ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.*, DOI: 10.1002/adhm.202101202

Functional siRNA Delivery by Extracellular Vesicle-Liposome Hybrid Nanoparticles

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Supplementary Table

Table S1: Oligonucleotide sequences

siRNA firefly luciferase	Sense: ‘5-GGA CGA GGU GCC UAA AGG AdCdG-3’
	Antisense: ‘5-UCC UUU AGG CAC CUC GUC CdCdG-3’
siRNA non specific	Sense: 5’-UGC GCU ACG AUC GAC GAU GdTdT-3’
	Antisense: 5’-CAU CGU CGA UCG UAG CGC AdTdT-3’

dT, dC, & dG indicate a deoxyribonucleic acid base

Supplementary Figures

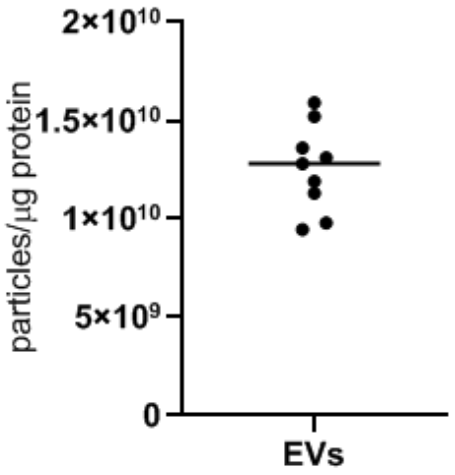

Figure S1: Batch to batch variability of EV purity.
EV purity is expressed as the number of particles per µg protein. Each datapoint represents an EV isolation. (n=9, biological replicates)

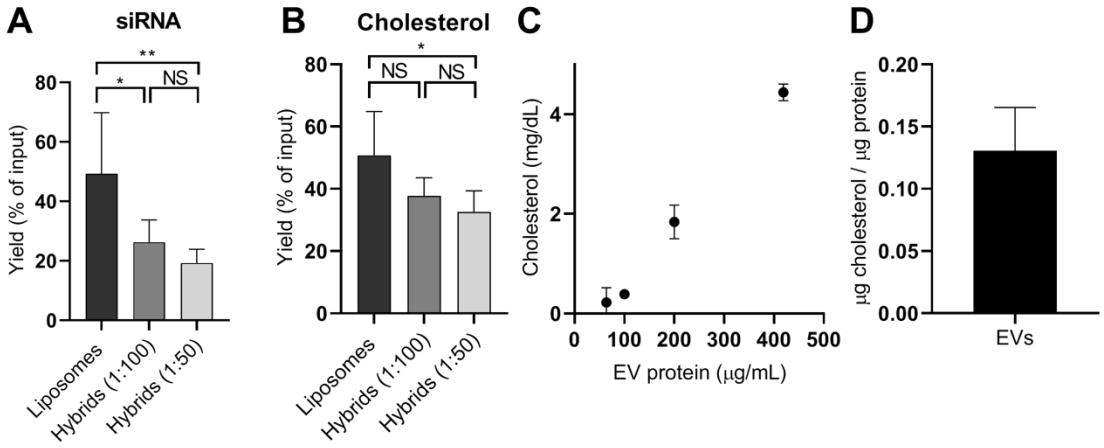

Figure S2: siRNA and cholesterol yield of production process.
The total amount of **A)** siRNA and **B)** cholesterol detected in liposomes and hybrids after dialysis expressed as percentage of the input amount. **C)** Cholesterol content of EVs at different EV-protein concentrations. **D)** Cholesterol content of EVs expressed per μg EV-protein. Data in A/B are shown as mean ± SD (n=6-7, biological replicates), One-way ANOVA with Tukey's post-hoc test, ns= not significant, * = $p<0.05$, ** = $p<0.01$. Data in C is shown as mean ± SD (n=3, technical triplicate). Data in D is shown as mean ± SD (n=2, biological replicate).

