Supplementary Material

Isolation and characterization of high affinity and highly stable anti-Chikungunya virus antibodies using ALTHEA Gold Libraries™

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CHIKV-033 Isolate Complete Genome Sequencing

Viral RNA of the CHIKV was extracted by using a QIAamp viral RNA minikit (Qiagen, Germany) and the sequencing library was constructed using the TruSeq Stranded mRNA Library Preparation Kit (Illumina Cat No. 20020594) and was sequenced by Illumina NextSeq™ 550 system. The raw sequence reads (10,970,848 reads) were processed using Geneious R10 version 10.2.6 software. Complete genome sequence assembly was achieved by mapping to a reference CHIKV genome sequence (strain S27, GenBank accession number AF369024.2) that included 5′ and 3′ untranslated terminal sequences, using default parameters and the low-sensitivity setting.

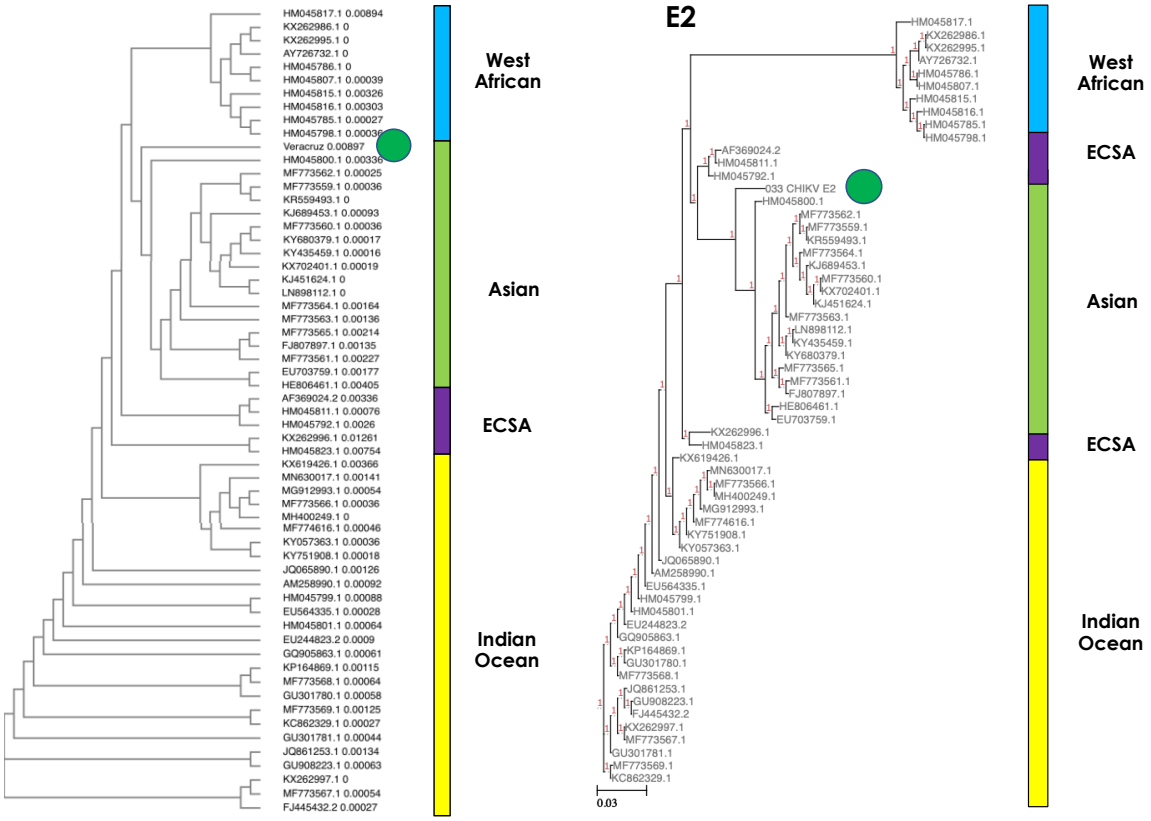

Figure S1. Phylogenetic tree from the whole genomes **(left)** and E2 protein **(right)** inferred for 59 CHIKV genome sequences from the West Africa, Asian and East/Central/South Africa-Indian Ocean lineages (Pyke AT, et al., 2020. Genome sequences of chikungunya virus strains from Bangladesh and Thailand. Microbiol Resour Announc 9:e01452-19.https://doi.org/10.1128/MRA.01452-19.). The sequence of CHIKV-033 isolate is marked with a green dot. Branch lengths are proportional to the number of substitutions per site.

