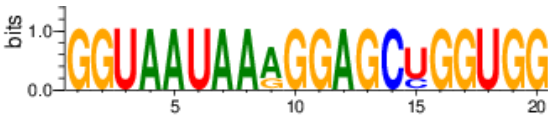


Figure S1

(a)

Selected PQSs in +gRNA

		Location +gRNA	
SARS-CoV-2	GGUAAUAAAGGAGCUGGUGG	644	
RaTG13	GGUAAUAAAGGAGCUGGUGG	644	100% identity
Bat_C21	GGUAAUAAAGGAGCUGGUGG	643	100% identity
Bat_C45	GGUAAUAAAGGAGCUGGUGG	643	100% identity
SARS-CoV	GGUAAUAAAGGAGCUGGUGG	643	90% identity



		Location +gRNA	
SARS-CoV-2	GGAGGAGGUGUUGCAGG	3,467	
RaTG13	GGAGGAGGUGUUGC- <b>GG</b>	3,464	94% identity
Bat_C21	GGAGGAGGUGUUGC <b>U</b> GG	3,385	94% identity
Bat_C45	GGAGGAGGUGUUGC <b>U</b> GG	3,451	94% identity



		Location +gRNA	
SARS-CoV-2	GGCUGGCAAUGGCGG-----	28,903	
RaTG13	GG <b>A</b> UGGC--UGGC <b>AAUGG</b>	28,880	66% identity
Bat_C21	GGCUGGCAAUGGCGG-----	28,739	100% identity
Bat_C45	GGCUGGCAAUGGCGG-----	28,808	100% identity
SARS-CoV	GGCU <b>A</b> GC <b>GGAGGUGG</b> -----	28,752	67% identity
MERS-CoV-2	--- <b>GGGUAUUGGCGGAGACAGG</b>	28,791	60% identity

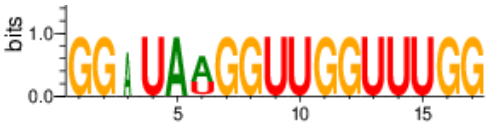


Figure S1

(b)

Selected PQSs in -gRNA

		Location -gRNA	Location +gRNA	
SARS-CoV-2	GGAUUAUGGUUUGGUUUGG	23,877	6,027	
RaTG13	GGAUAAAGGUUUGGUUUGG	23,832	6,024	100% identity
Bat_C21	GG-UAAGGUUUGGUUUGG	23,786	5,947	88% identity
Bat_C45	GG-UAAGGUUUGGUUUGG	23,790	6,013	88% identity



		Location -gRNA	Location +gRNA	
SARS-CoV-2	GGUGAUAGAGGUUUUGUGGUUGGUUGG-----	19,865	10,039	
RaTG13	GGUGAUAGAGGUUUUGUGGUUGG-----	19,820	10,036	84% identity
Bat_C21	-----GGUGGUUGGUAGAGG	19,789	9,944	36% identity
Bat_C45	-----GGUGGUUGGUAGAGG	19,793	10,010	36% identity
MERS-CoV	GGUUAUAGCUACAGUUGGUUGGUUGG-----	20,116	10,004	60% identity

		Location -gRNA	Location +gRNA	
SARS-CoV-2	GGAUCUGGGUAAGGAAGG	13,963	15,941	
RaRG13	GGAUCGGGUAAGGAGG	13,918	15,938	89% identity
Bat_C21	GGAUCUGGAUAAGGCAGG	13,871	15,862	89% identity
Bat_C45	GGAUCUGGAUAAGGCAGG	13,875	15,928	89% identity
SARS-CoV	GGAUCUGGGUAAGGCAGG	13,881	15,871	94% identity
MERS-CoV	GG-UCUGGAUAAGGAAGG	14,209	15,911	89% identity



Figure S1

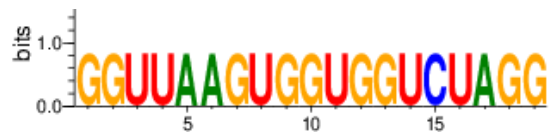
(c)

Not selected PQS

		Location +gRNA	
SARS-CoV-2	GGUAUGUGGAAAGGUUAUGG	13,385	
RaTG13	GGUAUGUGGAAAGGUUAUGG	13,382	100% identity
Bat_C21	GGAAUGUGGAAAGGUUAUGG	13,306	95% identity
Bat_C45	GGAAUGUGGAAAGGUUAUGG	13,372	95% identity
SARS-CoV	GGAAUGUGGAAAGGUUAUGG	13,315	95% identity

