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(54) **Title:** ENGINEERED NRG-1 VARIANTS WITH IMPROVED SELECTIVITY TOWARD ERBB4 BUT NOT AGAINST ERBB3

(57) **Abstract:** The present invention relates to engineered neuregulin-1 variants that selectively activate ErbB4 receptors but do not activate ErbB3 receptors. The invention also provides methods for using such variants to treat heart failure.

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ENGINEERED NRG-1 VARIANTS WITH IMPROVED SELECTIVITY TOWARD ErbB4 BUT NOT AGAINST ErbB3

FIELD OF THE INVENTION

[0001] The present invention relates to engineered neuregulin-1 variants that selectively activate ErbB4 receptors but do not activate, or weakly activate, ErbB3 receptors. The invention also provides methods for using such neuregulin variants in the treatment of heart failure.

SEQUENCE LISTING

[0002] This application contains a sequence listing, as a separate part of the disclosure, in computer-readable form (Filename:A-2828-WO-PCT Seq List_ST25.txt, created June 7, 2022, which is 334 KB in size), and which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] The epidermal growth factor receptor family, which comprises four members EGFR, ErbB2, ErbB3 and ErbB4, has been demonstrated to play an important role in multiple cellular functions, including cell growth, differentiation and survival. They are protein tyrosine kinase receptors, consisting of an extracellular ligand-binding domain, transmembrane domain and cytoplasmic tyrosine kinase domain. Multiple receptor ligands have been identified which mediate receptor homo- or hetero-dimerization upon binding. The specific receptor association results in different patterns of phosphorylation, complex signaling cascades and multiple biological functions, including cellular proliferation, prevention of apoptosis and promotion of tumor cell mobility, adhesion and invasion. Exemplary cells that express ErbB receptors include glial cells, glioblastoma cells, Schwann cells, hepatocytes, epithelial cells, and muscle cells. Glial cells are derived from the central nervous system and include oligodendrocytes and astrocytes. Muscle cells expressing ErbB receptors include muscle cell precursors (myoblasts) as well as the more specialized skeletal, cardiac, and smooth muscle cells.

[0004] Neuregulin, also known as heregulin, glial growth factor (GGF) and new differentiation factor (NDF), is an important growth factor, particularly for the heart and nervous system. Over 15 distinct isoforms of neuregulin -1 (NRG-1) have been identified and divided into two groups, known as alpha- and beta- types, on the basis of differences in the sequence of their essential EGF-like domains. Neuregulin-1 is a ligand of ErbB3 and ErbB4 receptors. It has been shown that the EGF-like domains of neuregulin-1, ranging in size from 50 to 64-amino acids, are sufficient to bind to and activate these receptors. See, e.g., Jones *et al.*, 1999, FEBS Lett. 26:447:227-231. Previous studies have shown that neuregulin-1β (NRG-1β) can bind directly to ErbB3 and ErbB4 with high affinity. The orphan receptor,

ErbB2, holds a preactivated conformation to facilitate hetero-dimerization with ErbB3 or ErbB4 with approximately 100-fold higher affinity than ErbB3 and ErbB4 homodimers. The heterometric receptors act in distinct cell types: ErbB2/ErbB3 in the peripheral nervous system and ErbB2/ErbB4 in the heart. Research in neural development has indicated that the formation of the sympathetic nervous system requires an intact NRG-1β, ErbB2 and ErbB3 signaling system. ErbB2/ErbB4 receptor activation promotes myocardial cell growth and survival. Targeted disruption of the NRG-1β, or ErbB2 or ErbB4 led to embryonic lethality due to cardiac development defects. Recent studies also highlighted the roles of NRG-1β, ErbB2 and ErbB4 in the cardiovascular development as well as in the maintenance of adult normal heart function.

[0005] Activation of ErbB4 by recombinant NRG-1 is a potential treatment option for heart failure because neuregulin stimulated ErbB2/ErbB4 heterodimerization is critical for myocardium function in early heart development and also prevents severe dysfunction of the adult heart. The short-term administration of a recombinant NRG-1β EGF domain significantly improves or protects against deterioration in myocardial performance in three distinct animal models of heart failure. More importantly, NRG-1β significantly prolongs survival of heart failure animals. See, e.g., De Keulenaer *et al.*, 2019, Circulation: Heart Failure 12:e006288. These effects make NRG-1β promising as a broad spectrum therapeutic or lead compound for heart failure due to a variety of common diseases.

[0006] There have been several drug candidates based on NRG-1 that have advanced in clinical trials for the treatment of heart disease through binding of ErbB4. However, binding through ErbB3 is thought to promote development or progression of certain cancers and also may cause gastrointestinal toxicity.

[0007] A 61-mer peptide (from S177 to Q237 of wild-type hNRG-1) has shown potent activity against both ErbB4 and ErbB3. See Liu *et al.*, 2006, J Amer Coll Cardiol 48:1438-1447; and U.S. Patent No. 7,226,907. See also International Patent Application Publication Nos. WO2010060265; WO2009007332; WO2009033373; WO2006030241; Jay *et al.*, 2013; Circulation 128:152-161; and Wali *et al.*, 2014, Mol Cancer Res 12:1140-1155. U.S. Pat. Nos. 7,115,554 and 7,063,961 describes heregulin β1 variants that show increased affinity for both ErbB3 and ErbB4 receptors. U.S. Patent Application Publication No. 2007/0213264 describes neuregulin-1β variants that show enhanced or decreased binding affinity to ErbB3 and/or ErbB4.

[0008] However, there is still a need for NRG-1 variants which show high selectivity for ErbB4 over ErbB3 for therapeutic use in heart failure.

SUMMARY OF THE INVENTION

[0009] The present disclosure provides polypeptide variants of neuregulin-1β that are selective against ErbB4 and not ErbB3 as compared to a wild-type sequence. In certain embodiments, the variant has increased binding affinity to ErbB4 and decreased binding affinity to ErbB3 compared to a wild type sequence. In certain embodiments, the variant has increased binding affinity to ErbB4 and similar binding affinity to ErbB3 compared to a wild type sequence. In certain embodiments, the variant has similar binding affinity to ErbB4 and decreased binding affinity to ErbB3 compared to a wild-type sequence.

[0010] The disclosure also includes NRG-1 variants that have greater specificity for the ErbB4 receptor, relative to the ErbB3 receptor, than the NRG-1 from which the NRG-1 variant is derived. In certain embodiments, the NRG-1 variant has a binding affinity for ErbB4 that is 2x, 3x, 4x greater than for ErbB3.

[0011] The disclosure also includes NRG-1 variants that have greater selectivity for the ErbB4 receptor compared to the ErbB3 receptor. In certain embodiments, the NRG-1 variant has a selectivity for ErbB4/ErbB3 greater than equal to 1000 or greater than equal to 10000.

[0012] In certain embodiments, the NRG-1 variant has agonist activity that is at least 50%, 60%, 70%, or 80% of the corresponding wild-type sequence.

[0013] In one embodiment, the disclosure provides a polypeptide variant comprising an amino acid sequence of the formula:

SHLVKCX_{183}E$X_{185}X_{186}$KX_{188}FCVNGGEC$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}SX_{204}PSRX_{208}$LCKCPNE FTGDRC$X_{222}X_{223}X_{224}X_{225}X_{226}ASX_{229}$ (SEQ ID NO:177)

wherein

X_{183} is A or G;

$X_{185}X_{186}$ is KD, KE, KH, ND, NE, NH, RD, RE, RH, RQ, SD, SE, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMIEHL (SEQ ID NO:180), FMVEDL (SEQ ID NO:181), FMVERS (SEQ ID NO:182), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), FVVEGL (SEQ ID NO:186), YMIEDL (SEQ ID NO:187), YMIEGL (SEQ ID NO:188), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEDL (SEQ ID NO:196), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QDDFM (SEQ ID NO:201), QDFFL (SEQ ID NO:202), QDFFM (SEQ ID NO:203), QDVFL (SEQ ID NO:204), QDVFM (SEQ ID NO:205), QEDFM (SEQ ID NO:206),

QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QKVFM (SEQ ID NO:216), QKVVM (SEQ ID NO:217), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is absent, F or FYKAEELYQ (SEQ ID NO:229).

[0014] In an aspect of this embodiment, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 114 to 176.

[0015] In one embodiment, the disclosure provides a polypeptide variant, wherein

X_{183} is A or G;

$X_{185}X_{186}$ is KD, KE, KH, ND, NH, RD, RE, RH, RQ, SD, SE, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMIEHL (SEQ ID NO:180), FMVEDL (SEQ ID NO:181), FMVERS (SEQ ID NO:182), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), YMIEDL (SEQ ID NO:187), YMIEGL (SEQ ID NO:188), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QDFFL (SEQ ID NO:202), QDFFM (SEQ ID NO:203), QDVFL (SEQ ID NO:204), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

[0016] In one aspect of this embodiment, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 114, 115, 117 to 124, 126 to 135, 138, 139, 144 to 147, 149 to 156, 160 to 168, or 171 to 176.

[0017] In one embodiment, the disclosure provides a polypeptide variant, wherein

X_{183} is A or G;

X_{185} is KE, KH, ND, RD, RH, RQ, SD, SE, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMVEDL (SEQ ID NO:181), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), YMIEDL (SEQ ID NO:187), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

[0018] In one aspect of this embodiment, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 117 to 124, 126 to 135, 144 to 147, 149 to 152, 154 to 156, 163, 167, 168, or 171 to 176.

[0019] In one embodiment, the disclosure provides a polypeptide variant

wherein

X_{183} is A or G;

$X_{185}X_{186}$ is KH, ND, RD, RH, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMVEDL (SEQ ID NO:181), FVIEDP (SEQ ID NO:184), YMIEDL (SEQ ID

NO:187), YMVEGS (SEQ ID NO:191), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is QAPHI (SEQ ID NO:200), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKTSL (SEQ ID NO:214), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

[0020] In one aspect of this embodiment, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 117 to 124, 126, 128 to 135, 146, 147, 150, 151, 163, 167, 168, or 171.

[0021] In another aspect of this embodiment, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 117, 118, 119, 120, 122, 123, 124, 126, 128, 129, 130, 131, 133, or 135.

[0022] In any of the embodiments, the polypeptide variant further comprises a second amino acid sequence to act as a signal sequence, increase the half-life, or aid in purification. In certain aspects, the polypeptide variant can be fused to an amino acid sequence to prolong its half-life. In one aspect, the amino acid sequence is an Fc region. In one aspect, the amino acid sequence is fused to the C-terminus of the polypeptide variant. In one aspect, the amino acid sequence is fused to the N-terminus of the polypeptide variant. In certain aspects, the amino acid sequence is fused to the polypeptide variant via a linker. In certain aspects, the polypeptide variant can be fused to His tag to aid in purification. In one aspect, the His tag is fused to the C-terminus of the polypeptide variant. In one aspect, the His tag is fused to the N-terminus of the polypeptide variant.

[0023] In other aspects, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 1 to 24, 29 to 54, 56 to 67, 69 to 80, 82 to 111. In other aspects, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 1 to 15, 19, 20, 22, 23, 29 to 34, 37, 38, 43 to 54, 56 to 59, 61 to 67, 69 to 80, 82 to 85, 87 to 93, or 97 to 111. In one aspect, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 3, 4, 6, 9, 11 to 13, 19, 22, 31 to 34, 43 to 48, 52, 54, 56 to 59, 61 to 63, 65 to 67, 71, 72, 75 to 80, 84, 85, 88, 100, or 104 to 106. In one aspect, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 3, 4, 6, 9, 11 to 13, 19, 22, 31 to 34, 43 to 48, 52, 54, 56 to 59, 61 to 63, 65 to 67, 71, 72, 75 to 80, 84, 85, 88, 100, or 104 to 106. In one aspect, the polypeptide variant comprises an amino acid sequence of SEQ ID NO:4, 11, 13, 32, 34, 45,

46, 48, 52, 72, 75, 76, 77, 78, 79, or 80. In one aspect, the polypeptide variant comprises an amino acid sequence of SEQ ID NO:56, 57, 58, 59, 61, 62, 63, 65, 66 or 67.

[0024] The disclosure also provides a pharmaceutical composition comprising the polypeptide variant described herein, and a pharmaceutically acceptable carrier.

[0025] The disclosure also provides a method of treating a cardiovascular disease or condition in a subject in need thereof, the method comprising administering a therapeutically effective amount of the polypeptide variant, or the pharmaceutical composition. In certain aspects of this embodiment, the cardiovascular disease or condition is heart failure, myocardial infarction, dilated cardiomyopathy, myocarditis, or cardiac toxicity. In certain aspects, the subject is a human.

[0026] The disclosure also provides the aforementioned polypeptide variants for use in treating a cardiovascular disease or condition or for the preparation of a medicament for treating a cardiovascular disease or condition. In one embodiment, the disclosure provides a neuregulin polypeptide variant for use in treating a cardiovascular disease or condition. In another embodiment, the disclosure provides the use of a neuregulin polypeptide for the preparation of a medicament for treating a cardiovascular disease or condition.

BRIEF DESCRIPTON OF THE DRAWINGS

[0027] Figures 1A-C show cardiac function assessment 1-week after ErbB4 agonist dosing. GraphPad One-way ANOVA was used to assess the statistical significance among groups. A) Terminal serum exposures. B) Cardiac function by ejection fraction (EF). C) Heart rate (HR) during echocardiography.

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention is based in part on the discovery that neuregulin variants can be designed that are highly selective for ErbB4 while having reduced or no binding affinity for ErbB3. Such variants are candidates for therapeutics for heart related diseases while minimizing cross-reactivity against other cell types. This invention arose, in part, from an effort to improve the efficacy of heart failure drugs. Accordingly, the disclosure is also directed to treating subjects with or at risk for development of heart disease and related conditions, e.g., heart failure.

Definitions

[0029] While the terminology used in this application is standard within the art, definitions of certain terms are provided herein to assure clarity and definiteness in the meaning of the claims. Units, prefixes, and symbols may be denoted in their SI (International System of Units) accepted form. Numeric ranges recited herein are inclusive of the numbers defining the

range and include and are supportive of each integer within the defined range. The methods and techniques described herein are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001) and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990).

[0030] As used herein, the terms "a" and "an" mean one or more unless specifically indicated otherwise. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

[0031] All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference. What is described in an embodiment of the invention can be combined with other embodiments of the invention.

[0032] As used herein, the terms "neuregulin-1" or "NRG-1" or "neuregulin" refer to proteins or peptides that can bind and activate ErbB2/ErbB4 or ErbB2/ErbB3 heterodimers protein kinases, such as all neuregulin isoforms, neuregulin EGF domain alone, neuregulin mutants, and any kind of neuregulin-like gene products that also activate the above receptors. Neuregulin also includes NRG-1, NRG-2, NRG-3, and NRG-4. These proteins and polypeptides can activate the above ErbB receptors and modulate their biological reactions, e.g., stimulate breast cancer cell differentiation and milk protein secretion; induce the differentiation of neural crest cell into Schwann cell; stimulate acetylcholine synthesis in skeletal muscle cell; and improve cardiomyocyte survival and DNA synthesis. Neuregulin also includes those variants with conservative amino acid substitutions that do not substantially alter their biological activity. Suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g., Watson *et al.* Molecular Biology of the Gene, 4th Edition, 1987. The Benjamin/Cummings Pub. Co. p. 224). The phrase "neuregulin protein" encompasses the full-length neuregulin protein as well as a neuregulin peptide (e.g.,

a truncated form of the full-length neuregulin protein. Neuregulin nucleic acid encompasses neuregulin nucleic acid and neuregulin oligonucleotide.

[0033] As used herein, "neuregulin variant" refers to neuregulin that has a modified sequence that alters or improves the selectivity of the neuregulin to ErbB4 and/or reduces the selectivity of the neuregulin to ErbB3.

[0034] As used herein, the terms, "epidermal growth factor-like domain" or "EGF-like domain refers to a polypeptide motif encoded by the neuregulin gene that binds to and activates ErbB2, ErbB3, ErbB4, or combinations thereof, and bears a structural similarity to the EGF receptor-binding domain as disclosed in International Patent Application Publication Nos. WO00/64400 and WO 97/09425, Holmes *et al.*, 1992, Science, 256:1205-1210; U.S. Pat. Nos. 5,530,109 and 5,716, 930; Hijazi *et al.*, 1998, Int. J. Oncol., 13:1061-1067; Chang *et al.*, 1997, Nature, 387:509-512; Carraway *et al.*, 1997, Nature, 387:512-516; Higashiyama *et al.*, 1997, J. Bio Chem., 122:675-680. EGF-like domains may be derived from NRG-1, NRG-2, NRG-3, or NRF-4. EGF-like domains may be C or B subtype.

[0035] As used herein, the terms "ErbB2", "ErbB2 (HER2)", "HER2" refer to the same protein (or the same gene when in reference thereto) and are used interchangeably herein. ErbB2 (erb-b2 receptor tyrosine kinase 2) is also known in the art as NEU, NGL, TKR1, CD340, HER-2, MLN 19 and HER-2/neu.

