

SARS-CoV-2 RNA VACCINES AND USES THEREOF**RELATED APPLICATION DATA**

The present application claims priority from United States Patent Application No. 5 63/511,340 filed 30 June 2023 entitled "SARS-CoV-2 RNA vaccines and uses thereof", the entire contents of which is hereby incorporated by reference.

SEQUENCE LISTING

The present application is filed together with a Sequence Listing in electronic 10 form. The entire contents of the Sequence Listing are hereby incorporated by reference.

FIELD

The present disclosure relates to SARS-CoV-2 RNA vaccines and uses thereof. The present disclosure also relates to conventional mRNA vaccines and self-replicating 15 RNA vaccines for the treatment of a SARS-CoV-2 infection or COVID-19.

BACKGROUND

Respiratory viral infections are a significant threat to human health and lives. Infections such as those caused by the influenza virus and severe acute respiratory 20 syndrome coronavirus (SARS-CoV) have been known to cause global pandemics, killing millions of people worldwide. More recently, SARS-CoV-2 has been responsible for causing the worldwide pandemic of the severely infectious coronavirus disease 2019 (COVID-19).

SARS-CoV-2, belonging to the family *Coronaviridae* and the subfamily 25 *orthocoronaviruses*, is an enveloped single-stranded positive-strand RNA virus which encodes non-structural proteins that play roles in viral replication and translation, and structural proteins including the spike protein (S protein), membrane protein (M protein), envelope protein (E protein) and nucleocapsid protein (N protein). The S protein is a transmembrane glycoprotein forming prominent homotrimers on the surface of the virus, 30 consisting of two functional subunits S1 and S2 which have become the main targets for current genetic engineering vaccine development.

SARS-CoV-2 has a high propensity to mutate, with a significant number of mutant strains now identified globally. The predominant mutant strains include: the Alpha (B.1.1.7) mutant, the Beta (B.1.351) mutant, the Gamma (P1) mutant, the Epsilon 35 (B.1.429) mutant, the Delta (B.1.617.2) mutant, the Kappa (B.1.617.1) mutant and the Omicron (B.1.1.529) mutant, which vary in their transmissibility, pathogenicity, and/or

immune escape capabilities. Typically, these mutant strains are identified based on the number and location of mutations in the viral genome, specifically in the genome encoding the S protein of the mutant SARS-CoV-2.

The Omicron strain contains up to 36 amino acid mutation sites and this strain in particular has been shown to have an improved affinity for the ACE2 target receptor, thereby enhancing its toxicity and infectivity, and accelerating virus escape. There are currently few vaccines available that target specific strains of SARS-CoV-2 such as the Omicron strain. Currently available vaccines, such as those developed against the Wuhan (original) strain, which are not specifically targeted to evolving mutant strains, are known to provide for a reduced protective effect and/or immunogenicity.

There is therefore a need for the development of new vaccines that can specifically target SARS-CoV-2 strains, such as the omicron strain of SARS-CoV-2.

SUMMARY

The present disclosure is based on the inventors' identification of a RNA comprising an antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus that is suitable for the treatment of a SARS-Cov-2 infection or coronavirus disease 2019 (COVID-19). In particular, the findings by the inventors provide basis for a RNA comprising an antigen from a SARS-CoV-2, for example a spike (S) protein from the omicron strain (or variants thereof) of a SARS-CoV-2, that is suitable for the treatment of a SARS-CoV-2 infection or COVID-19. Furthermore, the findings by the inventors provide basis for methods of treating or preventing or delaying progression of a disease or disorder such as a SARS-CoV-2 infection or COVID-19, as well as complications thereof including pneumonia, sepsis and acute respiratory distress syndrome (ARDS)) in a subject.

Accordingly, the present disclosure provides a polynucleotide comprising a nucleotide sequence encoding an antigen operably linked to a regulatory element, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

When discussing an "omicron strain" of SARS-CoV-2 herein, the term will be understood to include variants and sub-lineages arising from an omicron strain of SARS-CoV-2. For example, an omicron strain of SARS-CoV-2 includes BA.1 strains, BA.2 strains, XB strains, XBB strains, JN.1 strains, JN.2 strains, JN.3 strains, KP.1 strains, KP.2 strains.

In one example, the polynucleotide comprises a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 17.

In one example, the polynucleotide further comprises a nucleotide sequence
5 encoding a second antigen operably linked to a regulatory element. In one example, the second antigen is a nucleocapsid (N) protein from a SARS-CoV-2.

In one example, the polynucleotide comprises a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 18.

10 In one example, the polynucleotide comprises a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 19.

The present disclosure also provides a RNA comprising a first nucleotide sequence encoding an antigen operably linked to a regulatory element, wherein the
15 antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA further comprises a nucleotide sequence encoding a second antigen operably linked to a regulatory element. In one example, the second antigen is a nucleocapsid (N) protein from a SARS-CoV-2.

20 The present disclosure also provides a self-replicating RNA comprising a first nucleotide sequence encoding an antigen operably linked to a regulatory element, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA is encoded by a nucleotide sequence at
25 least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 17.

In one example, the self-replicating RNA further comprises a nucleotide sequence encoding a second antigen operably linked to a regulatory element. In one example, the second antigen is a nucleocapsid (N) protein from a SARS-CoV-2.

30 In an example, the self-replicating RNA comprises in 5' to 3' order:

a) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2; and

b) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2.

35 In another example, the self-replicating RNA comprises in 5' to 3' order:

a) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2; and

b) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2.

5 In an example, the self-replicating RNA comprises in 5' to 3' order:

a) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a SG promoter; and

b) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES).
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In another example, the self-replicating RNA comprises in 5' to 3' order:

a) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2 operably linked to a SG promoter; and

b) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES).
15

In one example, the self-replicating RNA is encoded a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 18.

20 In one example, the self-replicating RNA is encoded by a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 19.

The present disclosure further provides a polynucleotide comprising a nucleotide sequence encoding an antigen operably linked to a regulatory element, wherein the antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
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In one example, the polynucleotide further comprises a nucleotide sequence encoding a second antigen operably linked to a regulatory element. In one example, the second antigen is a spike (S) protein from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
30

The present disclosure also provides a RNA comprising a first nucleotide sequence encoding an antigen operably linked to a regulatory element, wherein the antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

35 In one example, the RNA further comprises a nucleotide sequence encoding a second antigen operably linked to a regulatory element. In one example, the second

antigen is a spike (S) protein from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure also provides a self-replicating RNA comprising a first nucleotide sequence encoding an antigen operably linked to a regulatory element,
5 wherein the antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA further comprises a nucleotide sequence encoding a second antigen operably linked to a regulatory element. In one example, the second antigen is a spike (S) protein from a severe acute respiratory syndrome
10 coronavirus 2 (SARS-CoV-2).

In an example, the self-replicating RNA comprises in 5' to 3' order:

- a) the nucleotide sequence encoding an N protein antigen from the omicron strain of a SARS-CoV-2; and
- b) the nucleotide sequence encoding an S protein antigen from a SARS-CoV-
15 2.

In another example, the self-replicating RNA comprises in 5' to 3' order:

- a) the nucleotide sequence encoding an S protein antigen from a SARS-CoV-2; and
- b) the nucleotide sequence encoding an N protein antigen from the omicron
20 strain of a SARS-CoV-2.

In an example, the self-replicating RNA comprises in 5' to 3' order:

- a) the nucleotide sequence encoding an N protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a SG promoter; and
- b) the nucleotide sequence encoding an S protein antigen from a SARS-CoV-
25 2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES).

In another example, the self-replicating RNA comprises in 5' to 3' order:

- a) the nucleotide sequence encoding an S protein antigen from a SARS-CoV-2 operably linked to a SG promoter; and
- 30 b) the nucleotide sequence encoding an N protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES).

In one example, the self-replicating RNA is encoded by a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical
35 to the sequence set forth in SEQ ID NO: 18.

In one example, the self-replicating RNA is encoded by a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 19.

In an example, the regulatory element is selected from the group consisting of a
5 subgenomic (SG) promoter and an internal ribosome entry site (IRES) and a Kozac consensus sequence or a combination thereof. In an example, the regulatory element is a SG promoter.

In an example, the nucleotide sequence encoding the second antigen is operably
10 linked to the same regulatory element as the nucleotide sequence encoding the first antigen. In an example, the nucleotide sequences encoding the second antigen is operably linked to the same regulatory element as the first antigen.

The present disclosure also provides a polynucleotide comprising: a) a first
nucleotide sequence encoding a first polypeptide of interest; and b) a second nucleotide
15 sequence encoding a second polypeptide of interest operably linked to a regulatory element selected from the group consisting of a subgenomic (SG) promoter and an internal ribosome entry site (IRES), wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first
20 nucleotide sequence encoding a first polypeptide of interest; and b) a second nucleotide sequence encoding a second polypeptide of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

25 In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first polypeptide of interest; and b) a second nucleotide sequence encoding a second polypeptide of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a nucleocapsid (N) protein from the omicron strain of a severe acute
30 respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure also provides a polynucleotide comprising: a) a first
nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide
sequence encoding a second antigen of interest operably linked to a regulatory element
35 selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory

syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide
5 sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first
10 nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

15 In an example, the polynucleotide is RNA or DNA. In one example, the RNA is messenger RNA (mRNA). In one example, the mRNA is conventional mRNA (cRNA) or self-replicating RNA.

Accordingly, the present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide
20 sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

25 In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome
30 coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a
35 nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure also provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide
5 is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide sequence
10 encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide
15 sequence encoding a first antigen of interest; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure further provides a self-replicating RNA comprising: a) a
20 first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory
25 syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a
first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide
30 sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a
first nucleotide sequence encoding a first antigen of interest; and b) a second nucleotide
35 sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first

antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the first nucleotide sequence encoding the first antigen of interest is operably linked to a regulatory element. In another example, the regulatory element is operably linked to the 5' end of the first nucleotide sequence. In one example, the regulatory element is selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof. For example, the regulatory element is a Kozak consensus sequence. For example, the regulatory element is an IRES. In another example, the regulatory element is a SG promoter.

10 In one example, the Kozak consensus sequence comprises or consists of a sequence set forth in SEQ ID NO: 6 (GCCACC). In one example, the Kozak consensus sequence consists of a sequence set forth in NO: 7 (ACCATGG).

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

25 In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

30 In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N)

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protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence,

an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the first nucleotide sequence encoding the first antigen of interest is operably linked to a Kozak consensus sequence.

In one example, the first nucleotide sequence encoding the first antigen of interest is operably linked to a Kozak consensus sequence and a SG promoter. For example, the Kozak consensus sequence is operably linked to the 5' end of the SG promoter which is operably linked to the 5' end of the first nucleotide sequence encoding the first antigen of interest.

In one example, the first nucleotide sequence encoding the first antigen of interest is operably linked to a Kozak consensus sequence and an IRES. For example, the Kozak consensus sequence is operably linked to the 5' end of the IRES which is operably linked to the 5' end of the first nucleotide sequence encoding the first antigen of interest.

In one example, the first nucleotide sequence encoding the first antigen of interest is operably linked to a SG promoter.

In one example, the first nucleotide sequence encoding the first antigen of interest is operably linked to an IRES.

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of

interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a polynucleotide comprising: a) a first nucleotide
5 sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2
10 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding
15 a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first
20 nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome
25 coronavirus 2 (SARS-CoV-2).

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of
30 a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak
35 consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group

consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first
5 nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2
10 (SARS-CoV-2).

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES,
15 wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter;
20 and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first
25 nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

30 The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute
35 respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the

omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the
5 first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a
10 second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide
15 sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.
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In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory
30 element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second
35 nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the

first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

10 In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

15 In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

20 The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

25 In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

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In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a

regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding a first antigen operably linked to a Kozac consensus sequence; and b) a second nucleotide sequence encoding a second antigen operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES), wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3', a) a first nucleotide sequence encoding a first antigen operably linked to a Kozac consensus sequence; and b) a second nucleotide sequence encoding a second antigen operably linked to an IRES or a SG promoter, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3', a) a first nucleotide sequence encoding a first antigen operably linked to a Kozac consensus sequence; and b) a second nucleotide sequence encoding a second antigen operably linked to an IRES or a SG promoter, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozac consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozac consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozac consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozac

consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a first antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding a second antigen of interest

operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The present disclosure provides a self-replicating RNA comprising: a) a first
5 nucleotide sequence encoding a first antigen of interest operably linked to an IRES; and
b) a second nucleotide sequence encoding a second antigen of interest operably linked to
a regulatory element selected from the group consisting of a SG promoter and an IRES,
wherein the first polypeptide is a spike (S) protein from the omicron strain of a severe
acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein
10 from the omicron strain of a SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a
first nucleotide sequence encoding a first antigen of interest operably linked to an IRES;
and b) a second nucleotide sequence encoding a second antigen of interest operably
linked to a regulatory element selected from the group consisting of a SG promoter and
15 an IRES, wherein the first antigen is a spike (S) protein from the omicron strain of a
severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a
first nucleotide sequence encoding a first antigen of interest operably linked to an IRES;
and b) a second nucleotide sequence encoding a second antigen of interest operably
20 linked to a regulatory element selected from the group consisting of a SG promoter and
an IRES, wherein the first antigen is a nucleocapsid (N) protein from the omicron strain
of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In one example, the polynucleotide is a bicistronic RNA. For example, the
polynucleotide is a bicistronic cRNA. In one example, the cRNA is a bicistronic cRNA.
25 In another example, the polynucleotide is a bicistronic self-replicating mRNA. For
example, the self-replicating RNA is a bicistronic self-replicating RNA.

In one example, the second nucleotide sequence encoding a second antigen is
operably linked to an IRES.

In one example, the second nucleotide sequence encoding a second antigen is
30 operably linked to a SG promoter.

In one example, the polynucleotide is a multicistronic RNA. For example, the
polynucleotide is a multicistronic cRNA. For example, the cRNA is a multicistronic
cRNA. In another example, the polynucleotide is a multicistronic self-replicating mRNA.
For example, the self-replicating RNA is a multicistronic self-replicating mRNA.

35 The present disclosure also provides a polynucleotide comprising: a) a first
nucleotide sequence encoding an antigen of interest; and b) a second nucleotide sequence

encoding an antigen of interest, operably linked to a regulatory element selected from the group consisting of a subgenomic (SG) promoter and an internal ribosome entry site (IRES), wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain
5 of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

10 In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2, operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.
15 In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2; and b) a
20 second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-
25 WA1/2020 strain of SARS-CoV-2.

In an example, the polynucleotide is RNA or DNA. In one example, the RNA is messenger RNA (mRNA). In one example, the mRNA is conventional mRNA (cRNA) or self-replicating RNA.

Accordingly, the present disclosure provides a RNA comprising: a) a first
30 nucleotide sequence encoding an antigen of interest; and b) a second nucleotide sequence encoding an antigen of interest, operably linked to a regulatory element selected from the group consisting of a subgenomic (SG) promoter and an internal ribosome entry site (IRES), wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain
35 of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the

N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure also provides a cRNA comprising: a) a first nucleotide sequence encoding an antigen of interest; and b) a second nucleotide sequence encoding an antigen of interest, operably linked to a regulatory element selected from the group consisting of a subgenomic (SG) promoter and an internal ribosome entry site (IRES), wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N

protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2; and b) a second
5 nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of
10 SARS-CoV-2.

The present disclosure further provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding an antigen of interest; and b) a second nucleotide sequence encoding an antigen of interest, operably linked to a regulatory element selected from the group consisting of a subgenomic (SG) promoter and an internal
15 ribosome entry site (IRES), wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); and b) a second nucleotide
25 sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In
35 an example, the N protein is from the omicron strain of a SARS-CoV-2. In another

example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the first nucleotide sequence is operably linked to a regulatory element. For example, the regulatory element is operably linked to the 5' end of the first
5 nucleotide sequence. In one example, the regulatory element is selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof. For example, the regulatory element is a Kozak consensus sequence. For example, the regulatory element is an IRES. For example, the regulatory element is a SG promoter.

10 In one example, the Kozak consensus sequence comprises or consists of a sequence set forth in SEQ ID NO: 6 (GCCACC). In one example, the Kozak consensus sequence consists of a sequence set forth in SEQ ID NO: NO: 7 (ACCATGG).

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected
15 from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the
20 omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

25 In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a
30 nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a Kozak consensus
35 sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and

combinations thereof; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

10 In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a
15 nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence
20 encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from
25 the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding
30 an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N)
35 protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of

a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the self-replicating RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the first nucleotide sequence is operably linked to a Kozak consensus sequence.

In one example, the first nucleotide sequence is operably linked to a Kozak consensus sequence and a SG promoter. For example, the Kozak consensus sequence is operably linked to the 5' end of the SG promoter which is operably linked to the 5' end of the first nucleotide sequence encoding the S protein.

In one example, the first nucleotide sequence is operably linked to a Kozak consensus sequence and an IRES. For example, the Kozak consensus sequence is operably linked to the 5' end of the IRES which is operably linked to the 5' end of the first nucleotide sequence encoding the S protein.

In one example, the first nucleotide sequence is operably linked to a SG promoter.

In one example, the first nucleotide sequence is operably linked to an IRES.

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting

of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

10 The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected
15 from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain
20 of SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a
25 nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence
30 encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

35 The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a SG promoter; and b) a

second nucleotide sequence encoding antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe
5 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first
10 nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a SG promoter; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

15 In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group
20 consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a polynucleotide comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to an IRES; and b) a second
25 nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence
30 encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe
35 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an IRES; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-

CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the polynucleotide comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably
5 linked to an IRES; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma
10 or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES,
15 wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from
20 the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a nucleocapsid (N)
25 protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a spike (S)
30 protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

35 The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence and a SG

promoter; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

10 In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element
15 selected from the group consisting of a SG promoter and an IRES.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

35 In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

5 In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the
10 group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a SG promoter; and b) a second
15 nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence
20 encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute
25 respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a SG promoter; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide
30 sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a
35 SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second
5 nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2.

10 In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an IRES; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG
15 promoter and an IRES.

In one example, the RNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to an IRES; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
20 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a cRNA comprising: a) a first nucleotide
25 sequence encoding an antigen of interest operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding an antigen of interest from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the
30 omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

35 In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence and a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected

from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a cRNA comprising: a) a first nucleotide
5 sequence encoding an antigen of interest operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the
10 omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

15 In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element
20 selected from the group consisting of a SG promoter and an IRES.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a Kozak consensus sequence and an IRES; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome
25 coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a cRNA comprising: a) a first nucleotide
30 sequence encoding an antigen of interest operably linked to a SG promoter; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe
35 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein

is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute
5 respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a SG promoter; and b) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide
10 sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a
15 SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

The present disclosure provides a cRNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to an IRES; and b) a second nucleotide sequence encoding an antigen of interest operably linked to a regulatory
20 element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein
25 is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an IRES; and b)
30 a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES.

In one example, the cRNA comprises, in order from 5' to 3': a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to
35 an IRES; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

5 The present disclosure provides a self-replicating RNA comprising: a) a first nucleotide sequence encoding an antigen of interest operably linked to a subgenomic (SG) promoter; and b) a second nucleotide sequences encoding an antigen of interest operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES, wherein the first and second nucleotide sequences are selected
10 from the group consisting of a nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain
15 of SARS-CoV-2.

 In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3', a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a SG promoter; and b) a second nucleotide sequence encoding a
20 nucleocapsid (N) protein from a SARS-CoV-2 operably linked to an IRES or a SG promoter. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

 In one example, the self-replicating RNA of the present disclosure comprises, in
25 order from 5' to 3', a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a SG promoter; and b) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an IRES or a SG promoter. In an example, the N protein is from the omicron strain of a SARS-CoV-2. In
30 another example, the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.

 In one example, the polynucleotide is a bicistronic RNA. For example, the polynucleotide is a bicistronic cRNA. In one example, the cRNA is a bicistronic cRNA. In another example, the polynucleotide is a bicistronic self-replicating mRNA. For
35 example, the self-replicating RNA is a bicistronic self-replicating RNA.

In one example, the second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 is operably linked to an IRES.

In one example, the second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 is operably linked to a SG promoter.

5 In one example, the polynucleotide is a multicistronic RNA. For example, the polynucleotide is a multicistronic cRNA. For example, the cRNA is a multicistronic cRNA. In another example, the polynucleotide is a multicistronic self-replicating mRNA. In another example, the self-replicating RNA is a monocistronic self-replicating mRNA. In another example, the polynucleotide is a multicistronic self-replicating RNA. In
10 another example, the self-replicating RNA is a monocistronic self-replicating RNA.

In one example, the SG promoter is a native SG promoter. For example, a native SG promoter is a promoter that is native to the RNA virus from which it is derived and/or based on (e.g., an alphavirus). In one example, the native SG promoter is a native alphavirus SG promoter.

15 In one example, the SG promoter is a minimal SG promoter or an extended SG promoter.

In one example, the SG promoter is a minimal SG promoter. In one example, the native SG promoter is a minimal SG promoter. For example, the minimal SG promoter is the minimal sequence required for initiation of transcription. In one example, the
20 minimal native SG promoter is 49 nucleotides in length. In one example, the minimal SG promoter is 49 nucleotides in length. In one example, the minimal native SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 1. In one example, the minimal SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 1.

25 In one example, the SG promoter is an extended SG promoter. In one example, the native SG promoter is an extended SG promoter. For example, the extended SG promoter is extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein (e.g., NSP4) of the RNA virus (e.g., an alphavirus). In one example, the extended SG promoter is extended at the 5' end with nucleotides occurring
30 in a sequence encoding an alphavirus NSP4. The addition of nucleotides to the 5' end of the SG promoter sequence did not interfere with expression of the non-structural protein and viral replicase, e.g., alphavirus NSP4.

