

Supporting Information

Surface Charge Influences Protein Corona, Cell Uptake and Biological Effects of Carbon Dots

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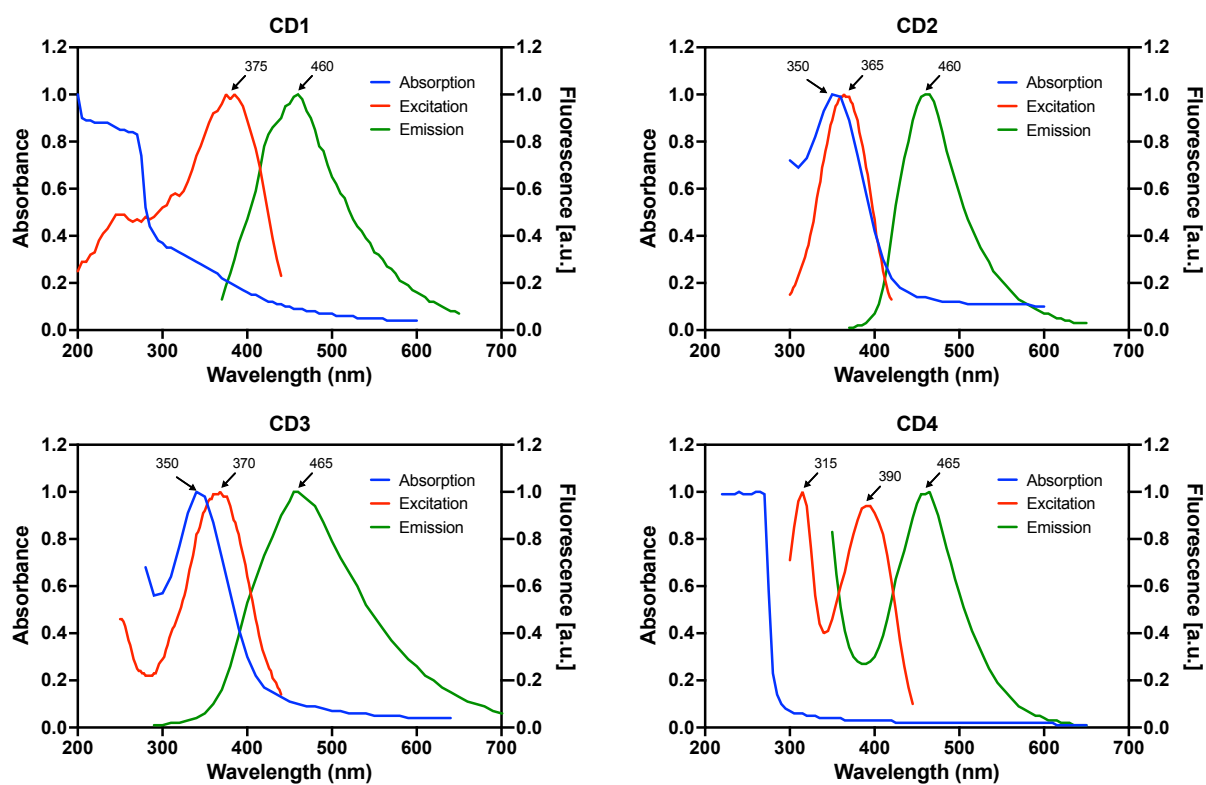


Figure S1. Optical properties of the CDs: absorption (blue), excitation (red), and emission (green; excitation at 375, 365, 370 and 315 nm for CD1, CD2, CD3 and CD4, respectively) normalized spectra.

