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(54) Title: TREATMENT USING HETERODIMERIC RELAXIN FUSIONS

(57) Abstract: The present invention relates to uses of heterodimeric Relaxin fusion polypeptides, in particular for the treatment of heart failure with pulmonary hypertension.

TREATMENT USING HETERODIMERIC RELAXIN FUSIONS

Field of the Invention

The present invention relates to methods of treatment using heterodimeric Relaxin fusions. In particular, the present invention relates to methods of treatment using Relaxin-2 fusions.

Background

Relaxin is a peptide hormone that belongs to the insulin superfamily. In humans, the Relaxin peptide family includes seven peptides of high structural but low sequence similarity: Relaxin 1, 2 and 3, and the insulin-like peptides INSL3, INSL4, INSL5 and INSL6. Naturally occurring Relaxins consist of A and B polypeptide chains covalently linked by two inter-chain disulphide bonds. The A chain has an additional intra-chain disulphide bond. The relaxin genes encode prohormones with structure B-C-A (B and A polypeptide chains linked by a C peptide). The prohormone undergoes endoproteolytic cleavage with PC1 and PC2 enzymes to remove the C peptide, before secretion of mature Relaxin.

Relaxin is a pleiotropic hormone known to mediate systemic haemodynamic and renal adaptive changes during pregnancy. Relaxin has also been shown to have anti-fibrotic properties and to have beneficial effects in heart failure e.g. with acute decompensated heart failure (ADHF). Heart failure is associated with significant morbidity and mortality. It is characterized by complex tissue remodelling involving increased cardiomyocyte death and interstitial fibrosis. Relaxin activates a number of signalling cascades which have been shown to be beneficial in the setting of ischemia-reperfusion and heart failure. These signalling pathways include activation of the phosphoinositide 3-kinase pathway and activation of the nitric oxide signalling pathway (Bathgate RA *et al.* (2013) *Physiol. Rev.* 93(1): 405-480; Mentz RJ *et al.* (2013) *Am. Heart J.* 165(2): 193-199; Tietjens J *et al.* (2016) *Heart* 102: 95-99; Wilson SS *et al.* (2015) *Pharmacology* 35: 315-327).

In heart failure patients, a significant subset also suffer from pulmonary hypertension (HF+PH patients). It was estimated that approximately 50% of heart failure patients with preserved ejection fraction also suffer from pulmonary hypertension, increasing to 60% of heart failure patients with reduced ejection fraction (Guazzi, (2014) *Circ Heart Fail.*, 7:367-

377; Miller et al., (2013) *JACC Heart Fail.*, 1(4):290-299). Patients suffering from heart failure with pulmonary hypertension have been shown to have reduced survival as compared with heart failure patients without pulmonary hypertension (Barnett and De Marco, (2012) *Heart Fail. Clin.* 8: 447–459). In heart failure patients, a 3 mmHg increase or decrease in Estimated Pulmonary Artery Diastolic Pressure (ePAD), equivalent to approximately 4 mmHg increase or decrease in mean Pulmonary Arterial Pressure (mPAP), was associated with a 24% increase or a 19% decrease in cardiovascular mortality respectively (Zile MR, et al. (2017) *Circ Heart Fail.*, 10:e003594). A 4 mmHg reduction in mPAP is also associated with dyspnea improvement in patients suffering from heart failure and pulmonary hypertension (Solomonica A, et al. (2013) *Circ Heart Fail.*, 6:53-60).

Clinical trials have been conducted using unmodified recombinant human Relaxin-2, serelaxin. Continuous intravenous administration of serelaxin to hospitalized patients improved the markers of cardiac, renal and hepatic damage and congestion (Felker GM *et al.* (2014) *J. Am. Coll. Cardiol.* 64(15): 1591-1598; Metra M *et al.* (2013) *J. Am. Coll. Cardiol.* 61(2): 196-206; Teerlink JR *et al.* (2013) *Lancet* 381(9860): 29-39). Serelaxin also demonstrated improvements in pulmonary artery pressures, cardiac output, and systemic and pulmonary vascular resistance in these patients with an approximate 20-hour continuous infusion (Ponikowski et al., (2014) *European Heart Journal* 35:431–441). However, due to the rapid clearance of serelaxin from the patients' circulation, the therapeutic effects were limited and the positive effects rapidly disappeared once intravenous injection stopped. Additionally, approximately one third of the patients experienced a significant blood pressure drop (>40 mm Hg) after receiving serelaxin intravenously, with the consequence that the dose had to be reduced by half or even more.

WO 2013/004607 and WO 2018/138170 describe recombinant Relaxin polypeptides in which the Relaxin A and Relaxin B are fused in a single chain with a linker peptide. WO2013/004607 describes recombinant Relaxin with a linker peptide of at least five amino acids and less than 15 amino acids. WO 2018/138170 describes recombinant Relaxin with a linker peptide of at least 15 amino acids.

Given the promising clinical studies conducted so far with unmodified recombinant Relaxin, there remains a need for further recombinant Relaxins which retain a Relaxin biological activity and provide advantages such as an extended half-life, convenient route of administration and convenient dosing.

Summary of Invention

The present invention relates to the use of heterodimeric fusions having Relaxin activity in the treatment of subjects suffering from heart failure with pulmonary hypertension (HF+PH). Treatment of HF+PH subjects specifically remains a significant unmet need.

Thus, in one aspect, the present invention provides a method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) a first heterodimerisation domain connected to at least one Relaxin A chain polypeptide or a variant thereof; and
(ii) a second heterodimerisation domain connected to at least one Relaxin B chain polypeptide or a variant thereof,

wherein the first heterodimerisation domain heterodimerises with the second heterodimerisation domain, and wherein the heterodimeric fusion has Relaxin activity.

Similarly, the invention provides a heterodimeric fusion for use in treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) a first heterodimerisation domain connected to at least one Relaxin A chain polypeptide or a variant thereof; and
(ii) a second heterodimerisation domain connected to at least one Relaxin B chain polypeptide or a variant thereof,

wherein the first heterodimerisation domain heterodimerises with the second heterodimerisation domain, and wherein the heterodimeric fusion has Relaxin activity.

Similarly, the invention provides the use of a heterodimeric fusion in the manufacture of a medicament for treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) a first heterodimerisation domain connected to at least one Relaxin A chain polypeptide or a variant thereof; and
(ii) a second heterodimerisation domain connected to at least one Relaxin B chain polypeptide or a variant thereof,

wherein the first heterodimerisation domain heterodimerises with the second heterodimerisation domain, and wherein the heterodimeric fusion has Relaxin activity.

In some embodiments, the Relaxin A chain and the Relaxin B chain of the heterodimeric fusion are covalently bound by one or more (e.g. two) inter-chain bonds, preferably one or more (e.g. two) inter-chain disulphide bonds. In some embodiments, the Relaxin A chain and the Relaxin B chain are not covalently linked to each other by an amino acid linker.

In some embodiments, the Relaxin A chain is a Relaxin-2 A chain and the Relaxin B chain is a Relaxin-2 B chain.

In preferred embodiments, the first and second heterodimerisation domains are derived from an immunoglobulin Fc region, *e.g.* an immunoglobulin G (IgG) Fc region, ("first Fc region" and "second Fc region"). The first and second Fc regions may comprise constant domains CH2 and/or CH3. Preferably, the first and second Fc regions comprise CH2 and CH3.

In alternative embodiments, the first and second heterodimerisation domains are derived from an immunoglobulin Fab region.

In yet further alternative embodiments, the first and second heterodimerisation domains heterodimerise to form parallel coiled coils.

In some embodiments, the Relaxin A chain is connected to the first heterodimerisation domain (e.g. first Fc region) via a connector and the Relaxin B chain is connected to the second heterodimerisation domain (e.g. second Fc region) via a connector. In preferred embodiments, one or preferably both connectors are polypeptides.

In some embodiments, at least one connector is a polypeptide having a length of between 6 and 40 amino acids. Preferably, both connectors are polypeptides having a length of between 6 and 40 amino acids. In preferred embodiments, at least one connector is a polypeptide having a length of 21 amino acids. In particularly preferred embodiments, both connectors are polypeptides having a length of 21 amino acids. In certain embodiments, both connectors have the sequence GGGGSGGGGSGGGGSGGGGGS [SEQ ID NO: 5].

In preferred embodiments, the C-terminus of the first heterodimerisation domain (e.g. first Fc region) is connected to the N-terminus of the Relaxin A chain and the C-terminus of the second heterodimerisation domain (e.g. second Fc region) is connected to the N-

terminus of the Relaxin B chain. In alternative embodiments, the N-terminus of the first heterodimerisation domain (e.g. first Fc region) is connected to the C-terminus of the Relaxin A chain and the N-terminus of the second heterodimerisation domain (e.g. second Fc region) is connected to the C-terminus of the Relaxin B chain.

In some embodiments, the first and second heterodimerisation domains (e.g. first and second Fc regions) comprise heterodimerisation-promoting amino acid mutations and/or modifications, preferably asymmetric heterodimerisation-promoting amino acid mutations and/or modifications. In preferred embodiments, the heterodimerisation-promoting amino acid mutations are “Fc Knob” and “Fc Hole” mutations. In particularly preferred embodiments, the “Fc Knob” and “Fc Hole” mutations are present in the CH3 domains. In preferred embodiments, the first Fc region comprises “Fc Knob” mutations and the second Fc region comprises “Fc Hole” mutations. Alternatively, the first Fc region has “Fc Hole” mutations, and the second Fc region has “Fc Knob” mutations. Preferably, the heterodimerisation-promoting amino acid mutations comprise “Fc Hole” mutations Y349C, T366S, L368A and Y407V, or conservative substitutions thereof, in one CH3 domain; and “Fc Knob” mutations S354C and T366W, or conservative substitutions thereof, in the other CH3 domain, wherein the amino acid numbering is according to the EU index as in Kabat.

In embodiments of any aspect of the invention, the Relaxin-2 A chain polypeptide comprises the sequence as set forth in SEQ ID NO: 1 or a variant thereof and the Relaxin-2 B chain polypeptide comprises the sequence as set forth in SEQ ID NO: 2 or a variant thereof. In some embodiments, the Relaxin-2 A chain polypeptide comprises the amino acid mutation K9H, K17M or K17I, preferably K9H.

Also provided by the present invention is a method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) an FcX-con-A fusion polypeptide; and

(ii) an FcY-con-B fusion polypeptide,

wherein:

A is a Relaxin A chain or variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

FcY is an immunoglobulin (e.g. IgG1) Fc region with “Fc Hole” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with “Fc Knob” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W or conservative substitutions thereof; and

con is a connector, e.g. a connector polypeptide preferably having the sequence GGGGSGGGGSGGGGSGGGGGS [SEQ ID NO: 5],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity.

Similarly, the invention provides a heterodimeric fusion for use in a method of treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) an FcX-con-A fusion polypeptide; and

(ii) an FcY-con-B fusion polypeptide,

wherein:

A is a Relaxin A chain or variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

FcY is an immunoglobulin (e.g. IgG1) Fc region with “Fc Hole” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with “Fc Knob” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W or conservative substitutions thereof; and

con is a connector, e.g. a connector polypeptide preferably having the sequence GGGGSGGGGSGGGGSGGGGGS [SEQ ID NO: 5],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity.

Similarly, the invention provides the use of a heterodimeric fusion in the manufacture of a medicament for treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) an FcX-con-A fusion polypeptide; and

(ii) an FcY-con-B fusion polypeptide,

wherein:

A is a Relaxin A chain or variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

FcY is an immunoglobulin (e.g. IgG1) Fc region with "Fc Hole" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W or conservative substitutions thereof; and

con is a connector, e.g. a connector polypeptide preferably having the sequence GGGGSGGGGSGGGGSGGGGGS [SEQ ID NO: 5],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity.

In particularly preferred embodiments, the heterodimeric fusion comprises a fusion polypeptide with the amino acid sequence of SEQ ID NO: 11 and a fusion polypeptide with the amino acid sequence of SEQ ID NO: 20.

In some embodiments of any aspect of the invention, the heterodimeric fusion further comprises one or more Fabs, optionally wherein the heterodimeric fusion comprises one Fab linked to the N-terminus of the first heterodimerisation domain (e.g. first Fc region) and a second Fab linked to the N-terminus of the second heterodimerisation domain (e.g. second Fc region).

In some embodiments of any aspect of the invention, the heterodimeric fusion further comprises a second Relaxin A chain polypeptide or variant thereof connected to the N-terminus of the first heterodimerisation domain (e.g. first Fc region) and a second Relaxin B chain polypeptide or variant thereof connected to the N-terminus of the second heterodimerisation domain (e.g. second Fc region), optionally wherein the second Relaxin A chain is connected to the first heterodimerisation domain (e.g. first Fc region) via a connector polypeptide and the second Relaxin B chain is connected to the second heterodimerisation domain (e.g. second Fc region) via a connector polypeptide.

In another aspect, the invention provides a method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) FcX-B-L-A and FcY, optionally FcY-B-L-A; or

(ii) FcY-B-L-A and FcX, optionally FcX-B-L-A;

wherein:

FcY is an immunoglobulin (e.g. IgG1) Fc region with “Fc Hole” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with “Fc Knob” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity. Alternatively, the FcX and the FcY are non-Fc heterodimerisation domains as described herein. In some embodiments, the Relaxin B chain is connected to FcX and/or FcY via a connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

Similarly, the invention provides a heterodimeric fusion for use in a method of treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) FcX-B-L-A and FcY, optionally FcY-B-L-A; or

(ii) FcY-B-L-A and FcX, optionally FcX-B-L-A;

wherein:

FcY is an immunoglobulin (e.g. IgG1) Fc region with "Fc Hole" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity. Alternatively, the FcX and the FcY are non-Fc heterodimerisation domains as described herein. In some embodiments, the Relaxin A chain is connected to FcX and/or FcY via a connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

Similarly, the invention provides the use of a heterodimeric fusion in the manufacture of a medicament for treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) FcX-B-L-A and FcY, optionally FcY-B-L-A; or

(ii) FcY-B-L-A and FcX, optionally FcX-B-L-A;

wherein:

FcY is an immunoglobulin (e.g. IgG1) Fc region with "Fc Hole" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity. Alternatively, the FcX and the FcY are non-Fc heterodimerisation domains as described herein. In some embodiments, the Relaxin A chain is connected to FcX and/or FcY via a connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

In yet another aspect, the invention provides a method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) FcX-A-L-B and FcY, optionally FcY-A-L-B; or

(ii) FcY-A-L-B and FcX, optionally FcX-A-L-B;

wherein:

FcY is an immunoglobulin (e.g. IgG1) Fc region with "Fc Hole" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity. Alternatively, the FcX and the FcY are non-Fc heterodimerisation domains as described herein. In some embodiments, the Relaxin A chain is connected to FcX and/or FcY via a connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

Similarly, the invention provides a heterodimeric fusion for use in a method of treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) FcX-A-L-B and FcY, optionally FcY-A-L-B; or

(ii) FcY-A-L-B and FcX, optionally FcX-A-L-B;

wherein:

FcY is an immunoglobulin (e.g. IgG1) Fc region with “Fc Hole” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with “Fc Knob” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity. Alternatively, the FcX and the FcY are non-Fc heterodimerisation domains as described herein. In some embodiments, the Relaxin A chain is connected to FcX and/or FcY via a

connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

Similarly, the invention provides the use of a heterodimeric fusion in the manufacture of a medicament for treating a subject with heart failure with pulmonary hypertension, the heterodimeric fusion comprising:

(i) FcX-A-L-B and FcY, optionally FcY-A-L-B; or

(ii) FcY-A-L-B and FcX, optionally FcX-A-L-B;

wherein:

FcY is an immunoglobulin (e.g. IgG1) Fc region with "Fc Hole" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;

FcX is an immunoglobulin (e.g. IgG1) Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity. Alternatively, the FcX and the FcY are non-Fc heterodimerisation domains as described herein. In some embodiments, the Relaxin A chain is connected to FcX and/or FcY via a connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

According to all aspects of the invention, the heart failure may be heart failure with reduced ejection fraction, heart failure with mid-range ejection fraction or heart failure with preserved ejection fraction.

According to all aspects of the invention, the subject may have a mean Pulmonary Arterial Pressure of about 25 mmHg or greater and/or a Right Ventricular Systolic Pressure of

about 40 mmHg or greater. Typically, this is prior to treatment with the heterodimeric fusions of the invention.

According to all aspects of the invention, the subject may have a Pulmonary Vascular Resistance of less than 3.0 wood units. Alternatively, the subject may have a Pulmonary Vascular Resistance of 3.0 or more wood units. Typically, this is prior to treatment with the heterodimeric fusions of the invention.

According to all aspects of the invention, the subject may have been fitted with a blood pressure monitoring device, preferably a pulmonary artery pressure monitoring device. Preferably, the pulmonary artery pressure monitoring device is a CardioMEMS pressure monitoring device.

In some embodiments of any aspect of the invention, the ratio of Relaxin activity of the heterodimeric fusion over the Relaxin activity of a reference Relaxin protein is between about 0.001 and about 10.

According to all aspects of the invention, the heterodimeric fusion may be administered as a pharmaceutical composition comprising the heterodimeric fusion of the invention.

Also described herein are nucleic acid molecules (e.g. DNA molecules) encoding a heterodimeric fusion of the invention, vectors comprising a nucleic acid molecule, host cells comprising a vector or nucleic acid, and methods of producing the heterodimeric fusions of the invention by culturing the host cells and collecting the fusion protein.

Aspects and embodiments of the invention are set out in the appended claims. These and other aspects and embodiments of the invention are also described herein.

Brief Description of Figures and Sequence Listing

Figure 1 shows exemplary formats of the heterodimeric fusions according to some embodiments of the invention. The format of each fusion polypeptide of the heterodimeric fusion is given in terms of FcX, FcY, A, B, con and L, wherein FcX ("Fc Knob") and FcY ("Fc Hole") are two Fc regions comprising heterodimerisation-promoting amino acid mutations and/or modifications; A ("Rlx A") and B ("Rlx B") are Relaxin A chain and Relaxin B chain polypeptides; "con" is a connector polypeptide; L is a linker polypeptide, HC X and HC Y – heavy chains of an antibody, LC – light chain of an antibody, hinge – the hinge region of an antibody and Fab is Fab fragment of an antibody.

Figure 2 shows LC-MS analysis of RELAX0019 and RELAX0023 A) RELAX0019 and RELAX0023 deglycosylated and non-reduced analysis showing the mass of intact molecules B) RELAX0019 and RELAX0023 deglycosylated and reduced analysis showing masses of individual Fc-fusion chains – Knob Relaxin Chain A and Hole Relaxin Chain B.

Figure 3 shows analysis of the C-terminal peptide of RELAX0019 and RELAX0023 by non-reduced peptide mapping using LC-MS. The amino acid sequence of the C-terminal peptide with predicted disulphide bonds represented by lines is shown in the top panel. Panels A and E - the extracted ion chromatogram of the C-terminal peptide in absence of the reducing agent (-DTT). Panels C and G - deconvoluted mass spectrum of the C-terminal peptide in absence of the reducing agent. Panels B and F - the extracted ion chromatogram in the presence of the reducing agent (+DTT) and Panels D and H - deconvoluted mass spectrum in the presence of the reducing agent. Figure 3 discloses SEQ ID NOS 75, 77, and 76, respectively in order of appearance.

Figure 4 shows the *in vitro* biological activity of some heterodimeric fusions of the invention measured by cAMP induction in cells expressing recombinant human RXFP1.

Figure 5 shows *in vivo* pharmacokinetic (PK) profiles from a series of ELISA experiments where heterodimeric fusions of the invention were administered to mice intravenously. Data is normalised as a % cMax at the 5 min time point (T1).

Figure 6 shows reversal of isoproterenol-induced cardiac fibrosis and hypertrophy in mice treated with RELAX0019 and RELAX0023. Levels of fibrosis and hypertrophy for (1) vehicle (baseline), (2) isoproterenol, (3) isoproterenol + Relaxin-2, (4) isoproterenol + RELAX0019, and (5) isoproterenol + RELAX0023 are shown.

Figure 7 shows the *in vitro* non-specific binding of heterodimeric fusions of the invention in Baculovirus (BV) ELISA assay.

Figure 8 shows the percentage of purity loss, aggregation and fragmentation of RELAX0023, RELAX0127 and RELAX0128 in solution upon storage.

Figure 9 shows the stability of RELAX0023, RELAX0127 and RELAX0128 in solution over time assessed by reduced LC-MS analysis. A) Total ion chromatograms B) Mass spectra of reduced molecules

Figure 10 shows the PK profile of RELAX0023 in cynomolgus monkeys following intravenous and subcutaneous injections.

Figure 11 shows the nucleotide sequences encoding some of the polypeptides of the present invention (SEQ ID NOS 80-140, respectively, in order of appearance).

Figure 12 shows the results of a chronic efficacy study of RELAX0023 in cynomolgus monkey (*Macaca fascicularis*) with heart failure and reduced left ventricular ejection fraction (LVEF). The effect of RELAX0023 on LVEF, heart rate (HR) and mean arterial pressure (MAP) is shown in (A), (B) and (C) respectively in said monkeys treated with a relative low, mid or high dose of RELAX0023 or vehicle control for 20 weeks, followed by an observational period. Each data point represents the average value of the group (n=8 for treatments groups and n=14 for vehicle group). The x-axis for each of (A), (B) and (C) represents weeks from the start of the treatment period.

FIGs. 13A-13F shows cardiac function, systemic vascular resistance, and organ perfusion in the MAD Cohorts following administration of RELAX0023: **FIG. 6A** shows ejection fraction (EF) in HFpEF subjects, **FIG. 6B** shows EF in HFrEF subjects, **FIG. 6C** shows cardiac output in pooled subjects, **FIG. 6D** shows systemic vascular resistance (SVR) in pooled subjects, **FIG. 6E** shows stroke volume (SV) in pooled subjects, and **FIG. 6F** shows estimated glomerular filtration rate (eGFR) in pooled subjects. HFpEF = heart failure with preserved ejection fraction. HFrEF = heart failure with reduced ejection fraction. Significant ($p<0.1$) compared to placebo.

