Title: Isolation of a potently neutralizing and protective human monoclonal antibody targeting yellow fever virus

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Abstract

Yellow fever virus (YFV) causes sporadic outbreaks of infection in South America and sub-Saharan Africa. While live-attenuated yellow fever virus vaccines based on three substrains of 17D are considered some of the most effective vaccines in use, problems with production and distribution have created large populations of unvaccinated, vulnerable individuals in endemic areas. To date, specific antiviral therapeutics have not been licensed for human use against YFV or any other related flavivirus. Recent advances in monoclonal antibody (mAb) technology have allowed for identification of numerous candidate therapeutics targeting highly pathogenic viruses, including many flaviviruses. Here, we sought to identify a highly neutralizing antibody targeting YFV envelope (E) protein as a therapeutic candidate. We used human B cell hybridoma technology to isolate mAbs from the circulating memory B cells from human YFV vaccine recipients. These antibodies bound to recombinant YFV E protein and recognized at least five major antigenic sites on E. Two mAbs (designated YFV-136 and YFV-121) recognized a shared antigenic site and neutralized the YFV 17D vaccine strain *in vitro*. YFV-136 also potently inhibited infection by multiple wild-type YFV strains, in part, at a post-attachment step in the virus replication cycle. YFV-136 showed therapeutic protection in two animal models of YFV challenge including hamsters and immunocompromised mice engrafted with human hepatocytes. These studies define features of the antigenic landscape on YFV E protein recognized by the human B cell response and identify a therapeutic antibody candidate that inhibits infection and disease caused by highly virulent strains of YFV.

Introduction

Yellow fever virus (YFV), the prototype and namesake member of the family *Flaviviridae*, is a historically important human pathogen. Yellow fever disease has been described in the New World since the 1600s, and YFV was first identified in 1927 [1]. The virus has caused numerous epidemics of human disease throughout the world. According to the World Health Organization, 47 countries in Africa and Central and South America currently have regions endemic for yellow fever, and the burden of yellow fever disease during 2019 was as high as 109,000 severe cases and 51,000 deaths [2]. Approximately thirty percent of infected individuals develop severe disease that includes hemorrhagic complications and multiorgan failure, half of which succumb to the infection [3]. Non-human primates serve as the primary reservoir for YFV, with mosquitoes in the *Haemagogus*, *Sabethes*, and *Aedes* genera serving as the vectors responsible for reservoir maintenance and spillover into humans, typically when humans encroach on primates' natural ecosystems, in what is referred to as the "sylvatic cycle." Once within the human population, YFV is spread by a different vector, the anthropophilic *Aedes aegyptii* mosquito, in an "urban cycle" [4]. Beginning in 2018, YFV epidemics began approaching coastal urban centers like Sao Paulo and Rio de Janeiro, sparking concerns that more severe YFV epidemics may occur in the future [5].

YFV is an enveloped virus with a positive-sense, single-stranded RNA genome. The YFV genome is translated as a single polyprotein, which is post-translationally cleaved by a combination of host and viral proteins into 3 structural (pr/M, E, C) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) proteins [6]. The envelope (E) protein is the primary surface-exposed protein on mature particles and is the principal target of the protective humoral immune system [7]. The E protein is comprised of three domains (domain I (DI), DII, and DIII). DII contains several immunodominant epitopes, including the fusion loop (FL), which is a hydrophobic peptide that mediates fusion of viral

and host membranes in the late endosome. Domain III on E contains the putative cellular attachment domain [8]. While several attachment factors have been postulated, specific entry receptors for YFV have not yet been identified. The virus enters host cells by receptor-mediated endocytosis, as the low pH of late endosomes triggers conformational changes in the E protein. These changes expose the FL, which inserts into the endosomal membrane, allowing penetration of the RNA genome into the host cytoplasm for translation and replication. Although E protein is the primary target of neutralizing antibodies [9-12], nonstructural 1 (NS1) proteins also can elicit protective antibodies [13-15]. Conversely, prM antibodies may confer the risk of antibody-dependent enhancement of infection by otherwise poorly infectious immature virions [16-18].

The YFV vaccine, based on a strain known as 17D, was created by serial passage and attenuation in the 1930s by Max Theiler [19] and is considered one of the most successful vaccines ever created. However, production of 17D has changed little since its inception, resulting in a system of manufacturing and distribution that have been unable to keep pace with demand: ~400 million people in endemic areas still require vaccination to achieve the herd-immunity threshold required to prevent urban spread of YFV [20]. Fractional dosing has been explored in outbreak settings when vaccine supply is insufficient, but its consequences on generation of durable, long-lasting protection are unknown [21, 22]. YFV vaccine shortages stem principally from the limitations inherent in the legacy methods of vaccine strain propagation still being used. When outbreaks do occur in the setting of vaccine insufficiency, specific licensed antiviral treatments targeting YFV are not available.

Recently, potent neutralizing mAbs against many viral targets have shown efficacy as potential treatments of highly pathogenic agents, including other flaviviruses. Several such antibodies targeting YFV have been described. A mAb designated A5 was identified using phage display technology and showed efficacy in an immunodeficient YFV-17D challenge model [23]. A humanized mAb

designated 2C9 showed benefit in hamsters against Jimenez strain [24] and in AG129 mice against YFV 17D-204 challenge [25], supporting the proof of principle for antibodies as a medical countermeasure for YFV. Fully human mAbs with native heavy and light chain pairing, however, are preferred for use in human therapy. A human antibody designated TY014 has been tested in a Phase 1 trial [26], and recently other groups have reported the isolation of human anti-YFV mAbs [27, 28]. Here, we isolated a panel of fully human mAbs targeting E protein to identify candidate therapeutic antibodies. Competition-binding studies mapped these antibodies to several antigenic sites, one of which elicits antibodies that neutralize YFV. *In vitro* studies of the most potent neutralizing mAb, designated YFV-136, revealed that this antibody exerts its neutralizing activity at least partially at a post-attachment step via binding to DII on YFV E protein. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) and neutralization escape virus selection established a key binding and functional epitope for YFV-136 in DII of the E protein. Passive transfer of YFV-136 mAb protected against lethal YFV challenge in a therapeutic setting in two small animal models, Syrian golden hamsters and immunocompromised mice engrafted with human hepatocytes. These studies identify a potentially neutralizing mAb targeting YFV and pave the way for further development of this human mAb YFV-136 as a possible candidate therapeutic agent.

