Supplementary Information (SI) for Nanoscale.

Metformin encapsulating immunoliposomes conjugated with anti-TROP 2 antibody fragments for the active targeting of triple negative breast cancer

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Supplementary information

Table S1. Met-ImmunoLP 3 characterization in terms of physical properties after incubation if FCS. The data is expressed as an average ± SD (n = 3).

Inucbation time in FCS	D_H (nm)	PdI	ζ (mV)
24 h	162.4 ± 1.9	0.236 ± 0.01	-16.6 ± 1.7
72 h	152.3 ± 2.6	0.225 ± 0.01	-14.7 ± 1.2
96 h	160.2 ± 1.9	0.256 ± 0.01	-18.1 ± 0.7

Abbreviations: D_H, average hydrodynamic diameter; FCS, fetal calf serum; PdI, polydispersity index; ζ, zeta potential

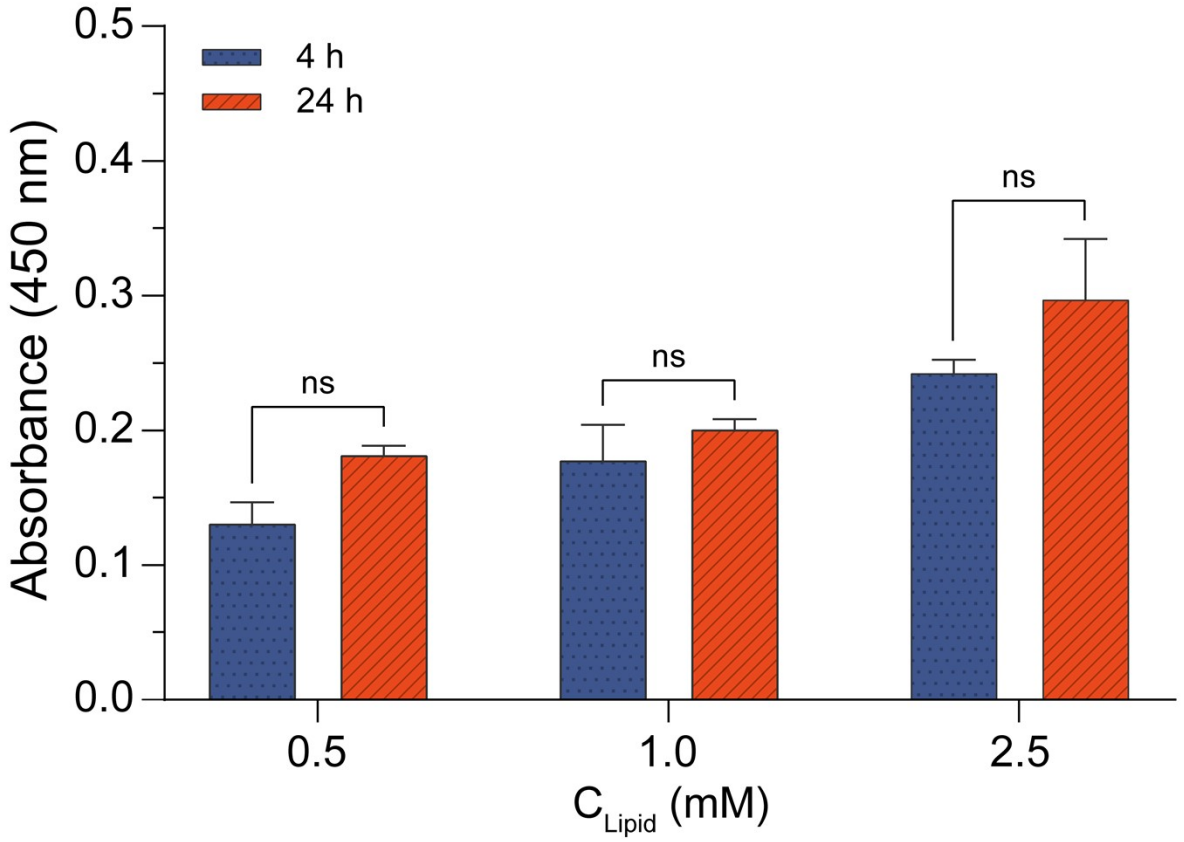

Figure S1. Indirect ELISA test showing the immunoreactivity of Met-ImmunoLPs 3 after incubation in FCS during 2 h and 24 h, in binding the TROP 2 protein, measured at varied lipid concentrations. The data is expressed as an average ± SD (n – 3). Statistical information was obtained using two-way ANOVA test (ns $p > 0.05$, * $p < 0.05$).

