Chapter 20

Isolation of Monoclonal Antibodies from Zika Virus-Infected Patient Samples

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Abstract

The combination of sorting antigen-specific memory B cells with determining immunoglobulin (Ig) genes at the single-cell level enables the isolation of monoclonal antibodies (mAbs) in individuals. This method requires a small amount of blood (usually 10 mL) and is rapid (less than 2 weeks to isolate antigen-specific mAbs). Due to the application of antigens as the bait to capture the specific memory B cells, the majority of isolated mAbs are true binders to the antigen, which increases the isolation efficiency. Here, applying this approach, we describe the characterization of mAbs against Zika virus from a convalescent patient sample. From 10 mL whole blood, we sorted 33 Zika envelope (E) protein-interacting single memory B cells. The Ig genes from 15 cells were determined, and 13 mAbs were found that bind to Zika E protein with varied binding affinities.

Key words Zika virus, Memory B cells, Monoclonal antibodies, Therapeutic antibodies, Convalescent patients

1 Introduction

Zika virus has caused global concern due to the accumulating evidence suggesting that infection is associated with microcephaly and neurological complications, such as Guillain-Barré syndrome [1–4]. However, there are currently no approved antivirals against Zika infection [5]. Administration of polyclonal or purified neutralizing monoclonal antibodies (mAbs) to pregnant mice helps to clear the virus and alleviates the neurological disorders in their fetuses [6, 7], providing proof of concept that neutralizing mAbs can be used to treat Zika virus infections. Currently, multiple strategies have been reported to generate human neutralizing mAbs against Zika infection, including sequencing antigen-specific memory B cells [8, 9] or generating Epstein-Barr virus-immortalized memory B cells from Zika patient samples [10], and identifying functional mAbs from phage display naïve antibody libraries

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[11]. In addition, murine mAbs against Zika virus have also been reported [12].

Here, we introduce a method to apply Zika envelope (E) glycoproteins, which play pivotal roles in virus entry and contain important neutralizing epitopes, to sort single memory B cells from a convalescent Zika patient. Subsequently, the immunoglobulin (Ig) genes encoded by the sorted cells were determined at the single-cell level. The approach to determine the Ig genes from single cells was first reported by Tiller et al. [13]. Later, Scheid et al. modified this approach to sequence single gp140-binding memory B cells, which were isolated from human immunodeficiency virus (HIV) patients [14]. Then the mAbs against human papillomavirus (HPV) or respiratory syncytial virus (RSV) were isolated from immunized or naturally infected human donors, respectively [15, 16]. During the epidemic of Zika virus, we and other groups also applied this strategy to isolate mAbs targeting Zika virus from Zika patients [8, 9].

From 10 mL whole blood, we were able to pair 15 mAbs from 33 collected single cells. As detected by surface plasmon resonance (SPR), 13 of the mAbs were true binders to Zika E protein [8].

Although the protocol depicted in this chapter focuses on the isolation of Zika mAbs from a Zika convalescent patient, it could also be expanded to other viruses, and we recently reported the isolation of human neutralizing mAbs against Rift Valley fever virus (RVFV) from a convalescent RVF patient, applying the same strategy [17].

2 Materials

2.1 Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

1. 10 mL Plastic K_2 EDTA Tubes.
2. Density gradient medium.
3. PBS (10× stock): dissolve 80 g of NaCl, 2 g of KCl, 14.4 g of Na_2HPO_4, and 2.4 g of KH_2PO_4 in 800 mL of ddH_2O. Adjust the pH to 7.4 with HCl and then add ddH_2O to 1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 15 min at 121 °C or by filter sterilization. Store PBS at room temperature.
4. 50 mL centrifuge tubes.
5. 15 mL centrifuge tubes.
6. FACS buffer: PBS with 1% fetal bovine serum (FBS) filtered through a 0.22 μm membrane for sterilization.
7. Freezing medium: FBS with 10% DMSO.
8. Freezing containers.
9. 2 mL freezing tubes.
10. Automated cell counter and adapted cell counting chamber.

Table 1
The staining master mix for isolation of antigen-specific memory B cells

Antigen	Amount (μL)
Human CD3	10
Human CD16	10
Human CD235a	10
Human CD19	10
Human CD27	4
Human CD38	10
Human IgG	10
His	20
FACS buffer	16
Total volume	100

2.2 Cell Sorting

1. Antibodies: anti-human CD3/PE-Cy®5 (BD Biosciences), anti-human CD16/PE-Cy®5 (BD Biosciences), anti-human CD235a/PE-Cy®5 (BD Biosciences), anti-human CD19/APC-Cy®7 (BD Biosciences), anti-human CD27/Pacific Blue® (BioLegend), anti-human CD38/APC (BD Biosciences), anti-human IgG/FITC (BD Biosciences), and anti-His/PE (Miltenyi Biotec).
2. Prepare a staining master mix in a 1.5 mL microcentrifuge tube. The antibody cocktail is listed in Table 1.

   Centrifuge the master mix at 15,000 × *g* at 4 °C for 1 min. Store staining master mix at 4 °C in the dark before use.
3. Antigen: Purified Zika E protein or MERS-RBD, both of which contain a 6× His tag at their C-terminus. Adjust the protein concentration to 1 mg/mL.
4. FACS tubes.
5. Compensation beads.
6. Cell sorter.
7. 37 °C water bath.
8. 70% ethanol.

2.3 Ig Gene Amplification

1. Reverse transcription (RT)-PCR synthesis system for the first-strand cDNA.
2. 96-Well PCR Plates.
3. PCR Plate Seal.
4. DEPC-treated ddH_2O.

5. DNA polymerase (for single-cell PCR).
6. 10× TAE buffer.
7. Agarose.
8. Ethidium bromide (EtBr) at 10 mg/mL.
9. 1.2% agarose gel: dissolve 0.36 g of agarose in 200 mL of 1× TAE buffer. Heat the solution to boiling in the microwave until no particles are observed. Add 6 μL of EtBr to the dissolved agarose and mix. Cool the solution to 60 °C prior to casting. Place spacers in the slots of a gel tank and pour the agarose solution into the area between the spacers. Then place a gel comb in position indicated by slots in the tank. Leave to set for approximately 30 min. Before using, remove the spacers and the comb from the gel (*see* **Notes 1** and **2**).
10. Gel Extraction Micro Kit for micro samples.
11. Gel electrophoresis instrument.

2.4 Primers Used for Ig Gene Amplification (See Notes 3 and 4)

1. Primers for reverse transcription (RT) are listed in Table 2.
2. Primers for the first round PCR (PCRa) to amplify V_H are listed in Table 3.
3. Primers for the PCRa to amplify V_κ are listed in Table 4.
4. Primers for the PCRa to amplify V_λ are listed in Table 5.
5. Primers for the second round PCR (PCRb) to amplify V_H are listed in Table 6.
6. Primers for the PCRb to amplify V_κ are listed in Table 7.
7. Primers for the PCRb to amplify V_λ are listed in Table 8.
8. Other primers used in this study are listed in Table 9.

