

Convergent immunological solutions to Argentine hemorrhagic fever virus neutralization

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Key words: Arenavirus, Glycoprotein, Structure, Antibody response, Hemorrhagic fever

Running title: Convergent solutions to Junín virus neutralization

Abstract

Transmission of hemorrhagic fever New World arenaviruses from their rodent reservoirs to human populations poses substantial public health and economic dangers. These zoonotic events are promoted by the specific interaction between the New World arenaviral attachment glycoprotein, GP1, and cell surface human transferrin receptor (hTfR1). Here, we present the structural basis for how a mouse-derived neutralizing antibody (nAb), OD01, disrupts this interaction by targeting the receptor-binding surface of the GP1 glycoprotein from Junín virus (JUNV), a hemorrhagic fever arenavirus endemic in central Argentina. Comparison of our structure with that of a previously reported nAb complex (JUNV GP1–GD01) reveals largely overlapping epitopes but highly distinct antibody binding modes. Despite differences in GP1 recognition, we find that both antibodies present a key tyrosine residue, albeit on different chains, that inserts into a central pocket on JUNV GP1 and effectively mimics the contacts made by the host TfR1. These data provide a molecular-level description of how antibodies derived from different germline origins arrive at equivalent immunological solutions to virus neutralization.

16 **Significance**

17 An estimated five million people are at risk of infection by Junín virus (JUNV), the causative
18 agent of Argentine hemorrhagic fever. JUNV displays a glycoprotein spike complex on the
19 surface of the viral envelope that is responsible for negotiating host-cell recognition and entry.
20 Here, we show that monoclonal antibodies that have gone through different germline selection
21 pathways have converged to target the host-cell receptor-binding site on the JUNV
22 glycoprotein spike. Immuno-focusing of the antibody response to mimic natural host-receptor
23 interactions reveals a key point of vulnerability on the JUNV surface.

/body

New World (NW) clade B arenaviruses (genus *Mammarenavirus*) comprise a number of human pathogens, including Junín virus (JUNV), Machupo virus (MACV), and Guanarito virus (GTOV) (1, 2). These viruses are endemic to rodent populations in rural areas of Argentina, Bolivia, and Venezuela, respectively, and viral spillover into human populations can result in severe hemorrhagic fever (HF) (3). Novel pathogenic NW arenaviruses continue to be identified (4-6), underscoring a wide-scale need for effective vaccines and therapeutics.

JUNV, the etiological agent of Argentine hemorrhagic fever (AHF), constitutes one of the most dangerous NW arenaviruses putting an estimated five million people at risk (7, 8). JUNV infection typically exhibits a rapid onset of disease (7–14 d) and high mortality rates (15-30%) (7, 9, 10). There are no internationally approved drugs for preventing or treating NW arenavirus HF. However, the successful development of a live, attenuated JUNV vaccine, Candid#1, has proven that AHF can be controlled (11). Similarly, virus-neutralizing immune plasma from convalescent individuals has been successfully used for the treatment of AHF (10, 12).

The JUNV envelope surface is decorated by trimeric multi-functional glycoprotein (GPC) spikes. Each protomer in the trimer consists of: a myristoylated (13) stable signal peptide (SSP), an attachment subunit (GP1), and a transmembrane fusion subunit (GP2) (14-19). Host-cell entry of JUNV and other clade B arenaviruses is initiated by the interaction between the arenaviral GP1 and the host transferrin receptor (TfR1) (20). The GP1 subunit interacts with the apical domain of TfR1, distal from natural transferrin and hereditary haemochromatosis protein recognition sites (21, 22). A primary determinant of zoonotic spread of clade B arenaviruses is the ability of the GP1 to recognize the human TfR1 orthologue (20, 23).

The JUNV GPC spike comprises the primary target for neutralizing immune responses (24) and three GPC-specific mouse-derived nAbs, GD01, OD01, and GB03 have shown promise in animal models of infection (25), raising hopes for the development of monoclonal antibody-based therapeutics. The structural basis for virus neutralization by one such monoclonal antibody (mAb), GD01, has been elucidated, revealing an epitope on the GP1 glycoprotein that overlaps with the hTfR1 binding site (26). Here, we sought to determine the molecular basis for JUNV neutralization by the similarly derived mAb OD01. Our X-ray crystallographic investigation reveals that although OD01 bears highly contrasting paratopes and exhibits a smaller binding surface on JUNV GP1 than GD01, both antibodies effectively mimic the contacts made by the host TfR1 during viral attachment. This analysis demonstrates that antibodies derived from different germplines can achieve this highly effective immunological solution.

