Cell Reports, Volume *35*

Supplemental information

SARS-CoV-2 genomic surveillance identifies naturally occurring truncation of ORF7a that limits immune suppression

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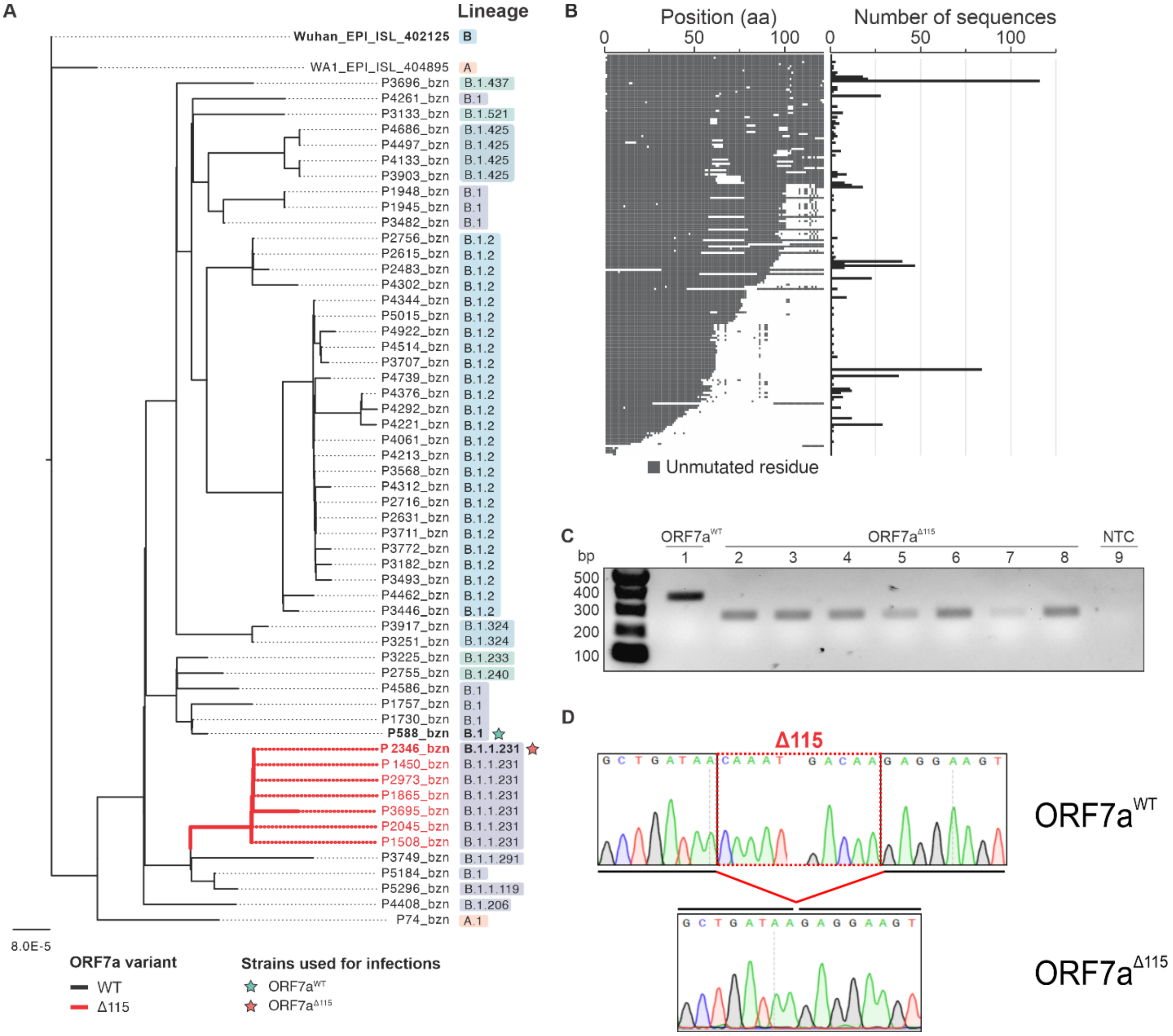
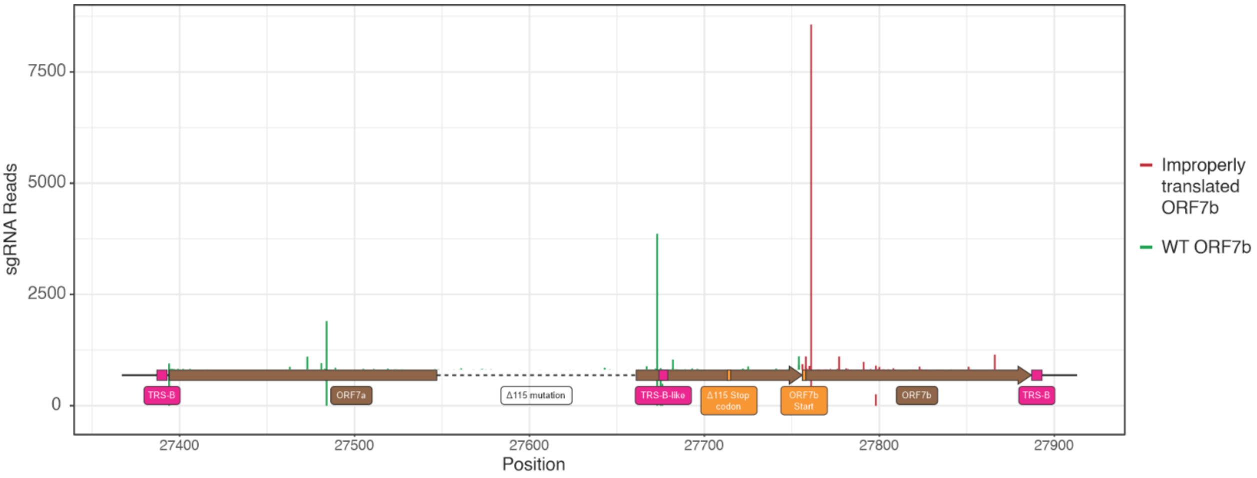
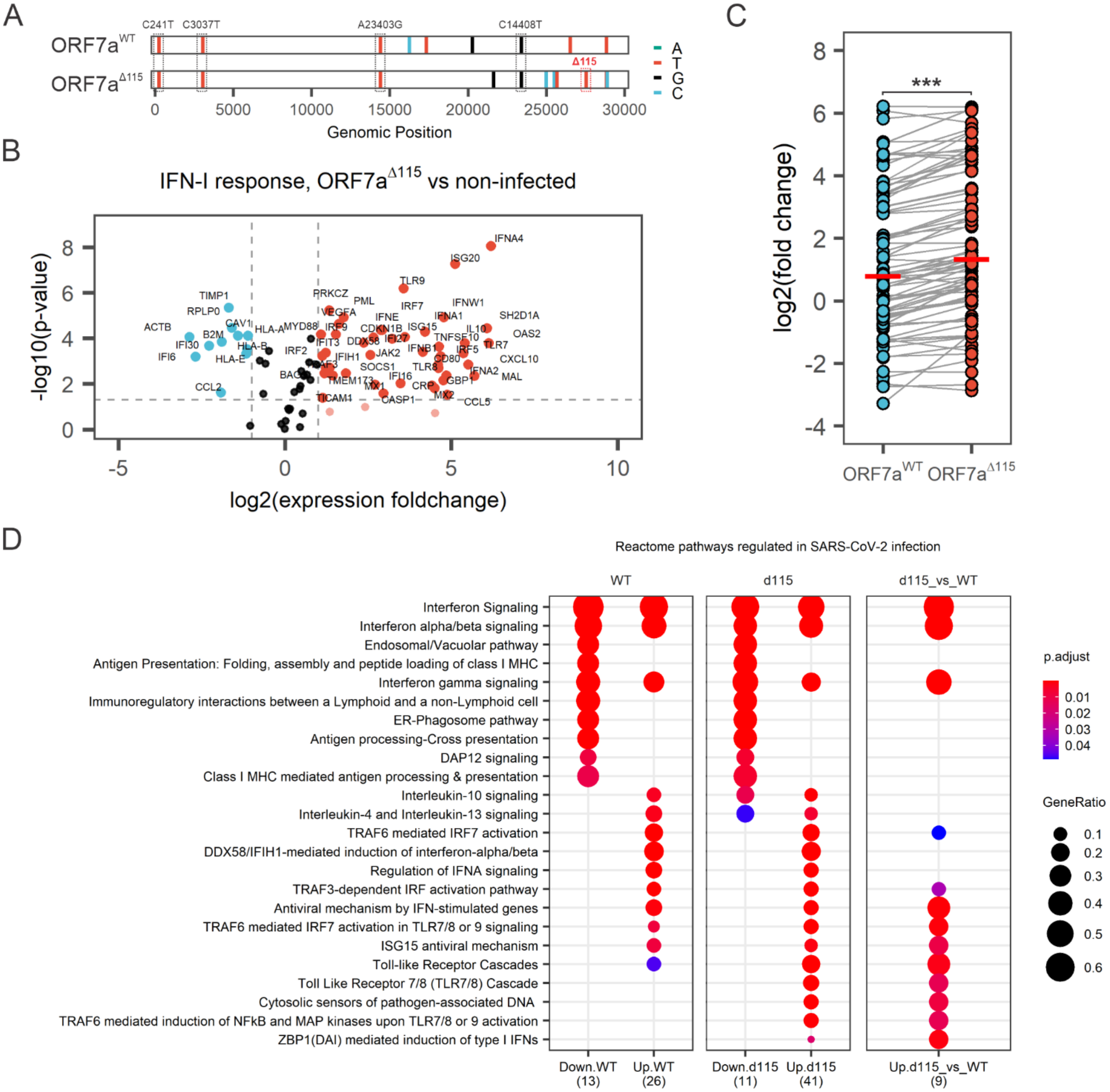


