

Mutations in VP1 and 5'-UTR affect enterovirus 71 virulence

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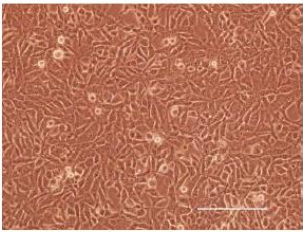
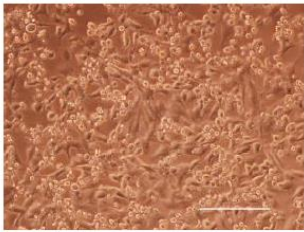
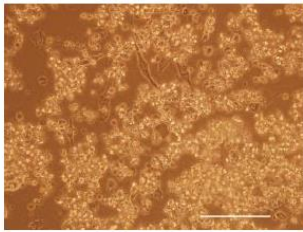
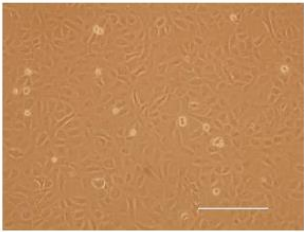
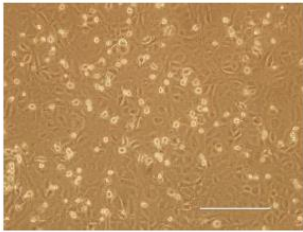
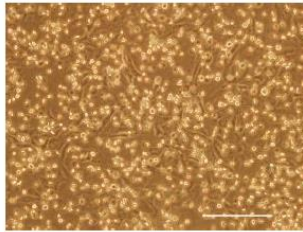
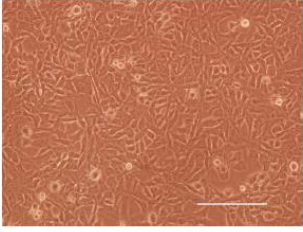
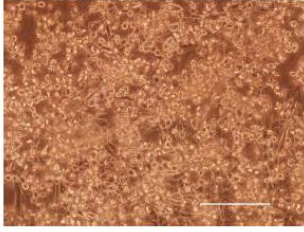
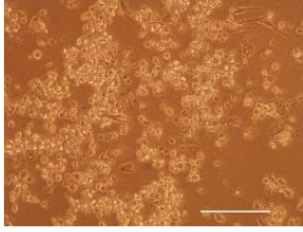
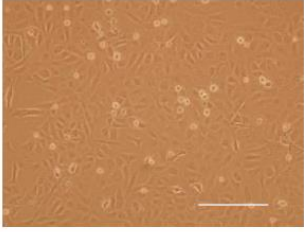
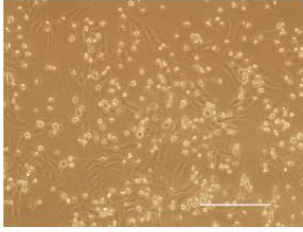
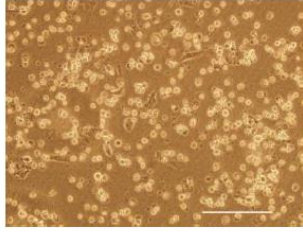
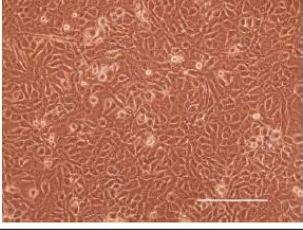
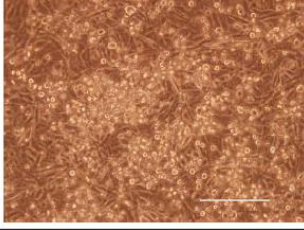
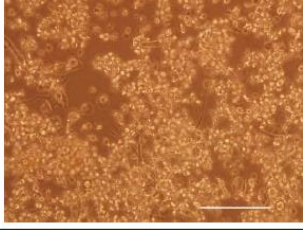
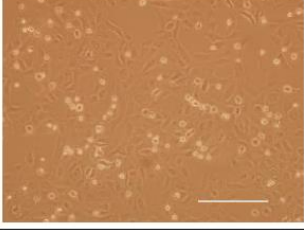

