

Supplementary information

PEGylated purpurin 18 with improved solubility: Potent compounds for photodynamic therapy of cancer

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1. CHEMICAL ANALYSIS

1.1. NMR spectra

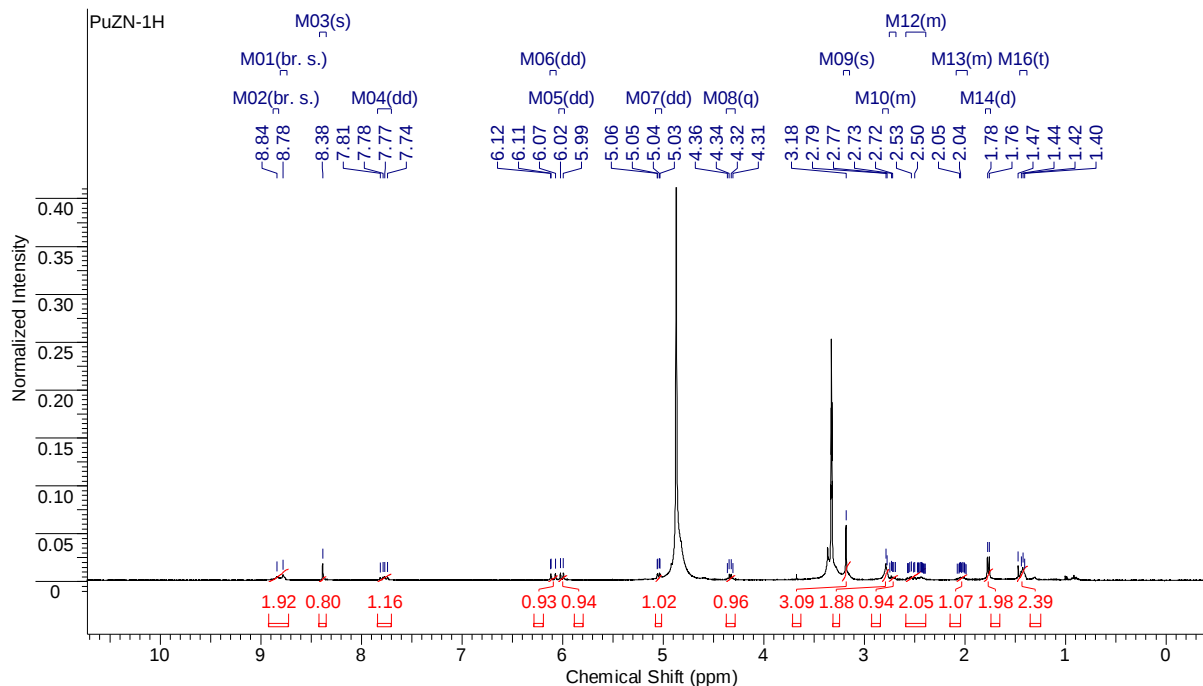


Figure S1: ^1H -NMR spectra of compound 2

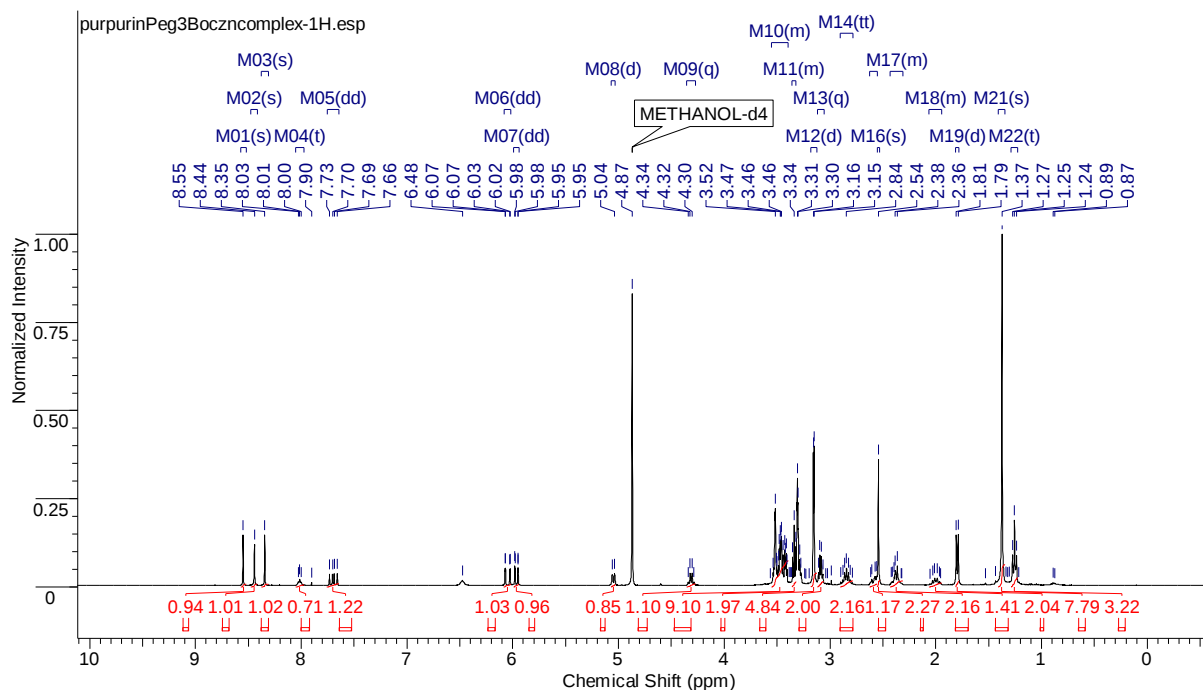


Figure S2: ^1H -NMR spectra of compound 3

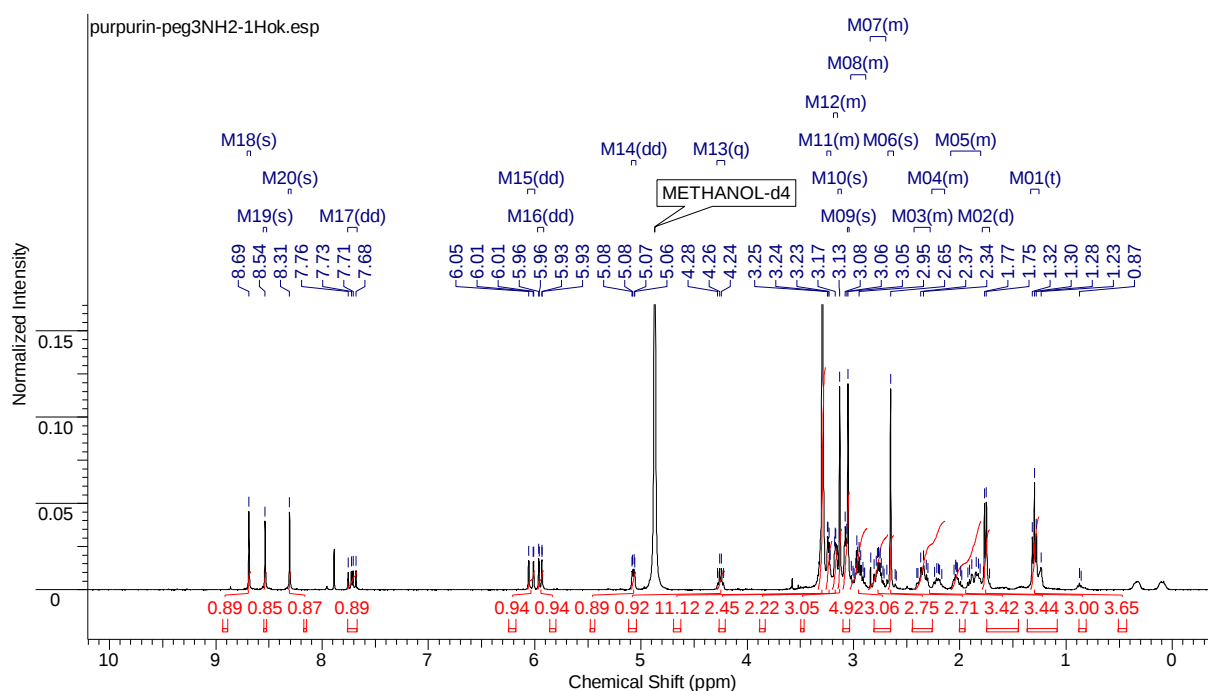


Figure S3-1: ^1H -NMR spectra of compound **4**

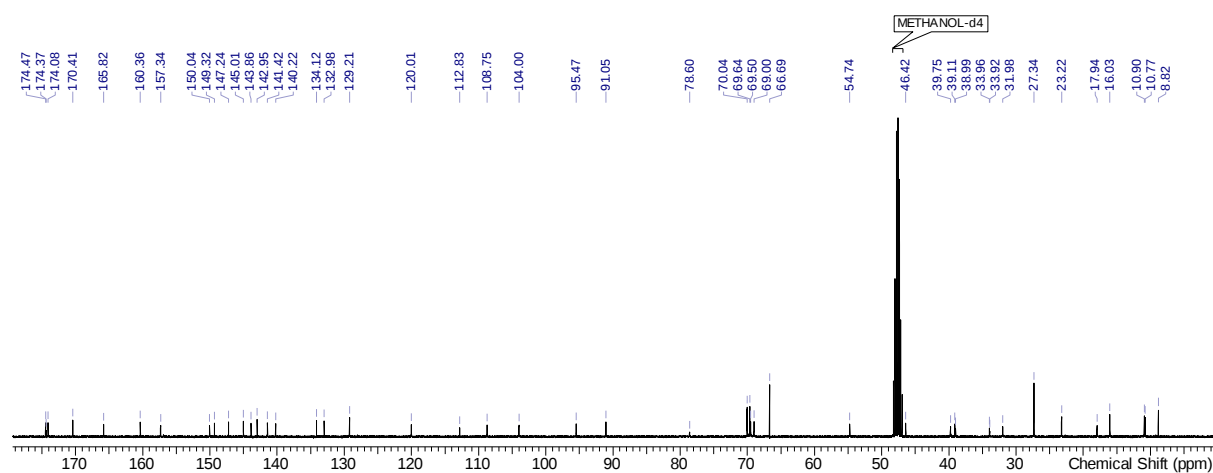


Figure S3-2: ^{13}C -NMR spectra of compound **3**

^{13}C NMR (101 MHz, CD_3OD) δ ppm: 8.82, 10.77, 10.90, 16.03, 17.94, 23.22, 27.34, 31.98, 33.92, 33.96, 38.99, 39.11, 39.75, 46.42, 54.74, 66.69, 69.00, 69.50, 69.64, 69.68, 70.01, 70.04, 78.60, 91.05, 95.47, 104.00, 108.75, 112.83, 120.01, 129.21, 132.98, 134.12, 140.22, 141.42, 142.95, 143.86, 145.01, 147.24, 149.32, 150.04, 157.34, 160.36, 165.82, 170.41, 174.08, 174.37, 174.47.

¹³C-NMR of compound 4

¹³C NMR (101 MHz, CD₃OD) δ ppm: *Major signals were determined from HSQC:* 8.10, 9.76, 9.78, 15.28, 17.21, 22.11, 28.24, 30.91, 30.92, 32.88, 37.94, 46.96, 45.56, 53.74, 57.89, 65.66, 68.85, 69.00, 69.51, 69.64, 69.90, 94.65, 103.44, 108.61, 119.14, 128.15. The carbons of methyls from Boc group at 27.34 ppm disappeared.

1.2.HRMS spectra

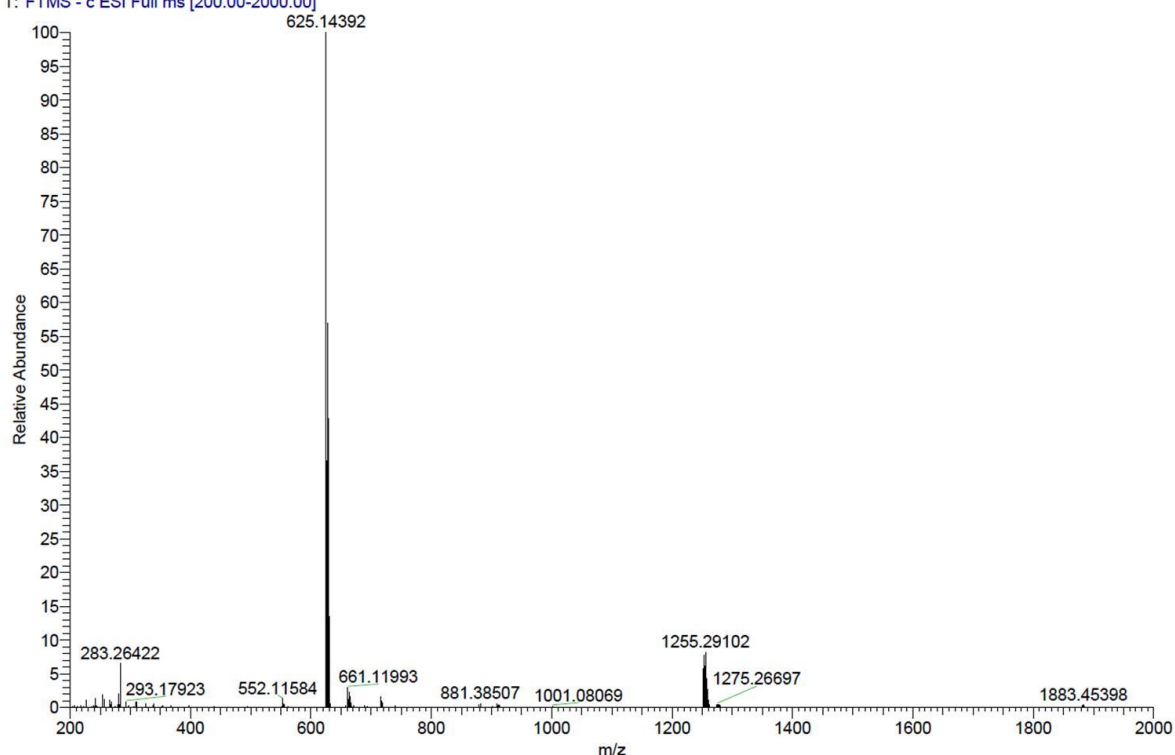
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8/8/2016 9:24:08 AM

MeOH

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T: FTMS - c ESI Full ms [200.00-2000.00]



265_ESIneg_MJ-TB-78_1

8/8/2016 9:24:08 AM

MeOH

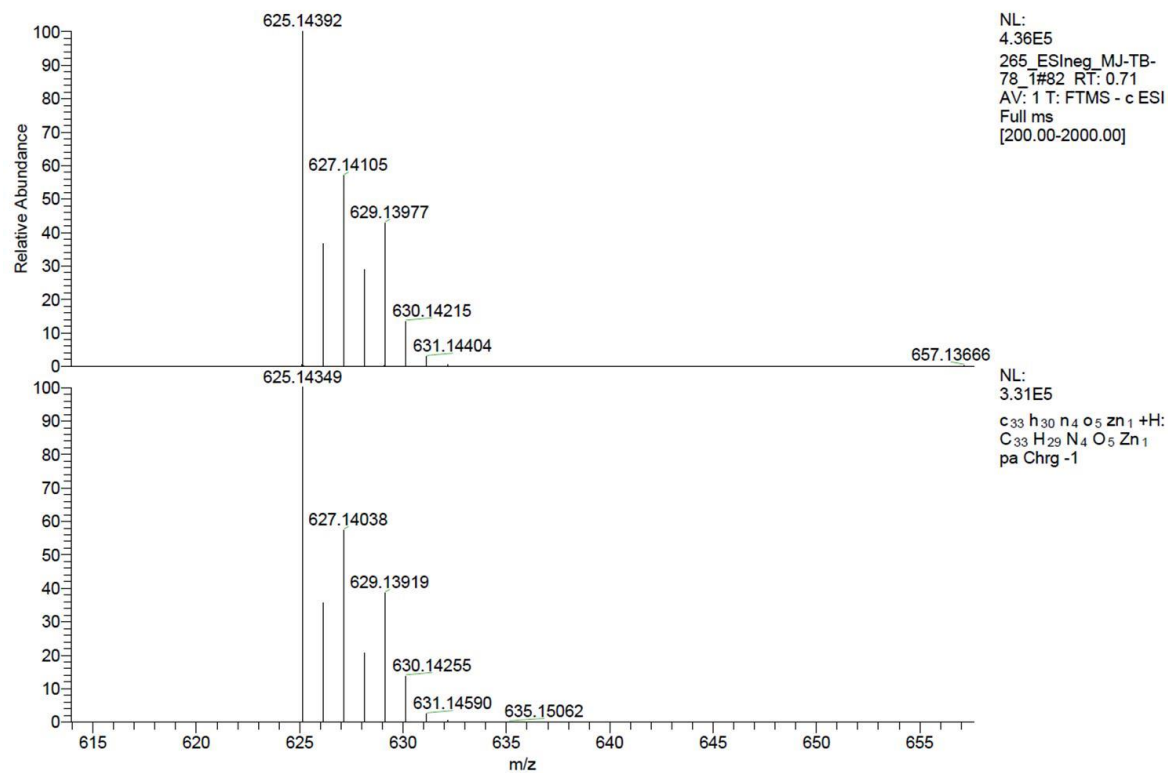


Figure S4: HRMS spectra of compound 2

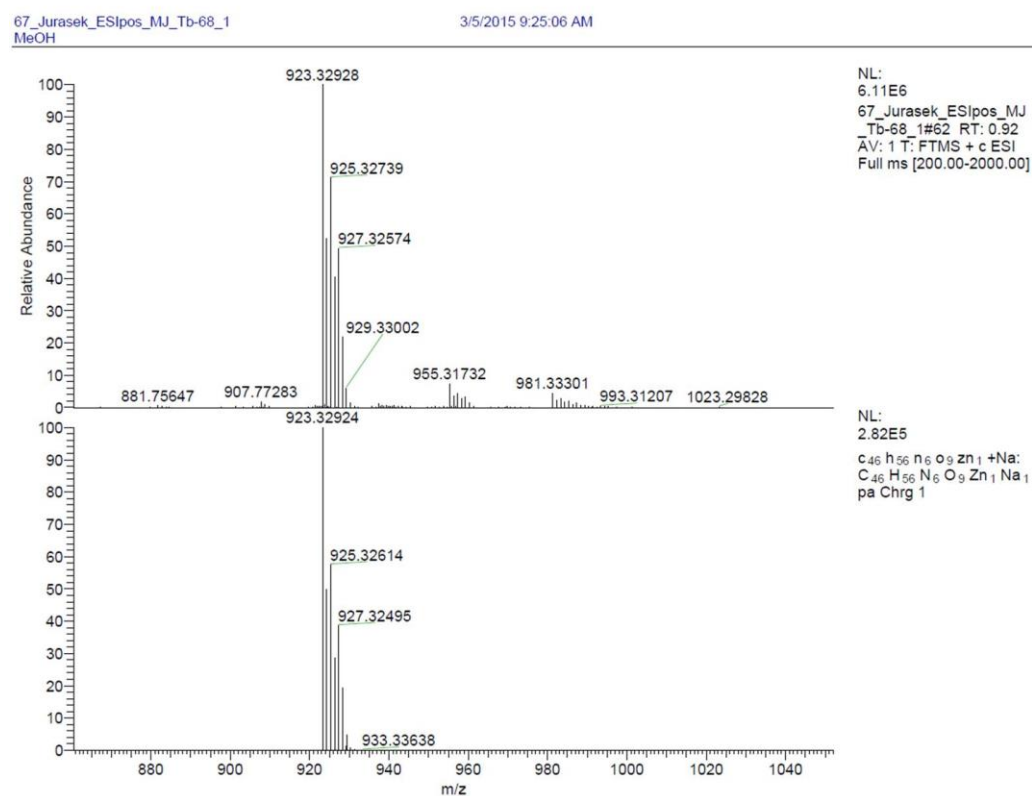
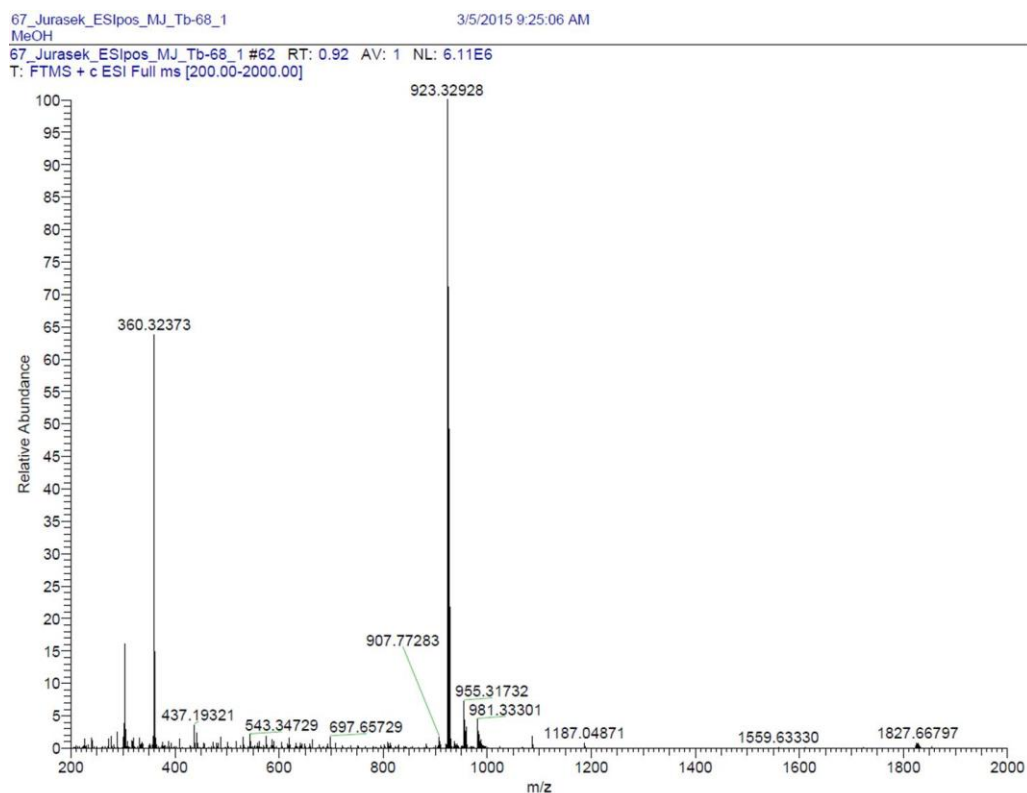


