

CLAIMS

WHAT IS CLAIMED IS:

1. A polynucleotide encoding a polypeptide construct comprising a S100 calcium-binding protein (S100) polypeptide, and a second functional polypeptide, wherein said second functional polypeptide is at least one of a cytokine, a chemokine, an angiogenic polypeptide, and any functional derivative thereof.
2. The polynucleotide of claim 1, wherein said S100 polypeptide is S100A1, fragment or variant thereof.
3. The polynucleotide of any one of claims 1 to 2, wherein said second functional polypeptide is an angiogenic polypeptide.
4. The polynucleotide of claim 3, wherein said angiogenic polypeptide is a vascular endothelial growth factor (VEGF) polypeptide.
5. The polynucleotide of claim 4, wherein said VEGF polypeptide is selected from a group consisting of VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF191, VEGF206, fragments and variants thereof.
6. The polynucleotide of any one of claims 4 to 5, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF165.
7. The polynucleotide of any one of claims 4 to 5, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF191.
8. The polynucleotide of any one of claims 1 to 2, wherein said second functional polypeptide is a chemokine.
9. The polynucleotide of claim 8, wherein said chemokine is a stromal cell-derived factor 1 (SDF) polypeptide.
10. The polynucleotide of claim 9, wherein said SDF polypeptide is selected from the group consisting of SDF1, SDF-1 α , SDF-1 β , fragments and variants thereof.
11. The polynucleotide of any one of claims 9 to 10, wherein said SDF polypeptide comprises a sequence with at least 70% identity to the sequence of SDF1.
12. The polynucleotide of any one of claims 1 to 11, wherein said S100 polypeptide is connected to said second polypeptide by a first polypeptide linker.
13. The polynucleotide of claim 12, wherein said linker is a cleavable linker.
14. The polynucleotide of any one of claims 1 to 13, wherein said polypeptide construct further comprises a third functional polypeptide.

15. The polynucleotide of claim 14, wherein said third functional polypeptide is at least one of a cytokine, a chemokine, an angiogenic polypeptide, and any functional derivative thereof.
16. The polynucleotide of any one of claims 14 to 15, wherein said third functional polypeptide is an angiogenic polypeptide.
17. The polynucleotide of claim 16, wherein said angiogenic polypeptide is a VEGF polypeptide.
18. The polynucleotide of claim 17, wherein said VEGF polypeptide is selected from a group consisting of VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF191, VEGF206, and fragments or variants thereof.
19. The polynucleotide of any one of claims 17 to 18, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF165.
20. The polynucleotide of any one of claims 17 to 18, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 191.
21. The polynucleotide of any one of claims 14 to 15, wherein said third functional polypeptide is a chemokine.
22. The polynucleotide of claim 21, wherein said chemokine is a SDF polypeptide.
23. The polynucleotide of claim 22, wherein said SDF polypeptide is selected from the group consisting of SDF1, SDF-1 α , SDF-1 β , fragments and variants thereof.
24. The polynucleotide of any one of claims 22 to 23, wherein said SDF polypeptide comprises a sequence with at least 70% identity to the sequence of SDF1.
25. The polynucleotide of any one of claims 14 to 24, wherein said third functional polypeptide is connected to at least one of said S100 polypeptide and said second functional polypeptide by a second polypeptide linker which is optionally cleavable.
26. The polynucleotide of any one of claims 12 to 25 wherein said first and second polypeptide linkers are independently selected from the list consisting of fp2a, p2a, GSG-p2a, and fmdv.
27. The polypeptide encoded by the polynucleotide of any one of claims 1 to 26.
28. A cell comprising a polynucleotide of any one of claims 1 to 26.
29. A method of treating a cardiac condition comprising contacting a cell with a therapeutically effective amount of a polynucleotide of any one of claims 1 to 26.
30. The method of claim 29, wherein said cell is a myocardial cell.
31. A method of treating cardiomyopathy in a subject comprising providing to the subject a therapeutically effective amount of a composition comprising a polynucleotide of any one of claims 1 to 26.
32. The method of claim 31, wherein said subject is a human subject.