Table 2
Primers for RT reaction

RT primer	5′–3′ sequence
IgM-RT	ATG GAG TCG GGA AGG AAG TC
IgD-RT	TCA CGG ACG TTG GGT GGT A
IgE-RT	TCA CGG AGG TGG CAT TGG A
IgA1-RT	CAG GCG ATG ACC ACG TTC C
IgA2-RT	CAT GCG ACG ACC ACG TTC C
IgG-RT	AGG TGT GCA CGC CGC TGG TC
Cκ-new RT	GCA GGC ACA CAA CAG AGG CA
Cλ-new-ext	AGG CCA CTG TCA CAG CT

Table 3
Primers for the PCRa to amplify V_H

Forward primer	5′–3′ sequence
V_H1-Ext	CCA TGG ACT GGA CCT GGA GG
V_H2-Ext	ATG GAC ATA CTT TGT TCC A
V_H3-Ext	CCA TGG AGT TTG GGC TGA GC
V_H4-Ext	ATG AAA CAC CTG TGG TTC TT
V_H5-Ext	ATG GGG TCA ACC GCC ATC CT
V_H6-Ext	ATG TCT GTC TCC TTC CTC AT
Reverse primer	**5′–3′ sequence**
IgG-exta	CGC CTG AGT TCC ACG ACA CC

Table 4
Primers for the PCRa to amplify V_κ

Forward primer	5′–3′ sequence
V_κ1/2-Ext	GCT CAG CTC CTG GGG CT
V_κ3-Ext	GGA ARC CCC AGC DCA GC
V_κ4/5-Ext	CTS TTS CTY TGG ATC TCT G
V_κ6/7-Ext	CTS CTG CTC TGG GYT CC
Reverse primer	**5′–3′ sequence**
Cκ-ext	GAG GCA GTT CCA GAT TTC AA

Table 5
Primers for the PCRa to amplify V_λ

Forward primer	5′–3′ sequence
V_λ1-Ext	CCT GGG CCC AGT CTG TG
V_λ2-Ext	CTC CTC ASY CTC CTC ACT
V_λ3-Ext	GGC CTC CTA TGW GCT GAC
V_λ3l-Ext	GTT CTG TGG TTT CTT CTG AGC TG
V_λ4ab-Ext	ACA GGG TCT CTC TCC CAG
V_λ4c-Ext	ACA GGT CTC TGT GCT CTG C
V_λ5/9-Ext	CCC TCT CSC AGS CTG TG
V_λ6-Ext	TCT TGG GCC AAT TTT ATG C
V_λ7/8-Ext	ATT CYC AGR CTG TGG TGA C
V_λ10-Ext	CAG TGG TCC AGG CAG GG
Reverse primer	**5′–3′ sequence**
C_λ-new-ext	AGG CCA CTG TCA CAG CT

Table 6
Primers for the PCRb to amplify V_H

Forward primer	5′–3′ sequence
V_H1-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG GTG CAG CTG GTR CAG TCT GGG
V_H2-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG RGC ACC TTG ARG GAG TCT GGT CC
V_H3-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** GAG GTK CAG CTG GTG GAG TCT GGG
V_H4-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG GTG CAG CTG CAG GAG TCG G
V_H5-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** GAR GTG CAG CTG GTG CAG TCT GGA G
V_H6-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG GTA CAG CTG CAG CAG TCA GGT CC
Reverse primer	**5′-3′ sequence**
IgG-extb	**GC TGT GCC CCC AGA GGT** GCT CYT GGA

Table 7
Primers for the PCRb to amplify V_κ

Forward primer	5′-3′ sequence
V_κ1-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAC ATC CAG WTG ACC CAG TCT C
V_κ2-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAT ATT GTG ATG ACC CAG WCT CCA C
V_κ3-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAA ATT GTG TTG ACR CAG TCT CCA
V_κ4-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAC ATC GTG ATG ACC CAG TCT C
V_κ5-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAA ACG ACA CTC ACG CAG TCT C
V_κ6-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAA ATT GTG CTG ACW CAG TCT CCA
V_κ7-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC**GAC ATT GTG CTG ACC CAG TCT
Reverse primer	**5′–3′ sequence**
Cκ-int	GGG AAG ATG AAG ACA GAT GGT

2.5 Expression Vector Cloning

1. High-Fidelity DNA Polymerase.
2. Gel Extraction Kit.
3. Universal DNA Purification Kit. Plasmids purification kit.
4. *Eco*RI.
5. *Kpn*I.

Table 8
Primers for the PCRb to amplify V_λ

Forward primer	5′–3′ sequence
V_λ1-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG TCT GTG YTG ACK CAG CC
V_λ2-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG TCT GCC CTG ACT CAG CC
V_λ3-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** TCY TAT GAG CTG ACW CAG CCA C
V_λ3l-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** TCT TCT GAG CTG ACT CAG GAC CC
V_λ4ab-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG CYT GTG CTG ACT CAA TC
V_λ4c-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CTG CCT GTG CTG ACT CAG C
V_λ5/9-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG SCT GTG CTG ACT CAG CC
V_λ6-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** AAT TTT ATG CTG ACT CAG CCC CAC T
V_λ7/8-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG RCT GTG GTG ACY CAG GAG
V_λ10-Int tag	**CTGGGTTCCAGGTTCCACTGGTGAC** CAG GCA GGG CWG ACT CAG
Reverse primer	**5′–3′ sequence**
C_λ-int	GGG YGG GAA CAG AGT GAC C

6. *Xho*I.
7. T4 DNA Ligase.
8. Competent cells.
9. DNA loading buffer.
10. Ampicillin (100 mg/mL): dissolve 500 mg of ampicillin in 4 mL ddH_2O. Mix until all ampicillin is completely dissolved. Adjust the volume to 5 mL with ddH_2O. Mix it again. Sterilize the ampicillin stock solution by filtering through a 0.22 μm membrane. Store at −20 °C.
11. LB agar plates containing ampicillin: dissolve 5 g of tryptone, 10.0 g of yeast extract, 10.0 g of NaCl, and 15.0 g of agar in 1 L of ddH_2O. The agar will not dissolve until autoclaved. Autoclave for 15 min and then allow it to cool until the bottle can be held with bare hands. Add 1 mL of ampicillin (100 mg/mL). Invert to mix thoroughly. Carefully pour out into sterile

Table 9
Other primers used in this study

Forward primer	**5′–3′**
SP	CTGGGTTCCAGGTTCC
C_H F new	ACCGTCTCCTCAGCCAGCACCAAAGGC
C_κ F new	CGAACTGTGGCTGCACCAAGCGTGTTTATC
C_λ F new	CCCAAGGCTGCCCCCTCCGTGACACTG
$LF_{h/l}$	CCGGAATTCGCCACCATGGAGACGGATACGCTGCTCCTG
$LF_{h/l}$	CCGGAATTCGCCACCATGGAGACGGATACGCTGCTCCTG
LF_κ	CCGGAGCTCGCCACCATGGAGACGGATACGCTGCTCCTG
LF2 new	GAGACGGATACGCTGCTCCTGTGGGTTTTGCTGCTGTGGGTTCCAGGTTCCAC TGGT
Reverse primer	**5′–3′**
V_H R new	GGTGCTGGCTGAGGAGACGGT
C_H R new	ATAAGAATGCGGCCGCTCATTTACCCGGAGACAG
V_κ R new	TGGTGCAGCCACAGTTCG
C_κ r new	ATAAGAATGCGGCCGCTTAGCTGCATTCGCCCCTG
V_λ R new	AGGGGGCAGCCTTGGG
C_λ r new	ATAAGAATGCGGCCGCTCAGCTGCACTCGGTTGGG

plastic petri dishes. Allow the dishes to set and then store wrapped in plastic in the cold room.

