

## SUPPLEMENTAL MATERIAL

# Conserved structural motif identified in peptides that bind to Geminivirus replication protein Rep

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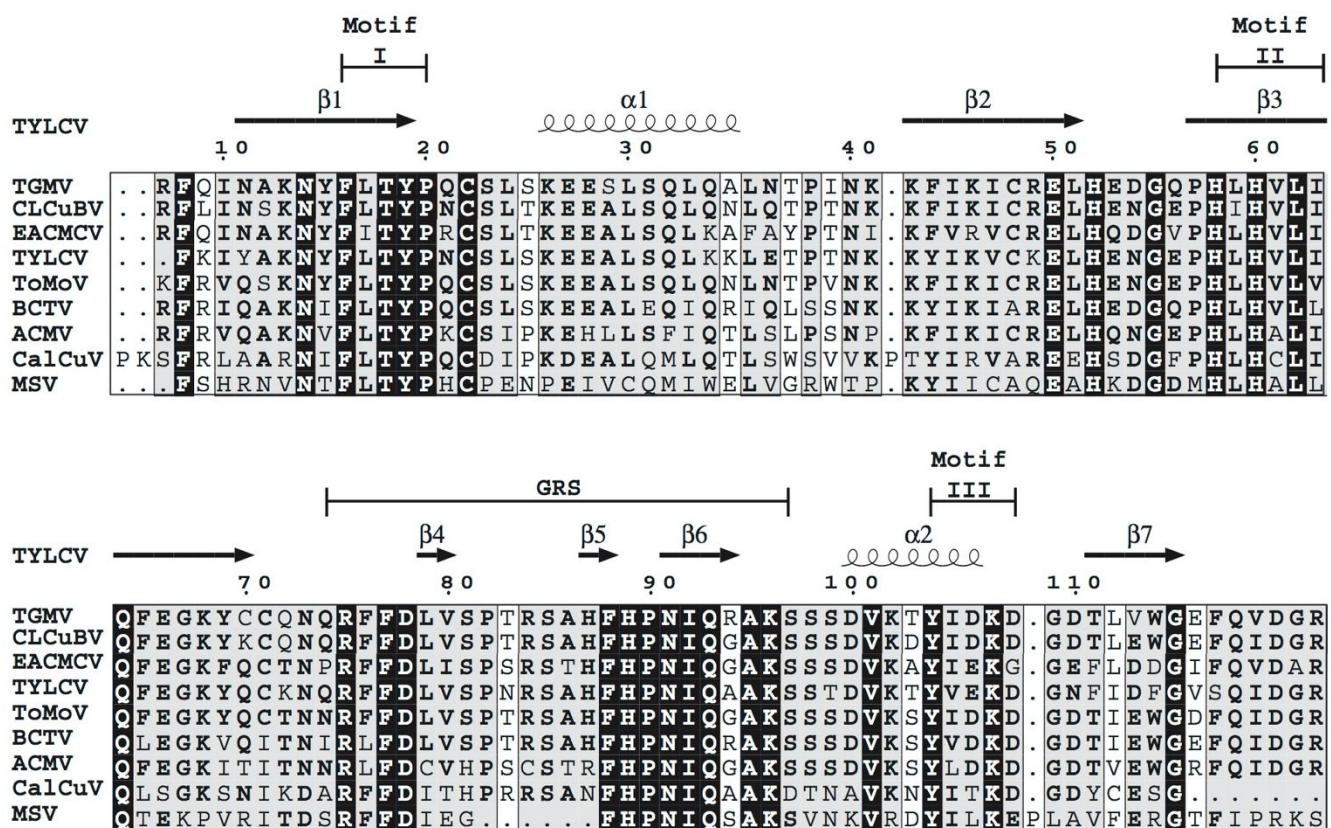
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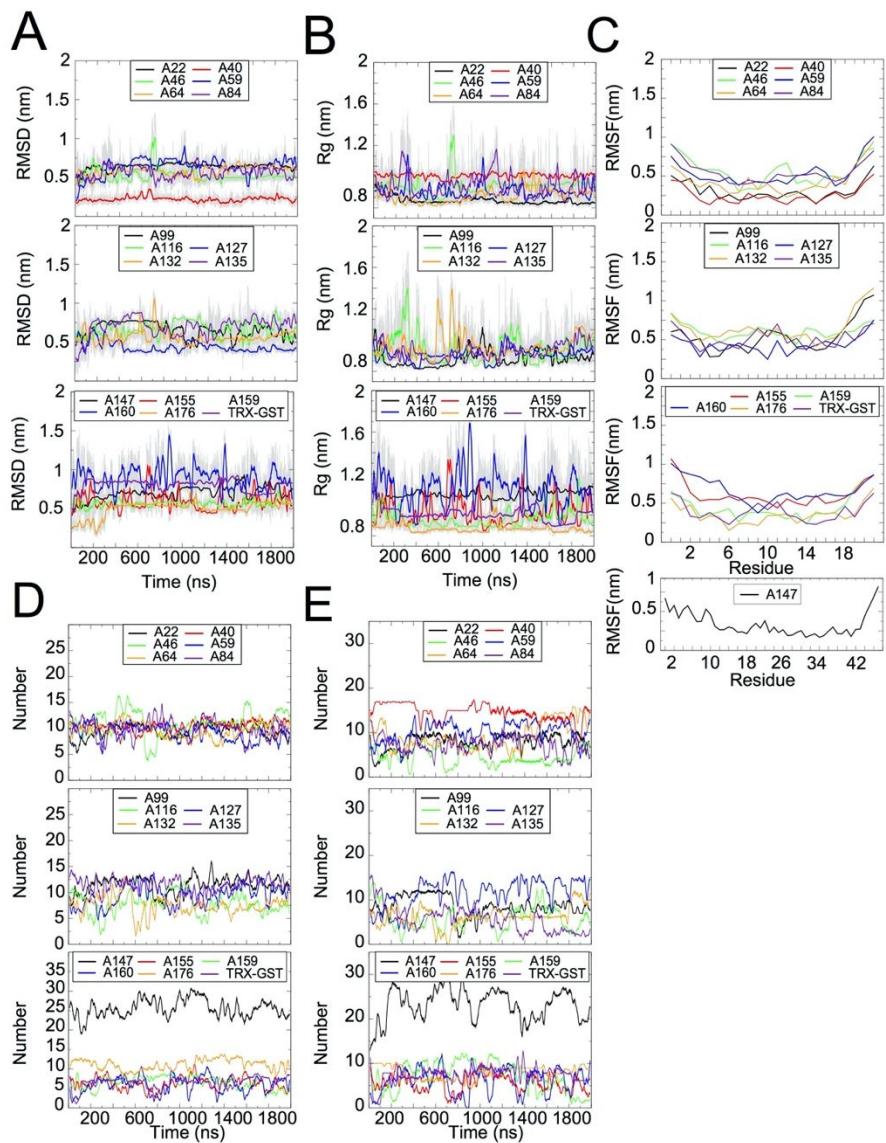
## Supplemental Figures

