sgDI-tector: defective interfering viral genome bioinformatics for detection of coronavirus subgenomic RNAs

Supplementary Information

Andrea Di Gioacchino, Rachel Legendre, Yannis Rahou, Valérie Najburg, Pierre Charneau, Benjamin D. Greenbaum, Frédéric Tangy, Sylvie van der Werf, Simona Cocco, Anastasia V. Komarova

	Replicate 1	Replicate 2	Replicate 3
Total number of reads	34470773	39467941	42751723
Human genome	18351424	18855674	20219414
(% of total reads)	(53.2%)	(47.7%)	(47.3%)
Human ribosomal RNA	119155	115706	156747
(% of total reads)	(0.3%)	(0.3%)	(0.4%)
Viral (SARS-CoV-2)	11461575	15714257	16776050
(% of total reads)	(33.2%)	(39.8%)	(39.2%)
Total DVG reads	81866	80598	94319
(% of viral reads)	(0.7%)	(0.5%)	(0.6%)
deletion DVG	59886	60854	70133
(% of total DVG)	(73.2%)	(75.5%)	(74.4%)
of which sgRNA	41751	41029	47858
(% of deletion DVG)	(69.7%)	(67.4%)	(68.2%)
insertion DVG	20577	18011	22409
(% of total DVG)	(25.1%)	(22.3%)	(23.8%)
copyback DVG	1403	1733	1777
(% of total DVG)	(1.7%)	(2.2%)	(1.9%)

