Synthetic virions reveal fatty acid-coupled adaptive immunogenicity of SARS-CoV-2 spike glycoprotein

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SARS-CoV-2 infection is a major global public health concern with incompletely understood pathogenesis. The SARS-CoV-2 spike (S) glycoprotein comprises a highly conserved free fatty acid binding pocket (FABP) with unknown function and evolutionary selection advantage1,2. Deciphering FABP impact on COVID-19 progression is challenged by the heterogeneous nature and large molecular variability of live virus. Here we create synthetic minimal virions (MiniVs) of wild-type and mutant SARS-CoV-2 with precise molecular composition and programmable complexity by bottom-up assembly. MiniV-based systematic assessment of S free fatty acid (FFA) binding reveals that FABP functions as an allosteric regulatory site enabling adaptation of SARS-CoV-2 immunogenicity to inflammation states via binding of pro-inflammatory FFAs. This is achieved by regulation of the S open-to-close equilibrium and the exposure of both, the receptor binding domain (RBD) and the SARS-CoV-2 RGD motif that is responsible for integrin co-receptor engagement. We find that the FDA-approved drugs vitamin K and dexamethasone modulate S-based cell binding in an FABP-like manner. In inflammatory FFA environments, neutralizing immunoglobulins from human convalescent COVID-19 donors lose neutralization activity. Empowered by our MiniV technology, we suggest a conserved mechanism by which SARS-CoV-2 dynamically couples its immunogenicity to the host immune response.

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Intriguingly, in A549 epithelial and HUVEC cells, we observed noticeable intracellular uptake of the MiniVs. To quantify S-mediated binding of MiniVs over time in high throughputs, we developed retention assays based on quantification of MiniV fluorescence (see methods) and compared MiniV retention to naïve SUVs, lacking S on the surface (Fig. 1g–i). S significantly increased vesicle binding over the observation time period. These MiniV interaction kinetics are in accordance with previous findings for natural hCoV virions.23 Increased binding was also observed for MiniVs presenting only a recombinant S1 domain (V16-R685) and with S ectodomains of other pathogenic hCoVs, e.g., SARS-CoV and MERS-CoV (Fig. 1j). We further performed competition assays between SARS-CoV-2 viruses and MiniVs. ACE2-expressing A549 human lung epithelial cells24 were pre-incubated with MiniVs and SARS-CoV-2 infection efficiency was measured by qRT-PCR amplification of Orf7a mRNA. We found that MiniVs are able to competitively block SARS-CoV-2 infection, underscoring the similarities in cell and receptor tropism between MiniVs and natural SARS-CoV-2 virions.

We next incubated MiniVs with ACE2-expressing human epithelial MCF-7 cells,22 A549 human alveolar basal epithelial cells and primary human umbilical vein endothelial cells (HUVEC). Interactions between MiniVs and cells were assessed by confocal microscopy via imaging fluorescent rhodamine lipids integrated into the MiniV membrane. Following the viral particle concentration found in the sputum and the upper respiratory track during SARS-CoV-2 infection, we applied 2.4 × 10^8 MiniVs mL^-1 as measured by nanoparticle tracking analysis. Initial single MiniV binding to the target cell membrane was observed after only 10 min (Fig. 1e and Movie S2). Longer incubation for several hours resulted in excessive binding of the MiniVs to the cells (Fig. 1f and Movie S3).

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SARS-CoV-2 virions (see Fig. S4). Comparing the retention of D614GS and S variants of the newly emerged B1.1.7 Variant of Concern (UK or alpha mutant), we observed increased binding for B1.1.7 MiniVs, which is in accordance with higher ACE2 affinity (Fig. 1k). Moreover, confocal microscopy assessment showed increased multiplicity of attachment for B1.1.7 MiniVs (see Fig. S5). Taken together, MiniVs mimic the binding of natural SARS-CoV-2 viruses to target cells, faithfully simulating an early event in COVID-19. Moreover, MiniVs represent a modular approach to adaptively mimic and study different hCoV S mutational variants and strains. Therefore, we applied MiniVs to systematically study FABP-regulated events in initial cell-binding.

