Supplementary files

Table 1.1: Summary of the intracellular morphological changes in B16 F10 melanoma cells after 48-hour exposure to various compounds.

Cell line	Compound	Intracellular organelles					
		Cell form and membrane	**Nuclei and nucleoli**	**Vacuoles and vesicles**	**Mitochondria**	**Golgi complex**	**Endoplasmic reticulum**
B16 F10	L-kynurenine	Unaffected	Unaffected	Myelin figures	Unaffected	Swollen	Unaffected
	Quinolinic acid	Apoptotic bodies	Unaffected	Increased amount of lysosomes and vacuoles. Myelin figures were present	Unaffected	Swollen	Unaffected
	Nocodazole	Rounded	Absent nuclei and nucleoli in the majority of the cells	Myelin figures	Unaffected	Swollen	Unaffected

Table 1.2: Summary of the intracellular morphological changes in RAW 264.7 macrophage cells after 48-hour exposure to various compounds.

Cell line	Compound	Intracellular organelles					
		Cell form and membrane	**Nuclei and nucleoli**	**Vacuoles and vesicles**	**Mitochondria**	**Golgi complex**	**Endoplasmic reticulum**
RAW 264.7	L-kynurenine	Unaffected	Unaffected	Unaffected	Unaffected	Unaffected	Unaffected
	Quinolinic acid	Unaffected	Unaffected	Lysosomes and vacuoles	Unaffected	Unaffected	Unaffected
	Nocodazole	Membrane blebbing	Unaffected	Increased amount of lysosomes and vacuoles	Swollen	Unaffected	Swollen SER

