International Journal of
Molecular Sciences

MDPI

Supplementary Material

Mercury Chloride but not Lead Acetate Causes Apoptotic Cell Death in Human Lung Fibroblast MRC5 Cells via Regulation of Cell Cycle Progression

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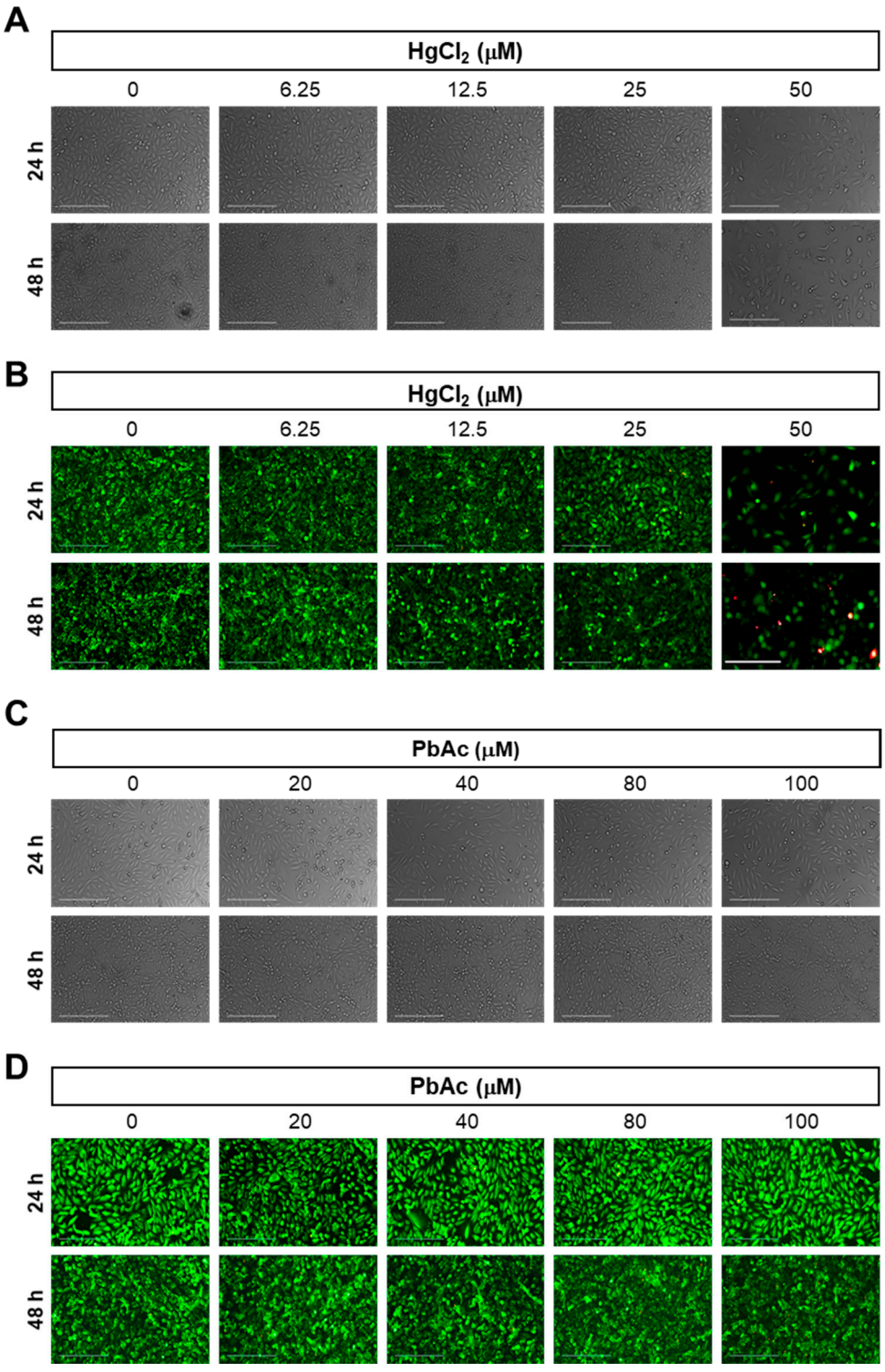

Figure S1. Effect of heavy metals on morphology of MRC5 cells. (**A and C**) The morphology of MRC5 cells was observed by phase-contrast microscopy after treatment with indicated concentration of $HgCl_2$ and PbAc for 24 or 48 h. (**B and D**) MRC5 cells were stained with calcein-AM (green) and ethidium homodimer (red) by the live/dead assay. EtOH were used as the negative control. Images are representative of three independent experiments. Scale bars represent 200 μm.

