

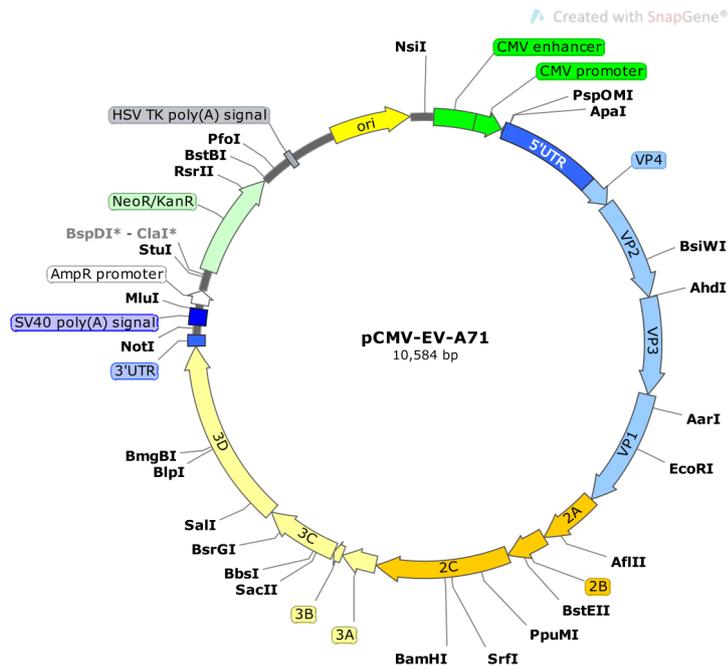
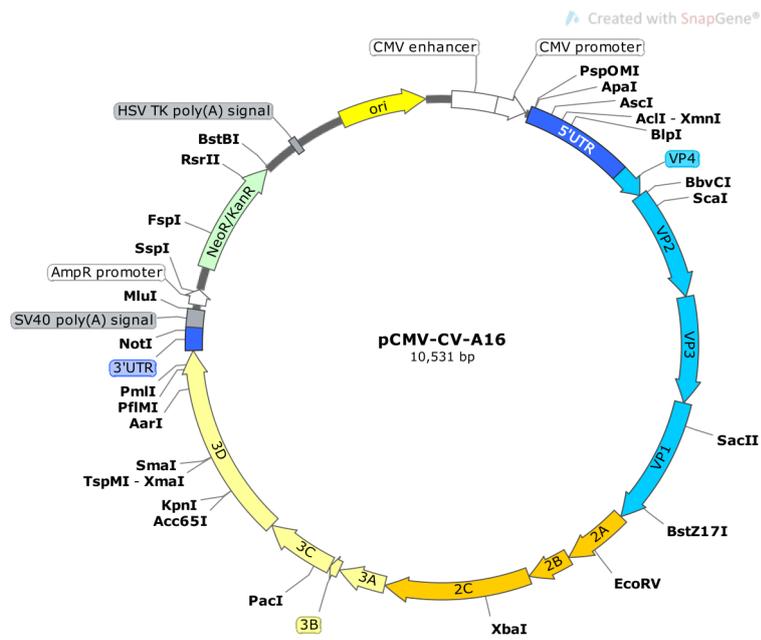
A**B**