126 **Results**

127

128 **Isolation of mAbs from YFV vaccine recipients**

129 Peripheral blood mononuclear cells (PBMCs) from four subjects who received a YFV vaccine
 130 previously (varying from months to years prior) were transformed *in vitro* with Epstein-Barr virus
 131 (EBV) to screen for YFV-reactive antibodies secreted by transformed memory B cells. We screened
 132 cell supernatants for binding to recombinant YFV E protein by ELISA and/or binding to YFV-17D-
 133 infected cells by flow cytometry. Cells secreting YFV-reactive antibodies were fused to a myeloma
 134 partner to generate hybridoma lines, which were cloned by flow cytometric cell sorting. Antibody was
 135 purified from serum-free hybridoma cell line supernatants by affinity chromatography. Using these
 136 methods, we isolated 15 mAbs from four YFV-immune subjects. These antibodies bound to
 137 recombinant E protein according to ELISA with varying half maximal effective concentrations (EC₅₀)
 138 for binding ranging from 29 to 15,600 ng/mL (**Figure 1A**). Each of the antibodies isolated was tested
 139 for ability to neutralize YFV-17D in a focus reduction neutralization test (FRNT) in Vero cells. While
 140 most antibodies did not neutralize YFV-17D infection, two mAbs showed inhibitory activity: YFV-
 141 121 was moderately neutralizing, with a half maximal inhibitory concentration of 202 ng/mL and
 142 YFV-136 showed exceptional potency, with an IC₅₀ below 10 ng/mL (**Figure 1B**).

143

144 **Competition-binding reveals antibodies target several antigenic sites on the E protein**

145 We used biolayer interferometry (BLI) to perform competition-binding studies that enable grouping of
 146 antibodies based on the major antigenic sites recognized (**Figure 2A**). In this platform, antigen is
 147 loaded onto a biosensor tip, with two antibodies sequentially flowed over the tip. If mAbs recognize
 148 non-overlapping antigenic sites, both bind to the coated sensors when applied in sequence. If binding
 149 of the first antibody applied to the antigen-coated sensor reduces or prevents binding of the second
 150 antibody, the pair of mAbs likely bind to the same or an overlapping antigenic site. We included the

previously described pan-flavivirus reactive murine mAb 4G2 targeting the fusion loop (FL) for comparison. The human antibodies recognized six antigenic sites. One group of mAbs, including YFV-39, -40, and -146, competed for binding with 4G2, indicating that these mAbs target regions near the FL epitope on YFV E. The neutralizing mAbs YFV-121 and -136 grouped together, indicating these mAbs target an overlapping antigenic site of neutralization vulnerability on YFV E. YFV-65 also competed for binding to E with YFV-121 and YFV-136, even though it did not neutralize YFV-17D when tested at concentrations as high as 10 µg/mL. These data suggest there are multiple antigenic sites on YFV E, with at least one site being a target of potentially neutralizing antibodies.

Neutralization of wild-type YFV strains by mAbs targeting YFV E protein

We next tested YFV-136 for its ability to neutralize wild-type YFV strains under BSL-3 conditions. YFV-136 neutralized Asibi and Kouma YFV strains, as well as a different 17D vaccine strain with high potency (**Figure 2B-D**). At lower antibody concentrations, we observed modest enhancement of infectivity in this assay, possibly due to aggregation of virions, as has been seen with other anti-flavivirus antibodies in cells lacking Fcγ receptors [29].

Identification of antigenic site for mAb YFV-136 using HDX-MS studies

Using HDX-MS, a technique in which antibody binding reduces deuterium labeling of surface-exposed viral protein residues, we identified peptides on the YFV-E protein that are occluded by binding of YFV-136 Fab fragments (**Figure 3A**). The start and end residues and the amino acid sequences of the representative E protein peptides that showed differential deuteration in the absence of presence of YFV-136 are shown in **Figure 3B**. The results are summarized in a Woods plot in which the peptides showing a significant decrease in HDX are marked in green lines, and the unaffected peptides are marked as gray solid lines (**Figure 3C**). The HDX protection profile is mapped onto a cartoon

representation of the YFV-E dimer (**Figure 3D**). The E protein domain II (DII) near the fusion loop showed the most protection following YFV-136 Fab binding. Some protection against deuteration also was observed in the dimer interface and in DIII.

YFV-136 escape mutation studies identify a substitution at H67 that abrogates neutralization capacity

To gain more insight into the epitope of YFV-136 in DII, we selected for neutralization escape variants to identify functionally important interaction residues. To identify mutations in the YFV envelope protein that allow escape from YFV-136 neutralization, we used a real-time cell analysis (RTCA) assay. This high throughput system monitors cell impedance and detects cytopathic effect (CPE) over time, allowing for identification of escape viruses by observation of decreased cell impedance/CPE at late time points after incubating virus with a neutralizing concentration of antibody. For these studies, YFV-17D was incubated with 5 µg/mL of YFV-136 in 16 wells of a 96-well plate. In 13 of 16 wells, complete neutralization and maintenance of cell monolayer integrity was observed throughout the study. However, 3 of 16 wells showed a late CPE phenotype, suggesting selection of variant viruses that subvert YFV-136 neutralization (**Figure 4A**). Supernatants from these wells were harvested and again incubated with 5 µg/mL of YFV-136 on the RTCA instrument to confirm escape. In this second round, CPE developed rapidly, similar to a virus-only control, confirming selection of a population of virus that is refractory to YFV-136 neutralization (data not shown).

Confirmation of viral escape using RTCA was followed by outgrowth of virus in the presence of 10 µg/mL YFV-136. Viral RNA was isolated, and prM and E genes amplified and sequenced. In all three escape viruses, a single histidine to tyrosine substitution at position 67 on DII of YFV E was identified (**Figure 4B**). This residue in the *b*-strand is conserved across all YFV genotypes, suggesting this escape phenotype likely would be recapitulated in wild-type YFV strains. Overall, escape mutation

studies identified a key residue in DII responsible for escape from YFV-136, suggesting this mAb functions by binding an epitope including H67 on DII, consistent with the dominant region of protection from deuteration in the HDX studies.

MAb YFV-136 neutralizes YFV-17D virus at a post-attachment step

Neutralizing antibodies can target different steps in the viral replication cycle, including, but not limited to, attachment, entry, or egress. To determine the mechanism of action for the most potently neutralizing antibody, YFV-136, we performed pre- and post-attachment neutralization assays (**Figure 4C**). In the pre-attachment inhibition assay, virus and antibody were pre-mixed prior to addition to Vero cell culture monolayers. In the post-attachment inhibition assay, virus first was incubated at 4°C with cells to allow attachment; afterwards, the excess, unbound virus was washed away, and subsequently antibody was added. YFV-136 neutralized infection in both assays, suggesting that at least part of its inhibitory activity occurs after viral attachment, albeit with some diminished potency.

