

Supplementary figures and tables

Augmenting the immune response against a stabilized HIV-1 Clade C envelope trimer by silica nanoparticle delivery

David Peterhoff^{1*}, Stefanie Thalhauser², Jan M. Sobczak^{3,4}, Mona O. Mohsen^{3,4}, Christoph Voigt¹, Nicole Seifert¹, Patrick Neckermann¹, Alexandra Hauser¹, Song Ding⁵, Quentin Sattentau⁶, Martin F. Bachmann^{3,4,7}, Miriam Breunig² and Ralf Wagner^{1,8*}

¹ Institute of Medical Microbiology and Hygiene, University of Regensburg, 93053 Regensburg, Germany; david.peterhoff@ur.de (D.P.), ralf.wagner@ur.de (R.W.), christoph.voigt@stud.uni-regensburg.de (C.V.), nicole.seifert@uni-hohenheim.de (N.S.), patrick.neckermann@klinik.uni-regensburg.de (P.N.), alexandra.hauser@klinik.uni-regensburg.de (A.H.)

² Institute of Pharmaceutical Technology, University of Regensburg, 93053 Regensburg, Germany; stefanie.thalhauser@gmail.com (S.T.), miriam.breunig@chemie.uni-regensburg.de (M.Br.)

³ Department for BioMedical Research, University of Bern, 3010 Bern, Switzerland; jan.sobczak@dbmr.unibe.ch (J.S.), mona.mohsen@dbmr.unibe.ch (M.M.), martin.bachmann@dbmr.unibe.ch (M.Ba.)

⁴ Department of Immunology RI, University Hospital Bern, 3010 Bern, Switzerland

⁵ EuroVacc Foundation, 1002 Lausanne, Switzerland; song.ding@eurovacc.org (S.D.)

⁶ Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom; quentin.sattentau@path.ox.ac.uk (Q.S.)

⁷ Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

⁸ Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Regensburg, Germany; ralf.wagner@ur.de (R.W.)

* Correspondence: david.peterhoff@ur.de and ralf.wagner@ur.de; Tel.: +49 941 94416459 (D.P.), +49 941 9446452 (R.W.)

Table S1: Exemplary expression yields as purified by Ni-affinity chromatography from 293Expi expression supernatants

Variant	Expression yield per liter cell culture / mg
BG505-SOSIP	3.12
BG505-NFL	3.76
BG505-NtCC	4.19
ConC-SOSIP	18.30
ConC-NFL	24.41
ConC-NtCC	15.22