33. The method of anyone of claims 31 to 32, wherein said subject is treated with at least one additional therapy.
34. A pharmaceutical composition comprising a polynucleotide of any one of claims 1 to 26, or a polypeptide encoded by said polynucleotide and a pharmaceutically acceptable excipient.
35. A polynucleotide encoding at least one polypeptide construct comprising at least a first polypeptide and a second polypeptide, wherein:
- said first polypeptide is an angiogenic polypeptide;
 - said second polypeptide is a chemokine or variant or fragment thereof.
36. The polynucleotide of claim 35, wherein said angiogenic polypeptide is VEGF polypeptide selected from a group consisting of VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF191, VEGF206, fragments and variants thereof.
37. The polynucleotide of claim 36, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 165.
38. The polynucleotide of claim 36, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 191.
39. The polynucleotide of claim 35, wherein said second polypeptide is a SDF polypeptide.
40. The polynucleotide of claim 39, wherein said SDF polypeptide is selected from the group consisting of SDF1, SDF-1 α , SDF-1 β , fragments and variants thereof.
41. The polynucleotide of any one of claims 39 to 40, wherein said SDF polypeptide comprises a sequence with at least 70% identity to the sequence of SDF1.
42. A method of improving vasculogenesis in a subject comprising providing to said subject a therapeutically effective amount of a composition comprising a polynucleotide of any one of claims 35 to 41, or a polypeptide encoded by said polynucleotide.
43. An expression vector comprising at least one promoter operably linked to at least two polypeptides selected from a SDF1 polypeptide, a S100A1 polypeptide, a VEGF polypeptide and fragments and variants thereof.
44. The expression vector of claim 43, wherein said at least two polypeptides are connected by a linker.
45. The expression vector of any one of claims 43 to 44, wherein said at least one promoter is selected from the group consisting of CAG promoter, CMV promoter, SV40 promoter, adenovirus promoter, Beta actin promoter, metallothionin promoter, EF1a promoter, myosin light chain promoter, myosin heavy chain promoter and NCX1 promoter.
46. The expression vector of any one of claims 43 to 45, wherein said expression vector is a cardiac expression vector.

47. A polypeptide construct comprising at least a first polypeptide, and a second polypeptide, wherein:
- said first polypeptide is a calcium binding protein, or variant or fragment thereof;
 - said second polypeptide is an angiogenic polypeptide, a chemokine, or variant or fragment thereof.
48. A polypeptide construct comprising at least a first polypeptide, a second polypeptide, and a third polypeptide wherein:
- said first polypeptide is an angiogenic polypeptide or variant or fragment thereof;
 - said second polypeptide is a chemokine, or variant or fragment thereof; and
 - said third polypeptide is a calcium binding protein, or variant or fragment thereof.
49. The construct of any one of claims 47 to 48, comprising S100A1, variant or fragment thereof.
50. The construct of any one of claims 47 to 49, comprising SDF1, variant or fragment thereof.
51. The construct of any one of claims 47 to 50, comprising VEGF, variant or fragment thereof.
52. A complex comprising a construct of any one of claims 47 to 51 complexed to at least one receptor.
53. A pharmaceutical composition comprising a construct of any one of claims 47 to 51 and a pharmaceutically acceptable excipient.
54. A method of treating cardiomyopathy in a subject comprising providing to said subject a therapeutically effective amount of a composition comprising a construct of any one of claims 47 to 51.
55. A method comprising: contacting at least one cardiac cell with a polynucleotide encoding a polypeptide construct comprising at least a first polypeptide and a second polypeptide, wherein: said first polypeptide is an angiogenic polypeptide variant or fragment thereof; and said second polypeptide is at least one of a chemokine, a calcium binding protein, or variant or fragment thereof.
56. The method of claim 55, wherein said angiogenic polypeptide variant or fragment thereof is a VEGF polypeptide selected from a group consisting of VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF191, VEGF206, fragments and variants thereof.
57. The polynucleotide of claim 56, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 165.

