Table S1. Programs that detected hotspot residues at the intraprotomer interface [1].

Protein	Residue	KFC2	ROBETTA	Hot Region	PredHS	PPCheck
VP1-VP2 Interface						
VP1	E6				■	■
	R94	■	■	■	■	
	W95	■	■	■	■	■
	V96	■	■	■		
	Y124	■	■	■	■	
	W202	■	■		■	■
	F203	■	■	■		
	N204	■	■	■	■	■
	W206	■	■	■	■	
	F215	■	■	■	■	■
	R249	■	■		■	■
	L252	■	■		■	
	F253	■	■		■	
	T260		■		■	
VP2	E133	■	■	■	■	
	F134		■	■		
	Y135	■	■	■		
	T136		■	■		
	E146		■		■	
	R168	■	■		■	
	Y169	■	■			
	F175	■	■	■	■	■
	F176	■	■	■	■	■
	M178	■		■	■	■
	Q185		■		■	
	Y189	■	■		■	
	V211		■		■	
VP1-VP3 Interface						
VP1	T31		■		■	■
	V33	■	■	■	■	
	F36	■	■	■		
	Y114		■	■		
	C118		■	■		
	F119	■	■	■	■	■
	F122	■	■	■	■	■
	F187	■	■	■		
	V189		■	■		
	R239	■	■			
	V245	■	■			

	F246					
	F254					
	W256					
	I272					
VP3	I25					
	F41					
	L44					
	L47					
	V98					
	R100					
	F102					
	R106					
	L112					
	V114					
	F159					
	N160					
	F161					
	D217					
	W229					
	Q232					
VP1-VP4 Interface						
VP1	K9					
	D128					
	K241					
VP2-VP3 Interface						
VP2	W184					
	Q185					
	R197					
	L230					
	S231					
VP3	F53					
	F115					
	T116					
	L209					
VP2-VP4 Interface						
VP2	R32					
	L33					
	Y36					
VP3-VP4 Interface						
No residues predicted to be important for stability of interface						

Table S1. Footnotes:

Grey blocks indicate where a program has predicted a residue to be a hotspot. Residues that were detected as hotspots by two or more programs are shown.

Table S2. Programs that detected hotspot residues at the interprotomer interface [1].

Protein	Residue	KFC2	ROBETTA	Hot Region	PredHS	PPCheck
VP1-VP1						
VP1	L160		■	■		■
	I161		■	■		■
	Y163		■		■	
	W176	■	■		■	■
	V178		■			■
	M45	■	■			
	L108		■	■		
VP1-VP2						
VP1	V24				■	■
VP1-VP3						
VP1	Q30			■		■
	F35				■	■
	P153				■	■
	R171		■			■
	P194				■	■
VP3	Q11		■		■	
	C13		■		■	
	S16		■		■	
	N103	■	■	■	■	
	Q104	■		■	■	
	Q173		■	■	■	
	I181		■			■
	M222	■	■	■	■	■
VP1-VP4						
VP4	Y29				■	■
	L35		■		■	■
VP2-VP3						
VP2	S47			■	■	■
	C48		■	■		
	Y104	■		■	■	
	L105	■	■	■	■	
VP3	P131				■	■
	F165	■	■	■	■	
	I166	■	■	■	■	■
	P168				■	■
VP3-VP3						
No residues predicted to be important for stability of interface						
VP3-VP4						
VP4	Y25				■	■

	Y29					
VP4-VP4						
VP4	G18					
	S26					

Table S2. Footnotes:

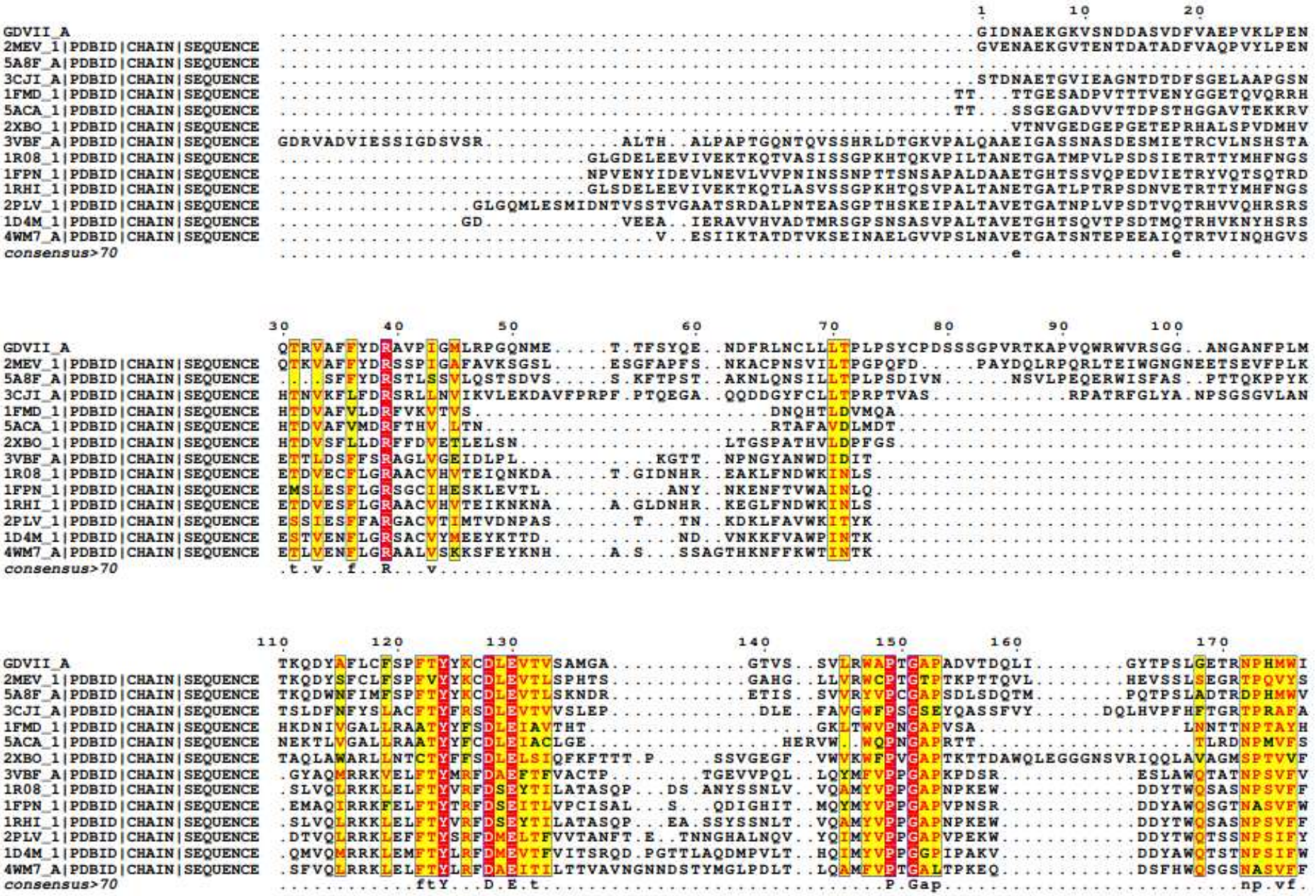
Grey blocks indicate where a program has predicted a residue to be a hotspot. Residues that were detected as hotspots by two or more programs are shown.