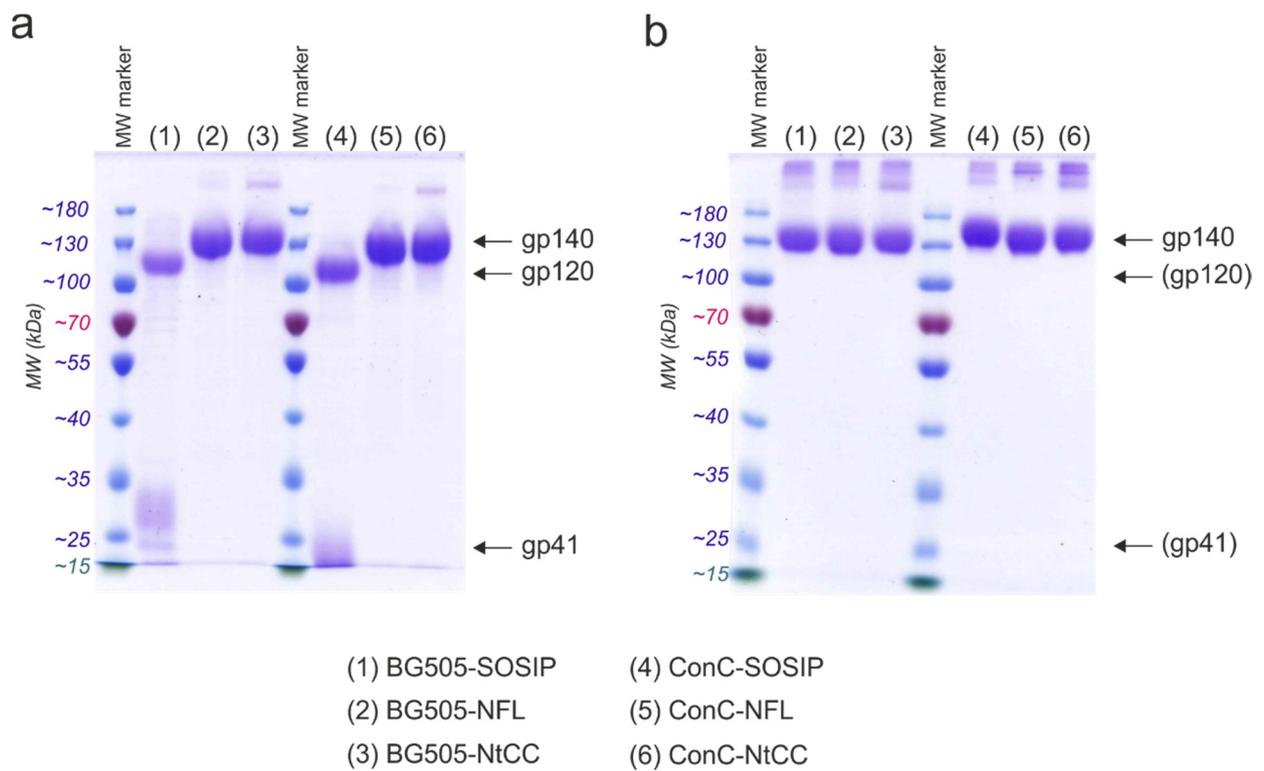


Fig. S1: SDS PAGE for all analyzed variants. 10% acrylamide gels were used. **(a)** reducing conditions; **(b)** non-reducing conditions. MW marker indicates sizes of protein bands. 5 μ g protein was loaded per lane.

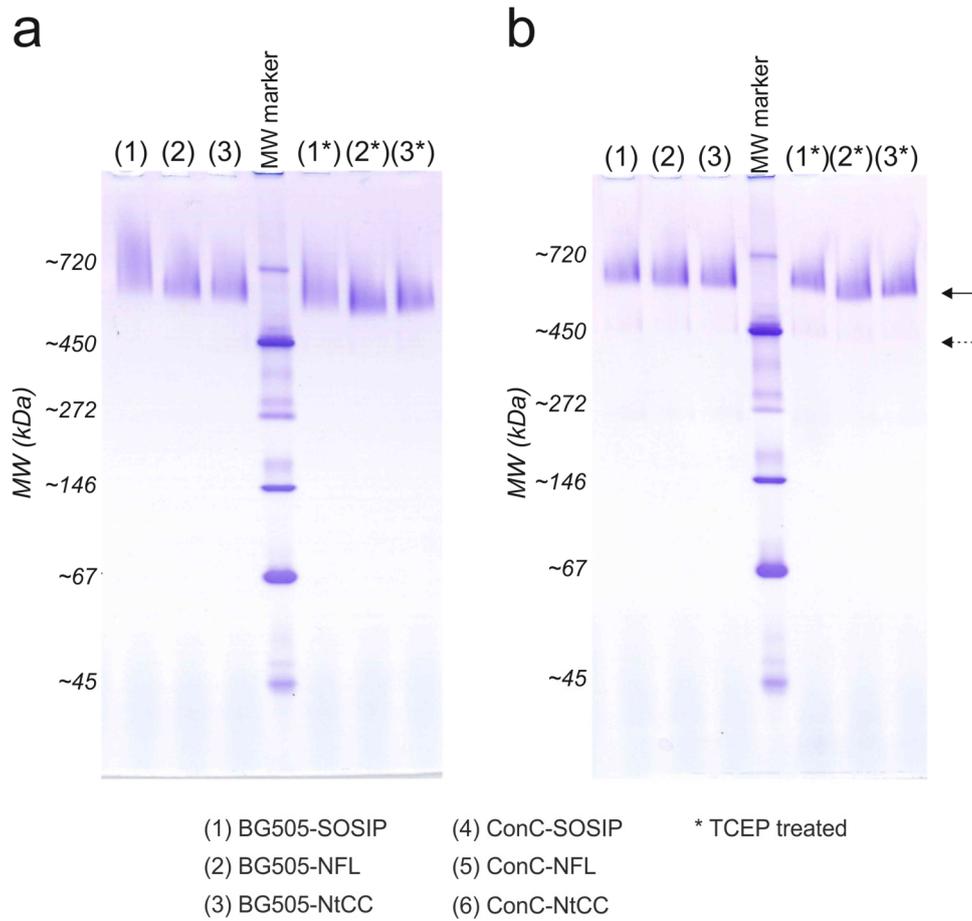


Fig. S2: Analysis of the influence of TCEP treatment to trimer integrity by Native PAGE.

To mimic the procedure during coupling of the protein to SiNPs, Env was treated with 1 mM TCEP for 1 h at ambient temperature, subsequently diluted to 0.5 mM TCEP and further incubated overnight at 4°C. 2.5 µg of protein was loaded on each lane of the 4–16% acrylamide native PAGE. ConC variants (**a**) and BG505 variants (**b**) were analyzed. Solid line arrow indicates trimer bands; dashed line arrow indicates dimer bands; MW marker indicates size of protein bands; asterisks mark TCEP treated samples.