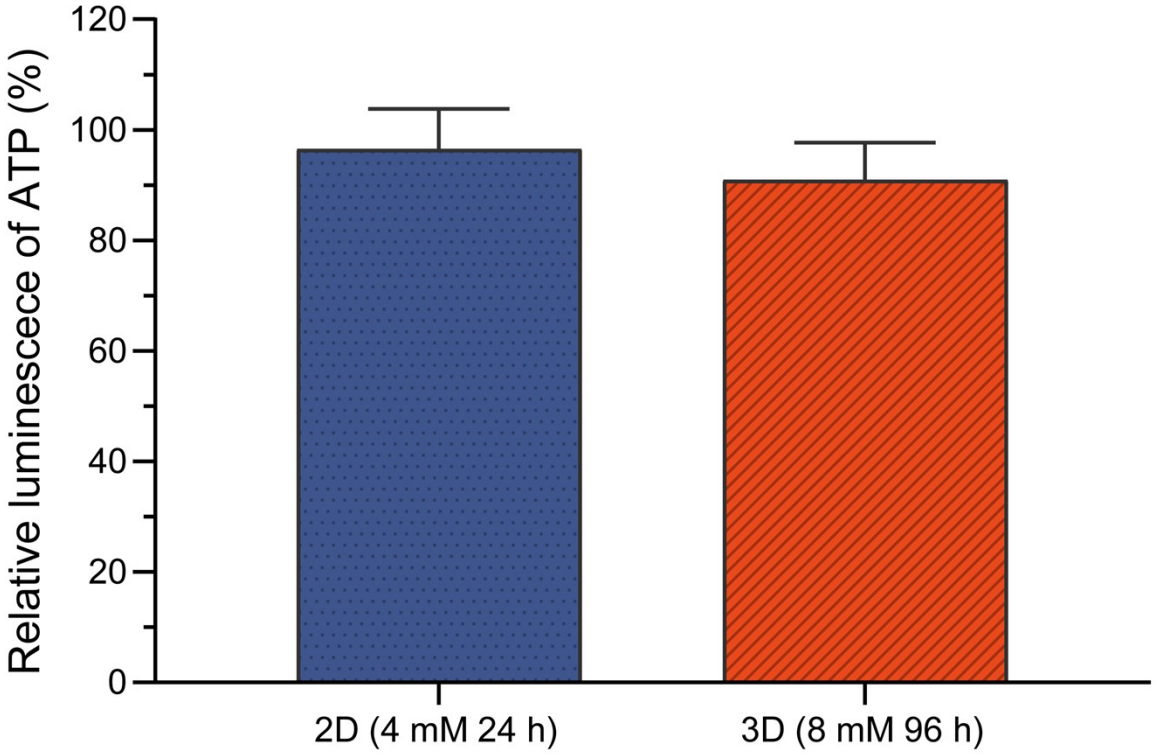

Figure S2. The effect of ImmunoLP, on the ATP expression of MDA-MB-468 cells cultured in 2D and 3D models. In the 2D model the cells, seeded at 5 x 10^3 cells/well, were treated 24 h with 4 mM ImmunoLPs during 24 h. In the 3D models, the spheroids were treated with 8 mM of ImmunoLPs during 96 h. The data is expressed as an average ± SD (n = 3).