Supplemental Figure 1.



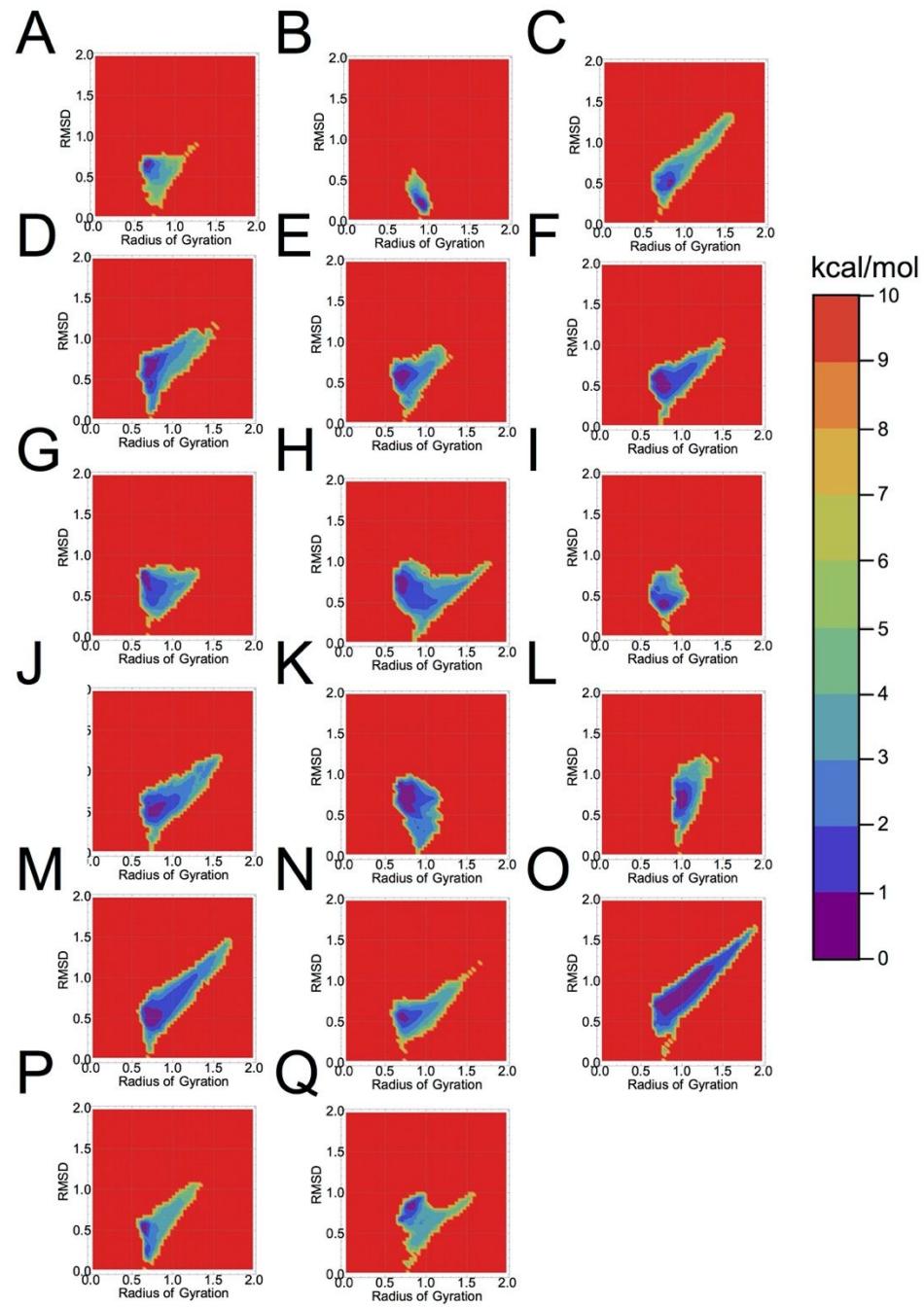
Supplemental Figure 1. A sequence alignment of N-term Rep proteins used in this study.  
TYLCV secondary structure as characterized in 1L5I PDB and conserved motifs are shown.

Supplemental Figure 2.



