Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Figures

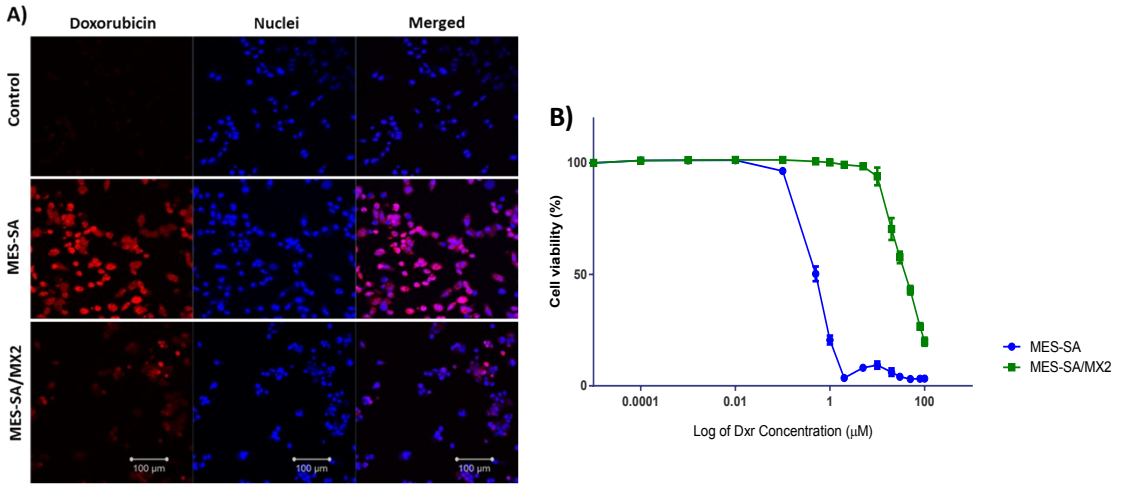

Figure S1. Sensitivity evaluation to Dxr of MES-SA and MES-SA/MX2 cell lines. A) Confocal images (20X) of 6x10^4 cells treated with 30 µg/ml of free Dxr, after 4 h of exposure. B) Cytotoxicity evaluation of both cell lines (2x10^4) exposed 4 h to 0-100 µM of free Dxr. Survival cells were determined by SRB assay at 72 h in triplicate along 3 independent studies. Survival percentage of each cell line was calculated as the relative percentage of their correspondent control (untreated cells), which was set at 100%. The concentration at which the half of cells are killed, Inhibitory Concentration 50 (IC_{50}), was determined by plotting survival versus the log of the concentration and fitting a non-linear regression curve using GraphPad Prism software v8.01. MES-SA/MX2 IC_{50}: 41.72 µM; MES-SA IC_{50}: 0.478 µM.

