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Supplemental Information

Conservation of Structure and Immune Antagonist Functions of Filoviral VP35 Homologs Present in Microbat Genomes

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Table S1. Crystallographic Data Collection and Structure Determination Statistics of batVP35 RR, Related to Figure 6.

Data Collection	
Wavelength (Å)	1.77110
Space group	$P3_2$ 2 1
Unit cell parameters	
Size (Å)	a=78.1, b=78.1, c=43.5
Angle (º)	α=γ=90.0, β=120
Resolution range (Å)	39.063-2.6 (2.693 - 2.6)
Unique reflections	4730 (409)
Completeness (%)	95.1 (86.5)
Average redundancy	6.8 (6.7)
mean $I/\sigma(I)$	11.97 (1.08)
R_{merge} (%)[a]	0.0873 (1.42)
$CC_{1/2}$ [b]	0.998 (0.609)
CC*[c]	1 (0.87)
Wilson B factors (Å^2)	61.8
Refinement Statistics	
Reflections	4662 (409)
Free reflections	200 (9)
R factor (%)[d]	17.10 (25.98)
R_{free} (%)[e]	22.27 (31.50)
RMSD bond lengths (Å)[f]	0.005
RMSD bond angles (º)	0.75
Mean B factors (Å^2)	67.76
Validation and stereochemistry [g]	
Number of protein residues	125
Number of waters	7
Most favored residues (%)	96.75
Generously allowed residues (%)	3.25
Outlier (%)	0
Molprobity Clashscore, all atoms	7.61 (83rd percentile)
Molprobity Score	1.92 (92nd percentile)

Parameters for the outermost resolution shell are shown in parentheses.
[a] $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of ith observation and $\langle I(hkl)\rangle$ is the mean value for reflection hkl.
[b] CC1/2: percentage of correlation between intersities from random half-datasets;
[c] $CC^* = \sqrt{[2CC1/2(1+CC1/2)]}$.
[d] R factor $= \sum_{hkl} ||F_{obs}| - |F_{calc}|| / \sum_{hkl} |F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure-factor amplitudes, respectively.
[e] R_{free} is equivalent to the R factor, but calculated with reflections excluded from the refinement process (5% of all reflections).
[f] RMSD: root-mean-square deviation from ideal values.
[g] The categories were defined by MolProbity.

Table S2. Filovirus-like *Myotis* VP35 residues with significant evidence of positive selection. Only codons with significant scores for three methods are considered, Related to STAR methods.

Codon[a]	SLAC dN-dS	SLAC p-value	FEL dN-dS	FEL p-value	MEME ω^+	MEME p-value	FUBAR dN-dS	FUBAR Post. Pr.
189	12.460	0.088	40.186	0.024	>100	0.036	2.903	0.994
209	11.280	0.134	40.657	0.032	>100	0.047	2.719	0.993
223	6.824	0.228	23.171	0.064	>100	0.083	0.972	0.930

Residues under selection determined using DataMonkey Rapid Detection of Positive Selection.

[a] Using batVP35 numbering

Table S3. Filovirus-like *Myotis* VP35 residues with significant evidence of negative selection. Only codons with significant scores for three methods are considered, Related to STAR methods.

Codon[a]	SLAC dN-dS	SLAC p-value	FEL dN-dS	FEL p-value	FUBAR dN-dS	FUBAR Post. Pr.[b]
94	-10.608	0.055	-37.318	0.012	-2.158	0.980
103	-15.607	0.030	-65.626	0.006	-4.684	0.980
132	-16.129	0.012	-60.765	0.002	-4.431	0.999
134	-19.129	0.062	-93.188	0.028	-7.325	0.978
176	-12.097	0.037	-38.523	0.011	-2.261	0.983
178	-12.097	0.037	-51.164	0.004	-3.903	0.995
181	-15.607	0.030	-66.189	0.006	-4.721	0.981
210	-15.607	0.030	-50.335	0.008	-3.400	0.972
273	-12.097	0.037	-49.807	0.006	-4.381	0.996

