

Capsid

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	M s n - x x K k g g r r x v N M L K R g r n R V S x l x G L - K R f s x g L L x G x G P x R I V L A f I A F x R F I A I - - - p P T a G I L k R W g s v k K s x A i K x L t g F K K E i g x M L n l i N
ILHV	M S K K P G K S A A K R T V N M L K R L - A S V S P L K G A - K K L F G E L L S G K G A I R L I L A L M A F F R F A A I - - - K P T L G L K K R W R S V N K T V A V K H L T N F K K E L T T M L D S V N
ROCV	M S K K P G G P A G R R V V N M L K R P - A S V S P I K G I - K R L I G N L T D G R G P L R V V L A F I A F F R F A A I - - - M P T Q G L L R R W R V M N K S E A L K H L T S F K K E I S N M L N I I N
WNV	M S K K P G G P G K S R A V N M L K R G M P R V L S L I G L - K R A M L S L I D G K G P I R F V L A L L A F F R F T A I - - - A P T R A V L D R W R G V N K Q T A M K H L L S F K K E L G T L T S A I N
JEV	M T K K P G G P G K N R A I N M L K R G L P R V F P L V G V - K R V V M S L L D G R G P V R F V L A L I T F F K F T A L - - - A P T K A L L G R W K A V E K S V A M K H L T S F K R E L G T L I D A V N
DENV1	M N N - Q R K K T G R P S F N M L K R A R N R V S T V S Q L A K R F S K G L L S G Q G P M K L V M A F I A F L R F L A I - - - P P T A G I L A R W G S F K K N G A I K V L R G F K K E I S N M L N I M N
DENV2	M N N - Q R K K A R N T P F N M L K R E R N R V S T V Q Q L T K R F S L G M L Q G R G P L K L F M A L V A F L R F L T I - - - P P T A G I L K R W G T I K K S K A I N V L R G F R K E I G R M L N I L N
DENV3	M N N - Q R K K T G K P S I N M L K R V R N R V S T G S Q L A K R F S R G L L N G Q G P M K L V M A F I A F L R F L A I - - - P P T A G V L A R W G T F K K S G A I K V L K G F K K E I S N M L S I I N
DENV4	- M N - Q R K K V V R P P F N M L K R E R N R V S T P Q G L V K R F S T G L F S G K G P L R M V L A F I T F L R V L S I - - - P P T A G I L K R W G L K K N K A I K I L T G F R K E I G R M L N I L N
ZIKV	M K N P K K K S G G F R I V N M L K R G V A R V N P L G G L - K R L P A G L L L G H G P I R M V L A I L A F L R F T A I - - - K P S L G L I N R W G S V G K K E A M E I I K K F K K D L A A M L R I I N
YFV	M S G - R K A Q G K T L G V N M V R R G V R S L S N K I - - - K Q K T K Q I G N R P G P S R G V Q G F I F F F L F N I L T G K K I T A H L K R L W K M L D P R Q G L A V L R K V K R V V A S L M R G L S

Ruler 1	110 120 130
Consensus	r R K r k x - - - - - l x x i x L l p t v m A - - -
ILHV	K R K E K K - - - K S F S T A L L W I T M I T A V A G - - -
ROCV	R R K A K R - - - - G N G S V L L W I A L V T G S M A - - -
WNV	R R S S K Q K K R G G K T G I A V M I G L I A S V G - - - A
JEV	K R G R K Q N K R G G N E G S I M W L A S L A V I I A C A G A
DENV1	R R K R S V - - - - - T M L M L L P T A L A - - -
DENV2	R R R R T A - - - - - G M I I M L I P T V M A - - -
DENV3	K R K K T S - - - - - L C L M M M L P A T L A - - -
DENV4	G R K R S T - - - - - M T L L C L I P T V M A - - -
ZIKV	A R K E R K R R - - G A D T S I G I I G L L L T T A M A - - -
YFV	S R K R R S H D V - - L T V Q F L I L G M L L M T G G - - -

Supplementary Figure S2: Alignment of Individual Proteins Across Multiple Flaviviruses. Individual proteins from representative flaviviruses were aligned using the Clustal Omega algorithm in MegAlign Pro v17.2.1 (DNASTAR, Madison, WI). Amino acids are colored by side chain chemistry: aromatic residues (F, W, and Y) are yellow, acidic residues (D and E) are red, basic residues (R, H, and K) are blue, nonpolar residues (A, G, I, L, M, P, and V) are orange, and polar residues (C, N, Q, S, and T) are green). Gaps are represented as dashes. Black rectangles designate cysteine residues predicted to form disulfide bridges.

prM

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	f h L x t r q G e p l M i V n k t D x G k s i l f x T x k G v N m C t x r A M D I G y M C e D T i T Y E C p x L t e g n E P E D I D C W C n l - t s x w V t Y G t C t q t g - e x R R s R R S V a L p p
ILHV	L K I S S H R D R P L L M V N K T D V S D A I P V P S V K G T N M C T I R A L D V G Y T C A Y D T T Y E C P H L E V T M D P E D I D C W C T L - E S V Y V N Y G L C K Q N H - H V R R G R R A I N I P H
ROCV	L R L G T Y Q G K V L M S I N K T D V A E I I P I P T T K G D N L C T V R A M D V G Y M C Q K D I T Y E C P R L E P G M D P E D I D C W C D R - E A I Y V H Y G L C T K N H - R E R R G R R S V N I P S
WNV	V T L S N F Q G K V M M T V N A T D V T D V I T I P T A A G K N L C I V R A M D V G Y M C D D T I T Y E C P V L S A G N D P E D I D C W C T K - S A V Y V R Y G R C T K T R - H S R R S R R S L T V Q T
JEV	M K L S N F Q G K L L M T V N N T D I A D V I V I P T S K G E N R C W V R A I D V G Y M C E D T I T Y E C P K L T M G N D P E D V D C W C D N - Q E V Y V Q Y G R C T R T R - H S K R S R R S V S V Q T
DENV1	F H L T T R G G E P H M I V S K Q E R G K S L L F K T S A G V N M C T L I A M D L G E L C E D T M T Y K C P R I T E - T E P D D V D C W C N A - T E T W V T Y G T C S Q T G - E H R R D K R S V A L A P
DENV2	F H L T T R N G E P H M I V S R Q E K G K S L L F K T X D G V N M C T L M A M D L G E L C E D T I T Y K C P X L R Q - N E P E D I D C W C N S - T S T W V T Y G T C T T T G - E H R R E K R S V A L V P
DENV3	F H L T S R D G E P R M I V G K N E R G K S L L F K T A S G I N M C T L I A M D L G E M C D D T V T Y K C P H I T E - V E P E D I D C W C N L - T S T W V T Y G T C N Q A G - E H R R D K R S V A L A P
DENV4	F H L S T R D G E P L M I V A K H E R G R P L L F K T T E G I N K C T L I A M D L G E M C E D T V T Y E C P L L V N - T E P E D I D C W C N L - T S A W V M Y G T C T Q S G - E R R R E K R S V A L T P
ZIKV	A E I T R R G S A Y Y M Y L D R S D A G K A I S F A T T L G V N K C H V Q I M D L G H M C A T M S Y E C P M L D E G V E P D D V D C W C N T - T S T W V V Y G T C H H K K G E A R R S R R A V T L P S
YFV	V T L V R K N R W L L L N V T S E D L G K T F - - - S V G T G N C T T N I L E A K Y W C P D S M E Y N C P N L S P R E E P D D I D C W C Y G V E N V R V A Y G K C D S A G - R S R R S R R A I D L P T

Ruler 1	110 120 130 140 150 160
Consensus	H g x s g L e T R t e t W M x S e x A t K y L q K V E n W i I R N P G F A l v A l x I A w m I G S n t t Q R V V f f I L L I L V A P A Y S
ILHV	H G E S H L E N R A T P W M D T T K T T K Y L T K V E N W V I R N P G Y A L V A L A T A W M L G S N T P Q R V V F M I M M M L I A P A Y S
ROCV	H G E S Q L E N R G T P W L D T A K T T K Y L T K V E N W M I R N P G Y A I V A V A A A W M L G S N T S Q K V I F T I M L L L I A P A Y S
WNV	H G E S T L A N K K G A W M D S T K A T R Y L V K T E S W I L R N P G Y A L V A A V I G W M L G S N T M Q R V V F V V L L L L V A P A Y S
JEV	H G E S S L V N K K E A W L D S T K A T R Y L M K T E N W I V R N P G Y A F L A A I L G W M L G S N N G Q R V V F T I L L L L V A P A Y S
DENV1	H V G L G L E T R T E T W M S S E G A W K Q I Q K V E T W A L R H P G F T V I A L F L A H A I G T S I T Q K G I I F I L L M L V T P S M A
DENV2	H V G M G L E T R T E T W M S S E G A W K H A Q R I E T W I L R H P G F T I M A A I L A Y T I G T T H F Q R A L I F I L L T A V A P S M T
DENV3	H V G M G L D T R T Q T W M S A E G A W R Q V E K V E T W A L R H P G F T I L A L F L A H Y I G T S L T Q K V V I F I L L M L V T P S M T
DENV4	H S G M G L E T R A E T W M S S E G A W K H A Q R V E S W I L R N P G F A L L A G F M A Y M I G Q T G I Q R T V F F V L M M L V A P S Y G
ZIKV	H S T R K L Q T R S Q T W L E S R E Y T K H L I K V E N W I F R N P G F A L V A V A I A W L L G S S T S Q K V I Y L V M I L L I A P A Y S
YFV	H E N H G L K T R Q E K W M T G R M G E R Q L Q K I E R W L V R N P F F A V T A L T I A Y L V G S N M T Q R V V I A L L V L A V G P A Y S

Supplementary Figure S2: Alignment of Individual Proteins Across Multiple Flaviviruses. Individual proteins from representative flaviviruses were aligned using the Clustal Omega algorithm in MegAlign Pro v17.2.1 (DNASTAR, Madison, WI). Amino acids are colored by side chain chemistry: aromatic residues (F, W, and Y) are yellow, acidic residues (D and E) are red, basic residues (R, H, and K) are blue, nonpolar residues (A, G, I, L, M, P, and V) are orange, and polar residues (C, N, Q, S, and T) are green). Gaps are represented as dashes. Black rectangles designate cysteine residues predicted to form disulfide bridges.

