Electronic Supplementary Information

In vitro and *ex vivo* nano-enabled immunomodulation by protein corona

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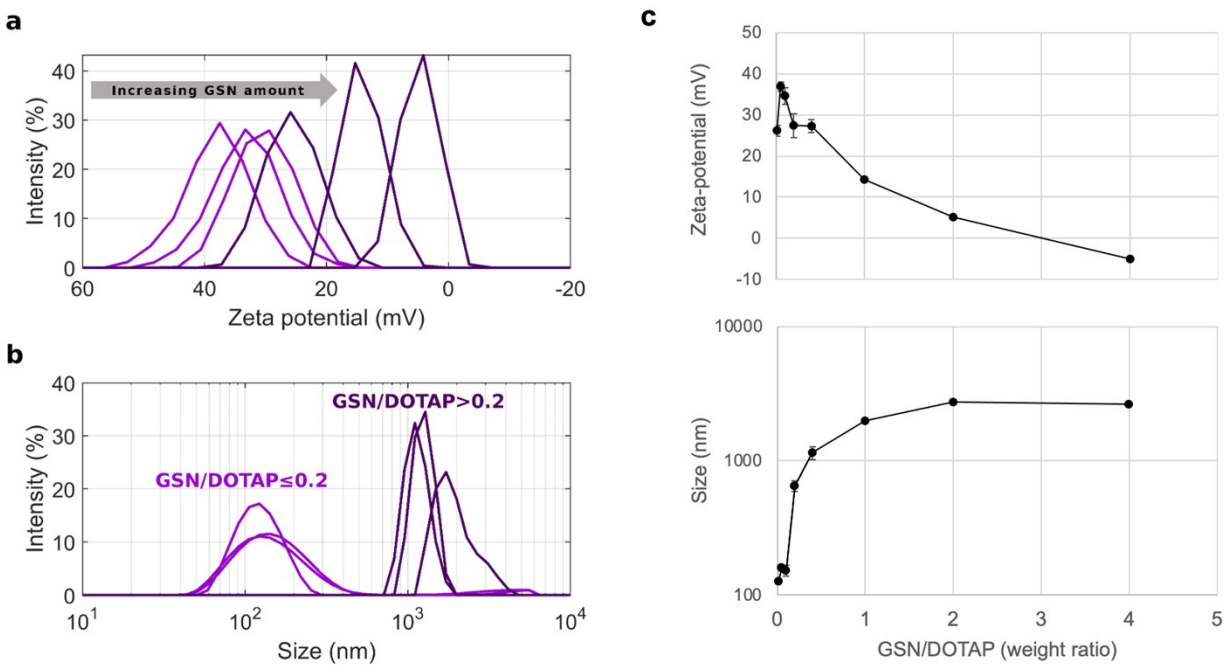

Fig. S1. Synthetic identity of DOTAP/GNS complexes as a function of the GSN/DOTAP weight ratio. (**a**) Representative zeta-potential distributions of DOTAP/GNS complexes at GSN/DOTAP weight ratios equal to 0,05, 0,1, 0,2, 0,4, 1, and 2 (from left to right). (**b**) Size distributions DOTAP/GNS complexes at GSN/DOTAP weight ratios equal to 0,01, 0,05, 0,1, 0,2, 0,4, 1, and 2 (from left to right). The coating data collapsed into two groups: 1) Low-density decoration (LD) for GSN/DOTAP weight ratio < 0,2 and corresponding to light purple distributions; 2) high-density decoration (HD) for GSN/DOTAP weight ratio > 0,2 and corresponding to deep purple distributions. (c) Zeta-potential and size values of DOTAP/GNS complexes as a function of the GSN/DOTAP weight ratio. Data are reported as the average of three independent measurements ± standard deviation.