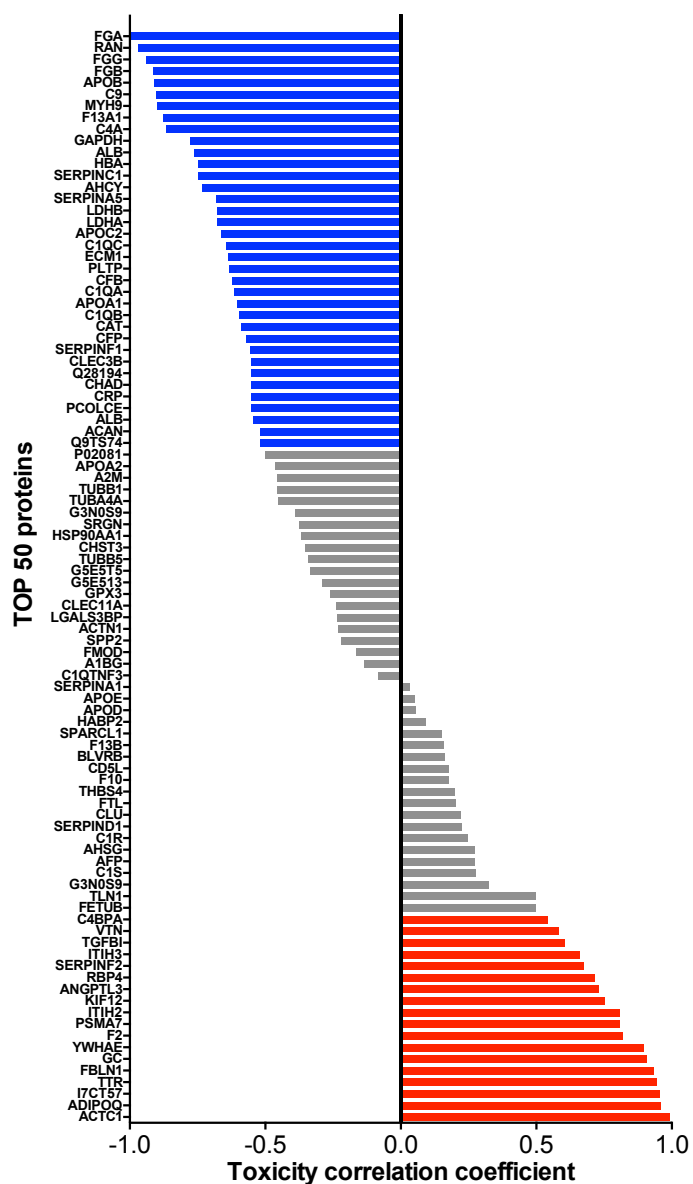


Figure S2. Correlation between the abundance (riBAQ) of the top 50 proteins and the CD1-CD4 toxicity. The toxicity marker that was chosen is the loss of viability induced by 125 $\mu\text{g}/\text{mL}$ CDs. The toxicity correlation coefficient is considered as significant when the absolute value is > 0.5 (labelled in blue and red for negative or positive correlation, respectively).

