

EXPLOITING THE ABILITY OF LinTT1-FUNCTIONALIZED LIPOSOMES TO TARGET CANCER CELLS AND TAMs TO IMPROVE BREAST CANCER THERAPY

Nicola D'Avanzo^{1,2}, Giulia Torrieri², Patrícia Figueiredo², Christian Celia³, Donatella Paolino⁴, Alexandra Correia², Karina Moslova⁵, Tabet Teesalu⁶, Hélder A. Santos^{2,7}, Massimo Fresta¹

¹ Department of Health Sciences and ⁴Department of Experimental and Clinical Medicine University of Catanzaro "Magna Graecia", Via "S. Venuta" s.n.c., I-88100 Catanzaro, Italy.

² Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy; ⁵Department of Chemistry; ⁷Helsinki Institute of Life Science, University of Helsinki, FI-00014, Helsinki, Finland

³ Department of Pharmacy, University of Chieti - Pescara "G. d'Annunzio", Via dei Vestini 31, I-66100 Chieti, Italy

⁶Laboratory for Cancer Biology, University of Tartu, Tartu 50411, Estonia

nicola.davanzo@unicz.it; fresta@unicz.it

Nowadays breast cancer is one of the most diagnosed cancer, accounting around 2 million of new cases per years worldwide[1]. The implication of tumor microenvironment (TME) in breast cancer progression is widely established, suggesting that the development of nanomedicine able to target specifically cancer cells as well as cancer associated cells, i.e. tumor associated macrophages (TAMs), could improve the current state of chemotherapies used in clinic.

In these attempts the aim of this work was to realize a targeted nanomedicine by the conjugation of LinTT1 peptide, a ligand of p32 receptor that results aberrantly expressed on cells' surface of both breast cancer cells[2] and TAMs[3]. Liposomes functionalization led to a higher cytotoxic effect of payloads (Doxorubicin and Sorafenib) in comparison with therapeutic bare liposomes on both mono-layer and spheroid breast cancer cellular models. The improved interaction rate of LinTT1-functionalized liposomes than bare liposomes with 3D breast cancer spheroids was also confirmed by flow cytometer and confocal laser scanning microscopy analysis. Furthermore, interaction studies between primary human M2-macrophages and LinTT1 functionalized-liposomes showed that nanovesicles that interacted with these cells were partially (ca. 50%) internalized while the other half part resulted only strongly associated to the cells' surface. This finding suggests the opportunity to exploit the associated but not internalized nanovesicles and the intrinsic ability of TAMs to accumulate themselves in the tumor core, to enrich the hypoxic area of tumor. These results highlight the potential use of LinTT1-functionalized liposomes as a new targeted nanomedicine in breast cancer therapy.

