Supplementary Materials for

The 3.8Å resolution Cryo-EM structure of Zika Virus

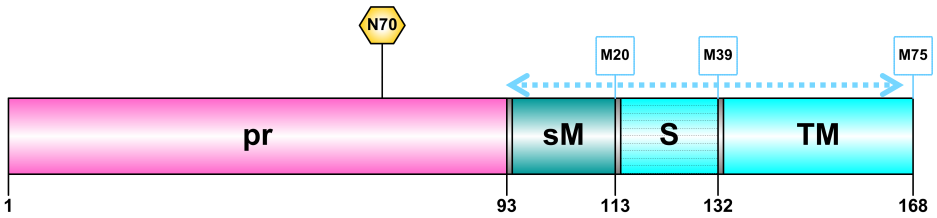
Devika Sirohi†, Zhenguo Chen†, Lei Sun, Thomas Klose, Theodore C. Pierson, Michael G. Rossmann*, Richard J. Kuhn*

† Equal contribution by these authors
*Correspondence to: mr@purdue.edu (M.G.R); kuhnr@purdue.edu (R.J.K)

This PDF file includes:

Figs. S1 and S2
Tables S1 and S2

A)



B)

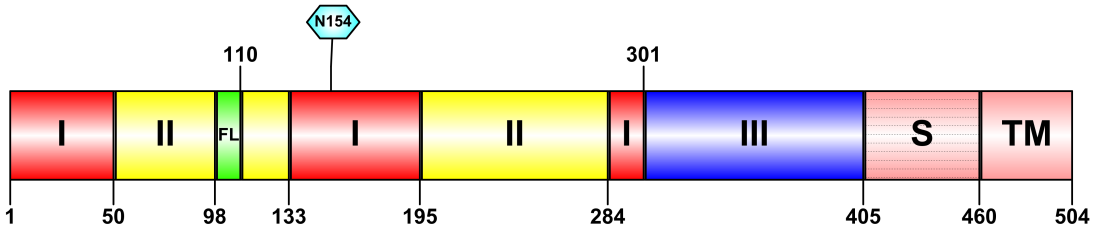
```
ZIKV_H/PF/2013              AEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDEGV    60
ZIKV_MR766                  AEITRRGSAYYMYLDRSDAGKAISFATTLGVNKCHVQIMDLGHMCDATMSYECPMLDEGV    60
DENV1_SG/07K3640DK1/2008    FHLTTRGGEPHMIVSKQERGKSLLFKTSAGVNMCTLIAMDLGELCEDTLTYKCPRITE-A    59
DENV2_16681                 FHLTTRNGEPHMIVSRQEKGKSLLFKTEDGVNMCTLMAMDLGELCEDTITYKCPLLRQ-N    59
DENV3_SG/05K863DK1/2005     FHLTSRDGEPRMIVGKNERGKSLLFKTASGINMCTLIAMDLGEMCDDTVTYKCPHITE-V    59
DENV4_SG/06K2270DK1/2005    FHLSTRDGEPLMIVAKHERGRPLLFKTTEGINKCTLIAMDLGEMCEDTVTYKCPLLVN-T    59
WNV_NY99                    VTLSNFQGKVMMTVNATDVTDVITIPTAAGKNLCIVRAMDVGYMCDDTITYECPVLSAGN    60
JEV_SA14                    MKLSNFQGKLLMTINNTDIADVIVIPTSKGENRCWVRAIDVGYMCEDTITYECPKLTMGN    60
YFV_Asibi                   VTLVRKNRWLLLNVTSEDLGKTF----SVGTGNCTTNILEAKYWCPDSMEYNCPNLSPRE    56
                              :        : :   :    :      *   *    ::    *  :: *:** :

ZIKV_H/PF/2013              EPDDVDCWCNTT-STWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREYT   119
ZIKV_MR766                  EPDDVDCWCNTT-STWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREYT   119
DENV1_SG/07K3640DK1/2008    EPDDVDCWCNAT-DTWVTYGTCSQ-TGEHRRDKRSVALAPHVGLGLETRTETWMSSEGAW   117
DENV2_16681                 EPEDIDCWCNST-STWVTYGTCTT-MGEHRREKRSVALVPHVGMGLETRTETWMSSEGAW   117
DENV3_SG/05K863DK1/2005     EPEDIDCWCNLT-STWVTYGTCNQ-AGEHRRDKRSVALAPHVGMGLDTRTQTWMSAEGAW   117
DENV4_SG/06K2270DK1/2005    EPEDIDCWCNLT-STWVMYGTCTQ-NGERRREKRSVALTPHSGMGLETRAETWMSSEGAW   117
WNV_NY99                    DPEDIDCWCTKS-AVYVRYGRCTK-TRHSRRSRRSLTVQTHGESTLANKKGAWMDSTKAT   118
JEV_SA14                    DPEDVDCWCDNQ-EVYVQYGRCTR-TRHSKRSRRSVSVQTHGESSLVNKKEAWLDSTKAT   118
YFV_Asibi                   EPDDIDCWCYGVENVRVAYGKCDS-AGRSRRSRRAIDLPTHENHGLKTRQEKWMTGRMGE   115
                            :*:*:****     . * ** *     . :*.:*:: :  *    * .:   *: .

ZIKV_H/PF/2013              KHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYS              168
ZIKV_MR766                  KHLIKVENWIFRNPGFALVAVAIAWLLGSSTSQKVIYLVMILLIAPAYS              168
DENV1_SG/07K3640DK1/2008    KQIQRVETWALRHPGFTVIALFLAHAIGTSITQKGIIFILLMLVTPSMA              166
DENV2_16681                 KHVQRIETWILRHPGFTMMAAILAYTIGTTHFQRALIFILLTAVTPSMT              166
DENV3_SG/05K863DK1/2005     RQVEKVETWALRHPGFTILALFLAHYIGTSLTQKVVIFILLMLVTPSMT              166
DENV4_SG/06K2270DK1/2005    KHAQRVESWILRNPGFALLAGFMAYMIGQTGIQRTVFFVLMMLVAPSYG              166
WNV_NY99                    RYLVKTESWILRNPGYALVAAVIGWMLGSNTMQRVVFVVLLLLVAPAYS              167
JEV_SA14                    RYLMKTENWIIRNPGYAFLAAVLGWMLGSNNGQRVVFTILLLLVAPAYS              167
YFV_Asibi                   RQLQKIERWLVRNPFFAVTALTIAYLVGSNMTQRVVIALLVLAVGPAYS              164
                            :   : * * .*.* ::. *  :.  :* .  *: :  :::  : *:
```