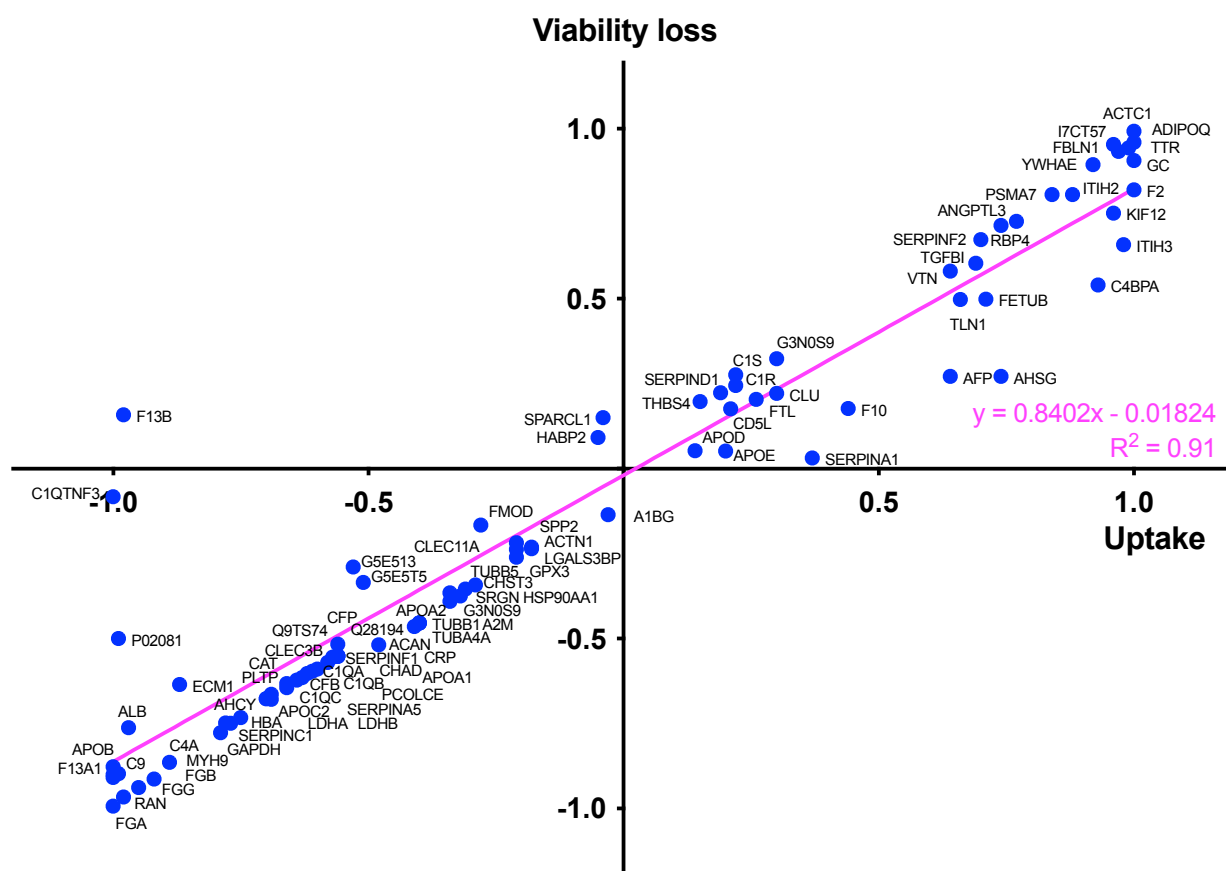


Figure S3. Role of corona proteins in CD cytotoxicity. Correlation between CD uptake and macrophage viability loss induced by 125 µg/mL CDs depending on each top 50 protein present in the NP corona. This analysis was conducted using FACS uptake data for cells exposed to CD2, CD3 and CD4.

ARTICLE

Table S1. Top 50 most abundant proteins in the CD corona.