Envelope

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	m r I G x x N R D F V E G V S G G T W V D x V L E H G s C V T T M A K D K P T L D I E L I k t E A k x I A x v R s C Y e A x x T x i x T d x R C P T Q G E A H L x x e q D x s Y V C K R t y V D R G
ILHV	L N C L G I S N R D F V E G L S G G T W V D I V L E G G S C V T V M A K D K P T L D I K L I R M E A K D L A T V R S Y C Y A T V T D S S T E A R C P T M G E A H N S K S L D A S Y V C K S S Y V D R G
ROCV	I N C L G V T N R D F V E G M S G G T W V D I V L E G D G C V T I M A K D K P T L D I R L L K M E A K D L A T V R S Y C Y H A T V T S V S S E A R C P T M G E A H N P K A L D S S Y L C K S T Y V D R G
WNV	F N C L G M S N R D F L E G V S G A T W V D L V L E G D S C V T I M S K D K P T I D V K M M N M E A A N L A E V R S Y C Y L A T V S D L S T K A A C P T M G E A H N D K R A D P A F V C R Q G V V D R G
JEV	F N C L G M G N R D F I E G A S G A T W V D L V L E G D S C L T I M A N D K P T L D V R M I N I E A V Q L A E V R S Y C Y H A S V T D I S T V A R C P T T G E A H N K K R A D S S Y V C K Q G F T D R G
DENV1	M R C V G I G N R D F V E G L S G A T W V D V V L E H G S C V T T M A K D K P T L D I E L L K T E V T N P A V L R K L C I E A K I S N T T T D S R C P T Q G E A T L V E E Q D T N F V C R R T F V D R G
DENV2	M R C I G I S N R D F V E G V S G S W V D I V L E H G S C V T T M A K N K P T L D F E L I K T E A K Q P A T L R K Y C I E A K L T N T T T D S R C P T Q G E P S L N E E Q D K R F V C K H S M V D R G
DENV3	M R C V G V G N R D F V E G L S G A T W V D V V L E H G G C V T T M A K N K P T L D I E L Q K T E A T Q L A T L R K L C I E G K I T N I T T D S R C P T Q G E A I L P E E Q D Q N Y V C K H T Y V D R G
DENV4	M R C V G V G N R D F V E G V S G G A W V D L V L E H G G C V T T M A Q G K P T L D F E L I K T T A K E V A L L R T Y C I E A S I S N I T T A T R C P T Q G E P Y L K E E Q D Q Q Y I C R R D V V D R G
ZIKV	I R C I G V S N R D F V E G M S G G T W V D V V L E H G G C V T V M A Q D K P T V D I E L V T T V S N M A E V R S Y C Y E A S I S D M A S D S R C P T Q G E A Y L D K Q S D T Q Y V C K R T L V D R G
YFV	A H C I G I T D R D F I E G V H G G T W V S A T L E Q D K C V T V M A P D K P S L D I S L E T V A I D G P A E A R K V C Y N A V L T H V K I N D K C P S T G E A H L A E E N E G D N A C K R T Y S D R G

Ruler 1	110 120 130 140 150 160 170 180 190
Consensus	W G N G C G L F G K G S i v T C A K F x C s k K a t G k t x Q p E N L k Y x V a i t V H g g x t h - - h g n y v g x e t a k h x a f x I T P q x P s x E A k L g e Y G e I T L D C E P R t G L D F n e
ILHV	W G N G C G L F G K G S I Q T C Y K F S C P G K A T G K S I Q R E N L N Y D A V Y V H G P I S A A A H G N Y T A Q L T G K Y A A K F S I T P S A P T Y T A N L G E Y G E A T M C E P R A A L D I D N
ROCV	W G N G C G L F G K G S L Q T C V K F G C T Q K A M G M T I Q R E N L D Y E L A I Y V H G P T S V A A H G N Y T T Q L G A K H A A K F S I T P S S P S T A N L G E Y G E A T V D C E P R A A L D I D N
WNV	W G N G C G L F G K G S I D T C A K F A C S T K A I G R T I L K E N I K Y E A I F V H G P T T V E S H G N Y S T Q V G A T Q A G R F S I T P A A P S Y T L K L G E Y G E V T V D C E P R S G I D T N A
JEV	W G N G C G L F G K G S I D T C A K F S C T S K A I G R T I Q P E N I K Y E V G I F V H G T T T S E N H G N Y S A Q V G A S Q A A K F T V T P N A P S T T L K L G D Y G E V T L D C E P R S G L N T E A
DENV1	W G N G C G L F G K G S L I T C A K F K C V T K L E G K I V Y E N L K Y S I V T V H T G D Q H - - - - Q V G N E T T E H G T T A T I T P Q A P T S E I Q L T D Y G A L T L D C E P R T G L D F N E
DENV2	W G N G C G L F G K G G I V T C A M F T C K K N M K G K V V Q P E N L E Y T I V I T P H S G E E H - - - - A V G N D T G K H G K E I K I T P Q S S I T E A E L T G Y G T V T M C E P R T G L D F N E
DENV3	W G N G C G L F G K G S L V T C A K F C L E S I E G K V V Q H E N L K Y T V I T V H T G D Q H - - - - Q V G N E T Q - - G V T A E I T S Q A S T A E A I L P E Y G T L G L E C E P R T G L D F N E
DENV4	W G N G C G L F G K G G V V T C A F S C S G K I T G N L V Q I E N L E Y T V V T V H N G D T H - - - - A V G N I S N H G V A T I T P R S P S V E V K L P D Y E E L T D C E P R S G M D F N E
ZIKV	W G N G C G L F G K G S L V T C A K F T C S K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G Y E T D E N R A K V E V T P N S P R A E A T L G G F G S L G L D C E P R T G L D F S D
YFV	W G N G C G L F G K G S I V A C A K F T C A K S M S L F E V D Q T K I Q Y V I R A Q L H V G A K Q E N W N T - - - - - D I K T L K F D A L S G S Q E A E F T G Y G K A T L E C Q V Q T A V D F G N

Ruler 1	210 220 230 240 250 260 270 280 290
Consensus	m Y x m t M x n K x W L V H R Q W F h D L p L P W T s G A x t - - x v W n n x E I L V E F x e x H A k k Q t V v x L G S Q E G A I H T A L x G A x e V e x S g x - - - x k l f s G H L K C R L K M D K
ILHV	Y Y V M S L N N K H W L V N R D W F H D L D L P W T G P A T - - - E S W K N R E S L I E F E E P H A T R Q T V V A L G N Q E G A L H T A L A G A I P V E V S S T T - - - L T L N S G H L K C R L K L D K
ROCV	Y Y V M S M N N K H W L V N R D W F H D L D L P W T G P A T - - - D V W K N R E S L V F E E A H A T R Q T V V A L A A Q E G A L H T A L A G A I P V T V A G T T - - - L T L T S G H L K C R M K L D K
WNV	Y Y V M T V G T K T F L V H R E W F M D L N L P W S S A G S - - - T V W R N R E T L M F E E P H A T K Q S V I A L G S Q E G A L H Q A L A G A I P V E F S S N T - - - V K L T S G H L K C R V K M E K
JEV	F Y V M T V G S X S F L V H R E W F H D L A L P W T P P S - - - T A W R N R E L L M F E E A H A T K Q S V V A L G S Q E G G L H Q A L A G A I V V E Y S - N S - - - V K L T S G H L K C R L R M D K
DENV1	M V L L T M E K K S W L V H K Q W F L D L P L P W T S G A S T S Q E T W N R Q D L L V T F K T A H A K K Q E V V V L G S Q E G A M H T A L T G A T E I Q T S G T - - - T T I F A G H L K C R L K M D K
DENV2	M V L L Q M E N K A W L V H R Q W F L D L P L P W L P G A D T Q G S N W I Q E T L V T F K N P H A K K Q D V V V L G S Q E G A M H T A L T G A T E I Q M S S G - - - N L L F T G H L K C R L R M D K
DENV3	M I L L T M K N K A W M V H R Q W F F D L P L P W T S G A T T E T P T W N R K E L L V T F K N A H A K K Q E V V V L G S Q E G A M H T A L T G A T E I Q T S G G - - - T S I F A G H L K C R L K M D K
DENV4	M I L M K M K K K T W L V H K Q W F L D L P L P W A A G A D T S E V H W N Y K E R M V T F K V P H A K K R Q D V T V L G S Q E G A M H S A L T G A T E V D S G D G - - - N H M F A G H L K C K V R M E K
ZIKV	L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E A L V E F K D A H A K R Q T V V V L G S Q E G A V H T A L A G A L E A E M D G A K - - - G K L F S G H L K C R L K M D K
YFV	S Y I A E M E K E S W I V D R Q W A Q D L T P W Q S G S G - - - G V W R E M H H L V E F E P P H A A T I R V L A L G N Q E G S L K T A L T G A M R V T K D T N D N N L Y K L H G G H V S R V K L S A

Ruler 1	310 320 330 340 350 360 370 380 390
Consensus	L x L K G x S Y x M C t g k F x F a K e P A x T g H G T v V x e V q Y e G t D g p K I P i s x - a D L n d I T p v G R L x T V N P x V t x s e - - s k V I I E I E P P F G D S Y I V V G v G d k q I k
ILHV	L K I K G T T Y A M C K G T F A F A Q T P V D T G H G T I V A E L T Y T G T D G P C K I P I S M T A D L R D M T P I G R L V T V N P I I P S S A K S Q K I L V E L E P P F G S S F I L V G Q E N N Q I K
ROCV	L K I K G S T Y L M C K D K F A F A K N P V D T G H G T I V E V Q Y A G S D G P C R I P I T M T E N L H D L T P I G R L V T V N P F V P S S E T A Q K I L I E L E P P F G T S F I L V G T G P N Q V K
WNV	L Q L K G T T Y G V C S K A F K F L G T P A D T G H G T V V L E L Q Y T G T D G P C K V P I S S V A S L N D T P V G R L V T V N P F V S V A T A N A K V L I E L E P P F G D S Y I V V G R E Q Q I N
JEV	L A L K G T T Y G M C T E K F S F A K N P A D T G H G T V V I E L S Y S G S D G P C K I P I V S V A S L N D M T P V G R L V T V N P F V A T S S A N S K V L V E M E P P F G D S Y I V V G R G D K Q I N
DENV1	L T L K G M S Y V M C T G S F K L E K E V A E T Q H G T V L V Q V K Y E G T A P C K I P F S S - Q D E K G V T Q N G R L I T A N P I V T D K E - - K P V N I E A E P P F G S Y I V V G A G E K A L K
DENV2	L Q L K G M S Y M C T G K F K V V K E I A E T Q H G T I V I R V Q Y E G D G S P C K I P F E I - M D L E K R H V L G R L I T V N P I V T E K D - - S P V N I E A E P P F G S Y I I G V E P G Q L K
DENV3	L E L K G M S Y A M C L N T F V L K K E V S E T Q H G T I L I K V E Y K G E D A P C K I P F S T - E D G Q G K A H N G R L I T A N P V V T K K E - - E P V N I E A E P P F G S N I V I G I G D K A L K
DENV4	L R I K G M S Y T M C S G K F S I D K E M A E T Q H G T T V V K V Y E G A G A P C K V P I E I - R D V N K E K V V G R I I S S T P F A E Y T N - - S V T N I E L E P P F G D S Y I V I V G D S A L T
ZIKV	L R I K G V S Y S L C T A A F T F T K V P A E T L H G T V T V E V Q Y A G T D G P C K I P V Q M A V D M Q T L T P V G R L I T A N P V I T E S T E N S K M M L E D P P F G D S Y I V I V G D K K I T
YFV	L T L K G T S Y K M C T D K M S F V K N P T D T G H G T V V M Q V K V P - K G A P C K I P V I V A D D L T A A I N K G I L V T V N P I A S T N D - - D E V L I E V N P P F G D S Y I I V G T G D S R L T

