Supplemental Information

High-density array of well-ordered HIV-1 spikes on synthetic liposomal nanoparticles that efficiently activate B cells

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Methods

Binding analysis of selected mAbs to JRFL SOSIP

Binding interactions between selected trimer-preferring and CDbs-directed antibodies to JRFL SOSIP trimers were examined by biolayer light interferometry (BLI) using Octet Red system (ForteBio). The mAbs were captured on the surface of the anti-human Fc sensors from a solution of 5μg/ml in PBS for 60s at 1,000 rpm. Bio-sensors were then immersed in a solution of the JRFL SOSIP trimers (diluted to 200nM) for 600s at 1,000 rpm to allow association of the immobilized antibodies with the analyte. Association was followed by dissociation in PBS for 600s at 1,000 rpm.

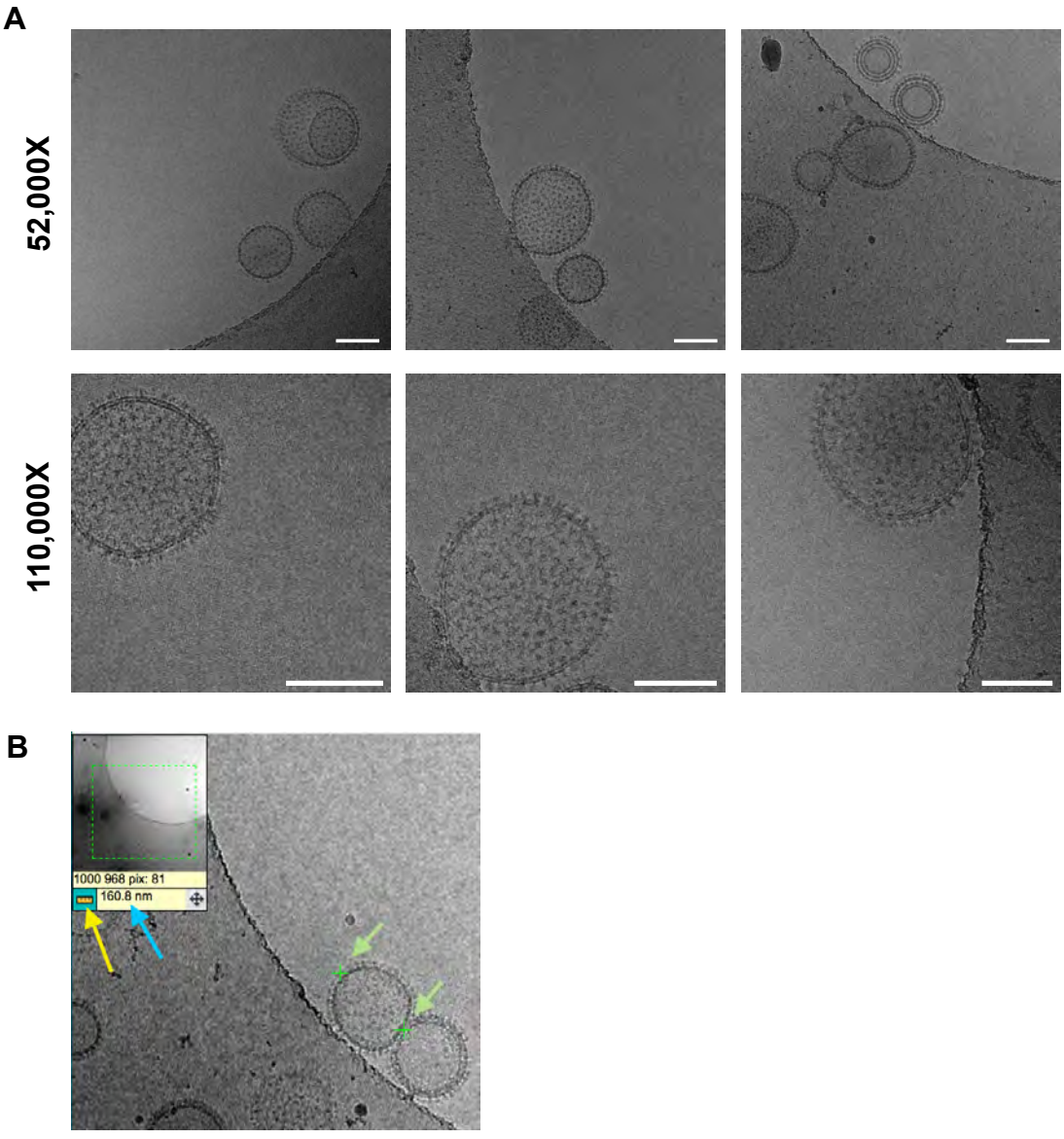
Electron Microscopy

JRFL SOSIP-conjugated liposomes were mixed at a 10% (v/v) with ISCOMATRIX and Adjuplex and incubated at 37°C for 1h. 5μl of the mixture was stained with phosphor tungstate on carbon-coated Cu grids. The grids were examined on a Philips CM100 electron microscope. (FEI, Hillsbrough OR) at 80kV and images were acquired with a Megaview III charge-coupled device (CCD) camera.

