Development of a novel peptide aptamer-based immunoassay to detect Zika virus in serum and urine

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Fig. S1. Construction of the Zika virus recombinant envelope (rE) protein and development of candidate monoclonal antibodies (mAbs).

Fig. S2. Alignment of amino acid sequence and location of the Z_10 envelope proteins from *Flavivirus*

Table S1. Binding score of 25 peptides with the Zika virus (ZIKV) and dengue virus (DENV) envelopes.

Table S2. Binding score of modified peptides derived from Z_10 with the Zika virus (ZIKV) and dengue virus (DENV) envelopes.

Fig. S3. Comparison of same antibodies pair (1A5/1A5 or 1G8/1G8)- and fluorescence-linked sandwich immunosorbent assays (FLISA).

Fig. S4. Sequence of binding sites using newly designed primers and probe.

Fig. S5. Prediction of the binding site between Z_10.8 and the Zika virus (ZIKV) envelope protein for each mode.

Fig. S6. Effect of human sera on an antibody pair (1A5 capture/1G8 detector)- and fluorescence-linked sandwich immunosorbant assay (FLISA).

Construct of recombinant Zika envelope protein

Vero cells were infected with Zika virus-MR 766 strain at a typical multiplicity of infection (MOI) of 0.01. After 4 to 6 days of incubation, total mRNA was extracted from the supernatant using an RNA extraction kit (Macherey Nagel, Düren, Germany). The full-length ZIKV envelop protein was amplified using a forward primer (5’-AAG CTT ATC AGG TGC ATT GGA GTC AGC A-3’) and reverse primer (5’-CTC GAG AGC AGA AAC AGC CGT GGA GAG-3’).

PCR was conducted using a CFX96 Touch™ Real-Time PCR detection system (Bio-RAD, Hercules, CA, USA); these products were then purified using the Quiagen-Gel extraction kit before ligating in pGEM-T easy and transforming into the DH5 alpha strain. DNA fragments were cloned into pET21b+ (Novagen, Madison, WI, USA). Recombinant plasmids were then transformed into BL21 DE3 (Agilent Technologies, Santa Clara CA, USA) and grown on lysogeny broth (LB) agar plates containing ampicillin (100 µg/mL) at 37°C overnight. The colonies were grown in 250 mL LB medium containing ampicillin (100 µg/mL) at 37℃ until the OD_{600} reached 0.5–0.6.

To induce antigen, 0.5 mM isopropyl β-d-1-thiogalactopyranoside (IPTG) was added to LB broth and continuously shaken at 18℃ for 20 h. The cells were centrifuged at 6,000 × g and 4℃ for 10 min and then washed with buffer A (20 mM Tris-HCl, pH 7.5 and 100 mM NaCl).

The cell pellet was then suspended in 10 mL buffer A with 10 mM imidazole and then sonicated for 15 min; total protein lysates were harvested after centrifugation at 4℃ and 27,000 × g for 15 min.

These lysates were incubated with a NI-NTA affinity column (Thermo Fisher Scientific, Waltham, MA, USA) for 2 h at 4℃ and washed with washing buffer (20 mM Tris buffer, pH 7.5; 300 mM NaCl; and 25 mM imidazole). Recombinant ZIKV envelope antigen was eluted with elution buffer (20 mM Tris-HCl, pH 7.5; 300 mM NaCl; and 250 mM imidazole).

Development of monoclonal antibodies

Six-week-old female BALB/c mice (Daehan Bio-Link, Eumseong, Korea) were immunized to develop monoclonal antibodies. First, mice were intraperitoneally injected with recombinant ZIKV envelope protein (50 μg/100 μL) mixed with an equal volume of Freund's complete adjuvant (Sigma-Aldrich). The mice were immunized three times intraperitoneally at 2-week intervals. After the third immunization, recombinant ZIKV envelope protein (5 μg/100 μL) was injected intravenously without adjuvant.

For cell fusion, splenocytes isolated from immunized mice were mixed with myeloma cell (F/o cell line) cultures in complete Dulbecco's modified Eagle's medium (DMEM) by adding 50% polyethylene glycol (Sigma-Aldrich) and culturing in a 96-well plate. Hybridoma cells were selected by sub-culturing in hypoxanthine-aminopterin-thymidine medium (Sigma-Aldrich) at 37 °C in a 5% CO_2 incubator for 3 days. After sub-cloning with limiting dilutions, selected hybridoma colonies were transferred into 75-cm^2 tissue culture flasks with DMEM containing 10% fetal bovine serum (FBS, Gibco/BRL, Grand Island, NY, USA). The general procedure for antibody purification with these ligands was conducted using a protein A agarose resin column (Amersham Biosciences, Uppsala, Sweden); the presence of antibodies was confirmed via western blotting and enzyme-linked immunoassays (ELISA).

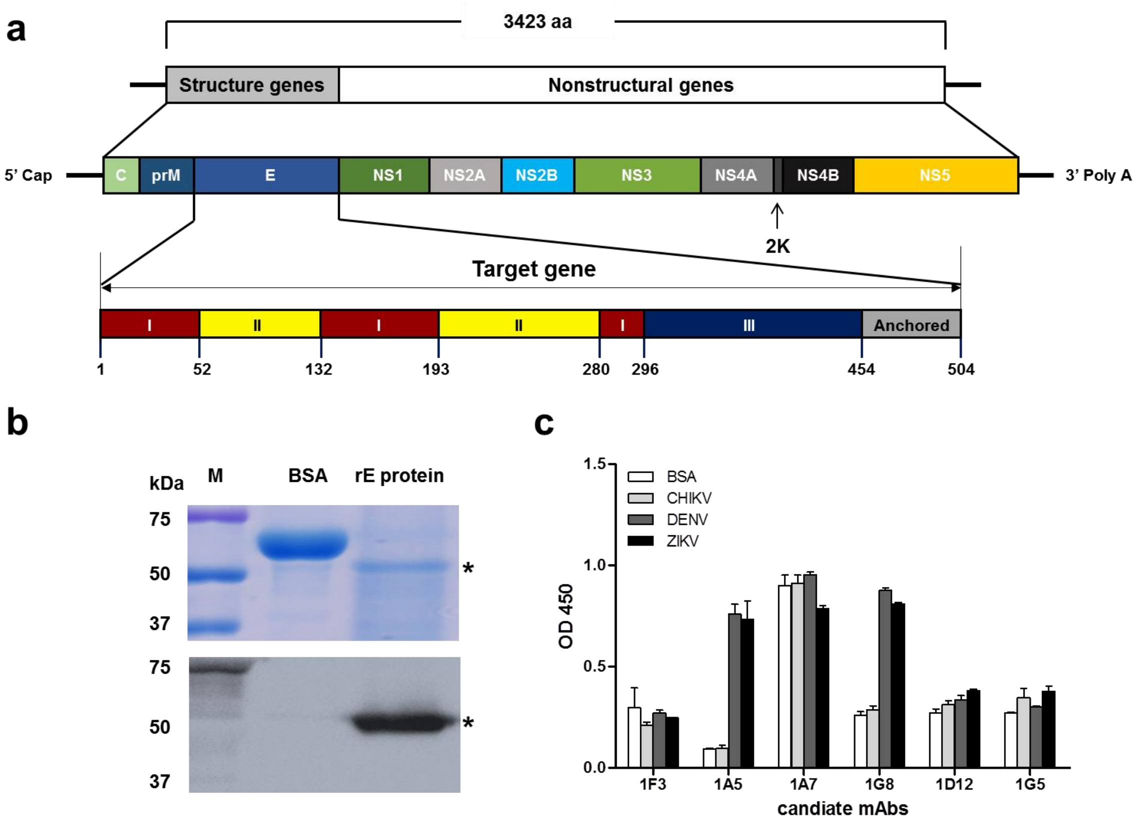


Figure S1. Construction of the Zika virus recombinant envelope protein and development of candidate monoclonal antibodies (mAbs). (a) A schematic diagram of the expressed envelope protein is shown. The cDNA coding 1–504 amino acids (aa) were selected for cloning. **(b)** Recombinant antigen was expressed in *Escherichia coli* and purified. The expression of antigen was confirmed via western blots of the purified antigen with anti-His×6 antibodies (bottom panel). rE: Zika virus recombinant envelope protein; *: target band; BSA: bovine serum albumin. **(c)** Antibody formation in 75T culture flasks after the final limiting dilution was confirmed via enzyme-linked immunoassay (ELISA). Six monoclones were selected and tested with virus at 1×10^5 tissue culture infective dose $(\text{TCID})_{50}$/mL via indirect ELISA.

