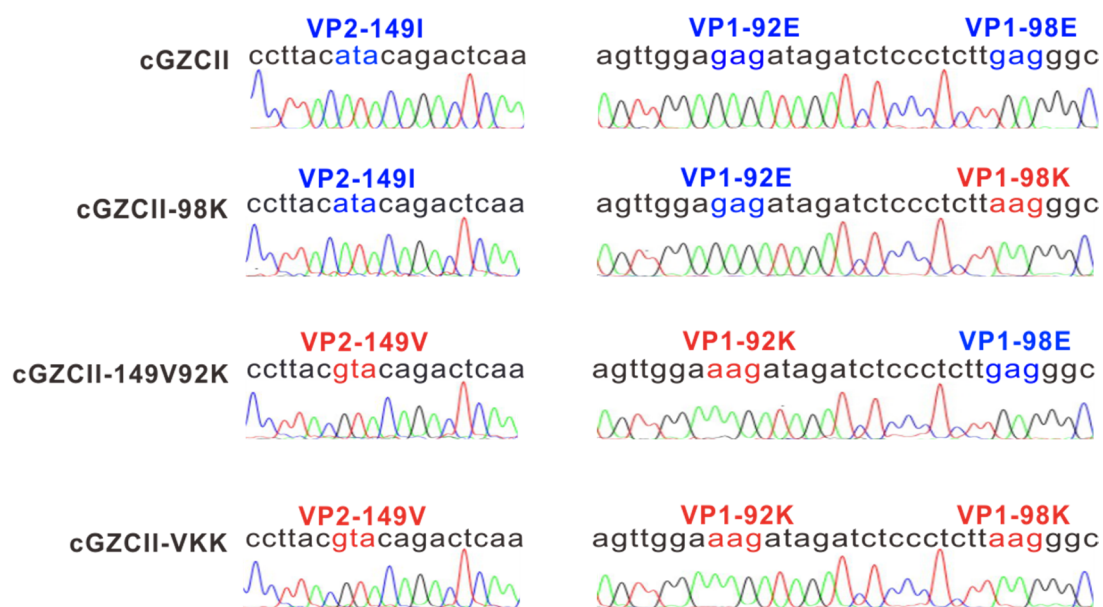


## Supplementary Information

**Figure S1**



**Figure S1.** Sequence analysis of the constructed GZCII variants with different mutations at the VP2-149, VP1-92 and VP1-98 residues. The VP1 and VP2 regions were amplified from viral cDNA and subjected to Sanger sequencing to confirm mutagenesis.

**Table S1.** DNA oligos used in this study

| <b>Oligos</b>       | <b>Sequence</b>                               | <b>Application</b>                                                    |
|---------------------|-----------------------------------------------|-----------------------------------------------------------------------|
| <b>Vec-F</b>        | gggggttaaattgttataaccagaatagca                | Construction of pEV71-GZCII                                           |
| <b>Vec-R</b>        | ccacaggcttttaacctatagtgagtcgtattaatacgtaatccg |                                                                       |
| <b>GZCII-1F</b>     | ttaaaacagcctgtgggttgacccactc                  |                                                                       |
| <b>GZCII-3470R</b>  | acaaccttgggcggtggtggat                        |                                                                       |
| <b>GZCII-3470F</b>  | accgccaaggttgatgatactattgcc                   |                                                                       |
| <b>GZCII-7404R</b>  | aacaaattacccccaccagtcacattcacg                |                                                                       |
| <b>149V-F</b>       | cccccttacgtacagactcaaccgggtgccgatg            | Site-directed mutagenesis to construct recombinant EV71-GZCII viruses |
| <b>149V-R</b>       | ctgtacgtaaggggggtgggtgtcttcg                  |                                                                       |
| <b>92K-F</b>        | ttagttggaaagatagatctccctcttgagggcacaact       |                                                                       |
| <b>92K-R</b>        | tatctttccaactaatccggcctgctgaaga               |                                                                       |
| <b>98K-F</b>        | cctcttaagggcacaactaacccgaatggt                |                                                                       |
| <b>98K-R</b>        | tgtgcccttaagaggagatctatcttccaactaatccggc      |                                                                       |
| <b>92K98K-F</b>     | ttagttggaaagatagatctccctcttaagggcacaact       |                                                                       |
| <b>92K98K-R</b>     | tatctttccaactaatccggcctgctgaaga               |                                                                       |
| <b>GZCII-Seq-1F</b> | ttaaaacagcctgtgggttgacc                       | Preparation of 2 DNA fragments covering the entire genome for NGS     |
| <b>GZCII-Seq-1R</b> | cttgtaggcagcagattgttcc                        |                                                                       |
| <b>GZCII-Seq-2F</b> | cgacatggttacctcactgca                         |                                                                       |
| <b>GZCII-Seq-2R</b> | gctattctggttataacaaattacccccacc               |                                                                       |
| <b>sgEXT1-S</b>     | caccggaccaggcaggacacatgc                      | sgRNA expression plasmid construction                                 |
| <b>sgEXT1-NS</b>    | aaacgcatgtgtcctgcctgggtcc                     |                                                                       |
| <b>sgSRB2-S</b>     | caccgagatgctgcttctacacggc                     |                                                                       |
| <b>sgSRB2-NS</b>    | aaacgccgtgtagaagcagcatctc                     |                                                                       |
| <b>SRB2KOid-F</b>   | ctctgctgctcattccggtatct                       | Amplification of the sgRNA target region for genotyping               |
| <b>SRB2KOid-R</b>   | gcaaacaagagctgtgtctgca                        |                                                                       |
| <b>EXT1KOid-F</b>   | cccacatgcgctggatcagt                          |                                                                       |
| <b>EXT1KOid-R</b>   | gtgcacgctggaatcctcgt                          |                                                                       |
| <b>qEV-F</b>        | aaatggcagcgccactgaag                          | qRT-PCR detection of EV71 RNA copies                                  |
| <b>qEV-R</b>        | gggcgcgttggtttatccac                          |                                                                       |
| <b>qEV probe</b>    | FAM-tccaccatcaccacgcaagaagcggct-TAMRA         |                                                                       |
| <b>mGAPDH-F</b>     | aggtcggtgtgaacggatttg                         | qPCR detection of the mouse GAPDH gene                                |
| <b>mGAPDH-R</b>     | tgtagaccatgtagttgaggtca                       |                                                                       |
| <b>Vec-F</b>        | gcagctgtggaatgtgtgcagt                        | Sequencing of pEV71-GZCII                                             |
| <b>630-R</b>        | caccggatggccaatcca                            |                                                                       |
| <b>1310-R</b>       | aggagtgtccttggtgg                             |                                                                       |
| <b>2030-R</b>       | gggtgtagtatccgcacaac                          |                                                                       |
| <b>2750-R</b>       | gttggcataaccattcgggt                          |                                                                       |

|               |                        |  |
|---------------|------------------------|--|
| <b>3470-R</b> | acaaccttgggcggtggtggat |  |
| <b>4110-R</b> | tagcggcgttggccatc      |  |
| <b>4820-R</b> | agtctgcacttcaatgtcac   |  |
| <b>5530-R</b> | tcacgagtttggtcaatcc    |  |
| <b>6230-R</b> | caggcctcctcatgctc      |  |
| <b>6930-R</b> | gggtagctagcgagcacatc   |  |
| <b>7570-R</b> | ggttattgtctcatgagcgga  |  |