Results

Structure determination of JUNV GP1–OD01 Fab complex. The mouse-derived neutralizing antibody, OD01 (clone OD01-AA09), has been shown to target the glycoprotein spike of JUNV (24). To further refine the specificity of the nAb OD01, we performed an ELISA (enzyme-linked immunosorbent assay) using our recombinantly-derived JUNV GP1 (residues D87–V231) as antigen (Fig. 1A). Although nAb OD01 bound to JUNV GP1 at concentrations as low as $0.01 \mu\text{g ml}^{-1}$, no cross-reactivity was observed with MACV GP1 or the lymphocytic choriomeningitis virus (LCMV) GP1 negative control. To facilitate structure determination, recombinant JUNV GP1 (Fig. 1B) was deglycosylated with endoglycosidase F₁ (endoF₁) (Fig. S1A) and crystallized in complex with the antigen binding fragment (Fab) of OD01. The structure of the complex was solved to 1.95 Å resolution (Table S1). The amino-acid sequence of OD01 was predicted crystallographically and used to create an engineered Fab fragment (herein referred to as eOD01) capable of stably and specifically binding JUNV GP1 (Figs. S1B

and C and S2). Recombinant expression, crystallization, and structural determination of eOD01 with JUNV GP1 to 1.85 Å resolution (Fig. 1C, Table S1) revealed identical binding modes for the JUNV GP1–OD01 and JUNV GP1–eOD01 complexes (0.2 Å root-mean-square-deviation (r.m.s.d.) over 569 Cα residues, Fig. S3). We note that it is possible for subtle amino-acid sequence differences that could not be distinguished by crystallographic analysis at this resolution (e.g. Asn versus Asp) to exist between OD01 and our engineered version. However, the near identical mode of JUNV GP1 recognition provides a realistic model for JUNV neutralization by the native antibody.

Structural characterization of JUNV GP1–eOD01. Two JUNV GP1–eOD01 Fab complexes were present in the asymmetric unit, with minimal structural differences observed between crystallographically related JUNV GP1 and eOD01 Fab pairs (Figs. S4A and B). As previously observed (26), JUNV GP1 forms a compact α/β fold (Fig. 1B). The two eOD01 Fabs in the asymmetric unit recognize JUNV GP1 nearly identically, with both binding to the convex face of their cognate GP1 molecules at a site overlapping that utilized for Tfr1 attachment (Fig. 1C and D) (0.6 Å r.m.s.d. over 575 equivalent JUNV GP1–eOD01 complex Cα atoms, Fig. S4C). The JUNV GP1–eOD01 interaction interface occludes approximately 1,200 Å² of solvent accessible surface area. Complementarity–determining regions (CDRs) from both the heavy and light chains of eOD01 form contacts with JUNV GP1, indicating that the chains are likely to be mutually required for antigen recognition (Figs. 1C and 2A and Table 1).

While the heavy chain CDR loops H2 and, especially, H3 make a sizeable contribution to the eOD01–GP1 interface, the interaction is dominated by the CDR1 from the light chain (CDR L1), which extends a fifteen amino-acid loop deep into a central pocket on the β-sheet of JUNV GP1 (Figs. 1C and 2A). Tyr30B from eOD01 CDR L1 (Chothia numbering scheme (27)) appears to be of chief importance to this interface, where the side-chain hydroxyl group

hydrogen bonds with JUNV GP1 side-chains Ser111 and Asp113 at the tip of the third strand of JUNV GP1 (Fig. 2A). From the opposite side of the central pocket, the Tyr30B^{eOD01} main chain is stabilized by a further hydrogen-bonding interaction with the guanidinium group of Arg165^{JUNV GP1} (Fig. 2A). Additionally, the aromatic ring of Tyr30B^{eOD01} is surrounded by the side chains of Ile115^{JUNV GP1}, Val117^{JUNV GP1}, Ile174^{JUNV GP1} and Lys216^{JUNV GP1}, which contribute to the hydrophobicity of the pocket (Fig. S5).