**FABP regulates S to cell-binding.** MiniVs allowed for a systematic assessment of changes in S-mediated cell interactions as a
function of FABP occupancy. Because recombinant native S binds on a priori undefined set of FFAs during expression and purification, we first generated FFA-depleted S (ApoS) by treatment of the purified native S samples with alkoxylated lipophilic columns (see Methods and Table S1). Compared to the native S, ApoS-decorated MiniVs displayed increased binding to human epithelial cells (Fig. 2a). This is consistent with a model where FFAs lock a closed RBD conformation, thereby reducing exposure of the receptor-binding motif (RBM). We then performed controlled loading of ApoS-MiniVs by incubating them with FFAs of differing length and saturation (i.e., palmitic acid (PA), oleic acid (OA), LA, and arachidonic acid (AA)). MiniVs were loaded with 2× molar excess of FFAs with respect to FABPs in S, equaling 1 µM, which is comparable to the FA levels in sera of COVID-19 patients. Based on the nanomolar affinity of S for FFAs, this concentration is expected to result in complete saturation of all binding pockets as partially supported by our mass spectrometry quantification (see below). Our choice of FFAs was based on an analysis of the most prominent changes in lipidomic profiles during COVID-19 infection. The binding of OA, LA, and AA, but not PA, in S was successfully verified by multiple reactions monitoring LCMS/MS (see Table S1). Of note, the addition of saturated PA did not significantly reduce MiniV-cell-binding compared to ApoS. However, we found that the (poly)-unsaturated FFAs OA, LA and AA were able to reduce MiniV binding compared to ApoS-MiniVs (Fig. 2a–c and Fig. S6). This is consistent with the FABP tube-like structure that features a kinked hydrophobic pocket accommodating cis-bond unsaturated FFAs and our MS analysis of FABP-binding by S. Importantly, when applying MiniVs presenting only the S1 domain without trimerization site, incubation with polyunsaturated FFAs did not significantly impact on cell-binding (see Fig. S7a). This is in agreement with the FABP structure, where a second adjacent RBD in the trimer is required to stabilize FABP-binding by coordinating the hydrophilic carboxy-group. Treatment of cells with 1 µM soluble FFAs alone, did not significantly decrease the binding of SUVs, demonstrating that FFAs directly act on S-mediated receptor interactions and that changes in MiniV binding are not based on cellular phenotypic alterations (see Fig. S7b). Moreover, loading of MiniVs with FFAs did not substantially change their zeta potential, indicating no alterations in charge-based MiniV interactions due to incorporation of the negatively charged FFAs into the SUV bilayer (see Fig. S7c). Upon initial S-mediated binding of SARS-CoV-2 to the target cell, S is cleaved by cellular membrane proteases (e.g., TMPRSS2) within the S2 domain. This induces fusion of the viral envelop with the target cell membrane, a process that is blocked by the serin protease inhibitor camostat mesylate. Interestingly, we did not measure any significant difference in the retention of FFA-loaded MiniVs upon camostat mesylate incubation (see Fig. S8). This indicates that the FABP is most crucial for the regulation of initial cell-binding rather than for S post-translational modification, fusogenic transformation and envelop fusion. Taken together, these results demonstrate that the FABP impacts S-mediated cell-binding, presumably via changes in the open-to-closed RBD equilibrium.

SARS-CoV-2 has 76% sequence identity to SARS-CoV and significantly higher infectivity, which is attributed to increased ACE2 binding affinity, more elaborate S posttranslational processing and newly emerged cell entry mechanisms. Among others, SARS-CoV-2 acquired a K403R mutation that introduces a RGD motif into the RBD nested next to the receptor-binding motif (Fig. 2d). This motif has been suggested to recruit cell surface integrins as co-receptors, potentially contributing to the increased infectivity compared to SARS-CoV. In our locked LA-bound S structure, the RGD motif is located above the FFA hydrophilic head supporting arginine residue, suggesting a FABP-regulated exposure (Fig. 2e). In order to assess the functional contribution of the RGD motif, we produced a SARS-CoV-2 SR403A. We found that S(R403A)-presenting MiniVs displayed significantly reduced cell-binding compared to native S MiniVs (Fig. 2f). This suggests that the RGD motif directly contributes to S-based cell-binding. To further verify a potential contribution of the RGD motif, we performed integrin blocking experiments with linear RGD (linRGD) peptides (see Fig. S9a, b). Incubation of native S MiniVs with 20 µM linRGD reduced binding to levels comparable to S(R403A) MiniVs. Importantly, integrin blocking did not affect the binding of MiniVs presenting SARS-CoV-2 S (see Fig. S9c). This indicates that SARS-CoV-2 S can engage integrins for cell entry, although the RGD motif is located proximal to the receptor-binding motif, suggesting a more sequential, rather than simultaneous binding of integrins and ACE2 (see Fig. S10) as observed for other enveloped virus (e.g., integrin β1, for mammalian reovirus). To assess if the FABP also regulates S-integrin binding, we measured changes in the binding efficiency of FFA-loaded ApoS-MiniVs blocked with linRGD (Fig. 2g). For this, we measured the native S-normalized differences in retention between linRGD-blocked cultures and control cultures incubated with MiniV that were loaded with FFAs (see methods for normalization of retention). As suggested by our cryo-EM structure, we found that unsaturated FFAs influence the engagement of integrins by S, most likely by modulation of the open-to-close RBD equilibrium. Taken together, this demonstrates that the FABP is also able to regulate RGD exposure and enhance integrin engagement by SARS-CoV-2 S.

In the search for therapeutic FABP antagonists that inhibit SARS-CoV-2 receptor-binding, a library of FDA-approved drugs has been screened by molecular simulations. These calculations of FABP binding energy suggested compounds with structural similarity to FFAs as FABP ligands (see Fig. S11). Towards an experimental assessment of these predictions and potential pharmacologic modulation FABP activity, we measured drug-normalized retention (see methods for normalization approach) of ApoS-MiniVs during 1 µM treatment with five of these compounds (Fig. 2h). We found two drugs, vitamin K and dexamethasone, that reduce S-mediated binding of the MiniVs in
a FABP-regulated manner. We found an IC50 concentration of 3.2 µM and 4.7 µM for MiniV retention in A549 human alveolar basal epithelial cells for dexamethasone and vitamin K, respectively. Interestingly, reduced vitamin K levels have previously been identified as a modifiable risk factor of severe COVID-1936, while dexamethasone is one of a few drugs approved for the treatment of ventilated COVID-19 patients37. This corroborates that the FABP is a potentially druggable regulator of SARS-CoV-2 cell-binding.

