Supporting Information

Opsonin-deficient nucleoproteic corona endows unPEGylated liposomes with stealth properties in vivo

Francesca Giulimondi,^{1#} Elisabetta Vulpis,^{1#} Luca Digiacomo,¹ Maria Valeria Giuli,¹ Angelica Mancusi,¹ Anna Laura Capriotti,² Aldo Laganà,² Andrea Cerrato,² Riccardo Zenezini Chiozzi,³ Carmine Nicoletti,⁴ Heinz Amenitsch,⁵ Francesco Cardarelli,⁶ Laura Masuelli,⁷ Roberto Bei,⁸ Isabella Screpanti,¹ Daniela Pozzi,¹ Alessandra Zingoni,¹ Saula Checquolo,^{9*} Giulio Caracciolo^{1*}

¹Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy

²Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy

³Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research, Utrecht Institute for Pharmaceutical Sciences Utrecht University, Heidelberglaan 8, 3584 CS Utrecht, The Netherlands

⁴Unit of Histology and Medical Embryology, Department of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University of Rome, Via. A. Scarpa 16, 00161 Rome, Italy

⁵Institute of inorganic Chemistry, Graz University of Technology, Stremayerg 6/IV, 8010 Graz, Austria

⁶NEST, Scuola Normale Superiore, Piazza San Silvestro 12, 56127 Pisa, Italy

⁷Department of Experimental Medicine, University of Rome "Sapienza", Viale Regina Elena 324, 00161 Rome, Italy

⁸Department of Clinical Sciences and Translational Medicine, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome Italy

⁹Department of Medico-Surgical Sciences and Biotechnology, Sapienza University of Rome, Corso della Repubblica 79, 04100 Latina, Italy [#] These authors contributed equally

*Correspondence: saula.checquolo@uniroma1.it (S.C.); giulio.caracciolo@uniroma1.it (G.C.)



Figure S1. Characterization of CL1 (light green) and CL2 (dark green). (a) Size and (b) zeta potential distributions, (c) synchrotron SAXS patterns with the corresponding fitting curves and (d) computed electron density profiles. Structural parameters of lipid bilayers are calculated according to [Caracciolo et al. Langmuir 2006, 22, 4267-4273] and are reported in Table S1.

Table S1. DLS and synchrotron SAXS parameters measurements for CL1 and CL2. The parameters of the Gaussians fitting the electron density profiles reported in Figure S1d are: ρ_h (electron density of polar headgroups); σ_h (width of the positive Gaussian representing lipid headgroups); ρ_c (electron density profile of hydrocarbon chains); σ_c (width of the negative Gaussian representing hydrophobic core); z_h (distance between polar headgroups of lipid monolayers).

	CL1	CL2
D _H (nm)	137	132
P.d.I.	0.14	0.11
Zeta potential (mV)	41.3	44.2
ρ _h (a.u)	0.56	0.53
σ _h (nm)	0.24	0.25
ρ _c (a.u.)	0.29	0.34
$\sigma_{\rm c} (\rm nm)$	0.75	0.75
z _h (nm)	1.84	1.81



Figure S2. Representative transmission electron microscopy (TEM) images of PLs (panels a and b) and DDLs (panels c and d). In both samples are visible nano-sized, rounded-shaped vesicles. In the inset of panel b, a vesicle is enlarged to make lamellar periodicity visible (indicated by a red arrow). The presence of DNA filaments on the surface is detectable in DDLs. Bars correspond to 200 nm.



Figure S3. Image for protein corona analysis by 1D SDS-PAGE. (blue) DDL1, (red) PL1, (cyan) DDL2, and (gold) PL2 at increasing amount of human plasma (HP). The corresponding electrophoretic profiles are reported in Fig. 2c.