C)

	ZIKV_H/PF	ZIKV_MR	DENV1	DENV2	DENV3	DENV4	WNV	JEV	YFV
1: ZIKV_H/PF/2013	100.00	94.05	42.17	40.36	42.17	45.78	41.92	39.52	36.81
2: ZIKV_MR766	94.05	100.00	42.77	40.36	42.77	45.18	42.51	39.52	38.04
3: DENV1_SG/07K3640DK1/2008	42.17	42.77	100.00	75.30	79.52	66.27	33.13	34.34	34.57
4: DENV2_16681	40.36	40.36	75.30	100.00	71.08	69.28	36.75	36.14	35.19
5: DENV3_SG/05K863DK1/2005	42.17	42.77	79.52	71.08	100.00	68.07	36.75	34.94	35.19
6: DENV4_SG/06K2270DK1/2005	45.78	45.18	66.27	69.28	68.07	100.00	42.17	39.76	35.19
7: WNV_NY99	41.92	42.51	33.13	36.75	36.75	42.17	100.00	74.25	38.65
8: JEV_SA14	39.52	39.52	34.34	36.14	34.94	39.76	74.25	100.00	36.20
9: YFV_Asibi	36.81	38.04	34.57	35.19	35.19	35.19	38.65	36.20	100.00

Fig. S1. Precursor membrane (prM) protein of ZIKV (H/PF/2013): A) Domain organization; B) Sequence alignment with other mosquito-transmitted flaviviruses and C) Percent identity matrix. The precursor peptide (pr) is indicated in pink, and the membrane protein (M) is highlighted in blue. sM stands for soluble ectodomain of M protein whereas S and TM are stem and transmembrane regions of the protein, respectively. The glycosylation site at residue N13/15 is represented in green, N29 in magenta, N51 in grey and N69/70 in yellow. The glycosylation motif N-X-S/T is underlined. The furin cleavage sequence (R-X-K/R-R) is colored in red. The sequences for various flaviviruses were obtained from the Virus Pathogen Database and Analysis Resource (ViPR). Virus strains for which structural information was available were chosen where possible. GenBank Genome Accession codes for these viruses are as follows: ZIKV_H/PF/2013: KJ776791; ZIKV_MR766: AY632535; DENV 1_SG/07K3640DK1/2008: GQ398255; DENV 2_16681: NC_001474; DENV 3_SG/05K863DK1/2005: EU081190; DENV4_SG/06K2270DK1/2005: GQ398256; WNV_NY99: DQ211652; JEV_SA14: D90194 and YFV_Asibi: AY640589. The domain map was constructed using Illustrator for Biological Sequences (IBS) *(37)*. The multiple sequence alignment and percent identity matrix was created by Clustal Omega tool (EMBL-EBI) *(38)*. The * (asterisk), : (colon) and . (period) denote consensus among the sequences. '*' indicates a completely conserved residue; ':' indicates conservation of amino acids with strongly similar properties (score of >0.5 in the Gonnet PAM 250 matrix) and '.' represents conservation of amino acids with weakly similar properties (score of =<0.5 in the Gonnet PAM 250 matrix).

