

Supporting Information

Interrogating the role of endocytosis pathway and organelle trafficking for Doxorubicin-based combination Ionic Nanomedicines

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Table S1. Different endocytosis inhibitors and their concentrations

Endocytosis inhibitor	Concentration
Chlorpromazine	21.9 μ M
Filipin	4.6 mM
Sucrose	0.3 mM
Chloroquine	100 μ M
AEBSF	0.5 mM
Imipramine	5 μ M
M β CD	2.5 μ M
Amiloride	12.6 μ M

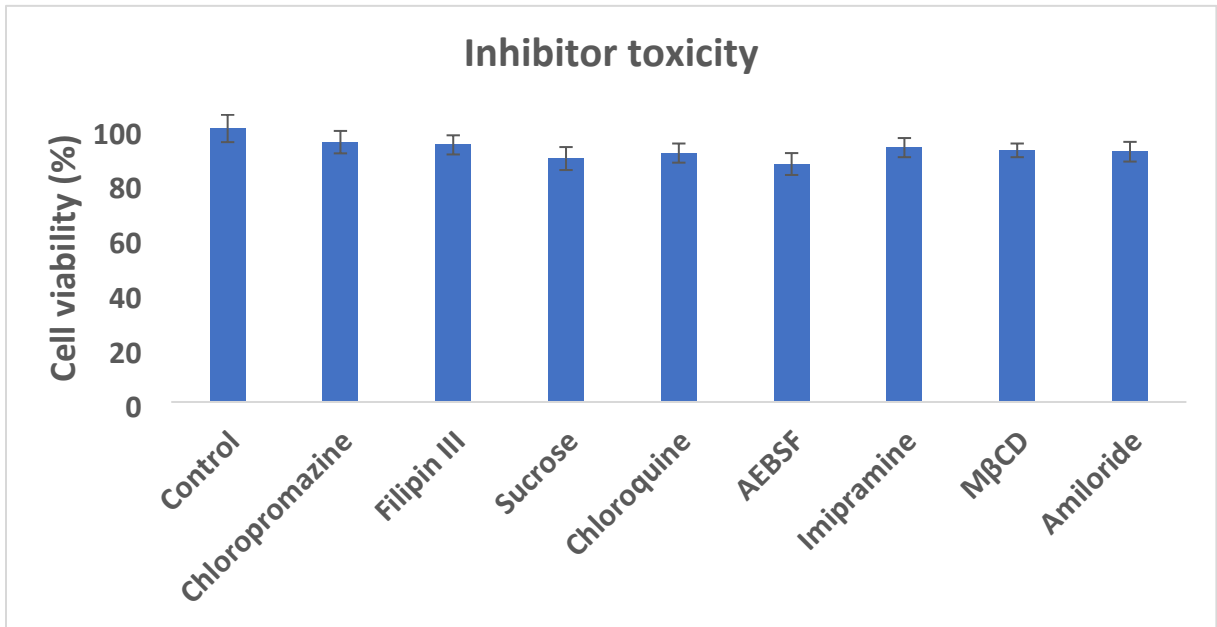


Figure S1. Cell viability endocytic inhibitors in MCF-7 cells pre-incubated for 2 hr at 37 °C prior to cell media introduction for 24 hr. Inhibitor concentrations are reported in Table S1 above. The results are represented as mean \pm SD (n=3), statistically significant $p \leq 0.05$ (*) was evaluated using the student t-test.

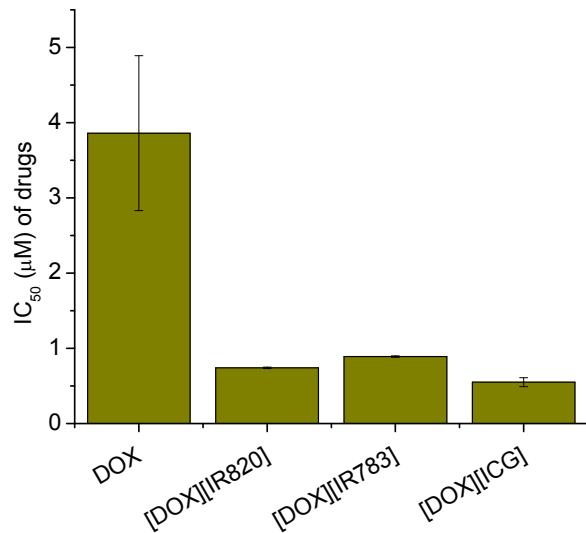


Figure S2: Bar graphs representing the IC₅₀ values for DOX and three chemo-PTT combination INMs on MCF-7 cells incubated for 24 hr. Bar graph obtained from IC₅₀ values generated from previous findings.^[5]