In one example, the SG promoter is extended at the 5' end by 51 or fewer nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus
35 NSP4). In one example, the extended SG promoter is a minimal SG promoter extended at the 5' end by no more than 51 nucleotides occurring in a sequence encoding a non-

structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 1 extended at the 5' end by no more than 51 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). For example, the extended
5 SG promoter is no more than 100 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 2 to 101 of SEQ ID NO: 5.

In one example, the SG promoter is extended at the 5' end by about 5 nucleotides to about 20 nucleotides, for example by about 5 nucleotides, or about 10 nucleotides, or
10 about 12, or about 15 nucleotides, or about 20 nucleotides, occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In another example, the SG promoter is extended at the 5' end by about 20 to about 35 nucleotides, for example, by about 25 nucleotides or about 27 nucleotides, or about 30 nucleotides, or about 35 nucleotides, occurring in a sequence encoding a non-structural protein (e.g., an
15 alphavirus NSP4).

In one example, the SG promoter is extended at the 5' end by about 12 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 1 extended at the 5' end by 12 nucleotides occurring in a sequence encoding a non-
20 structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 61 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 41 to 101 of SEQ ID NO: 5. In another example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 2.

In one example, the SG promoter is extended at the 5' end by about 31 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 1 extended at the 5' end by 31 nucleotides occurring in a sequence encoding a non-
30 structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 80 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 22 to 101 of SEQ ID NO: 5. In another example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 3.

In one example, the extended SG promoter comprises a repeat sequence
35 corresponding to nucleotides 66 to 75 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence comprising nucleotides 50 to 75 of SEQ ID NO: 5

and nucleotides 66 to 101 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 15.

In one example, the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF- κ B-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a (FGF1A), GTX, or a combination thereof.

In one example, the IRES is a wild-type IRES derived from encephalomyocarditis virus (EMCV). For example, the wild-type EMCV IRES comprises a sequence set forth in SEQ ID NO: 4.

In one example, the first and/or second nucleotide sequence and/or the one or more additional nucleotide sequences are codon optimized.

In one example, the G/C content of the first and/or second nucleotide sequence and/or the one or more additional nucleotide sequences are modified.

In one example, the G/C content of the first and/or second nucleotide sequence and/or the one or more additional nucleotide sequences are increased by at least 5% compared to the G/C content of the unmodified sequence. For example, the G/C content of the first and/or second nucleotide sequence and/or the one or more additional nucleotide sequences are increased by at least 10%, or 15%, or 20%, or 25%, or 30%, or 35%, or 40% compared to the G/C content of the unmodified sequence.

In one example, the polynucleotide comprises at least one chemically modified nucleotide.

In one example, the chemically modified nucleotide is selected from the group consisting of N⁶,2'-O-dimethyl-adenosine (m⁶Am), 5-methyluridine (m⁵U), N⁴-acetylcytidine (ac⁴C), 2-thiocytidine (s²C), 2-thiouridine (s²U), 5-methylcytidine (m⁵C), N⁶-methyladenosine (m⁶a), pseudouridine (ψ), 1-methylpseudouridine (m¹ ψ), and combinations thereof. For example, the chemically modified nucleotide is N⁶,2'-O-dimethyl-adenosine (m⁶Am). For example, the chemically modified nucleotide is 5-methyluridine (m⁵U). For example, the chemically modified nucleotide is N⁴-acetylcytidine (ac⁴C). For example, the chemically modified nucleotide is 2-thiocytidine (s²C). For example, the chemically modified nucleotide is 2-thiouridine (s²U). For example, the chemically modified nucleotide is 5-methylcytidine (m⁵C). For example,

the chemically modified nucleotide is N6-methyladenosine (m6a). For example, the chemically modified nucleotide is pseudouridine (ψ). For example, the chemically modified nucleotide is 1-methylpseudouridine (m1 ψ).

In one example, the first nucleotide sequence comprises the 5'-UTR of
5 haptoglobin (HP), fibrinogen beta chain (FGB), haptoglobin-related protein (HPR), albumin (ALB), complement component 3 (C3), fibrinogen alpha chain (FGA), alpha 6 collagen (Col6A), alpha-1-antitrypsin (SERPINA1), alpha-1-antichymotrypsin (SERPINA3) a fragment and/or a variant thereof.

In one example, the 5'UTR is a 5'UTR of a Venezuelan equine encephalitis virus
10 (VEEV) or modified forms thereof. For example, the 5'UTR comprises a sequence set forth in SEQ ID NO: 13.

In one example, the 5'-UTR, the fragment and/or the variant thereof is between 40 and 2000 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 40 and 100 nucleotides in length. For example, the 5'-UTR,
15 the fragment and/or the variant thereof is between 100 and 250 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 250 and 500 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 500 and 750 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 750 and 1000 nucleotides in length. For example,
20 the 5'-UTR, the fragment and/or the variant thereof is between 1000 and 1250 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1250 and 1500 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1500 and 1750 nucleotides in length. For example, the 5'-UTR, the fragment and/or the variant thereof is between 1750 and 2000
25 nucleotides in length.

In one example, the 5'-UTR, the fragment and/or the variant thereof comprises a nucleotide sequence at least 90% identical to a nucleotide sequence set forth in any one of SEQ ID NO: 9 to 12. For example, the 5'-UTR, the fragment and/or the variant thereof comprises a nucleotide sequence 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%,
30 or 97%, or 98%, or 99% identical to a nucleotide sequence set forth in any one of SEQ ID NO: 9 to 12.

In one example, the polynucleotide comprises a combination of two or more 5'-UTRs, fragments and/or variants thereof. In one example, the two or more 5'-UTRs are the same. In one example, the two or more 5'-UTRs are different.

35 In one example, the nucleotide sequence comprising the 5'UTR comprises at least one microRNA binding site, an AU rich element (ARE), a GC-rich element, a stem loop,

and combinations thereof. In one example, the nucleotide sequence comprises a microRNA binding site. In one example, the nucleotide sequence comprises an AU rich element (ARE). In one example, the nucleotide comprises a GC-rich element. In one example, the nucleotide sequence comprises a stem loop. For example, the stem loop is
5 a histone stem loop.

In one example, the polynucleotide further comprises a nucleotide sequence comprising a 3'UTR. In one example, the nucleotide sequence comprising the 3'UTR is located 3' of the second or the one or more additional nucleotide sequences. For example, the nucleotide sequence comprising the 3'UTR is located 3' of the second nucleotide
10 sequence. In one example, the 3'UTR comprises a 3'-UTR of arachidonate 5-lipoxygenase (ALOX5), alpha I collagen (COL1A1), tyrosine hydroxylase (TH) gene, amino-terminal enhancer of split (AES), human mitochondrial 12S rRNA (mtRNR1), a fragment and/or a variant thereof.

In one example, the 3'UTR is a 3'UTR of a Sindbis virus (SINV) or modified
15 forms thereof. For example, the 3'UTR comprises a sequence set forth in SEQ ID NO: 14 or 22.

In one example, the 3'UTR, the fragment and/or the variant thereof is between 40 and 400 nucleotides in length. For example, the 3'-UTR is between 40 and 50, or 50 and 60, or 60 and 70, or 70 and 80, or 80 and 90, or 90 and 100, or 100 and 125, or 125 and
20 150, or 150 and 175, or 175 and 200, or 200 and 225, or 225 and 250, or 250 and 275, or 275 and 300, or 300 and 325, or 325 and 350, or 350 and 375, or 375 and 400 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 40 and 50 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 50 and 60 nucleotides in length. For example, the 3'-UTR,
25 the fragment and/or the variant thereof is between 60 and 70 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 70 and 80 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 80 and 90 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 90 and 100 nucleotides in length. For example, the
30 3'-UTR, the fragment and/or the variant thereof is between 100 and 125 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 125 and 150 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 150 and 175 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 175 and 200 nucleotides in length. For
35 example, the 3'-UTR, the fragment and/or the variant thereof is between 200 and 225 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof

is between 225 and 250 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 250 and 275 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 275 and 300 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 300 and 325 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 325 and 350 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 350 and 375 nucleotides in length. For example, the 3'-UTR, the fragment and/or the variant thereof is between 375 and 400 nucleotides in length.

10 In one example, the polynucleotide comprises a combination of two or more 3'-UTRs, fragments and/or variants thereof. In one example, the two or more 3'-UTRs are the same. In one example, the two or more 3'-UTRs are different.

 In one example, the nucleotide sequence comprising the 3'UTR, the fragment and/or variant thereof comprises at least one microRNA binding site, an AU rich element (ARE), a GC-rich element, a triple helix, a stem loop, one or more stop codons and combinations thereof. In one example, the nucleotide sequence comprises a microRNA binding site. In one example, the nucleotide sequence comprises an AU rich element (ARE). In one example, the nucleotide sequence comprises a GC-rich element. In one example, the nucleotide sequence comprises a triple helix. In one example, the nucleotide sequence comprises a stem loop. For example, the stem loop is a histone stem loop. In one example, the nucleotide sequence comprises one or more stop codons. For example, the one or more stop codons are located at the 5' end of the 3'-UTR.

 In one example, the polynucleotide comprises a nucleotide sequence comprising one or more 3' tailing sequences located at the 3' end of the nucleotide sequence comprising the 3'UTR. In one example, the one or more 3' tailing sequences are selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof. For example, the 3' tailing sequence comprises a poly-A sequence. In one example, the 3' tailing sequence comprises a polyadenylation signal. In one example, the 3' tailing sequence comprises a G-quadruplex. In one example, the 3' tailing sequence comprises a poly-C sequence. In one example, the 3' tailing sequence comprises a stem loop. For example, the stem loop is a histone stem loop. In one example, the 3' tailing sequence comprises a poly-A sequence and a G-quadruplex. In one example, the 3' tailing sequence comprises a stem loop (e.g., a histone stem loop) and a poly-A sequence.

35 In one example, the one or more 3' tailing sequences comprises one or more poly-A sequences each comprising between 10 and 300 consecutive adenosine nucleotides.

For example, the poly-A sequences each comprises between 10 and 20, or 20 and 30, or 30 and 40, or 40 and 50, or 50 and 60, or 60 and 70, or 70 and 80, or 80 and 90, or 90 and 100, or 100 and 125, or 125 and 150, or 150 and 175, or 175 and 200, or 200 and 225, or 225 and 250, or 250 and 275, or 275 and 300 consecutive adenosine nucleotides.

5 For example, the one or more poly-A sequences each comprises between 10 and 20 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 20 and 30 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 30 and 40 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprise 36

10 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 40 and 50 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 50 and 60 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 60 and 70 consecutive adenosine nucleotides. For example, the one or more poly-A

15 sequences each comprises between 70 and 80 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 80 and 90 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 90 and 100 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 100 and 125 consecutive adenosine

20 nucleotides. For example, the one or more poly-A sequences each comprises between 125 and 150 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 150 and 175 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 175 and 200 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each

25 comprises between 200 and 225 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 225 and 250 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 250 and 275 consecutive adenosine nucleotides. For example, the one or more poly-A sequences each comprises between 275 and 300 consecutive adenosine

30 nucleotides.

In one example, the one or more poly-A sequence each comprises 10, or 20, or 30, or 40, or 50, or 60, or 70, or 80, or 90, or 100, or 125, or 150, or 175, or 200, or 225, or 250, or 275, or 300 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 10 consecutive adenosine nucleotides. For example,

35 the one or more poly-A sequence each comprises 20 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 30 consecutive adenosine

nucleotides. For example, the one or more poly-A sequence each comprises 40 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 50 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 60 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 70 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 80 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 90 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 100 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 125 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 150 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 175 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 200 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 225 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 250 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 275 consecutive adenosine nucleotides. For example, the one or more poly-A sequence each comprises 300 consecutive adenosine nucleotides.

In one example, the poly-A sequence comprises 36 consecutive adenosine nucleotides. For example, the poly-A sequence comprises a sequence set forth in SEQ ID NO: 16.

In one example, the one or more poly-A sequences is separated by an interrupting linker. For example, the 3'tailing sequence comprises, in order of 5' to 3': a poly-A sequence comprising consecutive adenosine nucleotides, an interrupting linker, and a further poly-A sequence comprising consecutive adenosine nucleotides.

In one example, the interrupting linker is from 10 to 50, or 50 to 100, or 100 to 150 nucleotides in length. For example, the interrupting linker is from 10 to 50 nucleotides in length. For example, the interrupting linker is from 50 to 100 nucleotides in length. For example, the interrupting linker is from 100 to 150 nucleotides in length.

In one example, the interrupting linker is 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 25, or 30, or 35, or 40, or 45, or 50, or 55, or 60, or 65, or 70, or 75, or 80, or 85, or 90, or 95, or 100, or 110, or 120, or 130, or 140, or 150 nucleotides in length. For example, the interrupting linker is 1 nucleotide in length. For example, the interrupting linker is 2 nucleotides in length. For example, the interrupting linker is 3 nucleotides in length. For

example, the interrupting linker is 4 nucleotides in length. For example, the interrupting linker is 5 nucleotides in length. For example, the interrupting linker is 6 nucleotides in length. For example, the interrupting linker is 7 nucleotides in length. For example, the interrupting linker is 8 nucleotides in length. For example, the interrupting linker is 9 nucleotides in length. For example, the interrupting linker is 10 nucleotides in length. For example, the interrupting linker is 11 nucleotides in length. For example, the interrupting linker is 12 nucleotides in length. For example, the interrupting linker is 13 nucleotides in length. For example, the interrupting linker is 14 nucleotides in length. For example, the interrupting linker is 15 nucleotides in length. For example, the interrupting linker is 16 nucleotides in length. For example, the interrupting linker is 17 nucleotides in length. For example, the interrupting linker is 18 nucleotides in length. For example, the interrupting linker is 19 nucleotides in length. For example, the interrupting linker is 20 nucleotides in length. For example, the interrupting linker is 25 nucleotides in length. For example, the interrupting linker is 30 nucleotides in length. For example, the interrupting linker is 35 nucleotides in length. For example, the interrupting linker is 40 nucleotides in length. For example, the interrupting linker is 45 nucleotides in length. For example, the interrupting linker is 50 nucleotides in length. For example, the interrupting linker is 55 nucleotides in length. For example, the interrupting linker is 60 nucleotides in length. For example, the interrupting linker is 65 nucleotides in length. For example, the interrupting linker is 70 nucleotides in length. For example, the interrupting linker is 75 nucleotides in length. For example, the interrupting linker is 80 nucleotides in length. For example, the interrupting linker is 85 nucleotides in length. For example, the interrupting linker is 90 nucleotides in length. For example, the interrupting linker is 95 nucleotides in length. For example, the interrupting linker is 100 nucleotides in length. For example, the interrupting linker is 110 nucleotides in length. For example, the interrupting linker is 120 nucleotides in length. For example, the interrupting linker is 130 nucleotides in length. For example, the interrupting linker is 140 nucleotides in length. For example, the interrupting linker is 150 nucleotides in length.

In one example, the interrupting linker is 10 nucleotides in length. In one example, the interrupting linker comprises or consists of the nucleotide sequence set forth in SEQ ID NO: 8. For example, the interrupting linker comprises or consists of a nucleotide sequence GCAUAUGACU.

In one example, the 3' tailing sequence comprises, in order of 5' to 3': a poly-A sequence comprising 30 consecutive adenosine nucleotides, an interrupting linker of 10

nucleotides, and a further poly-A sequence comprising 70 consecutive adenosine nucleotides.

In one example, the 3' tailing sequence comprises, in order of 5' to 3': a poly-A sequence comprising 30 consecutive adenosine nucleotides, an interrupting linker
5 comprising or consisting of the nucleotide sequence set forth in SEQ ID NO: 8, and a further poly-A sequence comprising 70 consecutive adenosine nucleotides.

In one example, the polynucleotide comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus
10 sequence, an IRES, a SG promoter and combinations thereof;
- c) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- d) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting
15 of a SG promoter and an IRES;
- e) a 3'-UTR, fragment and/or variant thereof; and
- f) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

20 In one example, the polynucleotide comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2;
25
- d) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- e) a 3'-UTR, fragment and/or variant thereof; and
30
- f) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the RNA comprises, in order from 5' to 3':

- 35 a) a 5'-UTR, fragment and/or variant thereof;

- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) a first nucleotide sequence encoding the a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- 5 d) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- e) a 3'-UTR, fragment and/or variant thereof; and
- f) one or more 3' tailing sequences selected from the group consisting of a poly-A
- 10 sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the RNA comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus
- 15 sequence, an IRES, a SG promoter and combinations thereof;
- c) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2;
- d) a second nucleotide sequence encoding the a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
- 20 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- e) a 3'-UTR, fragment and/or variant thereof; and
- f) one or more 3' tailing sequences selected from the group consisting of a poly-A
- 25 sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the cRNA comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus
- 30 sequence, an IRES, a SG promoter and combinations thereof;
- c) a first nucleotide sequence encoding the a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- d) a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- 35 e) a 3'-UTR, fragment and/or variant thereof; and

- f) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the cRNA comprises, in order from 5' to 3':

- 5 a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2;
- 10 d) a second nucleotide sequence encoding the a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- e) a 3'-UTR, fragment and/or variant thereof; and
- 15 f) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the self-replicating RNA comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- 20 b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- c) the first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);
- d) the second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- 25 e) a 3'-UTR, fragment and/or variant thereof; and
- f) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.
- 30

In one example, the self-replicating RNA comprises, in order from 5' to 3':

- a) a 5'-UTR, fragment and/or variant thereof;
- b) a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof;
- 35 c) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2;

- d) a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a regulatory element selected from the group consisting of a SG promoter and an IRES;
- 5 e) a 3'-UTR, fragment and/or variant thereof; and
- f) one or more 3' tailing sequences selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

In one example, the self-replicating RNA of the present disclosure comprises, in
10 order from 5' to 3':

- a) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG
15 promoter; or
- b) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to an extended SG
20 promoter; or
- c) a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to an a wild-type
25 EMCV IRES.

In one example, the self-replicating RNA of the present disclosure comprises, in
order from 5' to 3':

- a) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter; and a second nucleotide
30 sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter; or
- b) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter; and a second nucleotide
35 sequence encoding a spike (S) protein from the omicron strain of a severe acute

respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an extended SG promoter; or

- c) a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an a wild-type EMCV IRES.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter.

For example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1.

For example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1; and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter comprising a sequence set forth in SEQ ID NO: 1.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to an extended SG promoter.

5 In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an extended SG promoter.

10 In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter encoded by a sequence set forth in SEQ ID NO: 1; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a
15 SARS-CoV-2 operably linked to an extended SG promoter encoded by a sequence set forth in SEQ ID NO: 5.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter encoded by a sequence set
20 forth in SEQ ID NO: 1; and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to an extended SG promoter encoded by a sequence set forth in SEQ ID NO: 5.

In one example, the self-replicating RNA of the present disclosure comprises, in
25 order from 5' to 3': a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a wild-type EMCV IRES.

30 In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter; and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a wild-type
35 EMCV IRES.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a minimal SG promoter encoded by a sequence set forth in SEQ ID NO: 1 and a second nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a wild-type EMCV IRES encoded by a sequence set forth in SEQ ID NO: 4.

In one example, the self-replicating RNA of the present disclosure comprises, in order from 5' to 3': a first nucleotide sequence encoding a nucleocapsid (N) protein from a SARS-CoV-2 operably linked to a minimal SG promoter encoded by a sequence set forth in SEQ ID NO: 1 and a second nucleotide sequence encoding a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) operably linked to a wild-type EMCV IRES encoded by a sequence set forth in SEQ ID NO: 4.

In one example, the RNA further comprises a 5' terminal cap structure.

In one example, the 5' terminal cap structure is an endogenous cap or analogue thereof. For example, the 5' terminal cap structure is an endogenous cap. For example, the 5' terminal cap structure is an analogue of an endogenous cap.

In one example, the 5' terminal cap structure comprise a guanine or guanine analogue thereof. For example, the 5' terminal cap structure comprise a guanine. For example, the 5' terminal cap structure comprise a guanine analogue of a guanine.

In one example, the 5' terminal cap structure is selected from a group consisting of anti-reverse cap analogue (ARCA), N7,2'-O-dimethyl-guanosine (mCAP), inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, N6,2'-O-dimethyladenosine, 7-methylguanosine (m7G), Cap1, and Cap2. For example, the 5' terminal cap structure is anti-reverse cap analogue (ARCA). For example, the 5' terminal cap structure is N7,2'-O-dimethyl-guanosine (mCAP). For example, the 5' terminal cap structure is inosine. For example, the 5' terminal cap structure is N1-methyl-guanosine. For example, the 5' terminal cap structure is 2'fluoro-guanosine. For example, the 5' terminal cap structure is 7-deaza-guanosine. For example, the 5' terminal cap structure is 8-oxo-guanosine. For example, the 5' terminal cap structure is 2-amino-guanosine. For example, the 5' terminal cap structure is LNA-guanosine. For example, the 5' terminal cap structure is 2-azido-guanosine. For example, the 5' terminal cap structure is N6,2'-O-dimethyladenosine. For example, the 5' terminal cap structure is 7-methylguanosine

(m7G). For example, the 5' terminal cap structure is Cap1. For example, the 5' terminal cap structure is Cap2.

In one example, the 5' terminal cap structure is linked to the 5' end of the RNA by a 5'-5'-triphosphate linkage or a 5'-5' phosphorothioate linkage. For example, the 5' terminal cap structure is linked to the 5' end of the RNA by a 5'-5'-triphosphate linkage. For example, the 5' terminal cap structure is linked to the 5' end of the RNA by a 5'-5' phosphorothioate linkage.

In one example, the antigens (i.e., a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a nucleocapsid (N) protein from a SARS-CoV-2) are expressed at substantially the same level. For example, the antigens have a level of expression within about 10%, or about 5% or about 1% of each other. In another example, the antigens are expressed at different levels. For example, the antigens have a level of expression greater than about 10%, or about 15% or about 20% of each other. Methods for determining the level of expression are known in the art and/or are described herein.