Although *N*-linked glycans decorate the periphery of the JUNV GP1 β -sheet (Fig. 1B), the crystallographically observed antibody–antigen interaction is predominantly carbohydrate independent (Fig. S4C). Electron density corresponding to at least one *N*-acetylglucosamine moiety was observed at three out of the four *N*-linked glycosylation sequons: Asn105, Asn166, Asn178, but not Asn95. A chain of at least six *N*-linked glycan moieties (GlcNAc₂Man₄) was ordered at Asn178 in both molecules of the asymmetric unit, suggestive that the di-*N*-acetylchitobiose core of this glycan is protected by the surrounding proteinous environment from endoF₁ digestion (Figs. 1B and S6A). Upon overlay, we note that the extensions of the Asn178 glycans form subtly different conformations in the two molecules of the asymmetric unit (Fig. S6B), indicating that differential packing environments may play a role in stabilizing the termini of these otherwise flexible glycans.

Although glycans do not appear to play a role in eOD01–JUNV GP1 complex formation, the presence of glycosylation on the GP1 does affect the potency of antibody-mediated neutralization. For example, OD01 and GD01 neutralize rLCMV displaying XJ Clone 3 JUNV vaccine strain glycoprotein, which lacks the Asn166 glycosylation motif, more potently than the analogous virus, in which this glycosylation motif has been restored (28). Considering the proximity of Asn166 to the JUNV GP1–antibody interface (Fig. 1C), it seems possible that the presence of native *N*-linked glycosylation at this site may interfere with the antibody–glycoprotein interaction.

eOD01 and GD01 are distinct yet target overlapping epitopes. The structure of GD01 in complex with JUNV GP1 has been previously reported (26) and provides an opportunity to compare how JUNV is neutralized by two unique mouse-derived anti-JUNV nAbs (Figs. 2A and B and 3, Table 1). Structural comparison of these two complexes reveals that the antibodies exhibit differing footprint sizes on the GP1 surface (~600 and ~900 Å² for eOD01 and GD01, respectively), with both epitopes overlapping the predicted receptor binding site (RBS). Despite contacting the same region of the JUNV GP1 surface, the two nAbs exhibit highly contrasting modes of antigen recognition, where the light and heavy chain epitopes on JUNV GP1 are largely swapped (Figs. 2A and B and 3). For example, GP1 loop 3 residues Asp114 and Ala116 are stabilized by intermolecular hydrogen bonds in both GP1–nAb complexes. However, while these interactions are mediated by nAb heavy chain residues Arg95, Thr97, and Thr99 in the GP1–eOD01 complex, light chain residues Ser31, Ala32, and Ser92 provide these contacts in the GP1–GD01 interface (Figs. 2A and B).

These contrasting modes of antigen recognition are reflected in the amino-acid length and sequence of the dominant CDR regions (Table 1, Figs. S7 and S8). For example, while the CDR L1 loop of eOD01 is fifteen amino acids in length, characteristic of the mouse IGKV3 germline family, the corresponding CDR L1 region of GD01 is derived from a different mouse germline group IGKV6 and is eleven amino acids (Fig. S7), a length more commonly observed both in mouse and human antibodies (29) (Table 1). Interestingly, the eOD01 light chain protein sequence shows little deviation from the germline IGKV3-2*01 V-gene (Fig. S7), with only one mutation in the CDR L1 region. Instead of CDR L1, the GD01–GP1 interaction is dominated by the fifteen amino acid CDR H3, which is uncommonly long for the species, with only ~3% of the sequenced mouse CDR H3s displaying a length equal to or greater than 15 amino acids (29) (Table 1).

A shared feature of receptor mimicry. We note a striking commonality between eOD01 and GD01: Tyr30B from the L1 loop of eOD01 occupies a position in the central pocket of JUNV GP1 that largely overlaps with that of Tyr98 from the CDR H3 loop of GD01 (Figs. 2A and B). This is highly similar to the location of Tyr211^{hTfR1} in the MACV GP1–hTfR1 complex (22) (Fig. 2C and D), the only clade B arenavirus glycoprotein–receptor structure reported to date. Tyr211 is a key residue in the GP1–hTfR1 interface (22) and conserved across all TfR1 orthologues that support NW arenavirus entry (23, 30, 31). Although we note that sequence and structural variation between the MACV GP1 and JUNV GP1 RBS exist, the co-localization of Tyr30B^{eOD01} and Tyr98^{GD01} at the Tyr211^{hTfR1} recognition site indicates that both nAbs effectively mimic TfR1-mediated arenaviral attachment.