[a] Using batVP35 numbering

[b] Posterior Probability

Figure S1

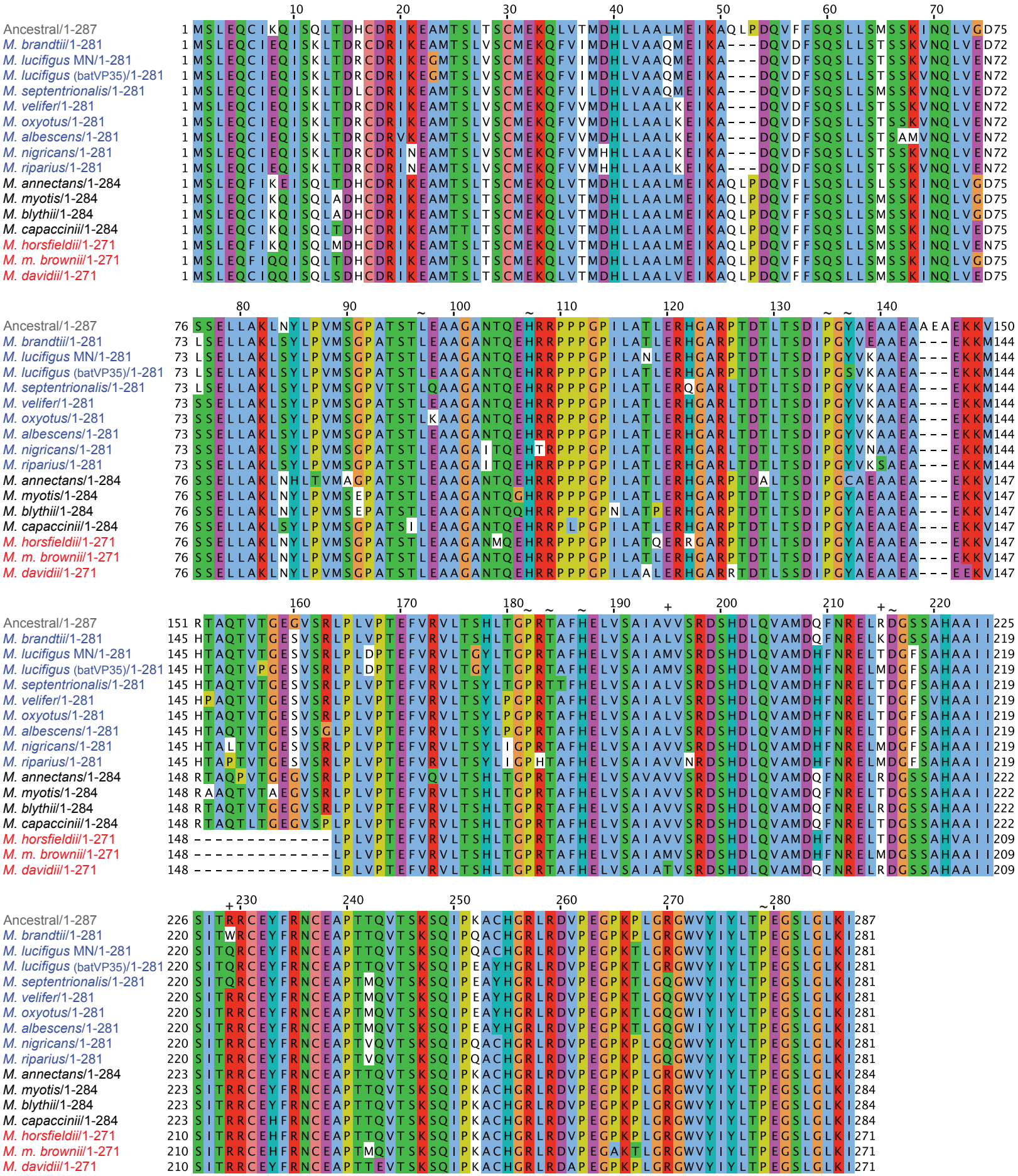

Figure S1. Alignment of Myotis VP35 sequences. Related to Figure 1. Amino acid sequences for each Myotis VP35 were aligned using MultAlin. Th imported into Jalview, and the default Clustalx coloring was applied. Residues under purifying selection are indicated with ~, residues under diversifying selection are indicated with +.