2.6 mAb Expression

1. NanoDrop 2000/2000c spectrophotometer.
2. Plasmids purification kit.
3. Dulbecco's Modified Eagle Medium (DMEM).
4. FBS.
5. Penicillin-streptomycin and liquid.
6. Culture medium: DMEM with 10% FBS, as well as 1× penicillin-streptomycin.
7. HEPES-buffered saline (HBS) buffer: Weigh 8.766 g NaCl and 5.2 g HEPES, and dissolve in 900 mL ddH_2O. Adjust the pH to 7.4 using 0.5 M NaOH. Sterilize the buffer by autoclaving for 15 min at 121 °C or by filter sterilization.
8. Trypsin-EDTA (0.05%) and phenol red.

9. 1 mg/mL polyethylenimine (PEI) in HBS buffer. Sterilize the PEI solution by filtering through a 0.22 μm membrane. Store at 4 °C.
10. Goat anti-Human IgG (H+L) Secondary Antibody, HRP.
11. Nitrocellulose membrane.
12. SDS-PAGE running buffer (10×): Dissolve 30.0 g of Tris base, 144.0 g of glycine, and 10.0 g of SDS in 800 mL of H_2O. Then add H_2O to 1 L. Store the running buffer at room temperature and dilute to 1× before use.
13. SDS-PAGE loading buffer (5×).
14. Transfer buffer: Dissolve 6.04 g of Tris base and 28.8 g of glycine together in 1.6 L of ddH_2O. Then add 200 mL of methanol and mix. At last, add ddH_2O to a final volume of 2 L.

2.7 Binding Characterization for the mAbs

1. Biacore T100 system.
2. Sensor Chip Protein A.
3. Biacore T100 evaluation software, version 1.0.
4. HBS-EP buffer (10×).

3 Methods

3.1 Isolation of PBMCs (0.5 Day)

1. Collect 10 mL of whole blood, using a K_2 EDTA Tube, from a convalescent Zika patient or a healthy donor with informed consent (*see* **Note 5**).
2. Dilute the anticoagulated blood with 10 mL PBS (one volume of the original blood).
3. Invert the density gradient medium bottle several times to ensure thorough mixing.
4. Add 13 mL of density gradient medium to a 50 mL centrifuge tube with a conical bottom.
5. Carefully layer the diluted blood sample onto the density gradient medium solution (*see* **Note 6**).
6. Centrifuge at 500 × *g* for 25 min at room temperature (*see* **Notes 7** and **8**).
7. Draw off the upper layer containing plasma and platelets using a sterile pipette, leaving the PBMC layer undisturbed at the interface. Aliquot 1 mL of the upper layer per vial, which contains the plasma, and store them at −80 °C (Fig. 1).
8. Transfer the layer of PBMCs to a sterile 50 mL centrifuge tube using a sterile pipette (*see* **Note 9**).

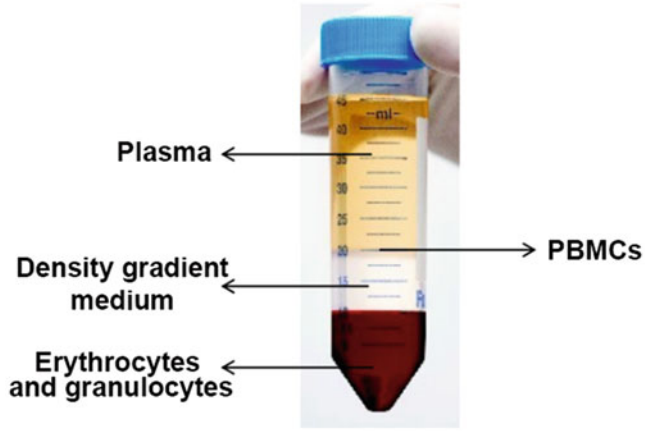

Fig. 1 Layers of density gradient medium solution and whole blood after centrifugation. Layer the diluted blood sample onto the density gradient medium solution. After centrifugation, four layers could be observed. They are, from top to bottom, plasma, PBMCs, density gradient medium solution, and erythrocytes and granulocytes, respectively

9. Add PBS until the total volume reaches 15 mL (1.5 volumes of the original blood). Resuspend the cells by gently drawing them in and out of a pipette.
10. Centrifuge at 500 × *g* for 15 min at room temperature, and discard the supernatant.
11. Add 15 mL PBS to resuspend the cells. Mix thoroughly and transfer 50 μL of cell suspension in a 1.5 mL tube for cell counting.
12. Load 10 μL of cell suspension into a cell counting chamber.
13. Insert the chamber into an automated cell counter and run the counting.
14. Repeat **step 10** in this section.
15. Quickly resuspend the cell pellet by adding freezing medium to a cell density of 1×10^7/mL (*see* **Note 10**).
16. Aliquot 1 mL of the cell-medium mixture per vial, and place in a freezing container.
17. Freeze the cells overnight at −80 °C.
18. Transfer vials to a liquid N_2 tank for storage (*see* **Note 11**).

3.2 Preparation of Cells for Flow Cytometry (0.5 Day)

1. Samples used for staining are designed as follows:
   (a) Zika patient PBMCs stained with Zika E protein, as well as a panel of memory B-cell markers (*see* **Note 12**).

(b) Healthy donor PBMCs stained with Zika E protein, as well as a panel of memory B-cell markers (negative control).

(c) Healthy donor PBMCs stained with MERS-RBD protein (positive control for anti-His antibody).

2. Transfer a vial containing the frozen PBMCs of either a convalescent Zika patient or a healthy donor from the liquid N_2 tank, and place them into a 37 °C water bath (*see* **Note 13**).
3. Gently swirl the vial in the 37 °C water bath, and quickly thaw the cells (<1 min) until there is just a small bit of ice left in the vial.
4. Transfer the vials into a hood. Before opening, wipe the outside of the vials with 70% ethanol.
5. Transfer the thawing cells into a centrifuge tube (15 mL) containing 10 mL of pre-warmed culture medium.
6. Centrifuge the cell suspension at 500 × *g* for 5 min at room temperature.
7. Decant the supernatant without disturbing the cell pellet.
8. Gently resuspend the cells in FACS buffer, 400 μL per 10^7 cells.
9. Transfer 1×10^5 cells (4 μL) into a FACS tube containing 200 μL of FACS buffer as control cells without staining.
10. Add purified Zika E protein to the cells to a final concentration of 100 nM.

    Calculation: Predicted molecular weight of Zika E protein = 45 kDa.
    Volume = 400 μL.

    Target molar concentration = 100 nM.

    Concentration of Zika E protein = 1 mg/mL.

    Thus, we need to transfer Zika E protein to the cells in a volume of as follows:

    $(400 \times 10^{-6}\ \text{L}) \times (100 \times 10^{-9}\ \text{mol/L}) \times (45 \times 10^3\ \text{g/mol}) / 1\ \text{g/L} = 1.8 \times 10^{-6}\ \text{L} = 1.8\ \mu\text{L}.$
11. Keep the mixture on ice for 1 h.
12. Add 10 mL of FACS buffer to the cells and pellet the cells by centrifuging at 500 × *g* for 5 min at 4 °C. Decant the supernatant without disturbing the cell pellet (*see* **Note 14**).
13. Resuspend the cells with 100 μL of FACS buffer per 10^7 cells (*see* **Note 15**).
14. Prepare staining master mix in a 1.5 mL microcentrifuge tube (*see* Table 1) and store them at 4 °C in the dark before use.
15. Add 100 μL of the staining master mix to cells that prepared in **step 13** in this section. Incubate them on ice for 30 min.