[0036] As used herein, the terms "ErbB3", "ErbB3 (HER3)", "HER3" refer to the same protein (or the same gene when in reference thereto) and are used interchangeably herein. ErbB3 (erb-b2 receptor tyrosine kinase 3) is also known in the art as FERLK, LCCS2, ErbB-3, c-erbB3, erbB3-S, MDA-BF-1, c-erbB-3, p180-ErbB3, p45-sErbB3 and p85-sErbB3.

[0037] As used herein, the terms "ErbB4", "ErbB4 (HER4)", "HER4" refer to the same protein (or the same gene when in reference thereto) and are used interchangeably herein. ErbB4 (erb-b2 receptor tyrosine kinase 4) is also known in the art as ALS19 and p180erbB4.

[0038] As used herein, the term "effective amount" or "therapeutically effective amount" means a dosage or amount sufficient to produce a desired result. The desired result may comprise an objective or subjective improvement in the subject of the dosage or amount (e.g., long-term survival, improved cardiac function, effective prevention of a disease state, etc.).

[0039] As used herein, the term "ejection fraction" refers to ejection fraction (EF), a measurement, typically expressed as a percentage, of how much blood the left ventricle pumps out with each contraction. For example, an ejection fraction of 50 percent means that 50 percent of the total amount of blood in the left ventricle is pushed out with each heartbeat.

[0040] As used herein, the term "heart failure" is meant as an abnormality of cardiac function where the output of the heart does not meet the requirements of metabolizing tissues. Heart failure includes a wide range of disease states such as congestive heart failure, myocardial infarction, tachyarrhythmia, familial hypertrophic cardiomyopathy, ischaemic

heart disease, idiopathic dilated cardiomyopathy, and myocarditis. The heart failure can be caused by any number of factors, including ischaemic, congenital, rheumatic, or idiopathic forms. Chronic cardiac hypertrophy is a significant disease state which is a precursor to congestive heart failure and cardiac arrest.

[0041] As used herein, the terms "polypeptide" and "protein" can be used interchangeably herein to refer to a polymer of amino acid residues. The terms also apply to amino acid polymers in which one or more amino acid residues is an analog or mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The terms can also encompass amino acid polymers that have been modified, *e.g.*, by the addition of carbohydrate residues to form glycoproteins, or phosphorylated. Polypeptides and proteins can be produced by a naturally-occurring and non-recombinant cell, or polypeptides and proteins can be produced by a genetically-engineered or recombinant cell. Polypeptides and proteins can also be produced by synthetic means. Polypeptides and proteins can comprise molecules having the amino acid sequence of a native protein, or molecules having deletions from, additions to, and/or substitutions of one or more amino acids of the native sequence.

[0042] The terms "polypeptide" and "protein" encompass molecules comprising only naturally occurring amino acids, as well as molecules that comprise non-naturally occurring amino acids. Examples of non-naturally occurring amino acids (which can be substituted for naturally-occurring amino acids found in any sequence disclosed herein, as desired) include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxyl-terminal direction, in accordance with standard usage and convention.

[0043] A non-limiting list of examples of non-naturally occurring amino acids that can be inserted into a protein or polypeptide sequence or substituted for a wild-type residue in a protein or polypeptide sequence include β-amino acids, homoamino acids, cyclic amino acids and amino acids with derivatized side chains. Examples include (in the L-form or D-form; abbreviated as in parentheses): citrulline (Cit), homocitrulline (hCit), Nα-methylcitrulline (NMeCit), Nα-methylhomocitrulline (Nα-MeHoCit), ornithine (Orn), Nα-Methylornithine (Nα-MeOrn or NMeOrn), sarcosine (Sar), homolysine (hLys or hK), homoarginine (hArg or hR), homoglutamine (hQ), Nα-methylarginine (NMeR), Nα-methylleucine (Nα-MeL or NMeL), N-methylhomolysine (NMeHoK), Nα-methylglutamine (NMeQ), norleucine (Nle), norvaline (Nva), 1,2,3,4-tetrahydroisoquinoline (Tic), Octahydroindole-2-carboxylic acid (Oic), 3-(1-naphthyl)alanine (1-Nal), 3-(2-naphthyl)alanine (2-Nal), 1,2,3,4-

tetrahydroisoquinoline (Tic), 2-indanylglycine (IgI), para-iodophenylalanine (pI-Phe), para-aminophenylalanine (4AmP or 4-Amino-Phe), 4-guanidino phenylalanine (Guf), glycyllysine (abbreviated "K(Nε-glycyl)" or "K(glycyl)" or "K(gly)"), nitrophenylalanine (nitrophe), aminophenylalanine (aminophe or Amino-Phe), benzylphenylalanine (benzylphe), γ-carboxyglutamic acid (γ-carboxyglu), hydroxyproline (hydroxypro), p-carboxyl-phenylalanine (Cpa), α-aminoadipic acid (Aad), Nα-methyl valine (NMeVal), N-α-methyl leucine (NMeLeu), Nα-methylnorleucine (NMeNle), cyclopentylglycine (Cpg), cyclohexylglycine (Chg), acetylarginine (acetylarg), α, β-diaminopropionoic acid (Dpr), α, γ-diaminobutyric acid (Dab), diaminopropionic acid (Dap), cyclohexylalanine (Cha), 4-methyl-phenylalanine (MePhe), β, β-diphenyl-alanine (BiPhA), aminobutyric acid (Abu), 4-phenyl-phenylalanine (or biphenylalanine; 4Bip), α-amino-isobutyric acid (Aib), beta-alanine, beta-aminopropionic acid, piperidinic acid, aminocaprioic acid, aminoheptanoic acid, aminopimelic acid, desmosine, diaminopimelic acid, N-ethylglycine, N-ethylaspargine, hydroxylysine, allo-hydroxylysine, isodesmosine, allo-isoleucine, N-methylglycine, N-methylisoleucine, N-methylvaline, 4-hydroxyproline (Hyp), γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, ω-methylarginine, 4-Amino-O-Phthalic Acid (4APA), and other similar amino acids, and derivatized forms of any of those specifically listed.

[0044] An "Fc" region, as the term is used herein, can comprise two heavy chain fragments comprising the C_H2 and C_H3 domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by hydrophobic interactions of the C_H3 domains. Proteins of interest comprising an Fc region, including antigen binding proteins and Fc fusion proteins, form another aspect of the instant disclosure.

NRG-1β Variants

[0045] The present disclosure provides polypeptide variants of NRG-1 that are selective for ErbB4. A neuregulin variant as disclosed herein has an amino acid sequence not found in nature in which one or more wildtype amino acid residues in a native neuregulin is substituted with different amino acid residues. At least one substitution is non-conservative so as to alter function, e.g., improve selectivity to ErbB4.

[0046] A functional human NRG-1 fragment which corresponds to amino acids 177-237 of human NRG-1 and contains the EGF-like domain has the amino acid sequence SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYK AEELYQ (SEQ ID NO: 112)

[0047] In some embodiments, a neuregulin fragment containing the EGF-like domain refers to amino acid residues 177-226, 177-228, 177-229, 177-237, or 177-240 of NRG-1 (SEQ ID NO: 112).

[0048] Representative neuregulin variants comprising a modified neuregulin sequence and one or more of linkers, Fc sequences and His tags are shown in Table 1 below.

Table 1

Name	Sequences	SEQ ID NO.
huNRG1(S177-F229)(NRGE1C)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	1
huNRG1(S177-F229)(NRGE1C)::1KmodT482V,M493L)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GSGSATGGSGSGVASSGSGSATHLDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	2
huNRG1(S177-F229)(NRGE1C)::(G4A)2:G4::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGAGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	3
huNRG1(S177-F229)(NRGE1C)((G4E)2)::G4::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4
huNRG1(S177-F229)(NRGE1C)::(G4S)2::G4::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGSGGGGSGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5
huNRG1(S177-F229)(NRGE1C)(G4E)::G4S::G4::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGSGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	6

huNRG1(S177-F229)(NRGE1C)(NRGmod)::G4::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**AAAEELYQGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK	7
huNRG1(S177-F229)(NRGE1D)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	8
huNRG1(S177-F229)(NRGE1D)::1KmodT482V,M493L)::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GSGSATGGSGSGSVASSGSGSATHLDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	9
huNRG1(S177-F229)(NRGE1D)::(G4A)2:G4::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGAGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	10
huNRG1(S177-F229)(NRGE1D)((G4E)2)::G4::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	11
huNRG1(S177-F229)(NRGE1D)::(G4S)2::G4::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGSGGGGSGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	12
huNRG1(S177-F229)(NRGE1D)(G4E)::G4S::G4S::G4::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGSGGGGSGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	13
huNRG1(S177-F229)(NRGE1D)(NRGmod)::G4::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**AAAEELYQGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK	14

huNRG1(S177-Q237)(3H8)::3xG4S::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	1 5
huNRG1(S177-F229)(3H8)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	1 6
huNRG1(S177-Q237)(3H8)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	1 7
huNRG1(S177-S228)(3H8)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMAS**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	1 8
huNRG1(S177-Q237)(NRG1C)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 9
huNRG1(S177-F229)(NRG1C)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	1
huNRG1(S177-Q237)(NRG1C)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**YKAEELYQGGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	2 0
huNRG1(S177-S228)(NRG1C)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMAS**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	2 1
huNRG1(S177-Q237)(NRG1D)::GG::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**YKAEELYQGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	2 2

huNRG1(S177-F229)(NRG1D)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	8
huNRG1(S177-Q237)(NRG1D)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**YKAEELYQGGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	2 3
huNRG1(S177-S228)(NRG1D)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMAS**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	2 4
huNRG1(S177-Q237)(wt)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	2 5
huNRG1(S177-F229)(wt)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASF**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK	2 6
huNRG1(S177-Q237)(wt)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGP SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK	2 7
huNRG1(S177-S228)(wt)::3x(G4Q)::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMAS**GGGGQGGGGQGGGGQDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	2 8
huNRG1(S177-Q237)(NRG1A)::GG::huFcSEFL2 (Pb)	**SHLVKCAENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	2 9
huNRG1(S177-Q237)(NRG1B)::GG::huFcSEFL2 (Pb)	**SHLVKCGENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	3 0

huNRG1(S177-Q237)(NRG1C)::GG::huFcSEFL2(Pb)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	19
huNRG1(S177-Q237)(NRG1D)::GG::huFcSEFL2(Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	22
huNRG1(S177-Q237)(NRG1E)::GG::huFcSEFL2(Pb)	**SHLVKCGENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	31
huNRG1(S177-Q237)(NRG1F)::GG::huFcSEFL2(Pb)	**SHLVKCAENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	32
huNRG1(S177-Q237)(NRG1G)::GG::huFcSEFL2(Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	33
huNRG1(S177-Q237)(NRG1H)::GG::huFcSEFL2(Pb)	**SHLVKCGESHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	34
huNRG1(S177-Q237)(NRG1I)::GG::huFcSEFL2(Pb)	**SHLVKCGENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	35
huNRG1(S177-Q237)(NRG1J)::GG::huFcSEFL2(Pb)	**SHLVKCGENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	36
huNRG1(S177-Q237)(NRG1K)::GG::huFcSEFL2(Pb)	**SHLVKCAEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	37

huNRG1(S177-Q237)(NRGE1L)::GG::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	3 8
huNRG1(S177-Q237)(NRGE1M)::GG::huFcSEFL2 (Pb)	**SHLVKCGENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDFFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	3 9
huNRG1(S177-Q237)(NRGE1N)::GG::huFcSEFL2 (Pb)	**SHLVKCGENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDFTMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	4 0
huNRG1(S177-Q237)(NRGE1O)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDFTMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	4 1
huNRG1(S177-Q237)(NRGE1P)::GG::huFcSEFL2 (Pb)	**SHLVKCAESHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDFFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	4 2
huNRG1(S177-Q237)(1D3)::GG::huFcSEFL2 (Pb)	**SHLVKCGENDKSFCVNGGECFVIEDPSIPSRYLCKCPNEFTGDRCQNDFLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	4 3
huNRG1(S177-F229)(NRGE1C)(D48T_F49Q_M50I) huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQETQIASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4 4
huNRG1(S177-F229)(NRGE1C)(E47A_D48P_F49H_M50I) huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQAPHIASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4 5

huNRG1(S177-F229)(NRG1C)(E47K_D48T_F49S_M50L) huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQKTSLASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4 6
huNRG1(S177-F229)(NRG1C)(E47Q_D48S_M50P) huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQQSFPASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4 7
huNRG1(S177-F229)(NRG1C)(E47S_D48A_F49L_M50T) huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQSALTASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4 8
huNRG1(S177-F229)(NRG1C)(E47S_D48T_F49R_M50V) huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQSTRVASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4 9
huNRG1(S177-F229)(NRG1C)huIgG1z SELF2 Fc	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5 0
huNRG1(S177-F229)(NRG1D)(E47K_D48L_F49D_M50I) huIgG1z SELF2 Fc	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQKLDIASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5 1
huNRG1(S177-F229)(NRG1D)(E47S_D48S_F49E_M50L) huIgG1z SELF2 Fc	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQSSELASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5 2

huNRG1(S177-F229)(NRG1D)(E47T_D48S_F49L_M50L) huIgG1z SELF2 Fc	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQTSLLASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5 3
huNRG1(S177-F229)(NRG1D) huIgG1z SELF2 Fc	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5 4
huNRG1(S177-F229) huIgG1z SELF2 Fc	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASF**GGGGEGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	5 5
huNRG1(S177-F229)(NRG1C)6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGAGGGGHHHHHH	5 6
huNRG1(S177-F229)(NRG1C)(D48T_F49Q_M50I) 6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQETQIASF**GGGGEGGGGAGGGGHHHHHH	5 7
huNRG1(S177-F229)(NRG1C)(E47A_D48P_F49H_M50I) 6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQAPHIASF**GGGGEGGGGAGGGGHHHHHH	5 8
huNRG1(S177-F229)(NRG1C)(E47K_D48T_F49S_M50L) 6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQKTSLASF**GGGGEGGGGAGGGGHHHHHH	5 9
huNRG1(S177-F229)(NRG1C)(E47Q_D48S_M50P) 6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQQSF**PASFGGGGEGGGGAGGGGHHHHHH	6 0
huNRG1(S177-F229)(NRG1C)(E47S_D48A_F49L_M50T) 6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQSALTASF**GGGGEGGGGAGGGGHHHHHH	6 1

huNRG1(S177-F229)(NRGE1C)(E47S_D48T_F49R_M50V)_6xHis	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQSTRVASF**GGGGEGGGGAGGGGHHHHHH	6 2
huNRG1(S177-F229)(NRGE1D)6xHis	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGAGGGGHHHHHH	6 3
huNRG1(S177-F229)(NRGE1D)(E47K_D48L_F49D_M50I)_6xHis	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQKLDIASF**GGGGEGGGGAGGGGHHHHHH	6 4
huNRG1(S177-F229)(NRGE1D)(E47S_D48S_F49E_M50L)_6xHis	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQSSELASF**GGGGEGGGGAGGGGHHHHHH	6 5
huNRG1(S177-F229)(NRGE1D)(E47T_D48S_F49L_M50L)_6xHis	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQTSLLASF**GGGGEGGGGAGGGGHHHHHH	6 6
huNRG1(S177-F229)(NRGE1D)(E47V_D48T_F49R_M50L)_6xHis	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQVTRLASF**GGGGEGGGGAGGGGHHHHHH	6 7
huNRG1(S177-F229)_6xHis	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASF**GGGGEGGGGAGGGGHHHHHH	6 8
:huFcSEFL2(desK):G4SG4:huNRG1(S177-F229)(NRGE1C)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGG**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQE** **DFMASF** DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGG**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQE** **DFMASF**	6 9

:huFcSEFL2(desK):G4SG4:huNRG1(S177-F229)(NRGE1D)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGS**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQE** **DFMASF**	70
:huFcSEFL2(desK):G4SG4:huNRG1(S177-Q237)(NRGE1C)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGS**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQE** **DFMASFYKAEELYQ**	71
:huFcSEFL2(desK):G4SG4:huNRG1(S177-Q237)(NRGE1D)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGS**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQE** **DFMASFYKAEELYQ**	72
:huFcSEFL2(desK):v131(+):G4SG4:huNRG1(S177-F229)(NRGE1C)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGS**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQE** **DFMASF**	73
	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVKGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	73

:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-F229)(NRGE1D)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGG**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQE** **DFMASF** DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	7 4
:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-Q237)(NRGE1C)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGG**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQE** **DFMASF**YKAEELYQ DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	7 5
:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-Q237)(NRGE1D)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGG**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQE** **DFMASF**YKAEELYQ DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	7 6
:huNRG1(S177-F229)(NRGE1C)::2X(G4A)G4::huFcSEFL2v131(+)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGAGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	7 7
:huNRG1(S177-F229)(NRGE1C)::2X(G4E)G4::huFcSEFL2v131(+)	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	7 8