Figure S2

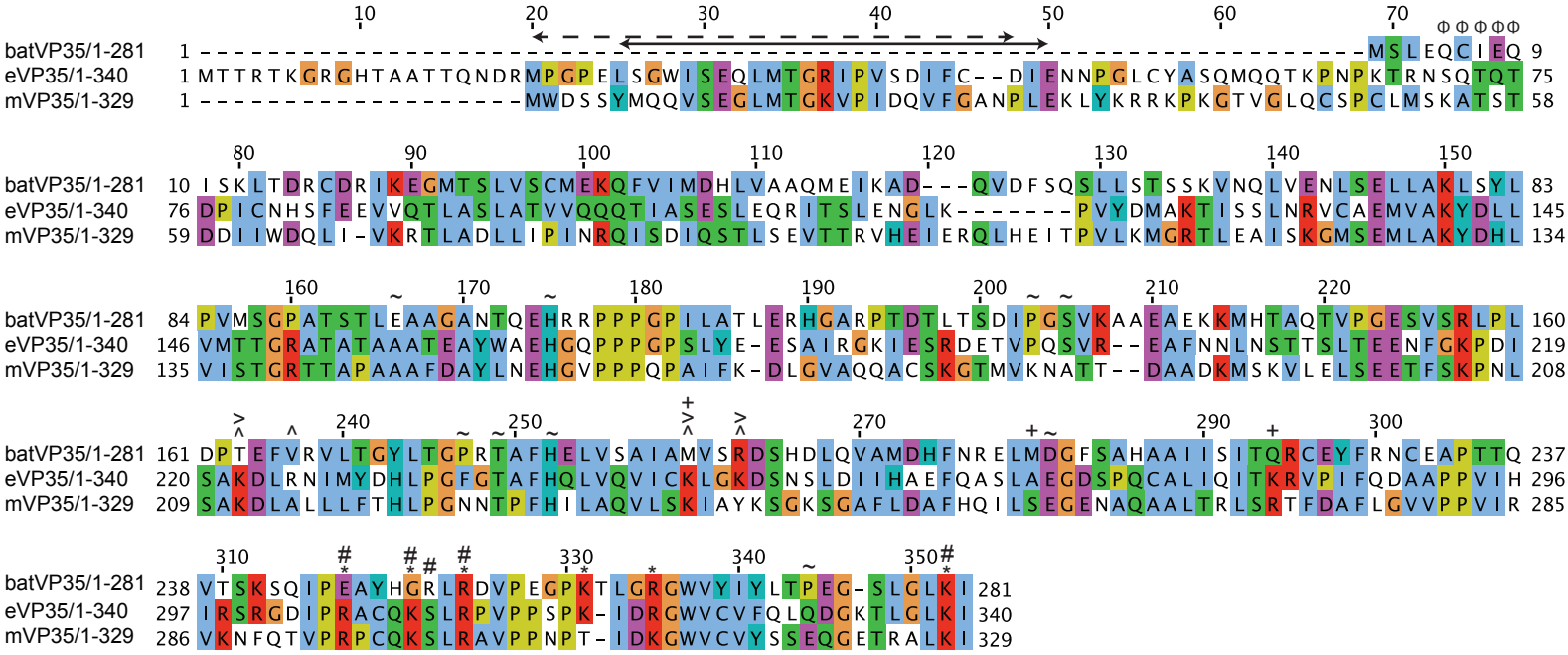

Figure S2. Alignment of batVP35 with extant filovirus VP35s. Related to Figure 2. Sequences were aligned using MultAlin and were imported into Jalview and the default Clustalx coloring was applied. Residues of interest are indicated using the following key: ~ residues under purifying selection in batVP35, + residues under diversifying selection in batVP35, # mVP35 central basic patch, * eVP35 central basic patch, > mVP35 first basic patch, ^ eVP35 first basic patch, and ϕ eVP35 LC8 binding motif. Solid line with arrowheads denotes eVP35 NP binding peptide (NPBP) and dashed line with arrowheads indicates mVP35 NPBP.

Figure S3

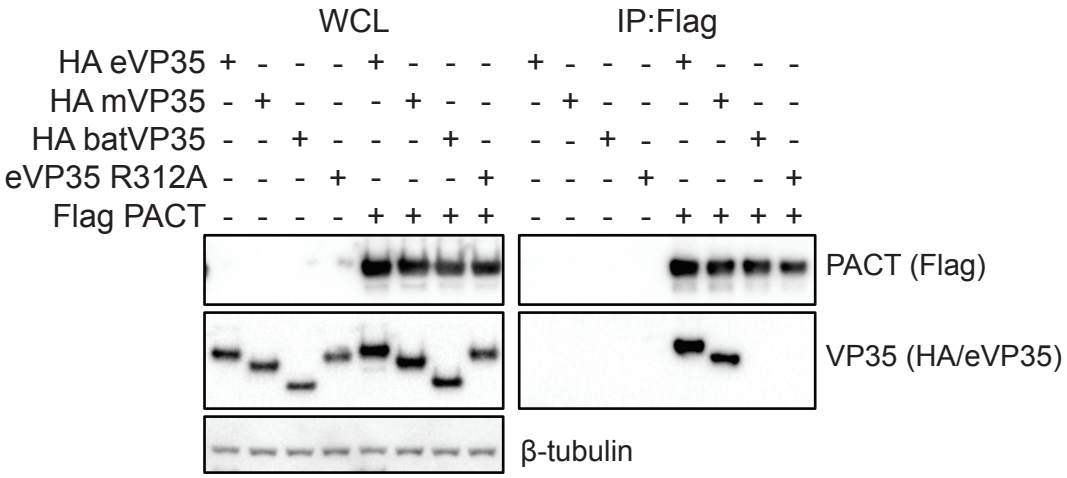

Figure S3. batVP35 does not interact with PACT. Related to Figure 2. Co-immunoprecipitation assay performed with Flag antibody on lysates of HEK293T cells expressing Flag-tagged PACT, HA-tagged eVP35, mVP35 and batVP35 and untagged eVP35 R312A as indicated. The co-immunoprecipitation was repeated three times, and a representative western blot is shown. Western blots were performed for the HA and Flag epitope tags and anti-eVP35 as indicated. WCL, whole cell lysate; IP, immunoprecipitation.