Molecular Pharmacology

Cyclopentenyl cytosine induces senescence in breast cancer cells through the nucleolar stress response and activation of p53

Min Huang, Patrick Whang, Patrick Lewicki, and Beverly S. Mitchell

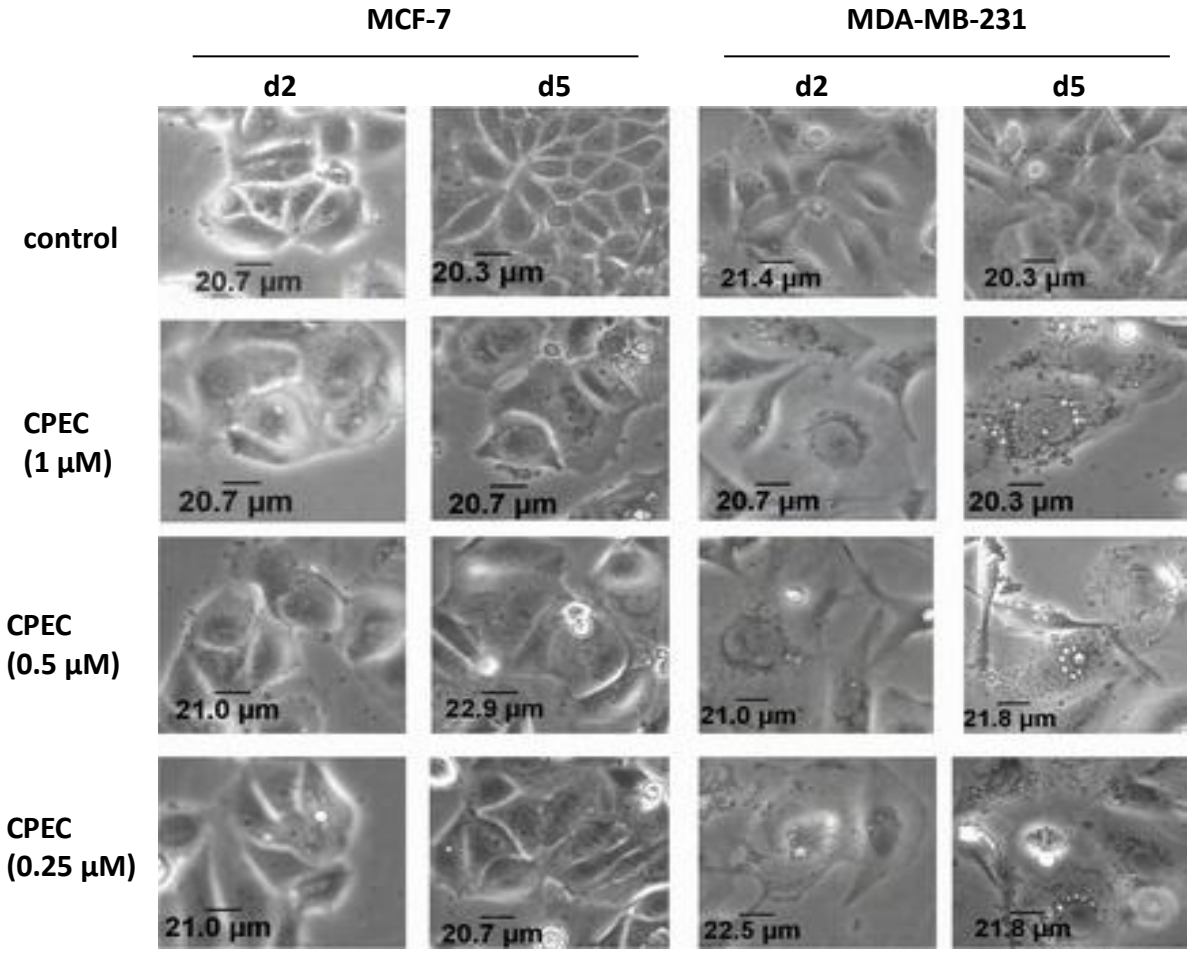

Fig. 1. Induction of morphological changes in MCF-7 and MDA-MB cell lines by CPEC. Cell lines were examined using a Nikon Eclipse TE200 microscope (40×) at days 2 and 5 after the addition of the indicated concentrations of CPEC. Scale bars ranged from 20.3 and 22.9 µm.

