X-ray Fluorescence Microscopy and X-ray Absorption Spectroscopy Reveal the Stability of the Plecstatin-1 Scaffold in Biological Model Systems: Comparison of Ru, Os and Ir Analogues

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Supplementary Information

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1. OPTICAL MICROSCOPY

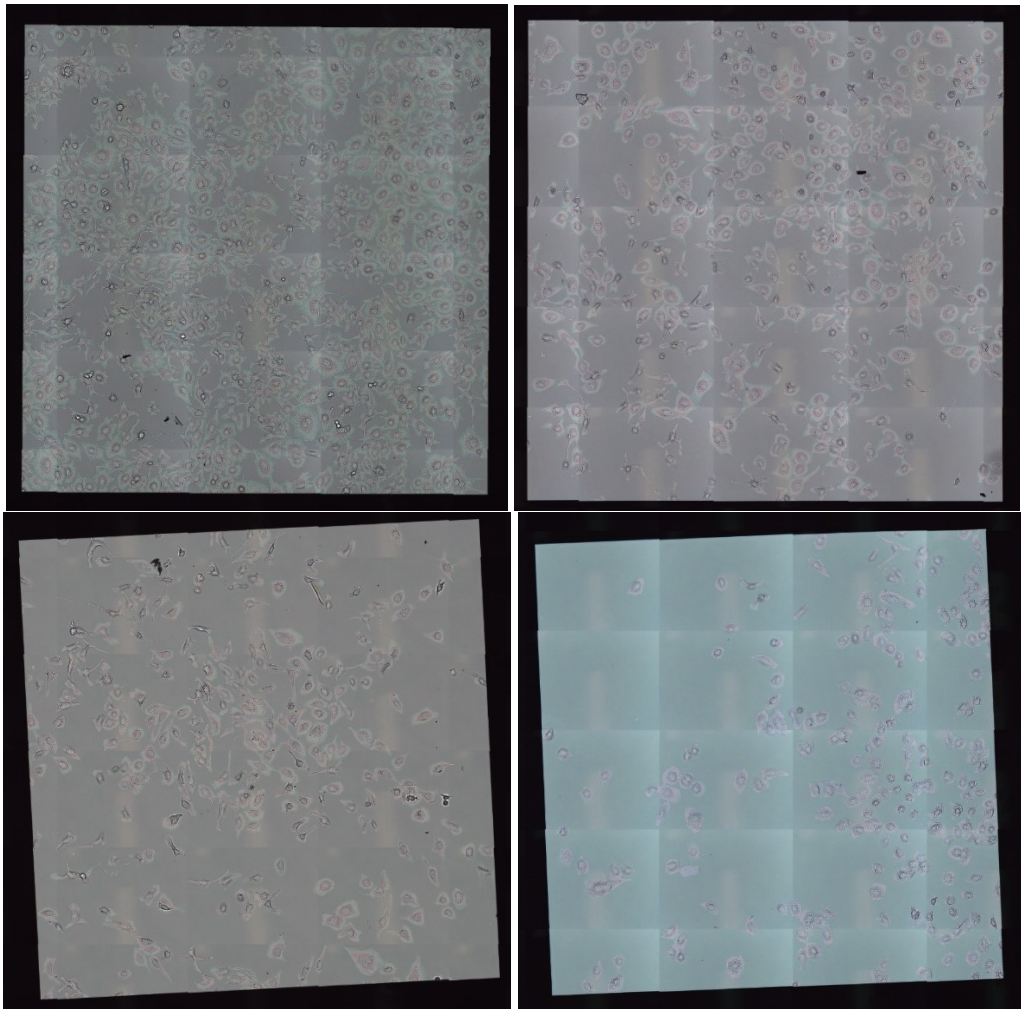
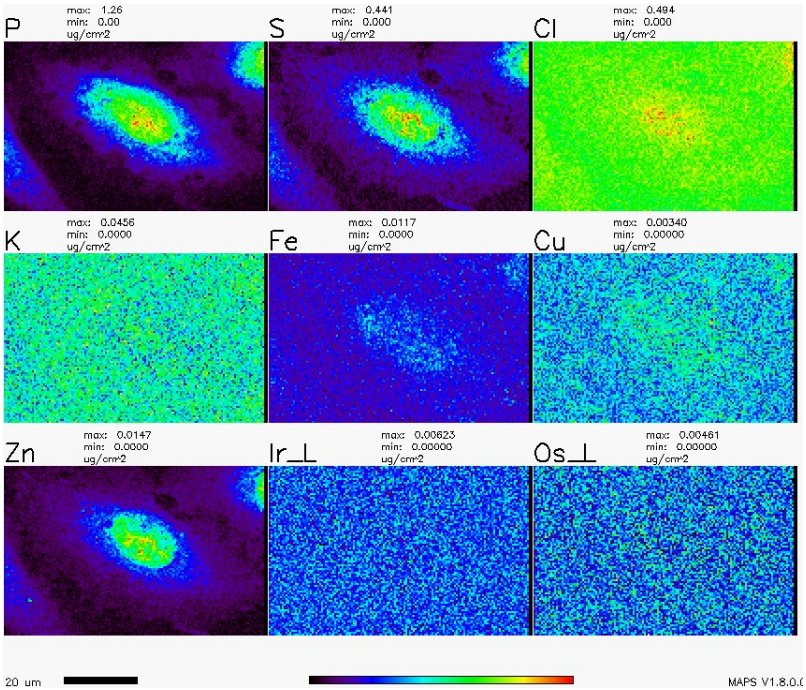


Figure S1. Optical micrograph of untreated SKOV-3 control cells (top left) and SKOV-3 cells treated with one of plecstatin-1 (20 μM; top right), plecstatin-Os (20 μM; bottom left) or plecstatin-Ir (30 μM; bottom right). Imaged at the Advanced Photon Source under a visual light microscope. Cells were grown on silicon nitride membranes (Silson).

2. X-RAY FLUORESCENCE MICROSCOPY



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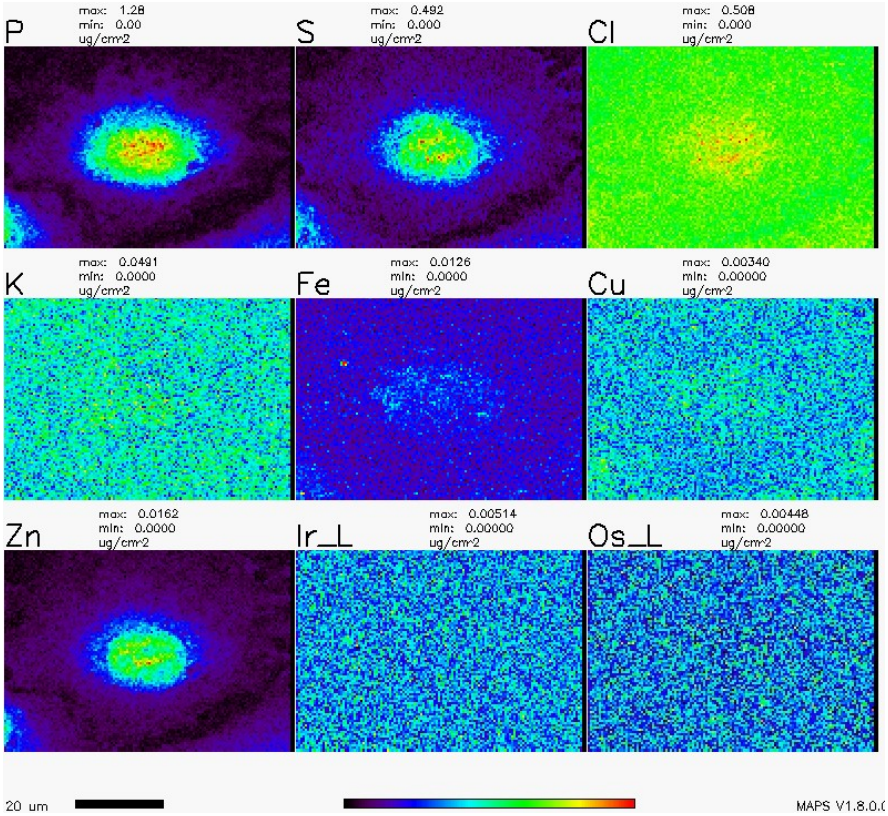
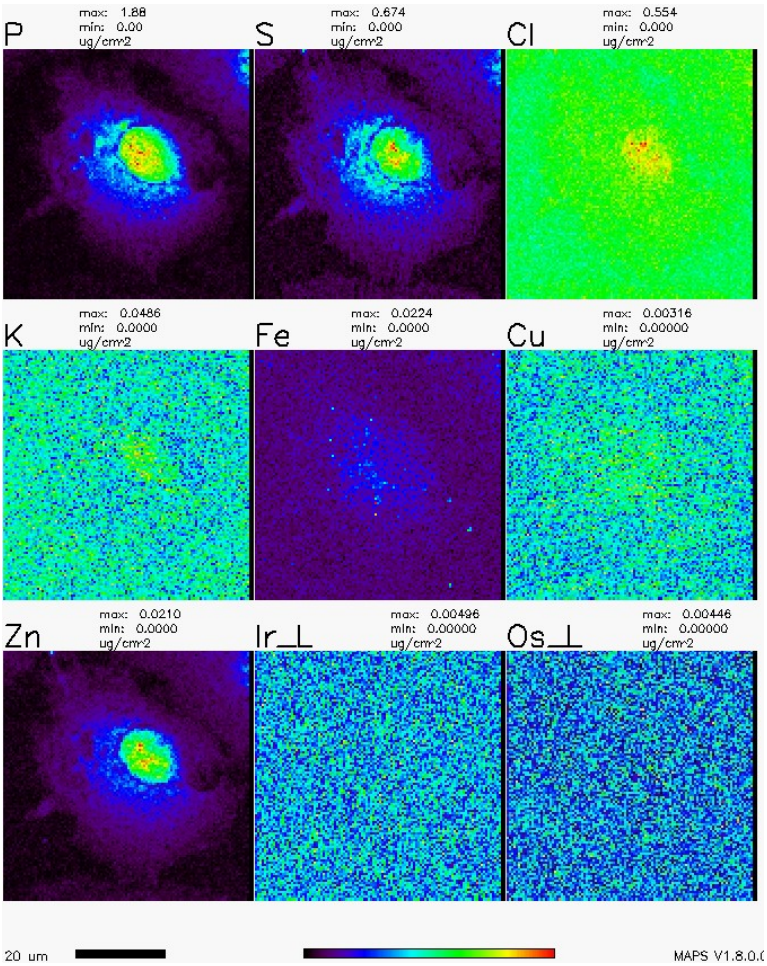


