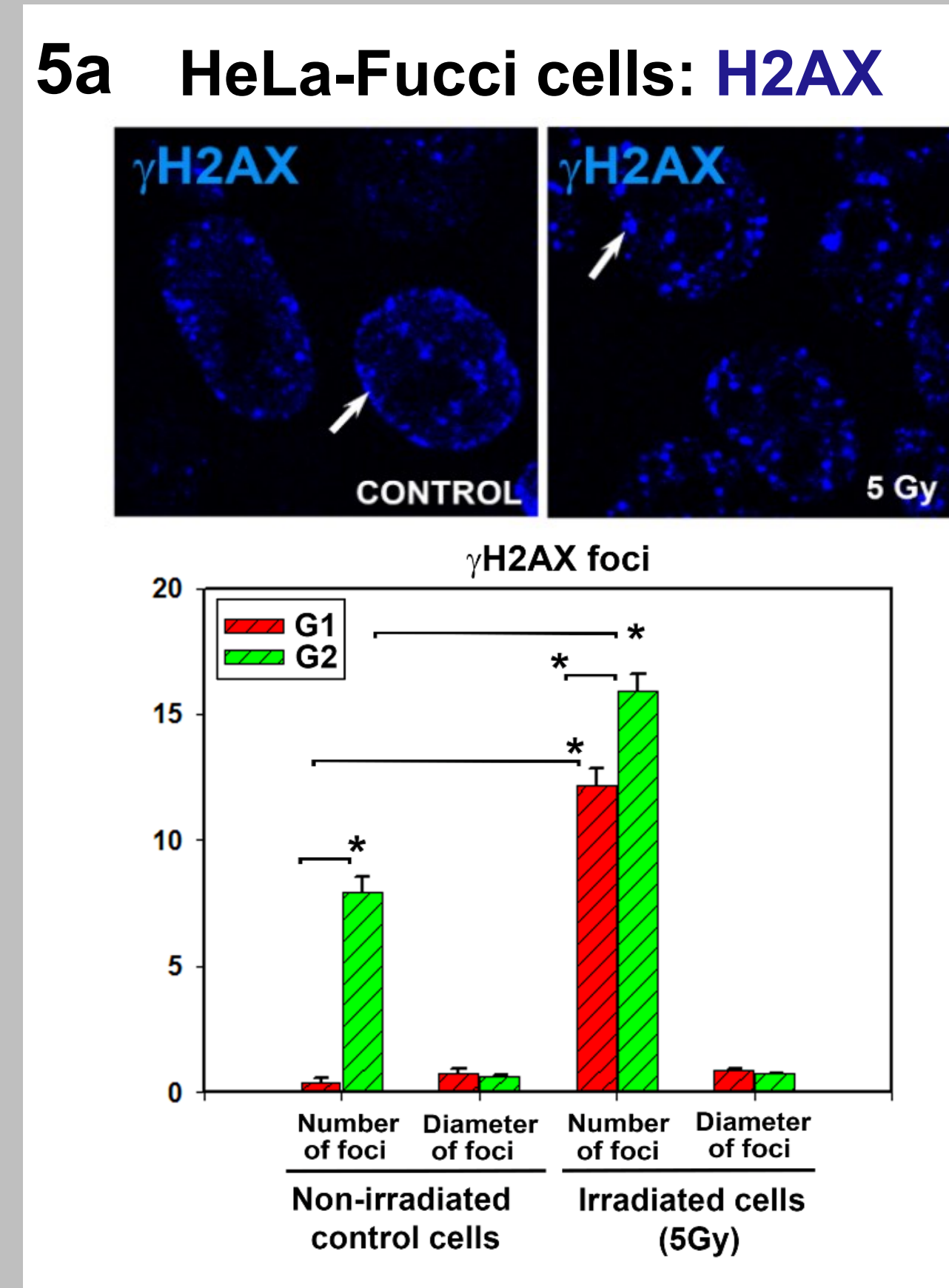


Fig. 5: Nuclear patterns of spontaneous repair foci and IRIF. Irradiation by γ -rays significantly increased the number of 53BP1- (Fig. 5a) and γ H2AX-positive IRIF (Fig. 5b), but cell cycle-dependent differences were only observed for γ H2AX-positive foci in both non-irradiated and γ -irradiated cells.