Supporting information

Cargo-Loaded Lipid-shielded breakable organosilica nanocages for enhanced drug delivery

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Characterization of the brakable ssOSCs

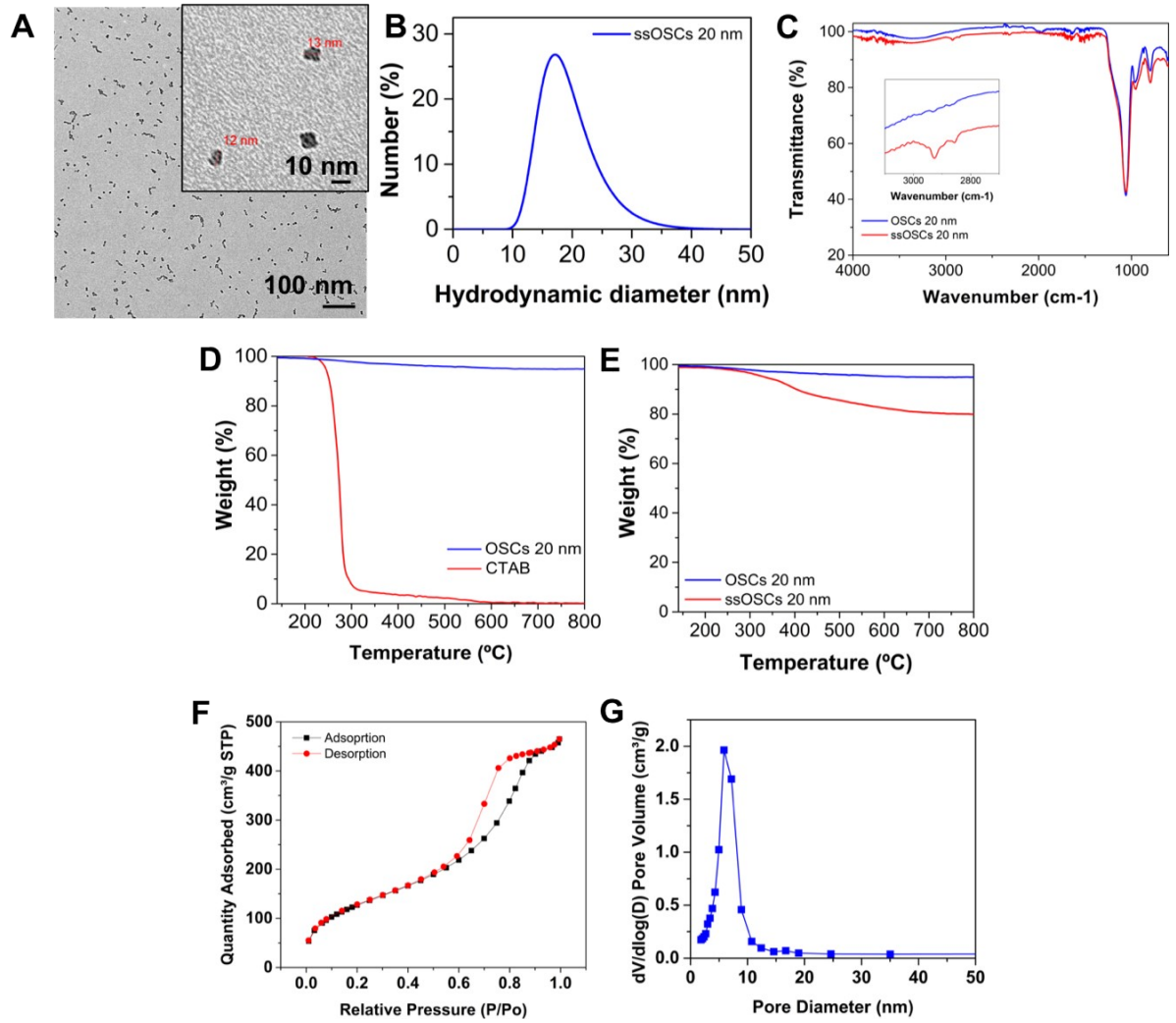

Figure S1. Characterization of the breakable ssOSCs. A) Transmission Electron microscopy images of the ssOSCs. B) DLS analysis, C) FTIR spectra, D) TGA evaluation of OSCs, E) TGA analysis of ssOSCs compared to OSCs, F) N_2-adsorption/desorption isotherms recorded on ssOSCs and, G) Pore size distribution calculated on ssOSCs.