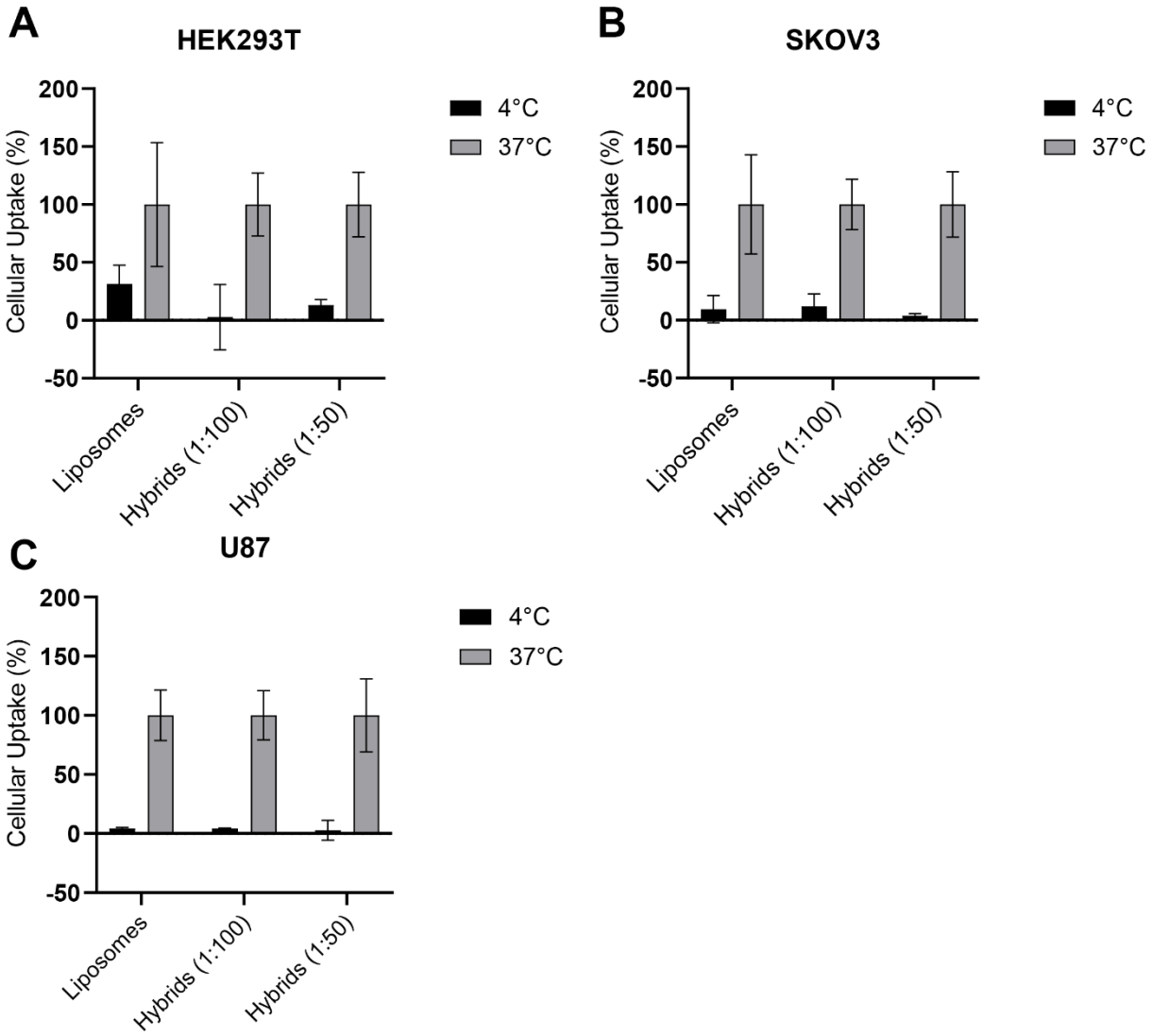

Figure S3: Cellular uptake analysis of liposomes and hybrids in different cell types
Cellular uptake in **A)** HEK293T-dluc. **B)** SKOV3-dluc. **C)** U87-MG-dluc as determined by flow cytometry and plotted as a percentage relative to the uptake observed at 37 °C. Data are plotted as mean ± SD (n=3, technical replicates).