Figure S5: HRMS spectra of compound 3

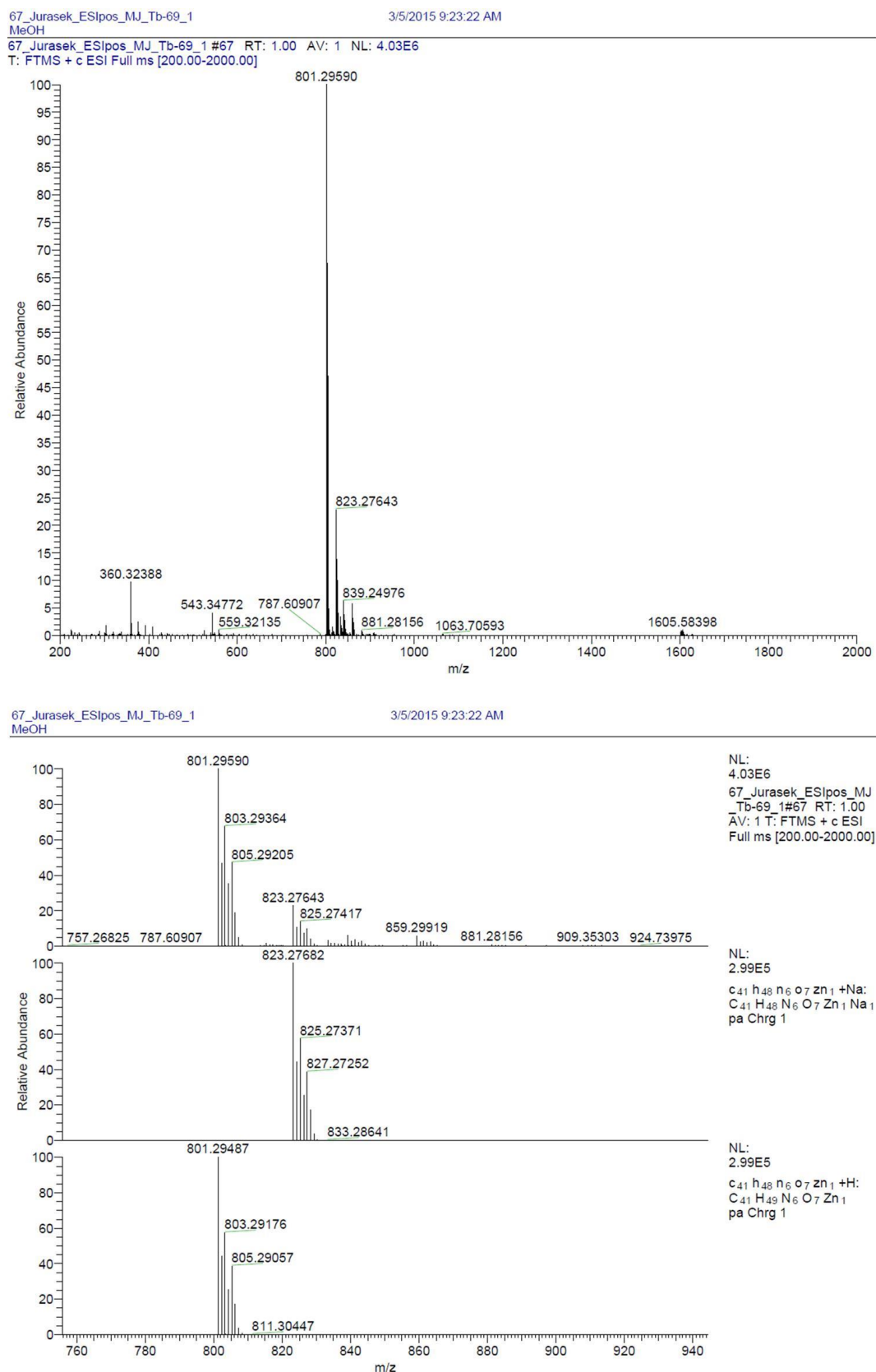


Figure S6-1: HRMS spectra of compound 4

Spectral properties of compounds 3 and 4

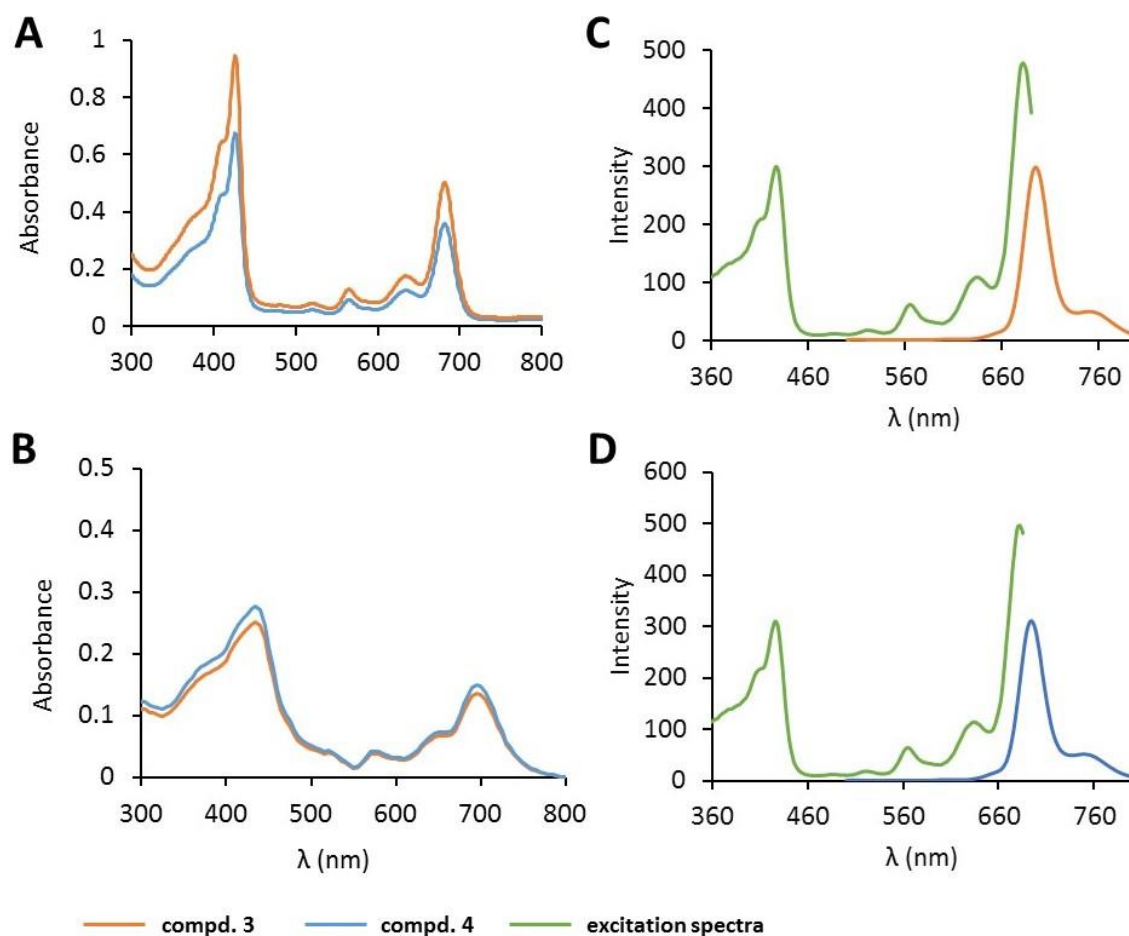


Figure S6-2: Absorption, excitation and emission spectra of compounds 3 and 4. Absorption spectra were measured in **A)** $\text{CHCl}_3\text{-MeOH}$, 8/2, **B)** phosphate buffered saline (PBS); amber color represents compound 3, blue color represents compound 4. **C, D)** Excitation and emission spectra were measured in $\text{CHCl}_3\text{-MeOH}$, 8/2. Each spectrum was the average of two repeated measurements. The emission and excitation wavelengths were following: **C)** 695 nm (green), 427 nm (amber, compound 3), **D)** 694 nm (green), 426 nm (blue, compound 4).

1.3. SINGLET OXYGEN GENERATION EFFICIENCY

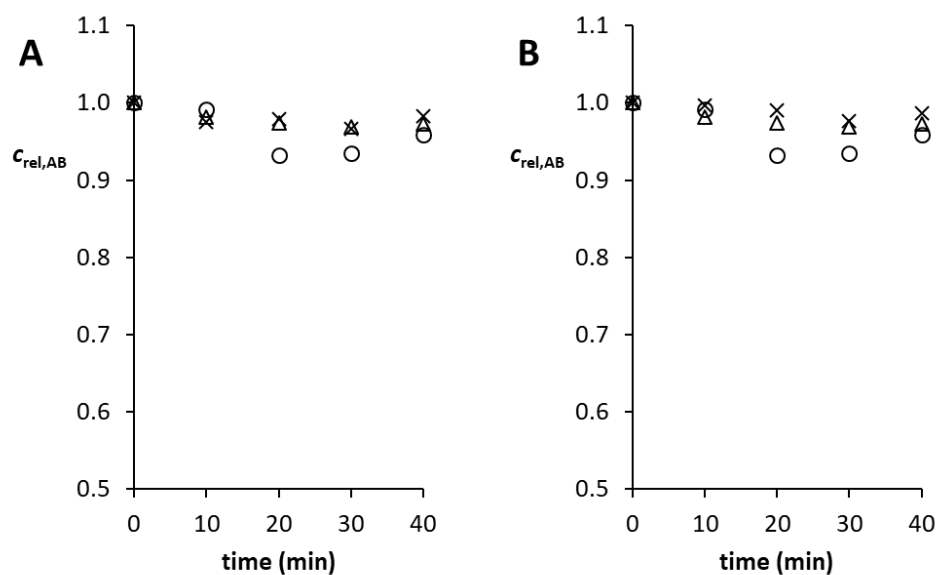


Figure S6-3: Depletion of 9,10-anthracenediyl-bis(methylene)dimalonic acid (AB , $7 \cdot 10^{-5}$ M) without presence of photosensitizer-generated singlet oxygen in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum. The experiments were triplicated. (A) solution exposed to light, (B) solution kept in dark. $c_{rel,AB}$ – relative concentration of AB (actual concentration with respect to concentration at experiment start).

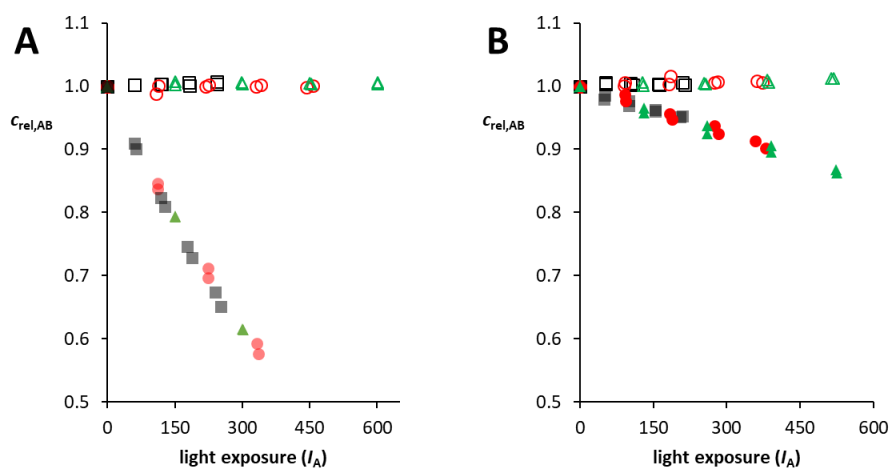


Figure S6-4: Depletion of 9,10-anthracenediyl-bis(methylene)dimalonic acid (AB , $7 \cdot 10^{-5}$ M) with RB -generated singlet oxygen in (A) PBS (concentrations of RB were: $1.3 \cdot 10^{-7}$ M, $2.6 \cdot 10^{-7}$ M, $3.1 \cdot 10^{-7}$ M) and (B) Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (concentrations of RB were $2.6 \cdot 10^{-7}$ M, $5.1 \cdot 10^{-7}$ M, $7.5 \cdot 10^{-7}$ M).

⁷ M). The experiments were duplicated. ■ ● ▲ – solution exposed to light, □ ○ △ – solution kept in dark.

$c_{\text{rel},\mathbf{AB}}$ – relative concentration of **AB** (actual concentration with respect to concentration at experiment start)

2. BIOLOGICAL ANALYSIS

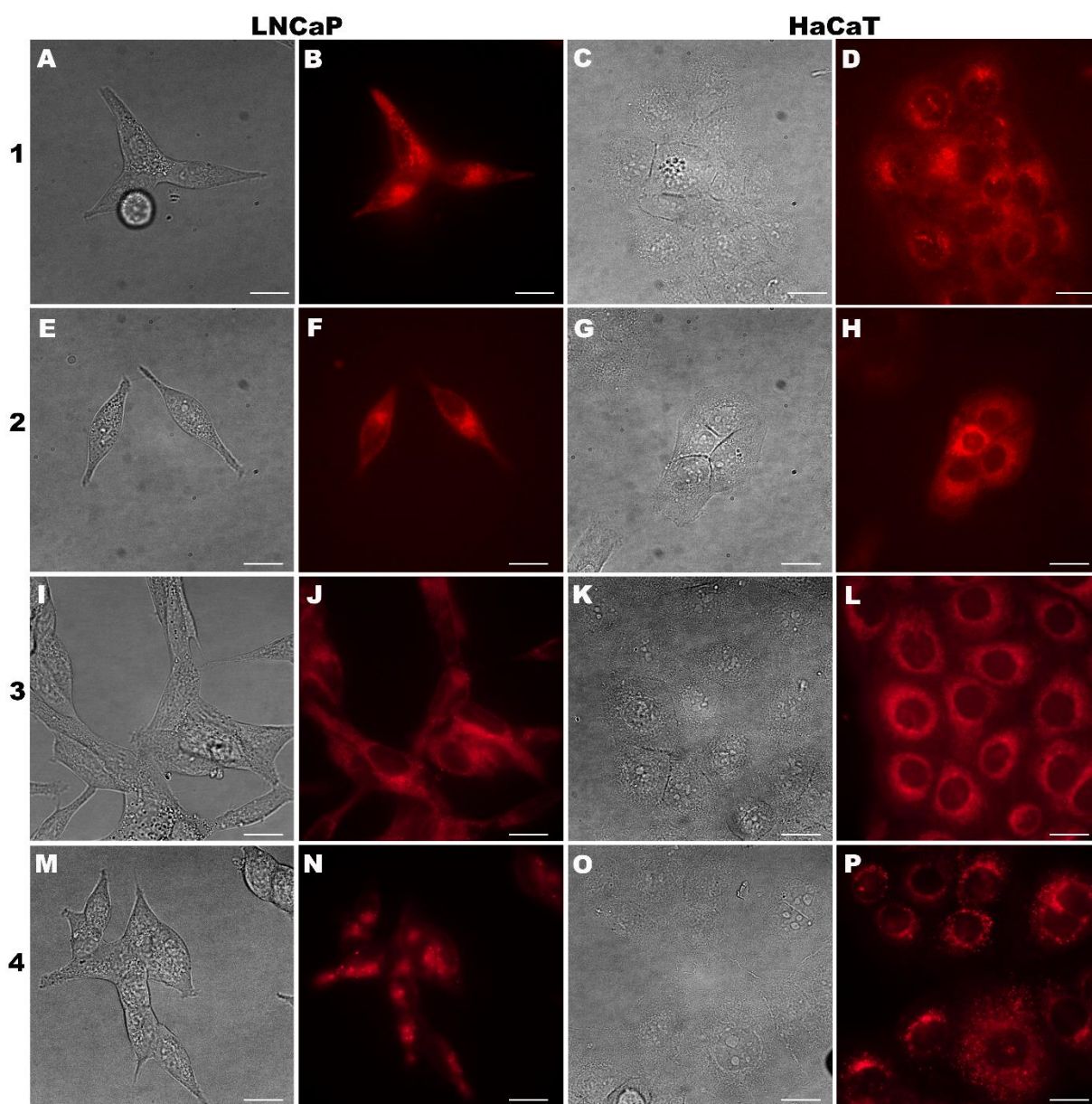


Figure S7: Fluorescence microscopy images of the intracellular localization of purpurin 18 (compound 1) and its derivatives (compounds 2–4) at 0.5 μM concentration in human cancer cell lines of LNCaP (prostate carcinoma) and HaCaT (keratinocytes) after 24 h incubation. In the first and third column, there are bright field images; in the second and fourth column, there is compound localization. The scale bars represent 20 μm .

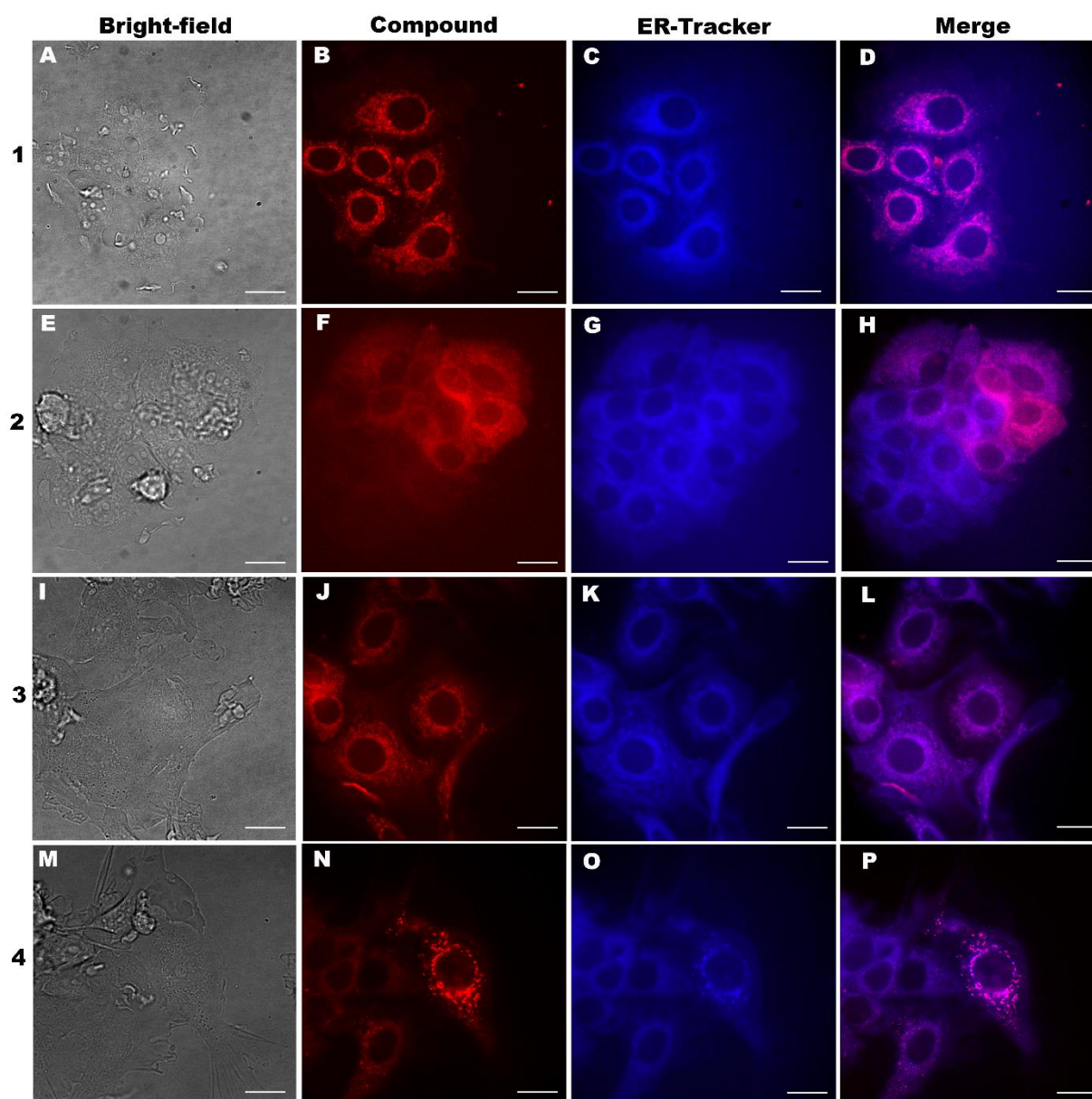


Figure S8: Fluorescence microscopy images of purpurin 18 (compound **1**) and its derivatives (compounds **2–4**) localization in the endoplasmic reticulum of human MCF-7 cells (breast carcinoma). Colocalization of compounds **1–2** (0.5 μ M, 24 h) or compounds **3–4** (0.5 μ M, 24 h) with ER-Tracker™ Blue-White DPX (70 nM, 30 min.). A, E, I, M) Bright-field images; B, F, J, N) localization of the tested compounds; C, G, K, O) ER-Tracker™ Blue-White DPX; D, H, L, P) merge of the fluorescent images. The scale bars represent 20 μ m.

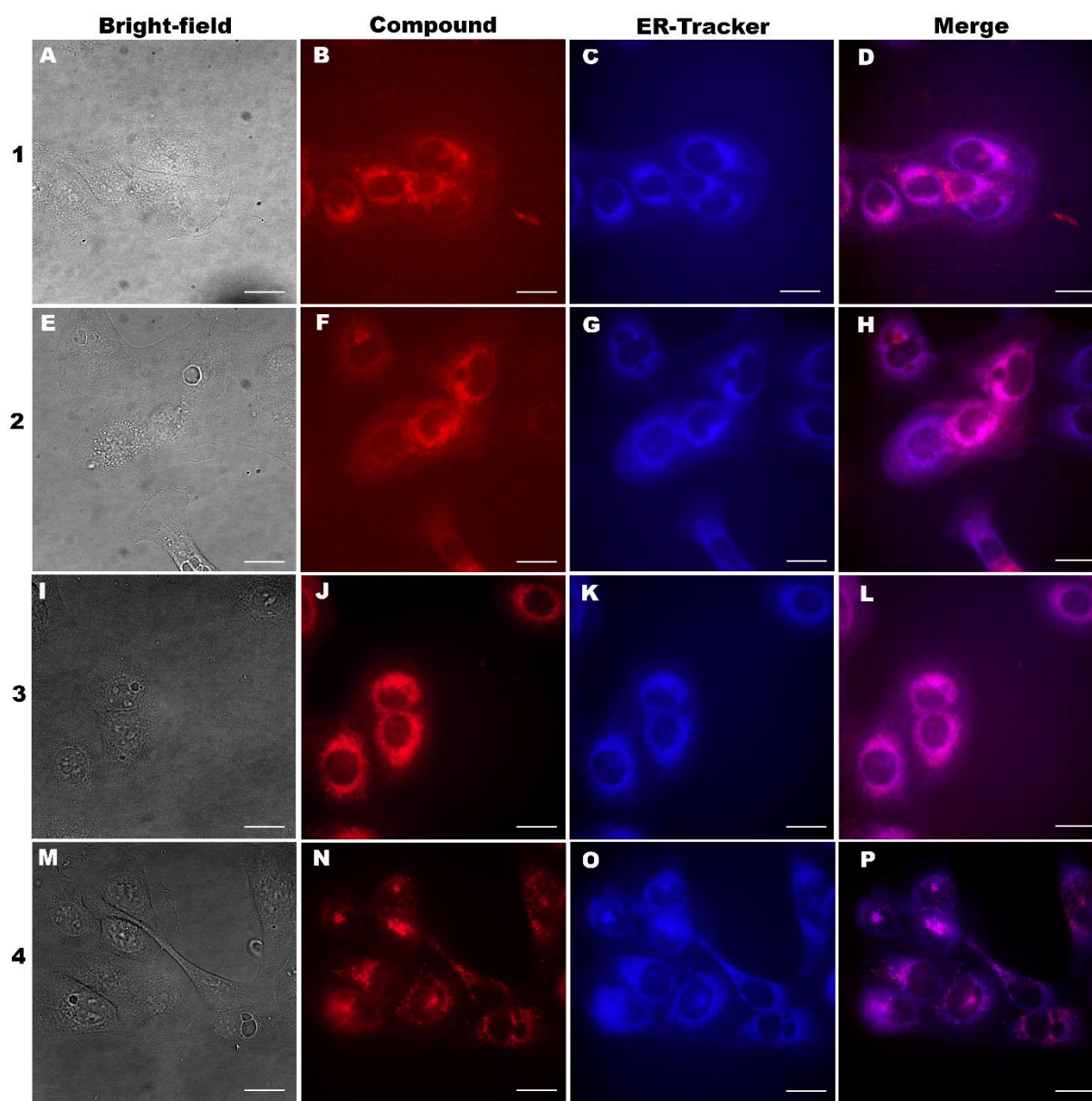


Figure S9: Fluorescence microscopy images of purpurin 18 (compound **1**) and its derivatives (compounds **2–4**) localization in the endoplasmic reticulum of human immortalized keratinocytes (HaCaT cells). Colocalization of compounds **1–2** (1 μ M, 24 h) or compounds **3–4** (0.5 μ M, 24 h) with ER-Tracker™ Blue-White DPX (70 nM, 30 min.). A, E, I, M) Bright-field images; B, F, J, N) localization of the tested compounds; C, G, K, O) ER-Tracker™ Blue-White DPX; D, H, L, P) merge of the fluorescent images. The scale bars represent 20 μ m.

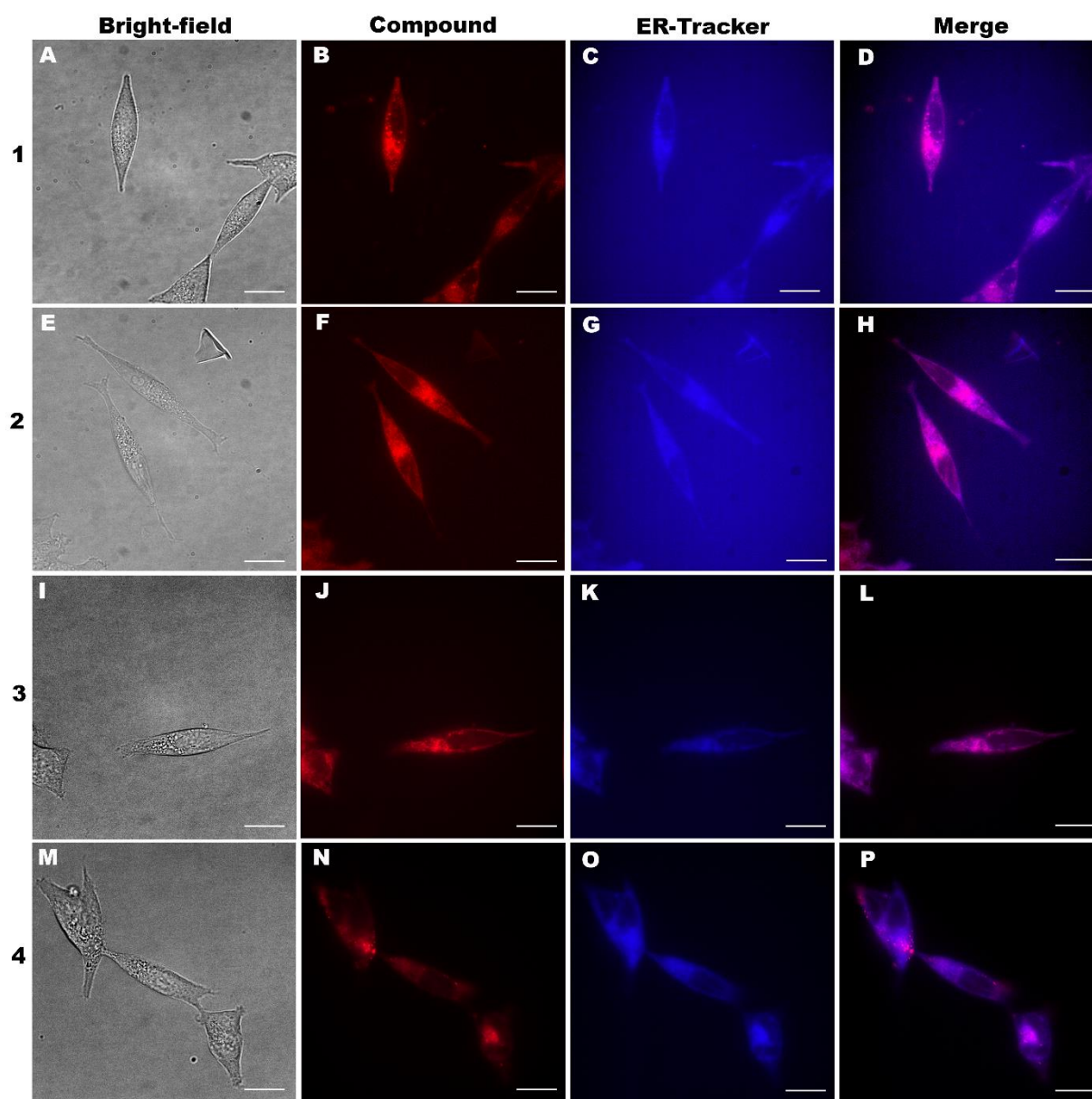


Figure S10: Fluorescence microscopy images of purpurin 18 (compound 1) and its derivatives (compounds 2–4) localization in the endoplasmic reticulum of human LNCaP cells (prostate carcinoma). Colocalization of compounds 1–2 (0.5 μ M, 24 h) or compounds 3–4 (0.5 μ M, 3 h) and ER-Tracker™ Blue-White DPX (70 nM, 30 min.). A, E, I, M) Bright-field images; B, F, J, N) localization of the tested compounds; C, G, K, O) ER-Tracker™ Blue-White DPX; D, H, L, P) merge of the fluorescent images. The scale bars represent 20 μ m.

