Supplementary materials

Figure S1

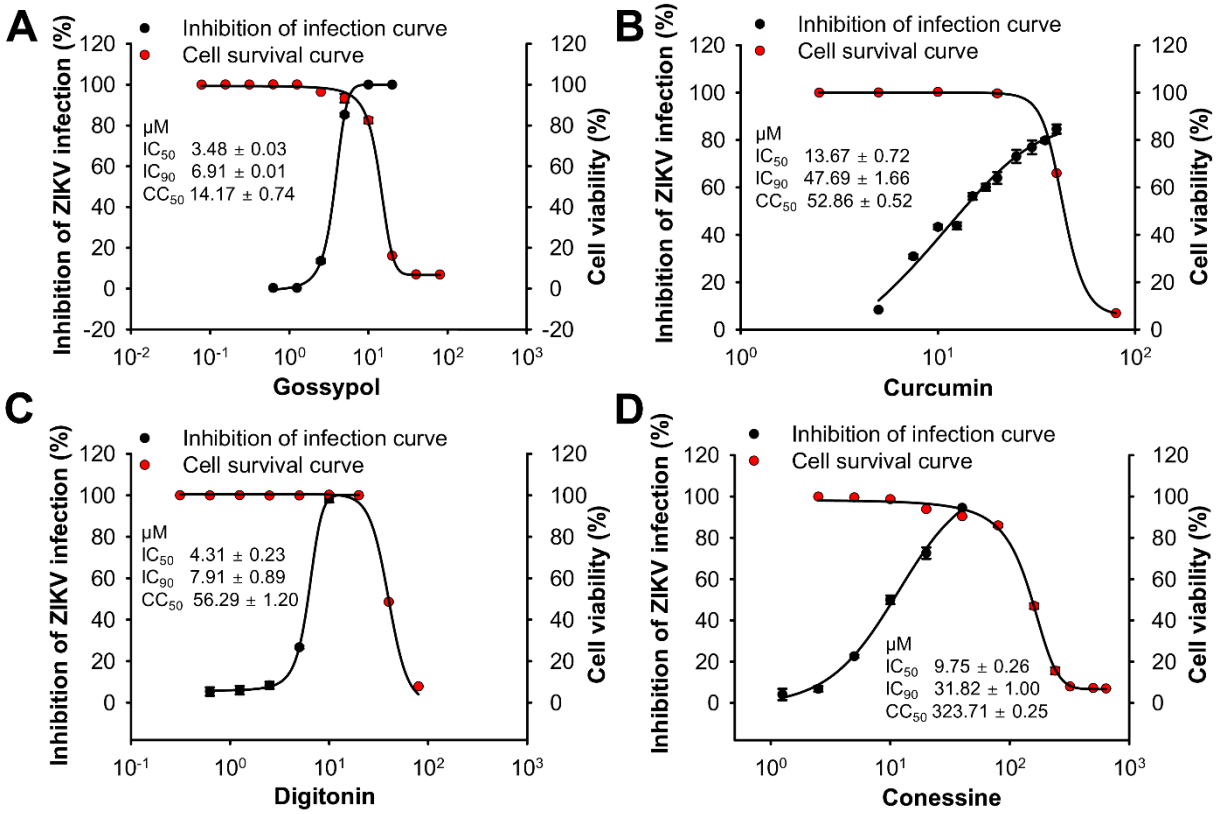


Figure S1. Association between inhibitory activity of natural products against ZIKV strain PAN2016 and their cytotoxicity. ZIKV stain PAN2016 (100 PFU) was incubated with each of the natural products at different concentrations at 37°C for 1 h. The compound-virus mixtures were then transferred to Vero E6 cells (10^5/well) and incubated at 37°C for 1 h. Plates were incubated at 37°C for 4-5 days. Viral titers at each concentration were calculated by plaque assay, which are expressed as percentage inhibition of the untreated virus (black circles). Cell viability was assessed by the Cell Counting Kit-8 (CCK8) assay, and expressed as a percentage relative to that of the untreated cells (red circles). The concentrations of natural products that inhibited 50% (IC_{50}) or 90% (IC_{90}) of plaque formation, or caused 50% cytotoxicity (CC_{50}) in Vero E6 cells are shown in the figure. The data are expressed as the mean ± standard error of the mean (s.e.m.) (n=2). The experiments were repeated twice with similar results.