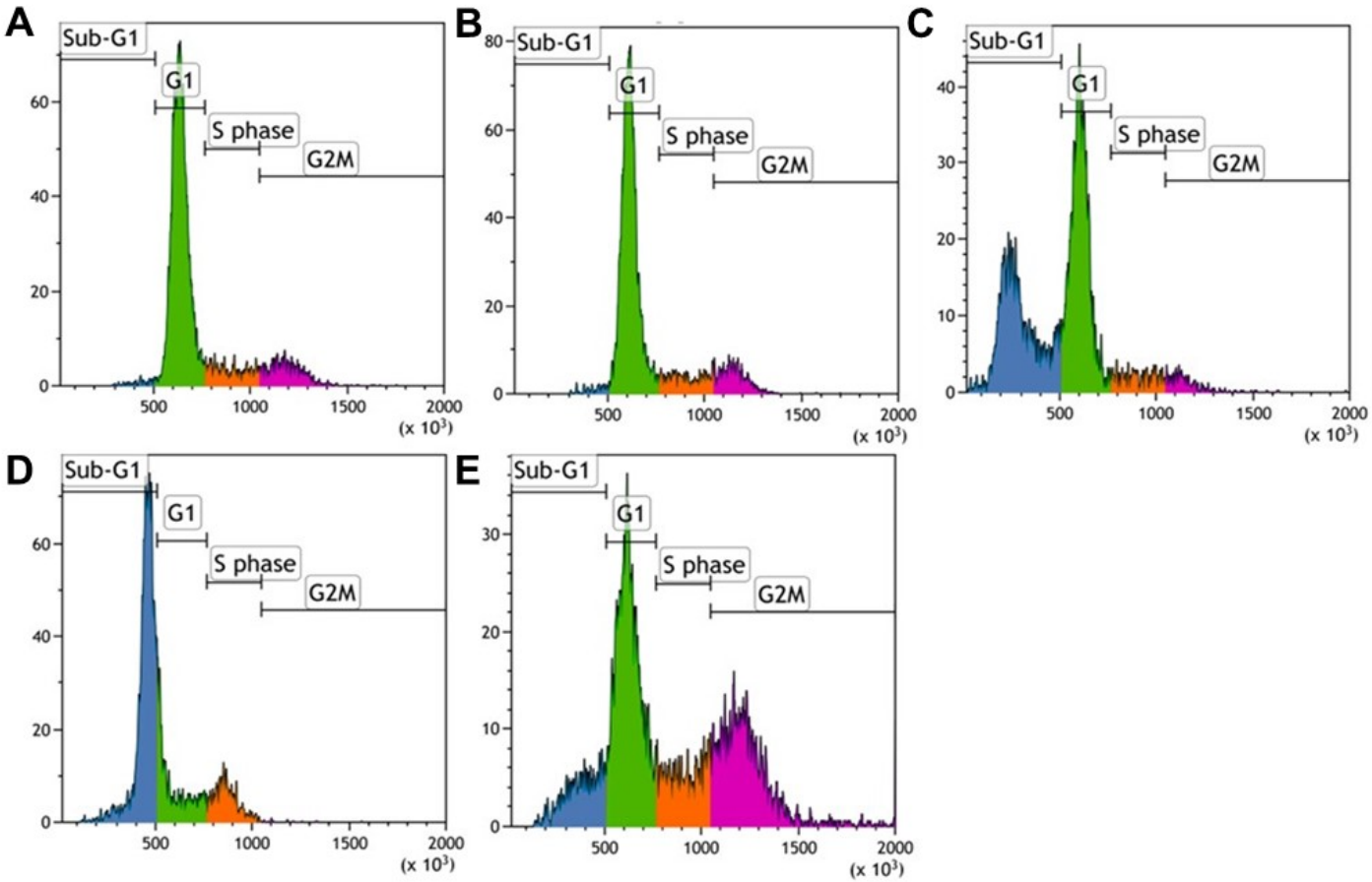

Figure 1.1: Flow cytometry histograms for cell cycle analysis in B16 F10 cells after 48 hours for (A) control cells treated with PBS, (B) control cells treated with ddH_2O, (C) L-kynurenine treated cells at 1.74 mM, (D) Quinolinic acid treated cells at 8.23 mM, (E) positive control cells treated with nocodazole at 1.30 mM. Cells in different phases of the cell cycle (sub-G1, G1, S and G2/M) were analysed using Kaluza C data analysis software (Version 1.1.00003.20057 Beckman Coulter).

