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Structural basis of Zika and Dengue virus potent antibody cross-neutralization

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Zika virus is a member of the flavivirus genus that had not been associated with severe disease in humans until the recent outbreaks, when it was linked to microcephaly in newborns in Brazil and to Guillain-Barré Syndrome in adults in French Polynesia. Zika virus is related to dengue virus, and we report here that a category of antibodies isolated from dengue patients and targeting a conformational epitope potently neutralize Zika virus. The crystal structure of two of these antibodies in complex with the envelope protein of Zika virus reveals the details of a conserved epitope, which is also the site of interaction of the envelope protein dimer with the precursor prM protein during virus maturation. Comparison of the Zika and dengue virus immunocomplexes provides a lead for a rational, epitope-focused design of a universal vaccine capable of eliciting potent cross-neutralizing antibodies to protect against Zika and dengue viruses simultaneously.

38 Zika virus (ZIKV) is an arthropod-borne enveloped virus belonging to the flavivirus
39 genus in the family *Flaviviridae*, which also includes the human pathogenic yellow fever,
40 dengue, West Nile and tick-borne encephalitis viruses¹. Flaviviruses have two structural
41 glycoproteins, prM and E (for precursor Membrane and Envelope proteins,
42 respectively), which form a heterodimer in the endoplasmic reticulum (ER) of the
43 infected cell and drive the budding of spiky immature virions into the ER lumen. These
44 particles transit through the cellular secretory pathway, during which the trans-Golgi
45 protease, furin, cleaves prM. This processing, required for infectivity, results in loss of a
46 large fragment of prM and reorganization of E on the virion surface. The mature
47 particles have a smooth aspect, with 90 E dimers organized with icosahedral symmetry
48 in a “herringbone” pattern^{2,3}.

49 Three-dimensional cryo-EM structures of the mature ZIKV particles have recently been
50 reported to near atomic resolution (3.8 Å)^{4,5}, showing that it has essentially the same
51 organization as the other flaviviruses of known structure, such as dengue virus (DENV)
52 ³ and West Nile virus^{6,7}. The E protein is about 500 amino acids long, with the 400 N-
53 terminal residues forming the ectodomain essentially folded as β-sheets with three
54 domains named I, II and III, aligned in a row with domain I at the center. The conserved
55 fusion loop is at the distal end of the rod in domain II, buried at the E dimer interface. At
56 the C-terminus, the E ectodomain is followed by the so-called “stem”, featuring two α-
57 helices lying flat on the viral membrane (the “stem” helices), which link to two C-
58 terminal trans-membrane α-helices. The main distinguishing feature of the ZIKV virion
59 is an insertion within a glycosylated loop of E (the “150” loop), which protrudes from
60 the mature virion surface^{4,5}.

61 Flaviviruses have been grouped into serocomplexes based on cross-neutralization
62 studies with polyclonal immune sera⁸. The E protein is the main target of neutralizing
63 antibodies, and is also the viral fusogen; cleavage of prM allows E to respond to the
64 endosomal pH by undergoing a large-scale conformational change that catalyzes
65 membrane fusion and releases the viral genome into the cytosol. Loss of the precursor
66 fragment of prM lets the E protein fluctuate from its tight packing at the surface of the
67 virion, transiently exposing otherwise buried surfaces. One surface exposed by this
68 “breathing” is the fusion-loop epitope (FLE), which is a dominant cross-reactive
69 antigenic site⁹. Although antibodies to this site can protect by complement-mediated
70 mechanisms, as shown for West Nile virus in a mouse model¹⁰, they are poorly
71 neutralizing and lead to antibody-dependent enhancement (ADE)¹¹⁻¹⁵ thereby
72 aggravating flavivirus pathogenesis and complicating the development of safe and
73 effective vaccines.

74 We recently reported the isolation and structural characterization of a panel of
75 antibodies isolated from dengue patients^{13,16}. Most of these antibodies target the FLE,
76 but others target a quaternary site readily accessible at the exposed surface of the E
77 protein on the virion, at the interface between the two E subunits in the dimer. These
78 broadly neutralizing antibodies (bnAbs), termed EDE for “E-dimer epitope”, potently
79 neutralize all four serotypes of DENV. Their binding site is conserved across serotypes
80 because it is also the interaction site of prM with E dimers during transport of the
81 immature virus particles through the Golgi apparatus of the cell. There were two
82 subsets of EDE Mabs, characterized by a differential requirement for glycosylation on
83 the 150 loop for binding. The EDE1 bnAbs bind better in the absence of glycan, whereas
84 EDE2 bnAbs bind better when the glycan is present.

85 In this study we show that the EDE Mabs neutralize ZIKV as potently as they neutralize
86 DENV. We also find that the FLE antibodies, which neutralize DENV - although not as
87 potently as the EDE Mabs - do not neutralize ZIKV at concentrations up to 1 μ M in spite
88 of a high affinity for the recombinant ZIKV E protein. We further describe the crystal
89 structures of the ZIKV E protein dimer alone and in complex with EDE1 C8 and EDE2
90 A11, identifying their binding determinants.

91 [A ZIKV-DENV super serogroup](#)

92 Phylogenetic analyses of the main human pathogenic flaviviruses using the amino acid
93 sequences of the viral RNA polymerase NS5 indicate a clustering of ZIKV with the group
94 of mosquito-borne encephalitic viruses (Fig. 1a). The clustering is different when the
95 amino acid sequences of the E protein are considered, with ZIKV branching with the
96 DENV group. If the sequence clustering extends to the antigenic surface of E, antibodies
97 that cross-react with several DENV serotypes should also bind ZIKV E. To test this
98 hypothesis we used bio-layer interferometry (BLI) to study the binding properties of a
99 poorly neutralizing, cross-reactive FLE antibody and the potently neutralizing EDE
100 mAbs for recombinant, soluble ZIKV E ectodomain (ZIKV sE) produced in insect cells
101 (see Online methods). The FLE mAb (P6B10) bound nearly 10fold more tightly than did
102 EDE1 C8 (apparent K_d ~1.5 nM vs. 9 nM) and nearly 1000 times more tightly than EDE2
103 A11 (Fig. 1b and ED Fig. 1a). Consistent with their affinities, we could isolate a complex
104 of ZIKV sE with a C8 Fab by SEC, but not with an A11 Fab (ED Fig. 1b).

105 Neutralization assays in African green monkey (VERO) cells using these and other
106 members of the three antibody subsets, showed that the EDE1 antibodies strongly
107 neutralize ZIKV, whereas the EDE2 were at least one log less potent. In spite of its
108 strong affinity, P6B10 did not neutralize in the concentration range used, nor did any of
109 the two other FLE antibodies tested (Fig. 2). The EDE1 bnAbs neutralized better the
110 African strain HD78788, which has over the years been cell-culture adapted and

111 passaged in suckling mice brain, and lacks E glycosylation. The IC₅₀ against the PF13
112 strain, isolated in French Polynesia in 2013 and in which the E protein is glycosylated at
113 position 154, was in the nanomolar range and comparable to - or lower than - that
114 against the four serotypes of DENV (Table 1). The EDE2 bnAbs showed no difference in
115 neutralization of the two strains, suggesting that the presence of the N154 glycan in the
116 ZIKV E protein did not enhance the interaction.

117 **The ZIKV / EDE bnAbs immune complexes**

118 We crystallized unliganded ZIKV sE and complexes of ZIKV sE with EDE1 C8 and EDE2
119 A11 with scFv and Fab fragments, respectively (ED Table1). In the structure of
120 unliganded ZIKV sE, the 150 loop is ordered, unlike the unglycosylated 150 loop in the
121 recently determined structure of the protein produced in bacteria and refolded in
122 vitro¹⁷. In contrast to our insect-cell secreted protein, which is a dimer (ED Fig. 1b) the
123 refolded protein was reported to be monomeric in solution, suggesting that the glycan
124 may help structure the loop and promote sE dimerization.

125 The antibodies recognize a quaternary epitope in the ZIKV sE dimer in the same way
126 they recognize the DENV serotype 2 (DENV-2) sE dimer described earlier¹⁶. The amino-
127 acid residues participating in the contacts, for both the ZIKV and DENV-2 structures, are
128 shown in ED Fig. 2. The pattern is, as expected, very similar, with the few differences
129 highlighted in red frames in ED Fig. 2b. Both epitopes in the sE dimer are occupied in
130 the case of the complex with C8 (Fig. 3a) whereas only one is occupied in the case of
131 A11 (Fig. 4a). Inspection of the crystal environment showed that a second Fab could not
132 be docked at this position without clashing with neighboring complexes in the crystal.
133 This observation indicates that crystal growth selected for incorporation of sE dimers
134 with a single Fab bound, which is facilitated by the low affinity of A11.

135 The bnAbs dock on ZIKV sE at different angles than they do on DENV-2 sE (see insets in
136 Figs. 3a and 4a). In the case of the C8 complex, the difference in docking results mainly
137 from an altered curvature of the sE dimer. We note that the conformation of ZIKV sE in
138 complex with the antibodies is very similar to the one it adopts on the virus particle,
139 with roughly 1.5 Å root mean square deviation (RMSD) for 790 C α atoms (see ED Table
140 2). The unbound ZIKV sE crystallized here displays a more distant conformation (2.5 Å
141 RMSD when comparing to both virion ZIKV E and either of the sE antibody complexes),
142 suggesting that the antibodies stabilize a conformation close to that on the viral particle.
143 In contrast, the same comparisons done for DENV-2 sE, alone or in complex with the
144 bnAbs result in RMSD values of 5-7 Å with respect to the E conformation on the DENV
145 virion observed by cryo-EM³. For comparison, superposition of the ectodomain of virion
146 E from ZIKV⁵ and DENV-2³ results in a similar 1.5 Å RMSD, indicating that they are
147 presented roughly in the same way, but that DENV sE is more deformable in solution.

148 This malleability may reflect the conformational breathing reported for DENV E¹⁸.
149 Instead, ZIKV sE remains in a similar conformation in the absence of the interactions
150 with the underlying stem α -helices and with the M protein (the membrane-anchored
151 remnant of prM after furin cleavage) on the virion, in line with the higher stability of the
152 ZIKV particles described recently⁴.

153 EDE1 C8 complex

154 The total buried surface area of EDE1 in the complex with ZIKV sE is about 900 Å²,
155 compared to about 1300 Å² in the DENV-2 sE complex (ED Table 3). Fig. 3d shows the
156 conservation of the epitope, and Figs. 3e and 3f compare the C8 footprint on ZIKV and
157 DENV-2 sE. The DENV-specific glycan at position N67, which is ordered in the DENV-2
158 sE structure (Fig. 3c), accounts for around two-thirds of the overall difference in
159 footprint area. The N67 glycan interacts with the framework region 2 of the heavy chain
160 (FRH2), and its absence in ZIKV sE shows that these contacts are not essential for
161 binding. The key cluster of interactions that is maintained is centered on β -strand *b* of
162 domain II, with side chains from CDRs H2, H3 and L3 recognizing all the available
163 hydrogen bond donors (NH atoms) and acceptors (main chain carbonyls) of the *bdc* β -
164 sheet edge (Figs. 3b and 3c). In addition, the fusion loop main chain (which contains
165 several glycine residues) and the disulfide bond between Cys74 and Cys105, are framed
166 by aromatic side chains of the CDRs L1 and L3 (see also ED Figure 3). Residues from
167 these two CDRs also recognize strictly conserved side chains of the fusion loop (Arg 99)
168 or nearby (Gln 77).

169 Across the dimer interface, and as in the complex with DENV-2, the 150 loop is partially
170 disordered, with no detectable density for the N154 glycan (Fig. 3a and ED Fig. 3d). As
171 shown in ED Fig. 3, the interacting residues across the dimer interface are different,
172 reflecting the more limited sequence conservation in these regions of the E protein: in
173 the DENV-2 sE complex, these contacts are with β -strands A and B of domain III, but in
174 ZIKV they mainly involve Lys 373 from β -strand E interacting with CDRs L1 and L2,
175 with a network of direct or water-mediated hydrogen bonds (ED Figs. 3b and 3c).
176 Similarly, a number of charged residues in domain I and from the nearby *kl* loop of
177 domain II across the interface, contribute to the binding and interact with the heavy
178 chain CDRs H2 and H3 (ED Figs. 3e and 3f). All the polar interactions between C8 and
179 ZIKV sE are listed in ED Tables 4 and 5, and the electrostatic surface of the epitope is
180 displayed in ED Fig. 4, left panel. In summary, these observations identify the conserved
181 cluster of contacts with the *b* strand and the fusion loop in domain II as the main
182 binding determinants of C8, with additional contacts from across the dimer interface -
183 or from the N67 glycan in DENV - further stabilizing but not determining the
184 interaction.

185 EDE2 A11 complex

186 ED Figure 4 compares the footprint of C8 and A11 on ZIKV sE, together with the surface
187 electrostatic potential of the complexes, which shows a strong basic patch on sE in the
188 C8 complex due to the disorder of the 150 loop. As shown in ED Fig. 5, C8 would clash
189 with the glycan had the loop remained in place, as was the case in the complex with
190 DENV-2 sE¹⁶. In the A11 complex the 150 loop remains in the same conformation as in
191 the cryo-EM structures of the virion (ED Fig. 5a) and in the X-ray structure of
192 glycosylated unliganded sE reported here. In the DENV-2 sE / A11 complex, the glycan
193 is recognized by an α -helix in the long CDR3 loop (Fig. 4e). The difference in length in
194 the 150 loop of E in ZIKV compared to DENV shifts the glycan position by about 6-7Å,
195 such that it cannot make the same interactions with the CDR H3 α -helix (Figs. 4d and e
196 and ED Fig. 5b). As a consequence, the A11 antibody docks at a different angle on ZIKV
197 sE than it does on DENV-2 sE, even accounting for the difference in sE dimer curvature
198 (Fig. 4a, inset). The contacts along the *b*-strand are preserved (Fig. 4b and c). Compared
199 to C8, the *b* strand is recognized only at its end (residues 71 and 73), whereas C8
200 recognizes it all along, from residue 68 (or from 67 in DENV).