FABP regulates S immunogenicity against neutralizing immunoglobulins. Intriguingly, all key FABP residues are conserved among the globally emerging SARS-CoV-2 Variants of Concern (Fig. 3a) as well as among previous highly pathogenic hCoV1 and corona viruses found in intermediate species38. This persistent structural reoccurrence is remarkable in the light of the fact that FABP apparently restricts rather than facilitates viral cell-binding. A complete absence of FABP disrupting mutations in all highly contagious variants hints at an evolutionary selection
advantage provided by FFA-binding. For sustainable infection and viral replication, non-genome-integrating RNA-viruses balance infectivity against immunogenicity during the inflammatory response, e.g., high initial contagiousness in the first days\textsuperscript{39} and subsequent transition into an immune-evasive “stealth phase” after the incubation period\textsuperscript{40}. In this regard, the RBD is the central immunogenic structure of SARS-CoV-2, accounting for ~90% of IgG neutralizing activity\textsuperscript{5,41}. Likely, the FABP regulates conformational changes in S immunogenicity as a function of FFA-binding. We applied MiniVs to systematically evaluate FFA-based changes in S immunogenicity and assessed changes in IgG epitope accessibility between open and closed RBD states. First, we computed the accessible surface area (ASA) of S residues in the open and FFA-locked states (see Fig. S12). Second, we calculated ASA ratios along RBD opening for individual residues to measure changes in the exposure of specific epitopes of neutralizing SARS-CoV-2 IgGs. To verify this strategy, we computed changes in epitope exposure of (i) IgG S2H14, which uniquely neutralizes an open RBD state by competitive blocking of the receptor-binding motif in S\textsuperscript{5} (Fig. 3b); (ii) IgG CR3022 that exclusively binds open RBD states and inhibits S-mediated entry by a disruptive mechanism\textsuperscript{42,43} (Fig. 3c); and (iii) S2H13 that binds a β-hairpin in the receptor-binding motif accessible in both open and closed states\textsuperscript{5} (Fig. 3d). For the CR3022 and S2H14 epitopes (open RBD only binders), we found an average open-to-close ASA ratio of 4.57 and 1.65, respectively. This shows that these IgG epitopes are more accessible in the open state, consistent with their neutralization pattern. In contrast, for the S2H13 epitope (open and closed RBD binder), the average open-to-close ASA ratio was 0.89. This indicates that the S2H13 epitope is even slightly more accessible in the closed conformation, consistent with S2H13 neutralization pattern. This endorses the ASA ratio as an indicator for IgG epitope accessibility in S.

General IgG neutralizing immunodominant sites (NIDS) in S have been identified by mutation mapping from 17 convalescent COVID-19 patient sera\textsuperscript{44}. To measure epitope exposure for polyclonal neutralizing human serum IgGs in open and closed states, we calculated the ASA ratio for NIDS in open and closed states (Fig. 3e). We found an average ASA ratio of 1.71, indicating that the key sites for S neutralization are more accessible in the open RBD state, which is in agreement with findings from previous molecular dynamics simulations of S\textsuperscript{5}. Importantly, NIDS residues largely overlap with the receptor-binding motif (see Fig. S13a, b). Thus, we raised a VH3 nanobody against the receptor-binding motif (ADAH11) in vitro using ribosome display\textsuperscript{40} to experimentally validate FABP-influenced neutralization by NIDS-targeting immunoglobulins. ADAH11 efficiently reduced the binding of native S MiniVs (Fig. 3f) with an ED\textsubscript{50} of 117 nM (see Fig. S13c). Importantly, ADAH11 neutralization was strongly dependent on S loading with FFA, where again only unsaturated FFAs reduced the neutralization efficiency (Fig. 3g and Fig. S14). This is in agreement with reduced neutralization for closed RBD states. In line with this, we found that CR3022 (open RBD only binder) is likewise able to reduce cell-binding of native S MiniVs (Fig. 3h) and CR3022 neutralization activity was sensitive to S loading with unsaturated FFAs (Fig. 3i). Taken together, this suggests that FFA-binding can regulate S neutralization by IgGs by reducing exposure to NIDS.

FFAs, particularly LA and AA, are essential eicosanoid precursors and tissue inflammatory regulators\textsuperscript{46}. Basal non-esterified ω-6 FFA levels are maintained below 0.1 µM under physiological conditions\textsuperscript{26,46} but can temporarily increase in a so-called FA-lipid storm over seven-fold during hCoV infection, lung inflammation and COVID-19\textsuperscript{12,27,47}. To test a possible link between altered FFA levels and S IgG immunogenicity in direct relevance for COVID-19 patients, we assessed MiniV neutralization by IgGs derived from the serum of six convalescent donors under “basal” (0.1 µM) and “inflammatory” (1.1 µM) LA/AA concentrations. We found that patient serum IgGs effectively neutralize MiniV binding under basal LA/AA concentration but lose neutralization activity under elevated LA/FA conditions (Fig. 3j). This reduction in neutralization activity was also found for live SARS-CoV-2 virus infection of human lung epithelial cells, were the IC\textsubscript{50} values of the COVID-19 convalescent serum-derived IgG dropped by approximately one order of magnitude (see Fig. S15). This suggests that FFA-binding by S modulates the exposure of immunodominant sites (see Fig. 3e), thereby coupling FFA concentrations to S immunogenicity.

Discussion

Our study introduces technology for the bottom-up assembly of synthetic SARS-CoV-2-like liposomes (see Fig. 1). MiniVs are modular, adaptive systems that allow quantitative and flexible assessment of different S-variants while precisely controlling the composition and biophysical properties of the particles. As programmable SARS-CoV-2 models, our MiniVs provide a modular framework for COVID-19 research and are particularly attractive as they can be deployed under biosafety level 1 condition. The SARS-CoV-2 MiniVs enabled us to perform systematic analyses of S binding to target cells under conditions exactly defined at the molecular level, in particular with respect to S-mediated cell-binding under FFA-free conditions and with defined FFA profiles as well as with respect to the newly acquired SARS-CoV-2 RGD motif (see Fig. 2e). We found that unsaturated FFA-binding reduced S-mediated cell-binding (see Fig. 2a) and that the RGD motif contributes to the enhanced cell attachment. We further assess the direct impact of FDA-approved drugs as potential FABP ligands on S’ cell-binding (see Fig. 2b), which could open up avenues for treating COVID-19 by locking S in a closed conformation.