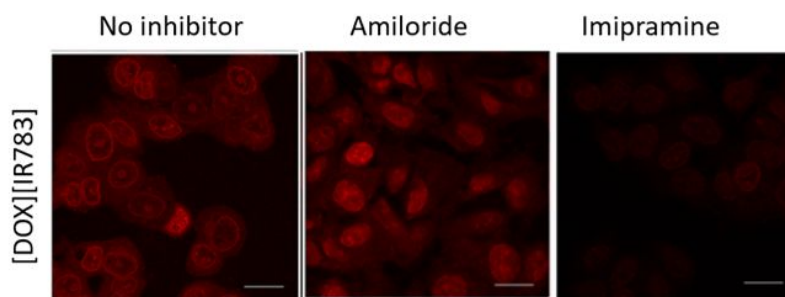


Figure S3. Confocal microscopy images of MCF-7 cells treated with [DOX][IR783] INMs in the presence of macropinocytosis inhibitors introduced at 5 µM and 12.6 µM for amiloride and imipramine. Scale bar represents 10 µm.

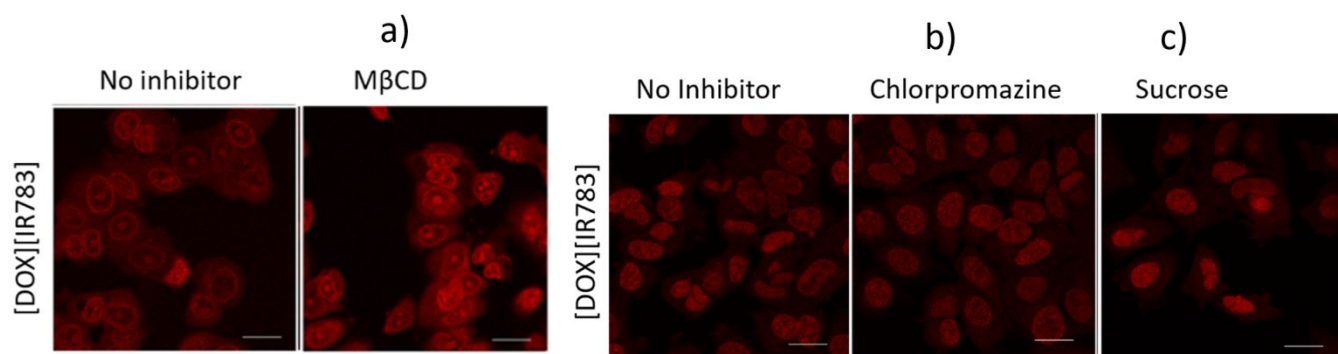


Figure S4. Confocal microscopy images of CME-related inhibitor treated MCF-7 cells incubated with [DOX][IR783] INMs. a) M β CD b) chlorpromazine c) sucrose. M β CD, chlorpromazine and sucrose introduced at 2.5 μ M, 21.9 μ M and 0.3 mM respectively. Scale bar represents 10 μ m.

