

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 in FCS. The data is expressed as an average \pm SD (n = 3).

| Inucbation time in FCS | D _H (nm) | Pdl | ζ (mV) |
|------------------------|---------------------|------------------|-----------------|
| 24 h | 162.4 \pm 1.9 | 0.236 \pm 0.01 | -16.6 \pm 1.7 |
| 72 h | 152.3 \pm 2.6 | 0.225 \pm 0.01 | -14.7 \pm 1.2 |
| 96 h | 160.2 \pm 1.9 | 0.256 \pm 0.01 | -18.1 \pm 0.7 |

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

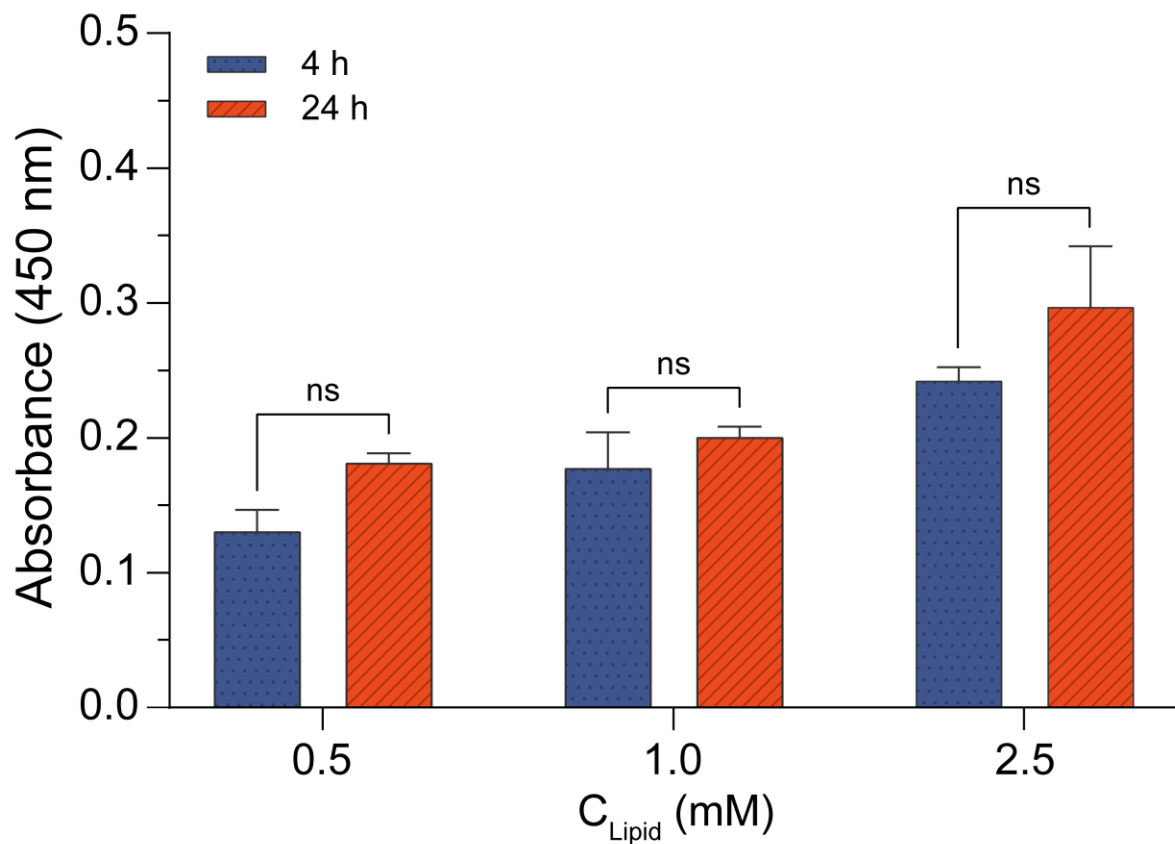


Figure S1. Indirect ELISA test showing the immunoreactivity of Met-ImmunoLPs 3 after incubation in FCS during 2 h and 24 h, in binding the TROP 2 protein, measured at varied lipid concentrations. The data is expressed as an average \pm SD ($n = 3$). Statistical information was obtained using two-way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

