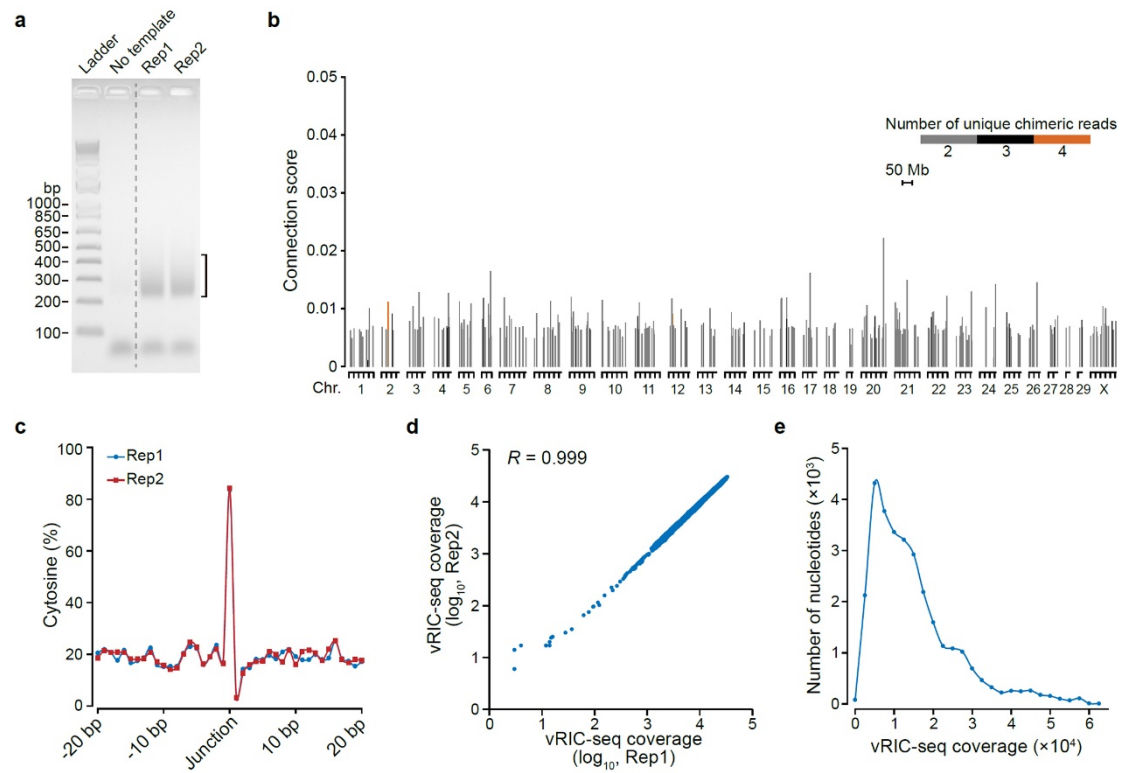
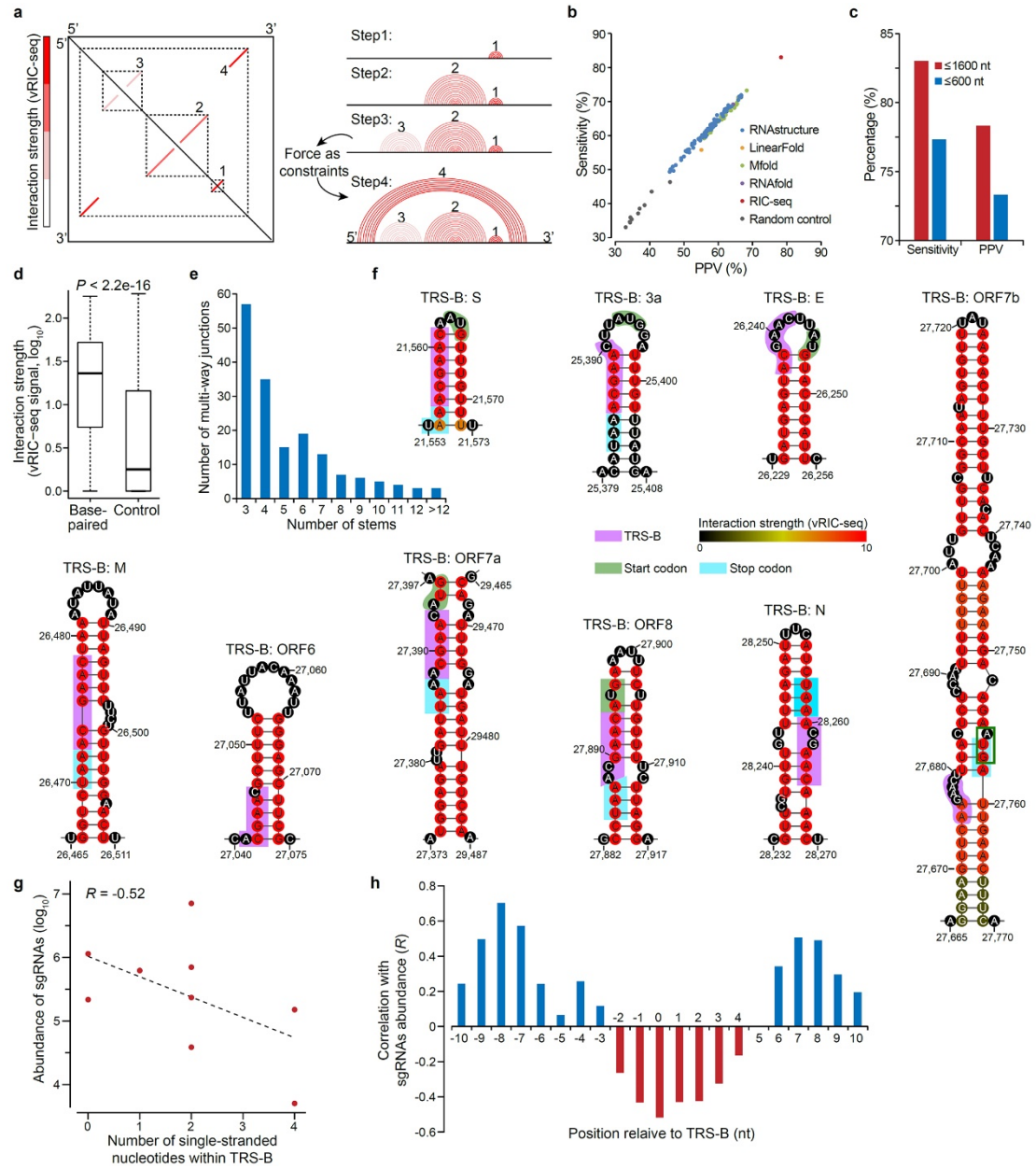