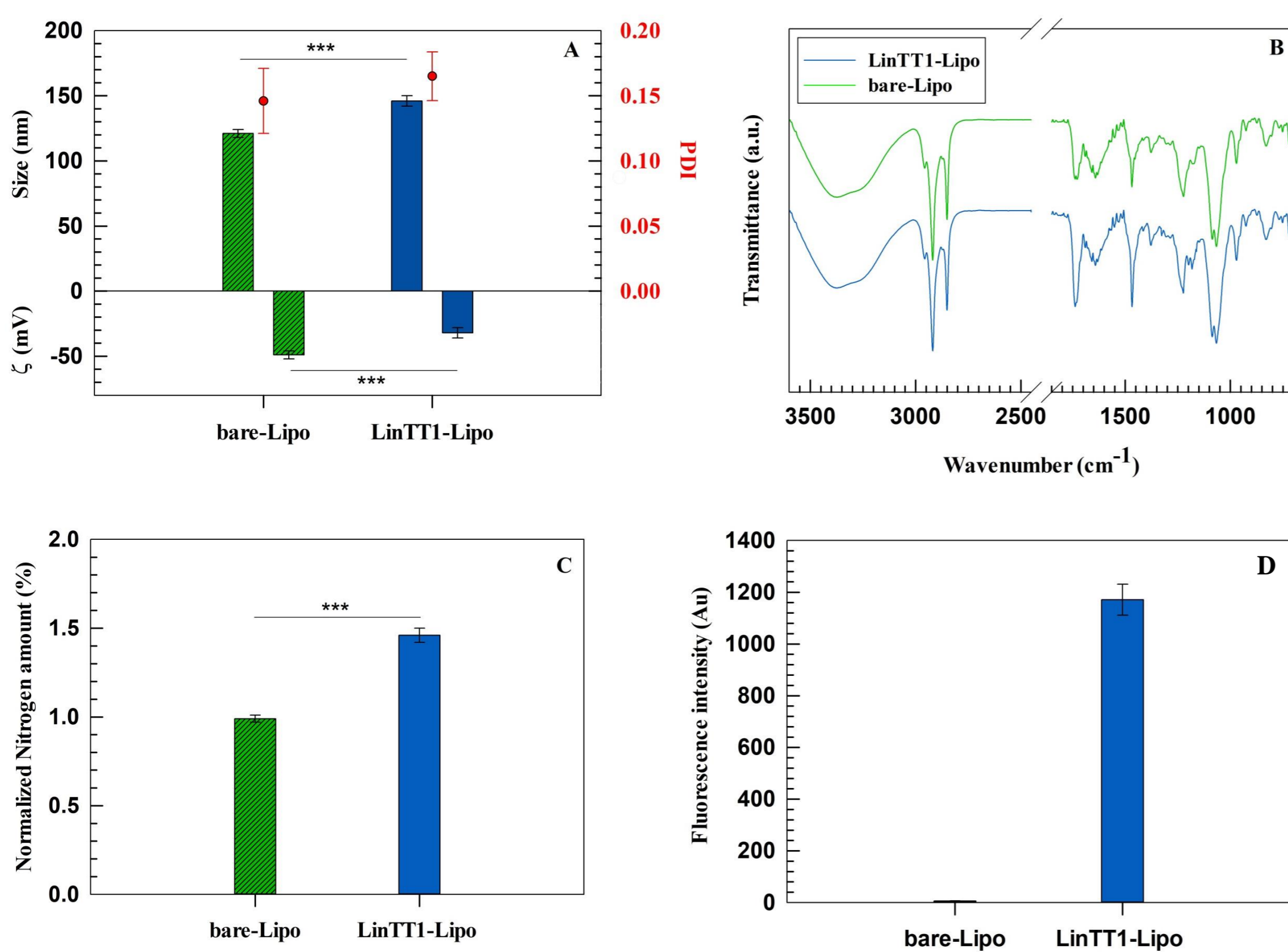


Fig. 1 - Physicochemical properties of liposomes evaluated before and after conjugation of LinTT1. (A) Average hydrodynamic diameter, PDI, and zeta-potential value (C). (B) ATR-FTIR spectra of bare and LinTT1-functionalized liposomes. (C) Nitrogen amount quantification by elemental analysis of liposomes. (D) Fluorescence intensity of bare and LinTT1-functionalized liposomes. Results are the average of three independent experiments \pm standard deviation (S.D.). Statistical significance was obtained by a * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