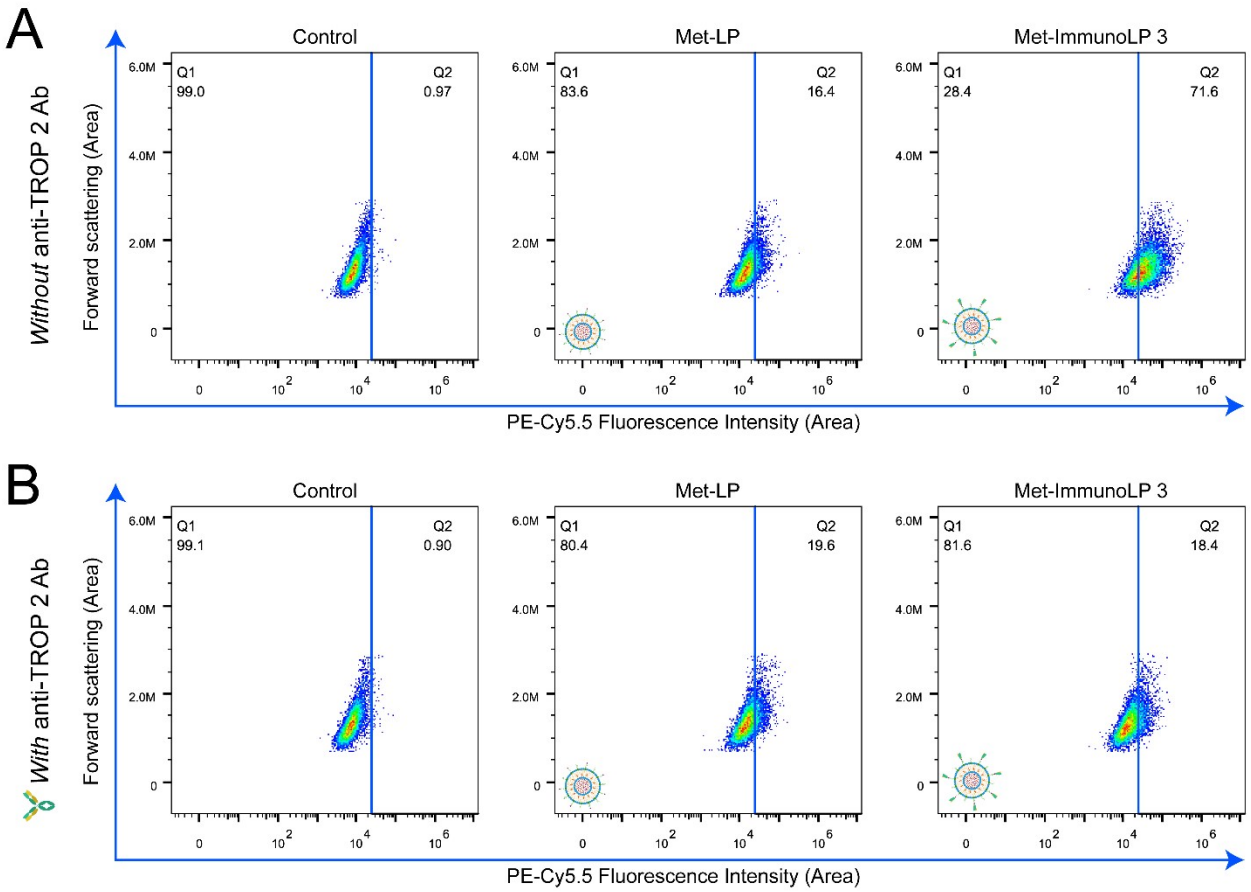

Figure S3. Flow cytometry results of MDA-MB-468 cells showing the cell population (9000 cells) distribution according to Cy5.5 fluorescence expression with or without prior TROP 2 blocking. The cells were treated with Met-LPs and Met-ImmunoLPs 3 during 24 h (lipid concentration – 2.5 mM). Cells incubated with Met-ImmunoLPs 3 without anti-TROP 2 antibody pre-incubation (**A**); cells incubated with Met-ImmunoLPs with anti-TROP 2 antibody pre-incubation (**B**).