



Extended Data Figure S1. Robust and efficient generation of an EGFP-reporter replication-competent VSV bearing SARS-CoV-2 spike (rcVSV-CoV2-S). (A) Schematic of the rcVSV-CoV2-S genomic coding construct and the virus rescue procedure. The maximal T7 promoter (T7prom) followed by a hammer-head ribozyme (HhRbz) and the HDV ribozyme (HDVRbz) plus T7 terminator (T7term) are positioned at the 3' and 5' ends of the viral cDNA, respectively. An EGFP(E) transcriptional unit is placed at the 3' terminus to allow for high level transcription. SARS-CoV-2-S is cloned in place of VSV-G using the indicated restriction sites designed to facilitate easy exchange of spike variant or mutants. (B) For virus rescue, highly permissive 293T cells stably expressing human ACE2 and TMPRSS2 (293T-[ACE2+TMPRSS2], F8-2 clone) cells were transfected with the genome coding plasmid, helper plasmids encoding CMV-driven N, P, M, and L genes, and pCAGS encoding codon-optimized T7-RNA polymerase(T7opt). 48-72 hpi, transfected cells turn EGFP+ and start forming syncytia. Supernatant containing rcVSV-CoV2-S are then amplified in Vero-TMPRSS2 cells at the scale shown. The blue arrows at the bottom indicate the timeline for production of each sequence verified stock.