:huNRG1(S177-F229)(NRGE1D)::2X(G4A)G4::huFcSEFL2v131(+)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGAGGGGAGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	7 9
:huNRG1(S177-F229)(NRGE1D)::2X(G4E)G4::huFcSEFL2v131(+)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREKMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLKSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTENQVSLTCLVEGFYPSDIAVEWESNGQPENNYETTPPVLDSDGSFFLYS DLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	8 0
huNRG1(S177-S228)(wt)((G4E)2:G4)::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASF**GGGGEGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	8 1
huNRG1(S177-F229)(NRGE1C)((G4E)2, GGT->GGC silent mutation before terminal lysine)::G4::huFcSEFL2	**SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	4
huNRG1(S177-F229)(NRGE1D)((G4E)2:G4)::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF**GGGGEGGGGEGGGGDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK	1 1
huNRG1(S177-Q237)(1A1)::GG::huFcSEFL2 (Pb)	**SHLVKCGESEKSFCVNGGECYVIEDSSIPSRFLCKCPNEFTGDRCQNVFLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 2

huNRG1(S177-Q237)(1A12)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKHKSFCVNGGECYVVERPSIPSRYLCKCPNEFTGDRCQKFFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 3
huNRG1(S177-Q237)(1A7)::GG::huFcSEFL2 (Pb)	**SHLVKCAERDKSFCVNGGECYVIEHLSNPSRFLCKCPNEFTGDRCQKDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 4
huNRG1(S177-Q237)(1B4)::GG::huFcSEFL2 (Pb)	**SHLVKCGERDKTFCVNGGECFMIEDSSNPSRYLCKCPNEFTGDRCQKDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 5
huNRG1(S177-Q237)(1B9)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKHKSFCVNGGECTMVEDLSIPSRYLCKCPNEFTGDRCQDVFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 6
huNRG1(S177-Q237)(1C11)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKEKTFCVNGGECYMVERPSIPSRFLCKCPNEFTGDRCQKFFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 7
huNRG1(S177-Q237)(1D10)::GG::huFcSEFL2 (Pb)	**SHLVKCAERDKTFCVNGGECYMIEDLSIPSRYLCKCPNEFTGDRCQKDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 8
huNRG1(S177-Q237)(1D3)::GG::huFcSEFL2 (Pb)	**SHLVKCGENDKSFCVNGGECFVIEDPSIPSRYLCKCPNEFTGDRCQNDFLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	4 3
huNRG1(S177-Q237)(1D4)::GG::huFcSEFL2 (Pb)	**SHLVKCGERDKTFCVNGGECYVVEHSSNPSRYLCKCPNEFTGDRCQKFTLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 9
huNRG1(S177-Q237)(1D4)::GG::huFcSEFL2 (Pb)	**SHLVKCGERDKTFCVNGGECYVVEHSSNPSRYLCKCPNEFTGDRCQKFTLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	8 9

huNRG1(S177-Q237)(2E3)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKEKSFCVNGGECYMIEGLSIPSRFLCKCPNEFTGDRCQDVFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	90
huNRG1(S177-Q237)(2E4)::GG::huFcSEFL2 (Pb)	**SHLVKCGESDKSFCVNGGECFMVKRPSNPSRYLCKCPNEFTGDRCQKVFLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	91
huNRG1(S177-Q237)(2F2)::GG::huFcSEFL2 (Pb)	**SHLVKCAESEKTFCVNGGECYMIEHPSNPSRFLCKCPNEFTGDRCQNVFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	92
huNRG1(S177-Q237)(2G3)::GG::huFcSEFL2 (Pb)	**SHLVKCGERQKSFCVNGGECYMVEDLSIPSRYLCKCPNEFTGDRCQKVFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	93
huNRG1(S177-Q237)(2G4)::GG::huFcSEFL2 (Pb)	**SHLVKCAENEKTFCVNGGECFVVEGLSIPSRYLCKCPNEFTGDRCQDVFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	94
huNRG1(S177-Q237)(2H7)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKEKSFCVNGGECYVVEDLSIPSRFLCKCPNEFTGDRCQKVVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	95
huNRG1(S177-Q237)(3A7)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKEKSFCVNGGECFVIEGSSIPSRFLCKCPNEFTGDRCQKVVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	96
huNRG1(S177-Q237)(3B8)::GG::huFcSEFL2 (Pb)	**SHLVKCGERDKSFCVNGGECFMVERSSIPSRYLCKCPNEFTGDRCQDFTLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	97
huNRG1(S177-Q237)(3D6)::GG::huFcSEFL2 (Pb)	**SHLVKCGESDKSFCVNGGECFVIEGSSIPSRFLCKCPNEFTGDRCQDFTMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	98

huNRG1(S177-Q237)(3D8)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKDKTFCVNGGECFMVEDLSIPSRFLCKCPNEFTGDRCQDFFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	9 9
huNRG1(S177-Q237)(3E6)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKHKSFCVNGGECYVIEGSSIPSRFLCKCPNEFTGDRCQKDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 0
huNRG1(S177-Q237)(3E9)::GG::huFcSEFL2 (Pb)	**SHLVKCAENHKTFCVNGGECFVIEGSSNPSRYLCKCPNEFTGDRCQDVFLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 1
huNRG1(S177-Q237)(3F2)::GG::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 2
huNRG1(S177-Q237)(3G3)::GG::huFcSEFL2 (Pb)	**SHLVKCGEREKTFCVNGGECFMIEHLSIPSRFLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 3
huNRG1(S177-Q237)(3G9)::GG::huFcSEFL2 (Pb)	**SHLVKCGESHKSFCVNGGECFMVEDLSNPSRFLCKCPNEFTGDRCQKDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 4
huNRG1(S177-Q237)(3H5)::GG::huFcSEFL2 (Pb)	**SHLVKCGERHKSFCVNGGECYVVERPSIPSRFLCKCPNEFTGDRCQKDFLASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 5
huNRG1(S177-Q237)(3H8)::GG::huFcSEFL2 (Pb)	**SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 6
huNRG1(S177-Q237)(_200E)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVEDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 7

huNRG1(S177-Q237)(_200E_KDF)::GG::huFcSEFL 2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVEDLSNPSRYLCKCPNEFTGDRCQKDFMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 8
huNRG1(S177-Q237)(_FVIEDPSI)::GG::huFcSEFL 2 (Pb)	**SHLVKCAEKEKTFCVNGGECFVIEDPSIPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 0 9
huNRG1(S177-Q237)(wt)::GG::huFcSEFL2 (Pb)	**SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	2 5
huNRG1(S177-Q237)(v80)::GG::huFcSEFL2 (Pb)	**SHLVKCAESHKSFCVNGGECFVIEGSSIPSRYLCKCPNEFTGDRCQKDVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 1 0
huNRG1(S177-Q237)(v80.3)::GG::huFcSEFL2 (Pb)	**SHLVKCAERHKSFCVNGGECFVIEGSSIPSRYLCKCPNEFTGDRCEKDVMASFYKAEELYQ**GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK	1 1 1

Neuregulin variants sequences are in bold.

[0049] The human neuregulin variant sequences (without linkers, Fc sequences or His tags) are presented in Table 2 below.

Table 2

Variant name	HuNRG1 variant sequence	SEQ ID NO:
NRG1(S177-Q237) (NRGE1A)	SHLVKCAENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ	114
NRG1(S177-Q237) (NRGE1B)	SHLVKCGENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ	115
NRG1(S177-S228) (NRGE1C)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMAS	116
NRG1(S177-F229) (NRGE1C)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASF	117
NRG1(S177-F229) (NRGE1C TQI)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQETQIASF	118
NRG1(S177-F229) (NRGE1C APHI)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQAPHIASF	119
NRG1(S177-F229) (NRGE1C KTSL)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQKTSLASF	120
NRG1(S177-F229) (NRGE1C QSP)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQQSFPASF	121
NRG1(S177-F229) (NRGE1C SALT)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQSALTASF	122
NRG1(S177-F229) (NRGE1C STRV)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQSTRVASF	123
NRG1(S177-Q237) (NRGE1C)	SHLVKCGEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ	124
NRG1(S177-S228) (NRGE1D)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMAS	125
NRG1(S177-F229) (NRGE1D)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF	126
NRG1(S177-F229) (NRGE1D KLDI)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQKLDIASF	127
NRG1(S177-F229) (NRGE1D SSEL)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQSSELASF	128
NRG1(S177-F229) (NRGE1D TSLL)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQTSLLASF	129

NRG1(S177-F229) (NRGE1D VTRL)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQVTRLASF	130
NRG1(S177-Q237) (NRGE1D)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ	131
NRG1(S177-Q237) (NRGE1E)	SHLVKCGENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ	132
NRG1(S177-Q237) (NRGE1F)	SHLVKCAENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ	133
NRG1(S177-Q237) (NRGE1G)	SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ	134
NRG1(S177-Q237) (NRGE1H)	SHLVKCGESHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQNDFMASFYKAEELYQ	135
NRG1(S177-Q237) (NRGE1I)	SHLVKCGENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ	136
NRG1(S177-Q237) (NRGE1J)	SHLVKCGENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ	137
NRG1(S177-Q237) (NRGE1K)	SHLVKCAEKHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ	138
NRG1(S177-Q237) (NRGE1L)	SHLVKCGESHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ	139
NRG1(S177-Q237) (NRGE1M)	SHLVKCGENDKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDFFMASFYKAEELYQ	140
NRG1(S177-Q237) (NRGE1N)	SHLVKCGENDKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDFFMASFYKAEELYQ	141
NRG1(S177-Q237) (NRGE1O)	SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQDFFMASFYKAEELYQ	142
NRG1(S177-Q237) (NRGE1P)	SHLVKCAESHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDFFMASFYKAEELYQ	143
NRG1(S177-Q237) (1A1)	SHLVKCGESEKSFCVNGGECYVIEDSSIPSRFLCKCPNEFTGDRCQNVFLASFYKAEELYQ	144
NRG1(S177-Q237) (1A12)	SHLVKCAEKHKSFCVNGGECYVVERPSIPSRYLCKCPNEFTGDRCQKFFMASFYKAEELYQ	145
NRG1(S177-Q237) (1A7)	SHLVKCAERDKSFCVNGGECYVIEHLSNPSRFLCKCPNEFTGDRCQKDFMASFYKAEELYQ	146
NRG1(S177-Q237) (1B4)	SHLVKCGERDKTFCVNGGECFMIEDSSNPSRYLCKCPNEFTGDRCQKDFMASFYKAEELYQ	147
NRG1(S177-Q237) (1B9)	SHLVKCAEKHKSFCVNGGECFMVEDLSIPSRYLCKCPNEFTGDRCQDVFMASFYKAEELYQ	148
NRG1(S177-Q237) (1C11)	SHLVKCGEKEKTFCVNGGECYMVERPSIPSRFLCKCPNEFTGDRCQKFFMASFYKAEELYQ	149
NRG1(S177-Q237) (1D10)	SHLVKCAERDKTFCVNGGECYMIEDLSIPSRYLCKCPNEFTGDRCQKDFMASFYKAEELYQ	150
NRG1(S177-Q237) (1D3)	SHLVKCGENDKSFCVNGGECFVIEDPSIPSRYLCKCPNEFTGDRCQNDFLASFYKAEELYQ	151
NRG1(S177-Q237) (1D4)	SHLVKCGERDKTFCVNGGECYVVEHSSNPSRYLCKCPNEFTGDRCQKFFLASFYKAEELYQ	152

NRG1(S177-Q237)(2E3)	SHLVKCGEKEKSFCVNGGECYMIEGLSIPSRFLCKCPNEFTGDRCQDVFMASFYKAEELYQ	153
NRG1(S177-Q237) (2E4)	SHLVKCGESDKSFCVNGGECFMVKRPSNPSRYLCKCPNEFTGDRCQKVFLASFYKAEELYQ	154
NRG1(S177-Q237) (2F2)	SHLVKCAESEKTFCVNGGECYMIEHPSNPSRFLCKCPNEFTGDRCQNVFMASFYKAEELYQ	155
NRG1(S177-Q237) (2G3)	SHLVKCGERQKSFCVNGGECYMVEDLSIPSRYLCKCPNEFTGDRCQKVFMASFYKAEELYQ	156
NRG1(S177-Q237) (2G4)	SHLVKCAENEKTFCVNGGECFVVEGLSIPSRYLCKCPNEFTGDRCQDVFMASFYKAEELYQ	157
NRG1(S177-Q237) (2H7)	SHLVKCGEKEKSFCVNGGECYVVEDLSIPSRFLCKCPNEFTGDRCQKVVMASFYKAEELYQ	158
NRG1(S177-Q237) (3A7)	SHLVKCAEKEKSFCVNGGECFVIEGSSIPSRFLCKCPNEFTGDRCQKVVMASFYKAEELYQ	159
NRG1(S177-Q237) (3B8)	SHLVKCGERDKSFCVNGGECFMVERSSIPSRYLCKCPNEFTGDRCQDFFLASFYKAEELYQ	160
NRG1(S177-Q237) (3D6)	SHLVKCGESDKSFCVNGGECFVIEGSSIPSRFLCKCPNEFTGDRCQDFFMASFYKAEELYQ	161
NRG1(S177-Q237) (3D8)	SHLVKCGEKDKTFCVNGGECFMVEDLSIPSRFLCKCPNEFTGDRCQDFFMASFYKAEELYQ	162
NRG1(S177-Q237)(3E6)	SHLVKCAEKHKSFCVNGGECYVIEGSSIPSRFLCKCPNEFTGDRCQKDFMASFYKAEELYQ	163
NRG1(S177-Q237)(3E9)	SHLVKCAENHKTFCVNGGECFVIEGSSNPSRYLCKCPNEFTGDRCQDVFLASFYKAEELYQ	164
NRG1(S177-Q237) (3F2)	SHLVKCGESHKSFCVNGGECFMIEGPSNPSRYLCKCPNEFTGDRCQDDFMASFYKAEELYQ	165
NRG1(S177-Q237) (3G3)	SHLVKCGEREKTFCVNGGECFMIEHLSIPSRFLCKCPNEFTGDRCQEDFMASFYKAEELYQ	166
NRG1(S177-Q237) (3G9)	SHLVKCGESHKSFCVNGGECFMVEDLSNPSRFLCKCPNEFTGDRCQKDFMASFYKAEELYQ	167
NRG1(S177-Q237) (3H5)	SHLVKCGERHKSFCVNGGECYVVERPSIPSRFLCKCPNEFTGDRCQKDFLASFYKAEELYQ	168
NRG1(S177-S228) (3H8)	SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMAS	169
NRG1(S177-F229) (3H8)	SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASF	170
NRG1(S177-Q237) (3H8)	SHLVKCGEKHKSFCVNGGECYMVEGSSIPSRYLCKCPNEFTGDRCQEDFMASFYKAEELYQ	171
NRG1(S177-Q237) (200E)	SHLVKCAEKEKTFCVNGGECFMVEDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ	172
NRG1(S177-Q237) (200E_KDF)	SHLVKCAEKEKTFCVNGGECFMVEDLSNPSRYLCKCPNEFTGDRCQKDFMASFYKAEELYQ	173
NRG1(S177-Q237) (FVIEDPSI)	SHLVKCAEKEKTFCVNGGECFVIEDPSIPSRYLCKCPNEFTGDRCQNYVMASFYKAEELYQ	174
NRG1(S177-Q237) (v80)	SHLVKCAESHKSFCVNGGECFVIEGSSIPSRYLCKCPNEFTGDRCQKDVMASFYKAEELYQ	175
NRG1(S177-Q237) (v80.3)	SHLVKCAERHKSFCVNGGECFVIEGSSIPSRYLCKCPNEFTGDRCEKDVMASFYKAEELYQ	176

[0050] In certain embodiments, the polypeptide variant comprises an amino acid sequence of the formula:

SHLVKCX$_{183}$EX$_{185}$X$_{186}$KX$_{188}$FCVNGGECX$_{197}$X$_{198}$X$_{199}$X$_{200}$X$_{201}$X$_{202}$SX$_{204}$PSRX$_{208}$LCKCPNEFTGDRCX$_{222}$X$_{223}$X$_{224}$X$_{225}$X$_{226}$ASX$_{229}$ (SEQ ID NO: 177)

[0051] wherein

[0052] X_{183} is A or G;

[0053] $X_{185}X_{186}$ is KD, KE, KH, ND, NE, NH, RD, RE, RH, RQ, SD, SE, or SH;

[0054] X_{188} is S or T;

[0055] $X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMIEHL (SEQ ID NO:180), FMVEDL (SEQ ID NO:181), FMVERS (SEQ ID NO:182), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), FVVEGL (SEQ ID NO:186), YMIEDL (SEQ ID NO:187), YMIEGL (SEQ ID NO:188), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEDL (SEQ ID NO:196), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

[0056] X_{204} is I or N;

[0057] X_{208} is F or Y;

[0058] $X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QDDFM (SEQ ID NO:201), QDFFL (SEQ ID NO:202), QDFFM (SEQ ID NO:203), QDVFL (SEQ ID NO:204), QDVFM (SEQ ID NO:205), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QKVFM (SEQ ID NO:216), QKVVM (SEQ ID NO:217), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

[0059] X_{229} is absent, F or FYKAEELYQ (SEQ ID NO:229).

[0060] In the above formula and formulae below, wild-type sequences are underlined.

[0061] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 114 to 176.

