

CLAIMS

1. A polynucleotide, comprising an expression cassette and optionally flanking adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein the polynucleotide comprises a polynucleotide sequence encoding Muscle LIM Protein (MLP) or a functional variant thereof, operatively linked to a promoter.
2. The polynucleotide of claim 1, wherein the promoter is a cardiac-specific promoter.
3. The polynucleotide of claim 1 or claim 2, wherein the promoter is a muscle-specific promoter.
4. The polynucleotide of any one of claims 1 to 3, wherein the promoter is a cardiomyocyte-specific promoter.
5. The polynucleotide of any one of claims 1 to 4, wherein the promoter is a MHCK7 promoter.
6. The polynucleotide of claim 5, wherein the MHCK7 promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 31.
7. The polynucleotide of any one of claims 1 to 4, wherein the promoter is a cardiac troponin T (hTNNT2) promoter.
8. The polynucleotide of claim 7, wherein the hTNNT2 promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 32.
9. The polynucleotide of any one of claims 1 to 8, wherein the expression cassette comprises exon 1 of the cardiac troponin T (hTNNT2) gene, wherein optionally the hTNNT2 promoter and exon 1 together share at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 32.
10. The polynucleotide of any one of claims 1 to 4, wherein the promoter is a ubiquitous promoter, optionally a CMV promoter or a CAG promoter.
11. The polynucleotide of any one of claims 1 to 10, wherein the expression cassette comprises a polyA signal.

12. The polynucleotide of claim 11, wherein the polyA signal is a human growth hormone (hGH) polyA.
13. The polynucleotide of any one of claims 1 to 12, wherein the expression cassette comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), optionally a WPRE(x).
14. The polynucleotide of any one of claims 1 to 13, wherein the Muscle LIM Protein (MLP) or a functional variant thereof is an MLP.
15. The polynucleotide of claim 14, wherein the MLP is a human MLP.
16. The polynucleotide of claim 14 or claim 15, wherein the MLP is MLP isoform A.
17. The polynucleotide of claim 15 or 16, wherein the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCMA CRKALDSTTVA
AHESIYCKVCYGRRYGPKGIGYGQGAGCLSTD TGEHLGLQFQQSPKPARSV
TTSNPSKFTAKFGESEKCPRCGKSVYAAEKVMGGGK PWHKTCFRCAICGKSL
ESTNVTDKDGELYCKVCYAKNFGPTGIGFGGLTQQVEKKE

(SEQ ID NO: 1).

18. The polynucleotide of claim 14 or claim 15, wherein the MLP is MLP isoform B.
19. The polynucleotide of claim 15 or 18, wherein the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCSPQSRHAQLPPATL
PNSLRSLES PRSALDVASQSMLLRRLWEVASLGTRPVSAVPSVGRVWSPQMS
LTKMGNFIAKFAMPKILAPRVLGLEALHNKWK RKNEEVRRFSDFLRA

(SEQ ID NO: 2).

20. The polynucleotide of claim 15, wherein the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCLC

(SEQ ID NO: 3).

21. The polynucleotide of claim 15, wherein the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCTLAQDLFPLCHLWE
ESGVHKC

(SEQ ID NO: 4).

22. The polynucleotide of any one of claims 1 to 21, wherein the polynucleotide sequence encoding MLP is a Cysteine And Glycine Rich Protein 3 (*CSRP3*) polynucleotide.

23. The polynucleotide of claim 22, wherein the *CSRP3* polynucleotide is a human *CSRP3* polynucleotide.

24. The polynucleotide of any one of claims 1 to 23, wherein the polynucleotide sequence encoding MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with

