

Table S1. Cytotoxic properties of **MM129**, **MM130**, and **MM131** presented as IC₅₀. Mean values and 95% confidence intervals (parentheses) are given in μ M [1].

Cell type	MM129	MM130	MM131
PBMCs	1.11 (0.98–1.26)	0.77 (0.71–0.84)	0.62 (0.52–0.72)
Hs27	1.15 (0.95–1.37)	0.81 (0.64–0.99)	0.62 (0.43–0.82)
HeLa	0.9 (0.73–1.06)	0.59 (0.51–0.67)	0.41 (0.28–0.54)
HCT 116	0.6 (0.58–0.62)	0.44 (0.43–0.45)	0.39 (0.37–0.41)
PC-3	0.36 (0.32–0.4)	0.22 (0.18–0.28)	0.17 (0.16–0.17)
BxPC-3	0.26 (0.2–0.32)	0.17 (0.13–0.21)	0.13 (0.11–0.15)

1. Flow Cytometry Assessment of Annexin V Binding

Figures S1-S12 show the exemplary flow cytometry (Annexin V-FITC/PI assay) dot-plots for depicting apoptotic and necrotic changes in examined cancer cell lines (Hela, HCT 116, PC-3, and BxPC-3) exposed to **MM129**, **MM130**, and **MM131** in concentrations of $\frac{1}{2}$ IC₅₀, IC₅₀, and 2×IC₅₀ for 24-h, 48-h, and 72-h. Q1 (upper left quadrant) – necrotic cells (Annexin V-, PI+); Q2 (upper right quadrant) – late apoptotic cells (Annexin V+, PI+); Q3 (lower right quadrant) – early apoptotic cells (Annexin V+, PI-); Q4 (lower left quadrant) – live cells (Annexin V-, PI-).

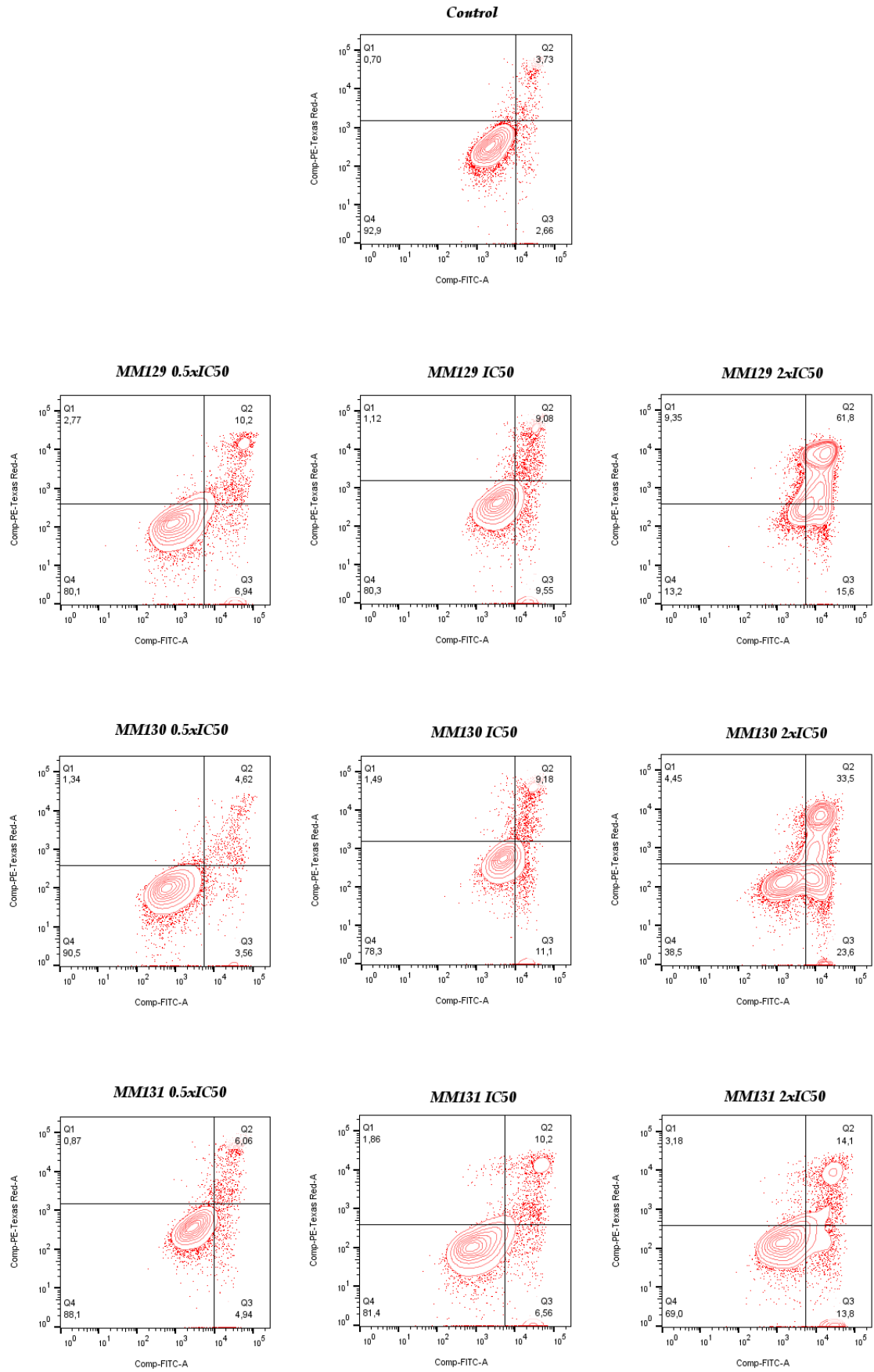


Figure S1. Hela cells after 24-h of exposure to MM-compounds.