16. Add 10 mL of FACS buffer to the mixture of cells and antibodies in the last step and pellet the cells by centrifugation at 500 × *g* for 5 min at 4 °C. Decant the supernatant without disturbing the pellet.
17. Resuspend the cells with 0.5 mL FACS buffer. Place the tube on ice and avoid light before cell sorting.

3.3 Preparation of Single-Color Compensation Controls (1 h)

1. Label a FACS tube for each of the six fluorochromes that will be used in the cell sorting. Add 0.5 mL of FACS buffer into each tube.
2. Mix compensation beads by vigorously inverting at least ten times.
3. Add 50 μL of compensation beads into each tube.
4. Add 1 μL of fluorochrome-conjugated antibody to the appropriately labelled tube. Mix well by flicking, inverting vigorously, or pulse vortexing (*see* **Note 16**).
5. Incubate at room temperature for 15 min in the dark.
6. Add 4 mL of FACS buffer to each tube and centrifuge at 500 × *g* for 5 min at 4 °C.
7. Decant the supernatant and add 0.5 mL of FACS buffer to each tube.
8. Mix briefly by flicking or pulse vortexing before analysis.

3.4 Isolation of Zika E-Specific Single Human Memory B Cells by Flow Cytometry (See Note 17) (0.5 Day)

1. Use the Instrument Setup feature to automatically calculate compensation settings. Load the samples as indicated by the instructions of the instrument.
2. After calculation of the compensation values, save and link them.
3. Load the negative control sample (healthy donor stained with Zika E protein, as well as a panel of memory B-cell markers) onto the cytometer and gate on the $CD3^{-}CD16^{-}CD235a^{-}CD19^{+}CD27^{+}CD38^{-}IgG^{+}$ memory B cells as shown in Fig. 2A (*see* **Notes 18** and **19**).
4. For the negative control, analyze the PE shift of the lymphocyte subpopulation as displayed in Fig. 2B.
5. Load the healthy PBMCs sample stained with MERS-RBD (Fig. 2C). Analyze the PE shift of the lymphocyte subpopulation (*see* **Note 20**).
6. Load the Zika PBMCs sample stained with Zika E protein and memory B-cell markers. Gate the target cells as in Fig. 2D.
7. Adjust the flow rate so that the event rate is approximately 8000 events/s.

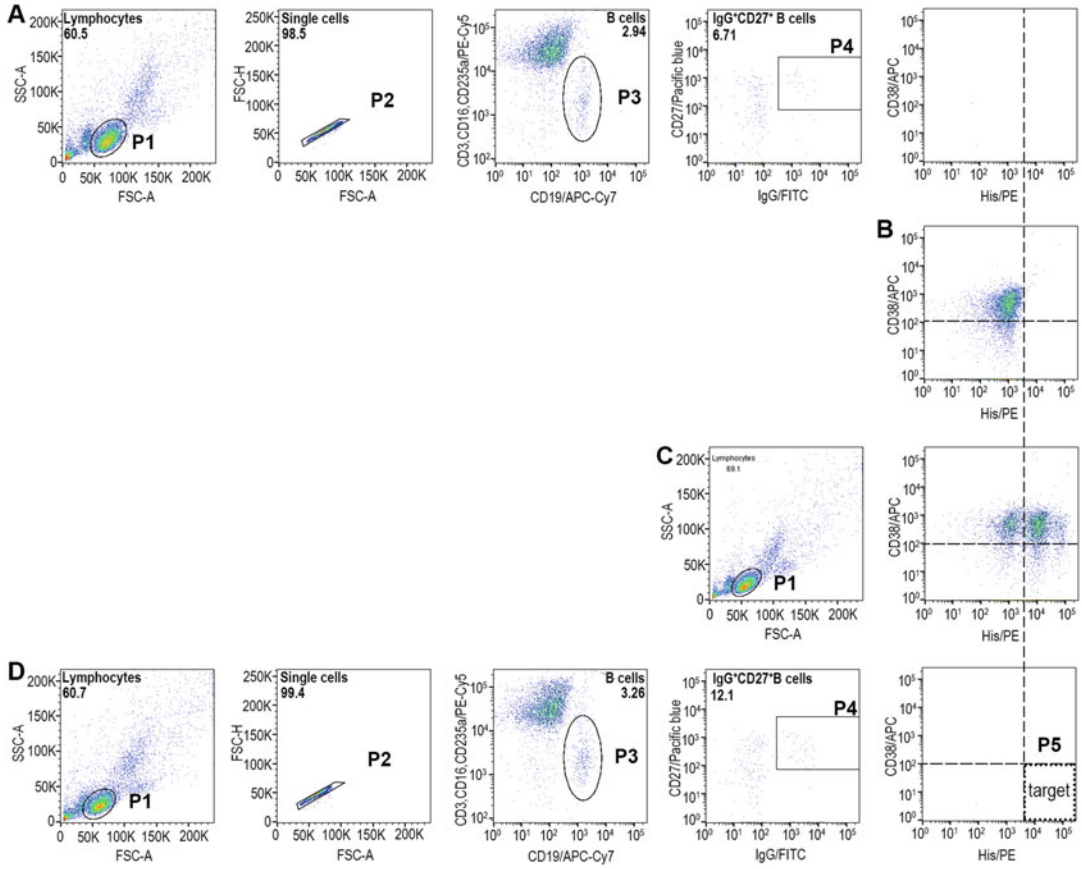

Fig. 2 Gating strategy for Zika E-specific memory B-cell population. (**A**) Healthy donor PBMCs stained with Zika E protein, as well as a panel of memory B-cell markers. Cells were first gated based on the FSC-A and SSC-A (P1). Single cells in P1 (P2) were further analyzed for marker expression. In particular, cells showing expression of CD19, but not CD3, CD16, or CD235a, were gated out (P3). B cells, represented by P3, were further analyzed for expression of CD27 and hIgG (P4). At last, cells in P4 were analyzed for the expression of CD38 and the PE shift. (**B**) P1 cells in panel (**A**) were analyzed for the expression of CD38 and the PE shift. (**C**) Healthy donor PBMCs stained with MERS-RBD, which contains a 6× His tag at its C-terminus. The lymphocyte subpopulation (P1) was analyzed for the expression of CD38 and the PE shift. (**D**) Zika patient PBMCs stained with Zika E protein and memory B-cell markers. The cells gated as those in panel (**A**). The target cells (P5) were sorted in the 96-well plate (one per well) for further analysis

8. Collect the cells (one per well) into a 96-well PCR plate that is preloaded with 8 μL DEPC-treated ddH_2O.
9. Plates are then sealed with an adhesive film and immediately frozen on dry ice before storage at −80 °C.

3.5 Determination of the Ig Genes in the Isolated Single Cells (2 Days)

1. Mix and briefly centrifuge each component in the RT-PCR synthesis system for the first-strand cDNA before use.
2. Combine the components in Table 10 in a 0.5 mL tube.