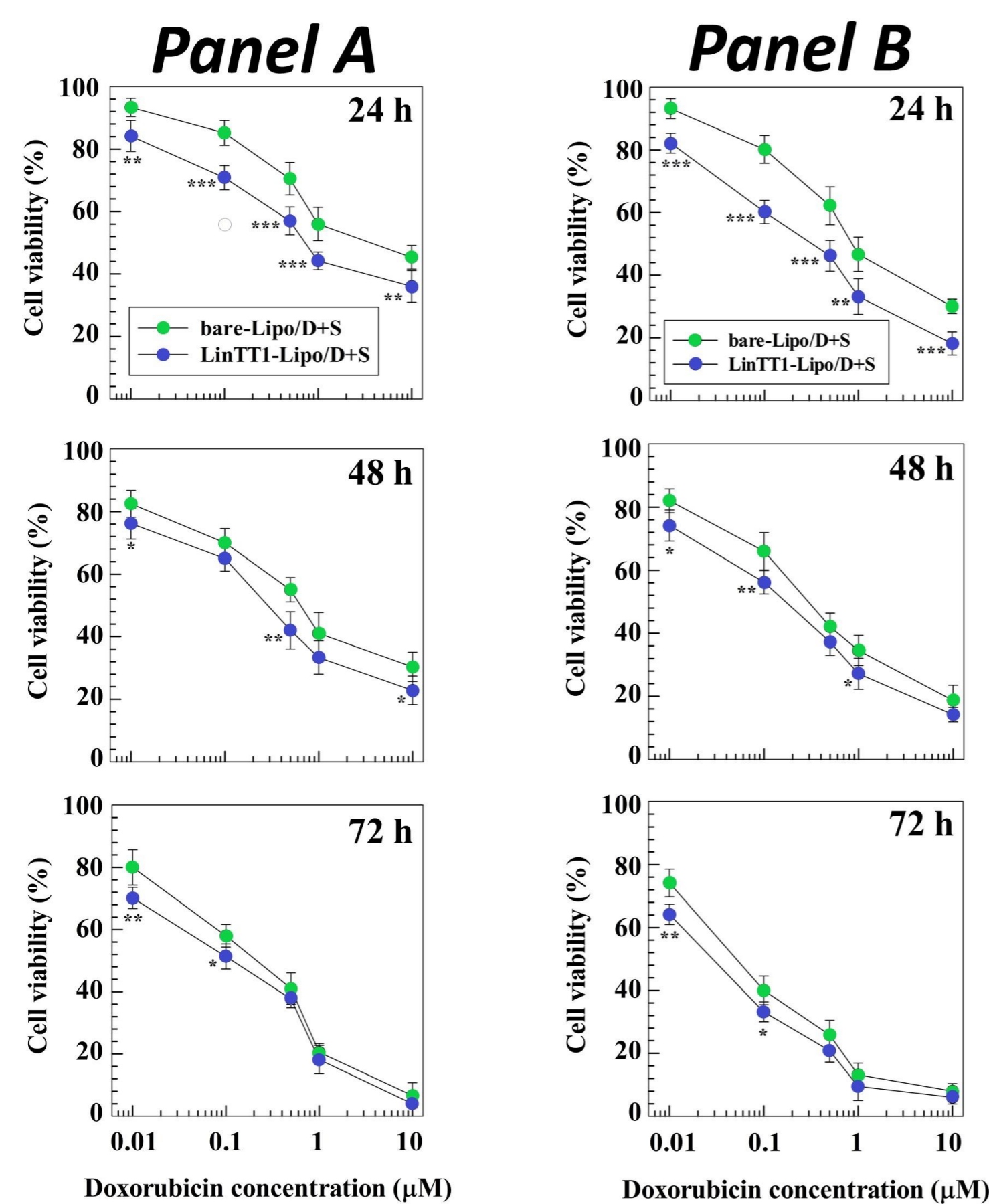


Fig. 2 - *In vitro* cytotoxic activity of DOX and SRF, co-loaded into bare- and LinTT1-functionalized liposomes in 3D spheroids of MDA-MB-231 cells using a monolayer cellular model. Antiproliferative effect was reported as cell viability percentage (%) and evaluated as a function of incubation times and drug concentrations. Drug concentration is reported as a ratio to the Doxorubicin concentration that, based on different entrapment efficiency is three fold higher than Sorafenib for all investigated time points. Cell viability percentage (%) of LinTT1-functionalized liposomes was compared to bare liposomes. Cellular viability percentage (%) was evaluated by using CellTiter-Glo luminescence assay. Cells that are treated with cell culture medium are the control and corresponds to 100% of cell viability for all tested concentrations at different times of incubation. Cells treated with empty liposomes demonstrated a cell viability over 90% for all tested concentrations (data not shown). Results are the average of three independent experiments \pm S.D. Statistical significance was set at: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Error bars, if not shown, are within symbols.

