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Supporting Information

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Cell-Derived Vesicles for Antibiotic Delivery—Understanding the Challenges of a Biogenic Carrier System

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SUPPLEMENTARY INFORMATION

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Overview of additional display items

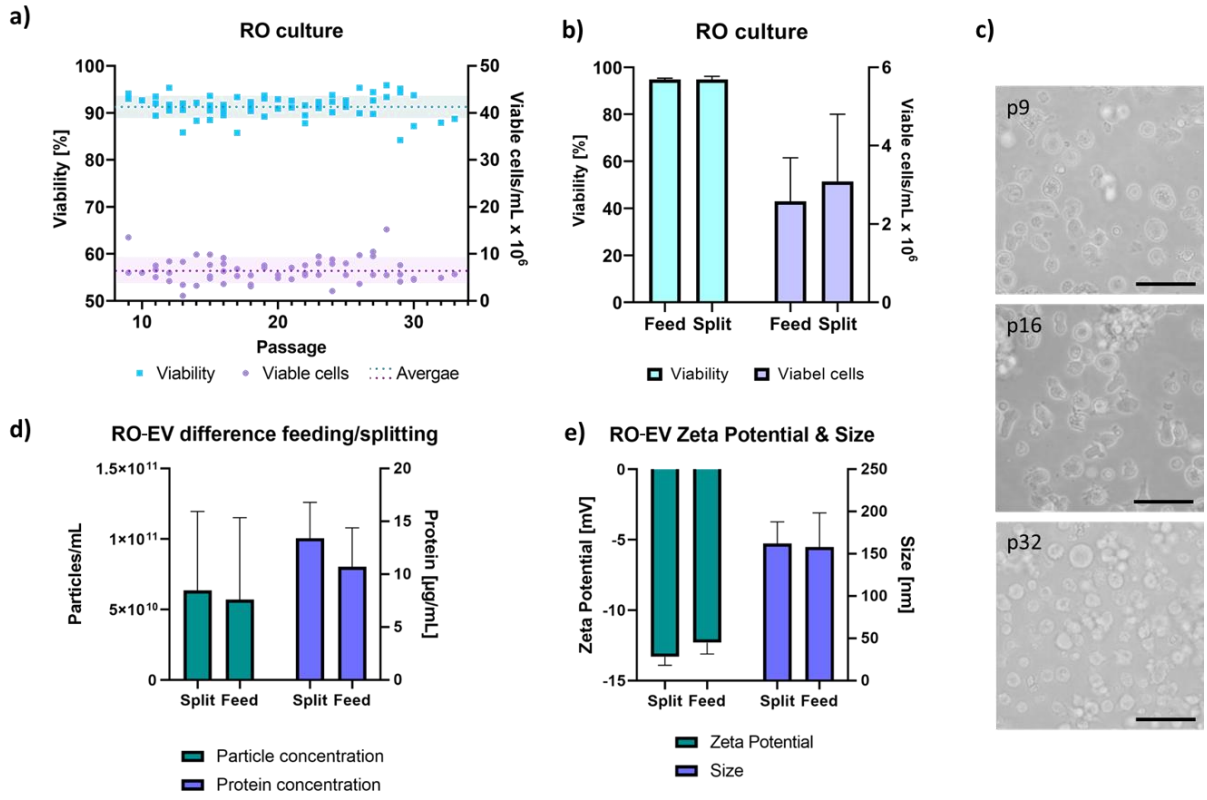

Figure S1 RO cell culture during vesicle isolation. *a) Cell viability and cell count from passage 8 to 35. b) Average cell viability and viable cell count on feeding and splitting days, before vesicle isolation. c) Light microscopy images of different passages of RO cells. Scale bar 50 µm d) Difference of RO-EVs in particle concentration and protein concentration when isolated on a feeding or splitting day. e) Difference in zeta potential and size between RO-EVs isolated on a feeding or splitting day. To remove non-EV components, a size exclusion chromatography using a 35 mL sepharose column was applied, respectively. Vesicles typically eluted after 12 to 15 mL.*

a)

Vesicle type	Protein/vesicle [µg/particle × 10^{-10}] n = 6 - 7	Size [nm] n = 6 - 7	Zeta potential [mV] n = 3 - 6
SB-OMVs	3.6 ± 2.9	194.0 ± 18.2	-6.8 ± 0.6
RO-EVs	6.5 ± 1.5	160.1 ± 2.0	-12.8 ± 0.5