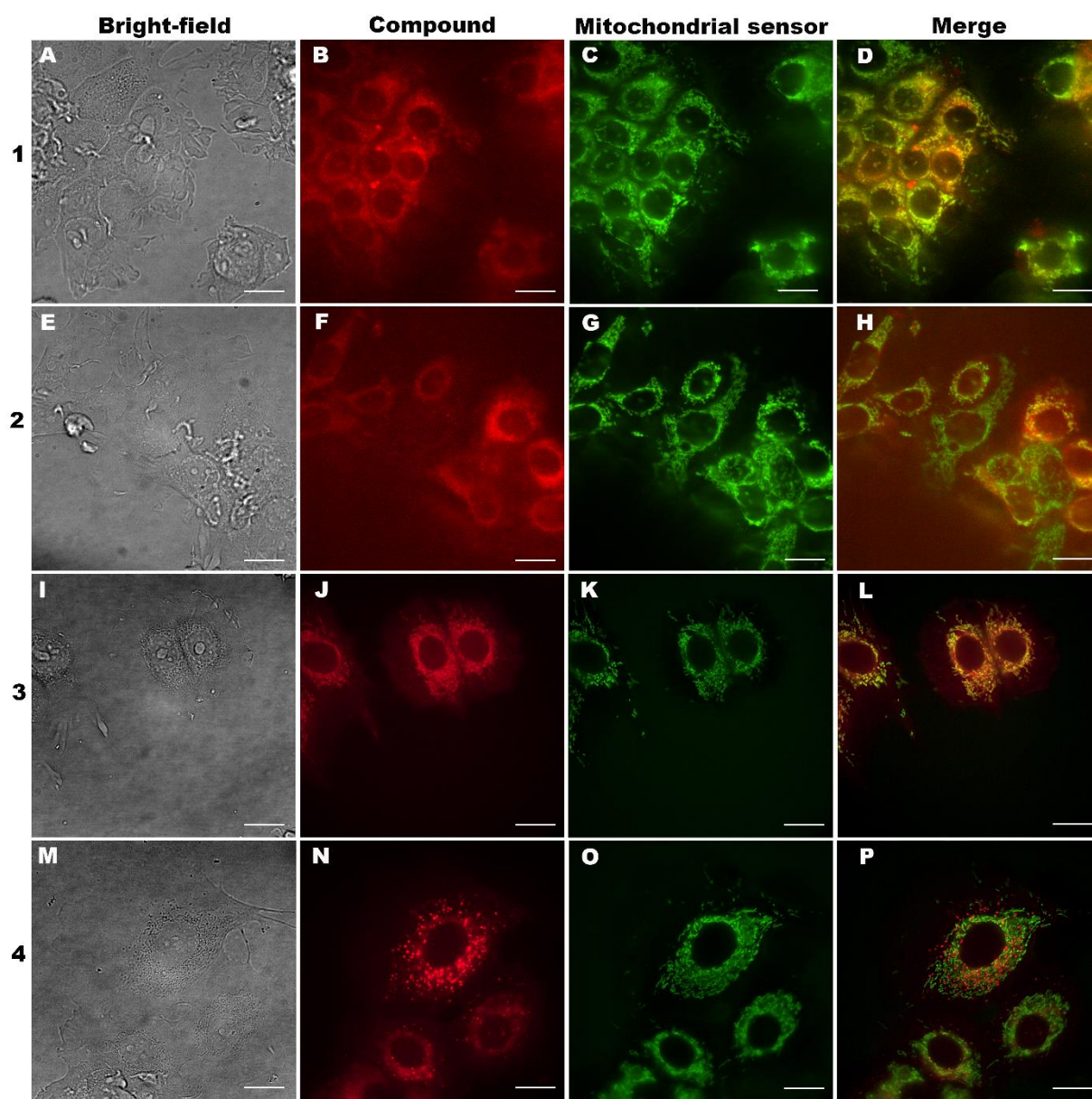


Figure S11: Fluorescence microscopy images of purpurin 18 (compound **1**) and its derivatives (compounds **2–4**) localization in the mitochondria of human MCF-7 cells (breast carcinoma). Colocalization of compounds **1–2** (0.5 μ M, 24 h) or compounds **3–4** (0.5 μ M, 24 h) with a mitosensor (70 nM, 10 min.) based on a dimethinium salt according to Bříza *et al.*¹. A, E, I, M) Bright-field images; B, F, J, N) localization of the tested compounds; C, G, K, O) mitosensor; D, H, L, P) merge of the fluorescent images. The scale bars represent 20 μ m.

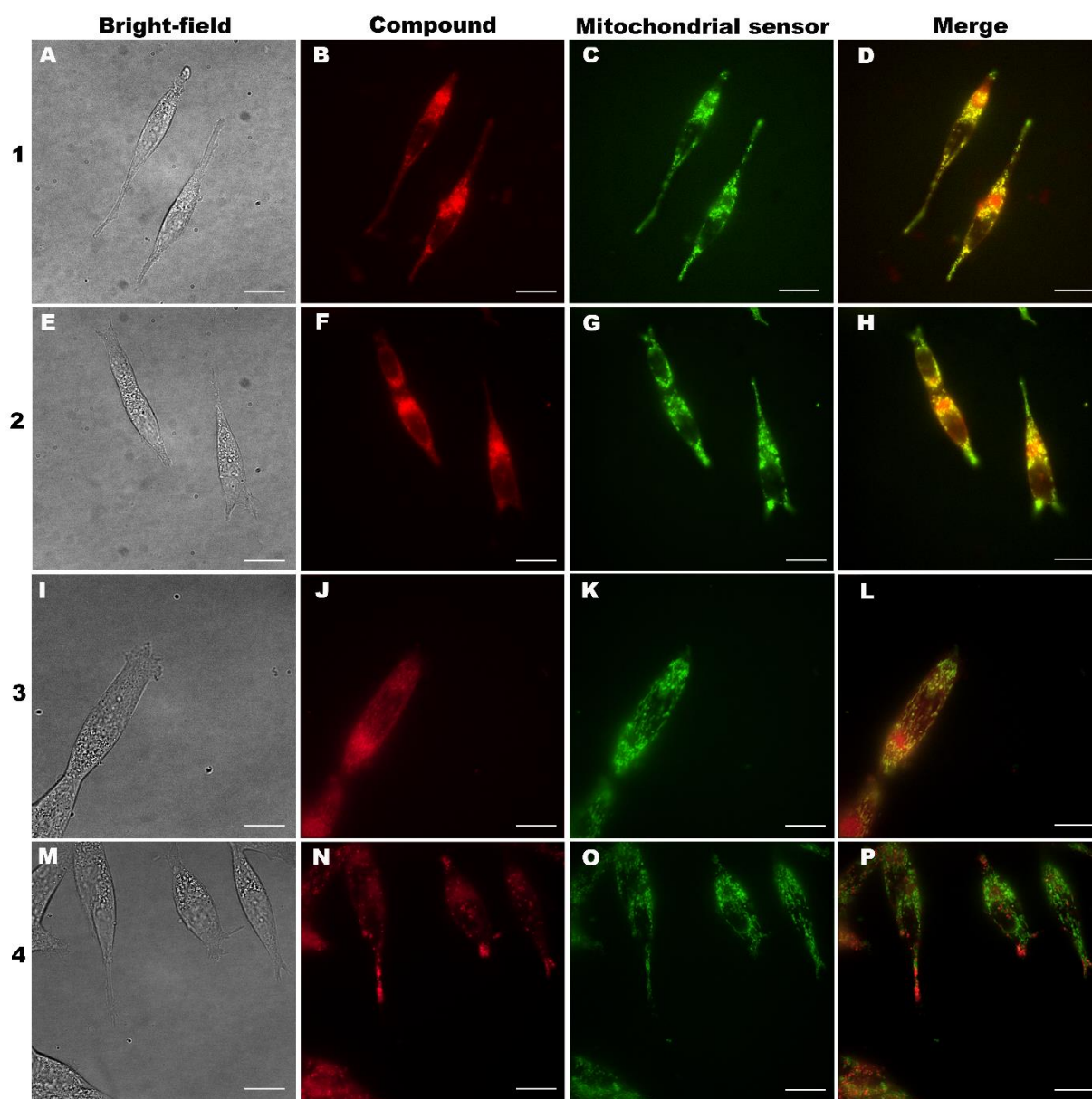


Figure S12: Fluorescence microscopy images of purpurin 18 (compound **1**) and its derivatives (compounds **2–4**) localization in the mitochondria of human LNCaP cells (prostate carcinoma). Colocalization of compounds **1–2** (0.5 μ M, 24 h) or compounds **3–4** (0.5 μ M, 24 h) with MitoTracker™ Green FM (70 nM, 20 min.). A, E, I, M) Bright-field images; B, F, J, N) localization of the tested compounds; C, G, K, O) MitoTracker™ Green FM; D, H, L, P) merge of the fluorescent images. The scale bars represent 20 μ m.

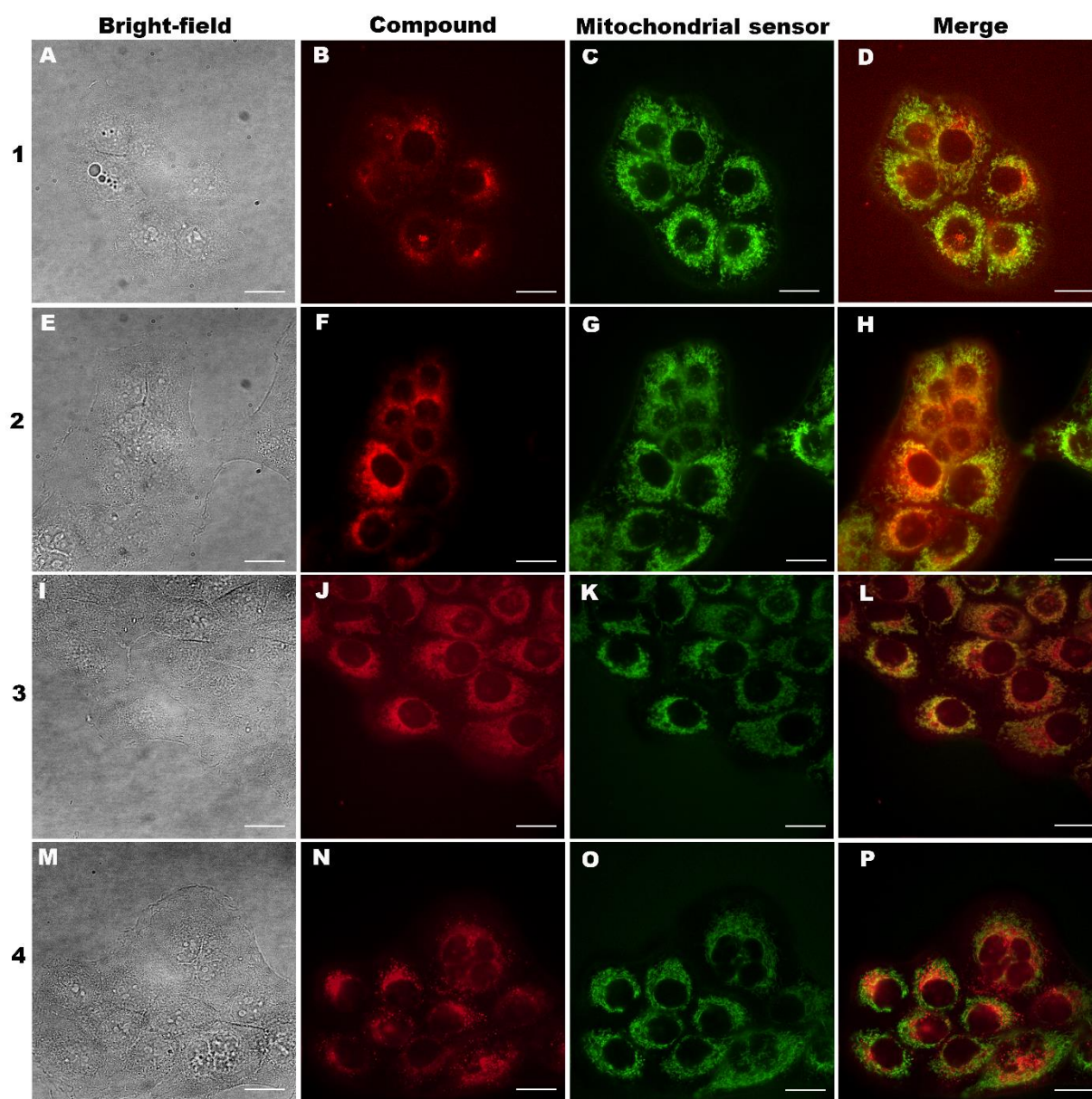


Figure S13: Fluorescence microscopy images of purpurin 18 (compound **1**) and its derivatives (compounds **2–4**) localization in the mitochondria of human keratinocytes HaCaT. Colocalization of compounds **1–2** (0.5 μ M, 3 h) or compounds **3–4** (0.5 μ M, 3 h) and MitoTracker™ Green FM (70 nM, 20 min.). A, E, I, M) Bright-field images; B, F, J, N) localization of the tested compounds; C, G, K, O) MitoTracker™ Green FM; D, H, L, P) merge of the fluorescent images. The scale bars represent 20 μ m.

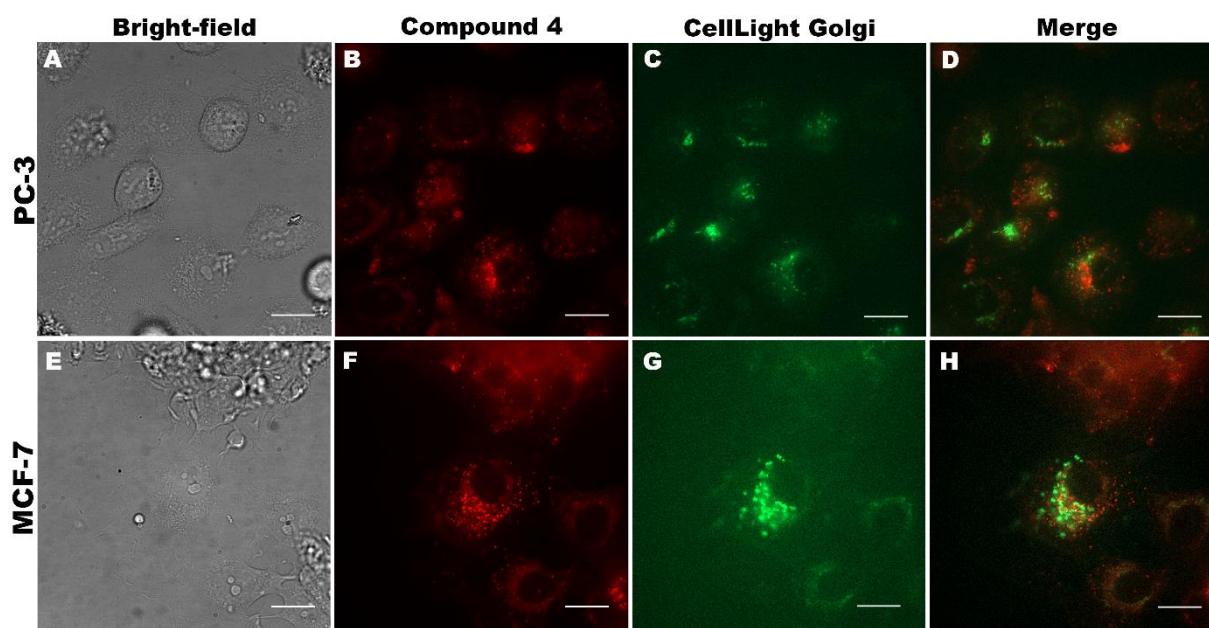


Figure S14: Fluorescence microscopy images of compound 4 localization in the Golgi apparatus in human PC-3 and MCF-7 cells. Colocalization of compound 4 (0.5 μM , 24 h) and CellLight Golgi-GFP, BacMam 2.0 ($2 \cdot 10^4$ particles per cell.). A, E) Bright-field images; B, F) localization of compound 4; C, G) CellLight Golgi-GFP, BacMam 2.0; D, H) merge of the fluorescent images. The scale bars represent 20 μm .

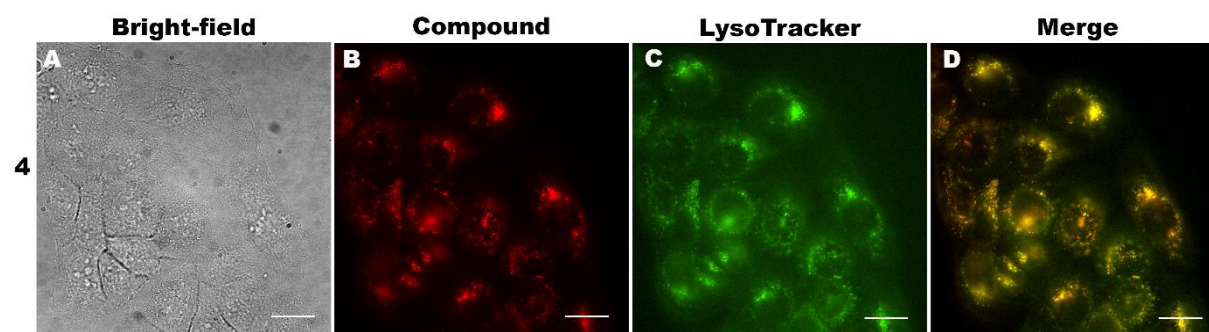


Figure S15: Fluorescence microscopy images of compound 4 localization in lysosomes of human immortalized keratinocytes (HaCaT cells). Colocalization of compound 4 (0.5 μM , 24 h) and LysoTracker Green DND-26 (70 nM, 20 min.). A) Bright-field images; B) localization of compound 4; C) LysoTracker Green DND-26; D) merge of the fluorescent images. The scale bars represent 20 μm .

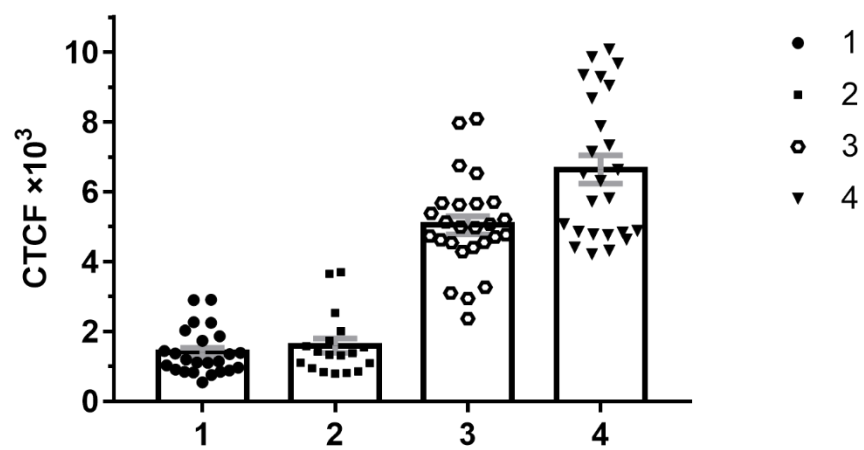


Figure S16: Corrected total cell fluorescence (CTCF) of compounds **1–4** (1 μ M, 24 h) localized in PC-3 cells

Table S1: Dose-dependent mechanisms of cell death in MCF-7 cells induced by compounds **1–4** after 24 h treatment and light induction (Light) measured by flow cytometry. Control represents untreated cells and cells incubated with the same compounds without illumination (Dark). The total light dose was of 4 J·cm⁻²

Compound	Concentration [μM]	Dark		Light	
		Apoptosis	Necrosis	Apoptosis	Necrosis
Control	0	9.5 ± 1.0	0	10.6 ± 0.6	0
1	0.10	7.6 ± 1.4	0	11.0 ± 2.4	0.1 ± 0.1
	0.25	8.5 ± 2.1	0	14.2 ± 2.7	0
	0.50	8.2 ± 1.1	0	32.6 ± 6.1	0.1 ± 0
	1.00	7.3 ± 0.2	0	51.7 ± 4.7	0.1 ± 0.1
2	0.10	10.5 ± 2.3	0	14.2 ± 2.1	0.1 ± 0.1
	0.25	12.1 ± 1.9	0.1 ± 0.1	18.2 ± 2.6	0
	0.50	12.8 ± 3.8	0	25.8 ± 4.0	0.4 ± 0.2
	1.00	14.3 ± 5.1	0	31.9 ± 4.1	0.3 ± 0.3
3	0.10	16.3 ± 3.5	1.7 ± 1.4	36.7 ± 12.1	0
	0.25	10.5 ± 0.8	0	56.1 ± 14.9	0
	0.50	13.7 ± 1.5	0	65.0 ± 9.5	0.1 ± 0
	1.00	21.2 ± 7.6	0	61.1 ± 7.6	0.3 ± 0.2
4	0.10	14.5 ± 3.7	0.1 ± 0.1	32.0 ± 2.6	0
	0.25	19.8 ± 7.7	0	49.8 ± 2.9	0
	0.50	19.0 ± 7.6	0.1 ± 0.1	57.8 ± 9.8	0
	1.00	22.4 ± 8.0	0.1 ± 0.1	68.0 ± 8.4	0

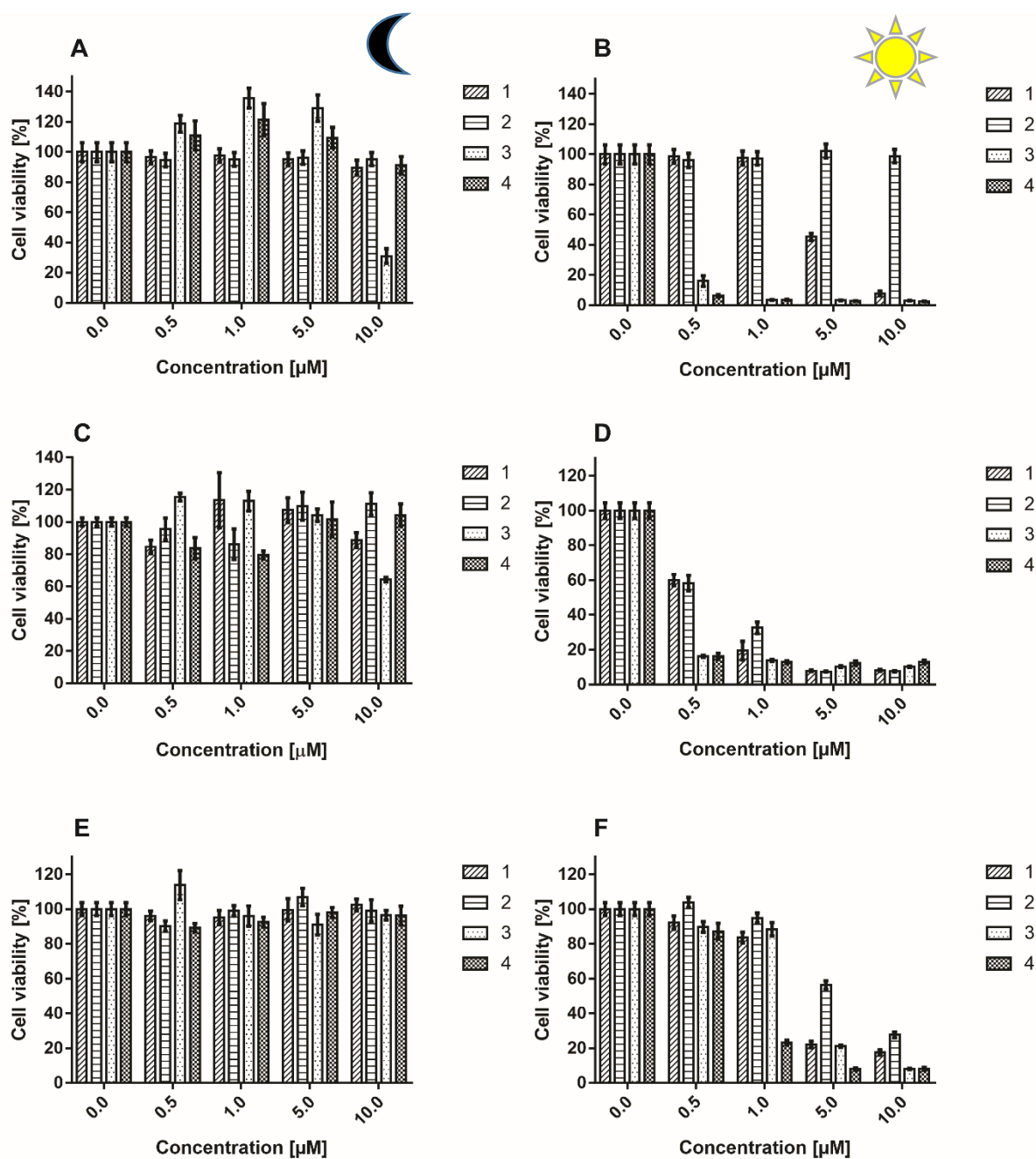


Figure S17: Photo- and dark toxicity of compounds 1–4 *in vitro*. Cell viability determined by WST-1 assay after 48 h of incubation with the tested compounds (24 h prior illumination + 24 h after illumination). Left panel dark toxicity (cells kept in the dark after the compound treatment), right panel phototoxicity (cell viability after compound photoactivation). A, B) HeLa, C, D) LNCaP, E, F) MCF-7 cells.