	DI	DL1	PL1	DI	DL2	PL2
Protein	HP=5%	HP=50%	HP=50%	HP=5%	HP=50%	HP=50%
APOC3	4.14%	3.77%	14.70%	3.19%	11.54%	13.61%
APOC2	4.12%	4.54%	20.95%	3.03%	5.22%	6.72%
APOA2	7.77%	2.10%	10.20%	3.01%	1.13%	2.04%
APOA1	7.38%	1.10%	1.93%	7.71%	1.12%	2.35%
APOE	2.25%	14.32%	13.61%	1.47%	4.08%	4.68%
APOC1	10.93%	1.37%	0.28%	14.07%	2.70%	2.39%
ALB	0.62%	3.81%	4.42%	0.75%	4.90%	9.12%
ACTG1	9.81%	4.15%	0.82%	13.27%	4.13%	0.87%
APOD	0.03%	0.23%	3.39%	0.59%	9.17%	15.25%
C1QC	0.22%	9.44%	0.61%	0.09%	7.91%	5.45%
C1QB	0.20%	8.64%	0.46%	0.05%	7.82%	5.70%
HIST1H2BL	5.94%	4.42%	0.42%	6.02%	2.15%	0.49%
IGKC	1.44%	3.19%	1.27%	0.77%	2.77%	4.20%
VTN	1.19%	1.41%	9.15%	0.55%	0.31%	0.72%
IGHM	1.09%	2.47%	0.63%	0.20%	1.36%	1.34%
HMGB1;HMGB1P1	5.63%	1.36%	0.34%	4.59%	0.82%	0.20%
HIST1H1E;HIST1H1D	3.67%	2.38%	0.19%	2.46%	1.04%	0.31%
APOA4	0.85%	0.68%	1.70%	0.99%	0.11%	0.27%
IGHG1	0.60%	2.14%	0.40%	0.23%	1.68%	2.30%
PF4	0.02%	1.26%	1.43%	0.01%	0.88%	3.73%
SERPINA1	0.01%	0.12%	0.13%	0.04%	0.17%	0.36%
IGLL5;IGLC1	0.76%	1.46%	0.51%	0.36%	1.23%	1.50%
KRT1	0.96%	0.56%	0.22%	1.36%	3.31%	0.37%
IGLC3;IGLC2;IGLC6	1.25%	1.46%	0.44%	0.30%	1.29%	1.42%
C4BPA	0.21%	0.22%	1.50%	0.15%	0.72%	2.00%
ACTBL2	1.75%	0.86%	0.10%	1.81%	0.98%	0.06%
C1QA	0.03%	2.59%	0.10%	0.02%	1.67%	1.37%
C3	0.13%	0.52%	1.10%	0.03%	0.20%	0.58%
HIST2H3A	1.43%	0.50%	0.11%	2.07%	0.30%	0.07%
CLU	0.22%	0.33%	0.67%	0.02%	0.35%	0.22%
FGG	0.15%	0.06%	0.06%	0.04%	0.08%	0.18%
IGHG3	0.43%	1.17%	0.13%	0.12%	0.83%	0.67%
FGB	0.05%	0.05%	0.05%	0.02%	0.08%	0.13%
HSPA8	1.04%	0.51%	0.07%	1.24%	0.43%	0.08%
IGHA1	0.10%	0.25%	0.25%	0.01%	0.28%	0.66%
APOB	0.15%	0.11%	0.28%	0.14%	0.08%	0.15%
HMGB2	1.41%	0.47%	0.06%	0.68%	0.16%	0.04%
HNRNPA	0.71%	0.24%	0.05%	1.60%	0.23%	0.03%
FGA	0.13%	0.09%	0.09%	0.07%	0.10%	0.18%
HIST1H2A	0.52%	1.05%	0.06%	0.57%	0.26%	0.08%
C4B	0.06%	0.14%	0.51%	0.01%	0.11%	0.36%
PPIA	0.75%	0.49%	0.06%	0.81%	0.42%	0.10%
KRT10	0.17%	0.15%	0.18%	0.62%	1.18%	0.21%

Table S2. Lists of plasma proteins identified in the coronas of DDLs and PLs by nano-LC-MS/MS.

