

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated RNA polynucleotide, the isolated RNA polynucleotide comprising a coding region having a coding sequence encoding one or more therapeutic polypeptides; and
template regions, wherein the template regions comprise two distinct regions, a left flanking region (“L region”) of a virus and a right flanking region (“R region”) of the virus, wherein the L region is adjacent to and contiguous with a 5' end of the coding region and the R region is adjacent to and contiguous with a 3' end of the coding region;
wherein the coding sequence is in an antisense orientation;
wherein the therapeutic polypeptide is heterologous to the virus;
wherein the template regions interact with and initiate RNA-dependent polymerase activity of a polymerase in a cell containing the RNA dependent polymerase; and
wherein the isolated RNA polynucleotide comprises at least one nucleoside modification.
2. The isolated RNA polynucleotide of claim 1,
wherein the template regions are nucleoside modified, wherein the percentage of modified nucleosides is not more than 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5%;
wherein the template regions are nucleoside modified, wherein the percentage of modified nucleosides is at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, or 100%;
wherein the nucleoside modification is a nonimmunogenic uridine modification, and the percentage of modified uridine modifications is not more than 40%, 35%, 30%, 25%, 20%, 15%, or 10%;
wherein the nucleoside modification is a nonimmunogenic uridine modification, and the percentage of modified uridine modifications is more than 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95%, or is 100%;
wherein the nucleoside modification is a nonimmunogenic cytidine modification, and the percentage of modified cytidine modifications is not more than 40%, 35%, 30%, 25%, 20%, 15%, or 10%;

wherein the nucleoside modification is a nonimmunogenic cytidine modification, and the percentage of modified cytidine modifications is more than 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95%, or is 100%; or

wherein the nucleoside modification is a nonimmunogenic adenosine modification, wherein the percentage of modified adenosine modifications is between 1% and 30%, and optionally wherein the percentage of modified cytidine modifications is about 1%, 5%, 10%, 15%, 20%, 25%, or 30%.

3. The isolated RNA polynucleotide of any one of claims 1 to 2, wherein the isolated polynucleotide comprises a 5' cap structure; wherein the 5' end of the L region comprises a 5' cap structure; wherein the 5' end of the L region comprises one or more variations associated with a 5' cap structure; wherein the 5' end of the isolated polynucleotide comprises a 5'-monophosphate; wherein the 5' end of the isolated polynucleotide does not comprise a 5'-phosphate (dephosphorylated); wherein the 5' end of the L region comprises a 5'-monophosphate; or wherein the 5' end of the L region does not comprise a 5'-phosphate (dephosphorylated).

4. The isolated RNA polynucleotide of claim 3, wherein the 5' cap structure is selected from the group consisting of Cap 0, Cap 0 (3'-O-Me), Cap 1, Cap 1 (3'-O-Me), Cap 2, Cap 2 (3'-O-Me), Anti-Reverse Cap Analog (ARCA), inosine, N1-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, locked nucleic acid guanosine (LNA-guanosine), and 2-azido-guanosine structure.

5. The isolated RNA polynucleotide of any one of claims 1 to 4, wherein the virus is selected from the group consisting of viruses in the orders of *Amarillovirales*, *Articulavirales*, *Blubervirales*, *Bunyavirales*, *Hepelivirales*, *Martellivirales*, *Mononegavirales*, *Nidovirales*, and *Picornavirales*,

wherein the virus is selected from the group consisting of viruses in the families of *Arenaviridae*, *Coronaviridae*, *Filoviridae*, *Flaviviridae*, *Hantaviridae*, *Hepadnaviridae*, *Matonaviridae*, *Nairoviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Phenuiviridae*, *Picornaviridae*, *Pneumoviridae*, *Rhabdoviridae*, and *Togaviridae*,

wherein the virus is selected from the group consisting of Alphacoronavirus 229E, Alphacoronavirus NL63, Alphacoronavirus WA2028, Avian metapneumovirus (AMPV), Betacoronavirus HKU1, Betacoronavirus HKU15, Betacoronavirus HKU33, Betacoronavirus OC43, Chikungunya virus, Crimean-Congo Hemorrhagic Fever Virus, Dengue Virus, Eastern Equine Encephalitis Virus (EEEV), Enterovirus D68 (EV-D68), Foot and Mouth Disease Virus, Hanta Virus, Hendra Virus, Hepatitis B Virus, Hepatitis C Virus, HMPV, Human Parainfluenzavirus 1 (HPIV1), Human Parainfluenzavirus 3 (HPIV3), Infectious Salmon Anemia Virus, Influenza A Virus, Influenza B Virus, Lassa Virus, Marburg Virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Newcastle Disease Virus (NDV), Nipah Virus, Norwalk Virus, Rabies Virus, Respiratory Syncytial Virus, Reston Ebola virus, Rhinovirus, Rift Valley Fever Virus, Rubella virus, SARS-CoV-1, SARS-CoV-2, Sudan Ebola virus, Venezuelan Equine Encephalitis Virus (VEEV), Vesicular Stomatitis Virus, Western Equine Encephalitis Virus (WEEV), Yellow Fever Virus, Zaire Ebola virus, and Zika Virus, or

wherein the virus is not an alphavirus.

6. The isolated RNA polynucleotide of any one of claims 1 to 5,
wherein the template regions are native to the virus,
wherein the template regions are variants of template regions native to the virus, and wherein the variants have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the template regions native to the virus;
wherein each of the L and the R regions of the template regions comprises fewer than 10, 9, 8, 7, 6, 5, 4, 3, or 2 variations relative to template regions native to the virus;
wherein each of the L and the R regions of the template regions varies from template regions native to the virus by not more than 10, 9, 8, 7, 6, 5, 4, 3, or 2 substitutions that are not involved in 5' capping; or
wherein each of the L and the R regions of the template regions varies from template regions native to the virus by not more than 1 substitution that is not involved in 5' capping.

