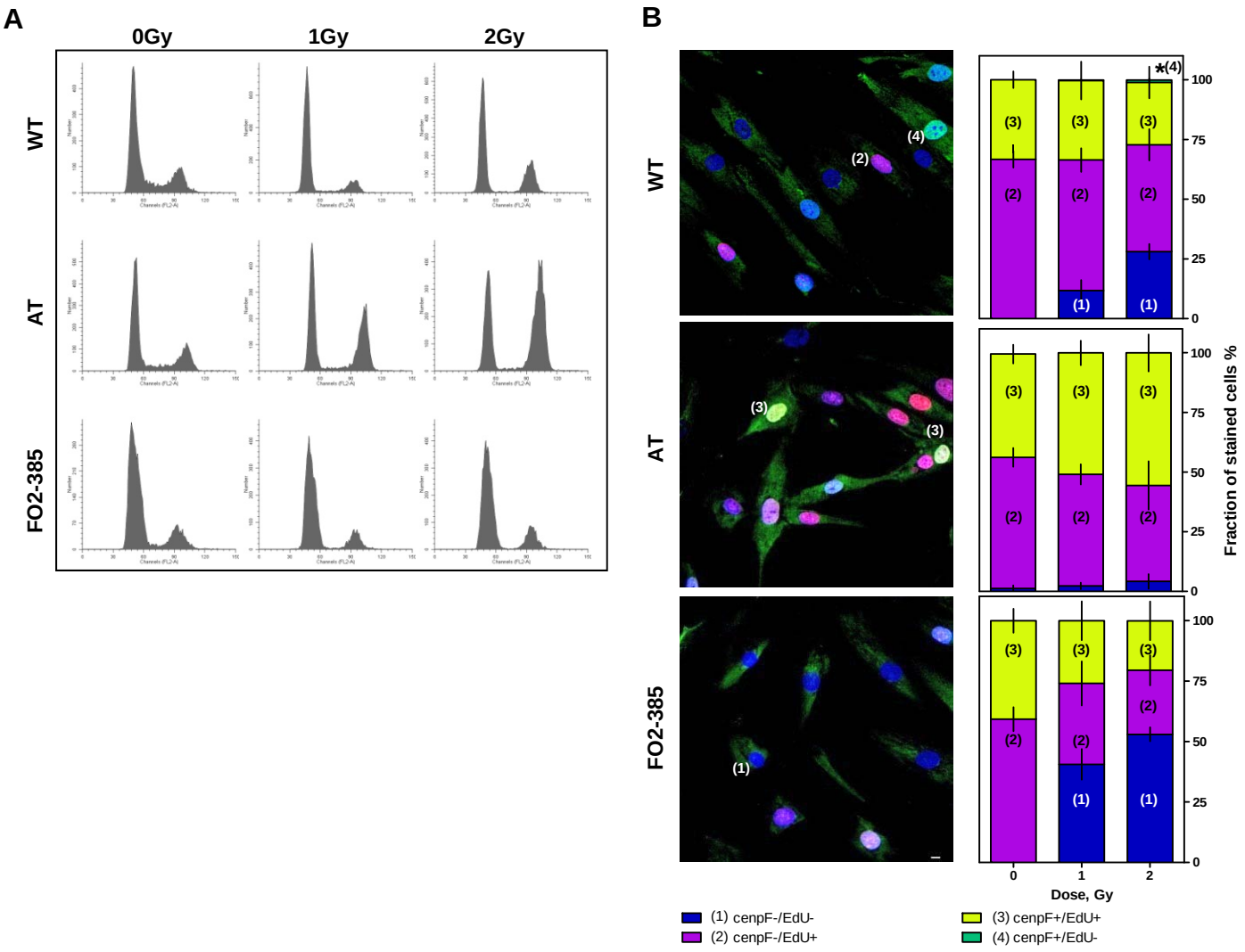


Supplementary Fig.1. Different cell cycle distribution in *AT* and *Artemis* cells

Cell cycle distribution in untreated cells and 24 h after X-irradiation. (A) Cells were fixed, stained with propidium iodine (PI) and analysed for their DNA content by FACS Cell cycle analysis by differential staining with EdU and Cenp-F. (B) Exponentially growing fibroblasts were irradiated with 1or 2 Gy, supplemented with the thymidine analogon EdU, to identify proliferating cells, and fixed after 24 h. Detection of S-phase passage by EdU, nuclear staining with Cenp-F and counterstaining with DAPI results in differentially stained subfractions:

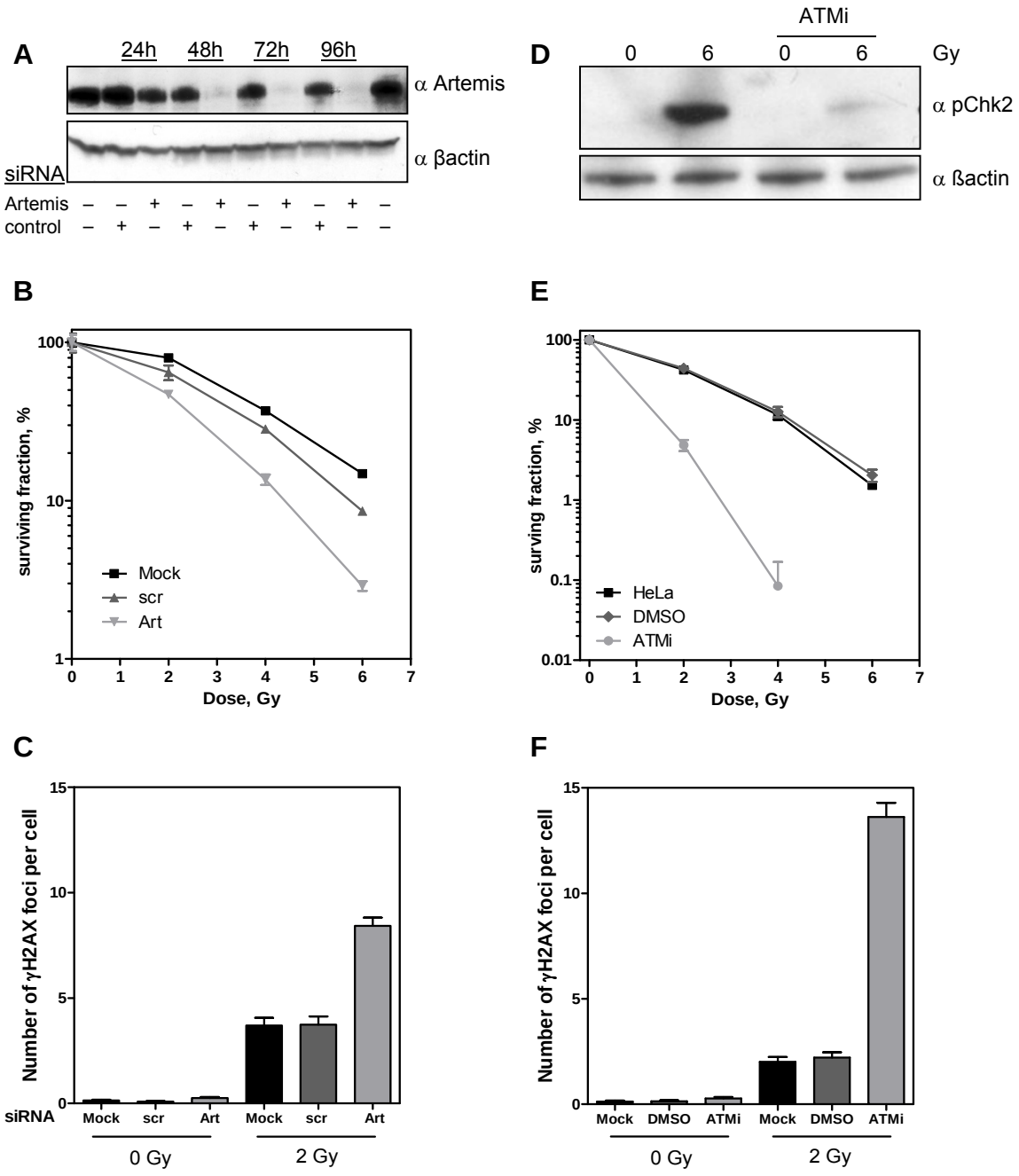
Example 1 (blue, Cenp-F-/EdU-): non-cycling cells in G1