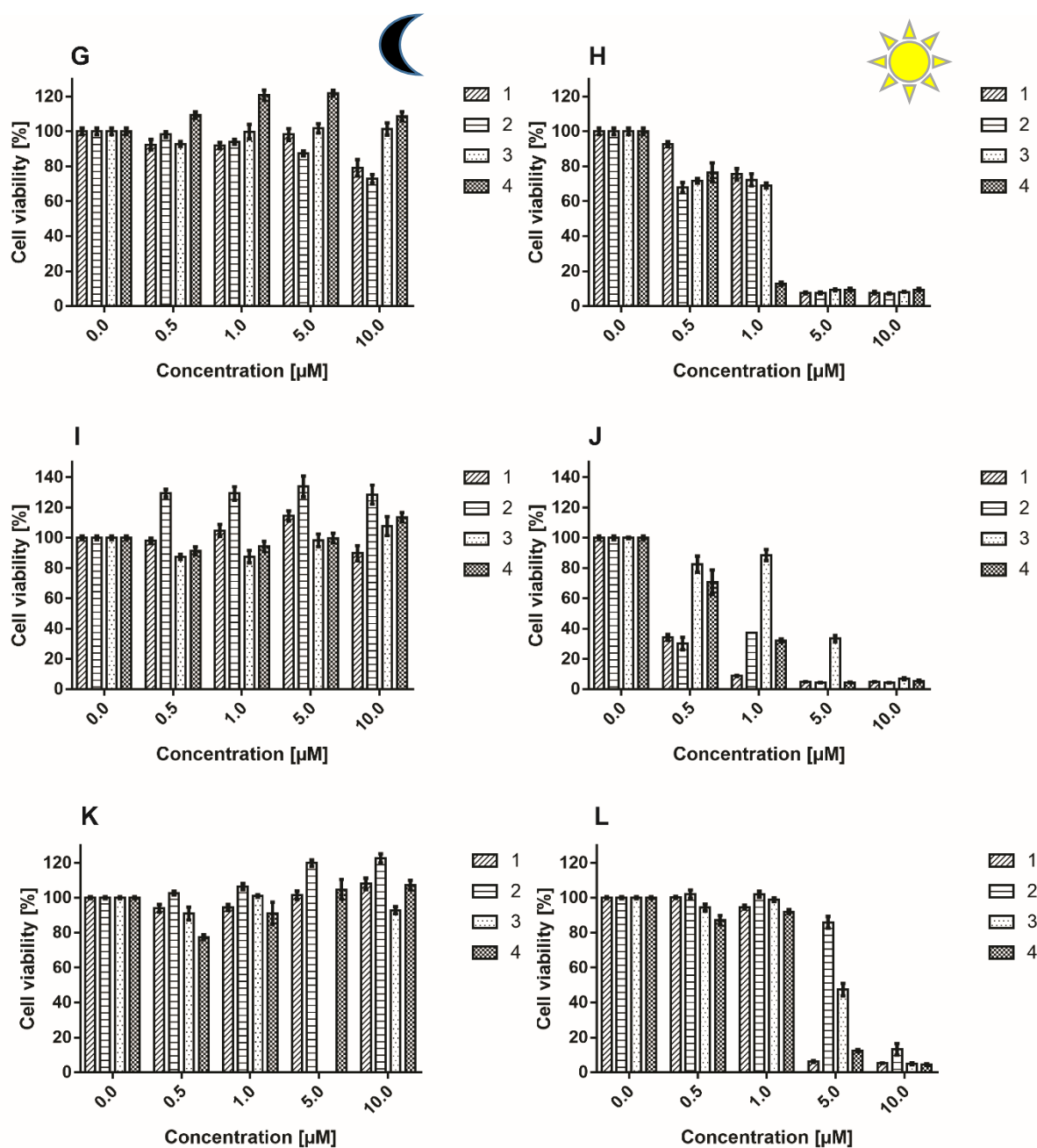
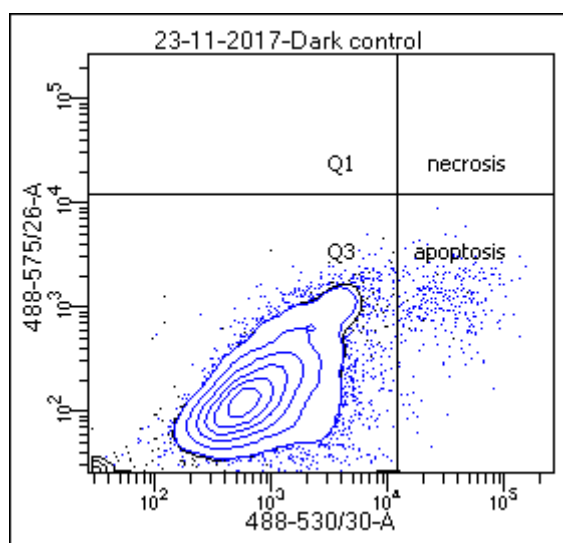


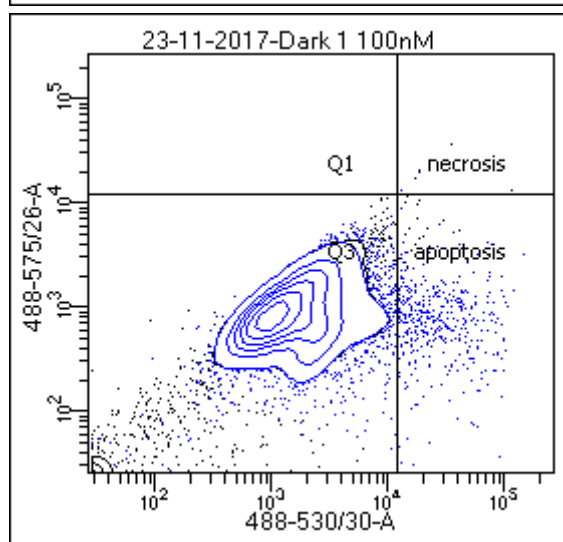
Figure S18: Photo- and dark toxicity of compounds 1–4 *in vitro*. Cell viability determined by WST-1 assay after 48 h of incubation with the tested compounds (24 h prior illumination + 24 h after illumination). Left panel dark toxicity (cells kept in the dark after the compound treatment), right panel phototoxicity (cell viability after compound photoactivation). A, B) MIA PaCa-2, C, D) PC-3, E, F) U-2 OS cells.

Flow cytometry – raw data



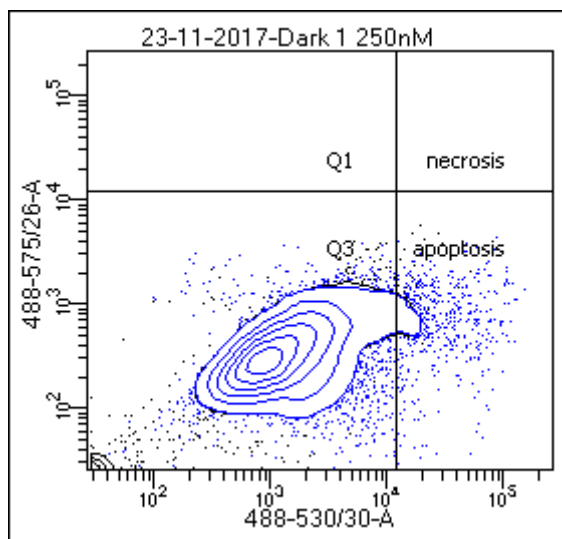
Tube: Dark control

Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
P1	9,319	93.2	93.2
P2	799	8.6	8.0
P3	2,980	32.0	29.8
Q1	0	0.0	0.0
necrosis	0	0.0	0.0
Q3	8,932	95.8	89.3
apoptosis	387	4.2	3.9



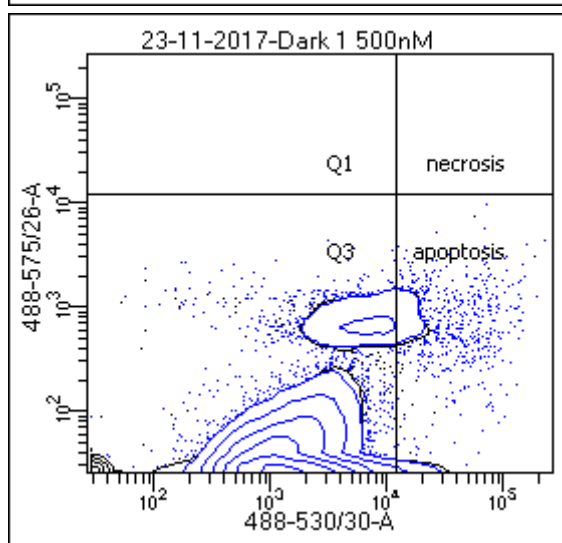
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Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
P1	9,192	91.9	91.9
P2	1,179	12.8	11.8
P3	8,920	97.0	89.2
Q1	0	0.0	0.0
necrosis	2	0.0	0.0
Q3	8,734	95.0	87.3
apoptosis	456	5.0	4.6



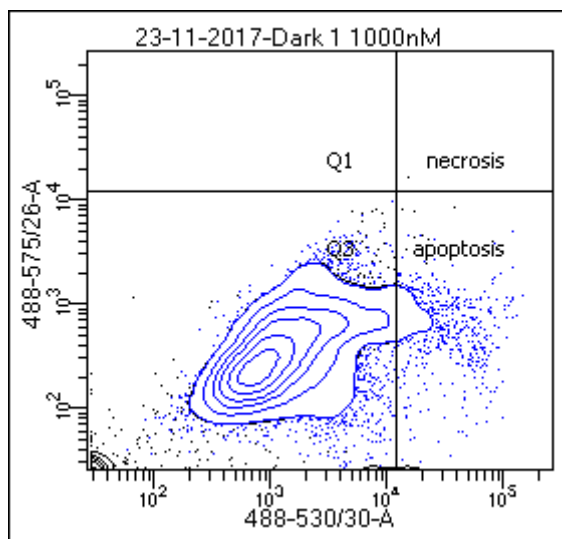
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Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,209	92.1	92.1
■ P2	1,413	15.3	14.1
■ P3	6,839	74.3	68.4
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	8,628	93.7	86.3
☒ apoptosis	581	6.3	5.8



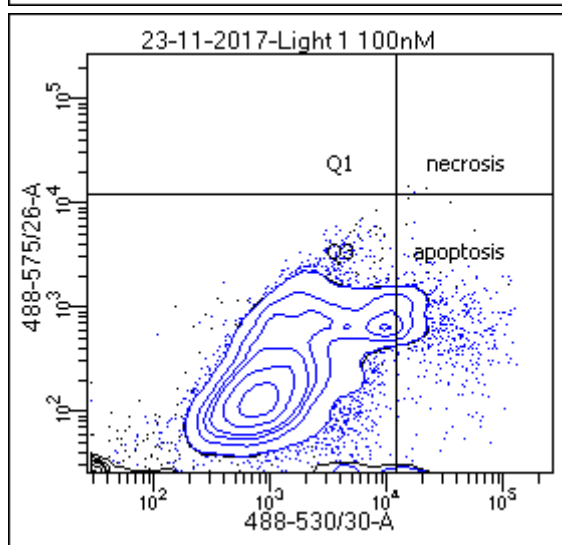
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■ All Events	10,000	####	100.0
■ P1	9,120	91.2	91.2
■ P2	2,090	22.9	20.9
■ P3	1,547	17.0	15.5
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	8,289	90.9	82.9
☒ apoptosis	830	9.1	8.3



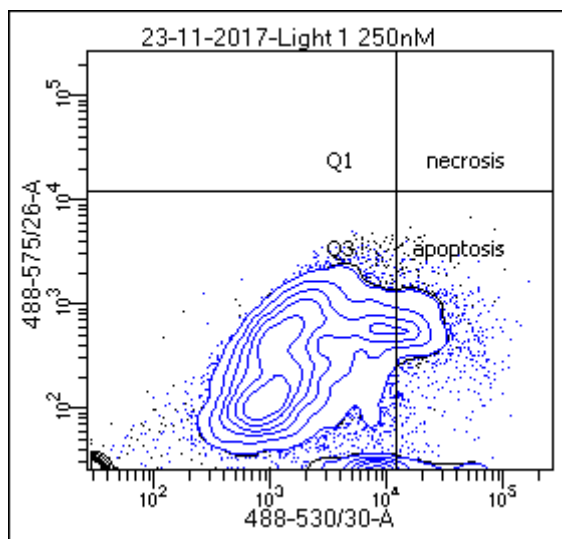
Tube: Dark 1 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,169	91.7	91.7
■ P2	1,698	18.5	17.0
■ P3	6,403	69.8	64.0
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	8,466	92.3	84.7
☒ apoptosis	703	7.7	7.0



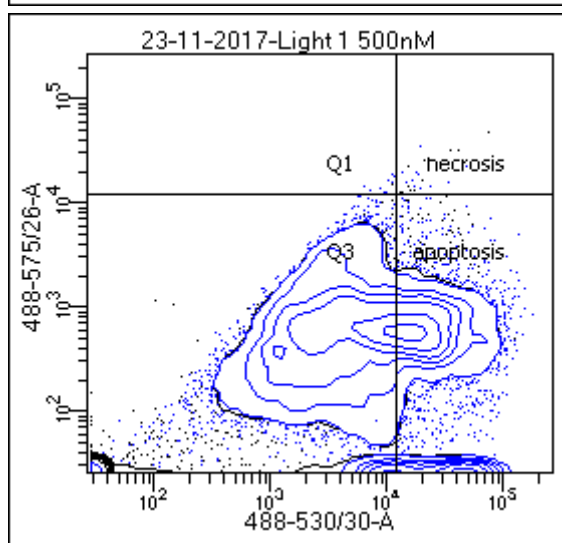
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Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,083	90.8	90.8
■ P2	1,929	21.2	19.3
■ P3	4,468	49.2	44.7
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	8,262	91.0	82.6
☒ apoptosis	820	9.0	8.2



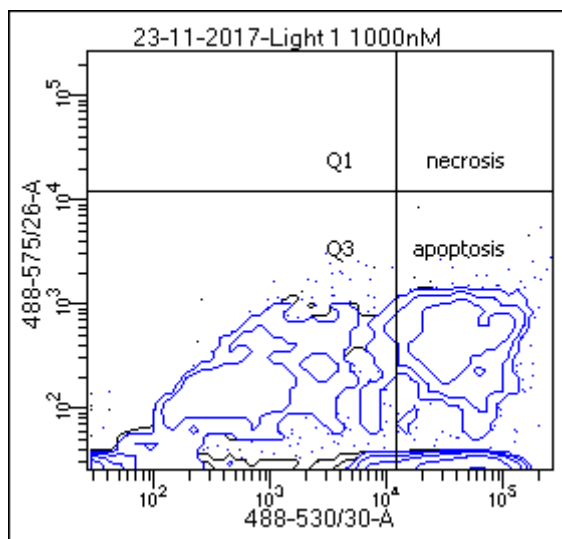
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Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,068	90.7	90.7
■ P2	2,863	31.6	28.6
■ P3	4,896	54.0	49.0
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,925	87.4	79.2
☒ apoptosis	1,143	12.6	11.4



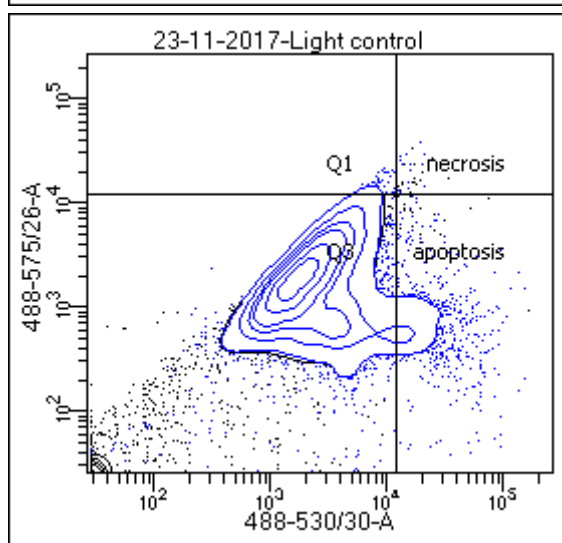
Tube: Light 1 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,260	82.6	82.6
■ P2	5,253	63.6	52.5
■ P3	5,671	68.7	56.7
☒ Q1	15	0.2	0.2
☒ necrosis	14	0.2	0.1
☒ Q3	5,111	61.9	51.1
☒ apoptosis	3,120	37.8	31.2



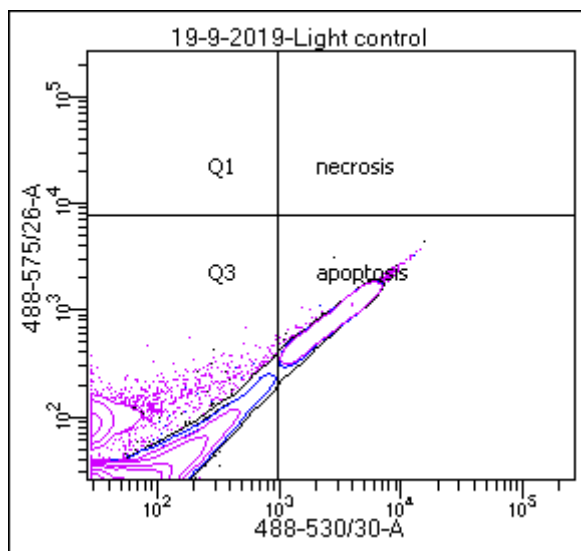
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■ All Events	3,054	####	100.0
■ P1	1,766	57.8	57.8
■ P2	1,175	66.5	38.5
■ P3	660	37.4	21.6
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.1	0.0
☒ Q3	718	40.7	23.5
☒ apoptosis	1,047	59.3	34.3



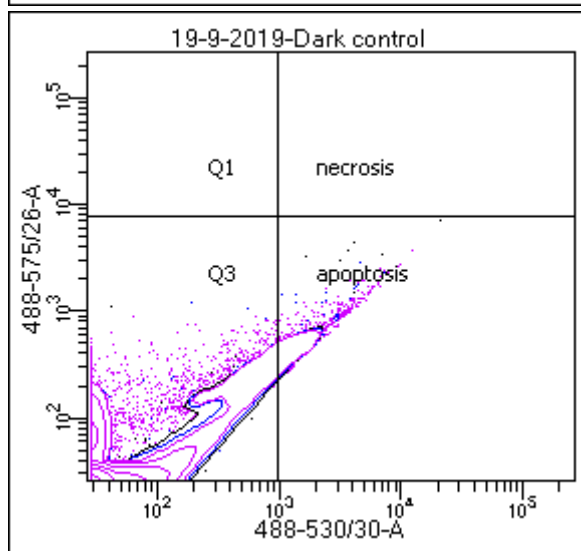
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,908	89.1	89.1
■ P2	2,563	28.8	25.6
■ P3	8,607	96.6	86.1
☒ Q1	88	1.0	0.9
☒ necrosis	14	0.2	0.1
☒ Q3	8,013	90.0	80.1
☒ apoptosis	793	8.9	7.9



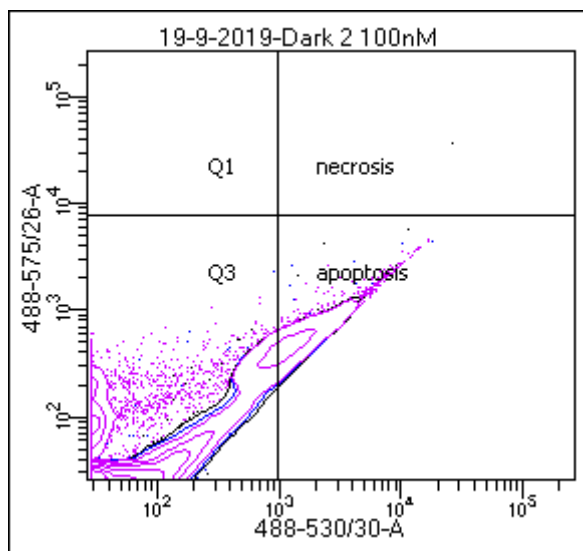
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,858	88.6	88.6
■ P2	7,383	83.3	73.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,555	88.8	65.6
☒ apoptosis	828	11.2	8.3



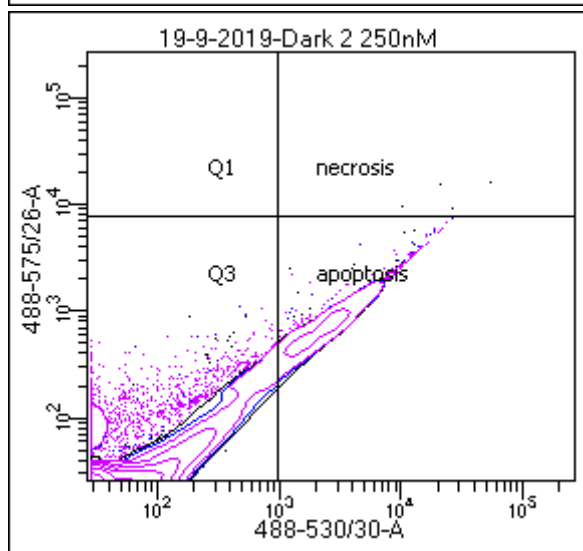
Tube: Dark control

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,147	91.5	91.5
■ P2	7,910	86.5	79.1
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,439	94.0	74.4
☒ apoptosis	471	6.0	4.7



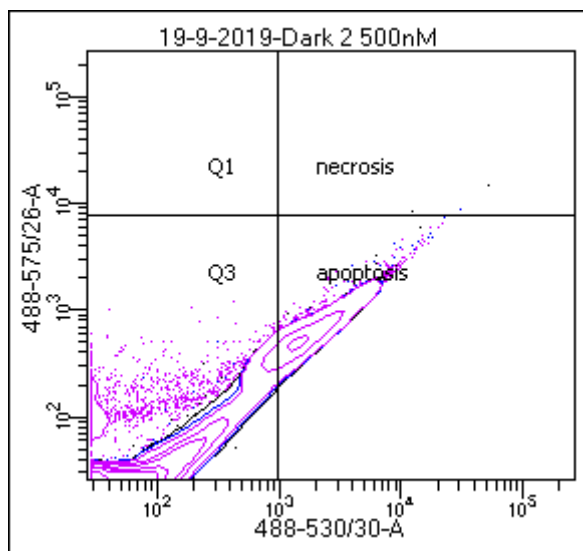
Tube: Dark 2 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,877	88.8	88.8
■ P2	7,541	84.9	75.4
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,587	87.3	65.9
☒ apoptosis	954	12.7	9.5



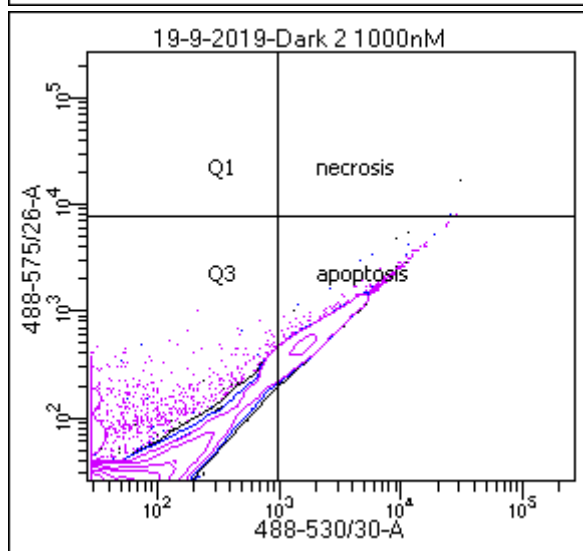
Tube: Dark 2 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,817	88.2	88.2
■ P2	7,419	84.1	74.2
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	6,300	84.9	63.0
☒ apoptosis	1,118	15.1	11.2



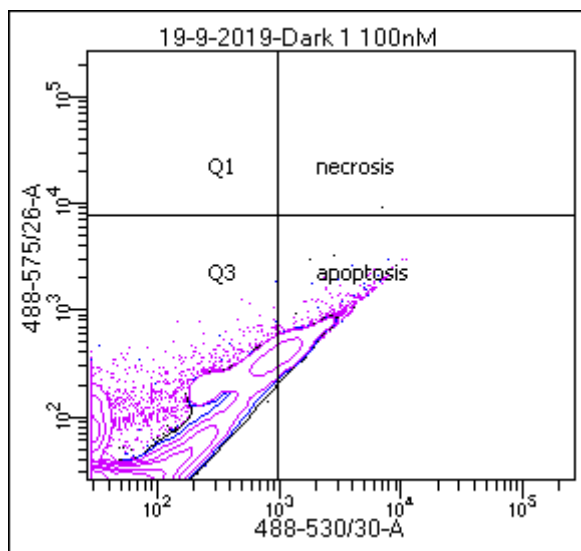
Tube: Dark 2 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,006	90.1	90.1
■ P2	7,750	86.1	77.5
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,277	81.0	62.8
☒ apoptosis	1,473	19.0	14.7



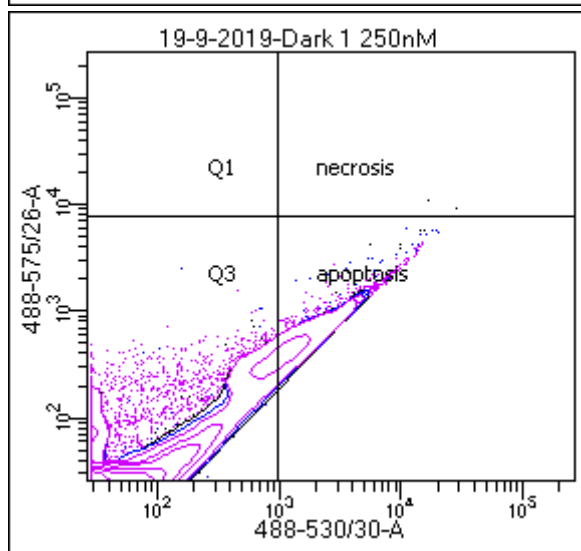
Tube: Dark 2 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,866	88.7	88.7
■ P2	7,377	83.2	73.8
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	6,421	87.0	64.2
☒ apoptosis	955	12.9	9.6



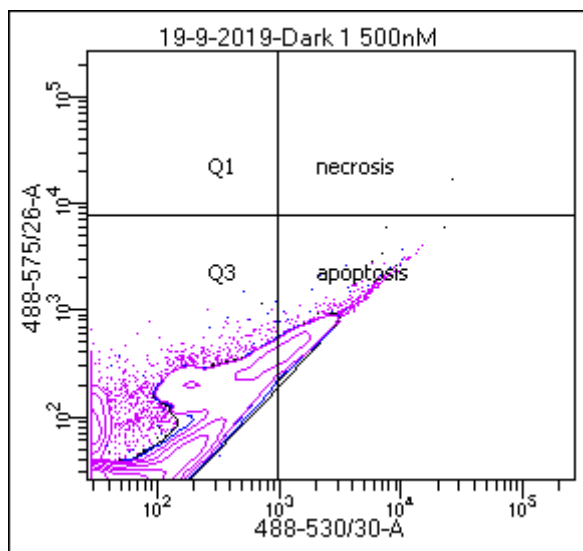
Tube: Dark 1 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,945	89.4	89.4
■ P2	7,589	84.8	75.9
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,873	90.6	68.7
☒ apoptosis	716	9.4	7.2