Estimation of amine groups on NPs surface obtained from Kaiser test

Table S1:Estimation of amine groups on NPs surface obtained from Kaiser test

NPs mass (mg)	Abs at 570 nm	molar ab	[M] (mol/L)	volume (L)	NH_2 molar	µmol	µmol/ mg	average
11.5	0.9	15000	6.04E-05	0.003	1.81E-07	0.18	0.015	0.015

SiO_2 density (g/mL)	2.65
nanoparticle diameter (nm)	20
radius (nm)	10
radius (m)	0.00000001
volume per nanoparticle (m^3)	4.18E-24
NH_2 concentration (µmol/mg)	0.015
1 mg nanopartilce NH_2 molar (mol)	1.57E-08
molar constant	6.02E+23
NH_2 number per mg	9.49E+15
1 mg nanoparticle volume (mL)	0.00037
1 mg nanoparticle volume (m^3)	3.77E-10
nanoparticle number per mg	9.01E+13
NH_2 number per nanoparticle	**105.39**

DLS and ζ potential

Table S2: DLS and ζ potential characterization results of uncoated and lipid-coated NPs.

Samples:	DLS size peaks (nm)	PdI	Zeta Potential (mV) ± SD
ssOSCs	25.8	0.46	-10.98 ± 0.56
ssOSCs - neutral lipids	50.43	0.54	-6.17 ± 0.12
ssOSCs -negative lipids	33.06 ; 114.02	0.44	-13.7 ± 0.30
OSCs - NH_2	28.07	0.40	23.57 ± 0.73
ssOSCs - NH_2 - neutral lipids	78.21	0.38	30.70 ± 1.55
ssOSCs - NH_2 - negative lipids 2:1	124.14	0.26	-48.60 ± 0.35
ssOSCs - NH_2 - negative lipids 1:1	107.85	0.37	-50.70 ± 4.78
neutral lipids control	28.19	0.39	-7.34 ± 2.37
negative lipids control	56.23 ; 245.66	1.00	-48,2 ± 1.77

Nanoparticle Tracking Analysis

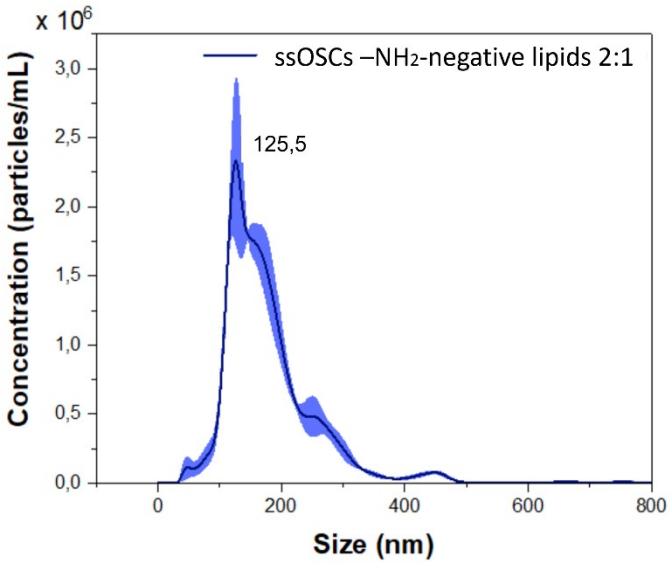

Figure S2. Nanoparticle Tracking Analysis of ssOSCs-NH_2 coated with negative lipids following the 2:1 nanoparticles to lipids mass ratio.

Fluorescence microscopy colocalization analysis results

ssOSCs

Table S3: Calculations of the colocalization percentage of ssOSCs samples.

Negative lipids					Neutral lipids				
ssOSC@DOPA-Chol-DSPE-PEG					ssOSC@DOPC-Chol-DSPE-PEG				
Image	Green channel (lipids) object count	Far-red channel (ssOSCs) object count	Merged image object count	Colocalization percentage	Image	Green channel (lipids) object count	Far-red channel (ssOSCs) object count	Merged image object count	Colocalization percentage
1	16	34	2	5.9%	1	23	17	10	58.8%
2	35	19	0	0.0%	2	28	8	7	87.5%
3	30	9	0	0.0%	3	19	11	9	81.8%
4	9	80	0	0.0%	4	20	19	9	47.4%
5	42	88	8	9.1%	5	15	9	7	77.8%
6	13	80	1	1.3%	6	14	19	7	36.8%
7	21	90	8	8.9%	7	60	21	17	81.0%
8	12	55	0	0.0%	8	14	12	7	58.3%
9	19	39	3	7.7%	9	46	45	29	64.4%
10	57	34	7	20.6%	10	38	22	14	63.6%
Average	25.4	52.8	2.9	**5.3%**	Average	27.7	18.3	11.6	**65.7%**
St. Dev.	15.4	29.9	3.4	**6.6%**	St. Dev.	15.6	10.7	7.0	**16.3%**