[0062] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 1 to 24, 29 to 54, 56 to 67, 69 to 80, or 82 to 111.

[0063] In certain embodiments, the polypeptide variant comprises an amino acid sequence of the formula:

$SHLVKCX_{183}EX_{185}X_{186}KX_{188}FCVNGGECX_{197}X_{198}X_{199}X_{200}X_{201}X_{202}SX_{204}PSRX_{208}LCKCPNEFTGDRCX_{222}X_{223}X_{224}X_{225}X_{226}ASX_{229}$ [SEQ ID NO: 177]

[0064] wherein

[0065] X_{183} is A or G;

[0066] $X_{185}X_{186}$ is KD, KE, KH, ND, NH, RD, RE, RH, RQ, SD, SE, or SH;

[0067] X_{188} is S or T;

[0068] $X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMIEHL (SEQ ID NO:180), FMVEDL (SEQ ID NO:181), FMVERS (SEQ ID NO:182), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), YMIEDL (SEQ ID NO:187), YMIEGL (SEQ ID NO:188), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

[0069] X_{204} is I or N;

[0070] X_{208} is F or Y;

[0071] $X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QDFFL (SEQ ID NO:202), QDFFM (SEQ ID NO:203), QDVFL (SEQ ID NO:204), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

[0072] X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

[0073] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 114, 115, 117 to 124, 126 to 135, 138, 139, 144 to 147, 149 to 156, 160 to 168, or 171 to 176.

[0074] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 1 to 15, 19, 20, 22, 23, 29 to 34, 37, 38, 43 to 54, 56 to 59, 61 to 67, 69 to 80, 82 to 85, 87 to 93, or 97 to 111.

[0075] In certain embodiments, the polypeptide variant comprises an amino acid sequence of the formula:

$SHLVKCX_{183}EX_{185}X_{186}KX_{188}FCVNGGECX_{197}X_{198}X_{199}X_{200}X_{201}X_{202}SX_{204}PSRX_{208}LCKCPNE$
$FTGDRCX_{222}X_{223}X_{224}X_{225}X_{226}ASX_{229}$ (SEQ ID NO: 177)

[0076] wherein

[0077] X_{183} is A or G;

[0078] X_{185} is, KE, KH, ND, RD, RH, RQ, SD, SE, or SH;

[0079] X_{188} is S or T;

[0080] $X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMVEDL (SEQ ID NO:181), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), YMIEDL (SEQ ID NO:187), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192),

YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

[0081] X_{204} is I or N;

[0082] X_{208} is F or Y;

[0083] $X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

[0084] X_8 is F or FYKAEELYQ (SEQ ID NO:229).

[0085] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 117 to 124, 126 to 135, 144 to 147, 149 to 152, 154 to 156, 163, 167, 168, or 171 to 176.

[0086] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 3, 4, 6, 9, 11 to 13, 19, 22, 31 to 34, 43 to 48, 52, 54, 56 to 59, 61 to 63, 65 to 67, 71, 72, 75 to 80, 84, 85, 88, 100, or 104 to 106.

[0087] In certain embodiments, the polypeptide variant comprises an amino acid sequence of the formula:

$SHLVKCX_{183}EX_{185}X_{186}KX_{188}FCVNGGECX_{197}X_{198}X_{199}X_{200}X_{201}X_{202}SX_{204}PSRX_{208}LCKCPNEFTGDRCX_{222}X_{223}X_{224}X_{225}X_{226}ASX_{229}$ [SEQ ID NO: 177]

[0088] wherein

[0089] X_{183} is A or G;

[0090] $X_{185}X_{186}$ is KH, ND, RD, RH, or SH;

[0091] X_{188} is S or T;

[0092] $X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMVEDL (SEQ ID NO:181), FVIEDP (SEQ ID NO:184), YMIEDL (SEQ ID NO:187), YMVEGS (SEQ ID NO:191), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), or YVVERP (SEQ ID NO:198);

[0093] X_{204} is I or N;

[0094] X_{208} is F or Y;

[0095] $X_{222}X_{223}X_{224}X_{225}X_{226}$ is QAPHI (SEQ ID NO:200), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKTSL (SEQ ID NO:214), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QQSFP (SEQ

ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and;

[0096] X_{229} is F or FYKAEELYQ (SEQ D NO:229).

[0097] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 117 to 124, 126, 128 to 135, 146, 147, 150, 151, 163, 167, 168, or 171.

[0098] In certain embodiments, the polypeptide variant comprises an amino acid sequence of any of SEQ ID Nos: 3, 4, 6, 9, 11 to 13, 19, 22, 31 to 34, 43 to 48, 52, 54, 56 to 59, 61 to 63, 65 to 67, 71, 72, 75 to 80, 84, 85, 88, 100, or 104 to 106.

[0099] In certain embodiments, the polypeptide variant has enhanced binding affinity to ErbB4 compared to the polypeptide of SEQ ID NO: 112.

[0100] In certain embodiments, the polypeptide variant has decreased binding affinity for ErbB3 compared to the polypeptide of SEQ ID NO:112.

[0101] In certain embodiments, the polypeptide variant has increased binding affinity for ErbB4 while having similar binding affinity to ErbB3 compared to the polypeptide of SEQ ID NO:112.

[0102] In certain embodiments, the polypeptide variant has increased binding affinity for ErbB4 while having decreased binding affinity for ErbB3 compared to the polypeptide of SEQ ID NO: 112.

[0103] In certain embodiments, the polypeptide variant has similar binding for ErbB4 while having decreased binding for ErbB3 compared to the polypeptide of SEQ ID NO: 124.

[0104] In certain embodiments, the polypeptide variant has a selectivity for ErbB4/ErbB3 that is at least 500, at least 1000, at least 5000, or at least 10,000. Selectivity for ErbB4/ErbB3 can be measured by methods known to those skilled in the art such as the Akt assay described in Example 1. For example, selectivity can be expressed at the ratio of EC50s in Schwann cells/cardiomyocytes. This ratio provides the activity in neural cells compared to heart cells.

[0105] In certain embodiments, the polypeptide variants have agonist activity that is at least 50%, 60%, 70%, or 80% of the activity against the wild-type sequence. This can be measured by phosphorylation of Akt.

[0106] Any of the above-described neuregulin variants can be modified by fusion to a heterologous polypeptide to produce a "chimeric neuregulin variant" or fusion protein. Typically, the heterologous polypeptide is fused at the N- or C terminus of the neuregulin variant to preserve the biological activity of the neuregulin variant. However, the heterologous polypeptide can also be introduced into regions of the neuregulin variant that are not critical for biological activity. Generally, chimeric neuregulin variants are produced by recombinant techniques or chemical synthesis. Examples of chimeric neuregulin variants include a neuregulin variant fused to a “signal sequence”, a “purification handle”, an

immunoglobulin sequence or any combination thereof. Linkers may be used to connect the neuregulin polypeptide to a heterologous polypeptide.

[0107] A “signal sequence” is an amino acid sequence that directs the secretion of a polypeptide fused thereto from a cell expressing the chimeric protein. Thus, fusion of a neuregulin variant to a signal sequence facilitates recombinant production of the neuregulin variant because the chimeric neuregulin variant is secreted into the host cell culture medium, from which the chimeric neuregulin variant can be recovered with relative ease. A suitable signal sequence can be obtained from any protein that has a signal sequence and is typically (but not always) fused to the N-terminus of the neuregulin variant. DNA encoding prokaryotic signal sequences can be obtained, for example, from lamB or ompF, MalE, PhoA, and other genes. Another suitable prokaryotic signal sequence is the E. coli heat-stable enterotoxin II (STII) signal sequence. Mammalian signal sequences are discussed below.

[0108] A “purification handle” is a portion of a polypeptide or a polypeptide sequence that binds another polypeptide, termed a “binding partner.” The fusion of a purification handle to a neuregulin variant confers on the variant the ability to bind the binding partner, which facilitates purification of the resultant chimeric neuregulin variant. Generally, the purification handle is selected so that the binding partner does not substantially cross-react with other components present in the mixture from which the chimeric neuregulin variant is to be purified. An exemplary “purification handle” is a His tag sequence. As used herein, the term "does not substantially cross-react" means that the affinity of the binding partner for the purification handle is at least about 20-fold, usually at least about 100-fold, more usually at least about 1000-fold, any affinity for any other components present in the mixture.

[0109] A chimeric neuregulin variant includes a neuregulin variant fused to an immunoglobulin sequence. In one embodiment, the immunoglobulin sequence is a Fc region of an IgG molecule (e.g., IgG1, IgG2, IgG3, or IgG4) that increases the in vivo serum half-life of the IgG. See, e.g., U.S. Patent Application Publication No. US2006/0140934.

[0110] In one embodiment, a neuregulin variant is attached to a linker via the first (1st) amino acid on the N-terminus of the neuregulin variant, which in one embodiment is a Serine (S or Ser) amino acid.

[0111] In certain embodiments, the Fc region has at least one mutation, preferably to reduce effector function and/or extend the half-life of the molecule. In certain aspects, the Fc region comprises at least one mutation in amino acids 234, 239, 434, or a combination thereof (numbered according to EU numbering), where in certain aspects, the amino acid mutations comprise at least one of the following substitution mutations: L234F, S239A, N434A or a combination thereof. Mutations to amino acids 234 and/or 239 knock down effector functions of the Fc region. Mutation to amino acid 434 extends the half-life of the fusion protein in a subject. Other mutations are known in the art such as the SEFL2.0 and SEFL2.2 mutations.

See, e.g., Jacobsen et al., 2017, J Biol Chem 292:1865-1875 and Yang et al., 2018, Front Immunol Vol. 8, Art. 1860.

[0112] In certain embodiments, the one or more mutations in the Fc region reduce effector function. In some embodiments, the reduced effector function comprises a reduced affinity of the fusion protein for one or more Fc Receptors. The FcRs can be FcγRI, FcγRIIa, FcγRIib, FcγRIIIa (158F), FcγRIIIa (158V) and Clq.

[0113] In one embodiment, a fusion protein comprises a neuregulin variant fused to or operably linked to the C-terminus of an Fc region via a GGGGSGGGGS (G4S) linker (SEQ ID NO: 113). In some embodiments, one or more copies of the linker may be used. In other embodiments, 2, 3, 4, or 5 copies of the G4S linker or any other linker known in the art and/or as described herein as being suitable for the composition disclosed herein may be used herein.

[0114] The term "linker" is art-recognized and refers to a molecule (including but not limited to unmodified or modified nucleotides or amino acids) or group of molecules (for example, 2 or more, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more) connecting two compounds, such as two polypeptides. The linker may be comprised of a single linking molecule or may comprise a linking molecule and at least one spacer molecule, intended to separate the linking molecule and a compound by a specific distance.

Preparation of Neuregulin Variants

[0115] The polypeptides of the invention may be produced by chemical synthesis or recombinant methods. Methods of chemically synthesizing polypeptides are well known in the art. Synthesizing polypeptides using recombinant methods are also well known in the art and are further described herein.

[0116] Methods and vectors for genetically engineering cells and/or cell lines to express, for example, a polypeptide, are well known to those of skill in the art; for example, various techniques are illustrated in Current Protocols in Molecular Biology, Ausubel *et al.*, eds. (Wiley & Sons, New York, 1988, and quarterly updates); Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (Cold Spring Laboratory Press, 1989); Kaufman, R.J., *Large Scale Mammalian Cell Culture*, 1990, pp. 15-69. The polypeptides generated may be tested for their binding affinity to the receptor and activation of the receptor using methods known in the art.

[0117] A vector may be any molecule or entity (e.g., nucleic acid, plasmid, bacteriophage, transposon, cosmid, chromosome, virus, virus capsid, virion, naked DNA, complexed DNA and the like) suitable for use to transfer and/or transport protein encoding information into a host cell and/or to a specific location and/or compartment within a host cell. Vectors can include viral and non-viral vectors, non-episomal mammalian vectors. Vectors are often

referred to as expression vectors, for example, recombinant expression vectors and cloning vectors. The vector may be introduced into a host cell to allow replication of the vector itself and thereby amplify the copies of the polynucleotide contained therein. The cloning vectors may contain sequence components generally include, without limitation, an origin of replication, promoter sequences, transcription initiation sequences, enhancer sequences, and selectable markers. These elements may be selected as appropriate by a person of ordinary skill in the art.

[0118] Vectors are useful for transformation of a host cell and contain nucleic acid sequences that direct and/or control (in conjunction with the host cell) expression of one or more heterologous coding regions operatively linked thereto. An expression construct may include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto. "Operably linked" means that the components to which the term is applied are in a relationship that allows them to carry out their inherent functions. For example, a control sequence, e.g., a promoter, in a vector that is "operably linked" to a protein coding sequence are arranged such that normal activity of the control sequence leads to transcription of the protein coding sequence resulting in recombinant expression of the encoded protein.

[0119] Vectors may be selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery, permitting amplification and/or expression of the gene can occur). In some embodiments, vectors are used that employ protein-fragment complementation assays using protein reporters, such as dihydrofolate reductase (see, for example, U.S. Pat. No. 6,270,964). Suitable expression vectors are known in the art and are also commercially available.

[0120] Typically, vectors used in host cells will contain sequences for plasmid maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences will typically include one or more of the following nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, transcriptional and translational control sequences, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, various pre- or pro-sequences to improve glycosylation or yield, a native or heterologous signal sequence (leader sequence or signal peptide) for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, internal ribosome entry site (IRES) sequences, an expression augmenting sequence element (EASE), tripartite leader (TPA) and VA gene RNAs from Adenovirus 2, a polylinker region for inserting the polynucleotide encoding the polypeptide to be expressed, and a selectable marker element. Vectors may be constructed from a starting vector such as a commercially available vector, additional elements may be individually obtained and ligated into the vector. Methods used for obtaining each of the components are well known to one skilled in the art.

[0121] Vector components may be homologous (*i.e.*, from the same species and/or strain as the host cell), heterologous (e.g.., from a species other than the host cell species or strain), hybrid (*i.e.*, a combination of flanking sequences from more than one source), synthetic or native. The sequences of components useful in the vectors may be obtained by methods well known in the art, such as those previously identified by mapping and/or by restriction endonuclease. In addition, they can be obtained by polymerase chain reaction (PCR) and/or by screening a genomic library with suitable probes.

[0122] A ribosome-binding site is usually necessary for translation initiation of mRNA and is characterized by a Shine-Dalgarno sequence (prokaryotes) or a Kozak sequence (eukaryotes). The element is typically located 3′ to the promoter and 5′ to the coding sequence of the polypeptide to be expressed.

[0123] An origin of replication aids in the amplification of the vector in a host cell. They may be included as part of commercially available prokaryotic vectors and may also be chemically synthesized based on a known sequence and ligated into the vector. Various viral origins (e.g., SV40, polyoma, adenovirus, vesicular stomatitus virus (VSV), or papillomaviruses such as HPV or BPV) are useful for cloning vectors in mammalian cells.

[0124] Transcriptional and translational control sequences for mammalian host cell expression vectors can be excised from viral genomes. Commonly used promoter and enhancer sequences are derived from polyoma virus, adenovirus 2, simian virus 40 (SV40), and human cytomegalovirus (CMV). For example, the human CMV promoter/enhancer of immediate early gene 1 may be used. See, e.g., Patterson *et al.*, 1994, Applied Microbiol. Biotechnol. 40:691-98. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites can be used to provide other genetic elements for expression of a structural gene sequence in a mammalian host cell. Viral early and late promoters are particularly useful because both are easily obtained from a viral genome as a fragment, which can also contain a viral origin of replication (Fiers *et al.*, 1978, Nature 273:113; Kaufman, 1990, Meth. in Enzymol. 185:487-511). Smaller or larger SV40 fragments can also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the BglI site located in the SV40 viral origin of replication site is included.

[0125] A transcription termination sequence is typically located 3′ to the end of a polypeptide coding region and serves to terminate transcription. Usually, a transcription termination sequence in prokaryotic cells is a G-C rich fragment followed by a poly-T sequence. While the sequence is easily cloned from a library or even purchased commercially as part of a vector, it can also be readily synthesized using methods for nucleic acid synthesis known to those of skill in the art.

[0126] A selectable marker gene encoding a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells; (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients not available from complex or defined media. Specific selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene. Advantageously, a neomycin resistance gene may also be used for selection in both prokaryotic and eukaryotic host cells.

[0127] Other selectable genes may be used to amplify the gene that will be expressed. Amplification is the process wherein genes that are required for production of a protein critical for growth or cell survival are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Examples of suitable selectable markers for mammalian cells include glutamine synthase (GS)/methionine sulfoximine (MSX) system, dihydrofolate reductase (DHFR), and promoterless thymidine kinase genes. Mammalian cell transformants are placed under selection pressure wherein only the transformants are uniquely adapted to survive by virtue of the selectable gene present in the vector. Selection pressure is imposed by culturing the transformed cells under conditions in which the concentration of selection agent in the medium is successively increased, thereby leading to the amplification of both the selectable gene and the DNA that encodes a protein of interest. As a result, increased quantities of a polypeptide of interest are synthesized from the amplified DNA.