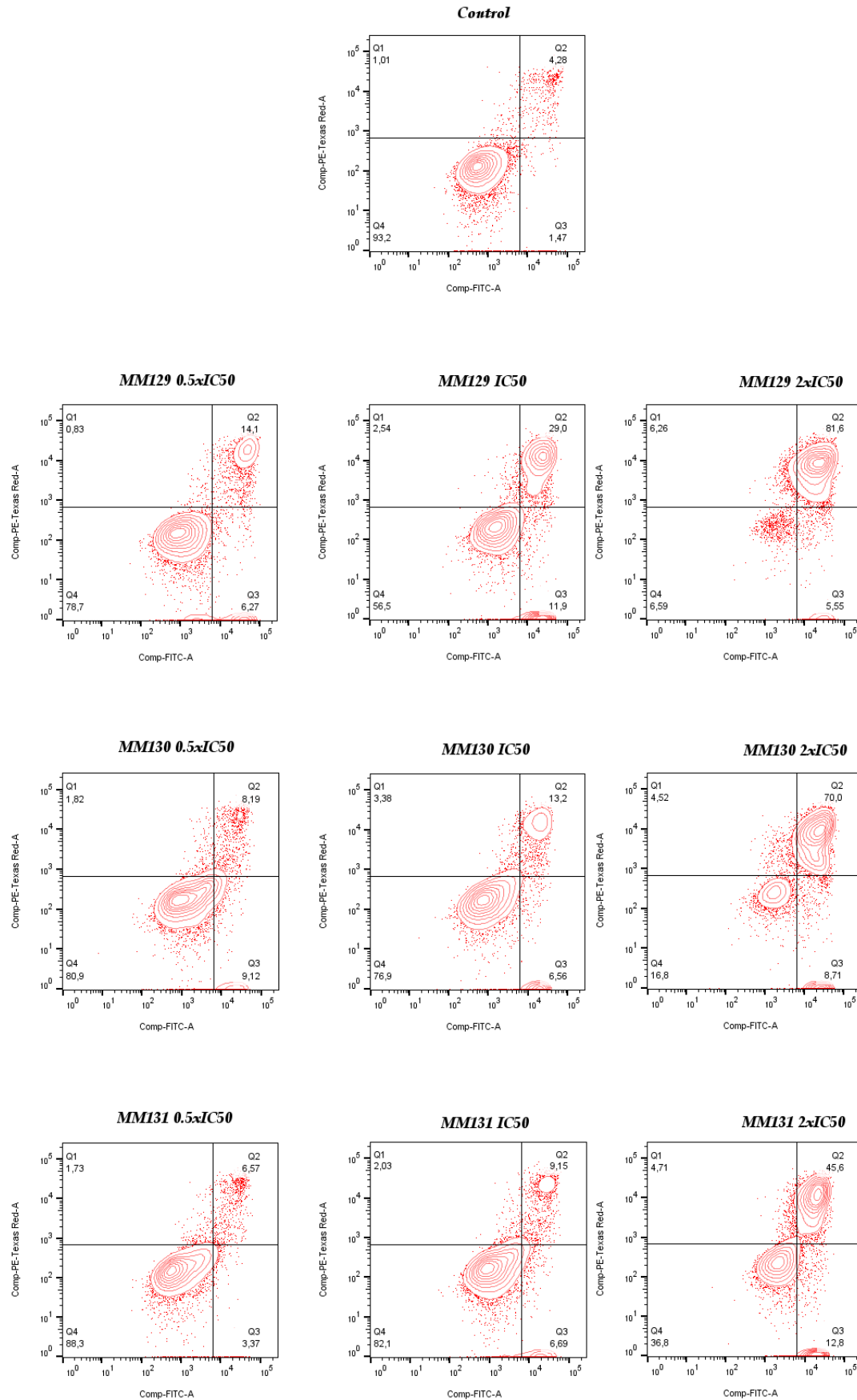


Figure S2. Hela cells after 48-h of exposure to MM-compounds.

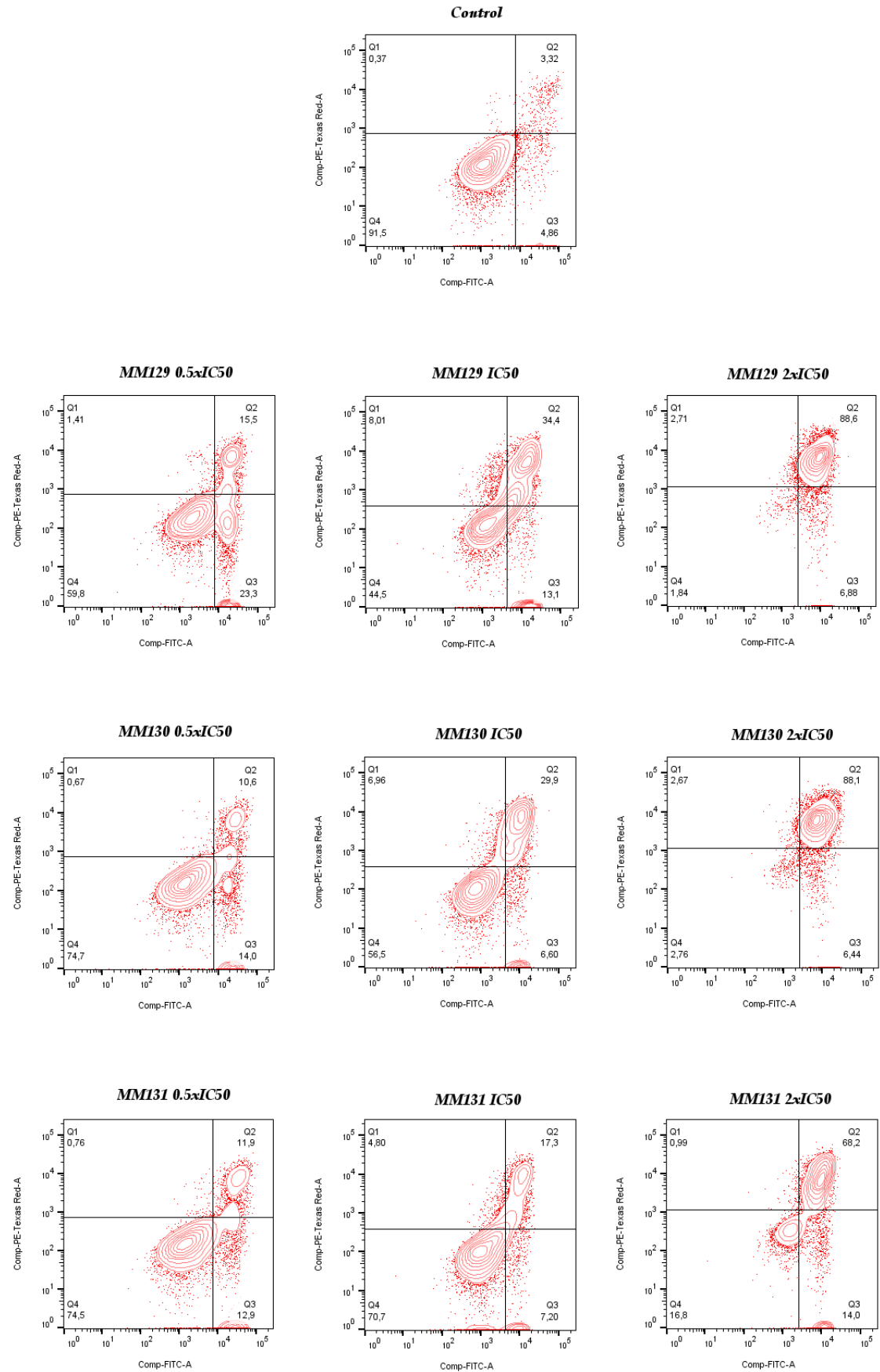


Figure S3. Hela cells after 72-h of exposure to MM-compounds.