MAb YFV-136 protects hamsters from lethal YFV challenge

Because YFV-136 is the most potently neutralizing antibody in our panel, we studied its activity *in vivo*. We first assessed the activity of YFV-136 in a model of YFV disease in Syrian golden hamsters. This model recapitulates many aspects of human YFV infections, including viscerotropism and liver infection and has been used to assess therapeutic efficacy of small-molecules and antibody drugs [24, 30, 31]. Prior to the *in vivo* study, we tested whether the YFV-136 neutralized the hamster-adapted YFV Jimenez strain. MAb YFV-136 exhibited a PRNT₅₀ value of 0.5 µg/mL when tested in a PRNT assay in Vero 76 cell monolayers using the hamster-adapted YFV Jimenez strain. Next, animals were administered a 6 × LD₅₀ dose of the hamster-adapted Jimenez strain of YFV by an intraperitoneal route. At 3 days post-infection (dpi), 10 animals were treated with 50 mg/kg of YFV-136, and 15

animals with 10 mg/kg of control antibody DENV-2D22 (**Table 1**). Whereas 12 of 15 animals in the control group succumbed to infection, all animals in the YFV-136 group survived the 21-day study (**Figure 5A**). Animals treated with YFV-136 showed transient weight loss after antibody treatment, but quickly recovered and gained weight throughout the remaining course of the study (**Figure 5B**). Viremia was assessed in all animals at day 6 after inoculation. While control mAb (DENV-2D22)-treated animals showed substantial viremia at day 6, animals treated with YFV-136 showed a significant reduction (**Figure 5C**). Finally, we assessed the ability of YFV-136 to prevent YFV-induced liver damage in hamsters by measuring serum alanine aminotransferase (ALT). Whereas animals treated with an isotype control showed markedly increased serum ALT, animals treated with YFV-136 had lower ALT levels (**Figure 5D**), suggesting YFV-136 protects hamsters from hepatic damage induced by the hamster-adapted YFV strain Jimenez.

YFV-136 protects humanized mice from lethal YFV challenge

We recently developed a YFV infection model in mice engrafted with human hepatocytes (hFRG mice) [32]. Immunodeficient hFRG mice have three genetic lesions (fumarylacetoacetate hydrolase [*Fah*]^{-/-}, *Rag2*^{-/-}, and *Il2r γ* ^{-/-} on a C57BL/6J background), which together with specifically-timed dietary modifications, facilitate the durable replacement of murine hepatocytes with transplanted human hepatocytes [33]. YFV-infected hFRG mice developed disease that recapitulates many features of YF in humans including massive hepatic infection and injury [32]. Here we tested the therapeutic activity of YFV-136 in this highly susceptible hepatotropic model. hFRG mice were administered a single 10 mg/kg dose of YFV-136 or isotype control mAb (DENV-2D22) at 8 hr after inoculation with 2×10^5 focus forming units (FFU) with *wt* YFV-Dakar (DakH1279), a highly pathogenic West African strain. By 4 dpi, all isotype mAb-treated hFRG mice displayed substantial signs of disease: two were dead, the other three were moribund, and all had lost 15 to 25% of their initial body weight (**Figure 6A-B**). In contrast, hFRG mice that were inoculated with YFV and treated with YFV-136 mAb

249 appeared healthy and exhibited minimal weight loss. The reduced disease observed in the YFV-136-
 250 treated group corresponded with significant (~1,000-fold) reductions in YFV burden in the serum and
 251 liver (**Figure 6C-D**), and normal levels of liver synthetic function (as measured by the prothrombin
 252 time [PT]), hepatocyte damage (alanine aminotransferase [ALT]), biliary function (bilirubin), and
 253 detoxification capacity (ammonium) (**Figures 6E-H**). Thus, YFV-136 therapy given 8 hr post-
 254 infection was highly protective in the susceptible hFRG mouse model of YFV infection and liver
 255 disease.
 256

Discussion

Yellow fever virus is a re-emerging arbovirus with epidemic potential. While a highly effective live-attenuated vaccine is available for human use, safety and manufacturing concerns warrant new countermeasure development. Here, we isolated a panel of naturally occurring fully human mAbs that bind to the primary target of anti-YFV functional humoral immunity, the E glycoprotein. Two mAbs, YFV-121 and YFV-136, showed neutralization activity against YFV-17D, with YFV-136 showing exceptional potency with $IC_{50} < 10$ ng/mL. The potency of YFV-136 represents one of the most potent mAbs against YFV ever isolated [23, 26], prompting us to study this mAb in detail. This mAb also neutralizes several wild-type strains of YFV. Both neutralizing mAbs YFV-121 and YFV-136 bind to overlapping antigenic sites as determined by competition binding, suggesting recognition of a shared site of neutralization vulnerability. Antibody escape mutant virus studies identified H67 on DII as a critical residue for the function of YFV-136 within an epitope region in DII identified by HDX-MS studies. We show that YFV-136 functions to inhibit infection at least in part at a post-attachment step in the viral lifecycle. Finally, this mAb was highly efficacious in two different small animal models of YFV infection even when administered after infection, suggesting YFV-136 warrants further development as a therapeutic mAb for use in humans.

Previous studies support the concept that administration of mAbs may be effective in reducing viral load and disease. The murine DII-specific mAb 2C9 and murine-human chimeric mAb 2C9-cIgG exhibited therapeutic activity when administered one day after infection in interferon alpha/beta/gamma receptor-deficient strain AG129 mice challenged with YF 17D-204 vaccine [25] and against virulent YFV infection in an immunocompetent hamster model [24]. A second murine-human chimerized mAb designated 864-cIgG that recognizes DIII and neutralizes the YFV 17D-204 vaccine substrain but did not protect AG129 mice against 17D-204 infection, probably due to its low

potency (reported as 10 µg/mL in a 90% plaque reduction neutralization test) [34]. Other investigators have isolated fully human mAbs with similar ultrapotent (<10 ng/mL IC₅₀) neutralizing activities [28], although animal protection studies are not reported for these mAbs. One human antibody has been tested in a Phase I clinical trial in which the mAb designated TY014 prevented viremia in 5/5 recipients, whereas only 1/5 placebo recipients lacked viremia at 48 or 72 hr following administration of live yellow fever vaccine (strain YF17D-204; Stamaril) [26]; neutralizing potency and protection in animal studies for this mAb were not reported.