In one example, the self-replicating RNA is from an alphavirus. For example, the alphavirus is selected from the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan equine encephalitis virus (VEE) and combinations thereof.

In one example, the self-replicating RNA is from a Semliki Forest virus (SFV).

In one example, the self-replicating RNA is from a Sindbis virus (SIN).

In one example, the self-replicating RNA is from a Venezuelan equine encephalitis virus (VEE).

In one example, the S protein comprises the mutation Q641R.

In one example, the antigen is from a S protein of an omicron variant selected from the group consisting of B.1.1.529, BA.1, BA.2, BA.4, BA.5, BA.2.12.1 and BA.2.75. In one example, the antigen is from an S protein from an omicron variant selected from the group consisting of B.1.1.529, a BA.1 strain, a BA.2 strain, a BA.4 strain, a BA.5 strain, BA.2.12.1, BA.2.75, a XB strain, a XBB strain, a JN.1 strain, a JN.2 strain, a JN.3 strain, a KP.1 strain and a KP.2 strain.

In one example, the antigen is from a S protein of omicron variant BA.1 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70.

In one example, the antigen is from a S protein of omicron variant BA.2 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In one example, the antigen is from a S protein of omicron variant BA.4 or BA.5 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In one example, the antigen is from an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, Δ144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K.

In one example, the antigen is from an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, V83A, Δ144, G142D, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K.

In one example, the antigen is from an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of 16insMPLF, T19I, R21T, Δ24-26, A27S, S50L, Δ69-70, V127F, Δ144, G142D, F157S, R158G, Δ211, L212I, V213G, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445P, G446S, N450D, L452W, N460K, S477N, T478K, N481K, Δ483, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, Q954H, N969K and P1143L.

In one example, the antigen is from an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, Δ144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, L455S, N460K,

S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K.

In one example, the antigen is from an S protein comprising one or more or all of the mutations R346T, F456L and T572I.

5 In an example, the nucleotide sequence encoding an antigen from the S protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 20.

10 In an example, the nucleotide sequence encoding an antigen from the S protein comprises a polynucleotide sequence set forth in SEQ ID NO: 20.

In an example, the S protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 20.

15 In an example, the S protein is encoded by a polynucleotide sequence set forth in SEQ ID NO: 20.

In an example, the nucleotide sequence encoding an antigen from the N protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 21.

20 In an example, the nucleotide sequence encoding an antigen from the N protein comprises a polynucleotide sequence set forth in SEQ ID NO: 21.

In an example, the N protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 21.

In an example, the N protein is encoded by a polynucleotide sequence set forth in SEQ ID NO: 21.

30 In one example, the self-replicating RNA comprises or consists of a sequence according to SEQ ID NO: 17. In another example, the self-replicating RNA comprises or consists of a sequence according to SEQ ID NO: 18. In another example, the self-replicating RNA comprises or consists of a sequence according to SEQ ID NO: 19.

35 The present disclosure provides an immunogenic composition comprising the polynucleotide of the present disclosure. The present disclosure further provides an immunogenic composition comprising the RNA of the present disclosure. For example, the present disclosure provides an immunogenic composition comprising the cRNA of

the present disclosure. The present disclosure also provides an immunogenic composition comprising the self-replicating RNA of the present disclosure. For example, the composition of the present disclosure, when administered, is capable of inducing an immune response in the subject. For example, administration of the composition induces
5 a humoral and/or a cell-mediated immune response. In one example, the composition induces a humoral immune response in the subject. For example, the humoral immune response is an antibody-mediated immune response. In another example, the composition induces a cell-mediated immune response. For example, the cell-mediated immune response includes activation of antigen-specific cytotoxic T cells.

10 In one example, the immunogenic composition of the disclosure comprises multiple polynucleotides, wherein each polynucleotide encodes different polypeptide antigen sequences. In another example, the immunogenic composition of the disclosure comprises multiple RNAs, wherein each RNA encodes different polypeptide antigen sequences. In a further example, the immunogenic composition of the disclosure
15 comprises multiple cRNAs, wherein each cRNA encodes different polypeptide antigen sequences. In one example, the immunogenic composition comprises multiple multicistronic self-replicating RNAs, wherein each multicistronic self-replicating RNA encodes antigen polypeptides from different SARS-CoV-2 strains (for example., a S protein from an omicron strain of a SARS-CoV-2 and an N protein from a SARS-CoV-
20 2 from a delta strain). In another example, the different polypeptide antigen sequences are from the same strain (e.g., S and N proteins both from an omicron strain of a SARS-CoV-2). In another example, the immunogenic composition of the disclosure comprises a plurality of self-replicating RNAs, wherein each self-replicating RNA encodes different polypeptide antigen sequences. In another example, the immunogenic
25 composition of the disclosure comprises a plurality of self-replicating RNAs, wherein each self-replicating RNA encodes the same polypeptide antigen sequences.

The present disclosure also provides a pharmaceutical composition comprising an immunogenic composition of the present disclosure and a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers suitable for use in the present disclosure
30 will be apparent to the skilled person and/or are described herein.

In one example, the pharmaceutical composition further comprises a lipid nanoparticle (LNP), a polymeric microparticle, and an oil-in-water emulsion. For example, the polynucleotide, the RNA, the cRNA or the self-replicating RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-
35 in-water emulsion. In one example, the polynucleotide is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. In another

example, the RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. For example, the cRNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle, and an oil-in-water emulsion. For example, the self-replicating RNA is encapsulated in, bound to or adsorbed on a
5 LNP, a polymeric microparticle, and an oil-in-water emulsion.

In one example, the pharmaceutical composition further comprises a LNP. For example, the polynucleotide is encapsulated in a LNP. In another example, the RNA is encapsulated in a LNP. For example, the cRNA is encapsulated in a LNP. For example, the self-replicating RNA is encapsulated in a LNP. For example, the polynucleotide is
10 bound to a LNP. In another example, the RNA is bound to a LNP. For example, the cRNA is bound to a LNP. In another example, the self-replicating RNA is bound to a LNP. For example, the polynucleotide is adsorbed on to a LNP. In another example, the RNA is adsorbed on to a LNP. For example, the cRNA is adsorbed on to a LNP. In a further example, the self-replicating RNA is adsorbed on to a LNP. In another example,
15 wherein each RNA is formulated together in the LNP. In another example, wherein each RNA is formulated separately in the LNP.

In one example, the LNP comprises a PEG-lipid, a structural lipid and/or a neutral lipid. For example, the LNP comprises a PEG-lipid, a structural lipid and a neutral lipid. In another example, the LNP comprises a PEG-lipid, a structural lipid or a neutral lipid.
20 In one example, the LNP further comprises a cationic lipid. In another example, the LNP does not comprise a cationic lipid.

In one example, the pharmaceutical composition further comprises a polymeric microparticle. For example, the polynucleotide is encapsulated in a polymeric microparticle. In another example, the RNA is encapsulated in a polymeric microparticle. For example, the cRNA is encapsulated in a polymeric microparticle. For example, the self-replicating RNA is encapsulated in a polymeric microparticle. For example, the polynucleotide is bound to a polymeric microparticle. In another example, the RNA is bound to a polymeric microparticle. For example, the cRNA is bound to a polymeric microparticle. In another example, the self-replicating RNA is bound to a
25 polymeric microparticle. For example, the polynucleotide is adsorbed on to a polymeric microparticle. In another example, the RNA is adsorbed on to a polymeric microparticle. For example, the cRNA is adsorbed on to a polymeric microparticle. In a further example, the self-replicating RNA is adsorbed on to a polymeric microparticle.

In one example, the pharmaceutical composition further comprises an oil-in-water emulsion. For example, the polynucleotide is encapsulated in an oil-in-water emulsion. In another example, the RNA is encapsulated in an oil-in-water emulsion. For example,
35

the cRNA is encapsulated in an oil-in-water emulsion. For example, the self-replicating RNA is encapsulated in an oil-in-water emulsion. For example, the polynucleotide is bound to an oil-in-water emulsion. In another example, the RNA is bound to an oil-in-water emulsion. For example, the cRNA is bound to an oil-in-water emulsion. In another example, the self-replicating RNA is bound to an oil-in-water emulsion. In a further example, the self-replicating RNA is adsorbed on to an oil-in-water emulsion. In a further example, the self-replicating RNA is resuspended in an oil-in-water emulsion.

The present disclosure also provides the immunogenic composition or the pharmaceutical composition of the disclosure for use as a vaccine.

10 In one example, the polynucleotide is DNA. In one example, the disclosure provides a DNA encoding a cRNA vaccine of the disclosure. In one example, the disclosure provides a DNA encoding a self-replicating RNA vaccine of the disclosure.

In one example, the DNA is a plasmid.

The present disclosure provides a method of treating or preventing or delaying
15 progression of a disease or condition in a subject, the method comprising administering the immunogenic composition or the pharmaceutical composition of the present disclosure to a subject in need thereof. In one example, the disclosure provides a method of treating a disease or condition in a subject, the method comprising administering the immunogenic composition or the pharmaceutical composition of the present disclosure
20 to a subject in need thereof. In another example, the disclosure provides a method of preventing a disease or condition in a subject, the method comprising administering the immunogenic composition or the pharmaceutical composition of the present disclosure to a subject in need thereof. In a further example, the disclosure provides a method of delaying progression of a disease or condition in a subject, the method comprising
25 administering the immunogenic composition or the pharmaceutical composition of the present disclosure to a subject in need thereof.

In one example, the present disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the
30 disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In another example, the disclosure provides use of a polynucleotide of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a polynucleotide of the
35 disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

In one example, the present disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for treating a disease
5 or condition in a subject in need thereof. In another example, the disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a RNA of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

10 In one example, the present disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In another example, the disclosure provides use
15 of a cRNA of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a cRNA of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

In one example, the present disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for treating or preventing or delaying progression of a disease or condition in a subject in need thereof. For example, the disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for treating a disease or condition in a subject in need thereof. In another example, the disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for preventing a disease or condition in a subject in need thereof. In a further example, the disclosure provides use of a self-replicating RNA of the disclosure in the manufacture of a medicament for delaying progression of a disease or condition in a subject in need thereof.

In one example, the subject suffers from a disease or condition. In one example, the subject has been diagnosed as suffering from a disease or condition. In one example, the subject is receiving treatment for a disease or condition.

In an example, the present disclosure provides a method of treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject in need thereof, the method comprising administering a RNA disclosed herein (e.g., self-replicating RNA),
35 a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject.

In an example, the present disclosure provides use of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject.

In an example, the present disclosure provides a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in the treatment or prevention or delaying progression of a SARS-CoV-2 infection.

In an example, the subject having a SARS-CoV-2 infection has at least one symptom of COVID-19. In an example, one such symptom of COVID-19 includes runny nose, cough, sore throat, fever, headache, muscle pain or fatigue. In another example, the at least one symptom is any one of the mild-COVID-19 symptoms described herein. In another example, the at least one symptom is any one of the moderate to severe COVID-19 symptoms described herein.

In one example, the subject is at risk of developing COVID-19 or a SARS-CoV-2 infection. For example, the subject is at risk of developing COVID-19. In another example, the subject is at risk of developing a SARS-CoV-2 infection.

In one example, the composition of the present disclosure is administered in an amount sufficient to reduce the severity of or prevent onset of one or more symptoms of a SARS-CoV-2 infection or COVID-19. Symptoms of a SARS-CoV-2 infection or COVID-19 will be apparent to the skilled person and/or are described herein.

In an example, the present disclosure provides a method of treating or preventing or delaying progression of COVID-19 in a subject in need thereof, the method comprising administering a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject.

In an example, the present disclosure provides use of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for treating or preventing or delaying progression of COVID-19 in a subject.

In an example, the present disclosure provides a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in the treatment or prevention or delaying progression of COVID-19 in a subject.

In an example, the present disclosure provides a method of inducing an immune response in a subject, the method comprising administering a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

5 In an example, the present disclosure provides use of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

10 In an example, the present disclosure provides a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in inducing an immune response in a subject in need thereof.

In one example, the composition induces a humoral immune response in the
15 subject. For example, the humoral immune response is an antibody-mediated immune response. For example, production of neutralizing antibodies. In another example, the composition induces a cell-mediated immune response. For example, the cell-mediated immune response includes activation of antigen-specific cytotoxic T cells. For example, the T cells are CD4 T cells and/or CD8 T cells. In one example, the T cells are CD4 T
20 cells. In another example the T cells are CD8 T cells. In a further example, the T cells are CD4 and CD8 T cells.

In one example, administration of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine of the present disclosure induces a CD4 T cell mediated
25 immune response.

In one example, administration of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition, an immunogenic composition disclosed herein or a vaccine of the present disclosure induces a CD8 T cell mediated immune response.

In one example, administration of a RNA disclosed herein (e.g., self-replicating
30 RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine of the present disclosure induces a CD4 and a CD8 T cell mediated immune response.

In one example, the CD4 T cell mediated immune response is a Th0, a Th1 and/or a Th2 response. For example, the CD4 T cell mediated immune response is a Th0
35 response. In another example, the CD4 T cell mediated immune response is a Th1 response. In a further example, the CD4 T cell mediated immune response is a Th2

response. In one example, the CD4 T cell mediated immune response is a Th0 and Th1 response. In another example, the CD4 T cell mediated immune response is a Th0 and Th2 response. In a further example, the CD4 T cell mediated immune response is a Th1 and Th2 response. In another example, the CD4 T cell mediated immune response is a
5 Th0, Th1 and Th2 response.

In one example, the Th0 response cytokines express interleukin 2 (IL2+) and/or tumor necrosis factor alpha (TNFα+); and/or are negative for interferon gamma (IFNγ-), IL5- and/or IL13-. For example, the cytokine is IL2+. In another example, the cytokine is TNFα+. In one example, the cytokine is IFNγ-. In another example, the cytokine is
10 IL5-. In a further example, the cytokine is IL13-.

In one example, the Th1 response cytokines express interferon gamma (IFNγ+); and/or are negative for IL5- and/or IL13-. For example, the cytokine is IFNγ+. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th2 response cytokines express IL5+ and/or IL13+; and/or
15 are negative for IFNγ. For example, the cytokine is IL5+. In a further example, the cytokine is IL13+. For example, the cytokine is IFNγ-.

In one example, the immune response is raised in response to the S protein from the omicron strain of a SARS-CoV-2 and to the N protein from a SARS-CoV-2, optionally the omicron strain of SARS-CoV-2. In another example, the immune response
20 is sufficient to treat, prevent or delay progression of at least one symptom of a SARS-CoV-2 infection caused by the omicron strain of a SARS-CoV-2. In another example, the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a SARS-CoV-2 infection caused by the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of the SARS-CoV-2. In another example, the subject
25 having a SARS-CoV-2 infection has at least one symptom of COVID-19. In an example, one such symptom of COVID-19 includes runny nose, cough, sore throat, fever, headache, muscle pain or fatigue. In another example, the at least one symptom is any one of the mild-COVID-19 symptoms described herein. In another example, the at least one symptom is any one of the moderate to severe COVID-19 symptoms described
30 herein.

In an example, the present disclosure provides a method for reducing SARS-CoV-2 viral load in a subject with COVID-19 comprising administering a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in
35 need thereof.

In an example, the present disclosure provides use of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for reducing SARS-CoV-2 viral load in a subject with
5 COVID-19.

In an example, the present disclosure provides a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in reducing SARS-CoV-2 viral load in a subject with COVID-19.

10 In an example, the present disclosure provides a method for treating, preventing or delaying progression of pneumonia in a subject with COVID-19 comprising administering a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

15 In an example, the present disclosure provides use of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of pneumonia in a subject with confirmed COVID-19.

20 In an example, the present disclosure provides a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of pneumonia in a subject with confirmed COVID-19.

In an example, the present disclosure provides a method for treating, preventing
25 or delaying progression of acute respiratory distress syndrome in a subject with COVID-19 comprising administering a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein or a vaccine disclosed herein, an immunogenic composition disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a RNA disclosed herein
30 (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of acute respiratory distress syndrome in a subject with COVID-19.

In an example, the present disclosure provides a RNA disclosed herein (e.g., self-
35 replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing

or delaying progression of acute respiratory distress syndrome in a subject with COVID-19.

In an example, the present disclosure provides a method for treating, preventing or delaying progression of sepsis in a subject with COVID-19 comprising administering
5 a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an
10 immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of sepsis in a subject with COVID-19.

In an example, the present disclosure provides a RNA disclosed herein (e.g., self-replicating RNA), a pharmaceutical composition disclosed herein, an immunogenic
15 composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of sepsis in a subject with COVID-19.

In an example, the present disclosure provides a method for preventing or decreasing mortality in a subject with COVID-19 comprising administering a RNA disclosed herein, a self-replicating RNA disclosed herein, a pharmaceutical composition
20 disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a RNA disclosed herein, a self-replicating RNA disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the
25 preparation of a medicament for preventing or decreasing mortality in a subject with COVID-19.

In an example, the present disclosure provides a RNA disclosed herein, a self-replicating RNA disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in
30 preventing or decreasing mortality in a subject with COVID-19.

In an example, COVID-19 has been caused by the omicron strain of the SARS-CoV-2. In another example, COVID-19 has been caused by the delta, beta, alpha or gamma strain 2019-nCoV/USA-WA1/2020 SARS-CoV-2 strain.

In an example, the subject is a human of 18 years of age or older. In another
35 example, the subject is a human of any age, e.g., from about 1 month to 100 years old, e.g., from about 2 months to about 80 years old, from about 6 months of age to about 3

years old, from about 3 years to about 18 years old, from about 12 years to about 18 years old, from about 18 years to about 55 years old, from about 50 years to about 75 years old, from about 40 years to about 65 years old. In another example, the subject is a human from 2 years of age. In another example, subject is a human from 18 years of age, a
5 human from 30 years of age, a human from 40 years of age, a human from 50 years of age, a human from 60 years of age, a human from 70 years of age, a human from 80 years of age or a human from about 90 years of age. In another example, the subject is less than 2 years of age, less than 18 months of age, less than 12 months of age, less than 6 months of age or less than 3 months of age.

10 In an example, a composition or vaccine described herein is administered in a one dose regimen. In another example, the composition is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.

The present disclosure also provides a polynucleotide that encodes the self-replicating RNA of the present disclosure. For example, the polynucleotide is a
15 recombinant DNA.

In one example, the polynucleotide comprises a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 17.

In one example, the polynucleotide comprises a nucleotide sequence at least 70%,
20 at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 18.

In one example, the polynucleotide comprises a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 19.

25 The present disclosure also provides a kit comprising at least one self-replicating RNA of the disclosure, optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions for use in treating or preventing or delaying progression of a disease or disorder (e.g., a SARS-CoV-2 infection, COVID-19 and/or ARDS) in a subject.

30 The present disclosure also provides a kit comprising at least one self-replicating RNA of the disclosure, optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions to administer the RNA to a subject who is suffering from or at risk of suffering from a disease or disorder (e.g., a SARS-CoV-2 infection, COVID-19 and/or ARDS).

35 In one example, the self-replicating RNA, the RNA, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a vial. In

another example, the RNA, the self-replicating RNA, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a syringe.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any
5 or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

10 **Figure 1 - sa-mRNA construct design:** Co96 includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an Spike (S) protein antigen from the omicron strain of SARS-CoV-2. Co97 includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an S protein antigen and a N protein antigen from the omicron strain of SARS-CoV-2, driven by a synthetic genomic promoter (SGP) v2.
15 Co99 includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an N protein antigen and a S protein antigen from the omicron strain of SARS-CoV-2, driven by a synthetic genomic promoter (SGP) v2. Co16 includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an S protein antigen from the original (Wuhan) strain of SARS-CoV-2. Co18 includes an alphavirus self-replicating
20 RNA comprising a polynucleotide encoding an S protein antigen and a N protein antigen from the original (Wuhan) strain of SARS-CoV-2, driven by a synthetic genomic promoter (SGP) v2.

Figure 2 – Detection of SARS-CoV-2 N and S protein expression *in vitro* when expressed from from sa-mRNA constructs Co96, Co97, Co99, Co18, Co16 and BHK-V.

25 **Figure 3 -** Measurement of specific potencies of sa-mRNA constructs Co96, Co97, Co99, Co18 and Co16 *in vitro*, determined according to dose of RNA (ng) when formulated in an LNP.

Figure 4 – Effect of sa-mRNA constructs Co96, Co97, Co99, Co18 and Co16 on LV microneutralisation titers, PV microneutralisation titers and ACE-2 binding
30 inhibition *in vitro*.

Figure 5 - Effect of sa-mRNA constructs Co96, Co97, Co99, Co18 and Co16 on LV microneutralisation titers, PV microneutralisation titers and ACE-2 binding inhibition *in vitro* of BA.1, BA.2 and BA.4/5 variants of the omicron strain of SARS-CoV-2.

