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Electronic Supplementary Material

Improved plasmid-based recovery of coxsackievirus A16 infectious clone driven by human RNA polymerase I promoter

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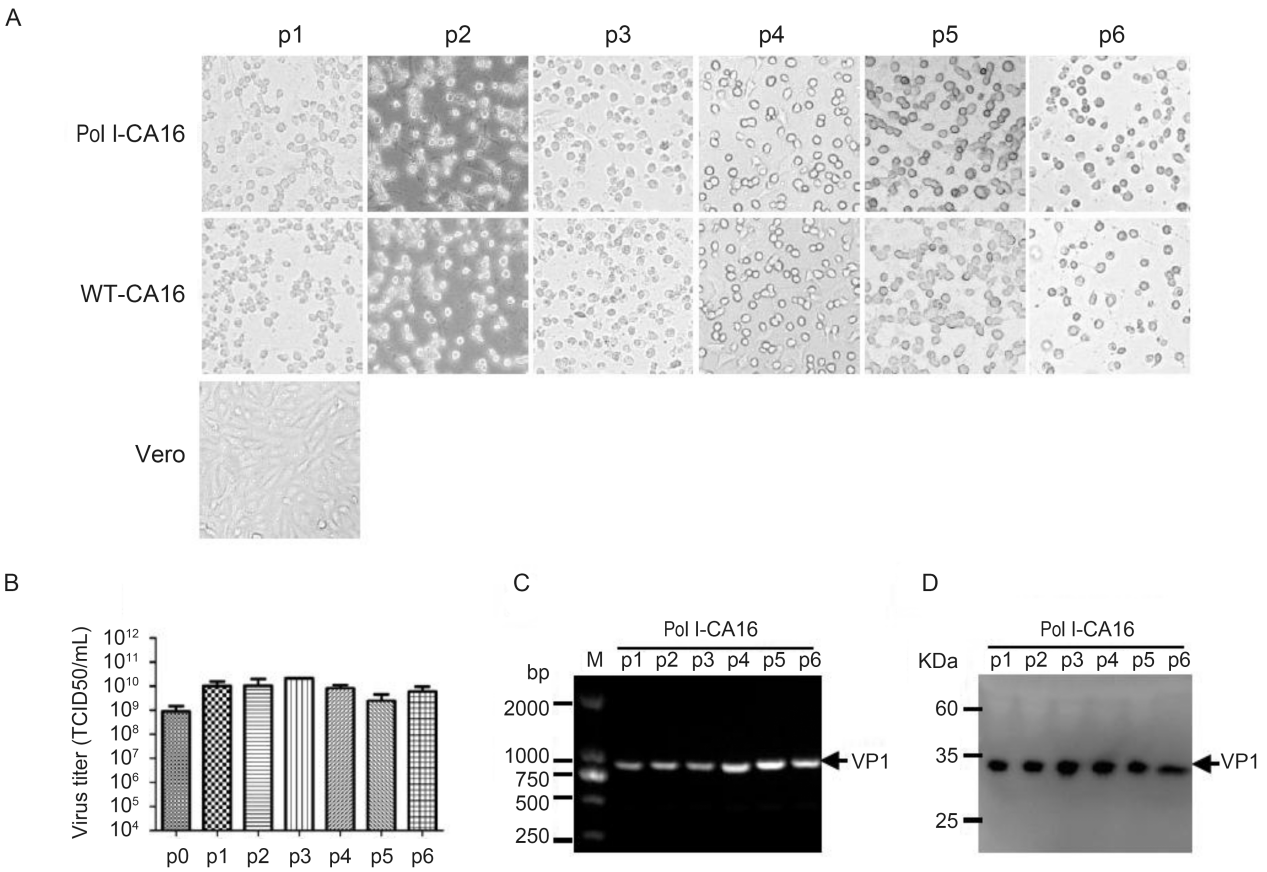

Figure S1. Stability of the recovered Pol I-CA16 particles. (A) Viral titers of Pol I-CA16. Each generation was titrated by TCID50 assay on Vero cells. Statistical analyses were performed with GraphPad Prism version 5. (B) Detection of Pol I-CA16 negative-sense RNA. RNA was extracted from viral particles from each generation and negative-sense RNA was detected by RT-PCR. Lane M,DNA marker; lane p1–p6, PCR products from the recovered infective virus. (C) Viral capsid proteins of Pol I-CA16. Proteins were detected using CA16 specific anti-VP1 antibody by western blotting (Liu et al., 2011). Lane p1–p6, Pol I-CA16 recovered from infected cell lysates. (D) Recovered Pol I-CA16 caused CPE upon passaging. The recovered Pol I-CA16 virus or WT-CA16 (equivalent MOIs) was passaged on Vero cells over six generations,and each generation resulted in CPE,as presented.

Table S1. Primers used in this study

Primer	Sequence (5'–3')	Enzyme site
P1	ACCGCCGGGAGGGCGTCCCC	None
P2	GCGGCCGCTCTAGAGAGCTCAAGCTTAATAACCCGGCGGCCCAAAA	*Not* I, *Xba* I, *Hind* III
P3	GGCCGCAGATCTCCCCCCCAACTTCGGAGGTCGACCAGTACTCC	*Xba* I
P4	GGCCGGAGTACTGGTCGACCTCCGAAGTTGGGGGGGAGATCTGC	*Xba* I
P5	GCCAAGCTTAAAACAGCCTGTGGGTTGTTCCCACCC	*Hind* III
P6	CGGGTCTAGAGCGTAGACTCTTTTGGCTTCAGTC	*Xba* I
P7	CTGGTCTAGAAAGAAGGATGAACAACTAC	*Xba* I
P8	TATTCTAGATTTTTTTTTTTTTTTTTTTTTTTT	*Xba* I