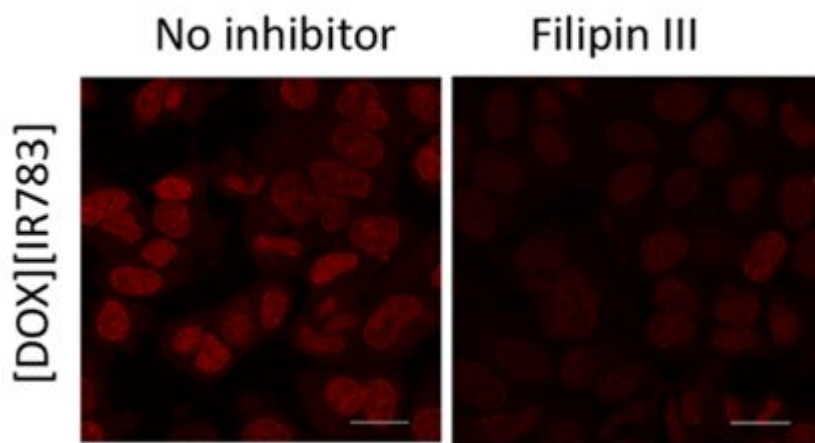


Figure S5. Confocal microscopy images of [DOX][IR783] INMs treated MCF-7 cells in the presence of filipin III at 4.6 mM. Scale bar represents 10 μ m.

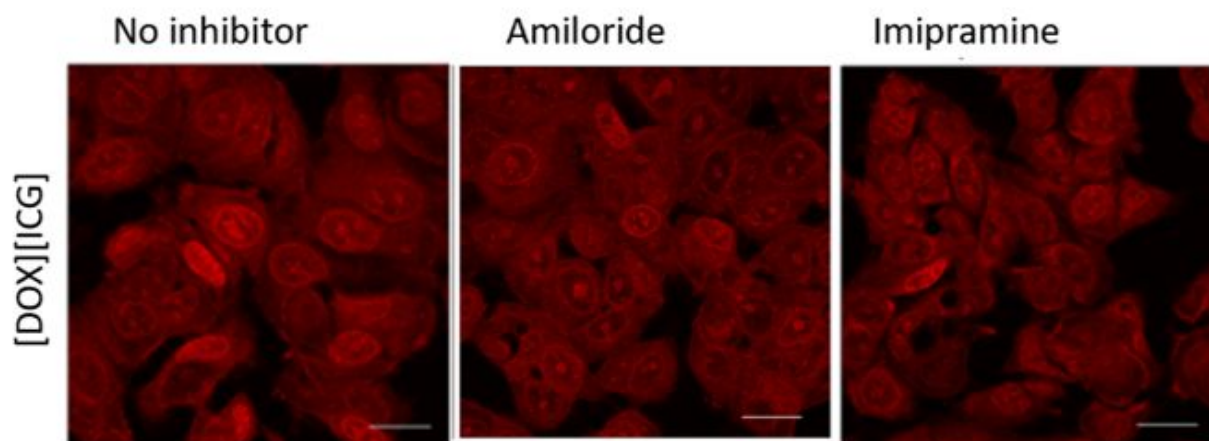


Figure S6. Confocal microscopy images of MCF-7 cells treated with [DOX][ICG] INMs pre-treated with macropinocytosis inhibitors introduced at a concentration of 5 μ M for amiloride and 12.6 μ M for imipramine. Scale bar represents 10 μ m.

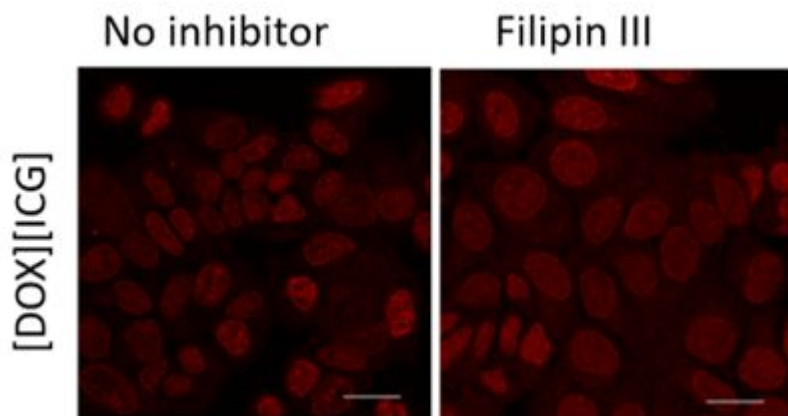


Figure S7. Confocal microscopy images of [DOX][ICG] INMs treated MCF-7 cells in the presence of Filipin III inhibitor introduced at 4.6 mM. Scale bar represents 10 μ m.