35

KEY TO SEQUENCE LISTING

Sequence	SEQ ID NO
Nucleotide sequence of alphavirus native subgenomic promoter (v1)	1
Nucleotide sequence of extended subgenomic promoter (v2)	2
Nucleotide sequence of extended subgenomic promoter (v3)	3
Nucleotide sequence of wild-type EMCV IRES	4
Nucleotide sequence of extended subgenomic promoter (v4)	5
Nucleotide sequence of a Kozak consensus sequence	6
Nucleotide sequence of a Kozak consensus sequence	7
Nucleotide sequence of an interrupting linker	8
Nucleotide sequence of a GC-rich element	9
Nucleotide sequence of a GC-rich element	10
Nucleotide sequence of a GC-rich element	11
Nucleotide sequence of a histone stem loop	12
Nucleotide sequence of 5'UTR of VEEV	13
Nucleotide sequence of 3'UTR of SINV	14
Nucleotide sequence of extended subgenomic promoter	15
Poly-A sequence	16
Self-replicating mRNA polynucleotide sequence comprising Spike (S) antigen from omicron strain of SARS-CoV-2 (Co96)	17
Self-replicating mRNA polynucleotide sequence comprising S and N antigens from omicron strain of SARS-CoV-2 (Co97)	18
Self-replicating mRNA polynucleotide sequence comprising comprising N and S antigens from omicron strain of SARS-CoV-2 (Co99)	19
Polynucleotide sequence of Spike (S) antigen from omicron strain of SARS-CoV-2	20
Polynucleotide sequence of Spike (N) antigen from omicron strain of SARS-CoV-2	21
Nucleotide sequence of 3'UTR	22

DETAILED DESCRIPTION

General

Throughout this specification, unless specifically stated otherwise or the context
5 requires otherwise, reference to a single step, composition of matter, group of steps or

group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter.

Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* to any other example of the disclosure unless specifically stated otherwise. Stated another way, any specific example of the present disclosure may be combined with any other specific example of the disclosure (except where mutually exclusive).

Any example of the present disclosure disclosing a specific feature or group of features or method or method steps will be taken to provide explicit support for disclaiming the specific feature or group of features or method or method steps.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual,

Cold Spring Harbour Laboratory, (1988), and J.E. Coligan *et al.* (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for
5 either meaning.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

10 As used herein the term “derived from” shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source. Similarly, the term “based on” shall be taken to indicate that a specified integer may be developed or used from a particular source albeit not necessarily directly from that source.

15

Selected Definitions

As used herein, the term “monocistronic” in reference to the polynucleotide, RNA, cRNA and/or self-replicating RNA, refers to a RNA that encodes one polypeptide.

As used herein, the term “multicistronic” (also known as “polycistronic”) in
20 reference to the polynucleotide, RNA, cRNA and/or self-replicating RNA, refers to a RNA that encodes two or more polypeptides. The term encompasses “bicistronic” (or “dicistronic”; i.e., encoding two polypeptides) and “tricistronic” (i.e., encoding three polypeptides) molecules. By “bicistronic” is meant a single nucleic acid that is capable of encoding two distinct polypeptides from different regions of the nucleic acid.

25 As used herein, the term “conventional mRNA” or “cRNA” or “non-amplifying RNA” refers to a construct that allows expression of heterologous RNA and proteins but the RNA that cannot amplify in host cells.

As used herein, the term “self-replicating RNA” refers to a construct based on an RNA virus that has been engineered to allow expression of heterologous mRNA and
30 proteins. Self-replicating RNA (e.g., in the form of naked RNA) can amplify in host cells leading to expression of the desired gene product in the host cell.

The term “naked” as used herein refers to nucleic acids that are substantially free of other macromolecules, such as lipids, polymers and proteins. A “naked” nucleic acid, such as a self-replicating RNA, is not formulated with other macromolecules to improve
35 cellular uptake. Accordingly, a naked nucleic acid is not encapsulated in, absorbed on,

or bound to a lipid nanoparticle (LNP), a liposome, a polymeric microparticle or an oil-in-water emulsion.

As used herein, the term “nucleotide sequence” or “nucleic acid sequence” will be understood to mean a series of contiguous nucleotides (or bases) covalently linked to a phosphodiester backbone. By convention, sequences are presented from the 5' end to the 3' end, unless otherwise specified. To facilitate a clear description of the nucleic acids, particular sequence components are referred to as e.g., a “first nucleotide sequence” and a “second nucleotide sequence”. It is to be understood that the first and second sequences can appear in any desired order or orientation, unless otherwise specified, and that no particular order or orientation is intended by the words “first”, “second” etc.

As used herein, the term “antigen” refers to a molecule or structure containing one or more epitopes that induce, elicit, augment or boost a cellular and/or humoral immune response. Antigens can include, for example, proteins and peptides from a pathogen such as a virus, bacteria, fungus, protozoan, plant or from a tumour.

As used herein the term “adjuvant” refers to a compound that, when used in combination with a specific immunogen (e.g. a VLP) in a formulation, augments or otherwise alters or modifies the resultant immune response. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses.

As used herein, the term “operably linked to” means positioning a subgenomic promoter or regulatory element (e.g., an IRES) relative to a nucleic acid such that expression of the nucleic acid is controlled or regulated by the element. For example, a subgenomic promoter can be operably linked to numerous nucleic acids, e.g., through another regulatory element, such as an internal ribosome entry site (IRES).

As used herein, the term “subgenomic promoter” (also known as ‘junction region’ promoter) refers to a promoter that directs the expression of a heterologous nucleotide sequence, regulating protein expression.

As used herein, the term “internal ribosome entry site” or “IRES” refers to a sequence of nucleotides within a mRNA to which a ribosome or a component thereof, e.g., a 40S subunit of a ribosome, is capable of binding. An IRES need not necessarily comprise nucleic acid that induces translation of a mRNA (e.g., a start codon; AUG).

The term “polypeptide” or “polypeptide chain” will be understood to mean a series of contiguous amino acids linked by peptide bonds. For example, a protein shall be taken to include a single polypeptide chain i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently

linked to one another (i.e., a polypeptide complex). The series of polypeptide chains can be covalently linked using a suitable chemical or a disulfide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

5 The term “recombinant” shall be understood to mean the product of artificial genetic recombination.

As used herein the term “substantially the same” in reference to the level of expression is meant that the first and second antigens (at least) have a level of expression within about 10% or less of each other unless the context implies otherwise.

10 As used herein, the terms “disease”, “disorder” or “condition” refers to a disruption of or interference with normal function, and is not to be limited to any specific condition, and will include diseases or disorders.

As used herein, a subject “at risk” of having developed or developing a SARS-CoV-2 infection may have nor may not have detectable disease or symptoms of a SARS-CoV-2 infection, and may have nor may not have displayed detectable disease or symptoms of a SARS-CoV-2 infection prior to the treatment according to the present disclosure. “At risk” denotes that a subject has one or more risk factors, which are measurable parameters that correlate with development of a SARS-CoV-2 infection, as known in the art and/or described herein.

20 As used herein, the terms “treatment” or “treating” of a subject includes the application or administration of a compound or composition of the disclosure to a subject (or application or administration of a compound of the disclosure to a cell or tissue from a subject) with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term “treating” refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms
25 or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating.

As used herein, “preventing” or “prevention” is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience
35 or display symptoms of the disease). Biological and physiological parameters for

identifying such patients are provided herein and are also well known by physicians.

As used herein, the phrase “delaying progression of” includes reducing or slowing down the progression of the disease or condition in an individual and/or at least one symptom of a disease or condition.

5 An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired result. For example, the desired result may be a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect treatment of a disease or condition as
10 hereinbefore described. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect a change associated with a disease or condition as hereinbefore described. The effective amount may vary according to the disease or condition to be treated or factor to be altered and also according to the weight, age, racial background, sex, health and/or physical condition and other factors relevant
15 to the mammal being treated. Typically, the effective amount will fall within a relatively broad range (e.g. a “dosage” range) that can be determined through routine trial and experimentation by a medical practitioner. Accordingly, this term is not to be construed to limit the disclosure to a specific quantity, e.g., weight or number of RNA. The effective amount can be administered in a single dose or in a dose repeated once or several times
20 over a treatment period.

A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disease or condition. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the RNA of the present
25 disclosure to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the RNA are outweighed by the therapeutically beneficial effects.

As used herein, the term “prophylactically effective amount” shall be taken to mean a sufficient quantity of the RNA of the disclosure to prevent or inhibit or delay the
30 onset of one or more detectable symptoms of a disease or disorder as described herein.

A “subject” can also be any animal that is susceptible to infection by a SARS-CoV-2. A subject of this disclosure can be a mammal and in particular embodiments is a human, which can be an infant, a child, an adult or an elderly adult. A “subject at risk of infection by SARS-CoV-2” or a “subject at risk of SARS-CoV-2 infection” is any subject
35 who may be or has been exposed to the SARS-CoV-2. The subject may be a primary contact of an individual diagnosed with a SARS-CoV-2 infection. “Subject” includes any

human or non-human animal. Thus, in addition to being useful for human treatment, the compounds of the present disclosure may also be useful for veterinary treatment of mammals, including companion animals and farm animals, such as, but not limited to dogs, cats, horses, cows, sheep, and pigs.

5 As used herein, the term “lipid nanoparticle” or “LNP” shall be understood to refer to lipid-based particles having at least one dimension on the order of nanometers (e.g., 1-1,000 nm) and which comprises a compound of any formulae described herein. In embodiments, LNPs are formulated in a composition for delivery of a polynucleotide to a desired target such as a cell, tissue, organ, tumor, and the like. For example, the lipid
10 nanoparticle or LNP any lipid composition, including, may be selected from, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles, wherein solid lipid nanoparticles lack lipid bilayers.

15

Polynucleotides

As used herein, the term “polynucleotide” refers a molecular chain of nucleotides chemically bonded by a series of ester linkages between the phosphoryl group of one nucleotide and the hydroxyl group of the sugar in an adjacent nucleotide. In one example,
20 the polynucleotide is a DNA. In one example, the polynucleotide is a RNA, e.g., mRNA. For example, the mRNA is a conventional mRNA (cRNA) or a self-replicating RNA.

As used herein, the term “fragment” refers to a portion of a nucleotide sequence or polypeptide of a reference nucleotide sequence or polypeptide disclosed herein which maintains a defined activity of the full length nucleotide sequence or polypeptide.

25 By way of non-limiting example, where the conventional mRNA, self-replicating RNA comprises a polynucleotide encoding more than one antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), said more than one antigen may be expressed by a monocistronic polynucleotide, or each of said SARS-CoV-2 antigens may be expressed by polycistronic (or multicistronic) polynucleotides. For example, the S and
30 N protein antigens may be expressed by a monocistronic polynucleotide or by polycistronic polynucleotides.

As used herein, the term “variant” refers to a nucleotide sequence with one or more substitutions, insertions, deletions and/or other modifications compared to the unmodified sequence. It will be apparent to the skilled person that any variant described
35 herein will have the same or similar expression of the encoded protein. For example, the

variant is a functional variant. Exemplary modifications to the nucleotide sequence and/or polypeptide will be apparent to the skilled person and/or described herein.

In one example, a modification is a chemical modification of one or more nucleotide(s) of the nucleotide sequence. For example, at least one naturally occurring
5 nucleotide of the polynucleotide is replaced with a chemically modified nucleotide (e.g. pseudouridine (ψ), and 1-methylpseudouridine ($m1\psi$)).

In one example, the modification comprises increasing the G/C content of the nucleotide sequence.

In one example, the modification comprises codon optimization of the nucleotide
10 sequence.

In one example, the substitution is a conservative substitution. A skilled person will appreciate that a conservative substitution with reference to a polypeptide involves replacement of an amino acid in the polypeptide with a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size). In one example, the
15 substitution is a non-conservative substitution.

As used herein, the term “encode”, “encodes” or “encoding” refers to a region of a polynucleotide capable of undergoing translation into a polypeptide.

The polynucleotide of the present disclosure includes DNA and RNA (e.g. mRNA).
20

Deoxyribonucleic acid (DNA)

In one example, the polynucleotide is a DNA (e.g. DNA vector).

It will be apparent to the skilled person that a DNA of the present disclosure further comprises an endonuclease restriction site at the 3' end of the 3' UTR. The skilled
25 person will appreciate that endonuclease restriction site allows for the insertion of one or more nucleotide sequence(s) (e.g. encoding an antigen of interest, a fragment and/or a variant thereof) without disrupting the remainder of the DNA.

As used herein, the term “restriction endonuclease site” refers to a sequence of DNA that binds to a restriction endonuclease. Typically, the restriction endonuclease
30 site is short sequence (e.g. of approximately 4-8 base pairs) recognised and cleaved by the restriction endonuclease.

As used herein, the term “restriction enzymes” or “restriction endonucleases” refers to a class of enzyme that occur naturally in bacteria and in some viruses. Restriction endonuclease bind specifically to and cleave double-stranded DNA at
35 specific sites within or adjacent to a restriction endonuclease site. Exemplary restriction endonuclease include, for example, BciVI (Bful), BclI (Spel), EcoRI, AatII, AgeI

(BshTI), Apal, BamHI, BglII, BlnI (Bpu1102I), BsrGI (Bsp1407), ClaI (Bsu15I), EcoRI, EcoRV (Eco32I), Eam1104I (EarI), HindIII, KpnI, MluI, NcoI, NdeI, NheI, NotI, NsiI, Mph1103I), PstI, PvuI, PvuII, SacI, SalI, ScaI, SpeI, XbaI, XhoI, SacII (Cfr42I) and XbaI.

In one example, the present disclosure provides a transcribable polynucleotide comprising the first nucleotide sequence encoding a first antigen of interest; and/or a second nucleotide sequence encoding a second antigen of interest operably linked to a regulatory element such as a SG promoter and an IRES. For example, the polynucleotide is the DNA plasmid comprising the first and second nucleotide sequences.

In one example, the DNA comprises a nucleotide sequence comprising a restriction endonuclease site located 3' of the 3'UTR. The presence of the restriction endonuclease site located 3' of the 3'UTR allows for production of a linearised DNA. Linearisation of DNA ensures defined termination of *in vitro* transcribed DNA to produce mRNA.

15 *Ribonucleic acid (RNA)*

In one example, the polynucleotide is a mRNA comprising a first nucleotide sequence encoding an antigen operably linked to a promoter, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

20 The mRNA of the present disclosure encompasses a non-replicating mRNA (also referred to as conventional mRNA (cRNA) or non-amplifying) in addition to a self-replicating RNA (also known as self-amplifying RNA or sa-mRNA).

Conventional (non-replicating) RNA

25 In one example, the polynucleotide is a cRNA comprising a first nucleotide sequence encoding an antigen operably linked to a promoter, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In an example, the cRNA of the present disclosure comprises in order from 5' to 3': a 5' cap structure, a 5'-UTR, a fragment and/or a variant thereof, a first nucleotide sequence encoding a first antigen of interest, a second nucleotide sequence encoding a second antigen of interest, a 3'-UTR and a 3' tailing sequence (e.g. a polyadenylation signal or one or more poly-A tails), wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

35 The cRNA of the present disclosure may further comprise an translation internal

ribosome entry site (e.g. Kozak consensus sequence or IRES) operably linked to the antigen of interest.

Self-replicating RNA

5 The present disclosure provides a self-replicating RNA (also known as a replicon).

 The skilled person will understand that the self-replicating RNA of the present disclosure is based on the genomic RNA of RNA viruses. The RNA should be positive (+)-stranded so that it can be directly translated after delivery to a cell without the need
10 for intervening replication steps (e.g., reverse transcription). Translation of the RNA results in the production of non-structural proteins (NSPs) which combine to form a replicase complex (i.e., an RNA-dependent RNA polymerase). The complex then amplifies the original RNA, producing both antisense and sense transcripts, resulting in production of multiple daughter RNAs which may subsequently be translated and
15 transcribed, enhancing overall protein expression.

 In one example, the self-replicating RNA of the present disclosure comprises the non-structural proteins of the RNA virus, the 5' and 3' untranslated regions (UTRs) and the native subgenomic promoter.

 In one example, the self-replicating RNA comprises one or more non-structural
20 proteins of the RNA virus. For example, the RNA comprises at least one or more genes selected from the group consisting of a viral replicase (or viral polymerase), a viral protease, a viral helicase and other non-structural viral proteins. For example, the self-replicating RNA comprises a viral replicase (or viral polymerase).

 In another example, the self-replicating RNA comprises a 5'- and a 3'-end UTR
25 of the RNA virus. It will be apparent to the skilled person that the terms 5' and a 3'UTR also encompasses the terms 5' and 3' conserved sequence elements (CSE). In one example, the self-replicating RNA comprises a 5'- and a 3'-end CSE.

 The self-replicating RNA of the present disclosure cannot induce production of infectious viral particles. For example, the self-replicating RNA of the present disclosure
30 does not comprise viral genes encoding structural proteins necessary for production of viral particles.

 In one example, the self-replicating RNA is derived from or based on an alphavirus. Suitable alphaviruses will be apparent to the skilled person and/or described herein.

35 In another example, the self-replicating RNA is derived from or based on a virus other than an alphavirus, for example, a positive-stranded RNA virus. Positive-stranded

RNA viruses suitable for use in the present disclosure will be apparent to the skilled person and include, for example, a picornavirus, a flavivirus, a rubivirus, a pestivirus, a hepacivirus, a calicivirus, or a coronavirus.

In one example, the self-replicating RNA is encoded by a nucleotide sequence at
5 least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 17.

In one example, the self-replicating RNA is encoded a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 18.

10 In one example, the self-replicating RNA is encoded by a nucleotide sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or identical to the sequence set forth in SEQ ID NO: 19.

Alphavirus

15 In one example, the self-replicating RNA of the present disclosure is derived from (or based on) an alphavirus.

Alphaviruses are the sole genus in the *Togaviridae* family and are an enveloped virus with a positive-sense, single-stranded RNA genome. The skilled person will understand that the alphavirus genome comprises two open reading frames (ORFs), non-
20 structural and structural. The first ORF encodes four non-structural proteins (NSP1, NSP2, NSP3 and NSP4) necessary for transcription and replication of viral RNA. The second encodes three structural proteins: the core nucleocapsid protein C, and the envelope proteins P62 and E1, which associate as a heterodimer. The viral membrane-anchored surface glycoproteins are responsible for receptor recognition and entry into
25 target cells through membrane fusion.

In one example, the self-replicating RNA of the present disclosure comprises a viral replicase (or viral polymerase). For example, the viral replicase is an alphavirus replicase, such as an alphavirus protein NSP4.

In one example, the self-replicating RNA of the present disclosure does not
30 encode one or more alphavirus structural proteins (e.g., capsid and/or envelope glycoproteins). For example, the self-replicating RNA is unable to produce RNA-containing alphavirus virions (i.e., infectious viral particles).

In one example, the self-replicating RNA comprises a native alphavirus SG promoter. For example, the native alphavirus SG promoter is a minimal SG promoter
35 (i.e., the minimal sequence required for initiation of transcription) and comprises a sequence set forth in SEQ ID NO: 1.

The skilled person will be aware of alphaviruses suitable for use in the present disclosure. Exemplary alphaviruses include, but are not limited to, Venezuelan equine encephalitis virus (VEE; e.g., Trinidad donkey, TC83CR), Semliki Forest virus (SFV), Sindbis virus (SIN), Ross River virus, Western equine encephalitis virus, Eastern equine encephalitis virus, Chikungunya virus, S.A. AR86 virus, Everglades virus, Mucambo virus, Barmah Forest virus, Middelburg virus, Pixuna virus, O'nyong-nyong virus, Getah virus, Sagiyama virus, Bebaru virus, Mayaro virus, Una virus, Aura virus, Whataroa virus, Banbanki virus, Kyzylagach virus, Highlands J virus, Fort Morgan virus, Ndumu virus, and Buggy Creek virus. The term alphavirus may also include chimeric alphaviruses (e.g., as described by Perri et al, (2003) J. Virol. 77(19): 10394-403) that contain genome sequences from more than one alphavirus.

Regulatory elements

The present disclosure provides a polynucleotide comprising a nucleotide sequence encoding an antigen operably linked to a promoter, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In an example, the regulatory element is selected from the group consisting of a subgenomic (SG) promoter and an internal ribosome entry site (IRES) and a Kozac consensus sequence or a combination thereof. In an example, the regulatory element is a SG promoter.

Kozak consensus sequence

As used herein, the term "Kozak consensus sequence" refers to a nucleotide sequence identified in eukaryotic genes that facilitates the translation of the gene by containing a start codon (also referred to as a translation initiation codon) which is recognised by a ribosome.

Exemplary Kozak consensus sequence are known in the art and/or described herein. In one example, the Kozak consensus sequence is set forth in SEQ ID NO: 6 (GCCACC). In another example, the Kozak consensus sequence is set forth in SEQ ID NO: 7 (ACCATGG).

Subgenomic Promoters

SG promoters (also known as 'junction region' promoters) suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein.

In one example, the SG promoter is derived from or based on an alphavirus SG promoter. For example, the SG promoter is a native alphavirus SG promoter. In one example, the native SG promoter is a minimal SG promoter. For example, the minimal SG promoter is the minimal sequence required for initiation of transcription. In one
5 example, the native SG promoter is an extended SG promoter. For example, the extended SG promoter is a minimal SG promoter extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein (e.g., NSP4) of the RNA virus (e.g., an alphavirus). In one example, the extended SG promoter is a minimal SG promoter extended at the 5' end with nucleotides occurring in a sequence encoding an alphavirus
10 NSP4.

In one example, the SG promoter is extended at the 5' end by about 31 nucleotides occurring in a sequence encoding a non-structural protein (e.g., an alphavirus NSP4). In one example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 1 extended at the 5' end by 31 nucleotides occurring in a sequence encoding a non-
15 structural protein (e.g., an alphavirus NSP4). For example, the extended SG promoter is no more than 80 nucleotides in length. In one example, the extended SG promoter is encoded by a sequence comprising or consisting of nucleotides 22 to 101 of SEQ ID NO: 5. In another example, the extended SG promoter is encoded by a sequence comprising or consisting of a sequence set forth in SEQ ID NO: 3.

20 In one example, the extended SG promoter comprises a repeat sequence corresponding to nucleotides 66 to 75 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence comprising nucleotides 50 to 75 of SEQ ID NO: 5 and nucleotides 66 to 101 of SEQ ID NO: 5. For example, the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 15.

25 In one example, the polynucleotide of the disclosure comprises a SG promoter from any alphavirus. For example, the RNA of the disclosure (e.g., cRNA or self-replicating RNA) comprises a SG promoter from any alphavirus.

In one example, the self-replicating RNA comprises a SG promoter from any alphavirus.