201 DISCUSSION

202 Our results identify the structural details of a quaternary epitope that provides a
203 previously unrecognized link of potent cross-neutralization between Zika and dengue
204 viruses, and thus identifies an antigenic flavivirus cluster beyond the traditional
205 serocomplexes. This relationship defines a super serogroup on the basis of strong cross-
206 neutralization through a conserved epitope that had not been recognized using
207 polyclonal sera⁸. This finding thus introduces the possibility of developing a universal
208 vaccine protecting against all the viruses from this group.

209 Vaccine design against dengue virus has been hampered by the heterogeneity of DENV
210 particles and the need to use polyvalent formulas to immunize against all four serotypes
211 ^{19,20}. One feature of DENV is that it undergoes incomplete furin maturation cleavage of
212 prM in many cell types, giving rise to heterogeneous mosaic particles with an immature-
213 like spiky patch on one side and a smooth mature-like region on the opposite side²¹.
214 These particles are infectious, as they can fuse with the cellular membrane through the
215 smooth, mature side. Because the FLE is exposed in immature regions²², most of the
216 antibody response in DENV infected patients is directed against it ²³. These cross-
217 reactive antibodies coat the particles on the "immature side"²² but neutralize only
218 weakly, because they can bind the "mature side" only when the E protein "breathes"²⁴⁻
219 ²⁶. A recently published structure of monomeric ZIKV sE in complex with an FLE-
220 specific monoclonal murine antibody of low neutralizing activity indeed shows that its
221 binding site would be occluded in the dimeric E protein on mature infectious virions¹⁷.

222 The observation that P6B10 and other FLE antibodies still neutralize DENV¹³ suggests
223 that E in the mature patches on DENV spends more time in conformations that expose
224 the FLE than does E in those patches on ZIKV. This inference is consistent with the high
225 thermal stability of ZIKV reported recently⁴.

226 Our results suggest that the epitope targeted by the EDE1 bnAbs is better suited for
227 developing an epitope-focused vaccine for viruses in the ZIKV/DENV super-serogroup
228 than is the FLE, which induces poorly neutralizing and strongly infection-enhancing
229 antibodies¹²⁻¹⁴. The EDE1 is also better suited than the related EDE2 epitope: although
230 the EDE1 Mabs require an E dimer to bind, the actual binding determinants are
231 centered on the *b* strand and on the highly conserved, E-dimer-exposed elements of the
232 fusion loop, as shown by the comparison between their binding to DENV-2 and ZIKV sE.
233 The fact that EDE2 bnAbs rely heavily in their contact points on the adjacent subunit -
234 on the variable 150 loop in which glycosylation is not always present - is a drawback, as
235 demonstrated by their poor affinity (Fig. 1) and by their strong induction of ADE¹².

236 Targeting the *b* strand and the E-dimer exposed elements of the fusion loop appears as
237 a powerful alternative to the multi-immunogen approaches against the DENV cluster
238 that have had limited success in clinical trials²⁷. As the E protein polypeptide chain
239 displays neither insertions nor deletions in the region of the *b* strand for any medically
240 relevant flavivirus, this region presents a low risk of inducing escape mutations, most
241 likely because it is also the interacting site with prM during virus maturation. Finally, in
242 a more immediate application, our study also suggests that the EDE1 antibodies -
243 perhaps carrying the "LALA" mutation ²⁸ if effector functions are to be avoided - could
244 be useful for immune prophylaxis for pregnant women at risk of contracting ZIKV
245 infection.

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344 Government's "Investissements d'Avenir" program). FAR and KS also acknowledge the
345 transnational ANR/FWF grant FlaviStem/I1378.

346 **Author contributions:** GBS did the first neutralization experiments and contributed
347 throughout the project. VMCL, AS and ESL provided ZIKV from both strains, PF13 and
348 HD78788. IM, KS and FXH provided the recombinant sE protein. AR and AS prepared
349 recombinant antibody fragments; AR, GBS and WD did neutralization and binding
350 experiments; AR grew the crystals and collected synchrotron data together with AH;
351 MCV collected synchrotron data, processed the data, built, refined and analyzed the
352 atomic models. ESL did the phylogenetic analyses; AR, AS and PE did the BLI
353 experiments with recombinant sE. AR, GBS, KS, JM, FXH, GRS and FAR planned the
354 experiments. FAR wrote the paper.

355 **Author Information.** Coordinates and structure factors amplitudes have been
356 deposited in the Protein Data Bank under accession numbers XXX1, XXX2 and XXX3 for
357 ZIKV sE/EDE1 C8 scFv, ZIKV sE/EDE2 A11 Fab complexes and ZIKV sE, respectively.

358 **Competing financial interests.** The EDE antibodies and epitope are the subject of a
359 patent application by Imperial College and Institut Pasteur on which G.S., J.M., F.R., A.R.,
360 M.C.V. and G.B.S. are named as inventors.

361 **FIGURE LEGENDS:**

362 **Figure 1:** ZIKV/DENV E protein phylogeny and reactivity with DENV-elicited
363 antibodies. **a)** Phylogenetic trees of the main human pathogenic flaviviruses based on
364 the amino acid sequences of the E protein (left panel) and of the polymerase NS5
365 protein (right panel). The arthropod vectors are differentiated by the background color.
366 **b)** ZIKV sE reactivity with human recombinant IgG mAbs FLE P6B10, EDE1 C8 and
367 EDE2 A11. Left panel: Binding properties were monitored by Biolayer interferometry
368 on Octet RED (ForteBio). The normalized response values expressed as fraction of
369 binding site occupancy are plotted against concentrations of ZIKV sE dimer shown at
370 logarithmic scale. Lines denote global curve fits used for Kd evaluation (see ED Fig. 1a
371 for linear concentration range showing concentration dependent saturation fits).
372 Normalized response values were deduced from individual sensograms of binding
373 monitored at different ZIKV sE concentration (see right panel for EDE1 C8).

374 **Figure 2:** Neutralization curves using three antibodies each from the three subsets FLE,
375 EDE1 and EDE2. The results represent the mean of four independent experiments done
376 each in triplicate for PF13 and duplicate for HD78788 strains. The two ZIKV strains are
377 in bright colors, red and blue. The neutralization data for the 4 DENV serotypes (dotted
378 lines in pale colors) were taken from ref. ¹³, and are given here for comparison. The
379 corresponding IC50 values are provided in Table 1. Note that the DENV4 strain used
380 was a natural isolate lacking the N153 glycosylation site.

381 **Figure 3:** EDE1 C8 / ZIKV sE complex. **a)** Overall view of the complex, with the sE
382 moiety colored by domains (I, II and III in red, yellow and blue, respectively, and the
383 fusion loop in orange); the antibodies in grey and dark green for light and heavy chains,
384 respectively. The CDRs are colored (H1 light blue, H2 sand, H3 pink, L1 light gray, L2
385 red, L3 orange). The inset shows a comparison with the corresponding DENV-2
386 complex. For clarity, the variable region of the C8 Fab fragment of the DENV-2 sE/C8
387 complex was superposed on the scFv in complex with ZIKV sE in order to draw the Fab
388 axis and better show the docking angles. **b)** Zoom of the ZIKV sE/C8 interaction to show
389 the recognition of the *b* strand. Hydrogen bonds are shown as dotted lines and
390 immobilized water molecules as red spheres. **c)** Same region on the DENV-2 sE/C8 Fab
391 complex. Note that the N67 glycan on DENV also interacts with the antibody. **d)** The
392 footprint of EDE1 C8 is outlined on ZIKV sE dimer shown in surface representation
393 (looking from outside the virion) colored according to conservation of surface exposed
394 amino acids. Atoms from the main chain and conserved side chains are orange, highly
395 similar side chains are yellow and all the others are white. **e, f)** Footprints of EDE1 C8
396 on a surface representation of ZIKV sE (**e**) and DENV-2 sE (**f**) shown in pink. The two
397 protomers of sE in the dimer are in light and dark gray. Relevant antigenic sE regions

398 are labeled. Note the more confined interacting surface in ZIKV sE dimer than DENV-2,
399 eg. N67 glycan is absent in ZIKV sE.

400 **Figure 4:** EDE2 A11 / ZIKV sE complex. Color coding is as in Fig. 3. **a)** Overall view of
401 the complex, with only one Fab bound per sE dimer, due to crystal packing. The dashed
402 ellipse represents the position of the missing A11 Fab. The inset compares the angle of
403 binding to the sE dimer in ZIKV and in DENV-2. **b,c)** Interactions at the *b* strand in ZIKV
404 (left panel) and in DENV-2 (right panel). Note the different angle of the *b* strand with
405 respect to the antibody (the antibody is exactly in the same orientation in both panels)
406 **d,e)** Zoom of the glycan on the 150 loop for ZIKV sE (d) and for DENV-2 sE (e), with
407 sugar residue numbers described in the key. The CDR H3 helix is too far to make
408 interactions with the glycan, as is the case in the DENV-2 structure (see ED Figs 2b and
409 5b).

410 **METHODS**

411 **Recombinant production of ZIKV sE protein.** Recombinant Zika virus sE protein
412 (strain H/PF/2013, GenBank accession no. KJ776791) was produced with a tandem
413 strep-tag in the Drosophila Expression System (Invitrogen) as described previously^{29,30}.
414 A chemically synthesized DNA fragment (GeneArt) containing the Zika sE sequence
415 (amino acid 1-408) was cloned into the expression vector pT389³¹ that encodes the
416 export signal sequence BIP, an enterokinase cleavage site and the strep-tag. Drosophila
417 Schneider 2 cells were stably transfected using blasticidin for selection. Protein
418 expression was induced by the addition of CuSO₄ and supernatants were harvested 7-10
419 days after induction. Antigens were purified via affinity chromatography with
420 Streptactin columns (IBA) according to the manufacturer's instructions. A final
421 purification gel filtration step used a Superdex increase 200 10/300 GL column
422 equilibrated in 50 mM Tris pH8, 500 mM NaCl.

423 **Production of antigen-binding (Fab) and single-chain Fv (scFv) fragments of the**
424 **bnAbs.** The bnAb fragments were cloned into plasmids for expression as Fab³² and
425 scFv³³ in Drosophila S2 cells. The constructs contain a tandem strep tag fused at the C-
426 terminus (only of the heavy chain in the case of the Fab) for affinity purification. The
427 purification protocol included a Streptactin affinity column followed by gel filtration as
428 described above.

429 **Expression of human monoclonal anti-DENV E antibodies.** Full IgG antibodies were
430 produced in 293T cells after co-transfection of plasmids containing heavy and light
431 chains of immunoglobulin G1 as described in ref.¹³.

432 **Immune complex formation and isolation.** The purified ZIKV sE protein was mixed
433 with Fab A11 or scFv C8 (in approximately twofold molar excess) in standard buffer
434 (500 mM NaCl, Tris 50 mM pH 8.0). The volume was brought to 0.5 ml by centrifugation
435 in a Vivaspin 10 kDa cutoff; after 30 min incubation at 4°C, the complex was separated
436 from excess Fab or scFv by size-exclusion chromatography (SEC) for ZIKV sE and scFv
437 C8. For ZIKV sE and Fab A11 no apparent complex formation could be seen in SEC;
438 therefore a solution containing sE at a concentration of 1.5 mg/ml and Fab A11 at a
439 concentration of 3 mg/ml (corresponding to a molar ratio ~ 1:2 antigen:antibody) was
440 directly used for crystallization. In all cases, the buffer was exchanged to 150 mM NaCl,
441 15 mM Tris, pH 8 for crystallization trials. The protein concentrations used for
442 crystallization, determined by measuring the absorbance at 280 nm and using an
443 extinction coefficient estimated from the amino-acid sequences, are listed in Extended
444 Data Table 1.

445 **Real-time biolayer interferometry binding assays.** The interactions of purified ZIKV
446 E protein with Mabs IgG FLE P6B10, IgG EDE1 C8, IgG EDE2 A11, and control Mabs IgG
447 28C (an anti-Influenza virus) were monitored in real-time using a Bio-layer
448 interferometry Octet-Red384 device (Pall ForteBio). Anti-human IgG Fc capture
449 biosensors (Pall ForteBio) were loaded for 10min at 1000 rpm shaking speed using
450 antibodies at 5µg/ml in assay buffer (PBS+0.2 mg/ml BSA + tween 0.01%). Unbound
451 antibodies were washed away for 1 min in assay buffer. IgG-loaded sensors were then
452 incubated for 15 min at 1200 rpm in the absence and presence of two fold serially
453 diluted ZIKV sE protein concentrations in assay buffer. Molar concentrations were
454 calculated for the sE protein in a dimeric form. For Mabs FLE P6B10, EDE1 C8 and
455 EDE2 A11, the following ZIKV sE concentration ranges : 50 - 0.78 nM, 200 - 3.125 nM
456 and 3200 - 50 nM, were respectively used. Reference binding experiments were carried
457 out in parallel on sensors loaded with control IgGs 28C. Dissociation of the complexes
458 formed was then monitored for 10 min by dipping sensors in assay buffer alone.
459 Operating temperature was maintained at 25 °C. The real-time data was analyzed using
460 Scrubber 2.0 (Biologic Software) and Biaevaluation 4.1 (GE Healthcare). Specific signals
461 were obtained by double-referencing, ie subtracting non-specific signals measured on
462 non-specific IgG-loaded sensors and buffer signals on specific IgG-loaded sensors.
463 Association and dissociation profiles, as well as steady-state signal vs concentration
464 curves, were fitted assuming a 1 :1 binding model.

