Supplementary Materials

CD44 Targeting Mediated by Polymeric Nanoparticles and Combination of Chlorine $TPCS_{2a}$-PDT and Docetaxel-Chemotherapy for Efficient Killing of Breast Differentiated and Stem Cancer Cells In Vitro

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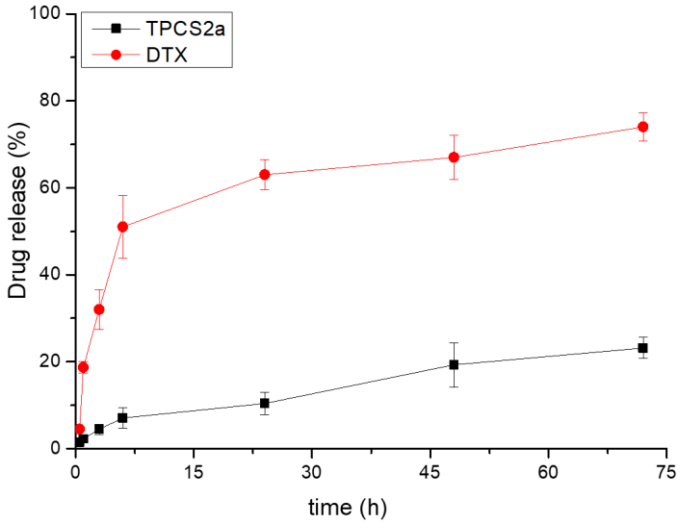

Figure S1. Release of TPCS2a and DTX from HA@DTX/TPCS2a-NPs in DMEM with 10% serum at 37 °C. Data are expressed as mean percentage ± SD of three independent experiments.

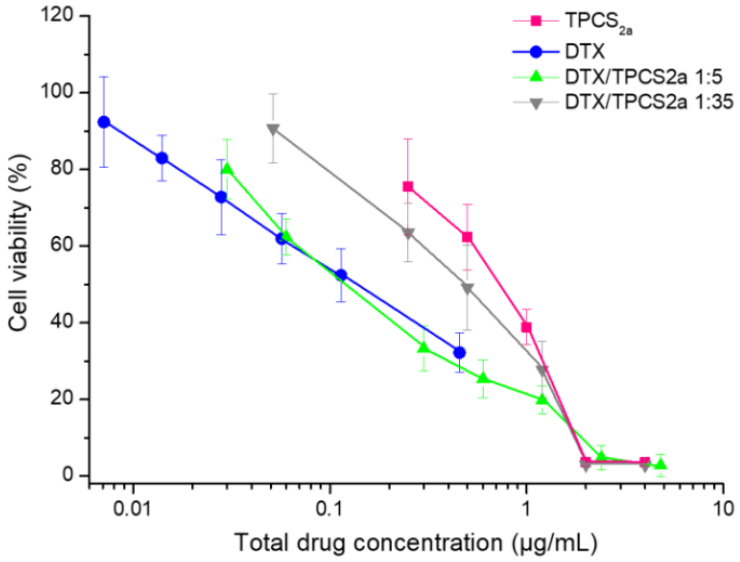

Figure S2. Cytotoxicity of free drugs delivered in the respective solvents in differentiated MCF-7 cells cultured as monolayers. Dose-response curves of cells incubated for 24 h with single drugs or their combination delivered in the free form, irradiated with 1 J/cm^2 of red light (600–800 nm) when PDT was part of the treatment. After additional 24 h in drug-free medium, cell viability was measured with the MTS assay. Total drug concentration is referred to DTX + TPCS2a concentration. Data are expressed as mean percentage ± SD of at least three independent experiments, carried out in triplicate.