Ruler 1	410 420 430 440 450 460 470 480 490
Consensus	x h W H K k G S S I G K a F e t T x x G A q R M A x L G D T A W D F G S V G G V F x S i G K a i H Q V F G x A F r x L F G G m S w i t q i L i G a L L I W I G x N s R n x S x S i t c I A V G G x x L F
ILHV	Y Q W H K T G S T I G N A L K T T W K G A Q R F A V L G D T A W D F G S V G G I F N S I G K T I H G V F G T A F R S L F G G M S W V T Q A L M G A L L L W L G I S A R E R T V S L I M L S V G G I L L F
ROCV	Y Q W H K S G S V I G S A F K T T I K G A Q R M A V L G E T A W D F G S V G G V F N S I G K I H G L F G G A F R T L F G G M S W V T Q A L M G A L L L W L G V S S R E R T V S I T L L A T G G I L L F
WNV	H H W H K S G S S I G K A F T T T L K G A Q R L A A L G D T A W D F G S V G G V F T S V G K A V H Q V F G G A F R S L F G G M S W I T Q G L L G A L L L W M G I N A R D R S I A L T F L A V G G V L L F
JEV	H H W H K A G S T L G K A F S T T L K G A Q R L A A L G D T A W D F G S I G G V F N S I G K A V H Q V F G G A F R T L F G G M S W I T Q G L M G A L L L W M G V N A R D R S I A L A F L A T G G V L V F
DENV1	L S W F K G G S S I G K M F E A T A R G A R R M A I L G D T A W D F G S I G G V F N S I G K I H Q I F G T A Y G V L F S G V S W T M K I G I G L L T W L G L N S R S T L S M T C I A V G M T L Y
DENV2	L N W F K K G S S I G Q M X E T T M R G A K R M A I L G D T A W D F G S L G G V F T S I G K A L H Q V F G A I Y G A A F S G V S W T M K I L I G V I I T W I G M N S R S T L S V S L V L V G V V T L Y
DENV3	I N W Y R K G S S I G K M F E A T A R G A R R M A I L G D T A W D F G S V G G V L N S L G K M V H Q I F G S A Y T A L F S G V S W I M K I G I G V L L T W I G L N S K N T S M S F S C I A I G I T L Y
DENV4	L H W F R K G S S I G K M L E S T Y R G A K R M A I L G E T A W D F G S V G G L T S L G K A V H Q V F G S V Y T T M F G G S W M V R L I G F L V L W I G T N S R N T S M A M T C I A V G D I T L F
ZIKV	H H W H R S G S T I G K A F E A T V R G A K R M A V L G D T A W D F G S V G G V F N S L G K I H Q I F G A A F K S L F G G M S W F S Q I L I G T L L V L W L G L N T K N G S I S L T C L A L G G V M I F
YFV	Y Q W H K E G S S I G K L F T Q T M K G A E R L A V M G D A A W D F S S A G G F F T S V G K I H T V F G S A F Q G L F G G L N W I T K V I M G A V L I W V G I N T R N M T M S M S M I L V G V I M M F

Ruler 1	
Consensus	L g v n V h A
ILHV	L A V N V H A
ROCV	L A M N V H A
WNV	L S V N V H A
JEV	L A T N V H A
DENV1	L G V M V Q A
DENV2	L G V M V Q A
DENV3	L G V V V Q A
DENV4	L G F T V H A
ZIKV	L S T A V S A
YFV	L S L G V G A

Supplementary Figure S2: Alignment of Individual Proteins Across Multiple Flaviviruses. Individual proteins from representative flaviviruses were aligned using the Clustal Omega algorithm in MegAlign Pro v17.2.1 (DNASTAR, Madison, WI). Amino acids are colored by side chain chemistry: aromatic residues (F, W, and Y) are yellow, acidic residues (D and E) are red, basic residues (R, H, and K) are blue, nonpolar residues (A, G, I, L, M, P, and V) are orange, and polar residues (C, N, Q, S, and T) are green. Gaps are represented as dashes. Black rectangles designate cysteine residues predicted to form disulfide bridges.

NS1

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	DtGCAIdwxrkELKCGSGGIFiHNDVeTWxqYKYqPesPxRLAKaIqkAheEGVGIRSVtRLExxMWKqlaxELNaIlEeNgVDLxVVVgdykGiyrG
ILHV	DTGCAIDMARRELKCGSGGIFIHNDVETWRNNYKYHPLTPRGFAKVIQMSKDKGVCIRSVGRLEHEMWEAIAPELNAIFEDNGVDLSVVVGQGTGIYKRA
ROCV	DTGCAIDITRRELKCGSGGIFIHNDVETWRDNYKYHPTPKNFAKIHKAYKEGICGVRASARLEHEMWWKIAPELNAILEDNEVDLSVVVEEHKGIYKKA
WNV	DTGCAIDISRQELRCGSGGVFIHNDVEAWMDRYKYYPETPQGLAKIIQKAHKEGVCGLRSVSRLHEQMWEAVKDELNTLLKENGVDLSVVVEKQEGMYKSA
JEV	DTGCAIDVTRKEMRCGSGGIFVHNDVEAWVDYKYLPETPRSLAKIVHKAHKEGVCGRSVTRLEHQMWEAVRDELNVLLKENAVDLSVVVNKPVGYSRA
DENV1	DSTGGVINWKGRELKCGSGGIFVNEVHTWTEQYKFQADSPKRLSAAIGKAWEEGVCGRSATRLLENIMWKQISNELNHILLENDMKFTVVVGVDVSGILAQQ
DENV2	DSTGGVVSWKRELKCGSGGIFITDNVHTWTEQYKFQPEPSKLSAAIQKAHEEGICGRSVTRLENLMWKQITPELNHILLENEVKLTIIMTGDIKGIMQAG
DENV3	DMGCVINWKGKELKCGSGGIFVNEVHTWTEQYKFQADSPKRLATAIAGAWENGVCGRSTTRMENLLWKQIANELNYILWENNIIKLTVVVGDIITGVLEQG
DENV4	DTGCAVSWSGKELKCGSGGIFVNDNVHTWTEQYKFQPESPARLASAILNAHKDGVCGRISTTRLENIMWKQITNELNYVLWEGGHDLTVVAGDVKGVLSKG
ZIKV	DVGCSVDFSKKETRCGTGVFIYNDVEAWDRYKYHPDSPRRLAAAVKQAWEEGICGIISSVSRMENIMWKSVEGELNAILEENGVDLTVVVGVSVKNPWMRG
YFV	DQGCALINFGKRELKCGSGGIFIFRSDDDLNLKYSYYPEDPVKLASIVKASFEKGKGLNSVDSLEHEMWRSSRADEINAILLEENEVDISVVVQDPKNVYQRG

Ruler 1	110 120 130 140 150 160 170 180 190
Consensus	pKRLtptpdELkyGWkTWGKskifSpExaNsTFxxDGPtKEPTenRAWNSIEVEDfGFGxFtTniWLKvRExyTxxCDsXImGxAVKgnxAVHsDIGY
ILHV	PKRLTETKDEMSFGWKNWGSKFIFSTETANSTFIVDGPESKEPTSDRAWNSLELEDfGFGIISTKIFLKVNEQRGNSCDASAVIGTAVKGNIAVHSDLGy
ROCV	PLRLENTSDEMhFGWKNWGSFLFKPTQMANSTFVVDGPEETPTERRAWNSLEIEDfGFGIMSTKVFLKVNGDKTECDSMVMGTAIKGNRAVHSDLGy
WNV	PKRLTATTEKLEIGWKAWGKSILFAPELANNTFVVDGPETKEPTQNRRAWNSLEVEDfGFGLTSTRMFLKVRESNTTECDSKIIGTAVKNNLAIHSDLSy
JEV	PKRLSMTQEKFEWGKAWGKSILFAPELANSTFVVDGPETKECPDEHRAWNSMQIEDfGFGITSTRVWLKIREESTDECDGAIIGTAVKGHVAVHSDLSy
DENV1	KKMIRPQPMEHKYSWKSWSGKAKIIGADVQNTTFIIDGPNTPEDPNQRAWNSIWEVEDYGFGIFTTNIIWLKLRDSYTQVCDHRLMSAAIKDSKAVHADMGy
DENV2	KRSLXPQPTLKYSWKTWGAKEMLSTESHNTQFTLIDGPTAECPNTNRRAWNSLEVEDYGFGVFTTNIIWLKLRKQDVFCDSKLMSAAIKDNRRAVHADMGy
DENV3	KRTLTPQPMELKYSWKTWGAKEIVTAETQNSSFIIDGPTSTPECPASRAWNSVWEVEDYGFGVFTTNIIWLKLRVYTTQLCDHRLMSAAIKDERAVHADMGy
DENV4	KRALAPPVNDLKYSWKTWGAKEIIFTPKAKNSTFLIDGPDTPKECPNERRAWNSLEVEDYGFGMFTTNIIWMKFRGSSVECDHRLMSAAIKDQKAVHADMGy
ZIKV	PQRLPPVYNELPHGWKAWGKSYYFVRAAKTNSFVVDGDTLKECPLEHRAWNSFLVEDYHGFVFTSNVWLKVRREDYSLECDPAVIGTAVKGRRAHSDLGy
YFV	THPFSSRIIDGLQYGWKTWGNLVSFSPGRKNGSFIIDGKSRKECPFSNRVWNSFQIEEFGTGVFTTRVYMDAVFEYTTIDCDGSILGAAVNGKKSAAHGSPTF

Ruler 1	210 220 230 240 250 260 270 280 290
Consensus	WIESx-KNdTWkLERAxLiEVKsCTWPkSHTLWxxGVxESdxIIPktIAGPxSqHNyRPGYkTQTxGPWHxg-kLEIdfdeCPGTTvtieExGnRGPSI
ILHV	WIESK-KNESWQLERAVLGEVKSCTWPESHTLWGDGYEESDLIIPITLAGPKSHHNMRRPGYKTQTQKPWHEETPLVIEFAECPGTTVTQEESCGGRGPSI
ROCV	WIESG-KNTSWRLERAVLGEVKSCTWPESHTLWNEGVEDSDLIIPPTLGGPRTHHNKREGYKTQLKGPWNEEGPIIEFGCEPGTGTVTQEESCRRRAASA
WNV	WIESR-LNDTWKLERAVLGEVKSCTWPETHLWGDGILESDLIIPVTLAGPNSHHNRPGYKTQNGQPWDEG-RVEIDFDYCPGTTVTLSESCGHRGPAT
JEV	WIESR-YNDTWKLERAVLGEVKSCTWPETHLWGDGYEESDLIIPHTIAGPKSKHNRREGYKTQNGQPWDEN-GIVLDFDYCPGTTVTITEDCGKRGPSV
DENV1	WIESE-KNETWKLARASFIEVKTCIWPKSHTLWSNGVLESEMIIPKIYGGPISQHNRRPGYHTQTAGPWHLG-KLELDFDCEGTTVVVDEHCGNRGPSL
DENV2	WIESA-LNDTWKIEKASFIEVKSCHWPKSHTLWSNGVLESEMIIPKNFAGPVPSQHNRRPGYHTQTAGPWHLG-KLEMDFFCEGTTVVVTEDCGNRGPSL
DENV3	WIESQ-KNGSWKLEKASLIEVKTCTWPKSHTLWSNGVLESDMIIPKSLAGPISQHNRRPGYHTQTAGPWHLG-KLELDFNCEGTTVVVISENCGTRGPSI
DENV4	WIESS-KNQTWQIEKASLIEVKTCLWPKTHTLWSNGVLESQMLIPKAYAGPFSQHNRRPGYHTQTAGPWHLG-KLEIDFGCEPGTTVTIQEDCDHRGPSL
ZIKV	WIESE-KNDTWRLKRAHLIEMKTCEWPKSHTLWTDGYEESDLIIPKSLAGPLSHHNTREGYRTQVKGPHSE-ELEIRFECEPGTKVYVEETCGTRGPSL
YFV	WMGSHEVNGTWMIIHTLEALDYKECEWPLTHTI-GTSVEESEMFMPRSIGGPVSSSHNIPGYKVQTNGPWWQV-PLEVEREACPGTSVIIDGNCDDGRGKST