465 **Crystallization and X-ray structure determinations.** The crystallization and cryo-
466 cooling conditions for diffraction data collection are listed in Extended Data Table 1.
467 Crystallization trials were performed in sitting drops of 400 nl. Drops were formed by
468 mixing equal volumes of the protein and reservoir solution in 96 wells Greiner plates,
469 using a Mosquito robot and monitored by a Rock-Imager. Crystals were optimized using

470 a robotized Matrix Maker and Mosquito setups on 400 nl sitting or hanging drops, or
471 manually in 24-well plates using 2–3 μ l hanging drops.

472 Because of the strong anisotropy of the crystals (see results for anisotropy in Extended
473 Data Table 1), an important number of crystals was tested at several beam lines at
474 different synchrotrons (SOLEIL, St Aubin, France; ESRF, Grenoble, France; SLS, Villigen,
475 Switzerland). The crystals having the less anisotropic diffraction data were used to
476 solve the structures. The datasets were indexed, integrated, scaled and merged using
477 XDS³⁴ and AIMLESS³⁵. A preliminary model of ZIKV sE protein was built from the DENV-
478 2 sE (4UTA) structure using the structure homology-modeling server SWISS-MODEL³⁶.
479 The structures of the complexes were then determined by molecular replacement with
480 PHASER³⁷ using the search models listed in Extended Data Table 1. AIMLESS and
481 PHASER programs were used within the CCP4 suite³⁸.

482 The DEBYE and STARANISO programs developed by Global Phasing Ltd. were applied
483 to the AIMLESS scaled data without truncation of the resolution, using the STARANISO
484 server (<http://staraniso.globalphasing.org/>). These softwares perform an anisotropic
485 cut-off of merged intensity data with a Bayesian estimation of the structure amplitudes,
486 and apply an anisotropic correction to the data. These corrected anisotropic amplitudes
487 were then used for further refinement of both structures with BUSTER/TNT³⁹. Please
488 note that the Extended Data Table 1 shows the refinement statistics for the full sets of
489 reflections truncated at the best high-resolution along h, k or l axis, values given by
490 AIMLESS before the anisotropic corrections computed by STARANISO software.

491 The models were then alternatively manually corrected and completed using COOT⁴⁰
492 and refined using BUSTER/TNT against the amplitudes corrected for anisotropy.
493 Refinements were constrained using non-crystallographic symmetry. The refined
494 structures ZIKV sE / EDE1 C8 scFv, ZIKV sE / EDE2 A11 Fab and ZIKV sE have a final
495 Rwork/Rfree (in %) of 19.2/22.1 and of 21.8/23.8 and of 20.8/23.6, respectively (see
496 Extended Data Table 1).

497 **Analysis of the atomic models and illustrations.** Each complex was analyzed with the
498 CCP4 suite of programs and the polar contacts were computed with the PISA website⁴¹.
499 For the intermolecular interactions shown in Extended Data Figures 2 and 3 and
500 Extended Data Tables 4 and 5, the maximal cutoff distances used were 4Å and 4.75Å for
501 polar and van der Waals contacts, respectively. Multiple sequence alignments were
502 calculated using Clustal W and Clustal X version 2⁴² on the EBI server⁴³. All protein
503 structure figures were prepared using ESPript⁴⁴ and the PyMOL Molecular Graphics
504 System, version 1.5.0.4 (Schrödinger) (pymol.sourceforge.net).

505 **Phylogenetic trees.** The Maximum likelihood phylogenetic trees were inferred using 12

506 representative amino-acid sequences of flaviviruses envelope protein E or RNA-
507 polymerase NS5 proteins, utilizing the LG model available in PhyML⁴⁵ and a
508 combination of SPR+NNI branch-swapping. Bootstrap values were calculated from 100
509 bootstrap replicates. The trees were visualized using Figtree
510 (<http://tree.bio.ed.ac.uk/software/figtree/>). The accession codes of sequences used in
511 the tree : Zika virus (ZIKV, KJ776791, strain H-PF-2013_French_Polynesia); dengue
512 virus serotype 1 (DENV-1, NC_001477); dengue virus serotype 2 (DENV-2, NC_001474);
513 dengue virus serotype 3 (DENV-3, NC_001475); dengue virus serotype 4 (DENV-4,
514 NC_002640); Saint Louis encephalitis virus (SLEV, NC_007580); Japanese encephalitis
515 virus (JEV, NC_001437; Murray Valley encephalitis *virus* (MVEV, NC_000943); West Nile
516 virus (WNV, NC_001563); yellow fever virus (YFV, NC_002031); tick-borne encephalitis
517 virus (TBEV, NC_001672); Powassan virus (POWV, NC_003687).

518 **Viral stocks.** The African strain Zika HD78788 was obtained from the Institut Pasteur
519 collection and the Asian strain Zika PF13, isolated from a patient during ZIKV outbreak
520 in French Polynesia in 2013, was obtained through the DENFREE (FP7/2007-2013)
521 consortium. Viral stocks were prepared from supernatant of infected C6/36 cells (ATCC
522 CRL-1660) clarified by centrifugation at 3000 g at 4°C and titrated on Vero cells (ATCC
523 CRL-1586) by a focus-forming assay. Stocks were kept at -80°C until use. All cell lines
524 were free from mycoplasma contamination.

525 **Neutralization Assays.** Virus neutralization by the tested human antibodies was
526 evaluated using a focus reduction neutralization test (FRNT). About 100 ffu (focus
527 forming unit) from virus stocks were incubated with a serial dilution of antibody for 1h
528 at 37°C. The mixture was then added to Vero cells and foci were let to develop in
529 presence of 1.5% methylcellulose for two days. Foci were then stained after fixation
530 with 4% formaldehyde using anti-E 4G2 antibody (ATCC HB-112) and anti-mouse HRP-
531 conjugated secondary antibody (ThermoFisher 31430). The foci were visualized by
532 diaminobenzidine (DAB) (Sigma D5905) staining and plates were counted using the
533 ImmunoSpot S6 Analyser (Cellular Technology Limited, CTL). Neutralization curves and
534 50% FRNT were calculated by non-linear regression analysis using Prism 6 software,
535 GraphPad software.

536

537 **METHODS REFERENCES**

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586

587

588 **EXTENDED DATA FIGURE LEGENDS:**

589

590 **Extended Data Figure 1.** Antibody binding to recombinant ZIKV protein.

591 **a)** Biolayer interferometry experiments plotted on a linear scale. The antibodies were
592 immobilized on the biosensor tip, and the ZIKV sE protein was in solution at the
593 indicated concentrations. The antibody used is indicated in each plot. Note that the
594 horizontal scale is different for the three antibodies. The estimated dissociation
595 constant (Kd) and the estimated dissociation rate (Koff) are indicated.

596 **b)** Size exclusion chromatography results for isolated sE, isolated Fab fragments, and
597 ZIKV sE + Fab fragments, as indicated.

598

599 **Extended Data Figure 2.** Residues involved in bnAb/antigen interactions.

600 **a)** Antibody contacts on the amino acid sequence alignment of ZIKV and DENV-2 sE. A
601 red background highlights identical residues. Secondary structure elements are
602 indicated together with their labels above (ZIKV) and below (DENV-2) the sequences.
603 The domain organization of ZIKV and DENV-2 sE is symbolized by a colored bar above
604 the sequences (domain I red, domain II yellow, domain III blue and the fusion loop
605 orange). Residues involved in polar and van der Waals protein-protein contacts are
606 marked using blue and green symbols, respectively, as indicated in the inset key,
607 displayed above and below the alignment for ZIKV and DENV-2 sE, respectively. Full
608 and empty symbols correspond to antibody contacts on the reference subunit of sE
609 (defined as the one contributing the fusion loop to the epitope) and the opposite
610 subunit of sE, respectively. Residues contacted only by the heavy or light chain are
611 marked with squares or triangles, respectively, and those contacted by both antibody
612 chains with circles. The details of the amino acid contacts are listed in the ED Tables 4
613 and 5. Dots above the sequences mark every 10 residues on the ZIKV sE sequence.
614 Disulfide bridges are numbered in green above the sequences.

615 **b)** Amino acid sequence of the heavy and light chains variable domains (vH and vL) of
616 bnAbs EDE1 C8 (top) and EDE2 A11 (bottom) with the framework (FRW) indicated by
617 black bars and IMGT CDR regions by thin dashed lines. The secondary structure
618 elements of the Ig vH and vL β -barrels are indicated above the sequences. Somatic
619 mutations are in red and residues arising from recombination at the V-D-J junction are
620 in green. Symbols above and below the sequences mark residues involved in contacts
621 with ZIKV and DENV-2 sE, respectively, coded for the contacted site in sE as indicated in
622 the key (inset at the bottom). Polar and van der Waals contacts are shown in blue and
623 green, respectively. The antibody residues contacting the reference sE subunit (defined
624 as the one contributing the fusion loop to the epitope) are marked by plain color
625 symbols while those making contact across the dimer interface by empty colored

626 symbols. Red boxes highlight the contacts found in the DENV-2 sE complex and absent
627 in the ZIKV sE complex, involving N67 glycan, *kl* and 150 loops. The details of the polar
628 contacts are listed in the Extended Data Tables 4 and 5 (see also Figs 3e and 3f). The
629 predicted vH and vL germline alleles are indicated with the corresponding CDR lengths
630 (see Table 1 in ref. 16).

631

632 **Extended Data Figure 3.** Details of EDE1 C8 bnAb contact across the dimer interface.

633 **a)** Overall view of the ZIKV sE/EDE1 C8 scFv complex. The box indicates the region
634 zoomed in b).

635 **b)** Details of the interactions of the C8 light chain with domain III across the dimer
636 interface.

637 **c)** Same region for the DENV-2 sE/EDE1 C8 Fab complex. Note that the sE residues
638 involved are different.

639 **d)** The complex rotated by 120 degrees (as indicated by the arrow) to show the
640 interaction in the *ij* loop, enlarged in e).

641 **e)** The *ij* loop is displayed in sticks, in order to show the interaction of its main chain
642 with the antibody. Domain II from the subunit across is colored green to distinguish
643 from domain II of the reference subunit; the dashed sticks for the Arginine shown is to
644 indicate that it has poor electron density in the crystal.

645 **f)** Same view of the complex with DENV-2. Note that the residues from across the dimer
646 interface that contact the antibody are different. The residues in the various CDRs are
647 colored coded, matching their label color (as in Figures 3 and 4).

648

649 **Extended Dta Figure 4.** Surface electrostatic potential on an open-book representation
650 of the immunocomplexes. The electrostatic potential is colored according to the bar
651 underneath. The antibody footprints are outlined in green. The disordered 150 loop in
652 the complex with C8 (left panels) results in a positive surface patch at one edge of the
653 epitope, which is counteracted by the residues in the 150 loop, as shown on the right
654 hand panel, in the complex with A11 where this loop is ordered.

655

656 **Extended Data Figure 5.** Details of the A11 interaction with the glycan on the 150 loop.

657 **a)** superposition of the ZIKV sE/A11 complex (in colors) on the E protein from the cryo-
658 EM structure of the mature virion (ref. 5) (PDB code 5IRE) in white. The E-protein was
659 superimposed on the tip of domain II of the reference subunit together with domain III
660 from the opposite subunit. It shows that the 150 loop adopts essentially the same
661 conformation, although fewer sugar residues are visible in the absence of the antibody.

662 **b)** Superposition of the ZIKV sE/A11 complex (in colors) on the DENV-2 sE/A11
663 complex (in white). The variable domains of the antibody from the two structures were
664 superimposed on each other. Note that in DENV-2 the glycan packs against the α -helix
665 of the CDR H3, whereas in ZIKV sE the glycan is too far to make the same interaction.

666 **c)** The ZIKV sE/C8 complex (in pink) was superimposed on the ZIKV sE/A11 complex
667 (in colors), to show the clash of the C8 light chain with the glycan, forcing it to move out
668 of the way and be disordered. The superposition also shows that EDE1 C8 reaches
669 further in to contact the *ij* loop and the *kl* loop of the adjacent subunit, as well as domain
670 III. As in a), the superposition was done using the tip of domain II of the reference
671 subunit and domain III of the adjacent subunit in the dimer as anchors. The two black
672 asterisks mark the places where the electron density of the 150 loop is lost, resulting in
673 no density in the ZIKV sE/C8 crystal for the short helix, nor for the glycan.