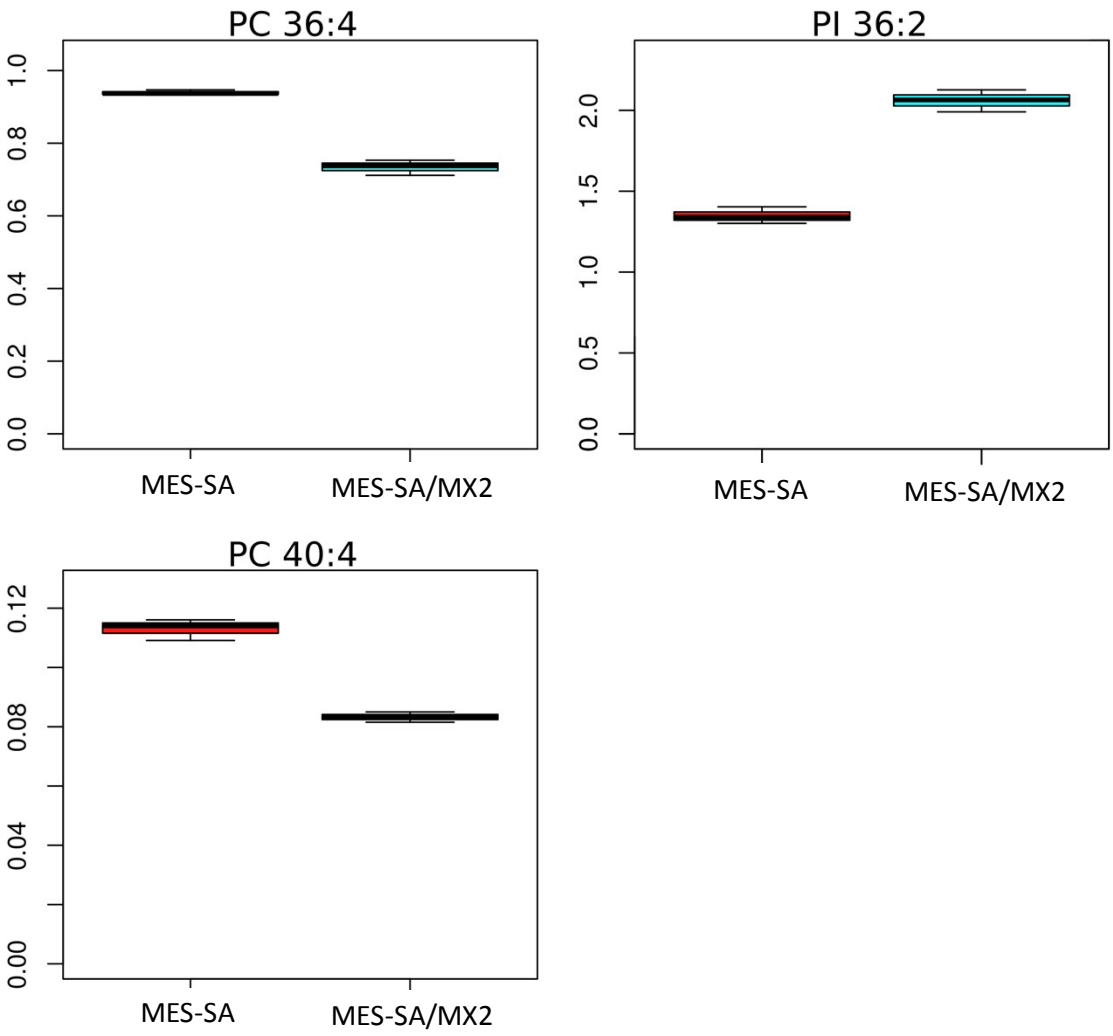

Figure S2. Detailed distribution of the unsaturation pattern in the lipid species that contributed most to cell line differences.

A)	MES-SA		
	10% FBS	5% FBS	0% FBS
Dxr	0.47 ± 0.02	0.27 ± 0.01[1.2]	0.50 ± 0.05[2]
Dxr SCS	0.47 ± 0.01	0.31 ± 0.01[1.2]	0.85 ± 0.13

	MES-SA/MX2		
	10% FBS	5% FBS	0% FBS
	41.60 ± 0.02[1.2.3.4.5]	10.60 ± 0.64[1.2.4.5]	5.27± 0.57[2.5]
	30.24 ± 4.24[2.4]	4.29 ± 0.83	0.46 ± 0.44

B)	Loss of resistance factor		
	10% FBS	5% FBS	0% FBS
MES-SA	1.00 ± 0.003	0.85 ± 0.024	1.55 ± 0.48
MES-SA/MX2	1.38 ± 0.10	2.50 ± 0.315	11.35 ± 6.53

C)	Resistance ratio		
	10% FBS	5% FBS	0% FBS
Dxr	86.88 ± 3.82	38.88 ± 2.45	6.25 ± 0.81
Dxr + SCS	63.21 ± 7.42	13.39 ± 2.35	0.65 ± 0.55

Figure S3. Different serum percentages were used to evaluate the effect of SCS on Dxr efficacy on MES-SA and MES-SA/MX2 cell lines after 72 h of continuous exposure. A) Average and standard deviation of IC_{50} values were calculated after 3 independent experiments. B) Loss of resistance Factor calculation dividing Dxr IC_{50}/Dxr+SCS IC_{50} values of the same tested condition. C) Resistance ratio was calculated dividing MES-SA/MX2 IC_{50} values by MES-SA IC_{50} values of the same tested condition.1: $p<0.05$ vs Dxr 0% FBS; 2: $p<0.05$ vs Dxr SCS 0%FBS; 3: $p<0.05$ vs Dxr 5% FBS; 4: $p<0.05$ vs Dxr SCS 5% FBS; 5: $p<0.05$ vs Dxr SCS 10% FBS.