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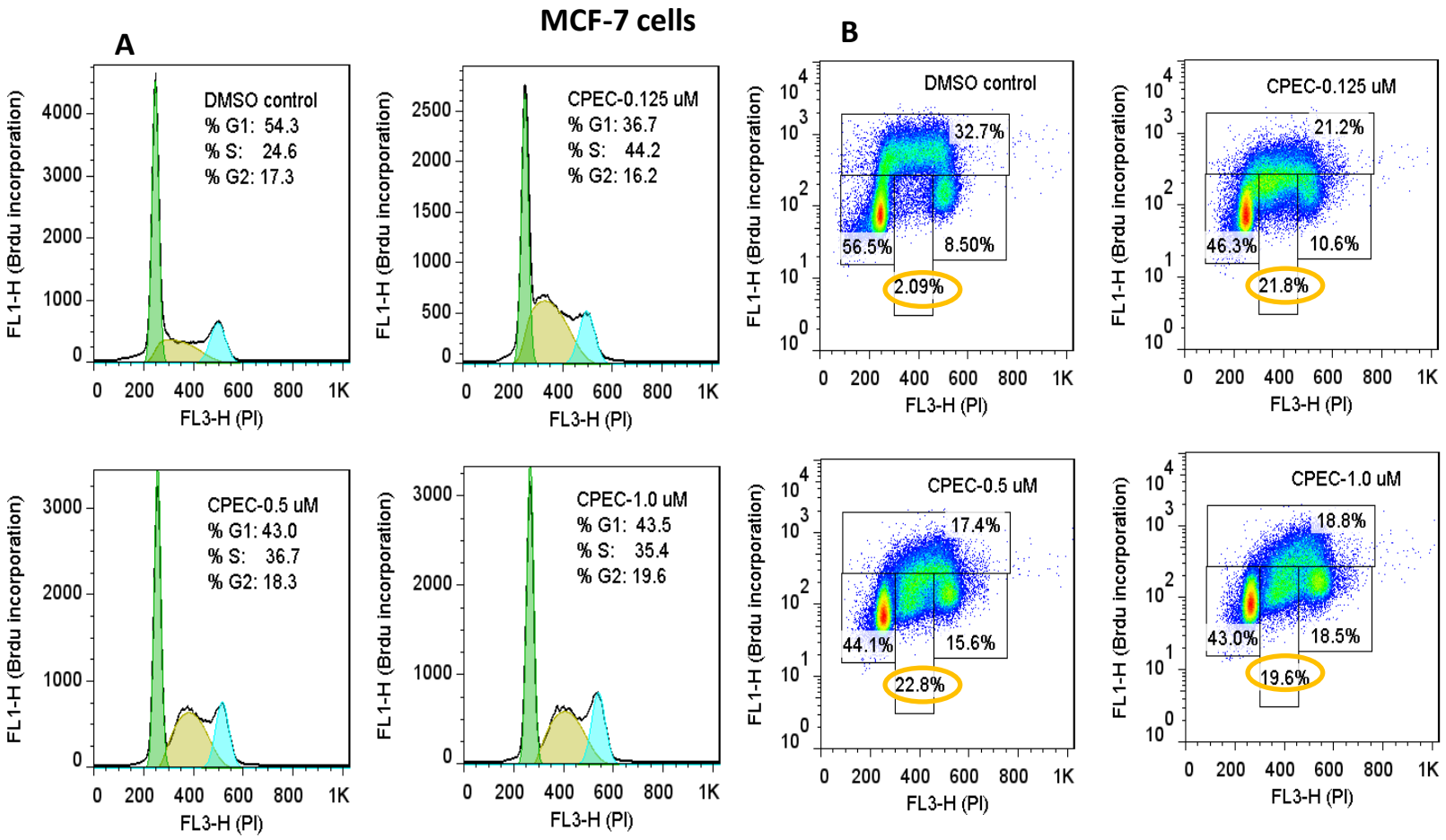

