Electronic Supplementary Material (ESI) for Nanoscale Horizons.

The Biomolecule Corona of Lipid Nanoparticles Contains Circulating Cell-free DNA

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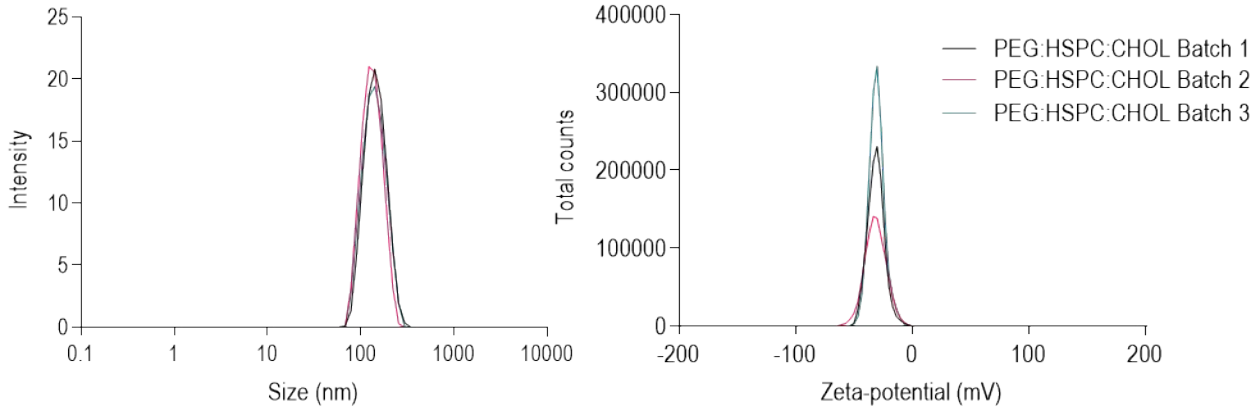
Supporting Information

A



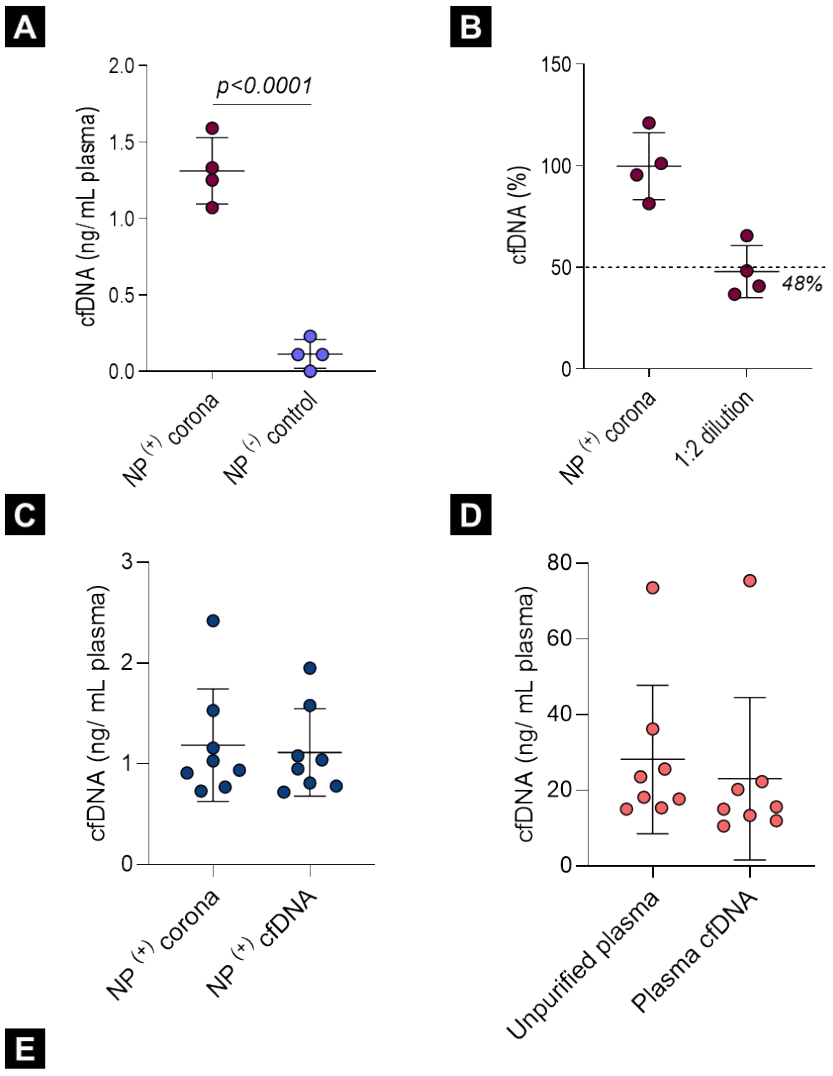
B

Sample	Size (nm)	PDI	ζ-potential (mV)
Batch 1	138.3 ± 3.061	0.054 ± 0.028	-32 ± 0.651
Batch 2	127.8 ± 2.312	0.043 ± 0.003	-31.4 ± 0.929
Batch 3	134 ± 1.365	0.059 ± 0.007	-30.4 ± 0.503