Table S3. Programs that detected hotspot residues at the interpentamer interface [1].

Protein	Residue	KFC2	ROBETTA	Hot Region	PredHS	PPCheck
VP1-VP2						
No residues predicted to be important for stability of interface						
VP2-VP2						
VP2	T53		■	■	■	■
	V56		■	■	■	■
	L57			■	■	■
	V95	■			■	■
	R102	■	■		■	■
	L259		■	■	■	■
VP2-VP3						
VP2	N25				■	■
	R61	■	■	■	■	
	Y62			■	■	
	Y63	■	■	■		
	T64	■	■	■	■	
	N117		■		■	■
	S240	■			■	
VP3	K124	■		■	■	■
	R140		■	■		
	M144	■		■	■	
	Y148	■	■	■	■	■
	I150	■	■	■	■	■
	D152	■		■	■	
	L153		■	■		
	T194	■	■		■	■
	P195	■			■	
	T197	■	■	■	■	
	Y198	■	■	■	■	■
VP2-4						
No residues predicted to be important for stability of interface						

Table S3. Footnotes:

Grey blocks indicate where a program has predicted a residue to be a hotspot. Residues that were detected as hotspots by two or more programs are shown.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3VBF_A|PDBID|CHAIN|SEQUENCE
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1FPN_1|PDBID|CHAIN|SEQUENCE
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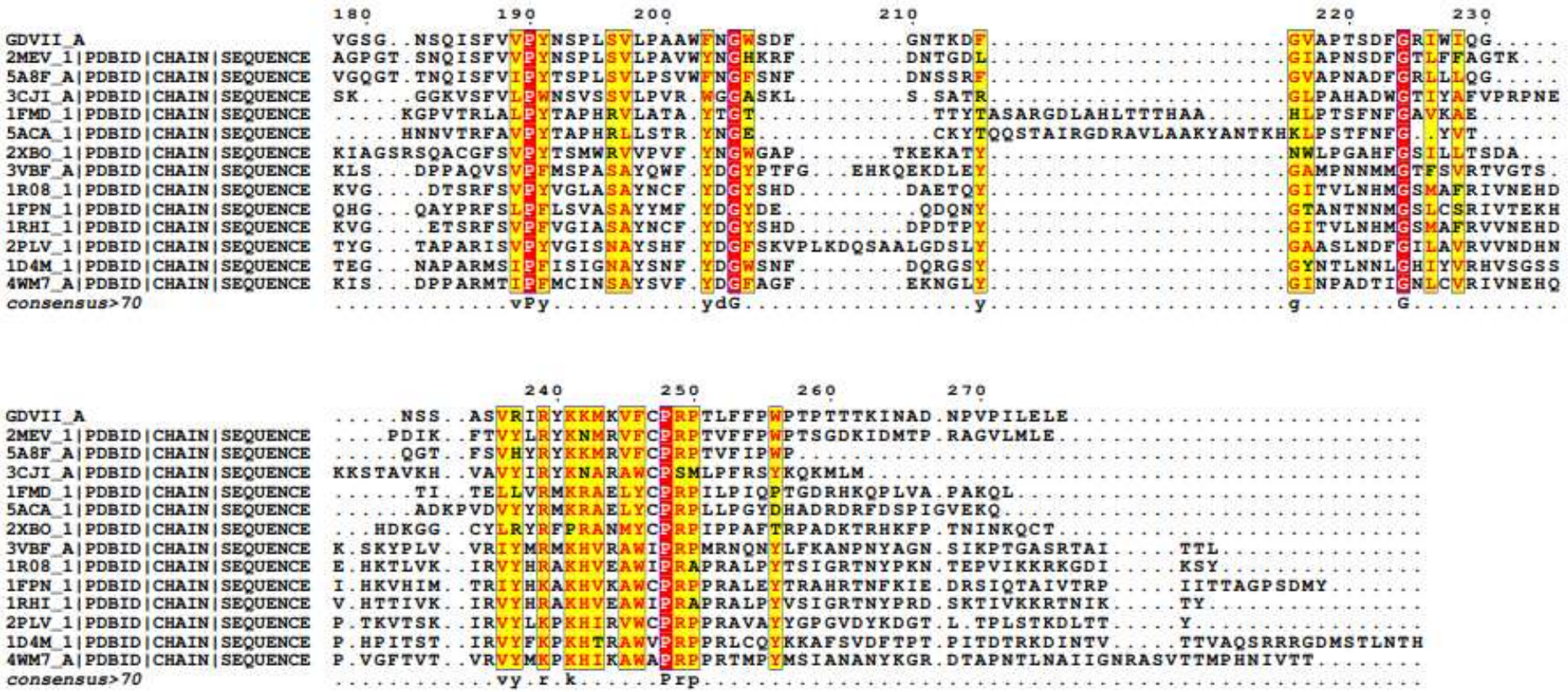
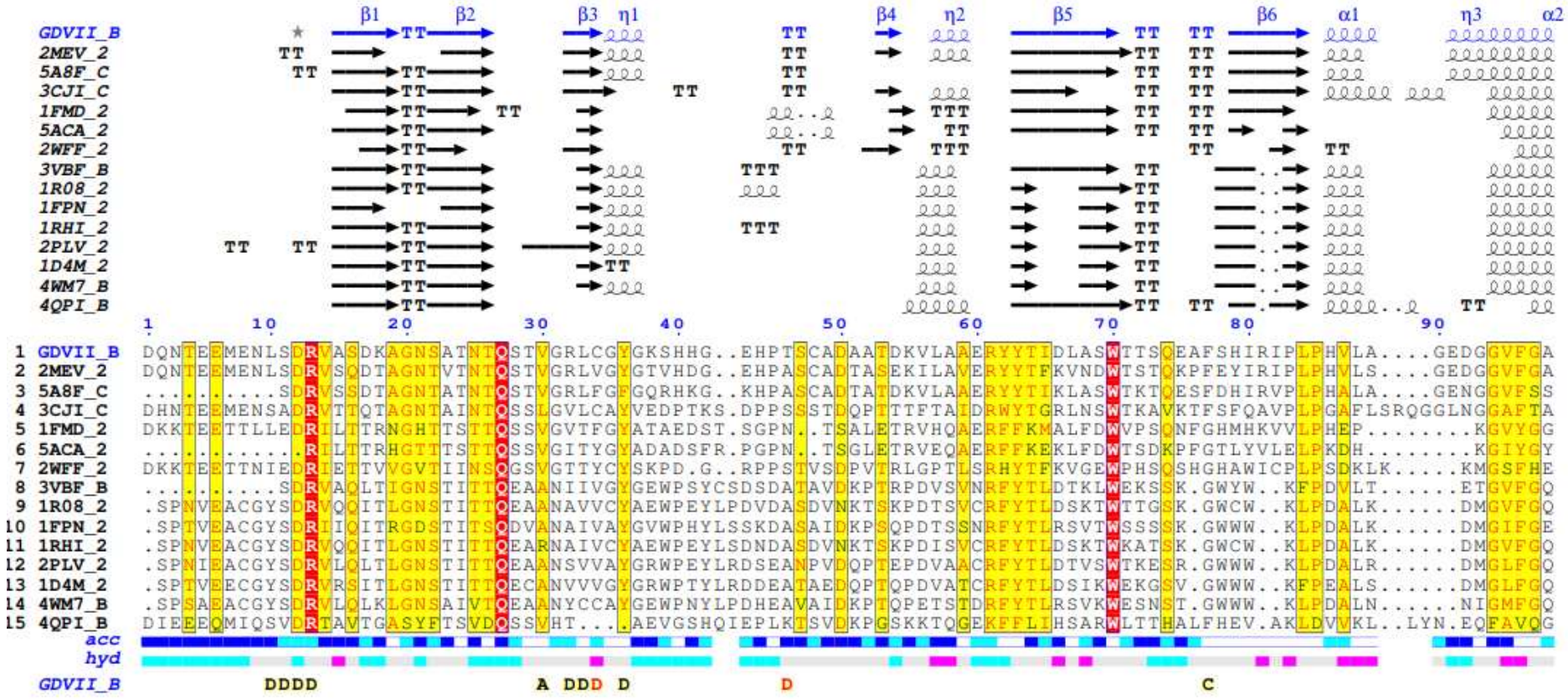


