Supplementary Information

Hot EVs – how temperature affects extracellular vesicles

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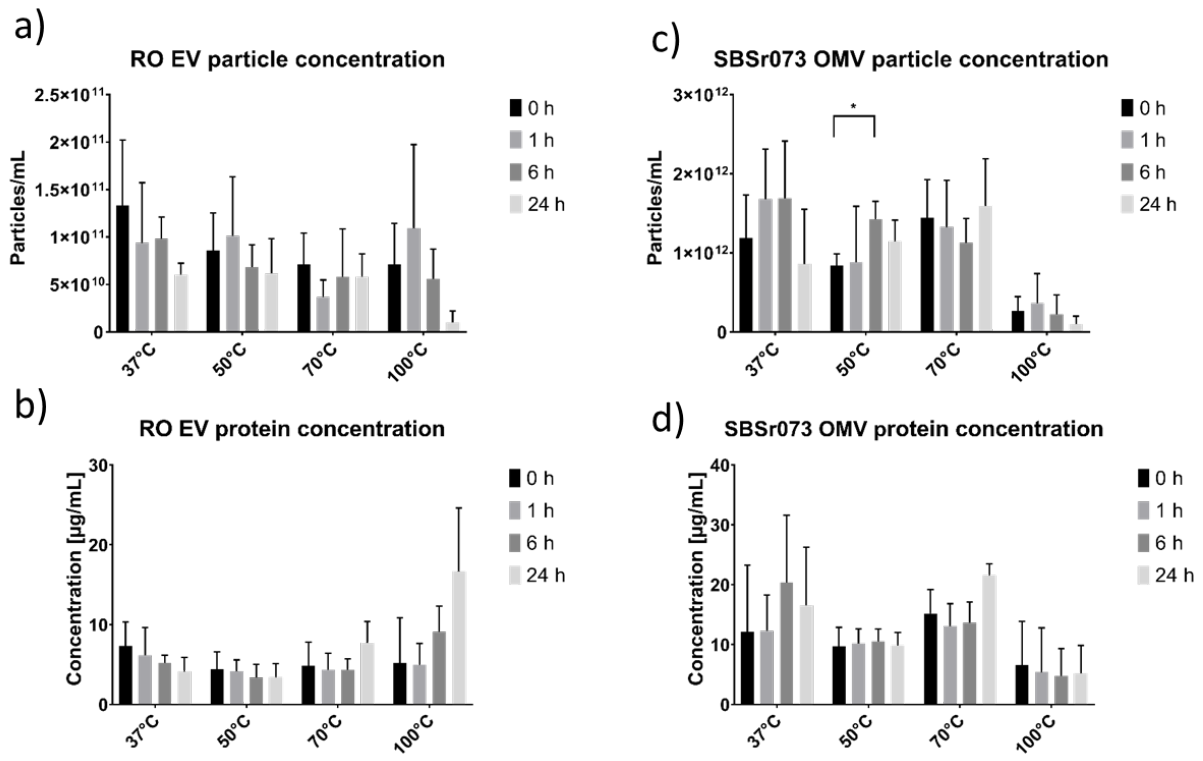

Figure S1 Physico-chemical alteration upon incubation at 37 °C, 50 °C, 70 °C, 100 °C for 1 h, 6 h and 24 h. a) RO EV particle concentration measured by NTA b) RO EV protein concentration determined by BCA c) SBSr073 OMV particle concentration d) SBSr073 OMV protein concentration. Mean ± SD, n = 3, *p < 0.05 (ANOVA followed by Tukey *post-hoc* test).

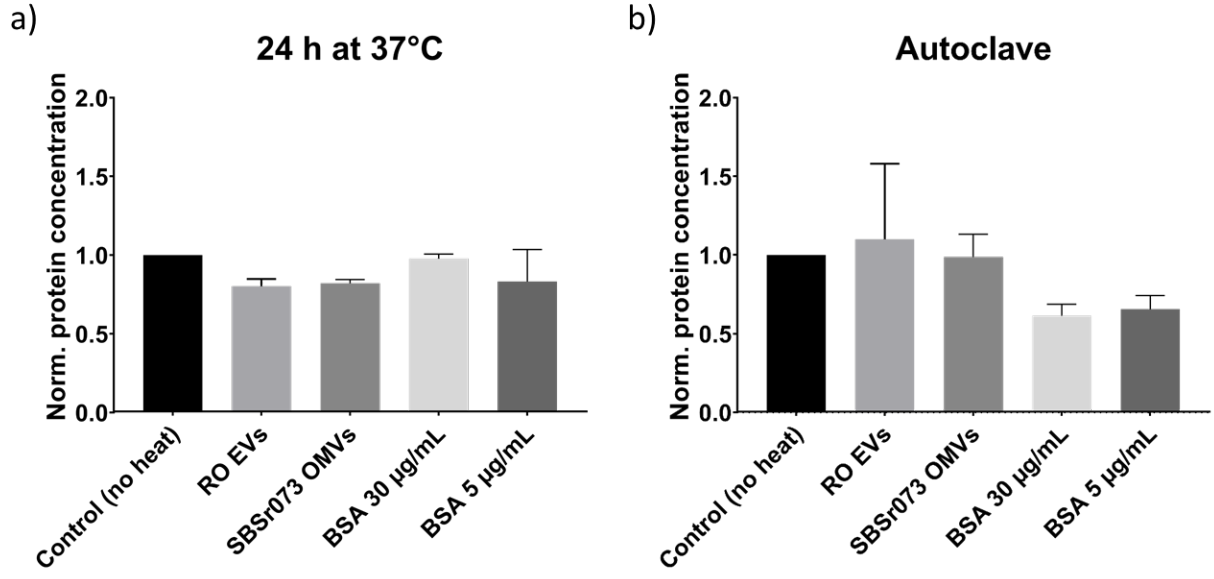

Figure S2 Total protein concentration of a) EVs and OMVs incubated at 37°C for 24 h b) autoclaved EVs and OMVs. Vesicles were heat-treated and afterwards lysed with RIPA buffer, a strong detergent, for 5 min, and the total protein concentration was determined by a bicinchoninic assay. As control and for data normalisation, non-heat-treated and RIPA buffer lysed vesicles were used. As an additional control, two different concentrations of bovine serum albumin (BSA) were treated at similar conditions. Mean ± SD, n = 3