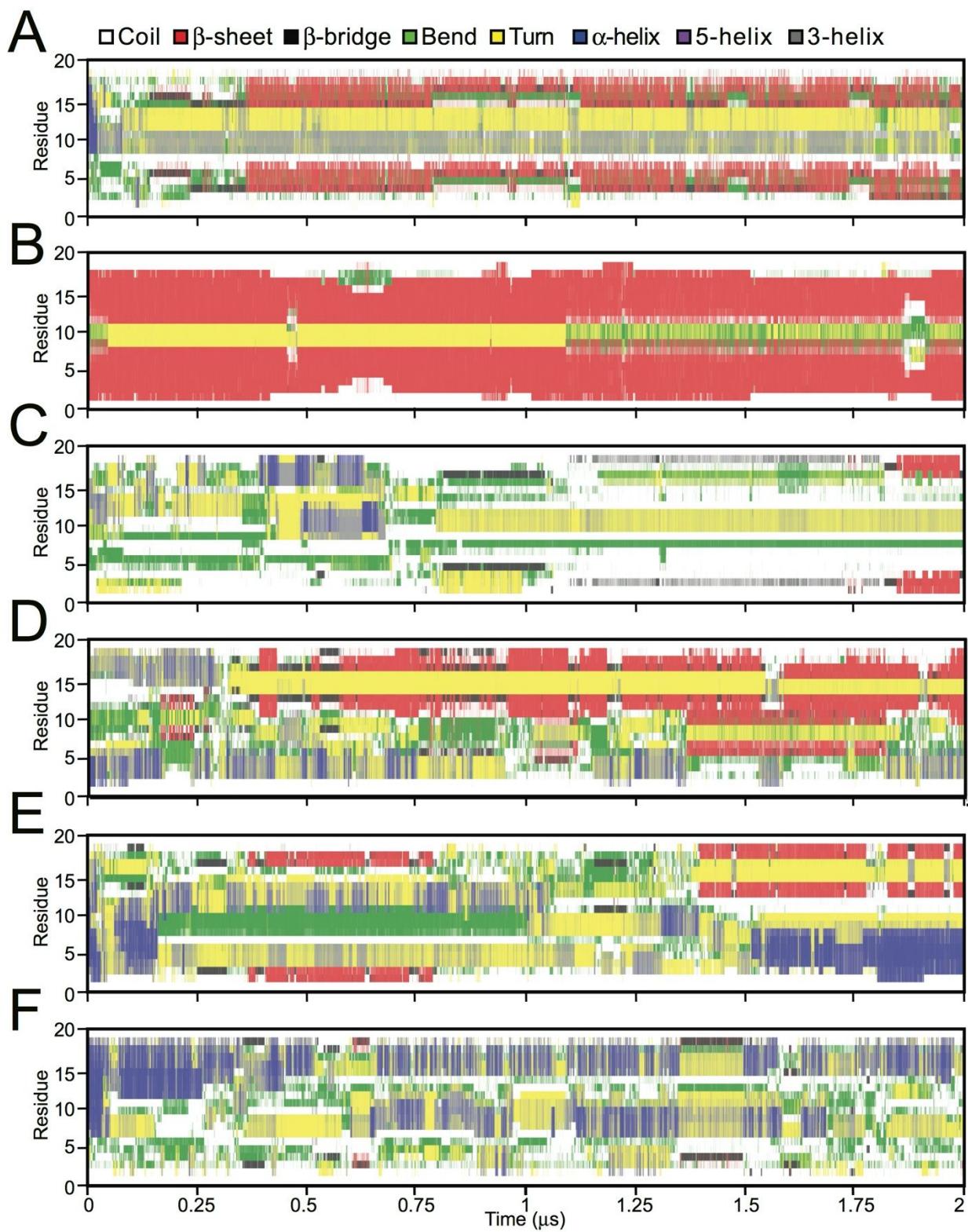
**Supplemental Figure 2. Peptide analysis of the 2 $\mu$ s peptide MD simulation.** (A),  $\text{Ca-RMSD}$  over the length of the simulation. (B),  $R_g$  (radius of gyration over the length of the simulation). (C), root mean square fluctuations (RMSF) per residue, A147 removed from the rest as there is 47 residues compared to the ~20 residues for each other peptide. (D), number of hydrogen bonds in total per peptide. (E), number of residues in a secondary structure confirmation per peptide ( $\alpha$ -Helix +  $\beta$ -Sheet +  $\beta$ -Bridge + Turn). The 18 peptides are divided up into 3 figures each within each panel: peptides A22 (black), A40 (red), A46 (green), A59 (blue), A64 (orange), and A84 (purple) (top); A99 (black), A116 (green), A127 (blue), A132 (orange), and A135 (purple) (middle); and A147 (black), A155 (red), A159 (green), A160 (blue), A176 (orange), and TRX-GST (purple) (bottom). Raw data is shown in grey lines while colored lines are the running averages in panels A and B.

