Supporting Information

Multilobed Magnetic Liposomes Enable Remotely Controlled Collection, Transport and Delivery of Membrane Soluble Cargo to Vesicles and Cells

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1. Characterization of Multilobed Magnetic Liposomes

The size of MMLs can be tuned by extrusion through membranes with varying pore sizes (400 nm, 800 nm, 1000 nm) and by sonication (**Figure S1**). The size distribution of MMLs after extrusion exhibits two local maxima with the position of the upper mode changing according to the membrane pore size, and the lower mode remaining at approximately 100 nm (Figure S1A). The maxima of the peaks and the polydispersity indices are shown in **Table S1**. From the TEM micrographs (Figure S1B) it can be seen that the whole sample after extrusion (800 nm) contains not only MMLs but also plain non-magnetic liposomes of various sizes. It can be concluded that the lower mode detected in DLS corresponds to the small plain liposomes, which are spontaneously created during the lipid hydration process and do not exhibit magnetic properties (they are removed by magnetic separation- see the main text). The MMLs exhibit weak reversible aggregation in the concentrated state; these aggregates spontaneously break up after 20x dilution (Figure S1C). The MMLs are stable for at least 14 days after the magnetic separation when stored in a fridge and their colloidal stability remains preserved also in cell culture media without any significant change (**Table S2**).

The ATR-FTIR spectra of MMLs contain characteristic bands of both pure (non-stabilized) iron oxide nanoparticles (IONPs) and pure (non-magnetic) giant liposomes. The spectrum of MML exhibits bands typical for phospholipids (2918 (s; $\nu_{as}(CH_2)$), 2851(s; $\nu_s(CH_2)$), 1738 (m; C=O), 1470 (m; $\delta(CH_2)$) cm^{-1}). The presence of the IONPs in the MMLs is reflected in a different ratio of several absorption bands (diffuse band around 3300 cm^{-1} vs. CH_2 or C=O bands), when compared to the spectrum of non-magnetic liposomes.