30 The polynucleotide of the present disclosure comprises two or more nucleotide sequences encoding two or more antigens of interest. In one example, the two or more nucleotide sequences are each operably linked to SG promoters. When two or more SG promoters are present in the RNA of the present disclosure, the promoters can be the same or different. For example, the two or more SG promoters are derived from the same
35 alphavirus. In another example, the two or more SG promoters are derived from different alphaviruses.

When two or more SG promoters are present in the self-replicating RNA of the present disclosure, the promoters can be the same or different. For example, the two or more SG promoters are derived from the same alphavirus. In another example, the two or more SG promoters are derived from different alphaviruses.

5 In another example, where the polynucleotide of the present disclosure comprises two or more nucleotide sequences encoding two or more antigens of interest, the two or more nucleotide sequences may be driven by the same promoter or by two or more promoters, which themselves may comprise the same sequence or a different sequence.

10 Internal Ribosomal Entry Site (IRES)

IRES suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein.

In one example, the IRES is derived from encephalomyocarditis virus (EMCV). For example, the IRES is a wild-type IRES from EMCV.

15 In one example, the IRES is derived from a fibroblast growth factor 1A (FGF1A) IRES.

In addition, synthetic IRES elements have been described, which can be designed, according to methods known in the art to mimic the function of naturally occurring IRES elements (see Chappell, SA et al. Proc. Natl Acad. Sci. USA (2000) 97(4): 1536-41).

20 In one example, the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF-κB-repressing factor
25 (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a (FGF1A), GTX, or a combination thereof.

In one example, the IRES is a wild-type IRES derived from encephalomyocarditis
30 virus (EMCV). For example, the wild-type EMCV IRES comprises a sequence set forth in SEQ ID NO: 4.

5'untranslated region (5'-UTR)

In an example, the polynucleotides described herein comprise a 5'-untranslated
35 region (5'-UTR).

As used herein, the term “5′-untranslated region” or “5′-UTR” refers to a non-coding region of an mRNA located at the 5′ end of the translation initiation sequence (AUG).

Exemplary 5′-UTRs include, for example, 5′-UTR of haptoglobin (HP),
5 fibrinogen beta chain (FGB), haptoglobin-related protein (HPR), albumin (ALB),
complement component 3 (C3), fibrinogen alpha chain (FGA), alpha 6 collagen (Col6A),
alpha-1-antitrypsin (SERPINA1), alpha-1-antichymotrypsin (SERPINA3) a fragment
and/or a variant thereof.

In one example, the 5′UTR is a 5′UTR of a Venezuelan equine encephalitis virus
10 (VEEV) or modified forms thereof. For example, the 5′UTR comprises a sequence set
forth in SEQ ID NO: 13.

In one example, the 5′UTR comprises at least one microRNA binding site, an AU
rich element (ARE), a GC-rich element, a stem loop, and combinations thereof.

15 microRNA binding site

As used herein, the term “microRNA binding site” refers to a sequence within a
polynucleotide (e.g. within a DNA or RNA transcript) that has sufficient complementarity
to all or one region of a miRNA to interact, associate or bind to the microRNA (miRNA).

As used herein, the term “microRNA” or “miRNA” refers to 19-25 nucleotide
20 long non-coding RNAs that bind to the 5′-UTR of polynucleotides and down-regulate
gene expression (e.g. by inhibiting translation). The presence of microRNA binding
site(s) in the 5′UTR of the present disclosure can function to inhibit translation of the 5′-
UTR.

Suitable miRNA binding sites for use in the present disclosure will be apparent to
25 the skilled person and/or described herein.

In one example, the miRNA binding site comprises a binding site for tissue
specific microRNA or those regulating biological processes. For example, miRNA of
the liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-
92, miR-126), myeloid cells (miR-142-3p, miR-142-5p, miR-16, miR-21, miR-223,
30 miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-id, miR-149), kidney
(miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126). For
example, microRNA that regulate biological processes such as angiogenesis (miR-132).
Further exemplifying miRNA and miRNA binding sites are disclosed in US patent
application US14/043,927.

35

AU rich element (ARE)

As used herein, the term “AU rich element (ARE)” or “AU rich elements (AREs)” refers to a region of a nucleotide sequence comprising stretches of Adenine (A) and Uridine (U). Exemplary AREs include, for example, ARE from cytoplasmic myc (c-myc), myoblast determination protein 1 (myoD), c-Jun, Myogenin, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor alpha (TNF- α), or a combination thereof.

In one example, the ARE comprises a human antigen R or “HuR” (also known as Elavl1) specific binding site. HuR is known to bind AREs increasing the stability of the mRNA.

10

GC-rich element

As used herein, the term “GC-rich element” refers to a nucleotide sequence with a high amount of Guanine (G) and/or Cytosine (C) compared to Adenine (A) and Thymine(T)/Uracil(U). The presence of GC-rich elements in a polynucleotide (e.g. mRNA) can stabilise the mRNA.

15

In one example, the GC-rich element comprises a sequence of 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 21, or 22, or 23, or 24, or 25, or 26, or 27, or 28, or 29, or 30 nucleotides in length.

In one example, the GC-rich element comprises between 30% and 40%, or 40% and 50%, or 50% and 60%, or 60% and 70% cytosine. For example, the GC-rich element comprises between 30% and 40% cytosine. For example, the GC-rich element comprises between 40% and 50% cytosine. For example, the GC-rich element comprises between 50% and 60% cytosine. For example, the GC-rich element comprises between 60% and 70% cytosine.

In one example, the GC-rich element comprises 30%, or 40%, or 50%, or 60%, or 70% cytosine. For example, the GC-rich element comprises 30% cytosine. For example, the GC-rich element comprises 40% cytosine. For example, the GC-rich element comprises 50% cytosine. For example, the GC-rich element comprises 60% cytosine. For example, the GC-rich element comprises 60% cytosine. For example, the GC-rich element comprises 70% cytosine.

25

In one example, the GC-rich element is at least 50% cytosine.

In one example, the GC-rich element is at least 60% cytosine.

In one example, the GC-rich element is at least 70% cytosine.

In one example, the GC-rich element comprises a nucleotide sequence CCCC GGCGCC. In another example, the GC-rich element comprises a nucleotide

30

sequence CCCCCGGC. In a further example, the GC-rich element comprises a nucleotide sequence GCGCCCCGCGGCGCCCCGCG.

In one example, the GC-rich element comprises a nucleotide sequence set forth in SEQ ID NO: 9 to 11. In one example, the GC-rich element comprises a nucleotide
5 sequence set forth in SEQ ID NO: 9. In another example, the GC-rich element comprises a nucleotide sequence set forth in SEQ ID NO: 10. In a further example, the GC-rich element comprises a nucleotide sequence set forth in SEQ ID NO: 11 (CCCCGGC).

Stem loop

10 As used herein, the term “stem loop” refers to a nucleotide sequence comprising an intramolecular base pairing of two neighbored entirely or partially reverse complementary sequences to form a stem-loop. A stem-loop can occur in single-stranded DNA or, more commonly, in RNA. The stem loop can also be referred to as a hairpin or hairpin loop which usually consists of a stem and a terminal loop within a consecutive
15 sequence, wherein the stem is formed by two neighbored entirely or partially reverse complementary sequences separated by a short sequence which builds the loop into a stem-loop structure.

The stability of the paired stem loop is determined by the length, the number of mismatched or bulges it contains, and the nucleotide composition of the paired region.

20 In one example, a loop of the stem loop is between 3 and 10 nucleotides in length. For example, the loop of the stem loop is between 3 and 8, or 3 and 7, or 3 and 6, or 4 and 5 nucleotides in length.

In one example, the loop of the stem loop is 4 nucleotides in length.

In one example, the stem loop is a histone stem loop. For example, the histone
25 stem loop comprises or consist of a nucleotide sequence set for in SEQ ID NO: 12.

3'untranslated region (3'-UTR)

In an example, the polynucleotides of the present disclosure comprise a 3'-untranslated region (3'-UTR).

30 As used herein, the term “3'-UTR” refers to a region of an mRNA located at the 3'end of the the translation termination codon (i.e. stop codon).

Exemplary 3'-UTRs include, for example, a 3'-UTR of arachidonate 5-lipoxygenase (ALOX5), alpha I collagen (COL1A1), tyrosine hydroxylase (TH) gene, amino-terminal enhancer of split (AES), human mitochondrial 12S rRNA (mtRNR1), a
35 fragment and/or a variant thereof.

In one example, the 3'UTR is a 3'UTR of a Sindbis virus (SINV) or modified forms thereof. For example, the 3'UTR comprises a sequence set forth in SEQ ID NO: 14 or 22.

5 In one example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of an albumin gene. In one example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of a vertebrate α -globin gene. For example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of a mammalian α -globin gene. For example, the 3'-UTR comprises or consists of a nucleotide sequence derived from a 3'-UTR of a human α -globin gene.

10 In one example, the 3'-UTR of the present disclosure further comprises at least one microRNA binding site, an AU rich element (ARE), a GC-rich element, a triple helix, a stem loop, one or more stop codons or a combination thereof.

Stop codon

15 As used herein, the term "stop codon" refers to a trinucleotide sequence within a mRNA that signals the stop of protein synthesis by a ribosome.

In one example, the polynucleotide of the present disclosure comprises at least one stop codon at the 5' end of a 3'-UTR. For example, the stop codon is selected from UAG, UAA, and UGA.

20 In one example, the polynucleotide comprises two consecutive stop codons comprising a sequence UGAUGA.

In one example, the polynucleotide comprises two consecutive stop codons comprising a sequence UAAUAG.

25 3' tailing sequence

In an example, the polynucleotide of the present disclosure comprises one or more 3' tailing sequences located at the 3' end of the 3'UTR.

As described herein, the term "3' tailing sequence" or "3' tailing sequences" refers to a nucleotide sequence (e.g. polyadenylation signal) which induces the addition
30 of non-encoded nucleotides to the 3' end of a mRNA or a nucleotide sequence (e.g. poly-A sequence) located at the 3' end of a mRNA. A skilled person will appreciate that the 3' tailing sequence and/or products of the 3' tailing sequence in a mRNA functions to stabilise the mRNA and/or prevent the mRNA from degradation.

As used herein, the term "interrupting linker" in reference to a poly-A or poly-C
35 sequence of the present disclosure refers to a single nucleotide or nucleotide sequence which are linked to, and interrupt, a stretch of consecutive adenosine or cytosine

nucleotides in the poly-A or poly-C sequence. For example, the interrupting linker in a poly-A sequence is a single nucleotide or a nucleotide sequence consisting or comprising a nucleotide other than an adenosine nucleotide. For example, the interrupting linker in a poly-C sequence is a single nucleotide or a nucleotide sequence consisting or comprising a nucleotide other than an cytosine nucleotide.

In one example, the one or more 3' tailing sequences are selected from the group consisting of a poly-A sequence, polyadenylation signal, a G-quadruplex, a poly-C sequence, a stem loop and combinations thereof.

10 Poly-A sequence

As used herein, the term "polyA sequence" refers to a nucleotide sequence of Adenine (A) located at the 3' end of a mRNA. In the context of the present disclosure, the polyA sequence may be located within the mRNA or DNA (e.g. a DNA plasmid serving as a template for generating the mRNA by transcription of the vector).

15 Suitable poly-A sequence for use in the present disclosure will be apparent to the skilled person and/or are described herein. In one example, the poly-A sequence comprises consecutive (i.e. one after the other) adenosine nucleotides of any length (e.g. to 10 to 300). In one example, the poly-A sequence comprises consecutive adenosine nucleotides separated by one or more interrupting linkers. In one example, the poly-A
20 sequence comprises consecutive adenosine nucleotides without an interrupting linker.

Polyadenylation signal

As used herein, the term "polyadenylation signal" refers to a nucleotide sequence which induces polyadenylation. Polyadenylation is typically understood to be the
25 addition of a polyA sequence to a RNA (e.g. to a premature mRNA to generate a mature mRNA). The polyadenylation signal may be located within a nucleotide sequence at the 3'-end of the polynucleotide (e.g. mRNA) to be polyadenylated.

Suitable polyadenylation signal for use in the present disclosure will be apparent to the skilled person and/or described herein.

30 In one example, the polyadenylation signal comprises a hexamer consisting of Adenine and Uracil/Thymidine nucleotides. In one example, the hexamer sequence comprises or consists of AAUAAA.

In one example, the 3'tailing sequence comprises a polyadenylation signal but does not comprise a polyA sequence.

35

G-quadruplex

As used herein, the term “G-quadruplex” or “G4” refers to a nucleotide sequence rich in guanine residues which forms a four stranded secondary structure. For example, the G-quadruplex is a cyclic hydrogen bonded array of four guanine nucleotides formed by G-rich sequences in both DNA and RNA.

- 5 In one example, the 3' tailing sequence comprises a polyA sequence and a G-quadruplex. For example, the 3' tailing sequence comprises a polyA sequence linked to a G-quadruplex to produce a polyA-G quartet.

Poly-C sequence

- 10 As used herein, the term “poly-C sequence” refers to a nucleotide sequence of Cytosine (C) located at the 3' end of a mRNA. In the context of the present disclosure, the polyC sequence may be located within the mRNA or DNA (e.g. a DNA plasmid serving as a template for generating the mRNA by transcription of the vector).

- 15 Suitable poly-C sequence for use in the present disclosure will be apparent to the skilled person and/or are described herein.

- In one example, the one or more 3' tailing sequences comprises one or more poly-C sequences each comprising between 10 and 300 consecutive cytosine nucleotides. For example, the one or more poly-C sequences each comprises between 10 and 20, or 20 and 30, or 30 and 40, or 40 and 50, or 50 and 60, or 60 and 70, or 70 and 80, or 80 and 20 90, or 90 and 100, or 100 and 125, or 125 and 150, or 150 and 175, or 175 and 200, or 200 and 225, or 225 and 250, or 250 and 275, or 275 and 300 consecutive cytosine nucleotides. For example, the one or more poly-C sequence each comprises 10, or 20, or 30, or 40, or 50, or 60, or 70, or 80, or 90, or 100, or 125, or 150, or 175, or 200, or 225, or 250, or 275, or 300 consecutive cytosine nucleotides.

- 25 In one example, the one or more poly-C sequences is separated by an interrupting linker. For example, the fourth nucleotide sequence comprising the one or more 3' tailing sequences comprises, in order of 5' to 3': consecutive cytosine nucleotides, an interrupting linker, and further consecutive cytosine nucleotides.

- In one example, the interrupting linker is from 10 to 50, or 50 to 100, or 100 to 30 150 nucleotides in length. For example, the interrupting linker is 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 25, or 30, or 35, or 40, or 45, or 50, or 55, or 60, or 65, or 70, or 75, or 80, or 85, or 90, or 95, or 100, or 110, or 120, or 130, or 140, or 150 nucleotides in length.

- 35 5' cap structure

In one example, the present disclosure provides a mRNA comprising a 5' terminal cap structure.

As used herein, the term "5' cap structure" refers to a structure at the 5' terminal end of a mRNA involved in nuclear export and binds a mRNA Cap Binding Protein (CBP). The 5' cap structure is known to stabilise mRNA through association of CBP with poly(A) binding protein to form a mature mRNA. Accordingly, the presence of a 5' cap structure in the mRNA of the present disclosure can further increase the stability of the mRNA compared to a mRNA without the 5' cap.

Exemplary 5' cap structure includes, for example, anti-reverse cap analogue (ARCA), N7,2'-O-dimethyl-guanosine (mCAP), inosine, N1-methyl-guanosine, 2'fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, 2-azido-guanosine, N6,2'-O-dimethyladenosine, 7-methylguanosine (m7G), Cap1, and Cap2.

Typically, an endogenous mRNA is 5' capped with a guanosine through a (5')-ppp-(5')-triphosphate linkage attached to the 5' terminal nucleotide of the mRNA. The guanosine cap can then be methylated to a 7-methylguanosine (m7G) generating a 7mG(5')ppp(5')N,pN2p (Cap0 structure), where N represents the first and second 5' terminal nucleotide of the mRNA. The cap0 structure can be further 2'-O-methylated to produce 7mG(5')ppp(5')NlmpNp (Cap1), and/or 7mG(5')-ppp(5')NlmpN2mp (Cap2).

In one example, the polynucleotide of the present disclosure comprises an endogenous cap.

As used herein, the term "endogenous cap" refers to a 5' cap synthesised in a cell. For example, endogenous cap is a natural 5' cap or a wild-type 5' cap. For example, the endogenous cap is a Cap0, Cap1, or Cap2 structure.

In one example, the polynucleotide of the present disclosure comprises an analog of an endogenous cap (also referred to as cap analog).

As used herein, the term "analogue thereof" in the context of an endogenous cap or "cap analog" refers to a synthetic 5' cap. The cap analog can be used to produce 5' capped mRNA in *in vitro* transcription reactions. Cap analogs may be chemically (i.e. non-enzymatically) or enzymatically synthesized and/or linked to a nucleotide (e.g. 5' terminal nucleotide of an mRNA). Exemplary cap analogs are commercially available and include, for example, 3''-O-Me-m7G(5')ppp(5')G, G(5')ppp(5')A, G(5')ppp(5')G, m7G(5')ppp(5')A, m7G(5')ppp(5')G (New England BioLabs). In one example, the cap analog is N7,3'-O-dimethyl-guanosine-5'-triphosphate-5'-guanosine (i.e. anti-reverse cap analogue (ARCA)).

In one example, the 5' cap structure is a non-hydrolyzable cap structure. The non-hydrolyzable cap structure can prevent decapping of the mRNA and increase the half-life of the mRNA.

In one example, the non-hydrolyzable cap structure comprises a modified
5 nucleotide selected from a group consisting of a α -thio-guanosine nucleotide, α -methyl-phosphonate, seleno-phosphate, and a combination thereof. In one example, the modified nucleotide is linked to the 5' end of the mRNA through an α -phosphorothiate linkage. Methods of linking the modified nucleotide to the 5' end of the mRNA will be apparent to the skilled person. For example, using a Vaccinia Capping Enzyme (New England
10 Biolabs).

Modifications

In one example, the polynucleotide of the present disclosure comprises one or more modification(s). Typically, modifications are introduced into a polynucleotide
15 (e.g. mRNA) to increase the translation efficiency and/or stability of the polynucleotide. Suitable modifications to the polynucleotide will be apparent to the skilled person and/or described herein.

In one example, the first nucleotide sequence comprising the 5'-UTR and/or the fragment thereof is modified. Modification of the first nucleotide sequences comprising
20 the 5'-UTR and/or the fragment thereof results in a variant of the 5'-UTR and/or the fragment thereof.

In one example, one or more nucleotide sequence(s) of the polynucleotide are codon optimized. Method of codon optimization will be apparent to the skilled person and/or described herein. For example, tools for codon optimization of polynucleotide
25 include, for example, GeneArt GeneOptimizer (ThermoFisher®) or GenSmart® (GeneScript®).

In one example, the polynucleotide is modified to increase the amount of Guanine (G) and/or Cytosine (C) in the polynucleotide. The amount of G/C in the polynucleotide (i.e. G/C content) can influence the stability of the polynucleotide. Accordingly,
30 polynucleotide comprising an increased amount of G/C nucleotides can be functionally more stable than polynucleotides containing a large amount of Adenine (A) and Thymine (T) or Uracil (U) nucleotides. The G/C content is increased by substituting A or T nucleotides with G or C nucleotides.

In one example, the G/C content is increased in the first and/or second nucleotide
35 sequence encoding the first and/or second antigen of interest. For example, the G/C content is increased in the first and/or second nucleotide sequence encoding the S protein

and/or N protein antigen. The modification(s) in the first and/or second and/or one or more nucleotide sequences takes advantage of the ability of substituting codons that contain less favourable combinations of nucleotides (in terms of mRNA stability) with alternative codons encoding the same amino acid, or encoding amino acid(s) of similar chemistry (e.g. conserved amino acid substitution). For example, the G/C content is increased by substituting codons containing A or T nucleotides with codons containing G or C nucleotides that encode for the same amino acid. For example, the G/C content is increased by substituting codons containing A or T nucleotides with codons containing G or C nucleotides that encode for an amino acid of similar chemistry.

10 In one example, the G/C content is increased in one or more nucleotide sequences of the polynucleotide which do not encode the antigen of interest. For example, the G/C content is increased in the 5'-UTR, the fragment and/or the variant thereof. For example, the G/C content is increased in the 3'-UTR, the fragment and/or the variant thereof.

15 In one example, the polynucleotide comprises at least one chemically modified nucleotide.

As used herein, the term "chemical modification" or "chemical modified" in the context of a nucleotide refers to a naturally occurring nucleotides (i.e. A, T, C, G, U) which are modified by replacement, insertion or removal of individual or several atoms or atomic groups compared to the naturally occurring nucleotides. In one example, at least one naturally occurring nucleotide of the polynucleotide is replaced with a chemically modified nucleotide. In one example, at least 10%, or 20%, or 30%, or 40%, or 50%, or 60%, or 70%, or 80%, or 90%, or 100% of naturally occurring nucleotides of the polynucleotide is replaced with a chemically modified nucleotides. Suitable chemical modified nucleotides for use in the present disclosure will be apparent to the skilled person and/or described herein. Exemplary chemically modified nucleotides include, for example, N6,2'-O-dimethyl-adenosine (m6Am), 5-methyluridine (m5U), N4-acetylcytidine (ac4C), 2-thiocytidine (s2C), 2-thiouridine (s2U), 5-methylcytidine (m5C), N6-methyladenosine (m6a), pseudouridine (ψ), and 1-methylpseudouridine (m1 ψ).

30

SARS-CoV-2 Antigens

The polynucleotide of the present disclosure comprises a nucleotide sequence encoding an antigen operably linked to a promoter, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In a further example, the polynucleotide of the present disclosure comprises a nucleotide sequence encoding a second antigen operably linked to a

promoter, wherein the antigen is a nucleocapsid (N) protein from a SARS-CoV-2. In the context of the present disclosure, the antigen is therefore a pathogenic antigen. For example, the antigen of interest is an antigen polypeptide, an immunogenic fragment and/or variant thereof which can induce an immune response in the subject.

