NET4mPLASTIC Project

Work Package 4.4

Deliverable 4.4.2

Supplementary Materials

DETERMINATION OF THE EFFECTS OF CONTAMINANTS AT SINGLE CELL LEVELS

June, 27, 2022 – Version 1.0

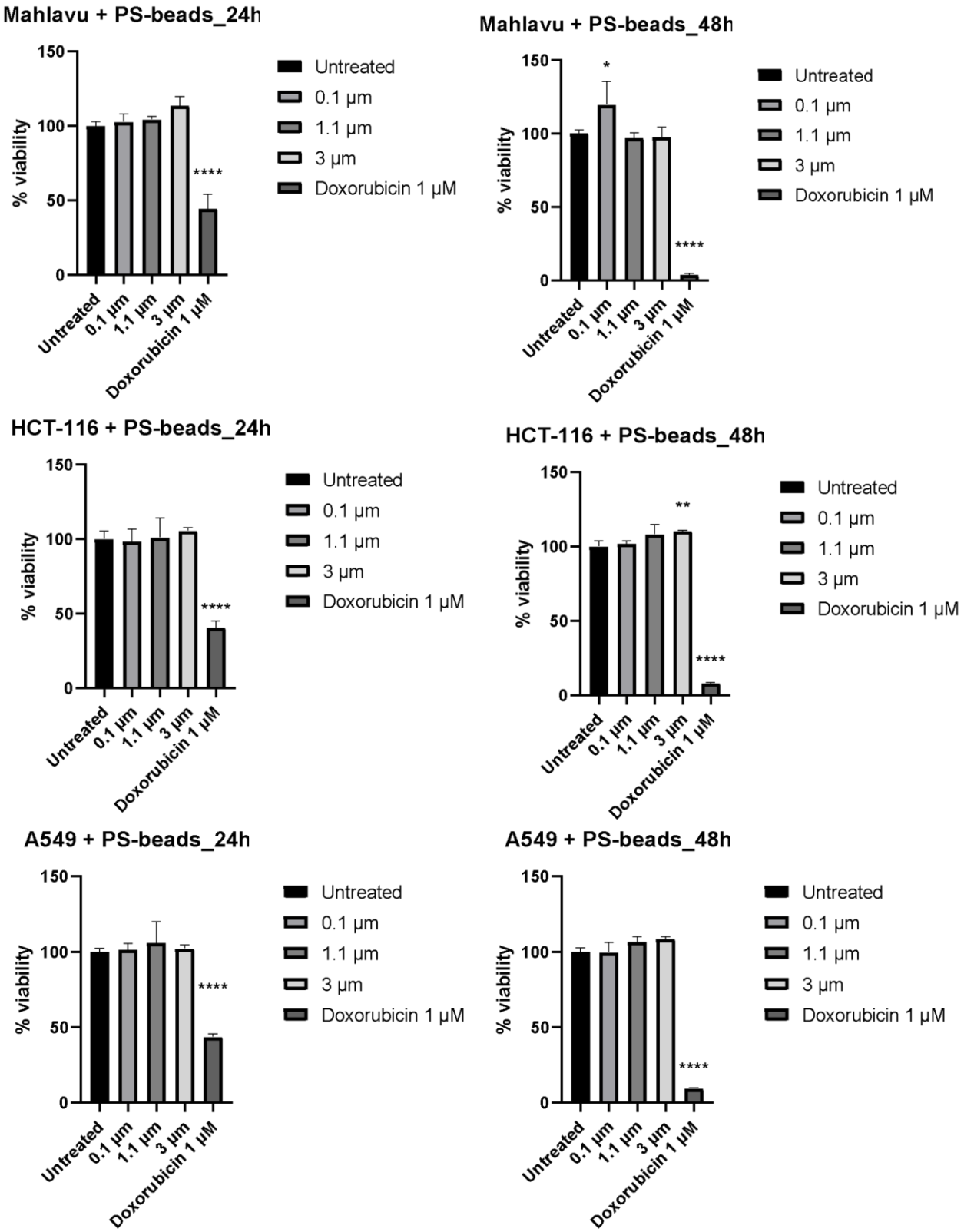

Figure 1. Cell viability of Mahlavu, HCT-116 and A549 cell lines exposed to 0.1 – 1.1 – 3 µm PS-MPs at the concentration of 20,000 beads/mm^2 for 24 and 48 h, using CCK8 assay. Data represent the percentage of PS-MPs treated live cells compared to the control (untreated cells). Samples treated with Doxorubicin 1 µM were used as control of cytotoxicity within the assays. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ (GraphPad Prism 8).