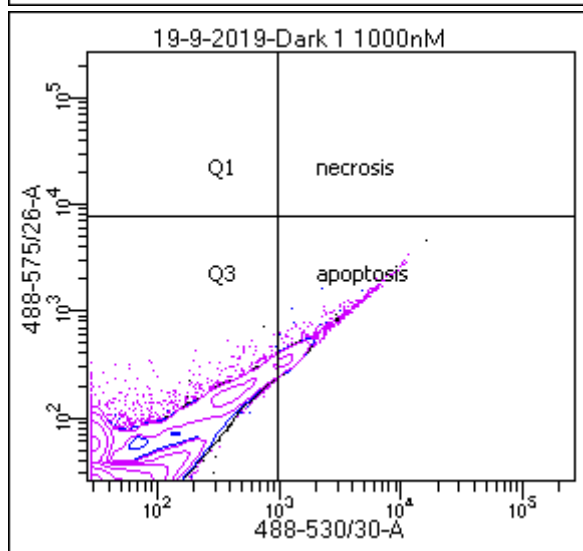
Tube: Dark 1 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,084	90.8	90.8
■ P2	7,803	85.9	78.0
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,821	87.4	68.2
☒ apoptosis	982	12.6	9.8



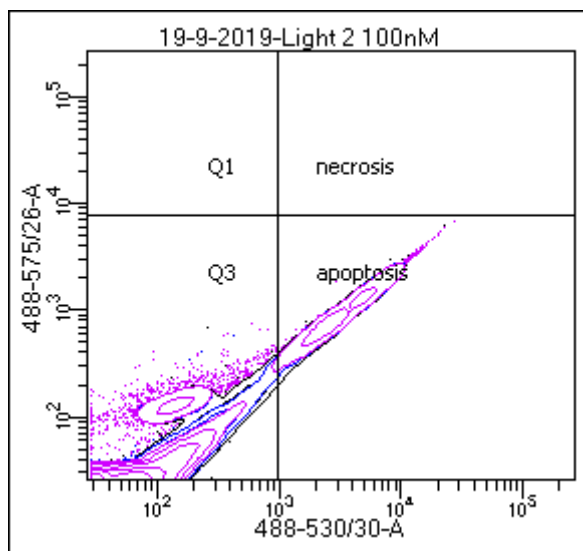
Tube: Dark 1 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,889	88.9	88.9
■ P2	7,524	84.6	75.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,810	90.5	68.1
☒ apoptosis	714	9.5	7.1



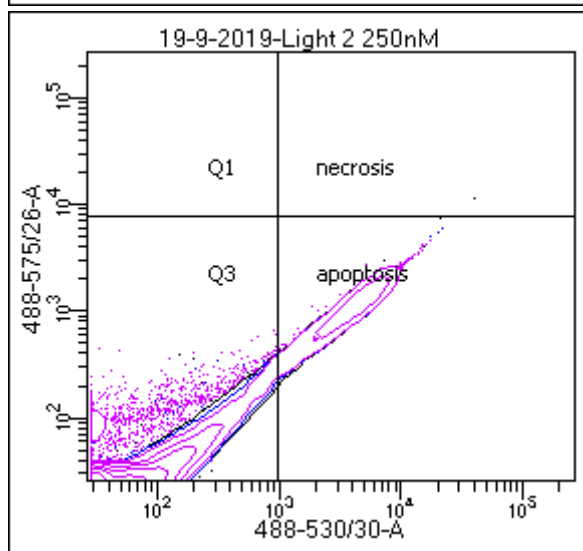
Tube: Dark 1 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,449	94.5	94.5
■ P2	8,314	88.0	83.1
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,700	92.6	77.0
☒ apoptosis	614	7.4	6.1



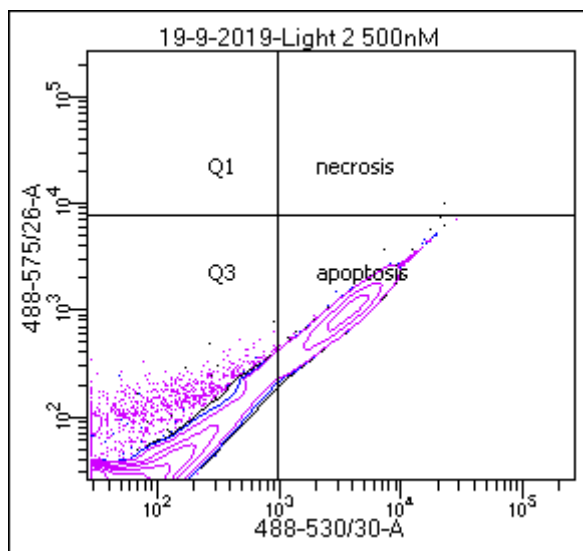
Tube: Light 2 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,759	87.6	87.6
■ P2	7,265	82.9	72.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,983	82.4	59.8
☒ apoptosis	1,282	17.6	12.8



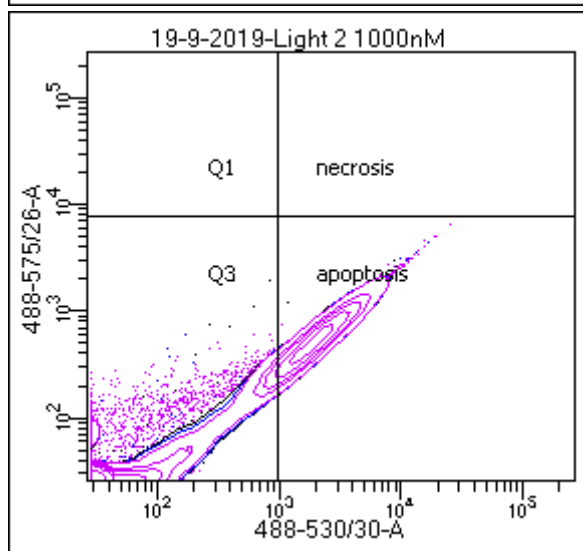
Tube: Light 2 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,229	92.3	92.3
■ P2	8,053	87.3	80.5
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,685	83.0	66.8
☒ apoptosis	1,368	17.0	13.7



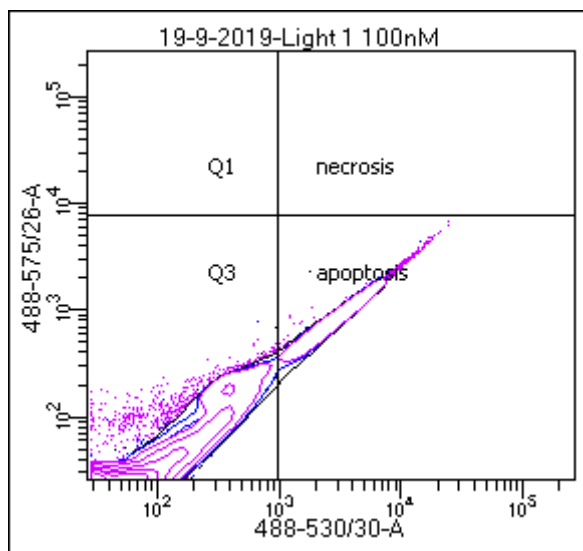
Tube: Light 2 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,154	91.5	91.5
■ P2	7,997	87.4	80.0
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,281	78.5	62.8
☒ apoptosis	1,716	21.5	17.2



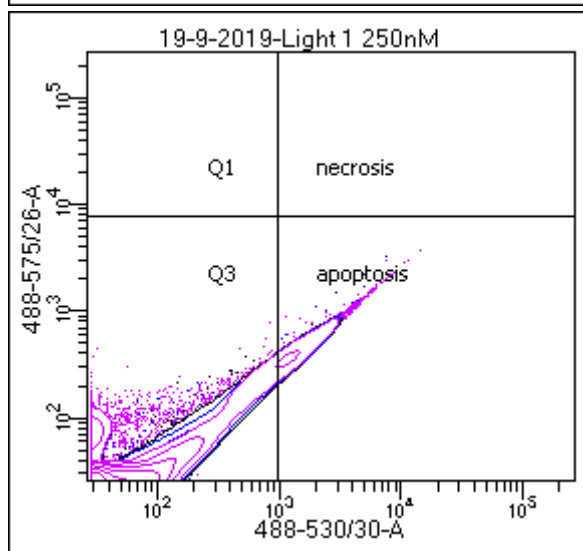
Tube: Light 2 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,476	94.8	94.8
■ P2	8,560	90.3	85.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,703	66.6	57.0
☒ apoptosis	2,857	33.4	28.6



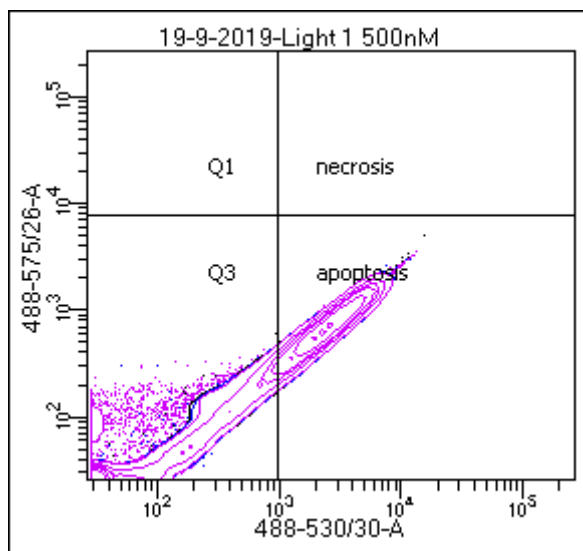
Tube: Light 1 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,508	85.1	85.1
■ P2	6,874	80.8	68.7
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,781	84.1	57.8
☒ apoptosis	1,093	15.9	10.9



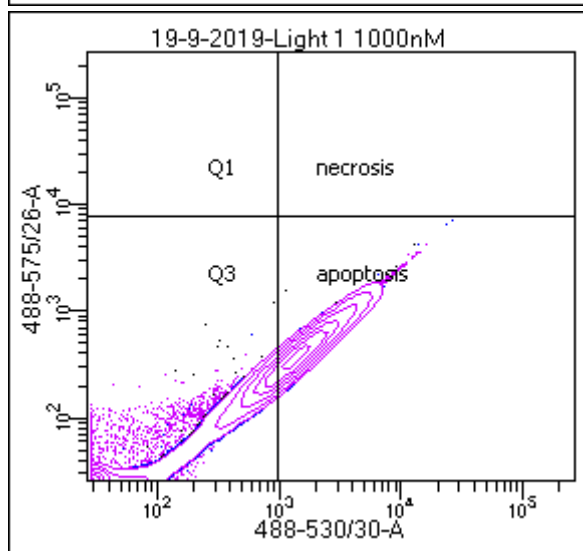
Tube: Light 1 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,967	89.7	89.7
■ P2	7,408	82.6	74.1
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,625	89.4	66.2
☒ apoptosis	783	10.6	7.8



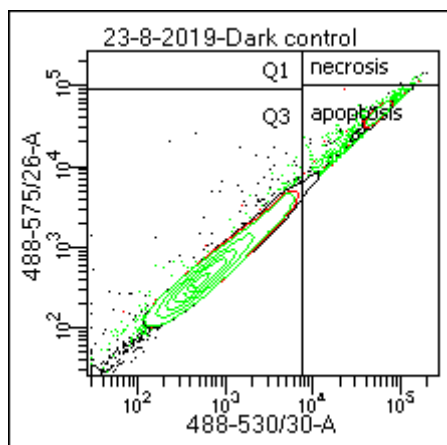
Tube: Light 1 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,744	97.4	97.4
■ P2	9,153	93.9	91.5
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,542	60.5	55.4
☒ apoptosis	3,611	39.5	36.1



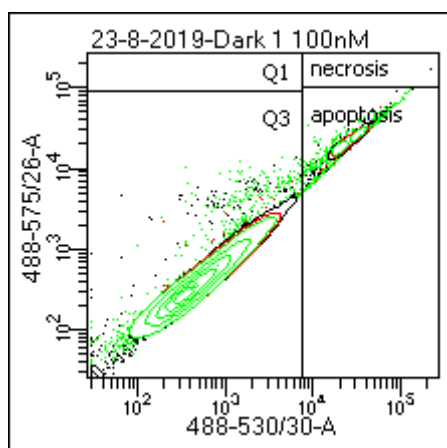
Tube: Light 1 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,923	99.2	99.2
■ P2	9,493	95.7	94.9
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,042	53.1	50.4
☒ apoptosis	4,451	46.9	44.5



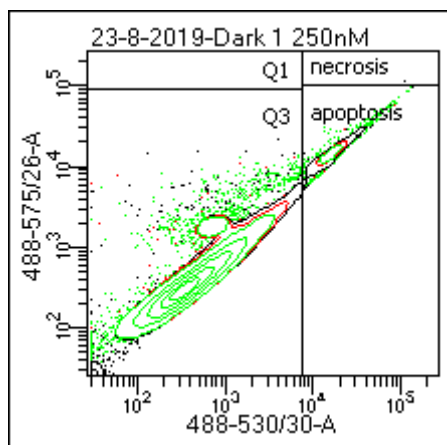
Tube: Dark control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,385	83.9	83.9
■ P2	6,835	81.5	68.4
☒ Q1	0	0.0	0.0
☒ necrosis	15	0.2	0.2
☒ Q3	6,081	89.0	60.8
☒ apoptosis	739	10.8	7.4



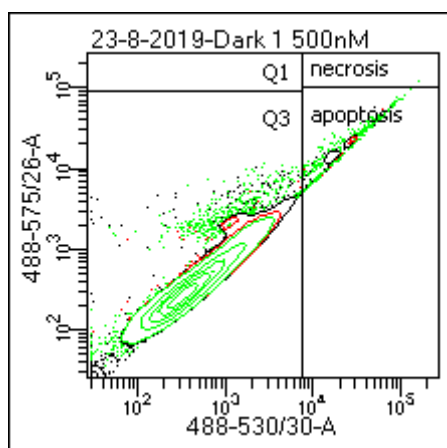
Tube: Dark 1 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,582	85.8	85.8
■ P2	7,147	83.3	71.5
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	6,557	91.7	65.6
☒ apoptosis	589	8.2	5.9



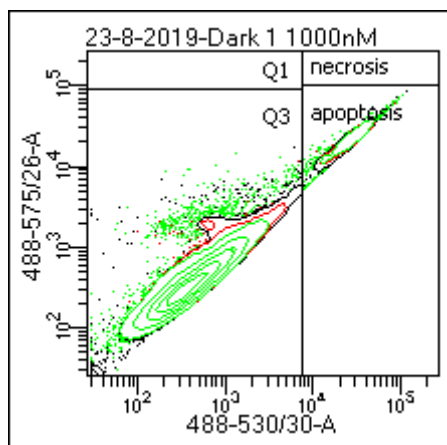
Tube: Dark 1 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,515	85.2	85.2
■ P2	7,004	82.3	70.0
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	6,576	93.9	65.8
☒ apoptosis	427	6.1	4.3



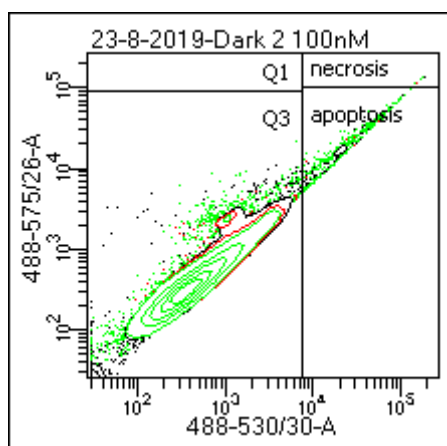
Tube: Dark 1 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,585	85.9	85.9
■ P2	7,063	82.3	70.6
☒ Q1	0	0.0	0.0
☒ necrosis	2	0.0	0.0
☒ Q3	6,654	94.2	66.5
☒ apoptosis	407	5.8	4.1



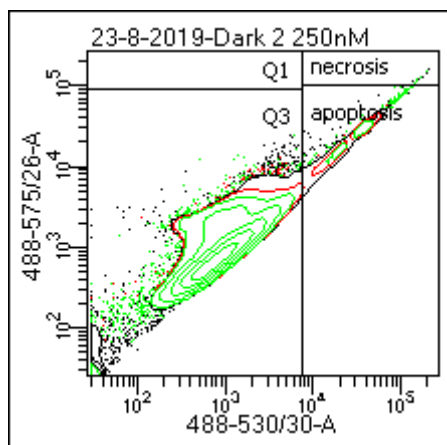
Tube: Dark 1 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,840	88.4	88.4
■ P2	7,505	84.9	75.0
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,006	93.4	70.1
☒ apoptosis	499	6.6	5.0



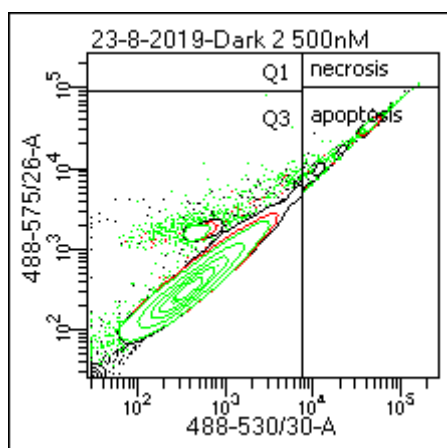
Tube: Dark 2 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,412	84.1	84.1
■ P2	6,881	81.8	68.8
☒ Q1	0	0.0	0.0
☒ necrosis	5	0.1	0.0
☒ Q3	6,491	94.3	64.9
☒ apoptosis	385	5.6	3.8



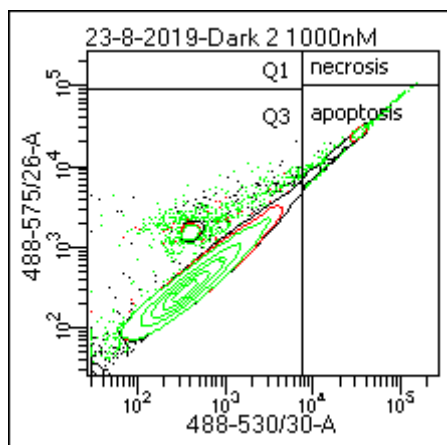
Tube: Dark 2 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,117	81.2	81.2
■ P2	6,661	82.1	66.6
☒ Q1	0	0.0	0.0
☒ necrosis	18	0.3	0.2
☒ Q3	6,109	91.7	61.1
☒ apoptosis	534	8.0	5.3



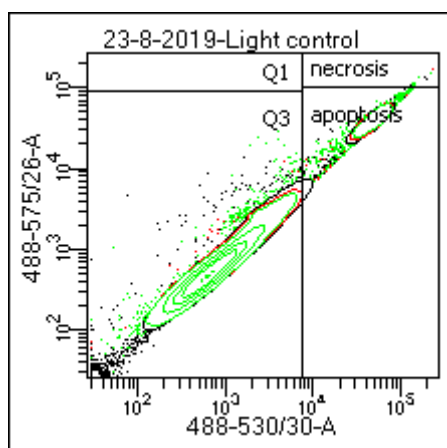
Tube: Dark 2 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,206	82.1	82.1
■ P2	6,687	81.5	66.9
☒ Q1	0	0.0	0.0
☒ necrosis	3	0.0	0.0
☒ Q3	6,325	94.6	63.2
☒ apoptosis	359	5.4	3.6



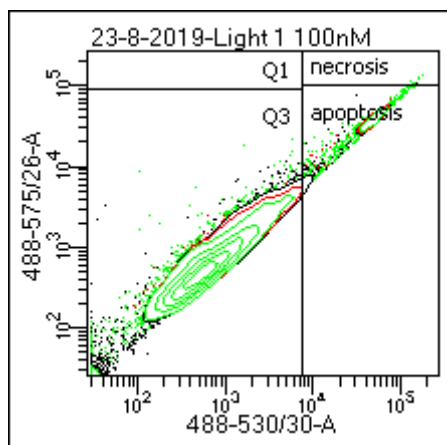
Tube: Dark 2 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,371	83.7	83.7
■ P2	6,699	80.0	67.0
☒ Q1	0	0.0	0.0
☒ necrosis	3	0.0	0.0
☒ Q3	6,308	94.2	63.1
☒ apoptosis	388	5.8	3.9



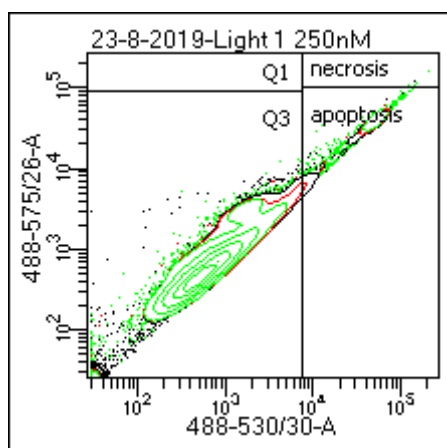
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	6,626	66.3	66.3
■ P2	5,485	82.8	54.8
☒ Q1	0	0.0	0.0
☒ necrosis	18	0.3	0.2
☒ Q3	4,874	88.9	48.7
☒ apoptosis	593	10.8	5.9



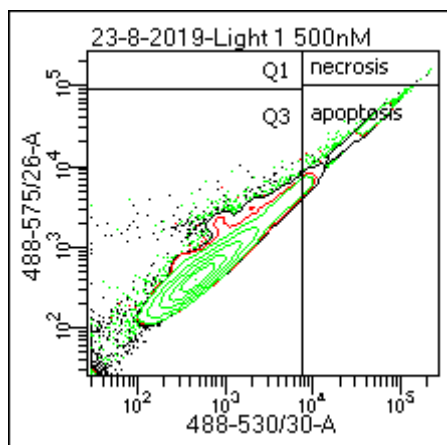
Tube: Light 1 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,702	77.0	77.0
■ P2	6,400	83.1	64.0
☒ Q1	0	0.0	0.0
☒ necrosis	16	0.2	0.2
☒ Q3	5,893	92.1	58.9
☒ apoptosis	491	7.7	4.9



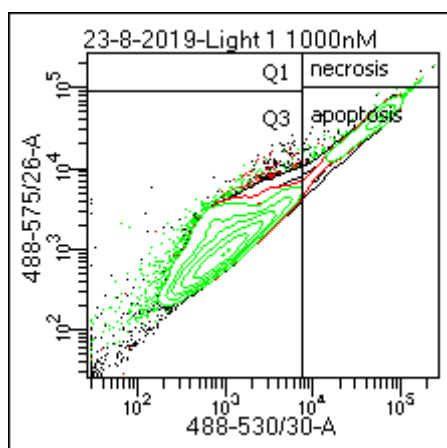
Tube: Light 1 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,595	75.9	75.9
■ P2	6,345	83.5	63.4
☒ Q1	0	0.0	0.0
☒ necrosis	9	0.1	0.1
☒ Q3	5,789	91.2	57.9
☒ apoptosis	547	8.6	5.5



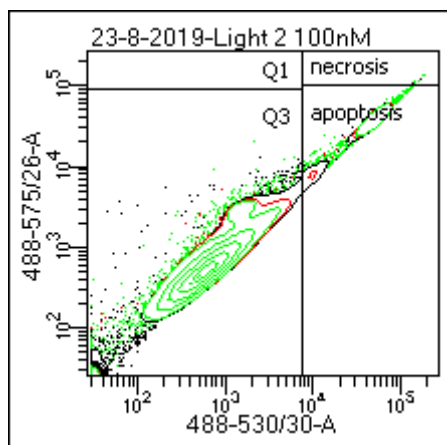
Tube: Light 1 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,633	76.3	76.3
■ P2	6,313	82.7	63.1
☒ Q1	0	0.0	0.0
☒ necrosis	9	0.1	0.1
☒ Q3	5,697	90.2	57.0
☒ apoptosis	607	9.6	6.1



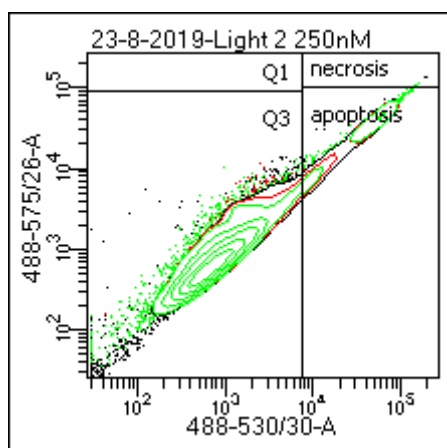
Tube: Light 1 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,207	82.1	82.1
■ P2	6,950	84.7	69.5
☒ Q1	0	0.0	0.0
☒ necrosis	31	0.4	0.3
☒ Q3	5,804	83.5	58.0
☒ apoptosis	1,115	16.0	11.2



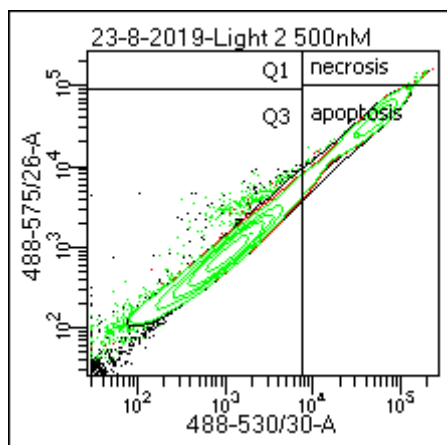
Tube: Light 2 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,130	71.3	71.3
■ P2	5,833	81.8	58.3
☒ Q1	0	0.0	0.0
☒ necrosis	11	0.2	0.1
☒ Q3	5,241	89.9	52.4
☒ apoptosis	581	10.0	5.8



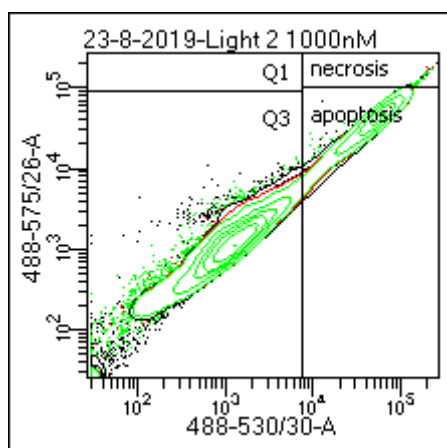
Tube: Light 2 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,129	81.3	81.3
■ P2	6,733	82.8	67.3
☒ Q1	0	0.0	0.0
☒ necrosis	17	0.3	0.2
☒ Q3	5,800	86.1	58.0
☒ apoptosis	916	13.6	9.2