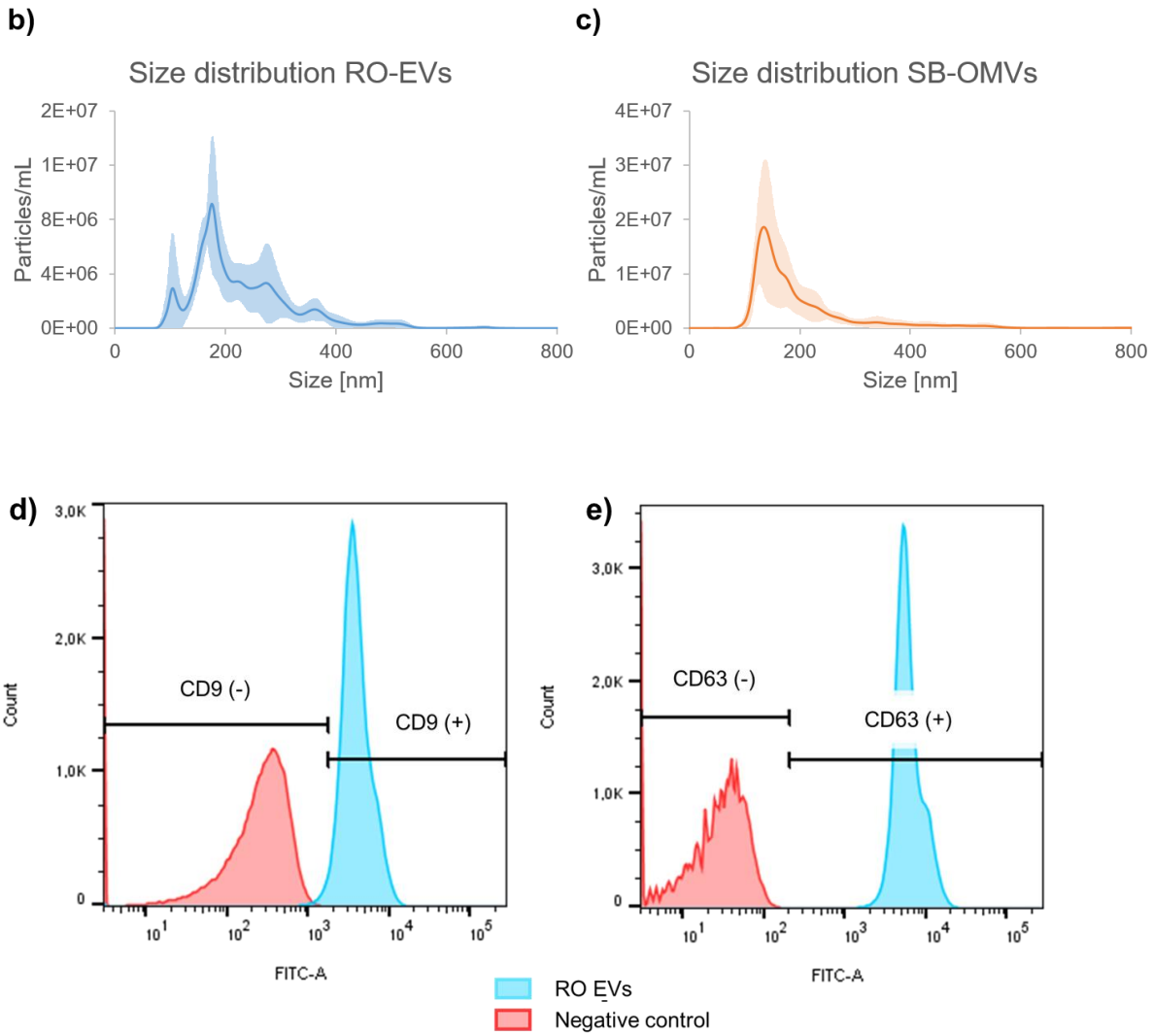

Figure S2 Physico-chemical characteristics and surface markers of RO-EVs and SB-OMVs. (a) *The protein concentration was measured using a bicinchoninic assay kit, the zeta potential using a zetasizer and,* ***(b,c)*** *size as well as the size distribution using nanoparticle tracking analysis. Data on SB-OMVs reproduced from Schulz […] Fuhrmann, EJPB 2020, https://doi.org/10.1016/j.ejpb.2019.11.010.* ***(d,e)*** *Surface markers CD9 and CD63 were analyzed using a bead array kit and detected by flow cytometry. Isotype antibodies were used as negative control. Data reproduced from Trenkenschuh […] Fuhrmann, Adv Healthcare Mater 2021, DOI: 10.1002/adhm.202100538*

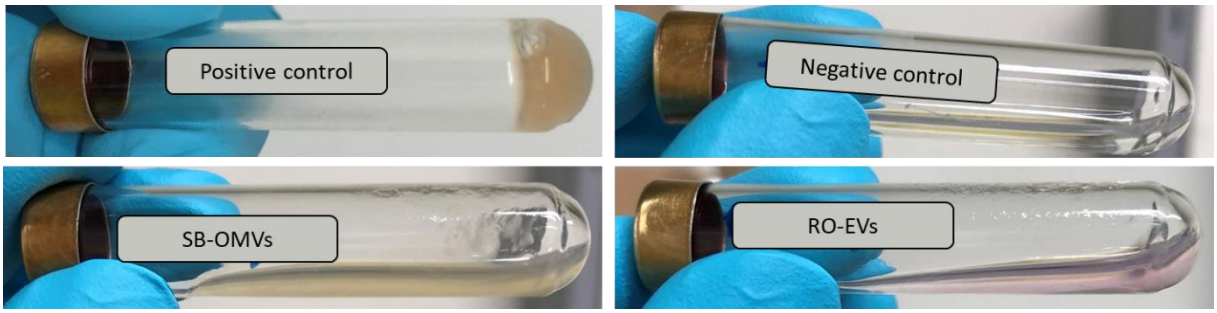

Figure S3 Gel clot endotoxin test. *When using the pharmacopeial test for endotoxins, vesicle pellets did not show any firm gel formation, similar to the negative control.*