CD1	Gene name	Protein name	RIBAQ	Contribution (%)	CD2	Gene name	Protein name	RIBAQ	Contribution (%)
1	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	0.1435	14.435	1	AHSG	Alpha-2-HS-glycoprotein	0.1238	12.448
2	KIF12	Protein AMBP	0.1180	11.874	2	ADIPOQ	Adiponectin	0.1203	12.097
3	VTN	Vitronectin	0.1130	11.373	3	VTN	Vitronectin	0.0903	9.082
4	FBLN1	Fibulin-1	0.0475	4.780	4	KIF12	Protein AMBP	0.0882	8.871
5	ADIPOQ	Adiponectin	0.0358	3.600	5	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	0.0841	8.461
6	AHSG	Alpha-2-HS-glycoprotein	0.0352	3.545	6	FBLN1	Fibulin-1	0.0579	5.818
7	HBA	Hemoglobin subunit alpha	0.0346	3.482	7	SERPINA1	Alpha-1-antitrypsinase	0.0435	4.376
8	G3NOS9	Uncharacterized protein	0.0282	2.835	8	G3NOS9	Uncharacterized protein	0.0343	3.445
9	F2	Prothrombin	0.0240	2.411	9	APOE	Apolipoprotein E	0.0234	2.356
10	LGALS3BP	Galectin-3-binding protein	0.0196	1.969	10	F2	Prothrombin	0.0215	2.159
11	SERPINC1	Antithrombin-III	0.0180	1.812	11	APOA1	Apolipoprotein A-I	0.0183	1.840
12	APOA1	Apolipoprotein A-I	0.0179	1.803	12	LGALS3BP	Galectin-3-binding protein	0.0171	1.717
13	SERPINA1	Alpha-1-antitrypsinase	0.0161	1.620	13	SERPINC1	Antithrombin-III	0.0156	1.565
14	P02081	Hemoglobin fetal subunit beta	0.0159	1.597	14	FETUB	Fetuin-B	0.0119	1.200
15	G5E5T5	Uncharacterized protein	0.0157	1.575	15	HBA	Hemoglobin subunit alpha	0.0109	1.093
16	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0.0101	1.017	16	ALB	Serum albumin	0.0083	0.832
17	APOE	Apolipoprotein E	0.0099	0.991	17	ALB	ALB protein	0.0079	0.799
18	G5E513	Uncharacterized protein	0.0091	0.921	18	TUBB1	Tubulin beta chain	0.0076	0.768
19	CLEC11A	C-type lectin domain containing 11A	0.0077	0.778	19	G5E5T5	Uncharacterized protein	0.0071	0.710
20	SERPIND1	SERPIND1 protein	0.0075	0.753	20	AFP	Alpha-fetoprotein	0.0070	0.705
21	TUBB1	Tubulin beta chain	0.0071	0.713	21	SERPINF2	Alpha-2-antiplasmin	0.0069	0.695
22	F10	F10 protein	0.0069	0.697	22	F13A1	Coagulation factor XIII A chain	0.0061	0.609
23	A2M	Alpha-2-macroglobulin	0.0066	0.665	23	ITIH2	ITIH2 protein	0.0057	0.572
24	ITIH2	ITIH2 protein	0.0059	0.590	24	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0.0056	0.565
25	F13A1	Coagulation factor XIII A chain	0.0058	0.583	25	CLEC11A	C-type lectin domain containing 11A	0.0046	0.465
26	SPARCL1	SPARC like 1	0.0054	0.547	26	ECM1	ECM1 protein	0.0043	0.436
27	ALB	ALB protein	0.0054	0.544	27	SERPIND1	SERPIND1 protein	0.0043	0.429
28	ALB	Serum albumin	0.0053	0.534	28	G5E513	Uncharacterized protein	0.0041	0.414
29	HABP2	Hyaluronan-binding protein 2	0.0052	0.521	29	TGFB1	Transforming growth factor-beta-induced protein ig-h3	0.0040	0.400
30	SERPINF2	Alpha-2-antiplasmin	0.0051	0.515	30	CLU	Clusterin	0.0039	0.395
31	TGFB1	Transforming growth factor-beta-induced protein ig-h3	0.0048	0.485	31	A2M	Alpha-2-macroglobulin	0.0037	0.376
32	HSP90AA1	Heat shock protein HSP 90-alpha	0.0045	0.456	32	TTR	Transthyretin	0.0035	0.357
33	E1B102	Fibromodulin	0.0043	0.434	33	GC	Vitamin D-binding protein	0.0033	0.337
34	C4A	Uncharacterized protein	0.0038	0.387	34	C4BPA	Complement component 4 binding protein. alpha chain	0.0032	0.320
35	THBS4	Thrombospondin-4	0.0037	0.371	35	FTL	Ferritin light chain	0.0029	0.290
36	ECM1	ECM1 protein	0.0036	0.366	36	GPX3	Glutathione peroxidase 3	0.0028	0.281
37	GPX3	Glutathione peroxidase 3	0.0034	0.340	37	YWHAE	14-3-3 protein epsilon	0.0025	0.253
38	TLN1	Talin 1	0.0033	0.337	38	TUBA4A	Tubulin alpha-4A chain	0.0025	0.247
39	FETUB	Fetuin-B	0.0033	0.333	39	C4A	Uncharacterized protein	0.0022	0.223
40	FGG	FGG protein	0.0029	0.292	40	I7CT57	Vitamin D binding protein	0.0022	0.222
41	Q9TS74	Pancreatic elastase inhibitor	0.0029	0.289	41	TLN1	Talin 1	0.0022	0.217
42	C1QTNF3	Adiponectin M	0.0028	0.283	42	ACTC1	Actin. alpha cardiac muscle 1	0.0021	0.216
43	CLU	Clusterin	0.0028	0.281	43	A1BG	Alpha-1B-glycoprotein	0.0020	0.198
44	SPP2	Secreted phosphoprotein 24	0.0026	0.265	44	ANGPTL3	Angiopoietin-like 3	0.0019	0.192
45	F13B	Coagulation factor XIII B chain	0.0026	0.263	45	C1R	Complement C1r	0.0019	0.187
46	BLVRB	Flavin reductase (NADPH)	0.0025	0.251	46	P02081	Hemoglobin fetal subunit beta	0.0018	0.185
47	CDS5L	CD5 molecule like	0.0024	0.241	47	PSMA7	Proteasome subunit alpha type-7	0.0018	0.184
48	FGB	Fibrinogen beta chain	0.0023	0.233	48	C1S	Complement C1s subcomponent	0.0017	0.173
49	TUBA4A	Tubulin alpha-4A chain	0.0023	0.230	49	Q9TS74	Pancreatic elastase inhibitor	0.0017	0.168
50	TTR	Transthyretin	0.0020	0.205	50	APOD	Apolipoprotein D	0.0016	0.160