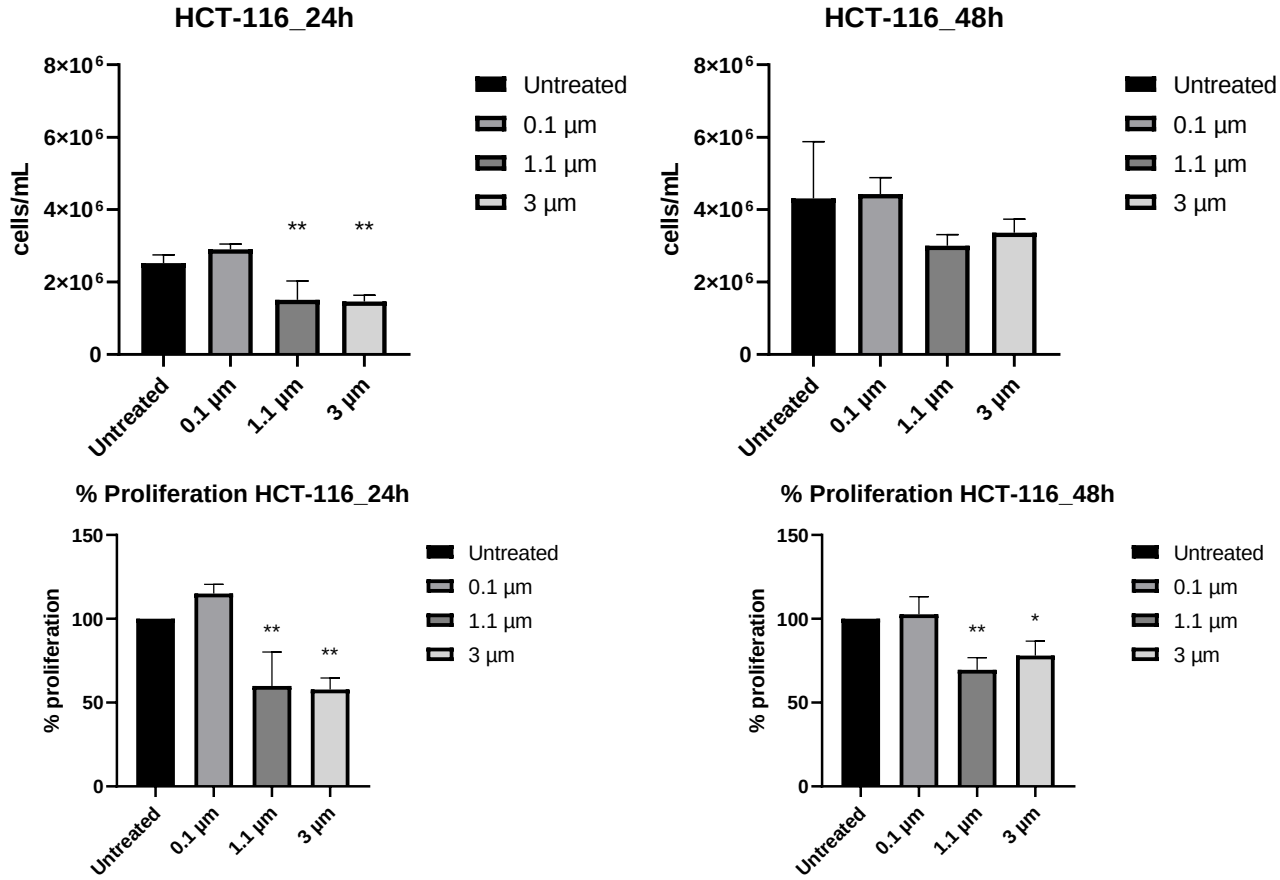

Figure 2. Analysis of HCT-116 cellular proliferation following treatment with 0.1 – 1.1 – 3 µm PS-MPs at the concentration of 20,000 beads/mm^2 for 24 and 48 h, by Trypan Blue. Both 1.1 and 3 µm treated HCT-116 showed reduction of cell number at 24 and 48 h compared to the control. Data represent means of three counts for each condition of a single experiment. * $p < 0.05$, ** $p < 0.01$ (GraphPad Prism 8).