The antigenic site recognized by YFV-136, which lies proximal to the fusion loop (FL) on E protein domain II, has been previously implicated as important for humoral immunity induced by YFV-17D vaccination [28]. This recognition pattern is not restricted to human immune responses, as Ryman *et al.* also showed that some mAbs isolated from mice bind to a site proximal to H67, suggesting broad immunodominance of this site [9]. MAbs characterized in Wec *et al.* display a propensity for pairings of heavy and light chain genes encoded by antibody variable genes *IGHV4-4* + *IGLV1-51*, suggesting a public clonotype is elicited by YFV-17D vaccination [28]. Our data complement these findings, as YFV-136 also uses this pairing. The neutralizing activity of YFV-136 is comparable to that of the most potent YFV antibodies reported [28]. It is possible that the efficacy of YFV-17D hinges on its ability to elicit antibodies to this site, since both neutralizing mAbs we isolated from these donors are members of this public clonotype. However, the number of mAbs isolated here is not sufficient to make definitive conclusions in this regard. To date, few studies probing the humoral immune response to YFV have studied the circulating B cells of survivors of natural infection.

The epitope mapping studies using HDX-MS and neutralization escape studies used here suggest that most likely that critical contacts of YFV-136 are focused on the H67 and the region surrounding it on

DII. This antigenic site is known in flaviviruses to be a site of vulnerability for potentially neutralizing antibodies. For instance, we have observed a similar pattern of binding for the DII-reactive human mAb ZIKV-117 that potentially neutralizes the related flavivirus Zika virus [35]; ZIKV-117 also binds detectably to monomeric E protein but it recognizes a quaternary epitope involving two protomers in the virus particle. Higher resolution structural studies are needed to understand the interaction of YFV-136 with virus particles better and to identify a more complete binding footprint for YFV-136.

It is important to note that the antibodies highlighted here bind to monomeric YFV E protein. While we attempted to identify antibodies targeting quaternary structural epitopes only present on virions using a flow-cytometric approach detecting antibodies binding to E protein expressed in YFV-infected cells, these efforts did not identify neutralizing mAbs, and the approach was not explored further. It is likely that functional mAbs targeting sites spanning one or more E protein dimers exist against YFV as has been observed for many flaviviruses. In future work, a multi-pronged approach that employs screens for binding to protein and whole virions, as well as front-end neutralization screens, might help to reveal if immune humans possess some of this rare class of antibodies that exclusively recognize quaternary epitopes on virus particles.

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AUTHOR CONTRIBUTIONS

Conceptualization M.P.D., J.R.G., A.L.B., and J.E.C.; Methodology M.P.D., J.R.G., A.L.B., N.K., J.G.J., M.S.D., J.E.C.; Investigation M.P.D., J.R.G., A.L.B., N.K., C.G., J.R., K.M.R., C.A.N., P.N.J., R.E.S., R.G.B., J.G.J.; Resources C.A.N., D.H.F. J.G.J., M.S.D., J.E.C.; Writing – Original Draft M.P.D., J.E.C., Writing – Review & Editing all authors; Supervision M.L.G., J.G.J., D.H.F., M.S.D., J.E.C.; Project Administration M.S.D., J.E.C.; Funding Acquisition M.P.D., M.S.D., J.E.C.

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353 **Figure Legends**

354

355 **Figure 1. ELISA binding and FRNT neutralization by human mAbs targeting YFV E protein.**

356 **A.** Half maximal effective concentrations (EC_{50}) of antibody binding to YFV E as determined by
 357 ELISA. Values were interpolated using a non-linear regression model in Prism software. Data from a
 358 single experiment are shown, representative of three independent experiments. **B.** Focus reduction
 359 neutralization test (FRNT) to assess neutralization of YFV-17D by YFV-121 and YFV-136.
 360 Neutralization values were fit to a non-linear regression model. Data from a single experiment are
 361 shown, representing at least two independent experiments.

362

363 **Figure 2. Competition binding and neutralization by human mAbs targeting YFV E protein.**

364 **A.** Octet biolayer interferometry competition binding of human mAbs against YFV E. Antibodies
 365 listed top to bottom were associated to immobilized YFV E protein, with antibodies shown left to right
 366 tested for their ability to bind in the presence of the first antibody. Binding is expressed as a percentage
 367 of residual binding, with black boxed indicating compete competition, gray boxed partial competition,
 368 and white boxes no competition. Antibodies were clustered based on their competition profiles and
 369 labeled A-F. **B-D.** Neutralization of diverse YFV strains assessed by a focus reduction neutralization
 370 test (FRNT). Values were fit to a non-linear regression model using Prism software. Three independent
 371 experiments were performed in technical triplicate, with data from a single representative experiment
 372 shown.

373

374 **Figure 3. Epitope mapping for Fab YFV-136 by HDX-MS. A.** Representative kinetic plots for the
 375 twelve different peptides showing effects of Fab binding by HDX. Black or green lines are for YFV-E
 376 protein in the absence or presence of the mAb136 antibody, respectively. At the top of each panel are
 377 the residue numbers and charge states of the peptide. **B.** Sequences and positions of each peptide are

378 given in the table. **C.** Woods plots showing accumulated difference in % deuteration (bound state -
 379 unbound state) across all time points for each analyzed peptide. The propagated error for cumulative
 380 difference was calculated for each respective peptide, and 99% confidence intervals were calculated.
 381 Peptides whose differential exchange exceeds the 99% confidence interval are considered to show
 382 significant differences between bound and unbound and involved in binding. Peptides that do not show
 383 any significant differences between bound and unbound are in gray whereas protected peptides are in
 384 green. The blue vertical line shows the location of H67Y escape mutation identified in studies shown
 385 in Figure 4. **D.** The protected peptides are shown in green color on a ribbon representation of the YFV-
 386 E dimer. The domains are indicated in red (DI), yellow (DII), or blue (DIII).