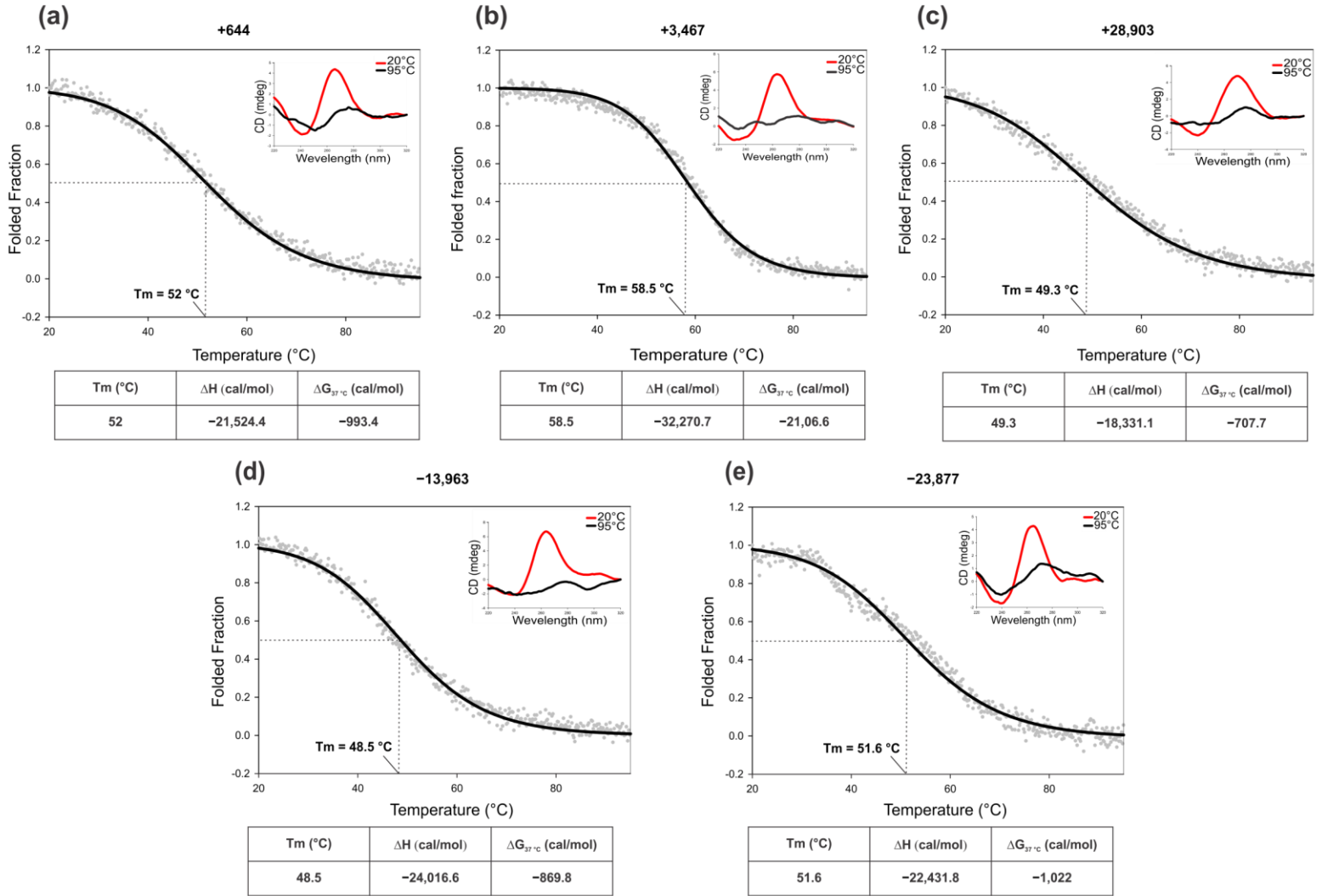


		Location -gRNA	Location +gRNA	
SARS-CoV-2	GGUUAAGUGGUGGUCUAGG	13,134	16,768	
RaRG13	GGUUAAGUGGUGGUCUAGG	13,091	16,765	100% identity



