

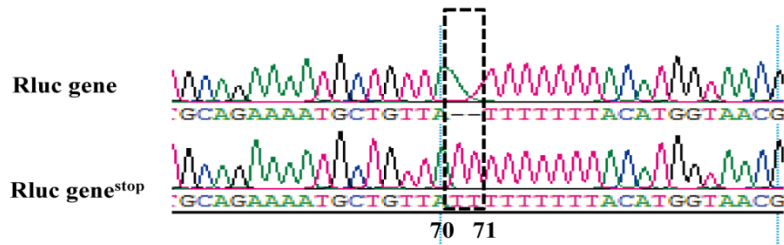
Figure legends

Fig. S1. Sequence analysis of the mutations in Rluc gene. The sequence of the 5' end of the Rluc expression cassette is shown. The asterisk represents a stop codon. Two-nucleotide insertion (TT) detected in Rluc gene of rOC43-ns2DelRluc (P11), rOC43-ns2FusionRluc (P4) and rOC43-ns2FusionRluc (P6) is shown in boldface.

Fig. S2. Virulence and stability of rOC43-ns2DelRluc *in vivo*. (A) Typical symptom of an infected BALB/C mouse at an advanced stage of disease when the animal presented a severe symptom of twitching limbs (3 days post-inoculation). (B) The survival curves of mice after intracerebral inoculated with either rOC43-ns2DelRluc or HCoV-OC43. Two groups of 12-day-old BALB/c mice (Four mice of each group) were intracerebral inoculation (IC) with 100 TCID₅₀ of rOC43-ns2DelRluc or HCoV-OC43-WT, another group was intracerebral inoculation with 20 µl of DMEM. (C) Illustration of the virus passage procedure in BALB/c mice. Mice were intracerebral inoculation with 500 TCID₅₀ of rOC43-ns2DelRluc (P0) in 20 µl of DMEM and sacrificed at 3 days post-inoculation, and then brains were homogenized in 500 µl of PBS. Then Brain homogenate were clarified by low-speed centrifugation at 3,000 rpm for 12 min to obtain passage 1 virus (P1). After 5 rounds of serial passages, the rOC43-ns2DelRluc was passaged to P5. (D) Analysis of genetic stability of the rOC43-ns2DelRluc after several passages in mice. Viral RNA was

extracted from each passage, and RT-PCR was performed with a primer set flanking the Rluc gene. The resulting RT-PCR products were resolved by 1% agarose gel electrophoresis. (E) Viral titers of rOC43-ns2DelRluc during passages in mice. BHK-21 cells were infected with rOC43-ns2DelRluc of each passage and titrated using the IFA-based viral titration assay. Data represent three independent experiments and are shown as means \pm standard deviation. (F) Rluc activity of reporter viruses of each passage. BHK-21 cells were infected with rOC43-ns2DelRluc (MOI = 0.01) of each passage in 48-well plates and assayed for the Rluc activity in RLUs at 72 h post-infection. Data represent mean values of three independent experiments with error bars representing the standard deviations of the means.

Figure S1



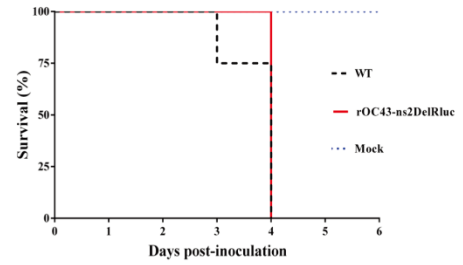
Rluc gene ATGAATGTTCTTGATTCA~~TTTATTA~~ATTATTATGATTCAGAAAAACATGCAGAAAATGCT
M N V L D S F I N Y Y D S E K H A E N A
Rluc gene^{Stop} ATGAATGTTCTTGATTCA~~TTTATTA~~ATTATTATGATTCAGAAAAACATGCAGAAAATGCT
M N V L D S F I N Y Y D S E K H A E N A
70 71
Rluc gene GTTATTTTTT TACATGGTAACGCGGCCTCTTCTTATTTATGGCGACATGTTGTGCCACAT
V I F L H G N A A S S Y L W R H V V P H
Rluc gene^{Stop} GTTATTTTTT TACATGGTAACGCGGCCTCTTCTTATTTATGGCGACATGTTGTGCCACAT
V I F E Y M V T R P L L I Y G D M L C H I
Rluc gene ATTGAGCCAGTAGCGGGTGTATTATAACCAGACCTTATTGGTATGGGCAAATCAGGCAA...
I E P V A R C I I P D L I G M G K S G K
Rluc gene^{Stop} ATTGAGCCAGTAGCGGGTGTATTATAACCAGACCTTATTGGTATGGGCAAATCAGGCAA...
L S Q *