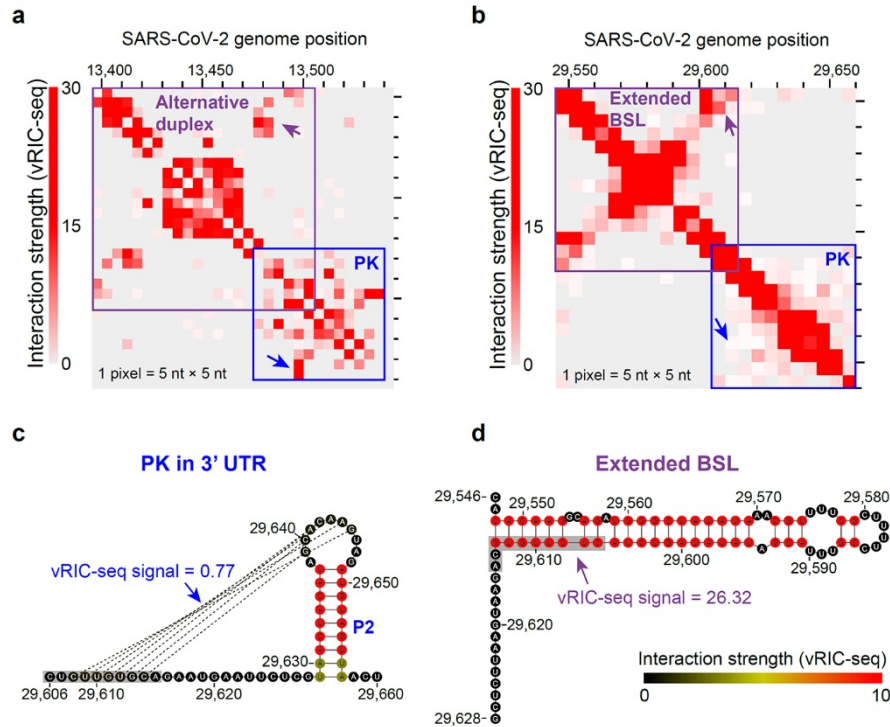
Supplementary Fig. 1 | Characterization of vRIC-seq technology. **a**, The PCR products were purified from the agarose gel (the bracketed region) for paired-end sequencing. The experiment was independently repeated twice with similar results. Source data are provided as a Source Data file. **b**, The chromosomal location of host RNA fragments ligated to viral RNA. The color scale indicates the number of unique chimeric reads supporting each interaction. Chr represents the chromosome. **c**, The percentages of cytosine around the junction of chimeric reads. **d**, Scatter plot showing the correlation for coverage of chimeric reads along the SARS-CoV-2 genome in two biological replicates (Rep1 and Rep2). R , Pearson correlation coefficient. **e**, The coverage of chimeric reads for each nucleotide of the SARS-CoV-2 genome.