Our study reveals a mechanism where FFAs function as molecular switches by which SARS-CoV-2 can adapt its immunogenicity to local inflammatory states and host immune response (Fig. 3k). Thus, the FABP represents a dynamic responsive element that provides an evolutionary advantage as it allows for a temporary escape from neutralizing IgG during peak
Fig. 3 FABP-regulated exposure of immunogenic S epitopes. a Sequence alignment of the FABP from five SARS-CoV-2 variants of concern and SARS-CoV. Residues of the hydrophobic pocket are highlighted in purple, the hydrophilic head-stabilizing residues in green and the gating helix tyrosine in orange. Residues differing in SARS-CoV are written in orange. b–e Molecular surface representation of open S (7BNN) with ASA open-to-close ratios for the S2H14, CR3022, S2H13 epitopes and NIDS shown in green (>1) and purple (<1). Diagrams show ASA ratios for single epitope residues. Average ASA ratio change over all epitope residues is given in %. f Retention assay for MiniVs presenting native S and incubated with MCF7 cells for 24 h. Reduction in retention by ADAH11 nanobodies was measured by addition of 1µM ADAH11 during the incubation period. g Native S-normalized neutralization of ADAH11 for MiniVs presenting FFA-loaded ApoS incubated with MCF7 cells for 24 h. h Retention assay for MiniVs presenting native S and incubated with MCF7 cells for 24 h. Reduction in retention by CR3022 IgG was measured by addition of 132 nM CR3022 during the incubation period. i Native S-normalized neutralization of CR3022 based for MiniVs presenting FFA-loaded ApoS incubated with MCF7 cells for 24 h. j Retention analysis with native S-normalized neutralization of MiniVs by convalescent COVID-19 patient serum-derived IgGs under low (0.1µM) and high (1µM) LA/AA concentrations. k Model of FFA as molecular switches that couple local inflammatory states to SARS-CoV-2 S immunogenicity. Results in f–j are shown as mean ± SD from at least n = 3 biological replicates in each experimental condition, *p < 0.05, **p < 0.005, ***p < 0.0005, unpaired two-tailed t-test. Source data are provided as a Source Data file.
inflammatory phases. This is achieved through coupling the RBD open-to-close equilibriu to the abundance of FFAs. In this context, the RBD’s major contribution to SARS-CoV-2 immunogenicity and its fundamental importance for COVID-19 vacination strategies has been recognized by several studies. Fat acids are exceptionally suitable metabolic markers for immunogen-host adaptation as altered fatty acid metabolism is an early indicator for beginning antiviral immune response against positively stranded RNA-viruses. This enables efficient viral replication and transmission during the initial incubation period until progressive activation of the host’s antiviral response. After immune recognition, FFA-binding could mediate immune-evasive adaptation, suppress SARS-CoV-2 immunogenicity and reduce viral infection via a stealth-like mechanism of reduced RBD exposure. Eventually, this could lead to increased viral titers because of reduced virus clearance. In summary, enabled by our MiniV technology, we identified a potentially immune-evasive link between FABP and FFAs in SARS-CoV-2 which could be exploited for future COVID-19 therapy.

Methods

Materials. 18:1 DOPC 1,2-dioleoyl-sn-glycero-3-phosphocholine, 18:1 DOPPE 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[lissamine rhodamine B sulfonyl], 18:1 DGS-NTA(Ni) 1,2-dioleoyl-sn-glycerol-3-[(N-(5-amino-1-carboxyethyl)iminodiacetic acid)succinyl] (nickel salt), 18:1 1,2-dioleoyl-sn-glycerol-3-phospho(1’-myo-inositol) (ammonium salt), 18:1 1,2-dioleoyl-sn-glycerol-3-phospho-L-serine (sodium salt), 1,2-Dioleoyl-sn-glycerol-3-phosphoethanolamin Atto488-conjugate, 18:1, cholesterol, 18:0 N-stearoyl-D-erythro-sphingophosphorylcholine, and estrade set with 50 nm pore size polycarbonate filter membranes were purchased from Avanti Polar Lipids, USA Dulbecco’s Modified Eagle Medium (DMEM) High Glucose, heat-inactivated fetal bovine serum, penicillin-streptomycin (10,000 U/mL), L-Glutamine (200 mM), trypsin-EDTA (0.05%), FluoroBrite DMEM, Cell-Tracker Green CMFDA dye, Hoechst 33342, phosphate-buffered saline (PBS) pH 8.0, 5 mM MgCl2) was added per gram of pellet and again incubated on a roller for 30 min. This supernatant was then incubated with 10 mL HisPur Ni-NTA Superflow Agarose (Thermo Fisher Scientific) per 4 l of expression culture for 1 h at 4 °C. Subsequently, the resin-bound with SARS-CoV-2 S protein was collected using a gravity flow column (Bio-Rad) and then eluted using a step gradient of elution buffer (65 mM NaH2PO4, 300 mM NaCl, 235 mM imidazole, pH 7.5). After analyzing elution fractions using reducing SDS-PAGE, fractions containing SARS-CoV-2 S protein were pooled and concentrated using 50 kDa MWCO Amicon centrifugal filter units (EMD Millipore) and finally buffer-exchanged in phosphate-buffered saline (PBS) pH 7.5. The protein was then subjected to size exclusion chromatography (SEC) using a Superdex 200 increase 10/300 column (GE Healthcare) in PBS pH 7.5. Peak fractions from SEC were then analysed using reducing SDS-PAGE and then fractions containing SARS-CoV-2 S protein were pooled and concentrated using 50 kDa MWCO Amicon centrifugal filter units (EMD Millipore) and finally aliquoted and flash-frozen in liquid nitrogen for storage at –80 °C until further use.