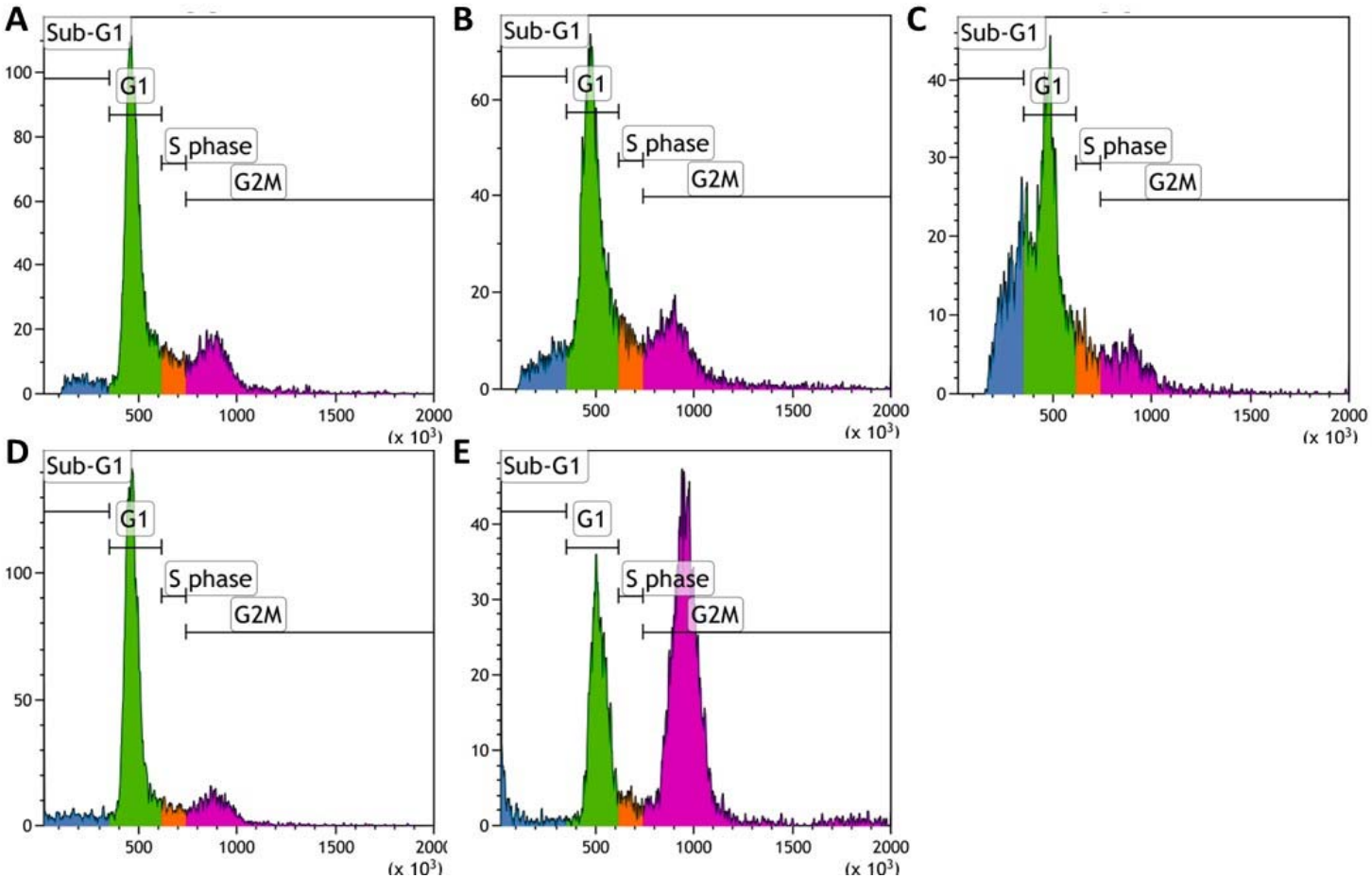

Figure 1.2: Flow cytometry histograms for cell cycle analysis in RAW 264.7 cells after 48 hours for (A) control cells treated with PBS, (B) control cells treated with ddH_2O, (C) L-kynurenine treated cells at 1.74 mM, (D) Quinolinic acid treated cells at 8.23 mM, (E) positive control cells treated with nocodazole at 1.30 mM. Cells in different phases of the cell cycle (sub-G1, G1, S and G2/M) were analysed using Kaluza C data analysis software (Version 1.1.00003.20057 Beckman Coulter).

Table 1.3: The average of the percentage of B16 F10 and RAW 264.7 cells in the different phases of the cell cycle (sub-G1, G1, S and G2/M) after 48 hours for control cells treated with PBS, control cells treated with ddH_2O, L-kynurenine treated cells at 1.74 Mm, quinolinic acid treated cells