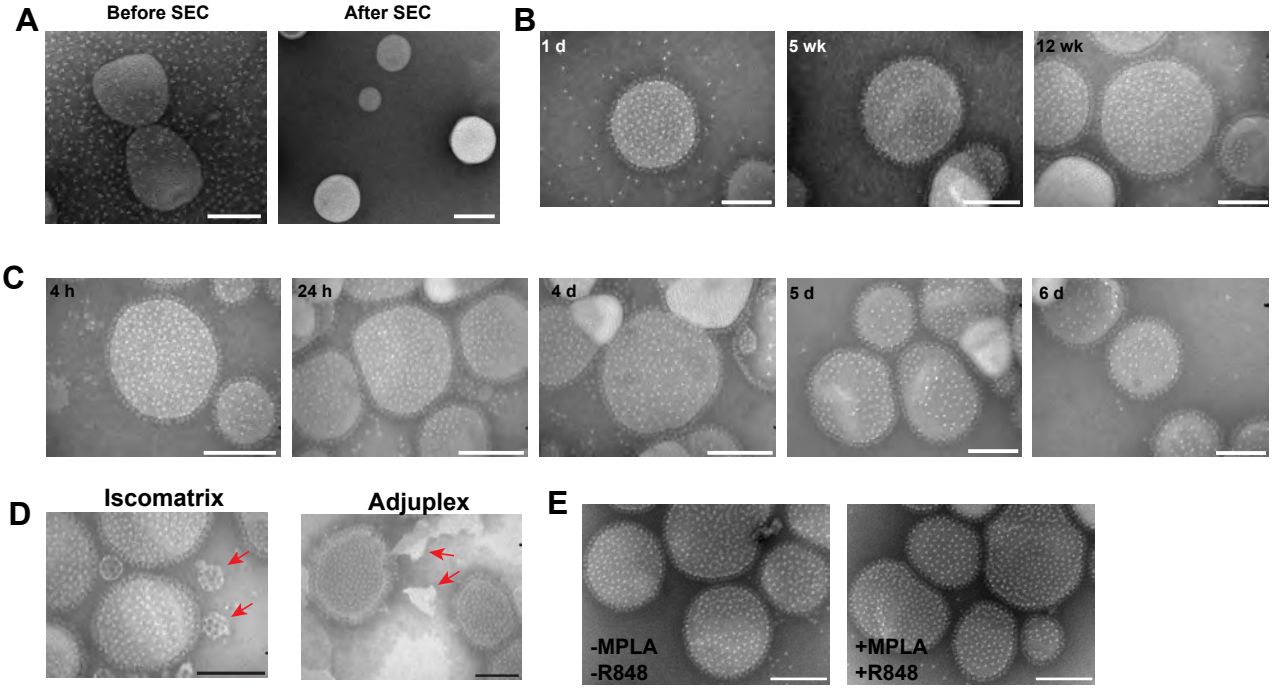
Avidity Measurements

For determination of antibody avidity, the ELISAs were performed as described previously, with an additional sodium isothiocynate (NaSCN) washing step. After incubation with sera, the plates were washed and incubated for 15 min with 1.5M NaSCN in PBS, while duplicate plates were incubated with an equal volume of PBS in parallel. The plates were then washed again to remove dissociated antibody. Detection of bound