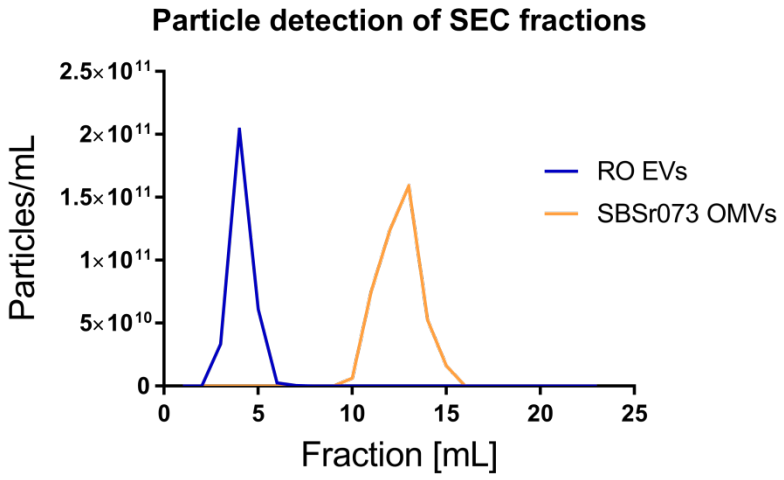

Figure S3 Representative size exclusion chromatography of RO EVs and SBSr073 OMVs. As the yield of RO EVs is considerably lower compared to bacterial OMVs from SBSr073, a 10 mL column was sufficient to separate purified vesicles from external proteins while minimising the dilution of the vesicles. SBSr073 were purified using a 35 mL column, to ensure adequate purification of vesicles. RO EVs typically eluted after 3-6 mL, SBSr073 after 10-15 mL.

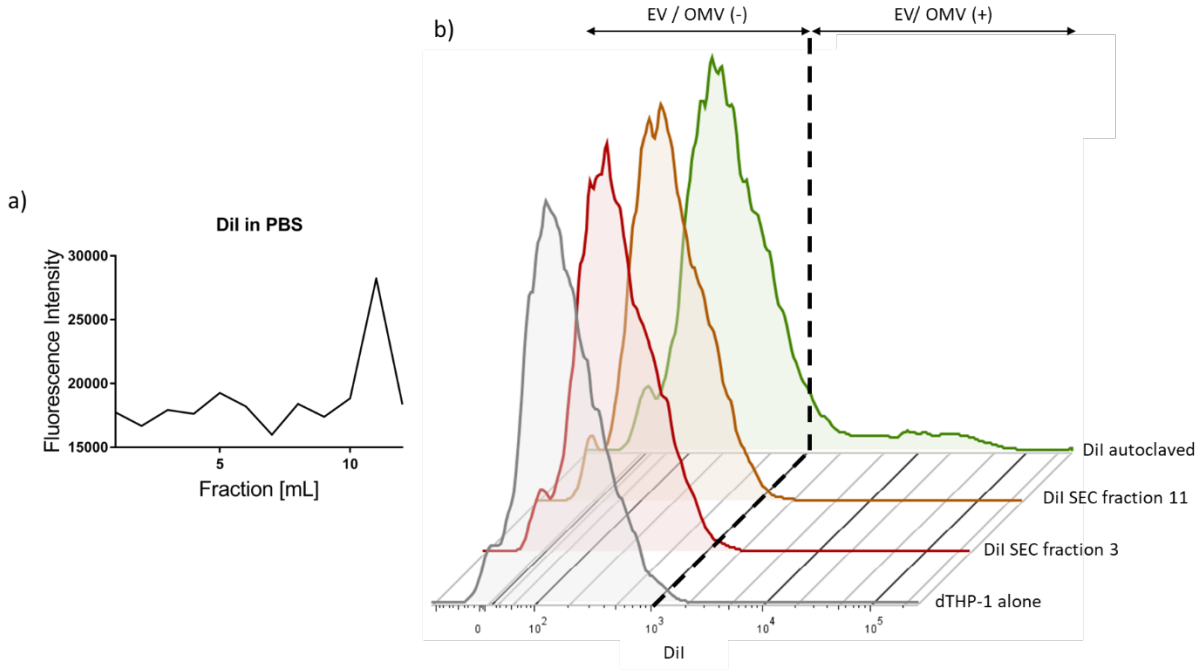

Figure S4 FACS signals of control samples a) 2 µL DiI were incubated with 1 mL PBS and loaded on to a 10 mL sepharose column. Fluorescence of each fraction was measured. b) Histogram plot of DiI positive dTHP-1 cells with DiI signal intensity (PE laser – x-axis). Different fractions (3 and 11) of DiI eluting from the SEC were also incubated with dTHP-1 and did not show a positive signal during FACS analysis.