Numbers next to the accession number indicate the bootstrap values. Multiple sequence alignment was performed using MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/). The tree was built with Simple Phylogeny (https://www.ebi.ac.uk/Tools/phylogeny/simple_phylogeny/) using the neighbor-joining method.

NGS and data mining to complement the ELSA-based screening

Plasmid of the rounds 2, 3 and 4 were purified using QIAGEN Plasmid Midi Kit (Cat No. 12143) and used as template to generate one amplicon of approximately 300 bp using the primers in Valadon and co-workers [21]. The PCR reactions were performed as follows: 5 min start at 95°C followed by 10 cycles of 1 min at 95°C, 1 min at 67°C, 1 min at 72°C and terminated by a 10-min extension at 72°C. The PCR fragments were gel-purified using QIAquick PCR Purification Kit (Cat No. 28104) and used as template to prepare the samples for the NGS following the manufacturer instructions. The NGS was performed in a Miseq platform from Illumina. FASTQ files were processed with the software AptaAnalyzer™ (AptaIT; Germany) using the BRC (B-cell receptors) functionality. Accepted output sequence files were further curated with in-house scripts to remove noticeably short or long fragments do not matching the sequence of the scaffolds. Truncated HCDR3 sequences do not having the conserved cysteine H88 and tryptophan H102 were removed from the analyses. The number of reads per round of panning and related statistics is shown below:

Round	Number of reads	Unique	U/T (%)
R2	362,964	102,458	28.23
R3	478,801	66,193	13.82
R4	726,561	14,520	2.00

Quality Control of the antibodies isolated from ALTHEA Gold Libraries™

Three hundred mL of HEK 293 were co-transfected with the plasmids having the heavy and light chains. After four days of incubation, the supernatants were purified using Protein A MabSelect SuRe column (5 mL, GE Healthcare). The IgGs were captured in 20 mM Phosphate buffer, 150 mM NaCl, pH 7.4 and eluted with 20 mM citrate buffer pH 3.5. The monomeric content of the purified IgGs was estimated by UPLC BEH200 150 mm SEC column (Waters) and the integrity was determined by SDS-PAGE.