HIST1H4A	0.78%	0.35%	0.04%	1.14%	0.16%	0.03%
CFP	0.01%	1.26%	0.26%	0.00%	0.80%	0.26%
SAA4	0.37%	0.43%	0.45%	0.32%	0.16%	0.25%
GAPDH	0.74%	0.43%	0.06%	0.75%	0.32%	0.05%
F2	0.01%	0.01%	0.39%	0.00%	0.02%	0.15%
SERPINA3	0.04%	0.22%	0.00%	0.01%	0.31%	0.13%
KRT9	0.12%	0.05%	0.06%	0.17%	1.52%	0.16%
HIST1H1B	0.70%	0.39%	0.02%	0.48%	0.20%	0.04%
ITIH2	0.04%	0.01%	0.08%	0.01%	0.01%	0.06%
HSP90AA1	0.53%	0.30%	0.04%	0.68%	0.30%	0.04%
ENO1	0.54%	0.26%	0.05%	0.73%	0.22%	0.04%
РКМ	0.38%	0.34%	0.07%	0.70%	0.31%	0.05%
GSN	0.70%	0.04%	0.02%	0.12%	0.06%	0.02%
MSN	0.43%	0.17%	0.04%	0.80%	0.16%	0.02%
НР	0.02%	0.21%	0.12%	0.05%	0.17%	0.41%
SAA1	0.04%	0.05%	0.48%	0.23%	0.24%	0.37%
PLG	0.26%	0.28%	0.05%	0.33%	0.38%	0.05%
CRP	0.00%	0.40%	0.58%	0.00%	0.16%	0.45%
HNRNPA3	0.32%	0.15%	0.02%	0.87%	0.12%	0.01%
CORO1A	0.31%	0.18%	0.04%	0.76%	0.20%	0.02%
TTR	0.00%	0.02%	0.04%	0.00%	0.01%	0.05%
PON1	0.01%	0.00%	0.17%	0.00%	0.00%	0.02%
HNRNPA2B1	0.27%	0.13%	0.04%	0.69%	0.13%	0.02%
EEF1A1P5	0.37%	0.14%	0.03%	0.55%	0.14%	0.03%
РТМА	0.31%	0.09%	0.07%	0.39%	0.01%	0.00%
CFH	0.02%	0.59%	0.02%	0.00%	0.19%	0.12%
АРОН	0.01%	0.06%	0.01%	0.02%	0.63%	0.43%
PROS1	0.01%	0.02%	0.16%	0.00%	0.06%	0.38%
AMBP	0.00%	0.01%	0.04%	0.00%	0.00%	0.02%
NME2;NME2P1	0.42%	0.09%	0.01%	0.46%	0.08%	0.01%
PGK1	0.26%	0.19%	0.03%	0.36%	0.18%	0.03%
RPS23	0.27%	0.07%	0.02%	0.55%	0.07%	0.00%
СР	0.00%	0.01%	0.03%	0.00%	0.01%	0.03%
IGHG2	0.09%	0.19%	0.07%	0.01%	0.14%	0.36%
IGKV3-20	0.10%	0.24%	0.03%	0.01%	0.21%	0.15%
TF	0.03%	0.12%	0.09%	0.03%	0.18%	0.37%
HSP90AB1	0.28%	0.14%	0.02%	0.33%	0.12%	0.02%
АРОМ	0.00%	0.04%	0.51%	0.02%	0.00%	0.07%
EEF2	0.22%	0.09%	0.02%	0.39%	0.08%	0.01%
PA2G4	0.25%	0.09%	0.01%	0.34%	0.09%	0.01%
CFL1	0.15%	0.22%	0.02%	0.21%	0.16%	0.03%
JCHAIN	0.06%	0.14%	0.02%	0.03%	0.08%	0.05%
PSMA6	0.21%	0.10%	0.02%	0.27%	0.09%	0.01%
ALDOA	0.24%	0.07%	0.01%	0.29%	0.08%	0.01%
AHSG	0.00%	0.0%	0.01%	0.00%	0.01%	0.01%
RPS3A	0.20%	0.09%	0.01%	0.35%	0.06%	0.01%
APOC4	0.10%	0.13%	0.08%	0.06%	0.13%	0.05%
FN1	0.02%	0.01%	0.01%	0.00%	0.02%	0.02%