674

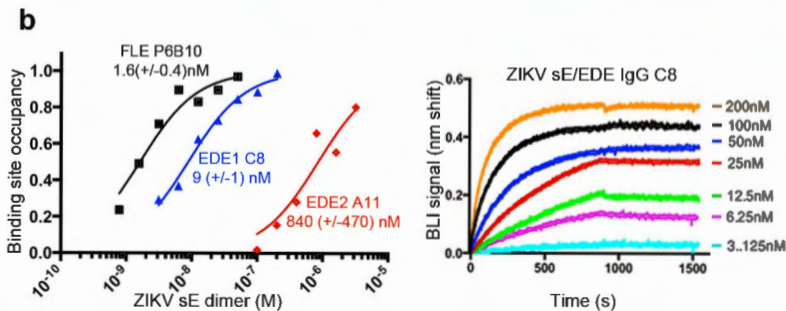
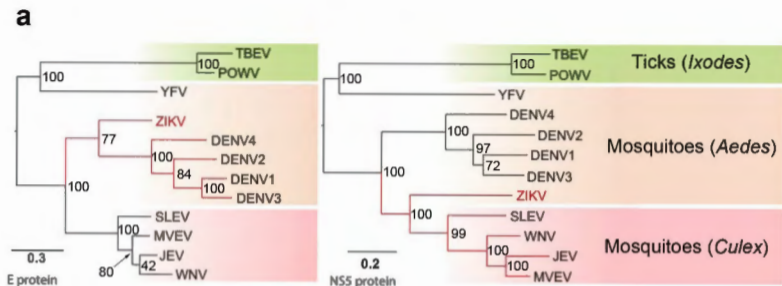
Figure 1

Figure 2

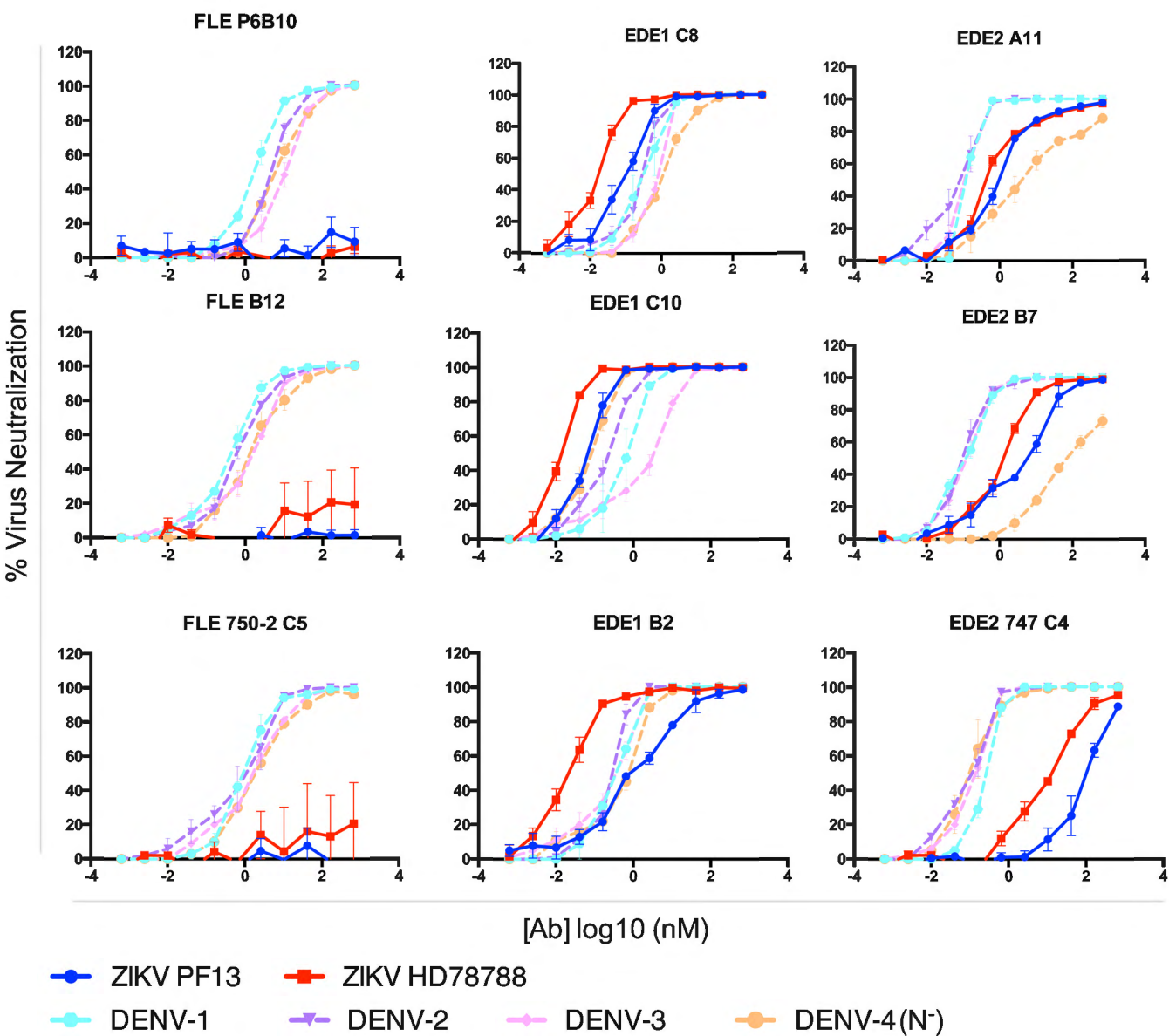
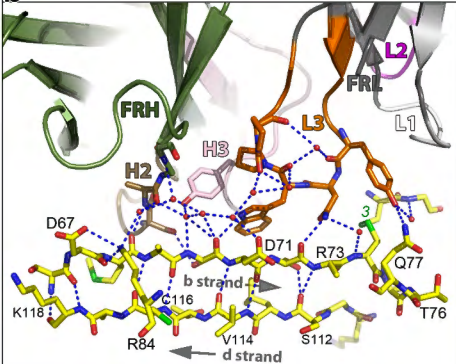


Figure 3. Structure of ZIKV sE / EDE1 C8 ScFv complex.

a

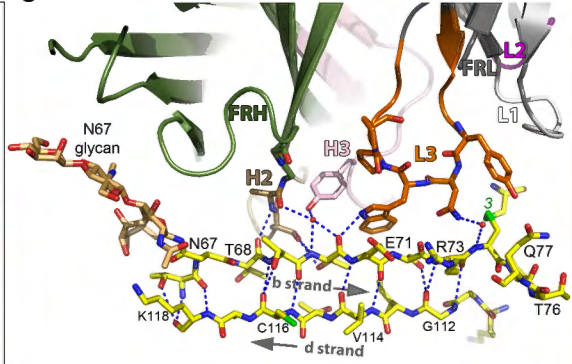


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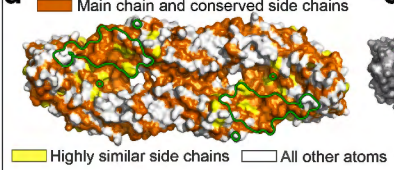
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c



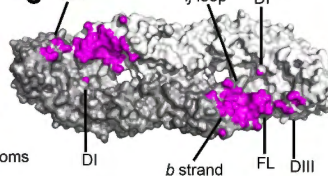
DENV-2 sE / EDE1 C8 Fab

d



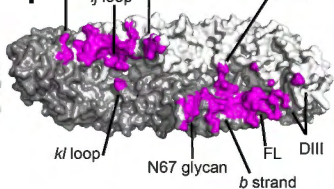
ZIKV/DENV amino acid sequence conservation of virion exposed residues

e



Footprint of EDE1 ScFv C8 on ZIKV sE

f



Footprint of EDE1 Fab C8 on DENV-2 sE

Figure 4. Structure of ZIKV sE / EDE2 A11 Fab complex.

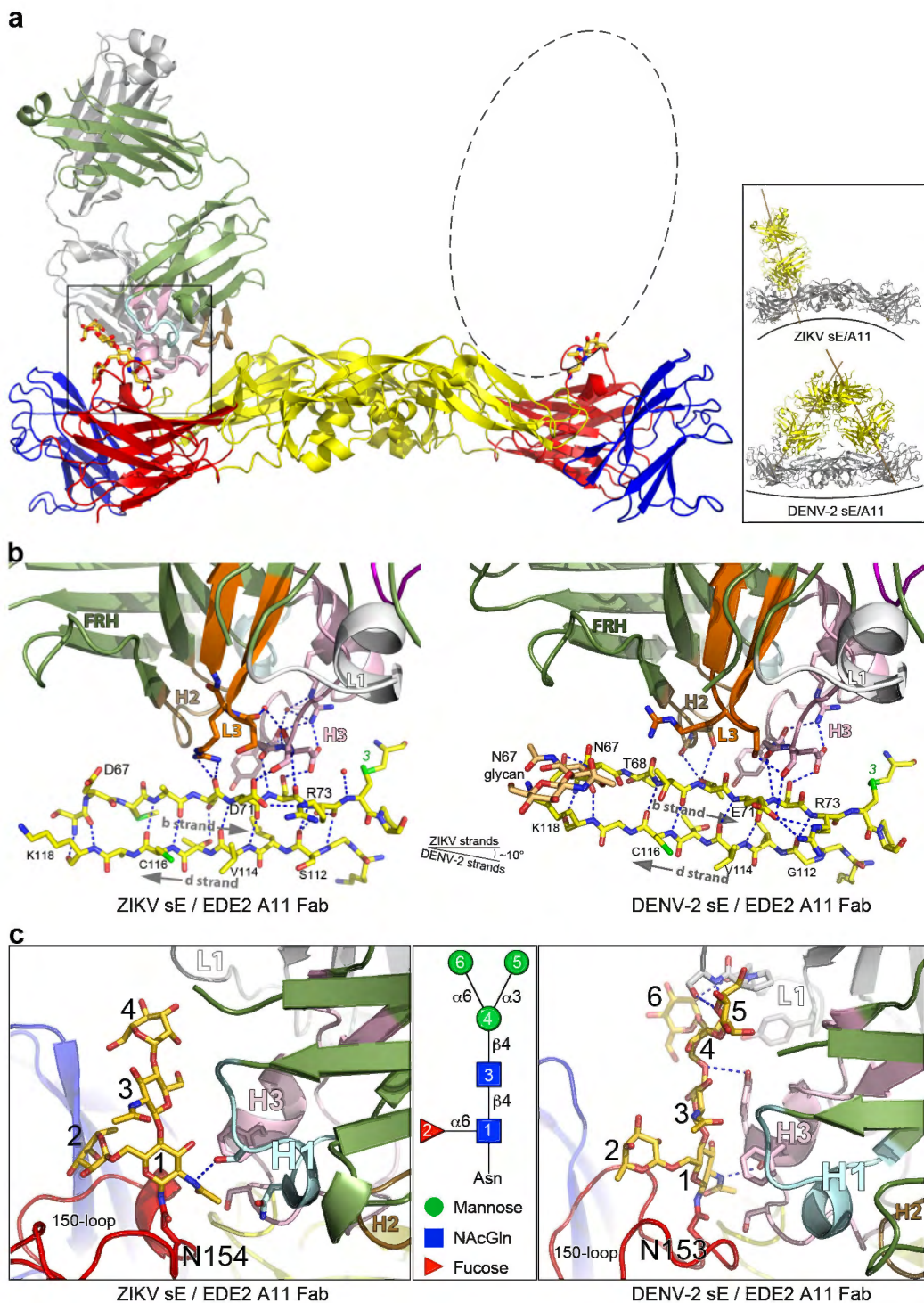
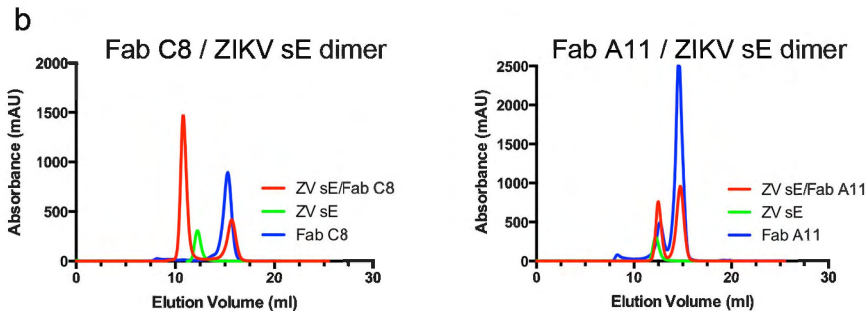
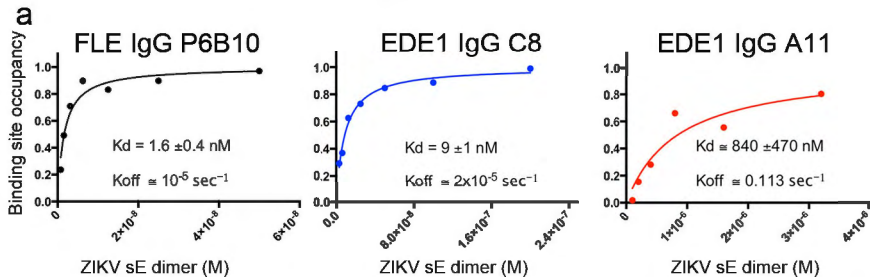