Table 1: *Sequence Listing. The upper hinge region is in Italics, Relaxin A is underlined, Relaxin B is double underlined, the FC region is bold.*

SEQ ID NO:	Construct	Amino acid sequence
1	Relaxin A	QLYSALANKCCHVGCTKRSLARFC
2	Relaxin B	SWMEEVIKLCGRELVRAQIAICGMSTWS
3	FcH01	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV**

		SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG
4	FcK01	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**
5	Con01	GGGGSGGGGSGGGGSGGGGGS
6	Con02	PAPAPAPAPAPAPAPAPAG
7	RELAX0009	SWMEEVIKLCGRELVRAQIAICGMSTWSGGGGSGGGG SGGGGSQLYSALANKCCHVGCTKRSLARFCAAAGGGG SGGGGSGGGGSGGGGSA**CPPCPAPEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG**
8	RELAX0010	*DKTHT***CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK**GGGGSGGGGSGGGGSQLYSALANKCCHVGCTKR SLARFCGGGGSGGGGSGGGGSSWMEEVIKLCGRELV RAQIAICGMSTWS
9	Rlx011	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV**

		KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARFC
10	Rlx011b	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP** **EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE** **QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS** **IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARFC
11	Rlx011DD	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE** **VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ** **YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI** **EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARFC
12	Rlx012	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP** **EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE** **QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS** **IEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARFC
13	Rlx012b	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP** **EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE** **QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS** **IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL**

		SPGGGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARFC
14	Rlx012DD	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
15	Rlx013	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
16	Rlx013b	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
17	Rlx013DD	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARFC

18	Rlx014	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP** **EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE** **QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS** **IEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
19	Rlx014b	*GGAGGA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP** **EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE** **QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS** **IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLSCAV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
20	Rlx014DD	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE** **VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ** **YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI** **EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
21	Rlx020	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE** **VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ** **YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI** **EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV** **KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY** **SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL** **SPG**GGGGSGGGGSGGGGSGQLYSALANKCCHVGCTK RSLARFC
22	Rlx021	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE** **VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ** **YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI**

		EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGGGGGSGGGGSGGGGGSSWMEEVIKLCGRELVRA QIAICGMSTWS
23	RIx022	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGGSQLYSALANKCCHVGCTKRSLAR FC
24	RIx023	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGGSSWMEEVIKLCGRELVRAQIAICG MSTWS
25	RIx024	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGGSQLYSALANKCCHVGCTKRSLARFC
26	RIx025	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGGSSWMEEVIKLCGRELVRAQIAICGMSTWS

27	Rlx026	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**APAPAPAPAPAPAPAPAPAGSQLYSALANKCCHVG CTKRSLARFC
28	Rlx027	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**APAPAPAPAPAPAPAPAPAGSSWMEEVIKLCGREL VRAQIAICGMSTWS
29	Rlx028	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**AAPAPAPAPAPAPAGSQLYSALANKCCHVGCTKR SLARFC
30	Rlx029	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**AAPAPAPAPAPAPAGSSWMEEVIKLCGRELVRAQI AICGMSTWS
31	Rlx030	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI

		EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGAPAPAPAPAGSQLYSALANKCCHVGCTKRSLARFC
32	RIx031	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**APAPAPAPAGSSWMEEVIKLCGRELVRAQIAICGM STWS
33	RIx041E	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANECC HVGCTKRSLARFC
34	RIx041H	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANHCC HVGCTKRSLARFC
35	RIx041L	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL**

		SPGGGGGSGGGGSGGGGSGGGGGSQLYSALANLCC HVGCTKRSLARFC
36	RIx041M	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANMCC HVGCTKRSLARFC
37	RIx044E	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKESLARFC
38	RIx044H	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKHSLARFC
39	RIx051A	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLAAFC

40	Rlx051I	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLAIFC
41	Rlx051M	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLAMFC
42	Rlx051Q	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLAQFC
43	Rlx051S	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLASFC

44	RIx052E	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLAREC
45	RIx052I	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARIC
46	RIx055	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWSGGGSGGGSGQLYSALANKCC HVGCTKRSLARFC
47	RIx056	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWSGGGSGGGSGQLYSALANKCC HVGCTKRSLARFC

48	RIx061H	SWMEEVIKLCGRELVRAQIAICGMSTWSAAAGGGGSG GGGSGGGGSGGGGSA**CPPCPAPEFEGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPASIEKTISKAKGQPREPQVCTLPPSREEMT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG**
49	RIx062K	QLYSALANKCCHVGCTKRSLARFCAAAGGGGSGGGGS GGGGSGGGGSA**CPPCPAPEFEGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPCREEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG**
50	RIx076	QLYSALANKCCHVGCTKRSLARFCAAAGGGGSGGGGS GGGGSGGGGSA**CPPCPAPEFEGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPASIEKTISKAKGQPREPQVYTLPPCREEMTKNQV SLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG**GGGGSGGGGSGGGGSGGGGGSQLYSA LANKCCHVGCTKRSLARFC
51	RIx077	SWMEEVIKLCGRELVRAQIAICGMSTWSAAAGGGGSG GGGSGGGGSGGGGSA**CPPCPAPEFEGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPASIEKTISKAKGQPREPQVCTLPPSREEMT KNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPG**GGGGSGGGGSGGGGSGGGGGSS WMEEVIKLCGRELVRAQIAICGMSTWS

52	Rlx014DDdel2aa	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCGRELVRAQIAICGMST
53	Rlx014DDdel3aa	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCGRELVRAQIAICGMS
54	R347 L	ELVLTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVSNRFSGSKSGNTASLTISGQAEDEADYYCSSYTSSSTLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQTHEGSTVEKTVAPTECS
55	R347Rlx011DD	EVQLLESGGGLVQPGGSLRLSCTTSGFTFNTYAMSWVRQAPGKGLEWLSGINNNGRTAFYADSVKGRFTISRDNSKNTLYLQINSLRADDTAVYFCAKDVRFIAVPGDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLVKGFYP**

		SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGG GGSGGGGSGGGGSGGGGGSQLYSALANKCCHVGCT KRSLARFC
56	R347RIx014D D	EVQLLESGGGLVQPGGSLRLSCTTSGFTFNTYAMSWV RQAPGKGLEWLSGINNNGRTAFYADSVKGRFTISRDNS KNTLYLQINSLRADDTAVYFCAKDVRFIAVPGDSWGQG TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC*DKTHT* **CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISK AKGQPREPQVCTLPPSREEMTKNQVSLSCAVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG**GGG GSGGGGSGGGGSGGGGGSSWMEEVIKLCGRELVRAQ IAICGMSTWS
57	RELAX0126	*DKTHT***CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK**GGSPQLYSALANKCCHVGCTKRSLARFCGGGSG GGSGSSWMEEVIKLCGRELVRAQIAICGMSTWS
58	RELAX0127	*DKTHT***CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS**

		PGKGGSGGSPQLYSALANKCCHVGCTKRSLARFCGG GSGGGSGSWMEEVIKLCGRELVRAQIAICGMSTWS
59	RELAX0128	*DKTHT***CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK**GGSGGSGGSPQLYSALANKCCHVGCTKRSLARFC GGGSGGGSGSWMEEVIKLCGRELVRAQIAICGMSTWS
60	Linker 01	GGGSGGGSGG
61	Rlx052A	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLARAC
62	RELAX0013 B chain	DSWMEEVIKLCGRELVRAQIAICGMSTWS
63	RELAX0013 A chain	QLYSALANKCCHVGCTKRSLARFC
64	RELAX0014 B chain	MRVSEEWMDGFIRMCGREYARELIKICGASVGR
65	RELAX0014 A chain	ESGGLMSQQCCHVGCSRRSIAKLYC
66	Rlx042R	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY**

		SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGGGGGSGGGGSGGGGSGGGGGSQLYSALANKCC RVGCTKRSLARFC
67	Rlx014d	*DKTHTA***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAS IEKTISKAKGQPREPQVCTLPPSREEMTKNQVSLSCAV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSSWMEEVIKLCG RELVRAQIAICGMSTWS
68	Rlx051Y	*DKTHT***CPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASI EKTISKAKGQPREPQVYTLPPCREEMTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPG**GGGGSGGGGSGGGGSGGGGGSQLYSALANKCC HVGCTKRSLAYFC

Detailed Description

Relaxin

The present invention is based, at least in part, on the finding that heterodimeric fusions described herein may exhibit Relaxin activity when the Relaxin A chain and the Relaxin B chain are not covalently linked to each other through an amino acid linker. This is surprising based on the disclosures of WO 2013/004607 and WO 2018/138170, which describe recombinant Relaxin in which the Relaxin A and Relaxin B are fused in a single chain. The present inventors have further found that heterodimerisation of the heterodimerisation domains induces correct folding and heterodimerisation of the Relaxin A and Relaxin B chains (see Example 2). In addition, unlike wild-type Relaxin proteins, the fusion polypeptides of the invention do not require endoproteolytic processing for biological activity.

As used herein, the term “heterodimeric fusion” refers to a heterodimer of fusion polypeptides, wherein one fusion polypeptide comprises a first heterodimerisation domain connected to a first subunit of a heterodimeric protein (e.g. Relaxin A chain), and the other fusion polypeptide comprises a second heterodimerisation domain connected to a second subunit of a heterodimeric protein (e.g. Relaxin B chain).

The heterodimeric fusions of the present invention may comprise Relaxin A and B chain polypeptides from the group of Relaxins selected from Relaxin-1, Relaxin-2 and Relaxin-3. In preferred embodiments, the Relaxin A chain polypeptide of the invention is a Relaxin-2 A chain polypeptide or a variant thereof; and the Relaxin B chain polypeptide of the invention a Relaxin-2 B chain polypeptide or a variant thereof. In particular embodiments, the Relaxin A chain polypeptide comprises a human Relaxin-2 A chain polypeptide or a variant thereof and a human Relaxin-2 B chain polypeptide or a variant thereof.

The terms “chain”, “polypeptide” and “peptide” may be used interchangeably herein to refer to a chain of two or more amino acids linked through peptide bonds.

In some embodiments, the Relaxin-2 A chain polypeptide has the sequence as set forth in SEQ ID NO: 1 or a variant thereof and the Relaxin-2 B chain polypeptide has the sequence as set forth in SEQ ID NO: 2 or a variant thereof. Variants may comprise one or more amino acid substitutions, deletions and/or insertions. In some embodiments, the Relaxin-2 A chain polypeptide comprises one or more amino acid mutations selected from K9E, K9H, K9L, K9M, R18E, R18H, R22A, R22I, R22M, R22Q, R22S, R22Y, F23E, F23A and F23I. In a preferred embodiment Relaxin-2 A chain comprises the amino acid mutation K9H.

Relaxin A and B chain variants are known in the art. In addition, guidance on the design of Relaxin A and B chain variants is available to the skilled person. For example, it will be understood that variants may retain those amino acids that are required for Relaxin function. For example, Relaxin-2 B chain variants may comprise the conserved motif Arg-X-X-X-Arg-X-X-Ile (Claasz AA *et al.* (2002) *Eur. J. Biochem.* 269(24): 6287-6293) or Arg-X-X-X-Arg-X-X-Val (Bathgate RA *et al.* (2013) *Physiol Rev.* 93(1): 405-480). Variants may comprise one or more amino acid substitutions and/or insertions. For example, Relaxin-2 B chain variants may have one or more additional amino acids for example K30 and R31 and N-terminal V-2, A-1 and M-1 compared to SEQ ID NO: 62. Alternatively or in addition, variants may comprise one or more amino acid derivatives. For example, the first amino acid of Relaxin-2 B chain variants may be pyroglutamate.

In preferred embodiments, the Relaxin A chain and the Relaxin B chain are covalently bound by two inter-chain disulphide bonds (see Example 2).

The Relaxin family of peptides mediate their biological effects, at least in part, through the activation of G protein-coupled receptors (GPCRs), and the subsequent stimulation or inhibition of the cAMP signalling pathway by the Gs or Gi protein subunit, respectively. Relaxin-2 is known to activate the GPCR RXFP1 (also known as LGR7) and, to a lesser degree, the GPCR RXFP2 (also known as LGR8), thus stimulating the Gs-cAMP-dependent signalling pathway, leading to an increase in the second messenger molecule cAMP.

As used herein, the term "Relaxin activity" refers to the ability of a Relaxin molecule to bind to a Relaxin receptor, and/or activate said Relaxin receptor and/or initiate a signalling cascade inside the cell. In embodiments in which the Relaxin activity is Relaxin-2 activity, Relaxin activity may refer to the ability to bind and/or activate the receptor RXFP1 and/or RXFP2. The term "Relaxin activity" may be used interchangeably with "biological activity".

Relaxin activity may be determined by measuring binding of a Relaxin molecule to a Relaxin receptor, and/or by measuring downstream events from binding to a Relaxin receptor.

Relaxin activity may be determined *in vitro* and/or *in vivo*. In some embodiments, Relaxin activity is determined *in vitro*.

Relaxin activity may be determined by measuring the amount and/or presence of a molecule downstream from Relaxin activation of a receptor. For example, Relaxin activity may be determined by measuring cAMP production following Relaxin activation of a receptor. Methods for the detection of Relaxin-induced cAMP generation are known in the art. Such methods include cAMP ELISA, HTRF cAMP assays and the HitHunter®cAMP assay. In some embodiments, Relaxin activity is determined by measuring Relaxin-induced cAMP production by HTRF cAMP assay, e.g. as performed in Example 3. Relaxin activity may also be determined by measuring nitric oxide (NO) production following Relaxin activation of a receptor. Relaxin activity may also be determined by measuring the activation of a molecule downstream from Relaxin activation of a receptor. For example, Relaxin activity may be determined by measuring activation of p42/44 MAPK.

Alternatively or in addition, Relaxin activity may be determined by measuring the activation of a known Relaxin target gene. For example, Relaxin activity may be determined by

measuring the activation of the transcription of the known Relaxin target gene, VEGF, in THP-1 cells. Methods to determine activation of transcription of a gene are known in the art and include quantitative PCR analysis of the mRNA. The relative expression of VEGF mRNA can be measured by quantitative real-time PCR induction of VEGF transcripts following incubation of THP-1 cells with Relaxin as described in Xiao *et al.* (2013) *Nat Commun.* 4: 1953.

Alternatively or in addition, Relaxin activity may be determined by measuring one or more downstream effects of Relaxin. For example, reduction of cardiac hypertrophy can be measured by echocardiography, left ventricular weight relative to body weight and/or tibia length according to standard methods. In another example, Relaxin activity may be determined by measuring fibrosis reduction by Masson's Trichrome stain. In another example, Relaxin activity may be determined by measuring modulation of connective tissue metabolism, such as the inhibition of profibrotic factors (such as TGF-beta), inhibition of fibroblast proliferation and differentiation, and/or activation of MMP-mediated extracellular matrix degradation (Bathgate RA *et al.* (2013) *Physiol Rev.* 93(1): 405-480).

In some embodiments, Relaxin activity is determined by measuring reversal of isoproterenol-induced cardiac hypertrophy (measured as heart weight relative to tibial length) and fibrosis (measured as collagen content relative to heart weight), e.g. as performed in Example 7.

The activity of the heterodimeric fusions of the invention may be determined in relation to a reference Relaxin protein. In some embodiments, the reference Relaxin protein is a recombinant protein. In preferred embodiments, the reference Relaxin protein is a Relaxin protein having the Relaxin A chain and Relaxin B chain array of a mature Relaxin protein. Recombinant Relaxins having the Relaxin A chain and Relaxin B chain array of a mature Relaxin protein are commercially available. For example, recombinant human Relaxin-2, murine Relaxin-1 and INSL3 are available from R&D systems (catalogue numbers 6586-RN, 6637-RN and 4544-NS, respectively).

In some embodiments, the reference Relaxin protein has the same Relaxin A and B chains as the heterodimeric fusion of the invention or differs from the Relaxin A and B chains of the heterodimeric fusion of the invention by up to 10 amino acids, for example 1 or 2 amino acids. In some embodiments, the first amino acid of the B chain of the reference Relaxin-2 is D and this amino acid is deleted in the Relaxin B chain of the heterodimeric fusion of the invention.

The reference Relaxin protein may be selected from:

(i) recombinant human Relaxin-2 (referred to herein as RELAX0013);and

(ii) recombinant murine Relaxin-1 (referred to herein as RELAX0014); and

(iii) recombinant Fc-fused Relaxin-2 in which the Relaxin A and Relaxin B are fused in a single chain, and wherein Fc is a half-life extending Fc region (referred to herein as RELAX0010 and described in WO2018/138170); and

(iv) recombinant Fc-fused Relaxin-2 in which the Relaxin A and Relaxin B are fused in a single chain, and wherein Fc is a half-life extending Fc region (referred to herein as RELAX0009 and described in WO2018/138170); and

(v) recombinant Fc-fused Relaxin-2 in which the Relaxin A and Relaxin B are fused in a single chain (referred to herein as RELAX0126 and described in WO 2013/004607); and

(vi) recombinant Fc-fused Relaxin-2 in which the Relaxin A and Relaxin B are fused in a single chain (referred to herein as RELAX0127 and described in WO 2013/004607); and

(vii) recombinant Fc-fused Relaxin in which the Relaxin A and Relaxin B are fused in a single chain (referred to herein as RELAX0128 and described in WO 2013/004607).

In particularly preferred embodiments, the reference Relaxin protein is a Relaxin-2 protein having the Relaxin-2 chain A and Relaxin-2 B chain array of a mature Relaxin-2 protein as disclosed under UniProtKB/Swiss-Prot Accession Number P04090.1.

The heterodimeric fusions of the invention may be considered to have Relaxin activity if they show at least a proportion of the activity of a reference Relaxin protein. For example, a fusion polypeptide may be considered to have Relaxin activity if it has at least about half of the activity of a reference Relaxin protein. A heterodimeric fusion of the invention may be considered to have Relaxin activity if the ratio of the activity of said fusion polypeptide over the activity of a reference Relaxin protein is between about 10^{-5} and about 1, between about 10^{-4} and about 1, between about 10^{-3} and about 1, between about 10^{-2} and about 1, between about 1/50 and about 1, between about 1/20 and about 1, between about 1/15 and about 1, between about 1/10 and about 1, between about 1/5 and about 1, or between about ½ and about 1. Alternatively, a heterodimeric fusion of the invention may be considered to have Relaxin activity if the ratio of the activity of said fusion polypeptide over the activity of a reference Relaxin protein is between about 1 and about 10^5, between about 1 and about 10^4, between about 1 and about 10^3, between about 1 about 100,

between about 1 and about 50, between about 1 and about 20, between about 1 and about 15, between about 1 and about 10, between about 1 and about 5, or between about 1 and about 2.

In some embodiments, the Relaxin activity of the heterodimeric fusion over the Relaxin activity of a reference Relaxin protein is between about 0.001 and about 10.

Relaxin activity may be determined as an EC50 value. As used herein the term "EC50" (half maximal effective concentration) refers to the effective concentration of a therapeutic compound which induces a response halfway between the baseline and maximum after a specified exposure time.

Heterodimerisation Domains

The heterodimeric fusions of the invention comprise a first heterodimerisation domain and a second heterodimerisation domain. In preferred embodiments, the first and second heterodimerisation domains are derived from an immunoglobulin Fc region.

The term "Fc region" defines the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain.

The first and second Fc regions may comprise the immunoglobulin domains CH2 and/or CH3. In preferred embodiments, the first and second Fc regions comprise the immunoglobulin domains CH2 and CH3.

The Fc region may be derived from an immunoglobulin (e.g. IgG) from any species, preferably human (e.g. human IgG). In embodiments in which the Fc region is derived from IgG, the Fc region may be derived from an IgG of any subclass (e.g. IgG1, IgG2, IgG3, IgG4), preferably IgG1. Preferably, the first and second Fc regions are derived from a human IgG1 immunoglobulin. In other embodiments, the first and second Fc regions are derived from a human IgG4 immunoglobulin.

In preferred embodiments, the first and second Fc regions comprise heterodimerisation-promoting amino acid mutations and/or modifications. Such modifications may include the introduction of asymmetric complementary modifications into each of the first and second Fc regions, such that both chains are compatible with each other and thus able to form a heterodimer, but each chain is not able to dimerize with itself. Such modifications may

encompass insertions, deletions, conservative and non-conservative substitutions and rearrangements. Incorporating such modifications provides a method for increasing the yield of heterodimers produced by recombinant cell culture over other unwanted end-products such as homodimers.

The first and second Fc regions may comprise any heterodimerisation-promoting amino acid mutations and/or modifications known in the art. A combination of modifications may be used to maximise the efficiency of assembly while minimising the impact on antibody stability.

In the "knob in hole" method, heterodimerisation may be promoted by the introduction of steric hindrance between contacting residues. A "protrusion' is generated by replacing one or more small amino acid side chains from the interface of one Fc region ("Fc Knob") with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the other Fc region ("Fc Hole") by replacing amino acid having large side chains with amino acids having smaller ones (e.g. alanine or valine). "Knob-in-holes" modifications are described in detail e.g. Ridgway JB *et al.* (1996) *Protein Eng.* 9(7) 617-621; Merchant AM *et al.* (1998) *Nat. Biotechnol.* 16(7): 677-681.

Other modifications which may be used to generate heterodimers include but are not limited to those which create favourable electrostatic interactions between the two Fc regions. For example, one or more positively charged amino acids may be introduced into one Fc region, and one or more negatively charged amino acids may be introduced into a corresponding position in the other Fc region. Alternatively or in addition, the Fc regions may be modified to include mutations that introduce cysteine residues capable of forming a disulphide bond. Alternatively or in addition, the Fc regions may comprise one or more modification(s) to the hydrophilic and hydrophobic residues at the interface between chains, in order make heterodimer formation more entropically and enthalpically favourable than homodimer formation.

Thus, in some embodiments, the heterodimerisation-promoting amino acid mutations and/or modifications create steric hindrance between contacting residues (e.g. by "knob-in-hole"), create favourable electrostatic interactions between the two Fc regions, introduce cysteine residues capable of forming a disulphide bond and/or modify the hydrophilic and hydrophobic residues at the interface between the two Fc regions.

In preferred embodiments, the heterodimerisation-promoting amino acid mutations are "Fc Knob" and "Fc Hole" mutations. In preferred embodiments, the "Fc Knob" and "Fc Hole" mutations are present in the CH3 domains.

In some embodiments, the first and second Fc regions are derived from a human IgG1 immunoglobulin and comprise "Fc X" and "Fc Y" with mutations in the CH3 domains, wherein the "Fc X" and "Fc Y" mutations are selected from the combinations set forth in Table 2 (or conservative substitutions thereof).

Table 2: *"Fc X" and "Fc Y" mutations*

Combination No.	Fc X mutation(s)*	Fc Y mutation(s)*
1	D399C	K392C
2	D399S	K392S
3	Y349C	S354C
4	Y349C	E356C
5	Y349C	E357C
6	L351C	S354C
7	T394C	V397C
8	T366W	T366S:L368A:Y407V
9	T366W:D399C	T366S:L368A:K392C:Y407V
10	T366W:K392C	T366S:0099C:L368A:Y407V
11	S354C:T366W	Y349C:T366S:L368A:Y407V
12	Y349C:T366W	S354C:T366S:L368A:Y407V
13	E356C:T366W	Y349C:T366S:L368A:Y407V
14	Y349C:T366W	E356C:T366S:L368A:Y41J7V
15	E357C:T366W	Y349C :T366S:L368A:Y407V
16	Y349C:T366W	E357C:T366S:L368A:Y407V
17	S364H/F405A	Y349T/T394F
18	T350V/L351Y/F405A/Y407V	T350V/T366L/K392L/T394W
19	K360D/D399M/Y407A	E345R/Q347R/T366V/K409V
20	K409D/K392D	D399K/E356K
21	K360E/K409W	Q347R/D399V/F405T
22	K360E/K409W/Y349C	Q347R/D399V/F405T/S354C
23	K370E/K409W	E357N/D399V/F405T

24	T366Y	Y407T

*wherein the amino acid numbering is according to the EU index as in Kabat.

In preferred embodiments the “Fc Y” is the “Fc Hole” with mutations Y349C, T366S, L368A and Y407V, or conservative substitutions thereof, and the “Fc X” is the “Fc Knob” with mutations S354C and T366W, or conservative substitutions thereof, wherein the amino acid numbering is according to the EU index as in Kabat.

The term "EU index as in Kabat" refers to the numbering system of the human IgG1 EU antibody described in Kabat EA *et al.* (1991) Sequences of Proteins of Immunological Interest, 5th ed. Public Health Service. National Institutes of Health. Bethesda, MD. All amino acid positions referenced in the present application refer to EU index positions.

In some embodiments, the first Fc region has “Fc Hole” mutations, and the second Fc region has “Fc Knob” mutations. In alternative and preferred embodiments, the first Fc region has “Fc Knob” mutations, and the second Fc region has “Fc Hole” mutations.

It will be understood that the Fc regions may further comprise other amino acid modifications relative to a wild-type Fc region. The Fc region may be modified to e.g. increase the affinity of the IgG molecule for the FcRn. WO 02/060919 discloses modified immunoglobulins comprising an Fc region having one or more amino acid modifications and is incorporated herein in its entirety by reference. Methods of making Fc regions with one or more amino acid modifications are known in the art.

In some embodiments, the first and/or second Fc region may comprise one or more amino acid modifications to reduce or abolish the effector function of the Fc region. In some embodiments, the amino acid modifications reduce or circumvent cytotoxicity, for example antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

In some embodiments, the first and/or second Fc region may comprise one or more amino acid modifications to increase the half-life of the heterodimeric fusion.

In some embodiments, the first and/or second Fc region comprises at least one of the following combinations of amino acid mutations:

(i) M252Y, S254T and T256E, or conservative substitutions thereof;

(ii) L234F, L235Q and K322Q, or conservative substitutions thereof;

(iii) L234F, L235E and P331S, or conservative substitutions thereof;

(iv) M252Y, S254T, T256E, L234F, L235Q and K322Q, or conservative substitutions thereof; or

(v) M252Y, S254T, T256E, L234F, L235E and P331S, or conservative substitutions thereof,

wherein the amino acid numbering is according to the EU index as in Kabat.

In some embodiments, the first and/or second Fc region may comprise the amino acid mutations L234F, L235E and P331S, or conservative substitutions thereof, wherein the amino acid numbering is according to the EU index as in Kabat.

In some embodiments, the Fc region comprising "Fc Hole" mutations has the sequence set forth in SEQ ID NO: 3 or variants thereof, and the Fc region comprising "Fc Knob" mutations has the sequence set forth in SEQ ID NO:4 or variants thereof.

In some embodiments, the Fc regions comprise a SEQ ID NO: 3 variant having the amino acid mutation Y349C reverted to Y349 and a SEQ ID NO: 4 variant having the amino acid mutation S354C reverted to S354, such that the Fc regions are unable to form a stabilising disulphide bond.

In some embodiments, the Fc regions comprise a SEQ ID NO: 3 variant and/or SEQ ID NO: 4 variant, wherein the first five residues DKTHTCPPC (SEQ ID NO: 69) are modified. In some embodiments, this region is replaced with the sequence DKTHTACPPC (SEQ ID NO: 70). In alternative embodiments, this region is replaced with the sequence GGAGGACPPC (SEQ ID NO: 71). In alternative embodiments, this region is replaced with the sequence ACPPC (SEQ ID NO: 72).

In alternative embodiments, the first and second heterodimerisation domains are derived from an immunoglobulin Fab region. In some embodiments, the heterodimerisation domains comprise CH1 and CL regions. It has been found that Fab regions comprising L and Fd chains mediate efficient heterodimerisation (Schoonjans R *et al.* (2000) J. Immunol. 165 (12): 7050-7057). Thus, in alternative embodiments, the heterodimerisation domains comprise L and Fd chains. In some embodiments, the L and Fd chains heterodimerise to form a disulphide-bridge stabilised heterodimer.

In yet further alternative embodiments, the first and second heterodimerisation domains heterodimerise to form parallel coiled coils. Heterodimeric coiled coils are described e.g. in Aronsson *et al.* (2015) *Sci. Rep.* 5: 14063. In some embodiments, the heterodimerisation domains comprise amino acid mutations and/or modifications to prevent formation of undesired folded assemblies and/or to promote formation of parallel coiled coils.

The first and second heterodimerisation domains (e.g. first and second Fc regions) may form a half-life extending moiety. Thus, in some embodiments the heterodimeric fusions of the invention have an extended half-life compared to a reference Relaxin.

As used herein, the term "half-life" is used to refer to the time taken for the concentration of fusion protein in plasma to decline to 50% of its original level. The "half-life" of a protein in plasma may depend on different factors such as the size of the protein, its stability, its clearance rate, turnover rate, *in vivo* proteolytic degradation, the rate of absorption by the body or specific tissues, etc. Methods to determine the half-life of proteins are known in the art and are described in the Examples below.