Discussion

NW hemorrhagic fever arenaviruses pose a significant threat to human health, underscoring the need for effective vaccines and antiviral therapies. While practical limitations of convalescent serum therapy exist, passive transfer of immunoglobulins remains an effective AHF treatment in a post-exposure context (10, 12), confirming that the neutralizing antibody response is crucial for controlling JUNV infection.

Our structural analysis reveals that antibodies derived from different germplines can be directed to mimic host receptor interactions on JUNV GP1. Similarly, neutralizing RBS-targeting antibodies of differing germline origins have been observed in other viruses, including influenza virus, HIV, and poliovirus (32-34). In the case of influenza virus, it has been possible to recognize a signature binding motif on the CDR H3 loop (33). Here, we demonstrate that a key immunoglobulin tyrosine residue, which mimics the critical Tyr211^{hTfR1}, can be located on the CDR loops of either heavy or light chains of anti-JUNV nAbs.

Although our structural analysis suggests that nAbs utilize the functionally conserved TfR1 binding site on the NW arenaviral GP1 as a major target, we note that OD01, GD01, and other monoclonal antibodies reported by Sanchez *et al.* (24) do not cross-react with MACV GP1 or other clade B arenaviruses (22, 24, 35) (Figs. 1A and S9). We suggest that the inability of these antibodies to cross-react with MACV GP1, the most closely related pathogenic NW arenavirus species, may be due to the specific sequence differences and structural variations among the GP1 glycoproteins (Fig. 3). Indeed, this hypothesis is supported by overlay analysis of our eOD01–JUNV GP1 complex with MACV GP1, which indicates major clashes with the elongated helical C-terminal region of the MACV GP1 that would interfere with OD01 recognition (Figs. 2D and S10). Cross-neutralizing antibodies capable of targeting both JUNV and MACV GP1 would likely need to accommodate for the differential presence of these helices, as well as for the sequence variation at the RBS.

Although few JUNV-specific nAbs have been reported to date, Mahmutovic *et al.*, have demonstrated that the hTfR1 binding site on the GP1 glycoprotein is a major target for antibodies generated during natural human infection (26). Indeed, the overlap of OD01 and GDO01 footprints (Fig. 3) is consistent with the existence of an immunodominant epitope at the RBS of JUNV GP1 (26, 35). As a result, the identification of nAbs targeting other neutralizing epitopes is an important consideration for developing synergetic, non-competing combinations of therapeutic anti-JUNV mAbs. Indeed, Robinson *et al.* (36) reported a range of neutralizing epitopes among human mAbs raised upon infection by Lassa virus (LASV), a more distantly related Old World arenavirus. Of the sixteen identified anti-LASV nAbs, thirteen require the assembled glycoprotein complex for binding, while three require only the GP1 (36). Thus, emerging techniques in mAb generation, such as the isolation of antigen-specific B-cells (37) against an intact pre-fusion GPC spike antigen, may reveal antibodies capable of targeting alternative non-RBS epitopes on the JUNV glycoprotein surface.

Materials and Methods

Protein Expression and Purification. The cDNA of JUNV (GenBank accession number ACO52428), MACV (AAS77647), and LCMV (CAC01231) glycoproteins was synthesized by GeneArt (Life Technologies). For crystallization, a construct of JUNV GP1 (D87–N232) was derived by high-throughput cloning into the pOPINTTGneo mammalian expression vector (38). Constructs of MACV GP1 (E87–F257), LCMV GP1 (M81–K256), and an additional construct of JUNV GP1 with an amino acid C-terminal truncation (D87–V231; to facilitate cloning) were cloned into the pHLsec vector (39) for ELISA experiments. Proteins were expressed in transiently transfected human embryonic kidney (HEK) 293T cells (ATCC CRL-1573) in the presence of the α -mannosidase inhibitor, kifunensine, as previously described (39). Cell supernatants were harvested 4 d after transfection, clarified, and diafiltrated against a buffer containing 10 mM Tris (pH 8.0) and 150 mM NaCl (ÄKTA Flux diafiltration system, GE Healthcare). Glycoproteins were purified by immobilized nickel-affinity chromatography (5 ml HisTrap FF crude column and ÄKTA FPLC system, GE Healthcare) followed by size exclusion chromatography (SEC) using a Superdex 200 10/300 Increase column (GE Healthcare), equilibrated in 10 mM TRIS pH 8.0, 150 mM NaCl buffer. To enable crystallogenesis, JUNV GP1 was partially deglycosylated by endoF₁ treatment. Following deglycosylation, JUNV GP1 was re-purified by SEC, as described above.