Fig. 2. Differential effects of CPEC on cell cycle progression and BrdU incorporation in MCF-7 cells. Analyses of cell cycle progression (A) and BrdU incorporation (B) in MCF-7 cells were performed as described in Materials and Methods after 48 h of culture in the absence or presence of CPEC at the concentrations indicated. The percentage of cells in each phases of the cell cycle is indicated for each sample in the histogram. Yellow circles represent S phase-arrested cells lacking BrdU incorporation.

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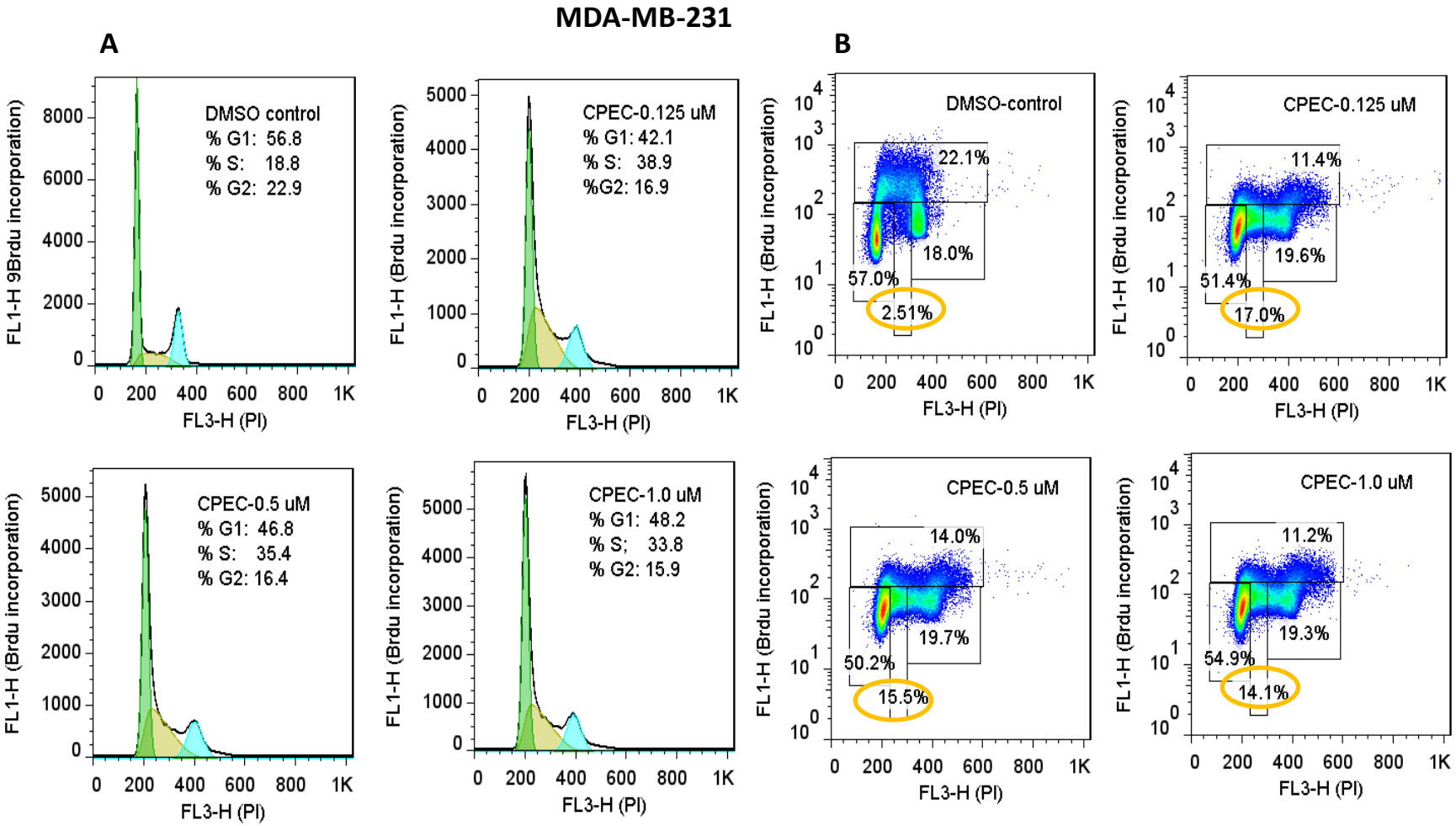