at 8.23 Mm and nocodazole at 1.30 mM. The values represent the average of at least 2 experimental repeats for each treatment condition ± SEM.

Cell line	Cell cycle phase	Control (PBS)	Control (H_2O)	L-kynurenine	Quinolinic acid	Nocodazole
B16 F10	**Sub-G1**	2.29 ± 0.62	2.58 ± 0.32	39.68 ± 0.63	67.91 ± 3.63	10.53 ± 1.68
	G1	72.84 ± 1.69	73.70 ± 0.84	49.40 ± 0.71	18.58 ± 3.14	40.29 ± 1.51
	S phase	11.28 ± 0.15	11.52 ± 0.10	6.92 ± 0.02	13.06 ± 1.02	15.52 ± 0.08
	G2/M	13.75 ± 0.77	12.87 ± 0.03	3.87 ± 0.40	0.39 ± 0.06	33.65 ± 2.91
RAW 264.7	**Sub-G1**	5.49 ± 0.07	9.38 ± 0.30	19.64 ± 2.72	7.47 ± 0.28	2.59 ± 1.69
	G1	61.93 ± 0.18	55.98 ± 0.40	56.53 ± 1.60	68.36 ± 1.35	28.24 ± 0.88
	S phase	8.53 ± 0.04	8.48 ± 0.22	8.35 ± 0.96	5.36 ± 0.16	3.45 ± 0.25
	G2/M	23.96 ± 0.15	26.04 ± 0.48	15.41 ± 0.18	18.46 ± 1.80	63.82 ± 2.36