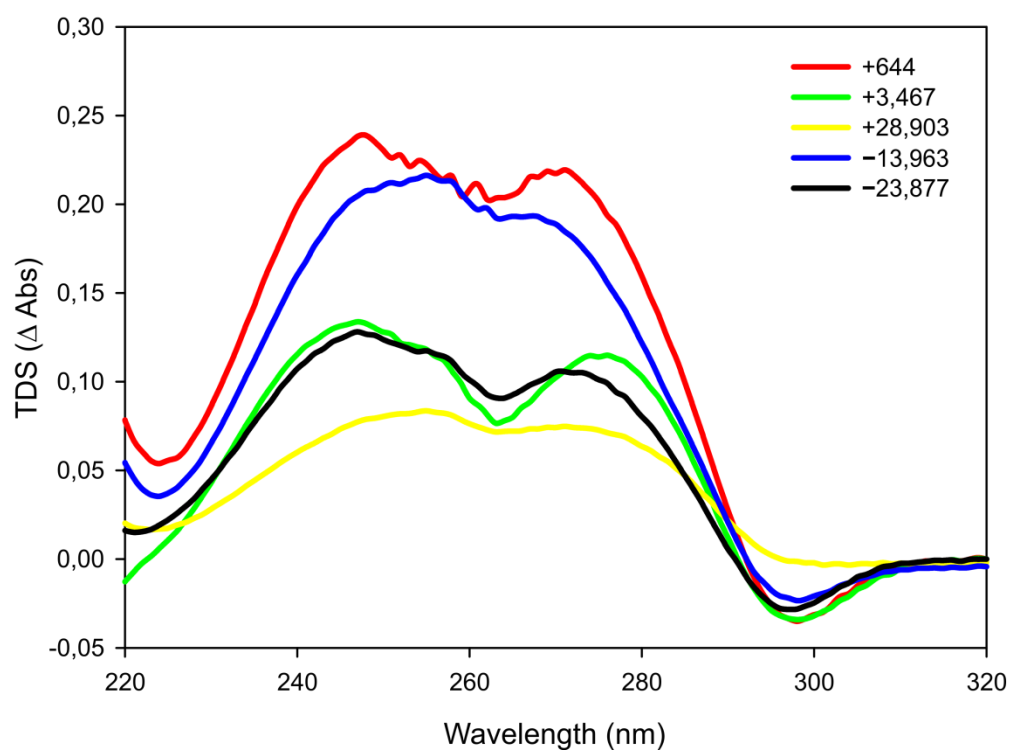
**Figure S1.** Multiple sequence alignments of the position-conserved selected SARS-CoV-2 PQSs identified in this study with their homologous PQSs in other members of the *Coronaviridae* family. (a) Selected PQSs in the +gRNA. (b) Selected PQSs in the -gRNA. (c) Not selected PQSs +13,385 of the +gRNA of SARS-CoV-2 (conserved in position and with significantly high cGcC scores, > 150) and -13,134 of the -gRNA of SARS-CoV-2 (conserved in sequence and position only in two viral genomes).