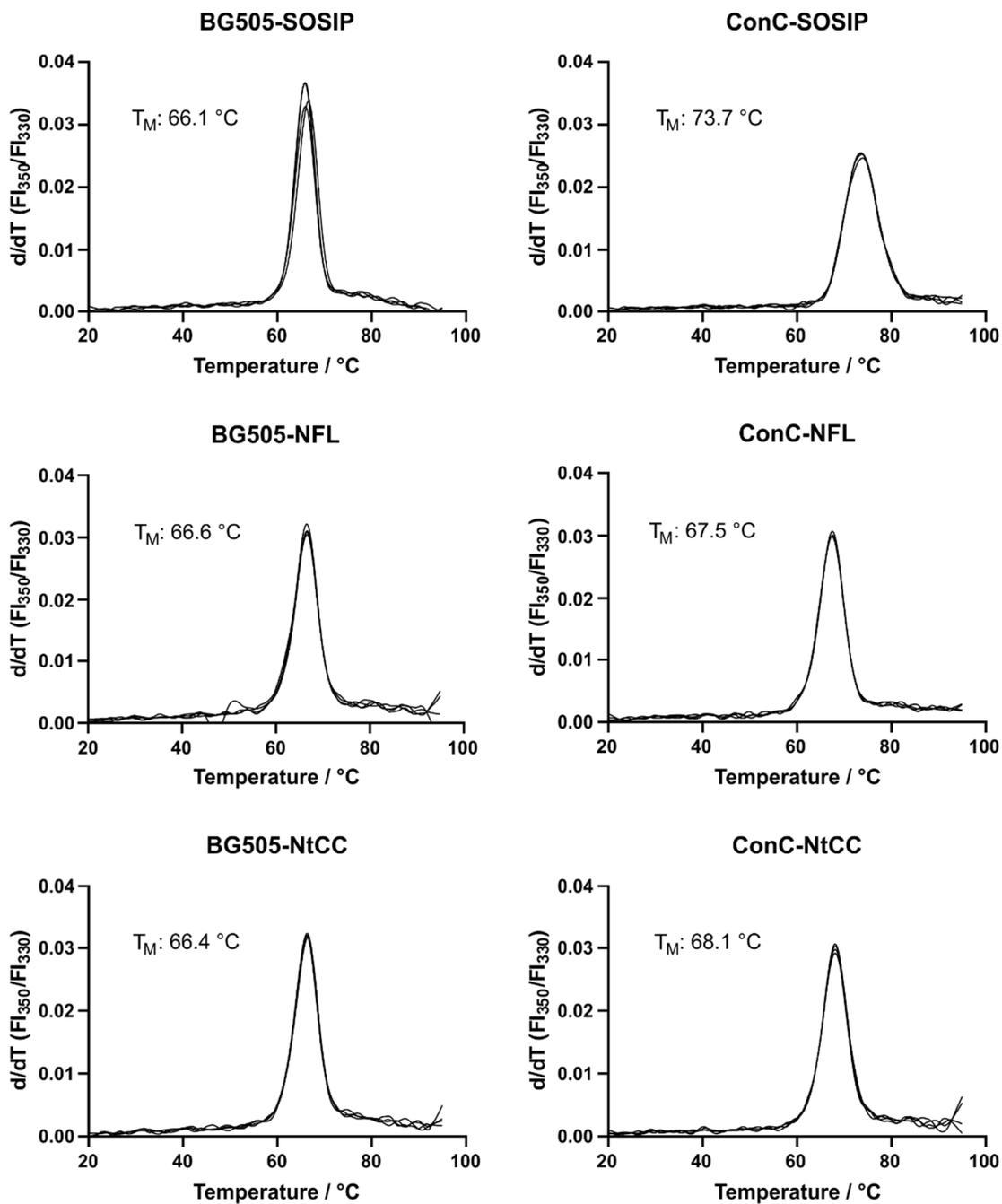


Fig. S3: Nano DSF analysis. 75 ng/mL protein in PBS was used for all measurements. Results from three replicate runs are shown. FI, Fluorescence intensity at 330 nm and 350 nm; T_M , mean melting temperature from the three replicates.