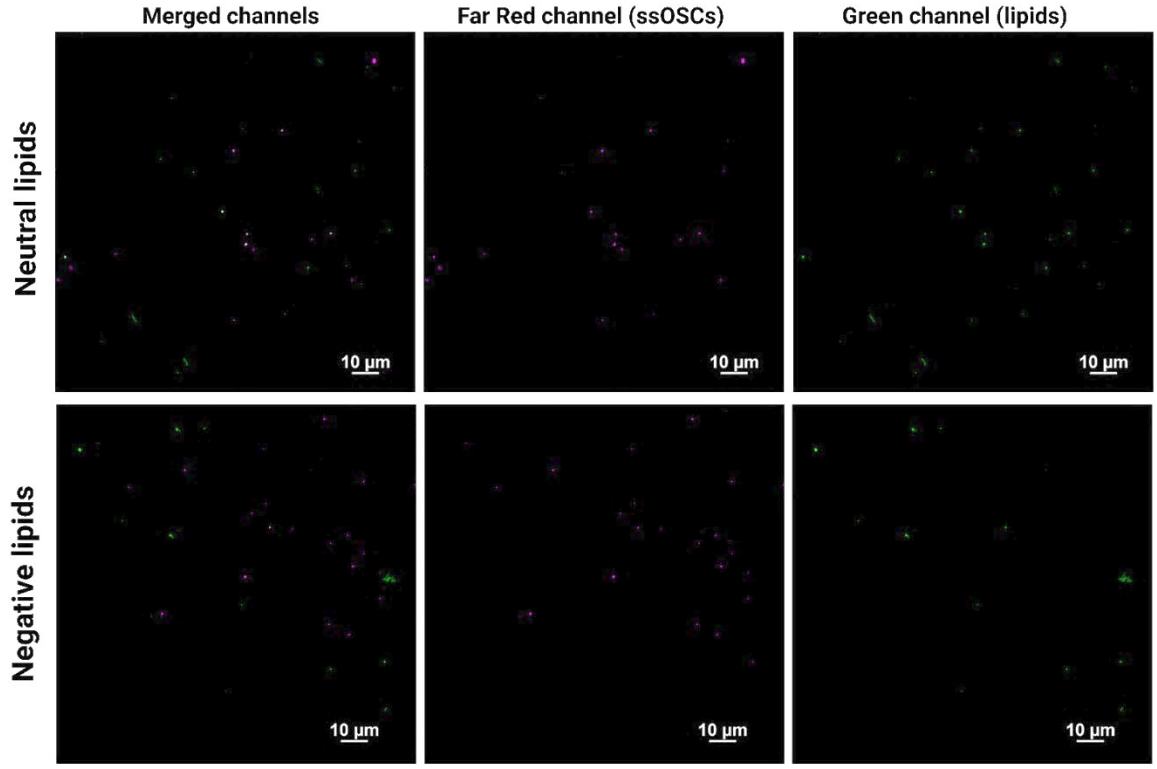

Figure S3. Multichannel epifluorescence microscopy images of the non-functionalized ssOSCs samples covered with neutral (top) and negative (bottom) lipid mixtures. Horizontally, Merged channel (left); Far-Red channel (centre), representing Cy5-labelled ssOSCs-NH_2; and green channel (right), representing DiO-labelled lipids. The colocalized particles appear as white spots in the merged image; the average colocalization percentages (# of colocalizing spots /total #of ssOSCs) is respectively 65.7 ± 16.3% for ssOSCs-Neutral lipids and 5.3 ± 6.6% for ssOSCs-Negative lipids. Scale bar = 10 µm; magnification =100X.

ssOSCs-NH_2

Table S4. Calculations of the colocalization percentage of ssOSCs-NH_2 samples.

Negative lipids					Neutral lipids				
ssOSC-NH2@DOPA-Chol-DSPE-PEG 2:1					ssOSC-NH2@DOPC-Chol-DSPE-PEG 2:1				
Image	Green channel (lipids) object count	Far-red channel (ssOSCs) object count	Merged imageobject count	Colocalization percentage	Image	Green channel (lipids) object count	Far-red channel (ssOSCs) object count	Merged imageobject count	Colocalization percentage
1	14	13	11	84.62%	1	32	45	26	57.8%
2	27	22	21	95.45%	2	21	23	20	87.0%
3	36	40	35	87.50%	3	33	36	13	36.1%
4	30	30	29	96.67%	4	15	23	9	39.1%
5	40	26	24	92.31%	5	101	159	55	34.6%
6	23	16	15	93.75%	6	28	36	17	47.2%
7	27	24	22	91.67%	7	26	31	13	41.9%
8	31	18	17	94.44%	8	28	23	11	47.8%
9	27	27	23	85.19%	9	27	50	17	34.0%
10	32	28	28	100.00%	10	50	32	24	75.0%
Average	28.7	24.4	22.5	**92.16%**	Average	36.1	45.8	20.5	**50.1%**
St. Dev.	7.1	7.8	7.1	**5.03%**	St. Dev.	24.5	40.8	13.3	**18.1%**