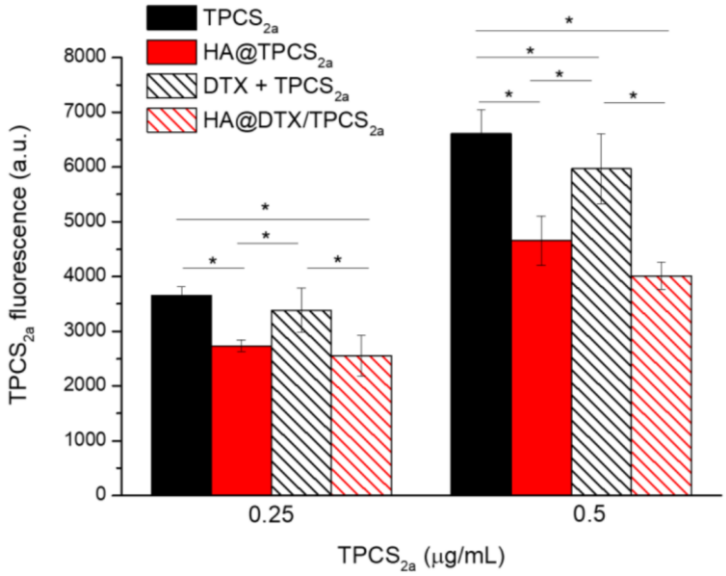

Figure S3. Intracellular uptake of TPCS2a measured in MCF-7 cell monolayers incubated with the photosensitizer delivered in the standard solvent or in HA-NPs, alone or in combination with DTX. The uptake was measured by flow cytometry after 24 h of cell incubation with the drugs. Data are expressed as mean percentage ± SD of at least two independent experiments, carried out in triplicate; * $p < 0.05$ (One-Way ANOVA, Bonferroni's correction).

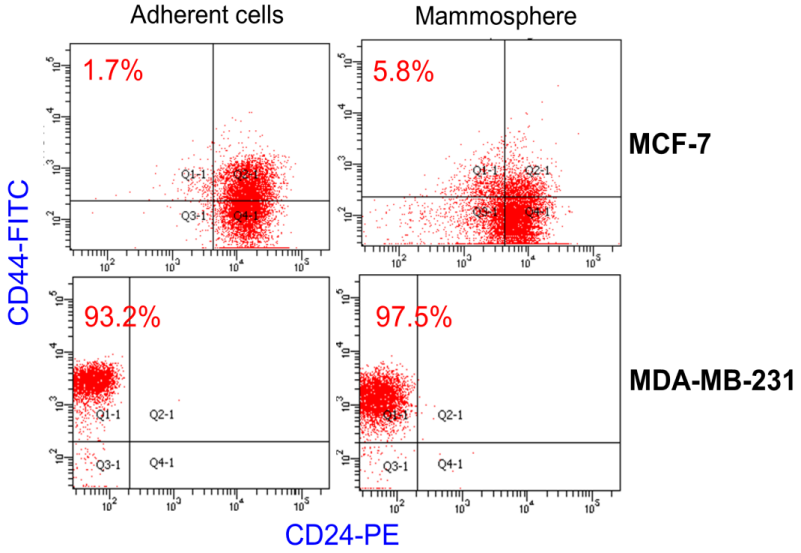

Figure S4. FACS representative plots of receptor profile (CD44/CD24) expression measured in MCF-7 and MDA-MB-231 cultured in adherent conditions or as mammospheres. Cells having a CD44high/CD24low profile, namely cancer stem-like cells, are indicated in Q1-1 with their percentages with respect to the total population indicated by the numbers in red.

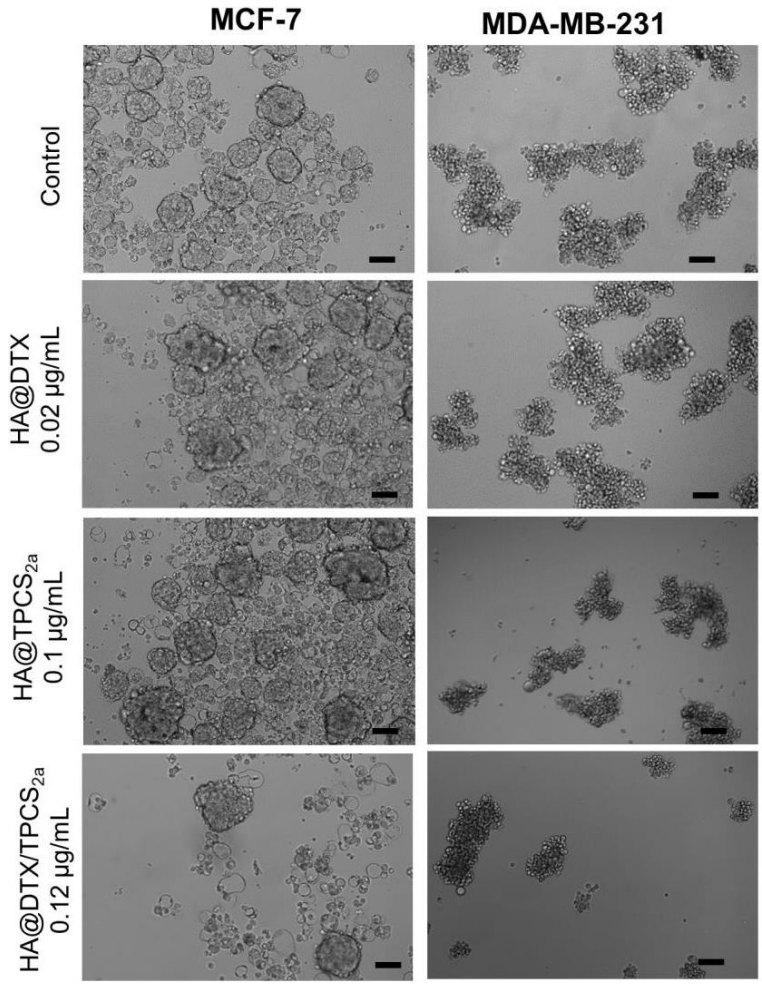

Figure S5. Representative bright field images of MCF-7 and MDA-MB-231 second-generation mammospheres derived from first generation mammospheres treated with HA-NPs. The images were acquired 7 or 4 days after the re-seed for MCF-7 and MDA-MB-231 cells, respectively. Scale bars: 100 µm.

