

## Supplementary Materials

### **Induction of endoplasmic reticulum stress-mediated apoptosis by aminosteroid RM-581 efficiently blocks the growth of PC-3 docetaxel-resistant prostate cancer cells and tumors**

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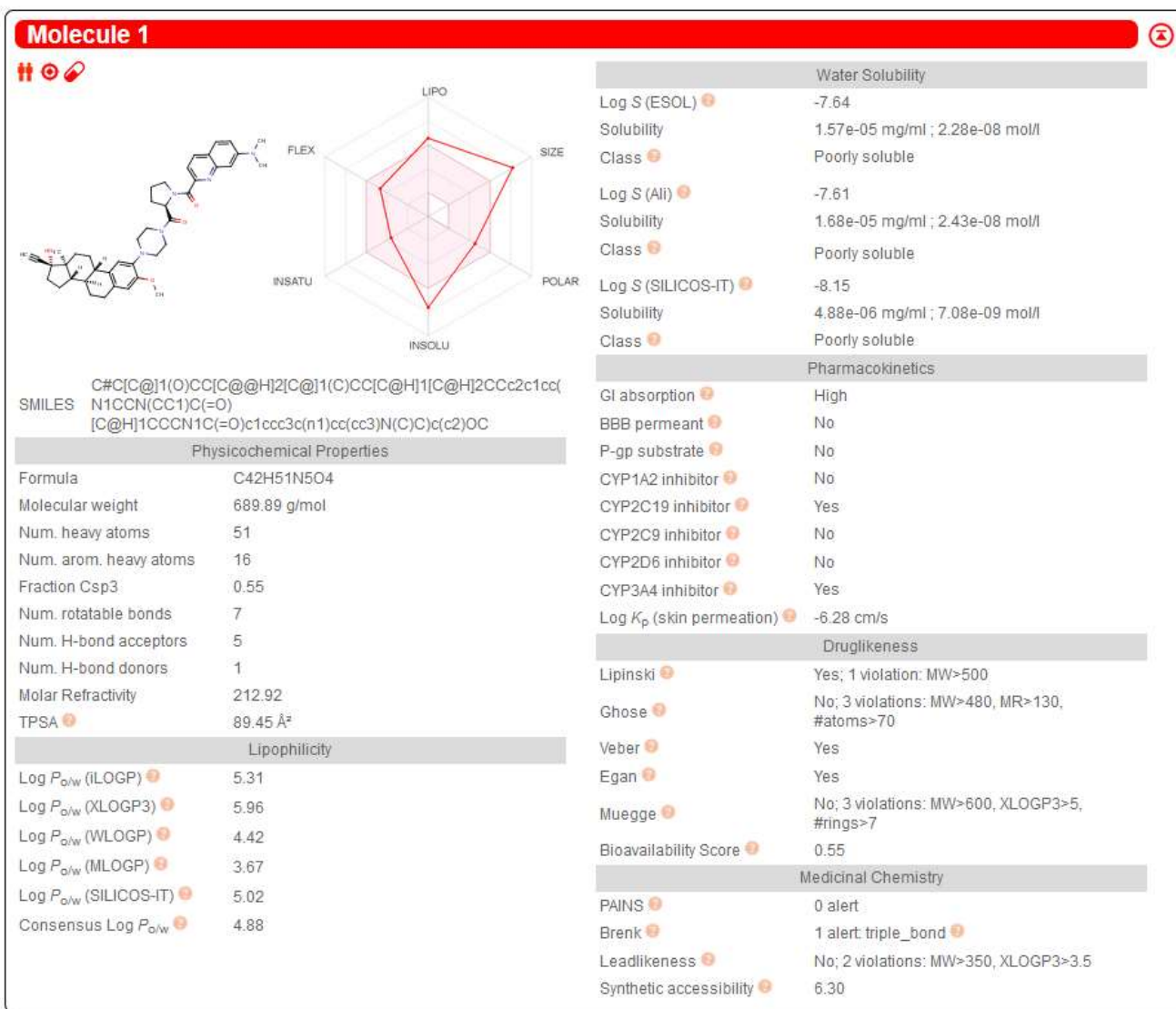
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## Supplementary Materials