The mAb, OD01 (clone OD01-AA09), was obtained through BEI Resources (Biodefense and Emerging Infections Research Resources Repository). The Fab fragment of OD01 was produced using the Pierce™ Mouse IgG1 Fab and F(ab')₂ Preparation Kit (Thermo Fischer Scientific) following the manufacturer's protocol. The eOD01 Fab fragment heavy and light chain genes were synthesized by GeneArt (Life Technologies), and the codon optimized cDNAs were cloned into the pHLsec vector (39). A C-terminal His₆-tag was included in the

heavy chain construct and both chains were co-expressed (1:1 (w/w) ratio of Fab heavy to light chain expressing plasmids) in HEK293T cells and purified, as described above.

For ELISA experiments, the eOD01 Fab fragment variable domains were cloned into human IgG heavy and kappa light chain full length constant region expression vectors (40). The recombinant antibody was expressed in HEK293 Freestyle cells co-transfected with heavy and light chain antibody expressing plasmids at a 1:1 (w/w) ratio with PEI_{max} (1:3 (w/w) PEI:total DNA, Polysciences). Transfections were performed according to the manufacturer's protocol, antibody supernatant was harvested 4 d following transfection and clarified. Chimeric eOD01 antibody was purified over a protein A column, eluted with 0.1 M glycine (pH 3.5), concentrated, and buffer exchanged into PBS.

Crystallization and Structure Determination. Prior to crystallization, Fab OD01 and JUNV GP1, were mixed in a 1:1.1 molar ratio. Initial OD01 Fab–JUNV GP1 complex crystals were obtained at room temperature using the sitting drop vapor diffusion method (41), by mixing 100 nL of protein (at a concentration of 6.0 mg mL⁻¹) in 10 mM TRIS pH 8.0, 150 mM NaCl buffer with 100 nL of precipitant containing 20 % (v/v) 2-propanol, 20 % (w/v) PEG 4000 and 0.1 M tri-sodium citrate (pH 5.6). Crystallization drops were equilibrated against 95 µL of a precipitant-containing reservoir. X-ray diffraction data were collected from several optimized crystals grown separately in the presence of the precipitant plus one of the following additives: 100 nL 6% (w/v) D-trehalose dihydrate (added to the crystallization drop), 100 nL 6% (w/v) D-galactose (added to the crystallization drop), or 20 µl 40% (v/v) 1-propanol (added to the reservoir).

For crystallization, recombinantly produced Fab eOD01 and JUNV GP1, were mixed at a 1:1.2 molar ratio. After complex formation, excess JUNV GP1 was removed by SEC and the complex was crystallized, as described above. X-ray diffraction data were collected from several optimized crystals grown separately in the presence of the original precipitant plus one

of the following additives: 20 μ l of 40% (v/v) 1-propanol (added to the reservoir) or 40% (v/v) acetone (added to the reservoir). Crystals were cryoprotected with 25% (v/v) glycerol and flash frozen in liquid nitrogen.

X-ray data were recorded at Beamline I02 at Diamond Light Source (Didcot, U.K.) on a Pilatus 6MF detector (Dectris). X-ray data were indexed, integrated, and scaled with XIA2 (42). The high resolution cutoff for the data was determined by analysis of $CC_{1/2}$, as defined by Karplus and Diederichs (43). The structure of the OD01–JUNV GP1 complex was phased by molecular replacement with PHASER (44) using the crystal structures of a mouse Fab fragment (PDB: 3WII (45)) and MACV GP1 (PDB: 2WFO (19)) as search models. Iterative model building was performed with COOT (46) to obtain a model of OD01 Fab with an amino acid sequence that was found to provide the best fit to the experimental electron density (eOD01 sequence). Structure refinement was performed with Refmac5 (47) in the CCP4 suite including translation–libration–screw-rotation restraints (48, 49) and locally defined non-crystallographic symmetry. The eOD01–JUNV GP1 complex structure was determined as described above, except the OD01–JUNV GP1 structure was used for phasing by molecular replacement. The final refined structure was validated with MolProbity (50). Conformational validation of carbohydrate structures was performed using the Privateer software (51).