CFB	0.06%	0.00%	0.00%	0.00%	0.01%	0.01%
KRT14	0.01%	0.00%	0.02%	0.06%	0.59%	0.01%
APCS	0.08%	0.02%	0.01%	0.01%	0.02%	0.01%
PRDX1	0.21%	0.12%	0.01%	0.21%	0.12%	0.02%
IGKV3	0.01%	0.09%	0.03%	0.04%	0.08%	0.09%
KNG1	0.01%	0.01%	0.03%	0.00%	0.01%	0.02%
ORM2	0.00%	0.01%	0.01%	0.00%	0.01%	0.02%
KRT2	0.03%	0.04%	0.05%	0.17%	0.27%	0.07%
A2M	0.01%	0.04%	0.02%	0.02%	0.02%	0.05%
PCNA	0.18%	0.07%	0.01%	0.23%	0.06%	0.00%
CAPZB	0.15%	0.07%	0.01%	0.24%	0.07%	0.01%
CD5L	0.02%	0.13%	0.04%	0.00%	0.08%	0.07%
YWHAZ	0.19%	0.06%	0.00%	0.21%	0.07%	0.01%
ITIH1	0.00%	0.00%	0.02%	0.00%	0.00%	0.01%
HNRNPU	0.16%	0.06%	0.01%	0.22%	0.05%	0.00%
EEF1G	0.15%	0.05%	0.01%	0.24%	0.05%	0.00%
ANP32B	0.19%	0.05%	0.02%	0.16%	0.06%	0.00%
APOL1	0.22%	0.03%	0.02%	0.06%	0.02%	0.01%
RHOA	0.14%	0.02%	0.00%	0.26%	0.02%	0.00%
HNRNPD	0.06%	0.06%	0.01%	0.25%	0.05%	0.01%
HPR	0.02%	0.01%	0.00%	0.00%	0.02%	0.00%
RNASE4	0.00%	0.03%	0.10%	0.00%	0.04%	0.29%
HBB;HBD	0.11%	0.16%	0.10%	0.01%	0.05%	0.02%
C4BPB	0.01%	0.00%	0.11%	0.03%	0.03%	0.18%
NCL	0.23%	0.06%	0.02%	0.10%	0.03%	0.01%
CAPZA2	0.12%	0.10%	0.01%	0.11%	0.10%	0.01%
SIGLEC16	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
ANP32A	0.16%	0.07%	0.01%	0.09%	0.04%	0.01%
IGHV3	0.04%	0.13%	0.03%	0.01%	0.11%	0.11%
FSCN1	0.14%	0.05%	0.00%	0.18%	0.04%	0.00%
RBBP4	0.14%	0.06%	0.01%	0.13%	0.06%	0.00%
PRH1	0.00%	0.00%	0.00%	0.13%	0.29%	0.00%
LDHB	0.11%	0.05%	0.01%	0.16%	0.05%	0.01%
WDR1	0.12%	0.07%	0.00%	0.15%	0.05%	0.01%
ARPC2	0.12%	0.03%	0.00%	0.21%	0.03%	0.00%
RPL11	0.13%	0.06%	0.01%	0.14%	0.04%	0.01%
MDH1	0.11%	0.05%	0.01%	0.17%	0.04%	0.01%
ORM1	0.00%	0.02%	0.01%	0.00%	0.01%	0.02%
SNRPD1	0.13%	0.08%	0.00%	0.09%	0.07%	0.00%
RPL22	0.19%	0.00%	0.00%	0.17%	0.00%	0.00%
SERPING1	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%
ACTA1	0.10%	0.05%	0.01%	0.12%	0.07%	0.02%
SPP2	0.00%	0.00%	0.28%	0.00%	0.00%	0.00%
RPL12	0.04%	0.02%	0.00%	0.04%	0.01%	0.00%
LCAT	0.00%	0.00%	0.06%	0.00%	0.06%	0.18%
PDIA3	0.11%	0.02%	0.00%	0.16%	0.03%	0.00%
TPI1	0.11%	0.03%	0.00%	0.14%	0.03%	0.00%
H1FX	0.12%	0.08%	0.00%	0.07%	0.03%	0.01%

