Supplementary Information

S1

The COMSOL Multiphysics® simulation is performed in a 3D geometry. The physics that are added to the simulation are: a. the laminar flow of a single-phase incompressible fluid, to describe fluid motion inside the platform and, b. the transport of a diluted species, to simulate the convective/diffusive behavior of a generic species i inside the platform. The addition of the transport of diluted species interface does not require the properties of the species i, except for the diffusion coefficient and the initial concentration c at the inlets. The diffusion coefficient (D) of a spherical particle in a viscous fluid is calculated with the Stokes-Einstein equation (Eq. 1), where k_B is the Boltzmann constant, T is the temperature, μ is the viscosity of the fluid and r is the radius of the particle:

$$D_i = \frac{k_B T}{6\pi\mu r} \quad (1)$$

The diffusion coefficient of EVs in water is calculated using Eq. 1 considering an average diameter of 100 nm and gives:

$$D_{EVs} = \frac{1.38 \cdot 10^{-23} \cdot 310}{6\pi \cdot 8.9 \cdot 10^{-4} \cdot 5.0 \cdot 10^{-8}} = 5.10 \cdot 10^{-12} \frac{m^2}{s} \quad (2)$$

To calculate the D of VP, its Stokes radius was first determined from the volume of the molecule, assuming it to be spherical. The volume per molecule was computed from VP Molecular Weight (M_W, 718.79 g/mol) and density (1.3 g/cm^3) using:

$$V = \frac{MW}{N_A \cdot \rho} = \frac{718.79}{6.022 \cdot 10^{23} \cdot 1.3} \approx 91.8 \cdot 10^{-23}\, cm^3 \approx 0.92\, nm^3 \quad (3)$$

The Stokes radius was then obtained using the volume formula for a sphere:

$$R_{VP} = \left(\frac{3V}{4\pi}\right)^{1/3} = \left(\frac{3 \cdot 0.92}{4\pi}\right)^{1/3} \approx 0.60\, nm \quad (4)$$

In Eq. 5 the D referred to VP was computed using the Stokes-Einstein equation:

$$D_{VP} = \frac{1.38 \cdot 10^{-23} \cdot 310}{6\pi \cdot 8.9 \cdot 10^{-4} \cdot 0.6 \cdot 10^{-9}} = 4.3 \cdot 10^{-10} \frac{m^2}{s} \quad (5)$$

The second information needed is the initial concentration *c* of the species at the inlets. At one inlet the concentration is set at an arbitrary value, while at the other one the concentration is set as zero. The final graphs are created by normalizing the concentration to the initial value. Several simulations

are performed in COMSOL, varying the inlet flow rates and the length of the mixing unit to determine the optimal configuration for the perfect mixing of the species.

The length of the delay-lines is calculated according to Eq. 6, that relates the delay time ϑ to volumetric flowrate $\dot{V}$ and l, w and h, respectively the length, the width, and the height of the delay-line geometry:

$$\vartheta = \frac{lwh}{\dot{V}} \tag{6}$$

The volumetric flowrate can be retrieved from COMSOL Multiphysics®, with a tool that returns the average velocity over a defined plane. By setting the ideal delay time t to 10 minutes, the calculated length of the delay lines is *l* = 264 mm.

S2

The mixing index (η), defined according to Eq. 7, is evaluated through a MATLAB® code, where COMSOL Multiphysics® retrieved concentration data are imported.

$$\eta = 1 - \sqrt{\frac{\gamma^2}{\gamma_{max}^2}} \tag{7}$$

where γ^2 is the variance calculated at the desired cross-sectional area, and γ_{max}^2 is the maximum variance.

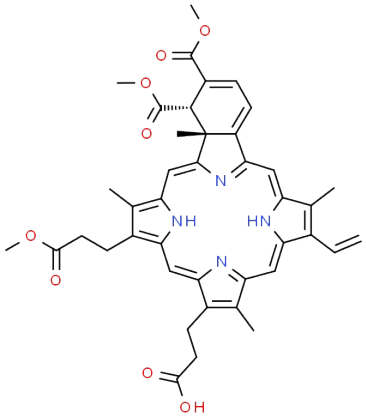
The variance is defined as:

$$\gamma^2 = \frac{1}{n}\sum_{i=1}^{n}\left(c_i - \bar{c}\right) \tag{8}$$

where n is the number of sampling points, c_i is the concentration of solute at the sampling point and $\bar{c}$ is the average concentration of solute. Values of mixing index η are 0 for entirely unmixed fluids and 1 for completely mixed fluids.

