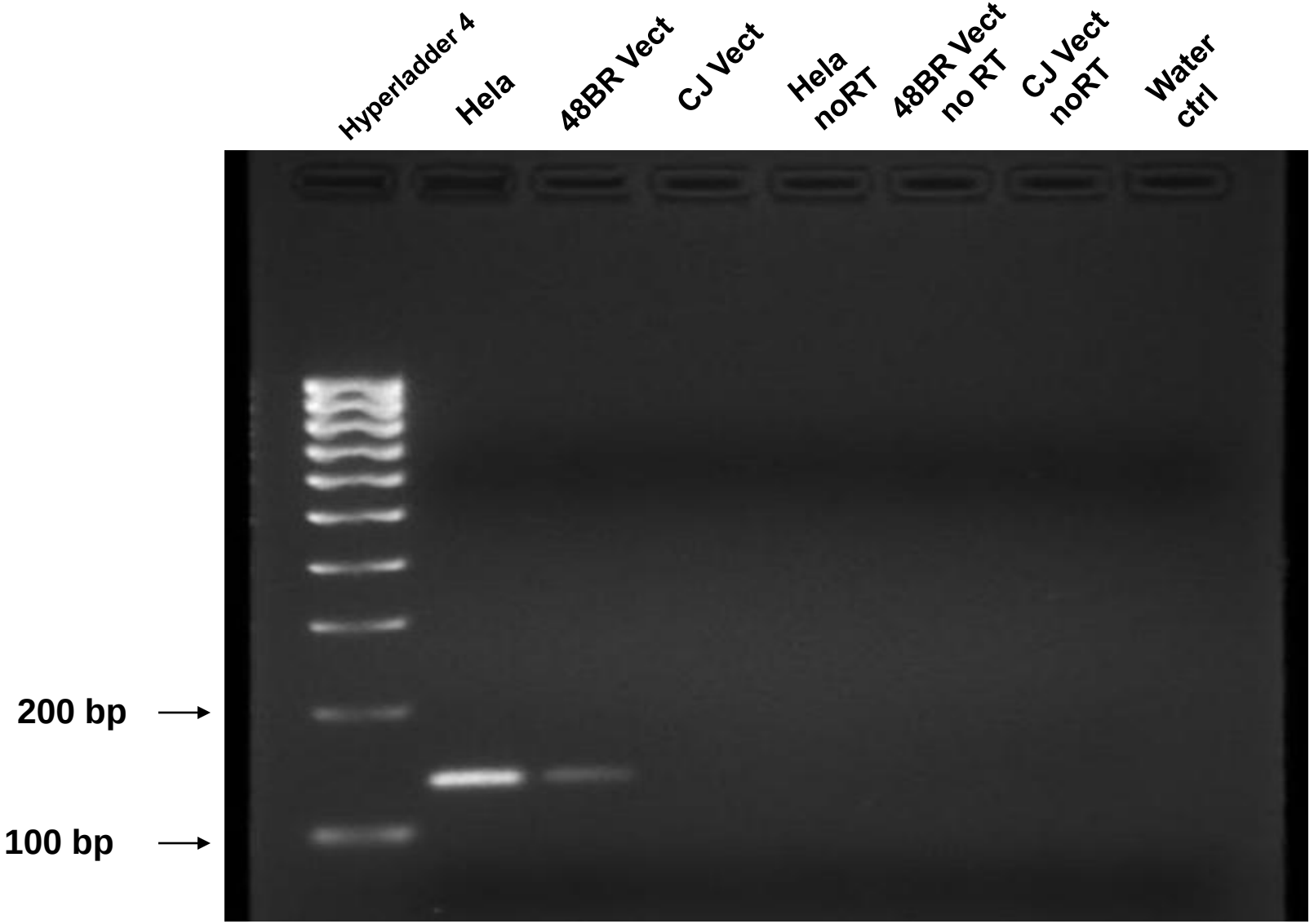
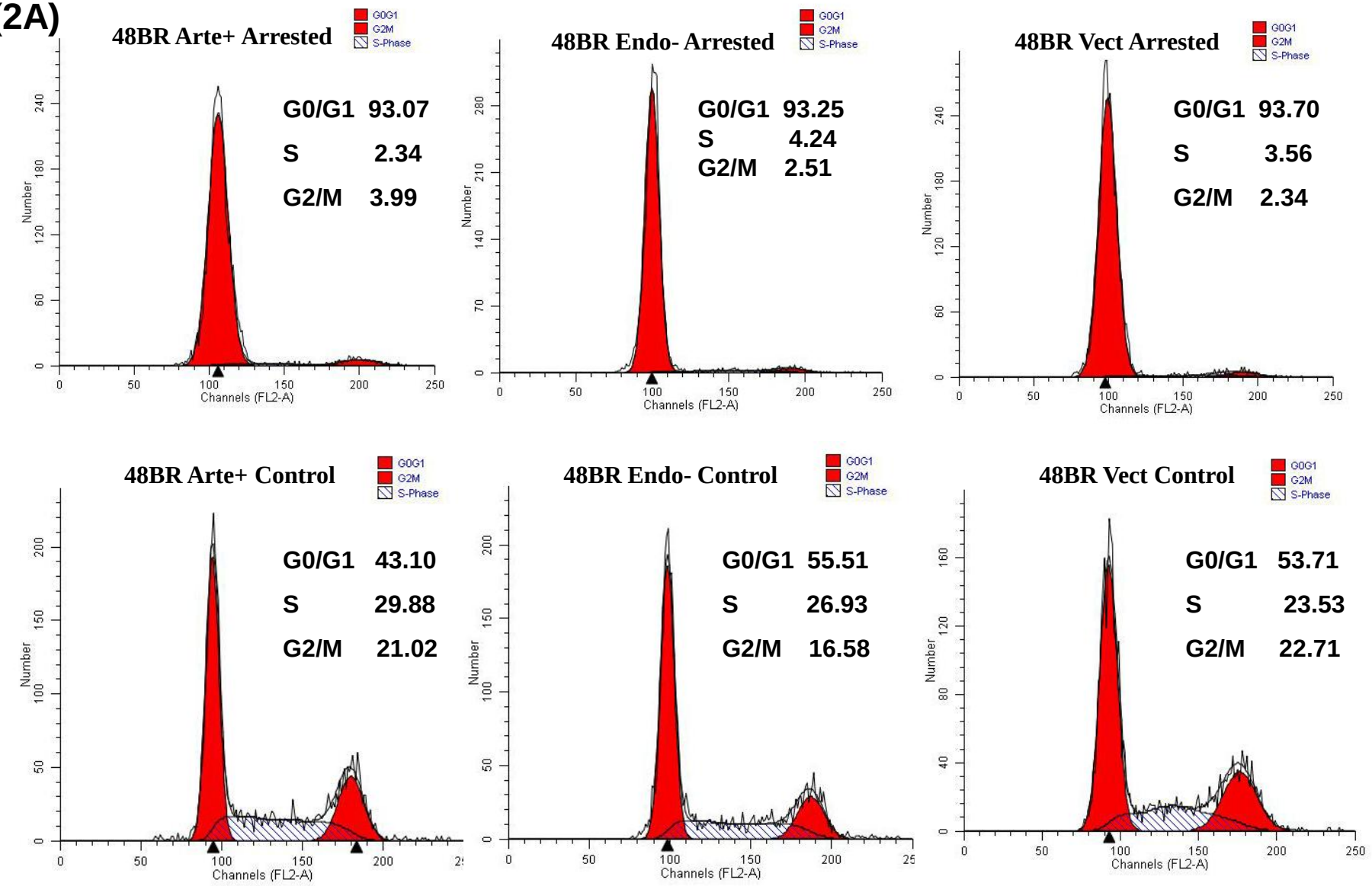
Supplemental Figure 1. Normal human fibroblasts (48BR) express low levels of Artemis transcript. Artemis cDNA from Normal (48BR Vect) and Hela cells was amplified using real time q-PCR as described for 40 cycles. 10 µl from a 25 µl reaction was run on 3% agarose gel with hyperladder 4 (Bioline) as reference. A specific amplification product 137 bp long was detected for both Hela and 48BR Vect cells but not in CJ179 Vect cells.