7. The isolated RNA polynucleotide of any one of claims 1 to 6,
wherein the therapeutic polypeptide is a secreted polypeptide, optionally an antibody;
wherein the therapeutic polypeptide is selected from the group consisting of an interferon, an interferon stimulated gene, a cytokine, a chemokine, an antibody, a signaling molecule, a cytotoxic protein, a protein that causes cell death, an antineoplastic protein, an

immunomodulatory protein, protein toll-like receptor agonist, or a dominant negative protein, optionally wherein the cytokine is an inflammatory cytokine, optionally wherein the inflammatory cytokine is TNF- α , optionally wherein the cytokine is an anti-inflammatory cytokine, optionally wherein the anti-inflammatory cytokine is an interleukin-1 receptor antagonist (IL-1RN);

wherein the therapeutic polypeptide is an interleukin or a caspase, optionally wherein the interleukin is IL-12A, IL-12B or IL-2; or

wherein the therapeutic polypeptide is an interferon, optionally an IFN- α , IFN- β , IFN- ϵ , IFN- κ , IFN- ω , IFN- γ , or IFN- λ , optionally IFN- α 1, IFN- α 2, IFN- α 4, IFN- α 5, IFN- α 6, IFN- α 7, IFN- α 8, IFN- α 10, IFN- α 13, IFN- α 14, IFN- α 16, IFN- α 17, IFN- α 21, IFN- β 1, IFN- ϵ , IFN- κ , IFN- ω 1, IFN- γ , IFN- λ 1 (IL28A), IFN- λ 2 (IL28B), IFN- λ 3 (IL29), or IFN- λ 4.

8. The isolated RNA polynucleotide of any one of claims 1 to 7,

wherein the coding sequence encodes more than one therapeutic polypeptide, which are separated by one or more ribosomal skipping sequences;

wherein the coding region further comprises one or more regulatory elements selected from the group consisting of a ribosomal binding site, Kozak sequence, Shine-Dalgarno sequence, ribozyme, riboswitch, promoter, microRNA binding site, and an internal ribosomal entry site (IRES), optionally wherein the one or more regulatory elements are operably linked to the coding sequence;

wherein the isolated RNA polynucleotide further comprises a polyadenylation signal and/or a 3' poly(A) tail;

wherein the RNA-dependent polymerase is an RNA-dependent RNA polymerase or an RNA-dependent DNA polymerase, optionally wherein the RNA-dependent polymerase is a polymerase from the virus;

wherein the isolated RNA polynucleotide is a single stranded RNA;

wherein the isolated RNA polynucleotide is in linear form; or

wherein the isolated RNA polynucleotide is in a covalently-closed circular form.

9. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is an influenza virus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 2; or a variant of SEQ ID NO: 2, wherein the variant of SEQ ID NO: 2 comprises a variation at one

or more nucleotide positions selected from the group consisting of positions 14-26 of SEQ ID NO: 2; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 20, 21, 22, or 23; or a variant of any one of SEQ ID NOs: 20, 21, 22, or 23, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-15 of any one of SEQ ID NOs: 20, 21, 22, or 23;

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 3; or a variant of SEQ ID NO: 3, wherein the variant of SEQ ID NO: 3 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-35 of SEQ ID NO: 3; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 24, 25, 26, or 27; or a variant of any one of SEQ ID NOs: 24, 25, 26, or 27, wherein

the variant of SEQ ID NO: 24 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-12 of SEQ ID NO: 24;

the variant of SEQ ID NO: 25 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-12 of SEQ ID NO: 25;

the variant of SEQ ID NO: 26 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-12 of SEQ ID NO: 26; or

the variant of SEQ ID NO: 27 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-12 of SEQ ID NO: 27;

(iii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 4; or a variant of SEQ ID NO: 4, wherein the variant of SEQ ID NO: 4 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-50 of SEQ ID NO: 4; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 28, 29, 30, or 31; or a variant of any one of SEQ ID NOs: 28, 29, 30, or 31, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-12 of any one of SEQ ID NOs: 28, 29, 30, or 31;

(iv) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 1 or 5; or a variant of SEQ ID NO: 1 or 5, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-37 of SEQ ID NO: 1 or 5; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 18 or 19; or a variant of SEQ ID NO: 18 or 19, wherein the variant comprises a variation at one or more

nucleotide positions selected from the group consisting of positions 8-20 of SEQ ID NO: 18 or 19;

(v) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 6; or a variant of SEQ ID NO: 6, wherein the variant of SEQ ID NO: 6 comprises a variation at one or more nucleotide positions selected from position 14 or 15 of SEQ ID NO: 6; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 32 or 33; or a variant of SEQ ID NO: 32 or 33, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-33 of SEQ ID NO: 32 or 33;

(vi) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 7; or a variant of SEQ ID NO: 7, wherein the variant of SEQ ID NO: 7 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-20 of SEQ ID NO: 7; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 34, 35, 36, or 37; or a variant of any one of SEQ ID NOs: 34, 35, 36, or 37, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 5-8 of SEQ ID NO: 34, 35, 36, or 37;

(vii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 8; or a variant of SEQ ID NO: 8, wherein the variant of SEQ ID NO: 8 comprises a variation at one or more nucleotide positions selected from position 14 or 15 of SEQ ID NO: 8; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 38, 39, 40, or 41; or a variant of any one of SEQ ID NOs: 38, 39, 40, or 41, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-13 of SEQ ID NO: 38, 39, 40, or 41;

(viii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 9; or a variant of SEQ ID NO: 9, wherein the variant of SEQ ID NO: 9 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-18 of SEQ ID NO: 9; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 42 or 43; or a variant of SEQ ID NO: 42 or 43, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-14 of SEQ ID NO: 42 or 43;