Supplemental Figure S1, Phylogenetic and mutational analysis of ORF7a gene sequences. Related to main Figure 1. A) A maximum-likelihood phylogenetic tree indicates that ORF7a$^{\Delta 115}$ strains form a monophyletic clade (red branches). Viral isolate P588 (teal star) was chosen as a "wildtype" control in our experiments. This strain shares a recent common ancestor with the predominant ORF7a$^{\Delta 115}$ clade. SARS-CoV-2 lineages were assigned using pangolin utility (cov-lineages.org, (Rambaut, Andrew et al., 2020)). **B)** Alignment of unique ORF7a protein sequences with five or more mutations as compared to the SARS-CoV-2 reference strain (Wuhan-Hu-1). Conserved residues are depicted in gray, while mutations and deletions are shown in white. The frequency of each genotype is shown on the right. **C)** RT-PCR with primers targeting regions that flank the ORF7a Δ115 deletion sampled in Bozeman, MT. **D)** All PCR products in (**C**) were Sanger sequenced. Representative sequencing chromatograms are shown**.** Deleted region is shown with dotted red box.



Supplemental Figure S2, Mapping TRS-B sites required for ORF7a and ORF7b discontinuous sgRNA transcription. Related to Main Figure 2F-H. Direct RNA sequencing data published by Kim et al was used to map TRS-B sites in SARS-CoV-2 ORF7a and ORF7b genes (Kim et al., 2020). Vertical lines indicate number of reads spanning the junction between TRS-L and "body" of the gene. Dashed line indicate region deleted in ORF7a$^{\Delta 115}$ strain. The deletion does not remove any of the mapped TRS-B sites suggesting that it has not affected ORF7b sgRNA transcription.



Supplemental Figure S3, Related to Main Figure 3. IFN-I response and gene set enrichment analysis. A) Genome sequences of ORF7a^{WT} and ORF7a$^{\Delta 115}$ are compared to Wuhan-Hu-1 reference sequence. Colored lines mark differences to reference genome. Mutations that define haplotype of B.1 SARS-CoV-2 lineage are boxed. There are 11 nucleotide positions that differ between WT and Δ115 and are outside ORF7a. Seven of these SNVs change amino acid. In ORF7a^{WT}: S194L in N protein and L1286F in ORF1ab. In ORF7a$^{\Delta 115}$: R195I, R203K, G204K in N protein; Q38P and L95I in ORF3a. **B)** Volcano plot depicting IFN-I response in HEK 293T-hACE2 cells infected with ORF7a$^{\Delta 115}$ SARS-CoV-2 strain at MOI = 0.05. RNA was extracted from infected cells 24 hpi and reverse transcribed. Expression of IFN-I response genes was studied using RT-qPCR array targeting 91 human transcripts (88 targets and 3 references). Experiment was performed in 3 biological replicates. Dashed lines show regulation (≥2-fold) and statistical significance thresholds (p-value < 0.05). Each dot represents mean (n = 3) normalized expression of a single gene relative to non-infected host. Genes that passed the threshold are labeled. **C)** Plot showing distribution of log2(expression fold change) values in ORF7a$^{\mathrm{WT}}$ and ORF7a$^{\Delta 115}$ infection vs non-infected control. Each dot represents relative normalized expression for one transcript targeted with the RT-qPCR array. Gray lines connect values for the same transcript in the infection with ORF7a$^{\mathrm{WT}}$ and ORF7a$^{\Delta 115}$ variants. Horizontal red bar shows median value. Median ISG activation between two infections was compared using Wilcoxon signed-rank test (***, p-value < 0.001) **D)** List of IFN-I response genes that were significantly regulated (p-value < 0.05, ≥ 2-fold change in expression) upon infection with ORF7a$^{\Delta 115}$ and ORF7a$^{\mathrm{WT}}$ (vs non-infected control) were analyzed for enrichment of associated Reactome Pathways (Jassal et al., 2019; Zhou, Z. et al., 2020). Gene set enrichment analysis (GSEA) was performed using clusterProfiler package in RStudio (Yu and He, 2016; Yu et al., 2012). *Down.WT* – genes downregulated during infection with ORF7a$^{\mathrm{WT}}$; *Up.WT* – genes upregulated during infection with ORF7a$^{\mathrm{WT}}$; *Down.d115* – Reactome pathways enriched in genes downregulated during infection with ORF7a$^{\Delta 115}$; *Up.d115* – genes upregulated during infection with ORF7a$^{\Delta 115}$. *Up.d115_vs_WT* – Reactome pathways enriched in genes upregulated during infection with ORF7a$^{\Delta 115}$ vs ORF7a$^{\mathrm{WT}}$ (**Figure 3E**).