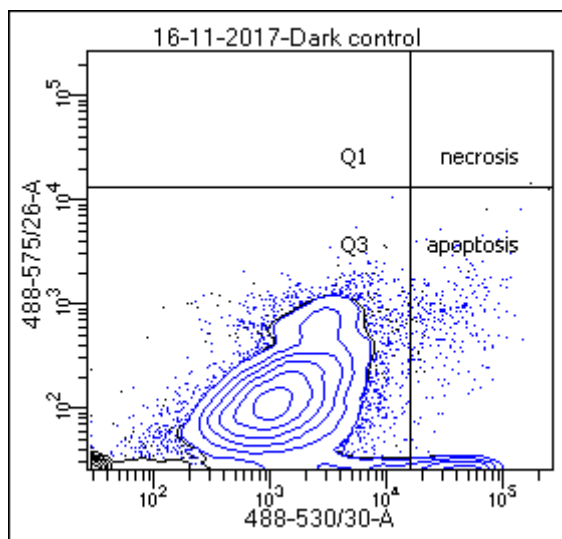
Tube: Light 2 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,719	77.2	77.2
■ P2	6,793	88.0	67.9
☒ Q1	0	0.0	0.0
☒ necrosis	60	0.9	0.6
☒ Q3	5,324	78.4	53.2
☒ apoptosis	1,409	20.7	14.1



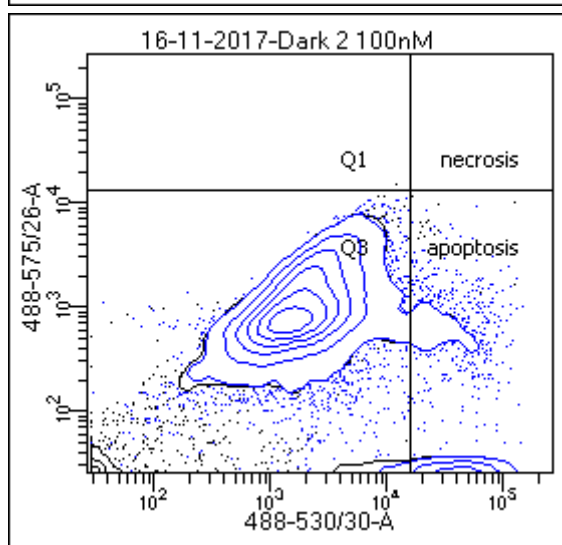
Tube: Light 2 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,764	77.6	77.6
■ P2	6,751	87.0	67.5
☒ Q1	0	0.0	0.0
☒ necrosis	70	1.0	0.7
☒ Q3	5,143	76.2	51.4
☒ apoptosis	1,538	22.8	15.4



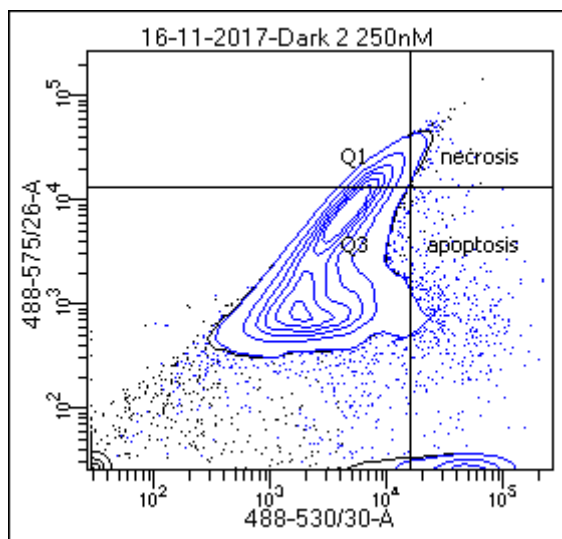
Tube: Dark control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,936	89.4	89.4
■ P2	1,971	22.1	19.7
■ P3	2,339	26.2	23.4
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,997	89.5	80.0
☒ apoptosis	939	10.5	9.4



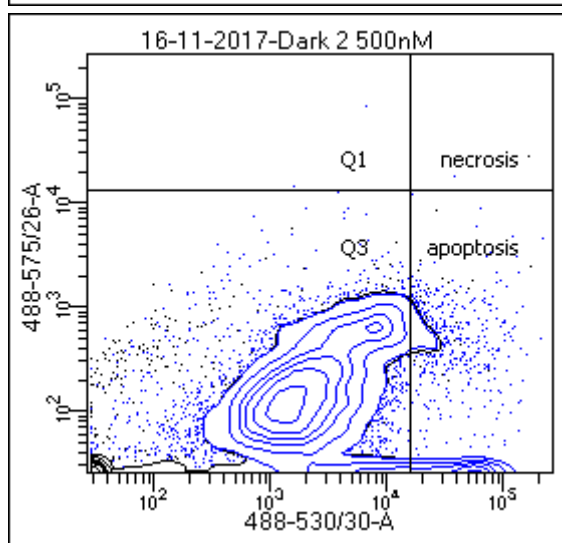
Tube: Dark 2 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,081	90.8	90.8
■ P2	2,426	26.7	24.3
■ P3	7,852	86.5	78.5
☒ Q1	1	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,921	87.2	79.2
☒ apoptosis	1,159	12.8	11.6



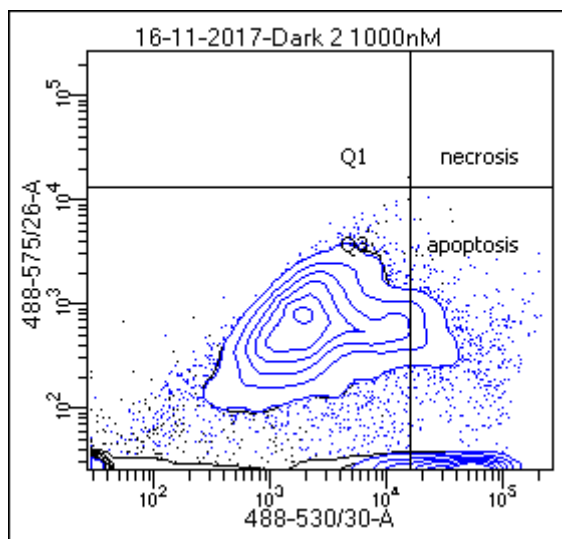
Tube: Dark 2 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,045	90.4	90.4
■ P2	4,138	45.7	41.4
■ P3	8,196	90.6	82.0
▣ Q1	762	8.4	7.6
▣ necrosis	117	1.3	1.2
▣ Q3	7,162	79.2	71.6
▣ apoptosis	1,004	11.1	10.0



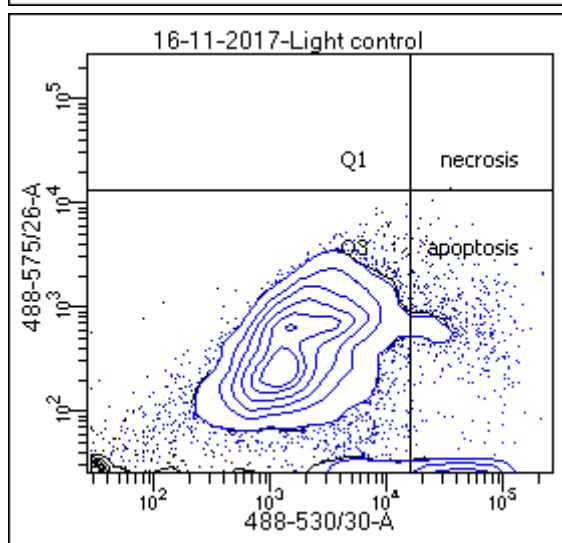
Tube: Dark 2 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,900	89.0	89.0
■ P2	3,169	35.6	31.7
■ P3	2,972	33.4	29.7
▣ Q1	3	0.0	0.0
▣ necrosis	1	0.0	0.0
▣ Q3	7,708	86.6	77.1
▣ apoptosis	1,188	13.3	11.9



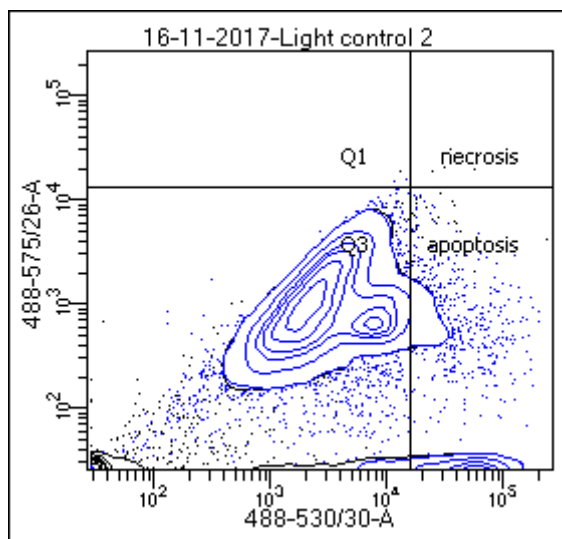
Tube: Dark 2 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,434	84.3	84.3
■ P2	3,766	44.7	37.7
■ P3	5,828	69.1	58.3
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,459	76.6	64.6
☒ apoptosis	1,975	23.4	19.8



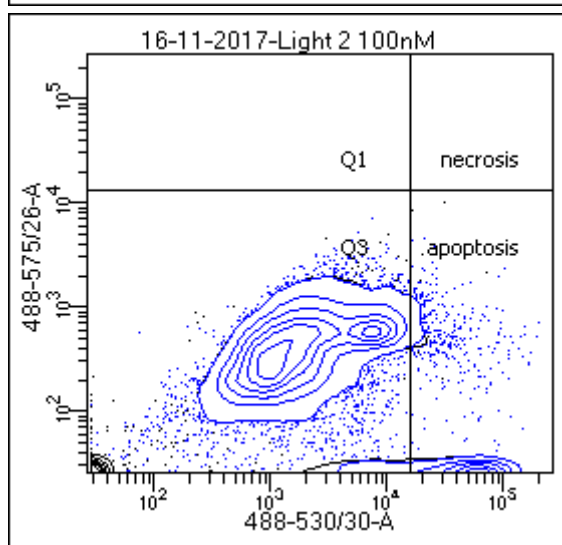
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,988	89.9	89.9
■ P2	2,496	27.8	25.0
■ P3	5,982	66.6	59.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,993	88.9	79.9
☒ apoptosis	995	11.1	10.0



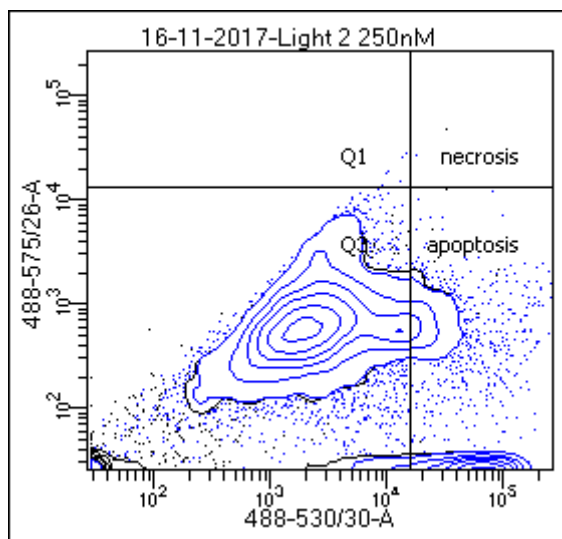
Tube: Light control 2

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,823	88.2	88.2
■ P2	3,935	44.6	39.4
■ P3	7,387	83.7	73.9
☒ Q1	7	0.1	0.1
☒ necrosis	0	0.0	0.0
☒ Q3	7,501	85.0	75.0
☒ apoptosis	1,315	14.9	13.2



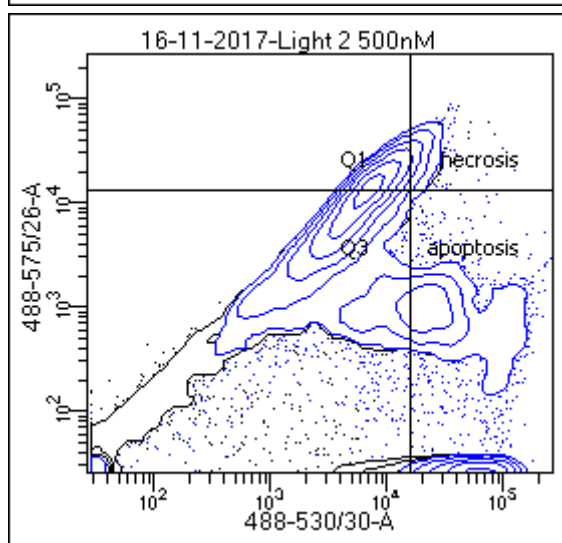
Tube: Light 2 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,981	89.8	89.8
■ P2	3,043	33.9	30.4
■ P3	5,979	66.6	59.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,710	85.8	77.1
☒ apoptosis	1,271	14.2	12.7



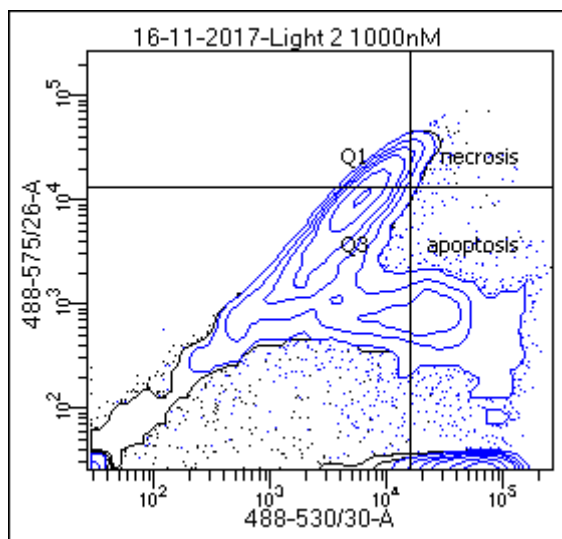
Tube: Light 2 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,743	87.4	87.4
■ P2	3,793	43.4	37.9
■ P3	6,522	74.6	65.2
□ Q1	7	0.1	0.1
□ necrosis	1	0.0	0.0
□ Q3	6,738	77.1	67.4
□ apoptosis	1,997	22.8	20.0



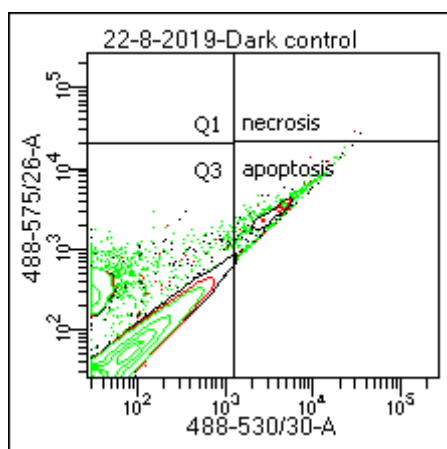
Tube: Light 2 500nM

Population	#Events	%Parent	%Total
■ All Events	8,051	####	100.0
■ P1	6,413	79.7	79.7
■ P2	4,839	75.5	60.1
■ P3	5,286	82.4	65.7
□ Q1	1,203	18.8	14.9
□ necrosis	228	3.6	2.8
□ Q3	3,049	47.5	37.9
□ apoptosis	1,933	30.1	24.0



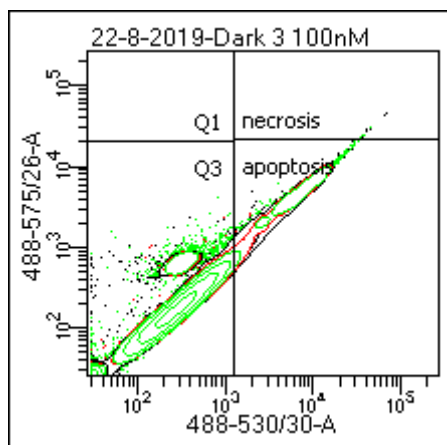
Tube: Light 2 1000nM

Population	#Events	%Parent	%Total
■ All Events	8,150	####	100.0
■ P1	6,653	81.6	81.6
■ P2	4,783	71.9	58.7
■ P3	5,028	75.6	61.7
☒ Q1	703	10.6	8.6
☒ necrosis	125	1.9	1.5
☒ Q3	3,432	51.6	42.1
☒ apoptosis	2,393	36.0	29.4



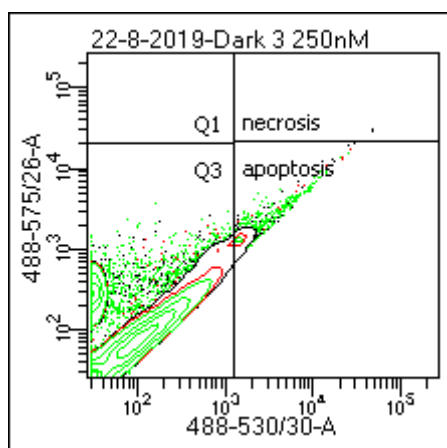
Tube: Dark control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,424	74.2	74.2
■ P2	6,228	83.9	62.3
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,834	93.7	58.3
☒ apoptosis	394	6.3	3.9



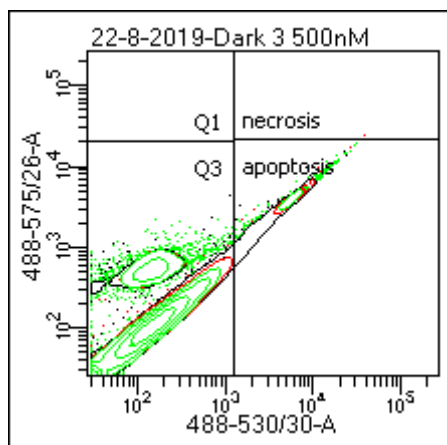
Tube: Dark 3 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	6,694	66.9	66.9
■ P2	5,704	85.2	57.0
☒ Q1	0	0.0	0.0
☒ necrosis	7	0.1	0.1
☒ Q3	4,727	82.9	47.3
☒ apoptosis	970	17.0	9.7



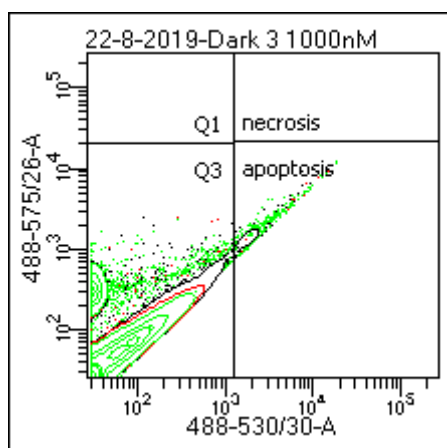
Tube: Dark 3 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,812	78.1	78.1
■ P2	6,586	84.3	65.9
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,240	94.7	62.4
☒ apoptosis	346	5.3	3.5



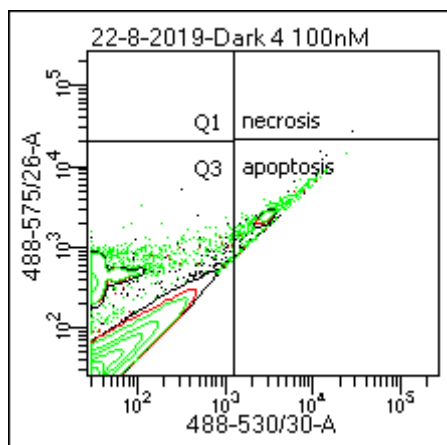
Tube: Dark 3 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,067	70.7	70.7
■ P2	5,948	84.2	59.5
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,388	90.6	53.9
☒ apoptosis	560	9.4	5.6



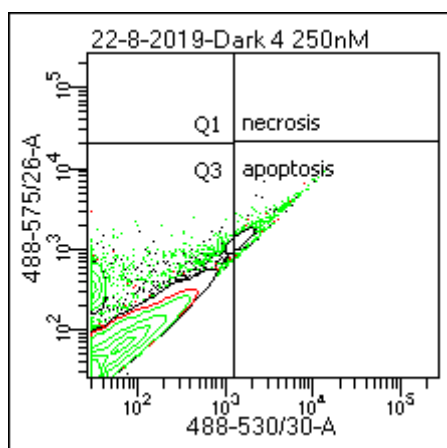
Tube: Dark 3 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,530	75.3	75.3
■ P2	6,353	84.4	63.5
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,068	95.5	60.7
☒ apoptosis	285	4.5	2.8



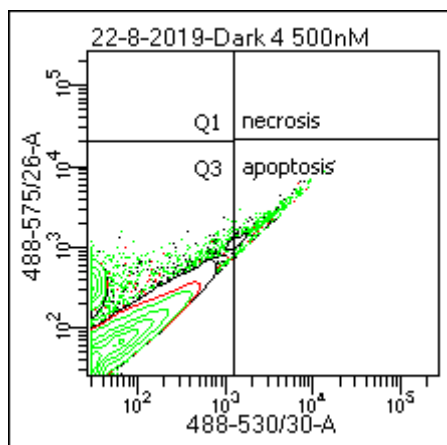
Tube: Dark 4 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,124	81.2	81.2
■ P2	6,723	82.8	67.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,375	94.8	63.7
☒ apoptosis	348	5.2	3.5



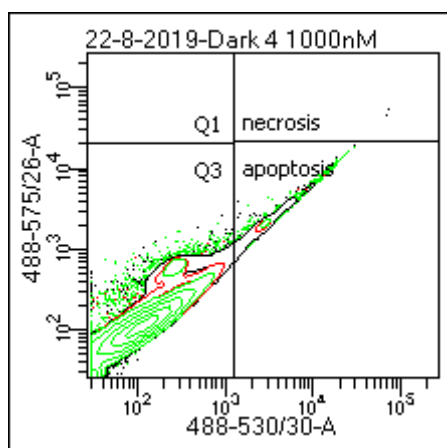
Tube: Dark 4 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,313	73.1	73.1
■ P2	6,264	85.7	62.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,980	95.5	59.8
☒ apoptosis	284	4.5	2.8



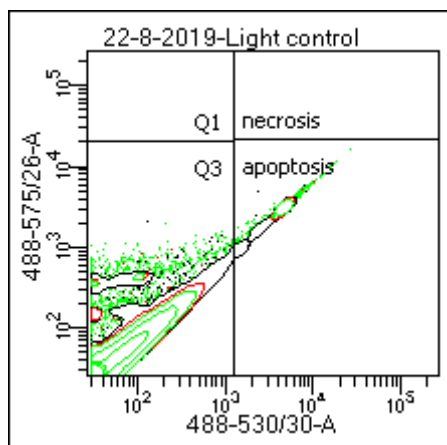
Tube: Dark 4 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	8,121	81.2	81.2
■ P2	6,880	84.7	68.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,616	96.2	66.2
☒ apoptosis	264	3.8	2.6



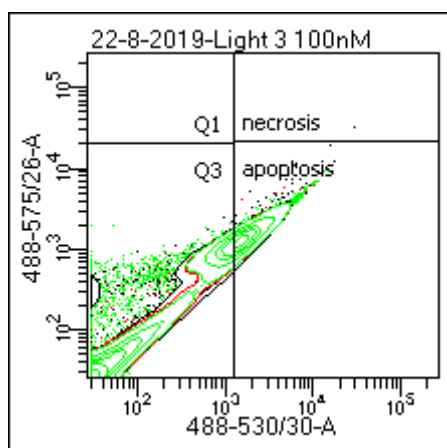
Tube: Dark 4 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,614	76.1	76.1
■ P2	6,386	83.9	63.9
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,778	90.5	57.8
☒ apoptosis	608	9.5	6.1



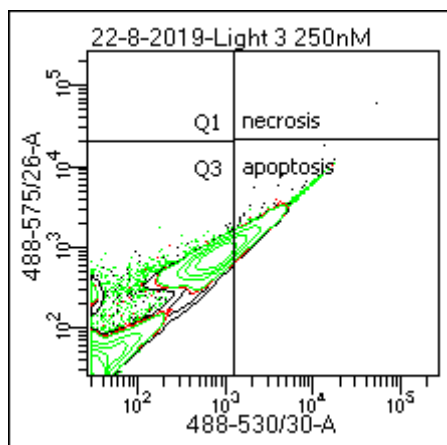
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	6,692	66.9	66.9
■ P2	5,662	84.6	56.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,334	94.2	53.3
☒ apoptosis	328	5.8	3.3



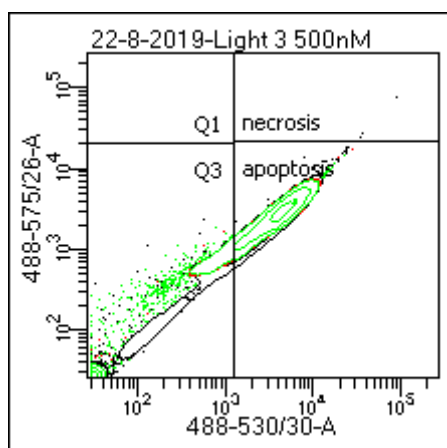
Tube: Light 3 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,590	75.9	75.9
■ P2	6,962	91.7	69.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,608	80.6	56.1
☒ apoptosis	1,354	19.4	13.5



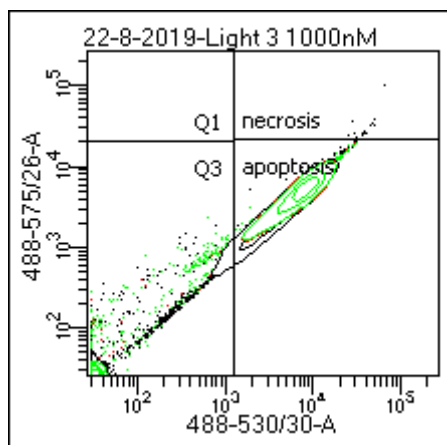
Tube: Light 3 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,986	79.9	79.9
■ P2	7,590	95.0	75.9
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,789	89.4	67.9
☒ apoptosis	801	10.6	8.0