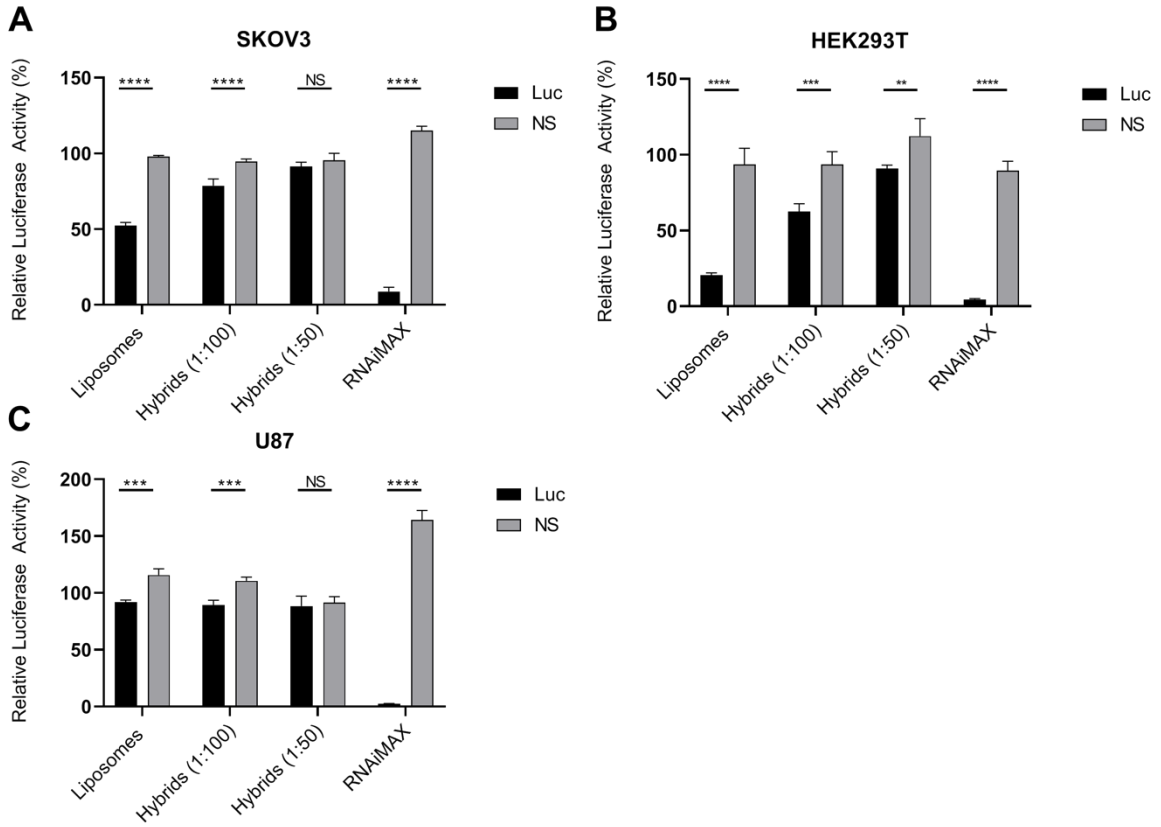

Figure S4: Gene silencing of firefly luciferase by liposome-, hybrid- or RNAiMAX mediated siRNA delivery in different cell types. siRNA targeting firefly luciferase (luc) or a non-specific siRNA (NS) was delivered via liposomes, hybrids or RNAiMAX and luciferase expression was measured after 48 hours incubation and normalized to renilla luciferase expression. Different cell types, **A)** SKOV3-dluc **B)** HEK293T-dluc **C)** U87-MG-dluc, were incubated with liposomes and hybrids at an siRNA concentration of 50 nM. For, RNAiMAX the concentration was 10 nM siRNA. Data are plotted as mean ± SD, n=3, technical replicates, two-way ANOVA with sidak's post-hoc test, ns = not significant, ** p = <0.01, *** = p <0.001, **** = p < 0.0001