Supplementary Table 1: Summary of results of RNAseq and alignment of the reads to the human genome, and SARS-CoV-2 viral genome. NGS library preparation was performed with an rRNA depletion step. The unmapped reads have been further processed with DI-tector and the resulting characterization of the DVG reads into deletions, insertions and copy-backs is given after the double horizontal line. To count sgRNA reads, we only considered standard subgenomic ORFs for SARS-CoV-2 (S, 3A, E, M, 6, 7a, 7b, N, 10). The DVG copyback row contains reads coming from both 3' and 5' copybacks and snapbacks.

ORF name	AUG start position in genome
U6cb1	27825
U54f0	21744
U633d	25405
U59dc	23004
U5345	21317
U7226	29222
U5757	22359
U5856	22614
U3dc4	15812
U11ee	4590
U5df3	24051

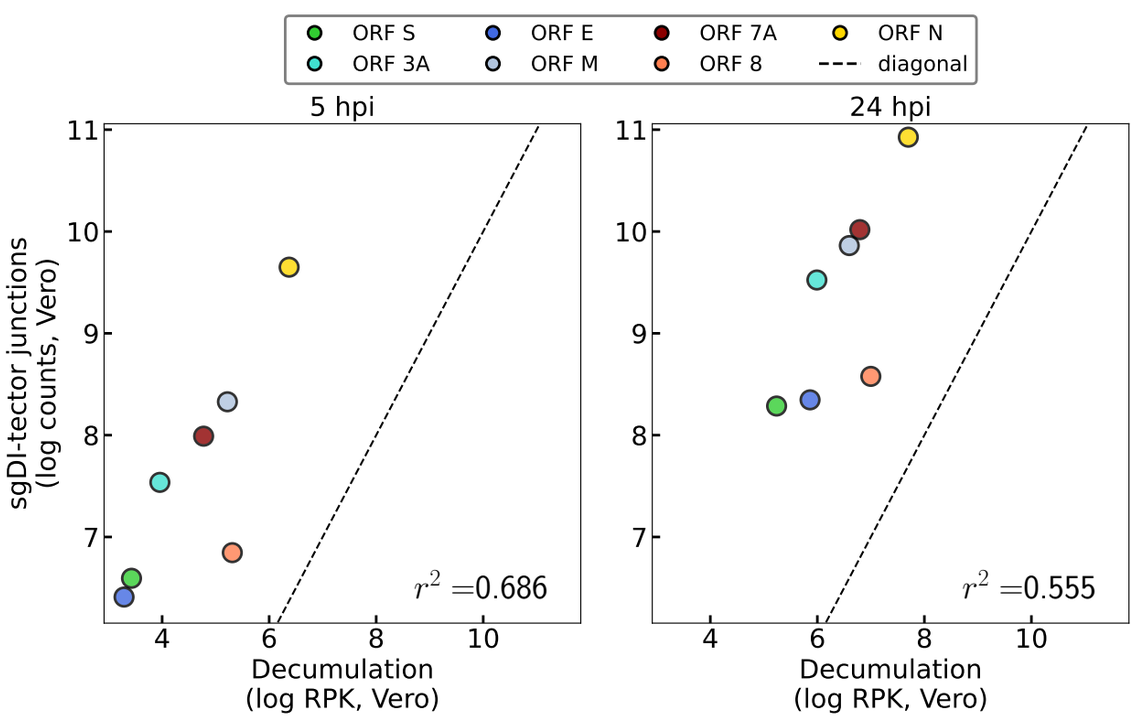
Supplementary Table 2: Conversion between names of the non-standard ORFs with highest counts obtained by sgDI-tector (blue bars in Figs. 4 and 5) and the corresponding position of the start codon (AUG) in the SARS-CoV-2 genome. The conversion is made by interpreting the alphanumerical characters after the U (which stands for unknown) in the ORF name as an hexadecimal number. The position in genome is given with respect to the SARS-CoV-2 strain hCoV-19/France/GES-1973/2020, with GISAID ID: EPI_ISL_414631.