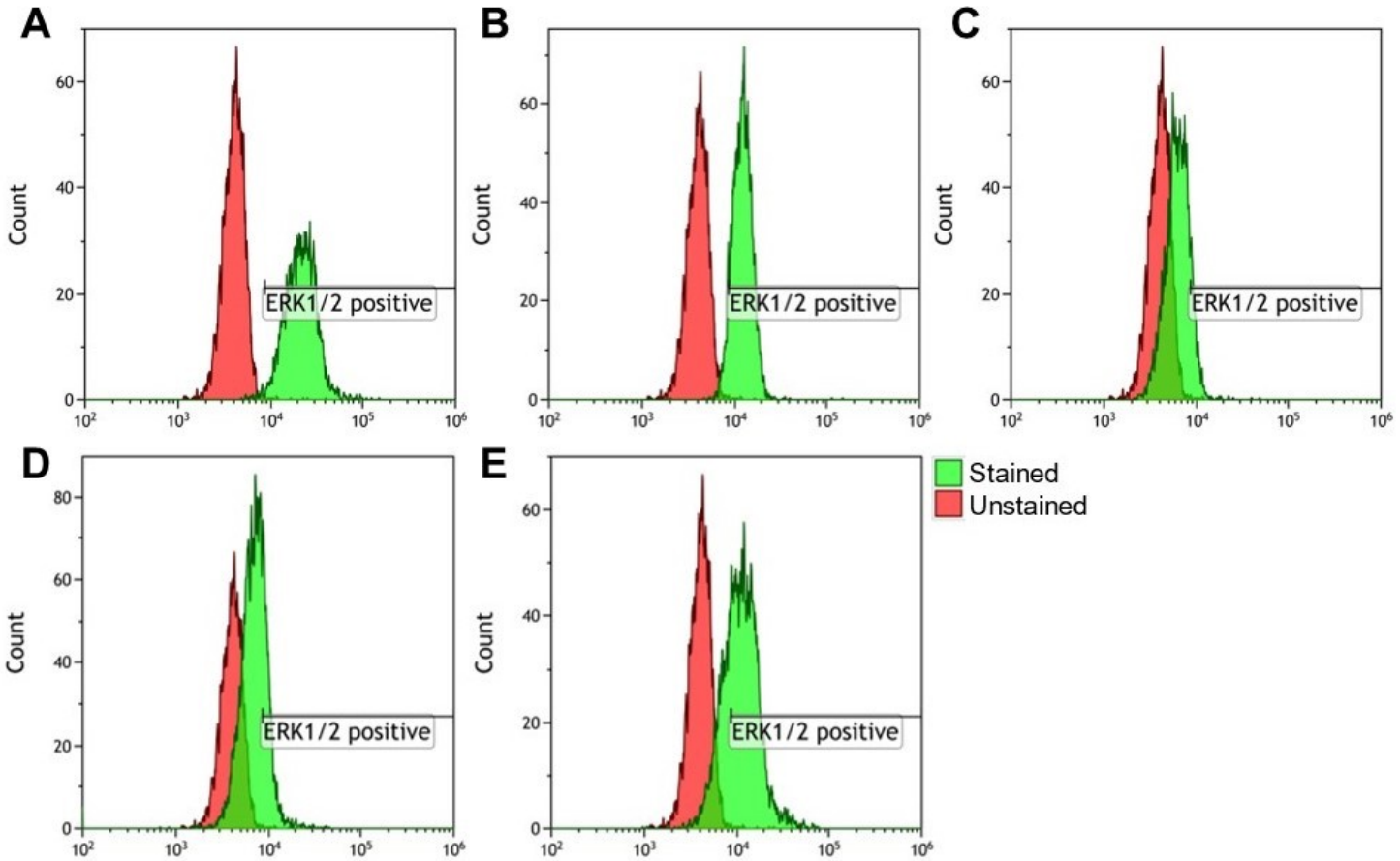

Figure 1.3: Flow cytometry overlay histograms for ERK1/2 activation quantification in B16 F10 cells after 48 hours for (A) control cells treated with PBS, (B) control cells treated with ddH_2O, (C) L-kynurenine treated cells at 1.74 mM, (D) Quinolinic acid treated cells at 8.23 mM, (E) positive control cells treated with nocodazole at 1.30 mM. Unstained control cells treated with PBS (red) was used to set positive gates for the cells treated with kynurenine exogenous compounds.