ARPC1B	0.10%	0.03%	0.01%	0.16%	0.02%	0.00%
TPM3;TPM1;TPM2	0.09%	0.03%	0.00%	0.12%	0.01%	0.00%
ATIC	0.08%	0.03%	0.01%	0.15%	0.03%	0.00%
CFHR1	0.00%	0.13%	0.00%	0.00%	0.06%	0.13%
RAN	0.06%	0.03%	0.00%	0.17%	0.04%	0.00%
HSPA1B;HSPA1A	0.09%	0.03%	0.01%	0.14%	0.03%	0.00%
IGKV2	0.01%	0.02%	0.02%	0.02%	0.03%	0.09%
IGFALS	0.13%	0.01%	0.00%	0.02%	0.01%	0.01%
SH3BGRL3	0.07%	0.10%	0.00%	0.02%	0.08%	0.03%
АНСҮ	0.09%	0.03%	0.00%	0.14%	0.02%	0.00%
ARHGDIA	0.09%	0.03%	0.00%	0.14%	0.03%	0.00%
ITIH4	0.01%	0.01%	0.00%	0.01%	0.01%	0.01%
EZR	0.05%	0.05%	0.00%	0.13%	0.03%	0.00%
PRDX4	0.08%	0.04%	0.00%	0.07%	0.04%	0.01%
HBA1	0.03%	0.08%	0.03%	0.03%	0.03%	0.03%
IGHV3-7	0.00%	0.10%	0.04%	0.00%	0.01%	0.04%
FABP5	0.07%	0.02%	0.00%	0.13%	0.04%	0.00%
RCC2	0.06%	0.02%	0.00%	0.13%	0.02%	0.00%
DEK	0.08%	0.02%	0.00%	0.09%	0.01%	0.00%
PSMB1	0.08%	0.04%	0.01%	0.07%	0.03%	0.00%
RPL5	0.06%	0.04%	0.00%	0.10%	0.02%	0.00%
PSMA2	0.08%	0.03%	0.01%	0.09%	0.02%	0.00%
SET;SETSIP	0.12%	0.01%	0.00%	0.06%	0.01%	0.00%
KRT16	0.01%	0.00%	0.01%	0.01%	0.20%	0.00%
KRT5	0.01%	0.00%	0.00%	0.04%	0.17%	0.01%
C1S	0.01%	0.01%	0.01%	0.00%	0.01%	0.02%
SNRPG;SNRPGP15	0.10%	0.02%	0.00%	0.08%	0.01%	0.00%
COTL1	0.10%	0.01%	0.00%	0.09%	0.01%	0.00%
ACTR3	0.03%	0.03%	0.00%	0.11%	0.02%	0.00%
PSMA5	0.07%	0.03%	0.00%	0.08%	0.02%	0.00%
ARHGDIB	0.05%	0.02%	0.01%	0.11%	0.02%	0.00%
NPM1	0.09%	0.03%	0.01%	0.06%	0.01%	0.01%
YWHAB;YWHAQ	0.06%	0.02%	0.00%	0.09%	0.02%	0.00%
SERPIND1	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
LYZ	0.01%	0.08%	0.01%	0.01%	0.07%	0.02%



Figure S4. Gating strategy of mouse phagocyte populations. Representative dot plots of phagocyte populations in the blood and in the spleen dissected by gating on CD11b⁺GR1^{low} (monocytes), CD11b GR1^{high} (neutrophils) and CD11b⁺GR1⁻F4/80⁺ (macrophages).