fragments shown in **(h)**. Bottom: the predicted local duplexes using the RNAstructure program. **k**, COMRADES reveals pervasive alternative RNA-RNA interactions in host cells, while vRIC-seq usually identifies one predominant interaction in the virion. **l**, COMRADES revealed in-cell pairwise interactions showed a higher value of Shannon entropy than in-virion interactions detected by vRIC-seq. The Shannon entropy was calculated for each 10-nt genomic window ($n = 2,986$). *P*-values in **f**, **k**, and **l** were determined by the two-tailed, unpaired *t*-test. For the box plots in **f**, **g**, and **l**, the centre line of the box plot represents the median, the box borders represent the first (Q1) and third (Q3) quartiles, and the whiskers are the most extreme data points within $1.5\times$ the interquartile range (from Q1 to Q3).

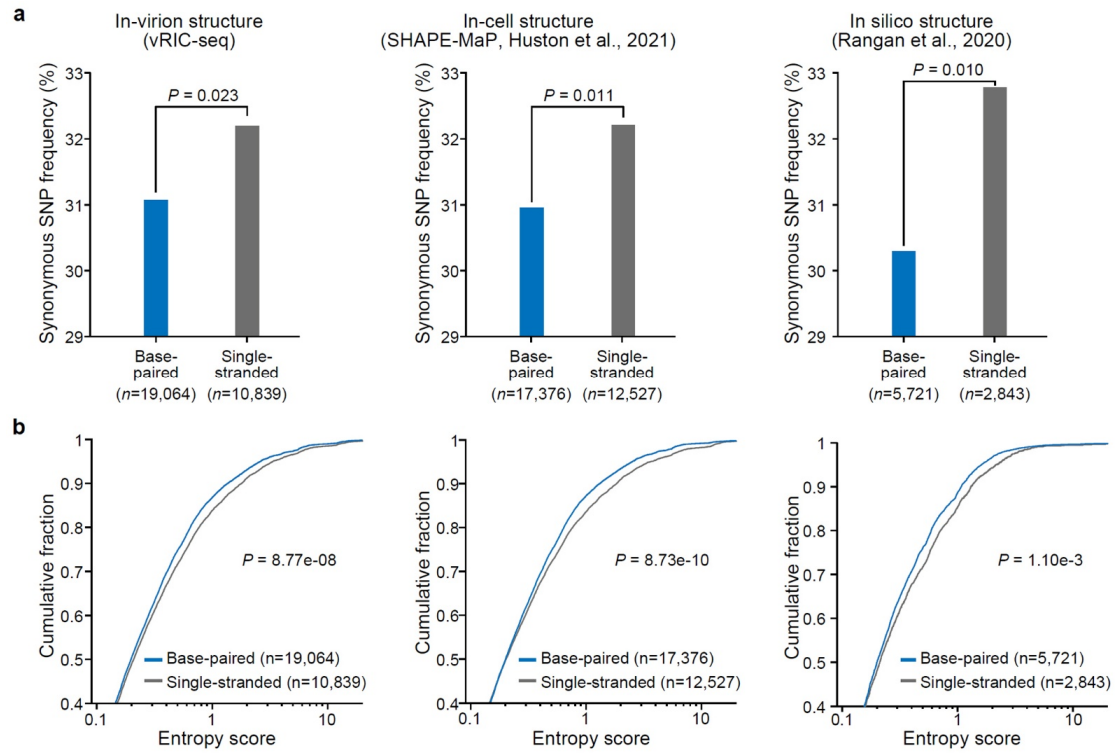


Supplementary Fig. 3 | Performance of the adaptive algorithm. **a**, The adaptive algorithm was developed to predict RNA secondary structure based on vRIC-seq data. Local duplexes were first predicted and used as a constraint for inferring long-range duplexes. **b**, Comparing the adaptive algorithm's performance and several computational software on predicting the secondary structure of 28S rRNA. PPV stands for positive predictive value. Control was chosen by randomly divide the 28S rRNA into size-matched fragments. **c**, Sensitivity and PPV increased if including the longer duplexes (≤ 1600 nt, red) for predicting the secondary structure of 28S rRNA by our adaptive strategy. **d**, Pairwise interacting RNA fragments ($n = 3,935$) showed more vRIC-seq signals than the random controls that have the same spanning distance ($n = 22,438$). P -value was determined by the two-tailed, unpaired t -test. The centre line of the box plot represents the median, the box borders represent the first (Q1) and third