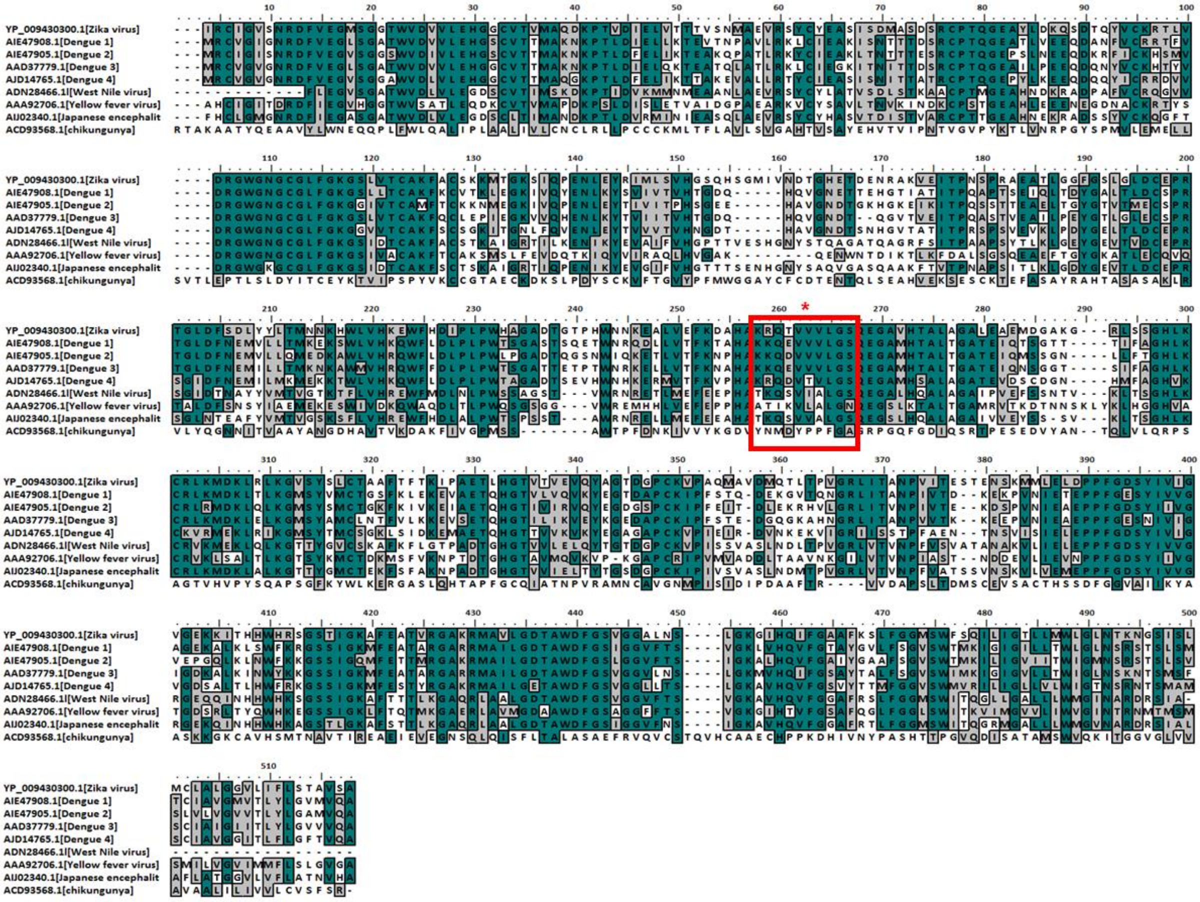


Figure S2. Alignment of amino acid sequence and location of the Z_10 envelope proteins from *Flavivirus*. Peptide Z_10 (KRQTVVVLGS) (*) in amino acids 258–267 was conserved in Zika virus (ZIKV).

Table S1. Binding score of 25 peptides

a. Docking with the Zika virus (ZIKV) envelope protein

Peptide Sequence	Mode	Binding Affinity (kcal/mol)	Average of binding energy	SD of binding energy	RMSD (u.b.)	RMSD (l.b.)	Average of RMSD
Z_1 [DVVLEHGGCV]	0	-6.7	-6.511111	0.1099944	0	0	8.7715
	1	-6.6			3.559	12.088	
	2	-6.6			2.958	6.605	
	3	-6.5			3.15	7.34	
	4	-6.5			2.631	4.467	
	5	-6.5			21.41	27.395	
	6	-6.5			2.699	5.018	
	7	-6.4			2.91	5.888	
	8	-6.3			22.202	27.567	
Z_2 [GWGNGCGLFG]	0	-6	-5.533333	0.25	0	0	11.277611
	1	-5.8			2.593	8.689	
	2	-5.7			2.937	10.283	
	3	-5.5			1.857	4.299	
	4	-5.5			4.99	9.416	
	5	-5.4			19.668	24.282	
	6	-5.3			2.973	12.268	
	7	-5.3			20.311	25.533	
	8	-5.3			23.104	29.794	
Z_3 [NKHWLVHKEW]	0	-6.6	-6.2	0.2645751	0	0	7.333
	1	-6.5			2.945	8.173	
	2	-6.4			3.565	9.906	
	3	-6.3			1.2	2.884	
	4	-6.2			3.054	9.993	
	5	-6			2.901	9.503	
	6	-6			3.708	8.493	
	7	-5.9			3.214	8.138	
	8	-5.9			24.732	29.585	
Z_4 [TQGEAYLDKQ]	0	-6.7	-6.433333	0.1322876	0	0	21.119167
	1	-6.5			22.854	25.568	
	2	-6.5			22.636	24.862	
	3	-6.5			22.946	26.233	
	4	-6.4			21.661	25.837	
	5	-6.4			22.459	23.979	
	6	-6.3			22.185	25.681	
	7	-6.3			22.502	23.207	