The inventors have shown that heterodimeric fusions of the invention having first and second heterodimerisation domains derived from an immunoglobulin Fc have a half-life of at least 5 hours in mouse models (see Example 6). In comparison, the half-life of human Relaxin-2 following IV administration is about 0.09 +/- 0.04 hours, i.e. 5.4 +/- 2.4 minutes in humans (Chen SA *et al.* (1993) *Pharm. Res.* 10(6): 834-838).

It will be recognised that an extended half-life is advantageous, as it permits the therapeutic proteins to be administered according to a safe and convenient dosing schedule, e.g. lower doses that can be administered less frequently. Moreover, the achievement of lower doses may provide further advantages such as the provision of an improved safety profile and/or the activation of multiple mechanisms of action *in vivo*.

Connectors

One or both of the Relaxin A and B chains may be connected to their respective heterodimerisation domains by a connector polypeptide. In some embodiments, the Relaxin A chain is connected to the first heterodimerisation domain (e.g. first Fc region) via a connector polypeptide, and the Relaxin B chain is connected to the second heterodimerisation domain (e.g. second Fc region) via a connector polypeptide.

The connector polypeptide may be any suitable length, for example between about 6 and 40 amino acids in length, preferably between about 6 and 21 amino acids in length. In some embodiments, the connector polypeptide is at least 6 amino acid residues in length, preferably at least 11 amino acids in length, preferably at least 16 amino acids in length. In some embodiments, the connector polypeptide is less than 40 amino acids in length. Connector polypeptides of different or the same lengths can be used for each arm of the heterodimeric fusions of the invention. In some embodiments, at least one connector polypeptide has a length of 21 amino acids. In preferred embodiments, both connector polypeptides have a length of 21 amino acids. The connector polypeptides can have any amino acid sequence. Connector polypeptides of different or the same amino acid compositions can be used for each arm of the heterodimeric fusions of the invention.

In some embodiments, one or preferably both connector polypeptides comprise proline and alanine repeats (PA)x (SEQ ID NO: 73), preferably wherein x is of between 3 and 15, preferably wherein the connector polypeptide has a length greater than 16 amino acids, preferably wherein the connector polypeptide is composed of the 21 amino acid sequence PAPAPAPAPAPAPAPAPAPAG (SEQ ID NO: 6).

In some embodiments, one or preferably both connector polypeptides comprise glycine and serine repeats such as those described in Chen X *et al.* (2013) *Adv. Drug. Deliv. Rev.* 65(10): 1357-1369. In some embodiments, one or both connector polypeptides comprise the motif (GGGGS)n (SEQ ID NO: 74), wherein n may be between 1 and 8, for instance wherein n is 4. In some embodiments, one or more connector polypeptide is composed of the 21 amino acid sequence GGGGSGGGGSGGGGSGGGGGS (SEQ ID NO: 5). In certain embodiments, both connector polypeptides are composed of the 21 amino acid sequence GGGGSGGGGSGGGGSGGGGGS (SEQ ID NO: 5).

In some embodiments, one connector polypeptide comprises proline and alanine repeats as described herein, and the other connector polypeptide comprises glycine and serine repeats as described herein.

Alternatively, one or both of the Relaxin A and B chains may be connected to their respective heterodimerisation domains by a synthetic connector polypeptide, such as a polyethylene glycol (PEG) polymer chain. Thus, the Relaxin A chain may be connected to the first heterodimerisation domain (e.g. first Fc region) via a synthetic connector, such as a polyethylene glycol (PEG) polymer chain, and the Relaxin B chain may be connected to the second heterodimerisation domain (e.g. second Fc region) via a synthetic connector,

such as a polyethylene glycol (PEG) polymer chain, wherein the synthetic connector may be covalently or non-covalently attached to the heterodimerisation domain (e.g. Fc region). PEGylation, that is the process of attaching PEG polymer chains to a molecule, can be carried out according to methods known in the art.

Stability

The present inventors have shown that heterodimeric fusions of the invention have unexpected superior physical and chemical stability. Thus, in some embodiments the heterodimeric fusions of the invention have superior physical and/or chemical stability compared to a reference Relaxin protein.

Physical stability of Relaxin may be determined by measuring purity and aggregation, for example by HP-SEC as in Example 9. Chemical stability of Relaxin may be determined by measuring fragmentation and modification of the molecule, for example by LC-MS as in Example 9.

Surprisingly, the present inventors have shown that heterodimeric fusions of the invention have superior physical and chemical stability compared to recombinant Fc-fused Relaxin in which the Relaxin A and Relaxin B are fused in a single chain (as opposed to Relaxin A and B in separate fusion polypeptides). WO 2013/004607 describes recombinant single chain Relaxin fusion polypeptides fused to an immunoglobulin Fc region, for example the fusion polypeptides referred to herein as RELAX0127 and RELAX0128. Thus, in some embodiments, the heterodimeric fusions of the invention have superior physical and/or chemical stability compared to RELAX0127 and RELAX0128.

The heterodimeric fusion may comprise a half-life extending moiety in addition to the first and second heterodimerisation domains. In some embodiments, the half-life extending moiety is a proteinaceous half-life extending moiety. The proteinaceous half-life extending moiety may be selected from the group consisting of an Fc region of an immunoglobulin, albumin-binding domain and serum albumin. In further embodiments, the half-life extending moiety is a chemical entity that is not a protein or peptide, such as a polyethylene glycol (PEG) polymer chain.

The half-life extending moiety may be attached at the N-terminus or the C-terminus of the first or second heterodimerisation domain. In some embodiments, the half-life extending moiety is attached at the N-terminus of the first or second heterodimerisation domain. In other embodiments, the half-life extending moiety is attached at the C-terminus of the first

or second heterodimerisation domain. Methods for attaching the half-life extending moiety to the heterodimeric fusion are known in the art. For example, the half-life extending moiety may be attached by chemical conjugation or recombinant technology. The half-life extending moiety may be attached to the heterodimeric fusion directly or through a connector (e.g. connector polypeptide). The use of a connector polypeptide may be particularly appropriate when the fusion polypeptide comprises a proteinaceous half-life extending moiety such as an Fc region.

Exemplary Embodiments

The heterodimeric fusions of the invention may have a variety of formats and/or sequences.

The term "fusion polypeptide of the invention" and "fusion polypeptides of the invention" may be used to refer to the first heterodimerisation domain fused to a Relaxin A chain, and/or the second heterodimerisation domain fused to a Relaxin B chain. The fusion polypeptides of the invention may be recombinant fusion polypeptides, i.e. which have been created by recombinant DNA technology.

In preferred embodiments, the C-terminus of the first heterodimerisation domain (e.g. first Fc region) is connected to the N-terminus of the Relaxin A chain and the C-terminus of the second heterodimerisation domain (e.g. second Fc region) is connected to the N-terminus of the Relaxin B chain. In some embodiments, the Relaxin A chain polypeptide and/or the Relaxin B chain polypeptide have a free C-terminus.

In alternative embodiments, the N-terminus of the first heterodimerisation domain (e.g. first Fc region) is connected to the C-terminus of the Relaxin A chain and the N-terminus of the second heterodimerisation domain (e.g. second Fc region) is connected to the C-terminus of the Relaxin B chain. In some embodiments, the Relaxin A chain polypeptide and/or the Relaxin B chain polypeptide have a free N-terminus.

The heterodimeric fusion of the invention may further comprise one or more Fabs. In some embodiments, the heterodimeric fusion comprises one Fab linked to the N-terminus of the first heterodimerisation domain (e.g. first Fc region) and a second Fab linked to the N-terminus of the second heterodimerisation domain (e.g. second Fc region).

The heterodimeric fusion of the invention may further comprise a second Relaxin A chain polypeptide or variant thereof and a second Relaxin B chain polypeptide or variant thereof. In some embodiments, the second Relaxin A chain polypeptide or variant thereof is

connected to the N-terminus of the first heterodimerisation domain (e.g. first Fc region) and the second Relaxin B chain polypeptide or variant thereof is connected to the N-terminus of the second heterodimerisation domain (e.g. second Fc region), optionally wherein the second Relaxin A chain is connected to the first heterodimerisation domain (e.g. first Fc region) via a connector (e.g. connector polypeptide) and the second Relaxin B chain is connected to the second heterodimerisation domain (e.g. second Fc region) via a connector (e.g. connector polypeptide).

Thus, in some embodiments, the format of the heterodimeric fusion is selected from:

(i) FcX-con-A/ FcY-con-B (e.g. see Figure 1);
(ii) FcX-con-B/ FcY-con-A (e.g. see Figure 1);
(iii) A-con-FcX/ B-con-FcY (e.g. see Figure 1);
(iv) B-con-FcX/ A-con-FcY (e.g. see Figure 1);
(v) Fab-FcX-con-A/ Fab-FcY-con-B (e.g. see Figure 1);
(vi) Fab-FcX-con-B/ Fab-FcY-con-A;
(vii) A-con-FcX-con-A/ B-con-FcY-con-B (e.g. see Figure 1);
(viii) B-con-FcX-con-B/ A-con-FcY-con-A;
(ix) FcX-con-B-L-A, and FcY, optionally FcY-con-B-L-A (e.g. see Figure 1);
(x) FcY-con-B-L-A, and FcX, optionally FcX-con-B-L-A;
(xi) FcX-con-A-L-B, and FcY, optionally FcY-con-A-L-B; and
(xii) FcY-con-A-L-B, and FcX, optionally FcX-con-A-L-B,

wherein:

FcY is an immunoglobulin Fc region with “Fc Hole” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V, or conservative substitutions thereof;
FcX is an Fc region with “Fc Knob” amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W, or conservative substitutions thereof;
“con” is a connector polypeptide;

B is a Relaxin B chain or a variant thereof;

A is a Relaxin A chain or a variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG (SEQ ID NO: 60).

In another aspect, the invention provides a heterodimeric fusion comprising

(i) X-B-L-A and Y, optionally Y-B-L-A; or
(ii) Y-B-L-A and X, optionally X-B-L-A,

wherein:

X and Y are heterodimerisation domains as described herein;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG (SEQ ID NO: 60),

wherein X heterodimerises with Y, and wherein the heterodimeric fusion has Relaxin activity.

In yet another aspect, the invention provides a heterodimeric fusion comprising

(i) X-A-L-B and Y, optionally Y-A-L-B or
(ii) Y-A-L-B and X, optionally X-A-L-B,

wherein:

X and Y are heterodimerisation domains as described herein;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin-2 B chain or variant thereof; and

L is a linker polypeptide, preferably with the amino acid sequence GGGSGGGSGG (SEQ ID NO: 60),

wherein X heterodimerises with Y, and wherein the heterodimeric fusion has Relaxin activity.

In particularly preferred embodiments according to all aspects of the invention, the heterodimeric fusion comprises the fusion polypeptides Rlx011DD as set forth in SEQ ID NO: 11 and Rlx014DD as set forth in SEQ ID NO: 20. This heterodimeric fusion may also be termed “RELAX0023” or “AZD3427”.

In alternative preferred embodiments, the heterodimeric fusion comprises the fusion polypeptides Rlx013DD as set forth in SEQ ID NO: 17 and Rlx012DD as set forth in SEQ ID NO: 14.

In an aspect of the invention, there is provided heterodimeric fusions comprising a fusion polypeptide combination selected from the FcX and FcY combinations set forth in Table 3.

Table 3: *Fusion polypeptide combinations in heterodimeric fusions of the invention*

Heterodimeric Fusion	FcX (knob) fusion polypeptide*	FcY (hole) fusion polypeptide*
RELAX0019	Rlx011	Rlx014
RELAX0020	Rlx013	Rlx012
RELAX0021	Rlx011b	Rlx014b
RELAX0022	Rlx12b	Rlx13b
RELAX0023	Rlx011DD	Rlx014DD
RELAX0024	Rlx013DD	Rlx012DD
RELAX0034	Rlx041H	Rlx014d
RELAX0039	Rlx041M	Rlx014DD
RELAX0040	Rlx041L	Rlx014DD
RELAX0041	Rlx041H	Rlx014DD
RELAX0043	Rlx041E	Rlx014DD
RELAX0046	Rlx042R	Rlx014DD
RELAX0052	Rlx044E	Rlx014DD
RELAX0053	Rlx044H	Rlx014DD
RELAX0054	Rlx028	Rlx029
RELAX0055	Rlx030	Rlx031
RELAX0056	Rlx026	Rlx027
RELAX0063	Rlx052A	Rlx014DD
RELAX0069	Rlx051M	Rlx014DD
RELAX0070	Rlx051I	Rlx014DD

RELAX0071	Rlx051Q	Rlx014DD
RELAX0072	Rlx051A	Rlx014DD
RELAX0073	Rlx051Y	Rlx014DD
RELAX0074	Rlx051S	Rlx014DD
RELAX0075	Rlx052I	Rlx014DD
RELAX0076	Rlx052E	Rlx014DD
RELAX0081	Rlx020	Rlx021
RELAX0082	Rlx022	Rlx023
RELAX0083	Rlx024	Rlx025
RELAX0084	Rlx026	Rlx014DD
RELAX0085	Rlx011DD	Rlx027
RELAX0086	Rlx020	Rlx014DD
RELAX0087	Rlx011DD	Rlx021
RELAX0088	Rlx055	FcH01
RELAX0091	Rlx062K	Rlx061H
RELAX0105	Rlx020	Rlx027
RELAX0106	Rlx022	Rlx027
RELAX0107	Rlx024	Rlx027
RELAX0109	Rlx020	Rlx029
RELAX0110	Rlx022	Rlx029
RELAX0111	Rlx024	Rlx029
RELAX0117	Rlx076	Rlx077
RELAX0122	Rlx055	Rlx056
RELAX0123	Rlx011DD	Rlx014DDdel2aa
RELAX0124	Rlx011DD	Rlx014DDdel3aa
RELAX0130**	R347Rlx011DD	R347Rlx014DD

*The sequences of the fusion polypeptides listed are set forth in Table 1.

**In this particular embodiment the heterodimeric fusion is an IgG and comprises an additional polypeptide corresponding to the Light Chain set forth in SEQ ID NO: 54.

According to all aspects of the invention, there is provided a heterodimeric fusion comprising the fusion polypeptides set forth in SEQ ID NO: 11 and SEQ ID NO: 20 for use

in a method of treating a subject with heart failure with pulmonary hypertension as described herein.

Alternatively, according to all aspects of the invention, there is provided a heterodimeric fusion comprising the fusion polypeptides set forth in SEQ ID NO: 17 and SEQ ID NO: 14 for use in a method of treating a subject with heart failure with pulmonary hypertension as described herein.

The fusion polypeptides of the invention may be produced by any method known in the art. In some embodiments, the fusion polypeptides of the invention are produced by recombinant expression of a nucleic acid molecule encoding a fusion polypeptide in a host cell.

Methods that are known to those skilled in the art can be used to construct expression vectors containing the nucleic acid molecules encoding the fusion polypeptides of the invention. Suitable vectors include, for example, plasmids, phagemids, phages or viral vectors.

Vectors containing the nucleic acid molecules encoding the fusion polypeptides of the invention may be transferred to a host cell by conventional techniques. Suitable host cells are known in the art. The host cells may be mammalian cells such as HEK293 cells or CHO cells.

The transfected cells may be cultured by conventional techniques to produce the fusion polypeptides of the invention.

Once a fusion polypeptide of the invention has been produced, for example by recombinant expression, it may be purified by any method known in the art. Exemplary protein purification techniques include chromatography (e.g. ion exchange, affinity and/or sizing column chromatography), centrifugation and differential solubility. The present disclosure provides isolated fusion polypeptides that have been separated from the cell culture, optionally by at least one purification step.

Therapeutic Methods

The fusion polypeptides of the invention may be provided in a pharmaceutical composition.

The pharmaceutical compositions of the invention may comprise one or more excipient(s). Pharmaceutically acceptable excipients are known in the art, see for instance Remington's

Pharmaceutical Sciences (by Joseph P. Remington, 18th ed., Mack Publishing Co., Easton, PA), which is incorporated herein in its entirety.

The present invention relates to methods of treating a subject with heart failure with pulmonary hypertension by administering a heterodimeric fusion (or pharmaceutical composition) as described herein, as well as uses of said heterodimeric fusions (or said pharmaceutical compositions) and said heterodimeric fusions (or said pharmaceutical compositions) for use in said methods. In particular, the subject may be an animal, preferably a mammal, more preferably a human.

The use or method may comprise administering a therapeutically effective schedule that has less frequent doses of the heterodimeric fusions / fusion polypeptides of the invention than the therapeutically effective dosing schedule of a wild-type Relaxin molecule.

As used herein, the term "heart failure" includes acute heart failure, chronic heart failure (CHF) and acute decompensated heart failure (ADHF). The term "heart failure" may also include more specific diagnoses such as heart failure with preserved ejection fraction (HFpEF), heart failure with mid-range ejection fraction or heart failure with reduced ejection fraction (HFrEF). This may also include heart failure due to hypertrophic cardiomyopathy or dilated cardiomyopathy.

As used herein, the term “pulmonary hypertension” may be defined as a subject with a mean Pulmonary Arterial Pressure of about 20mmHg or greater, preferably 25 mmHg or greater, typically when the subject is at rest. It may also be defined as a mean Pulmonary Arterial Pressure of about 30 mmHg or greater, typically when the subject is or has recently been exercising. Thus, the subject may have a mean Pulmonary Arterial Pressure in the range of about 20 mmHg to about 30 mmHg, preferably about 25 mmHg to about 30 mmHg, or greater. Alternatively or additionally, the subject may have:

a. a Right Ventricular Systolic Pressure of about 40 mmHg or greater;
b. a pulmonary artery wedge pressure (PAWP) greater than 15 mmHg; and/or
c. a Pulmonary Vascular Resistance of:
    i. less than 3.0 wood units; or
    ii. 3.0 or more wood units.

Thus, in some cases, the pulmonary hypertension may be classified as Group 2 pulmonary hypertension, as defined by the World Health Organisation. This may also be termed as “Heart Failure with Pulmonary Hypertension due to Left Heart Disease”. In other

cases, the pulmonary hypertension may be classified as Group 1 pulmonary arterial hypertension, as defined by the World Health Organisation (see Ryan *et al.*, 2012, *Pulm. Circ.* 2(1):107-121).

Parameters of pulmonary hypertension and heart failure may be measured or estimated using techniques known in the art. For instance, these include echocardiography, pulmonary artery catheter and implantable monitoring device. In certain embodiments, the subject may have been fitted with a blood pressure monitoring device, preferably a pulmonary artery pressure monitoring device, as are known in the art. In particular embodiments, the pulmonary artery pressure monitoring device is a CardioMEMS pressure monitoring device. Typically, the device is fitted prior to treatment with a heterodimeric fusion of the invention as described herein. Alternatively, the subject is fitted with the device during or after the period of treatment.

As used herein, the term "heart failure with pulmonary hypertension" refers to the subset of heart failure subjects who simultaneously suffer from pulmonary hypertension (HF+PH subjects).

"Treatment" refers to the amelioration and/or elimination of one or more symptoms or causes of the target disease. In some embodiments, this may involve modulating the levels of one or more biological markers or functions to within a non-diseased range (as compared against a healthy cohort). For instance, the heterodimeric fusions of the invention may reduce Pulmonary Vascular Resistance (PVR) in a subject. For example, PVR may be reduced after treatment by at least 1% to 10%, 1% to 20%, 1% to 30%, 1% to 40% or 1% to 50% or greater as compared to baseline PVR (prior to administration to the subject of the heterodimeric fusion of the invention). Thus, the heterodimeric fusions of the invention may reduce PVR in a subject by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50% or greater, as compared to baseline PVR (prior to administration to the subject of the heterodimeric fusion of the invention). In addition, or alternatively, the heterodimeric fusions of the invention may reduce mean Pulmonary Artery Pressure (mPAP) in a subject. For example, mPAP may be reduced by at least 1 mmHg to 15 mmHg or greater. Thus, the heterodimeric fusions of the invention may reduce mean Pulmonary Artery Pressure in a subject by at least 1 mmHg, at least 2 mmHg, at least 3 mmHg, at least 4 mmHg, at least 5 mmHg, at least 6 mmHg, at least 7 mmHg, at least 8 mmHg, at least 9 mmHg, at least 10 mmHg, at least 11 mmHg, at least 12 mmHg, at least 13 mmHg, at least 14 mmHg or at least 15 mmHg or greater. Equally, the heterodimeric fusions of the invention may reduce estimated Pulmonary Artery

Diastolic Pressure (ePAD) in a subject. For example, ePAD may be reduced by at least 1 mmHg to 15 mmHg or greater. Thus, the heterodimeric fusions of the invention may reduce estimated Pulmonary Artery Diastolic Pressure in a subject by at least 1 mmHg, at least 2 mmHg, at least 3 mmHg, at least 4 mmHg, at least 5 mmHg, at least 6 mmHg, at least 7 mmHg, at least 8 mmHg, at least 9 mmHg, at least 10 mmHg, at least 11 mmHg, at least 12 mmHg, at least 13 mmHg, at least 14 mmHg or at least 15 mmHg or greater. In addition, or alternatively, the heterodimeric fusions of the invention may increase percentage ejection fraction (EF%) in a subject, as a measure of cardiac output. For example, EF% may increase by at least 1% to 10%, 1% to 20%, 1% to 30%, 1% to 40% or 1% to 50% or greater. Thus, the heterodimeric fusions of the invention may increase percentage ejection fraction (EF%) in a subject by at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50% or greater. In addition, or alternatively, the heterodimeric fusions of the invention may, in a subject:

a) increase stroke volume (SV) of the heart;

(b) decrease systemic vascular resistance (SVR) and/or increase estimated glomerular filtration rate (eGFR);

(c) increase ejection fraction; and/or

(d) increase cardiac output;

as compared to baseline levels pre-administration. A combined decrease in SVR and increase in eGFR is indicative of improved organ perfusion.

Thus, the heterodimeric fusions of the invention may, in a subject:

a) reduce PVR;

(b) reduce mPAP;

(c) reduce ePAD;

(d) increase stroke volume (SV) of the heart;

(e) decrease systemic vascular resistance (SVR) and/or increase estimated glomerular filtration rate (eGFR);

(f) increase ejection fraction; and/or

(g) increase cardiac output;

as compared to baseline levels pre-administration. A combined decrease in SVR and increase in eGFR is indicative of improved organ perfusion. The change in one or more or all of these parameters may each result after 1-24 weeks of treatment. In some embodiments, the change in one or more or all of these parameters results after 24 weeks

of treatment.

In particular embodiments, a reduction in mPAP as described herein may cause an improvement in dyspnea, as described in Solomonica A, *et al.* (2013) *Circ Heart Fail.* 6:53-60.

The fusion polypeptides (thus including heterodimeric fusions) and/or pharmaceutical compositions of the invention are suitable for parenteral administration to a subject or patient. In some embodiments the subject or patient is a mammal, in particular a human.

Wild-type human Relaxin-2 has a half-life of minutes *in vivo*. As a consequence, it has to be administered by continuous intravenous infusion in hospitalized patients and presents severe side effects including blood pressure drop. In contrast, it will be understood that embodiments of the fusion polypeptides (thus including heterodimeric fusions) and/or pharmaceutical compositions of the invention may be administered by injection, such as by intravenous, subcutaneous or intramuscular injection, to a subject or patient. In some embodiments, the fusion polypeptides (thus including heterodimeric fusions) and/or pharmaceutical compositions are administered by subcutaneous injection. Administration by injection, such as by subcutaneous injection, offers the advantage of better comfort for the subject or patient and the opportunity to administer to a subject or patient outside of a hospital setting. In some embodiments the fusion polypeptide (thus including heterodimeric fusions) or pharmaceutical composition is administered by self-administration.

In some embodiments, the fusion polypeptides of the invention (thus including heterodimeric fusions) have an increased half-life compared to wild-type Relaxin, which permits lower overall exposure based on molar concentration. For example, the fusion polypeptides of the invention (thus including heterodimeric fusions) may be administered less frequently than wild-type Relaxin, thus providing a more convenient dosing schedule.

A kit comprising the pharmaceutical compositions of the invention may be provided. The kit may comprise a package containing the pharmaceutical compositions of the invention and instructions. In some embodiments, the pharmaceutical compositions of the invention are formulated in single dose vials or a container closure system (e.g. pre-filled syringe). Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or

biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

As used herein, the articles "a" and "an" may refer to one or to more than one (e.g. to at least one) of the grammatical object of the article.

"About" may generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

Embodiments described herein as "comprising" one or more features may also be considered as disclosure of the corresponding embodiments "consisting of" such features.

The term "pharmaceutically acceptable" as used herein means approved by a regulatory agency of the Federal or a state government, or listed in the U.S. Pharmacopeia, European Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

Concentrations, amounts, volumes, percentages and other numerical values may be presented herein in a range format. It is also to be understood that such range format is used merely for convenience and brevity and should be interpreted flexibly to include not only the numerical values explicitly recited as the limits of the range but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited.

The above embodiments are to be understood as illustrative examples. Further embodiments are envisaged. It is to be understood that any feature described in relation to any one embodiment may be used alone, or in combination with other features described, and may also be used in combination with one or more features of any other of the embodiments, or any combination of any other of the embodiments. Furthermore, equivalents and modifications not described above may also be employed without departing from the scope of the invention, which is defined in the accompanying claims.

In the context of the present disclosure other examples and variations of the fusion polypeptides and methods described herein will be apparent to a person of skill in the art.

Other examples and variations are within the scope of the disclosure, as set out in the appended claims. All documents cited herein are each entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited documents.