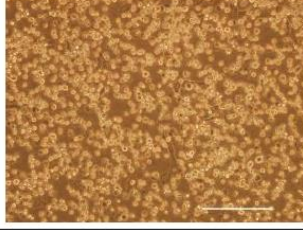
Supplementary Table S1. List of primer sets for Q-PCR

| Name | Sequence | Function |
|------------------|--------------------------|--------------------|
| Actin_F | ACCAACTGGGACGACATGGAGAAA | Actin gene |
| Actin_R | TAGCACAGCCTGGATAGCAACGTA | Actin gene |
| VP1_F | ACGCGCAAATGCGTAGAAAGGT | VP1 gene |
| VP1_R | TTAGTGGCAGTTTGCCATGCGA | VP1 gene |
| CCL3_F | TTCTGCTGACAAGCTCACCCCT | CCL3 gene |
| CCL3_R | ATGGCGCTGAGAAGACTTGGT | CCL3 gene |
| CXCL10_F | CTCTCTCCATCACTCCCCTTTAC | CXCL10 gene |
| CXCL10_R | ACTTAGAACTGACGAGCCTGAGC | CXCL10 gene |
| TNF- α _F | TCTCATGCACCACCATCAAGGACT | TNF- α gene |
| TNF- α _R | TTGCACCTCAGGGAAGAATCTGGA | TNF- α gene |

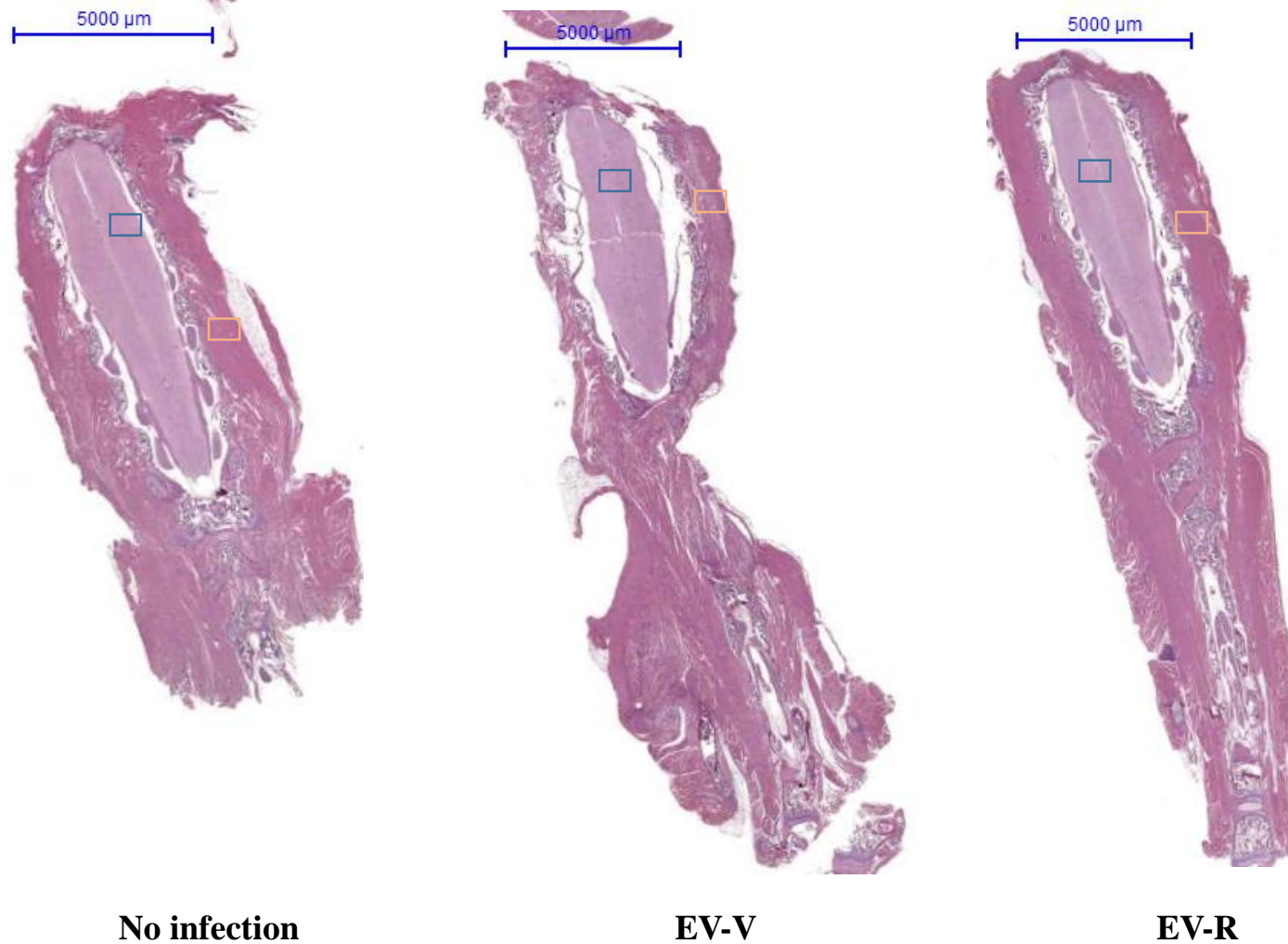
Supplementary Table S2. List of primers used for sequencing of the EV71 genome