Fig. 3. Differential effects of CPEC on cell cycle progression and BrdU incorporation in MDA-MB-231 cells. Analyses of cell cycle progression (A) and Brdu incorporation (B) in MDA-MB-231 cells were performed after 48 h of culture in the absence or presence of CPEC at the concentrations indicated. The percentage of cells in each phases of the cell cycle is indicated for each sample in the histogram. Yellow circles represent S phase-arrested cells lacking BrdU incorporation.

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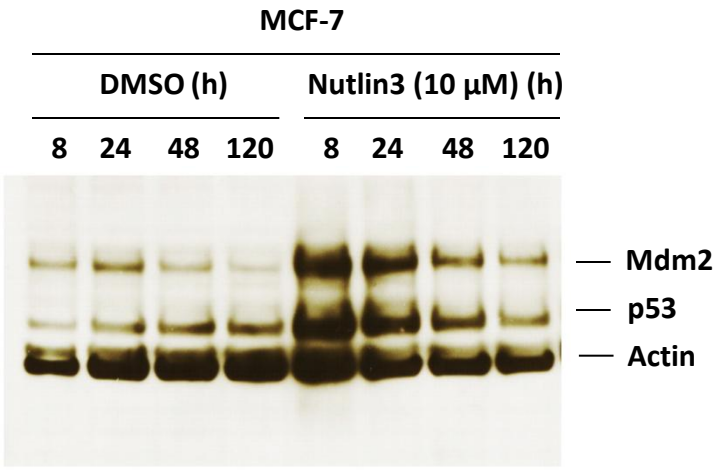

Fig. 4. Induction of p53 and Mdm2 by Nutlin 3 in MCF-7 cells. Immunoblot analysis of p53 and Mdm2 was performed in MCF-7 cells in the presence or absence of 10 μM Nutlin 3 for the time indicated. Beta-Actin was used as a loading control.

