Supplementary information

DLS analysis of DOX-loaded (C)TSLs

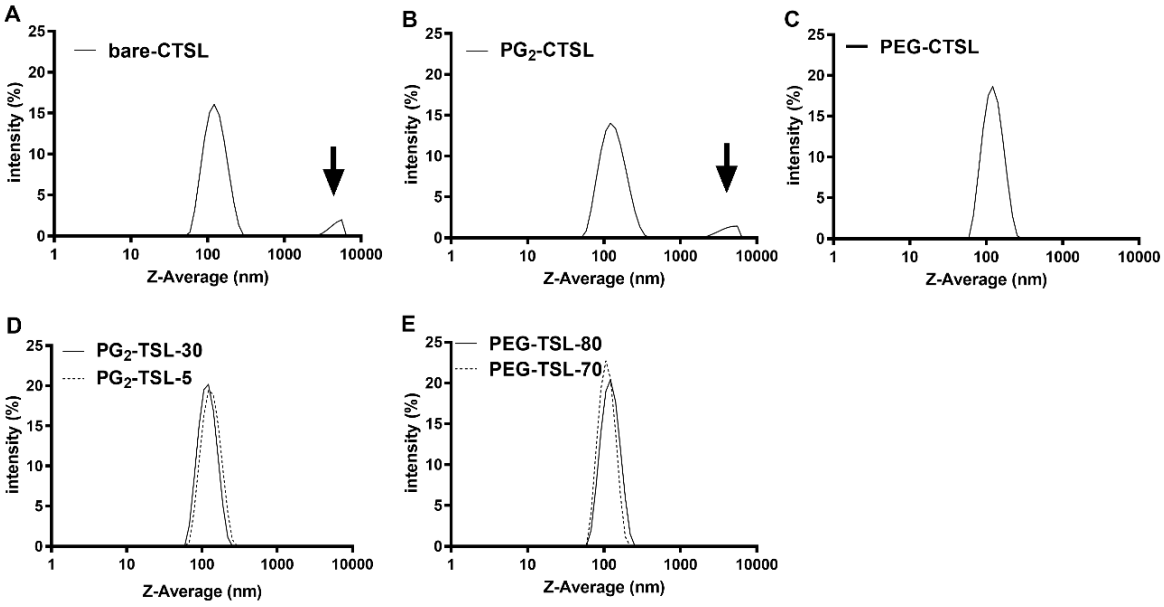

Figure 1 Representative size distribution by DSL analysis of bare-CTSL (**A**), PG_2-CTSL (**B**), PEG-CTSL 4 (**C**), PG_2-TSLs (**D**), and PEG-TSLs (**E**). DOX-loaded liposomes were diluted 1:50 (v/v) in NaCl 0.9% and 5 size measurement carried out via Zeta Sizer Nano SZ. Black arrows indicate presence of extra peaks 6 appearing in size measurements with intensity distribution.