Figure S1. Sequence-based alignment of TMEV, cardiovirus, senecavirus, aphthovirus and enterovirus VP1 proteins [1]. VP1 protein sequences were obtained from the PDB database: cardioviruses MEV and SAFV (PDB: 2MEV, 5A8F); senecavirus SVV-1 (PDB: 3CJI); aphthoviruses FMDV type C and SAT 2, ERAV (PDB: 1FMD, 5ACA, 2WFF); and enteroviruses EV-71, HRV-B14, HRV-2, HRV-3, PV-1, CV-A9, EV-D68 (PDB: 3VBF, 1R08, 1FPN, 1RHI, 2PLV, 1D4M, 4WM7); and were submitted to ESpript (built into ENDscript) to produce a multiple sequence alignment of the amino acid sequences.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3CJI_C
1FMD_2
5ACA_2
2WFF_2
3VBF_B
1R08_2
1FPN_2
1RHI_2
2PLV_2
1D4M_2
4WM7_B
4QPI_B
1 10 20 30 40 50 60 70 80 90
1 GDVII_B DQNTEEMENLSDRVASDKAGNSATNTQSTVGRLCGYGKSHHG..EHPTSCADAATDKVLAAERYYTIDLASWTTSQEAFSHIRIPLPHVLA....GEDGGVFGA
