VIROLOGICA SINICA

Electronic Supplementary Material

The Establishment of Infectious Clone and Single Round Infectious Particles for Coxsackievirus A10

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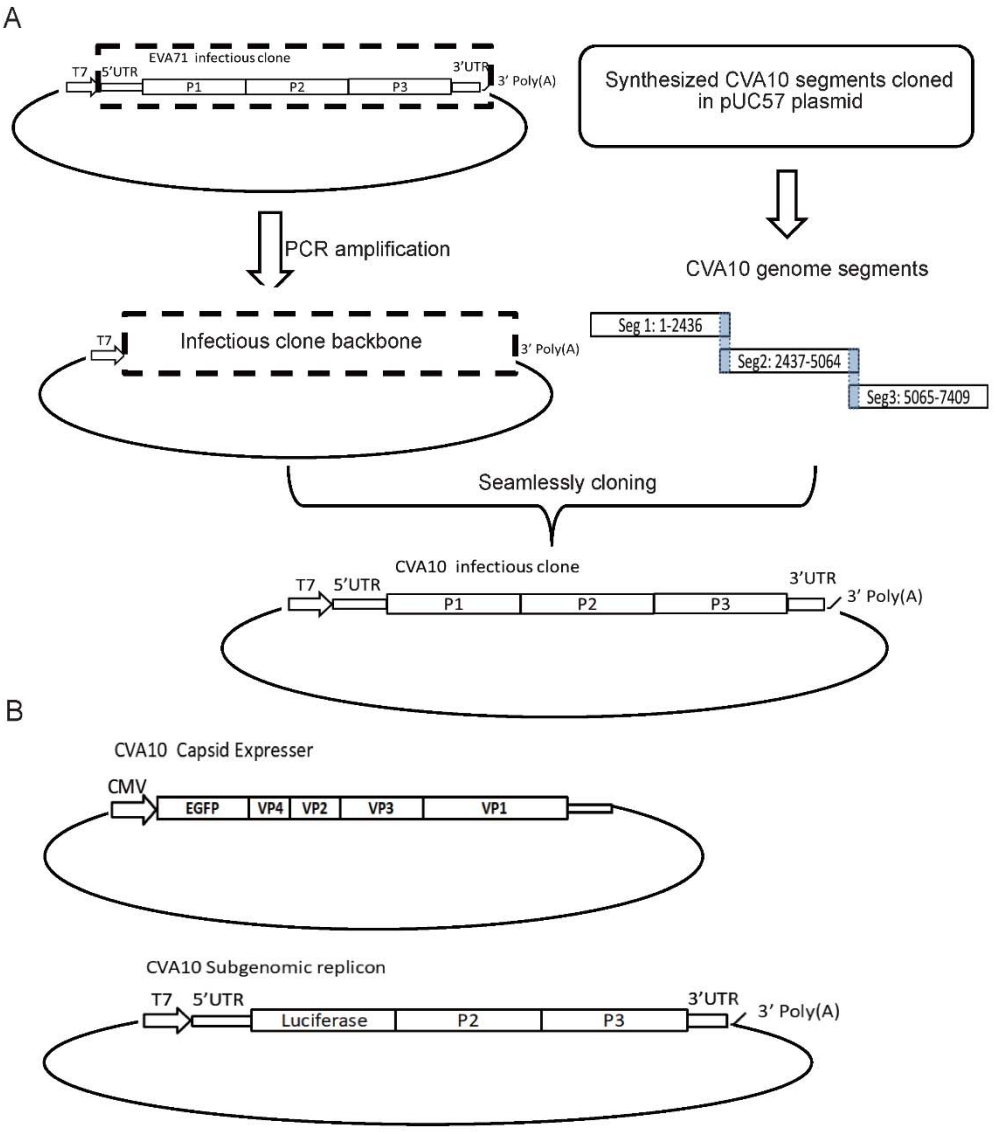

Figure S1. The cloning strategy and illustrations of the infectious clone, subgenomic replicon and capsid expresser. (**A**) The backbone was amplified from previous EVA71 infectious clone. Three CVA10 segments with overlapping end were amplified from pUC57 cloning vectors. Four DNA segments were seamlessly ligated together in transformed E.coli to form the CVA10 infectious clone. (**B**) Similar cloning strategies were used to generate CVA10 expressing vector and CVA10 subgenomic replicon. The cartoon shows the map of the constructs.