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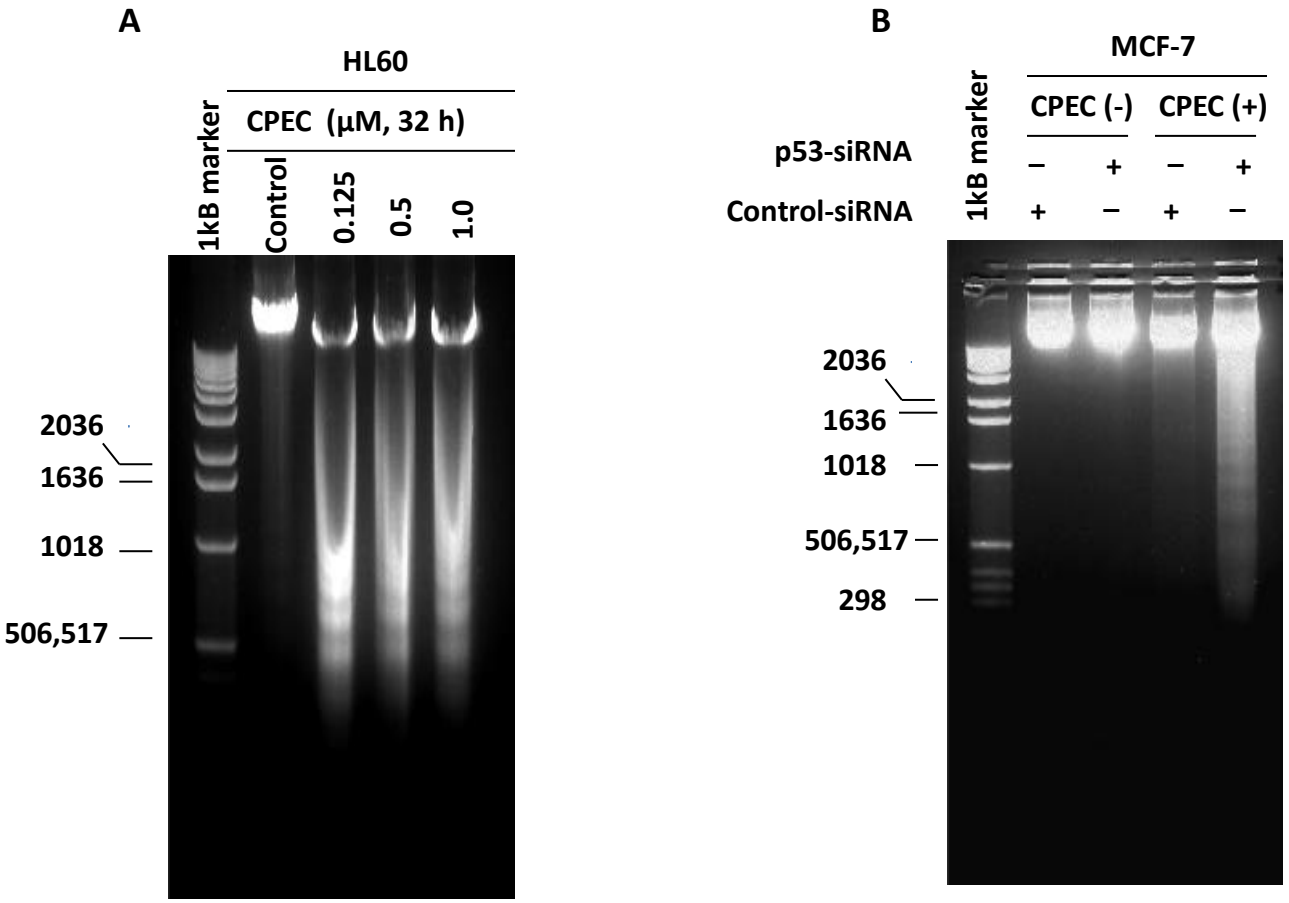

Fig. 5. Induction of DNA fragmentation by CPEC and effect of p53 depletion. (**A**) HL60 cells were treated with CPEC or control vehicle at doses indicated for 32 h. The DNA extracted was separated using agarose gel electrophoresis. Lane 1: 1 kB DNA marker; lane 2, HL60 cells: control; lane 3, HL60 cells: CPEC-0.125 μM, 32 h; lane 4, HL60 cells: CPEC-0.5 μM; Lane 5, HL60 cells: CPEC-0.5 μM. (**B**) MCF-7 cells were transfected with 100 nM p53-siRNA or control-siRNA by Amaxa electroporation. Twenty-four hours post-transfection, the cells were treated with 1 μM CPEC for five days prior to DNA extraction. Lane 1: 1kB DNA marker; lane 2: control-siRNA; lane 3: p53-siRNA; lane 4: control-siRNA +CPEC; Lane 5: p53-siRNA +CPEC.