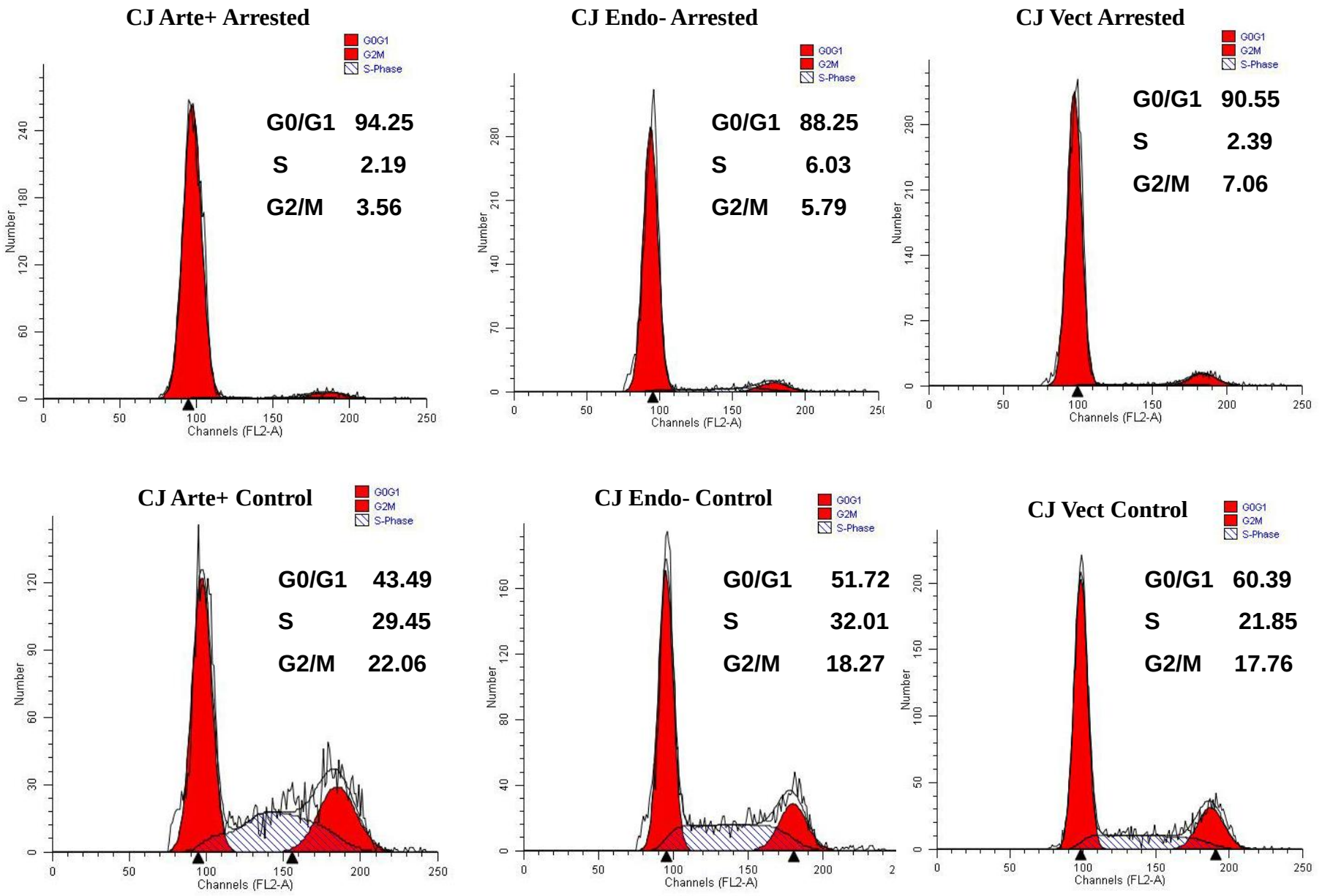


Supplemental Figure 2. Representative FACS plots of normal human fibroblasts (**A**) and Artemis deficient human fibroblasts (**B**) showing G1 arrest. Various cell lines were confluence arrested and serum starved (0.5% serum for 5 days) to synchronize them in G1 phase of cell cycle. Flow cytometric analysis to confirm cell cycle arrest was performed following Propidium iodide staining. Data was analysed using Modfit LT software. 90-95% cells were arrested in G1 phase of cell cycle for all cell lines.

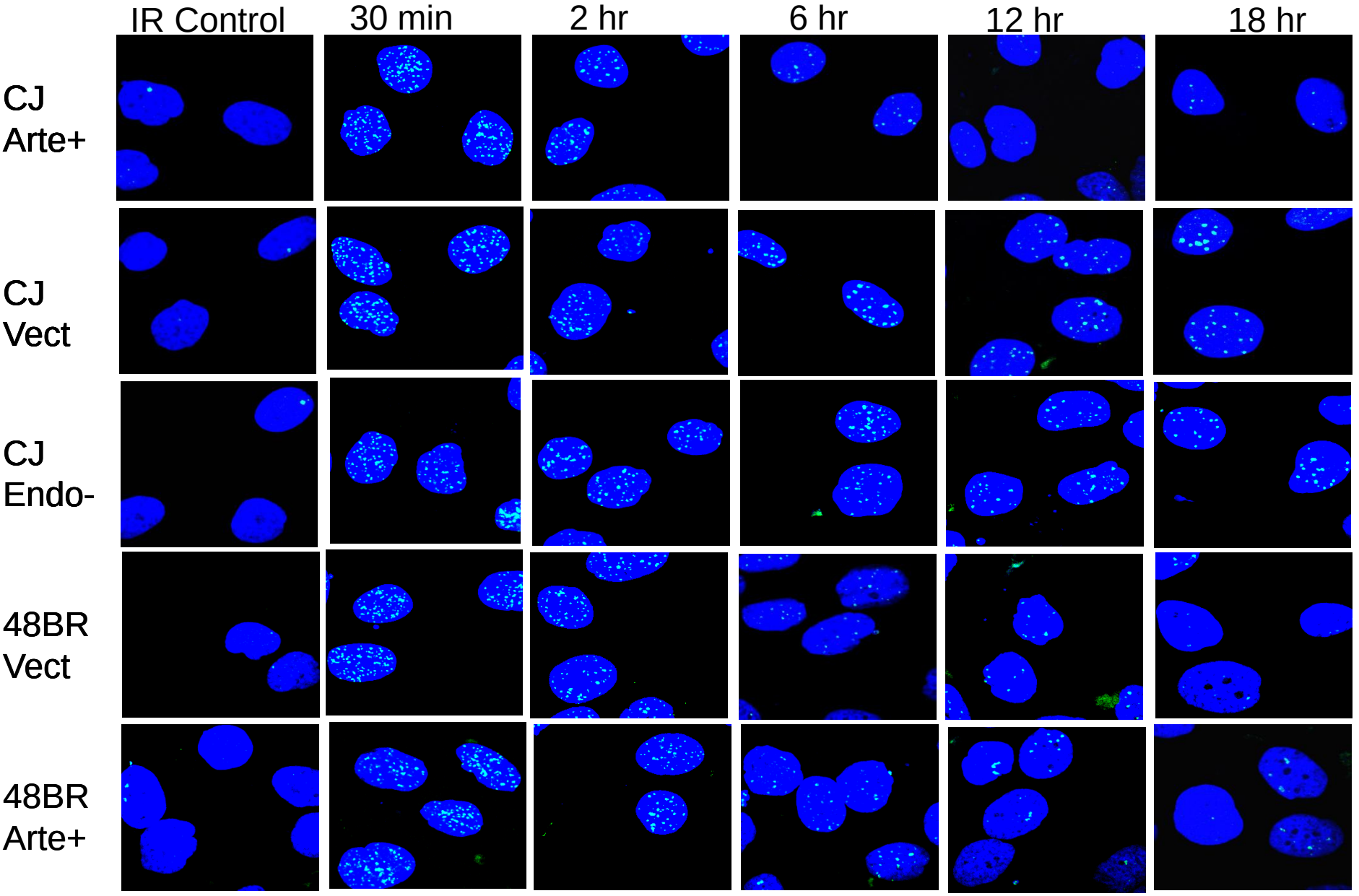
(2A)



