CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising a recombinant polynucleic acid construct, wherein the recombinant polynucleic acid construct is an RNA construct comprising:

   (i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target RNA; and

   (ii) at least one nucleic acid sequence encoding a gene of interest;

   wherein the target RNA is different from an mRNA encoded by the gene of interest, and

   wherein the at least one nucleic acid sequence encoding the gene of interest and the at least one nucleic acid sequence encoding or comprising the siRNA are comprised in a sequential manner and wherein the at least one nucleic acid sequence encoding the gene of interest and the at least one nucleic acid sequence encoding or comprising the siRNA are simultaneously expressed from a single transcript.

2. The composition of claim 1, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA and the at least one nucleic acid sequence encoding the gene of interest are separated.

3. The composition of claim 1, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is downstream of the at least one nucleic acid sequence encoding the gene of interest.

4. The composition of claim 1, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is upstream of the at least one nucleic acid sequence encoding the gene of interest.

5.2. The composition of claim 1, wherein the siRNA does not inhibit the expression of the gene of interest.

6. The composition of claim 1, wherein expression of the gene of interest is modulated, and optionally upregulated by expressing the mRNA or a protein encoded by the gene of interest.

7. The composition of claim 3, wherein expression of the gene of interest is increased compared to the expression of the gene of interest from a recombinant polynucleic acid construct comprising: at least one nucleic acid sequence encoding or comprising an siRNA capable of binding to a target RNA; and at least one nucleic acid sequence encoding the gene of interest, wherein the nucleic acid sequence encoding or

comprising the siRNA capable of binding to the target RNA is upstream of the nucleic acid sequence encoding the gene of interest.

8. The composition of claim 1, wherein the expression of the target RNA is modulated, and optionally downregulated by the siRNA capable of binding to the target RNA.

9. The composition of claim 3, wherein downregulation of the target RNA is enhanced compared to the downregulation of the target RNA from a recombinant polynucleic acid construct comprising: at least one nucleic acid sequence encoding or comprising an siRNA capable of binding to a target RNA; and at least one nucleic acid sequence encoding the gene of interest; wherein the nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is upstream of the nucleic acid sequence encoding the gene of interest.

10.3. The composition of any one of claims 1-29, wherein the recombinant polynucleic acid construct comprises two or more nucleic acid sequences encoding or comprising an siRNA capable of binding to a target RNA, wherein each of the two or more nucleic acid sequences encodes or comprises an siRNA capable of binding to a same target RNA or a different target RNA.

11. The composition of any one of claims 1-10, wherein the recombinant polynucleic acid construct comprises two or more nucleic acid sequences encoding a gene of interest, wherein each of the two or more nucleic acid sequences encodes a same gene of interest or a different gene of interest.

12.4. The composition of any one of claims 1-113, wherein the recombinant polynucleic acid construct further comprises a nucleic acid sequence encoding or comprising a linker.

13. The composition of claim 12, wherein the nucleic acid sequence encoding or comprising the linker connects (a) the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA and the at least one nucleic acid sequence encoding the gene of interest, (b) each of the two or more nucleic acid sequences encoding or comprising the siRNA capable of binding to the target RNA, and/or (c) each of the two or more nucleic acid sequences encoding the gene of interest.

14. The composition of claim 12 or 13, wherein the linker comprises a tRNA linker, a 2A peptide linker or a flexible linker.

15. The composition of any one of claims 12-14, wherein the nucleic acid sequence encoding or comprising the linker is about 6 to about 80 nucleic acid residues in length.

16. The composition of any one of claims 1-15, wherein the target RNA is an mRNA.

17.5. The composition of any one of claims 1-154, wherein the target RNA is an mRNA encoding a protein selected from the group consisting of: Interleukin 8 (IL-8), Interleukin 1 beta (IL-1 beta), Interleukin 17 (IL-17), Tumor Necrosis Factor alpha (TNF-alpha), Activin Receptor-like Kinase 2 (ALK2), and Superoxide Dismutase 1 (SOD1).

18.6. The composition of any one of claims 1-175, wherein the gene of interest is selected from the group consisting of Insulin-like Growth Factor 1 (IGF-1), Interleukin 4 (IL-4), and Erythropoietin (EPO).