A)



B)

```
ZIKV_H/PF/2013              IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYC    60
ZIKV_MR766                  IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYC    60
DENV_1_SG/07K3640DK1/2008   MRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEVTNPAVLRKLC    60
DENV_2_16681                MRCIGMSNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRKYC    60
DENV_3_SG/05K863DK1/2005    MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEATQLATLRKLC    60
DENV_4_SG/06K2270DK1/2005   MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTAKEVALLRTYC    60
WNV_NY99                    FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEAANLAEVRSYC    60
JEV_SA14                    FNCLGMGNRDFIEGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEASQLAEVRSYC    60
YFV_Asibi                   AHCIGITDRDFIEGVHGGTWVSATLEQDKCVTVMAPDKPSLDISLETVAIDGPAEARKVC    60
                             .*:*: .***:**  *.:**. .**   *:* *:  **::*. : .      *  *. *

ZIKV_H/PF/2013              YEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFA    120
ZIKV_MR766                  YEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFT    120
DENV_1_SG/07K3640DK1/2008   IEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGSLITCAKFK    120
DENV_2_16681                IEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGGIVTCAMFR    120
DENV_3_SG/05K863DK1/2005    IEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAKFQ    120
DENV_4_SG/06K2270DK1/2005   IEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGNGCGLFGKGGVVTCAKFL    120
WNV_NY99                    YLATVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVVDRGWGNGCGLFGKGSIDTCAKFA    120
JEV_SA14                    YHASVTDISTVARCPTTGEAHNEKRADSSYVCKQGFTDRGWGNGCGLFGKGSIDTCAKFS    120
YFV_Asibi                   YNAVLTHVKINDKCPSTGEAHLAEENEGDNACKRTYSDRGWGNGCGLFGKGSIVACAKFT    120
                              . ::.      **: **    :. :    *::   **************.: :** *

ZIKV_H/PF/2013              CSKKMTGKSIQPENLEYRIMLSVHGSQHS---GMIVNDTGHETDENRAKVEITPNSPRAE    177
ZIKV_MR766                  CSKKMTGKSIQPENLEYRIMLSVHGSQHS---GMI----GYETDEDRAKVEVTPNSPRAE    173
DENV_1_SG/07K3640DK1/2008   CVTKLEGKIVQYENLKYSVIVTVHTGDQH--------QVGNESTEHGTTATITPQAPTTE    172
DENV_2_16681                CKKNMEGKVVQPENLEYTIVITPHSGEEH--------AVGNDTGKHGKEIKITPQSSITE    172
DENV_3_SG/05K863DK1/2005    CLEPIEGKVVQYENLKYTVIITVHTGDQH--------QVGNET--QGVTAEITPQASTTE    170
DENV_4_SG/06K2270DK1/2005   CSGKITGNLVQIENLEYTVAVTVHNGDTH--------AVGNDTSSHGVTATITPRSPSVE    172
WNV_NY99                    CSTKAIGRTILKENIKYEVAIFVHGPTTVESHGNYSTQVG---ATQAGRLSITPAAPSYT    177
JEV_SA14                    CTSKAIGRTIQPENIKYEVGIFVHGTTTSENHGNYSAQVG---ASQAAKFTVTPNAPSIT    177
YFV_Asibi                   CAKSMSLFEVDQTKIQYVIRAQLHVGAKQENWN-----------TDIKTLKFDALSGSQE    169
                            *        :   :::* :    *                     .     .   :

ZIKV_H/PF/2013              ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN    237
ZIKV_MR766                  ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN    233
DENV_1_SG/07K3640DK1/2008   IQLTDYGALTLDCSPRTGLNFNEMVLLTMKEKSWLVHKQWFLDLPLPWTSGASTTQETWN    232
DENV_2_16681                AELTGYGTVTMECSPRTGLDFNEMVLLQMENKAWLVHRQWFLDLPLPWLPGADTQGSNWI    232
DENV_3_SG/05K863DK1/2005    AILPEYGTLGLECSPRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWASGATTETPTWN    230
DENV_4_SG/06K2270DK1/2005   VKLPDYGELTLDCEPRSGIDFNEMILMKMKKKTWLVHKQWFLDLPLPWTAGADTSEVHWN    232
WNV_NY99                    LKLGEYGEVTVDCEPRSGIDTNAYYVMTVGTKTFLVHREWFMDLNLPWSSAG---STVWR    234
JEV_SA14                    LKLGDYGEVTLDCEPRSGLNTEAFYVMTVGSKSFLVHREWFHDLALPWTSPS---STAWR    234
YFV_Asibi                   AEFTGYGKATLECQVQTAVDFGNSYIAEMEKESWIVDRQWAQDLTLPWQSGS---GGVWR    226
                              :  :*   ::*. ::.::        :  : ::*.::*  *: ***   .      *
```

```
ZIKV_H/PF/2013              NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAK---GRLSSGHLKCRLK     294
ZIKV_MR766                  NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAK---GRLFSGHLKCRLK     290
DENV_1_SG/07K3640DK1/2008   RQDLLVTFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGT----TTIFAGHLKCRLK     288