| Name | Sequence | Name | Sequence |
|-------------|--------------------------|-------------|-------------------------|
| EV71_1+ | TTAAAACAGCCTGTGGGTTGCA | EV71_4098+ | AATTCAACGATGCGGCGAGTGC |
| EV71_692+ | CACTGAAGTCTGTGATCACTCTCA | EV71_4142- | AGATCCACTCAAGCCCCTTC |
| EV71_779- | AACCGGAGCGTTGTGTGGATAC | EV71_4957+ | GGATAGGAAGTCCAAGGTGAGA |
| EV71_1029- | TGCGTAGTGATGGTAGAATTGC | EV71_5002- | ACTCACTACCGTGTCCCACTG |
| EV71_1036+ | ATGCACAGTTCCACTACCTC | EV71_5426+ | AGGAACATTAGGCAGGTCCA |
| EV71_1589+ | GGTCTGCTGGTTGTGCCTAT | EV71_5749+ | CCAGTATGGGTTTTTGAACCTT |
| EV71_1639- | CGTCGCACCTTGGTCATAAT | EV71_5796- | ATCATAGTCCTGTGAGTGGGCT |
| EV71_2011+ | CTTGTTGGGCCAGTTGTGC | EV71_6218+ | AAGATGAGCATGGAGGATGC |
| EV71_2439+ | AGGGAGATAGGGTGGCAGATGT | EV71_6376- | GTCCATGTAGAATTTTCATCTTG |
| EV71_2559- | TCTAATCGATGGCTGCTTACC | EV71_6561+ | ATCACTGGTTCAGCTGTT |
| EV71_2623+ | GAGTATGATTGAGACACGGT | EV71_6605- | TTGGTAACTTGCTCCAGAACAC |
| EV71_3196+ | CATGAGGATGAAACACGTCA | EV71_6962+ | CTGGAGTTGGCAAGAACAGG |
| EV71_3326- | TATCCACGCCCTGACGTGTTTC | EV71_7410- | GCTATTCTGGTTATAACA |
| EV71_3608+ | AGATACCAGTCACATCTCATGC | | |