ssOSC-NH2@DOPA-Chol-DSPE-PEG 1:1				
Image	Green channel (lipids) object count	Far-red channel (ssOSCs-NH_2) object count	Merged imaged object count	Colocalization percentage
1	20	18	15	83.3%
2	16	17	11	64.7%
3	104	57	52	91.2%
4	21	25	21	84.0%
5	33	35	28	80.0%
6	60	47	39	83.0%
7	54	40	37	92.5%
8	82	60	46	76.7%
9	192	119	102	85.7%
10	169	139	112	80.6%
Average	75.1	55.7	46.3	**82.2%**
St. Dev.	62.6	41.6	34.6	**7.8%**

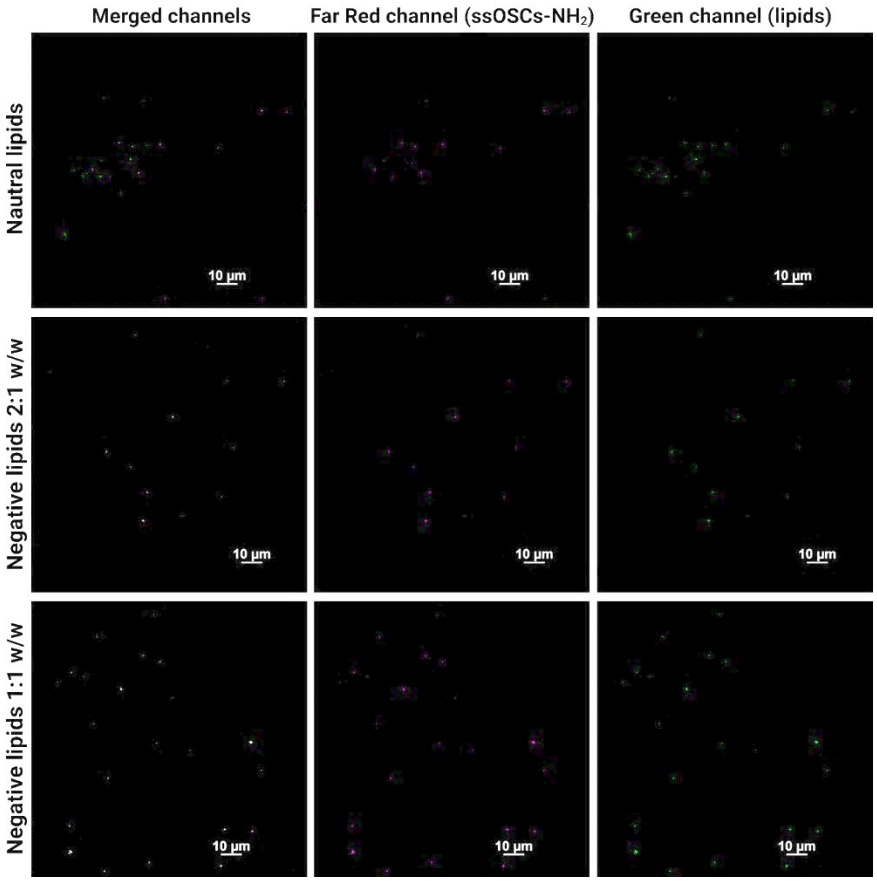

Figure S4. Multichannel epifluorescence microscopy images of ssOSCs-NH_2 samples covered with neutral (top) and negative (central and bottom) lipid mixtures and relative calculated colocalization percentage. Respectively, the central images represent ssOSCs-NH_2 covered with the negative lipid formulation at a 1:1 weight/weight ratio, the bottom images are related to the 2:1 ssOSCs-NH_2/lipids wight/weight ratio. Horizontally, left images: Merged channel; central images: Far-Red channel, representing Cy5-labelled ssOSCs-NH_2 and right images: Green channel, representing DiO-labelled lipids. The colocalized particles appear as white spots in the merged image; the average colocalization percentages (# of colocalizing spots /total #of ssOSCs) is respectively, from top to bottom: 50.1 ± 18.1% 92.16 ± 5.0% and 82.2 ± 7.8%. Scale bar = 10 µm; magnification =100X.