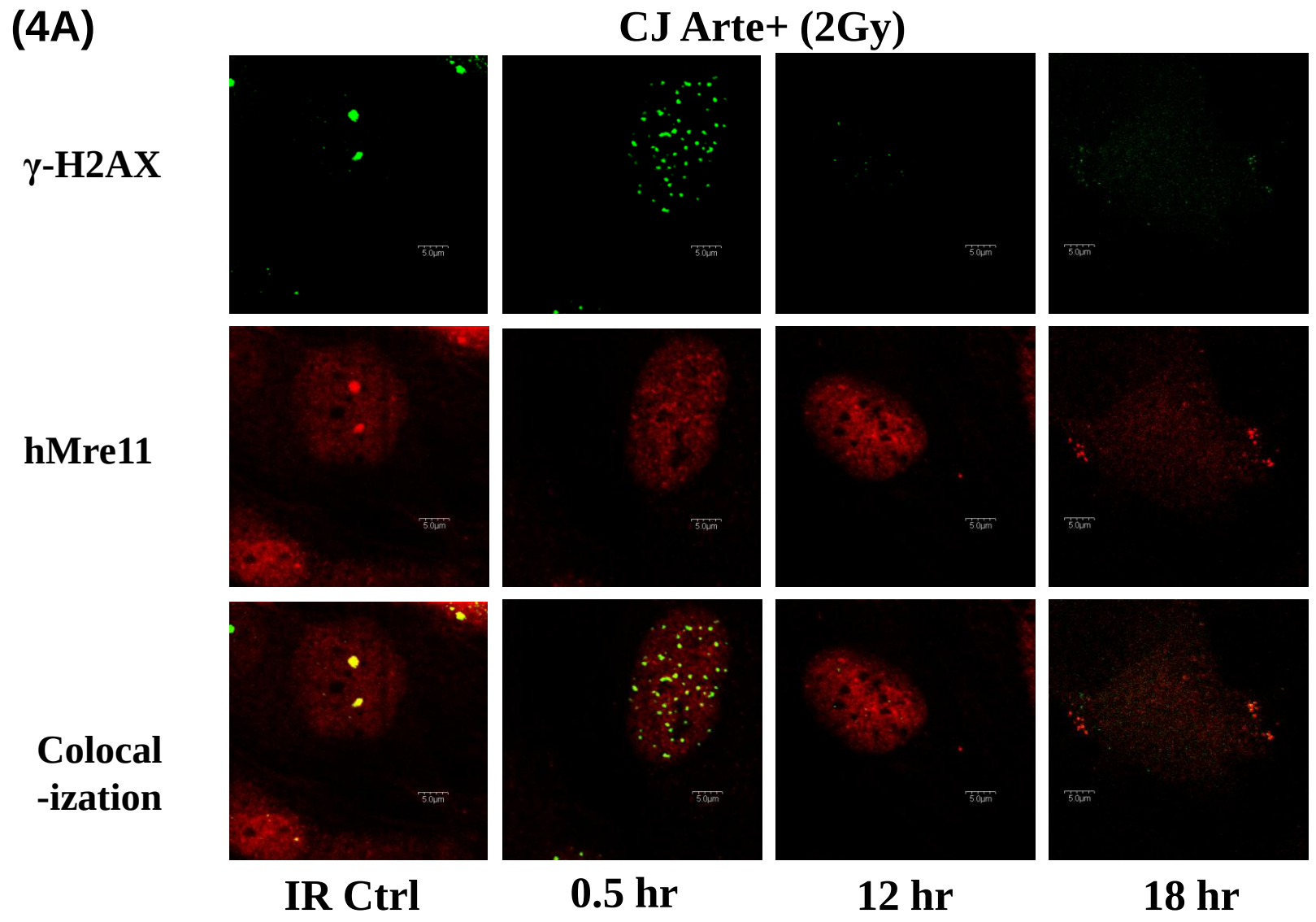
(2B)


CJ Arte+ Arrested
G0G1
G2M
S-Phase
G0/G1 94.25
S 2.19
G2/M 3.56
Number
Channels (FL2-A)
CJ Endo- Arrested
G0/G1 88.25
S 6.03
G2/M 5.79
CJ Vect Arrested
G0/G1 90.55
S 2.39
G2/M 7.06
CJ Arte+ Control
G0/G1 43.49
S 29.45
G2/M 22.06
CJ Endo- Control
G0/G1 51.72
S 32.01
G2/M 18.27
CJ Vect Control
G0/G1 60.39
S 21.85
G2/M 17.76