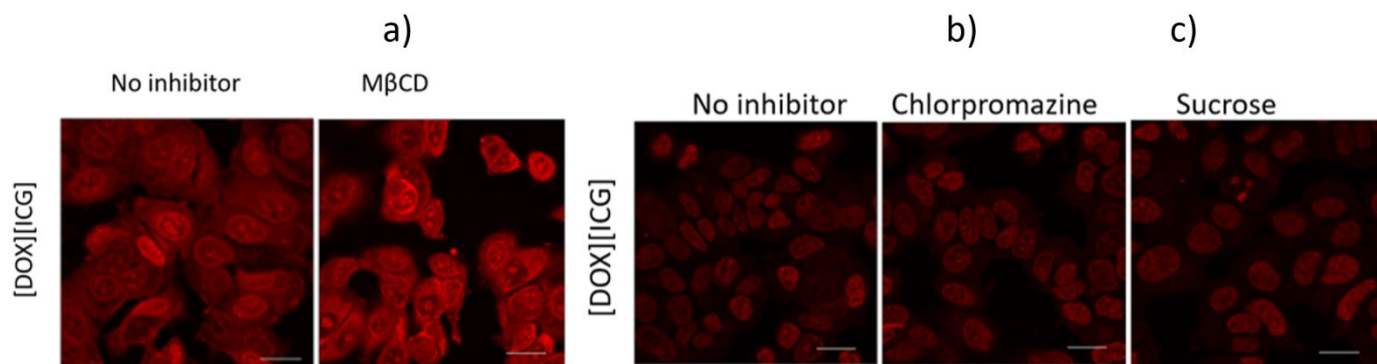


Figure S8. Confocal microscopy images of CME-related inhibitor treated MCF-7 cells incubated with [DOX][ICG] INMs. a) MβCD b) chlorpromazine c) sucrose. MβCD, chlorpromazine and sucrose introduced at 2.5 μM, 21.9 μM and 0.3 mM respectively. Scale bar represents 10 μm.