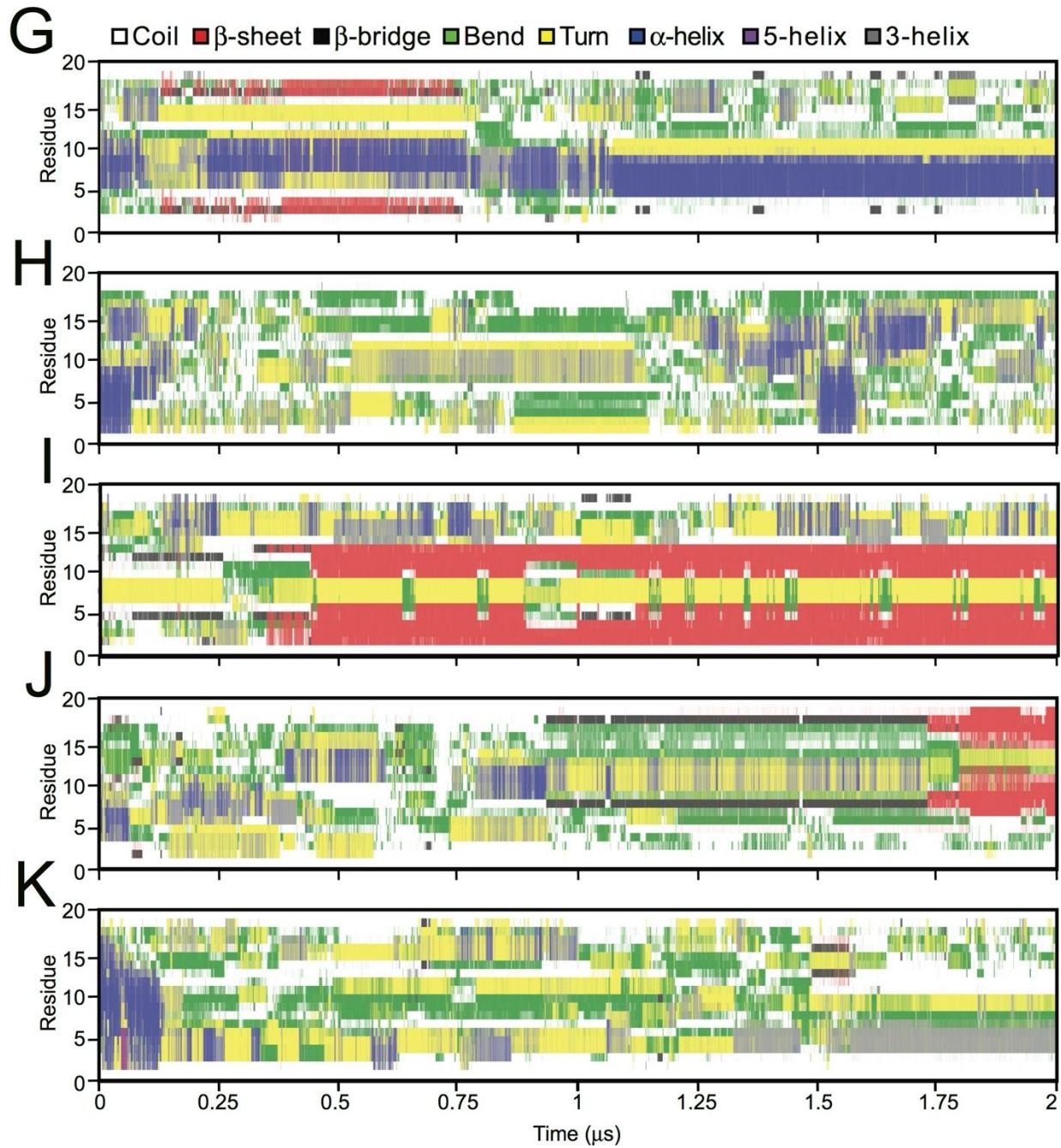
Supplemental Figure 3.

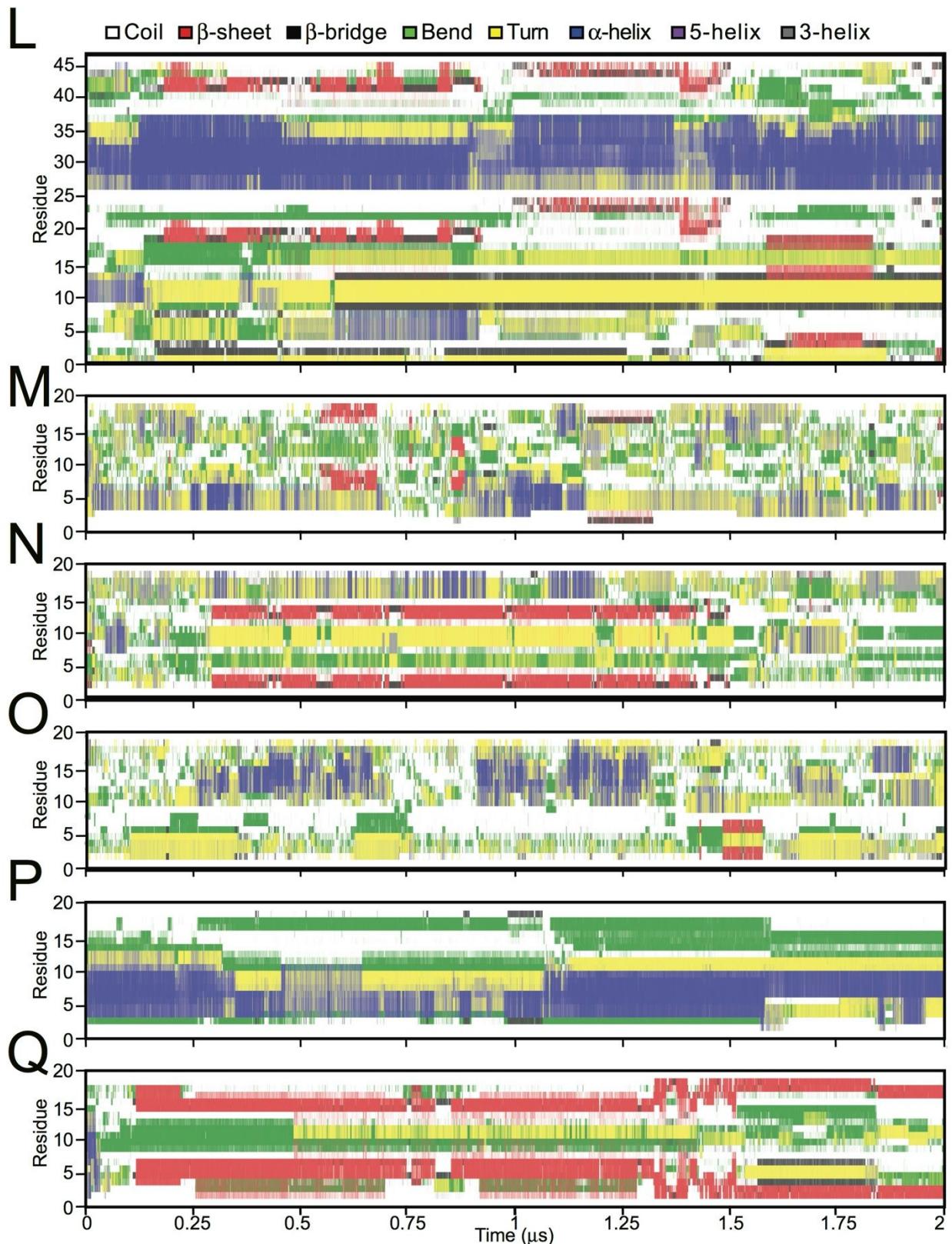


**Supplemental Figure 3. Free energy landscape analysis of the 2 $\mu$ s peptide MD simulation.**  
 Peptides A22 (A), A40 (B), A46 (C), A59 (D), A64 (E), A84 (F), A99 (G), A116 (H), A127 (I), A132 (J), A135 (K), A147 (L), A155 (M), A159 (N), A160 (O), A176 (P), and TRX-GST (Q) FEL analysis. The FEL values are constructed as a function of projections of the MD trajectory onto their own C $\alpha$ -RMSD and C $\alpha$ -Rg values, respectively. The color bar represents the relative free-energy value in kcal mol $^{-1}$ .