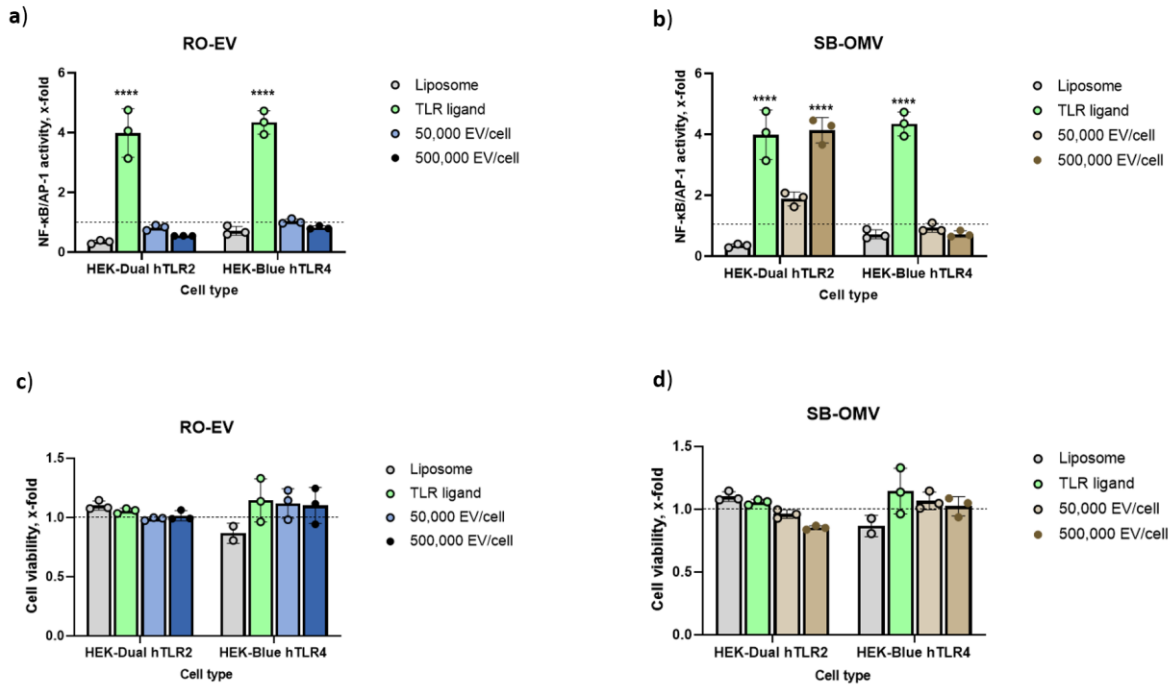

Figure S4 Incubation of TLR2- and TLR4-reporter cells with vesicles from RO cells and SB myxobacteria. *Reporter cells were treated with vesicles isolated from RO cells and SB myxobacteria at concentrations of 50,000 and 500,000 EVs per cell, and liposomes at a concentration of 500,000 per cell. Liposomes were prepared from dipalmitoylphosphatidylcholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) (molar ratio 3:2 and final concentration 5 mg/ml) using a thin-film hydration technique, followed by extrusion through a 0.2 µm polycarbonate filter at 40°C. The TLR2 ligand Pam3CSK4 and the TLR4 ligand LPS were used at concentrations of 10 ng/ml as positive controls for HEK-TLR2 and HEK-TLR4 cells respectively. (a,b) The activation of NF-κB/AP-1 was measured as activity of secreted embryonic alkaline phosphatase (SEAP) and expressed as fold of PBS-treated cells (indicated by the black line). (c,d) Cell viability was quantified by MTT assay. Mean ±SD, n=3; ****p<0.0001 compared with controls.*