19. The composition of any one of claims 1-18, wherein the recombinant polynucleic acid construct further comprises a nucleic acid sequence encoding a target motif operably linked to the at least one nucleic acid sequence encoding the gene of interest, wherein the target motif comprises a signal peptide, a nuclear localization signal (NLS), a nucleolar localization signal (NoLS), a lysosomal targeting signal, a mitochondrial targeting signal, a peroxisomal targeting signal, a microtubule tip localization signal (MtLS), an endosomal targeting signal, a chloroplast targeting signal, a Golgi targeting signal, an endoplasmic reticulum (ER) targeting signal, a proteasomal targeting signal, a membrane targeting signal, a transmembrane targeting signal, or a centrosomal localization signal (CLS).

20. The composition of claim 19, wherein the target motif is selected from the group consisting of:

(a) a target motif heterologous to a protein encoded by the gene of interest;

(b) a target motif heterologous to a protein encoded by the gene of interest, wherein the target motif heterologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid;

(c) a target motif homologous to a protein encoded by the gene of interest, wherein the target motif homologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid; and

(d) a naturally occurring amino acid sequence which does not have the function of a target motif in nature, wherein the naturally occurring amino acid

sequence is optionally modified by insertion, deletion, and/or substitution of at least one amino acid.

21. The composition of any one of claims 1-20, wherein the recombinant polynucleic acid construct further comprises a nucleic acid sequence encoding or comprising a poly(A) tail, a nucleic acid sequence encoding or comprising a 5' cap, a nucleic acid sequence encoding or comprising a promoter, or a nucleic acid sequence encoding or comprising a Kozak sequence.

22. The composition of any one of claims 1-21, wherein the recombinant polynucleic acid construct is an RNA construct.

23.7. The composition of any one of claims 1-216, wherein the recombinant polynucleic acid construct is a vector suitable for gene therapy.

24.8. A composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-8 and SEQ ID NOs: 152-158.

25.9. The composition of any one of claims 1-248, wherein the siRNA comprises a sense strand sequence encoded by a sequence selected from SEQ ID NOs: 80-92 and SEQ ID NOs: 140-145.

26. A composition comprising a recombinant polynucleic acid construct for treatment or prevention of a viral disease or condition in a subject, the construct comprising:

(i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target RNA; and

(ii) at least one nucleic acid sequence encoding a gene of interest;

wherein the target RNA is different from an mRNA encoded by the gene of interest.

27. The composition of claim 26, wherein the recombinant polynucleic acid construct comprises two or more nucleic acid sequences encoding or comprising an siRNA capable of binding to a target RNA, wherein each of the two or more nucleic acid sequences encodes or comprises an siRNA capable of binding to a same target RNA or a different target RNA.

28. The composition of claim 26, wherein the recombinant polynucleic acid construct comprises three or more nucleic acid sequences encoding or comprising an siRNA capable of binding to a target RNA, wherein at least two nucleic acid sequences encode or comprise an siRNA capable of binding to the same target RNA and at least one nucleic acid sequence encodes or comprises an siRNA capable of binding to a different target RNA .

29. The composition of any one of claims 26-28, wherein the target RNA is an mRNA.
30. The composition of any one of claims 26-28, wherein the target RNA is a noncoding RNA.
31. The composition of any one of claims 26-28, wherein the target RNA is an mRNA encoding a protein selected from the group consisting of: interleukin, Angiotensin Converting Enzyme-2 (ACE2), SARS-CoV-2 ORF1ab, SARS-CoV-2 S, and SARS-CoV-2 N.
32. The composition of claim 31, wherein the interleukin is selected from the group consisting of: IL-1-alpha, IL-1-beta, IL-6, IL-6R, IL-6R-alpha, interleukin IL-6R-beta, IL-18, IL-36-alpha, IL-36-beta; IL-36-gamma, and IL-33.
33. The composition of any one of claims 26-28, wherein the target RNA is an mRNA encoding a protein selected from the group consisting of: IL-6, IL-6R, IL-6R-alpha, IL-6R-beta, Angiotensin Converting Enzyme-2 (ACE2), SARS-CoV-2 ORF1ab, SARS-CoV-2 S, and SARS-CoV-2 N.
34. The composition of any one of claims 26-33, wherein the recombinant polynucleic acid construct comprises two or more nucleic acid sequences encoding a gene of interest, wherein each of the two or more nucleic acid sequences encodes a same gene of interest or a different gene of interest.
35. The composition of any one of claims 26-34, wherein the gene of interest of is selected from the group of genes encoding: IFN alpha-n3, IFN alpha-2a, IFN alpha-2b, IFN beta-1a, IFN beta-1b, ACE2 soluble receptor, IL-37, and IL-38.
36. The composition of any one of claims 26-34, wherein the gene of interest of is selected from the group of genes encoding: IFN beta and ACE2 soluble receptor.
37. The composition of any one of claims 26-36, wherein the recombinant polynucleic acid construct further comprises a nucleic acid sequence encoding a target motif operably linked to the at least one nucleic acid sequence encoding the gene of interest, wherein the target motif comprises a signal peptide, a nuclear localization signal (NLS), a nucleolar localization signal (NoLS), a lysosomal targeting signal, a mitochondrial targeting signal, a peroxisomal targeting signal, a microtubule tip localization signal (MtLS), an endosomal targeting signal, a chloroplast targeting signal, a Golgi targeting signal, an endoplasmic reticulum (ER) targeting signal, a proteasomal targeting signal, a membrane targeting signal, a transmembrane targeting signal, or a centrosomal localization signal (CLS).

