

SUMMARY ~~OF THE INVENTION~~

Disclosed herein are novel relaxin-2 compositions and methods of use thereof for modulating, *e.g.*, enhancing, relaxin-2 activity in a subject, *e.g.*, a human subject. The composition and methods disclosed herein provide a means to treat and/or prevent relaxin-2-associated diseases in a subject, such as a subject who can benefit from a modulated, *e.g.*, increased or decreased, level of relaxin-2.

The compositions and methods disclosed herein are particularly advantageous in that they employ various fusion proteins and polypeptides disclosed herein that provide superior properties. For example, the fusion proteins and polypeptides of the present ~~invention~~disclosure have improved pharmacokinetics, *e.g.*, longer circulating half-life, or improved activity, *e.g.*, enhanced activation of RXFP1 as compared to a native relaxin-2 protein. The fusion proteins and polypeptides of the present ~~invention~~disclosure have been shown to provide improved activation of RXFP1 on a cell, with an EC₅₀ of about 0.085 nM to about 465 nM; and exhibit enhanced circulating half-life of at least about 77.5 hours to at least about 130 hours.

Accordingly, in one aspect, the present disclosure refers to 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: 2; a peptide linker comprising the amino acid sequence DAAGANANAGAR (SEQ ID NO: 16) or a variant thereof; and a second peptide comprising an amino acid sequence that is at least about 90% 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. In another aspect, the present disclosure refers to 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. In another aspect, the present disclosure refers to 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. In another aspect, the present disclosure refers to 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), or a variant thereof.

In another aspect, the present disclosure refers to 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 any combination thereof. In another aspect, the present disclosure refers to 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. In another aspect, the present disclosure refers to 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 or a variant thereof; and a second peptide comprising a relaxin A amino acid sequence. In another aspect, the present disclosure refers to 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.

A polypeptide comprising an amino acid sequence that comprises SEQ ID NO:1 and SEQ ID NO:16. In another aspect, the present disclosure refers to 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. In another aspect, the present disclosure refers to a polynucleotide comprising a nucleotide sequence encoding the fusion protein as disclosed herein, or the polypeptide as disclosed herein, optionally wherein the polynucleotide is an RNA molecule. In another aspect, the present disclosure refers to an expression vector comprising the polynucleotide as disclosed herein. In another aspect, the present disclosure refers to a recombinant cell comprising the polynucleotide as disclosed herein or the expression vector as disclosed herein. In another aspect, the present disclosure refers to a method of producing the fusion protein as disclosed herein, or the polypeptide as disclosed herein, comprising culturing the recombinant cell as disclosed herein, and purifying the fusion protein. In another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of the fusion protein as disclosed herein, or the polypeptide as disclosed herein, or the polynucleotide as disclosed herein, or the expression vector as disclosed herein.

In another aspect, the present disclosure refers to the use of the fusion protein as disclosed herein, or the polypeptide as disclosed herein in the manufacture of a medicament for enhancing a relaxin-2-related activity in a cell. In another aspect, the present disclosure refers to the use of the fusion protein as disclosed herein, the polypeptide as disclosed herein, the polynucleotide as disclosed herein, the expression vector as disclosed herein, or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a relaxin-associated disorder in a subject in need thereof. In yet another aspect, the present disclosure refers to a kit comprising an effective amount of the fusion protein as disclosed herein, or the polypeptide as disclosed herein, the polynucleotide as disclosed herein, the expression vector as disclosed herein, or the pharmaceutical composition as disclosed herein, and an instruction of use.

~~in~~In one aspectexample, the present ~~invention~~disclosure features a fusion protein. The fusion protein comprises, from N-terminus to C-terminus, a first peptide comprising an amino acid sequence that is at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% 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%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% 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.