387

388 **Figure 4. Critical residue for neutralization escape, and mechanism of action studies for YFV-**
 389 **136. A.** Cell impedance measurements during a first round of YFV-17D escape mutant virus selection.
 390 Each box represents the cell impedance within a single well of a 96-well plate as a function of time.
 391 The * symbol indicates wells that exhibit a drop in cell impedance at late time points, suggesting viral
 392 escape in the presence of 5 µg/mL YFV-136. A single well marked with # was used as a control for
 393 cell culture adaptation. * and # wells were propagated once more in culture on the device, and finally
 394 in 6-well culture dishes in the presence of 10 µg/mL YFV-136 (or no antibody for #) to allow for viral
 395 outgrowth. Viral RNA was isolated and prM and E genes amplified by RT-PCR using primers flanking
 396 the prM and E genes. These same primers, and two other primers targeting internal prM and/or E
 397 sequences, were used to sequence virus isolated from * and # by Sanger sequencing. These sequences
 398 were aligned in Geneious software to identify point mutations. **B.** Escape mutant identified in panel **A**
 399 mapped onto the crystal structure of YFV E (PDB 6IW5). Colors denote domain I (red), domain II
 400 (yellow), or domain III (blue). **C.** Focus reduction neutralization test of YFV-136 at pre- or post-
 401 attachment of virus to host cells. Neutralization values were assessed using a non-linear regression

model in Prism software. Two independent experiments were performed in technical triplicate, with data from a single representative experiment shown.

Figure 5. Syrian golden hamster challenge studies to assess YFV-136 therapeutic efficacy. A.

Kaplan-Meier survival curves of animals (YFV-136, n=10; DENV 2D22 control, n=15; uninfected controls, n=5) treated with 50 mg/kg YFV-136 or 20 mg/kg isotype control 3 days post-inoculation with $200 \times \text{CCID}_{50}$ hamster-adapted YFV Jimenez strain. Statistical analysis was performed using a Wilcoxon Log-Rank test. **B.** Weights of YFV-infected animals treated with YFV-136 or isotype control mAb, or uninoculated animals throughout the course of the study. **C.** Serum virus titers (50% cell culture infectious doses [CCID_{50}]) were assessed 6 days after virus inoculation. A one-way ANOVA with Dunnett's multiple comparisons was used to assess statistical significance. **D.** Serum alanine aminotransferase (ALT) levels at 6 days after inoculation was assessed as a proxy for liver damage. A one-way ANOVA with Dunnett's multiple comparisons post-test was used to assess statistical significance.

Figure 6. hFRG challenge studies to assess YFV-136 therapeutic efficacy. FRG mice engrafted

with human hepatocytes (hFRG) were inoculated with 2×10^5 focus forming units (FFU) of *wt* YFV-DakH1279. Eight hr later, mice were administered a single 10 mg/kg dose of YFV-136 (n = 5, blue points) or isotype control (n = 5, black points). Note, two of the control mAb-treated YFV-infected mice succumbed to infection at 4 dpi (denoted by open circles), and thus some serum-based measurements were not available. **A-B.** Weight loss showing mean values (**A**) and individual animal profiles (**B**). **C-D.** Viral burden measures at 4 dpi in the liver (**C**) and serum (**D**) as measured by RT-qPCR. **E-H.** Serum biomarkers of hepatic injury at 4 dpi. Samples were tested for prothrombin time (**E**), alanine aminotransferase (ALT, **F**), total bilirubin (**G**), and ammonia (**H**) levels. **C-H.** Mann-

426 Whitney test (*, $p < 0.05$; **, $p < 0.01$). Bars denote median values. Dashed lines in **C and D** indicate the
427 (lower and/or upper) limit of detection of the assay. Grey boxes indicate the reference range for each
428 parameter in mice; for ALT a dashed line is shown to denote the upper limit of “normal” for healthy
429 hFRG mice at baseline.

430 **Methods**

431

432 **Generation of human mAbs**

433 Blood samples were obtained after written informed consent from four human subjects aged 20 to 47
 434 years who were previously vaccinated with a YFV vaccine prior to travel. The studies were reviewed
 435 and approved by the Institutional Review Board of Vanderbilt University Medical Center. Peripheral
 436 blood mononuclear cells (PBMCs) were isolated from whole blood and transformed using Epstein-
 437 Barr virus (EBV), as previously described [36]. Briefly, transformed B cells were expanded and co-
 438 cultured with irradiated human PBMCs in 96-well plates. Cell supernatants were screened by ELISA
 439 using recombinant YFV E protein (Meridian Life Sciences). Wells with positive reactivity were fused
 440 to a human-mouse myeloma cell line (HMMA 2.5) and plated by limiting dilution in 384-well plates.
 441 The resulting hybridomas were cloned by fluorescence-activated cell sorting (FACS) to produce clonal
 442 hybridoma cell lines. These clonal hybridoma cells were cultured in T-225 flasks containing serum-
 443 free medium, and mAb was purified from spent medium by affinity chromatography on an
 444 ÄKTATM pure Fast Protein Liquid Chromatography (FPLC) instrument (Cytiva).

445

446 **Recombinant antibody expression and purification**

447 For animal studies, large-scale recombinant antibody production of YFV-136 was performed. RNA
 448 was isolated from YFV-136 hybridoma line, and heavy and light chain genes were amplified using
 449 5'RACE (Rapid Amplification of cDNA Ends) and sequenced using a Pacific Biosciences Sequel
 450 instrument. Variable regions of YFV-136 were cloned into a monocistronic full-length, human IgG1
 451 expression vector (Twist Biosciences) for recombinant production. This expression vector then was
 452 transfected transiently into ExpiCHO cells for 7 to 8 days. Cell supernatants were harvested and
 453 filtered through 0.45-µm filters prior to purification. Purification was performed on an ÄKTATM pure

Fast Protein Liquid Chromatography (FPLC) instrument (Cytiva) using HiTrap MabSelect SuRe columns (Cytiva) as described above for hybridoma-derived mAbs.

ELISA binding of mAbs to YFV E protein

384-well plates were coated with 2 µg/mL of YFV E protein (Meridian Life Science) at 25 µL/well and incubated overnight at 4°C. Plates then were washed and blocked using Dulbecco's PBS with Tween 20 (DPBS-T) containing 2% milk and 1% goat serum for 1 hr at room temperature. Following a wash step, serial dilutions of antibody in DPBS were added to plates and incubated for 1 hr at room temperature. To detect bound antibodies, alkaline phosphatase conjugated goat anti-human IgG diluted 1:4,000 in DPBS-T containing 1% milk and 1% goat serum was added to plates for 1 hr at room temperature and developed using AP substrate tablets diluted in 1 M Tris, 0.3 mM magnesium chloride. Plates were developed in the dark for 1 hr and read on a BioTek plate reader at 405 nm. Binding curves were interpolated in Prism software (GraphPad) using a non-linear regression analysis.