Figure S2. Elemental maps of SKOV-3 ovarian cancer control cells (4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 200 ms.



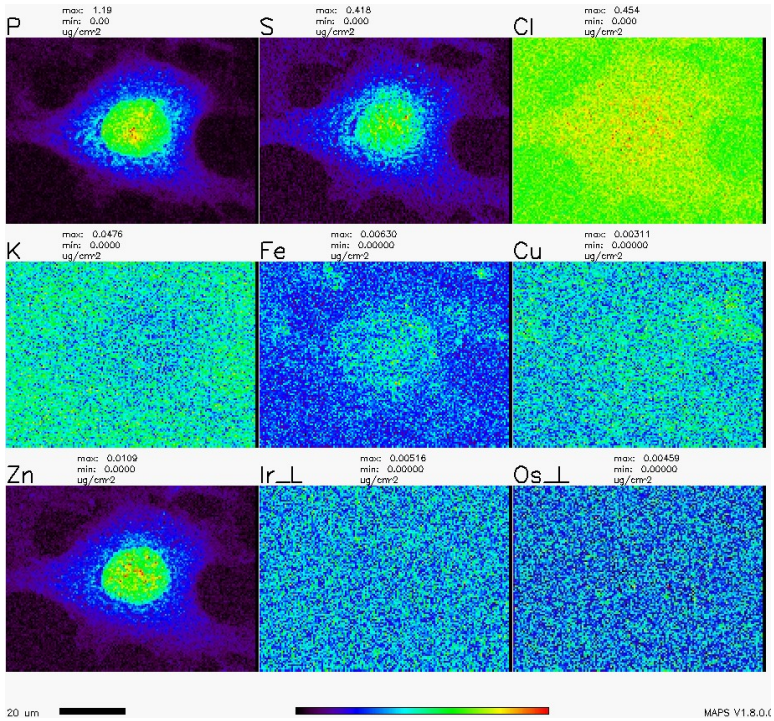


Figure S2 (cont). Elemental maps of SKOV-3 ovarian cancer control cells (4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 200 ms.

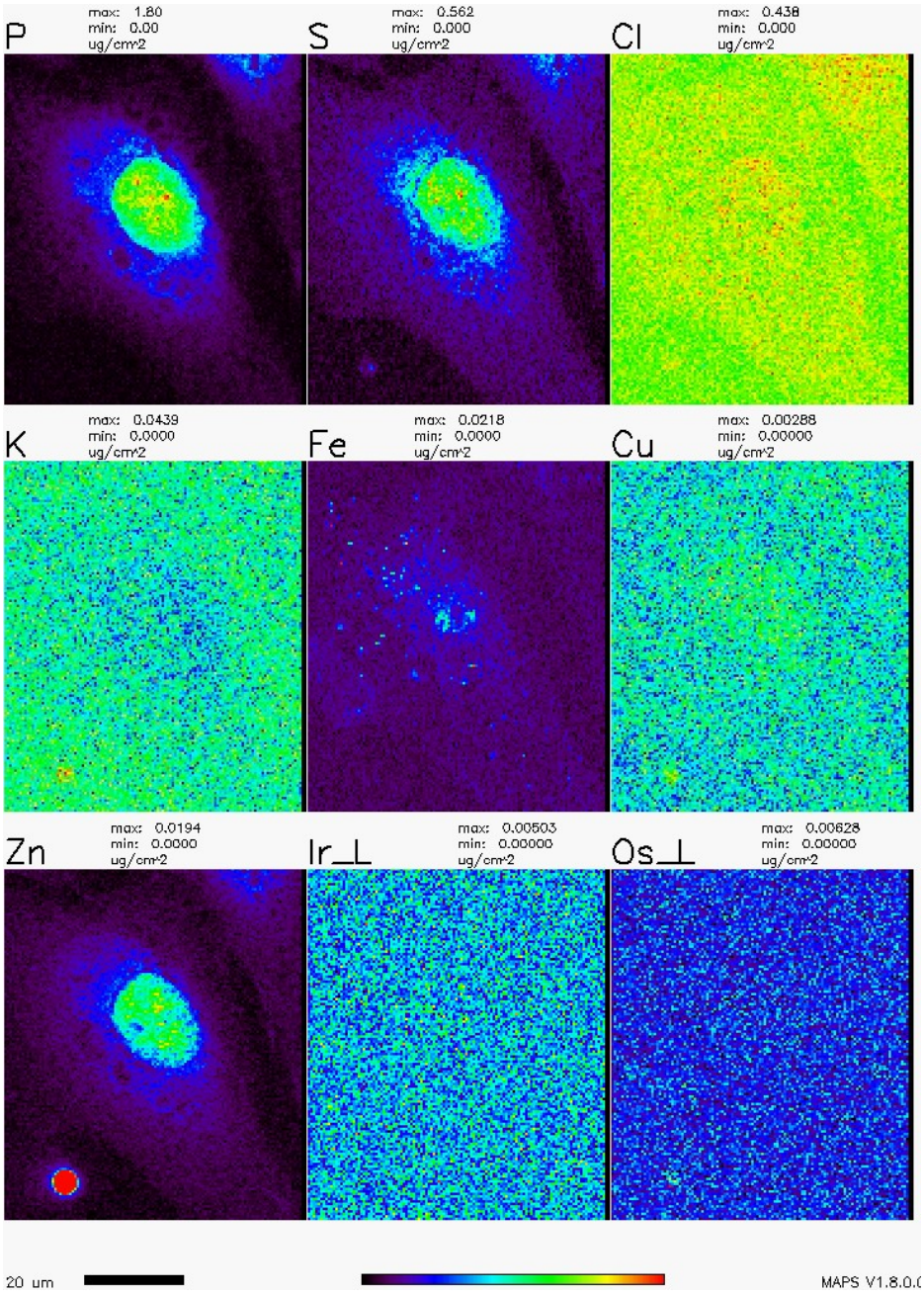


Figure S2 (cont). Elemental maps of SKOV-3 ovarian cancer control cells (4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 200 ms.

