SUPPORTING INFORMATION

Dengue Protease Substrate Recognition: Binding of the Prime Side

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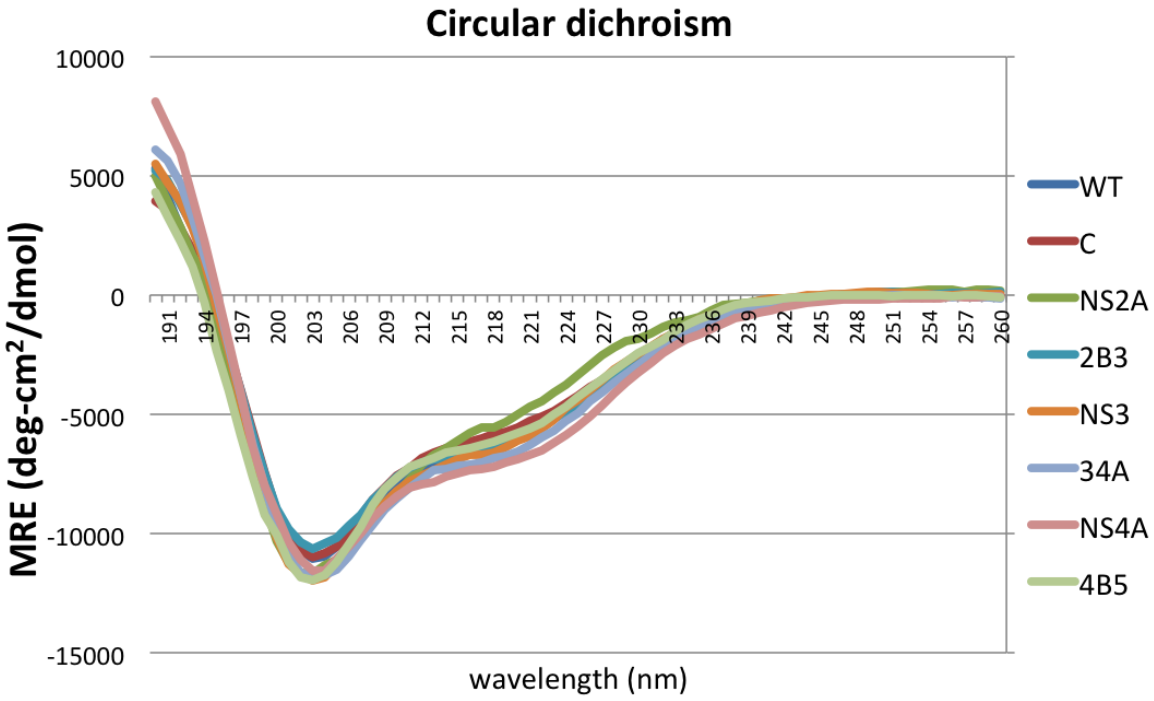


Figure S1. The secondary structure is conserved across all aprotinin constructs used in this study. Far-UV circular dichroism spectra of WT aprotinin and aprotinin constructs where the binding loop residues were replaced with DENV3 cleavage site sequences (see main manuscript Figure 1).