(Q3) quartiles, and the whiskers are the most extreme data points within $1.5\times$ the interquartile range (from Q1 to Q3). **e**, Summary of the number of multi-way junctions and the corresponding stems in on our secondary structure model. **f**, The secondary structure model of nine TRS-B elements. The core sequence (CS) of each TRS-B, the start codons, and the stop codons are marked by purple, green, and cyan boxes, respectively. **g**, The number of single-stranded nucleotides in the TRS regions showed negative correlations to the sgRNAs abundance. **h**, The number of single-stranded nucleotides surrounding the TRS regions showed positive correlations to the sgRNAs abundance.

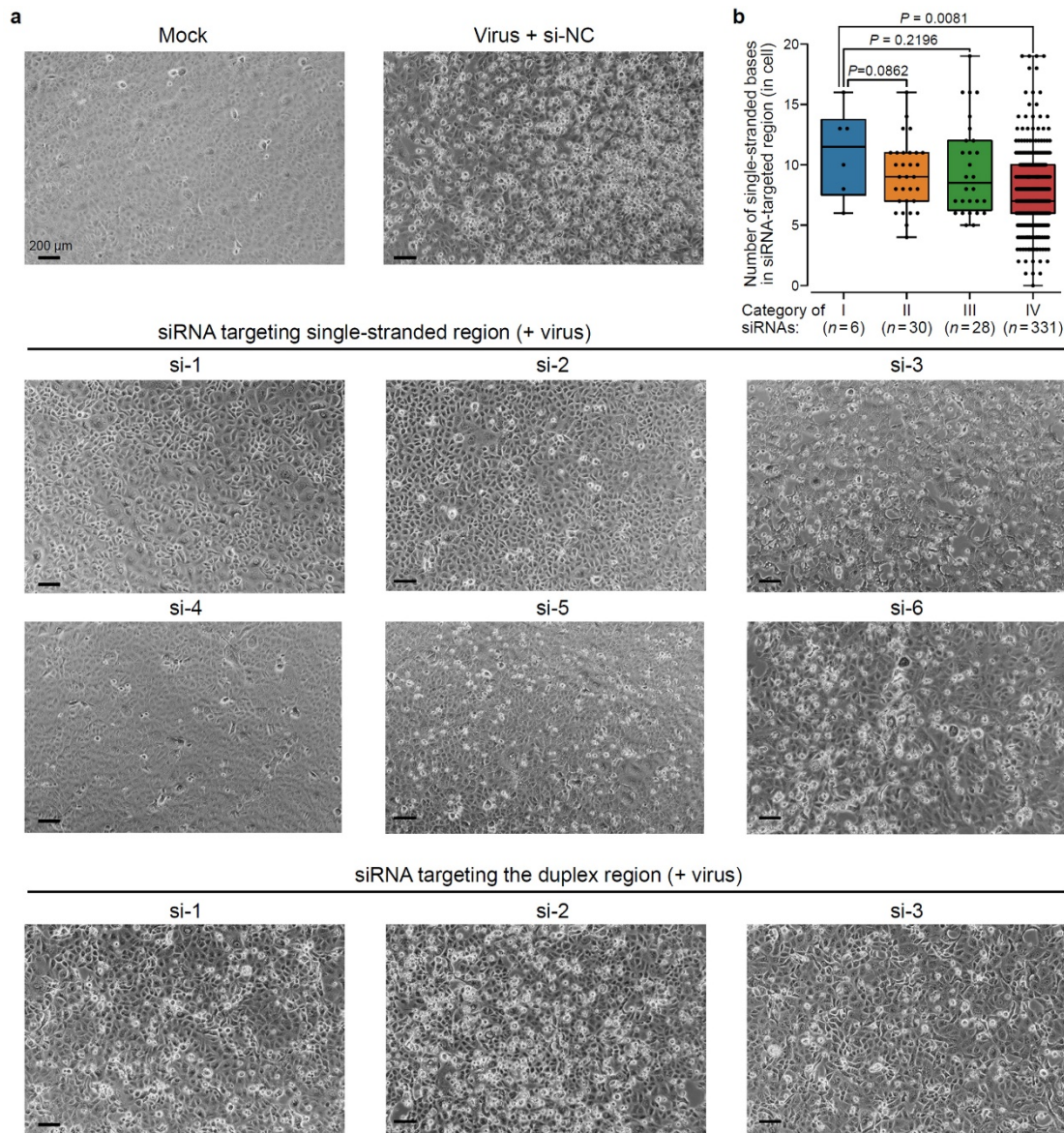


Supplementary Fig. 4 | Alternative conformation for pseudoknots in FSE and 3' UTR. **a**, The RNA map view of the frame-shifting element (FSE) surrounded regions. Two alternative conformations may mutually present in the virions. Arrowheads are showing two alternative interactions. **b**, The RNA map view of the bulged stem-loop (BSL) element and pseudoknot (PK) in the 3' UTR. The vRIC-seq data preferably supports an extended BSL conformation in the virions (see purple arrowhead). **c**, The 3' UTR pseudoknot structure is barely supported by vRIC-seq data. **d**, An extended BSL duplex is strongly supported by vRIC-seq data.



Supplementary Fig. 6 | Base-paired nucleotides are less mutated during evolution.

a, Synonymous single nucleotide polymorphisms (SNPs) more frequently happen in single-stranded regions than base-paired regions. P -value was determined by the one-sided Fisher's exact test. **b**, The entropy scores are significantly lower in the base-paired regions than single-stranded regions. A two-sided Kolmogorov-Smirnov test was used to calculate the P -value.