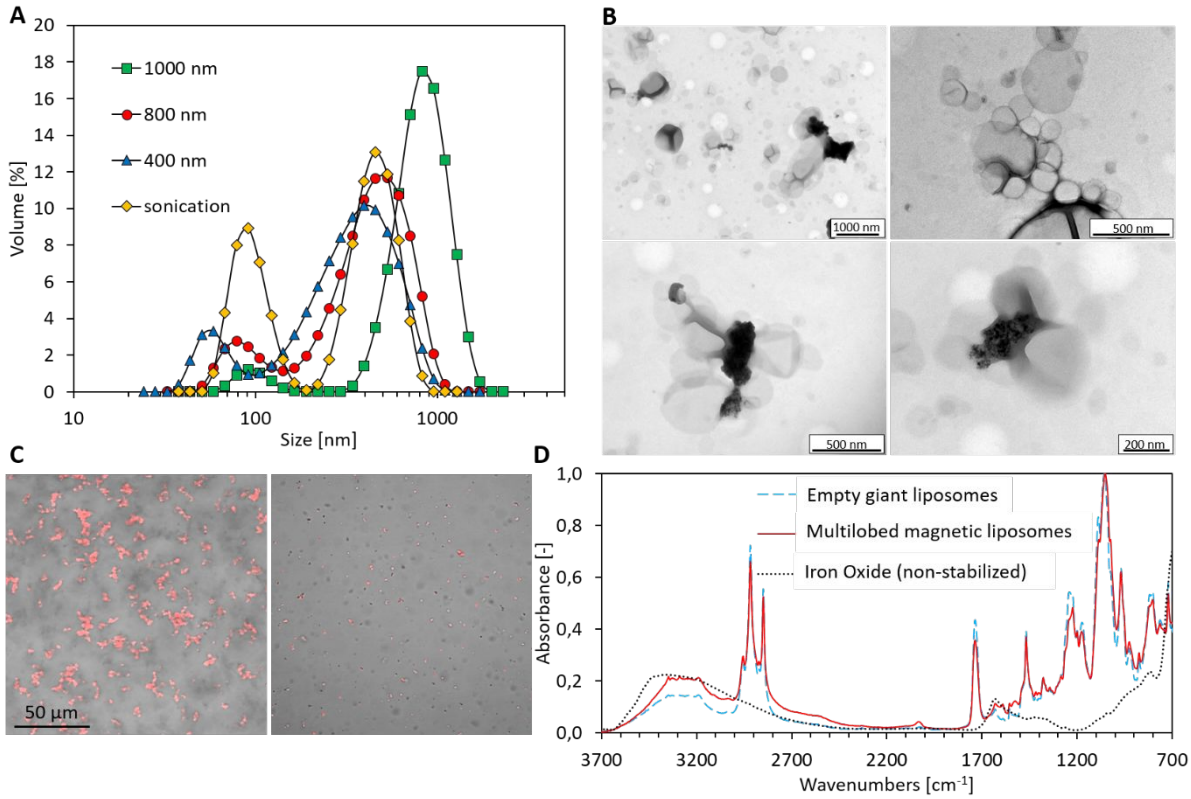

Figure S1: Particle size distribution (DLS) of different size classes of MMLs before magnetic sedimentation (**A**). TEM micrographs of MML sample extruded through 800nm membrane before magnetic sedimentation (**B**). MMLs loaded with Nile red in a concentrated state (left) and after 20x dilution (right) (**C**). ATR-FTIR spectra of MMLs, non-stabilized iron oxide nanoparticles and non-magnetic empty giant liposomes (**D**).

Table S1: DLS analysis of the MMLs prepared by extrusion or sonication.

Extrusion membrane	Main peak ± SD (nm)	Second peak ± SD (nm)	Z-Average (nm)	PDI
1000	863 ± 261	97 ± 20	529	0.318
800	484 ± 192	90 ± 25	304	0.284
400	394 ± 182	61 ± 14	253	0.209
Sonication	468 ± 125	95 ± 22	275	0.672

Table S2: DLS analysis of the MMLs after the magnetic separation measured in the isotonic buffer PBS and in the cell culture medium DMEM.

	Main peak ± SD (nm)	PDI
MMLs day 1 (PBS)	531 ± 135	0.192
MMLs day 14 (PBS)	461 ± 158	0.219
MMLs day 14 (DMEM)	506 ± 90	0.061

2. Control Experiments for Magnetic Delivery

Control experiments proving that the transport and delivery of membrane-soluble cargo occurs only in the presence of MMLs driven by a magnetic field are shown in **Figure S2**. From these experiments it is clear that the cargo (Nile red) is delivered to the giant liposomes in the acceptor compartment after the application of the magnetic field (Figure S2A-B), but there is no delivery when the magnetic field is not present (Figure S2C-D).

In a control experiment without MMLs, no cargo is delivered from the donor compartment (Figure S2E) to the acceptor compartment (Figure S2F). In a control experiment with MMLs but without magnetic field, the MMLs do pick up the cargo from giant liposomes in the donor compartment (Figure S2G), but there is no transport and therefore no delivery to the acceptor compartment (Figure S2H).