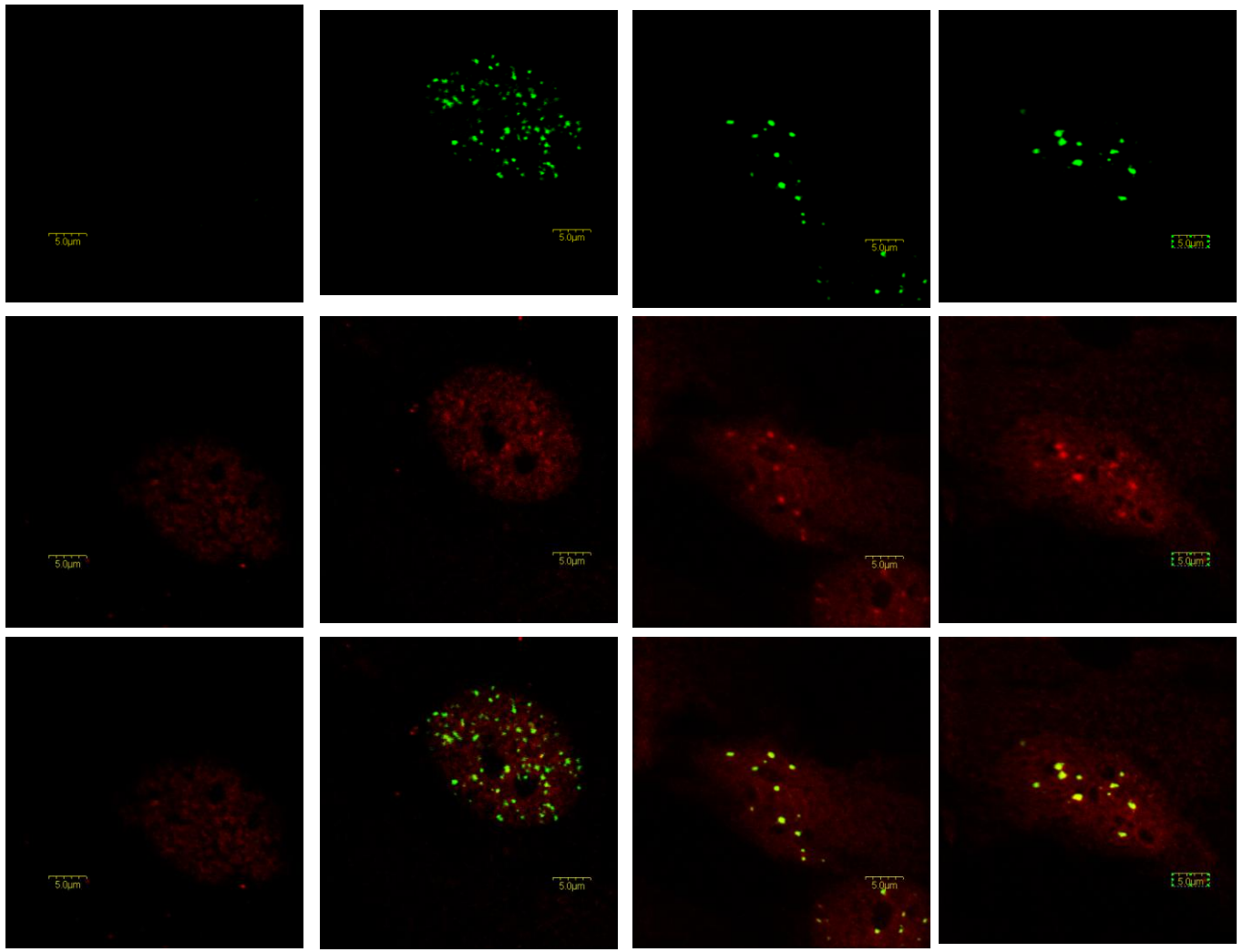
Supplemental Figure 3. Dependence of cellular DSB rejoining on Artemis endonucleolytic activity. Immunostaining was performed for phosphorylated γ-H2AX following exposure to 2Gy γ-radiation for the wildtype/D165N mutant Artemis expressing cell lines and the repair was followed at indicated times. Representative images for data quantified in Fig 2A are shown.