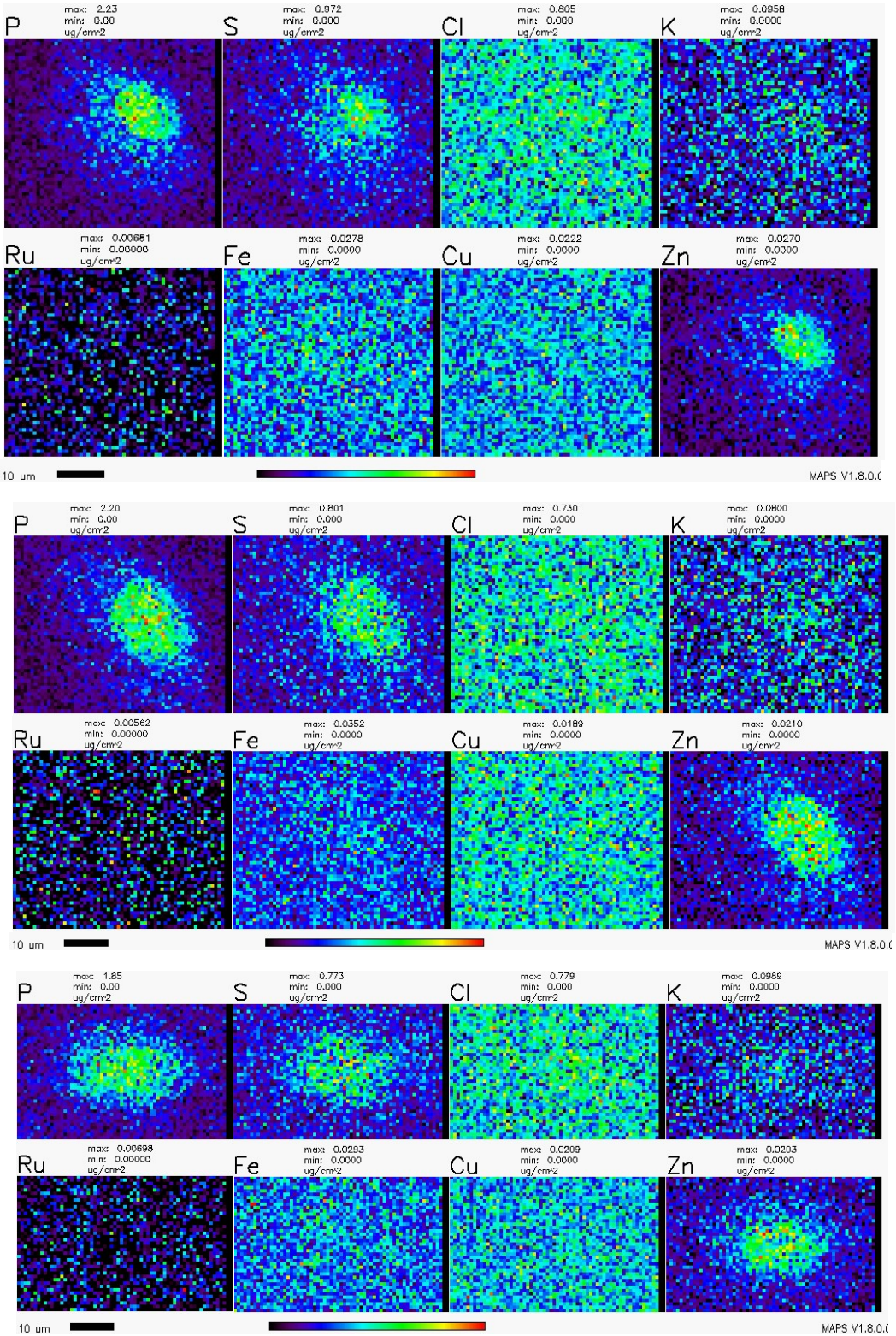


Figure S3. Elemental maps of SKOV-3 ovarian cancer control cells (4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 22.7 keV, the beam spot size was 0.75 µm x 0.75 µm and the dwell time was 500 ms.

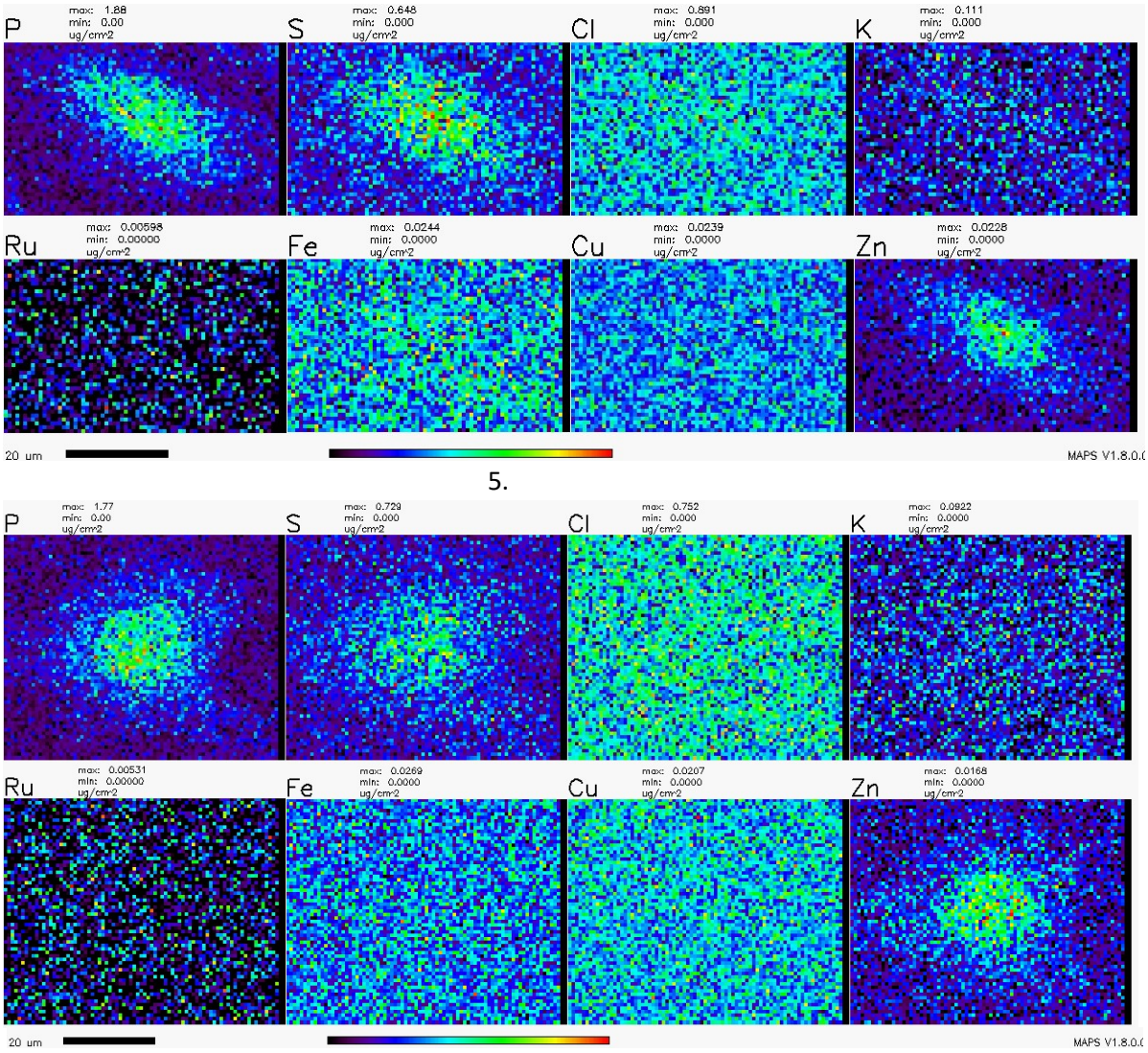


Figure S3 (cont). Elemental maps of SKOV-3 ovarian cancer control cells (4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 22.7 keV, the beam spot size was 0.75 µm x 0.75 µm and the dwell time was 500 ms.

