

CLAIMS

What is claimed is:

1. A fusion protein comprising, from N-terminus to C-terminus,
a first peptide comprising an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO: 2;
a peptide linker comprising an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of an amino acid sequence selected from the group consisting of DAASSHSHSSAR (SEQ ID NO: 14) and DAAGANANAGAR (SEQ ID NO: 16); and
a second peptide comprising an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO: 1;
wherein the first peptide, the peptide linker, and the second peptide are operably linked.
2. The fusion protein of claim 1, wherein the fusion protein has an activity of a native relaxin-2 protein.
3. The fusion protein of claim 1 or 2, wherein the fusion protein has at least about 50% activity of native relaxin-2 protein.
4. The fusion protein of claim 3, wherein the fusion protein has at least about 90% activity of native relaxin-2 protein.
5. The fusion protein of claim 4, wherein the fusion protein has at least about 100% activity of native relaxin-2 protein.
6. The fusion protein of claim 5, wherein the fusion protein has at least about 150% activity of native relaxin-2 protein.
7. The fusion protein of any one of claims 1-6, wherein the peptide linker comprises an amino acid sequence that is at least about 90% identical to the entire amino acid sequence of an amino acid sequence selected from the group consisting of DAASSHSHSSAR (SEQ ID NO: 14), DAASSHSHSSAA (SEQ ID NO: 15), and DAAGANANAGAR (SEQ ID NO: 16).

8. The fusion protein of claim 7, wherein the peptide linker comprises the amino acid sequence of DAASSSHSHSSAR (SEQ ID NO: 14), DAASSSHSHSSAA (SEQ ID NO: 15), or DAAGANANAGAR (SEQ ID NO: 16).
9. The fusion protein of any one of claims 1-8, wherein the first peptide has an amino acid sequence that is at least about 95% identical to the entire amino acid sequence of SEQ ID NO: 2, and the second peptide has an amino acid sequence that is at least about 95% identical to the entire amino acid sequence of SEQ ID NO: 1, wherein the fusion protein has a native relaxin-2 activity.
10. The fusion protein of claim 9, wherein the amino acid sequence of the first peptide is selected from the group consisting of SEQ ID NOs: 2, 7, 8, 9, and 10, and wherein the amino acid sequence of the second peptide is selected from the group consisting of SEQ ID NOs: 1 and 6.
11. The fusion protein of claim 9, wherein the first peptide comprises a substitution selected from the group consisting of M4K, M25K, W28A, and combinations thereof.
12. The fusion protein of claim 11, wherein the first peptide comprises the substitutions M4K, M25K, and W28A.
13. A fusion protein comprising a first peptide, a peptide linker, and a second peptide, wherein the amino acid sequence of the fusion protein is at least about 85% identical to the entire amino acid sequence of an amino acid sequence selected from the group consisting of SEQ ID NOs: 47, 48, 49, 50, 51, 52, 53, 54, and 55.
14. A fusion protein comprising a first peptide, a peptide linker, and a second peptide, wherein the amino acid sequence of the fusion protein is at least about 85% identical to the entire amino acid sequence set forth in SEQ ID NO: 55.
15. The fusion protein of claim 14, wherein the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 55.

16. The fusion protein of any one of claims 1-15, further comprising a first detectable label.
17. The fusion protein of claim 16, wherein the first detectable label is operably linked to the N-terminus of the first peptide or the C-terminus of the second peptide.
18. The fusion protein of claim 16 or 17, wherein the first detectable label is an immunoglobulin G (IgG) Fc peptide comprising an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO: 20.
19. The fusion protein of claim 18, wherein the first detectable label has an amino acid sequence of SEQ ID NO: 20 or 21.
20. The fusion protein of claim 18 or 19, wherein the first detectable label is operably linked to the N-terminus of the first peptide.
21. The fusion protein of claim 20, further comprising a second linker, wherein the second linker is operably linked to the C-terminus of the first detectable label and to the N-terminus.
22. The fusion protein of claim 21, wherein the second linker is selected from the group consisting of Gly-Gly-Ser, Ala-Ala-Ala, Pro-Pro-Pro, Gly-Ser-Gly, (Gly-Ser-Gly)₂ (SEQ ID NO: 57) and (Gly-Gly-Ser)₄ (SEQ ID NO: 17).
23. The fusion protein of any one of claims 18-22, wherein the fusion protein has an *in vivo* circulating half-life of greater than about 10 hours.
24. The fusion protein of claim 23, wherein the fusion protein has an *in vivo* circulating half-life of about 130 hours.
25. The fusion protein of claim 16 or 17, wherein the first detectable label is a polyhistidine tag having an amino acid sequence comprising an amino acid sequence that is at least about 85% identical to the entire amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 19.