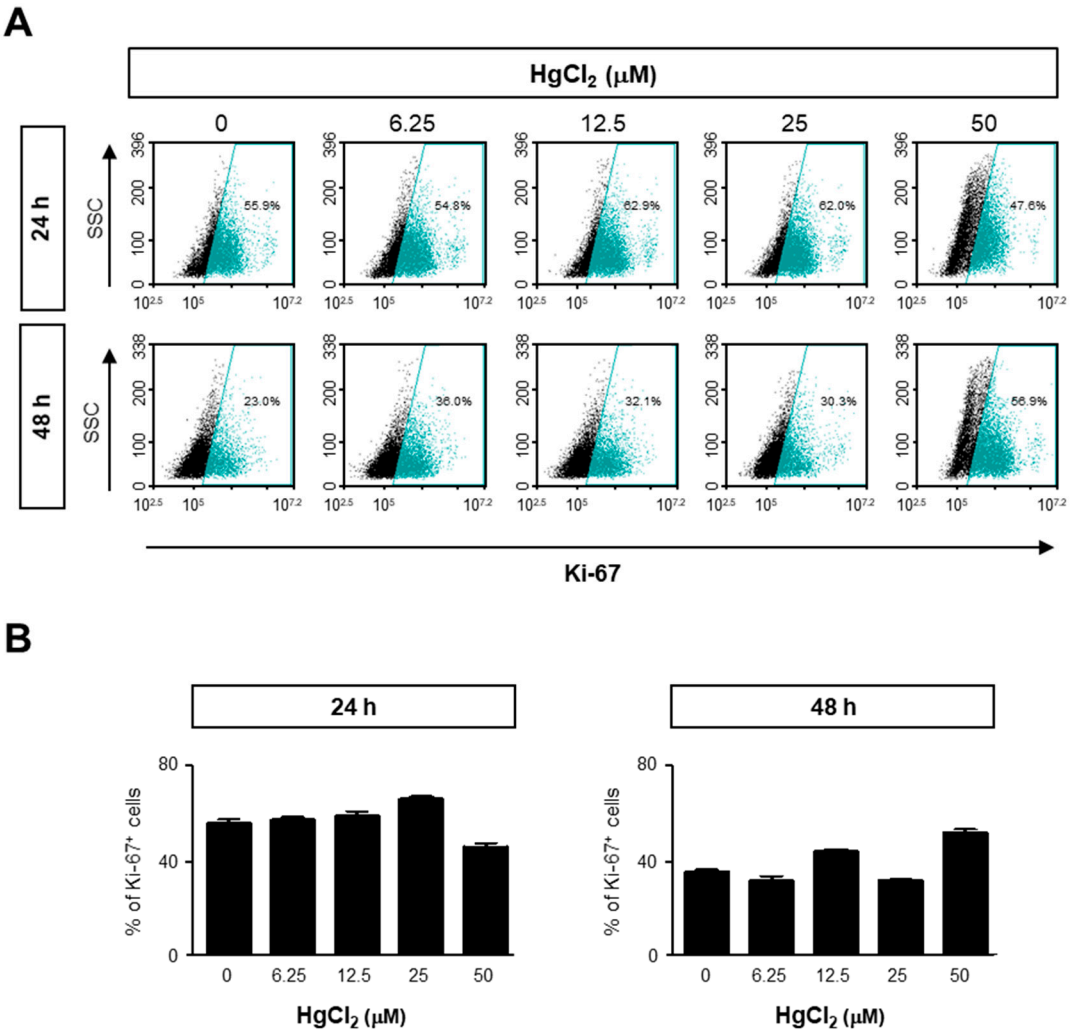

Figure S2. $HgCl_2$ treatment reduced the proliferation of MRC5 cells. (**A**) The heavy metals-treated MRC5 cells were immunostained with anti-Ki-67 antibodies. The cells were counted by FACS analysis. (**B**) The percentages of Ki-67-positive population are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate.

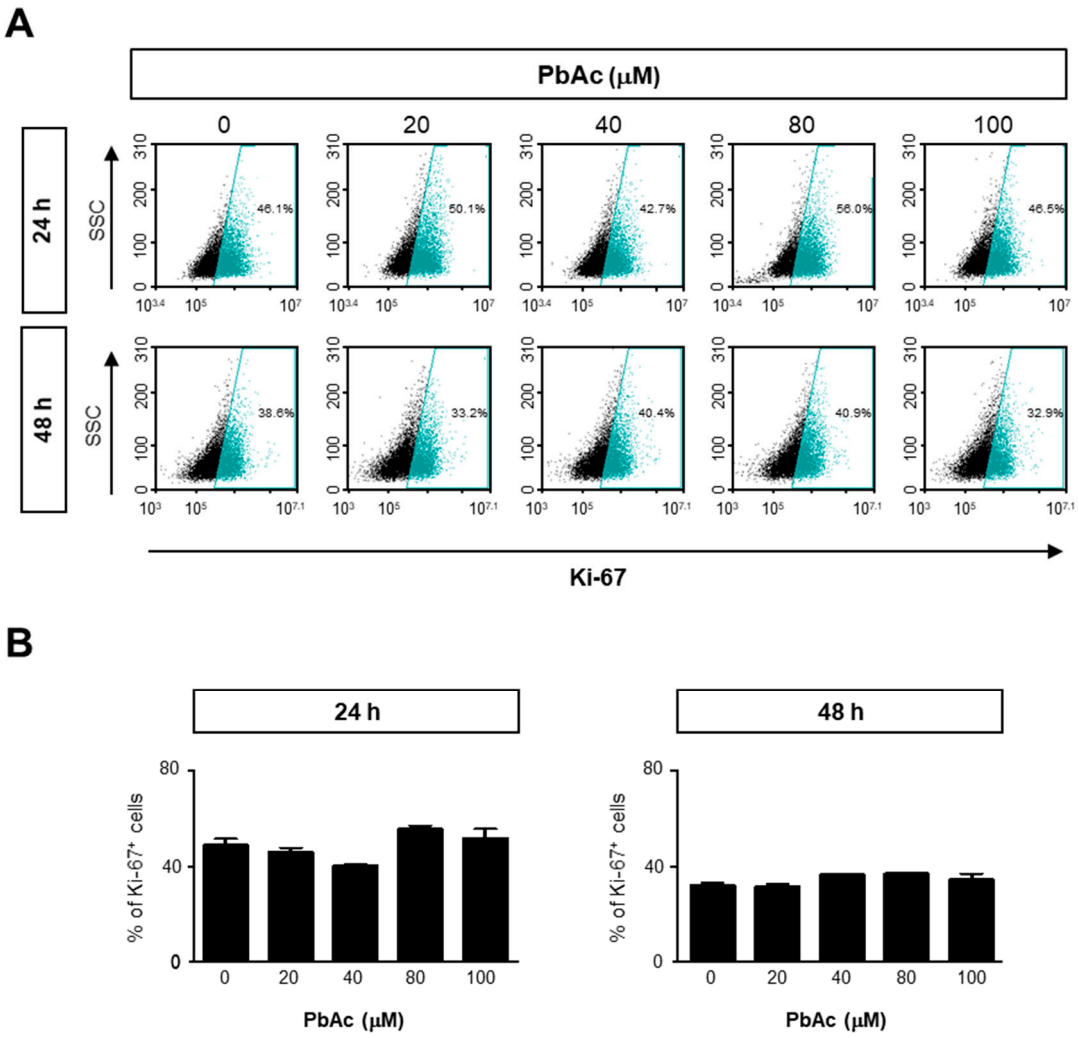

Figure S3. PbAc treatment reduced the proliferation of MRC5 cells. (**A**) The heavy metals-treated MRC5 cells were immunostained with anti-Ki-67 antibodies. The cells were counted by FACS analysis. (**B**) The percentages of Ki-67-positive population are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate.