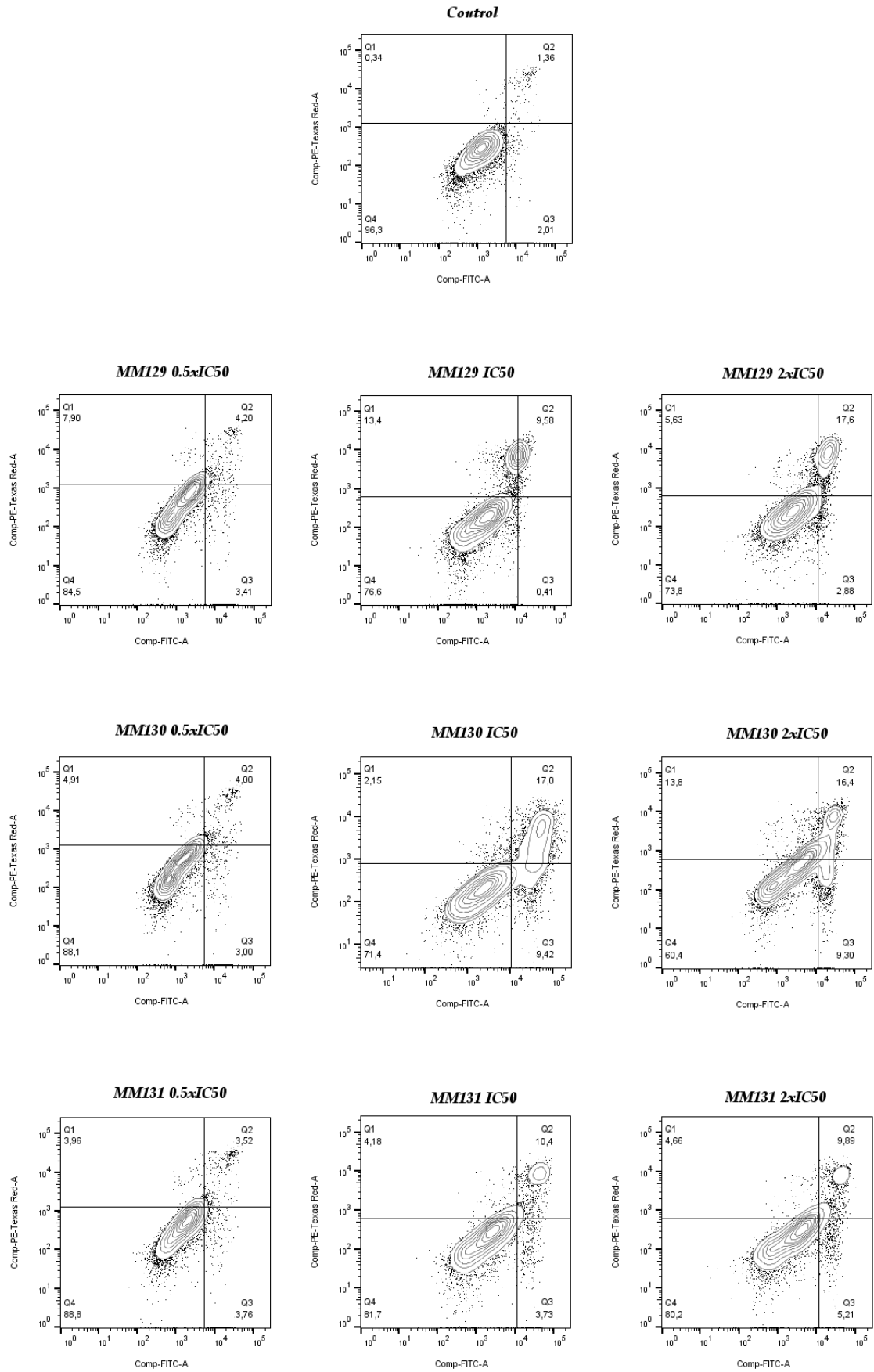


Figure S4. HCT 116 cells after 24-h of exposure to MM-compounds.

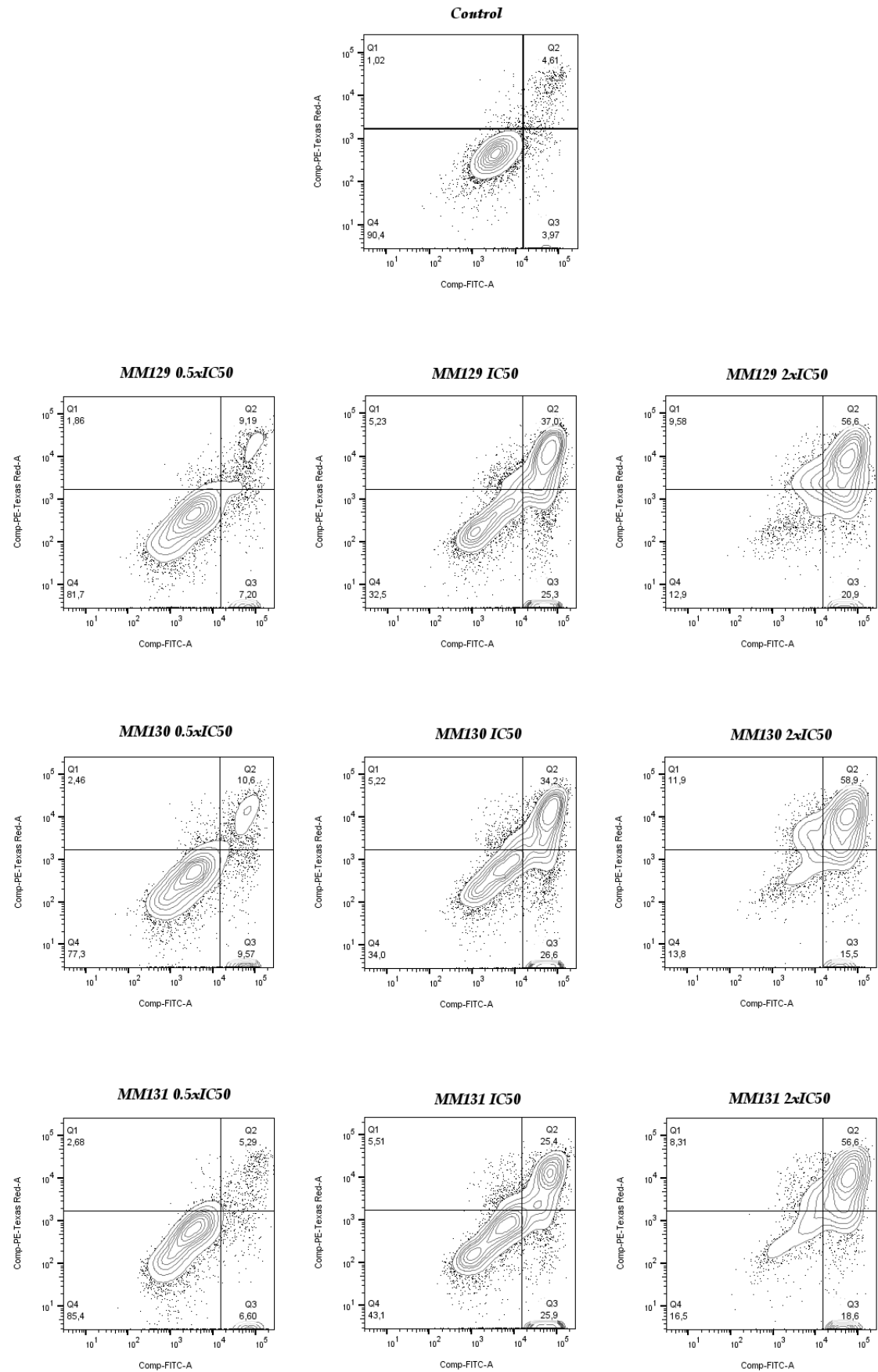


Figure S5. HCT 116 cells after 48-h of exposure to MM-compounds.