[0128] In some cases, such as where glycosylation is desired in a eukaryotic host cell expression system, one may manipulate the various pre- or pro-sequences to improve glycosylation or yield. For example, one may alter the peptidase cleavage site of a particular signal peptide, or add prosequences, which also may affect glycosylation. The final protein product may have, in the −1 position (relative to the first amino acid of the mature protein), one or more additional amino acids incident to expression, which may not have been totally removed. For example, the final protein product may have one or two amino acid residues found in the peptidase cleavage site, attached to the amino-terminus. Alternatively, use of some enzyme cleavage sites may result in a slightly truncated form of the desired polypeptide if the enzyme cuts at such area within the mature polypeptide.

[0129] Expression and cloning will typically contain a promoter that is recognized by the host organism and operably linked to the molecule encoding a protein of interest. Promoters are untranscribed sequences located upstream (i.e., 5′) to the start codon of a structural gene (generally within about 100 to 1000 bp) that control transcription of the structural gene. Promoters are conventionally grouped into one of two classes: inducible promoters and constitutive promoters. Inducible promoters initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, such as the

presence or absence of a nutrient or a change in temperature. Constitutive promoters, on the other hand, uniformly transcribe a gene to which they are operably linked, that is, with little or no control over gene expression. A large number of promoters, recognized by a variety of potential host cells, are well known.

[0130] Suitable promoters for use with mammalian host cells are well known and include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, retroviruses, hepatitis-B virus, and Simian Virus 40 (SV40). Other suitable mammalian promoters include heterologous mammalian promoters, for example, heat-shock promoters and the actin promoter.

[0131] Additional promoters which may be of interest include, but are not limited to: SV40 early promoter (Benoist and Chambon, 1981, Nature 290:304-310); CMV promoter (Thornsen *et al.*, 1984, Proc. Natl. Acad. U.S.A. 81:659-663); the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, Cell 22:787-797); herpes thymidine kinase promoter (Wagner *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1444-1445); glyceraldehyde-3-phosphate dehydrogenase (GAPDH); promoter and regulatory sequences from the metallothionine gene (Prinster *et al.*, 1982, Nature 296:39-42); and prokaryotic promoters such as the beta-lactamase promoter (Villa-Kamaroff *et al.*, 1978, Proc. Natl. Acad. Sci. U.S.A.75:3727-3731); or the tac promoter (DeBoer *et al.*, 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25). Also of interest are the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: the elastase I gene control region that is active in pancreatic acinar cells (Swift *et al.*, 1984, Cell 38:639-646; Ornitz *et al.*, 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); the insulin gene control region that is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122); the immunoglobulin gene control region that is active in lymphoid cells (Grosschedl *et al.*, 1984, Cell 38:647-658; Adames *et al.*, 1985, Nature 318:533-538; Alexander *et al.*, 1987, Mol. Cell. Biol. 7:1436-1444); the mouse mammary tumor virus control region that is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, Cell 45:485-495); the albumin gene control region that is active in liver (Pinkert *et al.*, 1987, Genes and Devel. 1:268-276); the alpha-feto-protein gene control region that is active in liver (Krumlauf *et al.*, 1985, Mol. Cell. Biol. 5:1639-1648; Hammer *et al.*, 1987, Science 253:53-58); the alpha 1-antitrypsin gene control region that is active in liver (Kelsey *et al.*, 1987, Genes and Devel. 1:161-171); the beta-globin gene control region that is active in myeloid cells (Mogram *et al.*, 1985, Nature 315:338-340; Kollias *et al.*, 1986, Cell 46:89-94); the myelin basic protein gene control region that is active in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, Cell 48:703-712); the myosin light chain-2 gene control region that is active in

skeletal muscle (Sani, 1985, Nature 314:283-286); and the gonadotropic releasing hormone gene control region that is active in the hypothalamus (Mason *et al.*, 1986, Science 234:1372-1378).

[0132] An enhancer sequence may be inserted into the vector to increase transcription by higher eukaryotes. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are relatively orientation and position independent, having been found at positions both 5′ and 3′ to the transcription unit. Several enhancer sequences available from mammalian genes are known (e.g., globin, elastase, albumin, alpha-feto-protein and insulin). Typically, however, an enhancer from a virus is used. The SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers known in the art are exemplary enhancing elements for the activation of eukaryotic promoters. While an enhancer may be positioned in the vector either 5′ or 3′ to a coding sequence, it is typically located at a site 5′ from the promoter.

[0133] A sequence encoding an appropriate native or heterologous signal sequence (leader sequence or signal peptide) can be incorporated into an expression vector, to promote extracellular secretion of the protein of interest. The choice of signal peptide or leader depends on the type of host cells in which the protein of interest to be produced, and a heterologous signal sequence can replace the native signal sequence. Examples of signal peptides that are functional in mammalian host cells include the following: the signal sequence for interleukin-7 described in U.S. Patent No. 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman *et al.*, 1984, Nature 312:768; the interleukin-4 receptor signal peptide described in EP Patent No. 0367 566; the type I interleukin-1 receptor signal peptide described in U.S. Pat. No. 4,968,607; the type II interleukin-1 receptor signal peptide described in EP Patent No. 0 460 846.

[0134] Additional control sequences shown to improve expression of heterologous genes from mammalian expression vectors include such elements as the expression augmenting sequence element (EASE) derived from CHO cells (Morris *et al.*, in Animal Cell Technology, pp. 529-534 (1997); U.S. Patent Nos. 6,312,951 B1, 6,027,915, and 6,309,841 B1) and the tripartite leader (TPL) and VA gene RNAs from Adenovirus 2 (Gingeras *et al.*, 1982, J. Biol. Chem. 257:13475-13491). The internal ribosome entry site (IRES) sequences of viral origin allows dicistronic mRNAs to be translated efficiently (Oh and Sarnow, 1993, Current Opinion in Genetics and Development 3:295-300; Ramesh *et al.*, 1996, Nucleic Acids Research 24:2697-2700).

[0135] Following construction, one or more vectors may be inserted into a suitable cell for amplification and/or polypeptide expression. The transformation of an expression vector into a selected cell may be accomplished by well-known methods including transfection, infection, calcium phosphate co-precipitation, electroporation, nucleofection, microinjection, DEAE-

dextran mediated transfection, cationic lipids mediated delivery, liposome mediated transfection, microprojectile bombardment, receptor-mediated gene delivery, delivery mediated by polylysine, histone, chitosan, and peptides. The method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan and are set forth in manuals and other technical publications, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001).

[0136] The term "transformation" refers to a change in a cell's genetic characteristics, and a cell has been transformed when it has been modified to contain new DNA or RNA. For example, a cell is transformed where it is genetically modified from its native state by introducing new genetic material via transfection, transduction, or other techniques. Following transfection or transduction, the transforming DNA can recombine with that of the cell by physically integrating into a chromosome of the cell or can be maintained transiently as an episomal element without being replicated, or can replicate independently as a plasmid. A cell is considered to have been "stably transformed" when the transforming DNA is replicated with the division of the cell.

[0137] The term "transfection" refers to the uptake of foreign or exogenous DNA by a cell. A number of transfection techniques are well known in the art and are disclosed herein. See, *e.g.*, Graham *et al.*, 1973, Virology 52:456; Sambrook *et al.*, 2001, Molecular Cloning: A Laboratory Manual, supra; Davis *et al.*, 1986, Basic Methods in Molecular Biology, Elsevier; Chu *et al.*, 1981, Gene 13:197.

[0138] The term "transduction" refers to the process whereby foreign DNA is introduced into a cell via viral vector. See Jones *et al.*, (1998). Genetics: principles and analysis. Boston: Jones & Bartlett Publ.

[0139] A wide variety of mammalian cell lines suitable for growth in culture are available from the American Type Culture Collection (Manassas, Va.) and commercial vendors. Examples of cell lines commonly used in the industry include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, (Graham *et al*, 1977, J. Gen Virol. 36:59); baby hamster kidney cells (BHK, ATCC CCL 10); mouse Sertoli cells (TM4, Mather, 1980, Biol. Reprod. 23:243-251); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human hepatoma cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, 1982, Annals N.Y Acad. Sci. 383:44-68); MRC 5 cells or FS4 cells;

mammalian myeloma cells, and a number of other cell lines and Chinese hamster ovary (CHO) cells.

[0140] Large-scale production of proteins for commercial applications is typically carried out in suspension culture. Therefore, mammalian host cells used to generate the recombinant mammalian cells described herein can, but need not be, adapted to growth in suspension culture. A variety of host cells adapted to growth in suspension culture are known, including mouse myeloma NS0 cells and CHO cells from CHO-S, DG44, and DXB11 cell lines. Other suitable cell lines include mouse myeloma SP2/0 cells, baby hamster kidney BF1K-21 cells, human PER.C6® cells, human embryonic kidney F1EK-293 cells, and cell lines derived or engineered from any of the cell lines disclosed herein.

[0141] CHO cells are widely used to produce complex recombinant proteins, including CHOK1 cells (ATCC CCL61). The dihydrofolate reductase (DHFR)-deficient mutant cell lines (Urlaub *et al*., 1980, *Proc Natl Acad Sci USA* 77: 4216-4220), DXB11 and DG-44, are desirable CHO host cell lines because the efficient DHFR selectable and amplifiable gene expression system allows high level recombinant protein expression in these cells (Kaufman R. J., 1990, *Meth Enzymol* 185:537-566). Also included are the glutamine synthase (GS)-knockout CHOK1SV cell lines, making use of glutamine synthetase (GS)-based methionine sulfoximine (MSX) selection. Other suitable CHO host cells could include, but are not limited to, the following (ECACC accession numbers in brackets): CHO (85050302), CHO (PROTEIN FREE) (00102307), CHO-K1 (85051005), CHO-K1/SF (93061607), CHO/dhfr- (94060607), CHO/dhfr-AC-free (05011002), RR-CHOKI (92052129).

[0142] The production of a recombinant protein begins with establishing a mammalian cell production culture of cells that express the protein, in a culture plate, flask, tube, bioreactor or other suitable vessel. Smaller production bioreactors are typically used, in one embodiment the bioreactors are 500L to 2000L. In another embodiment, 1000L – 2000L bioreactors are used. The seed cell density used to inoculate the bioreactor can have a positive impact on the level of recombinant protein produced. In one embodiment the bioreactor is inoculated with at least 0.5 $x10^6$ up to and beyond 3.0 x 10^6 viable cells/mL in a serum-free culture medium. In another embodiment the inoculation is 1.0 x 10^6 viable cells/mL.

[0143] The mammalian cells then undergo an exponential growth phase. The cell culture can be maintained without supplemental feeding until a desired cell density is achieved. In one embodiment the cell culture is maintained for up to three days with or without supplemental feeding. In another embodiment the culture can be inoculated at a desired cell density to begin the production phase without a brief growth phase. In any of the embodiments herein the switch from the growth phase to production phase can also be initiated by any of the afore-mentioned methods.

[0144] At the transition between the growth phase and the production phase, and during the production phase, the percent packed cell volume (%PCV) is equal to or less than 35%. The desired packed cell volume maintained during the production phase is equal to or less than 35%. In one embodiment the packed cell volume is equal to or less than 30%. In another embodiment the packed cell volume is equal to or less than 20%. In yet another embodiment the packed cell volume is equal to or less than 15%. In a further embodiment the packed cell volume is equal to or less than 10%.

[0145] Three methods are typically used in commercial processes for the production of recombinant proteins by mammalian cell culture: batch culture, fed-batch culture, and perfusion culture. Batch culture is a discontinuous method where cells are grown in a fixed volume of culture media for a short period of time followed by a full harvest. Cultures grown using the batch method experience an increase in cell density until a maximum cell density is reached, followed by a decline in viable cell density as the media components are consumed and levels of metabolic by-products (such as lactate and ammonia) accumulate. Harvest typically occurs at the point when the maximum cell density is achieved (e.g., $5x10^6$ cells/mL or greater, depending on media formulation, cell line, etc.). The batch process is the simplest culture method, however viable cell density is limited by the nutrient availability and once the cells are at maximum density, the culture declines and production decreases. There is no ability to extend a production phase because the accumulation of waste products and nutrient depletion rapidly lead to culture decline, (typically around 3 to 7 days).

[0146] Fed-batch culture improves on the batch process by providing bolus or continuous media feeds to replenish those media components that have been consumed. Since fed-batch cultures receive additional nutrients throughout the run, they have the potential to achieve higher cell densities (>10 to $30x10^6$ cells/ml, depending on media formulation, cell line, etc.) and increased product titers, when compared to the batch method. Unlike the batch process, a biphasic culture can be created and sustained by manipulating feeding strategies and media formulations to distinguish the period of cell proliferation to achieve a desired cell density (the growth phase) from the period of suspended or slow cell growth (the production phase). As such, fed batch cultures have the potential to achieve higher product titers compared to batch cultures. Typically, a batch method is used during the growth phase and a fed-batch method used during the production phase, but a fed-batch feeding strategy can be used throughout the entire process. However, unlike the batch process, bioreactor volume is a limiting factor which limits the amount of feed. Also, as with the batch method, metabolic by-product accumulation will lead to culture decline, which limits the duration of the production phase, about 10 to 21 days. Fed-batch cultures are discontinuous, and harvest typically occurs when metabolic by-product levels or culture viability reach predetermined levels. When

compared to a batch culture, in which no feeding occurs, a fed batch culture can produce greater amounts of recombinant protein. *See, e.g.,* U.S. Patent No. 5,672,502.

[0147] Perfusion methods offer potential improvement over the batch and fed-batch methods by adding fresh media and simultaneously removing spent media. Typical large scale commercial cell culture strategies strive to reach high cell densities, 60 – 90(+) x 10^6 cells/mL where almost a third to over one-half of the reactor volume is biomass. With perfusion culture, extreme cell densities of >1 x 10^8 cells/mL have been achieved and even higher densities are predicted. Typical perfusion cultures begin with a batch culture start-up lasting for a day or two followed by continuous, step-wise and/or intermittent addition of fresh feed media to the culture and simultaneous removal of spent media with the retention of cells and additional high molecular weight compounds such as proteins (based on the filter molecular weight cutoff) throughout the growth and production phases of the culture. Various methods, such as sedimentation, centrifugation, or filtration, can be used to remove spent media, while maintaining cell density. Perfusion flow rates of a fraction of a working volume per day up to many multiple working volumes per day have been reported.

[0148] An advantage of the perfusion process is that the production culture can be maintained for longer periods than batch or fed-batch culture methods. However, increased media preparation, use, storage and disposal are necessary to support a long-term perfusion culture, particularly those with high cell densities, which also need even more nutrients, and all of this drives the production costs even higher, compared to batch and fed batch methods. In addition, higher cell densities can cause problems during production, such as maintaining dissolved oxygen levels and problems with increased gassing including supplying more oxygen and removing more carbon dioxide, which would result in more foaming and the need for alterations to antifoam strategies; as well as during harvest and downstream processing where the efforts required to remove the excessive cell material can result in loss of product, negating the benefit of increased titer due to increased cell mass.

[0149] Also provided is a large-scale cell culture strategy that combines fed batch feeding during the growth phase followed by continuous perfusion during the production phase. The method targets a production phase where the cell culture is maintained at a packed cell volume of less than or equal to 35%.

[0150] In one embodiment, a fed-batch culture with bolus feeds is used to maintain a cell culture during the growth phase. Perfusion feeding can then be used during a production phase. In one embodiment, perfusion begins when the cells have reached a production phase. In another embodiment, perfusion begins on or about day 3 to on or about day 9 of the cell culture. In another embodiment perfusion begins on or about day 5 to on or about day 7 of the cell culture.

[0151] Using bolus feeding during the growth phase allows the cells to transition into the production phase, resulting in less dependence on a temperature shift as a means of initiating and controlling the production phase, however a temperature shift of about 36°C to about 31°C can take place between the growth phase and production phase. In one embodiment the shift is from 36°C to 32°C.

[0152] As described herein, the bioreactor can be inoculated with at least 0.5 x 10^6 up to and beyond 3.0 x 10^6 viable cells/mL in a serum-free culture medium, for example 1.0 x 10^6 viable cells/mL.

[0153] Perfusion culture is one in which the cell culture receives fresh perfusion feed medium while simultaneously removing spent medium. Perfusion can be continuous, stepwise, intermittent, or a combination of any or all of any of these. Perfusion rates can be less than a working volume to many working volumes per day. The cells are retained in the culture and the spent medium that is removed is substantially free of cells or has significantly fewer cells than the culture. Recombinant proteins expressed by the cell culture can also be retained in the culture. Perfusion can be accomplished by a number of means including centrifugation, sedimentation, or filtration, *See, e.g.,* Voisard *et al.*, 2003, Biotechnology and Bioengineering 82:751-65. An example of a filtration method is alternating tangential flow filtration. Alternating tangential flow is maintained by pumping medium through hollow-fiber filter modules. *See, e.g.*, US Patent No. 6,544,424; Furey, 2002, Gen. Eng. News. 22 (7):62-63.

[0154] "Perfusion flow rate" is the amount of media that is passed through (added and removed) from a bioreactor, typically expressed as some portion or multiple of the working volume, in a given time. "Working volume" refers to the amount of bioreactor volume used for cell culture. In one embodiment the perfusion flow rate is one working volume or less per day. Perfusion feed medium can be formulated to maximize perfusion nutrient concentration to minimize perfusion rate.