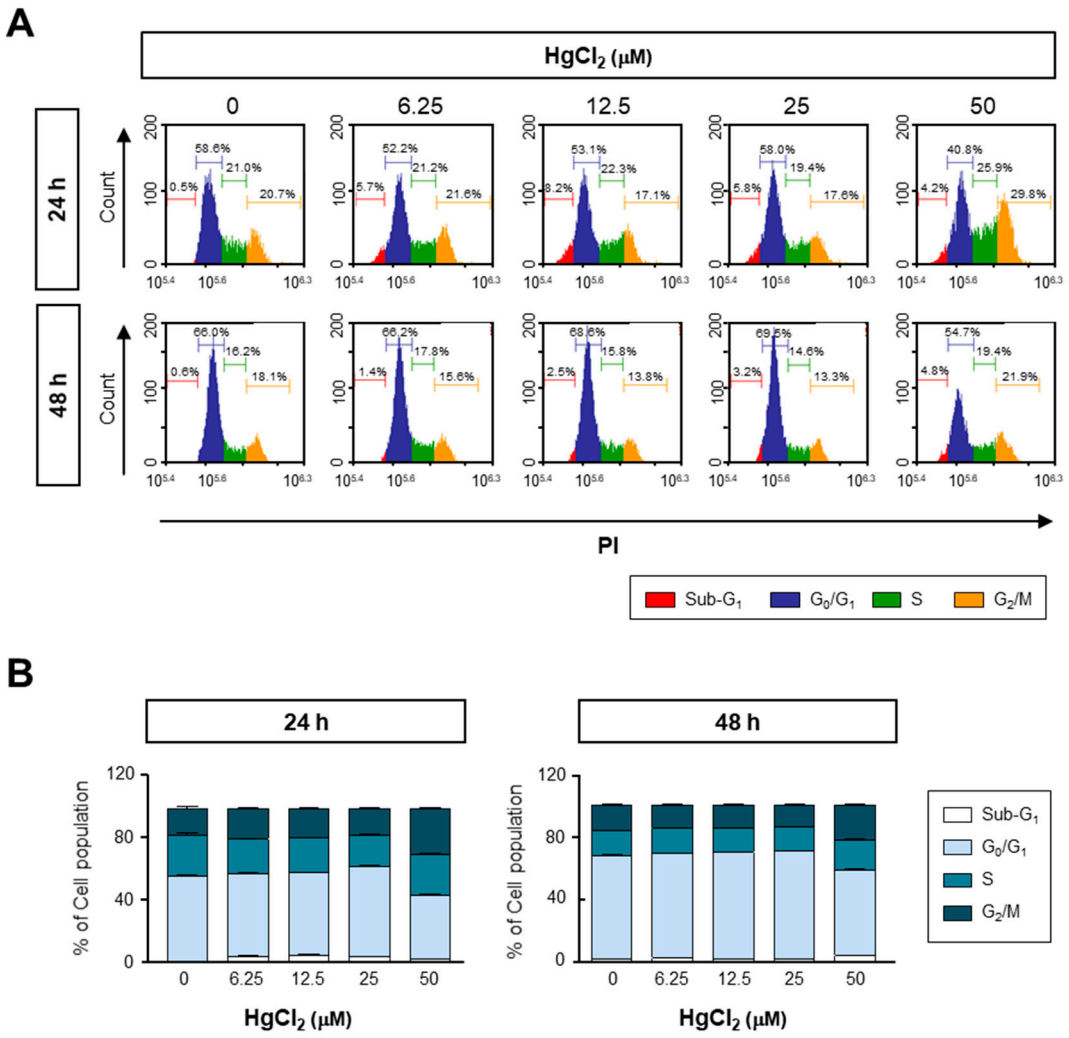

Figure S4. Cell cycle progression in $HgCl_2$ treatment in MRC5 cells. (**A**) The heavy metals-treated MRC5 cells were stained with propidium iodide (PI), and the cell cycle was measured using FACS analysis. (**B**) The percentages of population in the sub-G_1, G_0/G_1, S, and G_2/M-phases are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate.