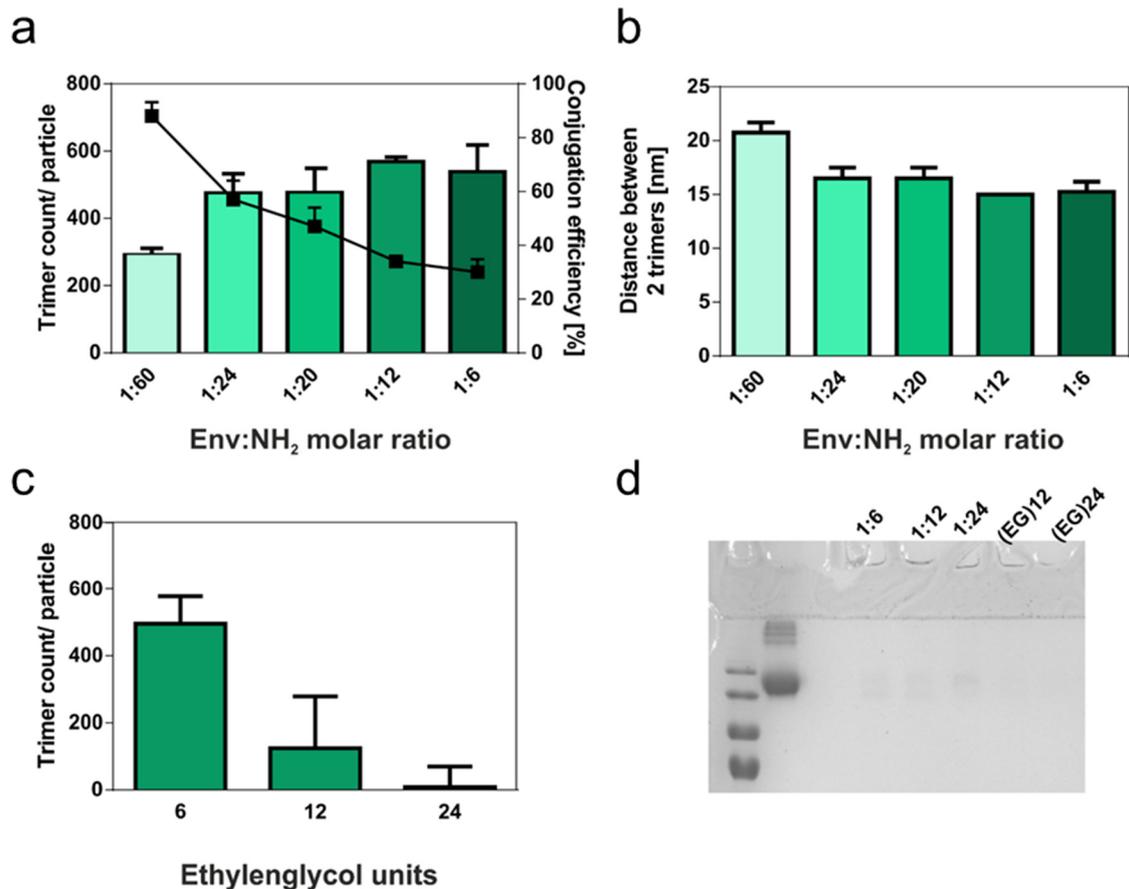


Fig. S4: Optimization of reaction conditions for the attachment of Env to 200 nm SiNPs. The number of trimers per particle (a) and distances (b) increased with increasing Env to NH₂ molar ratio corresponding to an increasing amount of Env added to the reaction, while conjugation efficiency decreased using SM(EG)₆ as linker. At molar ratios of 1:12 a sufficient Env loading of about 600 trimers per particle with acceptable conjugation efficiency of about 40% was achieved and was used for cell experiments. Applying a prolonged linker with a larger ethyleneglycol linker did result in lower Env attachment (c). Representative SDS PAGE of selected formulations. Lane 1: ladder, Lane 2: soluble Env, Lane 3: SiNPs, Lane 4-6: Env attached to SiNPs via SM(EG)₆ linker at different ratios. Lane 7+8: Env attached to SiNPs via different linkers. Only soluble Env was detected in the gel after Coomassie staining (d). Results are presented as mean ± standard deviation (n=4 samples).