CD3	Gene name	Protein name	RiBAQ	Contribution (%)	CD4	Gene name	Protein name	RiBAQ	Contribution (%)
1	VTN	Vitronectin	0.0824	8.343	1	HBA	Hemoglobin subunit alpha	0.1601	16.169
2	AHSG	Alpha-2-HS-glycoprotein	0.0728	7.371	2	AHSG	Alpha-2-HS-glycoprotein	0.1045	10.550
3	LGALS3BP	Galectin-3-binding protein	0.0630	6.377	3	SERPINF1	Pigment epithelium-derived factor	0.0909	9.182
4	HBA	Hemoglobin subunit alpha	0.0527	5.341	4	G3NOS9	Uncharacterized protein	0.0378	3.817
5	SERPINA1	Alpha-1-antiproteinase	0.0474	4.795	5	CLEC3B	Tetranectin (TN)	0.0352	3.550
6	KIF12	Protein AMBP	0.0350	3.540	6	SERPINA1	Alpha-1-antiproteinase	0.0304	3.069
7	A2M	Alpha-2-macroglobulin	0.0302	3.056	7	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0.0289	2.923
8	TUBB1	Tubulin beta chain	0.0287	2.907	8	APOA1	Apolipoprotein A-I	0.0288	2.905
9	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	0.0246	2.491	9	APOE	Apolipoprotein E	0.0269	2.717
10	APOA1	Apolipoprotein A-I	0.0192	1.939	10	SERPINC1	Antithrombin-III	0.0216	2.178
11	FBLN1	Fibulin-1	0.0191	1.935	11	APOA2	Apolipoprotein A-II	0.0184	1.857
12	ADIPOQ	Adiponectin	0.0182	1.847	12	C1QA	Complement C1q subcomponent subunit A	0.0171	1.729
13	CLEC11A	C-type lectin domain containing 11A	0.0179	1.813	13	ALB	ALB protein	0.0154	1.557
14	SERPINC1	Antithrombin-III	0.0173	1.755	14	CFB	Complement factor B	0.0141	1.425
15	G5E5T5	Uncharacterized protein	0.0154	1.559	15	APOC2	Apolipoprotein C-II	0.0137	1.387
16	G3NOS9	Uncharacterized protein	0.0148	1.498	16	VTN	Vitronectin	0.0136	1.370
17	APOE	Apolipoprotein E	0.0132	1.337	17	ALB	Serum albumin	0.0117	1.177
18	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	0.0131	1.326	18	CFP	Complement factor properdin	0.0112	1.133
19	ALB	ALB protein	0.0129	1.307	19	C1QB	Complement C1q subcomponent subunit B	0.0110	1.112
20	F2	Prothrombin	0.0123	1.249	20	P02081	Hemoglobin fetal subunit beta	0.0106	1.068
21	HSP90AA1	Heat shock protein HSP 90-alpha	0.0119	1.208	21	F2	Prothrombin	0.0104	1.050
22	APOA2	Apolipoprotein A-II	0.0107	1.085	22	F13A1	Coagulation factor XIII A chain	0.0098	0.987
23	FETUB	Fetuin-B	0.0100	1.017	23	C1QC	C1QC protein	0.0096	0.973
24	F13A1	Coagulation factor XIII A chain	0.0097	0.981	24	G5E5T5	Uncharacterized protein	0.0079	0.797