Additional characterizations of nanocages and lipid-coated nanocages

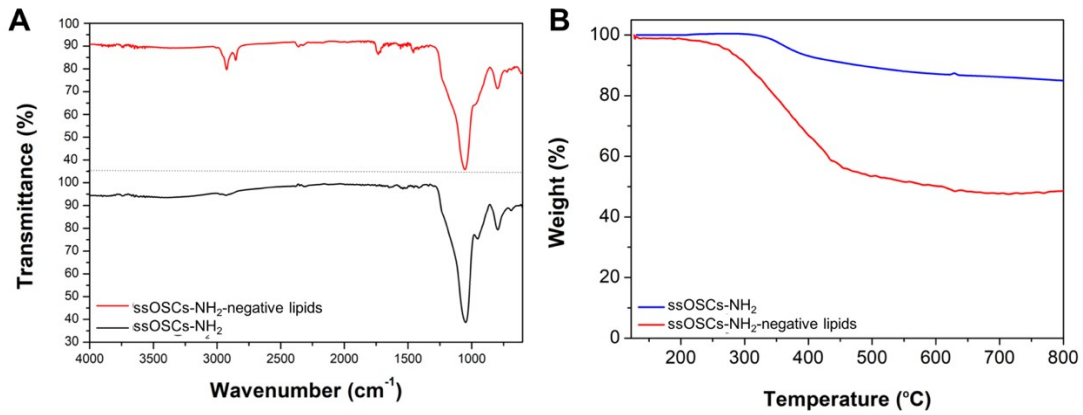

Figure S5. A) FTIR analysis of ssOSCs-NH_2 and ssOSCs-NH_2-negative lipids samples where the characteristic peaks coming from the lipids are clearly observed together with silica bands. B) TGA evaluation of both naked and lipid-coated ssOSCs-NH_2.

Hemocompatibility assay

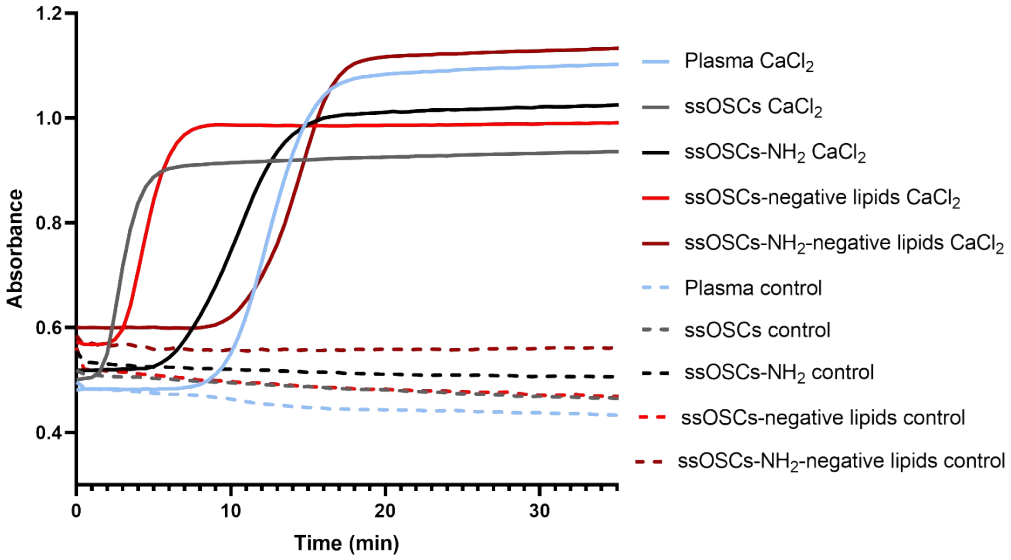

Figure S6. Results in terms of UV-absorbance of the plasma recalcification assay of pristine and negative lipid-coated ssOSCs and ssOSCs-NH2, suspended in physiological solution. The hemocompatibility was evaluated comparing samples in citrate plasma alone (controls, dashed lines) and the ones in citrate plasma treated with calcium chloride (sigmoid curves). The UV–vis absorption at 405 nm was recorded every 30 s. The coagulation time (t_c) was calculated as the time point correspondent to the central absorbance point of the sigmoid curves in which the plateau indicates the complete coagulation of plasma.