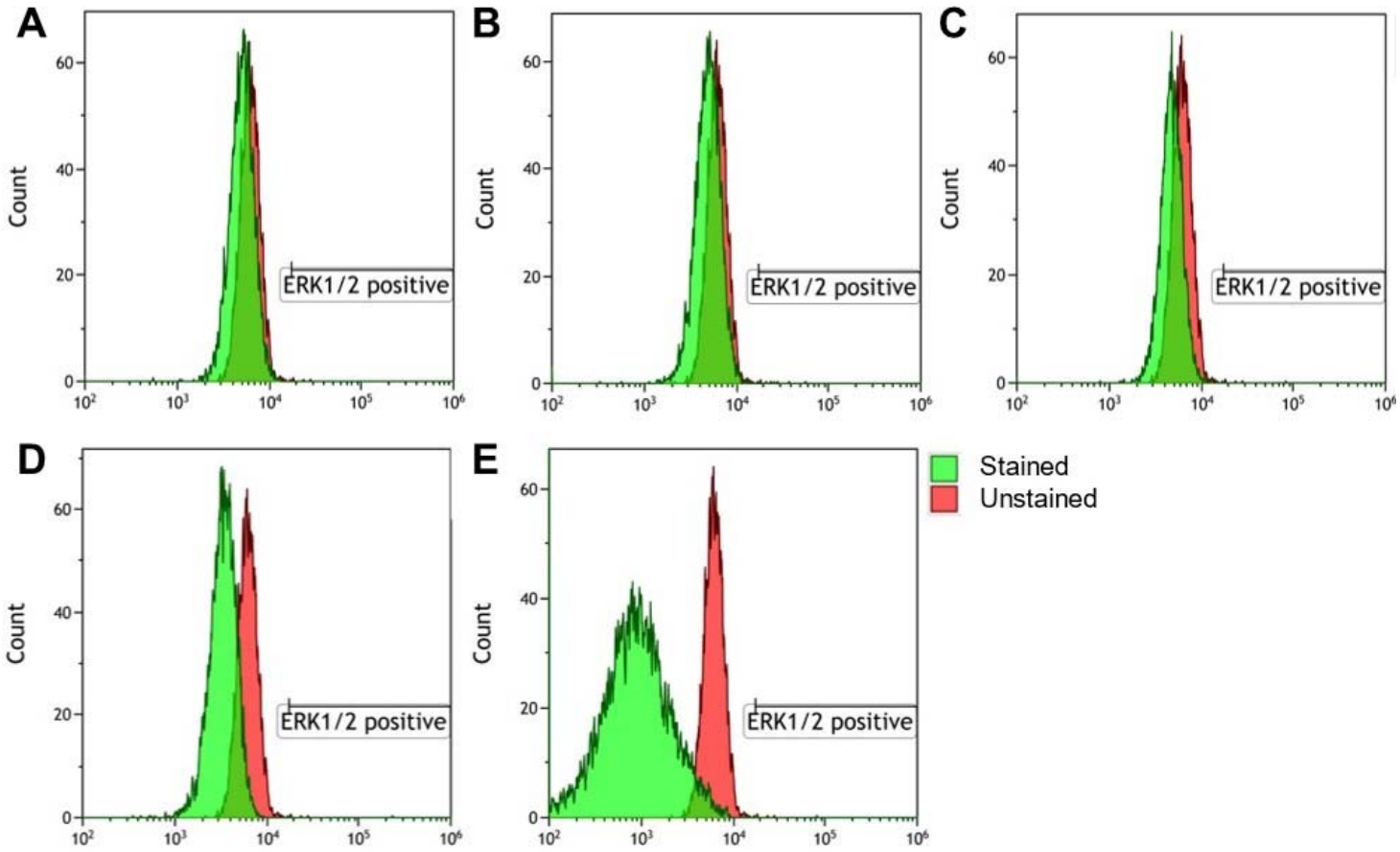

Figure 1.4: Flow cytometry overlay histograms for ERK1/2 activation quantification in RAW 264.7 cells after 48 hours for (A) control cells treated with PBS, (B) control cells treated with ddH_2O, (C) L-kynurenine treated cells at 1.74 mM, (D) Quinolinic acid treated cells at 8.23 mM, (E) positive control cells treated with nocodazole at 1.30 mM. Unstained control cells treated with PBS (red) was used to set positive gates for the cells treated with kynurenine exogenous compounds.

Table 1.4: The average of the percentage of ERK1/2 activation in B16 F10 and RAW 264.7 cells after 48 hours for control cells treated with PBS, control cells treated with ddH_2O, L-kynurenine treated cells at 1.74 Mm, quinolinic acid treated cells at 8.23 Mm and nocodazole at 1.30 mM. The values represent the average of at least 2 experimental repeats for each treatment condition ± SEM.