Figure S5. Characterization of CL2/oligonucleotide (ON) lipoplexes as a function of the cationic lipid/ON weight ratio, ρ . Size (black points) and zeta potential (grey points) of lipoplexes prepared by mixing CL2 and ON as a function of ρ . Comparing results of Fig. S5 with those reported in Fig. 2, we observe that both the curves are shifted to higher ρ values. This result is in full agreement with the conclusions of previous investigations showing that more cationic lipid is needed to complex ONs with respect to that needed to condense plasmid DNA (Munoz-Úbeda, Mónica, et al. "Why is less cationic lipid required to prepare lipoplexes from plasmid DNA than linear DNA in gene therapy?." Journal of the American Chemical Society 133.45 (2011): 18014-18017 ¹). Moreover, at fixed DNA mass, ONs bear double the nucleotides than plasmid DNA. Thus, to use the same moles of nucleotides in all the animal experiments, we employed double the mass of cationic lipid when the ON is used (i.e., $\rho = 2$). At $\rho = 2$ DDL2 were small in size ($D_H \approx 200 \text{ nm}$), and negatively charged (zeta potential $\approx -40 \text{ mV}$). ON-decorated lipoplexes from CL2 were indicated as DDL2* to differentiate them from those decorated with plasmid DNA) (i.e., DDL2).



Figure S6. Lipoplexes do not trigger TLR9 activity in reporter cells. HEK293/Luc reporter cells transfected or not with TLR9 were incubated for 5 hours with PL1, PL2, DDL1, DDL2 or specific TLR9 agonist. Data are expressed as luciferase activity. A representative experiment out of two is shown. Color code: untreated cells (white), TLR9 agonist (dark grey), DDL1 (blue), DDL2 (cyan), PL1 (red), PL2 (gold).

Supplementary Table S3. Material	Yes	No
characterization Question	-	
1.1 Are "best reporting practices" available for	Not applicable	
the nanomaterial used?		
1.2 If they are available, are they used? If not available	ole,	
ignore this question and proceed to the next one.		
1.3 Are extensive and clear instructions reported	$$	
detailing all steps of synthesis and the resulting		
composition of the nanomaterial?		
1.4 Is the size (or dimensions, if non-spherical) and		
shape of the nanomaterial reported?		
1.5 Is the size dispersity or aggregation of the		
nanomaterial reported?		
1.6 Is the zeta potential of the nanomaterial		
reported?		
1.7 Is the concentration (mass/volume) of the		
nanomaterial reported?		
1.8 Is the amount of any drug loaded reported?		
'Drug' here broadly refers to functional cargos (<i>e.g.</i> ,		
proteins, small molecules, nucleic acids).		
1.9 Is the targeting performance of the	Not applicable	
nanomaterial reported, including amount of ligand		
bound to the nanomaterial if the material has been		
functionalised through addition of targeting ligands?		
1.10 Is the label signal per nanomaterial/particle		
reported? For example, fluorescence signal per		
particle for fluorescently labeled nanomaterials.		
1.11 If a material property not listed here is varied,	Not applicable	
has it been quantified?		
1.12 Were characterizations performed in a fluid	$$	
mimicking biological conditions?		
1.13 Are details of how these parameters were	$$	
measured/estimated provided?		