38. The composition of claim 37, wherein the target motif is selected from the group consisting of:

(a) a target motif heterologous to a protein encoded by the gene of interest;

(b) a target motif heterologous to a protein encoded by the gene of interest, wherein the target motif heterologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid;

(c) a target motif homologous to a protein encoded by the gene of interest, wherein the target motif homologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid; and

(d) a naturally occurring amino acid sequence which does not have the function of a target motif in nature, wherein the naturally occurring amino acid sequence is optionally modified by insertion, deletion, and/or substitution of at least one amino acid.

39. The composition of any one of claims 26-38, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is upstream of the at least one nucleic acid sequence encoding the gene of interest.

40. The composition of any one of claims 26-38, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is downstream of the at least one nucleic acid sequence encoding the gene of interest.

41. The composition of any one of claims 26-40, wherein the recombinant polynucleic acid construct further comprises a nucleic acid sequence encoding or comprising a poly(A) tail, a nucleic acid sequence encoding or comprising a 5' cap, a nucleic acid sequence encoding or comprising a promoter, or a nucleic acid sequence encoding or comprising a Kozak sequence.

42. The composition of any one of claims 26-41, wherein the recombinant polynucleic acid construct further comprises a nucleic acid sequence encoding or comprising a linker.

43. The composition of claim 42, wherein the nucleic acid sequence encoding or comprising the linker connects (a) the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target mRNA and the at least one nucleic acid sequence encoding the gene of interest, (b) each of the two or more nucleic acid sequences encoding or comprising the siRNA capable of binding to the target mRNA, and/or (c) each of the two or more nucleic acid sequences encoding the gene of interest.

44. The composition of claim 42 or 43, wherein the linker comprises a tRNA linker, a 2A peptide linker, or a flexible linker.

45. The composition of any one of claims 42-44, wherein the nucleic acid sequence encoding or comprising the linker is about 6 to about 80 nucleic acid residues in length.

46. The composition of any one of claims 26-45, wherein the recombinant polynucleic acid construct is an RNA construct.

47. A composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 29-47.

48. The composition of any one of claims 26-47, wherein the composition is present in an amount sufficient to treat or prevent the viral disease or condition in the subject.

49. The composition of any one of claims 26-47, wherein the siRNA comprises a sense strand sequence encoded by a sequence selected from SEQ ID NOs: 93-109.

50.10. The composition of any one of claims 1-49 for use in simultaneously modulating the expression of two or more genes in a cell.

51.11. The composition of any one of claims 1-509, wherein the siRNA capable of binding to the target RNA binds to an exon of the target RNA.

52. The composition of any one of claims 1-51, wherein the siRNA capable of binding to the target RNA specifically binds to one target RNA.

53.12. The composition of any one of claims 1-529 and 11, wherein the siRNA capable of binding to the target RNA is not encoded by or comprised of an intron sequence of the gene of interest.

54.13. The composition of any one of claims 1-539 and 11-12, wherein the gene of interest is expressed without RNA splicing.

55. A composition comprising a recombinant polynucleic acid construct for treatment or prevention of a skin disease or condition in a subject, the construct comprising:

(i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target RNA; and

(ii) at least one nucleic acid sequence encoding a gene of interest;

wherein the target RNA is different from an mRNA encoded by the gene of interest.

