## A SARS-CoV-2 nucleocapsid protein TR-FRET assay amenable to high-throughput screening

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Supplementary information

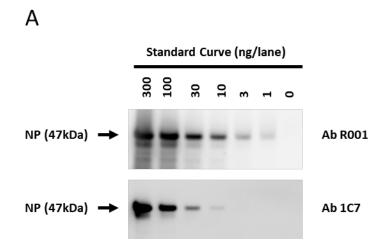
Supplementary Figure 1 - **Determination of NP concentration in viral samples** 

Supplementary Figure 2 - NP HTRF assay is able to detect recombinant SARS-CoV-2 NP

Supplementary Figure 3 - NP HTRF assay is able to detect SARS-CoV-2 NP in TCS and cell lysates

Supplementary Figure 4 - NP HTRF assay is able to detect transiently transfected SARS-CoV-2 NP

Supplementary Figure 5 - Sequence alignment of VoC Beta, Gamma, and Epsilon compared with SARS-CoV-2 USA-WA1/2020



**Supplementary Figure 1. Determination of NP concentration in viral samples.** Western blot of recombinant SARS-CoV-2 NP using donor antibody R001 and acceptor antibody 1C7 at 1:1000 dilution.

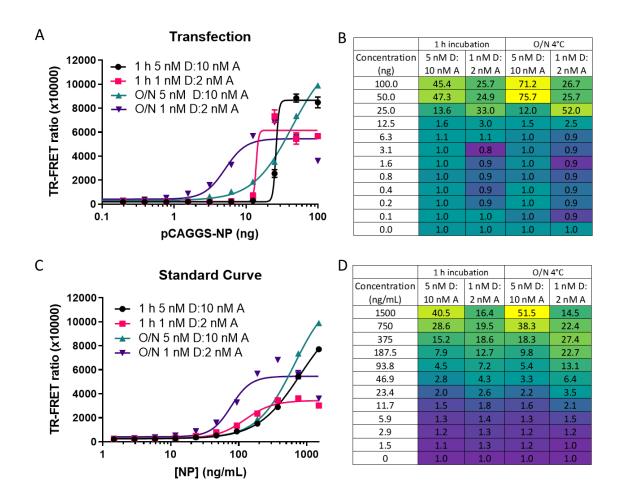
Δ			1h incubation			
^		Concentration	5 nM D:	1 nM D:	10 nM D:	2 nM D:
		(ng/mL)	10 nM A	2 nM A	10 nM A	2 nM A
		1500	12.6	43.5	23.5	55.0
		750	8.7	38.7	15.2	54.8
	Madia anly	375 187.5	5.2 3.2	23.3 12.8	8.7 4.9	37.1 21.8
	Media only	93.8	2.1	7.1	3.1	10.9
	1h	46.9	1.6	4.3	2.0	6.4
		23.4	1.4	2.6	1.6	3.9
		11.7	1.2	1.9	1.3	2.5
		5.9 2.9	1.1	1.5 1.3	1.1	1.7
		1.5	1.0	1.1	1.0	1.2
		0	1.0	1.0	1.0	1.0
В				0/N	14°C	
		Concentration	5 nM D:	1 nM D:	10 nM D:	2 nM D:
		(ng/mL)	10 nM A	2 nM A	10 nM A	2 nM A
		1500	9.2	35.1	17.7	45.0
		750	6.6	37.7 26.0	11.5	51.1 42.2
		375 187.5	4.2 2.7	12.8	6.4 3.8	24.6
	Media only	93.8	1.9	6.5	2.5	11.4
	O/N	46.9	1.5	3.8	1.7	6.1
	0/11	23.4	1.3	2.4	1.4	3.6
		11.7	1.1	1.7	1.2	2.4
		5.9	1.1	1.4	1.0	1.8
		2.9 1.5	1.0	1.2	1.0	1.4
		0		1.1	1.0	
						1.0
		- U	1.0	1.0	1.0	1.0
С			1.0		ubation	1.0
С		Concentration	5 nM D:			2 nM D:
С				1h incu	ubation	
С		Concentration (ng/mL) 1500	5 nM D:	1h incu 1 nM D:	10 nM D: 10 nM A 23.8	2 nM D: 2 nM A 45.2
С		Concentration (ng/mL) 1500 750	5 nM D: 10 nM A 15.0 11.3	1h incu 1 nM D: 2 nM A 39.9 36.5	10 nM D: 10 nM A 23.8 19.0	2 nM D: 2 nM A 45.2 50.8
С		Concentration (ng/mL) 1500 750 375	5 nM D: 10 nM A 15.0 11.3 7.2	1h inco 1 nM D: 2 nM A 39.9 36.5 29.5	10 nM D: 10 nM A 23.8 19.0 12.4	2 nM D: 2 nM A 45.2 50.8 39.6
С	Media + cells	Concentration (ng/mL) 1500 750 375 187.5	5 nM D: 10 nM A 15.0 11.3 7.2 4.6	1h inco 1 nM D: 2 nM A 39.9 36.5 29.5 18.5	10 nM D: 10 nM A 23.8 19.0 12.4 7.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6
С		Concentration (ng/mL) 1500 750 375	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9	1h inco 1 nM D: 2 nM A 39.9 36.5 29.5	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2
С	Media + cells 1h	Concentration (ng/mL) 1500 750 375 187.5 93.8	5 nM D: 10 nM A 15.0 11.3 7.2 4.6	1h inco 1 nM D: 2 nM A 39.9 36.5 29.5 18.5	10 nM D: 10 nM A 23.8 19.0 12.4 7.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6
С		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5	1h inco 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8
С		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2	1h incu 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9	10 nM D: 10 nM D: 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5
С		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1h incu 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7
С		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7
С		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1h incu 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7