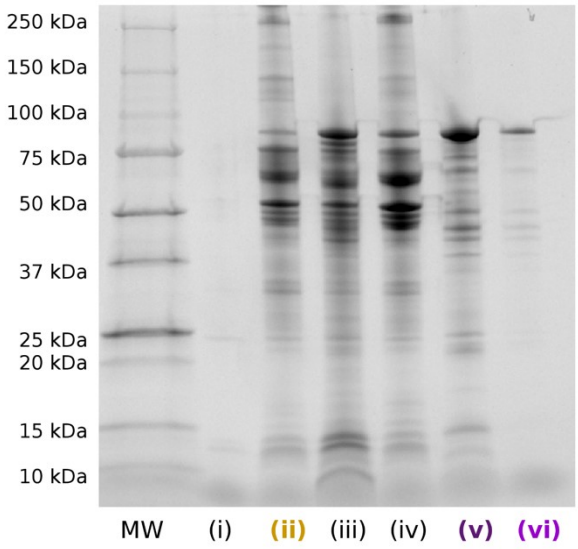

Figure S2. One-dimensional (1D) SDS-PAGE image of DOTAP-protein complexes.
i) Plasma sample (*i.e.*, not incubated with DOTAP) subjected to the same isolation procedure followed to isolate proteins from DOTAP. The empty lane indicates that the protein content of the "HP blank" sample is negligible with respect to that truly associated with cationic DOTAP. This is straight evidence that the centrifugation-based isolation method used in the present investigation resulted in minor if any, contamination by unbound proteins and biological NPs[50];
ii) DOTAP-HP complexes.
iii) High-density (HD) decoration DOTAP-GSN (GSN/DOTAP molar ratio = 1).
iv) Low-density (LD) decoration DOTAP-GSN (GSN/DOTAP weight ratio = 0,1).
v) HD DOTAP-GSN complexes exposed to HP for 1-hour at 37 °C.
vi) LD DOTAP-GSN complexes exposed to HP for 1-hour at 37 °C.
The Lane labeled with "MW" indicates the protein ladder.

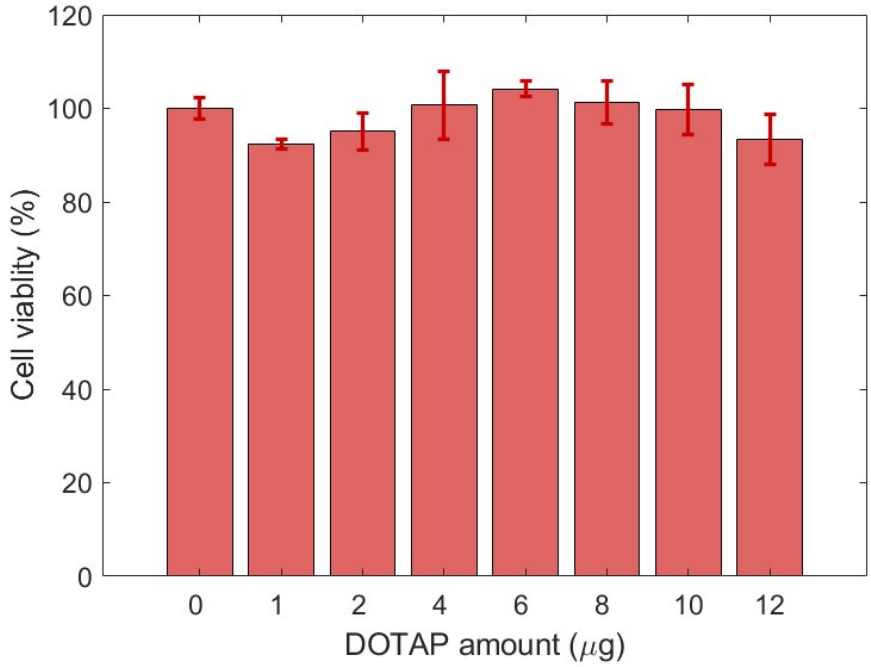

Figure S3. **Cell viability of human monocytic THP-1 cells as a function of lipid dose**. Cells were seeded on 96-well plates (50,000 cells/well) and were incubated with bare DOTAP at increasing amounts (1, 2, 4, 6, 8, 10, and 12 µg/well).