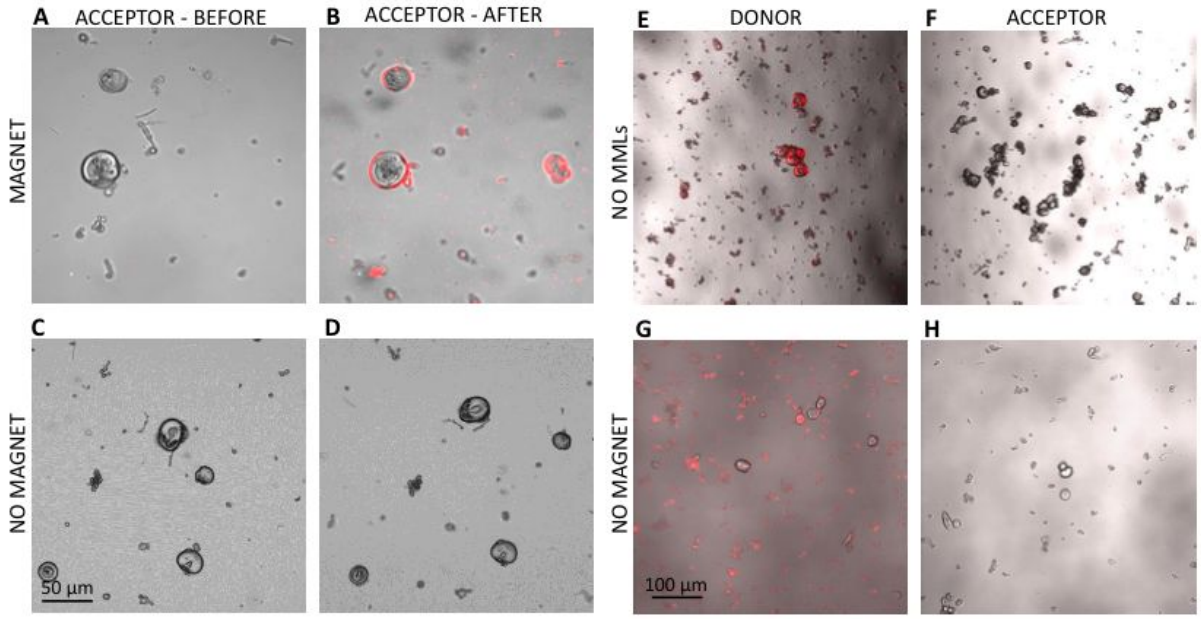

Figure S2: Confocal microscopy images confirming the successful cargo delivery when MMLs are magnetically driven to the acceptor compartment (**A-B**). Control experiment showing that no cargo is delivered do the giant liposomes in the acceptor compartment when the magnetic field is not applied (**C-D**). In a control experiment without MMLs, no cargo is delivered either (**E-F**). In a control experiment with MMLs but without magnetic field, the MMLs do pick up the cargo from giant liposomes in the donor compartment (**G**), but there is no transport and therefore no delivery to the acceptor compartment (**H**).

3. Characterization of Iron Oxide Nanoparticles

XRD analysis of dried IONPS@DPPC was carried out using PANalytical X'Pert PRO with High Score Plus software (**Figure S3**). The obtained diffraction pattern (**Table S3**) corresponds to magnetite (Fe_3O_4). However, it must be taken into account that the maghemite (Fe_2O_3) content bellow 50% cannot be distinguished from magnetite and therefore the sample can teoretically contain both forms. This fact does not limit its use in the creation of MML.

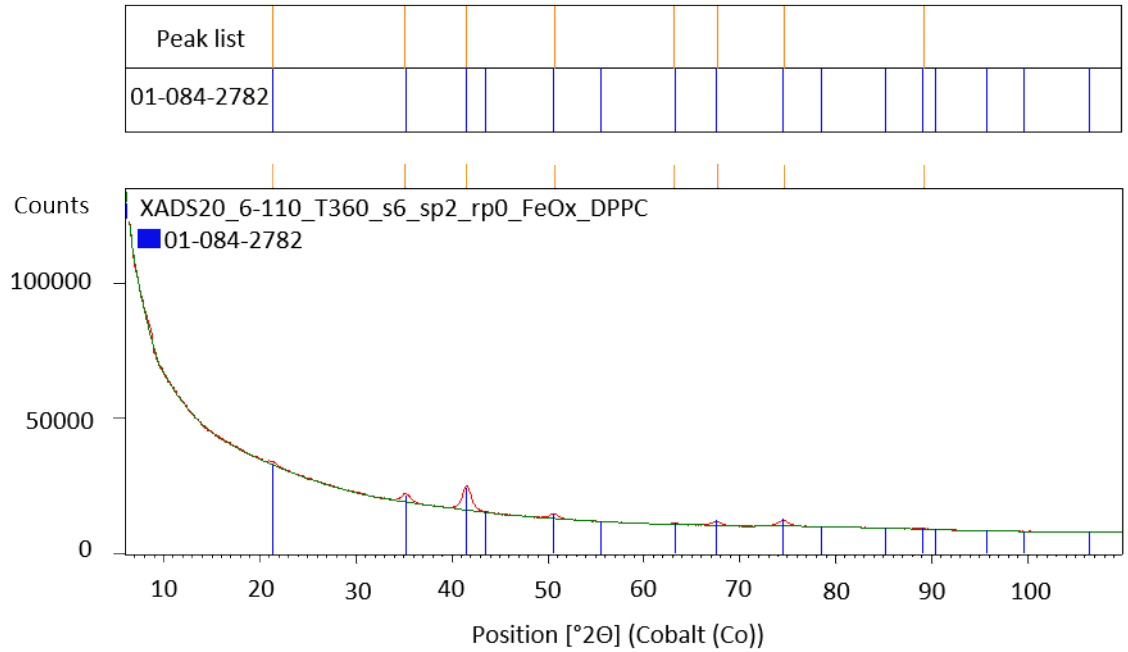

Figure S3: XRD results from IONPs@DPPC analysis.

Table S3: XRD peak list and the pattern details.

