

Supplementary Materials

Supplementary Table S1: Primers used for the whole genome sequencing of the EV-A71 genome

Name of Primer	Sequences (5→ 3)
5'-UTR Forward	TTAAAACAGCTGTGGGTTGTAC
5'-UTR Reverse	GTGGAGCCTTCTGTAGCTGAAT
VP4_VP2 Forward	CAATCAAACATGGGCTCACAGG
VP4_VP2 Reverse	TGATTCGTTCCCTGGTTTTGGCT
VP3 Forward	GATTTTGACCAAGGGGCAACTC
VP3 Reverse	GCTGGAAGCTTCACCAGTGTCTA
VP1 Forward	GCTTACATAATAGCACTAGCGGC
VP1 Reverse	GTAGCGAGGTGACGATTAACCA
2A_2B Forward	ACTGTGGGTTCATCATCAAAGTCCA
2A_2B Reverse	CTGCTGGAACAATCTTCTCCCT
2C Forward	AACCTGATTTCAGCGCTAGTGA
2C Reverse	AATCCAGCCTTGTTCCCTACAG
3A_3B_3C Forward	GTCGCAGGTTCTACATGGATTG
3A_3B_3C Reverse	AAACAGGGCTTGTTCAAAGTCG
3D_3'-UTR Forward	AAGAGGAGCTACTTTGCGAG
3D_3'-UTR Reverse	GCTATTCCGGTTATAACAAATTTAACC

Supplementary Table S2: PSI-BLAST of the VP1 protein sequence of the EV-A71 parental strain.

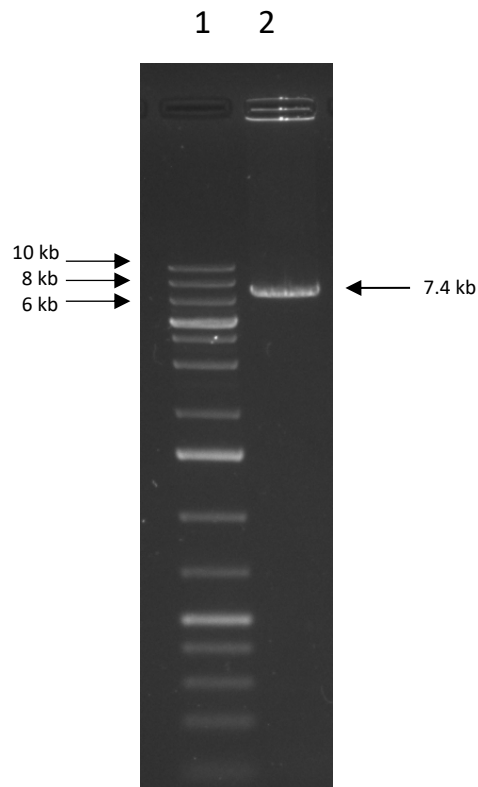
¹ The templates were chosen by protein blast of the VP1 protein sequence of the EV-A71 parental

No.	PDB ID	Percentage Identity (%)	Query Cover (%)	Resolution (Å)
1	4AED_A	99.33	100	3.80
2	612K_A	97.64	100	3.40
3	4CDQ-_A	95.96	100	2.65
4	3VBF_A	95.62	100	2.60

strain in NCBI using PDB database and PSI-BLAST (Position-Specific Iterated BLAST) algorithm.

Supplementary Table S3: Unpaired t-test analysis of the EV-A71/BP and the EV-A71/SP plaque variant using GraphPad Prism.

Unpaired t-test analysis:	MEAN PLAQUE SIZES RD	MEAN PLAQUE SIZES VERO
Column B	BP	BP
vs.	vs.	vs.
Column A	SP	SP
Unpaired t test		
P value	<0.0001	<0.0001
P value summary	****	****
Significantly different (P < 0.05)?	Yes	Yes
One- or two-tailed P value?	Two-tailed	Two-tailed
t, df	t=48.1 df=794	t=62.85 df=780
How big is the difference?		
Mean ± SEM of column A	293 ± 2.021, n=399	176.7 ± 1.68, n=408
Mean ± SEM of column B	518.6 ± 4.239, n=397	380.6 ± 2.851, n=394
Difference between means	225.6 ± 4.689	203.9 ± 3.245
95% confidence interval	216.4 to 234.8	197.6 to 210.3
R squared (eta squared)	0.7445	0.8351
F test to compare variances		
F, DFn, Dfd	4.376, 396, 398	2.639, 373, 407
P value	<0.0001	<0.0001
P value summary	****	****
Significantly different (P < 0.05)?	Yes	Yes