26. The fusion protein of claim 18, wherein the first detectable agent comprises the amino acid sequence of SEQ ID NO: 18 or 19.
27. The fusion protein of any one of claims 16-26, further comprising a second detectable label.
28. The fusion protein of claim 27, wherein the first detectable label is operably linked to the N-terminus of the first peptide, and the second detectable label is operably linked to the C-terminus of the second peptide.
29. The fusion protein of claim 27, wherein the first detectable label and the second detectable label are operably linked to the N-terminus of the first peptide.
30. The fusion protein of any one of claims 27-29, wherein the first detectable label and the second detectable label are different peptides.
31. The fusion protein of any one of claims 1-30, further comprising a cleavable linker.
32. The fusion protein of claim 31, wherein the cleavable linker is a peptide subject to the specific digestion of a protease.
33. The fusion protein of claim 32, wherein the protease is HRV 3C protease or thrombin.
34. The fusion protein of claim 33, wherein the cleavable linker is a peptide having the sequence of SEQ ID NO: 23, or a variant thereof.
35. The fusion protein of any one of claims 1-34, further comprising a signal peptide at the N-terminus of the fusion protein.
36. A fusion protein comprising a detectable label, a second linker, a first peptide, a peptide linker, and a second peptide, wherein the amino acid sequence of the fusion protein is at least about 85% identical to the entire amino acid sequence set forth in SEQ ID NO: 41, SEQ ID NO: 60, or SEQ ID NO: 61.

37. The fusion protein of claim 36, wherein the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 41.
38. The fusion protein of claim 36, wherein the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 60 or SEQ ID NO: 61.
39. A peptide linker comprising an amino acid sequence having at least about 85% amino acid identity to the entire amino acid sequence of an amino acid selected from the group consisting of DAASSHSHSSAR (SEQ ID NO: 14) and DAAGANANAGAR (SEQ ID NO: 16).
40. A fusion protein, comprising from N-terminus to C-terminus:
a first peptide comprising a relaxin B amino acid sequence;
a peptide linker; and
a second peptide comprising a relaxin A amino acid sequence,
wherein the fusion protein has an activity of a native relaxin-2 protein, and
wherein the fusion protein has a property selected from the group consisting of:
(i) activates the relaxin-2 receptor RXFP1 on a cell surface with EC50 of about 4.2 nM or less;
(ii) a melting temperature of at least about 57°C;
(iii) a circulating half-life of at least about 77.5 hours; and
(iv) any combination thereof.
41. A fusion protein comprising, from N-terminus to C-terminus,
a first peptide comprising an amino acid sequence that is at least about 90% identical to the entire amino acid sequence of SEQ ID NO:10;
a peptide linker; and
a second peptide comprising a relaxin A amino acid sequence.
42. The fusion protein of claim 41, wherein the amino acid in the first peptide corresponding to amino acid 4 of SEQ ID NO:10 is K; the amino acid in the first peptide corresponding to amino acid 25 of SEQ ID NO:10 is K; and the amino acid in the first peptide corresponding to amino acid 28 of SEQ ID NO:10 is A.

43. The fusion protein of claim 41 or 42, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO:16.
44. A fusion protein comprising, from N-terminus to C-terminus,
a first peptide comprising a relaxin B amino acid sequence;
a peptide linker comprising the amino acid sequence of SEQ ID NO:16; and
a second peptide comprising a relaxin A amino acid sequence.
45. The fusion protein of any one of claims 1-44, wherein:
the first peptide comprises the amino acid sequence of SEQ ID NO:10;
the amino acid sequence of the peptide linker consists of the amino acid sequence of SEQ ID NO:16;
the second peptide comprises an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO:1;
the second peptide comprises the amino acid sequence of SEQ ID NO:1;
the first peptide comprises an amino acid sequence that is at least about 90% identical to the entire amino acid sequence of SEQ ID NO:10; the peptide linker comprises the amino acid sequence of SEQ ID NO:16; and the second peptide comprises an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO:1; or
the first peptide comprises the amino acid sequence of SEQ ID NO:10; the peptide linker comprises the amino acid sequence of SEQ ID NO:16; and the second peptide comprises the amino acid sequence SEQ ID NO:1.
46. A polypeptide comprising an amino acid sequence that is at least about 90% identical to the entire amino acid sequence of SEQ ID NO:10.
47. The polypeptide of claim 46, wherein the amino acid corresponding to amino acid 4 of SEQ ID NO:10 is K; the amino acid corresponding to amino acid 25 of SEQ ID NO:10 is K; and the amino acid corresponding to amino acid 28 of SEQ ID NO:10 is A.
48. The polypeptide of claim 46 or 47, wherein the polypeptide comprises an amino acid sequence comprising SEQ ID NO:10.