ATGCCAAACTGGGGCGGAGGCGCAAAATGTGGAGCCTGTGAAAAGACCG
TCTACCATGCAGAAGAAATCCAGTGCAATGGAAGGAGTTTCCACAAGACG
TGTTTCCACTGCATGGCCTGCAGGAAGGCTCTTGACAGCACGACAGTCGC
GGCTCATGAGTCGGAGATCTACTGCAAGGTGTGCTATGGGCGCAGATATG
GCCCCAAAGGGATCGGGTATGGACAAGGCGCTGGCTGTCTCAGCACAGAC
ACGGGCGAGCATCTCGGCCTGCAGTTCCAACAGTCCCCAAAGCCGGCACG
CTCAGTTACCACCAGCAACCCTTCCAAATTTCACTGCGAAGTTTGGAGAGT
CCGAGAAGTGCCCTCGATGTGGCAAGTCAGTCTATGCTGCTGAGAAGGTT
ATGGGAGGTGGCAAGCCTTGGCACAAGACCTGTTTCCGCTGTGCCATCTG
TGGGAAGAGTCTGGAGTCCACAAATGTCACTGACAAAGATGGGGAACTTT
ATTGCAAAGTTTGCTATGCCAAAAATTTTGGCCCCACGGGTATTGGGTTTG
GAGGCCTTACACAACAAGTGGAAGAAAGAAAGAA

(SEQ ID NO: 5).

25. The polynucleotide of any one of claims 1 to 24, wherein the polynucleotide sequence encoding MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with

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ATGCCCAATTGGGGTGGAGGAGCTAAATGTGGAGCTTGTGAAAAAACAGT
TTATCATGCTGAAGAAATTCAATGTAATGGAAGATCTTTTCATAAAACATG
TTTTCATTGTATGGCTTGTAGAAAAGCACTTGATTCTACAACCTGTTGCAGC
ACATGAAAGTGAAATCTATTGTAAAGTATGTTATGGAAGAAGATATGGAC
CAAAAGGAATTGGATATGGACAAGGAGCAGGATGTCTTTCTACAGATACT
GGAGAACATTTGGGATTGCAATTTCAACAAAGTCCTAAACCAGCTAGATC
TGTTACAACAAGTAATCCATCAAAATTTACTGCTAAATTTGGAGAATCCG
AAAAATGTCCTAGATGTGGAAAATCAGTATATGCTGCTGAAAAAGTTATG
GGAGGTGGAAAACCATGGCATAAGACATGTTTTAGATGTGCAATTTGTGG
TAAATCTTTGGAATCTACAAATGTTACAGATAAAGATGGAGAATTGTATT
GTAAAGTTTGTTATGCTAAAAATTTGGACCTACAGGTATAGGATTTGGAG
GTTTGACACAACAAGTTGAAAAAAAAGAA
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(SEQ ID NO: 7).

26. The polynucleotide of any one of claims 1 to 25, wherein the polynucleotide comprises at least about 2.4 kb, at most about 2.6 kb, or between about 2.4 kb and about 2.6 kb.

27. The polynucleotide of any one of claims 1 to 26, wherein the polynucleotide comprises at least about 3.0 kb, at most about 3.3 kb, or between about 3.0 kb and about 3.3 kb.

28. The polynucleotide of any one of claims 1 to 27, wherein the polynucleotide comprises at least about 2.4 kb, least about 2.6 kb, least about 3.0 kb, at least about 3.3 kb, at least about 3.5 kb, at least about 3.7 kb, at least about 3.9 kb, at least about 4.1 kb., or at least about 4.3 kb.

29. The polynucleotide of any one of claims 1 to 28, wherein the polynucleotide comprises least about 2.6 kb, least about 3.0 kb, at most about 3.3 kb, at most about 3.5 kb, at most about 3.7 kb, at most about 3.9 kb, at most about 4.1 kb., at most about 4.3 kb, or at most about 4.5 kb.

30. The polynucleotide of any one of claim 1 to 29, wherein the expression cassette shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of SEQ ID NOs: 8-11.
31. The polynucleotide of any one of claim 1 to 30, wherein the polynucleotide shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of SEQ ID NOs: 12-15.
32. The polynucleotide of any one of claims 1 to 31, wherein the expression cassette is flanked by 5' and 3' inverted terminal repeats (ITRs), optionally AAV2 ITRs, optionally ITRs that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of SEQ ID NO: 20-26.
33. The polynucleotide of any one of claim 1 to 32, wherein the polynucleotide is self-complementary.
34. The polynucleotide of any one of claim 1 to 33, wherein the polynucleotide comprises the expression cassette and a reverse complement of the expression cassette.
35. The polynucleotide of claim 34, wherein the expression cassette and the reverse complement of the expression cassette are flanked by 5' and 3' inverted terminal repeats (ITRs), optionally AAV2 ITRs, optionally an ITR that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 23 or SEQ ID NO: 26.
36. A gene therapy vector, comprising the polynucleotide of any one of claims 1 to 35.
37. The vector of claim 36, wherein the gene therapy vector is a recombinant adeno-associated virus (rAAV) vector.
38. The vector of claim 37, wherein the rAAV vector is an AAV9 or a functional variant thereof.
39. The vector of claim 38, wherein the rAAV vector comprises a capsid protein that shares 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NO: 77.
40. The vector of claim 37, wherein the rAAV vector is an AAVrh10 or a functional variant thereof.