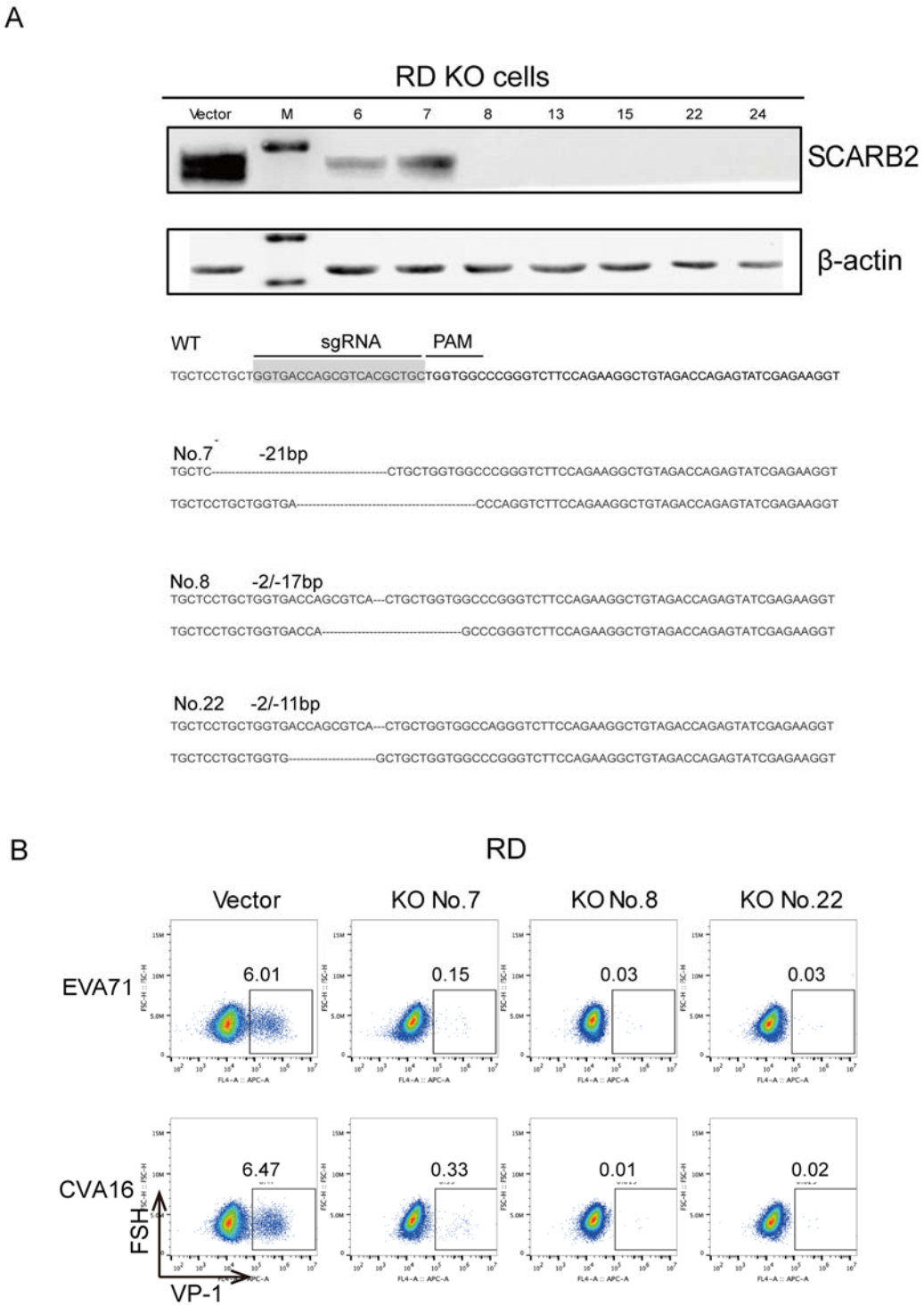

Figure S2. The establishment of RD SCARB2 KO cells. (**A**) SCARB2, the receptor for EVA71 and CVA16, was knocked out in RD cells using CRISPR/Cas9 technique. Several single-cell clones were harvested and examined for their SCARB2 expression by Western blot. And the genome editing in RD SCARB2 KO cells was confirmed by sanger sequencing, indicating double allele frameshift mutations in completely knockout clones. (**B**) EVA71 and CVA16 infection in RD SCARB2 WT and KO cell clones were monitored by VP1 expression measured by

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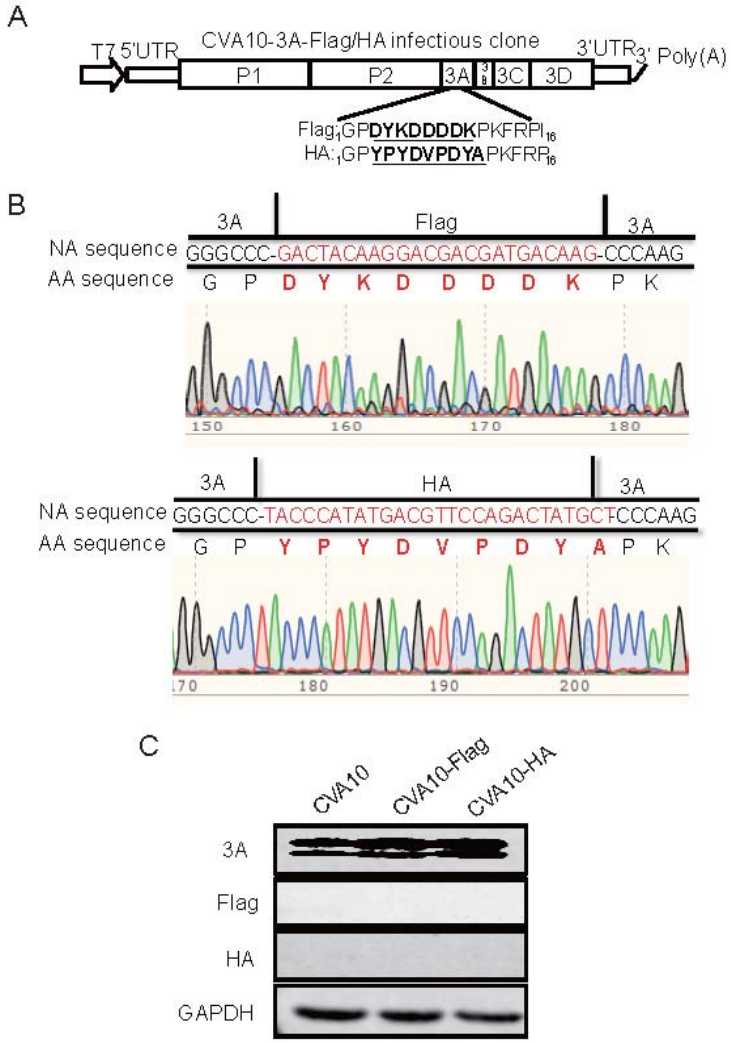

Figure S3. (**A**) The cartoon shows that a FLAG-tag or HA-tag sequence was inserted into the CVA10 infectious clone after the second amino acid (proline) of 3A. (**B**) The rescued CVA10 viruses with FLAG-tag or HA-tag were harvested in four continuous passages and the inserted FLAG-tag or HA-tag were sequenced. (**C**) RD cells were infected by CVA10, CVA10-FLAG or CVA10-HA at an MOI of 1. At 6 hpi, cells were harvested and lysed for Western blot. Anti-3A, anti-FLAG and anti-HA antibodies were probed. GAPDH was used as an internal control. Three independent experiments were conducted.