Supplementary Table 3. Biological	Yes	No
characterization Question	1	
2.1 Are cell seeding details, including number of	N	
cells plated, confluency at start of experiment,		
and time between seeding and experiment		
reported?		
designation and source provided?	V	
2.3 Is the passage number (total number of times a	Not applicable	
cell culture has been subcultured) known and	recuppitedete	
reported?		
2.4 Is the last instance of verification of cell line	No	
reported? If no verification has been performed, is		
the time passed and passage number since		
acquisition from trusted source (<i>e.g.</i> , ATCC or		
ECACC) reported? For information, see Science		
347 (2015) 938;		
http://doi.org/10.1126/science.347.6225.938		
2.5 Are the results from mycoplasma testing of	No	
cell cultures reported?		
2.6 Is the background signal of cells/tissue		
reported? (<i>E.g.</i> , the fluorescence signal of cells		
without particles in the case of a flow cytometry		
experiment.)		
2.7 Are toxicity studies provided to demonstrate	Not applicable	
that the material has the expected toxicity, and that		
the experimental protocol followed does not?		
2.8 Are details of media preparation (type of	\vee	
media, serum, any added antibiotics) provided?		
2.9 Is a justification of the biological model used	N	
provided? For examples for cancer models, see		
Cancer Res. 75 (2015) 4016;		
http://doi.org/10.1158/0008-54/2.CAN-15-1558,		
and Mol. Iner. 20 (2012) 882;		
11 (2017) 0504:		
11 (2017) 3334, http://doi.org/10.1021/acspano.7b0/1855		
2 10 Is characterization of the biological fluid (ar	1	
2.10 is characterization of the biological huld (ex vivo/in vitro) reported? For example, when	N	
investigating protein adsorption onto papoparticles		
dispersed in blood serum, pertinent aspects of the		
blood serum should be characterised ($\rho \sigma$ protein		
concentrations and differences between donors used		
in study)		
2.11 For animal experiments are the ARRIVE	\checkmark	
guidelines followed? For details, see <i>PLOS Biol.</i> 8		
(2010) e1000412;		
http://doi.org/10.1371/journal.pbio.1000412		

Supplementary Table 3. Experimental details	Yes	No
3.1 For cell culture experiments: are cell culture dimensions including type of well , volume of added media , reported? Are cell types (<i>i.e.</i> ; adherent <i>versus</i> suspension) and orientation (if non-standard) reported?	\checkmark	
3.2 Is the dose of material administered reported? This is typically provided in nanomaterial mass, volume, number, or surface area added. Is sufficient information reported so that regardless of which one is provided, the other dosage metrics can be calculated (<i>i.e.</i> using the dimensions and density of the nanomaterial)?	\checkmark	
3.3 For each type of imaging performed, are details of how imaging was performed provided, including details of shielding , non-uniform image processing , and any contrast agents added?	Not applicable	
3.4 Are details of how the dose was administered provided, including method of administration , injection location , rate of administration , and details of multiple injections ?	\checkmark	
3.5 Is the methodology used to equalise dosage provided?		
3.6 Is the delivered dose to tissues and/or organs (<i>in vivo</i>) reported, as % injected dose per gram of tissue (%ID g ₋₁)?	\checkmark	
3.7 Is mass of each organ/tissue measured and mass of material reported?	Not applicable	
3.8 Are the signals of cells/tissues with nanomaterials reported? For instance, for fluorescently labeled nanoparticles, the total number of particles per cell or the fluorescence intensity of particles + cells, at each assessed timepoint.	Not applicable	
3.9 Are data analysis details , including code used for analysis provided?		
3.10 Is the raw data or distribution of values underlying the reported results provided? For examples, see <i>R. Soc. Open Sci.</i> 3 (2016) 150547; http://doi.org/10.1098/rsos.150547, https://opennessinitiative.org/making-your-data- public/, http://journals.plos.org/plosone/s/data- availability, and https://www.nature.com/sdata/policies/repositories		

1. Munoz-Úbeda, M.; Misra, S. K.; Barrán-Berdón, A. L.; Aicart-Ramos, C.; Sierra, M. B.; Biswas, J.; Kondaiah, P.; Junquera, E.; Bhattacharya, S.; Aicart, E., Why is less cationic lipid required to prepare lipoplexes from plasmid DNA than linear DNA in gene therapy? *Journal of the American Chemical Society* **2011**, *133* (45), 18014-18017.