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

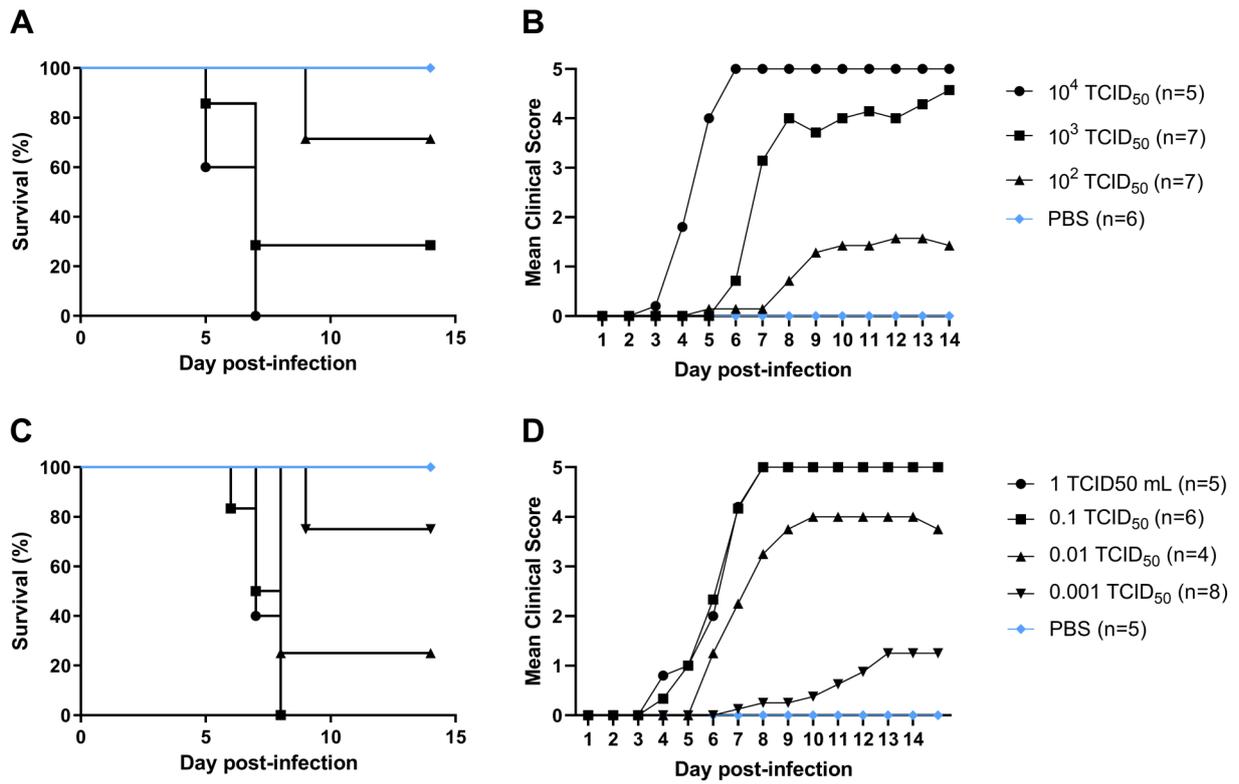


Figure S2: LD₅₀ determination of MP4 EV-A71 and WT CV-A16 in newborn mice. (A) Survival curve and (B) mean clinical score of mice inoculated with MP4 EV-A71. (C) Survival curve and (D) mean clinical score of mice inoculated with CV-A16. The mice were monitored for 14 days for survival and clinical symptoms. 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.