Ruler 1	310 320 330 340 350
Consensus	RTTTASGKIItEWCRSCSTLPLPLRFrgEDGQWYGMERPxkEkEENLVkSgVxA
ILHV	RTTTASGRITRDWCKNCTLPLPLRFMAAGENCWYGMERPRKRENEETLIKSKVSA
ROCV	RTTTASGKVIIRDWCKNCTMPPLRFTTKNGCWYGMERPKHESEETLIKSKVTA
WNV	RTTTESGKLIIDWCCRSCSTLPLPLRYQTDSGCWYGMERPRQRHDEKTLVQSQVNA
JEV	RTTTDSGKLIIDWCCRSCSLPLPLRFRTENGCWYGMERPRVRHDETTLVRSQVDA
DENV1	RTTTVTGKTIHEWCCRSCSTLPLPLRFKGEDGCWYGMERPVKEKEENLVKSMVSA
DENV2	RTTTASGKLIIEWCCRSCSTLPLPLRYRGEDGCWYGMERPLKEKEENLVNSLVTA
DENV3	RTTTVSGKLIIEWCCRSCSTLPLPLRYMGEDGCWYGMERIPINEKEENMVKSLSASA
DENV4	RTTTASGKLVITWCCRSCSTMPPLRFLGEDGCWYGMERPLPSEKEENMVKSQVSA
ZIKV	RSTTASGRVIEEWCCRSCSTMPPLSFRAKDGCWYGMERPRKPEENSLVRSMVTA
YFV	RSTTDSGKLIIEWCCRSCSTMPPVFSFHSGDGCWYPMERIPRKTHESHLVRSWVTA

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NS2A

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	- g g g x i D p F x L G L L x m f l f t x E V L R k R w T a K x x i p x v l x l L x v l i l G g x T y x D L x R y v l l V G A a F a E - N s G G D V t H L A L i A a F K i q P a f l V g f f L R k - W T
ILHV	G N G Q T I E P F Q L G I L M A F V F T Q E V L R R R W T A N L A L P T S A L L M A C F I F G G F T Y L D L F R Y F I L V G A A F A E A N S G G D V V H L A M I A A F N I Q P A L V T T F F R K N W T
ROCV	G T G N D I C R F Q L G L L M A F V F T Q E V L R K R W T A R L A L P T A A L L A C F V L G A F T Y S D M I R Y F V L V G C A F A E S N S G G D V I H L A L I A V F N I Q P A A L V S T F F R N R W T
WNV	Y N A D M I D P F Q L G L L V F L A T Q E V L R K R W T A K I S M P A I L I A L L V L V F G G I T Y T D V L R Y V I L V G A A F A E S N S G G D V V H L A L M A T F K I Q P V F M V A S F L K A R W T
JEV	F N G E M V D P F Q L G L L V M F L A T Q E V L R K R W T A R L T I P A V L G A L L V L M L G G I T Y T D L A R Y V V L V A A F A E A N S G G D V L H L A L I A V F K I Q P A F L V M N M L S T R W T
DENV1	- G S G E V D S F S L G L L C I S I M I E E V M R S R W S R K M L M T G T L A V F L L T M G Q L T W N D L I R L C I M V G A N A S D - K M G M G T T Y L A L M A T F R M R P M F A V G L L F R R - L T
DENV2	- G H G Q I D N F S L G V L G M A L F L E E M L R T R V G T K H A I L L V A V S F V T L I T G N M S F R D L G R V M V M V G A T M T D - D I G M G V T Y L A L L A A F K V R P T F A A G L L L R K - L T
DENV3	- G S G K V D N F T M G V L C L A I L F E E V M R G K F G K K H M I A G V L F T F V L L S G Q I T W R D M A H T L I M I G S N A S D - R M G M G V T Y L A L I A T F K I Q P F L A L G F F L R K - L T
DENV4	- G Q G T S E T F S M G L L C L T L F V E E C L R R V T R K H M I L V V V T L C A I I L G G L T W M D L L R A L I M L G D T M S G - R M G - G Q I H L A I M A V F K M S P G Y V L G I F L R K - L T
ZIKV	G S T D H M D H F S L G V L V I L L M V Q E G L K K R M T T K I M S T S M A V L V M I L G G F S M S D L A K L V I L M G A T F A E M N T G G D V A H L A L V A A F K V R P A L L V S F I F R A N W T
YFV	- - - G E I H A V P F G L V S M M I A M E V V L R K R Q G P K Q M L V G G V V L L G A M L V G Q V T L D L L K L T V A V G L H F H E M N N G G D A M Y M A L I A A F S I R P G L L I G F G L R T L W S

Ruler 1	110 120 130 140 150 160 170 180 190
Consensus	x R E n I L L x x G a A m x q m A - s d l p - - - i x l m e l l n x i A L a w M i L K A v T f f t t s q l a m P l A L L T P p x r x l l l - - d a w R x l l l i G v v s l i p x x x s s a k K k
ILHV	N R E N M I L I I A A A C T Q M A C M E L K - - - I E L F H V M N S L S L A W M I L K A L T T G T T S T A M P F L A A L S P P M N W L G L - - D V V R C L L I M A G V A A L I S E R R E S L A K K K
ROCV	N R E N L L V I A A A A Q M A W S D V G - - - I E I M P I M N A M A L W M I L K A V S I G T V S T I A M P I L S G L A P P M E W F G L - - D V L R C L L I V G V A A L I K E R K E N L A K K K
WNV	N Q E N I L L M L A A V F F Q M A Y H D A R Q I L L W E I P D V L N S L A V A W M I L R A I T F T T T S N V V V P L L A L L T P G L R C L N L - - D V Y R I L L M V G I G S L I R E K R S A A A K K K
JEV	N Q E N V V L V L G A A F F Q L A S V D L Q - - - I G V H G I L N A A A I A W M I V R A I T F P T T S S V T M P V A L L T P G M R A L Y L - - D T Y R I L L V I G I C S L L Q E R K K T M A K K K
DENV1	S R E V L L L T V G L S L V A S - - V E L P - - - N S L E E L G D G L A M I M M L K L L D F Q S H Q L W A T L L S L T F V K T T F S L H - - Y A W K T M A M I L S I V S L F P L C L S T T S Q K -
DENV2	S K E L M M T T I G I V L L S Q - - S T I P - - - E T I L E L T D A L A L G M M V L K M V R K M E K Y Q L A V T I M A I L C V P N A V I L Q - - N A W K V S C T I L A V V S V S P L F T S S Q Q K -
DENV3	S R E N L L L G V G L A M A A T - L R L P - - - E D I E Q M A N G I A L G M A L K L I T Q F E Y Q L W T A L V S L T C S N T I F T L T - - V A W R T A T L I L A G I S L L P V C Q S S M R K -
DENV4	S R E T A L M V I G M A M T T V - L S I P - - - H D L M E F I D G I S L G L I L L M V T H F D N T Q V G T L A L S L T F I R S T M P L V - - M A W R T I M A V L F V V T L I P L C R T S C L Q K Q
ZIKV	P R E S M L L A L A S C L L Q T A I S A L E - - - G D L M V L I N G F A L A W L A I R A M A V P R T D N I A L P I L A A L T P L A R G T L L - - V A W R A G L A T C G G I M L L S L K G K G S V K K N
YFV	P R E R L V L T L G A A M V E I A L G G M M - - - G G L W K Y L N A V S L C I L T I N A V A S R K A S N T I L P L M A L L T P V T M A E V R L A T M L F C T V V I I G - - V L H Q N S K D T S M Q K T

Ruler 1	210 220 230
Consensus	g a w L p x i a l a - t G x f s P l - l x x l i x l t r x n k K R
ILHV	G A L L I S A A L A L T G A F S P L V L Q G A L M F T Q S L G K R
ROCV	G A L L I S A G L A L T G A F S P L V L Q G A L M L S E C A T K R
WNV	G A S L L C L A L A S T G L F N P M I L A A G L I A C D P N R K R
JEV	G A V L L G L A L T S T G W F S P T T I A A G L M V C N P N K K R
DENV1	T T W L P V L L G S - L G C - K P L - T M F L I T E N K I W G R K
DENV2	A D W I P L A L T I - K G L - N P T - A I F L T T L S R T N K K R
DENV3	T D W L P M T V A A - M G V - P P L - P L F I F S L K D L K R R
DENV4	S H W V E I T A L I - L G A - Q A L - P V Y L M T L M K G A S K R
ZIKV	L P F V M A L G L T A V R V D P I N V V G L L L L T R S G K R -
YFV	I P L V A L T L S Y L G L T Q P F L G L C A F L A T R I F G R R

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NS2B

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	SWPx nEv l xAVGLv saLAGsLAKxDip -MAGPl aagGLL i v xYVxSGkSxDxwLE RAADV sWE e eAE I TG sSpRLDVeLDdDGDfKxxxdegvPm l t w l l
ILHV	GWPASEVLTA VGMTFALAGSVARLDGGTMAIPLATMAI LAVAYVLSGKSTDMWLE RCADISWINEAEITGTSPrLDVVELDNGDfKMINDPGVPMWMTWC
ROCV	GWPASEVLTA IGMTIALAGSVARLDsGTMAIPLATTSILFVS YVLSGKSTDMWIERTADISWESDAEITGSSERVDVRLDDDGfQLMNDPGAPWKIWMML
WNV	GWPATEVMTAVGLMFAIVGGLAELDIDSMAIPMTIAGLMFAAFVVISGKSTDMWIERTADISWESDAEITGSSERVDVRLDDDGfQLMNDPGAPWKIWMML
JEV	GWPATEFLSAVGLMFAIVGGLAELDIESMSIPFMLAGLMAVS YVVS GKATDMWLE RAADISWEMDAAITGSSRRLDVKLDDDGfDfHLIDDPGVPWKVWVVL
DENV1	SWPLNEGIMAVGIVSILLSSLLKNDVP -LAGPLIAGGMLIACYVISGSSADLSLEKAAEVSWEEAEHSGASHNILEVVEQDDGTMKIKDEERDDTLTILL
DENV2	SWPLNEA IMAVG MVSI LASSLLKNDIP -MTGPLVAGGLLTVCYVLTGRSADLELE RAADV KWEDQAEISGSSPILSITISEDGSMSIKNEEEEQILTILL
DENV3	SWPLNEGIMAVGLVSI LASSLLRNDVP -MAGPLVAGGLLTVCYVLTGTSADLTVEKAADV TWEEAEQTGVSHNLMITVDDDGTMRIKDDETENILTVLL
DENV4	SWPLNEGIMAVGLVSI LASSLLKNDVP -LAGPMVAGGLLLAAYVMSGSSADLSLEKAAENVQWDEMADITGSSPIIEVKQDEDDGSFSIRDIEETNMITLLV
ZIKV	SWPPSEVLTA VGLICALAGGFAKADIE -MAGPMAAVGLLIVSYVVS GKSVDMYIERAGDITWEKDAEVTGNSPrLDVALDESGDfSLVEEDGPPMREIIL
YFV	SIPVNEALAAAGLVGVLAG -LAFQEMENFLGPIAVGGILMMLVSVAGRV DGLLELKKLGEVSWEEAEISGSSARYDVALSEQGEfKLLSEEKVPWDQVVM