Examples

Example 1: Generation of recombinant heterodimeric Fc Relaxin-2 fusion proteins

The Fc Relaxin-2 fusion proteins described herewith have been designed using the heterodimerisation properties of the knob-in-hole Fc domains (Fc Knob and Fc Hole) to induce correct folding and heterodimerisation of chains A and B of Relaxin-2.

More precisely, Relaxin-2 chains A and B have been genetically fused to two complementary Fcs (at the N- and/or C-terminus of the Fc) via connectors, as illustrated in Figure 1. CHO cells were then co-transfected with two expression vectors comprising each of the single Fc-Relaxin chains (A and/or B). The two complementary Fc moieties assemble within the CHO cells and, thus, facilitate the assembly and correct folding of Relaxin-2. As demonstrated in the following Example 2, disulphide bonds are then formed between complementary Fc chains and between the chain A and the chain B, recreating the natural Relaxin-2 structure.

The heterodimeric Fc Relaxin-2 fusion proteins were secreted in the supernatant, then purified using an automated system by affinity chromatography, wherein the Fc region of the protein binds to the column matrix.

Example 2: LC-MS analysis of Fc Relaxin-2 knob-in-hole heterodimers

LC-MS analysis was performed on both non-reduced and reduced deglycosylated Fc-Relaxin-2 heterodimers. For deglycosylation, samples were diluted to 1 mg/ml and buffered at pH 7.80 using 10 mM Tris-Cl. PNGase F (Roche) was added to the sample at a concentration of 1 unit of enzyme per 50 µg of Fc-Relaxin-2 and incubated overnight at 37°C. For non-reduced analysis, the sample was diluted to 0.05 mg/ml in water and 20 µL loaded into an LC-MS-certified total recovery vial with a pre-slit cap (Waters part number: 186005663CV). For reduced analysis, 10 mM TCEP was added and the sample incubated at 37°C for a further 30 minutes prior to analysis.

Experiments were performed using an ACQUITY I-Class UPLC coupled to a Xevo G2-XS Q-TOF instrument (Waters, Milford, MA), both operated using UNIFI Scientific Information System. For the LC system, Solvent A was water with 0.1% formic acid and solvent B was acetonitrile with 0.1 % formic acid (both UPLC-MS grade, BioSolve). The UV detector was

set to measure at wavelengths of 220 nm and 280 nm and the vials placed in a sample chamber maintaining a temperature of 4°C. A volume of 1 µL was injected onto a reverse phase ACQUITY UPLC Protein BEH C4 Column, 300Å-pore column (Waters part number: 186004495) and proteins were eluted using an increasing gradient of solvent B from 5% to 75% over 6 minutes.

The mass spectrometer was calibrated from 500-5000 m/z by infusing 2 µg/µL sodium iodide in 50% 2-propanol and the lockspray was 200 pg/µL Leucine Enkephalin. The instrument was operated in positive ionisation mode and sensitivity analyser mode with the following key settings: capillary voltage = 3.0 V; sample cone voltage = 40 V; source temperature = 120 °C; desolvation temperature = 450 °C; cone gas flow = 120 L/h; desolvation gas flow = 1000 L/h; mass range = 500-5000 m/z, scan time = 1.0 sec.

Data were processed in UNIFI software. The spectra were combined from the retention time in the chromatogram where the protein of interest eluted. The raw data was background subtracted and deconvoluted using MaxEnt1 algorithm for large molecules. The experimental data was compared to the mass of theoretical sequences which took into consideration disulphide bonds for non-reduced analysis and free cysteines for reduced analysis. Deamidation of asparagine (+1 Da) was also considered following PNGasE F deglycosylation.

LC-MS analysis confirmed disulphide bonds are formed between complementary Fc chains and between the chain A and the chain B, recreating the natural Relaxin-2 structure. Figure 2A shows, as an example, LC-MS data for RELAX0019 and RELAX0023. Non-reduced analysis confirmed the formation of the heterodimers with expected masses of 58932 Da and 59361Da respectively for RELAX0019 and RELAX0023: no homodimers were detected. Reduced analysis (Figure 2B) confirmed the sequence identity of both chains and showed they had no modifications.

Non-reduced peptide mapping to identify disulphide bonds

Heterodimeric Fc-Relaxin (50 µg) was placed into a clean sample tube and diluted in 17 µL of 100 mM sodium phosphate pH 7.0. Alkylation of free cysteines was achieved by addition of 0.5 µL of 5 mg/ml iodoacetamide followed by incubation at room temperature for 20 minutes. Following the alkylation, a further 2.5 µL of 100 mM sodium phosphate buffer pH 7.0 was added and 2.5 µL of sodium chloride. The protein was denatured by addition of 40 µL 8.0 M Guanidine HCl and incubated at 37°C for 30 minutes. Dilution was

achieved by addition of 125 µL of 100 mM sodium phosphate buffer pH 7.0 followed by addition of 0.5 µL of 40 mM EDTA. Endoproteinase Lys-C (Wako Chemicals) was reconstituted in water at a concentration of 1 mg/ml and 5 µL added to Fc-Relaxin-2. Digestion was carried out at 37 °C for 2 hours after which time an additional 5 µL of Lys-C was added and the incubation continued for a further 2 hours. For peptide analysis, 42.5 µL of sample was transferred to a UPLC vial and 2.5 µL of water added. For reduction of disulphide bonds, 2.5 µL of 500 mM DTT was added to another 42.5 µL aliquot of sample and left at room temperature for 15 minutes before LC-MS analysis.

Analysis of the peptides was performed using an ACQUITY I-Class UPLC coupled to a Xevo G2-XS Q-TOF instrument (Waters, Milford, MA), both operated using UNIFI Scientific Information System. For the LC system, Solvent A was water with 0.1% formic acid and solvent B was acetonitrile with 0.1 % formic acid (both UPLC-MS grade, BioSolve). The UV detector was set to measure at a wavelength of 214 nm and the vials placed in a sample chamber maintaining a temperature of 4°C. A volume of 10 µl was injected onto a reverse phase ACQUITY BEH C18 300 Å-pore column (Waters part number: 186003687) and proteins were eluted using an increasing gradient of solvent B from 5 % to 37 % B over 73.5 minutes and then increased to 60 % B over a further 2.5 minutes. After 77.5 minutes the column was held at 95 % B for 5 minutes.

The mass spectrometer was calibrated from 100-2600 m/z by infusing 2 µg/µL sodium iodide in 50% 2-propanol and the lockspray was 200 pg/µL Leucine Enkephalin. The instrument was operated in positive ionisation mode and sensitivity analyser mode with the following key settings: capillary voltage = 3.0 V; sample cone voltage = 25 V; source temperature = 100 °C; desolvation temperature = 250 °C; cone gas flow = 0 L/h; desolvation gas flow = 500 L/h; mass range = 100-2600 m/z, scan time = 0.5 sec.

Data were processed in UNIFI software by importing the sequence with expected disulphide bonds and performing a search for matching Lys-C generated peptides. The chromatograms obtained in the absence and presence of reducing agent were overlaid to verify that the disulphide-bonded peptides identified were no longer observed once reduced.

A peptide matching the expected mass for the disulphide-bonded Relaxin-2 peptide incorporating both chains A and B was identified as depicted on the top of Figure 3 (SLSLSPGGGGGSGGGGSGGGGSGGGGGSQLYSALANKCCHVGCTK= LCGRELVRAQIAICGMSTWS=RSLARFC (SEQ ID NOS 75-77, respectively), expected

mass including 3 disulphide bonds 6836.23 Da). Figure 3 (A-D) shows the identification of this peptide for RELAX0019 and confirmation that the peptide was no longer observed when reducing agent was added: panels A and B show extracted ion chromatograms in the absence and presence of DTT and panels C and D show the corresponding mass spectrum of the peptide. Figure 3 (E-H) shows the identification of the same peptide for RELAX0023 and confirmation that the peptide was no longer observed when reducing agent was added: panels E and F show extracted ion chromatograms in the absence and presence of DTT and panels G and H show the corresponding mass spectra of the peptide. These data confirm that Relaxin chains A and B are interacting through disulphide bonds within the heterodimers RELAX0019 and RELAX0023.

Example 3: *in vitro* activity of Fc-Relaxin-2 fusion proteins (cell based cAMP activity assay)

The Relaxin-2 fusion polypeptides produced as described above were tested for biological activity, e.g. stimulation of one or more cellular receptor responses, by the following methods.

Stable cell lines expressing human or mouse receptors generated in CHO cells were purchased from DiscoverX.

- cAMP Hunter™ CHO-K1 RXFP1 Gs, cell line (DiscoverX catalogue number 95-0127C2)
- cAMP Hunter™ CHO-K1 RXFP2 Gs cell line (DiscoverX catalogue number 95-0140C2)
- cAMP Hunter™ CHO-K1 mRXFP1 Gs cell line (DiscoverX catalogue number 95-0180C2)

Activation of these receptors results in downstream production of cAMP second messenger that can be measured in a functional activity assay.

Routine cAMP assays were performed using bovine serum albumin (BSA)-based assay buffer: Hanks Balanced Salt Solution (Sigma # H8264) supplemented with 0.1% BSA (Sigma # A9418) and 0.5 mM IBMX (Sigma # I7018), adjusted to pH 7.4 with 1 M NaOH.

A frozen cryo-vial of cells expressing the receptor of interest was thawed rapidly in a water-bath, transferred to pre-warmed cell media and spun at 240xg for 5 minutes. Cells were

re-suspended in cell media at an optimized concentration (e.g. hRXFP1 at $3.33x10^4$ cells /mL), and 30 µL cell suspension was added to Poly-D-Lysine-coated 384-well plates (Greiner # 781946) and allowed to adhere overnight. The next day the media was flicked out of the plates and replaced with 5 uL assay buffer. Eleven-point serial dilutions of test recombinant peptide or Fc fusion samples were added to the cells using a non-contact liquid dispenser (ECHO™, Labcyte). All sample dilutions were made in duplicate. An additional 5 µL assay buffer was added to each well and the plates incubated at room temperature for 30 minutes.

cAMP levels were measured using a commercially available cAMP dynamic G_S HTRF kit (Cisbio, Cat # 62AM4PEJ), following the two-step protocol as per manufacturer's recommendations. In brief, anti-cAMP cryptate (donor fluorophore) and cAMP-d2 (acceptor fluorophore) were made up separately by diluting each 1/20 in conjugate & lysis buffer provided in the kit. 5 µL anti-cAMP cryptate was added to all wells of the assay plate, and 5 µL cAMP-d2 added to all wells except non-specific binding (NSB) wells, to which conjugate and lysis buffer was added. Plates were incubated at room temperature for one hour and then read on an Envision (Perkin Elmer) using excitation wavelength of 320nm and emission wavelengths of 620nm & 665nm. Data was transformed to % Delta F as described in manufacturer's guidelines and then transformed to percent activation of maximal native agonist response and analysed by 4-parameter logistic fit to determine EC50 values. The results are compared to corresponding results for recombinant hRelaxin-2 (R&D Systems Cat # 6586 RN) in the case of hRXFP1 cells, mRelaxin-1 (R&D Systems Cat # 6637 RN) in mRXFP1 cells and INSL3 (R&D Systems Cat # 4544 NS) in hRXFP2 cells.

Data analysis was performed using statistical analysis software (GraphPad Prism, V6).

The biological activity of the tested constructs is provided in Table 4 and in Figure 4. The average EC50 measurements for both the recombinant human Relaxin-2 and fusion polypeptides from several assays has been summarized in Table 4.

RELAX0013, RELAX0014 and RELAX0010 are proteins of reference, where RELAX0013 is the recombinant human Relaxin-2, RELAX0014 is the recombinant murine Relaxin-1 and RELAX0010 is a single chain fusion protein comprising chain A, linker of 15 amino acids, chain B, connector of 15 amino acids, and Fc, comprising the amino acid sequence of SEQ ID NO. 8, described in WO2018/138170.

Table 4: *Biological activity of heterodimeric Fc Relaxin fusion polypeptides (n: number of repeats).*

Name	n	EC50 hRXFP1 (M)	EC50 mRXFP1 (M)	EC50 hRXFP2 (M)
RELAX0013	23	1.15E-12	7.54E-13	1.75E-09
RELAX0014	23	4.47E-12	2.37E-12	1.78E-12
RELAX0010	10	8.3E-12	7.64E-12	2.88E-07
RELAX0019	8	3.57E-11	9.10E-12	3.42E-08
RELAX0020	4	4.41E-11	2.79E-11	3.54E-08
RELAX0023	11	3.77E-11	3.27E-11	3.24E-08
RELAX0024	2	4.60E-11	1.56E-11	4.26E-08
RELAX0021	4	8.27E-11	4.14E-11	Not tested
RELAX0022	2	4.74E-11	3.28E-11	Not tested
RELAX0091	2	5.88E-11	2.88E-11	>1.09E-7
RELAX0117	6	1.06E-11	1.74E-11	1.61E-08

From the results presented in Table 4, it can be concluded that the heterodimeric Fc Relaxin fusion proteins tested were less potent than the single chain fusion RELAX0010 or the recombinant human Relaxin-2 peptide, but they still retained high levels of biological activity (ranging from ~10 pM to ~ 80 pM in the human RXFP1 cell line).

These results show that the Relaxin A and B chains can be fused to either/both termini (connector can be attached to either N or C terminus of the Relaxin chain) and either chain of the heterodimeric Fc (X or Y) and retain biological activity. The format of the heterodimeric Fc Relaxin fusion proteins described herewith thus constitutes a robust format for generating a long half-life active Relaxin.

The presence of the disulphide bond to stabilise the heterodimeric Fc did not affect potency of the fusion protein (compare RELAX0023 versus RELAX0021, and RELAX0024 versus RELAX0022).

The two upper hinge regions used (GGAGGA (SEQ ID NO: 78) and native DKTHT (SEQ ID NO: 79)) did not affect potency (compare RELAX0023 versus RELAX0019, and RELAX0024 versus RELAX0020). The exact amino acid sequence of the upper hinge is not critical for the activity of the fusion protein.

Example 4: The effect of the connector composition and length in the heterodimeric Relaxin-2 Fc fusion proteins

The connectors can be composed of glycine and serine residues (GS) or can be composed of proline and alanine repeats (PA). The connectors used herewith had lengths between 6 and 21 residues. An example of a long GS connector is: GGGGSGGGGSGGGGSGGGGGS (SEQ ID NO: 5) (21 amino acids). An example of a long PA connector is: PAPAPAPAPAPAPAPAPAPAG (SEQ ID NO: 6) (21 amino acids).

Connectors of different lengths and compositions can be placed on each Fc-chain of the heterodimeric Relaxin-2 Fc fusion polypeptides.

Examples of heterodimeric Relaxin-2 Fc fusion proteins with a variety of connectors are shown in Table 5. This table also indicates information regarding developability/manufacturability (expression yield and percentage of monomeric/non-aggregated Relaxin-2 Fc fusion proteins after protein A capture from cell culture supernatant), and biological activity.

Table 5: *Effect of connectors on biological activity and developability properties of heterodimeric Fc Relaxin-2 fusion proteins during small scale expression.*

Name	Expression yield (mg/l)	% monomers	n	EC50 hRXFP1 (M)	EC50 mRXFP1 (M)	EC50 hRXFP2 (M)
RELAX0013			23	1.15E-12	7.54E-13	1.75E-09
RELAX0014			23	4.47E-12	2.37E-12	1.78E-12
RELAX0010	No data	No data	10	8.3E-12	7.64E-12	2.88E-07
RELAX0019	147	78	25	5.81E-11	2.24E-11	4.40E-08

RELAX0023	No data	No data	15	3.32E-11	1.36E-11	4.20E-08
RELAX0081	164	82	3	4.51E-11	4.73E-11	4.92E-08
RELAX0082	226	83	3	5.68E-11	4.90E-11	3.81E-08
RELAX0083	83	94	6	2.81E-11	1.34E-11	2.42E-08
RELAX0056	466	75	4	3.87E-11	3.27E-11	6.48E-08
RELAX0054	6	89	2	2.89E-11	1.59E-11	1.53E-08
RELAX0055	9	92	2	1.88E-11	1.51E-11	3.39E-08
RELAX0084	91	93	2	5.34E-11	3.48E-11	1.20E-08
RELAX0085	261	81	2	6.37E-11	3.09E-11	4.67E-08
RELAX0086	150	92	2	4.49E-11	2.68E-11	4.88E-08
RELAX0087	179	82	2	4.89E-11	3.48E-11	3.63E-08
RELAX0105	231	76	2	7.12E-11	1.49E-11	3.33E-08
RELAX0106	269	76	2	6.96E-11	1.98E-11	4.94E-08
RELAX0107	301	77	2	8.09E-11	3.87E-11	1.22E-07
RELAX0109	60	33	3	1.72E-09	8.22E-10	
RELAX0110	61	34	3	1.88E-09	1.11E-09	>6.07E-8
RELAX0111	60	36	3	1.93E-09	1.24E-09	>6.06E-8

The length and composition of the connectors does have an impact on the developability aspect of the molecules. As shown in Table 5, heterodimeric Relaxin-2 Fc fusion polypeptides with PA connectors of less than or equal to 16 amino acids did not express well. In contrast, a 21-residue long PA connector increased the expression yield significantly. Expression yields of constructs with GS connectors are more consistent.

Heterodimeric Relaxin-2 Fc fusion proteins with short and asymmetric (different) connectors retained potency. Reduction in biological activity was only observed in fusion proteins with low monomeric content (RELAX0109, RELAX0110 and RELAX0111).

Example 5: Point mutations in the Relaxin-2 sequence

Relaxin single point mutation analogues were made as heterodimeric Fc Relaxin-2 fusion proteins. Table 6 shows examples of such molecules which retained potency and favourable developability properties.

The native residues targeted are positively charged and could be liable to proteolysis but are not involved in the binding of Relaxin to its receptor.

For instance, R22X analogues of heterodimeric Fc Relaxin-2 fusion proteins seem to consistently have improved developability/manufacturability properties.

Table 6: *Examples of Relaxin-2 analogues which retain potency and favourable developability properties during small scale expression.*

Name	Expression yield (mg/l)	% Monomers	n	EC50 hRXFP1 (M)	EC50 mRXFP1 (M)	EC50 hRXFP2 (M)
RELAX0013			23	1.15E-12	7.54E-13	1.75E-09
RELAX0014			23	4.47E-12	2.37E-12	1.78E-12
RELAX0019	147	78	25	5.81E-11	2.24E-11	4.40E-08
RELAX0039	188.0	87	2	6.54E-11	4.25E-11	1.08E-07
RELAX0040	128.8	88	2	5.92E-11	2.92E-11	>1.27E-7
RELAX0041	162.5	82	2	6.22E-11	3.17E-11	1.18E-07
RELAX0043	160.2	79	2	7.98E-11	5.58E-11	>1.58E-7
RELAX0052	162.4	81	4	9.67E-11	5.69E-11	1.05E-07
RELAX0053	181.0	80	2	7.15E-11	4.36E-11	>1.79E-7
RELAX0063	157.2	84	2	1.96E-10	4.46E-11	>1.38E-7

RELAX0069	163.0	86	3	5.76E-11	3.69E-11	>1.62E-7
RELAX0070	145.5	91	3	6.67E-11	5.02E-11	1.07E-07
RELAX0071	174.7	85	3	6.87E-11	3.93E-11	1.15E-07
RELAX0072	232.3	78	2	8.53E-11	4.03E-11	>2.3E-7
RELAX0073	174.7	87	3	5.70E-11	4.15E-11	8.63E-08
RELAX0074	170.0	88	2	5.45E-11	4.53E-11	9.21E-08
RELAX0075	144.4	79	3	9.47E-11	6.14E-11	>1.43E-7

The results presented in Table 6 demonstrate that some variability in the amino acid sequence of the Relaxin-2 chain A is tolerated without the loss of potency while retaining favourable developability properties.

Example 6: PK profile of Fc-Relaxin-2 fusion proteins

The pharmacokinetic (PK) profiles of Relaxin-2 fusion polypeptides were determined using a Relaxin ELISA assay and/or cAMP assay. Relaxin-2 fusion polypeptides were administered to 6-10-week-old male C57BL/6J (Jax) mice (Jackson Laboratories) by either the subcutaneous (SC) and/or intravenous (IV) route at 6 mg/kg. For the IV route of administration, serum samples were collected at 5 minutes, 30 minutes and 60 minutes followed by either 3 hours and/or 6 hours and/or 8 hours and 24 hours followed by a series of minimum 1-day intervals to a maximum of 21 days post drug administration. A similar schedule was followed for the SC route of administration with less frequent collections within the first 8 hours; for example, collecting the first sample at 30 minutes then at 3 hours, 8 hours, 24 hours, 30 hours and 48 hours, followed by a series of minimum 1-day intervals to a maximum of 21 days. Samples were collected by cardiac puncture into a serum tube and were kept at room temperature for 15 to 30 minutes then centrifuged for 10 minutes at 10000 rpm within 30 minutes of collection. Aliquoted samples were stored at < -80°C and later tested by ELISA or cAMP activity assay.

For the majority of molecules, the PK samples were tested in an ELISA using an anti-hRelaxin-2 capture (pre-coated Human Relaxin-2 Quantikine ELISA Kit, R&D Systems Cat# DRL200) and anti-human Fc detection antibody (AU003 labelled with HRP), with the

exception of RELAX0010 (described in WO2018/138170) which was tested in an ELISA using anti-human Fc capture and anti-hRelaxin-2 detection (using the polyclonal HRP-labelled antibody from the Human Relaxin-2 ELISA kit, R&D Systems Cat# DRL200). In both assays, plates coated with the capture antibody were blocked with 100 µL RD1-19 assay diluent for one hour at room temperature. 50 µL of standard or sample was added to each well and incubated for two hours at room temperature. Samples were aspirated and wells washed three times with assay wash buffer. 50 µL of HRP-labelled detection antibody was added per well, diluted either 1:1000 in PBS/1% BSA in the case of anti-human Fc-specific detection or used undiluted in the case of anti-hRelaxin-2 detection. Following 1 hour incubation at room temperature and three washes, 50 µL per well TMB (SureBlue Reserve KPL 53-00-03) was added and once the colour change had occurred the reaction was stopped by adding 50 µL per well TMB stop solution (KPL 50-85-06).

Biological activity of PK samples in cell-based cAMP activity assay.

Serum samples collected from animals as outlined above were tested for biological activity in order to measure functional Relaxin-2 to assess integrity of Fc-Relaxin-2 fusion polypeptides. A stable cell line expressing human RXFP1 receptor generated in CHO cells was purchased from DiscoverX. Activation of this receptor results in downstream production of cAMP second messenger that can be measured in a functional activity assay.

cAMP assays were performed using bovine serum albumin (BSA)-based assay buffer: Hanks Balanced Salt Solution (Sigma # H8264) supplemented with 0.1% BSA (Sigma # A9418) and 0.5 mM IBMX (Sigma # I7018), adjusted to pH 7.4 with 1 M NaOH.

Dosing solutions of the Relaxin-2 fusion polypeptides or recombinant Relaxin-2 peptide (R&D Systems Cat# 6586-RN) were diluted in assay buffer and a non-contact liquid dispenser (ECHO, Labcyte) used to create 11-point standard curves in four matrix concentrations. The matrix used was blank serum from mock-dosed animals and was added manually to wells at twice the required concentration to allow for the addition of cells. Test samples were transferred from serum tubes to a 384-well source plate which was used by a non-contact liquid dispenser (ECHO, Labcyte) to set up four dilutions in assay buffer. All sample dilutions were made in duplicate.

A frozen cryo-vial of cells expressing hRXFP1 was thawed rapidly in a water-bath, transferred to pre-warmed cell media and spun at 240xg for 5 minutes. Cells were re-suspended in 8 mL cell culture medium, seeded in a T75 flask containing 10 mL culture medium and allowed to attach overnight. The following day the cells were detached using accutase and spun at 240xg for 5 minutes. The resulting cell pellet was resuspended at an optimized concentration, and 2.5 µL cell suspension was added to each well of the assay plates using a Combi-drop dispenser.

cAMP levels were measured using a commercially available cAMP dynamic 2 HTRF kit (Cisbio, Cat# 62AM4PEJ), following the two-step protocol as per manufacturer's recommendations. In brief, anti-cAMP cryptate (donor fluorophore) and cAMP-d2 (acceptor fluorophore) were made up separately by diluting each 1/20 in conjugate & lysis buffer provided in the kit. 2.5 µL anti-cAMP cryptate was added to all wells of the assay plate, and 2.5 µL cAMP-d2 added to all wells except non-specific binding (NSB) wells, to which conjugate and lysis buffer was added. Plates were incubated at room temperature for one hour and then read on an Envision (Perkin Elmer) using excitation wavelength of 320nm and emission wavelengths of 620nm & 665nm. Data was transformed to % Delta F as described in manufacturer's guidelines and sample values calculated from the linear part of the standard curves.

Results and conclusion

Figure 5 shows a summary of data from a series of in vivo PK experiments where Fc-Relaxin-2 polypeptides were administered to mice IV. Data is normalised for 5 minute time point.