Supplemental Figure 4.

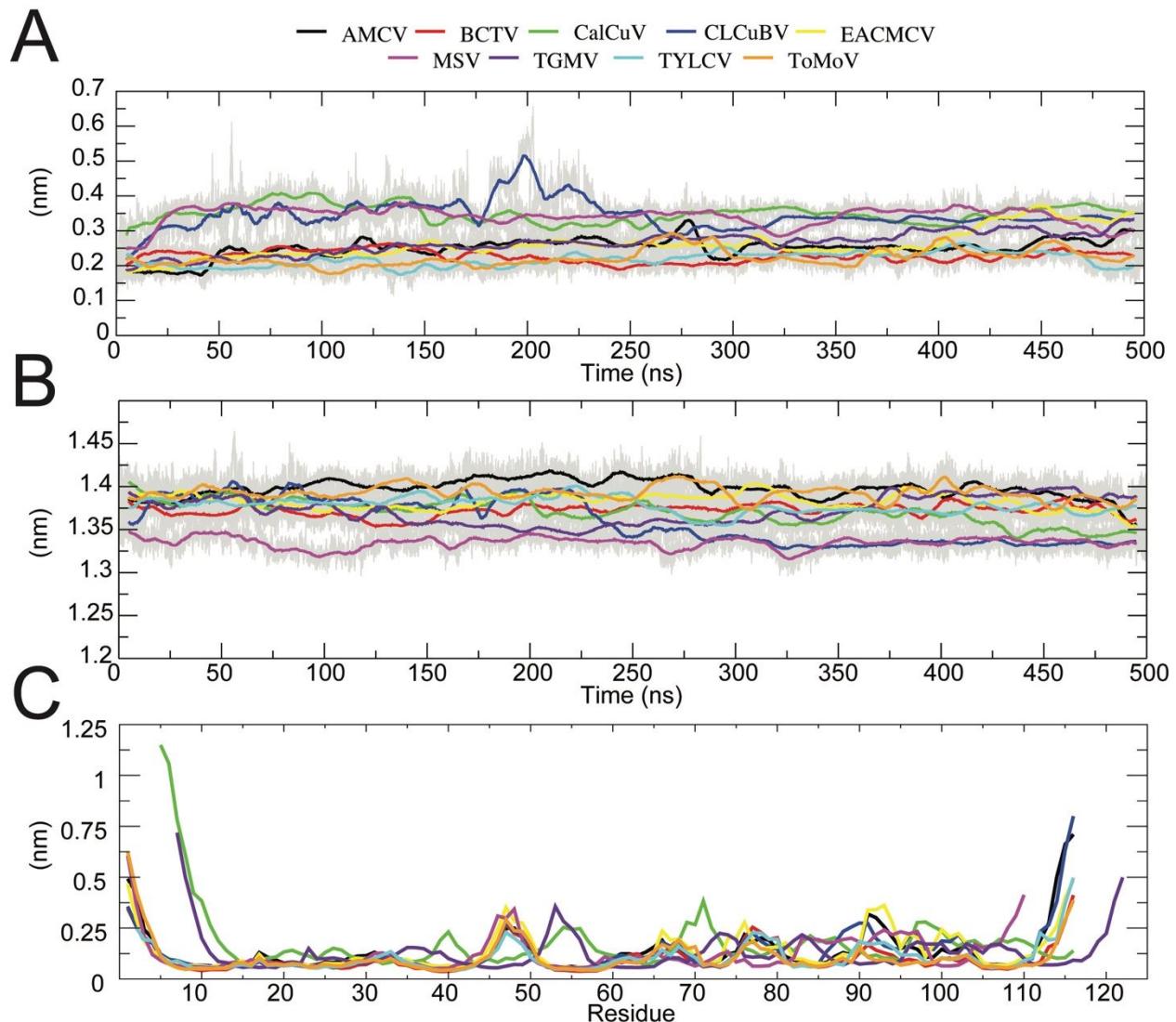






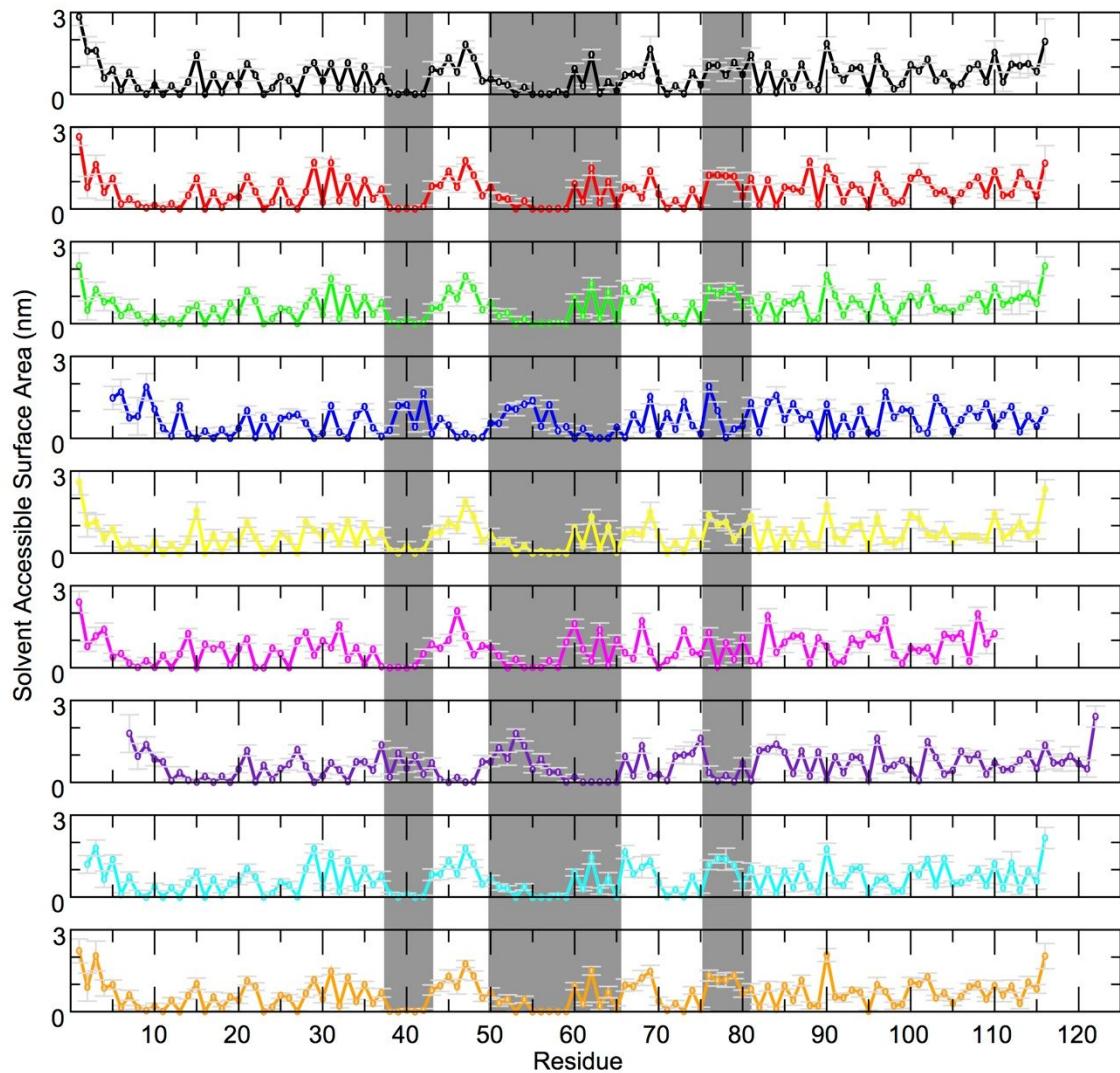
**Supplemental Figure 4. Peptide secondary structure analysis during the 2  $\mu$ s MD simulation.**  
Peptides A22 (A), A40 (B), A46 (C), A59 (D), A64 (E), A84 (F), A99 (G), A116 (H), A127 (I),  
A132 (J), A135 (K), A147 (L), A155 (M), A159 (N), A160 (O), A176 (P), and TRX-GST (Q)  
secondary structure analysis. Color output from DSSP: coil (white),  $\beta$ -sheet (red),  $\beta$ -bridge  
(black), bend (green), turn (yellow),  $\alpha$ -helix (blue), 5-helix (purple), and 3<sup>10</sup>-helix (grey).