(ix) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 11; or a variant of SEQ ID NO: 11, wherein the variant of SEQ ID NO: 11 comprises a variation at

one or more nucleotide positions selected from the group consisting of positions 14-81 of SEQ ID NO: 11; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 46 or 47; or a variant of SEQ ID NO: 46 or 47, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 5-9 of SEQ ID NO: 46 or 47;

(x) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 12; or a variant of SEQ ID NO: 12, wherein the variant of SEQ ID NO: 12 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-52 of SEQ ID NO: 12; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NO: 48 or 49; or a variant of any one of SEQ ID NO: 48 or 49, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-11 of SEQ ID NO: 48 or 49;

(xi) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 13; or a variant of SEQ ID NO: 13, wherein the variant of SEQ ID NO: 13 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-87 of SEQ ID NO: 13; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 50 or 51; or a variant of SEQ ID NO: 50 or 51, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-17 of SEQ ID NO: 50 or 51;

(xii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NO: 10; or a variant of any one of SEQ ID NO: 10, wherein the variant of SEQ ID NO: 10 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-86 of SEQ ID NO: 10; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 44 or 45; or a variant of SEQ ID NO: 44 or 45, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-21 of SEQ ID NO: 44 or 45;

(xiii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 14; or a variant of SEQ ID NO: 14, wherein the variant of SEQ ID NO: 14 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-93 of SEQ ID NO: 14; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NO: 52 or 53; or a variant of any one of SEQ ID NO: 52 or 53, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-48 of SEQ ID NO: 52 or 53;

(xiv) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 15; or a variant of SEQ ID NO: 15, wherein the variant of SEQ ID NO: 15 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-95 of SEQ ID NO: 15; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 54 or 55; or a variant of any one of SEQ ID NO: 54 or 55, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-34 of SEQ ID NO: 54 or 55;

(xv) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 16; or a variant of SEQ ID NO: 16, wherein the variant of SEQ ID NO: 16 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-81 of SEQ ID NO: 16; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NO: 56 or 57; or a variant of any one of SEQ ID NO: 56 or 57, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-12 of SEQ ID NO: 56 or 57; and

(xvi) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 17; or a variant of SEQ ID NO: 17, wherein the variant of SEQ ID NO: 17 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 14-22 of SEQ ID NO: 17; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NO: 58 or 59; or a variant of any one of SEQ ID NO: 58 or 59, wherein the variant comprises a variation at one or more nucleotide positions selected from the group consisting of positions 8-32 of SEQ ID NO: 58 or 59.

10. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is a sarbecovirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 137; or a variant of SEQ ID NO: 137, wherein the variant of SEQ ID NO: 137 comprises a variation at

one or more nucleotide positions selected from the group consisting of positions 40-1557 of SEQ ID NO: 137; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 128; or a variant of any one of SEQ ID NO: 128, wherein the variant of SEQ ID NO: 128 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-30 of SEQ ID NO: 128; and

(ii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 138, 139, 140, 141, 142, 143, or 144; or a variant of any one of SEQ ID NOs: 138, 139, 140, 141, 142, 143, or 144, wherein

the variant of SEQ ID NO: 138 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-312 of SEQ ID NO: 138;

the variant of SEQ ID NO: 139 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1567 of SEQ ID NO: 139;

the variant of SEQ ID NO: 140 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1488 of SEQ ID NO: 140;

the variant of SEQ ID NO: 141 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1593 of SEQ ID NO: 141;

the variant of SEQ ID NO: 142 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1570 of SEQ ID NO: 142;

the variant of SEQ ID NO: 143 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1488 of SEQ ID NO: 143; or

the variant of SEQ ID NO: 144 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1593 of SEQ ID NO: 144; and

the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 130, 136, 145, 146, or 147; or a variant of any one of SEQ ID NOs: 130, 136, 145, 146, or 147, wherein

the variant of SEQ ID NO: 130 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-320 of SEQ ID NO: 130;

the variant of SEQ ID NO: 136 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-33 of SEQ ID NO: 136;

the variant of SEQ ID NO: 145 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 50-1461 of SEQ ID NO: 145;

the variant of SEQ ID NO: 146 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60-1441 of SEQ ID NO: 146; or

the variant of SEQ ID NO: 147 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60-897 of SEQ ID NO: 147.

11. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is Respiratory Syncytial Virus (RSV),

wherein the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 158, 163, 165, 166, or 419; or a variant of any one of SEQ ID NOs: 158, 163, 165, 166, or 419, wherein

the variant of SEQ ID NO: 158 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–207 of SEQ ID NO: 158;

the variant of SEQ ID NO: 163 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 18–210 of SEQ ID NO: 163;

the variant of SEQ ID NO: 165 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–147 of SEQ ID NO: 165;

the variant of SEQ ID NO: 166 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–32 of SEQ ID NO: 166; or

the variant of SEQ ID NO: 419 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 18–35 of SEQ ID NO: 419; and

wherein the R region comprises the nucleotide sequence set forth as any one of SEQ ID NOs: 169, 170, 176, 177, or 420; or a variant of any one of SEQ ID NOs: 169, 170, 176, 177, or 420, wherein

the variant of SEQ ID NO: 169 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-78 of SEQ ID NO: 169;

the variant of SEQ ID NO: 170 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-80 of SEQ ID NO: 170;

the variant of SEQ ID NO: 176 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-36 of SEQ ID NO: 176;

the variant of SEQ ID NO: 177 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-33 of SEQ ID NO: 177; or

the variant of SEQ ID NO: 420 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-35 of SEQ ID NO: 420.

12. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is a parainfluenzavirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 181, 182, or 183; or a variant of any one of SEQ ID NOs: 181, 182, or 183, wherein

the variant of SEQ ID NO: 181 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-136 of SEQ ID NO: 181;

the variant of SEQ ID NO: 182 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-142 of SEQ ID NO: 182; or

the variant of SEQ ID NO: 183 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-136 of SEQ ID NO: 183; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 184; or a variant of SEQ ID NO: 184, wherein the variant of SEQ ID NO: 184 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-98 of SEQ ID NO: 184; and

(ii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 187, 188, or 189; or a variant of any one of SEQ ID NOs: 187, 188, or 189, wherein

the variant of SEQ ID NO: 187 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-95 of SEQ ID NO: 187;

the variant of SEQ ID NO: 188 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-101 of SEQ ID NO: 188; or

the variant of SEQ ID NO: 189 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-95 of SEQ ID NO: 189;
and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 190; or a variant of SEQ ID NO: 190, wherein the variant of SEQ ID NO: 190 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-93 of SEQ ID NO: 190.

13. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is a metapneumovirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 196, 197, or 199; or a variant of any one of SEQ ID NOs: 196, 197, or 199, wherein

the variant of SEQ ID NO: 196 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-224 of SEQ ID NO: 196;

the variant of SEQ ID NO: 197 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-230 of SEQ ID NO: 197;

the variant of SEQ ID NO: 199 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-140 of SEQ ID NO: 199;

and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 201; or a variant of SEQ ID NO: 201, wherein the variant of SEQ ID NO: 201 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-32 of SEQ ID NO: 201; and

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 195; or a variant of SEQ ID NO: 195, wherein the variant of SEQ ID NO: 195 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-224 of SEQ ID NO: 195; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 200; or a variant of SEQ ID NO: 200, wherein the variant of SEQ ID NO: 200 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-32 of SEQ ID NO: 200.

14. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is a henipavirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 204; or a variant of SEQ ID NO: 204, wherein the variant of SEQ ID NO: 204 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-77 of SEQ ID NO: 204; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 206; or a variant of SEQ ID NO: 206, wherein the variant of SEQ ID NO: 206 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-91 of SEQ ID NO: 206; and

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 209 or 210; or a variant of SEQ ID NO: 209 or 210, wherein

the variant of SEQ ID NO: 209 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17–77 of SEQ ID NO: 209; or

the variant of SEQ ID NO: 210 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17–83 of SEQ ID NO: 210; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 211; or a variant of SEQ ID NO: 211, wherein the variant of SEQ ID NO: 211 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-91 of SEQ ID NO: 211.

15. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is a filovirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 227, 228, 229, or 230; or a variant of any one of SEQ ID NOs: 227, 228, 229, or 230, wherein

the variant of SEQ ID NO: 227 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-710 of SEQ ID NO: 227;

the variant of SEQ ID NO: 228 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 23-713 of SEQ ID NO: 228;

the variant of SEQ ID NO: 229 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-707 of SEQ ID NO: 229; or

the variant of SEQ ID NO: 230 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-707 of SEQ ID NO: 230; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 231; or a variant of SEQ ID NO: 231, wherein the variant of SEQ ID NO: 231 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-449 of SEQ ID NO: 231;

(ii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 232, 233, 234, or 235; or a variant of any one of SEQ ID NOs: 232, 233, 234, or 235, wherein

the variant of SEQ ID NO: 232 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-678 of SEQ ID NO: 232;

the variant of SEQ ID NO: 233 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 23-681 of SEQ ID NO: 233;

the variant of SEQ ID NO: 234 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 23-678 of SEQ ID NO: 234; or

the variant of SEQ ID NO: 235 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 23-678 of SEQ ID NO: 235; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 236; or a variant of SEQ ID NO: 236, wherein the variant of SEQ ID NO: 236 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-437 of SEQ ID NO: 236;

(iii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 237, 238, or 239; or a variant of any one of SEQ ID NOs: 237, 238, or 239, wherein

the variant of SEQ ID NO: 237 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-605 of SEQ ID NO: 237;

the variant of SEQ ID NO: 238 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-606 of SEQ ID NO: 238; or

the variant of SEQ ID NO: 239 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-605 of SEQ ID NO: 239; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 240; or a variant of SEQ ID NO: 240, wherein the variant of SEQ ID NO: 240 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15-83 of SEQ ID NO: 240;

(iv) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 241; or a variant of SEQ ID NO: 241, wherein the variant of SEQ ID NO: 241 comprises a variation at

one or more nucleotide positions selected from the group consisting of positions 20-34 of SEQ ID NO: 241; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 242; or a variant of any one of SEQ ID NO: 242, wherein the variant of SEQ ID NO: 242 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 100-593 of SEQ ID NO: 242;

(v) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 243; or a variant of SEQ ID NO: 243, wherein the variant of SEQ ID NO: 243 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 30-45 of SEQ ID NO: 243; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 244; or a variant of SEQ ID NO: 244, wherein the variant of SEQ ID NO: 244 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 100-677 of SEQ ID NO: 244; and

(vi) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 245, 246, or 247; or a variant of any one of SEQ ID NOs: 245, 246, or 247, wherein

the variant of SEQ ID NO: 245 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 29-171 of SEQ ID NO: 245;

the variant of SEQ ID NO: 246 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 30-171 of SEQ ID NO: 246; or

the variant of SEQ ID NO: 247 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 29-171 of SEQ ID NO: 247; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 248; or a variant of SEQ ID NO: 248, wherein the variant of SEQ ID NO: 248 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20-91 of SEQ ID NO: 248.