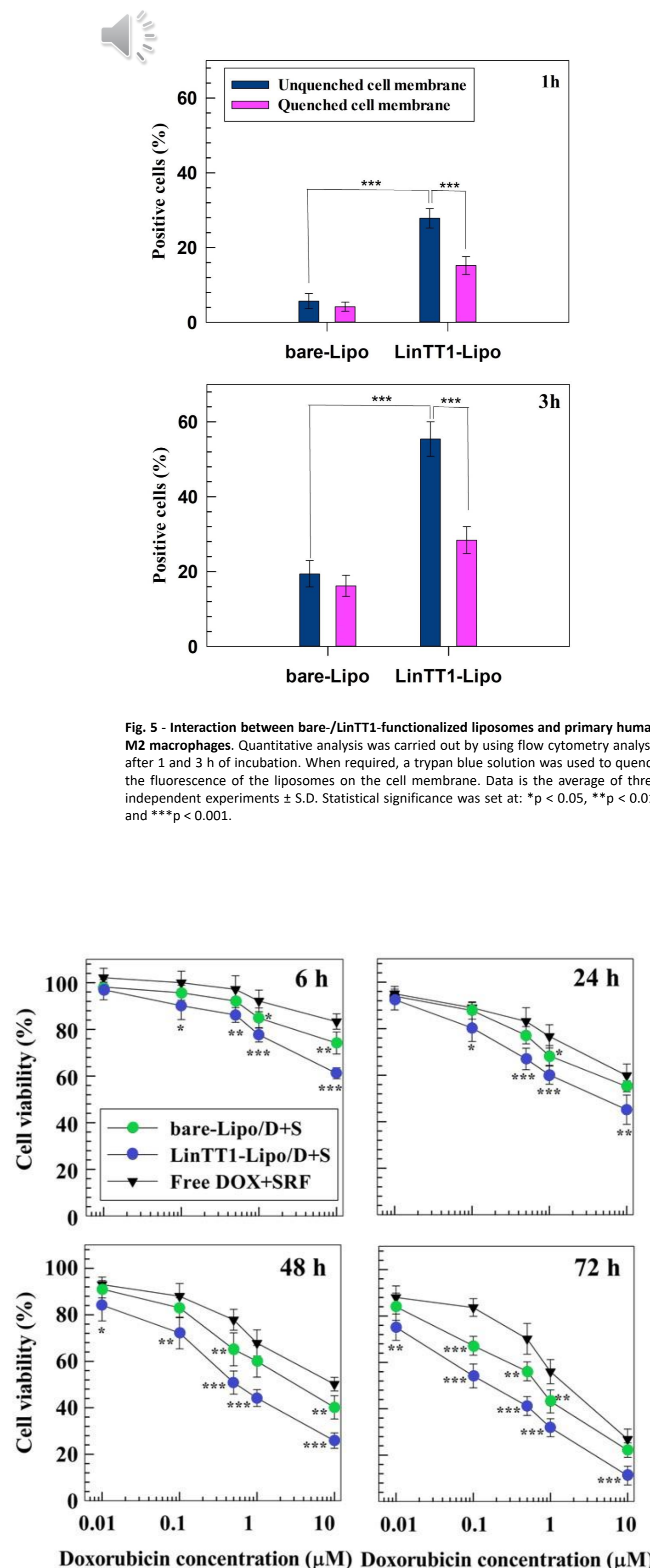


Fig. 3 - *In vitro* cytotoxic activity of combined free DOX and SRF or co-loaded into bare- and LinTT1-functionalized liposomes in 3D spheroids of MDA-MB-231 cells. Results are reported as cellular viability percentage (%) and were obtained by using RealTime-Glo MT Cell Viability Assay. Drug concentration is reported as a ratio to the Doxorubicin concentration that, based on different entrapment efficiency, is three fold higher than Sorafenib for all investigated time points. Data is the average of three independent experiments \pm S.D. Differences were evaluated between cytotoxic effect of combined free DOX and SRF and drugs co-loaded liposomes (both bare and functionalized ones). Statistical significance was set at: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Error bars, if not shown, are within symbols.