A)

Sample	Size ± SD (nm)	PDI ± SD	Zeta potential ± SD (mV)	EE (%)
LP-Dxr	82.56 ± 1.8	0.03 ± 0.02	-8.19 ± 0.8	99.91 ± 1.5
LP-SCS-Dxr	83.83 ± 1.1	0.06 ± 0.01	-8.95 ± 0.6	93.33 ± 3.2

B)

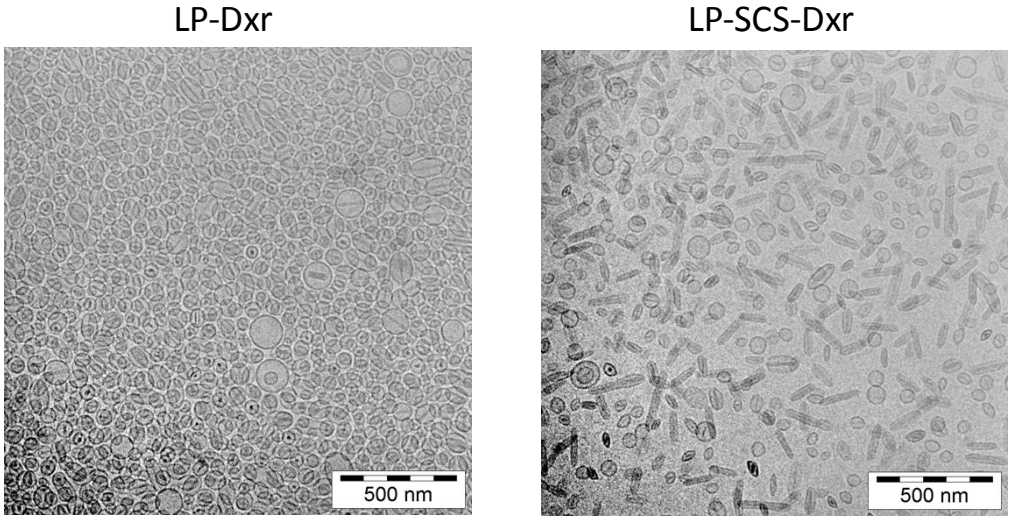

Figure S4. Physicochemical characterization of Doxil-like liposomes (LP-Dxr) and SCS liposomes (LP-SCS-Dxr). A) Liposomes were tested in terms of size, polydispersity index (PDI), surface charge or Zeta potential and encapsulation efficacy (EE). Data correspond to the average and standard deviation (SD) of 5 independent experiments measured in triplicate in water (1:100 dilutions); B) Cryo-TEM images of Dxr liposomes with or without SCS. Drug precipitate can be seen inside liposomes. LP-SCS-Dxr have more elongated rod-like structures upon loading with Dxr. The bar in the micrograph represents 500 nm. A 6,300X magnification was used.