58. The polynucleotide of claim 56, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 191.
59. The method of any one of claims 55 to 58, wherein said second polypeptide is a chemokine.
60. The method of claim 59, wherein said chemokine is a stromal cell-derived factor 1 (SDF) polypeptide.
61. The method of claim 60, wherein said SDF polypeptide is selected from the group consisting of SDF1, SDF-1 α , SDF-1 β , fragments and variants thereof.
62. The method of any one of claims 60 to 61, wherein said SDF polypeptide comprises a sequence with at least 70% identity to the sequence of SDF1.
63. The method of any one of claims 55 to 62, wherein said polynucleotide encoding a polypeptide is introduced *ex vivo*.
64. The method of any one of claims 55 to 62, wherein said polynucleotide encoding a polypeptide is introduced *in vivo*.
65. The method of claim 64, wherein said introduction *in vivo* is selected from a group consisting of percutaneous coronary artery catheterization, coronary venous blockade, cardiac recirculation, antegrade coronary artery infusion, retrograde perfusion, direct injection, ultrasound targeted microbubble destruction and any combination thereof.
66. The method of any one of claims 55 to 65, further comprising administration of at least one additional therapy.
67. A method of improving vasculogenesis in a subject comprising: administering to said subject an amount of a polynucleotide encoding a construct comprising a VEGF polypeptide, and at least one of a SDF polypeptide and a S100 polypeptide.
68. The method of claim 67, wherein said VEGF polypeptide is selected from a group consisting of VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF191, VEGF206, fragments and variants thereof.
69. The method of claim 68, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 165.
70. The method of claim 68, wherein said VEGF polypeptide comprises a sequence with at least 70% identity to the sequence of VEGF 191.
71. The method of any one of claims 67 to 70, wherein said SDF polypeptide is selected from the group consisting of SDF1, SDF-1 α , SDF-1 β , fragments and variants thereof.
72. The method of any one of claims 67 to 71, wherein said S100 polypeptide is S100A1, fragment or variant thereof.
73. The method of any one of claims 67 to 72, wherein said subject has a cardiomyopathy.

74. The method of claim 73, wherein said cardiomyopathy is selected from a group consisting of dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and any combination thereof.
75. A method of treating or preventing a cardiovascular condition in a subject comprising: administering to said subject an amount of a construct comprising a S100A polypeptide, and a second functional polypeptide, wherein said second functional polypeptide is at least one of a cytokine, a chemokine and an angiogenic polypeptide.
76. The method of claim 75, wherein said administration is performed preventively.
77. The method of claim 75, wherein said administration is performed therapeutically.
78. The method of any one of claims 75 to 77, further comprising administration of at least one additional treatment to said subject.
79. The method of any one of claims 75 to 78, wherein said second functional polypeptide is VEGF, SDF, or a combination thereof.
80. A method of gene therapy comprising administering to a subject at least one non-viral vector comprising:
- at least two genes encoding for functional proteins or portions thereof;
 - at least one promotor; and
 - at least one engineered recombination site;
- wherein said at least one promoter drives expression of said at least two genes.
81. The method of claim 80, wherein said at least one promotor is constitutive.
82. The method of claim 80, wherein said at least one promoter is tissue-specific.
83. The method of claim 80, wherein said at least one promoter is inducible.
84. The method of claim 82, wherein said at least one tissue-specific promoter is a myosin light chain (MLC) promoter.
85. The method of claim 83, wherein said inducible promoter is a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch.
86. The method of claim 83 or 85, wherein said inducible promoter utilizes a ligand for dose-regulated control of expression of said at least two genes.
87. The method of claim 86, wherein said ligand is selected from a group consisting of ecdysteroid, 9 cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines, oxadiazolines, dibenzoylalkyl cyanohydrazines, N-alkyl-N,N'-diaroylhydrazines, N-acyl-N-alkylcarbonylhydrazines, N-aryl-N-alkyl-N'-arylhydrazines, arnidoketones, 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, oxysterols, 22(R) hydroxycholesterol, 24(S) hydroxycholesterol, 25-epoxycholesterol, T0901317, 5-alpha-6-alpha-epoxycholesterol-3-

sulfate (ECHS), 7-ketocholesterol-3-sulfate, framesol, bile acids, 1,1-biphosphonate esters, juvenile hormone III, RG-115819 (3,5 -Dimethyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N'-(2-methyl-3-methoxy-benzoyl)-hydrazide-), RG-115932 ((R)-3,5-Dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide), and RG-115830 (3,5 -Dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide), and any combination thereof.