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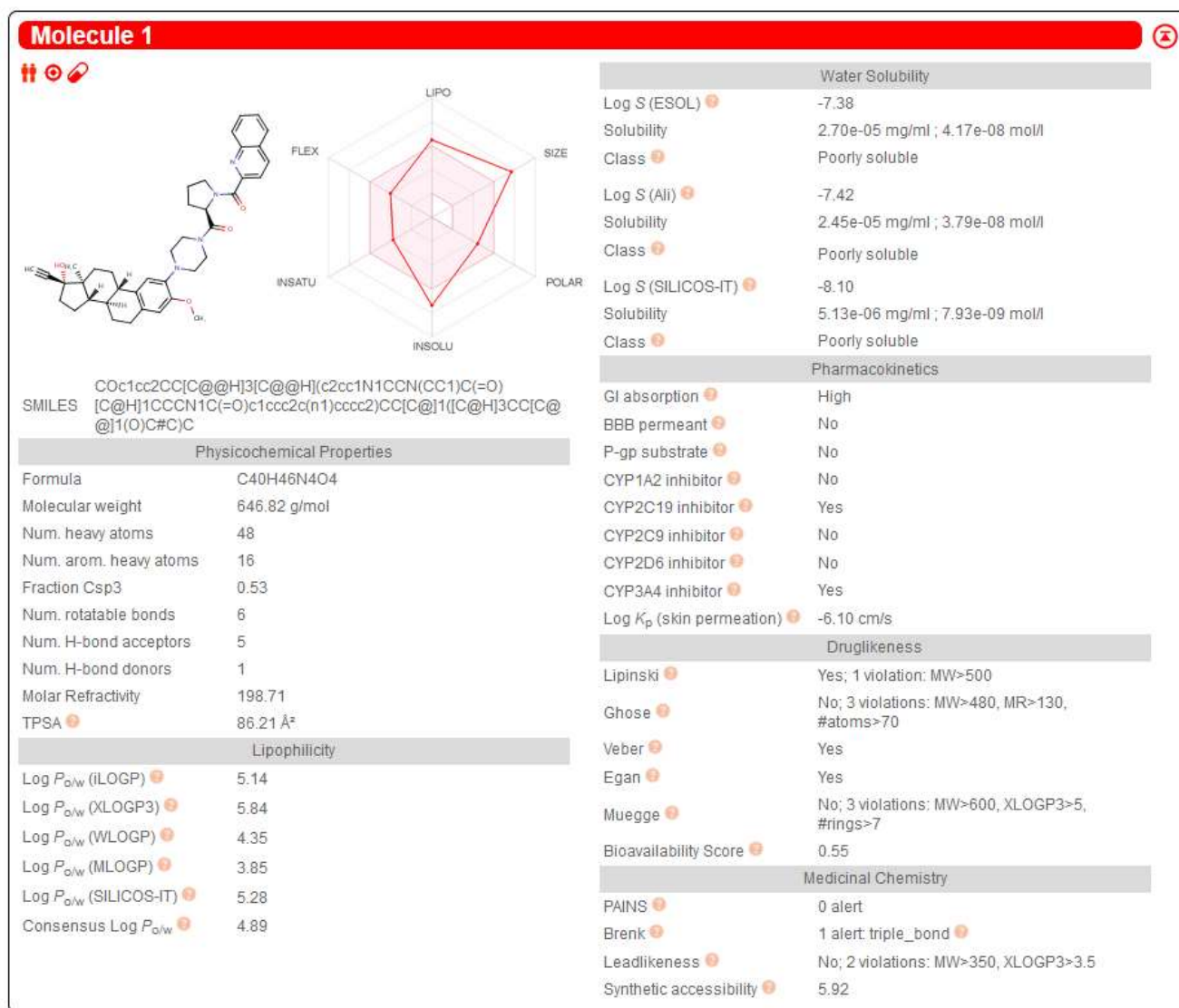
**Table S1.** Calculated physicochemical and ADME properties of RM-581-Fluo

[<http://www.swissadme.ch/faq.php>]. Copyright [2021] [SwissADME].



**Table S2.** Calculated physicochemical and ADME properties of RM-581

[<http://www.swissadme.ch/faq.php>]. Copyright [2021] [SwissADME].



**Table S3.** List of fatty acids dosed in human PC-3 tumors

Fatty acids (FA)	Common name	CTL group (PC-3 tumor)		RM-581 (PC-3 tumor)	
		%	mg FA/g of tissue	%	mg FA/g of tissue
<b>C8:0</b>		0.00	0.00	0.00	0.00
<b>C10:0</b>		0.00	0.00	0.00	0.00
<b>C12:0</b>		0.17	0.03	0.15	0.02
<b>14:0</b>	Myristic acid	1.48	0.22	1.35	0.16
<b>9t-14:1 n5</b>		0.00	0.00	0.00	0.00
<b>9c-14:1 n5</b>	Myristoleic acid	0.15	0.02	0.12	0.01
<b>C15:0</b>		0.15	0.02	0.17	0.02
<b>C16:0 DMA</b>		0.00	0.00	0.04	0.01
<b>C15:1 n5t</b>		0.17	0.03	0.19	0.02
<b>C15:1 n5c</b>		0.00	0.00	0.00	0.00
<b>C15:1 n1c</b>		0.00	0.00	0.00	0.00
<b>16:0</b>	Palmitic acid	19.59	2.98	18.71	2.22
<b>9t-16:1 n7</b>	Palmitelaidic acid	0.00	0.00	0.00	0.00
<b>C17:0 isobranched</b>		0.00	0.00	0.00	0.00
<b>9c-16:1 n7</b>	Palmitoleic acid	9.87	1.50	6.96	0.83
<b>C18:0 DMA</b>		0.00	0.00	0.00	0.00
<b>C17:1 n7t</b>		0.12	0.02	0.13	0.02
<b>C18:1 DMA</b>		0.18	0.03	0.20	0.02
<b>C17:1 n7c</b>		0.00	0.00	0.00	0.00
<b>18:0</b>	Stearic acid	2.77	0.42	3.30	0.39
<b>6t-18:1 n12</b>	Petroselaidic acid	0.00	0.00	0.00	0.00
<b>9t-18:1 n9</b>	Elaidic acid	0.01	0.00	0.00	0.00
<b>11t-18:1 n7</b>	Transvaccenic acid	0.07	0.01	0.13	0.02
<b>7c-18:1 n11/6c-18:1 n12</b>	/Petroselinic acid	0.00	0.00	0.00	0.00
<b>9c-18:1 n9</b>	Oleic acid	31.44	4.78	30.38	3.61
<b>11c-18:1 n7</b>	Vaccenic acid	2.05	0.31	2.05	0.24
<b>12c-18:1 n6</b>		0.00	0.00	0.00	0.00
<b>13c-18:1 n5</b>		0.03	0.00	0.03	0.00
<b>9t12t-18:2 n6</b>	Linolelaidic acid	0.00	0.00	0.00	0.00
<b>9c12t-18:2 n6</b>		0.09	0.01	0.11	0.01
<b>9t12c-18:2 n6</b>		0.00	0.00	0.00	0.00
<b>9c12c-18:2 n6 (LA)</b>	Linoleic acid	26.27	3.99	28.90	3.44
<b>9t12t15t-18:3 n3</b>		0.06	0.01	0.04	0.00
<b>20:0</b>	Arachidic acid	0.19	0.03	0.25	0.03
<b>6c9c12c-18:3 n6</b>	gamma-Linolenic acid	0.06	0.01	0.06	0.01
<b>9c12c15c-18:3 n3 (ALA)</b>	alpha-Linolenic acid	1.18	0.18	1.25	0.15
<b>8c-20:1 n12</b>		0.00	0.00	0.00	0.00
<b>11c-20:1 n9</b>	Gondoic acid	0.58	0.09	0.61	0.07