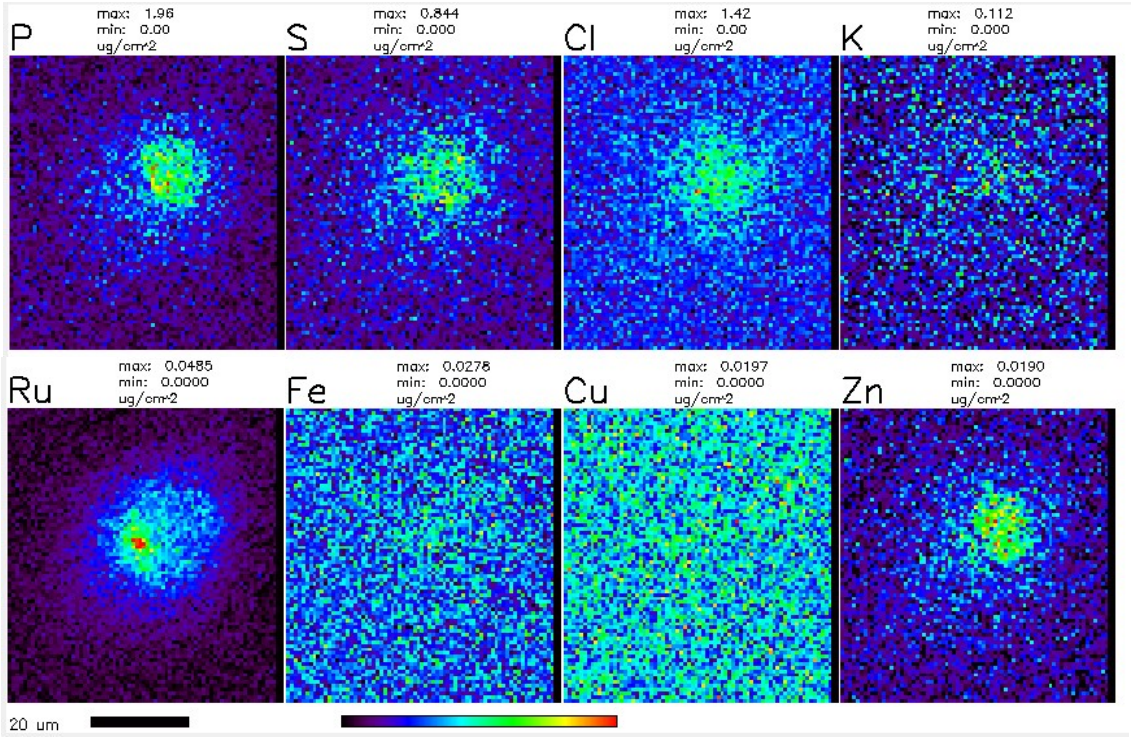
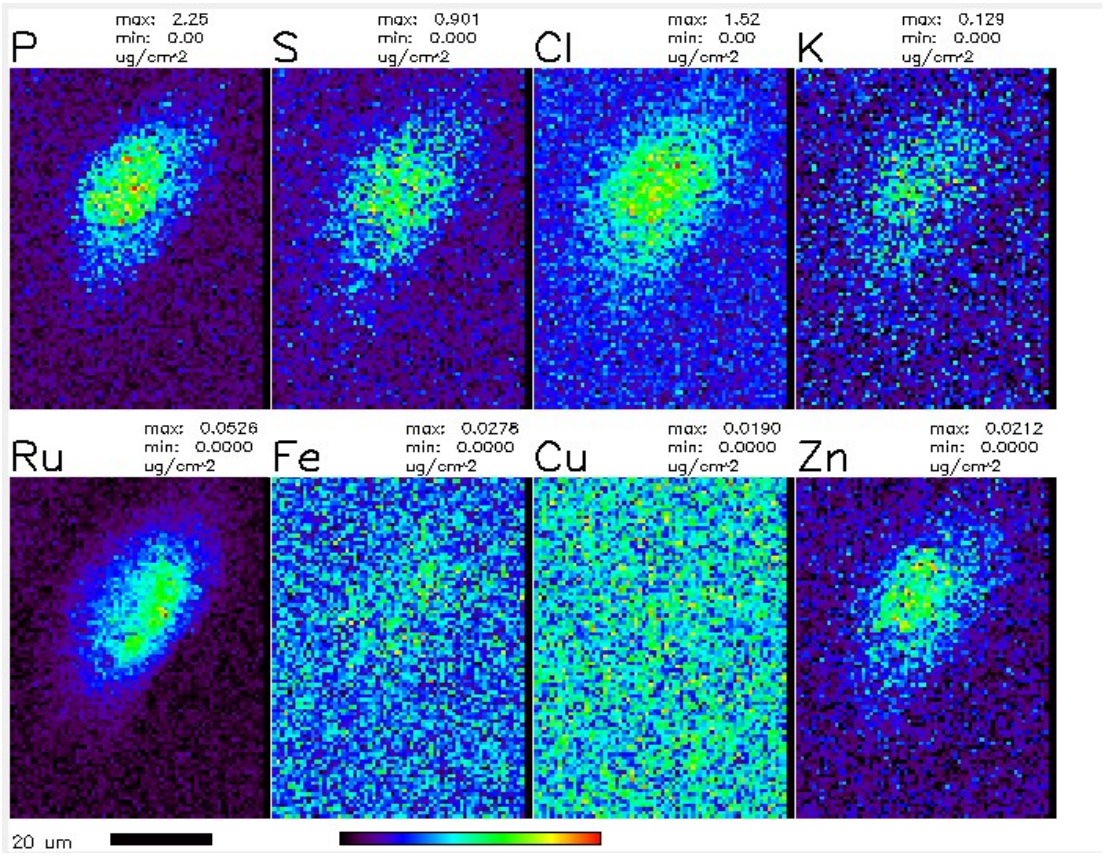
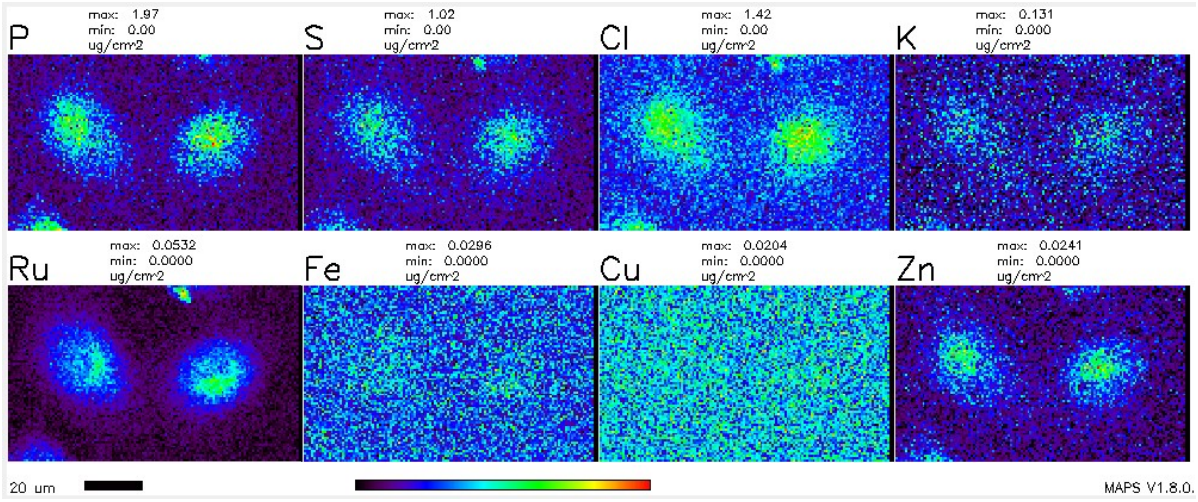

P
max: 1.96
min: 0.00
ug/cm^2
S
max: 0.844
min: 0.000
ug/cm^2
Cl
max: 1.42
min: 0.00
ug/cm^2
K
max: 0.112
min: 0.000
ug/cm^2
Ru
max: 0.0485
min: 0.0000
ug/cm^2
Fe
max: 0.0278
min: 0.0000
ug/cm^2
Cu
max: 0.0197
min: 0.0000
ug/cm^2
Zn
max: 0.0190
min: 0.0000
ug/cm^2
20 um

Figure S4. Elemental maps of SKOV-3 ovarian cancer cells treated with plecstatin-1 (20 µM; 4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 22.7 keV, the beam spot size was 0.75 µm x 0.75 µm and the dwell time was 500 ms.





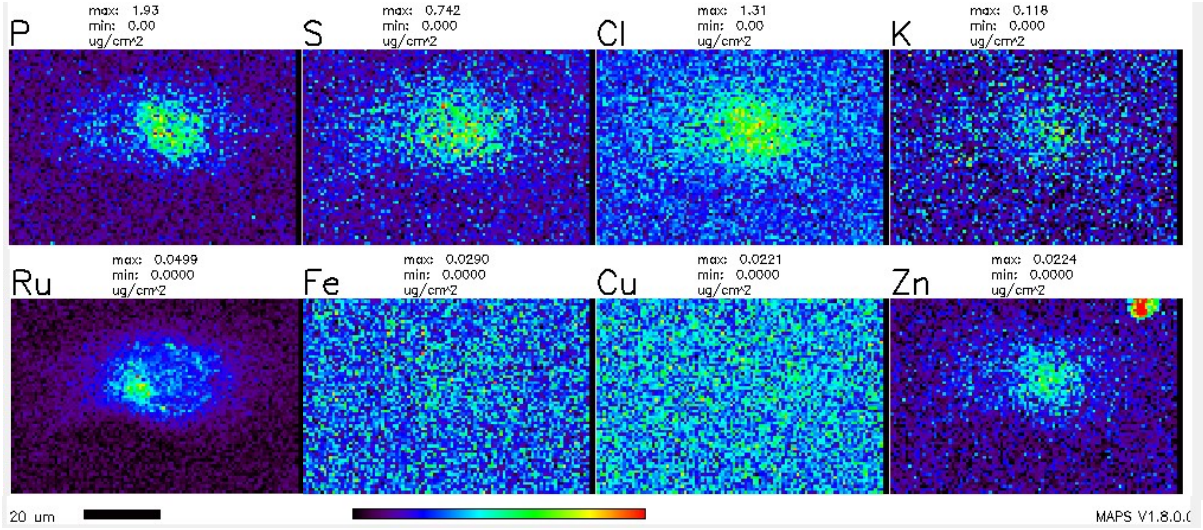
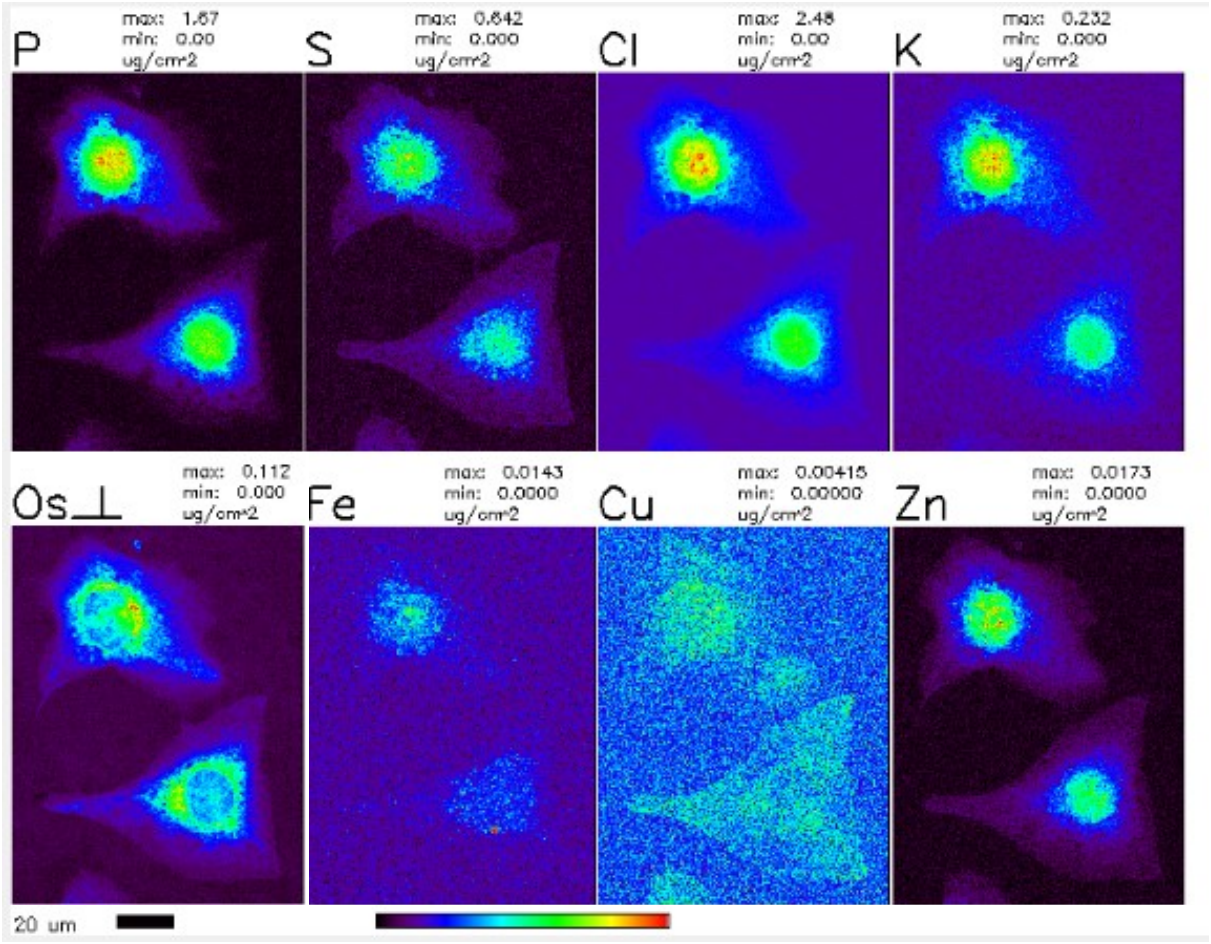