25	GPX3	Glutathione peroxidase 3	0.0094	0.949	25	Q9TS74	Pancreatic elastase inhibitor	0.0074	0.746
26	ALB	Serum albumin	0.0093	0.947	26	APOB	Apolipoprotein B	0.0073	0.740
27	P02081	Hemoglobin fetal subunit beta	0.0086	0.867	27	SERPINA5	Plasma serine protease inhibitor	0.0068	0.690
28	G5E513	Uncharacterized protein	0.0083	0.837	28	TUBB1	Tubulin beta chain	0.0066	0.671
29	TUBA4A	Tubulin alpha-4A chain	0.0072	0.733	29	PCOLCE	Procollagen C-endopeptidase enhancer	0.0059	0.594
30	LOC506828	Uncharacterized protein	0.0071	0.724	30	ECM1	ECM1 protein	0.0055	0.552
31	AFP	Alpha-fetoprotein	0.0066	0.670	31	FGG	FGG protein	0.0055	0.551
32	APOB	Apolipoprotein B	0.0066	0.665	32	C9	Complement component C9	0.0053	0.533
33	SERPINF2	Alpha-2-antiplasmin	0.0060	0.603	33	G5E513	Uncharacterized protein	0.0046	0.465
34	SERPIND1	SERPIND1 protein	0.0055	0.557	34	FGB	Fibrinogen beta chain	0.0041	0.417
35	CLU	Clusterin	0.0049	0.494	35	CRP	Pentaxin	0.0038	0.386
36	ECM1	ECM1 protein	0.0048	0.490	36	KIF12	Protein AMBP	0.0038	0.386
37	SPP2	Secreted phosphoprotein 24	0.0048	0.490	37	C4A	Uncharacterized protein	0.0038	0.379
38	C9	Complement component C9	0.0048	0.482	38	LDHA	L-lactate dehydrogenase A chain	0.0037	0.370
39	C4A	Uncharacterized protein	0.0047	0.478	39	MYH9	Myosin heavy chain 9	0.0033	0.338
40	E1BI02	Fibromodulin	0.0044	0.447	40	AFP	Alpha-fetoprotein	0.0033	0.336
41	ACAN	Aggrecan core protein	0.0043	0.439	41	PLTP	Phospholipid transfer protein	0.0032	0.326
42	FTL	Ferritin light chain	0.0043	0.434	42	CAT	Catalase	0.0032	0.320
43	SRGN	Proteoglycan 1-like	0.0039	0.398	43	ITIH3	Inter-alpha-trypsin inhibitor heavy chain H3	0.0030	0.307
44	FGG	FGG protein	0.0038	0.380	44	LDHB	L-lactate dehydrogenase B chain	0.0029	0.289
45	ACTN1	Alpha-actinin-1	0.0037	0.378	45	CHAD	Chondroadherin	0.0028	0.287
46	CHST3	Sulfotransferase	0.0037	0.372	46	AHCY	Adenosylhomocysteinase	0.0027	0.273
47	TGFBI	Transforming growth factor-beta-induced protein ig-h3	0.0036	0.361	47	A2M	Alpha-2-macroglobulin	0.0027	0.272
48	MYH9	Myosin heavy chain 9	0.0034	0.349	48	RAN	GTP-binding nuclear protein Ran	0.0026	0.264
49	A1BG	Alpha-1B-glycoprotein	0.0034	0.346	49	FGA	Fibrinogen alpha chain	0.0025	0.254
50	TUBB5	Tubulin beta-5 chain	0.0032	0.323	50	Q28194	Thrombospondin-1	0.0025	0.253