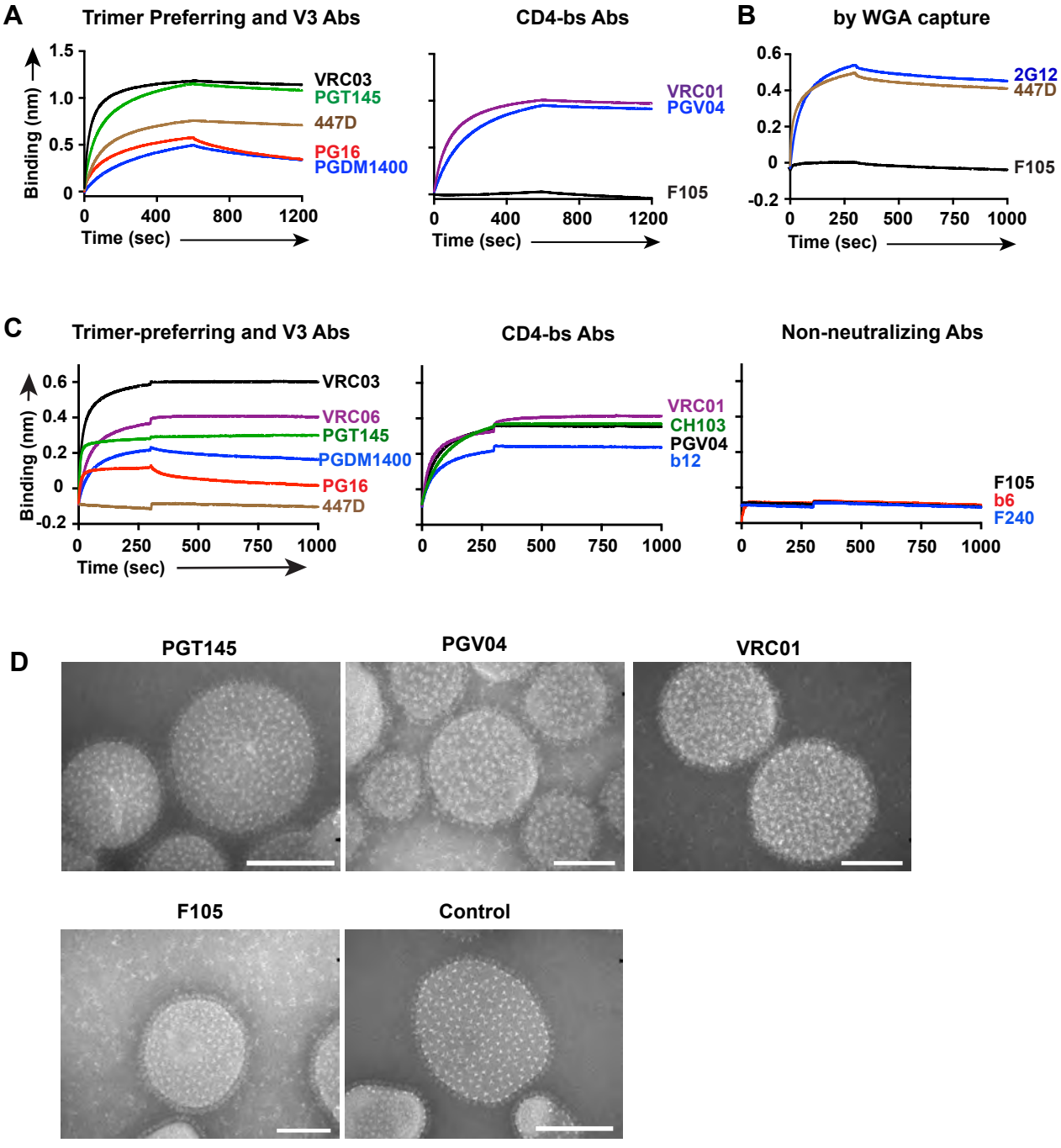
antibody was performed as described in the Methods of the main text. The avidity index was determined by calculating the (ED_{50} value with NaSCN treatment/ED_{50} value without NaSCN treatment)X100. P values were calculated using the two-tailed unpaired *t*-test with 95% confidence interval. Significance was defined as $P<0.05$.



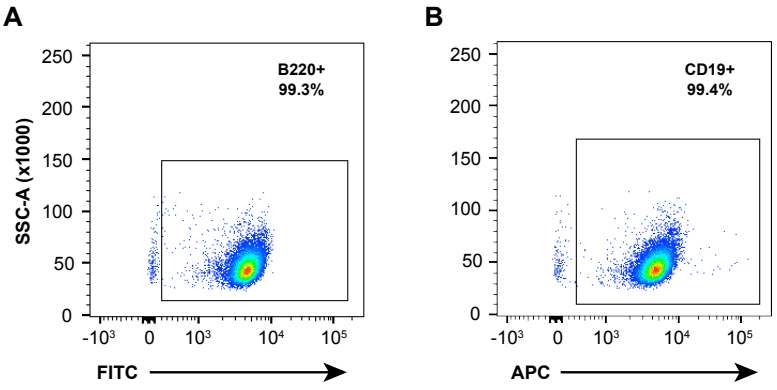
Supplemental Figure 1. Characterization of JRFL NFL-conjugated lipo-somes. (A) Cryo-electron microscope images of 4% Ni JRFL NFL-conjugated lipo-somes at 52,000 and 110,000X magnification. Scale bar = 100 nm. (B) Cryo-elec-tron microscope image measuring the diameter of the JRFL NFL-conjugated lipo-somes. Related to Figure 2.