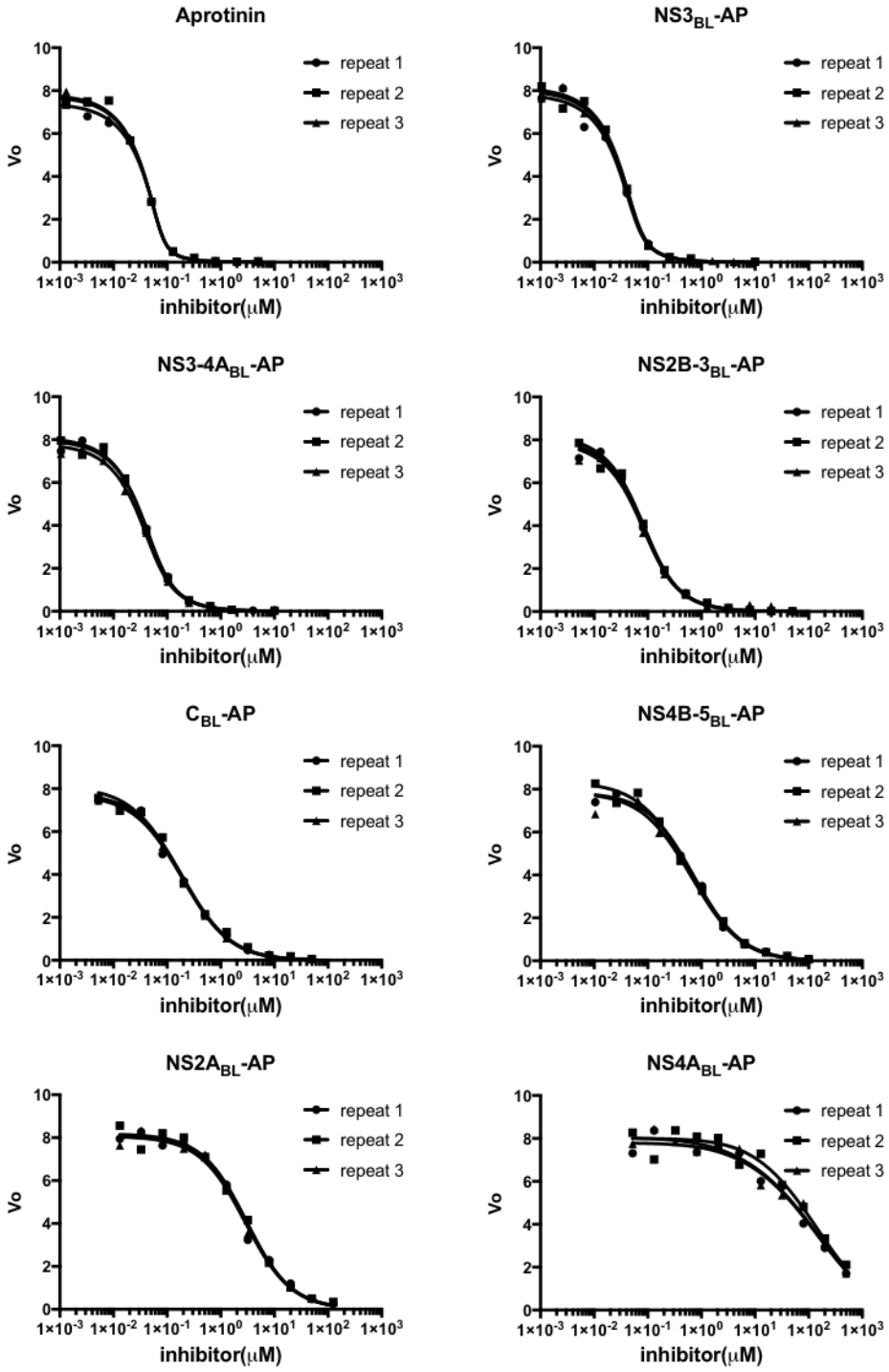


Figure S2. The curve fits for the inhibition data of aprotinin constructs and WT-AP against DENV3 WT protease in enzymatic assays, ordered from tightest to weakest binder.