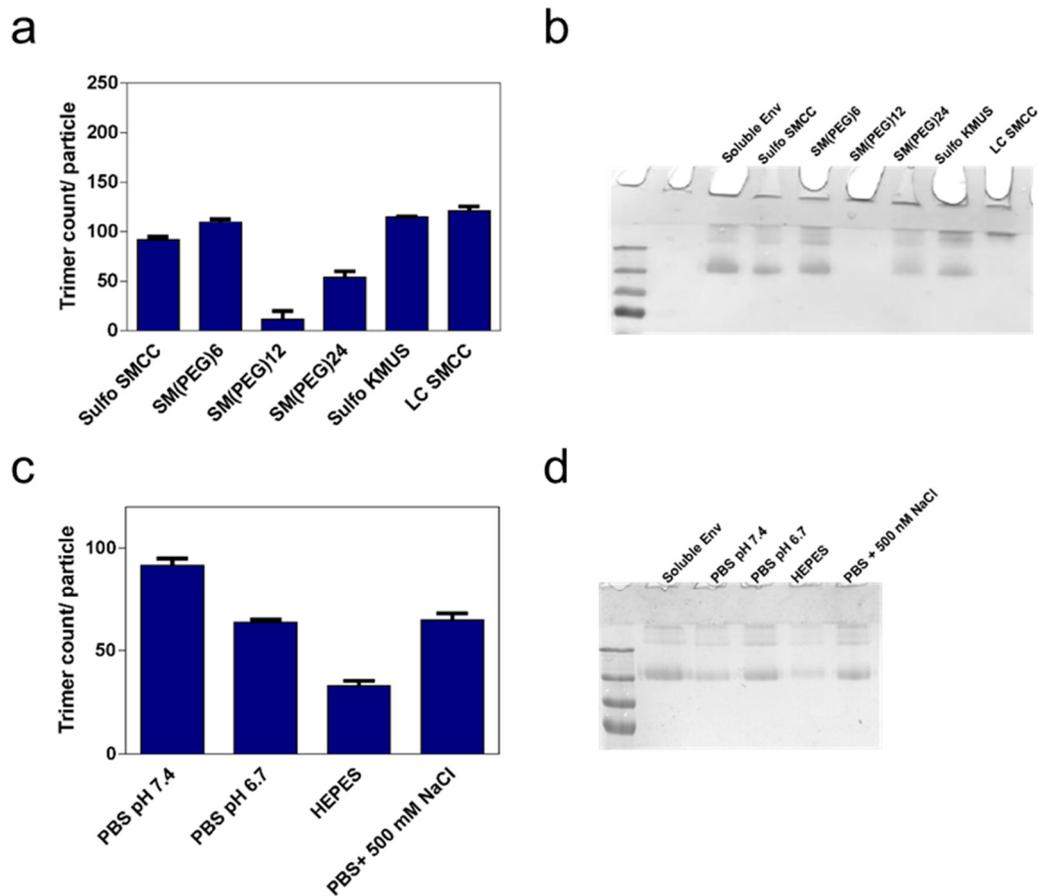


Fig. S5: Optimization of reaction conditions for the attachment of Env to 100 nm SiNPs.

For optimization of conjugation to 100 nm BG505 NFL-NtCC variants were used at an Env to NH₂ molar ratio of 1:3 Trimers per particle (a) after conjugation using Sulfo SMCC, SM(PEG)₆, SM(PEG)₁₂, SM(PEG)₂₄, Sulfo KMUS, LC SMCC. (b) SDS PAGE of BG505 NtCC attached to 100 nm SiNPs. BG505 amount was adjusted to 2µg. Lane 1: ladder, lane 2: SiNPs, lane 3: BG505 NtCC, lane 4: SiNP-(SulfoSMCC)BG505-NtCC, lane 5: SiNP-(PEG₆)BG505-NtCC, lane 6: SiNP-(PEG₁₂)BG505-NtCC, lane 7: SiNP-(PEG₂₄)BG505-NtCC, lane 8: SiNP-(Sulfo KMUS)BG505-NtCC, lane 9: SiNP-(LC SMCC)BG505-NtCC. SDS PAGE indicated strong non-specific adsorption during conjugation reaction. Among the applied linkers sulfo SMCC showed the lowest non-specific adsorption and was thus used for the subsequent experiments. (c) To decrease non-specific adsorption maleimide to thiol reaction was conducted in different media. Highest loading was achieved with PBS 7.4 while HEPES resulted in a decreased loading. (d) SDS PAGE of Env attached to 100 nm particles in different media. Coomassie staining indicated that with PBS 7.4 non-specific adsorption was reduced to about 20%. Hence, for subsequent experiments sulfo SMCC linker was used and as coupling medium PBS with pH 7.4 was used. Results are presented as mean ± standard deviation (n=4 samples).