41. The vector of claim 40, wherein the rAAV vector comprises a capsid protein that shares 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NO: 79.
42. The vector of claim 37, wherein the rAAV vector is an AAV6 or a functional variant thereof.
43. The vector of claim 42, wherein the rAAV vector comprises a capsid protein that shares 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NO: 78.
44. The vector of claim 37, wherein the rAAV vector is an AAVrh74 or a functional variant thereof.
45. The vector of claim 44, wherein the rAAV vector comprises a capsid protein that shares 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NO: 80.
46. The vector of any one of claims 36 to 45, wherein the rAAV vector is a self-complementary AAV vector.
47. A method of treating and/or preventing a disease or disorder in a subject in need thereof, comprising administering the vector of any one of claims 35 to 46 to the subject.
48. The method of claim 47, wherein the disease or disorder is a cardiac disorder.
49. The method of claim 47 or 48, wherein the disease or disorder is heart failure.
50. The method of any one of claims 47 to 49, wherein the disease or disorder is hypertrophic cardiomyopathy.
51. The method of any one of claims 47 to 49, wherein the disease or disorder is dilated cardiomyopathy.
52. The method of any one of claims 47 to 51, wherein the subject is a mammal.
53. The method of claim 52, wherein the subject is a primate.
54. The method of claim 53, wherein the subject is a human.

55. The method of any one of claims 45 to 54, wherein the subject has a mutation in the *CSRP3* gene that causes an amino acid substitution selected from C58G, L44P, S54R, E55G, and/or K69R, relative to a human *CSRP3* encoding a human MLP having the sequence of SEQ ID NO: 1.
56. The method of any one of claim 47 to 55, wherein the vector is administered by intravenous injection, intracardiac injection, intracardiac infusion, and/or cardiac catheterization.
57. The method of any one of claims 47 to 56, wherein the administration increases MLP expression by at least about 5%.
58. The method of any one of claims 47 to 56, wherein the administration increases MLP expression by at least about 30%.
59. The method of any one of claims 47 to 56, wherein the administration increases MLP expression by at least about 70%.
60. The method of any one of claims 47 to 56, wherein the administration increases MLP expression by about 5% to about 10%.
61. The method of any one of claims 47 to 56, wherein the administration increases MLP expression by about 30% to about 50%.
62. The method of any one of claims 47 to 56, wherein the administration increases MLP expression by about 70% to about 100%.
63. The method of any one of claims 47 to 62, wherein the method treats and/or prevents the disease or disorder.
64. A pharmaceutical composition comprising the vector of any one of claims 36 to 46.
65. A kit comprising the vector of any one of claims 34 to 46 or the pharmaceutical composition of claim 64 and optionally instructions for use.
66. Use of the composition of any one of claims 36 to 46 in treating a disease or disorder, optionally according to the method of any one of claims 47 to 63.

67. A composition according to any one of claims 36 to 46 for use in treating a disease or disorder, optionally according to the method of any one of claims 47 to 63.
68. A method of expressing Muscle LIM Protein (MLP) or a functional variant thereof, comprising contacting a cell with the vector of any one of claims 36 to 46.
69. The method of claim 68, wherein the cell is a cardiomyocyte.
70. The method of claim 69, wherein the cardiomyocyte is a human cardiomyocyte.
71. The method of any one of claims 68 to 70, wherein the promoter is an MHCK7 promoter and wherein the expression level of the MLP is at least 2-fold greater than the expression level of MLP in a cell transduced with a vector having an hTNNT2 promoter.
72. The method of any one of claims 68 to 70, wherein the promoter is an MHCK7 promoter and wherein the expression level of the MLP is between 2-fold greater and 10-fold greater than the expression level of MLP in a cell transduced with a vector having an hTNNT2 promoter.