Supplemental Figure 5.



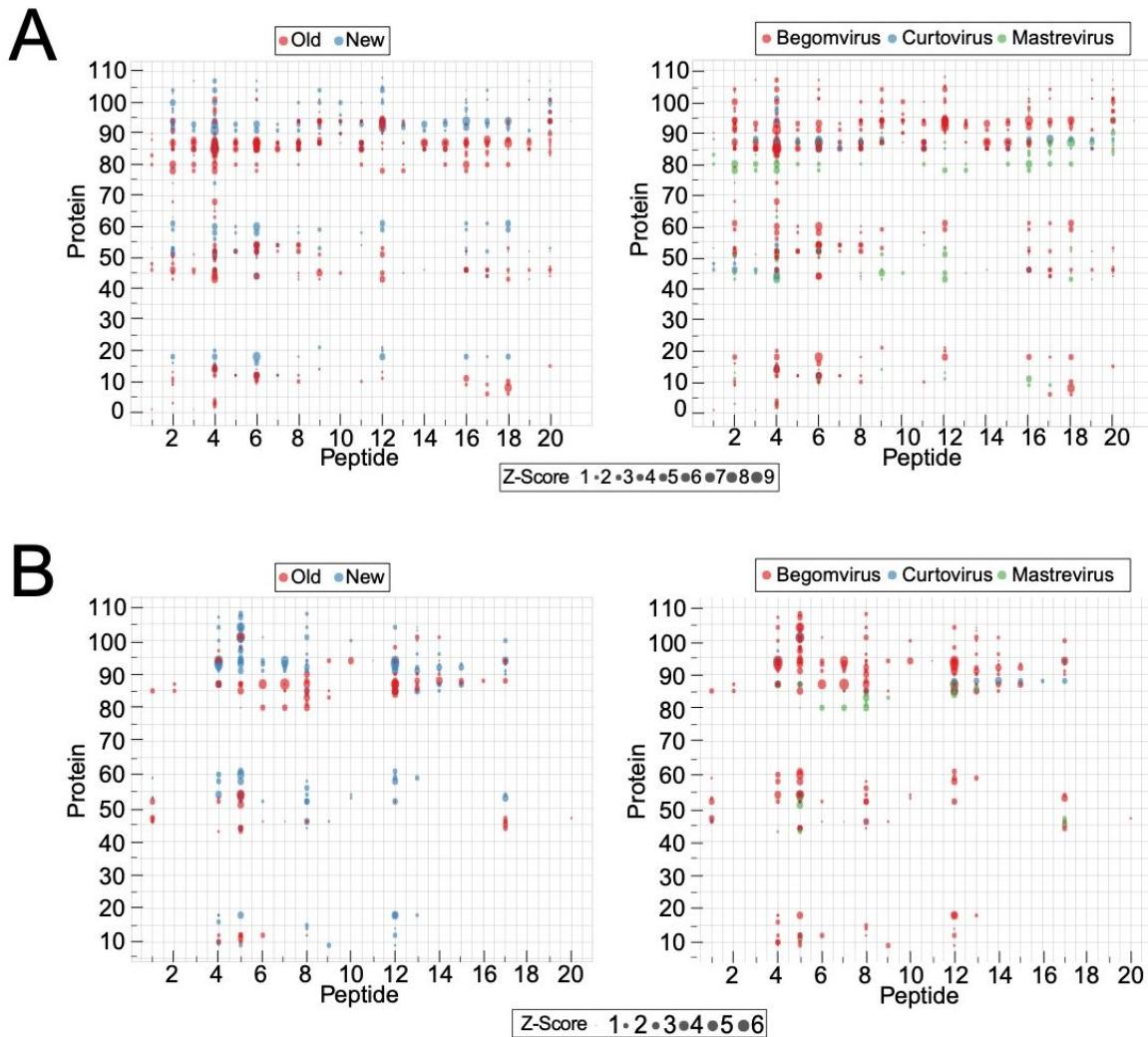
**Supplemental Figure 5. N-term Rep model analysis of the 500ns protein MD simulation.** (A) C $\alpha$ -RMSD, (B) the radius of gyration (Rg), and (C) the C $\alpha$ -RMSF per residue values over the length of the simulation. The lines are colored according to: AMCV (black), BCTV (red), CalCuV (green), CLCBuV (blue), EACMV (yellow), MSV (magenta), TGMV (purple), TYLCV (cyan), and ToMoV (orange). Raw data is shown in grey lines while colored lines are the running averages.