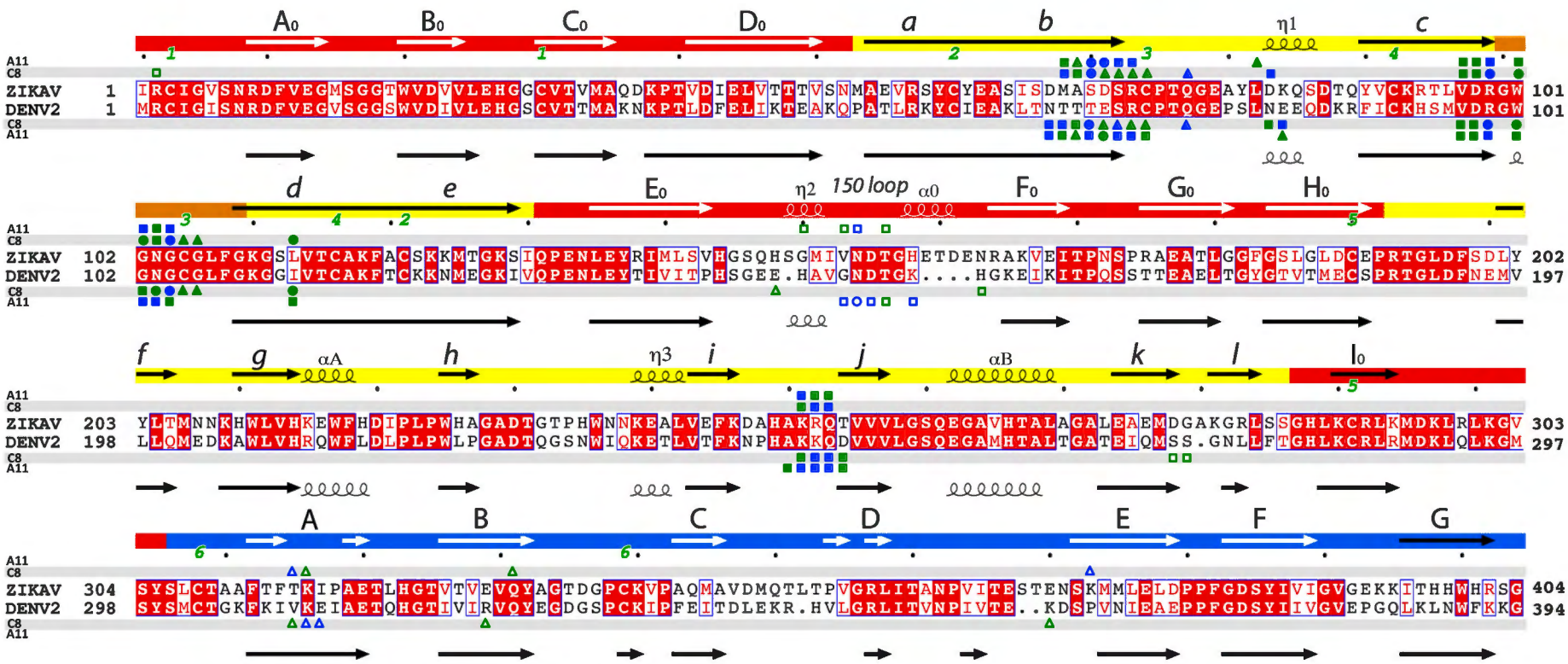
Table 1: 50% FRNT values of EDE1, EDE2, and FLE antibodies tested against ZIKV and DENV 1-4

	Epitope	ZIKV		DENV			
		50% FRNT (nM)		50% FRNT (nM)			
		PF13	HD78788	DENV1	DENV2	DENV3	DENV4
752-2-C8	EDE1	0.095 (±0.026)	0.015 (±0.003)	0.39 (±0.21)	0.24 (±0.06)	0.64 (±0.08)	1.13 (±0.14)
753(3) C10	EDE1	0.063 (±0.016)	0.013 (±0.025)	0.54 (±0.04)	0.18 (±0.02)	1.89 (±0.79)	0.08 (±0.03)
752-2 B2	EDE1	1.062 (±0.362)	0.021 (±0.004)	0.32 (±0.05)	0.23 (±0.02)	0.22 (±0.09)	0.44 (±0.14)
747(4) A11	EDE2	0.904 (±0.191)	0.506 (±0.102)	0.11 (±0.01)	0.07 (±0.03)	0.11 (±0.02)	7.79 (±3.19)
747(4) B7	EDE2	4.31 (±1.47)	1.17 (±0.180)	0.10 (±0.01)	0.11 (±0.02)	0.12 (±0.03)	93.19 (±19.15)
747 C4	EDE2	102 (±25.6)	11.6 (±2.6)	0.23 (±0.02)	0.08 (±0.01)	0.11 (±0.01)	0.12 (±0.05)
758 P6B10	FLE	No Neut.	No Neut.	1.85 (±0.44)	4.97 (±0.28)	9.40 (±2.83)	7.47 (±1.65)
749 B12	FLE	No Neut.	No Neut.	0.43 (±0.12)	0.73 (±0.20)	1.04 (±0.31)	1.80 (±0.64)
750-2 C5	FLE	No Neut.	No Neut.	1.08 (±0.21)	0.76 (±0.46)	1.40 (±0.25)	2.30 (±0.02)

ED Fig. 1

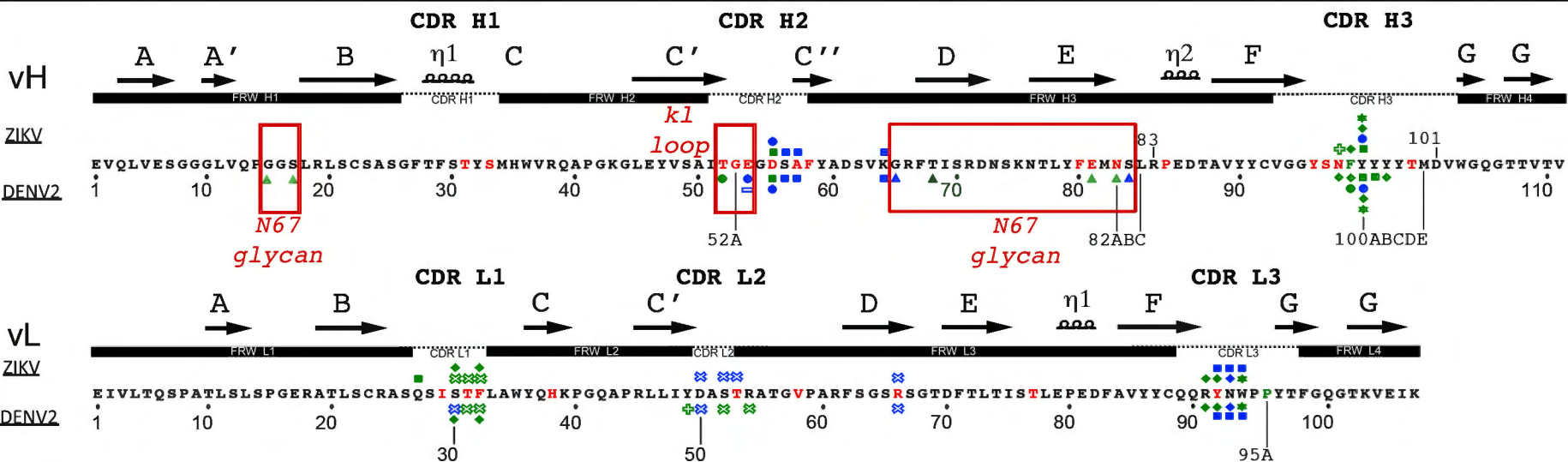


ED Figure 2

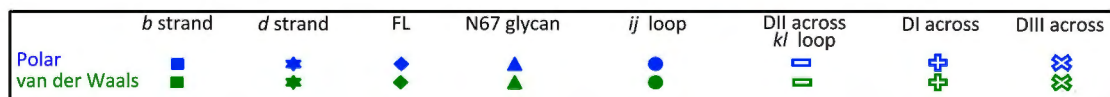
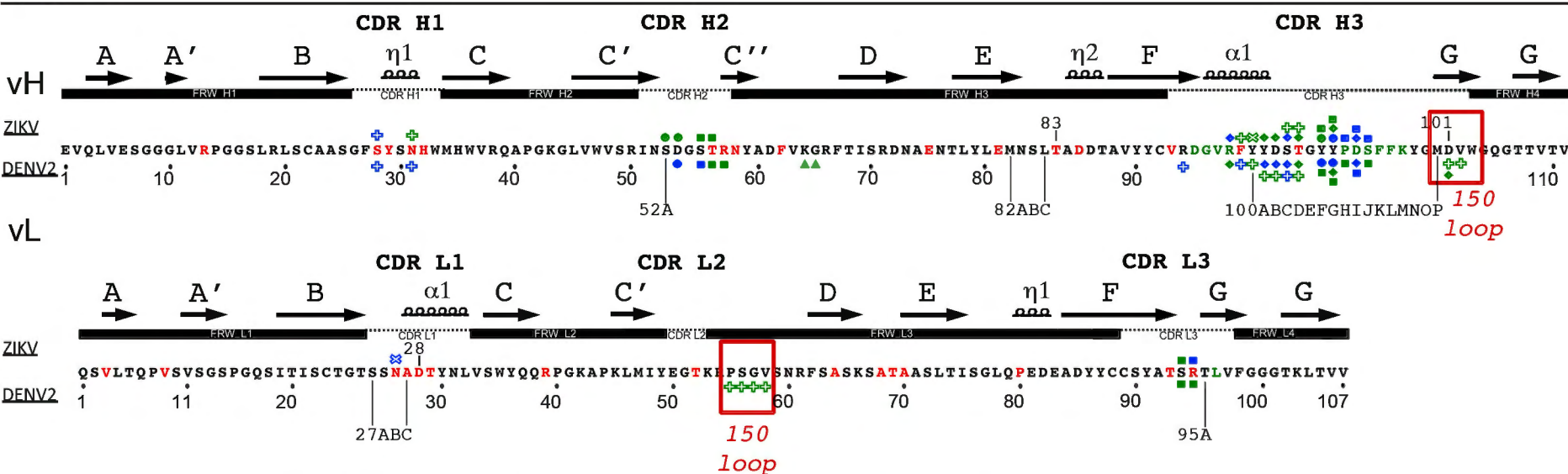


	Ref. E subunit			E subunit across		
	Heavy chain	Light chain	Heavy + Light chains	Heavy chain	Light chain	Heavy + Light chains
Polar	■	▲	●	□	△	○
van der Waals	■	▲	●	□	△	○

C8

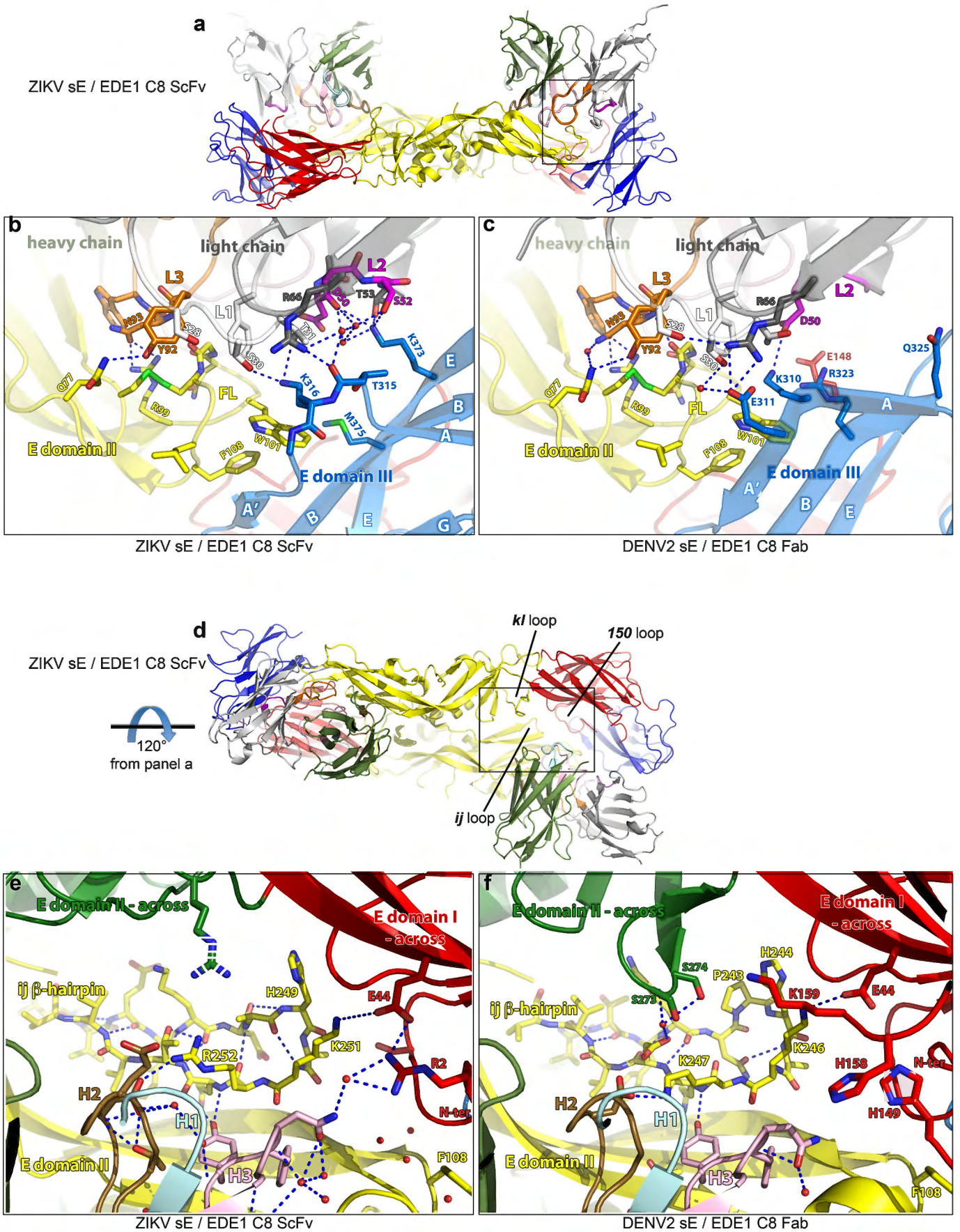


A11



ED Fig 4

Detailed interactions between ZIKA sE / EDE1 C8 ScFv and DENV-2 sE / EDE1 C8 Fab.