56. A composition comprising a recombinant polynucleic acid construct for treatment or prevention of a muscular disease or condition in a subject, the construct comprising:

(i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target RNA; and

(ii) at least one nucleic acid sequence encoding a gene of interest;

wherein the target RNA is different from an mRNA encoded by the gene of interest.

57. A composition comprising a recombinant polynucleic acid construct for treatment or prevention of a neurodegenerative disease or condition in a subject, the construct comprising:

(i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target RNA; and

(ii) at least one nucleic acid sequence encoding a gene of interest;

wherein the target RNA is different from an mRNA encoded by the gene of interest.

58. A composition comprising a recombinant polynucleic acid construct for treatment or prevention of a joint disease or condition in a subject, the construct comprising:

(i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target RNA; and

(ii) at least one nucleic acid sequence encoding a gene of interest;

wherein the target RNA is different from an mRNA encoded by the gene of interest.

59. The composition of any one of claims 55-58, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA and the at least one nucleic acid sequence encoding the gene of interest are comprised in a sequential manner.

60. The composition of claim 59, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is upstream of the at least one nucleic acid sequence encoding the gene of interest.

61. The composition of claim 59, wherein the at least one nucleic acid sequence encoding or comprising the siRNA capable of binding to the target RNA is downstream of the at least one nucleic acid sequence encoding the gene of interest.

62. The composition of any one of claims 55-61, wherein the target RNA is an mRNA encoding a protein selected from the group consisting of: Interleukin 8 (IL-8), Interleukin 1 beta (IL-1 beta), Interleukin 17 (IL-17), Tumor Necrosis Factor alpha

(TNF-alpha), Activin Receptor-like Kinase 2 (ALK2), and Superoxide Dismutase 1 (SOD1).

63. The composition of any one of claims 55-61, wherein the gene of interest is selected from the group consisting of Insulin-like Growth Factor 1 (IGF-1), Interleukin 4 (IL-4), and Erythropoietin (EPO).

64. The composition of any one of claims 55-63, wherein the subject is a human.

65.14. A The composition of any one of claims 1-9 and 11-13 for use in the treatment of method of treating a skin disease or condition, a muscular disease or condition, a neurodegenerative disease or condition or a joint disease or condition, wherein comprising administering to a subject in need thereof the composition of any one of claims 1-25 and 50-54is to be administered to a subject in need thereof.

15. Use of the composition of any one of claims 1-9 and 11-13 in the manufacture of a medicament for the treatment of a skin disease or condition, a muscular disease or condition, a neurodegenerative disease or condition or a joint disease or condition, wherein the medicament is to be administered in a subject in need thereof.

16. A method of simultaneously modulating the expression of two or more genes in a cell, comprising introducing into the cell a recombinant polynucleic acid construct, wherein the recombinant polynucleic acid construct is an RNA construct, comprising: (i) at least one nucleic acid sequence encoding or comprising a small interfering RNA (siRNA) capable of binding to a target messenger RNA (mRNA); and (ii) at least one nucleic acid sequence encoding a gene of interest; wherein the target mRNA is different from an mRNA encoded by the gene of interest, and wherein the expression of the target mRNA and the gene of interest is modulated simultaneously from a single transcript.

66. The method of claim 65, wherein the skin disease or condition comprises an inflammatory skin disorder.

67. The method of claim 66, wherein the inflammatory skin disorder comprises psoriasis.

68. A method of treating a muscular disease or condition comprising administering to a subject in need thereof the composition of any one of claims 1-25 and 50-54.

69. The method of claim 68, wherein the muscular disease or condition comprises a skeletal muscle disorder.

70. The method of claim 69, wherein the skeletal muscle disorder comprises fibrodysplasia ossificans progressiva (FOP).
71. A method of treating a neurodegenerative disease or condition comprising administering to a subject in need thereof the composition of any one of claims 1-25 and 50-54.
72. The method of claim 71, wherein the neurodegenerative disease or condition comprises a motor neuron disorder.
73. The method of claim 72, wherein the motor neuron disorder comprises amyotrophic lateral sclerosis (ALS).
74. A method of treating a joint disease or condition comprising administering to a subject in need thereof the composition of any one of claims 1-25 and 50-54.
75. The method of claim 74, wherein the joint disease or condition comprises a joint degeneration.
76. The method of claim 75, wherein the joint degeneration comprises intervertebral disc disease (IVDD) or osteoarthritis (OA).
77. The method of any one of claims 65-76, wherein the subject is a human.