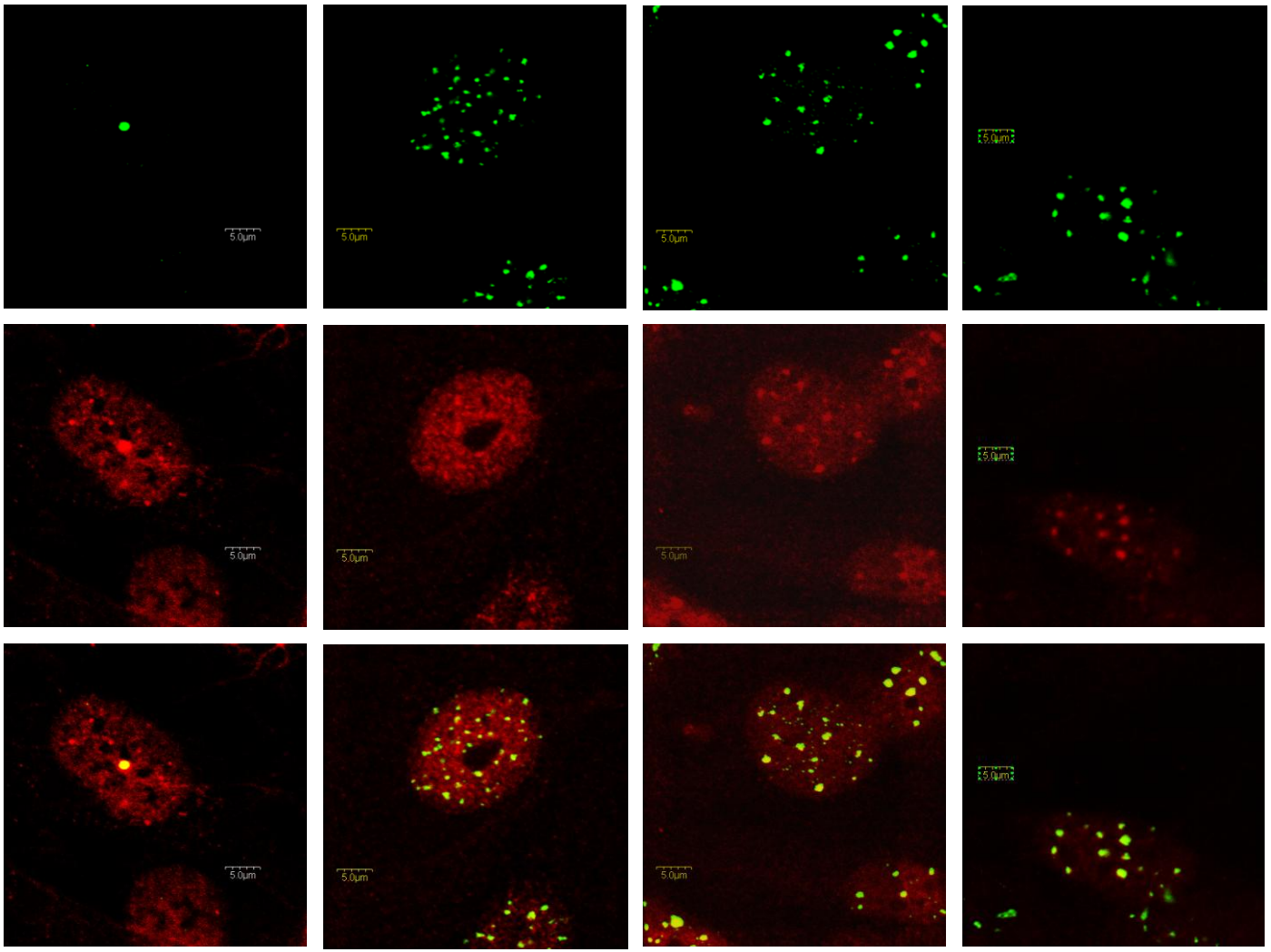
Supplemental Figure 4 (A, B, C). Mre11 and γ-H2AX co-localization following exposure to 2Gy γ-radiation. Double Mre11 : γ-H2AX staining was performed after paraformaldehyde fixation. Confocal analysis of representative