YFV-17D focus reduction neutralization test (FRNT)

A focus reduction neutralization test was performed as previously described with minor amendments. Briefly, 96-well plates were seeded with Vero cells at 2.5×10^4 cells/well and incubated overnight. The following day, serial dilutions of antibody were mixed with 10^2 FFU YFV-17D and incubated at 37°C for 1 hr. 30 µL/well of virus/antibody mixture then was added to Vero cell culture monolayers and incubated at 37°C for 1 hr. Without washing, 110 µL per well of overlay containing a 1:1 mixture of 2.4% methylcellulose and 2× Dulbecco's Modified Eagle Medium (DMEM) with 4% FBS was added to plates, which then were incubated for 72 hr at 37°C in 5% CO₂. To stain foci of virus infection, cells were fixed with 1% paraformaldehyde for 1 hr at room temperature, washed, and permeabilized using permeabilization buffer (0.1% saponin, 0.1% BSA in DPBS) for 10 min. Cells then were stained with 1

478 $\mu\text{g/mL}$ of pan-flavivirus murine mAb 4G2 in permeabilization buffer for 1 hr at room temperature.

479 After two washes, goat anti-mouse IgG- horseradish peroxidase (Southern Biotech) diluted 1:1,000 in

480 permeabilization buffer was added to cells and incubated for 1 hr at room temperature. Foci were

481 developed using TrueBlue peroxidase and counted using a spot counter instrument (ImmunoSpot;

482 CTL). Foci counts were normalized to that of a virus-only control, and neutralization curves were

483 interpolated in Prism software using a non-linear regression analysis.

484

485 **Wild-type and 17D YFV strain FRNT**

486 FRNT was performed as described above, with the following exceptions: 100 μL containing 200 FFU

487 of virus was mixed with 100 μL of serially-diluted mAb and incubated at 37°C for 1 hr. 100 μL of

488 virus:mAb mixture was then added to Vero cells in 96-well plate format and incubated at 37°C for 1

489 hr, and followed by addition of overlay and incubation at 37°C for two days. Cells were then fixed

490 with 4% paraformaldehyde (final concentration) for 30 min, permeabilized, stained, and analyzed as

491 described above.

492

493 **Pre- and post-attachment neutralization of YFV-17D**

494 Pre- and post-attachment neutralization assays were performed as previously described [37]. For pre-

495 attachment studies, 600 FFU YFV-17D was mixed with serial dilutions of antibody for 1 hr at 37°C.

496 Cells and virus:mAb mixtures were then pre-chilled for 15 min prior to addition of mixtures to cell

497 monolayers for 1 hr at 4°C. Cells then were washed three times and incubated with pre-warmed

498 DMEM for 15 min prior to addition of methylcellulose overlay containing DMEM. For post-

499 attachment studies, cell monolayers first were incubated with virus for 1 hr at 4°C. Cells then were

500 washed and incubated with serial dilutions of antibody for 1 hr at 4°C. Excess antibody then was

501 washed off, and cells were incubated for 15 min at 37°C with DMEM prior to addition of overlay. Foci

502 were enumerated as described for the focus reduction neutralization test described above.

503

504 **Biolayer interferometry competition-binding assay**

505 Competition-binding studies were performed using a biolayer interferometry instrument (FortéBio
506 Octet[®] HTX). HIS1K sensortips were pre-incubated in kinetics buffer (Pall) for 10 min. After a 60 sec
507 baseline step, his-tagged YFV E protein (Meridian Life Science) was associated to the tips at 5 µg/mL
508 for 60 sec. Readings again were set to baseline for 60 sec, followed by association of the first antibody
509 at 25 µg/mL for 600 sec to achieve complete saturation. Tip readings again were baselined, and then
510 tips were dipped into wells containing a second antibody at 25 µg/mL for 180 sec. Data were analyzed
511 using FortéBio data analysis software. Data from all steps were normalized to a buffer-only control,
512 and antibodies were grouped using a Pearson correlation statistical analysis.

513

514 **Hydrogen-deuterium exchange (HDX) mass spectrometry**

515 Soluble recombinant E protein used for HDX studies: A synthetic DNA fragment encoding
516 residues 123 to 680 (VTLV...EGSS) of yellow fever virus strain 17DD-Brazil (GenBank entry
517 AAZ07885.1) E protein was inserted downstream of a modified human IL-2 signal peptide
518 (MARMQLLSIALSLALVTNSV). The construct was also modified at the C-terminus of the
519 envelope region to contain a small linker, a TEV protease site, and a 6-HIS tag
520 (GSTGGSENLYFQGHHHHH). The fusion construct was inserted into an AgeI-NotI-cut pFM1.2R
521 vector [38] by Gibson assembly to lie downstream of the CMV promotor. Recombinant E protein was
522 produced by transient transfection of Expi293F cells using a ExpiFectamine 293 Transfection Kit
523 (Thermo Fisher Scientific). Cell supernatants were harvested 4 days after transfection and then
524 concentrated before exchange into 2× PBS at pH 6.5 and finally into 2× PBS at pH 8.0. The soluble
525 recombinant E protein was recovered by 6-HIS affinity chromatography on Ni-NTA agarose (G-

526 Biosciences) and purified by size exclusion chromatography on a Superdex S200 Increase column
527 (Cytiva).

528 Peptide mapping: To prepare for acquisition and analysis of HDX data, the YFV-E protein was
529 digested with two acid proteases (immobilized pepsin followed by immobilized acid protease from
530 fungal-XIII) to achieve better sequence coverage. To effectively reduce the protein and denature it, a
531 quench solution tris (2-carboxyethyl) phosphine hydrochloride, TCEP) and guanidine hydrochloride,
532 GdnHCl was added. The quenching conditions were 1:1 dilution of HDX reaction volume (100 μ L)
533 with quench buffer containing 500 mM TCEP and 4 M GdnHCl, pH 2.4 (resulting in a 250 mM TCEP,
534 2 M GdnHCl and pH 2.6), 3 min incubation, 25°C. A peptide map (in triplicate) of the digest was
535 generated by LC-MS/MS using a Maxis-II-HM mass spectrometer (Bruker Daltonics, Billerica, MA).
536 YFV-E protein (100 pmol) was injected in the LC-MS system where the protein was digested, and the
537 resulting peptides were captured and desalted by C-8 trap column (2.1 x 20 mm, Zorbax Eclipse XDB-
538 C8 trap (Agilent) followed by loading on to C-18 column (2.1 x 50 mm in size, 2.5 μ m Xselect-CSH
539 from Waters, Milford, MA) and elution into the mass spectrometer. The mass spectrometer was
540 operated in a data-dependent fragmentation mode with monitoring the high abundance peptides. Data
541 were analyzed by ByonicTM (Protein Metrics, Santa Carlos, CA, USA) for sequencing and accurate
542 precursor mass ($\pm$ 5 ppm), and the peptides also were curated manually.