Pos. [°2θ]	d-spacing [Å]	Height [cts]	Rel. Int. [%]	FWHM Left [°2θ]	Matched by
21.3834	4.82500	1337.41	14.84	0.6140	01-084-2782
35.1195	2.96703	2961.69	32.85	0.7675	01-084-2782
41.5590	2.52318	9014.88	100.00	0.4221	01-084-2782
50.7838	2.08754	1628.20	18.06	0.5373	01-084-2782
63.1475	1.70963	466.18	5.17	0.7675	01-084-2782
67.7683	1.60561	1239.70	13.75	0.6908	01-084-2782
74.6393	1.47652	2042.29	22.65	0.8443	01-084-2782
89.2480	1.27433	229.44	2.55	0.9210	01-084-2782

Pattern details

Ref.Code	Score	Compound Name	Mineral Name	Chem. Formula	SemiQuant[%]
01-084-2782	71	Iron Oxide	Magnetite, syn	Fe_3O_4	100

4. Confocal Microscopy

Figure S4 shows the co-localization of the fluorescent signals obtained from phospholipids (NBDPC) and hydrophilic (carboxyfluorescein) and lipophilic (Nile red) fluorescent dyes. For this purpose, two types of MMLs were prepared: (i) MMLs with Nile red and NBDPC (Figure S4A) and (ii) MMLs with Nile red and carboxyfluorescein (Figure S4B). Since the carboxyfluorescein and NDBPC have similar emission spectra, they cannot be distinguished one from another by confocal microscopy, and therefore this combination was not prepared. Prepared MMLs were repeatedly washed by magnetic sedimentation and only the magnetic particles were collected and analyzed by fluorescent microscopy, which provides information about their magnetic behavior and thus positively proves iron oxide content, since the plain liposomes are not magnetic. The MMLs were immobilized in 1% sodium alginate crosslinked with $CaCl_2$ in order to suppress Brownian motion. The pictures were taken using confocal microscope Olympus Fluoview FV 1000, 60x objective with immersion oil and the emitted light (after 488 nm excitation) was filtered to two channels – 505-540 nm (green; NBDPC / carboxyfluorescein) and 660-750 nm (red; Nile red). The overlapping of Nile red and NBDPC/carboxyfluorescein and their co-localization is demonstrated by plotting the same line segment in both images (red and green) and by comparison of the obtained intensity profiles shown below the fluorescent images (software ImageJ). Here it is clearly visible that both colors are present in the same regions of the image.

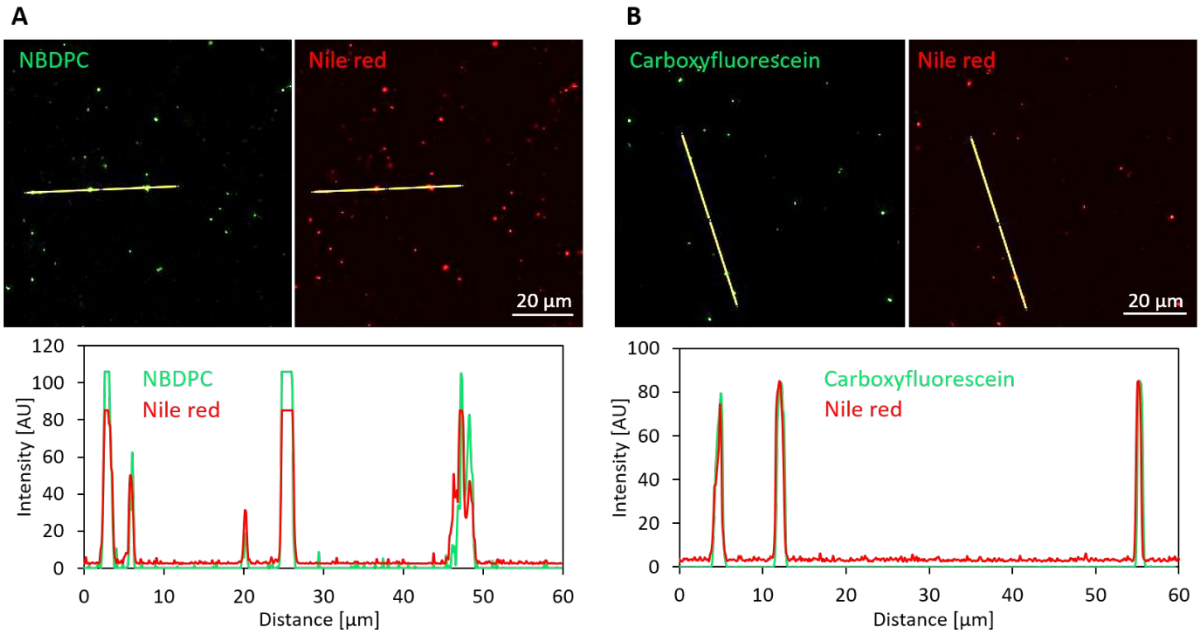

Figure S4: Confocal microscopy images of MMLs with covalently labelled phospholipid NBDPC and lipophilic fluorescent dye Nile red and the corresponding signal co-localization chart (**A**) and MMLs with hydrophilic carboxyfluorescein and lipophilic Nile red and the corresponding signal co-localization chart (**B**).