88. The method of any one of claims 80 to 87, wherein said linker is a cleavable linker.

89. The method of claim 88, wherein said linker comprises a sequence APVKQ (SEQ ID NO: 42), GPVKQ (SEQ ID NO: 43), VPVKQ (SEQ ID NO: 44), IPVKQ (SEQ ID NO: 45), MPVKQ (SEQ ID NO: 46), APIKQ (SEQ ID NO: 47), GPIKQ (SEQ ID NO: 48), VPIKQ (SEQ ID NO: 49), IPIKQ (SEQ ID NO: 50), MPIKQ (SEQ ID NO: 51), APAKQ (SEQ ID NO: 52), GPAKQ (SEQ ID NO: 53), VPAKQ (SEQ ID NO: 54), IPAKQ (SEQ ID NO: 55), MPAKQ (SEQ ID NO: 56), APVRQ (SEQ ID NO: 57), GPVRQ (SEQ ID NO: 58), VPVRQ (SEQ ID NO: 59), IPVRQ (SEQ ID NO: 60), MPVRQ (SEQ ID NO: 61), APIRQ (SEQ ID NO: 62), GPIRQ (SEQ ID NO: 63), VPIRQ (SEQ ID NO: 64), IPIRQ (SEQ ID NO: 65), MPIRQ (SEQ ID NO: 66), APARQ (SEQ ID NO: 67), GPARQ (SEQ ID NO: 68), VPARQ (SEQ ID NO: 69), IPARQ (SEQ ID NO: 70), MPARQ (SEQ ID NO: 71), APVKN (SEQ ID NO: 72), GPVKN (SEQ ID NO: 73), VPVKN (SEQ ID NO: 74), IPVKN (SEQ ID NO: 75), MPVKN (SEQ ID NO: 76), APIKN (SEQ ID NO: 77), GPIKN (SEQ ID NO: 78), VPIKN (SEQ ID NO: 79), IPIKN (SEQ ID NO: 80), MPIKN (SEQ ID NO: 81), APAKN (SEQ ID NO: 82), GPAKN (SEQ ID NO: 83), VPAKN (SEQ ID NO: 84), IPAKN (SEQ ID NO: 85), MPAKN (SEQ ID NO: 86), APVRN (SEQ ID NO: 87), GPVRN (SEQ ID NO: 88), VPVRN (SEQ ID NO: 89), IPVRN (SEQ ID NO: 90), MPVRN (SEQ ID NO: 91), APIRN (SEQ ID NO: 92), GPIRN (SEQ ID NO: 93), VPIRN (SEQ ID NO: 94), IPIRN (SEQ ID NO: 95), MPIRN (SEQ ID NO: 96), APARN (SEQ ID NO: 97), GPARN (SEQ ID NO: 98), VPARN (SEQ ID NO: 99), IPARN (SEQ ID NO: 100) or MPARN (SEQ ID NO: 101).

90. The method of any one of claims 80 to 89, wherein said at least one recombination site is a phage genomic recombination attachment site (attP) or a bacterial genomic recombination attachment site (attB).

91. The method of any one of claims 80 to 90, further comprising administering to said subject at least one secondary vector.

92. The method of claim 91, wherein said at least one secondary vector is an mRNA.

93. The method of claim 91, wherein said at least one secondary vector is a plasmid.

94. The method of any one of claims 91 to 93, wherein said at least one secondary vector comprises at least one recombinase.