Table 10
Primers and dNTP for the first-strand cDNA synthesis

Component	For 1 cell (μL)	For 100 cells (μL)
Primers (1.5 μg/μL)		
IgM-RT	0.1	10
Igd-RT	0.1	10
Ige-RT	0.1	10
Iga1-RT	0.1	10
Iga2-RT	0.1	10
Igg-RT	0.1	10
Cκ-new RT	0.1	10
Cλ-new-ext	0.1	10
10 mM dNTP mix	1	100
DEPC-treated water	0.95	20
Total	2.75	275

Table 11
cDNA Synthesis Mix for the first-strand cDNA synthesis

Component	For 1 cell (μL)	For 100 cells (μL)
10× RT buffer	2	200
25 mM $MgCl_2$	4	400
0.1 M DTT	2	200
RNase inhibitor (40 U/μL)	1	100
Reverse transcriptase (200 U/μL)	0.25	25
Total	9.25	925

3. Add 2.75 μL of mixed primers and dNTP (*see* Table 10) into each well containing the collected single cells prepared in **step 9** in Subheading 3.4.
4. Incubate the plate at 65 °C for 5 min and then place it on ice for at least 1 min.
5. Prepare the following cDNA Synthesis Mix in a 1.5 mL tube, adding each component in Table 11 in the indicated order.

Table 12
Primer mix for the PCRa

V_H (100 nM)	Amount (μL)	V_κ (100 nM)	Amount (μL)	V_λ (100 nM)	Amount (μL)
V_H1-Ext	10	V_κ1/2-Ext	10	V_λ1-Ext	10
V_H2-Ext	10	V_κ3-Ext	10	V_λ2-Ext	10
V_H3-Ext	10	V_κ4/5-Ext	10	V_λ3-Ext	10
V_H4-Ext	10	V_κ6/7-Ext	10	V_λ3l-Ext	10
V_H5-Ext	10	C_κ-ext	10	V_λ4ab-Ext	10
V_H6-Ext	10			V_λ4c-Ext	10
IgG-exta	10			V_λ5/9-Ext V_λ6-Ext V_λ7/8-Ext V_λ10-Ext C_λ-new-ext	10 10 10 10 10
DEPC-treated water	130		150		90
Total amount	200		200		200

6. Add 9.25 μL of cDNA Synthesis Mix (*see* Table 11) to each well, mix gently, and collect by centrifugation at 15,000 × *g* at 4 °C for 15 s.
7. The RT reaction is performed at 55 °C for 60 min and terminated at 85 °C for 5 min. Chill on ice and then store at −20 °C until use.
8. Prepare primers for the PCRa (*see* Tables 3–5). The amount for 100 reactions is listed in Table 12.
9. Perform the PCRa to amplify the Ig genes. Prepare the reaction system as indicated in Table 13 (*see* **Note 21**).

   The cycling conditions for the PCRa are shown in Table 14.

   Store the PCRa products at −20 °C until further use.
10. Prepare primers for the PCRb (*see* Tables 6–8). The amount listed in Table 15 is for 100 reactions.
11. Perform the PCRb to amplify the Ig genes. Prepare reaction system as indicated in Table 16 (*see* **Notes 21** and **22**).

    The cycling conditions for the PCRb are indicated in Table 17.
12. Load the samples onto an agarose gel (1.2%), and separate the DNAs by electrophoresis.
13. A typical band size for V_H, V_κ, and V_λ is approximately 400 bp, as displayed in Fig. 3.

Table 13
Reaction system for the PCRa

Component	For V_H (μL)	For V_κ (μL)	For V_λ (μL)
RT product	2	2	2
DNA polymerase (for single-cell PCR)	0.4	0.4	0.4
10 mM dNTP mix	0.4	0.4	0.4
Primers for V_H	2	--	--
Primers for V_κ	--	2	--
Primers for V_λ	--	--	2
10× PCR buffer	2	2	2
5× Q solution	4	4	4
ddH_2O	9.2	9.2	9.2
Total	20	20	20

Table 14
The cycling conditions for the PCRa

	Temperature	For V_H	For V_κ	For V_λ
	95 °C	5 min	5 min	5 min
35 cycles	95 °C 55 °C 50 °C 72 °C	30 s 30 s – 30 s	30 s 30 s – 30 s	30 s – 30 s 30 s
	72 °C	7 min	7 min	7 min

14. Cut out the ~400 bp bands and extract the DNA segments using a Gel Extraction Micro Kit according to the manufacturer's instructions.
15. Sequence the bands using primer SP.
16. Analyze the sequences by using the IgBlast tool (https://www.ncbi.nlm.nih.gov/igblast/index.cgi) or IMGT (http://www.imgt.org/) to identify germline V(D)J gene segments with the highest identity (*see* **Note 23**).

3.6 Construction of the Expression Vectors (See Note 24) (4 Days)

1. Perform the PCRc. Prepare reaction system as indicated in Table 18.

Table 15
Primer mix for the PCRb

V_H (100 nM)	Amount (μL)	Vκ (100 nM)	Amount (μL)	V_λ (100 nM)	Amount (μL)
V_H1-Int tag	10	Vκ1-Int tag	10	V_λ1-Int tag	10
V_H2-Int tag	10	Vκ2-Int tag	10	V_λ2-Int tag	10
V_H3-Int tag	10	Vκ3-Int tag	10	V_λ3-Int tag	10
V_H4-Int tag	10	Vκ4-Int tag	10	V_λ3l-Int tag	10
V_H5-Int tag	10	Vκ5-Int tag	10	V_λ4ab-Int tag	10
V_H6-Int tag	10	Vκ6-Int tag	10	V_λ4c-Int tag	10
IgG-extb	10	Vκ7-Int tag Cκ-int	10 10	V_λ5/9-Int tag V_λ6-Int tag V_λ7/8-Int tag V_λ10-Int tag C_λ-int	10 10 10 10 10
DEPC-treated water	130		120		90
Total amount	200		200		200

Table 16
Reaction system for the PCRb

Component	For V_H (μL)	For V_κ (μL)	For V_λ (μL)
PCRa product	1	1	1
DNA polymerase (for single-cell PCR)	0.4	0.4	0.4
10 mM dNTP mix	0.4	0.4	0.4
Primers for V_H	2	--	--
Primers for V_κ	--	2	--
Primers for V_λ	--	--	2
10× PCR buffer	2	2	2
5× Q solution	4	4	4
ddH_2O	10.2	10.2	10.2
Total	20	20	20

2. The PCR reaction is incubated at 98 °C for 2 min, followed by 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 60 s, with a final incubation at 72 °C for 5 min.
3. Load the samples onto an agarose gel (1.2%), and separate them by electrophoresis.

Table 17
The cycling conditions for PCRb

	Temperature	For V_H	For V_κ	For V_λ
	95 °C	5 min	5 min	5 min
35 cycles	95 °C	30 s	30 s	30 s
	58 °C	30 s	30 s	--
	60 °C	--	30 s	--
	64 °C	--	--	30 s
	72 °C	30 s	30 s	30 s
	72 °C	7 min	7 min	7 min