The half-life of human Relaxin-2 following IV administration is about 0.09 +/- 0.04 hours, i.e. 5.4 +/- 2.4 minutes in humans (Chen *et al.* 1993). Recombinant Relaxin Fc fusion polypeptides are all showing half-life improvements compared to native Relaxin-2. The Fc-Relaxin polypeptides where Relaxin A-chain and B-chain are connected to different heterodimeric Fc-chains (exemplified by RELAX0019, RELAX0023, RELAX0034, RELAX0046 and RELAX0117) have improved PK properties compared to those Fc-Relaxin polypeptides in which the Relaxin chains are connected with a linker (exemplified by RELAX0010 and RELAX0009). However, the presence of the connecting linker between Relaxin chain A and chain B by itself is not directly linked to quick in vivo

elimination of Fc-Relaxin polypeptides since linker-containing molecules RELAX0088 and RELAX0122 both show good in vivo stability.

Unexpectedly in this study, the heterodimeric Fc-Relaxin fusion polypeptides (RELAX0019, RELAX0023, RELAX0034, RELAX0046, RELAX0117, RELAX0088 and RELAX0122) all have significantly improved pharmacokinetic properties compared to the Fc-Relaxin fusion polypeptides RELAX0010 and RELAX0009.

Example 7: Reversal of established hypertrophy and fibrosis by RELAX0019 and RELAX0023

Isoproterenol was infused via minipump (15 mg/kg/day) into C57B6 mice for 10 days to induce cardiac hypertrophy and fibrosis. Mice infused with vehicle for the same duration were used as baseline controls. After 10 days, the minipumps were removed and mice were either given a new minipump containing rRelaxin-2 (500 ug/kg/day) or received the first of two, once-weekly (QW), subcutaneous injections of RELAX0019 (20 mg/kg) or RELAX0023 (20 mg/kg). After the 14-day treatment period, mice were sacrificed, and their hearts were collected for analysis of hypertrophy and fibrosis. Hearts from baseline control mice were collected after removal of the vehicle minipump. Hypertrophy was determined as a measure of heart weight relative to tibial length and fibrosis was established by quantitation of collagen content relative to heart weight. Infusion of isoproterenol significantly induced both hypertrophy and fibrosis in this model. QW dosing of RELAX0019 and RELAX0023 returned the isoproterenol-induced hypertrophy to baseline levels, as did constant infusion of rRelaxin-2. All Relaxin treatments also reduced cardiac fibrosis by more than 50%. N=8 for each group. **p<0.01, ***p<0.001, ****p<0.0001

Recombinant Relaxin Fc fusion proteins RELAX0019 and RELAX0023 were able to reverse hypertrophy and fibrosis in a similar manner to native hRelaxin-2 (Figure 6)

Example 8: Assessing non-specific binding of Fc-Relaxin-2 proteins using Baculovirus ELISA.

RELAX proteins were expressed in CHO cells and purified as described above. A Baculovirus ELISA developed for assessing non-specific binding of monoclonal antibodies (Ref: Hotzel et al 2012 mAbs 4:6, 753-760) was adapted to determine a non-specific binding of Fc-Relaxin polypeptides with the modification whereby instead of calculating a 'BV score' (Baculovirus plate absorbance/ blank plate absorbance) a non-specific binding was calculated separately for Baculovirus plate and blank plate as signal over background (where background is a value obtained in absence of Fc-Relaxin polypeptide). This

measure was introduced to reflect increased, when compared to monoclonal antibodies, non-specific binding of some Fc-peptides to both coated and un-coated (blank) plates. Preparations of each protein were made at either 100nM or 10nM in PBS (Gibco 14190-086) + 0.5% BSA (Sigma A9576) and used in duplicates in the ELISA assay on 96-well Nunc Maxisorp F plates coated overnight at 4°C with 50 µL/well of either 1% Baculovirus extract in 50mM sodium carbonate (BV plate) or with 50mM sodium carbonate (blank plate). Following a wash with PBS, plates were blocked with 300 µL/well of PBS + 0.5% BSA for 1 hour at room temperature and washed three times with PBS. 50 µL/well of either PBS + 0.5% BSA (background) or RELAX proteins dilutions were added and incubated for 1h at room temperature. Following three washes in PBS a detection antibody (anti-human Fc-specific -HRP Sigma A0170) diluted 1:5000 in PBS + 0.5% BSA was added at 50 µL/well. Samples were incubated for 1 hour at room temperature and plates were washed three times in PBS. The HRP substrate – TMB (SureBlue Reserve KPL 53-00-03) was then added at 50 µL/well and following the colour change, the reaction was stopped by adding 50 µL/well of 0.5M sulphuric acid. Absorbance was measured at 450nm and for each sample non-specific binding was determined. Non-specific binding (fold binding over background) was defined as a ratio of non-specific binding in the presence of Fc Relaxin-2 proteins and absence of Fc Relaxin-2 proteins (background). Data for Fc-Relaxin-2 proteins tested at 2 different concentrations of either 100nM or 10nM are shown in Table 7.

Table 7: *Binding of Fc-Relaxin fusion proteins in the Baculovirus ELISA at 100nM and 10nM (-001, 002, 003 refer to different batches of the same protein)*

Fusion name	non-specific binding BV plate (signal/ background) at 100nM	non-specific binding BLANK plate (signal/ background) at 100nM	non-specific binding BV plate (signal/ background) at 10nM	non-specific binding BLANK plate (signal/ background) at 10nM
RELAX0019-001	2.0	1.8	1.0	1.2
RELAX0019-002	1.5	1.9	1.1	1.1
RELAX0020	2.2	2.5	1.1	1.3

RELAX0021	2.7	5.3	1.0	2.0
RELAX0022	4.9	8.2	1.3	2.9
RELAX0023-001	1.7	1.8	1.0	1.0
RELAX0023-002	2.4	3.7	1.1	0.8
RELAX0024	1.8	5.3	0.9	1.5
RELAX0039	6.3	3.2	1.7	1.8
RELAX0040	7.5	3.0	2.6	2.1
RELAX0041	7.0	4.4	1.9	2.0
RELAX0043	3.7	1.6	1.3	1.3
RELAX0052	2.9	1.1	1.5	1.3
RELAX0053	5.5	3.8	1.7	2.2
RELAX0054	3.2	4.1	1.5	1.8
RELAX0055	1.4	4.6	0.7	1.7
RELAX0056	5.4	9.1	1.3	1.2
RELAX0069	1.7	1.8	1.1	6.5
RELAX0070	2.7	3.2	0.9	1.3
RELAX0071	1.3	1.7	0.8	0.9
RELAX0072	1.4	2.4	0.7	1.3
RELAX0073	1.7	1.6	0.7	1.1
RELAX0074	1.4	1.8	0.9	1.5
RELAX0075	4.7	7.9	3.3	4.8

RELAX0076	3.3	5.0	1.5	3.6
RELAX0081	3.2	4.9	0.8	1.5
RELAX0082	3.4	6.1	1.0	2.9
RELAX0083	2.9	5.7	2.6	1.5
RELAX0084	3.2	7.8	1.2	1.7
RELAX0085	5.4	12.3	1.4	2.2
RELAX0086	3.1	7.2	1.3	1.6
RELAX0087	4.1	17.3	1.4	2.7
RELAX0088-001	3.5	5.6	1.4	1.4
RELAX0088-002	1.9	2.2	1.1	0.8
RELAX0091	5.6	39.3	1.6	6.8
RELAX0105	12.9	8.3	2.4	1.1
RELAX0106	14.6	8.3	2.4	1.0
RELAX0107	11.6	7.0	1.8	0.9
RELAX0109	27.1	19.7	5.8	2.5
RELAX0110	26.8	23.9	8.3	2.6
RELAX0111	29.0	24.3	7.0	2.9
RELAX0117	18.5	47.4	3.0	8.2
RELAX0122	2.2	2.4	1.1	0.7
RELAX0123	2.5	4.8	1.1	0.9
RELAX0124-001	1.8	1.7	1.1	0.7

RELAX0124-002	6.4	4.6	1.5	0.9
RELAX0126-001	20.0	41.5	10.2	16.9
RELAX0126-002	21.3	40.4	10.9	14.3
RELAX0127	23.5	42.8	13.3	19.8
RELAX0128	23.5	42.4	13.2	19.2
RELAX0130	2.2	6.1	1.1	1.6
RELAX0010-001	6.3	13.7	1.5	5.0
RELAX0010-002	6.0	13.2	1.8	4.2
RELAX0010-003	2.4	21.0	0.8	7.7
RELAX0009	17.8	22.2	4.8	21.5

As shown in Table 7 and Figure 7, heterodimeric Relaxin-2 Fc fusion polypeptides exhibit lower non-specific binding when Relaxin chains are attached to the C-terminus using GS connectors. Some asymmetric and PA connectors, certain point mutations and positioning Relaxin chains at the N-termini, particularly in the context of a bivalent molecule (RELAX0117), increase non-specific binding to both blank and BV-coated plates. Some Fc-Relaxin proteins with particularly high non-specific binding exhibit greater non-specific binding to blank plates than to BV-coated plates at both high (100nM) and low (10nM) concentrations. Although the control molecules – the linker-containing bivalent RELAX0009, RELAX0010, RELAX0126, RELAX0127 and RELAX0128 all demonstrate high non-specific binding, neither the presence of the linker between chains A and B of Relaxin nor the bi-valency per se, drive high non-specific binding as can be demonstrated by low non-specific binding of RELAX0122.

Example 9: Stability in solution

Stability of RELAX0023 was assessed using High Performance Size Exclusion Chromatography (HP-SEC) and liquid chromatography-mass spectrometry (LC-MS) and compared to RELAX0127 and RELAX0128. HP-SEC with detection by absorbance at 280 nm can be used to measure purity, aggregation and fragmentation. The molecules were buffer exchanged into an optimised formulation composition and then concentrated up to 10 mg/mL. All samples were placed at a stressed temperature condition (40ºC) for up to 4 weeks. At the time points of 1, 2 and 4 weeks, the samples were collected and injected onto a size exclusion column and were eluted with an aqueous mobile phase isocratically at a fixed flow rate. Larger molecules are excluded from the pores of the size exclusion column to a greater extent than smaller molecules, and therefore elute earlier. Peaks eluting earlier than the monomer peak are recorded as aggregates. Peaks eluting after the monomer peak (excluding the buffer-related peak) are recorded as fragments. Results are reported as percent purity; percent aggregate; and percent fragment and shown in Figure 8. RELAX0023 is the most stable molecule with a %purity loss rate of only 0.1% per month compared to 7.7% and 9.3% respectively for RELAX0128 and RELAX0127. Both RELAX0127 and RELAX0128 showed signs of aggregation, however the aggregate level for RELAX0023 did not increase, indicating a better physical solution stability. Fragmentation appeared to be the main factor for the purity loss with RELAX0127 having a 6.6% fragmentation per month and 6.8% for RELAX0128. RELAX0023 only has a fragmentation rate of 0.7% per month. At the meantime, after 4 weeks of storage at 40ºC, the total peak area of RELAX0128 dropped from 22403 to 18216 (a decrease of 19%), and RELAX0127 dropped from 22225 to 18823 (a decrease of 15%). This significant loss in total peak area, together with a high fragmentation rate, indicated a potential high chemical degradation with these two molecules. It should be pointed out that, this loss in total areas had a strong impact to the chromatogram profiles of these two molecules. This explains why, despite an obvious increase in the aggregate peak areas after storage, RELAX0128 and RELAX0127 showed a lower percent aggregate at 4 weeks compared to previous timepoints. In contrast, the total peak area of RELAX0023 only dropped by 0.03%, from 21828 to 21761, indicating a better stability profile compared to RELAX0128 and RELAX0127.

The fragmentation of the molecules was further verified by LC-MS using reduced mass analysis which showed that the fragment peaks of RELAX0127 and RELAX0128 increased in intensity after storage at 40ºC (Figure 9A). In contrast, the fragment peak for RELAX0023 remained unchanged after stress. The mass spectra under reducing

conditions also showed modification of RELAX0127 and RELAX0128 over time which is evidenced by a shift of the peak to a larger mass and a broadening of the peak indicating greater heterogeneity (Figure 9B). In contrast, the intact mass spectra of RELAX0023 remained unchanged indicating no modification occurred. This study indicates that RELAX0023 has superior physical and chemical stability compared to RELAX0127 and RELAX0128.

Example 10: PK profile of RELAX0023 in cynomolgus monkeys

The pharmacokinetic (PK) profile of RELAX0023 in cynomolgus monkeys was determined using a sandwich ELISA-based immunoassay. RELAX0023 was administered to a total of 12 female cynomolgus monkeys that were randomly assigned to 4 groups of 3 animals per group. Animals in Groups 1, 2, and 3 were administered 0.1, 1, and 10 mg/kg of RELAX0023 SC, respectively. Animals in Group 4 were given 10 mg/kg IV bolus of RELAX0023. Serum samples were collected 0.25 hour, 1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 96 hours, 7 days, 14 days and 21 days post drug administration.

Assay plates were coated with goat anti-human IgG antibody and were incubated with cynomolgus monkey sera from group 1-4 animals. RELAX0023 bound to the plates was detected by an anti-relaxin antibody conjugated with HRP. Cynomolgus serum was diluted 1:10 prior to addition to plates. The lower limit of quantitation is 0.010 µg/mL and upper limit of quantitation is 0.300 µg/mL in 100% serum.

Results and conclusion

Figure 10 shows the mean serum concentration-time profiles of RELAX0023 in cynomolgus monkeys following a single dose. Following a single dose administered SC, RELAX0023 exhibited linear PK in a dose range of 0.01 to 10 mg/kg. A dose-proportional increase in C_{max} was observed. Mean C_{max} values were 0.400, 4.69, 34.8 µg/mL for 0.1, 1, and 10 mg/kg SC dose groups, respectively. A dose-proportional increase in $AUC_{0\text{-last}}$ values were also observed from 0.1 mg/kg to 10 mg/kg SC group. Mean $AUC_{0\text{-last}}$ values were 2.01, 25.5, 193 µg·day/mL for 0.1, 1, and 10 mg/kg SC dose groups, respectively. Overall, RELAX0023 PK is linear in the range of 0.1 mg/kg to 10 mg/kg with the mean CL/F of 51.0 mL/day/kg and mean $t_{1/2}$ of 3.07 days. SC bioavailability of RELAX0023 was estimated as 88.2%.

Example 11: Evaluation of chronic efficacy of RELAX0023, in cynomolgus monkey (*Macaca fascicularis*) with heart failure and reduced left ventricular ejection fraction (LVEF)

The chronic efficacy of RELAX0023 on cardiac function was evaluated in obese and aged cynomolgus monkeys (*Macaca fascicularis*). The cynomolgus monkey was selected as the test species of choice over other lower mammalian species because of its close relationship to humans, both phylogenetically and physiologically. Old cynomolgus monkeys fed with a high fat diet for at least 2 years share risk factors with human patients susceptible to cardiovascular disease and develop metabolic syndrome which can characteristically progress to heart failure and reduced LVEF. The effects of RELAX0023 on LVEF was evaluated when administered by subcutaneous (SC) injection at different dose levels for 20 weeks, with the first dose administered at week 1 of the study, followed by an 18-week observational period. From a pool of approximately 100 obese and aged cynomolgus monkeys aged 12–20 years with a body weight of 6–15 kg that had been fed a high-fat diet for at least 2 years, 38 monkeys were identified by 2D echocardiographic screening as having an LVEF between 30-60%. Healthy monkeys of this age weigh 5–8 kg and have an LVEF of 70–75%, and therefore LVEF of 60% or below represents an HFrEF model. Identified animals were selected and randomly assigned to 3 treatment groups with 8 monkeys each, and a vehicle group with 14 monkeys. The dosing period consisted of once-weekly subcutaneous administrations of RELAX0023 at 3 ascending dose levels (all lower than 10 mg/kg; so called “low”, “mid” and “high” dose respectively).

Cardiac functional measurements by 2D echocardiography were determined 9 times, at baseline week -2 and at week 5, 9, 13, 17, 21, 25, 29, 33 of the dosing and post-dose observation periods. A further 2D echocardiography is scheduled for week 39 (study end). Parameters, including LVEF, were based on apical two- and four-chamber views and the biplane method. HDO (High Definition Oscillometry) was used to measure parameters including mean arterial pressure (MAP) and heart rate (HR).

Results and conclusion

RELAX0023 was able to greatly improve LVEF at weeks 5, 9, 13, 17 and 21 at all RELAX0023 dose levels compared with vehicle control, without affecting heart rate or blood pressure (Figure 12). Remarkably, improved LVEF following treatment with RELAX0023 as compared with week 0 (baseline) was observed throughout the washout period after the end of treatment to week 33 of the study. These striking results indicate a remarkable improvement in hemodynamics in the treated animals and clearly demonstrate

efficacy of RELAX0023 in treating heart failure in this model. Moreover, the magnitude of the sustained response after treatment is something which, to the best of the inventors' knowledge, has not been achieved previously by any other known compound targeting this mechanism of action pathway. Monkeys continue to be monitored until week 39 of the study.

Example 12: Phase 1 (Ph1) Study in Healthy Volunteers and Heart Failure Patients

Study D8330C00001 was a Phase Ia/b, randomized, single-blinded, placebo-controlled, first-time-in-human (FTIH) study (ClinicalTrials.gov identifier NCT04630067). The primary objective of the study was to assess the safety and tolerability of single and multiple ascending doses of RELAX0023 (also termed "AZD3427"), and the secondary objectives were to evaluate (i) the pharmacokinetics (PK) and (ii) the immunogenicity of single and multiple ascending doses of AZD3427.

The study was performed in 2 parts, Part A and Part B. Part A was a single ascending dose (SAD) study in healthy participants (males and females of non-childbearing potential), and Part B was a multiple ascending dose (MAD) study in participants with HF (males and females of non-childbearing potential).

Part B included 48 patients across 6 cohorts (8 participants in each cohort). Of these, 3 cohorts were comprised of participants with HFrEF (Cohorts 1b, 3b, and 5b) and 3 cohorts were comprised of participants with HF with EF ≥41% (Cohorts 2b, 4b, and 6b). The dose levels in HFrEF and HF with EF ≥41% cohorts were 5 mg (Cohorts 1b, 2b), 15 mg (Cohorts 3b, 4b), and 45 mg (Cohorts 5b, 6b) administered once weekly (QW) for 5 weeks (i.e., a total of 5 doses).

For Part B, which included 48 patients across 6 cohorts, the inclusion criteria included: (i) All Cohorts: Have a known clinical diagnosis of Stage C HF (NYHA Class I to III) and be on stable medical therapy for at least 12 weeks prior to screening with no significant dose change or new medications added during that period, (ii) Cohorts 1b, 3b, 5b: Patients with a diagnosis of HFrEF defined as EF≤ 40%, (iii) Cohorts 2b, 4b, 6b: Patients with a diagnosis of HF with EF ≥ 41% (including patients with a diagnosis of HFpEF defined as EF ≥ 50%), (iv) All Cohorts: Have a BMI between 18 and 40 kg/m^2 (inclusive) and weigh at least 55 kg and no more than 120 kg (inclusive), and (v) All Cohorts: Prior recording of either NT-proBNP > 125 pg/mL or BNP > 35 pg/mL.

Example 13: Ph1 AZD3427 MAD study outcomes in HF patients

In the Part B MAD cohort, data were pooled for HFpEF and HFrEF patients. Trends suggest AZD3427 improved cardiac function, including improved cardiac output and stroke volume (SV), reduced systemic vascular resistance (SVR), and improved organ perfusion (SVR and eGFR) (FIGs. 13A - 6F).

Whilst the hypertensive status of the subjects was not determined in the MAD cohort, the observed improvements in cardiac output and stroke volume (SV), reduced systemic vascular resistance (SVR), increased estimated glomerular filtration rate (eGFR) and thus improved organ perfusion (SVR and eGFR) are expected to be of benefit in HF + PH patients.

Example 14: Ph2b Study - a randomized, placebo-controlled, multi-centre, dose-ranging study of AZD3427 in participants with heart failure and pulmonary hypertension due to left heart disease (World Health Organisation [WHO] Group 2).

This study (Study ID Number: D8330C00003) is intended to assess the ability of AZD3427 to reduce pulmonary vascular resistance (PVR) after 24 weeks of treatment in participants with heart failure (HF) and pulmonary hypertension (PH) Group 2.

Approximately 220 participants will be randomised to 4 treatment groups (in a 1:1:1:1 ratio) to receive a subcutaneous (SC) injection of AZD3427 or placebo every 2 weeks for 24 weeks. This study will evaluate 3 dose levels of AZD3427: Dose A, Dose B, and Dose C. Dose modification is not applicable for this study. The study will be conducted in approximately 60 study centres across an estimated 15 countries. The study will include approximately 16 study visits: 2 visits during the Screening Period,13 visits during the Treatment Period, and one visit during the Follow-up Period. The expected total duration of the study is 32 to 37 weeks, depending on the length of the Screening Period.

Participants will receive a single, subcutaneous dose of AZD3427 (Dose A, B or C) or placebo once every 2 weeks for 24 weeks from Day 1 to Day 155.

The Primary Outcome measure will be the change from baseline in Pulmonary Vascular Resistance (PVR) after 24 weeks of treatment. The effect of AZD3427 on PVR parameter will also be evaluated as compared with placebo as measured by right heart

catheterization (RHC) after 24 weeks of treatment in participants with HF and PH Group 2.

Secondary Outcome measures include:

- Change from baseline in Mean pulmonary arterial pressure (mPAP)
- Change from baseline in Pulmonary artery wedge pressure (PAWP)
- Change from baseline in cardiac output
- Change from baseline in Stroke Volume (SV)
- Change from baseline in Ejection fraction (EF)
- Change from baseline in left ventricular global longitudinal strain (LVGLS)
- Change from baseline in pulmonary arterial systolic pressure (PASP)
- Change from baseline in right ventricle/left ventricle (RV/LV) ratio
- Change from baseline in right ventricular outflow tract acceleration time (RVOT AT)
- Change from baseline in Tricuspid regurgitation velocity (TRV)
- Change from baseline in TAPSE/PASP [Tricuspid annular plane systolic excursion/ Pulmonary arterial systolic pressure]
- Change from baseline in right ventricular strain/pulmonary arterial systolic pressure (RVS/PASP)
- Change from baseline in inferior vena cava (IVC) diameter with inspiratory collapse
- Change from baseline in systemic vascular resistance
- Change from baseline in 6-minute walking distance (6MWD)
- Change from baseline in Kansas City Cardiomyopathy Questionnaire total symptom score (KCCQ TSS)
- Change from baseline in New York Heart Association Functional Class (NYHA FC)
- Change from baseline in serum creatinine
- Change from baseline in N-terminal prohormone of brain natriuretic peptide (NT-proBNP)
- Change from baseline in cystatin C
- Change from baseline in eGFR (estimated glomerular filtration rate)

Inclusion criteria: 1. Participant must be ≥ 18 years of age inclusive. 2. Participants must have a pre-existing diagnosis of HF, NYHA function class (FC) II to IV, and a pre-existing diagnosis of PH-LHD or likely or intermediate probability of Pulmonary hypertension due to left heart disease (PH-LHD) as per 2022 Pulmonary hypertension due to left heart disease European Society of Cardiology/European Respiratory Society (ESC/ESR)

guidelines. Participants must be on stable HF standard of care medication, including diuretics. 3. Participants must have a combination of echocardiographic parameters that show intermediate or high probability of PH as per 2022 ESC/ERS guidelines. 4. Participants must have an on-study elevated pulmonary artery pressure from RHC performed as per RHC manual provided by the Sponsor, at Screening Visit 2: (a) PAWP ≥ 15 mmHg (b) mPAP ≥ 20 mmHg 5. Minimum body weight of 50 kg (inclusive). 6. Capable of giving signed informed consent.

Exclusion Criteria 1. Diagnosis of PH in World Health Organization (WHO) Group 1, WHO Group 3, WHO Group 4, or WHO Group 5. 2. Historical or current evidence of a clinically significant disease or disorder. 3. Decompensated HF or any hospitalisation. 4. Any contraindications to RHC. 5. History of hypersensitivity to SC injections or devices. 6. History of hypersensitivity to drugs with a similar chemical structure or class to AZD3427 or any component of AZD3427 drug product, or ongoing clinically important allergy/hypersensitivity. 7. Known lung disease with Forced expiratory volume in the first second/Vital capacity (FEV1/VC) < 30%. 8. Congenital long QT syndrome. 9. Cardiac ventricular arrhythmia which requires treatment. Participants with atrial fibrillation or flutter and controlled ventricular rate are permitted. 10. History of or anticipated heart transplant or ventricular assist device implantation. 11. Any known planned (scheduled) highly invasive Cardiovascular (CV) procedure (eg, coronary revascularisation, ablation of atrial fibrillation/flutter, valve repair/replacement, aortic aneurysm surgery, etc). 12. Participants who have previously received AZD3427.

Claims

1. A method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) a first heterodimerisation domain connected to at least one Relaxin A chain polypeptide or a variant thereof; and

(ii) a second heterodimerisation domain connected to at least one Relaxin B chain polypeptide or a variant thereof,

wherein the first heterodimerisation domain heterodimerises with the second heterodimerisation domain, and wherein the heterodimeric fusion has Relaxin activity.

2. The method according to claim 1, wherein the Relaxin A chain polypeptide and the Relaxin B chain polypeptide of the heterodimeric fusion are covalently bound by at least one inter-chain disulphide bond.

3. The method according to claim 1 or 2, wherein the Relaxin A chain and the Relaxin B chain of the heterodimeric fusion are not covalently linked to each other by an amino acid linker.

4. The method according to any one of the preceding claims, wherein the Relaxin A chain of the heterodimeric fusion is a Relaxin-2 A chain and the Relaxin B chain of the heterodimeric fusion is a Relaxin-2 B chain.