**Figure S2**



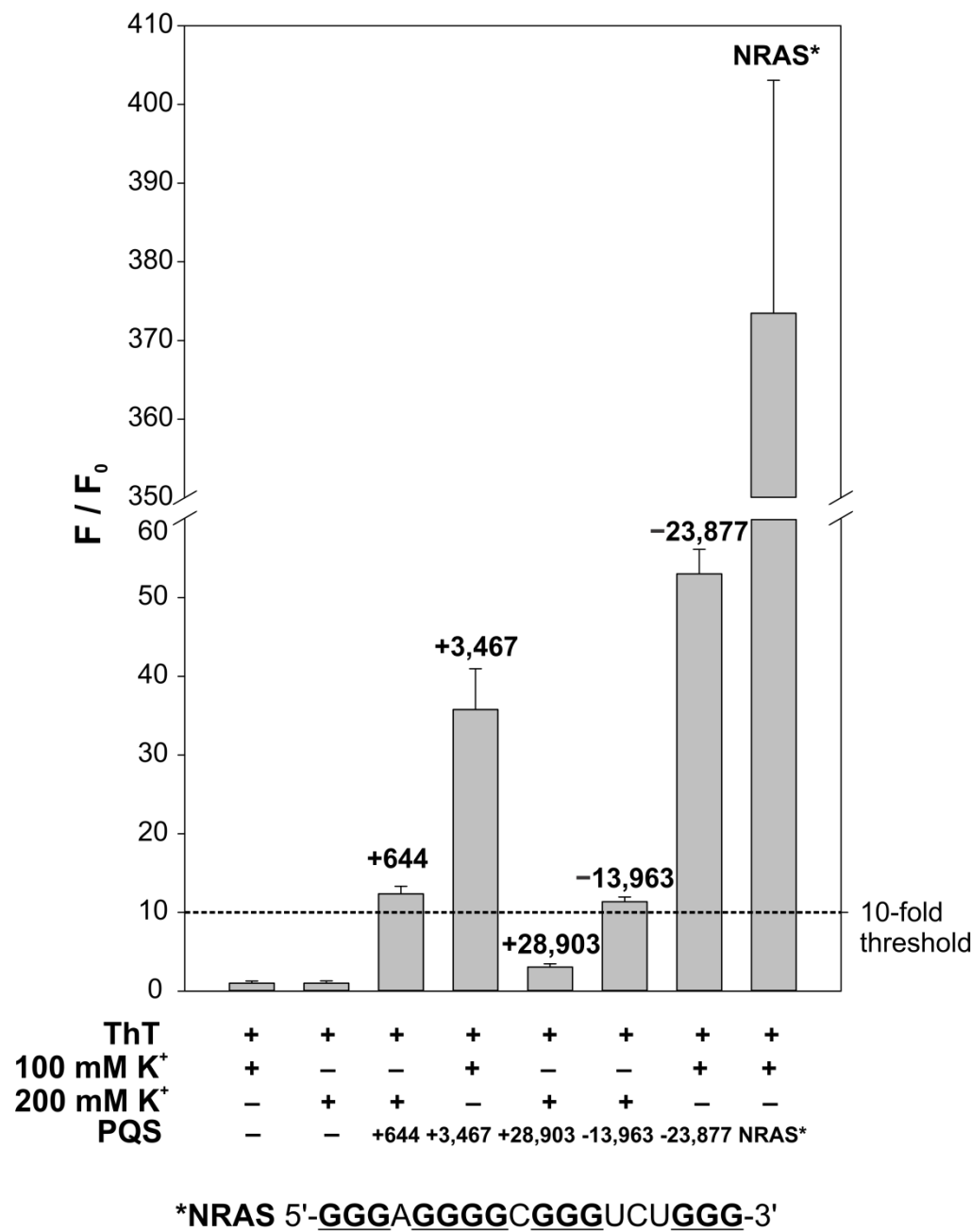
**Figure S2.** CD melting curves obtained for selected PQSs in the positive and negative-sense RNA genomes of SARS-CoV-2. (a), (b) and (c) show the results of selected PQSs in +gRNA of SARS-CoV-2: +644, +3,467 and +28,903, respectively. (d) and (e) show the results of selected PQSs in -gRNA of SARS-CoV-2: -13,963 and -23,877, respectively. Experimental data (dots) and fitted curves (solid lines) are represented.  $\text{K}^+$  concentrations were 100 mM (+3,467 and -23,877) or 200 mM (+644, +28,903 and -13,963). Estimated melting temperatures ( $T_m$ ), changes of enthalpies ( $\Delta H$ ) and change of free Gibbs energy at 37  $^{\circ}\text{C}$  ( $\Delta G_{37^{\circ}\text{C}}$ ) are informed below each graph. Insets in the top-right corners show CD spectra performed at the initial temperature (20  $^{\circ}\text{C}$ , red) and at the final temperature (95  $^{\circ}\text{C}$ , black) of the melting curve for each PQS.

**Figure S3**



**Figure S3.** TDS obtained for selected PQSs in the positive and negative-sense RNA genomes of SARS-CoV-2. TDS resulting from the subtraction of the 20 °C absorbance spectra from the 70 °C absorbance spectra.  $K^+$  concentrations were 100 mM (+3,467 and -23,877) or 200 mM (+644, +28,903 and -13,963).

Figure S4



**Figure S4.** ThT fluorescence assay for selected PQSs in the positive and negative-sense RNA genomes of SARS-CoV-2. Bar graph of fluorescence enhancement ( $F/F_0$ ) of ThT in the presence of the selected PQSs folded in the presence of 100 or 200 mM  $K^+$  as indicated. Each bar represents the mean of three technical repeats and error bars correspond to standard deviation (SD). The marked threshold of 10-fold was used for considering G4 formation. Below the graph the sequence of NRAS RNA oligonucleotide used as positive control for the assay is represented.