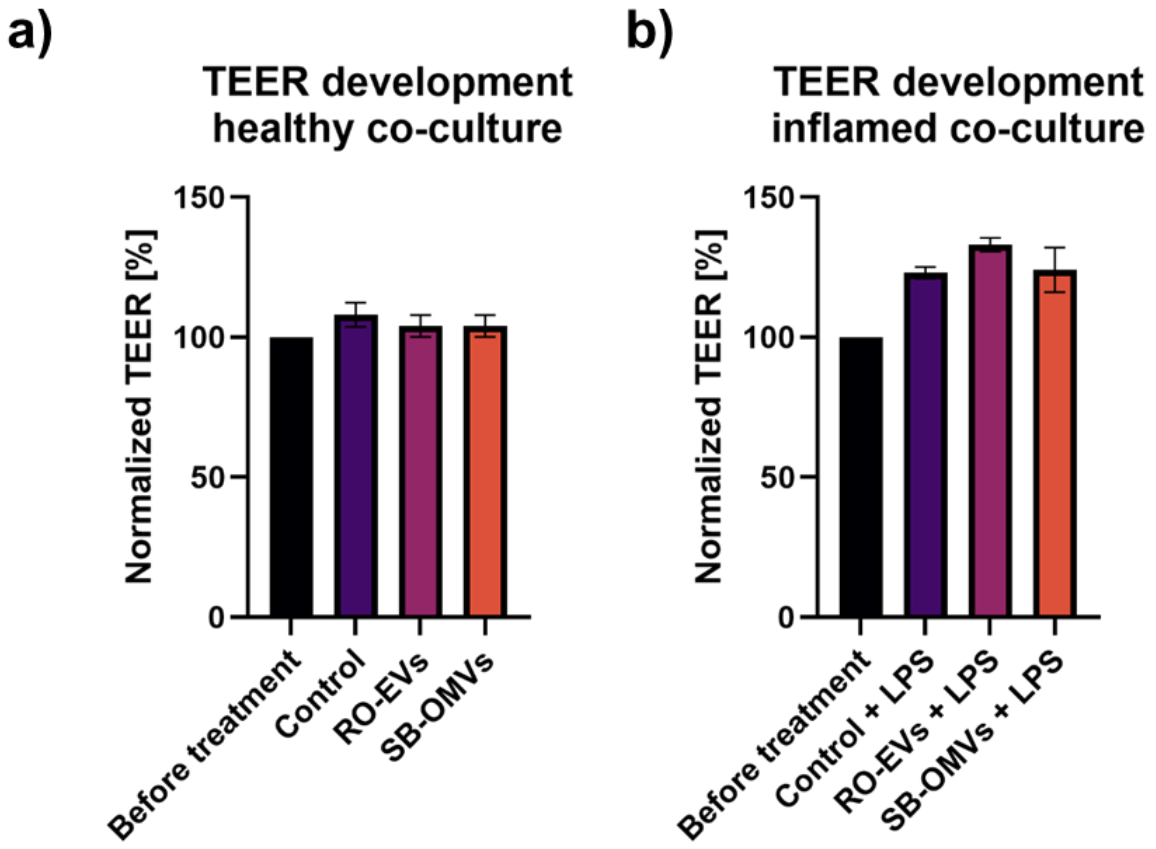

Figure S5 TEER values of the healthy and inflamed co-culture after vesicle or control incubation. *TEER values were normalized to values before the treatment. n = 2, N = 3*

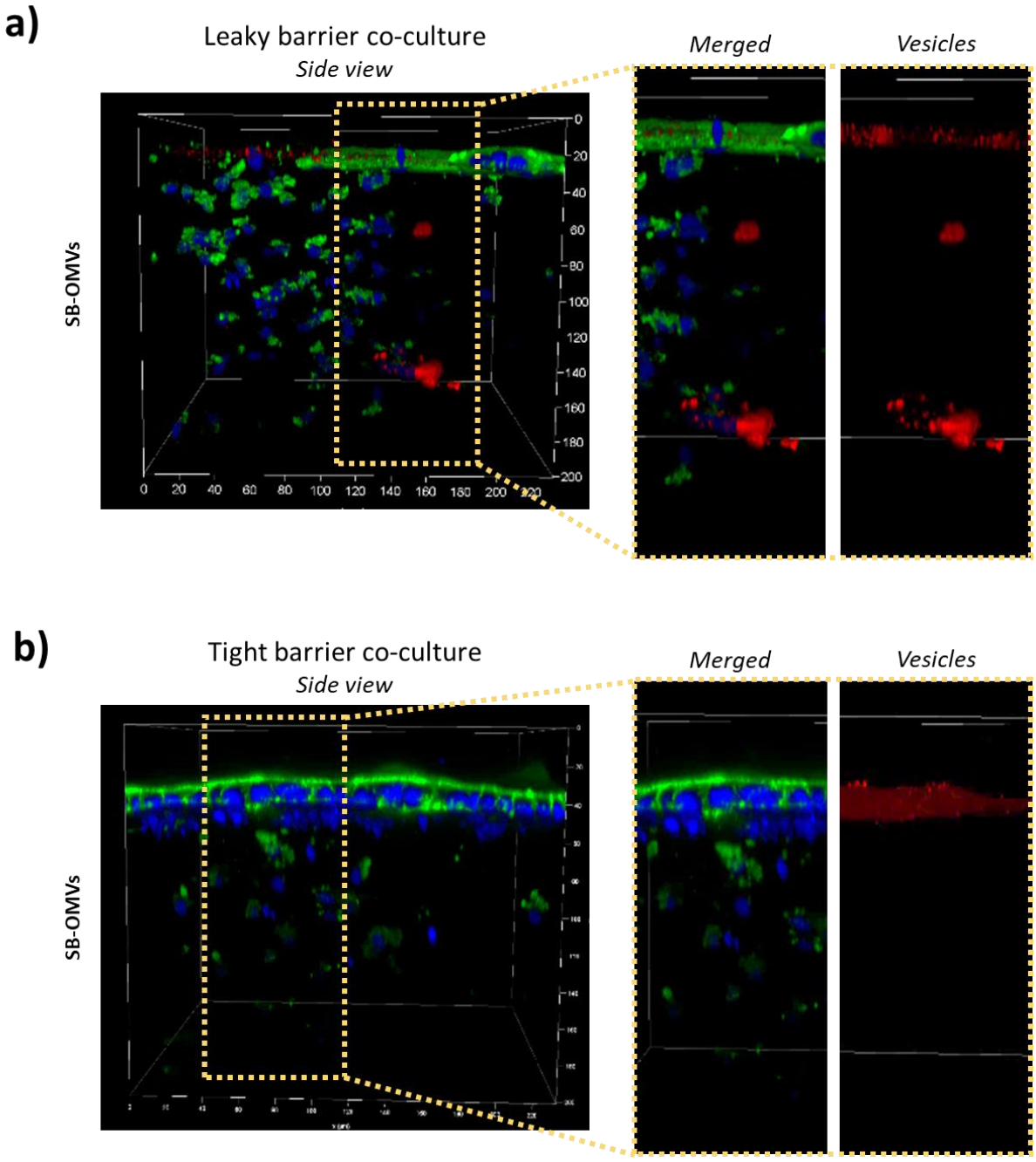

Figure S6 Magnification of the 3D co-culture exposed to SB-OMVs. *a) Leaky epithelial barrier revealed vesicle positive immune cells b) No vesicle positive cells were detected in the tight barrier model. Cells are stained in green, with blue nucleus; vesicles were labelled in red. All images were taken by confocal microscopy.*