Cell internalization by confocal microscopy

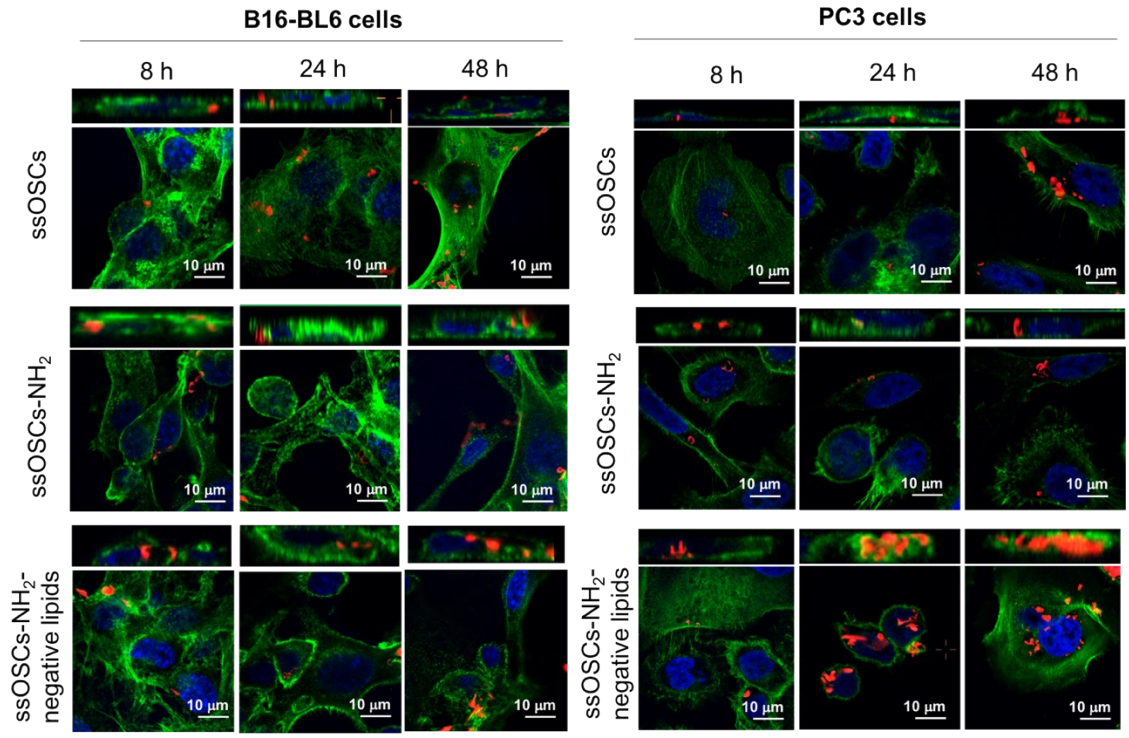

Figure S7. Confocal images of B16-BL6 melanoma cells (top) and PC3 prostate cancer cells (bottom). Cells were incubated with 0.1 mg/mL of ssOSCs, ssOSCs-NH2 and ssOSCs-NH2-negative lipids. Green: actin filaments, blue: nuclei, red: ssOSCs. Above every fluorescence image is reported the lateral projected view retrieved from the z-stacking and clearly proving the presence or absence of internalized nanoparticles.

Additional characterizations of nanocages loaded with calcein and doxorubicin

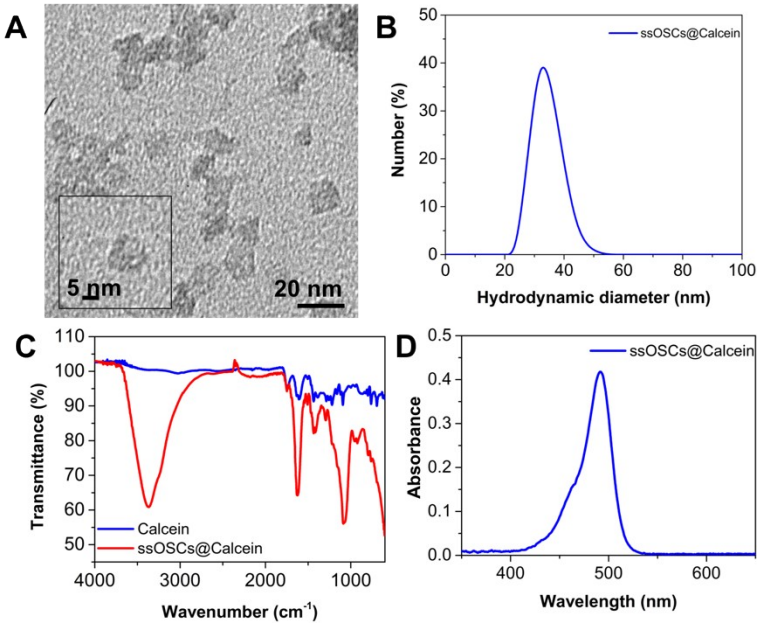

Figure S8. Characterization of ssOSCs@CAL. A) TEM images, B) DLS analysis, C) FTIR and D) Absorption spectrum.