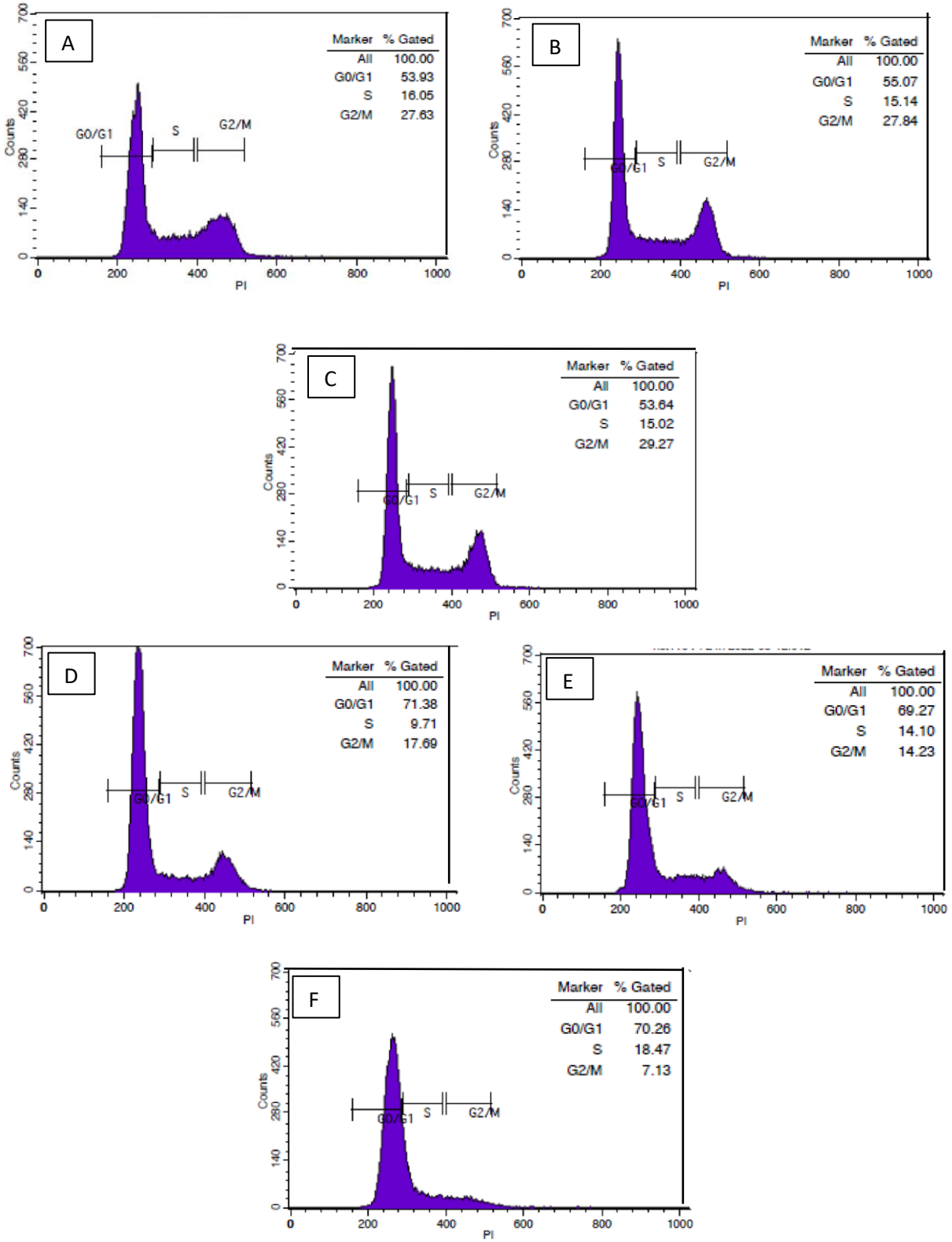

Figure 3. Cell cycle analysis of HCT-116 cells untreated and treated with 1.1 - 3 µm PS-MPs for 24 and 48 h: a) Untreated 24 h, b) 1.1 µm treated 24 h, c) 3 µm treated 24 h, d) Untreated 48 h, e) 1.1 µm treated 48 h, f) 3 µm treated 48 h. Percentage of gated cells in