5 The SARS-CoV-2 genome encodes for at least four main structural proteins: the spike (S), membrane (M), envelope (E), nucleocapsid (N) proteins and other accessory proteins which aid the replicative processes and facilitate entry into cells. The M protein is the most abundant component of the virus envelope, which directs the assembly of coronaviruses through interactions with all other structural proteins. The E protein is a
10 small membrane protein or viroporin that is thought to promote budding of virus particles by pinching off cellular membrane surfaces. The S protein is a class I fusion protein that mediates attachment of SARS-CoV-2 to the major cell surface receptor human Angiotensin Converting Enzyme 2 (ACE2). Due to its exposed conformation on the surface of the virus, the S protein is highly immunogenic and is the main focus of current
15 vaccine development. The N protein packages the RNA genome to form the nucleocapsid and whilst not necessarily required for envelope formation; it appears to play an important role in the assembly and stability of the complete virion, and in enhancement of VLP yields.

 The S protein comprises three domains: (i) a large ectodomain; (ii) a
20 transmembrane domain (which passes through the viral envelope in a single pass); and (iii) a short intracellular tail. The ectodomain consists of three receptor-binding subunits (3 x S1) and a trimeric stalk made of three membrane-fusion subunits (3 x S2). Thus, the SARS-CoV-2 S protein is a homotrimer. During virus entry, S1 binds to a receptor on the host cell surface for viral attachment, and S2 fuses the host and viral membranes,
25 allowing viral genomes to enter host cells. Receptor binding and membrane fusion are the initial and critical steps in the coronavirus infection cycle. There is significant divergence in the receptors targeted by different CoVs.

 The structure of the SARS-CoV-2 S protein is described, for example, in Cai et al. (Science (2020) 369:1586-1592)), which is herein incorporated by reference in its
30 entirety. Each S1 subunit of a SARS-CoV-2 S protein comprises an N-terminal domain (NTD), receptor binding domain (RBD), two C terminal domains (CTDs). Prior to fusion with the host cell membrane, the S1 subunits of the SARS-CoV-2 S protein protect the S2 subunits. On binding to ACE2, the SARS-CoV-2 S protein refolds in a "jack-knife" manner, forming a long-central coiled coil and ultimately leading to membrane fusion
35 and viral entry to a host cell.

Given the propensity of RNA viruses such as SARS-CoV-2 to mutate, the present inventors provide for polynucleotides encoding S protein sequences that may include mutations found in different strains of SARS-CoV-2 such that the vaccine compositions find particular utility in the treatment of targeted strains of SARS-CoV-2 such as the omicron strain of the SARS-CoV-2.

In one example, a polynucleotide of the disclosure encodes a S protein that includes mutations from the omicron strain of SARS-CoV-2. In one example, where the omicron strain is variant BA.1, the polynucleotide encodes a S protein that comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70.

In another example, where omicron strain is variant BA.2, the polynucleotide encodes a S protein that comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In another example, where the omicron strain is variant BA.4 or BA.5, the polynucleotide encodes a S protein that comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

The mRNA, conventional mRNA, self-replicating RNA, compositions and vaccines of the present disclosure may also be useful for the treatment of other variant SARS-CoV-2. In particular, the mRNA, conventional mRNA, self-replicating RNA, compositions and vaccines of the present disclosure find utility in the treatment of B.1.1.7 SARS-CoV-2 strain (also known as 201/501Y.V1, which was first detected in the UK, now known as the Alpha variant); the B.1.351 SARS-CoV-2 strain (also known as 20H/501.V2, which was first detected in South Africa, now known as the Beta variant), the P1 SARS-CoV-2 strain (also known as 20J/501 Y.V3, which was first detected in Japan and Brazil, now known as the Gamma variant), the B1.427 and B1.429 SARS-CoV-2 strains (first detected in California, now known as the Epsilon variant), and/or the B.1.617.2 SARS-CoV-2 strain (which was first detected in India, now known as the Delta

variant). The mRNA, conventional mRNA, self-replicating RNA, compositions and vaccines of the present disclosure may also be useful for the treatment of the Wuhan (original) strain of SARS-CoV-2.

According to the CDC (SARS-CoV-2 Variant Classifications and Definitions (cdc.gov)), the Alpha variant has been found to comprise the following mutations to the S protein: 69deletion, 70deletion, 144deletion, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, and (K1191N*) with the key mutations being deletion of residues 69/70 and 144Y, as well as N501Y, A570D, D614G and P681H substitutions. The Beta variant has been found to comprise the following mutations: D80A, D215G, 241deletion, 242deletion, 243deletion, K417N, E484K, N501Y, D614G, and A701V with the key mutations being K417N, E484K, N501Y and D614G substitutions. The Gamma variant has been found to comprise the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I with the key mutations being E484K, K417N/T, N501Y and D614G. The Delta variant has been found to comprise the following mutations: T19R, (G142D*), 156deletion, 157deletion, R158G, L452R, T478K, D614G, P681R, and D950N with the key mutations being L452R, E484Q and T478K. The Epsilon variant has been found to comprise the following mutations: S131, W152C, 30 L452R, D614G with the key mutation being L452R. Thus, the present disclosure encompasses self-replicating RNA comprising polynucleotides that encode antigens from a S protein which include or more or all of the above mutations.

In an example, the nucleotide sequence encoding an antigen from the S protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the S protein nucleotide sequence defined in SEQ ID NO: 17.

In an example, the nucleotide sequence encoding an antigen from the S protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the S protein nucleotide sequence defined in SEQ ID NO: 18.

In an example, the nucleotide sequence encoding an antigen from the S protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the S protein nucleotide sequence defined in SEQ ID NO: 19.

In an example, the antigen from the S protein is encoded by a polynucleotide sequence comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the S protein nucleotide sequence defined in SEQ ID NO: 17. In this example, the encoded sequence is self-replicating RNA.

In an example, the antigen from the S protein is encoded by a polynucleotide sequence comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the S protein nucleotide sequence defined in SEQ ID NO: 18. In this example, the encoded sequence is self-replicating RNA.

In an example, the antigen from the S protein is encoded by a polynucleotide sequence comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the S protein nucleotide sequence defined in SEQ ID NO: 19. In this example, the encoded sequence is self-replicating RNA.

In an example, the S protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 20.

In an example, the S protein is encoded by a polynucleotide sequence set forth in SEQ ID NO: 20.

In one example, the polynucleotide encodes antigens from each of the S protein, and N protein of a SARS-CoV-2. In one example, the polynucleotide encodes antigens from each of the S protein and N protein of the omicron strain of a SARS-CoV-2. In an example, the omicron variant may be BA.1 or BA.2.

In an example, polynucleotide encoding a second antigen may be from the delta, beta, alpha, gamma or strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. Thus, in an example, each of the S and N antigens are from the omicron strain of SARS-Cov2.

In one example, the S protein from the omicron strain of a SARS-CoV-2 is from:

- (a) the omicron variant BA.1 and the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or
- (b) the omicron variant BA.2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or
- (c) the omicron variant BA.4 or BA.5 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or
- (d) an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ 24-26, A27S, Δ 144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K; or
- (e) an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ 24-26, A27S, V83A, Δ 144, G142D, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K; or
- (f) an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of 16insMPLF, T19I, R21T, Δ 24-26, A27S, S50L, Δ 69-70, V127F, Δ 144, G142D, F157S, R158G, Δ 211, L212I, V213G, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445P, G446S, N450D, L452W, N460K, S477N, T478K, N481K, Δ 483, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, Q954H, N969K and P1143L; or
- (g) an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ 24-26, A27S, Δ 144,

G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, L455S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K; or

- (h) an omicron variant of a SARS-CoV-2 and the S protein comprises one or more
5 or all of the mutations R346T, F456L and T572I.

In an example, the nucleotide sequence encoding an antigen from the N protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the N
10 protein nucleotide sequence defined in SEQ ID NO: 18.

In an example, the nucleotide sequence encoding an antigen from the N protein comprises a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the N
15 protein nucleotide sequence defined in SEQ ID NO: 19.

In an example, the antigen from the N protein is encoded by a polynucleotide sequence comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or identical to the N protein nucleotide sequence
20 defined in SEQ ID NO: 18. In this example, the encoded sequence is self-replicating RNA.

In an example, the antigen from the N protein is encoded by a polynucleotide sequence comprising at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%,
25 at least about 98%, at least about 99% or identical to the N protein nucleotide sequence defined in SEQ ID NO: 19. In this example, the encoded sequence is self-replicating RNA.

In an example, the N protein is encoded by a polynucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about
30 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 21.

In an example, the N protein is encoded by a polynucleotide sequence set forth in SEQ ID NO: 21.

35 In one example, the polynucleotide encoding a second antigen from the N protein is from the omicron strain of a SARS-CoV-2.

Methods of Production

Suitable methods for the production of a polynucleotide, a cRNA and/or a self-replicating RNA of the present disclosure will be apparent to the skilled person and/or
5 described herein.

In one example, the polynucleotide is DNA. For example, the polynucleotide is a plasmid DNA.

In one example, the cRNA is produced using a plasmid DNA. In one example, the self-replicating RNA is produced using a plasmid DNA. The skilled person will
10 understand that plasmid DNA is relatively stable. Briefly, competent bacterial cells (e.g., *Escherichia coli*) cells are transformed with a DNA plasmid encoding a self-replicating RNA of the present disclosure. Individual bacterial colonies are isolated and the resultant plasmid DNA amplified in *E. coli* cultures.

In one example, the plasmid DNA is isolated following fermentation. For
15 example, the plasmid DNA is isolated using a commercially available kit (e.g., Maxiprep DNA kit), or other routine methods known to the skilled person. Following isolation, plasmid DNA is linearized by restriction digest (i.e., using a restricting enzyme). Restriction enzymes are removed using methods known in the art, including for example phenol/chloroform extraction and ethanol precipitation.

In one example, mRNA is made by *in vitro* transcription from a linearized DNA
20 template using an RNA polymerase (e.g., T7 RNA polymerase). Following *in vitro* transcription, the DNA template is removed by DNase digestion. The skilled person will understand that synthetic mRNA capping is performed to correct mRNA processing and contribute to stabilization of the mRNA. In one example, the mRNA is enzymatically
25 5'-capped. For example, the 5' cap is a cap0 structure or a cap1 structure. In one example, the 5' cap is a cap0 structure, for example, the 5'-cap (i.e., cap0) consists of an inverted 7-methylguanosine connected to the rest of the mRNA via a 5'-5' triphosphate bridge. In one example, the 5' cap is a cap1 structure, for example, the 5'-cap (i.e., cap1) consists of the cap0 with an additional methylation of the 2'O position of the initiating
30 nucleotide.

In one example, the mRNA is purified. Various methods for purifying mRNA will be apparent to the skilled person. For example, the mRNA is purified using lithium chloride (LiCl) precipitation. In another example, the mRNA is purified using tangential flow filtration (TFF). Following purification, the mRNA is resuspended in e.g., nuclease-
35 free water.

Compositions

The present disclosure provides an immunogenic composition comprising a polynucleotide of the present disclosure.

The present disclosure also provides an immunogenic composition comprising a
5 cRNA of the present disclosure.

The present disclosure further provides an immunogenic composition comprising a self-replicating RNA of the present disclosure.

The present disclosure also provides a pharmaceutical composition comprising an immunogenic composition of the present disclosure and a pharmaceutically acceptable
10 carrier.

It will be apparent to the skilled person and/or described herein, that the polynucleotide, cRNA and/or self-replicating RNA of the present disclosure may be present as naked RNA or in combination with lipids, polymers or other delivery system that facilitates entry into the cells.

15

Delivery systems

In one example, the pharmaceutical composition of the present disclosure further comprises a LNP, a polymeric microparticle and an oil-in-water emulsion. For example, the polynucleotide, the cRNA and/or the self-replicating RNA is encapsulated in, bound
20 to or adsorbed on a LNP, a polymeric microparticle, or an oil-in-water emulsion.

Lipid Nanoparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a LNP.

25 It will be apparent that the term “lipid nanoparticle” or “LNP” refers to any lipid composition, including, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar) micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles, wherein solid lipid nanoparticles lack lipid bilayers.

30 Lipid nanoparticles suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein. The lipids can have an anionic, cationic or zwitterionic hydrophilic head group.

In one example, the lipid nanoparticle comprises a PEG-lipid, a sterol structural lipid and/or a neutral lipid. In one example, the lipid nanoparticle further comprises a
35 cationic lipid. In one example, the lipid nanoparticle does not comprise a cationic lipid.

In one example, the LNP comprises a PEG-lipid. For example, the PEG-lipid is selected from the group consisting of PEG-c-DMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid and combinations thereof.

In one example, the LNP comprises a structural lipid. For example, the structural
5 lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, campesterol, stigmasterol, brassicasterol, ergosterol, tomatidine, tomatine, ursolic acid and alpha-tocopherol and combinations thereof.

In one example, the LNP comprises a neutral lipid. Exemplary phospholipids (anionic or zwitterionic) for use in the present disclosure include, for example,
10 phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, and phosphatidylglycerols. For example, the neutral lipid is selected from the group consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-
15 3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-3-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-
20 dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinenoyl-sn-glycero-3-phosphoethanolamine, 1,2-
25 diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin and combinations thereof.

In one example, the LNP comprises a cationic lipid. Exemplary cationic lipids include, but are not limited to, dioleoyl trimethylammonium propane (DOTAP), 1,2-
30 distearyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-dilinenyloxy-N,N-dimethyl-3-aminopropane (DLenDMA), 2,5-bis((9z,12z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). In one example, the phospholipid is 2,5-bis((9z,12z)-octadeca-9,12,dien-1-
35 yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). Exemplary zwitterionic lipids include, but are not limited to, acyl zwitterionic lipids and ether zwitterionic lipids, such

as dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and dodecylphosphocholine. The lipids can be saturated or unsaturated.

Polymeric microparticles

5 In one example, the pharmaceutical composition of the present disclosure further comprises a polymeric microparticle.

The skilled person will be aware that various polymers can form microparticles to encapsulate or adsorb the polynucleotide, the cRNA and/or the self-replicating RNA of the present disclosure. It will be apparent that use of a substantially non-toxic polymer
10 means that particles are safe, and the use of a biodegradable polymer means that the particles can be metabolised after delivery to avoid long-term persistence. Useful polymers are also sterilisable, to assist in the preparation of pharmaceutical grade formulations.

Exemplary non-toxic and biodegradable polymers include, but are not limited to,
15 poly(α - hydroxy acids), polyhydroxy butyric acids, polylactones (including polycaprolactones), polydioxanones, polyvalerolactone, polyorthoesters, polyanhydrides, polycyanoacrylates, tyrosine-derived polycarbonates, polyvinyl-pyrrolidinones or polyester-amides, and combinations thereof.

20 *Oil-in-water cationic emulsions*

In one example, the pharmaceutical composition of the present disclosure further comprises an oil-in-water cationic emulsion.

Suitable oils for use in an oil-in-water emulsion will be apparent to the skilled person and/or are described herein. For example, the emulsion comprises one or more
25 oils derived, for example, from an animal (e.g., fish) or a vegetable source (e.g., nuts, seeds, grains). The skilled person will recognise that biocompatible and biodegradable oils are preferentially used. Exemplary animal oils (i.e., fish oils) include cod liver oil, shark liver oils, and whale oil. Exemplary vegetable oils include peanut oil, coconut oil, olive oil, soybean oil, jojoba oil, safflower oil, cottonseed oil, sunflower seed oil, sesame
30 seed oil, corn oil.

In addition to the oil, the oil-in-water emulsion also comprises a cationic lipid to facilitate formation and stabilisation of the emulsion. Suitable cationic lipids will be apparent to the skilled person and/or are described herein. Exemplary cationic lipids include, but are not limited to, limited to: 1, 2-dioleoyloxy-3-(trimethylammonio)propane
35 (DOTAP), 3'-[N-(N',N'-Dimethylaminoethane)-carbamoyl] Cholesterol (DC Cholesterol), dimethyldioctadecyl-ammonium (DDA), 1,2-Dimyristoyl-3-Trimethyl-

AmmoniumPropane (DMTAP), dipalmitoyl[C16:0]trimethyl ammonium propane (DPTAP) and distearoyltrimethylammonium propane (DSTAP).

In some examples, the oil-in-water emulsion also comprises a non-ionic surfactant and/or a zwitterionic surfactant. The skilled person will be aware of surfactants suitable
5 for use in the present disclosure. Exemplary surfactants include, but are not limited to: the polyoxyethylene sorbitan esters surfactants (e.g., polysorbate 20 and polysorbate 80) and copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO).

10 *Pharmaceutically acceptable carrier*

Suitably, in compositions or methods for administration of the cRNA and/or the self-replicating RNA of the disclosure to a subject, the cRNA and/or the self-replicating RNA is combined with a pharmaceutically acceptable carrier as is understood in the art. Accordingly, one example of the present disclosure provides a composition (e.g., a
15 pharmaceutical composition) comprising the self-replicating RNA of the disclosure (and any delivery system) combined with a pharmaceutically acceptable carrier. Another example of the present disclosure provides a composition (e.g., a pharmaceutical composition) comprising the cRNA of the disclosure (and any delivery system) combined with a pharmaceutically acceptable carrier.

20 In general terms, by “carrier” is meant a solid or liquid filler, binder, diluent, encapsulating substance, emulsifier, wetting agent, solvent, suspending agent, coating or lubricant that may be safely administered to any subject, e.g., a human. Depending upon the particular route of administration, a variety of acceptable carriers, known in the art may be used, as for example described in Remington's Pharmaceutical Sciences (Mack
25 Publishing Co. N.J. USA, 1991).

The cRNA and/or the self-replicating RNA of the present disclosure is useful for parenteral, topical, oral, or local administration, intramuscular administration, aerosol administration, or transdermal administration, for prophylactic or for therapeutic treatment. In one example, the self-replicating RNA is administered parenterally, such
30 as intramuscularly, subcutaneously or intravenously. For example, the self-replicating RNA is administered intramuscularly. In another example, the cRNA is administered parenterally, such as intramuscularly, subcutaneously or intravenously. For example, the cRNA is administered intramuscularly.

Formulation of a cRNA and/or a self-replicating RNA to be administered will
35 vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. An appropriate pharmaceutical composition comprising a cRNA

and/or a self-replicating RNA to be administered can be prepared in a physiologically acceptable carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's
5 dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), dextrose solution and glycine. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally,
10 Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The cRNA and/or self-replicating RNA
15 can be stored in the liquid stage or can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques.

The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures known to the skilled artisan, and will
20 depend on the ultimate pharmaceutical formulation desired.

Upon formulation, compositions of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically/prophylactically effective. The dosage ranges for the administration of the cRNA and/or self-replicating RNA of the disclosure are those large enough to produce
25 the desired effect. For example, the composition comprises an effective amount of the self-replicating RNA. In one example, the composition comprises a therapeutically effective amount of the self-replicating RNA. In another example, the composition comprises a prophylactically effective amount of the self-replicating RNA. In one example, the composition comprises an effective amount of the cRNA. In one example,
30 the composition comprises a therapeutically effective amount of the cRNA. In another example, the composition comprises a prophylactically effective amount of the cRNA.

The dosage should not be so large as to cause adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual
35 physician in the event of any complication.

Dosage can vary from about 0.1 mg/kg to about 300 mg/kg, e.g., from about 0.2 mg/kg to about 200 mg/kg, such as, from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

In some examples, the cRNA and/or the self-replicating RNA is administered at an initial (or loading) dose which is higher than subsequent (maintenance doses). For example, the cRNA and/or the self-replicating RNA is administered at an initial dose of between about 10mg/kg to about 30mg/kg. The cRNA and/or the self-replicating RNA is then administered at a maintenance dose of between about 0.0001mg/kg to about 10mg/kg. The maintenance doses may be administered every 7-35 days, such as, every 7 or 14 or 28 days.

In some examples, a dose escalation regime is used, in which the cRNA and/or the self-replicating RNA is initially administered at a lower dose than used in subsequent doses. This dosage regime is useful in the case of subject's initially suffering adverse events

In the case of a subject that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

A subject may be retreated with the cRNA and/or the self-replicating RNA of the present disclosure. A subject may be retreated with the cRNA and/or the self-replicating RNA, by being given more than one exposure or set of doses, such as at least about two exposures of the binding protein, for example, from about 2 to 60 exposures, and more particularly about 2 to 40 exposures, most particularly, about 2 to 20 exposures.

In one example, any retreatment may be given when signs or symptoms of disease return.

In another example, any retreatment may be given at defined intervals. For example, subsequent exposures may be administered at various intervals, such as, for example, about 24-28 weeks or 48-56 weeks or longer. For example, such exposures are administered at intervals each of about 24-26 weeks or about 38-42 weeks, or about 50-54 weeks.

In the case of a subject that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

In another example, for subjects experiencing an adverse reaction, the initial (or loading) dose may be split over numerous days in one week or over numerous consecutive days.

Administration of the cRNA and/or the self-replicating RNA according to the methods of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the cRNA and/or the self-replicating RNA may be essentially continuous over a preselected period of time or may be in a series of spaced doses, e.g., either during or after development of a condition.

Screening Assays

Suitable methods for selecting a cRNA and/or a self-replicating RNA of the present disclosure are available to those skilled in the art. Assays may be conducted to assess the efficiency and efficacy of the RNA including, for example, serology and immune responses.

15 *Antigen expression*

In one example, the self-replicating RNA is assessed for expression of the polynucleotide/s of interest (i.e., a polynucleotide encoding a S protein antigen from the omicron strain of SARS-CoV-2). In another example, the cRNA is assessed for expression of the polynucleotide/s of interest (i.e., a polynucleotide encoding a S protein antigen from the omicron strain of SARS-CoV-2).