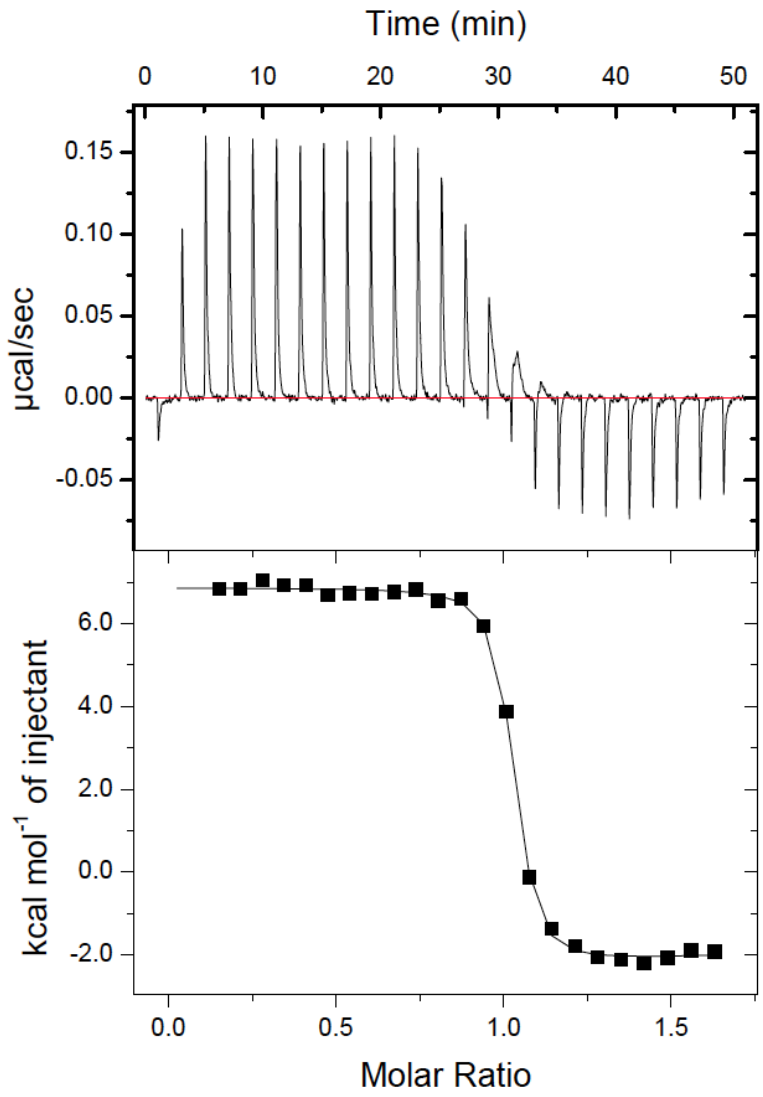


Figure S3. ITC titration of WT-AP with DENV3 WT PR.

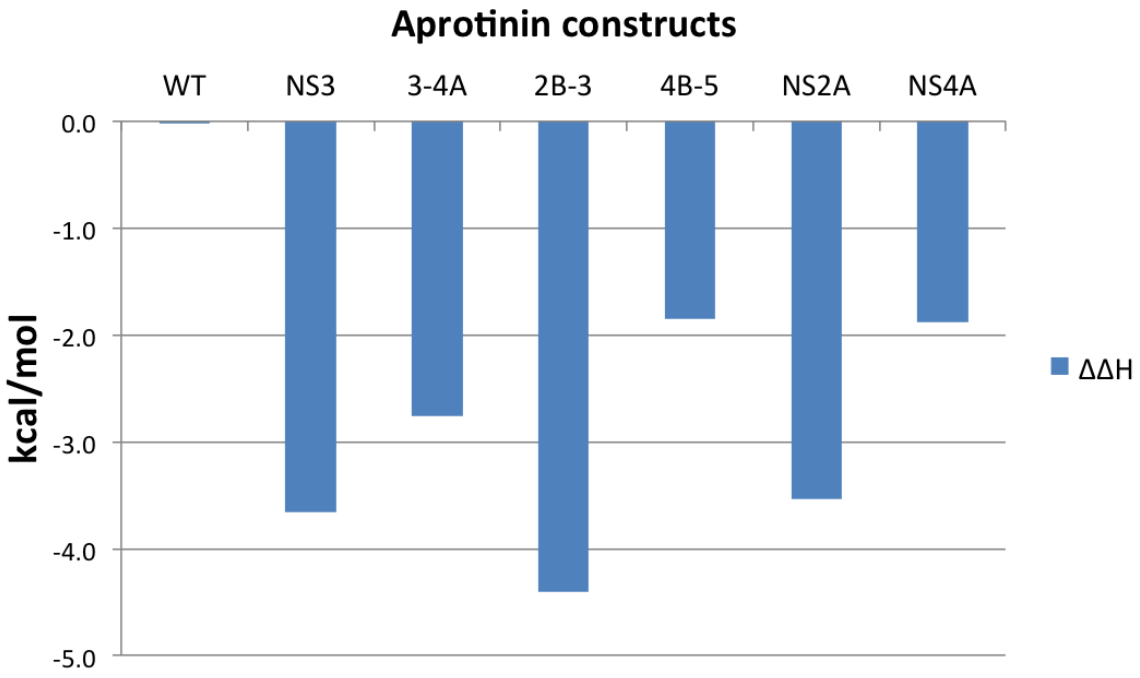


Figure S4. The change in enthalpic contribution to the free energy of binding to DENV3 protease for aprotinin constructs relative to WT aprotinin.