TSLs lipid composition analysis via thin layer chromatography

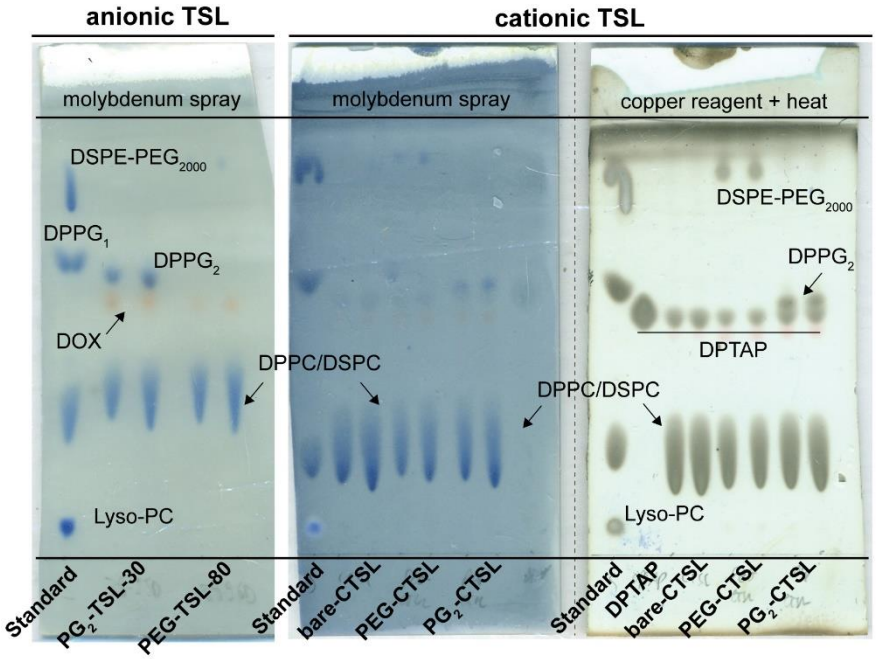

Figure 2 Representative thin layer chromatography analysis of all (C)TSL formulations used in the study. A mobile phase composed of chloroform/methanol/acetic acid/H_2O (100:60:10:5, vol:vol) was used to achieve lipid separation. A standard solution with Lyso-PC, DPPC/DSPC, DPPG and DSPE-PEG_{2000} was used for lipid identification**.** The plates were stained either with molybdenum spray or with copper reagent. The latter was used exclusively for cationic formulation to allow analysis also of non-phosphate containing lipids (e.g., DPTAP).

Effect of protein adsorption on (C)TSLs-cell targeting

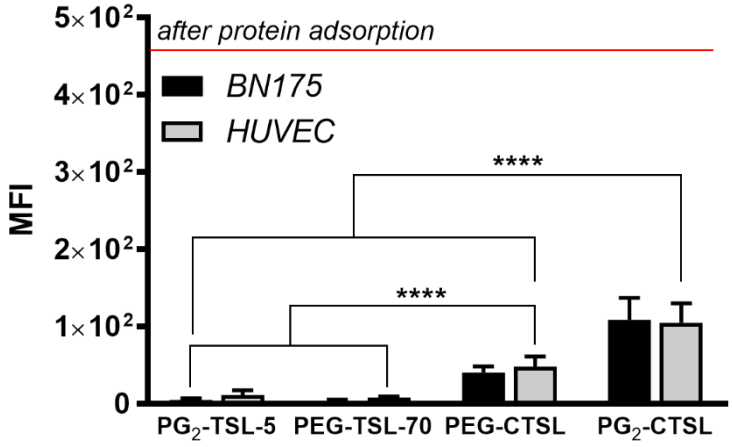

Figure 3 Mean fluorescence intensity (MFI) of Iso-PE shift of BN175 cells and HUVEC after incubation with rhodamine-labeled (C)TSLs after protein adsorption. Liposomes were diluted 1:12 (v/v) in pre-centrifuged (75,000 x g, 1 hour) full FCS for 30 min at 37 °C. (C)TSLs with surface-adsorbed proteins were recovered via centrifugation (75,000 x g, 1 hour) and used in cell binding assay. ISO-PE fluorescence of untreated cells was subtracted from each sample and final MFI plotted as mean value ± SD for three independent measurements. Groups were analysed via one-way ANOVA followed by Bonferroni test, and asterisks indicate significant differences between groups. **** $p < 0.0001$.

Live cell fluorescence microscopy and bright field analysis

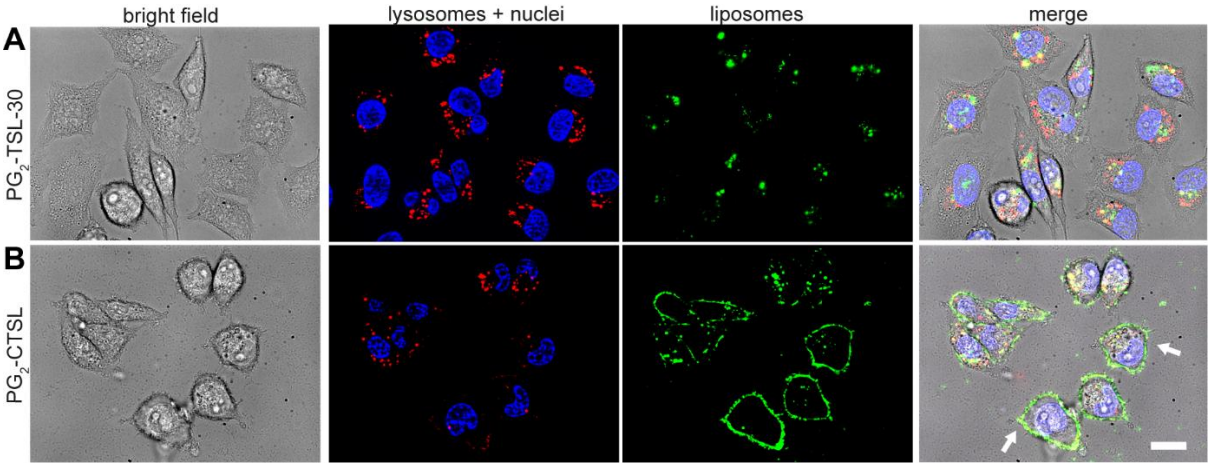

Figure 4 Live cell fluorescence microscopy of BN175 incubated with anionic (**A**) and cationic (**B**) NBD-labeled DPPG$_2$-TSLs. Cells were imaged via bright field, DAPI (blue; nuclei), DsRed (red; lysosomes), and GFP filter (green; liposomes). White arrows indicate presence of liposomes at the rim of the cells. Scale bar applied to all images is 20 µm.