C		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7
C		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7
C		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7 1.4
C		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 O/N 1 nM D: 2 nM A 34.7	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
C		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750	5 n M D: 10 n M A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 1.0 n M A 12.0 9.1	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM D:	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
C		Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375	5 n M D: 10 n M A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 n M A 12.0 9.1 5.6	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM A 22.4 16.6 9.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
C	1h	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5	5 n M D: 10 n M A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 9.1 15 n M A 12.0 9.1 5.6 3.7	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 14°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
C	1h  Media + cells	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 10 nM D: 10 nM D: 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
D	1h  Media + cells	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5	5 n M D: 10 n M A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 1.0 9.1 15 n M A 12.0 9.1 5.6 3.7	1h incc 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 14°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0
D	1h	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 14°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4 2.2	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 3.8 2.5 1.7 1.4 1.0
D	1h  Media + cells	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 9.1 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7 3.3 2.3 1.6	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 4°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 2.2 1.6 1.3 1.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7, 9.5 5.3 3.3 2.3
D	1h  Media + cells	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 2.9 2.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0 O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7 3.3 2.3 1.6 1.3	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 14°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 3.4 2.2 1.6 1.3 1.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7, 9.5 5.3 3.3 2.3 1.6
D	1h  Media + cells	Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9 2.9 1.5 0  Concentration (ng/mL) 1500 750 375 187.5 93.8 46.9 23.4 11.7 5.9	5 nM D: 10 nM A 15.0 11.3 7.2 4.6 2.9 2.0 1.5 1.2 1.1 1.0 1.0 1.0 1.0 9.1 5 nM D: 10 nM A 12.0 9.1 5.6 3.7 2.5 1.7	1h ince 1 nM D: 2 nM A 39.9 36.5 29.5 18.5 11.1 6.0 3.8 2.5 1.9 1.4 1.2 1.0  O/N 1 nM D: 2 nM A 34.7 42.8 37.2 20.8 10.6 5.7 3.3 2.3 1.6	10 nM D: 10 nM A 23.8 19.0 12.4 7.1 4.3 2.7 1.9 1.5 1.2 1.1 1.0 1.0 4°C 10 nM D: 10 nM A 22.4 16.6 9.1 5.4 2.2 1.6 1.3 1.1	2 nM D: 2 nM A 45.2 50.8 39.6 27.6 15.2 10.1 5.1 3.8 2.5 1.7 1.4 1.0 2 nM D: 2 nM A 36.8 48.0 49.3 35.1 17.7, 9.5 5.3 3.3 2.3

**Supplementary Figure 2. NP HTRF assay is able to detect recombinant SARS-CoV-2 NP. (A)** S/B for Vero E6 NP in media with 1h incubation. **(B)** S/B for Vero E6 NP in media with O/N incubation. **(C)** S/B for Vero E6 NP in media and cells with O/N incubation.