543 HDX experiment: YFV-E was equilibrated without or with antibody (1:2 antibody) in
544 phosphate buffered saline in H₂O (PBS, pH 7.4) overnight at 4°C and reequilibrated at 25°C for 30 min
545 before starting the HDX experiment. The exchanged-in with D₂O (PBS prepared in D₂O) in the
546 absence (10 μ M YFV-E) or presence of antibody (20 μ M antibody, (1:2 antigen: antibody ratio) was
547 initiated by diluting the protein solutions (10 μ L) by 10-fold with D₂O in PBS at 25°C (90 μ L, pH 7.4).
548 HDX was measured at 0 (undeuterated control), 10, 60, 300, and 2,700 s at 25 °C. For the undeuterated
549 control, the conditions were the same except the added buffer solution was H₂O instead of D₂O. The

550 HDX was quenched by adding an equal volume (100 μ L) of quench buffer equilibrated at 25°C
 551 followed by mixing and incubation at 25°C for 3 mins. The quenched sample was digested by passing
 552 it through a custom-packed column (2 mm x 20 mm) of immobilized pepsin beads followed by a
 553 column of immobilized Fungal XIII beads (2 mm x 20 mm) at 200 μ L/min flow rate. The resulting
 554 peptides thus generated were captured and desalted on a C-8 column by washing with 0.1% formic
 555 acid in water for 4.7 min. Desalted peptides were loaded on a C-18 analytical column where peptides
 556 were separated using a gradient of acetonitrile (ACN) in 0.1% formic acid (most peptides eluted during
 557 the linear part of gradient from 5 min (4% ACN) to 15 min (40% ACN)). To minimize back exchange,
 558 the trap and analytical columns were kept in an ice slush while protease columns were at room
 559 temperature. The isotope distributions of the exchanged peptides were measured with a Maxis-II-HM
 560 mass spectrometer (MS only mode) for duplicate samples.

561 HDX data analysis: LC-MS HDX data acquisition (retention time, isotopic distribution and
 562 observed m/z) was directed by the peptide map, and data were analyzed by HDExaminer (v 2.5.0, 64-
 563 bit, Sierra Analytics). The maximum deuterium level was set to 90%, and the data displayed as kinetic
 564 plots for each peptide for HDX. Only those peptides that provided good signal-to-noise ratio at all the
 565 time points and for both the states were included in the analysis. Ultimately, 107 unique peptides
 566 covering 95% sequence of YFV-E protein were analyzed. Average peptide length was 14 amino acids
 567 and average residue level redundancy was 4. To elucidate those regions where HDX changed with
 568 statistical significance upon antibody binding, a mean cumulative difference (bound - unbound) across
 569 all the time points for each peptide was calculated and plotted as a Woods plot. To identify significant
 570 differences upon binding, the propagated error for cumulative percent deuteration difference for each
 571 peptide was calculated using the standard error of mean, and a 99% confidence interval was
 572 determined (2 degrees of freedom, two tail distribution). Peptides that showed no change are marked in
 573 gray and peptides that showed significant differences between bound and unbound were highlighted
 574 based on protection (green) or exposure (red).

575

576 **Generation and analysis of YFV-17D escape mutations**

577 In a U-bottom 96 well plate, 25 μ L mAb YFV-136 IgG protein at 5 μ g/mL was pre-mixed with 25 μ L
 578 YFV-17D virus [39] diluted 1:10 (~5,000 FFU) in DMEM without FBS and incubated for 1 hr at
 579 37°C. This procedure was done in 16 separate wells within the 96-well plate. Virus also was mixed
 580 with DMEM alone and passaged throughout the study to control for substitutions that arise from cell
 581 culture adaptation. 50 μ L virus/antibody mixture and controls were added to HuH-7.5 cell culture
 582 monolayers in 96-well ePlates (Agilent) and incubated for 1 hr at 37°C. 100 μ L of DMEM containing
 583 5% FBS was then added to each well, plates placed back on an xCELLigence instrument (Agilent), and
 584 cell monolayers were monitored for delayed CPE. Cell supernatants in wells with a delayed CPE
 585 phenotype, as well as a virus-only control, were subjected to a repeat of this assay to confirm viral
 586 escape. Once escape was confirmed, 6-well plates containing confluent Huh7.5 cell monolayers were
 587 inoculated with 100 μ L/well escape virus or a virus control in the presence of 10 μ g/mL of YFV-136
 588 for outgrowth of escape virus. Virus was harvested from 6-well plates, and RNA isolated using the
 589 Qiagen virus RNA isolation kit. E and prM genes from isolated RNA were reverse-transcribed to
 590 cDNA and PCR-amplified using primers flanking the prM and E genes (One-step RT-PCR kit). Genes
 591 then were sequenced by GENEWIZ using overlapping primers that give coverage across prM and E.
 592 The control virus sequence was aligned to the 17D reference genome sequence to confirm that
 593 mutations did not result from adaptation to cell culture.

594

595 **Syrian golden hamster challenge studies**

596 The Syrian golden hamster model used for these studies has been described previously [24]. 30 female
 597 Syrian golden hamsters (LVG/Lak strain) supplied by Charles River were used. Hamsters were
 598 randomized by weight to experimental groups and individually marked with ear tags. For challenge
 599 studies, hamsters were challenged at day 0 with 200 of 50% cell culture infectious doses (CCID₅₀)

hamster-adapted YFV Jimenez strain by bilateral intraperitoneal injections in a total of 0.2 mL. At 3 days post-virus inoculation, hamsters were dosed at 50 mg/kg of recombinant YFV-136 (rYFV-136) (1 mL total volume) or 10 mg/kg of rDENV-2D22 control and monitored for weight loss and clinical manifestations for 21 days. Blood samples were taken at days 4 and 6 to assess viremia and ALT. Any surviving animals were humanely euthanized at the experimental endpoint.

Measurement of hamster serum aminotransferase

ALT (SGPT) reagent (Teco Diagnostics, Anaheim, CA) was used, and the protocol was altered for use in 96-well plates. Briefly, 50 μ L aminotransferase substrate was placed in each well of a 96-well plate, and 15 μ L of sample was added at timed intervals. The samples were incubated at 37°C, after which 50 μ L color reagent was added to each sample and incubated for 10 min as above. A volume of 200 μ L of color developer was next added to each well and incubated for 5 min. The plate was then read on a spectrophotometer, and aminotransferase concentrations were determined per manufacturer's instructions.