[0155] Cell cultures can be supplemented with concentrated feed medium containing components, such as nutrients and amino acids, which are consumed during the course of the production phase of the cell culture.

[0156] Concentrated feed medium may be based on just about any cell culture media formulation. Such a concentrated feed medium can contain most of the components of the cell culture medium at, for example, about 5X, 6X, 7X, 8X, 9X, 10X, 12X, 14X, 16X, 20X, 30X, 50X, 100x, 200X, 400X, 600X, 800X, or even about 1000X of their normal amount. Concentrated feed media are often used in fed batch culture processes.

[0157] The method described herein may be used to improve the production of recombinant proteins in multiple phase culture processes. In a multiple stage process, cells are cultured in two or more distinct phases. For example, cells may be cultured first in one or more growth

phases, under environmental conditions that maximize cell proliferation and viability, then transferred to a production phase, under conditions that maximize protein production. In a commercial process for production of a protein by mammalian cells, there are commonly multiple, for example, at least about 2, 3, 4, 5, 6, 7, 8, 9, or 10 growth phases that occur in different culture vessels preceding a final production culture.

[0158] The growth and production phases may be preceded by, or separated by, one or more transition phases. In multiple phase processes, the method according to the present invention can be employed at least during the growth and production phase of the final production phase of a commercial cell culture, although it may also be employed in a preceding growth phase. A production phase can be conducted at large scale. A large-scale process can be conducted in a volume of at least about 100, 500, 1000, 2000, 3000, 5000, 7000, 8000, 10,000, 15,000, 20,000 liters. In one embodiment production is conducted in 500L, 1000L and/or 2000L bioreactors.

[0159] A growth phase may occur at a higher temperature than a production phase. For example, a growth phase may occur at a first temperature from about 35°C to about 38°C, and a production phase may occur at a second temperature from about 29°C to about 37°C, optionally from about 30°C to about 36°C or from about 30°C to about 34°C. In addition, chemical inducers of protein production, such as, for example, caffeine, butyrate, and hexamethylene bisacetamide (HMBA), may be added at the same time as, before, and/or after a temperature shift. If inducers are added after a temperature shift, they can be added from one hour to five days after the temperature shift, optionally from one to two days after the temperature shift. The cell cultures can be maintained for days or even weeks while the cells produce the desired protein(s).

[0160] Samples from the cell culture can be monitored and evaluated using any of the analytical techniques known in the art. A variety of parameters including recombinant protein and medium quality and characteristics can be monitored for the duration of the culture. Samples can be taken and monitored intermittently at a desirable frequency, including continuous monitoring, real time or near real time.

[0161] Typically, the cell cultures that precede the final production culture (N-x to N-1) are used to generate the seed cells that will be used to inoculate the production bioreactor, the N-1 culture. The seed cell density can have a positive impact on the level of recombinant protein produced. Product levels tend to increase with increasing seed density. Improvement in titer is tied not only to higher seed density, but is likely to be influenced by the metabolic and cell cycle state of the cells that are placed into production.

[0162] Seed cells can be produced by any culture method. One such method is a perfusion culture using alternating tangential flow filtration. An N-1 bioreactor can be run using alternating tangential flow filtration to provide cells at high density to inoculate a production

bioreactor. The N-1 stage may be used to grow cells to densities of >90 x 10^6 cells/mL. The N-1 bioreactor can be used to generate bolus seed cultures or can be used as a rolling seed stock culture that could be maintained to seed multiple production bioreactors at high seed cell density. The duration of the growth stage of production can range from 7 to 14 days and can be designed so as to maintain cells in exponential growth prior to inoculation of the production bioreactor. Perfusion rates, medium formulation and timing are optimized to grow cells and deliver them to the production bioreactor in a state that is most conducive to optimizing their production. Seed cell densities of >15 x 10^6 cells/mL can be achieved for seeding production bioreactors. Higher seed cell densities at inoculation can decrease or even eliminate the time needed to reach a desired production density.

[0163] The invention also provides pharmaceutical compositions comprising any of the polypeptide variants of neuregulin or polynucleotides encoding the polypeptide variants described herein and a pharmaceutically acceptable excipient or carrier. As used herein, "pharmaceutically acceptable excipient or carrier" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990; and Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000). Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the carrier include saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of Solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of the polypeptide and the polynucleotide being administered.

Methods of Using Neuregulin Variants

[0164] The invention also provides methods for preventing, treating, or delaying development of diseases in an individual comprising administering to an individual a

pharmaceutical composition comprising a polypeptide variant of neuregulin described herein via activating ErbB2/ErbB4 receptors.

[0165] A neuregulin variant according to the invention can also be used to treat muscle cells and medical conditions affecting muscle cells. In particular, such neuregulin variant can be useful for treating muscle damage, decreasing atrophy of muscle cells, and increasing muscle cell survival, proliferation and/or regeneration. Examples of pathophysiological conditions of the musculature amenable to treatment with a neuregulin variant include skeletal muscle diseases (e.g., myopathy or dystrophy), cardiac muscle disorders (including atrial cardiac arrhythmias, cardiomyopathy, ischemic damage, congenital disease, and cardiac trauma), and smooth muscle disorders (such as arterial sclerosis, vascular lesion, or congenital vascular disease). A neuregulin variant can also be employed to reduce hypertension and to increase functional acetylcholine receptors on muscle cells (e.g., in individuals having myasthenia gravis or tachycardia).

[0166] The term “treating cardiovascular disease” as used herein, unless otherwise indicated, means inhibiting, suppressing, delaying, reversing, or alleviating, either partially or completely, the onset of a cardiovascular disease or condition in a subject, or the progression of a pre-existing cardiovascular disease or condition, or a symptom thereof, in a subject. Non-limiting examples of cardiovascular diseases that can be treated by the methods of the disclosure include chronic heart failure, congestive heart failure (CHF), acute heart failure, myocardial infarction (MI), left ventricular systolic dysfunction, reperfusion injury associated with MI, chemotherapy -induced cardiotoxicity (adult or pediatric), radiation-induced cardiotoxicity, adjunct to surgical intervention in pediatric congenital heart disease. Non limiting examples of symptoms of cardiovascular disease include shortness of breath, cough, rapid weight gain, swelling in legs, ankles and abdomen, dizziness, fatigue, weakness, dizziness, chest pain, fainting (syncope), tachycardia and bradycardia. Methods of determining the progression of cardiovascular disease and the effectiveness of treatment will be readily apparent to one of ordinary skill in the art. For example, the progression of various cardiovascular diseases can be determined by ejection fraction/electrocardiogram (ECG), ECG/Holter monitoring, stress test, cardiac catheterization, cardiac computerized tomography (CT) scan and cardiac magnetic resonance imaging (MRI).

[0167] In certain embodiments, the cardiovascular diseases that can be prevented, treated, or delayed for development via preferentially activating ErbB2/ErbB4 receptors include, but are not limited to, heart failure, myocardial infarction, dilated or hypertrophic cardiomyopathy, and myocarditis (e.g., viral myocarditis), cardiac toxicity.

[0168] As used herein, an “individual' or “subject” is a mammal, more preferably a human. Mammals include, but are not limited to, farm animals (such as cows, pigs, sheep, goats), sport animals, pets (such as cats, dogs, horses), primates, mice and rats.

[0169] According to the present invention, a polypeptide variant of neuregulin-1 described herein, or a nucleic acid encoding a polypeptide variant, alone or in combination with other agents, carriers or excipients, may be formulated for any suitable administration route such as subcutaneous injection, intravenous injection, intramuscular injection, intradermal injection, oral or topical administration. The method may employ formulations for injectable administration in unit dosage form, in ampules or in multidose containers, with an added preservative. The formulations may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, sterile pyrogen-free water or other solvents, before use. Topical administration in the present invention may employ the use of a foam, gel, cream, ointment, transdermal patch, or paste.

[0170] The magnitude of a therapeutic dose in the treatment or prevention will vary with the type and severity of the condition to be treated and the route of administration. The dose, and perhaps dose frequency, will also vary according to age, body weight, condition and response of the individual patient. It should be noted that the attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or adverse effects. Conversely, the physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

[0171] The dosage of a neuregulin variant composition to be employed therapeutically depends, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it is necessary for the clinician to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage can range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, but is typically between about 10 μg/kg/day to 10 mg/kg/day. Generally, the clinician begins with a low dosage of a pharmaceutical neuregulin variant composition and increases the dosage until the desired therapeutic effect is achieved.

[0172] In practical use, a polypeptide variant of neuregulin, a fusion protein comprising a polypeptide variant of neuregulin, or a nucleic acid encoding either of the foregoing, alone or in combination with other agents, may be combined as the active agent in intimate admixture with a pharmaceutical carrier or excipient, such as beta-cyclodextrin and 2-hydroxy-propyl-beta-cyclodextrin, according to conventional pharmaceutical compounding techniques. The carrier may take a wide form of preparation desired for administration, topical or parenteral. In preparing compositions for parenteral dosage form, such as intravenous injection or infusion, similar pharmaceutical media may be employed, water, glycols, oils, buffers, sugar, preservatives, liposomes, and the like known to those of skill in the art. Examples of such

parenteral compositions include, but are not limited to dextrose 5% w/v, normal saline or other solutions. The total dose of the polypeptide variant of neuregulin-1, or a nucleic acid encoding the polypeptide variant, alone or in combination with other agents to be administered may be administered in a vial of intravenous fluid, ranging from about 1 ml to 2000 ml. The volume of dilution fluid will vary according to the total dose administered.

[0173] Furthermore, a neuregulin variant can be capable of, enhancing the survival, proliferation, and or differentiation of cells having suitable ErbB receptors. The phrase "enhancing survival of cells" refers to increasing the period of existence of cells, either in vitro or in vivo, relative to the period of existence of cells that have not been exposed to the neuregulin variant ("untreated cells").

[0174] The expression "enhancing proliferation of cells" means increasing the rate or number of mitotic divisions, either in vitro or in vivo, relative to untreated cells. An increase in cell proliferation in cell culture can be detected by counting the number of cells before and after exposure to the neuregulin variant or by microscopic examination of the degree of confluency. Cell proliferation can also be quantified by measuring ^{3}H-thymidine uptake by the cells.

[0175] The phrase "enhancing differentiation of cells" refers to increasing the extent of cell specialization. Cell specialization is characterized by the acquisition of one or more characteristics that differ from those of the original cells. Thus, the extent of cell specialization is typically determined by screening for a change in the phenotype of the cell (e.g., identifying a change in cellular morphology).

[0176] The present invention is not to be limited in scope by the specific embodiments described herein that are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

Example 1: Yeast Display Engineering and Generation of NRG variants

[0177] The neuregulin variant sequences were displayed on the surface of yeast through a fusion to alpha agglutinin. The degenerate codons were introduced at structurally identified positions to alternate between NRG1 and NRG4 sequences or full 20 amino acid randomization through use of an NNK codon. Three visualization markers were used for

selection of binding competent/selective sequences. The first was an anti-HA antibody conjugated to Brilliant Violet 421 to measure surface display levels of neuregulin variants. The second was soluble recombinant ErbB3 receptor ECD conjugated to Alexafluor 488 for negative selection. The third was soluble recombinant ErbB4 receptor ECD conjugated to Alexafluor 647 for positive selection. Multiple rounds of positive sorting with constant ErbB4 concentrations (2.5 nM) and negative sorting against increasing ErbB3 concentrations (250, 500, 750 nM) resulted in obtaining selective neuregulin variants. Selected neuregulin variants were cloned into mammalian expression vectors using standard golden gate cloning methods from gblock DNAs. Final constructs incorporated various NRG domain truncation points, a variety of linkers, and different fusion domains and tags (Fc, scFc, 6xhis) as shown in Table 1. Stable expression was done using standard lipofectamine methods into a suspension adapted CHO K1 cell line under puromycin selection. After a 7-day production, proteins from filtered condition media were purified through a triple tandem LFAS system (ProA, In Line Dilution, SEC) or a serial individual column method of ProA (or NiNTA for 6xhis tagged proteins), Buffer exchange, CIEX, and/or HIC followed by a formulation into a 10 mM acetate 9% sucrose pH 5.2 buffer. Protein concentration was determined by A280.

In vitro phosphorylation of Akt in neonatal rat cardiomyocytes and rat Schwann cells by neuregulin variants

[0178] Specificity of neuregulin variants towards cardiomyocytes (ErbB4) versus Schwann cells (ErbB3) was tested by measuring Akt activity generated by formation of the ErbB2/ErbB4 and ErbB2/ErbB3 complexes. Upon activation by neuregulin, the ErbB4 and ErbB3 receptors dimerize preferentially with the ErbB2 co-receptor, forming the ErbB2/ErbB4 complex in cardiomyocytes and the ErbB2/ErbB3 complex in Schwann cells, both of which signal through the Akt pathway. Neonatal rat cardiomyocytes were isolated from hearts of Sprague Dawley rat pups at 0 to 4 days old according to manufacturer's protocol using the Neomyt kit and plated on Surecoat plates in NS media containing serum (all from Cellultron Life Technologies, Baltimore, MD). Rat Schwann cells were plated in Poly-D-Lysine coated plates with Dulbecco's modified Eagle's media (DMEM) supplemented with fetal bovine serum (Thermo Fisher) and forskolin (Millipore Sigma). After a 24-hour incubation in a humidified incubator maintained at 37°C and 5% CO_2, cardiomyocytes and Schwann cells were washed with serum free media and incubated an additional 16 hours in DMEM supplemented with bovine serum albumin (Sigma). Both cell lines were then treated with titrations of the neuregulin variant molecules for 15 minutes, upon which cells were lysed in lysis buffer (Meso Scale Discovery (MSD)). For detection of Akt phosphorylation, lysates were added to the Multi-Spot 96-Well 4-Spot Phospho (Serine 473)/Total Akt plates (MSD), incubated according to the manufacturer's protocol and read in

the SECTOR Imager 6000 (MSD). For both cell assays, the percent phosphorylation of Akt was calculated by multiplying the phospho Akt signal by 2, then dividing by the phospho Akt signal added to the total Akt signal and then multiplying by 100. The phosphorylation of Akt data points were fitted using a log (agonist) versus response, variable slope (4 parameters) analysis in GraphPad Prism to generate EC_{50} curves. The CM % agonism reflect the top value of the data points.

[0179] The results are presented in Table 3 below.

Table 3

	Cardio-myocyte (CM) (ErbB4)	Schwann cells (SC) (ErbB3)	Selectivity	CM % Agonism	SEQ ID NO:
	Avg EC_{50} (M)	Avg EC_{50} (M)	SC/CM	AVG	
huNRG1(S177-F229)(NRGE1C)::3x(G4Q)::huFcSEFL2 (Pb)	3.30E-10	>1E-5	>10000	77.3	1
huNRG1(S177-F229)(NRGE1C)::1KmodT482V,M493L)::huFcSEFL2 (Pb)	4.00E-10	>1E-5	>10000	75.8	2
huNRG1(S177-F229)(NRGE1C)::(G4A)2:G4::huFcSEFL2 (Pb)	4.90E-10	>1E-5	>10000	82.7	3
huNRG1(S177-F229)(NRGE1C)((G4E)2)::G4::huFcSEFL2 (Pb)	3.60E-10	>1E-5	>10000	81.9	4
huNRG1(S177-F229)(NRGE1C)::(G4S)2::G4::huFcSEFL2 (Pb)	6.20E-10	>1E-5	>10000	79.4	5
huNRG1(S177-F229)(NRGE1C)(G4E)::G4S::G4::huFcSEFL2 (Pb)	4.20E-10	>1E-5	>10000	82.2	6
huNRG1(S177-F229)(NRGE1C)(NRGmod)::G4::huFcSEFL2 (Pb)	8.70E-10	>1E-5	>10000	75.5	7
huNRG1(S177-F229)(NRGE1D)::3x(G4Q)::huFcSEFL2 (Pb)	2.90E-10	4.10E-07	1401	74.4	8
huNRG1(S177-F229)(NRGE1D)::1KmodT482V,M493L)::huFcSEFL2 (Pb)	3.40E-10	>1E-5	>10000	83.4	9
huNRG1(S177-F229)(NRGE1D)::(G4A)2:G4::huFcSEFL2 (Pb)	3.20E-10	>1E-5	>10000	78.8	10
huNRG1(S177-F229)(NRGE1D)((G4E)2)::G4::huFcSEFL2 (Pb)	2.80E-10	>1E-5	>10000	84.6	11
huNRG1(S177-F229)(NRGE1D)::(G4S)2::G4::huFcSEFL2 (Pb)	4.60E-10	>1E-5	>10000	80.5	12
huNRG1(S177-F229)(NRGE1D)(G4E)::G4S::G4::huFcSEFL2 (Pb)	3.50E-10	>1E-5	>10000	85	13
huNRG1(S177-F229)(NRGE1D)(NRGmod)::G4::huFcSEFL2 (Pb)	5.40E-10	>1E-5	>10000	74.9	14
Wild-type as control	2.80E-10	2.70E-10	1	100	
huNRG1(S177-Q237)(3H8)::3xG4S::huFcSEFL2 (Pb)	2E-10	>1E-5	>10000		15
huNRG1(S177-F229)(3H8)::3x(G4Q)::huFcSEFL2 (Pb)	2.8E-10	1.49E-7	527		16
huNRG1(S177-Q237)(3H8)::3x(G4Q)::huFcSEFL2 (Pb)	2.4E-10	1E-7	414		17
huNRG1(S177-S228)(3H8)::3x(G4Q)::huFcSEFL2 (Pb)	4.35E-08	2.77E-8	0.6		18
huNRG1(S177-Q237)(NRGE1C)::GG::huFcSEFL2 (Pb)	4.1E-10	>1E-5	>10000		19