Example 2 (purple, Cenp-F-/EdU+): cycling cells in G1 or S phase

Example 3 (yellow, Cenp-F+/EdU+): G2 cells after transit through S phase

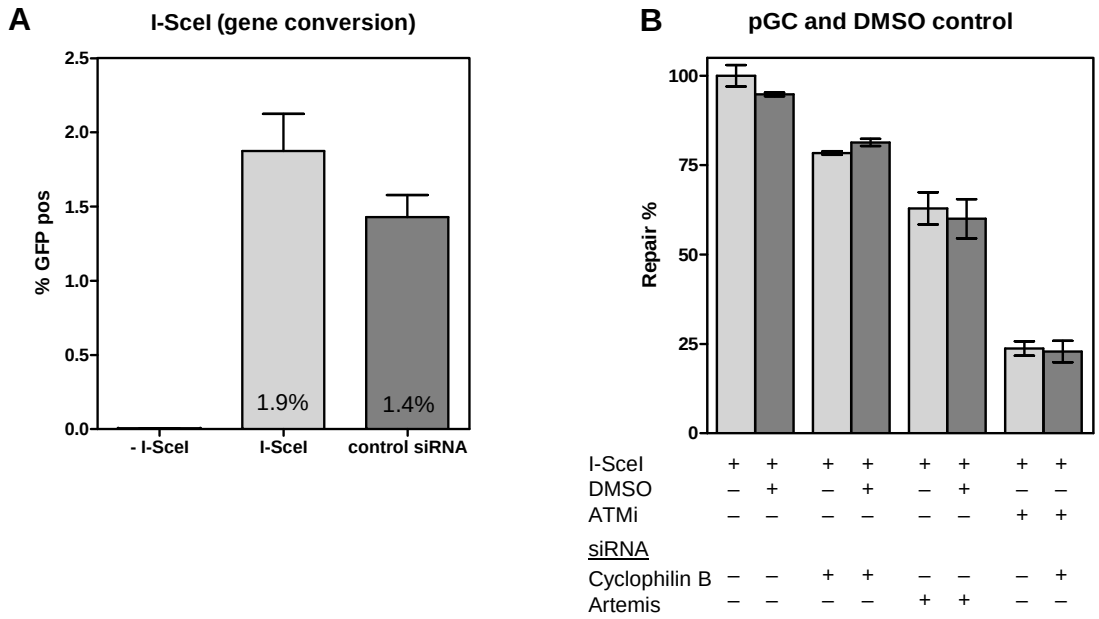
Example 4 (*green, Cenp-F+/EdU-): G2 cells directly arrested in G2

Untreated controls of all three strains included a certain fraction of non-cycling cells (7.1 ±2.7%, 23.5 ±1.2% and 19.6 ± 2.1 of WT, *AT* and *Artemis* cells, respectively, were both EdU- and Cenp-F-negative). In the following, these numbers were subtracted from the entire population which was then set to 100%. Only cells that were additionally arrested in G1 after IR were depicted (blue bars). After IR, WT and in particular *Artemis* (up to 52% after 2 Gy) but not *AT* cells accumulated in G1 without incorporating EdU. *AT* cells showed in contrast an increased G2 fraction after transit through S-phase (example (3), yellow). Remarkably, in WT cells a small fraction was irradiated in G2 and did not proceed through mitosis during the following 24 h (example (4) green). This fraction increased to 10% after 6 Gy in WT cells but was not found in *AT* or *Artemis* cells (not shown).