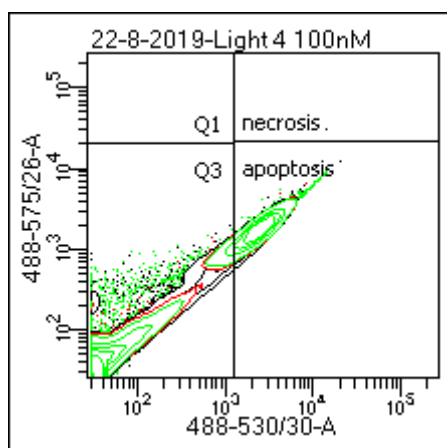
Tube: Light 3 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	2,940	29.4	29.4
■ P2	2,832	96.3	28.3
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	1,273	45.0	12.7
☒ apoptosis	1,559	55.0	15.6



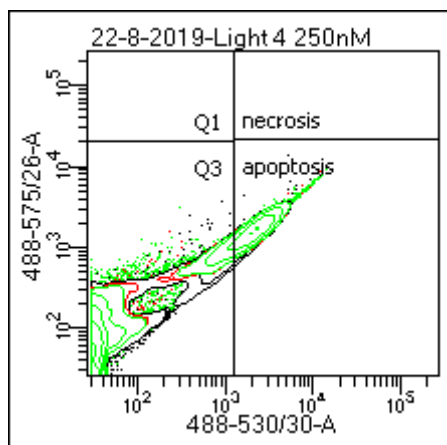
Tube: Light 3 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	3,976	39.8	39.8
■ P2	3,656	92.0	36.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	1,547	42.3	15.5
☒ apoptosis	2,109	57.7	21.1



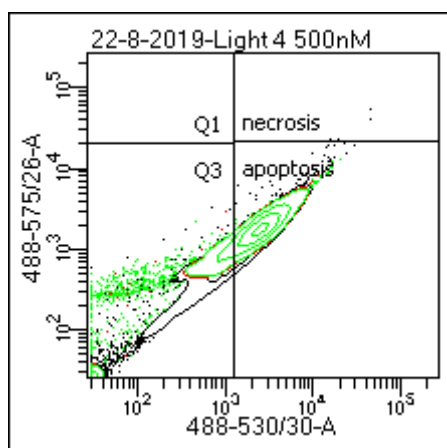
Tube: Light4 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	7,366	73.7	73.7
■ P2	6,728	91.3	67.3
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,216	77.5	52.2
☒ apoptosis	1,512	22.5	15.1



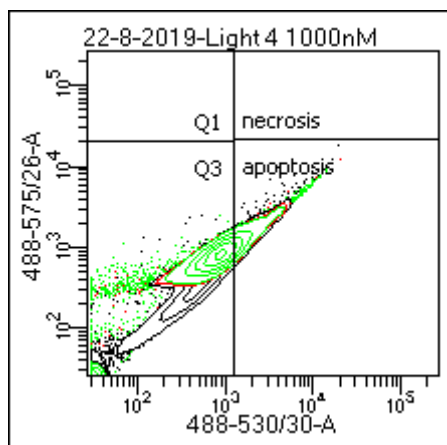
Tube: Light 4 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	6,823	68.2	68.2
■ P2	6,483	95.0	64.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,327	82.2	53.3
☒ apoptosis	1,156	17.8	11.6



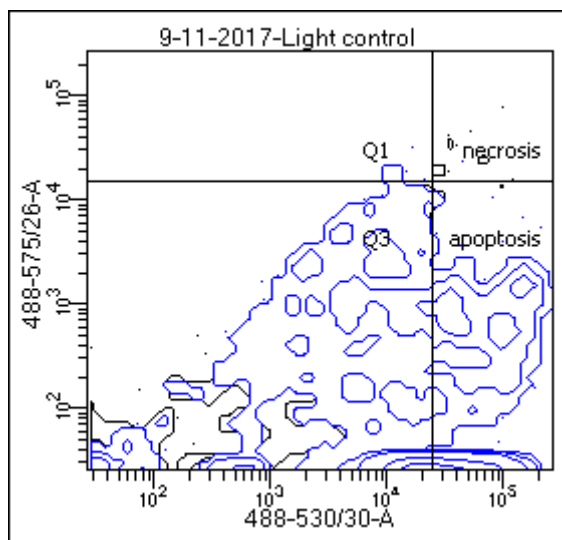
Tube: Light 4 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	4,521	45.2	45.2
■ P2	4,112	91.0	41.1
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	1,535	37.3	15.4
☒ apoptosis	2,577	62.7	25.8



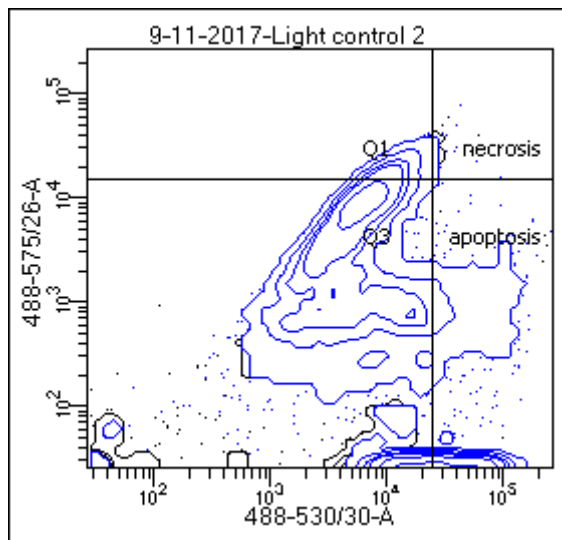
Tube: Light4 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	5,101	51.0	51.0
■ P2	4,465	87.5	44.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	3,633	81.4	36.3
☒ apoptosis	832	18.6	8.3



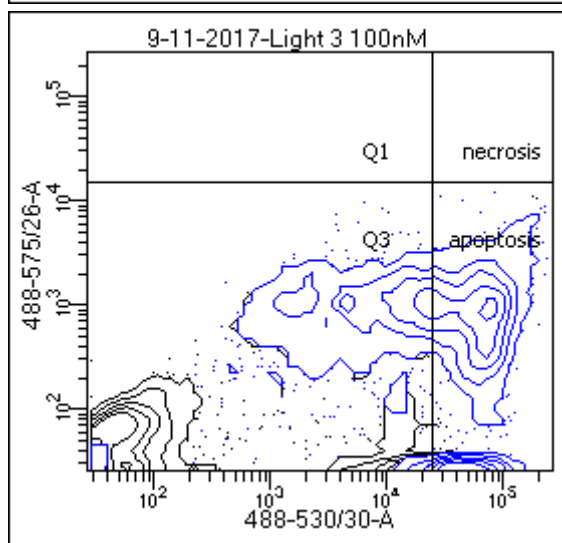
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	1,957	####	100.0
■ P1	1,471	75.2	75.2
■ P2	1,227	83.4	62.7
■ P3	473	32.2	24.2
☒ Q1	6	0.4	0.3
☒ necrosis	3	0.2	0.2
☒ Q3	777	52.8	39.7
☒ apoptosis	685	46.6	35.0



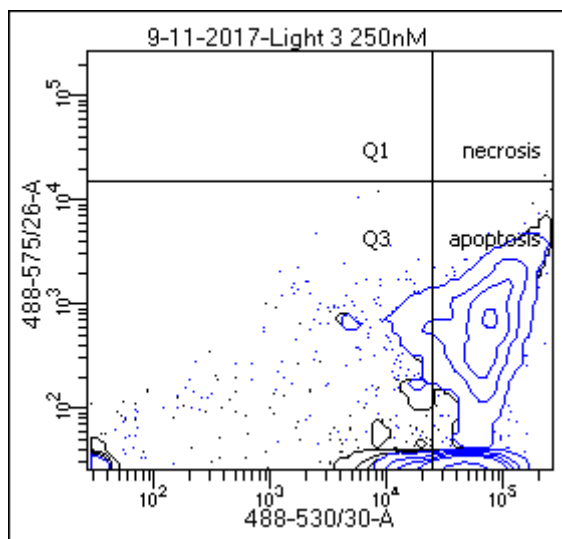
Tube: Light control 2

Population	#Events	%Parent	%Total
■ All Events	3,102	####	100.0
■ P1	2,682	86.5	86.5
■ P2	1,990	74.2	64.2
■ P3	1,792	66.8	57.8
▣ Q1	141	5.3	4.5
▣ necrosis	15	0.6	0.5
▣ Q3	1,880	70.1	60.6
▣ apoptosis	646	24.1	20.8



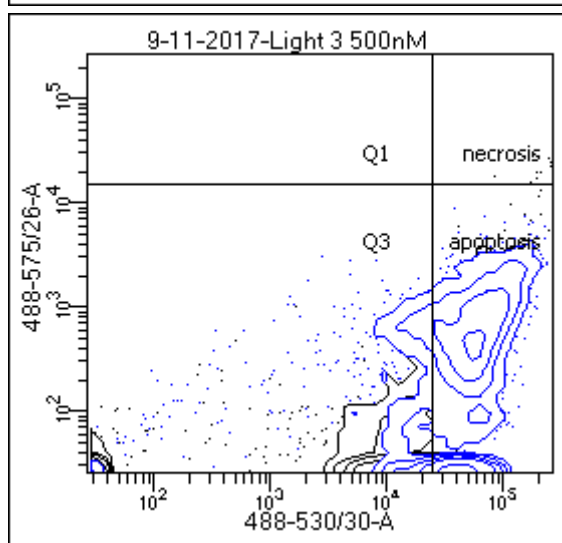
Tube: Light 3 100nM

Population	#Events	%Parent	%Total
■ All Events	3,400	####	100.0
■ P1	1,963	57.7	57.7
■ P2	1,609	82.0	47.3
■ P3	1,465	74.6	43.1
▣ Q1	0	0.0	0.0
▣ necrosis	0	0.0	0.0
▣ Q3	833	42.4	24.5
▣ apoptosis	1,130	57.6	33.2



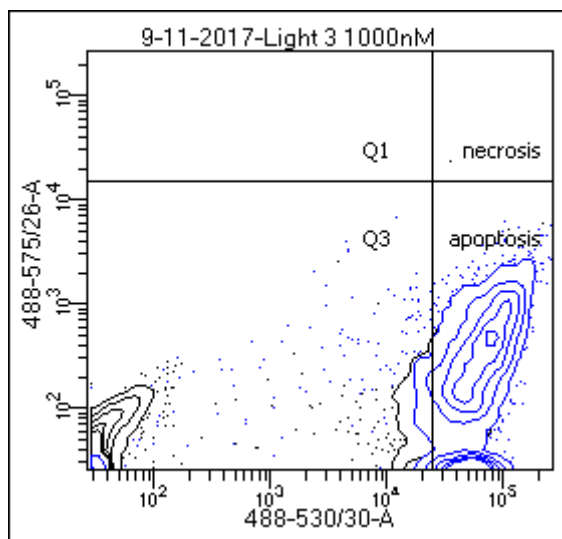
Tube: Light 3 250nM

Population	#Events	%Parent	%Total
■ All Events	3,103	###	100.0
■ P1	2,143	69.1	69.1
■ P2	2,034	94.9	65.5
■ P3	1,035	48.3	33.4
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	394	18.4	12.7
☒ apoptosis	1,749	81.6	56.4



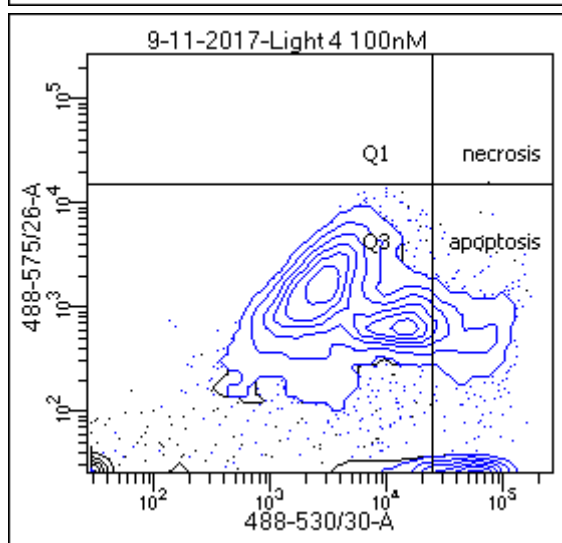
Tube: Light 3 500nM

Population	#Events	%Parent	%Total
■ All Events	3,185	###	100.0
■ P1	1,958	61.5	61.5
■ P2	1,845	94.2	57.9
■ P3	949	48.5	29.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	462	23.6	14.5
☒ apoptosis	1,496	76.4	47.0



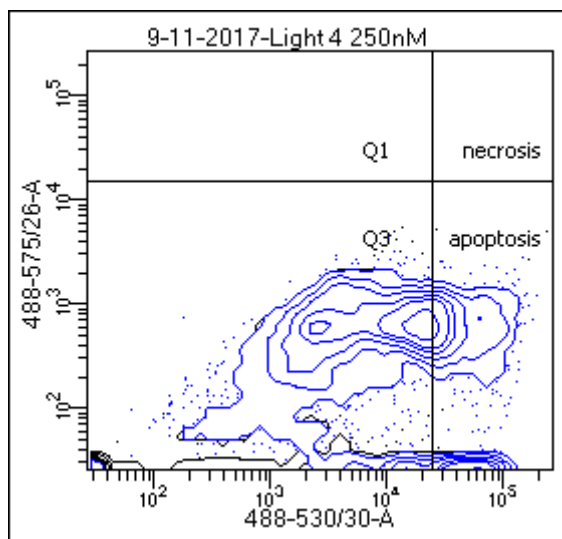
Tube: Light 3 1000nM

Population	#Events	%Parent	%Total
■ All Events	3,175	###	100.0
■ P1	1,842	58.0	58.0
■ P2	1,734	94.1	54.6
■ P3	941	51.1	29.6
□ Q1	0	0.0	0.0
□ necrosis	2	0.1	0.1
□ Q3	271	14.7	8.5
□ apoptosis	1,569	85.2	49.4



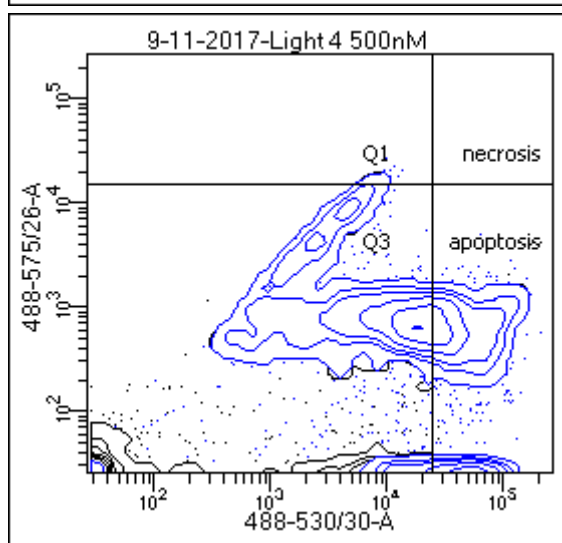
Tube: Light4 100nM

Population	#Events	%Parent	%Total
■ All Events	3,455	###	100.0
■ P1	3,140	90.9	90.9
■ P2	1,811	57.7	52.4
■ P3	2,587	82.4	74.9
□ Q1	0	0.0	0.0
□ necrosis	0	0.0	0.0
□ Q3	2,485	79.1	71.9
□ apoptosis	655	20.9	19.0



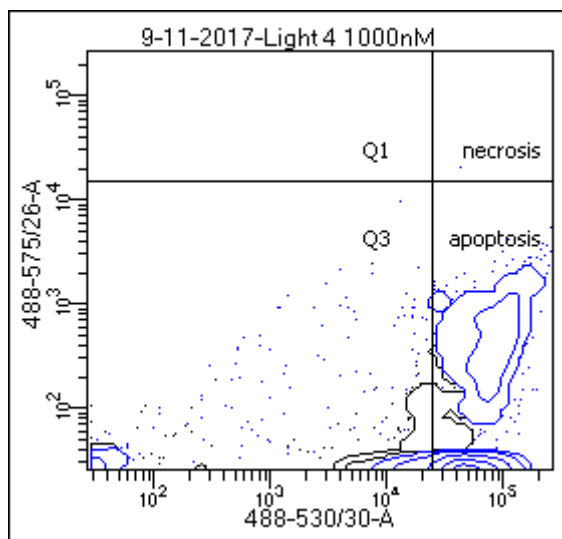
Tube: Light 4 250nM

Population	#Events	%Parent	%Total
■ All Events	3,073	####	100.0
■ P1	2,660	86.6	86.6
■ P2	1,782	67.0	58.0
■ P3	1,846	69.4	60.1
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	1,852	69.6	60.3
☒ apoptosis	808	30.4	26.3



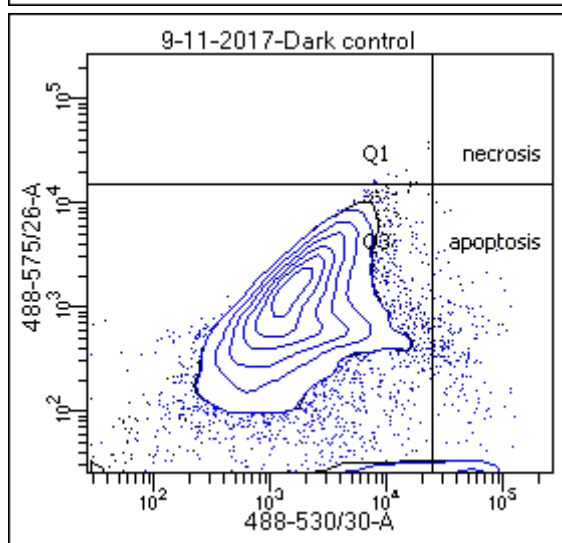
Tube: Light 4 500nM

Population	#Events	%Parent	%Total
■ All Events	3,493	####	100.0
■ P1	2,560	73.3	73.3
■ P2	1,917	74.9	54.9
■ P3	2,000	78.1	57.3
☒ Q1	25	1.0	0.7
☒ necrosis	0	0.0	0.0
☒ Q3	1,567	61.2	44.9
☒ apoptosis	968	37.8	27.7



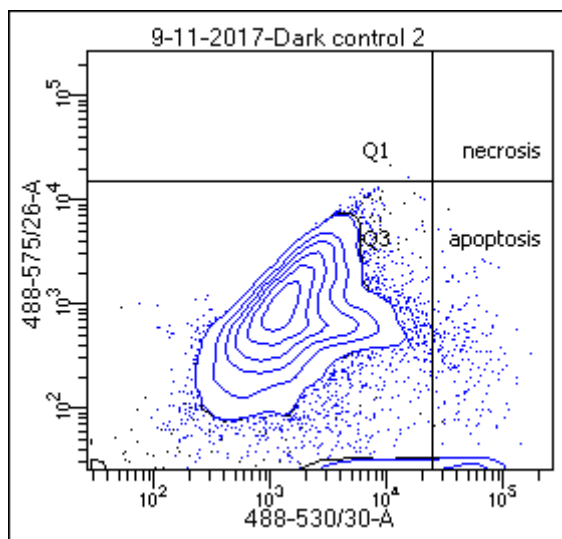
Tube: Light4 1000nM

Population	#Events	%Parent	%Total
■ All Events	2,446	####	100.0
■ P1	1,497	61.2	61.2
■ P2	1,373	91.7	56.1
■ P3	409	27.3	16.7
☒ Q1	0	0.0	0.0
☒ necrosis	2	0.1	0.1
☒ Q3	241	16.1	9.9
☒ apoptosis	1,254	83.8	51.3



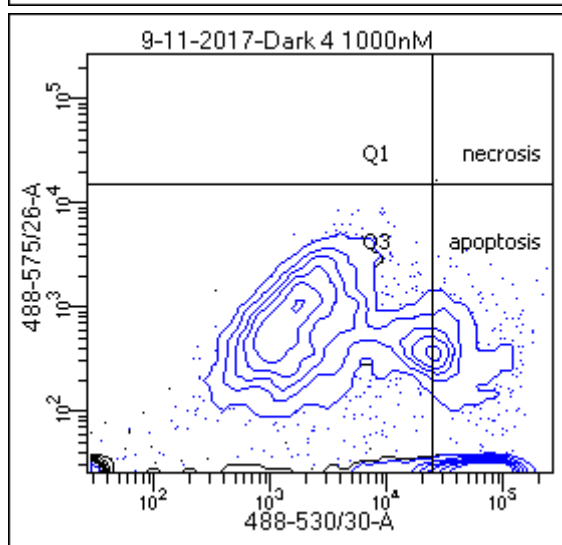
Tube: Dark control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,491	94.9	94.9
■ P2	1,929	20.3	19.3
■ P3	8,030	84.6	80.3
☒ Q1	4	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	9,050	95.4	90.5
☒ apoptosis	437	4.6	4.4



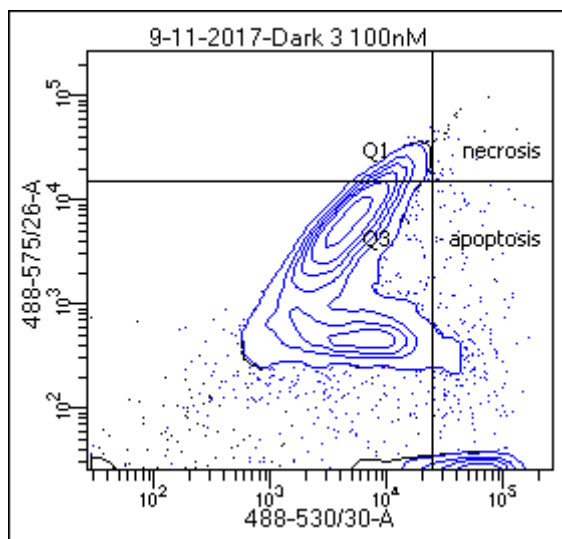
Tube: Dark control 2

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,533	95.3	95.3
■ P2	1,908	20.0	19.1
■ P3	7,745	81.2	77.4
☒ Q1	1	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	9,017	94.6	90.2
☒ apoptosis	515	5.4	5.1



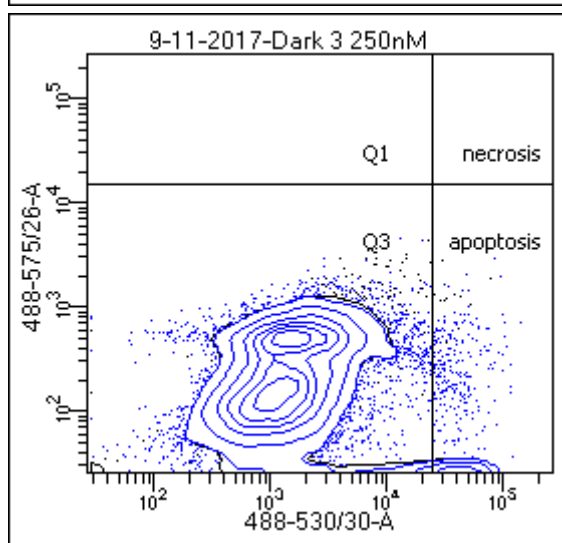
Tube: Dark 4 1000nM

Population	#Events	%Parent	%Total
■ All Events	3,049	####	100.0
■ P1	2,784	91.3	91.3
■ P2	1,346	48.3	44.1
■ P3	1,900	68.2	62.3
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	1,974	70.9	64.7
☒ apoptosis	810	29.1	26.6



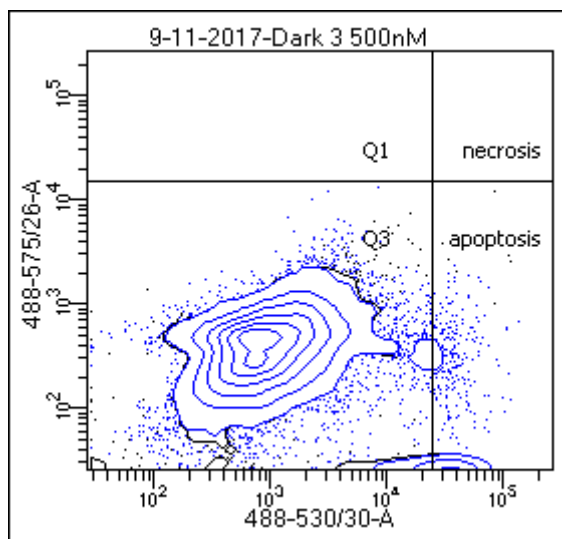
Tube: Dark 3 100nM

Population	#Events	%Parent	%Total
■ All Events	5,086	####	100.0
■ P1	4,758	93.6	93.6
■ P2	2,906	61.1	57.1
■ P3	4,076	85.7	80.1
☒ Q1	272	5.7	5.3
☒ necrosis	18	0.4	0.4
☒ Q3	3,856	81.0	75.8
☒ apoptosis	612	12.9	12.0



Tube: Dark 3 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,437	94.4	94.4
■ P2	1,784	18.9	17.8
■ P3	4,354	46.1	43.5
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	8,738	92.6	87.4
☒ apoptosis	699	7.4	7.0

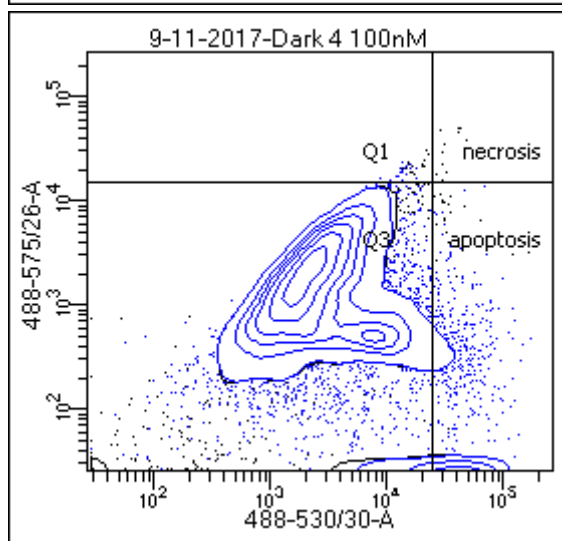


Tube: Dark 3 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,499	95.0	95.0
■ P2	1,403	14.8	14.0
■ P3	6,881	72.4	68.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	8,911	93.8	89.1
☒ apoptosis	588	6.2	5.9