huNRG1(S177-F229)(NRGE1C)::3x(G4Q)::huFcSEFL2 (Pb)	2.4E-10	>1E-5	>10000		1
huNRG1(S177-Q237)(NRGE1C)::3x(G4Q)::huFcSEFL2 (Pb)	2.2E-10	>1E-5	>10000		20
huNRG1(S177-S228)(NRGE1C)::3x(G4Q)::huFcSEFL2 (Pb)	1.48E-08	7.83E-7	53		21
huNRG1(S177-Q237)(NRGE1D)::GG::huFcSEFL2 (Pb)	3.3E-10	>1E-5	>10000		22
huNRG1(S177-F229)(NRGE1D)::3x(G4Q)::huFcSEFL2 (Pb)	3E-10	4.46E-7	1491		8
huNRG1(S177-Q237)(NRGE1D)::3x(G4Q)::huFcSEFL2 (Pb)	2.1E-10	>1E-5	>10000		23
huNRG1(S177-S228)(NRGE1D)::3x(G4Q)::huFcSEFL2 (Pb)	2.29E-07	>1E-5	>100		24
huNRG1(S177-Q237)(wt)::GG::huFcSEFL2 (Pb)	6E-10	1.5E-10	0.3		25
huNRG1(S177-F229)(wt)::3x(G4Q)::huFcSEFL2 (Pb)	3.4E-10	1E10	0.3		26
huNRG1(S177-Q237)(wt)::3x(G4Q)::huFcSEFL2 (Pb)	2.1E-10	7E-11	0.3		27
huNRG1(S177-S228)(wt)::3x(G4Q)::huFcSEFL2 (Pb)	2.6E-9	7E-11	0.03		28
huNRG1(S177-Q237)(NRGE1A)::GG::huFcSEFL2 (Pb)	1.84E-09	>1E-05	>5500	72	29
huNRG1(S177-Q237)(NRGE1B)::GG::huFcSEFL2 (Pb)	1.48E-09	>1E-05	>6700	76	30
huNRG1(S177-Q237)(NRGE1C)::GG::huFcSEFL2 (Pb)	4.76E-10	>1E-05	>10000	82	19
huNRG1(S177-Q237)(NRGE1D)::GG::huFcSEFL2 (Pb)	3.03E-10	>1E-05	>10000	83	22
huNRG1(S177-Q237)(NRGE1E)::GG::huFcSEFL2 (Pb)	9.95E-10	>1E-05	>10000	83	31
huNRG1(S177-Q237)(NRGE1F)::GG::huFcSEFL2 (Pb)	6.50E-10	>1E-05	>10000	90	32
huNRG1(S177-Q237)(NRGE1G)::GG::huFcSEFL2 (Pb)	2.18E-10	>1E-05	>10000	84	33
huNRG1(S177-Q237)(NRGE1H)::GG::huFcSEFL2 (Pb)	2.86E-10	>1E-05	>10000	88	34
huNRG1(S177-Q237)(NRGE1I)::GG::huFcSEFL2 (Pb)	2.29E-08	>1E-05	437	67	35
huNRG1(S177-Q237)(NRGE1J)::GG::huFcSEFL2 (Pb)	1.41E-08	>1E-05	710	68	36
huNRG1(S177-Q237)(NRGE1K)::GG::huFcSEFL2 (Pb)	7.54E-09	>1E-05	1320	66	37
huNRG1(S177-Q237)(NRGE1L)::GG::huFcSEFL2 (Pb)	2.29E-09	>1E-05	>4370	74	38
huNRG1(S177-Q237)(NRGE1M)::GG::huFcSEFL2 (Pb)	2.56E-08	1.01E-06	39	30	39
huNRG1(S177-Q237)(NRGE1N)::GG::huFcSEFL2 (Pb)	1.73E-09	7.51E-07	435	26	40
huNRG1(S177-Q237)(NRGE1O)::GG::huFcSEFL2 (Pb)	1.04E-09	5.46E-07	526	27	41
huNRG1(S177-Q237)(NRGE1P)::GG::huFcSEFL2 (Pb)	2.22E-09	5.10E-07	229	28	42
Wild-type as control	2.53E-10	6.52E-10	2.6	100	
huNRG1(S177-Q237)(1D3)::GG::huFcSEFL2 (Pb)	1.62E-09	>1E-5	>6170	78	43
huNRG1(S177-F229)(NRGE1C) (D48T_F49Q_M50I) huIgG1z SELF2 Fc	2.4E-10	1E-5	>10000	83.6	44
huNRG1(S177-F229)(NRGE1C) (E47A_D48P_F49H_M50I) huIgG1z SELF2 Fc	2.3E-10	1E-5	>10000	85.8	45
huNRG1(S177-F229)(NRGE1C) (E47K_D48T_F49S_M50L) huIgG1z SELF2 Fc	2.5E-10	1E-5	>10000	92.9	46
huNRG1(S177-F229)(NRGE1C) (E47Q_D48S_M50P) huIgG1z SELF2 Fc	3E-10	1E-5	>10000	83.2	47
huNRG1(S177-F229)(NRGE1C) (E47S_D48A_F49L_M50T) huIgG1z SELF2 Fc	4.8E-10	1E-5	>10000	87.8	48
huNRG1(S177-F229)(NRGE1C) (E47S_D48T_F49R_M50V) huIgG1z SELF2 Fc	1.9E-10	1E-5	>10000	76	49
huNRG1(S177-F229)(NRGE1C) huIgG1z SELF2 Fc	2.8E-10	1E-5	>10000	77.2	50
huNRG1(S177-F229)(NRGE1D) (E47K_D48L_F49D_M50I) huIgG1z SELF2 Fc	1.5E-10	9.33E-09	62.2	88.5	51

huNRG1(S177-F229)(NRGE1D) (E47S_D48S_F49E_M50L) huIgG1z SELF2 Fc	3.7E-10	1E-5	>10000	86.3	52
huNRG1(S177-F229)(NRGE1D) (E47T_D48S_F49L_M50L) huIgG1z SELF2 Fc	3.9E-10	1E-5	>10000	79.5	53
huNRG1(S177-F229)(NRGE1D) huIgG1z SELF2 Fc	4.8E-10	1E-5	>10000	82.2	54
huNRG1(S177-F229) huIgG1z SELF2 Fc	3.1E-10	1.4E-10	0.45161	91.4	55
huNRG1(S177-F229)(NRGE1C) 6xHis	1.444E-09	1E-5	>10000	106	56
huNRG1(S177-F229)(NRGE1C) (D48T_F49Q_M50I) 6xHis	7.123E-10	1E-5	>10000	105.7	57
huNRG1(S177-F229)(NRGE1C) (E47A_D48P_F49H_M50I) 6xHis	1.104E-09	1E-5	>10000	96.47	58
huNRG1(S177-F229)(NRGE1C) (E47K_D48T_F49S_M50L) 6xHis	8.251E-10	1E-5	>10000	91.82	59
huNRG1(S177-F229)(NRGE1C) (E47Q_D48S_M50P) 6xHis	#N/A	#N/A	#N/A	#N/A	60
huNRG1(S177-F229)(NRGE1C) (E47S_D48A_F49L_M50T) 6xHis	6.948E-10	1E-5	>10000	85.89	61
huNRG1(S177-F229)(NRGE1C) (E47S_D48T_F49R_M50V) 6xHis	5.795E-10	1E-5	>10000	87.47	62
huNRG1(S177-F229)(NRGE1D) 6xHis	3.896E-10	1E-5	>10000	90.65	63
huNRG1(S177-F229)(NRGE1D) (E47K_D48L_F49D_M50I) 6xHis	3.11E-10	1.723E-08	55.4019	91.65	64
huNRG1(S177-F229)(NRGE1D) (E47S_D48S_F49E_M50L) 6xHis	5.572E-10	1E-5	>10000	96.05	65
huNRG1(S177-F229)(NRGE1D) (E47T_D48S_F49L_M50L) 6xHis	3.295E-10	1E-5	>10000	99.83	66
huNRG1(S177-F229)(NRGE1D) (E47V_D48T_F49R_M50L) 6xHis	2.24E-10	1E-5	>10000	97.95	67
huNRG1(S177-F229) 6xHis	5.865E-10	3.616E-9	6.16539	97.51	68
:huFcSEFL2(desK):G4SG4:huNRG1(S177-F229)(NRGE1C)	2.90E-10	inactive	>10000	71.8	69
:huFcSEFL2(desK):G4SG4:huNRG1(S177-F229)(NRGE1D)	2.30E-10	inactive	>10000	76.2	70
:huFcSEFL2(desK):G4SG4:huNRG1(S177-Q237)(NRGE1C)	1.10E-10	inactive	>10000	84.9	71
:huFcSEFL2(desK):G4SG4:huNRG1(S177-Q237)(NRGE1D)	1.00E-10	inactive	>10000	90.5	72
:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-F229)(NRGE1C)	6.40E-09	inactive	>10000	62.7	73
:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-F229)(NRGE1D)	4.30E-09	inactive	>10000	72.3	74
:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-Q237)(NRGE1C)	3.00E-10	inactive	>10000	85.4	75
:huFcSEFL2(desK)v131(+):G4SG4:huNRG1(S177-Q237)(NRGE1D)	2.30E-10	inactive	>10000	88.7	76
:huNRG1(S177-F229)(NRGE1C) ::2X(G4A)G4::huFcSEFL2v131(+)	3.40E-09	inactive	>10000	91.6	77
:huNRG1(S177-F229)(NRGE1C) ::2X(G4E)G4::huFcSEFL2v131(+)	2.90E-09	inactive	>10000	94	78
:huNRG1(S177-F229)(NRGE1D) ::2X(G4A)G4::huFcSEFL2v131(+)	2.00E-09	inactive	>10000	87.1	79
:huNRG1(S177-F229)(NRGE1D) ::2X(G4E)G4::huFcSEFL2v131(+)	1.90E-09	inactive	>10000	91.9	80
Wild-type as control	1.50E-10	3.18E-10	2.12	100	
huNRG1(S177-S228)(wt)((G4E)2:G4)::huFcSEFL2 (Pb)	8.40E-11	2.22E-11	0.26429	92.6	81

huNRG1(S177-F229)(NRGE1C)((G4E)2, GGT->GGC silent mutation before terminal lysine)::G4::huFcSEFL2	7.30E-11	inactive	>10000	88.7	4
huNRG1(S177-F229)(NRGE1D)((G4E)2:G4)::huFcSEFL2 (Pb)	7.90E-11	inactive	>10000	88.2	11
huNRG1(S177-Q237)(1A1)::GG::huFcSEFL2 (Pb)	1.33E-10	9.11E-09	69	Full	82
huNRG1(S177-Q237)(1A12)::GG::huFcSEFL2 (Pb)	7.22E-10	4.79E-08	66	Full	83
huNRG1(S177-Q237)(1A7)::GG::huFcSEFL2 (Pb)	6.19E-10	1.00E-06	1616	Full	84
huNRG1(S177-Q237)(1B4)::GG::huFcSEFL2 (Pb)	9.96E-10	1.00E-05	>10000	Full	85
huNRG1(S177-Q237)(1B9)::GG::huFcSEFL2 (Pb)	1.92E-09	1.00E-06	520	Partial (<50%)	86
huNRG1(S177-Q237)(1C11)::GG::huFcSEFL2 (Pb)	1.10E-09	8.70E-08	79	Full	87
huNRG1(S177-Q237)(1D10)::GG::huFcSEFL2 (Pb)	7.41E-10	>3E-6	>4000	Full	88
huNRG1(S177-Q237)(1D3)::GG::huFcSEFL2 (Pb)	7.72E-10	1.00E-05	>10000	Full	43
huNRG1(S177-Q237)(1D4)::GG::huFcSEFL2 (Pb)	9.24E-10	2.26E-07	245	Full	89
huNRG1(S177-Q237)(1D4)::GG::huFcSEFL2 (Pb)	5.76E-10	3.23E-08	56	Full	89
huNRG1(S177-Q237)(2E3)::GG::huFcSEFL2 (Pb)	6.58E-10	1.00E-06	1521	Partial (<50%)	90
huNRG1(S177-Q237)(2E4)::GG::huFcSEFL2 (Pb)	4.29E-10	6.15E-08	143	Full	91
huNRG1(S177-Q237)(2F2)::GG::huFcSEFL2 (Pb)	2.44E-10	2.02E-07	826	Full	92
huNRG1(S177-Q237)(2G3)::GG::huFcSEFL2 (Pb)	2.08E-10	6.08E-08	292	Full	93
huNRG1(S177-Q237)(2G4)::GG::huFcSEFL2 (Pb)	2.42E-09	1.00E-06	414	Partial (<50%)	94
huNRG1(S177-Q237)(2H7)::GG::huFcSEFL2 (Pb)	3.00E-10	1.10E-07	368	Partial (<50%)	95
huNRG1(S177-Q237)(3A7)::GG::huFcSEFL2 (Pb)	3.66E-10	4.65E-08	127	Partial (<50%)	96
huNRG1(S177-Q237)(3B8)::GG::huFcSEFL2 (Pb)	1.40E-09	3.00E-06	2147	Partial (<50%)	97
huNRG1(S177-Q237)(3D6)::GG::huFcSEFL2 (Pb)	2.60E-09	>1E-5	>3800	Partial (<50%)	98
huNRG1(S177-Q237)(3D8)::GG::huFcSEFL2 (Pb)	5.10E-10	>3E-6	>5800	Partial (<50%)	99
huNRG1(S177-Q237)(3E6)::GG::huFcSEFL2 (Pb)	3.50E-10	>1E-5	>10000	Full	100
huNRG1(S177-Q237)(3E9)::GG::huFcSEFL2 (Pb)	2.68E-09	>3E-6	>8500	Partial (70%)	101
huNRG1(S177-Q237)(3F2)::GG::huFcSEFL2 (Pb)	7.38E-10	>1E-5	>10000	Partial (70%)	102
huNRG1(S177-Q237)(3G3)::GG::huFcSEFL2 (Pb)	1.84E-09	>1E-5	>5400	Partial (70%)	103
huNRG1(S177-Q237)(3G9)::GG::huFcSEFL2 (Pb)	5.81E-10	>1E-5	>10000	Full	104
huNRG1(S177-Q237)(3H5)::GG::huFcSEFL2 (Pb)	1.27E-09	>1E-5	>7800	Full	105
huNRG1(S177-Q237)(3H8)::GG::huFcSEFL2 (Pb)	2.76E-10	>1E-5	>10000	Full	106
huNRG1(S177-Q237)(_200E)::GG::huFcSEFL2 (Pb)	4.52E-10	4.82E-09	11	Full	107
huNRG1(S177-Q237)(_200E_KDF)::GG::huFcSEFL2 (Pb)	2.00E-09	1.21E-07	60	Full	108
huNRG1(S177-Q237)(_FVIEDPSI)::GG::huFcSEFL2 (Pb)	1.77E-10	4.27E-09	24	Full	109
huNRG1(S177-Q237)(wt)::GG::huFcSEFL2 (Pb)	2.75E-10	1.12E-10	0.4	Full	25
huNRG1(S177-Q237)(v80)::GG::huFcSEFL2 (Pb)	9.98E-10	6.65E-09	7	Full	110
huNRG1(S177-Q237)(v80.3)::GG::huFcSEFL2 (Pb)	3.36E-08	3.00E-06	89	Full	111
Wild-type as control	1.54E-10	3.28E-10	2	Full	

[0180] The neuregulin variants tested generally showed improved selectivity toward Erbb4, with many having a selectivity greater than 1000 and even 10000 as expressed by the ratios of SM/CM. Many of the neuregulin variants also showed agonism against cardiomyocytes that was at least 80% compared to the wild-type sequence.

[0181] These results demonstrate that neuregulin variants can be designed to be selective against heart cells. This suggests that these neuregulin variants are candidates for treatment of heart diseases and may show lower side effects against neural cells.

Example 2: ErbB4 selective agonist efficacy in rat myocardial infarction (MI) model.

[0182] An ErbB4 selective agonist (huNRG1(S177-F229)(NRGE1C)((G4E)2)::G4:: huFcSEFL2 (Pb)) was tested in a rat MI model to determine whether a benefit could be seen in improving cardiac function.