In one embodimentexample, the fusion protein has an activity of a native relaxin-2 protein. In another embodimentexample, the fusion protein has at least about 50% activity of native relaxin-2 protein. In still another embodimentexample, the fusion protein has at least about 90% activity of native relaxin-2 protein. In yet another embodimentexample, the fusion protein has at least about 100% activity of native relaxin-2 protein. In one embodimentexample, the fusion protein has at least about 150% activity of native relaxin-2 protein.

In another embodimentexample, the peptide linker comprises an amino acid sequence that is at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%,

about 97%, about 98%, about 99%, or about 100% 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). In still another [embodimentexample](#), the peptide linker comprises the amino acid sequence of

5 DAASSHSHSSAR (SEQ ID NO: 14), DAASSHSHSSAA (SEQ ID NO: 15), or DAAGANANAGAR (SEQ ID NO: 16).

In still another [embodimentexample](#), the first peptide has an amino acid sequence that is at least about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the entire amino acid sequence of SEQ ID NO: 2, and the second peptide has an amino acid sequence

10 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. In yet another [embodimentexample](#), 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. In one [embodimentexample](#), wherein the first peptide

15 comprises a substitution selected from the group consisting of M4K, M25K, W28A, and combinations thereof. In another [embodimentexample](#), the first peptide comprises the substitutions M4K, M25K, and W28A.

In another [aspectexample](#), the present [inventiondisclosure](#) provides a fusion protein. The fusion protein comprises a first peptide, a peptide linker, and a second peptide, wherein the amino

20 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.

In still another [aspectexample](#), the present [inventiondisclosure](#) provides a fusion protein. The fusion protein comprises a first peptide, a peptide linker, and a second peptide, wherein the

25 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. In one [embodimentexample](#), the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 55.

In various [embodimentexamples](#) of the above aspects or any other aspect of the [inventiondisclosure](#) delineated herein, the fusion protein further comprises a first detectable label.

30 In one [embodimentexample](#), the first detectable label is operably linked to the N-terminus of the first peptide or the C-terminus of the second peptide. In another [embodimentexample](#), 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. In still another [embodimentexample](#), the first detectable label has an amino acid sequence of SEQ ID

35 NO: 20 or 21.

In yet another [embodimentexample](#), the first detectable label is operably linked to the N-terminus of the first peptide.

In one [embodimentexample](#), the fusion protein further comprises a second linker, wherein the second linker is operably linked to the C-terminus of the first detectable label and to the N-terminus. In another [embodimentexample](#), 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).

In one [embodimentexample](#), the fusion protein has an *in vivo* circulating half-life of greater than about 10 hours. In another [embodimentexample](#), the fusion protein has an *in vivo* circulating half-life of about 130 hours.

In another [embodimentexample](#), the first detectable label is a polyhistidine tag having an amino acid sequence comprising an amino acid sequence that is at least about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100% identical to the entire amino acid sequence selected from the group consisting of SEQ ID NOs: 18 and 19. In still another [embodimentexample](#), the first detectable agent comprises the amino acid sequence of SEQ ID NO: 18 or 19.

In one [embodimentexample](#), the fusion protein further comprises a second detectable label. In another [embodimentexample](#), 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. In still another [embodimentexample](#), the first detectable label and the second detectable label are operably linked to the N-terminus of the first peptide. In yet another [embodimentexample](#), the first detectable label and the second detectable label are different peptides.

In various [embodimentexamples](#) of the above aspects, the fusion protein further comprises a cleavable linker. In one [embodimentexample](#), the cleavable linker is a peptide subject to the specific digestion of a protease. In another [embodimentexample](#), the protease is HRV 3C protease or thrombin. In still another [embodimentexample](#), the cleavable linker is a peptide having the sequence of SEQ ID NO: 23, or a variant thereof.

In various [embodimentexamples](#) of the above aspects, the fusion protein further comprises a signal peptide at the N-terminus of the fusion protein.

In one [aspectexample](#), the present [inventiondisclosure](#) provides a fusion protein. The fusion protein includes 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. In one [embodimentexample](#), the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 41, SEQ ID NO: 60, or SEQ ID NO: 61.