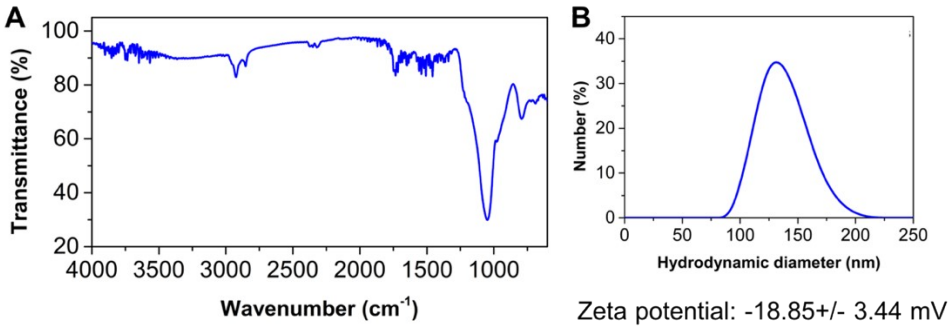

Figure S9. Characterization of ssOSCs@CAL-NH_2-Negative lipids. A) FTIR analysis B) DLS.

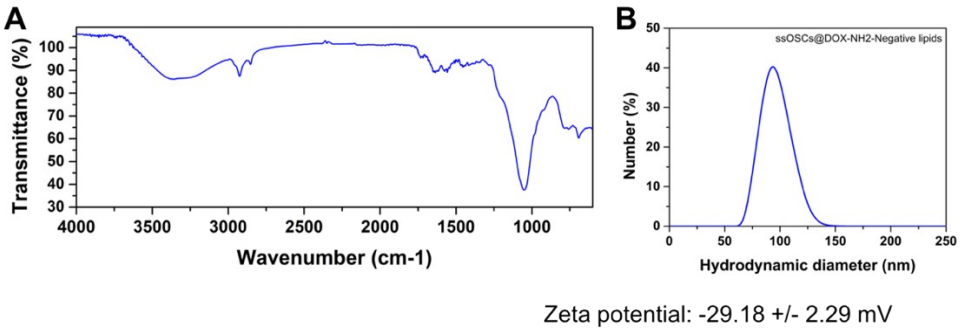

Figure S10. Characterization of ssOSCs@DOX-NH_2-Negative lipids. A) FTIR analysis B) DLS.

Release kinetics of naked and lipid-coated ssOSCs-NH_2

To examine if the lipid coating affects the NPs kinetics of degradation and thus the drug release process, a breakability test was performed. Naked and lipid-coated ssOSCs (both of them loaded with doxorubicin) were suspended in a PBS solution containing the reducing agent, GSH (10 mM), at a final concentration of 0.1 mg/mL. The solution was initially sonicated for 10 minutes and then kept at 37 ºC while stirring. Aliquots were taken at different time points (1, 3, 5, 7 and 24 h) for absorption spectroscopy analysis. Before the measurements, the aliquots were washed by centrifugation to allow the sedimentation of the intact particles, hence the supernatant fraction was collected and its absorption was measured. A progressive increase of the DOX absorbance signal intensity in the supernatant was detected in both naked and lipid-coated ssOSCs (Figure S11A and B). However, differences were observed between them: lipid-coated ssOSCs exhibited a slower and not completed dox release compared with the naked NPs (Figure S11C).