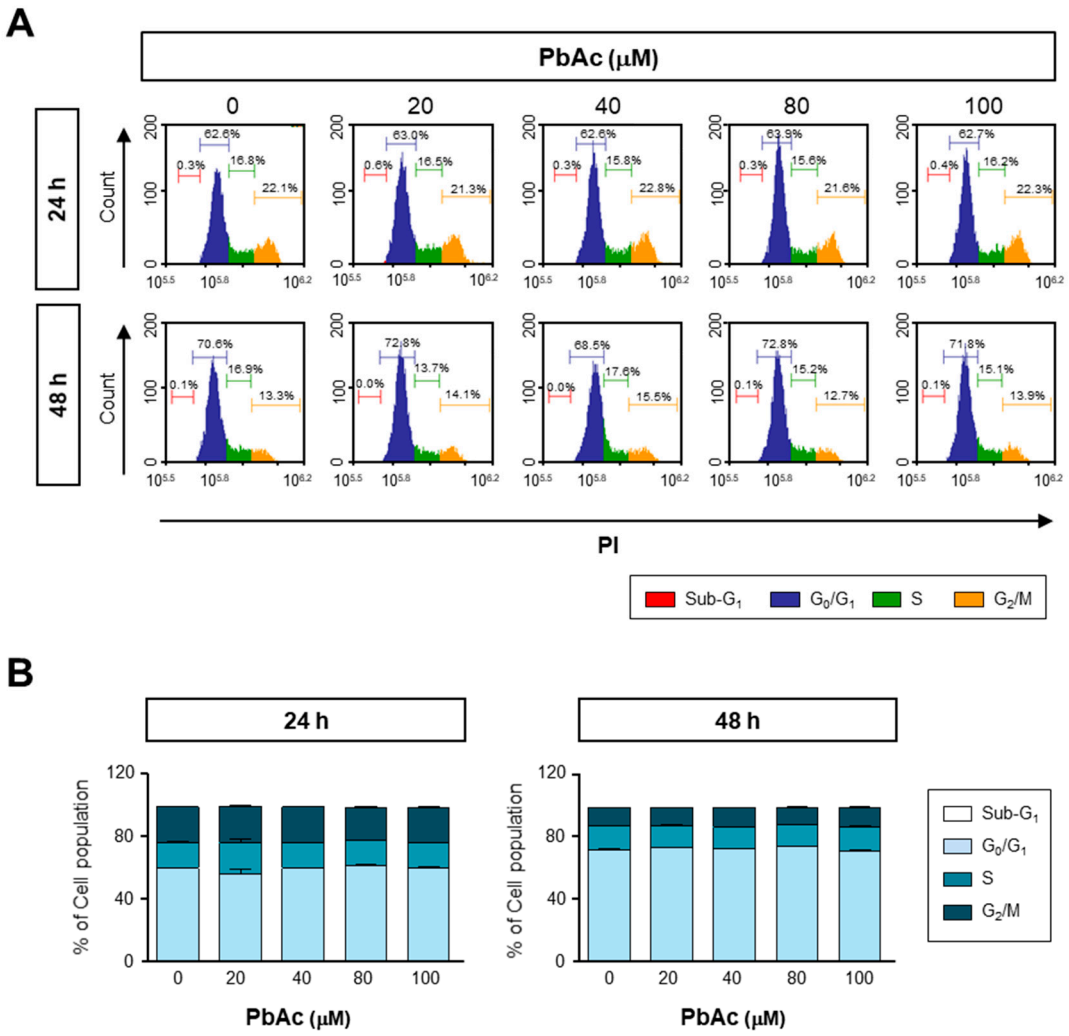

Figure S5. Cell cycle progression in PbAc treatment in MRC5 cells. (**A**) The heavy metals-treated MRC5 cells were stained with propidium iodide (PI), and the cell cycle was measured using FACS analysis. (**B**) The percentages of population in the sub-G_1, G_0/G_1, S, and G_2/M-phases are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate.

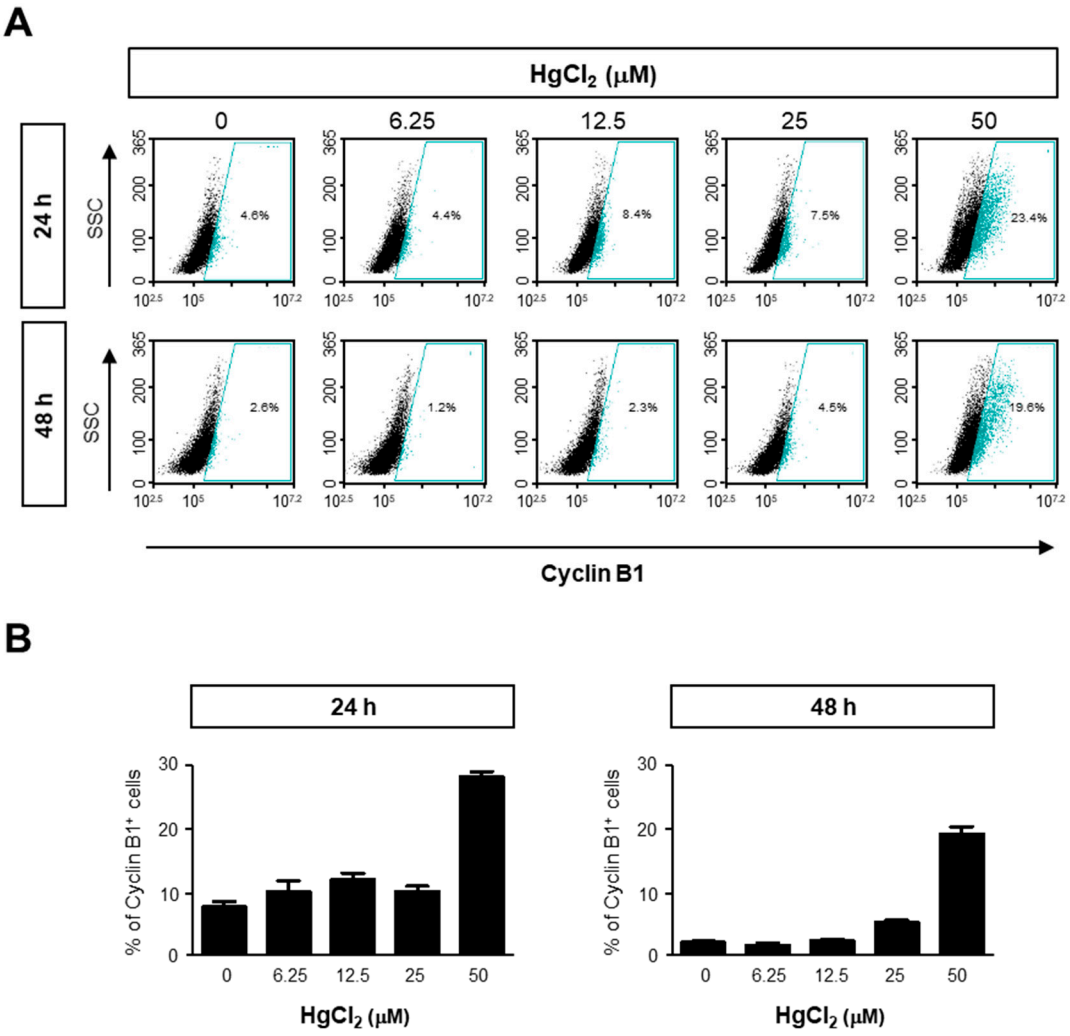

Figure S6. The effect of $HgCl_2$ on cyclin B1 expression in MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h. Cells were fixed with 1 % PFA and stained with anti-cyclin B1 antibody. The cyclin B1 expression were analyzed by FACS analysis. (**B**) The percentages of cyclin B1-positive cells are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate. EtOH was used as the negative control.