6c9c12c15c-18:4 n3	Stearidonic acid	0.04	0.01	0.04	0.00
11c14c-20:2 n6		0.31	0.05	0.48	0.06
22:0	Behenic acid	0.08	0.01	0.09	0.01
8c11c14c-20:3 n6	Dihomo-gamma-linolenic acid	0.47	0.07	0.76	0.09
C22:1 n9t		0.00	0.00	0.00	0.00
11c14c17c-20:3 n3 (ETE)		0.02	0.00	0.03	0.00
5c8c11c14c-20:4 n6 (AA)	Arachidonic acid	0.71	0.11	1.04	0.12
13c-22:1 n9	Erucic acid	0.07	0.01	0.08	0.01
8c11c14c17c-20:4 n3 (ETA)		0.01	0.00	0.04	0.00
13c16c-22:2 n6		0.03	0.00	0.05	0.01
5c8c11c14c17c-20:5 n3 (EPA)	Timnodonic acid	0.02	0.00	0.02	0.00
24:0	Lignoceric acid	0.30	0.05	0.32	0.04
13c16c19c-22:3 n3		0.00	0.00	0.00	0.00
15c-24:1 n9	Nervonic acid	0.05	0.01	0.06	0.01
7c10c13c16c-22:4 n6	Adrenic acid	0.54	0.08	0.82	0.10
4c7c10c13c16c-22:5 n6	Adrenic acid	0.14	0.02	0.20	0.02
7c10c13c16c19c-22:5 n3 (DPA)		0.15	0.02	0.22	0.03
4c7c10c13c16c19c-22:6 n3 (DHA)	Cervonic acid	0.37	0.06	0.58	0.07
<b>Total</b>		<b>100.00</b>	<b>15.20</b>	<b>100.00</b>	<b>11.89</b>

<b>n-3/n-6 (0,20 à0,33)</b>
<b>n-6 total</b>
<b>n-3 total</b>

0.06	0.06	0.07	0.07
28.63	4.35	32.42	3.85
1.81	0.28	2.18	0.26

<b>Saturated</b>
<b>P (n-3 &amp; 6)/S</b>
<b>Polyunsaturated n3 cis</b>
<b>Polyunsaturated n6 cis</b>
<b>Polyunsaturated n3 trans</b>
<b>Polyunsaturated n6 trans</b>

24.73	3.76	24.40	2.90
1.23	1.23	1.42	1.42
1.75	0.27	2.14	0.25
28.52	4.33	32.29	3.84
0.06	0.01	0.04	0.00
0.09	0.01	0.11	0.01
0.37	0.06	0.44	0.05
44.24	6.72	40.29	4.79
0.00	0.00	0.00	0.00
0.52	0.08	0.60	0.07
74.57	11.33	74.77	8.89

<b>Monounsaturated trans</b>
------------------------------

<b>Monounsaturated cis</b>
<b>Monounsaturated n6 cis</b>

<b>Total trans</b>
<b>Total cis</b>

<b>Total of omega-6 cis</b>
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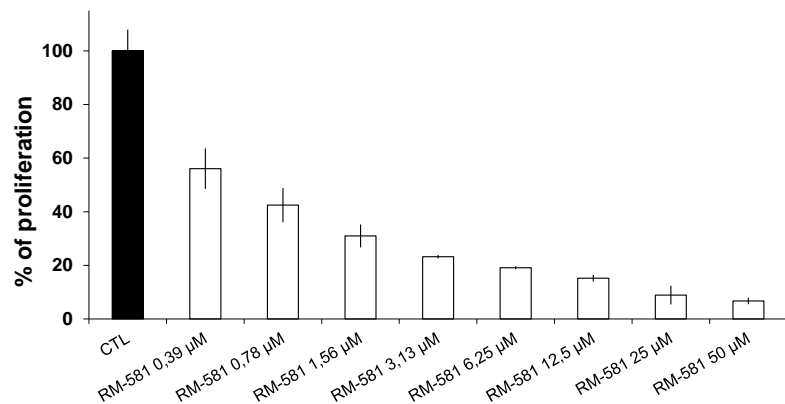
28.54	4.34	32.31	3.84
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**Table S4.** Primer sequences