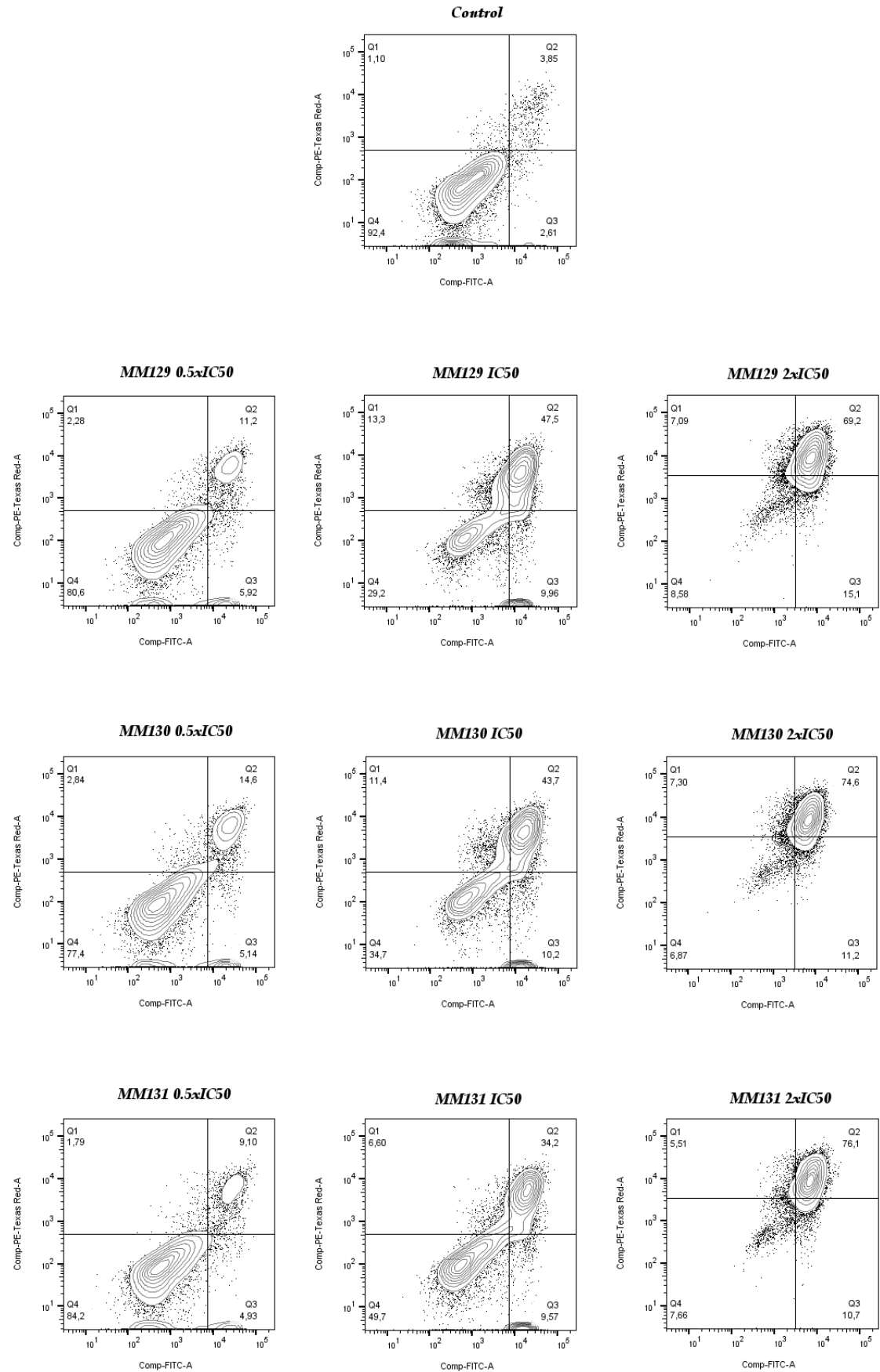


Figure S6. HCT 116 cells after 72-h of exposure to MM-compounds.

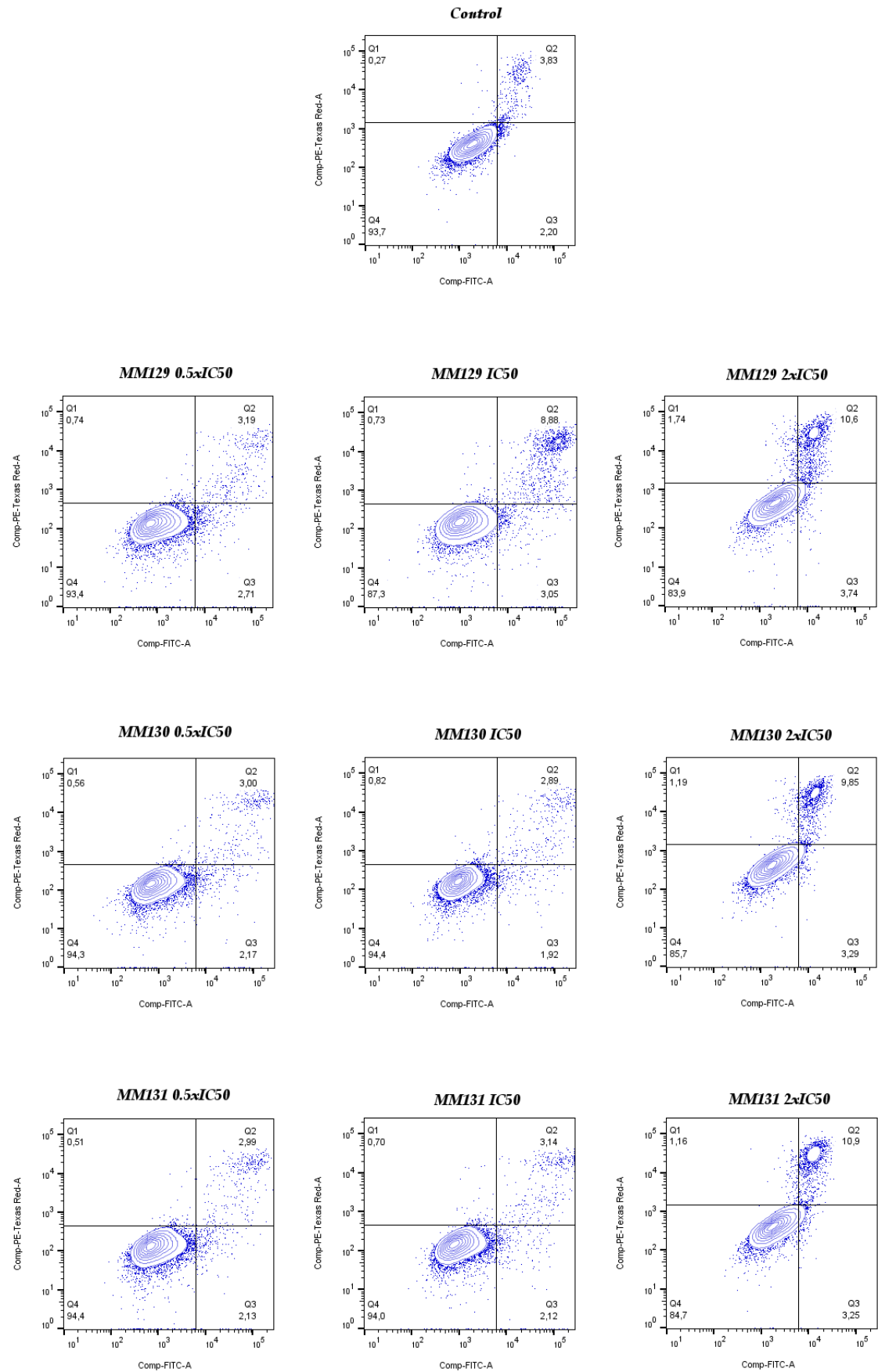


Figure S7. PC-3 cells after 24-h of exposure to MM-compounds.