For example, antigen expression is detected using antibodies against the polynucleotide/s of interest. In one example, the number of cells positive for antigen expression is measured by e.g., fluorescence-activated cell sorting (FACS). In another example the mean fluorescence intensity (MFI) is determined using e.g., FACS. In a further example, the specific potency value or the probability of successful transfection per unit mass of RNA is calculated.

Microneutralization Assay

In one example, the self-replicating RNA (naked and/or formulated) is assessed for antibody responses. In one example, the cRNA (naked and/or formulated) is assessed for antibody responses. For example, the cRNA and/or the self-replicating RNA is assessed using a microneutralisation assay. Methods of performing a microneutralization assay will be apparent to the skilled person. In one example, the microneutralization assay is a short form assay. For one example, a virus fluorescent focus-based microneutralization assay is performed. In another example, the microneutralization assay is a long form assay.

Antigen Specific T cell Responses

In one example, the self-replicating RNA is assessed for its ability to induce antigen specific T cell responses. In one example, the cRNA is assessed for its ability to induce antigen specific T cell responses. Methods of assessing induction of antigen specific T cell responses will be apparent to the skilled person and/or are described herein.

For example, antigen-specific T cell detection is performed on splenic cultures. Briefly, splenocyte cultures are established in T cell medium and cell cultures are either stimulated with antigenic peptides or unstimulated. In one example, antigen-specific T cell responses are determined using flow cytometry.

Neutralising assays

The self-replicating RNA of the disclosure may be screened *in vitro* for their ability to bind to a SARS-CoV-2 S protein and neutralises binding of the S protein to ACE2. Suitable assays will be apparent to the skilled person and include, for example, a Vero microneutralisation assay, a sVNT assay, or a pseudovirus neutralisation assay (using e.g., HEK-293T cells or HeLa-ACE2 cells).

In one example, the neutralization assay is a Vero microneutralization assay. Briefly, SARS-Cov-2 wild-type virus is passaged in Vero cells (i.e., the Vero lineage isolated from kidney epithelial cells extracted from an African green monkey). Serial two-fold dilutions of a test protein are incubated with 100 TCID₅₀ (i.e., median tissue culture infectious dose) of SARS-CoV-2 for 1 hour and residual virus infectivity is assessed in Vero cells; viral cytopathic effect is read, for example, on day 5. The neutralising antibody titre is calculated using the Reed/Muench method as previously described (Houser et al., 2016; Subbarao et al 2004).

In one example, the neutralization assay is a surrogate neutralization test (sVNT). Briefly, the wells of a plate are coated with hACE2 protein in carbonate-bicarbonate coating buffer (e.g., pH 9.6). HRP-conjugated SARS-CoV-2 and HRP-conjugated SARS-CoV-2 pre-incubated with test proteins is added to the hACE2 at different concentrations and incubated, for example, for 1h at room temperature. Unbound HRP conjugated antigens are removed by washing. Colorimetric signal is developed on the enzymatic reaction of HRP with chromogenic substrate, e.g., 3,3',5,5'-tetramethylbenzidine (TMB). In one example, the absorbance reading at 450 nm and 570 nm is acquired.

In one example, the neutralisation is a pseudovirus neutralisation assay. Briefly, HIV reporter virus pseudotyped with SARS-CoV-2 S protein is produced by co-transfection of SARS-2-CoV-2 spike plasmids together with a viral backbone plasmid (e.g., pDR-NL Δenv FLUC) into e.g., HEK-293T cells. Pseudovirus is harvested post
5 transfection and clarified by filtration. Virus stock titres, reported as Relative Luciferase Units infectious dose (RLU), are calculated by limiting dilution infections in Hela-hACE2 cells measuring luciferase activity as a read-out for viral infection.

Methods of Treatment or Prevention

10 The present disclosure provides, for example, methods of treating or preventing or delaying progression of COVID-19, caused by SARS-CoV-2. The present disclosure also provides, for example, methods of treating or preventing or delaying progression of a SARS-CoV-2 infection. In some examples of the present disclosure the subject has a SARS-CoV-2 infection but does not have clinically diagnosed COVID-19. Thus in an
15 example, the subject may exhibit one or more symptoms of a SARS-CoV-2 infection but COVID-19 is not yet clinically detectable.

Coronavirus Disease 2019 (COVID-19)

The present disclosure provides methods of treating, preventing or delaying the
20 progression of COVID-19 or a SARS-CoV-2 infection in a subject.

COVID-19 is an infectious disease caused by SARS-CoV-2. Common symptoms include fever, cough, fatigue, shortness of breath, and loss of smell and taste. While the majority of cases result in mild symptoms, some progress to ARDS or may result in one or more complications including pneumonia or sepsis. Thus the present disclosure
25 contemplates the treating, preventing or delaying the progression of mild COVID-19 or moderate to severe COVID-19 and includes treating, preventing or delaying the progression of ARDS, pneumonia or sepsis in a subject.

Mild COVID-19 may be considered to include symptoms including fever, cough, fatigue, shortness of breath, and loss of smell and taste. In particular, mild COVID-19 is
30 defined as a SARS-CoV-2 positive RT-PCR or molecular test result, and one of the following symptoms:

- fever;
- sore throat;
- headache;
- 35 -muscle pain (myalgia);
- gastrointestinal symptoms;

- cough;
 - chest congestion;
 - runny nose;
 - wheezing;
 - 5 -skin rash;
 - eye irritation or discharge;
 - chills;
 - new or changing olfactory or taste disorders;
 - red or bruised looking feet or toes;
 - 10 -shaking chills or rigors;
 - malaise (loss of appetite, generally unwell, fatigue, physical weakness).
- A case is considered mild when it meets the above case definition but not the moderate to severe/critical definition.
- 15 Moderate COVID-19 may be defined as: a SARS-CoV-2 positive RT-PCR or molecular test result, AND any one of the following new or worsening signs or symptoms:
- respiratory rate ≥ 20 breaths/minute;
 - abnormal saturation of oxygen (SpO_2) but still $\geq 93\%$ on room air at sea level;
 - 20 -clinical or radiologic evidence of pneumonia;
 - Radiologic evidence of DVT;
 - shortness of breath or difficulty breathing;
- or any 2 of the following new or worsening signs or symptoms:
- fever;
 - 25 -heart rate ≥ 90 beats/minute;
 - shaking chills or rigors;
 - new or changing olfactory or taste disorders;
 - sore throat;
 - malaise;
 - 30 -headache;
 - cough;
 - muscle pain (myalgia);
 - gastrointestinal symptoms;
 - red or bruised looking feet or toes.
- 35 Severe/critical COVID-19 is defined as: a SARS-CoV-2 positive RT-PCR or molecular test result; and any one or more of the following:

- clinical signs at rest indicative of severe systemic illness (respiratory rate ≥ 30 breaths/minute, heart rate ≥ 125 beats/minute, $\text{SpO}_2 \leq 93\%$ on room air at sea level, or $\text{PaO}_2/\text{FiO}_2 \leq 300$ mmHg);
- respiratory failure (defined as needing high-flow oxygen, non-invasive ventilation, mechanical ventilation, or ECMO [extracorporeal membrane oxygenation])
- evidence of shock (defined as systolic blood pressure ≤ 90 mmHg, diastolic blood pressure ≤ 60 mmHg, or requiring vasopressors);
- significant acute renal, hepatic, or neurologic dysfunction;
- admission to the ICU;
- death.

The time from exposure to onset of symptoms is typically around five days, but may range from two to fourteen days. Complications of a SARS-CoV-2 infection may include viral pneumonia, secondary bacterial pneumonia, sinus infections, and worsening of previous health problems such as asthma or heart failure. Viral pneumonia may also lead to acute respiratory distress syndrome (ARDS).

Thus, in some examples of the present disclosure, the methods or uses of the present disclosure can be used to treat, prevent or delay progress of ARDS in a subject suffering from COVID-19. In one example, the subject is at risk of having COVID-19 caused by a SARS-CoV-2. In one example, the methods of the present disclosure can be used to treat ARDS in a subject suffering from a SARS-CoV-2 infection. In one example, the methods of the present disclosure can be used to prevent ARDS in a subject suffering from SARS-CoV-2 infection. In one example, the methods of the present disclosure can be used to delay progression of ARDS in a subject suffering from SARS-CoV-2 infection.

A subject in need thereof may be an individual who is displaying a symptom of a SARS-CoV-2 infection or who has been diagnosed with a SARS-CoV-2 infection and/or has COVID-19. Further, a subject in need thereof may be one who has been clinically or biochemically determined to be infected with a SARS-CoV-2 infection or COVID-19. In one embodiment, the subject may be asymptomatic.

A reduction in SARS-CoV-2 infection may be determined using any method known in the art or described herein, including measuring viral load in a sample from the subject after treatment and comparing it to viral load in a sample from the same subject before treatment. Preferably, the sample is taken from the respiratory tract, preferably the upper respiratory tract, for example the nose or pharynx (i.e. throat). Alternatively,

responsiveness to a treatment may result in lessening of the severity of one or more of the symptoms described herein.

Acute Respiratory Distress Syndrome (ARDS)

5 The present disclosure provides methods of treating, preventing or delaying the progression of ARDS in a subject.

ARDS is a life-threatening condition characterized by bilateral pulmonary infiltrates, severe hypoxemia, and disruption of the alveolar-capillary membrane barrier (i.e., pulmonary vascular leak), leading to non-cardiogenic pulmonary edema. There is
10 currently no effective pharmacological therapy.

Infectious etiologies, including influenza, are leading causes of ARDS. Accordingly, in one example of the present disclosure, the ARDS is associated with an SARS-CoV-2 infection. For example, the ARDS is associated with SARS-CoV-2 or COVID-19.

15 ARDS is classified according to the Berlin Definition, which includes:

- (1) presentation within 1 week of clinical insult or onset of respiratory symptoms;
- (2) acute hypoxemic respiratory failure, as determined by a $\text{PaO}_2/\text{FiO}_2$ ratio of 300 mmHg or less on at least 5 cm of continuous positive airway pressure (CPAP) or positive end expiratory pressure (PEEP), where PaO_2 is the partial pressure of
20 oxygen in arterial blood and the FiO_2 is the fraction of inspired oxygen;
- (3) bilateral opacities on lung radiographs not fully explained by effusions, consolidation, or atelectasis; and
- (4) edema/respiratory failure not fully explained by cardiac failure or fluid overload.

In one example, the subject has or suffers from ARDS (i.e., the subject satisfies
25 the Berlin definition of ARDS). For example, the subject is in need of treatment (i.e., in need thereof).

In one example, the subject has or suffers from a symptom associated with ARDS. Symptoms associated with ARDS and methods of identifying subjects at risk of developing ARDS will be apparent to the skilled person and/or are described herein. For
30 example, the subject has one or more or all of the following symptoms:

- a) a respiratory frequency of greater than 30 breaths per minute;
- b) an oxygen saturation (SpO_2) of 93% or less on room air;
- c) a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) of less than 300 mmHg;
- 35 d) a $\text{SpO}_2/\text{FiO}_2$ ratio of less than 218; and
- e) radiographic lung infiltrates in an amount of greater than 50%.

Currently, ARDS is classified as mild, moderate or severe with an associated increased mortality. The severity of ARDS can be categorized according to the Berlin definition as follows:

- (i) Mild ARDS: $\text{PaO}_2/\text{FiO}_2$ of 200-300 mmHg on at least 5 cm CPAP or PEEP;
- 5 (ii) Moderate ARDS: $\text{PaO}_2/\text{FiO}_2$ of 100-200 mmHg on at least 5 cm PEEP; and
- (iii) Severe ARDS: $\text{PaO}_2/\text{FiO}_2$ of less than or equal to 100 mmHg on at least 5 cm PEEP.

In one example, the ARDS is mild ARDS. In another example, the ARDS is moderate ARDS. In a further example, the ARDS is severe ARDS.

- 10 The methods of the present disclosure can, in addition to treatment of existing ARDS, be used to prevent or delay the onset of ARDS. Thus, in one example, the subject does not have ARDS.

In one example, the subject is at risk of developing one or more symptom(s) associated with ARDS.

15

Kits

Another example of the disclosure provides kits containing a self-replicating RNA of the present disclosure useful for the treatment or prevention of a disease or disorder as described above.

- 20 Another example of the disclosure provides kits containing a cRNA of the present disclosure useful for the treatment or prevention of a disease or disorder as described above.

- 25 In one example, the kit comprises (a) a container comprising a self-replicating RNA optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent; and (b) a package insert with instructions for treating or preventing a disease or disorder (e.g., a SARS-CoV-2 infection, COVID-19 or ARDS) in a subject.

- 30 In one example, the kit comprises (a) a container comprising a cRNA optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent; and (b) a package insert with instructions for treating or preventing a disease or disorder (e.g., influenza, COVID-19, ARDS or sepsis or pneumonia associated with COVID-19) in a subject.

- 35 In accordance with this example of the disclosure, the package insert is on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for a disease or disorder of the disclosure and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic

injection needle). At least one active agent in the composition is the self-replicating RNA and/or the cRNA. The label or package insert indicates that the composition is used for treating a subject eligible for treatment, e.g., one having or predisposed to developing a SARS-CoV-2 infection, COVID-19, ARDS, or sepsis or pneumonia associated with COVID-19, with specific guidance regarding dosing amounts and intervals of treatment and any other medicament being provided. The kit may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and/or dextrose solution. The kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: Methods

15 *Generation of the self-replicating RNA*

DNA templates encoding self-replicating RNAs described herein were produced in competent *Escherichia coli* cells transformed with a DNA plasmid. Individual bacterial colonies were isolated and the resultant plasmid DNA amplified in *E. coli* cultures. Following fermentation, the plasmid DNA was isolated using Maxiprep DNA kit and linearized by restriction digest. Restriction enzymes were removed using phenol/chloroform extraction and ethanol precipitation.

mRNA was made by *in vitro* transcription from the linearized DNA template using a T7 RNA polymerase. Subsequently, the DNA template was removed by DNase digestion. Enzymatic capping was performed with Cap0 to provide functional mRNA. The resultant mRNA was then purified and resuspended in nuclease-free water. The self-replicating RNAs utilised in the experiments are outlined in **Figure 1** and include:

1. Co96 which includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an Spike (S) protein antigen from the omicron strain of SARS-CoV-2.
2. Co97 which includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an S protein antigen and a N protein antigen from the omicron strain of SARS-CoV-2, driven by a synthetic genomic promoter (SGP) v2.
3. Co99 which includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an N protein antigen and a S protein antigen from the omicron strain of SARS-CoV-2, driven by a synthetic genomic promoter (SGP) v2.

4. Co16 which includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an S protein antigen from the original (Wuhan) strain of SARS-CoV-2.
5. Co18 which includes an alphavirus self-replicating RNA comprising a polynucleotide encoding an S protein antigen and a N protein antigen from the original (Wuhan) strain of SARS-CoV-2, driven by a synthetic genomic promoter (SGP) v2.

In vitro characterisation of the compositions

The self-replicating RNAs were assessed for expression of the genes of interest that are expressed in the form of an antigen. Two-fold serial dilutions of unformulated (naked) or LNP-formulated self-amplifying mRNA constructs were either electroporated or transfected into a Baby Hamster Kidney (BHK) cell line. After about 17-19 hrs, cells were harvested and stained for antigen expression using suitable antibodies. The number of cells positive for antigen expression and the mean fluorescence intensities (MFIs) was measured by FACS. Data was analysed to calculate the specific potency values when formulated in an LNP (the probability of successful transfection per unit of mass of RNA) as outlined in **Figure 3** and the MFI generated.

In vitro activity and potency of unformulated RNA and LNPs was determined by FACS based on antigen co-expression and expressed in read-outs such as FACS potency, encapsulation efficiency, SAM recovery, size, PDI, Zeta potential, conductivity, concentration and endotoxin levels. Alternatively, expression of the various antigens in constructs Co96, Co97, Co99, Co16 and Co18 was determined by detection with antibodies for N and S proteins (**Figure 2**).

Antibody responses

To assess antibody responses, serum was collected at the end of study and tested by microneutralization assays.

For all serological assays, sera was treated in the same way, for example with *Vibrio cholerae* neuraminidase, also known as receptor-destroying enzyme (RDE) (Denka Seiken Co. Ltd., Tokyo, Japan) and diluted to a starting dilution of 1:10 with PBS. Sheep serum to H5N1 virus (FDA/CBER Kensington lot nu. H5-Ag-1115) can be used as positive control sera.

Microneutralization assays

Microneutralization assays, short and long form was performed in a qualified mammalian cell line (proprietary 33016-PF Madin-Darby Canine Kidney (MDCK)).

Microneutralization assay short form (MN Assay SF)

Virus fluorescent focus-based microneutralization (FFA MN) assay was performed using an in house developed protocol. RDE treated test mouse samples and positive control sera was heat inactivated, diluted to a starting dilution of 1:40 with PBS, and fourfold serial diluted using the U-Bottom 96 well plate (BD Falcon) in neutralization medium (comprised of minimum essential medium D-MEM (GIBCO), supplemented with 1% BSA (Rockland, BSA-30), 100 U/mL penicillin and 100 ug/mL streptomycin (GIBCO)). A/turkey/Turkey/1/2005 (H5N1) virus was diluted to ~ 1,000 – 1,500 fluorescent focus-forming units (FFU)/well (20,000 – 30,000 FFU/mL) in neutralization medium and added in a 1:1 ratio to diluted serum.

After incubation for 2 h at 37°C, 5% CO₂, plates (Half Area 96 well plate, Corning) containing MDCK 33016-PF cells were inoculated with this mixture and incubated overnight for 16 – 18 h at 37°C with 5% CO₂. MDCK 33016-PF cells are seeded as 3.0E4/well (3.0E6/plate) at 6-8h earlier in the cell growth medium (comprised of D-MEM, supplemented with 10% HyClone fetal bovine serum - FBS (Gibco), 100 U/mL penicillin and 100 ug/mL streptomycin). Following the overnight incubation and prior to immunostaining, cells were fixed with cold mixture of acetone and methanol.

The virus was visualized using separate 1 h incubations at room temperature of monoclonal antibodies specific to the virus proteins of interest and Alexa Fluor 488 Goat Anti-Mouse IgG (H+L) Ab (Invitrogen cat. no. A11001) diluted in PBS buffer containing 0.05% tween-20 (Sigma) and 2% BSA (Fraction V, Calbiochem, 2960, 1194C175). Viral protein was quantified by a CTL Immunospot analyzer (Cellular Technology Limited, Shaker Heights, Cleveland, OH), using a fluorescein isothiocyanate (FITC) fluorescence filter set with excitation and emission wavelengths of 482 and 536 nm. Fluorescent foci were enumerated by use of software Immunospot 7.0.12.1 professional analyzer DC, using a custom analysis module.

Microneutralization assay long form (MN Assay LF)

MN assay LF was performed using an in house developed protocol. RDE treated test mouse samples and positive control sera were heat inactivated, diluted to a starting dilution of 1:40 with PBS, and twofold serial diluted using the U-Bottom 96 well plate (BD Falcon) in neutralization medium (comprised of the 30% spent growth media (Irvine Scientific) and 70% infective media (protein free media – 33016 MDCK PFM; GIBCO) supplemented with 100 U/mL penicillin, 100 ug/mL streptomycin (GIBCO), and 0.33 ug/mL TPCK-trypsin (TPCK treated, Tosyl phenylalanyl chloromethyl ketone, Sigma).

A virus of interest was diluted to 100TCID₅₀ (tissue culture infectious dose) per well in neutralization medium and added in a 1:1 ratio to diluted serum. Serially pre-diluted serum samples were incubated with the virus and allowed to react for 1h at 37°C, 5% CO₂. In the inoculation step, plates (Cell Culture 96-well plate, Costar) containing
5 MDCK 33016-PF cells were seeded at 3.0^{E4}/well (3.0^{E6}/plate) a day before in the antibiotic free cell growth medium (Irvine Scientific) and washed with sterile PBS, then infected with this mixture and incubated for 1h at 37°C with 5% CO₂. Infection was stopped by aspiration of antibody/virus mixture and cells washed with sterile PBS, inoculated with neutralizing media (100ul/well) containing twofold serially diluted
10 antibodies and then incubated for 5 days at 37°C with 5% CO₂. In the final “read-out” step, detection of virus was performed by quantification of the virus using 0.5% turkey red blood cells (Lampire Biological Laboratories). The absence of infectivity constitutes a positive neutralization reaction and indicates the presence of virus-specific antibodies in the serum sample.

15

Results

The inventors sought to test the effect of the sa-mRNA constructs on LV microneutralisation, PV microneutralisation and ACE-2 binding inhibition *in vitro*. As shown in **Figure 4**, high neutralising titers were obtained for the various sa-mRNA
20 constructs that include Spike (S) or Nucleocapsid (N) protein antigens from the omicron strain of SARS-CoV-2, although better immunogenicity appeared apparent when the S protein was structurally positioned before the N protein antigen. N-specific CD4 T-cell responses for Co-97 and Co-99 were also observed (data not shown). An analysis was then conducted to determine the effect of the sa-mRNA constructs on neutralising titers
25 in different substrains of the omicron strain. As shown in **Figure 5**, the effect on neutralising titer was strongest against BA.1, followed by BA.2. These results demonstrate the utility of the the sa-mRNA constructs disclosed herein for use as vaccines to treat or prevent the omicron strain of SARS-CoV-2.