In one [aspectexample](#), the present [inventiondisclosure](#) provides a fusion protein. The fusion protein includes 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.

In another [aspectexample](#), the present [inventiondisclosure](#) provides 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).

In another [aspectexample](#), the present [inventiondisclosure](#) provides a fusion protein which includes, 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.

In another [aspectexample](#), the present [inventiondisclosure](#) provides 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.

In one [embodimentexample](#), 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.

In another [embodimentexample](#), the peptide linker comprises the amino acid sequence of SEQ ID NO:16.

In another [aspectexample](#), the present [inventiondisclosure](#) provides 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.

In one [embodimentexample](#), 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.

10 In another [aspectexample](#), the present [inventiondisclosure](#) provides 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.

In one [embodimentexample](#), 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.

In another [embodimentexample](#), the polypeptide comprises an amino acid sequence comprising SEQ ID NO:10. In yet another [embodimentexample](#), the amino acid sequence of the polypeptide consists of SEQ ID NO:10.

20 In yet another [embodimentexample](#), 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. In yet another [embodimentexample](#), the polypeptide further comprises an amino acid sequence comprising SEQ ID NO:16.

In yet another [embodimentexample](#), 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. In yet another [embodimentexample](#), the polypeptide further comprises an amino acid sequence comprising SEQ ID NO:1.

In another [aspectexample](#), the present [inventiondisclosure](#) provides a polypeptide comprising an amino acid sequence that comprises SEQ ID NO:1 and SEQ ID NO:16.

30 In another [aspectexample](#), the present [inventiondisclosure](#) provides 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.

In still another [aspectexample](#), the present [inventiondisclosure](#) provides a polynucleotide comprising a nucleotide sequence encoding the fusion protein or polypeptide of any [embodimentexamples](#) of the above aspects. In one [embodimentexample](#), the polynucleotide is an RNA molecule.

5 In yet another [aspectexample](#), the present [inventiondisclosure](#) provides an expression vector. The expression vector comprises the polynucleotide of the above [aspectexamples](#). In one [embodimentexample](#), the expression vector is a plasmid. In another [embodimentexample](#), the expression vector is a viral vector.

10 In one [aspectexample](#), the present [inventiondisclosure](#) provides a recombinant cell. The recombinant cell comprises the polynucleotide or the expression vector of the above [aspectexamples](#). In one [embodimentexample](#), the cell is a prokaryotic cell or a eukaryotic cell. In another [embodimentexample](#), the cell is a prokaryotic cell selected from the group consisting of *E. coli* cell and *Bacillus* cell. In still another [embodimentexample](#), the cell is a eukaryotic cell selected from the group consisting of yeast cell, insect cell, and mammalian cell. In yet another [embodimentexample](#),
15 the cell is a mammalian cell selected from the group consisting of CHO cell, HeLa cell, and 293 cell. In one [embodimentexample](#), the cell is an Expi293 cell.

In one [aspectexample](#), the present [inventiondisclosure](#) provides a method of producing the fusion protein of the above [aspectexamples](#), comprising culturing the recombinant cell of the above [aspectexamples](#), and purifying the fusion protein.

20 In another [aspectexample](#), the present [inventiondisclosure](#) provides a pharmaceutical composition. The pharmaceutical composition comprises an effective amount of the fusion protein of any one of the above [aspectexamples](#), or the polynucleotide of any one of the above [aspectexamples](#), or the expression vector of any one of the above [aspectexamples](#).

25 In still another [aspectexample](#), the present [inventiondisclosure](#) provides a method of enhancing a relaxin-2-related activity in a cell, comprising contacting the cell with the fusion protein of any of the above [aspectexamples](#), thereby enhancing relaxin-2-related activity in the cell. In one [embodimentexample](#), the fusion protein activates the relaxin-2 receptor, RXFP1, on a cell surface. In another [embodimentexample](#), the method elevates cAMP levels in the cell, inducing vasodilation, inducing the expression of angiogenic factors, inducing the expression of MMPs, and inducing
30 collagen degradation. In still another [embodimentexample](#), the cell is selected from the group consisting of endothelial cells, vascular smooth muscle cells, other vascular cells, cardiomyocytes, other cardiac cells, and fibroblasts.