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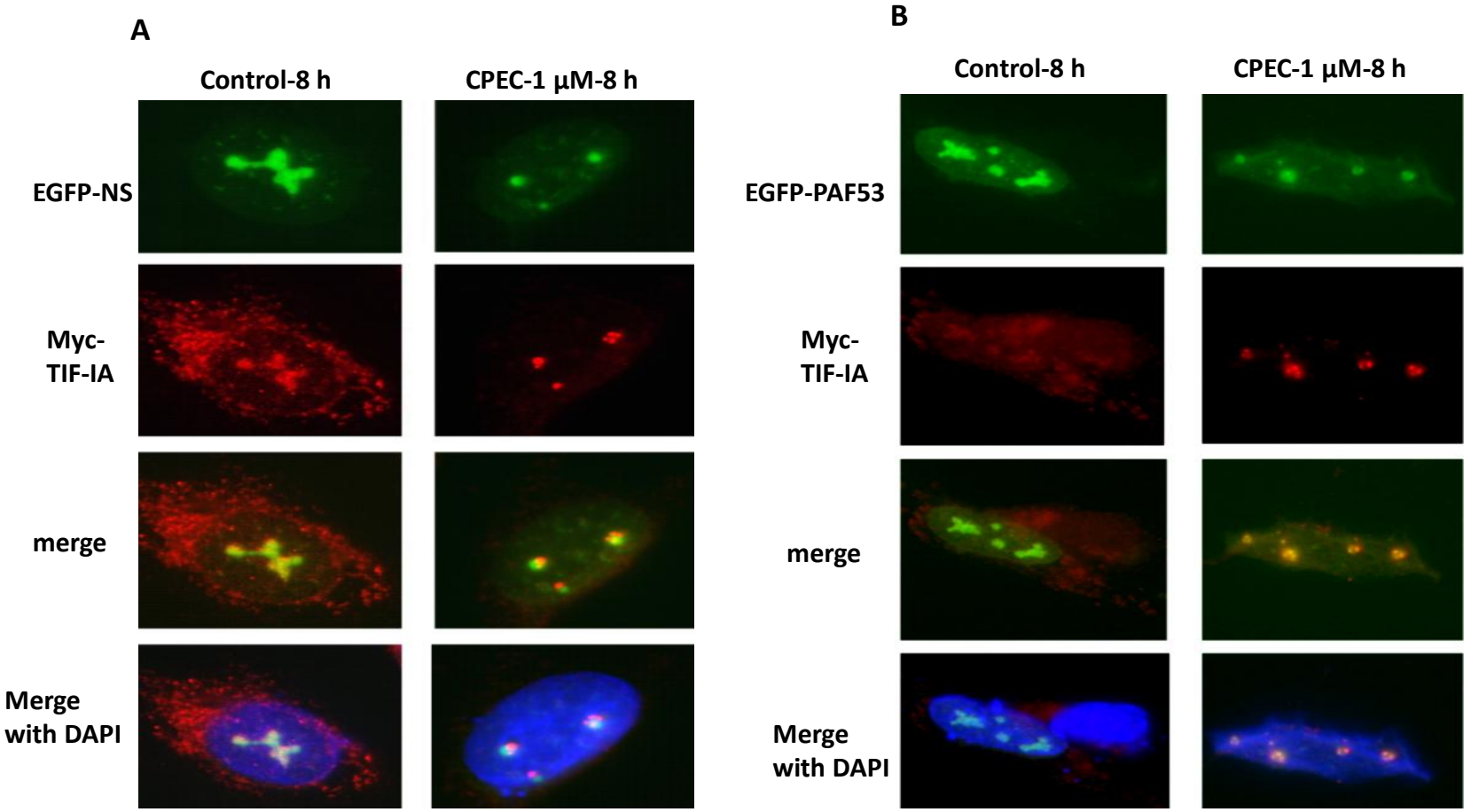

Fig. 6. CPEC-induced translocation of TIF-IA and PAF53 to the nucleolar cap. (A) U2OS cells were co-transfected with EGFP-NS and Myc- TIF-IA or **(B)** with EGFP-PAF53 and Myc-TIF-IA as described previously (Huang et al., 2008). Twenty-four hours post-transfection, cells were treated with 1 μM CPEC for 8 h and observed using fluorescent microscopy at 100× magnification.