Figure S4 (cont). Elemental maps of SKOV-3 ovarian cancer cells treated with plecstatin-1 (20 µM; 4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 22.7 keV, the beam spot size was 0.75 µm x 0.75 µm and the dwell time was 500 ms.



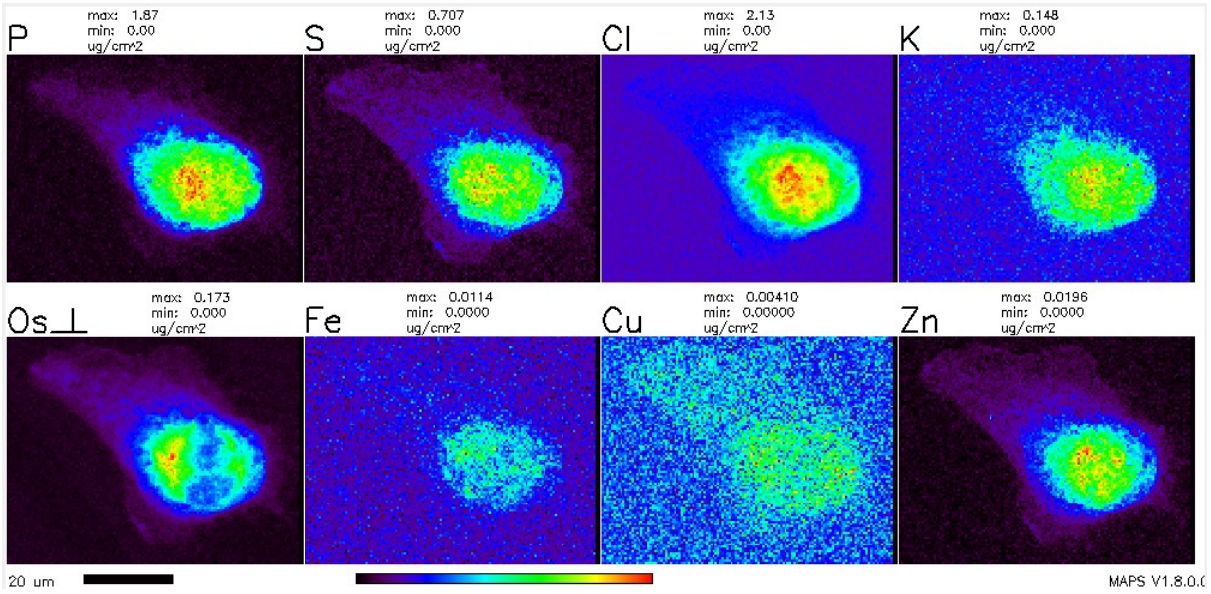
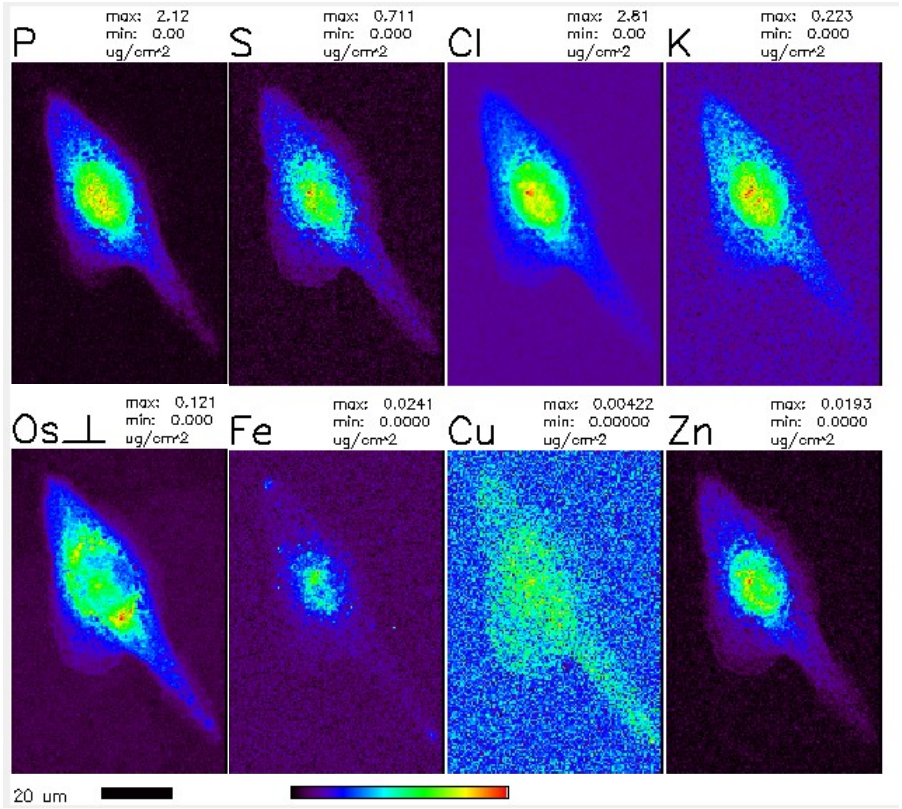


Figure S5. Elemental maps of SKOV-3 ovarian cancer cells treated with plecstatin-Os (20 µM; 4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 150 ms.