Supporting Figure 1

Figure S1: Physiochemical characterisation of liposome nanoparticles (NPs). **A)** Graphs representing the size (diameter in nm) and zeta-potential distribution (mV) of PEG:HSPC:CHOL liposome batches 1-3. **B)** Table listing the mean average size (nm), polydispersity index (PDI) and zeta-potential (mV) of each liposome batch including standard deviations.

Supporting Figure 2



Patient ID	Age	Histology	FIGO stage	BRCA status	Cytoreductive surgery	Prior lines of chemotherapy	Platinum sensitivity
OC 1	69	High-grade serous	3C	Wild-type	Optimal	2	Resistant
OC 2	70	High-grade serous	4B	Unknown	Optimal	1	Sensitive
OC 3	59	High-grade serous	3B	Wild-type	Complete	4	Resistant
OC 4	59	High-grade serous	3C	Wild-type	Suboptimal	3	Resistant
OC 5	62	High-grade serous	3C	Wild-type	Inoperable	2	Resistant
OC 6	63	High-grade serous	4B	Wild-type	Suboptimal	3	Resistant
OC 7	71	High-grade serous	3C	Wild-type	Suboptimal	2	Resistant
OC 8	76	High-grade serous	3C	Wild-type	Complete	1	Sensitive

Figure S2: Assessing the accuracy of direct real-time PCR cfDNA quantification in *ex vivo* healthy and disease nanoparticle corona samples. **A)** RNase P real-time qPCR quantification of in pooled healthy liposomal corona samples and liposome $^{(-)}$ plasma controls. **B)** Direct RNase P qPCR inhibition determined using 2-fold dilution of pooled NP corona samples. **C-D)** LINE-1 real-time qPCR quantification of cfDNA in late-stage serous ovarian cancer *ex vivo* biomolecule corona samples (n=8). Graph C represents cfDNA in NP corona samples and NP corona purified cfDNA, whereas graph D represents cfDNA in unpurified plasma (diluted 1:40) and purified plasma. All error bars represent mean and standard deviation. Groups were compared using a student t-test was performed (adjusted *p* values <0.05 were considered significant). **E)** Clinical details of eight late-stage ovarian cancer plasma samples included in graphs C and D.