	8	-6.3			22.264	25.271	
Z_5 [AGADTGTPHWNN]	0	-7	-6.833333	0.1118034	0	0	13.233389
	1	**-7**			**2.471**	**6.22**	
	2	-6.9			21.803	27.761	
	3	-6.8			3.327	12.212	
	4	-6.8			2.238	4.824	
	5	-6.8			21.472	27.38	
	6	-6.8			20.698	26.377	
	7	-6.7			3.571	10.508	
	8	-6.7			20.85	26.489	
Z_6 [NSPRAEATLG]	0	-7	-6.622222	0.1715938	0	0	16.4145
	1	**-6.7**			**2.43**	**11.048**	
	2	-6.7			22.725	26.598	
	3	-6.6			2.735	11.87	
	4	-6.6			3.314	8.244	
	5	-6.6			24.033	29.126	
	6	-6.5			23.513	27.542	
	7	-6.5			22.154	28.355	
	8	-6.4			22.319	29.455	
Z_7 [DRGWGNGCGLFG]	0	-6.4	-6.133333	0.1870829	0	0	15.6885
	1	**-6.4**			**22.286**	**27.146**	
	2	-6.3			22.576	27.947	
	3	-6.1			2.9	8.68	
	4	-6.1			2.707	6.073	
	5	-6			3.082	11.452	
	6	-6			21.544	26.72	
	7	-6			21.794	28.645	
	8	-5.9			21.465	27.376	
Z_8 [TGYETDENRAKVEVTPNSPRAEAT]	0	-6.7	-5.977778	0.3192874	0	0	14.180778
	1	**-6.2**			**3.851**	**9.801**	
	2	-6.1			21.04	27.667	
	3	-5.9			3.104	8.454	
	4	-5.9			1.928	2.816	
	5	-5.8			21.802	26.88	
	6	-5.8			2.923	9.263	
	7	-5.7			26.997	32.747	
	8	-5.7			24.814	31.167	
Z_9 [EATLGGFGSLGL]	0	-7.2	-6.977778	0.1563472	0	0	13.959167
	1	**-7.2**			**3.332**	**12.79**	
	2	-7.1			21.806	27.466	
	3	-7			2.698	5.359	

	4	-6.9			21.55	28.351	
	5	-6.9			22.415	27.719	
	6	-6.9			3.802	7.877	
	7	-6.8			22.023	33.267	
	8	-6.8			3.089	7.721	
Z_10 [KRQTVVVLGS]	0	-6.8	-6.522222	0.1563472	0	0	9.97
	1	**-6.7**			**1.933**	**5.671**	
	2	-6.6			3.646	10.513	
	3	-6.5			3.906	11.071	
	4	-6.5			22.655	28.686	
	5	-6.5			2.892	11.463	
	6	-6.4			3.302	10.468	
	7	-6.4			2.928	10.943	
	8	-6.3			22.191	27.192	
Z_11 [CPTQGEAYLDKQ]	0	-6.9	-6.633333	0.1322876	0	0	19.924278
	1	**-6.7**			**21.698**	**26.793**	
	2	-6.7			22.471	27.313	
	3	-6.7			22.379	28.131	
	4	-6.6			22.612	28.018	
	5	-6.6			4.133	8.046	
	6	-6.5			21.596	27.845	
	7	-6.5			21.316	26.345	
	8	-6.5			21.808	28.133	
Z_12 [TCSKKMTGKSIQ]	0	-5.2	-5.011111	0.1054093	0	0	10.547333
	1	**-5.1**			**3.101**	**8.287**	
	2	-5.1			21.047	26.899	
	3	-5			3.264	7.703	
	4	-5			2.072	2.575	
	5	-5			19.533	25.091	
	6	-4.9			3.28	7.555	
	7	-4.9			3.206	8.611	
	8	-4.9			20.943	26.685	
Z_13 [LEHGGCVTVMAQ]	0	-5.9	-5.6	0.1802776	0	0	9.2256667
	1	**-5.8**			**1.771**	**2.351**	
	2	-5.7			3.073	5.108	
	3	-5.6			2.901	9.092	
	4	-5.6			24.43	30.878	
	5	-5.6			23.573	29.542	
	6	-5.4			3.225	9.561	
	7	-5.4			3.24	7.142	
	8	-5.4			2.852	7.323	

Z_14 [YYLTMNNKHWLV]	0	-6.6	-6.266667	0.1870829	0	0	5.9967222
	1	-6.4			3.104	15.478	
	2	-6.4			2.684	8.924	
	3	-6.3			3.254	12.082	
	4	-6.3			2.835	11.492	
	5	-6.2			2.759	9.369	
	6	-6.1			3.03	12.361	
	7	-6.1			2.427	5.492	
	8	-6			2.839	9.811	
Z_15 [VVVLGSQEGAVH]	0	-6.9	-6.777778	0.0833333	0	0	14.157667
	1	-6.9			19.389	24.723	
	2	-6.8			14.412	18.584	
	3	-6.8			5.259	9.368	
	4	-6.8			20.093	24.064	
	5	-6.7			4.84	9.788	
	6	-6.7			18.694	23.872	
	7	-6.7			3.855	14.45	
	8	-6.7			19.672	23.775	
Z_16 [HAGADTGTPHWNN]	0	-6.7	-6.566667	0.1322876	0	0	12.047278
	1	-6.7			2.996	9.121	
	2	-6.7			2.467	6.088	
	3	-6.6			2.407	8.701	
	4	-6.6			22.649	29.333	
	5	-6.6			2.684	8.264	
	6	-6.4			17.054	21.683	
	7	-6.4			13.192	18.342	
	8	-6.4			22.808	29.062	
Z_17 [LGSQEGAVHT]	0	-7.2	-6.722222	0.2773886	0	0	10.612722
	1	-7			12.378	2.337	
	2	-6.9			16.205	11.969	
	3	-6.8			13.484	12.802	
	4	-6.7			12.876	12.37	
	5	-6.6			13.154	12.543	
	6	-6.5			10.35	2.926	
	7	-6.4			12.582	12.398	
	8	-6.4			18.191	14.464	
Z_18 [MTGKSIQPEN]	0	-6.3	-6.044444	0.1424001	0	0	18.314222
	1	-6.2			29.425	23.809	
	2	-6.1			26.049	20.468	
	3	-6.1			27.223	20.839	
	4	-6			25.812	20.24	

	5	-6			11.247	2.637	
	6	-5.9			10.768	2.516	
	7	-5.9			30.55	24.391	
	8	-5.9			29.972	23.71	
Z_19 [AYLDKQSDTQ]	0	-6.6	-6.2	0.25	0	0	10.510889
	1	-6.5			7.627	3.127	
	2	-6.4			7.533	2.432	
	3	-6.2			28.063	22.015	
	4	-6.2			5.582	2.419	
	5	-6			8.585	3.595	
	6	-6			23.474	19.366	
	7	-6			8.118	3.292	
	8	-5.9			24.771	19.197	
Z_20 [AKFTCSKKMT]	0	-5.7	-5.588889	0.0927961	0	0	12.918444
	1	-5.7			28.85	23.141	
	2	-5.7			8.385	2.829	
	3	-5.6			4.684	2.737	
	4	-5.6			27.997	22.557	
	5	-5.5			27.055	21.784	
	6	-5.5			24.459	19.108	
	7	-5.5			4.565	2.685	
	8	-5.5			8.411	3.285	
Z_21 [ASDSRCPTQG]	0	-5.8	-5.211111	0.3018462	0	0	4.4558333
	1	-5.5			5.372	2.652	
	2	-5.3			7.929	2.337	
	3	-5.3			7.382	3.812	
	4	-5.2			8.521	3.364	
	5	-5			1.927	1.467	
	6	-5			8.565	3.99	
	7	-4.9			8.616	3.774	
	8	-4.9			7.964	2.533	
Z_22 [LRLKGVSYSL]	0	-6.2	-5.922222	0.1481366	0	0	10.823889
	1	-6.1			25.51	19.607	
	2	-6			9.557	2.898	
	3	-5.9			4.374	2.145	
	4	-5.9			25.58	20.136	
	5	-5.8			9.777	2.378	
	6	-5.8			9.677	3.042	
	7	-5.8			7.244	2.777	
	8	-5.8			27.585	22.543	
Z_23 [RAKVEVTPNSPR]	0	-5.8	-5.122222	0.3192874	0	0	9.326