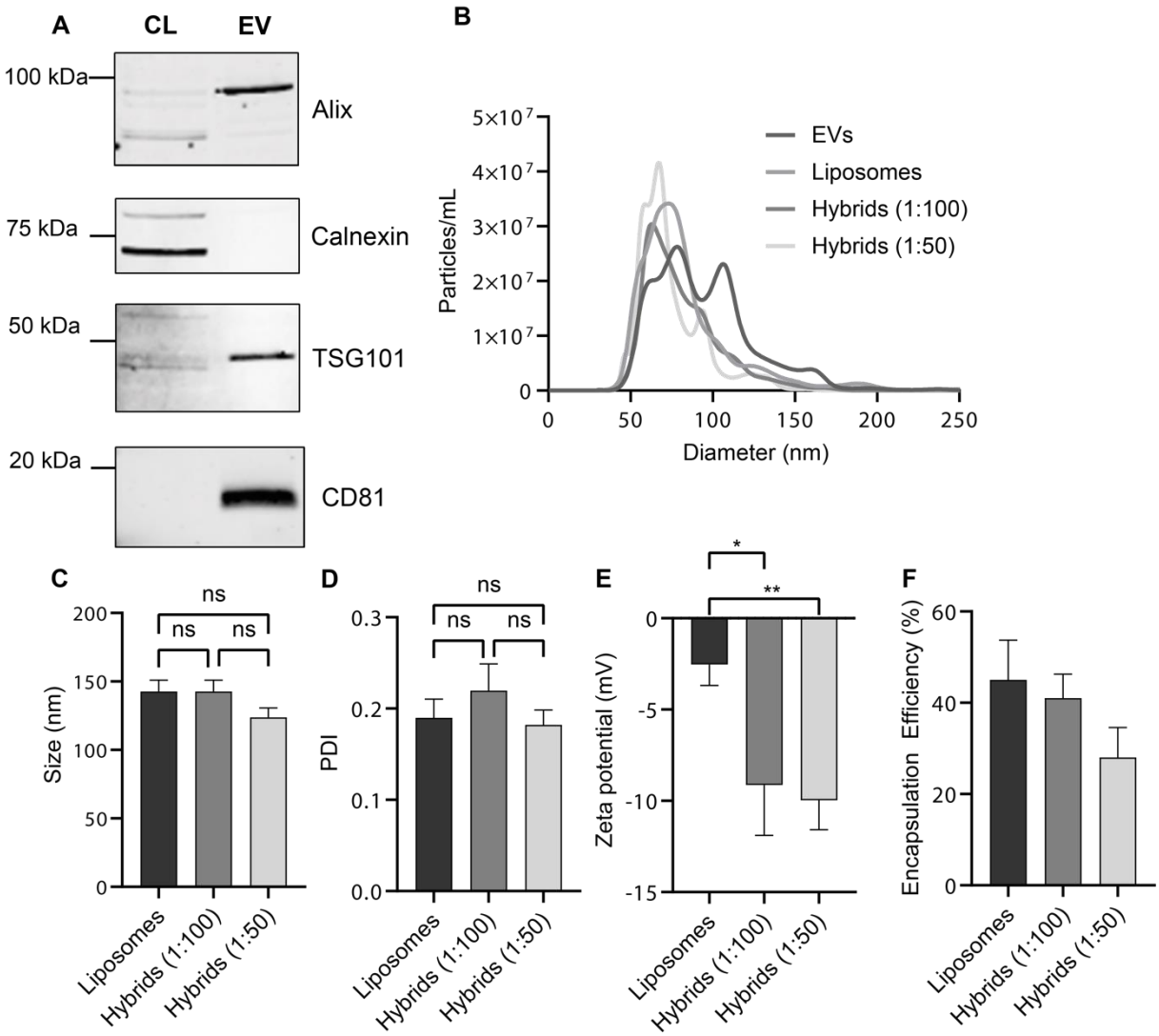

Figure S5: Characterization of CPC-derived EVs, liposomes and hybrids. A) Western Blot analysis of CPC cell lysate (CL) and CPC-derived EVs shows enrichment of typical EV markers Alix, TSG101 and CD81 and negative enrichment of a EV-negative marker, calnexin. **B)** NTA analysis of CPC-derived EVs, liposomes and hybrids. **C)** Nanoparticle size as determined by DLS. **D)** Polydispersity index of nanoparticles as measured by DLS. **E)** Zeta potential of nanoparticles as measured by laser doppler electrophoresis. **F)** RNA encapsulation efficiency of nanoparticles determined based on the cholesterol and siRNA concentrations before and after dialysis. Mean + SD is displayed for all samples (n=3, biological replicates), one-way ANOVA with tukey's post-hoc test, ns= not significant, * = $p<0.05$, ** = $p<0.01$.