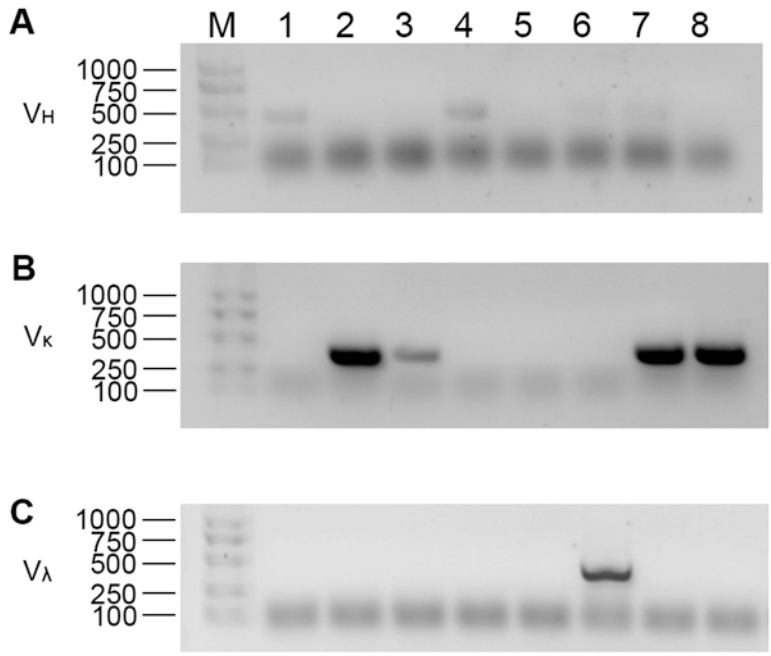

Fig. 3 Typical bands for V_H, V_κ, and V_λ after the second round PCR. After the second round PCR, the DNAs were separated by electrophoresis. The results for V_H, V_κ, and V_λ were displayed in A, B, and C, respectively

4. Cut out the ~400 bp bands and extract the DNA segments using a Gel Extraction Kit according to the manufacturer's instructions.
5. The PCR for amplification of the constant regions (*see* Table 19) is incubated at 98 °C for 2 min, followed by 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 60 s, with a final incubation at 72 °C for 5 min.

Table 18
Reaction system for the PCRc

Component	For V_H (μL)	For V_κ (μL)	For V_λ (μL)
PCRb product	2	2	2
LF2 new (10 μM)	1	1	1
V_H R new (10 μM)	1	--	--
V_κ R new (10 μM)	--	1	--
V_λ R new (10 μM)	--	--	1
10× PCR buffer	2	2	2
High-Fidelity DNA Polymerase	0.4	0.4	0.4
2.5 mM dNTP mix	1	1	1
ddH_2O	12.6	12.6	12.6
Total	20	20	20

Table 19
Reaction system for amplification of the constant regions

Component	For C_H (μL)	For C_κ (μL)	For C_λ (μL)
pCAGGS-H (100 ng/μL)	0.5	--	--
pCAGGS-K (100 ng/μL)	--	0.5	--
pCAGGS-L (100 ng/μL)	--	--	0.5
C_H F new	1	--	--
C_H R new	1	--	--
C_κ F new	--	1	--
C_κ R new	--	1	--
C_λ F new	--	--	1
C_λ R new	--	--	1
10× PCR buffer	5	5	5
High-Fidelity DNA Polymerase	1	1	1
2.5 mM dNTP mix	4	4	4
ddH_2O	37.5	37.5	37.5
Total	50	50	50

Table 20
Reaction system for overlapping PCR to generate the expression cassette

Component	For C_H (μL)	For C_K (μL)	For C_λ (μL)
V_H of PCRc	To 0.25 μg	--	--
C_H	To 0.75 μg	--	--
V_κ of PCRc	--	To 0.5 μg	--
C_K	--	To 0.5 μg	--
V_λ of PCRc	--	--	To 0.5 μg
C_λ	--	--	To 0.5 μg
$LF_{H/l}$	1	--	1
LF_κ	--	1	--
C_H R new	1	--	--
C_κ R new	--	1	--
C_λ R new	--	--	1
10× PCR buffer	5	5	5
High-Fidelity DNA Polymerase	1	1	1
2.5 mM dNTP mix	4	4	4
ddH_2O	To 50	To 50	To 50
Total	50	50	50

6. Load the samples onto an agarose gel (1.2%), and separate them by electrophoresis.
7. Cut out the ~1000 bp bands for C_H and ~400 bp for C_κ and C_λ. Extract the DNA segments using a Gel Extraction Kit according to the manufacturer's instructions.
8. Perform overlapping PCR to generate the expression cassette (*see* Table 20). The PCR reaction is incubated at 98 °C for 2 min, followed by 30 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 2 min, with a final incubation at 72 °C for 5 min.
9. Digest the PCR products from the last step with *Eco*RI and *Xho*I for both the heavy and lambda chains. Digest the kappa chain with *Kpn*I and *Xho*I (*see* Table 21).

   Mix gently and spin down for a few seconds. Incubate at 37 °C overnight.
10. Purify the digested PCR segment using a Universal DNA Purification Kit according to the manufacturer's instruction.
11. Ligate the expression cassette to the pCAGGS vector (*see* Table 22). For both heavy and lambda chains, the vector was

Table 21
Reaction system to digest the PCR products

Component	For heavy chain (μL)	For kappa chain (μL)	For lambda chain (μL)
Overlapping product	50	50	50
10× Tango buffer	20	10	20
*Eco*RI	2	--	2
*Xho*i	2	5	2
*Kpn*I	--	5	--
ddH_2O	26	30	26
Total	100	100	100

Table 22
Reaction system to ligate the expression cassette to the pCAGGS vector

Component	For heavy (μL)	For kappa (μL)	For lambda (μL)
Linear vector DNA	To 50 ng	To 50 ng	To 50 ng
Insert DNA	To 50 ng	To 25 ng	To 25 ng
10× T4 DNA ligase buffer	2	2	2
T4 DNA ligase	0.4	0.4	0.4
ddH_2O	To 20	To 20	To 20
Total	20	20	20

linearized with *Eco*RI and *Xho*I. While for kappa chain, the vector was digested by *Kpn*I and *Xho*I.

12. Incubate for 1 h at 22 °C.
13. Use 5 μL of the ligation mixture for transformation of 50 μL of competent cells according to the manufacturer's instruction.
14. Streak the transformed cells onto LB agar plates containing ampicillin. Incubate at 37 °C overnight.
15. Pick three single colonies for each construct for sequencing.
16. Align the sequences with the results in **step 16** in Subheading 3.5.
17. Extract the plasmids with the correct sequences. Mark the plasmid with Z, number, and H/K/L. Z represents Zika virus. The number indicates the sorted single cell. H/K/L stands for the chain encoded by the plasmid (e.g., the plasmid