	1	-5.5			8.516	2.636	
	2	-5.1			7.605	2.499	
	3	-5.1			9.119	2.809	
	4	-5			30.476	25.429	
	5	-4.9			24.9	20.062	
	6	-4.9			10.166	4.207	
	7	-4.9			7.461	2.605	
	8	-4.9			6.579	2.799	
Z_24 [LKGVSYSLCTAA]	0	-6.2	-5.855556	0.1666667	0	0	7.1197778
	1	-6			8.282	2.796	
	2	-5.9			9.499	3.086	
	3	-5.9			9.726	3.062	
	4	-5.8			8.03	2.82	
	5	-5.8			7.797	2.583	
	6	-5.7			5.412	2.716	
	7	-5.7			9.67	3.022	
	8	-5.7			27.22	22.435	
Z_25 [DMASDSRCPTQGEAVLDKQSDTQ]	0	-5.5	-5.244444	0.1666667	0	0	6.5372778
	1	-5.4			12.554	3.523	
	2	-5.4			12.522	3.615	
	3	-5.3			9.718	3.371	
	4	-5.2			12.381	3.54	
	5	-5.2			12.165	3.77	
	6	-5.1			13.17	2.953	
	7	-5.1			5.814	2.794	
	8	-5			12.162	3.619	

b. Docking with DENV envelope protein

Peptide Sequence	**Mode**	**Binding Affinity (kcal/mol)**	**Average of binding energy**	**SD of binding energy**	**RMSD (u.b.)**	**RMSD (l.b.)**	**Average of RMSD**
Z_1 [DVVLEHGGCV]	0	-6.2	-5.9	0.1699673	0	0	4.7445
	1	-6.2			2.016	1.363	
	2	-5.9			8.627	3.268	
	3	-5.9			7.087	2.818	
	4	-5.8			6.107	3.116	
	5	-5.8			9.307	4.191	
	6	-5.8			7.734	3.576	
	7	-5.8			11.105	3.377	
	8	-5.7			9.045	2.664	
Z_2 [GWGNGCGLFG]	0	-7.2	-6.744444	0.2114033	0	0	6.555

	1	-6.9			11.435	3.442	
	2	-6.8			23.004	16.903	
	3	-6.8			10.458	4.727	
	4	-6.7			10.119	2.748	
	5	-6.7			3.458	2.289	
	6	-6.6			11.324	3.24	
	7	-6.6			7.05	3.874	
	8	-6.4			2.053	1.866	
Z_3 [NKHWLVHKEW]	0	-7.8	-7.3	0.3333333	0	0	5.6839444
	1	-7.7			8.593	2.955	
	2	-7.6			8.813	2.804	
	3	-7.5			10.101	3.447	
	4	-7.2			11.594	2.673	
	5	-7.1			11.822	3.209	
	6	-7			7.072	2.778	
	7	-6.9			12.48	3.269	
	8	-6.9			7.596	3.105	
Z_4 [TQGEAYLDKQ]	0	-6.1	-5.877778	0.1030402	0	0	4.4717222
	1	-6			8.152	3.077	
	2	-5.9			2.114	1.384	
	3	-5.9			8.167	2.89	
	4	-5.8			7.134	2.281	
	5	-5.8			9.647	2.753	
	6	-5.8			8.14	2.617	
	7	-5.8			9.315	2.707	
	8	-5.8			7.311	2.802	
Z_5 [AGADTGTPHWNN]	0	-7.4	-7.044444	0.1706921	0	0	5.1682778
	1	-7.2			9.266	2.491	
	2	-7.1			8.652	2.63	
	3	-7.1			8.318	2.616	
	4	-7			8.603	2.633	
	5	-7			8.775	2.557	
	6	-6.9			10.019	2.725	
	7	-6.9			8.419	3.687	
	8	-6.8			9.04	2.598	
Z_6 [NSPRAEATLG]	0	-6.8	-6.444444	0.2114033	0	0	7.0666667
	1	-6.7			7.635	3.562	
	2	-6.7			10.498	2.632	
	3	-6.4			8.054	4.02	
	4	-6.3			7.997	3.647	
	5	-6.3			8.449	3.329	
	6	-6.3			24.41	19.348	
	7	-6.3			7.466	3.202	
	8	-6.2			8.695	4.256	
Z_7 [DRGWGNGCGLFG]	0	-7.1	-6.822222	0.1314684	0	0	5.5443333

	1	-6.9			8.473	3.308	
	2	-6.9			7.949	2.361	
	3	-6.9			6.67	4.199	
	4	-6.8			11.175	5.084	
	5	-6.7			10.497	2.925	
	6	-6.7			6.885	3.592	
	7	-6.7			10.302	3.171	
	8	-6.7			9.93	3.277	
Z_8 [TGYETDENRAKVEVTPNSPRAEAT]	0	-7.5	-7.255556	0.1342561	0	0	5.1361111
	1	-7.4			8.456	3.172	
	2	-7.3			8.896	3.146	
	3	-7.3			8.399	2.965	
	4	-7.3			9.537	2.718	
	5	-7.2			9.193	3.039	
	6	-7.1			11.885	4.952	
	7	-7.1			3.037	2.159	
	8	-7.1			8.305	2.591	
Z_9 [EATLGGFGSLGL]	0	-6.8	-6.355556	0.1892154	0	0	6.4503889
	1	-6.5			10.733	2.824	
	2	-6.4			7.617	2.271	
	3	-6.4			25.488	19.172	
	4	-6.3			10.359	2.768	
	5	-6.2			5.705	2.273	
	6	-6.2			6.948	2.437	
	7	-6.2			4.679	2.352	
	8	-6.2			7.801	2.68	
Z_10 [KRQTVVVLGS]	0	-6.5	-6.222222	0.1227262	0	0	5.8172222
	1	-6.3			9.786	3.55	
	2	-6.3			10.381	3.558	
	3	-6.2			10.323	3.469	
	4	-6.2			9.513	2.668	
	5	-6.2			10.513	3.601	
	6	-6.1			7.384	3.133	
	7	-6.1			9.987	5.318	
	8	-6.1			8.089	3.437	
Z_11 [CPTQGEAYLDKQ]	0	-5.9	-5.566667	0.1632993	0	0	4.8263333
	1	-5.8			8.06	2.61	
	2	-5.6			8.393	2.471	
	3	-5.5			7.925	2.685	
	4	-5.5			8.875	2.792	
	5	-5.5			6.725	2.499	
	6	-5.5			9.785	2.635	
	7	-5.4			5.824	2.777	
	8	-5.4			9.851	2.967	
Z_12 [TCSKKMTGKSIQ]	0	-6.1	-5.644444	0.2006163	0	0	4.7525556