95. The method of claim 92, wherein said recombinase is selected from the group consisting of a *Listeria monocytogenes* phage recombinase, a *Streptococcus pyogenes* phage recombinase, a *Bacillus subtilis* phage recombinase, a *Mycobacterium tuberculosis* phage recombinase, a *Mycobacterium smegmatis* phage recombinase, and SPBc2 recombinase.
96. The method of any one of claims 80 to 95, wherein said at least two genes are integrated into a genome of said subject by said recombinase.
97. The method of any one of claims 80 to 96, wherein said vector is good manufacturing practices (GMP) compatible.
98. A polypeptide comprising a sequence with at least 60% identity to a sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, and SEQ ID NO: 40.
99. The polypeptide of claim 98, wherein said polypeptide comprises a sequence with at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to a sequence selected from the group consisting of SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, and SEQ ID NO: 40.
100. A polypeptide comprising a sequence with at least 60% identity to a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 104 and SEQ ID NO: 106.
101. The polypeptide of claim 100, wherein said polypeptide comprises a sequence with at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to a sequence selected from the group consisting of SEQ ID NO: 102, SEQ ID NO: 104 and SEQ ID NO: 106.
102. A polypeptide comprising a sequence with at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity to a polypeptide sequence encoded by the nucleic acid sequence of SEQ ID NO: 108.
103. A polynucleotide encoding a polypeptide construct comprising a first functional polypeptide and a second functional polypeptide connected by a first linker comprising a sequence with at least 60% identity to the sequence APVKQ (SEQ ID NO: 42).
104. The polynucleotide of claim 103, wherein said linker sequence comprises an amino acid sequence of 80% or 100% identity to the sequence APVKQ (SEQ ID NO: 42).
105. A polynucleotide encoding a polypeptide construct comprising a first functional polypeptide and a second functional polypeptide connected by a first linker comprising a sequence with at least 60%, 80% or 100% identity to a sequence selected from the group consisting of: APVKQ, GPVKQ, VPVKQ, IPVKQ, MPVKQ, APIKQ, GPIKQ, VPIKQ, IPIKQ, MPIKQ, APAKQ, GPAKQ, VPAKQ, IPAKQ, MPAKQ, APVRQ, GPVRQ, VPVRQ, IPVRQ, MPVRQ, APIRQ, GPIRQ, VPIRQ, IPIRQ, MPIRQ, APARQ, GPARQ, VPARQ, IPARQ, MPARQ, APVKN, GPVKN, VPVKN, IPVKN, MPVKN, APIKN, GPIKN, VPIKN, IPIKN,

MPIKN, APAKN, GPAKN, VPAKN, IPAKN, MPAKN, APVRN, GPVRN, VPVRN, IPVRN, MPVRN, APIRN, GPIRN, VPIRN, IPIRN, MPIRN, APARN, GPARN, VPARN, IPARN, and MPARN, or any combination thereof.

106. The polynucleotide of any one of claims 103 to 105, wherein said first linker is a rigid linker, a flexible linker, a cleavable linker, a self-cleavable linker, a peptide linker, a linker cleavable via ribosome skipping, or any combination thereof.

107. The polynucleotide of claim 106, wherein said first linker is cleavable via proteolysis.

108. The polynucleotide of claim 107, wherein said first linker comprises from 1-10 cleavage sites.

109. The polynucleotide of any one of claims 107 to 108, wherein said cleavage is a reductive reaction, enzymatic reaction, or a combination of both.

110. The polynucleotide of claim 109, wherein said cleavage comprises an enzymatic reaction.

111. The polynucleotide of claim 110, wherein said cleavage comprising an enzymatic reaction is performed by a protease.

112. The polynucleotide of claim 111, wherein said protease is selected from a group consisting of: furin, and cathepsin B.

113. The polynucleotide of claim 112, wherein said protease is furin.

114. The polynucleotide of claim 113, wherein said furin cleaves RAKR

115. The polynucleotide of any one of claims 103 to 114, wherein said first functional polypeptide is different from said second functional polypeptide.

116. The polynucleotide of any one of claims 103 to 115, wherein at least one of said first and second functional polypeptides is a protein, hormone, glycoprotein or derivative or fragment thereof.