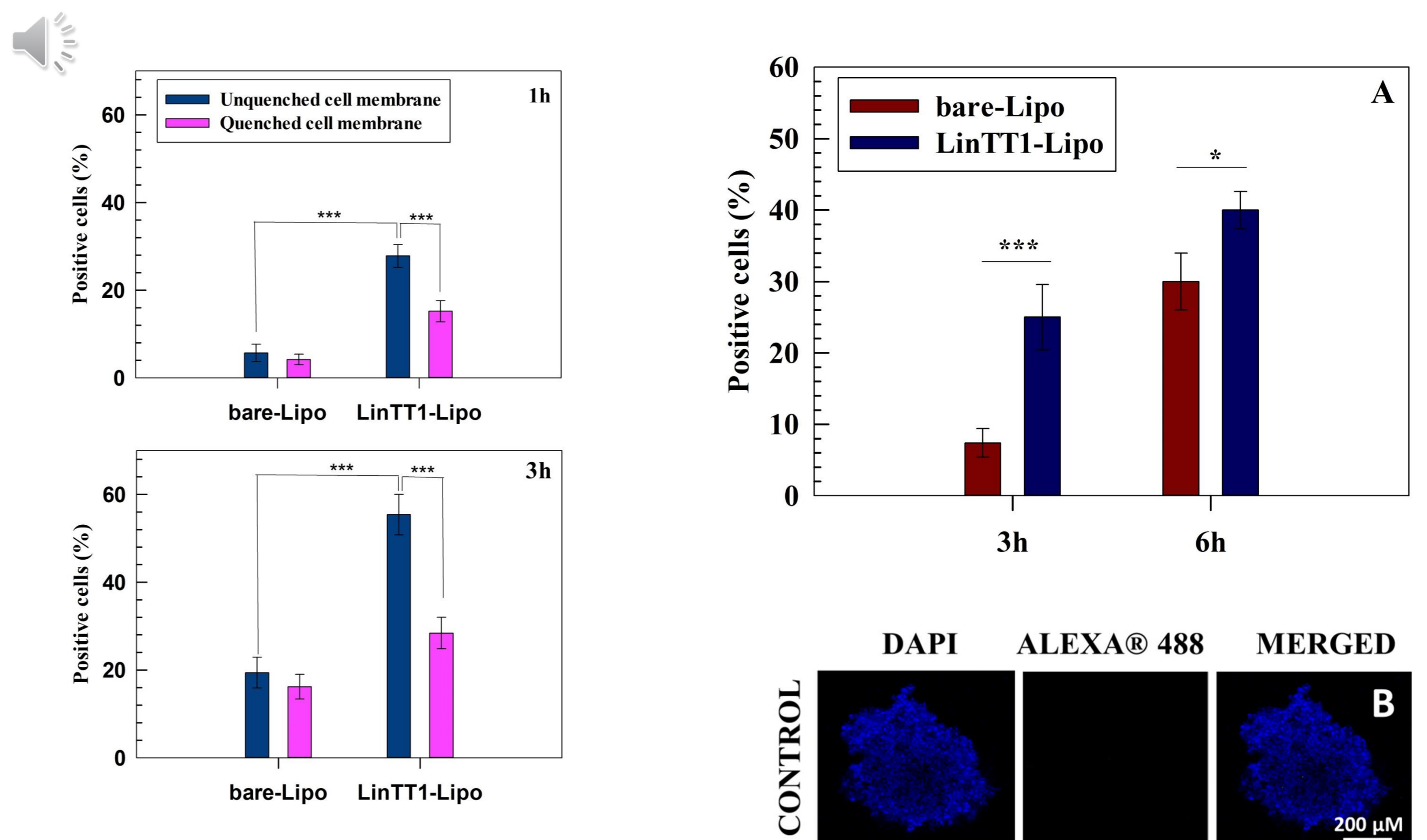


Fig. 4 - *In vitro* interaction between bare-/LinTT1-functionalized liposomes and 3D spheroids of MDA-MB-231 cells. Quantitative analysis of cellular uptake and internalization was evaluated by using flow cytometry analysis after 1 and 3 h of incubation. When required, a trypan blue solution was used to quench the fluorescence of the liposomes on the cell membrane. Data is the average of three independent experiments \pm S.D. Statistical significance was set at: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