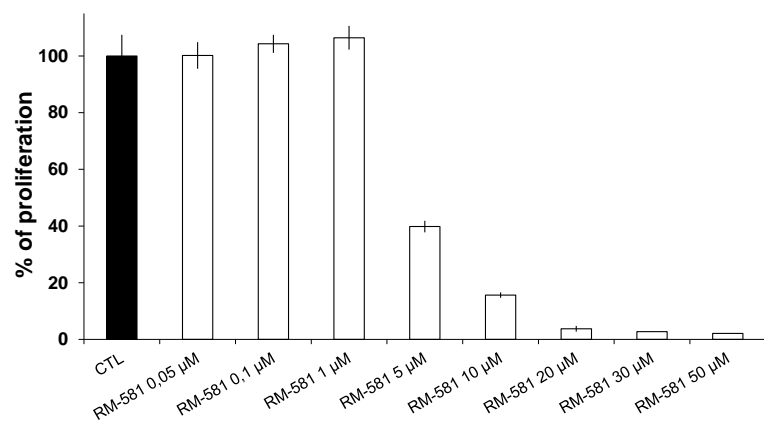
	<b>Forward sequence</b>	<b>Reverse sequence</b>
HMGR	TTCGGTGGCCTCTAGTGAGA	AAAGCTTCATTCAAGCCTGTCA
FASN	ATGGAGGAGGTGGTGATTGC	CTGGGCCCTCTGAAGTCGAA
BIP	CTTGGTATTGAAACTGTGGGAGGTG	TTCCAGTCAGATCAAATGTACCCAG
CHOP	GGAGGAGCCAGAACCAGCAGA	TTCCGTTTCCTGGTTCTCCCTT
HERP	TGCATCAGGGGCTTTTGTTC	AACCACTTGAGGAGCAGCATTCT

**Figure S1.** Cell proliferation and EC<sub>50</sub> values in LNCaP (A), DU-145 (B) and PC-3 (C) cells treated with RM-581. EC<sub>50</sub> values of 1.2, 4.4 and 1.2  $\mu$ M were obtained for LNCaP, DU-145 and PC-3 cells, respectively.