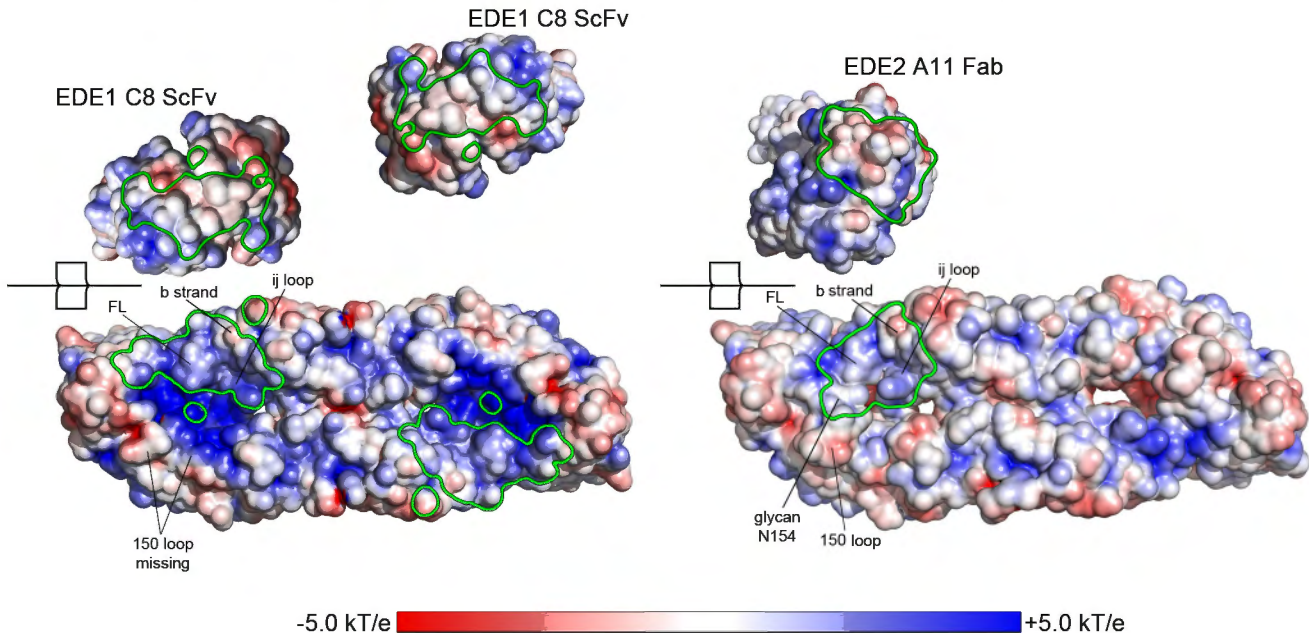


ED Fig. 5

Electrostatic potential of epitopes on ZIKV sE dimers and paratopes on EDE Abs.

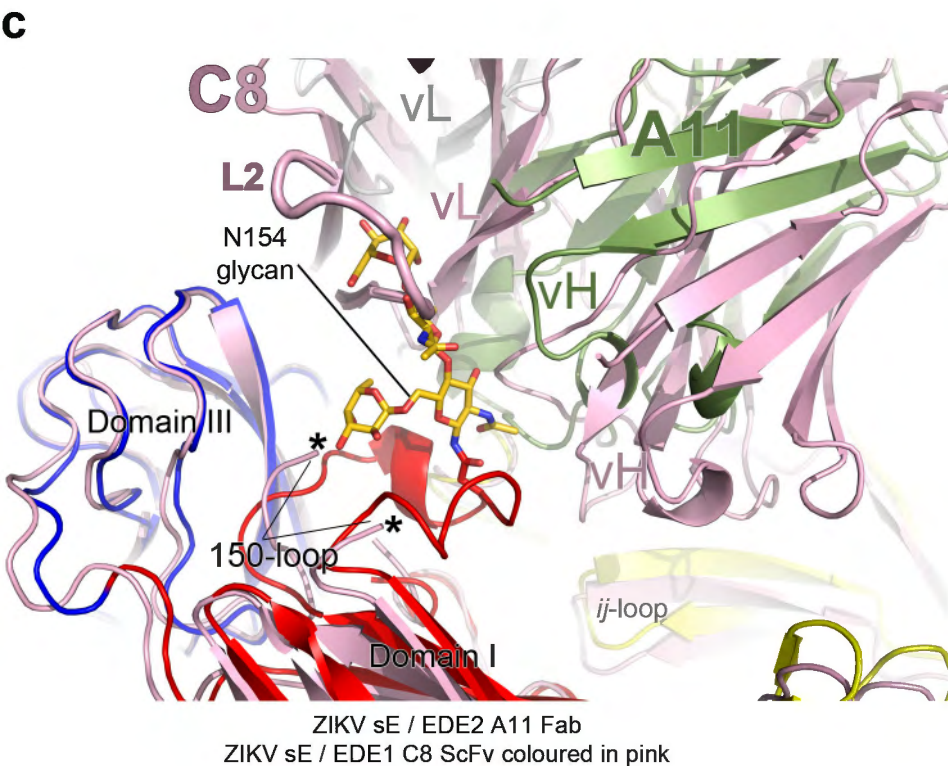
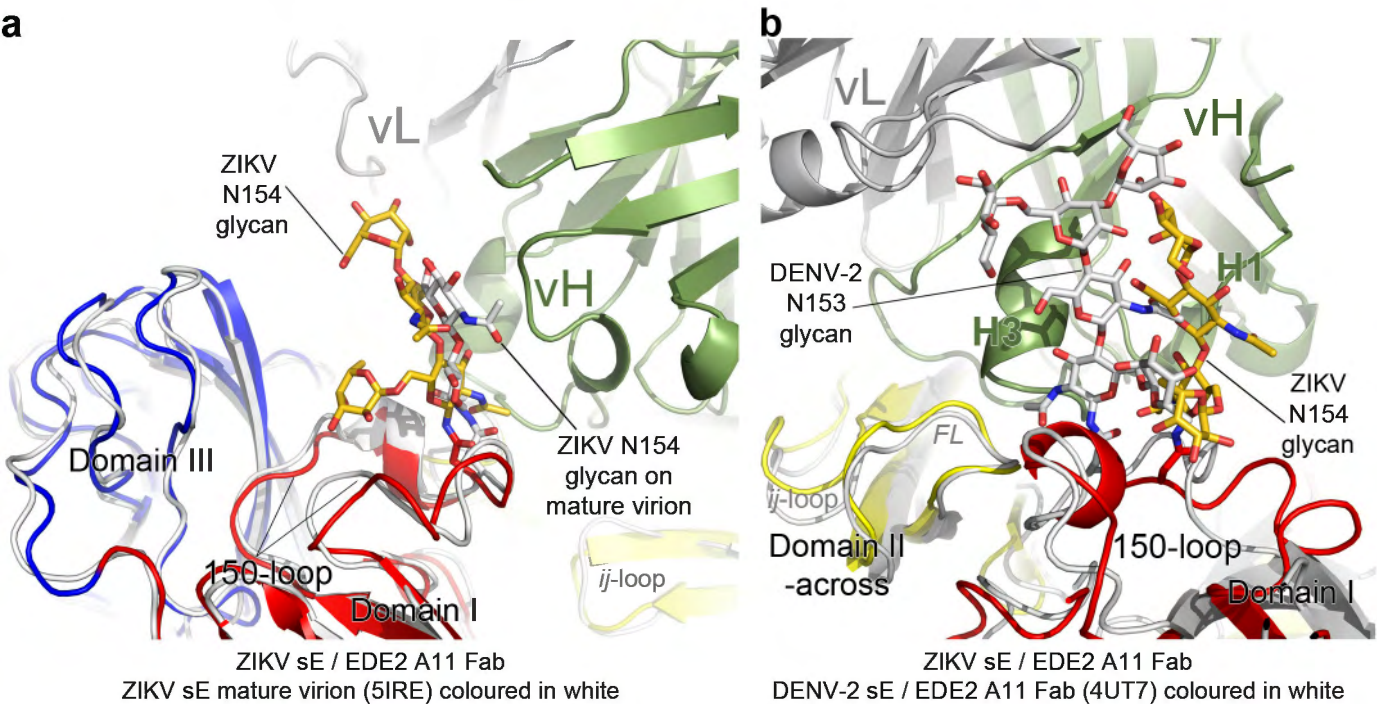
ZIKV sE / EDE1 C8 ScFv

ZIKV sE / EDE2 A11 Fab



ED Fig. 6

Zoom view of the glycans in ZIKV and DENV-2 complexes with EDE2 A11 Fab and in the ZIKV mature virion.



Extended Data Table 1. Crystallization conditions, data collection and refinement statistics.

	ZIKV sE / EDE1 C8 ScFv	ZIKV sE / EDE2 A11 Fab	ZIKV sE
Protein Data Bank code	to update	to update	to update
Crystallization conditions			
Protein conc. (mg/ml)*	1.3 (complex)	1.5 (ZIKV sE) 3 (EDE2 A11 Fab)	1.4
Crystallization buffer	1.26 M (NH ₄) ₂ SO ₄ , 0.1 M CHES pH 9.5, 0.2 M NaCl	3.5 M Na Formate, 0.1 M Tris pH 8.5	27% PEG 8K, 0.1M Hepes pH 8.3
Crystallization method	sitting drop at 18°C	sitting drop at 18°C	hanging drop at 18°C
Cryo-protectant	20% ethylene glycol in 67% of crystallization solution	16% glycerol in 67% of crystallization solution	16% ethylene glycol in 67% of crystallization solution
Data Collection ^{§§}			
Beamline	SOLEIL, Proxima 1	SOLEIL, Proxima 2	ESRF, ID23-2
Detector	Pilatus 6M	Eiger 9M	Pilatus3 2M
Space group	P 2 ₁ 2 ₁ 2 ₁	C 2 2 2 ₁	C 2 2 2 ₁
Unit cell: a, b, c (Å)	60.8, 121.3, 257.8	204.3, 207.3, 124.6	65.7, 215.3, 124.5
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	40.0-2.41 (2.46-2.41)	40.0-2.64 (2.69-2.64)	40.0-3.08 (3.29-3.08)
Anisotropy direction [¶]			
Resolution where CC _{1/2} > 0.50			
overall (Å)	2,69	2,87	3,08
along h axis (Å)	3,24	4,23	4,84
along k axis (Å)	2,95	2,64	2,99
along l axis (Å)	2,41	2,9	3,03
Measured reflections	560 284 (35 314)	760 210 (39 040)	124 201 (23 147)
Unique reflections	74 842 (4571)	77 483 (4 547)	16 828 (3 020)
Completeness (%)	100 (100)	99.8 (99.4)	99.9 (100)
Mn(I) half-set correlation	0.99 (0.15)	0.98 (0.19)	0.99 (0.61)
Mean I/σ(I)	6.7 (1.4)	4.9 (0.4)	7.2 (1.1)
Multiplicity	7.5 (7.7)	9.8 (8.6)	7.4 (7.7)
B wilson (Å ²)	31,2	50,4	77,9
Rmerge	0.31 (2.5)	0.51 (6.6)	0.2 (1.9)
Rmeas	0.36 (3.3)	0.52 (6.9)	0.21 (2.1)
Rpim	0.13 (1.2)	0.16 (2.3)	0.08 (0.7)
Structure Determination			
MR search models	ZIKV sE from ZIKV sE / EDE2 A11 Fab complex (this work)	ZIKV model sE from DENV-2 sE (4UTA) EDE2 A11 ScFv (4UT7)	ZIKV sE from refined ZIKV sE / EDE1 C8 ScFv complex
NCS restraint	EDE1 C8 Fab variable domain (4UTA)	EDE2 A11 Fab constant domain (4UTB)	
Targeting	2	2 applied only on sE dimer	2
Number of TLS groups	No targeting	EDE2 A11 Scfv (4UT7)	sE (from ZIKV sE / EDE1 C8 ScFv)
	12	12	12
Refinement [§]			
Resolution cut-off (Å)	40.0-2.41	40.0-2.64	40-3.08
Rwork (%) / Rfree (%)	18.7 / 22.0	21.8 / 23.8	22.9 / 27.5
N° of Work / Free reflections	74 785 / 3 719	76 253 / 3 840	16 099 / 843
 atomic factors (Å) ²	72,6	89,3	142,1
N° of protein atoms	9595	9495	6118
N° of heteroatoms	212	43	0
Rms deviation from ideal			
Bond lengths (Å)	0,01	0,01	0,01
Bond angles (°)	1,22	1,28	1,29
Ramachandran ^{¶¶}			
Favoured (%)	96,5	93,24	91,92
Allowed (%)	3,26	5,62	4,42
Outliers (%)	0,24	1,14	3,66

The ZIKV sE buffer used for all the crystallization experiments was: 150mM NaCl and 15mM Tris pH 8.