DENV_2_16681                QKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSG----NLLFTGHLKCRLR     288
DENV_3_SG/05K863DK1/2005    RKELLVTFKNAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGG----TSIFAGHLKCRLK     286
DENV_4_SG/06K2270DK1/2005   HKERMVTFKVPHAKRQDVTVLGSQEGAMHSALAGATEVDSGDG----NHMFAGHLKCKVR     288
WNV_NY99                    NRETLMEFEEPHATKQSVIALGSQEGALHQALAGAIPVEFSSNT---VKLTSGHLKCRVK     291
JEV_SA14                    NRELLMEFEGAHATKQSVVALGSQEGGLHQALAGAIVVEYSS-S---VKLTSGHLKCRLK     290
YFV_Asibi                   EMHHLVEFEPPHAATIRVLALGNQEGSLKTALTGAMRVTKDTNDNNLYKLHGGHVSCRVK     286
                            . . :: *:  **    * .**.***.:: **:**              :  **:.*:::

ZIKV_H/PF/2013              MDKLRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTP     354
ZIKV_MR766                  MDKLRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTP     350
DENV_1_SG/07K3640DK1/2008   MDKLTLKGMSYVMCTGSFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFST-QDEKGVTQ     347
DENV_2_16681                MDKLQLKGMSYSMCTGKFKVVKEIAETQHGTIVIRVQYEGDGSPCKIPFEI-MDLEKRHV     347
DENV_3_SG/05K863DK1/2005    MDKLELKGMSYAMCTNTFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFST-EDGQGKAH     345
DENV_4_SG/06K2270DK1/2005   MEKLRIKGMSYTMCSGKFSIDKEMAETQHGTTVVKVKYEGAGAPCKVPIEI-RDVNKEKV     347
WNV_NY99                    MEKLQLKGTTYGVCSKAFKFLGTPADTGHGTVVLELQYTGTDGPCKVPISSVASLNDLTP     351
JEV_SA14                    MDKLALKGTTYGMCTEKFSFAKNPADTGHGTVVIELSYSGSDGSCKIPIVSVASLNDMTP     350
YFV_Asibi                   LSALTLKGTSYKMCTDKMSFVKNPTDTGHGTVVMQVKVP-KGAPCKIPVIVADDLTAAIN     345
                            :. * :** :* :*:  : .    ::* ***  :.:.     . **:*     .

ZIKV_H/PF/2013              VGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEAT     414
ZIKV_MR766                  VGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEAT     410
DENV_1_SG/07K3640DK1/2008   NGRLITANPIVTDKE--KPVNIEAEPPFGESYIVIGAGEKALKLSWFKKGSSIGKMFEAT     405
DENV_2_16681                LGRLITVNPIVTEKD--SPVNIEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETT     405
DENV_3_SG/05K863DK1/2005    NGRLITANPVVTKKE--EPVNIEAEPPFGESNIVIGIGDNALKINWYKKGSSIGKMFEAT     403
DENV_4_SG/06K2270DK1/2005   VGRIISSTPFAENTN--SVTNIELEPPFGDSYIVIGVGESALTLHWFRKGSSIGKMFEST     405
WNV_NY99                    VGRLVTVNPFVSVATANAKVLIELEPPFGDSYIVVGRGEQQINHHWHKSGSSIGKAFTTT     411
JEV_SA14                    VGRLVTVNPFVATSSANSKVLVEMEPPFGDSYIVVGRGDKQINHHWHKAGSTLGKAFSTT     410
YFV_Asibi                   KGILVTVNPIASTND--DEVLIEVNPPFGDSYIIVGTGDSRLTYQWHKEGSSIGKLFTQT     403
                             * ::: .*.           :* :****:* *::*     :.  *.: **::*: *  *

ZIKV_H/PF/2013              VRGAKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMW     474
ZIKV_MR766                  VRGAKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVW     470
DENV_1_SG/07K3640DK1/2008   ARGARRMAILGDTAWDFGSIGGVFTSVGKLVHQIFGTAYGVLFSGVSWTMKIGIGVLLTW     465
DENV_2_16681                MRGAKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITW     465
DENV_3_SG/05K863DK1/2005    ARGARRMAILGDTAWDFGSVGGVLNSLGKMVHQIFGSAYTALFSGVSWVMKIGIGVLLTW     463
DENV_4_SG/06K2270DK1/2005   YRGAKRMAILGETAWDFGSVGGLFTSLGKAVHQVFGSVYTTMFGGVSWMVRILIGFLVLW     465
WNV_NY99                    LKGAQRLAALGDTAWDFGSVGGVFTSVGKAVHQVFGGAFRSLFGGMSWITQGLLGALLLW     471
JEV_SA14                    LKGAQRLAALGDTAWDFGSIGGVFNSIGKAVHQVFGGAFRTLFGGMSWITQGLMGALLLW     470
YFV_Asibi                   MKGAERLAVMGDAAWDFSSAGGFFTSVGKGIHTVFGSAFQGLFGGLNWITKVIMGAVLIW     463
                             :**.*:* :*::****.* ** :.*:** :* :**  :   *.*:.*  :  :* :: *

ZIKV_H/PF/2013              LGLNTKNGSISLMCLALGGVLIFLSTAVSA      504
ZIKV_MR766                  LGLNTKNGSISLTCLALGGVMIFLSTAVSA      500
DENV_1_SG/07K3640DK1/2008   LGLNSRSTSLSMTCIAVGLVTLYLGVMVQA      495
DENV_2_16681                IGMNSRSTSLSVTLVLVGIVTLYLGVMVQA      495
DENV_3_SG/05K863DK1/2005    IGLNSKNTSMSFSCIAIGIITLYLGAVVQA      493
DENV_4_SG/06K2270DK1/2005   IGTNSRNTSMAMTCIAVGGITLFLGFTVQA      495
WNV_NY99                    MGINARDRSIALTFLAVGGVLLFLSVNVHA      501
JEV_SA14                    MGVNARDRSIALAFLATGGVLVFLATNVHA      500
YFV_Asibi                   VGINTRNMTMSMSMILVGVIMMFLSLGVGA      493
                            :* *::. :::.  :  * : ::*.  * *
```