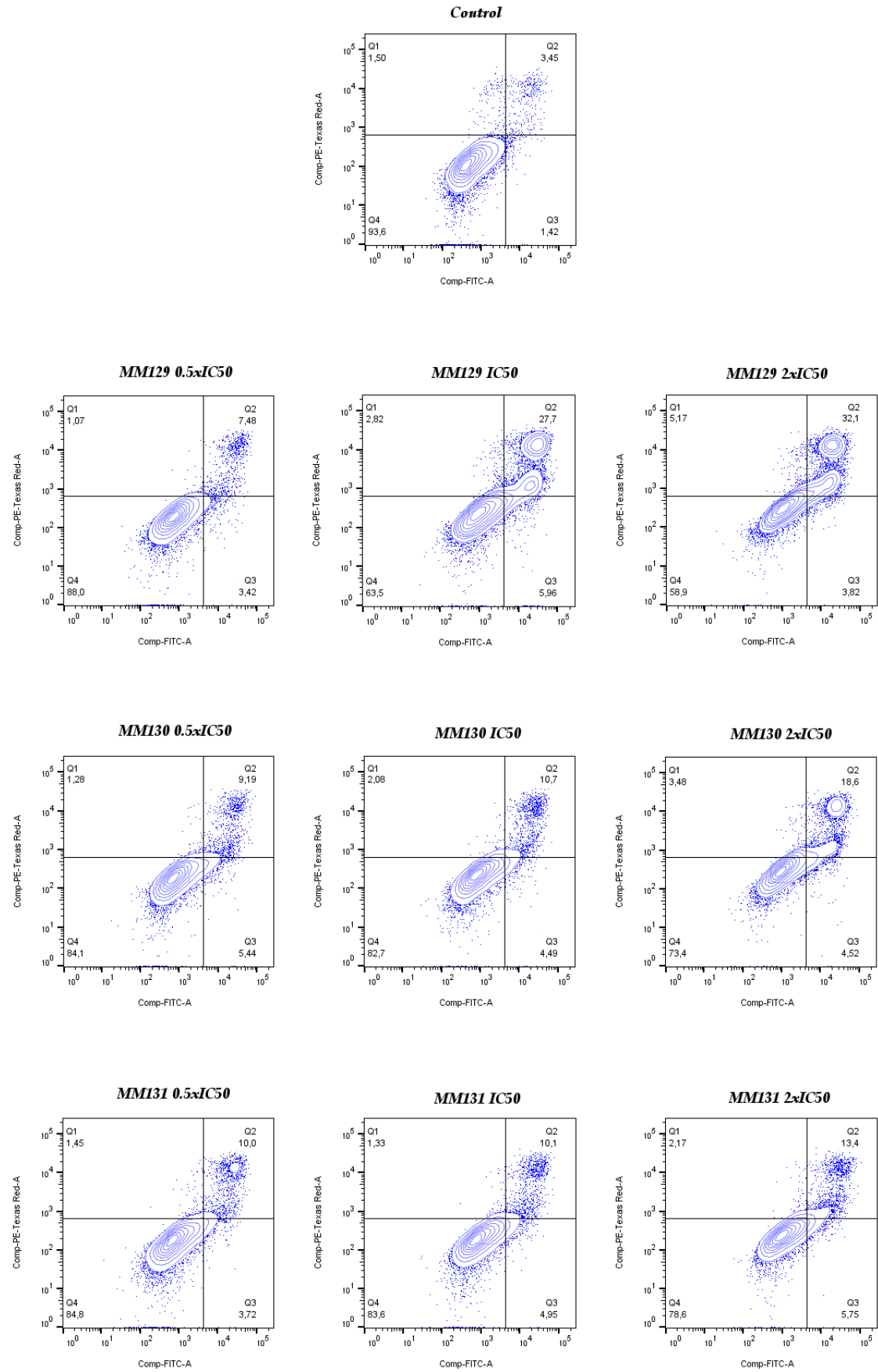


Figure S8. PC-3 cells after 48-h of exposure to MM-compounds.