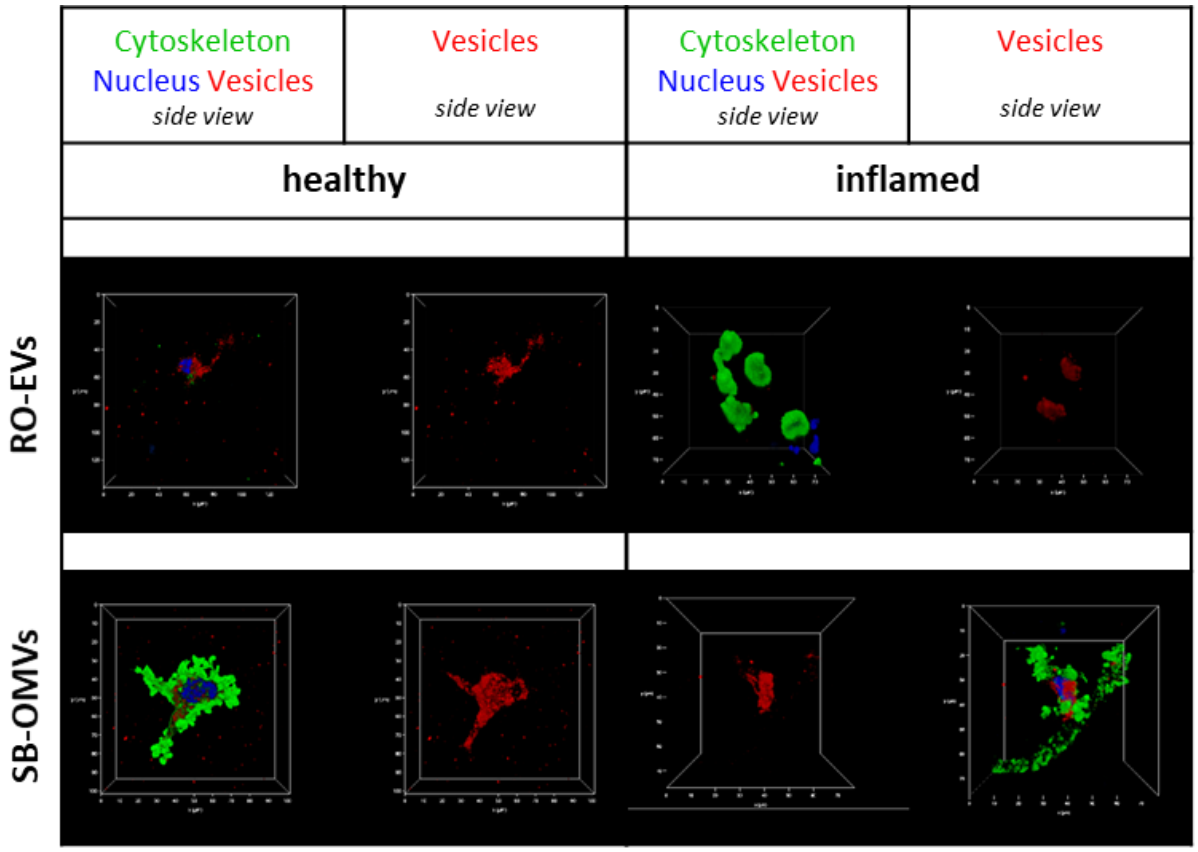

Figure S7 Vesicle positive macrophages. *Images were taken using confocal scanning microscopy. The cytoskeleton was labeled using FITC-phalloidin (green), DAPI for labeling the nucleus (blue) and the vesicles were stained using DiI (red).*

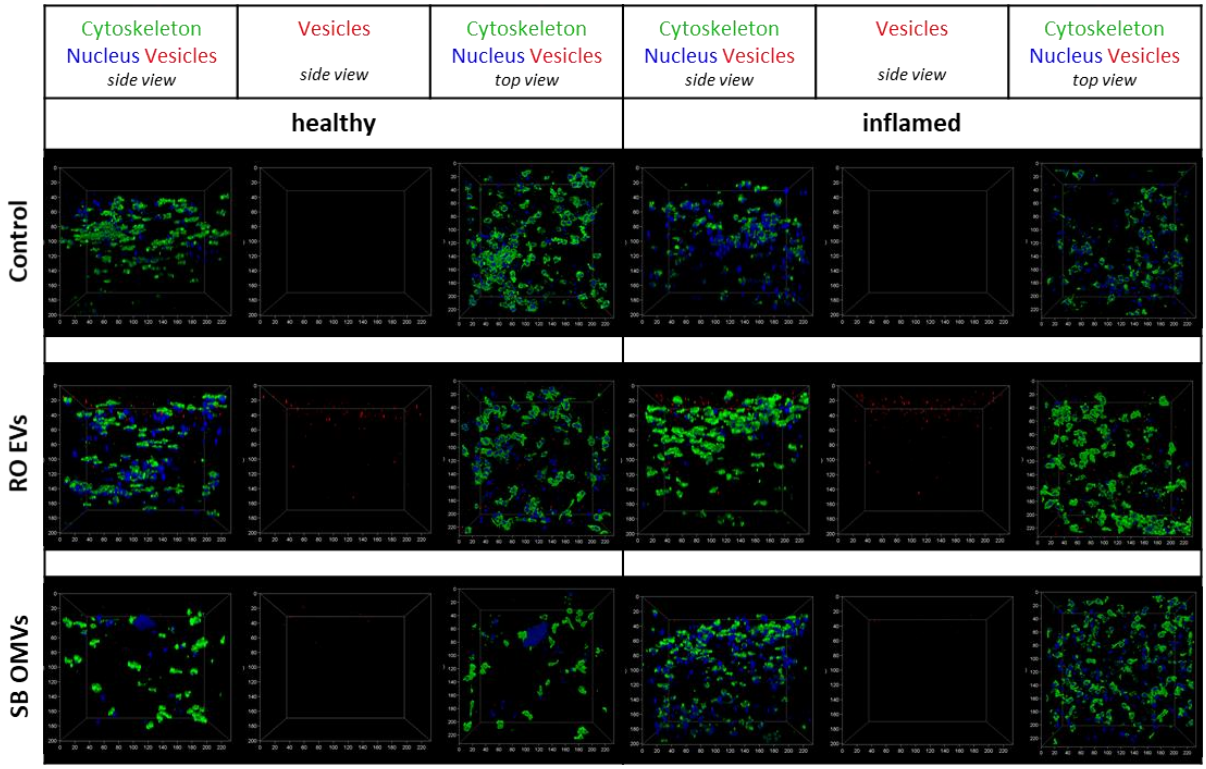

Figure S8 Mono-cultures of dendritic-like cells in a 3D model. *Confocal scanning microscopy revealed no vesicles positive cells as no co-localization was seen.*