Figure S2

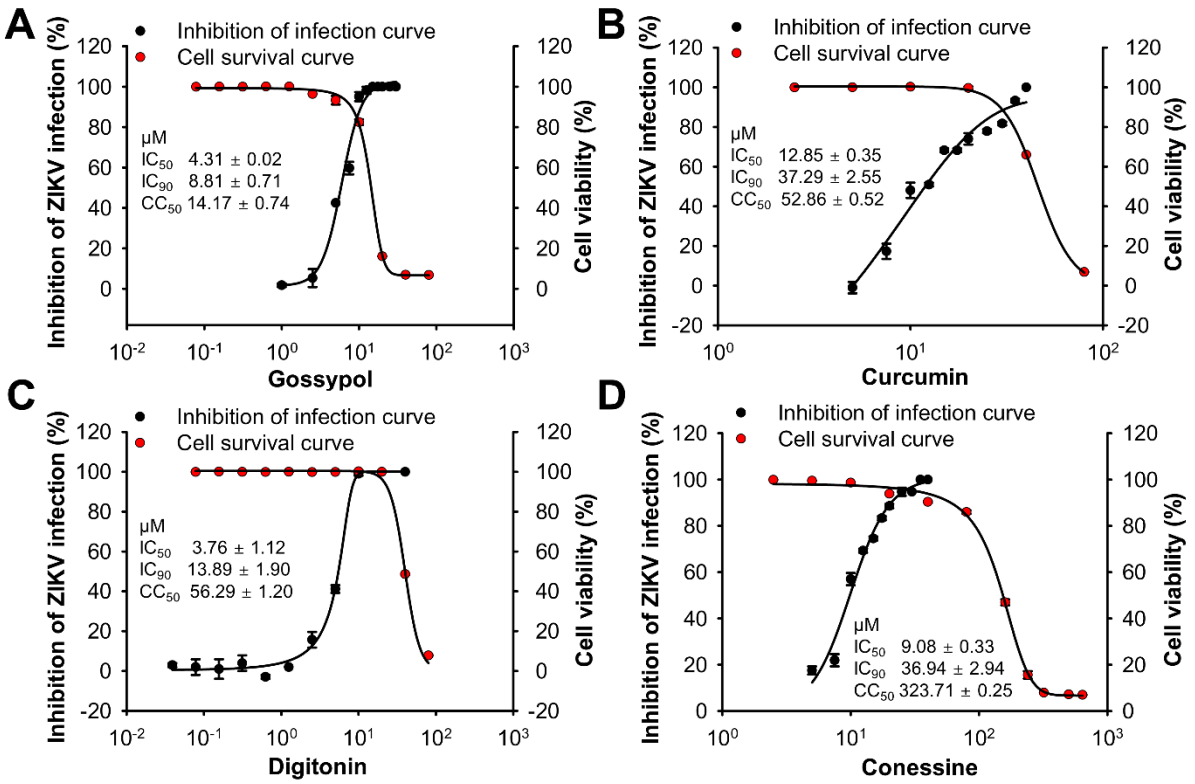
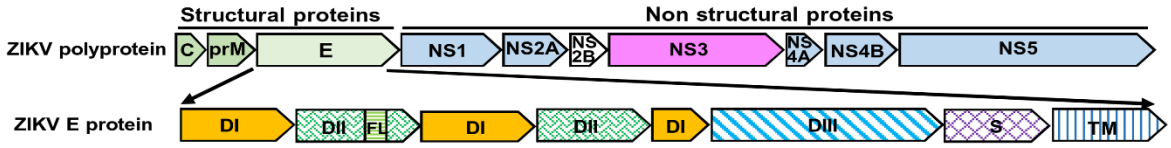


Figure S2. Association between inhibitory activity of natural products against ZIKV strain PRVABC59 and their cytotoxicity. ZIKV stain PRVABC59 (100 PFU) was incubated with each of the natural products at different concentrations at 37°C for 1 h. The compound-virus mixtures were then transferred to Vero E6 cells (10^5/well) and incubated at 37°C for 1 h. Plates were further incubated at 37°C for 4-5 days. Viral titers at each concentration were calculated by plaque assay, which are expressed as percentage inhibition of the untreated virus (black circles). Cell viability was assessed by the Cell Counting Kit-8 (CCK8) assay, and expressed as a percentage relative to that of the untreated cells (red circles). The concentrations of natural products that inhibited 50% (IC_{50}) or 90% (IC_{90}) of plaque formation, or caused 50% cytotoxicity (CC_{50}) in Vero E6 cells are shown in the figure. The data are expressed as the mean ± standard error of the mean (s.e.m.) (n=2). The experiments were repeated twice with similar results.