Cell line						lazole
B16 F10						± 1.63
RAW 264.7						0.00

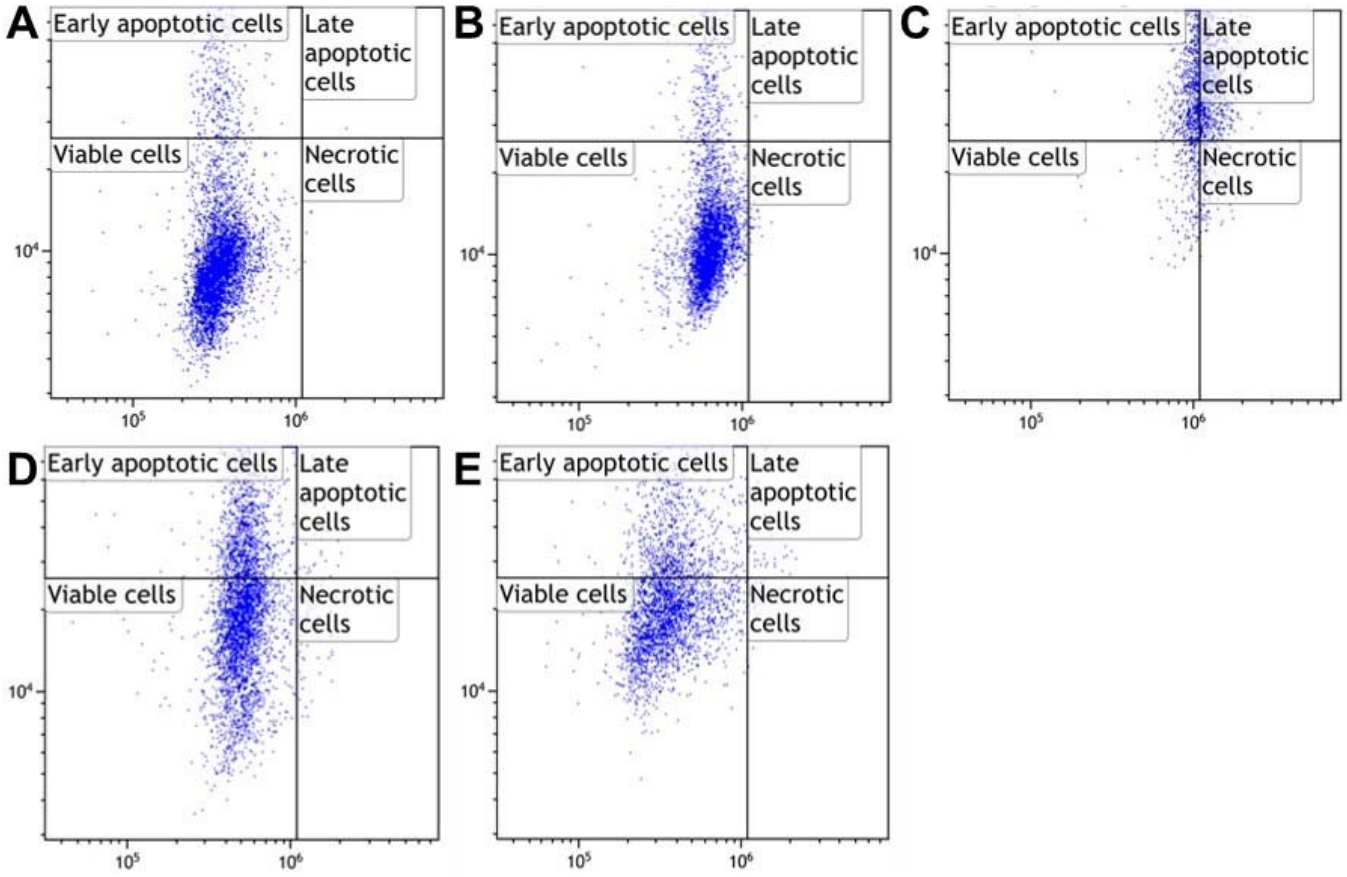

Figure 1.5: PI (FL3 log) versus Annexin V-FITC (FL1 log) dot-plots for B16 F10 cells after 48 hours for (A) control cells treated with PBS, (B) control cells treated with ddH_2O, (C) L-kynurenine treated cells at 1.74 mM, (D) quinolinic acid treated cells at 8.23 mM, (E) positive control cells treated with nocodazole at 1.30 mM. Cell death was analysed as follows: viable cells in the lower left quadrant, where FITC (-) and PI (-); necrotic cells in the lower right quadrant,

where FITC (-) and PI (+); early apoptotic cells in the upper left quadrant where FITC (+) and PI (-) and late apoptotic cells in the upper right quadrant where FITC (+) and PI (+). Samples were analysed using Kaluza C data analysis software (Version 1.1.00003.20057 Beckman Coulter).

