

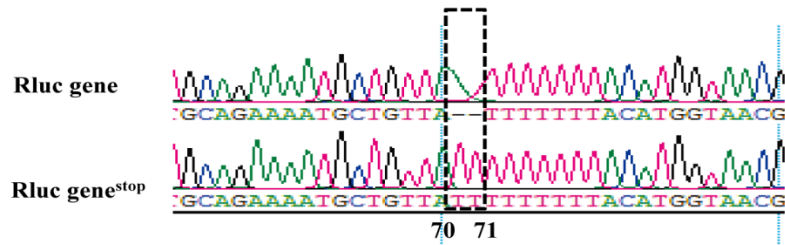
Figure legends

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	ATGAATGTTCTTGATTCATTATTAATTATTATGATTCAGAAAAACATGCAGAAAATGCT
	M N V L D S F I N Y Y D S E K H A E N A
Rluc gene^{Stop}	ATGAATGTTCTTGATTCATTATTAATTATTATGATTCAGAAAAACATGCAGAAAATGCT
	M N V L D S F I N Y Y D S E K H A E N A
	70 71
Rluc gene	GTTATTTTTTTTACATGGTAACGCGGCCTCTTCTTATTTATGGCGACATGTTGTGCCACAT
	V I F L H G N A A S S Y L W R H V V P H
Rluc gene^{Stop}	GTTATTTTTTTTACATGGTAACGCGGCCTCTTCTTATTTATGGCGACATGTTGTGCCACAT
	V I F E Y M V T R P L L I Y G D M L C H I
Rluc gene	ATTGAGCCAGTAGCGGGTGTATTATACCAGACCTTATTGGTATGGGCAAATCAGGCAAA...
	I E P V A R C I I P D L I G M G K S G K
Rluc gene^{Stop}	ATTGAGCCAGTAGCGGGTGTATTATACCAGACCTTATTGGTATGGGCAAATCAGGCAAA...
	L S Q *

Figure S2

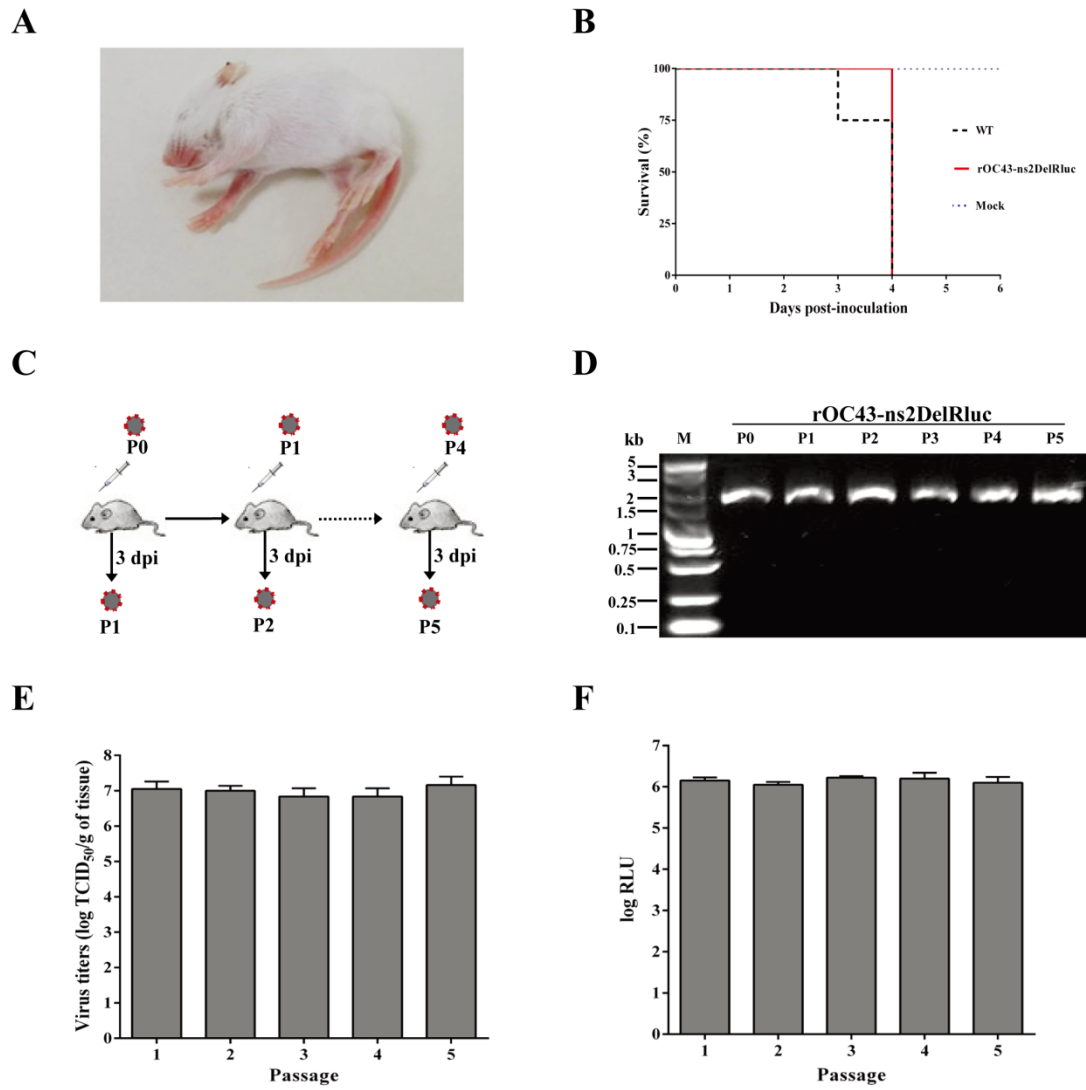


TABLE S1. Primers used for cloning and semi-quantitative RT-PCR

Primers	Sequences (5'-3')
Primers for cloning	
DDX3X-F	GCTCTAG <u>A</u> ATGAGTCATGTGGCAGTGGAA
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	GCTGTGAAACCATTATTACTC
q-Rluc-F	TCTGGTAATGGTTCTTATAGGT
q-Rluc-R	ATTCAATCACATCTACTACTT
q-GAPDH-F	GTGTTCTACCCCCAATGTGT
q-GAPDH-R	ATTGTCATACCAGGAAATGAGCTT
q-IFN- β -F	TAGCACTGGCTGGAATGA
q-IFN- β -R	GTTTCGGAGGTAACCTGTAAG
q-IFITM3-F	ATGTCGTCTGGTCCCTGTTC
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	TCCAGCACATTACCTCACC
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