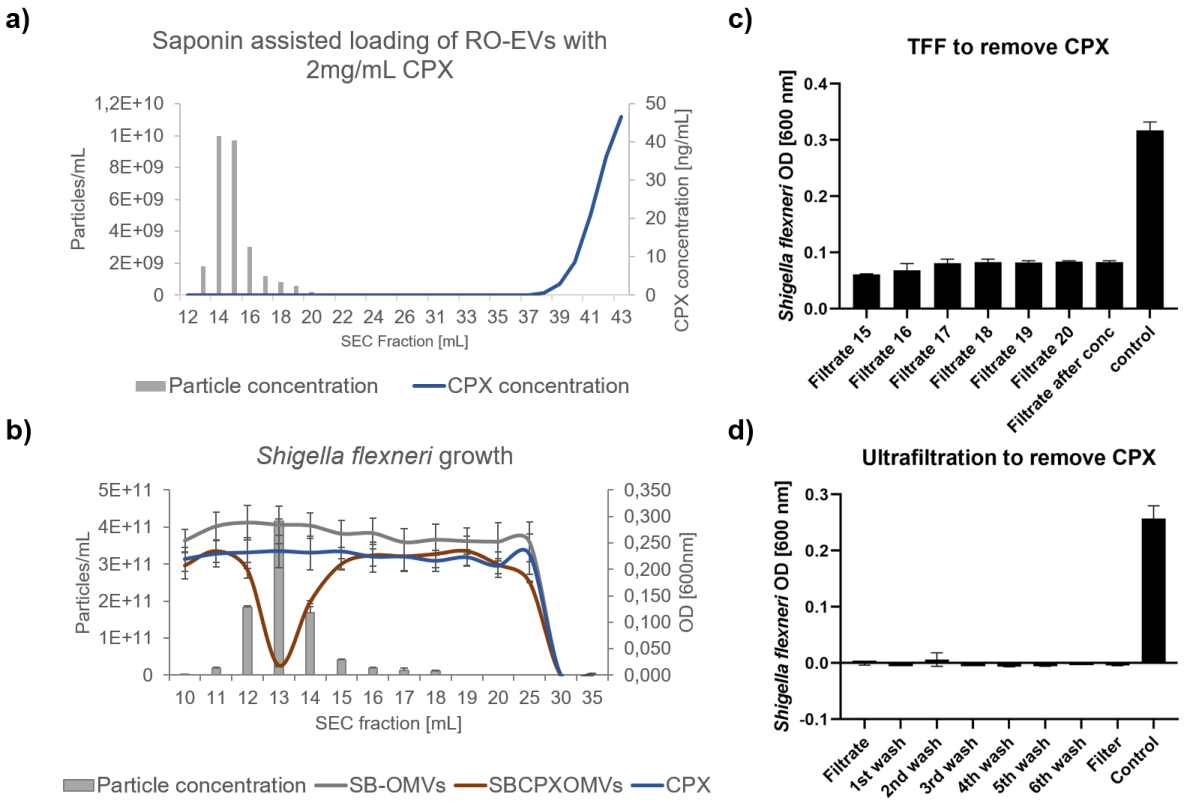

Figure S9 Different methods to separate free CPX from drug-loaded vesicles. *a) Loading RO-EVs with 2 mg/mL CPX was not successful, as no CPX in vesicles could be quantified b) Growth inhibition of Shigella flexneri using SB-OMVs, SB_{CPX}OMVs and CPX loaded onto a SEC column and the respective collected fractions. From fraction 30, free CPX was eluting from the column and induced an antibiotic effect. c) Removal of CPX using tangential flow field fractionation. Collected filtrates after several washing steps were still able to inhibit the growth of Shigella, thus free CPX was still present. d) Removal of CPX using ultrafiltration. Collected filtrates of washing steps still inhibited the growth of Shigella.*