2 2MEV_2 DQNTEEMENLSDRVSQDTAGNTVTNTQSTVGRLVGYGTVHDG..EHPASCADTASEKILAVERYYTFKVNDWTSTQKPFEYIRIPLPHVLS....GEDGGVFGA
3 5A8F_C .....SDRVSSDTAGNTATNTQSTVGRLFGFGQRHKG..KHPASCADTATDKVLAAERYYTIKLASWTKTQESFDHIRVPLPHALA....GENGGVFSS
4 3CJI_C DHNTEEMENSADRVTTQTAGNTAINTQSSLGVLCAYVEDPTKS.DPPSSSTDQPTTTFTAIDRWYTGRLNSWTKAVKTFSFQAVPLPGAFLSRQGGLNGGAFTA
5 1FMD_2 DKKTEETTLLEDRILTTRNGHTTSTTQSSVGVTFGYATAEDST.SGPN..TSALETRVHQAERFFKMALFDWVPSQNFGHMHKVVLPHEP.........KGVYGG
6 5ACA_2 .....RILTTRHGTTTSTTQSSVGITYGYADADSFR.PGPN..TSGLETRVEQAERFFKEKLFDWTSDKPFGTLYVLELPKDH.........KGIYGY
7 2WFF_2 DKKTEETTNIEDRIETTVVGVTIINSQGSVGTTYCYSKPD.G..RPPSTVSDPVTRLGPTLSRHYTFKVGEWPHSQSHGHAWICPLPSDKLK.....KMGSFHE
8 3VBF_B .....SDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDSDATAVDKPTRPDVSVNRFYTLDTKLWEKSSK.GWYW..KFPDVLT......ETGVFGQ
9 1R08_2 .SPNVEACGYSDRVQQITLGNSTITTQEAANAVVCYAEWPEYLPDVDASDVNKTSKPDTSVCRFYTLDSKTWTTGSK.GWCW..KLPDALK......DMGVFGQ