C)

	ZIKV_H/PF	ZIKV_MR	DENV1	DENV2	DENV3	DENV4	WNV	JEV	YFV
1: ZIKV_H/PF/2013	100.00	97.20	57.37	53.94	58.22	56.16	54.62	55.13	42.51
2: ZIKV_MR766	97.20	100.00	58.22	54.97	58.86	56.39	54.66	56.39	43.94
3: DENV_1_SG/07K3640DK1/2008	57.37	58.22	100.00	68.48	78.09	63.43	50.72	50.61	43.18
4: DENV_2_16681	53.94	54.97	68.48	100.00	68.97	63.84	48.67	48.16	45.25
5: DENV_3_SG/05K863DK1/2005	58.22	58.86	78.09	68.97	100.00	63.08	47.23	49.18	42.24
6: DENV_4_SG/06K2270DK1/2005	56.16	56.39	63.43	63.84	63.08	100.00	50.72	48.57	40.91
7: WNV_NY99	54.62	54.66	50.72	48.67	47.23	50.72	100.00	78.20	44.29
8: JEV_SA14	55.13	56.39	50.61	48.16	49.18	48.57	78.20	100.00	44.38
9: YFV_Asibi	42.51	43.94	43.18	45.25	42.24	40.91	44.29	44.38	100.00