Cellular uptake of DOTAP-GSN in THP1-cells

Another explanation for the superior cell uptake of HD DOTAP-GSN could be that non-specific particle-cell interaction triggered internalization. NP size and charge can both contribute to non-specific uptake. Previous studies demonstrated that macrophages were able to efficiently recognize and internalize NPs with a diameter above 100 nm.34 However, because all the NPs used in the present study were larger than 100 nm (Table S1 of the ESI), we were unable to correlate changes in NP internalization with size-dependent non-specific internalization. A second putative non-specific uptake mechanism by macrophages might be mediated by charge effects. Several previous investigations clarified that NP uptake is promoted by cationic charge34, 35. However, uptake results reported here (Figure 2) did not correlate with particle zeta-potential (Table S1 of the ESI). Indeed, the two formulations that displayed the lowest cell uptake (*i.e.*, DOTAP and LD DOTAP-GSN) were both positive in charge. In conclusion, our in vitro findings suggest that neither the particle size nor the charge was good predictors of uptake efficiency.

Table S1. Following exposure to human plasma (HP) for 1 hour at 37°C, DOTAP, LD DOTAP-GSN, and HD DOTAP-GSN are coated by a protein corona of HP proteins that provides them with an identity expressed in terms of size, polydispersity index (pdI), and zeta-potential.

	Size (nm)	pdI	Zeta-potential (mV)
DOTAP/HP	163 ± 14	0,3	-22.1 ± 1.3
LD DOTAP-GSN/HP	182 ± 7	0,2	-28 ± 4
HD DOTAP-GSN/HP	318 ± 14	0,5	-13 ± 3.5

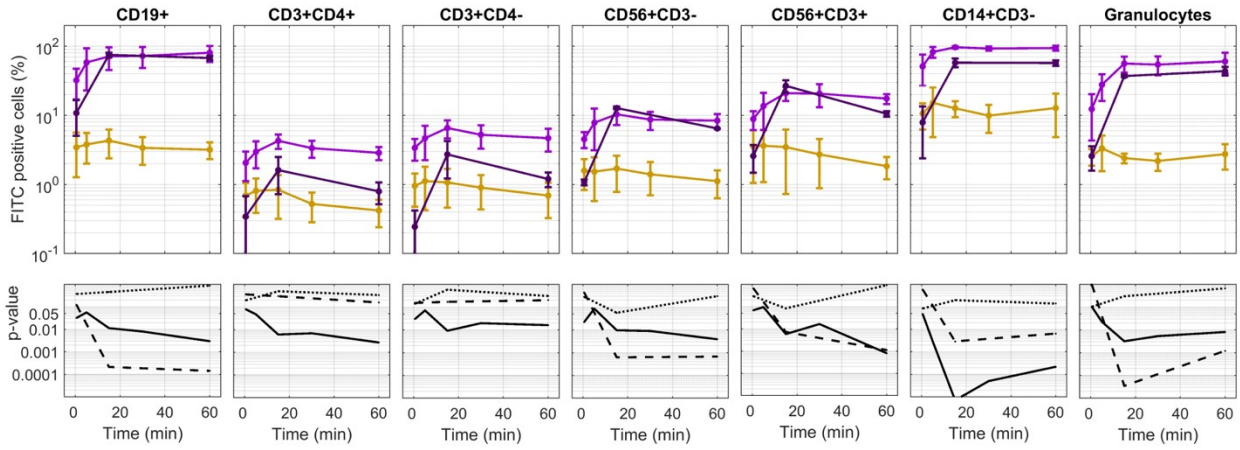

Figure S4. **Temporal evolution of leukocyte uptake**. DOTAP/HP (yellow), LD DOTAP-GSN/HP (light purple), HD DOTAP-GSN/HP (deep purple) were injected into whole blood and the leukocyte uptake was assessed as a function of time. The fluorescence signal of internalized nanoparticles was measured as the percentage of FITC-positive cells by gating on distinct leukocyte subpopulations. Data analysis was performed using FlowJo software, with results reported as mean ± standard deviation of three healthy donors. Statistical significance is reported as a p-value from Student's *t*-test as follows: DOTAP vs LD-DOTAP-GSN-HP (solid line); DOTAP vs HD-DOTAP-GSN-HP (dashed line); LD-DOTAP-GSN-HP vs HD-DOTAP-GSN-HP (dotted line).