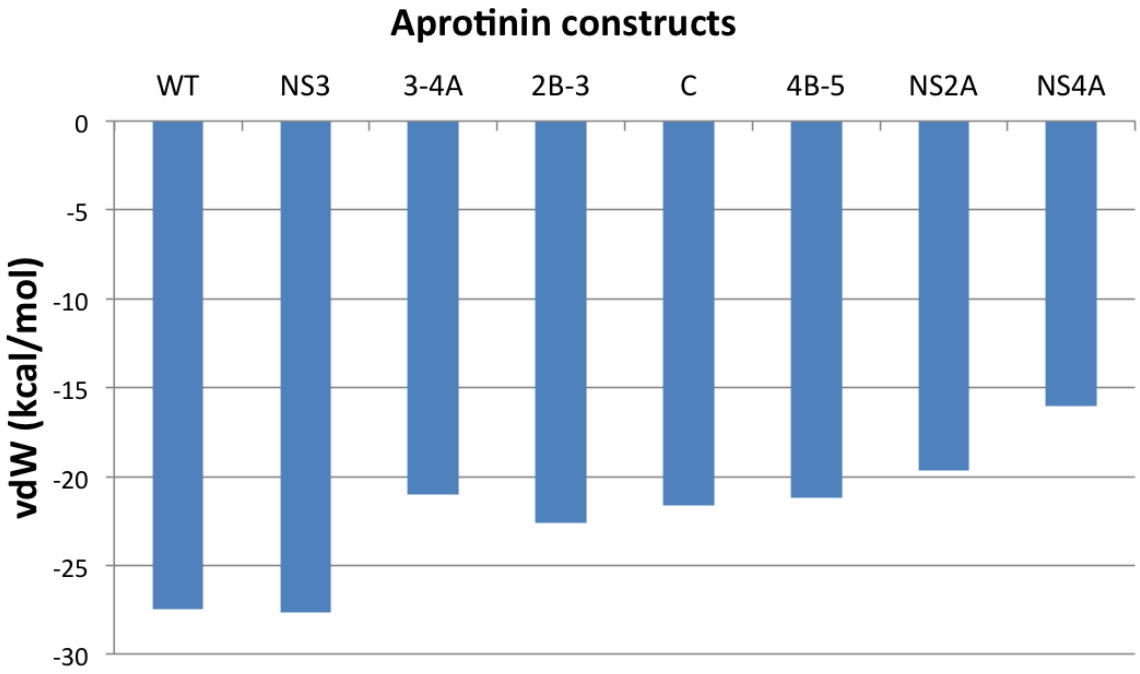
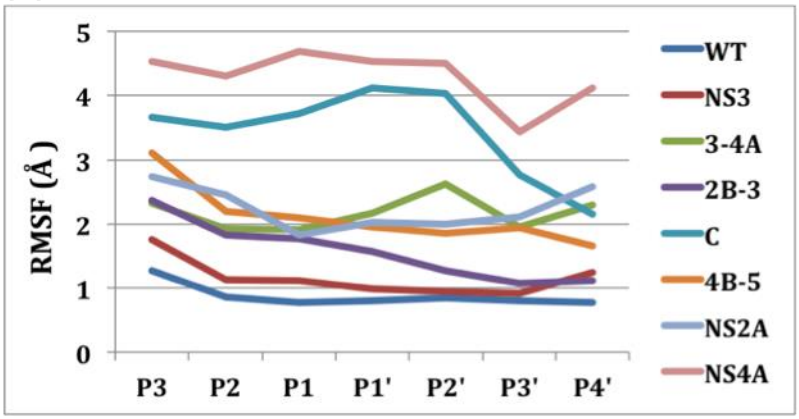


Figure S5. The overall binding loop vdW contact energy with DENV3 protease calculated from MD simulations of WT-AP and aprotinin constructs.

(a)



(b)

Aprotinin constructs	WT	NS3	3-4A	2B-3	C	4B-5	NS2A	NS4A
Binding loop RMSF (Å)	**0.9**	**1.2**	**2.2**	**1.6**	**3.4**	**2.1**	**2.3**	**4.3**

Figure S6. Binding loop fluctuations of WT-aprotinin and aprotinin constructs in MD simulations (a) RMSF values by residue (b) average RMSF values of the overall binding loop.

Table S1. The sequence alignments of dengue and Zika virus protease polyprotein cleavage sites. Amino acid identities shared with DENV3 are highlighted in yellow, and changes are indicated from green to red for more to less conservative.