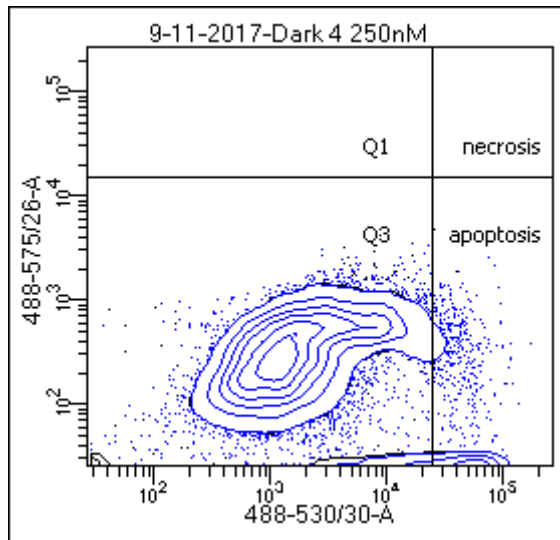
Tube: Dark 3 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,462	94.6	94.6
■ P2	3,649	38.6	36.5
■ P3	7,921	83.7	79.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	8,342	88.2	83.4
☒ apoptosis	1,120	11.8	11.2



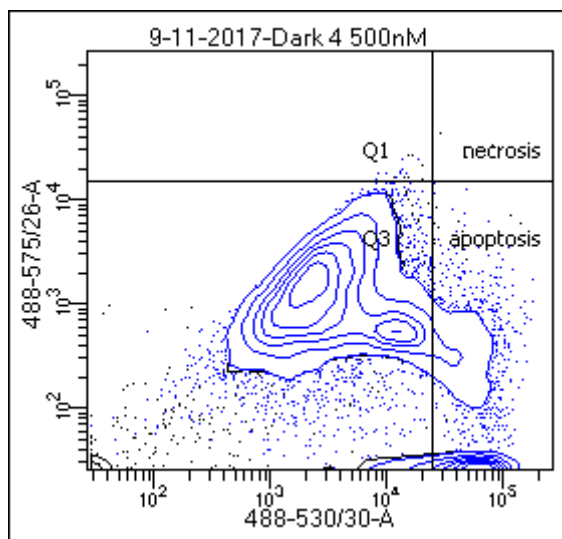
Tube: Dark 4 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,380	93.8	93.8
■ P2	3,567	38.0	35.7
■ P3	8,120	86.6	81.2
☒ Q1	25	0.3	0.2
☒ necrosis	0	0.0	0.0
☒ Q3	8,540	91.0	85.4
☒ apoptosis	815	8.7	8.2



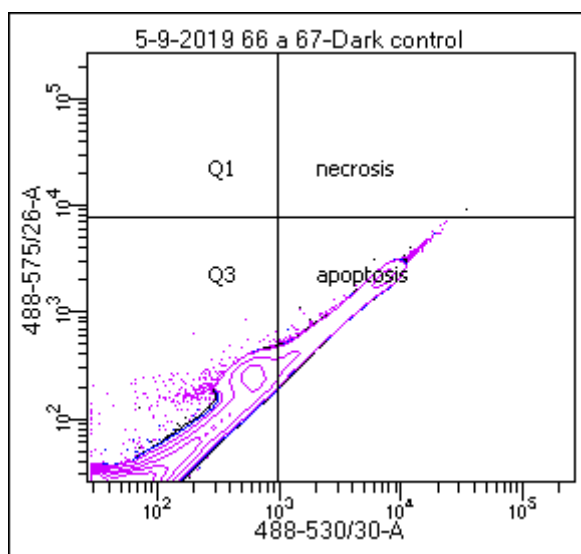
Tube: Dark 4 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,473	94.7	94.7
■ P2	2,642	27.9	26.4
■ P3	5,684	60.0	56.8
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	8,601	90.8	86.0
☒ apoptosis	872	9.2	8.7



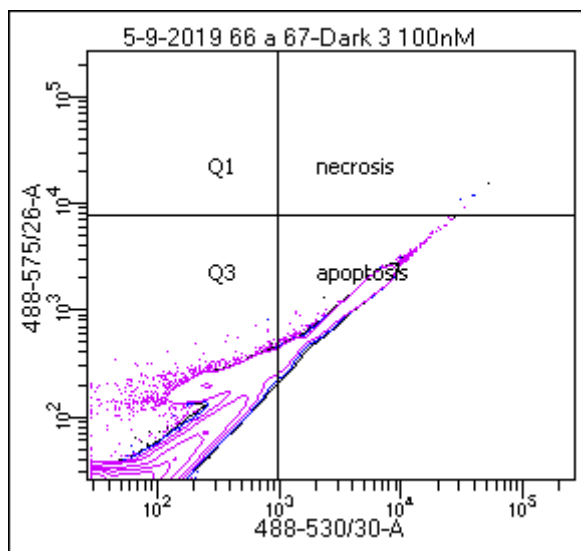
Tube: Dark 4 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,324	93.2	93.2
■ P2	4,755	51.0	47.6
■ P3	7,609	81.6	76.1
☒ Q1	21	0.2	0.2
☒ necrosis	1	0.0	0.0
☒ Q3	7,472	80.1	74.7
☒ apoptosis	1,830	19.6	18.3



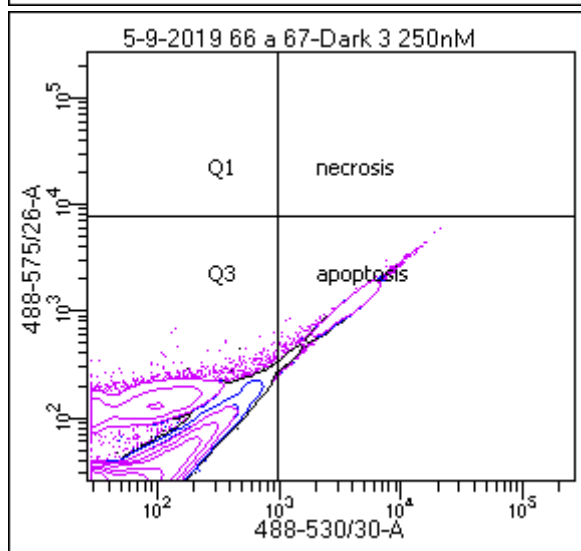
Tube: Dark control

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,357	93.6	93.6
■ P2	8,015	85.7	80.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,293	78.5	62.9
☒ apoptosis	1,722	21.5	17.2



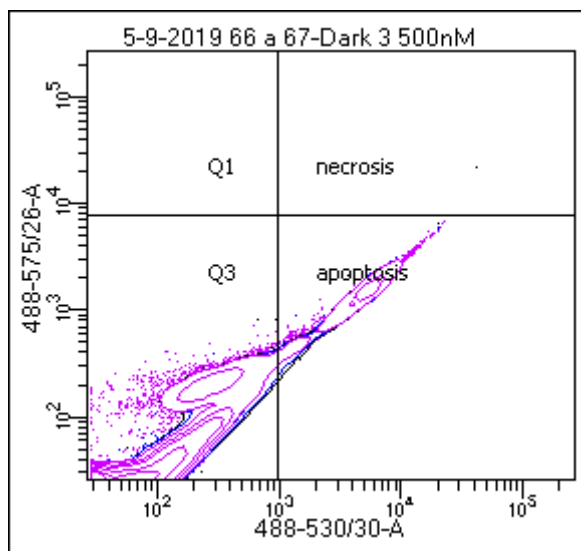
Tube: Dark 3 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,242	92.4	92.4
■ P2	7,751	83.9	77.5
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	6,703	86.5	67.0
☒ apoptosis	1,047	13.5	10.5



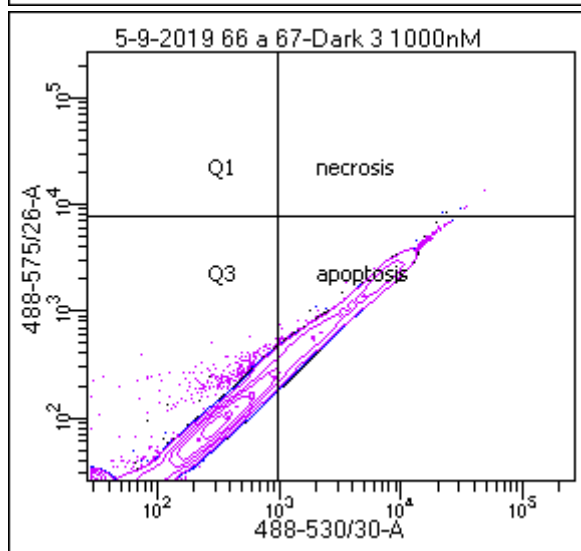
Tube: Dark 3 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,243	92.4	92.4
■ P2	7,759	83.9	77.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,053	90.9	70.5
☒ apoptosis	706	9.1	7.1



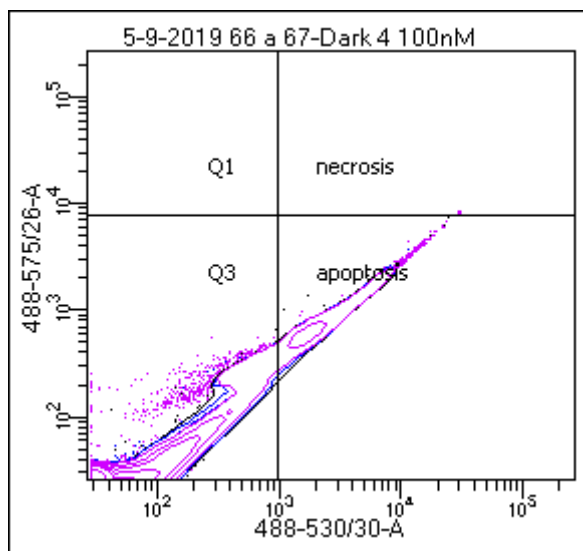
Tube: Dark 3 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,320	93.2	93.2
■ P2	7,958	85.4	79.6
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,751	84.8	67.5
☒ apoptosis	1,207	15.2	12.1



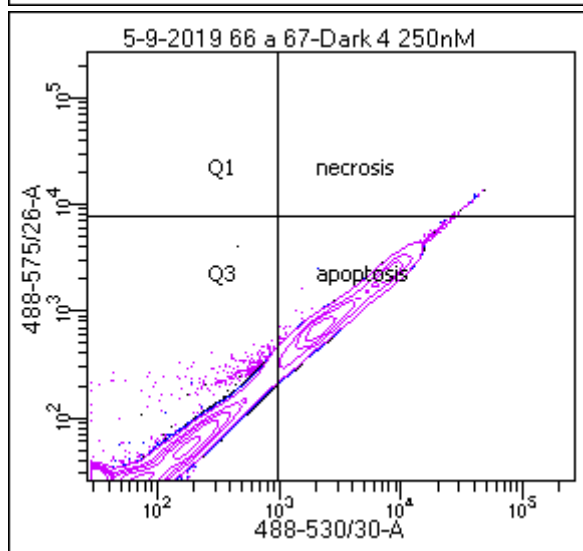
Tube: Dark 3 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,454	94.5	94.5
■ P2	8,186	86.6	81.9
☒ Q1	0	0.0	0.0
☒ necrosis	3	0.0	0.0
☒ Q3	5,389	65.8	53.9
☒ apoptosis	2,794	34.1	27.9



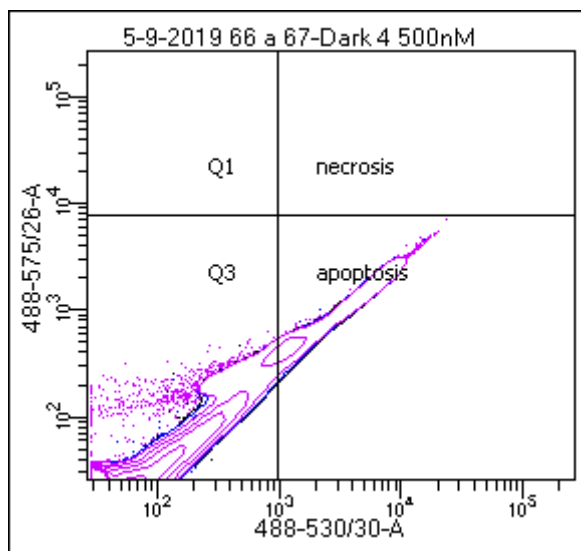
Tube: Dark 4 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,152	91.5	91.5
■ P2	7,743	84.6	77.4
☒ Q1	0	0.0	0.0
☒ necrosis	4	0.1	0.0
☒ Q3	6,362	82.2	63.6
☒ apoptosis	1,377	17.8	13.8



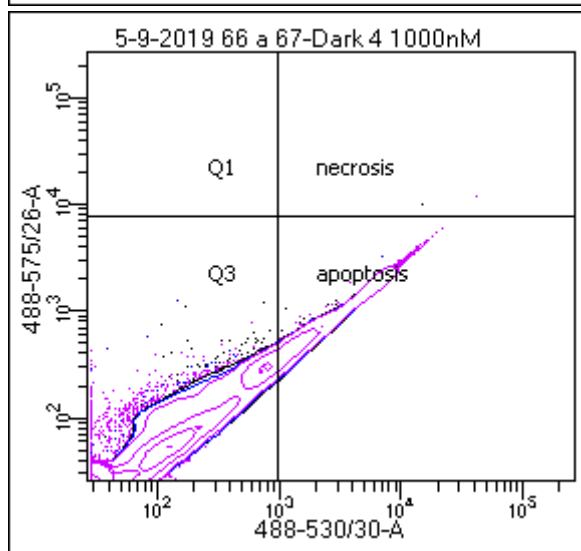
Tube: Dark 4 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,723	97.2	97.2
■ P2	8,840	90.9	88.4
☒ Q1	0	0.0	0.0
☒ necrosis	32	0.4	0.3
☒ Q3	5,781	65.4	57.8
☒ apoptosis	3,027	34.2	30.3



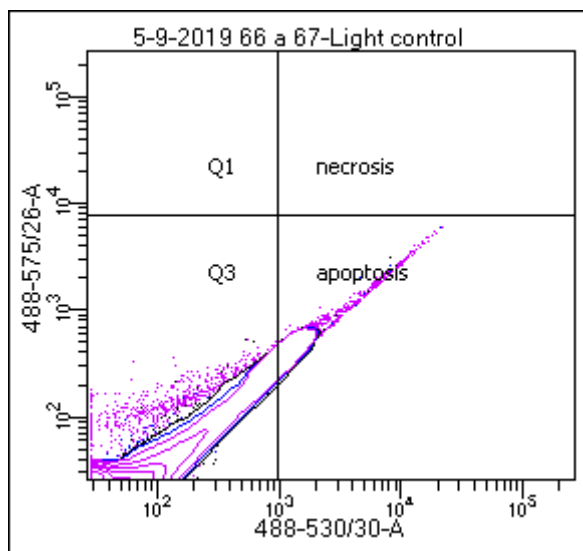
Tube: Dark 4 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,313	93.1	93.1
■ P2	7,936	85.2	79.4
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	6,547	82.5	65.5
☒ apoptosis	1,388	17.5	13.9



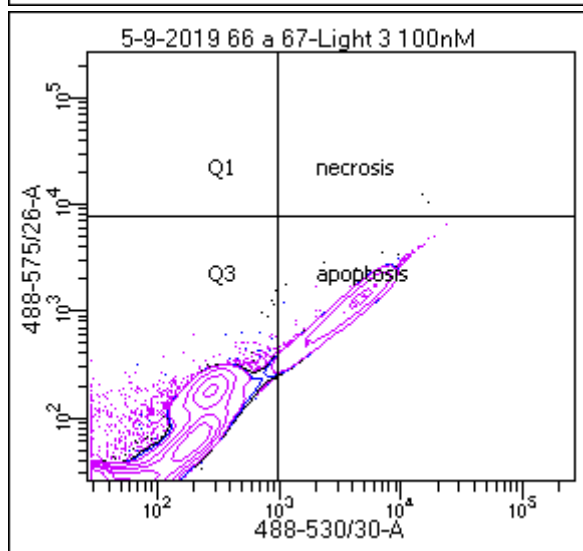
Tube: Dark 4 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,425	94.2	94.2
■ P2	8,265	87.7	82.6
☒ Q1	0	0.0	0.0
☒ necrosis	1	0.0	0.0
☒ Q3	7,044	85.2	70.4
☒ apoptosis	1,220	14.8	12.2



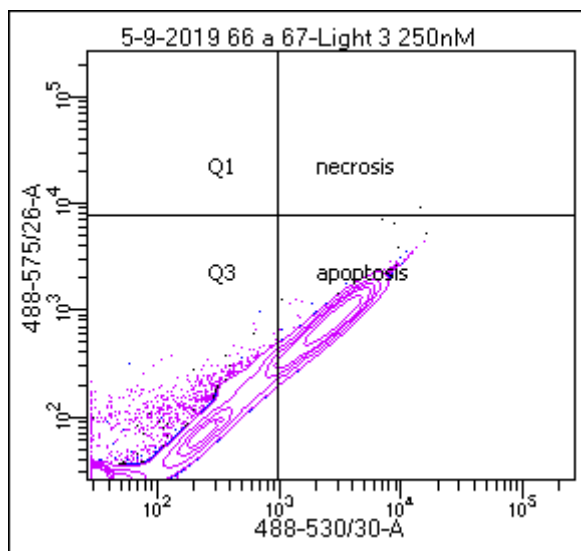
Tube: Light control

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	8,678	86.8	86.8
■ P2	6,921	79.8	69.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,175	89.2	61.8
☒ apoptosis	746	10.8	7.5



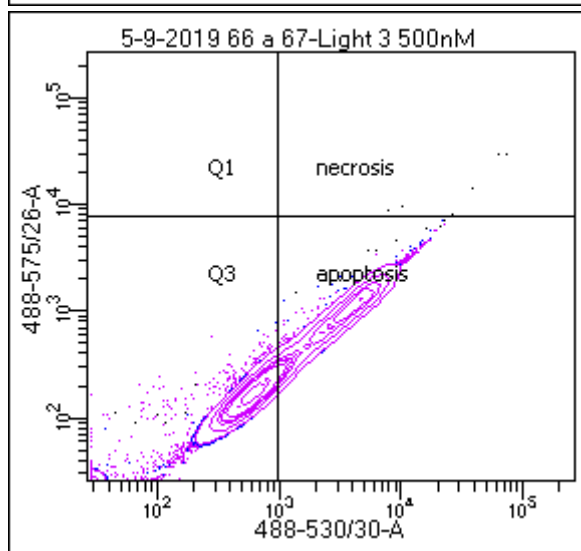
Tube: Light 3 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,620	96.2	96.2
■ P2	8,904	92.6	89.0
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	7,337	82.4	73.4
☒ apoptosis	1,567	17.6	15.7



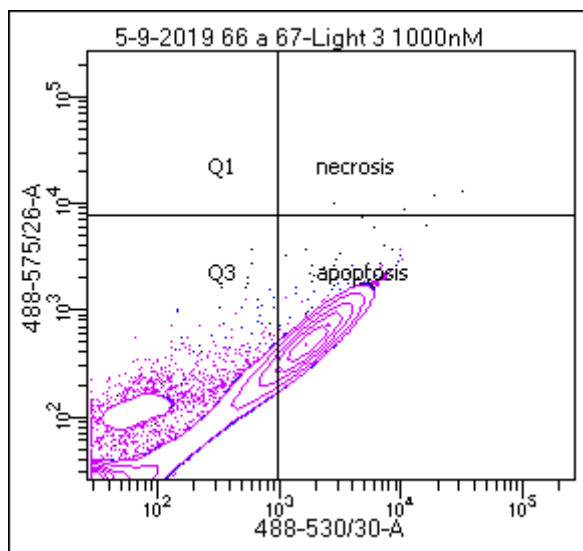
Tube: Light 3 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,935	99.4	99.4
■ P2	9,623	96.9	96.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	6,203	64.5	62.0
☒ apoptosis	3,420	35.5	34.2



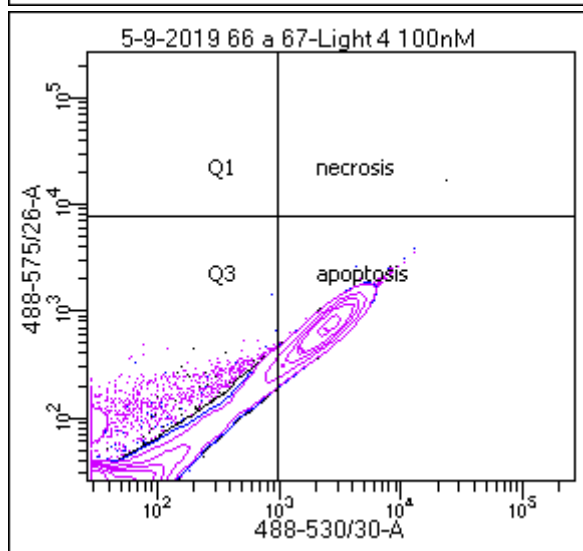
Tube: Light 3 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,968	99.7	99.7
■ P2	9,542	95.7	95.4
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	4,919	51.6	49.2
☒ apoptosis	4,623	48.4	46.2



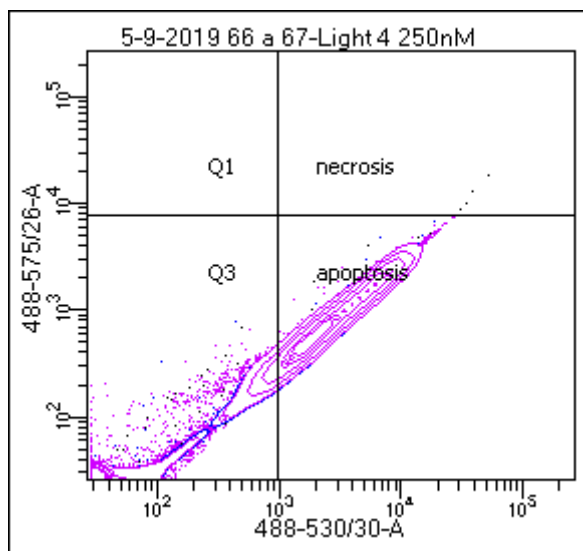
Tube: Light 3 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,939	99.4	99.4
■ P2	9,420	94.8	94.2
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,308	56.3	53.1
☒ apoptosis	4,112	43.7	41.1



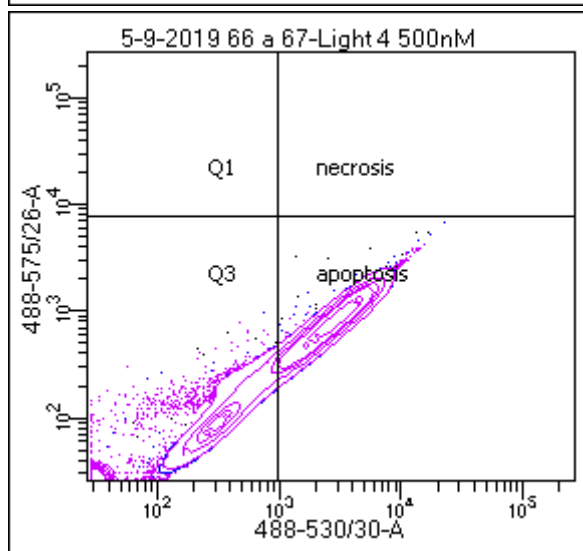
Tube: Light 4 100nM

Population	#Events	%Parent	%Total
■ All Events	10,000	####	100.0
■ P1	9,415	94.2	94.2
■ P2	8,327	88.4	83.3
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,860	70.4	58.6
☒ apoptosis	2,467	29.6	24.7



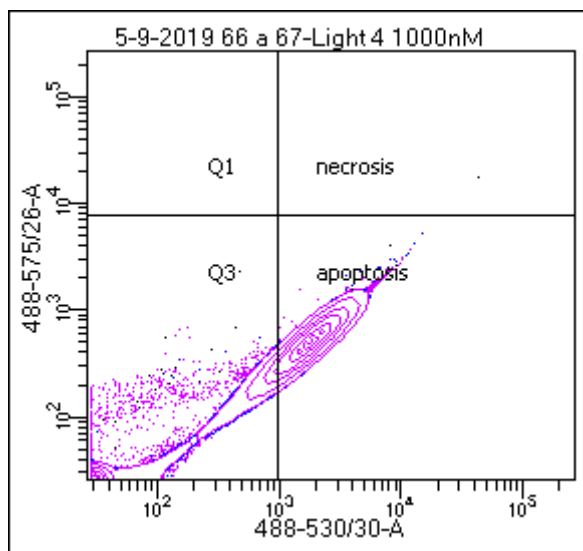
Tube: Light4 250nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,954	99.5	99.5
■ P2	9,613	96.6	96.1
☒ Q1	0	0.0	0.0
☒ necrosis	2	0.0	0.0
☒ Q3	4,339	45.1	43.4
☒ apoptosis	5,272	54.8	52.7



Tube: Light4 500nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,961	99.6	99.6
■ P2	9,492	95.3	94.9
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	5,527	58.2	55.3
☒ apoptosis	3,965	41.8	39.6



Tube: Light 4 1000nM

Population	#Events	%Parent	%Total
■ All Events	10,000	###	100.0
■ P1	9,964	99.6	99.6
■ P2	9,441	94.8	94.4
☒ Q1	0	0.0	0.0
☒ necrosis	0	0.0	0.0
☒ Q3	4,296	45.5	43.0
☒ apoptosis	5,145	54.5	51.4

References

1. Bříza, T.; Králová, J.; Rimpelová, S.; Havlík, M.; Kaplánek, R.; Kejík, Z.; Reddy, B.; Záruba, K.; Ruml, T.; Mikula, I.; Martásek, P.; Král, V. Dimethinium heteroaromatic salts as building blocks for dual-fluorescence intracellular probes. *ChemPhotoChem* **2017**, *1* (10), 442-450.