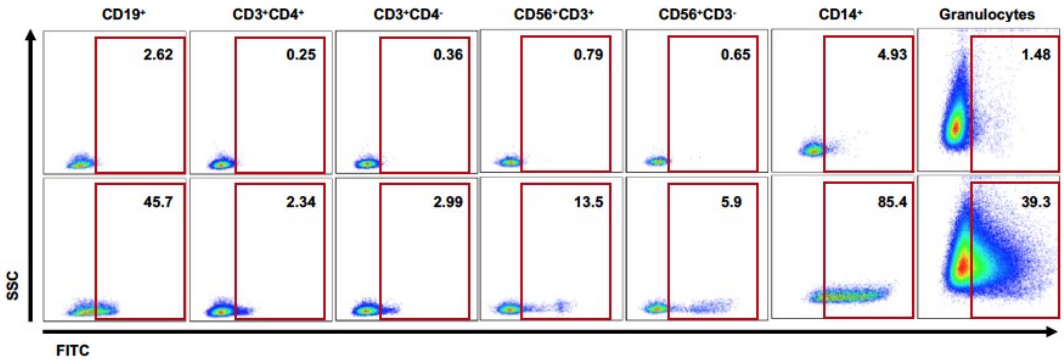

Figure S5. Leukocyte uptake of DOTAP/HP (top panels) and HD DOTAP-GSN/HP complexes (bottom panels) relative to a representative donor after 30 minutes of incubation. Data are shown as the percentage of FITC-positive cells in distinct leukocyte populations.