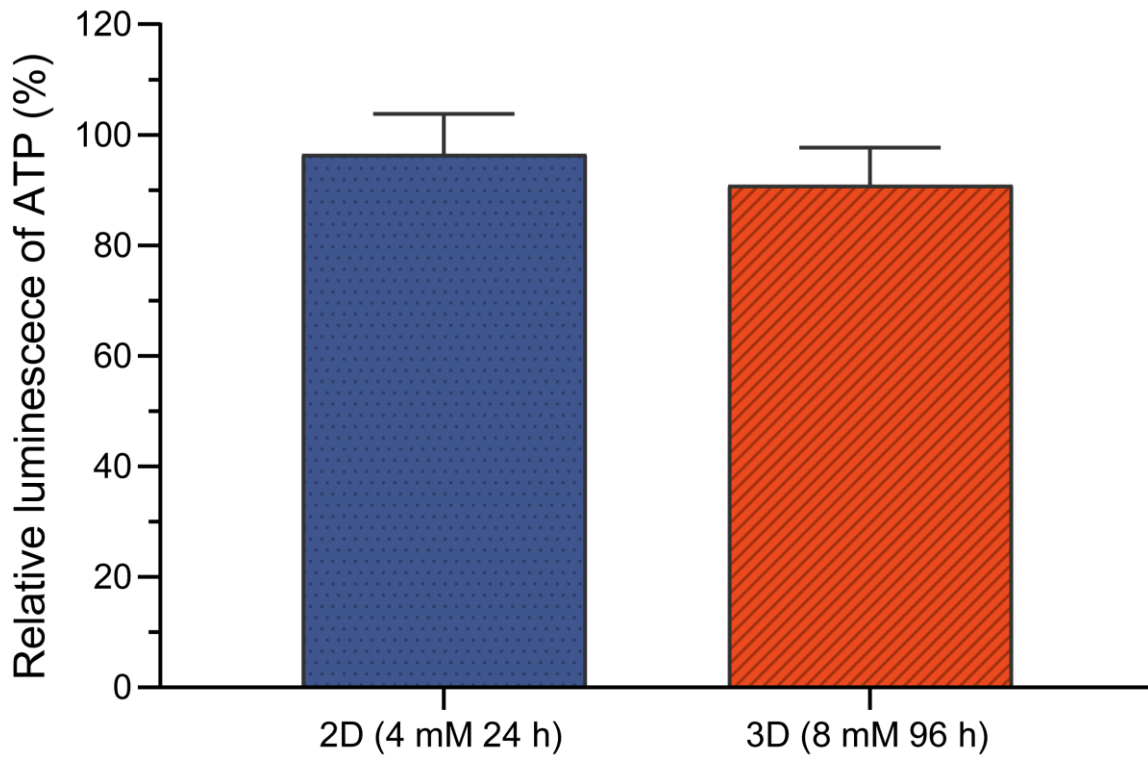


Figure S2. The effect of ImmunoLP, on the ATP expression of MDA-MB-468 cells cultured in 2D and 3D models. In the 2D model the cells, seeded at 5×10^3 cells/well, were treated 24 h with 4 mM ImmunoLPs during 24 h. In the 3D models, the spheroids were treated with 8 mM of ImmunoLPs during 96 h. The data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

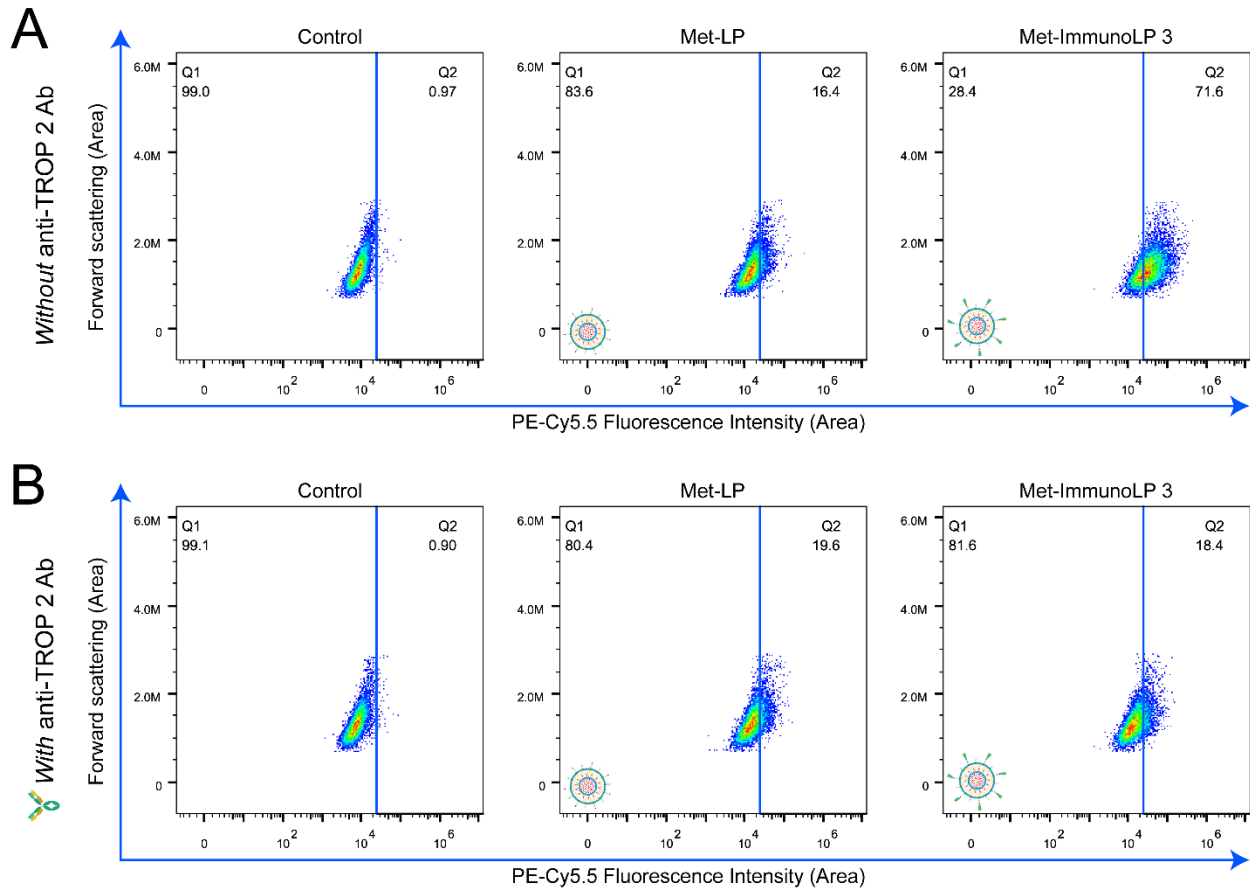


Figure S3. Flow cytometry results of MDA-MB-468 cells showing the cell population (9000 cells) distribution according to Cy5.5 fluorescence expression with or without prior TROP 2 blocking. The cells were treated with Met-LPs and Met-ImmunoLPs 3 during 24 h (lipid concentration – 2.5 mM). Cells incubated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