ELISA Experiments. ELISA plates (High Bind Microplate, Corning) were coated with viral glycoproteins in PBS (3 μ g mL⁻¹) overnight at 4°C. Plates were washed four times with PBS-T (PBS with 0.05% Tween-20) and blocked for 1h with 5% non-fat milk in PBS-T. The mouse-derived antibodies (OD01 (clone OD01-AA09), GD01 (clone GD01-AG02), QC03 (clone QC03-BF11), GB03 (clone GB03-BE08), and LD05 (clone LD05-BF09) (24), obtained through BEI Resources), and the purified eOD01 chimeric antibody were serially diluted in 5% nonfat milk/PBS-T and incubated with the viral glycoproteins for 2h, then plates were washed as above. Mouse-derived antibodies were detected using alkaline phosphatase conjugated goat

270 anti-mouse IgG (Fab specific) antibody (Sigma), and the chimeric eOD01 antibody was
271 detected using alkaline phosphatase conjugated goat anti-human F(ab')₂ antibody (Thermo
272 Fisher Scientific). Reactions were incubated with the secondary antibody for 1h and the plates
273 were washed as above. Binding was detected with the p-nitrophenyl phosphate substrate
274 (Sigma) and the plates were read at 405 nm.

275 **Accession Codes.** Atomic coordinates and structure factors of JUNV GP1–eOD01 have been
276 deposited in the Protein Data Bank (accession code 5NUZ).

277 **Acknowledgements**

278 We are grateful to Prof. David Stuart for helpful comments and discussions. We thank
279 Diamond Light Source for beamtime (proposal MX10627) and the staff of beamline I02 for
280 support. A.Z. is supported by the EU Horizon 2020 Marie Curie Fellowship (658363), T.A.B.
281 and K.D. are supported by the Medical Research Council (MR/J007897/1, MR/L009528/1,
282 MR/K024426/1, and MR/N002091/1), D.D.P. is supported by the Swiss National Science
283 Foundation (SNSF, grant No. 310030_149340/1), work in the M.C. laboratory is supported by
284 the International AIDS Vaccine Initiative (IAVI), the IAVI Neutralizing Antibody Center
285 CAVD grant and the Scripps CHAVI-ID (1UM1AI100663), J.N. is supported by NIH
286 (National Institutes of Health) grant R15 AI119803, The Wellcome Trust Centre for Human
287 Genetics is supported by Wellcome Trust Centre grant 090532/Z/09/Z. The following reagents
288 were obtained through the NIH Biodefense and Emerging Infections Research Resources
289 Repository, NIAID (National Institute of Allergy and Infectious Diseases), NIH: Monoclonal
290 Anti-Junin Virus, Clone OD01-AA09 (immunoglobulin G, Mouse), NR-2567; Monoclonal
291 Anti-Junin Virus, Clone GD01-AG02 (produced *in vitro*), NR-43776; Monoclonal Anti-Junin
292 Virus, Clone QC03-BF11 (produced *in vitro*), NR-43775; Monoclonal Anti-Junin Virus, Clone
293 GB03-BE08 (produced *in vitro*), NR-43227 and Monoclonal Anti-Junin Virus, Clone LD05-
294 BF09 (produced *in vitro*), NR-48833.

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Figure Legends

Figure 1. Reactivity and binding mode of the JUNV-specific neutralizing antibody, OD01. (A) ELISA analysis of nAb OD01 titrated against immobilized arenavirus GP1 glycoproteins. Wells were coated with JUNV GP1, MACV GP1, or LCMV GP1 (negative control). Error bars, s.d. (n = 3), not shown when smaller than symbol size. (B) *Upper panel:* domain organization of the JUNV glycoprotein precursor (produced using the DOG software (52)). The JUNV GP1 construct used for crystallization is highlighted as a rainbow. Diamond-shaped symbols designate *N*-linked glycosylation sequons (NXT/S, where X≠P) with sites observed to be occupied in the crystal structure colored yellow. SSP, stable signal peptide; GP1, attachment glycoprotein; GP2, fusion glycoprotein; TM, transmembrane domain; IV, intravirion domain; SKI-1/S1P, subtilisin-like kexin protease-1/site-1-protease. *Lower panel:* JUNV GP1 from the JUNV GP1–Fab eOD01 co-crystal structure. JUNV GP1 is shown as a cartoon and colored as a rainbow ramped from blue (N-terminus) to red (C-terminus). Primary interaction loops involved in eOD01 binding are labeled and *N*-linked glycans are shown as sticks. Glycosylation was not detected at Asn95, which is indicated as a white sphere. (C) Structure of JUNV GP1 in complex with eOD01. Complementarity–determining regions (CDRs) contributing to JUNV recognition are colored pink (heavy chain) and green (light chain). The side chain from residue Tyr30B of the eOD01 light chain, is shown in stick representation. V_H, V_L, C_H1, and C_L denote the antibody variable heavy, variable light, constant heavy 1, and constant light chain domains, respectively. The positions of crystallographically observed *N*-linked glycosylation on JUNV GP1 are indicated as yellow spheres. (D) Structure of MACV GP1 in complex with hTfR1 (22). Only the apical domain of hTfR1 (blue) is shown for clarity. Regions of hTfR1 that interact with MACV are marked as thick tubes and the side chain from the conserved tyrosine residue, Tyr211, is shown in stick representation.