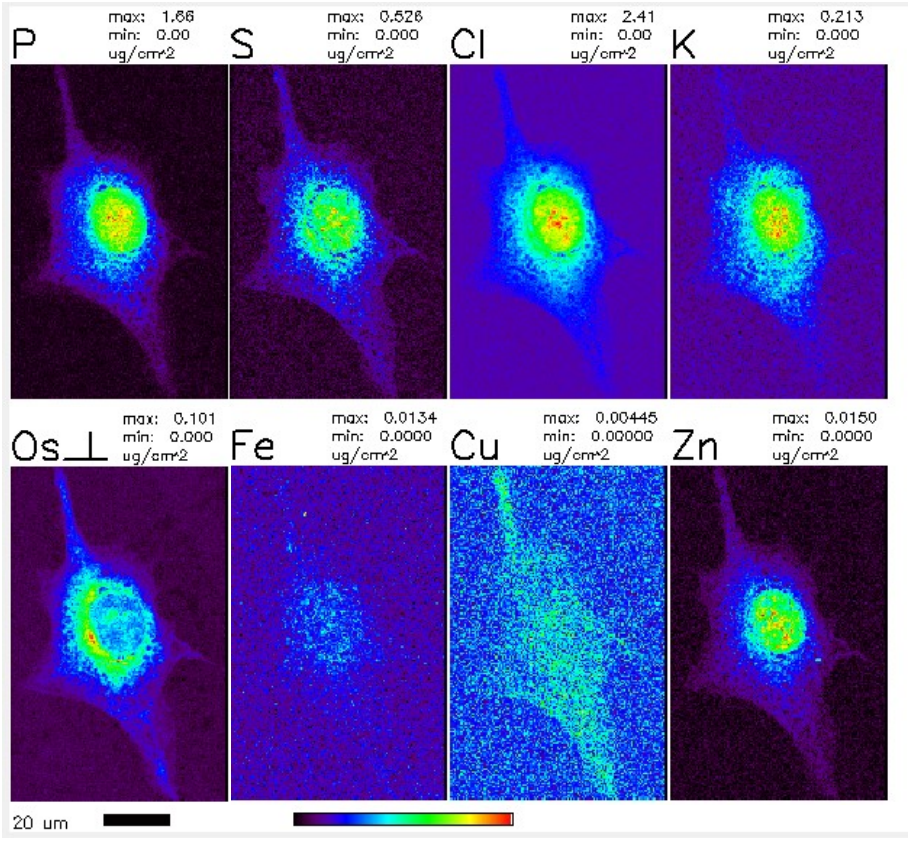
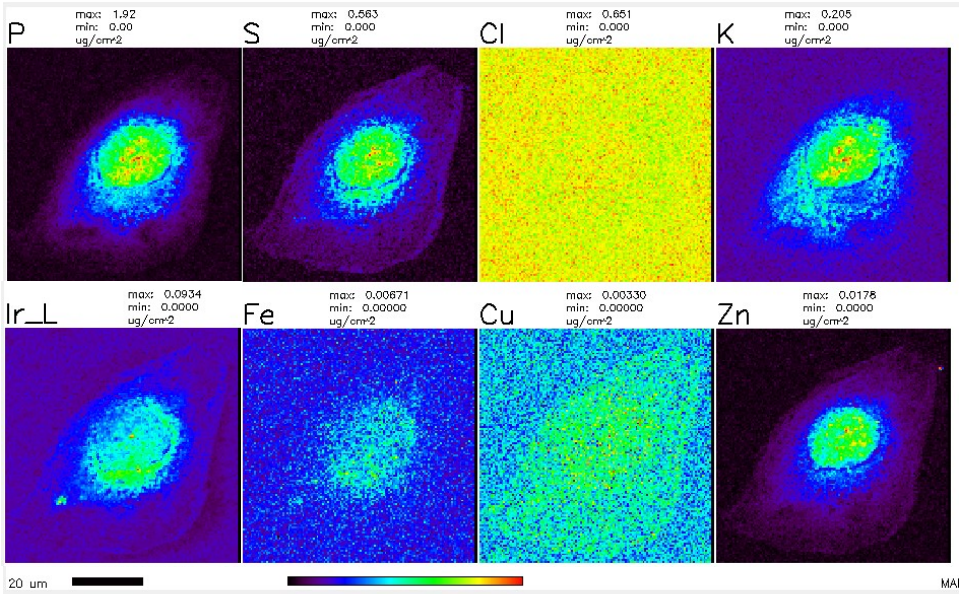
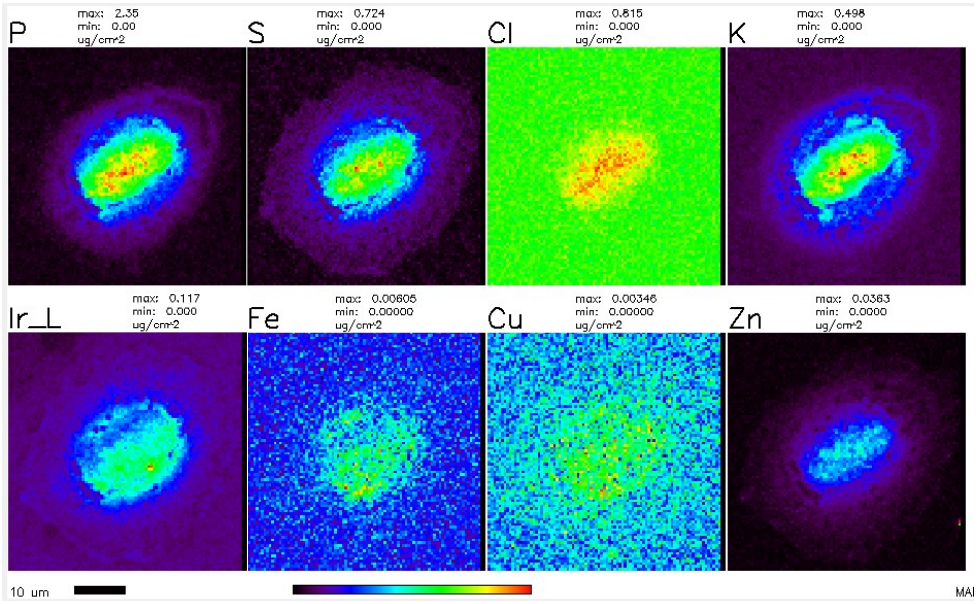


Figure S5 (cont). Elemental maps of SKOV-3 ovarian cancer cells treated with plecstatin-Os (20 µM; 4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 150 ms.





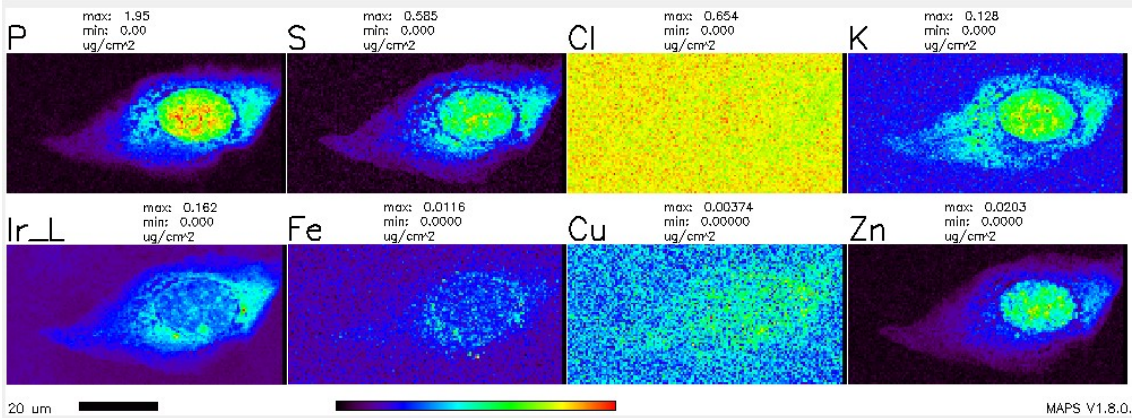
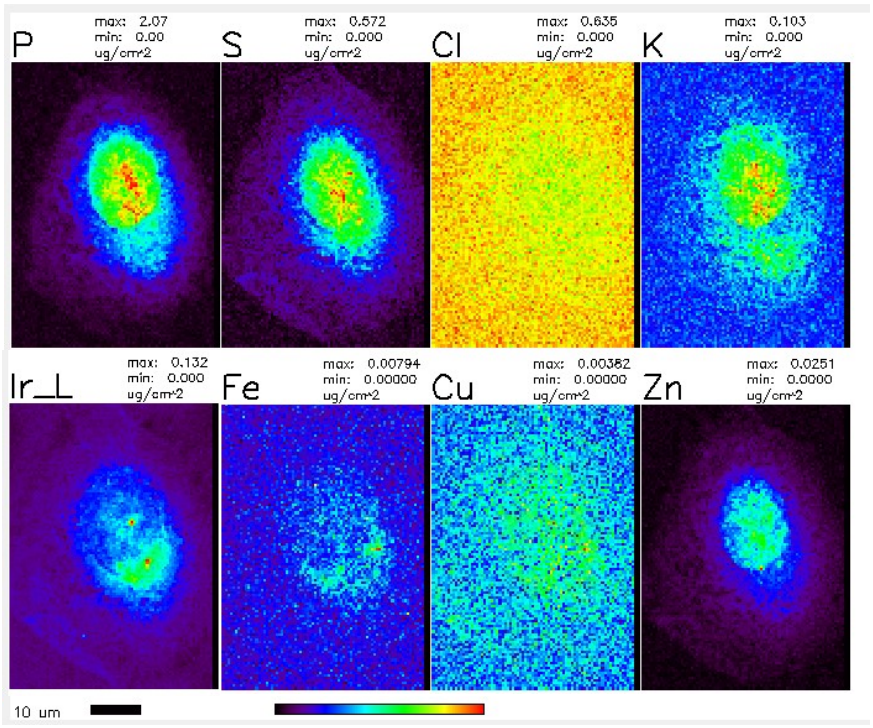


Figure S6. Elemental maps of SKOV-3 ovarian cancer cells treated with plecstatin-Ir (30 µM; 4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 250 ms.



