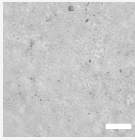
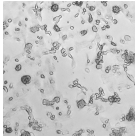
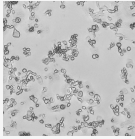
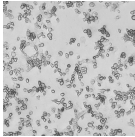
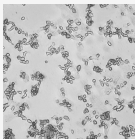
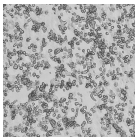
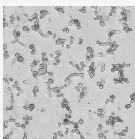
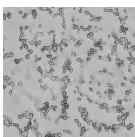
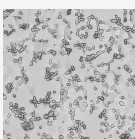
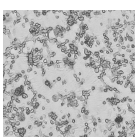
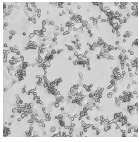
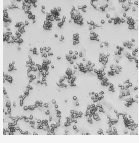
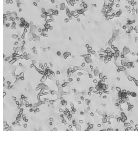
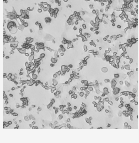
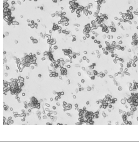
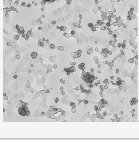
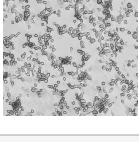
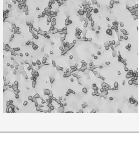
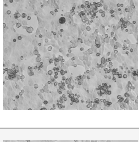
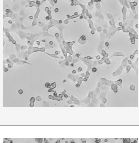
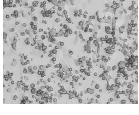


## Supplementary File 1. Infectivity assessment of recombinant virus on Vero E6 cells.

Number of fragments	Transfection method	Name	Cell line transfected	3 days post infection on Vero E6	Description
-	-	Vero E6 negative control	-		Uninfected Vero E6 cells.
8	electroporation	rCoV2-8fr	HEK293T		Rescue from eight fragments.
4	electroporation	rCoV2-4fr	HEK293T		Rescue from four fragments.
4	electroporation	rCoV2-293	HEK293		Rescue from HEK293.
4	electroporation	rCoV2-CHO	CHO		Rescue from CHO.
4	electroporation	rCoV2-BHK	BHK-21		Rescue from BHK-21.
4	electroporation	rCoV2-A549	A549-ACE2		Rescue from A549-ACE2.
4	jetPRIME	rCoV2-jP	HEK293T		Rescue after transfection with jetPRIME.
4	Lipofectamine-3000	rCoV2-3000	HEK293T		Rescue after transfection with Lipofectamine-3000.
4	Lipofectamine-LTX	rCoV2-LTX	HEK293T		Rescue after transfection with Lipofectamine-LTX.

5	electroporation	rCoV2-500bp	HEK293T		Rescue including one fragment to be only 500 bp in length.
5	electroporation	Wuhan <sup>BA.1 S</sup>	HEK293T		Chimeric virus, Omicron BA.1 S gene in the background of Wuhan.
5	electroporation	Wuhan <sup>BA.5 S</sup>	HEK293T		Chimeric virus, Omicron BA.5 S gene in the background of Wuhan.
5	electroporation	rCoV2-N501Y	HEK293T		Direct mutagenesis, incorporation of N501Y substitution in the S gene.
5	electroporation	rCoV2-G614D	HEK293T		Direct mutagenesis, incorporation of G614D substitution in the S gene.
5	electroporation	rCoV2-ΔORF3a	HEK293T		Direct mutagenesis, deletion of ORF3a.
5	electroporation	rCoV2-ORF8-FLAG	HEK293T		Direct mutagenesis, incorporation of a 3x-FLAG tag at the C-terminal of ORF8.
9	electroporation	rCoV2-link-Wuhan	HEK293T		Rescue from RNA directly using the linker fragment.
9	electroporation	rCoV2-link-Omicron BA.5 <sup>BA.1 S</sup>	HEK293T		Rescue of chimeric virus from RNA directly using the linker fragment. Omicron BA.1 S gene in the background of Omicron BA.5.
9	electroporation	rCoV2-link-Omicron BA.1 <sup>BA.5 S</sup>	HEK293T		Rescue of chimeric virus from RNA directly using the linker fragment. Omicron BA.5 S gene in the background of Omicron BA.1.
9	electroporation	rCoV2-link-Omicron BA.5 <sup>Wuhan S</sup>	HEK293T		Rescue of chimeric virus from RNA directly using the linker fragment. Wuhan S gene in the background of Omicron BA.5.