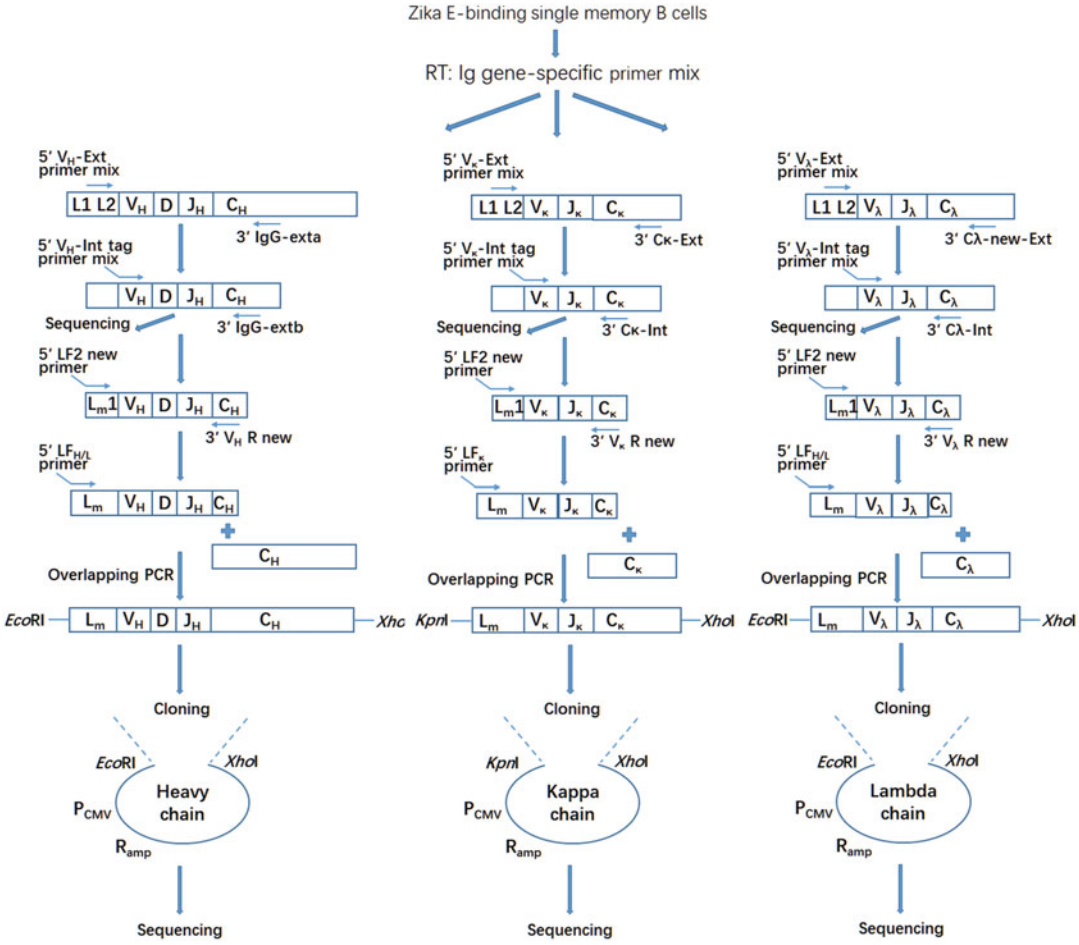

Fig. 4 Strategy to clone and express Zika E-specific human mAbs. The Ig genes from the sorted cells were determined and cloned into the expression vectors by a reported approach with some modifications [13, 18]. The Ig transcripts in the collected Zika E-interacting memory B cells were first reverse-transcribed into cDNA using the Ig gene-specific primer mix at the single-cell level. Then the variable regions for heavy, kappa, and lambda chains were amplified by nested RT-PCR. The first round PCRs were performed with the forward primer mix specific for the leader region and reverse primers specific for the constant regions of heavy, kappa, and lambda chain, respectively. The second round PCRs were performed with the forward primer mix specific for framework segment FR1 and respective nested reverse primers specific for the heavy, kappa, and lambda constant regions. Then the PCR products were separated by electrophoresis and sent for sequencing. In terms of the correct segments, another round of PCR (PCRc) was performed with the forward primer containing the signal peptide of mouse Ig_{κ} and reverse primer paired with the framework segment FR4. Then the resultant PCR segments were overlapped with the respective constant region to get the full expression cassettes for each chain, which were then ligated to the linearized pCAGGS vector. All expression plasmids were sequenced and aligned with those in PCRb

pCAGGS-Z23H encodes the heavy chain of the 23rd sorted cell).

18. The generation of vectors for mAb expression is displayed in schematic diagram in Fig. 4.

3.7 Expression of the Recombinant mAbs (4 Days)

1. Prepare the plasmids containing paired mAbs using a Plasmid Purification Kit according to the manufacturer's instruction.
2. Determine the concentration of each purified plasmid with a NanoDrop.
3. The day before transfection, plate 1×10^6 HEK 293T cells into 6-well plates in DMEM with 10% FBS.
4. For each mAb, mix 1.5 μg heavy chain together with 1.5 μg light chain in 125 μL DMEM medium. Add 9 μL PEI in another tube containing 125 μL DMEM medium.
5. Transfer the PEI solution into the tube with plasmids. Incubate the transfection mixture at room temperature for 20 min.
6. Add 750 μL of DMEM into each transfection mixture.
7. Aspirate the medium in the plates. Add the transfection mixture to the cells drop by drop. Gently rock the plate to ensure even distribution. Do not swirl.
8. Incubate at 37 °C for 4–6 h. Then, aspirate the medium, and add 3 mL culture DMEM. Cultivate for another 48 h at 37 °C.
9. Mix 10 μL of culture supernatant with SDS-PAGE loading buffer. Incubate in boiling water for 5 min. Centrifuge the samples in the tube at $15{,}000 \times g$ for 1 min.
10. Load the samples onto a precast SDS-PAGE gel (4–10%), and separate the proteins by electrophoresis.
11. Transfer the proteins onto a nitrocellulose membrane.
12. Perform western blotting to assess the expression of each mAb using anti-hIgG/HPR (diluted by 1:1000). As indicated in Fig. 5, two bands (indicating the heavy and light chain, respectively) are observed under reducing conditions (*see* **Note 25**).

3.8 SPR Analysis to Determine the Specificity and Binding Affinity of mAbs to Zika E Protein (See Notes 26 and 27) (1 Day)

1. Centrifuge the culture supernatant containing mAbs at $15{,}000 \times g$ at 4 °C for 10 min to avoid any cell debris. Transfer the supernatant into another 1.5 mL tube.
2. Insert the Sensor Chip Protein A to the instrument of Biacore T100. Prime with 1× HBS-EP buffer. Adjust the flow rate to 30 μL/min.
3. Load clarified supernatant from the first step in this section onto the Sensor Chip Protein A flow channel (Fc) 2 for 60 s (the amount of supernatant injected to the sensor is 30 μL). Leave Fc1 empty as negative control. Record the response unit (RU).
4. Inject 3 M $MgCl_2$ to both Fc1 and Fc2 to dissociate the mAb from protein A (regeneration).
5. Calculate the amount of supernatant that is needed to obtain ~500 RU in Fc 2. Dilute the supernatant or extend the

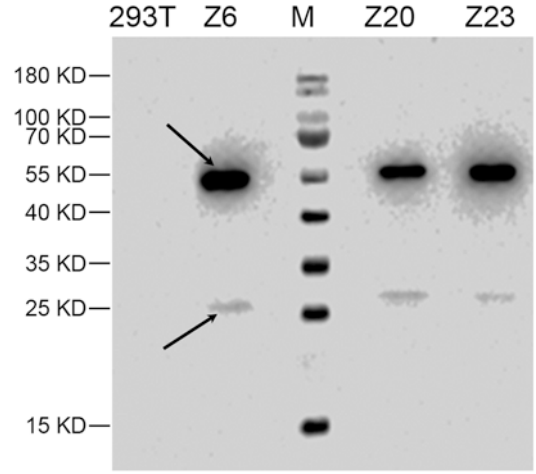

Fig. 5 Western blot to analyze the expression of mAbs. We loaded 10 μL of culture supernatant of HEK 239T cells, which were transfected with plasmids containing Ig genes for the indicated mAbs. Then, the goat anti-hIgG antibody conjugated with HRP (1:1000) was used to incubate the membrane transferred with the mAbs. Here, we displayed the results for Z6, Z20, and Z23. The upper arrow indicates the heavy chain, and the lower arrow indicates the light chain. 293T represents the parallel culture supernatant of HEK 293T cells without transfections

injecting time, if necessary (*see* **Note 28**). Then load the supernatant used in **step 3** in this section to Fc 2 again.