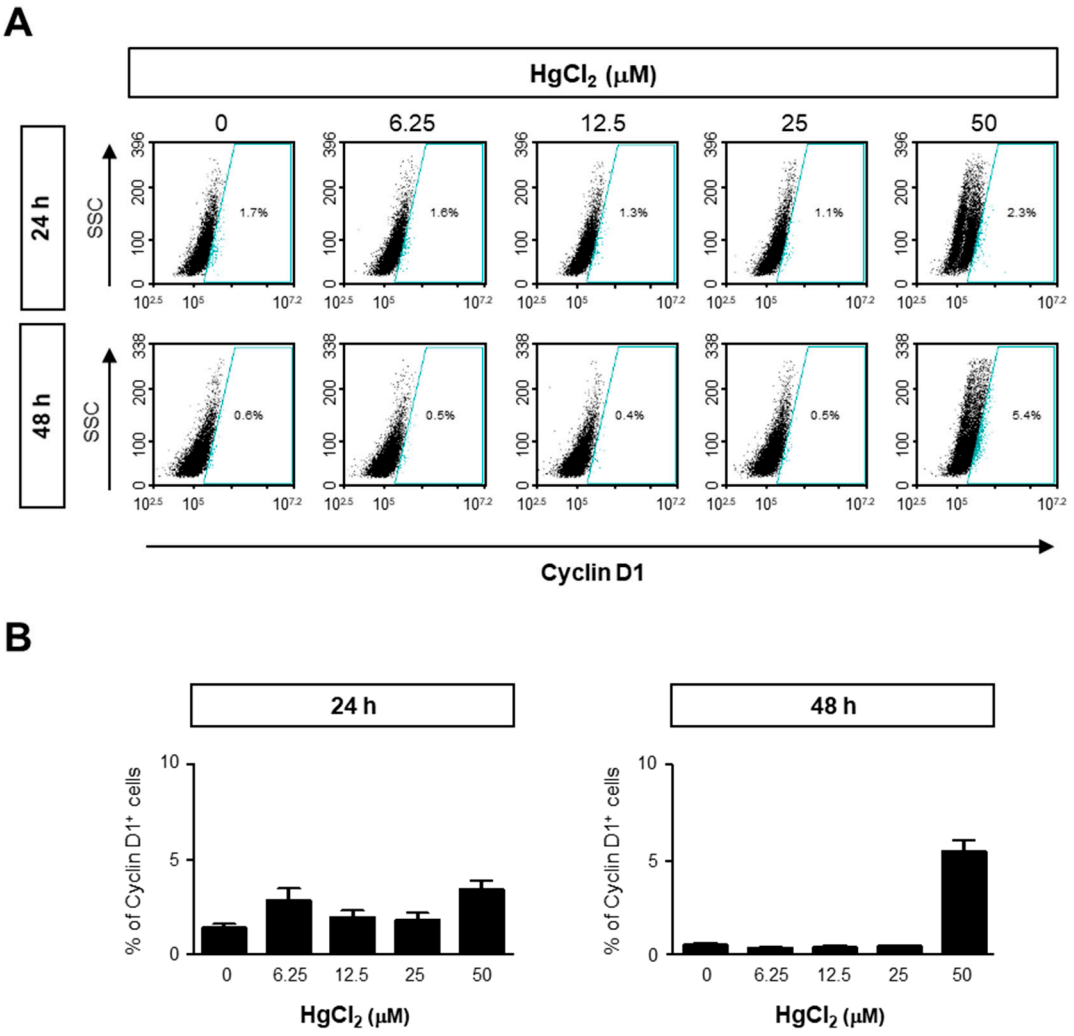

Figure S7. The effect of $HgCl_2$ on cyclin D1 expression in MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h. Cells were fixed with 1 % PFA and stained with anti-cyclin D1 antibody. The cyclin D1 expression were analyzed by FACS analysis. (**B**) The percentages of cyclin D1-positive cells are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate. EtOH was used as the negative control.

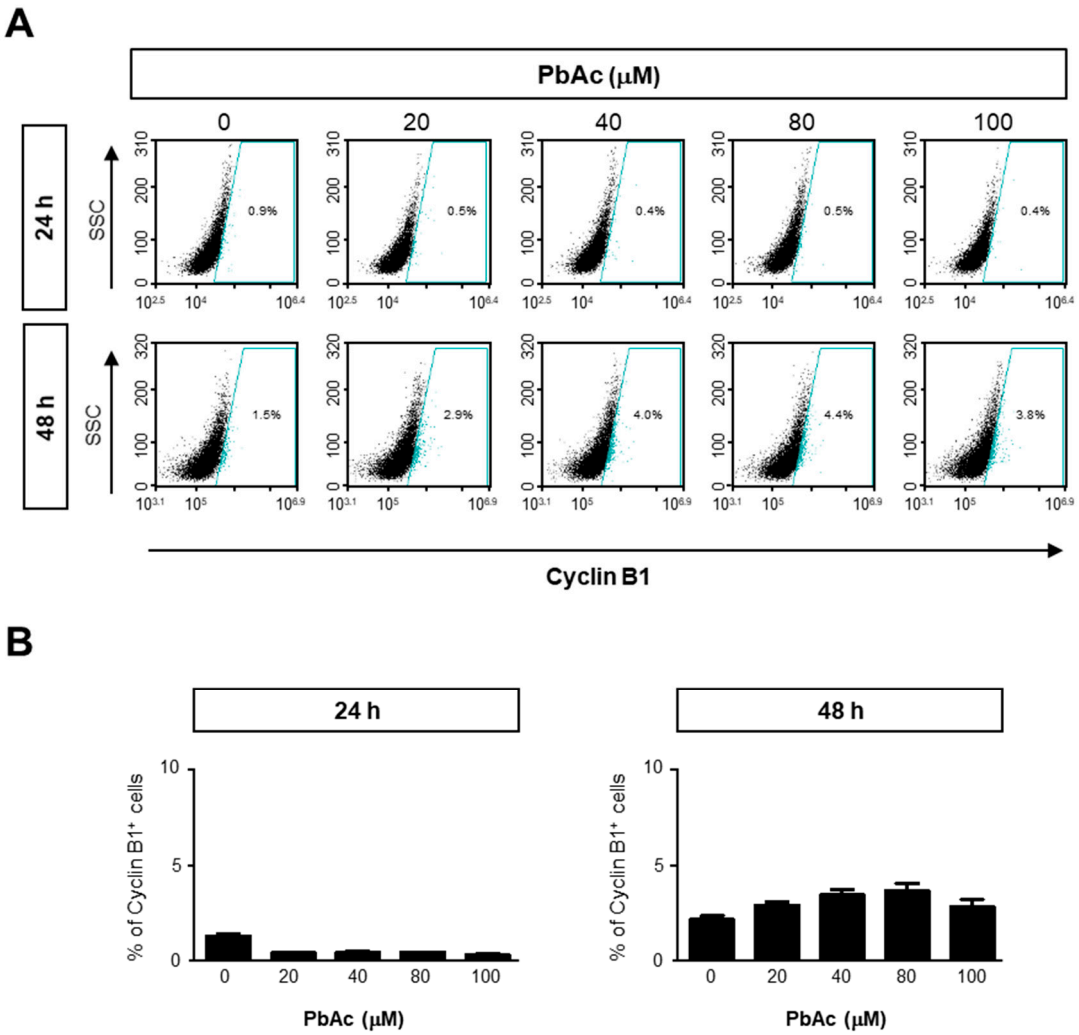

Figure S8. The effect of PbAc on cyclin B1 expression in MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h. Cells were fixed with 1 % PFA and stained with anti-cyclin B1 antibody. The cyclin B1 expression were analyzed by FACS analysis. (**B**) The percentages of cyclin B1-positive cells are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate. EtOH was used as the negative control.

