

Figure S1: Sequence maps of EV-A71 (A) and CV-A16 (B) infectious clones used in this study.

Α



Figure S2: LD₅₀ determination of MP4 EV-A71 and WT CV-A16 in newborn mice. (A) Survival curve and (B) mean clinical score of mice inoculated with MP4 EV-A71. (C) Survival curve and (D) mean clinical score of mice inoculated with CV-A16. The mice were monitored for 14 days for survival and clinical symptoms. Clinical scores were defined as: 0, healthy; 1, weak or less active; 2, hunched posture and lethargy; 3, one-limb paralysis; 4, two-limb paralysis; 5, moribund or dead.



Figure S3: RMSF of VP1 of ChiE71, WT EV-A71 and WT CV-A16. MD simulations were performed using AMBER 20 with trajectory analysis using the CPPTRAJ module. The y-axis of each graph is the average RMSF of each residue during 10 ns MD simulation. ChiE71, WT EV-A71 and WT CV-A16 are depicted in red, blue and orange, respectively.

Consensus Strain 41 Strain E59	MGSQVSTQRSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60 60 60
Consensus Strain 41 Strain E59	FTEMAAPLKSPSAEACGYSDRVAQLTIGNSTITTQEAANIIVGYGEWPSYCSDDDATAVD	120 120 120
Consensus Strain 41 Strain E59	KPTRPDVSVNRFYTLDTKLWEKSSKGWYWKFPDVLTETGVFGQNAQFHYLYRSGFCIHVQ	180 180 180
Consensus Strain 41 Strain E59	CNASKFHQGALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIP	240 240 240
Consensus Strain 41 Strain E59	ISQLTVCPHQWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPV	300 300 300
Consensus Strain 41 Strain E59	IPITITJAPMCSEFAGLRQAVTQGFPTEPKPGTNQFLTTDDGVSAPILPNFHPTPCIHIP LI	360 360 360
Consensus Strain 41 Strain E59	GEVRNLLELCQVETILEVNNVPTNATSLMERLRFPVSAQAGKGELCAVFRADPGRDGPWQ	420 420 420
Consensus Strain 41 Strain E59	STMLGQLCGYYTQWSGSLEVTFMFTGSFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIW	480 480 480
Consensus Strain 41 Strain E59	DFGLQSSVTLVIPWISNTHYRAHARDGVFDYYTTGLXSIWYQTNYVVPIGAPNTAYIIAL	540 540 540
Consensus Strain 41 Strain E59	AAAQKNFTMKLCKDTSHILQTASIQGDRVADVIESSIGBSVSRALTQALPAPTGQNTQVS D	600 600 600
Consensus Strain 41 Strain E59	SHRLDTGEVPALQAAEXGASSNTSDESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDL IVV.	660 660 660
Consensus Strain 41 Strain E59	PLEGTTNPNGYANWDIDITGYAQMRRKVELFTYMRFDAEFTFVACTPTGZVVPQLLQYMF EQ	720 720 720
Consensus Strain 41 Strain E59	VPPGAPKPESRESLAWQTATNPSVFVKLTDPPAQVSVPFMSPASAYQWFYDGYPTFGEHK	780 780 780
Consensus Strain 41 Strain E59	QEKDLEYGACPNNMMGTFSVRTVGSXKSKYPLVVRIYMRMKHVRAWIPRPMRNQNYLFKA SL.	840 840 840

Consensus	NPNYAGNSIKPTGTSRTAITTL	900
Strain 42		900
Strain E	9	900

Figure S4: Alignment of EV-A71 P1 amino acid sequence of strain E59 (JN874551.1) and strain 41 (AF316321). Difference of amino acids of the two strains are indicated respectively.