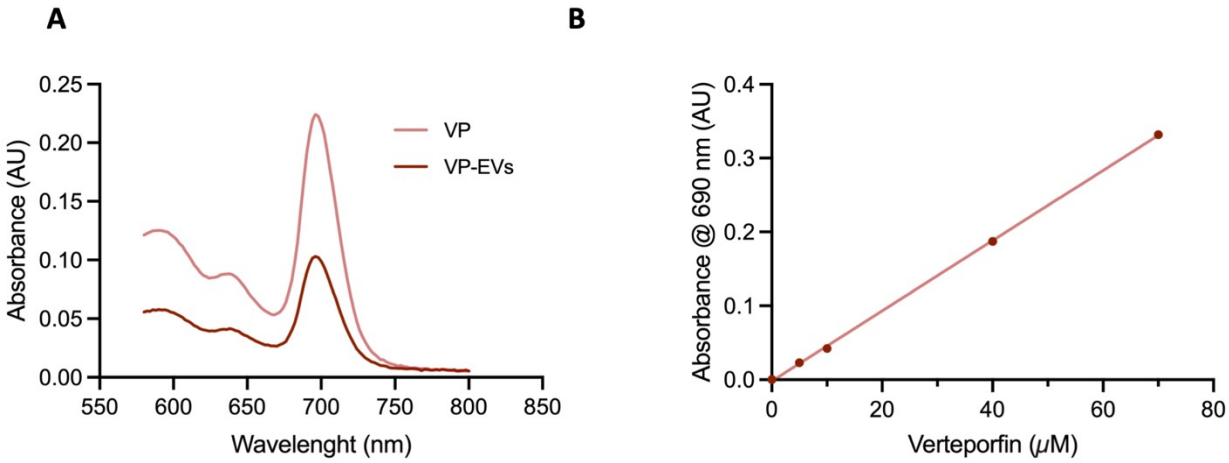
The mixing index computed for the proposed platforms is 0.99418.

S3



Chemical structure of Verteporfin (VP, M_w = 718.79)**.** (4R,4aS)-18-Ethenyl-4,4a-dihydro-3,4-bis(methoxycarbonyl)-4a,8,14,19-tetramethyl-24H,26H-benzo[b]porphine-9,13-dipropanoic acid monomethyl ester CSID:4515032, http://www.chemspider.com/Chemical-Structure.4515032.html (accessed 15:56, Feb 25, 2024).

S4



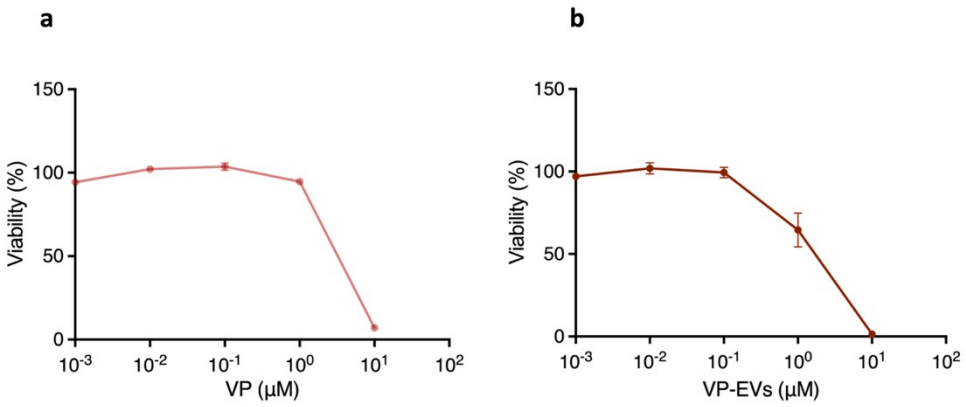
Loading of Verteporfin (VP) in Extracellular Vesicles (EVs). (A) Absorbance (scan: 450-800 nm; peak: 690 nm) of 40 μM free VP (light red) and ≋ 20 μM VP-loaded EVs (red). (B) Calibration curve based on absorbance at six different standard VP concentrations. R^2=0.9998.

S5

Video recording starting with time zero of perfusion into the microfluidics system, employing colored tracers and the PHD Ultra pump (Harvard Apparatus). The footage has been time-lapsed to enhance visualization and showcase the platform's functionality more effectively. This validation proved the correct mixing and incubation time required for the desired loading of Verteporfin into Extracellular Vesicles.

S6

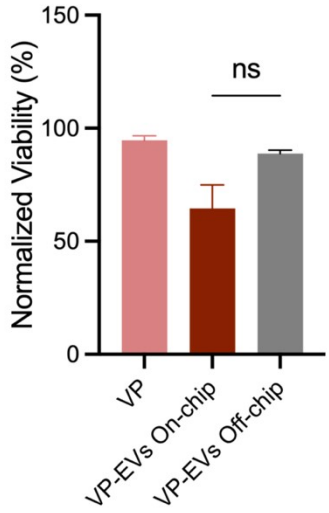
A



B

DMSO	EVs	VP	VP-EVs
0.8388	0.633	0.5228	0.1611
0.8791	0.608	0.5105	0.1749
0.7983	0.6143	0.5139	0.1854
0.8332	0.7674	0.553	0.1924
0.8979	0.6548	0.5191	0.1422
0.8873	0.6613	0.5126	0.1673

C



Verpeporfin-loaded Extracellular Vesicles (VP-EVs) reduce cell viability of a Neuroblastoma cell line (SK-N-AS). (A) Dose-response curves determined using the MTT assay of SK-N-AS treated for 48 h with VP-EVs (a) or free VP (b) at various VP concentrations (0.001–10 μM). Data are expressed as

mean ± SEM. (B) Representative raw cell viability data, expressed as absorbance read at 540 nm, of SK-N-AS treated for 48 h with DMSO, VP alone (1 µM), MSC-EVs alone (0.01 µg/µL, concentration approximately corresponding to the amount of EVs present in the 1 µM VP-EV condition) and VP-EVs (1 µM). (C) Cell viability was measured by MTT test after 48 h of incubation with 1 μM VP and 1 μM VP-EVs loaded using either the *on-chip* or *off-chip* method. Cell viability was normalized to DMSO (for VP) and EVs (for VP-EVs), respectively. Data are expressed as mean ± SEM.