Ruler 1	110 120 130
Consensus	rmgLLa i s x x nPva l P v t x a g W x m x x K k t k R
ILHV	RMGLMAMAA YNPVLIPVSMAGYWM TVKIHKR
ROCV	RMGLMCAA YNPVLIPVSVAGYWM TRKIHKR
WNV	RMVCLAISAYTPWAILPSVVGFWITLQYT KR
JEV	RMSCIGLAALTPWAI VPAAFGYWLTLKTTKR
DENV1	KATLLAISGVYPMSIPATL FVWYFWQKKKQR
DENV2	RTGLLVISGLFPVSIPI TAAAWYLVEVKKQR
DENV3	KTALLIVSGIFPYSIPATLLVWHTWQKQTQR
DENV4	KLALITVSGLYPLAIPVTMTLWYMWQVK TQR
ZIKV	KVVLMAICGMNP I AIPFAAGAWYVYVKTGR
YFV	TS LALVGAAIHPFALLLVLAGWLFHVRGAR R

Supplementary Figure S2: Alignment of Individual Proteins Across Multiple Flaviviruses. Individual proteins from representative flaviviruses were aligned using the Clustal Omega algorithm in MegAlign Pro v17.2.1 (DNASTAR, Madison, WI). Amino acids are colored by side chain chemistry: aromatic residues (F, W, and Y) are yellow, acidic residues (D and E) are red, basic residues (R, H, and K) are blue, nonpolar residues (A, G, I, L, M, P, and V) are orange, and polar residues (C, N, Q, S, and T) are green). Gaps are represented as dashes. Black rectangles designate cysteine residues predicted to form disulfide bridges.

NS3

Ruler 1	1102030405060708090
Consensus	-gGV LWDPSPK EvgKa-eL t dGVYRIMq rGxLG syQvGVGVmqEGVFHTMWHVTRGAaLmxgeGRLEPsWxSVKxDLISYGGGWKLs akWxGgEEVQvI
ILHV	-GGVMWDVPAPKQFGKT-ELKPGVYRVMTMGLGRYQSGVGVMWDGVFHTMWHVTTQGAALRNGEGRLNPTWGSVRDDLI SYGGKWKL SATWNGSEEVQMI
ROCV	-GGVLWDLPAPKQMGRS-DMKPGVYRVMTSGVLGSYQSGVGVMYDGVFHTMWHVTTQGAALRNGEGRLNPTWGSVRDDLI TYGGKWKL SATWDGTEEVQLI
WNV	-GGVLWDTPSPKEYKKG-DTTTGVYRIMTRGLLGSYQAGAGVMVEGVFHTLWHHTTKGAALMSGEGRLDPYWGVSVKEDRLCYGGPWWKLQHKWNGQDEVQMI
JEV	-GGVFWDTSPSPKPCSKG-DTTTGVYRIMARGILGT YQAGVGVMYENVFHTLWHHTTRGAAIMSGEGKLT PYWGSVKEDRIAYGGPWRFRDRKWNGTDDVQVI
DENV1	-SGVLWDTPSPPEVERA-VLDDGIYRILQRGLLGRSQVGVGVFQEGVFHTMWHVTRGAVLMYQGKRLPSWASVVKKDLISYGGGWRFGGSWNAGEEVQVI
DENV2	-AGVLWDVPSPPPVKGKAELEDGAYRIKQKGLIGYSQIAGGVYKEGTFHTMWHVTRGAVLMHKGKRIEPSWADVKKDLISYGGGWKLEGEWKEGEDEVQVL
DENV3	-SGVLWDVPSPPTTQKA-ELLEGVYRIKQQGIFGKTQVGVGQKEGVFHTMWHVTRGAVLTHNGKRLPENWASVVKKDLISYGGGWRLSAQWQKGEEVQVI
DENV4	-SGALWDVPSPAAQA-KLTEGVYRIMQRGLFGKTQVGVIHMEGVFHTMWHVTRGVSICHETGRLEPSWADVRNDMISYGGWRLGDKWDKEEDVQVL
ZIKV	-SGALWDVPAPKEVKKG-ETTDGVYRVMTRRLLGSTQVGVGVMQEGVFHTMWHVTKGAALRSGEGRLDPYWGVDVKQDLVSYCGPWWKLDAAWDGLSEVQLL
YFV	SGDVLWDIPTPKIIEECEHLEDGIYGI FQSTFLGASQRGVGVAQGGVFHTMWHVTRGAFLVRNGKKLIPSWASVKEDLVAYGGSWKLEGRWDGEEEVQLI

Ruler 1	110120130140150160170180190
Consensus	AVEPGKNpKNvQTKPGvFKTp-aGEIGAVxLDFPpGTSGPSI xNknGkVIGLYGNGVVtksGsYVSAI xQgErQeEgpp ee-fePeMLRKRqLTI LDLHP
ILHV	AVEPGKAAKNYQTKPGVFKTP-AGEIGAITLDFPKGTSGPSI INKAGEIIGLYGNGLVLASGAYVSAITQGERQEEETPEA-FTPDMLKKRRLTILDLHP
ROCV	AAEPGKPVKNFQTRPGVFKTP-AGEVGAITLDFPKGTSGPSI VNKAGAVIGLYGNGLVLSGAYVSAISQGERQEEEAPEA-FTPEMLRKRQLTILDLHP
WNV	VVEPGKNVKNVQTKPGVFKTP-EGEIGAVTLDFPTGTSGSPI VDKNGDVI GLYGNGVIMPNGSYISAIVQGERMDEPI PAG-FEPEMLRKKQITVLDLHP
JEV	VVEPGKAAVNIQTKPGVFRTF-FGEVGAVSLDYPRGTSGPSILD SNGDIIGLYGNGLVLDGGSYVSAIVQGRQEPEVPEA-YTPNMLRKRQMTVLDLHP
DENV1	AVEPGKNPKNVQTAPGTFTKTP-EGEVGAIALDFKPGTSGPSI VNRGKIVGLYGNGVVTS GTYVSAIAQAKASQEGPLPE-IEDEVFRKRNLTIMDLHP
DENV2	AL EPGKNPRAVQTKPGLF KTN-AGTIGAVSLDFS PGTSGPSI IDKKGKVVGLYGNGVVTRSGAYVSAIAQTEKSI E-DNPE-IEDDIFRKRRLTIMDLHP
DENV3	AVEPGKNPKNFQTMPGIFQTT-TGEIGAIALDFKPGTSGPSI INREGKVVGLYGNGVTKNGGYVSGIAQTNAEPDGP TPE-LEEEMFKKRNLTIMDLHP
DENV4	AIEPGKNPKHVQTKPGLF KTL-TGEIGAVTLDFKPGTSGPSI INRKGKVI GLYGNGVVTKSGDYVSAITQAERTGE-PDY E-VDEDIFRKRRLTIMDLHP
ZIKV	AVPPGERARNIQTLPGIFKTK-DGDIGAVALDYPAGTSGPSILD KCGRVI GLYGNGVVINKGSYVSAITQGKREEETPVEC-FEPSMLKKKQLTVLDLHP
YFV	AAVPGKNVNVQTKPSSLFKVRNGGEIGAVALDYPGTSGPSI VNRNGEVI GLYGNGLIVGDNSVSAISQTEVKEEGKEELQEIPTMLKKGMTILDFHP

Ruler 1	210220230240250260270280290
Consensus	GAGKTRRxLPqIVREAIKRRRLRTIILAPTRVVAAEMaEALRGLPIRYQTsAVkaEHxGrEIVDIMCHATxTmRLLSPPvRVPNYNLixMDEAHFTDPASIA
ILHV	GAGKTRRVIPQIVRECVKARLRTVILAPTRVVAAEMAEALRGLPIRYQTS AVKA EHS GNEIVDVMCHATLTQRL LTPAKVPNYNVFVMD EAHFTDPASIA
ROCV	GAGKTRRVIPQIVREAVKQRLRTVILAPTRVVAAEIAEALRGLPVRFQTS AVKA EHS GTEIVDVMCHATLTQRLMT PMRVPNYNV FVMD EAHFTDPASIA
WNV	GAGKTRRI LPQIIKEAINRRRLTAVLAPTRVVAAEMAEALRGLPIRYQTS AVPREHNGNEIVDVMCHATLTHRLMSPHRVPNYNLFVMD EAHFTDPASIA
JEV	GSGKTRKILPQIIKDAIQQLRTAVLAPTRVVAAEMAEALRGLPVRYQTS AVQREHQGNEIVDVMCHATLTHRLMSPNRVPNYNLFVMD EAHFTDPASIA
DENV1	GSGKTRRYLPAIVREAIRNVRTLVLAPTRVVASEMAEALKGMP IRYQT TAVKSEHTGKEIVDLMCHATFTMRL LSPVRVPNYNMIIMDEAHFTDPASIA
DENV2	GAGKTKRYLPAIVREAIKRGLRTLILAPTRVVAAEMEEALRGLPIRYQT PAIRAEHTGREIVDLMCHATFTMRL LSPVRVPNYNLIIMDEAHFTDPASIA
DENV3	GSGKTRKYLPAIVREAIKRRRLRTLILAPTRVVAAEMEEALKGLPIRYQT TATKSEHTGREIVDLMCHATFTMRL LSPVRVPNYNLIIMDEAHFTDPASIA
DENV4	GAGKTKRILPSIVREALKRRRLRTLILAPTRVVAAEMEEALRGLPIRYQT PAVKSEHTGREIVDLMCHATFTTTRL LSTTRVPNYNLIVMDEAHFTDPSSXA
ZIKV	GAGKTRRLPEIVREAIKRRRLRTVILAPTRVVAAEMEEALRGLPIRYQMTAVNTHSGREIVDLMCHATFTSLRLQPIRVPNYNLIIMDEAHFTDPSSIA
YFV	GAGKTRRFLPQILAECARRRLRTLVLAPTRVVLS EMK EAFHGLDVKFHTQAFSAHSGS GREVIDAMCHATLT YRMLEPTRVVNWEVIIMDEAHFLDPASIA