30 NUMBERED STATEMENTS OF THE DISCLOSURE

The present disclosure provides at least the following numbered statements:

1. A self-replicating RNA comprising a nucleotide sequence encoding an antigen
35 operably linked to a regulatory element, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

2. The self-replicating RNA of statement 1, further comprising a nucleotide sequence encoding a second antigen operably linked to a regulatory element.
- 5 3. The self-replicating RNA of statement 2, wherein the second antigen is a nucleocapsid (N) protein from a SARS-CoV-2.
4. The self-replicating RNA of statement 3, wherein the N protein is from the omicron strain of a SARS-CoV-2.
- 10 5. The self-replicating RNA of statements 1 to 4, wherein the regulatory element is selected from the group consisting of a SG promoter and an IRES.
6. The self-replicating RNA of any one of statements 2 to 5, wherein the self-replicating RNA comprises in 5' to 3' order:
15 a) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2; and
b) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2.
- 20 7. The self-replicating RNA of any one of statements 2 to 5, wherein the self-replicating RNA comprises in 5' to 3' order:
a) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2; and
b) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2.
- 25 8. The self-replicating RNA of any one of statements 2 to 5, wherein the self-replicating RNA comprises in 5' to 3' order:
a) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a SG promoter; and
30 b) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES), optionally encoded by the sequence set forth in SEQ ID NO: 18
- 35 9. The self-replicating RNA of any one of statements 2 to 5, wherein the self-replicating RNA comprises in 5' to 3' order:

- a) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2 operably linked to a SG promoter; and
- b) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES), optionally encoded by the sequence set forth in SEQ ID NO: 19.
10. The self-replicating RNA of any one of statements 2 to 3 or 5 to 9, wherein the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.
11. The self-replicating RNA of any one of statements 1 to 10, wherein the self-replicating RNA is a monocistronic self-replicating RNA.
12. The self-replicating RNA of any one of statements 2 to 10, wherein the self-replicating RNA is a polycistronic self-replicating RNA.
13. The self-replicating RNA of statement 2, wherein the regulatory element is the same as the regulatory element of statement 1.
14. The self-replicating RNA of any one of statements 1 to 13, wherein the regulatory element is a promoter, an internal ribosome entry site (IRES) or a KOSAC consensus sequence or a combination thereof.
15. The self-replicating RNA of statement 14, wherein the promoter is a subgenomic (SG) promoter.
16. The self-replicating RNA of statement 14, wherein the SG promoter is a minimal SG promoter or an extended SG promoter.
17. The self-replicating RNA of statement 16, wherein the extended SG promoter is extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein of an RNA virus.

18. The self-replicating RNA of statement 16, wherein the minimal SG promoter is encoded by a sequence set forth in SEQ ID NO: 1.
19. The self-replicating RNA of statement 16, wherein the extended SG promoter is
5 encoded by a sequence set forth in SEQ ID NO: 5.
20. The self-replicating RNA of statement 2, wherein the nucleotide sequence encoding a second antigen is operably linked to an IRES, optionally located 3' to the nucleotide sequence encoding a second antigen.
- 10 21. The self-replicating RNA of statement 20, wherein the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV),
15 Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5 (DAP5), cellular Myc (c-Myc), NF- κ B-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a
20 (FGF1A), GTX, or a combination thereof.
22. The self-replicating RNA of statement 21, wherein the EMCV IRES is a wild-type IRES encoded by a sequence set forth in SEQ ID NO: 4.
- 25 23. The self-replicating RNA of any one of statements 2 to 22, wherein the antigens are expressed at substantially the same level.
24. The self-replicating RNA of any one of statements 1 to 23, wherein the antigen is from a S protein of an omicron variant selected from the group consisting of B.1.1.529,
30 BA.1, BA.2, BA.4, BA.5, BA.2.12.1 and BA.2.75.
25. The self-replicating RNA of statement 24, wherein:
(a) the antigen is from a S protein of omicron variant BA.1 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected
35 from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K,

S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or

(b) the antigen is from a S protein of omicron variant BA.2 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected
5 from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or

(c) the antigen is from a S protein of omicron variant BA.4 or BA.5 and the S
10 protein encoded by the polynucleotide comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

15

26. The self-replicating RNA of any one of statements 1 to 24, wherein the the S protein is encoded by a polynucleotide sequence having at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 20.

20

27. The self-replicating RNA of any one of statements 1 to 24, wherein the the N protein is encoded by a polynucleotide sequence having at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 21.

25

28. The self-replicating RNA of any one of statements 1 to 24, wherein the self-replicating RNA is from an alphavirus.

29. The self-replicating RNA of statement 28, wherein the alphavirus is selected from
30 the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan equine encephalitis virus (VEE) and combinations thereof.

30. An immunogenic composition comprising the self-replicating RNA of any one of statements 1 to 29.

35

31. The immunogenic composition of statement 30, comprising a plurality of self-replicating RNAs of any one of statements 1 to 29, wherein each self-replicating RNA encodes different polypeptide antigen sequences.
- 5 32. The immunogenic composition of statement 31, comprising a plurality of self-replicating RNAs of any one of statements 1 to 29, wherein each self-replicating RNA encodes the same polypeptide antigen sequences.
- 33 A pharmaceutical composition comprising an immunogenic composition of any
10 one of statements 30 to 32 and a pharmaceutically acceptable carrier.
34. The pharmaceutical composition of statement 33, further comprising a lipid nanoparticle (LNP), a polymeric microparticle or an oil-in-water emulsion.
- 15 35. The pharmaceutical composition of statement 33, wherein the self-replicating RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle or an oil-in-water emulsion.
36. The pharmaceutical composition of any one of statements 33 to 35, wherein each
20 RNA is formulated together in the LNP.
37. The pharmaceutical composition of any one of statements 33 to 35, wherein each RNA is formulated separately in the LNP.
- 25 38. The immunogenic composition of any one of statements 30 to 32 or the pharmaceutical composition of any one of statements 33 to 37, for use as a vaccine.
39. A vaccine comprising the pharmaceutical composition of any one of statements 33 to 37 or the immunogenic composition of any one of statements 30 to 32.
30
40. A polynucleotide encoding the self-replicating RNA of any one of statements 1 to 29, optionally comprising a sequence set forth in SEQ ID NO:17, 18 or 19.
41. The polynucleotide of statement 40, wherein the polynucleotide is a recombinant
35 DNA.

42. The polynucleotide of statement 41, wherein the recombinant DNA is a plasmid.
43. A polynucleotide comprising:
- a) a first nucleotide sequence encoding a first antigen; and
 - 5 b) a second nucleotide sequence encoding a second antigen operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES),
- wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
- 10 44. The polynucleotide of statement 43, wherein the polynucleotide comprises, in order from 5' to 3':
- a) the first nucleotide sequence encoding a first antigen; and
 - b) the second nucleotide sequence encoding a second antigen operably linked to an
- 15 IRES or a SG promoter, optionally comprising a sequence set forth in SEQ ID NO: 18 or 19.
45. A conventional mRNA (cRNA) comprising:
- a) a first nucleotide sequence encoding a first antigen; and
 - 20 b) a second nucleotide sequences encoding a second antigen operably linked to a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES),
- wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
- 25 46. The cRNA of statement 45, wherein the cRNA comprises, in order from 5' to 3':
- a) the first nucleotide sequence encoding a first antigen; and
 - b) the second nucleotide sequence encoding a second antigen operably linked to an IRES or a SG promoter.
- 30 47. The polynucleotide of statement 43 or 44, or the cRNA of statement 45 or 46, wherein the first nucleotide sequence is operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof.
- 35

48. The polynucleotide of any one of statements 43, 44 or 47, or the cRNA of any one of statements 45 to 47, wherein the second nucleotide sequence encodes a nucleocapsid (N) protein from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), optionally from the omicron strain of SARS-CoV-2.

5

49. A method of treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject in need thereof, the method comprising administering the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 to the subject.

50. Use of the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 in the manufacture of a medicament for treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject.

51. The self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 for use in the treatment or prevention or delaying progression of a SARS-CoV-2 infection in a subject.

52. A method of inducing an immune response in a subject, the method comprising administering the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 to a subject in need thereof.

53. Use of the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

54. The self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 for use in inducing an immune response in a subject in need thereof.

55. The method of statement 52, the use of statement 53, or the self-replicating RNA, pharmaceutical composition, immunogenic composition or vaccine for use of statement 54, wherein the immune response is a humoral and/or a cell-mediated immune response.
- 5
56. The method, the use, or the self-replicating RNA, pharmaceutical composition, immunogenic composition or vaccine for use of statement 55, wherein the immune response is induced in response to at least one antigen from the omicron strain of a SARS-CoV-2.
- 10
57. A method for reducing SARS-CoV-2 viral load in a subject with COVID-19 comprising administering the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 to the
- 15 subject in need thereof.
58. Use of the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 in the preparation of a
- 20 medicament for reducing SARS-CoV-2 viral load in a subject with COVID-19.
59. The self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 for use in reducing SARS-
- 25 CoV-2 viral load in a subject with COVID-19.
60. A method for treating, preventing or delaying progress of acute respiratory distress syndrome in a subject with COVID-19 comprising administering the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any
- 30 one of statements 33 to 37, the immunogenic composition any one of statements 30 to 32 or 38 or the vaccine of statement 39 to a subject in need thereof.
61. Use of the self-replicating RNA of any one of statements 1 to 29, pharmaceutical composition of any one of statements 33 to 37, the immunogenic composition any one
- 35 of statements 30 to 32 or 38 or the vaccine of statement 39 in the preparation of a

medicament for treating, preventing or delaying progress of acute respiratory distress syndrome in a subject with COVID-19.

62. The self-replicating RNA of any one of statements 1 to 29, pharmaceutical
5 composition of any one of statements 33 to 37, the immunogenic composition any one
of statements 30 to 32 or 38 or the vaccine of statement 39 for use in treating, preventing
or delaying progress of acute respiratory distress syndrome in a subject with COVID-19.

63. The method of any one of statements 49, 52, 55 to 57 or 60 or the use of any one
10 of statements 50, 53, 55, 56, 58 or 61, or the for use of any one of statements 51, 54, 55,
56, 59 or 62, wherein the subject is a human of 18 years of age or older.

64. The method of any one of statements 49, 52, 55 to 57 or 60 or the use of any one
of statements 50, 53, 55, 56, 58 or 61, or the for use of any one of statements 51, 54, 55,
15 56, 59 or 62, wherein the self-replicating RNA, vaccine or composition is administered
in a one dose regimen.

65. The method of any one of statements 49, 52, 55 to 57 or 60 or the use of any one
of statements 50, 53, 55, 56, 58 or 61, or the for use of any one of statements 51, 54, 55,
20 56, 59 or 62, wherein the self-replicating RNA, vaccine or composition is administered
in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3
months apart.

66. A kit comprising:
25 (a) the self-replicating RNA of any one of statements 1 to 29, pharmaceutical
composition of any one of statements 33 to 37, the immunogenic composition
any one of statements 30 to 32 or 38 or the vaccine of statement 39;
(b) instructions for use thereof; and optionally
(c) a pharmaceutically acceptable carrier, excipient or diluent.

30

CLAIMS

1. A self-replicating RNA comprising a nucleotide sequence encoding an antigen operably linked to a regulatory element, wherein the antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
5
2. The self-replicating RNA of claim 1, further comprising a nucleotide sequence encoding a second antigen operably linked to a regulatory element.
- 10 3. The self-replicating RNA of claim 2, wherein the second antigen is a nucleocapsid (N) protein from a SARS-CoV-2.
4. The self-replicating RNA of claim 3, wherein the N protein is from the omicron strain of a SARS-CoV-2.
- 15 5. The self-replicating RNA of claim 1, wherein the regulatory element is selected from the group consisting of a SG promoter and an IRES.
6. The self-replicating RNA of claim 2, wherein the self-replicating RNA comprises
20 in 5' to 3' order:
 - a) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2; and
 - b) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2.
- 25 7. The self-replicating RNA of claim 2, wherein the self-replicating RNA comprises in 5' to 3' order:
 - a) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2; and
 - b) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2.
- 30 8. The self-replicating RNA of claim 2, wherein the self-replicating RNA comprises in 5' to 3' order:
 - a) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a SG promoter; and
 - 35 b) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2 operably linked to a regulatory element selected from the group consisting of a

SG promoter and an internal ribosome entry site (IRES), optionally encoded by the sequence set forth in SEQ ID NO: 18

9. The self-replicating RNA of claim 2, wherein the self-replicating RNA comprises
5 in 5' to 3' order:
- a) the nucleotide sequence encoding an N protein antigen from a SARS-CoV-2 operably linked to a SG promoter; and
 - b) the nucleotide sequence encoding an S protein antigen from the omicron strain of a SARS-CoV-2 operably linked to a regulatory element selected from the group
10 consisting of a SG promoter and an internal ribosome entry site (IRES), optionally encoded by the sequence set forth in SEQ ID NO: 19.
10. The self-replicating RNA of claim 2, wherein the N protein is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.
15
11. The self-replicating RNA of claim 1, wherein the self-replicating RNA is a monocistronic self-replicating RNA.
12. The self-replicating RNA of claim 2, wherein the self-replicating RNA is a
20 polycistronic self-replicating RNA.
13. The self-replicating RNA of claim 2, wherein the regulatory element is the same as the regulatory element of claim 1.
- 25 14. The self-replicating RNA of claim 1, wherein the regulatory element is a promoter, an internal ribosome entry site (IRES) or a Kozak consensus sequence or a combination thereof.
15. The self-replicating RNA of claim 14, wherein the promoter is a subgenomic (SG)
30 promoter.
16. The self-replicating RNA of claim 14, wherein the SG promoter is a minimal SG promoter or an extended SG promoter.

17. The self-replicating RNA of claim 16, wherein the extended SG promoter is extended at the 5' end with nucleotides occurring in a sequence encoding a non-structural protein of an RNA virus.
- 5 18. The self-replicating RNA of claim 16, wherein the minimal SG promoter is encoded by a sequence set forth in SEQ ID NO: 1.
19. The self-replicating RNA of claim 16, wherein the extended SG promoter is encoded by a sequence set forth in SEQ ID NO: 5.
- 10 20. The self-replicating RNA of claim 2, wherein the nucleotide sequence encoding a second antigen is operably linked to an IRES, optionally located 3' to the nucleotide sequence encoding a second antigen.
- 15 21. The self-replicating RNA of claim 20, wherein the IRES is an IRES from encephalomyocarditis virus (EMCV), poliovirus (PV), human enterovirus, foot-and-mouth disease virus (FMDV), hepatitis C virus (HCV), classical swine fever virus (CSFV), murine leukemia virus (MLV), simian immunodeficiency virus (SIV), Eukaryotic translation initiation factor 4G (eIF4G), Death-associated protein 5
20 (DAP5), cellular Myc (c-Myc), NF- κ B-repressing factor (NRF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), platelet-derived growth factor B (PDGF B), Antennapedia, X-linked inhibitor of apoptosis (XIAP or Apaf-1), immunoglobulin heavy-chain binding protein BiP, or fibroblast growth factor 1a (FGF1A), GTX, or a combination thereof.
- 25 22. The self-replicating RNA of claim 21, wherein the EMCV IRES is a wild-type IRES encoded by a sequence set forth in SEQ ID NO: 4.
23. The self-replicating RNA of claim 2, wherein the antigens are expressed at
30 substantially the same level.
24. The self-replicating RNA of claim 1, wherein the antigen is from a S protein of an omicron variant selected from the group consisting of B.1.1.529, BA.1, BA.2, BA.4, BA.5, BA.2.12.1 and BA.2.75.
- 35 25. The self-replicating RNA of claim 24, wherein:

(a) the antigen is from a S protein of omicron variant BA.1 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, 5 S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or

(b) the antigen is from a S protein of omicron variant BA.2 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, 10 N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or

(c) the antigen is from a S protein of omicron variant BA.4 or BA.5 and the S protein encoded by the polynucleotide comprises one or more or all of the mutations 15 selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

20 26. The self-replicating RNA of claim 1, wherein the the S protein is encoded by a polynucleotide sequence having at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 20.

25 27. The self-replicating RNA of claim 1, wherein the the N protein is encoded by a polynucleotide sequence having at least about 80%, at least about 85%, at least about 90% at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% to SEQ ID NO: 21.

30 28. The self-replicating RNA of claim 1, wherein the self-replicating RNA is from an alphavirus.

29. The self-replicating RNA of claim 28, wherein the alphavirus is selected from the group consisting of Semliki Forest virus (SFV), Sindbis virus (SIN), and Venezuelan 35 equine encephalitis virus (VEE) and combinations thereof.

30. An immunogenic composition comprising the self-replicating RNA of claim 1.
31. An immunogenic composition, comprising a plurality of self-replicating RNAs of claim 1, wherein each self-replicating RNA encodes different polypeptide antigen sequences.
32. An immunogenic composition, comprising a plurality of self-replicating RNAs of claim 1, wherein each self-replicating RNA encodes the same polypeptide antigen sequences.
33. A pharmaceutical composition comprising an immunogenic composition of claim 30 and a pharmaceutically acceptable carrier.
34. The pharmaceutical composition of claim 33, further comprising a lipid nanoparticle (LNP), a polymeric microparticle or an oil-in-water emulsion.
35. The pharmaceutical composition of claim 33, wherein the self-replicating RNA is encapsulated in, bound to or adsorbed on a LNP, a polymeric microparticle or an oil-in-water emulsion.
36. The pharmaceutical composition of claim 33, wherein each RNA is formulated together in the LNP.
37. The pharmaceutical composition of claim 33, wherein each RNA is formulated separately in the LNP.
38. The immunogenic composition of claim 30 for use as a vaccine.
39. The pharmaceutical composition of claim 33 for use as a vaccine.
40. A vaccine comprising the immunogenic composition of claim 30.
41. A vaccine comprising the pharmaceutical composition of claim 30.
42. A polynucleotide encoding the self-replicating RNA of claim 1, optionally comprising a sequence set forth in SEQ ID NO:17, 18 or 19.

43. The polynucleotide of claim 42, wherein the polynucleotide is a recombinant DNA.
- 5 44. The polynucleotide of claim 43, wherein the recombinant DNA is a plasmid.
45. A polynucleotide comprising:
- a) a first nucleotide sequence encoding a first antigen; and
 - b) a second nucleotide sequence encoding a second antigen operably linked to
- 10 a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES),
- wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
- 15 46. The polynucleotide of claim 45, wherein the polynucleotide comprises, in order from 5' to 3':
- a) the first nucleotide sequence encoding a first antigen; and
 - b) the second nucleotide sequence encoding a second antigen operably linked to an
- 20 IRES or a SG promoter, optionally comprising a sequence set forth in SEQ ID NO: 18 or 19.
47. A conventional mRNA (cRNA) comprising:
- a) a first nucleotide sequence encoding a first antigen; and
 - b) a second nucleotide sequences encoding a second antigen operably linked to
- 25 a regulatory element selected from the group consisting of a SG promoter and an internal ribosome entry site (IRES),
- wherein the first antigen is a spike (S) protein from the omicron strain of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
- 30 48. The cRNA of claim 47, wherein the cRNA comprises, in order from 5' to 3':
- a) the first nucleotide sequence encoding a first antigen; and
 - b) the second nucleotide sequence encoding a second antigen operably linked to an IRES or a SG promoter.

49. The polynucleotide of claim 45, wherein the first nucleotide sequence is operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof.
- 5 50. The cRNA of claim 47, wherein the first nucleotide sequence is operably linked to a regulatory element selected from the group consisting of a Kozak consensus sequence, an IRES, a SG promoter and combinations thereof.
51. The polynucleotide of claim 45 wherein the second nucleotide sequence encodes
10 a nucleocapsid (N) protein from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), optionally from the omicron strain of SARS-CoV-2.
52. The cRNA of claim 47, wherein the second nucleotide sequence encodes a
15 nucleocapsid (N) protein from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), optionally from the omicron strain of SARS-CoV-2.
53. A method of treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject in need thereof, the method comprising administering the self-replicating RNA of claim 1 to the subject.
20
54. Use of the self-replicating RNA of claim 1 in the manufacture of a medicament for treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject.
- 25 55. The self-replicating RNA of claim 1 for use in the treatment or prevention or delaying progression of a SARS-CoV-2 infection in a subject.
56. A method of inducing an immune response in a subject, the method comprising administering the self-replicating RNA of claim 1 to a subject in need thereof.
30
57. Use of the self-replicating RNA of claim 1 in the manufacture of a medicament for inducing an immune response in a subject in need thereof.
58. The self-replicating RNA of claim 1 for use in inducing an immune response in a
35 subject in need thereof.

59. The method of claim 56 wherein the immune response is a humoral and/or a cell-mediated immune response.

60. The method of claim 59, wherein the immune response is induced in response to
5 at least one antigen from the omicron strain of a SARS-CoV-2.

61. A method for reducing SARS-CoV-2 viral load in a subject with COVID-19 comprising administering the self-replicating RNA of claim 1 to the subject in need thereof.
10

62. Use of the self-replicating RNA of claim 1 in the preparation of a medicament for reducing SARS-CoV-2 viral load in a subject with COVID-19.

63. The self-replicating RNA of claim 1 for use in reducing SARS-CoV-2 viral load
15 in a subject with COVID-19.

64. A method for treating, preventing or delaying progress of acute respiratory distress syndrome in a subject with COVID-19 comprising administering the self-replicating RNA of claim 1 to a subject in need thereof.
20

65. Use of the self-replicating RNA of claim 1 in the preparation of a medicament for treating, preventing or delaying progress of acute respiratory distress syndrome in a subject with COVID-19.

25 66. The self-replicating RNA of claim 1 for use in treating, preventing or delaying progress of acute respiratory distress syndrome in a subject with COVID-19.

67. The method of claim 53, wherein the subject is a human of 18 years of age or older.
30

68. The method of claim 53, wherein the self-replicating RNA, vaccine or composition is administered in a one dose regimen.

69. The method of claims 53, wherein the self-replicating RNA, vaccine or
35 composition is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.

70. A kit comprising:
- (a) the self-replicating RNA of claim 1;
 - (b) instructions for use thereof; and optionally
- 5 (c) a pharmaceutically acceptable carrier, excipient or diluent.