Supplementary Figure S1: Agarose gel electrophoresis of the full-length genomic cDNA of EV-A71/WT. The cDNA was produced with the LongAmp DNA polymerase. (1) Lane 1: DNA ladder (2) Lane 2: cDNA of the full-length EV71 genome, 7400 bp in length.

Supplementary Table S4: Amino acid changes observed on the genomes of EV-A71/BP and EV-A71/SP variants.

Position of amino acid	EV-A71 Variant	Change in amino acids	Type of mutation
VP1 ⁹⁷	EV-A71/BP	Isoleucine (I) → Leucine (L)	Non-synonymous
VP1 ¹⁰⁴	EV-A71/BP	Asparagine (N) → Serine (S)	Non-synonymous
VP1 ²⁴⁶	EV-A71/BP	Serine (S) → Proline (P)	Non-synonymous
VP1 ²⁸²	EV-A71/BP	Asparagine (N) → Aspartic Acid (D)	Non-synonymous
VP1 ⁹⁷	EV-A71/SP	Isoleucine (I) → Threonine (T)	Non-synonymous
VP1 ²³⁷	EV-A71/SP	Asparagine (N) → Threonine (T)	Non-synonymous
VP1 ²⁹²	EV-A71/SP	Threonine (T) → Alanine (A)	Non-synonymous

*The amino acid sequence of both the EV-A71/BP and EV-A71/SP variants were compared against the genome of the EV-A71/WT.

The amino acid sequence of the EV-A71/WT (parental EV-A71 strain) against EV-A71/BP and EV-A71/SP. Analysis was performed via Geneious Software and Clustal Omega and significant mutations of EV-A71/BP are: I97L, N104S, S246P and N282D. Significant mutations of EV-A71/SP are: I97T, N237T and T292A.

BP1	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
BP2	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
BP3	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
BP4	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
WT	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
SP1	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
SP2	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
SP3	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60
SP4	GDRVADVIESSIGDSVSRALTQALPAPTQNTQVSSHRLDTGEVPALQAAEIGASSNTSD	60

BP1	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
BP2	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
BP3	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
BP4	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
WT	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
SP1	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
SP2	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
SP3	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120
SP4	ESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDLP[97/97]EGTTNPSGYANWDIDITGYAQMR	120

BP1	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
BP2	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
BP3	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
BP4	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
WT	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
SP1	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
SP2	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
SP3	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180
SP4	RKVELFTYMRFDAEFTFVACTPTGQVVPQLLQYMFVPPGAPKPESRESLAWQTATNPSVF	180

BP1	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
BP2	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
BP3	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
BP4	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
WT	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
SP1	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
SP2	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
SP3	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240
SP4	VKLTDPQAQVSVPFMSPASAYQWFYDGYPTFGEHKQEKDLEYGACPNNMMGTFSVRNVGS	240

BP1	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
BP2	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
BP3	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
BP4	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
WT	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
SP1	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
SP2	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
SP3	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297
SP4	SKSKY[246]LVVRIYMRMKHVRRAWIPRPMRNQNYLFKANPNYAG[282]SIKPTGTSRAAITTL	297

SMALL PLAQUE: [cyan], BIG PLAQUE: [green] AND WILD TYPE: [yellow]

Alignment of sequencing results for the EV-A71/BP variant:

VP4 alignment for big plaque isolates

WT	MGSQVSTQ RSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
BP1	MGSQVSTQ RSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
BP2	MGSQVSTQ RSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
BP3	MGSQVSTQ RSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
BP4	MGSQVSTQ RSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60

WT	FTEMAAPLK	69
BP1	FTEMAAPLK	69
BP2	FTEMAAPLK	69
BP3	FTEMAAPLK	69
BP4	FTEMAAPLK	69

VP2 alignment for the big plaque isolates:

WT	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDATAVDKPTRPDVSV	60
BP1	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDATAVDKPTRPDVSV	60
BP2	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDATAVDKPTRPDVSV	60
BP3	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDATAVDKPTRPDVSV	60
BP4	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDATAVDKPTRPDVSV	60

WT	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHYLYRSGFCIHVQCNASKFHHQ	120
BP1	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHYLYRSGFCIHVQCNASKFHHQ	120
BP2	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHYLYRSGFCIHVQCNASKFHHQ	120
BP3	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHYLYRSGFCIHVQCNASKFHHQ	120
BP4	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHYLYRSGFCIHVQCNASKFHHQ	120

WT	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
BP1	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
BP2	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
BP3	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
BP4	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180

WT	QWINLRNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
BP1	QWINLRNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
BP2	QWINLRNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
BP3	QWINLRNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
BP4	QWINLRNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240

WT	MCSEFAGLRQAVTQ	254
BP1	MCSEFAGLRQAVTQ	254
BP2	MCSEFAGLRQAVTQ	254
BP3	MCSEFAGLRQAVTQ	254
BP4	MCSEFAGLRQAVTQ	254

VP3 alignment for big plaque isolates:

WT	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
BP1	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
BP2	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
BP3	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
BP4	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60

WT	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
BP1	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
BP2	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
BP3	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
BP4	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120

WT	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
BP1	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
BP2	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
BP3	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
BP4	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180

WT	ARDGVFDYYTTGLVSIWYQTNYVVPVIGAPNTAYIIALAAQKNFTMKLCKDTSILQTS	240
BP1	ARDGVFDYYTTGLVSIWYQTNYVVPVIGAPNTAYIIALAAQKNFTMKLCKDTSILQTS	240
BP2	ARDGVFDYYTTGLVSIWYQTNYVVPVIGAPNTAYIIALAAQKNFTMKLCKDTSILQTS	240
BP3	ARDGVFDYYTTGLVSIWYQTNYVVPVIGAPNTAYIIALAAQKNFTMKLCKDTSILQTS	240
BP4	ARDGVFDYYTTGLVSIWYQTNYVVPVIGAPNTAYIIALAAQKNFTMKLCKDTSILQTS	240

WT	IQ	242
BP1	IQ	242
BP2	IQ	242
BP3	IQ	242
BP4	IQ	242
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Alignment of sequencing results for the EV-A71/SP variant:

VP4 alignment for all small plaque isolates:

WT	MGSQVSTQSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
SP1	MGSQVSTQSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
SP2	MGSQVSTQSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
SP3	MGSQVSTQSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
SP4	MGSQVSTQSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60

WT	FTEMAAPLK	69
SP1	FTEMAAPLK	69
SP2	FTEMAAPLK	69
SP3	FTEMAAPLK	69
SP4	FTEMAAPLK	69

VP2 alignment for all small plaque isolates:

WT	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDDATAVDKPTRPDVSV	60
SP1	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDDATAVDKPTRPDVSV	60
SP2	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDDATAVDKPTRPDVSV	60
SP3	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDDATAVDKPTRPDVSV	60
SP4	SPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDDATAVDKPTRPDVSV	60

WT	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHLYRSGFCIHVQCNASKFHQG	120
SP1	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHLYRSGFCIHVQCNASKFHQG	120
SP2	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHLYRSGFCIHVQCNASKFHQG	120
SP3	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHLYRSGFCIHVQCNASKFHQG	120
SP4	NRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHLYRSGFCIHVQCNASKFHQG	120

WT	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
SP1	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
SP2	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
SP3	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180
SP4	ALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIPISQLTVCPH	180

WT	QWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
SP1	QWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
SP2	QWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
SP3	QWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240
SP4	QWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPVIPITITLAP	240

WT	MCSEFAGLRQAVTQ	254
SP1	MCSEFAGLRQAVTQ	254
SP2	MCSEFAGLRQAVTQ	254
SP3	MCSEFAGLRQAVTQ	254
SP4	MCSEFAGLRQAVTQ	254

VP3 alignment for all small plaque isolates:

WT	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
SP1	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
SP2	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
SP3	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60
SP4	GFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIPGEVRNLELCQVETILEVNNVPT	60

WT	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
SP1	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
SP2	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
SP3	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120
SP4	NATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQSTMLGQLCGYYTQWSGSLEVTFM	120

WT	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
SP1	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
SP2	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
SP3	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180
SP4	FTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIWDFGLQSSVTLVIPWISNTHYRAH	180

WT	ARDGVFDYTTGLVSIWYQTNVVPPIGAPNTAYIIALAAAQKNFTMKLCKDTSFILQTAS	240
SP1	ARDGVFDYTTGLVSIWYQTNVVPPIGAPNTAYIIALAAAQKNFTMKLCKDTSFILQTAS	240
SP2	ARDGVFDYTTGLVSIWYQTNVVPPIGAPNTAYIIALAAAQKNFTMKLCKDTSFILQTAS	240
SP3	ARDGVFDYTTGLVSIWYQTNVVPPIGAPNTAYIIALAAAQKNFTMKLCKDTSFILQTAS	240
SP4	ARDGVFDYTTGLVSIWYQTNVVPPIGAPNTAYIIALAAAQKNFTMKLCKDTSFILQTAS	240

WT	IQ	242
SP1	IQ	242
SP2	IQ	242
SP3	IQ	242
SP4	IQ	242
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Supplementary Figure S2: Raw genome sequencing data of EV-A71/BP and EV-A71/SP.