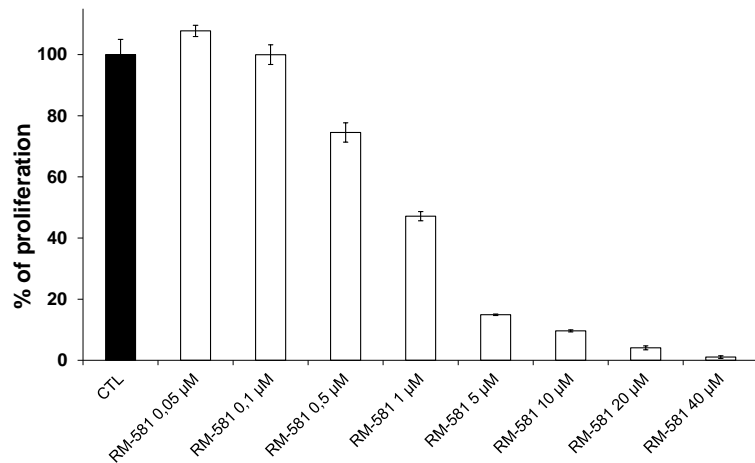
**A) LNCaP Cells**



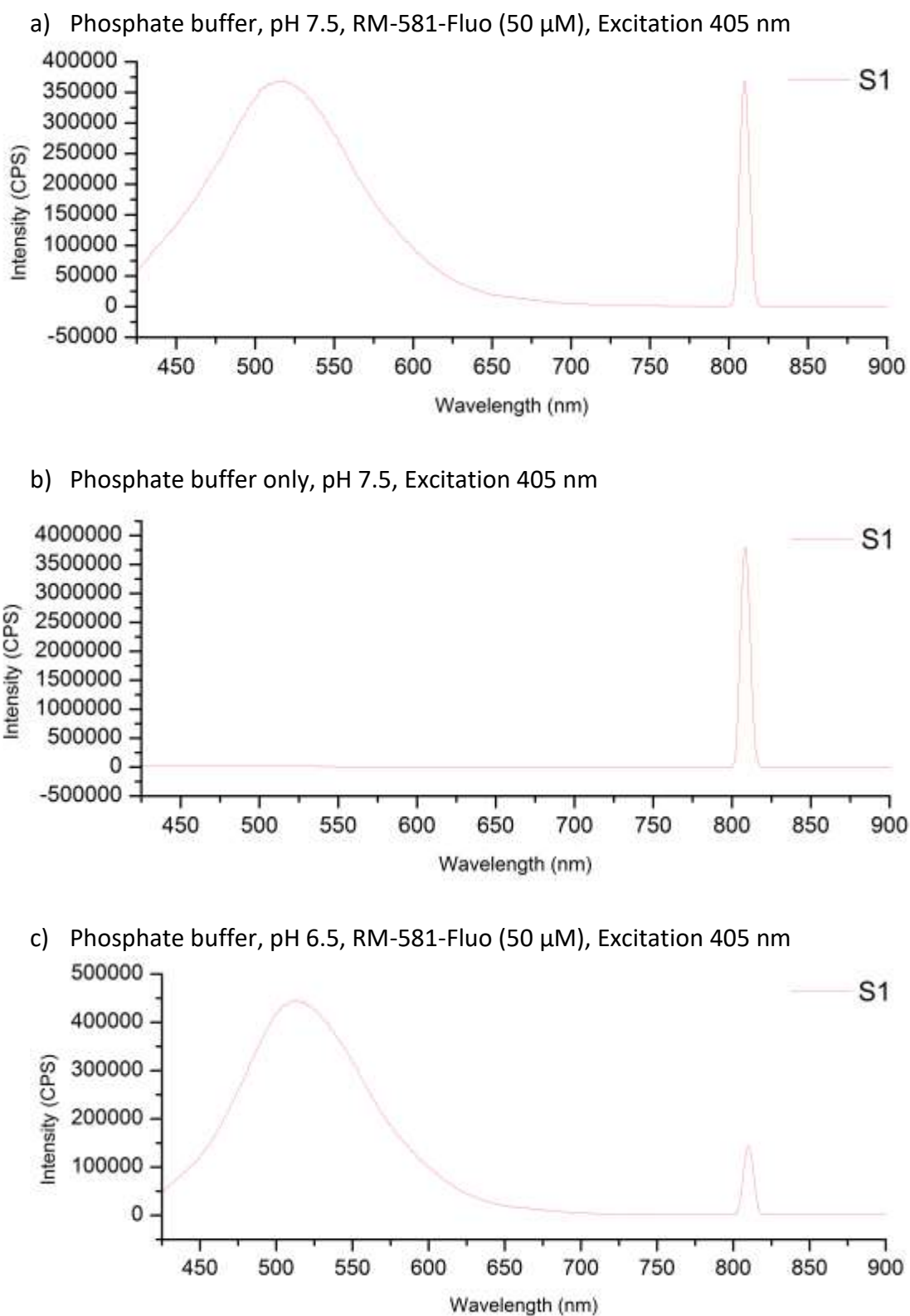
**B) DU-145 Cells**



**C) PC-3 Cells**

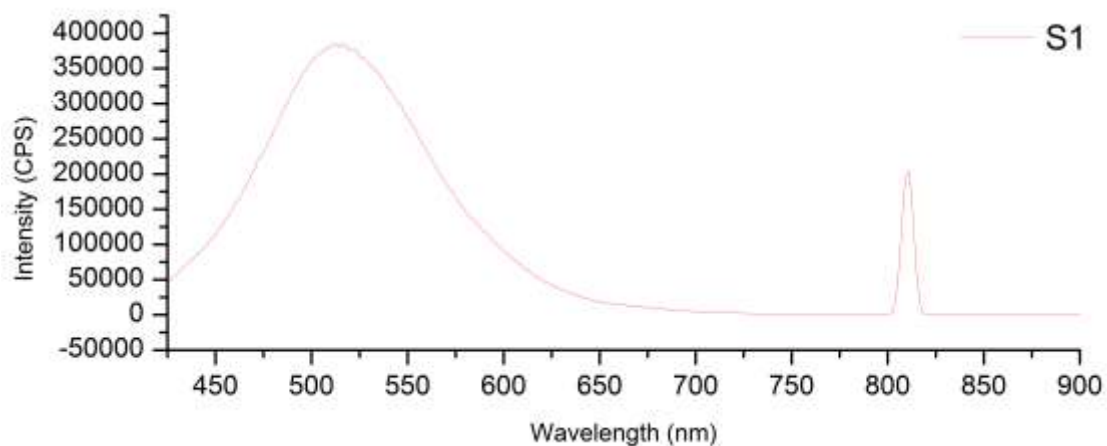


**Figure S2.** Emission spectra of RM-581-Fluo at different pH and concentrations

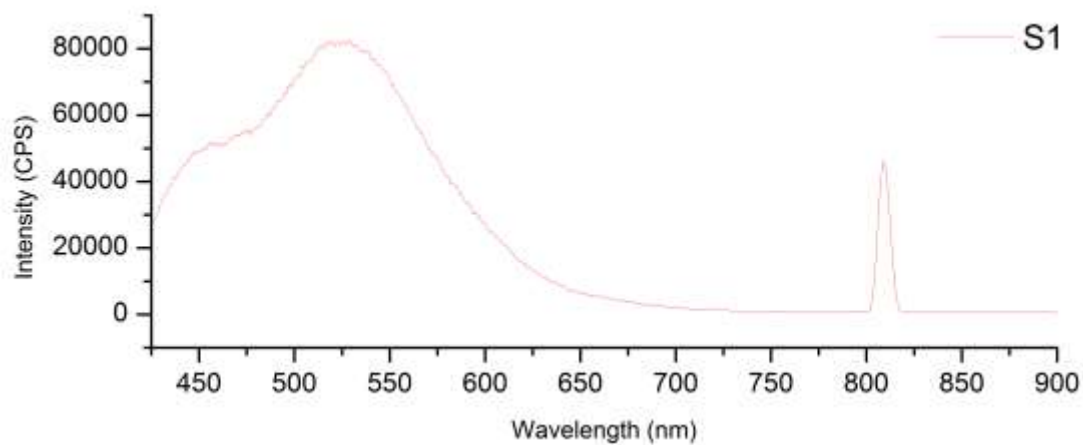




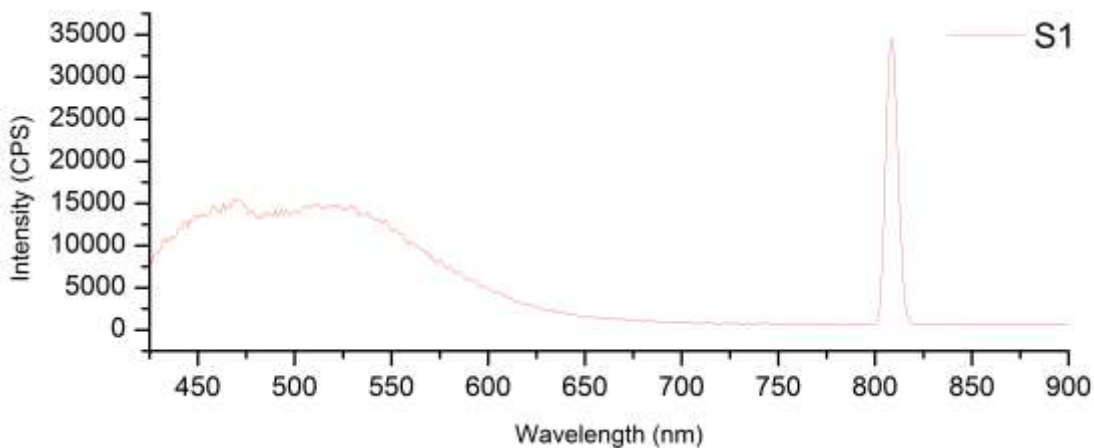
d) Phosphate buffer, pH 5.5, RM-581-Fluo (50  $\mu$ M), Excitation 405 nm