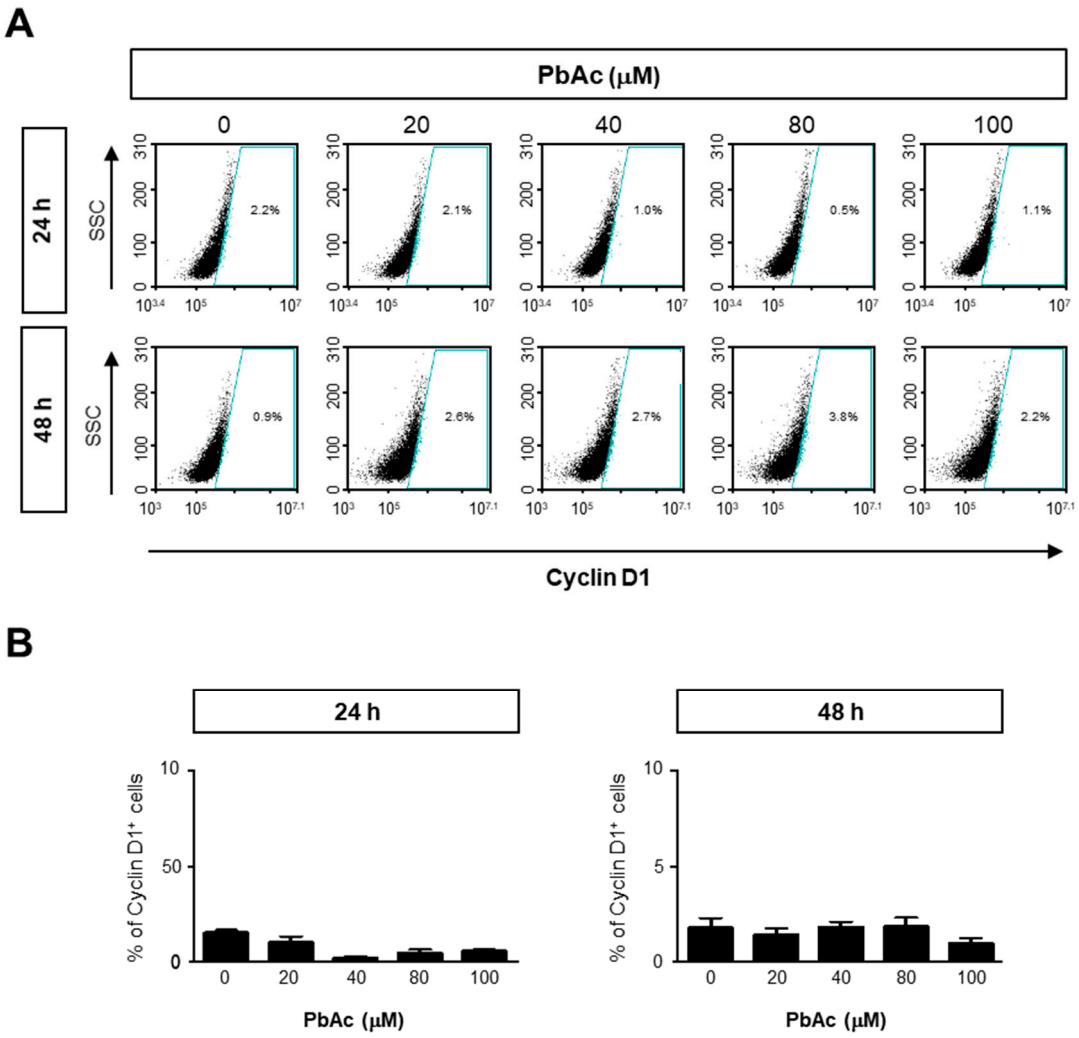

Figure S9. The effect of PbAc on cyclin D1 expression in MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h. Cells were fixed with 1 % PFA and stained with anti-cyclin D1 antibody. The cyclin D1 expression were analyzed by FACS analysis. (**B**) The percentages of cyclin D1-positive cells are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate. EtOH was used as the negative control.