Supporting Figure 3

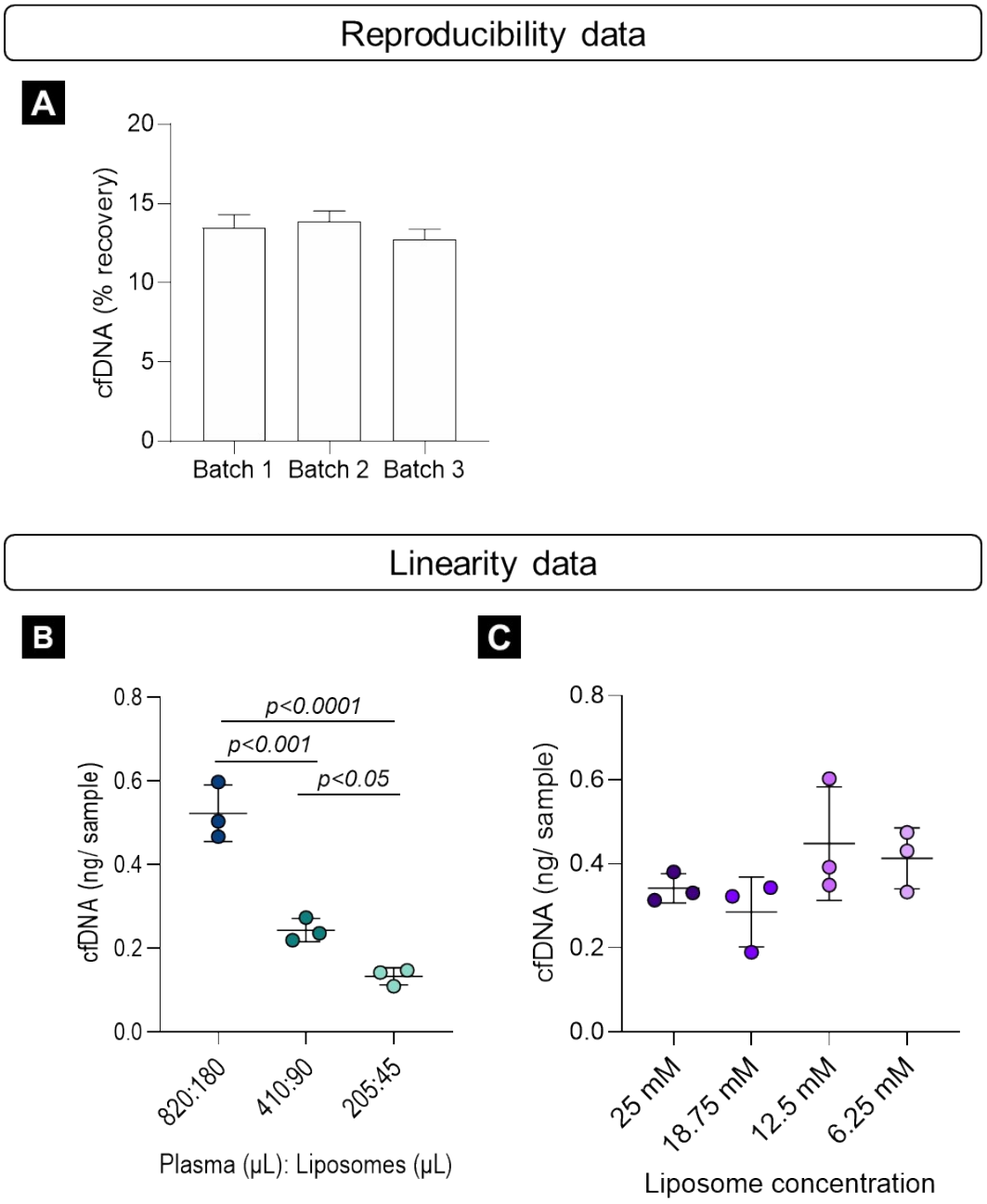
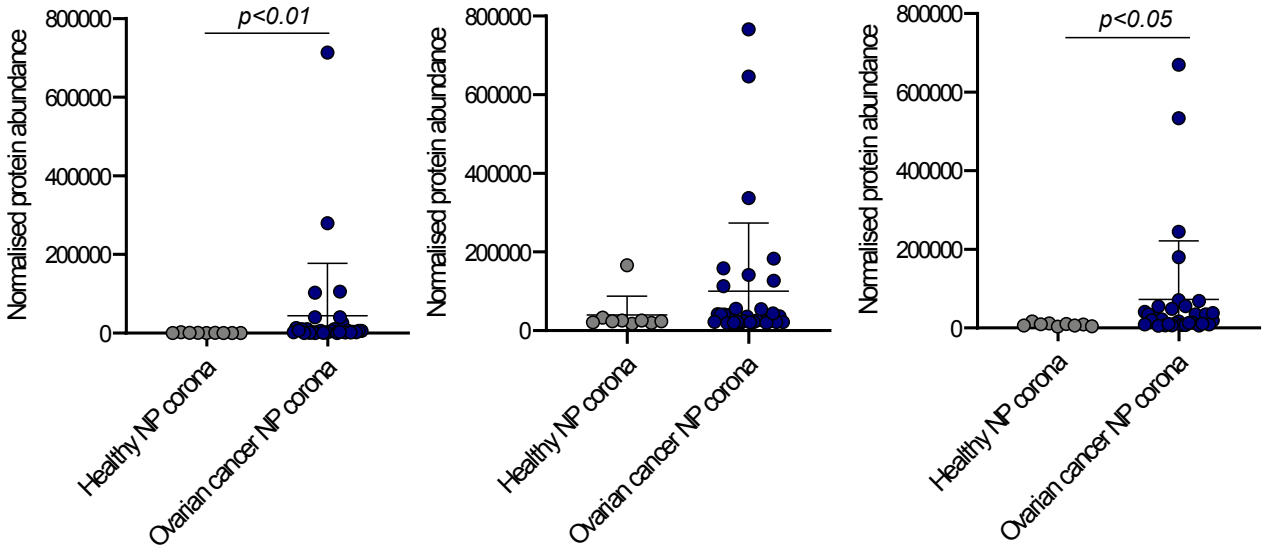