For immobilization of recombinant S ectodomains, the SUV solution was diluted to a final concentration of 100 µM in PBS, corresponding to 1 µM final DGS-NTA(Ni)2−. To this, 0.5 µM of recombinant histidine-tagged S was added and incubated for coupling for at least 15 min. For loading of FAs on MiniVs, FAs were dissolved in DMSO to a final concentration of 100 mg/mL. From these stocks, 100 µg/mL dilutions in PBS were prepared.

S proteins expression and purification. Recombinant wild-type (Wuhan) S protein with a mutated furin site was produced as described previously. The construct comprises amino acids 1–1213, lacks the native transmembrane domain, which was replaced by another C-terminal hexa-histidine tag followed by a C-terminal thrombin cleavage site and a T4-foldon from Avanti Polar Lipids, USA Dulbecco’s Modified Eagle Medium (DMEM) High Glucose, heat-inactivated fetal bovine serum, penicillin-streptomycin (10,000 U/mL), L-Glutamine (200 mM), trypsin-EDTA (0.05%), FluoroBrite DMEM, Cell-Tracker Green CMFDA dye, Hoechst 33342, phosphate-buffered saline (PBS) pH 7.5. The protein was then subjected to size exclusion chromatography (SEC) using a Superdex 200 increase 10/300 column (GE Healthcare) in PBS pH 7.5. Peak fractions from SEC were then analysed using reducing SDS-PAGE and fractions containing SARS-CoV-2 S protein were pooled and concentrated using 50 kDa MWCO Amicon centrifugal filter units (EMD Millipore) and finally aliquoted and flash-frozen in liquid nitrogen for storage at –80 °C until further use.

The UK (or ‘Kent’) B.1.1.7 variant 3 ectodomain gene sequence was synthesized and inserted into pACEBac plasmid (Genescript Inc, New Jersey USA). Expression and purification were carried out as described above for wild-type S.

The S(R403A) mutant was prepared by modifying the wild-type (Wuhan) S expression construct with the point mutation using the QuickChange site-directed mutagenesis kit (Qagen).

For fusion with the MiniV-SARS-CoV-2 S gene encoding was synthesized (Genescript Inc, New Jersey USA). The construct comprises amino acids 14 to 1193, preceded by a GP64 secretion signal sequence (amino acids MVSATYLVVLLAHHHSAFA) and contains a C-terminal thrombin cleavage site followed by a T4-foldon trimerization domain and a hexa-histidine affinity purification tag. The synthetic gene was inserted into pACEBac12. Protein was produced and purified as described above for SARS-CoV-2 S.

MERS-CoV S gene encoding was synthesized (Genescript Inc, New Jersey USA) and cloned into pACEBac12. This construct comprises amino acids 18–1294, preceded by the GP64 secretion signal sequence and contains a C-terminal thrombin cleavage site followed by a T4-foldon trimerization domain and a hexa-histidine affinity purification tag. Protein was produced and purified as described above for SARS-CoV-2 S.

ApoS protein lacking free fatty acid was produced from purified SARS-CoV-2 S protein by Lipidex treatment as described. Briefly, purified SARS-CoV-2 S protein was incubated with pre-equilibrated lipiddx 1000 resin (Perkin Elmer; cat no. 6083001) in PBS pH 7.5 overnight at 4 °C on a roller shaker. Following this, Lipidex-treated S protein was separated from the resin using a gravity flow column. The integrity of the protein was confirmed by size exclusion chromatography (SEC) using a S200 10/300 increase column (GE Healthcare) and SDS-PAGE.

ADAH11 selection, expression, and purification. Neutralizing nanobody ADAH11 against the RBM of S was selected from a synthetic library using vitro selection by ribosome display 32,33. Following selection, the ADAH11 coding sequence was cloned into plhEN6 plasmid containing a PeB signal sequence at the N-terminus, and a hexa-histidine and 3X FLAG tag at the C-terminus. ADAH11 was expressed in E. coli TG1 cells in 2x YT medium overnight at 30 °C induced with 1 mM IPTG (Isopropyl ß-D-1-thiogalactopyranoside). Cells were harvested by centrifugation at 3,000 x g for 10 min at 4 °C. Subsequently, the resin-bound with SARS-CoV-2 S protein was collected using a gravity flow Agarose (Thermo Fisher Scientific) per 4 l of expression culture for 1 h at 4 °C. The protein was then subjected to size exclusion chromatography (SEC) using a S200 10/300 increase column (GE Healthcare) in PBS pH 7.5. Peak fractions from SEC were then analysed using reducing SDS-PAGE and fractions containing ADAH11 were pooled and concentrated using 10 kDa MWCO Amicon centrifugal filter units (EMD Millipore) and finally aliquoted and flash-frozen in liquid nitrogen for storage at –80 °C until further use.
DLS + zeta potential. Size and zeta potentials of SUVs and Mini variants were measured with a Malvern Zetasizer Nano ZS system at a total lipid concentration of 100 µM. After equilibration time was set to 200 s at 25 °C, followed by three repeat measurements for each sample at a scattering angle of 173° using the built-in automatic run-number selection. The material refractive index was set to 1.4233 and solvent properties to η = 0.8882, n = 1.33 and ε = 79.0.