* The protein concentration was estimated using theoretical extinction coefficients of the complexes (ZIKV sE + Fab or scFv). Absorbance at 280nm (A_{280 nm}) of the protein solution was measured before crystallization. The theoretical extinction coefficients for individual component are: ZIKV sE: 1.345; bnAb EDE2 A11 Fab: 1.68 (see Methods for more details); bnAb EDE1 C8 ScFv: 1.9. Extinction coefficients were calculated without taking into account carbohydrate moieties.

One crystal was used to collect a diffraction dataset for each complex to determine the structure.

[¶] The anisotropy statistics were computed with AIMLESS (REF).

[§] Highest resolution shell is shown in parenthesis.

^{§§} Low-resolution for data processing and refinements was truncated to 40 Å.

^{¶¶} Ramachandran statistics were calculated with MolProbity (REF).

Abbreviations used: MR: molecular replacement; NCS: non-crystallographic symmetry; TLS: parametrization describing translation, libration and screw-motion to model anisotropic displacements (REF); CC_{1/2}: correlation coefficient (REF); h,k and l: indices that define the lattice planes; I/σ(I) empirical signal-to-noise ratio; Rmeas, multiplicity-corrected R; Rpim, expected precision; Rms, root mean square.

Extended Data Table 2. Root mean square deviations between sE dimers in the various structures of ZIKV and DENV-2.

ZIKV [§]	PDB code	ZIKV sE (this work)	ZIKV sE/EDE2 A11 Fab	ZIKV sE/EDE1 C8 ScFv [#]	ZIKV sE (Dai et al, 2016)	ZIKV mature virion sE dimer from au	ZIKV mature virion icosahedral sE dimer	ZIKV thermally stable mature virion sE dimer from au	ZIKV thermally stable mature virion icosahedral sE dimer
					5JHM	5IRE	5IRE	5IZ7	5IZ7
ZIKV sE (this work)		/							
ZIKV sE/EDE2 A11 Fab		3.33 (796)	/						
ZIKV sE/EDE1 C8 ScFv [#]		2.31 (776) 2.29 (764)	2.41 (773) 2.29 (761)	0.57 (764) ^{##}					
ZIKV sE (Dai et al, 2016)	5JHM	1.8 (769)	4.1 (768)	3.06 (771) 2.93 (761)	/				
ZIKV mature virion sE dimer from au	5IRE	3.22 (800)	1.72 (799)	1.57 (778) 1.69 (764)	3.72 (775)	/			
ZIKV mature virion icosahedral sE dimer	5IRE	2.88 (800)	1.76 (799)	1.60 (778) 1.71 (764)	3.74 (775)	0.46 (806) ¹ 0.45 (1002) ²	/		
ZIKV thermally stable mature virion sE dimer from au	5IZ7	3.03 (800)	1.80 (799)	1.75 (778) 1.88 (764)	3.89 (775)	1.36 (806) ¹ 1.57 (1002) ²	1.36 (806) ¹ 1.56 (1002) ²	/	
ZIKV thermally stable mature virion icosahedral sE dimer	5IZ7	2.94 (800)	1.84 (799)	1.67 (778) 1.80 (764)	3.78 (775)	1.36 (806) ¹ 1.56 (1002) ²	1.36 (806) ¹ 1.54 (1002) ²	1.41 (806) ¹ 1.43 (1008) ²	/

ZIKV/DENV-2 [§]	PDB code	ZIKV sE (this work)	ZIKV sE/EDE2 A11 Fab	ZIKV sE/EDE1 C8 ScFv [#]	ZIKV sE (Dai et al, 2016)	ZIKV sE mature virion sE dimer from au	ZIKV sE mature virion icosahedral sE dimer	ZIKV thermally stable mature virion sE dimer from au	ZIKV thermally stable mature virion icosahedral sE dimer
					5JHM	5IRE	5IRE	5IZ7	5IZ7
DENV-2 sE	4UTC	4.57 (766)	6.47 (768)	5.49 (756) 5.55 (742)	4.75 (753)	5.94 (772)	5.94 (772)	6.23 (757)	6.18 (757)
DENV-2 sE/EDE2 A11 Fab	4UTB	4.45 (773)	7.08 (774) 0.29/0.28 (241) ³	5.61 (763) 5.57 (744)	4.2 (754)	6.29 (773)	6.28 (773)	7.38 (763)	7.31 (763)
DENV-2 sE/EDE1 C8 Fab	4UTA	6.04 (745)	8.17 (744)	6.88 (748) 6.78 (736)	5.71 (747)	7.52 (751)	7.49 (751)	7.65 (738)	7.57 (738)
DENV-2 mature virion sE dimer from au	3J27	3.89 (779)	2.26 (778)	2.36 (769) 2.81 (755)	4.82 (766)	2.01 (785) ¹ 2.09 (979) ²	2.04 (785) ¹ 2.13 (979) ²	4.33 (770) ¹ 2.15 (988) ²	4.36 (770) ¹ 2.20 (988) ²
DENV-2 mature virion icosahedral sE dimer	3J27	3.59 (779)	2.45 (778)	2.25 (769) 2.68 (755)	4.49 (766)	1.99 (785) ¹ 2.07 (979) ²	1.99 (785) ¹ 2.07 (979) ²	4.31 (770) ¹ 2.14 (988) ²	4.33 (770) ¹ 2.15 (988) ²

DENV-2 [§]	PDB code	DENV-2 sE	DENV-2 sE/EDE2 A11 Fab	DENV-2 sE/EDE1 C8 Fab	DENV-2 mature virion sE dimer from au	DENV-2 mature virion icosahedral sE dimer
		4UTC	4UTB	4UTA	3J27	3J27
DENV-2 sE	4UTC	/				
DENV-2 sE/EDE2 A11 Fab	4UTB	2.34 (775)	/			
DENV-2 sE/EDE1 C8 Fab	4UTA	2.93 (747)	1.53 (741)	/		
DENV-2 mature virion sE dimer from au	3J27	5.26 (779)	6.78 (775)	6.99 (754)	/	
DENV-2 mature virion icosahedral sE dimer	3J27	4.92 (779)	6.37 (775)	6.56 (754)	0.89 (790) ¹ 0.88 (990) ²	/

rmsd, root mean square deviation (in Å) computed with PyMOL software using the carbon alpha atoms of the sE dimers. In parenthesis, the number of carbon alpha atoms used for the calculation; PDB code, Protein Data Bank accession number; au, asymmetric unit

[§] The rmsd is computed using residues 1 to 403 for ZIKV sE or residues 1 to 395 for DENV-2 sE, except when indicated^{1,2}.

[#] There are two independent half dimer (ZIKV sE/EDE1 C8 ScFv) in the asymmetric unit.

The rmsd is computed between the two dimers of sE generated by crystallographic symmetry for each sE in the asymmetric unit.

^{##} This rmsd is computed between the two independent half dimers of sE in the asymmetric unit.

¹ The rmsd is computed between the sE dimers excluding stem and TM regions of ZIKV (residues 1 to 403) and DENV-2 (residues 1 to 395).

² The rmsd is computed between the sE dimers including stem and TM regions of ZIKV and DENV-2 (residues 1 to C terminal).

³ The two rmsd values refer to the superposition of the variable domains of Fab A11 in ZIKV sE/EDE2 A11 Fab on each Fab A11 in DENV-2 sE/EDE2 A11 Fab.

	BSA BNA Fab or ScFv		Total	BSA sE dimer				Complex		
	vH	vL		Reference subunit (glycans [#])	Opposite subunit (glycans [#])	Total	Main chain atoms [‡]	Total glycan BSA [€]	BSA / molecule	SC
ZIKV sE / EDE1 C8 ScFv fragment*										
ZIKV sE dimer Epitope dimer-1	426.1	494.8	920.9	653.0 (NA)	237.2 (NA)	890.2	346.9 (39%)	NA	905.5	0.683
ZIKV sE dimer Epitope dimer-2	438.4	492.4	930.8	678.1 (NA)	223.2 (NA)	901.3	343.9 (38%)	NA	916.0	0.738
DENV-2 sE / EDE1 C8 Fab fragment (4UTA)										
DENV-2 sE dimer Epitope A	718.9 (516.8)"	471.0 (471.0)"	1189.9	919.1 (192.9)	222.2	1141.3	357.8 (31%)	192.9 (17%)	1165.6	0.693
DENV-2 sE dimer Epitope B	831.9 (570.3)"	528.3 (528.3)"	1360.2	945.5 (230.9)	340.0	1285.5	359.0 (28%)	230.9 (18%)	1322.8	0.687
ZIKV sE / EDE2 A11 Fab fragment[€]										
ZIKV sE dimer Epitope	718.5	75.7	793.4	570.0 (0.0)	217.4 (134.6)	787.4	253.7 (32.2%)	134.6 (17%)	790.4	0.674
DENV-2 sE / EDE2 A11 Fab fragment (4UTB)										
DENV-2 sE dimer Epitope A	923.9 (251.3)	189.2 (148.8)	1113.0	531.8 (14.2)	472.6 (342.4)	1004.4	221.5 (22%)	356.6 (35%)	1058.7	0.706
DENV-2 sE dimer Epitope B	954.1 (302.2)	185.1 (136.7)	1138.8	460.3 (58.5)	571.3 (341.4)	1031.6	219.3 (21.2%)	400.0 (39%)	1085.2	0.668

BSA, buried surface area (in Å²) of sE protein by the Fabs or ScFv (calculated with the program 'areaimol' in CCP4).

BSA/molecule, average buried surface area between one Fab or one ScFv and the sE dimer.

SC, shape complementarity coefficient (calculated with the program 'sc' in CCP4).

The dots density used to compute both BSA and SC was set to 15 dots/Å². The van der Waals probe radius was set to 1.4 Å.

NA, Non applicable.

[#] In parenthesis, contribution of glycan chains to buried surface area : N154 (for ZIKV sE) and N67 and/or N153 (for DENV-2 sE).

" In parenthesis, the BSA was computed removing the glycan chains N67 for DENV-2 sE, in order to compare with the BSA of ZIKV sE which do not carry the N67 glycan.

[‡] Contribution of main-chain atoms to buried surface area; In parenthesis, contribution indicated as percentages.

* There are two independent half dimer (ZIKV sE / EDE1 C8 ScFv) in the asymmetric unit.

The two dimers of ZIKV sE (dimer-1 and dimer-2) are generated by applying crystallographic symmetry for each sE in the asymmetric unit.

[€] Only one Fab A11 binds to the sE dimer in ZIKV sE / EDE2 A11 Fab complex.

Extended Data Table 4. Polar and salt bridges interactions for ZIKV sE/EDE1 C8 ScFv, ZIKV sE/EDE2 A11 Fab, DENV-2 sE/EDE1 C8 Fab and DENV-2 sE/EDE2 A11 Fab.