Supplementary Fig. 7 | Cells transfected with single-strand-specific siRNAs are more potent against SARS-CoV-2 infection. **a**, Vero cells treated by siRNAs targeting single-stranded regions survived better than those with siRNA targeting the duplex regions after the infection of SARS-CoV-2. Scale bar, 200 μ m. **b**, The number of single-stranded nucleotides within the siRNAs' target region in cells. Category I: six single-strand-specific siRNAs designed from our in-virion structure model. Category II: siRNAs designed by the ViennaRNA Web Services. Category III: siRNA targeting unstructured regions designed from the in silico model of SARS-CoV-2³⁶. Category IV: all possible siRNAs designed from the linear sequence SARS-CoV-2. The centre line of the box plot represents the median, the box borders represent the first (Q1) and third (Q3) quartiles, and the whiskers are the most extreme data points within 1.5 $\times$ the interquartile range (from Q1 to Q3). *P*-value was determined by the one-tailed, unpaired *t*-test.

Supplementary Table 1. List of siRNAs, primers, and probe used in this study.

siRNA sequences

siRNA	Sequence (5' to 3')
si-NC	UUCUCCGAACGUGUCACGUTT
single-stranded region si-1	CACACGUCCAACUCAGUUUTT
single-stranded region si-2	GCCCCUUUUCUCUAUCUUUTT
single-stranded region si-3	CCACCACAAACCUCUAUCATT
single-stranded region si-4	GGAGUCAAUUACAUAUACATT
single-stranded region si-5	CACCUGAAGAACAUUUUUAUTT
single-stranded region si-6	GAGCCCUAAUGUGUAAAAUTT
duplex region si-1	GGUUGGUGGUAUUUAUAAUTT
duplex region si-2	CUGCAAGAUAUAAUGAAATT
duplex region si-3	GACUGUGUUAUGUAUGCAUTT

Primer sequences and TaqMan probe used for qPCR

Primers	Sequence (5' to 3')
pcDNA3.1-dual-1-nCOV delete F	TGTACCGTCTGCGGTATGTGGAAACCCATGCTTCAGTCAGC TGATGCA
pcDNA3.1-dual-1-nCOV delete R	TGCATCAGCTGACTGAAGCATGGGTTTCCACATACCGCAGA CGGTACA
<i>GAPDH</i> -qF	GTCTCCTCTGACTTCAACAGCG
<i>GAPDH</i> -qR	ACCACCCTGTTGCTGTAGCCAA
SARS-CoV-2 <i>RdRp</i> -qF	CAAATTCTATGGTGGTTGGCACA
SARS-CoV-2 <i>RdRp</i> -qR	GGCATGGCTCTATCACATTTAGG
SARS-CoV-2 <i>N</i> gene-qF	AACACAAGCTTTCGGCAGAC
SARS-CoV-2 <i>N</i> gene-qR	ACCTGTGTAGGTCAACCACG
SARS-CoV-2 <i>N</i> gene probe	/5'-6-FAM/CAGCGCTTCAGCGTTCTTCGGAATGTCGC/3'-BHQ/