Hoechst staining and nuclei counting. To assess blocking of cell adhesion under liposomes, we imaged adherent cells after seeding and washing on fibronectin-coated well plates following previously developed protocols25,26. To this end, MCF-7 cells were seeded at a density of 50,000 cells/well in an 8-well plate in 100 µl culture medium. Cells were either incubated with 20 µM LiRNGD or with the addition of 20 µL PBS (mock) for 24 h. Subsequently, Hoechst33342 was added to a final concentration of 10 µM to the cell layers and incubated for 10 min. Cells were then washed twice with 200 µL PBS and fixed for 20 min with 4% paraformaldehyde (Sigma-Aldrich) at room temperature and ethanol (95%) at −20 °C for 15 min. Confocal microscopy was performed on a Leica DMi8 inverted fluorescence microscope equipped with a cCMSOS camera and 63x HC PL Fluor (NA 0.32, PH1) objective with DAPI emission/excitation filters. For automated nuclei counting, TIFF images from three wells (i.e., three replicates) were background segmented by global histogram thresholding and automated particle counting (particle analysis) with ImageJ software (NIH). Before particle counting, a watershed algorithm was applied to separated overlapping nuclei and nuclei counting was restricted to particles in the size range between 1 µm² and 100 µm².

Cryo-TEM tomography. For cryo-TEM imaging, MiniVs were diluted to a final particle concentration of 2 x 10⁹ particles/mL. The samples were applied to glow-discharged C-Flat 1.2/1.3/3.4 C grids (Protocols) and plunge-frozen using a Vitrobot (FEI). After a Vitrobot (FEI), the cryogrid was loaded into a Kulzer's temperature- controlled cryo-transfer system at −150 °C to −140 °C (Carl Zeiss AG). The vitrified samples were cryo-cut with an Agate knife, brought up to −196 °C, and kept at −196 °C until loading onto a Tecnai G2 T12 transmission electron microscope operated at 300 kV, equipped with a Falcon 3EC direct detector. Images were acquired with the software EPU (Thermo Scientific) in counting mode at a magnification of 820,000 ×. After thorough alignment of the original tilt series, the tomograms were generated with a filtered back-projection of the aligned micrographs. Tomogram slices were FFT band-pass filtered between 3 and 40 pixels and subsequently a 2 x 2 pixel Gaussian blur filter was applied. Images were contrast corrected by visual inspection.

QCM-D + imaging SLB. For QCM-D measurements, sensor crystals, AT-cut gold electrodes coated with a 50 nm thick layer of silicon oxide were used. SiO2 surfaces were nucleated using SLB formation was confirmed by the characteristic changes in frequency and dissipation as it is described elsewhere25. SLB membranes were deposited on the sensor as described elsewhere25. The subsequent LC-MS analysis was performed using a Shimadzu Nexera X2 system equipped with a Sciex QTRAP 4500 system. The LC system was equipped with water (A) and acetonitrile (B) in LCMS grade from Biosolve. The solvents were supplemented with 0.1% LCMS grade formic acid. 6 µL of the sample solutions were fractionated with a Supelco Titan C18 column 100 x 2.1 mm, 1.9 µm, at 0.25 mL/min. Whereas the following gradient was applied: 0 min—40% B; 1 min—40% B; 6.5 min—98% B; 8.0 min—98% B; 8.1 min—40% B and 9.5 min—98% B. The mass spectrometer was controlled using the Analyst 1.7 software and was operated in ESI positive mode. An optimal target compound ionization was achieved by setting the following source parameters: Curtain gas 35, temperature 350 °C, ionization voltage 550 V, drying gas 1000 L/min, nebulizer gas 30 L/min, sheath gas 10 L/min, collision gas 9.