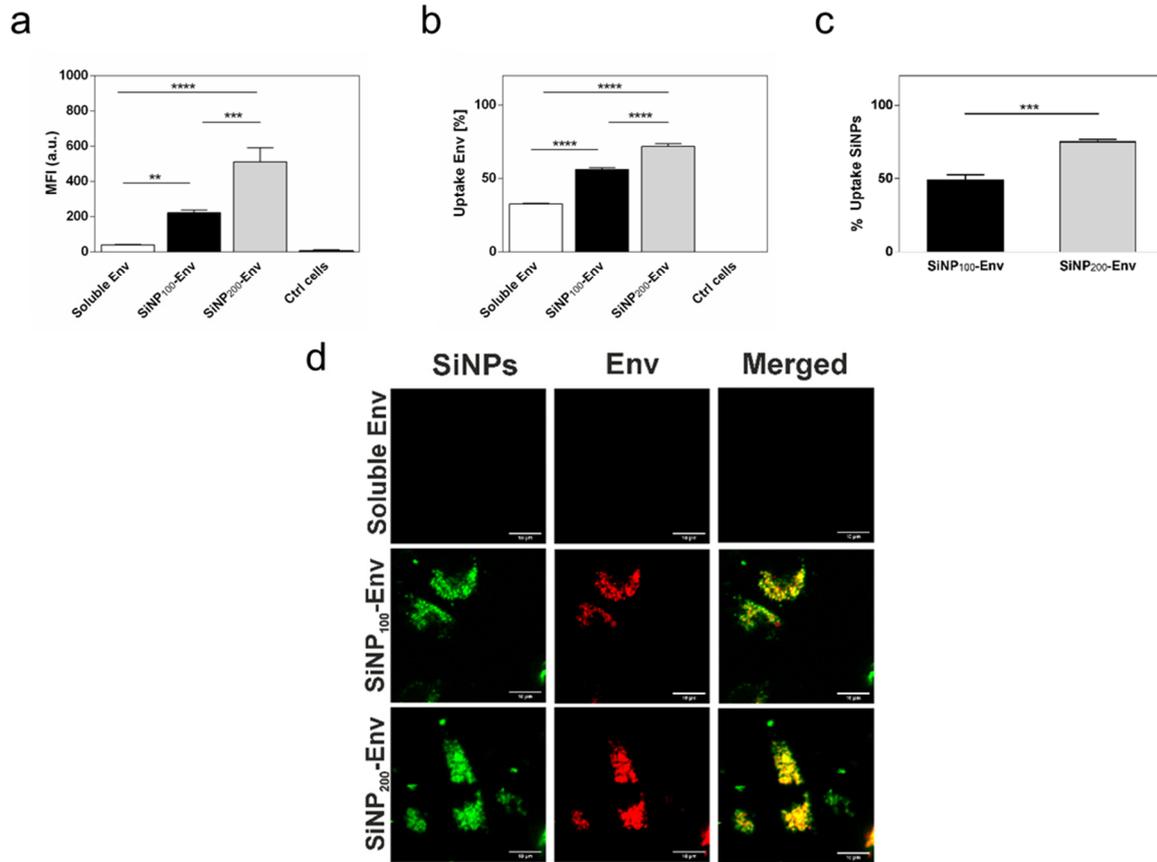


Fig. S6: Nanoparticle associated Env uptake by BMDCs. FACS analysis of the uptake of Env attached to SiNPs compared to the corresponding amount of soluble protein expressed as (a) mean fluorescence intensity (MFI) and (b) percentage of uptake. (c) Uptake of 100 nm SiNPs and 200 nm SiNPs by BMDCs. Results are presented as mean \pm standard deviation ($n=3$ samples, levels of statistical significance are indicated as $**p = 0.01$, $***p = 0.001$ and $****p = 0.0001$). (d) CLSM analysis of the uptake of Env attached to SiNPs compared to soluble protein. Co-localization of SiNPs (green) and Env (red) was indicated by the yellow spots of the merged channels (scale bar=10 μm).

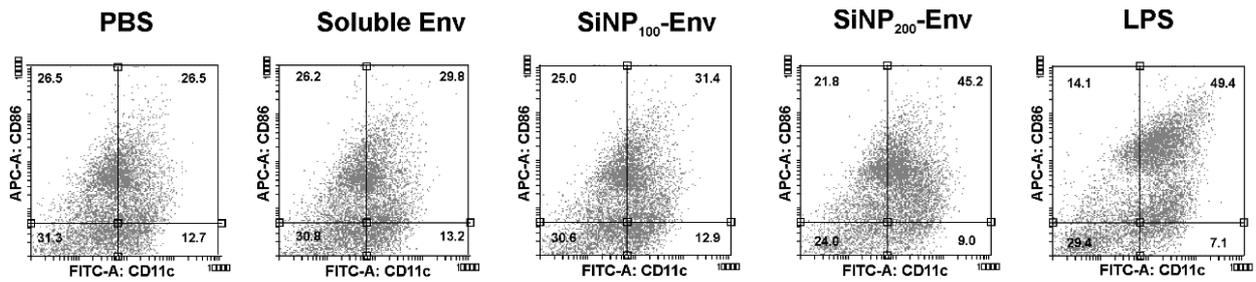


Fig. S7: Representative cytometry plots of the CD11c and CD86 phenotype of BMDC following activation.