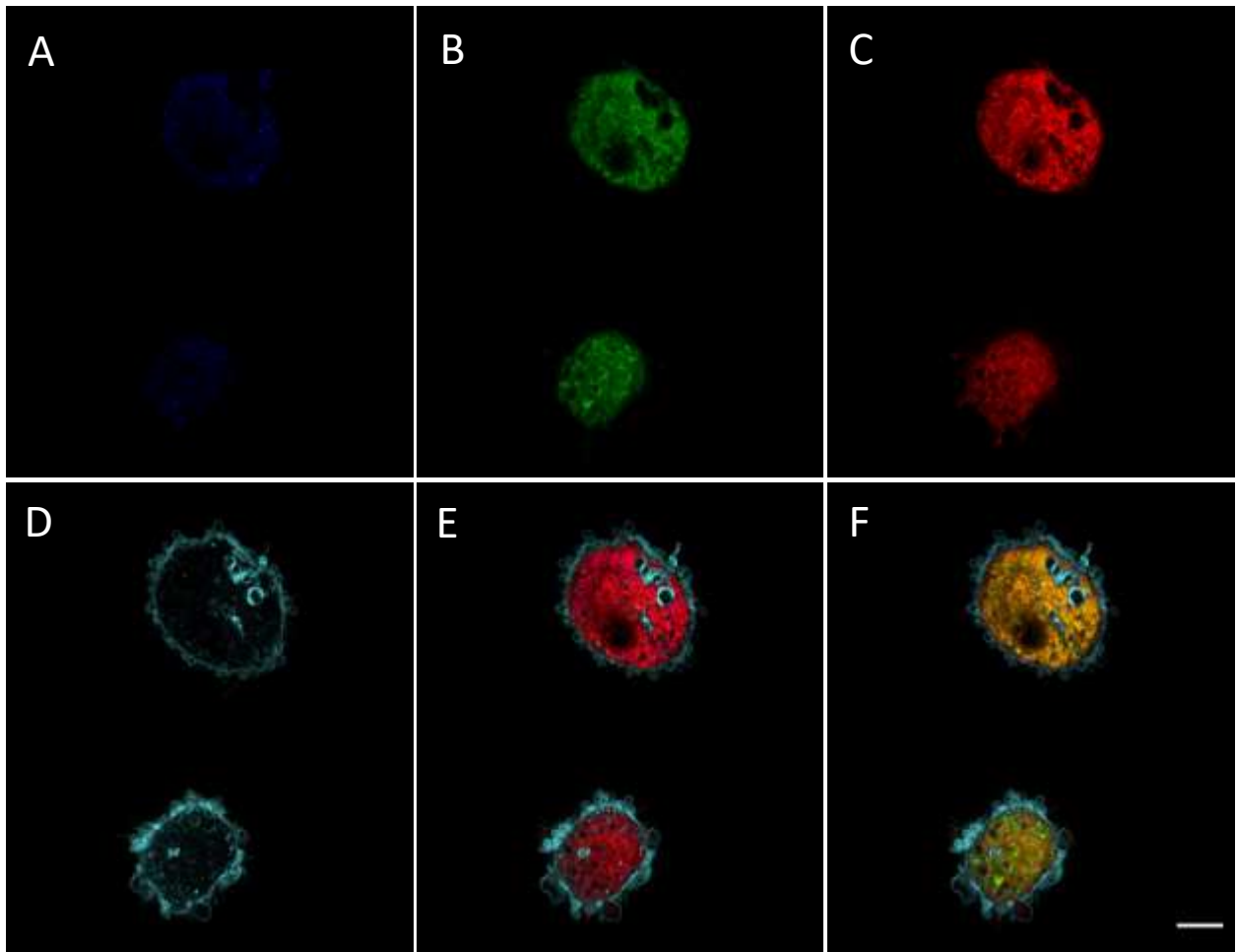
e) Phosphate buffer, pH 7.5, RM-581-Fluo (30  $\mu$ M), Excitation 405 nm