Replicate #2		Replicate #3	
Junction ORF	Motif	Junction ORF	Motif
ORF M	UAA**ACGAAC**U	ORF M	UAA**ACGAAC**U
ORF 3A	AA**ACGAAC**UU	ORF 3A	AA**ACGAAC**UU
ORF N	CUAA**ACGAAC**	ORF N	CUAA**ACGAAC**
ORF 7A	UAA**ACGAAC**	ORF 7A	UAA**ACGAAC**
ORF 8	CUAA**ACGAAC**	ORF 8	CUAA**ACGAAC**
ORF S	CUAA**ACGAAC**	ORF S	CUAA**ACGAAC**
U6cb1	GAACUUU	ORF E	**ACGAAC**UU
ORF E	**ACGAAC**UU	U6cb1	GAACUUU
U54f0	A**ACGAAC**	U54f0	A**ACGAAC**
ORF 3A-1	A**ACGAAC**UU	ORF 3A-1	A**ACGAAC**UU
U5757	AACUUUA	U3dc4	GAACUUUAA
U3dc4	GAACUUUAA	U5757	AACUUUA
		U5f5b	UUCUCUA
		U6d2e	UAA**ACGAAC**
		U744b	AACUUUAA
		U52c4	CUCUAAA

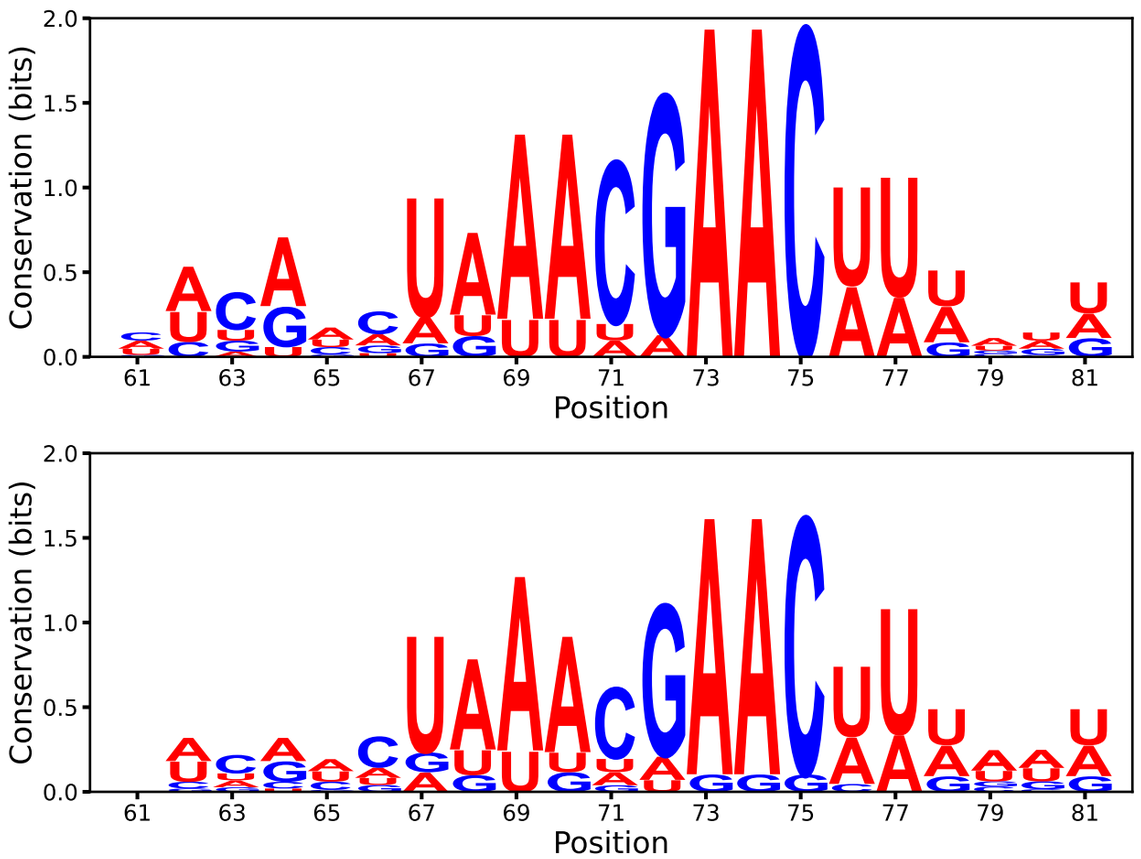
Supplementary Table 3: TRS and putative TRS obtained for the two biological replicates not discussed in the main text. The double vertical line divides the two biological replicates. The junction ORFs are sorted according to the number of times that junction was found by the algorithm (in decreasing order). As it is apparent, the results are consistent across replicates, especially for the junctions with more counts.

	Replicate #2		Replicate #3	
Target detected	SARS-CoV-2	Non-infected	SARS-CoV-2	Non-infected
U3dc4	23.72 ± 0.04	ND	22.1 ± 0.1	ND
ORF N	13.9 ± 0.1	ND	12.6 ± 0.1	ND
GAPDH	19.80 ± 0.03	18.8 ± 0.1	19.5 ± 0.1	18.5 ± 0.1