Ruler 1	310320330340350360370380390
Consensus	ARGYISTRVEmGEAAAI FMTATPPGTtDPPFxSNaPI xDeErEI PeRaWNSGfEWITDyTGKTVWFVPSxKaGNeIaXcLRKAGKKV IQLxRKTFDTEYq
ILHV	ARGYISTKVELGEAAAI FMTATPPGTTDPPFDSNAPIIDQEAEIPDRAWNSGF EWITEYTGKTVWFVPSVRMGNEIAMCLTKAGKKV IQLNRKSYDSEYQ
ROCV	ARGYISTKVESGEAAAI FMTATPPGTIDPPFDSNSPIIDQEAEIPDRAWNSGF EWITDYTGKTVWFVPSVRSNGNEIAMCLTKAGKKV IQLNRKSYETEYQ
WNV	ARGYISTKVELGEAAAI FMTATPPGTSDFPFSNSPIISDLQTEIPDRAWNSGYEWITEYTGKTVWFVPSVKMGNEIALCLQRAGKKV VQLNRKSYETEYP
JEV	ARGYIATKVELGEAAAI FMTATPPGTTDPPFDSNAPIHDLQDEIPDRAWSSGYEWITEYAGKTVWFVVASVKMGNEIAMCLQRAGKKV IQLNRKSYDTEYP
DENV1	ARGYISTRVGMGEAAAI FMTATPPGSVEAFPQSNNAVIQDEERDIPERSWNSGYDWITDFPGKTVWFVPSIKSGNDIANCLRKNGRKKVQLSRKTFDTEYQ
DENV2	ARGYISTRVEMGEAAGI FMTATPPGSRDPFQSNAPIIMDEEREIPERSWSSGHEWVTD FKGT VWFVPSIKAGNDIAACL RKNGKKV IQLSRKTFDSEYV
DENV3	ARGYISTRVGMGEAAAI FMTATPPGTADAFPQSNAPIQDEERDIPERSWNSGNEWITDFVGKTVWFVPSIKAGNDIANCLRKNNGKKV IQLSRKTFDTEYQ
DENV4	ARGYISTRVEMGEAAAI FMTATPPGTDPPFQSNNSPIIDEREIPERSWNTGFDWITDYQGKTVWFVPSIKAGNDIANCLRKNKSGKKV IQLSRKTFDTEYP
ZIKV	ARGYISTRVEMGEAAAI FMTATPPGTRDAFPDSNSPIMDTEVEVPERAWSGFDWVTDHSGKTVWFVPSVRNGNEIAACLTKAGKRV IQLSRKTFETEFQ
YFV	ARGWAAHRRANESATILMTATPPGTSDEFPHSNGEIEDVQTDIPSEPWNTGHDWILADKRPTAWFLPSIRAANVMAASLRKAGKSVVVLNRKTFEREYP

Ruler 1	410420430440450460470480490
Consensus	KtKnnDWDfFvTTDIS EGMANFkAdRVIDxRrCxKPVILtdGeeRVILaGpMPVTaSA460AQQRRRIGRNPnqeGDeYiYgGpxIedDedHAHWtEAKMLL
ILHV	KCKGNDWDFVITTDISEMGANFGAHRVIDSRCKVKPVIL-DGDDRVLMNGPAPITPASAAQRRRIGRDPQTQSGDEYFYGGPTTTDDTGHAHWIEAKILL
ROCV	KCKGNDWDFVVTDDISEMGANFGAHRVIDSRCKVKPVIINDGEGRVQLNGPLITASSAAQRRRGVRGRDPTQSGDEYFYGGPI TNDTGHAHWIEAKMLL
WNV	KCKNDWDVFVITTDISEMGANFASRVIDSRSVKPTIITEGEGRVILGEP SAVTASAAQRRRIGRNPQSQSGDEYCYGGHTNEDDSNFAHWTEAKIML
JEV	KCKGNDWDFVITTDISEMGANFGASRVIDCRKSVKPTILEEGEGRVILGNPSPITSA460AQQRRRGVRGNPNQVGDEYHYGGATSEDDSNLAHWTEAKIML
DENV1	KTKNNDWDFVVTDDISEMGANFRADRIDPRRCLKPVILKDGPERVILAGPMPVTVASAAQRRRIGRNPQKQEGDQYIYMGQPLNND EDAHWT EAKMLL
DENV2	KTRTNDWDFVVTDDISEMGANFKADRIDPRRCMKPVILT DGEERVILAGPMPVTHSSAAQRRRIGRNPKNENDQYIYMGEPLENDEDCAHWKEAKMLL
DENV3	KTCLNDWDFVVTDDISEMGANFKADRIDPRRCLKPVILTNGPERVILAGPMPVTVASAAQRRRGVRGNPQKENDQYIFMGQPLNND EDAHWT EAKMLL
DENV4	KTKLTDWDFVVTDDISEMGANFRAGRVIDPRRCLKPVILTDGPERVILAGPIPVTPASAAQRRRIGRNPQAQEDDQYVFGDPLKNDEDAHWT EAKMLL
ZIKV	KTKNQEWDFVITTDISEMGANFKADRIDSRRCLKPVILD--GERVILAGPMPVTHSSAAQRRRIGRNPKNKPGDEYMYGGGCAETDEGHAWLEARMLL
YFV	TIKQKKPDFILATDIAEMGANLCVERVLDCRTAFKPVLVDEG-RKVAIKGPLRISASSAAQRRRIGRNPNRDGDSEYYS EPTSEDNAHHVCWLEASMLL

Ruler 1	510520530540550560570580590
Consensus	DNIntPxGI IIAqLYgPEREKvAiDGEYRLRGEqRKfFVELMRrGDLPVWLxYKVA sAGI xYtDRRWCFDGP rNNxILEDNmEV-EIWTkeGExKkLRPR
ILHV	DN IQLQNLGLVAQLYGPREDKVFTT DGEYRLRGEQKKNFVEFLRTGDLPVWL SYKVAEAGYAYTD RRRWCFDGPANNTILEDNNEV-EIWT RQGEKRILRPR
ROCV	DN IQLQNLGLVAQLYGPREDKVFTT DGEYRLRGEQKKHFDVFLMRGTGDLPVWL SYKVAEAGIYTD RRRWCFDGPWNNTILEDNTEV-EIWT RQGERKVL RPR
WNV	DN I NMPNGLIAQFYQPEREKVYTMDGEYRLRGEERKNFLELLRTADLPVWLAYKVAAAGVSYH DRRWCFDGPRTNTILEDNNEV-EVITKLGERKILRPR
JEV	DN I HMPNGLVAQLYGPEREKAL TMDGEYRLRGEQKKNFLELLRTADLPVWLAYKVASNGI QYTD RKWCFDGPRTNAILEDNTEV-EIVTRMGERKILKPR
DENV1	DN INTPEGIIPALFEPEREKSAIDGEYRLRGEARKTFVELMRGDLPVWL SYKVASGEGFYSDRRWCFDGERNNQVLEENMDV-EIWTKEGERKKLRPR
DENV2	DN INTPEGIIPSMFEPEREKVDAIDGEYRLRGEARKTFV DLMRRGDLPVWLAYRVAAEGIN YADRRWCFDGI KNNQIILEENVEV-EIWTKEGERKKLKPR
DENV3	DN INTPEGIIPALFEPEREKSAIDGEYRLRGESRKTFVELMRGDLPVWL AHKVASG I KYTD RKWCFDGERNNQIILEENMDV-EIWTKEGEKKLRPR
DENV4	DN IYTPGEGIIPTLFGPEREKNSAIDGEFRLRGEQRKTFVELMRGDLPVWL SYKVASAGISYKDRWCFGTGERNNQIILEENMEV-EIWTREGEKKLRPK
ZIKV	DN IY LQDGLIASLYRPEADKVAIEGEFKLRTEQRKTFVELMRGDLPVWLAYQVASAGI TYTD RRRWCFDGTNTNTIMEDSVPA-EVWT KYGEKRVLKPR
YFV	DNMEVRGGMVAPLYGVEGKT PVS PGEMRLRDDQRKYFRELVRNCDLPVWL SWQVAKAGLKTND RKWCFEGPPEEHEILNDSGETVKCRAPGGAKKPLRPR

Ruler 1	610620
Consensus	WIDARVYS Dp qALKEfKEFAAGxR
ILHV	WSDARVYCDNQALRSFK EFAAGKR
ROCV	WSDARVYS DNQALRAFKEFAAGKR
WNV	WIDARVYS DHQALKAFKDFASGKR
JEV	WLDARVYADHQALKWFKDFAAGKR
DENV1	WLDARTYS DPLALREFKEFAAGRR
DENV2	WLDARTIYS DPLALKEFK EFAAGRK
DENV3	WLDARTIS DPLALKEFKDFAAGRK
DENV4	WLDARVYADPMALKDFKEFASGRK
ZIKV	WMDARVCS DHAALKSFKEFAAGKR
YFV	WCDERVSSDQSALSEFIKFAEGRR

Supplementary Figure S2: Alignment of Individual Proteins Across Multiple Flaviviruses. Individual proteins from representative flaviviruses were aligned using the Clustal Omega algorithm in MegAlign Pro v17.2.1 (DNASTAR, Madison, WI). Amino acids are colored by side chain chemistry: aromatic residues (F, W, and Y) are yellow, acidic residues (D and E) are red, basic residues (R, H, and K) are blue, nonpolar residues (A, G, I, L, M, P, and V) are orange, and polar residues (C, N, Q, S, and T) are green). Gaps are represented as dashes. Black rectangles designate cysteine residues predicted to form disulfide bridges.

NS4A and 2K

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	-sAlxli evlGRIPdhtxKtrxALDNLyVLxTaEkGGRAYRhALEELPxTLETxxLi alIgvxTgGxFIfIMxxKGIGKxgIGxlvIxaaxxLLWmAeV
ILHV	-SAGSVMEVVMGRMPDYFWTKTLNAADNLVVLATANKGGRAHQAALEELPDVTETILLMTMMCVASLGMFALMVHRRGLGKTGLGTLVLATVTVLLWISDV
ROCV	-SAGSMMDVVMARMPDYFWTKTMNAADNLVVLATTEKGGRAHRAALEELPDLTETVLLIAMMSLASCGMLALMMQRKGIGKTGMGTAVLTAVTILLWMADV
WNV	-SQIGLIEVLGKMPHFHMGKTWEALDTMYVVATAEKGGRAHRMALEELPDALQTIALIALLSVMTMGVFFLLMQRKGIGKIGLGGAVLG VATFFCWMAEV
JEV	-SAVSFIEVLGRMPFHFHMGKTREALDTMYLVATAEKGGKAHRMALEELPDAL ETITLIVAIVMTGGFFLLMMQRKGIGKMG LGALVLT LATFFLWAAEV
DENV1	SVSGDLILEIGKLPHLTQRAQNALDNLVMLHNSEQGGKAYRHAMEELPDITETLMLLALIAVL TGGVTLFFLSGRGLGKTSIGLLCVIASSALLWMA SV
DENV2	SLTLNLITETMGR LPTFTMQKARDALDNLAVLHTAEAGGRAYNHALSELPETLETLLLLTLLATVTGGIFLFLMSGRGIGKMTLGMCCIITASILLWYAQI
DENV3	SIALDLVTEIGRVP SHLAHRTRNALDNLVMLHTSEHGGRAYRHAVEELPETMETLLLLGLMILLTGGAMLFLISGKGIGKTSIGLICVIASSGMLWMADV
DENV4	SITLDILTEIASLPTVLYSSRAKLALDNLVMLHTTERGGRAYQHALLPESETLMLVALLGAMTAGIFLFFMQGKGIGKLSMGLIAIAVASGLLWVAEI
ZIKV	GAALGVMEALGTLPGHMTERFQEAIDNLAVLMRAETGSRPYKAAAAQLPETLETIMLLGLLGTVSLGIFFFVLMRNKGIGKMGFGMVTLGASAWLMWLSEI
YFV	-GAAEVLVVLSELDPFLAKKGGEAMD TISVFLHSEEGSRAYRNALSMMP EAMTIVMLFILA GLLTSGMVIFFMSPKGISRMSMAMGT MAGCGYLMFLGGV