Liposomes localization after HT application in cancer and endothelial cells

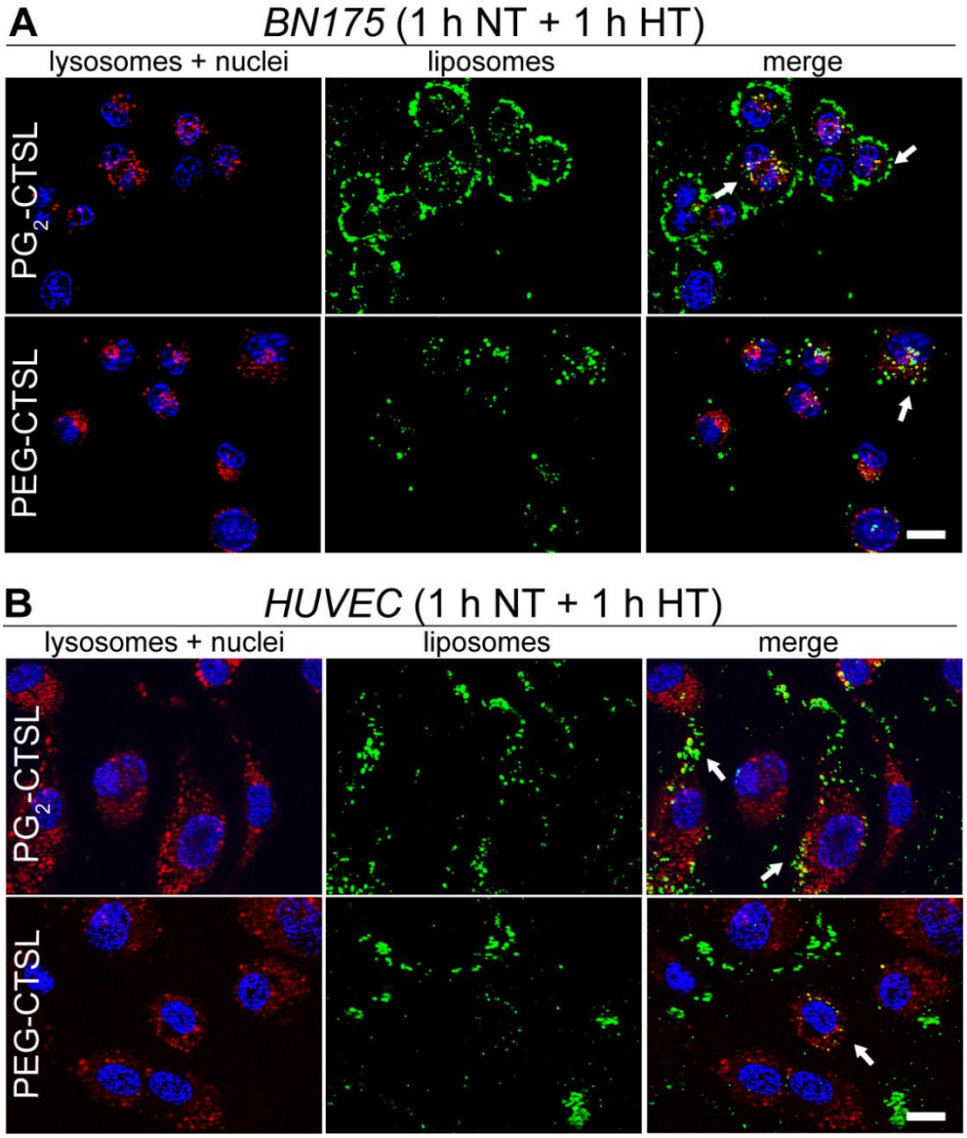

Figure 5 Live cell fluorescence imaging on BN175 (**A**) and HUVEC (**B**) after HT application. NBD-liposome were imaged using GFP filter (green color), lysosomes with DsRed filter (Lysotracker RED, red color) and nuclei with DAPI filter (Hoechst 33342, blue). Arrows indicate co-localization (yellow) of liposomes (green) and lysosomes (red). Images were taken after 1 h 37 °C (NT) and 1 h at 41 °C (HT). Scale bar applied to all images is 20 µm.