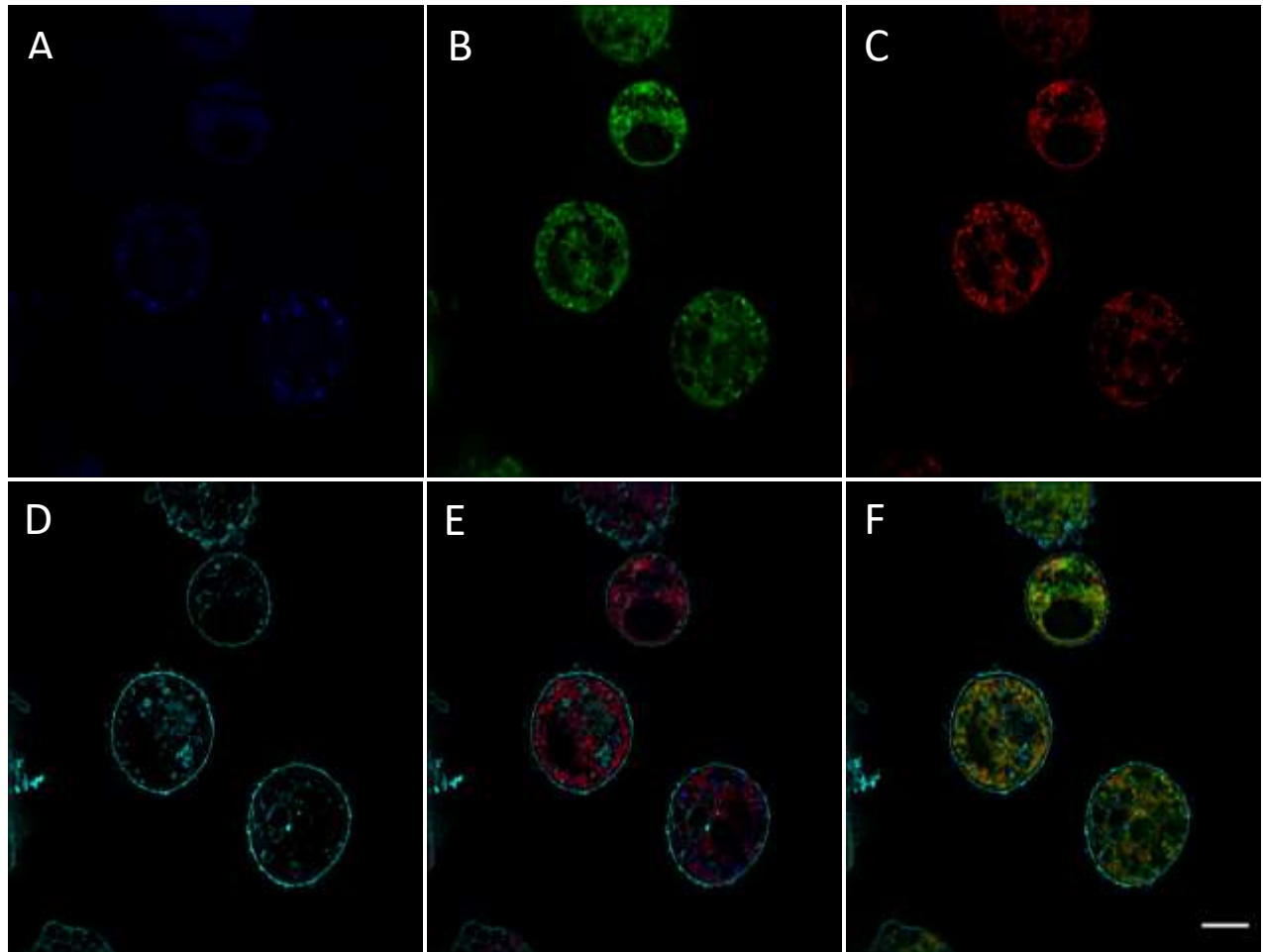
f) Phosphate buffer, pH 7.5, RM-581-Fluo (5  $\mu$ M), Excitation 405 nm



