Functional siRNA Delivery by Extracellular Vesicle-Liposome Hybrid Nanoparticles

Martijn J.W. Evers¹, Simonides I. van de Wakker², Ellis M. de Groot¹, Olivier G. de Jong¹,³, Jerney J.J. Gitz-François¹, Cor S. Seinen¹, Joost P.G. Sluijter²,⁴, Raymond M. Schiffelers¹, Pieter Vader¹,²*
Functional siRNA delivery by Extracellular Vesicle-Liposome Hybrid Nanoparticles

Martijn J.W. Evers¹, Simonides I. van de Wakker², Ellis de Groot¹, Olivier G. de Jong¹,³, Jerney J.J. Gitz-François¹, Cor S. Seinen¹, Joost P.G. Sluijter²,⁴, Raymond M. Schiffelers¹, Pieter Vader¹,²*  

Supporting Information
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 GuTdT-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 an 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.