Supplemental Figure 6.



**Supplemental Figure 6. Solvent accessibility surface area average and standard deviation as calculated through GROMACS over the length of the  $2\mu$ s MD simulation per residue in N-term Rep models.** From top to bottom: AMCV (black), BCTV (red), CLCBuV (green), CalCuV (blue), EACMV (yellow), MSV (magenta), TGMV (purple), TYLCV (cyan), and ToMoV (orange). Standard deviation is shown in light grey. Areas of notable difference of CalCuV and TGMV in comparison with the rest of the N-term Rep models are highlighted in grey.

**Supplemental Figure 7.**



Supplemental Figure 7. **3D bubble chart protein and peptide Z-score interaction pairs.** Each bubble represents an interaction between A22 (top) or A64 (bottom) with the protein of interest. The larger the bubble the bigger the Z-score and significance of the interaction. Sequences were aligned according to the alignment in **Supplemental Figure S1**. Legends for each chart are provided for clarity.

## Supplemental Tables

**Supplemental Table 1. Active residues during molecular docking**

Protein/Peptide	Active residues
ACMV	1,2,3,5,15,17,19,21,25,28,29,31,33,35,44,45,46,47,48,49,60,61,62,66,67,69,74,76,77,78,79,80,81,83,85,87,90,91,93,94,96,97,100,102,103,104,107,108,109,110,112,113,114,115,116
BCTV	1,3,5,15,17,21,25,29,31,33,35,44,45,47,48,49,60,62,64,66,67,69,76,77,78,79,81,83,85,86,88,90,91,93,96,97,100,101,102,103,104,107,109,110,113,114,116
CalCuV	5,6,8,9,13,21,23,25,27,31,35,39,40,42,44,52,54,55,56,57,67,68,69,71,73,76,79,80,83,84,86,87,90,92,94,97,98,99,100,103,104,107,109,112,115,116
CLCuBV	5,6,8,9,13,21,23,25,27,31,35,39,40,42,44,52,54,55,56,57,67,68,69,71,73,76,79,80,83,84,86,87,90,92,94,97,98,99,100,103,104,107,109,112,115,116
EACMV	1,3,5,15,17,21,25,28,29,31,33,35,45,47,48,49,60,62,64,66,67,69,76,77,80,81,83,85,87,90,91,93,94,96,100,101,102,103,107,109,110,113,115,116
MSV	1,2,3,4,14,16,18,21,28,30,31,32,34,43,44,45,46,47,48,49,59,60,61,63,65,68,73,74,76,78,80,83,84,85,86,87,90,93,96,97,104,105,106,108,110
TGMV	7,9,11,21,23,25,27,31,34,35,37,39,41,51,52,53,54,55,56,66,68,72,73,74,75,82,83,84,85,87,89,91,93,94,96,97,98,99,100,103,106,108,109,110,113,115,116,118,121,122
ToMoV	1,3,4,5,14,15,17,19,21,22,25,29,31,33,35,44,45,46,47,48,49,60,62,66,67,68,69,74,76,77,78,79,80,81,83,85,87,90,91,93,94,97,100,102,103,104,107,108,109,110,112,114,115,116
TYLCV	2,3,5,15,17,21,25,28,29,31,33,35,44,45,47,48,49,60,62,66,67,68,69,74,76,77,78,79,81,83,85,87,90,91,93,94,97,100,102,103,104,107,108,109,110,112,114,116,
A22	1,2,3,4,5,6,8,9,10,12,13,16,17,18,19,20
A40	1,2,3,5,7,8,9,10,11,13,14,15,16,17,19,20
A46	1,2,3,5,6,7,9,10,12,13,14,16,17,20
A59	1,2,3,4,6,8,9,11,12,13,14,15,16,17,19,20
A64	1,5,6,8,9,10,13,14,17,19
A84	1,2,3,4,5,6,7,8,9,11,12,13,15,16,17,18,19,20
A99	1,2,3,6,7,10,11,12,14,15,18,20
A116	1,2,3,4,5,6,7,9,10,12,14,15,16,17,18,19,20
A127	1,2,4,6,7,8,9,11,15,16,17,18,19,20
A132	2,3,4,6,8,9,11,12,13,14,15,16,17,18,19,20