35 In one [embodimentexample](#), the cell is within a subject. In another [embodimentexample](#), the subject has a relaxin-2-associated disorder. In still another [embodimentexample](#), the relaxin-2-associated disorder is selected from the group consisting of kidney diseases, fibrotic diseases, and

cardiovascular diseases. In yet another [embodimentexample](#), 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.

In one [aspectexample](#), the present [inventiondisclosure](#) provides a method of treating a relaxin-2-associated disorder in a subject in need thereof. The method comprises administering to the subject an effective amount of the fusion protein of any one of the above [aspectexamples](#), the polynucleotide of any one of above [aspectexamples](#), the expression vector of any one of above [aspectexamples](#), or the pharmaceutical composition of above [aspectexamples](#), thereby treating the relaxin-2-associated disorder. In one [embodimentexample](#), the relaxin-2-associated disorder is selected from the group consisting of kidney diseases, fibrotic diseases, and cardiovascular diseases. In another [embodimentexample](#), 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. In still another [embodimentexample](#), the method decreases arterial pressure, increases renal artery blood flow, increases cardiac filling at diastole, resolves established fibrosis, or suppresses new fibrosis development.

In another [aspectexample](#), the present [inventiondisclosure](#) provides a kit. The kit comprises an effective amount of the fusion protein of any one of the above [aspectexamples](#), the polynucleotide of any one of the above [aspectexamples](#), the expression vector of any one of the above [aspectexamples](#), or the pharmaceutical composition of any one of the above [aspectexamples](#), and an instruction of use.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A – 1C are images showing electrophoresis and Coomassie blue staining of recombinant relaxin-2 proteins, SE001, SE201, SE202, SE203, SE204, SE205, SE206, SE207, and SE301.

FIG. 2 is a graph showing the size exclusion chromatography of SE301.

FIG. 3 is a graph showing the determination of T_m of SE301 using differential scanning fluorimetry.

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~~90%
identical to the entire amino acid sequence of SEQ ID NO: 2;
a peptide linker comprising ~~an-the~~ 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)~~or a variant thereof; and
a second peptide comprising an amino acid sequence that is at least about ~~85~~90%
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:
(i) the fusion protein has at least about 50% activity of native relaxin-2 protein;
(ii) the fusion protein has at least about 90% activity of native relaxin-2 protein;
(iii) the fusion protein has at least about 100% activity of native relaxin-2 protein; or
(iv) the fusion protein has at least about 150% 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.~~

~~74.~~ The fusion protein of any one of claims 1-~~63~~, 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), optionally wherein the peptide linker comprises the amino acid sequence of DAASSHSHSSAR (SEQ ID NO: 14), DAASSHSHSSAA (SEQ ID NO: 15), or DAAGANANAGAR (SEQ ID NO: 16).

~~8.~~ ~~The fusion protein of claim 7, wherein the peptide linker comprises the amino acid sequence of DAASSHSHSSAR (SEQ ID NO: 14), DAASSHSHSSAA (SEQ ID NO: 15), or DAAGANANAGAR (SEQ ID NO: 16).~~

~~95.~~ The fusion protein of any one of claims 1-~~84~~, wherein:

(i) the first peptide has an amino acid sequence that is at least about 9592% 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; and/or

(ii) 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.

~~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.~~

~~116.~~ The fusion protein of claim ~~95~~, wherein the first peptide comprises a substitution selected from the group consisting of M4K, M25K, W28A, and combinations thereof, optionally wherein the first peptide comprises the substitutions M4K, M25K, and W28A.