10 1FPN_2 .SPTVEACGYSDRIIQITRGDSTITSQDVANAIVAYGVWPHYLSSKDASAIDKPSQPDTSSNRFYTLRSVTWSSSSK.GWWW..KLPDALK......DMGIFGE
11 1RHI_2 .SPNVEACGYSDRVQQITLGNSTITTQEARNAIVCYAEWPEYLSDNDASDVNKTSKPDISVCRFYTLDSKTWKATSK.GWCW..KLPDALK......DMGVFGQ
12 2PLV_2 .SPNIEACGYSDRVLQLTLGNSTITTQEAANSVVAYGRWPEYLRDSEANPVDQPTEPDVAACRFYTLDTVSWTKESR.GWWW..KLPDALR......DMGLFGQ
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14 4WM7_B .SPSAEACGYSDRVLQLKLGNSAIVTQEAANYCCAYGEWPNYLPDHEAVAIDKPTQPETSTDRFYTLRSVKWESNST.GWWW..KLPDALN......NIGMFGQ
15 4QPI_B DIEEEQMIQSVDRTAVTGASYFTSVDQSSVHT....AEVGSHQIEPLKTSVDKPGSKKTQGEKFFLIHSARWLTTHALFHEV.AKLDVVKL..LYN.EQFAVQG
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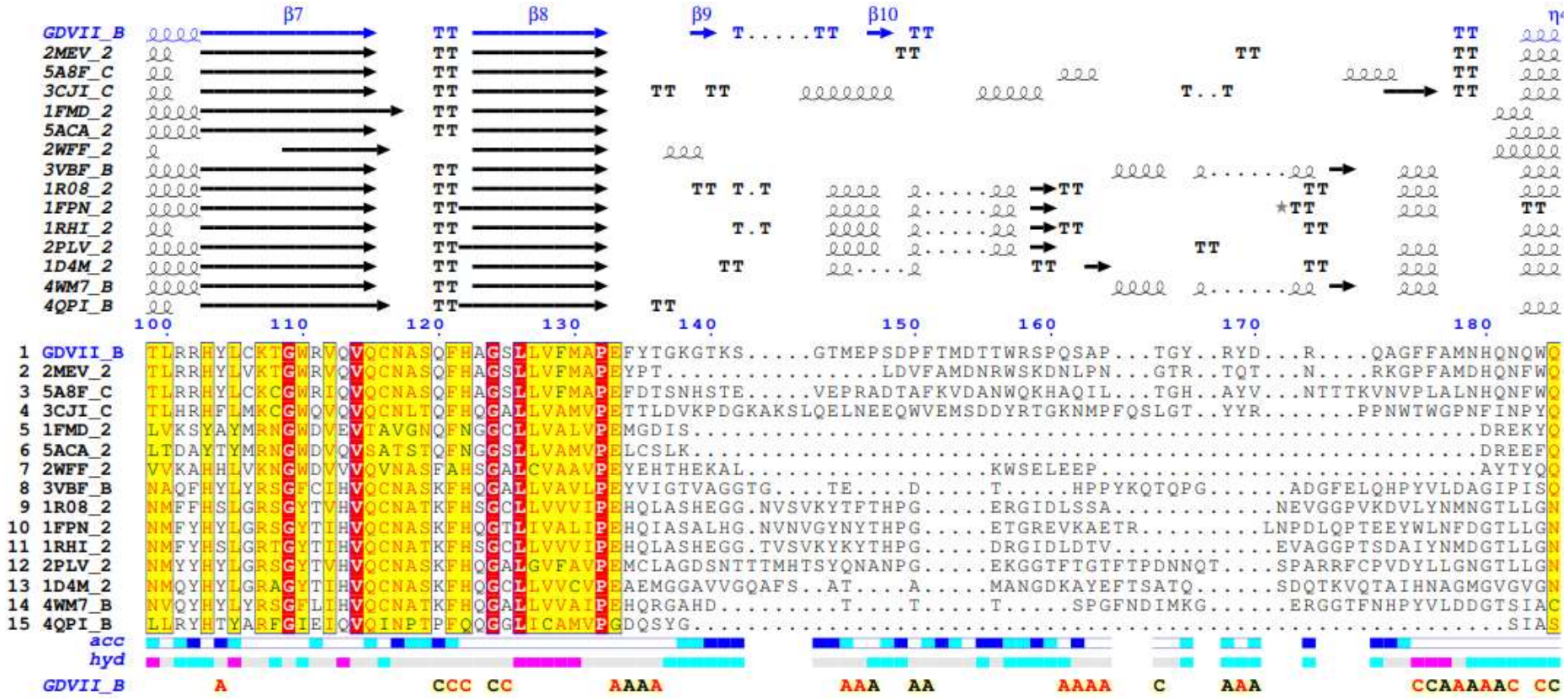
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3VBF_B
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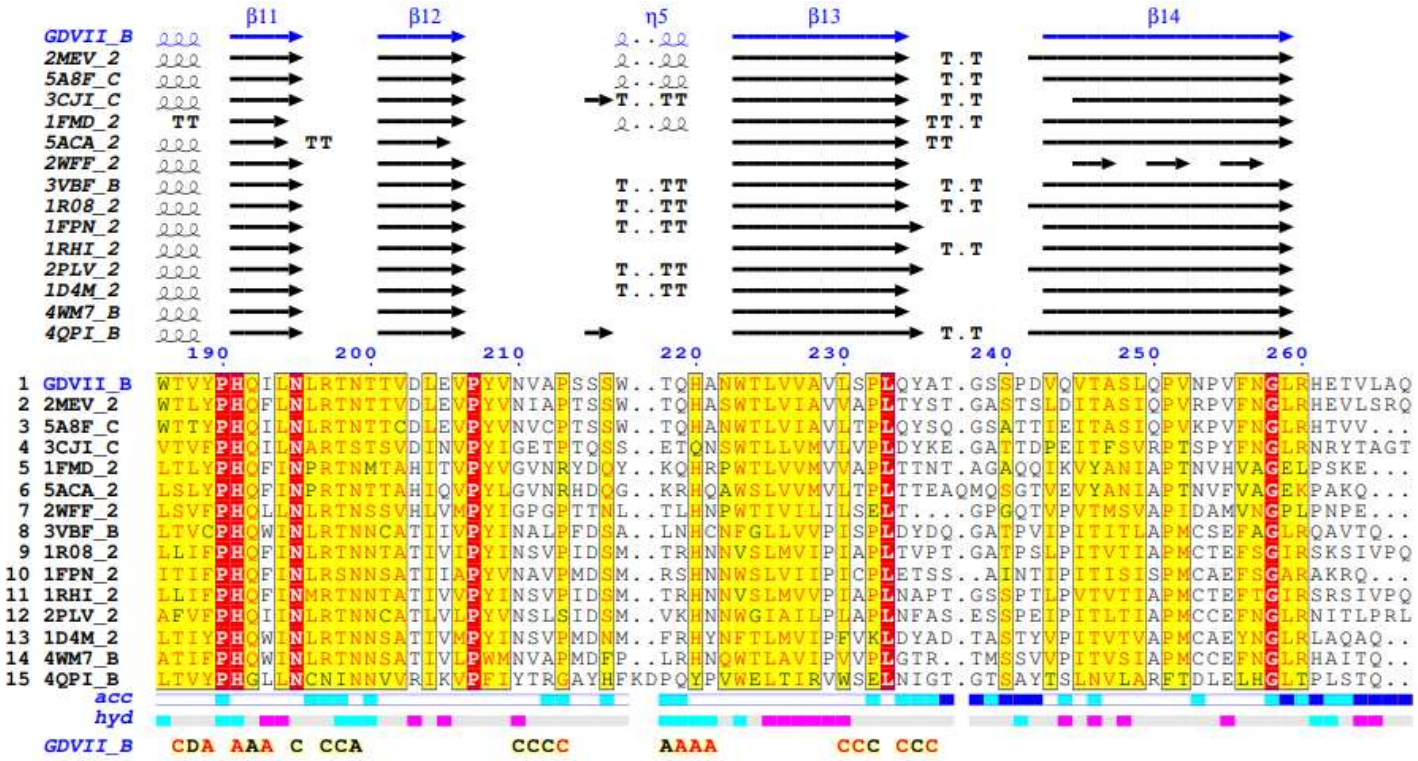
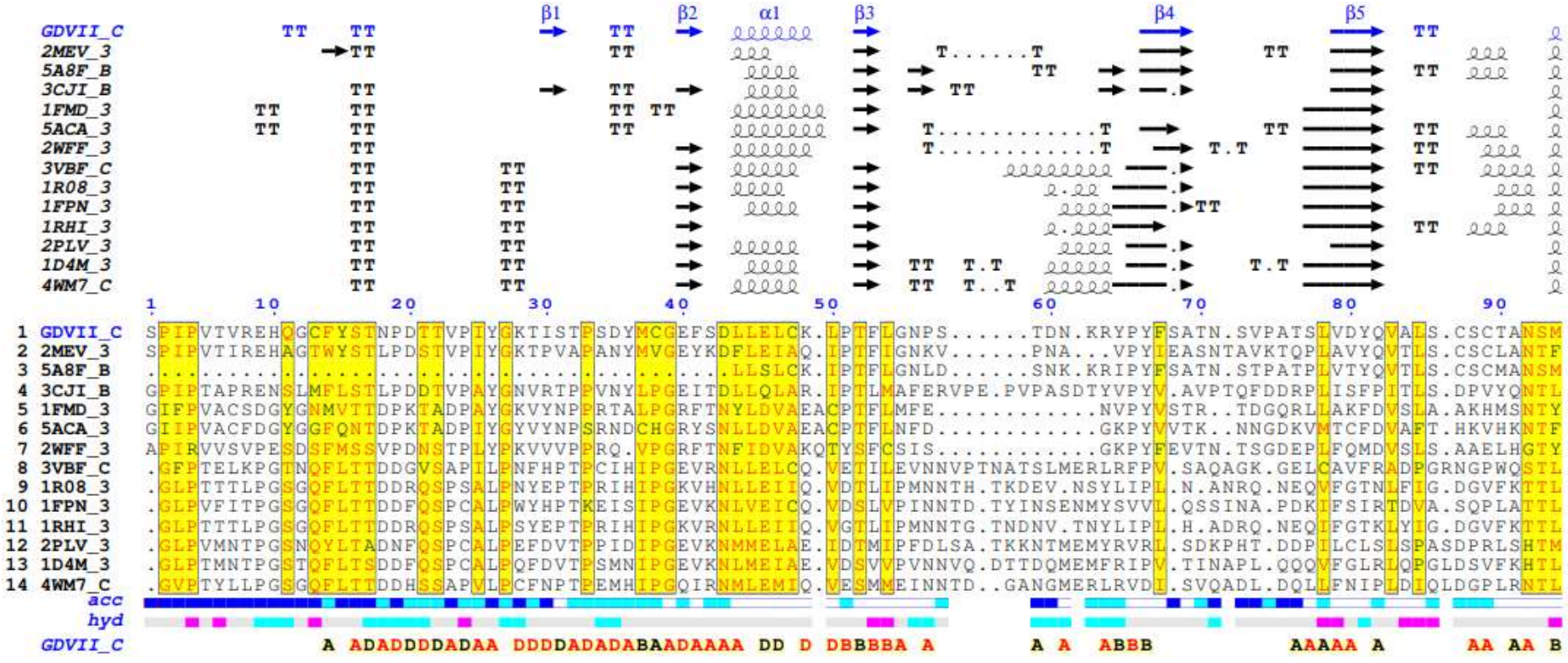