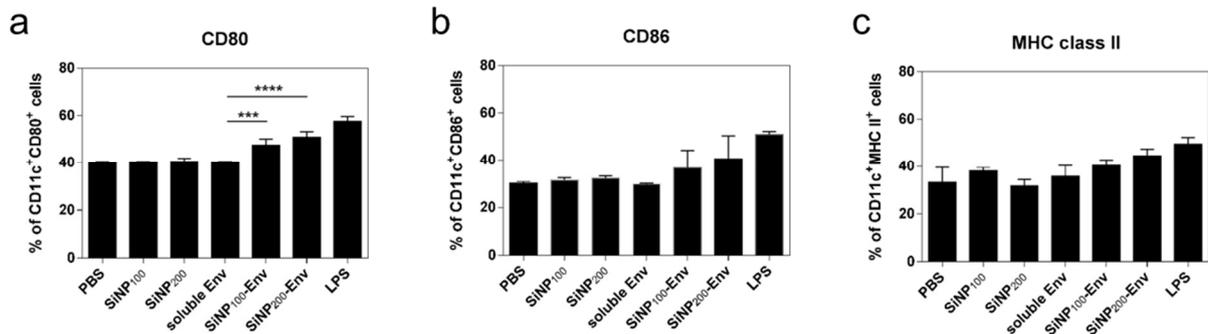


Fig. S8: Percentage of CD11c and CD80, CD86 and MHCII positive cells. (a)–(c) after stimulation of BMDCs for 16 hours. A slight increase of the amount of stimulated BMDCs was induced by a particulate Env presentation. Results are presented as mean \pm standard deviation ($n=3$ samples, levels of statistical significance are indicated as *** $p=0.001$ and **** $p=0.0001$).