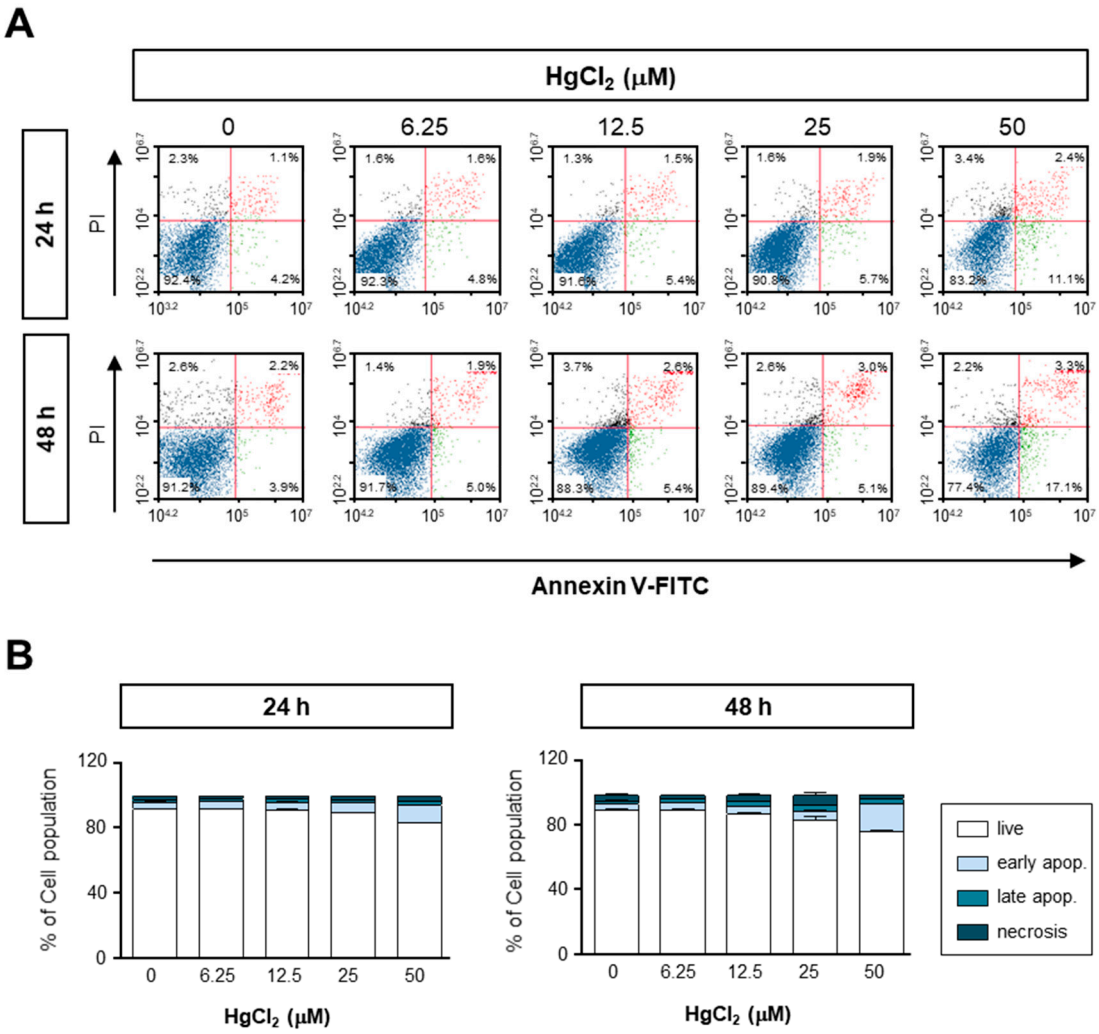

Figure S10. Effect of apoptotic cell death upon $HgCl_2$ treatment in MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h, and were double-stained with annexin V-FITC and PI. The proportion of apoptotic cells was assessed by FACS analysis. The scatter plots represent PI (Y-axis) and annexin V-FITC (X-axis). (**B**) The percentages of cells in the live, early- and late-apoptotic, and necrotic stages are expressed as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate.

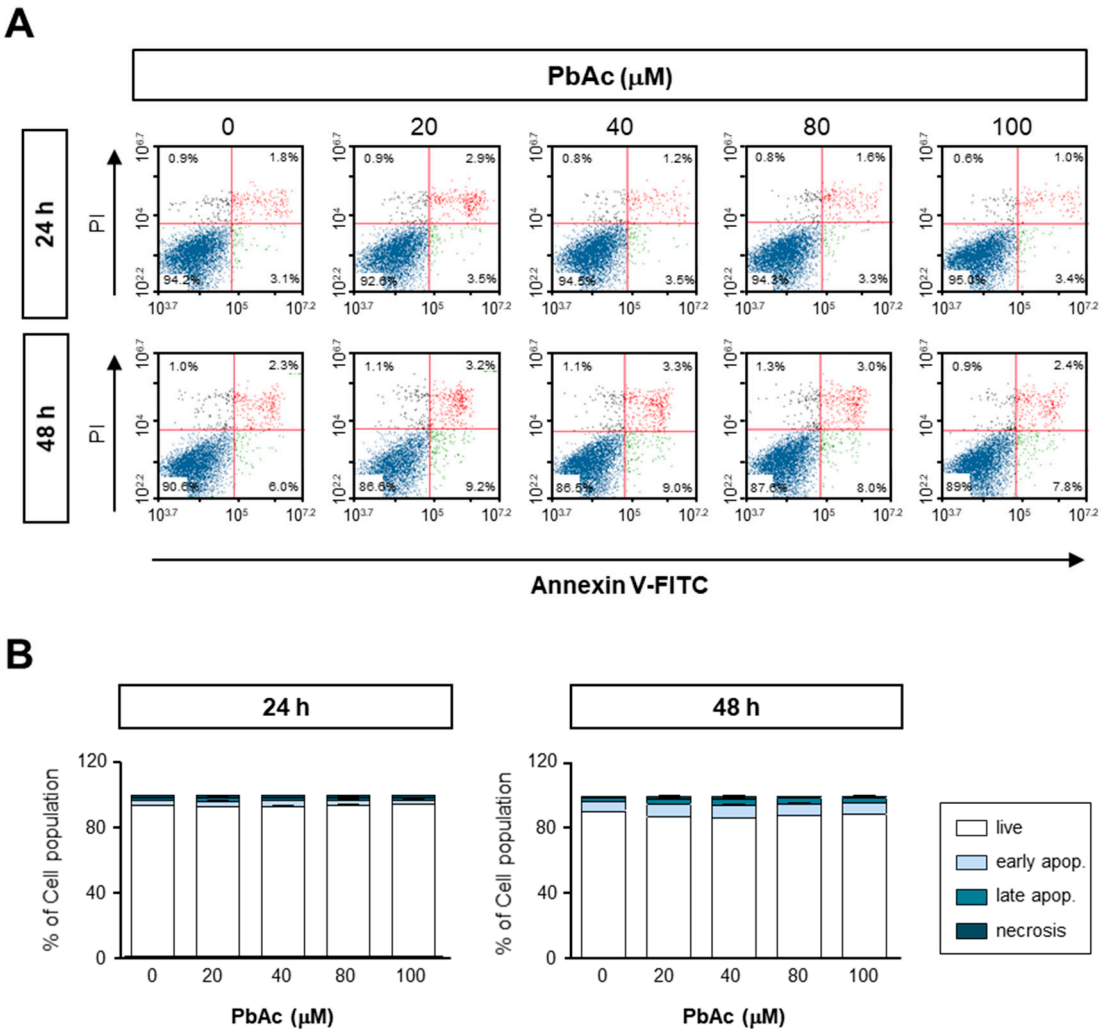

Figure S11. Effect of apoptotic cell death upon PbAc treatment in MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h, and were double-stained with annexin V-FITC and PI. The proportion of apoptotic cells was assessed by FACS analysis. The scatter plots represent PI (Y-axis) and annexin V-FITC (X-axis). (**B**) The percentages of cells in the live, early- and late-apoptotic, and necrotic stages are expressed as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate.