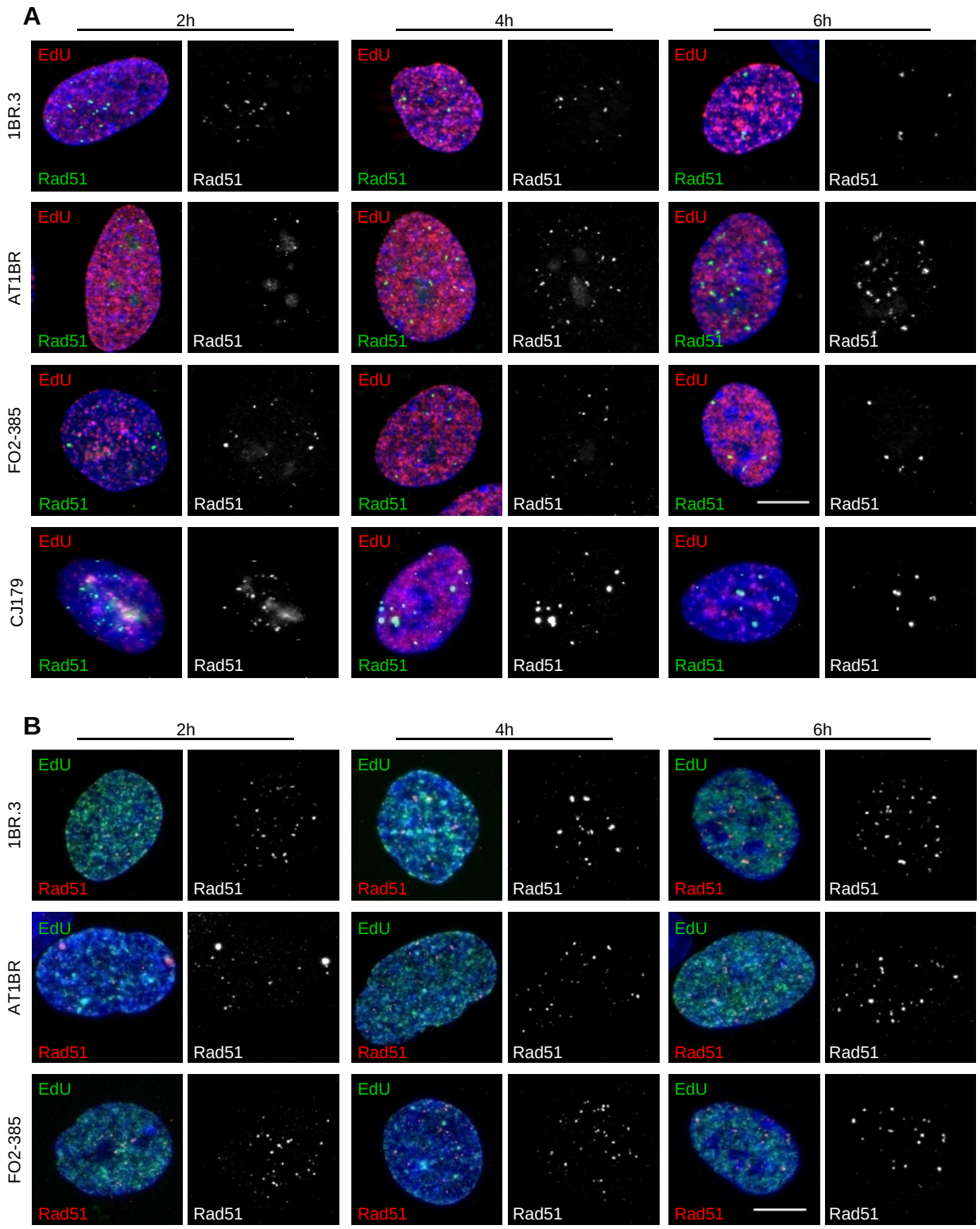
Supplementary Fig.2. Inactivation of Artemis and ATM reduced survival in HeLa cells and increased the number of residual DSBs

(A) HeLa cells were treated twice with Artemis or control siRNA (second time after 48 h) and Artemis expression was followed by Western blot for up to 96 h. (B) Artemis knockdown efficiently increased the radiosensitivity and (C) also the number of residual γH2AX foci 24 h after 2 Gy. (D) HeLa cells were treated with the ATM-inhibitor KU55933 (10 μM) which efficiently reduced Chk2 phosphorylation 1 h after IR with 6 Gy. (E) the ATM inhibitor drastically increased radiosensitivity and (F) the number of residual γH2AX foci.



Supplementary Fig.3. Gene conversion efficiency is not significantly affected by control treatments