cells, CJ Arte+ (**A**), CJ Vect (**B**) and CJ Endo^{-} (**C**) are shown. Green fluorescence: γ-H2AX (monoclonal anti γ-H2AX, Millipore); red fluorescence: Mre11 (Polyclonal anti-hMre11 Novus biologicals).



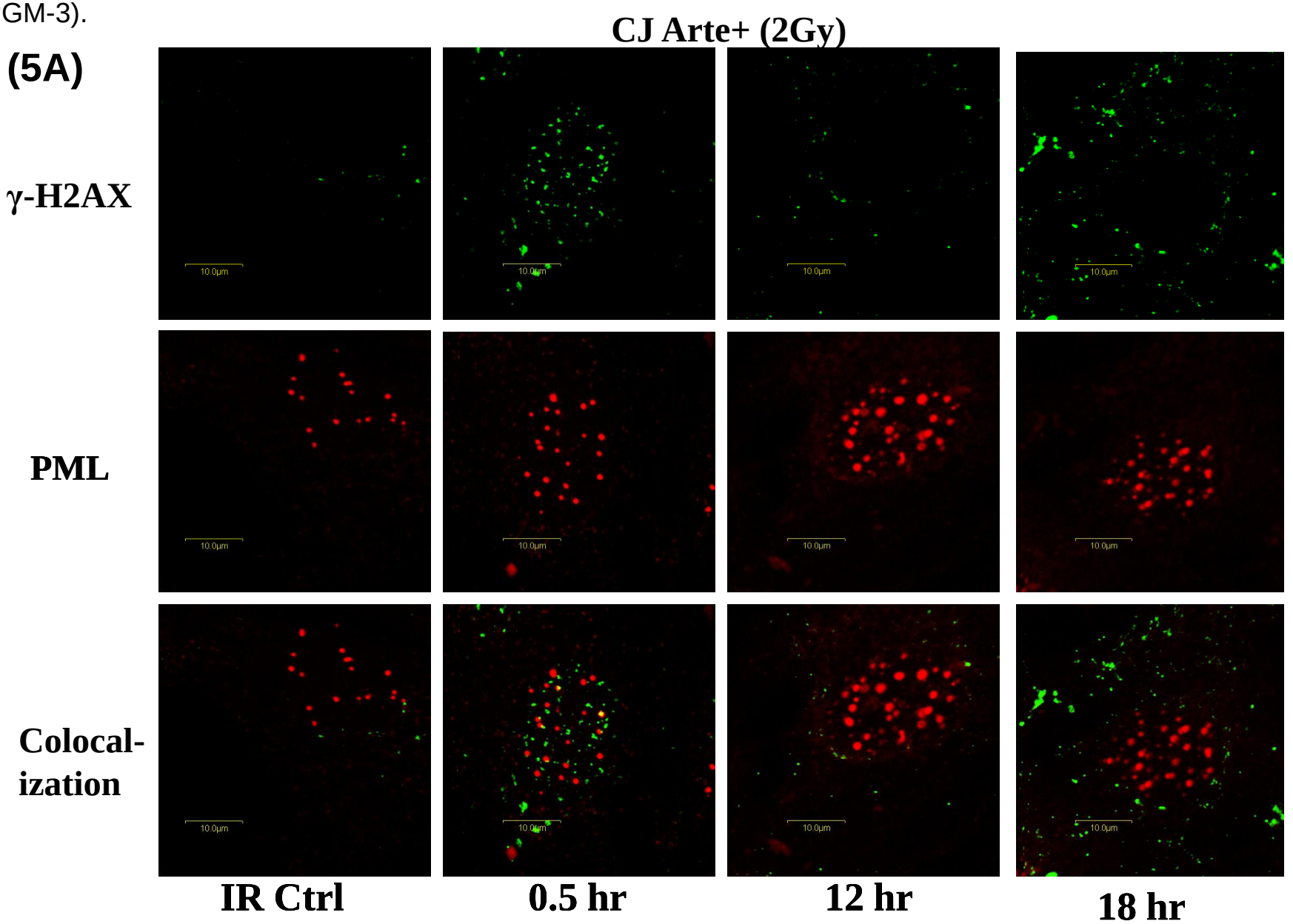
(4B)