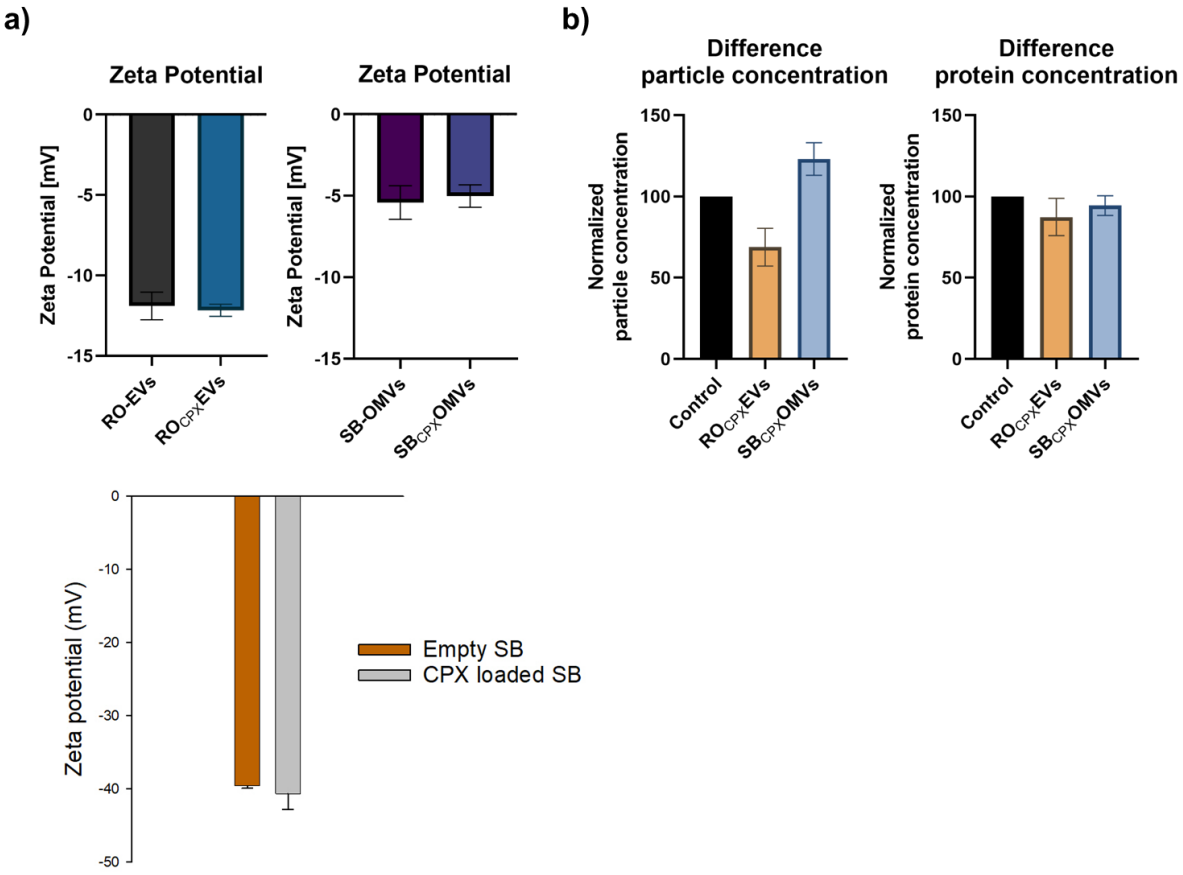

Figure S10 Difference in physico-chemical properties of vesicles upon drug loading. *a) Difference in zeta potential measured in PBS (top) and water (bottom), and compared to unloaded vesicles from RO cells and SB bacteria. Zeta potential in PBS was measured by dynamic light scattering, for measurements in water a Zetaview (Particle Metrix) was used. b) Difference in particle concentration and protein concentration normalized to respective unloaded vesicle types.*