Figure S3

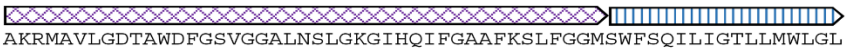



```
R116265          --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
PAN2016          --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
PAN2015          --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
FLR              --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
R103451          --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
PRVABC59         --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
PLCal_ZV         --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
IbH 30656        --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
MEX 2-81         --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
MR 766           --IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRS   58
DENV-1-V1792     MAMRCVGIGSRDFVEGLSGATWVDVVLEHGSCVTTMAKDKPTLDIELLKTEVTNPAVLRK   60
DENV-2-V594      --MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQPATLRK   58
DENV-3-V1043     --MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEATQLATLRK   58

R116265          YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
PAN2016          YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
PAN2015          YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
FLR              YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
R103451          YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
PRVABC59         YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
PLCal_ZV         YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
IbH 30656        YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
MEX 2-81         YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
MR 766           YCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAK   118
DENV-1-V1792     LCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGSLITCAK   120
DENV-2-V594      YCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFICKHSMVDRGWGNGCGLFGKGGIVTCAM   118
DENV-3-V1043     LCIEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGSLVTCAK   118

R116265          FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
PAN2016          FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
PAN2015          FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
FLR              FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
R103451          FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
PRVABC59         FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
PLCal_ZV         FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
IbH 30656        FTCSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIV------NDENRAKVEVTPNSPRAE   171
MEX 2-81         FACSKKMTGKSIQPENLEYRIMLSVH-GSQHSGMIVNDTGHETDENRAKVEITPNSPRAE   177
MR 766           FTCSKKMTGKSIQPENLEYRIMLSVH-GSQHSGM----IGYETDENRAKVEVTPNSPRAE   173
DENV-1-V1792     FKCVTKLEGKIVQYENLKYSVIVTVHTGDQHQ------VGNESTEHGTTATITPQAPTTE   174
DENV-2-V594      FTCKKNMEGKVVLPENLEYTIVITPHSGEEHA------VGNDTGKHGKEIKITPQSSITE   172
DENV-3-V1043     FQCLEPIEGKVVQYENLKYTVIITVHTGDQHQ------VGNET--QGVTAEITPQASTTE   170

R116265          ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
PAN2016          ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
PAN2015          ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
FLR              ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
R103451          ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
PRVABC59         ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
PLCal_ZV         ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
IbH 30656        ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHSGADTETPHWN   231
MEX 2-81         ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   237
MR 766           ATLGGFGSLGLDCEPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN   233
DENV-1-V1792     IQLTDYGALTLDCSPRTGLDFNEMVLLTMKEKSWLVHKQWFLDLPLPWTSGASTSQETWN   234
DENV-2-V594      AELTGYGTVTMECSPRTGLDFNEMVLLQMEDKAWLVHRQWFLDLPLPWLPGADTQGSNWI   232
DENV-3-V1043     AILPEYGTLGLECSPRTGLDFNEMILLTMKNKAWMVHRQWFFDLPLPWTSGATTETPTWN   230
```

```
R116265          NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
PAN2016          NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
PAN2015          NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
FLR              NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
R103451          NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
PRVABC59         NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
PLCal_ZV         NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
IbH 30656        NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  291
MEX 2-81         NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDK  297
MR 766           NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGKLFSGHLKCRLKMDK  293