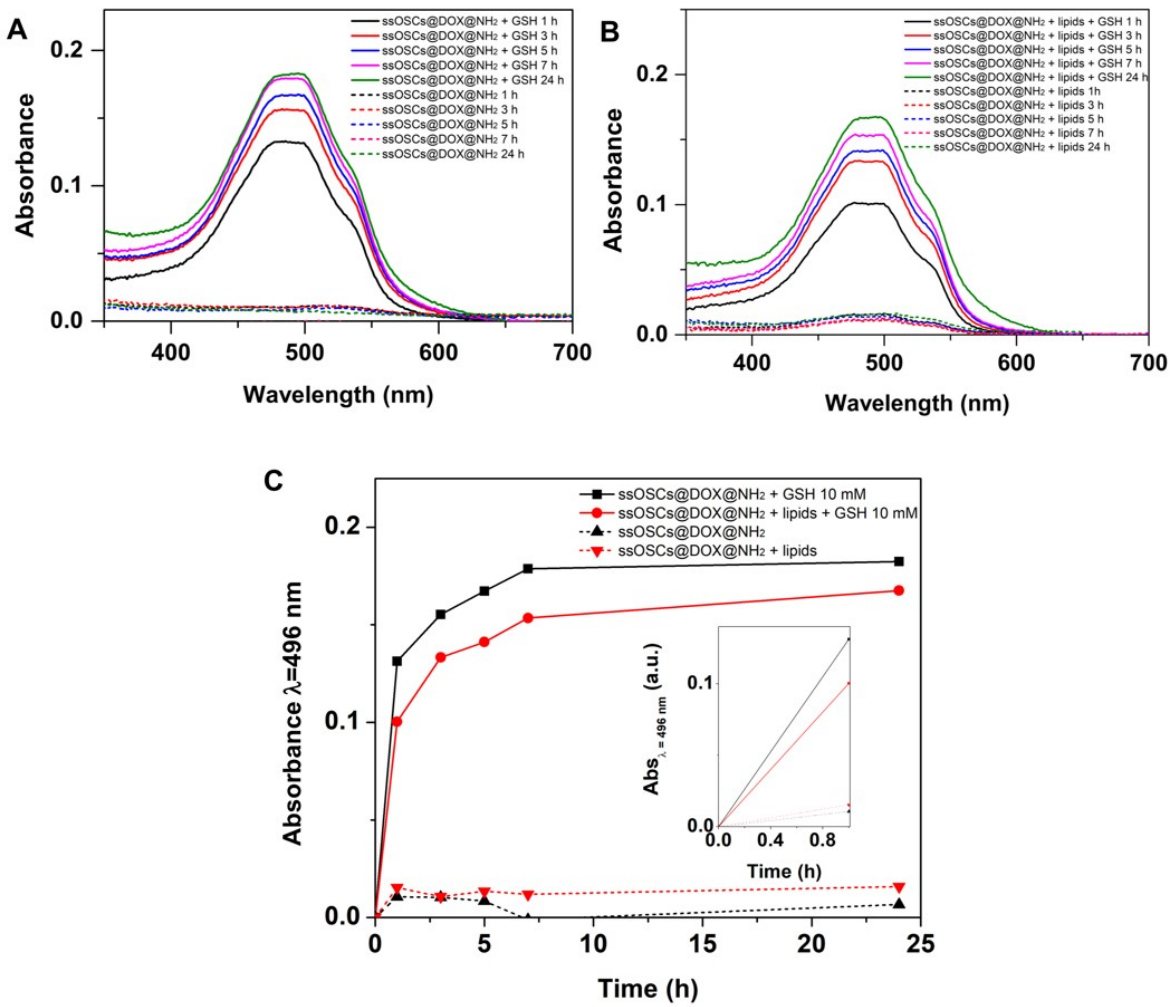

Figure S11. Release kinetics of naked and lipid-coated NPs ssOSCs@DOX-NH2. A) Absorption spectra of the supernatants recovered after treating ssOSCs@DOX-NH2with GSH 10 mM in PBs (pH= 7.4) for 1, 3, 5, 7 and 24 h. B) Absorption spectra of the supernatants recovered after treating ssOSCs@DOX-NH2+lipids with GSH 10 mM in PBS (pH= 7.4) for 1, 3, 5, 7 and 24 h. C) Release profile over time (maximum absorption wavelength = 496 nm.

Cy5 labelled ssOSCs-NH_2

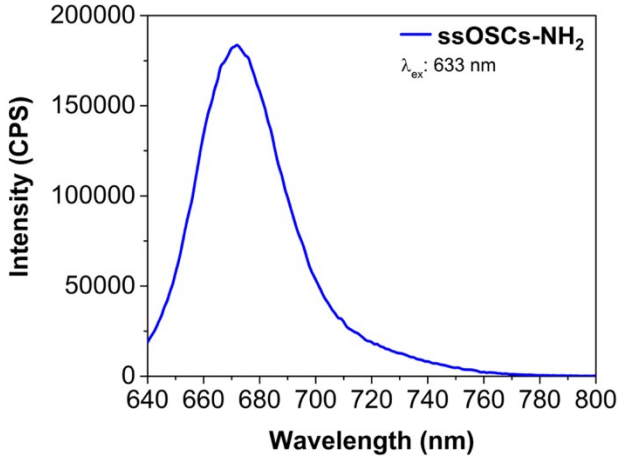

Figure S12. Emission spectra of ssOSCs-NH_2 covered with Cy5. λex= 633 nm

Videos of 3D reconstruction of lipid-coated nanocages internalization within cells – see attached files