6. Change the flow path to Fc 1–Fc 2. Then wash the sensor chip with 1× HBS-EP for 3 min.
7. Inject serially diluted Zika E protein that has been exchanged into 1× HBS-EP buffer into the sensor chip in the Fc 2-Fc 1 mode. The binding response is recorded (*see* **Note 29**).
8. Repeat **step 4** for regeneration.
9. Load another mAb supernatant, and test its interaction with Zika E as in **steps 3**–**7** in this section.
10. Calculate the binding kinetics between Zika E protein and the indicated mAbs, if there are interactions between them, using Biacore T100 evaluation software, version 1.0 (*see* **Note 30**).
11. Thus, using SPR, the true binders and their binding affinities to Zika E are determined.

4 Notes

1. EtBr stains DNA by intercalating between the bases of DNA. It will also intercalate into human DNA, so wear gloves to prevent contact with it. A separate space is also recommended for performing all experiments using EtBr-containing materials.

2. Do not pull the comb out too soon, as it causes the wells to collapse. It will take 15–20 min to gel. If the gel cannot be used in 1 h, it is recommended to transfer the gel without comb into a tank containing 1× TAE buffer.
3. The primers used for the amplification of Ig genes are the same as reported (*see* Tables 2–8) [18]. However, after sequencing, cloning of the expression vectors was designed based on the pCAGGS vector.
4. All primers are stored in small aliquots to avoid repeated freezing and thawing.
5. The blood sample of the convalescent Zika patient was collected 20 days post onset of fever, headache, and dizziness. It is reported that after immunization, antigen-specific B cells with a memory phenotype could be detected in the blood within 1 week [19]. In the presence of antigens, memory B cells undergo affinity maturation, and their B-cell receptors have increased affinities for the antigen. Studies on memory B cells after smallpox vaccination in humans indicated that antigen-specific memory B cells initially declined postimmunization (1 year) but then reached a plateau ~tenfold lower than peak and were stably maintained for >50 years after vaccination [20]. Thus, considering the time that needed for affinity maturation, we recommend collecting the blood 1 month–1 year post onset of symptoms to isolate specific mAbs.
6. When layering the sample, do not mix the density gradient medium solution and the diluted blood sample. Keep them in separate layers.
7. The break should be turned off at this step. For other steps using centrifuging, set the break on.
8. If the blood has been collected for >2 h, extend the centrifuging time to 30 min.
9. Usually, 3–4 mL of solutions containing PBMCs will be pipetted out from 10 mL of the blood.
10. For example, if the cell density is determined to be 1×10^6/mL at **step 13** in Subheading 3.1, the total cell number is $1 \times 10^6/\text{mL} \times 15\ \text{mL} = 1.5 \times 10^7$. Thus, we need to add 1.5 mL freezing medium to resuspend the cells.
11. Freshly prepared PBMCs exert higher efficiencies for Ig gene amplification than frozen cells. Thus, freshly prepared PBMCs are recommended for the isolation of mAbs. In case the following cell sorting cannot be performed immediately, the methods using the freezing cells are provided in Subheading 3.2.
12. Memory B cells express CD19 and CD27, but not CD38. In this study, we focus on the IgG^+ memory B cells.

13. If fresh PBMCs are used here, please skip to **step 8**. If using frozen cells, please follow **step 2**.
14. Before turning the tube right side up, it is recommended to aspirate the liquid left around the tube orifice.
15. It is difficult to decant the entire buffer. Usually, ~50 μL of buffer will be left. Thus, we add 50 μL FACS buffer for 10^7 cells or 150 μL for 2×10^7 cells.
16. There are three antibodies conjugated with PE-Cy®5. They are anti-human CD3/PE-Cy®5, anti-human CD16/PE-Cy®5, and anti-human CD235a/PE-Cy®5. Any of the three are suitable for preparation of the compensation beads for PE-Cy®5.
17. The steps in this section are specific to, but not limited to, a BD FACSAria III cytometer.
18. Here, we use the markers for T cells, NK cells, and platelets for negative selection, to exclude their disturbance.
19. Here, we include FSC-A and FSC-H to exclude cell aggregates.
20. We are not sure about the proportion of memory B cells that bind to Zika E protein. Thus, it is difficult to set the threshold to distinguish antigen-specific memory B cells from those targeting other antigens in the sample of Zika patient PBMCs. In previous work, we studied the interaction between the receptor-binding domain (RBD) of Middle East respiratory syndrome coronavirus (MERS-CoV) and its receptor CD26, which is widely expressed on lymphocytes, including T cells. Here, we used the MERS-RBD, which is also tagged at its C-terminus with 6× His like the Zika E protein, to stain PBMCs from a healthy donor. Then, anti-His/PE was applied to bind to the His tag. Through comparison between the results in Fig. 2B and C, we could determine the threshold to gate the Zika E-specific memory B cells, as indicated in Fig. 2D.
21. The PCR reactions are performed in 96-well plates.
22. The combined primers are used to amplify Ig genes in the PCRb. However, different primers in a single tube might disturb each other and reduce their annealing efficiency. Thus, in terms of the single cells that yield typical bands for the kappa or lambda chain but not for heavy chain in gel electroporation, we usually repeat PCRb using the separate primers. In addition, $MgCl_2$ exerts effects on the activities of DNA polymerase (for sing-cell PCR). Varied concentrations of $MgCl_2$, ranging from 1.5 to 4.5 mM, can also be applied to amplify the variable region of the heavy chain from single cell, whose light chains' variable regions have been sequenced.
23. From PMBCs isolated from 10 mL whole blood of a convalescent patient, we finally sequenced 15 paired mAbs, which have been published previously [8].

24. Here, we introduce the traditional method to double digest the PCR segments with two restriction enzymes and clone them into the same sites in the linearized pCAGGS vector using T4 ligase. However, other methods (e.g., In-Fusion reaction [21]) can be used.
25. Here we displayed the results for Z6, Z20, and Z23.
26. The steps in this section are specific to, but not limited to, a Biacore T100 system.
27. In addition to SPR, enzyme-linked immunosorbent assay is another typical method to assess the interaction between mAbs and antigens. However, for Zika E-specific mAbs, we found that some of them displayed relatively low response to the antigens coated on the plate (data not shown here). It is possible that Zika E proteins undergo some conformational changes when adsorbing to the plate, which results in the decreased binding to certain mAbs. In terms of SPR experiments, the mAbs were captured on the chip through interactions with protein A, which binds to the Fc region. Thus, the CDRs of mAbs orient to the buffer flowing over the chip surface. In addition, the Zika E proteins in the buffer of HBS-EP are label-free and more prone to be in its native conformation than those coated on the plate. Thus, we chose SPR assay to detect the interaction between mAbs and antigens.
28. For example, if the original supernatant reaches to 1000 RU with 30 μL, then shorten the injecting time from 60 to 30 s, or dilute the supernatant with equal volume of 1× HBS-EP buffer and load 60 s. If 30 μL of the original supernatant enables 200 RU, then extend the injecting time to 150 s.
29. Due to the different binding kinetics between an antigen and its mAb, we set varied dissociation time. For example, mAb Z6 dissociates with Zika E with very low rate; thus we set 600 s for their dissociation. However, for both Z20 and Z23, they are ready to dissociate with the antigen, and 60 s was used for their dissociation (Fig. S2 G, J, and L in the paper [8]).
30. Due to the different binding kinetics between mAbs and Zika E protein, the data could be fit by either a steady state affinity model or 1:1 (Langmuir) binding model.

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