	1	-5.9			8.315	2.787	
	2	-5.6			8.867	2.852	
	3	-5.6			6.826	2.963	
	4	-5.6			7.04	2.883	
	5	-5.5			6.51	3.335	
	6	-5.5			8.381	3.169	
	7	-5.5			7.239	3.044	
	8	-5.5			8.391	2.944	
Z_13 [LEHGGCVTVMAQ]	0	-6.3	-5.9	0.1699673	0	0	5.1402778
	1	-6			8.639	2.781	
	2	-5.9			9.84	2.919	
	3	-5.9			7.093	2.709	
	4	-5.9			10.24	2.727	
	5	-5.9			7.458	2.868	
	6	-5.8			8.797	4.306	
	7	-5.7			7.647	3.499	
	8	-5.7			7.784	3.218	
Z_14 [YYLTMNNKHWLV]	0	-7	-6.422222	0.2439389	0	0	5.6178889
	1	-6.6			8.334	2.71	
	2	-6.5			6.03	2.521	
	3	-6.4			11.984	3.596	
	4	-6.4			10.979	3.907	
	5	-6.3			6.325	2.782	
	6	-6.2			10.376	3.076	
	7	-6.2			11.651	3.641	
	8	-6.2			10.554	2.656	
Z_15 [VVVLGSQEGAVH]	0	-6.5	-6.188889	0.1662959	0	0	5.1364444
	1	-6.3			10.321	4.934	
	2	-6.3			8.102	2.617	
	3	-6.3			8.38	2.844	
	4	-6.2			10.224	2.344	
	5	-6.1			7.953	2.905	
	6	-6			7.979	2.767	
	7	-6			8.171	3.13	
	8	-6			7.439	2.346	
Z_16 [HAGADTGTPHWNN]	0	-7.5	-7.366667	0.0816497	0	0	5.3732222
	1	-7.5			6.201	2.474	
	2	-7.4			10.695	2.981	
	3	-7.4			9.092	2.4	
	4	-7.3			10.249	2.663	
	5	-7.3			9.96	3.274	
	6	-7.3			7.863	3.084	
	7	-7.3			10.089	2.759	
	8	-7.3			9.781	3.153	
Z_17 [LGSQEGAVHT]	0	-6.6	-6.4	0.0942809	0	0	6.6206667

	1	-6.5			6.87	2.739	
	2	-6.4			23.133	17.003	
	3	-6.4			7.494	2.705	
	4	-6.4			10.114	3.006	
	5	-6.4			8.917	3.48	
	6	-6.3			7.531	2.703	
	7	-6.3			8.98	3.298	
	8	-6.3			8.092	3.107	
Z_18 [MTGKSIQPEN]	0	-7.1	-6.933333	0.0942809	0	0	18.307722
	1	-7			29.192	22.443	
	2	-7			8.257	4.453	
	3	-7			29.353	23.429	
	4	-6.9			27.892	21.666	
	5	-6.9			27.878	20.779	
	6	-6.9			8.304	4.084	
	7	-6.8			28.089	22.956	
	8	-6.8			27.625	23.139	
Z_19 [AYLDKQSDTQ]	0	-6.8	-6.644444	0.106574	0	0	6.8914444
	1	-6.8			7.612	3.471	
	2	-6.7			10.842	3.112	
	3	-6.7			7.962	2.908	
	4	-6.6			10.628	3.707	
	5	-6.6			23.621	16.275	
	6	-6.6			7.711	3.361	
	7	-6.5			9.872	2.739	
	8	-6.5			7.594	2.631	
Z_20 [AKFTCSKKMT]	0	-5.9	-5.611111	0.2182987	0	0	5.1269444
	1	-5.9			8.444	3.399	
	2	-5.8			8.943	4.726	
	3	-5.7			8.399	3.633	
	4	-5.6			8.641	3.455	
	5	-5.5			6.747	3.611	
	6	-5.5			6.48	3.5	
	7	-5.3			7.665	4.034	
	8	-5.3			7.084	3.524	
Z_21 [ASDSRCPTQG]	0	-5.9	-5.722222	0.0916246	0	0	15.744111
	1	-5.8			11.981	2.776	
	2	-5.8			4.177	2.186	
	3	-5.7			24.818	19.325	
	4	-5.7			23.212	17.675	
	5	-5.7			24.246	18.629	
	6	-5.7			26.772	20.179	
	7	-5.6			24.709	18.755	
	8	-5.6			24.251	19.703	
Z_22 [LRLKGVSYSL]	0	-6.2	-6.066667	0.1054093	0	0	4.8847222

	1	-6.2			9.348	2.662	
	2	-6.1			6.725	2.536	
	3	-6.1			7.168	3.058	
	4	-6.1			8.727	2.871	
	5	-6.1			9.299	2.656	
	6	-6			9.969	2.998	
	7	-5.9			6.277	2.544	
	8	-5.9			8.193	2.894	
Z_23 [RAKVEVTPNSPR]	0	-6.4	-6.066667	0.2	0	0	5.0375
	1	-6.2			4.833	2.012	
	2	-6.2			10.354	2.529	
	3	-6.2			7.329	2.592	
	4	-6.1			9.025	2.475	
	5	-6			8.585	2.517	
	6	-5.9			10.564	2.423	
	7	-5.9			10.228	2.429	
	8	-5.7			10.283	2.497	
Z_24 [LKGVSYSLCTAA]	0	-6.6	-6.111111	0.2182987	0	0	4.3946111
	1	-6.3			6.29	2.533	
	2	-6.2			8.049	2.172	
	3	-6.1			7.233	3.141	
	4	-6.1			2.738	1.436	
	5	-6			6.589	2.603	
	6	-5.9			7.904	3.04	
	7	-5.9			8.841	3.607	
	8	-5.9			9.736	3.191	
Z_25 [DMASDSRCPTQGEAVLDKQSDTQ]	0	-6.1	-5.722222	0.1547599	0	0	12.375056
	1	-5.8			26.316	16.592	
	2	-5.8			17.346	9.071	
	3	-5.7			32.789	22.851	
	4	-5.7			16.814	8.513	
	5	-5.6			17.265	9.197	
	6	-5.6			2.687	1.803	
	7	-5.6			19.132	10.5	
	8	-5.6			8.652	3.223	

Table S2. Binding score of modified peptides derived from Z_10 with the Zika virus (ZIKV) and dengue virus (DENV) envelopes.

a. Docking with ZIKV envelope protein

Name of peptide	Peptide Sequence	Mode	Binding Affinity (kcal/mol)	Average of binding energy	SD of binding energy	RMSD (u.b.)	RMSD (l.b.)	Average of RMSD	Location of N-terminus
Z_10	KRQTVVVLGS	0	-6.8	-6.52222	0.156347	0	0	9.97	Out ward
		1	-6.7			1.933	5.671		Out ward
		2	-6.6			3.646	10.513		In ward
		3	-6.5			3.906	11.071		In ward
		4	-6.5			22.655	28.686		In ward
		5	-6.5			2.892	11.463		In ward
		6	-6.4			3.302	10.468		In ward
		7	-6.4			2.928	10.943		In ward
		8	-6.3			22.191	27.192		In ward
Z_10.1	KRQVVSCAWA	0	-7.1	-6.75556	0.224227	0	0	15.19728	Out ward
		1	-7			23.557	30.742		Out ward
		2	-6.9			26.585	33.986		In ward
		3	-6.8			3.408	10.73		In ward
		4	-6.8			26.271	33.75		Out ward
		5	-6.7			2.983	5.321		In ward
		6	-6.5			3.327	10.261		Out ward
		7	-6.5			20.998	27.758		In ward
		8	-6.5			2.95	10.924		In ward
Z_10.2	KRQTVVSCAEA	0	-5.6	-5.23333	0.206155	0	0	12.31083	In ward
		1	-5.4			1.732	2.363		Out ward
		2	-5.4			2.912	8.483		In ward
		3	-5.3			26.667	31.382		In ward
		4	-5.2			2.974	10.675		In ward
		5	-5.1			2.734	7.511		Out ward
		6	-5.1			25.941	30.566		In ward
		7	-5			25.587	30.047		In ward
		8	-5			2.907	9.114		In ward
Z_10.3	KRWQVVSCAE	0	-6.5	-6.28889	0.145297	0	0	5.248833	In ward
		1	-6.4			2.97	10.279		In ward
		2	-6.4			3.102	9.408		In ward
		3	-6.4			3.065	10.038		In ward
		4	-6.3			3.89	8.966		In ward
		5	-6.2			2.809	9.373		In ward
		6	-6.2			2.37	7.072		In ward
		7	-6.1			3.06	7.698		Out ward
		8	-6.1			2.829	7.55		In ward