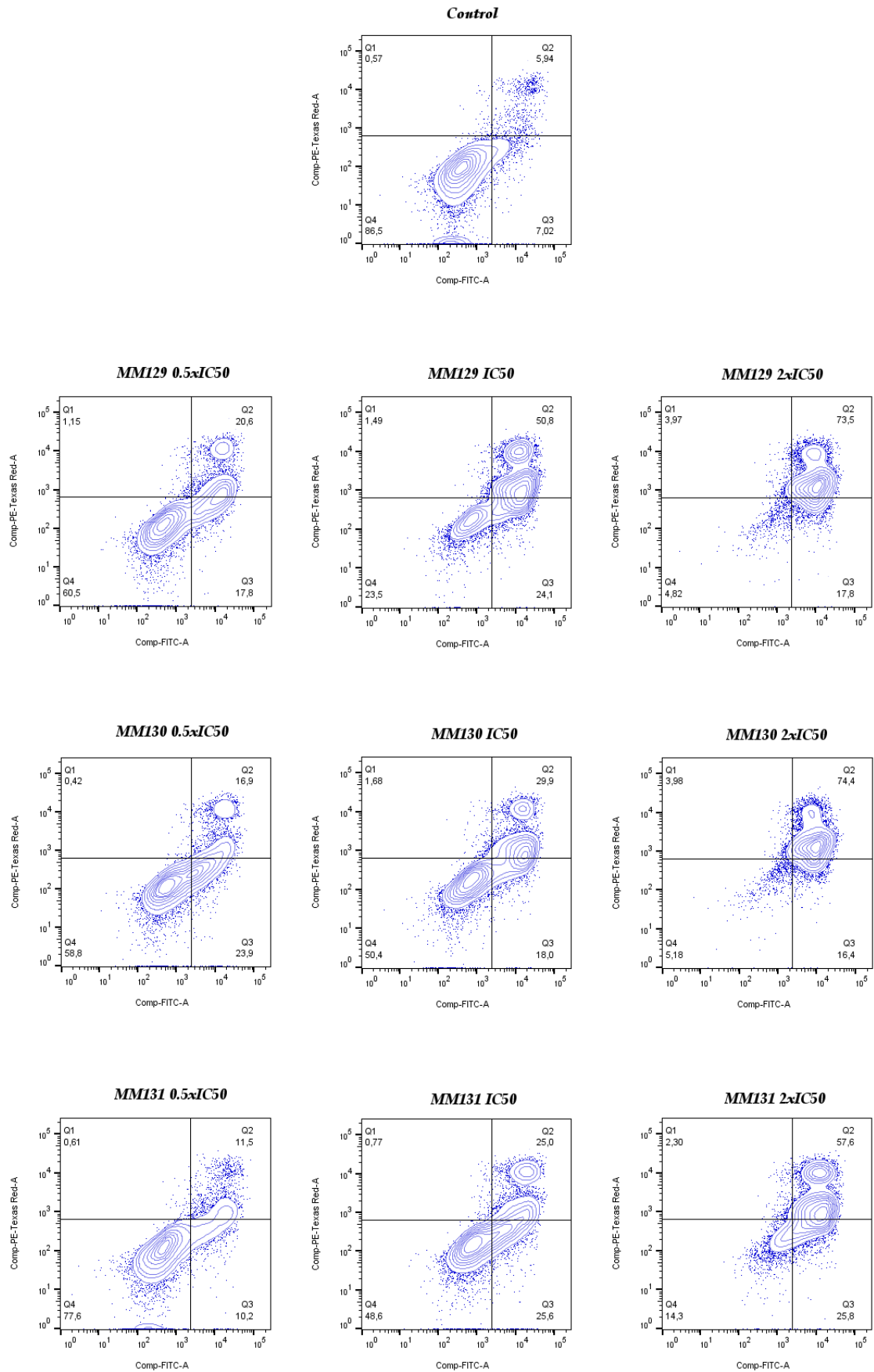


Figure S9. PC-3 cells after 72-h of exposure to MM-compounds.

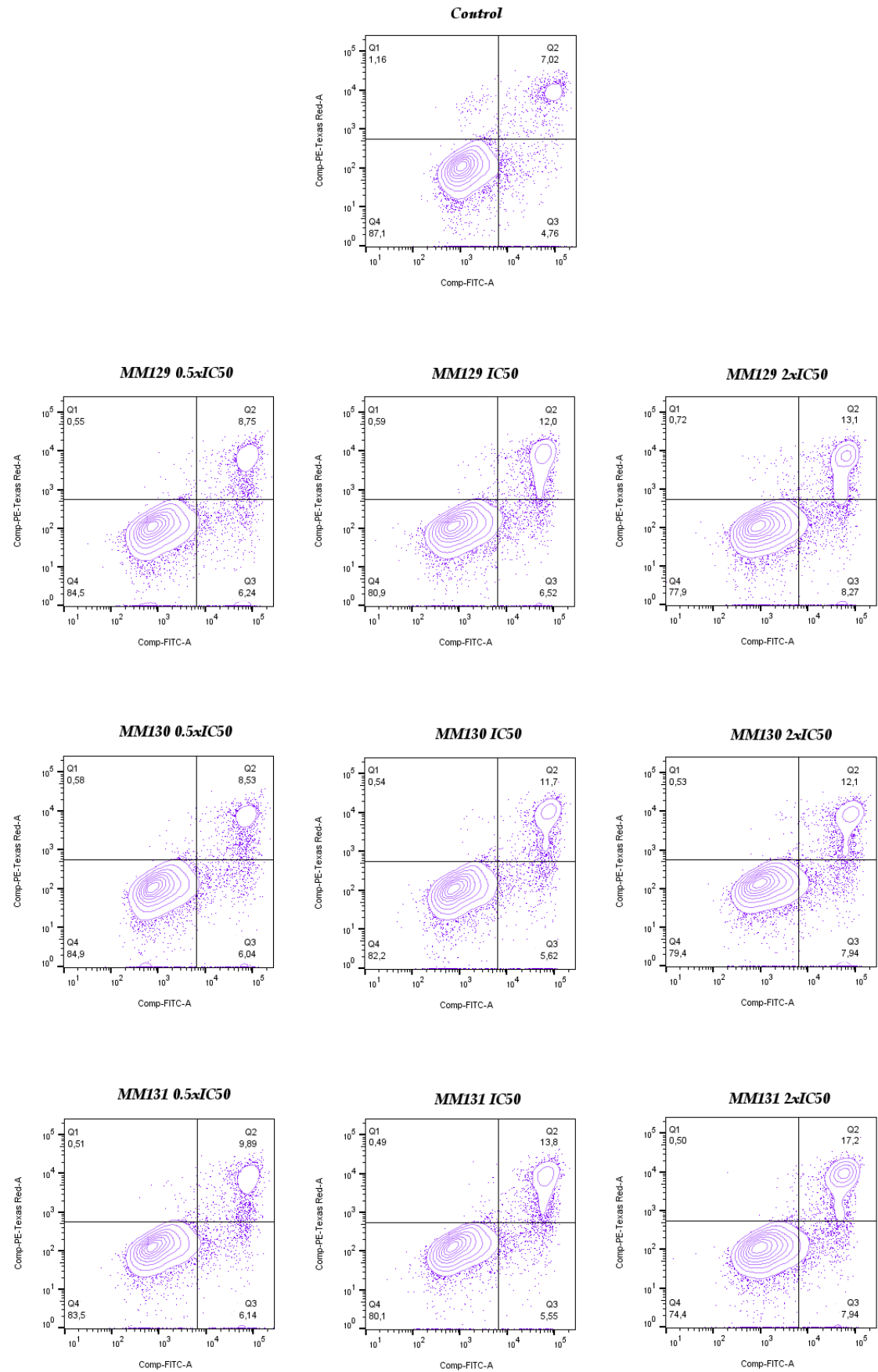


Figure S10. BxPC-3 cells after 24-h of exposure to MM-compounds.