G0/G1, S and G2/M phase were calculated maintaining the same gates for all the samples by CellQuest Pro Software (FACSCalibur, BD Biosciences).

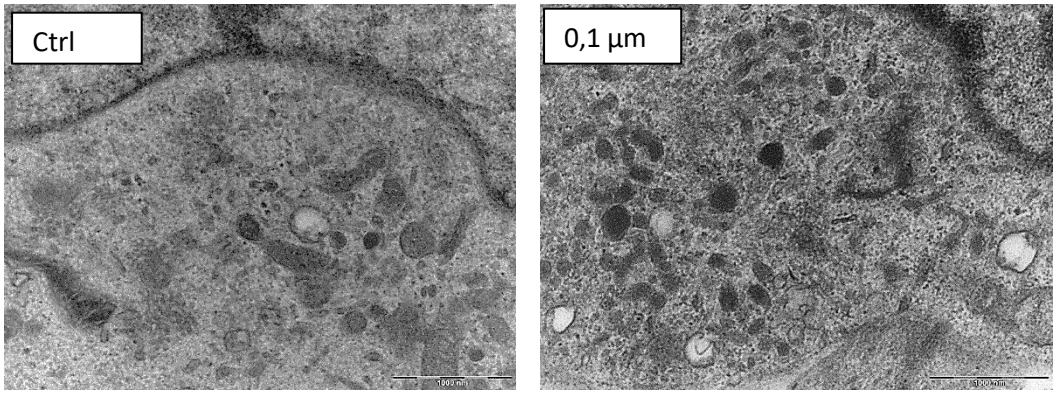

Figure 4. TEM images of Mahlavu cells untreated (ctrl, left) and 0.1 µm PS-MPs treated (0.1 µm, right). Magnification: 20,000x.