~~12.~~ ~~The fusion protein of claim 11, wherein the first peptide comprises the substitutions M4K, M25K, and W28A.~~

~~137.~~ 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.~~

~~168.~~ The fusion protein of any one of claims 1-~~157~~, further comprising a first detectable label.

~~179.~~ The fusion protein of claim ~~168~~, wherein:

(i) the first detectable label is operably linked to the N-terminus of the first peptide or the C-terminus of the second peptide;

(ii) 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;

(iii) the first detectable label has an amino acid sequence of SEQ ID NO: 20 or 21;

(iv) the first detectable label is operably linked to the N-terminus of the first peptide;

(v) 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; and/or

(vi) the first detectable agent comprises the amino acid sequence of SEQ ID NO: 18 or 19.

~~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~~10. The fusion protein of claim ~~20~~9, 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, optionally 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).

~~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~~11. The fusion protein of ~~any one of claims 18-229 or 10~~, wherein the fusion protein has an *in vivo* circulating half-life of greater than about 10 hours, optionally wherein the fusion protein has an *in vivo* circulating half-life of about 130 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~~12. The fusion protein of any one of claims ~~16~~8-2611, further comprising a second detectable label.

~~28~~13. The fusion protein of claim ~~27~~12, wherein:

(i) 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;

- (ii) wherein the first detectable label and the second detectable label are operably linked to the N-terminus of the first peptide; and/or
- (iii) the first detectable label and the second detectable label are different peptides.

~~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~~14. The fusion protein of any one of claims 1-~~30~~13, further comprising a cleavable linker.

~~32~~15. The fusion protein of claim ~~31~~14, wherein the cleavable linker is a peptide subject to the specific digestion of a protease, optionally wherein the protease is HRV 3C protease or thrombin, optionally wherein the cleavable linker is a peptide having the sequence of SEQ ID NO: 23, or a variant thereof.

~~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~~16. The fusion protein of any one of claims 1-~~34~~15, further comprising a signal peptide at the N-terminus of the fusion protein.

~~36~~17. 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~~18. The fusion protein of claim ~~36~~17, wherein the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 41.

~~38~~19. The fusion protein of claim ~~36~~17, wherein the amino acid sequence of the fusion protein is set forth in SEQ ID NO: 60 or SEQ ID NO: 61.

~~39~~20. 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 DAASSSHSSAR (SEQ ID NO: 14) and DAAGANANAGAR (SEQ ID NO: 16), or a variant thereof.

~~40~~21. 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~~22. 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~~23. The fusion protein of claim ~~41~~22, wherein:
(i) 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; and/or
(ii) the peptide linker comprises the amino acid sequence of SEQ ID NO:16.

~~43. The fusion protein of claim 41 or 42, wherein the peptide linker comprises the amino~~

~~acid sequence of SEQ ID NO:16.~~

~~4424.~~ 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 or a variant thereof; and
a second peptide comprising a relaxin A amino acid sequence.

~~4525.~~ The fusion protein of any one of claims 1-~~4424~~, 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.

~~4626.~~ 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.

~~4727.~~ The polypeptide of claim ~~4626~~, wherein:
(i) 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;
(ii) the polypeptide comprises an amino acid sequence comprising SEQ ID NO:10;
and/or
(iii) the amino acid sequence of the polypeptide consists of SEQ ID NO:10.

~~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~~28. The polypeptide of any one of claims ~~46-49~~26-27 wherein the polypeptide further comprises:

(i) an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO:16;

(ii) an amino acid sequence comprising SEQ ID NO:16;

(iii) an amino acid sequence that is at least about 85% identical to the entire amino acid sequence of SEQ ID NO:1; and/or

(iv) an amino acid sequence comprising SEQ ID NO:1.

~~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~~29. A polypeptide comprising an amino acid sequence that comprises SEQ ID NO:1 and SEQ ID NO:16.

~~55~~30. 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.