117. The polynucleotide of any one of claims 103 to 116, further comprising at least one sequence selected from (GS)_n, (SG)_n, (GSG)_n and (SGS)_n wherein n is a number from one to fifteen

118. The polynucleotide of any one of claims 103 to 116, wherein said polypeptide further comprises a second linker.

119. The polynucleotide of claim 118, wherein said second linker connects a third functional polypeptide to said first functional polypeptide or said second functional polypeptide.

120. The polynucleotide of claim 119, wherein said second linker that connects a third functional polypeptide to said first functional polypeptide or said second functional polypeptide is a self-cleavable linker.

121. The polynucleotide of claim 120, wherein said self-cleavable linker comprises a 2A sequence.
122. The polynucleotide of claim 121, wherein said 2A sequence is a F2A sequence or a P2A sequence.
123. The polynucleotide of any one of claims 103 to 122, further comprising a Kozak sequence.
124. The polynucleotide of any one of claims 103 to 123, further comprising the polypeptide encoded by said polynucleotide.
125. A polypeptide encoded by the polynucleotide of any one of claims 103 to 124, or derivative or variant thereof.
126. A pharmaceutical composition comprising the polynucleotide of any one of claims 103 to 124 and at least one pharmaceutically acceptable excipient.
127. A method of treatment comprising administering a polynucleic acid comprising a sequence with at least 60% identity to the sequence APVKQ (SEQ ID NO: 42) to a subject in need thereof.
128. The method of claim 127, wherein said sequence comprises 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to 100% identity to the sequence APVKQ (SEQ ID NO: 42).
129. The method of any one of claims 126 to 127, wherein said polynucleic acid encodes a functional polypeptide.
130. The method of claim 129, wherein said functional polypeptide is cleaved in vivo.
131. The method of any one of claims 129 to 130, wherein said functional polypeptide that is cleaved in vivo produces at least two functional proteins.
132. The method of any one of claims 129 to 130, wherein said functional polypeptide that is cleaved in vivo produces at least three functional proteins.
133. The method of any one of claims 130 to 131, wherein said at least two or at least three functional proteins are part of a combination therapy to treat said subject in need thereof.
134. The method of any one of claims 124 to 125, wherein said administration is preventative, curative, routine, or a combination thereof.
135. The method of any one of claims 127 to 134, wherein said administration is routine.
136. The method of claim 135, wherein said administration comprises hourly administrations, daily administrations, weekly administrations, monthly administrations, yearly administrations, or constant administrations.
137. The method of any one of claims 127 to 136, wherein said subject has an acute disease, a chronic disease, a terminal disease, or a combination thereof.

138. The method of any one of claims 124 to 128, wherein said subject has cancer, heart disease, diabetes, a neural disorder, an ocular disorder, a viral disorder, or any combination thereof.
139. The method of any one of claims 124 to 130, further comprising at least one secondary treatment.
140. A method of making a polynucleotide comprising a first functional polypeptide and a second functional polypeptide connected by a first linker comprising a sequence with at least 60% identity to the sequence APVKQ (SEQ ID NO: 42).
141. A method of expressing genes from a vector comprising a sequence with at least 60% identity to the sequence APVKQ (SEQ ID NO: 42) and efficiency of cleavage of said genes of at least 50% as measured by mass spectroscopy.
142. The method of claim 141, wherein said vector comprises genes for expression of two biologically active polypeptides, three biologically active polypeptides or more than three biologically active polypeptides.
143. The method of claim 142, wherein said vector is comprises genes for expression of three biologically active polypeptides.
144. The method of any one of claims 141 to 143, wherein said genes are functional after cleavage.
145. The method of any one of claims 141 to 144, wherein said efficiency of cleavage of said genes comprises at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or up to 100% as measured by mass spectroscopy.
146. A polynucleotide encoding a polypeptide construct comprising a furin polypeptide and a 2A polypeptide, wherein said furin polypeptide and said 2A polypeptide are connected by a polypeptide linker comprising at least three hydrophobic amino acids.
147. The polynucleotide of claim 146, wherein at least one of said at least three hydrophobic amino acids is selected from the list consisting of glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), methionine (Met), tryptophan (Trp).
148. The polynucleotide of claim 147, wherein said polypeptide linker comprises a sequence APVKQ, GPVKQ, VPVKQ, IPVKQ, MPVKQ, APIKQ, GPIKQ, VPIKQ, IPIKQ, MPIKQ, APAKQ, GPAKQ, VPAKQ, IPAKQ, MPAKQ, APVRQ, GPVRQ, VPVRQ, IPVRQ, MPVRQ, APIRQ, GPIRQ, VPIRQ, IPIRQ, MPIRQ, APARQ, GPARQ, VPARQ, IPARQ, MPARQ, APVKN, GPVKN, VPVKN, IPVKN, MPVKN, APIKN, GPIKN, VPIKN, IPIKN, MPIKN, APAKN, GPAKN, VPAKN, IPAKN, MPAKN, APVRN, GPVRN, VPVRN, IPVRN, MPVRN, APIRN, GPIRN, VPIRN, IPIRN, MPIRN, APARN, GPARN, VPARN, IPARN or MPARN.