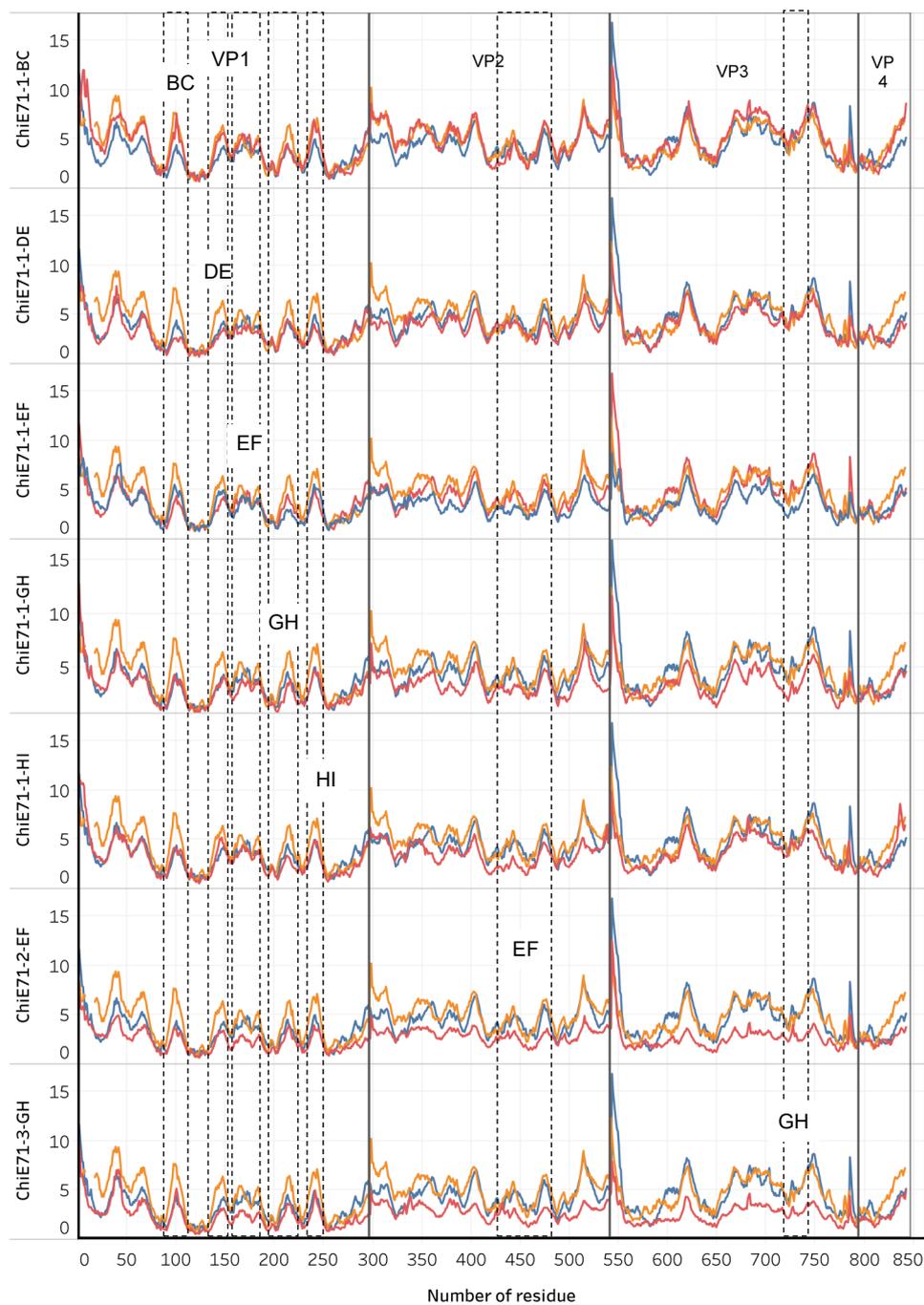


Figure S3: RMSF of VP1 of ChiE71, WT EV-A71 and WT CV-A16. MD simulations were performed using AMBER 20 with trajectory analysis using the CPPTRAJ module. The y-axis of each graph is the average RMSF of each residue during 10 ns MD simulation. ChiE71, WT EV-A71 and WT CV-A16 are depicted in red, blue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

Consensus	MGSQVSTQRSQSGSHENSNSATEGSTINYTTINYYKDSYAATAGKQSLKQDPDKFANPVKDI	60
Strain 41	60
Strain E59	60
Consensus	FTEMAAPLKSPSAEACGYSDRVAQLTIGNSTITTQEAAIIIVGYGEWPSYCSDDDATAVD	120
Strain 41	120
Strain E59	120
Consensus	KPTRPDVSVNRFYTLDTKLWEKSSKGWYWKFPDVLTTETGVFGQNAQFHLYRSGFCIHVQ	180
Strain 41	180
Strain E59	180
Consensus	CNASKFHQGALLVAILPEYVIGTVAGGTGTEDSHPPYKQTQPGADGFELQHPYVLDAGIP	240
Strain 41	240
Strain E59	240
Consensus	ISQLTVCPHQWINLRTNNCATIIVPYMNTLPFDSALNHCNFGLLVVPISPLDFDQGATPV	300
Strain 41	300
Strain E59	300
Consensus	IPITITJAPMCSEFAGLRQAVTQGFPTPEKPGTNQFLTTDDGVSAPILPNFHPTPCIHIP	360
Strain 41L.....	360
Strain E59I.....	360
Consensus	GEVRNLELQCQVETILEVNMVPTNATSLMERLRFVSAQAGKGELCAVFRADPGRDGPWQ	420
Strain 41	420
Strain E59	420
Consensus	STMLGQLCGYYTQWGSLEVTFMFTGSGFMATGKMLIAYTPPGGPLPKDRATAMLGTHVIW	480
Strain 41	480
Strain E59	480
Consensus	DFGLQSSVTLVIPWISNTHYRAHARDGVFDYYTGLXSIWYQTNVVPVIGAPNTAYIIAL	540
Strain 41V.....	540
Strain E59I.....	540
Consensus	AAAQKNFTMKLCKDTSILQTASIQGDRVADVIESSIGBSVSRALTQALPAPTQNTQVS	600
Strain 41D.....	600
Strain E59N.....	600
Consensus	SHRLDTGEVPALQAAEXGASSNTSDESMIETRCVLNSHSTAETTLDSFFSRAGLVGEIDL	660
Strain 41I.....	660
Strain E59V.....	660
Consensus	PLEGTTNPNGYANWDIDITGYAQMRRKVELFTYMRFDAEFTFVACTPTGZVVPQLLQYMF	720
Strain 41E.....	720
Strain E59Q.....	720
Consensus	VPPGAPKPESRESLAWQTATNPSVFVKLTDPPAQVSPVPMSPASAYQWFYDGYPTFGEHK	780
Strain 41	780
Strain E59	780
Consensus	QEKDLEYGACPNNMMGTFSVRTVGSXSKYPLVVRIYMRMKHVRAWIPRPMRNQNYLFKA	840
Strain 41S.....	840
Strain E59L.....	840

Consensus	NPNYAGNSIKPTGTSRTAITTL-----	900
Strain 41	900
Strain E59	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.