CJ Vect (2Gy)
γ-H2AX
hMre11
Colocal-
ization
IR Ctrl
0.5 hr
12 hr
18 hr

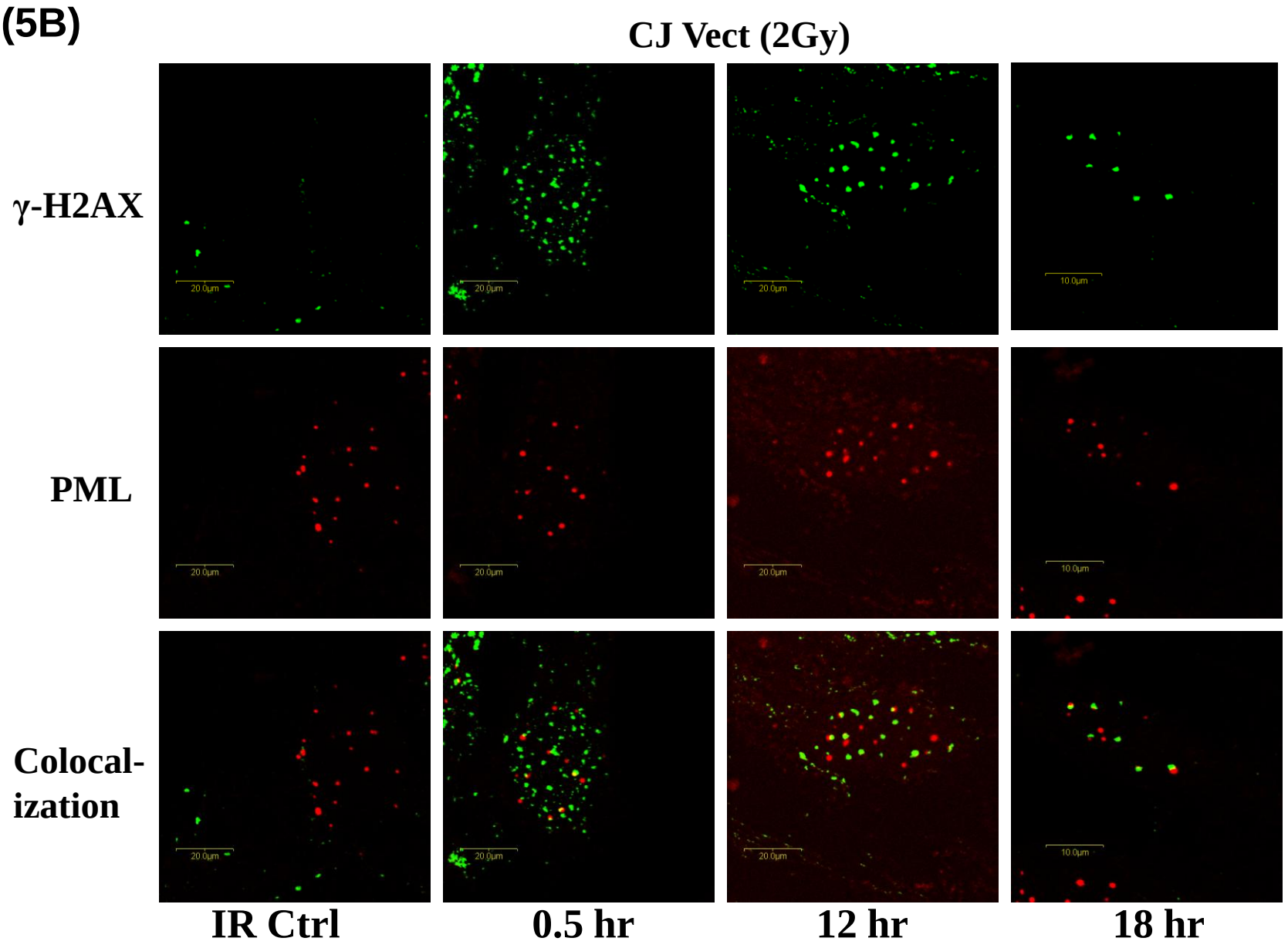
(4C)