~~5631.~~ A polynucleotide comprising a nucleotide sequence encoding the fusion protein of any one of claims 1-~~4525~~, or the polypeptide of any one of claims ~~4626-5530~~, optionally wherein the polynucleotide is an RNA molecule.

~~57.~~—The polynucleotide of claim 56, wherein the polynucleotide is an RNA molecule.

~~5832.~~ An expression vector comprising the polynucleotide of claim ~~5631~~.

~~5933.~~ The expression vector of claim ~~5832~~, wherein:

- (i) the expression vector is a plasmid; or
- (ii) the expression vector is a viral vector.

~~60.~~—The expression vector of claim 58, wherein the expression vector is a viral vector.

~~6134.~~ A recombinant cell comprising the polynucleotide of claim ~~5631~~ or ~~57~~ or the expression vector of ~~any one of claims 58-60~~ 32 or 33.

~~6235.~~ The recombinant cell of claim ~~6134~~, wherein:

- (i) the cell is a prokaryotic cell or a eukaryotic cell;
- (ii) the cell is a prokaryotic cell selected from the group consisting of *E. coli* cell and *Bacillus* cell;
- (iii) the cell is a eukaryotic cell selected from the group consisting of yeast cell, insect cell, and mammalian cell;
- (iv) the cell is a mammalian cell selected from the group consisting of CHO cell, HeLa cell, and 293 cell; and/or
- (v) the cell is an Expi293 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.~~

~~6736. A method of producing the fusion protein of any one of claims 1-4525, or the polypeptide of any one of claims 4626-5530, comprising culturing the recombinant cell of any one of claims 6134 or 6635, and purifying the fusion protein.~~

~~6837. A pharmaceutical composition comprising an effective amount of the fusion protein of any one of claims 1-4525, or the polypeptide of any one of claims 4626-5530, or the polynucleotide of claim 56 or 5731, or the expression vector of any one of claims 58-6032 or 33.~~

~~6938. Use of the fusion protein of any of claims 1-25, or the polypeptide of any one of claims 26-30 in the manufacture of a medicament for 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.~~

~~7039. The method-use of claim 6938, wherein:~~

- ~~(i) the fusion protein activates the relaxin-2 receptor, RXFP1, on a cell surface;~~
- ~~(ii) the use elevates cAMP levels in the cell, inducing vasodilation, inducing the expression of angiogenic factors, inducing the expression of MMPs, and inducing collagen degradation;~~
- ~~(iii) the cell is selected from the group consisting of endothelial cells, vascular smooth muscle cells, other vascular cells, cardiomyocytes, other cardiac cells, and fibroblasts; and/or~~
- ~~(iv) the cell is within a subject, optionally wherein the subject has a relaxin-2-associated disorder, optionally wherein the relaxin-2-associated disorder is selected from the group consisting of kidney diseases, fibrotic diseases, and cardiovascular diseases, optionally 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.

~~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.~~

~~7740. Use of the fusion protein of any one of claims 1-25, the polypeptide of any one of claims 26-30, the polynucleotide of claim 31, the expression vector of claim 32 or 33, or the pharmaceutical composition of claim 37 in the manufacture of a medicament for 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.~~

~~7841~~. The ~~method-use~~ of claim ~~7740~~, wherein:

(i) the relaxin-2-associated disorder is selected from the group consisting of kidney diseases, fibrotic diseases, and cardiovascular diseases;

(ii) the relaxin-2-associated 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; and/or

(iii) the use decreases arterial pressure, increases renal artery blood flow, increases cardiac filling at diastole, resolves established fibrosis, or suppresses new fibrosis development.

~~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.~~

~~8142~~. A kit comprising an effective amount of the fusion protein of any one of claims 1-~~4525~~, or the polypeptide of any one of claims ~~4626-5530~~, the polynucleotide of claim ~~5631~~ or ~~57~~, the expression vector of any one of claims ~~58-6032 or 33~~, or the pharmaceutical composition of claim ~~6837~~, and an instruction of use.