Supplementary information, Data S1

Materials and Methods

Protein Cloning and Expression

The gene sequences to express maltose binding protein (MBP) tagged ZIKVCC (residues 24 to 98) and ZIKVCL (residues 2 to 104) were constructed by overlapping PCR. The cDNA of ZIKV is from the strain Z1106033. The resulting coding sequences were cloned into the vector pET22b (Novagene) and pETDuet-1 (Novagene), separately. These plasmids were then transformed into *Escherichia coli* BL21 (DE3) strain. The cell cultures were grown in Luria–Bertani broth containing 100 mg/l ampicillin at 37°C. Protein expression was induced by 0.5 mM isopropyl β-D-thiogalactoside for 16-20 h at 16°C. Cells were harvested via centrifugation. The mutants of ZIKVCC (F56K and F84K), and the mutants of ZIKVCL (M1: K74E/K75E/K82E; M2: K31E/R32E/K74E/K75E/K82E, M3:K31E/R32E/R55E/K60E/K74E/K75E/K82E/ K83E/K85E/K86E/R93E) were constructed using Mut Express® Ⅱ Fast Mutagenesis Kit V2 (Vazyme™).

Protein Purification

For ZIKVCC, the cells were resuspended in lysis buffer A (50 mM Tris, pH 8.0, 300 mM NaCl) with DNase and RNase, and then lysed by high pressure homogenization. The resulting lysate was centrifuged at 30,000×g for 40 min at 4°C. The supernatant was initially purified by the amylose resin (NEB) affinity chromatography. The protein of interest was eluted using buffer A with 30 mM maltose. The elution was then diluted by buffer B (50 mM MES, pH 6.0, 50 mM NaCl) and loaded onto HiTrap SP HP column (GE) pre-equilibrated with buffer B. Afterward, it was eluted by a

linear NaCl concentration gradient. The concentrated protein of interest was subjected to a final gel-filtration purification step through a Superdex 200 10/300 column (GE) in buffer C (50 mM MES, pH 6.0, 150 mM NaCl, 5 mM maltose). The purification of ZIKVCL was similar to ZIKVCC except for different buffer B (50 mM Tris, pH 8.0, 50 mM NaCl) and buffer C (50 mM Tris, pH 8.0, 150 mM NaCl). The corresponding mutants were expressed and purified alike.

Electrophoretic Mobility Shift Assays (EMSA)

ZIKVCL and their mutants (M1, M2 and M3) were incubated with different amount of single-stranded M13mp18 DNA (NEB®) in the binding buffer (50 mM Tris, pH 8.0, 10 mM 2-mercaptoethanol). After incubation at 30°C for 30 min, 2 μl of DNA loading buffer was added. The reaction mixture was then analyzed by 0.6-0.8% agarose gel in TBE buffer (45 mM Tris, 45 mM Boric acid, 1mM EDTA), and DNA was visualized by ethidium bromide staining.

Crystallization, Data Collection and Structure Determination

The ZIKVCC was concentrated to 12-15 mg/ml and then subjected to crystallization screening using microbatch-under-oil method at 293 K. The high-resolution crystals were typically obtained in 10% v/v 2-Propanol, 0.1 M BICINE pH 8.5, 30% w/v Polyethylene glycol 1,500 overnight and the low-resolution crystals could be obtained in 0.1 M sodium acetate trihydrate pH 4.6, 8% w/v Polyethylene glycol 4,000 in 3-5 days. Both of the crystals were cryo-protected by using 30% glycerol before flash-frozen in liquid nitrogen. A complete data set diffracted to 2 Å resolution was collected on beamline BL18U at Shanghai Synchrotron Radiation Facility (SSRF) using a Pilatus3 6M detector at a wavelength of 0.97791 Å. The 2.9-Å resolution diffraction data were collected at 100 K at beamline I04 of DIAMOND Synchrotron

Radiation Facility (Britain) with a wavelength of 0.97950 Å. Diffraction data were processed using HKL3000[1]. The structure was solved by molecular replacement using the structure of WNV C protein (PDB ID: 1SFK) as well as the complex structure of MBP (PDB ID: 1EZ9) as searching models. In particular, two MBP monomers and one WNV C dimer were consecutively used to determine all the contents of one asymmetric unit. Initial model was mutated to ZIKV residues and further refined via rigid body refinement, Cartesian coordinate refinement as well as individual atom temperature factor refinement over several macro cycles with Phenix[2]. N-terminal structured loops region was manually rebuilt into the weighted *2mFo-DFc* omit difference Fourier map (2.8 σ) using Coot[3]. Further refinement was completed through three macro-cycles of individual coordinate and temperature refinement (Supplementary information, Table S1). Structural analysis and visualization was generated in open source PyMOL[4], while textual annotations were added during final figure assembly.

Supplementary References:

1. Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Methods in enzymology* 1997; **276**: 307-326.
2. Adams PD, Afonine PV, Bunkoczi G *et al.* PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta crystallographica. Section D, Biological crystallography* 2010; **66**: 213-221.
3. Emsley P, Cowtan K. Coot: model-building tools for molecular graphics. *Acta crystallographica. Section D, Biological crystallography* 2004; **60**: 2126-2132.
4 The PYMOL Molecular Graphics System, Version 1.7, Schrödinger, LLC.