Fig. S2. Envelope (E) protein of ZIKV (H/PF/2013): A) Domain organization; B) Sequence alignment with other mosquito-transmitted flaviviruses and C) Percent identity matrix. Domains I, II and III together constitute the surface portion of E protein and are represented in red, yellow and blue respectively. Fusion loop, part of domain II, is highlighted in green. S and TM are the stem and transmembrane regions of the protein and are displayed in light pink color. The conserved glycosylation site at N153/154 is highlighted in blue, the DENV specific glycosylation site (N67) is highlighted in pink whereas the predicted yellow fever glycosylation site (N309) is highlighted in grey. The glycosylation motif N-X-S/T is underlined. The sequences for various flaviviruses were obtained from the Virus Pathogen Database and Analysis Resource (ViPR). Virus strains that had structural information available were chosen where possible. GenBank Genome Accession codes for these viruses are as follows: ZIKV_H/PF/2013: KJ776791; ZIKV_MR766: AY632535; DENV 1_SG/07K3640DK1/2008: GQ398255; DENV 2_16681: NC_001474; DENV 3_SG/05K863DK1/2005: EU081190; DENV4_SG/06K2270DK1/2005: GQ398256; WNV_NY99: DQ211652; JEV_SA14: D90194 and YFV_Asibi: AY640589. The domain map was constructed using Illustrator for Biological Sequences (IBS) *(37)*. The multiple sequence alignment and percent identity matrix was created using Clustal Omega tool (EMBL-EBI) *(38)*. The * (asterisk), : (colon) and . (period) denote consensus among the sequences. '*' indicates completely conserved residue; ':' indicates conservation of amino acids with strongly similar properties (score of >0.5 in the Gonnet PAM 250 matrix) and '.' represents conservation of amino acids with weakly similar properties (score of =<0.5 in the Gonnet PAM 250 matrix).

Table S1. Primers used for verification of ZIKV

Primer	Genome Position	Protein Position	5'-3' Sequence	RT/qRT	Amplicon length	Reference
z-1A	228-250	Capsid	CCATCACTGGGTCTCATCAATAG	RT	741bp	-
z-1B	947-968	Envelope	ACCTGACATACCTTCCACAAAG	RT		-
z-2A	8554-8576	NS5	GGTCAGCGTCCTCTCTAATAAAC	RT	945bp	-
z-2B	9476-9498	NS5	GAACTTCCTCAGCCTCCATATTC	RT		-
z-3A	882-904	Membrane	TTGGTCATGATACTGCTGATTGC	qRT	77bp	*(22)*
z-3B	937-958	Envelope	CCTTCCACAAAGTCCCTATTGC	qRT		*(22)*
z-4A	1133-1149	Envelope	CCGCTGCCCAACACAAG	qRT	77bp	*(22)*
z-4B	1186-1209	Envelope	CCACTAACGTTCTTTTGCAGACAT	qRT		*(22)*

Table S2. Structure determination statistics

Cryo-EM reconstruction and refinement of Zika particles	
Number of particles in the final reconstruction	11,842
Pixel size in the super-resolution counting mode of K2 Summit (Å)	1.04
Pixel size corresponding to the physical detector sensor (Å)	2.08
Defocus range (μm)	-1 to -3
Electron dose (electrons/Å2)	23.6
Number of frames in each movie	70
Electron energy (kV)	300
Pixel dimension of single-particle image	236
Isotropic B-factor for amplitude correction (Å2)	-200
Pseudo-crystallographic refinement	
Resolution (Å)	3.8
R_{work} / R_{free}*	0.34/0.34
No. of residues E protein	501(1-501) (residues 502-504 missing)
M protein	75(1-75)
R.M.S. deviations	
Bond lengths (Å)	0.016
Bond angles (^{o})	1.355
Ramachandran plot values	
Most favored (%)	81.3
Generously allowed (%)	12.9
Disallowed regions (%)	5.8

*R_{free} was calculated for 5% of reflections randomly excluded from the refinement

References and notes:

22. R. S. Lanciotti *et al.*, Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* **14**, 1232-1239 (2008).
37. W. Liu *et al.*, IBS: an illustrator for the presentation and visualization of biological sequences. *Bioinformatics* **31**, 3359-3361 (2015).
38. F. Sievers *et al.*, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* **7**, 539 (2011).