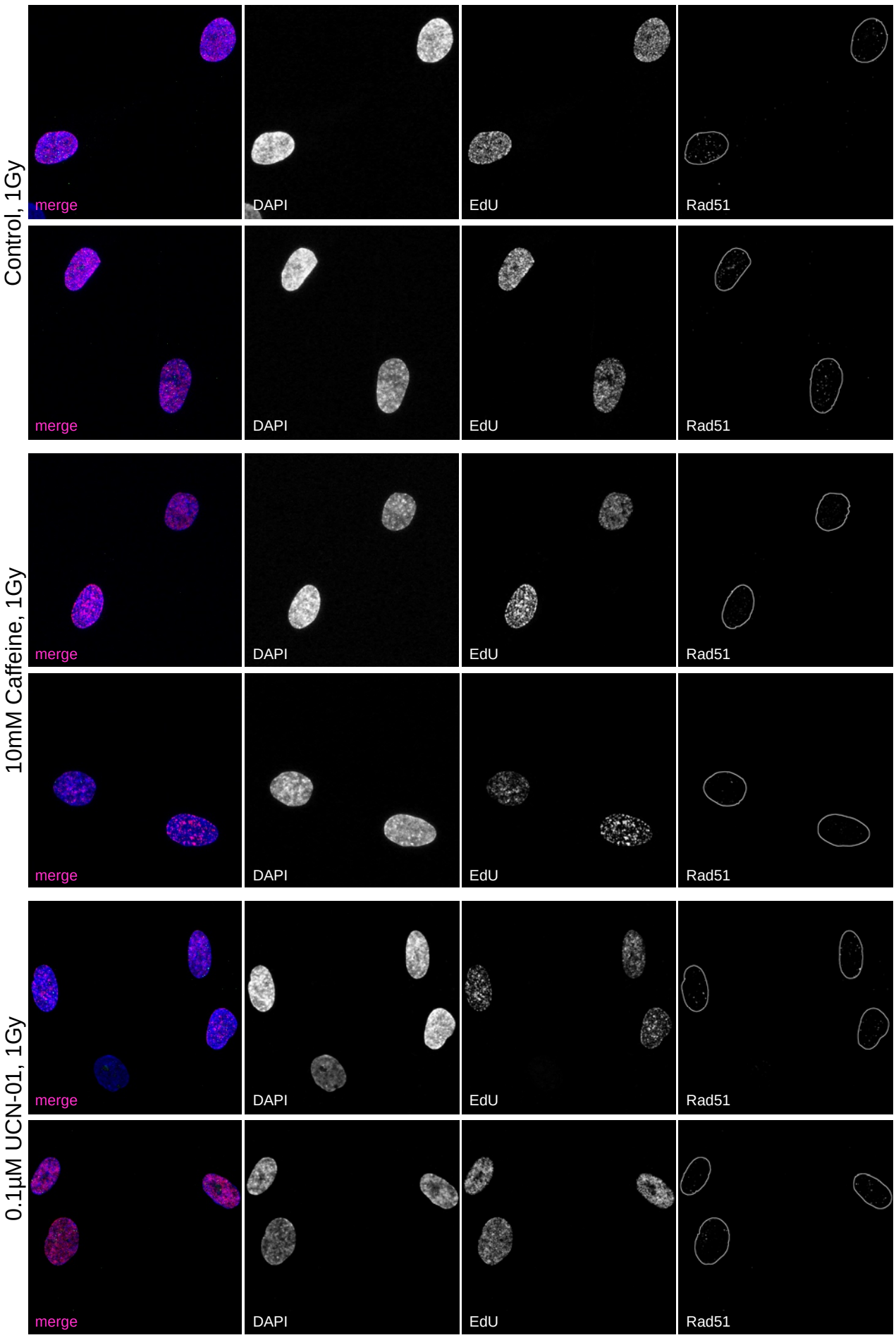
(A) The expression of GFP represents successful gene conversion at I-SceI induced DSBs. Pretreatment with control siRNA (scrambled, GAPDH, CyclophilinB) slightly reduced the efficiency to 1.4% GFP-positive cells compared to 1.9% in cells solely transfected with the I-SceI expression plasmid. (B) DMSO (dark bars) treatment did not significantly influence the rate of GFP positive cells in combination with Artemis or control siRNA. The effect of ATM-inhibition was not modulated by additional control siRNA.