Ruler 1	110 120 130 140
Consensus	pptxIAGxllleFILMVVLIPEPEKQRS pQDNQLAyxIliclTLvg aVAA
ILHV	PAPKIAGVLLIAFLLMIVLIPEPEKQRSQTDNHLAIFLVCVLLLIGAVSA
ROCV	PAPKIAGVLLISFLLMIVLIPEPEKQRSQTDNHLAVFLICALLLVSAVSA
WNV	PGTKIAGMLLLSLLMIVLIPEPEKQRSQTDNQLAVFLICVMTLVSAVAA
JEV	PGTKIAGTLLIALLLMVVLIPEPEKQRSQTDNQLAVFLICVLT TVVGMVAA
DENV1	EPHWIAASIIIEFFLMVLLIPEPDQRTPQDNQLAYVVIGLLFMILTAA
DENV2	QPHWIAASIIIEFFLVLLIPEPEKQRTTPQDNQLTYVVIAILTVVAATMA
DENV3	PLQWIAASIVLEFFMMVLLIPEPEKQRTTPQDNQLAYVVIGILTAAIVAA
DENV4	QPQWIAASIIIEFFLMVLLIPEPEKQRTTPQDNQLIYVILTILTIIGLIAA
ZIKV	EPARIACVLIVVFLLLLVLLIPEPEKQRS pQDNQMAIIMVAVGLLGLITA
YFV	KPTHISYIMLIFFFVLMVVLIPEPGQRS IQDNQVAYLIIGILT LVSVVAA

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NS4B

Ruler 1	1 10 20 30 40 50 60 70 80 90
Consensus	NEMGWLeXtTKDLGxIfggr-e---we-sxIDIDLPAAsAwTLyAvATTVLTPmLRHIIItNsYxNxSLtAlAnQAsvLfGLxKGwPFxkmDLgVpLL
ILHV	NEMGWLETTTKKDIGKLFRRSSGDTQEQSTWQSW--APEVRAATAWAGYAGLTVFLTPLFRHLITTTQYVSFSLTAITAQASALFGLSAGYPFVGI DLAVGFL
ROCV	NEMGWLDTTKRDIGKLFSGPSAV-TTSRWELPKLALALKPATAWAGYAGMTMLTLPFLRHLITTTQYVSFSLTAITSQASALFGLNSGYPFVGVDL SVVFL
WNV	NEMGWLDKTKSDISSLFGRIEVKENFSM--GEFLDLRPAWASLYAVTTAVLTPLKHLITSDYINTSLTSINVQASALFTLARGFPFVDVGVSAALL
JEV	NEYGMLEXTKADLKSMFGGKTQASGLTGL--PSMALDLRPAWALYGGSTVVLTPLKHLITSEYVTTSLASINSQAGSLFVLPRGVPFTDLDLTVGLV
DENV1	NEMGLLETTTKKDLGIGHAAA-ENH---HHAAMLVDLHPASAWTLYAVATTITPMMRHTIENTTANISLTAIANQAAILMGLDKGWPI SKMDIGVPLL
DENV2	NEMGFLEKTKKDLGLSITT-Q-Q---PESNILDLRPAWATLYAVATTFTVTPMLRHSIENSSVNSLTAIANQATVLMGLGKGWPLSKMDIGVPLL
DENV3	NEMGLLETTTKRDIGMSKEPG-V-V---SSTSYLDVLDHPASAWTLYAVATTITVTPMLRHTIENSTANVSLAAIANQAVVLMGLDKGWPI SKMDIGVPLL
DENV4	NEMGLIEKTKTDFGFYQVK-----TETTLVDLDRPASAWTLYAVATTITLTPMLRHTIENTSANLSLAAIANQAAVLMGLGKGWPLHRMDLGVPLL
ZIKV	NELGWLERTKNDIAHLMRREEGA-----TMGFSMDILDRPASAWIYAALTITPAVQHAVTTSYNNYSLMAMATQAGVLFMGKGMPFYAWDLGVPLL
YFV	NELGMLEKTKEDLFGKKNLIPS--SASPW--SWPDLDLKPGAATVVYGVITVMTLSPLMHWHIKVEYGNLSLSGIAQSASVLSFMDKGI PFMKMNI SVIIL

Ruler 1	110 120 130 140 150 160 170 180 190
Consensus	aIGCYsQvNpxTLxAAixLLvAHYAxiGPGIQAxAtrxAQKRTAAGIMKNPVDGIVATDxxevx-ydPkfEKKLGQVMLLVLCaaqxLImRTtwalcEa
ILHV	LLGCGYQYNLPTAVATGLLLLLAHYGYMIPGWQAAMRAAQRRTAAGVMKNNAVVDGIVATDIPEDVTATPI TEKKLGQILLILL CGASLLVVKFDTMVLVEA
ROCV	LVGCGYQYNLPTTMATIGLLLVGHYAFMIPGWQAAMRAAQRRTAAGVMKNNAVVDGIVATDIPEDMTATPI TEKKMGQVMLLI ISALAILLNPDTMTVVEG
WNV	AAGCGWQVTLTVTVTATLLFCHYAYMVPGWQAAMRSAQRRTAAGIMKNNAVVDGIVATDVPELERTTPI MQKKVQGIMLILVSLAAVVVNP SVKTVREA
JEV	FLGCGWQITLTTTTLTAMVLA TLHYGYMIPGWQAALRAAQRRTAAGIMKNNAVVDGMVATDVPELERTTPLMQKKVGVQLLIGVSVAAFLVNPNTTVREA
DENV1	ALGCGYQYNPLTLTAAVFMLVAHYAII GPGLQAKATREAAQRRTAAGIMKNPTVDGIVATDLDPPV-YDAKFELQGLQIMLLILCTSQILLMRTTWALCEA
DENV2	AIGCGYSQVNPITLTAALXLLVAHYAII GPGLQAKATREAAQRRTAAGIMKNPTVDGITVIDLDPPI-YDPKFEKQLGQVMLLVLCVTQVLMRTTWALCEA
DENV3	ALGCGYSQVNPITLAAAVLLLVTHYAII GPGLQAKATREAAQRRTAAGIMKNPTVDGIMTVIDLDPVI-YDSKFEKQLGQVMLLVLCAYQLLLMRTSWALCEV
DENV4	AMGCGYSQVNPITLIASLVMLLVHYAII GPGLQAKATREAAQRRTAAGIMKNPTVDGITVIDLEPIS-YDPKFEKQLGQVMLLVLCAGQLLLMRTTWAFCEV
ZIKV	MMGCGYQLTPTLTLIVAILLVLAHYMYLIPGLQAAAAAQAQRRTAAGIMKNPVDGIVTVDIDTMT-IDPQVEKKGQVLLIAYAISSAVLLRTAWGWGEA
YFV	LVSQWNSITVMPLLCGIGCAMLHWSLILPGIKAAQSKLAQRRTFHGVAKNPVDGNPTVDIEEAPEMPALYEKKLALYLLALLSLASVAMC RTPFSLAEG

Ruler 1	210 220 230 240 250
Consensus	gxLaTaAixTLWEGnpgkxWNxTiAVSmcni xRGSYLAGaxlaxtliKNxxxpxr--
ILHV	GVLTTSAMATLIEGNANTVWNSTVAVGVCHLMRGAWLAGPSIGWTIVRNLENP KLKR
ROCV	GVLITAAATLTLLEGNANTVWNSTVAVGVCHLMRGGWAAGPSIGWTIIRNLEAPKVKR
WNV	GILITAAAVTLWENGASVWNATTAIGLCHIMRGGWLSCLSIWTTLIKNMEKPG LKR
JEV	GVLVTAATLTLWDNGASAVWNSTTATGLCHVMRGSYLAGGSAWTLIKNADKPS LKR
DENV1	ITLATGPLTTLWEGSPGKFWNNTTIAVSMANIFRGSYLAGAGLAFSLMKSLGGRR--
DENV2	LTLATGPIITLWEGNPGRFWNNTTIAVSMANIFRGSYLAGAGLLFSIMKNTNTNTR--
DENV3	LTLATGPIITLWEGSPGKFWNNTTIAVSMANIFRGSYLAGAGLAFSIMKS VGTGR--
DENV4	LTLATGPVLTLWEGNPGRFWNNTTIAVSTANIFRGSYLAGAGLAFSLIKNAQT PRR--
ZIKV	GALITAAATSLWEGSPKNKYNSSTATSLCNIFRGSYLAGASLIVTVTRNAGLVKRR-
YFV	IVLASAALGPLIEGNTSLWNGPMASMTGVMRGNYAFVGVMYNLWKMKTGR--

Supplementary Figure S2: Alignment of Individual Proteins Across Multiple Flaviviruses. Individual proteins from representative flaviviruses were aligned using the Clustal Omega algorithm in MegAlign Pro v17.2.1 (DNASTAR, Madison, WI). Amino acids are colored by side chain chemistry: aromatic residues (F, W, and Y) are yellow, acidic residues (D and E) are red, basic residues (R, H, and K) are blue, nonpolar residues (A, G, I, L, M, P, and V) are orange, and polar residues (C, N, Q, S, and T) are green. Gaps are represented as dashes. Black rectangles designate cysteine residues predicted to form disulfide bridges.

NS5

Ruler 1	
Consensus	1102030405060708090 -GGa x Ge T L G E k W K r r LN Q L s re E F x x Y K x s G I i EV D RT E A x r A I K e G x x t g G H AV S R G x AK L R W x VE R GF v k P e G K V x DL G CG R GG W S Y Y c AT L K k v Q E
ILHV	-GGGS A PT L GE I W K AQ L N Q LT R EE F MA Y RR D GI E VD R T Q ARR A R R Q S GI T GG H P V S R GT A KL R WM V ER G F V RP I GV K VD L GC G RG G W S Y Y CA T L R H V Q E
ROCV	-GG I A A PT L GE I W K S R LN Q LT R EQ F ME Y R K DG I IE V DR T A A RR A RR R EG N RT GG HP V S R GT A KL R WL V ER G F V PL G K V VD L GC G RG G W S Y Y CA T L R H V Q E
WNV	-GG A K R TL G EV W K E R L N Q MT K EE F TR Y R K EA I IE V DR S AA K H A R K EG N VT GG HP V S R GT A KL R WL V ER R FL E P V G K VD L GC G RG G W C Y M AT Q K R V Q E
JEV	-GR PG GR T L G E K W K EL N AM S RE E F F K Y R R EA I IE V DR T E A RR A RR E NN I VGG H P V S R GS A KL R WL V E K G V SP I GV K VD L GC G RG G W S Y Y AA T L K K V Q E
DENV1	GT G AG Q ET L GE K W K R Q LN Q L S K S E F NT Y K R SG I IE V DR S E A KE G L K RGE P -TK H AV S RGT A KL R WF V ER N L V PE G K V ID L GC G RG G W S Y Y CA G L K K V T E
DENV2	GT G NI IG ET L GE K W K S R N A L G K S E F Q I Y K K S G I Q E VD R T L A K E G I K RGE T -DH H AV S RGS A KL R WF V ER N MT P EG K VVD L GC G RG G W S Y Y CG L K N V R E
DENV3	GT G SG Q ET L GE K W K KL N Q L S R KE F DL Y K K SG I TE V DR T E A KE G L K RGE I -TH H AV S RGS A KL Q W F VER N MT P EG R V I D L GC G RG G W S Y Y CA G L K K V T E
DENV4	GT G TT G ET L GE K W K R S NS L DR K E F E E Y K R S G I LE V DR T E A K S AL K DG S K- I K H AV S RGS S K I R W I V ER G MT P K G K V VD L GC G RG G W S Y Y MA T L K N V T E
ZIKV	-GGG T GET L GE K W K AR L N Q MS A LE F Y S Y K K S G I TE V CR E EA R RL K DG V AT GG H A V S RGS A KL R WL V ER G YL Q P Y G K VVD L GC G RG G W S Y Y AA T I R K V Q E
YFV	-GS AN G K TL G EV W K R EL N LL D K Q Q F EL Y K R TD I VE V DR T A RR HL A EG V DT G V A V S RGT A KL R WF H ER G V V K L E G R V ID L GC G RG G W C Y Y AA A Q K E V S G