Supplementary Table S3. The passage history of EV-V vs. EV-R

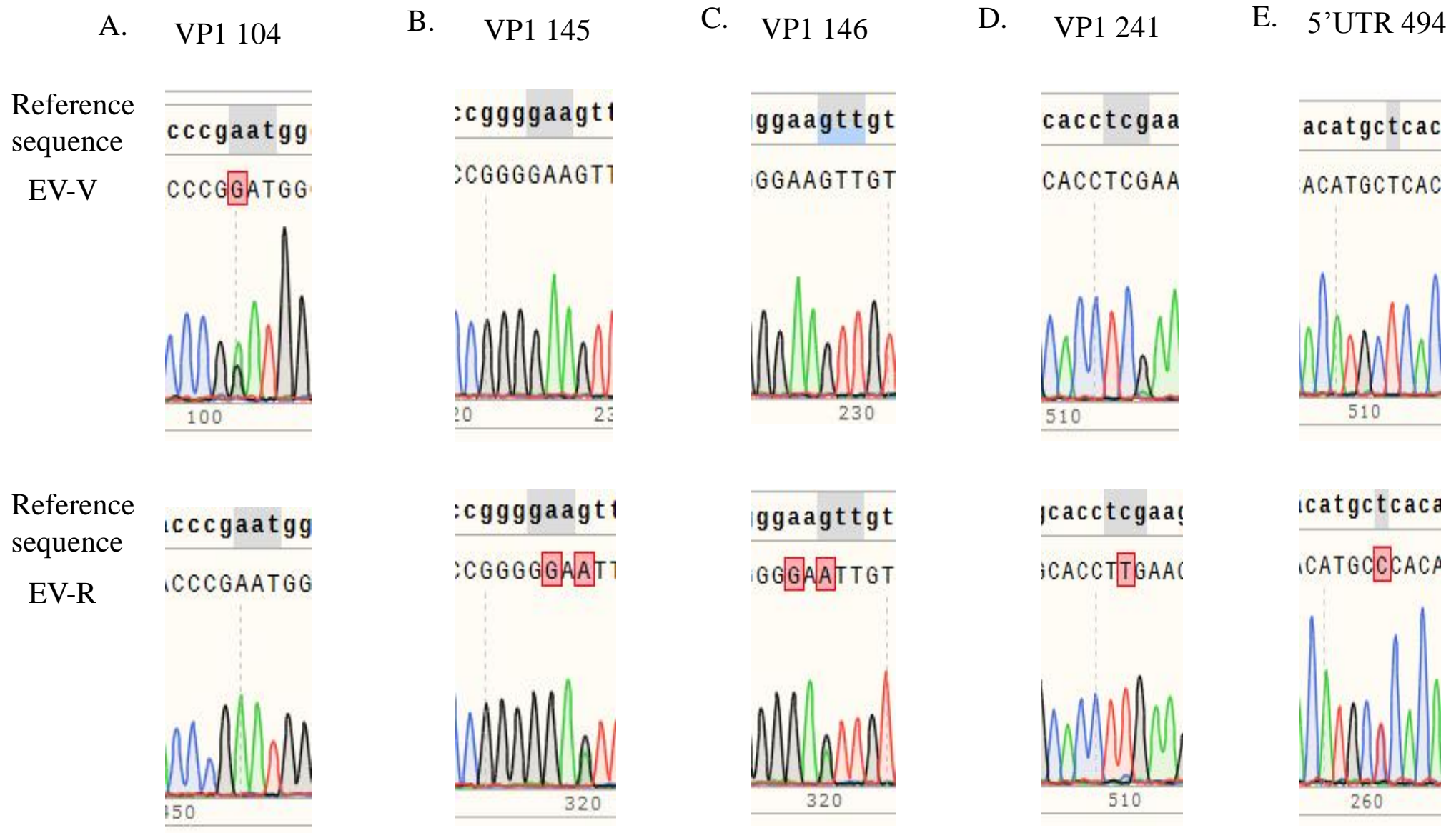
| | Day 1 | Day 2 | Day 3 | | Day 1 | Day 2 | Day 3 |
|---|--|--|---|---|--|--|--|
| *Produced virus/ titer (pfu/mL) | Cytopathic effect in RD | | | *Produced virus/ titer (pfu/mL) | Cytopathic effect in Vero | | |
| EV-R P1 / 4.0×10^5 |  |  |  | EV-V P1 / 5.2×10^5 |  |  |  |
| EV-R P2 / 4.3×10^6 |  |  |  | EV-V P2 / 8.9×10^5 |  |  |  |
| EV-R P3 / 6.9×10^6 |  |  |  | EV-V P3 / 7.8×10^5 |  |  |  |

* EV71 seed (GenBank: AF304457.1) propagated from Vero cells were subjected to infect RD or Vero cells cultured in 6-well plates with the multiplicity of infection (MOI) = 0.1 (day 0) and then cultured for 3 days. The pictures showing the cytopathic effect were taken by the microscope at 20x magnitude every day. The scale bar = 200 μ m were indicated. Produced EV-R P1 or EV-V P1 were collected from the supernatant of infected RD or Vero cells, respectively, at day 3 and then assayed their titer by plaque assay which performed in RD cells. EV-R P1 or EV-V P1 were used to infect RD or Vero cells to produce EV-R P2 or EV-V P2, respectively, as followed as the procedure described above. The third run of infection to produce EV-R P3 or EV-V P3, respectively, was performed as well.



Supplementary Figure S1. Isolation of the spine tissues from EV71-infected hSCARB2-Tg mice for histochemistry.

Whole spines from individual naïve and hSCARB2-Tg mice challenged with 5×10^6 pfu of EV-V or EV-R were isolated on day 4 pi. After sectioning, the spinal cord and surrounding muscle were consistently marked with grids for staining with dyes or antibodies as described in the Materials and Methods. The stained images are shown at 4x magnification. A scale bar of 5000 μm is indicated in the field.



Supplementary Figure S2. Alignment of the EV-V and EV-R nucleotide sequences.

The whole genomes of EV-V and EV-R were sequenced as described in the Materials and Methods. The graph shows the mutation sites VP1-104, 145, 146, 241, and 5'-UTR 494 from EV-V and EV-R. The respective reference sequences (GenBank: AF304457.1) are also included.