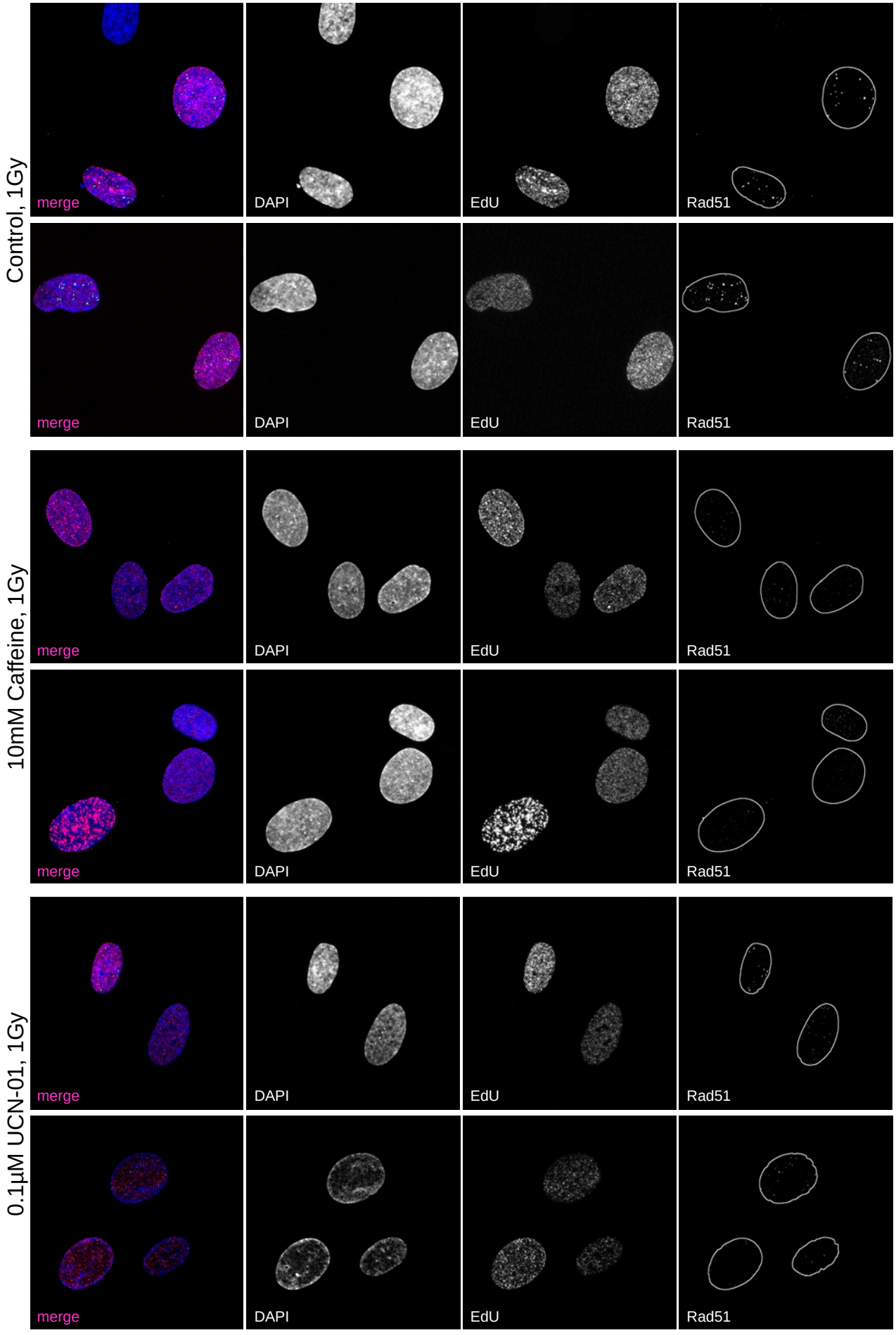
Supplementary Fig.4. Kinetics of Rad51 foci in S-phase are effected by ATM inhibition.

(A) Examples of discrete Rad51 foci in EdU-positive S-phase cells after 1 Gy of X-rays. The amount of Rad51 foci in *AT* cells steadily increases, whereas *Artemis* cells show fewer foci with time. Bars, 10 μm (B) WT, *AT* and *Artemis* cells were treated with ATM-inhibitor as described, pulse-labelled with EdU, irradiated (1 Gy) and stained for Rad51 at the time points indicated. Bars, 10 μm

Suppl.Fig. 5A


WT 2 h post 1 Gy
Control, 1Gy
10mM Caffeine, 1Gy
0.1µM UCN-01, 1Gy
merge
DAPI
EdU
Rad51

Suppl. Fig. 5B


AT 6 h post 1 Gy
Control, 1Gy
10mM Caffeine, 1Gy
0.1µM UCN-01, 1Gy
merge
DAPI
EdU
Rad51

Supplementary Fig.5. Rad51 focus formation in dependence of ATR / Chk1

Depicted are Rad51 foci in EdU-postive WT (1BR.3) cells (A) or *AT* (AT1BR) cells (B) 2 h and 6 h post irradiation with 1 Gy, respectively. Cells were either mock treated, treated with 10mM of caffeine, or using the Chk1 inhibitor UCN-01 (0.1 µM). Two examples are given each. Different channels for DAPI, EdU, and Rad51 are displayed. Caffeine treatment abolished Rad51 focus formation. Chk1 inhibition diminished Rad51 focus formation. For quantification see Figure 5E.