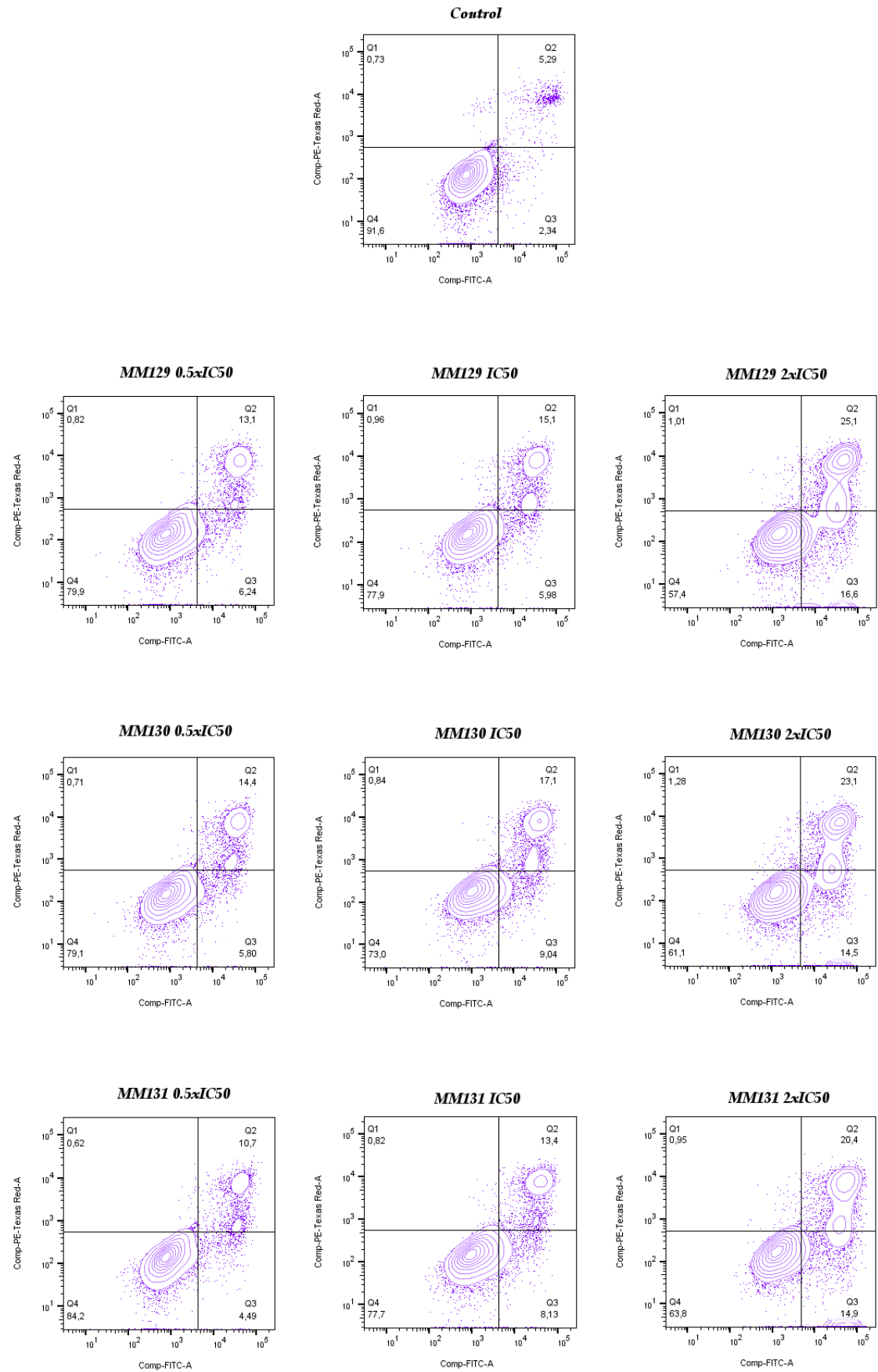


Figure S11. BxPC-3 cells after 48-h of exposure to MM-compounds.

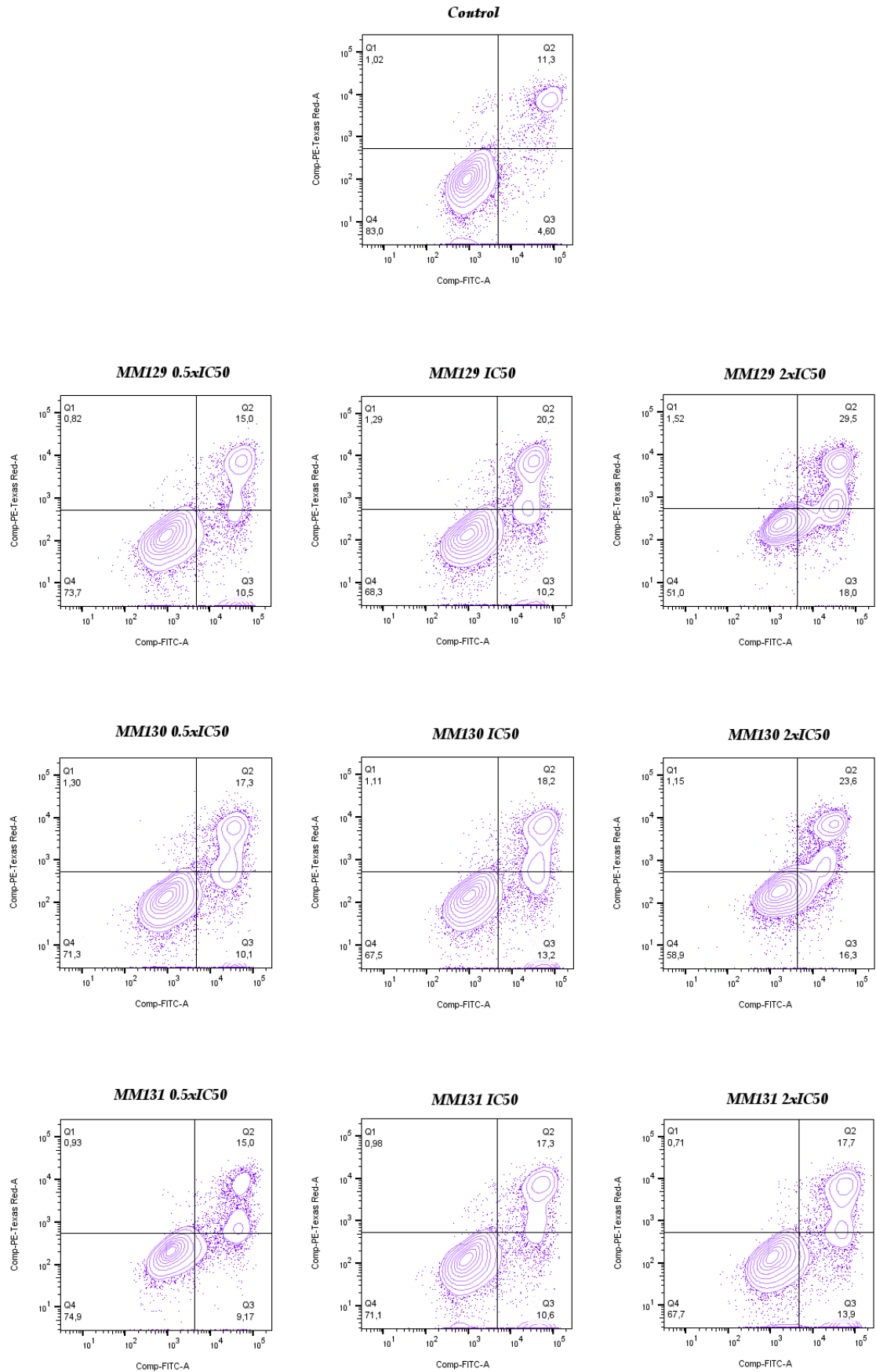


Figure S12. BxPC-3 cells after 72-h of exposure to MM-compounds.