domains	ZIKV sE / EDE2 Fab A11				DENV-2 sE / EDE2 Fab A11 (4UTB) epitope A				DENV-2 sE / EDE2 Fab A11 (4UTB) epitope B			
	sE	dist (Å)	Fab A11	CDR	sE	dist (Å)	Fab A11	CDR	sE	dist (Å)	Fab A11	CDR
domain I - Reference subunit	b-strand	S 70 [O]	2.96	R 95 [NH1]	L3							
		S 70 [O]	2.96	R 95 [NH2]	L3							
		D 71 [OD1]	3.72	R 95 [NH1]	L3							
		D 71 [OD1]	2.66	S 100J [OG]	H3							
		S 72 [OG]	3.21	D 100I [N]	H3							
		S 72 [OG]	2.71	D 100I [OD1]	H3							
		S 72 [O]	3.49	S 100J [N]	H3							
		S 72 [N]	3.39	P 100H [O]	H3							
	R 73 [N]	3.44	D 100I [OD1]	H3								
	R 73 [NE]	2.83	S 100J [OG]	H3								
	Fusion loop	R 99 [NH1]	2.78	D 100I [OD1]	H3							
R 99 [NH1]		3.47	D 100I [OD2]	H3								
R 99 [NH2]		3.60	D 100I [OD1]	H3								
R 99 [NH2]		2.70	D 100I [OD2]	H3								
G 102 [O]		3.16	S 100C [N]	H3								
G 102 [O]		3.25	S 100C [OG]	H3								
ij loop	G 104 [O]	3.78	R 98 [NH1]	H3								
	K 251 [O]	2.72	Y 100G [OH]	H3								
	K 246 [O]	2.77	Y 100G [OH]	H3								
	K 247 [NZ]	3.39	D 53 [OD1]	H2								
N67 glycan	no glycan at this position											
dom III	P 354 [O]	3.71	N 27B [ND2]	L1								
	V 153 [O]	3.5	S 100C [OG]	H3								
domain I - across	150 loop	G 152 [O]	3.62	S 100C [OG]	H3							
		D 154 [OD2]	2.79	N 31 [ND2]	H1							
	N153/ N154 glycan	D 154 [N]	3.17	S 100C [OG]	H3							
K 157 [NZ]		3.34	S 28 [OG]	H1								
	N154-1 [N2]	N153-1 [N2]	3.43	F 99 [O]	H3							
N154-1 [O3]		3.09	S 28 [OG]	H1								
	N153-4 [O3]	N153-3 [O3]	3.89	Y 100 [OH]	H3							
N153-4 [O4]		2.86	S 56 [OG]	L2								
N153-4 [O4]		3.64	S 56 [N]	L2								
N153-4 [O6]		3.69	S 56 [N]	L2								
N153-4 [O3]		3.89	Y 100 [OH]	H3								
N153-6 [O2]		3.54	R 94 [NH2]	H3								
	N153-6 [O5]	D 154 [OD2]	3.64	N 31 [ND2]	H1							
D 154 [N]		3.35	S 100C [OG]	H3								
	N153-1 [N2]	K 157 [NZ]	3.20	S 28 [OG]	H1							
N153-1 [N2]		3.28	F 99 [O]	H3								
	N153-3 [O3]	N153-3 [O3]	3.86	Y 100 [OH]	H3							
N153-4 [O4]		3.08	S 56 [N]	L2								
	N153-4 [O6]	N153-4 [O4]	3.55	S 56 [N]	L2							
N153-6 [O2]		3.81	R 94 [NH2]	H3								
	N153-6 [O5]	N153-6 [O5]	3.34	S 56 [OG]	L2							
E 84 [OE1]		3.83	K 64 [NZ]	H2								
domain II - reference subunit	b-strand	M 68 [O]	3.72	S 5 [OG]	H2							
		M 68 [O]	3.22	A 57 [N]	H2							
		S 70 [OG]	2.94	S 56 [OG]	H2							
		S 70 [N]	3.14	S 56 [OG]	H2							
		S 70 [O]	3.14	W 94 [NE1]	L3							
		S 72 [O]	3.12	N 93 [ND2]	L3							
		Q 77 [NE2]	3.34	Y 92 [OH]	L3							
		D 83 [OD1]	3.56	K 64 [NZ]	FH3							
	Fusion loop	R 99 [NH2]	3.65	N 93 [O]	L3							
		R 99 [NH1]	2.87	N 93 [OD1]	L3							
		R 99 [NH2]	3.02	N 93 [OD1]	L3							
	ij loop	G 104 [O]	2.74	N 93 [N]	L3							
R 252 [NH2]		3.37	D 55 [OD2]	H2								
	N67 glycan	Q 253 [N]	3.11	Y 100 [OH]	H3							
Q 253 [O]		3.37	Y 100 [OH]	H3								
150 loop	disordered loop				disordered loop				disordered loop			
N153/ N154 glycan	disordered glycan				disordered glycan				disordered glycan			
kI loop	disordered loop				disordered loop				disordered glycan			
K 310 [NZ]	3.69	D 50 [OD1]	L2									
E 311 [OE1]	3.94	R 66 [NH1]	FL3									
E 311 [OE2]	2.74	R 66 [NH1]	FL3									
E 311 [OE2]	3.28	R 66 [NH2]	FL3									
E 311 [OE1]	3.28	S 30 [OG]	FL1									
D 362 [OD2]	3.69	R 54 [NH1]	L2									

The polar contacts were computed with PISA server (REF).

In bold red: main chain atoms involved in contacts; In bold black: salt bridges; In bold blue: glycan interactions

Hydrogen bonds distances cut-off: 3.5Å; Salt bridges distances cut-off: ≤ 4Å

Extended Data Table 5. Polar and salt bridges interactions for ZIKV sE/EDE1 C8 ScFv, ZIKV sE/EDE2 A11 Fab, DENV-2 sE/EDE1 C8 Fab and DENV-2 sE/EDE2 A11 Fab.

		ZIKV sE / EDE2 Fab A11				DENV-2 sE / EDE2 Fab A11 (4UTB) epitope A				DENV-2 sE / EDE2 Fab A11 (4UTB) epitope B				
		sE	dist (Å)	Fab A11	CDR	sE	dist (Å)	Fab A11	CDR	sE	dist (Å)	Fab A11	CDR	
Light chain	L1	dom III	P 354 [O]	3.71	N 27B [ND2]	L1								
	L2	domain I - across - N154 glycan				N153-4 [O4]	2.86	S 56 [OG]	L2	N153-4 [O4]	3.08	S 56 [N]	L2	
						N153-4 [O4]	3.64	S 56 [N]	L2	N153-4 [O6]	3.55	S 56 [N]	L2	
						N153-4 [O6]	3.69	S 56 [N]	L2	N153-6 [O5]	3.34	S 56 [OG]	L2	
	L3	b-strand	S 70 [O]	2.84	R 95 [NH1]	L3								
			D 71 [OD1]	3.75	R 95 [NH1]	L3								
	H1	domain I - across - N154 glycan	N154-1 [N2]	3.24	S 28 [OG]	H1								
			N154-1 [O3]	3.03	S 28 [OG]	H1								
	Heavy chain	H2	ij-loop				D 154 [OD2]	2.79	N 31 [ND2]		D 154 [OD2]	3.64	N 31 [ND2]	H1
							K 157 [NZ]	3.34	S 28 [OG]		K 157 [NZ]	3.20	S 28 [OG]	H1
H2		b-strand				K 247 [NZ]	3.39	D 53 [OD1]						
						K 247 [NZ]	3.7	D 53 [OD2]						
Fusion loop		150 loop	S 72 [OG]	3.30	D 100I [N]	H3	T 70 [OG1]	3.5	S 55 [O]	H2	T 70 [OG1]	3.42	S 55 [O]	H2
			S 72 [OG]	2.69	D 100I [OD1]	H3	S 72 [OG]	3.73	D 100I [N]	H3	S 72 [OG]	3.61	D 100I [N]	H3
			S 72 [O]	3.50	S 100J [N]	H3	S 72 [OG]	2.78	D 100I [OD1]	H3	S 72 [OG]	3.01	D 100I [OD1]	H3
			S 72 [N]	3.43	P 100H [O]	H3					S 72 [O]	3.69	S 100J [N]	H3
			R 73 [N]	3.45	D 100I [OD1]	H3					S 72 [N]	3.45	P 100H [O]	H3
			R 73 [NE]	2.83	S 100J [OG]	H3					R 73 [NE]	3.59	S 100J [OG]	H3
	R 99 [NH1]		2.83	D 100I [OD1]	H3	D 98 [O]	3.51	Y 100G [OH]	H3					
	R 99 [NH1]		3.5	D 100I [OD2]	H3	R 99 [NH2]	2.64	D 100I [OD2]	H3	R 99 [NH2]	2.76	D 100I [OD1]	H3	
	R 99 [NH2]		3.64	D 100I [OD1]	H3	R 99 [NH2]	2.83	D 100I [OD1]	H3	R 99 [NH2]	2.49	D 100I [OD1]	H3	
	R 99 [NH2]		2.74	D 100I [OD2]	H3	G 102 [O]	3.49	S 100C [N]	H3	G 102 [O]	3.83	S 100C [N]	H3	
G 102 [O]	3.16	S 100C [N]	H3	G 102 [O]	2.88	S 100C [OG]	H3	G 102 [O]	3.02	S 100C [OG]	H3			
G 102 [O]	3.20	S 100C [OG]	H3	N 103 [N]	3.84	Y 100A [O]	H3							
G 104 [O]	3.84	R 98 [NH1]	H3	K 246 [O]	2.77	Y 100G [OH]	H3	K 246 [O]	2.76	Y 100G [O]	H3			
K 251 [O]	2.83	Y 100G [OH]	H3	Q 248 [O]	3.23	Y 100F [O]	H3	Q 248 [O]	3.70	Y 100F [OH]	H3			
ij-loop	150 loop	V 153 [O]	3.5	S 100C [OG]	H3	Q 248 [N]	3.18	Y 100F [OH]	H3	Q 248 [N]	3.85	Y 100F [OH]	H3	
					G 152 [O]	3.62	S 100C [OG]	H3						
N153/ N154 glycan					D 154 [N]	3.17	S 100C [OG]	H3	D 154 [N]	3.35	S 100C [OG]	H3		
					N153-1 [N2]	3.43	F 99 [O]	H3	N153-1 [N2]	3.28	F 99 [O]	H3		
					N153-4 [O3]	3.89	Y 100 [OH]	H3	N153-3 [O3]	3.86	Y 100 [OH]	H3		
					N153-4 [O3]	3.89	Y 100 [OH]	H3	N153-6 [O2]	3.81	R 94 [NH2]	H3		
					N153-6 [O2]	3.54	R 94 [NH2]	H3						

		ZIKV sE / EDE1 ScFv C8				DENV-2 sE / EDE1 Fab C8 (4UTA) epitope A				DENV-2 sE / EDE1 Fab C8 (4UTA) epitope B					
		sE	dist (Å)	ScFv C8	CDR	sE	dist (Å)	Fab C8	CDR	sE	dist (Å)	Fab C8	CDR		
Light chain	FL1					E 311 [OE1]	3.28	S 30 [OG]	FL1	E 311 [OE1]	3.28	S 30 [OG]	FL1		
	FL3	dom III - across - A strand	T 315 [O]	2.58	R 66 [NH1]	FL3	E 311 [OE1]	3.94	R 66 [NH1]	FL3	E 311 [OE1]	3.94	R 66 [NH1]	FL3	
						E 311 [OE2]	2.74	R 66 [NH1]	FL3	E 311 [OE2]	2.74	R 66 [NH1]	FL3		
						E 311 [OE2]	3.28	R 66 [NH2]	FL3	E 311 [OE2]	3.28	R 66 [NH2]	FL3		
	L2	dom III - across - A strand				K 310 [NZ]	3.69	D 50 [OD1]	L2	K 310 [NZ]	3.69	D 50 [OD1]	L2		
			E strand	K 373 [NZ]	2.85	S 52 [OG]	L2	D 362 [OD2]	3.69	R 54 [NH1]	L2	D 362 [OD2]	3.69	R 54 [NH1]	L2
				K 373 [NZ]	2.98	T 53 [OG1]	L2								
	K 373 [NZ]	3.56		D 50 [O]	L2										
	L3	b-strand	S 70 [O]	3.14	W 94 [NE1]	L3	T 70 [O]	2.76	W 94 [NE1]	L3	T 70 [O]	2.76	W 94 [NE1]	L3	
			S 72 [O]	3.12	N 93 [ND2]	L3	S 72 [O]	3.48	N 93 [ND2]	L3	S 72 [O]	3.48	N 93 [ND2]	L3	
Q 77 [NE2]			3.34	Y 92 [OH]	L3	Q 77 [NE2]	3.46	Y 92 [OH]	L3	Q 77 [NE2]	3.46	Y 92 [OH]	L3		
Fusion loop	150 loop	R 99 [NH2]	3.65	N 93 [O]	L3	R 99 [NH1]	3.1	N 93 [OD1]	L3	R 99 [NH1]	3.1	N 93 [OD1]	L3		
		R 99 [NH1]	2.87	N 93 [OD1]	L3	R 99 [NH2]	2.98	N 93 [OD1]	L3	R 99 [NH2]	2.98	N 93 [OD1]	L3		
		R 99 [NH2]	3.02	N 93 [OD1]	L3	G 104 [O]	2.86	N 93 [N]	L3	G 104 [O]	2.86	N 93 [N]	L3		
		G 104 [O]	2.74	N 93 [N]	L3	S274 [N]	3.3	E53 [OE2]	H1	S274 [N]	3.3	E53 [OE2]	H1		
H1	kl loop	M 68 [O]	3.72	S 56 [OG]	H2	T 68 [O]	3.11	A 57 [N]	H2	T 68 [O]	3.11	A 57 [N]	H2		
		M 68 [O]	3.22	A 57 [N]	H2	T 70 [N]	3.23	S 56 [OG]	H2	T 70 [N]	3.23	S 56 [OG]	H2		
		S 70 [N]	3.14	S 56 [OG]	H2	T 70 [OG1]	2.96	S 56 [OG]	H2	T 70 [OG1]	2.96	S 56 [OG]	H2		
		S 70 [OG]	2.94	S 56 [OG]	H2	E 84 [OE1]	3.67	K 64 [NZ]	H2	E 84 [OE1]	3.67	K 64 [NZ]	H2		
H2	b-strand				E 84 [OE2]	3.71	K 64 [NZ]	H2	E 84 [OE2]	3.71	K 64 [NZ]	H2			
		R 252 [NH2]	3.37	D 55 [OD2]	H2	K 247 [NZ]	3.84	E 53 [OE1]	H2	K 247 [NZ]	3.84	E 53 [OE1]	H2		
					K 247 [NZ]	2.75	D 55 [OD2]	H2	K 247 [NZ]	2.75	D 55 [OD2]	H2			
					N67-1 [O3]	3.0	G 65 [O]	H2	N67-1 [O3]	3.0	G 65 [O]	H2			
N67 glycan	b-strand	no glycan at this position			N67-1 [O3]	3.0	G 65 [O]	H2	N67-1 [O3]	3.0	G 65 [O]	H2			
		D 83 [OD1]	3.56	K 64 [NZ]	FH3	N67-4 [O2]	2.70	S 82B [OG]	FH3	N67-4 [O2]	2.70	S 82B [OG]	FH3		
FH3	N67 glycan	no glycan at this position			N67-4 [O2]	3.32	S 82B [OG]	FH3	N67-4 [O2]	3.32	S 82B [OG]	FH3			
		Q 253 [N]	3.11	Y 100 [OH]	H3	Q 248 [N]	3.03	Y 100 [OH]	H3	Q 248 [N]	3.03	Y 100 [OH]	H3		
H3	ij-loop	Q 253 [O]	3.37	Y 100 [OH]	H3										

In bold red: main chain atoms involved in contacts; In bold black: salt bridges
Hydrogen bonds distances cut-off: 3.5Å; Salt bridges distances cut-off: ≤ 4Å