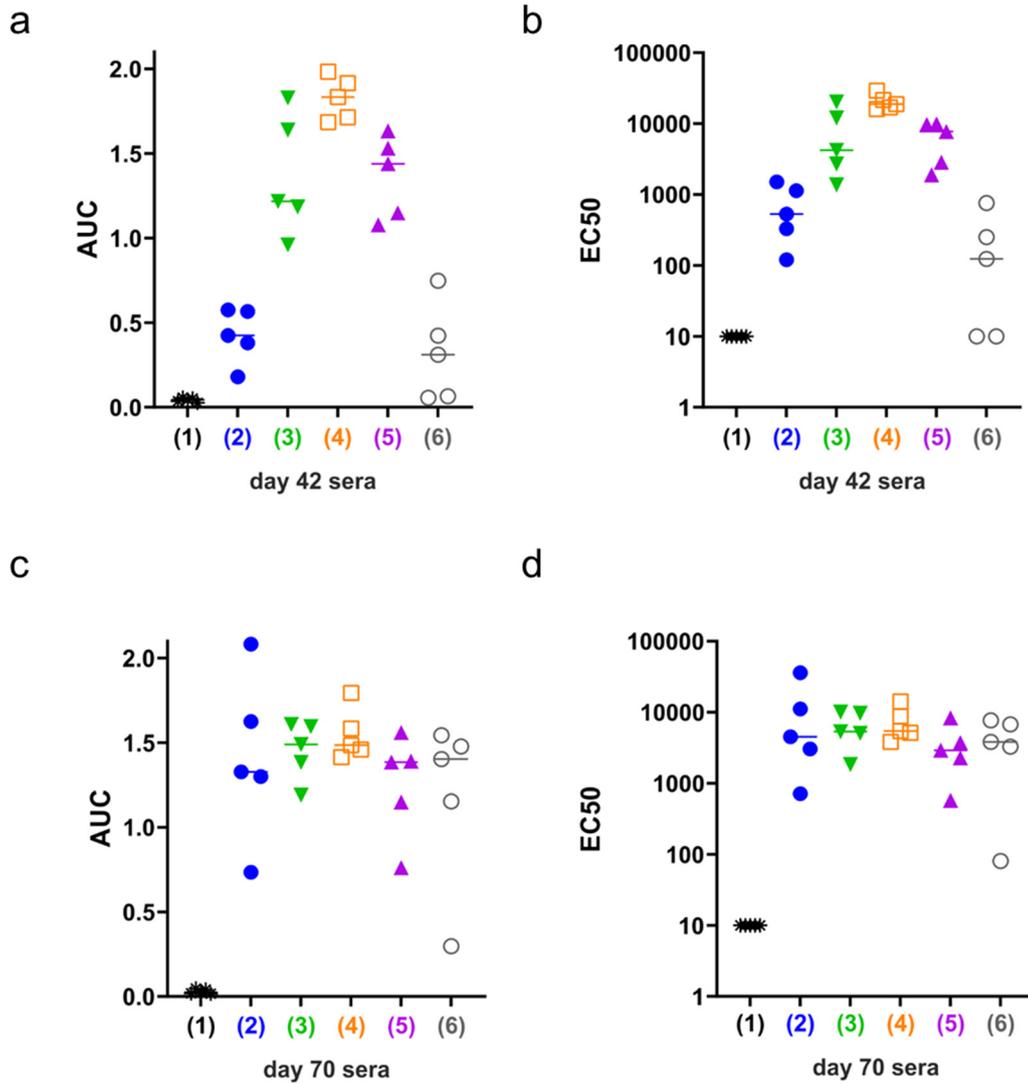


Fig. S9: Comparison between Area under curve (AUC) and Effective Concentration 50 (EC50) values. Results from the ELISA serum titration of each mouse, grouped per immunization group. (a)–(b) AUC and EC50 for bleed 3. (c)–(d) AUC and EC50 for bleed 4. Lines indicate the median for each group. Groups: (1), SiNP₁₀₀; (2), Env; (3), Env mixed with SiNP₁₀₀; (4), Env coupled to SiNP₁₀₀; (5), Env coupled to SiNP₁₀₀ at low dose; (6) Env coupled to SiNP₁₀₀ without adjuvant.

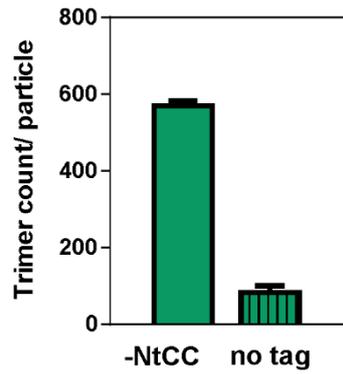


Fig. S10: Specificity of the coupling via the N-terminal tag. Env conjugation to SiNP₂₀₀ particles resulted in about 580 trimers per particle using Env with an N-terminal cysteine tag (NtCC), while Env without an N-terminal tag resulted only in 70 trimers per particle, indicating that the conjugation was formed between maleimides on the particle surface and thiols of the cysteine-tag. Results are presented as mean \pm standard deviation (n=4 samples).

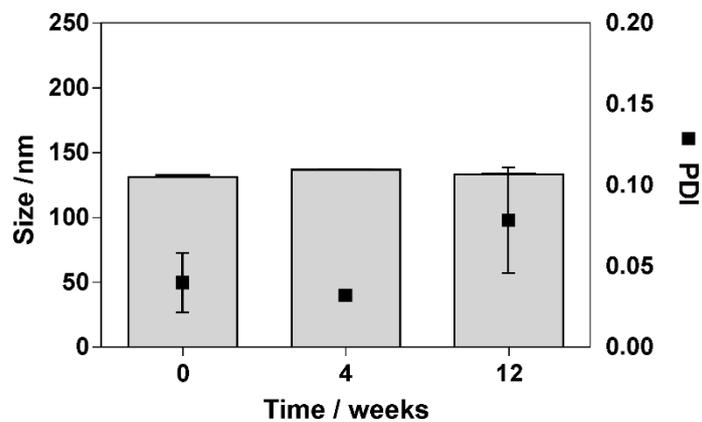


Fig. S11: SiNP₁₀₀-Env showed colloidal stability over a period of 12 weeks at 4°C. Results are presented as mean \pm standard deviation (n=3 measurements).

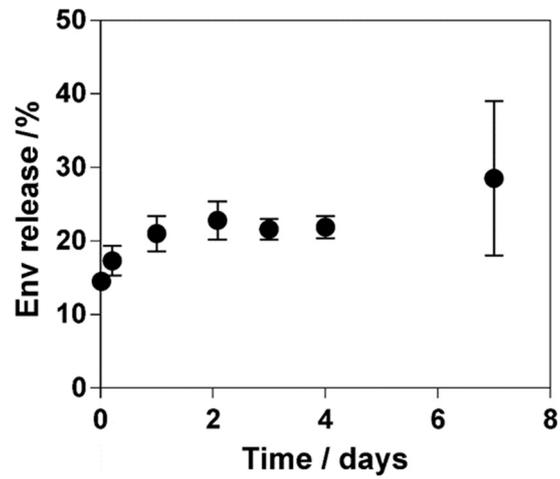


Fig. S12: Release of Env adsorbed to SiNP₂₀₀ particles. After an initial burst of about 15%, release slowed down reaching 30% after seven days in PBS at 37°C. Results are presented as mean \pm standard deviation (n=3 samples).