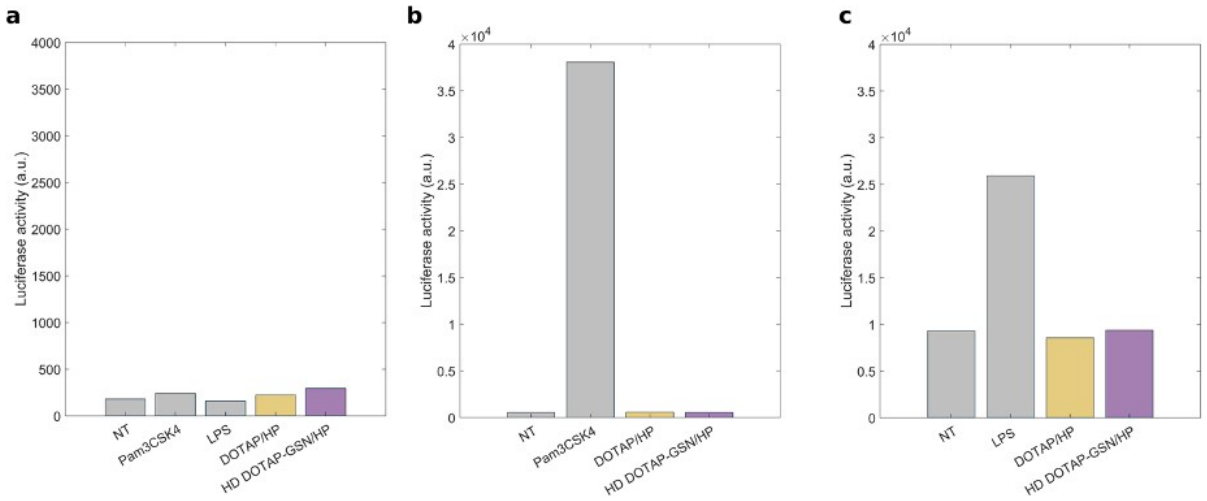

Figure S6. **DOTAP/HP and DOTAP-GSN/HP complexes do not trigger Toll-like receptor 2 (TRL2) and TLR 4 activation in reporter cells.** HEK293-NF-κB-luciferase reporter cells (a) transfected with TLR2 (b) or TLR4 (c) were incubated for 5 hours with DOTAP/HP, HD DOTAP-GSN/HP, and specific TLR2 or TLR4 agonists namely Pam3CSK4 and LPS, respectively. Data are expressed as NF-kB-induced luciferase activity (a.u.). NT: not treated. A representative experiment is shown.

Supplementary Table S2. Material characterization Question	**Yes**	**No**
1.1 Are "**best reporting practices**" **available** for the nanomaterial used?	Not applicable	
1.2 If they are available, **are they used**? If not available, 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) **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 (*e.g.*, proteins, small molecules, nucleic acids).	Not applicable	
1.9 Is the **targeting performance** of the nanomaterial reported, including **amount** of ligand bound to the nanomaterial if the material has been functionalised through addition of targeting ligands?	Not applicable	
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, has it been **quantified**?	Not applicable	
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 2. Biological	**Yes**	**No**

characterization Question		
2.1 Are **cell seeding details**, including **number of cells plated, confluency at start of experiment,** and **time between seeding and experiment** reported?	√	
2.2 If a standardised cell line is used, are the **designation and source** provided?	√	
2.3 Is the **passage number** (total number of times a cell culture has been subcultured) known and reported?	Not applicable	
2.4 Is the last instance of **verification of cell line** reported? If no verification has been performed, is the time passed and passage number since acquisition from trusted source (*e.g.*, ATCC or ECACC) reported? For information, see *Science* **347** (2015) 938; http://doi.org/10.1126/science.347.6225.938	No	
2.5 Are the results from **mycoplasma testing** of cell cultures reported?	No	
2.6 Is the **background signal of cells**/**tissue** reported? (*E.g.*, the fluorescence signal of cells without particles in the case of a flow cytometry experiment.)	√	
2.7 Are **toxicity studies** provided to demonstrate that the material has the expected toxicity, and that the experimental protocol followed does not?	Not applicable	
2.8 Are details of media preparation (**type of media, serum,** any **added antibiotics**) provided?	√	
2.9 Is a **justification of the biological model** used provided? For examples for cancer models, see *Cancer Res.* **75** (2015) 4016; http://doi.org/10.1158/0008-5472.CAN-15-1558, and *Mol. Ther.* **20** (2012) 882; http://doi.org/10.1038/mt.2012.73, and *ACS Nano* **11** (2017) 9594; http://doi.org/10.1021/acsnano.7b04855	√	
2.10 Is characterization of the **biological fluid** (*ex vivo*/*in vitro*) reported? For example, when investigating protein adsorption onto nanoparticles dispersed in blood serum, pertinent aspects of the blood serum should be characterised (*e.g.*, protein concentrations and differences between donors used in study).	√	
2.11 For **animal experiments**, are the ARRIVE guidelines followed? For details, see *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412	Not applicable	

Supplementary Table 2. Experimental details	**Yes**	**No**

Question		
3.1 For cell culture experiments: are **cell culture dimensions** including **type of well**, **volume of added media**, reported? Are cell types (*i.e.*; adherent *versus* suspension) and **orientation** (if non-standard) reported?	√	
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.e.*, using the dimensions and density of the nanomaterial)?	√	
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**?	√	
3.5 Is the methodology used to **equalise dosage** provided?	√	
3.6 Is the **delivered dose** to tissues and/or organs (*in vivo*) reported, as % injected dose per gram of tissue (%ID g_{-1})?	Not applicable	
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.	√	
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 *R. Soc. Open Sci.* **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	√	