[0183] Surgically induced myocardial infarction (MI) Sprague Dawley (SD) rats (bodyweight 180-200g) were purchased from ENVIGO (Indianapolis, Indiana, USA). 14 days before MI surgery, rats were subjected to echocardiography to assess the cardiac function by ejection fraction (EF%). Noninvasive parasternal long axis B mode cine loops were acquired using Vevo 2100 system (VisualSonics Inc., Toronto, Canada). EF was calculated using the manufacturer's recommended method. Rats were randomized into three groups based on the baseline ejection fraction numbers: 1. Vehicle; 2. ErbB4 agonist 10 μg/kg; 3. ErbB4 agonist 20 μg/kg. After randomization on week 2, 16 rats were dosed with a vehicle or 10 μg /kg or 20 μg /kg, ErbB4 agonist, subcutaneously (SC).

[0184] Anesthesia exposure is known to influence cardiac function so isoflurane was maintained at 2.5% during echocardiography to minimize its influence on cardiac function. Isoflurane exposure duration per animal was <10 min during echocardiography. Animals were closely monitored during the study. No adverse events (mortality/morbidity) were noticed throughout the study duration.

[0185] After randomization at week 2, all groups' baseline cardiac function and heart rates were similar. All the enrolled rats demonstrated heart failure condition. Terminal serum exposure was significantly higher in ErbB4 treated groups compared to the vehicle group (Fig. 1A). The plasma exposure levels of ErbB4 were markedly higher in the 20 μg/kg group than in the 10 μg/kg group (Fig. 2A). One week after subcutaneous (SC) dosing, the ErbB4 selective agonist improved cardiac function in ErbB4-selective agonist treated groups compared to the vehicle group (Fig. 1B). No statistically significant ErbB4-selective agonist effect was noticed on the heart rate (Fig. 1C), suggesting ErbB4-selective agonist mediated cardiac function improvement was not due to heart rate variability among the groups. Both treatment groups show similar cardiac functional improvements despite higher plasma

exposure of the 20 μg/kg than 10 μg/kg. This suggests that higher plasma ErbB4-selective agonist exposure (>1.9ng/mL) may be required to induce significantly higher cardiac function than observed with 10 μg/kg. No adverse events (mortality/morbidity) were not noticed after ErbB4-selective agonist treatment.

[0186] ErbB4 selective agonist treatment significantly improves cardiac function in rat myocardial infarction model. No significant impact was noticed on the heart rate after ErbB4-selective agonist treatment. ErbB4 treatment was not associated with mortality and morbidity in the myocardial infarction model

Example 3: ErbB4 selective agonist, without Fc, efficacy in rat myocardial infarction (MI) model.

[0187] An ErbB4 selective agonist (huNRG1(S177-F229)(NRGE1C) (E47S_D48A_F49L_M50T) 6xHis: SEQ ID NO: 61) was tested in the rat MI model to determine whether a benefit on cardiac function may be observed in response to treatment.

[0188] Sprague Dawley (Envigo, IN) rats (body weight 142-280g) underwent surgery to induce an MI. At 7 days post-MI surgery, rats were subjected to echocardiography to assess baseline cardiac function by ejection fraction (EF%). Noninvasive parasternal long axis B mode cine loops were acquired using Vevo 3100 system (VisualSonics Inc., Toronto, Canada). EF% was calculated using the manufacturer's recommended method. Rats were randomized into groups based on the baseline EF%: [1. Vehicle]; [2. ErbB4 agonist 50 μg/kg]; [3. ErbB4 agonist 150 μg/kg]; [4. ErbB4 agonist 500 μg/kg]. After randomization at 8 days post-MI surgery, all enrolled rats demonstrated cardiac dysfunction and underwent jugular vein cannulation surgery for IV dose administration and dosing was initiated per the treatment group labels: vehicle or 50 μg /kg/day or 150 μg /kg/day or 500 μg/kg/day of ErbB4 agonist, by cannulated jugular vein, once daily for 10 days.

[0189] Serum exposure is determined on blood samples collected at 10 minutes on day 10 post treatment and data from echocardiography performed at day 11 and approx. 4 weeks post initiation of dosing is analyzed to evaluate effects on cardiac function, EF%.

CLAIMS

What is claimed is:

1. A neuregulin variant comprising an amino acid sequence of the formula: SHLVKCX$_{183}$EX$_{185}$X$_{186}$KX$_{188}$FCVNGGECX$_{197}$X$_{198}$X$_{199}$X$_{200}$X$_{201}$X$_{202}$SX$_{204}$PSRX$_{208}$LCKCPNE FTGDRCX$_{222}$X$_{223}$X$_{224}$X$_{225}$X$_{226}$ASX$_{229}$ (SEQ ID NO:177)

wherein

X$_{183}$ is A or G;

X$_{185}$X$_{186}$ is KD, KE, KH, ND, NE, NH, RD, RE, RH, RQ, SD, SE, or SH;

X$_{188}$ is S or T;

X$_{197}$X$_{198}$X$_{199}$X$_{200}$X$_{201}$X$_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMIEHL (SEQ ID NO:180), FMVEDL (SEQ ID NO:181), FMVERS (SEQ ID NO:182), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), FVVEGL (SEQ ID NO:186), YMIEDL (SEQ ID NO:187), YMIEGL (SEQ ID NO:188), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEDL (SEQ ID NO:196), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

X$_{204}$ is I or N;

X$_{208}$ is F or Y;

X$_{222}$X$_{223}$X$_{224}$X$_{225}$X$_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QDDFM (SEQ ID NO:201), QDFFL (SEQ ID NO:202), QDFFM (SEQ ID NO:203), QDVFL (SEQ ID NO:204), QDVFM (SEQ ID NO:205), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QKVFM (SEQ ID NO:216), QKVVM (SEQ ID NO:217), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X$_{229}$ is absent, F or FYKAEELYQ (SEQ ID NO:229).

2. The neuregulin variant of claim 1, which comprises an amino acid sequence of any of SEQ ID Nos: 114 to 176.

3. The neuregulin variant of claim 1, wherein

X_{183} is A or G;

$X_{185}X_{186}$ is KD, KE, KH, ND, NH, RD, RE, RH, RQ, SD, SE, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMIEHL (SEQ ID NO:180), FMVEDL (SEQ ID NO:181), FMVERS (SEQ ID NO:182), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), YMIEDL (SEQ ID NO:187), YMIEGL (SEQ ID NO:188), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP (SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QDFFL (SEQ ID NO:202), QDFFM (SEQ ID NO:203), QDVFL (SEQ ID NO:204), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

4. The neuregulin variant of claim 3, which comprises an amino acid sequence of any of SEQ ID Nos: 114, 115, 117 to 124, 126 to 135, 138, 139, 144 to 147, 149 to 156, 160 to 168, or 171 to 176.

5. The neuregulin variant of claim 1, wherein

X_{183} is A or G;

X_{185} is KE, KH, ND, RD, RH, RQ, SD, SE, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMVEDL (SEQ ID NO:181), FMVKRP (SEQ ID NO:183), FVIEDP (SEQ ID NO:184), FVIEGS (SEQ ID NO:185), YMIEDL (SEQ ID NO:187), YMIEHP (SEQ ID NO:189), YMVEDL (SEQ ID NO:190), YMVEGS (SEQ ID NO:191), YMVERP

(SEQ ID NO:192), YVIEDS (SEQ ID NO:193), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), YVVEHS (SEQ ID NO:197), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is EKDVM (SEQ ID NO:199), QAPHI (SEQ ID NO:200), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKDVM (SEQ ID NO:210), QKFFL (SEQ ID NO:211), QKFFM (SEQ ID NO:212), QKLDI (SEQ ID NO:213), QKTSL (SEQ ID NO:214), QKVFL (SEQ ID NO:215), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QNVFL (SEQ ID NO:220), QNVFM (SEQ ID NO:221), QNYVM (SEQ ID NO:222), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

6. The neuregulin variant of claim 5, which comprises an amino acid sequence of any of SEQ ID Nos: 117 to 124, 126 to 135, 144 to 147, 149 to 152, 154 to 156, 163, 167, 168, or 171 to 176.

7. The neuregulin variant of claim 1, wherein

wherein

X_{183} is A or G;

$X_{185}X_{186}$ is KH, ND, RD, RH, or SH;

X_{188} is S or T;

$X_{197}X_{198}X_{199}X_{200}X_{201}X_{202}$ is FMIEDS (SEQ ID NO:178), FMIEGP (SEQ ID NO:179), FMVEDL (SEQ ID NO:181), FVIEDP (SEQ ID NO:184), YMIEDL (SEQ ID NO:187), YMVEGS (SEQ ID NO:191), YVIEGS (SEQ ID NO:194), YVIEHL (SEQ ID NO:195), or YVVERP (SEQ ID NO:198);

X_{204} is I or N;

X_{208} is F or Y;

$X_{222}X_{223}X_{224}X_{225}X_{226}$ is QAPHI (SEQ ID NO:200), QEDFM (SEQ ID NO:206), QETQI (SEQ ID NO:207), QKDFL (SEQ ID NO:208), QKDFM (SEQ ID NO:209), QKTSL (SEQ ID NO:214), QNDFL (SEQ ID NO:218), QNDFM (SEQ ID NO:219), QQSFP (SEQ ID NO:223), QSALT (SEQ ID NO:224), QSSEL (SEQ ID NO:225), QSTRV (SEQ ID NO:226), QTSLL (SEQ ID NO:227), or QVTRL (SEQ ID NO:228); and

X_{229} is F or FYKAEELYQ (SEQ ID NO:229).

8. The neuregulin variant of claim 7, which comprises an amino acid sequence of any of SEQ ID Nos: 117 to 124, 126, 128 to 135, 146, 147, 150, 151, 163, 167, 168, or 171.

9. The neuregulin variant of claim 8, which comprises an amino acid sequence of any of SEQ ID Nos: 117, 118, 119, 120, 122, 123, 124, 126, 128, 129, 130, 131, 133, or 135.

10. The neuregulin variant of any of claims 1 to 9, further comprising a second amino acid sequence comprising a signal sequence, a half-life extension moiety, or purification tag.

11. The neuregulin variant of claim 9, wherein the second amino acid sequence comprises an Fc region or a His Tag.

12. The neuregulin variant of claim 11, which comprises an amino acid sequence of any of SEQ ID Nos: 1 to 24, 29 to 54, 56 to 67, 69 to 80, 82 to 111.

13. The neuregulin variant of claim 11, which comprises an amino acid sequence of any of SEQ ID Nos: 1 to 15, 19, 20, 22, 23, 29 to 34, 37, 38, 43 to 54, 56 to 59, 61 to 67, 69 to 80, 82 to 85, 87 to 93, or 97 to 111.

14. The neuregulin variant of claim 11, which comprises an amino acid sequence of any of SEQ ID Nos: 3, 4, 6, 9, 11 to 13, 19, 22, 31 to 34, 43 to 48, 52, 54, 56 to 59, 61 to 63, 65 to 67, 71, 72, 75 to 80, 84, 85, 88, 100, or 104 to 106.

15. The neuregulin variant of claim 11, which comprises an amino acid sequence of any of SEQ ID Nos: 3, 4, 6, 9, 11 to 13, 19, 22, 31 to 34, 43 to 48, 52, 54, 56 to 59, 61 to 63, 65 to 67, 71, 72, 75 to 80, 84, 85, 88, 100, or 104 to 106.

16. The neuregulin variant of claim 15, which comprises an amino acid sequence of SEQ ID NO:4, 11, 13, 32, 34, 45, 46, 48, 52, 72, 75, 76, 77, 78, 79, or 80.

17. The neuregulin variant of claim 15, which comprises an amino acid sequence of SEQ ID NO:56, 57, 58, 59, 61, 62, 63, 65, 66 or 67.

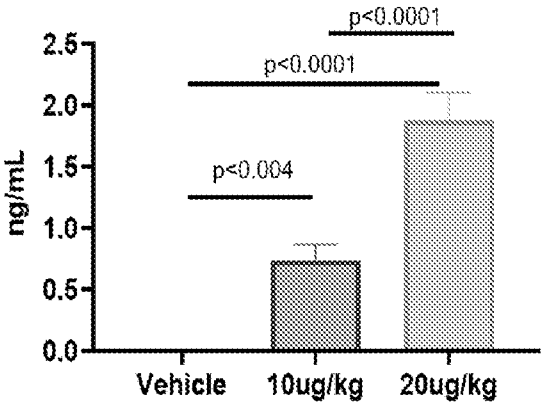
18. A pharmaceutical composition comprising the neuregulin variant of any of claims 1 to 17, and a pharmaceutically acceptable carrier.

19. A method of treating a cardiovascular disease or condition in a subject in need thereof, the method comprising administering a therapeutically effective amount of the neuregulin variant of any of claims 1 to 17, or the pharmaceutical composition of claim 18.

20. The method of claim 19, wherein the cardiovascular disease or condition is heart failure, myocardial infarction, dilated or hypertrophic cardiomyopathy, myocarditis, or cardiac toxicity.

21. The method of claim 19 or 20, wherein the subject is a human.

A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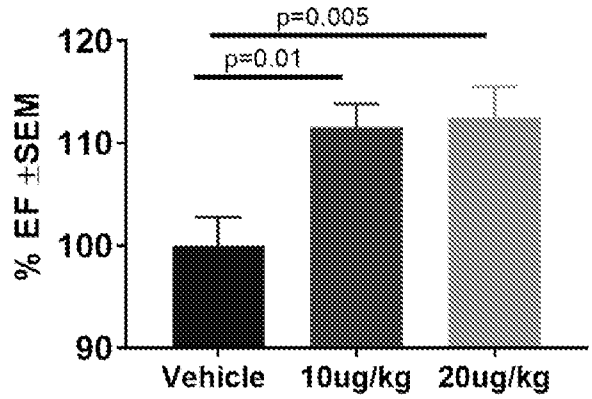
B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% EF ±SEM
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110
100
90
Vehicle
10ug/kg
20ug/kg

Figure 1

C

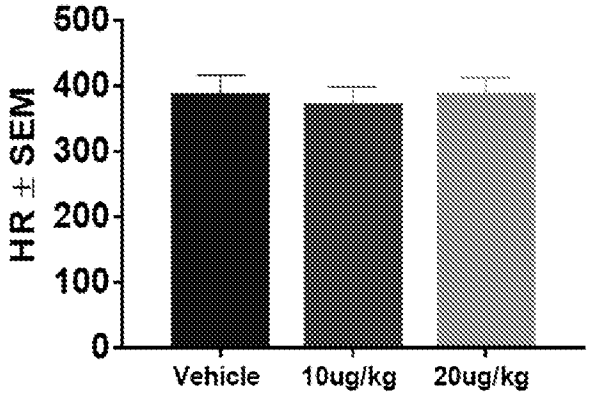

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/032801

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P9/10 C07K14/475
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/062594 A1 (ZENSUN SHANGHAI SCIENCE & TECH [CN]; ZHOU MINGDONG [CN]) 7 June 2007 (2007-06-07) abstract paragraph [0077]; claims 17-37; tables 2,4,; sequence 1	1-21
A	LUO C ET AL: "Computational Analysis of Molecular Basis of 1:1 Interactions of NRG-1beta Wild-Type and Variants With ErbB3 and ErbB4", PROTEINS: STRUCTURE, FUNCTION, AND BIOINFORMATICS, JOHN WILEY & SONS, INC, US, vol. 59, no. 4, 8 April 2005 (2005-04-08), pages 742-756, XP003013826, ISSN: 0887-3585, DOI: 10.1002/PROT.20443 abstract; figure 8; tables 2,4	1-21

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2022	21/10/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gurdjian, Didier

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/032801

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JONES J T ET AL: "Binding Interaction of the Heregulinbeta egf Domain with ErbB3 and ErbB4 Receptors Assessed by Alanine Scanning Mutagenesis", 19980508, vol. 273, no. 19, 8 May 1998 (1998-05-08), pages 11667-11674, XP003013824, abstract; figure 2	1-21
A	US 6 387 638 B1 (BALLINGER MARCUS D [US] ET AL) 14 May 2002 (2002-05-14) abstract; tables 4,5,9,12,13,14,; sequence 3	1-21

2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/032801

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/032801

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007062594 A1	07-06-2007	AU 2006319620 A1	07-06-2007
		BR PI0619136 A2	13-09-2011
		CA 2632152 A1	07-06-2007
		CN 101336251 A	31-12-2008
		EP 1963360 A1	03-09-2008
		JP 2009517055 A	30-04-2009
		US 2007213264 A1	13-09-2007
		US 2009203595 A1	13-08-2009
		US 2014031284 A1	30-01-2014
		US 2016297859 A1	13-10-2016
		WO 2007062594 A1	07-06-2007
US 6387638 B1	14-05-2002	AT 427353 T	15-04-2009
		AU 732027 B2	12-04-2001
		CA 2278151 A1	13-08-1998
		DK 0970207 T3	13-07-2009
		EP 0970207 A1	12-01-2000
		ES 2324320 T3	04-08-2009
		IL 130827 A	19-08-2007
		IL 174786 A	29-12-2011
		JP 4515542 B2	04-08-2010
		JP 2001521376 A	06-11-2001
		US 6387638 B1	14-05-2002
		US 2003129688 A1	10-07-2003
		US 2006183194 A1	17-08-2006
		WO 9835036 A1	13-08-1998