CJ Endo⁻ (2Gy)
γ-H2AX
hMre11
Colocal-
ization
IR Ctrl
0.5 hr
12 hr
18 hr

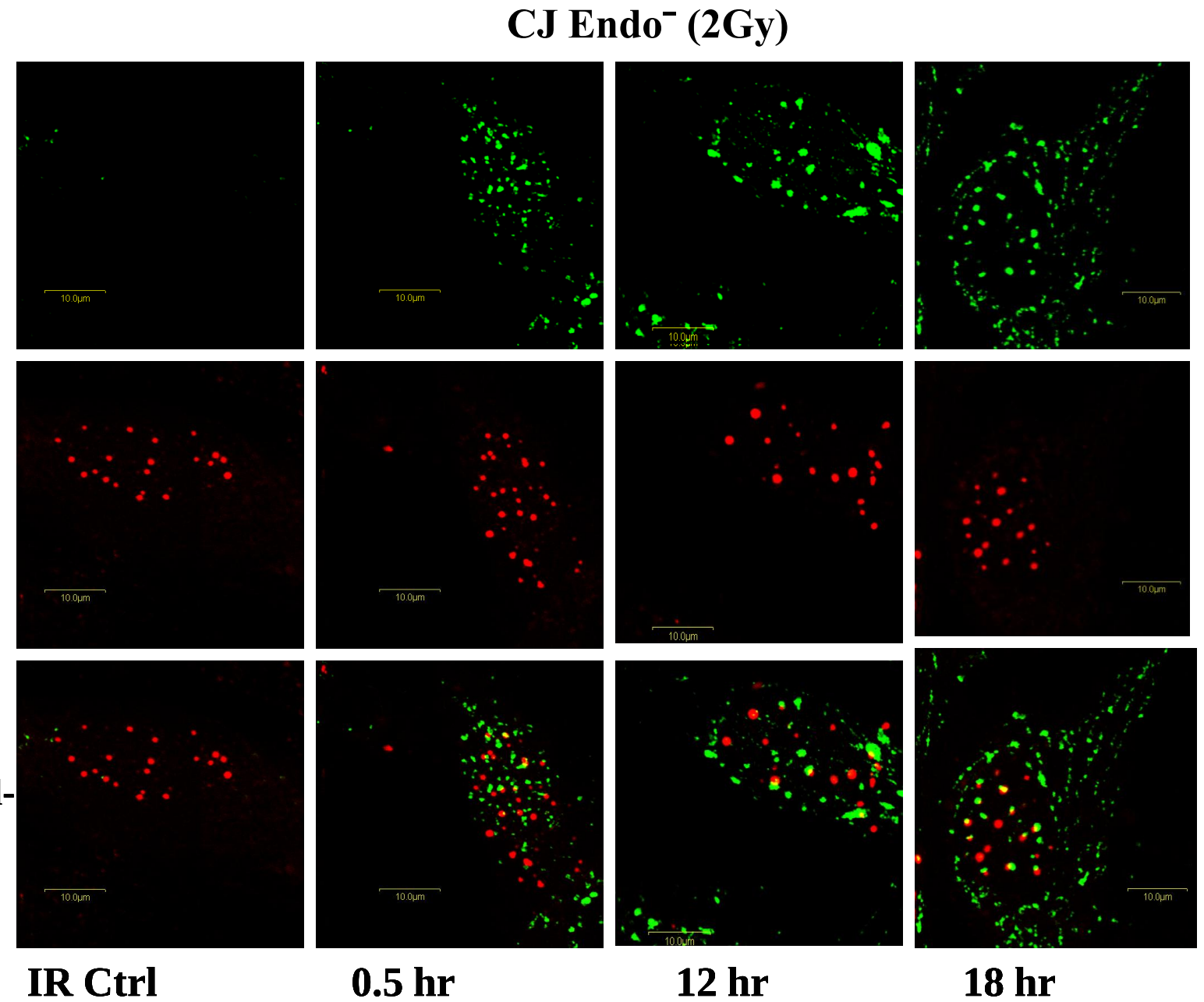
Supplemental Figure 5 (A, B, C). PML and γ-H2AX partial co-localization and juxtapositioning following ionizing radiation exposure. Double PML: γ-H2AX staining was performed after paraformaldehyde fixation. Confocal analysis of representative cells, CJ Arte+ (**A**), CJ Vect (**B**) and CJ Endo⁻ (**C**) are shown. Green fluorescence: γ-H2AX (polyclonal anti γ-H2AX, Novus biologicals); red fluorescence: PML (monoclonal anti-PML, Santa cruz, PGM-3).



(5B)



(5C)



Supplemental Figure 6. Overexpression of D165N Artemis has no effect on chemosensitivity of normal 48BR fibroblasts. Clonogenic survival was determined for confluence-arrested serum-starved cells overexpressing wild-type (Arte+) or endonuclease-deficient (Endo$^-$) Artemis from a lentivirus (see Fig. 1), following treatment with bleomycin, or neocarzinostatin. Vect cells are empty-vector controls. Although survival of 48BR Endo$^-$ cells was slightly lower than that of Vect cells following bleomycin treatment, the difference was not statistically significant. For bleomycin treatment, p = 0.56, 48BR Vect vs 48BR Endo$^-$ (1μg/ml, t test), p = 0.49, 48BR Vect vs 48BR Endo$^-$ (2μg/ml, t test). For NCS treatment, p = 0.62, 48BR Vect vs 48BR Endo$^-$ (3nM, t test), p = 0.93, 48BR Vect vs 48BR Endo$^-$ (6nM, t test).