Ruler 1	
Consensus	110120130140150160170180190 V R G Y T K GG P G H EE P mx M Q S Y G W N I V x L S G V D V F Y k P p E k C D T LL C D I GE S S x S P t V E E g R TL R VL x M V E P WL r -gp n q F C I K V L c P Y M P k V i E k L e R L Q
ILHV	V R G Y T K GG P G H EE P VM M Q S Y G W N I V TM K S G VD V F Y K P T E SC D TLL C D I GE S S S V G VE A ART L R V LD M VE P WL R -A A NS F C I K V L C P Y T P K V i E R L ER L Q
ROCV	V R G Y T K GG P G H EE P ML M Q S Y G W N I V SM K S G ID V F Y R P TE A CD T VL C D I GE S SP S PG VE E A RT L R V LE M IE P WL R -TA N Q Y CV K VL C P Y T P K V i E R L E K L Q
WNV	V R G Y T K GG P G H EE P QL V Q S Y G W N I V TM K S G VD V F Y R P SE C CD T LL C D I GE S SS A EE H RT I R V LE M VE D WL H R G P R E F CV K VL C P Y M P K V i E K M EL L Q
JEV	V R G Y T K GG A G H EE P ML M Q S Y G W N LV S L K S G VD V F Y K P SE P SD T LC D IGE S SP S PE VE EQ R T L R V LE M TS D WL H R G P R E F C I K V L C P Y M P K V i E K M EA V L Q
DENV1	V K G Y T K GG P G H EE P IM A TY G W N LV K LY S G D V F FT P PE K CD T LL C D I GE S SP N PT I EE G RT L R V L K M V EP W LR -G -NQ F C I K I L N P Y M S SV V ET L EQ M
DENV2	V K GL T K G GG P G H EE P IP M ST Y GW N LV R L Q S G VD V F FT P PE K CD TLL C D I GE S SP N TY E AG R T L R V LN L VEN W L -N NT F C I K V L N P Y M S SV I E K ME A V L Q
DENV3	V R G Y T K GG P G H EE P VP M ST Y GW N I V K L MS G K D V F YL P PE K CD T LL C D I GE S SP S PT VE ES R T I R V L K M V EP W LR -N -NQ F C I K V L N P Y M P TV I E H ER L L Q
DENV4	V K G Y T K GG P G H EE P IM A TY G W N LV K L H S G VD V F Y K P TE Q VD T LL C D I GE S SS N PT I EE G RT L R V L K M V EP W LS -S K P E F C I K V L N P Y M P TV I EE L E K L Q
ZIKV	V R G Y T K GG P G H EE P ML V Q S Y G W N I V R L K S G VD V F H M AA E P Q CD T LL C D I GE S SS PE VE ET RT L R V L S M V GD W LE K P G AF C I K VL C P Y T S TM M ET M ER L Q
YFV	V K G F TL G RD G H E K P M N V Q SL G W N I I TF K D K TD I HR L EP V K CD TLL C D I GE S SS S SV T EG E RT V R V LD T VE K WL AC G V D N F CV K V L A P Y M P D V L E K L EL L Q

Ruler 1	
Consensus	210220230240250260270280290 R k x GG G L V R n PL S R N ST H EM Y W V VS G a S GN I V s V N m T S q x LL n R m t x r h -r x P t Y E e D V D LG S GT R a V et e x p p x m d x l g x R I E R x K e H s t T W H Y D ee
ILHV	RA Y GG G L V R V PL S R N ST H EM Y W V VS G ASS N I N AV T VT S Q I L V QR M N K CG R H G PR Y EE D VC L GS G TR A VA T Q A SP S D H T K I K HR L ER L R K E F SA T W H I D LE
ROCV	R K YGG G L V R V PL S R N SN H EM Y W V VS E ASS N L I NAV N AT S Q V LL Q RL E K D HR K GP R YEE D V D LG S GT R SV A RR S PF M DT R K I HH R IE R L K S E FS T TW H Y D CE
WNV	RR Y GG G L V R N PL S R N ST H EM Y W V VS G AG N V H S V NMT S Q V LL G RM K RT W K G P Q YEE D N L GS G TR A VG K PL N SD S T S K I K N RI E RL R K E YS T W H HD E
JEV	RR F GG G L V RL L PL S R N SN H EM Y W V VS G AA G N V HAV N MT S Q V LL G R M DT Y W R GP K YEE D N L GS G TR A VG K VE S N O E K I K RI Q L K EE F AT T W H K D PE
DENV1	R K HGG M L V R N PL S R N ST H EM Y W V SC G T G N I VS A V N MT S R M L N RF T MA H -R K P T YER D VD L G A GT R H V AVE P E V AN L D I IG Q R I EN I K N G H K S T W H Y D E D
DENV2	R K YGG A L V R N PL S R N ST H EM Y W V VS N AS G N I VS S V N M I S R ML N RF T MR H -K K AT Y EP D VD L GS G TR N I G IE S I P N L D I IG K R I E K I K Q E H S T W H Y D Q D
DENV3	R K HGG M L V R N PL S R N ST H EM Y W I Y S NG T GN I VS S V N M V S R LL N RF T MT H -RR P T I E K D V D L G A GT R H V NA E PT P N M D V I G ER I K R I E E H S T W H Y D DE
DENV4	R K HGG S L V RC P L S R N ST H EM Y W V VS G VS G N I VS S V N T S K M L N RF T TR H -R K P T Y E K D VD L G A GT R SV S T E TE K PD M T I IG R RL Q RL Q E H E K T W H Y D Q E
ZIKV	RR H GG G L V R V PL S R N ST H EM Y W V VS L V N G V RL L S K P W D V VT G VT G I A MT D TT P Y G Q R Y F KE K VD T R V PD Q EG T R V M N I V SS W LV K EL G K R K R PR V CT K E
YFV	RR F GG T V I R N PL S R N ST H EM Y W V VS G ARS N VT F T V N Q TS R LL M RR M RR P T-G K V T LE A D V IL P IG T RS V ET D K G PL D KE A IE E R V ER I K S E Y MT S W F Y D ND

Ruler 1	
Consensus	310320330340350360370380390 H P Y R T W a Y H G S Y E V K P GS A SS M V N G V V x LL x K P WD v l p m V T x M A MT D TT P FG Q Q R V F KE K VD T R a Pe P x x GT x VM n i T x n W L W a f l g Re K r P r X CT R E
ILHV	HP Y RT W HY H GS Y EQ T GS A NS M V N G V VR L LL S K P WD A I T SV T TM A MT D TT P FG Q Q R Y F KE K VD T RA P D P AV G YA Q AL D IT T GW L W T FL A RS K K P RM CT RE
ROCV	HP Y RT W NY H GS Y EV K PT G S A SS M V N G V V K LS K PWD S I Q SV L TM A MT D TT P FG Q Q R Y F KE K VD T KA P E P AP G V K VLD L TT D WL V AL C RR S K K P RM CT K E
WNV	HP Y RT W NY H GS Y D V K P T G S A SS L V N G V VR L LL S K P WD T I T N V TT A MT D TT P FG Q Q R Y F KE K VD T KA P EP P EG V K V VL N ET T N W L W A F L A RE K R P MC S RE
JEV	HP Y RT W T Y HGS Y EV K AT G S A SS L V N G V V K LS K PWD A I A NY T TM A MT D TT P FG Q Q R Y F KE K VD T KA P E P P A GA K EV L NET T N W L W A H LS R E K R P RL CT KE
DENV1	HP Y K T W A Y H GS Y EV K PS G SA S SM V NG V VR L LT K PWD V I P MT V Q I AM T D T TP F G Q Q R Y F KE K VD T RT P KA K R G TA Q IME V TAR W L G W F LS R N K K P RI CT KE
DENV2	HP Y K T W A Y H GS Y ET K QT G SA S SM V NG V VR L LT K PWD V VP M VT Q AM T D T TP F G Q Q R Y F KE K VD T RT Q EP K EG T K K LM K I T AE W LV W KL G K K KT P RM CT RE
DENV3	NP Y K T W A Y H GS Y EV K AT G S A SS M ING V VR L LT K PWD V VP M VT Q AM T D T TP F G Q Q R Y F KE K VD T TR P MP GT R K YME I TA E W L W R T L GR N K R PL CT RE
DENV4	NP Y RT W AY H GS Y E A PT G SA S SM V NG V VR L LT K PWD V I P MT V Q I AM T D T TP F G Q Q R Y F KE K VD T RT P Q K PG T RM V MT T T A N W L W AL L G K K K N P RL CT KE
ZIKV	HP Y RT W AY H GS Y E A PT G SA S SM V NG V VR L LL S K P WD V VT G VT G I A MT D TT P Y G Q R Y F KE K VD T R V PD Q EG T R V M N I V SS W LV K EL G K R K R PR V CT K E
YFV	NP Y RT W HY C S Y VT K TS G SA A SM V NG V I K ILT P Y P WD R I EE VT R MA M T D TT P FG Q Q R Y F KE K VD T RA K D P P A GT R K I M K V V N R W L F R HL A RE K N P RL CT KE

Ruler 1	
Consensus	410420430440450460470480490 E F I x K V RS N AA L GA V F e x EQ W KS A RE A Y D ED r F W e L VD E R e I HL x G K C e TC V Y N MM G K R E K KL G EF G KA K GS R A I W Y MW L GA R FL E F E AL G FL N ED H W
ILHV	E F I A K V NS N AA L GA V FD E Q N Q W ST A RE A VED P AF W N L VD E ER K A H LAG R CE T C I Y N MM G K R E K KL G EF G KA K GS R A I W Y MW L GA R FL E F E AL G FL N ED H W
ROCV	E F I A K V NS H AA L GA I FE E Q N Q W AS T ARE A VED P GF W N L VD K ER Q A H L E GR CE T C I Y NN M G K R E KL G EF G KA K GS R A I W Y MW L GA R FL E F E AL G FL N ED H W
WNV	E F I R K V NS N AA L GA MF EE Q N Q W S ARE A Y ED PK F W E MV D EER E QA H LR GE CH T C I Y NN M G K R E K K P GE F G K A K GS R A I W F MW L GA R FL E F E AL G FL N ED H W
JEV	E F I K K X NS N AA L GA FA EE Q N Q W S TARE A Y DD PR F W E MV D EER E N H LR GE CH T C I Y NN M G K R E K K P GE F G K A K GS R A I W F MW L GA R FL E F E AL G FL N ED H W
DENV1	E F TR K V S NA A I GA V F DE Q N Q W S A K EA N I D RF W EL V HRE R EL H Q G K C AT C V Y NN M G K R E KL G EF G KA K GS R A I W Y MW L GA R FL E F E AL G FM N ED H W
DENV2	E F TR K V S NA A AL GA I F TD EN K W KS A RE A Y ED S R F W EL V D K ERN L H L E G K C AT C V Y NN M G K R E KL G EF G KA K GS R A I W Y MW L GA R FL E F E AL G FL N ED H W
DENV3	E F TR K V T NA A MG A VF T EN Q W S ARE