49. The polypeptide of any one of claims 46-48, wherein the amino acid sequence of the polypeptide consists of SEQ ID NO:10.
50. The polypeptide of any one of claims 46-49 wherein the polypeptide further comprises an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO:16.
51. The polypeptide of any one of claims 46-50, wherein the polypeptide further comprises an amino acid sequence comprising SEQ ID NO:16.
52. The polypeptide of any one of claims 46-51, wherein the polypeptide further comprises an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO:1.
53. The polypeptide of any one of claims 46-52, wherein the polypeptide further comprises an amino acid sequence comprising SEQ ID NO:1.
54. A polypeptide comprising an amino acid sequence that comprises SEQ ID NO:1 and SEQ ID NO:16.
55. A polypeptide comprising an amino acid sequence that comprises the amino acid sequences of SEQ ID NO:1, SEQ ID NO:10, and SEQ ID NO:16, wherein the amino acid sequence of SEQ ID NO:16 is interposed between the amino acid sequences of SEQ ID NO:1 and SEQ ID NO:10.
56. A polynucleotide comprising a nucleotide sequence encoding the fusion protein of any one of claims 1-45, or the polypeptide of any one of claims 46-55.
57. The polynucleotide of claim 56, wherein the polynucleotide is an RNA molecule.
58. An expression vector comprising the polynucleotide of claim 56.
59. The expression vector of claim 58, wherein the expression vector is a plasmid.

60. The expression vector of claim 58, wherein the expression vector is a viral vector.
61. A recombinant cell comprising the polynucleotide of claim 56 or 57 or the expression vector of any one of claims 58-60.
62. The recombinant cell of claim 61, wherein the cell is a prokaryotic cell or a eukaryotic cell.
63. The recombinant cell of claim 62, wherein the cell is a prokaryotic cell selected from the group consisting of *E. coli* cell and *Bacillus* cell.
64. The recombinant cell of claim 62, wherein the cell is a eukaryotic cell selected from the group consisting of yeast cell, insect cell, and mammalian cell.
65. The recombinant cell of claim 64, wherein the cell is a mammalian cell selected from the group consisting of CHO cell, HeLa cell, and 293 cell.
66. The recombinant cell of claim 65, wherein the cell is an Expi293 cell.
67. A method of producing the fusion protein of any one of claims 1-45, or the polypeptide of any one of claims 46-55, comprising culturing the recombinant cell of any one of claims 61-66, and purifying the fusion protein.
68. A pharmaceutical composition comprising an effective amount of the fusion protein of any one of claims 1-45, or the polypeptide of any one of claims 46-55, or the polynucleotide of claim 56 or 57, or the expression vector of any one of claims 58-60.
69. A method of enhancing a relaxin-2-related activity in a cell, comprising contacting the cell with the fusion protein of any of claims 1-45, or the polypeptide of any one of claims 46-55, thereby enhancing relaxin-2-related activity in the cell.
70. The method of claim 69, wherein the fusion protein activates the relaxin-2 receptor, RXFP1, on a cell surface.

71. The method of claim 69 or 70, wherein the method elevates cAMP levels in the cell, inducing vasodilation, inducing the expression of angiogenic factors, inducing the expression of MMPs, and inducing collagen degradation.
72. The method of any one of claims 69-71, wherein the cell is selected from the group consisting of endothelial cells, vascular smooth muscle cells, other vascular cells, cardiomyocytes, other cardiac cells, and fibroblasts.
73. The method of any one of claims 69-71, wherein the cell is within a subject.
74. The method of 73, wherein the subject has a relaxin-2-associated disorder.
75. The method of claim 74, wherein the relaxin-2-associated disorder is selected from the group consisting of kidney diseases, fibrotic diseases, and cardiovascular diseases.
76. The method of claim 75, wherein the disorder is selected from the group consisting of focal segmental glomerular sclerosis (FSGS), diabetic nephropathy, hepatorenal syndrome, scleroderma, idiopathic pulmonary fibrosis, renal fibrosis, cardiac fibrosis, NASH, dilated cardiomyopathy, diastolic heart failure, pulmonary arterial hypertension, chronic heart failure, acute heart failure, congestive heart failure, coronary artery disease, hypertension, and pre-eclampsia.
77. A method of treating a relaxin-associated disorder in a subject in need thereof, comprising administering to the subject an effective amount of the fusion protein of any one of claims 1-45, or the polypeptide of any one of claims 46-55, the polynucleotide of claim 56 or 57, the expression vector of any one of claims 58-60, or the pharmaceutical composition of claim 68, thereby treating the relaxin-associated disorder.
78. The method of claim 77, wherein the relaxin-2-associated disorder is selected from the group consisting of kidney diseases, fibrotic diseases, and cardiovascular diseases.
79. The method of claim 78, wherein the disorder is selected from the group consisting of focal segmental glomerular sclerosis (FSGS), diabetic nephropathy, hepatorenal syndrome, scleroderma, idiopathic pulmonary fibrosis, renal fibrosis, cardiac fibrosis, NASH, dilated

cardiomyopathy, diastolic heart failure, pulmonary arterial hypertension, chronic heart failure, acute heart failure, congestive heart failure, coronary artery disease, hypertension, and pre-eclampsia.

80. The method of any one of claims 77-79, wherein the method decreases arterial pressure, increases renal artery blood flow, increases cardiac filling at diastole, resolves established fibrosis, or suppresses new fibrosis development.

81. A kit comprising an effective amount of the fusion protein of any one of claims 1-45, or the polypeptide of any one of claims 46-55, the polynucleotide of claim 56 or 57, the expression vector of any one of claims 58-60, or the pharmaceutical composition of claim 68, and an instruction of use.