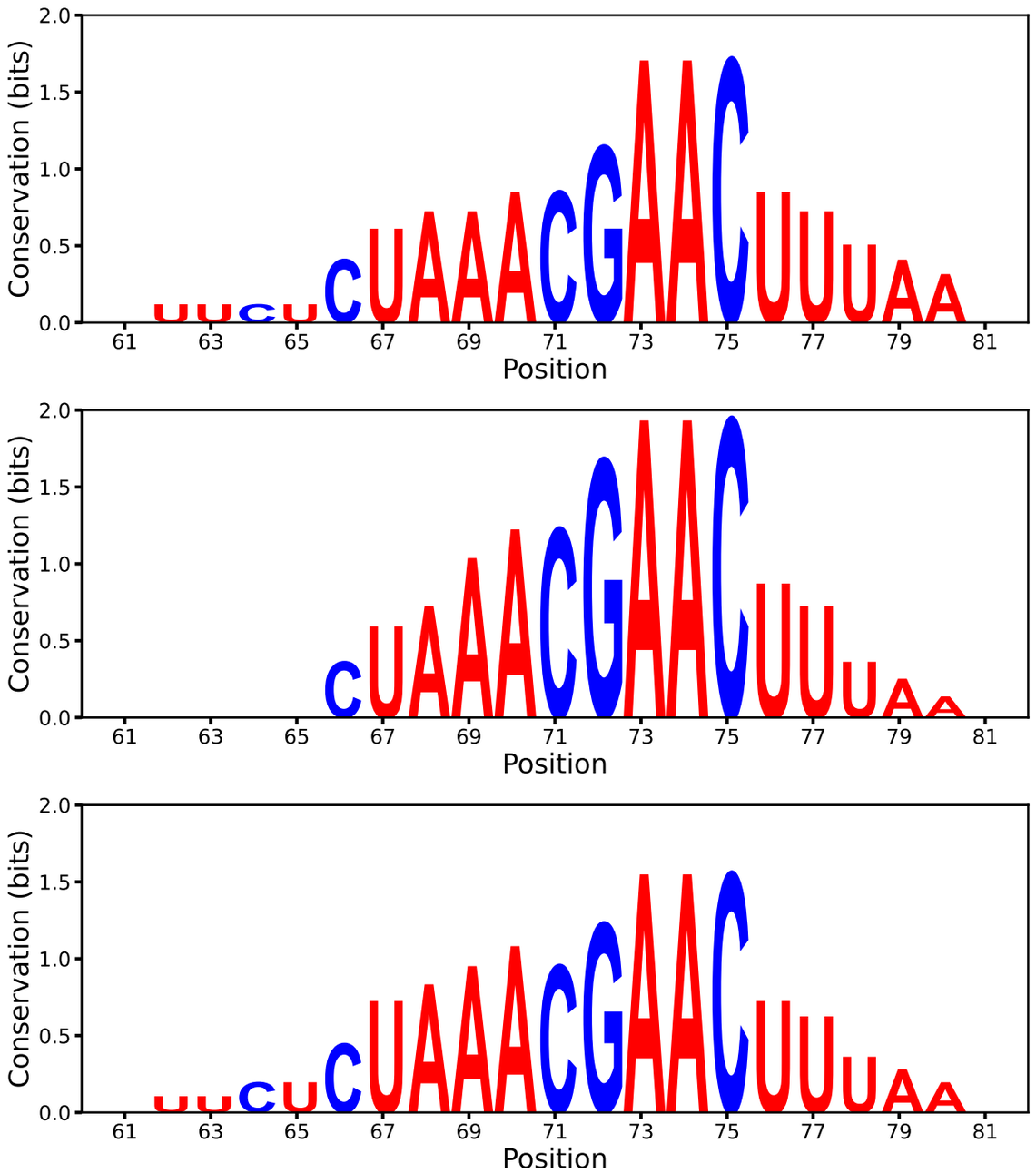
Supplementary Table 4: **RT-qPCR validation of U3dc4 sgRNA transcript.** RT-qPCR C_t values for U3dc4, ORF N, and GAPDH detection in cDNA equivalent of 50 ng of total RNA extracted from SARS-CoV-2 or mock-infected HEK293T cells are shown. Samples were analyzed in duplicates. ND: Not Determined ($C_t > 34$). Data are given as average $\pm$ standard deviation. Results obtained from the two biological replicates not shown in Table 2.



Supplementary Figure 1: sgDI-tector detected junctions are more correlated with the results of the decumulation method when the two strategies are applied to Finkel *et al.* 's data. In particular, this correlation is higher at 5 hpi, when less DVG are present.



Supplementary Figure 2: Sequence logo around RI positions aligned to the leader sequence, for the two other biological replicates (see Fig. 7).



Supplementary Figure 3: Sequence logo for the putative TRS sequences only, for the three biological replicates (see Tab. 2).