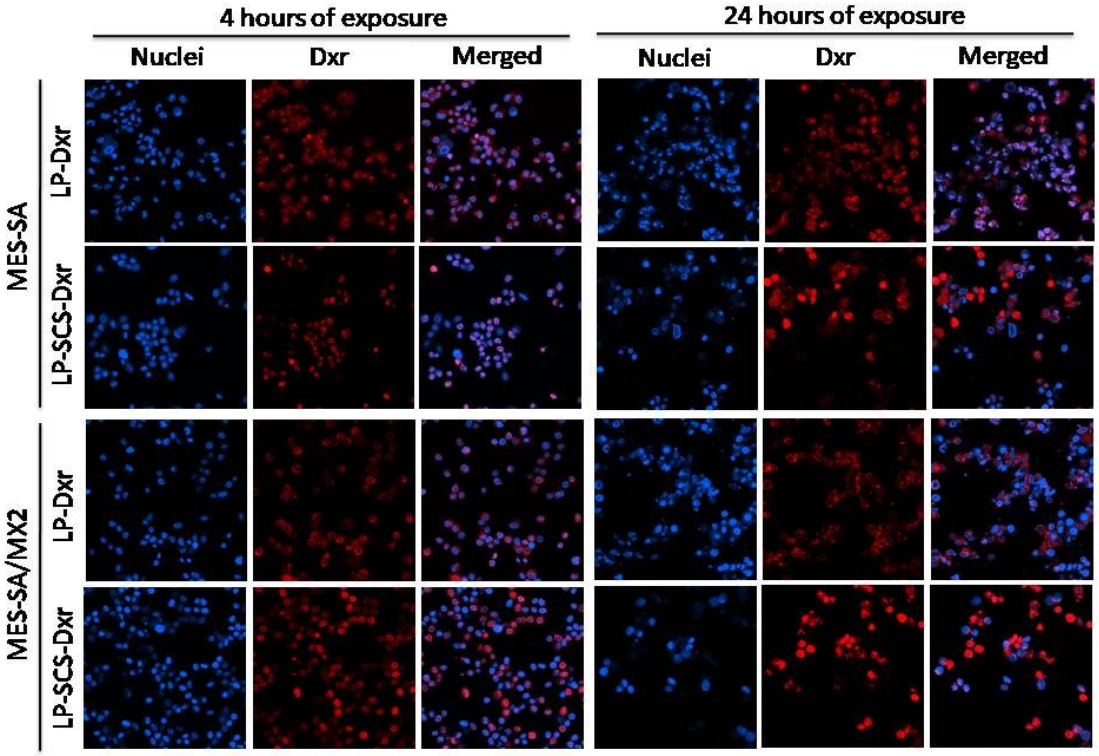

Figure S5. Confocal images of MES-SA and MES-SA/MX2 cell lines after 4 h and 24 h of exposure to LP-Dxr or LP-SCS-Dxr at 20X. Nuclei were stained in blue and Dxr signal is depicted in red in the images. The experiment was repeated 3 times with very similar results.

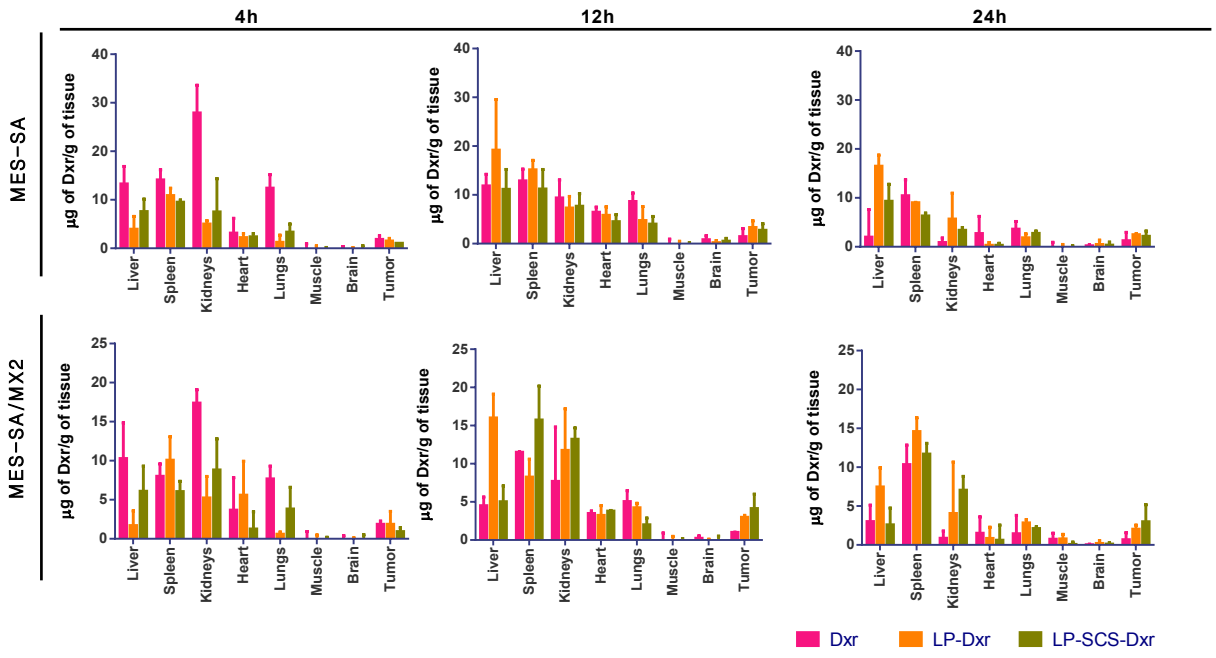

Figure S6. Dxr distribution profiles in different organs at 4, 12 and 24 h in sensitive (MES-SA) and resistant (MES-SA/MX2) tumor-bearing mice. Bars represent the average of 3 mice +/- SD.