16. The isolated RNA polynucleotide of any one of claims 1 to 8, wherein the virus is an alphavirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 249; or a variant of SEQ ID NO: 249, wherein the variant of SEQ ID NO: 249 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60-274 of SEQ ID NO: 249; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 250 or 251; or a variant of SEQ ID NO: 250 or 251, wherein

the variant of SEQ ID NO: 250 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60–183 of SEQ ID NO: 250; or

the variant of SEQ ID NO: 251 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60–375 of SEQ ID NO: 251;

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 255; or a variant of SEQ ID NO: 255, wherein the variant of SEQ ID NO: 255 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60-35 of SEQ ID NO: 255; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 256 or 257; or a variant of SEQ ID NO: 256 or 257, wherein

the variant of SEQ ID NO: 256 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 600–273 of SEQ ID NO: 256; or

the variant of SEQ ID NO: 257 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60–377 of SEQ ID NO: 257; and

(iii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 261; or a variant of SEQ ID NO: 261, wherein the variant of SEQ ID NO: 261 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60-215 of SEQ ID NO: 261; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 262 or 263; or a variant of SEQ ID NO: 262 or 263, wherein

the variant of SEQ ID NO: 262 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60–166 of SEQ ID NO: 262; or

the variant of SEQ ID NO: 263 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 60–379 of SEQ ID NO: 263.

17. An isolated RNA polynucleotide, the isolated RNA polynucleotide comprising a coding region having a coding sequence encoding one or more polypeptides; and template regions, wherein the template regions comprise two distinct regions, a left flanking region (“L region”) of a virus and a right flanking region (“R region”) of the virus, wherein the L region is adjacent to and contiguous with a 5' end of the coding region and the R region is adjacent to and contiguous with a 3' end of the coding region;
 - wherein the coding sequence is in a sense orientation;
 - wherein the polypeptide is heterologous to the virus;
 - wherein the template region interacts with and initiates RNA-dependent polymerase activity of a polymerase in a cell containing the RNA dependent polymerase;
 - wherein the isolated RNA polynucleotide comprises at least one nucleoside modification; and
 - wherein the virus is not an alphavirus.
18. The isolated RNA polynucleotide of claim 17,
 - wherein the template regions are nucleoside modified, wherein the percentage of modified nucleosides is not more than 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5%;
 - wherein the template regions are nucleoside modified, wherein the percentage of modified nucleosides is at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, 95%, or 100%;
 - wherein the nucleoside modification is a nonimmunogenic uridine modification, and the percentage of modified uridine modifications is not more than 40%, 35%, 30%, 25%, 20%, 15%, or 10%;
 - wherein the nucleoside modification is a nonimmunogenic uridine modification, and the percentage of modified uridine modifications is more than 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95%, or is 100%;
 - wherein the nucleoside modification is a nonimmunogenic cytidine modification, and the percentage of modified cytidine modifications is not more than 40%, 35%, 30%, 25%, 20%, 15%, or 10%;

wherein the nucleoside modification is a nonimmunogenic cytidine modification, and the percentage of modified cytidine modifications is more than 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95%, or is 100%; or

wherein the nucleoside modification is a nonimmunogenic adenosine modification, and the percentage of modified adenosine modifications is between 1% and 30%, and optionally wherein the percentage of modified adenosine modifications is about 1%, 5%, 10%, 15%, 20%, 25%, or 30%.

19. The isolated RNA polynucleotide of any one of claims 17 to 18, wherein the isolated polynucleotide comprises a 5' cap structure; wherein the 5' end of the L region comprises a 5' cap structure; wherein the 5' end of the L region comprises one or more variations associated with a 5' cap structure; wherein the 5' end of the isolated polynucleotide comprises a 5'-monophosphate; wherein the 5' end of the isolated polynucleotide does not comprise a 5'-phosphate (dephosphorylated); wherein the 5' end of the L region comprises a 5'-monophosphate; or wherein the 5' end of the L region does not comprise a 5'-phosphate (dephosphorylated).

20. The isolated RNA polynucleotide of claim 19, wherein the 5'-cap structure is selected from the group consisting of Cap 0, Cap 0 (3'-O-Me), Cap 1, Cap 1 (3'-O-Me), Cap 2, Cap 2 (3'-O-Me), Anti-Reverse Cap Analog (ARCA), inosine, N1-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, locked nucleic acid guanosine (LNA-guanosine), and 2-azido-guanosine structure.

21. The isolated RNA polynucleotide of any one of claims 17 to 20, wherein the virus is selected from the group consisting of viruses in the orders of *Amarillovirales*, *Articulavirales*, *Blubervirales*, *Bunyavirales*, *Hepelivirales*, *Mononegavirales*, *Nidovirales*, and *Picornavirales*,

wherein the virus is selected from the group consisting of viruses in the families of *Arenaviridae*, *Coronaviridae*, *Filoviridae*, *Flaviviridae*, *Hantaviridae*, *Hepadnaviridae*,

Matonaviridae, Nairoviridae, Orthomyxoviridae, Paramyxoviridae, Phenuiviridae, Picornaviridae, Pneumoviridae, and Rhabdoviridae, or

wherein the virus is from the group consisting of Alphacoronavirus 229E, Alphacoronavirus NL63, Alphacoronavirus WA2028, Avian metapneumovirus (AMPV), Betacoronavirus HKU1, Betacoronavirus HKU15, Betacoronavirus HKU33, Betacoronavirus OC43, Chikungunya virus, Crimean-Congo Hemorrhagic Fever Virus, Dengue Virus, Enterovirus D68 (EV-D68), Foot and Mouth Disease Virus, Hanta Virus, Hendra Virus, Hepatitis B Virus, Hepatitis C Virus, HMPV, Human Parainfluenzavirus 1 (HPIV1), Human Parainfluenzavirus 3 (HPIV3), Infectious Salmon Anemia Virus, Influenza A Virus, Influenza B Virus, Lassa Virus, Marburg Virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Newcastle Disease Virus (NDV), Nipah Virus, Norwalk Virus, Rabies Virus, Respiratory Syncytial Virus, Reston Ebola virus, Rhinovirus, Rift Valley Fever Virus, Rubella virus, SARS-CoV-1, SARS-CoV-2, Sudan Ebola virus, Vesicular Stomatitis Virus, Yellow Fever Virus, Zaire Ebola virus, and Zika Virus.