50% cell culture infectious doses (CCID₅₀) assays to assess hamster viral burdens

Virus titer was quantified using an infectious cell culture assay in which a volume of either tissue homogenate or serum was added to the first tube of a series of dilution tubes. Serial dilutions were made and added to Vero cell monolayer cultures. Ten days later, CPE was used to identify the endpoint of infection. Four replicates were used to calculate the CCID₅₀ of virus per mL of plasma or gram of tissues.

Mouse studies

All mouse experiments were conducted under a Washington University School of Medicine Institutional Animal Care and Use Committee approved protocol in compliance with the Animal

Welfare Act. Female hFRG mice were generated by Yecuris Corporation and maintained according to their specific care and use guidelines (see [32] for additional details). Only mice with human albumin levels of ≥ 5.0 mg/mL in plasma (indicative of $\geq 70\%$ engraftment) were used for this study. hFRG mice were continued on their regular diet of PicoLab High Energy Mouse Diet, 5LJ5 chow (LabDiet) and 3.25% w/v dextrose-water during infection experiments. Mice were inoculated with via retro-orbital injection of 50 μ L containing 2×10^5 FFU *wt* YFV-DakH1279, obtained from the World Reference Center for Emerging Viruses and Arboviruses and passaged once in Vero-CCL81 cells. Antibodies were given as a single treatment of 10 mg/kg dose, diluted in PBS in a 100 μ L total volume and given by the i.p. route, 8 hrs post-infection. The DENV2D22 mAb was used as a control. Euthanasia was performed via ketamine overdose and thoracotomy. Blood was collected via aspiration from the cardiac chambers and PT was measured on a Coagucheck (Roche). Perfusion of the entire vascular tree was then performed with saline prior to liver collection. After centrifugation, serum was mixed 1:9 with 10% Triton X-100 in HBSS (1% final volume of Triton-X-100), then incubated at room temperature for 1 h to inactivate infectious virus [32]. ALT, bilirubin, and ammonia were analyzed using the Catalyst Dx Chemistry Analyzer (IDEXX Laboratories). Some specimens required dilutions to achieve an ALT value within the analytical measurement range; in these instances, dilution was performed in HBSS and the value was corrected by the final dilution factor. RNA extraction and viral load analysis was performed as described previously [32] using the KingFisher Flex (Thermo Fisher) and the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher) on the QuantStudio 6 Flex Real-Time PCR System with the following primers: F: 5'-AGGTGCATTGGTCTGCAAAT-3'; R: 5'-TCTCTGCTAATCGCTCAAIG-3'; P: 5'-/56-FAM/GTTGCTAGGCAATAAACACATTTGGA/3BHQ_1/-3'.

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779

780 **Table 1. The effect of delayed treatment (3 dpi) with mAb YFV-136 in a Syrian golden hamster model of YFV Jimenez strain**781 **infection and disease**

782

MAb treatment	Dose of mAb given on 3 dpi (mg/kg)	Virus given i.p. on 0 dpi	Alive/total at 21 dpi	Mean day of death $\pm$ SD	Mean body weight change ^b (grams $\pm$ SD)	Serum virus titer on 4 dpi (CCID ₅₀ /mL $\pm$ SD)	Serum alanine aminotransferase test on 6 dpi (mean IU/L $\pm$ SD)
YFV-136	50	YFV ^a	10/10	$>21 \pm 0.0^{**}$	-5.2 ± 6.7	4.1 ± 2.2	$114 \pm 26^*$
Isotype control (DENV 2D22)	10	YFV ^a	3/15	7.5 ± 1.4	-8.9 ± 9.9	5.4 ± 2.3	214 ± 120
Normal controls	--	Sham	5/5	$>21.0 \pm 0.0^{**}$	$3.0 \pm 2.1^{**}$	$1.7 \pm 0.0^{**}$	$80 \pm 3^*$

783

784 ^a Hamster-adapted YFV Jimenez strain [24].785 ^b Difference between weight on 4 and 5 dpi, representing maximal weight change in this study.786 ^{**}P<0.001, ^{*}P<0.01, as compared to control treatment.

A YFV E half maximal binding

mAb	YFV E
YFV-59	28.7
YFV-142	33.4
YFV-132	34.6
YFV-59.2	43.6
YFV-40	52.6
YFV-155	56.7
YFV-149	73.7
YFV-147	87.1
YFV-39	100.3
YFV-83	157.9
YFV-136	269.7
YFV-108	292.7
YFV-121	311.1
YFV-146	1,769
YFV-99	15,600

EC₅₀ (ng/mL)

0-50

51-500

501-1,000

>1,000

B YFV-17D neutralization

Second antibody applied

First antibody applied

Group	mAb	YFV-83	YFV-132	YFV-59	YFV-39	4G2	YFV-40	YFV-146	YFV-121	YFV-136	YFV-65	YFV-142	YFV-147	YFV-108
A	YFV-83	5	1	6	177	151	147	175	117	49	93	116	100	105
	YFV-132	10	4	9	139	138	128	153	111	51	-72	108	131	117
	YFV-59	22	8	17	143	123	135	129	101	46	-145	114	91	94
B	YFV-39	131	37	129	12	14	11	17	99	47	84	111	98	96
	4G2	119	30	108	8	0	4	18	88	41	1	108	90	85
	YFV-40	124	36	135	28	19	14	24	115	48	157	125	125	105
	YFV-146	136	36	132	23	16	14	18	95	42	96	123	93	93
C	YFV-121	124	38	124	123	101	102	118	6	14	-242	98	94	87
	YFV-136	130	40	134	138	132	114	129	-2	6	-74	111	109	101
	YFV-65	113	32	103	127	117	107	115	84	41	171	131	86	75
D	YFV-142	95	27	98	98	93	85	111	69	37	-32	8	85	70
E	YFV-147	103	32	107	123	101	99	107	86	42	-223	89	11	87
F	YFV-108	129	37	143	145	128	128	151	100	49	173	121	101	1

Full Competition

Partial Competition

No Competition

B

YFV Asibi

C

YFV Kouma

D

YFV 17D

Concentration of mAb YFV-136 (log₁₀ ng/mL)

A Escape mutant generation

B Escape mutations mapped onto structure of the envelope protein extracellular domain dimer

C YFV-136 pre- vs. post-attachment neutralization

Weight

Viral Burden

Liver Markers