5. The method according to any one of the preceding claims, wherein the Relaxin A chain of the heterodimeric fusion is connected to the first heterodimerisation domain via a connector and the Relaxin B chain of the heterodimeric fusion is connected to the second heterodimerisation domain via a connector, optionally wherein one or preferably both connectors are polypeptides.

6. The method according to claim 5, wherein one or preferably both of the connectors of the heterodimeric fusion have a length of between 6 and 40 amino acids, e.g. one or preferably both connectors have a length of 21 amino acids.

7. The method according to any one of the preceding claims, wherein the first and second heterodimerisation domains of the heterodimeric fusion are derived from an immunoglobulin Fc region ("first Fc region" and "second Fc region", respectively), optionally wherein the first and second Fc regions comprise the constant domains CH2 and CH3.

8. The method according to claim 7, wherein the C-terminus of the first Fc region is connected to the N-terminus of the Relaxin A chain and the C-terminus of the second Fc region is connected to the N-terminus of the Relaxin B chain.

9. The method according to claim 7 or 8, wherein the first and second Fc regions comprise heterodimerisation-promoting amino acid mutations and/or modifications, optionally wherein the heterodimerisation-promoting amino acid mutations are "Fc Knob" and "Fc Hole" mutations, e.g. "Fc Knob" and "Fc Hole" mutations present in the CH3 domains.

10. The method according to any one of claims 7 to 9, wherein the first and second Fc regions are derived from a human IgG1 immunoglobulin.

11. The method according to claim 10, wherein the heterodimerisation-promoting amino acid mutations comprise:

a. "Fc Hole" mutations Y349C, T366S, L368A and Y407V in one CH3 domain; and

b. "Fc Knob" mutations S354C and T366W in the other CH3 domain,

wherein the amino acid numbering is according to the EU index as in Kabat.

12. The method according to claim 11, wherein:

a. the first Fc region comprises the “Fc Knob” mutations and the second Fc region comprises the “Fc Hole” mutations; or

b. the second Fc region comprises the “Fc Knob” mutations and the first Fc region comprises the “Fc Hole” mutations.

13. The method according to any one of claims 10 to 12, wherein the first and/or second Fc region comprises the amino acid mutations L234F, L235E, and P331S, wherein the amino acid numbering is according to the EU index as in Kabat.

14. The method according to any one of claims 4 to 13, wherein the Relaxin-2 A chain polypeptide of the heterodimeric fusion comprises the sequence as set forth in SEQ ID NO: 1 or a variant thereof and the Relaxin-2 B chain polypeptide of the heterodimeric fusion comprises the sequence as set forth in SEQ ID NO: 2 or a variant thereof.

15. The method according to claim 14, wherein the Relaxin-2 A chain polypeptide of the heterodimeric fusion comprises the amino acid mutation K9H, K17M or K17I.

16. The method according to any one of claims 5 to 15, wherein both connectors of the heterodimeric fusion have the sequence GGGGSGGGGSGGGGSGGGGGS [SEQ ID NO: 5].

17. A method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) an FcX-con-A fusion polypeptide; and

(ii) an FcY-con-B fusion polypeptide,

wherein:

A is a Relaxin A chain or variant thereof, e.g. a Relaxin-2 A chain or variant thereof;

B is a Relaxin B chain or variant thereof, e.g. Relaxin-2 B chain or variant thereof;

FcY is an Fc region comprising the constant domains CH2 and CH3 of a human IgG1 immunoglobulin and comprises "Fc Hole" amino acid mutations and/or modifications, preferably the amino acid mutations Y349C: T366S:L368A:Y407V;

FcX is an Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising the constant domains CH2 and CH3 of a human IgG1 immunoglobulin and comprises "Fc Knob" amino acid mutations and/or modifications, preferably the amino acid mutations S354C:T366W; and

con is a connector polypeptide, preferably having the sequence GGGGSGGGGSGGGGSGGGGGS [SEQ ID NO: 5],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity.

18. The method according to any one of the preceding claims, wherein the heterodimeric fusion comprises a fusion polypeptide with the amino acid sequence of SEQ ID NO: 11 and a fusion polypeptide with the amino acid sequence of SEQ ID NO: 20.

19. The method according to any one of claims 8 to 18, wherein the heterodimeric fusion further comprises one or more Fabs, optionally wherein the heterodimeric fusion comprises one Fab linked to the N-terminus of the first Fc region and a second Fab linked to the N-terminus of the second Fc region.

20. The method according to any one of claims 8 to 19, wherein the heterodimeric fusion further comprises a second Relaxin A chain polypeptide or variant thereof connected to the N-terminus of the first Fc region and a second Relaxin B chain

polypeptide or variant thereof connected to the N-terminus of the second Fc region, optionally wherein the second Relaxin A chain is connected to the first Fc region via a connector polypeptide and the second Relaxin B chain is connected to the second Fc region via a connector polypeptide.

21. A method of treating a subject with heart failure with pulmonary hypertension, the method comprising administering to the subject an effective amount of a heterodimeric fusion, the heterodimeric fusion comprising:

(i) FcX-B-L-A and FcY, optionally FcY-B-L-A; or

(ii) FcY-B-L-A and FcX, optionally FcX-B-L-A;

wherein:

FcY is an immunoglobulin Fc region with "Fc Hole" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations Y349C:T366S:L368A:Y407V;

FcX is an immunoglobulin Fc region with "Fc Knob" amino acid mutations and/or modifications, preferably comprising a CH3 domain having the amino acid mutations S354C:T366W;

B is a Relaxin B chain or a variant thereof, e.g. a Relaxin 2 B chain or variant thereof;

A is a Relaxin A chain or a variant thereof, e.g. a Relaxin 2 A chain or variant thereof; and

L is a linker polypeptide, preferably having the amino acid sequence GGGSGGGSGG [SEQ ID NO: 60],

wherein the amino acid numbering is according to the EU index as in Kabat, wherein FcX heterodimerises with FcY, and wherein the heterodimeric fusion has Relaxin activity.

22. The method according to claim 21, wherein the Relaxin B chain of the heterodimeric fusion is connected to FcX and/or FcY via a connector, optionally a connector polypeptide having a length of between 6 and 40 amino acids, e.g. a length of 21 amino acids.

23. The method of any one of the preceding claims, wherein the ratio of Relaxin activity of the heterodimeric fusion over the Relaxin activity of a reference Relaxin protein is between about 0.001 and about 10.

24. The method according to any one of the preceding claims wherein the heart failure is heart failure with reduced ejection fraction, heart failure with mid-range ejection fraction or heart failure with preserved ejection fraction.

25. The method according to any one of the preceding claims wherein the subject has a mean Pulmonary Arterial Pressure of about 25 mmHg or greater, a pulmonary artery wedge pressure (PAWP) greater than 15 mmHg and/or a Right Ventricular Systolic Pressure of about 40 mmHg or greater.

26. The method according to any one of the preceding claims wherein the subject has a Pulmonary Vascular Resistance of less than 3.0 wood units.

27. The method according to any one of claims 1-25 wherein the subject has a Pulmonary Vascular Resistance of 3.0 or more wood units.

28. The method according to any one of the preceding claims wherein the subject has been fitted with a blood pressure monitoring device, preferably a pulmonary artery pressure monitoring device.

29. The method according to claim 28 wherein the pulmonary artery pressure monitoring device is a CardioMEMS pressure monitoring device.

30. The method according to any one of the preceding claims wherein the heterodimeric fusion is administered as a pharmaceutical composition comprising the heterodimeric fusion and a pharmaceutically acceptable excipient.

31. The method according to any one of the preceding claims wherein the heterodimeric fusion or pharmaceutical composition is administered to the subject by subcutaneous injection.

32. The method according to any one of the preceding claims wherein the heterodimeric fusion or pharmaceutical composition is administered by self-administration.

33. The method of any one of the preceding claims, wherein administration of the heterodimeric fusion or pharmaceutical composition results in one or more of:

a) reduced PVR;

(b) reduced mPAP;

(c) reduced ePAD;

(d) increased stroke volume (SV) of the heart;

(e) decreased systemic vascular resistance (SVR) and/or increase estimated glomerular filtration rate (eGFR);

(f) increased ejection fraction; and/or

(g) increased cardiac output;

as compared to baseline levels pre-administration.

Fig 1

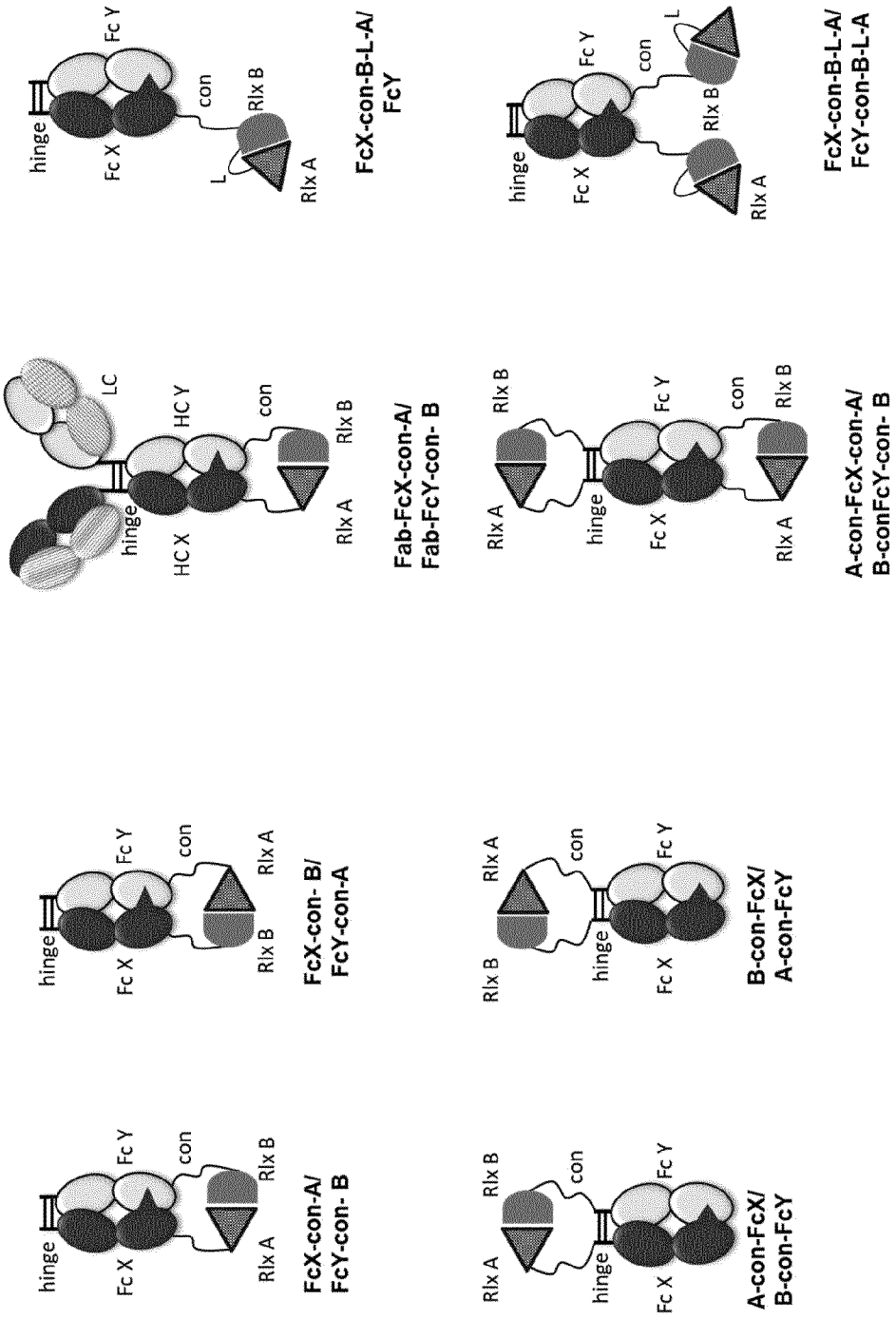

hinge
Fc X
Fc Y
con
L
Rlx A
Rlx B
FcX-con-B-L-A/ FcY
FcX-con-B-L-A/ FcY-con-B-L-A
LC
HC X
HC Y
Fab-FcX-con-A/ Fab-FcY-con- B
A-con-FcX-con-A/ B-conFcY-con- B
FcX-con- B/ FcY-con-A
B-con-FcX/ A-con-FcY
FcX-con-A/ FcY-con- B
A-con-FcX/ B-con-FcY

Fig 2

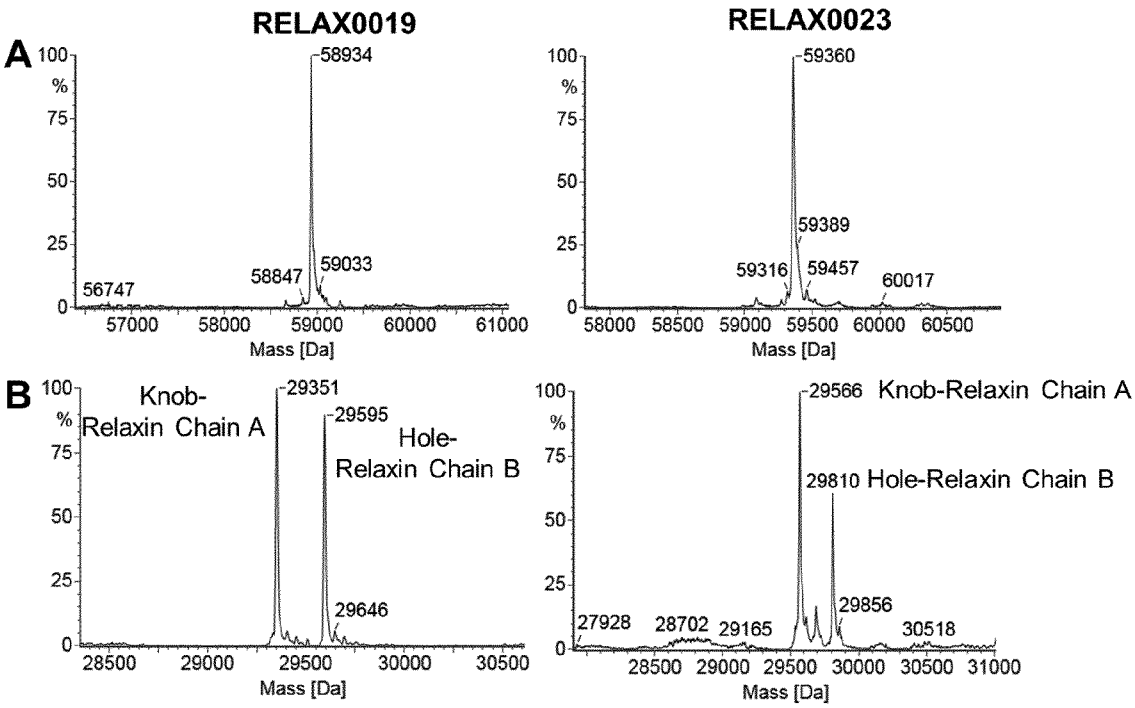
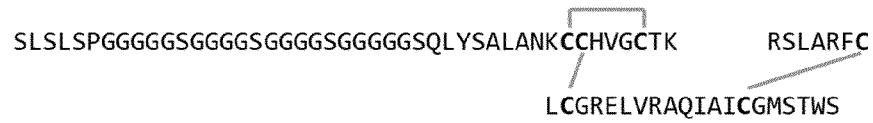

RELAX0019
RELAX0023
A
B
100
%
75
50
25
0
58934
56747
58847
59033
59360
59389
59316
59457
60017
57000
58000
59000
60000
61000
58000
58500
59000
59500
60000
60500
Mass [Da]
Knob-
Relaxin Chain A
29351
29595
Hole-
Relaxin Chain B
29646
29566 Knob-Relaxin Chain A
29810 Hole-Relaxin Chain B
27928
28702
29165
29856
30518
28500
29000
29500
30000
30500
31000

Fig 3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LCGRELVRAQIAICGMSTWS

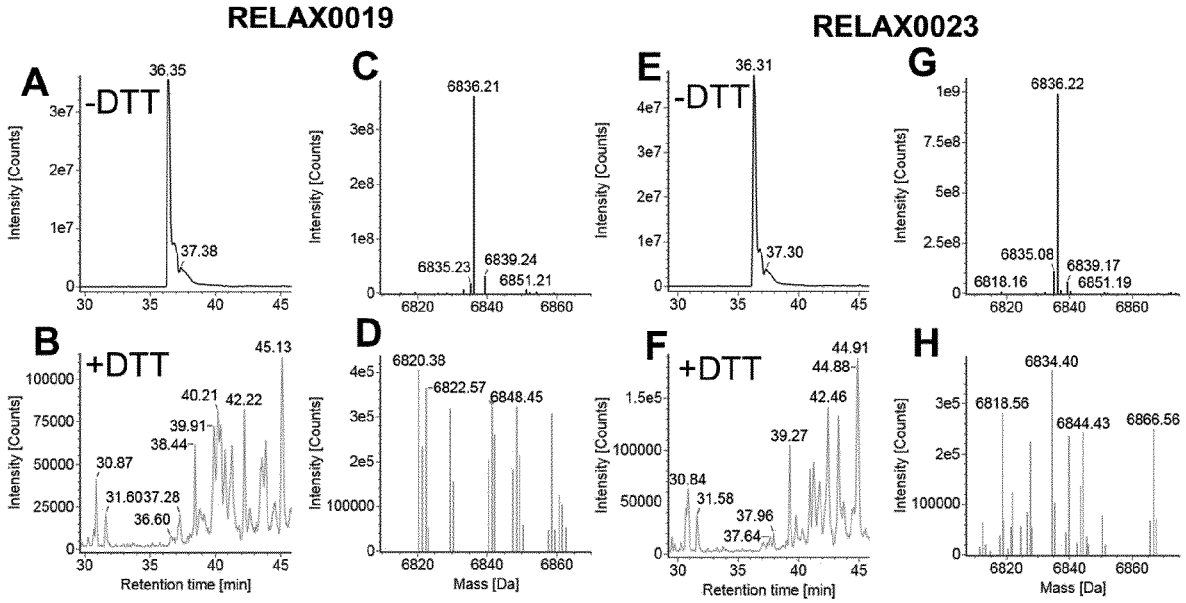

RELAX0019
RELAX0023
A
-DTT
36.35
37.38
Intensity [Counts]
B
+DTT
45.13
40.21
42.22
39.91
38.44
30.87
31.60
37.28
36.60
Retention time [min]
C
6836.21
6835.23
6839.24
6851.21
D
6820.38
6822.57
6848.45
Mass [Da]
E
-DTT
36.31
37.30
F
+DTT
44.91
44.88
42.46
39.27
30.84
31.58
37.96
37.64
G
6836.22
6835.08
6839.17
6818.16
6851.19
H
6834.40
6818.56
6844.43
6866.56

Fig 4

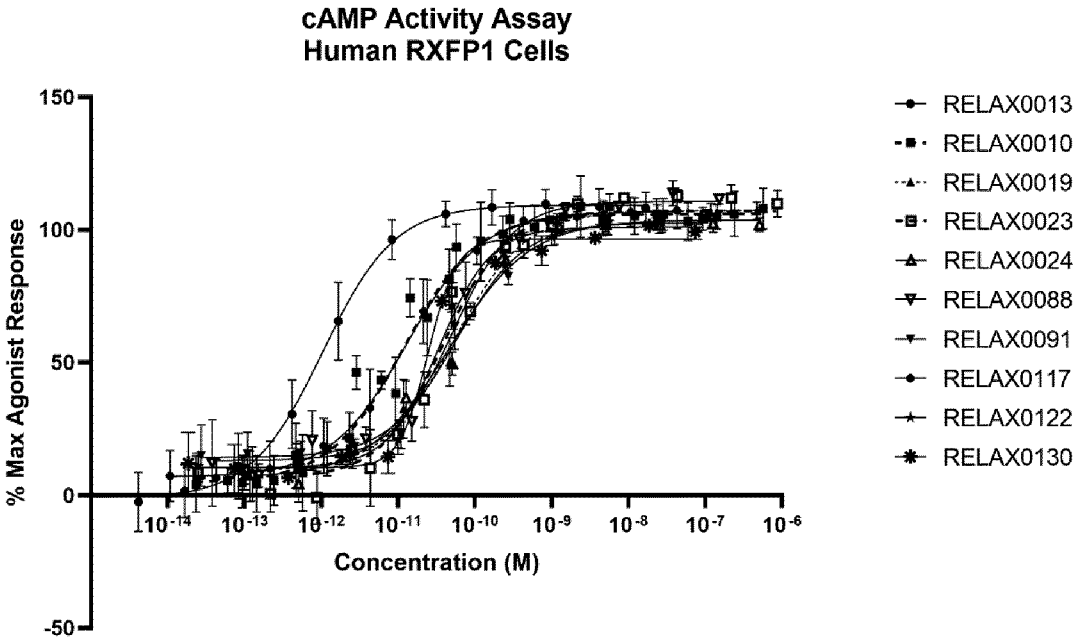

cAMP Activity Assay
Human RXFP1 Cells
% Max Agonist Response
150
100
50
0
-50
10^{-14}
10^{-13}
10^{-12}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
Concentration (M)
RELAX0013
RELAX0010
RELAX0019
RELAX0023
RELAX0024
RELAX0088
RELAX0091
RELAX0117
RELAX0122
RELAX0130

Fig 5

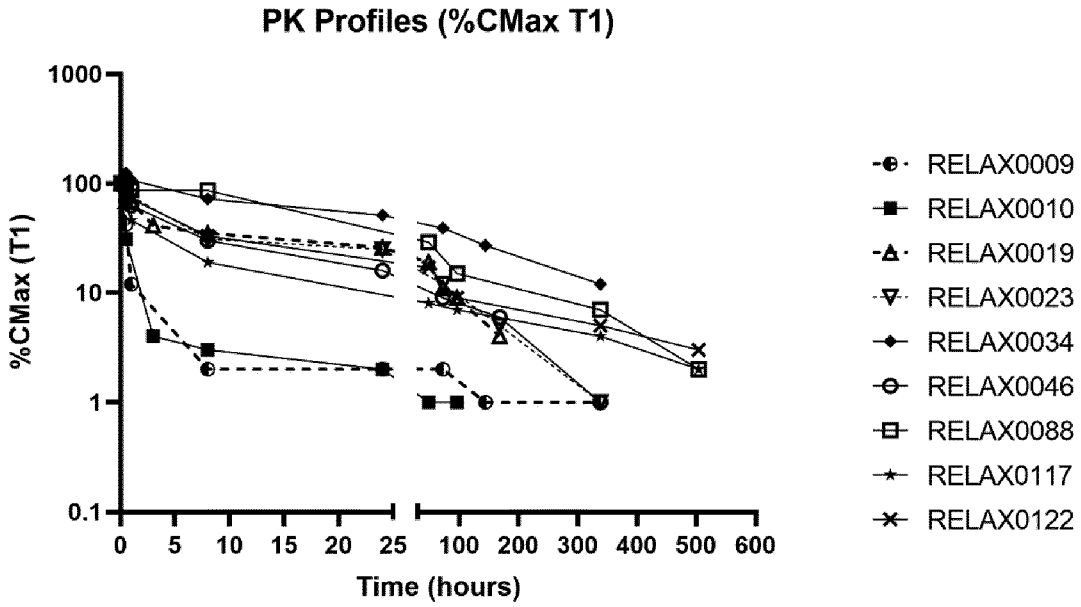

PK Profiles (%CMax T1)
%CMax (T1)
1000
100
10
1
0.1
0 5 10 15 20 25 100 200 300 400 500 600
Time (hours)
RELAX0009
RELAX0010
RELAX0019
RELAX0023
RELAX0034
RELAX0046
RELAX0088
RELAX0117
RELAX0122

Fig 6

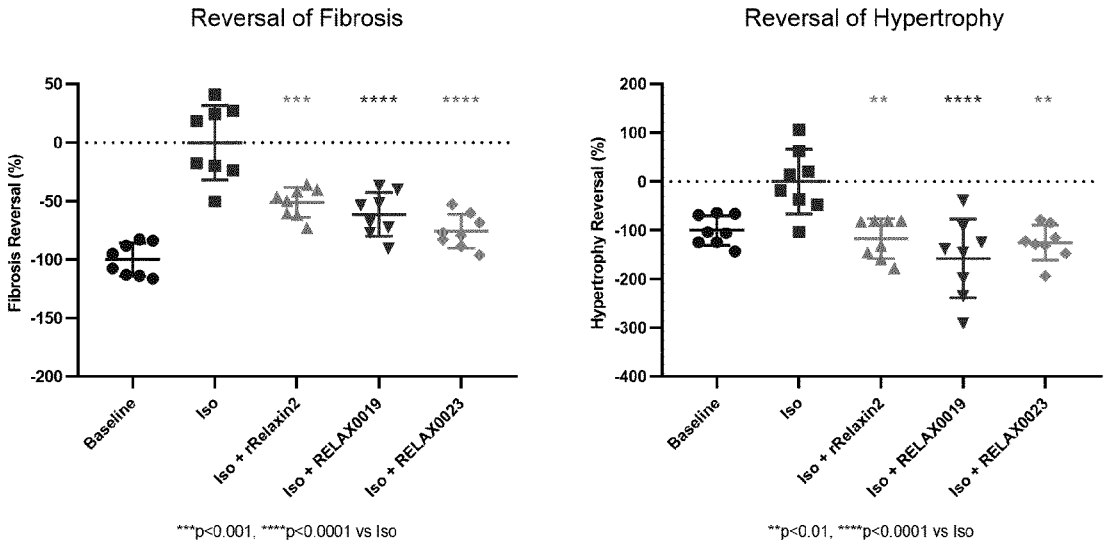

Reversal of Fibrosis
Fibrosis Reversal (%)
50
0
-50
-100
-150
-200
***
****
****
Baseline
Iso
Iso + rRelaxin2
Iso + RELAX0019
Iso + RELAX0023
***p<0.001, ****p<0.0001 vs Iso
Reversal of Hypertrophy
Hypertrophy Reversal (%)
200
100
0
-100
-200
-300
-400
**
****
**
Baseline
Iso
Iso + rRelaxin2
Iso + RELAX0019
Iso + RELAX0023
**p<0.01, ****p<0.0001 vs Iso