Figure S2. Sequence and structure-based alignment of TMEV VP2 with cardiovirus, senecavirus, aphthovirus and enterovirus representatives [1]. Chain B representing VP2 of the TMEV protomer was submitted to ENDscript2. A subset of structures provided by ENDscript2 was selected for the alignment and included: cardioviruses MEV and SAFV (PDB: 2MEV, 5A8F); senecavirus SVV-1 (PDB: 3CJI); aphthoviruses FMDV type C and SAT 2, ERAV (PDB: 1FMD, 5ACA, 2WFF); enteroviruses EV-71, HRV-B14, HRV-2, HRV-3, PV-1, CV-A9, EV-D68 (PDB: 3VBF, 1R08, 1FPN, 1RHI, 2PLV, 1D4M, 4WM7) and HAV (4QPI).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β2
α1
β3
β4
β5
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5A8F_B
3CJI_B
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3 5A8F_B .....LLSLCK.IPTFLGNLD.....SNK.KRIPYFSATN.STPATPLVTYQVTLS.CSCMANSM
4 3CJI_B GPIPTAPRENSLMFLSTLPDDTVPAYGNVRTPPVNYLPGEITDLLQLAR.IPTLMAFERVPE.PVPASDTYVPYV.AVPTQFDDRPLISFPITLS.DPVYQNTL
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6 5ACA_3 GIIPVACFDGYGGFQNTDPKTADPIYGYVYNPSRNDCHGRYSNLLDVAEACPTFLNFD.....GKPYVVTK..NNGDKVMTCFDVAFT.HKVHKNTF
7 2WFF_3 APIRVVSVPESDSFMSSVPDNSTPLYPKVVVPPRQ.VPGRFTNFIDVAKQTYSFCSIS.....GKPYFEVTN.TSGDEPLFQMDVSLS.AAELHGTY
8 3VBF_C .GFPTELKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLLELCQ.VETILEVNNVPTNATSLMERLRFPV.SAQAGK.GELCAVFRADPGRNGPWQSTL
9 1R08_3 .GLPTTTLPGSGQFLTTDDRQSPSALPNYEPTPRIHIPGKVHNLLEIIQ.VDTLIPMNNTH.TKDEV.NSYLIPL.N.ANRQ.NEQVFGTNLFIG.DGVFKTTL
10 1FPN_3 .GLPVFITPGSGQFLTTDDFQSPCALPWYHPTKEISIPGEVKNLVEICQ.VDSLVPINNTD.TYINSENMYSVVL.QSSINA.PDKIFSIRTDVA.SQPLATTL
11 1RHI_3 .GLPTTTLPGSGQFLTTDDRQSPSALPSYEPTPRIHIPGKVRNLLEIIQ.VGTLIPMNNTG.TNDNV.TNYLIPL.H.ADRQ.NEQIFGTKLYIG.DGVFKTTL
12 2PLV_3 .GLPVMNTPGSNQYLTADNFQSPCALPEFDVTPPIDIPGEVKNMMELAE.IDTMIPFDLSA.TKKNTMEMYRVRL.SDKPHT.DDPILCLSLSPASDPRLSHTM
13 1D4M_3 .GLPTMNTPGSTQFLTSDDFQSPCALPQFDVTPSMNIPGEVKNLMEIAE.VDSVVPVNNVQ.DTTDQMEMFRIPV.TINAPL.QQQVFGLRLQPGLDSVFKHTL