22. The isolated RNA polynucleotide of any one of claims 17 to 21,
wherein the template regions are native to the virus,
wherein the template regions are variants of template regions native to the virus,
wherein the variants have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the template regions native to the virus;
wherein each of the L and the R regions of the template regions comprises fewer than 10, 9, 8, 7, 6, 5, 4, 3, or 2 variations relative to template regions native to the virus;
wherein each of the L and the R regions of the template regions varies from template regions native to the virus by not more than 10, 9, 8, 7, 6, 5, 4, 3, or 2 substitutions that are not involved in 5' capping; or
wherein each of the L and the R regions of the template regions varies from template regions native to the virus by not more than 1 substitution that is not involved in 5' capping.
23. The isolated RNA polynucleotide of any one of claims 17 to 22,
wherein the therapeutic polypeptide is a secreted polypeptide, optionally an antibody;
wherein the therapeutic polypeptide is selected from the group consisting of an interferon, an interferon stimulated gene, a cytokine, a chemokine, an antibody, a signaling molecule, a cytotoxic protein, a protein that causes cell death, an antineoplastic protein, an immunomodulatory protein, protein toll-like receptor agonist, or a dominant negative protein,

optionally wherein the cytokine is an inflammatory cytokine, optionally wherein the inflammatory cytokine is TNF- α , optionally wherein the cytokine is an anti-inflammatory cytokine, optionally wherein the anti-inflammatory cytokine is an interleukin-1 receptor antagonist (IL-1RN);

wherein the therapeutic polypeptide is an interleukin or a caspase, optionally wherein the interleukin is IL-12A, IL-12B or IL-2; or

wherein the therapeutic polypeptide is an interferon, optionally an IFN- α , IFN- β , IFN- ϵ , IFN- κ , IFN- ω , IFN- γ , or IFN- λ , optionally IFN- α 1, IFN- α 2, IFN- α 4, IFN- α 5, IFN- α 6, IFN- α 7, IFN- α 8, IFN- α 10, IFN- α 13, IFN- α 14, IFN- α 16, IFN- α 17, IFN- α 21, IFN- β 1, IFN- ϵ , IFN- κ , IFN- ω 1, IFN- γ , IFN- λ 1 (IL28A), IFN- λ 2 (IL28B), IFN- λ 3 (IL29), or IFN- λ 4.

24. The isolated RNA polynucleotide of any one of claims 17 to 23,

wherein the coding sequence encodes more than one therapeutic polypeptide, which are separated by one or more ribosomal skipping sequences;

wherein the coding region further comprises one or more regulatory elements selected from the group consisting of a ribosomal binding site, Kozak sequence, Shine-Dalgarno sequence, ribozyme, riboswitch, promoter, microRNA binding site, an internal ribosomal entry site (IRES), optionally wherein the one or more regulatory elements are operably linked to the coding sequence;

wherein the isolated RNA polynucleotide further comprises a polyadenylation signal and/or a 3' poly(A) tail;

wherein the RNA-dependent polymerase is an RNA-dependent RNA polymerase or an RNA-dependent DNA polymerase; optionally, wherein the RNA-dependent polymerase is a polymerase is from the virus;

wherein the isolated RNA polynucleotide is a single stranded RNA;

wherein the isolated polynucleotide is in linear form; or

wherein the isolated polynucleotide is in a covalently-closed circular form.

25. The isolated RNA polynucleotide of any one of claims 15 to 24, wherein the virus is a sarbecovirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66, or 67; or a variant of any one of SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66, or 67, wherein the variant comprises a variation at one or more nucleotide positions

selected from the group consisting of positions 39–789 or 1426–1493 of any one of SEQ ID NOs: 60, 61, 62, 63, 64, 65, 66, or 67; and

the R region comprises the nucleotide sequence set forth SEQ ID NO: 129; or a variant of SEQ ID NO: 129, wherein the variant of SEQ ID NO: 129 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20–320 of SEQ ID NO: 129;

(ii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 68, 69, 70, 71, 72, 73, 74, 75, 76, or 77; or a variant of any one of SEQ ID NOs: 68, 69, 70, 71, 72, 73, 74, 75, 76, or 77, wherein

the variant of SEQ ID NO: 68 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1434–1501 of SEQ ID NO: 68;

the variant of SEQ ID NO: 69 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1434–1501 of SEQ ID NO: 69;

the variant of SEQ ID NO: 70 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1446–1513 of SEQ ID NO: 70;

the variant of SEQ ID NO: 71 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1455–1522 of SEQ ID NO: 71;

the variant of SEQ ID NO: 72 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1462–1529 of SEQ ID NO: 72;

the variant of SEQ ID NO: 73 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1469–1536 of SEQ ID NO: 73;

the variant of SEQ ID NO: 74 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1485–1552 of SEQ ID NO: 74;

the variant of SEQ ID NO: 75 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1686–1753 of SEQ ID NO: 75;

the variant of SEQ ID NO: 76 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1704–1771 of SEQ ID NO: 76; or

the variant of SEQ ID NO: 77 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1720–1787 of SEQ ID NO: 77; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 130; or a variant of SEQ ID NO: 130, wherein the variant of SEQ ID NO: 130 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20–320 of SEQ ID NO: 130;