Figure S2

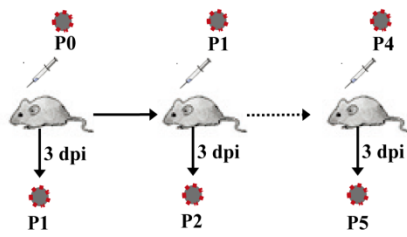
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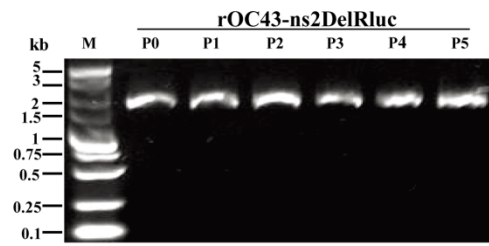
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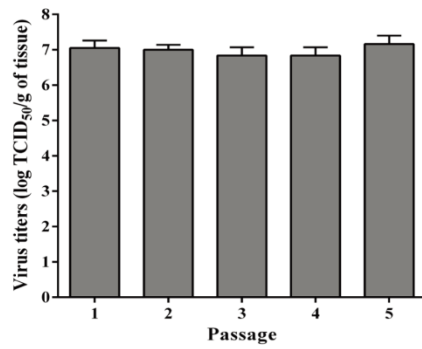
C



D



E



F

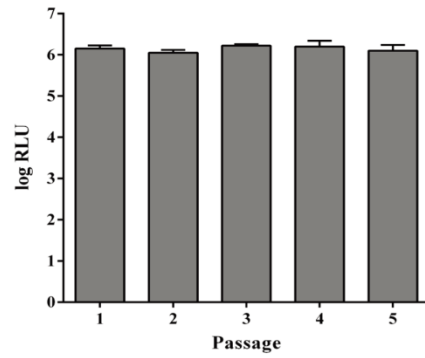


TABLE S1. Primers used for cloning and semi-quantitative RT-PCR

| Primers | Sequences (5'-3') |
|---|-----------------------------------|
| Primers for cloning | |
| DDX3X-F | GCTCTAGAAATGAGTCATGTGGCAGTGGAA |
| DDX3X-R | CGGGATCCTCAGTTACCCCACCAGTCAACC |
| ns2-EGFP-F | CGGGGTACCATGGCTGTCGCTTATGCAGAC |
| ns2-EGFP-R | CGCGGATCCTTAATATTCTTCAACTGAAGATTC |
| Primers for semi-quantitative RT-PCR | |
| q-HCoV-OC43-WT-ns2-F | CAATTTCAAATTCTCGATGAAGG |
| q-HCoV-OC43-WT-ns2-R | CTATGCATCTTCCTAGGATGTGA |
| q-HCoV-OC43-WT-ns129-F | CGGTTTTAATGTCTCAGAATTAG |
| q-HCoV-OC43-WT-ns129-R | GCTGTGAAACCATTTATTACTC |
| q-Rluc-F | TCTGGTAATGGTTCTTATAGGT |
| q-Rluc-R | ATTCAATCACATCTACTACTACTT |
| q-GAPDH-F | GTGTTCTACCCCAATGTGT |
| q-GAPDH-R | ATTGTCATACCAGGAAATGAGCTT |
| q-IFN- β -F | TAGCACTGGCTGGAATGA |
| q-IFN- β -R | GTTTCGGAGGTAACCTGTAAG |
| q-IFITM3-F | ATGTCTGCTGGTCCCTGTTC |
| q-IFITM3-R | GTCATGAGGATGCCCAGAAT |
| q-TRIM56-F | CATACCTACTGCCAAGAC |
| q-TRIM56-R | CCATTGACGAAGAAGTTG |
| q-OASL-F | AGGGTACAGATGGGACATCG |
| q-OASL-R | AAGGGTTCACGATGAGGTTG |
| q-TRIM22-F | ACCAAACATTCCGCATAAAC |
| q-TRIM22-R | TCCAGCACATTCACCTCACC |
| q-NRAMP-F | CCATCTCTACTACCCTAAG |
| q-NRAMP-R | GAGGAAGAGGAAGAAGAAG |
| q-EFTUD2-F | GCCTAATAAGAAGAACAAGA |
| q-EFTUD2-R | GAGTATCATCCACCAGAA |
| q-PKR-F | GATATTATCAGAAGAAACCTCAG |
| q-PKR-R | TTCTTCGTTGCTTATGAATG |
| q-DDX3X-F | TATATCTTCTTGGCTGTAGG |
| q-DDX3X-R | TCTGGAGGATTTCTTATACC |

Restriction enzyme sites are underlined.

TABLE S2. Genetic stability of rHCoV-OC43 during passages in BHK-21 cells

| Virus | Instability during passages | Percentage of clones positive for Rluc gene |
|------------------------|------------------------------------|--|
| rOC43-ns2DelRluc | P11 | 3/4 |
| rOC43-ns2FusionRluc | P4 | 3/4 |
| rOC43-ns12.9StopRluc | undetected | 4/4 |
| rOC43-ns12.9FusionRluc | P6 | 3/4 |