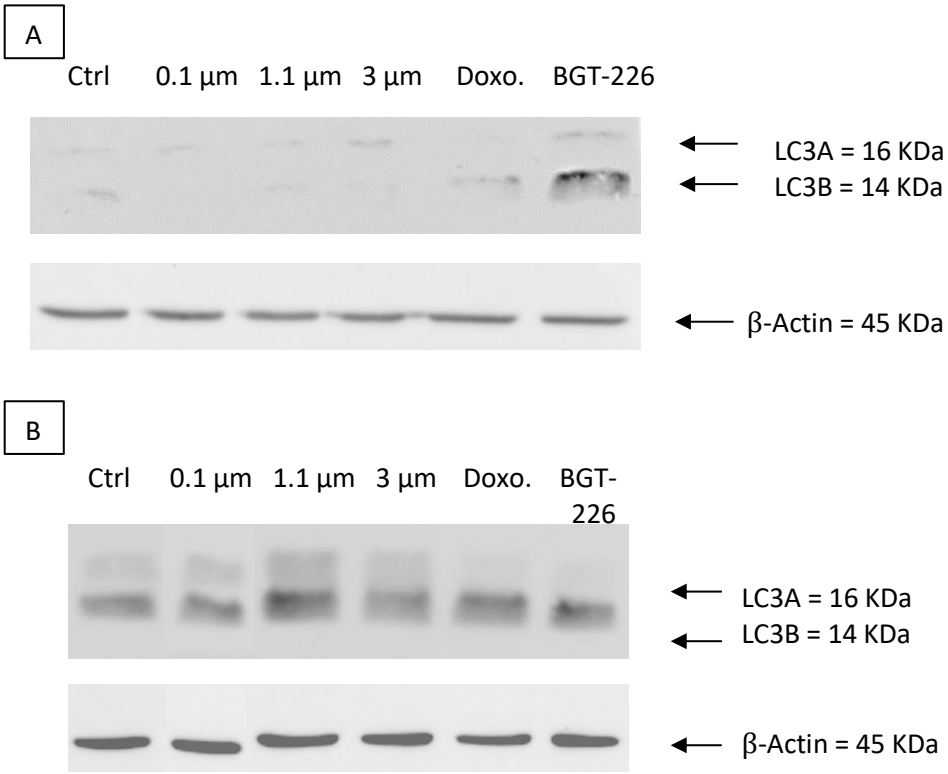

Figure 5. Western-blot assays to investigate autophagy response with LC3A/B isoforms detection in A549 treated with 10,000 beads/mm^2 and HCT-116 treated with 20,000 beads/mm^2 for 48 h: a) LC3A/B detection in A549 protein extracts, b) LC3A/B detection in HCT-116 protein extracts. Doxorubicin 1 µM and BGT-226 0.5 µM were used as controls for autophagy activation.

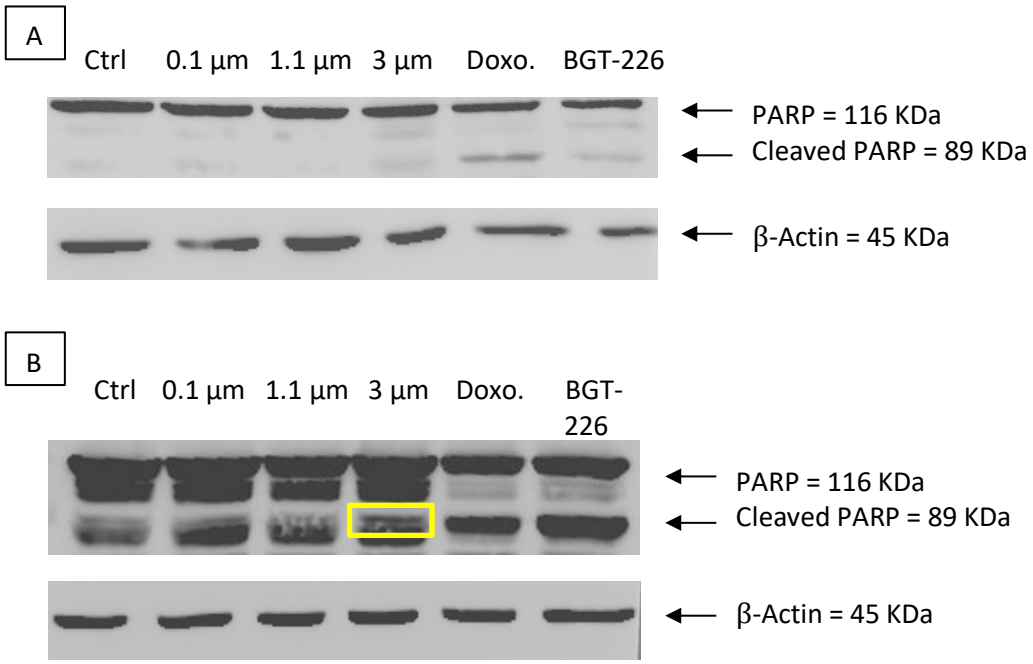

Figure 6. Western-blot assays to examine apoptosis by means of PARP cleavage in A549 treated with 10,000 beads/mm^2 and HCT-116 treated with 20,000 beads/mm^2 for 48 h: a) PARP/cleaved PARP detection in A549 protein extracts, b) PARP/cleaved PARP detection in HCT-116 protein extracts. Doxorubicin 1 µM and BGT-226 0.5 µM were used as controls for apoptosis activation. Yellow square denotes increasing band due to apoptosis pathway activation in PS-MPs treated HCT-116.