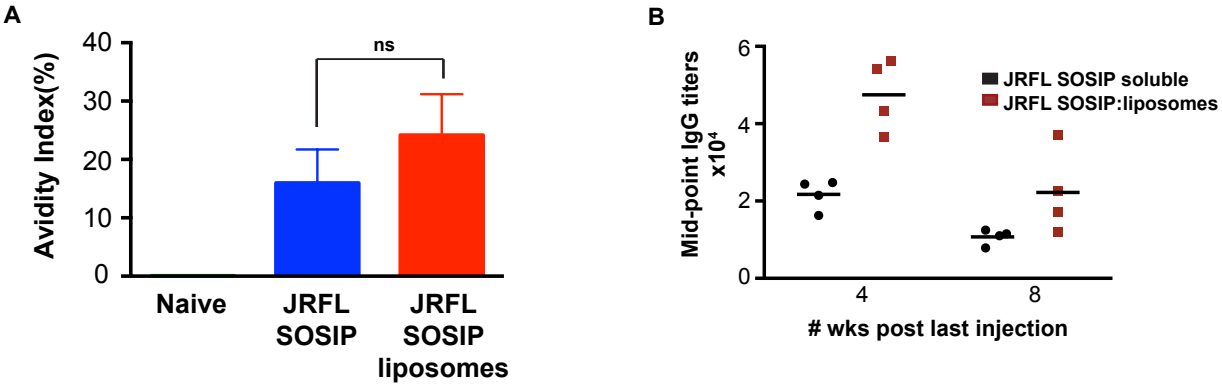
Supplemental Figure 2: Conjugation specificity and stability of liposomes. (A) Representative negative stain EM images of DGPC liposomes with no DGS-NTA(Ni) mixed with JRFL SOSIP trimer before and after size exclusion chromatography. (B) 2% DGS-NTA(Ni) liposomes with JRFL NFL trimeric protein were incubated at 4°C or (C) 37°C for varying times as indicated on the images and stained by phospho-tungstate for EM analysis. (D) 4% Ni DGPC liposomes with JRFL SOSIP trimeric protein were mixed and incubated at 37°C for 1 hour with Iscomatrix or Adjuplex and stained by phospho tungstate for EM analysis. Red arrows indicate the adjuvant in both cases. (E) 4% Ni DGPC liposomes without and with MPLA and R848 conjugated with JRFL SOSIP trimers. Scale bar = 100 nm. Related to Figure 3.



Supplemental Figure 3: Binding of HIV-1 antibodies to soluble JRFL SOSIP and liposome bound JRFL NFL. Binding of anti-HIV-1 monoclonal antibodies assessed by Bio-Layer Interferometry (BLI) using Octet. (A) Monoclonal antibodies were immobilized on human anti-Fc sensors and soluble JRFL SOSIP protein was used as an analyte. (B) JRFL NFL (10 μg/ml) was immobilized on WGA-captured streptavidin sensors and 20 μg/ml monoclonal antibodies (IgGs) were used as analyte. (C) 4% NTA-Ni liposomes (equivalent to 75 nmoles of phospho-lipids) conjugated to JRFL NFL were immobilized on WGA-captured streptavidin sensors and 20 μg/ml monoclonal antibodies (IgGs) were used as analyte. (D) 2% NTA-Ni liposomes with JRFL NFL were incubated with 10 molar excess of respective antibodies (IgG) at 37°C for 30 min and stained with phospho tungstate and viewed by EM. All images are at 180,000 magnification. Scale bar = 100 nm. Related to Figure 4.



Supplemental Figure 4. Purity of isolated B cells. Splenocytes from b12 knock-in mice were nega-tively selected for B cells and stained for cell surface markers (A) B220 and (B) CD19. Related to Figure 5.



Supplemental Figure 5. Immunogenicity of JRFL SOSIP trimer-conjugated liposomes. (A) Immunizations with JRFL SOSIP:liposomes elicit antibodies with higher avidity than soluble protein. New-Zealand white rabbits were immunized 4 times with 25 ug JRFL SOSIP protein as soluble or conjugated to 4% Ni DGPC liposomes**.** Sera after the 3rd boost was analyzed by ELISA with sodium isothio cynate (NaSCN) treatment for avidity measurements. Percentage avidity index is defined as (ED_{50} value with NaSCN treatment/ED_{50} value without NaSCN tratment)x100. *P* values were calculated with two-tailed unpaired t test. (B) Mid-point IgG titers of rabbits after 4 and 8 weeks post fourth inoculation analyzed by ELISA with JRFL SOSIP trimers captured on plate via the C-terminal His_6tag. Related to Figure 7.