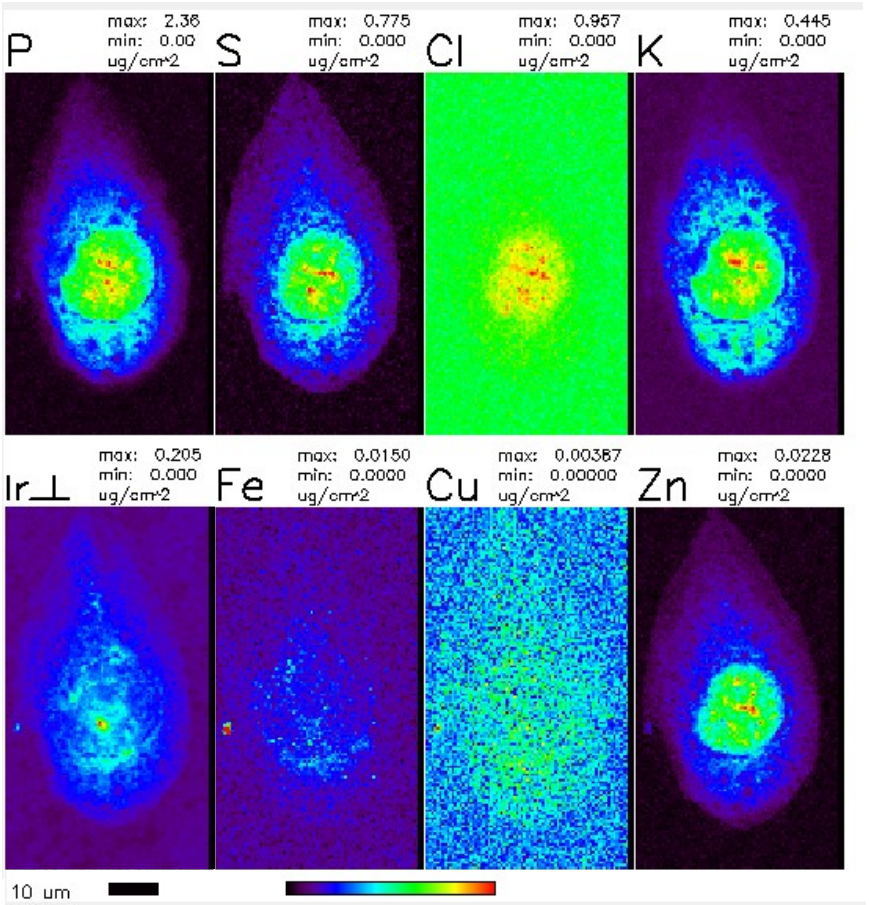


Figure S6 (cont). Elemental maps of SKOV-3 ovarian cancer cells treated with plecstatin-Ir (30 µM; 4 h, 37°C, 5% CO_2). Elemental maps were recorded at the 2-ID-D beamline at the Advanced Photon Source (Argonne National Laboratory, USA). The incident beam energy was 13.1 keV, the beam spot size was 0.50 µm x 0.50 µm and the dwell time was 250 ms.

3. ELEMENTAL QUANTITATION

Table S1. Average intracellular mass (fg) of elements in treated SKOV-3 cells and controls (n = 5 for all sample groups). Uncertainty in the mean is expressed as standard error of the mean (SEM). [1] Measurements were recorded using an incident beam energy of 13.1 keV. [2] Measurements were recorded using an incident beam energy of 22.7 keV. [3] Units of cell area are given in μm^2.

	Control[1]	*Plecstatin-Os*[1]	*Plecstatin-Ir*[1]	*Control*[2]	*Plecstatin-1*[2]
Cell areas[3]	2105 ± 205	2207 ± 216	1545 ± 203	1493 ± 101	1864 ± 80
Ru	-	-	-	10.9 ± 0.8	152 ± 4
Os	32 ± 3	171 ± 12	27 ± 3	-	-
Ir	40 ± 4	38 ± 4	489 ± 69	-	-
P	5229 ± 429	5527 ± 357	7179 ± 685	6690 ± 443	7530 ± 298
S	2015 ± 132	1956 ± 124	2150 ± 229	3094 ± 170	3796 ± 115
Cl	7096 ± 611	11504 ± 524	8342 ± 1009	5108 ± 307	8673 ± 310
K	457 ± 40	1138 ± 84	1149 ± 257	332 ± 17	483 ± 19
Ca	118 ± 4	58 ± 4	55 ± 6	300 ± 13	289 ± 10
Fe	51 ± 4	51 ± 5	33 ± 4	143 ± 9	169 ± 7
Mn	16 ± 1	18 ± 2	13 ± 2	63 ± 4	75 ± 3
Cu	29 ± 2	44 ± 4	25 ± 3	123 ± 8	153 ± 6
Zn	51 ± 4	47 ± 3	55 ± 6	73 ± 4	84 ± 3

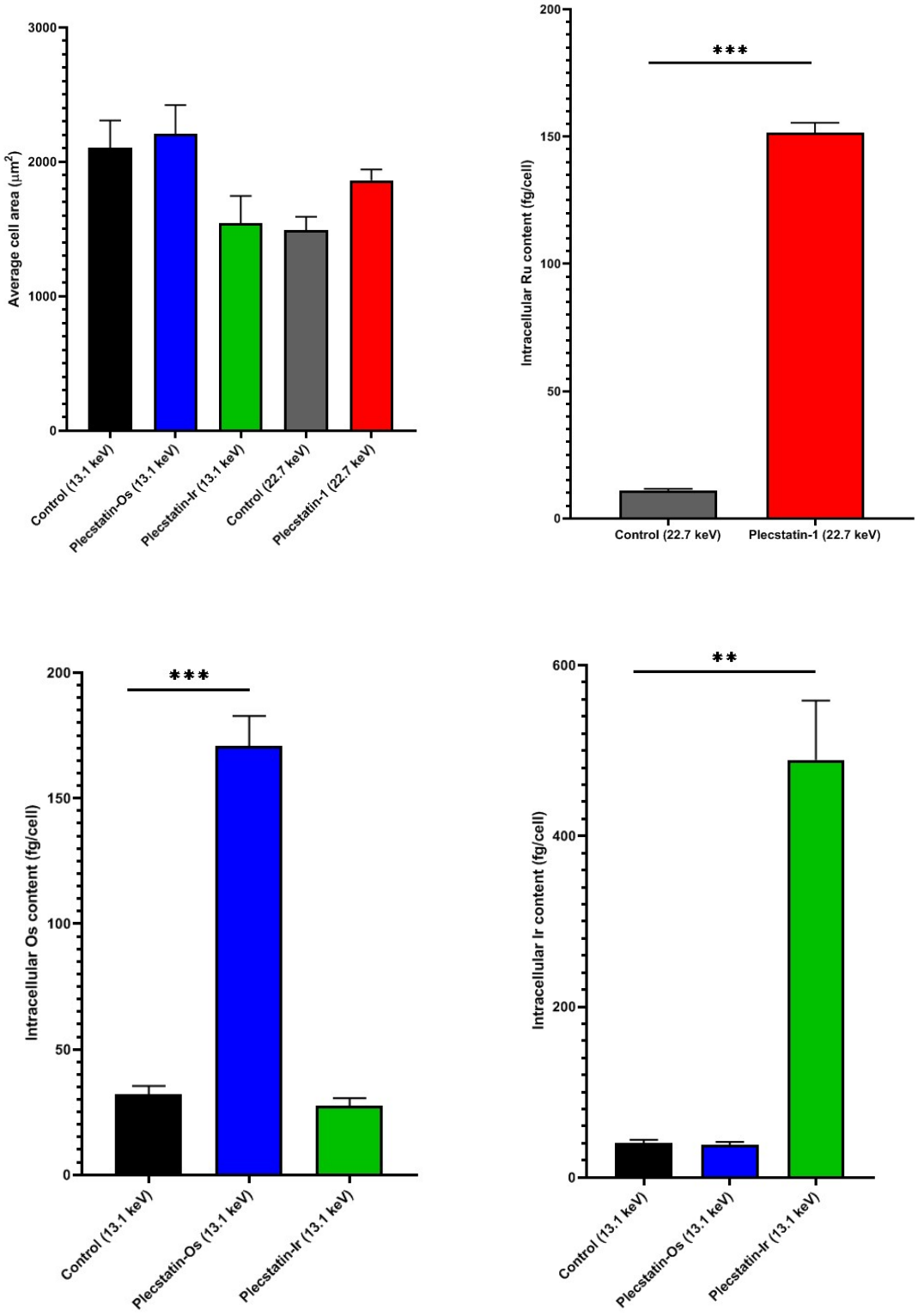


Figure S7. Results of elemental quantitation performed for plecstatin and its derivatives using the ROI Analysis tool in the XFM software analysis program MAPS. Top left; average cell areas calculated from the regions of interest drawn for each cell analysed across all sample groups. Top right; intracellular Ru content calculated of cells treated with plecstatin-1 and untreated control SKOV-3 cells measured with an incident beam energy of 22.7 keV. Bottom left; intracellular Os content of cells treated with plecstatin-Os, plecstatin-Ir or untreated control SKOV-3 cells measured with an incident beam energy of 13.7 keV. Bottom right; intracellular Ir content of cells treated with plecstatin-Ir, plecstatin-Os or untreated control SKOV-3 cells measured with an incident beam energy of 13.7 keV. Sample size n = 5 for all sample groups. Error bars are the standard error in the mean. ** $p < 0.01$, *** $p < 0.001$.