Fig 7

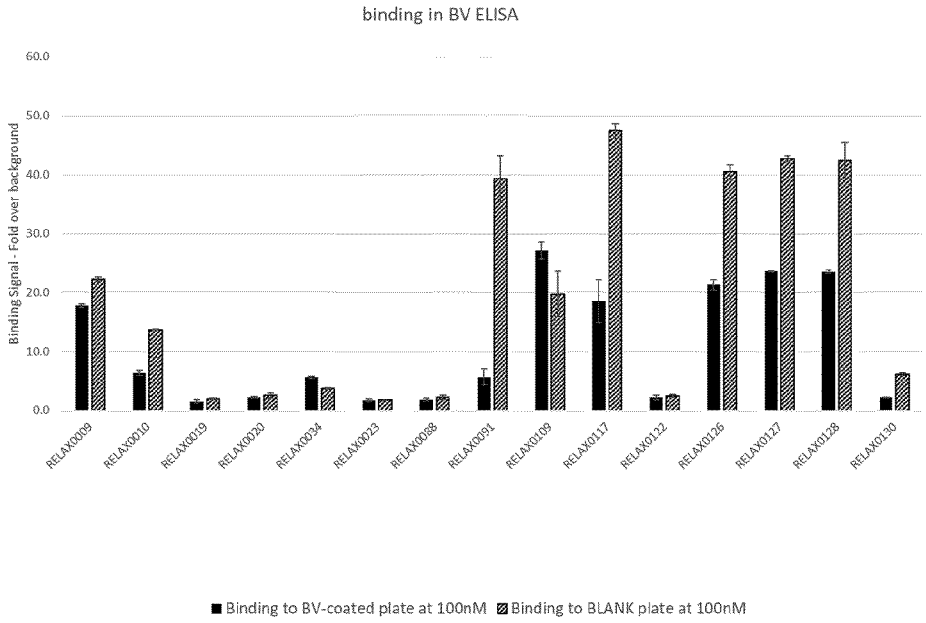

binding in BV ELISA
Binding Signal - Fold over background
60.0
50.0
40.0
30.0
20.0
10.0
0.0
RELAX0009
RELAX0010
RELAX0019
RELAX0020
RELAX0034
RELAX0023
RELAX0088
RELAX0091
RELAX0109
RELAX0117
RELAX0122
RELAX0126
RELAX0127
RELAX0128
RELAX0130
Binding to BV-coated plate at 100nM
Binding to BLANK plate at 100nM

Fig 8

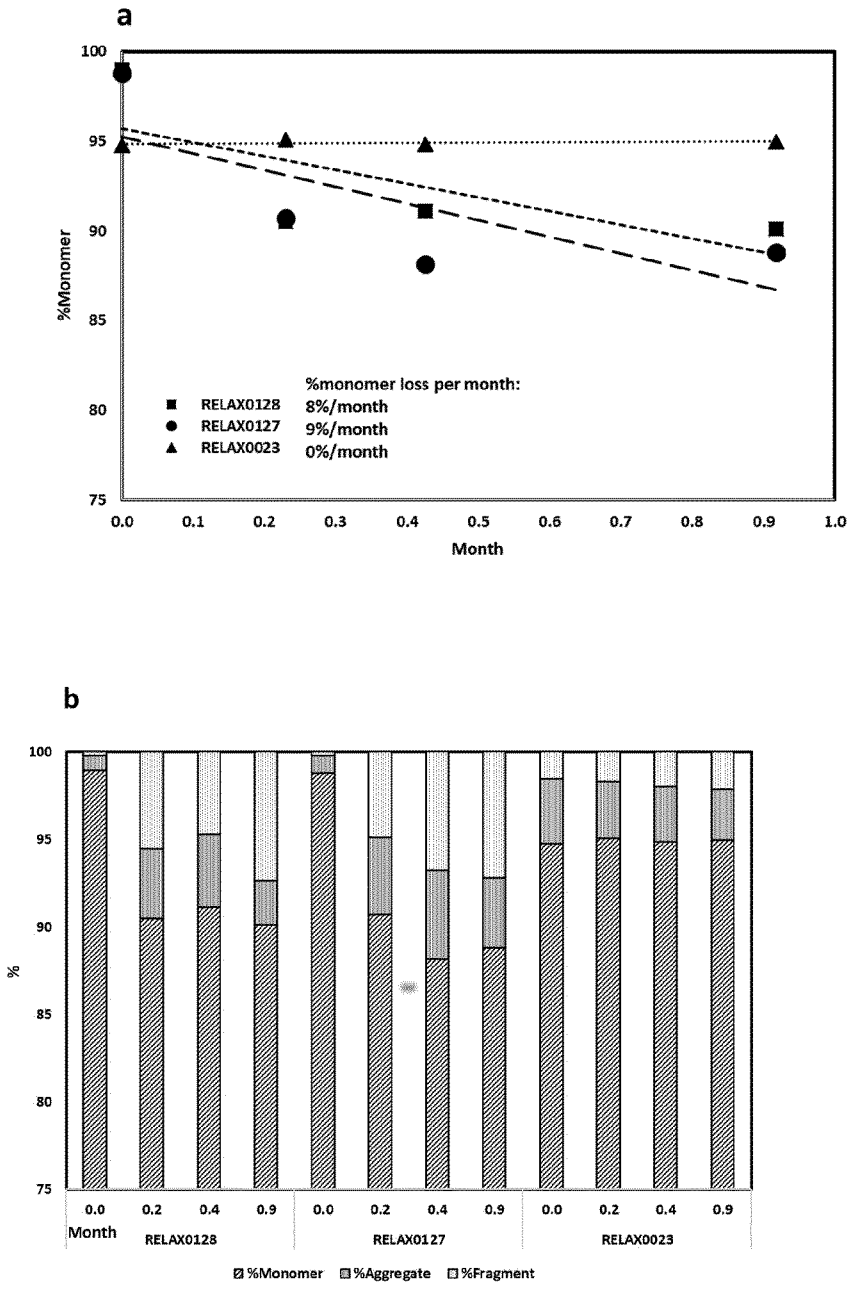
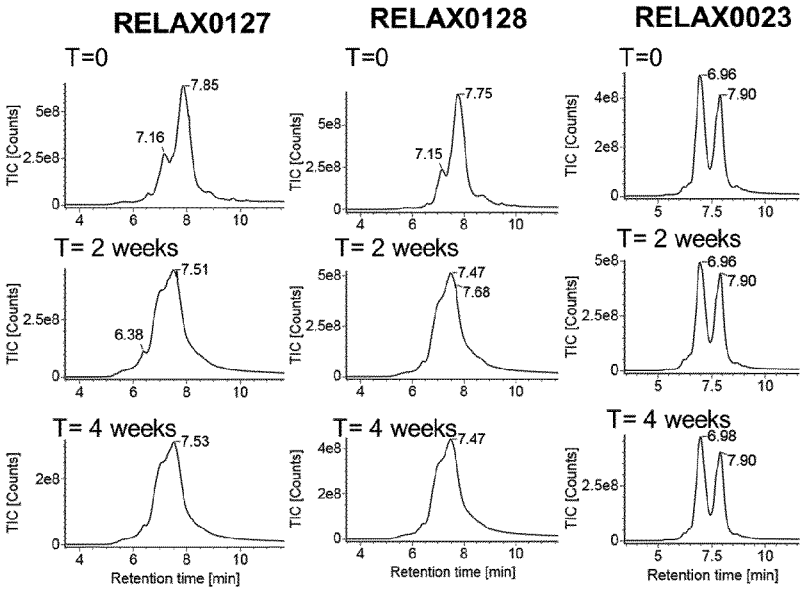
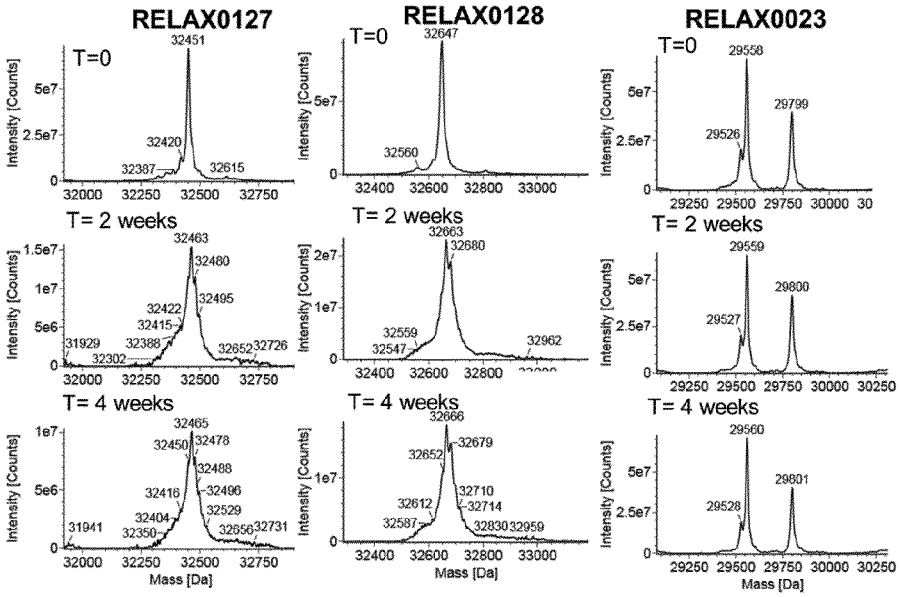

a
100
95
90
85
80
75
%Monomer
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Month
%monomer loss per month:
RELAX0128 8%/month
RELAX0127 9%/month
RELAX0023 0%/month
b
100
95
90
85
80
75
%
0.0
0.2
0.4
0.9
Month
RELAX0128
RELAX0127
RELAX0023
%Monomer
%Aggregate
%Fragment

Fig 9

A


RELAX0127
RELAX0128
RELAX0023
T=0
T= 2 weeks
T= 4 weeks
TIC [Counts]
Retention time [min]
7.85
7.16
7.75
7.15
6.96
7.90
7.51
6.38
7.47
7.68
7.53
6.98

B


RELAX0127
RELAX0128
RELAX0023
T=0
T= 2 weeks
T= 4 weeks
Intensity [Counts]
Mass [Da]
32451
32420
32387
32615
32647
32560
29558
29799
29526
32463
32480
32422
32495
32415
31929
32388
32302
32652
32726
32663
32680
32559
32547
32962
29559
29800
29527
32465
32450
32478
32488
32418
32496
32529
32404
31941
32350
32656
32731
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32679
32652
32710
32612
32714
32587
32830
32959
29560
29801
29528

Fig 10

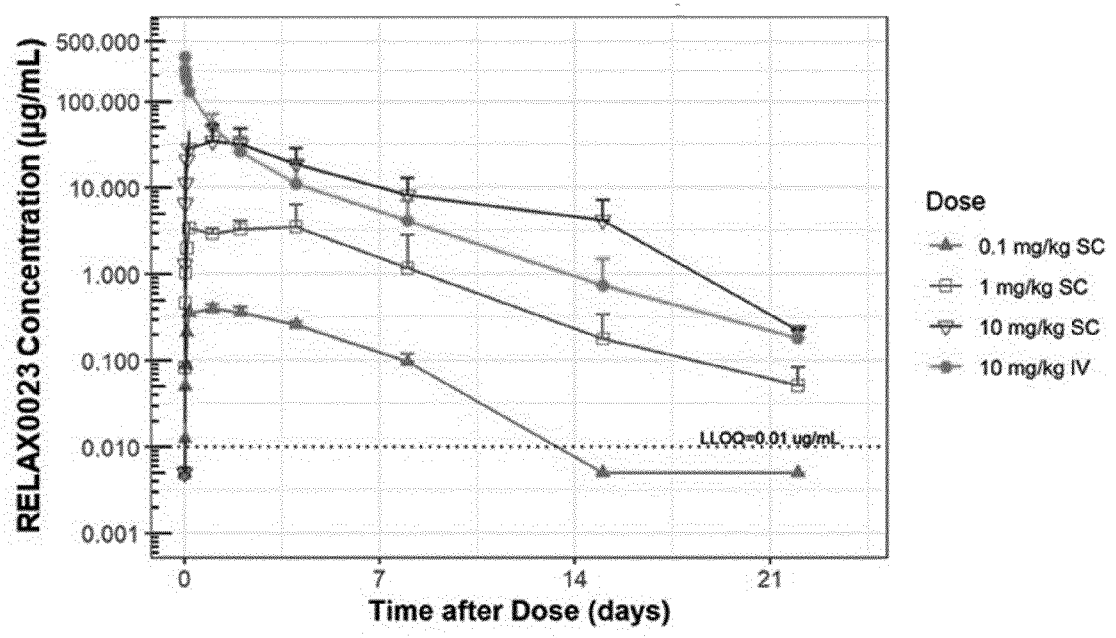
RELAX0023 Concentration (µg/mL)
500.000
100.000
10.000
1.000
0.100
0.010
0.001
LLOQ=0.01 ug/mL
0
7
14
21
Time after Dose (days)
Dose
0.1 mg/kg SC
1 mg/kg SC
10 mg/kg SC
10 mg/kg IV

Fig 11

Relaxin A

CAGCTCTACTCAGCGCTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTC
TGC

Relaxin B

AGCTGGATGGAAGAAGTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGC
ATGAGCACCTGGAGC

FcH01

GACAAGACCCATACATGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
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GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAAAGCTTGTCCCTGAGCCCCGGC

FcK01

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TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
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RELAX0010

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TGGCTGAACGGCAAAGAATATAAATGCAAAGTGAGCAACAAAGCGCTGCCGGCGCCGATTGAAAAAACC
ATTAGCAAAGCGAAAGGCCAGCCGCGCGAACCGCAGGTGTATACCCTGCCGCCGAGCCGCGATGAACTG
ACCAAAAACCAGGTGAGCCTGACCTGCCTGGTGAAAGGCTTTTATCCGAGCGATATTGCGGT

Fig 11 Continued

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CAGCGTGATGCATGAAGCGCTGCATAACCATTATACCCAGAAAAGCCTGAGCCTGAGCCCGGGCAAAGG
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CGGCAGCGGCGGCGGCGGCAGCAGCTGGATGGAAGAAGTGATTAAACTGTGTGGCCGCGAACTGGTGCG
CGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx011

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CTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx011b

GGAGGAGCGGGTGGAGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
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GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
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Rlx011DD

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Fig 11 Continued

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CCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTG
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CGCTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx012

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GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx012b

GGAGGAGCGGGTGGAGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
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ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCAGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx012DD

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAA

Fig 11 Continued

AGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAG
AGATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGG

AATGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCT
CATTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCT
CCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTG
GTGGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAG
AAGTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGA
GC

Rlx013

GGAGGAGCGGGTGGAGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAGAA
GTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx013b

GGAGGAGCGGGTGGAGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCAGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAGAA
GTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx013DD

GACAAGACCCAYACMTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACG

Fig 11 Continued

TGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGA
CCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGG
ACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGA
CCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGA

TGACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAAT
GGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCAT
TCTTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCG
TGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTG
GAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGC
TCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx014

GGAGGAGCGGGTGGAGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAGAA
GTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx014b

GGAGGAGCGGGTGGAGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCAGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAGAA
GTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Fig 11 Continued

Rlx014DD

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAGAAGTG
ATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx020

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGTGGAGGGGGCGGATCCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGT
CATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx021

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGTGGAGGGGGCGGATCCAGCTGGATGGAAGAAGTGATTAAACTGTGTGGC
CGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Fig 11 Continued

Rlx022

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGAGGTGGC
TCTGGTGGAGGGGGCGGATCCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGTCATGTGGGATGCACA
AAGCGGTCTCTCGCCAGATTCTGC

Rlx023

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGAGGTGGC
TCTGGTGGAGGGGGCGGATCCAGCTGGATGGAAGAAGTGATTAAACTGTGTGGCCGCGAACTGGTGCGC
GCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx024

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGTGGAGGGGGC
GGATCCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCC
AGATTCTGC

Fig 11 Continued

Rlx025

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGTGGAGGGGGC
GGATCCAGCTGGATGGAAGAAGTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATT
TGCGGCATGAGCACCTGGAGC

Rlx026

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGCACCTGCTCCC
GCACCAGCCCCTGCTCCCGCACCAGCCCCTGCTCCCGCACCAGCCGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx027

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGCACCTGCTCCC
GCACCAGCCCCTGCTCCCGCACCAGCCCCTGCTCCCGCACCAGCCGGATCCAGCTGGATGGAAGAAGTG
ATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Fig 11 Continued

Rlx028

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGCAGCTCCTGCT
CCCGCACCAGCCCCTGCTCCCGCACCAGCCGGATCCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGT
CATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx029

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGCAGCTCCTGCT
CCCGCACCAGCCCCTGCTCCCGCACCAGCCGGATCCAGCTGGATGGAAGAAGTGATTAAACTGTGTGGC
CGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx030

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGCACCAGCCCCT
GCTCCCGCACCAGCCGGATCCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGTCATGTGGGATGCACA
AAGCGGTCTCTCGCCAGATTCTGC

Fig 11 Continued

Rlx031

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGCACCAGCCCCT
GCTCCCGCACCAGCCGGATCCAGCTGGATGGAAGAAGTGATTAAACTGTGTGGCCGCGAACTGGTGCGC
GCGCAGATTGCGATTTGCGGCATGAGCACCTGGAGC

Rlx041E

GACAAGACCCACACCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATGAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx041H

GACAAGACCCACACCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATCACTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Fig 11 Continued

Rlx041L

GACAAGACCCACACCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC

GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATTTGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx041M

GACAAGACCCACACCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATATGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx044E

GACAAGACCCACACCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGGAGTCTCTCGCCAGATTCTGC

Fig 11 Continued

Rlx044H

GACAAGACCCACACCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC

GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCA
TTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCC
GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGT
GGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCG
CTCGCTAATAAGTGTTGTCATGTGGGATGCACAAAGCACTCTCTCGCCAGATTCTGC

Rlx051A

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCGCCTTCTGC

Rlx051I

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCATCTTCTGC

Fig 11 Continued

Rlx051M

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCATGTTCTGC

Rlx051Q

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCCAGTTCTGC

Rlx051S

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCTCCTTCTGC

Fig 11 Continued

Rlx051Y

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCTACTTCTGC

Rlx052E

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGAGAGTGC

Rlx052I

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
GCTAATAAGTGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGAATCTGC

Fig 11 Continued

Rlx055

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG

TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCTCCTGGATGGAGGAGGTT
ATCAAGCTGTGTGGACGCGAACTGGTGCGCGCTCAGATCGCGATATGCGGGATGTCCACATGGTCAGGC
GGCGGCAGCGGCGGCGGCAGCGGCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGTCATGTGGGATGC
ACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx056

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
TTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTG
ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCTCCTGGATGGAGGAGGTT
ATCAAGCTGTGTGGACGCGAACTGGTGCGCGCTCAGATCGCGATATGCGGGATGTCCACATGGTCAGGC
GGCGGCAGCGGCGGCGGCAGCGGCCAGCTCTACTCAGCGCTCGCTAATAAGTGTTGTCATGTGGGATGC
ACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx061H

TCCTGGATGGAAGAAGTGATCAAGCTCTGCGGCAGAGAACTCGTGCGGGCCCAGATCGCTATCTGCGGC
ATGTCTACTTGGAGCGCGGCCGCGGGTGGAGGTGGATCCGGAGGAGGTGGAAGCGGAGGAGGTGGAAGC
GGAGGAGGTGGAAGCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAG
ACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAG
ATGACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAA
TGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTC

Fig 11 Continued

CGACGGCTCATTCTTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTT
CTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTGTCCCTGAGCCCCGG
C

Rlx062K

CAGCTGTACTCTGCCCTGGCCAACAAGTGTTGCCACGTGGGCTGCACCAAGAGATCCCTGGCCAGATTC
TGTGCGGCCGCGGGTGGAGGTGGATCCGGAGGAGGTGGAAGCGGAGGAGGTGGAAGCGGAGGAGGTGGA
AGCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTCCCCCCAAAG
CCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAG
GACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGA
GAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAAC
GGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACCATCTCCAAG
GCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATGACCAAGAAC
CAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGGGAGTCCAAC
GGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTAC
TCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAG
GCCCTGCACAACCACTACACCCAGAAGTCTCTGTCCCTGAGCCCCGGC

Rlx076

CAGCTGTACTCTGCCCTGGCCAACAAGTGTTGCCACGTGGGCTGCACCAAGAGATCCCTGGCCAGATTC
TGTGCGGCCGCGGGTGGAGGTGGATCCGGAGGAGGTGGAAGCGGAGGAGGTGGAAGCGGAGGAGGTGGA
AGCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTCCCCCCAAAG
CCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAG
GACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAAGCCCAGA
GAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAAC
GGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACCATCTCCAAG
GCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCCTGCCGGGAAGAGATGACCAAGAAC
CAGGTGTCCCTGTGGTGTCTGGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGGGAGTCCAAC
GGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTAC
TCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAG
GCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGAAGCGGAGGA
GGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTCGCTAATAAG
TGTTGTCATGTGGGATGCACAAAGCGGTCTCTCGCCAGATTCTGC

Rlx077

TCCTGGATGGAAGAAGTGATCAAGCTCTGCGGCAGAGAACTCGTGCGGGCCCAGATCGCTATCTGCGGC
ATGTCTACTTGGAGCGCGGCCGCGGGTGGAGGTGGATCCGGAGGAGGTGGAAGCGGAGGAGGTGGAAGC
GGAGGAGGTGGAAGCGCTTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTG
TTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGAC
GTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAG
ACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG
GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCAT

Fig 11 Continued

CGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCG
GGAAGAGATGACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGC
TGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGA
CGGCTCATTCTTCCTGGTGTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTC
CTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGG

AGGTGGTGGAAGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGAT
GGAAGAAGTGATTAAACTGTGTGGCCGCGAACTGGTGCGCGCGCAGATTGCGATTTGCGGCATGAGCAC
CTGGAGC

Rlx014DDdel2aa

GACAAGACCCACACCTGTCCTCCATGCCCGGCGCCTGAGTTCGAGGGCGGACCCTCCGTGTTCCTGTTC
CCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTG
TCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACC
AAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGAC
TGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACC
ATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTGCACACTGCCCCCCAGCCGGGAAGAGATG
ACCAAGAACCAGGTGTCCCTGTCCTGTGCCGTGAAAGGCTTCTACCCCTCCGATATCGCTGTGGAATGG
GAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTC
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ATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCTCTAAGCTTGAGCCCCGGCGGAGGTGGTGGA
AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCAGCTGGATGGAAGAAGTG
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Rlx014DDdel3aa

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R347Rlx011DD

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Fig 11 Continued

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R347Rlx014DD

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Fig 11 Continued

R347 L

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RELAX0126

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RELAX0127

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Fig 11 Continued

RELAX0128

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RELAX0009

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TGCAAGGTCTCCAACAAGGCCCTGCCCGCCTCCATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCC
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Rlx014d

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Fig 11 Continued

Rlx042R

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Rlx052A

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AGCGGAGGAGGTGGCTCTGGAGGGGGTGGAAGCGGAGGTGGAGGTGGATCCCAGCTCTACTCAGCGCTC
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Fig 12