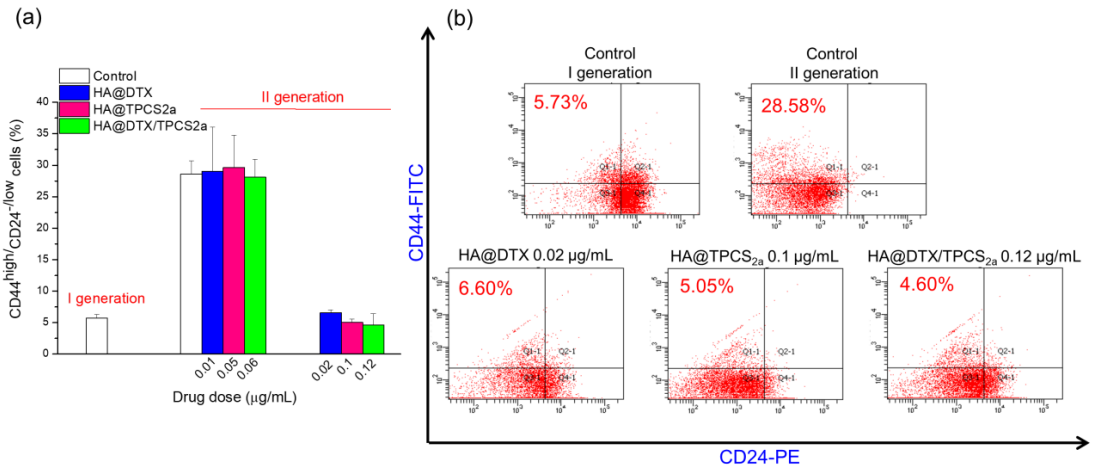

Figure S6. CD44/CD24 receptor profiles in MCF-7 mammospheres. (a) Proportion of CD44high/CD24low cells and (b) representative flow cytometry plots of receptor profile expression measured before (I generation) and after treatment (II generation) with HA@DTX-NPs, HA@TPCS2a-NPs and HA@DTX/TPCS2a-NPs. Mammospheres of the I generation were incubated for 24 h with NPs, irradiated with 1 J/cm2, and re-seeded in non-adherent condition to allow formation of II generation spheres. Before cytometer analysis, cells were stained with a combination of monoclonal antibodies against human CD44 (FITC-conjugated) and CD24 (PE-conjugated). In Q1-1 of the FACS

plots fall those cells having CD44high/CD24low cells receptor expression, namely cancer stem cells. Data are expressed as mean ± S.D. of at least two independent experiments, carried out in triplicate.

Table S1. IC_{50} or Dm value calculated by the Compusyn software in MCF-7 cells exposed to the different DTX and/or $TPCS_{2a}$ formulations. Values indicated drug dose expressed in μg/mL.

Drug Formulation	Total	$TPCS_{2a}$	DTX
$TPCS_{2a}$		0.553	
DTX			0.134
HA@$TPCS_{2a}$		1.108	
HA@DTX			0.144
DTX + $TPCS_{2a}$ (1:35)	0.340	0.330	0.009
DTX + $TPCS_{2a}$ (1:5)	0.137	0.114	0.023
HA@DTX/$TPCS_{2a}$ (1:35)	1.221	1.187	0.034
HA@DTX/$TPCS_{2a}$ (1:5)	0.272	0.226	0.045

Table S2. IC50 or Dm value calculated by the Compusyn software in MDA-MB-231 cells exposed to the different DTX and/or TPCS2a formulations. Values indicated drug dose expressed in μg/mL. These values have been already reported in our previous work ([16] of the main text).

Drug Formulation	Total	$TPCS_{2a}$	DTX
$TPCS_{2a}$		0.332	
DTX			0.016
HA@$TPCS_{2a}$		0.455	
HA@DTX			0.067
DTX + $TPCS_{2a}$ (1:35)	0.200	0.195	0.005
HA@DTX/$TPCS_{2a}$ (1:35)	0.055	0.054	0.001