2. Cell cycle analysis

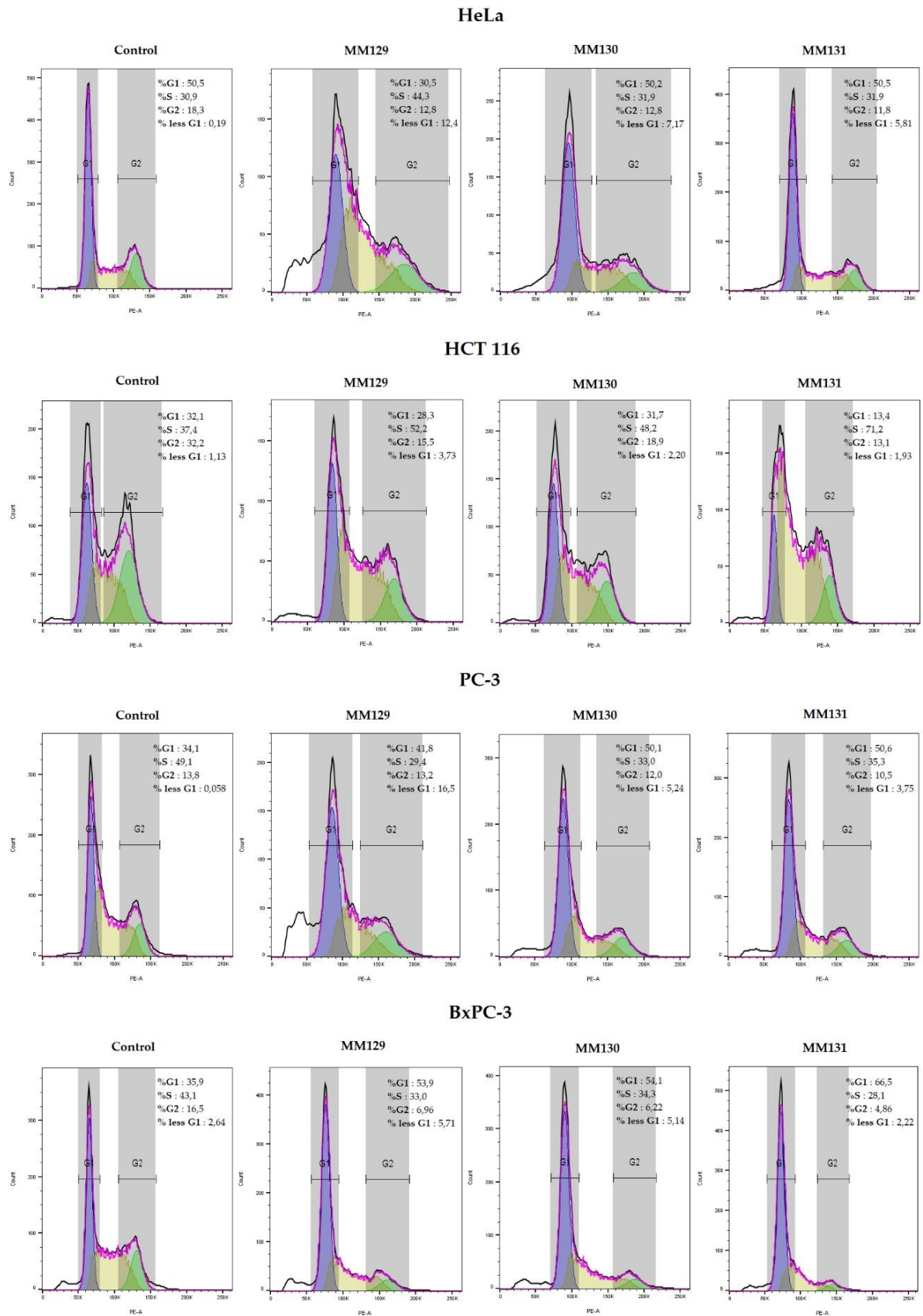


Figure S13. Representative dot-plots presenting the cell cycle distribution of HeLa, HCT 116, PC-3 and BxPC-3 cell lines after their 24-h incubation with **MM129**, **MM130**, and **MM131** in the concentration of IC₅₀.

Table S2. Comparison of anticancer activity of **MM129**, **MM130**, and **MM131** in the concentration of IC₅₀ toward HeLa, HCT 116, PC-3, and BxPC-3 cancer cells following 24-h and/or 48-h treatment. Significant differences ($p < 0.05$) compared with control (bold and *). MM compound that exhibited the highest activity (significant differences $p < 0.05$) among investigated sulfonamides in the given assay is blue color-coded.

Method	Exposure time	Cell line	Control	MM129	MM130	MM131
Annexin V binding ¹	24-h	HeLa	6.7 ± 0.7	17.4 ± 1.7 *	17.3 ± 2.6 *	15.5 ± 1.4 *
		HCT 116	6.9 ± 2.6	14.4 ± 2.7 *	16.8 ± 6.3 *	14.3 ± 3.3 *
		PC-3	9 ± 2.8	9 ± 3.4	5.9 ± 2.2	6.2 ± 2.7
		BxPC-3	13.8 ± 1.8	17.1 ± 1.2	18.1 ± 3.2	18.5 ± 1.3
	48-h	HeLa	7.1 ± 1.8	40 ± 0.82 *	20.6 ± 1.7 *	15.9 ± 0.66 *
		HCT 116	10.4 ± 2.3	61 ± 1.1 *	57.3 ± 3 *	50.2 ± 1 *
		PC-3	6.7 ± 1.6	23.3 ± 9 *	14.3 ± 0.8	14.4 ± 1
		BxPC-3	12 ± 3.4	19.4 ± 3.3 *	24.8 ± 4.4 *	21.2 ± 1.9 *
MitoTracker Red CMXRos ²	24-h	HeLa		85.5 ± 7.6	87.6 ± 2.2	87.5 ± 0.9
		HCT 116		83.6 ± 2.8 *	88.6 ± 3.5	85.4 ± 1.8
		PC-3		91.7 ± 8.5	91.2 ± 6.7	92.1 ± 1.4
		BxPC-3		90.5 ± 3.1	87.3 ± 3.7	89.8 ± 7
	48-h	HeLa		86.5 ± 5.3	88 ± 1.3	86.9 ± 6.9
		HCT 116		80.2 ± 7.9 *	82.8 ± 5.2 *	85.3 ± 2.3
		PC-3		80.2 ± 10.8	86.6 ± 2.1	83.2 ± 3.5
		BxPC-3		84.5 ± 8.1	87.4 ± 1.6	85.4 ± 8.7
AO/EB dual staining ¹	48-h	HeLa	1.3 ± 0.6	32 ± 6.1 *	23.3 ± 2.5 *	13.3 ± 1.5 *
		HCT 116	2.3 ± 2.1	23.3 ± 5.5 *	14.3 ± 4.5 *	21 ± 7.2 *
		PC-3	6 ± 2	8.7 ± 1.5	11.7 ± 1.5	7 ± 3
		BxPC-3	3 ± 2	16.3 ± 3.5 *	13 ± 2.6 *	14.7 ± 2.3 *
Cell cycle ³	24-h	HeLa		↑ subG1; ↓ G0/G1	—	—
		HCT 116		↑ S; ↓ G2/M	↑ S; ↓ G2/M	↑ S; ↓ G2/M; ↓ G0/G1
		PC-3		↑ subG1; ↓ S;	↑ subG1; ↓ S; ↑ G0/G1	↑ subG1; ↓ S; ↑ G0/G1
		BxPC-3		↑ G0/G1; ↓ G2/M	↑ G0/G1; ↓ G2/M	↑ G0/G1; ↓ G2/M

¹ Percentage of apoptotic cells shown as the mean ± SD.

² Changes in $\Delta\Psi_m$ presented as a mean ± SD relative to the control group normalized to 100%.

³ A significant ($p < 0.05$) increase (↑) or decrease (↓) in the percentage of cells in a particular cell cycle phase.

References:

1. Bukowski, K.; Marciniak, B.; Kciuk, M.; Mojzych, M.; Kontek, R. Pyrazolo[4,3-e]Tetrazolo[1,5-b][1,2,4]Triazine Sulfonamides as Novel Potential Anticancer Agents: Cytotoxic and Genotoxic Activities In Vitro. *Molecules* **2022**, *27*, 3761, doi:10.3390/molecules27123761.