149. A method of obtaining improved expression of a polypeptide construct comprising:
providing a polynucleotide encoding said polypeptide construct comprising a first functional polypeptide and a second functional polypeptide, wherein said first functional polypeptide and second functional polypeptide are connected by a polypeptide linker comprising a sequence with at least 60% identity to the sequence APVKQ; and
expressing said polynucleotide in a host cell, wherein said expressing results in an improved expression of the polypeptide construct as compared to a corresponding polypeptide construct that does not have a polypeptide linker comprising a sequence with at least 60% identity to the sequence APVKQ.
150. The method of claim 149, wherein said improved expression is at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% as compared to a corresponding polypeptide construct that does not have a polypeptide linker comprising a sequence with at least 60% identity to the sequence APVKQ.
151. A composition comprising:
at least one plasmid microbubble complex, wherein the microbubble comprises a lipid, a gas and a plasmid comprising a polynucleotide of any one of claims 1-27 and 35-41.
152. The composition of claim 151, wherein said lipid forms a shell enclosing said gas and plasmid.
153. The composition of any one of claims 151-152, wherein said gas is a perfluorocarbon gas.
154. The composition of claim 153, wherein said gas is perfluoropropane.
155. The composition of any one of claims 151-154, wherein said microbubble complex comprises at least one of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine glycerol.
156. A method comprising:
administering to a subject the composition of any one of claims 151-155;
contacting said subject with an ultrasonic energy sufficient to result in ultrasound disruption of the at least one microbubble at a predetermined tissue or organ.
157. The method of claim 156, wherein disruption of the at least one microbubble at said predetermined tissue or organ delivers said plasmid into said tissue or organ.
158. The method of claim 157, wherein said tissue or organ is an organ, and said organ is a heart.
159. A method of treating a cardiac disease or disorder in a subject comprising:

administering to the subject an amount of a composition comprising at least one microbubble, said microbubble comprising a lipid, a gas, and a plasmid comprising a polynucleotide of any one of claims 1-27 and 35-41;

contacting said subject with an ultrasonic energy sufficient to result in ultrasound disruption of the at least one microbubble and delivering said plasmid to the heart of said subject.

160. The method of claim 159, wherein said lipid forms a shell enclosing said gas and plasmid.

161. The method of any one of claims 159-160, wherein said gas is a perfluorocarbon gas.

162. The method of claim 161, wherein said gas is perfluoropropane.

163. The method of any one of claims 159-162, wherein said microbubble comprises at least one of 1,2-dipalmitoyl-sn-glycero-3 -phosphatidylcholine and 1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine glycerol.

164. The method of any one of claims 159-163 wherein said cardiac disease or disorder comprises at least one of congestive heart failure, cardiomyopathy, arrhythmia, pericardial disease, aorta disease, marfan syndrome and coronary artery disease.