(iii) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 78, 79, 80, 81, 82, 83, 85, 86, 87, or 88; or a variant of any one of SEQ ID NOs: 78, 79, 80, 81, 82, 83, 85, 86, 87, or 88, wherein

the variant of SEQ ID NO: 78 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1734–1801 of SEQ ID NO: 78;

the variant of SEQ ID NO: 79 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1687–1754 of SEQ ID NO: 79;

the variant of SEQ ID NO: 80 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1695–1762 of SEQ ID NO: 80;

the variant of SEQ ID NO: 81 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1434–1501 of SEQ ID NO: 81;

the variant of SEQ ID NO: 82 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1443–1510 of SEQ ID NO: 82;

the variant of SEQ ID NO: 83 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1459–1526 of SEQ ID NO: 83;

the variant of SEQ ID NO: 85 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1434–1501 of SEQ ID NO: 85;

the variant of SEQ ID NO: 86 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1434–1501 of SEQ ID NO: 86;

the variant of SEQ ID NO: 87 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1435–1502 of SEQ ID NO: 87; or

the variant of SEQ ID NO: 88 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1463–1530 of SEQ ID NO: 88; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 130; or a variant of SEQ ID NO: 130, wherein the variant of SEQ ID NO: 130 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20–320 of SEQ ID NO: 130;

(iv) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 89, 90, 91, 92, 96, 104, 105, 106, 107, or 108; or a variant of any one of SEQ ID NOs: 89, 90, 91, 92, 96, 104, 105, 106, 107, or 108, wherein

the variant of SEQ ID NO: 89 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1466–1533 of SEQ ID NO: 89;

the variant of SEQ ID NO: 90 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1425–1492 of SEQ ID NO: 90;

the variant of SEQ ID NO: 91 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1425–1492 of SEQ ID NO: 91;

the variant of SEQ ID NO: 92 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1425–1492 of SEQ ID NO: 92;

the variant of SEQ ID NO: 96 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–769 or 1471–1471 of SEQ ID NO: 96;

the variant of SEQ ID NO: 104 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1446–1513 of SEQ ID NO: 104;

the variant of SEQ ID NO: 105 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1455–1522 of SEQ ID NO: 105;

the variant of SEQ ID NO: 106 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1462–1529 of SEQ ID NO: 106;

the variant of SEQ ID NO: 107 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 39–789 or 1469–1536 of SEQ ID NO: 107; or

the variant of SEQ ID NO: 108 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 89–839 or 1485–1552 of SEQ ID NO: 108; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 130; or a variant of SEQ ID NO: 130, wherein the variant of SEQ ID NO: 130 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20–320 of SEQ ID NO: 130;

(v) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 109, 110, 111, 112, 113, 114, 115, 116, 117, or 118; or a variant of any one of SEQ ID NOs: 109, 110, 111, 112, 113, 114, 115, 116, 117, or 118, wherein

the variant of SEQ ID NO: 109 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1686–1753 of SEQ ID NO: 109;

the variant of SEQ ID NO: 110 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1704–1771 of SEQ ID NO: 110;

the variant of SEQ ID NO: 111 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1720–1787 of SEQ ID NO: 111;

the variant of SEQ ID NO: 112 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1734–1801 of SEQ ID NO: 112;

the variant of SEQ ID NO: 113 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1687–1754 of SEQ ID NO: 113;

the variant of SEQ ID NO: 114 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1695–1762 of SEQ ID NO: 114;

the variant of SEQ ID NO: 115 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 115;

the variant of SEQ ID NO: 116 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 116;

the variant of SEQ ID NO: 117 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 117; or

the variant of SEQ ID NO: 118 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 118; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 130; or a variant of SEQ ID NO: 130, wherein the variant of SEQ ID NO: 130 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20–320 of SEQ ID NO: 130;

(vi) the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 119, 120, 122, 123, 124, 125, 126, or 127; or a variant of any one of SEQ ID NOs: 119, 120, 122, 123, 124, 125, 126, or 127, wherein

the variant of SEQ ID NO: 119 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1443–1510 of SEQ ID NO: 119;

the variant of SEQ ID NO: 120 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1459–1526 of SEQ ID NO: 120;

the variant of SEQ ID NO: 122 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 122;

the variant of SEQ ID NO: 123 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 123;

the variant of SEQ ID NO: 124 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1434–1501 of SEQ ID NO: 124;

the variant of SEQ ID NO: 125 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1463–1530 of SEQ ID NO: 125;

the variant of SEQ ID NO: 126 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1466–1533 of SEQ ID NO: 126;

the variant of SEQ ID NO: 127 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 40–789 or 1425–1492 of SEQ ID NO: 127; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 130; or a variant of SEQ ID NO: 130, wherein the variant of SEQ ID NO: 130 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 20–320 of SEQ ID NO: 130.

26. The isolated RNA polynucleotide of any one of claims 17 to 24, wherein the virus is a Respiratory Syncytial Virus (RSV),

wherein the L region comprises a nucleotide sequence set forth as any one of SEQ ID NOs: 148, 149, 150, 151, or 152; or a variant of any one of SEQ ID NOs: 148, 149, 150, 151, or 152, wherein

(i) the variant of SEQ ID NO: 148 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–78 of SEQ ID NO: 148;

(ii) the variant of SEQ ID NO: 149 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–33 of SEQ ID NO: 149;

(iii) the variant of SEQ ID NO: 150 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–35 of SEQ ID NO: 150;

(iv) the variant of SEQ ID NO: 151 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 18–36 of SEQ ID NO: 151; or

(v) the variant of SEQ ID NO: 152 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–38 of SEQ ID NO: 152;
and

wherein the R region comprises the nucleotide sequence set forth as SEQ ID NO: 154 or 155; or a variant of SEQ ID NO: 154 or 155, wherein

(i) the variant of SEQ ID NO: 154 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–207 of SEQ ID NO: 154; or

(ii) the variant of SEQ ID NO: 155 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 15–32 of SEQ ID NO: 155.