А	Dilution Factor	Mock TCS	24h TCS	48h TCS
	no dilution	0.8	5.4	9.8
	15	1.1	2.0	8.1
	45	1.0	1.4	3.7
	135	1.1	1.1	2.0
	405	1.0	1.0	1.4
	1215	1.0	1.0	1.1
	3645	1.0	1.0	1.1
	10935	1.0	1.0	1.0
	32805	1.0	1.0	1.0
	98415	1.0	1.0	1.0
	295245	1.0	0.9	1.0
	885735	1.0	1.0	1.0

В				
Ь	Dilution Factor	Mock Lysate	24h Lysate	48h Lysate
	15	0.8	9.7	6.0
	45	1.1	26.8	41.0
	135	1.0	15.8	31.5
	405	1.0	6.0	16.6
	1215	1.0	2.6	6.2
	3645	1.0	1.5	2.5
	10935	1.0	1.2	1.4
	32805	1.0	1.0	1.0
	98415	1.0	1.0	1.0
	295245	1.0	1.0	1.0
	885735	1.0	0.9	0.9
	2657205	1.0	1.0	1.0

**Supplementary Figure 3. NP HTRF assay is able to detect SARS-CoV-2 NP in TCS and cell lysates. (A)** S/B for Vero E6 TCS. **(B)** S/B for Vero E6 cell lysate.



Supplementary Figure 4. NP HTRF assay is able to detect transiently transfected SARS-CoV-2 NP. (A) TR-FRET ratio from Vero E6 cells transfected with a pCAGGS plasmid encoding SARS-CoV-2 USA-WA1/2020 NP for 24h starting at 100 ng serially diluted 1:2. (B) S/B values for A. (C) TR-FRET ratio from Vero E6 cells treated with SARS-CoV-2 NP starting at 1500 ng/mL and serially diluted 1:2. (D) S/B values for C. Cells were incubated with HTRF reagents for 1h at RT or O/N at 4°C. N=3 wells in a half-area 96-well plate. Error bars indicate S.D.

## P80R

1

USA/WA-1	61	KEDLKFPRGQGVPINTNSS <b>P</b> DDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG 120
Beta	61	KEDLKFPRGQGVPINTNSS <b>P</b> DDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG 120
Gamma	61	KEDLKFPRGQGVPINTNSS <u>R</u> DDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG 120
Epsilon	61	KEDLKFPRGQGVPINTNSS <b>P</b> DDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAG 120

## R203K G204R T205I M234I ↓ ↓

USA/WA-1 181 QASSRSSSRSRNSSRNSTPGSS**RGT**SPARMAGNGGDAALALLLLDRLNQLESK**M**SGKGQQ 240

Beta 181 QASSRSSSRSRNSSRNSTPGSS**RGI**SPARMAGNGGDAALALLLLDRLNQLESK**M**SGKGQQ 240

Gamma 181 QASSRSSSRSRNSSRNSTPGSS**KRT**SPARMAGNGGDAALALLLLDRLNQLESK**M**SGKGQQ 240

Epsilon 181 QASSRSSSRSRNSSRNSTPGSS**KGI**SPARMAGNGGDAALALLLLDRLNQLESK I SGKGQQ 240

Supplementary Figure 5. Sequence alignment of VoC Beta, Gamma, and Epsilon compared with SARS-CoV-2 USA-WA1/2020. ClustalO alignment of amino acid sequences for NP regions that were different compared to the reference USA/WA-1 strain. Red, bolded, and underlined letters indicate mutations. Mutation P80R (Gamma), R203K (Gamma, Epsilon), G204R (Gamma), T205I (Beta, Epsilon), and M234I (Epsilon). Sequences were obtained from GISAID.