14 4WM7_C .GVPTYLLPGSGQFLTTDDHSSAPVLPCFNPTPEMHIPGQIRNMLEMIQ.VESMMEINNTD..GANGMERLRVDI.SVQADL.DQLLFNIPLDIQLDGPLRNTL
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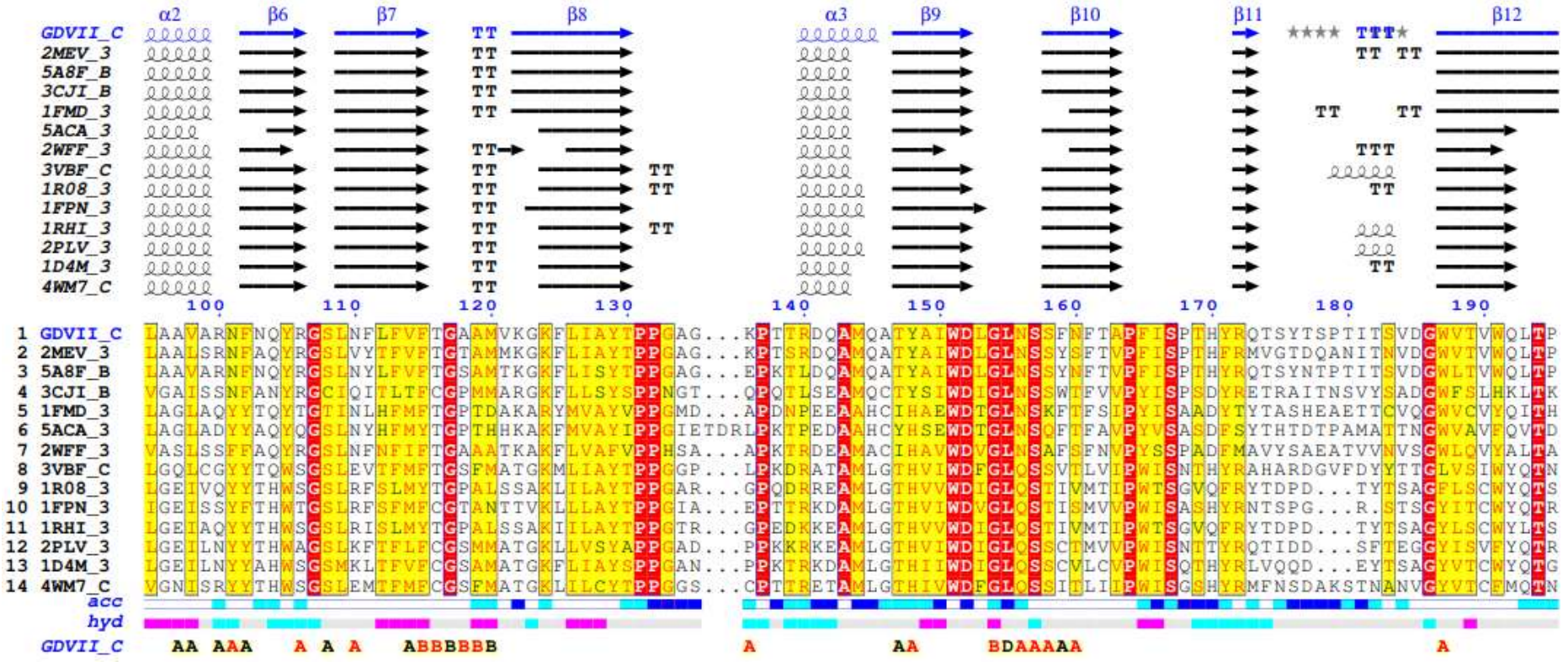
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3VBF_C
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1 GDVII_C LAAVARNFNQYRGSLNFLFVFTGAAMVKGKFLIAYTPPGAG...KPTTRDQAMQATYAIWDLGLNSSFNFTAPFISPTHYRQTSYTSPTITSVDGWVTVWQLTP
2 2MEV_3 LAALSRNFAQYRGSLVYTFVFTGTAMMKGKFLIAYTPPGAG...KPTSRDQAMQATYAIWDLGLNSSYSFTVPFISPTHFRMVGTDQANITNVDGWVTVWQLTP
3 5A8F_B LAAVARNFNQYRGSLNYLFVFTGSAMTKGKFLISYTPPGAG...EPKTLDQAMQATYAIWDLGLNSSYNFTVPFISPTHYRQTSYNTPTITSVDGWLTVWQLTP
4 3CJI_B VGAISSNFANYRGCIQITLTFCGPMMARGKFLLSYSPPNGT...QPQTLSEAMQCTYSIWDIGLNSSWTFVVPYISPSDYRETRAITNSVYSADGWFSLHKLTK
5 1FMD_3 LAGLAQYYTQYTGTINLHFMFTGPTDAKARYMVAYVPPGMD...APDNPEEAAHCIHAEWDTGLNSKFTFSIPYISAADYTYTASHEAETTCVQGWVCVYQITH
6 5ACA_3 LAGLADYYAQYQGSLNYHFMYTGPTHHKAKFMVAYIPPGIETDRLPKTPEDAAHCYHSEWDTGLNSQFTFAVPYVSASDFSYTHTDTPAMATTNGWVAVFQVTD
7 2WFF_3 VASLSSFFAQYRGSLNFNFIFTGAAATKAKFLVAFVPPHSA...APKTRDEAMACIHAVWDVGLNSAFSFNVPYSSPADFMAVYSAEATVVNVSGWLQVYALTA
8 3VBF_C LGQLCGYYTQWSGSLEVTFMFTGSFMATGKMLIAYTPPGGP...LPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAHARDGVFDYYTTGLVSIWYQTN
9 1R08_3 LGEIVQYYTHWSGSLRFSLMYTGPALSSAKLILAYTPPGAR...GPQDRREAMLGTHVVWDIGLQSTIVMTIPWTSGVQFRYTDPD...TYTSAGFLSCWYQTS
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11 1RHI_3 LGEIAQYYTHWSGSLRISLMYTGPALSSAKIILAYTPPGTR...GPEDKKEAMLGTHVVWDIGLQSTIVMTIPWTSGVQFRYTDPD...TYTSAGYLSCWYLTS
12 2PLV_3 LGEILNYYTHWAGSLKFTFLFCGSMMATGKLLVSYAPPGAD...PPKKRKEAMLGTHVIWDIGLQSSCTMVVPWISNTTYRQTIDD...SFTEGGYISVFYQTR
13 1D4M_3 LGEILNYYAHWSGSMKLTFVFCGSAMATGKFLIAYSPPGAN...PPKTRKDAMLGTHIIWDIGLQSSCVLCVPWISQTHYRLVQQD...EYTSAGYVTCWYQTG
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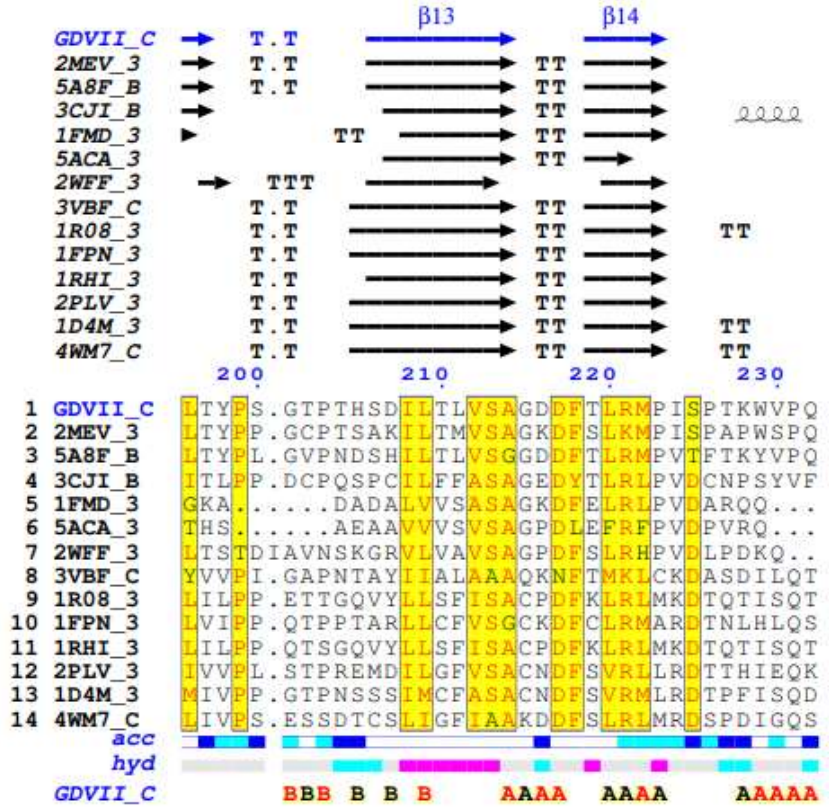