NS3

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	A	A	Q	R	R		G	R	I	G	R
DENV2	A	A	Q	R	R		G	R	I	G	R
DENV3	**A**	**A**	**Q**	**R**	**R**		**G**	**R**	**V**	**G**	**R**
DENV4	A	A	Q	R	R		G	R	I	G	R
Zika	A	A	Q	R	R		G	R	I	G	R

3-4A

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	A	A	G	R	R		S	V	S	G	D
DENV2	A	A	G	R	K		S	L	T	L	N
DENV3	**A**	**A**	**G**	**R**	**K**		**S**	**I**	**A**	**L**	**D**
DENV4	A	S	G	R	K		S	I	T	L	D
Zika	A	A	G	K	R		G	A	A	F	G

2B-3

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	K	K	K	Q	R		S	G	V	L	W
DENV2	V	K	K	Q	R		A	G	V	L	W
DENV3	**K**	**Q**	**T**	**Q**	**R**		**S**	**G**	**V**	**L**	**W**
DENV4	V	K	T	Q	R		S	G	A	L	W
Zika	K	T	G	K	R		S	G	A	L	W

C

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	N	R	R	K	R		S	V	T	M	L
DENV2	N	R	R	R	R		S	A	G	M	I
DENV3	**N**	**K**	**R**	**K**	**K**		**T**	**S**	**L**	**C**	**L**
DENV4	N	G	R	K	R		S	T	I	T	L
Zika	N	A	R	K	E		K	K	R	R	G

4B-5

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	G	G	G	R	R		G	T	G	A	Q
DENV2	T	N	T	R	R		G	T	G	N	I
DENV3	**G**	**T**	**G**	**K**	**R**		**G**	**T**	**G**	**S**	**Q**
DENV4	Q	T	P	R	R		G	T	G	T	T
Zika	G	L	V	K	R		R	G	G	G	T

NS2A

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	T	T	S	Q	K		T	T	W	L	P
DENV2	S	S	Q	Q	K		T	D	W	I	P
DENV3	**S**	**S**	**M**	**R**	**K**		**T**	**D**	**W**	**L**	**P**
DENV4	C	L	Q	K	Q		S	H	W	V	E
Zika	G	S	V	K	K		N	L	P	F	V

NS4A

	P5	P4	P3	P2	P1		P1'	P2'	P3'	P4'	P5'
DENV1	P	D	R	Q	R		T	P	Q	D	N
DENV2	P	E	K	Q	R		T	P	Q	D	N
DENV3	**P**	**E**	**K**	**Q**	**R**		**T**	**P**	**Q**	**D**	**N**
DENV4	P	E	K	Q	R		T	P	Q	D	N
Zika	P	E	K	Q	R		S	P	Q	D	N

Table S2. The thermodynamic parameters derived from ITC experiments of WT-AP and aprotinin constructs binding to DENV3 protease. The energy values are in kcal/mol.

Aprotinin	Kd (nM)	ΔG	ΔH	−TΔS
WT	1.7 ± 0.7	-11.8 ± 0.3	7.2 ± 0.2	-18.9
NS3	2.2 ± 0.5	-11.6 ± 0.1	3.5 ± 1.1	-15.1
3-4A	29.5 ± 6.5	-10.1 ± 0.1	4.4 ± 0.1	-14.5
2B-3	238.6 ± 21.0	-8.9 ± 0.1	2.8 ± 0.0	-11.7
4B-5	816.9 ± 56.9	-8.2 ± 0.0	5.3 ± 0.0	-13.5
NS2A	$9.5x10^3 \pm 3.0x10^3$	-6.7 ± 0.2	3.7 ± 1.1	-10.4
NS4A	$161.8x10^3 \pm 37.3x10^3$	-5.1 ± 0.0	5.3 ± 1.1	-10.4

Table S3. The values of binding loop van der Waals contacts calculated from MD simulations of WT-AP and aprotinin constructs bound to DENV3 protease.

Residue	WT	NS3	3-4A	2B-3	C	4B-5	NS2A	NS4A
P1	-12.4	-13.3	-10.8	-11.0	-9.7	-11.6	-11.2	-10.0
P1'	-4.4	-3.7	-4.6	-5.0	-4.4	-3.4	-3.8	-2.5
P2'	-7.1	-7.6	-3.7	-3.6	-4.9	-4.1	-2.5	-2.9
P3'	-1.5	-1.3	-0.3	-1.2	-0.4	-0.4	-1.7	-0.3
P4'	-2.1	-1.7	-1.5	-1.8	-2.2	-1.7	-0.4	-0.4
Overall	-27.5	-27.7	-21.0	-22.6	-21.6	-21.2	-19.6	-16.0