Z_10.4	KRATVVVLGS	0	-6.4	-6.14444	0.174005	0	0	18.07911	In ward
		1	-6.3			21.39	26.921		In ward
		2	-6.3			21.687	26.848		In ward
		3	-6.2			23.897	29.867		Out ward
		4	-6.2			24.035	30.805		In ward
		5	-6			20.121	26.24		In ward
		6	-6			21.602	27.209		In ward
		7	-6			4.816	9.736		Out Ward
		8	-5.9			2.913	7.337		Out Ward
Z_10.5	KRAVVLPCAEA	0	-6.2	-6	0.086603	0	0	14.70467	In ward
		1	-6			2.961	8.984		In ward
		2	-6			25.734	30.252		In ward
		3	-6			2.953	9.662		In ward
		4	-6			2.989	9.19		In ward
		5	-6			25.678	30.332		In ward
		6	-6			23.144	27.651		In ward
		7	-5.9			3.182	10.859		In ward
		8	-5.9			23.51	27.603		Out ward
Z_10_6	KRMVVSCLGS	0	-6.2	-6.07778	0.083333	0	0	10.15556	In ward
		1	-6.1			11.969	4.078		In ward
		2	-6.1			11.798	3.489		In ward
		3	-6.1			4.813	2.097		In ward
		4	-6.1			10.911	3.623		Out ward
		5	-6.1			9.773	4.848		Out ward
		6	-6.1			6.168	2.991		In ward
		7	-6			29.654	24.632		In ward
		8	-5.9			28.79	23.166		In ward
Z_10_7	KREVVSCLGS	0	-5.7	-5.43333	0.173205	0	0	18.72339	In ward
		1	-5.6			27.542	22.943		In ward
		2	-5.5			6.734	2.95		In ward
		3	-5.5			24.215	19.488		Out ward
		4	-5.5			26.582	22.92		Out ward
		5	-5.4			26.203	22.617		In ward
		6	-5.3			23.181	19.289		In ward
		7	-5.2			24.814	21.083		Out ward
		8	-5.2			25.055	21.405		In ward
Z_10.8	KRAVVSCAEA	0	-7.4	-7.02222	0.17873	0	0	3.807889	Out ward
		1	-7.2			1.675	3.381		Out ward
		2	-7.1			2.829	6.135		In ward
		3	-7			4.982	14.971		Out ward
		4	-6.9			2.136	5.018		Out ward
		5	-6.9			2.19	4.571		In ward
		6	-6.9			2.751	7.515		In ward
		7	-6.9			1.929	2.877		Out ward
		8	-6.9			1.91	3.672		Out ward

Z_10_9	KRSVVSCLGS	0	-6.3	-6.18889	0.116667	0	0	6.211833	Out ward
		1	-6.3			3.329	1.79		Out ward
		2	-6.3			2.059	1.795		In ward
		3	-6.3			2.847	1.941		In ward
		4	-6.2			6.955	3.719		In ward
		5	-6.1			29.72	23.181		In ward
		6	-6.1			4.763	2.381		In ward
		7	-6.1			9.536	5.094		Out ward
		8	-6			30.92	25.259		In ward
Z_10_10	KRPAVVSCLGS	0	-6.1	-5.95556	0.088192	0	0	15.52567	In ward
		1	-6			26.388	21.556		Out ward
		2	-6			25.886	20.766		In ward
		3	-6			26.708	21.812		In ward
		4	-6			7.508	2.9		In ward
		5	-5.9			11.288	4.563		In ward
		6	-5.9			27.402	21.722		In ward
		7	-5.9			26.272	21.133		Out ward
		8	-5.8			9.502	4.056		In ward
Z_10_11	KRHCVVSCPAE	0	-6.7	-6.52222	0.109291	0	0	14.24956	Out ward
		1	-6.6			6.871	2.731		Out ward
		2	-6.6			28.781	23.909		In ward
		3	-6.6			28.889	23.297		In ward
		4	-6.5			29.201	24.055		In ward
		5	-6.5			30.44	23.693		In ward
		6	-6.4			8.501	3.32		Out ward
		7	-6.4			7.257	3.9		In ward
		8	-6.4			8.231	3.416		In ward
Z_10_12	KRDEVVSCAEP	0	-6.4	-6.26667	0.086603	0	0	11.2545	In ward
		1	-6.4			27.077	21.048		Out ward
		2	-6.3			8.942	2.839		In ward
		3	-6.3			9.881	2.952		In ward
		4	-6.2			27.101	21.189		Out ward
		5	-6.2			5.582	2.456		In ward
		6	-6.2			10.035	2.916		In ward
		7	-6.2			26.618	20.51		In ward
		8	-6.2			9.662	3.773		In ward
P3	KPNDAINDAINF	0	-6	-5.85556	0.072648	0	0	12.09022	In ward
		1	-5.9			2.448	8.065		In ward
		2	-5.9			24.183	29.471		In ward
		3	-5.9			2.883	10.115		In ward
		4	-5.8			2.35	8.072		In ward
		5	-5.8			2.454	9.424		In ward
		6	-5.8			5.889	11.707		In ward
		7	-5.8			21.607	26.477		Out ward
		8	-5.8			23.476	29.003		Out ward

b. Docking with DENV envelope protein

Name of peptide	Peptide Sequence	Mode	Binding Affinity (kcal/mol)	Average of binding energy	SD of binding energy	RMSD (u.b.)	RMSD (l.b.)	Average of RMSD	Location of N-terminaus
Z_10	KRQTVVVLGS	0	-6.5	-6.22222	0.122726	0	0	5.817222	In ward
		1	-6.3			9.786	3.55		In ward
		2	-6.3			10.381	3.558		In ward
		3	-6.2			10.323	3.469		In ward
		4	-6.2			9.513	2.668		In ward
		5	-6.2			10.513	3.601		In ward
		6	-6.1			7.384	3.133		In ward
		7	-6.1			9.987	5.318		In ward
		8	-6.1			8.089	3.437		In ward
Z_10.1	KRQVVSCAWA	0	-6.6	-6.35556	0.166667	0	0	4.921611	In ward
		1	-6.5			4.129	2.491		In ward
		2	-6.5			10.83	3.013		In ward
		3	-6.4			7.05	3.245		In ward
		4	-6.4			9.554	3.847		In ward
		5	-6.3			5.477	2.734		In ward
		6	-6.2			9.791	3.341		In ward
		7	-6.2			7.929	2.693		In ward
		8	-6.1			9.364	3.101		In ward
Z_10.2	KRQTVVSCAEA	0	-5.7	-5.58889	0.092796	0	0	5.133833	In ward
		1	-5.7			8.606	3.429		In ward
		2	-5.7			9.249	2.812		In ward
		3	-5.6			5.864	2.476		In ward
		4	-5.6			9.512	3.028		In ward
		5	-5.5			7.93	2.939		In ward
		6	-5.5			8.402	2.904		In ward
		7	-5.5			10.354	2.609		In ward
		8	-5.5			9.374	2.921		In ward
Z_10.3	KRWQVVSCAE	0	-6.3	-6.08889	0.105409	0	0	4.577222	In ward
		1	-6.1			3.924	1.721		In ward
		2	-6.1			9.169	2.945		In ward
		3	-6.1			6.486	2.454		In ward
		4	-6.1			7.886	3.101		In ward
		5	-6.1			5.631	2.905		In ward
		6	-6.1			8.896	3.2		In ward
		7	-6			8.016	2.702		In ward
		8	-5.9			10.223	3.131		In ward
Z_10.4	KRATVVVLGS	0	-5.6	-5.45556	0.133333	0	0	5.033389	In ward