The targeted derivatized fatty acids were detected in MRM mode. Fragmentation of the monitored PA derivatized FA yielded a characteristic MSMS fragment with m/z 92. For a linoleic acid additional parent to fragment MSMS transitions were recorded. The following compound dependent MSMS parameters were used: MRCITY 39 (LAP: 92—571); 90 m/z, declustering potential (DP) 50, entrance potential (EP) 10, collision energy (CE) 43, cell exit potential (CXP) 11, LAP-109: 371.3—109.1 Da, Dwell 40 ms, DP 80, EP 10,
Retention assays. For quantification of MiniV-cell-binding, we developed retention assays to measure the amount of MiniVs retained within culture plates after incubation and washing. This assay is based on quantification of the rhodamine fluorescence form the SUV lipid membrane and can therefore not discriminate between attachment and uptake of MiniVs. For retention analysis, SUVs and MiniVs with different recombinant S ectodomains (as indicated in the figure legends), were added to MCF-7 cells in flat-bottom 96-well plates with 100 µl of 5% fetal bovine serum. The next day, cell medium was exchanged to the seeding medium (DMEM supplemented with phenol red, 4.5 g/1 glucose, 1% L-glutamine, 1% penicillin/streptomycin, 0.01 mg/mL recombinant human insulin, and 10% fetal bovine serum) to low-serum medium (DMEM supplemented without phenol red, 4.5 g/1 glucose, 1% L-glutamine, 1% penicillin/streptomycin, 0.01 mg/mL recombinant human insulin, and 0.5% fetal bovine serum) to reduce the amount of serum-derived FAs far below physiologically relevant concentrations. After incubation of MiniVs with cells for 24 h, rhodamine fluorescence was measured at 9 different positions and 1300 mm distance to well wall in each well using an Infinite M200 TECAN plate reader controlled by TECAN Control software with an in-built gain optimization and excitation/emission setting adjusted to 555/585 nm. Wells were then washed three times with PBS and subsequently fixed with 100 µL 4% paraformaldehyde. After 10 min fixation, rhodamine fluorescence was again measured in each well with the settings mentioned above. For retention analysis binding could then be deduced from residual fluorescence in each well (for assessment of patient IgG neutralization) or retention values could be calculated by dividing the residuals fluorescence to the initial fluorescence intensity before washing. All measurements were performed in triplicates. As retention show significant variations depending on cell seeding density, cell viability and vesicle preparation, a control of native S virus (i.e., without protein on the surface) was added to all experimental batches for normalization proposes. For time-resolved measurements of retention, separate wells for each time point were prepared and evaluated sequentially. For retention assay involving an assessment of FFA influence, stock of pure FFA (PA, OA, LA and AA) were prepared in DMSO at a final concentration of 100 mg/mL. From these stock, predilutions (100 µg/mL) in PBS were prepared. Individual FFAs, as indicated in the figures, were added to the MiniVs of the retention assays at a final concentration of 1 µM. For retention assays involving TMPrSS2 inhibition by camostat mesylate, camostat mesylate was added to the cell cultures at indicated concentration 2 h prior to MiniV addition.

Retention assays (RGG). For assessment of RGG-motif-mediated effects in MiniV-cell-binding, retention assay under integrin blocking conditions with linRGD was performed. For this, 100,000 MCF-7 cells/well were seeded in flat-bottom 96-well plates and allowed to form confluent monolayers overnight. Subsequently, the seeding medium was exchanged to low-serum (0.5%) cell culture medium and a final linRGD concentration of 20 µM was added to each well from a 10 mM stock solution. MiniVs (with S-configuration as indicated in the figure legends) were added to a final lipid concentration of 10 µM. Retention assays were performed as detailed above.

To quantify differences in FA-regulated MiniV-integrin engagement, retention was calculated as linRGD-normalized retention. For this, retention of MiniVs was measured with and without incubation of linRGD. Differences in retention were calculated and normalized by:

\[
R - R_{\text{linRGD}}
R_{\text{untreated}}
\]

Where \( R \) is the retention value of the respective MiniV S configuration without linRGD, \( R_{\text{linRGD}} \) is the retention value of the corresponding MiniV S configuration under the addition of 20 µM linRGD and \( R_{\text{untreated}} \) is the retention value MiniVs presenting native S.

Retention assays (FABP drug assessment). For assessment of drug-modulated S binding of potential pharmacologic FABP binders, retention assays under drug incubation were performed. For this, 100,000 MCF-7 cells/well were seeded in flat-bottom 96-well plates and allowed to form confluent monolayers overnight. Subsequently, the seeding medium was exchanged to low-serum (0.5%) cell culture medium and a final drug concentration of 1 µM was added to each well from DMSO stock solutions. MiniVs (with S-configurations as indicated in the figure legends) were added to a final lipid concentration of 10 µM and incubated for 24 h. For normalization purposes, also retention of naive SUVs under drug treatment was measured to account for any drug-induced changes in cellular phenotypes. Retention assays were performed as detailed above and drug-normalized retention was calculated from:

\[
\frac{R + R_{\text{app}}}{R_{\text{UVS}}}
\]

(1)

where \( R_{\text{corr}} \) is calculated by:

\[
\frac{R_{\text{UVS}} - R_{\text{APO}}}{R_{\text{apo}}}
\]

(2)

and \( R \) is the retention value of the respective MiniV ApoS configuration under drug incubation. \( R_{\text{apo}} \) is the retention value of MiniVs with ApoS without drug incubation, \( R_{\text{UVS}} \) is the retention value of SUVs without drug incubation and \( R_{\text{apo}} \) is the retention value of SUVs under drug incubation. IC50 values of MiniV retention for dexmethylone and vitamin k2 were measured by serial dilutions and IC50 values were calculated using nonlinear regression.

Protein structure visualization and ASA calculation. Previous studies have demonstrated the value of accessible surface area calculations for assessment of IgG epitope characterization in SARS-CoV-2 S and conformational changes upon protein binding\(^{64,65}\). For visualization of S cryo-TEM structures, the PDB 3D medium to a final lipid concentration of 10 µM. Before incubation, cell medium was exchanged to the seeding medium (DMEM supplemented with phenol red, 4.5 g/1 glucose, 1% L-glutamine, 1% penicillin/streptomycin, 0.01 mg/mL recombinant human insulin, and 0.5% fetal bovine serum) to reduce the amount of serum-derived FAs far below physiologically relevant concentrations. After incubation of MiniVs with cells for 24 h, rhodamine fluorescence was measured at 9 different positions and 1300 mm distance to well wall in each well using an Infinite M200 TECAN plate reader controlled by TECAN Control software with an in-built gain optimization and excitation/emission setting adjusted to 555/585 nm. Wells were then washed three times with PBS and subsequently fixed with 100 µL 4% paraformaldehyde. After 10 min fixation, rhodamine fluorescence was again measured in each well with the settings mentioned above. For retention analysis binding could then be deduced from residual fluorescence in each well (for assessment of patient IgG neutralization) or retention values could be calculated by dividing the residuals fluorescence to the initial fluorescence intensity before washing. All measurements were performed in triplicates. As retention show significant variations depending on cell seeding density, cell viability and vesicle preparation, a control of native S virus (i.e., without protein on the surface) was added to all experimental batches for normalization proposes. For time-resolved measurements of retention, separate wells for each time point were prepared and evaluated sequentially. For retention assay involving an assessment of FFA influence, stock of pure FFA (PA, OA, LA and AA) were prepared in DMSO at a final concentration of 100 mg/mL. From these stock, predilutions (100 µg/mL) in PBS were prepared. Individual FFAs, as indicated in the figures, were added to the MiniVs of the retention assays at a final concentration of 1 µM. For retention assays involving TMPrSS2 inhibition by camostat mesylate, camostat mesylate was added to the cell cultures at indicated concentration 2 h prior to MiniV addition.