Figure 2. Comparison of the JUNV GP1–eOD01, JUNV GP1–GD01, and MACV GP1–hTfR1 complex interfaces. (A) *Left* Interaction between JUNV GP1 and eOD01. JUNV GP1 is shown as a grey cartoon, CDR loops of eOD01 are colored as indicated (Chothia numbering scheme (27)). V_H , V_L , C_H1 , and C_L denote the antibody variable heavy, variable light, constant heavy 1, and constant light chain domains, respectively. *Upper right* CDR loop usage by eOD01 in the JUNV GP1 complex with the CDR loop carrying Y30B denoted with an asterisk; calculated using the PDBePISA server (53) and measured in buried surface area (\AA^2). *Lower right* Close-up view of the JUNV GP1–eOD01 interface with intermolecular hydrogen bonds (distance ≤ 3.5 \AA) highlighted with dashes and the participating residues shown as sticks. (B) *Left* Structure of JUNV GP1 in complex with GD01 (26), as presented in panel A. *Upper right* CDR loop usage by GD01 in the JUNV GP1 complex with the CDR loop carrying Y98 denoted with an asterisk, calculated as in panel A (53). *Lower right* Close-up view of the JUNV GP1–GD01 interface. Hydrogen bonds formed by the same JUNV GP1 residues as in panel A are shown, as well as bonds involving the JUNV GP1 loop 7. (C) Interaction between the apical domain of hTfR1 (blue) in complex with MACV GP1 (22) (pale green), with zoom-in panel as presented in panel B. (D) Relative orientation of the Y211^{hTfR1} (blue), Y98^{GD01 CDRH3} (pink), and Y30B^{eOD01 CDRL1} (dark green) residues with respect to JUNV GP1 (white cartoon) and MACV GP1 (pale green cartoon).

Figure 3. Footprints of hTfR1, eOD01, and GD01 plotted onto MACV and JUNV GP1. (A) Surface representations of MACV GP1 (*left panel*) and JUNV GP1 (*middle and right panels*). The footprint of hTfR1 on MACV GP1 is shown in blue. Dark blue represents amino acid residues conserved between MACV GP1 and JUNV GP1 (both identical and similar residues); not conserved residues are in light blue (including deletions and insertions). The antibody heavy (V_H) and light (V_L) chain footprints on JUNV GP1 are colored pink and green, respectively. Residues contacted by both chains are shown in grey. (B) Sequence alignment of

472 JUNV GP1 residues 87–231 with the corresponding residues of the MACV GP1 (determined
473 by Clustal Omega (54), plotted by ESPript (55), and adjusted by hand). Secondary structure
474 elements are shown with arrows representing β -strands ($\beta 1$ – $\beta 7$) and spirals representing alpha
475 ($\alpha 1$ – $\alpha 2$) and 3_{10} -helices ($\eta 1$ – $\eta 5$). Colored squares under the sequence alignment mark GP1
476 residues contacted by hTfR1, eOD01, and GD01; colored as in panel A. Black bordered squares
477 indicate residues not conserved between the JUNV GP1 sequences used for co-crystallization
478 with OD01 (shown) and GD01 (i.e. Q109K and Q121E). Black dots denote JUNV GP1
479 residues forming minor contacts with eOD01, which were only observed in one of the two
480 complexes in the asymmetric unit.