DENV-1-V1792     RQDLLVTFKTAHAKKQEVVVLGSQEGAMHTALTGATEIQTSGTT-TIFAGHLKCRLKMDK  293
DENV-2-V594      QKETLVTFKNPHAKKQDVVVLGSQEGAMHTALTGATEIQMSSGN-LLFTGHLKCRLRMDK  291
DENV-3-V1043     RKELLVTFKNAHAKKQEVVVLGSQEGAMHTALTGATEIQNSGGT-SIFAGHLKCRLKMDK  289
```

```
R116265          LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
PAN2016          LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
PAN2015          LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
FLR              LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
R103451          LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
PRVABC59         LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVELQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
PLCal_ZV         LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
IbH 30656        LRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGRDGPCKVPAQMAVDMQTLTPVGR  351
MEX 2-81         LRLKGVSYSLCTAAFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGR  357
MR 766           LRLKGVSYSLCTAAFTFTKVPAETLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGR  353
DENV-1-V1792     LTLKGMSYVMCTGSFKLEKEVAETQHGTVLVQIKYEGTDAPCKIPFSTQ-DEKGVTQNGR  352
DENV-2-V594      LQLKGMSYSMCTGKFKIVKEIAETQHGTIVIRVQYEGDGSPCKIPFEIM-DLEKRHVLGR  350
DENV-3-V1043     LELKGMSYAMCTNTFVLKKEVSETQHGTILIKVEYKGEDAPCKIPFSTE-DGQGKAHNGR  348
```

```
R116265          LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
PAN2016          LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
PAN2015          LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
FLR              LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
R103451          LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
PRVABC59         LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
PLCal_ZV         LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
IbH 30656        LITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSIIGKAFEATVRG  411
MEX 2-81         LITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRG  417
MR 766           LITANPVITESTENSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRG  413
DENV-1-V1792     LITANPIVTD--KEKPVNIEAEPPFGESYIVIGAGEKALKLSWFKKGSSIGKMFEATARG  410
DENV-2-V594      LITVNPIVTE--KDSPVNIEAEPPFGDSYIIIGVEPGQLKLNWFKKGSSIGQMFETTMRG  408
DENV-3-V1043     LITANPVVTK--KEEPVNIEAEPPFGESNIVIGIGDNALKINWYKKGSSIGKMFEATARG  406
```



```
R116265          AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
PAN2016          AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
PAN2015          AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
FLR              AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
R103451          AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
PRVABC59         AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
PLCal_ZV         AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
IbH 30656        AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGL  471
MEX 2-81         AKRMAVLGDTAWDFGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGL  477
MR 766           AKRMAVLGDTAWDFGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGL  473
DENV-1-V1792     ARRMAILGDTAWDFGSIGGVFTSVGKLVHQIFGTAYGVLFSGVSWTMKIGIGVLLTWLGL  470
DENV-2-V594      AKRMAILGDTAWDFGSLGGVFTSIGKALHQVFGAIYGAAFSGVSWTMKILIGVIITWIGM  468
DENV-3-V1043     ARRMAILGDTAWDFGSVGGVLNSLGKMVHQIFGSAYTALFSGVSWVMKIGIGVLLTWIGL  466
```

```
R116265          NTKNGSISLMCLALGGVLIFLSTAVSA     504
PAN2016          NTKNGSISLMCLALGGVLIFLSTAVSA     504
PAN2015          NTKNGSISLMCLALGGVLIFLSTAVSA     504
FLR              NTKNGSISLMCLALGGVLIFLSTAVSA     504
R103451          NTKNGSISLMCLALGGVLIFLSTAVSA     504
PRVABC59         NTKNGSISLMCLALGGVLIFLSTAVSA     504
PLCal_ZV         NTKNGSISLMCLALGGVLIFLSTAVSA     504
IbH 30656        NTKNGSISLTCLALGGVMIFLSTAVSA     498
MEX 2-81         NTKNGSISLMCLALGGVLIFLSTAVSA     504
MR 766           NTKNGSISLTCLALGGVMIFLSTAVSA     500
DENV-1-V1792     NSRSTSLSMTCIAVGLVTLYLGVMVQA     497
DENV-2-V594      NSRSTSLSVSLVLVGVVTLYLGVMVQA     495
DENV-3-V1043     NSKNTSMSFSCIAIGVITLYLGAVVQA     493
```

Figure S3. Multiple sequence alignment of amino acid (aa) sequences of E protein of 10 ZIKV strains and DENV-1-3 human strains used in this study. Schematic maps of ZIKV polyprotein and ZIKV E protein are listed on top of the alignment. Amino acids variable positions are highlighted. The sequence of DENV-4-PR 06-65-740 is not available, so its sequence alignment is not included. The alignment was performed using Clustal V method of MegAlign (v7.1) within the DNASTAR package. C: capsid; prM: precursor of membrane; E: envelope; NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5: nonstructural proteins. DI-DIII: domain I-III of E protein; FL: fusion loop; S: stalk region; TM: transmembrane domain.