Retention assays (FABP drug assessment). For assessment of drug-modulated S binding of potential pharmacologic FABP binders, retention assays under drug incubation were performed. For this, 100,000 MCF-7 cells/well were seeded in flat-bottom 96-well plates and allowed to form confluent monolayers overnight. Subsequently, the seeding medium was exchanged to low-serum (0.5%) cell culture medium and a final drug concentration of 1 µM was added to each well from DMSO stock solutions. MiniVs (with S-configurations as indicated in the figure legends) were added to a final lipid concentration of 10 µM. Three types of neutralizing immunoglobulins were tested (1) IgG CR3022 (2) ADAH11, and (3) purified IgG from convalescent COVID-19 donors. CR3022 neutralization was assessed by the addition of 132 nM purified IgG. ADAH11 neutralization was titrated in a concentration range of 7.4 nM–1.5 µM and retention assays for ADAH11 neutralization were performed at 1 µM ADAH11. Neutralization of purified donor IgGs was measured at a final concentration of 3.3 µg/mL.
For assessment of neutralization with different FPA profiles, immunoglobulin mediated reduction in retention was calculated by:

\[ R = \frac{R_{\text{immunoglobulin}}}{R_{\text{Native}}} \]  

where \( R \) is the retention value of the respective MiniV ApoA domain configuration without immunoglobulin, \( R_{\text{immunoglobulin}} \) is the retention value of the respective MiniV ApoA domain configuration with immunoglobulin, and \( R_{\text{Native}} \) is the retention value of MiniVs presenting Native S without immunoglobulin immunoblotting. For the assessment of MiniV neutralization as a function of FFAs, individual FFAs were added from DMSO stocks (100 mg/mL) to the culture medium at the retention assay at a final concentration of 1 \( \mu \)M. To mimic the basal, low FFA levels, for COVID-19 donor IgF neutralization, no additional FFAs were added to the culture medium as the 0.5% serum concentration of the retention assay culture medium already provide approximately 0.1 \( \mu \)M LA and AA.

**Competition assays.** 5 \times 10^5 A549-ACE2 cells were seeded in 24-well plates on the day prior to infection. Cells were incubated with 300 \( \mu \)L of SUV or MiniV dilution for 2.5 h. 100 \( \mu \)L of virus suspension containing 5 \times 10^5 infectious virus particles (multiplicity of infection of 1) were added to each well and incubated for 2 h. The medium was removed and washed twice with sterile PBS before the addition of 1 mL of fresh medium. Cells were harvested 18 h post-infection and total RNA extracted using NucleoSpin RNA Plus kit (Macherey-Nagel), following manufacturer’s instructions. cDNA was generated using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) following the manufacturer’s instructions. Expression levels of GAPDH and SARS-CoV-2 Orf7a mRNA were determined by using the iTaq Universal SYBR Green 2x (Bio-Rad). Reactions were performed on an iCycler (Bio-Rad) using the following program: 95 °C for 3 min and 45 cycles as follows: 95 °C for 10 s, 60 °C for 30 s. GAPDH mRNA level was determined by using the AACT method as previously described. The following primers were used:

**GAPDH-For 5′- GAAGTGAAAGTCCGAGCTC - 3′**

**GAPDH-Rev 5′- GAAGATGGTGATGGGATTTC - 3′**

**CoV-2 Leader_For 5′- TCCCAGGTAACAAACCAACCAACT - 3′**

**CoV-2 Orf7a_Rev 5′- AATTGTGTAATGGCTCTGTG - 3′**

**Live virus neutralization assays.** Purified IgG antibodies were serially diluted 2-fold in Opti-MEM, starting with a dilution of 1:20 and mixed with an equal volume of Opti-MEM containing 5 \times 10^5 pfu SARS-CoV-2 (final multiplicity of infection of 1). IgG/virus mixtures were incubated for 1 h at 37 °C and subsequently transferred to 24-wells containing 5 \times 10^5 A549-ACE2 cells seeded the day prior to infection. Cells were infected for 2 h at 37 °C, subsequently washed once with sterile PBS, and cultured for a further 6 h in a fresh medium. Cells were washed and harvested for RNA extraction and ORF7a mRNA expression levels were quantified by qRT-PCR as described above. Values were normalized to infection levels in the absence of IgG antibodies. Relative inhibitory concentration of 50% (IC50) values were calculated using nonlinear regression.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data generated in this study are available in the main text, the supplementary materials, the source data file or the corresponding authors upon reasonable request. Source data are provided as separate source data file. Protein structures used in this study were retrieved from the protein data bank under the accession codes 7BNN, 6ZB5, and 292.e5575 (2021).

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