Figure S3. Sequence and structure-based alignment of TMEV VP3 with cardiovirus, senecavirus, aphthovirus and enterovirus representatives [1]. Chain C representing VP3 of the TMEV protomer was submitted to ENDscript2. A subset of structures provided by ENDscript2 was selected for the alignment and included: cardioviruses MEV and SAFV (PDB: 2MEV, 5A8F); senecavirus SVV-1 (PDB: 3CJI); aphthoviruses FMDV type C and SAT 2, ERAV (PDB: 1FMD, 5ACA, 2WFF); and enteroviruses EV-71, HRV-B14, HRV-2, HRV-3, PV-1, CV-A9, EV-D68 (PDB: 3VBF, 1R08, 1FPN, 1RHI, 2PLV, 1D4M, 4WM7).

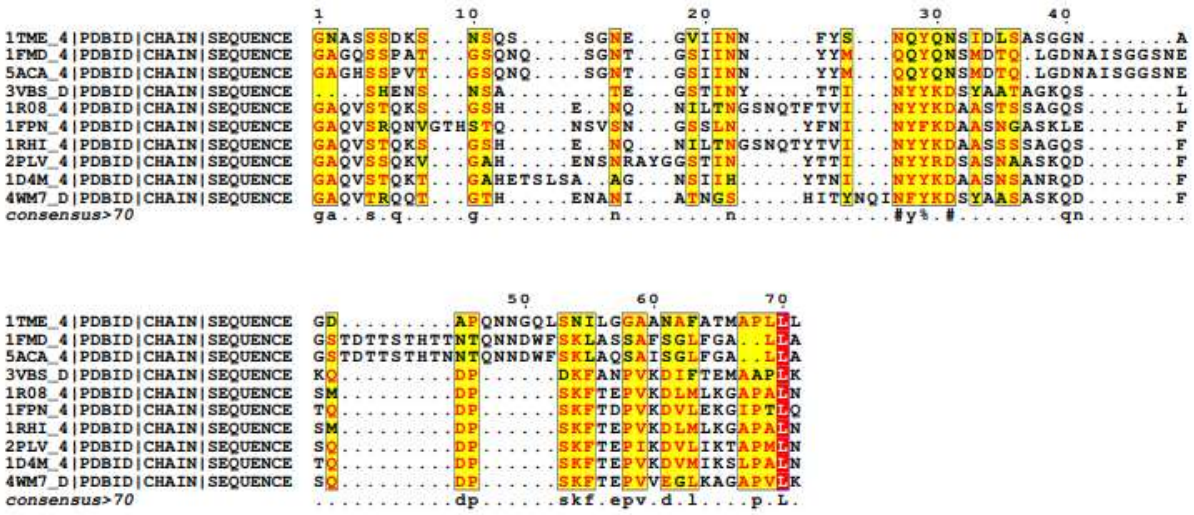

Figure S4. Sequence-based alignment of TMEV, aphthovirus, cardiovirus and enterovirus VP4 proteins [1]. VP4 protein sequences were obtained from the PDB database: TMEV (PDB: 1TME); aphthoviruses FMDV type C and SAT 2, ERAV (PDB: 1FMD, 5ACA, 2WFF); and enteroviruses EV-71, HRV-B14, HRV-2, HRV-3, PV-1, CV-A9, EV-D68 (PDB: 3VBS, 1R08, 1FPN, 1RHI, 2PLV, 1D4M, 4WM7) and were submitted to ESpript (built into ENDscript) to produce a multiple sequence alignment of the amino acid sequences.

Reference:

1. Upfold, N.S. Exploring the structural integrity of a picornavirus capsid, Rhodes University, 2019. http://hdl.handle.net/10962/131837.