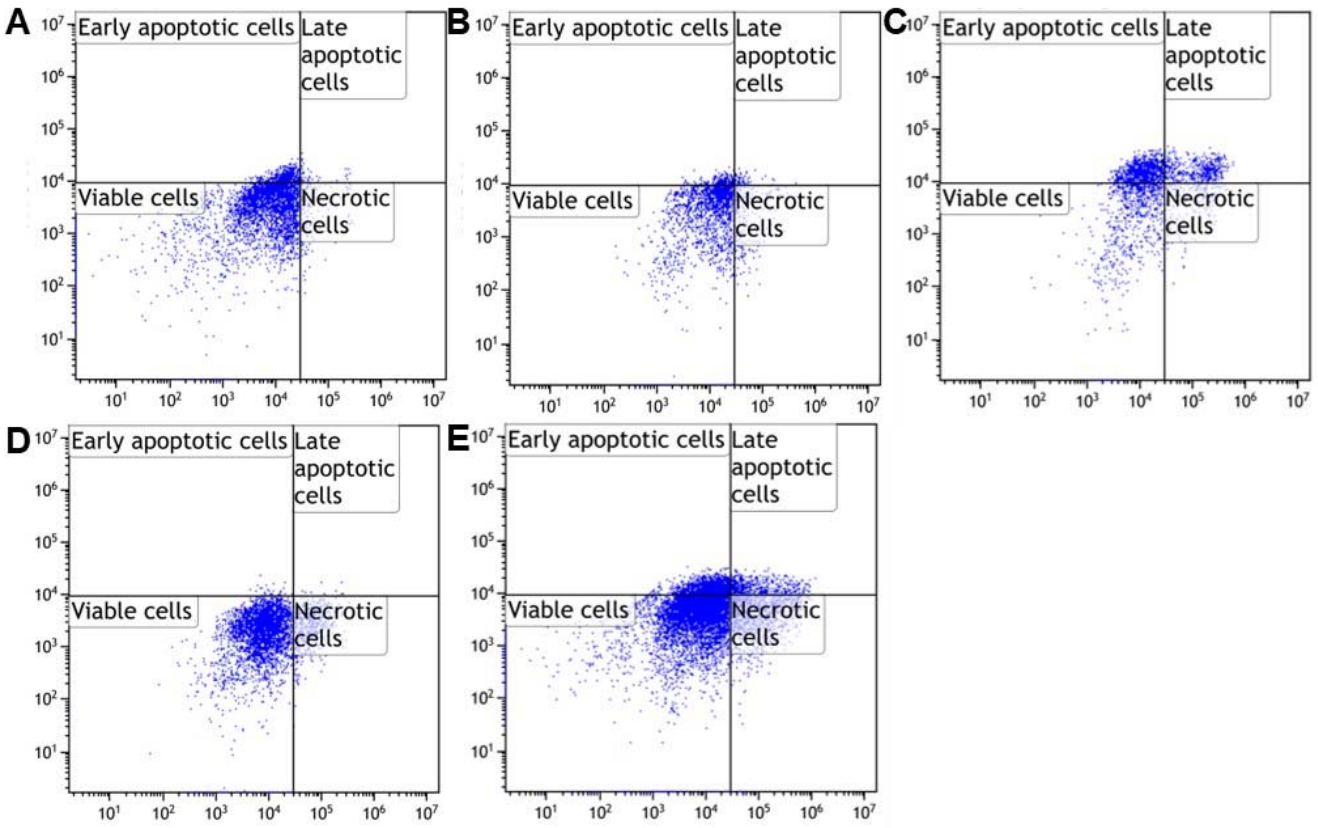

Figure 1.6: PI (FL3 log) versus Annexin V-FITC (FL1 log) dot-plots for RAW 264.7 cells after 48 hours for (A) control cells treated with PBS, (B) control cells treated with ddH_2O, (C) L-kynurenine treated cells at 1.74 mM, (D) quinolinic acid treated cells at 8.23 mM, (E) positive control cells treated with nocodazole at 1.30 mM. Cell death was analysed as follows: viable cells in the lower left quadrant, where FITC (-) and PI (-); necrotic cells in the lower right quadrant, where FITC (-) and PI (+); early apoptotic cells in the upper left quadrant where FITC (+) and PI (-) and late apoptotic cells in the upper right quadrant where FITC (+) and PI (+). Samples were analysed using Kaluza C data analysis software (Version 1.1.00003.20057 Beckman Coulter).

Table 1.5: The average of the percentage of cell death in B16 F10 and RAW 264.7 cells after 48 hours for control cells treated with PBS, control cells treated with ddH_2O, L-kynurenine treated cells at 1.74 Mm, quinolinic acid treated cells at 8.23 Mm and nocodazole at 1.30 mM. The values represent the average of at least 2 experimental repeats for each treatment condition ± SEM.

Cell line	Cell death	Control (PBS)	Control (ddH_2O)	L-kynurenine	Quinolinic acid	Nocodazole
B16 F10	**Viable cells**	90.31 ± 0.21	90.64 ± 0.41	9.39 ± 1.20	68.83 ± 0.09	66.73 ± 0.02
	Necrotic cells	0.06 ± 0.03	0.75 ± 0.01	10.73 ± 2.85	1.16 ± 0.47	0.59 ± 0.54
	Late apoptotic cells	0.01 ± 0.01	0.10 ± 0.03	47.58 ± 4.14	0.53 ± 0.13	0.75 ± 0.74
	Early apoptotic cells	9.63 ± 0.25	8.52 ± 0.37	32.30 ± 5.79	29.48 ± 0.51	31.90 ± 1.21
RAW 264.7	**Viable cells**	77.10 ± 0.43	74.68 ± 0.18	30.51 ± 1.23	83.07 ± 0.36	61.51 ± 0.05
	Necrotic cells	8.04 ± 0.48	13.72 ± 0.36	12.43 ± 0.33	15.88 ± 0.06	15.35 ± 0.49
	Late apoptotic cells	2.20 ± 1.03	3.16 ± 0.14	19.04 ± 1.86	0.31 ± 0.78	7.22 ± 0.46
	Early apoptotic cells	12.65 ± 1.06	8.46 ± 0.67	38.01 ± 0.28	0.43 ± 0.78	15.95 ± 1.01