27. The isolated RNA polynucleotide of any one of claims 17 to 24, wherein the virus is a parainfluenzavirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 180; or a variant of SEQ ID NO: 180, wherein the variant of SEQ ID NO: 180 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-136 of SEQ ID NO: 180; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 179; or a variant of SEQ ID NO: 179, wherein the variant of SEQ ID NO: 179 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-98 of SEQ ID NO: 179; or

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 186; or a variant of SEQ ID NO: 186, wherein the variant of SEQ ID NO: 186 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-95 of SEQ ID NO: 186; and

wherein the R region comprises the nucleotide sequence set forth as SEQ ID NO: 185; or a variant of SEQ ID NO: 185, wherein the variant of SEQ ID NO: 185 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 21-93 of SEQ ID NO: 185.

28. The isolated RNA polynucleotide of any one of claims 17 to 24, wherein the virus is a metapneumovirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 194; or a variant of SEQ ID NO: 194, wherein the variant of SEQ ID NO: 194 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-220 of SEQ ID NO: 194; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 192; or a variant of any one of SEQ ID NO: 192, wherein the variant of SEQ ID NO: 192 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-32 of SEQ ID NO: 192; or

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 193; or a variant of SEQ ID NO: 193, wherein the variant of SEQ ID NO: 193 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-220 of SEQ ID NO: 193; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 191; or a variant of SEQ ID NO: 191, wherein the variant of SEQ ID NO: 191 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-32 of SEQ ID NO: 191.

29. The isolated RNA polynucleotide of any one of claims 17 to 24, wherein the virus is a henipavirus, and wherein a combination of the L region and the R region satisfies one of the following:

(i) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 203; or a variant of SEQ ID NO: 203, wherein the variant of SEQ ID NO: 203 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-77 of SEQ ID NO: 203; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 202; or a variant of SEQ ID NO: 202, wherein the variant of SEQ ID NO: 202 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-91 of SEQ ID NO: 202; or

(ii) the L region comprises a nucleotide sequence set forth as SEQ ID NO: 207; or a variant of SEQ ID NO: 207, wherein the variant of SEQ ID NO: 207 comprises a variation at

one or more nucleotide positions selected from the group consisting of positions 17-77 of SEQ ID NO: 207; and

the R region comprises the nucleotide sequence set forth as SEQ ID NO: 208; or a variant of SEQ ID NO: 208, wherein the variant of SEQ ID NO: 208 comprises a variation at one or more nucleotide positions selected from the group consisting of positions 17-91 of SEQ ID NO: 208.

30. An RNA-protein complex, comprising
the isolated RNA polynucleotide of any one of claims 1 to 29, and
an RNA-binding protein, optionally a viral nucleocapsid protein (N) or viral capsid protein, or optionally a viral nucleocapsid protein (N) or viral capsid protein of the virus, optionally wherein the viral nucleocapsid protein or a viral capsid protein is from an influenza virus, sarbecovirus, pneumovirus, paramyxovirus, henipavirus, or hepadnavirus;
wherein the isolated RNA polynucleotide of the RNA-protein complex has increased stability as compared to the isolated RNA polypeptide without the RNA binding protein.

31. A composition comprising the isolated RNA polynucleotide of any one of claims 1 to 29 or the RNA-protein complex of claim 30, optionally further comprising a pharmaceutically acceptable carrier; or
a nanoparticle comprising the isolated RNA polynucleotide of any one of claims 1 to 29 or the RNA-protein complex of claim 30.

32. The isolated RNA polynucleotide of any one of claims 1 to 29, the RNA-protein complex of claim 30, or the composition or the nanoparticle of claim 31, for use as a medicament to be administered to a subject in need thereof;
optionally wherein the isolated RNA polynucleotide of any one of claims 1 to 29, the RNA-protein complex of claim 30, or the composition or the nanoparticle of claim 31 is to be administered to a subject in need thereof with a second isolated RNA polynucleotide of any one of claims 1 to 20, a second RNA-protein complex of claim 30, or a second composition or a second nanoparticle of claim 31;
optionally wherein the subject is a human, cow, pig, sheep, horse, deer, ruminants, rodent, fish, or fowl;
optionally wherein the subject has a disease or disorder resulting from a viral infection;

optionally wherein the subject has an infection with a virus;
optionally wherein the medicament is to be administered by intratracheal or inhalation, intranasal, oral, rectal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intradermal, transdermal (topical), intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, or intraperitoneal administration.

33. The isolated RNA polynucleotide of any one of claims 1 to 29, the RNA-protein complex of claim 30, or the composition or the nanoparticle of claim 31, for use in contacting a cell,

optionally wherein the contacting is *in vitro* or *ex vivo*.

34. A combination of:

(i) a therapeutically effective amount of the isolated RNA polynucleotide of any one of claims 1 to 29, the RNA-protein complex of claim 30, or the composition or the nanoparticle of claim 31; and

(ii) a second polynucleotide encoding a polymerase capable of interacting with and initiating the transcription or translation of the therapeutic polypeptide or polypeptide,

for use as a medicament to be administered to a subject in need thereof;

optionally wherein (i) and (ii) are to be administered sequentially or simultaneously;

optionally wherein (i) and (ii) are present on the same polynucleotide, or wherein (i) and (ii) are present on separate polynucleotides;

optionally wherein the combination of (i) and (ii) are to be administered to the subject with (iii) one or more accessory proteins associated with polymerase activity,

optionally wherein the accessory protein is a nucleocapsid protein;

optionally wherein the polymerase and/or accessory proteins are to be administered in the form of one or more nucleic acids encoding the polymerase and/or accessory proteins

optionally wherein the combination of (i) and (ii), and optionally (iii), are to be administered to the subject in need thereof with a therapeutically effective amount of a second isolated RNA polynucleotide of any one of claims 1 to 29, a second RNA-protein complex of claim 30, or a second composition or a second nanoparticle of claim 31;

optionally wherein the subject is a human, cow, pig, sheep, horse, deer, ruminant, rodent, fish, or fowl.