5. TEM validation

TEM microscopy was used to study morphology of IONPs@DPPC and MMLs (**Figure S5**). The TEM image of IONPs@DPPC presented in the main text shows a population of the particles which were also analyzed by image analysis represented by a histogram. Here we present images with higher magnification (Figure S5A-B). These nanoparticles can be clearly seen inside of the MMLs after the magnetic separation (Figure S5C-D). The size distribution of dark spots inside of the MML which are claimed to be IONPs@DPPC (Figure S5-D) is of a high similarity to the size distribution of IONPs@DPPC presented in the main text. The images with lower magnification show multiple MMLs in one frame (Figure S5E-F), which proves that the multilobal structures occur non-randomly. When comparing MMLs with plain liposomes (extruded through 800nm membrane) analyzed by the same procedure (sample drying and staining with 1% uranyl acetate), which are shown in Figure S5G-H, it can be concluded that the dark spots are indeed IONPs@DPPC, because no such structures appear in non-magnetic liposomes. Figure S5G-H also demonstrated that the sample preparation procedure did not result in the collapse of the spherical liposome structures.

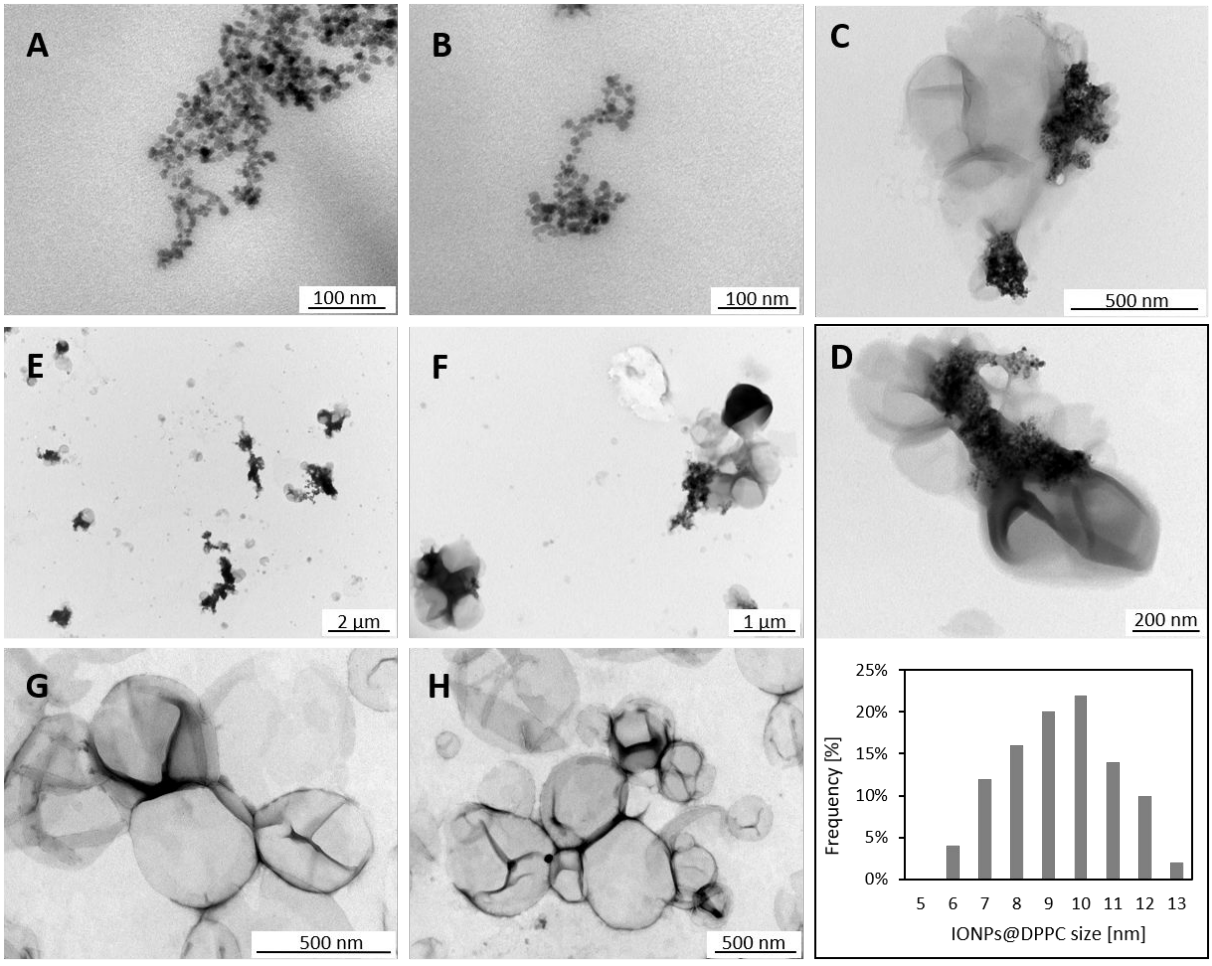

Figure S5: TEM images of IONPs@DPPC (**A, B**), magnified images of magnetically separated MML (**C**) and MML with an IONPs@DPPC size distribution chart (**D**), multiple MMLs in one frame (**E, F**) and plain liposomes (**G, H**).

6. Cytotoxicity Assay

The non-toxicity of the MMLs was confirmed by a cytotoxic assay presented in **Figure S6**. The HT-29 cells in DMEM medium were inoculated into 96-wellplate (20 000 cells/well) and grown for 24h, then the samples diluted in DMEM (500 µg/mL to 4 µg/mL) were added and incubated at 37°C, 5% CO_2 for another 24h. Then, the wells were washed with PBS and the number of living cells was established by spectrophotometric analysis using Cell Counting Kit-8 (Merck; 30min incubation at 37°C), measuring the absorbance at 450 nm and the reference at 650 nm. There was no statistically relevant reduction in the cell viability detected in case of MMLs exposition, when compared to both control and plain liposomes (ANOVA, $p<0.01$, $N = 3$).

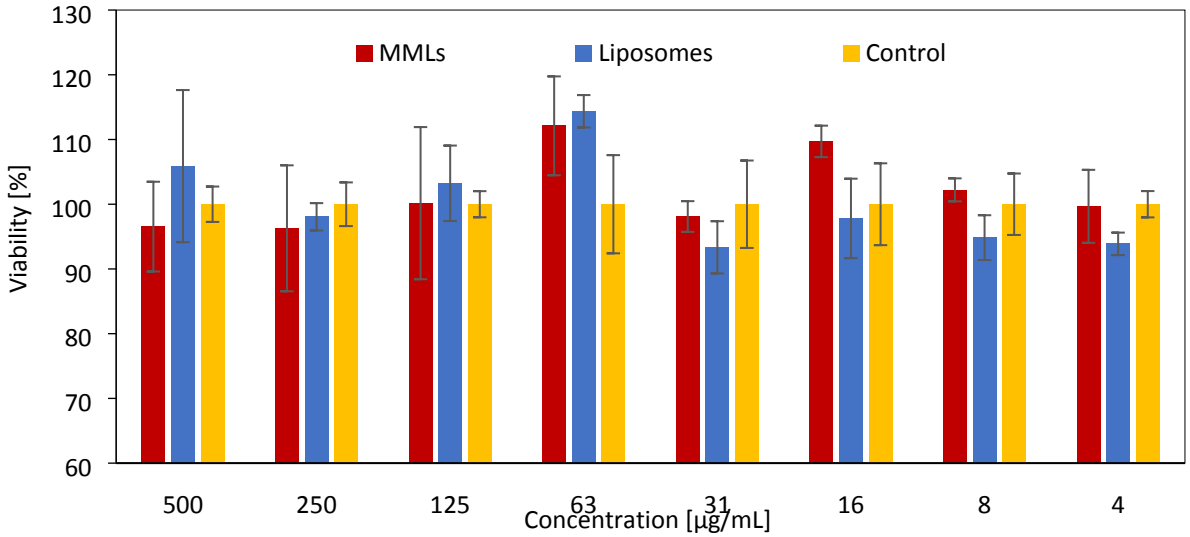

Figure S6: Cytotoxicity assay comparing the effect of MMLs and plain liposomes on HT-29 cells in a wide range of concentrations. No statistically relevant change in cell viability was detected.