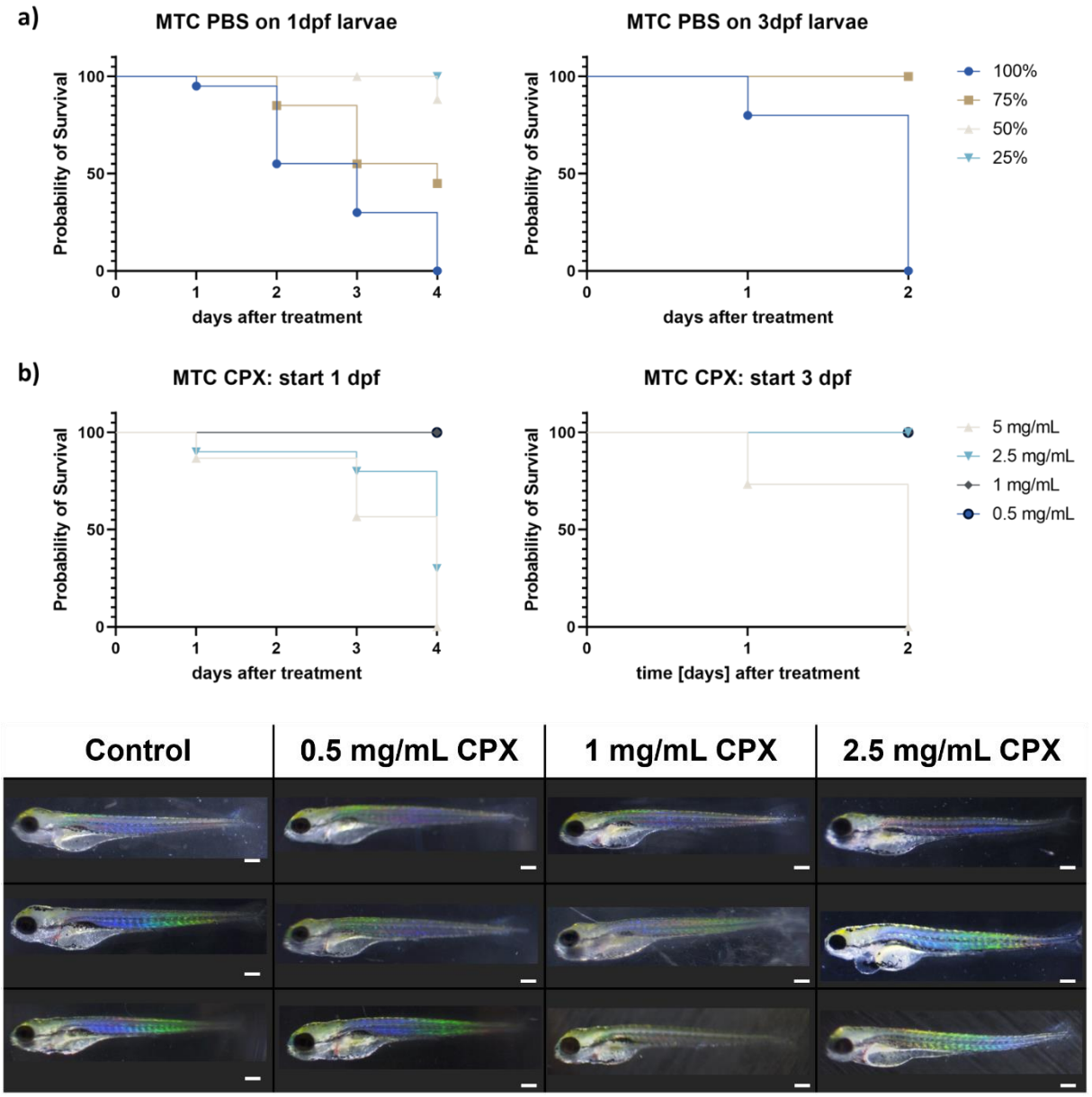

Figure S11 Maximum tolerated concentration of PBS and free CPX in zebrafish larvae. *a) Survival of larvae incubated with different concentration of PBS in water b) survival of larvae incubated with different concentration of ciprofloxacin in water. c) Representative 5dpf larvae exposed to various concentrations of CPX. n = 3, N = 10*

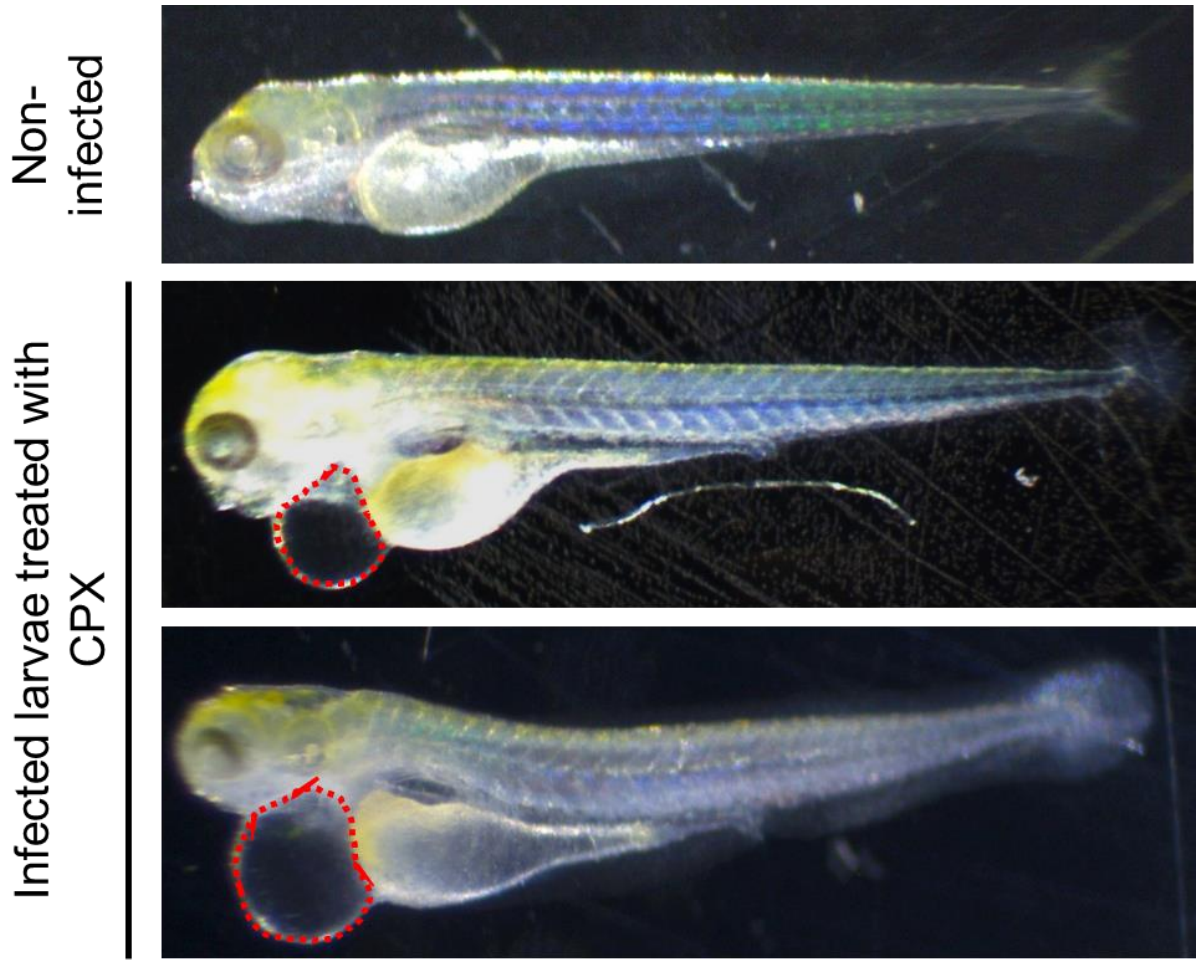

Figure S12 Side effects in infected larvae incubated with 1000 x MIC CPX. *After 4 h of infection, larvae were treated with 200 µg/mL CPX. After 24 h no infection was observed, however, after 3 days at 5dpf all larvae developed heart edema, whereas the control (not infected larvae treated with 1000 x MIC of CPX) showed no abnormalities.*