Table S2. Key corona proteins showing negative or positive correlation with CD cell uptake. The correlation analysis was carried out with the 4 CDs, or with CD2, CD3 and CD4 only.

	Protein	Analysis with the 4 CDs ^a	Analysis with CD2, CD3 and CD4 ^a		Protein	Analysis with the 4 CDs ^a	Analysis with CD2, CD3 and CD4 ^a
Negative correlation	FGA	-0.91	-1.00	Positive correlation	ADIPOQ	1.00	1.00
	FGG	-0.91	-0.95		GC	0.99	1.00
	C4A	-0.89	-0.89		TTR	0.99	0.99
	FGB	-0.87	-0.92		ACTC1	0.98	1.00
	RAN	-0.87	-0.98		I7CT57	0.90	0.96
	SERPINC1	-0.76	-0.78		FBLN1	0.81	0.97
	P02081	-0.74	-0.99		YWHAE	0.81	0.92
	APOB	-0.74	-1.00		C4BPA	0.77	0.93
	C9	-0.72	-1.00		PSMA7	0.69	0.84
	MYH9	-0.72	-0.99		SERPINF2	0.68	0.70
	GAPDH	-0.71	-0.79		FETUB	0.68	0.71
	F13A1	-0.68	-1.00		ITIH2	0.65	0.88
	HBA	-0.68	-0.77		RBP4	0.62	0.74
	AHCY	-0.67	-0.75		F2	0.61	1.00
	SERPINA5	-0.61	-0.69		ANGPTL3	0.60	0.77
	LDHB	-0.57	-0.70		KIF12	0.53	0.96
	LDHA	-0.57	-0.70		AFP	0.53	0.64
	Q9TS74	-0.56	-0.56		AHSG	0.52	0.74
	APOC2	-0.56	-0.69		TGFBI	0.42	0.69
	C1QC	-0.55	-0.66		VTN	0.42	0.64
	ALB	-0.54	-0.97		ITIH3	0.40	0.98
	CFB	-0.53	-0.64		TLN1	0.26	0.66
	C1QA	-0.53	-0.63				
	PLTP	-0.52	-0.66				
	C1QB	-0.52	-0.61				
	CAT	-0.50	-0.60				
	APOA1	-0.50	-0.62				
	G5E5T5	-0.50	-0.51				
	CFP	-0.49	-0.58				
	G5E513	-0.48	-0.53				
	Q28194	-0.48	-0.56				
	SERPINF1	-0.48	-0.57				
	CLEC3B	-0.48	-0.56				
	PCOLCE	-0.47	-0.56				
	CRP	-0.47	-0.56				
	CHAD	-0.47	-0.56				
ECM1	-0.40	-0.87					
C1QTNF3	-0.39	-1.00					
F13B	-0.15	-0.98					

^a) Pearson *r* correlation coefficient calculated from FACS data for cells exposed to CDs. The uptake correlation coefficient was considered as significant when the absolute value was > 0.5.