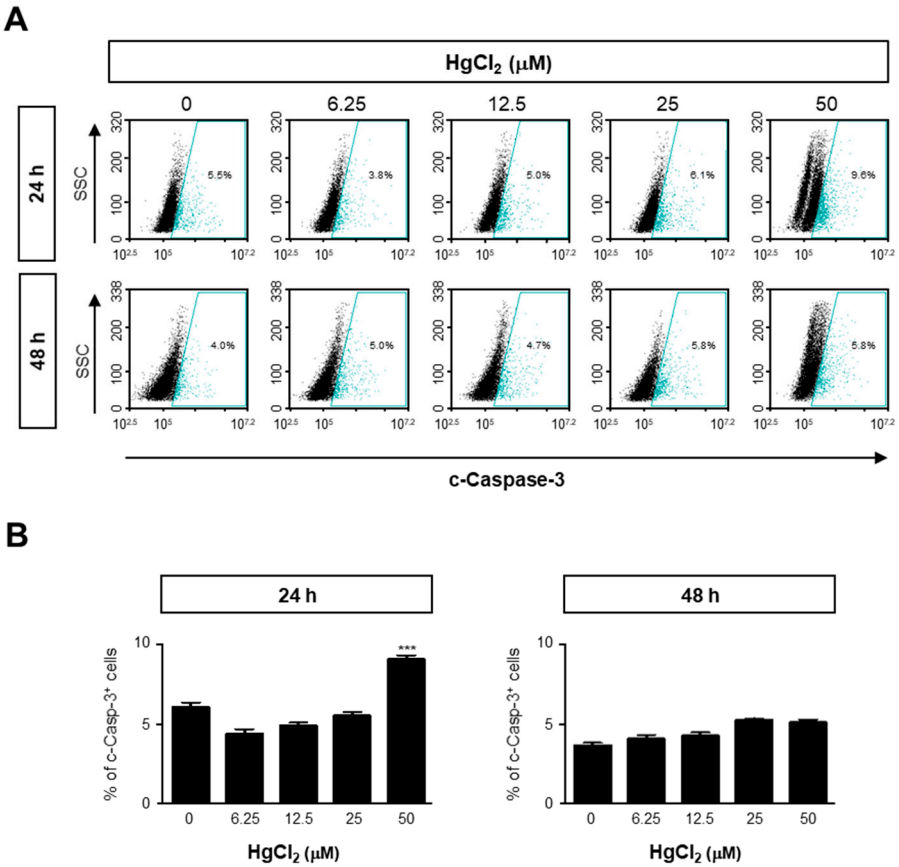

Figure S12. The effect of caspase-3 activity in $HgCl_2$-treated MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h. Cells were fixed with 1 % PFA and stained with anti-cleaved caspase-3 antibody. Expression levels of cleaved caspase-3 were analyzed by FACS analysis. (**B**) The percentages of cleaved caspase-3-positive cells are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate. EtOH was used as the negative control.

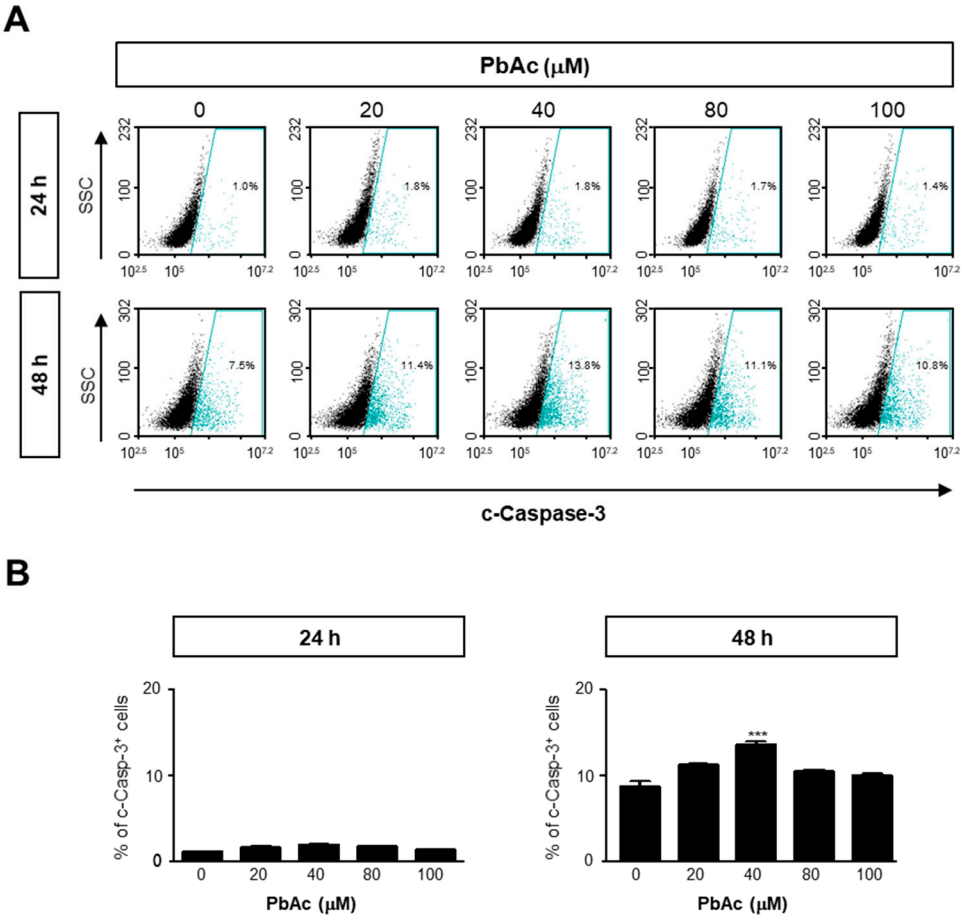

Figure S13. The effect of caspase-3 activity in PbAc-treated MRC5 cells. (**A**) Cells were treated with indicated concentration of heavy metals for 24 or 48 h. Cells were fixed with 1 % PFA and stained with anti-cleaved caspase-3 antibody. Expression levels of cleaved caspase-3 were analyzed by FACS analysis. (**B**) The percentages of cleaved caspase-3-positive cells are represented as the mean ± S.E.M. of three independent experiments (n = 6), each performed in triplicate. EtOH was used as the negative control.