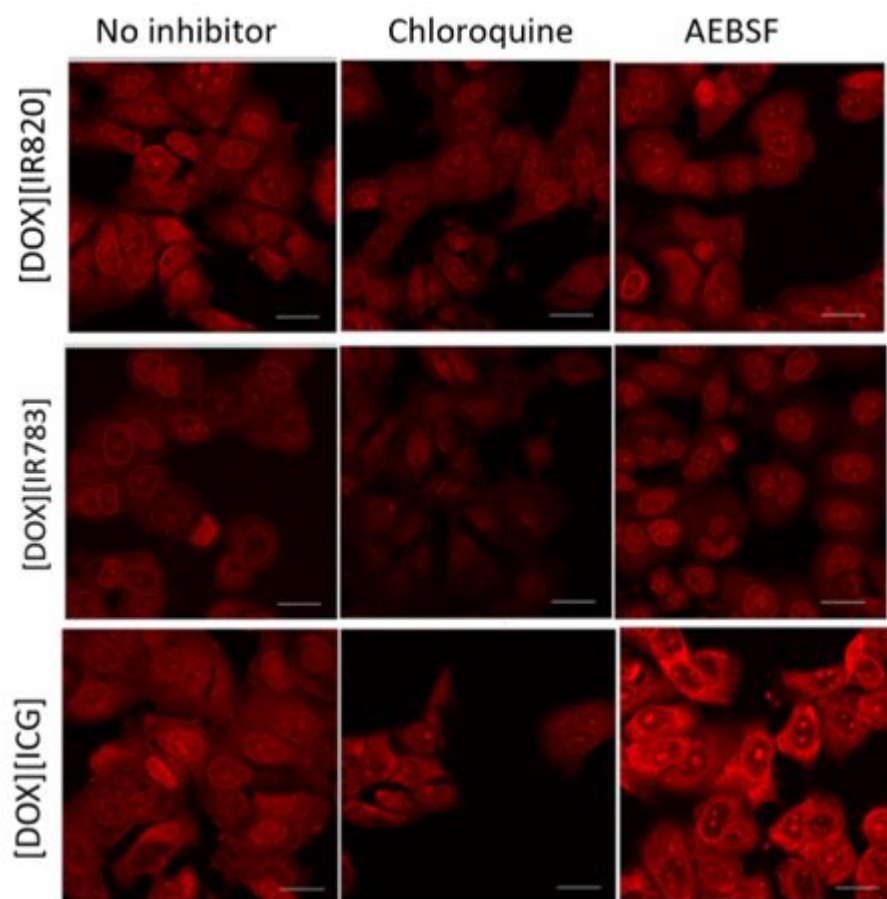


Figure S9. Confocal microscopy images of [DOX][IR820], [DOX][IR783] and [DOX][ICG] INMs pre-treated separately with chloroquine and AEBSF at 100 μ M and 0.5 mM respectively. Scale bar represents 10 μ m.

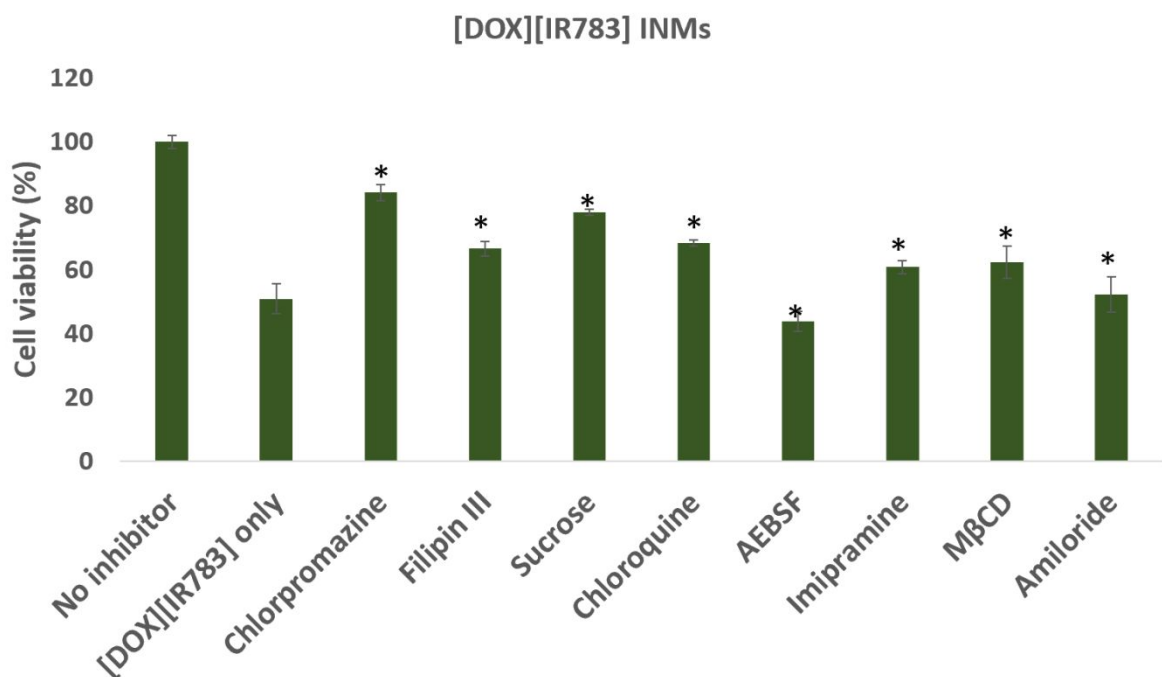


Figure S10. Cell viability of [DOX][IR783] INMs in MCF-7 cells in the presence of different endocytosis inhibitors pre-incubated for 2 hr at 37 °C prior to drug introduction for 24 hr. [DOX][IR783] INMs were introduced at 0.89 μM .¹ The results are represented as mean \pm SD (n=3), statistically significant $p \leq 0.05$ (*) was evaluated using the student t-test.