A135	1,4,8,9,10,11,12,15,16,17,18,19,20
A147	2,4,9,10,11,19,20,22,24,26,28,29,30,33,36,37,40,42,44,45,46
A155	1,4,5,6,8,9,10,11,12,13,14,15,16,17,18,19,20
A159	1,2,6,8,9,10,11,12,15,16,17,19
A160	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20
A176	1,2,5,9,10,12,14,15,16,17,18,19,20
TRX-GST	1,2,3,4,5,6,8,9,10,11,12,13,14,18,20

Supplemental Table 2. Active residues during molecular docking<sup>a</sup>

<b>Aptamer</b>	TGMV- Replication % (aprox)	<b>Binds to Rep</b>								
		TGMV	ToMoV	CaLCuV	CLCuV	EACMV	ACMV	TYLCV	BCTV	MSV
A5	30									
A6	25									
A22 <sup>bc</sup>	13	Y	Y	Y	Y	Y	Y	Y	Y	Y
A25	20									
A27	60									
A40 <sup>b</sup>	35	Y	Y	Y	Y	Y	N	Y	Y	Y
A46 <sup>b</sup>	30	Y	Y	Y	Y	Y	Y	Y	Y	Y
A53	75									
A59 <sup>b</sup>	49	Y	Y	Y	Y	Y	Y	Y	Y	Y
A63	75									
A64 <sup>bc</sup>	23	Y	Y	Y	N	Y	Y	Y	Y	Y
A67	60									
A71	35									
A84 <sup>b</sup>	20	Y	Y	Y	Y	Y	Y	Y	Y	Y
A94	35									
A99 <sup>b</sup>	20	Y/N	Y	Y	N	N	Y	Y	N	Y
A101	52									
A116 <sup>b</sup>	15	Y	Y	Y	N	Y	Y	Y	Y	Y
A127		Y	Y	Y	Y	Y	N	Y	Y	Y
A130	75									
A131	10									
A132 <sup>b</sup>	45	Y	Y	Y	Y	Y	N	Y	Y	Y
A135 <sup>b</sup>		Y	Y	Y	Y	Y	N	Y	Y	Y
A137	59									
A140	40									
A146	45									

A147 <sup>b</sup>	12	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
A153	75										
A155 <sup>b</sup>	28	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
A159 <sup>b</sup>	28	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
A160 <sup>b</sup>	14	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
A168	53										
A169	40										
A173	30										
A174	33										
A176 <sup>b</sup>	58	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
A177	53										

<sup>a</sup>References:

Lopez-Ochoa L, Ramirez-Prado J, Hanley-Bowdoin L. Peptide aptamers that bind to a geminivirus replication protein interfere with viral replication in plant cells. *J Virol.* 2006 Jun;80(12):5841-53. doi: 10.1128/JVI.02698-05. PMID: 16731923; PMCID: PMC1472579.

Reyes MI, Nash TE, Dallas MM, Ascencio-Ibáñez JT, Hanley-Bowdoin L. Peptide aptamers that bind to geminivirus replication proteins confer a resistance phenotype to tomato yellow leaf curl virus and tomato mottle virus infection in tomato. *J Virol.* 2013 Sep;87(17):9691-706. doi: 10.1128/JVI.01095-13. Epub 2013 Jul 3. PMID: 23824791; PMCID: PMC3754110.

<sup>b</sup>Used in this work

<sup>c</sup>Tested in transgenic plants

**Supplemental Table 3. Z-Scores higher than 3 for peptide:protein pairs**

Peptide	Organism	Peptide	Protein	Peptide	Organism	Peptide	Protein
A22	ACMV	3	85	A64	ACMV		
		4	68, 83, 85-87				
		5	85				
		6	12, 52, 85				
		11,14	87				
	BCTV	2	46		BCTV	4	87, 94
		3	88			5	54, 101
		4	44, 87, 88, 94, 101			12	85
		5	87			14	88
		6	85-88				
		7	85				
		8	85-87				
		11, 15	85				
		17, 18	88				
	CalCuV	2	61, 92, 94		CalCuV	4	92, 94
		12	18, 61, 92, 94, 104			5	61, 92, 94, 104
		16	18, 92, 94			7	94
		17	94			12	18, 92, 94
		18	18, 59, 61, 94			14	92
	CLCuBV	2	87, 91		CLCuBV		
		9	94				
		12	93, 94				
		18	8				
	EACMV	3	85, 87		EACMV	5	85
		4	85-87			6, 7	87
		5	87			8	87, 90
		6	12, 54, 85, 87			10	94
		8	52, 54				
		12	94				
		14, 15	87				
	MSV	2	51, 78, 80		MSV	4	93
		3	80			5	18, 58, 60, 91, 93
		4	43, 45, 51, 78, 80			7	93
		6	11, 87			12	58, 91, 93
		9	45, 87			17	53
		12	43, 51, 78				

		16	11, 80 ,87				
		17-19	87				
<b>TGMV</b>		2	100	<b>TGMV</b>		4	93
		3	93			5	18, 58, 60, 91, 93
		4	8, 58, 60, 91, 93, 100, 104			7	93
		6	16, 18, 58, 60, 91, 93			12	58, 91, 93
		14-15	93			17	53
		4	14, 46, 52, 85			4	54
<b>ToMoV</b>		7	12, 44, 52, 54			5	97, 101
		8, 9	87			8	46
		16	46			12, 13	85
		20	94			15	87
						17	94
<b>TYLCV</b>		4	14, 85	<b>TYCLV</b>		5	54
		6	12, 44, 52, 54			8	85
		20	94			12	85, 87