		1	-5.6			9.661	2.529		In ward
		2	-5.6			9.238	2.415		In ward
		3	-5.5			7.76	3.413		In ward
		4	-5.5			10.337	2.747		In ward
		5	-5.4			5.394	2.425		In ward
		6	-5.3			8.347	3.138		In ward
		7	-5.3			8.71	2.695		In ward
		8	-5.3			9.075	2.717		In ward
Z_10.5	KRAVVLPCAEA	0	-6.3	-5.85556	0.200693	0	0	8.378111	In ward
		1	-6			7.289	3.589		In ward
		2	-5.9			6.653	2.851		In ward
		3	-5.9			22.755	17.566		Out ward
		4	-5.8			9.194	3.244		In ward
		5	-5.7			22.744	17.818		In ward
		6	-5.7			9.295	2.6		In ward
		7	-5.7			10.214	3.774		In ward
		8	-5.7			7.606	3.614		In ward
Z_10_6	KRMVVSCLGS	0	-6.3	-6.05556	0.174005	0	0	5.363667	In ward
		1	-6.3			7.435	3.389		In ward
		2	-6.1			10.641	4.096		In ward
		3	-6.1			9.598	4.359		In ward
		4	-6.1			7.295	3.575		In ward
		5	-6			7.306	4.424		In ward
		6	-5.9			7.722	3.583		In ward
		7	-5.9			6.013	3.059		In ward
		8	-5.8			9.511	4.54		In ward
Z_10_7	KREVVSCLGS	0	-6.3	-5.98889	0.145297	0	0	5.998056	In ward
		1	-6.1			10.095	3.84		In ward
		2	-6			10.469	3.185		In ward
		3	-6			9.598	2.895		In ward
		4	-6			9.021	3.657		In ward
		5	-5.9			8.501	4.592		In ward
		6	-5.9			9.638	3.033		In ward
		7	-5.9			9.862	4.026		In ward
		8	-5.8			11.733	3.82		In ward
Z_10.8	KRAVVSCAEA	0	-5.9	-5.61111	0.153659	0	0	8.753111	In ward
		1	-5.8			22.687	16.802		In ward
		2	-5.7			8.999	4.176		In ward
		3	-5.6			7.864	3.297		In ward
		4	-5.5			7.668	2.723		In ward
		5	-5.5			8.315	3.136		In ward
		6	-5.5			7.589	2.818		In ward
		7	-5.5			9.444	3.576		In ward
		8	-5.5			26.688	21.774		Out ward
Z_10_9	KRSVVSCLGS	0	-6.2	-6.06667	0.1	0	0	6.211833	In ward

		1	-6.2			10.415	3.326		In ward
		2	-6.1			8.747	3.233		In ward
		3	-6.1			6.493	2.742		In ward
		4	-6.1			2.032	1.446		In ward
		5	-6			6.782	3.556		In ward
		6	-6			8.586	3.954		In ward
		7	-6			7.756	2.598		In ward
		8	-5.9			9.456	3.335		In ward
Z_10_10	KRPAVVSCLGS	0	-6.7	-6.04444	0.387657	0	0	4.588889	In ward
		1	-6.4			7.045	3.156		In ward
		2	-6.3			9.169	3.301		In ward
		3	-6.3			7.675	2.915		In ward
		4	-5.9			10.913	3.439		In ward
		5	-5.8			2.729	1.796		In ward
		6	-5.7			10.994	3.543		In ward
		7	-5.7			2.309	1.754		In ward
		8	-5.6			9.245	2.617		In ward
Z_10_11	KRHCVVSCPAE	0	-6	-5.76667	0.15	0	0	8.371056	In ward
		1	-6			9.621	3.012		In ward
		2	-5.8			6.568	3.073		In ward
		3	-5.8			9.208	3.171		In ward
		4	-5.7			9.394	3.154		In ward
		5	-5.7			6.187	2.353		In ward
		6	-5.7			9.592	3.007		In ward
		7	-5.6			23.399	17.496		In ward
		8	-5.6			24.252	17.192		Out ward
Z_10_12	KRDEVVSCAEP	0	-6.4	-6.11111	0.190029	0	0	5.292667	In ward
		1	-6.3			10.116	3.877		In ward
		2	-6.3			8.883	3.514		In ward
		3	-6.1			9.239	2.669		In ward
		4	-6.1			9.905	2.6		In ward
		5	-6.1			9.61	2.863		In ward
		6	-5.9			7.098	2.942		In ward
		7	-5.9			6.809	3.04		In ward
		8	-5.9			8.908	3.195		In ward
P3	KPNDAINDAINF	0	-6.4	-6.03333	0.206155	0	0	4.714	In ward
		1	-6.3			5.64	2.49		In ward
		2	-6.1			10.249	2.274		In ward
		3	-6			5.92	2.399		In ward
		4	-6			8.666	2.271		In ward
		5	-6			8.843	2.304		In ward
		6	-5.9			6.817	2.661		In ward
		7	-5.8			9.756	2.523		In ward
		8	-5.8			9.677	2.362		In ward

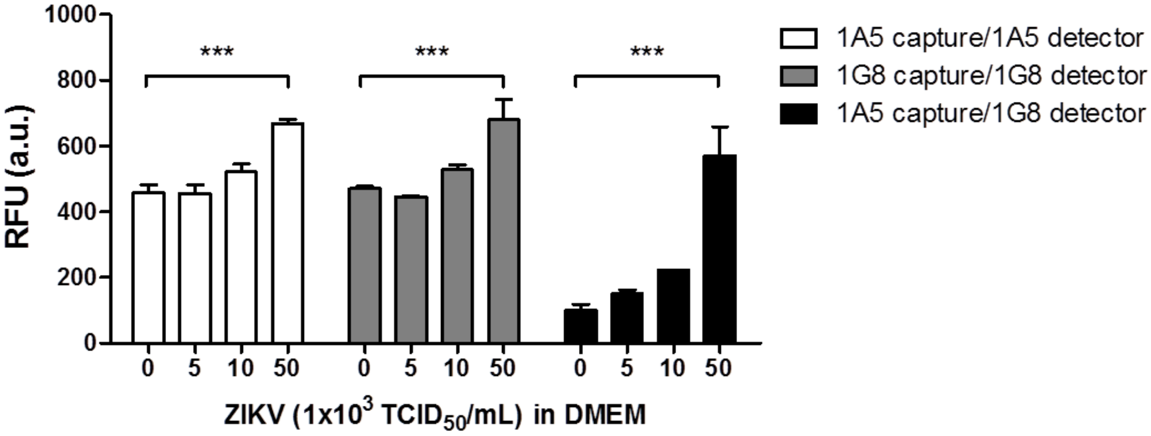
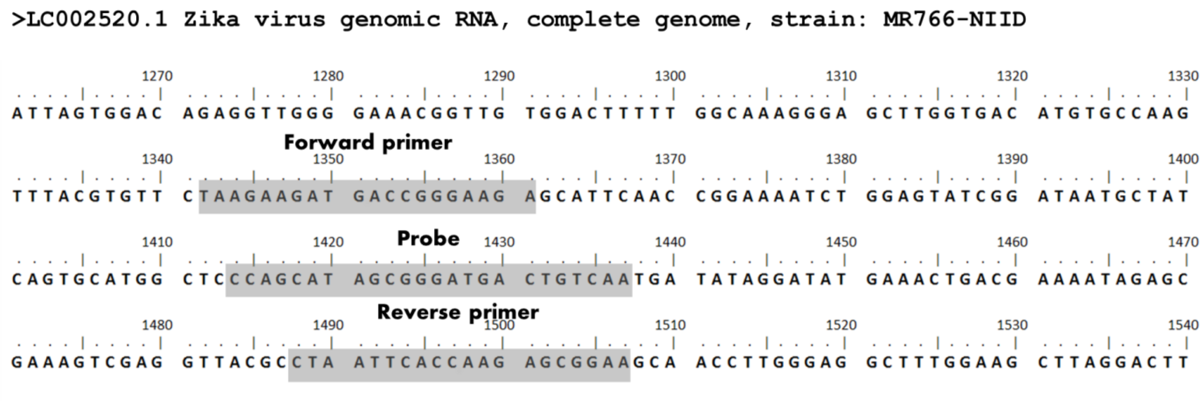