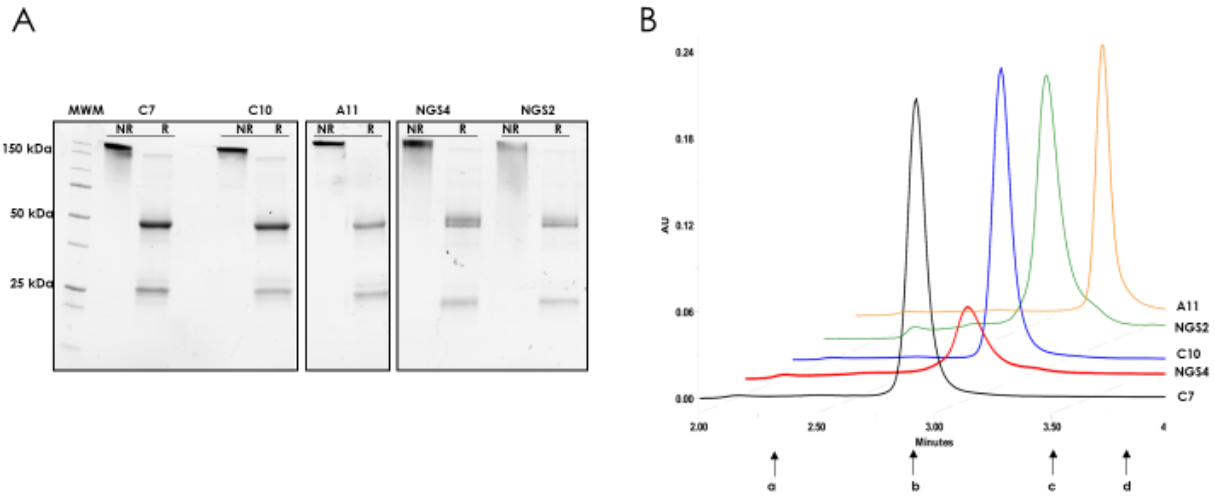

Figure S2. SDS-PAGE (A) and size-exclusion chromatography (SEC) profile (B) of the anti-CHIKV antibodies after Protein A purification. One band from ~150 kDa (complete IgG) is observed under non-reducing (NR) conditions. Two bands, ~50 kDa (heavy chains) and ~25kDa (light chains), were observed under reducing (R) conditions. Any kD™ Mini-PROTEAN® TGX Stain-Free™ Protein Gels (Biorad) and Precision Plus Protein Unstained Standards (molecular weight marker, Biorad) were used. The elution volumes of SEC molecular weight standards are shown in arrows: (a) thyroglobulin (670 kDa), (b) γ-globulin (158 kDa), (c) ovalbumin (44 kDa) and (d) myoglobin (17 kDa). The MWs from anti-CHIKV antibodies ranged from 154 to 172 kDa.

Thermal Stability (Tm)

The Tm was estimated using a Protein Thermal Shift Assay (Thermo Fisher, Cat No. 4466037). This assay measures thermostability using a fluorescent dye (SYPRO Orange) that binds to hydrophobic patches that are exposed as the unfolding process has been carried out. The amount of IgG used in the assay was 4 µg in PBS, pH 7.4. The fluorescence was read using a qPCR instrument with 480 nm excitation and 610 nm emission. Samples were heated from 25 ºC to 99 ºC at 5 ºC/minute. Unfolding transitions were calculated with the Protein Thermal Shift™ Software (Thermo Fisher, Cat No. 4466037).

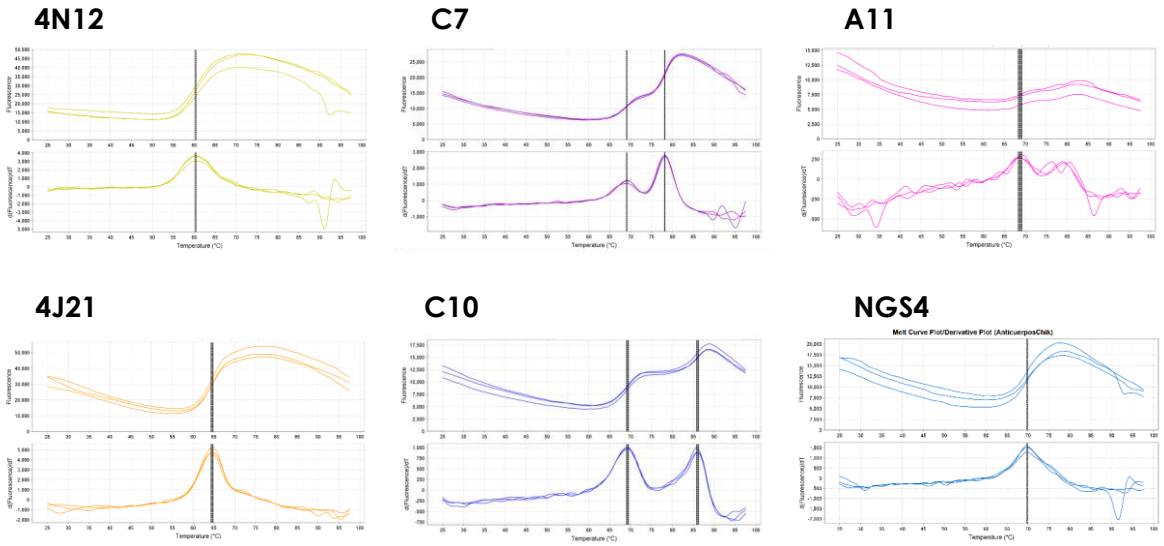

Figure S3. Unfolding profiles of the anti-CHIKV antibodies. The Boltzmann (upper panel) and the first derivative of the fluorescence emission as a function of temperature (dFluorescence/d*T*) (lower panel) melting profiles are shown to each antibody analyzed at a concentration of 4 µg in PBS. The T_m is indicated with a vertical arrow in each profile.