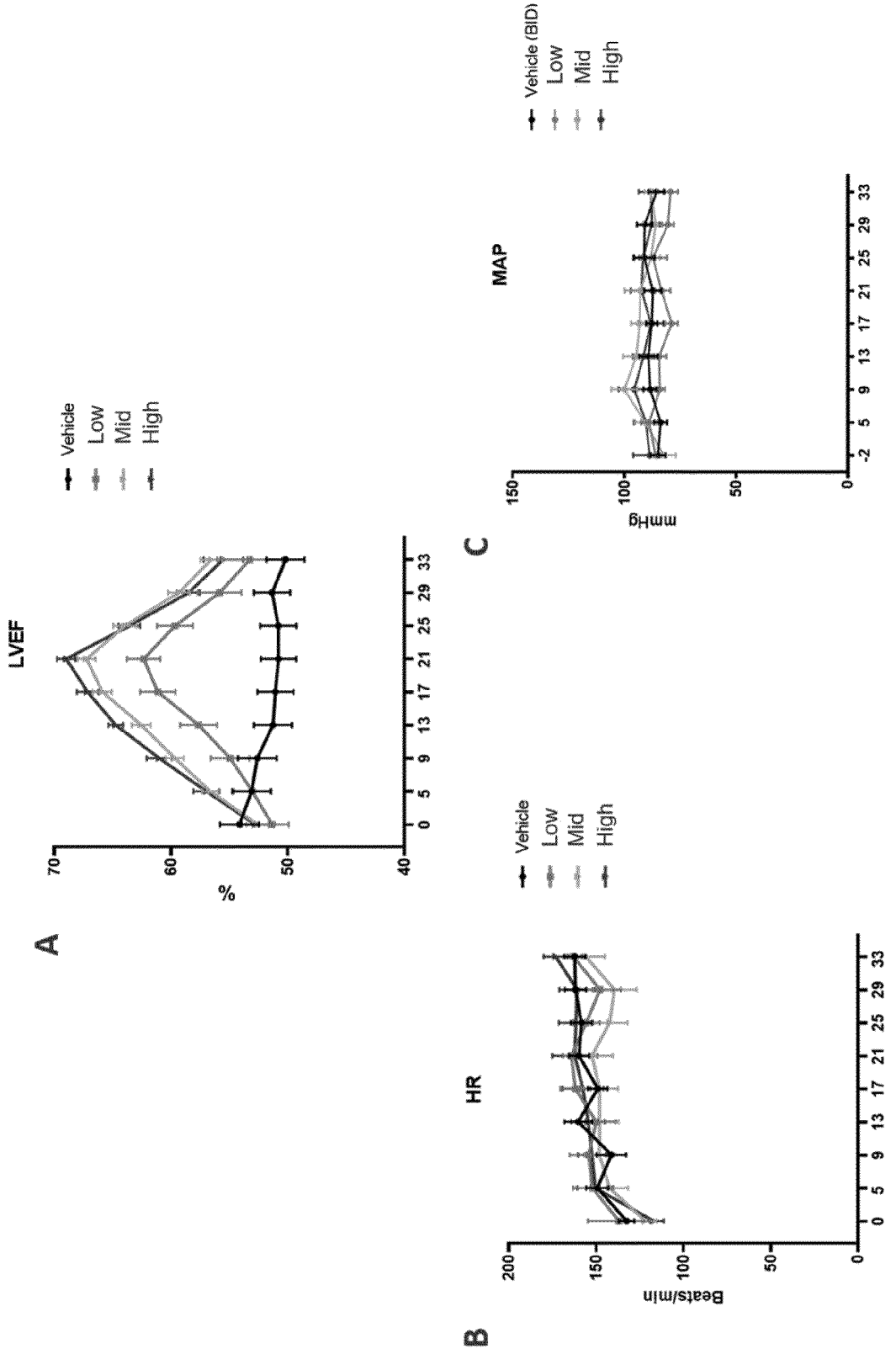

A
LVEF
%
70
60
50
40
0 5 9 13 17 21 25 29 33
Vehicle
Low
Mid
High
B
HR
Beats/min
200
150
100
50
0
0 5 9 13 17 21 25 29 33
Vehicle
Low
Mid
High
C
MAP
mmHg
150
100
50
0
-2 5 9 13 17 21 25 29 33
Vehicle (BID)
Low
Mid
High

Fig 13A

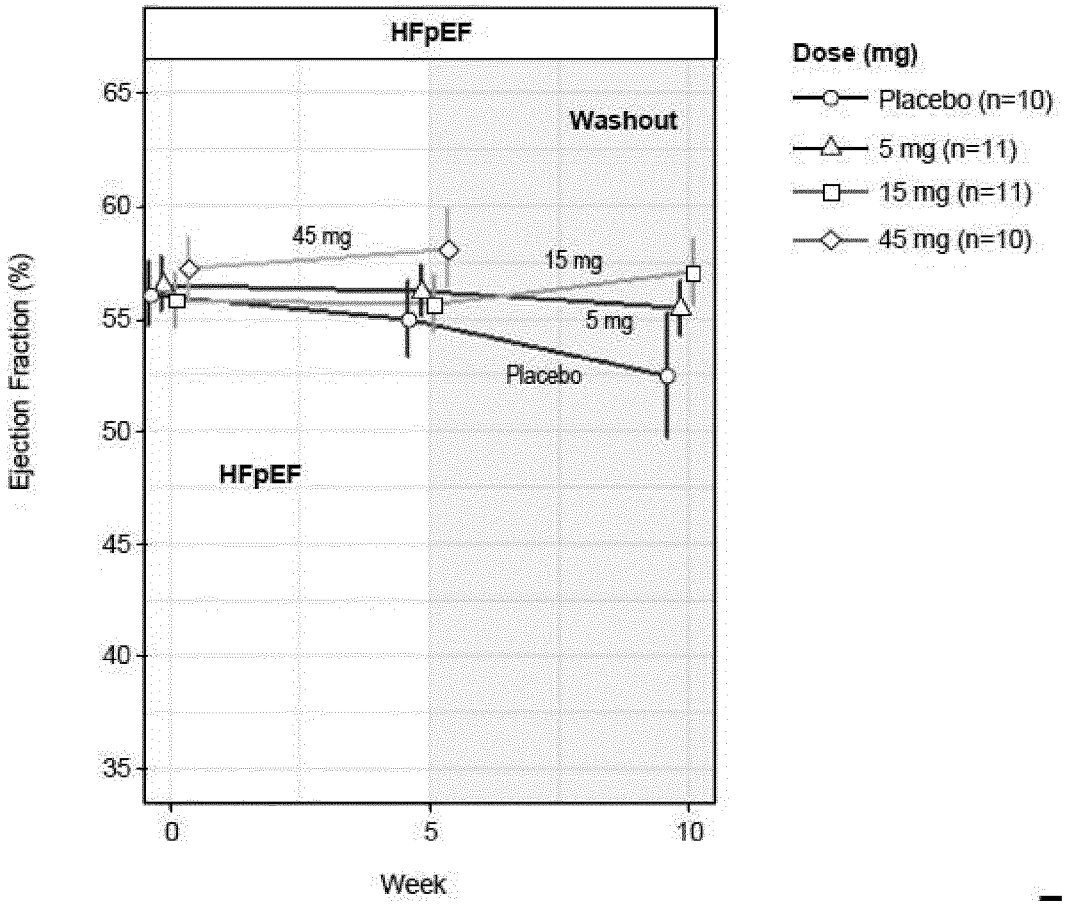

HFpEF
Washout
45 mg
15 mg
5 mg
Placebo
HFpEF
Ejection Fraction (%)
65
60
55
50
45
40
35
0
5
10
Week
Dose (mg)
Placebo (n=10)
5 mg (n=11)
15 mg (n=11)
45 mg (n=10)

Fig 13B

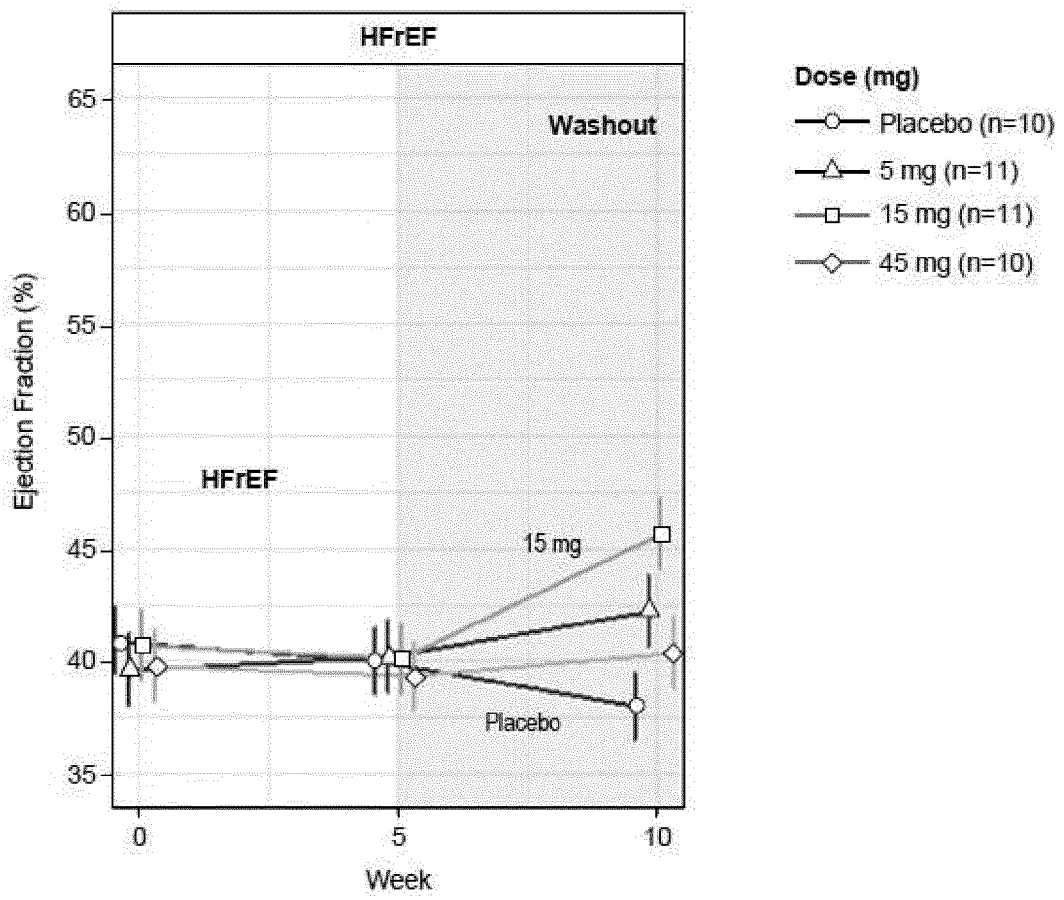

HFrEF
Washout
HFrEF
15 mg
Placebo
Ejection Fraction (%)
65
60
55
50
45
40
35
0
5
10
Week
Dose (mg)
Placebo (n=10)
5 mg (n=11)
15 mg (n=11)
45 mg (n=10)

Fig 13C

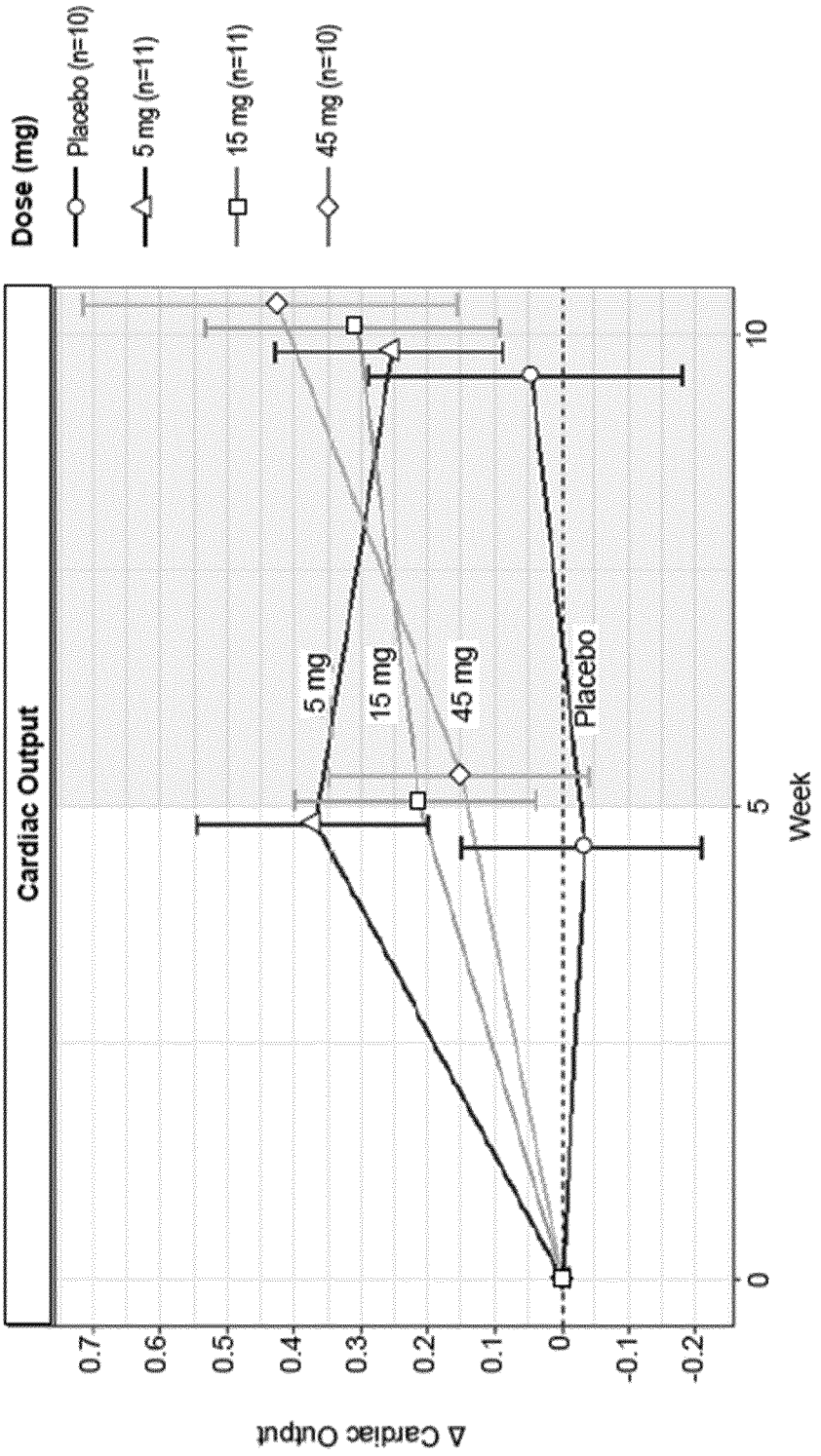

Dose (mg)
Placebo (n=10)
5 mg (n=11)
15 mg (n=11)
45 mg (n=10)
Cardiac Output
5 mg
15 mg
45 mg
Placebo
Week
Δ Cardiac Output
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0
5
10

Fig 13D

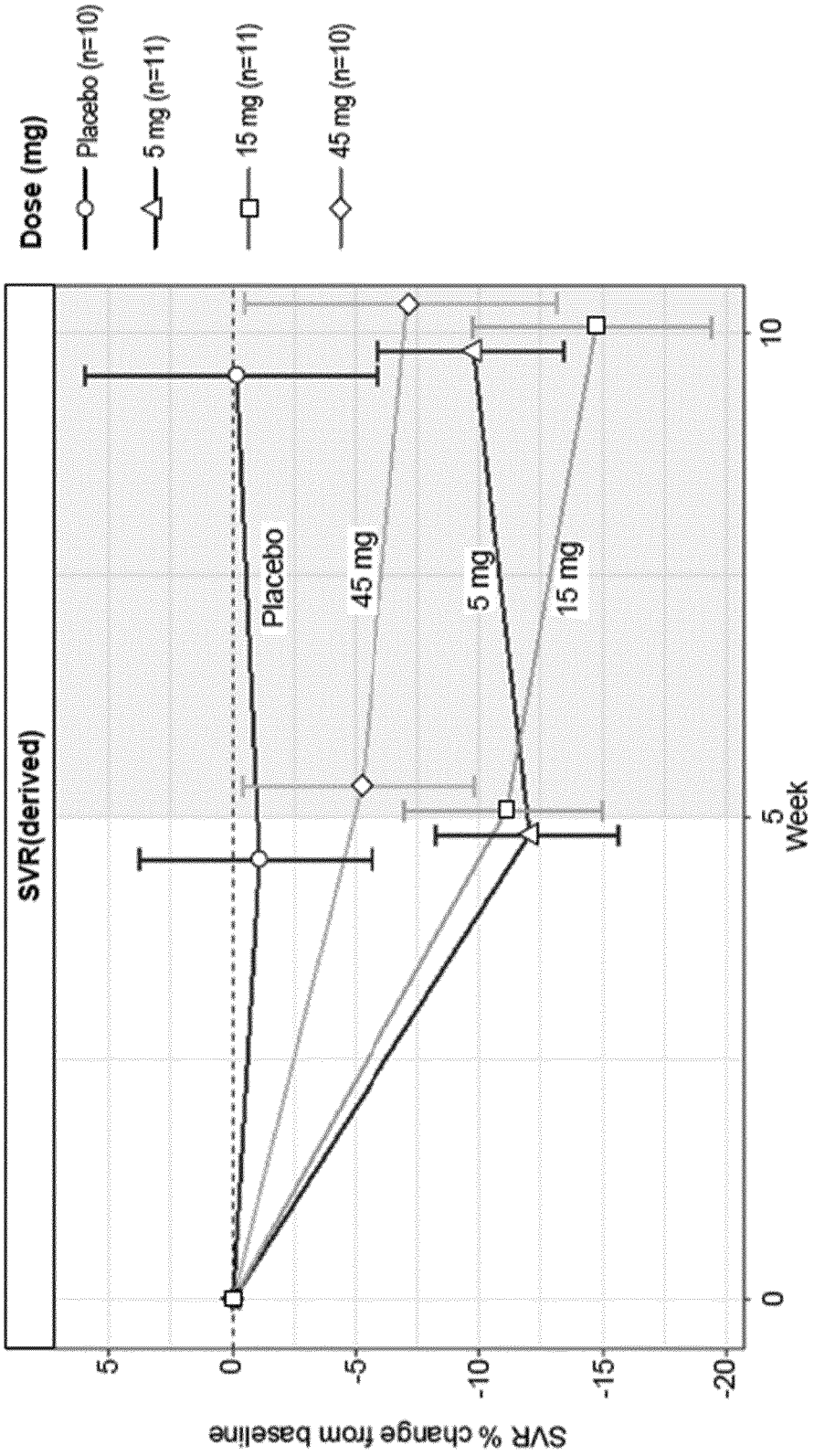

Dose (mg)
Placebo (n=10)
5 mg (n=11)
15 mg (n=11)
45 mg (n=10)
SVR(derived)
Placebo
45 mg
5 mg
15 mg
SVR % change from baseline
5
0
-5
-10
-15
-20
0
5
10
Week

Fig 13E

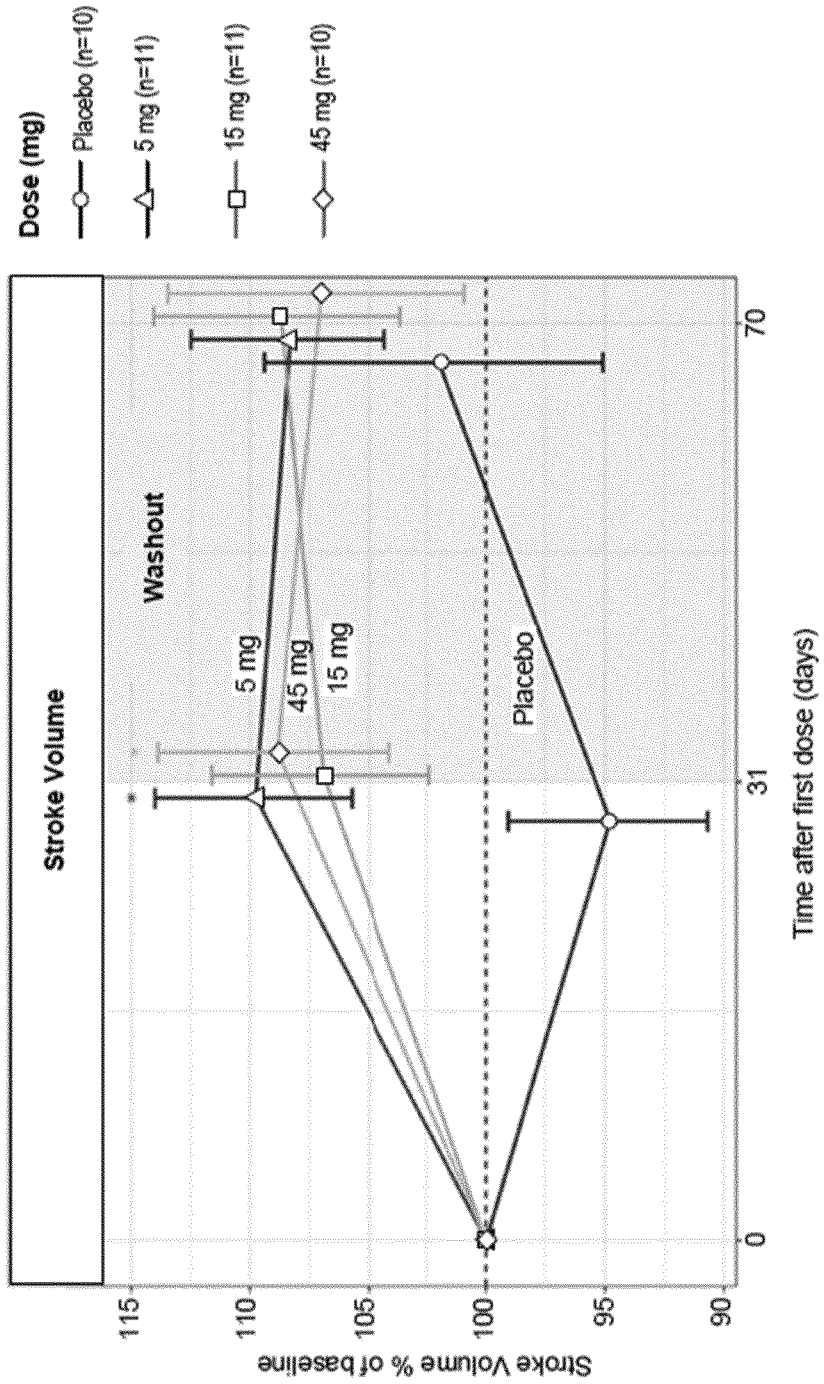
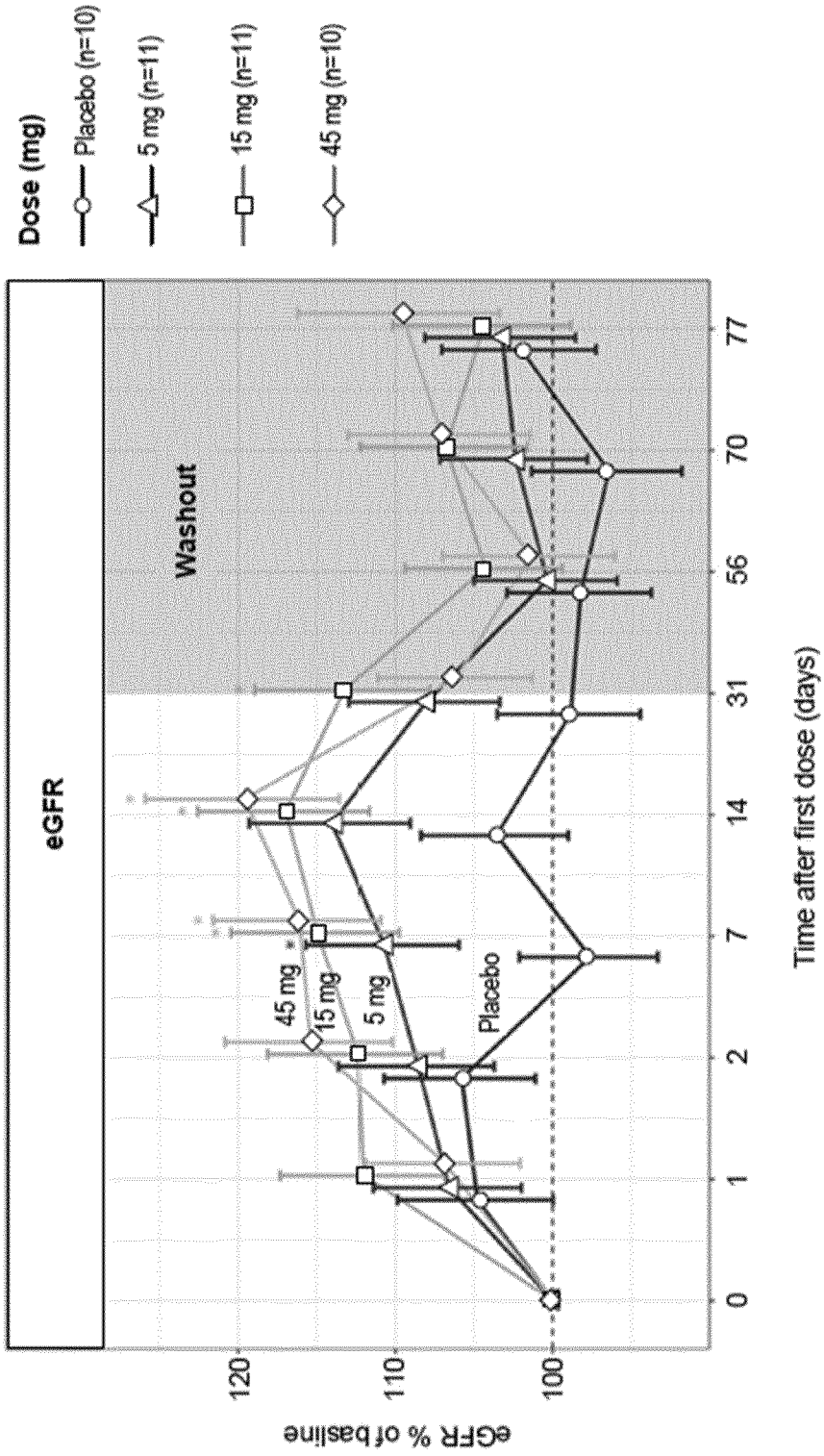

Dose (mg)
Placebo (n=10)
5 mg (n=11)
15 mg (n=11)
45 mg (n=10)
Stroke Volume
Washout
5 mg
45 mg
15 mg
Placebo
Stroke Volume % of baseline
115
110
105
100
95
90
0
31
70
Time after first dose (days)

Fig 13F


Dose (mg)
Placebo (n=10)
5 mg (n=11)
15 mg (n=11)
45 mg (n=10)
eGFR
Washout
45 mg
15 mg
5 mg
Placebo
eGFR % of baseline
120
110
100
0
1
2
7
14
31
56
70
77
Time after first dose (days)