Figure S3. Comparison of same antibodies pair (1A5/1A5 or 1G8/1G8)- and fluorescence-linked sandwich immunosorbent assays (FLISA). Different virus titers of ZIKV **(**5×10^3, 1×10^4, 5×10^4 tissue culture infective dose [TCID]$_{50}$/mL) was tested in the same antibodies pair-linked sandwich FLISA. RFU: relative fluorescent units;*** $P < 0.001$



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>LC002520.1 Zika virus genomic RNA, complete genome, strain: MR766-NIID

                1270        1280        1290        1300        1310        1320        1330
ATTAGTGGAC  AGAGGTTGGG  GAAACGGTTG  TGGACTTTTT  GGCAAAGGGA  GCTTGGTGAC  ATGTGCCAAG
                     Forward primer
                1340        1350        1360        1370        1380        1390        1400
TTTACGTGTT  CTAAGAAGAT  GACCGGGAAG  AGCATTCAAC  CGGAAAATCT  GGAGTATCGG  ATAATGCTAT
                                Probe
                1410        1420        1430        1440        1450        1460        1470
CAGTGCATGG  CTCCCAGCAT  AGCGGGATGA  CTGTCAATGA  TATAGGATAT  GAAACTGACG  AAAATAGAGC
                           Reverse primer
                1480        1490        1500        1510        1520        1530        1540
GAAAGTCGAG  GTTACGCCTA  ATTCACCAAG  AGCGGAAGCA  ACCTTGGGAG  GCTTTGGAAG  CTTAGGACTT
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Figure S4. Sequence of binding sites using newly designed primers and probe. Probe and primers of the envelope gene were newly designed in this study. Binding sites of the probe and primers are highlighted in grey.

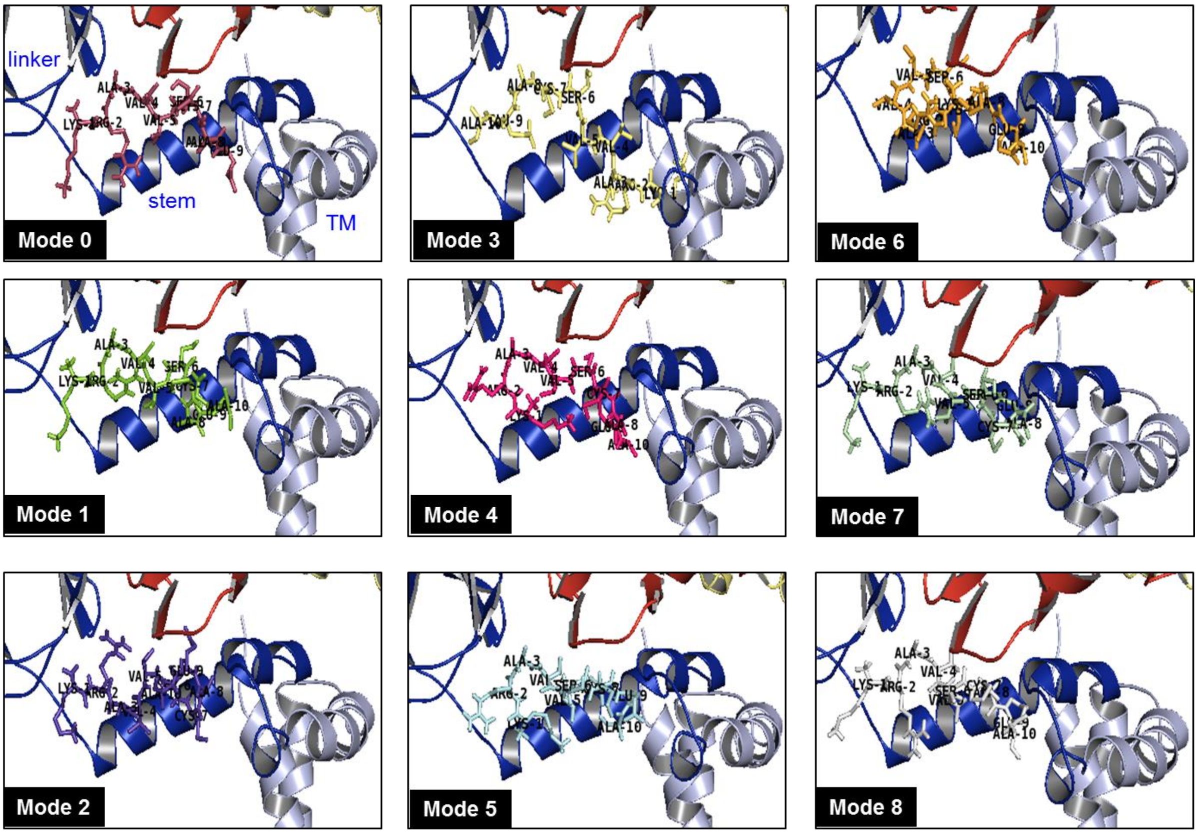

Figure S5. Prediction of the binding site between Z_10.8 and the Zika virus (ZIKV) envelope protein for each mode.

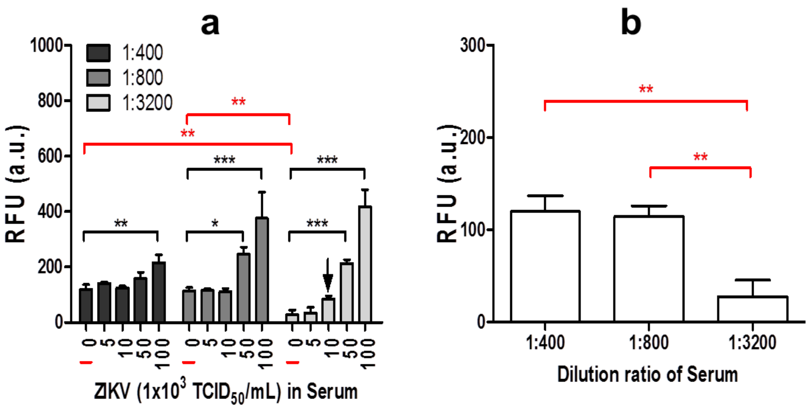

Figure S6. Effect of human sera on an antibody pair (1A5 capture/1G8 detector)- and fluorescence-linked sandwich immunosorbant assay (FLISA). (a) Zika virus (ZIKV) titers from 5×10^3 to 1×10^5 tissue culture infective dose (TCID)$_{50}$/mL were applied to a peptide pair-linked sandwich FLISA in the presence of human serum differentially diluted from 1:400 to 1:3200. The black arrow indicates the limit of detection (LOD) for the assay. **(b)** Only differentially diluted human sera were plotted to compare the relative fluorescent values representing non-specific reactions in the presence of sera. RFU: relative fluorescent units