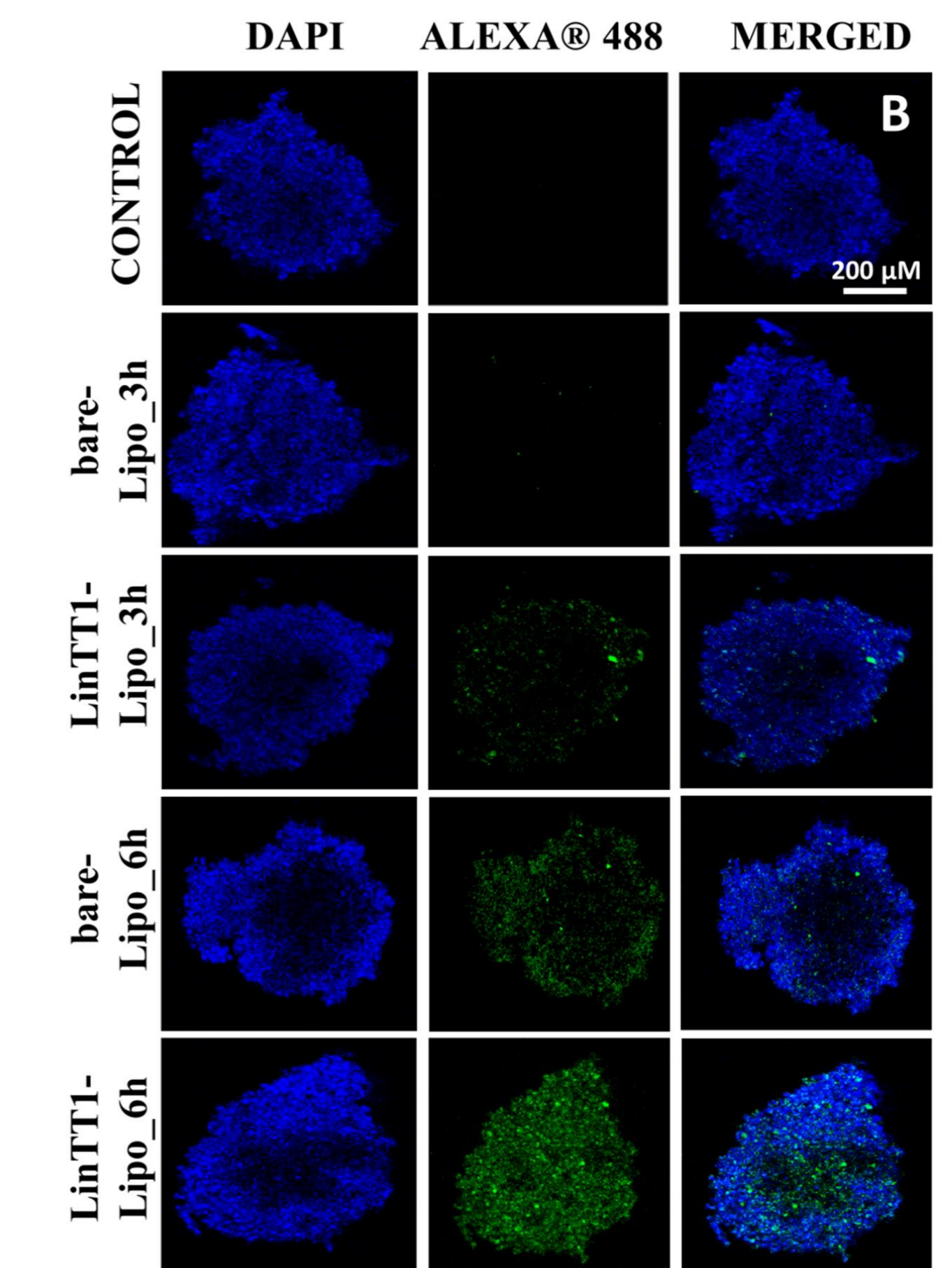


Fig. 5 - Interaction between bare-/LinTT1-functionalized liposomes and primary human M2 macrophages. Quantitative analysis was carried out by using flow cytometry analysis after 1 and 3 h of incubation. When required, a trypan blue solution was used to quench the fluorescence of the liposomes on the cell membrane. Data is the average of three independent experiments \pm S.D. Statistical significance was set at: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

REFERENCES
1. Bray, F., et al., *Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*. CA: a cancer journal for clinicians, 2018. 68(6): p. 394-424.
2. Saha, P. and K. Datta, *Multi-functional, multicompartmental hyaluronan-binding protein 1 (HABP1/p32/gC1qR): implication in cancer progression and metastasis*. Oncotarget, 2018. 9(12): p. 10784-10807.
3. Fogal, V., et al., *Mitochondrial/cell-surface protein p32/gC1qR as a molecular target in tumor cells and tumor stroma*. Cancer research, 2008. 68(17): p. 7210-7218.