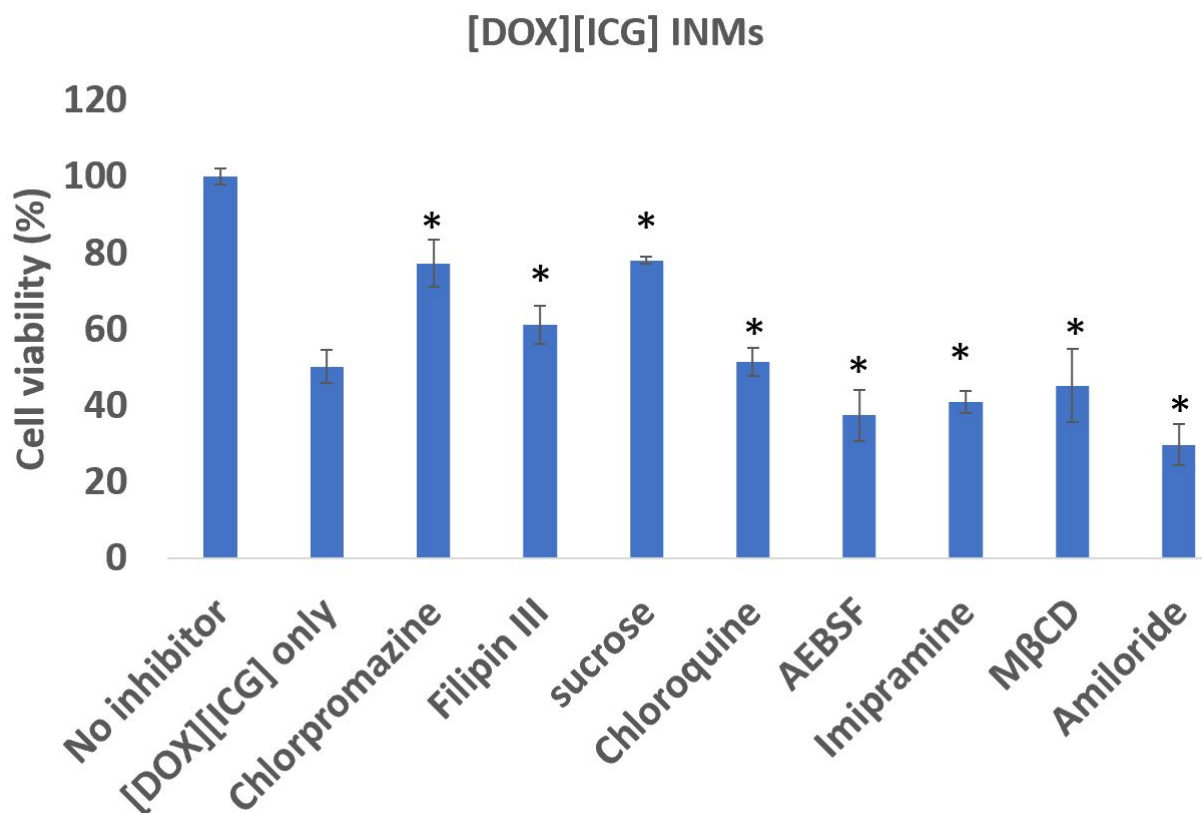


Figure S11. Cell viability of [DOX][ICG] INMs in MCF-7 cells in the presence of different endocytosis inhibitors pre-incubated for 2 hr at 37 °C prior to drug introduction for 24 hr. [DOX][ICG] INMs were introduced at 0.55 μM .¹ The results are represented as mean \pm SD (n=3), statistically significant $p \leq 0.05$ (*) was evaluated using the student t-test.

References

- (1) Bashiru, M.; Macchi, S.; Forson, M.; Khan, A.; Ishtiaq, A.; Oyebade, A.; Jalihal, A.; Ali, N.; Griffin, R. J.; Oyelere, A. K.; Hooshmand, N.; Siraj, N. Doxorubicin-Based Ionic Nanomedicines for Combined Chemo-Phototherapy of Cancer. *ACS Appl. Nano Mater.* **2024**, 7 (2), 2176–2189.
<https://doi.org/10.1021/acsanm.3c05464>.