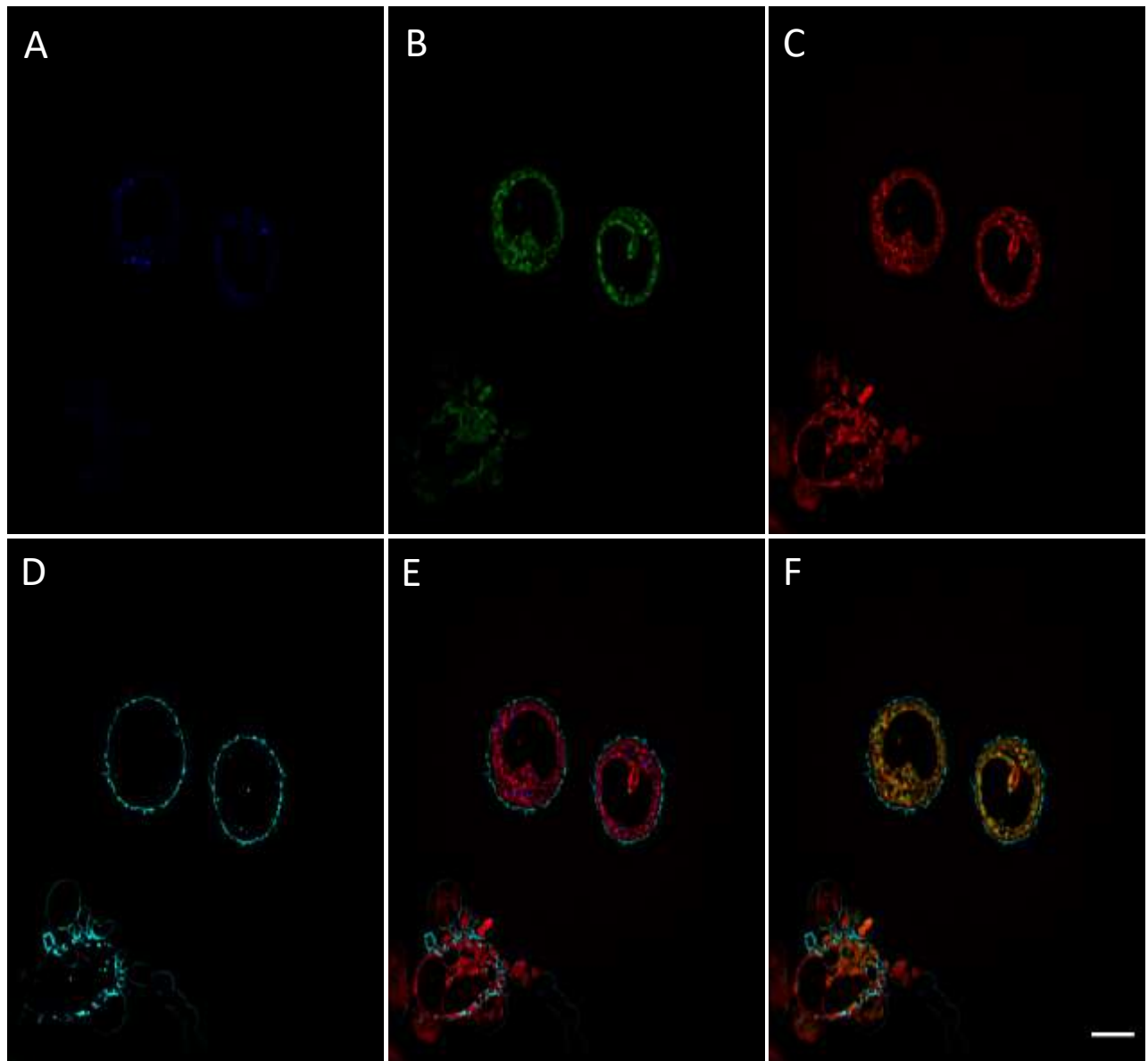
**Figure S3.** Three additional confocal imaging pictures from a second experiment with RM-581-Fluo in PC-3 cells. PC-3 cells were treated 2 h with 15  $\mu$ M of RM-581-Fluo prior microscopy. **(A)** Cellular localization of RM-581-Fluo in blue ( $\lambda_{\text{ex}}$  405 nm,  $\lambda_{\text{em}}$  460/50 nm); **(B)** Cellular localization of RM-581-Fluo in green ( $\lambda_{\text{ex}}$  405 nm,  $\lambda_{\text{em}}$  525/50 nm); **(C)** Cellular localization of ER-Tracker in red ( $\lambda_{\text{ex}}$  561 nm,  $\lambda_{\text{em}}$  593/40 nm); **(D)** Cellular localization of Cell-mask in cyan ( $\lambda_{\text{ex}}$  642 nm,  $\lambda_{\text{em}}$  700/75 nm); **(E)** Merged images A, C and D showing the localization of RM-581-Fluo (in blue), ER tracker (in red) and Cell-mask (in cyan); **(F)** Merged images B, C and D showing the localization of RM-581-Fluo (in green), ER tracker (in red), Cell-mask (in cyan) and a co-localization in yellow of RM-581-Fluo and ER-Tracker. Scale bar: 10  $\mu$ m.



**Figure S3 (follow)**

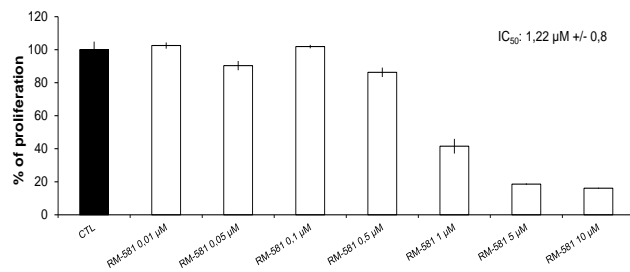


**Figure S3 (follow)**

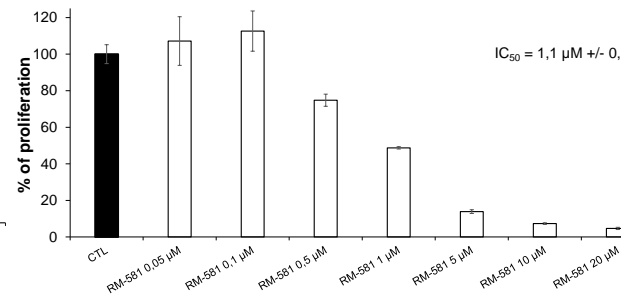


**Figure S4.** Cell proliferation and EC<sub>50</sub> values after continuous exposure to RM-581 in PC-3 cells. PC-3 cells did not develop resistance when treated with 0.8  $\mu$ M of RM-581. Cell proliferation and EC<sub>50</sub> values after 0 (A), 12 (B), 49 (C) and 119 (D) days of treatment with RM-581. EC<sub>50</sub> values are not significantly different.

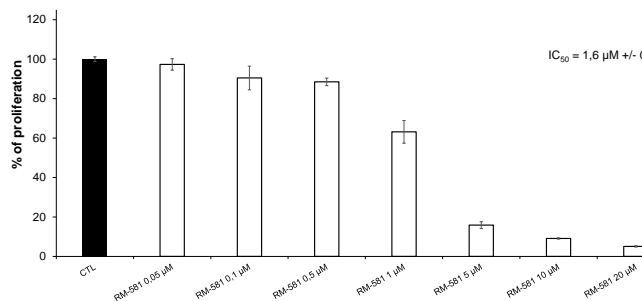
**A** (EC<sub>50</sub> = 1.2  $\pm$  0.8  $\mu$ M)



**B** (EC<sub>50</sub> = 1.1  $\pm$  0.5  $\mu$ M)



**C** (EC<sub>50</sub> = 1.6  $\pm$  0.6  $\mu$ M)



**D** (EC<sub>50</sub> = 0.8  $\pm$  0.4  $\mu$ M)