Figure S3: Reproducibility & linearity experiments of healthy plasma NP corona samples. **A)** Reproducibility data showing the percentage recovery (%) of QIAamp® purified NP corona cfDNA across liposome NP batches relative to QIAamp extracted plasma cfDNA (100%). **B-C)** Linearity data to investigate the effect of liposome concentration and plasma volume on cfDNA content in the liposome biomolecule corona. **B)** Graph highlighting the effect of plasma volume on cfDNA concentration (ng cfDNA/ sample). Standard protocol 820 µL plasma: 180 µL liposomes. **C)** Graph showing the effect of liposome concentration on cfDNA concentration (ng cfDNA/ sample). 12.5 mM liposomes represent standard protocol. All error bars represent mean and standard deviation. Three groups or more were compared using a one-way analyses of variance (ANOVA) test followed by the Tukey's multiple comparison test. Adjusted *p* values <0.05 were considered significant.

Supporting Figure 4

A



B

		Normalised abundance					
Protein class	**Protein name**	**Healthy control**	**Ovarian cancer**	**Fold-change**	***q* value (FDR)**	**UniProt ID**	**Peptide count**
Nucleosome-associated proteins	Histone H2A	671	44245	65.97	0.005	Q96QV6 (H2A1A_HUMAN)	2
	Histone H2B	39619	100365	2.53	0.087	Q96A08 (H2B1A_HUMAN)	4
	Histone H4	8788	73124	8.32	0.012	Q6B823 (Q6B823_HUMAN)	2
	Histone-lysine N-methyltransferase 2D	14405221	20832650	1.45	0.097	O14686 (KMT2D_HUMAN)	3
	Histone PARylation factor 1	20019	3288	6.09	0.151	Q9NWY4 (HPF1_HUMAN)	2

Figure S4: Histone proteins identified by LC-MS/MS in the biomolecule corona of healthy and ovarian cancer female plasma samples. **A)** LC-MS/MS normalised protein abundance of histones H2A, H2B and H4 in ovarian cancer corona samples and age-matched healthy corona controls. A one-way ANOVA was performed by the Progensis QI software with significance bars representing FDR-adjusted *p* values. **B)** Table summarising the relative abundance of proteins identified by LC-MS/MS associated with nucleosomes (DNA-histone complex) known to contain cfDNA. Max fold change between ovarian cancer corona samples and healthy corona controls is provided with FDR-adjusted *p* value from a one-way ANOVA in Progensis QI. Uniprot protein identifiers are provided along with LC-MS/MS peptide counts.

Supporting Table 1

		Ovarian cancer patients			
	Healthy	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**
Sample number	11	18	8	12	5
Age-range (median)	40-59 (51)	21-87 (59)	32-77 (60)	37-74 (62)	36-67 (48)
Histological subtype	N/A	Mucinous -11 (61%) Serous- 5 (28%) Clear cell-1 (5.5%) Endometroid- 1 (5.5%)	Serous- 6 (75%) Endometroid- 2 (25%)	Serous- 9 (75%) Adenocarcinoma (NOS)- 2 (17%) Carcinosarcoma- 1 (8%)	Serous- 5 (100%)
Germline BRCA status	N/A	Positive- 0 (0%) Negative- 1 (5.5%) Unknown- 17 (94.5%)	Positive- 0 (0%) Negative- 3 (37.5%) Unknown- 5 (62.5%)	Positive- 1 (8%) Negative- 0 (0%) Unknown- 11 (92%)	Positive- 1 (20%) Negative- 3 (60%) Unknown- 1 (20%)
Baseline CA125 (U/ mL)	N/A	Median 60 (12-550)	Median 29.5 (4-600)	Median 16 (7-358)	Median 15 (9-396)
Prior lines of chemotherapy	N/A	0 (94%) 2 (6%)	0 (62.5%) 1 (37.5%)	0 (50%) 1 (42%) 2 (8%)	0 (20%) 1 (80%)
Platinum sensitivity	N/A	Sensitive- 6 (33%) Resistant- 1 (6%) Unknown- 11 (61%)	Sensitive- 3 (37.5%) Resistant- 1 (12.5%) Unknown- 4 (50%)	Sensitive- 1 (8%) Resistant- 0 (0%) Unknown- 11 (92%)	Sensitive- 2 (40%) Resistant- 1 (20%) Unknown- 2 (40%)

Table S1: Table outlining clinical characteristics of ovarian cancer patient cohort and healthy normal volunteers (HNVs). Details include sample number (n), age-range (years), histological subtype, germline BRCA mutation status, baseline CA125 concentration (U/ mL), prior lines of chemotherapy and platinum sensitivity.