Cell Reports, Volume *36*

Supplemental information

No evidence of human genome integration of SARS-CoV-2 found by long-read DNA sequencing

Nathan Smits, Jay Rasmussen, Gabriela O. Bodea, Alberto A. Amarilla, Patricia Gerdes, Francisco J. Sanchez-Luque, Prabha Ajjikuttira, Naphak Modhiran, Benjamin Liang, Jamila Faivre, Ira W. Deveson, Alexander A. Khromykh, Daniel Watterson, Adam D. Ewing, and Geoffrey J. Faulkner

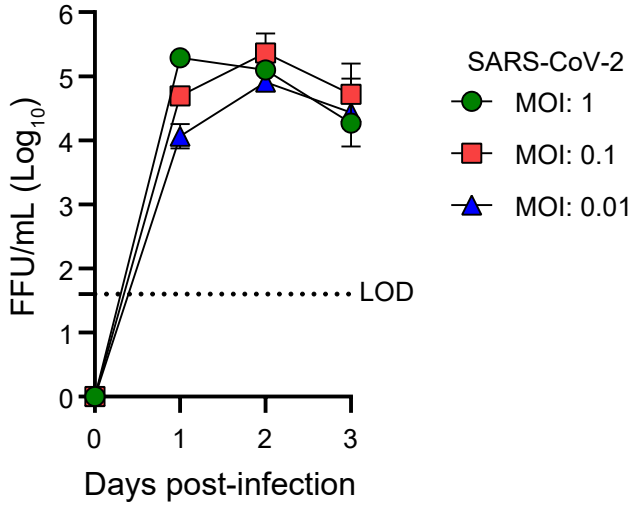

Figure S1. SARS-CoV-2 is replication competent in HEK293T cells, related to Figure 2. HEK293T cells were infected with SARS-CoV-2 isolate QLD02 at an MOI of 0.01, 0.1 and 1.0. Inoculum was removed after infection and cells were washed before the addition of growth media. Supernatant was collected at the indicated time points and viral titres were quantified as focus-forming units (FFU) per mL by immuno-plaque assay (iPA) (Amarilla et al., 2021) with a limit of detection (LOD) as indicated. Data are represented as the mean ± standard deviation of three replicates.

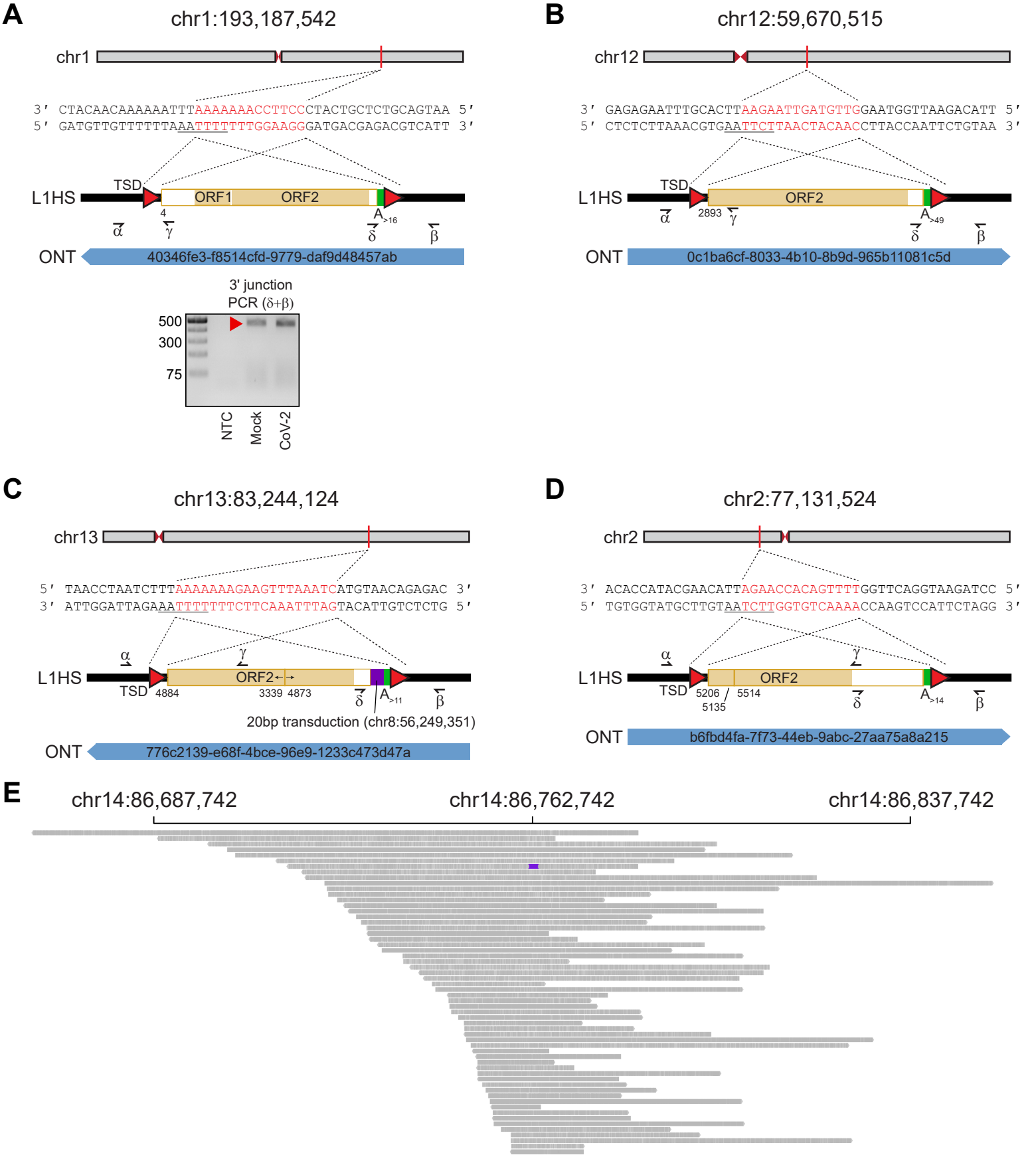

Figure S2. Additional L1HS insertions detected by ONT sequencing in HEK293T cells, related to Figure 2. **(A)** A near full-length L1. **(B)** A 5′ truncated L1. **(C)** A 5′ inverted/deleted L1 carrying a 3′ transduction (purple rectangle) traced to a non-reference source L1. **(D)** A 5′ inverted/deleted L1. **(E)** Integrative Genomics Viewer (Robinson et al., 2011) visualisation of read alignments spanning the L1 integration site displayed in Figure 2D. The L1 is coloured purple. Note: panels (A-D) show the genomic coordinates of an L1 insertion, as well as the sequence at the insertion site. Nucleotides highlighted in red correspond to the integration site TSD. Underlined nucleotides correspond to the L1 EN motif. Cartoons summarise the features of each L1, with the underneath numerals representing the 5′ end position relative to the mobile L1HS sequence L1.3 (Dombroski et al., 1993), TSDs shown as red triangles, and 3′ polyA tracts coloured as green rectangles. One spanning ONT read with its identifier is positioned underneath each cartoon. Symbols (α, β, δ, γ) represent the approximate position of primers used for empty/filled and L1-genome junction PCR validation reactions. The results of the L1-genome 3′ junction PCR are shown for panel (A). Ladder band sizes are as indicated, NTC; non-template control. The red filled triangle indicates an on-target product confirmed by capillary sequencing. No on-target products were observed for the corresponding 5′ junction PCR or the examples shown in panels (B-D).