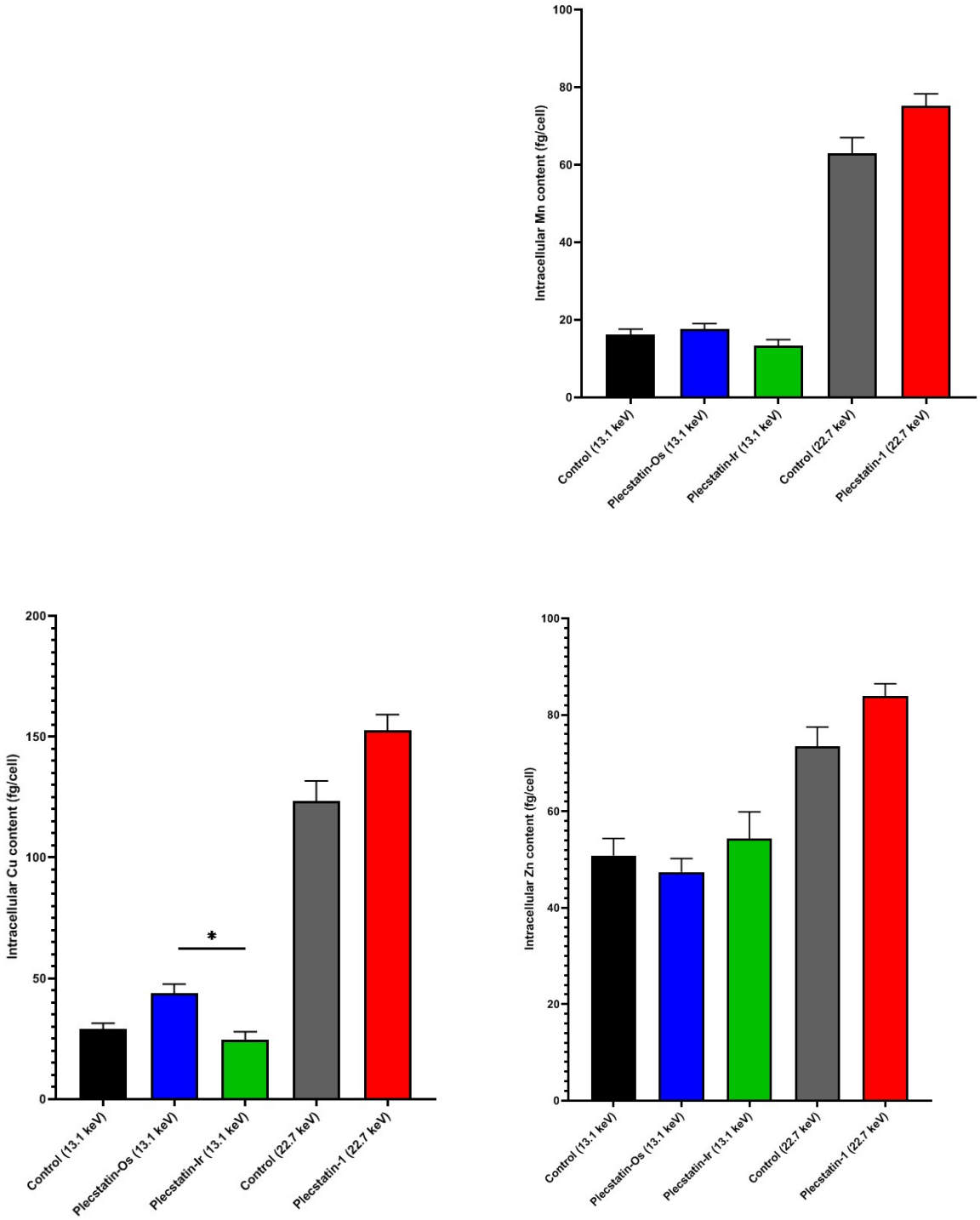
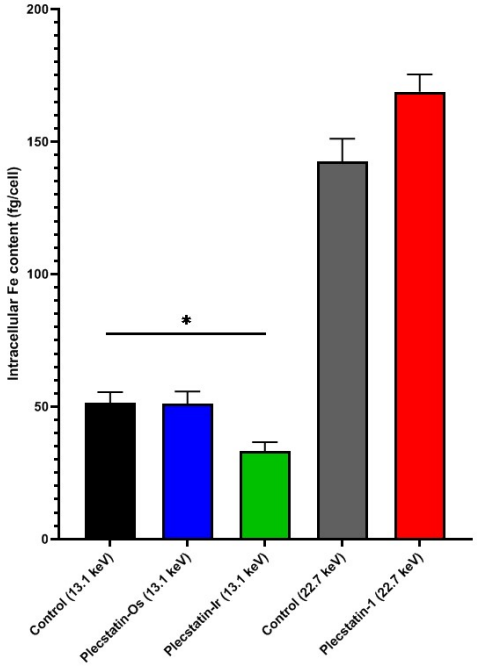


Figure S8. Endogenous metal (Mn, Fe, Cu, Zn) elemental quantitation performed using the ROI Analysis tool in the XFM software analysis program MAPS. Sample size *n* = 5 for all sample groups. Error bars are the standard error in the mean. * $p < 0.05$. NB: *p*-values have not been provided for pairs of samples measured with different beam energies as only measurements taken using the same incident energy are considered valid comparisons.


Intracellular Fe content (fg/cell)
200
150
100
50
0
*
Control (13.1 keV)
Plecstatin-Os (13.1 keV)
Plecstatin-Ir (13.1 keV)
Control (22.7 keV)
Plecstatin-1 (22.7 keV)

4. X-RAY ABSORPTION SPECTROSCOPY

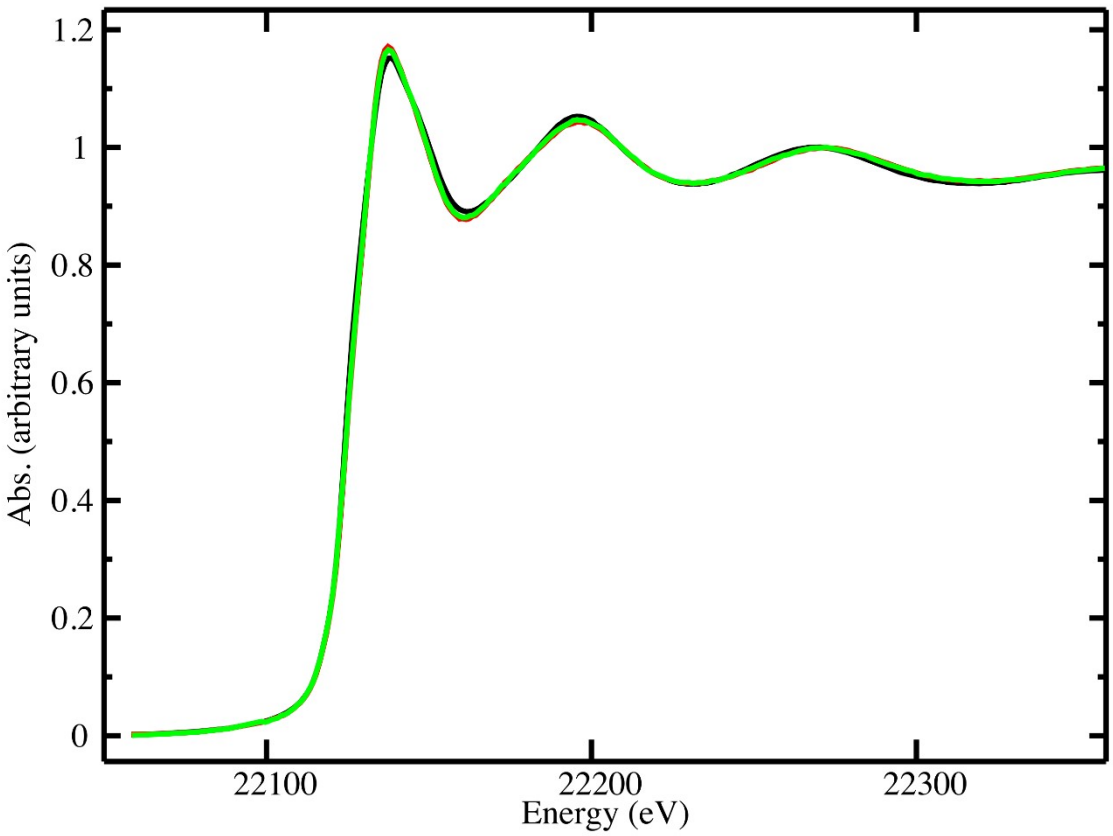


Figure S9. X-ray absorption near edge spectra of plecstatin-1 ground in cellulose (black) and plecstatin-1 incubated in media (1 mM, DMEM/F12, 10% DMSO) in the absence (green) or presence (red) of 10% foetal bovine serum.