In vitro cell toxicity of DOX-loaded (C)TSLs

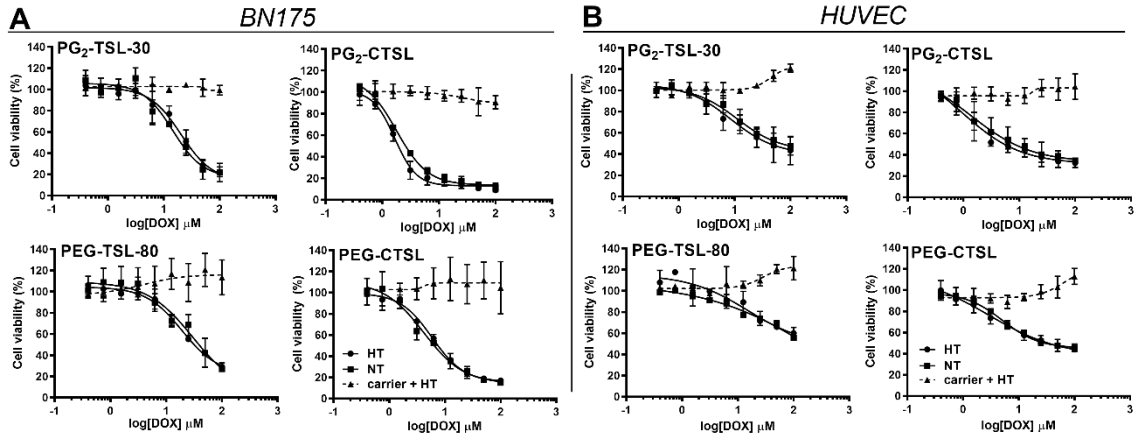

Figure 6 *In vitro* cell toxicity of DOX-loaded (C)TSLs on BN175 (**A**) and HUVEC (**B**) cells. Cells were treated with different concentrations of DOX (0.4 – 100 µM, log scale). Buffer-loaded (C)TSLs without DOX (carrier) were tested in combination with HT condition and dosed using the DOX-corresponding lipid concentration (0.04 – 2.22 mM; triangles, dashed line). Cell survival is expressed as percentage of untreated cells (control). Data are presented as mean value ± SD for three independent analyses and values were fit by a non-linear regression.

Hemocompatibility of DOX-loaded (C)TSLs

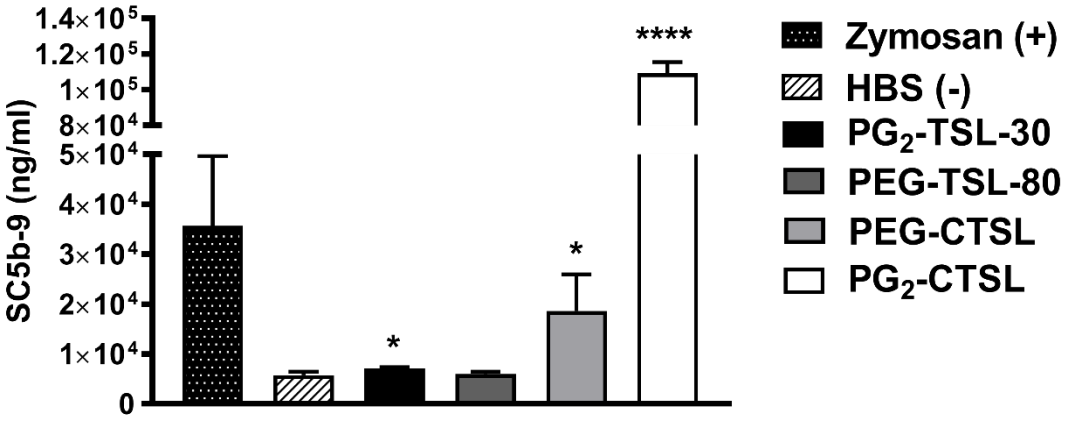

Figure 7 Liposome-mediated complement activation *in vitro*. DOX-loaded (C)TSLs were diluted in normal human serum for complement activation analysis (1:12 v/v). Incubation was carried out at 37°C for 30 min, Zymosan and HBS pH 7.4 were used as positive and negative controls, respectively. ELISA test for SC5b-9 was carried out using the manufacturer's instructions. Values are expressed as average + SD for 3 independent measurements. Groups were analyzed via one-way ANOVA Bonferroni test for multiple comparison against control (HBS pH 7.4), and asterisks indicate significant difference between groups. * $p < 0.05$, **** $p < 0.0001$.