>Wild type parental sequence 15 reads de novo assembled using Geneious Software

GGCTGTGGGTTGTACCCACTCACAGGGCCACGTTGGCGCTAGCACTCTGGTTCTGCGGAACCTTTGTGCGCCTGTTTACGCCCCCCCCCCAATTTGCAA
CTTAGAAGCAATACACAACACTGATCAACAGCAGGCATGGCGCACCAGCTATGTCTTGATCAAGCACTTCTGTTTCCCCGGGCGGAGTATCAATAGACTG
TTCACGCGGTTGAAGGAGAAAGCGCCGTTATCCGGCTAACTACTTCCGAGAAACCTAGTAGCACCATTGAAGCTGCAGAGTGTTTCGCTCGGCACTTCCC
CCGTGTAGATCAGGTTCGATGAGTCACTGCAATCCCCACGGGCGACCGTGGCAGTGGCTGCGCTGGCGGCTGCTATGGGGCAACCCATAGGACGCTCTA
ATGTGGACATGGTGCGAAGAGTCTATTGAGCTAGTTAGTAGTCTCCGGCCCTGAATGCGGCTAACTCCTAACTGTGGAGCACATGCCTTCAATCCAGAG
GGTAGTGTGTCGTAATGGGCAACTCTGCAGCGGAACCGACTACTTTGGGTGTCGGTGTTCCTTTTATCTTTACATTTGGTGCTTATGGTGACGATTATA
GAATTGTTACCATATAGCTATTGGATTGGCCATCCGGTGTGCAATAGAGCTATTATATACCTGTTTGTGGCTTTGTACCACTAACCTTAAATCTATAA
CCACCTTCGATTTTATATTAAACCTCAATACAATCAAACATGGGCTCACAGGTGTCTACTCAGCGATCCGGCTCCACAGAGAATCCAAATTCAGCTACAG
AAGGCTCCACCATTAAATACACTACCATCAACTATTACAAAGACTCCTATGCTGCGACAGCGGGCAACAGAGCCTCAAGCAAGACCTTGATAAATTTGC
TAACCTGTCAAGGACATTTTCACTGAAATGGCTGCACCCTGAAGTCTCCATCCGCTGAGGCTTGTGTTTACAGTGATCGCGTGGCACAACCTACCATTT
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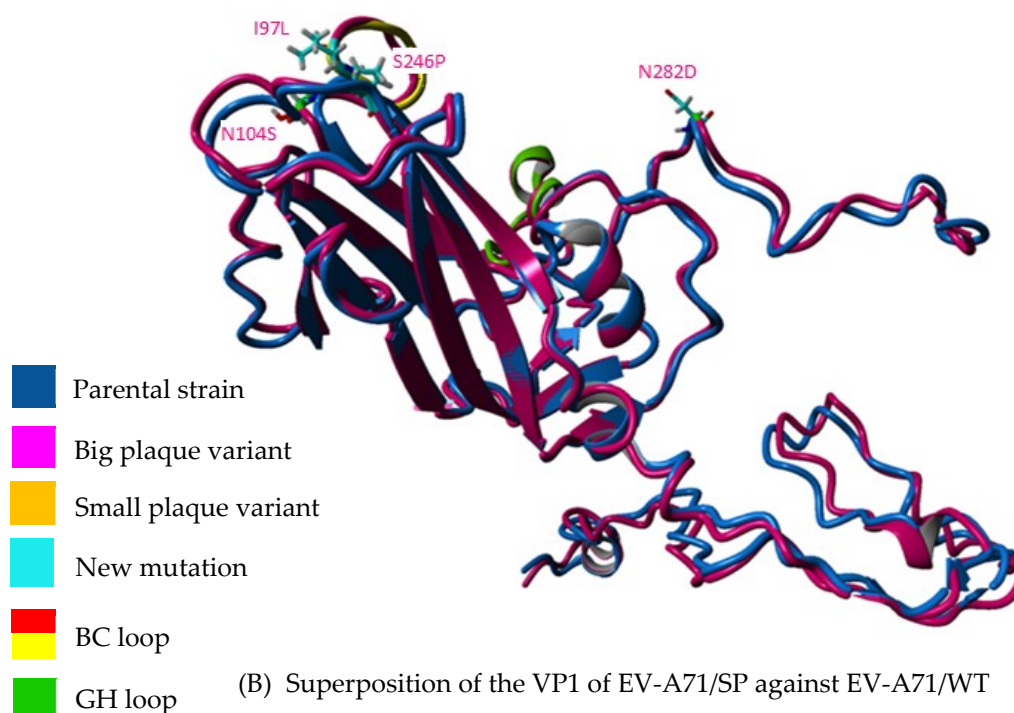
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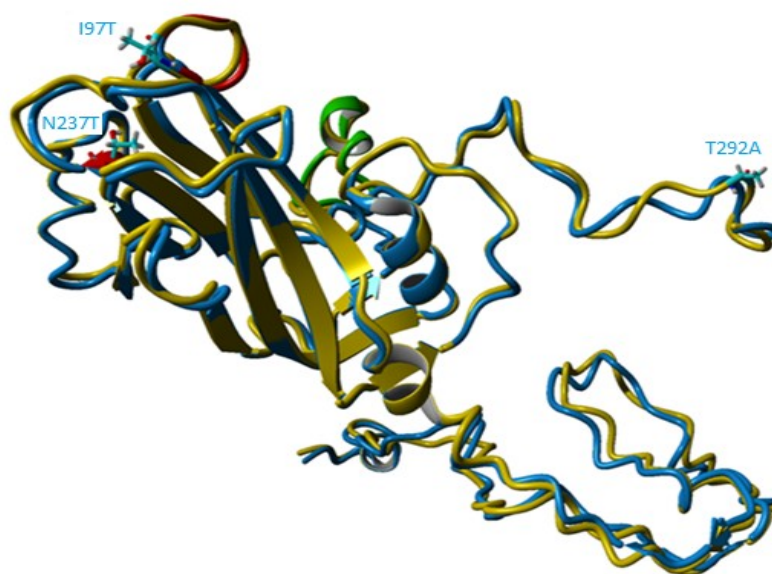
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(A) Superposition of the VP1 of EV-A71/BP against EV-A71/WT



(B) Superposition of the VP1 of EV-A71/SP against EV-A71/WT



Supplementary Figure S3: The 3D structure of the VP1 protein of the EV-A71/WT superposed against the EV-A71/BP and EV-A71/SP variants. The EV-A71/WT (blue) is showed against the **(A)** EV-A71/BP (magenta) and **(B)** EV-A71/SP variant (yellow) was performed using YASARA. The 4 amino acid substitutions in the EV-A71/BP variant (I97L, N104S, S246P and N282D) and the 3 amino acid substitutions in the EV-A71/SP variant (I97T, N237T and T292A).